Intracellular and Extracellular Microbial Enzymes and Their Role in Nanoparticle Synthesis

3

Aarif Hussain Shah and Mushtaq Ahmad Rather

Contents

Abstract

The synthesis of nanoparticles using green nanotechnology is fast emerging as a cleaner, economical, eco-friendly, stable, non-toxic, and biocompatible method when compared to conventional physical and chemical methods. Green and biosynthesized nanoparticles are globally being used in the areas of food industries, pharmaceuticals, personal-care products sector, biomedical engineering, and microbial nanotechnology. Plant extracts and micro-organisms like bacteria, yeast, algae, fungi, and cyanobacteria are most versatile "nanobiofactories" that have been studied for the synthesis of metallic nanoparticles. Micro-organisms use intracellular and/or extracellular mechanisms

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A. H. Shah (✉) · M. A. Rather
Department of Chemical Engineering, National Institute of Technology (NIT), Srinagar, Jammu and Kashmir, India
e-mail: aarif_shah@nitsri.ac.in

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M. A. Ansari, S. Rehman (eds.), *Microbial Nanotechnology: Green Synthesis and Applications*, https://doi.org/10.1007/978-981-16-1923-6_3

to synthesize nanoparticles. Nanoparticles synthesized using green pathways can be used for the treatment of wastewater containing dyes, pesticides, pharmaceutical residues, and heavy metals. The surface properties like particle size, shape, and monodispersity might be controlled by studying the effect of various parameters like type of organism/plant extract, growth medium, pH, source of intending nanoparticles, temperature, time, and presence of other ions. By optimizing these parameters, the green synthesis of nanoparticles would offer a great advantage over physical and chemical methods.

Keywords

Nanoparticles · Green synthesis · Extracellular and intracellular synthesis · Natural extracts · Anti-microbial activity · Microbial enzymes

3.1 Introduction

Nanotechnology is emerging as one of the most promising technologies in various arenas of science and technology including research and development. Green nanotechnology based nanoparticles have globally emerged as potent tools in the areas of food sector, pharmaceutical industries, cosmetics, biotechnology, and biomedical engineering. The global production of metallic nanoparticles is expected to cross 50,000 million US dollars by 2026 (Ovais et al. 2018a).

The conventional physical and chemical methods used for the synthesis of nanoparticles pose a serious threat to the environment and human health. Besides, the nanoparticles synthesized by these methods are expensive and hazardous for biomedical applications as they lack stability and biocompatibility (Patra et al. 2015; Salunke et al. 2014). The physical and chemical methods are operated under extreme conditions of temperature and pressure. Thus the focus has shifted to the use of biological methods for fabrication of nanoparticles that are non-toxic, cheap, biocompatible, and eco-friendly in nature. The biosynthesis or green synthesis of nanoparticles involves the use of plant extracts (Jalal et al. 2016; Ali et al. 2020a, b; Almatroudi et al. 2020; Ansari et al. 2020a; Farouk et al. 2020; Lakshmeesha et al. 2020; Ansari and asiri 2021; Alomary and Ansari 2021) and micro-organisms like bacteria, cyanobacteria, fungi, yeast, algae, etc. (Hulkoti and Taranath 2014; Jalal et al. 2018; Shobha et al. 2020; Sumanth et al. 2020). Although using plant extracts for green synthesis of nanoparticles is an economical and relatively simpler mode of synthesis, however, it generates polydispersed nanoparticles. The microbial synthesis of nanoparticles is fast becoming an indispensable method of generating metallic nanoparticles as they are easy to cultivate and grow in varying parameters of pH, temperature, pressure, and growth media.

Microbial nanotechnology uses micro-organisms as emerging potential “Nano Bio-factories” for economical, eco-friendly, and biocompatible synthesis of nanoparticles. Thus biosynthesis of metallic nanoparticles is an important and emerging “green synthesis” technique that interlinks microbiology, biotechnology, and nanotechnology sciences. Many studies have been conducted that report synthesis of different types of nanoparticles without using capping agents and stabilizers. Micro-organisms use intracellular and/or extracellular mechanisms to synthesize nanoparticles. In intracellular synthesis mode, negatively charged cell wall of the micro-organism attracts the positively charged metal ions. The bacterial cell wall also contains enzymes that cause bioreduction of the metal ions to their corresponding nanoparticles (Chokriwal et al. 2014). On the contrary, the extracellular synthesis mechanism involves secretion of reductase enzymes by the micro-organism cells that cause bioreduction of metal ions to appropriate nanoparticles (Baker et al. 2013). Micro-organisms can survive and flourish in environments having high concentration of toxic metals, high temperature, and salinity due to their specialized detoxification machinery as well as efficient membrane transport and anti-transport proteins. Thus the biosynthesis of nanoparticles is the most reliant and acceptable route of green synthesis.

3.2 Bio-Synthesis of Nanoparticles and Enzymes Involved

Green synthesis of nanoparticles is an efficient, versatile, and low cost method as compared to traditional physical and chemical methods. Green nanoparticles synthesized using plant based products pose no serious stress to the environment due to lack of toxic metabolites and also the reaction is carried out at room temperature within few minutes. The synthesis can be scaled up easily and toxicity of such nanoparticle reduced to a great extent. There is no need of capping and stabilizing agents as the properties like shape, size, charge, etc. are self-controlled by these biomolecules, thus making them more effective than traditional non-biologically synthesized nanoparticles (Makarov et al. 2014; Mukherjee et al. 2012; Ovais et al. 2018b).

Bacteria cells are potent “nanofactories” that have been used for the synthesis of various metallic nanoparticles using both intracellular and extracellular routes. The extracellular route is preferred as there are no downstream processes required for isolation of the final product. Bacterial biomass, culture supernatant, cell-free extracts are used for extracellular synthesis of nanoparticles that have mostly biomedical applications. Bacterial strains like *Bacillus brevis, Pseudomonas stutzeri,* and *Phormidium fragile* have been used for the synthesis of silver nanoparticles (Klaus et al. 1999; Saravanan et al. 2018; Satapathy and Shukla 2017).

Pseudomonas aeruginosa, Bacillus marisflavi, and *Rhodopseudomonas capsulate* were used for the synthesis of gold nanoparticles. *Lyngbya majuscule* and *Rhodococcus sp.* have been used for intracellular synthesis of gold nanoparticles that have shown anti-myocardial infraction properties (Ahmad et al. 2003; Bakir et al. 2018; Nadaf and Kanase 2019). In another study, iron oxide nanoparticles having anticancer activity were produced using *Bacillus cereus* and anti-microbial CuO nanoparticles were synthesized using *Halomonas elongate.* Similarly, anti-microbial ZnO nanoparticles have been synthesized from bacterial strains of *Serratia ureilytica, Lactobacillus plantarum,* and *Aeromonas hydrophila* (Dhandapani et al.

2014; Jayaseelan et al. 2012; Rad et al. 2018; Selvarajan and Mohanasrinivasan 2013).

Marine micro-organisms synthesize nanoparticles through different routes and mechanisms. The biosynthesis pathways are grouped into two main categories: (a) intracellular synthesis and (b) extracellular synthesis. In intracellular synthesis, nanoparticles are manufactured inside the micro-organism cell and they then diffuse out of the cell wall. On the contrary, extracellular synthesis involves various cellular constituents like proteins, peptides, amino acids, and enzymes that play role in the synthesis of nanoparticles (Mohanpuria et al. 2008; Sathiyanarayanan et al. 2017).

3.2.1 Intracellular Synthesis

Many hypotheses have been put forward to explain the mechanism of intracellular synthesis of different nanoparticles. However, the exact mechanism is not clear as different micro-organisms and biomolecules are used in the synthesis. The positively charged metal ions get trapped on the negatively charged cell wall or cytoplasmic enzymes and proteins. The trapped metal cations undergo reduction and form nanoparticles of various shapes and sizes inside the cell (Golinska et al. 2014; Manivasagan et al. 2016). Thus, in intracellular synthesis of nanoparticles, enzyme, and biomolecules mediated bioreduction of metal ions occurs inside micro-organism cells as shown in Fig. 3.1.

Gold nanoparticles were synthesized using alkaline microbes *Rhodococcus* sp. and *Thermomonospora* sp. Au nanoparticles of uniform size were produced by using solution of $HAuCl_4$ and mediated by cytoplasmic and mycelia surface enzymes. Higher amount of Au nanoparticles were synthesized on the membrane of cytoplasm than the mycelia surface, indicating the role of cytoplasmic membrane enzymes (Ahmad et al. 2003; Ovais et al. 2018a). In another study, algal biomass of *Tetraselmis kochinensis* was treated with $HAuCl_4$ solution and it showed higher amounts of Au nanoparticles on the cell wall, suggesting the role of cell wall enzymes. Lower levels of Au nanoparticles were observed on cytoplasmic membrane (Senapati et al. 2012).

Fungal biomass of *Verticillium* sp. was treated with silver ion solution and synthesis of Ag nanoparticles was observed under cell wall surface using electron microscope, indicating the role of cell wall enzymes in the intracellular reduction (Mukherjee et al. 2001). In another study, Au nanoparticles were synthesized inside the bacterial periplasmic space after treating the cells of *Pseudomonas stutzeri* (AG259) with $AgNO_3$ solution (Klaus et al. 1999). Au nanoparticles having size ranging from 10 to 100 nm were synthesized when *Phanerochaete chrysosporium* were treated with Au^{3+} solution. Extracellular reduction was achieved using enzyme laccase, while enzyme ligninase was found to be agent for intracellular reduction (Sanghi et al. 2011). Micro-organisms synthesizing nanoparticles through intracellular route are listed in Table 3.1 (Augustine and Hasan 2020; Chokriwal et al. 2014; Ovais et al. 2018a; Fang et al. 2019).

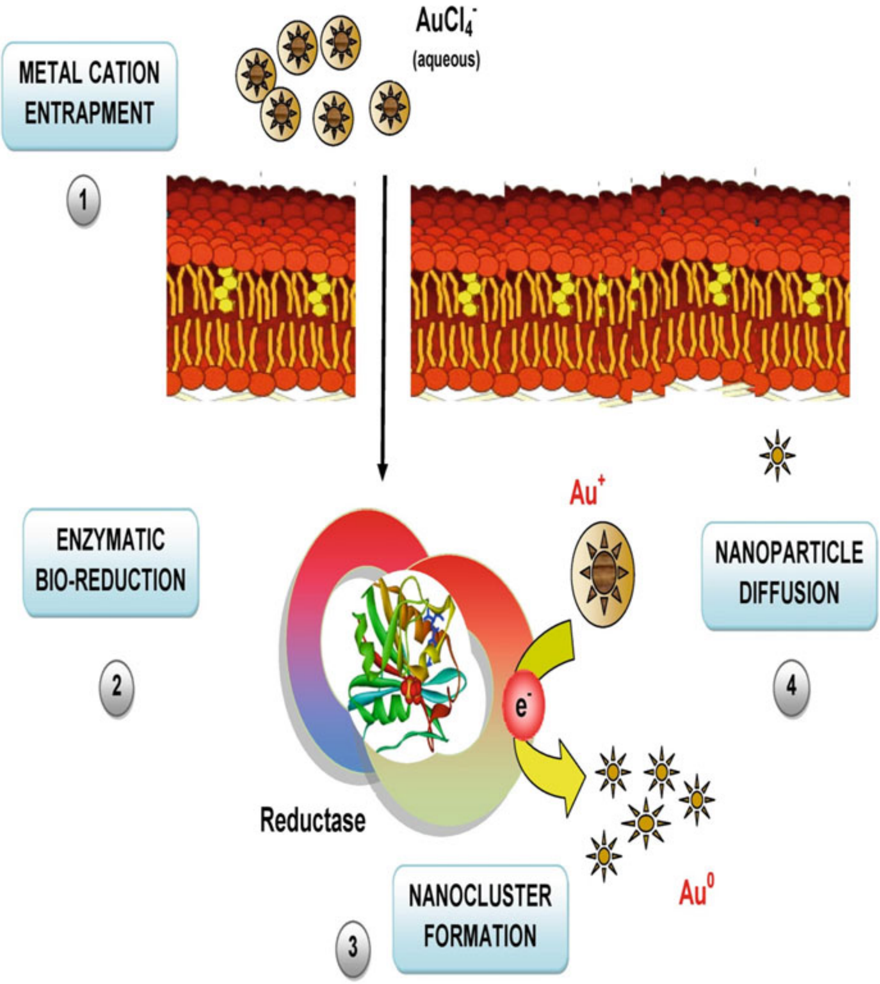

Fig. 3.1 Intracellular mechanism for biosynthesis of nanoparticles

3.2.2 Extracellular Synthesis

Extracellular synthesis of nanoparticles is mediated by surface proteins and enzymes that act as reducing agents. Nicotinamide adenine dinucleotide (NADH) and nicotinamide adenine dinucleotide phosphate (NADPH) act as cofactors in the process (Bose and Chatterjee 2016). Gold nanoparticles have been extracellularly synthesized using bacterium *Rhodopseudomonas capsulata*, mediated through NADH and NADPH-dependent enzymes that transfer electrons to gold ions (Au^{3+}) and form Au nanoparticles (He et al. 2007). Enzyme nitrate reductase (α-NADPH dependent) acts as an electron carrier and carries it from NADH to reduce $AgNO_3$ for synthesizing silver nanoparticles using fungus *Fusarium*

Table 3.1 Intracellular enzymes used for the biosynthesis of nanoparticles

Species	Enzyme source	Nanoparticle	Size (nm)
Rhodococcus sp.	Actinobacteria	Gold	5–15
Plectonema boryanum UTEX 485	Cyanobacteria	Silver	10
Idiomarina sp. PR58–8	Bacteria	Silver	26
Bacillus subtilis 168	Bacteria	Gold	5–25
Escherichia coli DH5α	Bacteria	Gold	113
Magnetospirillum magnetotacticum	Bacteria	Iron (II, III) oxide	47.1
Desulfosporosinus sp.	Bacteria	Uranium dioxide	1.5–2.5
Clostridium thermoaceticum	Bacteria	Cadmium sulfide	NR
Klebsiella pneumonia	Bacteria	Cadmium sulfide	5–200
Desulfovibrio desulfuricans NCIMB 8307	Bacteria	Palladium	50
Pseudomonas stutzeri AG259	Bacteria	Silver	<200
Lactobacillus sp.	Bacteria	Gold	20–50
Enterococcus faecalis	Bacteria	Palladium	<10
Shewanella sp. KR-12	Bacteria	Lead	3–8
Serratia marcescens	Bacteria	Bismuth	<150
Citrobacter freundii Y9	Bacteria	Selenium	500–600
Halococcus salifodinae BK3	Bacteria	Tellurium	10–40
Ochrobactrum anthropi	Bacteria	Silver	38–85
Vibrio alginolyticus	Bacteria	Silver	50–100
Shewanella oneidensis MR-1	Bacteria	Cadmium selenide	3–4
Desulforibrio caledoiensis	Bacteria	Cadmium sulfide	40–50
Magnetospirillum magneticum	Bacteria	Iron (II, III) oxide	10–60
Shewanella algae	Bacteria	Platinum	5
Bacillus cereus	Bacteria	Silver	4–5
Neurospora crassa	Fungi	Gold	32
Aspergillus sydowii	Fungi	Gold	8–15
Candida utilis	Fungi	Gold	NR
Aspergillus terreus	Fungi	Gold	NR
Penicillium sp. 1–208	Fungi	Gold	50
Verticillium (AAT-TS-4)	Fungi	Silver	13–37
Rhodosporidium diobovatum	Yeast	Lead sulfide	2–5
Yarrowia lipolytica CIM3589	Yeast	Gold	NR
Chlorella vulgaris	Microalgae	Gold	40–60
Euglena gracilis	Microalgae	Ferrihydrite	0.6–1
Euglena intermedia	Microalgae	Silver	6–24
Euglena gracilis	Microalgae	Silver	6–24
Scenedesmus sp. IMMTCC-25	Microalgae	Silver	5–10
Plectonema boryanum	Algae	Silver	10–25

NR not reported

oxysporum (Kumar et al. 2007a, b). *F. oxysporum* has also been used for synthesis of silver nanoparticles using nitrate reductase in the presence of NADPH, phytochelatin, and 4-hydroxyquinoline as an electron carrier. The results showed

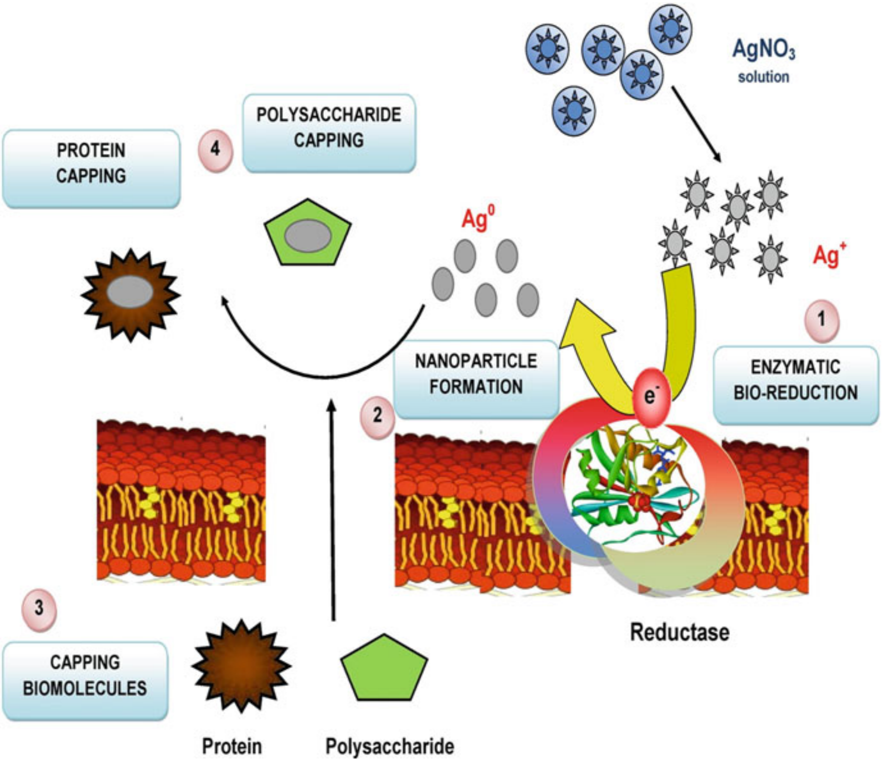

Fig. 3.2 Extracellular mechanism for biosynthesis of nanoparticles

excellent extracellular yield of Ag nanoparticles in oxygen free environment (Karbasian et al. 2008). Cadmium sulfide (CdS) and cadmium selenide (CdSe) luminescent nanoparticles have also been synthesized using reductase enzymes of *F. oxysporum* (Ahmad et al. 2002; Kumar et al. 2007a, b).

Various fungal species like *Fusarium semitectum*, *Fusarium solani, Aspergillus fumigates, Penicillium fellutanum*, *Cladosporium cladosporioides,* and *Coriolus versicolor* have been investigated for the extracellular synthesis of silver nanoparticles. *F. semitectum* and *F. solani* studied revealed the role of fungal proteins in addition to the enzymes in reducing Ag^+ ions to form silver nanoparticles. Fungal proteins, organic acids, and polysaccharides were found to play role in the extracellular biosynthesis of functionalized Ag nanoparticles using *C. cladosporioides* and *C. versicolor*. The general schematic mechanism for the extracellular biosynthesis of nanoparticles is shown in Fig. 3.2. These biomolecules also governed the morphology and growth of the nanoparticles (Ingle et al. 2009; Balaji et al. 2009). Treatment of *Aspergillus fumigates* and *Aspergillus niger* with $AgNO_3$ solution produced Ag nanoparticles in less time as compared to the conventional physical and chemical methods (Ovais et al. 2018b). Other fungal extracellular enzymes that play an important role in the biosynthesis of metallic nanoparticles include esterase (acetyl xylan esterase), hydrolase (cellobiohydrolase D), and glucosidase enzymes.

A list of micro-organisms that use extracellular mode of biosynthesis of nanoparticles is shown in Table 3.2 (Augustine and Hasan 2020; Chokriwal et al. 2014; Ovais et al. 2018a, b; Fang et al. 2019).

3.3 Applications of Biosynthesized Nanoparticles

Nanoparticles synthesized using green pathways can be used for the treatment of wastewater containing dyes, pesticides, pharmaceutical residues, and heavy metals. These pollutants which are present in large quantities in wastewater, groundwater, and soil pose a serious threat to the environment. The major applications of biosynthesized nanoparticles are discussed in this section and shown in Fig. 3.3.

3.3.1 Anticancer Tools

Metallic nanoparticles like gold, iron oxide, and silver have been recently used in the diagnosis and treatment of cancer and related diseases (Akhtar et al. 2019; Baig et al. 2020; Shah and Rather 2018) . These nanoparticles are potent anticancer tools owing to their magnetic, optical, and non-toxic properties. Gold nanoparticles (11 nm size) synthesized using *Nocardiopsis* species, a marine Gram-positive actinobacteria, were tested for anticancer properties against HeLa cells (cervical cancer cell lines). The HeLa cells showed morphological changes and condensation of genetic material indicating apoptosis. Also the cytotoxic activity was found to increase by increasing the dosage of gold nanoparticles (Manivasagan et al. 2016). In another study, silver nanoparticles synthesized from *Nocardiopsis* species showed anticancer properties against HeLa cells with characteristic apoptosis features like destruction of cell membrane, cell shrinking, and shape deformation (Manivasagan et al. 2015).

Silver nanoparticles synthesized using marine *Escherichia coli* VM1species showed anticancer activity against HeLa as well as lung cancer cells (A549). It was also observed that increasing the concentration of silver nanoparticles decreased the cellular growth of both HeLa and A549 cells (Maharani et al. 2016). The cytotoxic potential of the nanoparticles is dependent on factors like dosage, time, size of nanoparticles, and nature of cancer cell lines (Augustine and Hasan 2020; Rehman et al. 2019c).

3.3.2 Anti-Microbial Activity

The anti-microbial activity of nanoparticles is governed by two important factors: (a) nature and (b) size of the nanoparticles (Rehman et al. 2019b, 2020; Ansari et al. 2020b; Singh et al. 2018). Emergence of multi-drug resistant microbes has become a serious threat to the human health. Use of nanoparticles is a potent tool to combat the

Table 3.2 Extracellular enzymes used for the biosynthesis of nanoparticles

Species	Enzyme source	Nanoparticle	Size (nm)
Streptomyces sp. Al-Dhabi-87	Actinobacteria	Silver	10–17
Desulforibrio caledoiensis	Bacteria	Zinc sulfide	30
Enterococcus sp.	Bacteria	Cadmium sulfide	50–180
Escherichia coli VM1	Bacteria	Silver	10–15
Marinobacter pelagius	Bacteria	Gold	2–6
Pseudomonas putida MVP2	Bacteria	Silver	6–16
Streptomyces sp. 09 PBT 005	Bacteria	Silver	198–595
Exiguobacterium sp. KNU1	Bacteria	Silver	4.4
Bacillus sp	Bacteria	Silver	140
Stenotrophomonas sp. BHU-S7	Bacteria	Silver	12
Streptomyces ghanaensis VITHM1	Bacteria	Silver	30–50
Saccharophagus degradans ATCC 43961	Bacteria	Manganese dioxide	34
Vibrio alginolyticus	Bacteria	Silver	50–100
Shewanella loihica PV-4	Bacteria	Copper	10–16
Enterococcus faecalis	Bacteria	Palladium	10
Enterobacter cloacae Z0206	Bacteria	Selenium	100–300
Pseudomonas putida	Bacteria	Selenium	100–500
Citrobacter freundii Y9	Bacteria	Selenium	400–600
Erwinia herbicola	Bacteria	Tin (IV) oxide	15–40
Idiomarina sp. PR58–8	Bacteria	Lead (IV) sulfide	6
Escherichia coli	Bacteria	Cadmium telluride	62
Shewanella oneidensis	Bacteria	Uranium (IV)	NR
Klebsiella pneumonia	Bacteria	Silver	5–32
Thermomonospora sp.	Bacteria	Gold	30–60
Rhodobacter sphaeroides	Bacteria	Zinc sulfide	8
Gluconoacetobacter xylinus	Bacteria	Cadmium sulfide	30
Actinobacter sp.	Bacteria	Magnetite	10–40
Sulfurospirillum barnesii	Bacteria	Tellurium	<50
Brevibacterium casei	Bacteria	Cobalt (II, III) oxide	5–7
Plectonema boryanum UTEX 485	Cyanobacteria	Silver	1–200
Spirulina platensis	Cyanobacteria	Silver	7–16
Spirulina platensis	Cyanobacteria	Gold	6–10
Spirulina platensis	Cyanobacteria	Bimetallic	17–25
Aspergillus sydowii	Fungi	Gold	8.7–15.6
Aspergillus terreus	Fungi	Silver	1–20
Xylaria acuta	Fungi	ZnO	34–55
Penicillium brevicompactum KCCM 60390	Fungi	Gold	20–60
Penicillium sp. 1–208	Fungi	Gold	50
Magnusiomyces ingens LH-F1	Yeast	Gold	10–80

(continued)

Table 3.2 (continued)

Species	Enzyme source	Nanoparticle	Size (nm)
Candida sp. VITDKGB	Yeast	Silver	87
Candida glabrata	Yeast	Silver	2–15
Chlorella vulgaris	Microalgae	Palladium	2–15
Chlorella pyrenoidosa	Microalgae	Silver	2–15
Euglena intermedia	Microalgae	Silver	6–24
Euglena gracilis	Microalgae	Silver	15–60
Nannochloropsis oculata	Microalgae	Manganese dioxide	NR
Scenedesmus sp. IMMTCC-25	Microalgae	Silver	15–20
Tetraselmis suecica	Microalgae	Gold	79

NR not reported.

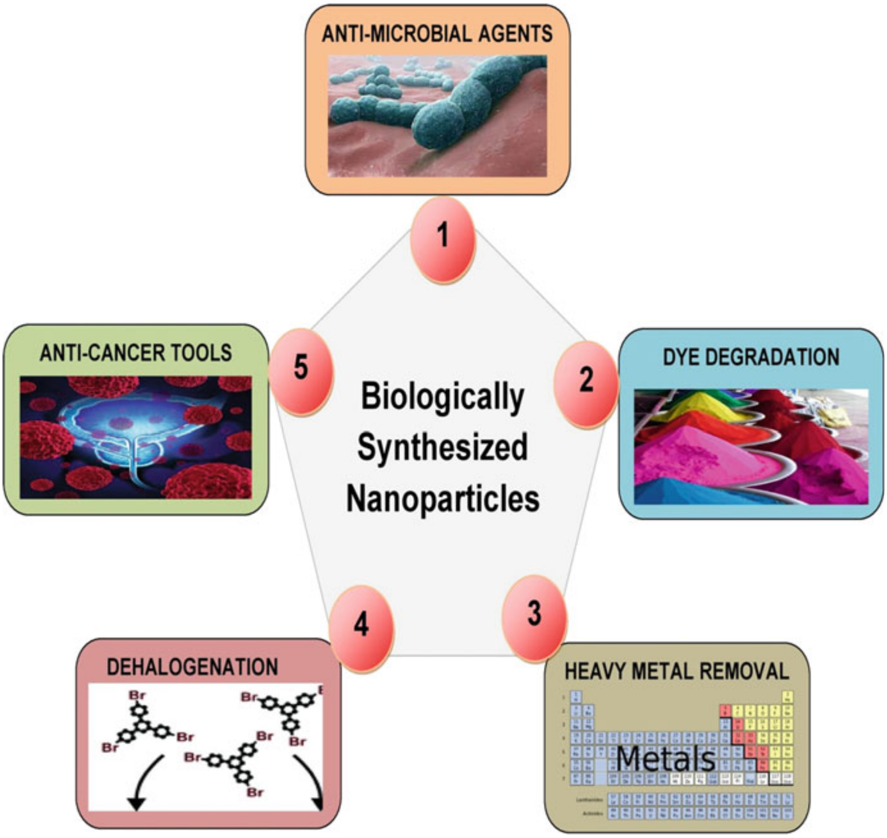

Fig. 3.3 Major applications of biosynthesized nanoparticles

Table 3.3 Mechanism of action of various metal-based nanoparticles against microbes

Nature of nanoparticles	Mode of action
TiO_2 based	• Generate highly reactive radicals like H_2O_2 and OH
Chitosan based	• Positively charged chitosan molecules bind DNA of bacteria and fungi and inhibit transcription and translation processes
Nitric oxide releasing	• Nitric oxide forms reactive intermediates that cause lipid peroxidation, inactivation of respiratory proteins and DNA strand breaks
Copper based	• Copper ions also produce reactive oxygen species at higher concentration and interact with amine and carboxyl groups
Silver based	• Ag^+ ions inhibit respiratory electron transport chain, damage DNA/RNA, inhibit DNA replication and cell division
Zinc oxide based	• Damage cell membrane lipids and proteins resulting in cell death. They also form reactive oxygen species that damage bacterial cells
Magnesium based	• Generate reactive oxygen species causing lipid peroxidation resulting in damage to cell membrane

development of microbial multi-drug resistance. These nanoparticles like metal-based nanoparticles, nitric oxide releasing nanoparticles, silver containing nanoparticles, chitosan based nanoparticles, use different mechanisms to fight microbial resistance. The microbial systems are unlikely to overcome these mechanisms as they lack such genetic machinery and mutations that could negate these nanoparticle operating mechanisms (Pelgrift and Friedman 2013). Different anti-microbial mechanisms of various nanoparticles are shown in Table 3.3. Silver (Ag) containing nanoparticles have been found to be the most effective agents against bacteria, fungi, and viruses. Ag nanoparticles disrupt the cell membrane of bacterial cells and interfere with the functioning of enzymes by binding with their disulfide and sulfhydryl containing amino acids, leading ultimately to cell death (Egger et al. 2009). Gold (Au) nanoparticles also act as effective anti-microbial agents but their activity does not involve generation of reactive oxygen species (Cui et al. 2012).

Effect of particle size on the anti-microbial activity of oxides of zinc (ZnO), copper (CuO), and iron (Fe_2O_3) nanoparticles was studied by Azam et al. (2012). The strongest activity was exhibited by ZnO nanoparticles (~19 nm size), while Fe_2O_3 nanoparticles (~35 nm size) showed weakest antibacterial property. Green synthesized nanoparticles have shown higher anti-microbial activity than chemically synthesized nanoparticles as the parent compounds involved in their synthesis have medicinal properties like plant extracts of tulsi plant (*Ocimum sanctum*) and neem tree (*Azadirachta indica*) leaves (Verma and Mehta 2016). Silver nanoparticles synthesized from marine pathogen *Streptomyces* sp. Al-Dhabi-87 have shown antibacterial activity against multi-drug resistant bacteria *Staphylococcus aureus* and *Escherichia coli* (Al-Dhabi et al. 2018).

Silver nanoparticles synthesized using *Nocardiopsis* sp. MBRC-1 were found to have dose-dependent antifungal activity against *Aspergillus niger, Aspergillus brasiliensis, Aspergillus fumigates,* and *Candida albicans* (Manivasagan et al.

2013). Cadmium sulfide nanoparticles synthesized from marine bacteria were also reported to show antifungal activity against *Aspergillus niger* and *Aspergillus flavus* (Rajeshkumar et al. 2014).

3.3.3 Degradation of Dyes

Dyes are used in food sector, paper mills, leather industries, printing press, textile industries, and pharmaceutical manufacturing, resulting in severe water and soil pollution. Once they reach water bodies, they cause increase in turbidity of water, resulting in reduced penetration of sunlight. This in turn affects the normal biochemical processes of the aquatic and marine life and disturbs the ecological balance (Dutta et al. 2014). Semi-conductor nanoparticles like TiO_2, ZnO, WO_3, and CuO have been used for the photocatalytic removal of dyes and other emerging contaminants from wastewater (Shah and Rather 2019; Rehman et al. 2019d; Qureshi et al. 2020). The mechanism for photocatalytic degradation of dyes and other organic contaminants is shown in Fig. 3.4. Green synthesized nanoparticles

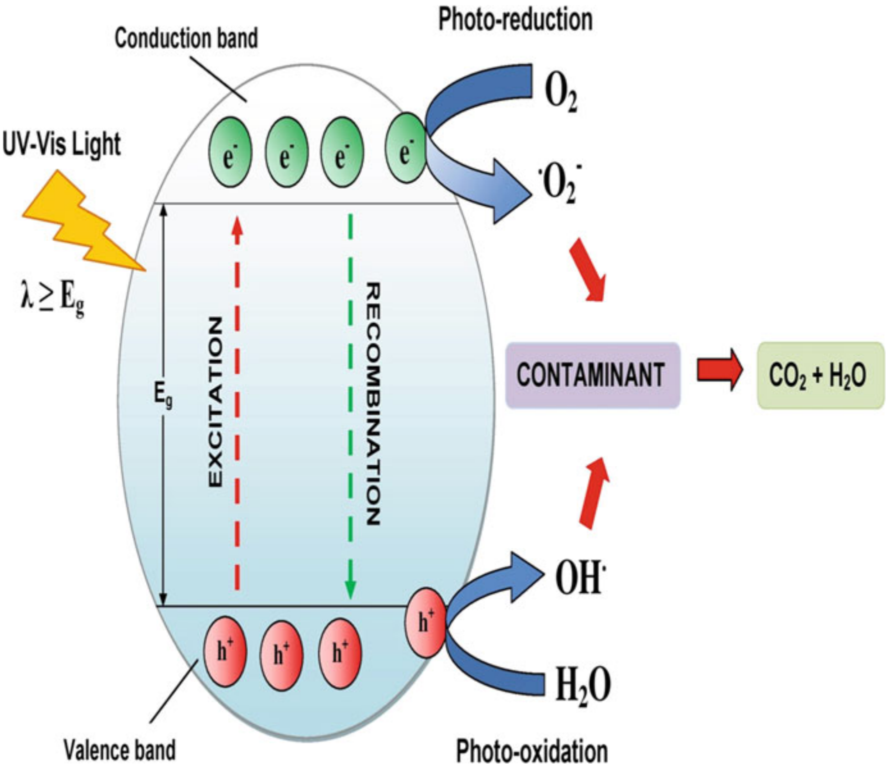

Fig. 3.4 General mechanism for photocatalytic degradation of dyes and other organic contaminants (Shah and Rather 2021)

show better catalytic efficiency due to their high specific area and more active sites compared to traditional nanoparticles synthesized through physical and chemical methods. PbS nanocuboid nanoparticles synthesized by biological methods showed better catalytic degradation of methylene blue (Yue et al. 2016). SnO_2 green nanoparticles were able to degrade >90% of methylene blue, methyl orange, and Eriochrome black T and all the nanoparticles could be easily separated from the reaction mixture by simple centrifugation (Srivastava and Mukhopadhyay 2014).

3.3.4 Dehalogenation

Chlorine containing aromatic chemical compounds are commonly used in various industries due to their high resistance against oxidation and flame. Their excess use has resulted in water, air, and soil pollution (Fang et al. 2019). Biosynthesized Pd nanoparticles using bacterial cells of *Desulfovibrio desulfuricans* and *Desulfovibrio vulgaris* were able to dechlorinate 30 times higher than chemically synthesized Pd nanoparticles. These biosynthesized Pd nanoparticles had better surface properties due to which catalytic efficiency was higher (Baxter-Plant et al. 2003). In another case, dehalogenation rate of tetrachlorobiphenyl was observed to be only 5% of that of biosynthesized Pd nanoparticles from *Desulfovibrio desulfuricans* (Baxter-Plant et al. 2004).

3.3.5 Heavy Metal Ions Removal

Wastewater released by mining and metal industries, vehicle exhaust emissions, coal, natural is laced with huge amounts of heavy metal ions (like Cr, Ni, Hg, Cd, Pb, Fe, Cu) that are toxic to the environment, aquatic life as well as human health. Some of these heavy metals like Pb, Cd, Hg, etc. are toxic even at trace concentrations (Singh et al. 2018; Zhang et al. 2012). Conventional methods of removal of these heavy metals from wastewater are costly, less effective, and have toxic side effects on the environment (Rehman et al. 2019a; Rudel et al. 2015; Shah and Rather 2020). *Shewanella loihica* PV-4 has been successfully used for the removal of vanadium and chromium ions from wastewater. The removal efficiency of both the heavy metal ions using this bacterial strain was >70% even after 27 days of operation (Wang et al. 2017). In another study, biosynthesized Pd nanoparticles showed better performance in the removal of chromium ions than chemically synthesized Pd nanoparticles due to comparatively small size and high surface to volume ratio (Ha et al. 2016).

3.4 Conclusion and Future Prospects in Research and Development

The synthesis of nanoparticles using biological sources has received tremendous response in the fields of agriculture, environment, and biomedical engineering. These nanoparticles provide non-toxic, eco-friendly, sustainable, and cost-effective solutions to the emerging global issues in areas of science. Besides being generally a simple route of synthesis, there are other advantages like better control on shape, size, and structure of nanoparticles, simple chemical synthesis, and non-toxic intermediates. Therefore, huge effort is being put to implement "green" production of nanoparticles at industrial scale using plants, plant extracts, fungi, bacteria, and other micro-organisms having medicinal value.

Conventional methods of nanoparticles syntheses involve use of toxic chemicals and consume high energy. Biosynthesis of nanoparticles requires low energy, is cheap, reliable, and eco-friendly method to fabricate efficient and stable nanoparticles. Some of the green synthesized nanoparticles showed better catalytic efficiency, stability, and surface properties in heterogeneous photocatalysis. The exact and detailed mechanism of synthesis, bioremediation, and bioreduction of many nanoparticles is still not known and therefore more studies are needed to address these knowledge gaps. Recent research has also focused on engineering of cells at the gene and proteome level to synthesize nanoparticles that are highly efficient and catalyze reactions in short time period.

The green synthesis of metal/metal oxide nanoparticles using marine organisms like algae, plants, etc. needs to be explored fully. Their potential in the areas of bioremediation, wastewater treatment, food sector, pharmaceutical, and personal-care products industries is still open for exploration. The surface properties like particle size, shape, and monodispersity might be controlled by studying the effect of various parameters like type of organism/plant extract, growth medium, pH, source of intending nanoparticles, temperature, time, and presence of other ions. By optimizing these parameters, the green synthesis of nanoparticles would offer a great advantage over physical and chemical methods. Thus with a detailed and proper understanding of the synthesis mechanism and reduced reaction time, the biosynthesis route will be more applicable, attractive, and preferred route of nanoparticle synthesis and will surely revolutionize the "nano-world."

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