

Environmental Applications of Microbial Nanotechnology

Emerging Trends in Environmental Remediation

Edited by
Pardeep Singh
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Preface

Nanotechnology has emerged as one of the most significant technologies in the world. The last two decades have witnessed tremendous growth in nanotechnology-based industrial, agricultural, and environmental applications. The applications of nanomaterials for environmental remediation have greatly increased studies of their synthesis and manufacturing process. Nanomaterials are mostly synthesized through chemical methods, which can lead to long term environmental implications. Manufacturing nanomaterials using a green synthesis mechanism can encourage environmental safety. The green synthesis method, which involves plants and microorganisms, is considered to be safer than chemical synthesis due to lower environmental impact. Furthermore, the use of microorganisms for the biosynthesis of nanomaterials is considered a new and viable prospect for the development of a safer and greener nano-manufacturing process.

This book, *Environmental Applications of Microbial Nanotechnology, Emerging Trends in Environmental Remediation*, explores applications of microbial nanotechnology for environmental remediation. The book focuses on the use of microbial nanoparticles synthesized through microbes and their relevant applications in the remediation of environmental contaminants. The main appeal of this book is its focus on the four areas of microbial nanotechnology in a systematic manner. The book explores the relevant theme through: (1) microbe-mediated synthesis of nanoparticles, (2) applications of microbial nanotechnology for environmental remediation, (3) pollutant degradation and adsorption using nanomaterials originated from microbes, and (4) environmental sustainability with microbial nanotechnology.

The book will appeal to researchers working in the fields of green nanotechnology, microbial nanotechnology, and environmental remediation. It will also be useful for future research and innovative prospects of nanomaterials with a microbial origin and their potential for the removal of contaminants from wastewater, degradation and adsorption of pollutants degradation, soil remediation and pathogen detection. It will also provide recommendations and future perspectives needed in the field of microbial nanotechnology and its applications. It would be gratifying if this work could contribute to microbial nanotechnology's challenging and emerging field.

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Nanotechnology as sustainable strategy for remediation of soil contaminants, air pollutants, and mitigation of food biodeterioration

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1.1 Introduction

The terms nanoparticle and nanomaterial refer to very tiny in nature in the scientific community which have variety of role in the different sectors including remediation of water, soil, heavy metals in environments, and also in agricultural applications (Gong et al., 2018). Nanomaterials are considered as prime catalysts and adsorbent for removal of contaminants based on their greater surface area, lower modification of temperature, maximum sites of sorption, and shorter interparticle distance (Cai et al., 2019). Nanoparticles are much attracted for elimination of heavy materials based on their interaction, mobilization and adsorption (Nasir et al., 2019). Food commodities are primarily contaminated by infestation of storage fungi during the postharvest periods along with their associated secondary metabolites especially termed as mycotoxins (Das et al., 2021a). Species of *Fusarium*, *Penicillium* and *Aspergillus* are maximally involved in contamination of foods by hyphal proliferation and spore production. They secrete hazardous mycotoxins viz. aflatoxins, zearalenone, fumonisins, patulin, deoxynivalenol, and tricothecene in foods which have adverse impact on human health with widespread cellular abnormalities, and toxicities (Reddy et al., 2010; Chaudhari et al., 2021). Most importantly, these mycotoxins are usual precursor of reactive oxygen species (ROS) production (Gauthier et al., 2020), and efficiently interact with cellular proteins, carbohydrates, fatty acids, and bioactive ingredients leading to loss of nutritional qualities (Wu et al., 2019). Recent investigation of Das et al. (2021b) demonstrated the heavy incidence of methylglyoxal in food commodities directly lead to production of aflatoxin, and generation of advance glycation end products. On the basis of toxicity, International Agency for Research on Cancer (IARC) classified aflatoxins as class 1 carcinogen, fumonisin, and ochratoxins as class 2B carcinogen, and zearalenone, and tricothecene as class 3 carcinogen (Chaudhari et al., 2019). The congenial climatic and environmental conditions favor infestation of fungal flora and production of mycotoxins in stored food commodities (Bhandari and Srivastava, 2020).

A number of synthetic fungicides are being applied for mitigation of fungal proliferation, and mycotoxin contamination; however, their indiscriminate application may cause negative impact on human health and maximum chance for development of resistant fungal species with more effects (Badr et al., 2021). Hence, in the current generation, researchers are completely focused on plant based chemicals to avoid the drawback of synthetic fungicides with maximum green image in food and agricultural industries. Among different plant based products, essential oils isolated from higher plants are involved in inhibition of microbial growth, mycotoxin production and lipid peroxidation with green image in food and agricultural industries (Bocate et al., 2021).

Indeed, the essential oils display promising preservation potentialities, but, their practical application exhibit several challenges like low water solubility, high volatility, and negative impact on food organoleptic attributes (Jamali et al., 2021). Nanoentrapment of essential oils has special advantage for improvement in bioefficacy in food system with long

term sustained release of volatile components (Zhu et al., 2020). Nanoencapsulation simply demonstrates the entrapment of essential oils into biodegradable polymers such as chitosan, gelatin, soy protein, β -cyclodextrin, sodium alginate, and starch with the involvement of several processes viz. ionic gelation, coacervation, nanoprecipitation, liposome, supercritical solution, freeze drying, and solid lipid nanoparticles (Guo et al., 2021).

Hence, the present article focuses updated details of application of nanoparticles for removal of soil and water pollutants along with remediation of hazardous heavy metals. More importantly, application of essential oil nanoparticles against fungal and mycotoxin contamination of foods has also been discussed. Furthermore, the biochemical and molecular mechanisms also strengthen the application of nanoencapsulated essential oils as effective green preservative with novel agro-ecological prospects.

1.2 Use of nanoparticle for soil and water purification/remediation

Contamination of soil and water (ground) are very closely linked. Methods that are used for the treatments of soil contamination are indirectly work for groundwater remediation's, and it has a vice versa effect (Mueller and Nowack 2010). Water is one of the most important components of human civilization, and is a basic necessity for human life. Water scarcity escalates due to high population, climate change, and it is also the reason of detritions of water-quality (Ali and Aboul-Enein, 2004; Nemerow and Dasgupta, 1991). Only 2.5% of the world's oceans and seas harness fresh water, However, 70% of fresh water (FW) is frozen as eternal ice, only <1% of FW can be used for drinking. According to WHO (2014), globally less than 700 million people don't have access to pure potable water. Therefore, the water treatment or improvement water quality must be implemented throughout the world.

A number of technologies are available for water purification, but that are reaching their limits in supplying sufficient amount of water to meet population, and environmental needs (Qu et al., 2013). Water contamination occur either by organic, inorganic, or by biological continents. Some water contaminants are most toxic and carcinogenic in nature (Ali and Aboul-Enein, 2004; Ali et al., 2009) and some contaminants have adverse effects on humans and whole environments (Ali, 2012). One of the most toxic components and deadliest elements in water is arsenic; however, other common water pollutants are chromium, mercury, cadmium, zinc, nickel, copper and lead (Ali, 2012). High concentration of phosphates, Nitrates, sulfates, chlorides, selenides, fluorides, and chromates in water showed hazardous effects in environment and human health (Damià, 2005). NPs based water purification is a classic technique for efficient removal of water contaminants.

Major processes involved in water purification are:

1.2.1 Adsorbent process

Here, nano-adsorbents are mainly used for removing of inorganic and/or organic pollutants from water. The properties of the nano-adsorbents are smaller in size, high level catalytic potential, reactivity, large surface area, easy separation procedure, and huge number of active sites that can easily adsorbed toxic materials of soil and wastewater (Ali, 2012). Some examples of adsorbent NPs materials are carbon-based nano-adsorbents, iron oxide, zinc oxide, alumina oxide, and titanium dioxide.

1.2.2 Membrane based process

In this process, a thin membrane is placed through which water is passed and contaminants are easily trapped by nanoparticle. Electrospun nanofiber, hydrophilic metal oxide NPs like Al_2O_3 , TiO_2 , and zeolite, nano-Ag and CNTs, and bimetallic NPs, TiO_2 are commonly used nanoparticles for removal of pollutants (Giwa et al., 2021).

1.2.3 Photocatalysis and antimicrobial NPs

Nanoparticles (NPs) are effectively used for the remediation or purification of soil and groundwater. The tiny particles (NPs) are highly reactive and have great adsorption capacity (Goyal et al., 2018). However, some technical challenges, such as the delivery process should have to solve. Moreover, the cost of NPs is huge for preparing large quantities for soil and water purification.

1.3 Nanotechnology in heavy metals (HMs) removal

Water and soil are the most important natural resource that required for survival, but random industrialization, cutting of forest, urbanization cause huge problem in the soil and water. Mostly soil and water are contaminated through heavy metal. This HMs come from different sectors like industries, agriculture chemicals, mining area, and chemicals plants. Some HMs like Pb, Zn, Cu, and Hg cause a severe threat to human and environmental health because this HM can be accumulated in the soil, water and human body through food chain. Therefore, removal or detoxification of HM is of great concern, till date, different technologies have been reported and developed to solve this HM contamination problem, these are included chemical precipitation (Gonzalez-Munoz et al., 2006), ion exchange (Verbych et al., 2004), adsorption process (Namasivayam and Sangeetha, 2006), membrane filtration techniques (Sudilovskiy et al., 2007) and electrochemical treatment (Tran et al., 2017), and so on. Different NPs have been used to remove the HM from the environments this included graphene oxide Nanocomposites, carbon nanotubes, nanosized metal oxides, carbon-based nanomaterials, zero-valent metal nanomaterials (Yang et al., 2019). Application of nanotechnology to remediate particular HM is a highly complicated task and depends on a number of factors like quality standard, efficiency and cost (Huang et al., 2008). Nanomaterials are potentially involved in adsorption and reaction for removal of hazardous HM from water and soil (Fujishima et al., 2000; Kamat et al., 2002; Masciaglioli Zhang, 2003). Recent investigation suggested the application of iron nanoparticle for scavenging As^{5+} , Pb^{2+} , Cr^{6+} , and Cd^{2+} (Alidokht et al., 2011; Zhu et al., 2009; Boparai et al., 2011; Zhang et al., 2011a,b). Wu et al. (2008) demonstrated the potential Fe_3O_4 nanoparticle for mitigation of Cr from aquatic waste. Chowdhury and Yanful (2010) synthesized maghemite and magnetite nanoparticles and studied their efficacy for adsorption of chromium and arsenic from aqueous solution. Checkol et al. (2018) reported nanoparticle consisting of poly(3,4- ethylenedioxythiophene)/polystyrene sulfonate (PEDOT/PSS) and lignin biopolymer for removal of Pb^{2+} from neutral solution. Zhang et al. (2011a,b) illustrated the effect of three different ZrP—Cl, ZrP—S and ZrP—N nanocomposites (prepared by encapsulation of zirconium phosphate nanoparticles (nano-ZrP) within macroporous polystyrene resins) for removal of Pb (II) based on the ionic group interaction.

1.4 Contamination of stored foods by fungi and mycotoxins

Postharvest food commodities are maximally infested and contaminated by fungi, and their associated mycotoxins because of congenial conditions like temperature, water activity, nutrition status, concentration of hydrogen ions, water activity, pH and specific solutes effects (Van Long et al., 2017). Moreover, these storage fungi mainly damage the stored food quality by reducing the nutrient availability, grain discoloration, cracking of grains and toxic effects on germination of seeds (Mohapatra et al., 2017; Pleadin et al., 2019). Mycotoxins are involved in generation of reactive oxygen species (ROS), leading to peroxidation of lipids into free fatty acids and their nutritional qualities (Da Silva et al., 2018). Dwivedy et al. (2017) demonstrated contamination of *Cucumis melo*, *Juglans regia*, *Citrus lanatus*, *Nelumbo nucifera*, *Buchanania lanzan*, and *Pistacia vera* seeds by *Aspergillus flavus*, *A. minutus*, *A. niger*, *A. sulphureus*, *Alternaria alternata*, *Curvularia lunata*, *Monilia brunia* and *Penicillium citrinum*. Achaglinkame et al. (2017) demonstrated aflatoxin contamination in legumes and cereals by toxigenic species of *Aspergillus flavus* in conducive pH, nutrient content and water activity of substrate. Fungal contamination of powdered spices viz. garam masala, tanduri masala, cumin, basil, ginger and turmeric by *A. fumigatus*, *A. terreus*, *Penicillium corylophilum*, and *P. chrysogenum* has been reported by Hammami et al. (2014). Recently, Singh and Cotty (2019) illustrated the contamination of dried red chilies from Nigeria and US by *A. parasiticus*, *A. aflatoxiformans*, *A. minisclerotigenes*, and *A. flavus* with resultant production of aflatoxins. In an investigation of Kumar et al. (2018), it has been observed that stored millets, especially *Sorghum bicolor* and *Pennisetum glaucum* were maximally contaminated with *A. flavus*, *A. sydowii*, *A. minutus*, *Penicillium italicum*, *P. purpurogenum*, and *Chaetomium spirale* leading to production of AFB_1 . Hashem and Alamri (2010) reported contamination of common spices such as cinnamon, green saffron, fenugreek, fennel, cardamom, aniseeds and cloves by *A. awamori*, *A. candidus*, *A. ochraceus*, *A. versicolor*, *A. tamari*, *Fennellia nivea* and *Humicola grisea* along with frequent contamination by AFB_1 and AFB_2 . Deepika et al. (2020) reported growth and proliferation of different fungal species like *A. luchuensis*, *A. fumigatus*, *A. flavus*, *A. humicola*, *P. spinulosum* and *A. terreus* during postharvest storage of *Fagopyrum esculentum*, *Macrotyloma uniflorum*, *Linum usitatissimum*, and *Salvia hiapanica* seeds. Kluczkowski (2019) demonstrated maximum contamination of chestnut, cashew nut, Brazil nut, almonds, peanuts, pecan nuts, and walnut by *A. ruber*, *A. chevalieri*, *Rhizopus stolonifer*, *A. fumigatus*, *Cladosporium cladosporioides*, *A. alternata* and *A. parasiticus* during storage. More importantly, peanuts, Brazil nuts, and cashew nuts were maximally contaminated with aflatoxins on the basis of environmental factors such as temperature and relative humidity of the storage conditions. Table 1.1 presents some major food commodities contaminated with fungi and mycotoxins and their hazardous effects on health.

TABLE 1.1 Some stored food commodities contaminated with fungal pathogens and mycotoxins with their adverse health effects.

Food commodities	Fungal pathogens	Mycotoxins produced	Hazardous effects on health	References
Rice	<i>Fusarium oxysporum</i> , <i>F. poae</i> , <i>Aspergillus niger</i> , <i>A. flavus</i> , <i>A. parasiticus</i> , and <i>Penicillium italicum</i>	Aflatoxins	Carcinogenic, teratogenic, mutagenic and hepatotoxic effects on health	Das et al. (2020a)
Maize	<i>Aspergillus flavus</i>	Aflatoxins	Mutagenic, liver cirrhosis and cellular abnormalities	Chaudhari et al. (2020a)
	<i>Fusarium verticilloides</i> , <i>Fusarium proliferatum</i> and <i>A. flavus</i>	Fumonisin and aflatoxins	Mutagenic effects on body	Chulze (2010)
	<i>Fusarium</i> , <i>Penicillium</i> and <i>Aspergillus</i> spp.	Zearalenone, ochratoxin A, and 3, 15-acetyl deoxynivalenol	Neurotoxic and nephrotoxic	Tarazona et al. (2020)
Dry fruits	<i>A. flavus</i> , <i>A. niger</i> , <i>A. minutes</i> , <i>A. sulphureus</i> , <i>Alternaria alternata</i> , <i>Curvularia lunata</i> , <i>Monilia brunia</i> and <i>Penicillium citrinum</i>	Aflatoxins	Carcinogenic and teratogenic effects	Dwivedy et al. (2017)
Spices (Red chili, Cinnamon, Clove, Cardamom, and black pepper)	<i>Aspergillus</i> , <i>Fusarium</i> and <i>Penicillium</i>	Citrinin, fumonisin B, nivalenol, deoxynivalenol, sterigmatocystin and ochratoxins	Hepatocarcinogenic symptoms	Thanushree et al. (2019)
<i>Cicer arietinum</i>	<i>A. niger</i> , <i>F. oxysporum</i> , <i>P. citrinum</i> , <i>A. oryzae</i> and <i>A. flavus</i>	AFB ₁	Toxic effects on immune system	Shukla et al. (2012)
Legume seeds (<i>Glycine max</i> , <i>Lens culinaris</i> , <i>Vigna mungo</i> , <i>Pisum sativum</i> , <i>Phaseolus vulgaris</i> and <i>Vigna aconitifolia</i>)	<i>A. flavus</i> , <i>Rhizopus stolonifer</i> , <i>Alternaria alternata</i> , <i>Rhizoctonia solani</i> , <i>Fusarium oxysporum</i> and <i>A. sydowii</i>	AFB ₁	Aflatoxicosis, abnormal liver growth and carcinogenesis	Shukla et al. (2009)
Meats	<i>A. flavus</i> , <i>A. parasiticus</i> , <i>P. chrysogenum</i> , and <i>P. verrucosum</i>	Aflatoxins and ochratoxins	Nephrotoxic, immunotoxic and hepatotoxic	Perrone et al. (2019)
Edible mushroom	<i>A. parvisclerotigenus</i> , <i>A. flavus</i> , and <i>Penicillium</i> spp.	AFB ₁ , AFB ₂ , deoxynivalenol, zearalenone and T-toxin	Toxic effects on cell growth	Ezekiel et al. (2013)
<i>Arachys hypogea</i>	<i>A. parasiticus</i> , and <i>A. flavus</i>	AFB ₁	Mutagenic and teratogenic effects	Goto et al. (2000)
<i>Triticum aestivum</i>	<i>Fusarium oxysporum</i>	Deoxynivalenol	Estrogenic effect on human	Ferrigo et al. (2016)
Coffee bean, grape and vine fruits	<i>Penicillium verrucosum</i> , and <i>A. carbonarius</i>	Ochratoxins	Neurotoxic effects on body	Magan and Aldred (2007)
<i>Sorghum bicolor</i> and <i>Pennisetum glaucum</i>	<i>A. flavus</i> , <i>A. sydowii</i> , <i>A. minutus</i> , <i>Penicillium italicum</i> , <i>P. purpurogenum</i> , and <i>Chaetomium spirale</i>	AFB ₁	Aflatoxicosis, abnormal liver growth and carcinogenesis	Kumar et al. (2018)

(Continued)

TABLE 1.1 (Continued)

Food commodities	Fungal pathogens	Mycotoxins produced	Hazardous effects on health	References
<i>Fagopyrum esculentum</i> , <i>Macrotyloma uniflorum</i> , <i>Linum usitatissimum</i> , and <i>Salvia hiapanica</i> seeds	<i>A. luchuensis</i> , <i>A. fumigatus</i> , <i>A. flavus</i> , <i>A. humicola</i> , <i>P. spinulosum</i> and <i>A. terreus</i>	AFB ₁	Carcinogenic, teratogenic, mutagenic and hepatotoxic effects on health	Deepika et al. (2020)
chestnut, cashew nut, Brazil nut, almonds, peanuts, pecan nuts, and walnut	<i>A. ruber</i> , <i>A. chevalieri</i> , <i>Rhizopus stolonifer</i> , <i>A. fumigatus</i> , <i>Cladosporium cladosporioides</i> , <i>A. alternata</i> and <i>A. parasiticus</i>	AFB ₁ and AFB ₂	Liver cirrhosis	Kluczkowski (2019)

1.5 Essential oils: a green chemical for preservation of stored foods

Essential oils are secondary metabolites of higher plants, especially isolated from flowers, fruits, buds, roots, leaves, twigs and seeds (Figueiredo et al., 2008). They contain terpenoids, sesquiterpenoids and phenolics as one of the major constituents (Froio et al., 2019), leading to active inhibition of fungal proliferation and mycotoxin contamination (Kumar et al., 2019). More importantly, terpenes are basic structural component of essential oils that cover greater than 50% of the total essential oil depending on extraction type and utilization of plant parts (Aziz et al., 2018). Aromatic, aliphatic, alcoholic, phenols, aldehydes, heterocycles alcohols and methoxy derivatives are also the minor components of essential oils (Falleh et al., 2020). The antioxidant capacity of essential oils also facilitates in scavenging of biodeteriorating free radicals which corresponds to their major effect for mitigation of fungal load and mycotoxin biosynthesis (Das et al., 2021c). The phenolic constituents of essential oils are mainly participated in antimicrobial and antifungal activities (Mandras et al., 2016). Alteration in efficacy of essential oils has been depended on geographical variation, maturity of plant, time of harvesting, plant organ and chemotypic variation (Lawrencet, 2001; Das et al., 2021a). Therefore, before recommendation of essential oils as ecofriendly food preservative, one should require standardizing the chemical compositions with their respective availabilities to protect foods from fungal and mycotoxin mediated biodeterioration.

1.6 Mechanisms involving antifungal and antimycotoxigenic activities

Majority of essential oil components such as phenylpropenes, terpenoids, phenolic and aldehyde components target different cellular sites viz. plasma membrane, cell wall, mitochondria, and other cell organelles (Hyldgaard et al., 2012). Different techniques have been involved for elucidation of mechanism of antifungal and antimycotoxigenic action in food system. Atomic force microscopy (AFM), transmission electron microscopy (TEM) and scanning electron microscopy (SEM) reveal the deformities in plasma membrane after interaction of essential oil components with membrane proteins (Tolouee et al., 2010; Miri et al., 2019; Jafri and Ahmad, 2020). Reduction in ergosterol content by essential oil fumigation may also be a possible reason for disintegration of membrane integrity and stability leading to premature cellular death (Das et al., 2021d). A number of target sites have been demonstrated, where, essential oils and their bioactive constituents are especially interact and disturb the normal functioning.

1.6.1 Effect on ergosterol biosynthesis

Ergosterol is prime sterol molecule in fungal cell membrane providing dynamicity, fluidity, permeability and integrity (Abe and Hiraki, 2009). Recent investigation of da Silva Bomfim et al. (2020) demonstrated the inhibition of ergosterol biosynthesis by *Rosmarinus officinalis* essential oil in *A. flavus* in a dose dependent fashion. Similar report with active potentiality of cinnamaldehyde for reduction of cellular ergosterol in *Fusarium sambucinum* has been illustrated by Wei et al. (2020). Tian et al. (2012a,b) reported dose dependent retardation of ergosterol content in *A. flavus* after fumigation with *Cinnamomum jensenianum* essential oil. Impairment in synthesis of ergosterol suggested plasma membrane as potential target site of action of essential oils for antifungal activity. Wang et al. (2019a,b) investigated toxic effect of

citral on ergosterol biosynthesis in *A. alternata* leading to cellular apoptosis. Retardation in ergosterol content by *Curcuma longa* essential oil in *Fusarium verticillioides* has been recently demonstrated by [Avanço et al. \(2017\)](#). They reported consequent reprogramming of genes involved in ergosterol synthesis after essential oil treatment which could be marked as possible mechanism of biochemical action for antifungal activity.

1.6.2 Effect on leakage of cellular constituents

Indeed, many essential oil components are reported to efflux major cellular constituents viz. Mg^{2+} , K^{+} and Ca^{2+} from fungal cells, suggesting impairment in membrane permeability and fluidity. [Singh et al. \(2021\)](#) reported excessive efflux of Ca^{2+} , Mg^{2+} and K^{+} ions from *A. flavus* cells after fumigation with *Cinnamomum cassia* essential oil. Effusion of Ca^{2+} , Mg^{2+} and K^{+} ions along with enhanced efflux of nucleic acids and proteins by *Apium graveolens* essential oil mixed linalyl acetate and geranyl acetate fumigation in *A. flavus* has been reported by [Das et al. \(2019\)](#). [Chaudhari et al. \(2020a\)](#) demonstrated antifungal activity of *Pimenta dioca* essential oil in *A. flavus* due to leakage of Mg^{2+} , K^{+} and Ca^{2+} ions leading to loss in cellular homeostasis. [Zhang et al. \(2019\)](#) reported antifungal activity of carvacrol and thymol due to excessive effusion of electrolytes from *Botrytis cinerea* along with destruction of membrane permeability. In another study of [Helal et al. \(2006\)](#) illustrated the effect of *Cymbopogon citratus* essential oil on leakage of Ca^{2+} , Mg^{2+} and K^{+} ions from *A. niger* cells. Moreover, leakage of larger molecules like carboxyfluorescein diacetate, and ATP and influx of ethidium bromide and propidium iodide also revealed the membrane dysfunction due to formation of pores and holes culminating to osmotic imbalances. Radio labeled amino acids and nucleotides detected the negative impact of essential oils on fungal DNA replications and protein synthesis ([Schneider et al., 2010](#)).

1.6.3 Effect of essential oils on energy metabolism

Essential oils/bioactive components are actively participated for inhibition of energy metabolism by blocking ATP synthesis. [Tian et al. \(2012a,b\)](#) reported disruption in mitochondrial membrane potential by *Anethum graveolens* essential oil leading to inhibition of ATP synthesis in *A. flavus*. Inhibition of mitochondrial ATPase and dehydrogenase activity culminating into reduced production ATP has been recently demonstrated by [Hu et al. \(2017\)](#). [Tatsadjieu et al. \(2009\)](#) described impairment in energy metabolism due to inhibition of H^{+} -ATPase pump and disturbed the active site of the enzyme leading to depletion in ATP pool. [Wang et al. \(2019a,b\)](#) reported antifungal effects of cinnamaldehyde due to effective inhibition of F_0 - F_1 complex of ATPase activity. Fumigation of *Penicillium roqueforti* cells by eugenol and citral synergistic formulation collapsed mitochondrial membrane potential with varying degree of distortions in mitochondrial inner layer ([Ju et al., 2020](#)). There are several components of essential oils that have indirect effect on ATP synthesis leading to inhibition of cell growth and sporulation.

1.6.4 Effect of essential oils on cellular methylglyoxal

Methylglyoxal, a cytotoxic component produced as respiratory byproduct act as inducer of cellular aflatoxin biosynthesis in *A. flavus*. Methylglyoxal mostly up-regulate the afl R and ver-1 genes for production of excessive aflatoxins. Recent investigation of [Das et al. \(2021e\)](#) reported prominent inhibition of methylglyoxal biosynthesis by eugenol in *A. flavus*, which directly correlated with reduction in AFB₁. Dose dependent retardation in methylglyoxal production by α -terpenol in *A. flavus* has been investigated by [Chaudhari et al. \(2020b\)](#). Recent study of [Singh et al. \(2019\)](#) suggested inhibition of methylglyoxal synthesis by *Ocimum sanctum* essential oil in *A. flavus*.

1.6.5 Molecular mechanism of antifungal and antimycotoxigenic activity

Molecular mechanisms reveal better understanding of antifungal and antimycotoxigenic effectiveness with proper mechanism of action and binding affinities. [Oliveira et al. \(2020\)](#) demonstrated down-regulation of lae A, met P and lip A genes leading to inhibition of fungal growth. [Das et al. \(2021d\)](#) reported in silico interaction of elemicine, p-cymene, α -pinene, apiol and fenchone with lanosterol 14- α -demethylase culminating into reduction of ergosterol biosynthesis and inhibition of ver-1 and polyketide synthase leading to reduced synthesis of AFB₁. [Murugan et al. \(2013\)](#) demonstrated interactive binding of 13 bioactive components (isolated from *Murraya koenigii* essential oil) with ver-1 protein which may cause interference in AFB₁ biosynthesis. [Badawy et al. \(2019\)](#) reported maximum antifungal activity of camphor, menthone, linalool, thymol, and citronellyl against *A. flavus* due to hydrogen bond and hydrophobic interaction with oxysterol binding protein (Osh4). [Das et al. \(2020b\)](#) observed interaction of thujanol, elemicine and

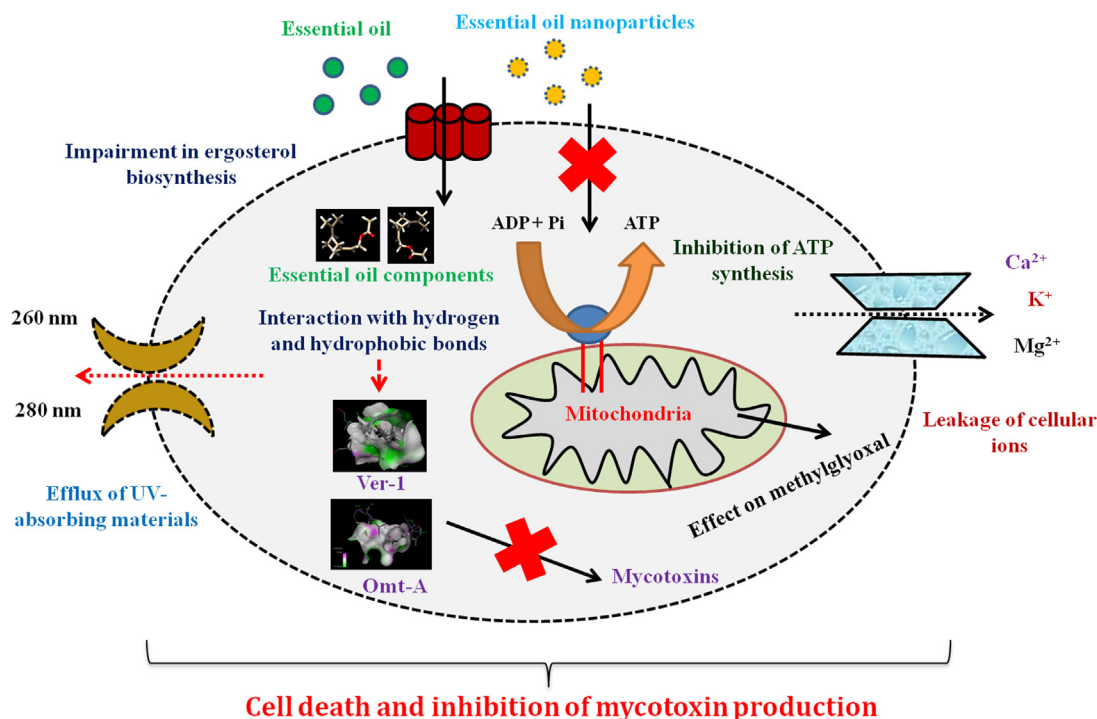


FIGURE 1.1 Mechanisms related to antifungal and antimycotoxigenic activity of essential oils.

myristicine with ver-1 and omt A proteins of *A. flavus* by hydrogen bonds (with amino acids Gly 22, Lys 252, Trp 190 and Gly 227) leading to inhibition of AFB₁ synthesis. Fig. 1.1 presents the antifungal and antimycotoxigenic mechanisms of action of essential oils.

1.7 Nanotechnology: novel sustainable green strategy to protect foods

Nanoencapsulation of essential oils into any biodegradable and biocompatible carrier matrix improve the bioefficacy in food system. The controlled release of essential oil components facilitates the long term stability and affectivity without altering the organoleptic properties of food (Chaemsanit et al., 2019). Different forms of nanoencapsulated essential oils viz. nanoemulsion, nanocapsule, nanoparticle, nanogel, nanotubes, nanoliposomes, nanosponge, and nanofibre have been synthesized after proper entrapment within any biocompatible and biodegradable polymer matrix (Bahrami et al., 2020). Antonioli et al. (2020) demonstrated antifungal affectivity of lemongrass essential oil loaded polylactic acid nanocapsules against pathogenic *Colletotrichum acutatum*. Effective inhibition of storage fungi along with mitigation of AFB₁ contamination in maize by anethole loaded chitosan nanoemulsion has been reported by Chaudhari et al. (2020c). Singh et al. (2020a,b) illustrated retardation in growth of food biodeteriorating fungi and AFB₁ secretion in masticatories by *Bunium persicum* essential oil nanoemulsion. Nanoencapsulated *Zataria multiflora* essential oil improved the fungitoxic potentiality against *Botrytis cinerea* causing gray mold disease in strawberries (Mohammadi et al., 2015). Hasheminejad et al. (2019) reported controlled delivery of clove essential oil from chitosan nanoemulsion with inhibitory activity against *A. flavus* infestation in strawberries over 56 days of storage. Enhancement in antifungal activity of clove and cinnamon essential oil against *A. niger*, *P. roqueforti*, and *Candida albicans* after encapsulation into chitosan nanocapsules has been reported by Mahdi et al. (2021). Table 1.2 presents nanoencapsulated essential oils/components with antifungal and antimycotoxigenic efficacy in food system.

1.8 Safety assessment of essential oils

Before broad scale recommendation of essential oils and nanoencapsulated green products in agri-food industries, one should assess the safety profile of essential oil in model mammalian system. De lima et al. (2013) investigated safety assay of *Croton argyrophylloides* and *C. sonderianus* essential oils through oral toxicity method and found the LD₅₀

TABLE 1.2 Nanoencapsulated essential oils/components with antifungal and antimycotoxigenic efficacy in food system.

Essential oils/ component	Biopolymer	Efficacy in food system	References
<i>Pimpinella anisum</i>	Chitosan	Preservation of stored rice against fungal and AFB ₁ contamination	Das et al. (2021b)
<i>Anethum graveolens</i>	Chitosan		Das et al. (2021a)
<i>Coriandrum sativum</i>	Chitosan		Das et al. (2019)
Anethole	Chitosan	Effective antifungal and antiaflatoxigenic agent in stored maize	Chaudhari et al. (2020b)
Eugenol	Chitosan	Effective inhibitor of <i>A. flavus</i> infestation and AFB ₁ secretion	Das et al. (2021e)
<i>Cuminum cyminum</i>	Chitosan	Quality control of sardine fillet by reducing lipid peroxidation and improved sensorial characters	Homayonpour et al. (2021)
<i>Satureja khuzestanica</i>	Chitosan	Reduced microbial growth, lipid peroxidation and sensorial characters in lamb meat	Pabast et al. (2018)
<i>Artemisia dracunculus</i>	Gelatin-chitosan	Preservation of pork slices against microbial contamination and lipid peroxidation	Zhang et al. (2020)
<i>Monarda citriodora</i>	Chitosan	Protection of stored functional foods against fungal infestation and AFB ₁ contamination	Deepika et al. (2020)
Clove essential oil	Chitosan	Quality improvement of strawberry fruits by inhibition of <i>A. niger</i> infestation	Hasheminejad et al. (2019)
<i>Zataria multiflora</i>	Chitosan	Inhibition of <i>Botrytis cinerea</i> growth in strawberries	Mohammadi et al. (2015)
<i>Petroselinum crispum</i>	Chitosan	Protection of stored chia seeds from fungal invasion and AFB ₁ contamination	Deepika et al. (2021)
<i>Pogostemon cablin</i>	Chitosan	Antifungal and AFB ₁ inhibition in stored maize kernel	Roshan et al. (2021)
<i>Cinnamodendron dinisii</i>	Chitosan	Preservation of ground beef against microbial deterioration	Xavier et al. (2021)
<i>Myristica fragrans</i>	Chitosan	Protection of stored rice against fungal and AFB ₁ contamination	Das et al. (2020b)
Cinnamon essential oil	Pullulan	Shelf life enhancer of stored strawberries by maintaining the physico-chemical parameters	Chu et al. (2020)
Oregano essential oil	Lecithin	Postharvest quality maintenance and enhancement of shelf life of tomatoes	Pirozzi et al. (2020)
Eugenol	Tween-80, Tween-20 and sesame oil	Inhibition of microbial infestation in orange juice	Ghosh et al. (2014)
<i>Illicium verum</i>	Chitosan	Mitigation of fungal growth and AFB ₁ secretion in dry fruits	Dwivedy et al. (2018)
<i>Mentha piperata</i>	Chitosan	Better antifungal activity against <i>Aspergillus flavus</i> in tomatoes	Beyki et al. (2014)
<i>Cuminum cyminum</i>	Chitosan	Superior antifungal activity against <i>Aspergillus flavus</i>	Zhavah et al. (2015)
<i>Zataria multiflora</i>	Glyceryl monostearate and Tween-80	Strong antifungal activity against <i>Aspergillus ochraceus</i> , <i>Aspergillus niger</i> , <i>Aspergillus flavus</i> , <i>Alternaria solani</i> , <i>Rhizoctonia solani</i> , and <i>Rhizopus stolonifer</i>	Nasseri et al. (2016)
D-limonene	Soy lecithin and starch	Preservation of pear and orange juices against microbial deterioration	Donsi et al. (2011)

value higher than 6000 mg/kg considering safe for practical utilization as food preservative. Jemaa et al. (2018) reported higher LD₅₀ value of *Thymus capitatus* essential oil in mice without abdominal contortion, piloerection and muscle tones. Dwivedy et al. (2018) demonstrated very high LD₅₀ value of *Illicium verum* essential oil (11,257.14 µL/kg body weight) in mice. Upadhyay et al. (2019) demonstrated high LD₅₀ (13,956.87 µL/kg body weight) in mice suggesting non-toxic effect to mammalian system. In different studies, it has been observed that nanoencapsulation reduced the LD₅₀ value, however, the values were found higher than OECD guideline (cut off value = 2000 mg/kg), hence they have been categorized as safe with green insight to preserve food commodities. In a study of Das et al. (2021a), LD₅₀ value of *Anethum graveolens* essential oil in mice was reported 18,714 µL/kg, while nanoencapsulation retarded the LD₅₀ value to 15,987 µL/kg. Authors also suggested the reduction of LD₅₀ value due to large surface/area ratio in nanoemulsion system and better binding affinity within cells. Ribeiro et al. (2014) demonstrated reduction in LD₁₀ and LD₅₀ values of *Eucalyptus citriodora* essential oil after encapsulation into chitosan nanomatrix without causing any vital toxicity in mice. Ragavan et al. (2017) illustrated non-toxic effect of garlic essential oil nanoemulsion in rats with improvement in dyslipidemia treatment. LD₅₀ value of *Origanum majorana* essential oil was found 14,117 µL/kg body weight of mice, while nanoencapsulation reduced the LD₅₀ value to 11,889 µL/kg body weight (Chaudhari et al., 2020d).

1.9 Conclusion and future prospective

Remarkable efficacy of nanoparticle for removal of water, soil and heavy metal pollutants facilitate their application in clean environments without any environmental hazards. The adsorbent, membrane and photocatalyst based methods are being usually used for removal of contaminants based on ionic interactions and bonding. Moreover, antifungal, antimycotoxigenic and antioxidant potentialities essential oils strengthen their utilization as green substitute to synthetic food preservative to avoid the hazardous effects on human, animals and surrounding environments. The biochemical and molecular mechanisms strongly support the binding mechanism and toxicity of essential oils into fungal cells. The efficacy of essential oils has been increased through sustainable nanoencapsulation strategy with broad scale agro-prospects in food industries to develop the nano-green smart food preservative. Additionally, the mammalian non-toxicity of essential oils and their nanoformulation could be helpful for large scale industrial commercialization with novel agro-ecological prospects. Although nanoparticles are of much interest in removing environmental contaminants but toxicity of nanoparticles in environment should also be focused. Effective nanomaterials without harming the environmental integrity should be practically used for creating green and clean environment. Essential oils and their nanoformulation showed prominent antifungal and antimycotoxigenic efficacy, however, most of the studies have been focused on in vitro investigation; the future direction should need to focus on in vivo studies in food system. Moreover, commercialization of nanoencapsulated plant essential oil as green food preservative is still not so popular, hence design of composite materials suitable for food application could facilitate the development of cost effective nanoformulations.

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Microbial nanobionics: future perspectives and innovative approach to nanotechnology

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2.1 Introduction

Nanobiotechnology is a unique fusion between nanotechnology and biotechnology (Ahmad et al., 2005; Niño-Martínez et al., 2019). Nanopowders, nanocrystals, and nanoclusters represent various types of nanoparticles (NPs) and atom/molecule aggregates within 1–100 nm in dimensions (Ball, 2002; Singh et al., 2016). Nowadays, the study of these nanoparticles is gaining the interest of scientists all over the world in research as they form effective connections amongst various bulky substances, atomic/molecular structures. One of the most unique methods in NPs synthesis is its occurrence in biological systems. Several organisms amongst unicellular and multicellular groups are identified to produce the nanoparticles, extra/intracellularly (Santhoshkumar et al., 2017; Simkiss and Wilbur, 2012). Richard Feynman (in 1960) received the Nobel Prize in physics for putting forward the idea of nanotechnology as its early vision (Grumezescu, 2017). Various applications of nanotechnology are in diagnostics, biomarkers, and contrast agents for cell labeling, biological imaging, antimicrobial agents, anticancer nanodrugs, drug delivery systems, and nanobased drugs for prevention and curing various diseases (Singh and Nalwa, 2011; Shafiq et al., 2020).

The biogenic approach or the synthesis of NPs from microbes is better than the synthesis through chemical methods in the eyes of green nanotechnology (Bhattacharya and Mukherjee, 2008). This approach combines microbial biotechnology with nanotechnology in order to produce NPs through microbes. This approach is considered as one of the positive step toward reducing global warming leading to sustainable development (Alghuthaymi et al., 2015). Microorganisms are the powerful nanofactories for the NPs synthesis. These microbes form various types of ions by generating element metal through the enzymatic conversion of metal ions. Based on the location of NPs synthesized by microbes, these can be differentiated as intracellular and extracellular (Mann, 2001; Grumezescu, 2017). This chapter focuses on various nanotechnology applications, their prospects, importance, and innovative approaches.

2.1.1 Biosynthesis of microbial nanoparticles

The biogenic approach involves various species of bacteria, fungi, actinomycetes, yeasts, and viruses for the biological synthesis of gold, silver, gold-silver alloy, selenium, tellurium, platinum, palladium, silica, titania, zirconia, quantum dots (QDs), magnetite, and uraninite NPs (Shafiq et al., 2020). The abundance of microorganisms in the ecosystem makes them preserved and reused in their natural habitat. In aqueous solutions, the microbes synthesizing nanomaterials can be easily filtered by separation is regarded as one of the most significantly proven techniques nowadays (Hulkoti and Taranath, 2014; Lugani et al., 2021).

Intra and extracellular are two methods to synthesize on the basis of the location of NPs development. The biological synthesis of NPs through targeting metal ions is an effective way of converting them to elemental metal using enzymes during cellular activities (Azmath et al., 2016). Metal ions are reduced to NPs within the microbial cell and at the surface during intracellular and extracellular methods, respectively, through enzymatic activity, for example, metallic nanoparticles (gold, silver, alloy), oxide nanoparticles (magnetic and nonmagnetic), and sulfide nanoparticles.

These NPs are drug carriers for targeted delivery, antibacterial agents, and biosensors, reaction rates enhancers (Li et al., 2013; Lugani et al., 2021). NPs like zinc oxide are food additives and antimicrobial agents synthesized using reproducible bacterium like *Aeromonas hydrophila* (Irvani, 2014; Shafiq et al., 2020).

2.1.2 Types of microbial nanoparticles

2.1.2.1 Metallic nanoparticles

Metallic NPs are of numerous kinds such as gold NPs, silver NPs, and alloy NPs (Fig. 2.1), as discussed below:

2.1.2.1.1 Biosynthesis of gold nanoparticles

Biosynthesis of nanoparticles is arising bionanotechnology (the connection of nanotechnology and biotechnology) has recognized significant awareness due to a developing need to increase environment-friendly technologies in resources invention. The extracellular production of gold nanoparticles is observed by fungal species such as *Fusarium oxysporum* and *Actinomyces*, *Thermomonospora* sp., respectively. They reported the intracellular invention of gold nanoparticles by fungus *Verticillium* sp. as well. Gold particles of nanoscale aspect may readily be precipitated inside bacterial cells by incubation of the cells with Au^{3+} ions (Niño-Martínez et al., 2019). Monodisperse gold nanoparticles are synthesized by using alkalotolerant *Rhodococcus* sp. under an extreme biological situations like alkaline and vaguely elevated temperature condition.

2.1.2.1.2 Biosynthesis of silver nanoparticles

The bacterium *Pseudomonas stutzeri* AG259, isolated from silver mine, was studied in a consistent aqueous solution of silver nitrate, shows a chief role in decreasing the silver ions and increases silver NPs of well-defined size with split topography within the periplasmic space of the bacteria (Santhoshkumar et al., 2017). Silver NPs synthesized during the film formation or shaped in solution, or concentrated at the exterior of its fungi cell association such as *Verticillium*, *Fusarium oxysporum*, and *Aspergillus flavus* (Table 2.1).

2.1.2.1.3 Biosynthesis of alloy nanoparticles

Alloy NPs are of vast significance suitable in applications like visual materials, electronics catalysis, and coatings. The construction of bimetallic such as Au-Ag alloy through species *Fusarium oxysporum* argued that the hidden cofactor NADH plays a crucial role in determining the Au-Ag alloy NPs composition. The characterization of this alloy through fluorescence microscopy and transmission electron microscopy indicates that the Au-Ag alloy nanoparticles were typically formed through extracellular progress and customarily existed within the sort of uneven polygonal nanoparticles. Electrochemical investigations reveal vanillin sensor based on Au-Ag alloy NPs customized glassy carbon electrode was able to improve the electrochemical reply of vanillin at least five times. The biosynthesis of these alloy NPs from *F. semitectum* shows that the NPs suspensions are steady for many weeks (Jandt and Watts 2020).

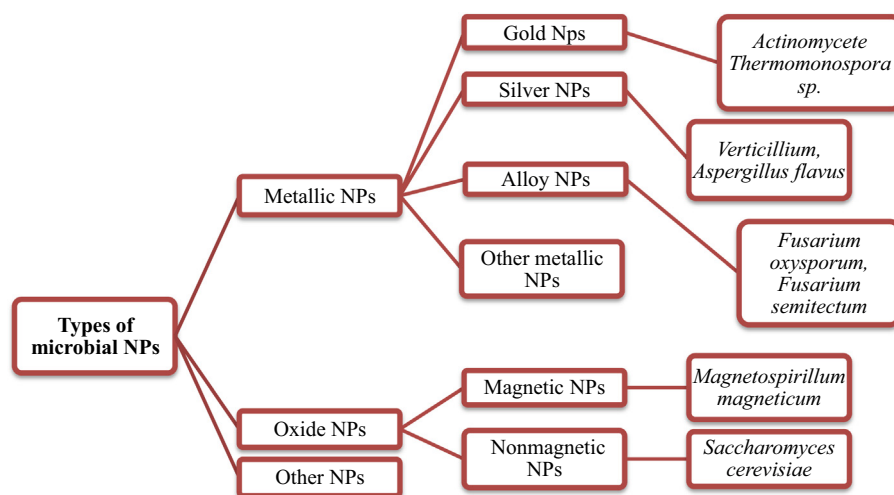


FIGURE 2.1 Classification and types of microbial nanoparticles and the microbes responsible for their extracellular and intracellular production.

TABLE 2.1 Biological synthesis of metal and oxide nanoparticles and various related parameters including type of nanoparticles (NPs), size of the NPs, culturing temperature in °C, and cellular/extracellular production details.

Microbial source of nanoparticles (NPs) synthesis	Type of NPs	Culturing temperature (in °C)	Size of NP (in nm)	Cellular/extracellular production localization	References
<i>Sargassum wightii</i>	Gold NP	—	8–12 nm	Extracellular	Niño-Martínez et al. (2019)
<i>Rhodococcus sp.</i>	Gold NP	37°C	5–12 nm	Intracellular	Ahmad et al. (2005), Singh et al. (2016)
<i>Shewanella oneidensis</i>	Gold NP	30°C	12 nm	Extracellular	Grumezescu (2017)
<i>Plectonema boryanum</i>	Gold NP	2°C–100°C	< 10–25 nm	Intracellular	Lugani et al. (2021)
<i>Trichoderma viride</i>	Silver NP	27°C	5–40 nm	Extracellular	Rana et al. (2016)
<i>Bacillus licheniformis</i>	Silver NP	37°C	50 nm	Extracellular	Kalimuthu et al., (2008)
<i>Escherichia coli</i>	Silver NP	37°C	50 nm	Extracellular	Gurunathan et al. (2009)
<i>Bacillus cereus</i>	Silver NP	37°C	4–5 nm	Intracellular	Lugani et al. (2021)
<i>Shewanella Oneidensis</i>	Ferrimagnetic magnetite NP (Fe ₃ O ₄)	28°C	40–50 nm	Extracellular	Grumezescu (2017)
Recombinant AMB-1	Ferrimagnetic magnetite NP (Fe ₃ O ₄)	28°C	20 nm	Intracellular	Amemiya et al. (2007)

2.1.2.1.4 Biosynthesis of other metallic nanoparticles

Heavy metals are famous to be cyanogenetic to life. Within the surroundings, microbic variation to most deadly significant metals is due to their matter detoxification additionally as attributable to energy-deprived particle effluence from the cell by membrane proteins to create straightforward additionally as ATPase or as chemiosmotic ion or nucleon anti-transporters. The excellence in solubility additionally plays a role in microbial variance. Platinum nanoparticles were achieved by the metal ion-dropping microorganism *Shewanella algae*. Platinum nanoparticles of about 5 nm were situated in the periplasm. Mercury nanoparticles will be synthesized by *Enterobacter sp.* Cells. The culture scenario (pH 8.0 and lower concentration of mercury) facilitate within the creating of uniform-sized 2–5 nm, spherical, and mono distinct intracellular mercury nanoparticles (Jandt and Watts 2020).

2.1.2.2 Oxide nanoparticles

Oxide nanoparticle is a significant type of composite nanoparticle synthesized by microbes. In this section, we reviewed the biosynthesized oxide nanoparticles from the two aspects: magnetic oxide nanoparticles and nonmagnetic oxide nanoparticles. Most of the examples of the magnetotactic bacteria used for the manufacture of magnetic oxide nanoparticles and organic systems for the development of nonmagnetic oxide nanoparticles (Jandt and Watts 2020).

2.1.2.2.1 Biosynthesis of magnetic NPs

Magnetic nanoparticles are recently urbanized new materials, due to their micro-agreement and properties like brilliant paramagnetic and significant coercive force, and their perspective for vast applications in standard sharing and biomedicine fields. Magnetic nanoparticles like Fe₃O₄ (magnetite) and Fe₂O₄ (maghemite) are known to be biocompatible. BacMPs, which are connected in chains within the bacterium, are postulated to efficiency as natural level needles that allow the bacterium to go all along oxygen gradients in aquatic environments, below the authority of the Earth's geomagnetic field (Singh et al., 2016).

Magnetotactic cocci, for example, have bare high variety and allocation and have been regularly known at the outside of marine sediments. The discovery of this bacterial type, with the only culture *Magnetotactic coccus* strain MC-1, recommended that they are micro aerophilic. *Magnetospirillum magneticum* AMB-1 inaccessible by facultative anaerobic *Magnetotactic spirilla* with several new magnetotactic bacteria have been set up in dissimilar marine environments since 2000 (Singh et al., 2016).

2.1.2.2.2 Biosynthesis of nonmagnetic oxide nanoparticles

Besides magnetic oxide nanoparticles, different oxide nanoparticles have additionally been deliberate as well as TiO_4 , Sb_2O_3 , SiO_2 , BaTiO_3 , and ZrO_2 . An inexperienced cheap and reproducible *Saccharomyces cerevisiae* mediated synthesis of Sb_2O_3 nanoparticles (Singh et al., 2016).

2.1.2.3 Other nanoparticles

In organic systems, a vast variety of organisms from organic/inorganic composites with ready structures by the utilization of biopolymers such as protein and microbe cells. In enhance to nanoparticles mentioned on top of, PbCO_3 , CdCO_3 , SrCO_3 , PHB, and $\text{Zn}_3(\text{PO}_4)_3$ nanoparticles were rumored to be synthesized by microbes. SrCO_3 crystals were obtained when demanding fungi were incubated with aqueous Sr^{2+} ions. The authors made-up that liberate of proteins through the expansion of the fungus *Fusarium oxysporum* is dependable for modulating the morphology of strontianite crystals and directing their hierarchical gathering into necessary order superstructures (Mukundan and Vasanthakumari, 2017).

2.1.3 Endophytic microbes as nanoparticle biofactories

Endophytic microbes secrete biologically crucial products, which show significant importance in modernized agricultural practices, anticancer properties, antidiabetic actions, antioxidant properties, immunosuppressants, medicinal properties (Fig. 2.2). A huge endophytic microbial diversity is yet to examine for its chief role in nanoparticle synthesis and its diverse applications (Rana et al., 2016). Compared to other microbial communities, endophytic microbes produce distinct

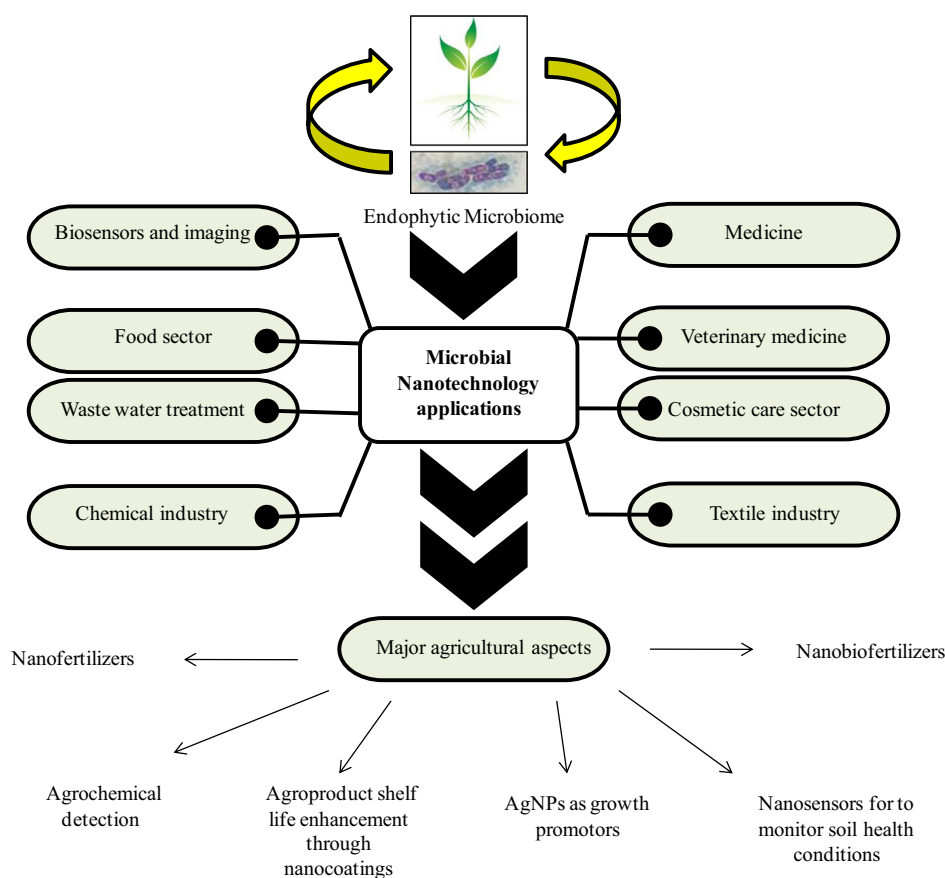


FIGURE 2.2 Applications of various endophytic microbes and synthesis of different types of nanoparticles in numerous industrial sectors.

metabolites with abundant biological activities that give rise to various applications of the endophytic secreted NPs (Baker and Satish, 2015). These microorganisms reside within plant tissues without harming the host (including bacteria, fungi, actinomycetes, and viruses). A huge fraction of endophytic microbes such as *Bacillus cereus* (Sunkar and Nachiyar, 2012), *Pseudomonas veronii* (Baker and Satish, 2015), *Colletotricum* sp. (Shankar et al., 2003), *Penicillium citrinum* (Honary et al., 2012), *Aspergillus fumigatus*, *Rhodococcus* sp. (Ahmad et al., 2005), and *Saccharomonospora* sp. (Verma et al., 2013) isolated from various origins that are recognized for their ability to synthesize NPs.

2.2 Future recommendations and applications of microbial nanoparticles

The synthesis of biologically synthesized nanomaterials has obtained immense recognition within a few years for its novel characteristic and numerous applications in the chemical and natural environment (Gouda et al., 2019; Pantidos and Horsfall, 2014). Microbial Nanotechnology proposes vast properties of the metals that reveal their diverse possible roles in the agriculture sector, as antimicrobial factors, diagnostics through biomedical agents, biomarkers, and bioimaging (Fig. 2.3) (Singh et al., 2016). Therefore, microbial nanoparticles have gained tremendous focus nowadays by scientists of various fields due to the novel property of nanoparticle synthesizing microbes thriving in extreme situations (Gouda et al., 2019).

2.2.1 Agriculture and food sector

2.2.1.1 Nanotechnology for food preservation and storage

Nanocomposites are ready of nanoparticles and polymers, and they are old in food areas for attractive the shelf life of food products, preservation of the products fresh, devoid of microbial action and provide gas obstacles to decrease the runaway of carbon dioxide from carbonated beverages (Shafiq et al., 2020). Guard IN Fresh is a nanocomposite-based commercialized artificial goods used for the ripening of fruits and vegetables by scavenging ethylene gas. NanoCeram PAC is an eco-friendly nanocomposite-based covering material that helps in the quick addition of revolting method of food, result in avoiding stinking odor, and foul taste (Table 2.2).

2.2.1.2 Nanotechnology in food packaging

One of the projections of growth of this ability is the use of polymer composites which provide dynamic, bendable, and smart packaging. Biologically synthesized silver and gold nanoparticles from *Fusarium* sp., *Pseudomonas struzeri*, and *Penicillium* sp. are for antimicrobial packaging (King et al., 2018).

2.2.1.3 Nanomaterials as antimicrobials

The antimicrobial characteristics of nanoparticles influence by the preserve of microbial growth on nonsterilized foods, and expectation of post contamination or pasteurized foods. Silver nanoparticles are quite stable with a broader variety of antimicrobial action, and these nanoparticles are reported to be confined for biological structure when integrated inside standard limits established by Food and Drug connection. The new nanoparticles which have been reported for antimicrobial are copper and copper oxide, chitosan, cadmium, magnesium oxide, selenium, single-walled carbon nanotubes, and telluride (Soren et al., 2018).

2.2.1.4 Nanotechnology in nutraceuticals production and their delivery

Nanoemulsions are also used in food processing in the form of proteins (milk, egg, and vegetable proteins), carbohydrates (alginate, pectin, carrageenan, dammar gum, guar gum, sucrose-acetate isobutyrate, and xanthan) for improving the excellence and reliability of ice creams, dipping creaming and sedimentation, allocation and convenience of food nutrients, and production of food products like sweeteners, salad dressing, beverages, and other processed foods (Soren et al., 2018).

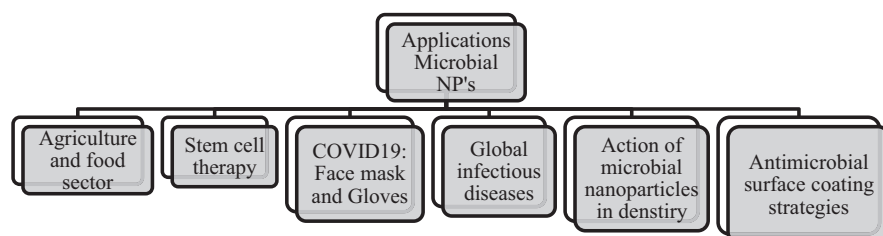


FIGURE 2.3 Various applications and future recommended areas of nanoparticles synthesized from microbes.

TABLE 2.2 Various applications of nanomaterials in food sector and their positive (direct/indirect) effects in nanodelivery, packaging, pathogen detection, and quality testing of food materials.

S. no.	Food application	Nanomaterials	Positive effect	References
1.	Nanodelivery of food ingredient	Nanoemulsion Nanoencapsulation	– Decreased chemical degradation – Acts as carriers for lipophilic bioactive constituents – Used as favoring agents – Antioxidants – Preservatives and drugs – Protection against environmental limiting factors – Used in food ingredients – Used to increase bioavailability – Control release – Enzyme kinetics – Minimize drug side effects	Ravichandran (2010) , Gouda et al. (2019)
2.	Packaging of food items	Nanocomposites Nanosensors	– Eco-friendly – Biodegradable – Growth of high obstacle properties – Screening of food pathogens – Transit processes in smart packaging	Pandey et al. (2013) , Pathakoti et al. (2017) Pathakoti et al. (2017)
3.	Pathogen detection	Specified protein on silica chip Immunogold nanoparticles	– Detects specific foodborne pathogens by luminescence – Detection of <i>Cronobacter sakazakii</i>	Horner et al. (2006) , Aly et al. (2018) Aly et al. (2018)
4.	Testing of food Quality	Nanobarcodes Gold (Au) and silver (Ag) NPs	– Detection of quality of agricultural products – Detection of food contaminants like melamine and malathion	Coles and Frewer (2013) , Aly et al. (2018) Paul et al. (2017)
5.	Nutritional drink	Iron (Fe) NPs	– Improve toddler health by increasing bioavailability and reactivity	Miller and Senjen (2019) , Paul et al. (2017)

2.2.1.5 Nanosensors in the food sector

A fluorescent nanobarcode detection system has been developed for introduction of some foodborne pathogens such as *E. coli*, *anthrax*, *tularemia* bacteria, *Ebola*, and severe acute respiratory syndrome (SARS) virus by varied color codes in a computer scanner. A Dip-pen nanolithography technique has been developed by NanoInk, Skokie, for discovery of food products and pharmaceutical pills. This officer tool is at nearby used by Barcode, a registered US company, for traceability to make sure union ([Soren et al., 2018](#)).

2.2.1.6 Nanoguarded pesticides

To develop the competence, feature, and yield of food crop pesticides are usually used. Lately, nanoformulated pesticides are used to defeat artificial pesticides. Nanopesticides are tiny particles of active ingredients having useful pesticidal properties engineered with tiny nanovariety structures ([Kranjc & Drobne, 2019](#)). These agricultural formulations are competently isolated from water, which acts as a hurdle for reducing needless progress of pesticides. Nanopesticides are delivered during several techniques such as nanobased emulsions, encapsulations, nanocages, and nanocontainers ([Gouda et al., 2019](#)).

2.2.1.7 Nanoguarded herbicides

Nanoherbicides are an efficiently feasible option, has the necessary needs to enlarge the yield of the crop and take out weeds that interrupt the crop development. Conservative herbicides are extremely efficient but lack of moisture limits their competence and convention. Therefore, silicon nanocarriers with diatom frustules are mainly utilized for

prohibited pesticides delivery and herbicides with particular discharge in plants and wastewater treatments (Abdelmonem 2020). Several herbicides are evaluated and reported by researchers based on mixture nanocomposites functions, anionic intercalation of two herbicides 2, 4-dichlorophenoxy acetate and 4-chloro phenoxy acetate with zinc–aluminum-layered double hydroxide.

2.2.1.8 Nanogels formulation

Nanogels are used in agrochemical industries particularly in fruit- producing orchards. Pheromones an eco-friendly, semiochemical, unstable complex acts as a pest scheming agent. This active complex is powerless in nanogels and exhibits efficiently in open orchards with improved remaining activity and reduces the adverse fruit fly and pest populations that minimize the feature and yield of the fruit manufacture (Lugani et al., 2021).

2.2.1.9 Nanoguarded fertilizers

Fertilizers formulated with nanostructured particles have formed a profound prospect by rising the collision on market and falling the environmental nitrogen failure that arises due to long-term contact with soil microorganism, release, and soil leaching. Recent research shows that the competence of nitrogen procedure by plants have become very low and about 50%–70% of the nitrogen is complete only through commercially accessible conservative fertilizers. During nanoencapsulated fertilizers, new nutrient delivery scheme is formulated to decrease the nitrogen loss in plants (Gouda et al., 2019).

2.2.1.10 Seed germination and plant growth

Nano- based composites and formulations are potentially used in seed germination and plant growth skill to accomplish the objective of promoting accurate agricultural farming applications. Titanium dioxide nanoparticles and bulk metal oxide titanium dioxide were treated with physically old spinach seeds to know germination and formation. Studies shows that nanotitanium dioxide treated spinach seeds formed plants with 73% dry weight and 45% improved chlorophyll substance along with a 3-fold increase in photosynthetic rate compared to bulk titanium dioxide uncovered for 30 days. So, the development rate of spinach seeds is inversely proportional to nanosize. Smaller size exhibits better photo-generation, photo-sterilization, and germination. Titanium dioxide nanoparticles also encourage oxygen and water intake for fast germination of seeds and effectively act as stress- resistant (Saleem and Zaidi, 2020).

2.2.1.11 Detection of residual pesticides

FDA has reported the discovery of more than 1045 residual pesticides in a variety of food crops globally. Previous gas or liquid chromatography- mass spectroscopy techniques were used in the discovery of 10 potential applications of agricultural nanotechnology remaining pesticides. In recent advancements of agricultural nanotechnology, nanosensors are a precise change in the exposure of accumulated pesticides in various food crops (Zulfiqar et al., 2019). Using Pesticide Data Program (PDA) analysis, the US Department of Agriculture (USDA) has evaluated more than 10,000 every year, which reported the gathering of some organophosphates such as organochlorines, carbamates, triazines, triazoles, pyrethroids, neonicotinoids, strobilurins (Gouda et al., 2019).

2.2.1.12 Nanobased technologies for water quality

The condition of clean water for human, animal, farming, and industrial usage globally has become the most daunting challenge. Over the next two decades, clean water can be availed as only one third than needed for a person (Saleem and Zaidi, 2020). This particular situation of water scarcity may condemn millions of people to premature death. Agriculture needs freshwater but in turn, due to excessive utilization of pesticides, fertilizers, and other crop-related chemicals the groundwater structure gets polluted. Then, an efficient measure is necessary on use of frequent sources by farmers with cost -efficient new technologies.

2.2.1.13 Nanoparticles in microbicidal action

Microorganisms sterile during the chemical and physical mode of procedures, such as chlorine dioxide, ozone, and ultraviolet rays are effective microbial disinfection systems. As pathogenic microbes are extremely increasing, it requires an efficient, cost-efficient, infrastructure and exchange skills. Oligodynamic metallic nanoparticles, such as silver nanoparticles act as capable nanomaterials with bactericidal and viricidal properties (Saleem and Zaidi, 2020).

2.2.1.14 Nanobased desalination

Conservative desalination skill requires a large quantity of energy to exchange seawater into freshwater using reverse osmosis (RO) membrane technology. To overcome huge power utilization, nanotechnology-based methods such as thin-film nanocomposite membranes, associated carbon nanotube membranes, and nanoprotein-polymer biomimetic membranes are urbanized. Especially, carbon nanotubes exhibit brilliant permeability of water than other nanomaterials and integrate with other functionalities such as disinfection, deodorizing, self-cleaning, and de-fouling actions (Saleem and Zaidi, 2020). Scale-up, manufacture, effectual desalination, and long-term constancy are some of the practical challenges to be sorted before its commercialization (Fig. 2.4).

2.2.1.15 Nanobased heavy metal removal systems

Heavy metal contaminants that live in high absorption can be efficiently adsorbed during the functionalization of ligand-based nanocoatings that are bonded to the matrix. The matrix used in the action is regenerated and additional biofunctionalized nanocoated media is used in the deletion of serious metals contaminants (Saleem and Zaidi, 2020).

2.2.2 Stem cell therapy

Nanotechnology has various applications in Stem cell (SC) therapy- from isolating SC to driving the cellular fate that acts as stem cells macromolecular delivery systems (Fig. 2.5). Isolation of cells is a very important part of SC therapies for which magnetic cell isolation method is widely used. In this cell isolation technique Magnetic NPs are utilized, SC is labeled using these NPs to distinguish the target cell types against multicellular mix. This method is called as MACS abbreviated for Magnetic Activated Cell Sorting (Rodrigues et al., 2014). The magnetic NPs are mixed with monoclonal antibodies for a specific antigen which results in the recognition of the cells having the particular antigen. Through this method, it has been demonstrated that magnetic NPs integrated with anti-CD34 antibodies can be used to disperse progenitor blood cell isolated from the human blood. These cells can be further optimized and utilized in SC regenerative therapy. Along with this, nanomaterials can also be used to regulate stem cell differentiation and proliferation. Mainly scaffold dependent nanomaterials are utilized for this process such as: Carbon nanotubes (CNTs), Titanium dioxide and few other nanomaterials (Table 2.3).

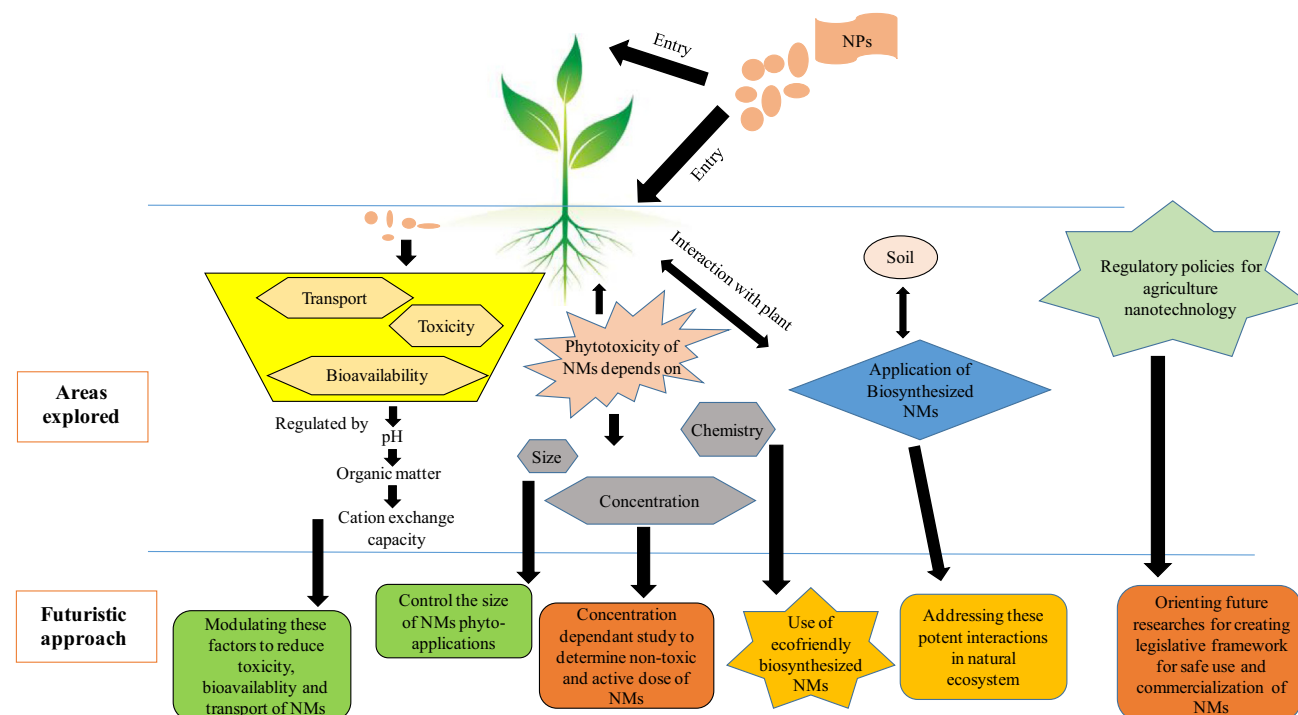


FIGURE 2.4 Role of microbial in agriculture and various food sectors.

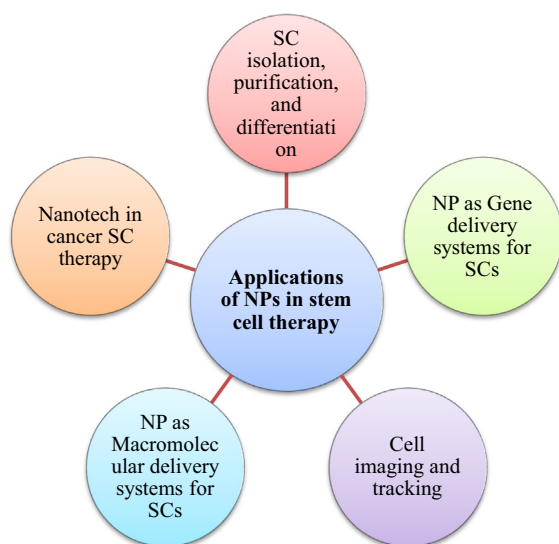


FIGURE 2.5 Various application of nanomaterials employed in stem cell therapy for instance gene delivery, cell imaging, and tracking.

TABLE 2.3 Nanomaterials used for stem cell and its differentiation ability.

Nanomaterials	Stem cell type	Effect on differentiation ability	References
Graphene/ graphene oxide	Mesenchymal stem cells (MSC)	Increase osteogenic differentiating potential	Lee et al. (2011) , Paul et al. (2017)
Gelatin-immobilized poly (L-lactide-co-caprolactone)	Human Mesenchymal stem cells (hMSC)	Increase Osteogenic differentiating potential	Shin et al. (2008) , Paul et al. (2017)
Graphene-oxide-patterned substrate	Human Neural stem cells (hNSC)	Improve integrin clustering, focal adhesion, and neuronal differentiation	Yang et al. (2016)
Gold NP-coated collagen-nanofiber	Placental derived MSCs	Increase neuronal differentiation and proliferation	Orza et al. (2011)
Nanopore patterned (NPo) polystyrene (PS) surfaces	Human ES cells	Distinction into endodermal cells	Kim et al. (2016)
AuNPs	hADSCs	Improved Osteogenic differentiating potential	Li et al. (2013)
Chitosan-conjugated AuNPs	Adipose tissue derived stem cells ADSCs (hADSCs)	Osteogenic differentiation	Choi et al. (2015)

Nanotechnology is also being applied to track the fate of the SC being transplant. Several imaging methods have been developed to do so, like radioactive cell imaging, photoacoustic imaging, Magnetic resonance imaging (MRI), fluorescent imaging. Nanoparticles used for the imaging, visualizing, and chasing stem cells are Quantum dots, Magnetic nanoparticles, gold nanorods. Quantum dots and magnetic NPs have been more prominently studied for this purpose. Quantum dots linked to anti-mortalin peptide antibody (AB) facilitate the formation of composite I-material. I-material is prone to get internalized by Mesenchymal SCs and labeled as Mesenchymal SC which get natural adipocyte, chondrocyte, and osteocyte differentiation. This presents the potential of QD for SC visualization and monitoring. Magnetic nanoparticles such as super- paramagnetic iron oxide NPs (SPIO) can also be used for stem cell tagging, Magnetic resonance imaging and tracking SC. For tracking engraftment phase and for characterization of the labeled Hematopoietic Stem Cells (HSCs), iron oxide nanoparticles dextran-covered iron oxide NPs encased in dextran covalently bound to fluorescent particles are used ([Meisel et al., 2020](#)).

NPs can also be utilized as gene delivery systems for SCs. Methods such as electroporation and nucleofection facilitate efficient working during transmission, but they may damage the Embryonic SCs. Therefore, polymeric NPs and liposomes

are extensively being researched. Polyamide-amine dendrimer-functionalized fluorescent multi-walled carbon-based nanotubes (dMNTs-C) are extremely efficient in penetrating the CCE embryonic SC line in mouse and Dendrimer-modified MNPs of polyamidoamine (PAMAM) are demonstrated to enhance the efficiency of gene delivering. Also, the tip or the nanoneedle of Atomic Force Microscope can be used for incorporating MSCs of human and HEK293 cells (Cui et al., 2008; Claxton et al., 2020).

Next challenge faced in SCs therapy is to distribute the biomolecules such as DNA, RNA, peptides, or proteins which regulate the proliferation and differentiation in the SCs. NPs used for this purpose comprises of numerous benefits that includes space for surface modification with target moiety to distribute the preferred SCs and reduction in toxicity risk. Mouse embryonic fibroblasts are programmed to pluripotency by using a plasmid having OSKM (pOSKM) - to make arginine-terminated polyamidoamine nanoparticle-based nonviral gene delivery system. Positively charged FMSN integrated with HNF3 β plasmid DNA (pHNF3 β) make a complex which is internalized by iPSCs and exhibit improvement in endoderm formation and differentiate to hepatocyte like stem cells (Sohn, et al., 2013).

Along with all the applications discussed nanotechnology is also applicable for providing cancer stem cell therapy by destroying cancer SCs (Tabassum et al., 2018). All-transretinoic acid entrapped albumin nanoparticles surface coated with hyaluronic acid can be used to strike the CD44 overexpressed cancer stem cell via a bond formed by hyaluronic acid on the surface which attract B16F10 cells enriched with CD44 making NPs applicable for suppressing stem cells (Li, et al., 2018).

2.2.3 COVID19: face mask and gloves

The current situation has revealed how the ongoing development of nanotechnology and nanomedicine can accelerate the fight against novel viruses. A comprehensive solution to this and future pandemic outbreaks includes preventing the spread of the virus through anti-viral personal protective equipment (PPE) and anti-viral surfaces, plus efforts to encourage the behavior to minimize risks.

There are various current opportunities for studies in biomaterials, nanotechnology, and cellular biology in COVID-19 research and provide impactful public health interventions. (Aydemir and Ulusu, 2020) have suggested synthesizing ACE2 coated/embedded nanoflowers or quantum dots to produce chewing gums, nose filters, and self-protective tools like masks, gloves, and clothes. This concept employs the production of long-lasting protective mechanisms to minimize infections. Since antibodies can neutralize viral antigens, immobilizing specific antibodies on air filters represents a potentially effective way to preventing SARS-CoV-2 transmission.

Several effective coating strategies such as hybrid coating, tin oxide nanowires, polysaccharide-coated NPs, zinc oxide tetrapod nanoparticles are to control the infection of enveloped viruses. For example, polymers displaying quaternary ammoniums coated on stainless steel showed more than 90% reduction of the coronavirus 229E within 10 min and by greater than 99.9% after two hours of contact (Ikner et al., 2020).

There is a global demand for rapid, mass-produced, and cost-effective methods of SARS-CoV-2 infection diagnosis. Biosensors are analytical devices used to detect analytes (e.g., biomolecules, species produced by microorganisms). In comparison with conventional virus detection methods, biosensors should be rapid, inexpensive, and sensitive (Metkar and Girigoswami, 2019), potentially incorporating nanomaterials that result in increased sensitivity, ease of processing, and higher signal/noise ratio; some examples of such SARS-CoV-2 sensors are discussed below in the Table 2.4.

2.2.4 Infectious diseases and microbial nanotechnology approach

Infectious diseases are a principal driver of morbidity and mortality globally (Aydemir and Ulusu, 2020). Treatment of malaria, tuberculosis and human immunodeficiency virus infection is particularly challenging, as indicated by the ongoing transmission and high mortality associated with these diseases. The formulation of new and existing drugs within nanosized carrier's promises to overcome several challenges associated with the disease treatment, including low on-target bioavailability, sub-therapeutic drug accumulation in microbial sanctuaries, reservoirs, and low patient adherence, due to drug-related toxicities and extended therapeutic regimens (Zhao et al., 2020). Nanocarriers used for formulating vaccines represent a significant weapon in our fight against infectious diseases (Table 2.5).

The aqueous dispersions of nanomilled drug crystals developed that follow the subcutaneous or intramuscular injection mode of drug administration (Zhao et al., 2020). The absorbed drug in the systemic circulation manages through the dissolution rate of the drug crystals into the interstitial fluid. Hence, the physicochemical properties of the drug and the size of the drug crystals play an important role in determining drug release kinetics (Schohy et al., 2020).

Nanoencapsulation of anti-infectives sought through two divergent goals (1) targeting drugs to macrophages and (2) infected tissues (Schohy et al., 2020). The mechanisms by which nanocarriers deposit drugs at these two sites and

TABLE 2.4 Recently developed detection methods and their parameters in SARS-CoV-2 detection.

Type	Target	Biomaterials	Advantages	Reference
Plasmonic fiber optic absorbance biosensor	N protein	Gold (Au) NPs	Label- free	Murugan et al. (2020)
Smartphone- based microfluidic	Nucleic acid	Complementary metal oxide	Fast	Farshidfar, Hamedani (2020)
Electrowetting-on-Dielectric	Nucleic acid	Indium tin oxide	Small testing volume, fast, safeguard against contamination	Jain, Muralidhar (2020)
Microfluidic ELISA	Monoclonal anti-S 1 antibodies	Glass capillary	Small testing volume, fast, point-of-care	Tan et al. (2020)
Lateral flow immunoassay	IgM/IgG antibody	Selenium NPs	Sensitivity of the kit is 94.74% and the specificity is 95.12%, portable, fast	Wang et al. (2020a,b)
Lateral flow immunoassay	IgG antibody	Colloidal gold NPs	Sensitivity of the kit is 69.1% and the specificity is 100%, portable, fast	Wen et al. (2020)
Lateral flow immunoassay	IgM antibody	Colloidal gold NPs	Sensitivity of the kit is 100% and the specificity is 93.3%, portable, fast	Huang et al. (2020)
Lateral flow immunoassay	IgM/IgG antibody	Colloidal gold NPs	The overall testing sensitivity is 88.66% and specificity 90.63%	Li et al. (2020)
Chemiluminescent immunoassays	IgM/IgG antibody	Magnetic microbeads	100% sensitivity for IgG and 88% sensitivity for IgM	Padoan et al. (2020)
Immunochromatography assay (GICA) and enzyme-linked immunosorbent assay (ELISA)	IgM antibody	Colloidal gold	Reducing false-positive results	Wang et al. (2020a,b)
Colorimetric assay	Nucleic acid	Gold (Au) NPs	Naked-eye detection	Moitra et al. (2020)
Flow- virometry	Virus particles	Magnetic NPs	Large-scale detection	Soni et al. (2020)
Immunochromatographic assay	Nucleoprotein antigen	Colloidal gold NPs	Sensitivity of the kit is 30.2% and the specificity is 100%, fast	Schohy et al. (2020)
Surface- enhanced Raman scattering (SERS)	Virus particles	Silver-nanorod array	Rapid and on-site diagnostic tool	Zhao et al. (2020)

therefore formulation strategies are distinct. Macrophages are common targets for bacteria (such as *Mycobacterium tuberculosis*), fungi (for example, *Aspergillus* species), and viruses (such as HIV).

As nanocarriers predominantly clear by the cells, they can be used for drugs targeting macrophages ([Moitra et al., 2020](#)). Administering the antiretroviral azidothymidine in poly (hexacyanoacrylate) nanoparticles improved its accumulation in reticuloendothelial systems (RES) organs such as the liver, lungs, and spleen. In rats, 60% of the drug dose was found in the RES organs 8 hours after treatment with nanoparticles. In contrast, after treatment with the soluble drug alone, only 12% of the drug was recovered in the RES tissues. Nanocarriers have been used to enhance drug accumulation at sites of infection other than the RES organs. This is based on the observation that blood vessels at infection sites are leaky, promoting greater nanocarrier entry ([Moitra et al., 2020](#)).

In this section, we highlight the most advanced nanotechnologies in major IDs—HIV infection, TB, and malaria. The reader will glean that nanotechnology has been most actively studied in the clinic and large animals for the treatment and prevention of HIV infection. In contrast, for malaria and TB, nanotechnology has been pursued with less gusto. For these IDs, work has mainly been limited to preclinical trials in rodents. We also hope to convey that the most advanced

TABLE 2.5 Nanotechnology employed in clinical and preclinical treatment of IDs, their limitations, and stage of testing.

Disease	Drug regimen	Technology	Motivation	Limitations	Stage of testing
Human Immunodeficiency virus (HIV) infection	Cabotegravir Rilpivirine Dolutegravir Lopinavir-ritonavir-tenofovir	Injectable NPs Injectable nanoparticles Injectable nanoparticles Injectable lipid nanoparticles	To reduce dosage recurrence To reduce dosage recurrence To reduce dosage recurrence; To target macrophages Avoids use of unfavorable excipients	Requires caregiver; common injection site reactions Requires refrigeration; Require caregiver Require caregiver Requires daily dosing	Phase III Phase III Preclinical Preclinical
Tuberculosis (TB) infection	Rifampicin-isoniazid-pyrazinamide Rifampicin-isoniazid-pyrazinamide Rifampicin-isoniazid-pyrazinamide-ethambutol-streptomycin BCG vaccine	Oral lipid NPs Nebulized NPs Oral polymer nanoparticles Aerosolized micro-nanoparticles	Bioavailability; To reduce dosing recurrence To improve bioavailability; Reduce dosing frequency Target alveolar macrophages; Improve bioavailability; Avoid first-pass metabolism Efficient delivery to the lung	Does not include ethambutol; Still requires frequent dosing Uses organic solvent Cost of synthesis higher than lipid NPs; Will require training patient Will require training patient	Preclinical (rodents) Preclinical (rodents) Preclinical (rodents) Preclinical (rodents)
Malaria infections	Chloroquine derivative; aminoalcohol derivative Artemether-tafenoquine Transmission-blocking vaccine antigen candidate Pfs25	Injectable immune-liposomes Microemulsion Injectable nanoliposomes	Target infected red blood cells; Improve solubility of lipophilic drugs Improve bioavailability; Ease of scale-up of microemulsions; Reduce dosing frequency Better bioavailability than oral formulation; Suitable for multiplexed immunization	Require caregiver Use of large concentrations of surfactant to stabilize microemulsions Will require caregiver	Preclinical (rodents) Preclinical (rodents) Preclinical (rodents) Preclinical (rodents)

Source: Adapted from Moitra, P., Alafeef, M., Dighe, K., Frieman, M.B. and Pan, D., 2020. Selective naked-eye detection of SARS-CoV-2 mediated by N gene targeted antisense oligonucleotide capped plasmonic nanoparticles. *ACS Nano*, 14(6), pp. 7617–7627.

nanotechnologies are noncomplex, yet highly impactful. Hence, perhaps for nanotechnology to benefit the patient, it needs to satisfy certain tenets such as simplicity in design, clinical need, and financial enthusiasm (Moitra et al., 2020).

The availability and correct use of safe and efficacious medications is imperative for treating IDs. Nanotechnology-based approaches are the topic of intensive preclinical evaluation to improve the therapeutic index of ID drugs and simplify their use. IDs are a significant driver of morbidity and mortality globally, and their impact on low SDI countries is particularly grave. Simplifying the use of medicines and making drugs safer and more efficacious can improve patients' quality of life and reduce disease burden.

2.2.5 Action of microbial nanoparticles in dentistry

The implication of nanomaterials in dentistry has been seen to significantly increased over time. Nanomaterials such as nanocomposites, antimicrobial nanomaterials, etc., have been reported yet. Nanomaterials in the dental sector have a

unique sign indicating their properties, and hurdles in their synthesis. In terms of understanding the physical principles of certain dental nanomaterials, it is important to understand their properties such as strength and limitations as this area have the potential for the future (Jandt and Watts 2020).

The principal purpose of NPs in dentistry is their use as fillers in nanocomposites. When designing a new particle-based composite material, the simple rule-of-mixtures enables us to predict Young's modulus or the strength of the material (Chawla et al., 2012; Moitra et al., 2020). For instance, during isostrain conditions, the stress of a composite can be measured as:

$$\sigma_c = \sigma_f V_f + \sigma_m V_m$$

Here,

σ denotes stress.

V denotes the volume fraction.

The subscript c, f and m denote the composite fibers (fillers) and matrix, respectively.

The chief dental materials reinforced with nanoparticles have some dental adhesive formulations. Virtually all restorative “nanocomposites” are actually “nanohybrids” that contain much larger volume fractions of nonnanosub-micron or micron-sized particles. From a packing perspective, nanoparticles in combination with larger particles allow a higher theoretical packing density. Dental nanocomposites simplified and enhanced esthetic properties such as high gloss and gloss stability, excellent polishability, and adaptability by their manufacturers (Jandt and Watts 2020).

Karabela and Sideridou (2011) studied the properties of dental resin composites with different nanosilica particles with average particle sizes of 40, 20, 16, 14, and 7 nm. They found that the prepared composites contain different amounts of silica filler, but with the same amount of silanized silica and organic matrix showed similar flexural strength and flexural modulus, except the composite with the smallest filler particle size, which showed lower flexural modulus. If these results can be generalized, it may mean that there is a lower limit of the particle size that affects the mechanical properties of a composite (Jandt and Watts 2020).

2.3 Advancements in antimicrobial surface coating strategies

Various implanted medical devices include a pacemaker, hip implants, catheters, and knee implants. The microbial adhesion and colonization on these medical implants are the preeminent source of health care problems that might affect the quality of a patient's life and may also manifest an immense risk of systemic as well as local infections following the implantations. The shelf life and functionality of the medical implants are directly affected by the microbial binding limit. Various physical and chemical surface-based properties are important for the working of medical implants/devices. Procedures including topographical and chemical surface modifications such as biofilm formation, covalently bound coatings, bacterial colonization have been overcome in order to reduce the probability of contamination developing over the surfaces of medical devices (Erkoc and Ulucan-Karnak, 2021).

The viable applications of various NPs as antimicrobial surface coatings are presented in pharmaceuticals, tissue replacements therapies, and medical devices/implants in order to manage cellular intervention-derived nanorobots. The advancements in nanotechnology in various sectors combined with numerous derivatives of polymers and nanocomposite surface coatings are being utilized for biomedical applications in order to provide antimicrobial surfaces (Erkoc and Ulucan-Karnak, 2021).

2.4 Conclusions

The synthesis of NPs using microbes is preferred over chemical synthesis. Microbial nanotechnology proposes vast properties of the metals that reveal their diverse possible roles in the agriculture sector, as antimicrobial factors, diagnostics through biomedical agents, biomarkers, and bioimaging (Jandt and Watts 2020). Therefore, microbial nanoparticles have gained tremendous focus nowadays by scientists of various fields due to the novel property of nanoparticle synthesizing microbes thriving in extreme situations (Erkoc and Ulucan-Karnak, 2021). One of the applications that include the role of NPs in dentistry has strengthened significantly over the past years. The persistence and colonial establishment of microorganisms on implanted medical equipment such as catheters, knee, hip implants, and pacemakers lead impose leading health care issues affecting patient's quality of life (Jandt and Watts 2020). Microbe's adhesive limits the shelf life and functionality of these devices. Their industrial applicability ranges from medical devices, pharmaceuticals, and tissue replacement therapies to engineered nanorobots generated for cellular intervention (Erkoc and Ulucan-Karnak, 2021).

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Application of biogenic nanoparticles in the remediation of contaminated water

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3.1 Introduction

Apart from food and shelter, clean water is an unavoidable requirement in human life. Due to the ever-increasing population growth and industrial development, the water sources in nature are facing severe pollution problems. As per recent studies, over 1.2 billion people have struggling to access clean drinking water worldwide (Zehra et al., 2020). The continuous discharge of various organic and inorganic contaminants to the water bodies have resulted in a detrimental effect on the aquatic ecosystems and thereby posed severe threats to natural ecosystems and human health (Safauldeen et al., 2019; Zhao et al., 2021). Water contamination with heavy metals is a serious concern, due to the persistence of heavy metals, and accordingly leads to long-term consequences in the biological system (Mishra et al., 2018). One of the significant consequences of water pollution is the unbridled proliferation of phytoplankton as a result of eutrophication, thereby the destruction of biodiversity of the aquatic ecosystem. Moreover, the use of contaminated water for livestock farming and agriculture and fishing in polluted waters results in the contamination of the food chain and the associated accumulation of toxic compounds into foods. Therefore, the wastewaters have to undergo adequate treatment before the discharge into the environment.

In conventional water treatment practices such as ion exchange, carbon adsorption, chemical precipitation, and membrane processes, there is no unique technique for treating the diverse toxic compounds in a single step (Rajasulochana and Preethy, 2016; Zamora-Ledezma et al., 2021). Therefore, the conventional practices used for wastewater treatment are not always effective in efficiently reducing and completely removing toxic heavy metals from polluted water. Thus, the residues of the contaminants present in the treated water result in toxicity symptoms in the cellular processes in plants and animals.

With the emergence of nanotechnology, application of nanoparticles has enhanced in different fields of science. Interestingly, the physical and chemical properties of these nanoparticles are entirely different from their bulk materials. The reduced size and enhanced surface area are the major attraction of nanoparticles, and morphology as well as size of the nanoparticles significantly contributes to the remarkable characteristics of the nanoparticles (Schwirn et al., 2014; Jain et al., 2020). Therefore, the exceptionally unique and tunable physicochemical characteristics of the nanoparticles are currently used in the field of agriculture and the environment for various applications (Amritha et al., 2021).

There are several physical, chemical, and biological techniques existing for the production of various types of nanoparticles. The physical and chemical methods are most popular due to their longer stability and best way to get uniform-sized nanoparticles (Li et al., 2011). However, both these techniques release toxic products to the environment and are expensive. In contrast, the synthesis of nanoparticles by biological methods is an eco-friendly and cost-effective alternative to the physical and chemical methods, with controlled toxicity and size characterization. There are various natural sources for nanoparticle biosynthesis, such as plant extracts, fungi, yeast, and bacteria. Biological particles such as viruses, proteins, peptides, and enzymes could also be exploited for nanoparticle biosynthesis.

Nowadays, nanoparticles are widely used in wastewater treatments. There are several reports regarding the nanoparticle-mediated removal of bacteria, heavy metals, inorganic anions, and organic pollutants from wastewater (Tang et al., 2014; Kalhapure et al., 2015; Yan et al., 2015; Lu et al., 2016; Venis and Basu, 2021). The extensively used nanomaterials in wastewater treatment include nanocomposites, carbon nanotubes, metal oxide nanoparticles,

and zero-valent metal nanoparticles (Lu et al., 2016). Because of the physicochemical properties, surface morphology, and nano-size, the nanoparticles can act as strong adsorbents and react with the contaminants in the wastewater (Parveen et al., 2016; Haghighat et al., 2020).

This chapter provides a detailed review of the synthesis of microbial nanoparticles and their potential applications in the remediation of various organic and inorganic contaminants in wastewater.

3.2 Different water remediation methods

There are three distinct steps concerned with conventional wastewater treatments, *viz.* primary, secondary, and tertiary wastewater treatments. The primary treatment involves the filtering of the larger particles and sedimentation of solid wastes. In secondary treatment, the sludge undergoes oxidation after primary treatment for further purification, which can be performed via biofiltration or aeration processes. In the case of tertiary treatment, the nutrients such as phosphates and nitrates, and other soluble organic and inorganic pollutants, and harmful microorganisms are removed from the water. Therefore, every step in wastewater treatment is vital for the achievement of the desired results. Generally, a combination of physical, chemical, and biological methods is implemented to remove colloids, organic pollutants, nutrient enrichment, and heavy metal ions from the effluents and wastewaters (Crini and Lichtfouse, 2019). However, most of these practices have various disadvantages in terms of cost-effectiveness, energy utilization, quality of final water, etc., and they are described in Table 3.1 in detail.

TABLE 3.1 Disadvantages of major conventional wastewater treatment processes.

Processes	Major characteristics	Disadvantages	References
Froth flotation	Removes oil, fat, grease, suspended solid wastes, microplastics and heavy metals	Initial cost is high with high maintenance and operation costs; requires chemicals to control the hydrophobicity between particles; the process is pH dependent	Kyzas and Matis (2018)
Electro-Fenton process	Removal of organic pollutants, especially aromatic compounds	Treatment of Fe-containing sludge is expensive and needs the use of chemicals; restricted to a narrow pH range (pH 2–3)	Nidheesh and Gandhimathi (2012)
Electrochemical technologies	Instead of chemical reagents, reactions at the anode surface produce oxidizing species such as $\text{OH}^\bullet$, possible for the recovery of heavy metals	Formation of halogenated byproducts such as oxyanions ClO_3^- , ClO_4^- , BrO_3^- , and organic compounds	Chaplin (2018)
Chemical Coagulation	Coagulation of particles by the addition of chemicals	Requires addition of chemicals such as alum, ferric chloride, ferrous sulfate, lime etc. to neutralize the charged particles; production of large volume of hazardous sludge; high disposal costs of the sludge	Nicholas (2020)
Chemical precipitation	Uptake of the pollutants and separation of the products formed	Consumption of chemicals such as lime oxidants etc.; fails to remove the metal ions present in low concentrations; requires oxidation for the removal of complexed metals; problems regarding the management of sludge and its cost	Crini and Lichtfouse (2019)
Advanced oxidation process	The driving force is $\text{OH}^\bullet$ radical, remove organic contaminants and some heavy metals, disinfect any pathogen present in the polluted water	Needs relatively high capital and maintenance costs; essential to maintain the optimum amounts of $\text{OH}^\bullet$ to achieve the maximum result, which needs highly skilled engineers; production of excess residual H_2O_2 , which is harmful to humans	Nicholas (2019)
Ion exchange	Softens the hard water, by exchanging the positively charged magnesium and calcium ions with sodium ions	The softening solution to be filled with calcium and magnesium ions; the entry of Na^+ increases the acidity of the softened water; high operational costs	Tripathi (2017)
Ozonation	Used for the degradation of organic and inorganic pollutants in the effluents and disinfection of drinking water	Due to the high reactivity and corrosive nature of ozone, the process requires corrosion resistant material; highly expensive; ozone is toxic and extremely irritating; not economical for poor quality wastewater	Brennan (2017)
Biosorption	Used for the removal of heavy metals and organic pollutants from wastewater using biosorbents, such as cyanobacteria, bacteria, algae, fungi and yeasts	Less effective as compared to conventional sorbents, the production as well as the maintenance of the living biomass is costly	Torres (2020)

3.3 Application of nanoparticles in wastewater treatment

Nanotechnology is a modern aspect of science where scientists design particles with superior qualities at the molecular level (Cai et al., 2020). Nanoparticles are tiny materials with a size range of 1–100 nm, and the morphology of these particles, including size and shape, determines their characteristic features (Khan et al., 2019). Novel nanotechnology-based solutions is a sustainable approach towards water remediation and the catalytic, optical, magnetic, and electric features of nanoparticle impart this remediation potential to these compounds. Carbon-based (graphene, graphene oxide-based nanomaterials, carbon and graphene quantum dots-derived nanomaterials, and carbon nanotubes), metal-based [nanoparticles based on nickel (Ni), cadmium (Cd), gold (Au), cobalt (Co), magnesium (Mg), manganese (Mn), silver (Ag), zinc oxide, titanium dioxide, iron oxide, and magnetic (Fe, Co and Ni) nanoparticles] and polymeric nano-adsorbents (low-cost and biocompatible chitin nanofiber and chitosan nanoparticle) for removing organic and metal contaminants; nano-photocatalysis for disinfection (TiO_2 , ZnO , SnO_2 as well as sulfides like ZnS); desalination of wastewater using nanomembranes are the advanced techniques for the implementation of nanotechnology in wastewater purification (Tourinho et al., 2012; Siahkamari et al., 2017; Villaseñor and Ríos, 2018; Hassan et al., 2020; Nasrollahzadeh et al., 2021; Cervantes-Avilés and Keller, 2021; Priya et al., 2021). The major roles of nanoparticles in wastewater treatment are diagrammatically represented in Fig. 3.1.

Oil pollution is a significant threat to life in marine water, and the application of magnetic sorbent-based iron oxide nanoparticles aid in remediating the excess hydrocarbons in the water (Qiao et al., 2019). When the bio-hydrothermal method using *Psidium guajava* leaf extract was adapted for the production of ZnO – ZnFe_2O_4 nanoparticles, this biosynthesis method aided in overcoming the environmental impact of other nanoparticle synthesis processes (Sahoo et al., 2021). Moreover, this ZnO – ZnFe_2O_4 nanoparticle potentially removed different dyes like congo red and methylene blue from the water sources (Sahoo et al., 2021). Immobilized nanoparticles have the potential to remediate contaminated groundwater and by removing the organic contaminants (Cai et al., 2020). Nanomaterials used for water remediation include both organic as well as inorganic nanomaterials, and these nanoparticles could oxidize, adsorb, or degrade the pollutants in water (Lu and Astruc, 2020).

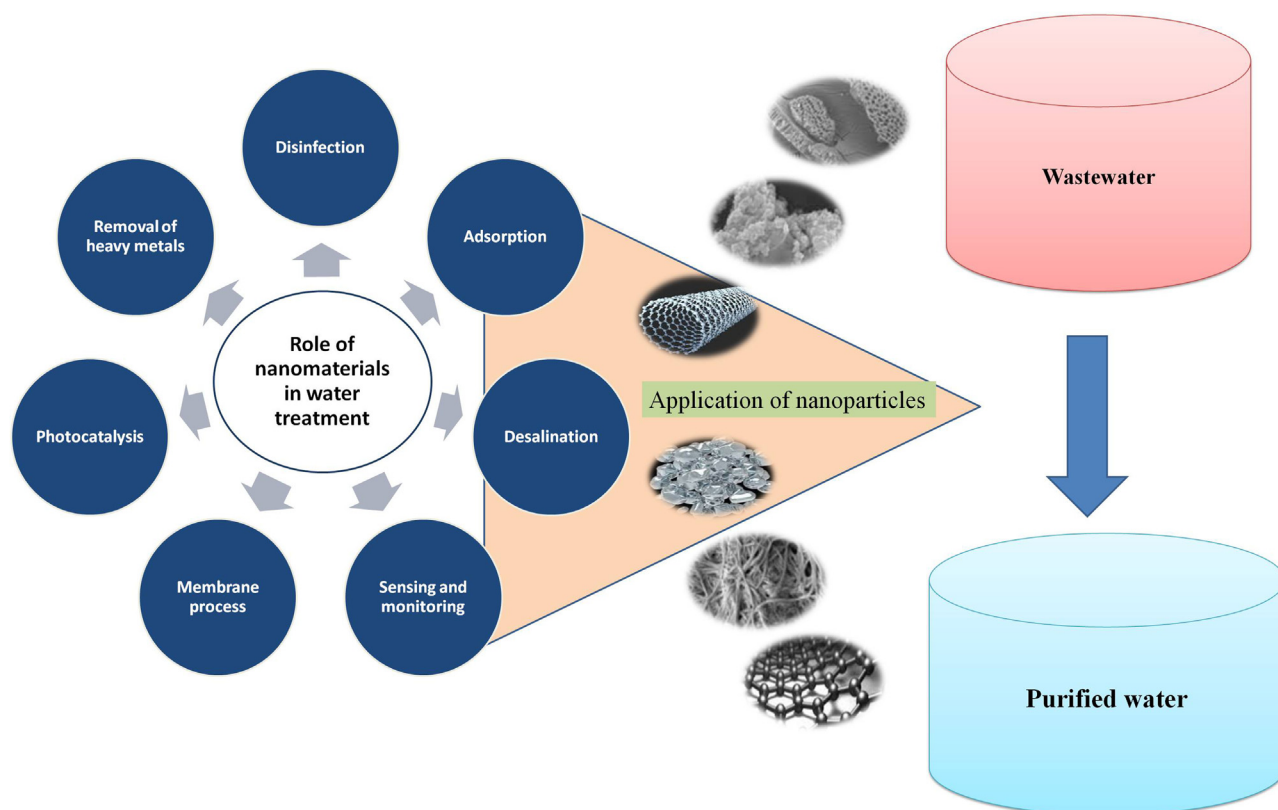


FIGURE 3.1 Role of nanoparticles in wastewater remediation.

Different nanoparticles can act as potential adsorbents and filter that aid the removal of contaminants from water; polysulfone hollow porous granules (PS-HPGs) are one among them (Posati et al., 2019). The treatment with PS-HPGs increased the removal rate of heavy metals (Zn and Ni) as well as antibiotics (ofloxacin) from contaminated water (Posati et al., 2019). Polydopamine nanoparticle-coated polysulfone porous granules could also act as a suitable adsorbent in water remediation processes (Posati et al., 2019). The high reactivity and eco-friendly nature of nano-sized zero-valent iron is extensively used in water decontaminating processes. Along with the size and morphology, the methodologies of nanoparticle biosynthesis processes also determined the kinetics of their actions. Two important strategies of nanoparticle biosynthesis are top-down and bottom-up methods. In the former one milling processes, pulsed laser ablation, and sputtering were used for nanoparticle production. In contrast, in the bottom-up method, dissolved iron salts, nano-sized iron oxides, or iron-containing molecules were used as the precursors.

It is necessary to clean the wastewater by removing the extensively multiplying microorganisms using antimicrobial nanomaterials, and for this screening of the disinfection potential of the nanoparticle is essential (Yusuf et al., 2021). A comprehensive analysis of the disinfection capacity of nanoparticles revealed that silver nanoparticles and ZnO nanoparticles are more efficient in removing microbes from water sources (Yusuf et al., 2021). These nanoparticles inhibit primary metabolisms of cells like enzyme activity, energy transfer, and DNA synthesis, and thus they can easily prevent the growth and multiplication of microbial cells (Li et al., 2008). TiO₂ nanoparticles have a specific role in the disinfection process due to photocatalytic disinfection, and this process does not generate any toxic compounds (He et al., 2021). The characteristics of wastewater and the nature of TiO₂ nanoparticles (composed of metal ions or carbon nanoparticles) together determine the efficiency of photocatalytic reaction (He et al., 2021). TiO₂ nanoparticles generate reactive oxygen species that aid to kill microbial cells in the contaminated water and supports water purification (Li et al., 2008).

Carbon-based nanomaterials are a promising technique in water remediation due to the efficiency in membrane separation, adsorption, and disinfection (Shafaqa Al-anzi and Chi Siang, 2017). These nanomaterials are helpful in water remediation owing the high water permeability, high specific surface area, chemical stability, the potential for size-controlled separation of pollutants etc. (Shafaqa Al-anzi and Chi Siang, 2017). Specific surface area, pore-volume, pore size distribution, easy separation, and reusability of metal-based nanoparticles have also attracted the scientists (Hassan et al., 2020).

However, there are some limitations to the extensive use of nanoparticles in wastewater treatment. The major drawbacks of nanoparticles include the introduction of toxic compounds, low selectivity, aggregation/sedimentation, and difficulty in separation (Irvani, 2021). However, the green biosynthesis of nanoparticles is a good remedy for these problems, and the fabrication of microbes with nanoparticles aid to improve the environmental sustainability of nanotechnology (Mandeep and Shukla, 2020).

3.4 Synthesis of microbial nanoparticles

Nanoparticle biosynthesis could be accomplished through various chemical, physical and biological processes. The physical and chemical methods direct to the synthesis of the uniform-sized nanoparticle. But, the hazardous compounds generated during this process, raise the alarming question of implementing these physical and chemical methods (Li et al., 2011). Bioprocess was invented to overcome this impact, and nanoparticles of silver, gold, copper, and platinum were prepared through biological way (Li et al., 2011). It is a sustainable step towards improved nanoparticle biosynthesis by utilizing microorganisms. Different microbes like bacteria, fungus, and algae are involved in nanoparticle biosynthesis processes (Mohanpuria et al., 2008). These microbes have varying mechanisms for the production of nanoparticles (Mohanpuria et al., 2008). However, the common method of nanoparticle biosynthesis includes both intracellular and extracellular, and is based on the nanoparticle forming location of microbes (Li et al., 2011).

The biosynthesis of microbial nanoparticle starts by accommodating metal ions on the surface or inside the microbes. Further, with the action of different biocatalysts, the metal ions become reduced to the nanoparticle. Here the microorganism involved in this bioprocess has two distinct ways to achieve nanoparticle biosynthesis (Shedbalkar et al., 2014). Microbial induced supersaturation and organic polymer production are the two important ways of nanoparticle biosynthesis (Li et al., 2011). In addition to this, a detailed analysis of microbial nanoparticle synthesis is essential to get more comprehensive knowledge in the field. For this purpose, bacterial-mediated Ag nanoparticle biosynthesis was explained here. In the first attempt of Ag nanoparticle biosynthesis, *Pseudomonas stutzeri* AG259 with a high level of Ag tolerance was utilized as the living system. The different salts of Ag have been experimented for microbial nanoparticle synthesis, and silver nitrate salt is the most common one (Singh et al., 2015). In bacterial-mediated extracellular nanoparticle biosynthesis, the surface of the bacterium acts as the location of nanoparticle formation (Fig. 3.2).

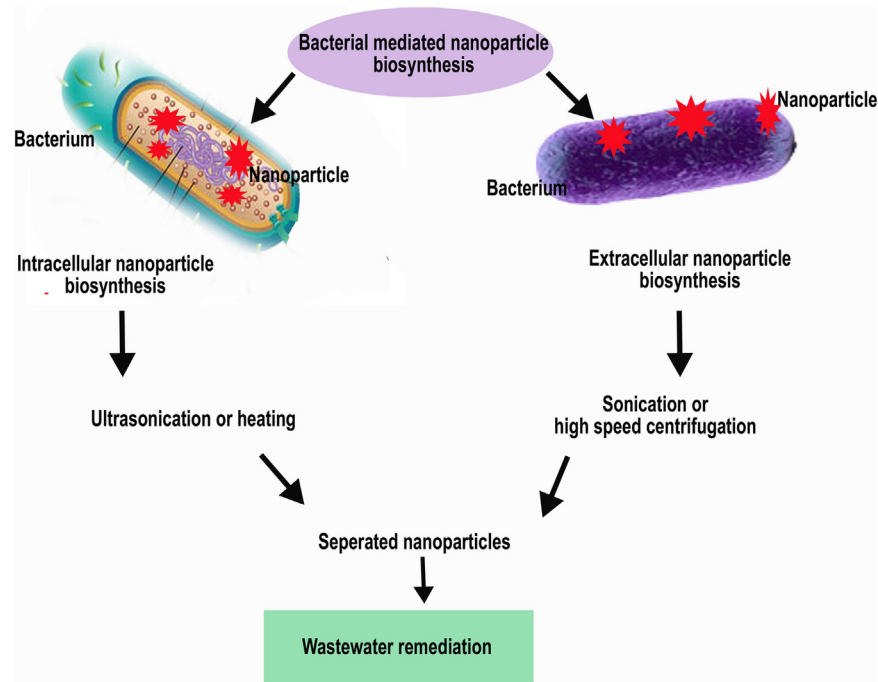


FIGURE 3.2 Overview of bacterial-mediated nanoparticle biosynthesis.

Extracellular Ag nanoparticle recovery needs sonication followed by high-speed centrifugation to separate the adsorbed material from the surface (Shedbalkar et al., 2014; Abu-Tahon et al., 2020).

Intracellular nanoparticle biosynthesis is different from this, where the formation of nanoparticles happens inside the cell (Fig. 3.2). Here, the bacterial cells were permitted to grow and multiply in a medium with silver salt with all other organic and inorganic nutrients. *Pseudomonas stutzeri* exhibited an intracellular mechanism of nanoparticle biosynthesis. The recovery of the intracellular nanoparticle is a time-consuming multi-step task, which includes ultrasonication or heating (Singh et al., 2015). Extracellular complexation, immobilization, fluctuation in homeostatic events, and intracellular precipitation are the actively involved mechanisms in the nanoparticle biosynthesis processes (Durán et al., 2011).

3.5 Application of microbial nanoparticles in wastewater management

Different bacteria were experimented with in water remediation by fabricating with nanomaterials, and *Bacillus subtilis*, *B. licheniformis*, *Kocuria flava*, *Pseudomonas aeruginosa*, *Deinococcus radiodurans*, *Serratia*, *Escherichia coli* were the well known bacterial candidates that exhibit remarkable potential for water decontamination. *Escherichia coli* fabricated Ag nanoparticles actively removed contagious microorganisms present in wastewater (Singh and Tiwari, 2021). Association of *Escherichia* sp. SINT7 with the Cu nanoparticle proved as an excellent strategy to clear contaminated water by the removal of heavy metals (Noman et al., 2020). This biogenic nanoparticle had high dye degradation potential, and it increased the removal of congo red, malachite green, direct blue-1, and reactive black-5 from the textile effluents (Noman et al., 2020). Similarly, *Bacillus marisflavi* TEZ7 was used for the green synthesis of Ag nanoparticles (Ahmed et al., 2020). This nanoparticle has photocatalytic degradation efficiency, and direct blue-1, methyl red, and reactive black-5 are the three dyes removed by the action of *Bacillus marisflavi* treated Ag nanoparticles (Ahmed et al., 2020). Both studies proved that microbial-assisted nanoparticles are efficient in clearing textile effluents and aid in balancing the pH, turbidity, and mineral content of the water (Ahmed et al., 2020; Noman et al., 2020).

The TiO₂ nanoparticle biosynthesis with *Bacillus subtilis* is an active nanomaterial with 66–77 nm size, and this could be utilized in the environmental cleanup programs (Kirthi et al., 2011). The radiation-resistant bacteria *Deinococcus radiodurans* was used to produce Ag nanoparticles, and this particle showed significant antibacterial activity. The high resistance of this bacteria towards hard environmental conditions makes the TiO₂ nanoparticle maintain its activity and efficiency even in highly polluted water sources (Yadav et al., 2020).

Actinomycetes are gram-positive mycelial bacteria, extensively exploits in the field of antibiotic production. These organisms are also used for the green synthesis of nanoparticles. *Rhodococcus* sp, *Streptomyces hygroscopicus*,

Streptomyces glaucus 71MD, *Streptomyces rochei*, *Streptacidiphilus durhamensis* are the major actinomycetes members used for the production of Au, Ag, Zn, and Cu nanoparticles (Manimaran and Kannabiran, 2017). Inhibited growth of *Pseudomonas aeruginosa*, *Bacillus cereus*, and *Staphylococcus aureus* indicates the antibacterial efficiency of Ag nanoparticles designed with *Streptomyces* sp. BDUKAS10 and thus this nanoparticle could be utilized in wastewater treatment (Sivalingam et al., 2012). Silver nanoparticles accommodating *Streptomyces* sp. and *Rhodococcus* sp. also had antibacterial efficiency (Sukanya et al., 2013).

Different fungal candidates were also involved in wastewater treatment as a part of nanotechnology. The antifungal activity of Ag nanoparticles prepared using *Trichoderma viride* is the best example for non-conventional nanoparticle biosynthesis (Mishra et al., 2014). Decontamination of the fungal pathogens like *Fusarium oxysporum* and *Alternaria brassicicola* was achieved using biologically prepared nanoparticles. When *Penicillium citreonigum* and *Scopulariopsis brumptii* was incorporated with Ag nanoparticles, it significantly improved the wastewater remediation potential of this nanoparticle (Moustafa, 2017). Application of white-rot fungi in wastewater treatment with nanoparticles increased the stability and bioremediation potential of nanoparticles (He et al., 2017). Instead of single species, a fungal consortium was found more effective in wastewater treatment. Silver nanoparticles synthesized from biomass of fungal consortium coated with chitosan have high stability in wastewater, and this nanoparticle could be utilized in batch wastewater treatment.

The production of metal (gold, silver, platinum, and palladium) nanoparticles by incorporating algae is a good strategy for improvising nanoparticles (Asmathunisha and Kathiresan, 2013). Algae such as *Turbinaria conoides*, *Stoechospermum marginatum*, *Pterocladia capillacea*, *Jania rubins*, *Portieria hornemannii*, *Ulva fasciata*, and *Colpomenia sinusa*, and *Sargassum muticum* were efficiently used for the green synthesis of nanoparticles (Brayner et al., 2007; Arockiya Aarthi Rajathi et al., 2012; El-Rafie et al., 2013; Fatima et al., 2020).

Polysulfone nanofibrous web immobilized with *Chlamydomonas reinhardtii* was utilized to remove dyes from textile effluents (San Keskin et al., 2015). Electrospun nanofiber mats fabricated with microalgal cells efficiently removed nitrogen from wastewater. In the future, they can play a crucial role in the environment cleanup by eliminating organic and inorganic wastes from contaminated water (Eroglu et al., 2012). It is essential to develop green nanoparticles as biosensors, and when the cells of *Chlorella pyrenoidosa* was stabilized with super magnetic nanoparticles (with poly (allylamine hydrochloride)), it functioned as an efficient biosensor for the detection of contaminants in wastewater (Fakhrullin et al., 2010). *Petalonia fascia*, *Colpomenia sinuosa*, and *Padina pavonica* were used to form iron oxide nanoparticles, and this nanoparticle exhibited high nitrogen and phosphorus reduction rate (El-Sheekh et al., 2021). Therefore, algae can be effectively utilized in wastewater treatment in connection with nanoparticles.

3.6 Conclusions

The application of advanced techniques for wastewater remediation is an excellent strategy to overcome the world's water scarcity. Nanotechnology is an efficient technique for wastewater treatment but is reported to have severe environmental impacts. At this moment, the synthesis of biogenic nanoparticles is essential, reducing the negative effects of nanoparticles on the environment. With the aid of a microbial system, this “green nontechnology” can improve nanoparticle efficiency in wastewater treatment. Different microorganisms like bacteria, actinomycetes, fungi, and algae were used to synthesize nanoparticles, disinfecting the wastewater. Moreover, these nanoparticles can remove excess mineral and toxic metal ions from water sources. However, the potential of microbes varies with the characteristics of wastewater. Therefore, an extensive understanding of the microbial candidates and nanomaterials is essential for the implementation of green nanotechnology. It thus could contribute significantly toward the implementation of advanced nano-techniques in wastewater remediation.

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Nanotechnology in biological science and engineering

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4.1 Introduction

Nanotechnology has changed the way we think about technology development by revolutionizing scientific research. The rapidly evolving field of nanotechnology has been discovered to have potential in a broad array of applications in recent decades (Mtibe et al., 2018). In domains such as medicinal, pharmacological, aesthetic, paint, material, electrical, food, catalysis and energy a wide spectrum of innovations, devices, methods, and applications are covered (Koopmans and Aggeli, 2010). Nanotechnology has long been a hotbed of research, and the field's popularity is expanding rapidly. This technology is described as a science or technology that enables the manipulation of materials with a diameter of less than 100 nanometer in at least one aspect of the dimension (Jonoobi et al., 2015; Kamel, 2007). Nanomaterials have superior qualities than bulk materials equivalents due to their smaller sizes combined with good mechanical, chemical, and physical properties (Jonoobi et al., 2015). Nanomaterials' characteristics are determined by their dimension, structure, and morphologies. The vast majority of the studies are currently emphasizing on regulating those parameters (Sirelkhatim et al., 2015).

Nanotechnology refers to processes, devices, and materials whose structure and function are significant at short length scales ranging from nanometers (10^{-9} m) to microns (10^{-6} m) in biomedical science (Whitesides, 2003). This size range is linked to fascinating occurrences in both natural and synthetic systems. Essentially the primary building blocks of life, such as biomolecules and cells, fall within this spectrum; cell membranes, for example, are sheet-like constructs with a 10 nm thickness. Artificial nanostructures, such as nanopores with an apertures of 2 nm, inorganic nanowires with a width of 10 nm, and spherical nanoparticles of 10 to 100 nm diameter, can be made with comparable dimensions (Fig. 4.1) (Stylios et al., 2005). Furthermore, this size range is linked to surprising physics and chemistry in which molecular effects might be significant (Wong et al., 2013).

Nanoparticles (NPs) are atom clusters having at least one dimension between 1 and 100 nanometers. Various types of NPs are generated and employed depending on the core substance. In biological applications of nanotechnology, metallic Nanoparticles such as gold (Au) (Frens, 1973), silver (Ag) (Ravindran et al., 2013), titanium dioxide (TiO_2) (Chen and Mao, 2007), iron oxide (Fe_2O_3) (Laurent et al., 2008), and others are often utilized. Complex structural NPs, such as crystalline structure and hollow aggregates (Wang et al., 2017) of two distinct metals, are created in addition to ordinary spherical and solid NPs. These NPs have exceptional characteristics and are employed in electronics, medicine, optics, catalysis and other fields. The inherent mechanical, chemical, optical, and physical features of these materials have been investigated to develop innovative approaches for a variety of applications (Borse et al., 2020).

Two broad themes can be used to establish a conceptual framework for nano-bio interactions. First, nanotechnology allows for novel in vitro and in vivo methods of measuring and detecting biology. Nanoscale devices, for example, can detect minute variations at the scale of individual cells and molecules. This highly exceptional sensitivity can be utilized to define single-cell heterogeneity at high throughput, exposing unique hierarchies and sub-populations, for example (Bayley and Cremer, 2001). Second, nanotechnology opens up new avenues for perturbing cells and treating patients. Nanomaterials, for instance, can be developed to transport medicines to specific regions while bypassing or escaping biological barriers, thus changing the drug's underlying pharmacokinetics and biodistribution. The

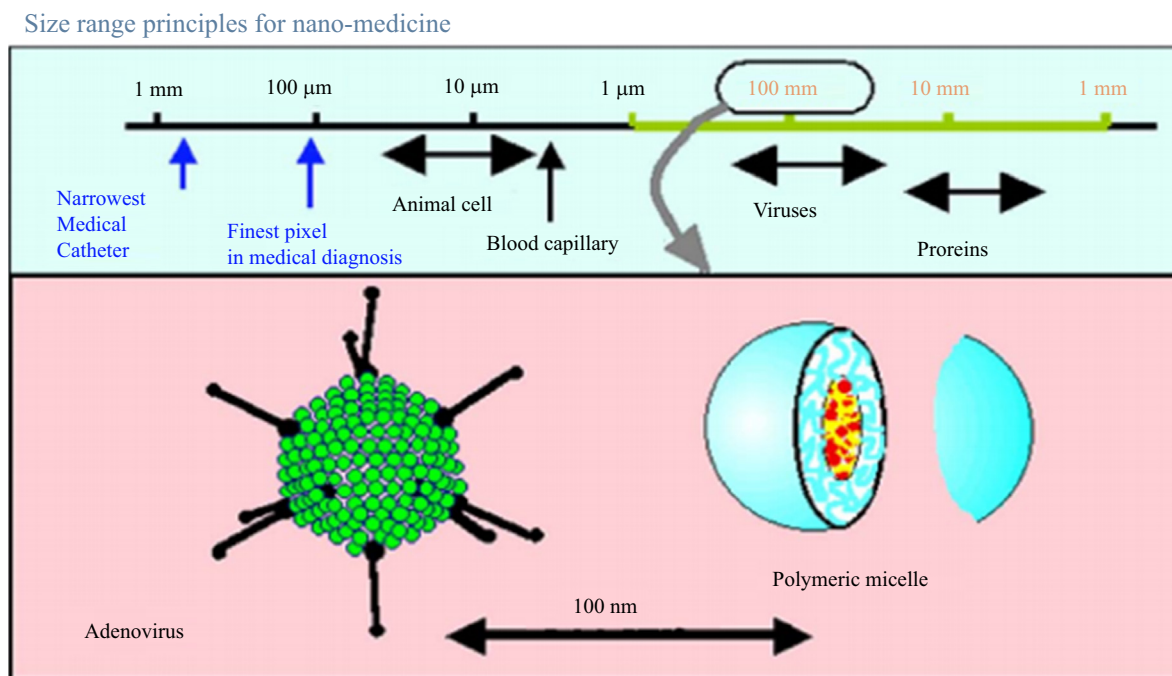


FIGURE 4.1 Illustration of the size range of nanoscale materials (Stylios et al., 2005). Adopted from Stylios G.K., Giannoudis, P.V., Wan, T., 2005. *Applications of nanotechnologies in medical practice*. *Injury*, 36 Suppl. 4, S6–S13. Permission obtained from Elsevier.

coordinating combination of these two concepts: nanotechnologies that can simultaneously monitor and perturb life, is an attractive prospect. Emergent behaviors such as amplification, adaptability, and self-organization will emerge from the design and fabrication of biomimetic nanosystems, which will be controlled by feedback between nanoparticles and their environment (Wong et al., 2013). These capabilities have the potential to revolutionize the way we deal with the spatial and temporal intricacy of biological systems.

4.2 Nanobiotechnology

Nanotechnology is a cutting-edge scientific field that includes materials and equipment capable of changing a material's physicochemical properties at the molecular scale. Biotechnology is the application of tools and biological understanding to change cellular, and molecular functioning in order to generate valuable products and services in a variety of fields, including health and agriculture (Gartland and Gartland, 2018). Nanomaterials have unique physical and chemical features, which when combined with biological materials, the resulting nano-bio complex can be used to create a variety of applications. Nanobiotechnology is a new hybrid of nanotechnology and biotechnology that allows traditional microtechnology to be combined with a molecular approach in real life (Qamar et al., 2019).

Nanobiotechnology is a new area of science and technology that has emerged as a result of the combination, integration, or linkage of biotechnology or bio-science with nanotechnology. Nanobiotechnology's purpose is to develop a variety of approaches that use nanomaterials and biological materials to conduct in-depth research (Borse et al., 2020). Nanobiosensing, nanoimaging, nanodiagnostics, nano-labeling, nanotherapeutics, and other approaches have resulted from the development and progressions of both nanotechnology and biotechnology.

Nanobiotechnology has evolved into a paradigm-shifting technology which is poised to transform the state of the twenty-first century by paving the way for a civilization that provides high-quality of life and is also sustainable. Nanobiotechnology has shown to be a particularly fertile research area with the majority of the themes being multidisciplinary in character. The subject domain can be seen as a field of applied sciences at the nanoscale focusing on natural, biologically-inspired, and biochemical process applications (Koopmans and Aggeli, 2010). Nanobiotechnology focuses on nanostructures and nanomachines, such as biological micro and nano molecules and cell machinery, that have evolved through natural selection over 3.8 billion years.

This new growing discipline of nanobiotechnology will replace the costly traditional manufacturing method with less expensive and environmentally friendly products that are resilient, flexible, and precise. This technology could be used to create materials that are both robust and lighter, like drug delivery, sensor systems, tiny robots, and surgical tools (Afsheen et al., 2020). By combining or mimicking biological processes, or synthesizing microscopic tools to modify varied features of the living system at the molecular level, atomic grade machines can be constructed using nanobiotechnology (Thirumavalavan et al., 2017). This is a cutting-edge technology that has the ability to blur the lines between physics, chemistry, and biology, as well as improve our current understanding and thoughts.

Bionanotechnology and nanobiotechnology are phrases that are frequently used synonymously. However, when a differentiation is done, it is dependent on whether the emphasis is on implementing biological ideas or executing research in biological science using nanotechnology. Bionanotechnology is the examination of how biological “machines” work and how these natural motifs might be used to improve current nanotechnologies or create new ones. Nanobiotechnology, on the contrary, is the use of nanotechnology to the creation of devices for studying biological systems (Nussinov and Alemán, 2006).

To put it another way, nanobiotechnology is basically miniaturized biotechnology, while bionanotechnology is an application or implementation of nanotechnology. Since they entail focusing on biomolecules on the nanoscale, DNA nanotechnology and cellular engineering, for example, would be categorized as bionanotechnology (Zadegan and Norton, 2012). Many innovative medical technologies that use nanomaterials as delivery or sensors systems, on the other hand, are illustrations of nanobiotechnology as they use nanotechnology to promote biological aims.

4.3 Bionanotechnology

Bionanotechnology applies molecular biology principles and tools to engineering goals, creating devices at the nanoscale and allowing us to mimic biological structures with molecular-level precision. Bionanotechnology is based on the idea of designing molecular machinery to atomic precision. Micro-observational investigations of the cells show atomically accurate molecule-sized motors, sensors, girders and a bunch of other useful systems, all ready for bionanotechnology to take complete advantage. Bionanotechnology is a branch of science that uses the knowledge of properties acquired by living organisms over time for technological purposes. Through the merger of biological systems and nanotechnology, it draws inspiration from human physiology to evolve complex artificial systems. Bionanotechnology applies molecular biology concepts and methods to engineering goals, creating devices at the nanoscale and allowing us to mimic biological structures with molecular-level precision. The creation of “artificial organs” manufactured from the cells of patients’ own bodies, for example, demonstrates the importance of bionanotechnology. Although synthetic materials have previously been used as implants, they have their own limitations in terms of availability, stability, feasibility, and compatibility issues (Bambole and Yakhmi, 2016).

The biological machines served as living proof that complex and functionally sophisticated systems might function at the nanoscale. These natural nanomachines are motivating in and of themselves, and their existence, as well as extensive studies of their operation, has sparked efforts to duplicate them with artificially planned and manufactured systems—a process known as bioinspired nanotechnology or biomimetic nanotechnology. Many structures, particularly devices created in living systems, are made up of biopolymers that have been engineered to fit constituents with high specificity and precision stoichiometry (Ramsden, 2016). One of the difficulties of biomimetic nanotechnology is recreating these characteristics with simpler artificial systems yet being robust, which has proven difficult till recently. Could one, for example, construct a synthetic oxygen transporter that functions similarly to hemoglobin but has a tenth or fewer atoms? Perhaps, but one has to ask if such a light and lean carrier would be as resistant to changes in its working environment (Takai, 2006).

4.4 Advantages of nanotechnology

Different morphological and pathological variances of damaged tissues may open up a lot of possibilities for developing targeted nano—structured products. The nanostructures are advantageous in a number of ways, including: (1) drug delivery that can be accomplished by different physical factors of damaged tissues (Hughes, 2005), (2) regular drug losses can be diminished using nanomaterials which can concentrate the drug dose at the area of target (Guo et al., 2018); (3) Increased microvascular permeability in tumors improves nanosystem efficiency in irritated tissues, developing improved retention and transport (Fenaroli et al., 2018); (4) Nanosystems are competent of preferential restraining of tumor and inflamed tissues (ElMeshad et al., 2014); (5) Nanomaterials can pass the blood-brain barrier, proving that they can be utilized to treat brain cancer (Saraiva et al., 2016); (6) Drug-loaded nanoparticles change particular cellular

transport, allowing for more effective drug administration while lowering the risk of side effects (Hua et al., 2015); (7) Nanodiamonds have the potential to be employed as fluorescent biomarkers for various disease detection, such as Alzheimer's disease (Morales-Zavala et al., 2018). The description and useful properties of various nanomaterials used in biotechnological applications are presented in Table 4.1 (Gilmartin and O'Kennedy, 2012).

The various advantages of nanotechnology in biological applications are listed below:

- Nanoparticles utilized in biotherapeutics can be used for cellular and molecular imaging, brain tissue engineering, and medication transport across the blood-brain barrier (BBB) (Nair et al., 2012).
- Nanoparticles can easily adjust to drug absorption by altering their shape and size (Sadrieh and Tyner, 2010).
- Nanoscale drug delivery processes have been proved to be very effective in the pharmacokinetic techniques of drug-molecules, like biodistribution, bioavailability, and drugs are released in a controlled and efficient manner, with tissue-specific drug delivery and a reduction in toxic effects (Allen and Cullis, 2004).
- Nanocarrier-based oral medication delivery, which incorporates specific proteins and enzymes, enhances drug absorption, increases water solubility and dissolution, and is free of enzymatic degradation (Vasir et al., 2005).
- Nanomaterials with strong thermal, mechanical, optical and electrical properties are up and coming for biosensor advancement in various cancer types (Vasir and Labhassetwar, 2005).

TABLE 4.1 Useful properties and description of various nanomaterials used for applications in biotechnology (Gilmartin and O'Kennedy, 2012).

Nanomaterial	Typical diameter	Description	Useful properties	Image
Quantum dots	2–10 nm	QD are colloidal semiconducting fluorescent nanoparticles consisting of a semiconductor material core (normally cadmium mixed with selenium or tellurium), which has been coated with an additional semiconductor shell (usually zinc sulfide)	Photostability and tunable emission spectra are utilized in assays in a number of modes, including fluorescence emission, fluorescence quenching, or as energy donors	
Magnetic nanoparticles	1–100 nm	Made of compounds of magnetic elements such as iron, nickel and cobalt and can be manipulated using magnetic fields	Used to concentrate particles in assays; excellent conductivity	
Carbon Nanotubes SWNT MWNT	0.4–3 nm 2–100 nm	Allotrope of carbon consisting of graphene sheets rolled up into cylinders; multiwalled nanotubes (MWNTs) are essentially a number of concentric single-walled nanotubes (SWNTs)	Exhibit photoluminescence; excellent electrical properties; semiconductors	
Au NPs	5–110 nm	Made of gold, may take the form of spheres, cubes, hexagons, rods or nanoribbons	Ability to resonantly scatter light; chemically highly stable; change color on aggregation from blue to red; excellent conductivity	

Source: Adopted from Gilmartin, N., O'Kennedy, R., 2012. Nanobiotechnologies for the detection and reduction of pathogens. *Enzyme and Microbial Technology* 50(2), 87–95. Permission obtained from Elsevier.

- Protein-based nanostructures are extensively utilized in biotherapeutics for encapsulating of active compounds due to their neutral and biodegradable characteristics (Vasir et al., 2005).
- Targeted delivery, in which cell surface indicators peculiar to tumor, such as tumor-specific receptors or antibodies, are coupled with drugs using nanoparticles, increases the effectiveness of anticancer therapies while reducing adverse effects (Feng et al., 2004).
- Nanoparticles are commonly employed for in vitro biomedical research because their sizes and structures are very similar to biological components and structures (Alyautdin et al., 1998).
- When compared with the conventional drug administration, in nanoscale drug delivery, the concentration of drug at the target site is higher (Chavda, 2019).

4.5 Biological applications of nanotechnology

Nanotechnology has numerous applications in a wide variety of disciplines. Targeted drug delivery, diagnosis of diseases, bioimaging, nanomedicines, nanoarrays, and gene therapy are all being investigated as nanobiotechnology applications in biomedical sciences as shown in Fig. 4.2. Using various in vitro and in vivo methodologies several new nanostructures are also being examined (Shen and Zhu, 2016; Bawarski et al., 2008). In the future, innovative nanobiotechnology applications to life processes will surely revolutionize the foundations of disease prevention, therapy and diagnosis.

4.5.1 Nanodiagnostics

The limits of molecular diagnostics have been refined and extended thanks to nanobiotechnologies. Nanomolecular diagnostics, often known as “nanodiagnostics” is nanobiotechnology’s application in molecular diagnostics (Jain, 2003). Most nanotechnology applications in molecular diagnostics can be broadly classified as nanochips and nanoarrays due to their small size. Nanotechnology-on-a-chip is a broad term that can be used for a variety of techniques, however some of them do not use nanotechnologies and simply examine nanoliter volumes of fluids. Due to the inherent nanoscale features, the creation of a new class of nanoscale devices will enable precise assessment and control of receptors, pores, and other functional parts of living cells. Biological tests detecting the existence or action of specific compounds become faster, more precise, and more versatile when certain nanoparticles are utilized as tags or labels.

Nanobiotechnology will boost analytical techniques’ sensitivity and coherence, resulting in a more comprehensive assessment of life and bio processes (Jain, 2007). Nanotechnology has the potential to improve the following molecular

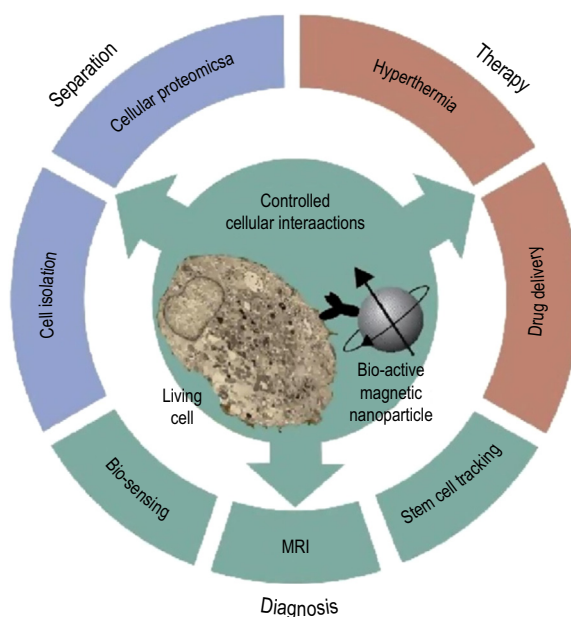


FIGURE 4.2 Schematic representing various biological applications of nanotechnology (Nath and Banerjee, 2013). Adopted from Nath, D., Banerjee, P., 2013. Green nanotechnology A new hope for medical biology. *Environmental Toxicology and Pharmacology* 36(3), 997–1014. Permission obtained from Elsevier.

diagnostics applications that are crucial to the development of personalized medicine: (1) point-of-care (POC) diagnosis, which includes diagnosis at the point-of-care or at premises and self-diagnostics to be used at household; (2) genetic screening; (3) sequencing; and (4) diagnostic-therapeutic integration.

Existing diagnostic approaches for a wide range of diseases rely on observable signs and symptoms before healthcare professionals can determine whether or not a patient has a certain type of disease (Augustine et al., 2021). The earlier an illness is detected, the better the chances of a cure. The most advantageous method is to diagnose and treat individuals before their symptoms appear. Diagnostics with DNA and RNA will play a critical part in the treatment and cure of various diseases, as they will allow identification of diseased cells and pathogens at an early stage, allowing for more effective treatment (Jain, 2011). Recent technologies, such as polymerase chain reaction, have led to the development of these devices, but nanobiotechnology is expanding previously available alternatives, resulting in improved cost and efficiency.

4.5.1.1 *Biomedical diagnostics using cantilever arrays*

Cantilevers are the most promising of the recently emerging technologies. Small complementing nucleic acid sections are bound to silicon based cantilevers with a thickness of 450 nm, that react with extreme sensitivity (Hegner and Arntz, 2004). Optical detection of physical bending happens when the specific gene transcript binds to the equivalent on one of the cantilevers. Nanomechanical techniques require no excitation, labels, laser or outside probes, and they are fast, extremely sensitive, specific, mobile, complementing and extending existing DNA and protein microarray approaches. Direct quantification of the transcripts, which denotes the intermediary stage and connect to synthesis of protein, is one example of a method for sensitive and rapid identification of active genes related to disorders and their therapy (Zhang et al., 2006). Another use is the detection of differential gene expression in a complicated setting, which could be a possible biomarker for the advancement of cancer or viral and infectious diseases.

The tests are highly sensitive to mismatches in base and offer data in very less time at the picomolar range without targeted amplification. By arranging properly coated cantilevers beside one another, an array of distinct gene transcripts can be monitored simultaneously (Sinensky and Belcher, 2007). Present molecular diagnostic tools such as the chip based on genes and RT-PCR can be complemented by this novel technology. It can be utilized as a real-time sensor to constantly monitor numerous clinical variables or to diagnose quickly replicating diseases that need to be diagnosed right away. These results establish the technology as a quick way to confirm biomarkers that signal disease progression, risk or medication response. The cantilever arrays have the potential to be used to assess the efficacy of treatment responses in personalized medicine (Lang et al., 2005).

4.5.1.2 *Biochips based on nanotechnology*

A nano-biochip, or nanotechnology on a chip, is a revolutionary change for total chemical analysis techniques. The potential to render biological and chemical information considerably more affordable and accessible is predicted to transform health care (Saji et al., 2010). Many structural variants in chromosomal DNA, ranging in length from kilobase pairs to megabase pairs, have been discovered, accounting for much of the variation between individual human genomes. Clinically, structural changes are linked to a variety of diseases and ailments, including Crohn's disease, morbid obesity, autism, schizophrenia and cancer. However, present means for detecting and analyzing structural changes, such as short DNA fragment sequencing, complicated analysis, and assembly procedures, are insufficient (Graham et al., 2004). To address this issue, nano Analyzer technology (BioNanomatrix) is able to unwind, sort, and contain native-state, lengthy genomic DNA fragments into an ordered, linear structure, which is a dramatic departure from current genomic analysis systems. The method eliminates the need for front-end amplification or shearing of source DNA into small pieces, keeping potentially clinically useful genomic structural information (Jain, 2011).

4.5.1.3 *Quantum dots for diagnostics*

Several currently used and traditional healthcare trials use precise antibodies tied to targeted disease to reveal the prevalence of a molecule, pathogenic organism, or microbe. In the past, inorganic or organic dyes were coupled with antibodies to allow imaging instruments such as electron or fluorescence microscopy to observe within the specimen. Synthetic dyes, on the other hand, tend to diminish the applicability and diagnostic specificity. Nanobiotechnology presents a feasible answer in the form of nanocrystals also known as quantum dots. A novel way to show a mono-color detection and binding in a plane is to mark unknown molecules and then recognize them in a system. Quantum dots have been used to create highly complex tags (Bhatia et al., 2016; Yan et al., 2015). Traditional fluorophores have a number of benefits over quantum dots-tagged compounds (Mongin et al., 2016; Chan et al., 2002). The absorption

spectra of quantum dots span a wide range. The size of the quantum dot and the core composition are used to determine the wavelength position. Emission is limited to a specific wavelength range (typically 20–40 nm), which is also due to the particle's wave nature. A single wavelength can be used to excite quantum dots, resulting in a range of colors with minimal photobleaching. Differentiation of color intensity, spectral breadth and emission can result in thousands of unique signatures (Pisanic et al., 2014).

4.5.1.4 Nanotechnology for sparse cell detection

Sparse cells differ from other locally situated cells in usual physiological conditions, such as HIV-infected T-cells, fetal cells, and cancer cells. These are important in the diagnosis of various genetic diseases. Even still, recognizing and separating these sparse cells is tough. As a result, nanobiotechnology presents unique potential for advancement in this discipline (LaGrow et al., 2018). Researchers have succeeded in developing nanotechnologies that can distinguish healthy tissues and sparse cells from blood. Nanobiotechnology demonstrates the unique properties of sparse cells, such as differences in charge density, deformation, and preference for a certain ligand and/or μ receptor. For example, precise separation can be achieved based on surface charges by implanting electrodes in microfluidic devices. Biocompatible materials with specific nanopores can also be used to classify sparse cells. Because of the tiny size range (30 nm–1 μ m) and large sample output for identification and separation, the use of microvesicles and exosomes as markers has been constrained in patient care. Nanobiotechnology is being used more frequently to separate and detect microvesicles and exosomes in biological and medical samples, as well as to monitor cancer detection. For rapid identification of nuclei on histopathological high-resolution images for breast cancer detection, a sparse autoencoder was applied (Xu et al., 2016).

4.5.1.5 Diagnosis with nanotubes

Healthcare professionals will continue to prefer calorimetric and optical detection over alternative detection methods such as magnetic detection. Nanosphere Inc. has developed certain ways that allow the medical community to visually recognize the genetic makeup of biological material. The analysis of the existence of certain genetic sequences is based on small DNA fragments tagged along with gold nanoparticles. If the relevant sequence existing in the samples bonds to the cDNA nanocarrier and form a dense network of gold balls, the target probes generation technique successfully enables pathogen detection (Su et al., 2017). This technique has shown promise in the in vivo detection of prostate cancer by using photo-thermal response to treatment (Lu et al., 2010) breast cancer cells (Beqa et al., 2011), all of which have significantly higher sensitivity than presently offered methods of test (Kim et al., 2013). Carbon nanotubes (CNTs) have the ability to improve DNA hybridization detection sensitivity. Field-effect transistor nanotubes are utilized to identify DNA hybridization at the molecular level (Sorgenfrei et al., 2011). A carbon nanotube was covalently bonded to the ssDNA probe. In the presence of specifically targeted cDNA, two-level variations in the carbon nanotube were detected. Fluorescence correlation spectroscopy was used to illustrate the non-Arrhenius behavior in the kinetics, which is analogous to hybridization of DNA. Carbon nanotubes (CNTs) can be utilized to study single molecule interactions on a microsecond timescale.

4.5.2 Therapeutic applications

Nanobiotechnology can help create new drug designs (also known as nanomedicines) with fewer side effects. The need for treatment and diagnosis of infectious diseases in public health domain is of interest continuously (Liu and Grodzinski, 2021). The number of nanoparticle-based medications available as commercially available medicinal treatments has dramatically expanded during the last few decades. As per a survey done by the European Science and Technology Observatory (2006), more than 150 businesses around the world are developing nanoparticle-based medicines (Wang et al., 2018). Schematic of various nanotechnology-based cancer therapeutic tools like nanocantilever, quantum dots, multifunctional nanoparticles, liposomes and carbon nanotubes are presented in Fig. 4.3 (Misra et al., 2010).

4.5.2.1 Drug delivery using nanocarriers

The transportation of drugs to the required target site is a crucial stumbling block in various illness treatments. Drug delivery control can be used to alleviate the problems and limits of traditional applications of few medications, such as low selectivity and efficacy, and poor biodistribution. Drug distribution to the site of action with a controlled drug delivery process can decrease the drug's negative effects on essential tissues (Cui et al., 2009). The drug-delivery

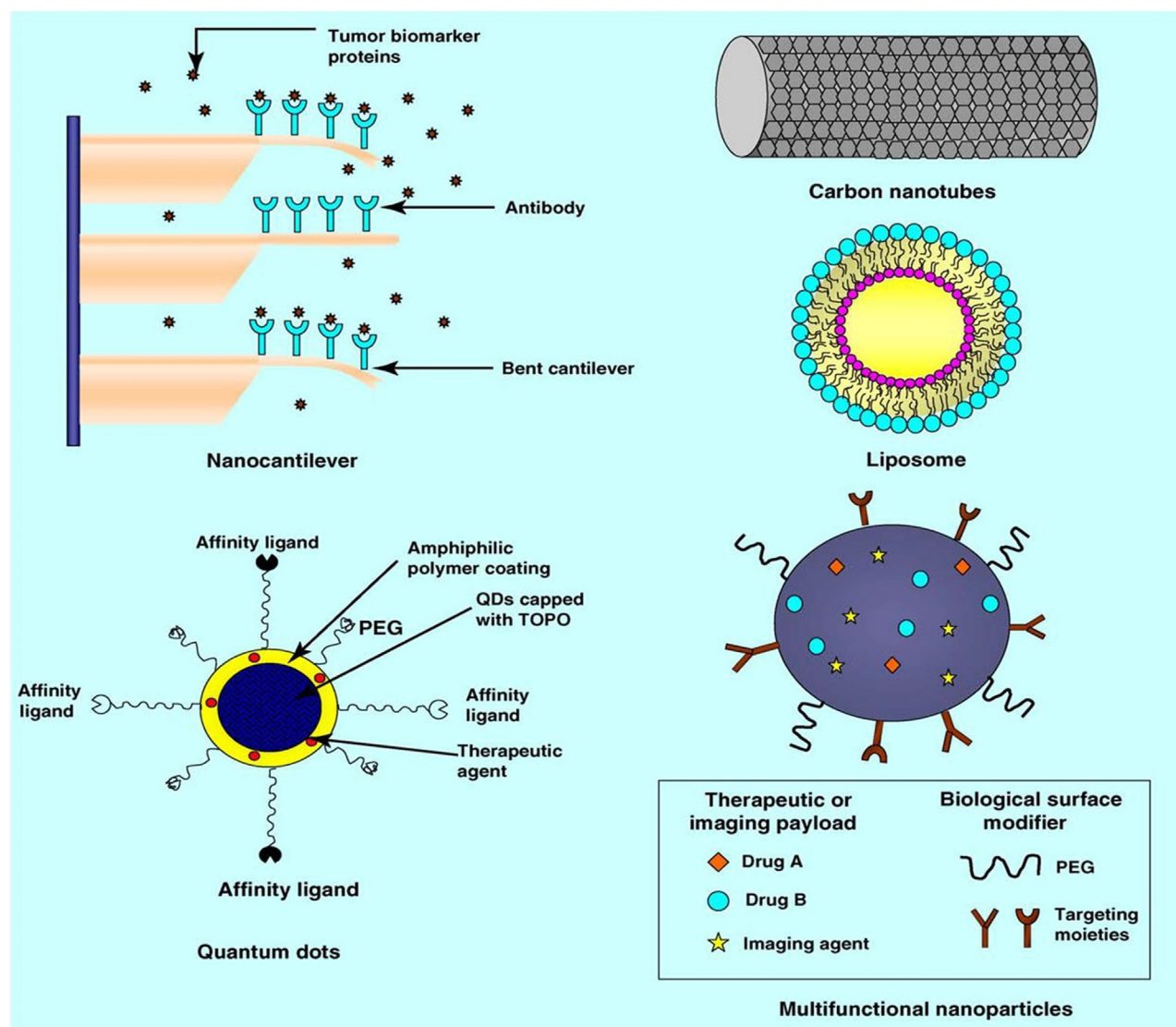


FIGURE 4.3 Schematic of various nanotechnology-based cancer therapeutic tools like nanocantilever, quantum dots, multifunctional nanoparticles, liposomes and carbon nanotubes (Misra et al., 2010). Adopted from Misra, R., Acharya, S., Sahoo, S.K., 2010. Cancer nanotechnology: application of nanotechnology in cancer therapy. *Drug Discovery Today*, 15(19–20), 842–850. Permission obtained from Elsevier.

method secures the drug against clearance or quick degeneration while also increasing its concentration in target sites of tissues (Nevozhay et al., 2007).

This modified technique of treatment has gained popularity when there is a disparity between concentration of the drug and therapeutic advantages. Cell specific targeting can be performed by connecting a specific drug to an independently created carrier. Another key terminology used to represent specific diagnosis with an emphasis on patient care and therapeutics is theranostics. For in vivo applications, several theranostics have been created and tested. Senapati et al. (2018) created nanoparticles that were doxorubicin-Ce6 conjugated.

Due to nanosized drug carriers and encapsulation, controlled drug release can be more precise than ever before. Encapsulations are used for delivering therapeutic payloads (chemo or radiation treatment, gene therapy), as well as for imaging purposes (Kesharwani and Iyer, 2015). Several medications that cannot be consumed orally due to a lack of availability will now be able to be used thanks to nanobiotechnology (Ramalingam and Ko, 2016). Antigen delivery for immunizations is another major application of nanobiotechnology (Zaric et al., 2015; Dölen et al., 2015). Research on the advancement of nanocarriers and encapsulations and appropriate clinical trials has shown that micro and nanomaterials possess immune-boosting properties (Benne et al., 2016).

4.5.2.2 *Nanoscale gene carriers*

Numerous human disorders caused by faulty genes, such as cystic fibrosis, muscle degeneration, cancer and Parkinson's disease, have been successfully treated via gene therapy (Loh et al., 2013; Kaplitt et al., 2007). Present gene therapy systems face challenges such as the possibility of mutant conversion back to its original form, as well as the processing and formulation of effective pharmaceuticals. A viable immunological response from the viral vector employed for the gene therapy is also a concern (Pluvinage and Wyss-Coray, 2017). Nanotechnology offers unique therapeutic strategies in human gene therapy and delivery, such as nanocarrier-based non-viral gene therapy, to address these challenges. Multifunctional inorganic nanocarriers (about size of 50–500 nm) have evolved as a adaptable and durable nanoscaffold for efficient gene treatment (Ghosh et al., 2008). Chemical and thermal stability, ease of functionalization, as well as scalable synthesis are all advantages of inorganic nanomaterials for practical applications. Sterilization, low toxicity (particularly for iron oxide, silica and gold nanoparticles), and availability in various forms and sizes are all important qualities of using nanoparticles. As a result, instead of using viral vectors, nanoscale gene carriers that show low immunogenic response can be used to replace or repair damaged genes in humans.

4.5.2.3 *Biopharmaceuticals based on nanotechnology*

Nanobiotechnology can also be used to develop nano-based therapeutics to treat disorders that are difficult to treat with traditional pharmaceuticals (Pelaz et al., 2017). Conventionally, the pharmaceutical industry has focused on the development of medications for a limited number of ailments. Around 70%–80% of individuals fail to progress to drug development, and this carelessness is frequently exposed late in the process, after millions of dollars have been spent on research and development. Pharmaceutical businesses would benefit from nanoscale drug development since they cannot afford to hire hundreds of chemists to synthesize and test thousands of different molecules (Chen et al., 2016; Jiang et al., 2017). Nanobiotechnology has the capability to physically manage molecules on solid substrates by attaching them to biomembranes and directing when and where chemical reactions occur in a quick, low-component process like solutions and reagents. Nanomedicine advancements will make highly targeted drug creation easier by lowering the cost of discovery with less time.

4.5.2.4 *Nanosurfaces*

Several instances of complicated interactions between surfaces and molecules may be seen in nature, such as the connection among brain and blood cells, and infections' ability to infect sites is dependent on complicated interactions between cells and substrate characteristics (Buttiglieri et al., 2003). With nanoscale determinations, nanofabrication can alter surface features, which could be a key component of a hybrid biological system. The hybrid nanomaterials can be employed as biosensors, implants, or biomedical equipment, as well as for drug screening (Park et al., 2014). A study described the surface modification of PLGA nanoparticles using a few surface modifiers such as folate, a peptide and a polymer. Surface-altered nanoparticles simulated desirable interactions while retaining no excess dopamine bioactivity or cytotoxicity.

Traditional biomolecular engineering has limited the accessibility of physiologically active compounds due to the time and cost needed. Nanoparticles can be used for immobilization, production of hybrid biomaterials with increased biocompatibility, and regulated production of nanostructures and nanomaterials. Rather than executing traditional solution-based processes, improvement can be done by the potential to carry out chemical and biological processes on solid surfaces (Sun et al., 2009). Solid matrix manipulation typically results in less waste and significantly more exact biomolecular manipulation. Different types of biomolecules, such as antibodies, enzymes and bioactive peptides can be immobilized on surfaces of solids, along with nanocarriers (Verma et al., 2013), as shown in Fig. 4.4 (Qamar et al., 2019).

4.5.2.5 *Drug development using nanoparticles*

Drug candidates include nanoparticles such as dendrimers and fullerenes, while nanobodies, the tiniest pieces of naturally existing heavy chain antibodies, can be made as therapeutics. Dendrimers are a new type of core-shell 3D nanoscale structures that may be accurately produced for a variety of purposes. Dendrimers can have their physical and chemical properties precisely controlled using specialized chemistry techniques (Abbasi et al., 2014). They're best for medication delivery, but they can also be employed to create new drugs with unique properties. Multiple pharmacological targets interact with polyvalent dendrimers at the same time. They could be turned into new cancer treatments that are specifically targeted. Anticancer medicines can be made from polymer–protein and polymer–drug conjugates (Wolinsky and Grinstaff, 2008).

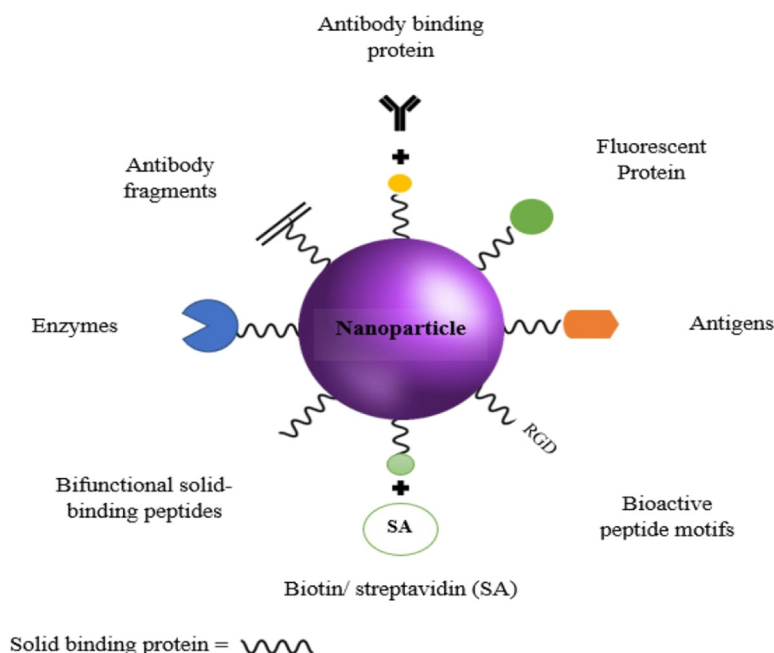


FIGURE 4.4 The immobilization of different kind of biomolecules, such as enzymes, antigens, proteins, antibodies, or bioactive peptides onto the nanoparticles' surface without affecting their functionality is depicted in this illustration (Qamar et al., 2019). Adopted from Qamar, S.A., Asgher, M., Khalid, N., Sadaf, M., 2019. *Nanobiotechnology in health sciences: current applications and future perspectives*. Biocatalysis and Agricultural Biotechnology, 22. Permission obtained from Elsevier.

The following are some of the benefits: Surface chemistry that is tailored to specific needs, nonimmunogenic, the body's inherent dispersion allows for precise tissue targeting, and is biodegradable. For enhancing pharmacokinetics, directing medications to specific areas, and aiding cellular absorption, dendrimer conjugation with low-molecular-weight pharmaceuticals has lately piqued interest. One study looked into the possibility of employing dendrimers to improve the ability of comparatively big therapeutic proteins like streptokinase (Wang et al., 2007). Position specific control in matching fullerene molecules to biological entities is enabled by this property, which is a hallmark of rational drug design. It is feasible to customize required pharmacokinetic qualities to drugs based on fullerene and maximize their therapeutic efficacy by combining them with further properties of fullerene, such as comparative inertness, size, potential for redox reactions in biological systems (Anilkumar et al., 2011). Nanobodies have excellent target specificity and minimal inherent toxicity, similar to traditional antibodies; nevertheless, like tiny molecule medicines, they can block enzymes and accessibility to receptor clefts. They integrate the benefits of traditional antibodies with the advantages of tiny molecular drugs. Nanobody coupled human trypanolytic factor for the cure of human African trypanosomiasis is an example of the usage of nanobodies as new pharmaceuticals (Jain, 2011).

4.5.2.6 Drug delivery using nanotechnology

One of the most significant factors in medication research and therapy is drug delivery (Sahu et al., 2021). For the creation and delivery of revolutionary formulations, new technologies are used. Targeted medication delivery is the focus, which is ideal for personalized medicine (Jain, 2011). There are various prerequisites for designing a device or tool tiny enough to escape the vasculature and get in cells quickly enough to conduct several, intelligent functions. However, size is an important consideration. Therapeutics can only exit through vascular pores if they are smaller than 50 nm in dimension, and cells will not internalize anything larger than 70 nm. Solubility, which is an important element for drug efficacy regardless of injection route, is one of the primary issues with drug delivery to the human body (Fahr and Liu, 2007). It is also a significant problem for pharmaceutical companies creating new medications, as roughly 50% of new drugs based on chemicals are not soluble in water or weakly soluble. Numerous promising chemicals never make it to the market. Others make it to market in a less-than-ideal formulation, with less or uncertain bioavailability or a higher risk of side effects. Reformulating such medications and increasing their economic potential can be done using a method that improves solubility. Some medications are available as nanoformulations, while others require delivery via nanoparticles (Arora et al., 2018).

Drug delivery difficulties can be solved using nanobiotechnology in the following ways: Nanoedge technology, for example, reduces particle size to the nanoscale range to enhance surface area and hence increase dissolving rate improving the drug's solubility (Salazar et al., 2014). The use of noninvasive modes of delivery eliminates the necessity for injectable medications. Novel nanoparticle synthesis with increased firmness and shelf-life are being developed. Improved bioavailability and release rates are possible with nanoparticle formulations for insoluble chemicals and macromolecules, thereby decreasing the quantity of essential dose and boosting security by reducing adverse effects. Nanoparticle formulations with regulated size of particles, quality of surface and morphology would be highly impactful and cost effective to produce than current technologies (Bailey and Berkland, 2009).

Patients' compliance with drug regimens can be improved by nanoparticle synthesis that provides continuous and prolonged release profiles of up to 24 hours. Direct conjugation of drugs to ligands of target limits the number of drug molecules that can be imported, whereas conjugation nanoplateforms carrying drugs to ligands permit thousands of drug molecules to be imported with just single ligand (Shi et al., 2009). Drugs can be coupled with novel disease-specific targets using nanosystems. Dendrimers are good scaffolds for delivery of drugs because of their distinctive features, such as increased degree of branching and well-defined molecular weight (Noriega-Luna et al., 2014). Functionalization has been used to rigorously characterized, commercially available dendritic polymers in order to create drug delivery platforms with minimal toxicity, highest loading proportions, the potential to target particular cells, and transportation from their membranes. Surface targeting ligands have been used to make the carriers specific to definite cells and PEG, ensuring stability, water solubility and circulation over long term. Furthermore, carriers make it easier for molecules to pass through the membrane of cell, while fluorescent sensors identify their intracellular location. Multivalency is a familiar characteristic of groups over surface, which significantly improves their bonding capacity with cell receptors. To these characteristics, it is essential to integrate the ability to achieve a high amount of active ingredient load combined with the release that is controlled as well as triggered (Paleos et al., 2009). Dendritic polymers with controlled release qualities can be made by including features of response to stimuli to dendrimers. These stimuli-responsive dendrimers could be used as medication carriers in the future.

4.5.3 Nanobiosensors

The demand for more accurate, compact sensors with increased functionality was unavoidable as a result of the world's scientific breakthroughs, the introduction of digital tools, and significant upheavals that happened in the previous several decades. New materials and devices must be discovered to improve the sensitivity, output, and accuracy of these sensors. Nanosensors are nanoscale sensors that have excellent precision and activity because of their small nanoscale dimensions, since they can identify and respond to physical stimulators on a nanoscale (Saylan et al., 2021). For instance, a nanosensor is designed which can be implanted on various surfaces like enamel and transmit data from those surfaces. The nanosensors, which are made from graphene, a sort of two dimensional carbon structure, are printed on a special silk which is water soluble and then shifted to the desired surfaces. This extremely small and fine circuit will be capable of reporting actions like breathing or the presence of bacteria in this way. The researchers have successfully retrieved data from the surface of an enamel by placing a nanosensor on it in preliminary trials (Mannoor et al., 2012). Advantages and Disadvantages of various different kinds of nanobiosensors are presented in Table 4.2 (Borse et al., 2020).

Nanofilters have sought a great attention recently due to their crucial and wide range of applications. Nanofiltration is a type of filtration occurring at pressures lower than inverse osmosis pressure, which results in a cheaper end cost and energy consumption. Nanofilters can recover viruses and bacteria from water, in addition to recycling salt and calcium. As a result, they can be utilized to remove contaminants from fresh water bodies and water utilized in agricultural activities (Van der Bruggen et al., 2001). Nanofilters can significantly speed up blood filtration. Blood poisoning is now one of the world's most dangerous concerns. Blood poisoning requires immediate disinfection of the blood to remove the contaminating element. Endotoxin and plasma must be separated in order to determine the infectious agent. Nanofilters can be used to do this, allowing for the determination of the component causing infection and subsequent blood disinfection (Burnouf and Radosevich, 2003). Nanofilters can also be used for biological isolation of viruses, bacteria, DNA, protein and nucleic acid absorption, ultrafiltration of foods and beverages, and sterilization of medical serums and biological fluids.

Nanotechnology helps us to analyze various factors with greater accuracy by manufacturing sensors in microscopic dimensions. The use of biological molecules allows us to fabricate nanosensors. Nanotechnology provides for more precise detection of many aspects by designing sensors with smaller dimensions. We will be able to make nanosensors using biomolecules (Stroble et al., 2009). Biological barcoding utilizing small fragments of DNA is one rapid diagnosis

TABLE 4.2 Advantages and disadvantages of various different kinds of nanobiosensors (Borse et al., 2020).

Types of nanobiosensor	Advantages	Disadvantages
Optical • Fluorescence • Colorimetric • Surface plasmon resonance	1. Rapid 2. Sensitive 3. Easy to handle (mostly strips) 4. No extra equipment required for analyzing the result	1. Lack of quantitative interpretation of the analyte
Electrochemical • Voltammetric • Amperometric • Conductometric	1. Quantitative interpretation of the analyte 2. Rapid 3. Sensitive and specific	1. The high ionic strength of biological buffers can interfere with electron transfer 2. Needs extra equipment for result interpretation
Magnetic	1. Trace amounts of analytes can also be detected using magnetic NP as the label	1. The inherent toxicity and chemical instability of metallic-magnetic NPs

Source: Adopted from Borse, V.B., Konwar, A.N., Srivastava, R., 2020. Nanobiotechnology approaches for miniaturized diagnostics. In: Handbook on Miniaturization in Analytical Chemistry. Elsevier, pp. 297–333. Permission obtained from Elsevier.

tool for a variety of illnesses, ranging from cancer to Alzheimer's disease (Nam et al., 2007). When compared to prior methods, this method is more exact, less expensive, and easy to implement.

Nanobiosensors are electronic devices that are used to study biochemical mechanisms. The electronic part contains the components required for signal transmission and recognition. The target analytes and receptors are part of the biological part. Nanomaterials are the primary signal generators, generating a signal depending on the interactivity of biological entities, which the electronic system translates into numerous forms (Lee et al., 2018). The nanomaterial must be in close proximity to the interaction system or physically bonded to any of the engaging biological components in order to do so. These nanoparticles are commonly used in the development of biosensors because they reduce the detection limit to single molecules. Gold and silver nanoparticles, Quantum Dots, carbon nanotubes (CNTs), graphene, magnetic Nanoparticles, and other nanomaterials are being explored extensively for the applications of biosensing. The typical mechanism of working of a biosensor representing the receptor and transducer components is presented in Fig. 4.5 (Borse et al., 2020).

Since many physical, chemical and biological sensors rely on interconnection between molecules and atoms at their surfaces, nanotechnology can be used to develop novel sensors and improve their potential. The ability of small groups of molecules to interact, process the data and transport by electrons, and store data in nanoscale structures, make nanotechnology an excellent instrument for responding to the needs of intelligent sensors. As a result, all of the ability and parameters of an intelligent network can be implemented in a nanosized system (Lu and Rosenzweig, 2000). The technology for manufacturing nanosensors from these nanostructures is rapidly evolving (Abdel-Karim et al., 2020).

4.5.3.1 Nanobiosensors and live cell evaluation

It is possible to quantify intracellular components without damaging them with this technology. Cell and molecular biologists can use this technology to study cells and molecules without damaging them. This technology was used for the first time in 2004 to observe cellular death inside a live cell. A 400-nm probe is affixed to the tip of these nanobiosensors, which is almost thousand times thinner than the hair of a human. Since this probe is very little, it can enter a cell without causing damage (Lu and Rosenzweig, 2000). Another noteworthy feature is background molecule interference, which is not present in living cells. Furthermore, only this method allows scientists to investigate cells in nanoscale without damaging them, unlike other systems, such as scanning electron microscopy (SEM) or other procedures, which result in death of a cell. An additional interesting feature of this approach is that it allows us to acquire information about any component of the cellular system in a dynamic manner, whereas other methods result in a large number of dead cells as well as partially non-dynamic information that is unrelated to the other cells in vicinity. Working with living cellular systems creates new avenues for comprehending and perceiving important and crucial data about living molecular systems (Lorenzelli et al., 2003). This can help us now better understand how harmful components reach the cell and the way biological pathogens trigger the right biological response inside the cell (Date et al., 2010).

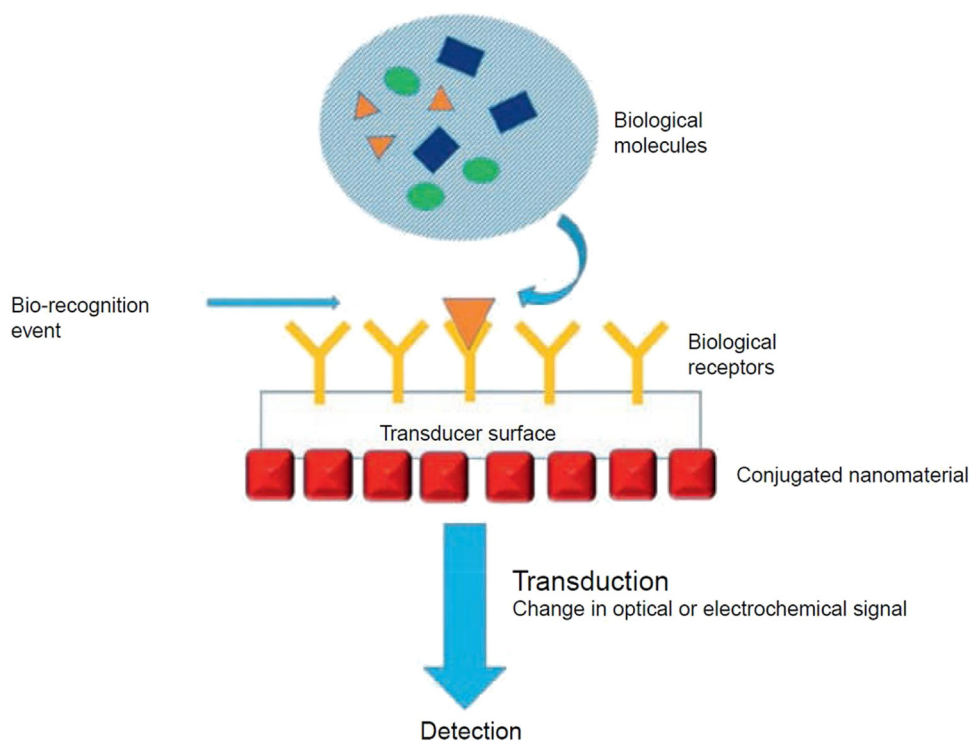


FIGURE 4.5 Illustration of the working mechanism of a biosensor representing the biological receptor and transduction components (Borse et al., 2020). Adopted from Borse, V.B., Konwar, A.N., Srivastava, R., 2020. Nanobiotechnology approaches for miniaturized diagnostics. In: *Handbook on Miniaturization in Analytical Chemistry*. Elsevier, pp. 297–333. Permission obtained from Elsevier.

4.5.3.2 Drugs feeding mechanism using nanoparticles

Bionanotechnology can help in drug encapsulation and delivery, particularly for pharmaceuticals that are not suited for a few specific systems. Nanopacks and microcrystals are well known drug delivery techniques, and the skin and lungs are considered as good drug administration routes. Microdevices have enabled cancer treatment by delivering drugs to tumors on a targeted basis (Elman et al., 2009). A few materials, which are among the very crucial drug delivery systems, coat the drugs to protect them as they move through the body. Liposomes and polymers (such as polylactideco- glycolide) as well as protein nanoparticles belongs to these materials (Malam et al., 2009). Upon transportation of these materials to the organs, they can build coatings surrounding the drugs and have regulated timed release. The drug can continue to release even if the coat is removed. Instead of microparticles, the coating materials will be nanoparticles having diameters of 1–100 nm after some time, which will have a larger surface area in a constant volume, fewer pores, better solubility, and different structural features. The qualities of the coatings linked to distribution and widening are improved as a result of this. Some natural and synthetic polymers, in addition to liposomes, can be used for encapsulation (Vilar et al., 2012). Silica and calcium phosphate are two examples of these materials, both of which have significantly improved characteristics when compared to their micro-dimension condition, making them ideal for drug administration (Trofimov et al., 2018).

4.5.3.3 Gold nanoparticles for low cost cancer diagnosis

Light absorption and scattering are both very efficient in gold. Researchers are now attempting to use the gold nanoparticles' ability to detect cancer quickly and easily (Cai et al., 2008). Numerous cancer cells contain a protein known as the epidermal growth factor receptor (EGFR) on their surfaces, whereas healthy cells do not. By attaching EFGR antibodies to gold nanoparticles, researchers were able to effectively deliver and attach these nanoparticles to cancer cells (Kao et al., 2013). Using a field dark microscopy equipment, they detected the cancer cells as bright dots. These nanoparticles are unable to attach themselves to healthy cells. The healthy cells appear darker in comparison to the malignant cells in this way. In comparison to other conventional approaches, this methodology for diagnosis of cancer is less expensive and rapid. This research revealed that the ability of these nanoparticles to attach to tumor cells is several hundreds times greater than that of other healthy cells. Since every nanoparticle absorbs and scatters light differently depending on its shape and dimension, it is feasible to analyze numerous molecules using several probes at the same

time. The attractive part of this technology is that it does not require any expensive devices, such as sophisticated microscopes; the operation can be carried out with only a simple optical microscope (Aliofkhazraei and Ali, 2014).

4.5.3.4 Nanoparticle based supersensitive biodetection

The limited sensitivity of classic antigen or protein detection techniques to the target is one of the most fundamental reasons for their failure. In order to detect and diagnose disease in its early stages, super-sensitive tests are required. These tests must identify extremely low levels of pathogen biomarkers. Supersensitive biobarcode studies for identification of protein or antigen analytes have recently been established (Bao et al., 2006). Biobarcode detection applies two different kinds of probes: Gold nanoparticle probes functionalized with several equal hybridized oligonucleotides—where DNA strands acts as a detecting signal—and polyclonal antibodies are used in biobarcode experiments (Lee, 2011). Particles of Polyamine having a diameter of 1 mm that have been functionalized with monoclonal antibodies are the second type of magnetic microparticle (Nam et al., 2003). Antibodies, both polyclonal and monoclonal, bind to particular protein targets, forming a protein sandwiched amid microparticles and nanoparticles. When the magnetization in the solution causes this sandwich to move, DNA strands with barcodes are liberated and transcribed using standard DNA detection methods. These tests have a higher sensitivity than those that rely on effective protein or antigen limitation and amplification of processes. Alzheimer's disease is detected using biobarcode test technology. Biobarcode test technique can screen HIV-infected samples of blood, certain types of cancer, and cardiac problems, among other diseases. Specific platforms have recently been developed which are quite convenient to handle and allow for automatic supersensitive protein and nucleic acid analysis and detection using a microfluidic chip (Wang et al., 2019).

4.5.3.5 Carbon nanotube sensors to measure and detect blood glucose level

Carbon nanotubes (CNTs) are interesting materials for applications as glucose sensors in blood and urine. Multiwalled CNTs (MWCNTs), can be used to create enzyme ammeter biosensors or biosensors for lighting applications (Cash and Clark, 2010). Glucose oxidase enzyme can catalyze glucose or byproducts of hydrogen peroxide and is found inside MWCNTs. Since the enzymes in ammeter biosensors are biologically immobile, delivery electrons can travel from one gold or platinum transmitter to another, creating reverse current. Biosensors based on fluorescence can be used in devices like near-infrared nanosensors as a new type of biological sensor. This sensor can be implanted into the organ and activated with a laser, allowing it to measure blood glucose levels in real time. CNTs encapsulated with proteins or functionalized ferrocyanide potassium, a chemical sensitive to hydrogen peroxide, are used in this sensor. Ions of ferrocyanide are adsorbed by single layers which are porous. Following this stage, hydrogen peroxide forms a complex molecule with ions, which alters the density of electrons and thus the optical properties of the carbon nanotube. Carbon nanotubes illuminate with more light when there are more glucose molecules present. The sensor can be implanted in the organ after being put into a capillary tube (Aliofkhazraei and Ali, 2014).

Prolonged monitoring applications are possible with optical nanoparticle sensors because carbon nanotubes, like organic molecules that do not glow, do not degrade. The results of these approved instances have been used to identify glucose levels in blood samples from laboratory mediums. Electrochemically, peptide nanotubes which are self-compatible can be used in a biosensor (Luo et al., 2016). These devices' sensitivity is improved by the addition of peptide nanotubes. Peptide nanotubes have several advantages compared to carbon nanotubes, including improved biocompatibility, water solubility and low cost, and ease of synthesis. They can also be chemically altered by targeting amine or carboxyl groups. Sensing technologies can be utilized to create a system for detecting chemical and biological substances with extreme sensitivity (de la Rica et al., 2011).

4.5.4 Nanotechnology for cancer: diagnosis and treatment

Nanooncology refers to the use of nanotechnology in cancer treatment, which encompasses both diagnostics and treatments (Jain, 2008). Nanobiotechnology is crucial in the discovery of cancer biomarkers, as well as in treatment and diagnosis. Nanobiotechnology is used in the progress of several cancer therapies, some of which have already been approved. Devices based on nanobiotechnology are being developed as cancer surgical aids. Finally, nanobiotechnology is a critical component of personalized cancer therapy, as well as early detection and the integration of diagnostics and therapeutic delivery (Jin et al., 2020). For the treatment of cancer, several nanotechnology techniques such as gene therapy, photodynamic therapy, radiotherapy, radiofrequency therapy, and cancer theranostics are being used. Fig. 4.6 shows how these technologies allow selectively the cancer cells to be targeted while leaving healthy cells alone (Misra et al., 2010).

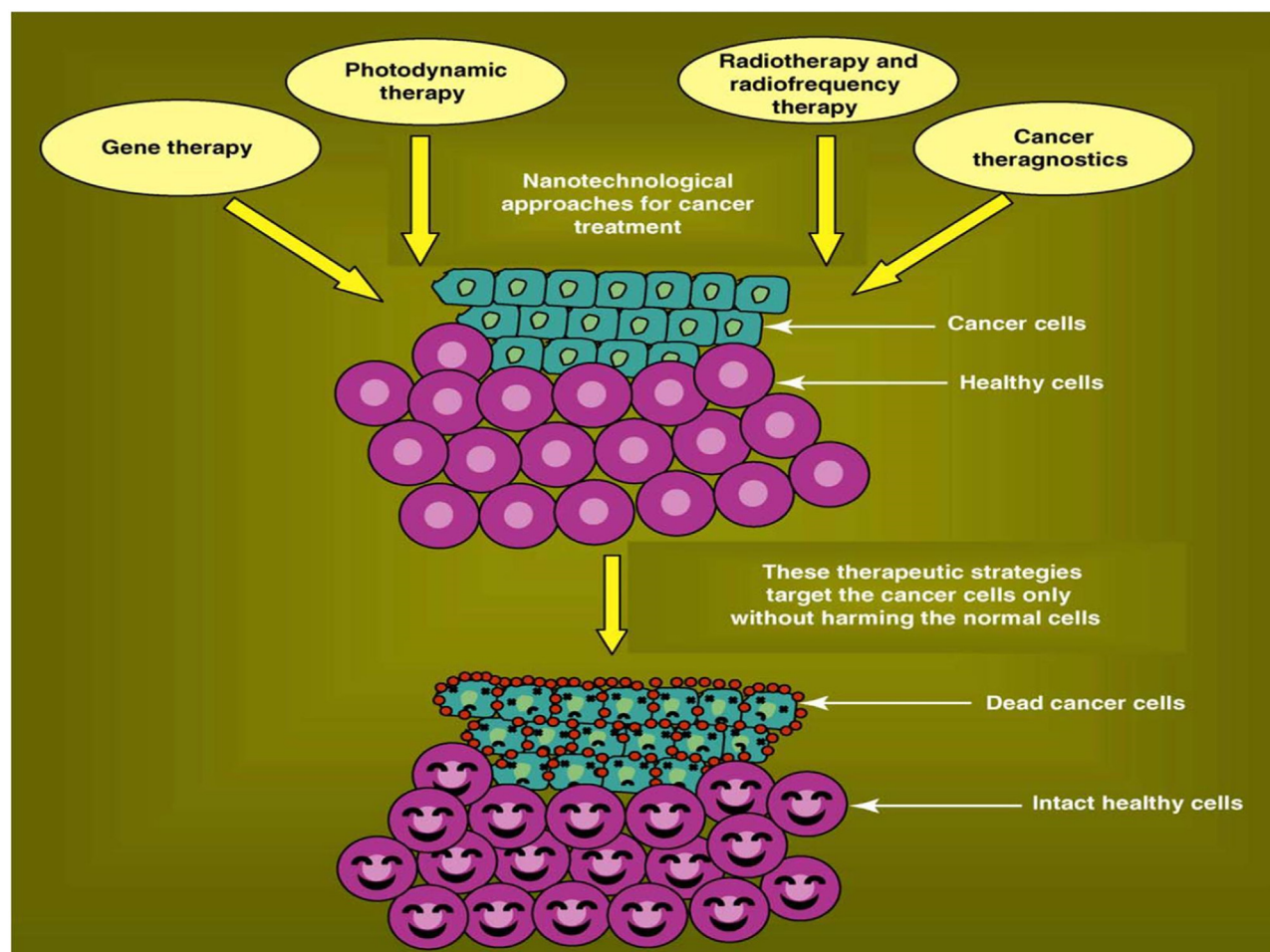


FIGURE 4.6 Nanotechnology techniques for treatment of cancer (Misra et al., 2010). Adopted from Misra, R., Acharya, S., Sahoo, S.K., 2010. Cancer nanotechnology: application of nanotechnology in cancer therapy. *Drug Discovery Today*, 15(19–20), 842–850. Permission obtained from Elsevier.

4.5.4.1 Early cancer detection using nanobiotechnology

When cancer is detected early on, it's simple to treat, barely prone to adopt medication resistance and reduces the mortality rate (Stephen et al., 2020). Early-stage cancer cells are not much likely to contain mutations that render them resistant to treatments. Tumor cells may be arduous to identify at first, but they create a footprint, or a specific pattern of change in protein biomarker that circulates in the blood, which can be detected. Multiple biomarkers may necessitate up to hundreds of measures, all of which should be taken from a pinprick of blood. As a result, nanoscale diagnostics will be crucial in this quest. Nanowire sensors are being developed for very early cancer detection, when only a few thousand cells are present (Doucey and Carrara, 2019).

Nanowires have the potential to identify a few molecules of protein, as well as some biochemical indicators that are early hallmarks of cancer, electronically. Every nanowire in the set is coated with a distinct compound which bonds to a specific biomarker and alters the conductivity of the nanowire, which could be measured. Several such nanowires are integrated on a single device to enable cancer type identification (Malsagova et al., 2021). Currently, such a device can identify between 20 and 30 biomarkers and is used to diagnose brain cancer early.

Prostate-specific antigen (PSA) detection using an autonomous gold nanoparticles biobarcode assay probe has been presented (Thaxton et al., 2009). PSA immunoassays are many times unable to identify antigen in the samples of patients who have undergone radical prostatectomy. This novel bio-barcode PSA assay is 300 times more sensitive than currently used immunoassays, as all of the participants in the trial had a detectable PSA level following radical prostatectomy. Since the patient's result is determined by PSA levels, this ultrasensitive test allows for (1) informing patients who have undetectable PSA levels with conventional assays but detectable and non-rising levels with the barcode assay

that their cancer will not recur; and (2) early identification of recurrence due to the potential to quantify growing levels of PSA.

4.5.4.2 *Combination of cancer diagnostics with therapeutics using nanobiotechnology*

The use of the single nanomaterial for diagnosis as well as therapeutics allows to couple two critical aspects of cancer treatment. Dendrimers could be employed as improved contrast agents in imaging technologies like MRI to target cancer cells specifically. Dendrimers can potentially be utilized to improve the safety and effectiveness of a range of cancer medicines. Dendrimers are being studied for use in capture therapy of boron neutron, photodynamic therapy, cancer gene therapy, for example (Baker, 2009). For targeted therapy of cancer, MRI and optical imaging a biocompatible, multimodal iron oxide nanoparticle has been developed. The co-encapsulation of an anticancer medication and NIR dyes is achieved using a modified solvent diffusion approach (Santra et al., 2009). The resulting folate-derived nanoparticles can be employed for detection by imaging as well as targeted killing of cancer cells which express folate, as they have diagnostic and therapeutic properties. Magnetic nanoparticles have shown potential in cancer treatments such as targeted drug delivery, hyperthermia, and MRI (Gobbo et al., 2015). The use of magnetic nanoparticles conjugated with aptamer regulated by an externally generated 3D rotating magnetic field has been established as a nanosurgical technique for selectively removing tumor cells from the core of a tissue without causing any collateral harm (Nair et al., 2010). By adding numerous ligands specific to target on magnetic nanoparticles, this approach could be improved for the judicious removal of complicated malignancies from various tissues.

4.5.4.3 *Imaging and targeting of tumor using radiolabeled carbon nanotubes*

Single-walled carbon nanotubes with multiple copies of tumor-selective monoclonal antibodies, chelates of radiometal-ion and fluorescent probes covalently attached can be used to target lymphomas and deliver both therapeutics as well as imaging agents (McDevitt et al., 2007). Each nanotube, which contained six antibodies and 114 radioactive atoms, was found to be viable in plasma samples for minimum of 96 hours and competent of attaching to tumor cells. Most importantly, the chemical association that bound the radioactive element indium-111 in plasma sample remained totally stable during the experiment. The nanotube structures favorably targeted tumors while ignoring normal healthy cells in a lymphoma mouse model. The ability of radiolabeled or fluorescent-labeled antibody coupled CNT structures to preferentially target tumors is promising, suggesting that they could be used as diagnostics and medication delivery for cancer (Jaymand et al., 2021). Imaging systems could provide distinctive in vivo capabilities to answer biological issues and enhance the effectiveness of cancer treatment (Liu et al., 2021).

4.5.4.4 *Thermal ablation of cancer using gold nanoshells*

Metal nanoshells are a type of nanoparticle with optical resonances which can be tuned, that have been utilized to treat cancer with thermally ablative therapy (Gobin et al., 2007). Nanoshells can be tailored to absorb light substantially in the near-infrared (NIR), which is when transmission of rays into tissue is best. Using moderately small extracorporeally delivered NIR exposures, nanoshells can be injected deep into tissues to administer a therapeutic level of heating. In solid tumors treated with nanoshells of metal, low dosages of NIR can attain temperatures competent of causing irreversible tumor destruction in minutes.

Gold nanoshells are 120 nm in size and a tumor cell is 170 folds larger. As a result, nanoshells can pass through tumor capillaries and become lodged in the tumor. NIR rays, that passes harmlessly via the skin, heats the inserted nanoshells and destroys the tumor cells (Vankayala et al., 2014). The cancer cells are improbable to create resistance to drugs because no drugs are used. The capacity to manipulate nanoshells' wavelength-dependent scattering and absorption opens up the possibility of designing nanoshells with treatment and diagnostic properties in a nanoparticle. All optical technologies based on nanoshells can merge tumor imaging and therapeutic applications. Immune targeted nanoshells are designed to scatter light in the NIR range, allowing for optical cancer imaging, as well as absorb light, allowing for photothermal therapy to selectively destroy cancer cells. Dual scanning immune targeted nanoshells were employed in a proof-of-concept experiment to identify and demolish breast cancer cells that overexpress HER2, a clinically crucial cancer biomarker. This technique has a number of important advantages over currently developed alternatives (Loo et al., 2005). Optical imaging, for example, is significantly rapid and low cost compared to other medical imaging methods. Other optically active nanoparticles, like Quantum Dots, are less biocompatible than gold nanoparticles. Nanoshell microparticles are delivered intravenously and gather in the tumor through the leaky vasculature associated with it. Following the accumulation of particles in a tumor, the region is illuminated with an NIR laser at wavelengths selected to permit highest light penetration in tissue. The microparticles, unlike solid metallic materials,

are intended to absorb that wavelength and transform the laser light into heat. As a result, the tumor is rapidly destroyed along its uneven boundaries (Morton et al., 2010). Some of the benefits of nanoshell-based tumor cell ablation are as follows:

- To avoid injury to neighboring tissue, specific cells and tissues are targeted.
- Compared to targeted chemotherapeutic drugs or photodynamic treatment, there are fewer side effects.
- Biocompatibility and repeatability due to a lack of tissue memory in therapy based on radiations.
- Ability to treat various cancer types like glioblastoma, metastases, and tumors that are inoperable.

4.5.4.5 *Imaging using nanoparticles for clinical trials in oncology*

In cancer treatment trials, computed tomography (CT) images are currently employed as surrogate end goals. The size of the tumor provides only a limited amount of information about the efficacy of treatment. Superior imaging results could assist oncologists better identify the most effective medicine for a given patient, as well as providing simultaneous information on whether a treatment is working, which could speed up the clinical trial process (Sharma et al., 2012). Oncologists and their patients currently have to wait for months to find out if a treatment is effective. Brief clinical trials would allow drug developers to concentrate their efforts on much promising medicines, allowing effective novel drugs to benefit patients at the earliest (Jain, 2011).

By applying gold nanoshell-based, multimodal contrast agents in the NIR range, a diagnostic and treatment probe for MRI, fluorescence optical imaging, and photo-thermal treatment for cancer of breast carcinoma cells in vitro is developed (Bardhan et al., 2009). In the near future, MRI techniques with customized enhanced ferrites as the MRI agent may be used to screen patients for breast cancer. Enhanced ferrites are a type of ferrite that has been developed to have improved electrical and magnetic functions and is made using a core-shell morphology (Bano et al., 2016). Nanoparticles based on magnet are connected to the MRI's radio frequency, which causes the radio frequency to be converted into heat. If a tumor is found, the physician can boost the MRI coils' strength, which will cause localized heating, which will destroy the tumor while causing no damage to the local healthy cells.

4.6 Future prospects

In terms of advancement, nanobiotechnology is still in its inception. Nanobiotechnology allows us to design miniaturized devices closer and closer to reality, allowing us to distribute dynamic molecules with greater ease and stability. In short, advancements in biotechnology will have a significant impact on science and technology. Nanobiotechnology has the ability to transform medicine and biotherapeutics. Drug delivery processes are just at the beginning of a new era, and it is worth forecasting that the untreatable will almost certainly become curable with nano biotherapeutics. Even though nanobiotechnology has a lot of potential benefits, its safety hasn't been clearly defined; in fact, authorities haven't been very clear in the form of recommendations that balance safety and risk aspects.

Nanotechnology has the ability to change and improve the capabilities of sensors, as well as build new ones. In spite of the progress made in this domain, realizing all of nanoscience's potential and capabilities will require a significant amount of labor and research. Building nano equipment and systems and using nanotechnology, on the other hand, has been sparked in a variety of disciplines, and its ever-increasing breakthroughs and developments point to a hopeful future. Drug delivery methods will progress even further, especially when specific groupings of nucleic acids or proteins are coupled to nanobiosensors. Nanomolecular systems will be viable to use in the future, and their use could result in significant improvements in science and technology. Another aspect is that nanotechnology is built on the transmission and conversion of various signal kinds. Nonetheless, nanoparticles' superior physical and chemical properties, as well as improved thermal, electrical and optical functioning, can be employed to identify or alter biological events locally. It's critical that artificial nanomaterials work well with biological systems. Artificial nanoparticles are frequently manufactured and created in very hazardous circumstances for cells and tissues. As a result, under complex physiological settings, these artificial nanoparticles may not perform as intended. To maintain and control the functioning and structure of both artificial as well as biological elements in vivo for long stretches of time is a hot topic of research.

4.7 Conclusions

Nanotechnology offers interesting new ways for both assessing and perturbing biological systems, like targeted drug delivery mechanisms which can bypass biological obstructions, as well as supplementing clinically useful biomarkers.

Several essential themes arise in this setting that encourage the further advancement of nanotechnology for biomedicine. Nanotechnology, for starters, allows for interfaces between humans' macroscale and the nanoscale of molecules and cells. Rather than the relatively crude mass procedures that have been employed in the past, these techniques can be used to inspect and modify individuals of the population with precision.

Recent years have seen the development of bioaffinity nanoparticle probes for cellular and molecular scanning, tailored nanoparticle medications for cancer treatment, and modified nanodevices for early cancer detection and monitoring. Nanobiotechnology has made major contributions to cancer detection and treatment, and by integrating multiple technologies, it will smoothen the path for creation of personalized cancer care. Nanobiotechnology has led to the discovery of markers that can be used to diagnose and treat cancer depending on the molecular characteristics of individual people. Nanoparticles enable cancer medicines to be delivered to specific locations, increasing efficacy while reducing side effects. Nanotechnology may one day play a crucial and unrivaled part in the diagnosis and therapeutics of life threatening diseases and hence will highly contribute to the quality as well as ease of life.

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Nanomaterials based sensors for detecting key pathogens in food and water: developments from recent decades

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5.1 Introduction

The diseases concerned to foodborne are generally caused because of the pathogens present in food, air and water. There is a significant contribution of pathogenic bacteria to different globally major diseases which results in millions of deaths every year. Hence, to avoid these issues earlier and accurate detection of food toxicity and pathogenic microbes causing food infection is needed. Current technological advancement has brought better understanding of bio-sensing, enhance sensitivity and specifications of pathogen detection by widely exploring through nano biosensors with various nanomaterials and composites application. Nanotechnology explored the materials having dimension 1–100 nm range by concerning its synthesis, fabrication and various field applications. Due to their dimension and surface effect nanomaterials show the specific physical and chemical properties. Recently, nanotechnology has been widely used in the field of biosensor by using various nanomaterials and nanocomposites materials (Kumar et al., 2020; Vo-Dinh et al., 2001; Jain, 2003; Haruyama, 2003). As per WHO (World Health Organization) due to food-borne infection and food contamination 600 million infection and 420,000 deaths has reported every year globally among which about 30% of death occurs among children. The present detection procedure of pathogenic bacteria of food takes up to 4–5 days to give the results and in many circumstances, it has been observed that the results are not even considerable.

Here comes the nanotechnology in the picture, the methods based on this technology can detect the pathogenic bacteria available in the complex food products with very high sensitivity. The basic working principle of biosensor is the interaction of biological molecules (i.e., an antibody, nucleic acid or receptor) with analyte where, by utilizing transducer the received response gets transformed into an electrical signal. With minute limitations the biosensor devices give specific response along with finding of non-targeted microorganisms. The recent advancements in technologies of signal transduction by use of nanomaterials in biosensor has largely transformed the domain of biological and chemical investigation to allow in vivo studies. Nanotechnology offers promising result in food microbial detection. The amount of materials and substrate used in microbial detection fewer used due to exceptional behaviors of nanomaterials for example higher surface to volume ratio and surface permeability, larger improved reactivity and penetrability. With relative to the bulk material, we tend to realize that nanomaterials functions more efficiently in chemical and physical reactions (Sastri et al., 2013). The percentage of pathogen required in the body to cause a life-threatening situation does not to be high enough; even minute amount can cause hazardous problems. Sepsis which is a severe blood infection is also a major public health concern and is caused due to the pathogens. It can only be treated if the identification of the responsible bacteria is done on time otherwise it may lead to a serious problem.

In current scenario, if issues like the melamine epidemic have to be avoided then it becomes very important to analyze understand and deal with the global consequences of foodborne diseases. In addition to the hazardous health effects

the contamination has also affected greatly to the economy. Conventional methods exercised for detecting the microorganisms can be very lengthy and quite tedious to perform. Sometimes it takes number of days to give the results hence, it was very necessary to look for alternate solutions. Researchers across the globe strived and presented time saving and more economical solutions about food and water borne pathogens detection (Mocan et al., 2017). The use of magnetic nanoparticles, gold nanoparticles, silver nanoparticles, nanorods, nanoshells and hybrid nanoparticles in the field of pathogen detection has proved to be a great discovery.

The size and properties of nanomaterials used for pathogen detection enables them to be act as remarkable floor for bacterial detection and identification in the biological samples (Wang et al., 2016; Duan et al., 2015; England et al., 2015; Jiang et al., 2015; Wang et al., 2016). In detection of pathogenic bacteria magnetic nanoparticles have been widely investigated by means of the nuclear magnetic resonance imaging (Bai et al., 2013). The widespread usage of carbon nanotubes, quantum-dot nanoprobe and magnetic nanoparticles have definitely paved the path swifter, with minimum interference and much more diligent diagnosis of dreadful diseases such as cancer. This has enabled an early-stage detection of the diseases and can be monitored more precisely throughout the course. The biomarkers related to diseases can be swiftly and directly can be identified by the nanostructure like, nanotubes, nanowires, nanorods, microarrays, cantilevers and nanoarrays comprises part of precise procedure also characterized by smaller consumption of sample and significantly high sensitivity (Ahn et al., 2014; Banu et al., 2015; Bucharskaya et al., 2016; Duan et al., 2015; Fan et al., 2016; Fratoddi et al., 2015; Lei et al., 2016; Li et al., 2015; Liu et al., 2015; Mocan et al., 2014; Mocan et al., 2015). Along with cell analysis, nanotechnology has open wide prospective for early detection of bacteria, virus and cancer cell (Zhou et al., 2014). In addition to it, recently an outburst has been observed in the pattern technique to assure pathogen targeting ligands through nanoscale arrays development which revolutionize the detection and identification of pathogens and infectious diseases. Antibiotics resistant bacteria has evolved as the major challenges altogether moreover, nanotechnology has accomplished the swift through susceptible bacterial drug preparation and resistance via new and exciting approaches, to name a few magnetic relaxations.

Due to paucity of infrastructure and resources the developing, underdeveloped along with the rural regions of developed countries, the pathogens which includes bacteria, fungi, viruses, and protozoa are the major cause for the loss of life and livelihood (Tallury et al., 2010). We must be aware of the fact that the pathogens causing infections may migrate and spread rapidly by means of humans, animals and plants (Kaittanis et al., 2010). The spread of pathogens in food and water if unchecked may also lead to an epidemic. There is no denying fact that infectious diseases are one of the leading and a major healthcare issue. The intoxication of food and contamination of water being a major contributor to the problem. Nanotechnology propounds a great platform to develop and fabricate rapid, precise, and low-cost diagnostics for the detection and identification of pathogenic infectious agents. Remarkable properties of nanomaterials help to prepare highly specific and capable devices for clinical or environmental pathogens detection. In comparison to bulk material the nanomaterials have contrast behavior owing to their high specific surface area, which further results in improved surface reactivity, quantum confinement effects, improved magnetic properties and electrical conductivity, etc.

5.2 Various contaminants in food and water

Food and water are one of the most vital parts to the well-being of humans since the beginning and its contamination is always a major concern. Around 8000 years ago the problem of food contamination was mentioned in the ancient testimony. There are several reasons for food and water contamination. Nonetheless, a new domain has been added in this new era i.e., globalization and international agribusiness has driven the problem with the food supply to expand across the globe all too quickly.

5.2.1 Contaminants in food

Contamination in food is a subject of grave concern, as the edible items may contain chemical of high concentration which can possess serious health issues. It has become an intimidating job to protect the human beings from the deadliness of the contaminated foods. There are number of reasons which lead to contamination of food (Ingelfinger, 2008). Contaminants present naturally in environment or induced by human artificially are the main reasons for food infection. There are various factors like contaminations during food processing, illicit additives and food transportation which may act as food contaminants. The preparation of food is a long chain progression like processing, packaging, transportation and storage and any mistake at any step may result the contamination of food. Under poor sanitary circumstances, transportation can be a potential contributor to food contamination. To enhance the shelf life many chemicals are

purposely mixed which at times can be harmful. The consequences of food contamination are quite fatal; it ranges from slight gastroenteritis to fatal hepatic cases, neurological and renal syndromes. Though, the government is taking crucial and necessary action to control the levels of chemical in the edible products by providing least limits which are enough secure for consumption of human beings. Recently, owing to the industrial progress and the resultant increase in the environmental pollution has further increased the contamination in food (Song et al., 2017). Environmental contaminants are referred to the adulterations that are moreover due to human incorporation or else present naturally in water, air or soil. The food processing steps like baking, heating, canning, roasting or fermentation may cause contamination in food (Schrenk, 2004). The proximity between the food items and its packaging material can also lead to chemical contamination as some foreign harmful particles may migrate to the food items.

5.2.1.1 Naturally occurring contaminants in food

A large number of bacteria, viruses and fungi naturally reside on the surfaces of the raw food. There are other reasons as well which lead to contamination of raw food such as sewage, external surface, soil and animals. Food obtained through chemical processing or chemical given as part of food or antibiotic to animals and poultry finally enter through the food chain and cause food contamination (Martin and Beutin, 2011). Due to the symbiotic relation in between the organisms and the parasites numerous parasites are available on the food. Several of them are capable of causing infections which further turns out into an epidemic. Enteric infections which are caused due to parasites can be transmitted via the fecal-oral way. This can take place due to intrinsic consumption of the contaminated food or can occur by direct swallowing of parasites that living freely from the environments. As the animals which produce food in either way could also be a carrier of infection if they are already infected themselves (Pozio, 1998) (Table 5.1).

5.2.1.2 Contamination taking place because of environmental influences

The several environmental pollutants responsible for food contamination get regulate through biosensor assay (Baeumner, 2003). Through industrial circumstances heavy metal food contamination like lead, cadmium, mercury, etc. arise. The base of the food chain consists of the plants, and they can readily soak toxic substances present in the soil, which not only contaminates the vegetables and fruits but seafood correspondingly (Peralta-Videa et al., 2009). Another origin of food contamination is the environment of soil. Industrial areas which eject out heavy metals in large quantity may enter in food chain and contaminate the food through penetrating the soil (Krishna and Govil, 2007). For the protection of plants, pesticides are extensively used which taking part in food chain when these chemicals are exposed to humans it leads to broad range of health issues such as, deterioration of immunity, declined intelligence, hormone disordering, cancer, and abnormalities in the reproductive system (Abhilash and Singh, 2009). Three billion kg pesticides used every year across the globe is the grave concern, as the chemicals present in them contaminate the raw food resources (Pimentel and Burgess, 2014). In addition to that, the maximum residue level (MRL) in the context of pesticides is a significant parameter for the human health impairment. The residue levels of pesticides in food are governed by regulating lower its exposure to the consumer (Nasreddine and Parent-Massin, 2002). Such legislations do not hold a good place or is poorly enacted in most of the under-developed nations. In the farm animals, often there is use of

TABLE 5.1 List of natural and anthropogenic food contaminants.

Natural food contaminants	Anthropogenic food contaminants
Viruses	Pesticides
Fungi	Herbicides
Bacteria	Rodenticides
Parasites	Food additives
Insects	Food packaging
Mycotoxins	Food storage
Alkaloids	Food processing

veterinary drugs so the residue of that is left behind in the meat and can threaten the health if consumed, there also exists threat of allergies (Reig and Toldrá, 2008).

5.2.1.3 Contamination during the food processing

In early raw stages food contamination may take place due to environmental pollutants. During food transportation, there are chances of cross-contamination due to some common sources such as exhaust of petrol and diesel from vehicles. The ships that are used for long distances are sometimes cross contaminated with the chemicals that are utilized for disinfection (Nerín et al., 2007). Contaminants can also storm in the cleaning and preparation stage of food production owing to the residues of decontaminators and cleaning agents on food handling surface (Nageli and Kupper, 2006; Villanueva et al., 2018). Another source of contaminant is the heating treatment in the process of production. The utilization of high temperature for cooking along with external parameters potentially results in the emergence of toxic compounds that creates an impact on the safety and quality of food. A prime source of toxic chemicals like chloropropanol, acrylamide or furans has been emerged while cooking food at high temperature which results in lowering the food safety and quality (Roccato et al., 2015). In addition to that, contamination of food can also be a consequence of microwave heating. The food packaging materials which can be used in microwave involves plastics, paperboard and composites, while cooking there is a chance of migration of these materials towards food items and food quality and safety reduction (Ehlert et al., 2008). The packaging of food items has many advantages but there is no denying fact that it can also cause a risk (Marsh and Bugusu, 2007). To enhance the properties of packaging materials several additives like antioxidants, slipping agents, plasticizers and stabilizers has been used. Corrosion behaves as a source of contamination in food when metallic cans are employed for packaging owing to metallic ions transfer to food. Nowadays on the way prevent this situation; the inner cover is generally layered with epoxy resin metallic cans (Buculei et al., 2012). The storage of food items is another benefactor of contamination of food. When packed food items come in direct contact with sunlight, the deuteriation and off-odors of food takes place. To increase the shelf life of food items various flavors and coloring compounds have been used which result the reduction in nutritive quality of food. Foods which contain high fatty acids are vulnerable to odor contamination (Fig. 5.1).

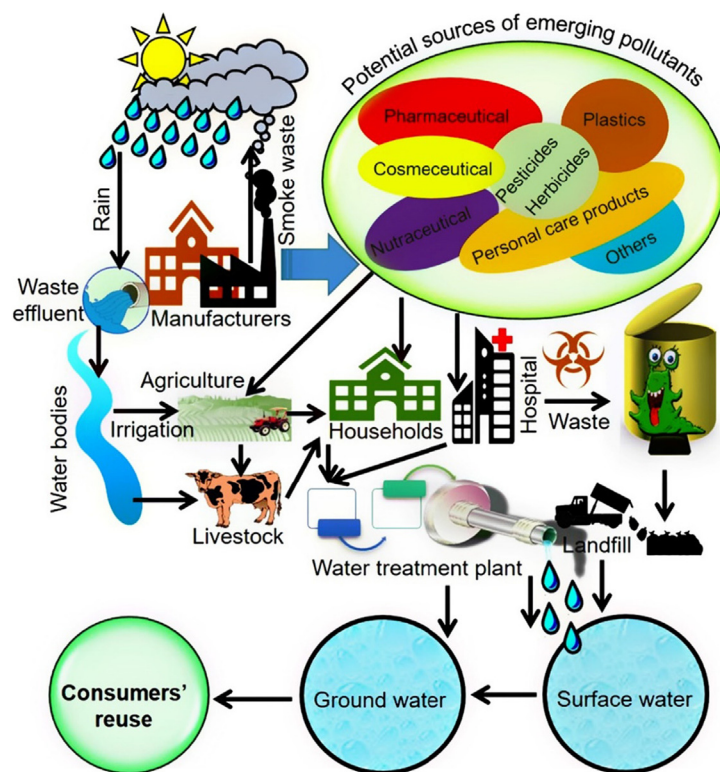


FIGURE 5.1 Food manufacture and processing contamination due to environmental influences. Reprinted with permission from Elsevier Rasheed, T., Bilal, M., Nabeel, F., Adeel, M., Iqbal, H.M.N., 2019. Environmentally-related contaminants of high concern: potential sources and analytical modalities for detection, quantification and treatment. *Environment International* 122, 52–66.

5.2.2 Contaminants in water

For healthful life the prime component is an ample reservoir of safe drinking water. Although the diseases occurred due to contamination of water has resulted in significant deaths across the globe since decades. Especially the water contamination has been a potential threat to the children. It has also resulted in significant economic crisis in subsistence economies. We all are aware that the major origins of drinking water are the surface water (lakes, reservoirs, rivers, etc.) and the ground water. Generally, the surface waters are more vulnerable to pollution than the groundwater. Generally national or international parameters has been used to measure the quality of drinking water in this regard WHO Guidelines for Drinking-Water Quality is widely accepted (WHO, 2003). According to data reported by WHO, about 765 million people in the world are deprived of simple drinking water, where about 144 million entirely depends on the surface water and about 2 billion people make use of contaminated water for their daily purpose. Because of which several epidemic diseases like typhoid, diarrhea, cholera, etc. raised which leads to about 500,000 deaths every year. These data transparently depicted the importance of enhancing the quality of drinking water for preventing several epidemics. The quality of drinking water and the health risks which are associated with them varies across the globe. The scenario of water contamination is not same everywhere. In some areas, the raise in arsenic level is major concern whereas in other places the water contamination due to pathogenic bacteria is the major issue. Due to water pollution apart from the thirst crisis this can also lead to food crisis (Prüss et al., 2002; Pruss-Ustun, 2014; Molinari et al., 2004; Al-Degs et al., 2006; Sadegh et al., 2015; Islam et al., 2017; Babel and Kurniawan, 2003; Karimi and Zohoori, 2013; Rath et al., 2020; Rath et al., 2018). Man-made contaminants have numerous possible sources which further are categorized into point and diffuse sources. Point sources refers to industrial effluents and the sewage treatment works. They can be easily identified and controlled. The runoff from the roads (hard surfaces) and the agricultural land cannot be readily controlled. These sources contribute significantly to the contamination load over time. The spills from the chemical factories, industries, agriculture and the slurry waste from the farms contain pathogens. Local industries, badly sited latrines and septic tanks also lead to water contamination. The swift bloom arises due to *Cryptosporidium parvum* and *Escherichia coli* 0157: H7 have created a major risk and challenges for drinking water industry which need immediate strategy to solve this potential risk (Table 5.2).

5.2.2.1 Contamination due to microbes

The major aspect of drinking water quality is the diarrheal disease caused by the water contamination by pathogens. The issue come into the picture because of infection caused by human waste matter which contains pathogenic organisms. A large part of both the developing and under-developed countries are suffering from this problem. It is hence very necessary to interrupt the fecal–oral cycle by avoiding the waste and fecal matter from getting into the water sources. It is very important to maintain our personal hygiene by washing hands regularly to prevent the person-to-person infections. In water the pathogens uncovering and control are not accurate in most of the cases as there are number of difficulties and requirement of resources. *E. coli* and *streptococci* are the major pathogens responsible for fecal contamination in water. The relevant step for treatment must be adopted if pathogens including viruses have been indicated by indicators. Nonetheless, the time which is taken to perform the analysis, and in case if the contamination or infection is detected then it is considered that the water has already been gulped down by the consumer.

TABLE 5.2 List of natural and anthropogenic water contaminants.

Natural water contaminants	Anthropogenic water contaminants
Arsenic	Irrigation and aquaculture
Mercury	Electricity generation
Chromium	Industrial use
Fluoride	Silviculture and pasture management
Cadmium	Oil and gas production
Chloride	Domestic wastewater
Copper	Animal Husbandry

5.2.2.2 Chemical contaminants

The subject of consumption of food started from short interaction chain between the producer and consumer but has evolved into a complicated network which includes different parties. Like food the drinking water has also the similar probability for getting contamination. It is not only dangerous for the human beings but also the entire aquatic ecosystem is at danger. Groundwater which is a source of drinking water may also be contaminated by the presence of heavy metals such as, mercury, copper, nickel and chromium which further results in increase in number of health problems due to toxic and carcinogenic nature (Wongsasuluk et al., 2014). A huge number of chemical contaminants are available in the environment; some of them are elaborately discussed below.

5.2.2.2.1 Arsenic

Arsenic which is waterborne is a prime reason of diseases and hazards in several area of the world especially in Indian sub-continent, South America, and the Far East region. Arsenic present in drinking water as a harmful contaminant has been observed to be the only major cause of cancer when exposed to humans. It is also responsible for a broad range of cancers related to skin, bladder, lungs and vascular diseases. It is also accredited by the huge poisoning of the masses in the world particularly in parts of India and Bangladesh. On the basis of practical boundary of viability, World Health Organization have assigned a provisional guideline value of 10 µg/L. Drinking water which has been contaminated by arsenic gives rise to a disease known as arsenicosis (Chen et al., 1988).

5.2.2.2.2 Fluoride

Presence of fluoride in water bodies emerge as a major cause of human illness in different parts of world. Mainly the sources of fluoride are either geological or anthropogenic. The exploitation of groundwater over and over again increases the concentration of fluoride. The pharmaceutical products are the anthropogenic sources of fluoride. It is widely used in toothpastes, medicine, insecticides, pesticides, disinfectants, supplements, etc. Children and elderly people are very much prone to the drastic effects of fluorides. The concentration of fluoride found in these places mounts up to 10 mg/L. Raise of fluoride consumption could tend to dental fluorosis, an unusual teeth brown mottling, and even upsurge consumption could consequently initiate skeletal fluorosis, a condition of increasing bone density which moreover results in fractures and crippling deformity in skeleton. A studying group of World Health Organization (WHO) have reported that a total portion of 14 mg fluoride day-to-day can give rise to skeletal fluorosis and enhances the probability of fracture in bones.

5.2.2.2.3 Mercury

Mercury also known as quicksilver, is a highly toxic metal, which is mainly found in the environment. The primary sources of mercury contamination in water are the run-off from agricultural land and effluents from the factories. Impairment in the functions of brain, disorders in the nervous system, inhibition of growth in children, abortion and endocrinal disorders are major fatal effects of presence of mercury in the drinking water (Clarkson, 1993; Counter and Buchanan et al., 2004).

5.2.2.2.4 Copper and chromium

The natural accumulations in rock and soil incorporate the copper in water bodies. The availability of copper in drinking water is also due to the corrosion in the plumbing pipes. There are short-term and long-term effects of presence of copper contamination in water. When it is exposed for a short span of time, it can lead to mild gastrointestinal distress where as if exposed for a large passage of time then it may result in permanent damage of kidney and liver. Chromium is tasteless and odorless metallic element. Chromium is usually found naturally in rocks, soil and volcanic dust. Contamination of groundwater can take place due to discharge of chromate mines or undesirable disposal of mining tools and supplies. If there is exposure of large amount of chromium then it may cause damages in liver and kidney, dermatitis and problems related to respiration.

5.2.2.2.5 Agricultural pollution

Agriculture is also a prime source of chemical contamination. Nitrate contamination occurs due to discharge of fertilizers in the water bodies. It enters to the water bodies through sewage and wastes from humans and farm animals. It is the prime cause of blue-baby syndrome in the infants. If a large amount of nitrate is present in drinking water and is consumed, then it can cause illness. Nitrate gets converted into nitrite inside the body and start interfering with the

transport of oxygen in the blood. In addition to this, it also shows symptoms of breathing issues and blue coloring of the skin. According to the research carried out, World Health Organization has laid down a recommendation to measured value of 50 mg/L nitrate concentration.

5.2.2.3 *Biological contaminants*

Biological contamination of water is a consequence of availability of bio-organisms, like bacteria, algae, viruses or protozoan in adequate quantity. Each one of these are capable enough of creating different problems in water. Algae are single celled and microscopic organisms. They are available in much abundance, and they are entirely relied on the nutrients (e.g., phosphorus) in water bodies. The domestic run-off and the industrial effluents provide the nutrients for the algae to survive. The excessive growth of algae does not only releases taste and odor issues in the water; but it also causes the blocks in filters and generates undesirable growth of slimes on the carriers. The blue-green algae are even known to release toxic elements which may lead to damage of liver, nervous system, and skin. The large number of microscopic pathogenic bacteria is the main reason for water contamination (Inamori and Fujimoto, 2009). Sometimes they can show adverse effects such as, dysentery, cholera, typhoid, and gastro related problems. Some of the non-pathogenic bacteria such as, crenothrix iron bacteria, sulfur, are not that dangerous, but can lead to taste and odor issues (Nwachuku and Gerba, 2004; Rusin et al., 1997). The bacteria that are noticed in tap water, packaged bottle water and various other sources of clean water are heterotrophic. For source of carbon, they need organic carbon instead of carbon dioxide. As per the reports of US Environmental Protection Agency (EPA) it has been reported that the number of heterotrophic bacteria in drinking water must not rise above five hundred (500) colony-forming units (CFU) per mL. Alike to pathogenic bacteria and algae, protozoans are also microscopic single-celled. The protozoans like Giardia and Cryptosporidium can be usually seen in rivers, lakes, water reservoirs and streams. They are being infected either with animal feces or waste generated from the sewage waste treatment plants. They can lead to diarrhea, stomach cramps, vomiting, headache, nausea, fatigue, and dehydration. Viruses are considered as the tiniest living organisms that are capable of producing infection and moreover cause a number of diseases. Hepatitis and viruses of polio are usually found in the contaminated or infected water.

5.2.2.4 *Radiological contaminants*

The radioactive elements like U^{226} , Ra^{226} , Ra^{228} and Rn^{228} are the source of radiological contamination. These elements are a major problem in the groundwater than the surface water. The frequent occurring erosions of natural deposits of radioactive elements emit radiation like α , β which are quite harmful. Every kind of radiological contamination is quite fatal and causes cancer. The areas where granitic rocks are found contains uranium in the groundwater present there. It causes toxicity in kidneys.

5.3 Designing and fabrication of nanomaterials-based sensors

With the discovery of nanosensors by Dr. Wolter in 1994 it has always been in the limelight and tremendous amount of research is going on. The research in advanced materials presently focuses on the design and fabrication of nanomaterial-based sensors. It has exhibited huge potential for the advancement of huge number of applications in different domain of nanotechnology. The noteworthy scientific and technological breakthrough in design and configuration of materials for nanomaterial-based sensor applications has broad range of economic consequences. From practical lens, nanosensor is categorized as either physical or chemical and generally consists of at least one component in nanometric range. Novel and exciting technologies of sensor relied upon nanoscale materials have a number of benefits over the traditional methods of sensors. The fabrication of nanosensors is a tedious and highly challenging task, but researchers have observed notable growth through their research and innovations, specifically in the context of fabrication modification methods for more particular applications. A variety of nanosensors are available and corresponding to that there are numerous ways of fabricating and manufacturing them. They are generally quite susceptible and may also detect small fluctuations in the concentrations of different varieties of particles, which includes single viruses, proteins, and molecules in diversified and complicated atmosphere (Mehrotra, 2016; Afsahi et al., 2018; Lerner et al., 2014). The integrated circuits having transistors, resistors, and capacitors as fundamental parts that comprise of such nanosensors. Henceforth, the potential to develop new and exciting nanoscale complicated materials and devices and moreover to integrate them into various configurations is completely based on the constructing strategies and synthesizing of highly complex structures (Burda et al., 2005; Chen et al., 2019; Longo and La Porta, 2017). The nanosensors used in the analysis of food merge knowledge and understanding of biology, chemistry and nanotechnology and can also be referred as

nano bio-sensors. The arrangement and configuration of nanosensors are same as the basic sensors, but the production and manufacturing are performed at nanoscale. The nanosensor can be referred as an incredibly small device that may integrate and design to detect desired requirement and revert a signal (Duncan, 2011). While designing a nanosensor the specific goals which are taken into account are sensitivity, specificity and ease of execution. Nanosensors can be prepared by using various methods. The five most common and frequently used methods are top-down lithography, bottom-up fabrication, molecular self-assembly, heat and pull method for fabrication of biosensors, etching for fabrication of nanosensors etc. Nanofabrication enables at the nanoscale to design and work with incredibly small models. Owing to their very small size, these models require extreme care and attention while handling. Nanosensors have the potential to merge the capabilities of computation and perception. Sensors perceive the environment, and the electronic part processes the sensory information and makes decision (Fig. 5.2).

Nanosensors utilize an exceptionally varied set of materials and processing techniques. The access to resources for the fabrication of nanosensors can be quite difficult and expensive. There is a need of very detailed technical knowledge and understanding to make good decisions. The important issues related to manufacturing of nanosensors are material properties, packaging, process of integration and designing of the tools. Nanosensor processes merges the Integrated Circuits (IC)-type processes with highly specialized and exclusive. The IC processes includes Chemical vapor deposition (CVD), photolithography, sputtering (RF and DC), ion implantation, etc. The sophisticated nanotechnology processes related to fabrication of nanosensors are exotic material deposition, exotic material etches, high-aspect ratio etches, deep reactive ion etching, nanomoulding, etc.

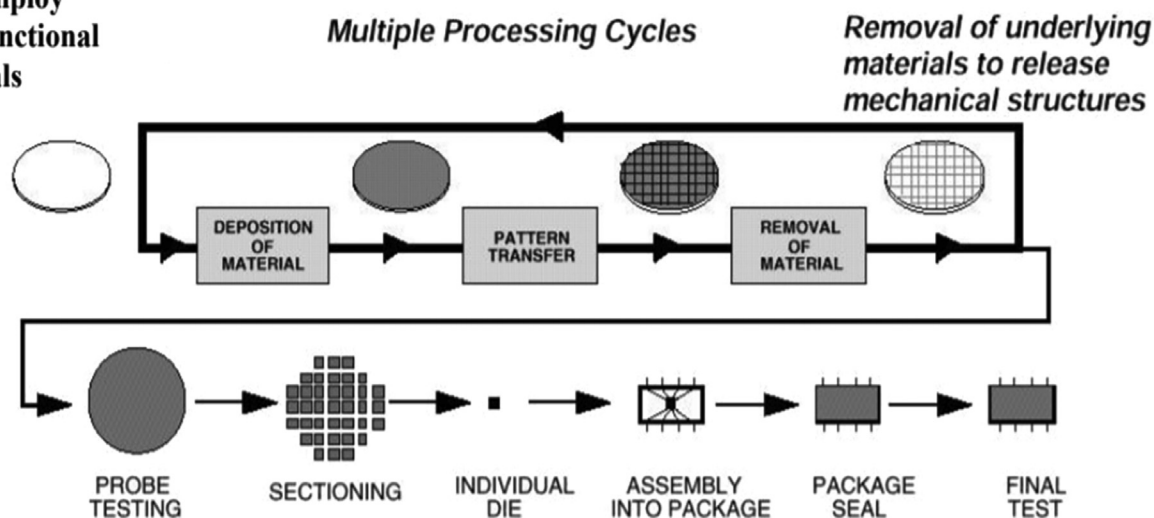
5.4 Applications of nanosensors in different sectors

Nanosensors are coming out to be promising and potential drive for the appliances in the field of food production, agriculture, defense and security, medicine and many more. Compared to traditional chemical and biological ways nanosensors provides tremendous enhancements in selectivity, speed and sensitivity. The use and implementation of nanosensors in the food industry is growing rapidly. The safety of food and quality has always been the prime concerns.

5.4.1 Agriculture

Nanosensors are used for monitoring the condition of soil (like moisture content and pH). It also monitors the level of pesticides and insecticides to be used and examines the growth of the crop. It can also be used for monitoring the environmental

**May Employ
Multifunctional
Materials**



Special probing, sectioning and handling procedures to protect released parts

Encapsulate some parts of device but expose others

Test more than just electrical functions

FIGURE 5.2 Typical process of fabrication of nanosensor. <http://www.memsexchange.org>.

condition of the farmland. It can also be employed in the capsules and then used for appropriate delivery of pesticides, vaccines and insecticides. Nanosensors are employed for early signaling and warning about the weather conditions. Nanochips are used for tracking and preservation of identity. Nanosensors have helped in the increase in productivity and intake of nutrients from the soil. It is also used to keep an eye on the signaling and to study the plant biology. They are also employed for the surveillance of the pathogens present in the plants. Nanofertilizers are readily absorbed by the plants.

5.4.2 Pollution

To curb the problem of environmental pollution, nanosensors can be used on a broad scale. It detects the various contaminants present in the air, water and soil. It monitors the level of different contaminants present in air. It can also be employed in the water bodies to detect the level of toxicity.

5.4.3 Food processing

Nano capsules enhance the flavor of food without causing harmful effects. They can be used in gelation and as viscosifying agents. They are used in appropriate attaching and removal of chemicals and bacterial pathogens from food.

5.4.4 Food packaging

Movable nanosensors can be employed to detect and identify pathogens, chemicals and toxins present in food items. The DNA biochips technology has been used to sense pathogens and identify different dangerous bacteria present in meat or fish or even the fungi which destroys the fruits. Nanosensors are assimilated into the packaging materials for the identification of the released chemical when the food is spoiled, it behaves as an electronic tongue. Ethylene can be detected by electromechanical nanosensors. Nanosensors can be incorporated as inner coatings or labels to include intelligent function to packaging of food. It confirms that the packet is safe from disintegration and leaks. Due to antimicrobial properties of nanomaterials researchers are focusing on the nanoparticles embedded food packaging materials.

5.4.5 Food transport

During distribution and storage, for monitoring the environmental conditions nanosensors are extensively used. The products which have a shorter shelf life it is necessary to keep record of traceability during the transportation and storage. To monitor the quality of grains, fruits and vegetables, dairy products, dry fruits smart-sensor technology can be used. It can also detect the type of spoilage and help to prevent it. The level of nutrition can also be maintained using this technology.

5.5 Recent developments in nanomaterials-based sensors for pathogen detection

In recent years the studies and investigation on nanomaterials-based bio sensors have gained momentum. A number of challenges has been addressed and overcome through the advancements and use of nanotechnology. The direct detection and identification in a very short span of time has been made possible through nanotechnology. Taking into consideration the peculiar properties (chemical, physical, magnetic, mechanical, etc.) different kinds of nanomaterials as discussed earlier such as magnetic nanoparticles, gold nanoparticles, quantum dots and carbon nanotubes have been amalgamated to biosensors. The advantages such as excellent sensitivity, high speed results and appropriate selectivity are highly desirable in the detection of the pathogens (Iqbal et al., 2000). Conventional methods have deliberately failed in achieving so but the alternate and the fast pace growing methodologies that include use of nanotechnology has filled the void. The recent advancements in nanotechnology offered to prepare electrodes on an extremely minute scale, nano-based sensors have made possible and lead to a new domain of diagnostic biosensors called Nano-biosensors. For significantly improving the applicability the material dimension has been decreased continuously. The properties of the bio sensors when reduced from bulk to considerably small in the range between 1 and 100 nm gets enhanced manifolds. The incredibly ratio of large surface to volume in the nano-size devices facilitates highly efficient surface interaction of analyte with the sensors. Hence, the materials at nanoscale exhibit unique features, effects and functionalities. Nanotechnology meant to bring advancement in pathogen detection technique by removing drawback, making cost effective and reducing time consumption. In addition to that, sensitivity and efficiency of biosensor has been enhanced by using nanomaterials. A highly efficient biosensor widely used for pathogens detection present in food and water has been designed by effective combination of biological elements and the transducer-based nanomaterials (Yockell-Lelièvre et al., 2016).

5.5.1 Quantum dots

Quantum dots (QDs) are basically 2–10 nm diameter nanoscale semiconductor crystals owning peculiar electrical and optical properties. Size dependent properties is exhibited by the QDs due to the confinement of electrons and holes further making them more desirable for fluorometric sensor applications. Due to their small size they have very high versatility. QDs have ability to emit visible light of various wavelengths by using UV radiation. The emitted wavelength depends on certain parameters such as size of QDs, as compared to the larger QDs the distance between energy bands in smaller QDs are higher. Expanding interest for the biological detection by Quantum Dot- based sensors, sets the ball rolling for the advancements of different ways of preparation of the QDs to name a few, plasma synthesis, colloidal synthesis, electrochemical assembly, viral assembly, etc. QDs have numerous novel and peculiar properties which includes broad absorption range, symmetric size photoluminescence, wide excitation range as well as high resistance to photo bleaching, long florescent lifetime, and multiplexed staining. The optical properties of quantum dots are going to help definitely in the future applications of detection and identification of pathogens present in food and water. We greatly hope that QDs will keep making tremendous contributions in the realms of pathogen detection. As the visible fluorescence of CdSe is very attractive for the biological imaging and analysis, so it is considered as one of the most investigated semiconductor QD. There are numerous advantages of semiconductor QDs over the traditional organic dyes regarding pathogen detection. The wide absorption spectra and the narrow emission spectra provides room for simultaneous excitation and detection. Colloidal QDs possess very high photostability. In the regime of toxicology and immunology, by exploiting the extraordinary optical properties of QDs it can surely impart numerous benefits in the future application of pathogen detection and identification.

5.5.2 Carbon nanotubes

Carbon nanotubes (CNTs) are absolute hollow tubes that consist of rolled graphite sheet. In accordance with the number of layers of graphite sheet, the CNTs have been mainly dividing up into single-walled carbon nanotubes (SWCNTs) and multi-walled carbon nanotubes (MWCNTs). The biosensors that are based on CNT are acknowledged to be the upcoming generation foundation for ultra-fast and ultra-sensitive bio-sensing systems. CNT has unique expeditious ability to analyze biological samples due to its high surface to volume ratio. CNTs possess numerous advantages which includes high sensitivity, fast response time, high stability and longer lifetime. CNTs provide a very fertile ground for research due to the specific mechanical, electrical, thermal, and chemical properties it holds. There is a particular engrossment in the field of biosensors of these carbon-based nanomaterials based on the biomedical utility of it. In biosensor fabrication CNTs play crucial role which make the biosensor effective to trace and detect target molecules even in trace amount also. The present strong facets of CNTs are instigated from physical chemical interactions transduction and high surface area to volume ratio (Yang et al., 2015). Although the recent advancements in CNTs that have made exciting advances in the field of pathogen detection, but the reality is that there are numerous scientific as well as economic challenges that has to be addressed. To mention a few of them, the first one is cost-effective. CNTs are highly expensive and reducing the cost is very complex challenge. It requires great understanding of the integration of both material science and nanotechnology (Chen et al., 2013). The second one is to have appropriate conditions such as temperature and pH for the functionalization of the CNTs. Moreover, to increase the thermal stability and the lifetime of CNTs is one of the key challenges that the researchers are facing across the globe.

5.5.3 Silver nanoparticles

The size varying from 1 to 100 nm range make the silver nanoparticle effective for biological applications. By continuously decreasing the size of Silver Nanoparticles (AgNPs), there is an effective rise in the surface to volume ratio which surprisingly results in observable changes in chemical, biological and physical activities (Varner et al., 2010). Recently silver nanoparticles have been extensively used in preparation of highly advance diagnostic devices like biosensor and immunosensor development. The AgNPs possess very good fluorescence characteristics which are used in optical biosensors with high sensitivity. To avoid infections AgNPs were initially utilized as antimicrobial agents. AgNPs are considered to be greatly toxic for the microorganism and hence possess antibacterial effects against a broad spectrum of microorganisms which includes fungi, bacteria and viruses. Being a good antimicrobial agent, AgNPs have been popularly utilized for the disinfection of water. Although the mechanism of the antimicrobial effects of AgNPs is not exactly known and still is a matter of debate. AgNPs are capable to adhere to the bacterial cell wall and then penetrate it, leading in structural variations of the cell membrane and hence increasing its permeability (Sondi and Salopek-Sondi,

2004). They possess the ability to damage the cell wall and are also regarded as a cause to the death of cells. In recent years, AgNPs have been profitably utilized in the water and wastewater disinfection. AgNPs may cause some serious threat if they have a direct application, owing to their propensity to aggregate in the aqueous media which eventually decreases their efficiency during prolonged usage (Li et al., 2012). To eradicate the existing problems, AgNPs have been attached to the filter materials and are regarded as a potential solution for water disinfection owing to its antibacterial activity and economical (Quang et al., 2013).

5.5.4 Gold nanoparticles

In the field of detection of virus gold nanoparticles (AuNPs) are extensively utilized due to their peculiar electrical and optical properties. They are mainly synthesized by either in an organic or aqueous environment. The UV–vis absorbance spectrum fluctuations reading in gold nanoparticles arise due to surface plasmon band shift which make AuNPs applicable for various biological applications. AuNPs are very much effective at detecting pathogen because of their capability to offer a simple and swift color variation whenever the environment around is altered. Needless to say, they are considered to be the most stable metallic nanoparticles. Pathogens responsible for food and water contamination as well as surface of hospital infection can be easily analyzed through gold nanoparticles (Agasti et al., 2010; Azzazy et al., 2012; Bunz and Rotello, 2010; Khanna, 2008; Saha et al., 2012; Tallury et al., 2010; Upadhyayula, 2012). A prime center of investigation is to enhance traditional methods of genomic analysis utilizing gold nanoparticles in a way that the assays have minor limits of detection and quick retaliation times. At the same time, many new ways of detection have flourished which is completely free from gene amplification. The alteration in gold nanoparticles surface accompanied by antibodies result a variety of commercially viable products which helps in affluent and precise pathogens analysis even in complicated sample also like body fluids, foods and plant extracts. The prominent reason for varying inherent surface properties of AuNPs and pathogens result electrostatic interactions and variation in color.

5.5.5 Magnetic nanoparticles

Magnetic nanoparticles (MNPs) exhibit outstanding performance and feasible entry into cells. The recent decade has observed swift advances in the transformation of present diagnostic methodologies to nanoscale by making use of magnetic nanoparticles. They have helped in shifting the limit of identification to early-phase disease diagnosis. The extensively used biosensors lie under different categories such as magnetoresistance (Mr) sensors, magnetic particle spectroscopy (MPS) platforms, and nuclear magnetic resonance (NMR) platforms. The magnetoresistance sensors depend on the surface behavior which is highly sensitive to the stray field from the MNPs assured to the close contact to the sensor surface. The MPS is technology derived from the magnetic particle imaging (MPI) where the tomographic images are regenerated by interplaying with the nonlinear magnetic responses of MNPs. For NMR platform a high homogenous field H_0 is required (Wu et al., 2020). *E. coli* O157:H7 the pathogenic bacteria responsible for drinking water and milk contamination were detected and investigated using a movable NMR platform (Luo and Alocilja, 2017). The benefits of using magnetic nanosensor platforms are simpler sample preparation, use of safer magnetic labels and even capable of homogenous detection relative to other techniques. The several advantages of using magnetic nanosensors include high sensitivity, availability of portable devices, capability of mass-production and low-cost.

5.5.6 Zinc oxide nanoparticles

ZnO is considered to be a bio-compatible and environment friendly material. ZnO have been used as an antibacterial agent. ZnO can be contemplated as the most dynamic due to its great variety of applications, of all the different materials that have been studied and investigated as nanosensors. In accordance with the Food and Drug Administration, ZnO has been currently reported as a “generally recognized as safe (GRAS)” substance and it can also be used as additive in food items. Nanoparticles of ZnO exhibit toxic effects on pathogenic bacteria (e.g., *Escherichia coli* and *Staphylococcus aureus*). Green synthesis of ZnO nanoparticles is of great interest. It involves the plant-based synthesis of nanoparticles. ZnO is considered to possess strong antimicrobial properties. Furthermore, ZnO is found to be stable under harsh and abrasive conditions which eventually make it appropriate for antimicrobial applications. It is important to note that as we decrease the particle size there is increase in the antimicrobial activity of ZnO. This can be accredited to the larger surface-to-volume ratio which leads in a more adequate means for antimicrobial activity (Baker et al., 2005). The usage of ZnO in water and wastewater disinfections shows promise. ZnO contains the benefits of addressing the drawbacks of conventional water treatment methods.

5.6 Future perspectives and challenges

Owing to their noteworthy morphological, structural and magnetic properties, nanotechnology has stirred a technological revolution in different areas of research. Since long time it has been observed that development of technology has been intricately concerned with the design and configuration of a specific material with basic functions and enhanced performance. It is very necessary to highlight the significance of understanding and to control the properties of nanostructures. It is a remarkable arena, which has spotted recognition in ongoing research in material sciences owing to its interesting outcome on the life of human beings and promotes life sciences, particularly biomedical, biotechnology and food technology. There are about 300 countries across the globe are involved in inducing nanotechnology in their food systems. Towards the sustainable move, nanotechnology can surely act as an intelligent weapon. From the perspectives of science and technology the upcoming future seems to be very fascinating and exciting. The advantages of nanotechnology will enable us to achieve things that we never contemplated before. Therefore, novel, and remarkable developments that have been attained in the basic understanding of the properties of the nanomaterials delivers new possibilities for fabricating the upcoming era of nanosensor technologies. Therefore, the whole focus should be on exploring the advanced materials at nano range and its integrated composition-structure-property relationship. For diagnostics purposes, as the nanomaterials are being embraced for the upcoming generation their practicality completely depends on assurance of quality. Although, if size of nanomaterial and surface properties are not consistent throughout the devices then phenomenon that serves to improve sensitivity can deteriorate the performance of the material. For the bulk-structured materials the well-defined and well-characterized material standards are very straightforward and relatively easy to follow as compared to the nanostructured materials. There are several challenges which has to be addressed in case of nanostructured materials as its very tedious to control the size dependent properties. The challenges are based on different shapes (e.g., rods, tubes, spheres, etc.), forms (e.g., single crystals, thin films, powder, etc.), and non-homogenous composition of elements (e.g., dopants, functional groups, etc.). Hence, to analyze the entire picture it is required to combine various characterization techniques. The toxicity of nanomaterials is a crucial consideration, as the integration of nanomaterials into the diagnostic systems are increasing day-by-day. The proper disposal of the diagnostics which contains nanomaterials is extremely important as its exposure can deteriorate the environment. Minimal research is being carried out on nanoparticle waste, which makes even difficult regarding the utilization of nanoparticles in the food industry. Owing to their incredibly small size, the nanomaterials are capable of migrating into the biological membranes and access cells and tissues which further can have hazardous effects. There are five principal requirements that should be followed by an ideal method of pathogen detection high sensitivity, high specificity, time saving, cost-effective and simple operation (Fig. 5.3).

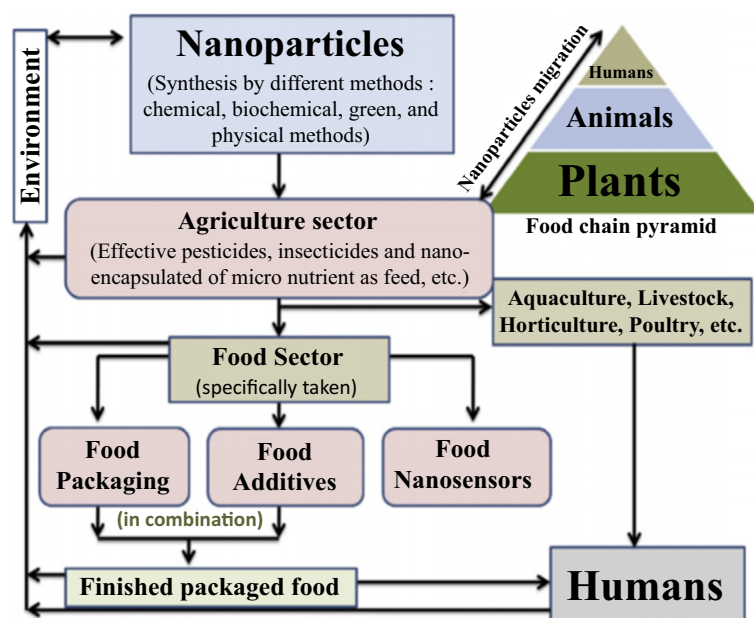


FIGURE 5.3 A continuous cycle of transfer of nanoparticles from their source of synthesis to the agriculture sector, and then finally gather inside the human body through the finished packaged food. Reprinted with permission from Kumar, P., Mahajan, P., Kaur, R., Gautam, S., 2020. Nanotechnology and its challenges in the food sector: a review. *Materials Today Chemistry*, 17, 100332.

5.7 Conclusions

In accordance with the European Commission, nanotechnology is considered to be an important component of its six “Key enabling technologies” which possess tremendous prospective to revolutionize different sectors. Improvement in the soil quality, increase in productivity, surveillance, and stimulation of proper growth of plant, the usage of detailed farming, monitoring the quality of food and novelty during production, food processing, distribution of edible items and food storage, are the few advantages of nanotechnology which must be extended to agricultural and food industry. Nanosensors enable an investigation of broad class of analytes, which includes heavy metal ions (such as fluoride, arsenic, etc.), toxins, pathogens (bacteria, viruses, fungi, protozoans, etc.) nucleic acids and proteins. Nanobiosensors have provided an outstanding deal of enthusiasm due to their ability to identify an immense range of materials at extremely minute concentrations. Nonetheless, this is at most beyond doubt that the nanomaterial-based sensors will enable the detection and identification of pathogens and diseases like never before. Nanotechnology is committed to transform the way of diagnostic tools for pathogen detection and infectious diseases in the ongoing 21st century. The upcoming shift in nanotechnology will carry on with the design and configuration of nanostructures to fabricate miniaturized devices, which requires quite small amount of sample volumes. Moreover, with the advancement in the domain of nanotechnology obsessed by the designing and configuration of smart 2-D and 3-D nanoassemblies, novel and exciting devices that offers rapid, effective and sensitive detection of pathogen even in extremely small amount of sample are anticipated. To conclude, developments and advancements in nanotechnology in the upcoming time will surely be a prominent headway in the domain of pathogen detection to prevent viral diseases and epidemics related to pathogens and offer a healthy and sound life to the infected patients.

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Chapter 6

Microbial nanostructures and their application in soil remediation

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6.1 Introduction

Nanostructures are structures that lie within 1 nm (molecular scale) to 100 nm in one or more dimensions. The majority of nanostructures are artificial and can be designed to have a variety of physical features. Some of the common nanostructures are nanosurfaces, cylindrical nanotubes, and nanospheres. They can be fabricated by either aggregating lone atoms or breakdown of sizable bulk materials (Patil and Chandrasekaran, 2020). In comparison to molecules of larger size, nanoparticles exhibit better mobility and due to a greater surface area per unit mass more reactivity (Sarkar et al., 2019; Yadav et al., 2017). They are usually classified into organic nanostructures and inorganic nanostructures. Examples of organic ones include graphene, polymers, fullerenes, protein aggregates, carotenoids, DNA, viruses, lipid bodies, liposomes, micelles, ferritin, dendrimers, etc. while inorganic ones include magnetic nanoparticles, metal nanoparticles (Au, Al, Bi, Ag, Co, Cu, Mo, Ni, Fe, Ti, In, W, Sn, Zn), metal oxide nanoparticles (CuO, ZnO, NiO, MgO, CeO₂, Cu₂O, SnO₂, ZrO₂, TiO₂, La₂O₃, In₂O₃, Al₂O₃), colloidal nanometer-sized crystal quantum dots and cylindrical graphene sheets as carbon nanotubes among others (Khalid et al., 2020; Rajput et al., 2018; Sarkar et al., 2019). Hybrid organic-inorganic nanomaterials are also synthesized to combine the properties of the two types (Khalid et al., 2020). Nanostructures have widespread biomedical, industrial, and electronic applications and are employed in several biotechnological practices, drug delivery systems, regenerative medicine, pollution control, and disinfection amongst others (Khalid et al., 2020). Engineered nanomaterials (ENMs) exhibit a high sorption capacity due to their small size along with better reactivity and cost-effectiveness which makes them ideal for use in soil remediation.

Soil remediation involves the elimination of contaminants from the soil through a range of chemical, physical and biological means that can be applied to carry it out. Some of the contemporary methods of soil remediation involve using engineered nanoparticles. Their use is usually associated with drawbacks like hazardous chemical and physical mechanisms of synthesis and possible accumulation in the environment (Sarkar et al., 2019). One key method of overcoming the side effects associated with the physicochemical synthesis of nanostructures is the use of biological systems. This pioneering technique helps to cleanly synthesize nanostructures of requisite morphology and size while also exhibiting superior applicability in biomedical, agriculture, and remediation fields (Yadav et al., 2017).

As with physicochemically produced nanostructures, the biogenically synthesized ones have also been shown to aggregate in soil ecosystems and adversely affect the soil flora and fauna in the long run. They are capable of entering food chains and groundwater resources thereby exerting a negative effect on human health and well-being (Rajput et al., 2020).

6.2 Biogenic synthesis of nanostructures

Nanotechnology as a concept was proposed in 1959 by Richard Feynman and involved the synthesis of different types of nanostructures (NS) via physical and chemical means. These procedures require the employment of several harmful chemicals, making large-scale nanostructure synthesis unsustainable. Green or biogenic methods are gaining prominence as they offer sustainable methods of nanostructure synthesis using microorganisms, plant parts, and plant extracts. Green synthesis methods are predominantly a bottoms-up approach (Patil and Chandrasekaran, 2020). Apart from these,

other plant materials like olive oil, eggshells, potato extracts, coconut coir dust, acacia gum, etc. can also be used for NS synthesis (Wang et al., 2019). The green synthesis mechanisms are usually straightforward and include the use of green reducing and capping agents followed by nanoparticle assembly. The future aim should be to focus on standardization of the various parameters that control green synthesis and assembly like temperature, pH, incubation time, solvent, and pressure that determine the NS size and shape (Singh et al., 2018; Wang et al., 2019). The focus of this chapter is predominantly on the microbial synthesis of NS and their applications in environmental remediation, particularly soil. Some of the commonly applied biogenic methods and organisms are listed below.

6.2.1 Biogenic synthesis using bacteria

Bacteria can manufacture a variety of nanoparticles because they can reduce metal ions and can be easily manipulated (Singh et al., 2018). These include nanoparticles of cadmium sulfide, titanium dioxide, gold, silver, palladium, platinum, and many more. Bacteria not only produce extracellular enzymes that aid in the synthesis of a variety of nanostructures in a fairly pure form but can also catalyze many reactions to produce nanoparticles. They utilize both extracellular and intracellular means for NP synthesis. The extracellular method is preferred since it is faster and without cellular debris therefore devoid of downstream processing. It involves the utilization of bacterial biomass, bacterial culture supernatant, or cell-free extracts for nanostructure synthesis (Ovais et al., 2018). The intracellular approach on the other hand requires enzymes like reductases that reduce metal ions to metal nanoparticles. Most of the nanostructures produced by bacteria are synthesized either by reduction or by biosorption. The reduction of the metal ion into the nanoparticle is accompanied by the oxidation of an enzyme. Further biogenic process optimization could aid in the manufacturing of nanostructures with the requisite shape and features without the dangers associated with chemical synthesis (Singh et al., 2020; Yadav et al., 2017). Au and Ag nanostructures produced by using bacteria are highly stable and exhibit superior antimicrobial properties (Ovais et al., 2018; Singh et al., 2020). A detailed account of nanostructures synthesized by bacteria and applied in soil remediation is studied in subsequent sections.

A detailed list of bacterial species that synthesize metallic and metal oxide nanoparticles is given below in Table 6.1 (Akintelu et al., 2020; Hosseinkhani and Emtiazi, 2011; Hussain et al., 2016; Koul and Taak, 2018; Ovais et al., 2018; Pal et al., 2019; Pantidos, 2014; Yadav et al., 2017).

6.2.2 Biogenic synthesis using fungi and yeast

Fungi, yeast, and different secondary metabolites and biomolecules produced by them can be employed to make nanoparticles at a reduced cost and in a lessened time frame. These were also found to be more stable in comparison to those produced from bacteria. Fungi have a variety of intracellular enzymes and other enzymes and reducing agents on their cell surface that are capable of carrying out the reduction of ions into their corresponding metal. Examples include conversion to silver metal by reduction of silver ions by the fungus *F. oxysporum* in the presence of NADH + (Singh et al., 2020). A much better nanoparticle yield is obtained from the use of isolated enzymes instead of fungal cultures. Optimization and genetic manipulations of the current methodologies can aid in improving synthesis via these organisms (Singh et al., 2020; Yadav et al., 2017). Mycosynthesis of nanoparticles therefore could be carried out using either electron shuttle quinones or action of nitrate reductase enzyme or both (Singh et al., 2016).

Nanostructures synthesized by fungi and yeast are listed below in Table 6.2 (Hussain et al., 2016; Koul and Taak, 2018; Pal et al., 2019; Pantidos, 2014; Pasinszki and Krebsz, 2020; Yadav et al., 2017).

6.2.3 Biogenic synthesis using plants

The utilization of plants and plant extracts for the synthesis of nanostructures is relatively unexplored even though they can synthesize nanoparticles by a single-step biosynthesis mechanism with natural capping and no pathogenicity. Also, they are readily available with a large number of metabolites like phytochemicals and polysaccharides that aid in synthesis without the need for toxic chemicals. They also do not require heat or very long incubation times, unlike microbes which reduce cost of synthesis. Extracts from plants and whole plants can be employed for nanostructure synthesis but comparatively use of whole plants is a much longer process. Manipulations in temperature help to produce different varieties of particles of varying shape and size (Pantidos, 2014; Yadav et al., 2017). Moreover, the synthesis of NS using extracts from plants and whole plants generates polydispersed nanoparticles due to the diverse photochemistry of plants (Ovais et al., 2018).

Nanostructures synthesized by plants and plant extracts are given in Table 6.3 (Hussain et al., 2016; Koul and Taak, 2018; Pantidos, 2014; Pasinszki and Krebsz, 2020; Yadav et al., 2017).

TABLE 6.1 Nanoparticles synthesized by bacteria.

Nanoparticle	Bacteria
Au	<i>Thermomonospora</i> sp., <i>Rhodococcus</i> sp., <i>Rhodopseudomonas capsulata</i> , <i>Pseudomonas aeruginosa</i> , <i>Alkalothermophilic actinomycete</i> , <i>Lactobacillus</i> strain, <i>Labrys</i> sp.
Ag	<i>Bacillus licheniformis</i> , <i>Bacillus cereus</i> , <i>Bacillus thuringiensis</i> , <i>Bacillus</i> sp., <i>Bacillus subtilis</i> 10833, <i>Bacillus amylococcus</i> 1853, <i>Bacillus brevis</i> , <i>Corynebacterium</i> , <i>Klebsiella pneumonia</i> , <i>Escherichia coli</i> , <i>Enterobacter cloacae</i> , <i>Lactobacillus</i> sp., <i>Enterococcus faecium</i> , <i>Pseudomonas stutzeri</i> , <i>Staphylococcus aureus</i> , <i>Lactococcus garvieae</i> , <i>Oscillatoria willei</i> NTDMO1, <i>Ureibacillus thermosphaericus</i> , <i>Pseudomonas stutzeri</i>
nZVI	<i>Bacillus cereus</i>
ZnO	<i>Lactobacillus plantarum</i> and <i>Aeromonas hydrophila</i> , <i>Serratia ureilytica</i> , <i>Pseudomonas aeruginosa</i> , <i>B. licheniformis</i> , <i>Lactobacillus sporogens</i> , <i>Rhodococcus pyridinivorans</i> , and <i>Sphingobacterium thalpophilum</i>
CuO	<i>Phormidium cyanobacterium</i> , <i>Morganella morganii</i> , <i>Halomonas elongate</i> , <i>Serratia</i> sp., and <i>Escherichia coli</i>
TiO ₂	<i>Bacillus mycoides</i> , <i>Bacillus subtilis</i>
MnO ₂	<i>Pseudomonas putida</i> strain GB-1, <i>Pedomicrobium</i> sp., <i>Bacillus</i> spore, <i>Leptothrix discophora</i> strain SS-1, <i>Acinetobacter</i> sp.
Pd, Pt	<i>Escherichia coli</i> , <i>Pseudomonas</i> , <i>Desulfovibrio desulfuricans</i> , NCIMB 8307
AsS	<i>Shewanella</i> sp.
U, Cu, Pb, Al, Cd	<i>Bacillus sphaericus</i> JG-A12
Se	<i>Shewanella</i> sp.
Magnetic nanoparticles	<i>Magnetosirillum magneticum</i> , <i>Sulfate-reducing bacteria</i>
PbS	Bacterial strains NS2 and NS6
CdS	<i>Clostridium thermoaceticum</i> , <i>Klebsiella aerogens</i> , <i>Escherichia coli</i> , <i>Rhodobacter</i> , <i>Sphaeroides</i> , <i>Rhodobacter sphaeroides</i>
ZnS	Sulfate-reducing bacteria of the family <i>Desulfobacteriaceae</i>

TABLE 6.2 Nanoparticles synthesized by fungi and yeast.

Nanoparticle	Fungi and yeast
Ag	<i>Silver tolerant yeast strains</i> MKY3, <i>Pediococcus pentosaceus</i> , <i>Cladosporium cladosporioides</i> , <i>Coriolus versicolor</i> , <i>Fusarium semitectum</i> , <i>Fusarium oxysporum</i> , <i>Fusarium solani</i> , <i>Phaenerochaete chrysosporium</i> , <i>Aspergillus niger</i> , <i>Aspergillus flavus</i> , <i>Aspergillus nidulans</i> , <i>Aspergillus fumigatus</i> , <i>Aspergillus terreus</i> , <i>Aspergillus oryzae</i> , <i>Pleurotus sajor-caju</i> , <i>Trichoderma viride</i> , and <i>Extremophilic yeast</i>
Au	<i>Fusarium oxysporum</i> , <i>Verticillium</i> sp., <i>Trichosporon montevidense</i> , <i>Aspergillus</i> sp.
nZVI	Yeast extract
ZnO	<i>Candida albicans</i> , <i>Aspergillus</i> strain, <i>Aspergillus terreus</i> and <i>Aspergillus fumigatus</i> TFR-8, <i>Aeromonas hydrophila</i>
TiO ₂	<i>Aspergillus flavus</i>
nCeO ₂	<i>Humicola</i> sps.
Pt	<i>Fusarium oxysporum</i> , <i>Neurospora crassa</i>
CdS	<i>Fusarium oxysporum</i> , <i>Candida glabrata</i> , <i>Schizosaccharomyces pombe</i> , <i>Coriolus versicolor</i> , <i>Saccharomyces cerevisiae</i>
PbS	<i>Torilopsis</i> species <i>Rhodospiridium dibovatum</i>
Bioactive nanoparticles	<i>Lichen fungi</i> (<i>Usnea longissima</i>)

TABLE 6.3 Nanoparticles synthesized by Plant and Plant Extracts.

Nanoparticle	Plants & plant extracts
Ag	<i>Aspergillus flavus</i> , <i>Elettaria cardamom</i> , <i>Ocimum</i> sp., <i>Desmodium triflorum</i> , <i>Coriolus versicolor</i> , <i>Acalypha indica</i> leaf extract, <i>Clerodendrum inerme</i> , <i>Pelargoneum graveolens</i> , <i>Coriandrum sativum</i> , <i>Ceriops tagal</i> , <i>Parthenium hysterophorus</i> , <i>Pongamia pinnata</i> , <i>Euphorbia hirta</i> , <i>Nerium indicum</i> , <i>Azadirachta indica</i> , <i>Brassica juncea</i> , <i>Gliricidia sepium</i> , <i>Opuntia ficus indica</i> , <i>Carica papaya</i> fruit, <i>Capsicum annum</i> , <i>Avicennia marina</i> , <i>Rhizophora mucronata</i> , <i>Rumex hymenosepalus</i> , <i>Pterocarpus santalinus</i> , <i>Sonchus asper</i> <i>Jatropha curcas</i> latex, <i>Aloe vera</i> extract, <i>Magnolia kobus</i> leaf broth, <i>Medicago sativa</i> seed exudate
Au	<i>Azadirachta indica</i> , <i>Macrotyloma uniflorum</i> (Lam) Verde, <i>Camellia sinensis</i> L., <i>Terminalia catappa</i> , <i>Amaranthus spinosus</i> , <i>Mucuna pruriens</i> , <i>Medicago sativa</i> , <i>Magnolia kobus</i> , <i>Dyopiros kaki</i> , <i>Cinnamomum zeylanicum</i> , <i>Allium cepa</i> L., <i>A. Juss.</i> , <i>Chenopodium album</i> L., <i>Justicia gendarussa</i> L., <i>Mentha piperita</i> L., <i>Syzygium aromaticum</i> (L), <i>Mirabilis jalapa</i> L., <i>Terminalia catappa</i> L., <i>Cymbopogon flexuosus</i> extract, Live Alfalfa plants, Banana peel
Au and Ag	<i>Coriandrum sativum</i> , <i>Pelargonium graveolens</i> , <i>Embllica officinalis</i> , <i>Phyllanthium</i> , <i>Hibiscus rosa sinensis</i> , <i>Diopyros kaki</i> (Persimmon), <i>Citrus sinensis</i> , Mushroom extract
nZVI	Apple, passion fruit, avocado, kiwi, eucalyptus, mulberry, lemon, medlar, apricot, mandarin, oak, peach, olive, pear, pine, orange, pomegranate, cherry, plum, raspberry, quince, strawberry, vine, tea-green & black, citrine juice wastes, oak leaves extract and walnut leaf extract
CuO	<i>Hylotelephium telephium</i> , <i>Kalopanax pictus</i> Leaf 5 <i>Pterolobium hexapetalum</i> , <i>Coriandrum sativum</i> L, Oak Fruit, <i>Albizia lebbbeck</i> , <i>Eichhornia crassipes</i> <i>Citrofortunella microcarpa</i> , <i>Eupatorium odoratum</i> , <i>Verbascum thapsus</i> , <i>Euphorbia pulcherrima</i> , <i>Acanthospermum hispidum</i> , <i>Sida Rhombifolia</i> , <i>Seidlitzia rosmarinus</i> Bark ashes, <i>Enicostemma axillare</i> (Lam.), <i>Terminalia catappa</i> L., <i>Punica granatum</i> Fruits Peel O. <i>Cochinchinense</i> <i>Rosa canina</i> , <i>Aloe barbadensis</i> , and <i>Sambucus nigra</i>
ZnO	Extract of <i>Aloe vera</i> , <i>Cassia auriculata</i> flower extract, green tea, the leaf extract of <i>Hibiscus rosa-sinesis</i>
CeO ₂	<i>Aloe barbadensis</i> gel, extract of <i>Gloriosa superba</i> leaf
Si-Ge	Freshwater diatom <i>Stauroneis</i> sp.
Pt	<i>Ocimum sanctum</i> L, <i>Diopyros kaki</i>
Pd	Soybean (<i>Glycine Max</i>) L. <i>Cinnamomum zeylanicum</i> Blume., <i>Gardenia jasminoides</i> Ellis., <i>Cinnamomum camphora</i> L., <i>Arabidopsis thaliana</i> .
Pb	<i>Jatropha curcas</i> L, <i>Vitis vinifera</i> L.
Ag, Ni, Co, Zn, Cu	<i>Brassica juncea</i> , <i>Medicago sativa</i> , and <i>Helianthus annuus</i>
CdS	Starch
Magnetic	<i>Aloe vera</i>

6.2.4 Advantages and applications of biogenic nanostructures

Some of the pronounced benefits of biologically produced nanostructures are reduced ecotoxicity, ease of synthesis, reduced contamination from by-products, rapid rate of manufacture, cost-effectiveness, and biocompatibility (Singh et al., 2016). Despite the benefits of the biological methodology of nanostructure synthesis, it is important to streamline processes to produce NPs of uniform size, stability, and capability while preventing aggregation. The important factors that should be taken into account are the choice of the most suited organism for NP synthesis, optimization of the reaction conditions for maximal enzyme activity and cell growth, standardization of the downstream process, and last but not the least the scaling up of the entire process to be able to manufacture NPs at an industrial scale (Irvani, 2019).

Since biogenically produced nanostructures have a wider scope of biomedical functions in comparison to their physicochemically produced counterparts mainly due to reduced toxicity and higher biocompatibility they are utilized for drug delivery, diagnostic tools for detection of drug molecules, bioimaging, antimicrobial and anticancer agents as well as in cosmetic and medical devices (Singh et al., 2016). Environmental remediation is another beneficial application of biological nanoparticles. They are already being widely used to counter groundwater and soil pollution. Some magnetic NPs such as zero valent iron (nZVIs) synthesized from *Camellia sinensis* extract, magnetite (Fe₃O₄) produced

from food waste like silky hair of corn (*Zea mays*), and outer leaves of *Brassica rapa* and maghemite (γ -Fe₂O₃) from extracts of oolong, green and black tea have shown great potential in the elimination of pollutants from soil (Iravani, 2019). The role played by nanostructures produced by microorganisms in soil remediation is discussed below in detail.

6.3 Environmental bioremediation

Nanostructures exhibit a broad spectrum of applications and can be extremely valuable in agricultural, biomedical, and remediation fields if they are biogenically produced. They are an important component of environmental remediation processes and are employed in limiting the damage caused by a variety of contaminants in the air, water, and land. Biogenically produced nanostructures are particularly important in reclaiming polluted soils since they are non-hazardous and eco-friendly along with being highly effective (Wang et al., 2019).

6.3.1 Soil pollution and bioremediation

Soil pollution is defined as the “existence of any chemical or substance out of place and/or present at a higher than the normal concentration that has adverse effects on any non-targeted organism” (Rodríguez Eugenio et al., 2018). It is caused by both natural and anthropogenic causes. Some of the natural causes of soil contamination include radionuclides, heavy metals like arsenic, chromium, cadmium, mercury, and lead, volcanic eruptions, forest fires, polycyclic aromatic hydrocarbons, naturally occurring asbestos, etc. Anthropogenic causes include chemicals from industries, domestic and municipal waste, agrochemicals, oil spills, untreated wastewater, semi-volatiles, volatiles, hydrocarbons, cyanides, leaching from landfills, sewage sludge, e-waste, etc. (Cachada et al., 2018; Rodríguez Eugenio et al., 2018; Sarkar et al., 2019). Industrial activities also degrade the soil by causing salination (Saha et al., 2017). Various man-made activities such as agriculture, urban development, deforestation, mining, transport, acid rain, and plastics also contribute significantly to the pollution of soil and water (Cachada et al., 2018; Havugimana et al., 2015). Various physical methods like air sparging, soil washing, thermal methods, electrokinetic techniques, phytoremediation, natural methods, chemical and bioremediation mechanisms can be applied for soil protection (Havugimana et al., 2015; Sarkar et al., 2019). These are chosen based on local conditions, their impact on other life forms in the vicinity, and the degree of deterioration of the soil along with other socioeconomic considerations like the cost of soil restoration (Cachada et al., 2018). Of the different remediation strategies available, bioremediation is far more sustainable and eco-friendly since microorganisms are naturally available and capable of taking up pollutants and employing them as energy and biomass sources leading to their elimination or conversion (Abatenh et al., 2017).

Bioremediation involves the adoption of biological operations like the application of growth stimulants, enzymes, aerobic and anaerobic bacteria, plants, and fungi for the process of removing impurities from a system via conversion, degradation, mobilizing, transforming, and sequestering contaminants present in the air, water or soil (Abatenh et al., 2017). It aims to make the environment less toxic and restore as closely as possible to its original state. The main proponents of bioremediation are bacteria, fungi, and archaea that act as biocatalysts via various enzymatic pathways. They can function well as mediators if they can exploit the substrate for their own multiplication needs. Apart from the existence of the appropriate microbe in the polluted environment, several other factors like temperature, pH, nutrients, oxygen, electron acceptors, and soil type along with the chemical composition of the pollutants are major determinants in the efficacy of the bioremediation process (Abatenh et al., 2017). The use of bioremediation processes becomes constrained in case of mixed wastes, low versatility, or low bioavailability of the microbe along with its inability to completely degrade a pollutant or a long timeline required for degradation (Dangi et al., 2019).

6.3.2 Bioremediation by engineered nanostructures

Even though bioremediation is an excellent choice for sustainable pollution control and helps restore the natural state of a degraded site, it is not suitable for a large number of contaminants and cannot be used for immediate application due to its long treatment time. These issues can be solved by using nanostructures instead of microorganisms for cleaning contaminated sites and a wide range of nanostructures are already being utilized for remediating soils.

Engineered nanostructures have many properties that are advantageous in soil remediation. They are broadly adopted for immobilization of inorganic and organic pollutants in the soil matrix or some like metal oxide nanostructures and nanocomposites use surface complexation to immobilize heavy metals and organic contaminants (Qian et al., 2020). Mostly they can act against many common soil pollutants like organic contaminants such as polycyclic aromatic hydrocarbons (PAHs), pesticides, poly-fluoroalkyl substances (PFASs), polychlorinated biphenyls (PCBs), heavy metals

like As, Cr, Pb, Zn, etc. and some inorganic contaminants (Iavicoli et al., 2017). Some of the commonly used engineered nanostructures are (Rizwan et al., 2014; Sarkar et al., 2019):

1. Nanoscale zero valent iron.
2. Nanoscale metal oxides.
3. Nanoscale calcium peroxide.
4. Carbon nanotubes (single or double-walled).
5. Bionanoparticles (virus, plasmids, proteins, etc.).
6. Polymeric nanoparticles.
7. Dendrimers (PAMAM).
8. Nanocomposites.

Nanostructures provide several beneficial ways to carry out soil remediation but the harmful chemicals associated with their synthesis are a deterrent to their widespread use. It is therefore imperative to employ eco-friendly biological means of nanoparticle synthesis like microorganisms and plant parts and extracts. Some common examples of the successful synthesis of nanostructures by green mechanisms include green tea extract to manufacture nanoscale zero valent iron and the fruit of *Terminalia Chebula* to produce gold, silver, iron, and palladium nanoparticles that can diminish Cr(VI) in soil (Wang et al., 2019).

6.3.3 Bioremediation by microbial nanostructures (nanobioremediation)

Nanobioremediation involves remediation by the use of nanostructures produced by biogenic means such as fungi, yeast, actinomycetes, bacteria, algae, plant parts, and plant extracts. The manufacturing of nanostructures using the abovementioned biological means offers several advantages like less toxicity, reduced cost, improved stability, faster manufacturing process with greater control on the type and variety of nanostructures along with better application in agriculture and biomedical fields (Ovais et al., 2018; Yadav et al., 2017). Microbial synthesis of nanostructures is beneficial since it employs non-toxic natural reagents and utilizes less energy. Such biogenically produced NPs have been shown to have positive effects on soil remediation and include examples like Fe₃O₄, Carbon-nZVI, Fe (clay supported) and are used to treat soils polluted with heavy metals such as Ni and Cr(VI). nZVI is also used to treat pharmaceutical contaminants in soil like Ibuprofen (Wang et al., 2019).

A Detailed description of the abovementioned biogenic nanoparticles (Table 6.4) and their role in soil remediation is given below.

TABLE 6.4 Commonly used biogenic nanoparticles for soil remediation.

S. no.	Nanoparticle	Soil contaminant
(i)	Nanoscale zero valent iron (nZVI)	Nitro explosives, polycyclic aromatic hydrocarbons (PAHs), heavy metals, certain macronutrients and halogenated organic compounds
(ii)	Copper oxide (CuO)	Organic pollutants, dyes like methyl orange and acid blue 62
(iii)	Zinc oxide (ZnO)	Nitro chlorobenzene, variety of dyes such as acidic red B, methyl orange, acidic black 234 and methylene blue
(iv)	Titanium oxide (TiO ₂)	Dyes such as Acid Orange7, Reactive Orange16, Malachite Green, direct red 23, methylene blue, acidic black 234, acidic red B and azo dyes, polymers such as PVC/PS, toxic metal ions, NO _x , aromatics, seawater crude oil, toluene, dichlorobenzene, benzene, organic pollutants, Nitrobenzene, Phenol, Procion Red MX-5B, Rhodamine B, and Parathion in agricultural soil
(v)	Cerium oxide (CeO ₂)	Heavy metals such as cadmium (Cd), arsenic (As), and lead (Pb)
(vi)	Manganese oxide (MnO ₂)	Toxic metal cations such as Mn, Zn, Pb, Co, Cu, Ni, and Cd and organic compounds like humic acids
(vii)	Gold (Au) and silver (Ag)	Dyes such as methyl orange, direct red 23 and chlorobenzene
(viii)	Palladium (Pd)	Azo dyes, polychlorinated dioxins and nitrate
(ix)	Lead sulfide (PbS)	Dyes like Malachite green
(x)	Cadmium sulfide (CdS)	Toxic metals

6.3.3.1 Nanoscale zero valent iron

Nanoscale zero valent iron (nZVI) is a manufactured NS that can also be synthesized using green technology employing microbes or extracts from various plants and yeast. It is constituted from a core of zero valent or metallic iron formed by reducing Fe^{2+} or Fe^{3+} and an oxide shell produced by oxidation of iron (Crespo et al., 2017; Li et al., 2021; Pasinszki and Krebsz, 2020). It can be easily introduced into contaminated soil in a variety of ways such as in the form of slurry produced with water and injected using nitrogen gas or by creating an emulsion with vegetable oil and water (Libralato et al., 2017). Zero valent iron (ZVI) has been profitably applied since 1996 to eliminate contaminants such as nitro explosives, polycyclic aromatic hydrocarbons (PAHs), heavy metals, certain macronutrients, and halogenated organic compounds from soil and water but recent studies show that the nanosize of ZVI or nZVI makes it more reactive against organic contaminants and heavy metals (Cecchin et al., 2017). nZVI has a reduced size that confers it with a large surface-to-volume ratio therefore a larger effective surface and a better rate of diffusion but lower transportability and aggregation. Capping, stabilization with the help of surfactants or macromolecules, sulfidation, and noble or transition metal doping can be used to improve the properties of nZVI even though each of these options has its drawbacks and reduce the efficacy of the NS for environmental remediation (Li et al., 2017). The ability of nZVI to remove contaminants from the soil is less than in water sources because of lower reactivity, slower diffusion rates, and more complex reactions. Factors like pH, temperature, and the presence of various soil ingredients such as minerals, organic matter, and moisture content play an important role in altering the rate of its reaction (Li et al., 2021).

1 Application of nanoscale zero valent iron for soil remediation

The use of nZVI is a preferable method for soil remediation due to its better reactivity, inferior cost, greater surface-to-volume ratio, and lower toxicity. The iron core of nZVI acts as an electron donor due to its slow oxidation to ferrous and these electrons react with a variety of contaminants leading to their transformation. To successfully apply it for remediation purposes it is important to prevent its destabilization due to aggregation and oxidation. Chemical modifications to synthesize bimetallic NPs by doping with metals like Cu, Ag, Ni, Pd & Pt (Tian et al., 2020), coating with different surfactants (nonionic, anionic, and cationic) (Tian et al., 2020), different macromolecules like carboxymethyl cellulose (CMC) (He et al., 2010) and biochar (BC) (Wang et al., 2019) and sulfidation to form S-nZVI (Guan et al., 2019) help to increase not only the stability of nZVI but also its reactivity and electrical conductivity. These properties enable controlling the electron transport capacity, dispersion, corrosion, and particle magnitude of nZVI (Wang et al., 2019). nZVI can directly carry out the removal of organic pollutants such as atrazine and molinate which are herbicides and chlorpyrifos which is a pesticide from soil (Rathoure, 2018). Some of the modifications of nZVI that are carried out to improve its employment for soil remediation are:

1. nZVI assisted by electrokinetics:

Employment of nZVI assisted by electrokinetics via permeable reactive barrier (PRB) to tetrachloroethene (PCE) contaminated soil removed 90% of it after 10 days of treatment while 83% of polychlorinated biphenyls (PCB) was removed from polluted soil as shown in a study (Gomes et al., 2015; Li et al., 2021). EK-PRB-nZVI technology aids in remediation by providing direct current, more electron sources, electrolysis of water, and oxidation of nZVI. It also brings about the agglomeration of the pollutant through its electric field thereby assisting nZVI action. Moreover, the nZVI precipitates are removed by electrophoresis to sustain their activity (Li et al., 2021). UV light and ultrasound can also be utilized along with nZVI to improve its reactivity. Ultrasound worked by breaking down the NP size and increasing the surface area. This was particularly useful for the removal of organic pollutants such as methyl orange and metronidazole (Yuan et al., 2016). UV light-assisted nZVI technology mostly occurs under aerobic circumstances. Even though beneficial, both UV and ultrasound have limited applications due to the greater energy requirement and operating noise considerations (Li et al., 2021).

2. Stabilized nZVI-coated, bimetallic, and sulfidated nZVI:

Stabilization of nZVI whether by coating with various macromolecules, surfactants, or the addition of sulfate improves its stability, mobility, and reactivity. The use of bimetallic NP exhibits better performance in comparison to nZVI and aids in better removal of soil pollutants like 2,2',4,5,5'-pentachlorobiphenyl and PCB. Studies show better results for nZVI and Pd/Fe in comparison to nZVI alone. Some of the bimetallic NPs that can be used for soil remediation are Fe/Cu, Fe/Pt, Fe/Pd, and Fe/Ni. Despite their better applicability, their large-scale use is limited due to their higher cost and lower mobility in the soil caused by their oxidation, aggregation, and interaction with various soil components. They have also been shown to be toxic to microorganisms and can cause cell death (Li et al., 2021).

Sulfidated nZVI, on the other hand, offers better outputs, under aerobic conditions it oxidizes some organic pollutants by functioning as an effective Fenton-like catalyst, and under anaerobic conditions, it acts on organic pollutants through its role as a reductant (Dong et al., 2019; Xu et al., 2020). It is also less expensive in comparison to bimetallic NPs and shows better longevity and mobility due to decreased agglomeration (Su et al., 2020; Vogel et al., 2019). Coating nZVI with surfactants (Tian et al., 2020) and different macromolecules like carboxymethyl cellulose (CMC) (He et al., 2010) and biochar (BC) (Wang et al., 2019), starch (He and Zhao, 2005), etc. cause stabilization of nZVI that exhibits better ability to remove TCE and PCB from contaminated soils at a much faster rate. Such nZVI particles also exhibit much lower toxicity to soil microorganisms. The surface coating also prevents the NPs from aggregating in the medium and helps maintain proper size, reactivity, and mobility within the soil matrix. Stabilized nZVI, therefore, has enhanced application in soil remediation and facilitates dechlorination reactions that are short-term abiotic and long-term biotic (Nunez Garcia et al., 2020). Despite these advantages, it is imperative to apply stabilized NPs in the optimal concentration to avoid environmental and microbial toxicity and attain maximum efficiency with minimal disturbance to the soil ecosystem.

3. nZVI integration with microbes:

nZVI stimulates anaerobic microbial growth of sulfate-reducing bacteria, organohalide-respiring bacteria, and iron reducing bacteria thereby improving long-term biotic degradation of organic halides and heavy metals and reduction of the iron (hydr)oxides (Dong et al., 2019). For the integration of nZVI and anaerobic bacteria to work well, nZVI species and dosage are important criteria to prevent cytotoxicity of nZVI. Moreover, nZVI corrosion provides electrons to hydrogenotrophic bacteria and improves the removal of pollutants like TCE (Honetschlägerová et al., 2018). The collaboration of nZVI and bacteria aids in soil bioremediation by creating favorable conditions such as lowered stability of organohalides, reducing the redox potential of the dehalogenation setting, shortening of the lag phase of PCB microbial dechlorination, and detoxification of chlorinated aliphatic hydrocarbons (CAHs) to completely degrade pollutants into a non-toxic mix of acetylene, ethane, and ethene (Li et al., 2021; Libralato et al., 2017). CMC-stabilized nZVI injection at contaminated field sites not only increased certain microbial populations such as *Dehalococcoides* and *Dehalogenimonas* spp but also reduced the corrosion rate and cytotoxicity of nZVI (Li et al., 2021).

2 Risks associated with nanoscale zero valent iron use for remediation

Even though the role of nZVI in organics-contaminated soil remediation is extremely promising, a variety of associated issues need to be addressed. Pure nZVI is incapable of migrating more than a few centimeters in soil mainly due to interaction with the surrounding environment and self-agglomeration. The stabilization of nZVI with various kinds of coatings and surfactants improves its migration capabilities, removing it from the remediation site and its gradual transformation into iron minerals can exert toxic effects (Libralato et al., 2017). nZVI is capable of coupling with microorganisms in the soil and in-depth studies are needed to evaluate its long-term impacts on microorganisms. It can cause ecotoxicity by altering the permeability of the biological membrane, creation of oxidative stress, or by associating with the cellular components. Increasing nZVI concentration results in a subsequent increase in Fe^{2+} toxicity to cells. It has also been shown to alter the photosynthetic ability of cyanobacteria due to Fe and Fe (III) hydroxide layering on cells (Libralato et al., 2017). Most in vitro studies carried out on the effects of nZVI exhibit that it alters the integrity, activity, and viability of bacterial cells. Gram-positive bacteria have been shown to get more adversely affected in contrast to Gram-negative bacteria mainly because of differences in their cell wall architecture which in turn influences the absorption of nZVI (Xue et al., 2018).

In fungi, the presence of a chitinous cell wall possibly makes it resistant to nZVI (Xue et al., 2018). The influence of nZVI on soil biota becomes an important criterion if it is to be widely used for soil remediation. A study carried out on macrophytes such as *Sinapis alba*, *Lepidium sativum*, and *Sorghum saccharum* did not report any significant effect of nZVI (Libralato et al., 2016). In general, it was seen that the toxicity associated with nZVI is due to its ability to create Fe^{2+} and reactive oxygen species (ROS) damaging both intracellular structures and membranes of the cell resulting in its death. Fe^{2+} released by nZVI can be internalized by living cells, where it reacts with hydrogen peroxide synthesized by the mitochondria to give rise to ROS (such as OH, O_2 , or FeO^{2+}) further damaging the cell's macromolecules.

Several studies have even shown that at greater concentrations and longer exposure times, the toxicity of nZVI decreases (Xue et al., 2018). Applying polymer coating such as CMC etc. on nZVI also decreases its toxicity due to lower adhesion with the bacterial wall and reduced oxidative stress response while the use of Ni and Ag with nZVI increased its toxicity. Elevated organic matter levels in the soil cause nZVI to inhibit microorganisms more effectively. Overall, nZVI toxicity in soil was controlled by parameters like type and quantity of organic matter in the soil and the

stabilizer utilized (Xue et al., 2018). nZVI is known to cause toxicity to live cells as mentioned above in the case of various soil organisms. Therefore there is a strong possibility that it may be exerting a similar effect on human cells. The limited studies that have explored the impact of nZVI on human cells indicate the ability of inhaled and ingested NS to cross biological membranes and be easily taken up by cells leading to damaged organs and tissues (Gornati et al., 2016). Human bronchial epithelial cells in phosphate-buffered saline (PBS) solution are vulnerable to nZVI exposure and exhibit oxidative damage leading to lung irritation (Keenan et al., 2009). Other *in vitro* experiments show that exposing cells to nZVI could cause neurotoxicity and increase apoptosis via various mechanisms (Libralato et al., 2017). It is therefore critical to weigh the hazards of nZVI exposure not only in the environment and to soil organisms but also on human health before focusing on the benefits of using nZVI for soil remediation.

ii Metal oxide nanostructures

Metal oxide nanostructures of transitional elements like Cu, Ti, Fe, and Ni have a broad range of functions in nanotechnology as they exhibit strong adsorption capabilities that are highly effective in various biomedical and environmental remediation fields. Green synthesis of CuO, ZnO and TiO₂ is done by employing plant extracts, bacteria, and fungi (Panhwar et al., 2020). The metal oxide nanostructures exhibit exceptional properties like antioxidants, antibacterial, anticancer, and antiviral agents (Panhwar et al., 2020).

3 Copper oxide (CuO) nanostructures

Biogenic synthesis of CuO NS is possible by various bacteria and plant-mediated routes as mentioned in Tables 6.1 and 6.3. Biogenic CuO NPs are antibacterial, anticancer, antioxidant along with being good photocatalytic agents, drug delivery agents, and useful in effluent treatment (Akintelu et al., 2020). They are also used in semiconductor devices and as industrial catalysts (Rajput et al., 2020). These nanostructures are important in soil remediation primarily due to their ability to degrade organic pollutants and dyes like methyl orange and acid blue 62 (Shan et al., 2009; Shi et al., 2018).

4 Risks associated with CuO NP use for remediation

In the soil, CuO NPs can break down quite rapidly particularly if the pH is acidic and releases ions from the inner core. These metal ions react with organic matter, microorganisms, and charged minerals forming stable complexes over time leading to toxicity in microorganisms and increased soil pH. The presence of organic matter in the soil reduces toxicity by the coating of the metal ions and limiting their uptake by microorganisms (Shi et al., 2018). In plants, copper is vital for mitochondrial respiration, electron transport, cell wall synthesis, and hormone signaling. Overexposure to copper released from biologically produced CuO NPs induces toxicity to plants by increased ROS production thereby altering the cells' redox mechanisms (Chung et al., 2019). CuO NPs can easily enter human cells via the food chain or by inhalation and cause toxicity and destruction of cell organelles such as lysosomes and mitochondria. Studies on a variety of human cell lines have shown their ability to induce ROS leading to oxidative stress and other cytotoxic influences (Rajput et al., 2020).

5 Zinc oxide (ZnO) nanostructures

ZnO NPs can be easily prepared by various green methods employing a variety of plant extracts, fungi, yeast, algae, and bacteria. Some of the commonly used microbes (bacteria and fungi) that are utilized for microbial synthesis of ZnO NPs of various shapes and sizes are listed in Tables 6.1 and 6.2. ZnO nanoparticles have strong antimicrobial, anticancer, antifungal, anticorrosive, antiallergic, biosensor, and photocatalytic properties that are widely used in pharmaceuticals, cosmetic and restorative fields (Ngoepe et al., 2018; Rahman et al., 2021). ZnO nanostructures are also used for environmental remediation of pollutants such as nitro chlorobenzene and various dyes such as acidic red B, methyl orange, and acidic black 234 (Shan et al., 2009). They are also widely employed to carry out the degradation of industrial dyes such as methylene blue (Chen et al., 2019).

6 Risks associated with ZnO-NP use for remediation

In the case of ZnO NPs, the risks associated with their use depends upon their shape & size. They can get easily adsorbed on soil particles and undergo degradation or get transported into groundwater resources. The amount of organic matter, pH, soil texture, and type of soil microorganisms majorly influence the impact of NPs on soil communities. Since ZnO NS have an affinity for soil colloids, they can attach to them thereby exhibiting lowered mobility and higher sorption in comparison to ionic Zn²⁺. Acidic soils have been shown to exhibit greater ZnO-NP toxicity and

several studies clearly show that toxicity is exhibited by microbes, plants, and animals including humans to varying degrees based on time and concentration of ZnO-NP exposure (Rajput et al., 2020, 2018). Certain plants such as *Glycin max* (L.) Merr. can accumulate ZnO NPs when exposed to it in amounts ≥ 500 mg/kg which prevents it from seed production and reduces total chlorophyll levels in inverse proportion to ZnO-NP levels (Zhu et al., 2019).

7 Titanium oxide (TiO₂) nanostructures

TiO₂ NPs exhibit high antimicrobial, UV absorption, and high photocatalytic activity. These nanoparticles can degrade widely used dyes such as Acid Orange7 and Reactive Orange16 while Sn⁴⁺-doped TiO₂ nanostructures are utilized for degrading Malachite Green dye (Sayilkan et al., 2008). They can be used to reduce white pollution caused by photocatalytic degradation of polymers such as PVC/PS in landfills (Shan et al., 2009). TiO₂ nanostructures are also applied for control of environmental pollution caused by toxic metal ions, NO_x, aromatics, seawater crude oil, and chemicals such as toluene, dichlorobenzene, and benzene as well as dyes like direct red 23, methylene blue, acidic black 234, acidic red B and azo dyes (Koul and Taak, 2018; Shan et al., 2009). TiO₂ nanoparticles can help in the remediation of various organic pollutants commonly found in agricultural soil (Koul and Taak, 2018). Some of the other contaminants that can be removed or degraded by TiO₂ and its various modified forms (Arginine-modified TiO₂, Anatase TiO₂, TiO₂/SrFe₂O₉ composite, Mesoporous TiO₂ nanopowder₃, Rutile TiO₂ nanoparticle, and TiO₂ nanoparticle and carbonized composite) include Nitrobenzene, Phenol, Procion Red MX-5B, Rhodamine B, Parathion, etc. from soil (Koul and Taak, 2018).

8 Risks associated with TiO₂ NP use for remediation

TiO₂ nanostructures have been shown to alter the toxicity of pollutants such as Cu in soil. The influence of the nanostructures on the toxicity of other pollutants shows a dependence on the amount of organic content of the soil being treated. Organic matter in the form of humic acid reduced both the remedial ability and the adsorption capability of TiO₂ nanoparticles leaving greater amounts of heavy metals contaminants like Cu free in the soil (Medina-Pérez et al., 2019).

9 Cerium oxide (CeO₂) nanoparticles

Cerium oxide nanoparticles can be synthesized biogenically by the fungus *Humicola* sps and from a variety of plant extracts as listed in Tables 6.2 and 6.3. They show antimicrobial properties and can be used for disease treatment via ROS production (Hussain et al., 2016). For remediation purposes, they are helpful in the removal of pollutants like heavy metals such as cadmium (Cd), arsenic (As), and lead (Pb) from the soil. Interactions between the nanostructures and the contaminants depend on various factors such as ionic strength, pH, or the amount of organic matter present in the soil (Duncan and Owens, 2019).

10 Risks associated with CeO₂ NP use for remediation

Improved plant growth, higher biomass, and better chlorophyll levels are seen with CeO₂ NP exposure in low amounts in *Triticum aestivum* L. but at higher levels, it can cause leaf necrosis and affect grain protein levels. They can also lengthen the harvest period of *Triticum aestivum* L. and harm the leaf biomass in *Glycin max* (L.) Merr. (Zhu et al., 2019).

11 Manganese oxide (MnO₂) nanostructures

Biogenic synthesis of MnO₂ nanostructures is possible by bacterial strains mentioned in Table 6.1. (Hosseinkhani and Emtiazi, 2011) have also shown an eco-friendly biosynthetic mechanism by *Acinetobacter* sp. Nanostructures of MnO₂ have wide applications as high-density magnetic storage media, batteries, catalysts, and in environmental remediation particularly of metal cations such as Mn, Zn, Pb, Co, Cu, Ni, and Cd and organic compounds like humic acids (Hosseinkhani and Emtiazi, 2011). The biogenically produced nanostructures exhibit higher sorption affinity for the toxic metal cations particularly for the soil contaminant Pb²⁺ over their synthetic counterparts (Villalobos et al., 2005). MnO₂ nanostructures also display environmental compatibility and are also naturally available (Hosseinkhani and Emtiazi, 2011).

12 Risks associated with MnO₂ NP use for remediation

High amounts of MnO₂ Nanostructures (400 mg/g VSS) exhibited inhibitory effects on propionate and butyrate degradation in microbes and reduced microbial manganese redox process during anaerobic digestion leading to abundant ROS production causing cell death (Tian et al., 2019).

iii Gold (Au) and silver (Ag) nanoparticles

Au and Ag NPs can be synthesized from cell-free extracts from various bacteria and fungi as mentioned in Tables 6.1 and 6.2. Both types of nanoparticles have applications in dentistry, biosensing, drug delivery and exhibit antimicrobial activity (Gour and Jain, 2019). *Volvariella volvacea* (fungus) can produce both Au and Ag NPs (Hussain et al., 2016). Biogenically produced AgNPs exhibit substantial antimicrobial action as compared to synthetically produced ones (Hussain et al., 2016; Ovais et al., 2018). They are also capable of environmental remediation by degradation of pollutants of dyes such as methyl orange, direct red 23, chlorobenzene, etc. (Shan et al., 2009). Composites of gold nanoparticles and mesoporous silica provide an enzyme immobilization matrix that offers better conductivity and biocompatibility along with a large surface area that aids in the functioning of the enzymes used in the bioconversion process for remediation (Shan et al., 2009).

13 Risks associated with Au and Ag NP use for remediation

Exposure to silver NPs caused a range of adverse effects on plants, microbes, and animals in the soil. Reduced biomass was seen in Rice (*O. sativa*), tomatoes, and basil plants along with a change in carotenoid and chlorophyll levels in rice decreased root length in tomatoes and accumulation of the NS in various plant parts like the leaf, stem, and root of basil. Depending on soil type and amount of exposure, earthworms showed either no effects or a reduction in cell viability, loss of weight, DNA damage, and lowered cell viability. In the case of microorganisms like *Escherichia coli* and *Bacillus subtilis*, the effects were dose-dependent and mostly consisted of cell membrane disruption and growth inhibition (Liu et al., 2019). There are opposing descriptions of the toxic influence of AuNPs but mostly they are shown to have high biocompatibility and inertness but a few studies do indicate instances of organ toxicity from AuNPs in animals such as rats (Patil and Chandrasekaran, 2020).

iv Other nanostructures with soil remediation properties

14 Palladium (Pd) nanostructures

Palladium nanostructures exhibit an affinity for H₂ and have catalytic properties therefore they are useful in the degradation of azo dyes used in the textile industry and dehalogenation reactions of polychlorinated dioxins that eventually make their way into soil and water resources (Pantidos, 2014; Wang et al., 2018). For the remediation of nitrate polluted water, nanoscale palladium is used in combination with iron and copper. The bimetallic nanoparticles help to convert nitrate to nitrite and ammonium with palladium aiding in the reduction of nitrite to non-toxic nitrogen gas (Tyagi et al., 2018).

15 Risks associated with Pd NP use for remediation

Leaves of Barley exhibited bioaccumulation of Pd NPs and kiwi fruit pollen morphology was modified resulting in plasma membrane damage and loss of calcium from them. Studies also showed that cytotoxic effects were shown by various human and animal cells upon exposure to palladium nanoparticles (Leso and Iavicoli, 2018).

16 Lead sulfide (PbS) nanoparticles

PbS nanoparticles have been successfully produced by using bacterial strains NS2 and NS6 by extracellular synthesis and exhibit photocatalytic absorption against Malachite green and antimicrobial activity against *S. aureus* (Hussain et al., 2016).

17 Risks associated with PbS NP use for remediation

In maize, PbS NPs have been demonstrated by (Ullah et al., 2020) to enter via the roots and bioaccumulate in the shoot where they lead to biomass reduction. They also exert toxic influences on seed germination and root elongation in maize. There is also a possibility that lead from PbS NPs accumulated in crops such as maize can enter the food chain and affect other organisms including humans (Mielke et al., 2019). Other detrimental impacts of PbS NPs are displayed by green algae *Dunaliella salina*, wherein they cause a reduction in the number of carotenoids and greater lipid peroxidation due to agglomeration of the NPs with the algal cells (Ubaid et al., 2020).

18 Cadmium sulfide (CdS) nanoparticles

Cadmium sulfide nanoparticles were produced by [Sanghi and Verma in 2009](#) using *Coriolus versicolor*, a fungus that successfully converts Cadmium to CdS nanoparticles without the utilization of any external source of sulfur. *Rhodobacter sphaeroides*, *Lactobacillus* sp., and *Saccharomyces cerevisiae* can also synthesize CdS nanoparticles. The Biogenic CdS nanoparticles exhibit outstanding ability to degrade toxic metals from soil ([Hussain et al., 2016](#)).

19 Risks associated with CdS NP use for remediation

The exposure of soil to CdS NPs results in size-dependent cytotoxicity in plants with NPs of the size <5 nm being more toxic than larger sized ones. The effects include inhibition of plant shoot and root growth. Moreover, the accumulation of CdS NP in the plant root can also lead to oxidative stress. Microorganisms such as *E. coli* and cell lines like HeLa cells were also susceptible to the toxic effects of the NP on cell division and growth ([Ubaid et al., 2020](#)).

6.4 Conclusion

A wide range of metal, metal oxide, and metal sulfide nanostructures are being synthesized successfully by using plants, plant extracts, and various microorganisms. In comparison to the use of fungi, yeast, bacteria, and algae, a higher number of studies and methodologies are focused on the biological synthesis of nanostructures from plants and plant extracts but the microbial synthesis of nanostructures is also fast gaining momentum. Biogenically produced nanostructures have a wider range of employment as antimicrobial, anticancer agents, in biomedicine, and environmental remediation among others. These nanostructures are particularly suited for bioremediation mostly due to their high photocatalytic and adsorption capabilities. Despite their immense contribution against soil pollution it is imperative to be aware of their long-term effects on soil and its resident communities. Recent studies in the field of nanobioremediation have emphasized their toxicological implications and have helped shift the spotlight to a more conservative approach. Using nanostructures in appropriate amounts with known threshold limits, availability of exhaustive studies on their influence on soil flora and fauna, and their movement through the food chain are some of the important criteria that need to be addressed before they can be widely utilized for soil remediation. In lower amounts, most biogenic nanostructures are known to be positive influencers on plant growth and photosynthesis but in greater amounts, most of them are now known to exert genotoxic and cytotoxic effects. If the maximum benefit is to be derived from their employment in soil remediation, safety evaluation and toxicological risk assessment studies are crucial for their safe and eco-friendly application. Since it is a relatively new field with extensive possibilities, having verifiable data on the long-term consequences of biogenic nanostructures utilization on both environment and organisms would help in creating awareness and understanding of all potential risks associated with their future adoption for safe soil remediation practices.

List of abbreviations

NS	nanostructures
NPs	nanoparticles
ENM	engineered nano material
nZVI	nano zero valent iron
PAH	polycyclic aromatic hydrocarbon pesticides
CMC	carboxymethyl cellulose
BC	biochar
PRB	permeable reactive barrier
PCE	tetrachloroethene
CAH	chlorinated aliphatic hydrocarbons
PCB	polychlorinated biphenyls
PFAS	poly-fluoroalkyl substances
ROS	reactive oxygen species
PVC	poly vinyl chloride
PS	polystyrene
VSS	volatile suspended solids

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Declarations

All tables are self-made.

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Green biosynthesis of nanoparticles: mechanistic aspects and applications

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7.1 Introduction

Nanotechnology is a cutting-edge technology that has achieved remarkable position in recent years owing to its exclusive properties and extensive applications in multiple sectors. Researchers have been observed to be oriented towards nanoparticles (NPs) and its novel synthesis because of emerging demand (Maurer-Jones et al., 2013). In present day scenario, novel NPs are essential for environmental applications, therefore considered as chief stays in budding scientific interventions (Wu et al., 2019). The presence of large surface area, pore size, short inter-particle diffusion, more adsorption sites, surface chemistry and optimal temperature requirements make NPs a marvelous absorbents as well as catalysts (Gong et al., 2018). Nanomaterials in the range of 1–1000 nm are generated with attractive shapes and applications (Arshad, 2017). Although, less than 1000 nm size is considered appropriate for industrial and biomedical applications owing to their easy penetration and size similarity with other biomolecules. Due to their myriad of opportunities within complex biological systems they attract the researchers and scientists. This emerging field has been observed to show its pertinence in drug delivery, discovery, treatment of cancer etc. due to their ability to cross blood-tissue barrier and efficacy at specific site (Pastorino et al., 2019). Nanocarriers also possess the ability to interact with biomolecules onto cell surfaces and in interior of cell in such a manner that they do not alter their biochemical properties (Pastorino et al., 2019; Stillman et al., 2020). It thereby provides rapid access to cell functioning with the perspective for research frontiers in biomedical fields *via* optical-based analytical strategies for bioimaging and biosensing (Elahi et al., 2018; Noori et al., 2020). For biomedical purpose, metal surface compatibility is imperative, therefore, metal NPs are synthesized by biological methods that provide metallic ions with high compatibility. Henceforth, due to their widespread biotechnological implications, NPs have showed drastic cutting-edge disposition along with anti-inflammatory, compatibility and anti-microbial characteristics along with efficient bioactivity, tumor targeting and absorption (Salem and Fouda, 2021).

It is noteworthy that multifaceted physical and chemical methods are developed for NP-synthesis but they are quite hazardous towards environment and cause several issues. Moreover, they require a large amount of energy and are less economical (Wageh et al., 2015). In present times, biological methods for NP-synthesis are best alternatives that are economical, eco-friendly, nontoxic, sustainable, safe and well scaled up for synthesis. Green synthesis of NPs surmount the disparaging effects of other methods. Apart from this, they offer various characteristics such as control on ambient pH, temperature, size, shape, pressure etc. along with avoiding external agents like reducing, stabilizing or capping (Usman et al., 2019). It is well-documented that there are numerous organisms that have potential to release inorganic materials *via* intracellular or extracellular synthesis (Senapati et al., 2012). Several microbes such as bacteria, yeasts, fungi, actinomycetes, viruses, algae etc. are most commonly used for the synthesis of NPs such as silver, gold, iron, titanium, copper, palladium etc. (Saxena et al., 2012; Saravanan et al., 2018). The presence of matrix in bacteria provides greater catalytic reactivity, enhanced enzyme and metal salt contact and higher surface area. The blend of biotechnology, nanotechnology and microbiology arises a novel field of nano-biotechnology. Extracellular and intracellular

biosynthesis are two major routes through which microbes produce NPs. In extracellular processes, the metal sorption occurs onto microbial surfaces and subsequent reduction *via* enzymes. On the other hand, intracellular synthesis, the transport of metal ions inside microbial cells takes place followed by its reduction and expulsion of NPs generated in extract.

Interestingly, soil and water pollution is most prevalent issue of environment. During optimal geo-environmental conditions, the pollutants being recalcitrant are most hazardous due to its non-viability for natural attenuation (O'Connor et al., 2018). For this reason, the NPs have evolved as most efficient technological advancement. They are useful in many containment sites for their remediation efficiency. For instance, FeNPs synthesized from microbes are useful in the degradation of toxic dyes *via* adsorption and also in the wastewater treatment (Jin et al., 2016; Sharma et al., 2017; Fazlzadeh et al., 2018). *Rhodococcus* sp. has showed its paramount importance for NP-synthesis for environmental remediation purposes. Regardless of overcoming restraints of conventional strategies, the use of NPs for wastewater treatment also shows remarkable results. And biological or microbial synthesis of NPs offers an array of advantages towards environmental issues (Anitha, 2016). The present chapter unravels the microbial-mediated synthesis of various NPs in detail. Particularly, various methods of NP-synthesis encompassing bacteria, fungi, algae, yeasts, viruses and actinomycetes have been explored in detail.

7.2 Microbial enzymes in nanoparticle synthesis

Nanotechnology has gained huge accomplishment in terms of technological advancement since last few decades. Enormous development of microbial-synthesized NPs play a vital role in various sectors of biological sciences, food and agricultural industry, engineering, electronics, cosmetics, biomedical and pharmaceutical sciences. Therefore, they have reached the momentous rank owing to its specific physicochemical properties and substantial biotechnological applications (Slavin et al., 2017). The market report depicts that global production of microbial-synthesized NPs values 13.7 US billion dollars that have been accountably expected to attain 50 billion dollars by 2026. The extensive utilization of NPs has significantly caused robust development, thereby enhancing its demand in different regions of the world (Future Market Insights, 2018). Since decades, researchers synthesize NPs by conventional physical and chemical protocols. The limitations of these methods comprises of its expensive synthesis process and lesser production and yield. Likewise, chemical synthesis are also toxic and hazardous towards environment as chemicals are significantly attached onto NP surfaces causing detrimental issues during biomedical and pharmaceutical purposes (Mukherjee et al., 2017).

Keeping in view the abovementioned issues, the scientists have been oriented towards the discovery of novel methods of biological origin that are economical, compatible, environment friendly, nontoxic and safe (Mukherjee et al., 2013). Biological synthesis of NPs by microbes is relatively simple and it synthesizes polydispersed NPs owing to its varied photochemistry (Ovais et al., 2016). Microorganisms are known as prospective bio-factory in green synthesis of NPs (Fig. 7.1). They have gained a vast recognition in present times due to their indispensable and technological significance. The wide array of microbes react differently among metal ions for the synthesis of NPs. Microbial synthesis of

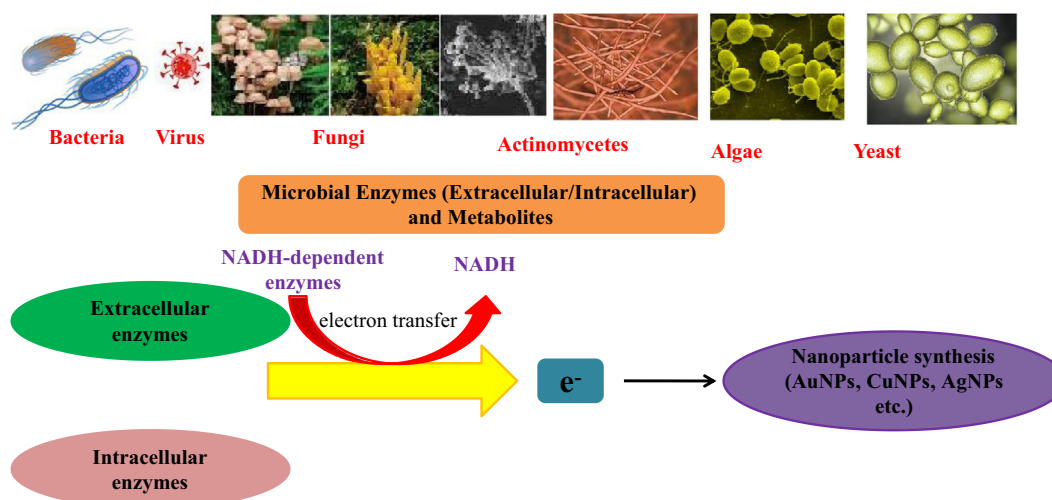


FIGURE 7.1 Nanoparticle synthesis through microbial enzymes.

NPs have been observed varying in sizes, shapes and functions in different species of bacteria, fungi, actinomycetes, yeasts, viruses etc. All these possess improved growth rate, easy cultivation method and capability to get establish in various conditions of temperature, pH and pressure (Barabadi et al., 2017). The NP-biosynthesis and their alloys such as uraninite, gold, copper, silver, titanium, platinum, magnetite, palladium, selenium etc. have been observed in microbes. However, the synthesis of NPs in microbes may occur both intracellular and extracellular. In case of intracellular method, the microbe cell contain remarkable ion transport mechanism. Owing to electrostatic interactions, the cell wall of microbial cell with negative charge attracts the metal ions with positive charge. In addition, the cell wall comprising enzymes also reduce the metal ions into their respective NPs. Contrastingly, in extracellular method, the microbial cell releases reductases that is used in the reduction of metal ions in NPs (Hulkoti and Taranath, 2014). In the following section, the intracellular and extracellular enzyme synthesis of NPs have been elaborated. (Fig. 7.2).

7.2.1 Extracellular enzymes

Extracellular enzymes secreted by microbes play an essential role in NP-synthesis (Subbaiya et al., 2017). The studies conducted suggest that co-factors like nicotinamide adenine dinucleotide (NADH) and nicotinamide adenine dinucleotide phosphate (NADPH) enzymes play crucial role as reducing agents through transfer of electrons and acting as electron donors (Bose and Chatterjee, 2016). Moreover, AuNPs biosynthesis have been favored by bacterium *Rhodopseudomonas capsulata* and is also mediated by secretion of NADH and NADH-based enzymes. Bioreduction of gold is also mediated through electron transfer from NADH via NADH reductases enzymes carried by bacterium. Subsequently, Au-ions accept electrons and are reduced from Au^{3+} to Au^0 respectively (He et al., 2007). Various other factors like concentration of predecessor, temperature, pH and time duration of reaction effects the size of NP to be synthesized. Alongside, few compounds such as naphthoquinones, anthraquinones and hydroquinones also favors NP-biosynthesis (Patra et al., 2014). The microbial underlying mechanisms also include solubility, biosorption, complexation, precipitation, toxicity, oxidation/reduction mechanism, transporters, ionic and efflux pumps (Mukherjee et al., 2017). Many fungal strains also stimulate the production of extracellular enzymes such as xylan esterases, glucosidases, cellobiohydrolases D etc. that play pivotal role in NP-biosynthesis (Ovais et al., 2018a,b).

Interestingly, the mechanism involved in AgNPs synthesis by extracellular enzymes is by the use of nitrate reductases released by fungi in order to boost bioreduction while synthesizing NPs (Kumar et al., 2007a,b). The use of nitrate reductases discs available commercially depicted that NADH-based reductases are substantially indulged in Ag^+ reduction into Ag^0 respectively for formation of AgNPs (Ingle et al., 2008). Alongside, *Fusarium oxysporum* was used as reducing agents in Au and AgNPs. Extracellular reductases released by fungi reduce Au^{3+} and Ag^+ into Au/Ag NPs. Besides, nitrate-based reductases and quinone released by this fungus is also used for extracellular NP-synthesis (Senapati et al., 2005). Contrastingly, few fungal species like *F. moniliforme* did not released AgNPs after the synthesis of reductases, depicting that Ag^+ reduction occurs through conjugation of oxidation/reduction of electron carriers via NADP-dependent nitrate reductase (Duran et al., 2005). Additionally, nitrate reductase from *F. oxysporum* utilized in vitro under anaerobic conditions with the existence of NADPH as cofactor, protein, phytochelatin as stabilizer and

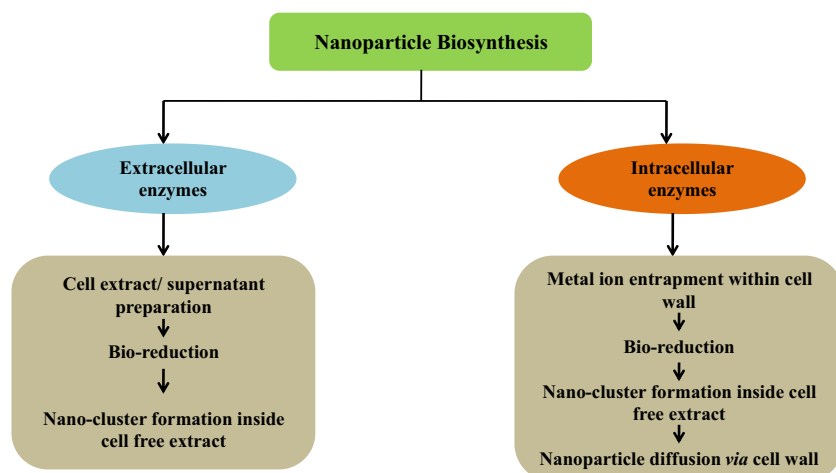


FIGURE 7.2 Flowchart depicting sequential steps of nanoparticle synthesis by microbial extracellular and intracellular enzymes.

4-hydroxyquinoline as electron carrier also result in AgNPs synthesis. This fungal species have good potential for extracellular synthesis of NPs and could be tagged as brilliant applicant for extracellular NP-synthesis (Karbasian et al., 2008). Hitherto, in several studies, *F. oxysporum* was utilized in extracellular synthesis of CdSNPs whilst, CdSeNPs were synthesized by reductase enzymes released by fungi (Kumar et al., 2007a,b). Enzymes from various other fungal species such as *F. semitectum*, *F. solani* etc. were used for AgNPs synthesis through extracellular enzymes. Results also encapsulated that various proteins may be the probable reason for Ag^+ reduction for AgNP production. Furthermore, *Cladosporium cladosporioides* and *Coriolus versicolor* were used in AgNP extracellular synthesis with the active role of fungal proteins, polysaccharides and organic acids. All these factors affect the final shape and growth of nanocrystals (Balaji et al., 2009). A study reported that inoculation of *Aspergillus niger* in AgNO_3 lead to extracellular AgNP synthesis stabilized through fungal proteins (Gade et al., 2008). Similarly, *A. fumigatus* produce AgNP extracellularly in limited time in contrast to other physical and chemical methods (Bhainsa and D'souza, 2006). Henceforth, *A. fumigatus* is considered to be potential candidate for producing NPs on industrial scale. On the other hand, *Penicillium fellutanum* also reduces Ag^+ ions in a lesser time. Additionally, the studies depicted that protein encoding nitrate reductase also reduces Ag^+ ions (Kathiresan et al., 2009). Also, *P. brevicompactum* led to reduction of Ag^+ through liberating NADH-dependent enzymes nitrate reductases (Shaligram et al., 2009). Nanotechnology as a new field of science has also introduced algae, *Sargassum wightii* for reducing Au^{3+} to generate AuNPs of 8–12 nm size (Singaravelu et al., 2007). Amongst algae, another species namely, *Chlorella vulgaris* also produces AuNPs respectively (Lengke et al., 2006).

7.2.2 Intracellular enzymes

In case of intracellular enzymatic mechanism underlying metal bioreduction, microbial cells along with sugars play a critical role. Specifically, when intracellular enzymes and positively charged ions interact among each other, the metal ions are gripped from medium followed by reduction within the cell (Dauthal and Mukhopadhyay, 2016). After critical observations, it has been observed that NPs get accumulated within the periplasmic space, cell wall and membranes. This is mainly due to diffused metal ions within the membranes, consequently causing enzymatic reduction for synthesis of NPs. In the midst of actinomycetes, *Rhodococcus* sp. (alkalophiles) and *Thermomonospora* sp. (alkalo-thermophile) are efficient AuNPs synthesizers (Ahmad et al., 2003). Intracellular synthesis of AuNPs is done by inoculating *Rhodococcus* sp. with AuCl_4 aqueous solution. The reduction of Au^{3+} ion is mediated by enzymes present on the mycelial surfaces and cytoplasmic membranes. The contact of *Verticillium* biomass to Ag^+ ions result in intracellular reduction followed by AgNP synthesis. Results visualized by electron microscopy determined that AgNPs are generated beneath the cell wall via enzymatic bioreduction and this process is completely nontoxic towards fungi (Mukherjee et al., 2001). A similar kind of protocol was seen for AgNP biosynthesis through *Verticillium* as a resource of reduction enzyme. AuNPs were observed to be entrapped within cell wall and membranes of fungi, depicting that Au^{3+} undergo bioreduction by reductase enzymes (Mukherjee et al., 2001).

Furthermore, it was depicted that AuNPs were synthesized and precipitated within bacteria cell after incubating Au^{3+} solution (Southam and Beveridge, 1996). The exposure of *Pseudomonas stutzeri* to AgNO_3 lead to reduction of Ag^+ ions to form AgNPs within periplasmic spaces of the bacterial cells (Klaus et al., 1999). Moreover, the filamentous bacterium *Plectonema boryanum* when treated with AuCl_4 and $\text{Au}(\text{S}_2\text{O}_3)_2$ synthesized AuNPs in membranal surfaces along with gold sulfide within intracellular spaces (Lengke et al., 2006). Similarly, *Phanerochaete chrysosporium* incubated with Au^{3+} formed AuNPs of 10–100 nm size. Besides, laccase enzyme was also utilized as extracellular element, while ligninase was known for reducing Au^{3+} ions (Sanghi et al., 2011). Several factors such as incubation time, stage of fungal species, concentration of AuCl_4 solution, temperature etc. also influence the shape of AgNPs. Mesophile *Shewanella*, an algal species is also known to be an efficient bioreducer of AuCl_4 ions to elemental gold particles. The AuNPs were formed residing in the periplasmic spaces through intracellular enzymes (Konishi et al., 2007). However, *Brevibacterium casei* when treated with Au^{3+} and Ag^+ ions undergo reduction to intracellular enzymes via spherical shaped Au and AgNPs (Kalishwaralal et al., 2010).

7.3 Microbe-mediated biosynthesis of nanoparticles: mechanism of action

Because of rapid growth, easy cultivation and potential to grow at ambient pH, temperature and pressure the microbial communities such as bacteria, fungi, actinomycetes, yeast, viruses, algae etc. are of paramount importance for NP-synthesis. The synthesis of NPs mainly occurs by different metal solutions either through intracellular or extracellular processes as explained above. The microbes produce several kinds of inorganic components as well as mechanisms depending on type of microbes to synthesize NPs (Fariq et al., 2017; Saravanan et al., 2020). Various metal ions are

entrapped onto cell surfaces subsequently after the reduction of metallic ions into NPs by the aid of enzymes secreted by microbes. Basically, there are two different mechanisms of NP-synthesis, biosorption and bioreduction. Biosorption includes the binding of metal ions in aqueous media onto cell wall of the microbes. Owing to the presence of cell wall or peptides the NP-synthesis takes place in a stabilized manner (Pantidos and Horsfall, 2014). The key mechanisms namely, physisorption, complexation, ion-exchange method and precipitation are some fundamental modes of biosorption of metal ions onto microbial cells. The microbes release many extra-polysaccharides, glycoproteins, lipids, lipopolysaccharides, glycoproteins with anionic groups and with the possibility to attract cationic groups from solutions. Particularly, bacteria with peptidoglycan, phospholipids, lipopolysaccharides and teichoic acids within cell wall possess negative group that bind onto positive group of metal ions. Within fungal cells, chitin is the crucial component that is responsible for complexation of metal ions for NP-biosynthesis (Wang et al., 2018). The synthesis of CuNPs *via* biosorption method through dead biomasses of *Rhodotorula mucilaginosa* was reported. NPs synthesized were spherical and amenable for NP-synthesis as well as bioremediation. Also, *Clostridium pasteurianum* synthesized molybdenum NPs as depicted by few researchers (Nordmeier et al., 2018).

Nevertheless, bioreduction mechanism mainly comprises of chemical reduction of metallic ions into stable forms through use of microflora and their enzymatic interactions (Jamkhande et al., 2019). Alongside, various components like amines, amides, proteins, alkaloids, carbonyl groups, various reducing agents and pigments also modulate the NP-synthesis (Asmathunisha and Kathiresan, 2013). The chemicals have strong oxidizing or reducing ability for metal ions to produce zero valent and magnetic NPs released by microbes namely, bacteria, yeasts, algae, fungi etc. Genetic engineering of such microbes are conducted for better results and they are quite easy to handle. As described in the above sections the inorganic NPs are produced either through intracellular or extracellular processes. In intracellular process, on the basis of localization of reduced cell components the NPs of varied dimensions are accumulated and dispersed. This process also requires additional steps, therefore, extracellular processes of NP-synthesis are preferred owing to their enormous applications (Gahlawat and Choudhury, 2019). Microbial-mediated NP-synthesis mainly includes amalgamation of complexed biochemical attributes like complexolysis, redoxolysis, alkyl reactions and acidolysis reactions. Moreover, the phytochemical constituents there in algae are efficient in stabilizing NPs as well as for their immobilization. Further in case of yeast based NP-synthesis, the plasma membrane plays a vital role for encapsulating subsequent for production of metallic NPs. Due to their excellent catalytic characteristics, yeast cells operate as superior biosystems for metallic NP-synthesis within them along with their remediation potential. Alongside, algae are also considered as rapid machineries for NP-synthesis due to their efficiency and their ability to produce excess of amino acids and negative charges in contrast to yeasts. They also enhance more nucleation along with crystal growth. Strikingly, algal cells enable easy disruption as well as economic scaling during NP-synthesis. Due to this, they have gained much popularity for NP-biosynthesis (Gautam et al., 2019). However, there are still some challenging factors such as shape and size control, lower production, quality issues, scale-up process, and elucidation of specific mechanism involved during biological NP-biosynthesis (Hosseini and Sarvi, 2015).

7.3.1 Nanoparticle biosynthesis by bacteria

Bacteria have been known as best source for synthesizing NPs owing to its numerous qualities such as easy purification, higher yields, optimal production conditions. Henceforth, they are known as nano-factories for synthesis of metal NPs. They employ both extracellular as well as intracellular techniques for the synthesis process. In former, the synthesis takes place exterior to cell *via* different techniques using bacterial biomasses, supernatant, and cell-free extracts. This method is often preferred over other due to avoid any hindrance during downstream processing (Singh et al., 2013). Since decades, *B. thuringiensis* have been used for the biosynthesis of AgNPs (44–142 nm) (Zhao et al., 2018). Additionally, *B. licheniformis*, *Klebsiella pneumonia*, *P. aeruginosa*, *B. subtilis*, *R. capsulata* etc. are involved in NP-biosynthesis (titanium NPs, CuONPs, CdNPs) (Zhao et al., 2018; Alghuthaymi et al., 2015). Bacterial synthesis of NPs is remarkable due to their ability to adapt varying environmental conditions. Due to this, bacterial supernatants, *P. meridiana*, *P. antarctica*, *Arthrobacter gangotriensis*, *A. kerguelensis* act as factories/reducing agents in AgNPs synthesis (Singh et al., 2015). Bacterial population could be utilized as biocatalysts for inorganic NP-synthesis and also operate as bio-scaffold for mineralization during NP-synthesis (Iqtedar et al., 2019). However, the synthesis may occur both in broth media during incubation either through intracellular or extracellular process. Bacterial-mediated NP-biosynthesis is quite reasonable, appropriate and flexible approach for broad scale production of NPs. Table 7.1 represents various reports of NPs synthesized by bacteria.

NPs are used for different applications such as AgNPs are used as antimicrobial against multidrug-resistant *Staphylococcus aureus*, and other strains like *Bacillus brevis*, *Salmonella typhi* etc. (Saravanan et al., 2018). Few other

TABLE 7.1 Bacterial-mediated biosynthesis of NPs and their applications.

S. no	Bacterial source	Type of NPs	Size	Application	References
1.	<i>Bacillus cereus</i>	Ag	5–7 nm	Antimicrobial activity against multidrug-resistant microbial strains	Ibrahim et al. (2021)
2.	<i>Vibrio alginolyticus</i>	Au	100–150 nm	Antioxidant, anticancer and free radical scavenging activity through DPPH (1, 1-diphenyl 2-picrylhydrazyl) and metal chelating assay	Shunmugam et al. (2021)
3.	<i>Marinospirillum alkaliphilum</i>	Ag	30–70 nm	Antimicrobial activities along with biodegradation properties	Nazari and Kashi (2021)
4.	<i>Streptomyces albidoflavus</i>	Ag	40 nm	Microbicidal effect and biodegradation	Saada et al. (2021)
5.	<i>Staphylococcus pasteurii</i>	Ag/AgCl	20–85 nm	Antimicrobial activity against pathogenic organisms	Fakher and Kashi (2021)
6.	<i>Pseudomonas</i> sp.	Ag	20–70 nm	Antimicrobial activity against pathogens	John et al. (2020)
7.	<i>Escherichia coli</i> , <i>Exiguobacterium aurantiacum</i> , <i>Brevundimonas diminuta</i>	Ag	5–50 nm	Antimicrobial activity	
8.	<i>Sphingobium</i> sp.	Ag	7–22 nm	Anti-microbial activity against drug-resistant pathogenic microorganisms	Akter and Huq (2020)
9.	<i>Pseudoduganella eburnea</i>	Ag	8–24 nm	Antimicrobial activity against multidrug-resistant pathogenic microbes	Huq (2020)
10.	<i>Azospirillum brasilense</i>	Se	5–10 nm	Biomedical applications	Tugarova et al. (2020)
11.	<i>Pseudomonas fluorescens</i>	Cu	10–70 nm	Insecticidal activity against grain pest <i>Tribolium castaneum</i>	El-Saadony et al. (2020)
12.	<i>Serratia nematodiphila</i>	ZnO	10–50 nm	Antimicrobial and antifungal activity with photocatalytic properties	Jain et al. (2020)
13.	<i>Pseudomonas putida</i>	ZnO	44.5 nm	Antimicrobial and antibiofilm activity.	Jayabalan et al. (2019)
14.	<i>Shewanella loihica</i>	Cu	10–16 nm	Antimicrobial activity	
15.	<i>Caldicellulosiruptor changbaiensis</i>	Au	19 nm	Antibacterial, Antibiofilm	Bing et al. (2018)
16.	<i>Streptomyces griseoplanus</i>	Ag	19–20 nm	Antifungal	Vijayabharathi et al. (2018)
17.	<i>Nocardioopsis flavescens</i>	Ag	5–50 nm	Cytotoxicity	Ranjani et al. (2018)
18.	<i>Micrococcus yunnanensis</i>	Au	54 nm	Antibacterial, Anticancer activity	Jafari et al. (2018)
19.	<i>Bacillus endophyticus</i>	Ag	5.1 nm	Antimicrobial activity against <i>Candida albicans</i> , <i>Escherichia coli</i> , <i>Salmonella typhi</i> and <i>Staphylococcus aureus</i>	Gan et al. (2018)
20.	<i>Vibrio natriegens</i>	Se	100–400 nm	Biocatalyst for bioremediation of selenite	Fernández-Llamosas et al. (2017)
21.	<i>Klebsiella pneumoniae</i>	Au	10–15 nm	Antibacterial activity	Prema et al. (2016)

microbial strains such as *Pseudomonas stutzeri*, *Bacillus* sp. etc. accumulate NPs intracellularly. Besides, *P. aeruginosa*, *Rhodopseudomonas*, *Lactobacillus plantarum* and *Serratia ureilytica* were also found to synthesize AuNPs and ZnONPs of different shapes and sizes with antimicrobial activity against pathogens (Dhandapani et al., 2014). Moreover, *Aeromonas hydrophila*, a Gram-negative bacteria is utilized for synthesizing ZnONPs and CuONPs were effectively synthesized by *Halomonas elongate* respectively (Rad et al., 2018). Similarly, paramagnetic FeNPs were also synthesized by *B. cereus* with potential anti-cancerous activities (Fatemi et al., 2018). Meanwhile, bimetallic Ag-Au NPs are also produced using bacterial strains. Strikingly, bacteria residing in gold mines show high resistance against Au-toxicity (Srinath et al., 2018). To elucidate, *Acinetobacter* when incubated with AuCl₃ and varying cell density depict variation in AuNPs color encompassing colloidal solution of different sizes and shapes. Amino acids are utilized in Au-salt reduction whereas, amide groups in AuNPs stabilisation (Wadhwani et al., 2016). In addition, lactic acid bacterial cells different NPs namely Au, Ag, CuO etc. are synthesized (Nair and Pradeep, 2002). Apart from this, another suitable method for Mn and Zn-based NPs is through reduction of MnSO₄ and ZnSO₄ by use of *Streptomyces* sp. via intracellular process (Waghmare et al., 2015). A study reported that *B. amyloliquifaciens* secreted surfactant in order to enable CdSNPs (3–4 nm) while *Escherichia coli* and *K. pneumoniae* released CdSNPs (3.5–45 nm) with highest antimicrobial activity against *B. subtilis*, *S. aureus*, *E. coli* and many other pathogenic organisms (Singh et al., 2011; Dhandapani et al., 2014). Likewise antimony sulfide NPs of size <35 nm were synthesized by *Serratia marcescens* and SeNPs of 96 nm were synthesized by *P. aeruginosa* respectively (Kora and Rastogi, 2018).

Interestingly, various researchers have also untangled the role of cyanobacteria in NP-biosynthesis. It has been determined that owing to the presence of various bioactive metabolites that stabilize and help in proper functioning of NPs along with limited biosynthetic steps, cyanobacteria are considered as most suitable candidates for NP-synthesis. Also, they possess very high growth rate with maximum biomass for NP-synthesis. It has been estimated that cell-free extracts are best for NP-production. And aqueous extract of *Oscillatoria limnetica* has also been used for AgNPs (3.5–18 nm) through reduction followed by stabilization (Hamouda et al., 2019). For instance, different cyanobacterial strains were isolated to explore their NP-generation ability. Resultantly, it was estimated that cyanobacteria produced AgNPs under light as well as dark conditions (Patel et al., 2015). Further, ammonia-releasing cyanobacteria *Phormidium fragile* and *Nostoc* synthesized AgNPs with cytotoxic activities (Sonker et al., 2017). Similarly, AgNPs (60–80 nm) were also synthesized by *Microchaete* sp. by the use of aqueous extract to obtain spherical, polydispersed NPs (Husain et al., 2019). Therefore, cyanobacteria possess most promising platform for biogenesis of NPs to be utilized in numerous applications.

7.3.2 Nanoparticle biosynthesis by fungi

Fungi have been predominantly used to synthesize NPs owing to its abundant characteristics in terms of efficiency of metabolites released by them for fabrication of NPs (Fouda et al., 2019). Due to their flexible nature, they have been added into the present catalogue of NP-biosynthesis. Their use have been consistently increased due to their potential for synthesizing numerous proteins, metabolites and enzymes along with easy to handle nature (Spagnoletti et al., 2019). The abundance in their characteristics for synthesizing NPs outskirts many other microbes because of the advantages over other. Like ease scale-up methods along with downstream processing and many other features of economic friendly, mycelium with large surface area are certain advantages offered by fungi over other microbes (Fouda et al., 2017). In addition, they also possess high tolerance towards metal ions due to which mainly attributed for biological synthesis (Sastry et al., 2003). Moreover, fungi are active involvers for extracellular enzymes/proteins synthesis thereby effective in terms of economic feasibility, livability for utilising biomasses in green synthesis of NPs from fungal cells or their metabolites. Sidewise, many fungal species show rapid growth and form huge biomass making them easy to maintain under laboratory conditions (Fouda et al., 2018).

Fungal synthesis is quite similar to bacteria or cyanobacteria by intracellular or extracellular routes. In intracellular process, metal ions in fungal mycelium are changed into nontoxic forms (Rajeshkumar and Sivapriya, 2020). Also, this process is more resourceful due to secretion of active molecules, higher aggregation rate and enhanced production and yield (Alghuthaymi et al., 2015). They synthesize metal NPs of varied structures and shapes in the form of meso/nano structures through enzymatic reduction or intracellular synthesis of biomimetic mineralization (Duran et al., 2005). Fungi mediated NP-synthesis have several biotechnological applications, in medical fields and this technique is called myco-nanotechnology. Numerous fungal species of fungi such as *Phanerochaete chrysosporium*, *Schizophyllum commune*, *Pleurotus sajorajju*, *S. radiatum*, *Fusarium* sp., *F. keratoplasticum*, *F. oxysporum*, *Aspergillus terreus*, *Coriolus versicolor*, *A. niger*, *Alternaria alternata*, *Helminthosporium tetramera*, *A. sydowii*, *Penicillium aurantiogriseum*, *P. citrinum*, *P. waksmanii* synthesize Au, Ag, ZnO and Fe₂O₃ based NPs (El Domany et al., 2018; Elamawi et al., 2018).

Moreover, *F. oxysporum* is used for biosynthesis of ZnS, PbS, CdS and MoS based NPs after the addition of salt into growth media (Ahmad et al., 2002). It has been further reported that the biosynthetic processes takes place within vacuoles of fungi after enzymatic reduction of sugars for tailoring the shapes and sizes of NPs. And most importantly, metabolites containing proteins/enzymes or various other biomolecules function as capping agents for stabilization of the ongoing processes (Zhang et al., 2011). Furthermore, the metal reduction through nitrate reductases and extracellular quinone was verified using various techniques such as UV–visible, enzymatic analysis and fluorescence analysis (Durán et al., 2007). Moreover, AuNPs of size 10–25 nm were formed and stabilized by using NADPH as cofactor, phytochelatin, nitrate reductases (capping agent) and 4-hydroxyquinoline respectively (Kumar et al., 2007a,b). Furthermore, the characterization of fungi synthesized NPs is done using X-ray diffraction analysis, Energy dispersive X-ray analysis, Transmission electron microscopy, Fourier transform infrared spectroscopy from which the exact shape and size of NPs is revealed. Table 7.2 represents various reports of NPs synthesized by fungi along with their applications.

7.3.3 Nanoparticle biosynthesis by actinomycetes

Actinomycetes are well known nano-machineries amongst microbial species as they have the potential to synthesize various metallic nanoparticles both extracellularly or intracellularly. Actinomycetes are Gram positive, aerobic, and filamentous bacteria. Several members of the Actinomycetes are well known for their distinctive capacity to secrete secondary metabolites having biological properties like antimicrobial, anti-parasitic, antioxidant, anticancer and anti-biofouling etc. (Zotchev, 2012; Manivasagan et al., 2013). They have gained special attention for nanomaterials due to the production of various bioactive constituents and enzymes through their saprophytic activities which act as strong reductants (Kumar et al., 2008; Kumari et al., 2020). In actinomycetes, extracellular synthesis of NPs involves the cell wall secretions and associated reductants. Intracellular synthesis takes place through the reduction of metal ions on the surface of mycelia together with cytoplasmic membranes (Ahmad et al., 2003). Extracellular synthesis is gaining much importance commercially as compared to intracellular production since polydispersity is act as vital factor (Kumari et al., 2020). The NPs biosynthesized by actinomycetes display significant stability and polydispersity. Actinomycetes can be genetically manipulated easily to accomplish required size of NPs to be synthesized. A summarized list of size and applications of NPs synthesized by various actinomycetes is depicted in following Table 7.3.

7.3.4 Nanoparticle biosynthesis by yeast

Amongst the eukaryotic microorganism, yeast strains of numerous genera are known to employ various strategies for synthesis of NPs having significant variations in size, monodispersity, particle position, and other properties. Yeast cells in the environs of toxic metals can amend the absorbed metal ions into complex polymer compounds which are not toxic to the cell. The NPs produced in the yeast are known as “quantum semiconductor crystals” or “semiconductor crystals.” Yeast cells are principally renowned for their capability to synthesize semiconductor crystals, mainly that of cadmium sulfide (Ghosh et al., 2021; Narayanan and Sakthivel, 2010). There are several reports on the production of NPs using yeast. *Candida glabrata* and *Saccharomyces pombe* have been used for the production of intracellular synthesis of cadmium sulfide, gold, silver, selenium and titanium nanoparticles (Boroum and Moghaddam et al., 2015; Feng et al., 2017). Extracellular synthesis silver nanoparticles was carried out using *Candida utilis*. AgNPs are spherical in shape, having size in the range of 20–80 nm and showed antibacterial activity against pathogenic organisms such as *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* (Waghmare et al., 2014). A summarized list of size and applications of NPs synthesized by yeast is shown in following Table 7.4.

7.3.5 Nanoparticle biosynthesis by algae

Among various microorganisms used for the synthesis of NPs, the use of algae is also increasing for the biosynthesis of NPs (Sanaeimehr et al., 2018). As algae belong to sea microorganisms, so they are used both for the uptake of the metal NPs and their biosynthesis (Luangpipat et al., 2011). Several microalgae that are utilized in the synthesis of NPs process include *Sargassum muticum*, *Sargassum crassifolium*, *Cystoseira trinodis*, *Sargassum iicifolium*, *Turbinaria conoides*, *Sargassum tenerriumum*, *Laminaria japonica* that synthesize ZnONPs, AuNPs, CuONPs, and AuNPs, respectively (Sanaeimehr et al., 2018; Maceda et al., 2018; Gu et al., 2018; Koopi and Buazar 2018; Ghodake and Lee, 2011; Ramakrishna et al., 2016 and Swaminathan et al., 2011). Maceda et al. (2018) during their study have found that as the concentration of *Sargassum crassifolium* was increased, there was reduction in the size of the NPs which results in the

TABLE 7.2 Fungal-mediated biosynthesis of NPs and their applications.

S. no	Fungal source	Type of NPs	Size	Application	References
1.	<i>Aspergillus terreus</i>	MgO	≥ 300 nm	Antimicrobial activity, biodegradation of tanning effluent, and chromium ion removal	Saied et al. (2021)
2.	<i>Aspergillus fumigatus</i> , <i>Byssoschlamys spectabilis</i> , <i>Cladosporium xanthochromaticum</i>	Ag	40 nm	Microbicidal effect and biodegradation	Saada et al. (2021)
3.	<i>Penicillium chrysogenum</i>	ZnO, CuO	9.0–35.0 nm and 10.5–59.7 nm	Antimicrobial activities against Gram positive and negative bacteria, pathogenic organisms and antibiofilm properties against <i>Staphylococcus aureus</i>	Mohamed et al. (2021)
4.	<i>Penicillium oxalicum</i>	Ag	60–80 nm	Applications as bactericidal agent against resistant bacteria, preventing infections, healing wounds, and anti-inflammation	Feroze et al. (2020)
5.	<i>Ganoderma lucidum</i>	Ag	15–22 nm	Antimicrobial activity against both Gram positive and negative bacteria and fungal species	Aygün et al. (2020)
6.	<i>Piriformospora indica</i>	Ag	6–15 nm	Antioxidant, antimicrobial, and anticancerous activity along with applications in nanomedicine and fabrication coating of ambulatory and non-ambulatory medical devices	Aziz et al. (2019)
7.	<i>Penicillium</i> sps. <i>Fuzarium Oxysporum</i>	Ag	30–45 nm	Antibacterial activity	Ukkund et al. (2019)
8.	<i>Macrophomina phaseolina</i>	Ag/ AgCl	5–30 nm	Antibacterial activity	Spagnoletti et al. (2019)
9.	<i>Fusarium oxysporum</i>	Pt	25 nm	Photocatalytic and biological activities such as antioxidant and antimicrobial properties against both bacteria and fungus	Gupta and Chundawat (2019)
10.	<i>Macrophomina phaseolina</i>	Au	14–16 nm	Medical applications such as drug delivery system	Sreedharan et al. (2019)
11.	<i>Trichoderma longibrachiatum</i>	Ag	10 nm	Antifungal against phyto-pathogenic fungi	Elamawi et al. (2018)
12.	<i>Ganoderma sessiliforme</i>	Ag	45 nm	Antibacterial, Antioxidant, Anticancer	Mohanta et al. (2018)
13.	<i>Nemania</i> sp.	Ag	34 nm	Antibacterial activity	Farsi and Farokhi (2018)
14.	<i>Rhodotorula mucilaginosa</i>	Ag	14–15 nm	Antifungal, Dye degradation, Cytotoxicity	Cunha et al. (2018)
15.	<i>Penicillium chrysogenum</i>	MgO	10.28 nm	Antimicrobial agents against <i>Enterococcus faecalis</i> , <i>Candida albicans</i> , and <i>Klebsiella pneumoniae</i> and applications in biomedicine, food control, pharmaceuticals, and cosmetics	El-Sayyad et al. (2018)
16.	<i>Trichoderma atroviride</i>	Ag	15–25 nm	Antibacterial, antioxidant, anticancer activity to induce cancer cell death	Saravanakumar and Wang (2018)

(Continued)

TABLE 7.2 (Continued)

S. no	Fungal source	Type of NPs	Size	Application	References
17.	<i>Aspergillus oryzae</i>	Se	55 nm	Applications in biomedicine, cosmetics, pharmaceuticals, antimicrobial agent and for preventing food spoilage	Mosallam et al. (2018)
18.	<i>Cladosporium cladosporioides</i>	Au	60 nm	Antioxidant, Antibacterial activity	Joshi et al. (2017)
19.	<i>Aspergillus</i> sp.	Au	3–7 nm	Nitrophenol reduction	Shen et al. (2017)
20.	<i>Aspergillus terreus</i>	Ag	2 nm	Antibacterial activity	Velhal et al. (2016)

TABLE 7.3 Actinomycetes-mediated biosynthesis of NPs and their applications.

S. no	Actinomycetes	Type of NPs	Size	Application	References
1.	<i>Streptomyces antimycoticus</i>	Ag	13–40 nm	Bactericidal activity and cytotoxic efficacy	Salem et al. (2020)
2.	<i>Streptomyces</i> sp.	Ag	—	Antibacterial activity	Bizuye et al. (2020)
3.	Endosymbiotic marine actinomycete	Ag	8–35 nm	antimicrobial and cytotoxic activity	Hamed et al. (2020)
4.	Marine Actinomycetes	Ag	40–44 nm	Antifungal activity	Ravi et al. (2020)
5.	<i>Streptomyces laurentii</i>	Ag	7–15 nm	Cytotoxic activity	Eid et al. (2020)
6.	<i>Nocardiopsis alba</i>	Ag	20–60 nm	Antibacterial activity	Avilala and Golla (2019)
7.	<i>Streptomyces spp</i>	CuO	80 nm	Antimicrobial activity	Hassan et al. (2019)
8.	<i>Streptomyces griseoruber</i>	Se	100–250 nm	Cytotoxic activity	Ranjitha and Ravishankar (2018)
9.	VITBN4	CuO	61.7 nm	Antibacterial activity	Nabila and Kannabiran (2018)
10.	Marine actinomycetes	Cu	Nanorange size	Antibacterial efficacy	Rasool and Hemalatha (2017)
11.	<i>Streptomyces griseoruber</i>	Au	5–50 nm	Catalytic activity	Ranjitha and Rai (2017)
12.	<i>Gordoniaaamicalis</i>	Au	5–25 nm	Antioxidant scavenging activity	Sowani et al. (2016)
13.	<i>Gordoniaamarae</i>	Au	15–40 nm	Sensing of copper ions	Bennur et al. (2016)
14.	<i>Streptomyces</i> sp. VITPK1	Ag	20–45 nm	Anticandidal activity	Sanjenbam et al. (2014)

blue shift of the UV absorption spectra. Another study conducted by [Gu et al. \(2018\)](#) observed that CuONPs synthesized by *Cystoseira trinodis* have methylene blue degrading potential along with the antibacterial and antioxidant properties. Likewise there are many nanoparticles that are biosynthesized by different species of micro and macroalgae. These NPs produced are of different sizes and also possess various applications which are compiled in the [Table 7.5](#).

TABLE 7.4 Yeast-mediated biosynthesis of NPs and their applications.

S. no	Yeast source	Type of NPs	Size	Application	References
1.	<i>Saccharomyces cerevisiae</i>	Ag ₂ O	9 to 85 nm	Antibacterial activity	Eddy et al. (2021)
2.	Baker's yeast	Ag	13.8 nm	Antibacterial activity	Shu et al. (2020)
3.	<i>Yarrowiali polytica</i> DSM 3286	Ag	12.4 nm	Antibacterial activity	Bolbanabad et al. (2020)
4.	<i>Magnusiomyces ingens</i>	Se	70–90 nm	Antimicrobial activity	Lian et al. (2019)
5.	<i>Candida albicans</i>	CdS	50–60 nm	Antibacterial activity	Kumar et al. (2019)
6.	<i>Saccharomyces cerevisiae</i>	Ag	16.07 nm	Antibacterial activity	Olobayotan and Akin-Osanaiye (2019)
7.	<i>Rhodotorula</i> sp. strain ATL72	Ag	8.8 to 21.4 nm	Antimicrobial activity	Soliman et al. (2018)
8.	Baker's yeast	TiO ₂	6.7 ± 2.2 nm	Antibacterial activity	Peiris et al. (2018)
9.	<i>Saccharomyces cerevisiae</i>	Ag	10–60 nm	Antimicrobial activity	Sowbarnika et al. (2018)
10.	<i>Saccharomyces cerevisiae</i>	Pd	32 nm	Photocatalytic activity	Sriramulu and Sumathi (2018)
11.	<i>Rhodotorulam ucilaginosa</i>	Ag	13.7 ± 8.21 nm	Antifungal, catalytic and cytotoxic activities	Cunha et al. (2018)
12.	<i>Candida glabrata</i>	Ag	2–15 nm	Antibacterial and antifungal activity	Jalal et al. (2018)
13.	<i>Pichiakudriavzevii</i>	ZnO	10–61 nm	Free radical scavenging activity, cytotoxicity and antibacterial activity	Moghaddam et al. (2017)
14.	<i>Candida lusitaniae</i>	Ag	35 nm	Antibacterial activity	Eugenio et al. (2016)

7.3.6 Nanoparticle biosynthesis by viruses

Due to biocompatibility, capacity of mass production, biodegradability, programmable scaffolds and manipulation of desired genes, viruses are emerging as new field for the production of NPs. NPs that are synthesized from viruses have many applications in drug delivery, gene delivery, theranostics and imaging. Drug delivery is done by the mammalian viruses whereas theranostics and imaging is done by plant virus and bacteriophage (Steinmetz, 2010). There is another class of NPs called as Virus-Like Particles and is derived from the protein coating of the virus. There are various steps that are involved in the production of viral NPs. The first step involves its generation in the body of host which may be plant, bacteria or animal. It is then followed by tuning and chemical conjugation and subsequent evaluation under both in vivo and in vitro conditions (Steinmetz, 2010). As viral nanoparticles are toxic for humans, so plant bacteriophage are preferred in comparison with the mammalian virus (Bruckman et al., 2008).

Viral nanoparticles and virus-like particles are also exploited for the delivery of the chemotherapeutic drugs (Ashley et al., 2011). It was observed by Franke et al. (2017) that the viral NPs that are derived from Tobacco Mosaic Virus are used in the platinum-resistant ovarian cancer cells. Viral NPs are also applied as agents in MRI contrast (Steinmetz, 2010). These NPs are also explored for their use to produce vaccines against HIV, hepatitis B and *Neospora caninum* (Oh and Han, 2020). Table 7.6 represents various reports of NPs synthesized by viruses along with their applications.

TABLE 7.5 Algal-mediated biosynthesis of NPs and their applications.

S. no.	Algae	Nanoparticle	Size	Application	References
1.	<i>Chaetomorpha linum</i>	Ag	70–80 nm	Acts as an efficient anticancer agent	Acharya et al. (2021)
2.	<i>Chlorella ellipsoidea</i>	Ag	220.8 ± 31.3 nm	Shows catalytic, photophysical, and antibacterial activity	Borah et al. (2020)
3.	<i>Spirulina platensis</i>	Au	15.60–77.13 nm	Antiviral activity	El-Sheekh et al. (2020)
4.	<i>Chlorella vulgaris</i>	Pd	70 nm	Shows catalytic activity	Mishra et al. (2020)
5.	<i>Amphiroa rigida</i>	Ag	25 nm	Inhibit cytotoxicity, larvicidal efficiency and posses antibacterial activity.	Gopu et al. (2020)
6.	<i>Chlorella vulgaris</i>	CuFe ₂ O ₄ @Ag	20 nm	Shows antibacterial activity, antibiofilm activity, inhibit efflux pump genes in <i>Staphylococcus</i> respectively	Kahzad and Salehzadeh (2020)
7.	Red algae <i>Portieria hornemannii</i>	Ag	60–70 nm	Antibacterial activity against fish pathogens was observed	Fatima et al. (2020)
8.	Marine macroalgae <i>Padina</i> sp.	Ag	~ 25–60 nm	Antibacterial and antioxidant activities were observed	Bhuyar et al. (2020)
9.	<i>Anabaena flos-aquae</i>	Ag	5–25 nm	Includes anticancer and cytotoxic activity against T47D cell lines	Ebrahimzadeh et al. (2020)
10.	<i>Ulva armoricana</i> sp.	Ag	33 nm	Bactericidal activity	Massironi et al. (2019)
11.	<i>Microchaete</i> NCCU-342	Ag	60–80 nm	Dye decolorization ability	Husain et al. (2019)
12.	Macroalgae (<i>Ulva lactuca</i> L.)	Ag	31 ± 8 nm	Used in cancer therapy	González-Ballesteros et al. (2019)
13.	<i>Spirulina platensis</i>	Fe ₃ O ₄ @Ag	30–68 nm	Showed modulatory effect on the expression of norA and norB genes in <i>Staphylococcus aureus</i>	Shokoofeh et al. (2019)
14.	Brown algae <i>Padina pavonia</i>	Ag	49.58–86.37 nm	One-pot method for synthesis	Abdel-Raouf et al. (2019)
15.	<i>Gracilaria birdiae</i>	Ag	20.3 nm	Antibacterial activity	de Aragao et al. (2019)
16.	<i>Polysiphonia algae</i>	Ag	5–25 nm	Anticancer activity against MCF-7 cell lines	Moshfegh et al. (2019)
17.	Marine algae <i>Gelidiella acerosa</i>	Au	5.8–117.6 nm	Possess biological potential	Senthilkumar et al. (2019)
18.	<i>Gelidium amansii</i>	Ag	27–54 nm	Antimicrobial property	Pugazhendhi et al. (2018)

7.4 Applicability of biologically synthesized nanoparticles

Owing to abundant unique characteristics in terms of controlled size, distinctive properties, compatibility, nontoxic nature etc. microbial-mediated synthesis of NPs comprises of plethora of applications in various realms specifically

TABLE 7.6 Virus-mediated biosynthesis of NPs and their applications.

S. no.	Virus	Nanoparticle	Size	Application	References
1.	Tobacco mosaic virus	Au nanowires	50 nm in diameter and 150–400 nm in length	Properties of nanowires	Kurgan and Karbivskyy (2020)
2.	Hepatitis E virus	Nanoconjugates	27–34 nm	Used in cancer therapy	Chen et al. (2018)
3.	Bacteriophage	Au	20–50 nm 50–150 nm 150–500 nm	Applied in biosensor electrode	Ahiwale et al. (2017)
4.	M13 virus	Au-DNs	-	Used in biosensor platform	Seo et al. (2017)
5.	Potato virus X	Nanocarriers	12 nm	Used for the delivery of doxorubicin in cancer therapy	Le et al. (2017)
6.	M13 virus	TiO ₂	20–40 nm	Inhibit photo-electrochemical properties	Chen et al. (2015)
7.	Cowpea mosaic virus and Tobacco mosaic virus	Metal nanoparticles	≤ 100 nm	Used in nanotechnology industry	Love et al. (2014)
8.	Cucumber mosaic virus	Nanoassemblies	~ 29 nm	Used for anticancer activity, and drug delivery	Zeng et al. (2013)
9.	Tobacco mosaic virus (TMV)	Pd	2.9–3.7 nm	Used as multiwalled carbon nanotubes catalyst	Yang et al. (2013)
10.	Cowpea mosaic virus (CPMV)	Ni, Co, Fe, Pd, Co–Pd, Ni–Fe	≤ 35 nm	One-pot method for synthesis	Aljabali et al. (2010)

medical applications. They have been extensively employed in pharmaceutical as well as biomedical industries in order to be used as anti-microbial agents, antibiofilm agents, as antioxidants, anticancer/antitumor agents, drug delivery system, diagnostic and imaging purposes and various other biomedical facilities. The applicability of biologically synthesized NPs have been described in the following sections (Fig. 7.3).

7.4.1 Antimicrobial agents

Antimicrobial characteristics are known to exist in a variety of metallic nanoparticles, including silver, copper, zinc, magnesium, gold, and titanium. Antimicrobial properties are quite important based on the size and morphology of the particles, with finer, more compact NPs being more effective monodisperse NPs (high surface to volume ratio) with a higher antibacterial proclivity (Duran et al., 2010). In case of metal oxide NPs, distortion of lipid bilayer, pore creation on the bacterial cell wall, suppression of biofilm formation, and, generation of reactive oxygen species (ROS) are some of the modes of antibacterial action attributed to nanoparticles (Busi and Rajkumari, 2019). The increase of multidrug-resistant (MDR) among infectious agents has pushed the search for new antibacterial NPs. The inclusion of natural stabilizing or capping agents such as polysaccharides or proteins on the NP surface during synthesis is a significant advantage of biogenic synthesis, which significantly lowers post production procedures. Traditional antibiotic molecules have been proven to be more effective when conjugated to NPs. The discharge of Ag⁺ ions from silver NPs disrupts bacterial membranes and interferes with DNA and protein synthesis. Gold NPs, can be prepared in combination with photosensitizers for antimicrobial photodynamic treatment due to strong photocatalytic activity. Antibacterial activity was also demonstrated against *Bacillus subtilis*, *Pseudomonas* sp., *Trichophytonmentha grophytes*, *K. pneumonia*, *Trichophytonsimii*, *Trichophytonrubrum*, *E. coli*, *B. subtilis*, and *Klebsiella planticola*, respectively, using AgNPs synthesized intracellularly by the mushroom fungus *Schizophyllum commune* and *Trichoderma viride* (Arun et al., 2014;

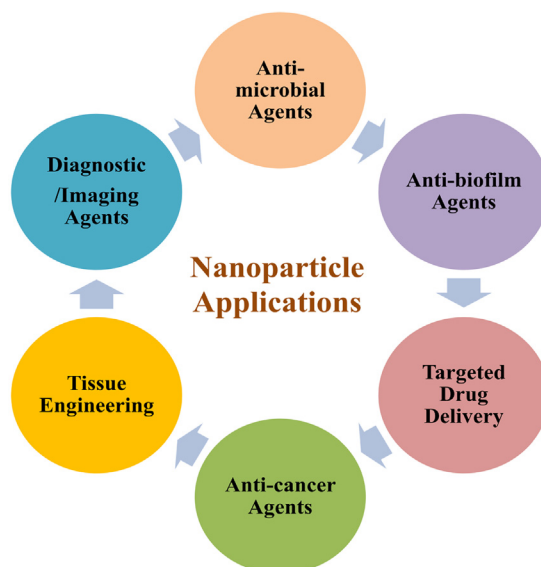


FIGURE 7.3 Applications of biologically synthesized nanoparticles (NPs).

Chitra and Annadurai, 2013). When bacterial cells are exposed to near-infrared radiation (NIR), the heat generated damages the bacterial cell wall (Busi and Rajkumari, 2019). A study conducted by Fayaz et al. (2011), suggested that binding of vancomycin-AuNPs to the *S. aureus* transpeptidase, in place of terminal peptides of the glycopeptidyl precursors. Also the facile transport across lipid bilayer in the case of *E. coli*, cell wall lysis was reported in vancomycin resistant *S. aureus* and *E. coli*. Ciprofloxacin, gentamycin, vancomycin, and rifampicin drugs loaded on AuNPs biogenically produced from *B. subtilis*, suppressed growth in *S. haemolyticus* and *S. epidermidis*, owing to the increased surface area supplied by the NPs for the medications to bind (Roshmi et al., 2015). The NPs created using one microorganism's extracts are effective in killing other microbial species and boost the efficacy of current antibiotics to overcome antimicrobial resistance traits, as shown in the instances above.

7.4.2 Antibiofilm agents

Development of antibiotics and antimicrobials is a hard process because of resistance to antibiotics. The microbial population to create biofilms, which make them impervious to drugs, is one of the main causes of bacterial infection and multidrug resistance. Biofilm production by *Staphylococcus aureus*, *Acinetobacter baumannii*, *Escherichia coli*, and *Pseudomonas aeruginosa* are known to induce opportunistic infections and therefore suppressing it is a crucial issue addressed in the case of biogenic nanoparticles. Furthermore, due to biofilm formation, biomedical and dental equipment are at a significant risk of spreading infections, and NP coating has been investigated as a viable approach for avoiding it. Cell staining with crystal violet and absorbance measurements, or observation under electron microscopes, are used in the majority of research to assess biofilm formation. Biofouling induced by microbial consortia present in the wastewater slurry affects the efficacy of the bioreactor, which is an intriguing detrimental influence of biofilm formation. In an activated sludge bioreactor *Lactobacillus fermentum* LMG 8900 generated microbial silver NPs (bio-Ag0) (around 11 nm size) embedded in polyethersulfone (PES) membranes were tested on (*E. coli* and *P. aeruginosa*) and another mixed culture for 9 weeks and the results showed significant antibiofilm and antibacterial potential (Zhang et al., 2011). A study conducted by Dhandapani et al. (2012) revealed that the biomass of *Bacillus subtilis* was used to make TiO₂ NPs. Following that, microbe-rich pond water was being used to produce biofilm in solution or on microscope slide containing the NPs, which was then irradiated with polychromatic light to restrict biofilm growth. The TiO₂ NPs served as a photocatalyst, releasing H₂O₂ to suppress biofilm formation. *Stenotrophomonas maltophilia* SeITE02 and *Ochrobactrum* sp. MPV1 synthesized Se and Te microbial nanoparticles have antimicrobial and antibiofilm abilities against *E. coli* JM109, *P. aeruginosa* PAO1 and *S. aureus* ATCC 25923 biofilms cells and planktonic cells (Zonaro et al., 2015). Similarly, studies on silver NPs extracted from *B. licheniformis* biomass have effective antibiofilm potential for *P. aeruginosa* and *S. epidermidis* whereas gold-silver NPs synthesized from *Shewanella oneidensis* Mr-1 have the significant ability to inhibit the biofilm production of *Enterococcus faecalis*, *S. aureus*, *E. coli* and *P. aeruginosa*.

(Zonaro et al., 2015; Ramasamy and Lee, 2016). Selenium NPs synthesized from SeO_2 and isolated *Bacillus licheniformis* have been observed to have antimicrobial and antibiofilm properties. They are used to control biofilm formation and growth of pathogens like *Bacillus cereus*, *Staphylococcus aureus*, *Salmonella typhimurium*, *Salmonella enteritidis*, *Escherichia coli* and *Enterococcus faecalis* respectively (Khiralla and El-Deeb, 2015).

7.4.3 Drug delivery system

In order to overcome the limitations caused by the conventional drug delivery system various studies had been conducted to find any appropriate system. It has been studied that any ideal drug delivery system must be controlled and must have targeted delivery. As a result the use of NPs for the same has been emerged. Due to their controlled and targeted delivery actions NPs have evolved as an effective drug delivery system. Drugs are beneficial when they are present in optimum concentration. When NPs are produced, its major point is to control the size and surface properties of NPs synthesized, so that drug is delivered properly at the specific site. NPs are very small in size and as their size can be controlled, so they get easily penetrated in the body of the cells and are also very reactive with the biological system (Zhang et al., 2010). The use of NPs for the drug delivery system in place of conventional system has many advantages over the commercial system (Ranghar et al., 2014) (1) As nanoparticles do not show any chemical reactions, so drug is preserved and is delivered properly without any loss. (2) The bioavailability of the drug is enhanced for the prolonged period at any specific site. (3) The therapeutic efficiency of the drug is enhanced because of its sustained and controlled delivery at the infection site. (4) The degradation profile and release of drug can be modified easily by altering the size of NP to the size of drug. (5) The serum solubility of the poor soluble drugs can be improved and multiple drugs can also be delivered in the same cell for the purpose of combined synergistic therapy.

Various studies has been conducted to study the use of biologically synthesized NPs in the drug delivery system. It has been studied by Ahmad et al. (2019) that magnetic Fe_3O_4 and magnetic greigite Fe_3S_4 are converted to magnetosomes (which are bilayer bound membrane structures) with the help of magnetotactic bacteria. These are used for the encapsulation of drugs and also help to carry them. Tang et al. (2019) studied that magnetosomes that are produced with the help of *Magnetospirillum gryphiswaldense* has been used against cancer in TC-1 mouse models. Biologically produced gold nanoparticles also have the potential to cross the barrier in the blood brain in order to deliver the drugs in the brain (Tripathi et al., 2015). Another gold nanoparticles that are produced from the fungi *Humicola* sp. are used for antitumor drug delivery system (Khan et al., 2014).

7.4.4 Anticancer and medical purposes

Without any kind of drug fill and immaculately biosynthesized NPs have also been widely used to build up anticancer agents (Fig. 7.3) The reasonable mechanism of action of NPs is all the way through DNA impairment, mitochondrial apoptosis and also influenced detention of the cytokinesis (El-Batal and Tamie, 2015). Gold nanoparticles were synthesized from *Streptomyces cyaneus* showed anticancer activity against MCF-7 breast cancer cells and HEPG-2 human liver cancer cells respectively. *Saccharomyces boulardii* is a source for platinum NPs and those were established to be very effectual against MCF-7 breast cancer cell lines and A431 epidermoid carcinoma (Borse et al., 2015). The water extract of endophytic fungi, *Cladosporium perangustum* synthesized the silver NPs and has also been found to lessen the viability of MCF-7 cells via augmentation in the levels of caspase-9, caspase-9, caspase-7, and caspase-3 expression respectively (Govindappa et al., 2020). The ZnONPs were produced from *Rhodococcus pyridinivorans*, laden with anthra-quinone illustrated cell death in HT-29 colon carcinoma cells in comparison to the normal cells and can accordingly find function as an anticancer agent (Vimala et al., 2019). The biomass of fungus *Fusarium oxysporum* is very useful as it was used to synthesize the terbium oxide NPs and was biocompatible and efficient in dose-dependent cytotoxicity in Saos-2 and MG-63 cell-lines although being nontoxic to main osteoblasts; here, the assembly of reactive oxygen species was improved and the apoptosis was verified with NPs action (Iram et al., 2016). Furthermore, as compared to doxorubicin alone, the conjugation of golden particles to doxorubicin, found from *Helminthosporium solani* fungi had elevated uptake as well as equivalent cytotoxicity in the HEK293 cells (Mukherjee et al., 2017). Similarly, both the gold and gadolinium oxide NPs from *Humicola* species could be conjugated to doxorubicin or taxol for anticancer applications (Lu et al., 2016; Capuzzo, 2021). Another, noteworthy study used the magnetotactic bacteria for obtaining the magnetic NPs (biomineralized) directed by MRI to change the energy from infrared light to heat thus ensuing in the ablation of tumor cells with no toxicity. This process was known as a photothermal effect where, the bacterial NPs acted as theranostic (Chen et al., 2016). In one more finding, the bacterial magnetosomes were inoculated in BALB/C mouse and examined for their immune response, but here no significant response was obtained, thus

demonstrated their effectual drug delivery potential (Shen et al., 2020). Endophytic extract was used for the synthesis of gold nanoparticles by using *Cladosporium* sp. (Myco AuNPs) those were isolated from *Commiphora wightii*. These Myco AuNPs possess very strong *in vivo* antitumor activity and persuasive *in vitro* cytotoxicity with excellent catalytic activities such as degradation of Rhodamine-B and Methylene blue in consequence proving versatile functioning (Munawer et al., 2020). Leaf extract of *Pongamia pinnata* were also used for the synthesis of AuNPs and the characterization was done using different analytical techniques such as SEM, TEM, UV–visible spectroscopy etc. The anticancer activities of these gold NPs were studied in HeLa cells by numerous assays and outcomes were significant with reduction in the capability of wound healing, alteration in cell morphology and also inactivation of mitochondria due to loss of membrane potential. In the results, the observation was made in AuNPs as these showed dose-dependent toxicity towards the HeLa cells and also nontoxic in response to the human embryonic kidney cell line (HEK293) (Khatua et al., 2020). The gold ions (Au^{3+}) were reduced by using the fruit juice of *Citrus macroptera* and stabilized as the AuNPs. These CM-AuNPs were used against three different human cancer cell line and their cytotoxic effect illustrated that they are somewhat abler in regulating HepG-2 (liver cancer cell line) growth than MDA-MB 468 (breast cancer cell) and A 549 (human alveolar basal epithelial cell which is adenocarcinogenic). These results confirmed that CM-AuNPs can play role as a potential anticancer agent (Majumdar et al., 2019). *Klebsiella oxytoca* biogenerated the AgNPs where, these AgNPs were embedded in a specific polysaccharide and the generation was done under both aerobic and anaerobic conditions. By using cytotoxic activity, both types of these silver nanoparticles (raised under different conditions) were tested by using MTT assay against the colon (HT-29, HCT 116 and Caco-2) and human breast (SKBR3 and 8701-BC) cancer cell lines. The findings exhibited in terms of IC₅₀ that AgNPs of aerobic conditions were active most, with more prominent effectiveness against the breast cancer cell lines (Buttacavoli et al., 2018). Furthermore, the AuNPs were achieved from *Salacia chinensis* from extract of bark where this extract acted as a green source for the reduction of silver nitrate to AgNPs. Against blood erythrocytes as well as fibroblasts no cytotoxic effect was noticed after use of SCAuNPs and this validated the biocompatible nature of AuNPs (green synthesized). Afterwards, *in vitro* anticancer assay confirmed their role against pancreas (MIA-Pa-Ca-2), liver (HepG2), breast (MDA-MB-231), prostate (PC-3) cancer cell lines, verifies its efficient anticancer action to diminish the limitations of active conventional cancer chemotherapeutics (Jadhav et al., 2018). ZnONPs were synthesized green from *Aspergillus niger* and characterized and evaluated for antimicrobial and anticancer activity. The detailed study of this species demonstrated that ZnONPs can be used for healing application in the future as an anticancer or antimicrobial compound (Gao et al., 2019). Also, by using the solution combustion technique ZnONPs were synthesized from *Ricinus communis* and its antifungal, antioxidant, and anticancer activities were studied (Shobha et al., 2019).

7.4.5 Diagnostic imaging and other medical purposes

In broad-spectrum, the NPs uncover growing applications in diagnostics and also as biosensors which are frequently conjugated to diagnostic enzymes (Ghosh et al., 2018a,b). Furthermore, in imaging the modalities like MRI, the biogenic nanoparticles have also been investigated as biosensors. Numerous Gram-negative magnetotactic bacteria (MTB) synthesizes contrast agents comprising of magnetites in the form of magnetosomes, enfolding crystals of magnetic iron oxides, are very useful in MRI (Uebe and Schüler, 2016). Relaxivity is defined as assess of how susceptible or sensitive a contrast agent is. For very alike compounds, molecule with advanced relaxivity would offer equal contrast at a minor dose compared to a low relaxivity compound. A lower dose may decrease the danger of NP-toxicity. The synthesized NPs exhibit r_2 relaxivity but the bacterial magnetosomes illustrate superior r_2 relaxivity via showing appliance in targeting the human epidermal growth factor receptor-2 (HER2) articulating tumor cells (Anderson et al., 2015). In the orthotopic breast cancer models, the intravenous management of HER2 marking bacterial magnetosomes, demonstrated the increased contrast in Mr signals (Zhang et al., 2018). A treatment of cancer is established via using magnetic NPs of *Magnetospirillum magneticum* AMB-1 strain beneath the supervision of MRI and known as theranostic photothermal therapy was attained both *in vitro* and *in vivo* (Chen et al., 2016). An exciting study engaged magneto endosymbionts as the existing contrast agent in iPSC-developed cardiomyocytes, which could be followed by MRI and cleaned out within one week, resulting in amplified biocompatibility (Mahmoudi et al., 2016). Another finding produced *Magnetospirillum magneticum* AMB-1 strain by generic engineering having RGD-peptide which expresses the magnetosomes and targets the avb3 integrins-overexpressing tumor cells of brain in gliomas as apparent in MRI (Boucher et al., 2017; Zhao, 2017). Afterwards, several bacteriogenic metal NPs such as palladium, copper and gold have also been surveyed for their role and potential in biosensing (Rai et al., 2016; Ghosh, 2018). In a remarkable study, *Candida albicans* synthesized AuNPs, were conjugated to the liver cancer cell surface defined antibodies. Consequently, when these golden particles were used to explore into liver cancer cells, they might distinctively bind to the cancer definite

surface antigen in the liver and thus differentiating them from the common cells. Such kind of NPs could thus locate purpose as a carrier or diagnostic of anticancer drugs (Mioc et al., 2019). Precipitation method was followed to synthesize $\text{Zn}_{0.5}\text{Ni}_{0.5}\text{Fe}_2\text{O}_4$ NPs and also their coating was done with dextrin to enhance solubility and biocompatibility. For more findings, size, structure, morphology and some other properties of these nanoparticles were also examined. Results showed their tremendous paramagnetic properties with slim and very fine size distribution. The use of phantom agar for MRI study demonstrated their capability to use as an efficient contrast agent for imaging (Sattarahmady et al., 2016). Lai et al. (2015), illustrated AuNPs can be used as contrast agents for fine and explainable high resolution 3D X-ray as well as also for fluorescence imaging analysis of the relation between angiogenic (tumor-induced) microvasculature and xenografted glioma cells.

As is obvious, the pharmaceutical applications of microbial-synthesized NPs are much more than above explained. One earlier finding employed the biomass of *Brevibacterium casei* to diminish AgNO_3 and HAuCl_4 to achieve the silver and gold NPs from intracellular removes and these were further additionally investigated and discovered as an anti-coagulant of human plasma (Li et al., 2016). In opposition to the cestode parasite *Raillietina* sp. the gold NPs nanoparticles were derived from fungal species *Nigrospora oryzae*, by the displayed anthelmintic activity (Tomar and Preet, 2017). Whereas by following the hydrothermal method, cell-free supernatant of *Lactobacillus acidophilus* were used in synthesis of antimicrobial carbon dots and these showed antimicrobial activity against *Listeria monocytogenes* (Gram-positive) and *Escherichia coli* (Gram-negative) (Kousheh et al., 2020). Another nano-scale material which is mainly synthesized by bacteria is nano-cellulose. These nano-celluloses are scaffold based and have essential applications in the tissue engineering as in improving, replacing or repairing the injured tissues as well as organs, those counting skin, nerve, heart, blood vessel, skeletal muscle and liver, primarily due to their biocompatibility, optical transparency, water absorption and retention and chemo-mechanical properties (Luo et al., 2019). Many of these nano-celluloses have been also medically approved and accessible in markets in the variety of patents for burn treatment, injury curing and decorative applications (Brown et al., 2015). AuNPs have a good place in research now, by having attention towards nanomedicine for several years due to their physicochemical properties such as biocompatibility; superior stability; advanced magnetic and electronic properties and also trouble-free surface chemistry. All of these properties distinguish gold nanoparticles as advantageous carriers to be exploited. According to the transversal and longitudinal plasmonic resonances in gold nanorods, there is the presence of two absorption peaks and that is why these are the most deliberated anisotropic gold nanoparticles among all. Because, the longitudinal surface plasmonic resonance affords the absorption near-infrared region which is a significant characteristic of gold nanorods for the medical purposes (Onaciu et al., 2019). Besides these, use of silver AgNPs are also many, diverse and promising in biomedical and pharmaceutical. The most developed feature is their anti-inflammatory and antimicrobial capacity. But it is strongly recommended that the higher concentrations of AgNPs are lethal and if released into the environment, can prompt several ecological harms that can further lead to numerous diseases. Varied uses of the silver NPs are in the custom of coatings for medical devices, wound dressings, etc., as there is constant release of silver ions and hence coating of different devices from both outer and inner sides, improves the antimicrobial efficiency (Mathur et al., 2018). Copper nanoparticles (CuNPs-C) were condensed by carbon and synthesized by mixing glucose and cupric chloride in a solution, followed by carbonization. The antibacterial properties of CuNPs-C were characterized by XRD, TEM, nitrogen adsorption and antibacterial activity tests by using the samples. It was found that the layers of carbon are fully responsible for seizing the bacteria and copper ions on getting loose from the copper nanoparticles kill these bacteria. Additionally, the outer carbon layers defend the metallic copper inside from oxidation very efficiently. These outcomes specify the role of CuNPs-C in biomedical as a stable antibacterial agent (Chen et al., 2019). ZnONPs and Ag-ZnO-NPs were biosynthesized and characterized by using infrared and ultraviolet–visible spectroscopy, high resolution electron microscopy and X-ray diffraction etc. The results were so positive as Ag-ZnO-NPs showed more developed antimicrobial potential than ZnONPs. These NPs directed the stimulating biological properties and should be subjected to more research to find out their pharmacological significance (Hameed et al., 2019).

7.5 Challenges associated with microbial synthesis of nanoparticles: a possible path to solution

Nanotechnology is an integrative field that requires close collaboration between engineers, scientists, and others. To discover and develop possible treatment possibilities, chemists and biologists collaborate and use microorganisms to synthesize NPs has sparked a lot of curiosity. The growing interest of NPs in biology and medicine has necessitated the development of low-cost, simple, and environmentally friendly microbial-synthesized NPs preparation methods. Microbial-synthesized NPs have emerged as an ideal agents for biomedical applications because of their unique

physicochemical, optical, electrical, and biological properties (Mody et al., 2010). In order to design the microbial-synthesized NPs, the fundamental challenge is the selection of the most appropriate options, based on the inherent qualities of bacteria such as growth rate, replication, and metabolic activity. Another key criterion for achieving the required therapeutic effects of biosynthesized microbial-synthesized NPs is controlling the form, size, and monodispersity, which can be controlled by adjusting biomolecule concentrations, reduction time, temperature, and other variables. Uncovering the critical elements required for the synthesis and stability of microbial-synthesized NPs from precursor salts from a broad pool of biomolecules acquired from microbial resources as well as maintenance of optimal conditions for optimum growth is also a challenging task. The effectiveness of microbial-synthesized NPs biosynthesis utilising microorganisms is considerably improved by optimizing circumstances such as providing critical nutrients for development, the quantity of the inoculum, the temperature, the pH, and the amount of light (Korbekandi et al., 2009). Another set of significant characteristics is to consider the yield of the production. However the production yield with the biosynthesis method is $1/3^{\text{rd}}$ of the yield produce from chemical processes. Synthesis of microbial-synthesized NPs by bottom-up or top-down methods also differs greatly from industrial manufacture. As a result, it is reasonable to assume that, in the long term, the overall production cost of NPs can be decreased by roughly a tenth when compared to chemical synthesis methods as it does not require any organic solvents, heat or any chemical agent and any tedious technique. It is noteworthy to mention that when microbial-synthesized NPs are produced by chemical synthetic methods were compared with biosynthesis method, they showed that biosynthetically synthesized are more stable and agglomeration-free, even at ambient temperature for lengthy periods of time. To successfully transfer this bioinspired technique of Microbial-synthesized NPs synthesis for large-scale industrial production, a number of significant difficulties and technological aspects must be addressed (Ovais et al., 2018a,b).

7.6 Conclusion and future perspectives

Nanotechnology is the most promising field of science that offers abundant applications in present times. Metal and metal oxide based NPs are widely synthesized for agricultural, biomedical, and industrial sectors along with the motive for environmental remediation. In general top-down and bottom-up strategies are used for NP-synthesis but these methods prove to be toxic. NP-synthesis by microbes prove to be advantageous over traditional methods. Basically, two different protocols are classified as extracellular and intracellular for NP-synthesis. The diverse biomolecules secreted by microbes are involved in reduction reactions. The reductases enzymes such as nitrate reductase play a vital role for reducing metal ions into NPs. The biosynthetic processes of metal NPs are also safe and eco-friendly. Moreover, the microbial-synthesized NPs are emerging in biomedical as well therapeutic use owing to their unique characteristics. These methods act as beneficial due to the fact that NPs are coated with biomolecules or lipid bilayers for physiological solubility and stability. Certain factors should be kept in mind during the biosynthesis namely, organism type, genetic factors, optimal growth conditions, enzymatic reactions, reaction conditions as well biocatalysts.

The green synthesis of NPs with the aid of microbial entities namely, bacteria, viruses, fungi, actinomycetes, algae, yeasts etc. are gaining attention and considered substantial part of biotechnology. Synthesis of NPs by green technology is advantageous in many aspects in terms of cost-effectiveness, easy scale-up method, environmental friendly, thereby, overcoming all the limitations of chemical and physical methods. To enhance our understanding about green routes of NP-synthesis essential biotechnological considerations should be kept in mind. The primary methods for green NP-synthesis possess different utilities as antimicrobial, antitumor, remediation purpose, biocontrol of pathogens as well in textiles and food industries. The metal NPs synthesized for remediation purposes are extensively reviewed for commercial applications. Research has been mostly inclined for regulation of particle size, shape and other morphological attributes of NPs along with easy protocols for inducing yield and stability of NPs by microbes. Future research is mainly aimed for understanding the mechanistic pathway, various cellular, biochemical processes and industrial scale production and applications of NPs in more detail. The future exploration is also focused on screening the appropriate microbial species for the synthesis of high quality and productivity of NPs. So, better optimization attributes, reaction parameters and improved stability of NPs along with explicating microbial communities for NP-synthesis is mainly oriented. This is the crucial practice that also covers the sustainability aspect and offers a lot to explore further.

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Microorganism assisted synthesized metal and metal oxide nanoparticles for removal of heavy metal ions from the wastewater effluents

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8.1 Introduction

The removal of metal toxicants from water sources using low-cost materials is a challenging task especially in the developing world. The modern and technological advanced human race is facing one of the major threats to its survival in the form of environmental pollution especially heavy metals induced pollution (Ali and Khan, 2017; Ali et al., 2013; Hashem et al., 2017). The rates of dispersal and mobilization of heavy metals in the ecosystem have escalated after the industrial revolution (Khan et al., 2004; Merian, 1984), both intense urbanization and industrialization are responsible for the environmental pollution. Metals are widespread in the aquatic environment emanating from both natural and anthropogenic sources.

Although clean and fresh water is necessary for survival of all beings but the different types of pollution have degraded the water resources world-wide when the demand for water has been increasing ever year (UN-Water, 2018). It is estimated that water demand globally would increase by 50% by 2030 (<https://inweh.unu.edu/projects/water-related-sustainable-development-goals>). The sustainable developmental goal of water is “universal access to safe drinking water, sanitation and hygiene, improving water quality, sustainable use of water resources, improved water quality and waste water management leading to reduced risk of water related disasters” (<https://www.un.org/waterforlifedecade> (2015)). Exposure of water containing toxins or pathogens can cause harmful effects on humans, even if polluted water is used for recreational purposes (Schwarzenbach et al., 2010). The health of the people living in the developing countries are generally affected by using contaminated water directly due to absence of resources to treat polluted water or lack of access to clean drinking systems. According to the World Health Organization almost 1.6 million people die annually from water-related diseases which are preventable and majority (90%) are children below 5 years (Pandit and Kumar, 2015). Moreover, 844 million are lacking in basic drinking water source (World Health Organization, 2017).

The drinking water resources are also getting proliferated with heavy metals along with microbial agents which is posing a considerable challenge to the sustainable development water goal. The water bodies are getting contaminated by untreated effluents released from various industries, power plants using coal (Demirak et al., 2006), mining (Archundia et al., 2017), and solid waste disposal (Perkins et al., 2014; Wu et al., 2015). Contaminated urban stormwater and agricultural runoff, acidic rain water are additional contributors to the burden of water pollution (Lye, 2009). The toxic effects and associated health hazards of heavy metals have been documented by many workers (Chakraborty et al., 2013; Gleason et al., 2016; Järup, 2003; Schwartzbord et al., 2013). Common heavy metals found in contaminated water in the developing countries are Pb, Cd, As, Cr, and Hg. Since the heavy metals are hazardous scientists and researchers are in pursuit of developing effective methods of

removing these metal toxicants from drinking water sources, and wastewater emanating from industries and other sources.

The various methods explored include electrocoagulation (Bazrafshan et al., 2015), microbial remediation (Rajendran et al., 2003), activated carbon adsorption (Agarwal and Singh, 2017), membrane filtration and carbon nanotechnology (Xu and Chai, 2018) to name a few. All these methods have some shortcomings and hence this chapter focusses on cost effective nano bioremediation method of removing heavy metals from waste water. The use of nanomaterials has been effectively applied in other fields like catalysis, electronics, agriculture, medical science, etc. Waste water can be purified at a low cost, with high efficiency and reusability using the nanotechnology. To resolve the water crisis world-wide researcher have used nanomaterials in waste water treatment due to its exemplary properties like size, great surface area, hydrophilicity, mechanical strength, strong mobility in solutions, dispersibility, etc. Studies have shown that by use of nanoparticles both organic and inorganic pollutants, microbes and heavy metals like Pb, Mo, etc. have been successfully removed (Umar, 2018; Kalhapure et al., 2015; Fang et al., 2017; Yang et al., 2019; Mallikarjunaiah et al., 2020; Parvin, et al., 2019; Fang et al., 2018; Tahoon et al., 2020). Fig. 8.1 shows the utility of metal-based nanoparticles in environmental clean-up.

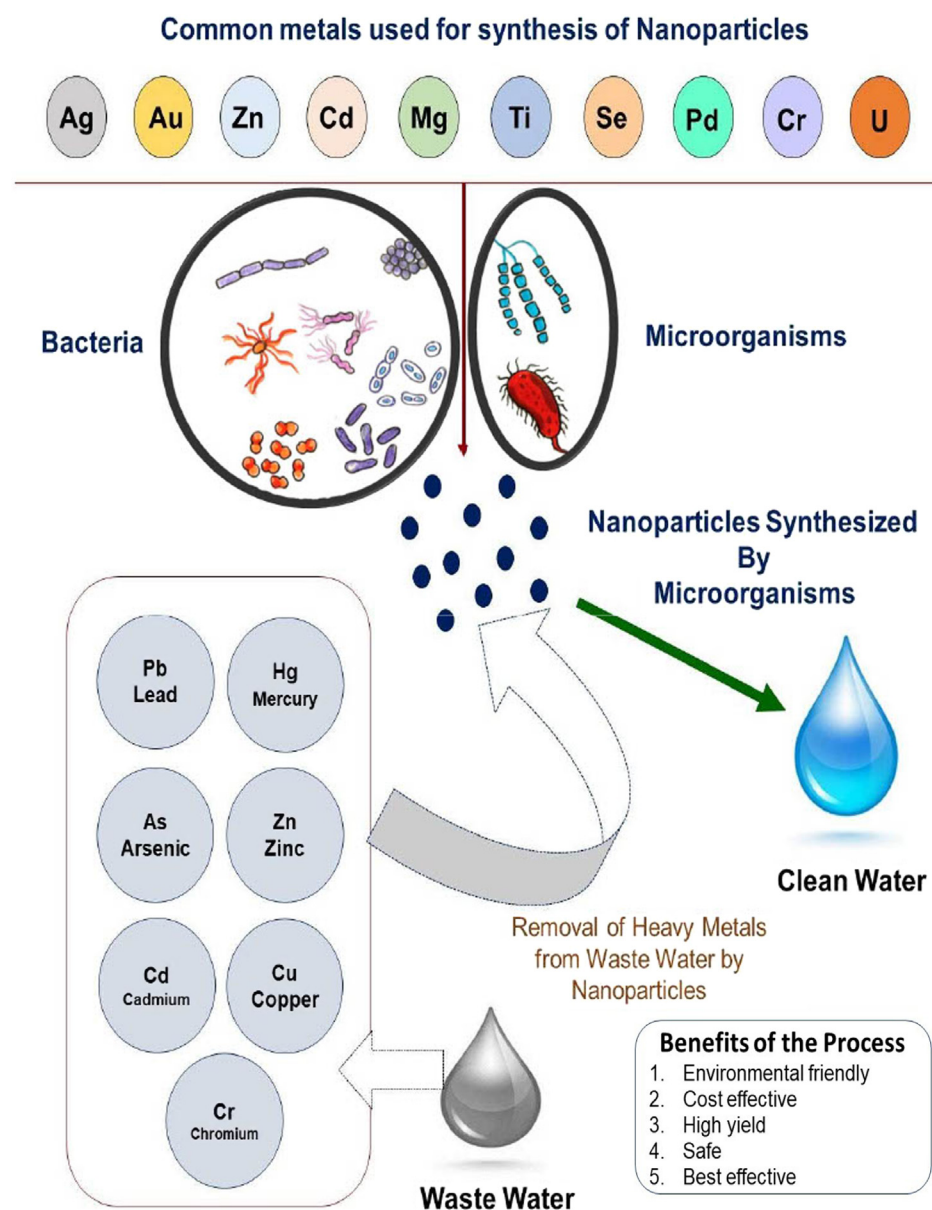


FIGURE 8.1 Metal-based nanoparticles and its application in toxin removal.

8.2 Metals and their requirement for existence

8.2.1 Definition of metals

Heavy metals have been defined based on various parameters such as density, atomic number or weight, toxicity or some chemical properties. The common definition is based on density, if elements have density more than 5 g/cm^3 they are termed as heavy metals. This group includes elements lighter than carbon and excludes some of the heaviest metals too. According to Duffs et al. (2002) the term heavy metal has no meaning, he further concluded that the use of the term heavy metal and the density has no relation. Arsenic is also included in heavy metals although it is a metalloid which implies heaviness is associated with toxicity, has ability to produce toxicity at low levels. Some metals have biochemical and physiological importance in living organism and their scantiness or excessiveness leads to metabolic disturbances and various diseased conditions.

8.2.2 Classification of heavy metals

On the basis of the biological activity of the metals in living organisms they are classified as essential and non-essential. The metals required for indispensable biological functions are termed as essential heavy metals namely Cu, Zn, Ni, Fe, Mn (Cempel and Nikel, 2006; Göhre and Paszkowski, 2006; Mertz, 1981) whereas those heavy metals which are dysfunctional for any biological functions like As, Pb, Cd, Cr and Hg (Clemens et al., 2002; Dabonne et al., 2010; Kärenlampi et al., 2000; Peng et al., 2009; Sánchez-Chardi et al., 2009; Suzuki et al., 2001) are non-essential heavy metals.

On the basis of their usage in the biosorption field (Bishop, 2002; Volesky, 1990) the metals are classified into three categories namely toxic metals, precious metals and radionuclides. Pb, Cd, Cr, Hg, As, Co, Cu, etc. are major toxic metals. Pt, Ag, Au, Ru are precious metals whereas U, Th, Ra, Am are the radionuclides. According to some researchers from China, the five heavy metals namely Pb, Cd, As, Cr and Hg are termed as the highest priority pollutants for control and prevention of pollution (Fu et al., 2017). Other heavy metals are toxic and their toxicity is dependent on dose and period of exposure.

8.2.3 Sources of heavy metals

Naturally, heavy metals are components of earth's crust and sources of heavy metals in the environment are both natural and anthropogenic. Volcanic eruptions and weathering of rocks containing the metals are the natural sources of heavy metals. The anthropogenic sources are varied and include emissions from industries, burning of fossil fuels, mining, smelting and agricultural activities.

8.2.4 Adverse effects of heavy metals

Heavy metals are non-biodegradable, they bioaccumulate, enter the food chain and are associated with various health disorders and chronic exposure is a major challenge to living beings (Fu and Xi, 2020; Wieczorek-Dąbrowska et al., 2013). The fertility of the soil is affected by presence of heavy metals above the threshold values which in turn disturbs the microbiological balance of the soil (Anyanwu and Orisakwe, 2020; Barbieri, 2016). The higher concentration of heavy metals in the biome of riverine ecosystem adversely impacts the health of aquatic species (Malik and Maurya, 2014) as they are neurotoxins and some heavy metals are responsible for causing deformities in the fishes (Sfakianakis et al., 2015). The central nervous system, cardiovascular system, gastrointestinal tract and renal systems all are impacted by heavy metals (Sarker et al., 2020). Various parameters like metal speciation, duration of exposure, type of heavy metals involved and age of the individual are important determinants in severity of toxicity due to heavy metals. The five commonly occurring toxic heavy metals Pb, Cd, Hg, As and Cr have been discussed below:

8.2.4.1 Lead

Lead is one of the most common heavy metal and has been in use for over 5000 years. It occurs naturally and found in combination with other elements like sulfur and oxygen. In early days it was used as materials for buildings, pigments for ceramic glazing, decorative fixtures, ammunitions, brass and in water pipes. With growth in population and economy the usage of lead increased and lead is used in lead acid-batteries, cable sheaths, machinery manufacturing, shipbuilding, light industry, lead oxide, radiation protection and other industries (<https://www.usgs.gov/centers/national-minerals-information-center/lead-statistics-and-information>). The annual consumption of lead in the year 2003 was

10.3 million tons. In the year 2012, China with 44.5% of total consumption in the world was the largest consumer followed by US with 15%, 4.8% India and 4% South Korea. Lead exists in organic as well as inorganic form and in several valence states ranging from 0 to IV represented as Pb (0), Pb(I), Pb (II), and Pb (IV). Lead enters water bodies from various industrial effluents, mining, smelting discharges or dissolved lead from old leads plumbing. Inhalation, ingestion, transfer via placenta and dermal contact are the routes through which lead is absorbed by the body. Young children absorb lead approximately 4–5 times more compared to adults with a biological half-life in blood and bones is 16–40 days and 17–27 years respectively. It is an accumulative toxin for the body and impacts the kidneys, central nervous system and the gastrointestinal tract. In children exposure to lead lowers IQ, impairment in development, short period of attention and mental retardation. Other symptoms observed in adults are irritation, nausea, memory loss, joint weakening, and inhibition of heme synthesis and in severe cases encephalopathy. The fall of mighty Roman empire is linked to lead poisoning. Organic lead is more toxic than inorganic lead and once in the body it replaces the calcium in the bones and remains there for long-term release.

8.2.4.2 Cadmium

Cadmium is naturally found in ores along with other elements, it is divalent, and resembles zinc. It is used in plastics, paint pigments, silver-cadmium batteries, electroplating, photography, transportation equipment, machinery and baking enamels. It is being used in manufacturing of solar cell and thin-film photovoltaic containing telluride. Cadmium effects the kidneys causing nephrotoxicity, infertility, alteration in calcium metabolism, physiological and gastrointestinal disorders, deficiency of the immune system, DNA impairment, osteoporosis, renal dysfunction and Itai-Itai disease. An estimated 23,000 metric tons of total global refinery cadmium was produced in 2020 which shows the use of cadmium products are expanding. Cadmium is discharged in the water body due to electroplating industries, nickel-cadmium battery industries, smelting operations and municipal waste. Volcanic eruptions and weathering of rocks also release cadmium in the environment and it can travel long distance before settling down. The uptake and eventually the toxicity is affected by environmental factors like temperature, salinity and acidity. With increase in temperature, uptake increases and thus the toxic impact whereas with increase in salinity or hardness the uptake decreases. The organisms in fresh water are affected even at low cadmium concentration compared to marine ones. Cadmium once absorbed remains in an organism for many years, it is bio persistent. Cadmium reduces the enzymatic activities of many enzymes such as alcohol dehydrogenase, arylsulfatase, delta-aminolaevulinic acid synthetase whereas activities of enzymes such as pyruvate dehydrogenase, pyruvate decarboxylase and delta-aminolaevulinic acid dehydratase are enhanced. The chronic accumulation of cadmium in the kidneys is associated with renal dysfunction and is a major threat to human health, other toxic symptoms being lung emphysema and proteinuria. Cadmium enters the body through inhalation and ingestion. Plants absorb cadmium through their roots or deposition of cadmium containing aerosols on plant surfaces. One of the most absorbed metals by living cells is cadmium and accumulated by plants.

8.2.4.3 Mercury

Mercury is liquid at room temperature and rare to find it in its natural state. Its common ore is cinnabar with highest concentration of mercury is found in sedimentary rock and lowest in igneous rock. It finds application in thermometers, pharmaceuticals, dental preparation, fungicides, in ultraviolet and fluorescent lamps. Significant amount of mercury is used in paper and pulp industry in the form of phenyl mercuric acetate and in caustic soda. The disease caused by mercury poisoning is Minamata and it has been responsible for many deaths around globe and crippled many. The salts and inorganic compounds of mercury are formed when mercury combines with sulfur, chlorine or oxygen and on combination with carbon forms organic mercury compounds. Mercury exists as monovalent (Hg_2^{2+}) and divalent ions (Hg^{2+}) along with the most reduced metal form Hg^0 . The organic compounds of mercury especially the methyl mercury is one of the most deadly substances in our environment. It is persistent in the environment and bioaccumulates in fishes, animals and humans. The extent of toxicity is dependent on the redox state of mercury and the type of compound. Mercury interferes with the electron transport in mitochondria and chloroplasts thereby affecting the photosynthesis and oxidative metabolism in plants. Mercury poisoning causes neurological and renal disturbances and affects the brain. Mercury inhalation causes lung and eye irritation, damages DNA and chromosomes, congenital and learning disabilities, etc. The inorganic mercury gets converted to organic mercury compounds by the action of microorganisms, methyl mercury being the most common. Methyl mercury has affinity for lipids and can distribute to the fatty tissues. Mercury gets solubilized in aquatic environment and reaches the higher organisms of the food chain through incorporation in the lowest ones and biomagnifying through successive trophic. Thus, even a small amount of mercury contamination could be sufficient to cause significant health hazards. The extent of toxicity due to mercury is dependent on the

speciation of mercury, type of mercuric compound, ionization potential, route of administration, duration and levels of interacting elements like selenium in the diet. Kidney is the primary target organ for accumulation of inorganic mercuric compounds.

8.2.4.4 Arsenic

Arsenic is ubiquitous element and exists in two inorganic forms as trivalent arsenite and pentavalent arsenate and as monomethylarsenic acid, dimethylarsinic acid and trimethylarsine oxide in organic forms. The sources of arsenic pollution are natural and anthropogenic, the former includes soil erosion and volcanic eruptions and later includes manufacturing and industrial processes which use arsenic and its compounds. Arsenic compounds have medical applications too. FDA had approved arsenic trioxide an anticancer agent in treating acute promyelocytic leukemia (Rousselot et al., 1999). A large population in countries like Chile, Mexico, Taiwan, Bangladesh, Uruguay and India have been affected by arsenic toxicity due to presence of high content of arsenic in ground water. The route of exposure can be dermal, ingestion, inhalation and parenteral in some instances (Tchounwou, 1999; Centeno et al., 2006; Rousselot et al., 1999). The level of arsenic in soil naturally is found to vary between 1–40 mg/kg which increases to a higher-level due waste disposal or application of pesticides (Tchounwou et al., 2004). People are exposed to arsenic mostly through the food intake averaging almost 50 µg each day compared to intake through water, soil or air except in places where contamination due to arsenic is high. The workers who use arsenic compounds in their occupation are also at risk of high exposure. Arsenic is implicated in various health effects including cancer. It affects the nervous, renal, respiratory, hepatobiliary cardiovascular and gastrointestinal systems (Tchounwou et al., 2003). The chemical speciation of arsenic along with time and quantity are determinant factors in severity of health effects (Yedjou et al., 2006). It has been reported that inorganic arsenic causes toxicity in humans and the toxicity of trivalent arsenite is almost 2–10 times more compared to the pentavalent arsenate. Arsenic has affinity towards sulfur and bind to the sulfhydryl group or thiol on proteins inactivating more than 200 enzymes effecting many organs and their systems. Phosphate is required in various biochemical pathways which can be replaced by pentavalent form of arsenic interfering with many essential syntheses. The inorganic arsenic is methylated and this is the primary metabolic pathway, arsenic trioxide is first converted to monomethylarsonic and then to dimethyl arsenic acid before being excreted in urine and the process of methylation is non-enzymatic.

8.2.4.5 Chromium

Chromium compounds are colored as chroma means color in Greek and their oxidation state varies from chromium (II) to chromium (VI) (Jacobs and Testa, 2005). Exposure to chromium can happen through skin contact, breathing, eating or drinking substances containing chromium. Cr (III) is most stable and occurs in the form of ores namely ferrochromate in nature and Cr (VI) is the next most stable form. Both natural and anthropogenic sources release chromium into the different components of environment like soil, air and water which ultimately is the cause of various toxic effects on plants, animals and humans. The tanneries, stainless steel welding, metal processing and pigment industries release chromium in hexavalent state in the environment, which has been categorized as a human carcinogen (US EPA, 1992). The oxidation state of chromium is important to ascertain the toxicity levels which ranges from low to high, the hexavalent state causes highest toxicity. Chromium compounds are commercially used in chrome plating, pigments and dyes, preservation of wood, tanning and industrial welding as well in boilers and cooking systems. Humans are exposed to chromium by the intake of food and water containing chromium and also through inhalation which is means of occupational exposure. In trivalent state chromium has a regulatory role in metabolism of fats, proteins and glucose. Although the main exposure route is inhalation primarily affecting the respiratory system especially the lungs but exposure through skin also takes place (Shelnutt et al., 2007). Workers in construction sector have been found to suffer from dermatitis which is attributed to chromium exposure (Shelnutt et al., 2007). Hexavalent chromium compounds are implicated in toxicity of various organs namely kidneys and lungs in human as well allergic reaction, irritation in the nose, ulcers in nose and respiratory tract cancer (WHO/IPCS, 1988). High doses of hexavalent chromium can result in chronic hepatic, renal, respiratory, gastrointestinal neurological and cardiovascular effects leading to death (Agency for Toxic Substances and Disease Registry ATSDR, 2008). The differences in toxicity of trivalent and hexavalent chromium are dependent on the solubility. Hexavalent chromium can cross the cell membrane and gets reduced to reactive intermediates but trivalent chromium is absorbed poorly. Major factors governing the toxicity of chromium compounds are oxidation state and solubility. Table 8.1 summarizes the sources of some of the heavy metals (Zn, Hg, As, Cd, Cu, Cr, Hg, and Pb) their effects on humans and microbes, and standard permissible limit (Malik, 2004; Dixit et al., 2015; Sankarammal et al., 2014; Cervantes et al., 2001; Fashola et al., 2016; Prabhu and Poulouse, 2012).

TABLE 8.1 Heavy metal sources, their effects on humans and microbes, and standard permissible limit.

1.	Zinc (Zn)	Sources	Cosmetics, paint, toys, furniture, air and soil, rubber products, aerosol, deodorant
		Clinical effects	Stomach pain, Skin edema, anemia, convulsion, ocular damage, vomiting, and headache
		Standard permissible limit (μg)	3000
		Effects on Microbes	Biomass alteration, inhibition of growth, death
2.	Mercury (Hg)	Sources	Industrial runoff, electronic devices, medical devices, coal combustion, cosmetics, medicines, pesticides, laboratory chemicals and apparatus, insecticide and fungicides
		Clinical effects	Effects on immunological system, reproductive system, circulatory system, cardiovascular system and GI system. Deposition of Hg in brain causes death
		Standard permissible limit (μg)	6
		Effects on Microbes	Membrane damage, inhibition of enzyme action, alteration in protein structure
3.	Arsenic (As)	Sources	Drinking water, electronic equipment, glass apparatus
		Clinical effects	Deleterious effects on kidney, liver, lung, skin, brain. Damage in cardiovascular system. Immunological alteration and hormonal imbalance
		Standard permissible limit (μg)	10
		Effects on Microbes	Inhibition of enzymatic action, nuclear damage, alter transcription processes
4.	Cadmium (Cd)	Sources	Fossil fuel combustion, Iron and steel production, plastics, battery, galvanized pipes, paint
		Clinical effects	Immunological alteration, effects on cardiovascular system, hepatic system, renal system, GI system, neurological system and reproductive system
		Standard permissible limit (μg)	3
		Effects on Microbes	Inhibit cell division, denature protein, nucleic acid damage
5.	Copper (Cu)	Sources	Electronic machineries, battery, plumbing, cabal apparatus, ayurvedic medicine
		Clinical effects	Effects on hematological system, neurological system, renal system, immunological system, respiratory system, hepatic system
		Standard permissible limit (μg)	2000
		Effects on Microbes	Inhibition of enzymatic activity, suppression of cellular growth and function
6.	Chromium (Cr)	Sources	Food sources like broccoli, liver and brewer's yeast. Potatoes, whole grains, seafood, and meats Tanneries and Steel and pulp mills
		Clinical effects	Effects of GI system, reproductive system, renal system, immune system
		Standard permissible limit (μg)	50
		Effects on Microbes	Breakdown of protein structure, nucleic acid damage, cell membrane damage
7.	Lead (Pb)	Sources	●Paint, air and soil, folk medicines, ayurvedics, and cosmetics, Children's jewelry and toys, Workplace and hobbies
		Clinical effects	Effects on renal system, immunological system, respiratory system, hepatic system, hematological system, neurological system
		Standard permissible limit (μg)	10
		Effects on Microbes	Inhibit protein and enzyme action, inhibit transcription process

8.3 Nanotechnology and environmental remediation

Eutrophication along with heavy metals, agro-industrial waste and chemicals have endangered the health and safety of the ecosystem and humans by polluting the water bodies. As a result, the need of the hour is to restore and remediate the environment of pollutants which is a daunting and challenging task (Chauhan et al., 2020). Bioremediation has become one of the most preferred technique for restoration of the environment (Gouma et al., 2014; Adams et al., 2014; Adenigba et al., 2020). Bioremediation technique together with nanoparticle incorporation helps in breaking and reducing pollutants to substances in favorable form which could respond to natural biodegradation (Cecchin et al., 2017; Singh et al., 2018). Nanobioremediation is the method of removing the multifarious contaminants like organic or inorganic pollutants and heavy metals from the environment with the help of nanoparticles which are produced by microorganisms or plants using both biological and physicochemical methods (Goutam, and Saxena, 2021; Deshpande et al., 2020; Sachan et al., 2021). Nanobioremediation is gaining importance as a versatile technique for contaminant removal from the environment keeping the agenda of sustainability (Koul and Taak, 2018). Fig. 8.2 illustrates the uses of nanotechnology in the various applications related to clean-up, energy savings and other fields (Annu et al., 2018)

8.3.1 Advantages of conventional treatment methods

Scientists and researchers have been working extensively to develop efficient and low-cost technologies with focus on addressing the environmental challenges. Various techniques have been developed and continuously modified for treating wastewater, ground water, leachate and soil using ex situ (Tomei and Daugulis, 2013) and in situ (Jørgensen, 2007) methods. Ex situ methods classically involve removal of toxicants and treating through conventional techniques which are intensive energy requiring making it a costly process. Moreover, the residual contaminated substances need further treatment and disposal. Compared to the ex situ processes, the in situ methods utilize the nanomaterials for the treatment and eliminating the need of transportation to other places for further treatment and disposal. The nano size and

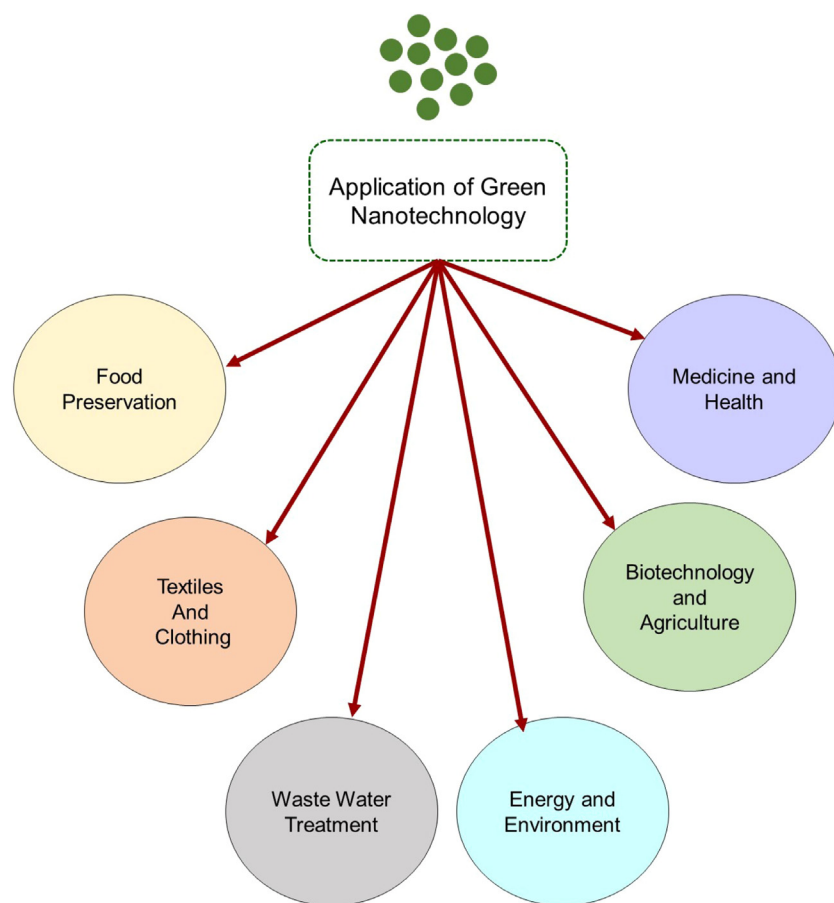


FIGURE 8.2 Application of nanotechnology in various fields.

novel surface fabrication which allows the particles to invade the subsurface of the contaminant, increasing the mobility and achieving wide spread distribution (Tratnyek and Johnson, 2006). Therefore, soil is not required to be transported to other sites in situ for furthering processing in comparison to the ex situ method. (Otto et al., 2008). The nanomaterials are reactive, have better mobility and can be produced from various bulk-materials (Davis et al., 2017). The nano-bioremediation works out to be effective, low-cost and eco-friendly technique to remove environmental toxicants (Fang et al., 2019). Various studies have documented the role played by nanoparticles in environmental clean-up (Adam et al., 2021; Aloulou et al., 2020; Baragaño et al., 2020; Khan et al., 2020; Nizamuddin et al., 2019; Patil et al., 2016; Tamjidi et al., 2019; Yadav et al., 2020). The synthesis of nanoparticle using microorganism like bacteria (Fig. 8.3) and fungi

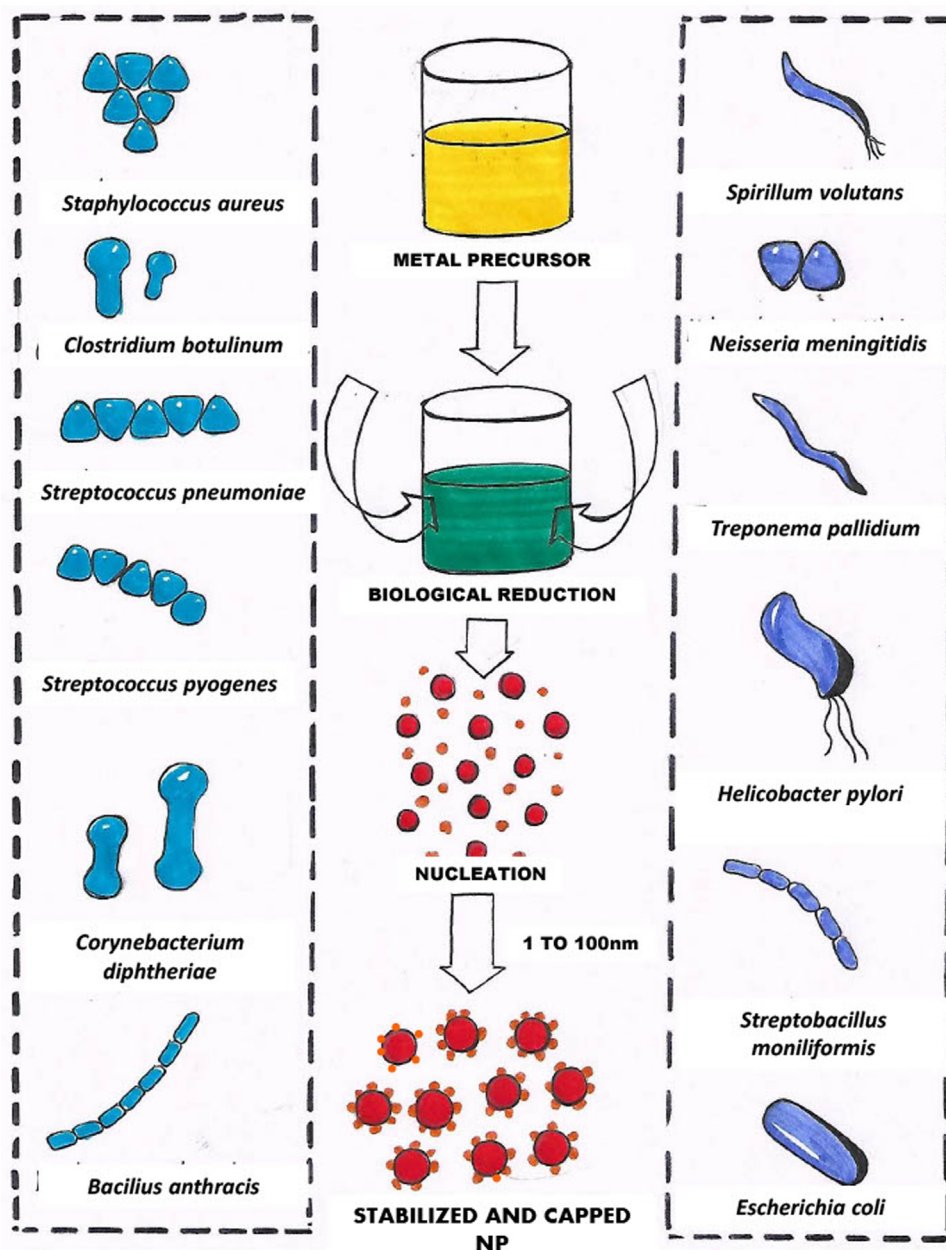


FIGURE 8.3 Representative diagram of metal-based nanoparticles synthesized by using microbes.

Metal Based Nanoparticles synthesized by using Bacteria

would be effective, safe, and an alternate method for remediation (Corral-Bobadilla et al., 2019; Durán et al., 2007; Rajendran and Gunasekaran, 2007).

Several workers have documented positive results in environmental remediation by using microbes assisted nanoparticles (Agam et al., 2020; Ibrahim et al., 2019). The unique properties of microbes like filtering, super-hydrophobic nature, high surface to volume ratio, adjustable functionality, and sensitive affinity membranes have led to the acceptance of these organisms in nanoremediation (Wang et al., 2016). The microorganisms help in degrading, detoxifying and transforming the pollutants to less hazardous waste form which gets easily degraded naturally (Joutey et al., 2013). The process to obtain the desired characteristics like shape and size of the nanoparticles can be optimized to get the requisite output (Irvani, 2014).

8.3.2 Bacteria in nanoparticle synthesis

Bacteria are endowed with extraordinary potential of reducing heavy metals and hence they are chosen for synthesizing nanoparticle. Some bacterial strains for instance *Pseudomonas aeruginosa*, *Pseudomonas stutzeri* (Bridges et al., 1979; Haefeli et al., 1984) can survive at high concentration of metal ions. As observed by some researchers that some bacterial species (*Sulfolobus acidocaldarius*, *Thiobacillus thiooxidans*, *Thiobacillus ferrooxidans*) have the remarkable capability of reducing Fe (III) to Fe (II) state while using elemental sulfur as energy source (Brock and Gustafson, 1976). The forward reaction which is the reduction of ferric ion to ferrous was carried out by *T. thiooxidans* under aerobic conditions and at low pH but the backward reaction which is reversible conversion of ferrous to ferric state was not feasible by *T. thiooxidans*. *T. ferrooxidans* was not able to reduce ferric iron under aerobically as presence of oxygen favors the reoxidation of ferrous ion (Brock and Gustafson, 1976). The other organisms like *Escherichia coli* K12, *Shewanella putrefaciens* and *Geobacter metallireducens* are involved in the formation of tellurium and direct enzymatic reduction of Tc (VII). The cells of *Bacillus cereus*, *B. subtilis*, *E. coli*, and *P. aeruginosa* are capable of binding large quantities of metallic cations like Ag⁺, Cd²⁺, Cu²⁺, and La³⁺ from solutions as reported by some researchers (Mullen et al., 1989). Nanoparticles of silver, gold, zinc, copper, etc. have been explored for their reactivity, cost-effectiveness, specificity, and eco-friendliness. Table 8.2 shows the various nanoparticles synthesized by different species of bacteria.

8.3.2.1 Silver nanoparticles

Bacillus subtilis has been used for green biosynthesis of silver nanoparticles (~5–50 nm) by group of researchers (Bhuyar et al., 2020; Saifuddin et al., 2009) using microwave radiation for uniform heating which helped in producing no clumping and aggregation by digestive ripening of particles. The synthesis of silver nanoparticles (~40 nm) was done extracellularly by using the culture supernatant of *Bacillus licheniformis* which reduced the aqueous Ag⁺ ions has also been documented (Kalishwaralal et al., 2008). Other researchers have also used *Bacillus licheniformis* to prepare nanoparticles of silver (~50 nm) (Kalimuthu et al., 2008). Some workers have studied the synthesis of silver nanoparticles (~14.6 nm) using silver nitrate, cell-free extracts of *Bacillus amyloliquefaciens* and irradiating with solar light (Wei et al., 2012). The synthesis of silver nanoparticles could be influenced by the intensity of solar light, concentration of extract (Gupta et al., 2020), and addition of sodium chloride. Other microorganisms like *Bacillus cereus* were used in the synthesis of silver nanoparticle (~20–40 nm), these microbes were isolated from the *Garcinia xanthochymus* (Sunkar and Nachiyar, 2012).

8.3.2.2 Gold nanoparticles

When the bacterial cells of *Bacillus subtilis* 168 were incubated with gold chloride under ambient pressure and temperature it was observed that the Au³⁺ ions were reduced to octahedral gold nanoparticles (~5–25 nm) (Lee et al., 2020; Beveridge and Murray, 1980; Southam and Beveridge, 1994). Although the microorganism used are same i.e. *B. subtilis* but the process of reduction is different in chloroaurate and silver ions for synthesis of gold and silver nanoparticles respectively. The difference between the synthesis of gold and silver nanoparticles is that while silver nanoparticles were formed extracellularly exclusively, the gold nanoparticles were biosynthesized both intracellularly and extracellularly (Akintelu et al., 2020). A strong potential adsorption capability of Au³⁺ was demonstrated by *Bacillus megaterium* D01 (Wen et al., 2009) on exposing the bacterial biomass to the aqueous solution of HAuCl₄, it was found that the spherical gold nanoparticles (1.9 ± 0.8 nm sizes) were synthesized extracellularly which were capped with self-assembled monolayer of thiol (Balasubramanian et al., 2020). The biosynthesized gold nanoparticles were found to be stable for several weeks and they did not get aggregated.

TABLE 8.2 Using bacterial in the green biosynthesis of nanoparticles.

Bacterial species	Type of nanoparticle	Citation
<i>Aeromonas</i> sp.	Silver NP	Rai, et al. (2006)
<i>Bacillus cereus</i>	Silver NP	Sunkar and Nachiyar (2012)
<i>Bacillus subtilis</i>	Silver NP	Saifuddin et al. (2009)
<i>Corynebacterium</i> sp.	Silver NP	Zhang et al. (2005)
<i>Escherichia coli</i>	Silver NP	Mahanty et al. (2013)
<i>Lactobacillus</i> strains	Silver NP	Nair and Pradeep (2002)
<i>Lactobacillus casei</i> subsp. <i>casei</i>	Silver NP	Nair and Pradeep (2002)
<i>Pseudomonas putida</i>	Silver NP	Thamilselvi and Radha (2013)
<i>Pseudomonas stutzeri</i> A	Silver NP	Haefeli et al. (1984)
<i>Nocardiopsis</i> sp.	Silver NP	Manivasagan et al. (2013)
<i>Bacillus megatherium</i>	Gold NP	Wen et al. (2009)
<i>Bacillus subtilis</i>	Gold NP	Beveridge and Murray (1980), Southam and Beveridge (1994)
<i>Desulfovibrio vulgaris</i>	Gold NP	Labrenz et al. (2000)
<i>Escherichia coli</i>	Gold NP	Kashefi et al. (2001)
<i>Geobacillus</i> sp.	Gold NP	Correa-Llantén et al. (2013)
<i>Geovibrio ferrireducens</i>	Gold NP	Southam and Beveridge (1994)
<i>Lactobacillus</i> strains	Gold NP	Labrenz et al. (2000)
<i>Plectonema boryanum</i>	Gold NP	Lengke et. al. (2006a, b)
<i>Pseudomonas fluorescens</i>	Gold NP	Lengke et. al. (2006a, b), Hussein et al. (2007)
<i>Pseudomonas aeruginosa</i>	Gold NP	Rajasree and Suman (2012)
<i>Rhodopseudomonas capsulata</i>	Gold NP	He et al. (2008)
<i>Clostridium thermoaceticum</i>	Cadmium sulfide NP	Cunningham and Lundie (1993)
<i>Escherichia coli</i>	Cadmium sulfide NP	Sweeney et al. (2004)
<i>Klebsiella aerogenes</i>	Cadmium sulfide NP	Holmes et al. (1995)
<i>Desulfovibrio magneticus</i>	Magnetite NP	Pósfai et al. (2006)
<i>Magnetospirillum magnetotacticum</i>	Magnetite NP	Philipse and Maas (2002)
<i>Desulfobacteraceae</i>	Zinc sulfide NP	Southam Beveridge (1994)
<i>Rhodobacter sphaeroides</i>	Zinc sulfide NP	Bai et al. (2006)
<i>Lactobacillus</i> strains	Titanium NP	Prasad et al. (2007)
<i>Enterobacter cloacae</i>	Selenium NP	Kessi et al. (1999)
<i>Desulfovibrio desulfuricans</i>	Selenium NP	Kessi et al. (1999)
<i>Desulfovibrio desulfuricans</i>	Palladium NP	Yong et al. (2002)
<i>Desulfovibrio vulgaris</i>	Uranium NP	Kessi et al. (1999)
<i>Desulfovibrio vulgaris</i>	Chromium NP	Yong et al. (2002)

In order to produce monodispersed spherical gold nanoparticles addition of dodecanethiol which serves as the capping ligand was an essential requirement. The ability of *Escherichia coli* DH5 α in the synthesis of gold particles of size $\sim 25 \pm 8$ nm has been reported by some workers (Du et al., 2007), in this case uniformity in size and shape was not obtained with predominantly spherical shape was observed along with few quasi-hexagons and triangles. Moreover, as

observed by the TEM micrographs the gold nanoparticles were found adhered to the surface of the bacteria. Microorganisms such as *Rhodospseudomonas capsulata* could produce stable nanoparticles of gold extracellularly and the nanoparticles produced were found to be of variety of sizes and pH variation could be used to control the shape of the biosynthesized gold nanoparticles (He et al., 2007). The AuCl_4 ions concentrations effect the morphology and size of the synthesized gold nanoparticles, it was found that spherical gold nanoparticles of size ranging from 10–20 nm were formed at lower concentration while network of gold nanowires were obtained at a higher concentration (He et al., 2008). The bacterial cells *R. capsulata* secrete some NADH dependent enzymes which play a regulatory role in the bioreduction of AuCl_4 ions and are the potential binding sites of the AuCl_4 ions. The reduction process could possibly be mediated by the electron transfer through the NADH dependent reductase.

8.3.2.3 Magnetite nanoparticles

A sulfate-reducing bacteria *Desulfovibrio magneticus* strain RS-1 grows anaerobically with fumarate and acts as the terminal electron acceptor (Taufiq et al., 2020) and this bacterial strain accumulate magnetite nanoparticle intracellularly. The nanoparticles produced were slightly larger than 30 nm mostly (Pósfai et al., 2006). It was observed by Klaus-Joerger that the other metals like Co, Cr and Ni could be substituted into magnetite crystals synthesized by *Thermoanaerobacter ethanolicus*, which is thermophilic iron-reducing bacteria (Klaus-Joerger et al., 2001) which led to the formation of magnetite nanoparticle of size <12 nm, octahedral in shape along with crystalline magnetite phase near the bacterial surface (Roh et al., 2001). The inorganic geochemistry of sediments can be influenced by the Fe (III) and Mn (IV) due to the fact that Fe (III) acts as an oxidant in aquatic sediments. It is instrumental in increasing the concentration of dissolved manganese, phosphate, trace metals and iron (Lovley, 1987). The sulfate-reducing bacteria could synthesize magnetic iron sulfide nanoparticles, which could adsorb radioactive metals owing to their high surface area (400–500 m²/g) thus providing a safe and long-term storage of hazardous radioactive pertechnetate ion (Watson et al., 2001). It was reported that metals like Cr (VI), Co (III), U (VI), Tc (VII), and Mn (IV) could be reduced by the suspended cells of *P. islandicum* in presence of hydrogen and thus *P. islandicum* could help in metal removal and thus bioremediating the contaminated water due to its reducing potential. Heavy metals like As (V) or Se (VII) could not be reduced by *P. islandicum* (Kashefi and Lovley, 2000).

8.3.2.4 Palladium and platinum nanoparticles

When the electron donors like lactate, formate, pyruvate or hydrogen was used it was observed that *Shewanella oneidensis* (Anju et al., 2020) which is metal ion reducing bacteria and *Desulfovibrio desulfuricans* (Yong et al., 2002), which is sulfate-reducing bacteria were able to reduce soluble palladium (II) into insoluble palladium (0). The researchers further documented that the bacterial cells could adsorb 12% platinum (IV) ions when a platinum chloride 2 mM solution was used (Yong et al., 2002). Researchers have demonstrated that resting cells of *S. algae* were able to reduce PtCl_6^{2-} ions at standard temperature of 25°C and pH 7 within 1 hr time period to synthesize platinum nanoparticles (Konishi et al., 2007). The synthesized platinum particles were present in periplasmic space and the appearance to black color was the indicator of formation of the metallic platinum nanoparticle by the microbes (Tan et al., 2020). Although the PtCl_6^{2-} ions were not reduced by lactate but in the absence of lactate the reduction was not caused by the *S. algae* cells. In *P. boryanum* UTEX 485 a Gram negative cyanobacterium, was able to produce extracellularly the metallic platinum nanoparticles while maintaining the temperature between 25°C–100°C for a period of 28 days and for 1 day at 180°C. the nanoparticle produced were found to be morphologically different in the form of spheres, beads and dendritic with size ranging from 30 nm–0.3 µm (Lengke et al., 2007).

8.3.2.5 Selenium and tellurium nanoparticles

Microelectronic circuit devices and photocopiers are made of materials like selenium which is a semiconductor and has photo-optical properties. It was seen that selenite (SeO_3^{2-}) could be transformed to selenium in the elemental form by the action of *Stenotrophomonas maltophilia* SELTE02 and the selenium granules accumulated accumulating in the extracellular matrix or in cell cytoplasm (Zambonino et al., 2021). Additionally, some other species like *Desulfovibrio desulfuricans*, *Rhodospirillum rubrum* and *Enterobacter cloacae* SLD1a-1, could also reduce selenite to selenium and the process of biotransformation could take place intracellularly as well as extracellularly giving various morphological shapes of nanoparticles such as fibrillar, spherical and granular. The elemental selenium has been found to aggregate too. Microorganisms like *P. stutzeri* could also reduce selenite to elemental selenium under aerobic conditions (Narayanan and Sakthivel, 2010). Other workers have also reported that other microorganisms like *E. coli* and *Tetrathibacter kashmirensis* are also involved in reducing selenite and the selenium deposits are found both in the

cytoplasmic matrix and periplasmic space too. *T. kashmirensis* has the potential to reduce selenite to selenium and it was reported that a 90-kDa protein present in the extract that had been cell free was responsible for bioreduction under aerobic conditions (Hunter and Manter, 2008).

P. aeruginosa SNT1 could help in transformation of selenium oxyanions to elemental red selenium which was amorphous and spherical in structure and shape respectively. The selenium nanostructured allotropes of selenium bioproduced both extracellularly and intracellularly (Yadav et al., 2008). Moreover, they further showed that *P. aeruginosa* SNT1 biosynthesized nanostructured selenium by biotransforming selenium oxyanions to spherical amorphous allotropic elemental red selenium both intracellularly and extracellularly. In addition, other microbes like *Bacillus selenitireducens*, *Sulfurospirillum barnesii*, and *Selenihalanaerobacter shriftii* could also biosynthesize crystalline form of elemental selenium nanospheres of size ~ 300 nm (Oremland et al., 2004). This synthesis gave a unique compacted but complex nanostructured arrangement of selenium atoms. *Bacillus selenitireducens* and *Sulfurospirillum barnesii* are responsible for bioreducing tellurite to tellurium in elemental form tellurium under anaerobic conditions. Firstly, the microorganism *B. selenitireducens* forms 10 nm diameter nanorods of 200 nm length which clustered to form 1000 nm large rosettes. In case of *S. barnesii* it is observed that the nanospheres formed are irregular and small as well as formed in extracellular matrix.

8.3.2.6 Zinc oxide nanoparticles

Zinc oxide nanoparticles have been used widely in various sectors pharmaceuticals, cosmetics, food industry, water treatment, paints and pigments, etc. *Aeromonas hydrophila* has been able to synthesize zinc oxide nanoparticles with an average size of 57.72 nm and in a simple and cost effective process, the zinc oxide nanoparticles produced were crystalline in nature and morphology was spherical and oval which was confirmed by the X-ray diffraction and atomic force microscopy respectively (Akbar et al., 2020).

8.3.2.7 Zinc sulfide nanoparticles

Desulfobacteraceae, a sulfate-reducing bacteria was found to produce zinc sulfide nanoparticles of spherical shape aggregating 2–5 nm in diameter from zinc sulfide (Labrenz et al., 2000) within the natural biofilms. Both microbial and geochemical factors are responsible for the biomineralization of zinc sulfide (Morshedtalab et al., 2020). This zinc sulfide nanoparticle was able to reduce the zinc concentration below the permissible limit in drinking water samples. Zinc sulfide nanoparticles could be synthesized using *Rhodobacter sphaeroides* which could produce nanoparticles of 8 nm diameter (Biruntha et al., 2020; Bai et al., 2006). Other workers could produce zinc sulfide nanoparticles using *Rhodobacter sphaeroides* having an average size of ($\sim 10.5 \pm 0.15$ nm) (Bai and Zhang, 2009).

8.3.2.8 Titanium and titanium dioxide nanoparticles

The culture filtrate of *Lactobacillus* sp. was used to prepare the titanium nanoparticles, the synthesized nanoparticles were spherical in shape with dimensions varying between 40–60 nm and produced extracellularly at room temperature (Ahmad et al., 2020). The synthesized nanoparticles were corrosion resistant and light weight and can be utilized in various filed such as submarines, automobiles, desalting plants, etc. (Prasad et al., 2007). *Bacillus subtilis* was used to synthesize titanium dioxide nanoparticle which would find application biomedical field like biosensing, contrasting agents, targeted drug delivery, etc. (Babitha and Korrapati, 2013)

8.3.2.9 Cadmium sulfide nanoparticles

Yeasts such as *Candida glabrata* and *Schizosaccharomyces pombe* and bacteria namely *Klebsiella aerogenes* (Shenton et al., 1997; Holmes et al., 1995) have been used to produce cadmium sulfide nanoparticles could be microbially produced in *Klebsiella aerogenes* (Holmes et al., 1995) and the yeasts such as *Candida glabrata* and *Schizosaccharomyces pombe* (Dameron et al., 1989; Williams et al., 1996). The cadmium sulfide nanoparticles were found in the intracellular matrix of the bacterium *K. aerogenes* with size varying between 20–200 nm and the formation of nanocrystals are dependent on the buffer composition of the growth media.

8.3.3 The mechanism

The microorganisms have the capability and inerrant ability to grow as well as survive under conditions of stress and this can be attributed to their specific resistance mechanisms. The mechanisms which resist the stress encompasses

various factors such as impermeability to metals, complexation and inactivation of metals, metal efflux systems, efflux pumps, extracellular precipitation of metals, solubility alteration, changes in toxicity due to oxidation/reduction and enzymes induced volatilization of metals (Rouch et al., 1995; Beveridge, et al., 1996). As for instance the microorganism *Pseudomonas stutzeri* AG 259 which was isolated from silver mines has shown the ability of synthesizing silver nanoparticles (Mohanpuria et al., 2008). Numerous examples are available in the literature which highlight the importance of interaction of microorganisms with metals and has pivotal role in biotechnological fields like bioremediation, biomineralization, microbial-influenced corrosion and bioleaching. Another area which is gaining focus is process of microbial-influenced corrosion and is helping researchers to understand the phenomenon causing local changes mediated by microbes on the surface of copper alloys, carbon steel, stainless steel, etc. (Angell, 1999). Bacteria have the capability to oxidize minerals (Harvey and Crundwell, 1997) and intervene in precipitation of minerals by two processes namely direct or indirect process, the later response is through geochemical reactive solids (Zierenberg and Schiffmant, 1990) and the former through precipitating minerals by acting as catalysts in chemical reactions in aqueous phase. These techniques are used in the bioleaching process as for instance the use of bacterial leaching in pre-treating gold ores containing arsenopyrite (Harvey and Crundwell, 1997). The ex situ and in situ remediation of wastes and other toxicants from waste water can be carried out using the process of microbial assisted metal reduction and help in achieving the sustainable development goals. The key to understanding the microorganism-assisted nanoparticle synthesis process and their application in the process of bioremediation, it is important to investigate the enzymes and proteins present in the microorganisms and the biochemical mechanism which helps in reducing the metal ions. The role of these reducing agents have become critical to understand the utilization of the bacterial strains (natural and/or genetically engineered) and other microbes in removal of hazardous heavy metals and other toxicants from the ecosystem. The factors which regulate the bioremediation of metals (Boopathy, 2000) are represented in Table 8.3. The microbes could immobilize and mobilize the metals as well as reduce metal ions by precipitating at nano scale. Thus, researchers have used genetically engineered microbes which could help in over expressing certain reducing agents and bio-produce

TABLE 8.3 Factors influencing bioremediation of heavy metals.

Sl. no.		Factors that influence bioremediation of heavy metals
1.	Factor	Microbes (microbial contamination)
	Activity	a. Inhibition of enzymatic activity b. Generates various poisonous toxic metabolites c. Transfer of genetic substances and mutation d. Enhancements of microbial growth
2.	Factor	Temperature
	Activity	a. Increased normal water temperature b. Inhibit growth and development of aquatic animals c. Stimulates microbial growth d. Decline normal water quality
3.	Factor	Substrate
	Activity	a. Chemical nature and structure of pollutants b. Very low pollutant concentration c. Toxicity of pollutants d. Dissolving power of pollutants
4.	Factor	pH
	Activity	a. Acidic and alkaline pH decreased water quality b. Alteration of pH inhibit growth and development of fishes c. Variation of pH effects on normal cellular functions
5.	Factor	Mass transfer limitations
	Activity	i. Dissolvability and capacity of oxygen diffusion ii. Ability to dissolve or mix in water iii. Diffusion of supplements

(Continued)

TABLE 8.3 (Continued)

Sl. no.		Factors that influence bioremediation of heavy metals
6.	Factor	Anaerobic or aerobic processes
	Activity	i. Depending on the population of microorganism's present ii. Reduction or Oxidation potential iii. Availability of electron acceptors
7.	Factor	Environment factor
	Activity	i. Alters different environmental condition ii. Decline nutrition storage iii. Depletion of various water substrates
8	Factor	Built-up of substrate against co-digestion
	Activity	a. Interaction of microbes (Succession, competition, succession, and predation) b. Concentration c. Presence of alternate source of carbon

nanoparticle controlling the dimensions, morphology, yield and stability of nanoparticle. The hydrogenases present in the periplasm and cytoplasm play an important role in reduction of metal ions (Nair and Pradeep, 2002; Bowman et al., 1997).

The genetically engineered *E. coli* was used to synthesize CdS nanocrystals to produce phytochelatin which serve as a site for binding/nucleation of metal ions as well as to stabilize the nanocrystal by reducing aggregation (Kang et al., 2008). Numerous genetically engineered microbial strains have been developed and studied by scientists to control shape, size and the expression of the reducing agents in the microbes for the synthesis of desired nanoparticles.

8.4 Challenges in nanoparticle synthesis

The biosynthesis of nanoparticles using microorganisms is a laborious process as it requires elaborate purification steps and moreover, the understanding of the mechanism is also not very clear. Another challenge is that controlling the morphology and size of the nanoparticles. The biosynthesis generally yields polydisperse particles hence achieving monodispersity is a major concern. This green based synthesis of nanoparticles is very promising having myriad applications and by overcoming the technical aspects this could be a substitute to the conventional chemical synthesis of nanoparticles. Scaling up of the production is also a challenge. The mechanistic aspects of the nanoparticles have to be further studied in details which is important in terms of rational and economic development in the biosynthesis process. The following factors need to be optimized synthesis process while using microbes.

8.4.1 Bacteria selection

Depending on the type of nanoparticle to be synthesized one needs to choose the bacterial candidate which is best suited taking into account their intrinsic properties which include enzymatic activities, rate of growth and biochemical pathway. Also focus has to be on the type of application of the synthesized nanoparticle which would be the guiding factor in the selection of the suitable bacterial strain.

8.4.2 Selection of reducing agents

The main agents in nanoparticle synthesis are the biocatalysts or the bacterial enzymes which can be used in purified form or in crude form or in whole cells. It has been observed that the rate of reaction increases when the cell extract or the supernatant of the culture is used but in these cases the stability of synthesized nanoparticles is not as desired. In case the nanoparticles are intracellularly produced, their release from the cells needs to be considered. As we are aware that synthesis of nanoparticles involved bioreduction process and hence coenzymes like NADH, FAD, NADPH, etc. need

to be supplied from outside if whole cells are not used. Therefore, to make the process cost effective live whole cells are preferred as the coenzymes are recycled in biological system. (Korbekandi et al., 2009).

8.4.3 Optimizing the conditions for growth and enzymatic reactions

The nanoparticle synthesis is dependent on enzymes and increasing the concentration of enzymes is possible by escalating the biomass hence growth conditions need to be optimized to get requisite yield. The other growth conditions like nutrient load, size of the inoculum, temperature, light, buffer strength, pH and speed also need to be maximized. The enzymes responsible for the reduction need to be induced as well as the substrates in subtoxic levels at the start of the process for increasing the activity. The time of harvest is crucial while using crude enzymes and whole cells. To summarize the enzymatic activity plays an important role and needs to be monitored in the growth period. Harvesting time is important in case of using whole cells and crude enzymes. Therefore, it might be necessary to monitor the enzyme activity during the time course of growth (Korbekandi et al., 2009). The yield and rate of production of nanoparticles are important parameters for commercial scaling up during biosynthesis. Hence the bioreduction conditions need to be maximized and the undesired, unwanted residual by-products and nutrients need to be removed for better analysis. The use of complementary factors like irradiation using microwave or visible light or boiling could help in achieving the desired shape and size as well as stability. Additionally, it is important to isolate, purify and obtain stable nanoparticles which can be challenging. Determination of optimal reaction conditions and cellular mechanisms is the focal point of the biosynthesis of metal nanoparticles (Korbekandi et al., 2009; Korbekandi et al., 2013).

8.4.4 The process of extraction and purification

The biosynthesized nanoparticles can be produced intracellularly, extracellularly or by both processes. Hence in case of nanoparticles present in the intracellular matrix, extra processing is required to release them, which might be carried out by treating with a suitable detergent or using ultrasound waves. While other methods like heating processes, osmotic shock and freeze-thaw techniques can be used to extract the synthesized nanoparticle but they might disturb the morphology or size and, in some cases, cause precipitation, sedimentation or aggregation.

The enzymatic lysis of cells can release the nanoparticles but it is a costly method and not suitable in scaling and commercial production. The chemicals like organic solvents and surfactants can also be used for extracting and stabilizing the nanoparticles but they are hazardous and expensive. Centrifugation is employed to extract and purify nanoparticles from the extracellular matrix but aggregates might be formed during this process.

8.4.5 The process of stabilization

To achieve stable nanoparticles workers have demonstrated the use of green methods and these biosynthesized nanoparticles are found to be stable for many weeks at the room temperature (Wen et al., 2009). The stability might be attributed to the reducing enzymes and proteins released by the microorganism.

8.4.6 The process of scaling

The enhanced nanoparticle synthesis requires the scaling of the process from bench top to industrial production, which would require maximization of reaction conditions taking into account types of organisms, inheritable properties of organisms, selection of the enzyme or protein, and selection of the state of biocatalyst. Shape and size of the nanoparticles can be optimized by controlling the reaction conditions. Industrial scaling requires the use of biomass and other processing which includes inoculation of the seed, cell harvesting, seed culture, addition of metal ions for nanoparticle synthesis, separation, homogenization, stabilization, formulation of the product and controlling the quality of the product (Iravani et al., 2014; Korbekandi et al., 2009; Korbekandi et al., 2013).

8.4.7 Safety issues

The application of nanoparticles in the field of bioremediation and biomedical is a cause of concern because of nanotoxicity arising due to the interaction of metal nanoparticle with the biological systems. The biological systems are controlled by various factors like the route of uptake and cell types which can cause some toxic effect. Some studies have revealed that the toxicity of nanoparticles is governed by their physio-chemical properties.

8.5 Conclusion

Removal of heavy metals from waste water throughout the world is a big challenge. Variety of effluents emanating from multifarious and heterogenous sources like industry, domestic, institute, hospital, etc. contains varied heavy metals at various concentration levels. Nanotechnology derived materials have a great potential to remove the heavy metal ions from the waste water and are the future tools. Development of eco-friendly, low-cost, non-toxic, biocompatible nanoparticles have huge commercial value which also increases the economic feasibility. Biotechnologically derived nanoparticle has the capacity to remove the heavy metals from the waste effluent and provide the clean water and thus is a prospective potential tool. Metal-based nanoparticles synthesized through green route by using microorganisms mainly bacteria successfully remove the heavy metals from waste water. This scientific methodology of microorganism-assisted nanoparticle synthesis has changed the whole scenario in waste water treatment technology. Silver nanoparticle synthesized by *Aeromonas* sp., *Bacillus subtilis*, *Corynebacterium* sp., *Corynebacterium* sp., *Escherichia coli*, *Lactobacillus* strains, *Lactobacillus casei* subsp. *Casei*, *Pseudomonas putida*, *Pseudomonas stutzeri* A, and *Nocardiopsis* sp. successfully absorbed the heavy metal ions from the waste water have a potential future prospect. Using gold nanoparticle synthesized by *Bacillus megatherium*, *Bacillus subtilis*, *Desulfovibrio vulgaris*, *Escherichia coli*, *Geobacillus* sp., *Geovibrio ferrireducens*, *Lactobacillus* strains, *Plectonema boryanum*, *Pseudomonas aeruginosa* and *Rhodopseudomonas capsulate* through green route also cleans the waste water and removes the toxicants from water bodies. The other metals and its oxides like cadmium sulfide, Magnetite, zinc sulfide, Titanium, Selenium, Palladium, Uranium and chromium are also effectively synthesized using various microorganisms such as *Clostridium thermoaceticum*, *Escherichia coli*, *Klebsiella aerogenes*, *Desulfovibrio magneticus*, *Magnetospirillum magnetotacticum*, *Rhodobacter sphaeroides*, *Lactobacillus* strains, *Enterobacter cloacae*, *Desulfovibrio desulfuricans* and *Desulfovibrio vulgaris*.

The synthesis of the bio assisted nanoparticles is still in developmental stage and more such studies need to be undertaken to synthesize the desired nanoparticle based on their requirement keeping in mind the various factors like stability, aggregation, growth of the crystal as well as shape and size.

8.6 Future recommendations

The removal of heavy metal from wastewater is of incredible importance in adhering to the sustainable development goal which ultimately takes care of the health and well-being of the ecosystem as well as all living beings. The use of nanotechnology in the field of environmental remediation and water purification have shown encouraging and promising results. The microorganism assisted nanoparticles offer many advantages viz-a viz traditional methods including cost-effectiveness and energy requirement. Additionally, many nano-based processes may produce fewer toxic metabolites having less space requirement and can be reused. The development of nanomaterials has widened the scope of remediation and it appears to be a promising and potential option in contrast to the customary adsorbents for eliminating heavy metals which are hazardous and toxic to health and safety of all.

The biosynthesized nanoparticles are more polydisperse than the ones synthesized using chemical which could be a challenge too. In order to control the properties of the synthesized nanoparticles it is important to optimize the growth of the microorganisms along with its cellular activities including reducing agents which are essential for the reaction. Since, the biological system are very complex elaborate studies are required to point out the exact reaction mechanism and specific proteins and enzymes responsible for the synthesis of metal nanoparticles. The advantages of large-scale bacterial synthesis of nanoparticles are that the process of synthesis and stabilization does not require toxic, hazardous and expensive chemicals. These are natural nano factories and by improving the reaction conditions and choosing the suitable microorganism the shape, size and composition of the nanoparticles can be controlled to get the desired output. Although the use of nanomaterials in water remediation and treatment is an alternative and potential method but some challenges need to be addressed which includes uncertainty regarding the fate of nanomaterials in the ecosystem, regulatory hurdles, scaling, technical issues, and unavailability of comparative cost to benefit analysis data in comparison to present methods.

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Microbial metallonanoparticles—an alternative to traditional nanoparticle synthesis

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9.1 Introduction

Nanotechnology is an interdisciplinary and multidisciplinary area of science that deals with the study and application of immensely small particles ranging from 1–100 nm in size. The extreme small size of the nanoparticles (NPs) imparts them with unique physicochemical and biological properties beneficial than their conventional counterparts (Gatoo et al., 2014; Guerrini et al., 2018; Khan et al., 2019; Wu et al., 2020). Due to the incredible superiority in the optical, mechanical, electric, electromagnetic, catalytic and biologic properties of nanoparticles, they have attracted wider applications (Jeevanandam et al., 2018; Madkour, 2019). Its omnipresence in every sector of economy has led to the increased research activities on nanoparticle synthesis and applications in the past two decades.

However, nature is an adept nanotechnologist, as there occur a number of nanoparticles in the environment. They are generated by the biogeochemical cycles (Chi and Yu, 2021), biomineralization (Min et al., 2018; Nanda et al., 2020), volcanic eruptions (Griffin et al., 2017; Jeevanandam et al., 2018) and are important for the dynamics of the ecosystem. Bottom-up or top-down are the two approaches, traditionally used for the production of nanoparticles via chemical and physical methods (Cele, 2020; Nam and Luong, 2019; Singh et al., 2020). Bottom-up approach involves the amalgamation of the raw materials together followed by self-assembly to get the next order of nanostructure. This can be done using spinning (Gu et al., 2017; Mohammadi et al., 2014), sol–gel method (Parashar et al., 2020; Yarbrough et al., 2020), pyrolysis (Gao et al., 2020; Rahemi Ardekani et al., 2019; Saravanakkumar et al., 2018), and chemical vapor deposition (CVD) (Lan et al., 2018; Manawi et al., 2018) methods. On the contrary, the top-down approach is a destructive process and disintegrates the larger particles into smaller entities in a controlled environment. Mechanical milling (Wei et al., 2020; Zhang et al., 2018), laser ablation (Lin et al., 2020; Piatto et al., 2020), nanolithography (Li et al., 2021; Tiwale et al., 2019), thermal decomposition (Vernaya et al., 2020; Wang et al., 2020) and sputtering (Ishida et al., 2017; Verma et al., 2018) are the preferred methods of top-down approach (Khan et al., 2019). They involve use of harsh chemicals, extreme physical conditions and therefor over-priced methods.

9.1.1 Advantages and disadvantages of nanoparticles

The higher surface to volume ratio of nanoparticles contributes much beneficial baggage to the industrial applications of nanoparticles. The larger surface area increases the carrier capacity, stability of nanoparticles and they can take various routes of administration. The smaller size of NP favors the contained material to be manufactured in miniature formats that are especially advantageous in electronics (He et al., 2020; Liu et al., 2018) and biomedical sectors (Cardoso et al., 2018; Han et al., 2019). It increases the bioavailability (Peñalva et al., 2018; Talegaonkar and Bhattacharyya, 2019) and helps in specific drug targeting (Akhter et al., 2018; Ghazy et al., 2021) for the nanomedical applications.

Though the infinitesimal size of the nanoparticles is the reason for the numerous advantages of nanoparticles, the same physical trait also leads to the toxicity of the nanoparticles (Li et al., 2018; Naqvi et al., 2019). In addition to this the surface area, chemical composition, agglomeration state, surface morphology, solubility, surface charge and

individual chemistry influences the detrimental effects of nanoparticle. Therefore the intended or unintended leakage of nanoparticles during its production and usage are harmful to the health and balance of the ecosystem (Gatoo et al., 2014; Sukhanova et al., 2018).

Natural occurrence of nanoparticles in microorganisms at mines, oceans, etc. have inspired the mankind to exploit it more and get the benefit of eco-friendly synthesis of nanoparticles. The biogenic synthesis methods include the use of plants and microorganisms as a system for the production of nanoparticles.

9.1.2 Microorganisms as an alternative to the traditional nanoparticle synthesis

The green synthesis of nanoparticles does not require strident conditions and therefore is explored to get environment-friendly, cost-effective method of nanoparticle formation. The living organisms from prokaryotic to higher order eukaryotes can synthesize nanoparticles by different mechanisms and serve for the benefit of ecological health (Fairbrother et al., 2012; Griffin et al., 2017; Luo et al., 2015; Zhang et al., 2020). Microorganisms by and large play a key role in the maintenance of earth's ecosystem. They are equally harmful and beneficial in nature. They include viruses, bacteria, archaea, protozoa, algae, yeasts and fungi. Advancements in biotechnology have exploited most of the microorganism groups in the field of drug manufacturing, enzyme catalysis, agriculture products, cosmetics, biomedicine, bio-warfare, etc. Similarly, microbes are capable of utilizing their metabolic processes, enzymes for the transformation of metal precursors to their corresponding nanoparticles. They can be classified mainly into intracellular and extracellular synthesis. The ease of large-scale cultivation, fast growth, cost-effective downstream processes and eco-friendly growth conditions are some of the factors that make microbes a favorable system for nanoparticle synthesis. The biogenic synthesis is commonly done by bottom-up method. As a general methodology, the microbial culture is grown for 16–72 hours at ambient culture conditions as a pre-inoculum. The culture as a whole (for intracellular production) or the cell free extract (for extracellular production) is then seeded with the corresponding metallic salt and incubated at 25°C–40°C for 1–4 days, where after the nanoparticle will be deposited at the bottom of the vessel. The change of color and pH is regarded as the indication for nanoparticle formation (Taran et al., 2018) (Fig. 9.1). Herein, we give the details of microbe mediated synthesis of different nanoparticles like metal NPs, organic NPs, quantum dots, nano-composites made of metal and scaffolding proteins. The focus is mainly on the methods and mechanism of nanoparticle synthesis using microorganisms.

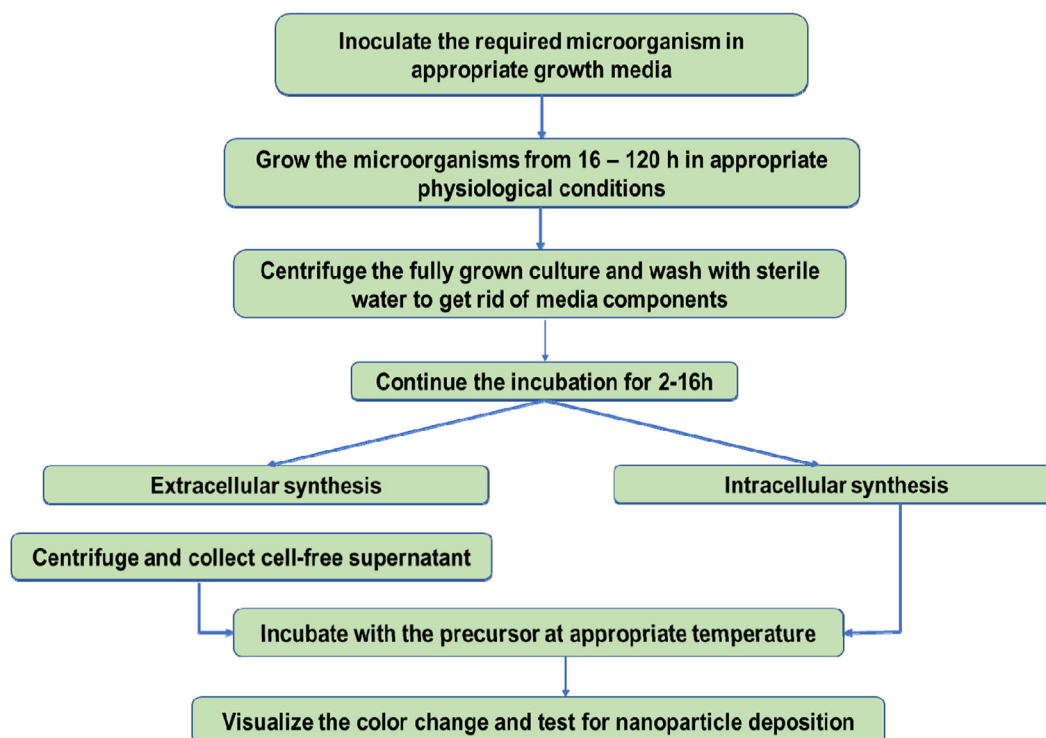


FIGURE 9.1 A general flowchart for the microorganism mediated synthesis of nanoparticles.

9.1.3 Bacteria mediated synthesis

Bacteria are one of the primitive organisms present on earth's crust and are the most exploited industrially. They are used in food industry, drug manufacturing, enzyme production, bioremediation, paints, textiles, fertilizers and pesticides industry. The fast growth, ease of genetic manipulation has opened the way to its rhetorical industry use (Tosato and Bruschi, 2004). The biogenic synthesis and fabrication of metal and metal oxide NPs are predominantly accomplished using different genera of bacteria. The below given Table 9.1. quotes a list of bacteria used for the synthesis of nanoparticles. The details of synthesis are explained in the following paragraphs below.

9.1.3.1 Titanium nanoparticles

Titanium is one of the strongest and non-reactive metals, widely used in automobile, aerospace engineering, paints, jewelry and medical industry (Froes, 2015). *Lactobacillus* sp. has been used to synthesize TiNP by combining the fully grown culture

TABLE 9.1 List of bacteria producing different types of nanoparticles.

Bacterium	Nanoparticle synthesized	References
<i>Lactobacillus</i> sp.	TiO ₂	Jha et al. (2009)
<i>Lactobacillus plantarum</i> VITES07	ZnO	Selvarajan and Mohanasrinivasan (2013)
<i>Lactobacillus acidophilus</i>	Ag	Rajesh et al. (2015)
<i>Lactobacillus rhamnosus</i> GG	Ag	Aziz Mousavi et al. (2020)
<i>Bacillus subtilis</i> (FJ460362)	TiO ₂	Dhandapani et al. (2012)
<i>Bacillus subtilis</i>	Au	Srinath et al. (2018)
<i>Halomonas elongata</i> IBRC-M 10214	Cu	Taran et al. (2016)
	TiO ₂ , ZnO	Taran et al. (2018)
<i>Pseudomonas aeruginosa</i>	Au	Singh and Kundu (2014a)
<i>Pseudomonas aeruginosa</i> ATCC 27853	Se	Kora and Rastogi (2016)
<i>Pseudomonas fragi</i>	CdS	Gallardo et al. (2014)
<i>Deinococcus radiodurans</i>	Au	Li et al. (2016)
	Au, Ag, Au-Ag	Li et al. (2018)
<i>Shewanella loihica</i> PV-4	Pt, Pd, Au	Ahmed et al. (2018)
<i>Shewanella oneidensis</i> Mr-1 (Mr-1) and <i>Shewanella xiamenensis</i> BC01 (SXM)	Au	Wu and Ng (2017)
<i>Acinetobacter calcoaceticus</i> LRVP54	Ag	Chopade et al. (2013)
<i>Acinetobacter</i> sp. KCS11	ZrO ₂	Suriyaraj et al. (2019)
<i>Rhodococcus</i> NCIM 2891	Ag	Otari et al. (2015)
<i>Oscillatoria limnetica</i>	Ag	Hamouda et al. (2019)
<i>Zooglea ramigera</i>	Se	Srivastava and Mukhopadhyay (2013)
	Au	Srivastava and Mukhopadhyay (2015)
<i>Streptomyces minutiscleroticus</i> M10A62	Se	Ramya et al. (2015)
<i>Desulfovibrio desulfuricans</i>	ZnS	Pinto da Costa et al. (2012)
<i>Serratia nematodiphila</i>	CdS	Malarkodi et al. (2013)
<i>Staphylococcus aureus</i>	CdSSe	Xiong et al. (2014)

of *Lactobacillus* and 25 mM of $\text{Ti}(\text{OH})_2$. The mixture was then heated to 60°C till the deposition of TiNP is visible at the bottom of the flask. The X-Ray diffraction (XRD) and transmission electron microscopy (TEM) analysis demonstrated the spherical shaped NPs of 8–35 nm size. The low pH that supports membrane dependent oxidoreductases and the high oxidation potential controlled by the nutrients in the culture medium were responsible for the TiO_2 nanoparticles formation (Jha et al., 2009; Prasad et al., 2007). *Bacillus subtilis* (FJ460362) isolated from the soil rich in rare elements was used to prepare TiO_2 nanoparticles. The pre-inoculum grown for 72 hours was incubated with Potassium hexafluoro titanate to get TiO_2 crystalline nanoparticles. The TEM and XRD data showed the size of TiNP to be 15–20 nm. The authors have also shown the successful anti-biofilm activity of the TiNPs by photocatalytic mechanism (Dhandapani et al., 2012). In another study, the extracellular production of TiO_2 nanoparticles was mediated by *Halomonas elongata* IBRC-M 10214. The culture supernatant was incubated with different $\text{Ti}(\text{OH})_2$ concentrations for 2–4 days. The scanning electron microscopy (SEM) analysis of TiNP showed spherical shaped NPs with 104.63 ± 27.75 nm size. The use of toxic capping agents were not necessary for the stabilization of NPs (Taran et al., 2018).

9.1.3.2 Gold nanoparticles

Gold is one of the precious, yellow metal having highly malleable and ductile properties. It is also one of the ancient metals used by mankind in the preparation of coins, sculptures, ornaments, medicines, etc. the oldest biomedical application of gold nanoparticles is its use as a supplemental feed in neo-natal care called as swarnaprashana (Jyothy et al., 2014). Many researchers have explored green synthesis of gold NP. *Rhodopseudomonas capsulate* and *Pseudomonas aeruginosa* mediated the extracellular biosynthesis of AuNP using hydrogen tetrachloroaurate (HAuCl_4) as precursor (Singh and Kundu, 2014b). They have studied the effects of different pH conditions on the obtained nanoparticles, where acidic pH of 4–6 led to the synthesis of triangular nanoplates of 50–400 nm size and the increase in pH from acidic to neutral gave rise to spherical nanoparticles of 10–40 nm size. This variation could be because of the positive ions present in the acidic pH which tends to lower the reducing power of the biomass and thereby forming larger nanoplates. The higher pH increases the reducing power, the reaction rate and therefore thermodynamically favored spherical nanoparticles are formed. The authors also attribute the reduction of gold to the NADH-dependent reductases that are present in the electron transport chain of the bacterium. Though this method doesn't involve the extraction of nanoparticles from the cells, it is a slow process and the mechanism of AuNP formation still needs to be resolved. *Deinococcus radiodurans* is a bacterium isolated from the irradiated meat that can biologically eliminate toxic heavy metals such as chromium, uranium and technetium. *D. radiodurans* grow in extreme conditions and possess efficient antioxidant and DNA repair systems as its survival process. This intracellular process was examined for the formation of gold nanoparticles and Au(III) reduction (Li et al., 2016), which was a fast process with the accumulation of AuNPs within 8 hours. The equilibrium of the reaction was reached within 16 hours. The size of the gold NPs formed were of 43.75 nm with irregular, spherical, and triangular shapes. This group has also investigated the effects of initial precursor concentration, pH and bacterial growth period on the biosynthesis of AuNPs. They have found that a precursor concentration of 1 mM and neutral pH to be optimal for gold nanoparticle formation. The TEM and SEM analysis showed the distribution of AuNPs on the cell envelope, cytosol and the extracellular space. It also shows the presence of carbon, nitrogen, oxygen and phosphorus peaks indicating the capping of AuNPs formed by the interaction of AuNPs with the surrounding proteins and free amino groups. This suggests that there exists a mechanism for the transport of gold NPs into and out of the cell. It also proves the participation of intracellular enzymes and the microenvironment in the transformation of Au(III) to AuNPs in the cells of *D. radiodurans*.

In yet another approach, the ultra-small gold, palladium and platinum nanoparticles were produced from electrochemically active biofilm (EAB) of *Shewanella loihica* PV-4. The EAB possess the capacity to produce extracellular electrons by the biological oxidation of electron donors (e.g., sodium lactate) and transfer electrons to reduce metal precursors. Ahmed et al. (2018) have utilized this mechanism to generate Pd, Pt, and Au NPs. Further, they have also examined the effects of concentration the metal precursor and pH of the reactants on the production of nanoparticles. The increased reducing ability of EABs in comparison with other bacteria helps in the production of smaller nanoparticles of size 2–7 nm.

9.1.3.3 Silver nanoparticles

Silver metal is widely used for its antibacterial properties in the field of medicine. It is also used in textiles, dentistry, healthcare products, etc. (Calderón-Jiménez et al., 2017). The rhizosphere soil of wheat plant was sourced to isolate *Acinetobacter calcoaceticus* LRVP54 that was positive for AgNP synthesis out of the eighteen strains. The cell free supernatant was used to prepare AgNP at an elevated temperature of 70°C and pH-7. 1 mM AgNO_3 was used as the

metal precursor. The color change to reddish brown solution pinpointed the completion of AgNP synthesis and the TEM examination showed the presence of AgNPs with 8–12 nm size spherical shape. The authors report the involvement of reducing and stabilizing agents in the cell free supernatant can be accountable for the formation of silver nanoparticles (Chopade et al., 2013). To quote an example of intracellular AgNP synthesis, *Rhodococcus* sp. was used by Otari et al. (2015) to obtain spherical AgNPs of size ranging from 5 to 50 nm. The TEM analysis revealed the intracellular distribution of Ag NP and Energy-dispersive X-ray spectroscopy (EDS) analysis revealed carbon and oxygen peaks. This indicated the capping and stabilization of AgNPs with the intracellular amide molecules. The author also suggests that the reduction of AgNO₃ to Ag NPs involves the participation of cytoplasmic NADH-dependent nitrate reductase. Another blue-green alga *Cyanobacteria* has also been explored to synthesize silver nanoparticles. The aqueous extract from *Oscillatoria limnetica*, was able to produce quasi-spherical Ag NPs of 3.30–17.97 nm size. The phyco-biliproteins and other sulfur containing enzymes present in the crude extract absorb light energy and consequently excites the chromospheres which might have caused the AgNO₃ reduction (Hamouda et al., 2019).

9.1.3.4 Selenium nanoparticles

Selenium is a rare metalloid element known for its photovoltaic and photoconductive properties. It is a trace element essential for the human body as selenium plays a vital role in the activity of enzymes like thioredoxin reductase, glutathione peroxidase, etc. *Bacillus subtilis* was used to synthesize spherical Se nanoparticles ranging from 50–400 nm. Sodium selenite at 4 mM concentration was used as a metal precursor at 35°C to get monoclinic SeNP by extracellular synthesis. This was incubated further to get trigonal-SeNP nanowires based on the Ostwald ripening process (Wang et al., 2010). *Zooglea ramigera*, an aerobic bacteria growing in organically rich environment such as sewage water and sludge is widely used for biosorption and bioremediation of metals (Abbas et al., 2006; Sağ and Kutsal, 2000; Zouboulis et al., 2004). *Z. ramigera* is also explores for its capability to biosynthesize SeNPs with 3 mM sodium selenite precursor (Srivastava and Mukhopadhyay, 2013). They obtained spherical SeNPs of size 30–150 nm. The group was also able to synthesize trigonal-SeNP nanorods by Ostwald ripening. The membrane surface proteins of the bacterium that are involved in electron transport chain, namely NADH-dependent oxidoreductases are said to reduce SeO₃²⁻ ions. In a similar manner, (Ramya et al., 2015) have synthesized SeNPs from the actinobacteria *Streptomyces minuscule* M10A62 obtained from the magnesite mine, Forootanfar et al. (2014) has synthesized spherical SeNPs of 80–220 nm from *Bacillus* sp. MSh-1, Kora and Rastogi (2016) have synthesized spherical SeNPs with 47–165 nm size from *Pseudomonas aeruginosa* ATCC 27853. Along with this many more researchers (Kamnev et al., 2017; Sonkusre and Singh Cameotra, 2015; Srivastava and Kowshik, 2016; Wang et al., 2019, 2017) have biosynthesized SeNPs from bacteria using selenite as the metal precursor.

9.1.3.5 Zinc nanoparticles

Sulfur reducing bacteria (SRB) are primitive anaerobic microorganisms that can accept sulfate (SO₄²⁻) as the terminal electron acceptor, utilize the energy and reduce it to sulfide. Several researchers have extended this capacity of SRB and used as a tool for bioremediation (Ayangbenro et al., 2018). A consortium of SRB, mainly consisting of *Desulfovibrio desulfuricans* was used as the sulfide donor to produce predominantly spheroidal zinc sulfide (ZnS) nanoparticles of 20–30 nm size (Pinto da Costa et al., 2012). In another study ZnS nanoparticles were biosynthesized using the immobilized *Rhodobacter sphaeroides* which is a photosynthetic bacterium capable of aerobic chemoheterotrophy in the case of unavailability of light (Bai et al., 2006). The immobilized bacterium was incubated with ZnSO₄ precursor for 35 hours at 30°C to get spherical ZnSNP of 2.5–8 nm. The increase in incubation time caused the nucleation effect and large sized agglomerates were formed. In another case of ZnO nanoparticle production, the cell free extract of *Halomonas elongata* IBRC-M 10214 grown for one week was incubated with ZnCl₂ precursor. The ZnO NPs were 18.11 ± 8.93 nm in size with multiform shapes (Taran et al., 2018).

9.1.3.6 Quantum dots

Quantum dots (QDs) are artificial atoms with semiconductor properties, consisting of group II to VI or III to V elements. They have unique electronic and optical characteristics which can be regulated by varying the size. Therefore, QDs find many applications in Light Emitting diodes (LED), photovoltaics, photoconductors, photodetectors, biomedical imaging, markers, etc. Recently they are biosynthesized using microorganisms and plants. The major challenge in the preparation of QDs is to mix the two appropriate elements so as to get their correct valence states (Gu et al., 2012). The cells of *Staphylococcus aureus* at the stationary phase of growth were incubated with 5 mM of Na₂SeO₃ followed by harvesting and addition of 1 mM CdCl₂ salt. These cells were further incubated for 12 hours to obtain intracellular

fluorescent $\text{CdS}_{0.5}\text{Se}_{0.5}$ QDs. *S. aureus* cells initially reduce Na_2SeO_3 to selenocysteine and other organo-selenium compounds, after which the reduced organo-selenium compound reacts with the incoming Cd^{+} precursor ions to form the fluorescent CdSSe QDs. The characterization of QDs by TEM analysis revealed monodispersed nanocrystals of 1.8 ± 0.5 nm diameter (Xiong et al., 2014). In another study, psychrotolerant *Pseudomonas fragi* strains were used for the synthesis of cadmium sulfide (CdS) QDs at a low temperature of 15°C . The low temperature is advantageous as it provides enhanced control over the distribution of size in the obtained nanoparticles (Gallardo et al., 2014). Another case of CdS QD biosynthesis at low temperature is from psychrotolerant *Pedobacter* strain with high cadmium tolerance (Carrasco et al., 2021).

9.1.4 Fungus-mediated synthesis

Fungi, regarded as principal decomposer, have a major share of responsibility in the sustainability of the ecosystem. They were the conventional workhorses for brewing, production of antibiotics like penicillin, enzyme manufacturing, secondary metabolite production, etc. (Mukherjee et al., 2018). Nanotechnology has also utilized the capacity of these lower eukaryotes intensively. A small number of examples are stated in Table 9.2 and the details are explained in the following paragraphs.

TABLE 9.2 List of fungus synthesizing different types of nanoparticles.

Fungus	Nanoparticle synthesized	References
<i>Fusarium oxysporum</i>	ZrO_2	Bansal et al. (2004)
	Si and Ti	Bansal et al. (2005)
	Ag	Birla et al. (2013), Hussein et al. (2015)
	Au	Naimi-Shamel et al. (2019)
	Pt	Gupta and Chundawat (2019)
<i>Fusarium oxysporum</i> (PTCC 5115)	ZnS	Mirzadeh et al. (2013)
<i>Aspergillus flavus</i>	TiO_2	Rajakumar et al. (2012)
<i>Aspergillus terreus</i> (BC0603)	Ag	Li et al. (2012)
<i>Aspergillus terreus</i>	PbSe	Mary Jacob et al. (2014)
<i>Aspergillus oryzae</i>	Se	Mosallam et al. (2018)
<i>Aspergillus parasiticus</i> NRRLY 2999	Si	Zielonka et al. (2018)
<i>Aspergillus sydowii</i>	Au	Vala (2015)
<i>Aspergillus sydowii</i>	Ag	Wang et al. (2021)
<i>Aspergillus niger</i>	ZnO	Kalpna et al. (2018)
<i>Verticillium</i> sp.	Au	Mukherjee et al. (2001)
<i>Trichoderma viride</i>	Ag	Elgorban et al. (2016)
<i>Trichoderma</i> sp. WL-Go	Se	Diko et al. (2020)
<i>Trichoderma hamatum</i> SU136	Au	Abdel-Kareem and Zohri (2018)
<i>Helminthosporium solani</i>	CdSe	Suresh (2014)
<i>Helminthosporium tetramera</i>	Au	Shelar and Chavan (2014)
<i>Guignardia mangiferae</i>	Ag	Balakumaran et al. (2015)
<i>Alternaria</i> sp.	Au	Niranjan Dhanasekar et al. (2015)
<i>Ganoderma enigmaticum</i> and <i>Trametes ljubarskyi</i>	Ag	Gudikandula et al. (2017)
<i>Cladosporium cladosporioides</i>	Ag	Manjunath Hulikere and Joshi (2019)

9.1.4.1 Titanium nanoparticles

Several species of *Fusarium oxysporum*, a plant pathogenic fungus is largely used in biogenic production of nanoparticles (Bansal et al., 2004; Birla et al., 2013; Mirzadeh et al., 2013; Rai et al., 2021; Syed and Ahmad, 2013). In one of the studies to generate TiO₂ nanoparticles, the fully grown biomass of *F. oxysporum* was incubated with K₂TiF₆ at 27°C shaker for 24 hours. They obtained spherical TiO₂ NP with 10 nm average size. They have further analyzed that *F. oxysporum* secretes extracellular enzymes that hydrolyze the hexafluoro titanium ion complexes at room temperature to form Ti nanoparticles. *Aspergillus flavus* is used in the TiO₂ nanoparticle generation (Rajakumar et al., 2012). The five-day grown culture of *A. flavus* is challenged with 1 mM TiO₂ to form spherical and oval shaped nanoparticles of 62–74 nm size range. The proteins of *A. flavus* acted both as capping and reducing agents of TiO₂ nanoparticles. The quinones and hydroxyquinones that exist on the cell membrane and the oxygenase available in the cytosol might support the reduction of TiO₂. (Jha et al., 2009) suggests a similar mechanism would take place in yeast for the formation of TiO₂ nanoparticles.

9.1.4.2 Gold nanoparticles

Gold nanoparticles are produced using a variety of fungi. *Verticillium* sp. exposed to HAuCl₄ solution. The AuNPs obtained were of size 20–25 nm, with mostly spherical shape, a few triangular, hexagonal and quasi-hexagonal shapes were also observed. The TEM analysis showed that the AuNPs are concentrated on the cell wall as well as cytoplasmic membrane. The authors put forth the theory that AuCl₄[−] ions are trapped by the positively charged amino acids present on the cell wall, which is then reduced by the enzymes present mostly on the cell wall and few of the diffused particles are reduced by the membrane enzymes. They also note that the amount of nanoparticles concentrated on the cell wall are more in number compared to that of a bacterial nanoparticle biosynthesis (Mukherjee et al., 2001).

The extracellular, intracellular, and autolysate fractions of thermophilic fungi were used to produce AuNPs. Out of the 29 thermophilic fungi tested, all were positive when used for AuNP biosynthesis along with the growth media. When the mycelia were washed and tested for AuNP synthesis, only few of them were positive for AuNP synthesis. This shows that the chemicals present in the growth media affects the biosynthesis of AuNPs. The group has also analyzed the fungal chemicals responsible for AuNP formation and has concluded that the factors responsible for Au(III) to Au(0) reduction are less than 3 kDa (e.g., glucose, amino acids, co-factors, and the capping agents acting as stabilizers of the nanoparticles belong to the larger group of biomolecules like proteins) (Molnár et al., 2018).

9.1.4.3 Silver nanoparticles

The cell filtrate of *Fusarium oxysporum*, isolated from decayed banana produces silver nanoparticles using 1 mM AgNO₃ (silver nitrate) as the precursor. They were able to produce spherical AgNPs of 10–20 nm size using optimal conditions. The researchers studied the effect of distinct parameters including temperature, media components, pH, intensity of light, quantity of the biomass, volume of the filtrate and salt concentration on the yield of AgNP. They found that MGY medium (malt extract, glucose, yeast extract, peptone - pH 9–11) with physiological growth conditions at 40°C–60°C, 190.7 Lux and sunlight were the optimal conditions to get good yield of silver nanoparticles using *F. oxysporum* (Birla et al., 2013). Likewise, yeasts are used for the biosynthesis spherical AgNPs of 6–20 nm size (Jha et al., 2008).

9.1.4.4 Selenium nanoparticles

The intracellular and extracellular extracts of fungi are also used for the synthesis of selenium nanoparticles. Vetchinkina et al. (2018) have used basidiomycetes such as *Lentinus edodes*, *Pleurotus ostreatus*, *Ganoderma lucidum* and *Grifola frondosa* for the synthesis of selenite NPs. *L. edodes* and *P. ostreatus* yielded regular spherical nanoparticles of 50–150 nm diameter SeNP, whereas the intracellular extracts of *G. lucidum* and *G. frondosa* produced 200–300 nm spherical NPs. But their corresponding extracellular fungal extracts yielded smaller NPs of 20–30 nm in size. *Trichoderma* sp. WL-Go could yield spherical nanoparticles of 20–220 nm (Diko et al., 2020). SDS-PAGE analysis showed two proteins of size ~15kDa and ~19kDa in the extracellular extracts are responsible for reducing and capping of SeNPs.

9.1.4.5 Zinc nanoparticles

ZnS nanoparticles were prepared using ZnSO₄ precursor from the fungus *Fusarium oxysporum* (PTCC 5115). The extracellular filtrate was able to produce spherical shaped ZnSNP with an average size of 42 nm. The polysaccharides and enzymes found in the extract were found to interact with the nanoparticles and provide stability (Mirzadeh et al., 2013). *Aspergillus niger* was used to prepare zinc oxide nanoparticles (ZnO NP) using 5 mM zinc nitrate precursor at 32°C. The morphology studies showed the presence of spherical ZnO NP with 53–65 nm size (Kalpana et al., 2018).

9.1.4.6 Quantum dots

Lead selenide (PbSe) quantum rods were biosynthesized using *Aspergillus terreus* extracellularly at room temperature. TEM and SEM analysis revealed the presence of PbSe nanorods of ~ 57.94 nm size (Mary Jacob et al., 2014). Spherical shaped, monodispersed, CdSe QDs were synthesized using the fungus *Helminthosporium solani*. The product did not require any other downstream process except for filtration, as it was synthesized extracellularly. Cadmium chloride and selenium tetrachloride were used as precursors to get nanospheres of 2–10 nm diameter (Suresh, 2014).

9.1.5 Algae-mediated synthesis

The technical advantages such as easy scale-up, low manufacturing costs, non-requirement for a big growing space have made algae a suitable candidate for number of industrial products (Fabris et al., 2020). The Table 9.3 shows some examples where algae are used for the synthesis of nanoparticles.

The alga *Tetraselmis kochinensis* is used for the intracellular production of AuNP of 5–35 nm size. The TEM analysis shows the concentration of gold NP more upon the cell wall rather than the cytoplasmic membrane, where it is easily accessible for the downstream processing (Senapati et al., 2012). Another study demonstrates the intracellular biosynthesis of gold NP using the diatom *Stephanopyxis turris* (Pytlik et al., 2017). Two groups of spherical AuNPs of 30 and 10 nm were obtained, but the mechanism of synthesis has not been elucidated.

Similarly, in another study of AgNPs using the diatoms *Chaetoceros* sp., *Skeletonema* sp., *Thalassiosira* sp., they attribute AgNO_3 reduction to the photosynthetic pigments such as chlorophyll-C and fucoxanthin (Mishra et al., 2020). They were able to produce square shaped and non-spherical AgNPs of 148.3 ± 46.8 , 238.0 ± 60.9 and 359.8 ± 92.33 nm size with *Chaetoceros* sp., *Skeletonema* sp. and *Thalassiosira* sp. respectively.

The aqueous extract of the microalgae *Chlorella* and zinc acetate precursor is mixed at optimal ratio and raised to 58°C with constant mixing for 1 hour to biosynthesize ZnO nanoparticles. The authors predict the reduction mechanism to be of a donor-acceptor model, with the carbohydrate moiety donating an electron to the electrophile zinc species. This results in the oxidation of the hydroxyl moiety of the carbohydrate and the reduction of the zinc atoms (Khalafi et al., 2019).

The carbon dots, that is, Carbon nanoparticles that have semiconductor and fluorescence properties, were biosynthesized using algal biomass. (Guo et al., 2017) used a hydrothermal process in which the microalgae suspension and the aqueous formaldehyde were mixed and subjected to 180°C for 10 hours in a Teflon reactor. This gave rise to nano spherical carbon dots of 1–8 nm. Ramanan et al. (2016) collected the algal bloom from a eutrophic pond which consisted of fresh water algae belonging to *Cyanophyceae*, *Chlorophyceae*, *Bacillareophyceae*, and *Euglenophyceae*. The algae were crushed into a fine powder and utilized as a starting material for the biosynthesis of carbon QDs using a domestic microwave method. The TEM analysis confirmed the size of CDs as 8.5 ± 5.6 nm in diameter.

TABLE 9.3 List of algae used for the synthesis of various nanoparticles.

Algae	Nanoparticle synthesized	References
<i>Tetraselmis kochinensis</i>	Au	Senapati et al. (2012)
<i>Spirulina platensis</i>	Au	Uma Suganya et al. (2015)
<i>Stephanopyxis turris</i>	Au	Pytlik et al. (2017)
<i>Sargassum polycystum</i>	Cu	Ramaswamy et al. (2016)
<i>Chaetoceros</i> sp., <i>Skeletonema</i> sp., <i>Thalassiosira</i> sp.	Ag	Mishra et al. (2020)
<i>Chlorella</i>	ZnO	Khalafi et al. (2019)
Microalgae	Carbon dots	Guo et al. (2017)
<i>Sargassum myriocystum</i>	Ag	Balaraman et al. (2020)
<i>Caulerpa racemosa</i>	Ag	Edison et al. (2016)

9.1.6 Viral mediated synthesis

The highly symmetrical, robust protein capsids with unique structural architecture and its amenability to genetic manipulations have made viruses an attractive scaffold for nanoparticle biosynthesis. They offer a template for directed synthesis and self-assembly of nanoparticles (Kale et al., 2013). The genetically engineered viral capsid of cowpea chlorotic mottle viruses (CCMV) provided a template for the reduction of HAuCl_4 . The tyrosines present in the viral sequence coupled with deprotonation/ electron transfer mechanisms were able to reduce Au^{3+} to Au^0 nanoparticles (Slocik et al., 2005). In another case, the tobacco mosaic virus (TMV) was decorated with citrate covered iron oxide and gold nanoparticles. pH controlled protonation of TMV attracts the negatively charged citrate-coated gold NPs to bind irreversibly and results in the formation of decorated TMV particles. This is a simple and quick process, yet to be researched further with other nanomaterials (Khan et al., 2013). The coat protein and the scaffolding protein of the wild-type bacteriophage P22 was co-expressed recombinantly in *E. coli* BL21(DE3). The pre-synthesized CdS QDs and the recombinant pro-capsid shells were mixed in a thermo-mixer operated at 25°C for 24 hours to get a directed self-assembly of pentameric and hexameric CdS NP (Kale et al., 2013). Though the principle of binding interaction is not fully understood, the simple approach for the ordered assembly of nanoparticles can be experimented with other inorganic nanoparticles also.

9.1.7 Nanoparticle synthesis using protein and DNA scaffolds

The organic and carbon-based nanoparticles are usually arranged in ordered arrays which enhance their unique structural and physical properties. These self-assembled molecules have interesting applications in diverse areas of molecular electronics, biosensing, bio-imaging, photonics, etc. (Mark et al., 2006). The process of self-assembly necessitates a protein or DNA scaffold molecule. The previously synthesized platinum nanoparticles were self-assembled into a 2-dimensional array of dendrimer encapsulated Pt NPs (Pt-DEN). The prokaryotic surface-layer (S-layer) protein from acidothermophilic archaeon *Sulfobolus acidocaldarius* and the bacteria *Deinococcus radiodurans* were used as bio template (Mark et al., 2006). In another study, the chaperone protein γ -prefoldin (γ -PFD), extracted from *Methanocaldococcus jannaschii*, was used to create a scaffold, along with the capping mutant protein of γ -PFD called TERM (thermophilic extension resistant mutant). The group has generated 1D gold arrays using nickel-nitrilotriacetic acid (Ni^{2+} -NTA)-gold nanoparticles and γ -PFD (Glover et al., 2016). Plasmid DNA from *Bacillus* was utilized as a scaffold for the synthesis of silver NPs by using photo-irradiation method. The plasmid helped in reducing Ag^+ to Ag^0 and also acted as a scaffold for metallic Ag to form the nanoparticles (Liu et al., 2012).

9.1.8 Applications of nanoparticles synthesized via microbial route

The increasing urge for the use of safer, sustainable technologies that do not harm the environment has enhanced the research on microbial and plant mediated synthesis of nanoparticles. The property of being more efficient with miniature size has allowed nanotechnology to dominate its implementation in all the divisions from textiles to electronic goods, paints to ceramics, food products to pharmaceutical drugs (Nasrollahzadeh et al., 2019). Majority of the green synthesized nanoparticles are used for its antimicrobial, cytotoxic benefits in biomedicine. Recent researches have exploited the semiconducting properties of nanoparticles and applied them as photosensitizers, semiconductor diodes, etc. Some of the applications of nanoparticles developed via microbial route are listed in Table 9.4.

9.1.9 Future perspectives

The extensive application of nanotechnology in day to day life has posed the risk environmental pollution and ultimately health hazard for the living beings (Boxall et al., 2007). As an alternative, the green synthesis of nanoparticles via microbes and plant reduces the toxic effects of chemical and physical synthesis. The ease of growing and genetic manipulations has provided microorganisms to take an upper hand in the biosynthesis of NP. In spite of using the green technology for the manufacture of nanoparticles, the genotoxic and cytotoxic effects caused by nanoparticles are not to be ruled out. Hence, the regulatory bodies around the globe have addressed the safety issues concerning nanotechnology. The European Union (EU) has developed a regulatory framework concerning different sector like consumer products, chemicals, health, environment, and energy) (Rauscher et al., 2017). The Food and Drug administration, United States (USFDA) has formed a Nanotechnology Task Force in August 2006 and releases guidelines for the industries involved in the manufacture of nanotechnology products (Commissioner of Food and Drugs, 2020). In India, the Department of Biotechnology, Government of India, has constituted an Inter-ministerial Expert Committee and

TABLE 9.4 List showing the applications of the specific nanoparticles and the corresponding microorganism used for their biosynthesis.

Type of nanoparticle	Microorganism	Applications	References
Titanium dioxide	<i>Bacillus subtilis</i> (FJ460362)	Photocatalysis of aquatic biofilm	Dhandapani, Maruthamuthu and Rajagopal (2012)
Titanium dioxide	<i>Aspergillus flavus</i>	Antimicrobial against pathogens	Rajakumar et al. (2012)
Titanium	<i>Lactobacillus crispatus</i>	Antibacterial (tested against urinary tract infection causal bacteria)	Ibrahim, et al. (2014)
Titanium dioxide and zinc oxide	<i>Halomonas elongata</i> IBRC-M 10214	Antibacterial	Taran, Rad and Alavi (2018)
Gold	<i>Fusarium oxysporum</i> f. sp. <i>cubense</i> JT1	Antibacterial	Thakker, Dalwadi and Dhandhukia (2012)
Gold	<i>Trichoderma viridae</i> (MTCC 5661) and <i>Hypocrea lixii</i> (MTCC 5659)	Antibacterial (against pathogenic bacteria, biocatalytic)	Mishra et al. (2014)
Gold	<i>Deinococcus radiodurans</i>	Antibacterial	Li et al. (2016)
Gold	<i>Bacillus cereus</i> and <i>Fusarium oxysporum</i>	Cytotoxic	Pourali et al. (2017)
Gold, Palladium, Platinum	<i>Shewanella loihica</i> PV-4	Catalysis in dye degradation	Ahmed et al. (2018)
Silver	<i>Trichoderma viridae</i>	Antibacterial	Fayaz et al. (2010)
Silver	<i>Acinetobacter calcoaceticus</i>	Antibacterial	Chopade et al. (2013)
Silver	<i>Fusarium oxysporum</i>	Antibacterial, Antitumor	Husseiny, Salah and Anter (2015)
Silver	Actinobacteria <i>Rhodococcus</i> NCIM 2891	Antibacterial	Otari et al. (2015)
Silver	<i>Oscillatoria limnetica</i>	antibacterial against multidrug-resistant bacteria, cytotoxic against cancer cell-line, hemolytic	Hamouda et al. (2019)
Silver	<i>Chaetoceros</i> sp., <i>Skeletonema</i> sp., and <i>Thalassiosira</i> sp.	Antibacterial	Mishra, Saxena and Tiwari (2020)
Magnetite	<i>M13 virus</i>	Bio-imaging	Ghosh et al. (2012)
Selenium	<i>Bacillus subtilis</i>	H ₂ O ₂ biosensor	Wang et al. (2010)
Selenium	<i>Shewanella putrefaciens</i> 200	Bioremediation of mercury	Jiang et al. (2012)
Selenium	<i>Bacillus</i> sp. <i>MSH-1</i>	Anti-parasitic (<i>Leishmania major</i>)	Beheshti et al. (2013)
Selenium	<i>Pseudomonas stutzeri</i> NT-I	Biovolatilization, Selenium recovery	Kagami et al. (2013)
Selenium	<i>Bacillus</i> sp. <i>MSH-1</i>	Antioxidant, cytotoxic	Forootanfar et al. (2014)
Selenium	<i>Streptomyces minutiscleroticus</i> M10A62	Antioxidant, cytotoxic, Anti-biofilm, wound healing, Anti-viral	Ramya, Shanmugasundaram and Balagurunathan (2015)
Selenium	<i>Bacillus</i> sp. <i>MSH-1</i>	Biofilm inhibition	Shakibaie et al. (2015)

(Continued)

TABLE 9.4 (Continued)

Type of nanoparticle	Microorganism	Applications	References
Selenium	<i>Bacillus licheniformis</i> JS2	Prevents adhesion of <i>S. aureus</i> on different surfaces	Sonkusre and Singh Cameotra (2015)
Selenium	<i>Idiomarina</i> sp. PR58–8.	Anti-neoplastic	Srivastava and Kowshik (2016)
Selenium	<i>Burkholderia fungorum</i>	Bioremediation of selenite	Khoei et al. (2017)
Selenium	<i>Stenotrophomonas maltophilia</i> SeITE02	Bioremediation of selenite	Lampis et al. (2017)
Selenium	<i>Enterococcus faecalis</i>	Antibacterial	Shoeibi and Mashreghi (2017)
Selenium	<i>Citrobacter freundii</i> Y9	Mercury bioremediation	Wang et al. (2017)
Tellurium	<i>Bacillus</i> sp.	Antibacterial	Zare et al. (2012)
Tellurium	Anaerobic biomass in up flow anaerobic sludge bioreactor	Detoxification of sludge	Ramos-Ruiz et al. (2017)
Zinc Sulfide	<i>Thermoanaerobacter</i> , X513	Formation of functional thin films	Moon et al. (2014)
Zinc oxide	<i>Aspergillus niger</i>	Antimicrobial textiles, dye degradation	Kalpna et al. (2018)
Zinc oxide	<i>Chlorella</i>	Photocatalysis and degradation of dibenzothiophene (organosulfur pollutant)	Khalafi, Buazar and Ghanemi (2019)

finalized the “Guidelines for Evaluation of Nano pharmaceuticals in India” (Chowdhury, 2006; Rashi and Sharvil, 2020). However microbial synthesis of nanoparticles offers a greater platform to circumvent the disadvantages and toxic effects of conventional methods.

9.2 Conclusion

The review sheds light on the cost-effective, greener technology of nanoparticle synthesis using microorganisms from different taxonomic groups. The use of biomolecules for the NP synthesis is also mentioned. Increased dependency on technology and gadgets in our daily life has made it inevitable to the exposure risks of nanoparticles. Hence, there is a need to create sustainability and balance in the ecosystem. Microorganisms are one of the best renewable natural resources that can be exploited for the production of biochemicals (enzymes, proteins, metabolites, co-factors, small molecules, nanomaterials, polymers, etc.), biodegradation, bioremediation and innumerable bioprocesses happening inside. More research should be paced to enrich our knowledge on the mechanism of nanoparticle synthesis, feasible ways of synthesis using microorganisms. Deeper knowledge on the scale-up of microbial mediated nanoparticle synthesis will enhance its usage in electrical and electronic gadgets, thereby reducing the extent of harmful leakage of nanoparticles into the environment.

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Further reading

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Microbial-based synthesis of nanoparticles to remove different pollutants from wastewater

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10.1 Introduction

Nanoparticles (NPs) have started a great deal of consideration as a result of their interesting provisions, which open up a lot of entryways for new specialized arrangements and inventiveness. The advancement of different nanoparticle-based innovations has been helped by their capacity to work on a wide scope of technologies. Over the last few decades, technological advancements have resulted in an increased use of NPs in commercial products for a variety of applications (Santos et al., 2015). Indeed, even close to home consideration products like beauty care products, sunscreens, and toothpastes currently contain them in significant amounts (Gupta and Xie, 2018). This change has encouraged the advancement of antimicrobial materials incorporated into packaging materials, refrigerators, plate, enzyme immobilization technologies, and other food-contact surfaces in the agri-food business (Kadam and Kaur, 2018; Sun-Waterhouse and Waterhouse, 2016). In agriculture, NPs have been proven to promote germination, plant growth, and pest and disease resistance. Water maintenance definitions based on nano-dirts and zeolites, as well as Bouisol, a Cu-NPs containing fungicide that has been used since 1931, are all examples of existing economically accessible NPs-containing commodities in the agribusiness sector (Sekhon, 2014). Emerging nanotechnologies using generated NPs also provide recommendations for water and waste treatment because they are found in remediation sludge that is reused to nourish farming areas (Deschênes and Ells, 2020).

A “top down” or “bottom up” method is frequently used to generate and stabilize NPs. These NPs are made by a procedure known as “bottom up,” which involves the self-assembly of atoms into nuclei, which then evolve into tiny particles. Bulk material is broken down into small particles using different chemical and physical techniques in the “bottom up” strategy (Ahmed et al., 2016; Kharisov et al., 2016; Shedbalkar et al., 2014). Physical procedures include milling, grinding, and thermal ablation, to name a few. On the other hand, the chemical method to NP synthesis comprises techniques such as electrochemistry, chemical reduction, and photochemical reduction. Physical techniques necessitate a significant quantity of energy, making these processes more costly. Actual methodologies have another drawback: nanoscale materials have a lower production yield (Shedbalkar et al., 2014). Chemical methods have recently become the most popular method for synthesis of NPs due to the less energy requirements during the reduction process and the creation of homogeneous particles with incredible size and shape precision. Nevertheless, synthetic techniques are naturally hazardous as a result of the utilization of different unsafe chemicals like potassium bitartrate or hydrazine which are liable for cancer-causing nature, cytotoxicity and genotoxicity (Nath and Banerjee, 2013). Owing to their low biocompatibility, toxicity and instability, synthesis techniques for amalgamating NPs for biomedical applications have been limited. Therefore, developing an ecofriendly methodology that viably balances the morphology, characteristics, size, and stability is as of now the primary focus point of consideration area on nanoparticle synthesis. The use of microorganisms in a bio-assisted synthesis has emerged as a possible alternative to traditional nanoparticle manufacturing approaches (Gahlawat and Choudhury, 2019; Singh et al., 2016). Microbial synthesis is a green methodology that uses biological things such as microbes, fungus, actinomycetes, viruses, algae, and yeast to produce nanoparticles. The microbial pathway provides a low-cost, nontoxic, and dependable method for producing NPs with a wide range of size, composition, shape, and physico-chemical properties. This “green” method of nanoparticle synthesis is intriguing since it involves synthesis in a liquid

environment with little expenses and energy consumption, and it can easily be scaled up to a larger scale (Dhuper et al., 2012). The potential of these microbial agents to act as templates for the generation and organization of nanoscale particles into specific forms is another essential aspect (Gahlawat and Choudhury, 2019).

These nanoparticles hold great potential in pharmaceuticals, biotechnology, nanobiotechnology, infection nanotechnology, biomedicine, catalysis, and wastewater management, among other domains. They can be used to eliminate and reuse heavy metals from wastewaters, for example, without degrading their stability much (Goutam et al., 2020). These biogenic NPs may also be useful in early tumor detection, anticancer activity, drug/gene delivery, bioimaging, and photothermal treatments (Barabadi et al., 2017). Because of their increasing accomplishment and the simplicity with which nanoparticles may be assembled, organisms are increasingly being used in this arena. Nanoscientists and specialists have altered several essential components, such as pH, substrate and biomass concentrations, exposure time to substrate, cellular activities, temperature, and enzymatic processes, to optimize nanoparticle synthesis with controlled sizes and morphologies (Gericke and Pinches, 2006a; Gericke and Pinches, 2006b). The length of the reaction and the use of dodecanethiol as a capping agent, for example, were both critical factors in changing the sizes and morphologies of gold NPs when mixing gold ions with *Bacillus megatherium* cells (Iravani and Varma, 2020). Additionally, Nanda and Saravanan (2009) supported silver NP synthesis via bioreduction of silver ions with *Staphylococcus aureus* aqueous culture supernatants, with the resulting NPs having high antibacterial action. When compared to traditional procedures, bacterial-designed technologies can be applied as cost-effective, and ecologically friendly frameworks. Due to difficulties with biosystems equipment, bacterial applications are now uncommon, and more study is needed to ensure reproducibility of the NP generation process, bacterial survival, and the synthesis of morphologically distinct and pure NPs with controlled sizes and stabilities. Bacteria, on the other hand, have distinct benefits in terms of cost and ease of NP production (Iravani and Varma, 2020).

This chapter focuses on the use of different microbiological agents for metal NPs synthesis, as well as studying the possible mechanisms involved in metal nanoscale particle formation. Further, this chapter also will discuss about different benefits and uses of microbial methodologies in NPs synthesis and its application in water remediation.

10.2 Preparation of nanomaterials

Nanoscience is the study, control, and restructuring of matter on a nanoscale scale (size range 1–100 nm) to generate materials with novel properties and functions that differ significantly from their bulk counterparts (Gallardo-Benavente et al., 2019). The major functional technology of the twenty-first century is bio-nanotechnology. It is a term that refers to the functional usage of biomolecules in nanotechnology and is based on science and nanotechnology-based principles and chemical pathways of live life forms (Bagchi et al., 2012). The synthesis method can be divided into two categories: intracellular and extracellular (Ahmad et al., 2005). A huge range of organic compounds accessible in nature comprising plants and plant items, algae, bacterial yeast, fungi, and viruses could all be utilized for synthesis of NPs. The usage of chemical and bacterial based NPs is similar in most circumstances, however the yields of these two approaches may differ. Diagnostic gadgets, different agents, analytical instruments, physical therapy applications, and drug delivery carriers have all improved as a result of the integration of nanomaterials with biology. There is an extending commercial interest for NPs on account of their complete real nature in various areas like devices, catalysis, science, energy, and drug. Moreover, microorganism can adjust the NPs and constrain their aggregate and likewise are proper for formation of nontoxic and stable particles (Behera and Debata, 2011; Ghashghaei and Emtiazi, 2015).

As the size of the materials building blocks expands from the nanoscale (10–9), to the micro scale, and finally to bulk structures, their characteristics and functionalities may alter. Conventional synthesis of NPs includes various chemical and physical strategies containing chemical reduction in nonaqueous or aqueous solution, sonochemical, template, micro emulsion, and microwave-assisted techniques. All of these processes, however, require energy. Microorganisms such as fungus and bacteria have the natural capability to reduce and oxidize metal ions into metallic or metal oxide NPs, so acting as nano-factories (Raliya et al., 2013).

Over the last decade, there have been enormous advancements in the field of microorganism-made NPs and their applications. Biosynthesis of NPs has several advantages, including benign and ecologically friendly manufacturing, biocompatibility of mixed NPs and cost-effectiveness (Iravani and Varma, 2019; Singh et al., 2016). Instead of physicochemical methods, biosynthesized nanoparticles are free from toxic chemical contaminant which is mainly a useful characteristic for biomedical applications (Ahmed et al., 2016).

Biogenic synthesis has the advantage of not requiring the formation of a coating or the connecting of bioactive combinations to the nanoparticle surface to generate consistent and pharmacologically effective particles, as physicochemical synthesis does (Gahlawat and Choudhury, 2019). Moreover, NP biosynthesis takes substantially less time than physicochemical

methods. Arsiya et al. (2017), for example, used *Chlorella vulgaris* to demonstrate palladium NP production. At room temperature, the transformation of palladium particles into NPs took only 10 minutes.

The polyol and amide groups in *C. vulgaris* extract operate as reducing and stabilizing agents, according to FTIR analysis. Using specific algal concentrates, a number of researchers have developed a fast biosynthetic process with high NP output. Algal concentrations, for example, fused silver NPs in 2, 15 minutes, and 1 hour. Gold NPs were also molded employing biogenic subject matter specialists in 5 and 10 minutes, depending on the significance of the NPs mix. Leaving aside the benefits provided by the natural path for the association, the polydispersity and size of the NPs are still significant and provide testing challenges. Furthermore, considerable effort is based on the utility of mix, as well as particle size and morphological control. As a result, a couple of recent studies have developed a consistent framework for NP biosynthesis that is monodispersity-free. The size and condition of metal NPs could be mandated by either updating or altering the cycle limits (Gahlawat and Choudhury, 2019). El Domany et al. (2018), for example, used *Pleurotus ostreatus* extracellular filtrate to consolidate stable gold NPs with moderate dispersion. The AuNPs combination rate increased as the HAuCl₄ salt concentration increased, lengthening the duration and disturbing the process, whereas pH and temperature had a negative association with AuNPs mix rate, indicating higher productivity at lower temperatures. In the case of organic entities, a change in pH causes an adjustment in the total charge of bioactive molecules. *Arthrobacter sp.* pushes silver NPs mixtures at pH 7.0 and 8.0, but no AgNPs mix was detected at pH 5.0 due to considerable electrostatic offensiveness between silver molecules and EPS under acidic conditions, according to Yumei et al. (2017). Indeed, despite the high electronegativity under basic circumstances, no AgNPs improvement was observed, which is not incredible for decrease in Ag⁺ molecule due to the presence of the -COOH group. This necessitates a more thorough investigation of bio-mediated NP synthesis for high-viability manufacturing. Various regulating elements are involved with the nucleation and course of action of offset NPs for beneficial bio-mediated of metallic NPs (Gahlawat and Choudhury, 2019). The use of biomolecules as a reducing agent in nanoparticle synthesis is a rapidly expanding field of study. In comparison to conventional harmful inorganic reducing agents such as sodium borohydride, the numerous biosynthetic methods used so far have opened the prospect of establishing an affordable and environmentally acceptable technique (Hietzschold et al., 2019). In the following part, synthesis of NPs, numerous parameters such as pH, reactant centers, reaction time, and temperature are discussed.

10.2.1 Components affecting the synthesis of green nanoparticles

Controlling nanomaterial parameters, such as size, shape, and composition, is a major problem in biogenic nanomaterial synthesis. The shape and size of nanomaterials can be controlled by biomolecules including glycolipids and proteins (Chellamuthu et al., 2019). A deeper understanding of the biochemical processes and biomolecules involved in controlling nanomaterial qualities would aid in the development of a biological system with tunable circuits to control nanomaterial properties. Do we need live cells to process the precursors, or can we regulate the nanomaterial characteristics with cell extracts, purified proteins, or glycolipids alone? Purified proteins and biomolecules were found to be sufficient for some nanomaterials, whereas an active microbial cell was required in others. When comparing rod and spherical-shaped copper oxide nanoparticles to star-shaped copper oxide nanoparticles, star-shaped copper oxide nanoparticles had the best catalytic activity for reduction of 4-nitrophenol by NaBH₄. The optical characteristics of gold nanoparticles were similarly controlled by the geometry of the nanoparticles. The size of the nanomaterial is another important factor in determining its optical characteristics. When working with nanoscale materials, the quantum confinement effect is crucial for controlling optical properties. For example, when the size of the nanomaterial grows, the bandgap energy decreases. By increasing the incubation time with *E. coli* cells, investigators noticed an increase in the size of the nanomaterials and a corresponding shift in photoluminescence emission of spherical CdTe quantum dots toward higher wavelength (Red-shift). Biological pathway of preparation presents novel strategies for controlling nanoparticles properties (Engineering bacteria for biogenic synthesis of chalcogenide nanomaterials)

The synthesis, characterization, and application of NPs are all influenced by a number of parameters. Several studies have shown that the nature of synthesized NPs changes depending on the kind of adsorbate and the activity of the substances used in the synthesis process (Ajayan, 2004; Somorjai and Park, 2008). Some of them have NPs that are dynamic in character, with many sorts of symptoms and concepts that alter with environment and time and so forth (Pennycook et al., 2012). The temperature, pH of the solution, concentrations of the extracts utilized, concentrations of the raw constituents used, size, and, most importantly, the synthesis methods all have an impact on the synthesis of NPs (Baker et al., 2013).

The pH of the NPs synthesized by green development methods is a vital aspect. According to researchers, the size and surface of manufactured NPs are affected by the pH of the solution medium. Changes in the pH of the solution

media can influence the size of nanoparticles in this way. [Soni and Prakash \(2011\)](#) demonstrated the influence of pH on the shape and size of silver nanoparticles produced. Furthermore, temperature and pressure are important factors in the synthesis of NPs. The form and size of the synthesized NPs are influenced by the pressure applied to the reaction media. At ambient pressure, the rate of metal ion reduction utilizing biological agents was shown to be substantially faster ([Tran and Le, 2013](#)). Temperature is another significant limiting factor that affects the synthesis of NPs utilizing any of the three techniques. The physical procedure necessitates the maximum temperature ($> 350^{\circ}\text{C}$), while chemical approaches necessitate a temperature of under 350°C . Overall, adopting green technology to synthesize NPs necessitates temperatures below 100°C or ambient temperature. The nature of the nanoparticle generated is determined by the temperature of the reaction medium ([Patra and Baek, 2014](#)). Furthermore, the time period for which the response medium is incubated has a major impact on the quality and kind of nanoparticles created via green innovation ([Darroudi et al., 2011](#)). Additionally, the properties of the synthesized NPs changed over period and were impressively influenced by the synthesis method, exposure to light, and capacity conditions, among other factors ([Kuchibhatla et al., 2012](#)). Differences in time can happen in a variety of ways, including particle aggregation as an outcome of long-term storage; particles may shrink or develop as an outcome of long-term storage; and they may have a shelf life, which influences their potential. To work with the expected use of NPs in current applications, the costs of their synthesis must be handled and controlled. As an outcome, the manufacturing process cost-effectiveness has a big influence on nanoparticle production. Chemical synthesis delivers a high yield in a short amount of time, while it is not cost-effective. As a consequence, chemical and physical synthesis of NPs could be constrained; nevertheless, biological synthesis of NPs is less expensive and scalable.

The particle has a significant impact on the characteristics of NPs. For example, when the size of NPs was reduced to the nanoscale scale, the melting point of the NPs was observed to fall. Because NPs with varied configurations have identical energy, it is simple to improve their shape ([Patra and Baek, 2014](#)). The type of energy often utilized to evaluate NPs induces the nanoparticle's form to change. The chemical characteristics of produced NPs are greatly influenced by their remarkable nature and shape. The porosity of the synthesized NPs has a noteworthy influence on the quality and usage of the NPs. The immobilization of biomolecules onto NPs has been achieved, allowing them to be used in medication delivery and biological applications ([Patra and Baek, 2014](#)). The surrounding environment has a big influence on how the synthesized NPs are designed. A single nanoparticle can quickly form core shell NPs by absorbing materials or reacting with other components in the environment through oxidation or corrosion in a variety of situations ([Sarathy et al., 2008](#)). The manufactured NPs form a covering in a biological system, which makes them thicker and larger ([Lynch et al., 2007](#)). The physical structure and chemistry of the produced NPs are also influenced by the surrounding environment. There are just a few models that show how the environment affects the ability of synthesized NPs to form. When the zinc sulfide NPs' environment was changed from wet to dry, the crystalline nature of the NPs changed rapidly. Similarly, when peroxide is present in the solution in which cerium nitrate NPs are floating, their chemical composition changes ([Kuchibhatla et al., 2012](#)). Individual or isolated NPs are observed to modify their properties when they come into touch with or near the exterior layer of other NPs in the majority of cases ([Baer et al., 2008](#)). The NPs' shifting behavior can be used to create more finely tuned NPs. Secondary metabolites are abundant in many living organisms, including as plants, and act as reducing and stabilizing agents in the synthesis of NPs. Nevertheless, the content of these metabolites differs based on the sort of plant, plant component, and extraction method used. Basically, a variety of microorganisms create different internal and extracellular enzymes in varied concentrations, which affect nanoparticle formation ([Patra and Baek, 2014](#)).

10.2.2 Mechanistic aspects

In the literature, several mechanistic theories regarding NP biosynthesis have indeed been offered. The majority of the research have suggested that nitrate reductase is the primary reducing agent, as well as having a stabilizing effect. A viable biosynthesis pathway, on the other hand, might entail more than one biological component. Pathways involved in the green synthesis of nanomaterials are critical for both the commercialization of nanotechnology and the long-term sustainability of the environment. Environmental pollutants will benefit from improved bioremediation and biomineralization processes thanks to synthesis mechanisms. We attempted to compile a list of the mechanisms for green NP synthesis that have been published in recent years. The latest research will help researchers better comprehend the molecular pathways that are engaged in biochemical processes at complicated surfaces ([Ali et al., 2019a,b](#)).

Efflux pumps, inactivation and complexation of metals, metal efflux systems, impermeability to metals and the shortage of particular metal transport systems, extracellular precipitation of metals, change of solubility and toxicity in changes in the redox state of metal ions, and volatilization of toxic metals by enzymatic reactions might all contribute

to bacteria's capability to survive and develop in unfavorable conditions (Beveridge et al., 1996; Rouch et al., 1995). *Pseudomonas stutzeri* AG259, for example, has been found to transport silver NPs when isolated from silver mines (Mohanpuria et al., 2008). In biotechnological applications, such as bioleaching, biomineralization, and microbial-influenced utilization (MIC) measurements, there are a few instances of microorganisms-metal interaction that are quite important. The degree to which microbially mediated binding changes in the surface study of stainless steel, carbon steel, copper alloys, or other materials are gaining popularity is determined by MIC (Angell, 1999). Bacteria have also demonstrated the ability to oxidize minerals and interfere in mineral precipitation reactions, both directly as aqueous chemical catalysts and indirectly as geochemically reactive solids (Zierenberg and Schiffman, 1990). Commercially, these procedures are used in bacterial leaching activities, such as the processing of gold ores with arsenopyrite (FeAsS) (Harvey, and Crundwell, 1997). Microbial metal reduction can help with both in-place and ex-place remediation of metal pollutants and wastes. To know and understand more about the significance of nanoparticle synthesis and metal reduction, biorecovery of heavy metals, and bioremediation of toxic metals, scientists looked into the components of nanoparticle synthesis and bioreduction, focusing on reducing agents in bacteria (e.g., proteins and enzymes) and biochemical pathways resulting metal ion reduction.

There were more assessments in understanding the work and application of natural and genetically modified bacterial strains and diverse microorganisms in bioremediation of hazardous metals and radionuclide-polluted terrestrial ecosystems because of the vital role that these agents play. Furthermore, these microbes had the power to mobilize and immobilize metals (Stephen and Macnaughton, 1999), and bacteria that might decrease metal ions had the capability to precipitate metals at the nanoscale scale on rare occasions (Stephen and Macnaughton, 1999).

These investigations would lead to a determination of the likelihood of genetically modified microbes overexpressing certain reducing particles and the development of a microbial nanoparticle production technique that may potentially control the form, stability, size, and yield of NPs. The most likely reducing agent is genetically modified microbes, which have been created to enhance protein secretion in general. For example, Kang et al. (2008) investigated a method for adjustable manufacture of semiconductor CdS nanocrystals using genetically engineered *E. coli* for the first time. A strain was produced that could generate phytochelatin (PCs) by expressing the *S. pombe* PC synthetase to test if *E. coli* could be used as a biofactory for the controlled synthesis of CdS nanocrystals (SpPCS). PCs serve to keep the nanocrystal focus from aggregating further by acting as a binding nucleation location for metal ions. The intended *E. coli* structure was shown to be appropriate for a standard metal NP combination (Park et al., 2010). To generate more effective organisms for in vivo NP synthesis, recombinant strains have been explored. Recombinant *E. coli* strains expressing *Arabidopsis thaliana* phytochelatin synthase (AtPCS) and/or *Pseudomonas putida* metallothionein (PpMT) were used to manufacture Se, Cd, Te, Cs, Zn, Fe, Sr, Ni, Co, Au, Mn, Pr, Gd, and Ag NPs. The size of metal NPs might be adjusted by adjusting the concentrations of supplied metal ions. Metal NPs can be biologically synthesized using a modified *E. coli* system (Park et al., 2010).

10.3 Advantages of microbial-based nanomaterials in water remediation

Heavy metal pollution is a critical concern since even extremely low concentrations can have harmful consequences. Heavy metals are nonbiodegradable, bioaccumulate in tissues, and are bio magnified as trophic levels rise (Kalaimurugan et al., 2020; Kapahi and Sachdeva, 2019). Heavy metals can be removed from tannery waste using bioremediation, which is a cost-effective and environmentally benign process. Metal toxicity is a severe environmental hazard due to its nonbiodegradability and bioaccumulation in nature. Bacteria could be used as green biofactories to produce metallic NPs and heavy metal bioremediation in an environmental-friendly way. pH, temperature, reaction mixture, redox potential, and nutritional circumstances, as well as metal chemical composition and moisture, all affect and limit bioremediation efficiency (Iravani and Varma, 2020). Due to a multitude of issues, including low competitiveness and high heavy metal concentrations, bacterial applications have been limited in their usefulness (Kapahi and Sachdeva, 2019). Significant changes in inorganic nutrients, biosurfactants, bulking agents, compost, and charcoal could also improve efficacy (Wiszniewska et al., 2016). Extracellular confiscation, extracellular barrier, and effective movement of metallic ions (efflux), intracellular confiscation, and bioreduction of metals in their ionic state are some of the protecting mechanistic features of heavy metal resistance by bacterial cells (Choudhury and Srivastava, 2001). Chemical entity ionization on the cell wall, sorption sites, and bacterial cell wall layout, all contribute to the stability of a bacterium metal complex (Kapahi and Sachdeva, 2019). The outcome of deterioration progression is determined by the substrate materials and includes a variety of additional factors. Bacteria capable of enzymatic activities, biotransformation, and the generation of exopolysaccharide and metallothioneins be able to interact and endure in the presence of harmful metals. Bacteria have evolved effective metal detoxification and resistance mechanisms in response to metal toxicity in the environment, employing a number of processes including ion exchange, electrostatic interaction, precipitation, redox

procedure, and surface complexation. Heavy metal resistance in bacteria is mediated by methylation, metal organic complexation, intracellular and extracellular metal sequestration, demethylation, metal oxidation, metal efflux pumps, permeability barrier prohibition, and the generation of chelators for metals like metallothioneins and biosurfactants. Extracellular volatilization, valence conversion, and chemical precipitation are all methods used by bacteria to remediate metals (Iravani and Varma, 2020). Because they have negative charge on their cell surfaces in the form of anionic structures, they can bind cationic metal species. The phosphoryl, sulfhydryl, carboxyl, thioether, hydroxyl, amine, ester, alcohol, sulfonate, and thiol groups are all negatively charged sites for adsorbing metals in microorganisms. In the medium model system, for example, heavy metals were removed using *Halomonas elongata* and *Tetragenococcus halophilus* at concentrations of 0.5, 1, and 3 mg/L, respectively (Asksonthong et al., 2018). The effects of pH and incubation time on metal elimination in both microorganisms were also examined, with $Pb > Cd > Hg$ being the order of metal absorption potential in both bacteria. *H. elongata* removed 69.5281.11%, 97.8298.47%, and 74.1789.16% of Hg, Pb, and Cd when the cultivated pH was 5. Hg and Cd removal were highest when *T. halophilus* was cultivated in media pH 7, at 8.6412.69% and 93.5095.12%, respectively, whereas Pb removal was highest in media pH 6.81, at 91.6096.54%. Due to its harmful heavy metal content, fly ash, a substantial byproduct from coal-fired power plants, causes significant environmental degradation. Because arsenic released from fly ash can quickly raise arsenic levels in drinking water above the maximum allowed limit of 10 ppb, arsenic leaking from such ash ponds is unsettling. As a result, arsenic-resistant bacteria could be used for arsenic bioremediation. As a result, researchers observed that two exopolysaccharide-producing strains can absorb arsenic from their biomass. Fly ash was found to be abundant in arsenic-resistant heterotrophs when arsenic-resistant heterotrophs were counted against overall heterotrophs. Because of its interaction with other metals, chromium is considered toxic. In one investigation, strains of *Bacillus safensis* and *Pseudomonas fluorescens* were shown to be capable of degrading chromium at rates of more than 84% and 72%, respectively, among 300 bacteria isolates. Using bioinformatics, the phylogenetic association of 16 S rRNA was confirmed, and the bacterial species YKS2 and YPS3 were identified with 100% sequence similarity. These bacterial-mediated metal degrading technologies could be more environmentally friendly ways to reduce pollution (Iravani and Varma, 2020; Kalaimurugan et al., 2020).

The use of microorganisms to synthesize diverse inorganic metal/metal oxide NPs has emerged as a unique technology. Particle form, size, and monodispersity are all controlled by process factors. The remarkable optical, chemical, photo electrochemical, and electronic characteristics of inorganic metal NPs synthesized with living entities have piqued researchers' interest. *Bacillus* sp., in particular, is quickly adapted to heavy metals and can create inorganic NPs of unique size and shape via intracellular or extracellular mechanisms. *Lactobacillus* sp. and *Bacillus* sp. were used in the microbial-mediated production of TiO_2 NPs. Fungal species have also been used to produce silica and titania NPs, according to Bansal et al. (2006). Fungi secreting extracellular 21 and 24 kDa proteins carry out the hydrolytic conversion of hexafluorotitanate complexes into spherical titania NPs. As a result, it's likely that microbially produced nanomaterials might be used in a variety of industrial applications while increasing their effectiveness (Dhandapani et al., 2012).

10.4 Application of microbial-based nanomaterials wastewater treatment

Heavy metals, pigments, hazardous textile dyes, halogenated recalcitrant pollutants, pharmaceutical and personal care products, organic contaminants, pesticides, and pathogenic microorganisms have all been removed using BNPs made from various bacterial species (Zhou et al., 2016). Also, power age and asset recuperation (for example metal particles) can be accomplished from residue and wastewaters. Sediments and wastewaters can also be used to generate electricity and recover resources (such as metal ions). Microbes' ability to shift metal oxidation states has shown significant promise for resource regaining from polluted streams (Ali et al., 2019a,b; Kim et al., 2012). Instead of using metal ions solution to produce BNPs, wastewater might be used (Ahluwalia et al., 2016). These synthesized BNPs can be used for a variety of applications, including as a catalyst, adsorbent, antibacterial agent for the production of antibiofouling membranes, and so on, thanks to their high specific surface area, charge opposite to contaminants, high reactivity, unique size, and the availability of bacterial cell matrix. As a consequence, the research concentrated on the effectiveness of BNPs as an adsorbent and catalyst in the removal and biodegradation of hazardous contaminants from wastewaters (Ali et al., 2019a,b). Because the rate controlling stage in adsorption provides an indicator of the removal mechanism, the adsorption method has been used in most research studies to evaluate removal performance and processes. The results were compared using linear regression correlation coefficient (R^2) values and adsorption isotherms such as Freundlich, Langmuir, and D-R, as well as kinetic models such as pseudo first-order, pseudo second-order, Elovich and Boyd's, and Weber-Morris (Kim et al., 2016; Jain et al., 2016a,b). The pseudo second-order kinetic model and Langmuir isotherm were shown to be the best fit for adsorption kinetic and isotherm data in the majority of studies. According to the findings of the published research, elimination primarily supports the chemisorption hypothesis. BNP

functional groups including hydroxyl, carboxyl, amino, and others were also important in the adsorptive removal of contaminants through ion exchange mechanism and electrostatic interactions (Jain et al., 2016a, b; Pei et al., 2013). Adsorption could be generated by chemical monovalent ion exchange/intraparticle diffusion microorganism co-metabolism, surface precipitation, and oxidation/reduction, according to a few studies (Kim et al., 2016; Watts et al., 2015a, b). The Langmuir model, which assumes monolayer adsorption of pollutants on a solid surface, was supported by almost all of the studies that looked at adsorption isotherm data. Several researchers have employed BNPs to adsorb dangerous heavy metals such as Pb(II), Cu(II), Cr(IV), Ag⁺, Zn²⁺, Mn²⁺, Co(II), Ni(II), and Cd²⁺. It was determined that the presence of particular functional groups such as carboxylate, methyl, hydroxyl, and amide I, II, and II, as well as a variety of reducing chemicals produced by bacterial cells with charge opposite to metallic ions, were responsible for elimination (Kim et al., 2016; Jain et al., 2016a, b). –OH-groups were largely responsible for the elimination of metallic ions by electrostatic attraction and ion exchange. Furthermore, carboxylate groups were found to be accountable for the surface precipitation of hazardous metal ions. Electrostatic interaction has been discovered to cause H⁺ ions departing bacterial cell groups to form connections with metallic ions on the adsorbent surface (Watts et al., 2015a, b). Due to the capability of the unique functional group and the existence of various cell metabolites, the reactivity of numerous BNPs synthesized utilizing different microorganisms and salt solutions suggested varied adsorptive elimination against the same metallic ion (Iwahori et al., 2014). Regardless, the Langmuir isotherm model was utilized to determine each heavy element's maximum adsorptive capacity and elimination. Furthermore, the negative charge on the surface of BNPs caused elimination efficacy to vary depending on metal type and application conditions (Pei et al., 2013). When the relevant metal ionic radius had a lower ionic radius and a greater electronegativity or higher ratio of ionization potential, selective adsorption of harmful metal ions onto BNPs adsorbents occurred in a complicated system (Kim et al., 2016). It is worth noting that relative preference can be assessed by looking at how easily metal hydroxyl species or acetate complexes can be generated (Tuo et al., 2013). Contact time, pH, initial contaminants concentration, and adsorbent dosage were all taken into consideration. BNPs can operate in almost all pH levels, while their efficiency varies depending on the types of BNPs and pollutants utilized. At pH 5, for example, 95% adsorptive elimination of Cu was attained using biogenic selenium NPs. Furthermore, only 64% adsorptive removal of Cr(VI) was seen when bio-Pd was used at a pH of 12. According to some studies, pH changes may impact adsorptive removal capacity because to the attraction and repulsion between contaminants and BNP surface areas (Kim et al., 2016; Pei et al., 2013).

Contact time and adsorbent dose impact BNPs' capacity to remove certain contaminants (Xiao et al., 2017). Tuo et al. (2013) investigated the reduction of Cr(VI) utilizing biogenic Pd(0) produced by *Geobacter sulfurreducens* and found that over 96% of Cr(VI) was eliminated in just 24 hours. The researchers discovered that the metabolic activity of *G. sulfurreducens* cells on the surface of biogenic Pd(0) was crucial in lowering Cr levels (VI). Enhanced cell dry weight, Pd ratio, and the addition of anthraquinone-2,6-disulfonate (AQDS), which increased the quantity of biogenic Pd(0), all improved Cr(VI) removal. Pei et al. (2013) used biogenic Mn oxide (BMO) biosynthesized by *Marinobacter* sp. MnI to recover silver ions (Ag⁺) from synthetic wastewater and examined the elimination mechanism using SEM and XPS methods. Without injecting silver ions solution, SEM analysis indicates that BMO had a shuttle shape, however after adsorbing silver ions, BMO size quickly increased and was covered by flocculi. As previously demonstrated in an isotherm experiment, the existence of electrostatic interaction among negatively charged BMO and passivity charge silver ions (Ag⁺) caused this. The percentages of Ag(0) and Ag(I) on the surface of BMO after adsorption were 15.29 and 84.71 percent, respectively, according to XPS examinations. Furthermore, the XPS data showed that chemical processes were active during BMO adsorption since a small quantity of Ag(I) was changed to Ag(0) on the surface during the adsorption process. That might be because there are reducing chemicals present (such as hydroxyl groups from glucose residues) that can reduce Au³⁺, Pd²⁺, Pt⁴⁺, and Rh³⁺ (Pei et al., 2013).

In a few tests, BNPs were found superior in degrading and removing hazardous colors and organic contaminants through adsorptive elimination. Toxic dyes (e.g., MO, MB, MG, and CV) and refractory contaminants (e.g., EE2 and diatrizoate) were successfully removed employing BNPs through electrostatic contact and ion exchange mechanisms, along with reduction by bacterial cell co-metabolism (Zhou et al., 2016; Kim et al., 2012).

In a few tests, BNPs were found superior in degrading and removing hazardous colors and organic contaminants through adsorptive elimination. Toxic dyes (e.g., MO, MB, MG, and CV) and refractory contaminants (e.g., EE2 and diatrizoate) were successfully removed employing BNPs through electrostatic contact and ion exchange mechanisms, along with reduction via bacterial cell co-metabolism. According to the findings, EE2 was primarily eliminated, and surface-dependent reactions proceeded, enhancing reactive sites on the surface of biogenic Mn oxide. Furthermore, no pseudo first-order or pseudo second-order kinetics were detected, indicating that the buildup of chemical intermediates or the sorption of released Mn²⁺ ions onto BMO had no effect on the reactivity of biogenic Mn oxide (Ali et al., 2019a,b). The following are some examples of microbial-based nanomaterials for polluted water cleanup. Table 10.1 shows some examples of microbial-based nanomaterials for wastewater treatment

TABLE 10.1 Different microbial based nanoparticles used for the wastewater treatment.

Nanomaterials	Microbial species	Application	References
Polysulfone nanofibrous web	<i>Chlamydomonas reinhardtii</i>	Decolorization of dyes (Remazol Black 5 and Reactive Blue 221)	San Keskin et al. (2015)
Algae-TiO ₂ /Ag bionano hybrid material	<i>Chlorella vulgaris</i>	Photocatalytic reduction of Cr(VI)	Wang et al. (2017)
Microalgae immobilized on chitosan nanofiber mats	<i>C. vulgaris</i>	Nitrate removal from liquid effluents	Eroglu et al. (2012)
Magnetic Chitosan Nanocomposites	Chitosan	Heavy metal ion removal	Liu et al. (2009)
Nano zirconia	<i>Pseudomonas aeruginosa</i>	Adsorption driven bioremediation of tetracycline from wastewater	Debnath et al. (2020)
Biogenic iron (siderite and magnetite)	Iron compounds generated by a natural consortium	To remove copper, zinc, arsenate and chromate from aqueous solutions	Castro et al. (2018)
ZnO	<i>Bacillus licheniformis</i>	Photocatalytic degradation of methylene blue	Tripathi et al. (2014)
Silica nanoparticles	<i>Actinomyces</i>	To decolorize textile effluent	Mohanraj et al. (2020)
TiO ₂ nanoparticles	<i>Bacillus subtilis</i>	Photocatalytic generation of H ₂ O ₂	Dhandapani et al. (2012)

10.4.1 Titanium dioxide

Applications for environmental cleanup have been one of the most fiercely contested topics in photocatalysis in recent years. For large-scale industrial applications, photocatalysis is a straightforward, low-cost, and simple-to-handle technology. Nano Titanium dioxide (TiO₂) with a higher photocatalytic efficacy can be utilized to decontaminate, purify, and decolonize air and water from a variety of environmental sources, as well as remove dye pollution and bio-waste organic compounds. In both cooling water and drinking water systems, biofilm growth causes major difficulties with cleanliness, odor, and taste. The growth of biofilm on the walls of metal surfaces promotes pitting corrosion of pipeline materials such as mild steel, copper, and stainless steel, finally leading to material failure. The corrosive bacterial biofilm amplifies the corrosion products on the metal surface. It is possible that toxic components will leak or release into drinking water, providing a health concern.

As a result, one of the cooling water industry's current priority areas is investigating pipeline materials—bacterial interactions to prevent bacterial species from corroding the material. The formation of nano-crystalline semiconductor thin films of titanium dioxide on a variety of substrates, such as glass slides, stainless steel, and PVC materials, has attracted interest. Following the application of TiO₂ coatings to substrates, corrosion resistance, surface hardness, and bacterial adhesion all increased dramatically. Photocatalytic treatment of aquatic biofilms, pathogenic bacteria, and antibiotic-resistant microorganisms has recently been discovered to be efficient using various sizes and shapes of TiO₂ particles. When bacteria are exposed to light, they will adhere to the optical catalyst (TiO₂) in the substrate's center. The production of free radicals on the particle surface damages bacterial cell walls, resulting in a decrease in bacterial population within the biofilm. For a variety of applications, such as wastewater treatment, biosensors, solar cell panels, lithium-ion batteries, antibacterial activities, and anticancer therapies, a number of technologies for generating TiO₂ particles and enhancing their efficiency are available. Only a few applications employ biosynthesized NPs. In this study, *Bacillus* sp. was isolated from a rare earth element soil environment and used to perform bio-mediated TiO₂ NPs synthesis. The biocidal/photocatalytic activity of these TiO₂ NPs (anatase) on aquatic biofilms was tested using a glass slide ([Dhandapani et al., 2012](#)).

10.4.2 Silica nanoparticles

Silica is a versatile material that has a wide range of industrial and commercial uses. Resins, catalytic supports, molecular filters, medical uses, rubber fillers, and construction materials, among other applications, have successfully generated and employed high surface area silica NPs. Silica NPs are made from a number of sources, including silicon

sludge, rice husk (RH), and rice husk ash, using a variety of processes and materials (RHA) (Bansal et al., 2006; Pineda-Vasquez et al., 2014). RHA, an industrial waste produced by burning RH as a renewable biomass energy source, is one of the most prevalent sources of silica. Rice mills create RH, which is the hard protective shell of rice grains. Rice husk was used in a variety of processes, including combustion, gasification, and pyrolysis, in 2012, with approximately 243 million tonnes generated globally (Pineda-Vasquez et al., 2014). Almost 15% of rice husks are ashes, which might be exploited as a low-cost source of silica in the industrial production of a number of products. Rice husk includes amorphous silica that has been hydrated. To make silica micro and NPs, thermal and chemical techniques were applied (Pineda-Vasquez et al., 2014). These processes, on the other hand, might be costly and inefficient. The fungus *Fusarium oxysporum* was employed by Bansal et al. (2005) to provide a biological method for the synthesis of silica NPs from rice husk. This technology has a lot of potential because it can work in low-temperature and low-pressure environments while also being ecologically benign. Pineda et al. (2012) also used cultures of *F. oxysporum* to generate silica particles from RHA. The shape of RHA silica particles changed before and after contact with the fungus, according to the researchers, and their average size shrank from 600 to 5 m. Depending on the circumstances, microorganisms can alter their interactions with minerals. Process parameters involving *F. oxysporum*, such as substrate and protein concentrations, as well as the addition of silica-containing minerals to the growth media, may have an impact on the biotransformation process (Narayanan and Sakthivel, 2010).

F. oxysporum can leach and change the amorphous silica in RHA into crystalline silica NPs at the same time. The biotransformation step is linked to the generation of organic acids, whereas the leaching step is linked to a hydrolysis process driven by the activity of certain proteins (Bansal et al., 2005). Both processes are influenced by growing circumstances and culture media, which differ in composition, particularly in terms of sugar/protein ratio. The goal of this research was to see how the ability of *F. oxysporum* to produce nanosilica particles in this environment. The ideal conditions for the synthesis of silica particles were determined after comparing the individual growth curves. The effects of organic acids on the hypothesized first phase of the leaching process were given considerable attention (Pineda-Vasquez et al., 2014).

This study looks on the efficacy of silica NPs in the treatment of textile wastewater. The production of silica NPs using magnesium trisilicate hydrate as the silica substrate was made possible by indigenous actinomycetes species isolated from the effluent polluted location. The presence of functional groups and physical features in silica NPs was established using characterization techniques. Encapsulating procedures were used to test the viability and stability of the produced NPs for use in wastewater treatment. The immobilized silica NPs treatment reduced the quantities of solids and hazardous chemicals in the effluents. For encapsulated native silica NPs, the investigations demonstrated an 80% decolorization effectiveness. As a result of its adsorbent and decolorization properties, the present work proposes that immobilized silica NPs in the form of encapsulation could be used to decolorize textile effluent.

In the wastewater collected for this study, toxic substances were identified in high proportions. Out of magnesium trisilicate hydrate, *Streptomyces* sp. was employed to generate silica NPs. UV–VIS spectrometer data, TEM images, and PSA all verified the utilization of magnesium trisilicate hydrate as a favorable substrate for silica nanoparticle conversion. When biosynthesized silica NPs were added to textile effluent, the treated water samples had lower toxicity and color intensity than the untreated samples, indicating that textile effluent can be treated more cheaply and effectively in the future (Mohanraj et al., 2020).

10.4.3 Zinc oxide

The proposed numerous examples a system for making biologically activated ammonia using urea broth in the presence of the ureolytic bacterial species *Serratia ureilytica*, which may then be used to make Zinc oxide (ZnO) NPs on cotton fabric (HM475278). The cotton fabric was immersed in a biogenic zinc ammonium complex medium and heat treated for various periods of time (30, 60, 90 min) at an ideal temperature of 50°C. Analytical techniques such as SEM, XRD, CHNS, EDAX, TGA, and UV–visible spectra were used to evaluate antibacterial efficacy against *E. coli* and *Staphylococcus aureus*. The binding of ZnO NPs to cotton fabric was confirmed by morphology and crystallographic studies. With increasing time periods of 30–90 minutes, spherical to nanoflower shaped particles were produced. Loaded cotton fabrics have significantly stronger antibacterial activity than bare cotton samples. Nanoparticle interaction at the bio-interface has been demonstrated to increase antibacterial activity in wet film interfacial contact experiments, as detected by Epi-fluorescent microscopic observations. This paper describes how antimicrobial textile textiles containing ZnO NPs were made via a biochemical precipitation process mediated by ureolytic bacteria. pH and ammonia content measurements have been used to track the bacterial species' production of biogenic ammonia and its subsequent precipitation to create zinc ammonium complexes. Analytical approaches were used to investigate the loading of ZnO NPs on cotton fabric. The crystallographic orientation of wurtzite type ZnO NPs

was confirmed using XRD. The attachment of ZnO NPs to cotton fabric was demonstrated using SEM and EDAX observations. The structural shape of ZnO NPs changes from spherical to flower-like as treatment time's increase. The nanoparticle laden fabric had higher tensile strength and maximal elongation at breakage, demonstrating its applicability for regular biological and textile uses. Due to continuous interfacial interaction among bacterial species and nanocrystals on the cotton fabric, antibacterial activity experiments on the ZnO NPs loaded cotton fabric expose substantial killing efficacy against *E. coli* and *S. aureus* (Dhandapani et al., 2014).

The straightforward and cost-effective method for producing nanosized ZnO flowers at room temperature were prepared. By incubating *B. licheniformis* bacterial biomass with zinc precursors, ZnO nanoflowers have been created. XRD, TEM, SEM, EDX, and FTIR spectroscopy were used to evaluate the biosynthesized ZnO nanoflowers. The biosynthesized nanoflowers have multiple nanopetals with a width of 40 nm and a length of 400 nm, and are three-dimensional in appearance with a size range of $200\text{ nm}^{-1}\text{ lm}$. ZnO nanoflowers have an average mean size of 620 nanometers. With hexagonal wurtzite structure, XRD indicates excellent crystallinity. Bacterial biomass was discovered to be a significant factor in the biosynthesis of ZnO nanoflowers, according to FTIR studies. The findings reveal that ZnO nanoflowers may be made readily at room temperature using *B. licheniformis* biomass. In the presence of UV irradiation, the produced nanoflowers have excellent photocatalytic activity against MB, with an 83% degradation in 60 minutes. The larger content of oxygen vacancies on the surface of ZnO nanoflowers was responsible for this significant increase in photocatalytic activity. Even after three repeating cycles, the photocatalytic stability of the biosynthesized ZnO nanoflowers was determined to be satisfactory. As a result of our findings, ZnO nanoflowers appear to be a promising choice for photocatalytic destruction of organic contaminants (Tripathi et al., 2014).

10.4.4 Graphene

Physical adsorption and covalent bonding were used to immobilize *Aspergillus oryzae* laccase enzymes on graphene nanosheets. Microscopy techniques were used to study the morphological aspects of the graphene sheets. Contacting graphene with such a solution of laccase enzyme dissolved in deionized water resulted in adsorption immobilization. Within the range of values used, the adsorption process followed a Freundlich model with no trend to saturation. The nitration of graphene was following by sodium borohydride reduction and glutaraldehyde crosslinking in the processes of covalent bonding immobilization. Both approaches enhanced the laccase enzyme's pH range and working temperature when compared to the free enzyme. When immobilized by physical adsorption, the enzyme immediately loses efficiency during the second reaction cycle, however when immobilized using covalent bonding, the enzyme sustains roughly 80% of its effectiveness after six cycles.

Laccase enzymes from the *Aspergillus oryzae* fungus were adsorbed on graphene nanosheets and immobilized. The findings revealed that the adsorption technique provided the enzyme greater stability as a function of pH and medium temperature, which is significant for using the enzyme in industrial operations with varied operating environments. The strategy of immobilization via adsorption, on the other hand, did not ensure the stability of immobilized enzymes in a variety of reaction cycles. In this way, covalent immobilization allowed the enzyme's operational activity to be preserved longer, allowing it to be used in several reaction cycles (Skoronski et al., 2017).

10.4.5 Iron nanoparticles

Trigonella foenumgraecum is a plant with a variety of biological and chemical elements that have a number of applications, including the prevention and treatment of diabetes and other chronic diseases like cancer. Metal cations can be reduced to NPs thanks to the mild reduction characteristics of specific biological and organic components in the organic and aqueous phases of *Trigonella foenumgraecum*. *Trigonella foenumgraecum* was detected in the seed extract for the first time in an aqueous media in this investigation. In an aqueous medium, we were able to successfully synthesize and stabilize zerovalent iron NPs (Fe⁰). The ability of *Trigonella foenumgraecum* in an aqueous extract to stabilize Fe NPs was investigated. UV–visible spectrometry, XRD, thermogravimetric analysis TGA/DTG, magnetization, FTIR spectroscopy, and TEM images were also used to characterize Fe NPs. The Debye-Scherrer equation and TEM were used to measure the size of the NPs, finding that the largest particle distribution number was only about 11 nm. With a rate constant of 0.025 min^{-1} and pseudo first-order kinetics, Fe NPs are extremely effective at degrading methyl orange dye under UV radiation. Antibacterial activity of Fe NPs was also tested against gram-negative *E. coli* and gram-positive *S. aureus* infections. For *E. coli* and *S. aureus*, Fe NPs showed MICs of 32 and 64 g/mL, respectively. Fe NPs were successfully generated at room temperature using aqueous extract of *Trigonella foenumgraecum* and ferric chloride metal salt. To establish the stability and functionality of the nanoparticle surface, Fe NPs were also subjected to a range of tests. After analyzing XRD data

and TEM pictures, the Debye-Scherrer equation was used to compute the Fe NPs size, which was found to be around 11 nm. The current approach for making aqueous phase-stabilized Fe NPs is simple, inexpensive, and efficient. When exposed to UV light, Fe NPs were found to be effective photocatalysts for the breakdown of methyl orange dye. Using pseudo first-order kinetics, the dye degradation reaction rate and order were approximated, and the rate constant k_{app} was found to be 0.025 min. The reactive surface effects of Fe NPs were discovered to cause predictable methyl orange dye degradation. Fe NPs have also been used to treat gram-positive and gram-negative bacteria like *E. coli* and *S. aureus*. In *E. coli* and *S. aureus*, Fe NPs had MICs of 32 and 64 g/mL, respectively (Radini et al., 2018).

The enzyme immobilization approach has the potential to improve the enzyme's overall stability and recoverability, allowing it to be used in new fields. In contrast to the most complex and time-consuming procedure of enzyme immobilization, we provide a simple, moderate, and successful technique for immobilizing laccase on magnetic carrier, which may then be employed to decompose chlorophenol. Self-assembly was used to create $Fe_3O_4@Chitosan$ ($Fe_3O_4@CS$) composite NPs as enzyme carriers. Laccase was successfully covalently bound on $Fe_3O_4@CS$ composite NPs, which effectively removed chlorophenol from water. The specific activity of IM-laccase (immobilized laccase) is 112.4 U, with a 51.8% activity recovery. When compared to free laccase, storage stability, thermostability, pH stability, and operational stability all improved. The effectiveness of 2,4-DCP (2,4-Dichloro-Phenol) and 4-CP (Chlorophenol) degradation by IM-laccase was tested, and the results revealed that 91.4% and 75.5% of 2,4-DCP and 4-CP were degraded after 12 hours, respectively. After ten rounds of deterioration, IM-laccase performed well in a reusability test, with degradation rates of 75.8% and 57.4%, respectively.

Bioreduction of ferric citrate by a natural microbial community from an abandoned mine produces iron oxide and siderite. Because of its high surface area and composition, this precipitate was able to adsorb a variety of heavy metals from dilute solutions. The electrostatic interaction between metallic anions and the positively charged surface of biogenic iron compounds causes pH 4 to be the ideal pH for chromate and arsenate adsorption. The adsorbent has a greater affinity for arsenate than chromate and has a larger sorption capacity. The organic molecules connected to the NPs were able to absorb zinc and copper. Because of the possible interest in treating acid mine drainage caused by mixed sulfide minerals, researchers looked into the bimetallic system As-Cu. When compared to monometallic systems, the presence of other heavy metals in solution influences metal removal. The adsorbent's maximum adsorption absorption remained steady at roughly 0.4 mmol/g despite this. Biotechnological water treatment applications could benefit from these biogenic chemicals (Castro et al., 2018).

10.4.6 Zirconia nanoparticles

The green synthesis of ZrO_2 NPs have been represented by using aqueous leaf extract of *Acalypha Indica* (Tharani, 2016); *Eucalyptus globulus* leaf extract (Balaji et al., 2017); *Azadirachta indica* (neem) leaf extract (Nimare and Koser, 2016); different plant extracts like *Capsicum annum*, *Allium cepa* and *Lycopersicon esculentum* (Jalili et al., 2017); lemon juice and sucrose (Majedi et al., 2016); *Fusarium oxysporum* fungus (Bansal et al., 2004). The bacterial cell free supernatant obtained from bacterial growth media has yet to be employed to synthesize ZrO_2 NPs, which is crucial at this time because bacteria cultivation is one of the most cost-effective and simple methods. With these considerations in mind, ZrO_2 NPs were produced for the first time utilizing free culture supernatant from *Pseudomonas aeruginosa* bacteria in our work to remove tetracycline from wastewater using an adsorption approach. In the not-too-distant future, environmentalists may rely largely on ZrO_2 NPs to purify antibiotic-contaminated water.

In this study, *P. aeruginosa* bacteria were employed to synthesize zirconia NPs using green technology for tetracycline bioremediation using adsorption. Dynamic light scattering (DLS), FE-TEM, XRD, FTIR spectroscopy, and point of zero charge analysis were used to characterize manufactured nano zirconia. The zirconia NPs had an average particle size of 15 nm, a monoclinic and tetragonal crystal structure with a crystallite size of 6.41 nm, elemental zirconium and oxygen, and functional groups such as O-Zr-OH, Zr-O-Zr, and Zr-O-O bonds, and a monoclinic and tetragonal crystal structure with a crystallite size of 6.41 nm. At a pH of 6.0 and a contact time of only 15 minutes, zirconia NPs were found to be effective at adsorbing tetracycline. A strong electrostatic contact between tetracycline's zwitterionic form and the protonated surface of zirconia NPs is the major adsorption mechanism in our study. The kinetic analysis of the tetracycline adsorption process indicated a pseudo second-order kinetic, showing that tetracycline chemisorption over zirconia NPs is achievable. The Langmuir isotherm model proved to be the most accurate of all isotherm models, implying that monolayer tetracycline uptake on the surface of zirconia NPs is involved. In addition, the Langmuir isotherm model calculates a maximum tetracycline adsorption capacity of zirconia NPs of 526.32 mg/g. This finding suggests that zirconia NPs could be employed as a replacement adsorbent to reduce tetracycline contamination in wastewater (Debnath et al., 2020).

10.5 Future recommendations

The following are some research proposals for bacterial-assisted nanomaterial production in the future. Poor quality, biotoxicity and low yield of the utilized precursors, as well as insufficient procedural controls, limit bacterial NP synthesis on an industrial scale. Because of their particular ability to transport electrons outside of the cell, dissimilatory metal-reducing bacteria can be employed to synthesize NPs in this scenario. The inactive extracellular electron transfer method, as well as issues with metabolic pathways and the environment matrix, have a negative impact on the yield and quality of NPs produced; as a result, more optimization and assessment research is needed to rationally create high-quality stable nanomaterials with desirable morphologies. Furthermore, genetically modified bacteria have shown amazing ability in the removal of heavy metals and designed nanomaterials, but the hazards, limitations, and obstacles connected with these abilities must be assessed. For bacterial-assisted NP synthesis processes and heavy metal removals, standardized practical approaches should be fully laid out. Novel methods for treating contaminated industrial wastewaters containing heavy metals, such as the use of novel nano- and biotechnologies for biological toxicity reduction concerns, must be studied to achieve technology-based treatment criteria. Despite the fact that chemical and biological approaches to NP synthesis have gotten a lot of attention, there is still a lot of opportunity for improvement in terms of underlying mechanistic variables, yields, manufacturing speed, separation processes, and final quality control. Whole cells, crude enzymes, refined enzymes, cellular extracts, and culture supernatant can all be used in biosynthesis. Synthetic methods can be sped up, but the NPs produced may not have the long-term stability required. Because bioreduction methods using coenzymes are more expensive, it appears that employing entire cells may be a better option, as coenzymes may be regenerated along pathways in living cells. To improve NP portrayals, enhanced biochemical, microbiological, and biotechnological procedures, along with hereditarily designed plans, are required, including such cell feasibility estimation during and after NP formation, ideal advancement and blending conditions, optimal development pH, and temperature ideal reaping circumstances, and equipped extraction methods.

10.6 Conclusion

Bacteria could help with heavy metal ion bioreduction and biorecovery, as well as the synthesis of metallic nanoparticles. However, further research appears to be needed to improve NP biosynthesis efficiency and reduce particle size. These microorganisms can be used to generate environmentally friendly, nontoxic, and low-cost NPs because they proliferate quickly. Nevertheless, certain essential hurdles must be overcome, such as NP purification and extraction, precise mechanistic features, and NP monodispersity and morphologies management. As a result, NP biosynthesis involving various species of bacteria might be developed in the near future, and their associated mechanistic aspects could be completely researched and explored. To establish the appropriate composition and size, future study will need to consider biosynthesized NPs from these biofactories. It is critical to identify the proteins and enzymes involved in the creation of NPs. Low-cost alternatives should also be developed in order for these synthesis techniques to be economically and commercially feasible. By modifying production parameters, operational consistency and alteration of sizes, morphologies, and monodispersity must be examined and thoroughly verified. Because bacterial synthesis of NPs with varied compositions is currently limited, it is necessary to develop this technology and undertake a complete review of the NPs generated.

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Implementation of microbe-based metal nanoparticles in water remediation

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11.1 Introduction

Water is the most quintessential substance on earth. Anthropogenic activity has destroyed nature's precious gift and has rendered it unfit for consumption. United Nations recognizing the importance of safe drinking water has emphasized the its need and has put this as sustainable goal number 6 amongst the 17 SDGs to be achieved by 2030 (United Nations, 2018). Existing technologies using traditional methods of treatment have low efficiency in removal of emerging pollutants and fail to maintain the strict standards of water quality (Qu et al., 2012). Research is being undertaken to develop new technologies to purify potable water and treat wastewater to reuse it. Nano-based filtration techniques are much more effective than the traditional methods of water purification (Khan et al., 2021). Nanoparticles (NPs) have unique properties which differ from the bulk material. Recent innovation and advancement in nanoscience and technology has led to pathbreaking revolution in water purification, desalination and waste water treatment. The nano-technology based nanofilters are very simple and easy to use, less pressure is required to pass water across the filter. Nanomaterials are effective in water treatment because of their ability to interact with other particles and their high surface to volume ratio (Kapoor et al., 2021). The potential application of NPs for water treatment is based on three systems: disinfection, membrane biofouling control, biofilm control on other relevant surfaces (Das et al., 2013). NPs show antimicrobial activity which is utilized in disinfection of wastewater (Rani et al., 2022).

Depending on their physicochemical characterization nanomaterials can be categorized into metallic nanoparticles (Ag, Au, Pt NPs), metal-based nanoadsorbents (CuO, ZnO, TiO₂, Fe₂O₃ NPs), mixed oxide nanoparticles (Fe-Ti NPs), natural and modified nanoclays, carbon nanotubes, nanofibers, polymer-based nanoabsorbents, etc. (Patanjali et al., 2019). Nanoparticles made of carbon, metals and oxides of metals are used for the process of cleaning up toxic chemicals from different wastewater. Nanofiltration membranes are used for recovery of nutrients from industrial effluent. However very little is known about the impact of nanomaterials on human health and environment. Physically and chemically synthesized nanoparticles can agglomerate and form non-dispersive large particles which can be hazardous for environment (Moustafa, 2017). Also, since nanoparticles behave differently in environment than the bulk materials, they might be toxic and a potential hazard for all living organisms. There is still a lack of laws regarding nanowaste disposal and most of the it lands up in water streams. Life cycle assessment studies on nanomaterials have been able to give a clear picture on the fate of nanoparticles (Arts et al., 2015).

Green manufacturing of nanoparticles has gained acceptance as it is non-toxic, cheaper, environmentally friendly, and requires less time for synthesis. It uses the bottom-up approach of nanoparticle synthesis which is a novel, energy efficient process with less or no sludge production (Kapoor et al., 2021). Studies have shown that nanoparticles generated by the biogenic green processes have higher catalytic reactivity, greater specific area and better contact between enzyme and metals due to the microbial carrier matrix (Li et al., 2011). Bacterial and other microbes take up metal ions from the environment and convert them into element metal with the aid of enzymes generated during cell activity. Nanoparticles may be formed both intracellularly or on extracellular surface (Zhang et al., 2011). Several nanoparticles have been synthesized using bacterial, fungi, algae, etc. (Table 11.1).

TABLE 11.1 Mechanism of microbe-based nano particles for water remediation.

Microorganism involved	Name of microbe	Type of nanoparticle	Size of Nanoparticle	Synthesis method	Mode	Mechanism	References
Bacteria	<i>Shewanella loihica</i>	Copper	10–16 nm	Extracellular	Water pollution treatment	- Antimicrobial activity by disrupting cellular components - Cu induced cytotoxicity is primarily caused by reactive oxygen species formed from ionic Cu in solution via catalytic reaction intermediated by reduced Cu(I)	Wang et al. (2017), Chen et al. (2014), Clar et al. (2016)
	<i>Desulfovibrio desulfuricans</i>	Gold, Palladium	20–50 nm	Periplasmic space, Cell surface	Water pollution treatment	- Gold NPs reduce the pollutants in the form of heavy metals, fertilizers, detergents and pesticides - Palladium NPs causes catalytic degradation of contaminants	Fang et al. (2019), Qian et al. (2013), Wang et al. (2021)
	<i>Desulfovibrio vulgaris</i>	Gold, Palladium	20–50 nm	Cell surface	Water pollution treatment	- Gold NPs reduce the pollutants in the form of heavy metals, fertilizers, detergents and pesticides - Palladium NPs causes catalytic degradation of contaminants	Fang et al. (2019), Qian et al. (2013), Wang et al. (2021)
Fungi	<i>Penicillium citreonigum</i> Dierck	silver	6–26 nm	Extracellular	Water pollution treatment	Antimicrobial activity by generating free radicals. Antimicrobial Ag ⁺ ions will interact with thiol groups of enzymes and inactivate cells	Moustafa (2017), Prabhu and Poulouse (2012)
	<i>Scopulaniopsisosbrumptii</i> Salvanet-Duval	silver	4.24–23.2 nm	Extracellular	Water pollution treatment	Antimicrobial activity by generating free radicals. Antimicrobial Ag ⁺ ions will interact with thiol groups of enzymes and inactivate cells	Moustafa (2017), Prabhu and Poulouse (2012)
	<i>Fusarium oxysporium</i>	Gold	2–20 nm	Intracellular	Water pollution treatment	Gold NPs reduce the pollutants in the form of heavy metals, fertilizers, detergents and pesticides	Das et al. (2017), Qian et al. (2013)
	<i>Verticillium luteoalbum</i>	Gold	2–10 nm	Intracellular	Water pollution treatment	Gold NPs reduce the pollutants in the form of heavy metals, fertilizers, detergents and pesticides	Gericke and Pinches (2006), Qian et al. (2013)

	<i>Aspergillus niger</i>	silver	2–10 nm	Extracellular	Water pollution treatment	Antimicrobial activity by generating free radicals. Antimicrobial Ag + ions will interact with thiol groups of enzymes and inactivate cells	Jiang et al. (2018), Prabhu and Poulouse (2012)
	<i>Aspergillus tubingensis</i>	Iron	Less than 100 nm	Extracellular	Water pollution treatment	Fenton reaction results in generation of hydroxyl radicals that have strong oxidizing ability toward organic compounds	Mahanty et al. (2020), Muradova et al. (2016)
Yeasts	<i>Yarrowia lipolytica</i>	Gold	15 nm	Intracellular and extracellular	Water pollution treatment	Gold NPs reduce the pollutants in the form of heavy metals, fertilizers, detergents and pesticides	Pimprikar et al. (2009), Qian et al. (2013)
	<i>Candida albicans</i>	Silver	5 nm	Extracellular	Water pollution treatment	Antimicrobial activity by generating free radicals. Antimicrobial Ag + ions will interact with thiol groups of enzymes and inactivate cells	Soliman et al. (2018), Prabhu and Poulouse (2012)
	<i>Candida utilis</i>	Silver	5 nm	Extracellular	Water pollution treatment	Antimicrobial activity by generating free radicals. Antimicrobial Ag + ions will interact with thiol groups of enzymes and inactivate cells	Soliman et al. (2018), Prabhu and Poulouse (2012)
	<i>Saccharomyces boulardii</i>	Silver	5 nm	Extracellular	Water pollution treatment	Antimicrobial activity by generating free radicals. Antimicrobial Ag + ions will interact with thiol groups of enzymes and inactivate cells	Soliman et al. (2018), Prabhu and Poulouse (2012)
Algae	<i>Chlorella vulgaris</i>	Silver	Less than 15 nm	Extracellular	Water pollution treatment	Antimicrobial activity by generating free radicals. Antimicrobial Ag + ions will interact with thiol groups of enzymes and inactivate cells	Mohseniazar et al. (2011), Prabhu and Poulouse (2012)
	<i>Dunaliella salina</i>	Silver	Less than 15 nm	Extracellular	Water pollution treatment	Antimicrobial activity by generating free radicals. Antimicrobial Ag + ions will interact with thiol groups of enzymes and inactivate cells	Mohseniazar et al. (2011), Prabhu and Poulouse (2012)

(Continued)

TABLE 11.1 (Continued)

Microorganism involved	Name of microbe	Type of nanoparticle	Size of Nanoparticle	Synthesis method	Mode	Mechanism	References
	<i>Nannochloropsis oculata</i>	Silver	Less than 15 nm	Extracellular	Water pollution treatment	Antimicrobial activity by generating free radicals. Antimicrobial Ag + ions will interact with thiol groups of enzymes and inactivate cells	Mohseniazar et al. (2011) , Prabhu and Poulose (2012)
Actinomycetes	<i>Nocardia farcinica</i>	Silver	11–20 nm	Extracellular	Water pollution treatment	Antimicrobial activity by generating free radicals. Antimicrobial Ag + ions will interact with thiol groups of enzymes and inactivate cells	Sheng et al. (2018) , Prabhu and Poulose (2012)
	<i>Rhodococcus sp</i>	Silver	Less than 25 nm	Intracellular	Water pollution treatment	Antimicrobial activity by generating free radicals. Antimicrobial Ag + ions will interact with thiol groups of enzymes and inactivate cells	Sheng et al. (2018) , Prabhu and Poulose (2012)
	<i>Streptomyces viridogens</i>	Silver	Less than 50 nm	Extracellular	Water pollution treatment	Antimicrobial activity by generating free radicals. Antimicrobial Ag + ions will interact with thiol groups of enzymes and inactivate cells	Sheng et al. (2018) , Prabhu and Poulose (2012)
	<i>Streptomyces hygroscopicus</i>	Silver	Less than 50 nm	Extracellular	Water pollution treatment	Antimicrobial activity by generating free radicals. Antimicrobial Ag + ions will interact with thiol groups of enzymes and inactivate cells	Sheng et al. (2018) , Prabhu and Poulose (2012)
	<i>Thermoactinomyces sp</i>	Silver	Less than 50 nm	Intracellular and Extracellular	Water pollution treatment	Antimicrobial activity by generating free radicals. Antimicrobial Ag + ions will interact with thiol groups of enzymes and inactivate cells	Sheng et al. (2018) , Prabhu and Poulose (2012)
	<i>Thermomonospora</i>	Silver	Less than 50 nm	Intracellular and Extracellular	Water pollution treatment	Antimicrobial activity by generating free radicals. Antimicrobial Ag + ions will interact with thiol groups of enzymes and inactivate cells	Sheng et al. (2018) , Prabhu and Poulose (2012)

Marine microbes	<i>Pterocladia capillacea</i>	Silver	Less than 50 nm	Intracellular and Extracellular	Water pollution treatment	Antimicrobial activity by generating free radicals. Antimicrobial Ag + ions will interact with thiol groups of enzymes and inactivate cells	Azizi et al. (2013) , Prabhu and Poullose (2012)
	<i>Jania rubens</i>	Silver	Less than 50 nm	Intracellular and Extracellular	Water pollution treatment	Antimicrobial activity by generating free radicals. Antimicrobial Ag + ions will interact with thiol groups of enzymes and inactivate cells	Azizi (2013) , Prabhu and Poullose (2012)
	<i>Ulva fasciata</i>	Silver	28–41 nm	Extracellular	Water pollution treatment	Antimicrobial activity by generating free radicals. Antimicrobial Ag + ions will interact with thiol groups of enzymes and inactivate cells	Azizi (2013) , Prabhu and Poullose (2012)
	<i>Colpomenia sinusa</i>	Silver	Less than 50 nm	Intracellular and Extracellular	Water pollution treatment	Antimicrobial activity by generating free radicals. Antimicrobial Ag + ions will interact with thiol groups of enzymes and inactivate cells	Azizi (2013) , Prabhu and Poullose (2012)
Virus	<i>Tobacco mosaic virus</i>	Quantum dots	10–40 nm	surface	Water pollution treatment	Mostly degrade the colored pollutants upon irradiation with infrared and visible light	Zeng et al. (2013) , Salunke et al. (2015)

The current review focuses on role of nanotechnology in water and waste water treatment with emphasis on microbe-based nanoparticles or materials. Comparison of technologies (artificially synthesized and microbe-based nanoparticles) for water purification will be made. Lastly, limitations of microbe-based technologies and possible intervention methods, in order to make these technologies available to the larger population at low cost, will be discussed.

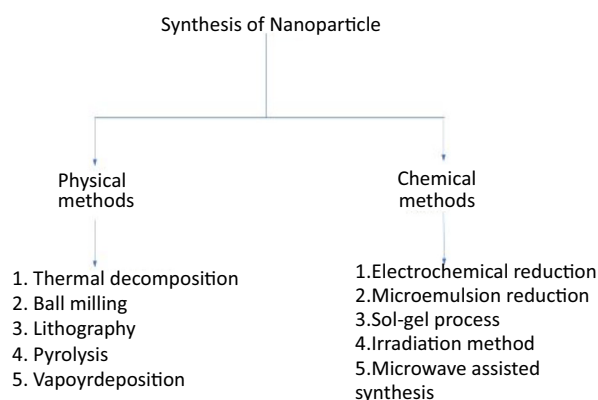
11.1.1 Nanomaterials used in water remediation

Treatment of water and wastewater using nanomaterial is one of the latest innovative technologies being used. Nanomaterials have strong reactivity and very high ability to adsorb due to their small size and large surface area (Lu et al., 2016). In addition, the mobility of nanoparticles in solutions is high which aids in the effective removal of ions, metals, organics, pathogens, etc. It is less toxic to humans and can be used easily. Several metals, metal oxides, composites are now widely being used in water purification and treatment. In the context of water and waste water treatment, NPs and NMs (nanomaterials) can be used as disinfectants (removal of pathogens), as catalyst (removal of pesticides), as sorbents (removal of heavy metals) and as filtering agents (removal of all other contaminants) (Vijaya Lakshmi et al., 2001). NPs can also help in removal of dyes from industrial waste water (Sabouri et al., 2019).

Antimicrobial activity of silver is well known since ancient times and it has been found to disrupt the microbial cell by different mechanisms. Ag can disrupt bacterial cell membrane and alter its permeability (Lu et al., 2016). Studies have revealed that silver ions interact with the phosphorus of DNA causing damage (Dhanalekshmi and Meena, 2016). It also interacts with the thiol group of enzymes and effectively inactivates it (Prabhu and Poulose, 2012). Silver nanoparticles on ceramic membrane and materials has found use in household water treatment units as they help in disinfection and reduction of biofouling (Ren and Smith, 2013). Several other zero valent nanoparticles derived from iron, aluminum, nickel, zinc, titanium, gold, etc. are now in common use. Iron, besides being low cost has exceptional adsorption properties, oxidation and precipitation which makes it a choicest element for application in water treatment (Lu et al., 2016). Magnetic biosorbents derived from iron, cobalt, nickel, etc., have high capacity for removing metal contaminants and organic matter from water (Hassan et al., 2020).

Carbon nanomaterials (CNMs) or carbon nanotubes (CNTs) synthesized from graphene sheets are the material of interest in water and waste water treatment. CNTs have large specific surface area and abundant porous structure which helps it to absorb a large number of contaminants and also aromatic compounds (Khin et al., 2012). Despite the outstanding properties application of CNTs has been marred by the high cost of production. To improve properties of CNTs, they are combined with metals or other types of support to form nanocomposites. Waste water treatment has been revolutionized with advent of nanocomposites (Tesh and Scott, 2014; Yang et al., 2021) which are heterogenous materials produced by a combination of inorganic solids and polymers at the nano level (Sen, 2020). Functional polymer-based nanocomposites are now being used in the ultrafiltration of water (Saleh et al., 2019) and bioremediation of polluted water (Scaffaro et al., 2017). Nanofiltration technique which uses nano membranes has huge commercial demand as their energy requirement and carbon emissions are low, making them very cost-effective (Shahzad et al., 2017). Most common types of nanofilter membranes used are polymeric membranes and ceramic membranes (Jain et al., 2021). Nanostructured titanium oxide and zinc oxide films and membranes can be activated by UV and visible light irradiation to enhance photo degradation and photocatalysis of contaminants removal (Prachi et al., 2013). Nanosorbents such as zeolites, carbon tubes, metal and metal oxide nanosorbent, etc. have also found wide application in treatment of polluted water (El-Sayed, 2020).

The most popular methods for large scale synthesis of NPs are that by physical and chemical techniques (Fig. 11.1). However, use of toxic chemicals during the manufacturing process greatly reduce their application (Li et al., 2011). Physical methods (vapor deposition, lithographic, etc.) and chemical methods which use borohydrides and chemical reduction are harmful for environment and result in aggregation of nanoparticles forming larger particles with reduced monodispersity (Mukherjee et al., 2008). Limitations of this technology include-less reusable, requirement of high energy, reaction selectivity and lack of disinfection of residues (Gehrke et al., 2015). One big concern is that the size of NPs is almost similar in size to biomolecules, they can enter biological systems and mimic the biomolecules (Nagar and Pradeep, 2020). Metallic nanoparticles can be cytotoxic as well as genotoxic (Schins, 2013). Application of nanoparticle may result in dumping and their introduction in soil and water systems. Nanoparticles may enter in organisms via ingestion or inhalation and get translocated to different organs (Khan et al., 2017). Once inside the cell nucleus, NPs may directly interact with DNA or may aid in formation of reactive oxygen species (ROS) which damage nucleic acids (Schins, 2013). NPs may eliminate beneficial microbes from the environment. In a study done by Zhang et al. (2011), it was observed that higher concentrations of Zinc NPs damaged the anammox process which is essential in removal of nitrogen from industrial wastes. Similar research done with titanium oxide NPs reported detrimental effects

**FIGURE 11.1** Physical and chemical methods of nanoparticle synthesis.**TABLE 11.2** Comparison of microbial synthesis vs physical/chemical synthesis of nano particle.

Parameters	Microbial synthesis	Physical/chemical synthesis	Remarks
Reaction conditions	Organisms and their optimum growth conditions, biocatalyst, etc.	Concentration of reactants, different physicochemical conditions, catalysts	Physical and chemical reaction conditions are harsher
Mechanism of synthesis	Biological reduction, mostly enzymatic modifications involving whole cell or cellular extracts	Pyrolysis, Chemical reduction, sol-gel processing, irradiation, electrolysis	Though physical/chemical processes are better optimized, microbial synthesis of nano is less polluting
Time required	Longer duration for microbial cell growth	Lesser than biological synthesis	Optimum cell growth requires time
Environmental concerns	Harsh chemical not used. Process is environment friendly	Toxic chemicals are used which cause soil and water pollution	Biological synthesis is more environment friendly
Energy input and capital requirement	Very low	Quite high compared to microbial synthesis	Microbial synthesis is a low-cost process

on populations of nitrifiers (Yang et al., 2018). The study concluded that the inhibition was due to the accumulation of Zinc NPs in the microbes involved in anammox. Interestingly, in another study on activated sludge, it was observed that lower concentrations of AgNPs actually helped in maintaining microbial diversity (Sheng et al., 2018). Biological or green synthesis of nanoparticles counters the ill effects of physically/chemically synthesized NPs.

The greatest advantage of microbe-based nanoparticles is that it can be produced using natural sources like a biological system, enzymes and biodegradable polymers (Koul et al., 2021). The mechanisms applied by microbes for NPs synthesis include- acidification, bioconjugation, enzyme catalysis, bioreduction, mineralization, metal ion capping, etc. (Khan and Khan, 2017). The biological methods of nanoparticle synthesis are economical and it can be applied for large scale production of NPs (Gour and Jain, 2019; Ali et al., 2019). Microbial NP syntheses occur with aid of enzymes and various reducing agents like terpenoids, proteins, polysaccharides and electron shuttle quinines (Gahlawat and Roy Choudhury, 2019) hence, is essentially considered environment friendly. The production process does not generate any toxic material hence it is largely perceived to be safe (Arya, 2010). Nanoparticles produced by microbes have the capacity to convert toxic pollutants to lesser toxic compounds (Wasi et al., 2008). Microbes synthesize nanoparticles by detoxification process i.e., absorbing precursor metal ions (Mohseniazar et al., 2011). The biological synthesis of metal nanoparticles is a safe and cost-effective method (Ovais et al., 2018). A comparison of biological/green synthesis vs physical/chemical synthesis has been shown in Table 11.2.

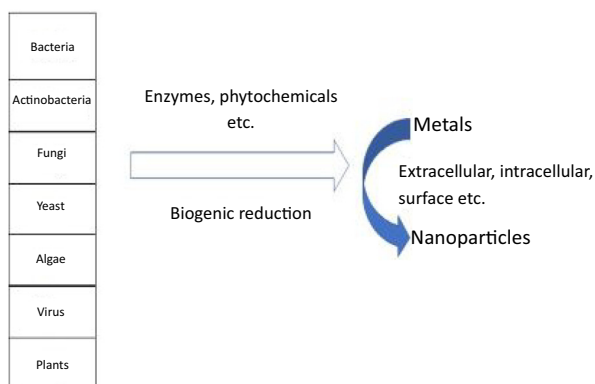


FIGURE 11.2 Green synthesis of nano particles.

Microbe-based nanoparticles can be made in two ways—intracellularly and extracellularly. Metal ions are captured via electrostatic interaction on the surface or inside the cell (Sneha et al., 2010). Enzymes reduce the trapped metals to nanoparticles (Fig. 11.2). The composition of the solution is modified by the microbes, making the solution supersaturated or more supersaturated than it was previously. Microbes can form organic polymer which can impact nucleation by favoring the stabilization of very first mineral seeds (Benzerara et al., 2010). Sneha et al. (2010) stated that enzymes reduced the metal ions to form metallic nuclei such as gold and silver nuclei, which subsequently grow through reduction and accumulation. NADH and NADH-dependent nitrate reductase enzymes are also important for metallic nanoparticle biosynthesis (Husseiny et al., 2007). Microbes produce extracellular and intracellular polymeric substances which can readily interact with NPs in wastewater and can be removed easily (Huangfu et al., 2019). Microbial nanoparticle synthesis depends on pH, temperature, raw material, size, procedure, etc. (Baker et al., 2013). The size and composition of nanoparticles greatly depends on pH of solution or medium (Patra and Baek, 2014). The synthesis of nanoparticles by physical, chemical and biological methods are temperature-dependent. Green synthesis of nanoparticle requires temperature below 100°C (Rai et al., 2006). Tran et al. (2013) stated that at ambient pressure condition, metal ion reduction with biogenic sources was fast.

11.2 Types of microbial nano particle used in water remediation

11.2.1 Nanoparticle from filamentous fungi

The proteins from mycelium of filamentous fungi are used for nanoparticle synthesis (Mohanpuri et al., 2008). Maintenance of filamentous fungi and their growth in laboratory condition is easy (Fouda et al., 2018). Gold nanoparticles synthesized by *Fusarium oxysporum* in presence of aqueous AuCl_4 ions with NADH-dependent enzyme are stable due to protein binding capacity by linkage of cysteine and lysine residues (Das et al., 2017). *Aspergillus niger*, *Penicillium* sp. can synthesize NPs by absorbing heavy metals from polluted sites (Say et al., 2003). They can also remove heavy metals from contaminated wastes (Jiang et al., 2018). *Aspergillus tubingensis* made iron nanoparticles is effective in removal of heavy metals (Mahanty et al., 2020). Salvadori et al., 2013 stated that dead cells of *Hypocrealixii* can uptake Cu (II) to produce copper nanoparticle (Table 11.1). Filamentous fungi can uptake metals. They can be cultured by solid substrate fermentation. Gold nanoparticles are synthesized in the vacuoles of filamentous fungi and their proteins have functional role in capping of gold nanoparticle (Zhang et al., 2011). Khandel and Shahi (2018) stated that filamentous fungi have different enzymes in their cells and they can be easily handled. Synthesis of gold nanoparticle by *Verticillium luteoalbum* was found by Gericke and Pinches (2006). Enhancement of nanoparticle properties can be done by genetic modification methods (Saxena et al., 2014). Fungal metabolites are good resource for synthesis of nanoparticles (Singh et al., 2016).

Fungus such as *F. oxysporum* when exposed to metal ions releases enzymes and causes reduction of metal ions generating nanoparticles (Sharon et al., 2017).

11.2.2 Nanoparticles from yeast

Yarrowia lipolytica was used to generate gold nanoparticles via intracellular and extracellular synthesis (Pimprikar et al., 2009) (Table 11.1). *Yarrowia lipolytica* helps in hydrocarbon degradation and resists heavy metals (Bankar et al.,

2009). *Candida albicans*, *Saccaromycesboulardii* and *Candida utilis* are used to synthesize silver nanoparticle (Soliman et al., 2018). Selenium nanoparticles were generated using *Magnesiomycesingens* LHF1. The synthesized nanoparticle was stable (Lian et al., 2019).

Both intra and extracellular synthesis of nanoparticle occurs in yeasts. Intracellular synthesis is by reduction of metal salts involving passive diffusion of metal salts in aqueous solution into cells, removal of extracellular salts and reduction mediated by transport of reducing reagents into cells (Ma et al., 2016). Membrane bound oxidoreductase are responsible for making nanoparticles (Salunke et al., 2015). Phytochelatin (PCs) and glutathione are essential for bioreduction of metal ions (Sharon et al., 2017).

11.2.3 Nanoparticle from algae

Castro et al. (2013) stated that algae can synthesize nanoparticles as they are aquatic oxygenic photoautotrophs. Silver nanoparticles of approximately 15 nm size can be synthesized inside the cells of *Chlorella vulgaris*, *Dunaliella salina* and *Nannochloropsisoculata* within 48 hours. Algae has significant potential in green synthesis of nanoparticles such as gold, silver, platinum, palladium, copper oxide and zinc oxide. Algae also has bio reduction ability (Konishi et al., 2007; Xie et al., 2007; Oza et al., 2012; Momeni and Nabipour, 2015). Inorganic nanomaterials can be produced by algae both intracellularly and extracellularly (Sau and Murphy, 2004) (Table 11.1). Electrostatic interactions between ions and negatively charged carboxylate groups are responsible for attachment of metal ions on cell surface (Parial et al., 2012).

Algal nanoparticle synthesis involves bioreduction process involving metal ions reduction, enzyme secretion, nucleation and finally color change of the solution (Prasad et al., 2016). Algal nanoparticle synthesis is of two types— intra and extracellular. Intracellular synthesis occurs via NADPH or NADPH dependent reductase released during metabolism (Sharma et al., 2016; Dahoumane et al., 2014). Senapati et al. (2012) demonstrated intracellular synthesis of gold nanoparticles in *Tetraselmiskochinensis*. Extracellular synthesis of nanoparticles occurs when metal ions get attached to the algal cell surface and reduction results by the metabolites (Vijayan et al., 2014).

11.2.4 Nanoparticles from bacteria

Ability of bacteria to adapt in adverse environmental conditions helps in the fabrication of nanoparticles (Wang et al., 2017). Bacteria are capable of converting toxic inorganic ions to non-toxic insoluble metal nanoparticles (Garole et al., 2018; Fang et al., 2019). Bacteria acts as biological platform for mineralization and can be utilized as biocatalyst (Iqtedar et al., 2019). Nanoparticles can be synthesized from dead and live bacteria. *Desulfovibrio desulfuricans* and *Desulfovibrio vulgaris* can convert toxic inorganic ions to insoluble metal nanoparticles which are non-toxic (Fang et al., 2019) as they can catalyze different reactions due to their enzymes (Irvani, 2014) (Table 11.1). Bacterial cells and S layers are capable of metal binding, so they can be used for bioremediation. In an extracellular process the metal ions are reduced by biomolecules present in the medium or cell wall components and electrostatic interactions are responsible for intracellular process.

Vesicle formation, accumulation of metallic ions into vesicles, oxidation reduction system and finally the triggering of crystal nucleation are the steps involved in the synthesis of nanoparticles in bacteria (Arakaki et al., 2008).

Natural metallic nanoparticle synthesis can be of two types- bioreduction and biosorption (Deplanche et al., 2010). In *Delftiaacidovorans*, a small non-ribosomal peptide named Delftibactin, is involved in synthesis of gold nanoparticle (Johnston et al., 2013). *Rhodopseudomonas capsulate* synthesizes gold nanoparticle extracellularly via an NADH-dependent reductase reported He et al. (2007).

11.2.5 Nanoparticles from actinobacteria

Metallic nanoparticles can be produced by actinomycetes intracellularly or extracellularly (Manivasagan et al., 2016; Hassan et al., 2018). Składanowski et al. (2016) reported synthesis of gold nanoparticles by *Nocardia farcinica*, *Rhodococcus* sp., *Streptomyces viridogens*, *Streptomyces hygroscopicus*, *Thermoactinomyces* sp., and *Thermomonospora* sp. *Streptomyces* sp. are used to synthesize copper, zinc, manganese and silver nanoparticles (El-Gamal et al., 2018) (Table 11.1).

Actinobacteria are able to synthesize nanoparticles both intracellularly and extracellularly involving enzymatic reduction and formation of metallic nanoparticle synthesis forming nuclei (Sunitha et al., 2013).

11.2.6 Nanoparticles from marine microbes

Marine microbes that populate the bottom of the sea and can reduce huge amounts of inorganic elements. *Lyngbya majuscula* can generate gold nanoparticle intracellularly (Bakir et al., 2018). CuO nanoparticle synthesized by *Cystoseira trinodis* was found to degrade methylene blue dye and possess antioxidant activity (Gu et al., 2018). Silver nanoparticle are also synthesized by *Pterocladia capillacea*, *Jania rubens*, *Ulva fasciata* and *Colpomenia sinusa* (Azizi et al., 2013) (Table 11.1). *Hypnea musciformis* seaweed can be used as a stabilizing agent in silver nanoparticle synthesis (Roni et al., 2015).

11.2.7 Nanoparticles from virus

Quantum dots are synthesized with the use of viruses (Zeng et al., 2013) (Table 11.1). Inorganic nano crystals in 3D materials can be changed by nanoparticles produced by genetically modified tobacco mosaic viruses (Shenton et al., 1999). Cowpea chlorotic mottle virus and Cowpea mosaic virus are used for mineralization of inorganic materials (Douglas et al., 2002).

11.3 Feasibility of implementation of microbe-based nano in water remediation

Microbe-based NPs synthesis is safer, easier and low cost. However, as with any other microbe-based technology, there are certain drawbacks as well. Achieving uniform shape, size, surface area, composition, symmetry, etc. depends on conditions such as temperature, pH, growth medium, etc. (Hulkoti and Taranath, 2014). Though microbes have tremendous potential to be used in NPs production yet, upscaling it for commercial production can be a huge challenge. Providing laboratory conditions in industry might not be feasible hence the output might vary to a large extent. Moreover, proper toxicity tests and life assessment of NPs should be conducted. A lot of research still needs to be undertaken on the biosafety aspect of NPs. Studies have shown that unregulated deposition of nanoparticles especially in soil and water can lead to the destruction of beneficial microbes and can also destroy the natural microbiome (Ameen et al., 2021).

Use of engineered microbes can be one of the techniques to produce uniform shaped and sized NPs. Genes responsible for fabrication of NPs have been discovered, which when introduced into the wild type microbial strains, generate NPs of desired characteristics (Iravani and Varma, 2019). Researchers have found that genetically modified *Escherichia Coli* could enhance AgNPs production (Yuan et al., 2019). Though genetically modified microbes may synthesize better quality NPs but on the other hand they are difficult to maintain and may not be genetically stable (Iravani and Varma, 2019). Public acceptance of such technology is also an issue which needs to be addressed.

In the context of water or waste water treatment, technology used should have the following features: (1) The technology should be low cost and within the purchase limits of the common man. (2) It should be environment friendly (i.e., low carbon footprint, zero emission, etc. during production). (3) It should not add to the quantum of waste that is already being generated. (4) Should be reusable and recyclable. Following the above principles, scientists at University of New South Wales (UNSW) and Royal Melbourne Institute of Technology have developed a low-cost portable water filter which uses aluminum oxide nano-sheets for filtration (<https://www.indiatoday.in/education-today/gk-current-affairs/story/low-cost-portable-filter-1350778-2018-09-28> dated 28.09.2018). Several such innovation are on the way however the main challenge lies in transferring the technologies from laboratory to the base of the pyramid (BoP) (Vijaya Lakshmi et al., 2001). For that the production cost should be low and risks should be minimum. The nano-based technology should be easy to use, maintenance cost should be low and it should be disposable.

Biomimetic or bio-inspired membranes may also be a solution for water purification. Biomimetic or aquaporin-based membranes can form selective water channels which reject ions. While many of these membranes carry biological nine components, extensive work is being done to develop synthetic alternates like nanochannels which mimic transport or channel proteins (Porter et al., 2020). They are now being used for desalination of water (Gehrke et al., 2015). However, these membranes have been found to be unstable sometimes during actual operations (Giwa et al., 2017) and their efficiency is not at par with biological membranes. Much research is being performed to improve this technology. In future water purifiers will be IoT (Internet of Things) enabled and they will be intelligent devices making data on water quality available at point-of-use (Nagar and Pradeep, 2020). However, sustainable consumption and conservation of water will still hold the key to making water available to the large populations.

11.4 Conclusions

Nanotechnology can provide sustainable solution to the water crisis that looms over humanity. From the above review we can conclude that green synthesis of NPs is a technology of the future. Green synthesis reduces the carbon footprint of NP synthesis and its environmental impact is much lower. Nano-based water purifiers can be used at an individual level as well as for large scale water purification and waste water treatment. Toxicity and end of life assessment studies of NPs can give a clear perception about the safety issues regarding the impact of NPs or NMs on health and environment. Ongoing research has focused on the development of better nanomaterials which addresses all safety concerns.

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Microbial nanoproducts in “waste compost”: a “quality-check” for sustainable “solid-waste management”

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12.1 Introduction

“Nanotechnology” is an innovation for different scientific developments and holds important possibilities for further improvements in the protection of the environment (Faisal et al., 2018). “Nanoparticles” are materials with an exterior dimension of 1–100 nm (Faisal et al., 2018; ISO/TS 27687, 2008; ISO/TS 80004–1, 2010). There are different origins of nanoparticles by which their characters and fate in the environment are controlled. There are mainly four types of nanoparticles viz. organic, inorganic, composite, and carbon nanoparticles. These nanoparticles have different specialized physical, chemical, and optical characteristics at the nanoscale which changed abruptly thus differ from the characters on the macro-scale. For example, gold (Au) nanoparticles are highly reactive show catalytic activities but behave as inert material on a macro-scale (Faisal et al., 2018). Day by day, the utilization of nanoparticles is increasing in various fields of research. There are some knowledge gaps regarding the result of nanoparticle incorporation and their impacts on human health as well as on the ecosystem (Maynard & Michelson, 2006; Stamou & Antizar-Ladislao, 2016) thus elevating the research interest. Nanoparticles are highly reactive as they carry a lot of reaction sites due to higher surface area as well as their mass and surface area ratio (Faisal et al., 2018). Different metal NPs and metal oxide NPs have different uses in different industries to produce essential things like pacifiers, fillers, lubricants, textiles, electronic goods, pharmaceutical, cosmetics, and other personal or environmental care products (Bystrzejewska-Piotrowska et al., 2009; Khan et al., 2013; Whitley et al., 2013; Geertsen et al., 2014; Guvenc et al., 2017). The broad range of their use results in increased NPs production. NPs have immense effects on microorganisms. They have antimicrobial activity especially in silver nanoparticles, thus used in water plants, treatment of wastewater. Different NPs with different characters have different impacts on microbes thus resulting in a variety of responses among the organisms. Like magnetite nanoparticles (Fe₃O₄ NPs) stimulate methane production by enhancing microbial growth (Faisal et al., 2018). The biological synthesis of different metal NPs is performed by different microorganisms like bacteria, fungi, actinomycetes, algae, etc., even by viruses also (Li et al., 2011; Grasso, 2019). They produce NPs of different shapes in both extra and intracellular ways. Most of these NPs synthesized by bacteria have antimicrobial activities like anti-migration, anti-coagulant, antioxidant, etc., for pathogenic bacterial strains (Kalishwaralal et al., 2009, 2010; Torres et al., 2012; Grasso, 2019). These nanoparticles are synthesized by various microbial enzymes. There are potential uses of NPs in biomass like MSW (Municipal Solid Waste) or agricultural waste management. Sewage waste is also purified with the NPs application (Zhang et al., 2017). Composting is a low-cost and environment-friendly way for waste management where solid organic wastes are biologically degraded into humus and can be used as fertilizer (Ayilara et al., 2020). The use of nanoparticles makes difference in the metabolic reaction rate of the composting process. Nanoparticles cause an increase in carbon mineralization thus facilitating humification (Stamou & Antizar-Ladislao, 2016). It also enhances nitrogen utilization in organic composting. Loss in the total organic matter also indicates the maturity and efficiency of

composting. Experiments show that the loss of organic matter is high in the composting associated with nanoparticles than the control one (Zhang et al., 2019) (Fig. 12.1).

In this chapter a brief overview is provided about the biosynthesis of nanoparticles, different organisms, their culture conditions, biosynthetic pathways and morphology of NPs; different ways of NPs synthesis (top-down and bottom-up); aerobic and anaerobic digestions for composting, and notably the application of NPs on solid waste composting.

12.2 Biosynthesis of different nanoparticles

For synthesizing different nanoparticles various methods are there. For example, chemical, physical, biological, or hybrid methods among which chemical and physical methods are well approved but due to toxicity, their application is problematic in the biological field. Thus, the synthesis of NPs through microorganisms has a great impact as this process is non-toxic and eco-friendly. Though the chemical method has several benefits like NPs of defined shape and size are produced on a large scale very fast but become out-dated as this is very expensive, complicated, and harmful to the environment and mankind also (Li et al., 2011). In biological synthesis, the hazards of such toxic and expensive chemicals can be avoided. NP synthesis through microorganisms is inexpensive, safe as well as supported by the certitude that microorganisms inhabit in different conditions with varying temperature, moisture, pH, pressure, and other factors. The NP synthesized by the microbes has a higher surface area and reaction site thus more catalytically active and that improves contacts between the metal salt and enzymes due to bacterial matrix (Bhattacharya & Mukherjee, 2008; Li et al., 2011). Microorganisms grab the metal ion and enzymatically turn them into element metal both extracellular and intracellular (Mann, 2001). In the intracellular method, the ions are transferred into the cell of the microbe and become metal NPs enzymes and extracellular production of NPs depends on enmeshing of the metal ions and their reduction by the microbial enzyme on the cell surface (Li et al., 2011; Zhang et al., 2011).

12.2.1 Gold nanoparticles

Gold has been being used since historic times. Gold NPs are used in medical purposes since 2500 BC among Chinese and In India Red colloidal gold is still used in ayurvedic medicine as “Swarnabhasma” (Swarna, i.e., Gold; Bhasma means ash) (Mahdihassan, 1971; Mahdihassan, 1981; Bhattacharya & Mukherjee, 2008). Radioisotopes of gold are also used in the remedy of different kinds of cancers (Bhattacharya & Mukherjee, 2008). The gold nanoparticle synthesis in modern time had started around 150 years ago. The properties of colloidal gold solutions differ from bulk gold was possibly first inferred by Michael Faraday (Hayat, 1989; Li et al., 2011). Among different microbes, bacteria have a massive use for the synthesis of AuNPs. Bacteria synthesize the NPs by different biochemical mechanisms both in THE intracellular and extracellular ways. The details of these mechanisms are under investigation. These biochemical mechanisms are basically related to microbial resistance. Cellular transporters and oxidoreductase enzymes are mainly involved in this biosynthesis of nanoparticles (Grasso, 2019). The gold nanoparticles may be of different shapes like

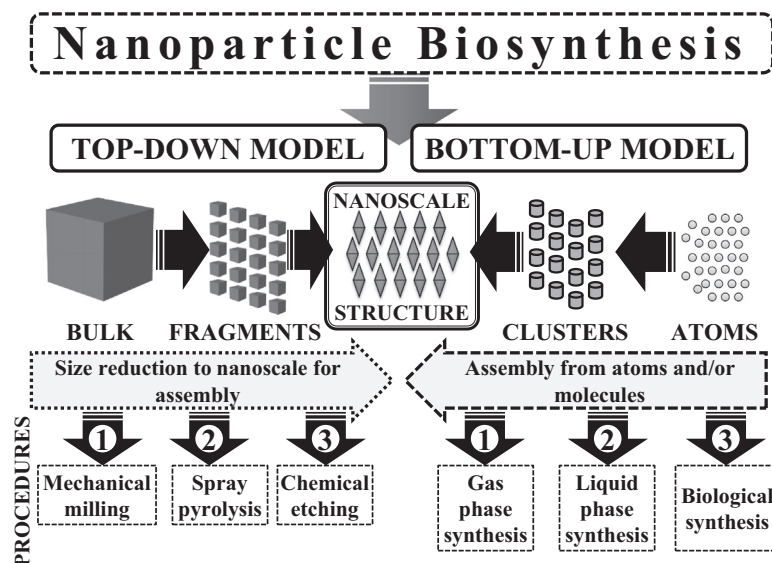


FIGURE 12.1 Top- down and bottom-up approach in synthesis of nanomaterials.

cubical, spheroidal, or octahedral. *Lactobacillus*, *Rhodococcus*, *Escherichia coli*, *Pseudomonas aeruginosa* etc. are AuNPs producing bacteria (Nair & Pradeep, 2002; Du et al., 2007; Husseiny et al., 2007; Li et al., 2011). Fungi are also capable of nanoparticle synthesis. The enzymes, proteins, and metabolites secreted from fungal mycelium take part in the NPs production and required low production cost, low maintenance, and fast-growing thus considered as a better option for the production of nanoparticles (Fouda et al., 2018; Spagnoletti et al., 2019; Kapoor et al., 2021). *Fusarium oxysporum*, *Verticillium luteoalbum*, *Rhizopus stolonifera*, etc., are AuNP producing fungi (Li et al., 2011; Kapoor et al., 2021). Microalgae, yeast, actinomycetes, and virus also participate in the production of AuNPs. Here is brief information in the table that represents different organisms that produce gold nanoparticles (Table 12.1).

12.2.2 Silver nanoparticles

Biosynthesis of AgNPs with controlled shape size and mono-disparity has an immense effect in different disciplines of nanotechnology. Due to unique shape, size, morphology, and distribution Ag nanoparticles show many improved characters (AbdelRahim et al., 2017). Silver nanoparticles have effective antimicrobial properties as in the macroscale. They show antimicrobial activity against many bacterial strains which are multi-resistant like *Staphylococcus aureus*

TABLE 12.1 Details of AuNPs producing microorganisms.

Sl. no.	Organisms	Biosynthetic pathway	Temperature (°C)	Shape	Size (nm)	Reference
A.	<i>Bacteria</i>					
1.	<i>Rhodopseudomonas capsulata</i>	Extracellular	30	Spherical	10–20	He et al. (2007)
2.	<i>Pseudomonas aeruginosa</i>	Extracellular	37	Not available	15–30	Husseiny et al. (2007)
3.	<i>Escherichia coli</i>	Extracellular	37	Tringles, Hexagon	20–30	Du et al. (2007)
4.	<i>Brevibacterium casei</i>	Intracellular	37	Spherical crystalline	10–50	Kalishwaralal et al. (2010)
B.	<i>Filamentous Fungi</i>					
1.	<i>Verticillium luteoalbum</i>	Intracellular	37	Not available	Not available	Gericke and Pinches (2006)
C.	<i>Yeast</i>					
1.	<i>Candida albicans</i>	Extracellular	25	Spherical	5	Ahmad et al. (2013)
2.	<i>Candida utilis</i>	Intracellular	37	Not available	Not available	Gericke and Pinches (2006)
D.	<i>Actinomycetes</i>					
1.	<i>Rhodococcus sp</i>	Extracellular	37	Planar	5–16	Ahmad et al. (2003a,b)
2.	<i>Streptomyces hygroscopicus</i>	Intracellular	35	Spherical	10–20	Waghmare et al. (2014)
E.	<i>Algae</i>					
1.	<i>Tetraselmis kochinensis</i>	Intracellular	28	Spherical and Triangular	5–35	Senapati et al. (2012)
F.	<i>Virus</i>					
1.	TMV	–	–	Spherical	5	Kobayashi et al. (2012)

TABLE 12.2 Details of AgNPs producing microorganisms.

Sl. no	Organisms	Biosynthetic pathway	Temperature (°C)	Shape	Size (nm)	Reference
A.	<i>Bacteria</i>					
1.	<i>Pseudomonas putida</i> NCIM2650	Extracellular	37	Spherical	70	Thamilselvi and Radha (2013)
2.	<i>Bacillus methylotrophicus</i>	Extracellular	28	Spherical	10–30	Wang et al. (2016)
3.	<i>Escherichia coli</i>	Extracellular	Not available	Spherical	10–100	Ghorbani (2013)
B.	<i>Filamentous Fungi</i>					
1.	<i>Aspergillus flavus</i>	Extracellular	25	Spherical	8.92 ± 1.61	Vigneshwaran et al. (2007)
2.	<i>Verticillium sp</i>	Extracellular	25	Spherical		Senapati et al. (2004)
3.	<i>Fusarium oxysporum</i>	Extracellular	25	Spherical		Senapati et al. (2004)
C.	<i>Yeast</i>					
1.	<i>Chlorococcum humicola</i>	Cell extract	Room temp.	Spherical	16	Jena et al. (2013)
2.	<i>Caulerpa racemosa</i>	Cell extract	Room temp.	Spherical and Triangular	5–25	Kathiraven et al. (2014)
D.	<i>Actinomycetes</i>					
1.	<i>Streptomyces kasugaensis</i> NH28 strain	Cell filtrate	27	Rounded	4.2–65	Skladanowski et al. (2016)
E.	<i>Algae</i>					
1.	<i>Scenedesmus sp</i>	Extracellular	28	Spherical	3–35	Jena et al. (2014)

which is methicillin-resistant (Panacek et al., 2006; Li et al., 2011). Biosynthesis of AgNPs is an eco-friendly approach. Various microorganisms are capable of reducing Ag^+ ion for producing spherical AgNPs (Mukherjee et al., 2001a,b; Ahmad et al., 2003a,b; Li et al., 2011). *Verticillium*, *Fusarium oxysporum*, *Aspergillus flavus* are the fungi produce AgNPs in the solution or as film or accumulate on the cell surface (Senapati et al., 2004; Bhainsa & D'Souza, 2006; Vigneshwaran et al., 2007; Jain et al., 2011; Li et al., 2011). *Pseudomonas putida*, *Bacillus methylotrophicus*, *Escherichia coli*, etc. are silver nanoparticle-producing bacteria (Thamilselvi & Radha, 2013; Ghorbani, 2013; Wang et al., 2016). Along with filamentous fungi many yeasts, actinomycetes, and algae also produce silver nanoparticles (Table 12.2).

12.2.3 Other nanoparticles

Along with gold and silver, microorganisms produce different heavy metals, metal alloys, oxides, and sulfide nanoparticles which have different importance. Heavy metals are poisonous to microbes.

Resistant strains are capable of heavy metal detoxification and metal ion efflux which is an energy-consuming process associated with a membrane protein. Different heavy metals like platinum, chromium, cobalt, manganese, cadmium, mercury, etc., are synthesized by a variety of microorganisms (Li et al., 2011). Production of different alloy NPs has different importance. They are used as catalysts, used in electronic industries, used as coating material, etc. (Senapati et al., 2005; Zheng et al., 2010). Yeast (*Fusarium oxysporum*) is capable of producing Au-Ag alloy nanoparticles (Senapati et al., 2005; Zheng et al., 2010). Alloy NPs may be of mixed nanoalloy with random or evenly arranged structure, or with two little sub-cluster, or metal, or intermixed core surrounded by another metal shell, or even may carry multiple shells covering the core (Huynh et al., 2020). Oxide nanoparticles synthesis is another important section

in nanobiotechnology. Mainly two types of oxide particles are synthesized by microorganisms, that is,—magnetic oxide nanoparticles and non-magnetic oxide nanoparticles (Li et al., 2011). Magnetic nanoparticles are comparatively new with distinct properties like high coercive and paramagnetic force, unique micro configuration. For these unique properties, they have wide use in biological separation and bio-medicinal areas. Magnetite (Fe_3O_4), maghemite (Fe_2O_3) are biocompatible magnet NPs that are used in Gene therapy, cancer treatment, MRI, and DNA analysis (Fan et al., 2009; Li et al., 2011). Non- magnetic oxide nanoparticles like TiO_2 , SiO_2 , Sb_2O_3 , CuO , ZnO , are also synthesized by various

TABLE 12.3 Details of microorganisms producing different nanoparticles (heavy metals, metallic alloys, oxides, and sulfides).

Sl. no	Organisms	Items	Biosynthetic pathway	Temperature (°C)	Shape	Size (Nm)	Reference
1.	<i>Shewanella algae</i>	Pt	Intracellular	25	Not available	5	Konishi et al. (2007)
2.	<i>Enterobacter sp</i>	Hg	Intracellular	30	Spherical	2–5	Sinha and Khare (2011)
3.	<i>Shewanella sp</i>	Se	Extracellular	30	Spherical	181 ± 14	Lee et al. (2007)
4.	<i>Desulfovibrio desulfuricans</i>	Pd	Extracellular	25	Spherical	50	Lloyd et al. (1998)
5.	<i>Streptomyces capillaris</i>	Cu	Extracellular	35	Spherical	3.6–59	Hassan et al. (2018)
6.	<i>Rhodospiridium diobovatum</i>	Pb	Intracellular	25	Spherical	2–5	Seshadri et al. (2011)
7.	<i>Fusarium oxysporum</i>	Au-Ag alloy	Extracellular	25	Spherical	8–14	Senapati et al. (2005)
8.	<i>Neurospora crassa</i>	Au-Ag alloy	Extracellular	28	Spherical	20–50	Castro-Longoria et al. (2011)
9.	<i>Saccharomyces cerevisiae</i>	Sb_2O_3	Intracellular	25–60	Spherical	2–10	Jha et al. (2009a)
10.	<i>Lactobacillus sp.</i>	TiO_2	Extracellular	25	Spherical	8–35	Jha et al. (2009b)
11.	<i>Shewanella oneidensis</i>	Fe_3O_4	Extracellular	28	Rectangular, hexagonal, rhombic	40–50	Perez-Gonzalez et al. (2010)
12.	<i>Shewanella oneidensis</i> Mr-1	Fe_2O_3	Intracellular	25	Pseudo-hexagonal / irregular or rhombohedral	30–43	Bose et al. (2009)
13.	TMV	SiO_2 Fe_2O_3	Surface	—	Nanotubes	10–40	Shenton et al. (1999)
14.	<i>Escherichia coli</i>	CdS	Intracellular	25	Wurtzite crystal	2–5	Sweeney et al. (2004)
15.	<i>Rhodobacter sphaeroides</i>	ZnS	Extracellular	Not Available	Spherical	10.5 ± 0.15	Bai and Zhang (2009)
16.	TMV	CdS, PbS	Surface	—	Nanotubes	10–40	Shenton et al. (1999)

microorganisms (Jha et al., 2009a,b; Li et al., 2011; Yang et al., 2021). Due to their unique optical and electronic properties sulfide nanoparticles also have immense importance in the area of fundamental and technical research (Yang et al., 2005). CdS, ZnS, PbS, FeS, etc., are the typical sulfide nanoparticles synthesized by the microorganisms (Li et al., 2011). Brief information of these different nanoparticles mentioned above, their production, morphology and other details are elaborated below (Table 12.3).

12.3 Effect of microbial enzyme on nanoparticle synthesis

An enzyme is contemplated as an organic impetus that has a significant capacity in biochemical responses and assimilation of cellular organisms. The enzymatic action relies on its potentiality to bind with the substrate at explicit called the active site and the enzyme changes over into the product (Brahmachari et al., 2017). The enzyme active site is explicit for a specific substrate with no impedance of others. In nature, the catalysts have a specific job in the blend or biodegradation of mixtures during catabolic or anabolic procedures. Various researches have been done with regards to enlistment and portrayal of microbial catalysts running from small to large scale manufacturing (Abada et al., 2017). The microbial enzymes could be utilized as free or in immobilized structures which relies upon the enzyme particularity. Based on the quick advancement of biotechnology, numerous microbial enzymes have been planned or designed utilizing a molecular approach (Chirumamilla et al., 2001). The production of nanosized particles utilizing microbial cells is an arising pattern in the area of nanoscience. Micro-organisms including fungi, bacteria, viruses, yeast, and actinomycetes go about as potent bioreactors for the decrease of gold, silver, selenium, cadmium, gold-silver amalgams, silica, palladium, platinum and different metals ensuing nanoparticles for organic applications (Narayanan & Sakthivel, 2010). Enzymes effectuate the blend however not get intricate in the reactions. The procedures may likewise get catalyzed by the entire catalyst or amino acids delivered after denaturation of the catalysts by reaction circumstances (Adelere & Lateef, 2016). The nanoparticle can be synthesized by extracellular and intracellular enzymes present in microorganisms as portrayed beneath.

12.3.1 Extracellular enzymes

The microbial extracellular enzyme functions as a diminishing envoy and plays a vital role in metallic nanoparticle synthesis (Subbaiya et al., 2017). For extracellular production emitted enzymes or those existing on the plasma membrane are included (Fariq et al., 2017). The extracellular enzymes like cellobiohydrolase D, acetyl xylan esterase, and glucosidase existing in fungi partakes in formation of metal NPs (Ovais et al., 2018). Extracellular microbial enzymes are referred to assume a huge part as decreasing envoys in the synthesis of metallic nanoparticles (Subbaiya et al., 2017). Studies proposed that co-factors, for example, nicotinamide adenine dinucleotide (NAD) and decreased type of nicotinamide adenine dinucleotide phosphate (NADH) subordinate enzymes both assume imperative parts as reducing envoy through the exchange of electrons from NADH by NADH dependent enzymes which acts as electron transporters (Bose & Chatterjee, 2015). *Fusarium oxysporum* was used as a reducing envoy for AuNPs and AgNPs procreation. The transporter quinone and nitrate-dependent reductase acquired from *Fusarium* species were utilized underway of nanomaterials extracellular (Senapati et al., 2005). *Sargassum wightii* decreased gold ions to structure AuNPs (Singaravelu et al., 2007). *Chlorella vulgaris* also orchestrated AuNPs (Lengke et al., 2006). Enzymes from *Fusarium solani* and *Fusarium semitectum* were used to produce AgNPs extracellularly (Ingle et al., 2009). AgNPs were extracellularly synthesized by *Aspergillus fumigatus* just in 10 minutes when contrasted with chemical and physical procedures. Hence, *Aspergillus fumigatus* has an optimal possibility for pilot scale production of an assortment of nanoparticles (Bhainsa & D'Souza, 2006). Fungal nitrate reductase produces the AgNPs extracellularly. It helps in the bio-reduction and production of metal NPs. Various reports delineated the association of this enzyme in the extracellular production of metal nanoparticles (Kumar et al., 2007a,b). *Penicillium fellatum* was additionally perceived to diminish silver particles in an exceptionally short measure of time. Further investigations state that a protein of nitrate reductase was accountable for the decrease of silver particles (Kathiresan et al., 2009). *Penicillium brevicompactum* was revealed to cause the decrease of silver particles through the release of NADH- dependent enzyme nitrogen reductase (Shaligram et al., 2011).

12.3.2 Intracellular enzymes

In the case of the intracellular system of metal bio-reduction, fungal and bacterial cells alongside sugar particles have a vital role. Predominantly the connections of intracellular enzymes and emphatically charged groups are used in holding metallic particles from the medium and ensuing decrease inside the cell (Thakkar et al., 2010; Dauthal et al., 2016).

When noticed minutely, metallic nanoparticles amassed in the periplasmic space, the cell wall, and the cytoplasmic space. Other than the generally detailed reductase, laccase and ligninase have likewise been accounted for the intracellular synthesis course (Ovais et al., 2018). Among the actinomycetes, alkalo-linient (*Rhodococcus* sp.) and alkalo-thermophilic (*Thermonospora* sp.) species were utilized for the intracellular production of gold nanoparticles (Ahmad et al., 2003a,b). The intracellular synthesis of gold nanoparticles with uniform measurements was done by retaliating *Rhodococcus* sp. with an aqueous solution of tetrachloroaurate ions. The decrease of gold particles was viably interceded by enzymes at the outer layer of the cytoplasmic membrane and mycelia (Ovais et al., 2018). A similar method was taken on the synthesis of AuNPs utilizing *Verticillium* sp. for the reducing enzymes. Gold nanoparticles were captured in the cell wall and plasma membrane of the fungi showing that gold particles were bio-diminished by reductase catalysts that were available there (Mukherjee et al., 2001a,b). The disclosure of *Verticillium* biomass to silver ionic arrangement brought about intracellular decrease and the resulting arrangement of silver nanoparticles. Perception utilizing electron microscopy displayed that the AgNPs were cast underneath the cell wall surface because of enzymatic bio-reduction which is not at all harmful to the fungi (Mukherjee et al., 2001a,b). *Pseudomonas stutzeri* when presented to concentrated silver nitrate solution has diminished silver particles with the development of AgNPs in the periplasmic space of bacteria (Klaus et al., 1999).

Apart from these in the occurrence of palladium nanoparticles, hydrogenases have been found to assume a significant part in decreasing palladium chloride with the accumulation of palladium nanoparticles relying upon the limitation of hydrogenases in *Desulfovibrio fructosivorans*. The chemical fills in as the site of nucleation, for the reduction of palladium by giving atoms (Mikheenko et al., 2008). Moreover, the following enzymes have been reckoned in the reduction of various metals like uranium by *Micrococcus lacticus*, selenium by *Cladosporium pasteurianum*, and gold by *Shewanella* algae (Iravani, 2014).

12.4 Model for formation of nanoparticles

Different physical and chemical mechanisms are there for the production of different nanoparticles and with a lot of research program, these mechanisms have been established by which the physical and chemical properties of the nanoparticles can be tailored as per requirements (Drummer et al., 2021).

Two different mechanisms are there for nanoparticle biosynthesis (Abid and Khan, 2022; Drummer, 2021; Yadav, 2012).

1. Top-down model.
2. Bottom-up model.

12.4.1 Top-down model

The top-down methodology includes more mechanical and physical procedures like mechanical processing. The size of the particles is decayed by processing from the miniature measurements to the nanoscale with solid mechanical shear powers and post-toughening in a static atmosphere (Yadav et al., 2012). Top-down method encompasses the separation of the mass material into nanosized particles. Top-down fusion procedures are an expansion of those that have been employed to produce micron-measured particles. Top-down methodologies are intrinsically straightforward and rely either upon expulsion or division of mass material or on scaling down of mass creation cycle to deliver the ideal construction with suitable properties (Abid and Khan, 2022). In this strategy, the ruinous methodology is utilized. Beginning from a colossal molecule, which deteriorated into modest units, and afterward, these units are changed over into nanoparticles. Illustrations of this strategy are physical vapor deposition, grinding, and other deterioration procedures. These techniques showed the impact of processing time on the general size of the nanoparticles through various portrayal strategies. It was resolved that with time increments the size of nanoparticles crystallite decreases (Iravani, 2011). The fundamental issue of this technique is the defilement of the nanomaterial from the processing media and additionally the climate with powder union, particularly for exceptional dynamic mills. For example, persistent granulating utilizing high-power shaker mills can cause over 10% iron pollution from steel balls and holders. Besides, in case of processing is to be completed under barometric pressing factor, air (i.e., nitrogen and oxygen) can undoubtedly respond with the processing media like metallic tin, aluminum. Subsequently, there are limits to the sort of nanomaterials that can be fabricated utilizing this technique (Yadav et al., 2012). The most concerning issue with the top-down methodology is the fault of surface construction. For instance, lithographically produced nanowires are not so smooth and may accommodate a ton of debasements and imperfections on their surface (Abid and Khan, 2022). A basic top-down route was utilized to integrate colloid carbon circular particles with control size. The blended strategy depends on

the ceaseless compound adsorption of polyoxometalates on the carbon interfacial surface (Khan, et al., 2017). Adsorption made the carbon dark and aggregates into generally more modest round particles with high scattering limits also tight size dispersion (Garrigue et al., 2004). Spray pyrolysis is then again utilized in the industry for large-scale manufacturing of nanoparticles. Such strategy also includes consuming a forerunner in one or the other fluid or fume structure at high pressing factor as well as the temperature where the antecedent is taken care into the furnace through an opening. The benefit of utilizing pyrolysis is that the device set-up can be very straightforward; the production cycle can be very efficient and savvy. Along with that, the nanomaterials can be made under a constant interaction with high manufacture yield (Kammler et al., 2001). Although these strategies can be appropriately settled for huge scope fabrication measures, it requires huge energy utilization and concentrated cleaning convention to create ideal materials in a profoundly unadulterated state. It was additionally reported that materials created utilizing such a methodology were bound to agglomerate and be powerless to surface contamination (Yang et al., 2021).

12.4.2 Bottom-up model

The elective methodology, which has the capability of making less waste and subsequently more orthodox, is the method of a bottom-up approach. Granular perspective alludes to the development from the base. A large number of these methods are as yet being worked on are simply starting to be utilized for commercial manufacture of nanopowders (Abid and Khan, 2022). The bottom-up methodology is utilized backward as nanoparticles are framed from generally more straightforward substances; thus, this methodology is moreover hit developing methodology. Instances of this case are sedimentation and also reduction methods. It incorporates sol-gel, spinning, green amalgamation, and biochemical production (Iravani, 2011). The bottom-up approach can be broadly classified into two categories namely gas-phase synthesis and liquid phase synthesis and biological synthesis (Yang et al., 2021). Generally, in gas-phase synthesis nanoparticles are produced through the reciprocity of gaseous progenitor constituent over an impetus or arranged surface. For instance, chemical vapor deposition is a technique that includes the testimony of a dainty film of the gaseous reactant onto a substrate. A slender film of product is synthesized on the substrate when it is warmed at surrounding temperature by consolidating gas molecules (Bhaviripudi et al., 2007). The upsides of chemical vapor density are in creating profoundly unadulterated, uniform, hard, and solid nanoparticles. Though chemical vapor density requires unique hardware and the flatulent by-products can be poisonous (Adachi et al., 2003). Nanometal layers produced utilizing these strategies were ended up being dynamic against a lot of bacterial strains. For instance, the antimicrobial property investigations of silver nanoparticles, and copper nanoparticles accumulated on the outer layer of biomedical materials like titanium and steel affirmed the inhibitory impact of *Staphylococcus aureus* (Wan et al., 2006). Two regular procedures are utilized to synthesize nanomaterials through liquid-phase synthesis: they are “sol gel” and “microwave assist” strategies. Nanomaterials are framed by “sol gel” measure (Charitidis et al., 2014). Sol-gel amalgamation generally utilizes metal alkoxides as the progenitors or other reactants that would shape a homogeneous medium with the concerned solvent. The interaction initially goes through hydrolysis to frame a colloidal suspension (“sol”). Then it is trailed by complete dissolvable calcination to permit nanoitems to be shaped by employing precipitation. By controlling the factors and drying conditions during precipitation interaction various types of nanoparticles can be made by utilizing this technique (Raab et al., 2011; Rashid et al., 2019). Research information proposes that there has been an expanded interest in using microwave radiation for nanoparticle synthesis (Huang et al., 2004). The manipulation of microwave force and radiation time can likewise control the morphology of nanoparticles manufactured (Hu et al., 2008). Through biological synthesis, nanoparticles can be orchestrated without using poisonous synthetic compounds or worries overages of destructive results. It is feasible to supplant the diminishing substrate utilized in compound strategies with innocuous micro-organisms or plant extracts (Hernandez-Diaz et al., 2021). Although there are various benefits of utilizing biological synthesis for nanoparticle production, there are a couple of downsides. As a large number of systems engaged with these biosynthetic measures stay hazy and it is not facile to maneuver the constituents in microorganisms or plant extracts to enhance the quality and amount of nanomaterial synthesis. Subsequently, nanoparticles devised utilizing such synthetic strategies regularly result in low manufacturing rates and yield (Yang et al., 2021).

12.5 Different conditions for composting

We can split the microbial composting as:

1. Aerobic digestion.
2. Anaerobic digestion.

12.5.1 Aerobic digestion

Aerobic digestion is the process of debasement of organic waste into humus-like stable items utilizing high-impact microorganisms in wet and self-warming conditions. The technique where high-impact absorption is utilized for a change of biowaste into helpful items is prominently known as composting (Srivastava & Bora, 2009). Though compost strength comprises a significant and presumably the most disputable part of in general compost quality as far as definition and assessment. The quality and strength of compost are reliant upon its crude material. During the composting procedure different aspects such as C:N proportion, temperature while composting, pH of the completed item, moisture content along with the presence of potential microbes for example coliform microbes are utilized to survey the quality of the compost (Pan et al., 2011). The main focus should be on the recognizable proof of reasonable however modest crude materials, measures that utilization negligible energy, and the determination of legitimate microorganisms to deliver quality compost. Organic waste is a blend of various amalgams. Regardless of the heterogeneous combination of the organic source materials, it tends to separate into the following significant components such as carbohydrates, fats, proteins, cellulose, hemicellulose, lignin and mineral substituent. The initial three gatherings of organic matter which are truly liable to disintegration incorporate mixtures like sugar, starch, unsaturated fat, gelatin, amino acids, nucleic acid, and lipid. On the other half lignin, hemicellulose and cellulose are considerably more impervious to decay and mineral matter are unaffected by the procedure (Srivastava & Bora, 2009). Oxygen is one more fundamental part essential for metabolic action for vigorous micro-organisms in a composting pit. Oxygen is provided by dynamic air circulation, connective wind stream (passive air circulation) and actual turning of the manure mass. Unnecessarily wet manure material becomes anaerobic, which represses the development of aerobic organisms. On the other hand, flat moisture content can likewise restrict microbial movement since water is the fundamental medium where supplements diffuse and accessibility of the supplement might become restricted (Steger, 2006). While composting carbon dioxide and water are delivered as deterioration items. Respiratory carbon dioxide is developed during microbial movement and the change in carbon dioxide emanations mirrors the metabolic action during the composting process. In the first place accessible carbon is used and delivered carbon dioxide increments to a pinnacle all simultaneously along with pinnacle of temperature and delivered moisture. As the procedure continues nonetheless the pace of the carbon dioxide development diminishes as the accessibility of the carbon decreases prompting a decline in metabolic action (Kulcu & Yaldiz, 2004). The micro-organisms deteriorate the organic matter into a steady amendment for further developing the soil quality and richness. The accomplishment of the treating the composting procedure depends on the capacity of the microbial community to assist with its fundamental requirement for oxygen, moisture, temperature control and supplement accessibility (Borken et al., 2001). Distinctive microbial communities overwhelm during these different composting stages, each adjusted to a specific environment. It is imperative to specify that an enormous assortment of thermotolerant, thermophilic and mesophilic aerobic micro-organisms have been broadly used in composts and other self-warming organic materials.

With the expansion of nanotechnology, nanoparticles have gotten a worldwide concern due to their expanding production and usage. Environmental observation review has acknowledged that nanoparticles are now present in different conditions for example innate water and soil. Toxicological endeavors have reported the unfavorable impacts of nanoparticles on micro-organisms, plants, invertebrates, etc. (Brayner et al., 2006). The high impact of aerobic digestion is well known inferable from its low functional overhead and straightforward functional administration. In any case, it remains muddled to date whether the presence of metallic nanoparticles causes negative consequences for aerobic digestion processing as the presence of nanoparticles in aerobic digester diminished the plenitudes of key microorganisms associated with organic debasement. Overall nanoparticles change the qualities of waste-activated sludge, bringing about the further developed solubilization during high-impact processing; yet it stifled the resulting hydrolysis harshly. This way causes deteriorative execution of waste-activated sludge aerobic digestion (Wei et al., 2021).

12.5.2 Anaerobic digestion

Various elementary studies on the digestion of solid wastes have been ventured. The most important prospect is carving the pattern of the mechanism. In anaerobic digestion, methane and carbon dioxide are produced from the organic matter with the help of various micro-organisms in oxygen gratuitous circumstances (Srivastava & Bora, 2009). The accessibility of a powerful anaerobic digestion representation, permitting the best working boundaries for ideal control to delineate would be significant. This measure happens normally in swamps, rice fields, landfills, and anaerobic bioreactors. Bioreactors are generally landfills where non- precarious fluid waste or water is prepended in a controlled way: in this manner upgrading the speed of waste debasement and landfill gas generation (Kaszubska, 2017). Bioreactor landfills

are additionally regular landfills where microbial measures are strengthened prompting the alteration and adjustment of biodegradable parts of waste inside 5–10 years. There are numerous difficulties in demonstrating the anaerobic digestion of wastes (Mata-Alvarez et al., 2000). In this aspect, micro-organisms play a vital role in breaking down the organic matter. Generally, these micro-organisms exploit nitrogen, phosphorus, and auxiliary supplements for their metabolism but scale down the organic nitrogen into organic acids and ammonia. This underused carbon from the organic amalgamate is fundamentally redeemed in the form of methane or carbon dioxide. The total anaerobic debasement of organic matter is a complicated process including various advances and micro-organisms with distinct metabolic limits. This deterioration is executed by the activity of extracellular catalysts created by agitated and hydrolytic micro-organisms. In this contrast, methanogenesis is one of the major steps involved in the production of methane by anaerobic digestion involving two primary prospects. Firstly, it implicates methanogens which utilize acetate as a substrate whereas various methanogens utilize the carbon dioxide and hydrogen produced (Srivastava & Bora, 2009). In this process not the entirety of the organic matter is debased and winds terminate to a leftover item termed as digestate that is additionally prosperous within inanimate supplements. This generates the leftover item a magnificent supplement to fertilizer, furthermore financial manures on agricultural soils (Oldare, 2005). This digestate accommodates organic matter and plant supplements such as nitrogen, phosphorus, potassium, magnesium and these emphatically influence the quality of soil through further development of the grime architecture, expanding the water-retaining limit and invigorating the action of microbes. In any case, it is significant that the reused digestates does not contain any microbes or potentially substance pollutants that may move towards injurious level in the soil. Sterilization of biowaste at 70°C for an hour is a powerful method of warmth treatment to decrease most pathogens (Bagge et al., 2005). At the inauguration of the fermentative digestion span, no warming or automated blending was enforced to fermenters, bringing about low change proficiency and gas yield. The advancement of fermenters working at thermophilic (50°C–60°C) and mesothermic (30°C–40°C) conditions was a result of expanded information with reference to response figure and also development peak for the fermenting micro-organisms dynamic in the absorption cycle (McHugh et al., 2005).

Apart from the pathogens several nanoparticles also release toxicity during anaerobic digestion of the sludge (Mu, et al., 2011). Previously researchers use only a small dosage of nanoparticles to check their toxicity but it was not prominent enough hence some researchers suggested that it is necessary to increase the amount of nanomaterial dosage to check the toxicity of the nanomaterial (Nyberg et al., 2008). As we have previously discussed the role of methane production in anaerobic digestion, it comprises of dispersible sludge- specific organic compounds, acidification, methanation and hydrolysis (Mu, et al., 2011). The poisonousness of metal oxide nanoparticles is in some cases accepted to apply to the delivered metal particles. Hence micro-organisms are used in bioreactors to reduce the toxicity of nanomaterial and used in the mineralization of the sludge. After digestion the effluents are not suitable to dump directly into the land as they are wet; carrying an outstanding measure of unstable, unsaturated fatty acids which are to some extent phytotoxic. Consequently, it is, for the most part, acknowledged that post-treatment after anaerobic digestion is expected to acquire a top-notch. After a progression of estimation at fertilizer plants, researchers tracked down that the methane outflows were more prominent than they had expected to be simply anaerobic assimilation enjoyed the upper hand over treating the soil, burning or again blend of processing and fertilizing the soil chiefly due to its further developed energy balance (Mata-Alvarez et al., 2000). Finally, it can be presumed that anaerobic digestion will turn out to be a lot more significant in the future for many ecological reasons. In reality, the fate of anaerobic digestion ought to be looked at with regards to a generally feasible waste-management point of view.

12.6 Application of nanoparticles in composting solid waste

Waste can be defined as any surplus rejected things that need to be treated or purified (Lamb et al., 2012). As per the European Association (EU) Waste Framework Directive 2008/98/EC, “any substance or object which the holder discards or intends or is required to discard is defined as waste” (Halkos et al., 2016). The generation of wastes and their proper management is a burning issue nowadays. Waste Management refers to all the necessary actions taken from start to end for controlling the waste. It includes all the steps from production, collection, transport, treatment, and final deposition (Vijayalakshmi, 2020). Several management procedures are followed for solid waste, many of these are very much expensive and problematic also. Among them composting of organic waste material is a safe non-hazardous and environment-friendly method (Singh et al., 2011; Vyas et al., 2021). Composting is a bio-oxidative microbial digestion that ultimately produces stabilized organic matter with high nutrients (Stamou & Antizar-Ladislao, 2016). Microbial composting produces humus with full of minerals which can be further used in agricultural field (Ayilara et al., 2020). Different nanoparticles like AuNP, AgNP, different alloy, and oxide NPs are used in various sectors. Likewise, nanoparticles for their unique properties are also used in composting procedures.

Agricultural solid organic waste like vegetable, rice straw, bran, and also sewage sludge management are done by composting with the AgNP association (Zhang et al., 2017). AgNPs are coated in PVP (polyvinylpyrrolidone) and applied in composting (Zhang et al., 2017) as the stability of AgNP is well maintained in the coating (Gitipour et al., 2013). AgNPs promote carbon mineralization. According to the result of the experiment, the amount of carbon mineralization is higher in the composting pile containing PVP-AgNP but it reduces total nitrogen losses (Zhang et al., 2017).

AgNP and Ag-TiO₂ nanoparticle solutions were founded to stimulate composting in an artificial mixture of solid organic waste (grass, sawdust, wheat straw, vegetables) (Stamou & Antizar-Ladislao, 2016). With a higher concentration of Ag and Ag-TiO₂ nanoparticles, a higher carbon mineralization value is resulted in waste composting. That indicates an impactful presence of nanoparticles for humification. AgNP induces the humins formation in nature and humins accumulation slows down the mineralization of organic matter as the heterogeneous organic components need a large enzymatic assemblage to perform the mineralization properly (Rice, 2001; Stamou & Antizar-Ladislao, 2016; Zhang et al., 2017).

Iron is an important nutrient both for plants and microorganisms which involves in cell growth as well as various metabolisms like N₂ fixation, synthesis of nucleic acid, respiration, etc. (Zhang et al., 2019). A positive response of the enzymatic activity of urease and dehydrogenase enzyme from the iron oxide (Fe₃O₄, Fe₂O₃ etc.) treated soil has been described (He et al., 2011; He et al., 2018). These two enzymes state the performance of organic matter degradation and composting maturity though they are also subjected to environmental factors (Ye et al., 2017; Ren et al., 2018; Zhang et al., 2019). In an experiment, Rice straw, bran, vegetables are dried and cut to make the substrate for composting. Rice straw, soil, vegetables and bran are mixed at a weight ratio 30:28:8:5 and homogenized and moisture content kept 55%. C/N ratio was about 30. The sample was divided as a control (without NP), with Fe₂O₃ and Fe₃O₄. The ambient temperature was 17°C–28°C. During the composting period, the temperature of the compost increases digestion of organic matter due to the activities of microbes. In this setup, the duration of high temperature (about 55°C) of the NP treated pile was longer. Between the 20–40 days of composting, the temperature of the control pile was higher than the NP treated pile and this might be a result of slower rate of organic degradation in the control pile (Zhang et al., 2019).

In case of pH value, three piles have a final average pH of 8–8.2 indicating maturation of composting (Zhang et al., 2018). The IONPs treated piles show a higher pH between 10–43 days attributed to NH₄⁺-N in both the piles due to ammonification of organic nitrogen. After that period, the NH₄⁺-N concentration decreases due to nitrification and ammonia emission. At the end of this procedure, result shows a remarkably high concentration of NH₄⁺-N in the pile compost treated with Fe₂O₃ nanoparticles while remarkably lower concentration of NO₃-N and higher mineral nitrogen than rest of the two. This result suggests that Fe₂O₃ NP is very much efficient for nitrogen utilization in organic composting and their efficiency is greater than Fe₃O₄ nanoparticles (Zhang et al., 2019).

The loss of organic matter is also a good indication for the evaluation of composting. In this experiment, the result about the utilization of organic matter also indicates the efficiency of Fe₂O₃ NP (Zhang et al., 2019).

Dehydrogenase which is an intracellular enzyme catalyzes various metabolic procedures. These are associated with the degradation of organic matter and production of ATP. DHA is an enzyme which is a reflector of the biochemical reaction rate of composting as well as the microbial activity as it involves in the respiratory chain (Li et al., 2015). DHA increases in the three piles during the primary phase of composting and reaches to its maximum value due to the organic digestion and then decreases with the time of composting. The value of DHA is higher in the IONPs treated composting than the control one and height in Fe₂O₃ which is again an indication for the IONPs efficiency and shows that Fe₂O₃ facilitates the composting more (Zhang et al., 2019).

Similarly in case of another intracellular enzyme urease, the same result has been shown. UA catalyzes the production of amide from ammonium. The activity of UA reflects nitrogen mineralization. Like DHA it is also increased in all the piles and then decreases along with the depletion of organic matter by microorganisms (Sudkolai & Nourbakhsh, 2017; Zhang et al., 2019). The average UA is greater in IONPs treated composting pile and the result is similar to DHA (Fe₂O₃ > Fe₃O₄). Iron nanoparticles are suggested as toxic due to the formation of ROS. The positive effect of Fe₃O₄ NP is counteracted by the Fe₂⁺ ion. On the other hand, for being stable and totally oxidized Fe₂O₃ generates very lower amount of ROS and induces an improved microbial activity (He et al., 2011; Zhang et al., 2019). So, it is clear from the experiment that IONPs have a great impact on composting process and they also cause better seed germination and seedling growth when applied in fields (Zhang et al., 2019). Nanotechnology has a great impact on recycling as they are also used as a physical tag in nanoscale. Such tags help the identification of the materials from their start to end. Nanoparticles help the product to keep their original structure. Optical detection, magnetic or electric methods are used to detect the NPs as they are never detached from the product due to their extreme nanosize (Vyas et al., 2021).

12.7 Conclusions

Nanotechnology becomes a booming sector for research of waste management, medical ground, and different industries. Production of nanoparticles through bacteria, actinomycetes, fungi, algae, virus promotes a toxin-free environment-friendly green synthesis of nanoparticles. The morphology of the nanoparticle could be changed by controlling the culture conditions. For the production of desirable nanoparticles, research about genomic and proteomic manipulation of microbes is going on (Li et al., 2011). The use of nanoparticles in the field of waste management is a growing practice. Several experiments indicate that the nanoparticles with their unique properties help the organic composting. They enhance the microbial enzyme for the degradation of organic wastes, increase the rate of composting, carbon mineralization, and nitrogen utilization (Zhang et al., 2019). Further researches is going on this matter to find out more utilities about NPs in this sector as well as in medical sectors and researchers are hopeful about the implementation of NPs in these sectors.

List of abbreviations

NPs	nanoparticles
AuNPs	Au or gold nanoparticles
AgNPs	Ag or silver nanoparticles
PVP	polyvinylpyrrolidone
PVPAgNP	polyvinylpyrrolidone coated Ag nanoparticle
IONPs	iron oxide nanoparticles
DHA	dehydrogenase enzyme
UA	urease enzyme

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Microbial nanotechnology: a potential tool for a sustainable environment

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13.1 Introduction

One of the major constraints in the Anthropocene era is to furnish the basic amenities to every human being while having least impact on the environment and climate. Because of escalating anthropogenic necessities, there is an increasing imbalance between the needs and the natural resources available in environment. Therefore sustainability is the prerequisite for all the studies to be done in the future. What is environmental sustainability? The word sustainability is derived from the Latin *sustinere* (tenere, to hold; sus, up).

Sustainable development has many connotations, explanations, and interpretations in terms of development discourse. According to Brundtland Commission Report, it is defined as the development which meets the requirements of the present generation without threatening future generations' ability to fulfill their requirements (Schaefer and Crane, 2005). It can be considered as a method of advancement that harnesses resources in a manner that these resources should continue to exist for the benefit of societies (Mohieldin, 2017). Evers (2018) connects the idea to establish the principles for accomplishing human development goals while also conserving natural resources and its ability to provide its services to boost the economy while preserving society's trust. The current viewpoints advocates for sustainable development to achieve community improvement, ecological balance, and economic growth (Gossling-Goldsmiths, 2018; Zhai & Chang, 2019). One should underline the essential strategies that diverge away from deleterious socioeconomic activities and apply methods that could support environmental, economic, and social benefits while investigating sustainable development demands (Ukaga et al., 2011; Mensah, 2019).

The following three important features of the sustainable development paradigm were identified:

1. the notion of development (socio-economic development following ecological restrictions),
2. the concept of requirements (resource redistribution to assure the quality of life for everyone), as well as,
3. the idea of future generations (the probability of long-term resource usage while ensuring the essential quality of life for forthcoming generations).

Furthermore, sustainable development outlined key principles such as: providing for the needs and care of the existing and forthcoming generations, continuously improving the global quality of life, preserving the environment, species diversity, and ecosystems, using renewable resources wisely, reducing depletion of nonrenewable resources, reshaping production and consumption according to/with ecological principles, utilizing renewable energy technologies to diminish the climatic changes and its adverse effects, improving national, regional, local collaboration, and creating an institutional framework to encourage the implementation of sustainable development concepts (Tomislav, 2018).

A holistic sustainable development must integrate the three pillars of social, economic, and environmental sustainability (Olawumi and Chan, 2018). Sustainability means limiting human consumption activity of resources, like energy, land, and water, etc., below the carrying capacity of prevailing ecosystems, with the emphasis on the life-sustaining components such as air quality, pure water, and human health. Furthermore, economic sustainability involves maximizing profitability as well as market value by maximizing the efficient use of resources. Besides substituting natural resources for man-made ones, recycling and reusing are also addressed. A social sustainability approach, on the other

hand, balances the needs of an individual with those of the group (equity), educates the public, and mobilizes effort to create coherence and consensus involving local labor and firms. [Sartori et al. \(2014\)](#) concluded that sustainability approaches vary according to the area of implementation such as engineering, management, ecology, etc. Sustainability assessment has been described as a good way to determine whether or not sustainability measures are being implemented at all ([Sala et al., 2015](#)). For an assessment of sustainability, policy formulation and decision-making will be undertaken based on the results ([Hacking and Guthrie, 2008](#)).

Sustainable development combines capitalism and ecology (two antagonistic ideas) into one to improve the system's quality (sustainability). ([Sachs, 1993](#)) made a similar point, arguing that sustainable development attracted a strong following from different areas, bringing ecology, which is the basic concept of sustainability, and economics closer together in the pursuit of sustainability. [Jabareen \(2008\)](#) claims that sustainable development cannot adversely affect economic relations without resolving the ecological crisis. As such, it is intended to resolve the paradox between environmental (sustainable) and economic (development) ([Feil & Schreiber, 2017](#)).

In practice, sustainable development entails the alignment of commercial, environmental, and societal goals across different sectors, boundaries, and generations. This aspect results to achieve truly sustainable development, eliminating any fragmentation; with incorporation of environmental, societal, and economic concerns throughout the policymaking procedures.

13.2 Nanomaterials as an alternative for sustainable development

Growing population caused over-consumption of natural resources, as well as high emissions of damaging gases, which has entrusted the scientific community with the responsibility of establishing a sustainable and safe environment so as to conserve natural resources by protecting our ecosystems to support health and wellbeing. Therefore a tool, which can establish a balance between these two, leading to achieving sustainable development, is required; and nanotechnology has appeared as a technology that can be the answer to the challenges faced by the world for sustainability ([Diallo and Brinker, 2011](#); [Brinker and Ginger, 2011](#)).

Nanotechnology uses materials that possess dimensions in the nanometer range (<100 nm) with distinct chemical and characteristic features like high surface-to-volume ratios and explicit surface plasma resonance when compared to bulk materials, which boosts their potential for a variety of applications ([Khan and Rizvi, 2014](#)). Nanotechnology is a recent revolutionary technique that involves the synthesis of materials at the atomic size for usage in nanoproducts ranging from cosmetics to clothes, plastics to photonics, pharmaceuticals to food, and energy. However, the earlier synthetic route adopted for the development of such nanoparticles imparts an increase in toxicity to the environment. The nanoparticles which are produced chemically have disadvantages as the use of chemicals and self-accumulation in an aqueous solution. Further, the cost involved in making synthetic nanoparticles was another tailback.

Moreover, to give society a friendly environment, "green nanotechnology" is now being considered as an alternate tool to produce nanoproducts and the procedures which are capable of combating large energy consumption and rising environmental toxicity. Green nanotechnology has established a role in the conception of a sustainable environment by eliminating the usage of harmful chemicals in the production of nanomaterials. It is being used to develop cost-effective methods for producing nanoparticles that pose no damage to humans or the environment ([Razack and Durairasan, 2020](#)).

As nanoparticles are increasing into industrial applications and consumer products, it is critical to acquire knowledge of how these substances impact the environment. The nanomaterials have inimitable physiochemical properties that can make them functional materials for sustainable development. However, the extensive breadth and complexity of nanomaterials necessitate the development of molecular-level design specifications. The major challenge like to harness the energy of chemistry to ensure that nanoenabled technology can be developed in an environmentally responsible manner ([Hamers, 2017](#)). To their enhancements, biogenic nanomaterial synthesis employing plants and microbes as biofactories also broaden their application because these methodologies reduce the toxicity associated with chemical methods of nanoparticle manufacture, making their adoption easier. The transition towards these sustainable methods of nanostructure production, microbial nanomaterial production methodologies seems to be one of the promising tools that will ultimately benefit in their safe, non-toxic, efficient, and eco-friendly application in a wide range of industries and agricultural practices in the future.

With an increase in demand and the idea of a sustainable environment, microbes-mediated fabrication of nanoparticles connotes a green approach. Unlike the synthetic method, the microbial approach is eco-friendly, cost-effective, and non-toxic and can be carried out in both intra- and extracellular modes. Thus the use of microbial nanotechnology represents a cleaner production method that is both toxin-free, natural resource-based, and cost-effective.

In the present scenario, the field of microbial nanotechnology has become more effective and striking for agriculture, industry, health, and the environment. The integration of microbial nanotechnology and environmental biotechnology has shown encouraging results in many areas which include environmental issues. The present chapter will focus on some areas such as a brief idea of microbial synthesis of nanoparticles and their role in the sustainability of the environment. Further, the advantages, disadvantages, and future prospective of nanoparticles are discussed in the chapter.

13.3 Microbial synthesis of nanoparticles

Microorganisms and biological systems are the best-known options to achieve this aim i.e. the synthesis of non-toxic nanoparticles which have minimum impact on the environment, more reliable, non-toxic, eco-friendly, and non-chemical based experimental procedures to synthesize nanoparticles (Iravani, 2011; Korbekandi et al., 2012, 2013). They are considered to be an effective bio-factory for the biofabrication of nanoparticles like gold, silver, platinum, titanium, etc. To biosynthesize diverse forms of NPs, a microbial cell acts as a proficient bioreactor. This microbial nanostructure offers a green and efficient way for synthesizing biocompatible particles with striking physicochemical and optoelectronic properties. Therefore an understanding of the microbial cellular mechanism is essential for designing a nanoparticle with the desired size, shape, and properties. Microbes of different environmental origins interact with minerals and heavy materials present in their niche which enable them to develop unique characters either cellular or physiological through which they can alter and modify in the forms of nanostructures. Microbes that are thermophilic, acidophilic, halophilic are being explored for their nanobiotechnological applications and are genetically modified to design a unique procedure for the synthesis of nanoparticles. For example, microbes living in high metal contamination areas have gained tolerance to the surrounding metals which can lead to the conversion of toxic form to the non-toxic form of metals. Similarly, microbes growing in industrial discharge, polluted waters have the capability of recovering metals and converting them into a new nanoparticle/structure. Due to these attractive and exotic features of microbes, these are preferred over the conventional methods to construct the nanoparticles. Further, these microbial nanoparticles can be harvested as drug delivery vehicles, as they have large surface areas. Various drug molecules and ligands show their efficiency on the surface of nanoparticles where they show a sustained and triggered release of the drug. This not only prevents the accumulation of non-specific drug but also decrease the amount of drug to be used. This approach is important to overcome the drug resistance faced in medical sciences. In the current chapter, we will focus on outlines of scientific literature discussing the microbes used in the synthesis process of different nanomaterials and their approach towards sustainability (Table 13.1).

13.4 Application of microbial nanoparticles in different sectors

13.4.1 Microbial nanoparticles for integrated pest management and agricultural practices

Apart from any development, the first and foremost necessity is to feed the global population, and to fulfill this demand sustainable agriculture is essential to, achieve “Zero Hunger,” one of the United Nations’ 17 sustainable development

TABLE 13.1 Examples of nanoparticles synthesized by microorganisms (Bahrololom et al., 2021; Li et al., 2011).

S. no.	Microorganism	Nanoparticle synthesized
1.	<i>Brevibacterium casei</i> , <i>Rhodopseudomonas capsulate</i> , <i>Yarrowia lipolytica</i> , <i>V. luteoalbum</i> , <i>Plectonema boryanum</i> , <i>Shewanella oneidensis</i> , <i>Sargassum wightii</i> , <i>Shewanella algae</i> , <i>Pseudomonas aeruginosa</i> , <i>Escherichia coli</i> , <i>Candida utilis</i> , <i>Plectonemaboryanum</i> , <i>Rhodococcus</i> sp.,	Gold (Au)
2.	<i>Fusarium oxysporum</i> , <i>Aspergillus fumigatus</i> , <i>Fusarium solani</i> , <i>Bacillus cereus</i> , <i>Trichoderma viride</i> , <i>Escherichia coli</i> , <i>Phaenerochaete chrysosporium</i> , <i>Trichoderma viride</i> , <i>Bacillus licheniformis</i> , <i>Corynebacterium glutamicum</i> , <i>Aspergillus flavus</i> , <i>Verticillium</i> sp., <i>Neurospora crassa</i>	Silver (Ag)
3.	<i>Fusarium oxysporum</i> , <i>Lactobacillus acidophilus</i>	Cadmium Sulfide (CdS)
4.	<i>Lactobacillus casei</i>	Copper (Cu)
5.	<i>Bifidobacterium</i> sp., <i>Lactobacillus casei</i> , <i>Lactobacillus acidophilus</i>	Selenium (Se)

objectives. Food production and distribution around the world are under enormous strain as a result of the rising world population, climatic changes, contamination of water and other resources, penicillin-resistant, and growing energy demands.

Agricultural practices now consume a startling amount of resources. For example, worldwide annual crop production exceeds 3 billion tons, while requiring 187 million tonnes of fertilizer, 4 million tonnes of pesticides, 2.7 trillion cubic meters of water (which is approximately 70% of all freshwater for consumption at the global level), and more than two quadrillion British thermal units (BTU) of energy (Kah et al., 2019). These circumstances necessitate significant modifications in worldwide agricultural and other food production systems.

As Food and Agriculture Organization (FAO) estimated that the global population will increase to 10 billion by 2050, and this expansion will create an upsurge of food demand by 50%, especially in developing countries (Usman et al., 2020). To achieve this demand, the global food production system needs a profound and strategic refurbishment. Fortunately, recent findings have revealed that nanotechnology has the potential to improve the agricultural sector by enhancing the efficacy of agricultural practices and can offer remedial solutions to combat agricultural and environmental constraints like improving crop yields as well as a healthy ecosystem.

Moreover, worldwide agricultural land is frequently disturbed by different pathogens and pests which very badly affects the growth of plants and overall agriculture production leading to greater loss at economic levels and developing warnings for food security at the global level (Ingale and Chaudhari 2013). To overcome these notorious agents, farmers indiscriminately used a variety of synthetic agrochemicals which lead to soil and water and environmental contamination through mechanisms like biomagnification and bioaccumulation at different trophic levels. These harmful chemicals enter into the food chain and get stored in living organisms leading to a variety of diseases, species destruction, and habitat destruction. As an alternative option, the microbial production of NPs through the green synthesis procedure offers simple, environmentally safer, and cost-effective pathways side by side, also ensuring a sustainable approach to development. The new category of nanopesticides produces non-toxic and better results of pesticides delivery system in accordance of global food production via reducing the total number of applications in comparison to applied classical formulation (Kah and Hofmann, 2014).

Employment of nanotechnology in agriculture as nanofertilizers, nanoinsecticides, and nanosensors has demonstrated substantial benefits in plant growth and crop production. These NPs can be further employed for the biosensors field because of sensitivity and performance-like properties which are helpful in the detection of pests in the crop field, analysis of soil, and stress factors as drought (Fraceto et al., 2016). For analytical purposes, the transduction properties of NPs had been explored. Examples include AuNPs showing transducing properties used in agricultural products improvements (Kandasamy and Prema, 2015). For an enhanced and efficient delivery system of pesticides, fertilizers and plant growth regulators, and much more, nanoscale carriers can be used. Microbial NPs of copper and copper oxide bio-fabricated using *Streptomyces* sp. found to be high antifungal properties for harmful fungi (*Pythium ultimum*, *Fusarium oxysporum*, *Alternaria alternata*, and *Aspergillus niger*) (Hassan et al., 2018, 2019). During an experiment Kaur et al. (2018) found the antifungal property of microbial NPs AgNPs (*Pseudomonas* sp. and *Achromobacter* sp. used in this synthesis) acts on *Fusarium* sp. as an infective agent in chickpea.

It has also enhanced the efficacy of agricultural products and delivered promising results to protect agriculture as well as the environment by not only improving food production but also sustainably providing stability. They act as effective tools for smart agriculture systems due to their nanosized sustained release and site-targeted distribution. Within particular concentration levels, certain metal and metal oxide nanoparticles improved plant growth, seed germination rates, root and shoot elongation, and plant biomass (Chhipa, 2019). Sustainable agricultural growth may use nanotechnology to meet the world's increasing food demand and focus on these options since they are not only eco-friendly but also secure a high crop yield (Kumar et al., 2020).

In addition to enhancing crop yield, a nanotechnological conceptual framework can be used for early detection, tracking, and controlling of insect pests, as well as in targeted delivery systems that aid in slow-release with greater agro-input efficiency. Nanotechnology provides a foundation for agricultural sector transformation at all levels of production, preservation, manufacturing, and storage. The judicious application of nanotechnological breakthroughs can meet post-Green Revolution demands in developing countries such as India to reduce the use of larger concentrations of fertilizers and pesticides to safeguard both agricultural production demands as well as the environment. As a result, by utilizing nanotechnology, the difficulty of balancing agricultural output with environmental conservation can be met (Thangadurai et al., 2020).

13.4.2 Microbial nanoparticles for medicine and drugs

Application of nanotechnology in the medical field (nanoscience) explored in various areas such as nanobiosensors (Mohanpuria et al., 2008), fluid detox processes using biological methods (Singh et al., 2016), gene and drug delivery

ligands (Li et al., 2011), diagnosis and detection of harmful organisms (Nath and Banerjee, 2013), combating human disease through tissue fabrication and manipulation (Gurunathan et al., 2009), DNA analysis (Razavi et al., 2015), destroying tumors through heat or hyperthermia (Shinkai et al., 1999), enhanced magnetic resonance imaging (Weissleder et al., 1990) and phagokinetic examination (Parak et al., 2002).

For the use of a drug delivery system, the basic requirement is accurate targeting of drugs to their accurate sites in the desired dosage at the given time period. Therefore, consumption of the drug and side effects had to be reduced for safer drug delivery with enhanced therapeutic impact (Dhillon et al., 2012). Carrier for drug delivery can be achieved using NPs (Gref et al., 1994). An example includes AgNPs as drug conveyors because of their tiny size it reaches targeted places through the narrow epithelial joints and blood-brain barrier. It has the property to reduce poison through the assembly at the target side and provides a higher surface area vs volume ratio hence, enhancing biodistribution and pharmacokinetics of drugs (Moghaddam et al., 2015). Metals NPs also used in the 3-dimensional analysis of various fatty acids, biomolecules, lipids, metabolites, peptides, nucleic acids, glycosphingolipids, and drug molecules (Li et al., 2011). Damaged tissue repair and reproduction can be also done using Microbial NPs. Examples include Fe₂O₃ (Magnetite) and Fe₂O₃ (Maghemite) are known for target cancer therapy, gene therapy, drug delivery, DNA analysis, stem cell sorting, and magnetic resonance imaging (Xiang et al., 2007); AgNPs used for gloves used in surgery and covers, bed lines, dressing of injury happening in presence of antibacterial and so on.

Sondi and Sondi (2004) explored the possibility of effective application of NPs against bacteria by the formation of pits at cellular surfaces which caused extensive damage to the plasma membrane and various vital cellular components which leads to the bacterial cells death. Microbial NPs used in combination with traditional antibiotics enhance the outcome of that drug examples are studied of Banu et al. (2011) study of AgNPs (using *R. stolonifera*) and antibiotics (ciprofloxacin, carenicillin, and nitrofurantoin) showed enhanced activity against ESBL-strains (Enterobacteriaceae) bacterial species. Sunkar and Nachiyar (2012) found the activity against bacteria using microbial-silver NPs (synthesized from *Bacillus cereus* by agar-well diffusion method) against *Klebsiella pneumonia*, *Staphylococcus aureus*, *Salmonella typhi*, *Escherichia coli*, and *Pseudomonas aeruginosa*.

Similarly, various microbial NPs have been found to show antibacterial activity for example AuNPs synthesized using *Norcardiopsis sp.* MBRC-48 against *S. aureus*. Titanium oxide formed using *Planomicrobium sp.* was used for the study of anti-microbial action against both the gram +ve and gram -ve bacteria. Recently, Jain et al. (2020) synthesized ZnO-NPs using *Serratia nematodiphila* and found their high activity against *Xanthomonas oryzae*, a penicillin-resistant proteobacterial strain. Kundu and colleagues in 2014 found ZnO-NPs efficacy obtained through biofabrication of *Rhodococcus pyridinivorans* as nanocarriers for anthraquinone (Kundu et al., 2014). On HT-29 colon carcinoma cells as a model, when the ZnO-NPs loaded anthraquinone was assayed for its cytotoxicity using concentration as a parameter, it is concluded that these NPs can be harvested for targeted drug delivery system. The hydrophilic property of NPs, if present, will enable the drugs to get enhanced absorption and cytotoxicity due to the efficient diffusion process. Kumar et al. (2020) conducted a bioassay on biofabricated AuNPs bounded *Doxorubicin* on cancer cell line HEK293 observed a higher diffusion rate of the drug into the cell line. AuNPs bounded with *doxorubicin* also showed similar effects on hepatic cancerous cell lines (Syed et al., 2013). When anticancer drug *Taxol* was coupled with biosynthesized Gd₂O₃ NPs (gadolinium oxide NPs), it performed efficiently as treatment of cancerous cell line (Khan et al., 2014). Biofabricated AuNPs obtained using *Candida albicans* were also shown to be effective for working as a probe in hepatic cancer cells. These NPs bind with cancer cells and help in differentiation from non-cancerous normal cells.

Nanomaterials often provide significant prospects to promote public health in a variety of other fields such as healthcare, biosensors, pharmaceuticals, cosmetology, power storage, catalysts, photovoltaic conversion, and environmental cleanup. Their distinct qualities make them particularly valuable as antioxidants, antibacterial, antifungal, and antiviral agents, pharmaceuticals, DNA sequencing, genetic manipulation, penicillin-resistant and anticancer drugs, among several other applications. (Lateef et al., 2019).

13.4.3 Microbial nanoparticles for building construction material

It has been reported that the application of microbial NPs also enhances the binding properties by providing a base of cement materials in constructions. Nanoscale material used as the cementing agent's preparations offers benefits like safer use, quicker binding, and low cost (Sanchez and Sobolev, 2010). When hematite (Fe₂O₃) or nanosilica (SiO₂) NPs were combined with the usual concrete, it showed improved strength, mechanical properties and made them durable (Shah et al., 2009). Using the same technique, steel qualities are also enhanced through nanotechnological applications. Nanosized steel provides more strong steel cables used in bridge construction. Glass is another essential construction material modified using TiO₂ for self-cleaning, antifouling, and sterilizing properties through glass coating glazing

which functions through better blockage of heat and light via windows (Sobolev et al., 2009). Examples include SiO₂ and aluminum oxide, titanium oxide, and quartz used in the modification of materials to increase their functional capacity in the construction industry.

13.4.4 Microbial nanoparticles in research

Usage of microbial NPs has received much attention and their applications are emphasized during recent research especially in the medical field because of the huge abundance of micro-organisms showing resistance to antibiotics. These NPs had utility in various antimicrobial purposes such as antiviral, antifungal, anti-inflammatory factors, and antibacterial (Fayaz et al., 2010). Some NPs (AgNPs) by default had high aspect ratios which make it easier to interact with few particles and hence, enhanced their antimicrobial property (Thakkar et al., 2010). Gold NPs derived using fungal sp. had powerful bactericidal potential due to dephosphorylation of key peptide substrate on tyrosine residues, penetrating and anchoring the cell wall of bacteria in both the strains of gram-negative and gram-positive (Sadhasivam et al., 2010; Singh et al., 2008).

Because of the optical and electronic properties of NPs, they are frequently used under the category of biosensors. Examples include Se (Selenium) NP crystals used in horseradish peroxidase biosensors for the reduction of H₂O₂. Similarly, AuNPs showed various categories as labels for biosensors, glucose injections for glucose determinants, biological tissues stains, and biomolecules estimation (Moghaddam et al., 2015). Therefore, microbial NPs also worked as novel biosensors having very sensitive with unarmful motives to the environment providing a way for sustainable development.

Cancer as a characteristic feature of uncontrolled cell growth has been tried to treat using traditional methods of chemotherapy, surgery, and radiation which shows adverse side effects. A crucial step has to be taken to design certain alternative methods for the diagnosis and treatment of cancer. Through clinical research experiments, it has been found that nanomedicines are helpful for the diagnosis and treatment of tumors with a targeted drug delivery system. studies conducted by Borse et al. (2015), showed in vitro anticancer activity of biosynthesized PtNPs from *Saccharomyces boulardii* against A431 and MCF-7 cell lines. Ortega et al. (2015) also reported the efficacy of silver-NPs synthesized using *Cryptococcus laurentii* against breast cancer for antitumor and anti-cancerous properties. Biosynthesized Se (Selenium) nanorods using *Streptomyces bikiniensis* strains showed a death rate of MCF-7 and Hep-G2 cancer cell lines (Ahmad et al., 2015). Studies also have been done on AuNPs efficacy against human hepatic and mammary carcinoma where it is observed that it caused apoptosis of mitochondria, DNA damage system, cytokinesis detention, and destruction of the cancerous nucleus in cell lines (El-Batal et al., 2015). There are a large number of research reports where NPs studied and reported for their anti-cancerous properties in laboratory conditions but various factors as toxicity, doses response and other vital factors are also still to be explored extensively before their commercialization.

13.4.5 Microbial nanoparticles in industrial use

Microbial NPs show a variety of usage in different industries such as the food industry, cosmetics industry, etc. In the food industry NPs involvement leads to quality enhancement by increasing the shelf-life of food materials as well as reducing food wastage due to microbial infestation during production, processing, protection, and packaging of food materials (Pradhan et al., 2015). The NPs work as nanocarriers for the delivery systems of food enhancers such as nanocomposite coating used in food packages can work as antimicrobial substance and added also a sheath from external environmental mechanical and thermal shocks (Pinto et al., 2013). An example under this category is nanodrops, frequently placed under the canola oil manufacturing industry which helps in the transfer of minerals and vitamins to the food (Sekhon, 2014). A new technique of nanofiltration was introduced in the dairy and food industry to exclude solids including bacteria and other parasites through the principle of membrane filter technique for water purification.

In the cosmetics industry nanocarriers such as nanoemulsions, lipid carriers, nanocapsules, solid lipid NPs are showing self-cleansing, skin compatibility, anti-microbial and dermatological behavior which are used extensively to condition the skin, nail, hair, aging, hyperpigmentation, and lip care (Singh et al., 2016). NPs show a lesser wavelength range than visible light which leads to their transparency and this property makes them suitable for use in the cosmetics industry (Raj et al., 2012). Examples that fall under this category include, zinc and titanium oxide NPs which are frequently used in suntan lotion because of their ability to absorb UV rays and are transparent (Pierfrancesco, 2010).

13.4.6 Microbial nanoparticles in energy sectors

Electronic gadgets are modified to less-power consuming, large-sized, bright display techniques using NPs. Modern computer monitors and television uses a variety of NPs including cadmium sulfide (CdS), lead telluride (PbTe),

titanium dioxide (TiO₂), zinc selenide (ZnSe), and zinc sulfide (ZnS) for LED (light-emitting diodes) based displays. Portable electronic equipment as mobile phones, computers, and laptops needed compact, lightweight and batteries that have the capacity of more power storage. In these batteries, a foam-like (aerogel) structure is found to increase their efficiency. NPs are frequently used for making separator plates such as nickel, palladium, and nanowires with carbon-coating and metal hydrides. NPs can increase the surface area several folds which lead to increased energy density and results in reduced charging duration as well as longer storage capacity (Lu et al., 2010).

13.4.7 Microbial nanoparticles in environmental protection

The micro-organisms having the capacity for converting inorganic metallic ions into other forms such as metal NPs using their environmental conditions are still to be explored extensively. Songara et al. (2018) observed that ZnO NPs obtained from *Pseudomonas putida*, a bacteria has time and dose-dependent photocatalytic activity and a high transformation rate on benzyl butyl phthalate (BBP), photodegraded products, and environmental pollutant. Debnath et al. (2020) successfully synthesized zirconia NPs for bioremediation of tetracycline from wastewater through adsorption process (526.32 mg/g) and they found it's another use in caffeine degradation which is a pharmaceutical active pollutant compound. Recent studies show that intra and extracellular synthesized microbial NPs can detoxify contaminants through redox reactions (Liu 2006; Shah et al., 2015) and this field has been under survey for such micro-organisms populations who can become eco-friendly for a variety of environmental remediations.

13.4.8 Microbial nanoparticles in fuel processing

With the increasing demand of the world population for the utilization of conventional energy sources like coal, petroleum leads to shifting towards a new issue for more need of fuel production which requires skyrocketing. So, the scientists are in the search to devise efficient alternative methods for the production of fuel using micro-biota. Bio methanation is one such way that could be used in a modified way for reliable energy sources including sustainable development. A wider substrate is available for the nutritional basis of microflora used in biogas production. Biogas yield is dependent upon the type of nutrients available in the substrate for microbial growth thereby directly proportional to the yield of the process (Cheng, 2009; Cooke, 2014; De Clercq et al., 2016). The substrate involved is agriculture waste and energy crops, fruits and vegetable waste, municipal waste, sewage sludge, and industrial waste. Different groups of microflorae are involved in an anaerobic manner to degrade and production of biogas (Amani et al., 2010). A lot of anaerobic micro-organisms growing in a syntropic manner make it difficult to identify and isolate the organisms involved in bio methanation. Bio methanation is an advanced process providing an efficient, and cost-effective route but still, to increase its applications more focus is required in this field.

Moreover, nanostructured materials, particularly 1D, 2D, and 3D nanostructures, and their tailored designs are constantly being exploited. They hold great promise to accomplish sustainability in the energy and environmental domains, thereby addressing a variety of global concerns. A significant amount of recent research has been devoted to the fine-tuning of nanoarchitectures to achieve innovative technologies in energy storage and modifications such as batteries, superconductors, fuel cells, solar cells, and electroactive devices, functionalized catalysts for ORR and OER, gas to fuel sources, liquid to fuel sources, and photoelectrochemical, abrasion, electrochemical devices along with environmental damage and toxins removal (Naushad et al., 2020).

13.5 Environmental issues associated with microbial nanoparticles

Nanopollution is the pollution caused by nanostructures as their usage increases worldwide and their subsequent emergence as environmental contaminants. Due to the extremely small size of nanostructures, nanopollution is often referred to as “invisible” pollution which is extremely difficult to manage and control (Biswas and Sarkar, 2019). Nanoparticles have a wide range of applications and are touted to be the solution to a large number of concerns including environmental remediation. Synthetically produced nanostructures utilize chemicals and therefore are a source of pollution from the time of their production till their disposal. Even though nanoparticles produced by green methods such as micro-organisms, plant parts, and extracts do not utilize polluting chemicals for synthesis, there are indications that their use and disposal may also be polluting and eco-toxic. The reactions undergone by the biogenic NPs upon their release into the environment involve oxidation, adsorption, and aggregation among others, and lead to an altering of their properties. Transformable green nanoparticles get converted into other forms by oxidation or reduction reactions and release their more toxic metal ions or exhibit greater solubility and bioaccessibility leading to invasion of biological cells.

Non-biodegradable green NPs on the other hand show aggregation and accumulation in the environment. Catalytic NPs interact with other components of the environment such as pollutants and macromolecules and disturb the ecological balance of the affected area by modifying various parameters such as pH, salinity, etc. (Turan et al., 2019).

To sustainably utilize the benefits of nanostructures it's important to be aware of all implications associated with their use including any drawbacks. Studies point towards their genotoxic and cytotoxic behaviors due to their ability to enter living cells as they show properties similar to biomolecules like DNA, enzymes, and proteins. They can cause extensive damage to cells and organs by destroying cell membranes, affecting the electron transport chain, ROS production, and causing oxidative damage to DNA (Biswas and Sarkar, 2019; Turan et al., 2019). In contrast to physicochemically synthesized NPs very little detail is available on the impact of green nanostructures on the environment and biota (Rana et al., 2020).

It is postulated that the shape, size, crystal structure, surface energy, smoothness, solubility, and chemical makeup along with the presence or absence of surface coatings on the nanoparticles affect their toxicity. Spherical NPs exhibit greater endocytosis ability over star, triangular or tubular shapes, and coating of NPs with organic compounds alters their charge, reactivity, and pore structure while their aggregation ability was exhibited to be under the control of surface charge. The size was an important determining character of toxicity and NPs of size <50 nm were found to be toxic to most cells (Gatoo et al., 2014; Turan et al., 2019). Toxic manifestations of nanoparticles include hematological, neurological, splenic, hepatological and pulmonary toxicities, allergic reactions, organ failure, fibrosis, and genotoxicity in the form of DNA damage due to strand breakage, point mutations, changes in gene expression among other effects (Gatoo et al., 2014; Singh et al., 2009).

13.6 Toxicity of biogenic nanoparticles in the environment

The Royal Society and Royal Academy of Engineering (2004) first put forth the idea that there is a scarcity of information on the impact of nanostructures on human health and environment ("Nanoscience and nanotechnologies: opportunities and uncertainties." Available from <http://www.nanotec.org.uk/finalReport.htm>; 2004), and since then a significant amount of interest has been generated about their impact.

One of the most commonly used NPs is ZnO whose applicability widens if it is biogenically synthesized. It can be efficiently produced from several plant extracts and microorganisms such as bacteria, fungi, yeast, and algae (Saravanan et al., 2021; Sharma et al., 2020). ZnO NPs are utilized in soil and water remediation, as antimicrobial agents, in photocatalysis, and in various pharmacological and cosmetic preparations and biomedicine due to their non-toxic and biocompatible properties (Kalpana and Rajeswari, 2018; Sharma et al., 2020). Exposure to ZnO NPs causes dose-dependent toxicity and inflammatory response (Alghsham et al., 2019; Kalpana and Rajeswari, 2018) while inhalation of uncoated and triethoxycaprylsilane-coated ZnO nanoparticles could have acute toxic effects on the lungs of humans (Hadrup et al., 2019). ZnO NPs induced several hematological changes in rats in the form of decreased RBC and platelet counts but significantly higher WBC counts exhibiting signs of hematotoxicity (Yahya et al., 2019). In aquatic ecosystems, at higher CO₂ concentrations they are toxic to aquatic life forms such as goldfish (Yin et al., 2017). They can create a growth inhibition zone on a variety of microbes studied as clinical isolates showing that they may be destructive towards useful bacteria in natural environments (Rajput et al., 2018).

Another widely used NP is the AgNPs that exhibit unique biocidal properties and are easily produced by biogenic means (Saravanan et al., 2021). When released into the environment these particles undergo several modifications such as oxidation to form Ag₂S, Ag₂O, and AgCl that can release Ag⁺, aggregate under the influence of environmental pH or in the presence of divalent cations like Ca²⁺ and Mg²⁺ and exhibit adsorption of different substances on their surface (Jorge de Souza et al., 2019). These modifications alter the properties of the NPs and influence their toxicity. Cells can internalize AgNPs by pinocytosis and phagocytosis which releases toxic ions in the cytoplasm leading to increased reactive oxygen species (ROS) production, cell lesions, inactivation of various enzymes, lysosomal destruction, and DNA damage ultimately causing cell death (Jorge de Souza et al., 2019). On the other hand, the cytotoxic effects of AgNPs can be beneficially adopted for anticancer, antimicrobial, antiviral, and anti-inflammatory, and wound healing applications in biomedicine (Jorge de Souza et al., 2019; Patil and Chandrasekaran, 2020).

CuONPs are also widely adopted for various industrial, domestic, medical, or environmental applications due to their antimicrobial and catalytic properties. Their environmental use ultimately causes their release into aquatic systems where they exhibit considerable toxicity to aquatic organisms (Miao et al., 2019). Cu ions released from the NPs are particularly toxic to native soil bacterial populations (Rajput et al., 2018).

nZVI is another abundantly used nanostructure particularly for soil and water remediation and displays exceptional ability to degrade heavy metals and polychlorinated organic compounds. Despite its benefits, bare nZVI cannot be used

to its full potential due to its toxic influences and needs to be stabilized by coatings and surfactants. In its pure form, it can destabilize membranes, create oxidative stress, damage macromolecules and produce Fe^{2+} toxicity in cells (Xue et al., 2018).

Most biogenically synthesized Nanostructures when applied for environmental remediation purposes ultimately find their way into either soil or water bodies or both where they transform exposure to different environmental conditions that greatly alter their properties. These alterations can lead to increased toxicity, aggregation, and greater nanoparticle accumulation at the site of injection. A large body of evidence exists for toxicity of physicochemically engineered nanomaterial (ENM) but similar studies are lacking for biologically synthesized nanoparticles. Moreover, the ecotoxic effects of ENMs cannot be correlated to the biogenic ones without experimental data to back the similarities. It is therefore imperative that in-depth studies are taken up to study the effect of biogenic nanomaterials independent of the data available for the physicochemical ENMs.

Another area that deserves attention is the interaction of nanostructures with soil microorganisms. Studies carried out on several ENMs show that NPs viz CuO-NPs, ZnO-NPs, Ag-NPs, TiO₂-NPs, and FeO-NPs exhibit a range of toxic effects on non-target soil microbes when used for remediation purposes (Ameen et al., 2021). It would be beneficial if similar studies focusing on the interactions between biogenic nanostructures and soil microbes are carried out to understand the differences if any in their interactions and influence on soil microbial populations. The focus should also be on the effect of various transformations shown by the biogenic nanostructures and their corresponding change in properties that in turn determine their toxicity. Therefore, to guarantee their safe usage it is imperative to be fully cognizant of their toxic side effects and long-term cytotoxic and genotoxic studies via different in vitro and in vivo routes should be carried out.

13.7 Future prospects towards sustainable environment and impact of Government's and NGOs initiatives towards sustainable development with green nanotechnology

Nanotechnology is identified as one of the key technologies of the future that can address the world's paramount issues like water depletion and contamination, energy deficit and requirement, wellness & health sustenance, agriculture yield and biodiversity management also covered under the title "WEHAB" i.e., water, energy, health, agriculture, and biodiversity. These are the main areas focused on by the United Nations Johannesburg Summit on "Sustainable Development" in 2002 and are critical for nations to achieve millennium development goals (Dasgupta et al., 2020).

Nanomaterials have far-reaching applications in fields ranging from materials science to biotechnology and biomedicine. It is a pioneering technology and with its ever-increasing usage, it is here to stay. The use of nanomaterials produced via physicochemical means is a major deterrent to their large-scale utilization. Green nanotechnology on the other hand aids in the sustainable application of nanomaterials in a variety of fields since the manufacture of nanostructures using plants and microorganisms does not utilize toxic chemicals, unlike chemical-based synthesis methods. Green nanotechnology follows the principles of green chemistry which despite limitations and challenges offers various advantages towards sustainable development (Khan, 2020).

It helps to alleviate environmental issues such as excessive use of non-renewable resources, production of greenhouse gases, exorbitant use of fossil fuels, and the resulting pollution. Due to their greater surface area and specificity along with their higher reactivity as nanocatalysts, nanomaterials help in reducing the above-mentioned environmental problems by improving process efficiency (Nasrollahzadeh et al., 2019). Biogenic nanostructures are therefore being extensively used in drug and gene delivery systems, biomedical fields, pharmacology and cosmetics, biosensing, bioimaging, environmental remediation, and photocatalysis among other functions.

As with all new technologies, nanotechnology is also regulated by various governmental and scientific agencies to maximize benefits and minimize potential risks. With the emergence of new technology with unknown risks, most governments issue voluntary regulations, directives, or guidelines but once extensive studies are complete governmental regulations become mandatory to mitigate the potential hazards associated with the technology. Similar steps were taken concerning nanotechnology before and after 2010 which mark the emergence and wide-scale application of nanotechnology respectively (Soltani and Pouypouy, 2019). There are several organizations such as ISO, IEC, and CEN that have formulated technical committees for nanotechnology. International NGOs like ISO acts as an independent agency to devise voluntary, consensus-based, market-oriented International Standards while IEC is working towards standard setting in the field of electrical and electronic related technologies. Whereas, CEN a European Committee for Standardization, made up of 34 European countries to look for technical aspects of nanotechnology since 2005

(Soltani and Pouypouy, 2019). Other organizations include the Standardization Administration of China (CAS) that drafted a technical commission for nanotechnology (SAC/TC279) in 2005, BSI devised committee NTI/1 in 2004, several committees in the USA such as ANSI-NSP (American National Standards Institute constituted the Nanotechnology Standards Panel), ASTM (American Society for Testing and Materials), NIST, NIOSH and NCL, Iran Nanotechnology Innovation Council (INIC) formed in 2006 and committees in other countries such as Malaysia, Thailand, Russia, Taiwan, etc. (Soltani and Pouypouy, 2019). United Nations International Training and Research (UNITAR) was formulated in 2009 to lend a helping hand to developing nations on both nanosafety and trade of nanomaterials across borders while World Health Organization (WHO) also has prepared the “WHO guidelines on protecting workers from potential risks of manufactured nanomaterials (MNMs)” to ensure the safety of workers from the risks associated with nanostructures (Soltani and Pouypouy, 2019).

These committees and organizations established at various international, national, and regional levels carry out some tasks associated with the standardization and regularization of nanomaterials.

The shift towards green production of nanomaterials was the focus of the Gordon Research Conference on Environmental Nanotechnology in 2011 and led to the formation of the Sustainable Nanotechnology Organization (SNO) (Falsini et al., 2018). SNO is concerned with the employment of sustainable nanotechnology taking into account its impact on health, safety, and the environment. European Chemicals Agency (ECHA) presented directions on the safe use of nanomaterials under European Community Regulation on chemicals and their safe use (REACH) in April 2012, Likewise, the European NanoSafety Cluster emphasizes nanosafety as issued by their report on “*Nanosafety in Europe 2015–2025: Towards Safe and Sustainable Nanomaterials and Nanotechnology Innovation.*” The effect of the SNO treaty has led to the National Nanotechnology Initiative (NNI) in the United States and the Asia Nano Forum (ANF) to focus on shaping the future of nanotechnology (Falsini et al., 2018).

Before green nanotechnology can be fully adopted and utilized to its full potential various regulations and mandates need to be fulfilled as laid down by the various regulatory bodies. These include studying like Life Cycle Assessment (LCA) and Risk Assessment (RA) to assess the environmental impact of nanomaterials. These tools are crucial as they provide critical information not only on the fate of the nanostructures in the environment but also on their effect on soil, water, air, and organisms. LCA (described by ISO standards 14040 and 14044) emphasizes the energy and resources utilized by a particular function, emissions produced as a result of the process, and their effect on various parameters. Since LCA focuses on both resources and emissions it is a tool that can help in designing early-stage processes for the function with maximal eco-efficiency and provide guidance for sustainable usage (Tsang et al., 2017). Risk Assessment or RA is another important tool that studies the risks associated with a product in terms of the pollutants/chemicals released by the product into the environment and its potential effect on human health. Currently, there is a paucity of both data and methodologies for studying the impact of sustainable nanotechnological processes that limits the application of these structures. Both LCA and RA must be integrated to study the fate and impact of nanostructures on both the environment and human health so that regulations can be drafted accordingly by various international and national agencies for safe and sustainable adoption of nanomaterials and their maximal utilization (Tsang et al., 2017).

13.8 Conclusions

In the present chapter, we have discussed the importance of the microbial synthesis of nanoparticles which are required to avoid the production of toxic by-products and environmental sustainability. This technology offers the opportunity to find a solution to global problems which are being faced due to increasing population, pollution, and demand for necessities. The major advantage of these microbial methods of synthesis of nanoparticles is the availability of secondary metabolites in the synthesis process, the wide range of available resources in the form of microbes, stability, and density of the nanoparticles (69 silver). Microbial nanotechnology is an emerging area but has some limitations like toxicity-related issues of nanoparticles, life cycle analysis, and economic barriers. These limitations should be considered for sustainable development although this technology develops products that reduce pollutants and are eco-friendly. Also, the life cycle assessment of the nanoproducts through microbial nanomanufacturing must be taken into account before making their use commercial and widely available.

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Environmental applications of microbial nanotechnology based sustainable wet waste management techniques adopted by Bruhat Bengaluru Mahanagarapalike, Bangalore—a case study

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The objective of the case study:

1. Effective implementation of biomethanation and biocomposting, technologies in Bengaluru.
2. Microbial nanotechnology based sustainable wet waste management techniques.
3. Social and environmental impact, Employment generation.

14.1 Introduction

Solid waste is a chunk, due to scarcity of resourceful waste management systems MSW produced in Indian cities remains untreated. As per the publication by the MoHUA “Swachhata Sandesh Newsletter” as of January 2020, 147,613 metric tons of solid waste is generated per day in India (Singh, 2020). At present, the current global volume of waste is 67–70 billion tons (Song and Wu, 2021). Bengaluru is a third most densely inhabited city in India along with 12 million populations; the city has situated in the south-eastern of India. It is located at 12.97 N 77.56E and occupies a district of 2,190 sq. kilometers at a standard advancement of 920 meters with 198 wards. The location map of Bengaluru has as shown in Fig. 14.1. Population of Bengaluru according to the Census of India, 2019 is 12.9512 million. The city generates 5757 TPD of MSW. Municipal solid waste management is the challenging issue for the town. The stated, Solid waste resources in addition to organic/inorganic domestic waste contains with medical waste, hotel/restaurant, institutional, and vegetable/fruit market waste and other wastes like street sweepings (dry leaves, paper, plastics), accumulated sediment from the drainage, crop (agriculture) waste, building construction and demolition waste and treated biomedical waste, not including harmful wastes from industrial, biomedical waste and e-waste form MNCs, govt offices generated in an metropolitan. Parallel the energy demand is also increasing in the city to fulfill the energy requirement the technical communities adapted renewable methods by reduce, reuse & recycling of waste. MSW consist mainly biodegradable material >70%, as per the survey reported that 91.01 ± 45.5 g/day per capita waste generated is about with the 74 ± 35 g/person/day per capita organic waste generated (Ramachandra et al., 2018). Untreated Municipal solid waste becomes an issue for promotion for immeasurable illness causes public health hazards like malaria, dengue, chikungunya, etc. MSW, a crucial segment in the direction of development of urban city, as waste

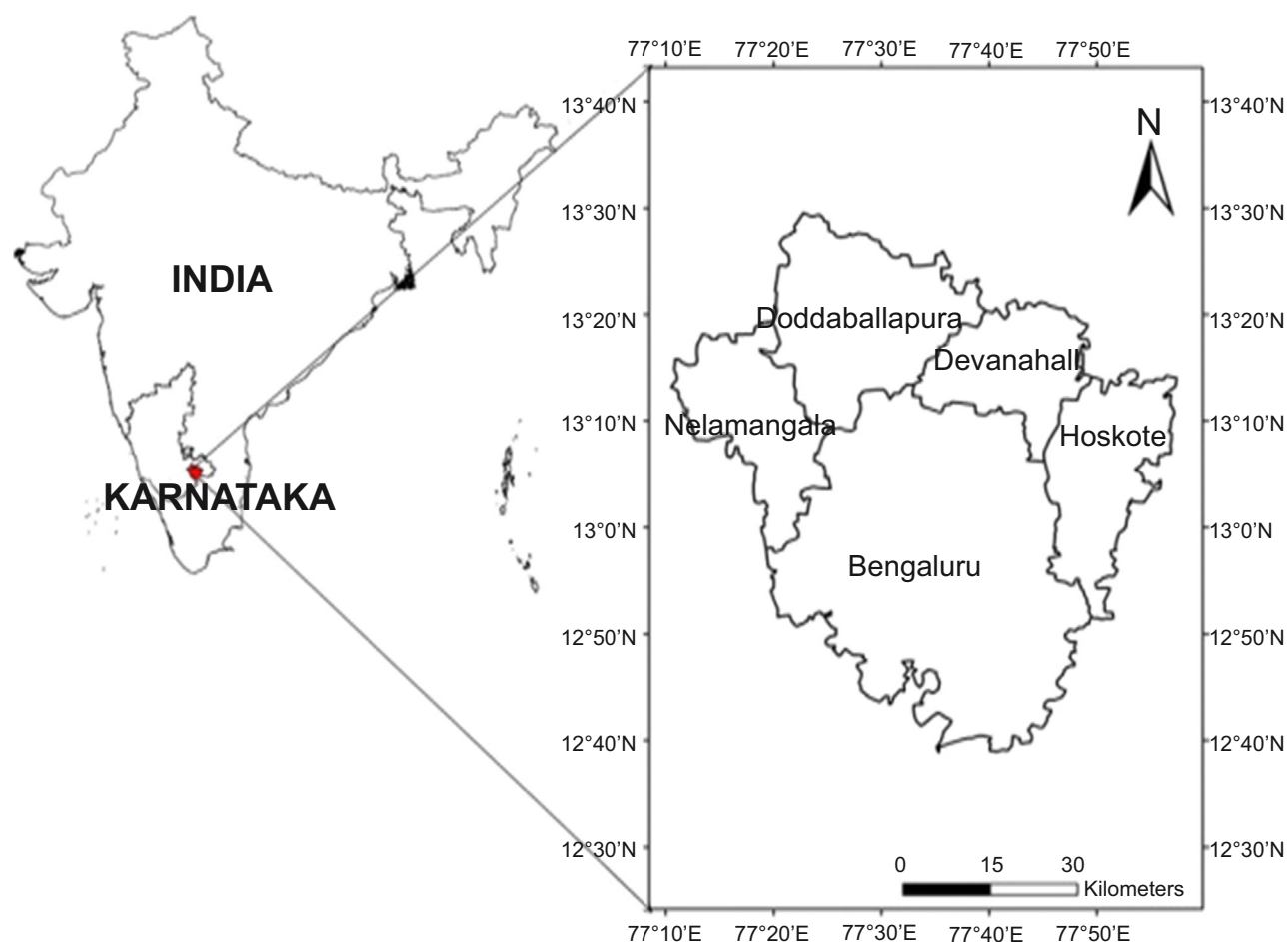


FIGURE 14.1 Location map of Bengaluru city, India.

TABLE 14.1 Generation of waste gram per capita per day (Kumar et al., 2009).

	Mean	Skewness	Std errors
Organic	74.09 ± 34.94	0.72	0.81
Paper	19.18 ± 22.22	2.88	0.65
Metal	10.66 ± 11.87	1.94	0.71
Glass	6.8 ± 5.01	0.67	0.39
Others	4.53 ± 1.74	5.11	0.04

need to be segregated at the source and stored, collected by the waste pickers from door to door, relocated to the processing centers; here it reduces the volume of the waste to keep down the side effects on the environment (SWM manual).

In many Industries, biogas plants have been installed to treat kitchen waste/water waste and also reclaim biogas. Energy demands are growing and the declining of the fossil fuels, additional consciousness need to be given to the available various inexhaustible energy sources. From among these, biogas is the promising and more flexible technology; here the most important stage is anaerobic digestion of organic waste which is efficient to produce multiple sources of energy like electricity, vehicle fuel, steam and heat (Table 14.1).

Using of biomass to generate energy is one correspondent substitute that has turned out to be attractive globally as a sustainable source of energy. There is a trouble of efficient disposal of Municipal Solid Waste (MSW), as Indian MSW

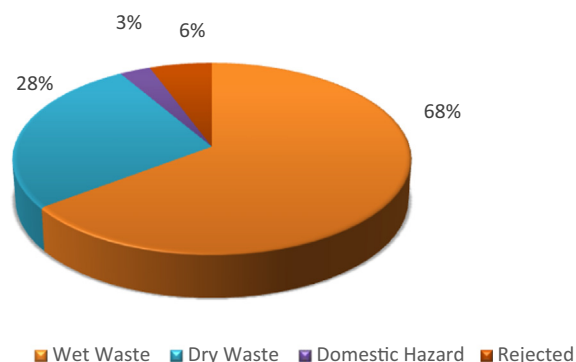


FIGURE 14.2 Waste management in Indian cities.

contains higher moisture as compared to other western countries it also emits unpleasant odor and methane gas which also contribute for global warming when dumped untreated in open land fill. Due to rapid urbanization, the Solid Waste Management policy has been adopted by the Bengaluru Mahanagara Palike (BBMP). As instructed in Bangalore's SWM Manual Part I, in integrated solid waste management (ISWM) hierarchy methodize, generation of waste can be diminishing by separation and reusing of dissipate at the source because this is the greatest function to minimize the amount of waste. It also cost huge budget for the transporting of waste to recycling center even it impacts on environment. Bulk Generators (who generates more than 100 kg in a day) contribute to 25% of the city's waste as per BBMP notification of 25/07/2013, BBMP mandates Bulk Generators to keep apart waste into three categories as wet waste, dry waste & medical waste and manage their waste either at the source by adopting biomethanation or composting even they can take help of Service Providers from BBMP to dispose the waste in more technical way without harming the environment.

As shown in Fig. 14.2, a pie chart shows the wt.% of waste generation of MSW in Bengaluru. Public awareness about the waste segregation is the major challenging issue for the Urban local bodies (ULBs) (Swachh Bharat Mission, 2016). Organic waste is the most extensive material in the generation of biogas, and Bengaluru, annually, produces estimated 5757 TPD of MSW out of this 68% is an organic fraction. Treatment of organic fraction waste at anaerobic condition changes its chemical composition mainly CH₄ (65%–75%), CO₂ small percentage of N₂, H₂, NH₃, and H₂S. Methane % is high in biogas which is also flammable can be used as renewable fuel (Lee et al., 2013; Chen et al., 2014). The vital processing techniques for organic waste include composting aerobic composting or vermi composting; treatment produces dark brown colored earthy odor manure which is used as soil nutrient. In urban cities like Bengaluru, compost plants are underused due to unsegregated waste is poor quality of input to produces manure doesn't acquire required nutrients that results in reduced demand from end users (Ramachandra, 2011). The study has reported groundwater contamination due to leachate produced from land filling of organic waste as it has high moisture content in it. To prevent the issue, it requires site redress and anticipatory actions need to take to avoid contamination of the leachate. In India only 3.59 km² (0.08%) of the total land is well appropriate to dump waste (Santhosh and Sivakumar Babu, 2018). WHO has stated that 22 types of health issues can be stopped in India by getting better MSWM system? Methodical direction of MSWM will guard, massive fund at present used up on medical aid and the health treatments of our county inhabitants (Kasturirangan, 2014).

Feasting without esteem for source preservation generates additional requirement for withdrawal and production of materials begins untreated resources, all of those sponsors to greenhouse emission in fluctuating volumes on various phases of manufacture also utilization. Combining of wet/organic waste included in dry waste/E waste at the origin of production outcomes with numerous adverse downstream effects. The amplified capacity of untreated assorted waste increases to conveyance requirement which in turn rises consumption of fossil fuel for gathering and carriage of waste from the supply of generation to the dumping yards (Ahluwalia and Patel, 2018).

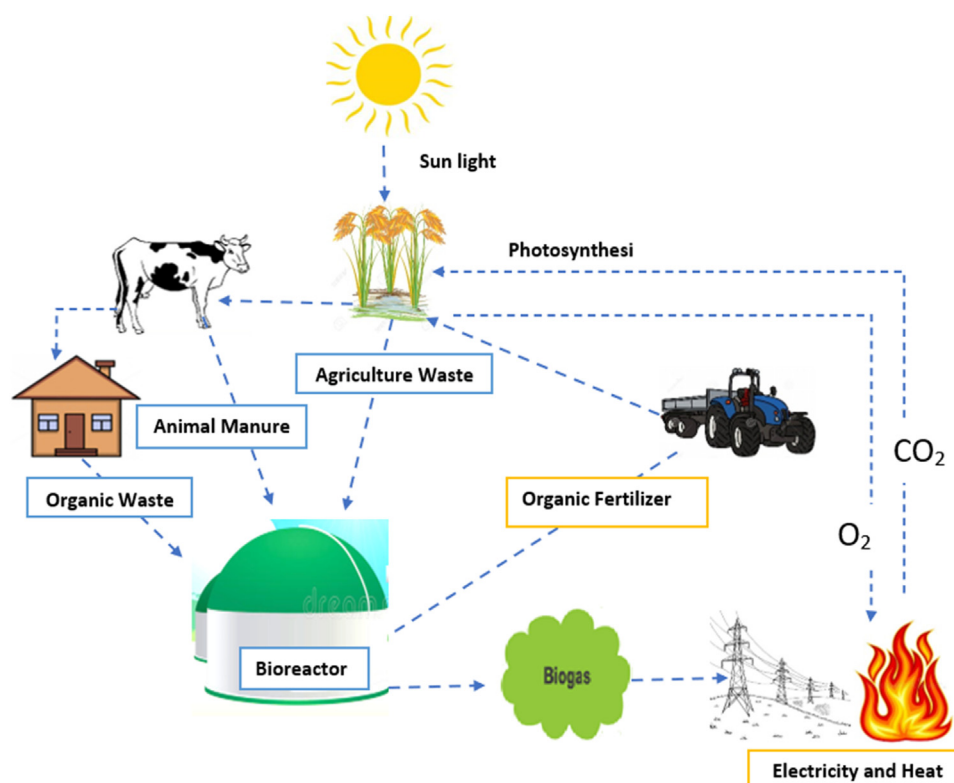
14.1.1 Biomethanation

Biogas is a renewable supply of strength produced with the aid of using the AD of animal manures, cultivated residues, and natural waste from food, one-of-a-kind strength crops, sewage sludge and Table 14.2 represents for one-of-a-kind styles of feedstock and their biogas yield. The content of proteins, fats, and carbohydrates substrates depends on production of methane yield of the AD. Even though AD, the primary process for biogas production, resulted withinside the

TABLE 14.2 In AD, diverse substrates produce methane (Teodorita Al Seadi et al., 2008).

Substrate	Methane yield (%)	Biogas yield (m ³ /t FF)
Organic waste	61	100
Liquid cattle manure	60	25
Liquid pig manure	65	28
Cattle manure	60	45
Poultry manure	60	80
Grass silage	54	172
Corn silage	52	202
Pig manure	60	60
Distiller grains with soluble	61	40
Beet	53	88
Forge beet	51	111

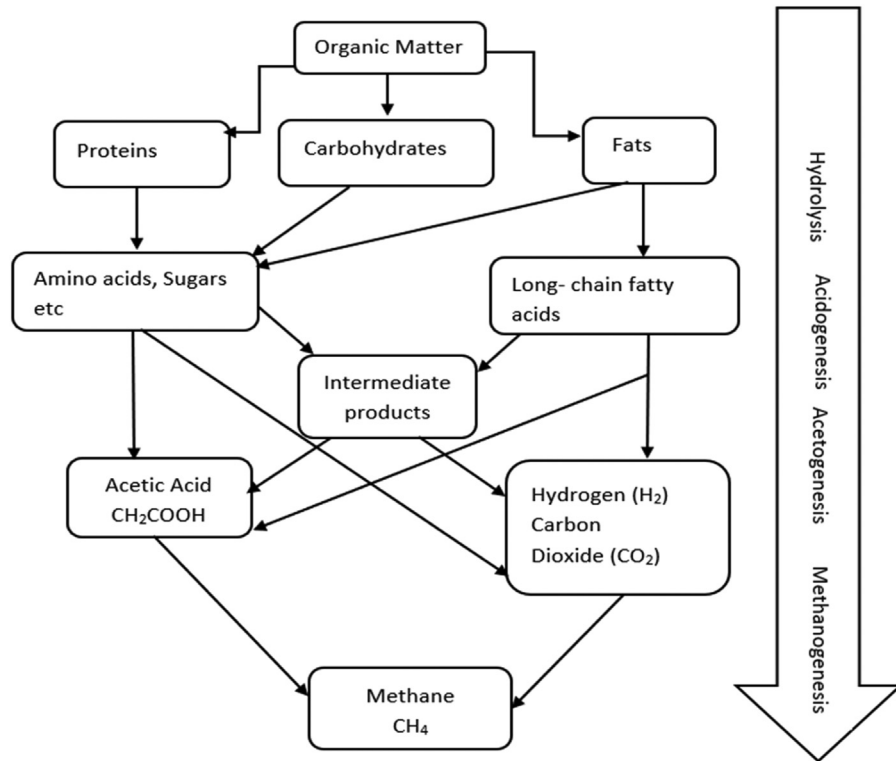
FF, fresh feedstock.

**FIGURE 14.3** The closed cycle of biogas production from anaerobic digestion process.

formation of CO₂ as certainly considered one among its by-products, this CO₂ is eating up in a closed cycle through photosynthesis and brought once more to AD as agricultural wastes and animal manures (Teodorita Al Seadi et al., 2008), as proven in Fig. 14.3. biochemical procedure of AD is a complex that changes complicated organics into biogas, a collective of methane, carbon dioxide gas along with different residuary, as shown in Table 14.3 within the non-availability of oxygen beneath the impact of anaerobic microorganisms. The procedure of anaerobic digestion comes about over four succeeding ranges as (1) hydrolysis, (2) acidogenesis, (3) acetogenesis, and (4) methanogenesis.

TABLE 14.3 The biogas configurations (Teodorita Al Seadi et al., 2008).

Compounds	Chemical symbol	Yield (%)
Methane	CH ₄	50–75
Carbon dioxide	CO ₂	25–45
Water vapor	H ₂ O	2(200 C)–7(400 C)
Oxygen	O ₂	<2
Nitrogen	N ₂	<2
Ammonia	NH ₃	<1
Hydrogen	H ₂	<1
Hydrogen sulfide	H ₂ S	<1

**FIGURE 14.4** Decomposition of organic matter in the absence of oxygen.

The anaerobic digestion procedure is reliant at the interactions among the various microorganisms which can be capable of perform the four aforesaid ranges. In single-degree batch reactors. All wastes are loaded immediately, and all four techniques are permissible to arise withinside the equal reactor serially, the compost is released after certain period of storage or the cessation of biogas production (Verma, 2002). Fig. 14.4 portrays as easy guide of the four digestion ranges defined below. The central constituent of a biogas plant is the digester/AD reactor, that is followed through some of different constituents as mentioned. The procedure of anaerobic digestion comes about over four succeeding ranges as (1) hydrolysis, (2) acidogenesis, (3) acetogenesis, and (4) methanogenesis. The anaerobic digestion procedure is reliant at the interactions among the various microorganisms which can be capable of perform the four aforesaid ranges. In single-degree batch reactors. All wastes are loaded immediately, and all four techniques are permissible to arise withinside the equal reactor serially, the compost is released after certain period of storage or the cessation of

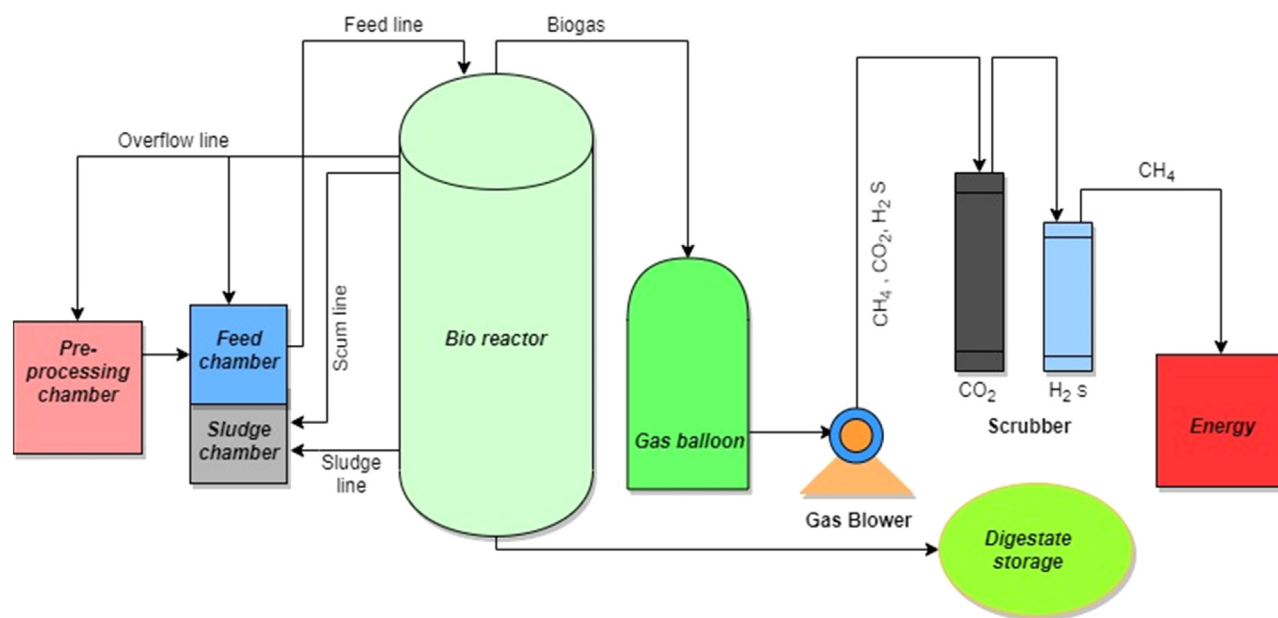
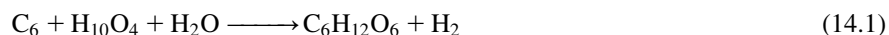


FIGURE 14.5 Biogas production process flowchart (AD-UASB).

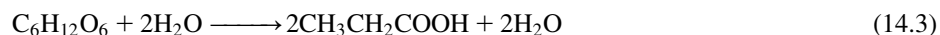
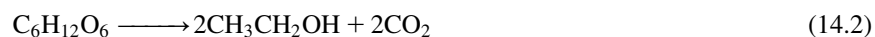
biogas production (Verma, 2002). The central constituent of a biogas plant is the digester/AD reactor, that is followed through some of different constituents as mentioned in Fig. 14.5.

AD biogas plants function with four diverse process steps: (1) Collection of organic waste, transportation, delivery to the site, storage, (2) pre-treatment of feedstock at pre-processing chamber and biogas production in the anaerobic biogas reactor. (3) Storage of biogas in gas balloon, post processing of biogas by removing CO_2 and H_2S using scrubber, conditioning and consumption. (4) In final stage digestate collection, eventual processing and consumption on the fields as fertilizer.

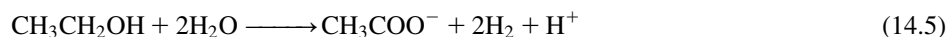
Hydrolysis is the initial step withinside the AD method, wherein big polymers react with water to shape smaller natural compounds with the resource of hydrolytic bacteria. In this hydrolysis process, hydrolytic bacteria are up to excrete exoenzyme that can “undergo metamorphosis lipids, carbohydrates and proteins into sugars, long chain fatty acids (LCFAs), and amino acids, respectively. Subsequently enzymatic breakdown, the outcomes of hydrolysis are up to scatter along the cell membranes of acidogenic microbes”.



The second step in AD is acidogenesis, the quickest step of the process wherein the hydrolysis step products are transformed under the action of acidogenic bacteria into organic acids.



In the third step called acetogenesis in AD, higher VFA so far need to be complete accessible to methanogenic bacteria. Acetogenesis is that the phase by acetogenic microbes that converts these higher VFAs, alcohol and different intervenes within acetate, H_2 and CO_2 additionally produced. Products from acidogenesis, which can't be immediately transformed to methane via way of means of methanogenic bacteria, are transformed into methanogenic substrates for the duration of acetogenesis.



The last stage of anaerobic digestion is methanogenesis, where methane is produced available intervened are consumed by methanogenic bacteria. Methanogens takes an essential part as the terminator of acetogenesis products such as acetic acid, hydrogen and carbon dioxide produced can dismiss action of acetate-forming microorganisms it transforms into CH_4 and CO_2 . In this methanogenesis phase there are two important alleyways to produce CH_4 , processes called hydrogenotrophic methanogenesis and acetoclastic methanogenesis. The way in hydrogenotrophic methanogenesis can function from format or H_2/CO_2 , procurement of dropping probable from format or H_2 , correspondingly as shown in the following reaction.



In the acetoclastic alleyway gains electrons from the oxidation of carbon monoxide released by the breakdown of acetate.



These four phases are conducted in a single reactor, anaerobic digester is often named as Single-Phase Anaerobic Digester (SPAD).

14.1.2 Biocompost

Biocomposting is a productive and quite inexpensive process of organic waste use (Thi et al., 2015). Organic waste is transformed both in aerobic or anaerobic process. When transformation takes place under aerobic environments, compost is developed (Lasaridi et al., 2018). Biocomposting is a habitual progression of decaying of organic product by microbes under precise conditions. Composting permits reducing the amount of waste that is being handled towards dumping sites. This way a reduction of concentrated, toxic leachates and methane fuel line that is being launched into the atmosphere, which equates to a lower in regular pollutants. Composting additionally cuts down on using chemical fertilizers, which damages water supply. Biocompost is the harmless technique of organic waste management. The temperature, aeration, C:N ratio, pH and moisture content, are physical—chemical parameters affect in this process. Organic wastes are classified into three types, which are municipal solid waste, kitchen waste and agriculture waste (Dhokhikah and Trihadiningrum, 2012).

14.1.2.1 Municipal solid waste

The study conducted by the Manohara and Belagali on quality of composting through three downpours which are premonsoon, monsoon and post-monsoon. In the course of composting, water and bioinoculum have been delivered to preserve the dampness in the pile. The highest temperature in the compost process was increased in pre-monsoon was up to 68°C , high temperature is dominant in removal of the weed seeds and eradicating the occurrence of pathogens in the solid wastes. The pH of the compost was acidic in monsoon, the moisture content 44%–64%, pH values were 6.88–7.67, organic carbon content 10%–21% during monsoon and nutrients were N-1.75%, P-3.4%, and K-0.57% (Manohara and Belagali, 2014). The metal ions, copper was 700 ppm, zinc was 510 ppm, lead was 9.8 ppm, chromium was 29 ppm and nickel were 11 ppm. Another study done by Syamala and Belagali in Mysore Karnataka, India, suggested compost as the indication for waste control. In the course of compost process, the pH values logged had been among 7.13–8.76. The pH values which had been better than ordinary variety because of creation of carbon dioxide (CO_2) from natural acids and the dropping of N. The total organic carbon (TOC) reduced at some point of the composting system and this changed into associated with the decomposition of waste via way of means of microbial populations. And the moisture content reduced because of high evaporation rates from 85.19% to 29.87%. The heavy metal ions were analogized to Ohai EPA standards. There is an occurrence metals ions in the range of copper-146.8–325 mg/kg, cadmium-0.1–1.4 mg/kg, lead-1–8 mg/kg and mercury-0–3 mg/kg.

Alternatively, Rawat et al. (2013) composted municipal solid wastes from selected cities of India are Delhi, Bangalore and Ahmadabad. The compost values have been in comparison to the Standards of Indian. Throughout the composting process, the dampness was between 23.83% to 31.61%. The dampness for the compost became inadequate, however revolving the compost at regular intervals had fasten the procedure of composting. The values of pH remained 7.82–8.19 and the C:N ratios were 18.99–25.36. The N and P contents were 0.85%–1.13% and 2.52%–2.9% respectively. Whereas the heavy metal ions concentration was logged to be less than the standard provided those were, copper-300 mg/kg, chromium-50 mg/kg, nickel-50 mg/kg, lead-100 mg/kg and cadmium-5 mg/kg. This study has recommended that to improve the compost quality, by addition of cow dung, bagasse and garden wastes was suggested.

14.1.2.2 Agricultural waste

Agricultural waste is described as the leftovers after the developing and handling of organic cultivated and related merchandise together with yields, vegetables, fruits, dairy, meat, poultry and fishery. They are the unproductive productions and proceeding of farming merchandise which can advantage to a man however whose monetary values are much less as compare to the value of collection, transportation, and processing for beneficial/secure use. These wastes are frequently controlled imperfectly due to the restricted get entry to the discarding provision. For this reason, maximum of agriculture wastes is gone up in smoke. Agricultural waste can be classified as (1) farm yield leftovers (2) agro-waste from industries. Farm yield leftovers similar twig, shoot, straw, stalks, weeds, seeds, etc. and waste after disposing like hull, roots, bagasse, molasses, seeds, etc. it also takes in fruit peels, pulp, cakes (oil), coir, nuts, etc. The agriculture leftovers as it may be cultivated straight into the field as well transformed into manure. Adequate control of agro waste may grow the productivity of hydration of the field, control of soil erosion, recover soil aerify, and soil fertility. Agro yield waste holds compositions are carbon, oxygen, hydrogen, potassium, nitrogen, phosphorus and sulfur. Yield residue contains carbonate in high quantity, such residues are utilized as resource for biofuel production. The study conducted by [Asadi and Zilouei \(2017\)](#) “used ethanol organosolv by pre-processing rice straw to generate biohydrogen by making use of *Enterobacter aerogenes*.” Crops like cereal straw releases nutrients 10%–15% and pea plant remains releases 35% of their nutrients over a period of one year. The factors effect on speed of neutralization are nitrogen value, moisture of the soil, temperature and ratio of combining with the soil.

The discharged substance from living animals is normally taken into consideration as animal waste. The residue additionally consists of natural debris, straw, hay and wooden flakes. Using of excreta to earth carries aids for instance of enhanced soil health, expanded hydrating capacity, adds plants mineralization. Poultry farm and livestock generates products are milk, egg and meat, and also produce huge amount of animal excrete and water which are useful as well as injurious to the atmosphere. Animal waste is useful in case reprocessed efficiently. As animal compost abundant in N, P and K can be used as manure for crops also for energy generation ([Sutanu et al., 2018](#)).

14.1.2.3 Kitchen waste

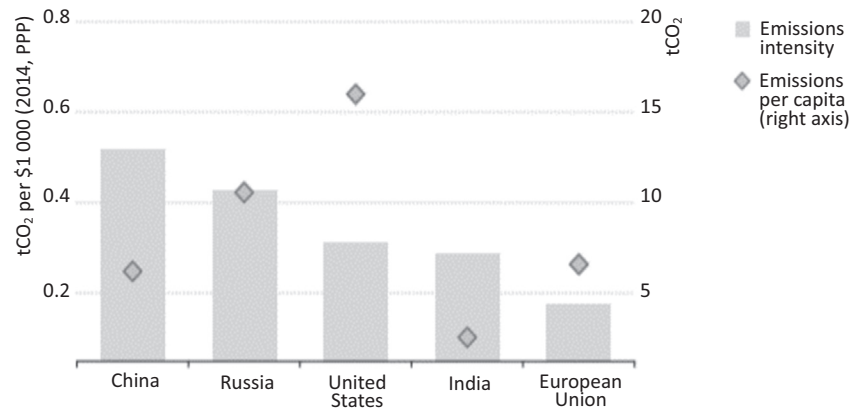
In most countries, food wastes contribute almost half of the total municipal wastes whereas; this percentage may be higher in developing countries. Organic components of food wastes include fruits, peelings, vegetables trimmings, cooked food or uncooked food wastes, meat and so forth. Food wastes are generated during production, handling, storage, processing and consumption. Because of the presence of extreme moisture content in the food leftovers may be applied in composting, aerobic digestion etc. It is essential to improve management of food waste to reduce the risk for human life as well as environment. As study conducted by Shwetha Chaudhary on kitchen waste, collected from university campus, analyzed physico-chemical characteristics, pH value was very high on the beginning days (pH = 8.8) due to presence of ammonia in high amount, after 60 days of decompose of waste it became neutral due to release of organic acids from the fermentation of fats and carbohydrates (pH = 7.6). Electric conductivity of the compost increases gradually were highest around 60 days of composting. In this study moisture content ranges from 86.23% to 61.28%. Moisture content of compost range between 50%–60% characterizes constructive environments for bacteria to cultivate and reproduce along with their best transport medium.

14.1.3 Greenhouse gas emission

Carbon-dioxide emissions in India can be perceived over two lens system. Designed as a per capita base, discharges are tremendously short, positioning at 1/4th of China and the European state's and tenth part the measure of the United States ([Fig. 14.6](#)), although India as well contributing in individual a minor portion of collective greenhouse gas discharges. At the same time, India is that the third largest nation in volume terms of GHG discharges within the world, behind solely China and therefor the United States. Substantial necessity of depending on coal for the production of the power and the utilization of incompetent subcritical plants to burn coal impulse the C concentration of India's energy division to 791 grams of carbon dioxide per kilowatt-hour g CO₂/kWh, associated to a world average of 522 g CO₂/kWh ([Birol and Gould, 2015](#)).

Methane emission (kt CO₂e/yr)

$$= MSWf \times MSWf \times MCF \times DOC \times DOCf \times [(F \times MCR) - R] \times [1 - OF] \times 25 \dots \dots \quad (14.10)$$



Note: tCO₂ = tonnes of carbon dioxide; PPP = purchasing power parity.

FIGURE 14.6 Carbon intensity of GDP and CO₂ emission per capita on selected regions (Birol and Gould, 2015).

TABLE 14.4 CO₂ Emission from the land fill sites in 2016 (Ahluwalia and Patel, 2018).

	Total MSW (tones/day)	MSW dumped	CO _{2e} emission (tons/day)	CO _{2e} emission (kilotons/year)	Equivalence to passenger vehicles (thousands/yr)
Delhi	9620	50%	1764	643.7	137
Mumbai	8600	80%	2523	920.8	196
Chennai	5000	80%	1467	535.3	114
Bengaluru	4200	60%	924	337.3	72
Pune	1600	35%	205	74.9	16
Iudore	700	60%	154	56.2	12
Chandigarh	450	60%	99	36.1	8

where:

MSW_t = total mass of municipal solid waste generated in kilo tonne/year.

MSW_f = fraction of MSW disposed at landfill sites.

MCF = methane correction factor for aerobic decomposition in the year of deposition (=0.4).

DOC = degradable organic carbon in the year of deposition (Cgms/wastegms ~0.11) 17.

DOC_f = fraction of degradable organic carbon which decomposes = 0.5.

F = fraction of methane generated in landfill gas = 0.5.

MCR = methane to carbon molecular weight ratio ⇒ 16/12 = 1.33.

R = methane recovery (kilo tonne/year) no recovery = 0.

OF = oxidation factor (unmanaged disposal = 0).

“IPCC suggested standards specified in brace, multiplied by 25 for methane to carbon dioxide global warming potential correspondence alteration” (Birol and Gould, 2015).

In approximating greenhouse gas footmark of several areas of seven main capitals in India, in 2015 applied same procedure to obtain carbon dioxide corresponding discharges after throwing away of solid waste in the year 2009. By referring Eq. (14.10), GHG emissions from MSW discarding in dumping spots are considered for chosen seven Indian metropolises, given in Table 14.4. The unprocessed throwing away of assorted MSW at dumping spots in Mumbai and Chennai about 80%, Delhi and Bengaluru 50%–60%, and in Pune 35%. It suggests that in 2016, Mumbai discharged maximum CO₂ around 921 kilotons of gases from its dumping yard up to yearly discharges from 196,000 typical traveler automobiles. For Delhi, the estimation is 137,000 cars (Ahluwalia and Patel, 2018).

14.2 Methodology

The survey is grounded on the literature overviews and site visits. Primarily, articles on solid waste management are reviewed in detail to comprehend the position of waste management structure in Bengaluru by SWM Manual, mainly pointing on the organic/wet waste of MSW. The MSW handling methodologies in Bengaluru were recognized and the achievement stories were googled. Throughout the site visits, the hazards of the processes were also discussed. To understand how MSWM working in Bengaluru, numerous firms, housing complex were recognized by internet search and BBMP networks. Those firms, housing complex were visited and many features were surveyed to know the process and maximum knowledge and data are collected. Based on the literature review and field visit results, a sustainable structure of reusing and discarding of MSW was projected.

14.2.1 Case Study 1: biomethanation plant at BEL campus Bengaluru

Feed material: Organic Canteen Waste.

Operating Mode: Privately operated.

Capacity: 2 tons.

Product: produces 160 CUM Biogas per day, supply to the canteen kitchen with 0.4 kg/cm² pressure.

Bharat Electronics Ltd (BEL) has installed an Up flow Anaerobic Sludge Blanket based biogas plant which makes use of the biomethanation technology for transforming refractory food waste into inexhaustible energy. This plant cost of Rs. 40 lakhs, Maihem Ikos, a Pune based waste management company has installed the plant, and this is able to digest 2 tons of food waste every day. Food/organic waste produced in the company mess are able to produce of 160 m³ of biogas every day. The biogas typically utilized for electricity production, bio CNG or heat. The biogas has replaced around 19,167 kg of LPG consumed per year at the factory kitchen. Gas supplied to the mess kitchen maintaining of constant pressure of 0.4 kg/cm² to produce effective flaming. It's an environmentally friendly method of recycling of food waste; other companies in the city can follow the footsteps in dropping environment pollution measure.

14.2.2 Feed stock

Kitchen waste have calorific value of 23,000 kJ/kg, 74% CH₄ of (Apte, Cheernam et al., 2013) nutritive, produces bio-gas by anaerobic digestion method which is suitable environment for microbial activity which produces gas, mainly this gas contains methane (CH₄) and carbon dioxide (CO₂). In Table 14.5 describes the characteristic of natural gas and biogas. The calorific value of biogas is 21.48 MJ/m³ (60% CH₄, 38% CO₂, 2% other impurities) much lower as compared to natural gas at 36.14 MJ/m³. As additional to kitchen waste cow dung slurry is inoculums to maintain the pH value and alkalinity. The low calorific value is sufficient for cooking and for electricity generation. Impurities in the biogas like H₂S, water vapors are reduced by the scrubbing and some applied techniques of cleaning (Vélez, Segovia et al., 2012). H₂S has high corrosive property which damages components of the combustion equipment, while combustion of biogas in the presence of H₂S it forms SO₂ and SO₃ those are additional contaminated comparatively H₂S (Bolin et al., 2009). Biogas composition is influenced by the feed stock, and anaerobic biogas production technologies.

Mixed food and green wastes: C₆H_{9.6}O_{3.5}N_{0.28}S_{0.2} (Meegoda et al., 2013).

TABLE 14.5 Parameters of natural gas and biogas (Wellinger and Linberg, 2000).

Parameter	Unit	Natural gas	Biogas 60% CH ₄ , 38% CO ₂ , 2% other impurities
Low calorific value	MJ/m ³	36.14	21.48
Density	kg/m ³	0.82	1.21
Max ignition velocity	m/s	0.39	0.25
Theoretical air requirement	m ³ air/m ³	9.53	5.71

14.2.3 Plant data analysis

The data is consisting over period of 12 months of monthly account of organic matter loading over and above energy produced in the form of biogas. Fig. 14.7 indicates the total inputs of organic matter, kitchen waste and biogas generated per month in CUM. Table 14.6. Shows LPG consumption in canteen to cook food to feed 10,000 employs. Table 14.7 represents the production of biogas, consumption for cooking in main canteen by using 448,641 kg of kitchen waste over 12 months.

14.2.4 Case study 2: Bio CNG plant

Company name: Carbonlites Bengaluru, Karnataka, India.

Feed material: Municipal Organic Waste.

Operating mode: Partnership with BBMP.

Capacity: 4 TPD.

Product: Produces bio CNG & Organic Manure.

The plant is located in Koramangala Bengaluru city. Carbonlites is a first branded bottled Bio CNG is sold in India. The plant works in partnership with BBMP. Wet waste is collected by the BBMP collectors from residences and apartment associations, hotels, local vegetable and fruit market in BTM layout and Koramangala. The majority of waste is supplied by waste management collectives like Saahas and Hasiru Dala. The collected waste i.e., food waste and vegetable waste transported to the decentralized plant located in BBMP waste processing center in ward No 5. The food processing plant has a capacity of 10 tonnes per day. The collected wastes (around 4 tons) are placed on the

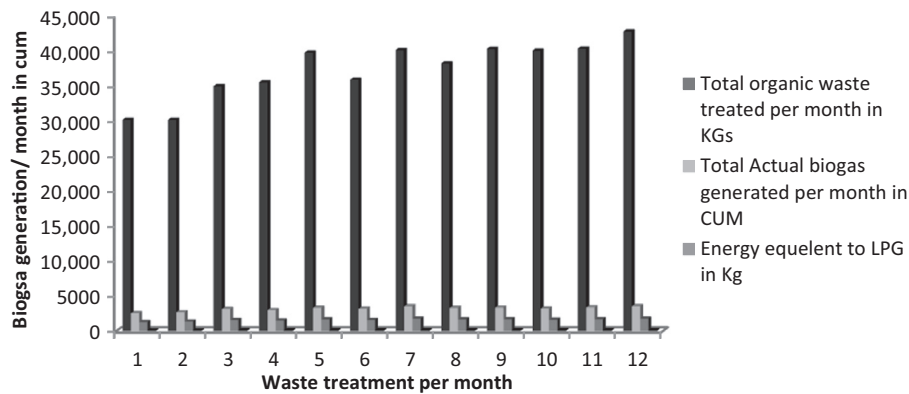


FIGURE 14.7 Production of Biogas by using food waste from common dining.

TABLE 14.6 LPG Consumption at Canteen.

Capacity of staff canteen	No. of LPG cylinders required per year (per day usage × no. of days)	Price of single cylinder	Total price Per year
10,000	4 × 300 = 1200	1446 Rs/-	17,35,200/-

TABLE 14.7 Canteen waste biogas plant comparison with LPG.

Canteen kitchen waste for 12 months in Kg	Total Biogas Generated per year in CUM	Energy Equivalent to LPG in Kg	Equivalent to LPG cylinders	Price of single Cylinder (19 kg)	Total price Per year
448641	38,331CUM	19167	1008	1446 Rs/-	14,57,568/-



FIGURE 14.8 (A) Anaerobic Digester (refurbished 40 ft shipping container), (B) Shredder.

sorting table to remove nonbiodegradable materials like paper, piece of metal, rubber etc. After the segregation wet wastes are fed to the shredder through screw conveyor later shredded waste been collected in the slurry tank by mixing with water, partly fresh water and partly recycled water is used to make slurry, later slurry has collected in the slurry tank. The slurry is injected in the anaerobic digester. The biodigester is housed inside refurbished 40 ft shipping containers. The biogas collected from the digester into the balloon of capacity of 200 kg, the biogas is purified by removal of moisture, passing the gas over scrubber H_2S gases are removed from the biogas. After the purification pure methane (78%) has been compressed and pressure is maintained of 2 bar. Compressed gas is collected in the 2 separate cylinders 80 kg each. The compressed gas is supplied 100 kg/day to the nearest restaurant Truffles through the pipeline. CNG replaces LPG in restaurants. Slurry from digester is treated to produce enriched organic manure. The clarified water and some portions of sludge are recycled to the slurry tank to make microbe population in the biodigester (Fig. 14.8).

14.2.5 Case Study 3: Aerobic compost, purvankara venezia apartment, Bengaluru

Feed material: Kitchen waste collected from the 1332 flats.

Operating Mode: Private.

Capacity: Produce 1 ton of organic compost per month.

Product: Organic Compost.

This is another success story of the production of organic compost by decomposing kitchen waste followed by aerobic method. In operation from 2013 to till date. Purvankara Venezia is a housing complex has 22 acres of land in that 12 acres is built up area with 1332 flats and 12 acres landscaped area, with varieties bushes, big trees, shrubs and variety of grasses like Mexican and buffalo, located in Attur layout Bengaluru. Waste is segregated by the residents as per the guidelines of BBMP. Everyday 1–1.5 tons of organic waste is generated this waste is processed in house gets converted to manure. Dry waste (paper, card board, plastic bottles/containers, metal) and E- waste is produced amount of 8–9 ton/month has been Sold/sell to the authorized recyclers for further processing, 2.6 tons/month of Biomedical/ Sanitary Waste sent to authorized incinerator service to destroy. Finally Rejected Waste (Broken Glass, Cloths beyond repair, Soiled cover, cat poop, Sludge, plastics refused by vendors) 6.61 tons/month Sent to land fill (Fig. 14.9). In Table 14.8 waste segregation methodology is discussed.

In this particular housing society Aerobic composting is done by using Tray method. The main resource is kitchen waste, dry leaves from their own garden, extensive use of dry leaves and twigs to compost pile for better C/N, microbial coco peat is added to increase the microbial activity and small portion of neem powder is also added to restrict flies and other pests. Everyday 1–1.5 tons of kitchen waste is collected by the house keeping employs. All residents of the apartment have to segregate the waste in three different bins as organic, dry and sanitary waste. The collected organic waste

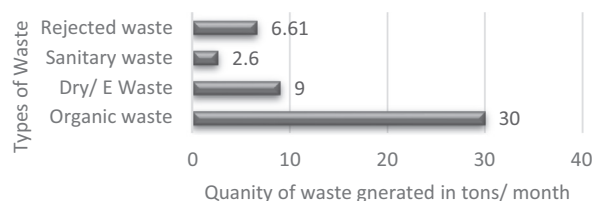


FIGURE 14.9 Types of waste generated with quantity.

TABLE 14.8 Waste segregation method followed.

Type of waste	Solution
Wet waste (food waste)	Processed in house gets converted to manure.
Dry waste-E waste	Sold/sell to the authorized recyclers for further processing
Biomedical/sanitary waste	Sent to authorized incinerator service to destroy
Rejected waste (broken glass, cloths beyond repair, soiled cover, cat poop, sludge, plastics refused by vendors)	Sent to land fill



FIGURE 14.10 Schematic representation of kitchen waste and garden residues aerobic composting process. (A) collected kitchen and garden waste. (B) preprocessing, combining and chopping of residues. (C) aerobic composting at ware house (D) organic compost, recycled product with rich in nutrition.

bought to the composting site, weighed and chopped in small pieces showed in Fig. 14.10. Later mixed in 50% of food waste, 20% of dry leaves, 20% coco peat and 10% of old compost and fills the crates kept in the rack for curing for 10 days. After 10 days the mixture is shredded again in OWC to reduce the size and stored in bin for another 20 days. After 30 days of curing the mixture is turned in to dark brown to black color with earthy odor. This plant yields 1 tons of organic compost per month. The compost has 0.65% of N, 0.14% P, 1.16% K, pH (1:10) 7.75, 22% of OC. Same compost is used in their gardens and more than 240 tons of compost sold to local farmers to increase the fertility of the soil, buy the organic compost for yielding fruits, vegetable crops. Organic compost is selling in Rs 4000/- for one load of tractor.

14.3 Microbial nanotechnology application and role in biomethanation and biocomposting

In enhancing environmental safety techniques nanotechnology is emerging generation for scientific development and holds significant possible developments. The implementation of nanotechnology in protection of environment safety is

receiving greater interest and is being implemented in the field of waste water treatment, biomass treatment, groundwater remediation, soil remediation, waste management. Biomethanation is taken into account as effective and economical technology for handling natural waste sustainably and at the same time produces bioenergy. However, it's far regularly restrained with the aid moderate reaction in account of various pathogens syntrophic organic digestion. Rapid improvement and utilization of nanotechnology have led to huge use of numerous nanoparticles in biogas enhancement due to their amplified physical, chemical and mechanical properties (Baek et al., 2016, 2018). In spite of massive efforts dedicated to the implementation of nanoparticles in unique areas together with rectification of numerous pollutants from soil and water. The materials are classified into three type (1) metal oxides, (2) zero valent metals, and (3) carbon-based materials.

Beak-Jaai et al., reviewed the “fast interspecies electron transfer (IET) between volatile fatty acid-oxidizing bacteria and hydrogenotrophic methanogens is crucial for efficient methanogenesis.” By this syntrophic reaction electrons are exchanged through redox mediators which includes hydrogen and formate. The important thing to improving biomethanation performance is to systematize and accelerate microbial actions (Kim et al., 2013). Interspecies electron transfer (IET) among syntrophic companions performs an important function in oxidizing better natural subjects and lowering CO_2 to CH_4 in AD atmosphere (Batstone et al., 2006). To balance the condition for syntrophic relation to degrade carboxylic acid indirect interspecies electron transfer (IIET) uses hydrogen and formic acid as electron carriers, interruption of this syntropy can cause the gathering of arbitrates like unstable VFAs in addition to excessive hydrogen partial pressure; those can cause a substantial downturn the productivity of AD (Beak & Kim). Over IIET direct interspecies electron transfer (DIET) has been advised as an opportunity in AD condition. DIET is active additionally beneficial as it does now no longer want hydrogen to be used as a carrier of electrons (Cheng and Call, 2016). DIET has recommended to be quicker and dynamically more productive over IIET. DIET has benefit, no longer want complicated enzymatic phases from thermodynamic point of view to diffuse redox mediators (Jing et al., 2017). DIET commonly entails organic electrical connections, that is, cytochrome/pili among microbes. DIET can also arise via conductive substances as nonbiological electric powered conduits, like iron oxide, biochar and activated charcoal, carbon fibers, other conductive materials as carbon nanotubes, Graphene (Chen et al., 2014).

The study conducted by Ambuchi et al. (2016), investigated the responsibility of Iron Oxide nanoparticles (IONOs) and multi wall carbon nanotubes (MWCNTs) in amplifying biogas productivity. The seed sludge (anaerobic granular sludge) acclimation of volume of 6.5 L for 30 days was done under mesophilic condition at 360 C, 12 h of HR time and 3.2 kg COD m^{-3}/day OLR. COD concentration reduces quickly in early 24 h in all reactors with IONPs-151 mg/L, MWCNTs-226 mg/L and controlled reactor-189 mg/L. After 84–96 h leveler off, MWCNTs- 51.0 mg/L, IONPs-60.5 mg/L, and controlled reactor-64.5 mg/L COD concentrations corresponding to 97.0%, 96.5% and 96.3% removal efficiency respectively as shown in Fig. 14.11(A) and (B) shows anaerobic digestion process is demonstrated by the production of methane. Following 96 h of digestion in the reactor at anaerobic condition methane gas was produced with higher rate, that is, MWCNTs – 151.8 mg/L VSS, IONPs- 146.5 mg/L VSS and at controlled reactor was recorded as lowermost – 106.0 mg/L VSS. mg/L VSS. The output illustrations that MWCTs at a concentration 1500 mg/L encouraged scale up in methane production as estimated to IONPs at concentration of 750 mg/L.

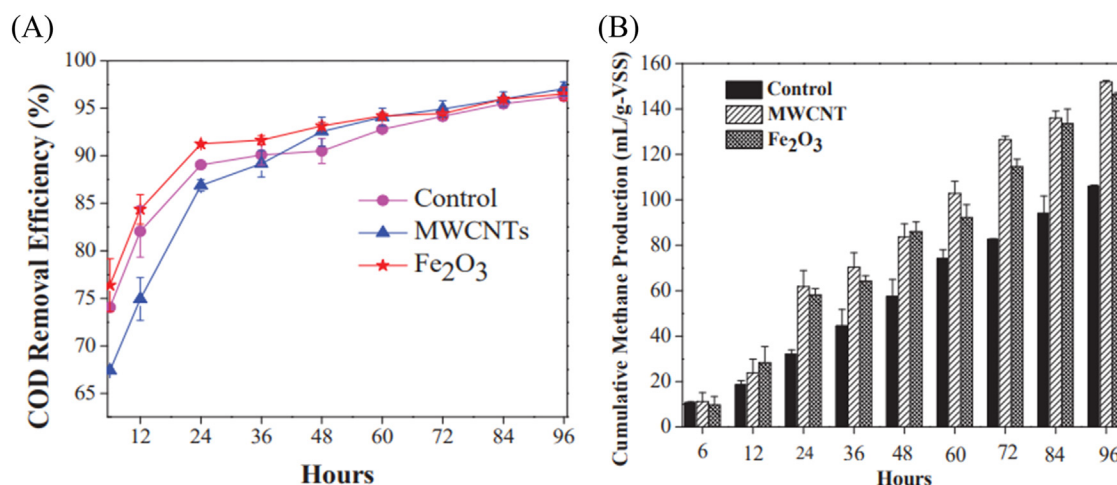


FIGURE 14.11 (A) Efficiency of removal of COD under IONPs, MWCNTs and Controlled reactors. (B) Methane production under anaerobic condition in the presence of MWCNTs and IONPs (Ambuchi et al., 2016).

Faisal S, reviewed on NPs as benefit for biogas, states that “In connection to microorganisms, there are two characteristics to the influence of Fe: (1) it serves as an essential trace element for anaerobe microbes and improves competition with sulphate reducing bacteria (SRB) leading to the growth and reproduction of methane producing microbes; (2) activities of the enzymes involved in methanogenesis and acidogenesis can be stimulated by iron due to its ability to improve basic elements in metallo-enzymes. The above-mentioned analysis strongly suggests that both metabolically and technically, enhancement of AD by nanoions is feasible. Energy recovery through iron-based anaerobic digestion is a sustainable and promising strategy that covers many cross disciplinary fields. This technique can result in a novel industrial chain because it can interlink wastewater treatment, the steel industry and energy generation” (Faisal et al., 2019).

Over 2000 years, (Gitipour et al., 2013) silver has been identified that it has protective properties. Recently silver has turned into engineered nanoparticles, with specific physiochemical properties, structure scaling from 1–100 nm in dimensions (one dimension). In the existing study very steady, PVP-AgNPs were utilized. PVP-AgNPs withstand accretion in high ionic solution along with high valence background electrolyte. In the study nine reactors used with 130 L volume. Three batches of reactor are made which has three reactors each, batch (1) No treatment, batch (2) treated with Ag, and batch (3) treated with AgNPs-PVP. In the composting process microbial respiration is analyzed by collecting gas samples of CO₂, H₂, O₂, CH₄ and N₂O. CH₄ and H₂ concentrations were below the method detection limits i.e., 0.3% for CH₄ and 0.04% for H₂. O₂ and CO₂ concentrations are correlated due to the microbial respiration. The variation showed in Fig. 14.12 the microbial groups in composted samples have been extremely various and in the main ruled with the aid of using Clostridia-48.5%, Bacilli-27.9%, and beta-Proteobacteria-13.4%. Bacterial diversity research confirmed that the general bacterial network structure withinside the composters modified in reaction to the Ag-primarily based totally treatments. he information additionally imply that even as the floor transformation of AgNPs to AgCl and Ag₂S can lessen the toxicity, complexation with natural rely can also additionally play a primary role. The outcomes of this work addition propose that at notably low concentrations, the organically wealthy waste control systems capability won't be influenced with the aid of using the existence of AgNPs.

The study conducted by Waqas et al. (2019), aimed to inspect the reaction of zeolites in augmenting the food waste composting process. Raw and modified natural zeolites were used at 10% and 15% (w/w) of the total waste and analogized with nontreated control samples. Composting process affected by both raw and modified natural zeolite. Modified natural zeolite were noticed with prominent outcomes. At thermophilic phase was sustained for three weeks, for 10% of modified zeolite concentration was recorded the maximum temperature of 55.3°C, 10% raw natural zeolite recorded 52°C. Likewise, for nonamended zeolite sample the temperature was reached 36.7°C subsequently 30 days shown in Fig. 14.13(A and B). Because of high porosity in zeolite permit them to catch extra condensation and create aerobic environment for faster O₂ uptake to microbial degradation of organic matters efficiently which produces heat. As shown in Fig. 14.13(C and D) moisture contents was lowest in 15% modified natural zeolite-33.4%, 10% modified natural zeolite-38.9%, 15% raw natural zeolite-34.1%, 10% raw natural zeolite-403.8% and Nonzeolite amended sample – 57.2%. pH variation represented in Fig. 14.14(A and B), 10% raw natural zeolite-7.5, 15% raw natural zeolite-8.0, 10% modified natural zeolite-7.7, and 15% modified natural zeolite-8.3, this shows that zeolite amended samples has highest pH values compared with other samples. The electrical conductivity of samples after 90 days 3.4%–10% raw natural zeolite, 3.5%–15% raw natural zeolite, 3.5%–10% modified natural zeolite and 3.7%–15% modified natural zeolite [Fig. 14.14(C and D)]. The study shows that steadiness was attained later 60 days. The maturity and steadiness of the produced compost were conforming with rules of the international compost standards recommended by various countries.

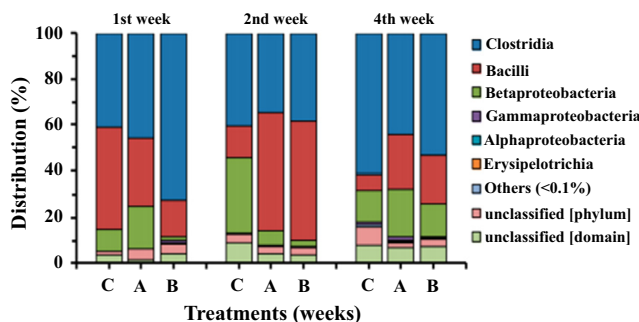


FIGURE 14.12 Relative distribution of bacterial classes. C: No treatment, A: AgNPs, B: AgNPs-PVP (Gitipour et al., 2013).

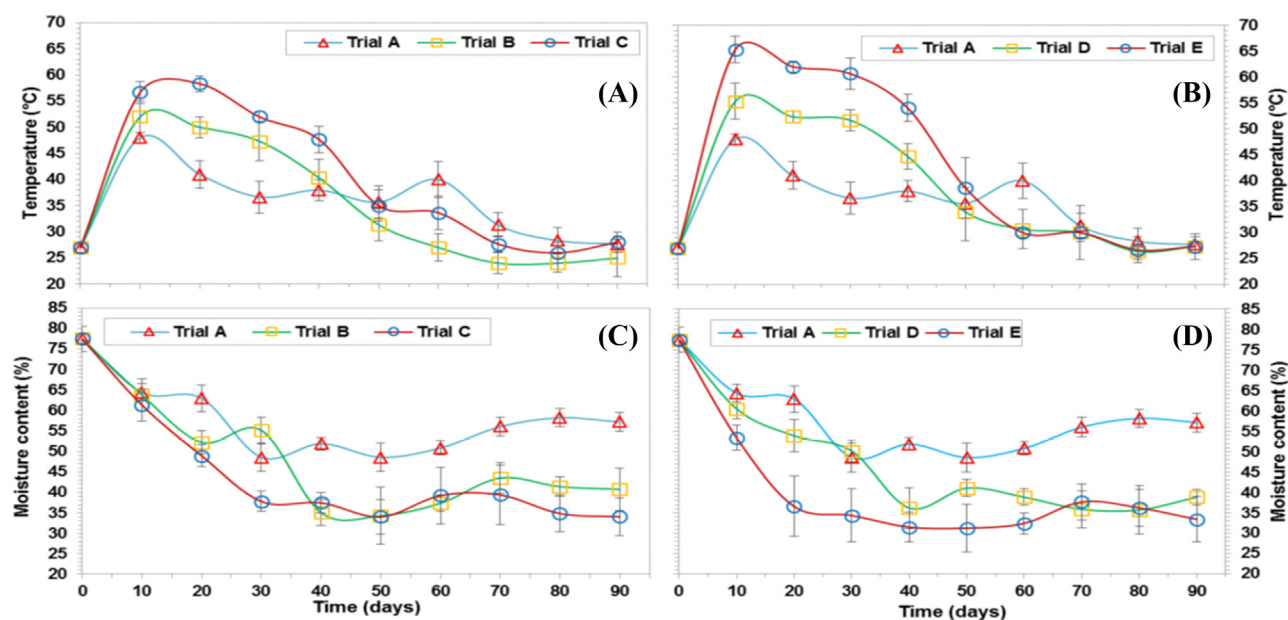


FIGURE 14.13 Temperature and moisture contents variation in food waste aerobic composting process. Trial A: Nonzeolite amended sample, Trial B: 10% raw natural zeolite, Trial C: 15% raw natural zeolite, Trial D: 10% modified natural zeolite, Trial E: 15% modified natural zeolite (Waqas et al., 2019).

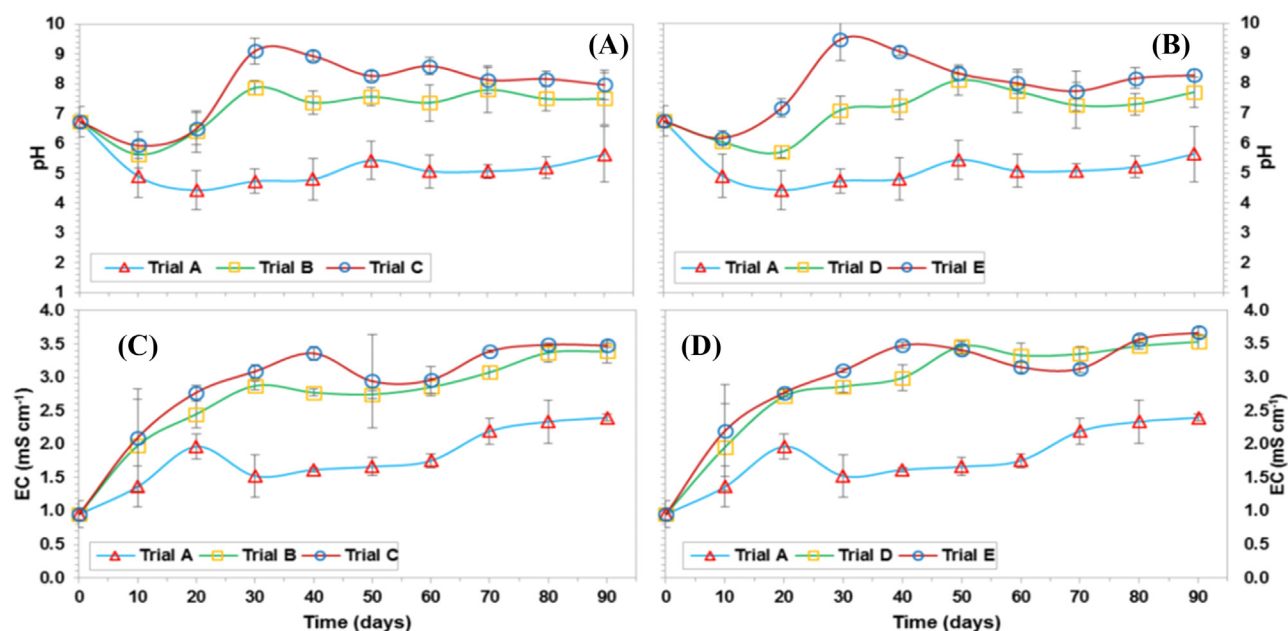


FIGURE 14.14 pH and EC variation in food waste aerobic composting process. Trial A: Nonzeolite amended sample, Trial B: 10% raw natural zeolite, Trial C: 15% raw natural zeolite, Trial D: 10% modified natural zeolite, Trial E: 15% modified natural zeolite (Waqas et al., 2019).

14.4 Discussion on sustainability of the WM techniques and economical challenges

14.4.1 Statistics on waste generation and recycling: sustainability of WM techniques

As per the report published by the Ministry of Health and Family Welfare Indian in 2019 population is predicted to grow from 121.1 crores to 151.8 crores (Population Projections for India and States, 2019). Table 14.9 shows the population growth in India from 1901–2011. Indian population was 238.4 million at the beginning of the 20th century over

TABLE 14.9 Indian population growth from 1901 to 2011 (Census of India, 2011).

Census years	Population X 10 ⁶	Decadal growth (%)	Average annual growth (%)	Progress growth rate compared with 1901 (%)
1901	238.4	—	—	—
1911	252.0	5.75	0.56	5.57
1921	251.3	0.31	−0.03	5.42
1931	278.9	11.0	1.04	17.02
1941	318.6	14.22	1.33	33.67
1951	361.1	13.31	1.25	51.47
1961	439.2	21.64	1.96	84.25
1971	548.1	24.80	2.20	129.94
1981	683.3	24.66	2.22	186.64
1991	846.4	23.87	2.16	255.05
2001	1028.7	21.54	1.97	331.52
2011	1210.2	17.64	1.64	407.64

TABLE 14.10 Citywise per capita waste generation in India.

City	^a Population X 10 ⁶	^b Waste generation (TPD)	Per capita waste generation (kg/day)	^c Waste generation (TPD) 2016
Mumbai	18.4	6500	0.35	11000
Delhi	16.3	5800	0.41	8700
Kolkata	14.1	3670	0.26	4000
Chennai	8.6	4500	0.52	5000
Bengaluru	8.4	3700	0.44	3700
Hyderabad	7.7	4200	0.54	4000
Ahmedabad	6.3	2300	0.36	2500

^aPopulation of major cities of India (census of India–2011).^bwaste generation per capita (CPCB report–2011).^cwaste generation TPD (CPCB report–2016).

a period of 110 years it has increased four times and reached 1210 million by 2011 (Census of India, 2011). Major contributors to MSW in India are its population. The population growth and per capita waste generation is shown in Table 14.10. Existing operational facilities in Indian status are presented in Table 14.11. House to house waste collection has implemented in all states, it has 98.5% efficiency for collection of generated waste. Majorly five states are at work on segregation of waste at the source, and processing of waste at composting, vermi-composting, biogas, and RDF/palletization facilities. Around 1768 Composting plants are working of which 721 are from state of Kerala, 420 vermi-composting, 383 small and medium scale biogas plants are operating, 22 RDF/palletization under performance. Few plants have been shut down due to insufficient resource (poor quality of waste/nonsegregated waste), technical issues, and lack of coordination between UDDs, ULBs, SPCB and private agencies to implement the SWM 2016 rules (CPCB, 2018–19).

As per the guidelines listed in the by ministry of environmental department, BBMP has also instructed to follow by the citizens of Bengaluru to over the garbage issue. Out of them some industries and some gated communities come forward to take up the challenge of decentralized waste management system. The MSW has to be controlled by adopting

TABLE 14.11 MSW existing operational facilities in major status of India (CPCB, 2018–19).

States	Composting	Vermi-composting	Biogas	RDF/Palletization
Maharashtra	302	75	52	13
Karnataka	143	38	05	02
Kerala	721	16	216	0
Tamil Nadu	539	261	107	6
Telangana	63	31	03	01

TABLE 14.12 Environmental impacts from the recycling plants on Bengaluru.

Treatment method	Location	Feed stock	Process parameters	Impact on the environment
Biogas	Bharat Electronics Ltd., Bengaluru	Canteen Food Waste	NH ₄ 70%–78%, C/N ratio-23–30, Digestion time 45 days, Temp 30°C–35°C	Reduces environmental impact by utilizing NH ₄ as resource, freshwater ecotoxicity, global warming, acidification, nitrification (Bolin et al., 2009)
Bio CNG	Carbonlites,	MSW		
Aerobic Composting	Purvankara VENEZIA Apartment	Organic Kitchen Waste	Duration 40 days N-0.65%, P-0.14% K-1.16%	Global warming, CO ₂ emission, NH ₄ emission, acidification, nitrification ozone depletion, Greenhouse Gas emission (Andersen et al., 2010)

technologies and suitable practice that facilitate maintaining our city spotless, avoid contamination and which also nature friendly. It also minimizes the cost through recovery of resource and energy. As described in the case study 3.3 and in Table 14.12, aerobic composting is also favorable technology for the city along vermicomposting and windrow composting as organic fraction is rich in domestic waste comparing with the proportion of lignin, the vermicomposting plant is more appropriate uses *Eichhornia crassipes* that has high polymer content, but the windrows composting plants take away any high polymer half conjointly throughout last screening as it leftovers a minor volume affected and is habitually handled by discarding. On the other hand, these technologies need if the organic fraction is wealthy in municipal, residential, bazaar, and vegetable solid wastes. This can be a loss of source. Hence, it's been instructed within the context that prime organic fraction municipal solid waste should be subjected to biomethanation and low lignin OFMSW to be composted using aerobic try method. As found in each aerobic composting process and biogas, windrow composting is a centralized plant also working in the city for setting up for decentralized facilities are not available. But centralized processing of waste becomes predictable, such services should be adequately bulky, it needs huge funding for design, construction, operation, maintenance and transportation of waste.

Bengaluru tracked the appropriate model of decentralized biomethanation process and set up 10 biomethanation plants in the city by the municipal corporation of the Bengaluru, but hardly few of them are in action, primarily due to poor separation of waste. For 4 plants are effectively in process, feedstock waste must be methodically kept apart and shifted on scheduled time to biomethanation plant. Imperfect separation planes and substandard quality of feedstock retains the plants from sustainable operating at their maximum capacity. Regular develop of fault in the plant get effect from the existence of nonbiodegradable things in feedstock waste. The processing consequently consumes ample extended process as lower-level categorizing is implemented at the biomethanation plant.

The feedstock segregation process subsequent the unpleasant odor makes refusal from inhabitants of the neighborhood where the plant is situated. Absence of firm observance to standard working systems and consistent conservation of the plants has too caused in regular failures of processes. Arrangements for retrieval of liquid slurry or manure have also not constantly stood in place, consequential in absence of support from the resident community. The well-known challenges have been the faced by some of biomethanation plants in Bengaluru. The bio-CNG plant processes hardly an uncertain 4 TPD of biodegradable waste collected from restaurants, catering, Slaughter house waste, street sweeping

and slit from drains. The divided action thru a distance of 2 km between the two locations imposes over the financial charge of processes. The sheer deterioration of the cost and subsidy from the Indian government on natural gas in recent years has also unpleasantly exaggerated the economic estimation for this medium-scale biomethanation plant.

14.4.2 Financial and economic support for MSWM

According to the Indian constitution solid waste management is a part of public health and sanitation. Meanwhile the movement is important, universal and unmatched, the responsibility is lies in the public domain. As municipal government is local in nature to commended the moment. The Municipal made a budget from the income, revenue and funds sanctioned by the Central and State Government, to carry out the essential activities. As mentioned in the economics and financial considerations by the CPHEEO 2016, “Municipal agencies have to manage various civic services. The number of activities managed by these agencies increase with the size of city. It is observed that smaller towns where the main activity is Solid Waste Management spend up to 70% of their budget on Solid Waste Management. Metropolitan cities on the other hand due to wider resources base and responsibility of provision of larger number of services spend only around 10% of their budget on Solid Waste Management. A majority of urban centers however spend 5%–40% of their budget on Solid Waste Management. This is approximately Rs. 50–250 per capita per year. It is observed that a large proportion of this expenditure is incurred on salaries and only a limited amount is spent on Operation & Maintenance (O&M) and development works.” For a strong waste management system would require economic source for its ease operation. It is suggested that MSWM is handled as a detailed special progressive mission, that involves a huge amount of capital expenditure and repeatedly growing process and maintenance expenses over the next few years. Various factors concerned in the monetary making plans are discussed here. (1) SWM tax benefits are introduced, (2) Capital investment loans from suitable agencies. According to the tool kit, [Public Private Partnership \(2016\)](#), the major gap in the challenges of project development r, Institutional and financing policy issues, Policy related challenges, public awareness of segregation of waste are included.

[Mani and Singh \(2016\)](#) reviewed in their study “The Ministry of Environment, Forest and Climate Change also provides financial support of up to 50% of the capital cost to set up pilot demonstration plants on MSW composting. The Ministry also extends limited financial assistance for waste characterization and feasibility studies. The Ministry of New and Renewable Energy (MNRE) has designed schemes to promote waste to energy projects. The Ministry also promotes all technological options for setting up projects for recovery of energy from urban, industrial and agricultural wastes. Currently only five pilot projects based on MSW to energy are being supported. Under this scheme, a minimum amount of Rs. 2 crore and a maximum of Rs. 10 crore per project are available as capital subsidy. The entire capital subsidy amount is released to the beneficiary’s loan account. Some of State governments of Andhra Pradesh, Haryana, Gujarat, Karnataka, Maharashtra, Madhya Pradesh, Rajasthan, Tamil Nadu and Uttar Pradesh have announced policy measures pertaining to allotment of land, supply of garbage, and facilities for evacuation, sale and purchase of power to encourage the setting up of waste to energy projects. Land for the facilities is provided by the ULB at a nominal rent. Although recent announcement by the Power Ministry to purchase power generated from WTE plants at a higher rate than earlier, the cost of monitoring and mitigating emissions and pollutants from such incineration based WTE plants has made them unviable. Greater incentivization and operational support is required for promoting biomethanation based WTE. Swachh Bharat Mission is currently making available Viability Gap Funding (VGF) up to 20% to states and individual municipal corporations. However, ULBs are still not able to raise the rest of 80% investment required for SWM improvement in their cities and towns. Greater convergence of schemes and sustainable models need to be explored.”

In India, the fundamental economic issues facing by municipal local administration bodies is that, they are economically actual relying on leading high-ranking government/administrations for transactions whichever neither undertook nor foreseeable. In 2012–13, in India the over-all urban local administration incomes stand just over 1% of GDP, while this proportion was 4.5% in Poland, 6% in South Africa and 7.4% in Brazil ([Birol and Gould, 2015](#)). Under this circumstance of very shallow quantity of entire urban local body returns, their particular returns have been deteriorating and were just a slight over 50% of the entire incomes in 2012–13. Municipal corporations in India are typically not authorized to deploy economic funds over increasing taxes or imposing user charges or exposing property worth charges. Final point, which is hypothetically much significant is ruled out since the state governments to local governments has typically not been proceeded the urban development occupation. Also, there is an essential to get agreement from the state government to raise the handling price, tax to wrap the process and sustentation cost of a plant. By the High-Powered Expert Committee proceeding Indian Urban Infrastructure and Services was last estimated from 2012 to 2031 over the period 20-year for the venture necessity to get the connection between the municipal infrastructure discrepancy

in the municipal solid waste management division for entirely metropolitan cities and townships of India. At 2016–17 values, High-Powered Expert Committee valued a total needed funding to Rs 70,000 crore (excluding the cost of land). Municipal corporation bodies are facing it very problematic to encounter the requirements of the principal investment fund, that shows the very scrawny condition of urban bodies economic means. However, this is only 1.5% of the entire fund demand for the urban infrastructure segment during the period of 20-year. Further addition to this, the municipal corporations are likewise not in a condition to figure the establishment of business venture with profitable usage fee possibly could make adequate profit to empower the urban local bodies to cover its operation and maintenance charges and it also help in enticing private venture as proprietorship in the development mission even in taking the capital from the market.

The Government of India has dedicated to pay out Rs 14,620 crore under the Swachh Bharat Mission out of an entire Mission cost of Rs 62,000 crore for all of the current 4041 approved cities of the country over a period of 5-year. The Mission is mainly projected to be funded by way of the resources of state/urban local bodies, private ownership endowment, boost up by user charges and revealing property charge. Diminishes the demand for city compost due to huge subsidy has provided by the Government on chemical, synthesized fertilizers as like Urea makes misrepresentations in comparative values. If there is good demand for city organic compost then only the implementation of aerobic, vermi and windrow composting of organic, biodegradable waste would boost up.

By the installation of decentralized biomethanation plants giving an impression on a favorable resolution by way of all about 50% of a city's organic fraction solid waste could be handled. The challenge in the decentralized plants, these renewable power stations which includes conveyance charge for consuming the left-over and huge funding of principal amount for the equipment. It requires financial sustainability because the biogas is utilized largely for cooking (heat), bottled up as compressed biogas (CNG) which had been using as fuel for the vehicles as well for cooking, even biogas is used to produce electricity to light up the street lights and finally the sludge is assorted, dehydrated and sold as organic fertilizer (attentive improvement). Private ownership contribution also needs to be encouraged further in the expansion or progress and process of biomethanation and/or bio-CNG plants and composting plants, since these plants provide excellent redemption on carriage of waste from the resource to the centralized processing units or dumping sites and even these plants prevent GHG discharge hitches prevent the sequence of municipal solid waste management especially can through feasibility breach sponsoring.

14.5 Conclusion

This paper reviews the challenges, barriers and opportunities associated with improving waste management in India. The organic waste to recycle as fertilizer and even used as substitute energy like biogas to generate electricity, used for cooking purpose, bio CNG and biodiesel can be blend in diesel to run the vehicles. In the view of current situation of MSWM in the country can minimize the dumping of waste by ensuring resource recovery/recycling of waste at in-situ. Emphasizing type and quality of waste available is an input to adopt technology. Decentralized waste management is convenient for WtoE and recycling is good step as collection and transportation of waste needs financial support. The choice of technology, like composting plants (1–2 tons/day) which takes less composting time and anaerobic digestion (2–20 tons/day) is way out for Municipal Solid waste as it has mixed waste which produce leachate and bad odor if store a long period, it also releases methane which is major greenhouse effect for global warming. As the India has demand of energy production for country development along with that Indian fields need fertilizers to get a quality yield so the production of biogas and organic composting need to be replicated all over the country.

Directives necessitate trades producing high capacities of organic waste to pursue more sustainable waste discarding resolutions. Trades have dedicated to sustainability and are now crawling to go along with the directions by the urban local governing bodies. Anaerobic digestion (AD) of organic solid waste has not been successfully implemented in the India to day in marketable scale. Successful technology development and commercialization of MSW requires: Research revolution, machinery, mechanization development, Public and private venture and partnership needs more reimbursements from the leading government, the plants need capable and operative methodological/technique, administration, and business evolution squad along with commending merchandise atmosphere. In the world renewable energy market, Indian renewable energy sector is in the fourth most attractive sector. The Indian government committed to increase production and utilization of clear energy. Is undertaking various large-scale projects. The MNRE has targeted around 5000 compressed biogas plants to be setup across India by 2023. These projects have the potential to create employments in all levels mainly in rural areas. That helps to decrease the unemployment rate, which supports to country's economy growth. The City has best waste policy, trained employs and best waste management technologies that improving public health and environment.

14.6 Future scope

As stated by the research discussed here, complementary contrary forces of NPs on AD biogas production, methane content and biocomposting were described. On that account, it's far essential to acquire additional thorough perceptions into the effects of NPs on the microbial community and waste digestion technique with an intention to enhance methane production yield in the course of the AD techniques. Study of commercial-scale is way intricated due to the complex microbial community. NPs are appropriate for numerous reactions as NPs has high volume ration, potential of excessive reactivity. Thus, varieties and concentrations of NPs should be converted or tune on the consequences acquired on commercial production. Hence, NPs in biocomposting investigation results the stability and maturity with nutrient rich compost with international standers.

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Application of microbial nanotechnology in sustainable agriculture through soil remediation

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15.1 Introduction

As Charles E. Kellogg quoted in USDA Yearbook of Agriculture, 1938, “Essentially, all life depends upon soil. There can be no life without soil and no soil without life; they have evolved together,” it has been evident to human race over again that soil bears the strongest connection to life. Soil holds the key to sustainability of every minute living being that exists on this planet by maintaining the ecological balance between nature and its consumers. However unfortunately, soils are the worst sufferers of the inhuman behavior persisting in this world. Vast quantity of contaminants belonging to different types and classes are deposited and accumulated in the soil. Most of these soil contaminants can be categorized under inorganic pollutants such as heavy metals in particular and organic pollutants such as organophosphorus pesticides, chlorinated solvents, polycyclic aromatic hydrocarbons (Wang et al., 2015; Chen et al., 2015). While some of the pollutants are nonpersistent in nature, others are quite persistent in nature which tend to accumulate over the years and degrade the soil nutrition and quality. Moreover, they also adversely affect the ecological balance that is well maintained otherwise. As reports suggest that by 2050, it will be critical for the mankind to produce and consume adequate quantity of food crop and if immediate steps are not taken as an initiative to combat this crucial situation, it is expected to get worse. As a result, extensive research has been conducted for years to find an effective solution that can remediate and replenish contaminated soil in order to pave a way for sustainable agriculture. Among the various experiments conducted, biotechnology has been an important part of them which has proved its effectiveness in remediation of polluted soil over the years of study. Most recently, focus has shifted towards the development of few novel materials like bio-sorbent, graphene-derived materials along with nanomaterials which has proven to be more promising in comparison to others (Gong et al., 2018). Thus, this field of using nanoparticles (NPs) for various purposes has come to be known as nanotechnology.

NPs can be defined as micro-atomic particles or molecules that usually exist individually or in the form of aggregates. They have variable sizes within the range of 1 to 100 nm (Roco, 2003) in a minimum of one dimension (Lin et al., 2014). This property of small size renders NPs to be versatile in nature, have high SSA and reactivity (Zhang et al., 2019), thereby extending its use in various fields of life like agriculture, environmental science, energy, medicine, material sciences, cosmetics, food industry, etc. (Kah, Tufenkji and White, 2019). Moreover, NPs are characterized by unique physicochemical properties that enhance its potential to function as a means towards remediation of soil. Generally, these NPs originate naturally from a diverse range of sources like weathering, meteoric dust, volcanic eruptions and action of microbiota on organic soil (Morales-Díaz et al., 2017). Other times, these NPs are also produced biologically or through physicochemical processes in the presence of microbes, known as engineered NPs, whose sources are termed as anthropogenic (Rathinamoorthy and Senthilkumar, 2009). NP production in assistance with microbes is carried out in two dynamic steps whereby the microbial system is utilized to trap the metallic ions followed by reduction of the same metallic ions to NPs mediated by enzymes. Conventionally, these microbe-mediated NPs can be produced by following two approaches: “bottom-up” and “top-down.” In “bottom-up” approach, the molecules follow the molecular recognition principle by chemically arranging themselves to form NPs. Riveting and welding are two of the widely employed techniques under “bottom up” approach. On the other hand, “top-down” approach generally uses

larger molecules rather than lower level of atoms that includes methods like grinding, drilling and milling. Comparing both the approaches, it has been concluded that “bottom-up” approach is cost-effective, less time-consuming, and is suitable for large-scale production than “top-down” approach (Rathinamoorthy and Senthilkumar, 2009). Once produced, these NPs are applied in soil from where they are absorbed into the soil ecology as well as the plant systems. However, one crucial fact that necessarily demands attention is overapplication of NPs in soil can indeed negatively influence the regular metabolism of soil accompanied by hampering the physiological apparatus and leading to NP accumulation in plants which predictably flows through the food chain through consumption of the accumulated NPs in plants by animals and in turn humans, thus leading to biomagnification in higher class of organisms (Table 15.1).

For centuries, soil has continued to suffer the deficiency of micronutrients like Zn, S, Se, etc., and macronutrients like K, N, P, etc., along with invasion by pest and foreign organisms. Hence recently, more focused research is being carried out in the agricultural sector to use nanotechnology as an intermediate for effective gene delivery in plant breeding systems Gladkova and Terekhova, (2013) by improving the soil nutrition and quality. The most common NPs used for soil remediation are $\text{Fe}_3\text{O}_4/\text{Fe}_2\text{O}_3$ (iron oxides), CuO (copper oxide), Ag (silver), CeO_2 (cerium dioxide), ZnO (zinc oxide) and TiO_2 (titanium dioxide). In soil, NPs are absorbed through various pathways like sedimentation via leaf abscission, aerosols and dust, precipitation and through gaseous absorption which, in fact, helps to newly discover soils as NP storage systems. NP absorption by soil is regulated by anthropogenic activities as well. This can be well understood by studying the entry routes, time of residence, pattern of accumulation, migration and action of NPs in soil Gladkova and Terekhova (2013). Conducted experiments have confirmed the delivery, uptake and accumulation of NMs in plants (Schwab et al., 2016). Due to the micro-size of NPs, it becomes easier for the soil to absorb them quickly and collectively, the plants uptake them through their roots which is then propagated to all the vegetative parts. It has been observed that on administration of TiO_2 NP has enhanced the seed growth of spinach plant (Zheng et al., 2005). A study conducted with pumpkin as the model system has indicated the accumulation of NPs around the roots as well as leaves (Zhu et al., 2008). On the other hand, few experiments involving six different species of plants—rape, lettuce, cucumber, radish, ryegrass and corn were done where five different NPs—alumina, aluminum, zinc oxide, zinc and multi-walled nanotube carbon—were provided. The results revealed that there was no such effect on seed germination, however, there was little inhibition in root elongation. The results of another evaluation where tetramethylammonium hydroxide (TMA-OH) coated NP was applied on *Zea mays* plant species demonstrated that excessive amount is capable of inducing toxic or adverse effects (Zhu et al., 2008). To understand the potentiality of plants to provide entry routes for NPs, Hischemöller et al. performed an experiment with *Arabidopsis* and *Phalaenopsis* where the results were studied using a confocal laser-scanning microscope. The microscopy showed the consecutive steps of NP uptake by the roots that could be observed from the velamen radium, followed by movement to the exodermis, then parenchyma and gradually reached the vascular tissues (Hischemöller et al., 2009) (Table 15.2).

Owing to the environmental pollution suffered by environment, soil in particular, demanded a new innovation using a harmonious activity portrayed by biotechnology and nanotechnology which exhibits a potential to eradicate pollutants from the contaminated soil. This made the research world to think about the effect and interaction between NMs, soil, plant system and also microorganisms present in the soil which also raised the issue regarding the optimal conditions that are essential to be maintained during the application of NPs in the soil system to enhance agriculture. Intriguingly, these NPs bearing unique properties also allow them to openly interact with soil and its population of biological systems, thereby showcasing both positive and negative regulations. Current research has focused on designing a nanotechnology-associated-biotechnological practices that promise to decipher polluted soil into healthy and nutrient-rich soil in the near future. One such example that has been witnessed is the administration of nano-titanium oxide in degrading cadmium from contaminated soil, synergistically with phytoremediation (Singh and Lee, 2016). Not to overlook any boon or bane of NP application in soil, research has been conducted to find the advantages as well as the ill-effects of NPs under conditioned factors integrally related to soil atmosphere. On one hand, it has been indicated that NPs portray a dual characteristic property of functioning as a macro- and micro-particle as well as carriers of nutrients that increased the possibility of NPs positively regulating the growth of plants along with enhancing crop productivity (Ditta and Arshad, 2016) while on the other, it has also been evident that exposing plants to concentrations of NPs equal to 100 ppm or higher can adversely affect plant systems (Reddy et al., 2016). Inclusively, it has also been predicted from reports that microorganism-NP interaction can certainly mediate the efficacy of bioremediation in contaminated soils. Controversially, fullerene NP application in soil has been observed to upregulate the passage of contaminated, organic form of chlorine from soil (De La Torre-Roche et al., 2013). Hence, no conclusive statement regarding the application of NPs in soil remediation has been straightly put-forward, however, various remediation techniques are being applied in permutation to observe the benefit offered by NPs, depending on its size and type, in establishing sustainable agriculture (Table 15.3).

15.2 Synthesis of nanoparticles mediated by microbes

Modernized technology paves the way for exploiting some of the vital characteristics exhibited by various microorganisms that includes rapid growth under extreme conditions of atmospheric pressure, pH and temperature, thereby enhancing the ease of cultivation. This allows the favorable involvement of microbes such as yeast, bacteria, algae and fungi for the synthesis of a wide variety of NPs. The synthesis of NPs from microbial sources is an emerging field of study and processing that is estimated to pave ways to greater opportunities in the near future. However, one noticeable factor that determines efficient NP synthesis is the behavior portrayed by different NPs on interacting with varying biological agents. The general protocol followed for NP synthesis is initial trapping of metal ions on microbial cell surface followed by an enzyme-catalyzed step where the metal ions are reduced to form NPs.

The synthesis of NPs from microbial sources is considered to be a “biogenic” approach or “green” approach due to the very fact that most microorganisms have the ability to cope up with ambient conditions of varying parameters like pH, pressure and temperature (Bhattacharya and Mukherjee, 2008). Hence, the NPs formed from these microorganisms exhibit greater specificity in surface area, higher catalytic activity and enhanced interaction between the ion salts and the enzyme in the presence of bacterial carrier matrix (Mann, 2001). The microbial NPs are synthesized when they come in contact with the target metal ions and turn them into element metals with the help of enzymes produced within the cellular components. This can be achieved in two ways depending on the location where the process is taking place (Zhang et al., 2011):

1. Extracellular—Trapping of metal ions on the cell surface and reducing them into metal elements mediated by cellular enzymes is known as the extracellular method of NP synthesis.
2. Intracellular—Transportation of metal ions into the microbial cell characterized by enzyme presence for the synthesis of NP synthesis is known as the intracellular method of NP synthesis (Zhang et al., 2011).

TABLE 15.1 Summary of different nanoparticles synthesized by different microorganisms.

Types of nanoparticles	Microorganisms involved	Location of action	References
AgNPs	<i>Fusarium oxysporum</i>	Extracellular	Ahmad et al. (2003a)
	<i>Aspergillus flavus</i>	Extracellular	Sadowski et al. (2008)
	<i>Aspergillus fumigatus</i>	Extracellular	Sadowski et al. (2008)
	<i>Verticillium sp.</i>	Extracellular	Sadowski et al. (2008)
	<i>Escherichia coli</i>	Extracellular	Gurunathan et al. (2009)
	<i>Trichoderma viride</i>	Extracellular	Fayaz et al. (2010)
	<i>Bacillus cereus</i>	Intracellular	Babu and Gunasekaran (2009)
	<i>Bacillus licheniformis</i>	Extracellular	Kalimuthu et al. (2008)
	<i>Phaenerochaete chrysosporium</i>	Extracellular	Vigneshwaran et al. (2006)
	<i>Corynebacterium glutamicum</i>	Extracellular	Sneha et al. (2010)
	<i>Pseudomonas strutzeri</i>	Extracellular	Sadowski et al. (2008)
	<i>Enterobacter cloacae</i> (Enterobacteriaceae)	Intracellular	Shahverdi et al. (2007)
	<i>Klebsiella pneumonia</i>	Intracellular	Shahverdi et al. (2007)
AuNPs	<i>Rhodopseudomonas capsulate</i>	Extracellular	He et al. (2007)
	<i>Bacillus subtilis</i>	Intracellular	Beveridge and Murray (1980)
	<i>Shewanella oneidensis</i>	Extracellular	Suresh et al. (2011)
	<i>Aspergillus fumigatus</i>	Extracellular	Sadowski et al. (2008)
	<i>Colletotrichum sp.</i>	Extracellular	Sadowski et al. (2008)
	<i>Thermomonospora sp.</i>	Extracellular	Mukherjee et al. (2002)
	<i>Fusarium oxysporum</i>	Extracellular	Ahmad et al. (2003b)
	<i>Verticillium sp.</i>	Intracellular	Mukherjee et al. (2001)
	<i>Rhodococcus sp.</i>	Intracellular	Ahmad et al. (2003a)
	<i>Pseudomonas fluorescens</i>	Extracellular	Rajasree and Suman (2012)
	<i>Alternaria alternata</i>	Extracellular	Sarkar et al. (2012)
	<i>Aspergillus niger</i>	Extracellular	Bhambure et al. (2009)
	<i>Candida utilis</i>	Intracellular	(Gericke and Pinches, 2006)
	<i>P. aeruginosa</i>	Extracellular	Husseiny et al. (2007)
Au-Ag alloy	<i>Fusarium oxysporum</i>	Extracellular	Senapati et al. (2005)
	<i>Fusarium semitectum</i>	Extracellular	Sawle et al. (2008)
	Yeast cells	Extracellular	Zheng et al. (2010)

(Continued)

TABLE 15.1 (Continued)

Types of nanoparticles	Microorganisms involved	Location of action	References
CdSNPs	<i>Clostridium thermoaceticum</i> <i>Klebsiella pneumoniae</i> <i>Escherichia coli</i> <i>S. glabrata</i> <i>S. pombe</i>	Extracellular Extracellular Intracellular Intracellular Intracellular	Li et al. (2011) Li et al. (2011) Sweeney et al. (2004) Li et al. (2011) Kowshik et al. (2002)
Fe ₃ S ₄ NPs	<i>Uncultured magnetotactic bacteria</i>	Extracellular	Li et al. (2011)
ZnS PbS	<i>Rhodobacter sphaeroides</i> <i>Rhodobacter sphaeroides</i>	Intracellular	Bai and Zhang (2009) Bai et al. (2006)
FeSNPs	Sulfate-reducing bacteria	Extracellular	Watson et al. (1999)
PdNPs	<i>Desulfovibrio desulfuricans</i>	Extracellular	(Lloyd et al., 1998)
PtNPs	<i>Shewanella algae</i>	Intracellular	(Konishi et al., 2007)
SeNPs	<i>Shewanella sp</i>	Extracellular	Lee et al. (2007)
HgNPs	<i>Enterobacter sp</i>	Intracellular	Sinha and Khare (2011)
CdTeNPs	<i>Escherichia coli</i>	Extracellular	Bao et al. (2010)
TiO ₂ NPs SiO ₂ NPs ZrO ₂ NPs CdSeNPs SrCO ₃ NPs	<i>Fusarium oxysporum</i>	Extracellular	Bansal et al. (2005) Bansal et al. (2005) Bansal et al. (2004) Kumar et al. (2007) Rautaray et al. (2012)
Sb ₂ O ₃ NPs	<i>Saccharomyces cerevisiae</i>	Intracellular	Jha et al. (2009a,b)
Zn ₃ (PO ₄) ₂ NPs	Yeast cells	Extracellular	Pandian et al. (2009)
Fe ₃ O ₄ NPs FePO ₄ NPs	<i>Shewanella oneidensis</i> Yeast cells Yeats cells	Extracellular Extracellular Extracellular	(Perez-Gonzalez et al., 2010) He et al. (2009) He et al. (2009)
TiO ₂ NPs	<i>Lactobacillus sp.</i> <i>Fusarium oxysporum</i>	Extracellular Extracellular	Jha et al. (2009,b) Bansal et al. (2005)
BaTiO ₃ NPs	<i>Fusarium oxysporum</i> <i>Lactobacillus sp.</i>	Extracellular Extracellular	Bansal et al. (2006) Jha et al. (2009,b)
Fe ₃ S ₄ NPs	<i>Multicellular prokaryotes</i>	Intracellular	Lefèvre et al. (2010)

Among all the aforementioned microbes, bacteria has been identified as one of the regulators capable of behaving as ecofriendly catalysts for the synthesis of different materials along with catalyze the process of bioremediation. One of the many bacterial genera is *Shewanella* has been recorded to synthesize metal oxide via the process of microbial respiration. Monitoring the diverse species of *Shewanella*, it has been observed that *S. oneidensis* Mr-1 and *S. loihica* PV-4 bear the potential to carry out Pd NP biosynthesis having increased catalytic activities. Comparatively, *S. oneidensis* Mr-1 individually is held responsible for mediating the biosynthesis of not only the Te NPs in the form of rod formulations by using soluble, toxic tellurite with the ability of accepting electrons but also Cu NPs via Cu (II) bio-reduction and enhanced antibacterial activity possessing Ag NPs. Te nanorods can also be extracellularly and intracellularly synthesized as crystalline structures in the shapes of needle. Bio-reduction process extracellularly catalyzed by genus *Shewanella* leads to the production of flavins and nanowires. Moreover, Gram-negative bacteria, under aerobic environment also contributes towards the formation of biogenic selenium NPs. While on the other hand, in cases of dissimilatory microbial anaerobic respiration, the organic compounds release electrons thus getting reduced and these released electrons are accepted by inorganic compounds which get oxidized. This in turn facilitates the process of bioremediation in association with crystal formation. *Shewanella* has been found to actively participate in this process by functioning as both electron donors as well as electron acceptors. Another significant study has revealed that *Enterobacter cloacae* MF₀₁ crude extracts promote the biosynthesis of NPs containing tellurium and gold elements under both anaerobic as well as aerobic environment. Other than bacteria, fungi has also been employed for NP synthesis and in fact,

with time it has been explored that fungal instrumentation is preferred over all other microbial systems for the same. This is majorly because of the fact that presence of mycelial mesh in fungi is characterized by high resistance to rapid agitation and higher rate of flow when put in any bioreactor. Further investigation on this matter disclosed that since fungal cell wall is composed of chitin, it gets indulged in synthesizing diverse NPs by forming heavy metal complexation. Additionally, it has also been proposed that fungal systems can be more resourceful as compared to bacterial systems for NP biosynthesis owing to the presence of numerous metabolites that provides a few implicit benefits including increased efficacy along with stronger and robust aggregation. Furthermore, it has been presumed that the compounds and various other media elements secreted by fungi results in stabilization of the synthesized NPs. Accordingly, some filamentous fungi are believed to promote biosynthesis of Au NP in particular. Another type of widely biosynthesized NP is silver NPs (Ag NPs). The Ag NPs can be formulated in various ways such as *Fusarium Chlamydosporum* NG₃₀ and *Penicillium chrysogenum* NG₈₅ successfully assist in formulating Ag NPs where cell-free lysate, containing several proteins and enzymes, is derived from autolyzed biomass. The involved proteins and enzymes regulate the formation of silver atoms from silver ions through reduction which are henceforth stabilized by capping via proteins serving as capping agents. The other process involves *Phomopsis liquidambaris* whereby extracellular filtrate is basically separated out from the healthy leaves of *Salacia chinensis* consisting of proteins that exhibits the potential of acting as a capping agent and carries out reduction, hence formulating Ag NPs that possess bactericidal activity against any pathogen (Ghosh et al., 2021) (Fig. 15.1).

- a. Silver NP synthesis—There are several published scientific literature studies that focuses on the biosynthesis of silver NPs primarily using *Fusarium oxysporum* due to the characteristic properties of cell mass along with the leached components capable of reducing the concentration of silver ions in silver NPs, thus rendering it less harmful for the nature. One such study highlights the fact that on exposure of aqueous silver ions to the fungus *Fusarium oxysporum*, they were reduced to a dimensional range of 5–15 nm. They get stabilized by the fungus-secreted proteins, particularly reductase release, in the solution which establishes a NP formulation of an extraordinarily stable silver hydrosol (Ahmad et al., 2003a). Through in silico studies, it has been found that silver NPs synthesized from *F. oxysporum* also proved to be effective as a strong antibacterial agent against selected pathogens including *Pseudomonas aeruginosa* and *Escherichia coli* (Srivastava et al., 2019). Another study reveals that bacteria *Pseudomonas strutzeri* which are predominantly available among materials of silver mining, is quite effective in reducing the size of silver ions. This mediates the accumulation of silver NPs into a size range of 16–40 nm with an average of 40 nm in most cases. Extracellular silver NP synthesis can also be carried out using *Aspergillus fumigatus* and *Colletotrichum* sp. Another novel two-step method of silver NP production using *Vericillum* was proposed where the first step involves silver ion trapping on fungal cell surface followed by the second step in which the cellular enzymes reduce the silver ions (Sadowski et al., 2008). The reduced forms of silver ions in the formation of silver NP can also be formed by some other microorganisms like *Escherichia coli*, *Enterobacter cloacae* (Enterobacteriaceae) and *Klebsiella pneumonia*. In this process of NP formation, the silver ions were exposed to cell filtrate which immediately facilitated the formation of silver particles within 5 minutes. Moreover, it has also been found that the process of silver ion reduction by Enterobacteriaceae can be partially inhibited by piperitone (Shahverdi et al., 2007).

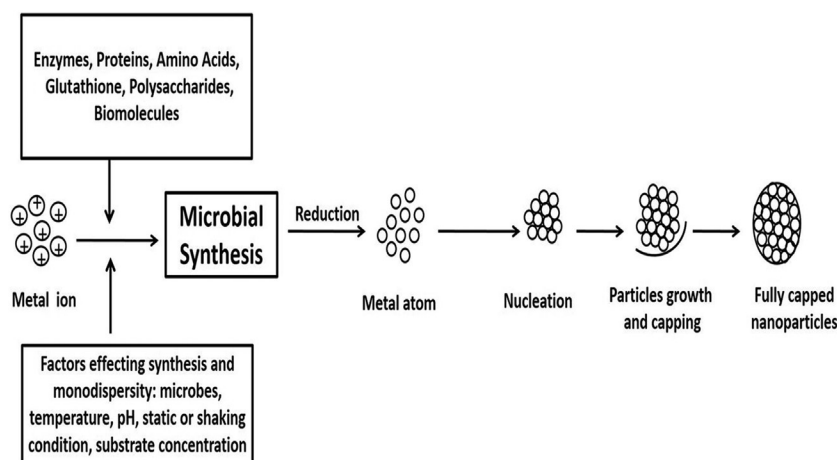


FIGURE 15.1 Schematic diagram showing the mechanistic synthesis of nanoparticles using microbes (Ghosh et al., 2021). From https://www.frontiersin.org/files/Articles/638068/fmicb-12-638068-HTML/image_m/fmicb-12-638068-g001.jpg.

- b. Gold NP synthesis—The administration of AuNPs dates back to 150 years when Michael Faraday discovered that colloidal gold solution is distinguishable from bulk gold in properties. Biosynthesis of different sized and shaped gold NPs depending on the pH is mediated by a bacterium *Rhodospseudomonas capsulata*. The process involves incubation of aqueous HAuCl₄ solution and R. capsulate biomass at pH values of range 4 to 7 which resulted in the formation of nanoplates at pH 4 and 10–20 nm spherical shaped gold NPs at pH 7 (He et al., 2007). Additionally, *Bacillus subtilis* 168 (Beveridge and Murray, 1980) and *Shewanella algae* are utilized in the reduction of gold ions and synthesis of 5–25 nm and 10–20 nm gold NPs respectively. Along with other microorganisms, extracellular gold NP synthesis can also be carried out using *Aspergillus fumigatus* and *Colletotrichum* sp. (Sadowski et al., 2008). Moreover, actinomycete *Thermomonospora* sp. and fungus *Fusarium oxysporum* facilitate extracellular synthesis of gold NPs (Mukherjee et al., 2002, Ahmad et al., 2003c) whereas fungus *Verticillium* sp. mediate intracellular gold NP synthesis (Mukherjee et al., 2001). Certain alkalotolerant microbes such as *Rhodococcus* sp. under extreme conditions of alkalinity and slightly high temperatures play vital role in synthesis of monodisperse gold NPs with 5–15 nm size range (Ahmad et al., 2003b). Intriguingly, marine microbiome has been collectively referred to as “nano-factories” because of its potential to engage in gold NP synthesis. Among all other marine bacterium, RS 11 strain of *Marinobacter pelagius* which is a Gram-negative and nonendospore forming bacteria was found to synthesize gold NPs with much efficiency. Other marine microbial strains include RS15 (*Halomonas ventosae*), RS8 (*Marinobacter pelagius*), LD17 (*Alteromonas macleodii*), LD16 (*Alteromonas* sp.), LD14 and LD4 (*Pseudoalteromonas mariniglutinos*), K20 (*Marinobacter salsuginis*), K4 (*Halomonas ventosae*), M6 (*Photobacterium ganghwense*) and PR5 (*Marinobacter vinifermus*) (Sharma et al., 2012). Cell supernatants of bacteria *P. aeruginosa* ATCC 90271 was found to reduce gold ions and form AuNPs in the range of 15–30 nm (Husseiny et al., 2007). Treating culture supernatant of *Aspergillus niger* NCIM 616 with aqueous gold ions resulted in the production of gold NPs with an average size of 12.79 ± 5.61 nm extracellularly (Bhambure et al., 2009). AuNPs are also synthesized by chloroauric acid bio-reduction (HAuCl₄) utilizing the fungal culture filtrate of *Alternaria alternata* (Sarkar et al., 2012). Moreover, a highly stable Gram-negative soil bacterium *Pseudomonas fluorescens* is employed for the extracellular synthesis of gold NP. Gold NPs synthesized from *P. fluorescens* are quite effective considering the act that *P. fluorescens* can produce a diverse range of antibiotics including 2,4–diacetylphloroglucinol, pyrrolnitrin, pyoluteorin along with pyoverdine, pyochelin and hydrogen cyanide. All of these synergistically suppress or inhibit the target pathogens present in the rhizosphere through competition for iron absorption (Rajasree and Suman, 2012). Gold nanostructure can also be formed in different shapes such as spherical, octahedral and cubic from Au (III)-chloride and Au (I)-thiosulphate complexes by filamentous cyanobacteria (Lengke et al., 2006a,b).
- c. Synthesis of alloy NPs—Alloy NP synthesis is essential owing to the fact that they have higher applications in fields of electronics and catalysis as coatings and optical materials. It has been reported that bimetallic Au-Ag alloy NP can be synthesized by *F. oxysporum* (Senapati et al., 2005) as well as by yeast cells (Zheng et al., 2010). During the biosynthesis process, cofactor NADH is released which plays a crucial role in composition determination of Au-Ag alloy NPs (Senapati et al., 2005). Fungal strains such as *Fusarium semitectum* is also responsible for the biosynthesis of core-shell Au-Ag alloy NP which have been found to be quite stable for weeks (Sawle et al., 2008).
- d. Synthesis of sulfide NP—One of the most typical types of sulfide NPs is CdS nanocrystal which can be precipitated by *Clostridium thermoaceticum* on the surface of cells as well as in a medium containing CdCl₂ and cysteine hydrochloride where cysteine is assumed to function as a sulfide source. CdS nanocrystals of the size 20–200 nm can be formed by *Klebsiella pneumoniae* when exposed to medium containing Cd²⁺. Moreover, *Escherichia coli* is also capable of intracellularly synthesizing CdS nanocrystals that is mostly composed of phase of wurtzite crystal only when it is incubated with Na₂SO₄ and CdCl₂. Additionally, *S. glabrata* and *S. pombe* are efficient in forming intracellular CdS nanocrystals on exposure to cadmium salt solution. On the other hand, formation of Fe₃S₄ NPs is promoted by uncultured magnetotactic bacteria. Magnetic FeS NPs are produced by sulfate-reducing bacteria (Li et al., 2011). *Rhodobacter sphaeroides* are implemented for the intracellular synthesis of PbS and ZnS NPs (Bai et al., 2006, Bai and Zhang, 2009) of variable size and 8 nm, respectively.
- e. Synthesis of other NPs—There are several NPs other than the ones mentioned above which have been little indulged in research. For instance, TiO₂ and SiO₂ NPs are produced by *F. oxysporum* from aqueous anionic TiF₆²⁻ and SiF₆²⁻ complexes, respectively (Bansal et al., 2005). *F. oxysporum* also mediates the formation of quasi-spherical ZrO₂ (Bansal et al., 2004) and tetragonal BaTiO₃ of sizes 3–11 nm and 4–5 nm respectively (Bansal et al., 2006). Biosynthesis of Sb₂O₃ NPs of size 2–10 nm is promoted by reproducible and low cost green *Saccharomyces cerevisiae* (Jha et al., 2009a,b). In association with these, a diverse range of other microbes are also involved in the synthesis of PHB, PbCO₃, SrCO₃, CdCO₃, CdSe and Zn₃(PO₄)₂ NPs. CdSe NPs in the form of high luminescence dots are synthesized by *F. oxysporum* (Kumar et al., 2007). Yeasts have been demonstrated to synthesize Zn₃(PO₄)₂

nano-powders as bio-templates which normally takes the shape of butterfly-like microstructure of 80–200 nm in length and 10–80 nm in width (Pandian et al., 2009). On incubating fungi *F. oxysporum* with aqueous Sr^{2+} ions, SrCO_3 crystals were obtained (Rautaray et al., 2012).

15.3 Nanoparticles as an aid towards sustainable agriculture

The core reason for finding alternatives for sustainable agriculture is the detrimental impacts posed by currently employed chemical pesticides, fertilizers, fungicides, herbicides and other chemical rich ingredients as a demand to excessively increase the annual crop yield that the respective crop plants do not have the ability to harvest under such conditions. To add, reports have very well indicated a possibility of food scarcity by 2050 considering the overwhelming population, if proper preventive measure are not being taken in order to promote sustainable agriculture. The question that arises is to why put so much effort into sustainable agriculture? It can be simply answered by stating the fact that the fundamental existence of higher order animals, human beings and every other creature on this planet solely depends on these autotrophic plants without which living would become extremely difficult. Crop plants are mostly on the verge of getting infected by various abiotic and biotic factors including alarming pollution rate in the environment, climatic change, nutrient scarcity, pathogens, pests or disease-causing organisms, weeds, to name a few. All these can combinedly lead to a significant damage to the plant health along with a critical reduction in the crop productivity and fruit and flower yield. As a result, it becomes our chief concern to maintain and balance plant health as well as increase the productivity per annum. Normally, the application of pesticides and fertilizers aims at providing benefits and protecting the plant kingdom by keeping the disease-causing pathogens and pests at bay as well as to provide essential nutrients so as to facilitate increased plant growth, development which in turn leads to enhanced crop yield. However recently, it has been evident that instead of benefitting the plants, these chemically synthesized fertilizers and pesticides, on overuse, are causing lethal and damaging effects on plants that adversely impacts the health of not only the plant biosystem but also the other beings (animals and human) following the food chain after photosynthetic plants due to bioaccumulation and biomagnification. It has also been found that certain pathogens undergo certain mutations on their own which have allowed themselves to tolerate the applied chemicals and therefore, these chemicals have gradually started to make less or no impact on these pests or pathogens. Conclusively, many countries have so far banned the usage of chemically produced pesticides and fertilizers while others still continue to permit their utilization. In the meanwhile, agriculturists and plant-related scientists have been attempting to create new formulations that can be administered to plants as fertilizers and pesticides and can cause minimum toxicity to plants, however, through the process it has been realized that constructing a near-to-perfect composition of fertilizers and pesticides is quite difficult. Even in the presence of adversities, it has been possible to employ the emerging nanotechnology to pave a way for sustainable agriculture in the near future. Nanotechnology has been quite flourishing in various sectors including pharmaceutical, cosmetics, food, etc., and has been extended to agricultural industry where implementation of produced NP-mediated fertilizers and pesticides has not only been limited towards pest control but also been engaged in rising stable food production globally, bare-minimum waste production, plant growth and development promotion along with food quality enhancement (Bhowmik and Gupta, 2017). Till date, NPs that have been widely studied and put under trials are in the form of nanofertilizers, nanopesticides which have been again subdivided into classes that include nanonematicides, nano herbicides, nano fungicides, and nano insecticides.

15.3.1 Nanoparticle-encapsulated fertilizers

As reported by the Food and Agricultural Organization of the United Nations, the demand for fertilizers is about to suffer a major demand which prompted to the discovery and production of eco-friendly nanofertilizers but their application is still restricted to the systematic machineries before final administration to local farms. The principal aim behind the formulation of these nanofertilizers is to provide with a substitute that can not only ensure a potential increase in the soil nutrient (nitrogen and phosphorous, in particular) quantity and quality (Dimkpa et al., 2017) but also can contribute to a significant growth in nutrient supply to the plants even in the presence of minimally applied fertilizers (Rameshaiah, Pallavi and Shabnam, 2015). The manufacturing of these nanofertilizers is quite simple in nature. The fertilizers are usually encapsulated with NPs that help in governing the gradual supply of nutrients to the targeted plants as and when required (Singh, Singh and Prabha, 2016). Along with maintaining a proper balance of nutrients in the soil and plant biosystems, it also causes minimum toxicity and degradation to the surrounding ecosystems. Highly ordered and volume of pores accompanied by large surface area/volume size ratio are few of the specialized characteristic

properties of nanofertilizers (Patil et al., 2011). Nanofertilizers have been chiefly categorized into three diverse forms (Chhipa, 2017):

- a) Nanoparticulate nanofertilizers—This type of nanofertilizers normally consist of a combination or exclusive NPs such as SiO_2 and TiO_2 and carbon nanotubes (CNTs). CNTs along with mesoporous silica NPs (Patil et al., 2011) are being administered in order to achieve efficient nutrient delivery to soil, germinating seeds, young as well as mature plants. In addition, CNTs have been prominently observed to take part in enhancing water absorption and its retention through an experiment where seeds of tomato plants were kept in incubation for two days after inoculating with CNTs. The result showed a peculiar rise in the percentage of moisture content level, an approximate of 19%, in comparison to the tomato seeds that were not treated with CNTs. While investigating the mechanism involved, it was theoretically speculated that perhaps, CNTs interact with aquaporins (AQPs) which are the core membrane protein regulators of water uptake and retention in the cell membrane and are present in the seed coats. This interaction is presumed to induce the formation of microspores in the proximity of cell membrane (Sanborn et al., 2018) and influences the permeation of water channels which has affected the growth of plants as well as seed germination (Khodakovskaya et al., 2009), however, the exact practical mechanism is not yet known. SWCNTs (single-walled CNTs) are sometimes applied to plants where they penetrate and accumulate near the lipid layer of the targeted plant followed by effecting the electron transport system affirmatively in sync with amplified photoabsorption pathway. This has shown to facilitate photosynthesis rate in such a way that it increased three times as compared to controlled conditions (Giraldo et al., 2014). Another carbon coated nanostructure with conical shape is carbon nanohorn which are one-dimensional, single-walled and very similar to SWCNTs and hence, they are also known as SWCNHs (Karfa et al., 2019). Treating a diverse array of six varied species of crop plants in cell cultures including tomato, barley, wheat, rice, tobacco, switchgrass, maize and soybean with SWCNHs regulated a remarkable acceleration in seed germination (Milewska-Hendel et al., 2016). On the other hand, the most studied and applied form of nano-fertilizer is TiO_2NP due to its exceptional quality of photocatalytic activity as well as its contribution towards activating redox reactions associated with transfer of charge between TiO_2NPs and light harvesting complex II (Ali, Mehmood and Khan, 2021). It also been observed to enhance growth, germination of seeds and efficient nitrogen fixation cycle mediated by combined application of $\text{TiO}_2/\text{SiO}_2\text{NP}$ has been observed in soyabean plants specifically (Dimkpa and Bindraban, 2016). $\text{TiO}_2/\text{SiO}_2\text{NP}$ administration accelerates the formation of nitrite (NO_2^-) from nitrate (NO_3^-) by reduction though an improved functioning of nitrogen reductase along with enhanced potential of the plants to absorb water and fertilizers applied (Ali, Mehmood and Khan, 2021). SiO_2 alone, in tomato plants, has been reported to facilitate accumulation of proline followed by improving fresh along with dry weight of leaves and content of chlorophyll under conditions of high NaCl concentration. It also plays a major role in seed germination as observed in tomato (Mushtaq et al., 2019). On application of nano-coated TiO_2 at a concentration of 10ppm on wheat in comparison to controlled conditions, it has been reported that it took the slowest duration of germination and comparatively, at a concentration of nano-based TiO_2 ranging within 2–10 ppm showed a prominent increase in the length of seedling and shoot (Feizi et al., 2012). Similarly, administration of properly concentrated nano-based TiO_2 has been recorded to induce seed germination in mature spinach plants. Conversely, overapplied nano- TiO_2 concentrations implied null or inhibitory effect on wheat (Zheng et al., 2005). Therefore, from all these observation sit can be concluded that nano-coated TiO_2 can be of worth only when administered in correct proportions and its overutilization can indeed have adverse effects on the health and ecological balance of soil and plant systems. However, CNT application on *Phaseolus mungo* and *Brassica juncea* has shown to parallelly increase the length of root and root hair quantity at a concentration of 20 ppm but increasing the concentration to 40 ppm leads to a modest decrease in the quantity of root hairs whereas it results in a 100% of seed germination at any concentration (Milewska-Hendel et al., 2016). Furthermore, TiO_2NPs have been demonstrated to promote accumulation of nitrogen and in turn accelerates root growth, increases absorption of light which further enhances the transport of light energy along with transformation by regulating LHCIIb gene expression and prevents premature ageing of chloroplasts. Specifically in spinach plants, TiO_2NPs have been found to improve light absorbing by positively regulation the action of Rubisco activase class of enzymes accompanied by providing shield against oxidative stress produced from ultraviolet radiation emitted during the process of photosynthesis (Lyu et al., 2017). Not only this, these TiO_2NPs also exist in the form of anatase crystals which on application has been found to influence the synthesis of carotene and chlorophyll in the species of *Cucumis sativus* (Ali, Mehmood and Khan, 2021).
- b) Micronutrient nanofertilizers—Micronutrient nanofertilizers are nothing but the encapsulated forms of the well-known plant micronutrients including copper (Cu), nickel (Ni), zinc (Zn), iron (Fe), molybdenum (Mo) and manganese (Mn), with NPs. This type of nanofertilizers have been recorded to work most efficiently when applied in

combination, for instance, the composite form of CuO, Be₂O₃ and ZnO which has the ability to provide resistance during drought stress as studied in soyabean model of plants and thus further lending a helping-hand in fortification. Zn is considered as one of the most essential plant micronutrients which makes its application of utmost importance. The most utilized nano-coated form of zinc in agriculture is zinc oxide (ZnO) and has been quite effective in enhancing numerous factors. To begin with, it has been well associated with increased chlorophyll content in peanuts (Prasad et al., 2012). ZnO NPs have also been observed to reduce the parameter values of energy flux (TR0/RC, ET0/RC, ABC/RC, DI0/RC) in addition to boosting the kinetics of PSII fluorescence (PIABS, Phi-E0 and Psi-E0) provided that an overall increment in the quantitative and qualitative features of crop plants, especially in rice (Singh, Prasad and Singh, 2018). Particularly in tomato plants, implementation of ZnO NPs have been observed to upregulate carbonic anhydrase action in accompany with increased chlorophyll content and photosynthesis rate (Faizan et al., 2018). Summarizing, ZnO NPs have been found to be involved in providing support for tolerance during stress conditions by revamping the functions of antioxidant enzymes including peroxidase, glutathione reductase, superoxide dismutase, catalase and nitrate reductase, render efficacy to plants in accumulating adequate amount of nutrients and water for sequential metabolic functioning apart from free proline and other amino acids (Elsakhawy et al., 2018). Fe is another micronutrient that has been comprehensively researched and administered on plants in the form of iron (II) sulfate (FeSO₄). Its foliar application on cultivars of sunflower has extensively increased tolerance under extreme salinity conditions. In addition to amplifying the leaf area, iron and chlorophyll content, CO₂ concentration in the substomatal cavity, highest photochemical efficacy of photosystem II (Fv/Fm), dry shoot weight, it also adversely diminished sodium content in the leaf region (Torabian, Farhangi-Abriz and Zahedi, 2018; Gowayed, Al-Zahrani and Metwali, 2017). Another form of applied nano-sized Fe is iron oxide NPs (IONPs) which as evidence have shown to play significant roles in different activities. For instance, it uniquely regulates dissolution of iron which in turn enhances elongation of roots in pumpkin while comparatively, it boosts the dry weight of leaves and pods of soybean. Intriguingly, they also escalate iron photosynthate transfer in leaves of peanut, thus proving themselves to be effective facilitators of iron (Ali, Mehmood and Khan, 2021). Alongside, CuO NPs applied as nanofertilizers are observed to get easily translocated from the roots passing through the xylem to shoots as well as back to roots through the phloem system (Wang et al., 2012). Additionally, foliar spraying of MnO NPs resulted in an upregulation of the contents of pigments involved in photosynthesis along with improvement in the characteristics of vegetative growth, fruits and thus improvising on the overall yield (Shebl et al., 2019). Hence, it can be concluded that formulations of micronutrient nanofertilizers applied in formulations increases efficacy of nutrient supply and thereby, enhancing its bioavailability as well as has regulated lesser degradational loss in the environment. Often, it has been observed that these are required in minimal quantities to promote maximum functioning in plants (Dimkpa and Bindraban, 2016).

- c) **Macronutrient nanofertilizers**—Macronutrient nanofertilizers are those composed of a diverse range of elements including magnesium (Mg), phosphorous (P), calcium (Ca), potassium (K) and nitrogen (N) (Ditta and Arshad, 2016). These are manufactured by encapsulating one, two or more specific nutrients with very particular nanomaterials. Keeping in mind the benefits required to be provided by these nanofertilizers, these have been either administered exclusively or in combination. This type of nanofertilizers is being crafted in an aim to accelerate adequate uptake of nutrients and their utilization by plants along with added advantages of minimal transportation and purchase costs (Chhipa, 2017). Foliar application of Ca NPs in association with humic acids on peanuts expressed an approximate 30% improvement in seedling growth followed by a probable 5.78 g increase in dry biomass (Xiumei et al., 2005). A more synthesized, complex form of applied calcium NPs with 16 nm size is the hydroxyapatite NPs [Ca₅(PO₃)₃] was applied on soybean (*Glycine max*) plant. The comprehensive results cleared showed an overall increase in the growth and yield of the plant (Liu and Lal, 2014). Together with hydroxyapatite NPs, mesoporous silica and urea-modified zeolites NPs delivered as a source of nitrogen have shown promising effectiveness as a control/slow release nanofertilizers (Liu and Lal, 2015; Monreal et al., 2016). Similarly, foliar application of Mg NPs in addition to Fe NPs upgraded potential of photosynthesis along with increased seed weight which in turn improved yield in black-eyed peas (*Vigna unguiculata*) (Delfani et al., 2014). Phosphorous applied in the form of biosafe nano-fertilizer phosphate has been nano-structurally prepared phosphorite suspension in water of the particle size ranging between 90–120 nm. This phosphatic suspension was the first nanofertilizer obtained from the raw phosphorite deposits of Tatarstan's Syundyukovskoe via ultrasonic material dispersion (Patra et al., 2016).

In general, nanofertilizers are applied either directly to plants or indirectly to soil from where they are translocated to the plant systems. This application is often administered in either of the two ways: foliar spray and root entry.

Among these, foliar spray can be carried out at any point of time, even during the unfavorable climatic and soil conditions. One of the most important points to be considered here is the fact that foliar spray reduces unnecessary wastage of nanofertilizers provided that it gets directly absorbed by plants via leaf system. Thereby, increasing nutrient use efficiency and promoting crop growth and productivity are some of the valid advantages to be noticed. However, this allowance of nanofertilizer entry into the plant systems via the stomata, lenticels, through cuticle, hydathodes or some surface wounds if present and this integrally depends on the leaf stromal openings, trichome base and the nano-pores itself induced by these applied nanofertilizers which dominantly regulates nanofertilizer absorption and translocation. Conversely, nanofertilizers applied to the soil in close association with the plant roots have been estimated to lead to a small amount of nanofertilizer loss due to indirect contact with the plant system. This entry through the roots is also primarily governed by spray technique. On nanofertilizer spray on the soil, these nanofertilizers begin interacting with the root system, predominantly by inducing pores surrounding the root region such as root hairs, root tips, rhizodermis and lateral roots which permit root entry of fertilizers (Kumar and Nagesh, 2019). One noticeable boon of root entry is that it allows passage for greater nutrient uptake in a shorter period of time because of the pores formed in comparison to chemically synthesized or traditional fertilizers or manures. Subsequently after root entry, these nanofertilizers travel apoplastically or symplastically until they reach the xylem vascular system from where they get distributed to different aerial parts such as stem and leaves (Sun et al., 2016). On the other hand, the nanofertilizers applied through foliar spray follow the symplastic or apoplastic pathway through the plasmodesmata of various cells till they reach the cytoplasm from where they get allocated to separate organelles for their participation in abundant metabolic pathways (Rai et al., 2015).

Nanofertilizers, also labeled as smart fertilizers have nowadays gained popularity which has magnificently unfolded their utilization over the traditional fertilizers or manures (Dimkpa and Bindraban, 2018). Provided the indistinctive features exhibited by NPs including release of kinetics in controlled manner along with sorption capacity and high surface-to-volume ratio, nanomaterials-coated fertilizers are consistently emerging as one of the promising substitutes of manures or conventional fertilizers (Feregino-Perez et al., 2018). On record, extrinsic as well as intrinsic factors in addition with exposure route simultaneously and equally determine the efficiency of nanofertilizer absorption by the plants, accumulation and translocation within the plants. To list the extrinsic factors, it most importantly includes pH and texture of soil and composition of organic matter in soil whereas intrinsic parameters largely involve the structural architecture of NPs and their efficacy rate on administration (El-Ramady et al., 2018; Ma et al., 2018). As an initiative to reduce the pressure of adequate nutrient-rich and healthy food production on the agricultural sector, the application of NPs in the form of nanofertilizers was proposed which is indeed proving to be tremendously beneficial so far only when implemented in the appropriate proportion. Till date, nanofertilizer administration has been witnessed to allow slow or gradual release of nutrients from the soil biosystem into the plant (Rai et al., 2015) which has been advantageous to a great extent in terms of controlled and consistent, long-term availability of nutrients, thus ensuring a substantial growth in crop productivity. For instance, supply of the same amount of nutrients for a longer period of time, around 40–50 days by means of nanofertilizers rather than a shorter period of delivery like 4–10 days with traditional fertilizers (Chen and Wei, 2018). Besides, controlled nutrient supply has also prevented the clogging of soil due to the excessive presence of salts from applied fertilizers and henceforth, facilitates better interaction between soil, micro-organisms and plants. This has also further upregulated nutrient uptake given that the root hairs get timely exposed to sufficient nutrients and has led to minimal wastage of fertilizers lost because of leaching. As already well-known, individual plant species respond to various macro- and micro-nutrients differently (Srivastava and Malhotra, 2017) and so, production of nanofertilizers provides an opportunity to craft each nanofertilizers as per the need of the plant species in question during seed germination, growth and development of different plant parts (Kah et al., 2018). Nanofertilizers were also administered in six week old plants to estimate their effects on the normal activities of enzymes like acid phosphatase and dehydrogenase, chlorophyll content, total soluble leaf protein and dry biomass. On application of ZnO NPs on *Cicer arietinum* showed an increase in the level of indole acetic acid in the root region which in turn enhanced the growth rate of the plant species (Yadav, 2021). Though it might be hectic considering a large-scale production but looking at the added advantages of decreased application and transportation expense can nullify other disadvantages to some extent. Reports indicate that much of the agricultural lands invested in horticultural farming suffer a deficiency of nutrients, particularly iron and zinc among many others. Hence, balanced application of nanofertilizers is expected to act as savior in the future years with all its effectiveness that have been evident until now. They are also hoped to function as fortification elements to elevate the manufacturing of ample quantity of food crop and other agricultural products which, in turn, would cater towards a flourishing industry for sustainable agriculture.

TABLE 15.2 List of different nanofertilizers and their applications on various crops.

Nature of nanofertilizers	Crop species	Effect on crop species	References
TiO ₂ /SiO ₂	<i>Glycine max</i>	- Accelerates nitrite (NO₂⁻) from nitrate (NO₃⁻) formation by reduction through an improved functioning of nitrogen reductase. - Enhances potential of the plant to absorb water and fertilizers applied. - Enhances growth, germination of seeds and efficient nitrogen fixation cycle.	Ali, Mehmood and Khan (2021); Dimkpa and Bindraban (2016)
SiO ₂	<i>Solanum lycopersicum</i>	- Facilitates proline accumulation. - Improves fresh and dry weight of leaves and chlorophyll content under conditions of high NaCl concentration. - It also plays a major role in seed germination	Mushtaq et al. (2019)
TiO ₂	<i>Spinacia oleracea</i> <i>Triticum</i> <i>Cucumis sativus</i>	- Induces seed germination. - Improves light absorption by positively regulating Rubisco activase. - Provides shield against oxidative stress produced from ultraviolet radiation emitted during the process of photosynthesis. - Implies null or inhibitory effect. - Influences carotene and chlorophyll synthesis	Zheng et al. (2005); Lyu et al. (2017); Zheng et al. (2005); Ali, Mehmood and Khan (2021)
CNT	<i>Phaseolus mungo</i> , <i>Brassica juncea</i> <i>Solanum lycopersicum</i>	- Increases root length and root hair quantity at a concentration of 20 ppm but increasing the concentration to 40 ppm leads to a modest decrease in the quantity of root hairs whereas it results in a 100% of seed germination at any concentration. - Enhances water absorption and its retention	Milewska-Hendel et al. (2016); Sanborn et al. (2018)
ZnO	<i>Arachis hypogaea</i> <i>Oryza sativa</i> <i>Solanum lycopersicum</i> <i>Cicer arietinum</i>	- Increases chlorophyll content. - Reduces the parameter values of energy flux (TR0/RC, ET0/RC, ABC/RC, DI0/RC) - Boosts the kinetics of PSII fluorescence (PIABS, Phi-E0 and Psi-E0) provided that an overall increment in the quantitative and qualitative features. - Upregulates carbonic anhydrase action - Increases chlorophyll content and photosynthesis rate. - Increases indole acetic acid (IAA) levels in the roots.	Prasad et al. (2012); Singh, Prasad and Singh (2018); Faizan et al. (2018); Yadav (2021)
FeSO ₄	<i>Helianthus sp.</i>	- Increases tolerance under extreme salinity conditions. - Amplifies leaf area, iron and chlorophyll content, CO₂ concentration in the substomatal cavity, - Promotes highest photochemical efficacy of photosystem II (Fv/Fm), dry shoot weight and adversely diminished sodium content in the leaf region	Torabian, Farhangi-Abriz and Zahedi (2018); Gawayed, Al-Zahrani and Metwali (2017)
FeO	<i>Cucurbita pepo L.</i> <i>Glycine max</i> <i>Arachis hypogaea</i>	- Enhances elongation of roots. - Boosts the dry weight of leaves and pods. - Escalates iron photosynthate transfer in leaves	Ali, Mehmood and Khan (2021)
CuO	<i>Zea mays</i>	Gets easily translocated from the roots passing through the xylem to shoots as well as back to roots through the phloem system	Wang et al. (2012)
MnO	<i>Cucurbita pepo L.</i>	- Upregulates pigment contents involved in photosynthesis - Improves the characteristics of vegetative growth and fruits	Shebl et al. (2019)

(Continued)

TABLE 15.2 (Continued)

Nature of nanofertilizers	Crop species	Effect on crop species	References
CuO/Be ₂ O ₃ / ZnO	<i>Glycine max</i>	• Provides resistance during drought stress. • Helps in fortification.	Prasad et al. (2012)
SWCNH	<i>Solanum lycopersicum</i> , <i>Hordeum vulgare</i> , <i>Triticum</i> , <i>Oryza sativa</i> , <i>Nicotiana tabacum</i> , <i>Panicum virgatum</i> , <i>Zea mays</i> and <i>Glycine max</i>	• Remarkably accelerates seed germination	Milewska-Hendel et al. (2016)
Ca/humic acids	<i>Arachis hypogaea</i>	• 30% improvement in seedling growth followed by a probable 5.78 g increase in dry biomass	Xiumei et al. (2005)
Ca ₅ (PO ₃)	<i>Glycine max</i>	• Overall increases growth and yield of the plant	Liu and Lal (2014)
Mg/Fe	<i>Vigna unguiculata</i>	• Upgrades photosynthesis potential • Increases seed weight.	Delfani et al. (2014)

15.3.2 Nano-structured pesticides

Nanopesticides are, as well, prepared through the same sequential stages like nanofertilizers. Nano-coated pesticides are often applied in forms of nano-fungicides, nano-nematicides, nano-herbicides, nano-emulsions and nano-insecticides. Among all the engineered NPs employed in agricultural sector, nanostructured alumina (NSA) is the finest example of nano-insecticides that performs similarly to an insecticide. NSA has been designed using advanced nanotechnology which involves metal oxidation demonstrating constant electric charge. The insecticidal property of NSA particle highly depends on sorbative feature, small size, large surface area and intrinsic electric charge. This provides an upper hand to NSA for interaction with insects that displays triboelectrification-mediated electric charges. By definition, triboelectrification depicts any kind of electrification or electricity generated through contact between two uncharged materials or elements as a result of friction produced between them. This remarkable phenomenon allows the anionic NSA particles to coherently interact by forming strong electrical bonding with the cationic charges present in insects. Extensive studies on insects have shown that they bear a fibrous cuticle layer consisting of chitin. The primary consequence of NSA and insect interaction is the detachment of fibrous cuticle layer of insects from them caused as a result of dehydration by the NSA particle, predominantly due to its characteristic sorption property. Attachment of NSA particle to the insect cuticle not only disturbs the water balance but also increases humidity as hypothetically speculation. This finally results in complete dehydration of the insect and thereby, leads to the elimination of all unnecessary insects harboring near the soil region. Hence, it can be said that the triboelectrification property of insects is taken into benefit by the NSA particle (converted into powdered form) which sequentially brings about all the mechanical changes (Stadler et al., 2018). Engineered nano-pesticide application is not limited to certain spheres anymore but has been recently extended to insect-resistant cotton (Bt cotton), developed in order to provide protection to the plants from bollworm, or conventional cotton. On comparative study between nano-pesticides and *Bacillus thuringiensis*-coded Bt toxin individually and in combination, it has been interpreted from the observations that presence of Cu NPs, applied in nano-pesticide form, has essentially enhanced exogenous microbial protein expression of Bt due to its insecticidal activity which, in turn, has been recorded to upregulate resistance of crops against bollworm in comparison to discrete applications (Zhao et al., 2020).

The major aim concerning the formulation of nano-pesticides is largely dependent on the parameters like nano-pesticide-soil insect interaction, effective penetration into the organism's body, potential target site delivery along with few other factors like cost-effectiveness, packaging and transportation ease, controlled release of nano-pesticides as required over a prolonged period of time, minimal risk of contamination, improved pest control and least cynical threats to the environment. Producing nano-pesticides facilitating all the aforementioned advantages can help overcome the cytotoxic and carcinogenic effects inflicted by conventional fertilizers on crop plants as well as the surrounding ecosystem. In an attempt, nano-silver has been administered to fight and protect crop plants against pathogens (Yasur and Rani, 2013). Nowadays, more advanced formulations of nano-pesticides are being manufactured from conventional pesticides to enhance effective pest control. These formulations are also termed as nano-carriers which primarily function as vehicles to regulate proper transportation and controlled release of the essentially active compounds over an extended time period.

which not only reduces the unnecessary usage of the compounds at the inappropriate time but also gives a chance to preserve an adequate quantity of these compounds while the insects undergo growth and development from larval to mature stage. Sometimes, these nanopesticides, employed as nanocarriers, are coated with polymers. These polymer-coated nanopesticides are quite robust in terms of polyesters and polysaccharide reservoirs. Over the years, they have relatively gained attention in regards to their flexibility owing to their design, biocompatible and biodegradable. Furthermore, these nanoformulations have likely increased the dispersion capacity in aqueous media that, in turn, influences the controlled delivery of pesticides in requisite manner (Nuruzzaman et al., 2016). Another type of NPs that has been recently evidenced to be used as pesticides is dendrimer NPs. Dendrimer NPs are a class of proprietary NPs belonging to the class of polymeric NPs but are an exception due to their unique properties. They are very precisely synthesized from branched layers globular molecules because of which the resultant dendrimers are monodisperse molecules. These are surface-modified by engineering numerous functional groups like NH_2 , COOH , OH or COONa on the outer surface which renders them to be specific in their action and function (Carvalho, Reis and Oliveira, 2020). These dendrimer NPs have been extensively exploited in the form of pesticide nanocarriers in order to improve effect of cytotoxicity of pesticides. PDI-core fluorescent dendrimers are the first examples of this type that have been administered on an insect model and are being explored as pesticide nanocarriers. This new kind is expected to pave ways for improved insecticidal action on targeted pests, enhanced effective pesticidal functions shown by nanopesticides in addition to minimal application of chemically synthesized pesticides or insecticides (Liu et al., 2015). Starpharma is one of the many global biopharmaceutical companies that believes in extending the application of nanotechnology in establishing a secured future by developing sustainable agriculture. They have been potentially utilizing dendrimer NPs for this purpose. In some of the studies performed, Priostar dendrimer technology of Starpharma has demonstrated quite fascinating results. It has been exclusively applied to enhance agrochemical delivery to plant systems that in turn has enabled plants to fight against several diseases and promote growth of healthier plants. In addition to increasing efficacy, controlled chemical delivery, lesser application frequency and quantity, such an innovation also promises to reduce impacts of such practices on environment and make the lives of worldwide farmers simplified in the long run (Biospace, <https://www.biospace.com/article/releases/starpharma-holdings-ltd-to-expand-agricultural-program-through-250-000-funding-/>). This business is being carried out in partnership with Agrium in order to develop better agrochemical formulations (Starpharma, <https://starpharma.com/news/318>).

Intriguingly, there are some other NPs which do not substantially act as nanocarriers but are quite fruitful in their solid form existence. Because of their characteristic pesticidal/antibacterial/insecticidal activities, such varieties of solid NPs including clay, silica and alumina in inert dust form have been employed as nano-structured pesticides. Among these, nano-structured type of alumina pesticides was discovered to be applied as an insecticide on two major grain pests, namely *S. oryzae* and *R. dominica* (Stadler, Buteler and Weaver, 2010). Nano-clays are being manufactured from molten ashes of volcanoes in forms of thin silicate sheets which have been found to potentially control the release of 2,4-dichlorophenoxyacetate bin (Bin Hussein et al., 2005). These solid nanopesticides mechanistically function by damaging the inner wax coating of the cuticle layer of the insects which leads to desiccation and water loss and therefore finally resulting in death of toxic insects (Shah and Khan, 2014). For instance, Si NPs, through physisorption gets attached to the lipids and act on the epidermal layer, and thereby causing damage to it and finally, death (Barik, Sahu and Swain, 2008). This technique contributes to an additional advantage which pertains to the fact that this mechanism restricts the insects from developing any kind of resistance against these nano-pesticides thus ensuring confirmed death of the insects. Undoubtedly, nanopesticides claim to the next generation varieties of conventional pesticides with the additional benefits of maximum benefit with minimal pesticide application, durability and high efficiency (Abd-Elsalam, 2015).

TABLE 15.3 List of nanopesticides and their effectiveness on different insects, pests and unnecessary microbes in the soil.

Nature of nanopesticides	Effectivity	References
Nanostructured alumina (NSA)	Mediates detachment of fibrous cuticle layer of insects as a result of dehydration due to its sorption property. Attachment of NSA particle to the insect cuticle disturbs the water balance and increases humidity as hypothetically speculation. This finally results in complete dehydration of the insect and thereby, leads to the elimination of all unnecessary insects harboring near the soil region	Stadler et al. (2018)

(Continued)

TABLE 15.3 (Continued)

Nature of nanopesticides	Effectivity	References
Cu	Enhances exogenous microbial protein expression of Bt due to its insecticidal activity which, in turn, upregulates crop resistance against bollworm	Zhao et al. (2020)
PDI-core fluorescent dendrimers	- Improves insecticidal action on targeted pests - Enhances effective pesticidal functions - Reduces application of chemically synthesized pesticides or insecticides	Liu et al. (2015)
Si	- It gets attached to the lipids and act on the epidermal layer through physisorption, causes damage to it and finally, death - It is administered as nanocarriers due to its property of pH sensitivity	Barik, Sahu and Swain (2008); Usha Rani, Madhusudhanamurthy and Sreedhar (2014)
Ag/Cu	Inhibits the normal growth and functioning of <i>Botrytis cinerea</i> and <i>Alternaria alternata</i>	Mittal et al. (2020)
Ag	- It functions as disinfectants. - It has inhibitory effect on the action of <i>B. cinerea</i> to some extent	Baker et al. (2005); Malandrakis, Kavroulakis and Chrysikopoulos (2019)
MgO/ZnO	It deactivates the growth and multiplication of <i>Rhizopus stolonifera</i>, <i>Mucor plumbeus</i>, <i>Alternaria alternata</i> and <i>Fusarium oxysporum</i>	Wani and Shah (2012)
CuO	It induces inhibition in mycelial growth	Malandrakis, Kavroulakis and Chrysikopoulos (2019)
Poly epsilon-caprolactone (PCL) capsules	It acts as carrier that has potent post-emergence herbicidal action in comparison to standard atrazine composition on mustard plants	Oliveira et al. (2015)

15.3.2.1 Nano-fungicides

One of the common diseases observed in plants as an entire community is provoked by harmful fungal pathogens. The fungal diseases caused are responsible for destroying more than 70% of the world's dietary crops including barley, rice, cotton, wheat and groundnut (Godfray, Mason-D'croz and Robinson, 2016) which can be anytime during the entire growth period of plants. In particular, certain fungal pathogens such as *Botrytis cinerea* have the ability to specifically attack around 200 cultivars of plant species and negatively affect the green tissues and fruits and account for any damage inflicted on fruits at the time of storage, transportation or distribution of the same. Not only this, *B. cinerea* alone is also held responsible for 10% of the world's plant fungal diseases owing to their fungicidal characteristic feature. On the other hand, some other pathogenic fungi like *Fusarium* sp., *Rhizoctonia solani*, *Phytophthora* sp., are accountable for infecting the soil and aerial parts of the plants (Abd-Elsalam, 2015). As an attempt to reduce the destructive impacts of fungi on plants, nano-fungicides are being constructed. In regards to this above mentioned point, the natural question that arises is regarding the necessity of designing nano-fungicides even after the production and application of conventional fungicides on diseased plants and even normal plants as a precaution. As a matter of fact, it has been observed that in place of promoting improvement, conventional fungicides attack the nontargeted beneficial organisms which adversely hampers the ecological balance of the soil-plant biosystem. Hence, the alternative method proposed was to establish NPs possessing antifungal properties as an effective fungicide to protect the plants from catastrophic impacts. Reports suggest that a combined application of Ag NPs and Cu NPs have the capacity to inhibit the normal growth and functioning of two important fungal pathogens that involve *Botrytis cinerea* and *Alternaria alternata* (Mittal et al., 2020). Similarly, findings indicate that silver NPs applied as nano-fungicide critically diminished the seriousness of fungal diseases thus proving itself to be quite potent. To include, Ag NPs also manifested its worth as disinfectants as a result of their unique antimicrobial properties (Baker et al., 2005). Furthermore, it has also been noticed that MgO NPs and ZnO NPs can deactivate the growth and multiplication of *Rhizopus stolonifera*, *Mucor plumbeus*, *Alternaria alternata* and *Fusarium oxysporum* (Wani and Shah, 2012). An experiment was conducted to record the suppressive properties exhibited by various metal ions, particularly composed of zinc (ZnO-NPs), copper (CuO-NPs, Cu-NPs) and silver (Ag-NPs) on contact with seven major soil-borne and foliar plant diseases in vitro. It was

exclusively observed that Ag-NPs could pose inhibitory effect on the action of *B. cinerea* to some extent. Controversially, the fungal species were almost insensitive and irresponsive to CuO-NPs application. However, CuO-NPs showed to have the capability to induce inhibition in mycelial growth, thus suggesting their maximum efficacy which was followed by ZnO-NPs displaying mean rates of mycelial growth inhibition (Malandrakis, Kavroulakis and Chrysikopoulos, 2019). This further highlighted the fact that various formulations of nano-fungicides showcasing antimicrobial, antibacterial or antifungal properties can fight against several detrimental pathogenic microorganisms and protect the soil and plants, thereby rejuvenating and replenishing them.

15.3.2.2 Nano-herbicides

While focusing on herbicides, the first and fundamental threat one is reminded of is weeds. Weeds are those undesirable plants that grow along and among the desirable crop plants which tend to absorb the essential macro- and micro-nutrients, water, major gaseous compounds and other necessities that are predominantly required for normal enrichment of crop plants. Speaking of physical eradication of weeds by hand, it is tedious, time-consuming and might also lead to overlooking and missing out on some of the weeds growing in a definite field. As a solution to physical weed removal technique, conventional herbicides came into existence as a substitute solution which are presently available in the market at cheaper price. However, on examining, it has been found that traditional herbicides not only instigate killing of the unwanted weeds but also interferes with the nutrient quantity and quality of the crops, thus imposing menace to the plants. It was also noticed that consistent administration of herbicides on weeds increased their ability of resistance towards these traditional forms. To come up with a better solution, nano-herbicides are being designed in order to promote better weed control without harming the environment in any means, even without disposing the toxic elements in soil (Pérez-de-Luque, 2017). Nano-herbicides are basically the advanced forms of the conventional herbicides applied with a NP coating, for instance, the nano-structured pesticide form of traditional herbicide atrazine is poly epsilon-caprolactone (PCL) capsules that are commonly termed as polymeric NPs. This formulation has been administered as carriers which demonstrated potent post-emergence herbicidal action in comparison to standard atrazine composition on mustard plants (Oliveira et al., 2015). The developed nano-herbicides by exploiting the conventional herbicide formulations and nanotechnology, on introduction to the roots of weeds, they travel into the root system and traverse to the targeted cytoplasm where glycolysis takes place. On entering the cytoplasm, it facilitates inhibition of glycolysis in the weed root system which results in starvation followed by death of the weed plants. These nano-herbicides are designed in such a way that these operates only by targeting specific receptors present in the root system of the weeds. Nano-herbicides also prevents the weeds from developing resistance against them which otherwise occurs during consistent herbicide application. Rain can be as destructive as beneficial for farmers. This is because, while sufficient amount of rainfall can enrich the soil with necessary water and nutrients, excessive rainfall can wash away the soil that, in turn removes all the applied herbicides or sometimes gets vaporized due to insufficient rainfall. However, due to encapsulation, nano-formulated herbicides can timely control the release of herbicides into the soil which has been quite a combat to the issue (Ivanova et al., 2016). Inorganic herbicides such as silica dioxide (Si) NPs have been administered as nanocarriers due to their property of pH sensitivity. It is being currently assumed that nano-herbicides as carriers can be possibly utilized by sustained delivery and mechanism of adsorption (Usha Rani, Madhusudhanamurthy and Sreedhar, 2014). Another practical attempt taken was to encapsulate a nano-sized rice husk with 2,4-dichlorophenoxyacetic acid (2,4-D) through surface adsorption to formulate a nanocarrier herbicide. Conclusions drawn were that rice husk-derived nano-herbicide utilization can be a boon in the near future provided that it makes productive use of wastes with reduced environmental contamination, hence paving a possibility for the substantial use of nano-herbicides to strengthen sustainable agriculture (Abigail et al., 2016).

15.3.2.3 Nano-emulsions

Dispersion of nano-sized pesticides as droplets in water is termed as nano-emulsions. Nano-emulsions are also called by the names of “ultrafine emulsion,” “mini-emulsion” and “sub-micron emulsion.” These are often available in the size range of 20–500 nm and is colloidal in kind. In general, nano-emulsions are composed of surfactant, oil and water which are formulated in three distinct manners: (1) water-in-oil (W/O), (2) oil-in-water (O/W) and (3) bi-continuous system where surfactant layer induces interfacial tension that separates aqueous phase and oil/organic phase, as per requirement. The surfactants involved in this technique are also known as emulsifiers that exist in four types: amphoteric, cationic, nonionic, and anionic. Among all these types, nonionic surfactants are the most common forms that are employed for nano-emulsion formulation owing to their relative capacity tolerate any alteration in ionic strength and pH. One of the governing factors that determine the selection of surfactant type and kinetically stable nano-emulsions is the value of hydrophilic-lipophilic balance (HLB). Under conditions of higher HLB value (normally <10 HLB value), affinity of

surfactant to dissolve in water is expected to considerably increase, thus portraying the possibility of favorable oil-in-water emulsion (Mustafa and Hussein, 2020). However, findings represent that combined nonionic surfactants that includes nonionic (single) and a fusion of nonionic and anionic (complex) surfactants, have the potential to give higher HLB value which manifests greater stability of the manufactured nano-emulsions, surfactant flexibility and partition ability on countering the interface of oil-water (Du et al., 2016). Moreover, application of surfactants has been crucially associated with low aggregation because of their ability to alter electrostatic charge (Feng et al., 2016) in addition to their ability to cause reduction in contact angle and improve leaf wettability depending on the applied surfactant nature (Feng et al., 2018). In view of the plant leaves exhibiting negative charge, the suitable identified surface-active agents are ionic liquids that aid in developing nano-emulsions with positive charge. It has also been conjectured that surfactants administered only in requisite proportion while producing nano-emulsions can marvelously their stability, antimicrobial and viscosity properties and desired particle size and excessive regulation of surfactant can unfavorably pose lethal impacts (Zhao et al., 2017) along with no such guarantee that it will provide improved contact angle to the abaxial (facing away from the stem) and adaxial (facing towards the stem) leaves (Feng et al., 2018). The main motive behind producing such nano-emulsions is to surge the solubility and efficacy of the active pesticidal elements. Also, it has been evident from recent experiments that pesticides are gradually released from these colloidal formulations which is of much benefit (Fig. 15.2).

In conclusion, a diverse array of NPs is applied in encapsulated forms as nanofertilizers, nanopesticides, nanofungicides and much more which has been quite effective when administered in sufficient quantities. However, as

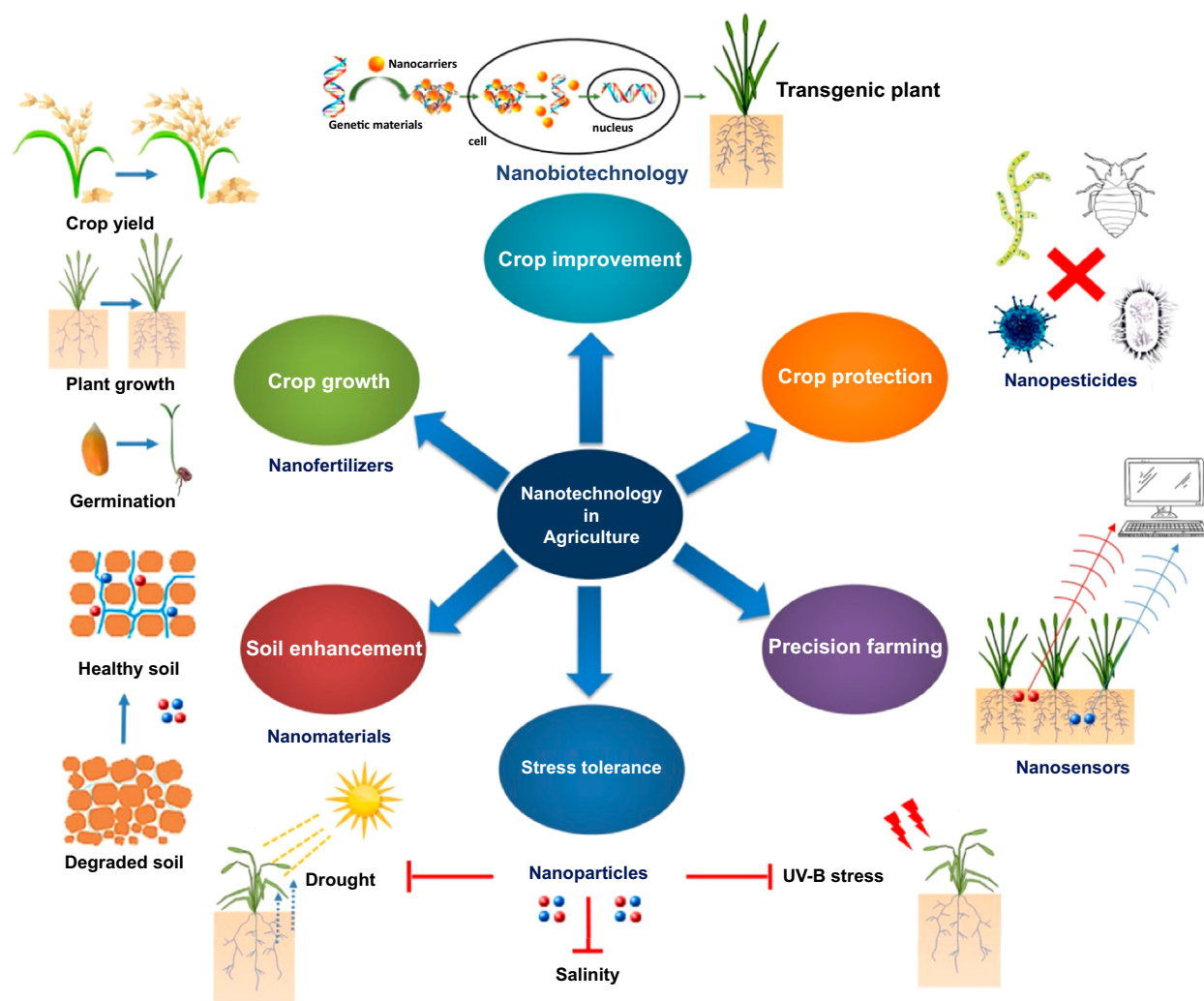


FIGURE 15.2 Pictorial depiction exhibiting the positive effects caused on application of nanoparticles in different forms to the soil-plant systems (Shang et al., 2019). From <https://www.mdpi.com/1420-3049/24/14/2558>.

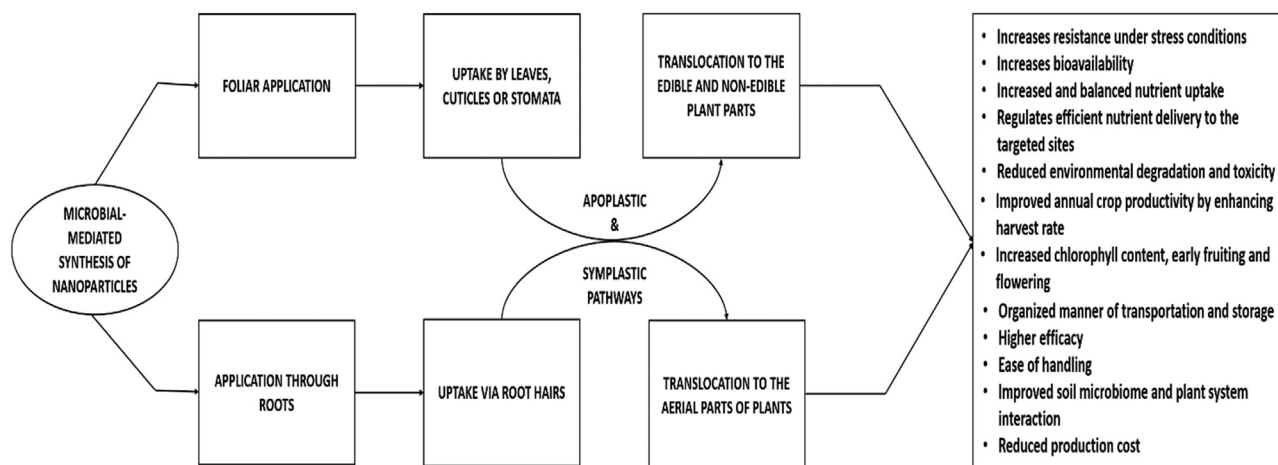


FIGURE 15.3 Schematic diagram showing an overview of nanoparticle application and the benefits provided by nanoparticles in replenishing and remediating soil which henceforth leads to increased crop productivity and yield.

beneficial as these encapsulated nanoparticulated formulations of conventional fertilizers, pesticides or manures, they can also pose an immense measure of toxicity to the crop plants by disturbing the coordinated balance maintained by the various physiological and metabolic pathways within the internalized plant systems which further affect other enormously important parameters associated with plants as discussed in the next section (Fig. 15.3).

15.4 Conclusions

Findings demonstrate that nanotechnology has been successful in different dimensions of life such as cosmetics, medicine, and recently being extended to the arena of agriculture. The extent of toxicity and probable biochemical impacts inflicted by NPs to the plants are critically defined by the characteristic features including concentration, size, aggregation, morphology and surface modification. Not to mention, the proposed impacts can also be governed by certain extrinsic factors such as soil pH to which the NPs have been exposed, species, stage of lifecycle, characteristics of selected growth media, age of the concerned plant. Despite of being aware of some concerning issues related to nano-encapsulated fertilizer and pesticide application, holistic information regarding the wide effects inflicted on agricultural plants on administering these NPs is till elusive. Deficiency in establishing the proper parameters for measuring the extent of toxic impacts on crops as well as the surrounding environment under NP proximity can cost huge loss. Considering this sensitive background, nature, various concentrations, extent and duration of exposure of NPs, NP-soil-plant interaction and consequences of their application in the real field have to be focused on in future studies. Hence, knowing the precise effects developed due to interaction between diverse NPs and various types of plants and soil systems would bestow us with an opportunity to avoid misuse and supply of adequate NPs to the soil biosystem. Additionally, acquiring rightful knowledge regarding the action of different contaminants at trophic levels and their response in the presence of nanomaterials has to be addressed on an urgent basis. In the light of this urgency, another immediate requirement is the study revolving around the long-term administration of NPs to which a wide range of crop plants and soil microorganisms are directly exposed which can work miraculously to indicate the standard parameters to be followed and concerns needing attention. It has to be necessarily kept in mind that renewing and replenishing soil nutrients and quality not only enriches the crop yield but also is integrally linked with a secure and protected food industry.

15.5 Future perspectives

With regard to the necessity of promoting sustainable agriculture for securing a worldwide healthy and nutritious food source in the near future, it becomes the responsibility of the present generation to utilize and implement the modern technologies to bring about revolutionary impact as a take a step towards the aim. A combination of nanotechnology and biotechnology field is being expected to be advantageous in establishing a stable food chain, energy balance in association with accorded abiotic and biotic systems. Along with employing the modernized technologies in the agricultural and food industries, one must be aware of one important fact that NPs can be equally detrimental to the

agricultural sector and can indirectly poses threat to the food industry. As an imply to overcome such hurdles, certain ecofriendly and safe delivery systems have been innovated in order to assure mechanized and proper administration of diverse plant nutrients and food enhancing compounds. Majorly, the unique properties of NPs including desirable shape, size, surface chemistry, exposure time, accumulation, immune response, retention time, structures and other factors for which they are considered to be critically beneficial, should be kept under evaluation. For the same reason, new analytical methods capable of detecting, accessing, evaluating, re-evaluating and validating the impacts of NPs are being developed. Most recently, nano-based biosensors have been designed for determining favorable pest control methods and valuable food products. Additionally, clear guidelines, rules and regulations have to be formulated for limiting the use of NPs so as to reduce probabilities of adverse effects caused to the agricultural plants by the authorities. New arrays of discussion and communicating panels should be opened whereby people from every sphere of life has to be encourage to participate that can help to discuss and measure the different advantages and disadvantages related to the application of nanobiotechnology in sustainable agriculture. This can be indispensable in the future owing to the economical, nutritional and agricultural benefits that it promises. Furthermore, the cost of these nano-structures fertilizers and pesticides should be kept within the reach of the common people and the poor farmers so that everyone is benefited from this emerging field. In view of the interactions among the soil microbiome, plant systems and NPs, it is advisable to put emphasis on developing few adaptive models. Another notable shift that has begun shaping in regards to the production of nanofertilizers and nanopesticides from natural resources such as alginate derived from brown algae, chitin and chitosan derived from exoskeletons of crustaceans, can be a promising step towards building up new avenues for sustainable agriculture. Stepping ahead, economic feasibility, social acceptance, transportation and storage costs, market supply and regulatory norms are some other dimensions that need attention in order to build up a strong community for sustainable agriculture.

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Green synthesized nanonutrients for sustainable crop growth

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16.1 Introduction

Increase in population is leading to shortage of food which is an alarming situation. The reason for shortage of food includes soil infertility, climatic conditions and various plant diseases. To elude this universal problem, chemicals and higher doses of fertilizers, pesticides and herbicides has been used. These harsh chemicals have polluted water streams and soil microflora. Also, these chemicals are harmful for human health. Excess use of fertilizer has resulted in disruption of biogeochemical cycle (Chhipa, 2017). Chemical fertilizer increases the soil fertility but are not able to meet the global need. Recently nanotechnology has become an effective tool to benefits agriculture by increasing the nutritional demands, soil quality that results in growth of healthy crop and lessened the use of chemicals fertilizers, pesticides and herbicides (Zulfiqar et al., 2019; Kopittke et al., 2019). Nanoparticles are vividly used in agriculture for promoting yield, plant growth, and disease suppression. The nanofertilizer role is well played by zinc oxide nanoparticles as there are no side effects caused by them, unlike the chemical fertilizer.

Nanotechnology is the combination of two words nano and technology, “nano” which means billionth (1×10^{-9}) in size. Nanotechnology can be elaborated as something that can concept, can control, and rebuild a substance of nanometers with enhanced functionality (Lowry et al., 2019). Nanotechnology has always attracted researchers for its wide application not just in physics and chemistry but also in biology. In physics, nanotechnology is used for making semiconductor and also nanoscale devices are employed for making nanotubes, etc. (Khosravi and Hosseini, 2020). Likewise in Chemistry, nanotechnology uses are very relevant like personal care items, foods, pharmaceuticals by making use of nanocrystalline cellulose (Peng et al., 2011) or can be used in the treatment of air and water (Mobasser and Firoozi, 2016; Qu et al., 2013).

Nanoparticles can be synthesized by several processes like physical, chemical and biological methods (Fig. 16.1) (Parveen et al., 2016). The newest approach of synthesis in nanotechnology is biological synthesis or green synthesis. Green synthesis is preferred because it makes use of eco-friendly, nontoxic reagents for synthesis of nanoparticles. Green synthesized nanoparticles are more stable and stricter to dimension as they are made by a one-step process (Gour and Jain, 2019).

Green synthesis involves plants and microorganisms (Dhiman et al., 2021a). Nanoparticles can be synthesized from plant parts such as leaf, stem, seed, fruit and root (Dhiman et al., 2021b). Bacteria has property to reduce metals and this reduction mechanism has been exploited in the manufacturing of nanomaterials (Menon et al., 2017). Fungi considered to produce nanoparticles. Fungi are more efficient producers of nanoparticles than bacteria (Zhao et al., 2018). Yeasts are also a great manufacturer of nanoparticles. Studies have been reported so far that this unicellular microorganism is an efficient producer of many nanoparticles. (Sivaraj et al., 2020).

Nanotechnology has emerged out as a boon for biologist. With the help of nanotechnology treatment of fatal diseases like cancer is also possible. They are potent antimicrobial agents which can be used in the pharmaceutical industry for the manufacturing of drugs (Nie et al., 2007). Nanoparticles have multipurpose features and have a diverse role in various streams like health care, nutrition, agriculture, etc. (Chandran et al., 2006). More specifically, if agricultural importance is considered then nanoparticles play a very outstanding role. Nanoparticles are used as nanofertilizers or plant growth promoters, pesticides and also as antimicrobial agents (Fig. 16.2).

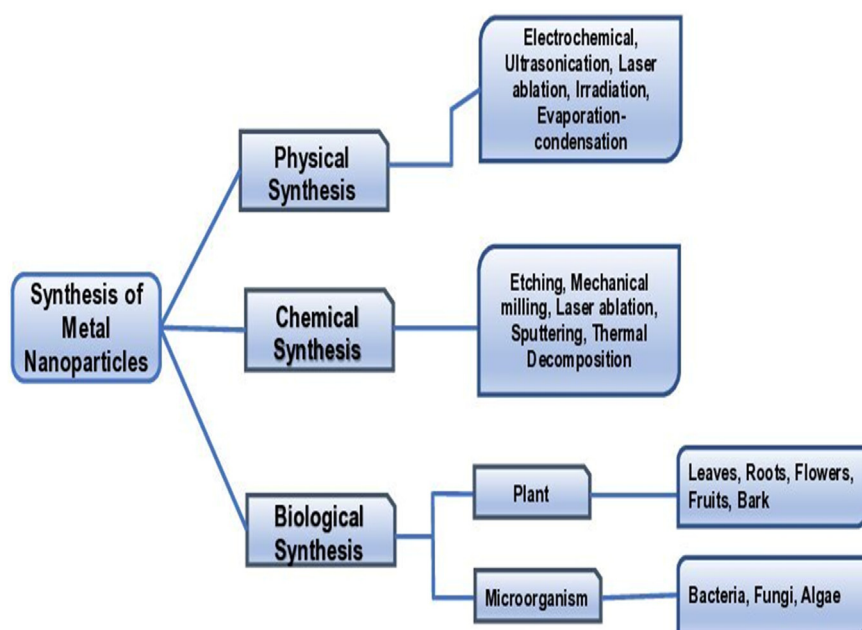


FIGURE 16.1 Different methods of synthesis of nanoparticles.

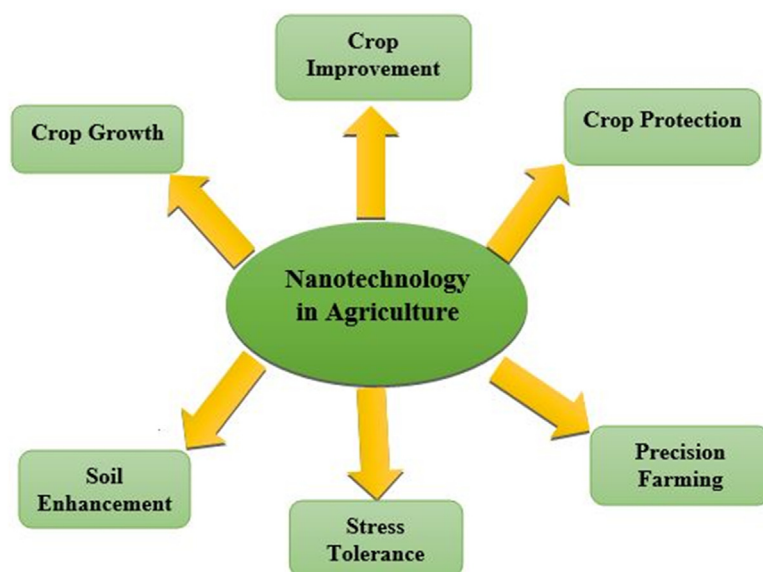


FIGURE 16.2 Different applications of nanotechnology in agriculture.

16.2 Nanoparticles in crop growth

Nanoparticles are used in sustainable agricultural practices like controlled and targeted delivery to fill the void of chemical fertilizer (Kumar et al., 2021). Nanofertilizers are formed by combining the function of nanomaterial and can deliver the plant with several nutrients at a time and hence improve plant growth. Nanoparticles can directly enter plant cells having size smaller than the size of the cell wall pore. It plays an active role in the cytoplasm of the cell. The nutrients are obtained when nanoparticles are dissolved in solution and leaching of ions progressed. These ions are taken by plant root by osmotic pressure, capillarity, or epidermal root transfer (Lin and Xing., 2008) and delivered to

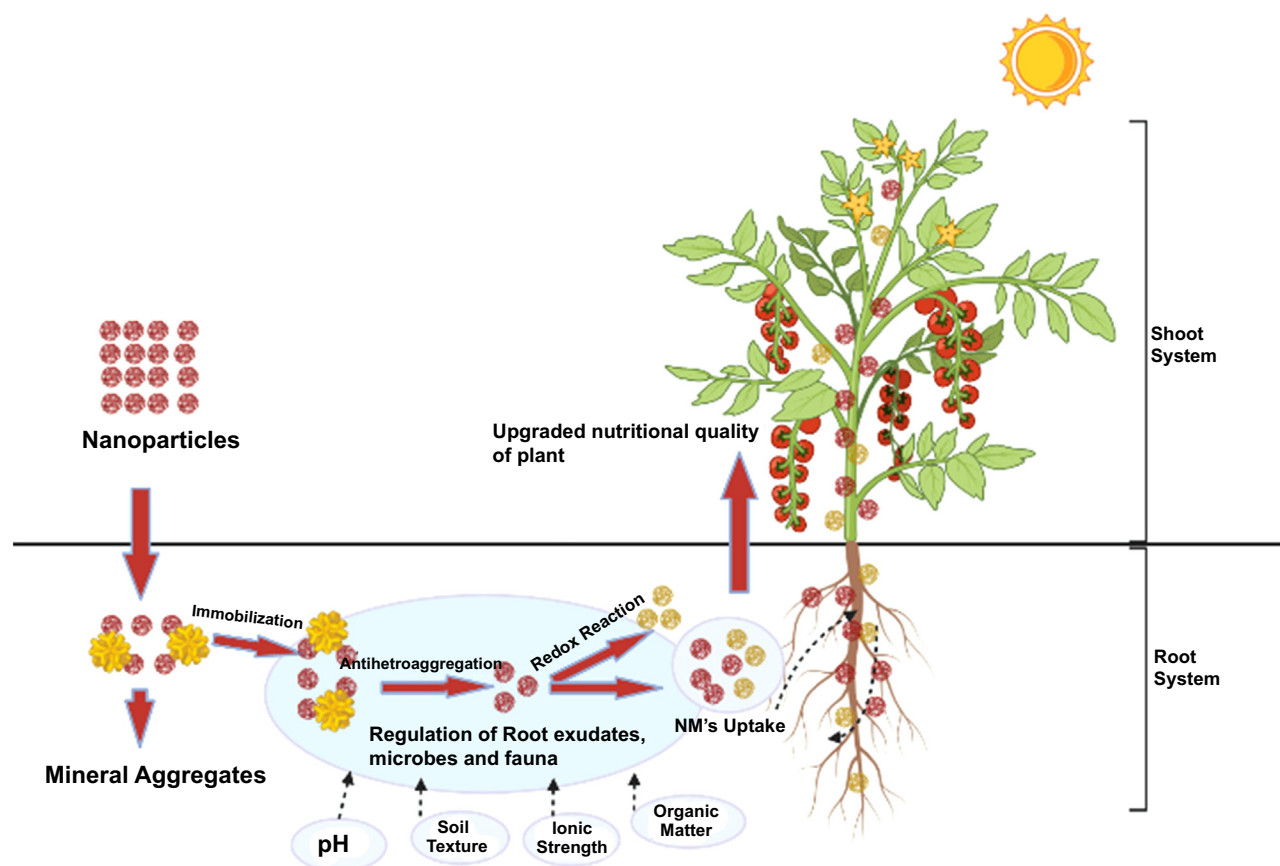


FIGURE 16.3 Uptake of nanonutrients by the plants.

the aerial parts and finally to plasmodesmata in the cytoplasm (Tripathi et al., 2017). In short, nanoparticles can make their way to plants by numerous paths (Fig. 16.3). The small dimension of nanoparticle makes the uptake of nutrients by plant pores easy (Lv et al., 2019).

Nanoparticle uptake mechanism is a critical step in plant physiology, it may activate defensive response against them. Nanoparticle uptakes enhance the nutritional benefit of plant and synthesize only those material that claim the objective (Odzak et al., 2017).

Accumulation of nanoparticles can directly act as food for plants and humans. Many times, this accumulation can even form toxicity which can harm human wellbeing (Pullagurala et al., 2018). Hence, correct amount of nanonutrient application is recommended (Prakash & Mathur 2020). Nanoparticles has been used for seed germination. Translocation and uptake of nanonutrient in the plant is always as major public concern (Zhao et al., 2014). The physiochemical property of soil could be modified by the nanonutrient before the plant, mainly pH and the ionic strength. In the rhizospheric region of the plant, a large no. of microbes (bacteria and fungi) and soil fauna (e.g., earthworms) which maintains the biotic and abiotic environment, for example, root secretion, microbial growth, and earthworm activity (Berendsen et al., 2012). these all process such as aggregation, anti-aggregation, redox reaction, and nanomaterial uptake sequentially maintain the translocation of nanonutrients into the plant as shown in Fig. 16.3.

Many research claimed that seeds used with nanoparticles demonstrated rapid seed germination. After treatment with nanoparticles, seed absorbed water retention property that results in faster seed germination (Khodakovskaya et al., 2009). It is evidenced that nanoparticles enhance the seed growth (Gopinath et al., 2014), high regeneration of shoot, root, length, healthy point yield, good metabolic content (Kole et al., 2013). For healthy crop growth, nanoparticles slowly release the nutrients to the plants to keep the flow regular and hence reduce the loss of nutrients (El-Ramady et al., 2018). Many times, environmental factors influence the growth of plants and to fight this phase nanonutrients activate antioxidant enzyme and reduce oxidative metabolism (Sharma et al., 2019).

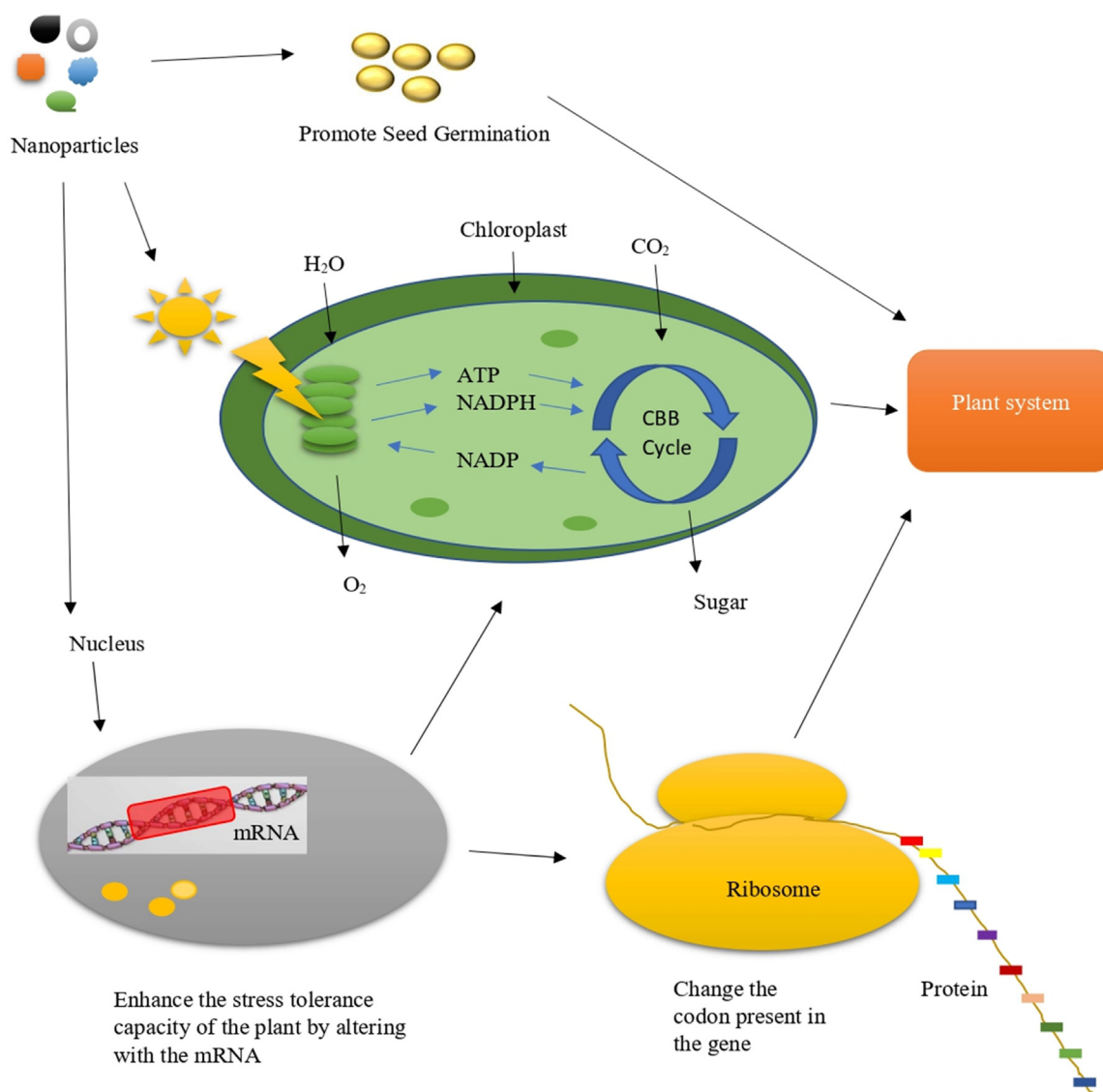


FIGURE 16.4 Nanoparticle affecting different region of plant and promote its growth and yield.

There is a several positive effect of nanoparticles on the growth and development. The optimum amount of nanoparticle causes different type of effect on plant as shown in Fig. 16.4, the nanoparticle alters different physiological processes such as seed germination and rapid photosynthesis in chloroplast for food formation. Further, the nanoparticle changes the gene expression of several gene and miRNA synthesis. This led to plant survive under abiotic stress and increase in the plant biomass.

Studies have proved that along with Zn and Ag other nanonutrients like Boron, Manganese, Iron, Copper, Molybdenum and Chloride have great potential towards promoting healthy crop growth in plants (Bhardwaj et al., 2022). Table. 16.1 shows the various nanoparticles and their different effect on the plant.

16.2.1 Nanonutrients in crop growth

Following plants requirements, various macro and micronutrients can be provided in the form of nanofertilizers or nanonutrients to the plants (Liu and Lal, 2015; Chhipa, 2017). Iron (Fe), Copper (Cu), Silicon (Si), Cobalt (Co), Manganese (Mn), Nickel (Ni), play a very crucial role in disease management by activating enzymes and boosting up

TABLE 16.1 Types of nanoparticles and their effect in plants.

Nanoparticles	Plants	Beneficial effects	Reference
Titanium	<i>Brassica oleracea</i>	High Yield in Crop	Pais (1983)
	<i>Capsicum annuum</i>		Pais (1992)
	<i>Malus domestica</i>		Pais (1983)
	<i>Solanum lycopersicum</i>	Weight of fruit increases	Pais (1983)
	<i>Ribes uva-crispa</i>	High Yield	
	<i>Prunus Persia</i>		
	<i>Zea mays</i>	Yield Increases	Berahmand et al. (2012)
Titanium-ascorbate	<i>Pisum sativum</i>	Uptake of minerals increased	István et al. (1991)
Nanohydroxyapatite	<i>Glycine max</i>	Growth and yield increases	Liu, Lal (2014)
Cobalt	<i>Glycine max</i>	Yield increases	Ngo et al. (2014)
Zinc	<i>Pennisetum americanum</i>	Grain Production Increases	Tarafdar et al. (2014)
Silver	<i>Triticum aestivum</i>	Quality of crop improves	Razzaq et al. (2016)

energy building by photosynthesis (Vuong 2019). Macronutrients are needed in larger quantities by plants for their growth and development, such as nitrogen, potassium, phosphorus, calcium, magnesium, and sulfur. The demand for food by population is directly proportional to the use of macronutrients (Ditta and Arshad, 2016). Nitrogen increases important enzyme secretion by upgrading microbial population. When Nitrogen nanoparticle applied on plant like pearl millet led to appreciable growth of root area, length, perimeter, etc. (Thomas et al., 2016).

Phosphorous is the least available plant nutrient so, phosphorus nanoparticle is provided to soil to increase the agricultural productivity. It also increases growth and biomass. In many cases, it also increases physiological efficiency of shoots and roots (Miranda-Villagómez et al., 2019). Deficiency of phosphorus leads to stunting growth, thinning and pigmentation of plants.

Carbon Nanoparticles like fullerenes, carbon nanotube enhances germination of seed, chlorophyll, and protein content. It also increases the shoot and root length. In many cases it was evidenced that carbon nanoparticles increase barley root elongation (Panova et al., 2016).

When potassium nanoparticles are applied to plants, they show greater dry weight of the shoot, improved root length. It also leakage of electrolyte, increased protein content. Hence resulted in salt tolerance and efficient antioxidant activity (El-Sharkawy et al., 2017).

Calcium nanoparticle direct genetic modification in plants (Rajput et al., 2021). This nanoparticle is also used as foliar nanoparticle and helps in nutrient uptake, etc. This increases protein content in plants. Calcium help plants for photosynthesis, hormonal systems, cation exchange and other important mechanisms (Tombuloglu et al., 2019).

Various studies have shown magnesium remarkable impact on plant growth with peroxidase activity and nutrient accumulation higher magnesium content which thus increase chlorophyll amount in plants (Cai et al., 2018).

Boron nanoparticle is important for the growth of pea. It provides antioxidant properties, mineral uptake, and many more (Sutulienė et al., 2022). It significantly increases plant height, shoot dry weigh, photosynthesis rate, chlorophyll content and stress. Boron nanoparticle also play a crucial role in salinity stress over potato plant. It reduces the salt concentration in the soil and help the plant to grow in high salt concentration (Mahmoud et al., 2020).

Zinc oxide nanoparticle affect the root and shoot length of wheat (Singh et al., 2019). It improved shoot dry matter and increase the leaf area index. It also improves the corn growth and yield in mineral poor soils (Taheri et al., 2016).

Manganese nanoparticles help in nutrient acquisition in wheat. Application of manganese nanoparticle affect the plant growth, grain yield, and improve the nutritional requirement in the soil (Dimkpa et al., 2018). Manganese nanoparticles have a potential to improve the morphology, physiology and secondary metabolism of shoot tip (Tian et al., 2018).

Soyabean plant benefitted by the iron nanoparticle by increase in the growth and nutrient content. It increases the colonization of nodule forming nitrogen fixing bacteria (Burke et al., 2015).

There is an increase in plant growth and productivity of several economically important crop plant using copper nanoparticle. It also increases the content of anthocyanin, chlorophyll and carotenoid in the leaves of plant (Van Nguyen et al., 2022). It also improves the germination of some plant at lower concentration of nutrients (Kasana et al., 2017).

The Molybdenum nanoparticle help in uptake, bioaccumulation of nutrients from the soil in rice. It increases growth and protein level of rice for its better germination (Sharma et al., 2021).

16.2.2 Nanonutrients in stress tolerance

When the plant grows, it needs several factors for its desirable growth like soil, water, light, temperature and other environmental factors. During the growth, there may be conditions like drought heat, salinity, cold is a few abiotic stresses that a crop needs to tolerate (Mujtaba et al., 2021). This stress ultimately destroys the productivity of crop. In further times, abiotic stress will be a great issue, hence nanonutrients use is able to fight against these factors, stress tolerance that can hamper seed germination, availability of nutrients to roots can be obstructed and so on (Soliman et al., 2015). Under stressed condition nanoparticle act as nanosupplement for better survival of plant by improving the gene expression of several antioxidant enzymes and stabilize the activity of catalase and peroxidase enzyme. Nanonutrients like zinc and iron help to fight against abiotic stress by preventing membrane damage (Tarafdar et al., 2018).

As shown in Fig. 16.5 that the impact of nanoparticles enhances several growing effects such as shoot growth, root growth, chlorophyll, carotenoids, water content which finally improve the plant growth and its productivity. Various studies are being conducted in wide sphere to combat the environmental stress (Salem et al., 2021). Success rate is pretty in pressive and can be applied to crops in large scale.

16.2.3 Nanonutrients enhances soil quality

As discussed earlier, prolonged chemical fertilizer can deteriorate the soil quality to a large extent. Slow nutrient delivery support plant growth without altering the pH of soil (Singh et al., 2018). The use of nanofertilizer restores the microbiome and keeping soil environment healthy. These all helps to fight against biotic and abiotic stress.

Bioremediation is an important phenomenon to shatter the toxins from soil and water. Nanoparticles are used as zeolites which improves delivery system. Soil is made herbicide resistant using these nanofertilizers in addition it also helps in detecting toxic material in soil. Zinc and Iron nanoparticles helps to improve soil quality by retaining moisture and humidity and has carbon content in arid soil. Nanofertilizers improve nutrient mobilization in soil (Tarafdar, 2021).

16.3 Nanonutrients in disease management

Microbes such as bacteria, fungi, and the vast variety of zooplankton/phytoplankton are micro-sized agents. Studies shown that nanoparticles interact with all types of microorganisms in a positive way or in negative way. Nanoparticles

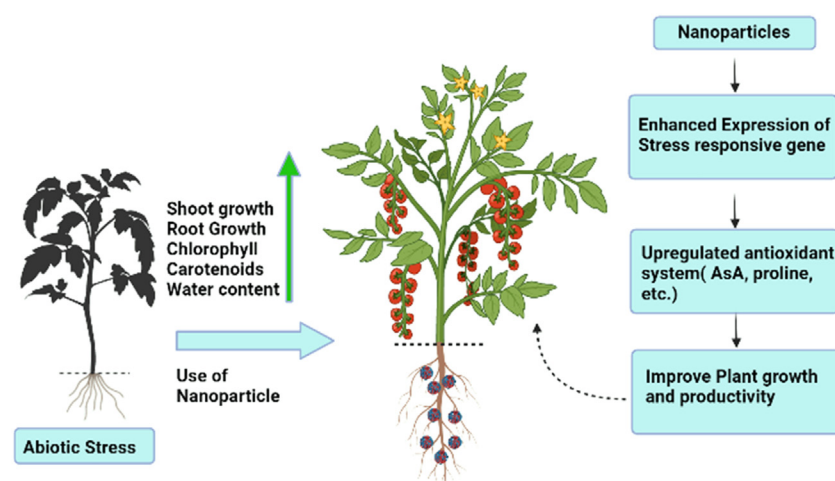


FIGURE 16.5 Stress tolerance developed due to introduction of nanonutrients.

has been reported as antimicrobial agent and therefore can be used for disease suppression in plants. [Beadle \(1993\)](#) said that due to the enhanced surface area of nanoparticles, retaining capacity of nutrients become more efficient that perform a durable and better mineral store for the crops. Hence, with the help of nanoparticles the management of disease in plants and their growth can be achieved simultaneously in a novel, green, and eco-friendly way. Disease suppression by plant pathogen inhibition, cheaper because used in small quantities. Some agriculturally important nanoparticles are zinc oxide nanoparticles, silver nanoparticles, iron oxide nanoparticles, copper-based nanoparticles, titanium oxide nanoparticles, etc. ([Singh et al., 2021](#)).

For now, nanoherbicides, nanopesticides, and nanofungicides are used for the application in agricultural practices ([Dwivedi et al., 2016](#)). The consequences of nanoparticles that are adjuvanted, depend upon the variety of plant and the type of nanoparticles. For example, some nanoparticles such as Ag, ZnO, Mg, Si, and TiO₂ have been evidenced to suppress various crop diseases by their antimicrobial properties ([Prasad et al., 2014](#)).

Metal oxide nanoparticles like ZnO also restrict the growth of the pathogen ([Dhiman et al., 2022](#)). It has several benefits including lesser toxicity than silver nanoparticles ([Dimkpa et al., 2013](#)). Carbon Nanoparticles include C60/70 fullerene, organic nanoparticles as carbon, fullerene, single and multiwalled carbon nanotubes. The nonfunctionalized carbon nanotubes can reduce chlordan matter in the roots and shoots up to 88%. After several experiments the amino-functionalized tubes was more reasonable because they showed 57% repression in roots and 23% repression on shoot as compared to control ([Table 16.2](#)).

TABLE 16.2 Microbes interacting with nanoparticles and their target site.

Nanoparticle	Organism	Target site	Reference
Magnesium oxide	<i>Escherichia coli</i>	Cell membrane	Stoimenov et al. (2002)
	<i>Bacillus megaterium</i>		
Silver	<i>Escherichia coli</i>	Cell membrane	Morones et al. (2005)
	<i>Pseudomonas. aeruginosa</i>		
	<i>Vibrio cholera</i>		
	<i>Scrub typhus</i>		
Copper–silica	<i>Staphylococcus aureus</i>	Interaction with protein	Kim et al. (2006)
	<i>Escherichia coli</i>		
	<i>Enterobacter cloacae</i>		
Zinc oxide	<i>Escherichia coli</i>	Cell membrane	Zhang et al. (2007)
Fe ₃ O ₄ silver	<i>Escherichia coli</i>	Cell membrane	Gong et al. (2007)
	<i>Staphylococcus epidermidis</i>		
	<i>Bacillus subtilis</i>		
Styrene–acrylic acid/ silver nanoparticles	<i>Escherichia coli</i> <i>Staphylococcus aureus</i>	Cell membrane	Da Silva et al. (2009)
Silver	<i>Candida spp.</i>	Cell membrane	Panáček et al. (2009)
Silver	<i>Trichophyton rubrum</i>	Cell membrane	Noorbakhsh et al. (2011)
Zinc oxide	<i>Botrytis cinerea</i> , <i>Penicillium expansum</i>	Cell membrane	He et al. (2011)
Silver	<i>Cryptococcus neoformans</i>	Cell wall and cytoplasmic membrane	Ishida et al. (2013)
Silver	<i>Candida albicans</i>	Cell membrane and sulfur	Bhimba (2014)
	<i>Candida parapsilosis</i>		
	<i>Aspergillus niger</i>		

Nanomaterials have been seen as antimicrobial agents against bacteria viruses and fungi (Wang et al., 2017). There are several reasons behind the fact that nanoparticle is a good antimicrobial agent. Some of those reasons can be enlisted as, a physical structure that can damage the membrane, release of ions from metals and surface to volume ratio makes them possess broad-spectrum antimicrobial activity (Fernando et al., 2018). Like Copper oxide nanoparticles, it shows a cytotoxic effect on the cells of bacteria due to the ROS formation by metal oxide nanoparticles when adhered to bacterial cells which in turn call off oxidative stress in the cell hence causing cell death (Fig. 16.6) (Applerot et al., 2012).

There are several properties that nanoparticle inherit to act as an antimicrobial agent such as disordering of the plasma membrane by charges, disturbance in metal ion balance, increment of oxidation number and reactive oxygen species (ROS), enzymatic misfunction, photokilling, molecular inhibition technique signal transduction inhibition etc. (Raghunath and Perumal, 2017). Alkalinity is well understood in research to enhance the antimicrobial activity of nanoparticles for, for example, calcium oxide nanoparticles and manganese oxide (Sawai et al., 2005). Likewise, the positively charged cerium oxide nanoparticle show significant potential for killing the microbial cells (Thill et al., 2006). Titanium dioxide nanoparticle has a photocatalytic property which stops UV resistant species to grow (Sadiq et al., 2010).

As shown in the figure that the use of nanoparticle disintegrates the fungal cell it degrades the cell wall and plasma membrane, it also interacts with the mitochondria to interrupt its functioning and enters the nucleus and denatures the Double stranded structure of DNA which result in killing of the fungal cell, it also attacks the endoplasmic reticulum of the cell and denatures the protein structure.

Mycelial dysfunction and spore's distortions of fungi by the action of nanoparticles of zinc oxide and silver oxide has also been reported (Fig. 16.7) (He et al., 2011). Silver oxide nanoparticles interrupt the cell wall and inhibit general budding procedure (Kim et al., 2009; Fernando et al., 2018). This antimicrobial property of nanoparticles makes them outstanding materials to be used in agriculture for the healthy plant development. Also, nanomaterials can act as plant growth stimulators when exposed to the soil environment. The use of nanoparticles increases the pH of the soil hence making them capable to hold water, enhance ion-holding capacity all of which create fertility in the soil and nutrient absorbance capacity of plants (Das et al., 2018).

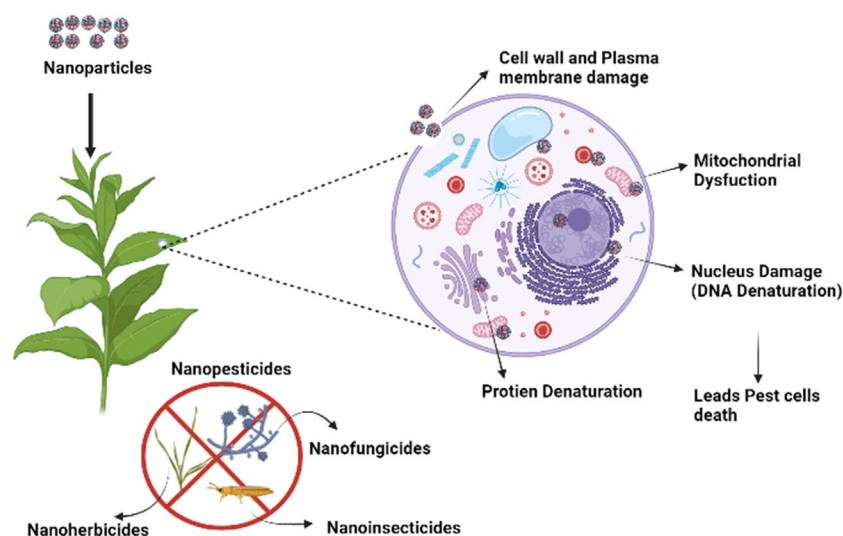


FIGURE 16.6 Antifungal properties of nanoparticles leads to catastrophe of fungal cell.

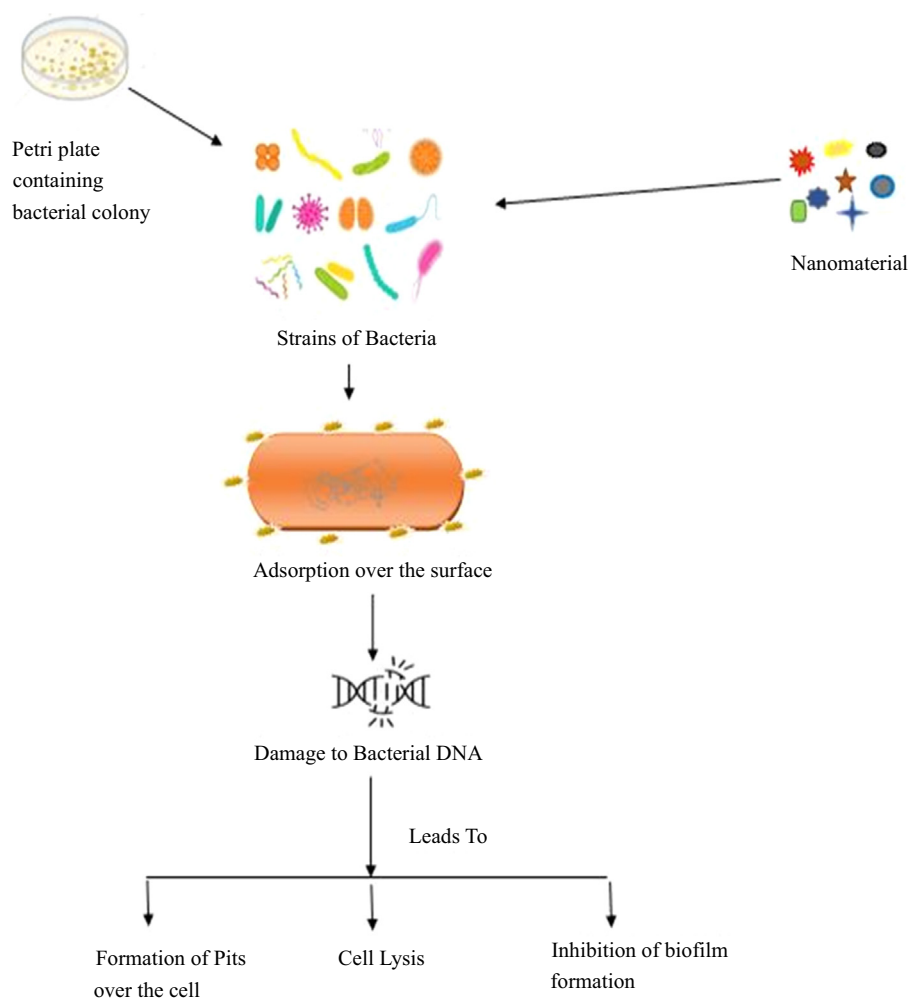


FIGURE 16.7 Antibacterial activity of nanoparticles causing bacterial cell damage in different ways.

16.4 Conclusion and future perspective

The nanofertilizers made successful impact on enhancing the productivity and resistance towards the abiotic stress. The potential of nanofertilizer increases the production potential of agricultural crops under the current scenario. The tremendous potential of nanofertilizers despite the exciting outcomes by increasing the profit margin of farmers. So, future studies should basically focus on the interaction of nanonutrients with the environment and human health, then should be used to novel frontier for the sustainable development.

Enhanced evaluation of nanofertilizer will result in characterization of different physiochemical properties that are necessary for use of recommended crop and soil type.

Nanotechnology has gained widespread acceptance due to its numerous applications and its promise of creating new products and processes (Bhushan, 2017; Nasrollahzadeh et al., 2019). Understanding the inter-relation of nanoparticles can help improve the efficiency and profitability of crop production (Wang et al., 2016; Singh et al., 2019). The vast development in nanotechnology at various sectors such as food, agricultural, and smart devices like nanosensors, nanopesticides, and nanofertilizers. Several nanomaterials are developed for fixing food index, crop development, and environmental factors (He et al., 2019). India's agriculture growth is reducing at a very high rate due to the lack of technological development in the agricultural. Agricultural practices can be improved through the use of nanotechnology which can be used to enhance the growth and development of plants. Nanotechnology can distribute agrochemicals precisely to improve disease resistance, plant development, and target specific pest, insect, herbicides for a cleaner environment (Subramani et al., 2019).

Nanotechnology can give us a sustainable agriculture system and thus many major problems can be solved that causes damage to the environment and humans. The use of nanofertilizer which has many agricultural advantages over conventional fertilizers is the better idea for improving agricultural practices. Today the use of agrochemicals has polluted the ground, water, and fertile soil to increase crop productivity. So, using nanoparticles, the productivity of soil will get increased by minimizing the damage to the environment. Silica nanoparticles leads to better performance than conventional fertilizer. Also, the green nanoparticle can tolerate abiotic stress and biotic conditions (Snehal and Lohani, 2018). The green nanoparticle is an integrative field that is a rapidly developing research area, that has the capability of making useful products that are eco-friendly in contrast to using physiochemical methods for nanosynthesis. The biogenic process is a process that produces green nanoparticles without involving hazardous chemicals or energy inputs. The exquisiteness of the green synthesized nanoparticles has important applications in various industries such as food processing medicine and agriculture (Saratale et al., 2018).

Hence, nanotechnology is a very important field that inter-relates many different streams like medicine, sustainable agriculture, energy source, food packaging, and many more to count. This mostly focused on the fact that nanoparticles are used as nanonutrients for crop growth and development. There has been a very in-depth discussion about the potential of nanomaterials for plant growth, disease suppression, antimicrobial agents. Use of nanomaterials in agriculture has shown its effect both as disease suppressor because of which plant stayed healthy and thus observed increased crop yield at the same time. The efficiency of nanoparticles as nanofertilizer is outstanding and the concept of using them is also appreciated with wide arms because of the economic-ness, less use of artificial chemical fertilizer, and reduction of loss of nutrition. The concept of the book chapter is emerging trends in environmental remediation where we chose to show how green synthesized nanonutrients used for sustainable crop growth like in crop productivity by uptaking micro and macronutrients, disease suppression by acting as antimicrobial agent, etc.

The metals and metal oxide nanoparticles are in air for their multiple uses in agriculture and practices of using them in croplands will be increased at a high rate in near future. The research done to date is not just enough, there are yet some stones left to unturn in this stream for making use out of it in a better way. One of them is nanoparticles being directly used in the fields. Due to their toxic effects nanoparticles are not directly used in fields. Researchers can make efforts in this area to either recognize the range of toxic concentration and then use them on field accordingly or work on reducing the toxicity of nanoparticles so that they can be used for the better agricultural purpose.

Nanotechnology is a point of interest for budding scientists and fundings in this area is even very fair. So, works can be done for easy recovery of useful materials from the soil. Better standardization can be planned out for making a proper design of a research idea.

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Environment sustainability with microbial nanotechnology

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17.1 Introduction

The term “nanotechnology” was coined by Prof. Norio Taniguchi to describe materials with nanometer dimensions (Bulovic et al., 2004) and the word nano refers to one billionth of a 1 m length. No doubt, the boundaries of nanotechnology have significantly broadened with the introduction of modern technologies but when compared to other disciplines, nanotechnology has found better applicability in electronic and materials engineering (Prasad et al., 2017). The environmental sustainability of nanomanufacturing processes is one of the primary issues for the global development of the nanotechnology industry. The development of efficient ecologically sound nanotechnologies for nanoparticle synthesis is critical for future exploitations of a wide range of nanostructured-based technological solutions, such as therapeutics, in vivo or in vitro scanning, and the development of antibacterial and anticancer drugs (Petros and DeSimone, 2010; Kiessling et al., 2014; Dragone et al., 2017). Further, the more advanced “green nanotechnology” is based on the use of microbial processes to synthesize nanoparticles for enhancing environmental sustainability. Microorganisms produce nanoparticles by assembling a range of inorganic materials either inside or outside the cells. Although a wide range of microorganisms, including bacteria and fungi, are known to produce metal nanoparticles, the methods of microbial production are crucial. Microbe-catalyzed nanoparticle production is a new green chemistry technology (Alghuthaymi et al., 2015). The ability of fungus, bacteria, actinomycetes, and yeasts to synthesize different metal nanoparticles has been widely demonstrated (Kapoor et al., 2021). The nanoparticles biosynthesis has gained popularity due to its low toxicity, energy efficiency, biocompatibility, and eco-friendliness, which allows it to be used in biomedical applications and the pharmaceutical industry (Mohammadlou et al., 2016; Ijaz et al., 2020; Goutam and Saxena, 2021). The problem of environmental contamination has raised public awareness globally and has created interest in today’s scientists and the general public for green nanotechnology. Nanoparticles are also used in environmental remediation activities, such as contaminant removal, pollutant detection, etc. Biosynthesized NPs have broad applications in biosensing, bioimaging, diagnosis, drug delivery, pest management, environmental sustainability, electronic component fabrication, remediation, catalysis, biofuel generation, wastewater treatment, textiles, cosmetics, sports equipment and other fields (Malik et al., 2017). This chapter explores the microbial-assisted synthesis of nanoparticles and their applications in sustainable agriculture, bioremediation, environmental monitoring, detection and management. Finally, the limitations and opportunities of biosynthesized NPs in terms of development and mitigating drawbacks have been addressed.

17.2 Microbial nanotechnology

Chemically generated nanoparticles may have several drawbacks of chemical consumption and aqueous solution self-agglomeration. As a result, green nanoparticle manufacturing using plant extracts, fungal and bacterial enzymes could be a viable option and nanotechnology can be made more sustainable and environmentally friendly through their bio-fabrication and the concomitant use of microbes. Mahanty et al. (2020) obtained *Aspergillus tubingensis* (STSP 25)

biofabricated iron oxide nanoparticles from the soil samples (rhizosphere) of *Avicennia officinalis* in the Sundarbans (WB), India. Similarly, exopolysaccharides (EPS) obtained from *Chlorella vulgaris* were utilized to co-precipitate with iron oxide (Fe_3O_4) nanoparticles in another research. The effective alteration of nanoparticles by functional groups of EPS was discovered using Fourier-transform infrared spectroscopy. Furthermore, the nanocomposite was capable of removing 91% of PO_4^{3-} and 85% of NH_4^+ (Govarthanan et al., 2020). The use of microbes to synthesize nanoparticles has proven to be a cost-effective and environmentally beneficial technique. Studies were carried on copper nanoparticles made from copper resistant bacteria, *Escherichia* sp. SINT7. It was discovered that biogenic nanoparticles degraded azo dye and textile wastewater. The azo dyes, such as Congo red, malachite green, direct blue-1, and reactive black-5 were 97.07, 90.55, 88.42 and 83.61, respectively, at a lower concentration of 25 mg/L, whereas at a higher concentration of 100 mg/L these were reduced to 76.84%, 83.90%, 62.32% and 31.08%, respectively. The industrial effluent(s) was also treated, and the suspended particles, chloride, and phosphate ions were also reduced by these biogenic nanoparticles. The synthesized nanoparticles remove more than 90% of heavy metals [Pb (II), Cu (II), Ni (II) and Zn (II)] from wastewater with a regeneration capability of up to five cycles. In endothermic processes, metal ions were adsorbed chemically on the surface of the nanoparticles (Mahanty et al., 2020). Cheng et al. (2019) used no additional sulfur to make iron-sulfur nanoparticles. The extracellular transport of electrons allowed these nanoparticles to break down naphthol green B dye. The utilization of *Pseudoalteromonas* sp. CF10–13 in nanoparticle manufacturing gives a sustainable biodegradation approach. The generation of hazardous gases and metal complexes was suppressed by the endogenous synthesis of nanoparticles. The utilization of biogenic particles in the cleanup of industrial effluents is a superior method. Apart from directly producing nanoparticles, microorganisms can aid in the advancement of nanotechnology in several other ways. Microorganisms, for example, could supply catalytic enzymes that, in combination with nanoparticles, aid in wastewater cleanup and play a key role in converting industrial waste into useful products.

17.3 Synthesis of nanoparticles from microbes

Microbes produce nanoparticles using a bottom-up method in which the majority of reactions involve reduction or oxidation. The underlying idea behind bioremediation is that toxic contaminants are converted into less hazardous products. Microbe-produced nanoparticles could convert contaminants into products with lower toxicity, mobility and solubility (Wasi et al., 2008). At room temperature, biologically synthesized metallic nanoparticles are much more stable than chemically generated metallic nanoparticles for longer periods (Balakrishnan et al., 2017). By using correct methodologies, the overall cost of nanoparticle production could be reduced to 1/10th of the cost of chemical synthesis processes. With a very little amount of biogenic nanoparticles, a significant quantity of pollutants may be eliminated. The biosynthetic processes are stabilized by the capping of microbial proteins over the metallic particle surfaces. These biogenic nanoparticles have a wide surface area and high catalytic reactivity. Microbes are capable of producing nanoparticles both intracellularly and extracellularly. Zhang et al. (2011) discovered gold nanoparticles synthesized in filamentous fungi vacuoles and thus, described the role of fungal proteins in gold nanoparticle capping. According to Mishra et al. (2014a,b), extracellular biosynthesis is preferred since it is a low-cost procedure and does not require downstream processing. With the use of a detoxification mechanism, microorganisms can absorb precursor metal ions and generate the corresponding nanoparticles (Mohseniazar et al., 2011). The use of microorganisms in the synthesis of the nanoparticle is also a cost-effective technique, as it does not require, high energy and capping or stabilizing agents (Makarov et al., 2014). It's difficult to create fine, homogeneous, and useful nanoparticles under normal conditions (Tang et al., 2017) but fabrication of metallic nanoparticles can be done in a cost-effective, safe, and eco-friendly manner using biogenic resources (Ovais et al., 2018). For scientists, the benefits of using natural biological resources as nano factories include well-defined morphologies ease of manufacturing, scaling, and higher biocompatibility (Singh et al., 2016a,b). As mentioned below and included in Tables 17.1, 17.2, 17.3, 17.4 and 17.5, microorganisms, such as fungi, viruses, yeast, algae, bacteria and actinomycetes have already been widely employed to produce nanoparticles with silver, gold, silicon, copper, nickel, lead and cadmium.

17.3.1 Metallic nanoparticle production assisted by filamentous fungi

Filamentous fungi can be employed as a source of nanoparticles. Fungi mycelium is comprised of a large surface area and secretes a large variety of proteins that can directly contribute to nanoparticle production (Mohanpuria et al., 2008). Because of their ability to secrete proteins, metabolites and enzymes along with scaling up and downstream handling, economic feasibility, increased surface area due to mycelia, and low-cost production procedures, filamentous fungi are preferred for nanoparticle production (Fouda et al., 2018; Spagnoletti et al., 2019). Due to the presence of several

TABLE 17.1 Biological synthesis of metal nanoparticles using fungi species.

S. no.	Fungi	Metal nanoparticles	References
1.	<i>Aspergillus niger</i>	Silver	Gade et al., (2008)
2.	<i>Pleurotussajor-caju</i>	Silver	Nithya and Raghunathan, (2009)
3.	<i>Fusarium solani</i>	Silver	Ingle et al., (2009)
4.	<i>Coriolus versicolor</i>	Silver	Sanghi and Verma, (2009)
5.	<i>Penicillium fellutanum</i>	Silver	Kathiresan et al., (2009)
6.	<i>Trichoderma viride</i>	Silver	Fayaz et al., (2010)
7.	<i>Epicoccum nigrum</i>	Silver	Qian et al., (2013)
8.	<i>Fusarium oxysporum</i>	Silver	Husseiny et al., (2015)
9.	<i>Guignardia mangiferae</i>	Silver	Balakumaran et al., (2015)
10.	<i>Arthroderma fulvum</i>	Silver	Xue et al., (2016)
11.	<i>Colletotrichum</i> sp. ALF2–6	Silver	Azmath et al., (2016)
12.	<i>Fusarium oxysporum</i>	Silver	Hamed et al., (2017)
13.	<i>Rhizopus stolonifera</i>	Gold	AbdelRahim et al., (2017)
14.	<i>Trichoderma ongibrachaitum</i>	Silver	Elamawi et al., (2018)
15.	<i>Aspergillus fumigatus</i> BTCB10	Silver	Shahzad et al., (2019)

TABLE 17.2 Biological synthesis of metal nanoparticles using yeast species.

S. no.	Yeast	Metal nanoparticles	References
1.	<i>Pichia jadinii</i>	Au	Gericke and Pinches (2006)
2.	<i>Candida glabrata</i>	CdS	Dameron and Winge (1990)
3.	<i>Yarrowia lipolytica</i> NCIM3589	Au	Agnihotri et al., (2009)
4.	<i>Saccharomyces cerevisiae</i>	Au	Sen et al., (2011)
5.	<i>Saccharomyces cerevisiae</i>	TiO ₂	Jha et al., (2009)
6.	<i>Rhodotorula mucilaginosa</i>	Ni/NiO	Salvadori et al. (2016)
7.	<i>Aphanotroche</i> sp.	Silver	Sudha et al., (2013)
8.	<i>Sargassum muticum</i>	Silver	Azizi et al., (2013)
9.	<i>Caulerpa cernosa</i>	Silver	Kathiraven et al., (2015)
10.	<i>Sargassum longifolium</i>	Silver	Rajeshkumar et al., (2014)
11.	<i>Bifurcaria bifurcata</i>	Copper oxide	Abboud et al. (2014)
12.	<i>Sargassum bovinum</i>	Palladium	Momeni and Nabipour (2015)
13.	<i>Sargassum ilicifolium</i>	Aluminum oxide	Koobi and Buazar, (2018)
14.	<i>Sargassum tenerrimum</i>	Gold	Ramakrishna et al., (2016)
15.	<i>Chlorococcum humicola</i>	Silver	Jena et al. (2013)
16.	<i>Rhodotorula mucilaginosa</i>	Ni/NiO	Salvadori et al. (2016)

TABLE 17.3 Biological synthesis of metal nanoparticles using algae.

S. No.	Algal species	Metal nanoparticles	References
1.	<i>Bifurcaria bifurcata</i>	Copper oxide	Abboud et al. (2014)
2.	<i>Caulerpa racemose</i>	Silver	Kathiraven et al. (2015)
3.	<i>Chlorella vulgaris</i>	Gold	Luangpipat et al. (2011)
4.	<i>Chlorococcum humicola</i>	Silver	Jena et al. (2013)
5.	<i>Chondrus crispus</i>	Gold, Silver	Castro et al. (2013)
6.	<i>Fucus vesiculosus</i>	Gold	Mata et al. (2009)
7.	<i>Galaxaura elongata</i>	Silver	Abdel-Raouf et al. (2013)
8.	<i>Gracilaria dura</i>	Silver	Shukla et al. (2012)
9.	<i>Kappaphycus alvarezii</i>	Gold	Rajasulochana et al. (2010)
10.	<i>Padina pavonica</i>	Silver	Sahayaraj et al. (2012)
11.	<i>Pichia capsulata</i>	Silver	Manivannan et al., (2010)
12.	<i>Rhodospiridium diobovatum</i>	Lead	Seshadri et al. (2011)
13.	<i>Sargassum plagiophyllum</i>	Silver chloride	Dhas et al. (2014)
14.	<i>S. wightii</i>	Gold	Singaravelu et al. (2007)
15.	<i>S. wightii</i>	Silver	Singaravelu et al. (2007)
16.	<i>Spirulina platensis</i>	Gold/ Silver	Govindaraju et al. (2009)
17.	<i>Spyrogira insigni</i>	Gold, Silver	Castro et al. (2013)
18.	<i>Tetraselmis kochinensis</i>	Gold	Senapati et al. (2012)
19.	<i>Turbinaria conoides</i>	Gold	Rajeshkumar et al. (2013)
20.	<i>Ulva fasciata</i>	Silver	Rajesh et al., (2012)

TABLE 17.4 Biological synthesis of metal nanoparticles using bacterial species.

S. no.	Bacterial species	Metal nanoparticles	References
1.	<i>Lactobacillus</i> species	Titanium	Prasad et al., (2007)
2.	<i>Pseudomonas putida</i> NCIM2650	Silver	Thamilselvi and Radha, (2013)
3.	<i>Escherichia coli</i> (DH5a)	Silver	Ghorbani, (2013)
4.	<i>Serratia nematodiphila</i>	Silver	Malarkodi et al., (2013)
5.	<i>Bacillus</i> strain CS11	Silver	Das et al., (2014)
6.	<i>Bacillus methylotrophicus</i>	Silver	Wang et al., (2016)
7.	<i>Pseudomonas fluorescens</i> CA417	Silver	Syed et al., (2016)
8.	<i>Novosphingobium</i> species	Silver	Du et al., (2017)
9.	<i>Bacillus amyloliquefaciens</i>	Titanium dioxide	Khan and Fulekar, (2016)
10.	<i>Pseudomonas</i> sp. ef1	Silver	John et al., (2020)
11.	<i>Brevibacillus formosus</i>	Gold	Srinath et al., (2017)

TABLE 17.5 Biological synthesis of metal nanoparticles using Actinomycetes species.

S. no.	Actinomycetes	Metal nanoparticles	References
1.	<i>Streptomyces hygroscopicus</i>	Gold	Waghmare et al., (2014)
2.	<i>Streptomyces kasugaensis</i> NH28 strain	Silver	Składanowski et al. (2016)
3.	<i>Streptomyces capillispiralis</i>	Copper	Hassan et al., (2018)
4.	<i>Streptomyces</i> species	Silver	El-Gamal et al. (2018)
5.	<i>Rhodococcus</i>	Gold	Lengke et al. (2007)
6.	<i>Brevibacterium casei</i>	Gold	Kalishwaralal et al. (2010)
7.	<i>Thermonospora</i> sp.	Gold	Sastry et al. (2003), Ahmad et al. (2003)
8.	<i>Arthrobacter globiformis</i> 151B	Gold	Kalabegishvili et al. (2012)

enzymes in their cells, filamentous fungi are considered a better choice for the production of metallic nanoparticles (Khandel and Shahi, 2018). Filamentous fungi have metal absorption capacity and can be cultured in large quantities using solid substrate fermentation. Different filamentous fungus species grow rapidly and are easy to maintain in laboratory conditions (Fouda et al., 2018). Nanoparticles having nanoscale dimensions synthesized from fungi have more monodispersity than those made by bacteria. In aqueous AuCl_4 ions, *Fusarium oxysporum*, releases reducing agents into the solution through an NADH-enzyme-mediated process, resulting in the production of gold nanoparticles. The protein binding ability of the synthesized nanoparticles, which is achieved by linking lysine and cysteine residues, ensures long-term stability (Das et al., 2017). The filamentous fungi have a strong regenerative capacity along with eco-friendly manufacturing for the synthesis of metal nanoparticles in large quantities with commercial viability (Bansal et al., 2005). Heavy metals were absorbed by, *Aspergillus niger*, *Aureobasidium pullulans*, *Cladosporium resinae*, *Funalia troglia*, *Ganoderma lucidum*, *Penicillium species*, *Rhizopus arrhizus* and *Trametes versicolor* from polluted sites when used to make nanoparticles (Say et al., 2003). Salvadori et al. (2013) reported the Cu(II) absorption by dead biomass and synthesis of copper nanoparticles *Hypocrea lixii*. Both extracellular and internal NiO nanoparticles were produced by the same bacterium (Salvadori et al., 2015). According to (Salvadori et al., 2014), the dead biomass of *Aspergillus aculeatus* produces Nickel oxide (NiO) nanoparticles that were arranged in the form of a film. Similarly, Au-Ag nanoparticles can also be synthesized extracellularly from *Fusarium oxysporum* when it was treated with an equimolar mixture silver nitrate and tetrachloroaurate ion (Senapati et al., 2005) and it can also produce platinum nanoparticles with hexachloroplatinic acid (Riddin et al., 2006). *Verticillium luteoalbum* was discovered by Gericke and Pinches (2006) to synthesize gold nanoparticles. After being exposed to chloroauric acid solution, *Verticillium* species generated gold nanoparticles within their cells. The structure of the gold nanoparticles was unaffected by age, but the number of nanoparticles was considerably reduced when older cells were used. The *Fusarium oxysporum* biomass was used to make silver (Ag) nanoparticles (Karbasiyan et al., 2008).

17.3.2 Synthesis of metallic nanoparticles using yeast

The majority of yeast genera are known to accumulate substantial amounts of heavy metals as the presence of metallothioneins, glutathione and phytochelatins present inside their cells are involved in the detoxification process. Because yeast cells can create semiconductor nanoparticles like cadmium and lead sulfide, Dameron and Winge (1990) referred to them as semiconductor crystals or quantum semiconductor crystals. The cellular enzymes of *Pichia jadinii* were used to make gold nanoparticles by reducing gold ions (Gericke and Pinches, 2006). *Yarrowia lipolytica* is also known to produce gold nanoparticles both intracellularly and extracellularly (Pimprikar et al., 2009). Dameron and Winge (1990) reported the production of cadmium nanoparticles by *Candida glabrata* and *Schizosaccharomyces pombe*. *Rhodospiridium diobovatum* has been utilized to produce intracellularly stable lead sulfide nanoparticles (Seshadri et al., 2011). *Magnusiomyces ingens* LHF1 was used by Lian et al. (2019) to produce stable selenium nanoparticles. *Saccharomyces boulardii*, *Candida albicans* and *Candida utilis* can also be utilized for the synthesis of silver nanoparticles (Soliman et al., 2018). Because of the presence of peptide coating, the biogenic nanoparticles did not clump together and had very high stability than nanoparticles synthesized through chemical procedures. The yeast

Schizosaccharomyces pombe and *Candida glabrata* generated quantum crystallites when grown in cadmium salts (Williams et al., 1996). Nickel and cadmium in lower concentrations caused a considerable accumulation of metal-binding proteins in *Yarrowia lipolytica* (Strouhal et al., 2003). Apart from resistance to heavy metals, it also aids in the breakdown of hydrocarbons (Bankar et al., 2009). Further, it can be utilized to produce metallic nanoparticles as well as to remove environmental contaminants like heavy metals. Benedito Correa and his colleagues were awarded two patents for the production of copper nanoparticles and the application of fungal species for wastewater cleanup and industrial level synthesis of copper nanoparticles was achieved in a cheap and environmentally friendly manner.

In a nutshell, the processes involves soaking the dead biomasses of *Rhodotorula mucilaginosa*, as well as the *Hypocrea lixii* and *Trichoderma koningiopsis* in aqueous solution containing copper, removing the metal and simultaneously synthesizing metallic copper nanoparticles. Thus this process produces nanoparticles of metallic copper as a final product, as well as facilitate the removal of the contaminated transition metal (copper) from the affected area. Being a low-cost and environmentally beneficial procedure. *Hypocrea lixii*, *Rhodotorula mucilaginosa* and *Trichoderma koningiopsis* had copper removal capabilities and concurrent transformation into metallic copper NPs of 26.2, 19.0, and 21.1 mg/g, respectively (Correa et al., 2014). Salvadori et al. (2016) explained how the dead organic matter of *Rhodotorula mucilaginosa* produces magnetic spherical nanoparticles of nickel (Ni) or nickel oxide (NiO). The formation of magnetic metallic nanoparticles from liquid waste with harmful metals contamination, which can result in effluent detoxification and safe environmental release, can make this technology commercially valuable. Table 17.2 summarizes the results of yeast-mediated metallic nanoparticle production.

17.3.3 Synthesis of metallic nanoparticles using algae

Algae are oxygenic photoautotrophs that can be exploited to make nanoparticles in water (Castro et al., 2013). Algae's bio reduction capacity revealed tremendous potential in the green synthesis of several metal oxide nanoparticles, including gold, silver, platinum, copper oxide, palladium and zinc oxide (Konishi et al., 2007; Xie et al., 2007; Oza et al., 2012; Momeni and Nabipour, 2015). These can create both internal and extracellular complex inorganic nanomaterials (Sau and Murphy, 2004) such as the gold nanoparticles can be synthesized both intracellularly and extracellularly in *Lyngbya majuscula* and *Rhizoclonium fontinale* as well as in *Spirulina subsalsa* (Chakraborty et al., 2009). Electrostatic interactions between metal ions and negatively charged carboxylate groups can attach metallic ions to the cell surface. Later, enzymes can reduce ions, resulting in nucleus formation and growth as metal ions are reduced (Parial et al., 2012). The accumulation of 9–20 nm gold particles in cell solution of dried *Chlorella vulgaris* has also been reported (Hosea et al., 1986). The gold (I)–sulfide nanoparticles were also recovered from the cell wall of *P. boryanum* in aqueous AuCl₃ solution. Cyanobacteria reduce gold (III)–chloride to metallic gold to form Au (I) and gold (I)–sulfide in the process (Lengke et al., 2006). Within 48 hours, *Dunaliella salina*, *Chlorella vulgaris* and *Nannochloropsis oculata* can create silver nanoparticles intracellularly with a diameter of less than 15 nm (Mohseniazar et al., 2011). The synthesis of metallic nanoparticles by algal species is highlighted in Table 17.3.

17.3.4 Metallic nanoparticle synthesis assisted by bacteria and actinomycetes

The use of bacteria to produce nanoparticles is a profitable approach since it does not necessitate the use of expensive and harmful chemicals in the synthesis and stabilizing processes. Table 17.4 lists bacteria as prospective bio-resources for the production of nanoparticles, such as silver, gold, platinum, titanium, titanium dioxide, palladium, cadmium sulfide, magnetite and others. Bacteria are significant for nanoparticle manufacturing as they show an ability to adapt to harsh environmental conditions (Wang et al., 2017). Some of these can convert or precipitate, soluble harmful inorganic ions into non-hazardous insoluble metallic nanoparticles (Garole et al., 2018). Under various physicochemical parameters such as exposure time,

TABLE 17.6 Biological synthesis of metal nanoparticles using virus.

S. No.	Virus	Metal nanoparticles	References
1.	Tobacco mosaic virus	CdS, PbS, SiO ₂ and Fe ₂ O ₃	Shenton et al. (1999)
2.	M13 bacteriophage	ZnS, CdS	Mao et al. (2003)
3.	Tobacco mosaic virus	Gold	Kobayashi et al., (2012)

temperature, pH, bacterium concentration and metal salts, bacteria can create nanoparticles with metals and metalloids both intra or extracellularly. In an extracellular process, biomolecules in the medium, and cell wall and its components can decrease metal ions. However, in the intracellular process, metal and metalloids are attracted to functional groups of the cell wall by electrostatic interactions, where metal ions interact with protein to produce nanoparticles. Extracellular reduction looks to be more beneficial than intracellular reduction due to the ease of extraction and high efficiency. A dead bacteria can also be used to synthesize nanoparticles in the same way that live bacteria can (Fang et al., 2019). As a biological platform for mineralization, bacteria can be used as a biocatalyst (Iqtedar et al., 2019). Metals can be mobilized or immobilized, and metal ions can be reduced or precipitated by bacteria. Bacteria may catalyze a variety of processes and create inorganic nanoparticles because of their enzymes (Iravani, 2014). Using the extracellular secretion of bacterial enzymes, vast amounts of nanoparticles (100–200 nm in size) can be created in a pure form. The ability of bacterial cells and the S-layer to bind metals makes them effective in bioremediation. Metals may seep into the cytoplasm via the cell wall and then be transported back for extracellular secretion, making the cell wall of bacteria extremely important. Chemical processes for certain groups, such as amines and carboxyl groups, can modify the cell wall with metal-binding sites, converting positive charge to negative.

Actinomycetes can play an important role in the manufacturing of metallic nanoparticles. Under extreme environmental circumstances, such as alkaline conditions, high temperature, and certain *Thermomonospora* species are known to create gold ions. Alkali-tolerant actinomycetes from *Rhodococcus* species were driven to produce gold nanoparticles with a diameter of 5–15 nanometers (Ahmad et al., 2003). In comparison to the cell membrane, the cell wall has a high concentration of nanoparticles. Because of their enormous surface area and ability to secrete secondary compounds, actinomycetes are a good source for metallic nanoparticle and can be produced both inside and outside of cells (Hassan et al., 2018; Manivasagan et al., 2016). *Rhodococcus* sp., *Nocardia farcinica*, *Streptomyces hygroscopicus*, *Thermoactinomyces* sp., *Streptomyces viridogens* and *Thermomonospora* species were used to generate gold nanoparticles (Składanowski et al., 2016). *Streptomyces* species were also used to produce zinc, copper, manganese and silver nanoparticles (El-Gamal et al., 2018). The creation of metallic nanoparticles by actinomycetes is highlighted in Table 17.5.

17.3.5 Synthesis of metallic nanoparticles using virus

The utilization of viruses in nanoparticle synthesis is a promising method for producing inorganic nanomaterials, such as cadmium sulfide, iron oxide, silicon dioxide and zinc sulfide and their use in the synthesis of quantum dots was described by Zeng et al. (2013). Table 17.6 summarizes the data on virus-mediated metallic nanoparticle generation. The usage of protein frameworks, DNA recognizing linkers and surfactant designed pathways has caused a challenge in the manufacture of inorganic nano-crystals in bacteria and fungus. However, these limitations can be overcome by using modified viruses to create self-assemble surface(s) having quantum dot structures having monodispersity in form and size, as well as nanoparticle length. Nanoparticles produced by the genetically engineered tobacco mosaic virus can modify inorganic nano-crystals in 3D materials (Shenton et al., 1999). With their application in the pharmaceutical business, the manufactured viral films can be preserved for longer times and stored with high-density designed DNA (Mao et al., 2003). Douglas et al. (2002) observed the mineralization of inorganic materials using cowpea mosaic virus and chlorotic mottle virus. Tobacco mosaic virus (TMV) aids in the mineralization of sulfide and crystalline nanowires (Shenton et al., 1999).

17.4 Microbial/green synthesis of nanoparticles and advantages over nonbiological synthesis

Several scientific breakthroughs have been made in the field of nanotechnology, and several methods for nanoparticle synthesis have been established and proposed (Sabri et al., 2016). Currently, nanotechnology is concentrating on a variety of biological ways for producing nontoxic and ecologically friendly nanoparticles. As a result, bionanotechnology was born, which uses a wide range of microbes, such as bacteria, algae, fungi and plant extracts. Carbohydrates, proteins, and lipids produced from plants have also been reported to catalyze the creation of nanoparticles. The Green technology is based on fundamental rules for nanoparticle production and is exclusively reliant on techniques that produce fewer byproducts, nontoxic compounds, and less expensive resources while using the least amount of energy (Iravani et al., 2017). Nanotechnology research and development has so far mostly centered on the manufacture of molecules in the nanoscale range, with nanoparticle synthesis taking place by physical, chemical, and biological processes. The methods used to make them are usually determined by the properties of the bulk material being employed. Physicochemical methods, compared to biological processes, have several limitations, including the use of huge volumes of poisonous chemicals, high cost, the generation of unnecessary

secondary products, the necessity for high heat conditions and a high input of continual energy supply (Ghorbani, 2016). Because of their high toxicity, chemically produced nanomaterials cannot be used in health management (Khan, 2013). In a few circumstances, however, physicochemical strategies for particular nanoparticle creation are required because biological procedures are currently unavailable.

17.5 Microbial nanoparticles and sustainable agriculture

Nanotechnology has been receiving enormous attention in the agricultural sector because it shows the potential to increase agricultural output while reducing energy and cost input. Nanoparticles have already been employed in several applications due to their size specificity, in the form of nano-pesticides, nano-fertilizers, nanosensors, nano-herbicides and innovative delivery systems for controlled and slow release of agrochemicals (Oliveira et al., 2014; Grillo et al., 2016). In addition, current agricultural research is focusing on expanding the use of nanotechnology in major crop improvement disciplines like genetic engineering and plant breeding by producing nanotechnology-based devices, among other things (Jiang et al., 2018). In agriculture, the use of nanoparticles has been demonstrated, specifically in the major areas of disease management and crop productivity (Mishra et al., 2014a,b; Mishra and Singh, 2016). Further, Nano-encapsulation of seeds, nanoparticle-mediated genetic material, pesticides, carbon nanotube-assisted seed germination, nano-fertilizer, nano-pesticides and nano-herbicides for weed elimination are all promising applications that are helpful in crop improvement program and disease management (Campos et al., 2015; Liu and Lal, 2015). Among nanoparticles, silver (Ag) nanoparticles, have been widely recognized in plant pathology for their antibacterial capabilities against a variety of phytopathogen-caused plant illnesses, and are highly effective in reducing fungal and bacterial pathogens in plant disease management. In vitro testing revealed that silver nanoparticles inhibited the growth of tested pathogens, but had a substantial impact on *Magnaporthe grisea* proliferation. Silver ions and nanoparticles significantly reduced the growth of perennial ryegrass (*Lolium perenne*) when tested in vivo. When applied 3 hours before pathogen entrance, there is a reduction in disease severity (Jo and Kim, 2009). *Bipolaris sorokiniana*, which causes spot blotch disease in wheat, was successfully controlled when biogenic nanoparticles were produced from *Serratia* sp. BHU-S4 was used similarly (Mishra et al., 2014a,b).

17.5.1 Applications in agriculture

In agriculture, NPs are used as, nano-pesticides, nano-fungicides, nano-fertilizers and nano-insecticides. The following are some of the most important applications of NPs in agriculture.

17.5.1.1 Nanoparticles as fungicides

Metal nanoparticles and metal oxide nanoparticles have good antifungal activities. Fungi are among the most destructive plant pathogens, causing a variety of diseases in crops. Copper and copper oxide NPs synthesized from *Streptomyces* spp. have been reported to have strong antifungal properties against a variety of pathogenic fungi, including *Alternaria alternata*, *Aspergillus niger*, *Fusarium oxysporum* and *Pythium ultimum*, (Hasan et al., 2018; Hassan et al., 2019). Kaur et al. (2018) investigated the antifungal activity of silver NPs (derived from *Achromobacter* sp. and *Pseudomonas* sp.) in chickpea against *Fusarium oxysporum* infection. In this study, biosynthesized silver NPs were used for in vivo and in vitro pot studies against *F. oxysporum* and it was discovered that they had a very significant antifungal activity. As a result, NPs can be employed as fungicides to treat fungal infections in plants. However, the dosage and toxicity of NPs remain major concerns for their commercialization as fungicides.

17.5.1.2 Nanoparticles as fertilizers

Overuse of chemical fertilizers has resulted in ever-decreasing soil fertility, which is a major challenge for the agriculture sector. Alternative nano-fertilizers made from NPs are harmless, effective and ecologically friendly (Salem and Fouda, 2021). Bisinoti et al. (2019) employed carbon-based nanomaterials as soil fertilizers in a recent paper, concluding that nanomaterial-based fertilizers can reduce the need for chemical fertilizers. Subbarao et al. (2013) revealed that covering nanomaterial on potash fertilizer led it to release slowly, which can reduce fertilizer loss and chemical fertilizer misuse (Duhan et al., 2017). Guo et al. (2018) discovered that zeolites and nanoclay crystals can be employed as fertilizers in other investigations.

17.5.1.3 Nanoparticles as pesticides

Nanoparticles can be employed to make nano-pesticides. NPs can be found in the form of particles, micelles, nanopolymers (organic constituents) and metal oxides in nano-pesticides (an inorganic constituent). Several new formulations incorporating NPs have been developed and utilized as insecticides in recent years. Many studies have shown that metallic NPs can be employed as effective insecticides against many insects and pests that cause significant loss in agricultural production (Duhan et al., 2017; Rai and Ingle, 2012; Goswami et al., 2010). Wang et al. (2007) for example, demonstrated that a new oil-water (O/W) based nanoemulsion may be employed to successfully transport the insecticide beta-pypermethrin (beta-PP). In their study they noticed that beta-PP coated on O/W nanoemulsion spread more rapidly than regular beta-PP, reducing overall pesticide consumption. Goswami et al. (2010) found that several NPs, such as silver, zinc oxide, aluminum oxide and titanium oxide NPs, can suppress illnesses induced by the baculovirus nuclear polyhedrosis virus in *Sitophilus oryzae* and *Bombyx mori*. As a result, NPs can be utilized as insecticides. However, the dosage of NPs and their plant toxicity remain significant barriers to their commercialization as pesticides.

17.6 Environmental applications of microbial nanoparticles

Nanobioremediation refers to the process of using nanomaterials to boost microbial activity to clean up the environment. Nanomaterials have unique physicochemical features for the removal of heavy metals, such as a low melting point, magnetization, strong surface reactivity, and a comparatively greater surface area. For environmental cleanup, a biological strategy that assists the eco-friendly path of nanoparticle synthesis was used. In heavy metal removal, the surface area and kind of nanoparticle play a major role. Iron oxide nanoparticles have been extensively used for heavy metal removal from contaminated water due to their high surface area, superparamagnetic characteristics and small size (Yadav et al., 2017; Thekkudan et al., 2017). The process of chromium ion removal by myco-synthesized iron oxide nanoparticles was investigated using X-ray photoelectron spectroscopy and *Aspergillus niger*-derived iron oxide nanoparticles were successfully employed in chromium remediation. Adsorption of ions onto the surface of nanoparticles, iron (Fe (II)) reduces Cr (VI) to Cr (III) and synthesis of iron-chromium complex and precipitation of $\text{Cr}(\text{OH})_2$ and Cr_2O_3 are three proposed methods for removing chromium ions from aqueous solutions. Batch adsorption experiments revealed the optimum parameters, which included a pH of 3, a temperature of 40°C, and an adsorbent dosage of 2.5 g/L (Chatterjee et al., 2019). At adsorbent concentrations of 1 g/L and pH of 2, iron oxide nanoparticles generated from *Penicillium pinitouense* effectively excluded chromium ions from synthetic solution for 120 minutes, with a maximum adsorption capacity of 4.62 mg/g. The thermodynamic analysis found that nanoparticle adsorption is both spontaneous and endothermic (Mahanty et al., 2019). Palladium nanoparticles made from *Shewanella loihica* and electrochemically active biofilms (EAB) showed good catalytic activity in methyl orange dye degradation (Ahmed et al., 2018). The mineralization of Bismarck Brown dye and the loss of color occurred as a result of the creation of zinc oxide nanoparticles by *Aspergillus niger*. These nanoparticles hold industrial applications such as waste water treatment due to the catalytic effectiveness of Bismarck brown dye degradation. After 72 hours of incubation with the nanomaterials, maximum degradation was discovered (Kalpana et al., 2018). With sodium borohydride (NaBH_4) as a reducing agent, cell-linked gold and silver nanoparticles produced from *Pseudomonas lipolytica* were used to decolorize methylene blue and Congo red dyes. AuNPs facilitate the transport of electrons from BH_4 (donor) to methylene blue dye and were found to have better catalytic efficiency about Congo red decolorization. *Pseudomonas lipolytica*-associated nanoparticles stabilized by peptides efficiently decolorized two hazardous dyes (Kulkarni et al., 2018). The recovery of oil has been studied by using biosynthesized silica nanoparticles from *Saccharomyces cerevisiae* with a precursor, sodium silicate. The oil recovery efficiency of these nanoparticles was higher than that of commercial silica nanoparticles. The enhanced viscosity of the nanofluid and the lowering of interfacial tension resulted in higher production efficiency (Zamani et al., 2020). The reduction of 4-nitrophenol by catalysis is essential since it is one of the environmental contaminants generated during the manufacturing of insecticides, pesticides, and herbicides. Because 4-aminophenol has a wide range of applications as a corrosion inhibitor, anticorrosion lubricant, etc. thus, reducing 4-nitrophenol to 4-aminophenol is a major environmental and economic concern. When 4-nitrophenol is reduced in the presence of sodium borohydride, then 4-nitrophenolate ion is formed. Gold nanoparticles from *Sporosarcina koreensis*, this ion is transformed to 4-aminophenol (Singh et al., 2016a,b). Similarly, AuNPs generated by *Magnusiomyces ingens* cells were an effective nitrophenol reduction catalyst (Zhang et al., 2016). For the treatment of drinking water, copper oxide nanoparticles made from *Anabaena cylindrica* extract were used. The maximum potential number of colonies/100 mL was decreased to nil after infected drinking water was treated with nanoparticles at a concentration of 40 mg/mL. It also had antibacterial activity in food due to positively charged copper oxide to the negatively charged

TABLE 17.7 Environmental uses of microbial synthesized nanoparticles.

S. no.	Type of nanoparticle	Organism	Environmental applications	References
1.	Iron oxide	<i>Penicillium pimiteouiense</i>	Chromium removal	Mahanty et al. (2019)
2.	Copper	<i>Escherichia</i> sp.	Azo dye degradation	Noman et al. (2019)
3.	Silica	<i>Saccharomyces cerevisiae</i>	Oil recovery	Zamani et al. (2020)
4.	Silver	<i>Aspergillus flavus</i>	Malachite green dye Nano-sorption	Karthikeyan et al., (2016)
5.	Silver	<i>MKH1 bacterium</i>	Hydrazineelectro-oxidation	Rostami et al., (2018)
6.	Silver	<i>Leuconostoc lactis</i>	Methyl Orange and Congo Red dye degradation	Saravanan et al., (2017)
7.	Silver	<i>Macrophomina phaseolina</i>	Crop protection	Spagnoletti et al. (2019)
8.	Gold, Silver	<i>Pseudoalteromonas lipolytica</i>	Methylene and Congo red decolorization	Kulkarni et al. (2018)
9.	Copper oxide	<i>Anabaena cylindrical</i>	Disinfection of drinking water	Bhattacharya et al. (2018)
10.	Palladium	<i>Shewanella loihica</i>	Methyl orange decomposition	Ahmed et al. (2018)
11.	Tellurium	<i>Raoultella</i> sp., <i>Escherichia</i> sp.	Tellurite reduction	Ahmed et al. (2018)

peptidoglycan found in bacteria's cell walls (Bhattacharya et al., 2018). Similarly, harmful bacteria have been removed from wastewater using AgNPs from the filamentous fungus *Penicillium citreonigum* Dierck and *Scopulaniopsis brumptii* Salvanet-Duval. Silver nanoparticles (AgNPs) are coated onto the surface of polyurethane foams after treatment with a silver nanoparticle solution. With 15 and 120 minute contact intervals, a concentration of 676.9 mg/L was sufficient to reduce the bacterial population to zero. Both gram-positive and gram-negative bacteria were effectively reduced by nanoparticles (Moustafa, 2017). Mercury ions (Hg^{2+}) were detected using gold nanoparticles made from the fungus *Trichoderma harzianum*. Mercury, a heavy metal, harms the environment. As a result, detecting Hg^{2+} is critical for pollution control. Due to the high cost of detection instrumentation, a quick method of detecting mercury ions using gold has been developed. As a result, detecting Hg^{2+} is critical for pollution control. Due to the high cost of detection equipment, a fast approach to detecting mercury ions using gold nanoparticles has been investigated. Cysteine molecules with thiol groups are used to cap biosynthesized AuNPs. The thiol group forms a bond with Au due to its high affinity, leading to the Au-S bond formation. SH group bonds with mercury ions in the presence of Hg^{2+} ions, which may then be detected for Hg^{2+} using a colorimetric approach, which is a quick and selective procedure (Tripathi et al., 2014). Copper nanoparticles produced from *Escherichia* sp. were used to study the photocatalytic degradation of dyes. For methyl red, methylene blue, and Congo red dye, the decolorization percentages were around 73.89%, 91.53% and 84.89%, respectively. They were also utilized for the wastewater effluent from the textile industry (Noman et al., 2019). Following the addition of *Clostridium pasteurianum* culture, a reduction of molybdenum ions to roughly 88% was observed during the formation of molybdenum nanoparticles. Furthermore, rather than employing palladium nanoparticles, the produced Mo nanoparticles could serve as an excellent catalyst for the rapid degradation of azo dyes like methyl orange within 6 minutes for a 155 mM dye concentration (Nordmeier et al., 2018). The photocatalytic degradation of methyl violet was studied using silver nanoparticles produced by *Bacillus amyloliquefaciens*. Within 20 minutes of incubation, 85.56% of the methyl violet was degraded. The steps in a proposed mechanism are as follows. By colliding nanoparticles, electrons present in the surface of the particles are energized due to exposure to sunlight. The electrons are converted into oxygen anion radicals by dissolved oxygen present in the reactive media. As a result, the radicals generated are involved in the breakdown of complex organic dyes into simpler molecules, resulting in dye degradation (Jishma et al., 2018). Due to the high surface area to volume ratio, silver nanoparticles produced by

cyanobacteria were found to be effective in the degradation of methyl red, an azo dye, with a maximum removal percentage of roughly 84.6% (Husain et al., 2019). Graphite carbon nitride based catalytic materials have been used exclusively in the breakdown of organic contaminants (Yang et al., 2020a). The photocatalytic elimination of oxytetracycline pollutants from the environment was investigated using carbon nitride. The degradation of oxytetracycline was achieved using a newly synthesized 2-hydroxy-4,6-dimethylpyrimidine grafted carbon nitride. Engineered material showed the most degradation, at 79.3% (Yang et al., 2020b). The chromium and oxytetracycline hydrochloride were reduced and degraded using black phosphorous quantum dots coupled tubular carbon nitride (Wang et al., 2020). For the cleanup of pharmaceutical contaminants, several green manufactured nanoparticles have been used. Nano-zero valent iron with activated carbon, nickel iron nano composite from pomegranate, copper nanoparticles from tilia extracts and iron oxide nanoparticles from citrus fruits are only a few examples of nanomaterials. The production of carbon quantum dots by *Tamarindus indica* proved effective in detecting mercury ions in pond samples (Kumar et al., 2018; Nasrollahzadeh et al., 2021). Environmental uses of microbially synthesized nanoparticles are shown in Table 17.7.

17.6.1 Applications in bioremediation

Nano-biotechnology has recently made numerous contributions to the remediation of soil, water, and air contaminants into ecologically friendly products and can be divided into three categories: (1) treatment and remediation (2) sensing and detection (3) pollution prevention (Carata et al., 2017). Most importantly, the advent of nanobioremediation has the potential to benefit the environment by removing pollution and implementing cleanup approaches. As previously stated, combining traditional bioremediation with nanobiotechnological approaches or using direct nano-remediation techniques could be a viable solution for eliminating contaminants from the ecosystem. Heavy metals, pesticides, herbicides and insecticides (organic and inorganic pollutants) are degraded or sequestered by nanoparticles from the contaminated environment. In general, research indicates that nanoparticles can be used to mitigate pollution in both direct and integrated ways. Organic pollutants, such as herbicides (i.e., atrazine, molinate) and pesticides (i.e., chlorpyrifos) are destroyed directly utilizing nZVI (Zerovalent nano ions). Long-chain hydro- and halocarbons that resisted bioremediation were broken down through a nanoparticle-mediated process. The conversion of nZVI/ nanoscale FeO to FeO is one proposed process that implies enhanced reactivity of nanomaterials for the breakdown and detoxification of pollutants (Sakulchaicharoen et al., 2010). As a result, using this reaction mechanism to detect, remove and degrade environmentally harmful pollutants like halo and hydrocarbons, in general, could be a unique way to achieve a more sustainable environment. Highly persistent water pollution and xenobiotics, such as cationic dyes, anionic dyes, azo dyes, and other xenobiotic pollutants, must be remedied for wastewater treatment and future prospective. These pollutants worsen water contamination and harm aquatic life. Because NPs have a bigger surface area and are smaller, they can act as a catalyst or absorb pollutants due to their larger surface area. Many studies have looked at the catalytic characteristics of certain NPs combined with biological components for decreasing hazardous contaminants (Kumari et al., 2019; Mandeep, 2020). These NPs may be employed as a high-efficiency catalyst for the degradation of organic dyes in industries (Modi et al., 2015; David and Moldovan, 2020). Sharma et al. (2021) discovered that AgNPs decolorize organic dyes efficiently by catalytic action. Both Ag and AuNPs have been shown to have acceptable catalytic activity in the elimination of organic dyes. These NPs reduce the amount of time it takes to remove the dye and increase the rate of response (David and Moldovan, 2020). AuNPs can also be employed as an adsorbent for organic dyes (Bhargava et al., 2016). The adsorption of rhodamine-B organic dye was enhanced by AuNPs with surface proteins produced from the fungus *Cladosporium oxysporum* AJP03. The contributions of TiO₂NPs, FeNPs, bimetallic NPs, magnetic NPs, nanotubes, nanoclays and nanosponges in soil bioremediation were recently reported and this green synthesis of NPs, could be an effective strategy to address soil and water pollution (Koul and Taak, 2018).

17.6.2 Valorization of waste

The use of technology to convert waste materials into usable products fascinates researchers all over the world. We can reduce the amount of waste while also producing useful products by adopting this approach. This process is frequently utilized in the synthesis of adsorbents, biogas, clinker, biomolecules, biohydrogen and a variety of other products (Gupta and Shukla, 2020). Nanotechnology has aided in the improvement of production rates for effective waste-to-resource conversion. Kumar and his colleagues published a paper in 2019 on using nanoparticles to boost dark fermentation reactions and yield more biohydrogen (Kumar et al., 2019). The addition of nanoparticles to fermentative bacteria has opened up new opportunities for biohydrogen production from wastewater. To make biohydrogen, Elreedy and colleagues used mixed culture bacteria as well as single, dual, and multiple nanoparticles. When many nanoparticles were

employed, they discovered that biohydrogen generation was at its peak (14% higher than single nanoparticles utilized). The various nanoparticles boost the activity of hydrogenase and dehydrogenase, resulting in more biohydrogen generation (Elreedy et al., 2019). Similarly, the combination of both nickel oxide and hematite nanoparticles boosted biohydrogen generation by 1.2–4.5 times compared to the solo nanoparticles. The combination of nanoparticles produced the maximum hydrogen output of 8.83 mmol/g COD. The enhanced activity of hydrogenase and ferredoxin oxidoreductase enzymes is responsible for this increase (Gadhe et al., 2015). Thus, Nanotechnology can be employed to provide green energy for long-term industrial growth and ecologically sustainable production.

17.6.3 Applications in environmental management

The environment has been deteriorating as a result of industrialization, population growth and exposure to various toxic materials, such as heavy metals, poly-aromatic hydrocarbons, and other pollutants, either as a result of natural or anthropogenic activities. Nanosciences are a safer and effective technology for reducing the toxicity of xenobiotics and other waste materials in the surroundings. Our natural resources have been contaminated with unwanted organic and inorganic materials as a result of rapid industrialization, urbanization and some anthropogenic activities. Large amounts of these substances in water have created health risks that should be resolved using environmentally friendly technology. Groundwater along with other water bodies has been found to contain organochlorine pesticides, as well as several heavy metals. CNTs, for example, have been widely used in water purification due to their antimicrobial activity and lack of side effects (Gehrke et al., 2015). Silver (Ag) nanoparticles embedded in zeolites are profoundly used in sanitary applications because they prevent harmful microbes from increasing in water (Dutta et al., 2011). Other nanoparticles, such as TiO₂, are widely used as disinfecting and decontaminating agent because they provide a long-lasting coating and are stable throughout the purification process (Savage and Diallo, 2005). Iron sulfide nanoparticles stabilized by biomolecules produced from the fungus *Itajahia* sp. have been reported to aid in the breakdown of chlorinated chemicals to detoxify drinking water (Paknikar et al., 2005). After 8 hours of incubation in a medium containing iron and sulfur, the cells isolated from lindane-rich locations were efficient for mineralizing the lindane. Using *Desulfovibrio vulgaris*, a sulfate-reducing bacteria iron sulfide-based nanomaterials can be effectively designed to control the pollution problem resulting from heavy metals (Watson et al., 1999). This intriguing potential for treating waste water with extremely high concentrations of heavy metals/or metalloids has industrial implications. Das et al. (2009) proposed using gold nanoparticles generated in vitro by *Rhizopus oryzae* could regulate pesticide contamination caused by a variety of chlorinated insecticides. The good antibacterial property related to synthesized nanoparticles could be used to decontaminate pathogens from water. Recently, Qu et al. (2018) published a study on the effectiveness of spherical-shaped gold nanoparticles generated by *Magnusiomyces ingens* (LH-F1) for nitrophenol reduction. In the presence of sodium tetra hydroborate, the catalytic reduction using culture-free supernatant was found to be dose dependent. Certain metal oxidizers, such as bacteria that produce iron nanoparticles, are also extremely important (Singh et al., 2018). Arsenic, manganese, iron, chromium, copper, zinc, lead and cadmium can all be remedied with biologically synthesized nanoparticles. Matsushita et al. (2018) reported that in a reactor system the use of manganese oxidizing bacteria can remove cobalt, nickel, and manganese, from contaminated systems. The metal-laden waste water can be treated under ideal conditions. Bedi et al. (2018) suggested the use of *Aspergillus aculeatus* (a fungus) for the remediation of discarded trash accumulated by mining activities. Under normal environmental conditions, the fungus showed extraordinary efficiency in the extracellular creation of protein coated iron nanoparticles. In vitro investigations with iron oxide nanoparticles enhanced the growth of test plants. Biologically generated nanoparticles can be used to remediate industrial dye effluents, break down waste cooking oil and to develop biosensors for detecting very low levels of environmental contaminants, and. However, further study is required for field-scale applications.

17.6.3.1 Application as biosensors

A biosensor, is an analytical device, which in combination with a biological recognition element signal transducer, convert analyte's reaction into a quantifiable signal proportional to the analyte's concentration (Mohanty and Kougianos, 2006; Lei et al., 2006). A microbial biosensor uses microorganisms as bioelements and contains a large number of enzymes. The enzymes in living cells can create a particular and specific response to analytes without the need for time-consuming and expensive purification or the unfavorable impacts of the operating environment (Lei et al., 2006; Su et al., 2011). The primary condition for developing a reliable microbial biosensor is to integrate the microbes onto the transducer (D'souza, 2001a,b; Su et al., 2011; Lei et al., 2006). The immobilization of bioelements to the transducers must be stable to transmit the responses from recognition elements to transducers. The quality of the signal

conveyed from the microorganisms to the transducer is determined by immobilization, as is the microbial biosensor's reusability. As a result, immobilization is critical in the development of a microbial biosensor (D'souza, 2001). Adsorption, encapsulation, trapping, cross-linking and covalent binding are examples of traditional immobilization methods. Nanomaterials such as nanotubes, nanoparticles, and fiber optics, as well as advances in nanotechnology, provide an alternative for improved immobilization by promoting stronger bioelement dependability and stability (Tuncagil et al., 2011). All of these techniques, however, have either poor long-term stability or adverse outcomes when subjected to strong reaction conditions (D'souza, 2001).

Another important component of the microbial biosensor is the transducer, which converts the biological reaction into a quantifiable signal (Mohanty and Kougianos, 2006). Microbial fuel cells (MFC) have recently been presented as a new approach for microbial biosensors that previously depended on optical transducers as the primary transducer. MFCs have selective sensing capability and a high sensitivity and can produce sustainable electricity from biodegradable organic molecules through microbial metabolism (Tuncagil et al., 2011; Choi and Chae, 2013). The microbial biosensors are widely used in many industries ranging from food and fermentation industry, environmental monitoring, and clinical diagnostics owing their benefits of low cost, high stability, and fast response. It is required to develop a simple, cost-effective, fast and field-portable screening approach for monitoring diverse organic and inorganic chemical pollutants that pose a risk to human health (Rogers, 2006). Further, the food and fermentation industries require fast, low-cost, and reliable technologies to ensure quality standards and process monitoring (Arora et al., 2011). In clinical diagnostics, there is also a pressing need for precise, quick, and low-cost instruments that may be used regularly to monitor clinically significant parameters (Ivnitski et al., 1999). Traditional approaches in those applications often necessitate analytical procedures, specialized laboratories and experienced operators, all of which are costly, inefficient and time-consuming (Malhotra and Chaubey, 2003). Low cost, higher sensitivity, mobility and rapid response, are all advantages of microbial biosensors (Arora et al., 2011; Rogers, 2006).

17.6.3.2 Environmental monitoring

Monitoring of the environmental pollutants in the environment poses a serious threat to human health. Due to the widespread use of heavy metals by the industry, they have become a major toxicant of waste water and the non-biodegradability these metal ions allow them to accumulate in living organisms, causing a variety of illnesses (Gammoudi et al., 2010). Heavy metal ions can function as an acute enzyme inhibitor, causing alterations that can be exploited as a signal for heavy metal ion detection. For heavy metal monitoring, a low-cost, specialized, simple and efficient tool is required. This challenge can be solved with the use of a microbial biosensor. Two general methodologies for constructing a microbial biosensor for detecting heavy metal toxicity are constitutive (light-off) and inducible (light-on) (Ivask et al., 2009). The lux gene exists constitutively in a constitutive manner. The presence of a toxic heavy metal diminishes light density by altering the expression of the lux gene (Su et al., 2011). This microbial biosensor is nonspecific since it can respond to any molecule that is poisonous to the organism (Ramanathan et al., 1997). More complex and sensitive biosensors relying on inducible promoters coupled to reporter genes have been developed (Xu and Ying, 2011). For in-situ pollutant measurement, only the specified biosensor can be utilized. Because mercury inhibits the action of alkaline phosphate enzymes found in *Chlorella* spp. cell walls, Singh and Mittal (2012) constructed a mercury biosensor by immobilizing *Chlorella* sp. on a glassy carbon surface. The best method for detecting heavy metals is to employ genetically engineered bacteria that can produce detectable signals when interacting with biocomponents (Yong and Zhong, 2009). P and cusC promoters were fused with a dual-labeling reporter protein as an interacting biocomponent for copper and zinc to generate a signal from the developed biosensor (Ravikumar et al., 2012). Further, Ravikumar et al. (2012) construct a biosensor for detection of zinc or copper on engineered bacteria. For precise detection of zinc, a promoterless enhanced green fluorescent protein (egfp) gene with the czcR3 promoter was fused, which can respond quantitatively to zinc (Liu et al., 2012). The use of dead biomass to passively ingest heavy metals is a more efficient, cost-effective, and simple method of detecting them. Dead biomass, compared to living cells, requires no nutrition, and is simple to handle, store and resistant to toxic and harsh reaction conditions (Vijayaraghavan and Yun, 2008). *Pseudomonas aeruginosa* was employed to make a microbial biosensor for detection of the heavy metal Pb (II), which had a linear response to Pb (II) (1.0–2.0 M) with detection limit of 0.6 M (Yüce et al., 2011).

Another major contaminant in the environment that is hazardous to humans is organic toxicity. Organophosphorus (OP) pesticides, such as methyl parathion, are widely used in agriculture, but show toxicity in insects and humans. Based on the principle that organophosphorus hydrolase (OPH) can hydrolyze methyl parathion to p-nitrophenol (an electrochemical or colorimetric detectable product), an optical microbial biosensor using *Sphingomonas* sp. immobilized on the lower surface of the microplate wells was developed for the detection of methyl parathion pesticide

(Kumar and D'Souza, 2010). Trichloroethylene (TCE), another major organic contaminant employed in industry, as an organic solvent and degreasing agent, has been associated with central nervous system damage. In toluene-grown *Pseudomonas putida* F1, the toluene dioxygenase enzyme may degrade TCE into the conductometrically detectable metabolites glyoxylate and formate ions (PpF1). An impedimetric biosensor, based on the immobilization of PpF1 strain on gold microelectrode, can detect trichloroethylene based on their reaction mechanism (Hnaïen et al., 2011). It is necessary to develop a speedy, low-cost and specific approach for detecting different organic toxicities. Microbial biosensors offer a potential solution to this issue. The microbial biosensor for phenol and nitrophenol detection based on *Pseudomonas fluorescens* is widely being used (Liu et al., 2011).

17.6.4 Application in food and fermentation

Customers and the government both expect high-quality products, thus quick and low-cost methods to ensure product quality and process controls are essential (Mello and Kubota, 2002). Fermentation is widely utilized in the manufacture of foods and beverages, and it necessitates meticulous fermentation system operation (Kim et al., 2009). Monitoring nutritional parameters and food pollutants necessitate the use of quick and particular analytical tools (D'souza, 2001), and microbial biosensors serve as a quick and cost-effective way to ensure product quality. Caffeine must be identified sensitively and quickly as an index in determining the quality of coffee. Contaminants should also be thoroughly detected to ensure product quality. Mycotoxins from the zearalenone family are prevalent pollutants in milk that can cause mycotoxicoses (Bryden, 2007). To manage the fermentation process, microbial biosensors are utilized to monitor the ingredients. Due to the importance and necessity of ethanol in many fermentation processes, microbial biosensors for sensitive ethanol determination have been utilized for monitoring the process. The determination of ethanol in the range of 0.5–7.5 mM was achieved using an amperometric biosensor from *Candida tropicalis* cells immobilized in gelatin and glutaraldehyde (Akyilmaz and Dinçkaya, 2005). Valach et al. (2009) had also developed a new microbial amperometric biosensor in flow injection studies for ethanol measurement, which demonstrated a linear response to ethanol in the range of 10–1.5 mM in 3 minutes. Furthermore, an ethanol biosensor with a linear range of 0.050–7.5 mmol/L and a detection limit of 0.035 mmol/L was developed using *Methylobacterium organophilum* immobilized on an oxygen electrode and even in eggshell membrane (Wen et al., 2012). By immobilizing *Pseudomonas alcaligenes* (MTCC 5264) on a cellophane membrane, Babu et al. (2007) had also produced an amperometric biosensor for the measurement of caffeine that responded linearly to caffeine over a range of 0.1–1 mg/mL within 3 minutes. Both consumers and the food business are becoming increasingly interested in food quality and freshness control (Mello and Kubota, 2002). The two optimal sweeteners and nutritional agents that are extensively employed in food are D-glucose and D-xylose. A voltametric biosensor for the detection of D-glucose and D-xylose was created based on the co-immobilization of glucose oxidase and xylose dehydrogenase exhibited XDH-bacteria on multiwalled CNT nanocomposite films modified electrode (Li et al., 2013). For the detection of zearalenone family mycotoxins in milk genetically engineered *Saccharomyces cerevisiae* strains were employed as the bioelement of a microbial biosensor to ensure milk quality (Välimaa et al., 2010).

17.6.5 Biomedical applications of microbial nanoparticles

Antibiotic resistance is acquired by pathogenic bacteria by altering their metabolic processes and target sites (Seil and Webster, 2012). Furthermore, as a result of their continued exposure, these microorganisms become more pathogenic and resistant to medications (Fariq et al., 2017). As a result, it is critical to discover alternative, unconventional, potent antimicrobial drugs that are both less expensive and safer. Biofabricated NPs are smaller and have a larger surface area. These features enable NPs to effectively interact with microbial cell membranes and infiltrate cells to disrupt metabolic pathways and DNA replication (Fariq et al., 2017). Many studies have looked into the antibacterial properties of various NPs. The antibacterial effects of NPs, according to (Sondi and Salopek-Sondi, 2004), involve the breakdown of the cell membrane and cellular machinery via the development of pits, which leads to the death of microbial cells. Furthermore, if NPs are utilized in conjunction with standard medicines, the antibacterial efficacy of NPs can be amplified. (Banu et al., 2011) investigated the combined action of AgNPs and antibiotics (carbenicillin, ciprofloxacin and nitrofurantoin) against *Enterobacteriaceae* bacteria (ESBL-strains). (Sunkar and Nachiyar, 2012) evaluated the antimicrobial activity of AgNPs generated from *Bacillus cereus* against disease-causing bacteria such as *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Staphylococcus aureus*. In comparison to conventional antibiotics (amoxicillin, ofloxacin and streptomycin), AgNPs produce larger inhibitory zones. The synergetic activity of AgNPs derived from *Brevibacterium frigoritolerans* and antibiotics, like lincomycin, novobiocin, oleandomycin, penicillin G, rifampicin, and vancomycin was studied by

(Singh et al., 2015). When antibiotics were combined with AgNPs, their antibacterial activity against disease-causing organisms of *Candida albicans*, *Vibrio parahaemolyticus*, *Bacillus anthracis*, *Salmonella enterica*, *Escherichia coli* and *Bacillus cereus* was increased. (Elbeshehy et al., 2015) used three strains of *Bacillus licheniformis*, *B. pumilus* and *B. persicus* strains to make AgNPs and tested their antimicrobial activities against pathogens such as *Candida albicans* ATCC 1021, *Staphylococcus epidermidis* ATCC 1228, *Escherichia coli* ATCC 25922, *Aspergillus flavus*, *Streptococcus bovis* ATCC 49147, *Shigella sonnei* ATCC25931, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* ATCC 700603 and *Staphylococcus aureus* (methicillin-resistant strain) The AgNPs produced using *B. licheniformis* had the highest antibacterial activity. (Mahmoud et al., 2016) had also produced AgNPs from *Bacillus pumilus* and also studied their antibacterial potential against pathogenic bacteria, such as *E. coli*, *Shigella aureus*, *S. bovis*, *S. sonnei*, *S. typhimurium* and *Klebsiella pneumoniae*. Similarly, (Roychoudhury et al., 2016) synthesized AgNPs with the *Lyngbya majuscula* (CUH/AI/MW-150) bacteria and discovered that these AgNPs have significant antibacterial activity against *P. aeruginosa*. These AgNPs were also found to have antiproliferative properties in leukemic cells and the REH cell line. AgNPs produced from *Streptomyces* sp. Al-dhabi-87 showed high antibacterial activity against *B. subtilis*, *S. epidermidis* and *E. faecalis*, and the multidrug-resistant *S. aureus* strain (Al-Dhabi et al., 2018). There are numerous reports on the use of AgNPs as antibacterial agents. However, just a few publications on the utilization of additional NPs as antibacterial agents are published. For example, AuNPs extracellularly produced from *Nocardiopsis* sp. MBRC-48 has been shown to have potent antibacterial activity against *Candida albicans* and *Staphylococcus aureus* (Manivasagan et al., 2015). Malarkodi et al. (2013) investigated the antibacterial activity of titanium oxide NPs (TiO₂NPs) derived from *Planomicrobium* sp. gram-positive *B. subtilis*, against gram-negative *K. planticola*, and a fungus *Aspergillus niger*. In this study, TiO₂NPs showed the maximum suppression of both bacterial and fungal growth at a 0.4 ppm concentration when compared to the antibiotic kanamycin, while fungal growth was inhibited at a 1 mL concentration of TiO₂NPs. Antibacterial activities of zinc oxide NPs have also been reported against several disease-causing bacteria (*Bacillus subtilis*, *Enterobacter aerogenes*, *Klebsiella pneumoniae*, *Pseudomonas alcaligenes*, *Proteus mirabilis*, *P. vulgaris*, *Streptococcus pyogenes*, *Shigella flexneri*, *Salmonella typhimurium* and *Staphylococcus epidermidis*). (Jain et al., 2020) biosynthesized zinc oxide NPs with the help of zinc-resistant bacterium *Serratia nematodiphila* and examined their antibiotic activity against *Xanthomonas oryzae* and was found resistant to penicillin.

17.6.6 Application in clinical diagnostics and drug delivery

Traditional diagnostic approaches for a variety of disorders are a time-consuming process, have a slow reaction time, and are a convoluted process, all of which make critical care challenging in an emergency (Malhotra and Chaubey, 2003). Microbial biosensors are better as compared to enzyme-based biosensors, as they do not require purification, which is time-saving and cost-effective. Microbial biosensors offer a quick, accurate and low-cost method of diagnosing hormones, infections and DNA, all of which are significant parameters in a living person. A new microbial biosensor was also generated for the determination of epinephrine with a linear range and a detection limit of 5–100 and 1.04 M, respectively (Akyilmaz et al., 2011). Through a redox activity in the fungal cells mediated by lactase, epinephrine was converted to epinephrine quinone in this biosensor, generating an increase in the current. Pathogen identification is vital in clinical diagnostics since pathogens are the cause of viral illnesses. Mast cells from rat basophilic leukemia (RBL) were employed to create a microbial biosensor for pathogen detection (Curtis et al., 2008). RBL mast cells could elicit a strong exocytotic response within a few minutes of antigen addition. Many diseases, including cancer, are caused by DNA damage, which affects the replication and repair of DNA and also gene expression. For DNA damage detection, an *E. coli* SOS-EGFP based on the SOS reaction was designed. To create fluorescent protein controlled by the recA gene promoter, the SOS response could be initiated by toxic chemicals for DNA (Chen et al., 2012).

Cancer, which is defined by uncontrolled cell growth and proliferation, is currently one of the most serious diseases. Traditional cancer treatment procedures (chemotherapy, radiation and surgery) are not possible without side effects. Furthermore, targeted therapeutic delivery to the damaged organ and early illness detection are still in their infancy (Jabir et al., 2012). As a result, developing alternate means of detection and therapy for this disease is critical. Nanomedicines are effective in the diagnosis of tumors and the treatment of tumors via. targeted medication delivery (Suthradhar et al., 2014). Borse et al. (2015) investigated the anticancer activity of biofabricated PtNPs derived from *Saccharomyces boulardii* against MCF-7 and A431 cell lines in vitro. Different types of cancer cell lines have been treated with nanomedicines. Women with breast cancer have a high recurrence rate. (Ortega et al., 2015) investigated the efficacy of AgNPs made from *Cryptococcus laurentii*, finding that biofabricated AgNPs had effective anticancer and antitumor capabilities against cancer or breast cancer cell lines. Biosynthesized SeNPs had anticancer properties as well. (Ahmad et al., 2015) investigated the effects of biofabricated Se nanorods (SeNrs) from the *Streptomyces bikiniensis* strain EssamA-1 on MCF-7 and Hep-G2 cancer cells. SeNPs extracellularly synthesized from the bacterium

Streptomyces minutiscleroticus M10A62 demonstrated significant antiproliferative action against Hep-G2 and HeLa cell lines, according to (Ramya et al., 2015). (El-Batal and Al Tamie, 2015) investigated the efficacy of AuNPs against breast carcinoma (MCF7) cell lines and human liver (HEP-G2), finding that AuNPs cause mitochondrial apoptosis, cytokinesis arrest, DNA impairment and nuclei degradation in cancer cell lines. As a result, NPs have been used in a variety of studies for their anticancer properties under controlled conditions. However, issues including toxicity, dosages, and the host immunological response during treatment must also be addressed before their commercialization. Nanomaterials can also be utilized as a vehicle for targeted drug administration and are a valuable tool for improving medication bioactivity, bioavailability and stability. Liposomes, nanoemulsions, NP-coated natural antibodies, nanospheres, polymeric micelles and water-soluble polymers, are some of the most common drug delivery nanomaterials (Salouti and Ahangari, 2014; Omlor et al., 2015). Nanomaterials have many advantages over standard medication delivery systems. Targeted medication delivery can lower the risk of drug toxicity-related side effects in patients. Further, Kundu et al. (2014) investigated the efficiency of ZnO NPs made from *Rhodococcus pyridinivorans* as anthraquinone nanovehicles. ZnO NPs loaded with anthraquinone showed concentration-dependent cytotoxicity against HT-29 colon cancer cells, leading to the conclusion that ZnO NPs loaded with anthraquinone could be used for targeted drug administration. Moreover, through improved diffusion, nanomaterials with hydrophilic nature can boost drug absorption and enhance drug cytotoxicity. Kumar et al. (2008) investigated biofabricated AuNPs in combination with the drug doxorubicin in HEK293 cancer cell lines and discovered that the drug combined with AuNPs diffused more quickly into the cells. Tomuleasa et al. (2012) reported comparable findings against hepatic cancer cells utilizing AuNPs in combination with the medication doxorubicin. Khan et al. (2014) reported a new drug delivery method in combination with anticancer medication taxol and biosynthesized gadolinium oxide NPs, which could be utilized to treat malignancies successfully. The traditional techniques of disease diagnosis are time-consuming, costly and inefficient. Pathogens and chronic diseases can be detected with NPs in a cost-effective, quick, specific manner. Fluorescent nanoparticles, magnetic nanoparticles (quantum dots and dye-loaded NPs) and metallic nanoparticles can all be utilized to identify, image and track infections and disease progressions (Tallury et al., 2010). Fluorescent NPs can be utilized for imaging chronic diseases in their early stages, such as cancer. Magnetic nanoparticles can be employed in advanced detecting methods like MRI. Biofabricated *Candida albicans* AuNPs, for example, have been employed for probing liver cancer cells. The AuNPs only attached to cancer cells and made it easy to differentiate cancer cells from normal cells (Chauhan et al., 2011). As a result, microbe-based NPs play an important role in disease diagnosis; nevertheless, their application in disease detection and diagnosis is still in its early stages, and more research is needed.

17.7 Limitations

The microbial nanoparticles synthesis has gained a lot of attention because of its simplicity, environmental friendliness, and low cost of production. They are employed in environmental cleanup because of their unique features. Nowadays, there has been an increase in interest in using microorganisms to remove and degrade contaminants. The selection of suitable microorganisms is an important aspect of the production of metal nanoparticles and thus poses a little difficulty. The synthesis is also influenced by the pace of growth, biochemical functioning, and replication process. According to several studies, changing the recombinant strains of microbial species enhances the synthesis of metal nanoparticles. The cost of developing recombinant strains can increase the production cost of nanoparticles. Monodispersity, size, and structure of the NPs should also be considered during environmental applications on an industrial scale. The identification of particular biomolecules responsible for the stabilization and reduction of metal NPs from its precursor is another problem. The majority of the research was conducted on a laboratory level due to this it was easy to maintain the microbial growth parameters. However, on an industrial scale, maintaining those parameters becomes a difficult operation, increasing the cost of nanoparticle production (Korbekandi et al., 2012; Hulkoti and Taranath, 2014). The usage of pathogenic organisms can also harm the ecosystem. Various limitations and constraints should be addressed properly for the large-scale implementation of nanomaterial biosynthesis for environmental cleanup. Nanoparticles have emerged as a promising candidate for environmental pollution treatment and detection. Metal nanomaterials can detect pollutant molecules several heavy metal ions, like aluminum, cadmium, copper, iron, gold, silver, titanium and silica nanoparticles are some of the most common nanoparticles used in the remediation of environmental contaminants. Hybrid nanomaterials have been employed for the adsorption of biological molecules, dyes and heavy metals, due to their excellent thermal and chemical stability. Toxicological effects, on the other hand, have an impact on the use of nanoparticles in environmental concerns. Metal ions in nanoparticles could potentially penetrate cells, causing oxidative stress. The toxicity of finer metal nanoparticles was shown to be higher than that of bulk materials.

17.8 Conclusion and future approach

Nanotechnology, as one of the newest fields, has a lot of potential in terms of environmental and economic problems. For the manufacturing of nanoparticles, commonly top-down and bottom-up methods are used. Nanoparticle production by various microorganisms has significant advantages over traditional approaches. For efficient metal nanoparticle biogenesis, current research inclinations such as managing nanoparticle form and particle size, difficulty in obtaining higher nanoparticle production from microorganisms and reduced stability must be overcome. Metal nanoparticle biogenesis has mostly favorable environmental consequences, such as removal of metal ions, biological substances and dyes from a contaminated environment. Though, the toxicity of finer nanoparticles that penetrated the cells has been found in some cases. The exact mechanism, cellular and metabolic processes, applications of biosynthesized nanoparticles, and large-scale production should be the focus of future research. Fungal-mediated synthesis has been shown to synthesize more protein than bacteria, resulting in increased productivity. For a better understanding of nanoparticle synthesis, different metal nanoparticle synthesized from bacteria, fungus, algae and yeast has been studied. Biosynthesized metal nanoparticles, that are widely used for environmental cleanup, are being thoroughly examined to expand their commercial applications. As a result, future research should concentrate on optimizing reaction parameters, enhancing nanoparticle stability, and exploring the wide range of microbes for nanoparticle synthesis, as this is crucial for the practical sustainability of biosynthesized nanomaterials.

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Nanobioremediation: a novel technology with phenomenal clean up potential for a sustainable environment

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18.1 Introduction

Nanobioremediation is a contemporary consolidated technology which includes both nanotechnology and bioremediation. It has got best of both technologies which makes nanobioremediation highly efficient and environment friendly approach (Singh et al., 2020). Toxicity of Nanoparticles for microorganisms is very well cited in the literature, but the right dose of nanoparticles plays critical role (Li et al., 2010). It was reported by Le et al. (2015) that with the help of nano-bio approach, polychlorinated biphenyls (PCBs) were effectively bioremediated with *B. xenovorans* and Pd/Fe nanoparticles. Another similar study conducted by Němeček et al. (2016), stated that nZVI and whey generated microbes removed 97%–99% of Cr (VI) in an integrated system. On basis of the sequence for applying the nanoparticle and bioagent, mainly two methods are designed namely sequential method and concurrent or combined method. In sequential method, contaminant is first treated with nanoparticles followed by bioagent. Whereas, in concurrent or combined method both the components i.e., bioagent and nanoparticles are added simultaneously (Singh et al., 2020). Biogenic nanoparticles are synthesized from Algal, Fungal and Bacterial agents. They are synthesized using intracellular or by extracellular processes. In intracellular processes, diffusion of positively charged metal ions takes place into negatively charged cell wall by electrostatic interactions. While in extracellular processes, enzymes secreted by fungus convert metal ions into metal nanoparticles (Menon et al., 2017). In another study, Sundaram et al., 2012 reported production of iron oxide nanoparticles by *Bacillus subtilis*, isolated from rhizosphere soil samples. Similarly, Menon et al. (2017) stated that fungal cells are capable of producing gold nanoparticles (AuNPs). Brown algae are engaged in biosynthesis of nanoparticles, since it is rich in mucilaginous polysaccharides and carboxyl groups. They assist in metal uptake and hence are used for production of nanoparticles (Khandel and Shahi, 2016). Microorganisms being biological entity, serves as a cost-effective renewable source for environmental remediation (Narayanan and Sakthivel, 2011). Various laboratory-based studies and field-level application of biogenic nanoparticles are conducted to clear away environmental toxins. Microbe mediated nanobioremediation is used to target heavy metals, hydrocarbons, dyes, pharmaceuticals as they adversely pollute natural resources. In recent study, it was reported that iron nanoparticles synthesized from natural consortium had ability to efficiently absorb copper, zinc and chromium from wastewaters (Castro et al., 2013). Biogenic manganese oxides (BMO) were manufactured using *Pseudomonas putida* MnB1 for eradication of heavy metals. Zhou et al., 2015 reported that BMO adsorbed heavy metals better than the chemically synthesized manganese oxide. Aziz et al., 2015 stated that biogenic AgNPs (silver nanoparticle) synthesized using *Chlorella pyrenoidosa* productively degrades Methylene blue (MB) dye, an obstinate toxic organic compound. Another study came up

demonstrating use of *Pseudomonas aeruginosa* mediated Fe_3O_4 nanoparticles to get rid of picric acid (Yousefi et al., 2020). Studies conducted by Wang et al. (2018a,b) reported that palladium nanoparticles effectively resulted in reduction of azo dyes that were synthesized using *Klebsiella oxytoca*. The present chapter includes information on Nanobioremediation technique along with the sources for production of biogenic nanoparticles and their application in pulling out organic and inorganic contaminants effectively from environment.

18.2 Application methods of nano-bio technique

18.2.1 Sequential method

A sequential hybrid treatment system which employed bimetallic nanoparticle (Pd/nFe) and an enzyme was established for the degradation of triclosan (TCS), an antimicrobial agent used in personal care goods. Firstly, triclosan was reduced anaerobically with Pd/nFe nanoparticles which dechlorinates TCS to 2-phenoxyphenol. Further, nanoparticles were separated, and the oxidation of dechlorinated product was carried out in presence of syringaldehyde by laccase enzyme isolated from *Trametes versicolor*. Transformation of TCS to nontoxic oligomers was done through redox reaction (Bokare et al., 2012). Similar strategy was successfully used for the degradation of polybrominated diphenyl ethers using nZVI along with diphenyl ether-degrading bacteria *Sphingomonas* sp. in aqueous solution. Effective debromination of deca-BDE was done using sequential system with nZVI anaerobically. After 20 days, reaction mixture was supplemented with the bacterial strain and further incubated for 4 days (Kim et al., 2012). Sequential treatment of 2, 2', 4, 5, 5'-pentachlorobiphenyl with nZVI anaerobically and further transformation with bacterium H1 aerobically was also reported by He et al. The degradation of gamma isomer of 1,2,3,4,5,6-hexachlorocyclohexane (γ -HCH) in soil was conducted by Singh et al. (2013) using stabilized Pd/Fe0 bimetallic nanoparticles (CMC-Pd/nFe0) and a *Sphingomonas* sp. strain NM05 in batch experiments in glass beakers containing 5 g soil having 25 $\mu\text{g/g}$ γ -HCH concentration. Reaction was started with the inoculation of 1 mL of NM05 followed by the addition of 0.40 g of nFe0 or CMC-Pd/nFe0. The degradation efficiency was greater in integrated system in comparison to system having either NM05 or CMC-Pd/nFe0 alone.

18.2.2 Combined/concurrent method

When both nanoparticles and biological agents are administered to the system at the same time, this is referred to as a concurrent or mixed technique (Singh et al., 2020). Using trichloroethylene (TCE) as a model compound, Xiu et al. (2010) studied the influence of nZVI on dechlorinating microflora in a microcosm investigation. In reaction vials containing TCE, nZVI and *Dehalococcoides* spp. were introduced concurrently, along with mineral salt support. After that, the reaction mixture was shaken at 200 rpm. Two further trials were conducted under comparable conditions, one with only nZVI and the other with only *Dehalococcoides* spp. nZVI was first discovered to impede microbial dechlorination, but it was later discovered to have a biostimulatory impact on dechlorinating bacteria, potentially increasing the total rate of contaminant breakdown which was possible due to the hydrogen produced by nZVI during cathodic corrosion can be used as an electron donor by dechlorinating bacteria. In another study carried out on nanoparticles which were coated with a carboxymethyl cellulose polymer to prevent direct contact with bacterium cells reported that prevention of direct contact decreases the bacterial cell growth (Singh et al., 2013). The degradation of -HCH was observed in both single and combination systems of carboxymethyl cellulose polymer and *Sphingomonas* strain NM05. In comparison to systems containing either the NM05 strain or carboxymethyl cellulose alone, the combined system's -HCH degradation efficiency was 1.7–2.1 times higher.

18.3 Designing new age biogenic nanoparticles

A number of physical and chemical methods can be employed for the synthesis of nanoparticles but these are uneconomical and at the same time cause serious threat to the environment. Therefore, the concept of “biogenic nanoparticles” is gaining more attention in the present era. Biogenic nanoparticles as the name suggests are those nanoparticles which are produced from living organisms. The process of synthesis of biogenic nanoparticles is eco-friendly, cost-effective and is highly beneficial due to its enormous applications in field of biomedical sciences and this makes it as superior approach over the chemical and physical synthesis of nanoparticles (Saravanan et al., 2018). Usually, plants and microbes are being utilized for the production of biogenic nanoparticles. As microbes are able to grow at faster rates under easily available growth environments, they are usually preferred for synthesizing nanoparticles. Micro-organisms engage in the synthesis

of biogenic nanoparticles by following either of the two mechanisms: Extracellular and Intracellular synthesis. The former involves adsorption of metal ions and their enzymatic reduction on the surface of microbial cells while the latter occurs through the movement of metal ions into interior of microbial cells and discharge of the synthesized nanoparticles into the surrounding medium (Fariq et al., 2017). Almost all kinds of microbes i.e., bacteria, fungi as well as algae show potent application in the synthesis of different varieties of nanoparticles such as gold, silver, palladium, iron and copper nanoparticles (Sathishkumar et al., 2019a,b). Production of biogenic nanoparticles from microbes encompasses the techniques of microbiology, biotechnology and nanotechnology into a novel arena of nano-biotechnology. Utilization of different microbes i.e., bacteria, algae and fungi for the production of nanoparticles has been discussed in detail in the subsequent sections.

18.3.1 Bacterial synthesis of nanoparticles

Among the different categories of microorganisms, Bacteria are considered to be a potent candidate for the synthesis of nanoparticles. Easy cultivation and rapid multiplication are the outstanding properties of bacteria making it liable for nanoparticle production (Saratale et al., 2018). In addition to this, bacteria exhibit excellent metal-chelating properties enhancing their potential for nanoparticle production. Bacterial carrier matrix provides large surface area and higher catalytic reactivity between metal ion and the enzyme thus enabling the rapid production of nanoparticles. Almost all the bacterial species adopt the common mechanism of synthesis of reductases enzymes for bio reduction of the metal ions. Variety of nanoparticles i.e., gold (Au), silver (Ag), iron (Fe), copper (Cu) manganese (Mn), zinc (Zn), palladium (Pd) etc have been reported to be synthesized by different species of bacteria (Table 18.1).

Most of research studies evidence that silver nanoparticles (AgNPs) can be produced at large scale from bacterial cell cultures, filtrates and cell pellets. Different bacterial species have adopted different ways of reducing Ag^+ ions into AgNPs. The most common mechanism involves the synthesis of AgNPs from bacterial biomass and silver nitrate solution under controlled temperature and pressure. The process is catalyzed by NADH-dependent reductase enzyme which reduces Ag^+ ions from silver nitrate into AgNPs by oxidizing NADH into NAD^+ . Depending upon the place of action of reductase enzyme, the AgNPs may be synthesized on or within the bacterial cell surface (Javaid et al., 2018). Also, the cell free extracts of certain bacterial strains such as *Cupriavidus*, *Bacillus brevis* (NCIM 2533), *Pseudomonas*

TABLE 18.1 Biogenic nanoparticles synthesized using various bacterial species.

S. no.	Biogenic nanoparticle	Most common substrate used by bacteria for synthesis of nanoparticle	Bacterial species synthesizing it	Reference
1.	Ag NPs	AgNO_3	<i>Bacillus subtilis</i> , <i>B. megaterium</i> , <i>B. flexus</i> , <i>B. methylotrophicus</i> , <i>Pseudomonas deceptionensis</i> , <i>B. cereus</i> , <i>B. licheniformis</i> , <i>WeisseNalla oryzae</i> , <i>B. stearothermophilus</i> , <i>B. marisflavi</i>	Omole et al. (2018), Wang et al. (2015), Priyadarshini et al. (2013), Subramaniyam et al. (2015), Sunkar and Nachiyar (2012)
2.	Au NPs	AuO	<i>B. cereus</i> , <i>Brevibacillus formosus</i>	Srinath et al. (2017), Singh et al. (2011)
3.	Fe NPs	FeS	<i>Streptomyces</i> sp., <i>Thiobacillus thioparus</i> , <i>Microbacterium marinilacus</i>	Sharma et al. (2016), Elcey et al. (2014), Mukherjee (2017)
4.	Cu NPs	CuS	<i>Pseudomonas stutzeri</i> , <i>P. fluorescens</i> , <i>Escherichia coli</i>	Varshney et al. (2010), Shantkriti and Rani. (2014), Singh et al. (2011)
5.	Zn NPs	ZnO	<i>Aeromonas hydrophila</i> , <i>P. putida</i>	Jayaseelan et al. (2012), Jayabalan et al. (2019)
6.	Pd NPs	Pd mines	<i>Desulfovibrio vulgaris</i>	Martins et al. (2017)
7.	Cd NPs	CdS	<i>Rhodopseudomonas palustris</i> , <i>B. amyloliquifaciens</i>	Singh et al. (2011), Torimiro et al. (2021)

aeruginosa etc have also been proven useful for the production of AgNPs. The extracellular polymeric substances released by bacteria may be present in these extracts which act as capping and stabilising agents for the reduction of Ag^+ ions and hence prevent clumping of AgNPs (Quinteros et al., 2019). Certain bacteria such as *Geobacter sulfurreducens* use metals as final electron acceptor during respiration. Hence, metal ions such as Ag^+ present in the vicinity of bacterial cultures get reduced by accepting electrons delivered by c-type cytochromes from periplasm to bacterial cell wall and thus the reduced Ag ions grow as AgNPs (Tanzil et al., 2016).

By adopting similar extracellular mechanisms, bacterial species have also been found to be involved in the production of gold nanoparticles (AuNPs). AuNPs are synthesized in cell free suspension of *Bacillus marisflavius* in time period of 96 hours, bluish-violet color was developed. Certain proteins and exopolysaccharides secreted by bacterial cells persist in cell free suspension and may be considered to catalyze and stabilize the formation of AuNPs (Vidya et al., 2016). Also, enzyme-catalyzed production of AuNPs has been reported in *Acinetobacter* sp. SW30 wherein Lignin peroxidase enzyme aids the synthesis of AuNPs (Jafari et al., 2018).

Das et al. (2018) reported that one of bacterial species *Desulfovibrio* is involved in the synthesis of Fe nanoparticles by utilizing FeS as substrate which is reduced by a specific reductase enzyme secreted by bacterial cell. Similarly, different bacterial species assist the synthesis of different biogenic nanoparticles by more or less similar mechanisms (Fig. 18.1).

18.3.2 Algal based synthesis of nanoparticles

The amalgamation between medical sciences and engineering has opened up new horizons in the ever-expanding new domain of nanotechnology targeted at the origin, implementation, and application of nanomaterials (NMs) in biological research. As an alternative to chemical and physical synthesis methods, the biological synthesis of (nanoparticles) NPs using algae has been proposed. Though still in its infancy, phyconanotechnology is emerging as an exciting and promising field with greater potential in the synthesis of algae-based NPs. Algae, the most abundant photoautotrophic group of microorganisms, are a potential source of a wide range of secondary metabolites, proteins and pigments, polyunsaturated

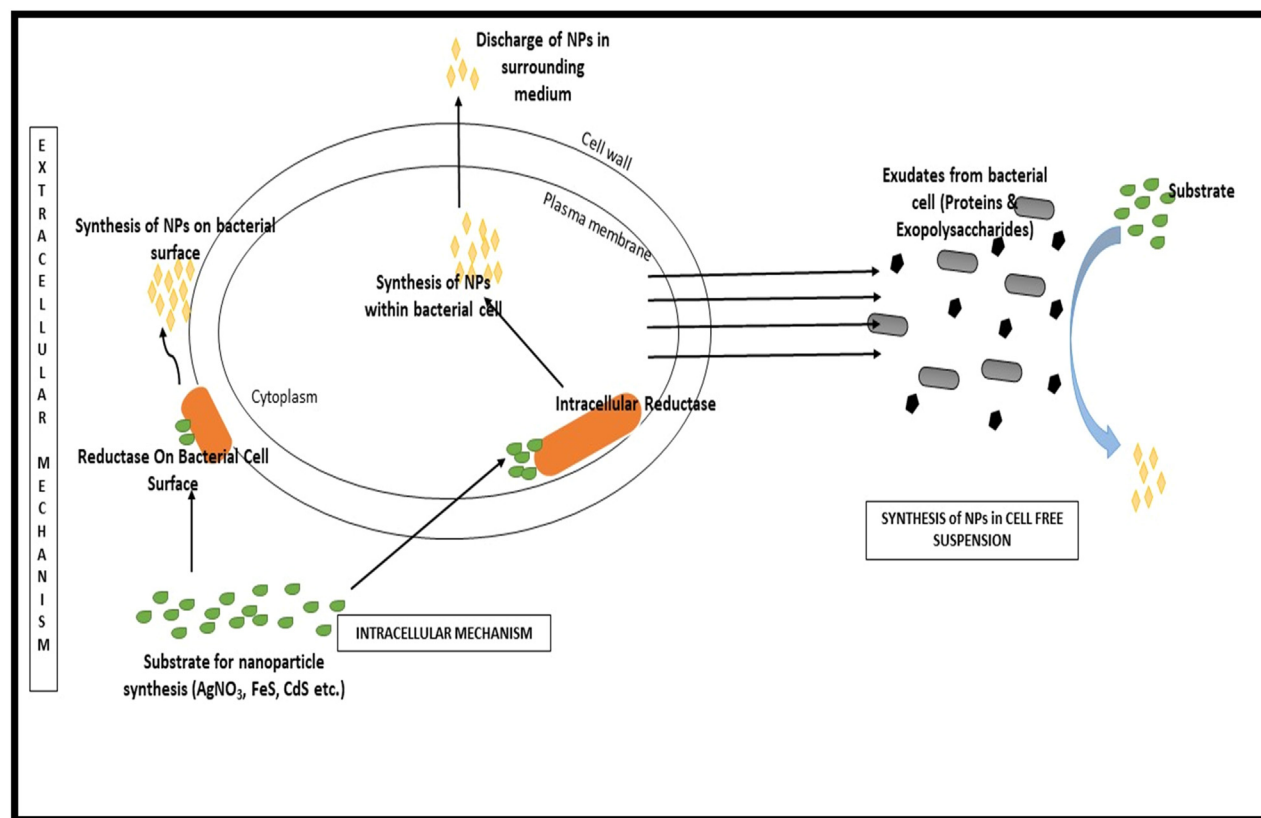


FIGURE 18.1 Mechanisms of bacterial synthesis of biogenic nanoparticles.

fatty acids (PUFAs), and other bioactive compounds like antioxidants (e.g., tocopherols and polyphenols) that can be used as/capping/stabilizing and reducing agents (Khanna et al., 2019). Because of these overall characteristics, algae are utilized as a model organism for the eco-friendly production of diverse NMs such as metallic NPs (González-Ballesteros et al., 2017). A list of common algae species utilized in the production of NPs is provided in the Table 18.2 below.

18.3.2.1 Synthesis of nanoparticles using algae

The synthesis of NPs using algae can be accomplished in three steps: (1) preparation of the algal extract in an organic solvent or water by boiling or heating it for a set period of time, (2) preparation of ionic metallic compounds molar solutions (3) incubation of algal solutions and ionic metallic compound molar solutions under controlled conditions, either with continuous stirring or without stirring for a set period of time (Rauwel et al., 2015). It should be noted that the final reaction generates a color change, which determines nucleation that occurs when adjacent nucleon particles bind together to create thermodynamically stable NPs of varied shapes and sizes (Rai and Posten, 2013). The production of NPs is dosage-dependent, as well as depending on the species of algae used. Algae may produce NPs both intracellularly and extracellularly (Aboelfetoh et al., 2017). An intracellular mechanism of NP production is dependent on metabolic pathways and does not involve algal pre-treatment (Sharma et al., 2016). To begin the production of intracellular NPs, algal biomass is collected and rinsed with distilled water. After that, the algal biomass is treated with particular metallic solutions for instance AgNO_3 solution. The combination is then incubated for bioreduction at a particular pH,

TABLE 18.2 Nanoparticles produced using algal species.

S. no.	Nanoparticle type	Source organism	Pollutant (Heavy metal, Dyes in textiles, hydrocarbons pharmaceuticals)	Reference
1.	Pd NPs	<i>Padina boryana</i>	Antibacterial, antibiofilm, and anticancer activities	Sonbol et al. (2021)
2.	Cu_2O NPs	<i>Sargassum latifolium</i>	Antibacterial and anticancer activity	Soureshjani et al. (2021)
3.	Ag NPs	<i>Amphiroa rigida</i>	Antibacterial activity	Gopu et al. (2021)
4.	Au NPs	<i>Acanthophora spicifera</i>	Pharmaceutical uses	Babu et al. (2020)
5.	Ag NPs	<i>Sargassum myriocystum</i>	Anticancerous, control the vector borne diseases	Balaraman et al. (2020)
6.	ZnO NPs	<i>Ulva lactuna</i>	Bactericidal activity, mosquito larvicide	Ishwarya et al. (2018)
7.	ZnO NPs	<i>Sargassum muticum</i>	Anticancer, anti-inflammatory, antioxidant, antidiabetic and anti-allergic	Sanaeimehr et al. (2018)
8.	Pd NPs	<i>Sargassum wightii</i>	Insecticidal activity	Moorthi et al. (2015)
9.	Au NPs	<i>Galaxaura elongata</i>	Antibacterial agent	Abdel-Raouf et al. (2017)
10.	Au NPs	<i>Hypnea musciformis</i>	Nanofungicides	Murugesan et al. (2015)
11.	Au NPs	<i>Laurencia papillosa</i>	Antibacterial activity	Montasser et al. (2017)
12.	Ag NPs	<i>Ulva fasciata</i>	Antibacterial agent	Hamouda et al. (2018)
13.	Ag NPs	<i>Sargassum ilicifolium</i>	Nano-biofertilizer	Roy and Anantharaman (2018)
14.	Ag NPs	<i>Caulerpa racemosa</i>	Catalytic degradation of methylene blue	Edison et al. (2016)
15.	Ag NPs	<i>Hypnea musciformis</i>	Antibacterial activity	Vadlapudi and Amanchy (2017)

temperature, and time conditions. Sonication and centrifugation are then used to extract the stable NPs (Sadhasivam et al., 2020).

During the extracellular production of NPs, firstly algal biomass is collected and then washed with distilled water. During the subsequent procedure, the three methods mentioned below are frequently used: (1) the algal biomass is dried in the shade for a specific period of time before being treated with distilled water and filtered out; (2) To get a cell-free extract, the algal biomass is sonicated by using distilled water; or (3) then algal biomass is rinsed with distilled water and incubated for 8–16 hours, and the resulting product is then filtered (Dahoumane et al., 2017). Furthermore, recent research provides a novel technique for synthesizing AgNPs (Silver nanoparticles) and AuNPs (Gold nanoparticles) using ultrasonic irradiation-assisted synthesis (UIAS) with the cyanobacteria *Calothrix* (Kumar et al., 2016). This eco-friendly and cost-effective technique enables the acceleration of a wide range of chemical reactions and extraction processes due to the resulting cavitation collapses. In reality, cavitation collapse causes substantial local heating (5000°C) and high pressures (2000 atmospheres) in the liquid reaction mixture, resulting in considerably shorter lives (Kumar et al., 2016).

The above Fig. 18.2 depicts the general mechanism for algal based AuNPs synthesis, algal biomass is mixed with deionized water and then heated at a definite temperature. The resultant algal extract is then incubated at room temperature with a metal precursor for a period of time. The formation of NPs is indicated by a change in color. The following processes of nucleation and condensation (accumulation) during metal ion bioreduction ensure the production (growth) of stable NPs surrounded by capping agents. The biosynthesized NPs can then be characterized using a variety of physical methods. UV-Vis spectroscopy may therefore be used to detect the solution color change and SPR, while Dynamic Light Scattering (DLS) and/or Scanning Electron Microscopy (SEM) and/or Transmission Electron Microscopy (TEM) can be used to identify the size, shape, and morphology of NPs. Furthermore, FTIR spectroscopy may reveal their structure (functional groups), and XRD spectroscopy can determine their crystalline nature.

Besides, marine algae have received less attention in terms of NP synthesis. *Chlorella vulgaris* has a high affinity for tetrachloroaurate ions, it produces algal-bound gold that can be reduced to Au (0). Around 90% of algal-bound gold became metallic and gold crystals with tetrahedral, decahedral, and icosahedral structures accumulated in the inner and outer portions of cell surfaces (Xie et al., 2007). Extracellular Au, Ag and Au/Ag bimetallic NPs have been synthesized using *Spirulina platensis* (Chakraborty et al., 2009). *Tetraselmis kochinensis* was used by Senapati et al. (2012) to

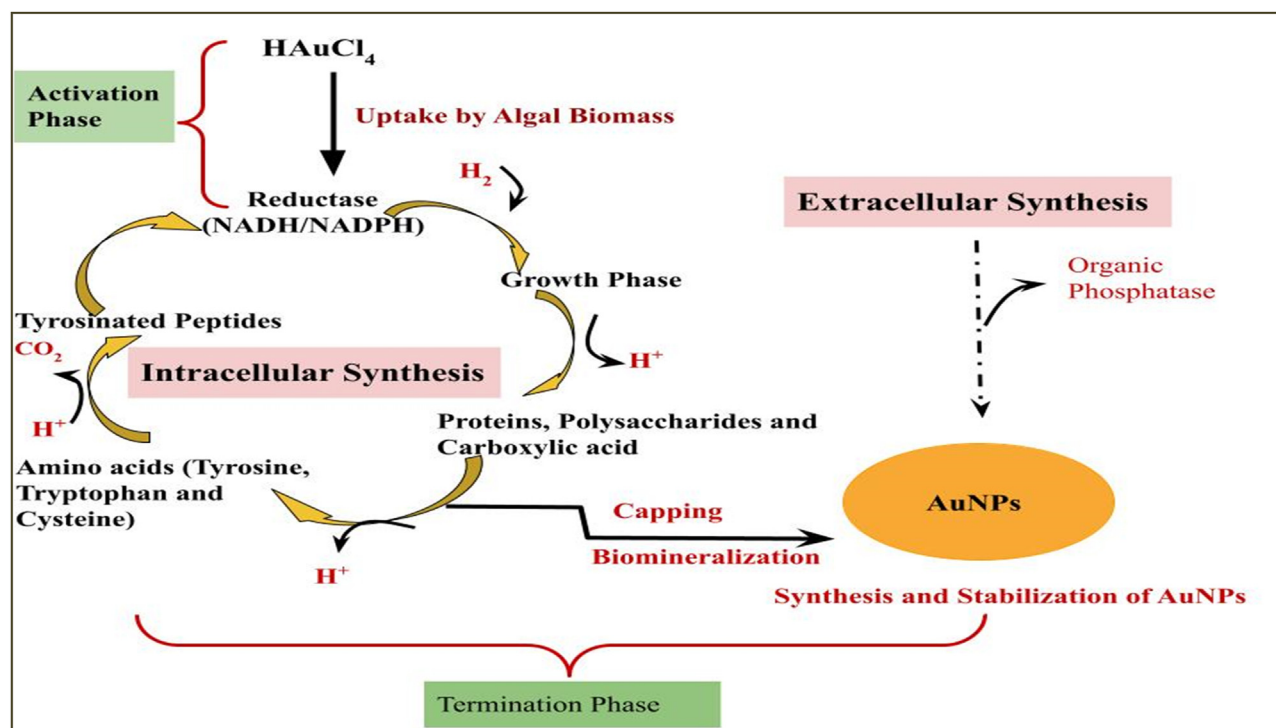


FIGURE 18.2 The extracellular and intracellular mechanism involved in the production of algal-mediated gold nanoparticles (AuNPs).

produce intracellular gold nanoparticles. Aside from seaweeds, microalgae like diatoms (*Navicula atomus* and *Diadlesmis gallica*) produce gold nanoparticles, gold, and silica -bionanocomposites (Mubarak Ali et al., 2013).

18.3.3 Fungal based synthesis of nanoparticles

Apart from algae, fungi have also been used for biosynthesis of NPs (Sadhasivam et al., 2020). The synthesis of NPs using fungi is known as mycosynthesis and is being addressed by myconanotechnology (Khandel and Shahi, 2018). Fungi-derived NPs have good dispersion and stability properties. Myconanotechnology is an environment-friendly and cost-effective option. It is due to easy availability of large number of specific enzymes, simplicity of dealing with them in the laboratory, scalability, and financially feasible development of fungi even on an industrial scale (Youssef et al., 2017). Furthermore, filamentous fungus may produce a variety of MtNPs (metallic NPs), including silver (Ag), iron oxide (Fe_2O_3), gold (Au), and even bimetallic NPs (Molnár et al., 2018). According to research, numerous different fungus species may be employed in the green synthesis of MtNPs with the required size, shape, surface charge, as well as desirable characteristics (Khan et al., 2017). The Table 18.3 given below summarizes some of the most recent studies on the synthesis of NPs using fungus.

18.3.3.1 Synthesis of nanoparticles using fungi

The process of biogenic synthesis of NPs falls into two main categories: intracellular or extracellular. In terms of the mechanism of extracellular mycosynthesis, it is proposed that the metabolites secreted by fungus for survival when exposed under different environmental stresses, such as proteins, enzymes, polysaccharides, alkaloids, flavonoids, organic acids, and phenolic and so on, are primarily dependable for the reduction of metal ions to metallic NPs via the catalytic effect (Srivastava et al., 2019). Furthermore, the same metabolites function as reducing and stabilizing agents, which contribute to the development and stability of biogenic metal NPs (Gudikandula et al., 2017). One of the recently presented hypotheses is that AgNPs were produced in the presence of purified form of enzyme nitrate reductase. They extracted enzyme from fungus (for example, *Fusarium oxysporum*) on selective medium and purified it using ion exchange chromatography and ultrafiltration. The synthesis of Ag NPs by nitrate reductase was NADPH-dependent,

TABLE 18.3 Nanoparticle synthesized using fungal species.

S. no.	Nanoparticle type	Source organism	Pollutant (heavy metal, dyes in textiles, hydrocarbons pharmaceuticals)	Reference
1.	Ag NPs	<i>F. oxysporum</i>	Pharmaceuticals	Rai et al. (2021)
2.	Fe_2O_3 NPs	<i>Aspergillus tubingensis</i>	Heavy metal remediation	Mahanty et al. (2020)
3.	Au, Ag NPs	<i>Pleurotus sajor-caju</i>	Nanopesticides	Chaturvedi et al. (2020)
4.	ZnO NPs	<i>Aspergillus niger</i>	Antimicrobial textiles and Dye	Kalpana et al. (2018)
5.	Ag NPs	<i>Trichoderma harzianum</i>	Antifungal	Guilger et al. (2017)
6.	Ag NPs	<i>P. nagiovense</i> AJ12	Nanopesticides	Maliszewska et al. (2014)
7.	Au NPs	<i>Cylindrocladium floridanum</i>	Antifungal, Antiparasitic combined with Triclabendazol, Insecticide	Narayanan and Sakthivel (2013)
8.	Au NPs	<i>Trichoderma Koningii</i>	Antibacterial activity Vegetable and fruit preservation	Maliszewska (2013)
9.	Ag NPs	<i>Alternaria alternata</i>	Enhancement in antifungal activity of fluconazole against Phoma- glomerata and water quality monitoring	Sarkar et al. (2012)
10.	Ag NPs	<i>Aspergillus terreus</i>	Antimicrobial activity	Li et al. (2012)

with gelatin acting as a capping agent (Baymiller et al., 2017). The authors of the previous study described the function of anthraquinone and NADPH-nitrate reductase in AgNPs biosynthesis, and it was put forward that both quinone and NADPH donated the electron required to fulfill the deficiency of aqueous silver ions (Ag^+) and convert it into Ag neutral (Ag^0 , i.e., AgNPs) (Rai et al., 2021). On the other hand, later research found that the reduction of NADPH to NADP^+ and the hydroxyquinoline may function as an electron shuttle, transporting the electron produced during the reduction of nitrate to Ag^+ ions and converting them to Ag^0 . Some reports suggested that several hydroquinones serve as electron shuttles and they reduce metal ions. *F. oxysporum* f. sp. *cubense* JT1 was reported to be capable of reducing Au ions to AuNPs (Thakker et al., 2013).

Besides extracellular processes, a few pathways for intracellular mycosynthesis of metal NPs have been postulated. Metal NPs are often produced below the cell surface in the case of *Fusarium*-mediated intracellular mycosynthesis, which may be owing to metal ion reduction by metabolites (i.e., enzyme) that is present in the cell membrane. In general, a two-step process for intracellular mycosynthesis of NPs had been proposed. In the case of first step, electrostatic interaction between lysine residues and metal ions (M^+) attaches aqueous metal ions to the fungal cell surface. On the other hand, the actual mycosynthesis of NPs take place in the second phase via the enzymatic reduction of metal ions (M^0), resulting in aggregation and production of NPs (Rajput et al., 2016). Furthermore, various studies on mycosynthesis hypothesized the involvement of numerous additional enzymes and proteins. Among all of these extracellular mycosynthesis pathways, the proposed mechanism involving the NADH-dependent nitrate reductase enzyme is the most well-known.

In addition to the metal NPs, fungi can also produce metal oxide NPs. Magnetite is a ubiquitous iron oxide that displays magnetic characteristics. *Aspergillus* fungi, for example, are capable of generating such NPs (Kumari et al., 2020). Because of their high (and quick) surface area to volume ratio, NMs are being utilized to remove heavy metals and radioactive contaminants (Ding et al., 2015). Magnetite NPs are more capable than other metallic NPs because of their superparamagnetic nature, which allows them to easily separate from wastewater by electrostatic and surface complexation. Magnetite NPs have a wide range of applications, including oscillation damping, MRI, recording devices, and position sensors (Yan et al., 2017). There are numerous advantages to intracellular formation as well. As a remedy, the environmental contaminants such as depleted metals (platinum and copper) must be removed from the environment. Fungi that are formed intracellularly are employed due to the ease with which contaminants may be extracted from the sample (Abdel-Aziz et al., 2020). These findings pave the way for more research into the application of fungus NPs in agriculture.

18.4 Microbe mediated nanobioremediation of pollutants

Nanobiotechnology based remediation is being applauded in recent years. The use of microbes along with metal nanoparticle is a profitable approach owing to renewable, easy to handle, cost-effective and high metal tolerance of microbes (Kumari et al., 2019). Many reports came up stating the beneficial effect of microbe-mediated nanoparticles in remediation processes (Wang et al., 2016a,b). Microbes serves as capable candidates for the process due to their unique physical, biological, chemical, and optical properties such as adjustable functionality, super-hydrophobic, filtering nature and high surface-to-volume ratio (Anjum et al., 2016). Recent times have witnessed wide scale applications of nanobioremediation in removal of inorganic, organic pollutants and heavy metals from soil and water bodies. Nanoparticles that are of biogenic origin serves better than chemically synthesized nanoparticles as they play multiple roles: catalysts, adsorbents, oxidants and reductants (Kumari et al., 2019). Iron oxides act on contaminants by both oxidation and reduction processes. Heavy metal removal is dependent on the factors such as surface area and type of nanoparticle. For heavy metal removal iron oxide nanoparticles are extensively utilized (Yadav et al., 2017). Additionally, biogenic iron oxides also reduce organic contaminants and stimulate dechlorination of aliphatic compounds (Castro et al., 2013). It was reported by Aziz et al. (2015) that silver nanoparticle effectively degrades toxic organic contaminants from the environment. Palladium nanoparticle produced from *Klebsiella oxytoca* GS-4–08 was used for removal of azo dye from wastewater (Wang et al., 2018a,b). Similarly, it was stated by Shi et al. (2017) that use of gold nano-catalyst prepared from *Saccharomyces cerevisiae* resulted in reductive degradation of quinclorac.

18.4.1 Nanobioremediation of heavy metals

Kumar et al. (2019) reported that magnetic oxide nanoparticles from different microbe productively removed hexavalent chromium in the environment. They also stated that application of exopolysaccharides (EPS) matrix from *Lysinibacillus sphaericus* provided better binding sites for various metal ions. Hence, they adsorbed Cr (VI) more effectively. Iron

nanoparticles produced from algae *Chlorococcum* sp. exhibit appreciable reduction of about 92% of 4 mg/L Cr (VI) to Cr (III) (Subramaniyam et al., 2015). Another method involves synergistic approach of algae (*Chlorella vulgaris*) and TiO₂/Ag hybrid nanomaterial. This setup led to photo-removal of Cr (VI) under UV irradiation (Wang et al., 2017a,b). Similar approach was targeted at pharmaceutical effluents containing heavy metals mainly Chromium (Cr) and Lead (Pb) (Kumari et al., 2020). It was stated by Raj et al. (2016) that *Pseudomonas aeruginosa* possess ability to oust cadmium from cadmium contaminated water and also stimulate biosynthesis of cadmium sulfide nanoparticles. Likewise, another study conducted on removal and bioremediation of cadmium polluted soil in which it was observed that co-treatment of nanohydroxyapatite (NHAP) and *Bacillus subtilis* productively removed cadmium (Liu et al., 2018). Mercury polluted soil was bioremediated with selenium nanoparticles which were produced with the presence of *Citrobacter freundii* (Wang et al., 2017a,b). Nickel toxicity in industrial effluents was tackled by introducing *Microbacterium* sp. which also led to recovery of Ni by production of nickel oxide nanoparticles (Sathyavathi et al., 2014). Recently it was reported that combined application of *Escherichia coli* and metallic nanoparticles are best suited for bioremediating both cadmium and lead present in soil (Zhu et al., 2020). There are many reports stating higher concentrations of arsenic, Fe (II) and Mn (II) in groundwater and surface waters. To tackle the problem biogenic Fe–Mn oxides (BFMO) were synthesized by employing *Pseudomonas* sp. QJX-1. BFMOs were able to perform oxidation and adsorption of arsenic (Bai et al., 2016). It was verified by Zhou et al. (2015) that Biogenic manganese oxide (BMO) synthesized from *Pseudomonas putida* MnB1 performed better in heavy metal removal than chemically synthesized manganese oxide (birnessite). Alsharari et al. (2018) potential of chitosan nanoparticles (NCt) from chitosan (bioactive polymer) and *Cunninghamella elegans* as they had capacity to retain Pb and Cu ions in polluted environment. In related study, Kumari et al. (2020) reported that silver nanoparticles produced using *Bacillus cereus*, led to 98.13% and 98.76% reduction in Cr and Pb respectively from the waste effluents from pharmaceutical industries. Kumar et al. (2019) documented in his study that exopolysaccharides (EPS) matrix obtained from *Lysinibacillus sphaericus* possessed binding sites for diverse forms of metallic ions. These exopolysaccharides (EPS) matrix productively absorb Cr (VI). Likewise, Biogenic iron sulfides also removed zinc ions from mine drainage water, recommending there utility in remediating real wastewater too (Jencarova and Luptakova, 2012). Table 18.4 comprises nanobioremediation methods used for removal of heavy metals from the environment.

18.4.2 Nanobioremediation of dyes in textiles

A large number of dyes are being used for the cloth coloration in textile and paper industries. Some of them are methyl red, methylene blue, methyl orange, Congo red etc. though these dyes provide beautiful colors to the cotton and woolen fabrics but the waste generated from dye processing and utilization is being released into water or soil directly or

TABLE 18.4 Remediation of heavy metal using nanobioremediation.

S. no.	Nanoparticle	Organism	Remediation	Reference
1.	Bio-Pd nanoparticle	<i>C. pasteurianum</i> BC1	Cr(VI) reduction	Chidambaram et al. (2010)
2.	Biogenic manganese oxide (BioMnOx)	<i>Pseudomonas putida</i> MnB1	Heavy metals removal	Zhou et al. (2015)
3.	Nanoparticle synthesized using sulfate reducing bacteria (SRB)	Sulfate-reducing bacteria	Chromium (VI), sulfate removal from wastewater	Verma et al. (2015)
4.	Biogenic Fe–Mn oxide (BFMO)	<i>Pseudomonas</i> sp. QJX-1	Arsenic removal from contaminated groundwater	Bai et al. (2016)
5.	nZVI-C-A beads	<i>Bacillus subtilis</i> , <i>E. coli</i> , and <i>Acinetobacter junii</i>	92% Cr(VI) removed	
6.	Nanoparticle synthesized using sulfate-reducing bacteria (SRB)	Sulfate-reducing bacteria	Arsenic removal from groundwater	Saunders et al. (2018)
7.	Carbon nanotubes	<i>Shewanella oneidensis</i> Mr-1	92% removal of Cr(VI)	Yan et al. (2013)

TABLE 18.5 Microbe-mediated nanobioremediation of dyes in textiles.

S. no.	Nanoparticle type	Source organism	Dyes in textile pollutants	Reference
1.	Copper	<i>Escherichia</i> sp.	Photocatalytic degradation of methylene blue, congo red and methyl red dyes	Noman et al. (2020)
2.	Silver	<i>Aspergillus flavus</i> , <i>Bacillus amyloliquefaciens</i> , <i>Leuconostoc lactis</i> , <i>Pseudoalteromonas lipolytica</i> , <i>Chlorella pyrenoidosa</i> , <i>Chromobacterium violaceum</i> , <i>Bacillus pumilis</i> , <i>Caulerpa serrulata</i>	Adsorption of malachite green dye Photocatalytic degradation of methyl orange, methyl violet and congo red dyes	Pandian et al. (2016), Saravanan et al. (2017), Kulkarni et al. (2018), Aziz et al. (2015), Jishma et al. (2018), Modi et al. (2015), Aboelfetoh et al. (2017)
3.	Gold.	<i>Aspergillum</i> sp., <i>Pseudoalteromonas lipolytica</i>	Degradation of aromatic pollutants and azodyes	Qu et al. (2017), Kulkarni et al. (2018)
4.	Palladium	<i>Shewanella loihica</i> , <i>Klebsiella oxytoca</i> , <i>Saccharomyces cerevisiae</i>	Decomposition of methyl orange dye and other azo dyes	Ahmed et al. (2018), Wang et al. (2018a,b), Sriramulu and Sumathi (2018)
5.	Zinc	<i>Aspergillus niger</i>	Mineralization & discoloration of Bismarck brown dye	Kalpna et al. (2018)
6.	Molybdenum	<i>Clostridium pasteurianum</i>	Degradation of Azo dyes like methyl orange	Nordmeier et al. (2018)
7.	Titanium	<i>Micrococcus lylae</i> , <i>Micrococcus aloeverae</i> , <i>Cellulosimicrobium</i> sp.	Photocatalytic degradation of Methyl orange dye	Fulekar et al. (2018)

indirectly. These dyes are not biodegradable and therefore their entry into food chain through any means may pose serious threat to the health of living organisms. Therefore, an eco-friendly strategy like nanobioremediation can prove accountable for the removal of such harmful pollutants from environment or even may cease their entry into the ecosystem by early degradation (Asaduzzaman et al., 2016). Several biogenic nanoparticles have been utilized for the elimination of harmful dyes from environment and are listed in Table 18.5.

18.4.3 Nanobioremediation of hydrocarbon

Organic compound like hydrocarbons has been reported one of the persistent pollutant in this modern era. Microbe-mediated bioremediation was one of the recent modern technique to diminish, detoxify and transform the pollutants into less toxic form called as bioaugmentation. Specific microbes and fungal cultures were modified with nanoparticles for effective biological augmentation for degeneration of contaminants. It was reported that the electrostatic synergy exists between the microbes and magnetic nanoparticles. It has been documented the role of *Sphingomonas* sp. with nanoparticles (non-Zerovalent Iron) in remediating PBDEs (Polybrominated Diphenyl Ethers) hydrocarbons in aqueous medium (Kim et al., 2012). Another microbe species i.e., *Sphingomonas wittichii* was combined with bi-metallic nanoparticles (nFe/Pd) for mitigating the 2,3,7,8-Tetrachlorodibenzo-p-dioxin hydrocarbon. This integrated form facilitated the detoxification process, resulting in production of Dibenzo-p-dioxin (Bokare et al., 2012). Further the *Sphingomonas* species was used as biofunctionalization mechanism for stabilized CMC (Carboxymethyl Cellulose)-cum-Fe/Pd nanoparticles. Lindane which was commonly used in cosmetics detoxified by nanohybrid and successfully diminishes the γ -Hexachlorohexane (lindane).

Another study revealed the dechlorination of pollutant (Aroclor 1248- a congener of Polychlorinated Biphenyls) when combined with Fe/Pd bimetal nanoparticles, converted it into biphenyl. Then these biphenyls were remediated via using *Burkholderia xenovorans* for decreasing the persistent hydrocarbons (Le et al., 2015). Other hydrocarbons (PAH, anthracene) were cleaned up via using nanoparticles modified with lipid layer of microbes (*Pseudomonas aeruginosa*, *Trametes versicolor*) (Wang et al., 2015). The lipid bilayer was found to play an interesting role in evicting and

adsorbing the hydrocarbons when combined with nanoparticles. Polyallylamine Hydrochloride (polymer) coated nanoparticles further biofunctionalized by combining with *Alcanivorax borkumensis* results in degradation of polymer (Konnova et al., 2016).

Some studies showed the effectiveness of microbial chemical agent in bioremediation of oil pollutants. It was documented that *Alkaligenes faecalis* and iron oxide nanoparticles together ameliorated the contaminants such as crude oil (Oyewole et al., 2019). The biofunctionalization of $Zn_5OH_8Cl_2$ modified Iron oxide nanoparticles combined with microbe (*Bacillus licheniformis*) resulted in breakdown of crude oil into degraded compound (El-Sheshtawy and Ahmed, 2017). Different concentrations of microbes-nano hybrid were observed for the bioremediation of pollutants.

18.4.4 Nanobioremediation of pharmaceuticals (antibiotics and antiseptics)

The discharge of pharmaceuticals compounds including antibiotics and antiseptics into the surroundings is a severe issue these days. The chance of eliminating these toxic compounds via using nanobioremediation integrated with microbial sp. was evaluated in many studies (Table 18.6). Platinum nanoparticles were biofunctionalized with *Desulfovibrio vulgaris* for the efficient removal of pharmaceuticals. Manganese Oxide which was biosynthesized from *Bacillus* and

TABLE 18.6 Enlists examples of microbe mediated bioremediation of pollutants.

S. no	Pollutant		Microbes used	Microbe-mediated bioremediation of pollutants	Reference
1.	Hydrocarbons	Crude oil	<i>Alkaligenes faecalis</i>	Maintained the Iron Oxide nanoparticles	Oyewole et al. (2019)
		PAH	<i>Alcanivorax borkumensis</i>	Modified the Polyallylamine Hydrochloride layered magnetic nanomaterial	Konnova et al. (2016)
		PAH (Anthracene)	<i>Trametes versicolor</i>	Biofunctionalization of nanoparticles of Graphene Oxide	Patil et al. (2016)
		PAH (Benzo[a]pyrene)	<i>Pseudomonas aeruginosa</i>	Silica nanoparticles modified by adding microbes for the removal of PAHs	Wang et al. (2015)
		PCBs (Polychlorinated biphenyls)	<i>Burkholderia xenovorans</i>	Biofunctionalization of Fe/Pd (bi-metallic) nanoparticles	Le et al. (2015)
		Lindane	<i>Sphingomonas</i> sp.	Biofunctionalization of CMC-Pd/Fe (Carboxymethyl Cellulose) maintained bi-metallic nanoparticles	Singh et al. (2013)
		Polybrominated diphenyl ethers (PBDEs)	<i>Sphingomonas</i> sp.	Modification of nZVI (non-Zerovalent Iron)	Kim et al. (2012)
2.	Antibiotics/ Antiseptics	2,4,6-Trinitrophenol	<i>Pseudomonas aeruginosa</i>	Modification of many layered carbon tubes with iron oxide nanoparticles	Yousefi et al. (2020)
		Pharmaceutical substances	<i>Desulfovibrio vulgaris</i>	Biosynthesis of platinum nanoparticles	Martins et al. (2017)
		1,2,4-Triazole	<i>Pseudomonas</i> and <i>Bacillus</i> sp.	Production of MnO_2 (Manganese Oxide) nanoparticles	Wu et al. (2017)
		Triclosan	<i>Candida</i> sp.	Modification of MgO (Magnesium Oxide) nanoparticles	Adikesavan and Nilanjana (2016)
		Hydrogen peroxide (H_2O_2)	<i>Sargassum bovinum</i>	Formation of the Palladium nanoparticles	Momeni and Nabipour (2015)
		Cefdinir	<i>Turbinaria conoides</i>	Production of Silver and Gold nanoparticles	Vijayan et al. (2014)

Pseudomonas sp. had potentially removed the 1, 2, 4-Triazole and other pollutants from industrial water. Another report stated that the antiseptic which is commonly called as picric acid (2,4,6-trinitrophenol) poses risk to the environment (Wu et al., 2017). For the remediation of picric acid, *Pseudomonas aeruginosa* integrated with Iron oxide (Fe_3O_4) nanoparticles (multi-layered nanotubes of carbon) used for the synthesis of nano-composite material which further nanobio-mediated the antiseptic (Yousefi et al., 2020).

A fungal species, *Trametes versicolor*, used as a biosynthesized agent for the production of Fe/Pd nano-hybrids removed the tricloan (antiseptic/antibiotic agent) from industrial effluents.

The mechanism of detoxification and degradation of tricloan includes the dechlorination process and oxidation of 2-Phenoxyphenol via laccase enzyme synthesized from the *Trametes versicolor*. Further there was another report that documented the modification of MgO (Magnesium Oxide) nano-particles by using yeast species (Adikesavan and Nilanjana, 2016). This nano-hybrid strategy was implied for the detoxification of cefdinir pollutant. Silver and Gold nanoparticles derived from an algal species, *Turbinaria conoides*, were used as an antibiofouling agent (Vijayan et al., 2014). H_2O_2 (Hydrogen Peroxide) a potent pollutant was removed via electrochemical reduction of the molecule mediated by Palladium nano-particles bio functionalised by *Sargassum bovinum* from industrial effluents (Momeni and Nabipour, 2015).

18.5 Conclusions

Nanotechnology had been very promising in the field of pollution control. Manufacturing of nanoparticles using biotic agents is a very expedient approach taking into consideration the zero emission of toxic chemicals. From the reviewed literature it can be summed up that these biogenic nanoparticles are effective in treating several pollutants from water and soil resources. This study also highlights the role of various microbes (algae, fungi, bacteria) in production of such nanomaterial that have been immensely effective in remediation processes. They are eco-friendly and economically viable substitute to the ones prepared by chemical methods. They dispose of numerous contaminants such as pesticides, halogenated compounds, heavy metals, dyes, and pharmaceutical wastes. Moreover, a better understanding is required to create effective and sustainable environmental clean-up strategies. In future, bioremediation innovations are more desirable than physical and chemical approaches used at present. The research in upcoming years should also take into account the doubts among communities with the regards to use of genetically engineered microbes. The technology can be improved by using highly functional genes than the entire microorganism. A more understanding is required regarding the reaction mechanisms involved, to widen the horizons for application at the field scale.

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Application of microbially-synthesized nanoparticles for adsorptive confiscation of toxic pollutants from water environment

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19.1 Introduction

Presently, the detection of conventional hazardous and priority contaminants is progressively incapacitating the endurance of traditional water treatment technologies (Rout et al., 2021). The removal of emerging pollutants, which include inorganic or organic microcontaminants, personal care products, pharmaceuticals, pigments, and infectious microorganisms has been reported by several investigators (Cheng et al., 2021; Egea-Corbacho et al., 2019). The intensity and quantity of these contaminants are growing endlessly owing to the swift surge in industrial, domestic, and agricultural activities (Li et al., 2020). However, the strict thresholds of these contaminants are making constraints for the prevailing wastewater treatment technologies to meticulously sequester these pollutants from the aquatic environment (Khan et al., 2022; Siddiqui et al., 2022). With the recent progress in the nanotechnology, the nanomaterials have aroused much attention for their nanosize, high surface area and unusual physicochemical characteristics and amazing surface properties. In addition to their miscellaneous usage in different areas, the nanomaterials exhibit significant potential as nano-sorbents for environmental abatement of various hazardous substances like heavy metals, dyes, pesticides, and pharmaceuticals from the aquatic phase. However, the synthesis of nanoparticles employing physical or chemical strategies involves elevated consumption of energy and consequently costly, lower percentage yield, and use of toxic chemicals (Patil and Chandrasekaran, 2020). In recent times, the microbes-mediated preparation of nanoparticles has attained considerable popularity (Menon et al., 2017) owing largely to its cost-effectiveness, biocompatibility, eco-friendliness and minimum energy consumption. Due to their exceptional assets, the biogenic nanoparticles find useful applications in biomedical, electronics, and in environmental remediation (Zhang et al., 2017). Microbes induced synthesis utilizes bacteria, yeast, fungi, and viruses, which provide economical, high-yield, and environmentally viable synthesis of nanoparticles (Durán et al., 2005).

Biogenic fabrication of nanoparticles is a green method and is deemed as a stimulating area of research for future application (Mandal et al., 2006). The rate of biogenic nanoparticles preparation along with properties could be enhanced by employing rich biodiversity of microbes under cellular, biochemical, and molecular mechanism. The facets of nanoparticles as well as nanostructured mineral crystals made by biogenic approach are analogous to chemically produced nanomaterials (Roy et al., 2019). The development of biogenic nanomaterials can be swayed for the form and extent by influencing the culture factors to an extent. The formation mechanism of biogenic nanoparticles (intracellular and extracellular) is diverse across different microbial strains (Hulkoti and Taranath, 2014).

In addition to the robust synthetic protocol, biogenic nanoparticles have been extensively utilized for remediation of wastewaters (Martins et al., 2017). They have been used in many ways like biocatalysts, oxidants/reductants, and adsorbents for the abatement of contaminants from wastewater. Various investigations have revealed the existence of functional moieties (i.e., carboxylate, amine, hydroxyl, and methyl groups) and the range of reducing element produced by the bacterial cells are principally liable for the elimination of pollutants through electrostatic interactions and ion exchange (Qu et al., 2017a). In this chapter, we have attempted to convey a thorough summation of adsorptive applications of biogenic nanoparticles. Additionally, synthetic approaches and contaminant confiscation mechanisms are also reviewed in detail.

Lastly, some major concerns that require consideration of professionals for the workable performances of biogenic nanoparticles-based technology for wastewater treatment are emphasized.

19.2 Bio-mediated synthesis of nanoparticles and their characterization

Inorganic materials and biological units have been in frequent contact with one another since initiation of life forms on the earth. Owing largely to this common interaction, life could nurture on earth with a well-organized accumulation of minerals. Green nanotechnology is a developing arena to model novel nanomaterials utilizing green chemistry procedure. Bio-mediated fabrication of nanomaterials present a new prospect of producing nanomaterials employing natural reducing and stabilizing mediators. It is cost-conscious and ecologically benign substitute to physical and chemical tactics with minimum utilization of power and noxious substances. Microbial fabrication of nanomaterials falls under bottom-up methodology that entails usage of unicellular to intricate multicellular biotic units like yeast, fungi actinomycetes, bacteria, and viruses (Gahlawat and Choudhury, 2019). Broadly, these unicellular entities exhibit metal-tolerant competence and flourish at extreme environmental circumstances (Fig. 19.1). These intrinsic characteristics are exploited by microorganisms to endure, amass, and result in the formation of nanostructured counterparts from parent metal solution. Each unit represents a distinct metabolic processing mechanism to produce nanomaterials, such as the oxidation/reduction of metallic ions via sugars, polyphenol, carboxyl, and aldehyde groups, proteins, and enzymes (Ali et al., 2019). Diverse biogenic nanomaterials can be produced employing various metal salts solution (silver, gold, selenium, antimony, titanium, and palladium) and distinct biological entities (Qu et al., 2017b). More emphasis is being paid to microorganisms that create nanomaterials in the environment through biological processes or biomineralization. For instance, biologically controlled mineralization (BCM) and biological induced mineralization (BIM) are well documented to produce biogenic nanomaterials (Bloch et al., 2021). Biological controlled mineralization processes involve the synthesis of biogenic nanomaterials into the cells under explicit environment with the nucleation and development of the nanomaterials entirely dominated by the biological entity. But, in BIM process metal ions solution is treated with bacterial cell wall/membrane filtrate, resulting in the formation of biogenic nanomaterials via precipitation and/or reduction (Chen et al., 2019). The microbial entities can also produce biogenic nanomaterials through methods such as biosorption, complexation, and intra- or extracellular bioaccumulation/ biomineralization. In the beginning, the interaction of metal ions solution (electron acceptor) and organic matter (electron donor) in the presence of microbes play a crucial part in the fabrication of these nanomaterials under anaerobic or aerobic conditions. Metal ions will be reduced on cell walls because of metabolites such as carboxyl, amino, imidazole, thiol, and guanidine, among others. Consequently, these nanomaterials will propagate due to surface development and nucleation method. The morphological characteristics might, however, be regulated by fluctuating temperature, pH, and oxygen levels etc. (Deplanche et al., 2014). Various biogenic nanomaterials constituting different metal ions have been fabricated. Gold nanoparticles have been synthesized using actinomycetes, *Thermomonospora* and *Fusarium oxysporum* (Ahmad et al., 2003), and filamentous cyanobacteria (Lengke et al., 2006). Silver nanoparticles were obtained by *Staphylococcus aureus* (Panáček et al., 2006), and *Verticillium*, *Fusarium oxysporum*, or *Aspergillus flavus* (Vigneshwaran et al., 2007). Other nanoparticles like SrCO_3 , $\text{Zn}_3(\text{PO}_4)_2$ were fabricated using *Fusarium oxysporum* (Rautaray et al., 2004) and yeast (Yan et al., 2009), respectively. Protozoan species have also been utilized for the synthesis of biogenic NPs like nano-Se using *Tetrahymena thermophila* (Cui et al., 2016), and foraminifera species was employed for the development of magnetic nanocomposite (Magnabosco et al., 2019). Archaea, which are single cell prokaryotes have been ubiquitously exploited for the synthesis of NPs including Ag and Au NPs by *Haloferax volcanii* (Costa et al., 2020), tungsten NPs by *Metallosphaera sedula* (Milojevic et al., 2019) and Pd NPs by *Sulfolobus tokadaii* (Peskova et al., 2019). Ag NPs were obtained using *Pseudomonas* sp. ef 1 (50 nm) (John et al., 2020) and *Streptomyces* sp. (2.3–85 nm) (El-Gamal et al., 2018). *Brevibacillus formosus* (Srinath et al., 2017) and *Streptomyces capillisspiralis* (Hassan et al., 2018) have been utilized for synthesizing Au NPs (2–5 nm) and Cu NPs (3.6–59 nm), respectively. The biosynthesis of Ag NPs was

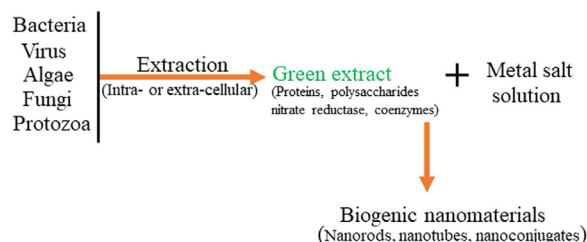


FIGURE 19.1 Graphical description of fabrication of biogenic nanoparticles (Gahlawat and Choudhury, 2019).

reported by [Soliman et al. \(2018\)](#) with *Candida albicans*, *Candida utilis* and *Saccharomyces boulardii*. Se NPs was generated through *Magnusiomyces ingens* LHF1 ([Lian et al., 2019](#)). Biosynthesis of Ag NPs by *Streptomyces xinghaiensis* OF1 strain ([Wypij et al., 2018](#)) and *Bacillus mojavensis* BTCB15 ([Iqtedar et al., 2019](#)) was reported recently.

The representation of the biogenic synthetic approaches is pictorially depicted in [Fig. 19.1](#).

The obtained nanoparticles are generally characterized by means of FTIR, XRD, EDX, SEM, TEM and BET analyses in order to obtain information with regard to surface functional groups, elemental composition, morphology, particle size, porosity and surface area etc.

19.3 Factors affecting the synthesis of biogenic nanomaterials

There are several features which can have a dominating impact on the formation of biogenic nanoparticles that include, pH, temperature, microbe type, concentration of precursor, reducing agent, and contact time. However, limited literature focusing on these facets is available ([Tuo et al., 2013](#)).

19.4 Impact of pH on the synthesis of biogenic nanomaterials

The impact of pH on the synthesis of biogenic nanomaterials is huge, which not only influences the size but have a commanding impact on the shape of nanoparticles. A study ([Velgosová et al., 2016](#)) reported irregular shape of the nanoparticles with the tendency to aggregate at lower pH. Further, they demonstrated that the desired size and uniform shape can be achieved at higher alkaline pH, wherein the nanomaterials form cluster distribution in the colloidal phase inhibiting aggregation ([Anigol et al., 2017](#)). The effect of pH on the shape of gold nanoparticles were demonstrated by [Armendariz et al. \(2004\)](#) who illustrated that acidic pH (2, 3, and 4) was responsible for the formation of cubic, tetrahedral, and hexagonal gold nanoparticles.

19.5 Impact of precursor and reducing agents' concentration on biogenic nanomaterials synthesis

Concentration of precursors and reducing agents have substantial impact on the size of biogenic nanoparticles ([Shankar et al., 2005](#)). This action could be attributed to reducing chemicals attaching to the surface of performed nuclei, enhancing secondary metal ion reduction. Subsequently, formation ratio of prominent biogenic nanomaterials is enhanced. Contrarily, numerous reducing techniques, on the other hand, may enhance the bridging effect between generated nanomaterials, which leads to the aggregation phenomena. A study reported ([Rao and Tang, 2017](#)) the formation of maximum spherical nanoparticles by escalating the amount of extract. Thus, the optimal concentration of precursor as well as reducing agents is vital to get nanoparticles of preferred size and shape.

19.6 Impact of temperature on the fabrication of biogenic nanomaterials

Temperature has a commanding effect on the production of large size to small size biogenic nanomaterials. Nucleation for the creation of larger nanoparticles is usually facilitated by high temperatures ([Kredy, 2018](#)). Low temperature is also beneficial to produce nanoparticles, but it was reported that the kinetics of the reaction is enhanced with rise in temperature ([Liu et al., 2020](#)). Temperature has varied effects on the size of nanoparticles when there are sufficient and insufficient precursors, due to its effect on nucleation and developmental kinetics ([Molnár et al., 2018](#)). With the augmentation in reaction temperature the rate in reduction surges, which results in the formation of nuclei with the consumption of all metal ions and preventing secondary reduction on the nuclei's surface. Consequently, small, very well dispersed nanoparticles are produced with improved yield ([Ndikau et al., 2017](#)).

19.7 Adsorptive removal of environmental contaminants employing biogenic nanomaterials

Biogenic nanomaterials produced from various types of microbial species have been utilized for remediating wastewater laden with heavy metals, dyes, emerging contaminants, halogenated recalcitrant pollutants, pathogenic microorganisms ([Xiao et al., 2015](#)). They have been effectively exploited as adsorbents owing to unique size, high specific surface area, high reactivity, charge opposite to pollutants and the availability of microbial cell matrix.

19.8 Removal of inorganic pollutants

Rapid industrialization has boosted the requirement of metals in different sectors like automobile, coating tools, storage batteries, fuels, photographic films, aeronautics, and explosives (Khan and Khan, 2021). Nonetheless, discharging of these toxic metal contaminants into the environment becomes a greater cause of global epidemic. The heavy metals (Hg^{2+} , Cd^{2+} , Pb^{2+} , Cu^{2+}) and their oxyanions ($\text{Cr}_2\text{O}_7^{2-}$, SeO_3^{2-} , CrO_4^{2-} , AsO_4^{3-}) are persistent to degradation rendering them to bioaccumulate, instigating plentiful concerns for the human health such as respiratory difficulties, dysfunctions of brain, liver, kidneys, dermal irritations, injuries to the basic cellular processes (Jacob et al., 2018), ocular problems, joint and muscle discomfort besides having mutagenic and carcinogenic characteristics (Ge and Li, 2018). Because of these health and environmental problems, effective removal of toxic metals from wastewater is critical. Different approaches of toxic metal removal have been probed, amongst which adsorption has been highly efficient route. Biosorption method exploits the capability of various microbial species to adsorb heavy metals. Application of microbial entities either living, dead, or their products have demonstrated to be very effective biosorbents for cleansing wastewater from toxic metal contaminants whether in soluble form or as particulates.

Pei et al. (2013) used Mn oxide nanoparticles produced by *Marinobacter sp.* MnI7–9 to evaluate silver ions (Ag^+) adsorption. They illustrated the adsorption of silver (Ag^+) on the biogenic nanoparticles employing XPS and SEM techniques. Biogenic Mn oxide nanoparticles size increased after adsorption and was covered by floccule as portrayed by SEM analysis indicating the prevalence of electrostatic attraction among positively charged silver ions (Ag^+) and negatively charged Mn oxide nanoparticles. XPS assessment revealed that during adsorption, a minor quantity of Ag(I) was reduced to Ag(0) , indicating that chemical processes were involved. The reduction might be owing to the presence of hydroxyl groups which have the power to reduce Pd^{2+} , Au^{3+} , Rh^{3+} , and Pt^{4+} . Watts et al. (2015a) demonstrated the removal of alkaline Cr(VI) polluted leachates utilizing varieties of biogenic nanoparticles produced by bacterial strain (*Geobacter sulfurreducens*) and assessed its adsorption efficiency with synthetic nanoparticles. The outcomes of this investigation revealed a complex relationship between biogenic nanoparticles and Cr(VI) oxyanions at various pH levels, with primary confiscation being Cr(VI) anion co-adsorption and reactivity with H_2 or formate. When H_2 gas was applied in acidic medium (pH 2 and 4) as electron donor there was a rise in contacts between Cr(VI) anion and biogenic nanoparticles. But a complex performance was detected when formate was utilized as donor of electrons. Further, the saturation capacity of biogenic magnetite nanoparticles for Cr(VI) was 75 mg/g, which was quite high than the synthetic magnetite nanoparticles (32 mg/g). Jain et al. (2015) investigated the adsorption potential of biogenic selenium nanoparticles for Zn^{2+} at different pH values. The study disclosed that adsorption of Zn^{2+} on biogenic selenium nanoparticles was pH dependent. At pH value (6.5), the adsorption of Zn^{2+} exhibited a double plateau isotherm. XPS revealed the adsorption of Zn^{2+} occurred via precipitation as ZnO , Zn(OH)_2 and $\text{Zn}_3(\text{PO}_4)_2$ over the nanoparticle exterior in alkaline pH. The mechanism responsible for this sequestration chiefly was electrostatic interactions prevalent between the positive Zn^{2+} and negative surface of biogenic selenium nanoparticles. In acidic pH, the removal was governed by ligand like adsorption mechanism. In another investigation (Jain et al., 2016), the authors demonstrated that the adsorption of Cu^{2+} on biogenic selenium nanoparticles occurred via binding with carboxyl/ hydroxyl groups rather than precipitation or ligand complexation. Kim et al. (2016) prepared a biogenic manganese oxide nanoparticle from *Pseudomonas putida* and immobilized on a zeolite. The adsorption of Pb^{2+} , Cd^{2+} , and Zn^{2+} on this adsorbent was thoroughly investigated. The maximum saturation capacity reached 17.09 mg/g, which was 36.4%–70.5% greater than reported for NaCl-zeolite adsorbent. Further, the adsorption capacity was not affected by temperature, pH, and ionic strength. Adsorption isotherm was governed by Langmuir model, while the dynamics of adsorption was controlled by pseudo-second order model. Iwahori et al. (2014) synthesized biogenic magnetite nanoparticles using *Geobacter* strain and subsequently utilized it for the adsorption of Co^{2+} , Ni^{2+} , and Zn^{2+} . The corresponding adsorption capacities were found to be 0.7, 0.6, and 2.2 mmol/mol, which was 30–40 times more than the synthetic counterpart. This result obtained could be ascribed to higher specific surface area ($103 \text{ m}^2/\text{g}$) of the biogenic magnetite nanoparticles which is 20-fold higher than synthetic magnetite nanoparticles ($5.4 \text{ m}^2/\text{g}$). Castro et al. (2018) synthesized biogenic iron nanoparticles employing a natural microbial consortium. The synthesized material was probed as an adsorbent for arsenate, chromate, zinc, and copper removal. The influence of pH on the adsorptive efficiency of the biogenic iron nanoparticles was thoroughly studied. The maximum adsorption capacity of iron nanoparticles was obtained at pH 4 for arsenate and chromate, pH 5, and pH 6 for zinc and copper, respectively. Langmuir adsorption capacity was found to be 1.32, 1.23, 0.44 and 3.53 mg/g for arsenate, chromate, zinc, and copper, respectively. Pseudo-second order and Langmuir model governed the dynamics and isotherm, respectively. Biosynthesized iron oxide NPs using *Avicennia officinalis* rhizosphere derived *Aspergillus tubingensis* (STSP 25) removed >90% of Pb^{2+} , Ni^{2+} , Cu^{2+} , and Zn^{2+} ions from aqueous solution (Mahanty et al., 2020). The adsorption involved chemisorption and the process was endothermic in nature. A study (Watts et al., 2015b)

described the biofabrication of Pd-BnM/H₂ for Cr(VI) sequestration with maximum adsorption capacity of 55 mg/g indicating good adsorptive efficiency of the biogenic NPs for heavy metal sequestration.

19.9 Removal of organic pollutants

Organic pollutants mainly consisted of dyes and emerging contaminants, which find their utilization in almost every field. The application of these organic compounds has been increased briskly with the industrial revolution (Azari et al., 2020). However, the contamination of aquatic sources by these compounds is immense. The breakdown products of these organic moieties are noxious to all organisms triggering severe problems like dysfunction in vital internal organs (Abbasi et al., 2021), affecting the basic life process and having carcinogenic and mutagenic impacts (Hussain et al., 2021). Furthermore, due to their complex chemical properties, these chemicals have a retentive half-life and are more persistent in the environment. Consequently, their elimination from aquatic resources necessitates an effective approach, which is substantially offered by adsorption. Biosorption way to confiscate organic moieties is envisioned to be effective in terms of environmental and economical perspective. Liu et al. (2015) employed *Shewanella oneidensis* Mr-1 for the bio-reduction of graphene oxide and ferrihydrite and subsequently used it for the synthesis of eco-friendly magnetite/reduced graphene oxide nanocomposite (MRGO). The synthesized nanocomposite was probed as an adsorbent for methylene blue (MB) removal. The study depicted that dose of adsorbent has a dominating effect on %removal. The MRGO dose of 0.25 g/L was enough to decontaminate 99% of MB from water. The Langmuir adsorption capacity of MRGO reached 144.9 mg/g and the kinetics followed pseudo-second order model. Thermodynamic studies revealed the process to be endothermic, spontaneous, and feasible. The adsorbent could be regenerated via Fenton-like reaction, which demonstrated potential in successive processes. Pandian et al. (2016) utilized *Aspergillus flavus* for the synthesis of silver nanoparticles and employed it for the removal of malachite green (MG). The impact of process parameters was studied using response surface methodology (RSM) and it was observed that increase in *Aspergillus flavus* concentration enhances the production of silver nanoparticles, which reached a maximum at the concentration of 2.5 mL. The maximum Langmuir adsorption capacity was 44.21 mg/g with D-R model appropriating the whole data. Intraparticle diffusion controlled the overall rate of malachite green adsorption onto the silver nanoparticles. Another study (Kubendiran et al., 2021) demonstrated the fabrication of bimetallic core-shell Pd/Fe nanoparticles on the surface of aerobic microbial granules and its efficiency in scavenging methyl orange (MO) from aquatic environment. The microbial granules of size 1.5 ± 0.32 mm was developed in 3-L glass sequencing batch reactor for 70 days and the H₂ gas produced by it was used to catalyze the fabrication of bimetallic Pd/Fe nanoparticles. The optimization was done using RSM and it was found that at optimal conditions, 99.33% of MO was adsorbed by Pd/Fe nanoparticles. The studied parameters depicted that removal of dye decreased with increase in initial concentration and pH but increased with nanoparticle dosage. Ravikumar et al. (2018) revealed the fabrication of biogenic nano zerovalent iron (Bio-nZVI) and its subsequent utilization in decontamination of methyl orange from aquatic phase. Central composite design was used for the purpose of optimization. Under the optimized conditions (dosage: 30 mg; interaction time: 240 min and initial methyl orange concentration: 10 mg/L), the %efficiency was 99%. Pseudo-second order model was found to be governing the process dynamics. The adsorptive elimination of congo red, safranin T and methylene blue by Se(0) NPs, obtained from *Escherichia coli* strain overexpressed Selenite Reductase CsrF, has been reported with enhanced adsorption capacities (Xia et al., 2018). The fungus *Cunninghamella echinulate* chitosan NPs removed chicao sky blue 6 B, remazol brilliant blue, methyl orange, naphthol blue black, and disperse red 13 (Shajahan et al., 2017). Fungal et al. (2015) produced an in situ biogenic manganese oxide nanoparticles (BioMnOx) via *Pseudomonas putida* and used it for the removal of an array of micro-pollutants at environmentally appropriate concentrations. The removal was also done in situ, and it was found that BioMnOx was able to sequester steroid hormones (estrone and ethinylestradiol), but pharmaceutical pollutants (diclofenac and ibuprofen) were not efficiently removed. The study also revealed that BioMnOx had good workability even in the presence of organic carbon in wastewater. But as the content of organic carbon was increased the potential to sequester emerging contaminants declined. Debnath et al. (2020) fabricated zirconia nanoparticles via greener technology employing *Pseudomonas aeruginosa* bacterial strain and utilized it for the biosorption of tetracycline from aqueous phase. Tetracycline adsorption onto ZrO₂ NPs was rapid attaining an equilibrium within 15 min and was most effective at pH 6. Electrostatic interaction prevalent between the protonated surface of ZrO₂ NPs and the zwitterionic species of tetracycline was responsible for the adsorption. Langmuir model and pseudo-second order model governed the adsorption phenomena, and the maximum adsorption capacity was found to be 526.32 mg/g. ZrO₂ NPs demonstrated excellent adsorption-desorption potential with 81.5% removal efficiency in the fifth cycle. Biogenic nanoparticles obtained via microbe-assisted synthetic method have shown great potential for attenuation of

organic pollutants from wastewater through photocatalytic degradation mechanism (Martins et al., 2017; Noman et al., 2020; Rajkumar et al., 2021; Mohanraj et al., 2020; Mandeep, 2020; Saravanan et al., 2017; Xiao et al., 2017; Cheng et al., 2019). The adsorptive performance in terms of maximum adsorption capacity (Q_m)/removal efficiency of some reported biogenic nanoparticles utilized for the sequestration of different pollutants are summarized in Table 19.1.

19.10 Impact of counter ions on the adsorptive efficiency of biogenic nanoparticles

The evaluation of the impact of co-existing components on the surface-assimilative operation is prerequisite to execute biogenic nanoparticles for industrial and workable applications. Jain et al. (2015) probed the removal of Zn^{2+} in presence of various counter ions (Na^+ , Ca^{2+} , Mg^{2+} , Fe^{2+}) on BioSe NPs. It was found that Na^+ has no influence on the adsorption, whereas other cations followed the order: $Fe^{2+} > Zn^{2+} > Ca^{2+} > Mg^{2+}$, in accordance with the ratio of ionization enthalpy and ionic radius. In another study (Jain et al., 2016), the elimination of Cu^{2+} in presence of Cd^{2+} and Zn^{2+} cations was investigated. This investigation demonstrated the adsorptive affinity of BioSe NPs in the order of $Cu^{2+} > Cd^{2+} > Zn^{2+}$. The superior uptake of Cu^{2+} was ascribed to the presence of $-COOH$ groups in higher concentration, which have greater binding affinity with Cu^{2+} rather with Cd^{2+} and Zn^{2+} ions. Pb^{2+} , Cd^{2+} , and Zn^{2+} were sequestered using biogenic manganese oxide (BMO) immobilized on zeolite (Kim et al., 2016). Impact of K^+ as counter ion on the confiscation of metal ions was appraised. The work revealed that increase in K^+ concentration from 0.05 to 0.5 mol/L declined the uptake of metal ions that was attributed to the reaction prevalent between zeolite and metal ions resulting in the formation of inner-sphere complex. Kim et al. (2012) synthesized biogenic manganese oxide using *Pseudomonas putida* strain and employed it for the removal of 17 α -ethinylestradiol. The influence on adsorptive efficacy of BMO in presence of electrolytes, metal ions and humic acid was assessed. It was disclosed that Na^+ (1–100 mM) did not exert any kind of influence on the %uptake, while Ca^{2+} declined the %efficiency at lowest concentration (10 mM). Because of the increased interaction with the functional sites of BMO, these findings suggested that bivalent ions have a greater influence on sequestration than univalent ions. The removal of 17 α -ethinylestradiol was also appraised in presence of Pb^{2+} , Cd^{2+} and Zn^{2+} and it was observed that %uptake declined with increasing concentration of the metal ions. The functional sites of BMO were dominated by positive cations and negatively manganese oxide due to electrostatic contact, which appeared to be the origin of this discovery, thus declining the 17 α -ethinylestradiol removal owing to the least availability of binding sites. Humic acid at low concentration declined the removal but as its concentration increased, there was a surge in 17 α -ethinylestradiol removal. This trend could be attributed to the formation of Mn^{2+} complexes with humic acid. The removal of 17 α -ethinylestradiol on BMO release Mn^{2+} ions via reduction of MnO_2 and the adsorption of these ions onto the adsorbent could possibly decline the accessibility of functional sites for 17 α -ethinylestradiol uptake. As a result, the complex formation would reduce BMO's self-inhibitory function by providing free binding sites for 17 α -ethinylestradiol.

19.11 Reusability studies of biogenic nanoparticles

Along with effective adsorption potential and economic viability, the regeneration and reusability of an adsorbent is also vital to prevent secondary pollution from the adsorbate-packed adsorbent when disposed in environment. Biogenic nanoparticles can be regenerated effectively and be reused repeatedly in various cycles. The colloidal characteristics of biogenic nanoparticles allowed them to be easily removed from discharges using gravity filtration, sedimentation, or coagulation-flocculation procedures. Adsorbed contaminants can, therefore, be rinsed away, or zerovalent ions can be recuperated from separated biogenic nanoparticles using regeneration or desorbing agents (HCl, NaOH, HNO_3 , ethanol, acetone, or EDTA). Biogenic gold NPs (Das et al., 2009) was used as an adsorbent for the removal of various organophosphorus pesticides. The adsorbent demonstrated the potential of regeneration up to six cycles with less effect on %removal. Similarly, the *Aspergillus tubingensis* mediated iron oxide NPs, which successfully removed Pb^{2+} (98%), Ni^{2+} (96.45%), Cu^{2+} (92.19%), and Zn^{2+} (93.99%) demonstrated good regeneration capacity up to five adsorption/desorption cycles (Mahanty et al., 2020).

19.12 Modeling of adsorption data

Adsorption modeling is fundamental way of gaining insight regarding various interactions prevalent between the adsorbate and the adsorbent. Generally, isotherm and kinetic modeling is appraised which furnish details about equilibrium relations between adsorbate-adsorbent and elucidate the mechanism involved in the process. Table 19.2 encapsulates the linear and non-linear sets of equations on which the various isotherm and kinetic models are based.

TABLE 19.1 Adsorptive performance of various biogenic nanoparticles.

Biogenic NPs	Microbe	Contaminant	Q_m (mg/g)	Size	Removal mechanism	Ref
Mn oxide NPs	<i>Marinobacter</i> sp. Mnl7–9	Ag(I)	8.09		Redox, Electrostatic attraction	Pei et al. (2013)
Nano magnetite	<i>Geobacter sulfurreducens</i>	Cr(VI)	32	5–20	Electrostatic interaction, precipitation	Watts et al. (2015a)
Selenium NPs	<i>Anaerobic granules</i>	Zn(II)	60	160–180	Electrostatic interaction, precipitation	Jain et al. (2015)
Selenium NPs	<i>Anaerobic granules</i>	Cu(II)	48.4		Electrostatic interaction, precipitation	Jain et al. (2016)
Mn oxide NPs/ Zeolite	<i>Pseudomonas putida</i>	Pb(II)	17.09	242–185	Ion exchange, surface precipitation	Kim et al. (2016)
Nano magnetite	<i>Geobacter</i> sp.	Co(II)	26	30	Ion exchange, electrostatic interaction	Iwahori et al. (2014)
Iron NPs	<i>Geobacter metallireducens</i>	As(III) Cr(VI) Zn(II) Cu(II)	1.32 1.23 0.44 3.53	10	Electrostatic interaction	Castro et al. (2018)
Pd-BnM/H ₂	<i>Geobacter sulfurreducens</i>	Cr(VI)	55	–	Electrostatic interaction, surface precipitation	Azari et al. (2020)
Palladium(0)	<i>Geobacter sulfurreducens</i>	Cr(VI)	36	20	Reduction via metabolic activity	John et al. (2020)
Fe ₂ O ₃ NPs	<i>Aspergillus tubingensis</i> (STSP 25)	Pb(II), Ni(II), Cu(II), Zn(II)	9.206 9.666 8.335 9.106	73.05	Electrostatic interaction	Mahanty et al. (2020)
CuS	<i>Shewanella oneidensis</i> Mr-1	Cr(VI)	94.1		Electrostatic attraction, Reduction	Xiao et al. (2017)
MRGO	<i>Shewanella oneidensis</i> Mr-1	Methylene blue	144.9		Electrostatic attraction	Liu et al. (2015)
AgNPs	<i>Aspergillus flavus</i>	Malachite green	44.21	–	Electrostatic attraction	Pandian et al. (2016)
Pd/Fe NPs	<i>Microbial granules</i>	Methyl orange	99.33%	–	Redox	Kubendiran et al. (2021)
Bio-nZVI	<i>Anaerobic granules</i>	Methyl orange	168	–	–	Ravikumar et al. (2018)
Se(0) NPs	<i>Escherichia coli</i>	congo red, safranin T, methylene blue				Xia et al. (2018)
<i>Cunninghamella echinulate</i> Chitosan NPs	<i>Cunninghamella echinulate</i>	Chicago sky blue 6 B, remazol brilliant blue, methyl orange, naphthol blue black, disperse red	–	–	–	Shajahan et al. (2017)
ZrO ₂ NPs	<i>Pseudomonas aeruginosa</i>	Tetracycline	526.32	15	Electrostatic attraction	Debnath et al. (2020)

TABLE 19.2 Linear and non-linear equation sets for isotherms and kinetics modeling.

	Non-linear form	Linear form	
Mass balance equation	$q_e = \frac{(C_0 - C_t)V}{m}$		q_e : adsorption capacity (mg/g) C_0 : initial adsorbate concentration (mg/L) C_t : adsorbate concentration at t time C_e : equilibrium adsorbate concentration V : volume of adsorbate solution (L) m : mass of adsorbent (g) R : removal efficiency (%)
%Removal (R)	$R = \frac{(C_0 - C_e)}{C_i} \times 100$		
Langmuir	$q_e = \frac{q_m b C_e}{1 + b C_e}$	$C_e/q_e = C_e/q_m + 1/q_m b$	Q_m : Langmuir saturation capacity (mg/g) b : Langmuir constant (L/g)
Freundlich	$q_e = (K_f C_e)^{1/n}$	$\log q_e = \log K_f + 1/n \log C_e$	K_f : Freundlich constant (mg/g)(L/mg) ^{1/n} n_f : heterogeneity factor
Temkin	$q_e = \beta_t \ln (K_t C_e)$	$q_e = (RT/b_t) \ln A_T + (RT/b_t) \ln C_e$	K_t : binding constant (L/g) b_t : enthalpy of adsorption (= RT/β_t) (kJ/mol)
Dubinin-Radushkevich	$q_e = (q_D) \exp[-K_{ad} \{ + \frac{1}{C_e} \}]^2$	$\ln q_e = \ln q_D - \beta_{ad} - R \varepsilon^2$	q_D : adsorption capacity (mg/g) K_{ad} : activity coefficient (= $\beta_{ad} R^2 T^2$) ε^2 : Polanyi potential (= $RT \ln (1 + 1/C_e)$)
Energy of adsorption	$E = \frac{1}{\sqrt{2\beta_{ad}}}$		E : energy of adsorption (kJ/mol)
Pseudo-first order (PFO)	$q_t = q_e(1 - e^{-k_1 t})$	$\log(q_e - q_t) = \log q_e - k_1/2.303 \cdot t$	k_1 : PFO rate constant (1/min)
Pseudo-second order (PSO)	$q_t = \frac{k_2 q_e^2 t}{(1 + k_2 q_e^2 t)}$	$t/q_t = 1/k_2 q_e^2 + t/q_e$	k_2 : PSO rate constant (g/mg/min)
Intraparticle diffusion	$q_t = k_i t^{0.5} + C_i$		k_i : intraparticle diffusion rate constant (mg/g/min ^{0.5}) C_i : boundary layer thickness
Liquid film diffusion	$-\ln(1 - \frac{q_t}{q_e}) = k_A \cdot t$		k_A : Liquid film diffusion rate constant (mg/g min)

The appropriate model is usually chosen on the basis of higher coefficients of correlation (R^2) and lower standard error of estimate (SEE) and other statistical parameters (Khan et al., 2021). The nature of the process whether it is endothermic or exothermic is quantified from the adsorption capacity calculated from Langmuir model (Langmuir, 1918). The significance of the process is associated with $1/n$ values of Freundlich model (Freundlich, 1906). Temkin (Temkin and Pyzhev, 1940) and D-R (Dubinin, 1947) models indicate whether the process follows physisorption or chemisorption on the basis of the values of b_t and E (kJ/mol). Kinetic modeling, which includes pseudo-first (Lagergren, 1898) and pseudo-second order models (Ho and McKay, 1999) elucidates the rates of adsorbate uptake. In case of biogenic nanoparticles, intraparticle (Weber and Morris, 1963) and liquid film diffusion (Boyd et al., 1947) both synergistically govern the adsorption process. Moreover, electrostatic interaction, precipitation and ion-exchange dominate the removal of contaminants on these adsorbents (Watts et al., 2015a). The different isotherms and kinetics equations generally used for the modeling of adsorption data along with other relationships are summarized in Table 19.2.

19.13 Environmental problems

Nanoparticles are associated with environmental issues owing to their noxiousness in relation to various organisms, including humans. Since antiquity, humans as well as other organisms have always been imperiled by the exposure of tiny particles arising from several natural activities. The last century witnessed a brisk escalation in the fabrication of nanoparticles, which rendered them as environmental contaminants. Owing to their nanosize, these NPs can translocate from the physical parts to circulatory, lymphatic systems, and eventually to tissues and soft organs where they are potentially dangerous (Das et al., 2017). The peril nature of metallic nanoparticles could be attributed to several reasons

like size, dosage, surface area, crystalline nature, and functionalization/ modification (Tyagi et al., 2013). It is worth mentioning that not all nanoparticles possess toxic nature and the noxiousness rely on chemical composition, shape along with particle aging. Thus, it is mandatory, to lay emphasis on physical facets, particle aging before fabrication process, which will enable to develop nanoparticles with viable characteristics.

19.14 Conclusions and perspectives

This book chapter evaluated the substantial influence of biogenic nanoparticle-based adsorbents for mitigation of pollutant laden wastewater. The exploitation of microbial strains for the fabrication of different nanoparticles renders the process to be green and reduces the cost of chemicals, which makes it feasible environmentally and economically. Biogenic nanoparticles (BNPs) have been established to be exceptional adsorbents for the uptake of different pollutants (especially heavy metals) from the aquatic phase. Owing to their physical facets like shape, size, and high specific area they have commendable potential for the decontamination of wastewaters from the various pollutants. Process parameters such as dosage, time, pH, and concentration have a dominating effect on the adsorptive performance of these adsorbents. Co-existing ions have both synergetic as well as antagonistic impact on the %uptake. Many mechanisms such as electrostatic interaction, precipitation, ion-exchange, and redox reaction are responsible for the sequestration of pollutants on these biogenic nanoparticles. These nanoparticles have the greater potential for regeneration and can be reused for various adsorption-desorption cycles without much loss in adsorption capacity. The fabrication of BNPs is still ambiguous, thus needs further clarification along with the comparative studies between biologically and chemically synthesized nanoparticles. The BNPs synthesized are usually purified using chemical reagents, which should be avoided to maintain the green status. The adsorption studies using BNPs have been appraised via batch mode with thorough emphasis on isotherm and kinetic modeling. It is recommended to implement continuous column approach for commercial applications. Green technology should be implemented in the synthetic procedure, which will be a great step towards sustainability.

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Nanomaterials originated from microbes for the removal of toxic pollutants from water

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20.1 Introduction

Water plays vital role for every form of life known on Earth. It is essential for energy, economy, health, and food. So, it should be available in pure form to all living organisms. The advances in science and technology, unfortunately, along with ever growing population, industrialization, urbanization and the demand for goods and services, is ultimately leading to the production and release of huge amount of hazardous water pollutants which are recalcitrant also, like heavy metals, textile dyes, plasticizers along with pharmaceutical and personal care products and endocrine disrupting compounds. The removal and treatment of such pollutants is one of the priority issues of global concern. It is necessary for the sustainable development of industries as well as environment (Zangeneh et al., 2015; Mara, 2003; Singh and Mishra, 2020). With this globally rising concern and demand for availability of clean and potable water, it also becomes necessary to not only upgrade the available techniques but also develop new technologies and methods which are of low cost, consumes less energy, are more efficient as well as are environmentally safe (Malato et al., 2009; Bousselmi et al., 2004; Mozia et al., 2007; Belgiorio et al., 2011; Rizzo et al., 2009). Amongst the conventional approaches available for the remediation of various toxic water pollutants, the most adopted technique is adsorption, which is economic, efficient, versatile as well as eco-friendly.

In recent years, nanotechnology, which is related to manipulating the materials and systems to nanoscale, that is, <100 nm, has evolved as a powerful platform for remediation of water pollutants. In this regard, various nanoadsorbents have been developed and explored. Nanoadsorbents have high “surface area to volume” ratio and that is why are advantageous in providing higher adsorption capacity. They might also be designed and modified to target the specific pollutants (Kumar et al., 2014). Hence, promoting the use of nanoadsorbents offers greater opportunity to develop local and practical solutions to tackle the global concern of water pollution. Nanoadsorbents are prepared via various physical, chemical, biological and hybrid methods. The chemical and physical methods of nanoadsorbent synthesis are more common but associated with the use of toxic chemicals limiting their application in fields like biomedical and pollutant remediation. To expand the use of nanomaterials for such applications, it becomes necessary to encourage their synthesis through greener approaches, that is, the use of biological methods for biosynthesis (green synthesis) of nanomaterials. Green synthesis of nanoadsorbents, through microorganisms and plant extracts, is not only reliable and nontoxic but also environment friendly. Microbes act as “nanofactories” which can accumulate metals onto them and then convert them into nanoparticles. Recent researches are being focused on to exploit the use of microbes to synthesize nanoadsorbents (Baharulolum et al., 2021).

The present chapter is focused to provide the concepts, fundamentals, mechanisms and theories of the adsorptive remediation of toxic water pollutants using nanoadsorbents synthesized using microorganisms. The topics covered include adsorption and its basics, nanotechnology and nanoadsorbents in field of water treatment, classification, properties and factors affecting nanoadsorbent synthesis, insight into green synthesis of nanoadsorbents by microorganisms, mechanism of synthesis and mechanism of adsorption by nanoadsorbents, issues, challenges and future prospects of using nanoadsorbents originated from microbes.

20.2 Adsorption for remediation of toxic pollutants

Over the last few decades, several remediation methods have evolved for treatment of toxic water pollution. Generally, the water remediation approaches are combination of several physicochemical and biological operations and processes. Overall, the methods can be categorized as conventional methods, established recovery methods and emerging remediation methods (Crini and Lichtfouse, 2018) as shown in Fig. 20.1. The most commonly used techniques for removal of toxic pollutants are ion-exchange, electrodialysis, reverse osmosis and membrane separation. These conventional techniques however, are associated with several drawbacks in terms of efficiency, incomplete and low rate of elimination of pollutants, high cost of instruments, difficult maintenance, short life, less surface area and low mechanical strength. Many conventional methods also require chemicals, expertise and infrastructure, high cost of maintenance and employ fairly expensive minerals matrix. Some of the techniques are even incapable to overcome variations of pH, hardness or microbial strength, that ultimately affects the quality of water. The above-mentioned factors render the conventional approaches of being costly and time taking, which minimize concentration of toxic pollutants to a certain limit. Thus, if a technique has few advantages on one hand, it might be associated with some disadvantages on the other hand. Of all the methods adsorption has attracted the most interest and is the most adopted method of removal of pollutants in ancient as well as today's world because of its efficiency, versatility, low cost, design and operational flexibility, simple and convenient operation. In general adsorption is a separation process that involves the removal of a substance (i.e., adsorbate) from one phase (liquid or gas) and then its accumulation at the other surface (adsorbent) (Kumar et al., 2014; Yamamura et al., 2011; Rajasulochana and Preethy, 2016; Zinicovscaia, 2016).

For efficient removal of toxic water pollutants, various types of commercial adsorbents and/or bioadsorbents are available. Various minerals, organic and inorganic or biological materials like activated clay, activated carbon, industrial and agricultural by-products and wastes, zeolite, polymers, biofuels, etc., have been applied as adsorbents, and have shown varying adsorption capacity towards the targeted pollutants for their removal from water effluents. The surface of these conventionally available adsorbents has also been modified with the help of chemicals to increase the adsorption capacity (Younas et al., 2021). The use of most commonly used adsorbent, that is, activated carbon began during 1900–1901 to replace bone char in sugar refining industry. However, activated carbon being synthesized using coal, are expensive. It is also not cheap to regenerate them and hence it is not an attractive adsorbent. This promoted synthesis of activated carbon using cheap and abundant agricultural wastes and by-products (Anirudhan and Sreekumari, 2011; Renu et al., 2017).

20.2.1 Bioadsorption

Biosorption is another cost effective, simple and eco-friendly option for remediation of toxic pollutants from water. It has gained popularity during the last decade over conventional methods. It is metabolically passive physicochemical process. Various biosorbents include microbes, plant-derived materials, industrial and agricultural waste/by-products, biopolymers etc. Apart from being economic and eco-friendly, biosorption has the advantage of removing toxic contaminants even in very dilute concentrations. The ease and low cost of operation, no additional

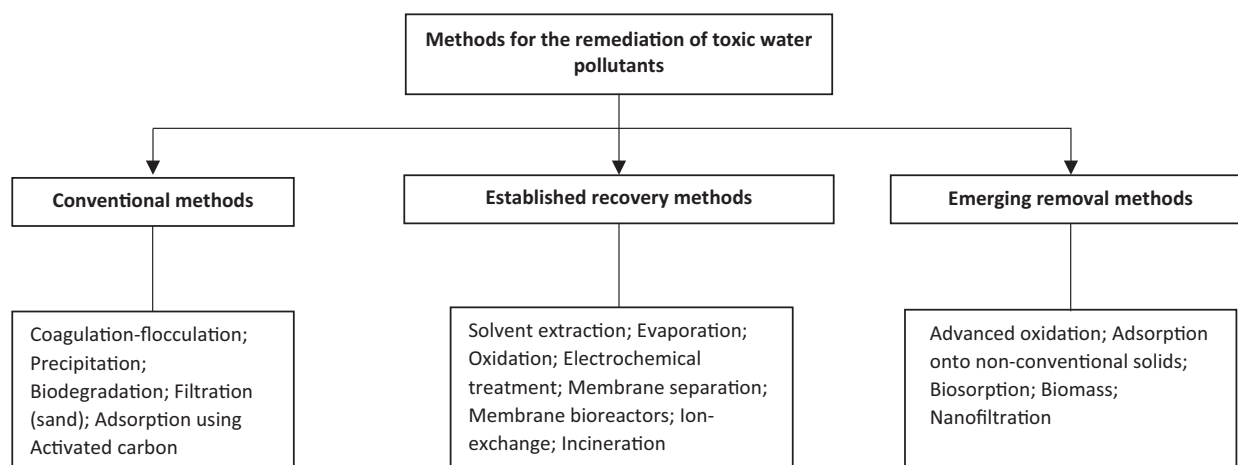


FIGURE 20.1 Categorization of methods for the remediation/removal of toxic water pollutants.

nutrient requirement, low quantity of sludge generation, higher efficiency, regeneration of biosorbents and no increase in chemical oxygen demand of water, has all made biosorption the very popular approach towards toxic pollutants remediation (Derco and Vrana, 2018; Davis et al., 2003; Chojnacka, 2010).

20.3 Nanotechnology in water treatment

Nanotechnology is the field of nanoscience, which is related to manipulating the materials at nanometer (10^{-9} m) scale. These materials have various applications in industries, medicines as well as environmental remediation. Nanomaterials have their one dimension in the range of 1–100 nm. Depending upon how many dimensions are in this range, nanomaterials could be nanoparticles, nanotubes and nanoplates (nanosheets) (Chaturvedi et al., 2012).

In comparison to their microscale size, materials when reduced to nanoscale suddenly start showing unique properties, due to two effects (1) surface effects, and (2) volume effects. As compared to the bulk material, nanomaterials show different atomic, electronic, magnetic, physical and chemical properties. These high specific surface area and discontinuation properties allow the nanomaterials to behave as improved water and wastewater treatment processes, namely nanocatalysts, nanomembrane and nanoadsorbents (Bose, 2020; Yakoob et al., 2020; Lu et al., 2016; Gehrke et al., 2015).

Nanocatalysis (or nanophotocatalysis) is actually the process of photocatalysis, that is, decomposition in light's presence with the help of nanomaterials. This method is used to destroy microorganisms and organic compounds by oxidation. Zero-valent metals, semiconductors and few bimetallic are examples of nanomaterials which act as nanocatalyst for treatment of toxic pollutants. Nanocatalysts have shown good results at laboratory level for purification of air and water. However, it lacks at commercial level due to several reasons one of them is toxicity and recovery of catalysts from water (Bose, 2020; Yakoob et al., 2020; Lu et al., 2016; Gehrke et al., 2015; Anjum et al., 2019).

On the other hand, nanomembranes are unique type of membranes made up of nanotubes, nanoribbons and nanofilters. It is a pressure driven process used to separate unwanted nano pollutants from water. Nanomembranes reject particles in the range nanometers (from 0.5 to 1 nm). These membranes mostly consist of some porous support along with a composite layer (like graphene oxide or carbon nanotubes (CNT)) providing significant and promising in fouling prevention and aqueous transport. The traditional membranes require another ion to compensate serving as exchanges, however in nanomembrane it is not required. Use of nanomembrane however, is also associated with few issues, one of them is fouling after few cycles of application, making it expensive and inefficient. Other major issue is stability of membrane. The process thus is less reliable, expensive, has slower operation, lesser selectivity, and low efficiency after few cycles of use (Bose, 2020; Yakoob et al., 2020; Lu et al., 2016; Gehrke et al., 2015; Anjum et al., 2019).

20.4 Adsorption using nanoadsorbents

Nonconventional low-cost adsorbents have received much attention as an alternative to expensive adsorbents, to improve the performance of cost ratios and the existing water clean-up techniques. In this regard nanomaterials offer great advantages as cost-effective, technoeconomical and universal adsorbents. Nanoadsorbents possess two main properties: innate, that is, inherent surface properties and external surface modification or functionalization properties. The physical as well as chemical properties are defined by the external surface structure, and internal chemical components. Nanomaterials because of their smaller size inherent comparable specific surface area which was a long-lasting bottleneck for conventional adsorbents. They thus offer high porosity, small size and active surface sites for adsorption of pollutants with varying molecular size, hydrophobicity, and speciation behavior. Nanoadsorbents also have higher potentiality and efficiency towards novel pollutants. A faster removal capacity is observed for various organic, inorganic and micropollutants. Also, more compact water treatment devices are possible with nanomaterials having smaller footprints, in addition to saving of adsorbent materials. The frequently used nanoadsorbents are based on metal and metal oxides, carbon (such as carbon black, graphite, graphene oxide). Polymeric nanoadsorbents are also in application (Bose, 2020; Yakoob et al., 2020; Lu et al., 2016; Gehrke et al., 2015; Anjum et al., 2019; Khajeh et al., 2013).

20.4.1 Classification of nanoadsorbents

Nanomaterials can be of different shapes and sizes. The various shapes are rod, globular, conical, hollow, coiled, plane, cylindrical and asymmetrical also. They also may be amorphous or crystalline. Nanomaterials which are used in adsorption should be highly effective and efficient for remediation of toxic pollutants from water. Atoms of those elements can be used to synthesize nanoadsorbents whose surface is chemically active and show high efficiency of adsorption.

Various metals and their oxides, materials like activated carbon and clay, silica, and other chemically or physically improved compounds can be used to prepare nanoadsorbents.

Generally, nanomaterials are classified as nanoparticles, nanoclays and nanoemulsions. Depending on material production and their role in adsorption process nanomaterials and thus nanoadsorbents are classified as organic, inorganic (metallic), mixed oxide nanoadsorbents, magnetic nanoadsorbents, carbon based nanoadsorbents, silicon based nanoadsorbents. Research now-a-days is also focused on to use nanoclays, polymers' based nanoadsorbents, nanofibers as well as aerogels (Gehrke et al., 2015; Saeed et al., 2019; Nik-Abdul-Ghani et al., 2021).

20.4.2 Properties of nanoadsorbents

It is necessary to understand the properties of nanoadsorbents as their applicability depends on them. The physicochemical nature of nanomaterials depends upon the innate properties of nanoadsorbents including internal chemical composition, external surface structures, apparent size etc. The fundamental innate factors which affect the overall functioning of nanoadsorbents are the larger surface area of nanomaterials, providing multiple active sites of adsorption which ultimately leads to high adsorption efficiency; high chemical activity; short intraparticle diffusion distances; high surface binding energies. All these factors influence the atoms and molecules present on the surface as well as interface of the nanoadsorbent and increase the number of active adsorption sites present of surface of nanoadsorbent. The factors also increase the surface affinity, surface binding energy of nanoadsorbents.

Sometimes, nanomaterials do not behave as good adsorbent when applied in their pure form. To overcome the limitations external functionalization or surface modification is done. Some organically functionalized nanoadsorbents like organo-clays, functionalized silica gel and polymers nanocomposites have been developed in this regard (Najafi et al., 2012). External functionalization renders nanomaterials versatility so that they can be applied to a wide variety of pollutants. It is done in order to enhance their adsorption capacity and efficiency by customizing and modifying their bulk properties and surface properties like charge density, reactivity, functionality, biocompatibility, dispensability, stability and inertness towards harsh environmental conditions. In magnetic nanoparticles external functionalization has been reported to prevent aggregation. In addition to providing high porosity, superior surface area, high adsorption capacity; functionalization also provides impressive mechanical strength, high surface binding energies, low temperature modification (Singh et al., 2011; Khajeh et al., 2013).

20.4.3 Characteristics of an ideal nanoadsorbent

The overall factors that govern the nature and hence applicability of nanoadsorbents are surface as well as internal chemical composition, solubility, particle size, geometric dimensions and shape, state of agglomeration, structure of nanocrystal (OECD, 2010; Perera and Ayres, 2020; Shen et al., 2019; Xiong et al., 2020). The standards which are sought in an ideal nanoadsorbent for the remediation of varied variety of pollutants from waters and wastewaters under different physical, chemical, biological and microbial conditions are as follows:

1. the first and foremost is that the nanomaterial used should be nontoxic.
2. it should show good adsorption capacity even if pollutant concentration is very low in ppb.
3. it should have chemically, thermally stable and adequate selectivity.
4. the recovery rate of spent nanoadsorbents should be high.
5. it should have regeneration and recyclability and reusability.
6. pores should be adequately tunable.
7. the nanoadsorbent surface should be easily regenerated and modified by using specific type of functionalization.
8. nanoadsorbents should have high mechanical strength, structural integrity and shape recovery potential.
9. it should show self-healing and self-cleaning qualities.
10. low-cost production should be possible.
11. it should be easily and readily available in bulk.
12. large-scale application for pollutant remediation should be possible.
13. possibility of being produced through green synthetic routes.
14. it should be environment friendly.

The ideal nanoadsorbent should be universally applicable and as compared to conventionally available adsorbents should be able to resolve the technical, economical and secondary pollutant issues.

20.4.4 Factors affecting overall adsorption processes by nanoadsorbents

There are many factors that affect the adsorption of toxic pollutants on nanoadsorbents such as temperature, pressure, pH, pH of zero-point charge (pH_{zpc}), adsorbent dose, contact or incubation time and coexisting ions. pH of solution (water and/or wastewater) plays very important role in the adsorption process (Srivastava et al., 2015; Shirsath and Shirivastava, 2015; Tarigh and Shemirani, 2013). It influences the ionization, speciation of adsorbate in solution and also the nature of surface of adsorbent as H^+ and OH^- ions at the active sites of adsorbent. pH_{zpc} is the pH at which the surface charge is equal to zero. The electrostatic force of attraction among oppositely charged adsorbate species increases the adsorption efficiency. Temperature also affects the adsorption efficiency by altering the stability of adsorbate, characteristics of adsorbent, and interaction between adsorbent and adsorbate. The adsorption reaction could be exothermic or endothermic, spontaneous or random. On the other hand, an increase in pressure may intensify the adsorption till the surface of adsorbent become saturated. After attaining equilibrium, pressure no longer plays any active role in adsorption process. It has been reported that adsorption increases when contact time increase, as more time of adsorption is provided. Initially the adsorption increase with time as high concentration of pollutants is present, then it starts decreasing as the active sites are blocked. The presence of coexisting ions in aqueous environment also affects the adsorption capacity of adsorbent as there be an increased competition between the targeted adsorbate particles and coexisting ion to occupy the active site of adsorbent surface. Factors which affect the adsorption process also depend on the characteristics of nanoadsorbents. These include surface area and charge, pore size, particle size, hydrophobic nature of surface. Surface modification via different functional groups also affects adsorption. Adsorption efficiency shown by smaller particles is more as compared to the larger ones, as they have large surface area. The pore size and surface area are also inversely related. That means for a given volume of nanoadsorbent if the pores are of smaller size, the surface area will be large, which will enhance the adsorption efficiency. Moreover, modification of nanoadsorbent surface with the help of various favorable functional groups is also done to enhance its adsorption capacity.

20.4.5 Routes of synthesis of nanoadsorbents

There are two main approaches for the synthesis of nanomaterials viz. Bottom-up and Top-down. Top-down approach is the traditional approach it is based on synthesizing nanomaterials from bulk materials by reducing the particle size mainly through physical forces. Techniques like reactive milling, mechanical alloying etc. come under this approach. On the other hand, bottom-up approach is the modern approach where smaller components are arranged into more complex assemblies by applying chemical and physical and biological forces. It is building the nanomaterials from atomic or molecular level. Chemical/physical vapor deposition, sol-gel are the most common bottom-up approaches. Biological synthesis of nanomaterials also comes under this category. The main disadvantage of top-down approach is that during reduction of particle size through milling like techniques, there is a damage of crystallography and surface structure. However, in spite of the deficiency this approach is still used in the preparation of nanomaterials. The two main issues associated with bottom-up approach is difficulty in large scale synthesis, and requirement of chemical purification. Despite these issues, the bottom-up is the preferred approach for nanomaterial synthesis, as with this approach nanomaterials with specifically designed properties can be prepared by choosing the route of synthesis, so that it can be used for desired purposes (Tulinski and Jurczyk, 2017; Kapoor et al., 2021; El-Sayed, 2020; Zhang et al., 2020; Cele, 2020; Ali et al., 2016). All these techniques under top-down and bottom-up approach are clubbed into three categories, that is, physical, chemical and biological techniques. Table 20.1 shows different techniques which come under these categories.

TABLE 20.1 Different methods of synthesis of nanoadsorbents.

Physical methods	Chemical methods	Biological methods (green synthesis method)
Mechanical methods: High energy ball milling Melt mixing Vapor methods: Physical vapor deposition Laser ablation Sputter deposition Electric arc deposition Ion implantation	Sol gel process Chemical reduction Irradiation Electrochemical Microemulsion Photoreduction Hydrothermal Polyol synthesis	Plants extracts or enzymes Microorganism like fungi (eukaryotes), bacteria, actinomycetes (prokaryotes) Templates like DNA, membranes, viruses and diatoms

As compared to biological methods, physical and chemical methods are more popular for synthesizing nanomaterials. Amongst all of them, the chemical methods are most adopted as they produce more yield at lower cost. All methods have their own advantages and disadvantages. However, the search and development of nontoxic, reliable, economical and eco-friendly methods for synthesis of nanomaterials is given more concern now-a-days to expand their applications in fields like biomedical and pollutant removal. Greener approach to synthesize nanomaterials is one of the options to achieve this goal (Tulinski and Jurczyk, 2017; Kapoor et al., 2021; El-Sayed, 2020; Zhang et al., 2020; Cele, 2020; Ali et al., 2016).

20.4.6 Advantages and disadvantages of physical and chemical methods of nanoadsorbents synthesis

The physical methods use physical forces to degrade the bulk material to nanoscale level, it has the advantage that it avoids solvent contamination of nanomaterials. But it is associated with large energy consumption for the evaporation and condensation of particles. Application of very high temperature and pressure in physical methods, not only takes longer time for nanomaterial synthesis but also indirectly increases the cost of synthesis. It is also difficult to control the particle size in nanoscale range as physical methods are very elaborate (Tulinski and Jurczyk, 2017; Kapoor et al., 2021; El-Sayed, 2020; Zhang et al., 2020; Cele, 2020; Ali et al., 2016).

As compared to physical methods, chemical methods give fast response and are cost effective. They also have advantages of being simple; using inexpensive instrument; involving low temperature synthesis; large scale synthesis is possible; size, shape and composition of nanomaterials are manageable through changing salt used, pH and ionic strength; allowing doping of foreign atoms (ions) during synthesis. However, in order to regulate the particles size and shape, to synthesize pure and stable nanomaterials, and to avoid agglomeration, there is use of different types of chemical reagents like strong reducing agent, protective agent and soft chemical reagent for stabilization of nanomaterials. The application of large quantity of strong chemicals leads to the contamination and toxicity of nanomaterials, which limits the use of nanomaterials in many fields like biomedical and water pollution remediation (Tulinski and Jurczyk, 2017; Kapoor et al., 2021; El-Sayed, 2020; Zhang et al., 2020; Cele, 2020; Ali et al., 2016).

Apart from above mentioned method-based limitations, when the prepared nanomaterials are actually subjected to field applications, they suffer from various challenges like their stability in the hostile environment, difficulty in understanding the fundamental modeling factors and mechanism, bioaccumulation of chemicals in food chain and toxicity in environment, requirement of extensive analysis, skilled operators are needed, issues related to assembly and structure of device, recycle/reuse and regeneration of nanoadsorbents.

20.5 Biological methods of synthesis of nanoadsorbents (green synthesis)

Keeping these limitations in mind, it becomes mandatory to improve the types, quality and behavior of synthesized nanomaterials. To overcome these limitations a new approach of biological synthesis of nanomaterials, that is, “Green synthesis” is gaining much concern and creating new challenges and opportunities in the field of research. Green synthesis is an environmentally benign technique for stable nanomaterial synthesis with the help of regulated, control, clean up and remediation process. Collectively the products, that is, nanomaterials are known as biogenic nanomaterials. Synthesis of nanoadsorbents via green synthesis route is a bottom-up technique, which mostly involves the oxidation and/or reduction reactions. The use of green solvents and organic natural resources is a mandate for green synthesis. Greener approach will help in extending the application of nanomaterials in fields like biomedical, pharmaceutical and environmental remediation. Nanoadsorbents produced through biological synthesis help in transforming the toxic, soluble pollutants to their nontoxic or less toxic, insoluble and immobile form. This approach involves synthesizing nanomaterials through plant extracts, microbes and templates like DNA, membranes, viruses and diatoms (Purohit et al., 2019; SongYan et al., 2011; Goutam et al., 2020; Pal et al., 2019).

Extract from different parts of plant such as leaves, fruits, stems, roots are used for synthesizing nanomaterials using plants. Detailed mechanism of synthesis is still under investigation but it involves proteins, vitamins, organic acids, alkaloids, terpenoids, flavonoids, polysaccharides and other heterocyclic compounds.

Microbes are considered to be the nanofactories. They hold wide potential as an efficient, economic and eco-friendly technique. It avoids the use of toxic and harsh chemicals and energies. In microorganisms’ various types of reductase enzymes are present which help in reducing the metallic particles into nanomaterials. Over last few years various bacteria, fungi, yeasts have been used to synthesize nanomaterials.

Also, protein, templates like DNA and s-layers are also used to synthesize nanomaterials. Protein nanomaterials are biodegradable, metabolizable and easy surface modification is possible. They can be prepared from water soluble as well as insoluble proteins and are used in various targeted therapies (Verma et al., 2018; Samantaab and Medintz, 2016).

20.5.1 Advantages of biological or green methods of synthesis of nanoadsorbents

- Green approach is extremely welcomed to synthesize nanomaterials in larger context and is widely accepted for removal and remediation purpose, as it is safe, nontoxic and eco-friendly (Saeed et al., 2019; Kapoor et al., 2021; El-Sayed, 2020; Purohit et al., 2019)
- It is an advanced, simple and feasible method to produce variously designed nanostructures like nanorods, nanosheets, nanoparticles
- As compared to the chemical routes of synthesis, the composition of biologically prepared nanoadsorbents is more homogeneous. They are also more stable due to reduced Gibb's free energy
- Larger surface area of nanoadsorbents
- No need to add capping or stabilizing agent
- The optimal operating conditions of nanomaterial synthesis such as temperature and humidity are easily manageable
- Highly efficient, as significantly much quantity of contaminants can be removed with lesser number of nanoadsorbents.
- Energy efficient
- Cost of production is low
- Easy production
- Fewer accidents
- Lesser waste is generated
- Can be used in biomedical and pharmaceutical industries.

20.5.2 Factors influencing green synthesis of nanoadsorbents

During the environmental interaction with metal species, plants and microbes are subjected to various conditions like pH, temperature, contact time etc. hence it becomes necessary to understand the influence of such parameters as it affects the biological synthesis of nanomaterials.

1. **pH:** The first and the most important is pH. pH of aqueous medium affects the morphology, that is, the size and shape of nanoadsorbents. Synthesis of large size nanoparticles from metals have been reported in acidic medium (Dubey et al., 2010; Sathishkumar et al., 2010).
2. **Temperature:** It is also a crucial feature that affects nanomaterials' biosynthesis. The physical methods require temperature above 350°C whereas chemical methods work at temperatures below 350°C. However, the microbes synthesize nanomaterials at temperature as low as 100°C. It is an advantage over the physical and chemical methods. Temperature has important role in determining the size, shape and production rates of nanomaterials synthesized (Sathishkumar et al., 2010; Rai et al., 2006).
3. **Pressure:** It is also an essential factor that affects biosynthesis of nanoadsorbents. Overall, pressure has been shown to alter the dimensions and form of nanomaterials. It has been reported by Tran et al. (2013) that reduction of metal ions was speedy at normal pressure conditions.
4. **Reaction time:** The reaction time or exposure period or the contact time plays important role in the shape, size and more importantly in the adsorption capacity of the nanoadsorbents. In many studies, it was found that the color of nanoparticles formed changed rapidly and nanoparticles were synthesized within 2 min of reaction time. However, the color change was slow after 5 min of contact (Ahmad, 2012).
5. **Concentration of biological molecules:** The synthesis of nanoparticles has been found to be influenced by the concentration of microbes or plant extracts used for synthesizing the nanoscale materials. Concentration has been reported to alter the morphology specially shape of synthesized nanomaterials. Nanoparticles of various shapes triangular, spherical, hexagonal, decahedral were prepared by altering the concentration of biomolecules (Chandran et al., 2006; Huang et al., 2007).

Overall, size and shape of nanomaterials affects their various physical (like melting point) and chemical properties, which ultimately affect the adsorption capacity. Green synthesis helps in preparing the tuned nanomaterials.

20.6 Microorganism for the synthesis of nanoadsorbents

Microorganisms are the microflora which predominate the marine as well as terrestrial ecosystem. The microbes are involved in performing various phenomenon like the degradation, decomposition, precipitation of metals and thus are integral part of biogeochemical cycle of metals. During this interaction of microbes with metals in nature, metal is mobilized, distributed and chemically modified, causing untimely toxicity of microbes. This microbial metal toxicity may lead to the death of microorganisms, if not managed. The metal toxicity responses are observed in microbes and metallic nanomaterials are synthesized as a consequence of it. That is nanomaterial synthesis through microbes is a result of metal resistance mechanism, where the microorganisms alter the chemical nature of metal to lower their toxicity or to make them nontoxic and inert. Nanoscale range metal clusters of definite shape and size are precipitated as a result (El-Sayed, 2020; Zhang et al., 2020).

20.6.1 Various microbial components for green synthesis of nanoadsorbents

This property of microbes to convert the metals to their nanoscale sizes in order to avoid their toxicity has been exploited and various algae, bacteria, fungi, yeast, marine microbes are harnessed for the synthesis of nanomaterials from metals like Au, Ag, Cu, Si, Fe, Ni, Cd, Pb and many others as mentioned in detail below (Hulkoti and Taranath, 2014; Kapoor et al., 2021; Pal et al., 2019):

1. **Bacteria:** Bacteria behave as potential factories for the synthesis of nanomaterials from metals in toxic environmental conditions. The mechanism involved to do so involves reduction or precipitation of toxic soluble metal ions to their inert/less toxic/nontoxic insoluble forms either extracellularly or intracellularly. Due to this potential, bacteria have wide application in various fields like bioremediation, biomedical etc. Prokaryotes as well as actinomycetes are used at large scale for synthesis of metal and metal oxide nanomaterials. Few examples of bacterial species used for synthesis of Ag and Au nanoadsorbents are *Lactobacillus casei*, *Escherichia coli*, *Aeromonas* sp. SH10, *Bacillus cereus*, *Pseudomonas proteolytica*, *Phaeocystis antarctica*, *Bacillus indicus*, *Bacillus amyloliquefaciens*, *Arthrobacter gangotriensis*, *Enterobacter cloacae*, *Shewanella oneidensis*, *Nocardia farcinica*, *Streptomyces viridogens*, *E. coli DH5a*, *Desulfovibrio desulfuricans*, *Bacillus subtilis* 168, *Shewanella alga* and *Rhodospseudomonas capsulate*. Nanomaterials of Cu, Zn, Mn have also been prepared by various species of *Streptomyces*.
2. **Fungi:** Fungi act as template for synthesizing nanoadsorbents with well-defined morphologies. Like bacteria they also metal resistance mechanism, high binding capacity. As compared to other microorganisms, fungi have advantage of having enzymes, proteins and other biomolecules on their cell surface. They grow more quickly and are simpler to handle in laboratory conditions. Many fungal species like *Fusarium oxysporum*, *Verticillium species*, *Aspergillus aculeatus*, *Colletotrichum sp.*, *Phaenerocheate chrysosporium*, have been employed for preparation of variety of nanomaterials of Au, Ag, Pt, NiO, TiO₂, ZnO etc.
3. **Yeast:** Yeast are single celled microbes and are present in eukaryotic cells. Yeast cells are employed successfully for the synthesis of nanomaterials as a result of detoxification mechanism by enzymatic oxidation or reduction, cell wall sorption, chelation, cell wall transfer of metal ions or their efflux from the cell. Yeast cells are mainly known for synthesizing semiconductor nanoparticles of CdS specially. Recently it has been found that yeast can synthesize other nanoparticles as well. Yeast species like *Pichia jadinii*, *Yarrowia lipolytica* 3589, *Saccharomyces cerevisiae* have been reported to synthesize nanomaterials from Au, TiO₂, Sb₂O₃ respectively.
4. **Algae:** Algae are a group of plant kingdom which are well employed in the preparation of nanomaterials as a result of their capability to reduce metallic ions. *Chlorella vulgaris*, *Dunaliella salina*, and *Nannochloropsis oculata*, *Rhizoclonium fontinales*, *Lyngbya majuscula* and *Spirulina subsalsa*, are reported to produce Ag, Au, Pt, Pd, CuO, ZnO, nanoparticles.
5. **Marine microbes:** They are found at the bottom of sea level and can produce inorganic nanomaterials by reducing metal ions. *Penicillium fellutanum*, *Thraustochytrids*, *Aspergillus niger* strain and *Escherichia coli* strain, *Pseudomonas* sp. 591786, *Spirulina platensis* and many other marine microorganisms are reported to synthesize nanomaterials.

20.6.2 Mechanism of microbe mediated synthesis of nanoadsorbents

Microorganisms serve as potential nanofactories for the synthesizing nanoadsorbents via green route. Along with other advantages microbe mediated synthesis of nanomaterials has gained popularity, due to the ease of cultivation, high growth rate, ability to grow in ambient conditions of pH, temperature, and pressure. Microbial biosynthesis of

nanomaterials is carried out by trapping the metal present in vicinity of the microorganism and converting them to their elemental form followed by reducing them. Actually, in nature synthesis of nanomaterials by microorganism is a resultant of resistance mechanism to avoid toxicity of metals. However, not each and every variety of microbes is able to synthesize nanomaterials, as it takes place via metabolic routes and enzymatic actions that may not be present in many microbes. It also depends upon the microbes' ability to tolerate the metal toxicity. High metal stress can affect various microbial metabolic activities which results in reducing metal ions to the elemental form. Generally, microbes under very high stress of toxic metal concentration become resistant to metal toxicity as the proteins present intracellularly or extracellularly act as chelates and uptake the metal ions. Thus, the resistance mechanism ranges from using the redox enzymes which converts metals to their inert nontoxic forms; involvement of structural proteins which binds metals; or via involvement of efflux proteins which transports ions of metals through proton motive force, chemiosmotic gradients or ATP hydrolysis. All these mechanistic routes coordinate to synthesize the nanoscale materials by microorganisms. The biological method of synthesis imitates the natural process of biomineralization to synthesize the metallic nanomaterials which are used in various applications. The overall mechanism of microbe mediated synthesis of nanomaterials and thus nanoadsorbents thus is categorized as extracellular and intracellular which are explained as follows (Kapoor et al., 2021; Purohit et al., 2019; Pal et al., 2019; Gadd, 2010; Nies, 2003; Rangarajan et al., 2014).

1. **Intracellular biosynthesis:** Intracellular synthesis involves, nanomaterials' synthesis inside microbial cell, when metal ions are reduced to their inert forms by enzymes present inside microbial cell. Nanomaterials thus produced gets collected in microbial periplasmic space. Positively charged toxic metal ions first gets deposited on negatively charged microbial cell wall. This occurs via electrostatic attraction between them. Ions are then transported inside microbial cell, where, with the help of metabolic enzymes, such as nitrate reductase, ions are reduced to finally produce metallic nanomaterials.
2. **Extracellular biosynthesis:** The extracellular mechanism involves the synthesis of metallic nanomaterials through reduction of metal ions outside the microbial cell. The reductase enzymes or soluble secreted enzymes are extracted outside the microbial cell and reduces the metal ions. Nitrate reductase is one of those enzymes which helps to reduce metal ions during extracellular synthesis.

Nitrate reductase, one of the metabolic enzymes which reduces the metal ions to their elemental form, is a NADH (nicotinamide adenine dinucleotide) and NADPH (nicotinamide adenine dinucleotide phosphate) dependent enzyme. According to a mechanism proposed by He et al. (2007), this enzyme reduces the Au^{3+} to Au^0 which then ultimately forms the gold nanoparticles. The enzyme functions by transferring the electron from NADH- to NADPH-dependent reductase which acts as electron carrier. The proteins secreted by the microbes then act as stabilizers which prevents the agglomeration of synthesized nanomaterials by providing the colloidal stability (Kato and Suzuki, 2020; Hulkoti Ali et al., 2020; Singh et al., 2015).

This natural process of converting the metal ions to their nanoscale materials by microorganisms can be imitated under laboratory conditions to biosynthesize the tailored nanomaterials of desired morphology for targeted purpose applications, by varying and optimizing the various experimental conditions, as in the case of nature. In case of intracellular synthetic approach, the microbes are first cultivated in the culture medium with optimum conditions of nutrients, pH and temperature. After a suitable period of incubation, the microbial biomass is harvested, washed with distilled water to avoid any kind of contamination caused by culture media. The microbial cells are then exposed to solution containing metal salts. It is then incubated at favorable temperature, pH and contact time to obtain the nanomaterials (Kato and Suzuki, 2020; Hulkoti Ali et al., 2020; Singh et al., 2015).

The other approach is cell free approach done either by using extracts which are cell free or supernatants of cell culture. During cell culture supernatant approach, the microbes are cultured in liquid medium which after culture is centrifuged to collect the supernatant. Supernatant is then incubated with metal salt solutions for synthesis of nanomaterials. The supernatant has the components of culture medium containing enzymes and other biomolecules secreted by the microbes, which then reduce and synthesize the nanomaterials from their metal ion solutions. In the cell free extract approach, microbes and culture medium are separated by repeatedly washing and resuspending in distilled and sterile water for few moments. Cell free extract, obtained this way, is centrifuged and then incubated with desired metal salt solution. Extract contains only the biomolecules secreted by microorganisms which reduces the metal ions and stabilizes the synthesized the nanomaterials. The change in color indicates the synthesis of nanomaterials in the cell free approaches. This color change depends upon the exact nature of the nanomaterial under synthesis (Kato and Suzuki, 2020; Hulkoti Ali et al., 2020; Singh et al., 2015).

Since, the advantage of microbial synthesis is to obtain nanomaterials with desired morphology, size, chemical composition. In order to achieve desired nanomaterials, experimental factors like temperature, pH, metal ion concentration,

pressure, source of microbes and its concentration, composition of nutrients in microbial culture are varied, adjusted and optimized accordingly for a specific period of incubation and static or shaking condition. It is also mandatory to purify and enrich the prepared nanomaterials by washing repeatedly and centrifuging them. The microbes which are selected, for nanomaterial synthesis purpose, should have good growth rate. Also, they should be capable of producing different kinds of reducing agents in the form of various enzymes, proteins and other metabolites. The harvesting time of microbes or the molecules produced by them is also very crucial factor to optimize the process of reduction. As it was found that the protein function and kinetics of enzymes do vary with the period of incubation. Hence, to obtain precise nanomaterials of desired size, morphology, color and composition etc. it is mandatory to optimize the variables (Kato and Suzuki, 2020; Hulkoti Ali et al., 2020; Singh et al., 2015).

20.6.3 Characterization of nanoadsorbents originated from microbes

It is important to characterize the nanomaterials synthesized using microorganisms. The characterization is performed with the help of various analytical techniques including UV–Visible spectroscopy, Fourier Transform Infra-Red Spectrometry (FTIR), Transmission Electron Microscopy (TEM), Field Emission Scanning Electron Microscopy (FESEM), Energy Dispersive X–ray Spectroscopy (EDS), Dynamic Light Scattering (DLS) analyzer, Selected Area Electron Diffraction (SAED) analyzer, X-ray Powder Diffractometer (XRD), Zeta analyzer, and Vibrating—Sample Magnetometer (VSM). These techniques are used for characterizing various properties like nanomaterials' stability in an aqueous interface, magnetic properties, if any, of synthesized nanomaterials. Chemical composition and, size and shape of crystalline nanomaterials are also characterized. UV-Visible spectroscopy is generally employed to measure the concentration and hence to confirm the formation and stability of nanomaterials. FTIR is used for studying surface chemistry and its properties, that is, the functional groups present on surface, chemical concentration, atomic arrangements. TEM, SEM and AFM helps in determining the size and morphology of nanomaterials. XRD determines the crystal structure, while EDS determines the elemental composition. DLS technique is used for the evaluation of size and charges present on surface of nanomaterials (Mahanty et al., 2020).

20.6.4 Mechanism of adsorption of toxic pollutants by nanomaterials originated from microbes

Nanomaterials, because their particularly remarkable chemical and physical qualities, are good choice for the remediation of toxic pollutants from different environments be it water, air and soil, since last few decades. Nanomaterials are reported to remediate heavy metals, organic pollutants, fertilizers, pesticides, oil spills, toxic gases, sewage (Liu, 2006). Microorganism utilize their inherent metabolic process and convert toxic metals present in the surrounding environment into their inert forms. This property of microbes has opened new unexplored field of research.

Adsorption is a surface phenomenon, in which pollutants bind onto the surface of adsorbent. Nanomaterials synthesized by microbes have also been explored for the adsorptive removal of toxic water pollutants. The efficiency of nanoadsorbent is depended on its inherent surface properties as well as external surface functionalization. There are various mechanisms with which the nanoadsorbents adsorb the toxic pollutants. The process of adsorption has been classified into physisorption and chemisorption. Physisorption involves binding of adsorbate particles on adsorbent surface via weak van der Waals forces. While, chemisorption occurs via covalently bonding and electrostatically attracting the adsorbate molecules on adsorbent surface. There are various factors like pH, temperature, particle size etc. which affect both physical and chemical adsorption processes. Chemisorption is monolayer and is slower than physisorption, while physisorption in multilayer adsorption (Nik-Abdul-Ghani et al., 2021; Vázquez-Guerrero et al., 2021; Messaoudi et al., 2021; Katibi et al., 2021; Arayne and Sultana, 2006; Kecili and Hussain, 2018).

1. Physical mechanism: There are various ways which favors adsorption through physical mechanism like van der Waals forces, Hydrogen bonds, high surface to volume ratio. The equilibrium of adsorption can be calculated using following equation:

$$Q_e = V(C_o - C_e)/m$$

where, V , volume of solution in L; m , mass of adsorbent; C_o and C_e , adsorbate's initial and equilibrium concentration respectively.

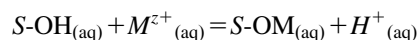
Various interactions are mentioned below (33, 68–72):

- a. **π - π interaction:** The π - π interaction arise between adsorbate acting as π electron acceptor and the adsorbent acting as π electron rich region. This interaction is common in two attractive aromatic rings.

- b. Hydrogen bonding:** Hydrogen bonds are the electrostatic force of attraction between a hydrogen atom, which is covalently bonded to an electronegative atom (F, O, N), and the electronegative atom of another molecule. Aliphatic hydroxyl group forms stronger hydrogen bonds than phenolic groups.
- c. Electrostatic interaction:** It depends on the pH difference between solution and nanoadsorbents. If there is a difference in the pH then the surface of nanoadsorbent acquires either positive or negative charge. Simultaneously the pollutants could be either protonated or deprotonated and attains charge at different pH. This results in the electrostatic interaction between nanoadsorbent and pollutant adsorbate.
- d. Hydrophobic interaction:** It may be another mechanism path for the adsorption of hydrophobic organic compounds. When net charge on adsorbent surface is zero and the pollutant is in the molecular state then hydrophobic interaction is largest and adsorption capacity is highest.

2. Chemical mechanism:

- a. Complexation:** The main process is covalent bond formation through inner sphere complexation by which the metals bind with the nanoadsorbents. The complexation reactions are initiated by functional groups such as hydroxyl and carboxylic groups present on the surface of the nanoadsorbents over the spheres. It is one the dominant mechanism of adsorption via nanoadsorbents. The complexation reaction may occur as given below:



The inner sphere complexation is affected by the magnetic behavior of nanoadsorbents like Fe_3O_4 etc. It is the major cause of complexation which further can be enhanced by addition of polymers and mild acids, making them a good choice as adsorbent. The adsorption efficiency is very less when pH of the solution is less than pH_{zpc}, whereas, in alkaline medium, negative charge is created, when carboxyl sites are converted into carboxylate anions and adsorption efficiency increases until pH is greater than pH_{zpc}.

- b. Redox reaction and Acid-base properties:** The application of metal oxides for adsorption is possible as they have oxidation-reduction and acid-base properties. The surface of nanoadsorbent can be modified using organic and inorganic compounds which stabilizes them and prevents oxidation. The functional groups present on nanoadsorbent surface provides active sites for the increased adsorption efficiency.
- c. Immobilization of pollutants:** It has been found that the stabilized nanoadsorbents have more affinity to bind and immobilize the metallic contaminants for longer time. The stabilized nanoadsorbents have higher adsorption efficiency as compared to nonstabilized.

The mechanism of adsorption is deduced by using various isotherms, kinetic and thermodynamic models are given, which are briefed as follows (Mahanty et al., 2020):

- 1. Adsorption isotherm:** For any adsorption system, isotherm is a curve, obtained at constant temperature, between pressure and adsorbate amount adsorbed on the surface of adsorbent. Various isotherms are proposed:

- a. Langmuir isotherm** According to this model all active sites of adsorption on adsorbent surface are identical, that is, homogeneous. Adsorption is monolayer on adsorbent surface and adsorbed molecules do not interact with each other. The Langmuir adsorption model is demonstrated by equation given below:

$$C_e/q_e = 1/K_L q_{\max} + C_e/q_{\max} \quad (20.1)$$

where, C_e (mg/L) = concentration of the molecules adsorbed at equilibrium, q_e (mg/g) = amount of molecules adsorbed on adsorbent surface at any time. $Q_{\max}$ = maximum adsorption capacity (mg/g) and K_L = Langmuir constant (L/mg).

- b. Freundlich isotherm:** This model describes that the adsorbent surface is heterogeneous and adsorption occurs in multilayers. Model Equation is given as follows:

$$\log q_e = \log K_F + 1/n \log C_e \quad (20.2)$$

where, K_F = Freundlich constant.

- c. Temkin isotherm:** This model considers the effect of heat of adsorption on the adsorbed molecules layer. It is given as follows:

$$q_e = (RT/b_T) \ln (AC_e) \quad (20.3)$$

where, R = ideal gas constant (8.314 J/mol/K), T = temperature (Kelvin), b_T = Temkin constant (J/mol), depending on the heat of adsorption, A = equilibrium adsorption constant, corresponding to maximum adsorption energy (L/mg).

- d. Dubinin–Radushkevich isotherm (D-R isotherm):** D-R model describes that adsorption occurs in the micropores and not on the adsorbent surface which may lead to the formation of monolayer or multilayer. Model is given as follows:

$$\ln q_e = \ln q_m - \beta \epsilon^2 \quad (20.4)$$

where q_m = adsorption capacity (mg/g), β = activity coefficient (mol^2/J^2), representing adsorption energy, ϵ = Polanyi potential.

- e. Harkins–Jura and Halsey isotherms:** The isotherm describes adsorption process to be multilayer on the adsorbent surfaces having heterogeneous pore distribution. It is given by following equation:

$$(1/qe^2) = (B_{\text{HJ}}/A_{\text{HJ}}) - (1/A_{\text{HJ}})\log C_e \quad (20.5)$$

where, B_{HJ} and A_{HJ} = Harkins–Jura constants, determined by the linear plot of $(1/qe^2)$ versus $\log C_e$.

- f. Redlich–Peterson isotherm (R-P isotherm):** The combined features of both Langmuir and Freundlich isotherms are represented in Redlich–Peterson isotherm. At higher adsorbate concentrations, the isotherm shows the features of Freundlich isotherm however, at lower adsorbate concentrations, it obeys Henry's law. The model is represented below:

$$\ln\{K_R(C_e/q_e) - 1\} = b_R \ln C_e + \ln a_R \quad (20.6)$$

where, K_R = adsorption capacity constant, a_R = isotherm constant, and b_R = exponent between 0 and 1.

2. Adsorption kinetics and thermodynamics:

- a. Pseudo-first-order kinetic model:** This kinetic model explains the presence of specific binding sites on the adsorbent surface and that adsorption occurs only at these sites. The adsorbed molecules do not interact with each other and the energy of adsorption does not depend on the formation of a layer on the adsorbent surface. It is given as below:

$$\log(q_e - q_t) = \log(q_e) - (k_1/2 - 303)t \quad (20.7)$$

where, q_t and q_e = adsorbate amount adsorbed per mass of adsorbent (mg/g) at any time “ t ” and at equilibrium, respectively, k_1 = first order rate constant (min^{-1}).

- b. Pseudo-second-order kinetic model:** This model assumes chemisorption to be the rate determining step. It predicts the behavior over the whole adsorption range. The model is represented as follows:

$$t/q_e = 1/k_2 q_e^2 + (1/q_e)t \quad (20.8)$$

where, k_2 = rate constant of second-order sorption ($\text{concentration}^{-1} \text{ min}^{-1}$).

- c. Intraparticle diffusion:** The model considers internal pore diffusion of adsorption to be the slowest and hence rate determining step. Model equation is given below:

$$q_t = k_p t^{1/2} + C \quad (20.9)$$

where C = intercept, k_p = intra-particle-diffusion rate constant ($\text{mg/g h}^{1/2}$), evaluated from the slope of the linear plot of q_t versus $t^{1/2}$.

- d. Estimation of thermodynamic parameters:** Gibbs free energy ΔG° (kJ/mol), Enthalpy ΔH° (kJ/mol) and Entropy ΔS° (J/mol K) are monitored during adsorption process to understand its mechanism. The equations to determine these parameters are given as follows:

$$K_c = q_e/C_e \quad (20.10)$$

$$\Delta G^\circ = -RT \ln K_c \quad (20.11)$$

$$\ln K_c = \Delta S^\circ/R - \Delta H^\circ/RT \quad (20.12)$$

A linear plot (Van't Hoff plot) between $\ln K_c$ and $1/T$ is drawn to find out the slope as well as intercept which provides values of ΔH° and ΔS° respectively.

20.6.5 Application of nanoadsorbents synthesized by microbes for remediation of toxic pollutants

Nanoadsorbents prepared via green synthesis are urgently needed to be given more attention to employ them for removal of toxic organic as well inorganic pollutants as it is an easy, efficient, economical as well as environmental

friendly option. Many microbes ranging from prokaryotes to eukaryotes are applied as nanofactories for the synthesis of nanoadsorbents and have been successfully employed to remediate environmental pollutants via adsorption from polluted sites.

Various metal, metal oxide and metal sulfide nanoparticles are reported to act as good adsorbent for heavy metals as well as organic pollutants. Magnetic nanoparticles are also used for the remediation of large-scale treatment of water. *Rhodotorula mucilaginosa* yeast was used for adsorption of Cu metal via synthesizing its nanoparticles (Salvadori et al., 2014a). Dead biomass of the filamentous fungus *Aspergillus aculeatus* was used for synthesis of NiO nanoparticles. It was reported to give high adsorption capacity of Ni as compared to live and dried biomass (Salvadori et al., 2014b). *Pseudomonas aeruginosa* has been studied for zirconia nanoparticles synthesis and was employed for adsorption of tetracycline from water (Debnath et al., 2020). Extracellular synthesis of CdS nanoparticles was done by marine bacterium and was used to explore the adsorption of Cd from aqueous medium. The adsorption efficiency of CdS nanoparticles incorporated with functionalized extracellular polymeric substance was found to be 88.66% (Raj et al., 2016). Iron oxide nanoparticles synthesized from *Aspergillus tubingensis* (STSP 25), was reported to adsorb around 90% of Pb (II), Ni(II), Cu(II), Zn (II) from wastewater up to regeneration capacity of five cycles (Mahanty et al., 2020). The nanocomposite obtained from coprecipitating iron oxide nanoparticles with exopolysaccharides from *Chlorella vulgaris* was shown to adsorb 91% of phosphate and 85% of ammonia ions (Govarthanan et al., 2020). Dead biomass of *Hypocrea lixii* was used to biologically prepare Cu nanoparticles which showed maximum Cu biosorption of 19.0 mg/g (Salvadori et al., 2013).

20.6.6 Stability and reusability of biosynthesized nanoadsorbents

Nanoadsorbents biosynthesized using plants and microbes have great potential in terms of safety, nontoxicity, simplicity, efficiency, eco-friendliness for purpose of remediation of toxic pollutants. The application of biosynthesized nanoscale adsorbents, in remediation of pollutants and water/wastewater treatment on large scale, should be examined for their regeneration and reusability features. It is necessary to study these factors as they are important in analyzing not only the cost benefit but also its feasibility, stability and sustainability. Hence, the recycling, reusability and adsorption stability are the essential factors to be kept in mind for economic concerns and commercial success, when using biosynthesized nanoadsorbents. There are reports which show that there is no effluent produced on application of nanoadsorbents (Wang et al., 2013; Srivastava et al., 2017). Nanoadsorbents can be regenerated as various functional groups are present on its surface. But these groups may degrade with time, which in turn minimizes the possibility of their regeneration and reuse with same efficiency. According to a study the magnetic nanoparticles synthesized by *Lagerstroemia speciosa* bark (LB) extract showed good adsorption efficiency of Cr(VI) and it was reported to be around 94% even after repeatedly regenerating and reusing for eleven times (Srivastava et al., 2017). There are some more studies on nanoadsorbent synthesized by plant extract of *Eucalyptus globulus* and seed extract of *Cnidium monnieri* L. Cuss (CLC) which showed the effective use of biosynthesized nanoadsorbents after the adsorbent was recycled and reused and retained its stability as well adsorption capacity (Martínez-Cabanas et al., 2016; Lingamdinne et al., 2017). More research is needed in this field in order to successfully employ the nanoadsorbents originated from microbial entities for the remediation of toxic pollutants.

20.6.7 Challenges and future prospects

Recently several nanoscale materials are synthesized and utilized for adsorptive remediation of water from toxic contaminants and pollutants (Kapoor et al., 2021; Purohit et al., 2019; Goutam et al., 2020; Pal et al., 2019). However, the scientists are of different opinion on using the nanomaterials for water remediation. In general, all types of nanoadsorbents, be it synthesized using physical, chemical or biological pathways, exhibit extremely large surface area and provide promising results in terms of high adsorption capacity. There are certain limitations which need to be addressed in order to successfully employ them for large scale applications for real water samples.

The biosynthesis of nanomaterials involves the utilization of plants and microbes. The biological route of synthesis, where the size and shape of generated nanoadsorbents can be controlled, would definitely offer significant advantage over chemical and physical synthetic routes. Very recently there has been tremendous development in using microorganism for the synthesis of nanoadsorbents. The use of microbes however may be risky because of issues related to their pathogenicity. Despite few studies on the functioning of biosynthesized nanoadsorbents, it remains mostly unclear as to why and how the morphology of nanomaterials is affected by microbial systems. Much work and better understanding is required in this regard and also in terms of cellular, molecular and biochemical mechanism of synthesis of

nano-adsorbents by microbes, in order to enhance the production rate and control the size, morphology, color and composition of nano-adsorbents. The synthesis of nano-adsorbents is quite a slow process and may take several hours to few days, as compared to the conventional physical and chemical methods. Research in this regard to reduce the synthesis time will make the biosynthetic nano-adsorbents more attractive. Thus, for commercial production, the methods of microbial biosynthesis of nano-adsorbents needs to be modified, and the synthesis of nano-adsorbents needs to be scaled up to match the traditional methods. In depth research is required to study biocompatibility, toxicity and potential negative effect of nanomaterials on human health as well as environment. Now-a-days focus is also given to fabricate the nanomaterials for acquiring desired shape and size by genetically engineering the microbes. However, despite having several advantages over traditional way of biosynthesis, the use of recombinant microbes is still very rare for preparation of nanomaterials, as there may be issues of purity, reproducibility, survival of genetically engineered strains.

There are few studies which show that nano-adsorbents synthesized by microbes may decompose after a certain period of use. Thus, more study and focus should be given to enhance their stability and thus reusability, which is an essential factor to make the method cost effective. The study of regeneration and reusability of nano-adsorbents after recycling many times while maintaining its adsorption efficiency should be given focus. Thus, cost-based analysis is very important for large scale applicability. Thus, the current focus of research in this field is how to change the physicochemical factors and select the variety of microbes to get monodisperse nanoscale adsorbents of enhanced stability. However, it still needs much exploration to select the microbes, which have the potential to be exploited for biologically synthesizing nano-adsorbents, owing to their rich biodiversity. As compared to bacteria, fungi produce larger amounts of nanomaterials as a result of larger secretion of biomolecules such as proteins.

It is also a challenge that in order to employ microbe originated nano-adsorbents for large scale water remediation, large microbial cultures need to be maintained, which is a tough task. Although, the large-scale synthesis of nano-adsorbents is not easy to maintain, but it is interesting as it does not require toxic, hazardous and expensive chemicals for its preparation as well as stabilization.

20.7 Conclusions

The green synthesis provides a simple, nontoxic, efficient, clean and environment friendly approach for producing nanomaterials. Also, the method has gained much attention because of its stability and economic prospects. Several microbes as well as plant extracts have been employed for the efficient biosynthesis of nanomaterials, which have varied applications including remediation of toxic pollutants from environment through adsorption. Although, comparatively it is easy to generate nano-adsorbents through plant extracts but employing microbes to synthesize nano-adsorbents is more cost effective. Keeping in mind the sustainable development, efforts for improving environmental conditions and generating minimum toxic anthropogenic waste, provides a promising future to the microbe mediated synthesis of nanomaterials, which can be employed successfully for the adsorption of toxic pollutants.

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Application of microbial nanobiotechnology for combating water pollution

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21.1 Introduction

Water is a quintessential natural resource for the survival of all life forms on the earth; however, approximately only 1% of freshwater is existing for anthropological purposes (Grey et al., 2013; Adeleye et al., 2016). At present, drinking water is scarce, and conditions becoming more severe, especially in developing countries. Therefore, providing safe drinking water to the human population is a nightmare and a worldwide challenge of the 21st century (Qu et al., 2013). It is believed that approximately 1.1 billion people or even more are devoid of getting pure drinking water (World Health Organization, 2015), due to excessive upsurge of the human population as well as variable changes in the environment (Adeleye et al., 2016). In India, a developing country is also facing scarcity of drinking water and about 80% of the population is affected by water-borne diseases due to consumption of unhygienic water contaminated with disease-causing pathogens. The impediment of water contamination against toxic chemicals and biologically hazardous microbes used as bioweapons is also facing a critical threat in water resources management.

The water pollutants can be organic, inorganic, or biological in origin (Fig. 21.1) which may cause deleterious effects on the human body or even be carcinogenic (Ali et al., 2009; Laws, 2000) and exhibit undesirable impacts on an ecosystem (Ali, 2012). Some heavy metals such as arsenic, mercury, cadmium, lead, etc. present in water as contaminant is



FIGURE 21.1 Major constituents of water pollution.

highly toxic and causes dreadful diseases (Ali, 2012). Other pollutants such as chlorides, fluorides, nitrates, phosphate, sulfates, and several others are hazardous when present at high concentrations in contaminated water. In addition, organic pollutants like fertilizers, hydrocarbon compounds, detergents, pesticides, phenols, plasticizers, etc. are also responsible for causing damage to the human body and environment (Damià, 2005).

Natural organic matter (NOM), a total sum of carbon-based compounds as well as dissolved organic carbon, is present everywhere and includes animal/plant origins remnants (Matilainen et al., 2011). It counteracts the efficiency of treatment methods like filtration, flocculation, and carbon adsorption. Further, it causes the formation of unwanted oxidation products during chlorination and provides the breeding site for several microorganisms in an aquatic system and became unfit for drinking purposes (Gledhill, 1987).

Globally, enhanced human activities have made a worse condition of drinking water and increased its scarcity by polluting the natural sources of freshwater. The continuously increasing strict standard of water quality, the emergence of new contaminating compounds have forced the development of new alternating methods to counter the present water treatment techniques and distribution systems. Moreover, currently available technology for wastewater treatment and infrastructure are at the level of saturation for providing sufficient quality of pure drinking water to meet human needs.

Pollutants that are present in wastewater can be categorized into six major types, namely, organic degradable pollutants, inorganic salts, nutrients, suspended solids, heavy metals, and disease-causing microbes (Abdel-Raouf et al., 2012). These contaminants exhibit a very high risk of environmental damage and are also responsible for increased levels of pollution in water bodies. These pollutants are either native to the specific area or are continuously added to the water body at various stages of water discharge. Various nutrients such as carbohydrates, fat, and proteins are also available in the organic pollutants which also include organic-rich humans' & animals' excretions, moreover, heavy metals could also be found in wastewater if any industry is situated close to a water body. Usually, organic components of pollutants provide dietary support to the microbes present in the water body, through which these microbes grow, increase their metabolic activity, and propagate as well. However, when the microbes grow exponentially and their load increases in wastewater along with an increase in organic components, they exhibit an adverse effect on the system as well as become the opportunistic carrier of transmittable diseases. The biological oxygen demand increases several folds that lead to depletion of oxygen in the surrounding environment that result in hypoxia condition which causes great loss to native biodiversity. Similarly, Inorganic pollutants such as Ca^{2+} , Na^+ , K^+ , SO_4^{2-} etc. dissolved impurities in water make it unfit for consumption. To overcome the problems of water contaminants, numerous treatment strategies are applied to reduce the chemical load of water such as removal of solid impurities through the process of sedimentation, froth floatation, or precipitation process. Each process helps in the reduction of toxic material from wastewater implicit towards the chemical diversity of impurities.

The available classical water treatment methods exhibit several disadvantages viz need of high-energy, insufficient removal of pollutants as well as generation of toxic sludge. The filtration method yields a concentrated sludge which is both toxic and creates problems while its disposal (Catalkaya et al., 2003). The employment of biologically based water treatment plants is highly popular; however, these are much slower and face constraints when non-biodegradable pollutants are present in the wastewater and also it exhibits an adverse effect on microbes (Zelmanov and Semiat, 2008). Therefore, an urgent alternative method is required for the eco-friendly, highly efficient, cost-effective, powerful, and energy-efficient treatment of polluted water (Burkhard et al., 2000; Parsons and Jefferson, 2006; Ferroudj et al., 2013). Out of various emerging technologies, nanotechnology is at the utmost position due to its unique properties and implausible potential for cleaning polluted water (Sanchez and Sobolev, 2010).

Nanotechnology is the branch of nanoscience in which the size of particles exists in a few nanometers scale (Chaturvedi et al., 2012) which provides a great horizon for the development of water treatment technology intended for the next generation. The nanomaterials can be synthesized in several shapes and structures such as nanotubes, nanowires, biofilms, colloids, and quantum dots. These nanomaterials with astonishing properties having high surface area, catalytic, photosensitivity, tunable pore size, and antimicrobial activity make them highly efficient and provide useful features for efficient and unbeaten tools for wastewater treatment (Edelstein and Cammaratra, 1998; Lubick and Betts, 2008). The modular and multifunctional processes of these nanomaterials provide a promising and potential route to develop high-performance as well as low-maintenance decentralized systems for water treatment.

The nanoparticles are being synthesized through several methods that harness physical, chemical, and biological components (Fig. 21.2). NPs synthesized from chemical methods exhibit several disadvantages such as usage of harmful chemicals and self-agglomeration in aqueous solution. Therefore, the synthesis of NPs through green technology such as the use of plants, bacteria, fungus, algae, viruses, and other microbes are more sustainable and environment-friendly (Fig. 21.3). Among them, microbial-based nanoparticle synthesis such as the use of bacteria, yeast, and fungi are highly appreciated because of their exponential growth rate and easy culture at ambient conditions. These microbes can act as

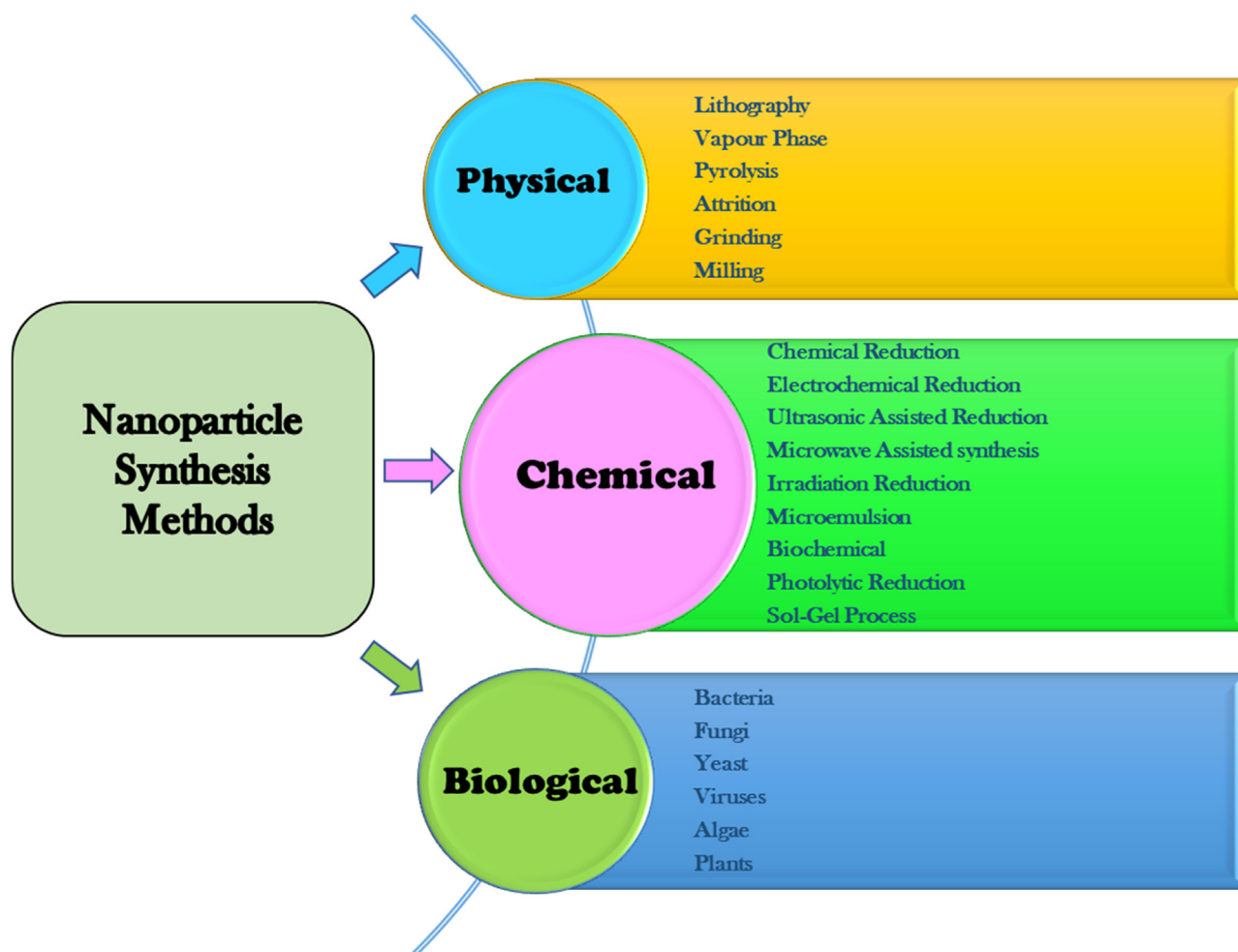


FIGURE 21.2 Different methods of synthesis of NPs.

reducing agents and are capable to synthesize metallic nanoparticles which achieve greater solidity in an aqueous system. It can be achieved because of their co-precipitation or by releasing bioactive and proteinaceous compounds on the outer surface of NPs. For example, the fungus, *Aspergillus tubingensis* capable of bio-fabricating iron oxide NPs from (STSP 25), which were extracted from *Avicennia officinalis*'s rhizosphere (Mahanty et al., 2020). The fungus *A. flavus* produces extracellular AgNPs (Vighneshwaan et al., 2007) which can clean the water, contaminated with bacteria, viruses, and other microbes. The efficiency of these NPs primarily depends on a high surface area: volume ratio, apart from its excellent physicochemical and biological properties (Duran et al., 2010; Jain and Pradeep, 2005; Nair and Pradeep, 2007; Piksova et al., 2009). Several other nanoparticles with varied shapes and sizes have been synthesized from different species of fungus such as, *A. fumigates*, *Fusarium oxysporum*, and *F. semitectum* (Dhillon et al., 2012; Shedbalkar et al., 2014).

The NPs are known to be ideal for minimizing the chemical complexity of oxygenated aggregations of wastes and assist the proper disposal of pollutants (Chen et al., 2015). Amongst metallic Nanoparticles, Au, Ag, Cu, and Fe whereas, oxides NPs such as TiO₂, ZnO, nanozerovalent iron, silica, etc. have been the commonly used for the purpose (Ruttkey-Nedecky et al., 2017). The utilization of microbial-based NPs for the management of wastewater solely depends upon the interaction that enhances the NPs efficiency. Iron-NPs have more reactive properties as compared to Cu-NPs and Ag-NPs, this makes the nanozerovalent iron NPs more efficient to remove several chemical pollutants, such as oxidized heavy metals, chlorinated compounds, and aromatic nitro compounds, efficiently from wastewater (Yang et al., 2019). However, under aerobic conditions, water can cause rapid oxidation of nanozerovalent iron NPs, and results in dissolved oxygen oxidizing Fe²⁺ to Fe³⁺ (Yang et al., 2019). Therefore, the pH and availability of dissolved oxygen can be regulatory factors that determine the reducing efficiency of nanozerovalent FeNPs (Park and Dempsey, 2005).

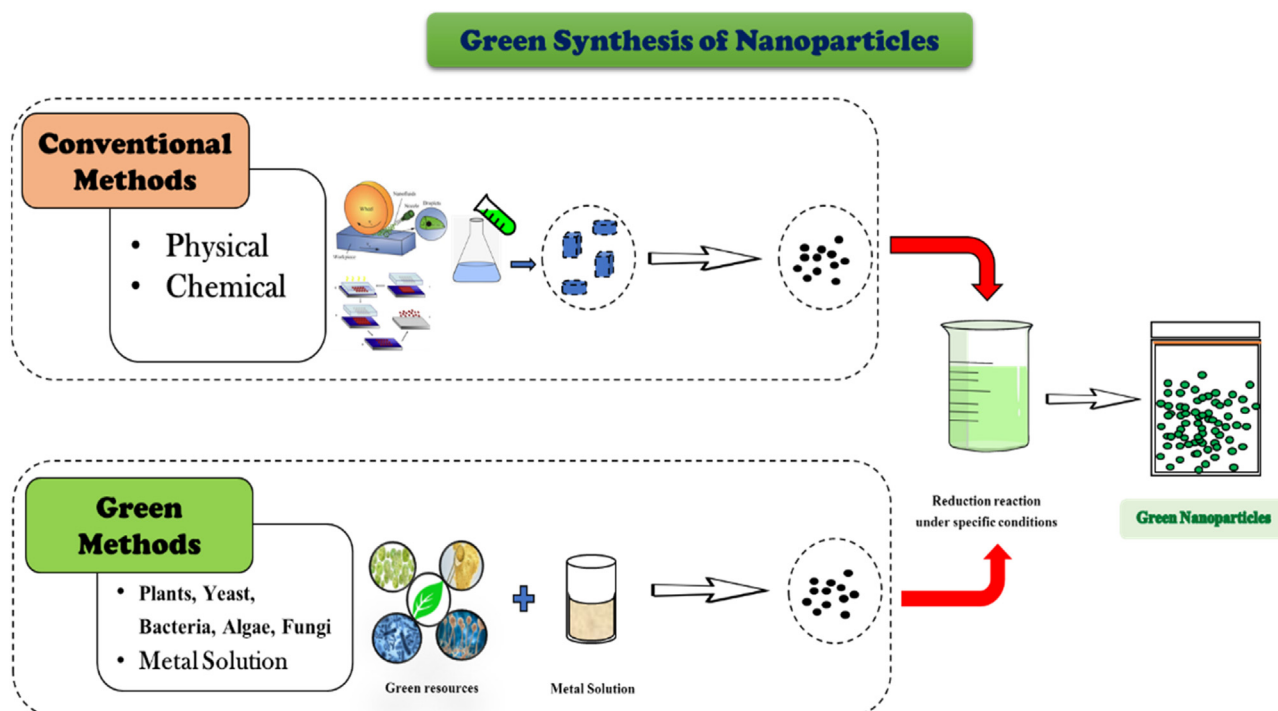


FIGURE 21.3 Comparison of conventional method and green method for NPs synthesis.

The present chapter emphasizes the microbial-based green synthesis of nanoparticles and their implementation in wastewater treatment, their benefits, limitation as well as future perspective in sustainable water management systems.

21.2 Classification of nanoparticles

Nanoparticles can be categorized based on the nature of nanomaterials as **nanoadsorbents**, **nanocatalysts**, and **nanomembranes**.

21.2.1 Nanoadsorbents

Effective and large numbers of nanoadsorbents have been synthesized for the separation of water pollutants (Tang et al., 2014; Kyzas and Matis, 2015). These NPs can be synthesized from elements such as metal oxides, clay, activated carbon, and silica, which have chemically active atoms with high adsorption efficacy (El-Saliby et al., 2011; Kyzas and Matis, 2015). The large surface area of NPs favors increased chemical activity as well as adsorption efficacy on their outer surface (Kalfa et al., 2009). Ionic structural transformation of NPs is favored by redox reactions for removing persistent inorganic pollutants (Gupta et al., 2015). Heavy metal removal through the adsorption method harnesses mostly and frequently the nanoparticles such as carbon nanotubes, activated carbon, zinc oxide, manganese oxides, graphene, ferric oxide, and titanium oxide (Gupta et al., 2015). These NPs bear dual properties of inner surface adsorption as well as exterior functionalization. The physicochemical properties of these NPs are also associated with their size, exterior surface morphology, and intrinsic composition (Mirkin et al., 1996). In a liquid medium, the adsorption process is influenced by chemical activity, greater surface area, adsorption efficacy, reduced or negligible resistance against internal diffusion, and high surface binding energy (Khajeh et al., 2013). Moreover, these nanoadsorbents are biosynthesized for the elimination of pollutants from wastewater, must be nontoxic, have high efficiency of pollutant adsorption even in low concentration, are recyclable, and can easily remove adsorbed pollutants from its surface (Cloete, 2010).

Many carbon nanotubes (CNTs) form aggregates because of entanglement which results in enlarged external surface area for the maximum adsorption of analytes. Moreover, hollow internal surfaces of CNTs, boundary grooves, interstitial pore spaces, and external surfaces are important sites that can be utilized to adsorb the pollutants of both organic and inorganic origin. For functionalization of CNTs, non-covalent bonding is preferred to bring distinct changes in chemical properties of CNTs which enhances their adsorption efficiency.

Microbial-based TiO_2 NPs also have been successfully exploited to clean organic impurities present in wastewater. The performance of TiO_2 NPs depends upon the surface area, crystallite size, types of impurity, and concentration. The pH of TiO_2 NPs also plays a key role in the distribution of various active sites on metal oxides, whereas surface hydroxyl groups provide the binding efficiency of the metals.

Hence, the efficacy of nanoadsorbents to adsorb heavy metallic contaminants from wastewater as influenced by pH, adsorbent dose, temperature, and incubation time (Srivastava et al., 2015). Furthermore, an increased incubation period increases the adsorption time of heavy metals from an aqueous medium by providing longer duration to interact.

21.2.2 Nanocatalysts

There are various nanocatalysts such as electro-catalysts, photocatalysts that have been employed to degrade different pollutants present in wastewater (Dutta et al., 2014), e.g., catalysts based on Fenton as well as catalysts exhibiting anti-microbial activities (Chaturvedi et al., 2012; Kurian and Nair, 2015).

The nanomaterials produced from noble metals like Pd, Au, and Pt are known to exhibit very effective catalytic properties to degrade various types of organic as well as inorganic contaminants (Liu et al., 2013). However, these nanocatalysts may have certain limitations which include the high cost of manufacturing as well as the problem in full recovery of NPs for reuse in the system.

21.2.3 Nanomembranes

The third class of nanomaterials is **nanomembranes**. It is considered an efficient strategy for wastewater treatment (Ho et al., 2012). It is a pressure-driven technique and is supposed to be ideal for increasing water quality (Rao, 2014). Out of several membrane filtration techniques, the nanofiltration (NF) technique is highly popularized and vastly used for removing various pollutants from industrial wastewater as this methodology has some unique properties like small pore size, increased efficiency, eco-friendly approach, and inexpensive feasibility (Petrinic et al., 2007; Rashidi et al., 2015). This is a widely used technique as it has several benefits such as quality of purified water, its effectiveness against infection as well as the need for less space (Jang et al., 2015; Guo et al., 2016). It can be effectively used to treat wastewater contaminated with heavy metals, various organic as well as inorganic dyes, various other pollutants (Jie et al., 2015), and the decomposition of organic pollutants (Yang et al., 2015).

21.3 Microbial synthesis of nanoparticles

The defined mechanism of microbial-based synthesis of NPs is not completely understood because different microorganisms interact differently during NP synthesis. There are large numbers of microorganisms that can synthesize NPs either through intracellular or extracellular machinery by using both organic and inorganic materials (Mukherjee et al., 2001) (Fig. 21.4) while the mechanism of biosynthesis of NPs is unique to each microbe.

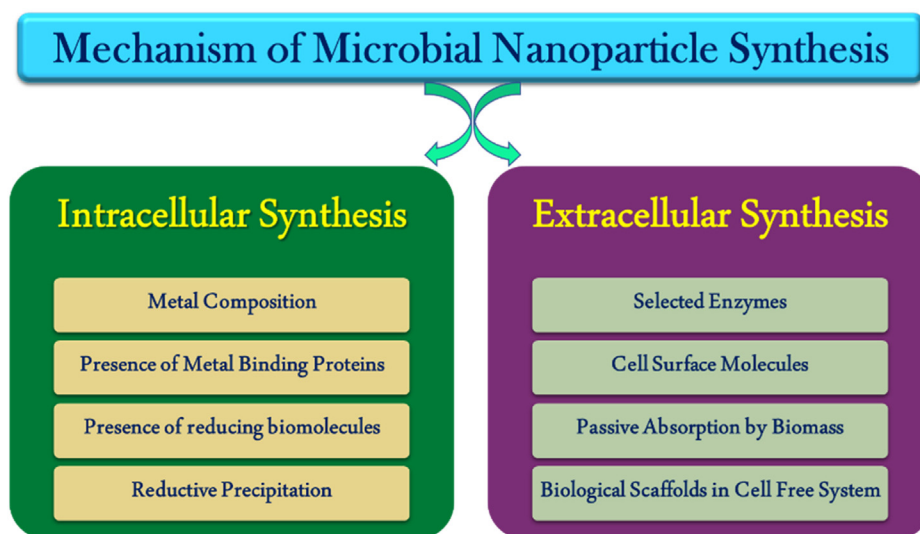


FIGURE 21.4 Mechanisms involved in microbial-based nanoparticles syn-thesis.

21.3.1 Intracellular biosynthesis of NPs

The intracellular mode of synthesis of NPs involves a particular ion-transport system where the cell wall displays a significant role. During the synthesis of NPs, electrostatic interaction occurs between metal ions bearing positive charge and negatively charged microbial cell walls. During this interaction, there is the conversion of metal ions into their elemental form occurring through different enzymes and their diffusion from the cell wall.

Actinomycetes such as *Rhodococcus* species (alkali-tolerant) and *Thermo monospora* species (alkali-thermophilic) have been used for gold nanoparticles (AuNPs) biosynthesis through intracellular mechanism (Ahmad et al., 2003; Ahmad et al., 2005). When *Verticillium* species was exposed to an AgC ion solution, caused an intracellular reduction that resulted in the formation AgNPs. A similar procedure can be followed to biosynthesize AuNPs by *Verticillium* spp. acting as a source of reducing enzymes (Ovais et al., 2018a,b). Several other metallic NPs can be obtained by using yeast in the same manner, as in yeasts' cell wall there could be possible enzymes that can elicit an electrostatic interaction with metal cations and amide groups of enzymes present in a yeast cell. The enzymatic metal ion reduction causes their accumulation that leads to the nanoparticle's synthesis (Salvadori et al., 2017).

21.3.2 Extracellular biosynthesis of NPs

Nanoparticle synthesis through extracellular mechanisms is mediated through different enzymes which are released either in culture medium or through the enzymes present in cell membranes themselves. The extracellular enzymes of microbes act as reducing agents which promote the metallic nanoparticles synthesis (Subbaiya et al., 2017). These mechanisms of extracellular synthesis of NPs causes reduction of positively charged metal ions which get entrapped either in the cytoplasm of the cells or on the cellular wall due to the presence of negatively charged biomolecules available there and lead to nanoparticle formation (Golinska et al., 2014). The site for nanoparticles production is species-specific and it is formed at the intracellular level at the surface either on the plasma membrane or beneath the cell wall (Mukherjee et al., 2001; Ahmad et al., 2003). Likewise, the extracellular synthesis of metallic nanostructures involves surface proteins or secreted enzymes. Synthesis of nanoparticles through an extracellular mechanism by *Fusarium oxysporum*, a fungus, and *Rhodopseudomonas*, a bacterium can be documented through the secretion of α -NADPH dependent nitrate reductase enzyme (Khandel and Shahi, 2018).

In addition, yeast and molds can also be used to synthesize NPs with specific morphology through an extracellular mechanism by employing the enzymes located on their cell surfaces to produce mono-dispersed metal-oxide-NPs, examples are, mesoporous magnetite (Fe_3O_4), titanium dioxide (TiO_2), and antimony trioxide (Sb_2O_3). Nanoparticles were synthesized using the yeast, *Saccharomyces cerevisiae* (Zhou et al., 2009). Similarly, *Aspergillus fumigatus* and *Candida albicans*, *Sargassum muticum*, *Chlamydomonas reinhardtii*, and *Gracilaria gracilis* Can be harvested to produce possible ZnO-NPs (Mahdavi et al., 2013; Zikalala et al., 2018).

It is also possible to synthesize metallic NPs from extracellular fungal enzymes like glucosidase, acetyl xylan esterase, and cellobiohydrolase D (Ovais et al., 2018a,b). AuNPs synthesized by an extracellular process in *Rhodopseudomonas capsulata* through electron transfer from NADH by the action of NADH-dependent reductase enzymes resulting in a reduction of gold ions to form AuNPs (He et al., 2007). Similarly, silver nanoparticles (AgNPs) have been biosynthesized from the extracellular mechanisms by enzymes present in fungal species such as *Fusarium semitectum*, *Fusarium solani* (Ingle et al., 2009), *Cladosporium cladosporioides*, *Coriolus versicolor* (Balaji et al., 2009), and *Aspergillus fumigates* (Bhainsa and D'Souza, 2006). Gold nanoparticles (AuNPs) were synthesized from the extracellular mechanism of reducing Au_3C ions through the algae *Chlorella vulgaris* and *Sargassum wightii* (Lengke et al., 2006; Singaravelu et al., 2007). During NP biosynthesis, metal ion interacts with enzymes of filamentous fungi at the cell wall leading to the metal ions reduction and yielding of nanoparticles (Salvadori et al., 2013).

Microorganisms contain a large variety of biomolecules such as polysaccharides, enzymes, and organic acids that act as key components during nanoparticle synthesis. In addition, these available biomolecules also help in the encapsulation process of nanoparticles which prevents aggregation through functioning as a capping agent.

Microorganisms can form almost all categories of NPs by reducing metals to form metallic nanoparticles from Cu, Au, Ag, Fe, Se, Fe, etc. (Das et al., 2017), metal oxides like TiO_2 , Fe_2O_3 , ZnO, etc. as well as metal sulfides such as CdSe, PbS, ZnS CdS, etc.

21.4 Why microbial-based nanotechnology?

Green sources form the backbone of biocompatible NP synthesis due to the availability of varieties of biomolecules that are capable to reduce metal ions as well as exhibiting several advantages over other conventional methods (Fig. 21.5).

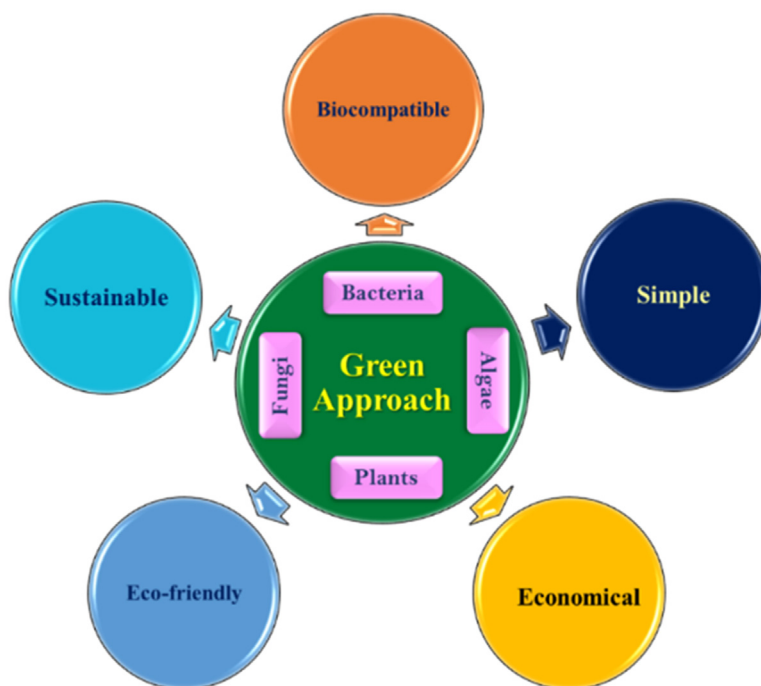


FIGURE 21.5 Advantages of green approach for NPs synthesis.

The microbial-based synthesis of NPs is beneficial in several terms. It is emerging as a cost-effective technique for NP synthesis when compared with other methods and does not require expensive and sophisticated instruments or physical conditions. This is because it uses a variety of biological systems such as microbes. It is also not dependent on toxic or harmful chemicals needed for NP synthesis hence making it eco-friendly. It requires water as a key solvent for green synthesis and no capping agent is needed for NPs stabilization. The enzymes or other biomolecules present in biological systems are self-reliant to perform the reaction and stabilization of NPs.

Microbial-based NP synthesis provides nontoxic, clean, ecologically compatible, and environmentally friendly technology. These NPs exhibit a wide variety of shape, size, composition, physical and chemical properties (Mohanpuria et al., 2008; Prasad et al., 2016). Biologically produced NPs have the potential to treat polluted wastewater and can remove several pollutants like pesticides, heavy metals, dyes as well as organic and inorganic pollutants (Das et al., 2017). These NPs have smaller sizes with larger surface/volume ratios (SVR) which makes them indispensable in the treatment of wastewater (Shah et al., 2015). Moreover, their production with the help of microbes provides them with some special characteristics like capping which checks their aggregation along with providing various functional active sites useful in the adsorption of various pollutants from contaminated water.

Polluted water streams lead to an increased risk of environmental degradation which in turn becomes a threat for the flora and fauna growing in polluted water bodies (Bhateria and Jain, 2016). NPs bear high surface areas which initiate various multiple interactions to reduce this pollutant load to a large extent in comparison to other conventional technologies (Lin et al., 2014).

21.5 The implication of microbial-based nanoparticles in bioremediation of wastewater

A huge amount of waste water coming from various industries and agricultural waste is dumped into water bodies regularly which not only impacts human health but is also very toxic to aquatic life and enhances environmental degradation (Sangeetha et al., 2017a,b,c). The NPs with the small size and large surface area provide them unique physicochemical properties that make them the first choice in the sustainable management of wastewater. They are also eco-friendly, cost-effective, and practical options for the removal of pollutants (Singh and Walker, 2006) from water. These NPs clean the environment by removing xenobiotic pollutants such as acid dyes, cationic dyes, azo dyes, and several other pollutants including heavy metals, pesticides, fertilizers, herbicides, industrial effluents, oil spills, and other organic

compounds. In comparison with older traditional methods of remediation, the microbial-based nanoparticles have unique properties through which they can either behave as catalysts or can adsorb various pollutants over their substantial surface area (Vanalakkar et al., 2018). Therefore, these NPs can be used to degrade water pollutants without facing any unpleasant effects (Naim et al., 2016).

21.6 Degradation of organic and inorganic contaminants from wastewater

The biogenic NPs possess a large surface area that results in a notable number of active binding sites that enhance their catalytic activities. It has been observed that biosynthetic production of different types of nanoparticles from different microorganisms acts as a prominent tool for the degradation of harmful dyes used at the industrial level. Their biosynthetically active nanoparticles show various modes of actions such as photolysis, surface adsorption due to their active sites available in surface proteins, some act in anaerobic conditions and some produce additional benefits like inhibition of H₂S release and metal sludge accumulation during cleaning processes (Table 21.1).

Due to the advantageous utility of the chlorinated aromatic compounds like flame resistance, low solubility in water, and oxidation rate, these are used in most of the industries, but due to their overuse, contamination of soil, water, and the air is also caused extensively.

However, nature has also provided a possible solution in the form of biosynthetically derived Pd-based nanoparticles extracted from organisms like *Desulfovibrio desulfuricans*, *D. vulgaris*, and *D. sp.* “Oz-7” showed the dehalogenation ability of aromatic compounds to combat pollution (Fang et al., 2019). It has been observed that cell surfaces of these microorganisms can be used for the biosynthesis of Pd NPs which were found to be thirty times more effective in dechlorination than synthetic Pd NPs.

The nitroaromatic compounds 4-nitrophenol present in synthetic insecticides and dyes adversely affects the central nervous system of various organisms including humans. This may lead to potential environmental hazards. However, reduction of these 4-nitrophenol compounds has been reported through Pd and Au NPs which were synthesized using *Bacillus* sp. since these NPs exhibited catalytic activity. Nitroaromatic compounds (Zhang and Hu, 2018) and these microbial-based Au-NPs have also been observed for high catalytic degradation of nitrobenzene (Cumbal et al., 2003). Further, the Au NPs, biosynthetically obtained from the bacterial population of *Staphylococcus warneri*, have been testified with higher efficiency towards Surface-Enhanced Raman Scattering (SERS), a tool to identify harmful compounds. Biogenic Au NPs have also been reported as a potential candidate for the complete degradation of highly toxic nitroaromatic pollutants such as 4-nitroaniline, 4-nitrophenol, 2-nitroaniline, and 2-nitrophenol (Nag et al., 2018). In another study, it was found that Au nanoparticles synthesized from *Trichoderma viride* could degrade the compounds like para-nitrophenol to amino phenol as early as half-hour only (Mishra et al., 2014).

Likewise, other biogenic nanoparticles have also shown their promising and futuristic usable activities for combating water pollution. Some of the potent examples are here like Fe NPs which have been observed in remediation of ground-water contaminated with carbon tetrachloride compound (Liou et al., 2007). Songara et al. (2018) claimed another candidate nanoparticle that is ZnO NPs, when synthesized from bacteria *Pseudomonas putida* has shown its photocatalytic activity against a prominent environmental pollutant i.e., benzyl butyl phthalate (BBP).

TABLE 21.1 List of microorganisms producing effective degrading nanoparticles.

Microorganism	Type of nanoparticle synthesized	Mode/activity	Dye	Reference
<i>Erwinia herbicola</i>	SnO ₂	Photocatalysis	Methylene Blue, Methyl Orange, and Erichrome black	Srivastava and Mukhopadhyay (2014)
<i>Cladosporium oxysporum</i> AJP03	Au	Adsorption	Rhodamine B dye	Bhargava et al. (2016)
<i>Pseudoalteromonas</i> sp.	FeS	Anaerobic degradation, Inhibited H ₂ S release, and metal sludge accumulation	Naphthol Green B dye	Cheng et al. (2019)
<i>Escherichia</i> sp. SINT7	Cu	Adsorption	Azo dyes	Mandeep and Shukla (2020)

Green synthesis of metallic nanoparticles like Fe, Fe-Co, Ag, Pt, Pt-Co, Pt-Ni, etc. extracellular and/or intracellular methods aids in actively detoxifying water contaminants by involving their redox reaction mechanisms (Liu, 2006; Shah et al., 2015). There are protuberant examples of harvesting of various biogenic nanoparticles at industries that are involved in dye production which need such efficient and active NPs that not only clear the pollutants but work in a time-bound manner so that they don't become a fiscal burden to them. Some of these can be discussed here like Ag-NPs which can efficiently decolorize organic dyes and have the potential to be used as biocatalysts for organic dyes degradation at the industrial level (Sharma et al., 2019). Apart from AgNPs, Au NPs can also exhibit a catalytic level of activity acceptable in industries to get rid of the organic dyes due to their high catalytic and time-bound mode of action which reduces the time required to remove the dyes as well as enhances the rate of reaction. Further, in one of the studies, it was observed that these biogenic Au-NPs obtained from *Cladosporium oxysporum* AJP03 fungus, has shown the ability of better adsorption of organic dyes due to their surface protein structure, and it has been reported for improved adsorption of the organic dye, rhodamine-B (Bhargava et al., 2016).

Industries involved in the synthesis of dyes may also harness the biogenic nanoparticles like FeS-NPs, which have been synthesized with a lesser amount of sulfur and have been reported to degrade Naphthol Green B dye via an extra-cellular electron transfer mechanism (Cheng et al., 2019). A bacterial strain like *Pseudoalteromonas* sp. CF10–13 can be an appropriate weapon for the eco-friendly production of biogenic nanoparticles to attain the successful application of biodegradation. These species carry out the production of nanoparticles to resist producing detrimental gases and toxic metal complexes. Such NPs when applied to industrial effluents, cause a comprehensive depletion of suspended solids and ions of chloride and phosphate in the treatment specimens. Moreover, promising observations regarding these biogenic nanoparticles are indicative of possible improvement towards lucrative and sustainable industrial production (Noman et al., 2020).

21.7 Elimination of ions of heavy metals

Pollution caused by heavy metals in the water body is another serious environmental concern. The nuisance of heavy metals has reached everywhere at a rapid rate due to injudicious agricultural and metal industrial practices, nonplanned disposal of waste, synthetic fertilizers, and pesticides. Their biological intrusion and disruption of vital functioning as well as causing many serious diseases have been very well documented (Briffa et al., 2020). Several traditional techniques have been used in the past and these are now realized to be ineffective, costly and they add secondary pollutants as well. These remedial technologies have been continuously evolving and adapting various new and advanced tools to contest this heavy metal pollution and nowadays many researchers and scientific organizations are looking for a blended approach of utilizing biogenic nanoparticles with an approach of green harnessing get promising, reliable, biocompatible, cost-effective, and alternate methods to remove heavy metals and other adulterants from contaminated water (Kapoor et al., 2021).

There are some examples of biogenic NPs which have been observed to fulfill these expectations. To name a few like *Shewanella loihica* PV-4 has been observed to reduce the toxic influence, up to 71% and 91% respectively, of metals like vanadium and chromium when applied for 27 days (Wang et al., 2017).

Biogenic Pd-NPs were found to be more capable in catalysis than synthetic palladium nanoparticles while eliminating hexavalent chromium as a water contaminant. It was observed that it has more surface-to-volume ratio compared to synthetic Pd-NPs. It was demonstrated that the bacterium *Clostridium* was capable of successful biosynthesis of metallic Pd NPs from palladium, Pd (II) ions (Johnson et al., 2013). Biogenic syntheses of *E. coli*-mediated Au-NPs have also been shown to be efficient, bioactive, environment-friendly to degrade organic pollutants like 4-nitrophenol. Another nanoparticle i.e., Ag-NPs biosynthesized from *Aspergillus niger* has shown time-bound efficiency with decolorization of up to 86% of dye in the first 24 h followed by 100% efficiency within 48 h. Further examples are there of FeO-NPs obtained from *Aspergillus tubingensis* which have been observed to successfully remove heavy metals like Pb, Ni, Cu, Zn (up to 98%, 96.45%, 92.19%, and 93.99%, respectively) from contaminated water. In addition, it was observed that these iron-nanoparticles have five-times high regeneration ability and reusability (Mahanty et al., 2020).

21.8 Other nanoparticles use

21.8.1 Microbial-based nanoparticles as biosensors

Nanostructures have distinctive electrochemical as well as optical properties which make them unique for applications in varieties of biosensors (Faraz et al., 2018). Microbial-based synthesis of biosensors exhibits high sensitivity that is

reported as non-hazardous to the environment. These biosensors have been utilized for monitoring a range of pollutants (organic and inorganic) present in soil or wastewater. They are also very successful in determining traces of heavy metals, pesticides, and other toxic materials present in contaminated water (Catrinel et al., 2010). Various types of sensing devices such as biosensors, optical and electrochemical sensors have been successfully implemented for sensing environmental contaminants. These biosensors are integrated with a recognition element that helps to bind the target elements and a transducer component that facilitate signaling the downstream event. Electrode modified with biosynthesized Au NPs bears good stability and electrochemical detection ability. These modified electrodes were demonstrated for analyzing the availability of nitroaromatic compounds present in polluted water (Emmanuel et al., 2014).

Nanomaterial-based biosensors have been also found to detect heavy metals in polluted water with very high sensitivity. Ag and Au NPs have a tunable size and high extinction coefficients with distance-dependent optical properties that make them very effective for metal detection (Ray, 2010; Aragay et al., 2011). The application of chelating agents on the surface of Au NPs, provides an efficient sensor system for the recognition of metal ions through colorimetric sensing. The metallic ions present in the sample cause accumulation of NPs that result in the formation of a complex of multidentate interparticle with a chelating ligand that caused a color change (Saha et al., 2012).

Biogenic composite NPs of Au-Ag-yeast have been demonstrated for their efficiency in the detection of vanillin from various samples (Zheng et al., 2010). SeNPs are known to bear a large surface-to-volume ratio, biocompatibility along with excellent adhering and electro-catalytic activity. Selenium NPs produced from *Bacillus subtilis* and *Bacillus pumilus* sp., have been reported as the potential biosensor to detect horseradish peroxidase production in a variety of food materials, industrial byproducts, pharmaceutical industries as well as environmental pollutants analysis (Li et al., 2011; Prasad et al., 2015; Wang et al., 2010a). Biosensors made of AuNPs are utilized for curing hyperthermia, determination of glucose level, and estimation of various biomolecules (Moghaddam et al., 2015).

Biosynthesized AuNPs with tunable size and optical properties were reported detecting metallic ions such as Cu^{2+} , Pb^{2+} , Hg^{2+} , Ca^{2+} & Zn^{2+} through plasmonic and fluorescent sensing methods. Nag et al. (2018), showed the high surface-enhanced detection of toxic compounds like 4-nitroaniline, 2-nitroaniline, 2-nitrophenol, and 4-nitrophenol when they applied the Raman scattering method on AuNPs derived from bacterial strain *Staphylococcus warneri*. Similarly, Wang et al., 2010b, detected Hg^{2+} by a sensor prepared from AgNPs and mercury-specific oligonucleotides. 11-mercaptopundecanoic acid-functionalized AuNPs were also reported for detecting heavy metals in the water bodies. There are several other NMs/NPs are reported for sensing organic and inorganic water pollutants.

21.8.2 Antimicrobial activity

Water pollution not only consists of organic or inorganic pollutants but several microbial pathogens present in the surface water or groundwater which have been proved to pose serious health concerns to humans as well as a great menace to aquatic systems (Seo et al., 2012).

Several pathogenic bacteria are known to cause serious diseases such as cholera, typhoid, intestinal infections, and many more. Water polluted with cyanobacteria releases microcystins that are reported fatal to humans. Several protozoans are responsible for gastroenteritis and other digestive system-related problems, whereas fungi like mycotoxins can cause inflammation in the digestive and respiratory systems (Kumar et al., 2014a, b). In addition, the resistance of microbes against different drugs also became a serious concern to identify alternate methods. Their ability to cause devastating effects on human health and water bodies, emphasize the urgent need for undeniable water disinfecting systems across the globe (Ong, 2016).

In recent years, the preparation of nanoparticles (NPs), mediated by microbes, attracted researchers due to their massive abundance. These biosynthesized NPs could be effectively used to control the population of different microbes such as fungi, bacteria, protozoa, helminths, viruses, and anti-inflammatory effects as well (Fayaz et al., 2010; Moustafa, 2017).

Properties of microbial-assisted synthesis of AgNPs, such as high aspect ratio increase its efficiency of interaction with different particles which enhances its antimicrobial and antiviral efficacy several times (Birla et al., 2009; Thakkar et al., 2010; Goodman et al., 2013).

AgNPs synthesized from a fungus *Trichoderma viride*, when combined with different antibiotics such as ampicillin, erythromycin, kanamycin, and chloramphenicol for the assessment of any effect against microbes, it showed enhanced functioning of antibiotics-AgNPs complex in comparison to antimicrobe activity shown by individual antibiotics (Fayaz et al., 2010; Aziz et al., 2016). Biofabricated AgNPs from fungus, *Fusarium oxysporum*, and *Trichoderma* sp. Have been used as anti-infection agents in textile fabrics and inhibit the growth of pathogens like *Staphylococcus aureus* (Durán et al., 2007). AgNPs are known manipulators of several bacterial functions like solubility and surface adhesion

properties (Campoccia et al., 2013; Romanò et al., 2013). AgNPs are attached to and enter into the cell wall of bacteria and modify signaling pathways by dephosphorylation of relevant peptides present on tyrosine (Sadhasivam et al., 2010; Singh et al., 2008) and it causes the damage of bacterial membranes due to reactive oxygen species produced there, ultimately it forms free radicals that enhance bacterial mortality (Wu et al., 2014). The NPs such as cadmium sulfide and zinc sulfide NPs were reported to act as antimicrobial agents against several pathogens like *Streptococcus* sp., *Candida albicans*, *Lactobacillus* sp., and *Staphylococcus* sp. (Malarkodi et al., 2014).

Biofabricated NPs are also reported to exhibit strong antifungal properties. CuO-NPs synthesized from *Streptomyces* spp. demonstrated excellent antifungal activity against *Aspergillus niger*, *Fusarium oxysporum*, *Alternaria alternate*, and *Pythium ultimum* (Hassan et al., 2018, 2019). Similar increased antifungal activity was found with the microbial-based synthesis of AgNPs (Kaur et al., 2018).

21.9 Challenges and future prospects

Despite all the expected and promising results observed, there are some limitations associated with the synthesis of biogenic nanoparticles in the form of less production, difficulty in the extraction process of nanoparticles from bioactive compounds as well as the chances of getting a contaminated population of these biological agents. So, there is an essentiality of an extensive survey of microbial diversity for the selection of suitable and sustainable microbial and green flora for the synthesis of green nanoparticles (Singh et al., 2018). There is an emphasis to conduct more and more research especially focused on the identification, isolation, and selective culture/breeding as well as acquisition of eco-friendly mechanisms in biosynthesis of biogenic nanoparticles with cautious and controlled utilization of their morphology, size, and dispersity.

However, in comparison to conventional treatment and remedial methods, the biological agents like microbes and their byproducts, if exploited meticulously, can be a game-changer in the process of biogenic synthesis of nanoparticles and in their strategical use in combating pollution. Due to their substantial potential like small size, user friendly, cost-effective, eco-friendly, and with high degradation ability, the microbial assisted metal nanoparticles synthesis has gained huge interest. However, it is still mandatory to understand the various mechanism associated with the biological synthesis of nanoparticles, though there are many examples of synthesis of biogenic and stable nanoparticles because of the selection of suitable microorganisms under optimized conditions.

Biogenic synthesis of nanoparticles does not require specialized physiological conditions as that of synthetic nanoparticles procedures that require specific temperature, high pressure, continuous energy, numerous stabilizers, and unwanted toxic chemicals. They use their physiological arena and because of these exceptional properties, they can be turned into noteworthy tools in various fields of energy, environment, and agriculture. If we can understand this microbial wealth, we can produce a wide range of nanoparticles just by doing small manipulations in microbial biological processes to use against any specific or multi-pollutants present in wastewater (Kapoor et al., 2021).

It is noteworthy here that the biogenic synthesis of metallic nanoparticles has been accomplished at the laboratory level, however, industrial level appreciation is yet to be achieved and is also much needed. For mass production at the industrial level, various hurdles need to be defeated like the commercialization of nanotechnology materials, their efficiency of synthesizing nanoparticles in terms of long incubation/contact time, cost-effective production of microbial nanoparticles. The stability of biogenic nanoparticles is one more concern that should be resolved by promoting in-depth research in such an area.

Biogenic nanomaterials should also be analyzed for cost-benefit parameters to explore the avenues in commercial exploitation as information is scarce in this context. This will create the pathways to decide the reproducibility of these green components. Moreover, detailed studies should be conducted, on leftover or residual products arising during biosynthesis of nanoparticles, so that estimate of costs in terms of economy as well as environmental health can be assessed to avoid any accumulation of undesired products that can bring the method to become non-reproducible. Apart from this, it should not be against environmental and other safety issues which can result in undesirable consequences (Briffa et al., 2020).

The global appeal of sustainable water management generates demand for nanoscale materials, machinery, and manpower, if these all can be fulfilled keeping in mind the health and environmental impacts, there are the possibility of huge opportunities and cause industrial prosperity in the field of manufacturing biogenic NPs. Extensive studies on microbial understanding can create the demand for advanced computational tools in the field of omics, where one can exploit the derived data for the betterment of synthesis microbial NPs. This will strengthen the pathways leading towards the implementation of green nanotechnology. Moreover, it can open various avenues in terms of nano factories

for the investigation of microbes and their biosynthetic pathways to develop competitive strategies towards the bioremediation of contaminated water.

List of abbreviations

NOM	Natural organic matter
CNT	Carbon nanotubes
NF	Nanofiltration
SVR	Surface/volume ratio
NPs	Nanoparticles
SERS	Surface-Enhanced Raman Scattering

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A review on azo dye degradation by exopolysaccharide-mediated green synthesis of stabilized silver nanoparticles

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22.1 Introduction

Nanoparticles have made multiple breakthroughs in various disciplines, including electronics, biotechnology, chemistry, medicine, etc. Presently, nanoparticles are the center of interest for numerous research communities due to their unusual characteristics and prospective applications (Bayda et al., 2019). Nanoparticles exhibit significantly distinct characteristics in comparison to their larger body counterparts. The word “nanoparticles” refers to a wide spectrum of solid particulate dispersions with size ranging from 0.1 to 100 nm.

Nanoparticles occur naturally and can be synthesized by few commonly used methods like top-down and bottoms-up approaches, as shown in Fig. 22.1 (Nadeem et al., 2021). Specifically, in the top-down method, largely organic and inorganic particles are broken down into nanoparticles. And the bottoms-up method employs self-assembly and

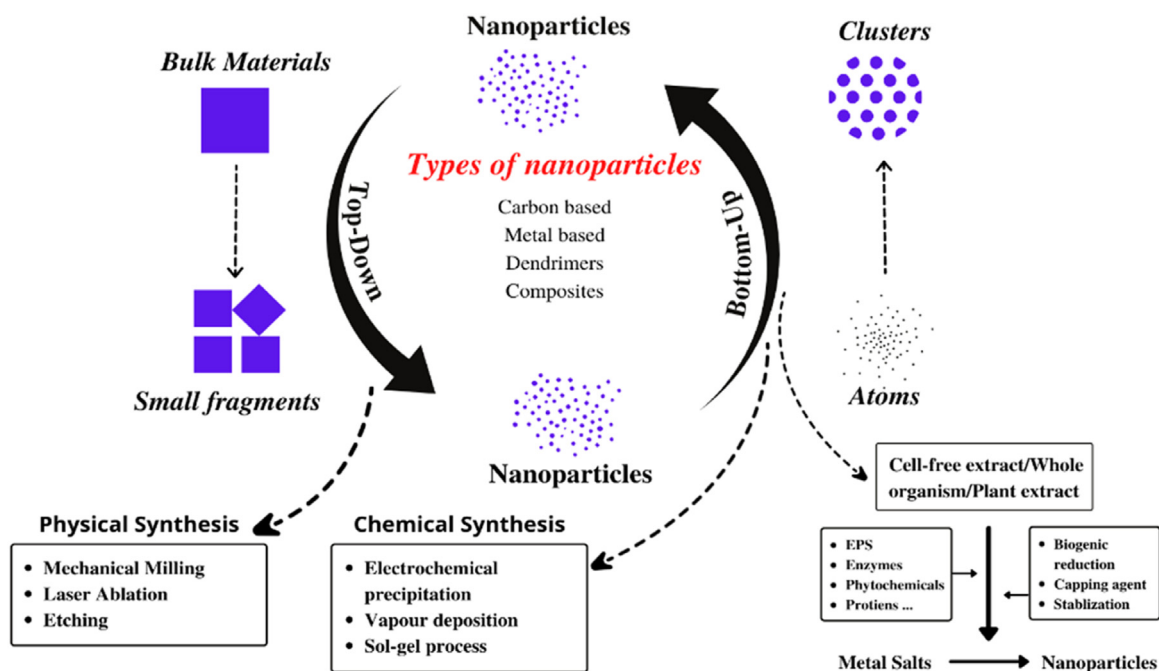


FIGURE 22.1 Various approaches for fabrication of metal nanoparticles.

supramolecular chemistry to generate nanoparticles from smaller molecules or atoms (Cao and Wang, 2011). In addition, the bottoms-up method includes chemical and biological processes.

The use of non-renewable, non-cost effective reducing/capping agents and other organic solvents in the chemical production of nanoparticles pose a greater risk to the whole bio-diverse and the earth's environment. In contrast, the biological approach of nanoparticle synthesis is developing as a newer and eco-friendly strategy (Sharma et al., 2019). Biological synthesis is a cost-effective method for metal reduction and nanoparticle stabilization that uses renewable resources (Thakkar et al., 2010; Kumari et al., 2021a). For example, microbes and microbial metabolites have been utilized to make a variety of metal nanoparticles, with bacteria, actinomycetes, fungus, and yeasts frequently used for colloidal-based nanoparticle production (Narayanan and Sakthivel, 2010).

Gold, Silver, Iron, Platinum, Palladium, titanium, and many other nanoparticles have been produced using microorganisms in the past. Among all other metals, silver nanoparticles (AgNPs) are being used in numerous applications, including biological labeling, catalysis, biosensors, optoelectronics, and photography because of their characteristic to produce comparatively stable nanoparticles (Abou El-Nour et al., 2010; Dhand et al., 2016; Kumari et al., 2021b). In addition, due to their remarkable antibacterial and catalytic properties, silver nanoparticles are frequently used compared to other types of metal nanoparticles (Manivasagan et al., 2014). However, the silver nanoparticles synthesized using the general methods mentioned above are not stable enough. Carbohydrate polymers like exopolysaccharides (EPS), derived from various sources, as shown in Fig. 22.2, can be employed to reduce and stabilize synthesized nanoparticles (Emam and Ahmed, 2016).

Enzymes involved in the synthesis of EPSs can be divided into four major groups:

- In the internal carbohydrate metabolism.
- In the production and conversion of nucleotide sugars.
- Glycosyltransferases.
- In the polymerization and translocation of carbohydrates.

EPS is well known among other stabilizing agents due to its property as a metal reductant and its ability to prevent aggregation. Microbial EPS plays a prominent role in biofilm formation, attachment to cell surfaces, predator defense, and protection from unfavorable environmental circumstances, as mentioned in Fig. 22.3 (Emam and Ahmed, 2016). The evolution of extremophilic organisms has led to the development of their adaptation strategies like cell adhesion, the concentration of nutrients, retention of water, and so on, which is possible due to the synthesis of novel EPS. These organisms have the potential to be biotechnologically useful. For instance, the *Halomonas maura* has shown the ability to produce an EPS called muran that can stabilize gold nanoparticles (Arias et al., 2003; Poli et al., 2010). Recently, a *Pseudomonas* sp. having the potential to produce EPS was reported with good metal removal and reduction property in an Arctic soil sample (Ali et al., 2020). However, the current knowledge on the structural and functional properties of EPS from alkaliphiles is limited, and

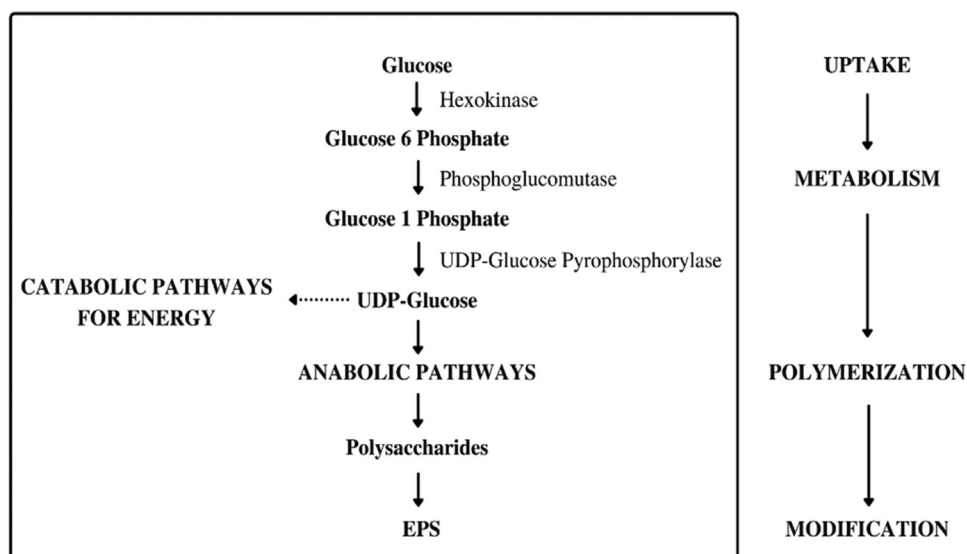


FIGURE 22.2 General mechanism of bacterial EPS production. EPS, exopolysaccharides.

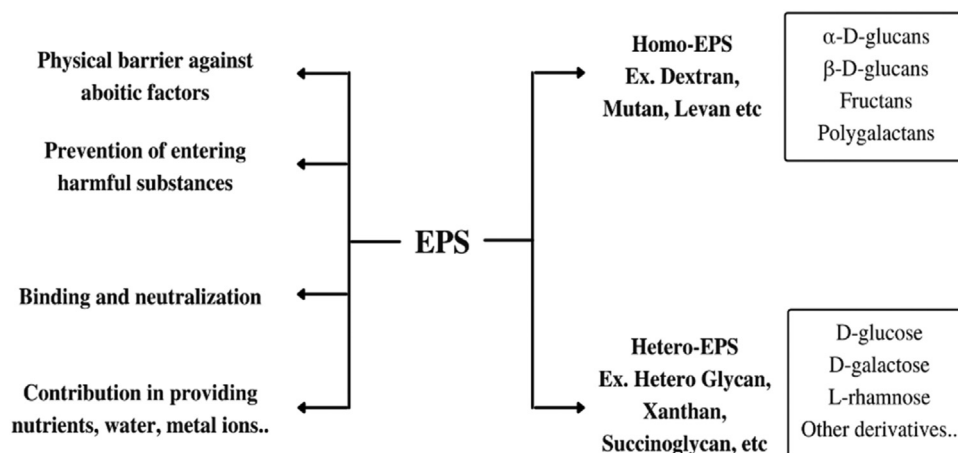


FIGURE 22.3 Types and applications of microbial EPS. EPS, exopolysaccharides.

so is their application. The most often used carbon supplies for large-scale EPS manufacturing are conventional sugar derivatives. Low-cost carbon sources, such as agro/food waste, palm sugars, and plant sprouts, have currently been identified as cost-effective substrates for the production of EPS (Sathiyarayanan et al., 2013).

Capsular and slimy polysaccharides are the two primary types of bacterial EPS. Capsular polysaccharides are firmly attached to the bacterial cell surface by ionic or covalent contacts, making it difficult to extract the produced EPS and use it for further applications. On the other hand, slime EPS is loosely connected and is periodically discharged into the surrounding medium (Moradali and Rehm, 2020).

The film-forming properties of these exopolysaccharides can be wisely applied to store various metal nanoparticles for a longer period without any cluster formation. As a result, they can easily replace the chemical reducing agents such as acid citrate, sodium borohydride, trisodium citrate, sodium hydroxide, and other organic compounds (Jian et al., 2016). Few popular EPSs such as bacterial alginates, levan, dextran, gellan, xanthan, curdlan, and many more marketed or used in various fields such as industrial, biomedical, and biotechnological applications (More et al., 2014).

22.1.1 Preparation of metal nanoparticles using polysaccharides and exopolysaccharides

Metallic nanoparticles that are produced biologically have a number of advantages compared to the physical and chemical methods but still includes a longer reduction time, the formation of aggregates, and more sophisticated downstream procedures. To address these issues, researchers are looking at using various EPS as reducing and capping agents in the synthesis of various metal nanoparticles (Vaseghi et al., 2018).

Polysaccharides and exopolysaccharides mainly differ in their composition like “polysaccharides are made up of carbohydrates, for example, starch a polymer of D-glucose; alginate is composed of β -D-mannuronate and α -L-guluronate; carboxymethyl cellulose is a polymer of D-glucose with carboxymethyl groups bonded to the hydroxyl groups; the pectin is mainly composed by galacturonic acid, linked to other components like acetic acid, D-apiose, glucuronic acid, etc.” Mainly hydroxyl and hemiacetal groups of these polysaccharides get involved in reducing metal precursor salts during the synthesis process (Park et al., 2011). During the synthesis of metallic nanoparticles, alkaline conditions are generally maintained to maintain pH for enhanced nanoparticle production and the availability of reducing sugars by hydrolyzing the given polysaccharides (Ahmed et al., 2016).

In comparison exopolysaccharides, primarily are made up of carbohydrates along with organic and inorganic substituents. Few cyanobacteria produce EPS that are primarily heteropolysaccharides with ‘S’ functional groups attached to the sugar backbone (Tiwari et al., 2020), similar to a few cases of *Bacillus* strains (Manca et al., 1996; Pereira et al., 2009). In few reports, the introduction of N acetylamino sugars in the reducing end of EPS makes them capable of complexing and stabilizing much better metallic nanoparticles (Nadkarni et al., 1994; Singh et al., 2011).

For example, EPS have been discovered to influence the surface stability of metal nanoparticles as well as the suppression of aggregation formation in colloidal solution that are produced by Gram-positive or Gram-negative bacteria (Raveendran et al., 2013) mainly because of hydrogen bonds present on the surface of EPS.

Zhang and Edgar (2014), studied about the bacterial curdlan which is a neutral and water-soluble EPS mainly produced by *Agrobacterium* and *Rhizobium* sp. Carboxylated and oxidized bacterial curdlan were used extensively in

formation of stable nanoparticles where the negatively charged hydroxyl and carboxylic groups involves in reduction of silver ions with a very small sized AgNPs were synthesized. According to [Dhar et al. \(2012\)](#), one- step magnetic AgNPs were produced using gellan and xantham gum with greater stability at high temperature of about 800°C and at varying pH.

Not only the bacterial EPS, plant and animal derived EPS were also been used in fabrication of metal nanoparticles ([Table 22.1](#)). For example, [Venkatesham et al. \(2014\)](#), used chitosan derivatives like methyl cellulose, hydroxyl cellulose, carboxymethyl cellulose and so on as green stabilizers and reducing agents during production of various nanoparticles. A plant cell wall containing heteropolysaccharide pectin was also used to fabricate a variety of nanoparticles includes silver, lead and gold nanoparticles with enhanced catalytic, antimicrobial and anticancerous activities ([Krishna Rao et al., 2015](#)).

The characterization of various metal nanoparticles fabricated using EPS as a template was studied using microscopic and spectroscopic techniques includes Fourier transform infrared spectroscopy (FTIR) where the absorption frequencies of the functional groups are detected that are transferred to metal nanoparticles from EPS used during reduction process ([Vignesh et al., 2015](#)). And also, Zeta potential analyzer can be used to measure the net charge of EPS stabilised metal nanoparticles mostly as negatively charged species due to the presence of polyanionic groups on EPS template during reduction of metal ions ([Sudheer Khan et al., 2011](#)). The other techniques include Transmission electron microscopy (TEM), Atomic force microscope (AFM) and Scanning electron microscope (SEM) are widely used to measure the size and 3D structure of various metal nanoparticles. Few studies shown that along with SEM, energy dispersive X-ray spectroscopy (EDX or EDS) were also used to check the elements quantitatively on the metal surface area. The degree of crystallinity of metal nanoparticles was also studied using X-ray powder diffraction (XRD).

22.1.2 Mechanism of exopolysaccharide-mediated reduction and stabilization of green nanoparticles

As exopolysaccharides act as eco-friendly components due to their properties like biocompatible, non-toxic, renewable, and contain functional groups that allow functionalization, they are extensively used as colloidal stabilizers as they allow surface modification during synthesis of metal nanoparticles ([Escárcega-González et al., 2018](#)).

The various functional groups of EPS containing reducing sugars get involved in chelation, reduction and finally stabilize the metal ions once they are in contact with each other ([Sathiyarayanan et al., 2017](#)). The well-known polyanionic groups can synthesize and stabilize the metal nanoparticles. An advantage during the synthesis of metal nanoparticles is mainly the electrostatic interactions between an anionic group and a metal cationic ion involving the end groups like hydroxyl, carboxyl, phosphoric, hemiacetal, and amino in reducing the respective metal ions ([Nadkarni et al., 1994](#); [Raveendran et al., 2014](#)).

Oxidation of various groups like hydroxyl, alcohol, and aldehyde to carbonyl and carboxylic groups were respectively a significant component during the reduction process of metal salts into nanoparticles. In [Fig. 22.4](#), it is showed that the film produced by the EPS matrix acts as a capping and stabilizer after the nanoparticles are created, which in the end produces metal nanoparticles with a small surface area distribution, preventing agglomeration ([Shah et al., 2015](#)). The adsorption of EPS on the nanoparticle surface promotes steric repulsion, which prevents the nanoparticles from aggregating ([Pooja et al., 2014](#)).

TABLE 22.1 List of few plants and animal-based EPS used for fabrication of various metal nanoparticles.

Exopolysaccharide (EPS)	Metal nanoparticles	References
Starch	AgNPs, AuNPs	Manno et al. (2008) , Engelbrekt et al. (2009)
Cellulose	AgNPs, FeONPs	Yu et al. (2013) , Cheng et al. (2013)
Pectin	AgNPs, AuNPs, PbNPs	Balachandran et al. (2015) , Khazaei et al. (2013) , Moharana et al. (2014)
Xylan	AgNPs	Harish et al. (2015)
Chitosan	AgNPs, AuNPs, CuNPs	Tran et al. (2010) , Chen et al. (2013)
Heparin	AgNPs, AuNPs	Kemp et al. (2009a) , Kim et al. (2013)
Agar	AgNPs, AuNPs	Shukla et al. (2012) , Rhim and Kanmani (2015)

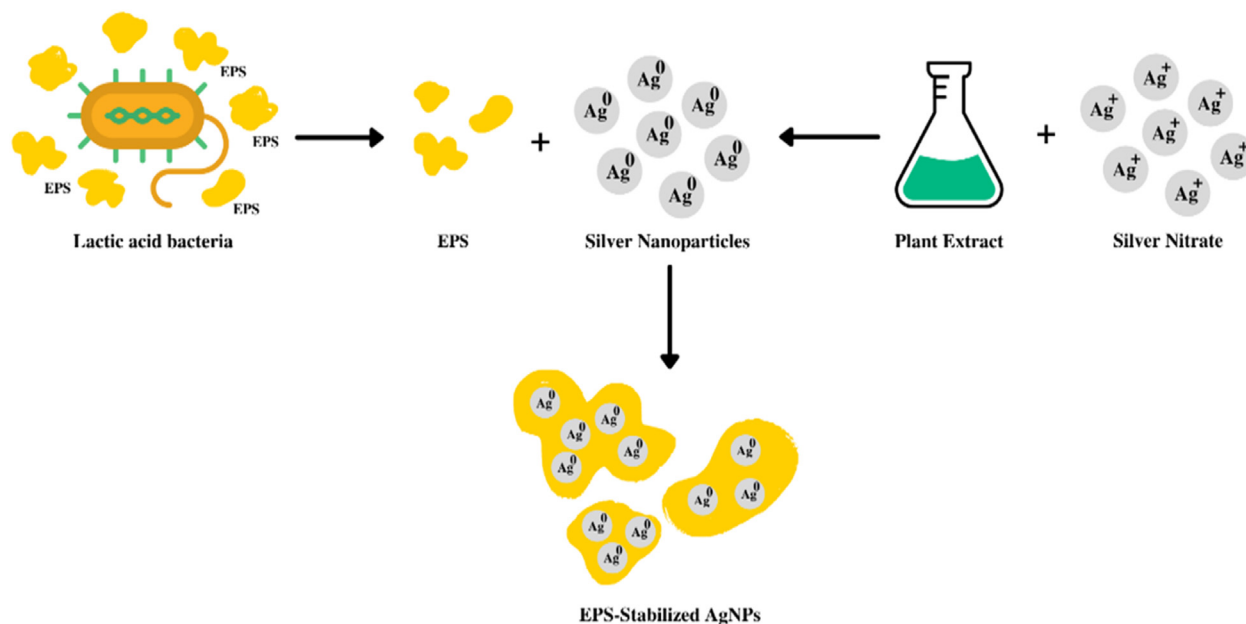


FIGURE 22.4 Green synthesis of EPS stabilized AgNPs. EPS, exopolysaccharide.

The EPS that act as capping agents impact metal nanoparticle properties, including surface charge and colloidal stability (Jain et al., 2015). The helical structure of xanthan gum, one of the EPS, with numerous hydroxyl groups, is capable of metal ion reduction. Furthermore, through hydrogen bonding, this structure generates a network for the nanoparticles to be stabilized. In addition, because of the acetyl groups and pyruvic acid connected to mannose, xanthan gum contains a negative charge that is transferred to the nanoparticle surface, creating a steric repulsion among them (Xu et al., 2014).

Another example is dextran, a glucose polymer produced by *Weissella confusa* and other LAB (Díaz-Montes, 2021). Combined with *Aloe Vera*, dextran is used as a good reducing and stabilizing agent for the green synthesis of AgNPs (Chaitanya Kumari and Naga Padma, 2020) and as a chemical sensor for cysteine (Davidović et al., 2017). Dextran helps to produce metal nanoparticles due to the interaction of its various functional groups, including hydroxyl, ketone, aldehydes, and carboxyl groups, allowing it to reduce Ag⁺ ions, which results in the production AgNPs while avoiding agglomeration (Cakić et al., 2016).

22.1.3 Applications of exopolysaccharide stabilized green silver nanoparticle in dye degradation

Increasing globalization, urbanization, and industrialization have led to a wide range of environmental pollution in recent decades. Textile dyeing is a major environmental problem. More specifically, the textile industry is one of the most chemical-intensive industries globally and a major polluter of water (Pandey et al., 2012). Dyes are chemicals used to color clothes, foods, and other products to add beauty and uniqueness to them. They adhere to textiles permanently and are resistant to water, sunlight, soap, acids, and alkalis due to the presence of chromophores and auxochromes in their structure. The fabric absorbs only 80% of the color during the dyeing process, with the remaining 20% in the water. As illustrated in Fig. 22.5, the release of such wastewater, which contains synthetic dye residues, tends to cause serious harm to aquatic biota, with direct consequences on photosynthesis and other natural processes. The toxicity of these compounds and their resistance to natural degradation result in permanent staining (Slama et al., 2021).

Dyes are grouped as azo, phthalein, triphenylmethane, indigoid, anthraquinone, and nitro based on the structural units that make them up. Azo dye is one of the most widely used dyes in the textile industry, yet it has a stronger tolerance to natural degradation than other colors. Because most azo dyes are water-soluble, they are easily absorbed by the body through inhalation, ingestion, and skin contact, causing cancer (Shah et al., 2015). Azo dye toxicity also poses a threat to aquatic creatures and has long-term negative consequences in the aquatic environment.

In general, azo dyes contain one to three azo bonds, connecting phenyl, naphthyl rings, which are generally substituted by certain functional groups, triazine amine, chlorine, hydroxyl, etc. (Bell et al., 2000). There are over 3000 azo

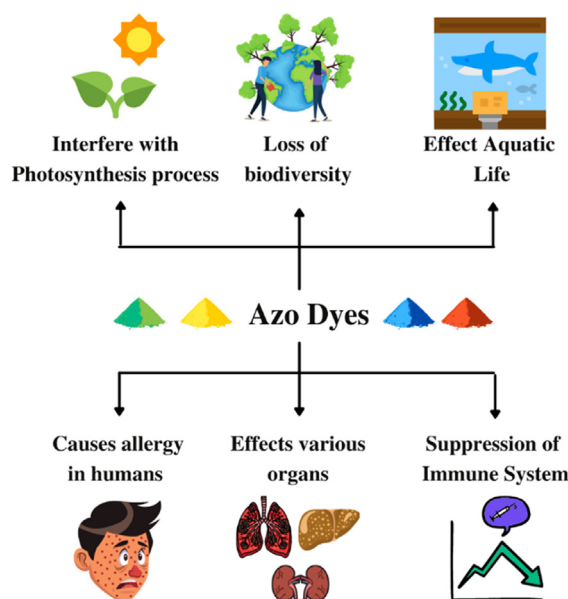


FIGURE 22.5 Toxicology of dyes on environment.

dyes, including Astrazon GTLN Red, Maxilon GRL Blue, and Sandolan Yellow, which are widely used in the textile, leather, and paper industries. About 80%–90% of the textile industry uses azo dyes.

Organic pollutants have a significant persistence, even at extremely low concentrations, making the water more hazardous to use similarly in the case of dyes that are not readily biodegradable. It is therefore important to remove or minimize dyes to an acceptable concentration before application. As an emerging technique, degradation induced by nanoparticles is one of the best techniques to detoxify azo dyes. Over the past few decades, various physical and chemical treatment techniques, including biological methods, have been employed to eliminate toxic dye color from the disposed water bodies (Levard et al., 2013; Kent et al., 2014; Zhang et al., 2018). To treat dye industry effluents, wastewater treatment typically includes various treatment steps. Physical treatment approaches like stripping, sedimentation, filtering, flotation, reverse osmosis, ion exchange, and adsorption (Kaur et al., 2018) removed both dissolved and undissolved organic pollutants without necessarily changing their chemical structures; however, the main disadvantage of this method is the sludge formation (Cáceres-Vélez et al., 2018). Chemical coagulation and flocculation lead to a larger accumulation of sludge material, hazardous to nature, and difficult to get rid of. One of the physical treatment approaches, i.e., adsorption, requires dye to be absorbed by absorbent and regenerated regularly, making it an expensive approach. Membrane technologies like ultrafiltration, nanofiltration and reverse osmosis have been used to complete water and recycle, but these processes have many operative problems with high capital costs (Zhao et al., 2018).

Physical and chemical procedures for breaking down colors are more expensive than biological processes. Biodegradation methods such as microbial degradation, adsorption by microbial biomass, and bioremediation systems are often used in industrial wastewater treatment because microorganisms can accumulate and breakdown a variety of pollutants (McMullan et al., 2001; Fu and Viraraghavan, 2001; Shah et al., 2019). However, technological constraints, such as the necessity for a large work area and less design and operating flexibility, limit their application (Robinson et al., 2001). While many organic molecules are broken down, many others are resistant due to their structure's intricacy and their synthetic organic or inorganic nature (Kumar et al., 1998). Due to their xenobiotic nature, azo dyes are not completely broken down.

The method of reducing organic dyes uses the ability to donate electrons to nanoparticles of transition metals such as silver, gold, iron, nickel, palladium, and platinum in the presence of reducing agents such as LiAlH_4 , NaBH_4 , or H_2O_2 . Due to the presence of properties like minute dimensions with high surface area to volume ratio, high dispersity and the ability to transfer electrons between electron relay systems donors and acceptors made AgNPs to act as good catalytic agents (Ghosh et al., 2002; Pal et al., 1998). As a result, biogenic silver nanocatalysts made using various microbial EPS are widely used to remove dye contaminants effectively due to their higher stability.

In previous investigations, the degradation efficiency of EPS stabilised AgNPs, characterised by UV-Vis spectroscopy, was studied. Initially, during the process, the concentration of the EPS-mediated AgNPs was changed by keeping other parameters such as pH, incubation time, and aeration constant, mainly to determine the amount of the

EPS-mediated AgNPs on the rate of degradation. It was observed that the rate of degradation increases significantly with the amount of EPS-mediated AgNPs up to a certain degree of saturation. A further increase showed no influence on the degradation process in the 4 hours. As a result, the breakdown of azo dyes using AgNPs that are stabilised by EPS were comparatively showed good results than using bacterial EPS produced by various LAB group of bacteria and others (Chen et al., 2016; Ganesan et al., 2017).

A higher rate of degradation of these EPS stabilized AgNPs may be due to the adsorption of azo dyes on the large area of EPS, which leads to weakening of the azo double bond by conjugation in turn by binding with oxygen and sulfur atoms of the dye. And oxygen atoms in EPS could contribute to the number of AgNPs. Finally, EPS networks containing more heteroatoms should show hydrophilic interactions with azo dyes that help get the dye molecules close to the catalytic centers. Accordingly, these EPS stabilized AgNPs can be used as an environmentally friendly and inexpensive strategy to degrade harmful dyes with potential applications in the textile industry.

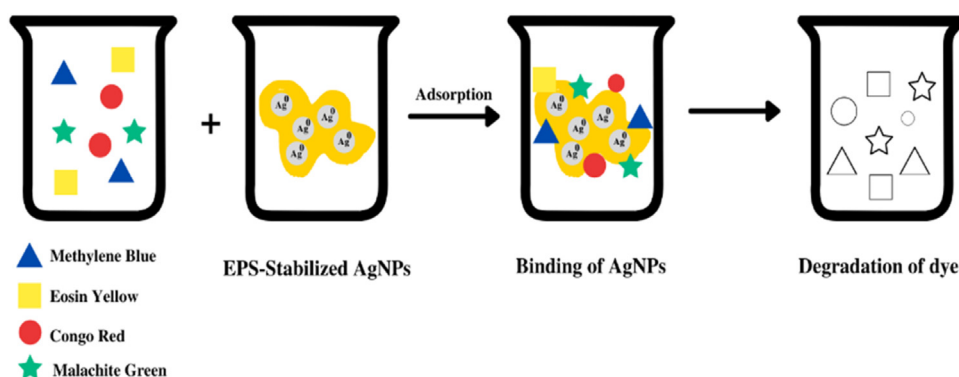


FIGURE 22.6 Degradation of Azo dye by EPS stabilized AgNPs. EPS, exopolysaccharide; AgNPs, green silver nanoparticles.

TABLE 22.2 List of few bacterial EPS (exopolysaccharide) in synthesis of metal nanoparticles and their applications in textile industry.

Bacterial EPS	Bacteria	Application	Metal nanoparticles	References
Curdlan	<i>Alkaligenes faecalis</i> <i>Agrobacterium</i> sp. <i>Rhizobium</i> sp.	Degradation of Sudan, Synazol red	AuNPs, AgNPs	Dhar et al. (2012), Leung et al. (2010)
Dextran	<i>Leuconostoc mesenteroids</i> <i>W.Confusa</i> <i>Streptococcus</i> sp.	Degradation of Coomassie brilliant blue, Azo dye	AuNPs, AgNPs, CuNPs, FeONPs	Raveendran et al. (2014)
Levan	<i>Acetobacter xylinum</i> , <i>Aerobacter</i> , <i>Bacillus</i> , <i>Pseudomonas</i> , <i>Zymomonas</i>	Degradation of Azo dye	AgNPs, AuNPs	Ahmed et al. (2016)
Pullula	<i>Aureobasidium pullulans</i>	Degradation of Erichrome, Black direct red 80 (polyazo dye)	AgNPs	
Muran	<i>Halomonas maura</i>	Degradation of Acid orange 7	AuNPs	Raveendran et al. (2014)
Xanthan	<i>Xanthomonas campestris</i>	Degradation of Direct blue, direct red 81, direct black 22	AuNPs	Pooja et al. (2014)
Gellan gum	<i>Sphingomonas</i> sp.	Degradation of Metanil yellow	AuNPs, AgNPs	Dhar et al. (2012), Ahmed et al. (2016)
Fuco pol	<i>Enterobacter</i> sp.	Degradation of Azo dye	AuNPs	

22.1.4 Mechanism of dye degradation using exopolysaccharide stabilized green silver nanoparticles

During metal degradation, the catalytic process takes place on the metal surface, resulting in a massive increase in surface availability and, as a result, an increase in catalyst efficiency (Geeta and Anirban, 2019). The reducing agent and dye molecules can be adsorbed on the AgNPs' large surface area without altering their activity, as demonstrated in Fig. 22.6. When the reducing agent (EPS) is adsorbed on AgNPs, its reduction potential is reduced. However, when dye molecules adsorb on nanoparticles, their reduction potential increases, and when both species adsorb on nanoparticles, the dye molecules' reduction potential increases (Table 22.2). During the electron transfer reaction between the reducing agent and the azo dye via nanoparticles, the dye's structure is damaged, resulting in small species such as CO₂, H₂O, which are less hazardous than the organic pollutant (Pradhan et al., 2002).

22.2 Conclusions

The unique features of metal nanoparticles compared to their bulk have become fascinating towards research in this scope. In particular, green synthesis involving a biological compound, like bacterial EPS, performs chelation and stabilizes reduced metal ions due to their chemical composition. Utilizing a few bacterial EPS permits the synthesis of metal nanoparticles, with a wide assortment of utilizations, in an eco-accommodating and non-toxic way, giving intriguing qualities through the functionalization of these nanomaterials. But more to be focussed on toxicology and environmental studies to evaluate the effect of these synthesized nanoparticles on the environment.

Until now, the green synthesis of metal nanoparticles using EPS is still at the laboratory level, which significantly needs to increase production at a large scale. The synthesis of nanoparticles still needs to focus on the cost-effective production of EPS at the industrial level. As a result, an effective fermentation strategy that is economic and viable ventures need to be explored.

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