

Microorganisms as efficient biosystem for the synthesis of metal nanoparticles: current scenario and future possibilities

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Abstract Nanoparticles, the elementary structures of nanotechnology, are important materials for fundamental studies and variety of applications. The different sizes and shapes of these materials exhibit unique physical and chemical properties than their bulk materials. There is a great interest in obtaining well-dispersed, ultrafine, and uniform nanoparticles to delineate and utilize their distinct properties. Nanoparticle synthesis can be achieved through a wide range of materials utilizing a number of methods including physical, chemical, and biological processes with various precursors from liquids and solids. There is a growing need to prepare environmentally friendly nanoparticles that do not produce toxic wastes in their process synthesis protocol. This kind of synthesis can be achieved by green environment benign processes, which happen to be mostly of a biological nature. Microorganisms are one of the most attractive and simple sources for the synthesis of different types of nanoparticles. This review is an attempt to provide the up-to-date information on current status of nanoparticle synthesis by different types of microorganisms such as fungi, yeast, bacteria, cyanobacteria, actinomycete, and algae. The probable biosynthesis mechanism and conditions for size/shape control are described. Various applications of microbially synthesized nanoparticles are summarized. They include antibacterial, antifungal, anti-cancer, larvicidal, medical imaging, biosensor, and catalytic

applications. Finally, limitations and future prospects for specific research are discussed.

Keywords Biosynthesis · Nanoparticles · Microorganisms · Mechanism · Applications

Introduction

Nanotechnology, a multidimensional science devoted to study fundamental properties of nanomaterials, has attracted interest in recent years. Nanotechnology is defined as the manipulation of matter with at least one dimension sized from 1 to 100 nm. There is increasing research activity in the field of nanotechnology, which has influenced many areas such as energy, medicine, electronics, and space industries. Different types of nanomaterials such as nanoparticles, nanotubes, and nanowires are useful in different applications. Nanoparticles (NPs) have attracted considerable scientific interest in recent years (Wang et al. 2014; Polte 2015). Nanoparticles usually referred as particles between 1 and 100 nm in size. They are constituted of several tens or hundreds of atoms or molecules. Nanoparticles can have a variety of sizes and morphologies such as amorphous, crystalline, spherical, needles, etc. Nanoparticles display distinctive physical, chemical, and biological properties than their bulk materials (Lee et al. 2014). Depending on size and shape, nanoparticles show different useful properties. Nanoparticles have been used for applications in medicine, healthcare, electronics, and agriculture (Sun et al. 2000; Vilchis-Nestor et al. 2008; Mohanpuria et al. 2008; Lee et al. 2014; Borase et al. 2014).

Different methods like physical, chemical and biological processes are being used for the synthesis of nanoparticles. Some chemical molecules such as anionic polysaccharides

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like polyglucuronic acid, oligosaccharide derivatives, and chitosan have been investigated for their bioactivities and their abilities to be functionalized as interesting nano or film materials (Tavernier et al. 2008; Elboutachfai et al. 2011; Mati-Baouche et al. 2014; Delattre et al. 2015; Elchinger et al. 2015). However, nanomaterial synthesis protocols of some chemical and physical methods involve use of toxic chemicals (Azim et al. 2009; Tapan and Andrey 2009; Borase et al. 2014). Green processes for nanoparticle synthesis are therefore needed. Synthesis of nanoparticles by biological methods using microorganisms or plant extracts offers a reliable, eco-friendly alternative to chemical and physical methods. Biological approach of nanoparticle synthesis is advantageous than physical and chemical methods because of simplicity of synthesis method without requirement of high temperature, pressure, and energy besides eco-friendly, cost-effective, scalable processes (Borase et al. 2014). Many microorganisms have resistance against toxic metals and have abilities of remediation of toxic metals through reduction of metal ions. This property makes them useful for nanoparticle synthesis. Microorganisms with their enzymes can select metal ions from the precursor solutions and accumulate them in elemental form of reduced metal thereby synthesizing nanoparticles.

As per the location of nanoparticle synthesis, the microbial mediated synthesis is generally categorized into intracellular and extracellular synthesis (Simkiss and Wilbur 1989; Mann 1996). The nanoparticles in intracellular approach are synthesized in the presence of enzymes where ions are transported inside the microbial cell for the

formation of nanoparticles. Whereas in extracellular synthesis, metal ions are trapped on the surface of the cells and are reduced to form nanoparticles with the help of enzymes. Different types of microorganisms have been used for the synthesis of mineral nanocrystals and metallic nanoparticles. The microorganisms include fungi (Ahmad et al. 2003a; Bruins et al. 2000; Salunkhe et al. 2011a, b), yeast (Mithila et al. 2009; Salunke et al. 2015), S-layer bacteria (Pum and Sleytr 1999), magnetotactic bacteria (Blackmore 1982), algae (Castro et al. 2013; Aziz et al. 2015), diatoms (Mann 2001), actinomycete (Ahmad et al. 2003b, c), and lichen (Mie et al. 2014). The control of the size, shape, composition, and monodispersity of particles has also been studied.

The present review summarizes the current status on the use of microbes for nanoparticle biosynthesis, possible biosynthesis mechanism, control of size/shape, limitations, future prospects, and diverse applications of nanoparticles.

Types of microorganisms in nanoparticle synthesis

Many microbes including fungi, yeast, bacteria, and actinomycetes have been found to be capable of intracellularly or extracellularly synthesizing mineral crystals and metallic nanoparticles. The general scheme of nanoparticle synthesis is displayed in Fig. 1. Different species of microorganisms such as fungi (Table 1), yeast (Table 2), bacteria (Table 3) cyanobacteria, actinomycete, and algae (Table 4) have been used for the synthesis of diverse types of nanoparticles. The type of nanoparticles includes Ag, Au, lead, Ag₂O, zirconia, CdS, barium titanate, CdSe quantum dots, silica, titanium, nickel oxide, iron, iron oxide (Fe₂O₃), copper, MnO₂, ZnO,

Fig. 1 General scheme of synthesis of nanoparticles by microorganisms

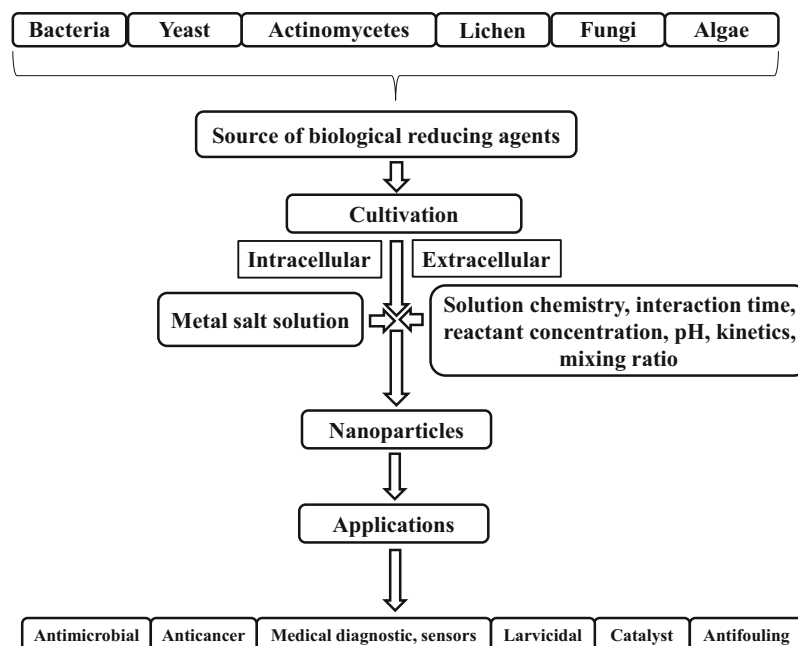


Table 1 Synthesis of different nanoparticles by fungi

Microbial species	Nanoparticle type	Size (nm)	Shape	Way of synthesis	Application	Reference
Fungi						
<i>Alternaria solani</i>	Ag	25	Spherical	Extracellular	–	Ghazwani (2015)
<i>Alternaria</i> sp.	Au	7–18	Spherical, rod, square, pentagonal, and hexagonal morphologies	Extracellular	–	Dhanasekar et al. (2015)
<i>Aspergillus fumigatus</i>	Ag	5–25		Extracellular	–	Bhainsa and D'souza (2006)
<i>Aspergillus niger</i>	Ag	20	Spherical	Extracellular	–	Ghazwani et al. (2015)
<i>Aspergillus niger</i>	Ag	43–63	Spherical	Intracellular and Extracellular	Antibacterial activity	Vanaja et al. (2015)
<i>Aspergillus niger</i> PFR6	Ag	8.7 ± 6	Spherical	Extracellular	–	Devi and Joshi (2015)
<i>Aspergillus oryzae</i>	Ag	6–37	Spherical	Extracellular	–	Bhimba et al. (2015)
<i>Aspergillus oryzae</i>	Ag	76.14	Spherical	Extracellular	Antifungal activity	Pereira et al. (2014)
<i>Aspergillus</i> sp.	Ag	–	–	Extracellular	Antimicrobial activity	Prabavathy et al. (2015)
<i>Aspergillus</i> sp.	Lead	5–20	Spherical	Extracellular	–	Pavani et al. (2012)
<i>Aspergillus tamarii</i> PFL2	Ag	3.5 ± 3	Spherical	Extracellular	–	Devi and Joshi (2015)
<i>Aspergillus terreus</i> VIT 2013	Ag ₂ O	–	–	Extracellular	Antibacterial	Sangappa, and Thiagarajan (2015)
<i>Cochliobolus lunatus</i>	Ag	14	Spherical	Intracellular and Extracellular	–	Salunkhe et al. (2011a)
<i>Cochliobolus lunatus</i>	Ag	3–21	Spherical	Intracellular and Extracellular	Mosquito larvicidal	Salunkhe et al. (2011b)
<i>Colletotrichum</i> sp.	Au	20–40		Extracellular	–	Shankar et al. (2003)
<i>Fusarium oxysporum</i>	Magnetite	20–50		Extracellular	–	Bharde et al. (2006)
<i>Fusarium oxysporum</i>	Ag	5–15	Spherical	Extracellular	–	Ahmad et al. (2003a, b, c)
<i>Fusarium oxysporum</i>	Au	20–40		Extracellular	–	Mukherjee et al. (2002)
<i>Fusarium oxysporum</i>	Zirconia	3–11		Extracellular	–	Bansal et al. (2004)
<i>Fusarium oxysporum</i>	CdS	5–20		Extracellular	–	Ahmad et al. (2002)
<i>Fusarium oxysporum</i>	Barium titanate	4–5		Extracellular	–	Bansal et al. (2006)
<i>Fusarium oxysporum</i>	CdSe quantum dots	–		Extracellular	–	Kumar et al. (2007b)
<i>Fusarium oxysporum</i>	Silica and Titanium particles (SiF ₆ ^{2–} and TiF ₆ ^{2–})	5–15		Extracellular	–	Bansal et al. (2005)
<i>Fusarium oxysporum</i>	Ag	5	Spherical	Extracellular	–	Ghazwani et al. (2015)
<i>Fusarium oxysporum</i> Nitrate reductases	Ag	10–25		Extracellular	–	Kumar et al. (2007a)

Table 1 continued

Microbial species	Nanoparticle type	Size (nm)	Shape	Way of synthesis	Application	Reference
<i>Humicola</i> sp.	Ag	5–25	Spherical	Extracellular	Cytotoxicity	Syed et al. (2013)
<i>Hypocrea lixii</i>	Nickel oxide	1.25–3.8	Spherical	Intracellular and Extracellular	–	Salvadori et al. (2015)
<i>Penicillium chrysogenum</i>	Ag	100.6	Spherical	Extracellular	Antifungal activity	Pereira et al. (2014)
<i>Penicillium ochrochloron</i> PFR8	Ag	7.7 ± 4.3	Spherical	Extracellular	–	Devi and Joshi (2015)
<i>Phanerochaete chrysosporium</i> and enzyme	Au	10–100	Spherical	Intracellular and Extracellular	–	Sanghi et al. (2011)
<i>Pleurotus</i> sp.	Iron	–	–	Intracellular	–	Mazumdar and Haloi (2011)
<i>Pycnoporus sanguineus</i>	Au	10–500	Spherical, pseudo-spherical, triangular, truncated triangular, pentagonal, and hexagonal	Intracellular	Catalysis in degradation of 4-nitroaniline	Shi et al. (2015)
<i>Rhizopus stolonier</i>	Ag	5–50	Spherical	Extracellular	–	Banu and Rathod (2011)
<i>Trichoderma Reesei</i>	Ag	5–50	Spherical	Extracellular	–	Vahabi et al. (2011)
<i>Trichothecium</i> sp.	Au	–	–	Extracellular and Intracellular	–	Ahmad et al. (2005)
<i>V. luteoalbum</i>	Au	Few to 100	–	Intracellular	–	Gericke and Pinches (2006b)
<i>Verticillium</i>	Au	20	–	Intracellular	–	Mukherjee et al. (2001a)
<i>Verticillium</i>	Ag	25 ± 12	–	Intracellular	–	Mukherjee et al. (2001b), Senapati et al. (2004)
<i>Verticillium</i> sp.	Magnetite	20–50	–	Extracellular	–	Bharde et al. (2006)

Table 2 Synthesis of different nanoparticles by yeast

Microbial species	Nanoparticle type	Size (nm)	Shape	Way of synthesis	Biological activity	Reference
Yeast						
Baker's Yeast	Ag	5–25	Spherical	Extracellular		He et al. (2013)
Baker's Yeast	Ag	6–20	Spherical	Extracellular		Jha et al. (2008)
Baker's Yeast <i>Saccharomyces cerevisiae</i>	Ag	60–80	Spherical	Extracellular	Antimicrobial activity	Saravanan et al. (2013)
<i>Candida albicans</i>	Iron oxide (Fe ₂ O ₃)	80	Spherical	Extracellular	Antimicrobial activity	Hassan et al. (2015)
<i>Candida glabrata</i>	CdS	2		Intracellular		Dameron et al. (1989)
Extremophilic yeast strain Portugal	Ag	20 nm		Extracellular	–	Mourato et al. (2011)
Extremophilic yeast strain Portugal	Au	30–100 nm		Extracellular	–	Mourato et al. (2011)
MKY3	Ag	2–5		Extracellular		Kowshik et al. (2002)
<i>P. jadinii</i>	Au	Few to 100		Intracellular		Gericke and Pinches (2006b)
<i>Rhodotorula mucilaginosa</i>	Copper	10.5	Spherical	Intracellular and Extracellular	–	Salvadori et al. (2014)
<i>Saccharomyces cerevisiae</i>	MnO ₂	15–70	Hexagonal and spherical	Extracellular		Salunke et al. (2015)
<i>Saccharomyces cerevisiae</i>	ZnO	52.72	–	Extracellular		Pavani et al. (2015)
<i>Saccharomyces cerevisiae</i>	Au	–	–	–		Stegenga et al. (2011)
<i>Saccharomyces cerevisiae</i>	Ag	10	Spherical	Extracellular	Photocatalytic activity	Roy et al. (2014)
<i>Schizosaccharomyces pombe</i>	CdS	1–1.5		Intracellular		Kowshik et al. (2002)
<i>Schizosaccharomyces pombe</i>	CdS	2		Intracellular		Dameron et al. (1989)
<i>Yarrowia lipolytica</i> NCIM 3589	Au	9–27	Spherical hexagonal, triangular nanoplates	Intracellular and Extracellular	–	Pimprikar et al. (2009)
<i>Yarrowia lipolytica</i> NCIM 3589	Au	15	Hexagonal, triangular, Spherical	Intracellular and Extracellular	–	Agnihotri et al. (2009)
<i>Yarrowia lipolytica</i> NCYC 789	Au	–	–	Intracellular and Extracellular	Antibacterial and anti-biofilm activity	Nair et al. (2013)
<i>Yarrowia lipolytica</i> NCYC 789 and its melanin	Ag	15	–	Intracellular and Extracellular	Antibiofilm activity	Apte et al. (2013)
Yeast cells	Iron phosphate (FePO ₄)	50–200	Spherical aggregations	Extracellular		He et al. (2009)

Table 3 Synthesis of different nanoparticles by bacteria

Microbial species	Nanoparticle type	Size	Shape	Way of synthesis	Biological activity	Reference
Bacteria						
<i>Arthrobacter nitroguajacolicus</i>	Au	40	Spherical	Extracellular		Dehnad et al. (2015)
<i>Bacillus pumilus</i> , <i>B. persicus</i> , and <i>Bacillus licheniformis</i>	Ag	77–92	Spherical	Extracellular	Antimicrobial and antiviral	Elbeshehy et al. (2015)
<i>Bacillus subtilis</i>	Ag	5–60	Spherical	Extracellular	–	Saifuddin et al. (2009)
<i>Deinococcus radiodurans</i>	Ag	4–50	Spherical	Extracellular	Antibacterial, Anti-biofouling, Anticancer	Kulkarni et al. (2015)
<i>Desulfovibrio desulfuricans</i>	Palladium	–	–	–	–	Yong et al. (2002)
<i>E.coli</i>	Ag	20–50	Spherical	Extracellular	Antibacterial	Kushwaha et al. (2015)
<i>Geobacillus</i> sp. strain ID17	Au	5–50	Quasi-hexagonal	Intracellular	–	Correa-Llantén et al. (2013)
<i>Geobacter</i> sp.	Iron sulfide (FeS) and siderite (FeCO ₃)	40–60 and 10 nm –3 µm	–	–	–	Kim et al. (2015)
<i>K. pneumonia</i>	Ag	1–6	Spherical	Extracellular	–	Mokhtari et al. (2009)
<i>Klebsiella aerogenes</i>	Cadmium sulfide	20–200	Crystalline	Extracellular	–	Holmes et al. (1995)
<i>Lactobacillus</i>	TiO ₂	8–35	Spherical and other shapes	Extracellular	–	Jha et al. (2009)
<i>Lactobacillus</i> sp.	Titanium	40–60	Spherical	Extracellular	–	Prasad et al. (2007)
<i>Magnetotactic bacteria</i>	Magnetic Fe ₃ O ₄ , reigte Fe ₃ S ₄)	–	–	–	–	Roh et al. (2001)
<i>Nocardopsis</i> sp. MBRC-1	Ag	45 ± 0.15	Spherical	Extracellular	Antimicrobial and Anticancer	Manivasagan et al. (2013)
<i>Pseudomonas aeruginosa</i>	Au	15–30	–	Extracellular	–	Husseiny et al. (2007)
<i>Pseudomonas stutzeri</i>	Ag	Up to 200	–	–	–	Joerger et al. (2000), Klaus et al. (2001)
<i>Rhodobacter sphaeroides</i>	Zinc sulfide	Average diameter of 8	Spherical	Extracellular	–	Bai et al. (2006)
<i>Rhodopseudomonas palustris</i>	Cadmium sulfide	8.01 ± 0.25	Crystalline, face-centered cubic	Intracellular	–	Bai et al. (2009)
<i>Rhodopseudomonas capsulata</i>	Au	10–20	Spherical and nanowires	Extracellular	–	He et al. (2008)
<i>Serratia nematodiphila</i>	Ag	10–31	Spherical	Extracellular	Antibacterial	Malarkodi et al. (2013)
<i>Shewanella algae</i>	Pt	Around 5	–	Intracellular and Extracellular	–	Konishi et al. (2007)
<i>Shewanella putrefaciens</i> (Gs-15)	Magnetite	10–50	Fine-grained crystal	Intracellular	–	Lovley et al. (1987)
<i>Staphylococcus aureus</i>	Ag	160–180	Spherical	Extracellular	Antibacterial	Nanda and Saravanan (2009)

Table 4 Synthesis of different nanoparticles by Cyanobacteria, Actinomycete, and Algae

Microbial species	Nanoparticle type	Size (nm)	Shape	Way of synthesis	Biological activity	Reference
Cyanobacteria						
<i>Plectonema boryanum</i> UTEX 485 (Cyanobacterium)	Au platlets	6–10	Octahedral	At the cell wall		Lengke et al. (2006a, b)
<i>Plectonema boryanum</i> (Cyanobacterium)	Palladium	≤30	Spherical and elongate	Intracellular and Extracellular	–	Lengke et al. (2007)
Actinomycete						
<i>Rhodococcus</i> sp.	Au	5–15		Intracellular		Ahmad et al. (2003a)
<i>Streptomyces clavuligerus</i>	Au	8.25 ± 0.64	Spherical	Extracellular	Anticancer	Kumar et al. (2015)
<i>Streptomyces fulvissimus</i>	Au	20–50	Spherical, Trinagular	Extracellular	–	Soltani et al. (2015)
<i>Thermomonospora</i> sp.	Au	8		Extracellular		Ahmad et al. (2003b)
Algae						
<i>Aphanothece</i> sp, <i>Oscillatoria</i> sp, <i>Microcoleus</i> sp, <i>Aphanocapsa</i> sp, <i>Phormidium</i> sp, <i>Lyngbya</i> sp, <i>Gleocapsa</i> sp, <i>Synechococcus</i> sp, <i>Spirulina</i> sp	Ag	40–80	Spherical	Extracellular	Antibacterial	Sudha et al. (2013)
<i>Chlorella pyrenoidosa</i>	Ag	5–30	Spherical	Extracellular	Antibacterial and Photocatalytic	Aziz et al. (2015)

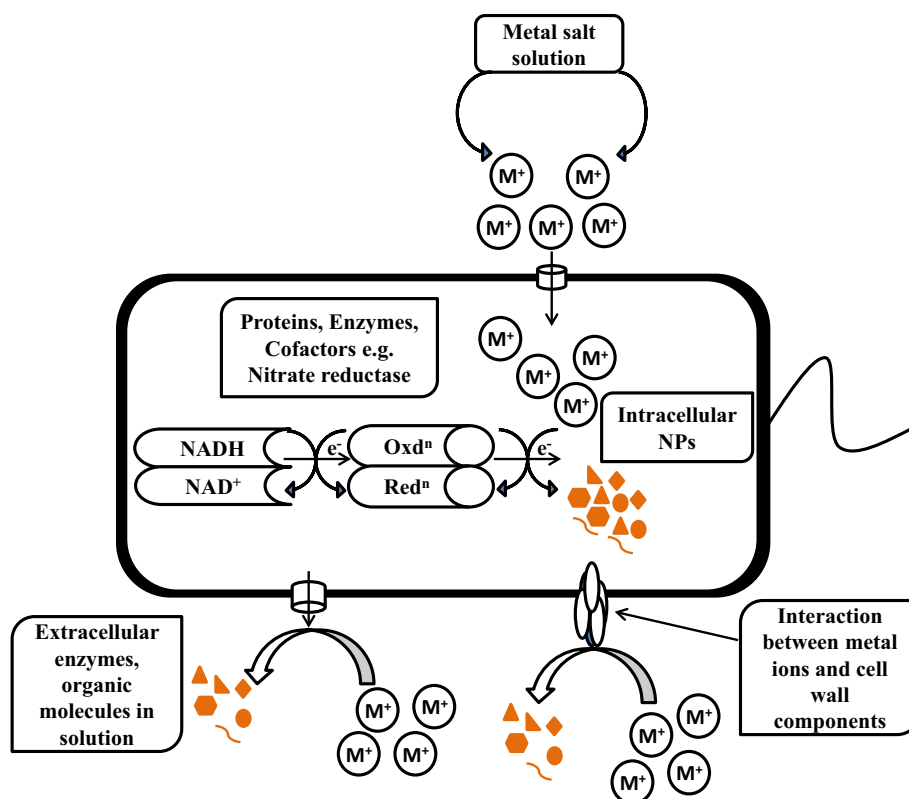
iron phosphate (FePO₄), palladium, iron sulfide (FeS), siderite (FeCO₃), cadmium sulfide, TiO₂, titanium, magnetite (Fe₃O₄), greigite (Fe₃S₄), zinc sulfide, and Pt. AgNPs have been most studied due to their good antimicrobial and catalytic activities. The size of the synthesized nanoparticles ranges from 1 to 500 nm. The shape is predominantly spherical, but other shapes such as pseudo-spherical, rod, triangular, truncated triangular, square, pentagonal, hexagonal, nanoplate, fine-grained crystal, and nanowire morphologies have also been observed. The synthesized nanoparticles have been utilized for antimicrobial, antibacterial, anti-biofilm, anti-biofouling, antiviral, anticancer, and photocatalytic activities (Tables 1, 2, 3 and 4).

Mechanism of nanoparticle synthesis by microorganisms

Different studies have shown the role of cell wall, cell membrane, different enzymes, proteins, and other organic molecules in nanoparticle synthesis (Fig. 2). Salunke et al.

(2015) suggested the role of *S. cerevisiae* cell wall components, alcoholic compounds, and proteins in MnO₂ nanoparticle synthesis and stabilization. *S. cerevisiae* cell walls are composed of chitin, glucan, and mannoprotein (Kollár et al. 1997; Lipke and Ovalle 1998). The components of the cell walls collectively may be responsible for the rapid synthesis of MnO₂ nanoparticles (Salunke et al. 2015). Proteins released by yeast cells have been reported to play an important role in the synthesis of AgNPs, whereas the cell wall was involved in the synthesis of AuNPs (Mourato et al. 2011). Proteins may have an active role in the synthesis of MnO₂ nanoparticles along with sugars and chitin. Priyabrata et al. (2001) observed majority of the particles were attached on the surface of the cytoplasmic membrane in their AgNP synthesis study. They suggested that electrostatic interaction on the cell surface traps the silver ions and further reduces it to form silver nuclei through action of unknown enzymes in the cell wall, which lead to the formation of nanoparticles on the mycelia after accumulation at nuclei. Reducing sugars formed by some

Fig. 2 Mechanism of nanoparticle synthesis by microorganisms



fungi were found responsible for synthesis of nanoparticles. Ahmad et al. (2003a) suggested the role of NADH-dependent reductase in nanoparticle synthesis. Nitrate reductase is apparently essential for metal ion reduction, but Salunkhe et al. (2011a) found that 72 h fungal culture of *Cochliobolus lunatus* was negative for nitrate reduction during silver resistance and AgNP formation. Their results indicated that other mechanisms for Ag reduction may be present. They suggested that the mechanism of AgNP synthesis was linked to cell wall polymer or electron shuttle quinones or both. The growth phase of microorganisms has an important role in nanoparticle biosynthesis. *Verticillium luteoalbum* cells harvested from different growth phase produced different numbers of particles (Gericke and Pinches 2006a, b). The cells obtained from the early exponential phase showed better ability to synthesize a number of nanoparticles than the cells from late exponential phase. Gericke and Pinches (2006a, b) gave a possible explanation for this phenomenon for gold nanoparticles that cells in early exponential phase of growth produce very high concentrations of enzymes and proteins that are actively involved in accumulation and reduction of the gold ions.

The synthesis mechanism of nanoparticles is not well understood yet. The provision of electrons is needed when the ions are converted to the corresponding elemental metals. The activities of microorganisms to reduce ions to their metallic particles may lead to generation of reducing

agents. As heavy metallic ions are hazardous to microbes, the microbes will have to have protective mechanisms to resist toxicity of the metallic ions (Hallmann et al. 1997). The microbial cells need to trap the ions by some way. Electrostatic interaction and/or secretion of substances such as extracellular polymeric substances that can adhere to the ions are some of these mechanisms. Most of the bacteria have negative charge on cells (Albert et al. 1997; Manti et al. 2008). The electrostatic interactions are believed to happen between the positively charged ions and the negatively charged groups such as carboxylate groups on the cells. The secretion of adhesive materials as a result of their stickiness may possibly stabilize the ions in the cell. The ions are involved in the nutrient exchange and/or substance diffusion during the intracellular synthesis of nanoparticles. The microbial cells prevent damage by producing specific enzymes such as NADH-dependent reductase and electrons, which can reduce ions. The nuclei grow and thereby form nanoparticles intracellularly or extracellularly. Enzymes may be important in the whole process. The reduction reaction on the cell surface or inside the cells may be catalyzed by enzymes. Nanoparticle synthesis ability of most of the microorganisms has been correlated with a specific type of secreted enzyme(s), but the exact information about the different enzymes involved in nanoparticle synthesis is not fully established yet.

Size and shape control of nanoparticles

As compared to the bulk materials, their nanoforms possess some unique chemical, optical, and electronic properties. The properties of the nanoparticles change with size, shape, and monodispersity of the particles. Therefore, controlling these parameters is an important task during biosynthesis. The type of microorganism, growth medium, and synthesis conditions are important factors in controlled synthesis of nanoparticles (Holmes et al. 1995; Murali et al. 2003; Ahmad et al. 2003a; Gericke and Pinches 2006a, b; Hussein et al. 2015). Fungi, yeast, and actinomycetes are found to be good for synthesis of uniform nanoparticles of controlled size (Murali et al. 2003; Ahmad et al. 2003b; Hussein et al. 2015). After parametric optimization, Hussein et al. (2015) found smallest particle size when *Fusarium oxysporum* was incubated for 72 h with 10^{-2} M silver nitrate (metal ion concentration) at 50 °C with 11 g wet biomass at pH 6 with fungal age 7 days. AgNPs produced were characterized by transmission electron microscopy (TEM) which revealed the formation of spherical, well-dispersed nanoparticles with size between 5 and 13 nm. Kathiresan et al. (2009) found that AgNP size increased with the increase of pH by *Penicillium fellutanum*. Change of Ag^+ concentration was found to control the size and dispersion of particles using *Hormoconis resinae* (Varshney et al. 2009). The original precursor salt (e.g. silver nitrate or silver chloride) showed synthesis of different sized particles (Narges et al. 2009). Temperatures have effect on microbial activity and ion movement. The distinctive morphologies of AgNPs and size were found with change of temperature during synthesis using cyanobacteria (Maggy et al. 2007). Reaction time showed effect on particle sizes and monodispersity on nano-gold particle synthesis (Gericke and Pinches 2006b). Controlled particle size of AgNPs was achieved using irradiation of the mixture of *B. subtilis* cells and 1 mM silver ions (Narges et al. 2009). Precise controlling particle size and monodispersity using microbes are still not known. More extensive investigations on optimization of different parameters described are needed to achieve controlled synthesis. Extensive studies considering factors such as selection of specific strain of microbe, phase of growth, growth medium, synthesis conditions, pH, substrate concentrations, precursor salts, temperature, reaction time, irradiation, agitation, ultrasonication, and many different factors will need to be investigated and optimized to discover a reliable method of synthesizing particles with uniform shape and size.

Application of nanoparticles

Nanoparticles synthesized by different sources have been reported for various applications including antimicrobial,

optics, biosensors, catalysis, and diagnosis (Sintubin et al. 2012; Borase et al. 2014). The applications of microbially synthesized nanoparticles are summarized below.

Antibacterial

Nanoparticles are coming up as new class of compounds as antibacterial. Microorganisms have developed resistance against many antibiotics. AgNPs are found to be good antiseptics in this regard. AgNPs have been investigated for activities against Gram-positive bacteria such as *Bacillus subtilis*, *Bacillus cereus*, *Enterococcus hirae*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Streptococcus pyogenes* (Nanda and Saravanan 2009; Saravanan et al. 2013; Manivasagan et al. 2013; Malarkodi et al. 2013; Sudha et al. 2013; Kulkarni et al. 2015; Kushwaha et al. 2015; Prabavathy et al. 2015; Vanaja et al. 2015; Sangappa and Thiagarajan 2015) and Gram-negative bacteria such as *Escherichia coli*, *Klebsiella planticola*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Pseudomonas* sp., *Salmonella typhi*, *Shigella flexneri*, and *Vibrio cholerae* (Nanda and Saravanan 2009; Saravanan et al. 2013; Manivasagan et al. 2013; Malarkodi et al. 2013; Sudha et al. 2013; Kulkarni et al. 2015; Kushwaha et al. 2015; Prabavathy et al. 2015; Vanaja et al. 2015). They are found to enhance antibacterial effect when used in combination with different antibiotics. *F. oxysporum* synthesized AgNPs integrated into textile fabrics were able to avoid or reduce survival of pathogenic bacteria like *Staphylococcus aureus* (Durán et al. 2007). Antibacterial activities of different nanoparticles have been shown in different studies by researchers (Nanda and Saravanan 2009; Nair et al. 2013; Malarkodi et al. 2013; Sudha et al. 2013; Vanaja et al. 2015; Sangappa and Thiagarajan 2015; Kulkarni et al. 2015; Kushwaha et al. 2015; Aziz et al. 2015). Vanaja et al. (2015) found that *E. coli* was most susceptible bacteria to the action of AgNPs with maximum zone of inhibition (11.53 ± 0.145 mm) compared to *K. planticola*, *Pseudomonas* sp., *B. subtilis*, and *B. cereus* (9.43 ± 0.219 , 11.43 ± 0.067 , 9.43 ± 0.219 , and 11.47 ± 0.273 mm), respectively.

Antifungal activity

Antifungal activities of nanoparticles synthesized by microorganisms have been reported (Gajbhiye et al. 2009; Musarrat et al. 2010; Pereira et al. 2014). The biosynthesized nanoparticles were effective in combination with fluconazole (a triazole antifungal drug) with increase in diameter of inhibition zones and fold area against fungi *Phoma glomerata*, *P. herbarum*, *Fusarium semitectum*, *Trichoderma* sp., and *Candida albicans* (Gajbhiye et al. 2009). The antifungal activity of fluconazole increased significantly in presence of

AgNPs. The maximum antifungal activity was observed against *C. albicans* followed by *Trichoderma* sp. and *P. glomerata*. However, no enhancement of the antifungal activity was recorded in the cases of *F. semitectum* and *P. herbarum*. This was also shown through the assessment of increases in fold area of activity. It was observed that *C. albicans* had the highest increase in the fold area (3.69), while a much smaller increase in fold area (0.15) was observed for both *F. semitectum* and *P. herbarum*. *Amylomyces rouxii* synthesized AgNPs were active against fungi *F. oxysporum* and yeast *Candida albicans* (Musarrat et al. 2010). Antifungal susceptibility of the eight clinical strains of *T. rubrum* and the reference strain *T. rubrum* ATCC MYA-4438 were tested against three commercial antifungal drugs (terbinafine, itraconazole and fluconazole), and Chem-AgNPs and Bio-AgNPs produced by *P. chrysogenum* MUM 03.22 and *A. oryzae* MUM 97.19 (Pereira et al. 2014). The authors reported that the reference strain *T. rubrum* ATCC MYA-4438 was the most susceptible fungus against itraconazole, fluconazole, Chem-AgNPs, and Bio-AgNPs. In contrast, it was the less susceptible to terbinafine. Among the commercial antifungal drugs evaluated, terbinafine exhibited the lower minimum inhibitory concentration (MIC) values (0.063–0.25 µg/ml) for all clinical strains. Chem-AgNPs showed antifungal activity in the range from 0.25 to 2.5 µg/ml. Furthermore, Chem-AgNPs exhibited a stronger inhibitory effect against all *T. rubrum* strains evaluated, which MIC values were at least twofold higher than the MIC values of Bio-AgNPs. Bio-AgNPs produced by *P. chrysogenum* MUM 03.22 showed an antifungal activity in the range from 0.5 to 5.0 µg/ml. The Bio-AgNPs produced by that fungi exhibited higher antifungal activity than fluconazole, but lower activity than terbinafine, itraconazole, and Chem-AgNPs. In contrast, Bio-AgNPs produced by *A. oryzae* MUM 97.19 did not exhibit antifungal activity in concentrations up to 7.5 µg/ml. Regardless of the process used, Chem-AgNPs and Bio-AgNPs produced by *P. chrysogenum* MUM 03.22 (geometric mean of MIC values = 1.19 and 2.89 µg/ml, respectively) exhibited a stronger inhibiting activity than fluconazole (geometric mean of MIC values = 5.65 µg/ml).

Cytotoxicity

Cytotoxicity of different nanoparticles has been investigated in different studies (Syed et al. 2013; Kulkarni et al. 2015; Kumar et al. 2015). Monodispersed AuNPs produced by *Humicola* spp. were found to show cytotoxicity. The nanoparticles were conjugated with the anticancer drug doxorubicin for targeted drug delivery to liver (hepatic) cancer. Syed et al. (2013) assessed cytotoxicity of AgNPs against the cells (NIH3T3 mouse embryonic fibroblast cell line and MDA-MB-231 human breast carcinoma cell line)

by treating with varying concentrations of AgNPs (50, 250, 500, and 1000 µg/ml) for 24 h. The authors did not find significant difference on cell viability at concentration of 50 µg/ml. The cell viability was reduced in a dose-dependent manner in both cell lines and the cytotoxicity of the nanoparticles was observed at concentrations of 250 µg/ml and higher in both cell lines. The cell viability was reduced by 20.83 % in the case of NIH3T3 and 42.18 % for MDA-MB-231 cell line at 1000 µg/ml concentration. Kulkarni et al. (2015) studied cell viability and metabolic activity using MTT assay by exposing MCF-7 cells to AgNPs at 0–30 µg concentrations for 24 h. The results of MTT assay showed a dose-dependent decrease in percent viability of the cells. The authors fitted cytotoxicity data to a sigmoidal curve, and used a four-parameter nonlinear logistic model to calculate the LD₅₀ of nanomaterials that caused a 50 % inhibition in comparison to untreated controls, which was 7.9 µg/ml. Exposure to increasing concentrations of AgNPs showed a dose-dependent cytotoxicity on the cancer cell line. LD₅₀ of AgNPs synthesized using *D. radiodurans* was reported to be 7–8 µg/ml, which suggests that these nanoparticles are effective at a lower concentration against MCF-7 human breast cancer cell line compared to AgNPs synthesized using other sources. The AgNPs in their studies were of small in size (average size of 16.82 nm). The cytotoxicity of nanoparticles is size dependent with smaller nanoparticles entering the cells more easily and thus are more efficient than larger ones. Therefore, AgNPs have promise for potential applications in cytotoxicity and anti-cancer activity.

Medical imaging

AgNPs synthesized by *Trichoderma viride* were demonstrated to be suitable for labeling and imaging (Sarkar et al. 2010). The cadmium telluride quantum dots associated with folic acid were utilized for in vitro imaging of cancer cells, and were recognized to have biocompatibility in a cytotoxicity assay (Bao et al. 2010a, b). The authors developed an efficient bacterial synthesis method to harvest cadmium telluride (CdTe) quantum dots (QDs) with tunable fluorescence emission using *E. coli* and yeast cells. Ultraviolet–visible, photoluminescence, X-ray diffraction, and TEM analysis confirmed the superior size-tunable optical properties, with fluorescence emission from 488 to 551 nm, and the good crystallinity of the as synthesized QDs. A surface protein capping layer was confirmed by hydrodynamic size, zeta potential, and Fourier transform infrared spectroscopy measurements, which could maintain the viability (92.9 %) of cells in an environment with a QD concentration as high as 2 µM. After functionalization with folic acid, the QDs were used to image cultured cervical cancer cells in vitro. Investigations of bacterial growth and

morphology and the biosynthesis of CdTe QDs in Luria–Bertani medium containing *E. coli*-secreted proteins showed that extracellular synthesis directly relied on the *E. coli*-secreted proteins. The authors suggested that the biosynthesized QDs may have great potential in broad bio-imaging and bio-labeling applications.

Enhancing reaction rates

Shewanella oneidensis synthesized palladium nanoparticles were effective in dehalogenate polychlorinated biphenyl reduction (De Windt et al. 2005). The authors carried out microbial reduction of soluble Pd^{2+} by cells of *Shewanella oneidensis* MR-1 and of an autoaggregating mutant, which resulted in precipitation of palladium Pd^0 nanoparticles on the cell wall and inside the periplasmic space (bioPd). As a result of biosorption and subsequent bioreduction of Pd^{2+} with H_2 , formate, lactate, pyruvate, or ethanol as electron donors, recoveries higher than 90 % of Pd^0 associated with biomass could be obtained. The bioPd nanoparticles thus obtained had the ability to reductively dehalogenate polychlorinated biphenyl (PCB) congeners in aqueous and sediment matrices. Bioreduction was observed in assays with concentrations up to 1000 mg Pd^{2+} /l with depletion of soluble Pd^{2+} of 77.4 % and higher. More than 90 % decrease of PCB 21 (2,3,4-chloro biphenyl) coupled to formation of its dechlorination products PCB 5 (2,3-chloro biphenyl) and PCB 1 (2-chloro biphenyl) was obtained at a concentration of 1 mg/l within 5 h at 28 °C. Bioreductive precipitation of bioPd by *S. oneidensis* cells mixed with sediment samples contaminated with a mixture of PCB congeners, resulted in dechlorination of both highly and lightly chlorinated PCB congeners adsorbed to the contaminated sediment matrix within 48 h at 28 °C. Fifty milligrams per litre of bioPd resulted in a catalytic activity that was comparable to 500 mg/l commercial Pd^0 powder. The high reactivity of 50 mg/l bioPd in the soil suspension was reflected in the reduction of the sum of seven most toxic PCBs to 27 % of their initial concentration. Magnetic Fe_3O_4 nanoparticle coated microbial cells of *Pseudomonas delafieldii* were good to accomplish desulfurization of dibenzothiophene (Shan et al. 2005). The authors coated microbial cells of *Pseudomonas delafieldii* with magnetic Fe_3O_4 nanoparticles and then immobilized by external application of a magnetic field. Magnetic Fe_3O_4 nanoparticles were synthesized by a co-precipitation method followed by modification with ammonium oleate. The surface-modified Fe_3O_4 nanoparticles were monodispersed in an aqueous solution and did not precipitate in over 18 months. Using TEM, the average size of the magnetic particles was found to be in the range from 10 to 15 nm. TEM cross section analysis of the cells showed further that the Fe_3O_4 nanoparticles were for the most part strongly absorbed by the surfaces of the cells and coated the

cells. The coated cells had distinct superparamagnetic properties. The coated cells not only had the same desulfurizing activity as free cells but could also be reused more than five times. Compared to cells immobilized on Celite, the cells coated with Fe_3O_4 nanoparticles had greater desulfurizing activity and operational stability. Integrated nanosized zero-valent iron (NZVI) and microbial process demonstrated great advantage compared to solo NZVI and solo microbial process application on nitrate reduction (Choe et al. 2000). Nanoscale iron particles with a diameter in the range of 1–100 nm, which were characterized by the large BET specific surface area to mass ratio ($31.4 \text{ m}^2/\text{g}$), removed mostly 50, 100, 200, and 400 mg/l of nitrate within a period of 30 min with little intermediates. Compared with microscale (75–150 μm) Fe^0 , endproduct was not ammonia but N_2 gas. Kinetic analysis from batch studies revealed that the denitrification reaction with nanoscale Fe^0 appeared to be a pseudo first-order with respect to substrate and the observed reaction rate constant (k_{obs}) varied with iron content at a relatively low degree of application. The effects of mixing intensity (rpm) on the denitrification rate suggest that the denitrification appears to be coupled with oxidative dissolution of iron through a largely mass transport-limited surface reaction (<40 rpm).

Catalytic activity

Nanoparticles have been tested for catalytic activities (Shi et al. 2015; Roy et al. 2014; Aziz et al. 2015). Shi et al. (2015) synthesized of AuNPs using intracellular protein extract (IPE) of *Pycnoporus sanguineus*. The catalysis results showed that 0.019 mg of AuNPs with average size of 6.07 nm could catalyze the complete degradation of 12.5 μmol of 4-nitroaniline within 6 min and the degradation rate increased drastically with the addition of AuNPs. Roy et al. (2014) used AgNPs synthesized by yeast (*S. cerevisiae*) extract for photocatalytic activity under solar irradiation. These biosynthesized nanoparticles showed efficacy in degrading the organic dye, methylene blue within a few hours of exposure. Aziz et al. (2015) evaluated the photocatalytic activity by separately adding 10 mg of biosynthesized AgNPs, commercial AgNPs (8 nm diameter, Sigma-Aldrich), and bulk Ag powder (Sigma-Aldrich) to 100 ml of aqueous solution containing 5 mg/l of methylene blue (MB) dye. The solution was continuously stirred in the dark for 1 h to ensure adsorption/desorption equilibrium between the AgNPs and the MB solution. Subsequently, the suspension was irradiated using a 500 W xenon lamp. The suspensions were taken from the reactor and centrifuged at 10 min intervals, and their absorption spectra were recorded on a UV–vis spectrophotometer using deionized water as a reference. In the absence of AgNPs, the MB dye concentration remains almost unchanged after 30 min under visible

light irradiation, indicating that MB does not self-degrade under these conditions. The kinetics of MB degradation using different catalysts was found to follow a pseudo-first-order reaction and the biosynthesized AgNPs display superior photocatalytic activity toward the degradation of MB as compared to the commercially procured AgNPs and bulk silver powder. The authors reported that the superior activity may be due to their inherent porosity and therefore larger surface area.

Biosensor

Au–Ag alloy nanoparticles synthesized by yeast were effective for sensing vanillin (Zheng et al. 2010a). The authors biosynthesized Au–Ag alloy nanoparticles by yeast cells and applied to fabricate a sensitive electrochemical vanillin sensor. Fluorescence microscopic and TEM characterizations indicated that the Au–Ag alloy nanoparticles were mainly synthesized via an extracellular approach and generally existed in the form of irregular polygonal nanoparticles. Electrochemical investigations revealed that the vanillin sensor based on Au–Ag alloy nanoparticles modified glassy carbon electrode was able to enhance the electrochemical response of vanillin for at least five times. Under optimal working conditions, the oxidation peak current of vanillin at the sensor linearly increased with its concentration in the range of 0.2–50 μM with a low detection limit of 40 nM. This vanillin sensor was successfully applied to the determination of vanillin from vanilla bean and vanilla tea sample, suggesting that it may have practical applications in vanillin monitoring system. Biosensing of glucose oxidase was successful using AuNP-based biosensors (Zheng et al. 2010b). The authors used a facile green biosynthesis method to prepare AuNPs of various core sizes using a natural biomaterial, eggshell membrane (ESM) at ambient conditions. In situ synthesis of AuNPs-immobilized ESM was conducted in a simple manner by immersing ESM in a pH 6.0 aqueous solution of HAuCl_4 without adding any reductant. The formation of AuNPs on ESM protein fibers was attributed to the reduction of Au^{3+} ions to Au^0 by the aldehyde moieties of the natural ESM fibers. Energy dispersive X-ray spectroscopy, scanning electron microscopy, X-ray photoelectron spectroscopy, and X-ray powder diffraction unambiguously identified the presence of AuNPs on ESM. The effect of pH on the in situ synthesis of AuNPs on ESM was investigated in detail. The pH of the gold precursor (HAuCl_4) solution can influence the formation rate, dispersion and size of AuNPs on ESM. At $\text{pH} \leq 3.0$ and ≥ 7.0 , no AuNPs were observed on ESM while small AuNPs were homogeneously dispersed on ESM at $\text{pH} 4.0$ – 6.0 . The optimal pH for AuNP formation on ESM was 6.0. AuNPs/ESMs were used to immobilize glucose oxidase for glucose

biosensing. AuNPs on ESM can increase the enzyme activity of glucose oxidase. The linear response range of the glucose biosensor was 20 μM to 0.80 mM glucose with a detection limit of 17 μM . The biosensor was successfully applied to determine the glucose content in commercial glucose injections.

Mosquito larvicidal

Salunkhe et al. (2011b) used *Cochliobolus lunatus* mycosynthesized AgNPs against larvae of vectors *Aedes aegypti* and *Anopheles stephensi*. The efficacy of mycosynthesized AgNPs at all the tested concentrations (10, 5, 2.5, 1.25, 0.625, and 0.3125 ppm) against second, third, and fourth instar larvae of *A. aegypti* (LC_{50} 1.29, 1.48, and 1.58; LC_{90} 3.08, 3.33, and 3.41 ppm) and against *A. stephensi* (LC_{50} 1.17, 1.30, and 1.41; LC_{90} 2.99, 3.13, and 3.29 ppm) was observed, respectively. The mortality rates were positively correlated with the concentration of AgNPs. Significant ($P < 0.05$) changes in the larval mortality were also recorded between the period of exposure against fourth instar larvae of *A. aegypti* and *A. stephensi*. The possible larvicidal activity may be due to penetration of nanoparticles through membrane.

Current status and future prospects

There is great progress in microorganisms based synthesis of nanoparticles. Still, a lot of research needs to be done for improvement in processes of nanoparticle biosynthesis and applications. The process of nanoparticle synthesis by microorganisms is relatively slow as compared to physical and chemical approaches. Use of pathogenic microbes is not advisable for the nanoparticle synthesis as it can limit the use of produced particles for applications involving human or animal contacts. The produced nanoparticles should be non-toxic to living organisms and environment so investigation of toxicity using model organisms is vital. Stability of nanoparticles produced by microbes can be cause of concern. The investigation of microbes with ability to produce stable nanoparticles can be useful for developing reliable production processes. Some microbes can produce nanoparticles intracellularly with good features. However, obtaining such intracellular particles for applications may not be economical. The microbes with ability to synthesize nanoparticles extracellularly can be advantageous in this regard. The understanding on mechanism of microbial synthesis of nanoparticles can help to devise strategy for tailor made synthesis of nanoparticles according to desired shape and size. The rate of nanoparticle synthesis can differ with different species of microbes. Identification and characterization of microbial species with ability to synthesize nanoparticles at rapid rate are valuable. The growth medium,

synthesis conditions, substrate concentrations, pH, source compound of target nanoparticle, temperature, reaction time, irradiation, and agitation can have impact on rate of synthesis and control of size, shape, and uniformity of nanoparticles. Rapid synthesis of nanoparticles can be accomplished by choice of most suitable microbial strain and optimization of different conditions required for microbial synthesis. Scaling up of microbe based nanoparticle production processes from laboratory scale to large scale production processes can be important for increasing availability of nanoparticles for variety of applications. Therefore, a large scale screening of non-pathogenic microbes with ability to synthesize stable nanoparticles at rapid rate and development of large scale production processes are urgently needed.

Conclusion

In the present review, the synthesis of nanoparticles by microorganisms including probable biosynthesis mechanism, conditions for size/shape control, reaction rate enhancement, and various applications of nanoparticles, limitations and future prospects were briefly discussed. Different types of microorganisms such as fungi, yeast, bacteria, and actinomycetes have abilities to produce diverse nanoparticles such as Ag, Au, lead, Ag₂O, zirconia, CdS, barium titanate, CdSe quantum dots, silica, titanium, nickel oxide, iron, iron oxide (Fe₂O₃), copper, MnO₂, ZnO, iron phosphate (FePO₄), palladium, iron sulfide (FeS), siderite (FeCO₃), cadmium sulfide, TiO₂, titanium, magnetite (Fe₃O₄), greigite (Fe₃S₄), zinc sulfide, and Pt. The size of nanoparticles can range from 1 to 500 nm and shapes were mostly spherical, but other shapes such as pseudo-spherical, rod, triangular, truncated triangular, square, pentagonal, hexagonal, nanoplate, fine-grained crystal, and nanowires morphologies have also been observed. The microbially synthesized nanoparticles display antimicrobial, antibacterial, anti-biofilm, anti-biofouling, antiviral, anticancer, and photocatalytic properties. Considering the usefulness of nanoparticles for these various applications, they have promise to be used in food, cosmetic, or pharmaceutical field.

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