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# DIVERSITY OF MICROBES IN SYNTHESIS OF METAL NANOPARTICLES: PROGRESS AND LIMITATIONS

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## 1.1. INTRODUCTION

Nanotechnology is a widely emerging field involving interdisciplinary subjects such as biology, physics, chemistry, and medicine (Bankar et al., 2010; Zhang, 2011; Rai and Ingle, 2012). Nanotechnology involves the synthesis of nanoparticles using the top-down and bottom-up approach (Kasthuri et al., 2008; Bankar et al., 2010; Nagajyothi and Lee, 2011). However, due to the growing environmental concern and the adverse

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effects of physical and chemical synthesis, most researchers are looking to the biological protocols for nanoparticle synthesis (Rai et al., 2008). The biological method of synthesis involves a wide diversity of biological entities that could be harnessed for the synthesis of metal nanoparticles (Sharma et al., 2009; Vaseeharan et al., 2010; Zhang et al., 2011a; Gupta et al., 2012; Rajesh et al., 2012). These biological agents emerge as an environmentally friendly, clean, non-toxic agent for the synthesis of metal nanoparticles (Sastri et al., 2003; Bhattacharya and Gupta, 2005; Riddin et al., 2006; Duran et al., 2007; Ingle et al., 2008; Kumar and Yadav, 2009; Vaseeharan et al., 2010; Thakkar et al., 2011; Zhang et al., 2011b; Rajesh et al., 2012).

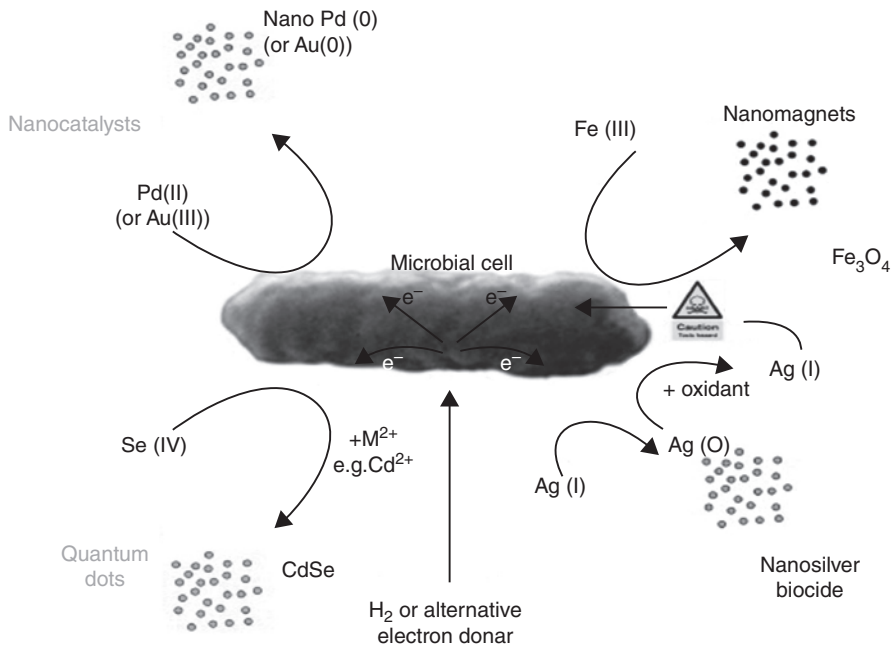
A wide array of microorganisms such as bacteria, fungi, yeast, algae, and actinomycetes are majorly employed as biological agents for the synthesis process (Kumar and Yadav, 2009; Satyavathi et al., 2010). The synthesis of metal nanoparticles employs both intracellular and extracellular methods (Sharma et al., 2009; Mallikarjuna et al., 2011). Some examples of these microbial agents include bacteria (Husseiny et al., 2007; Shahverdi et al., 2007, 2009), fungi (Kumar et al., 2007; Parikh et al., 2008; Gajbhiye et al., 2009), actinomycetes (Ahmad et al., 2003a; Golinska et al., 2014), lichens (Shahi and Patra, 2003), and algae (Singaravelu et al., 2007; Chakraborty et al., 2009). These diverse groups of biological agents have many advantages over physical and chemical methods such as easy and simple scale-up, easy downstream processing, simpler biomass handling and recovery, and economic viability (Rai et al., 2009a; Thakkar et al., 2011; Renugadevi and Aswini, 2012). These different biological agents such as bacteria, fungi, yeast, algae, and actinomycetes therefore demonstrate immense biodiversity in the synthesis of nanoparticles and lead to green nanotechnology (Vaseeharan et al., 2010; Singh et al., 2011, 2013; Thakkar et al., 2011).

The present review also deals with the diversity of microbes involved in the synthesis of metal nanoparticles. The possible mechanisms and different applications for the synthesis of metal nanoparticles are also discussed.

## 1.2. SYNTHESIS OF NANOPARTICLES BY BACTERIA

Although it is known that bacteria have the ability to produce various inorganic nanoparticles (e.g., metal, calcium, gypsum, silicon), research in this area is usually focused on the formation of metals and metals sulfide/oxide (Fig. 1.1).

Different bacteria from different habitats and nutritional modes have been studied for the synthesis of metallic nanocrystals, as summarized in Table 1.1. Some of the earliest reports on the reduction and accumulation of inorganic particles in bacteria can be traced back to the 1960s, where zinc sulfide was described in sulfate-reducing bacteria (Temple and Le-Roux, 1964). Later studies in this area date back to the 1980s, when Beveridge and Murray (1980) described how the incubation of gold chloride with *Bacillus subtilis* resulted in the production of octahedral gold nanoparticles with a dimension of 5–25 nm within the bacterial cell. It is believed that organophosphate compounds secreted by the bacterium play an important role in the formation of these nanostructures (Southam and Beveridge, 1996). Another example



Current opinion in biotechnology

**Figure 1.1.** Mechanisms of microbial fabrication of nanobiominerals, catalyzed by enzymatic reductive biotransformations of redox active metals, driven by a suitable electron donor such as hydrogen. In some cases, for example transformations of Fe(III) minerals and Se(IV), redox mediators such as AQDS (anthraquinone-2,6 disulfonate) are utilized to increase the kinetics of metal reduction and hence nanobiomineral formation. Source: Lloyd, J.R., Byrne, J.M., Coker, V.S. 2011. Biotechnological synthesis of functional nanomaterials. *Current Opinion in Biotechnology* 22: 509–515. Copyright © 2011, Elsevier. See insert for color representation of the figure.

of bacterial reduction and precipitation of gold was described by Kashefi and co-workers (2001). These authors demonstrated that iron-reducing anaerobic bacteria *Shewanella algae* can reduce gold ions in the presence of  $H_2$  gas, which results in the formation of 10–20 nm gold nanoparticles. It was further hypothesized that specific hydrogenase might be involved in the reduction of gold ions when hydrogen was used as an electron donor.

Karthikeyan and Beveridge (2002) observed microbial reduction of gold ions by *Pseudomonas aeruginosa*, resulting in intracellular accumulation of gold nanoparticles with a particle diameter of 20 nm. In another study, it was shown that bacterium *Rhodospseudomonas capsulate* was capable of reducing gold ions into gold nanoparticles (He et al., 2007). When the biomass of *R. capsulate* was incubated with gold ions at neutral pH, gold nanoparticles of 10–20 nm size were formed. Further, when the same reaction was carried out under acidic pH conditions, triangular gold particles

TABLE 1.1. List of Different Metallic Nanoparticles Synthesized by Bacteria

Metallic material	Bacteria (reference)
Au <sup>0</sup>	<i>Bacillus subtilis</i> (Beveridge and Murray, 1980); <i>Shewanella algae</i> (Kashefi et al., 2001); <i>Rhodopseudomonas capsulate</i> (Kashefi et al., 2001; He et al., 2007, 2008); <i>Pseudomonas aeruginosa</i> (Karthikeyan and Beveridge, 2002); <i>Lactobacilli</i> strains (Nair and Pradeep, 2002); <i>Thermomonospora</i> sp. (Ahmad et al., 2003b); <i>Rhodococcus</i> sp. (Ahmad et al., 2003a); <i>Ralstonia metallidurans</i> (Reith et al., 2006); <i>Actinobacter</i> sp. (Bharde et al., 2007); <i>Streptomyces viridogens</i> strain HM10 (Balagurunathan et al., 2011); <i>Streptomyces griseus</i> (Derakhshan et al., 2012); <i>Streptomyces hygrosopicus</i> (Sadhasivam et al., 2012); <i>Streptomyces</i> sp. ERI-3 (Zonooz et al., 2012)
Ag <sup>0</sup>	<i>Pseudomonas stutzeri</i> A259 (Klaus et al., 1996; Joerger et al., 2000); <i>Corynebacterium</i> sp. SH09 (Zhang et al., 2005); <i>Enterobacteriaceae</i> ( <i>Klebsiella pneumoniae</i> , <i>E. coli</i> and <i>Enterobacter cloacae</i> ) (Shahverdi et al., 2007); <i>Morganella spp.</i> (Parikh et al., 2008); <i>Bacillus licheniformis</i> (Kalishwaralal et al., 2008); <i>Lactobacillus fermentum</i> (De-Gusseme et al., 2010); <i>Morganella psychrotolerans</i> (Ramanathan et al., 2011); <i>Escherichia coli</i> AUCAS 112 (Kathiresan et al., 2010); <i>Idiomarina</i> sp. PR58-8 (Seshadri et al., 2012)
Fe <sub>3</sub> S <sub>4</sub>	<i>M. magnetotacticum</i> (Mann et al., 1984; Philipse and Maas, 2002); <i>Magnetospirillum</i> (Farina et al., 1990); Sulfate-reducing bacteria (Mann et al., 1990); <i>M. gryphiswaldense</i> (Lang et al., 2006); <i>Acinetobacter</i> sp. (Bharde et al., 2008)
Fe <sub>3</sub> O <sub>4</sub> , Fe <sub>2</sub> O <sub>3</sub>	Magnetotactic bacteria (Blakemore, 1975; Mann et al., 1984); <i>Geobacter metallireducens</i> (Vali et al., 2004); <i>Actinobacter</i> sp. (Bharde et al., 2005)
Pt <sup>0</sup>	<i>Shewanella algae</i> (Konishi et al., 2007)
Pd <sup>0</sup>	<i>Desulfovibrio desulfuricans</i> (Yong et al., 2002a,b)
Cu <sup>0</sup>	<i>Serratia</i> sp. (Hasan et al., 2008); <i>E. coli</i> (Singh et al., 2010)
Co <sub>3</sub> O <sub>4</sub>	Marine cobalt-resistant bacterial strain (Kumar et al., 2008)
CdS	<i>Clostridium thermoaceticum</i> (Cunningham and Lundie, 1993); <i>R. palustris</i> (Bai et al., 2009)
ZnS	Sulfate-reducing bacteria (Labrenz et al., 2000)
Se <sup>0</sup>	<i>Thauera selenatis</i> (DeMoll-Decker and Macy, 1993; Bledsoe et al., 1999; Sabaty et al., 2001); <i>Rhizobium selenitireducens</i> strain B1 (Hunter and Kuykendall, 2007; Hunter et al., 2007); <i>E. coli</i> (Avazeri et al., 1997); <i>Clostridium pasteurianum</i> (Yanke et al., 1995); <i>Bacillus selenitireducens</i> (Afkar et al., 2003); <i>Pseudomonas stutzeri</i> (Lortie et al., 1992); <i>Wolinella succinogenes</i> (Tomei et al., 1992); <i>Enterobacter cloacae</i> (Losi and Frankenberger, 1997); <i>Pseudomonas aeruginosa</i> (Yadav et al., 2008); <i>Pseudomonas alkaphila</i> (Zhang et al., 2011a)
Te <sup>0</sup>	<i>Sulfurospirillum barnesii</i> , <i>B. selenireducens</i> (Baesman et al., 2007)
Ti <sup>0</sup>	<i>Lactobacillus</i> sp. (Prasad et al., 2007), <i>Bacillus</i> sp. (Prakash et al., 2009)
UO <sub>2</sub>	<i>Micrococcus lactilyticus</i> (Woolfolk and Whiteley, 1962); <i>Alteromonas putrefaciens</i> (Myers and Nealson, 1988); <i>G. metallireducens</i> GS-15 (Lovley et al., 1991); <i>S. oneidensis</i> MR-1 (Marshall et al., 2006); <i>Desulfosporosinus</i> sp. (O'Loughlinej et al., 2003)

with an edge length of c. 500nm were obtained as well as the spherical gold nanoparticles (He et al., 2008).

The ability of bacterium *Ralstonia metallidurans* to precipitate colloidal gold nanoparticles from aqueous gold chloride solution has recently been reported, but the exact mechanism of this process is not yet clear (Reith et al., 2006). In a series of reports, Sastry's group screened different bacterial strains for the biosynthesis of gold and silver nanoparticles with control over morphologies and size distribution. It was shown that alkalothermophilic actinomycete, *Thermomonospora* sp., synthesized spherical gold nanoparticles of size 8nm with a narrow size distribution (Ahmad et al., 2003b). In other case, an alkalotolerant actinomycete, *Rhodococcus* sp., was reported for the formation of nearly monodisperse 10nm gold nanoparticles (Ahmad et al., 2003a). Further study demonstrated that the size and shape of gold nanoparticles could be controlled by adjusting the reaction parameters. Gold nanoparticles with variable size and shape were obtained using a strain of actinomycetes, *Actinobacter* sp. (Bharde et al., 2007). When this strain reacted with gold ions in the absence of molecular oxygen, gold nanoparticles of triangular and hexagonal shapes were produced along with some spherical particles. It was consequently concluded that protease enzyme secreted by *Actinobacter* sp. was responsible for the reduction of gold ions. Moreover, it was described how molecular oxygen slows down the reduction of gold ions in an unknown manner, possibly by inhibiting the action of protease secreted by this strain. Other bacterial systems able to reduce gold ions to make nanoparticles include: *Streptomyces viridogens* strain HM10 (Balagurunathan et al., 2011), *Streptomyces griseus* (Derakhshan et al., 2012), *Streptomyces hygroscopicus* (Sadhasivam et al., 2012), and *Streptomyces* sp. ERI-3 (Zonooz et al., 2012). The reduction appears to be initiated via electron transfer from NADH-dependent enzymes as an electron carrier.

Some bacteria have been reported for the formation of more than one metal nanoparticle and bimetallic alloy. Nair and Pradeep (2002) synthesized nanoparticles of gold, silver, and their alloys using different *Lactobacillus* strains, lactic acid-producing bacteria, and the active bacterial component of buttermilk. It is well known that ionic silver is highly toxic to most microbial cells. Nonetheless, several bacterial strains have been reported to be silver resistant. *Pseudomonas stutzeri* A259, isolated from a silver mine in Utah (USA), was the first bacterial strain with reductive potential to form silver crystals (Klaus et al., 1996; Joerger et al., 2000). This bacterium produced a small number of crystalline  $\alpha$ -form silver sulfide acanthite ( $\text{Ag}_2\text{S}$ ), crystallite particles with the composition of silver and sulfur at a ratio of 2:1. The resistance mechanism of *P. stutzeri* towards silver ions is poorly understood however; several groups postulated that efflux cellular pumps might be involved in the formation of silver nanoparticles (Silver, 2003; Ramanathan et al., 2011). Parikh and co-workers (2008) used *Morganella* sp., a silver-resistant bacterium for the synthesis of silver nanoparticles, and established a direct correlation between the silver-resistance machinery of this bacteria and silver nanoparticles biosynthesis. Three silver-resistant homologue genes (*silE*, *silP*, and *silS*) were recognized in *Morganella* sp. The presence of these genes suggested that this organism has a unique mechanism for protection against the toxicity of silver ions that involves the formation of silver nanoparticles. Similarly, the intracellular

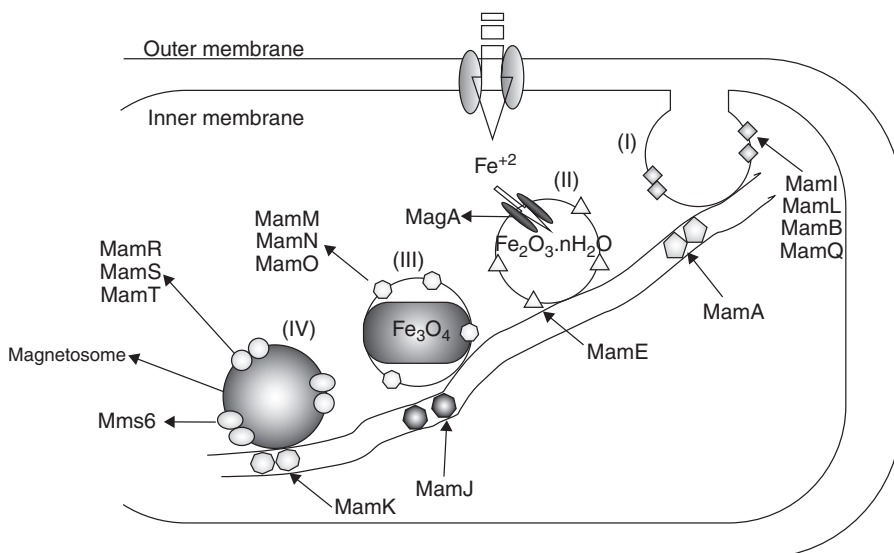
production of silver nanoparticles by the highly silver-tolerant marine bacterium, *Idiomarina* sp. PR58-8, was described by Seshadri et al. (2012). The strain of *Escherichia coli* AUCAS 112 isolated from mangrove sediments is also capable of reducing the silver ions at a faster rate (Kathiresan et al., 2010).

Zhang and co-workers (2005) demonstrated that dried cells of *Corynebacterium* sp. SH09 produced silver nanoparticles at 60 °C in 72h on the cell wall in the size range of 10–15 nm with diamine silver complex  $[\text{Ag}(\text{NH}_3)_2]^+$ . The culture supernatants of *Enterobacteriaceae* (*Klebsiella pneumoniae*, *E. coli*, and *Enterobacter cloacae*) also formed silver nanoparticles by reducing  $\text{Ag}^+$  to  $\text{Ag}^0$ . With the addition of piperitone, silver ions reduction was partially inhibited, which suggested the involvement of nitro-reductase in this process (Shahverdi et al., 2007). Similarly, the formation of silver nanoparticles by *Bacillus licheniformis* (Kalishwaralal et al., 2008), *Lactobacillus fermentum* (De-Gusseme et al., 2010), and psychro-tolerant bacteria *Morganella psychrotolerans* (Ramanathan et al., 2011) was described.

Attempts to synthesize metallic nanoparticles such as iron, platinum, palladium, copper, selenium, and uranium have only been made recently. Most of the work in this area has been oriented towards synthesis of magnetite nanoparticles. For instance, a simple and green approach for the synthesis of iron sulfide has been shown in bacteria such as *Magnetospirillum* (Farina et al., 1990), *M. magnetotacticum* (Mann et al., 1984; Philipse and Maas, 2002), sulfate-reducing bacteria (Mann et al., 1990), *M. gryphiswaldense* (Lang and Schüler, 2006), and *Acinetobacter* sp. (Bharde et al., 2008). Nanoparticles formed by these strains showed predominant morphologies of octahedral prism, cubo-octahedral, and hexagonal prism in the size range 2–120 nm. The results obtained indicated that bacterial sulfate reductases are responsible for the process. The possibility of using bacteria for the formation of iron oxide nanoparticles has also been studied. Magnetotactic bacteria (Blakemore, 1975; Mann et al., 1984), iron-reducing bacteria *Geobacter metallireducens* (Vali et al., 2004), and *Actinobacter* sp. (Bharde et al., 2005) were able to form magnetite ( $\text{Fe}_3\text{O}_4$ ) or maghemite ( $\gamma\text{-Fe}_2\text{O}_3$ ).

The process of magnetic nanoparticles mineralization can be divided into four steps: (1) vesicle formation and iron transport from outside of the bacterial membrane into the cell; (2) magnetosomes alignment in a chain; (3) initiation of crystallization; and (4) crystal maturation (Fig. 1.2; Faramarzi and Sadighi, 2013).

Platinum and palladium nanoparticles have also been explored as they are interesting materials with a wide range of applications. Konishi et al. (2007) demonstrated platinum nanoparticle synthesis by the resting cells of *Shewanella algae* in the presence of  $\text{N}_2\text{-CO}_2$  gas mixture. *S. algae* were able to reduce  $\text{PtCl}_6^{2-}$  ions to platinum nanoparticles during 60 minutes of reaction time at neutral pH and 25 °C. It should be noted that the underlying biochemical process as to how these organisms are able to synthesize platinum nanoparticles is still unclear and remains an open challenge. The first report for the synthesis of palladium nanoparticles appeared in early 2000 using a sulfate-reducing bacterium *Desulfovibrio desulfuricans* (Yong et al., 2002a,b). The formation of palladium nanoparticles of approximately 50 nm in size in the presence of molecular hydrogen or formate as an electron donor when reacted with aqueous palladium ions at pH 2–7 was demonstrated.



**Figure 1.2.** Magnetosome bio-mineralization in magnetotactic bacteria (MTB). (I) MamI, MamL, MamB, and MamQ proteins initiate the membrane invagination and form a vesicular membrane around the magnetosome structure. (II) The protease-independent function of MamE recruits other proteins such as MamK, MamJ, and MamA to align magnetosomes in a chain. (III) Iron uptake occurs via MagA, a transmembrane protein, and initiation of magnetic crystal bio-mineralization occurs through MamM, MamN, and MamO proteins. (IV) Finally, MamR, MamS, MamT, MamP, MamC, MamD, MamF, MamG, the protease-dependent function of MamE, and Mms6, a membrane tightly bounded by GTP-ase, regulate crystal growth and determine morphology of the produced magnetic nanoparticles. Source: Faramarzi, M.A., Sadighi, A. 2013. Insights into biogenic and chemical production of inorganic nanomaterials and nanostructures. *Advances in Colloid and Interface Science* 189–190: 1–20. Copyright © 2013, Elsevier. See insert for color representation of the figure.

Gram-negative rod, such as *Serratia* sp. (Hasan et al., 2008) isolated from insect gut and *Escherichia coli* (Singh et al., 2010) when challenged with aqueous copper precursors, were able to synthesize a mixture of copper and copper oxide quasi-spherical nanoparticles of size range 10–40 nm. It was postulated that two proteins/peptides of molecular weight 25 kDa and 52 kDa might be involved in the reduction and stabilization of these nanoparticles. Kumar et al. (2008) reported the extracellular synthesis of ferromagnetic  $\text{Co}_3\text{O}_4$  nanoparticles by a marine cobalt-resistant bacterium isolated from the Arabian Sea.

Different bacterial strains have also been explored for the synthesis of semiconductors (so-called quantum dots), such as CdS and ZnS. Production of CdS nanocrystals was observed in the case of *Clostridium thermoaceticum* (Cunningham and Lundi, 1993), *K. pneumoniae* (Smith et al., 1998), *E. coli* (Sweeney et al., 2004), and *R. palustris* (Bai et al., 2009). It is believed that bacterial cysteine desulfhydrase is responsible for the formation of CdS nanoparticles. Labrenz et al. (2000) has demonstrated that

sphalerite crystals (ZnS) are produced within natural biofilms, which are dominated by the sulfate-reducing bacteria.

Selenium and tellurium respiration studies have fascinated scientists as these ions are rarely in contact with microbes in their natural environment. Some studies have suggested that  $\text{SeO}_3^{2-}$  reduction may involve the periplasmic nitrite reductase in *Thauera selenatis* (DeMoll-Decker and Macy, 1993; Bledsoe et al., 1999; Sabaty et al., 2001) and *Rhizobium selenitireducens* strain B1 (Hunter and Kuykendall, 2007; Hunter et al., 2007), nitrate reductase in *E. coli* (Avazeri et al., 1997), hydrogenase I in *Clostridium pasteurianum* (Yanke et al., 1995), and arsenate reductase in *Bacillus selenitireducens* (Afkari et al., 2003) or some of the non-enzymatic reactions (Tomei et al., 1992). Most research on the biogenesis of selenium nanoparticles are based on anaerobic systems. However, there are also a few reports in the literature on the aerobic formation of these nanostructures by bacteria such as: *Pseudomonas stutzeri* (Lortie et al., 1992), *Enterobacter cloacae* (Losi and Frankenberger, 1997), *Pseudomonas aeruginosa* (Yadav et al., 2008), *Bacillus* sp. (Prakash et al., 2009), and *Pseudomonas alkaphila* (Zhang et al., 2011a). Another metalloid semiconductor, tellurium (which belongs to the chalcogen family), has been reduced from tellurite to elemental tellurium by two anaerobic bacteria, *Sulfurospirillum barnesii* and *B. selenitireducens*. Interestingly, the two different species yielded a different morphology of tellurium nanoparticles. *S. barnesii* formed extremely small nanospheres of diameter less than 50 nm that coalesced to form large aggregates, while *B. selenitireducens* initially formed nanorods of approximately 10 nm in diameter by 200 nm length, which clustered together forming large rosettes (c. 1000 nm) made from individual shards of 100 nm width by 1000 nm length (Baesman et al., 2007). Titanium nanoparticles of spherical aggregates of 40–60 nm were also produced extracellularly using the culture filtrate of *Lactobacillus* sp. at room temperature (Prasad et al., 2007).

Another interesting metal, especially in its nanoparticulate form, is uranium. In the same way as for gold, uranium is soluble in the oxidized form, U(VI); the reduced form of uranium, U(IV), is insoluble however. Among the first reports of U(IV) synthesis, Woolfolk and Whiteley (1962) found that the cell-free extracts of *Micrococcus lactilyticus* reduced U(VI) to U(IV). *Alteromonas putrefaciens* grown in the presence of hydrogen as an electron donor and U(VI) as an electron acceptor also reduced U(VI) to U(IV) (Myers and Nealson, 1988). In line with these observations, Lovley et al. (1991) demonstrated that *G. metallireducens* GS-15, when grown anaerobically in the presence of acetate and U(VI) as electron donor and electron acceptor, reduced soluble uranium U(VI) to insoluble U(IV) oxidizing acetate to  $\text{CO}_2$ . Marshall and co-workers (2006) found out that c-type cytochrome (MtrC) on the outer membrane and extracellular of dissimilatory metal-reducing bacterium *S. oneidensis* MR-1 was involved in the reduction of U(VI) predominantly with extracellular polymeric substance as UO<sub>2</sub>-EPS in cell suspension and intracellularly in periplasm. *Desulfosporosinus* sp., a gram-positive sulfate-reducing bacterium isolated from sediments when incubated with mobile hexavalent uranium U(VI) reduced to tetravalent uranium U(IV) which precipitated uraninite. These uraninite ( $\text{UO}_2$ ) crystals coated on the cell surface of *Desulfosporosinus* sp. were within the size range  $1.7 \pm 0.6$  nm (O'Loughlinej et al., 2003).



### 1.3. SYNTHESIS OF NANOPARTICLES BY FUNGI

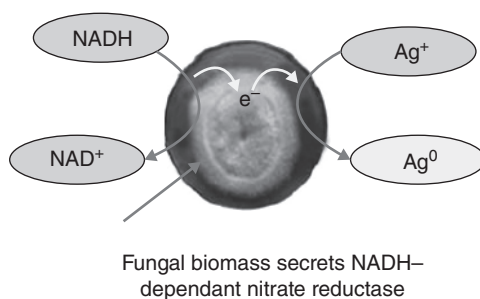
Fungi have also been used for the synthesis of different kinds of metal nanoparticles (Basavaraja et al., 2008; Bawaskar et al., 2010; Raheman et al., 2011). Rai et al. (2009b) proposed the term “myconanotechnology” to describe research carried out on nanoparticles synthesized by fungi, the integrated discipline of mycology and nanotechnology. Many fungal species have so far been exploited for the synthesis of metal nanoparticles, including endophytic fungi.

Ahmad et al. (2003c) exploited *Fusarium oxysporum* for the synthesis of silver nanoparticles. They reported that when exposed to the fungus *F. oxysporum*, aqueous silver ions were reduced in solution thereby leading to the formation of an extremely stable silver hydrosol. The silver nanoparticles were in the range of 5–15 nm in dimensions and stabilized in solution by proteins secreted by the fungus. Exposure of *F. oxysporum* to an aqueous solution of  $K_2ZrF_6$ , resulting in the protein-mediated extracellular hydrolysis of zirconium hexafluoride anions at room temperature, leading to the formation of crystalline zirconia nanoparticles, was reported by Bansal et al. (2004). They concluded that as *F. oxysporum* is a plant pathogen which is not exposed to such ions during its life cycle, it secretes proteins capable of hydrolyzing  $ZrF_6^{2-}$ .

Bansal et al. (2005) reported the synthesis of silica and titania nanoparticles by *F. oxysporum* when the fungal cell filtrate was challenged with their respective salts, that is,  $K_2SiF_6$  and  $K_2TiF_6$ . The resulting nanoparticles formed were in the range of 5–15 nm and had an average size of  $9.8 \pm 0.2$  nm. Duran et al. (2005) studied the production of metal nanoparticles by several strains of *F. oxysporum*. They found that when aqueous silver ions were exposed to the cell filtrate of *F. oxysporum*, they reduced to the formation of silver nanoparticles. The resulting silver nanoparticles were in the range of 20–50 nm. They hypothesized that the reduction of the metal ions occurs by a nitrate-dependent reductase enzyme and a shuttle quinone extracellular process. *F. oxysporum* f. sp. *lycopersici*, causing wilt in tomato, was exploited for intracellular and extracellular production of platinum nanoparticles (Riddin et al., 2006). Bharde et al. (2006) reported the synthesis of magnetite nanoparticles from *F. oxysporum*. *F. acuminatum* isolated from infected ginger was successfully exploited for the mycosynthesis of silver nanoparticles by Ingle et al. (2008). Ingle and coworkers (2008) also proposed the hypothetical mechanism for the synthesis of silver nanoparticles. According to them, NADH-dependent nitrate reductase played an important role in the synthesis of nanoparticles (Fig. 1.3).

They reported the formation of polydispersed, spherical nanoparticles in the range of 4–50 nm with average diameter of 13 nm. Ingle et al. (2009) used *F. solani* isolated from infected onion for the synthesis of silver nanoparticles; again, polydispersed, spherical nanoparticles were synthesized in the size range of 5–35 nm.

Soil-born fungus *Aspergillus fumigatus* is also reported to produce the silver nanoparticles extracellularly when the cell extract was challenged with aqueous silver ions (Bhainsa and D'Souza, 2006). Gade et al. (2008) reported the biosynthesis of silver nanoparticles from *A. niger* isolated from soil, and also suggested the mechanism for the action of silver nanoparticles on *E. coli*. Similarly, Jaidev and Narsimha



**Figure 1.3.** Possible mechanism of enzymatic reduction of the silver ions. Source: Ingle, A., Gade, A., Pierrat, S., Sonnichsen, C., Rai, M.K. *Mycosynthesis of silver nanoparticles using the fungus *Fusarium acuminatum* and its activity against some human pathogenic bacteria. Current Nanoscience 4: 141–144. Copyright © 2008, Bentham Science Publishers.*

(2010) reported the synthesis of silver nanoparticles using *A. niger*. Vigneshwaran et al. (2007) reported the potential of *A. flavus* for the intracellular production of silver nanoparticles; when treated with aqueous silver ions, the silver nanoparticles were synthesized in the cell wall. Moharrer and co-workers (2012) reported the extracellular synthesis of silver nanoparticles using *A. flavus* which was isolated from soil of Ahar copper mines. Extracellular biosynthesis of silver nanoparticles was studied using the fungus *Cladosporium cladosporioides* (Balaji et al., 2009). The transmission electron microscope (TEM) image showed polydispersed and spherical particles with size ranges of 10–100 nm.

Mukherjee et al. (2001) were the first to report the use of fungal systems for the synthesis of silver and gold nanoparticles. They observed for *Verticillium* sp. that the reduction of the metal ions occurred intracellularly, leading to the formation of gold and silver nanoparticles in the size range of 2–20 nm. Sastry et al. (2003) also used fungal systems and actinomycetes for the synthesis of silver nanoparticles and gold nanocrystals by intra- and extracellular processes, respectively. They reported a number of silver nanoparticles on the surface of the mycelial wall of *Verticillium*. The mycelia also showed an individual *Verticillium* cell with silver particles clearly bound to the surface of the cytoplasmic membrane, confirming the intracellular formation of the silver nanoparticles. TEM analysis showed the formation of the silver nanoparticles in *Verticillium* cells of average diameter  $25 \pm 12$  nm. In case of *Thermomonospora* sp. when exposed to aqueous gold ions, the metal ions were reduced extracellularly, yielding gold nanoparticles with a much-improved polydispersity in the range of 7–12 nm. Bharde et al. (2006) reported the formation of nanoparticulate magnetite at room temperature extracellularly, after the treatment of cell filtrate of fungus *Verticillium* sp. with mixtures of ferric and ferrous salts. Extracellular hydrolysis of the anionic iron complexes by cationic proteins secreted by the fungi results in the room-temperature synthesis of crystalline magnetite particles that exhibit the signature of a ferromagnetic transition with a negligible amount

of spontaneous magnetization at low temperature. TEM analysis revealed a number of cubo-octahedrally shaped iron oxide particles ranging in size from 100 to 400 nm.

Chen et al. (2003) studied the extracellular formation of silver nanoparticles using *Phoma* sp. 3.2883. Another species of *Phoma* used for the synthesis of silver nanoparticles was *P. glomerata* (Birla et al., 2009). In this study, the authors reported extracellular synthesis of silver nanoparticles when the fungal cell filtrate was treated with aqueous silver ions (silver nitrate, 1 mM) and incubated at room temperature. The silver nanoparticles synthesized were found to be in the range of 60–80 nm when the colloidal solution of these silver nanoparticles was analyzed using scanning electron microscopy (SEM).

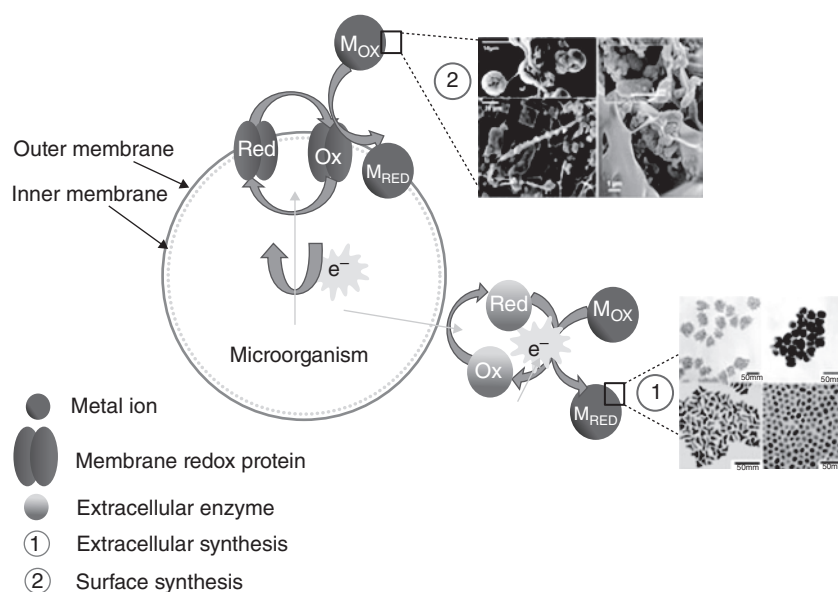
An endophytic fungus *Colletotrichum* sp., growing in the leaves of Geranium, produced gold nanoparticles when exposed to chloroaurate ions. These particles are predominantly decahedral and icosahedral in shape, ranging in size from 20 to 40 nm (Shivshankar et al., 2003). Lichen fungi (*Usnea longissima*) have shown synthesis of bioactive nanoparticles (usnic acid) in specified medium (Shahi and Patra, 2003).

Ahmad et al. (2005) reported the ability of fungus *Trichothecium* sp. for the synthesis of gold nanoparticles both intra- and extracellularly. When kept in a stationary condition, the fungal biomass resulted in rapid extracellular synthesis of gold nanoparticles with spherical, rod-like, and triangular morphology; when the biomass was shaken on a rotary shaker it resulted in the intracellular synthesis of nanoparticles. The authors therefore concluded that the enzymes and proteins which are released in the stationary phase are not released under shaking conditions, resulting in the intracellular and extracellular synthesis of gold nanoparticles.

The agriculturally important fungus *Trichoderma asperellum* was used to depict the capacity of extracellular synthesis of silver nanoparticles. The silver nanoparticles were in the range of 13–18 nm with stability of up to 6 months due to the binding of proteins as capping legands. Synthesis of silver nanoparticles by yeast strain MKY<sub>3</sub> has been reported, leading to particles ranging 2–5 nm in size. The extracellular synthesis of nanoparticles was useful, as nanoparticles could be obtained in large quantities (Kowshik et al., 2003).

Das and Marsili (2010) reviewed the role of bacteria and fungi in the synthesis of metal nanoparticles, and also suggested the possible mechanism of the synthesis of nanoparticles using microorganisms (Fig. 1.4). According to them, natural processes such as biomineralization may be mimicked to design efficient nanoparticle synthesis techniques. Biomineralization processes exploit biomolecular templates that interact with the inorganic material throughout its formation, resulting in the synthesis of particles of defined shape and size.

Many successful attempts have been made for the synthesis of metal nanoparticles using different fungi such as *A. foetidus*-MTCC-8876 (Roy et al., 2013), *Penicillium citrinum* (Honary et al., 2013), and *Epicoccum nigrum* (Qian et al., 2013) for the synthesis of silver nanoparticles and *Phanerochaete chrysosporium* (Sanghi et al., 2011) and *F. oxysporum* f. sp. *cubense* JT1 (Thakker et al., 2013) for gold nanoparticles synthesis.



**Figure 1.4.** Biomineralization process for nanoparticle synthesis. Source: Das, S.K., Marsili, E. A green chemical approach for the synthesis of gold nanoparticles: characterization and mechanistic aspect. *Reviews in Environmental Science and Biotechnology* 9: 199–204. Copyright © 2010, Springer. See insert for color representation of the figure.

## 1.4. SYNTHESIS OF NANOPARTICLES BY ALGAE

The synthesis of metal nanoparticles using algae is relatively unexplored, but it is a more biocompatible method than the other biological methods. The process of synthesis of metal nanoparticle using algal extract was found to be a rapid and non-toxic process (Jena et al., 2013). Many scientists therefore became interested in the use of algal systems for successful synthesis of metal nanoparticles. Merin et al. (2010) exploited four different micro algae – *Chaetoceros calcitrans*, *Chlorella salina*, *Isochrysis galbana*, and *Tetraselmis gracilis* – for the synthesis of silver nanoparticles. Synthesized nanoparticles were also checked for their antibacterial potential against human pathogens (*Klebsiella* sp., *Proteus vulgaricus*, *Pseudomonas aeruginosa*, and *E. coli*). The silver nanoparticles produced by the above mentioned algal species showed significant activity with all four bacteria (Merin et al., 2010). In recent years, Kalabegishvili et al. (2012) reported the reduction of chloroaurate ( $\text{HAuCl}_4$ ) into gold nanoparticles when expose to blue-green algae *Spirulina platensis*. Similarly, Mahdieh et al. (2012) used the same algae (*Spirulina platensis*) for the synthesis of silver nanoparticles by treating an aqueous solution of silver ions with a live biomass of *S. platensis*. The transmission electron studies carried out for nanoparticles showed the production of silver nanoparticles with an average size of ~ 12 nm.

Jena and co-workers (2013) described how not only the extract but also whole cells of algal biomass can be used for the synthesis of silver nanoparticles. In their

study, they used fresh extract and whole cell of microalga *Chlorococcum humicola*. The *in vivo* and *in vitro* formation of nanoparticles were characterized using different analytical methods including ultraviolet–visible (UV-vis) spectroscopy, scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), and transmission electron microscopy (TEM), confirming the synthesis of nanoparticles of 16 nm in size. These biosynthesized silver nanoparticles were found to be significantly active against *E. coli*.

There are also some other reports suggesting the potential of crude extracts of some microalgae such as *Kappaphycus alvarezii* and *Gelidiella acerosa* for the production of metallic nanoparticles, which could be used for medicinal purposes (Rajasulochana et al., 2010; Vivek et al., 2011). These investigations imply that the vast types of algae most commonly found can be efficiently used for the synthesis of nanoparticles in an environmentally friendly approach.

Cyanobacteria, also called blue-green algae, are a well-known group of aquatic and photosynthetic microorganisms. They are also the source organisms for the synthesis of several types of nanoparticles. Very few studies have yet been performed on cyanobacteria-mediated synthesis of nanoparticles. However, these bacteria are a good source of the enzymes responsible for the catalysis reaction that leads to the reduction of ions to their corresponding nanoparticles (Mubarak-Ali et al., 2011). Various cyanobacteria have been found to be capable of synthesizing nanoparticles. Lengke et al. (2006a) investigated the synthesis of silver nanoparticles by using *Plectonema boryanum*. The investigators proposed the involvement of the cyanobacterial metabolites in the synthesis of silver nanoparticles from its salt.

Cyanobacteria are thought to synthesize silver nanoparticles intracellularly. During this synthesis, the silver ions become reduced to neutral silver with the help of the protein molecule present inside the cell, and the nanoparticle becomes released outside the dead cell. The released nanoparticles are capped with the protein (Mubarak-Ali et al., 2011). Recently, Sudha et al. (2013) reported the synthesis of silver nanoparticles by using *Aphanothece* sp., *Oscillatoria* sp., *Microcoleus* sp., *Aphanocapsa* sp., *Phormidium* sp., *Lyngbya* sp., *Gleocapsa* sp., *Synechococcus* sp., and *Spirulina* sp. The silver nanoparticles synthesized were found to be spherical in shape, without aggregation, and with good antibacterial activity.

From a synthesis point of view, it is obvious that some components of algal biomass or the cell are used to reduce the ions to the nanoparticles. In this respect, X-ray absorption spectroscopy (XAS) results for the *Chlorella-vulgaris*-mediated synthesis of gold nanoparticles showed that the reduction of gold ions to gold nanoparticles was coordinated with the conversion of sulfhydryl residues into sulfur atoms (Watkins et al., 1987). On the other hand, the carbonyl groups ( $C=O$ ) of cellulosic materials of *Sargassumnatans* cell wall were reported to be the main functional group in gold precipitation (Kuyucak and Volesky, 1989). However, the cell wall components such as hydroxyl ( $OH^-$ ) groups of saccharides and carboxylate anion ( $COO^-$ ) of aminoacids residues also interacts with gold (Lin et al., 2005). Collectively, it can be said that various molecules or their components play an important role in the formation of various types of nanoparticles, especially metal nanoparticles.

Brayner et al. (2007) used commonly occurring cyanobacteria such as *Anabaena*, *Calothrix*, and *Leptolyngbya* for intracellular synthesis of gold (Au), silver (Ag), palladium (Pd), and platinum (Pt) nanoparticles. The study also indicated the role of the intracellular nitrogenase enzyme in the reduction of corresponding metal ions and which, after release in the surrounding medium, were stabilized by polysaccharides produced by the same algae. Likewise, *Lyngbya majuscula* and *Spirulina subsalsa* have also been exploited for the synthesis of gold nanoparticles, both intracellularly and extracellularly (Chakraborty

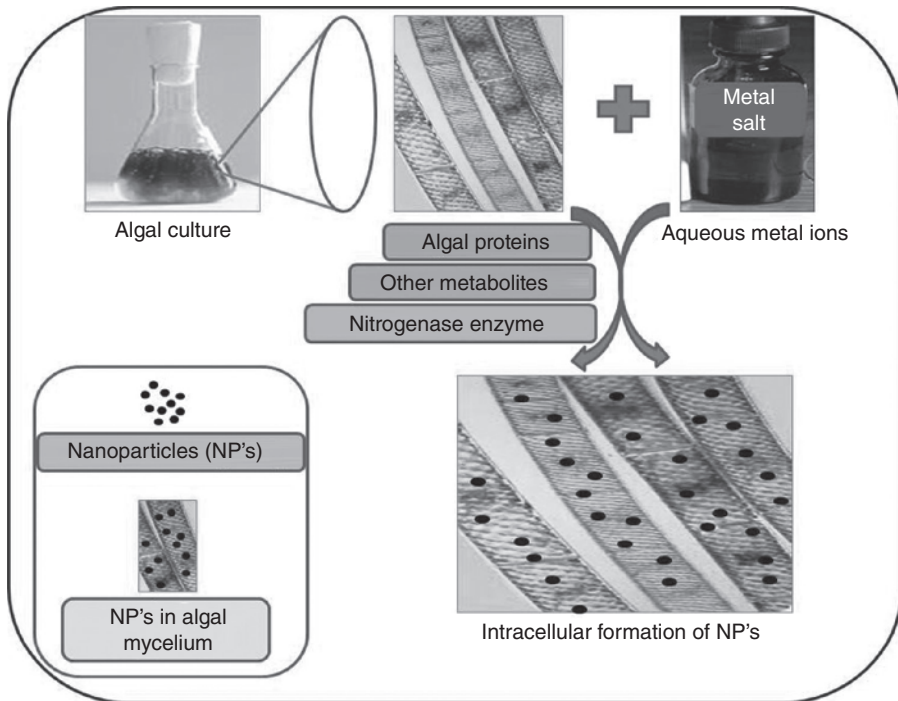
TABLE 1.2. Synthesis of Metal Nanoparticles using Different Algae

Serial No.	Name of fungi	Type of nanoparticle synthesized	Size (nm)	Shape	Reference
1	<i>Plectonema boryanum</i>	Gold	10–6000	Octahedral platelets	Lengke et al., 2006a
2	<i>Plectonema boryanum</i>	Platinum	<300	Spherical	Lengke et al., 2006b
3	<i>Plectonema boryanum</i>	Paladium	<30	Spherical and elongate	Lengke et al., 2007
4	<i>Sargassum wightii</i>	Gold	8–12	Spherical	Singaravelu et al., 2007
5	<i>Spirulina platensis</i>	Silver, Gold Au core and Ag shell	7–16 6–10 17–25	All spherical	Govindraju et al., 2008
6	<i>Chaetoceros calcitrans</i> <i>Chlorella salina</i> <i>Isochrysis galbana</i> <i>Tetraselmis gracilis</i>	Silver	Not mentioned	Not mentioned	Merin et al., 2010
7	<i>Oscillatoria willei</i> NTDM01	Silver	100–200	Not mentioned	Mubarak-Ali et al., 2011
9	<i>Oscillatoria willei</i>	Silver	100–200	Spherical	Mubarak-Ali et al., 2011
10	<i>Spirulina platensis</i>	Silver	11.6 (avg)	Mostly spherical	Mahdieh et al., 2012
11	<i>Aphanothece</i> sp. <i>Oscillatoria</i> sp. <i>Microcoleus</i> sp. <i>Apanocapsa</i> sp. <i>Pormidium</i> sp. <i>Lyngbya</i> sp. <i>Gleocapsa</i> sp. <i>Syneococcus</i> sp. <i>Spirulins</i> sp.	Silver	40–80	Spherical	Sudha et al., 2013

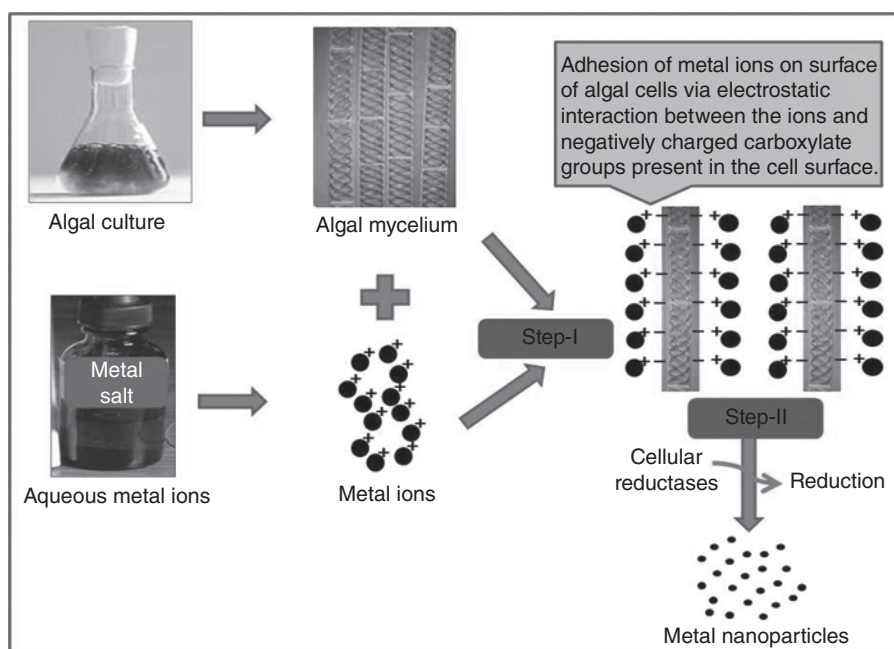
et al., 2009). In an interesting study, Mubarak-Ali et al. (2012) reported the synthesis of pigment-stabilized CdS nanoparticles. They first extracted C-phytoerythrin (C-PE) from a marine cyanobacterium *Phormidium tenue* NTDM05, and later used these C-PE to synthesize CdS nanoparticles. All of these studies signify that cyanobacteria are promising biological agents for the synthesis of stable nanoparticles. However, as very few reports have described their use in the synthesis of nanoparticles, there is a great need for further research. Some of the algal species used for the synthesis of metal nanoparticles to date are listed in Table 1.2.

After summarizing the above mentioned studies on the mechanism for nanoparticle synthesis using different algae, we propose a general mechanism for the intracellular synthesis of metal nanoparticles. In this mechanism different compounds or metabolites such as proteins or nitrogenase enzyme, present intracellularly in the algal mycelium itself, are responsible for the reduction of metal ions to its nanoparticulate form (Fig. 1.5).

Figure 1.6 depicts the general mechanism for the extracellular synthesis of metal nanoparticles, based on the hypothesis given by Mahdiah et al. (2012). In this two-step mechanism, the aqueous metal ion first adheres to the surface of algal cells due to the electrostatic attraction between the positively charged metal ions



**Figure 1.5.** General mechanism for the intracellular synthesis of metal nanoparticles using algae. See insert for color representation of the figure.



**Figure 1.6.** General mechanism for the extracellular synthesis of metal nanoparticles using algae. See insert for color representation of the figure.

and negatively charged carboxylate ions present on the cell surface of algae. The ions are then reduced to metal nanoparticles as a result of the secretion of reductase enzyme (cellular reductases) by algal cells.

## 1.5. APPLICATIONS OF METAL NANOPARTICLES

Metal nanoparticles offer applications in many disciplines including agriculture, catalysis, and biomedical biosensors.

### 1.5.1. Nanoparticles as Catalyst

The application of nanoparticles as a catalyst is a rapidly expanding field in nanotechnology. Due to their distinctive properties, nanoparticles are an ideal material for catalyst. Platinum and gold bimetallic nanoparticles have been used as electrocatalyst for polyelectrolyte fuel cells for the conversion of exhaust heat to energy (Toshima, 2013). Titanium and silver heterostructures have also demonstrated electrochemical properties, and could be used as photocatalysts (Zhang et al., 2013). Similarly, gold nanoparticles also demonstrate properties of photocatalysis (Kawamura et al., 2013).



### 1.5.2. Nanoparticles as Bio-membranes

Nanotube membranes with molecular dimension  $<1$  nm could be used as channels for the separation of molecules and ions between solutions. These nanotube membranes could separate nano-sized molecules based on their size while membranes of dimension 20–60 nm could be used to separate proteins (Gupta et al., 2012).

### 1.5.3. Nanoparticles in Cancer Treatment

Gold nanoparticles have shown potential in the treatment of cancer (Bhattacharya and Mukherjee, 2008; Chauhan et al., 2011). Vascular endothelial growth factor (VEGF) acts as a potential angiogenic factor and blood vessel permeabilizing agent after ligand binding to VEGF receptors (VEGFRs) on endothelial cells. Blocking the interaction of the VEGF with its receptors could be a possible way to inhibit angiogenesis. Quantum dots are luminescent crystals that allow specific drugs such as proteins, oligonucleotides, and siRNA (small interfering RNA) to penetrate targeted cancer cells in the central nervous system; they are therefore utilized for imaging in biological crystals. However, the toxicity issues of quantum dots is a major obstacle in its medical application to humans (Dikpati et al., 2012).

### 1.5.4. Nanoparticles in Drug Delivery

Metal nanoparticles with magnetic properties work as an effective molecular carrier for gene separation and also show promising application in drug delivery (Bava et al., 2013). For drug delivery, magnetic nanoparticles are injected into the drug molecule which is to be delivered; these particles are then guided towards the chosen site under a localized magnetic field. These magnetic carriers can carry large doses of drugs (Lu et al., 2007; Perez-Martinez et al., 2012). Similarly, silica-coated nanoparticles are also used in drug delivery due to their high stability, surface properties, and compatibility. Silica nanoparticles are also used in biological applications such as artificial implants (Dikpati et al., 2012; Perez-Martinez et al., 2012).

### 1.5.5. Nanoparticles for Detection and Destruction of Pesticides

Pesticides are hazardous to both human beings and the environment, contaminating drinking and surface water. The unique properties of nanoparticles allow their use in the detection and destruction of pesticides. The large-surface-area-to-volume-ratio property of nanoparticles plays a crucial role in the catalytic reactions used to degrade pesticides (Aragay et al., 2012). The optical properties of nanoparticles are related to their size and surface-induced changes in electronic structure, which helps in the detection of pesticides. For the destruction of pesticides, a photocatalytic oxidation method employing titanium nanoparticles is used (Aragay et al., 2012).

### 1.5.6. Nanoparticles in Water Treatment

Polyethylenimine-derived (PEI) nanoparticles and dendrimers have a number of applications such as gene delivery, catalysis, and electronics (Dikpati et al., 2012; Perez-Martinez et al., 2012). Magnetite nanoparticles are used in wastewater treatment and the removal of heavy metals from water. Das et al. (2009) reported the use of nanogold-bioconjugate in water hygiene management. The authors described the single-step removal of some model organophosphorus pesticide from water along with some microorganisms. Magnetite nanoparticles can be harnessed as adsorbents for separating and removing contaminants in water by applying an external magnetic field (Carlos et al., 2013).

## 1.6. LIMITATIONS OF SYNTHESIS OF BIOGENIC NANOPARTICLES

Nanotechnology in general and nanomaterials in particular have the potential to revolutionize different sectors such as pharmacy and medicine, electronics and agriculture and allied sectors such as food processing, packaging, and storage with modern tools (Rai and Ingle, 2012). There are different approaches for the synthesis of nanoparticles and each approach – whether physical, chemical, or biological – has its own limitations. In this chapter we have discussed some of the major limitations of biological (biogenic) synthesis of nanoparticles.

In nanotechnology, the synthesis of monodisperse nanoparticles is always a challenge for researchers and is one of the major limitations of biogenic approaches. It was reported that biogenic synthesis of nanoparticles failed to synthesize monodisperse nanoparticles, while chemical and physical methods are found to be significant for the synthesis of monodisperse nanoparticles (Tran et al., 2013). Another major limitation of the biogenic approach regards the mechanism of the synthesis of metal nanoparticles. There are many different mechanisms proposed for the elucidation of biogenic synthesis of metal nanoparticles using different biological systems; a survey of the literature suggests that many scientists have proposed a hypothetical mechanism based on their studies (Duran et al., 2005; Huang et al., 2007; Kumar et al., 2007; Li et al., 2007; Ingle et al., 2008). Some studies proposed that NADH-dependent nitrate reductase is responsible for the reduction of aqueous silver ions (Duran et al., 2005; Kumar et al., 2007; Ingle et al., 2008). Duran et al. (2005) further suggested that anthraquinone pigments and their derivatives synthesized by certain fungi were responsible for the reduction of metal ions into their respective nanoparticles. Gade et al. (2011) proposed a three-step mechanism for the synthesis of silver nanorods using fungus *Phoma sorghina* and reported that anthraquinone pigments played a key role in the formation of silver nanorods. Similarly, the exact mechanism for the formation of nanoparticles using bacteria and plants is not yet known; many investigators have suggested possible mechanisms however (Chandran et al., 2006; Huang et al., 2007; Li et al., 2007).

Fourier transform infrared spectroscopy (FTIR) studies carried out for the characterization of synthesized nanoparticles showed the presence of capped proteins

(Ingle et al., 2008, 2009; Birla et al., 2009; Gajbhiye et al., 2009; Bawaskar et al., 2010; Gade et al., 2010). Jain et al. (2010) studied the sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) profiles of the extracellular proteins secreted by *Aspergillus flavus*, which has been used for the synthesis of silver nanoparticles. They reported the presence of two proteins having a molecular weight of 32 and 35 kDa. Further, they proposed a two-step mechanism for synthesis and stabilization of nanoparticles. The first step involves a 32 kDa protein which may be a reductase and is responsible for the reduction of silver ions into silver nanoparticles. The second step involves 35 kDa proteins which bind with nanoparticles and confer stability. This is the only study which determines the exact protein involved in the capping of nanoparticles, playing a key role in their stabilization. Due to the lack of other publications on this topic, we believe that further extensive studies are needed to determine the exact proteins responsible for nanoparticle capping and their stabilization.

## CONCLUSION

In summary, there are three types of methods applied for the synthesis of nanoparticles, viz. physical, chemical and biological. Physical methods are tedious and time consuming, whereas many chemical methods make use of toxic chemicals for the purpose. On the other hand, biological methods of synthesis are quite safe and has the capability to produce nanoparticles with good biocompatibility.

Among biological methods, various types of microorganisms including bacteria, fungi and algae can be used. Biological systems, especially those using bacteria, have the potential to offer cheap and scalable, green, synthetic production of the latest generation of nanomaterials; it should however, be emphasized that, in many bacterial mediated synthesis methods, the actual biochemical mechanism remains poorly investigated. Likewise, many studies are upholding the use of fungi and algae for the size controlled and stable nanoparticle synthesis.

The metal nanoparticles are getting recognition for treatment of dreadful diseases like cancer, AIDS and tuberculosis. Moreover, they have potential to help in cleaning the environment by destructions of hazardous material like pesticide.

Although the biogenic nanoparticles have many advantages, toxicity issues associated with them are also important. The reports showing the toxic effects of the biogenic nanoparticles are emerging with slower pace. However, there is need of exhaustive study concerning the hazardous effects of nanoparticles.

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