Chapter 12
Mycosynthesized Nanoparticles: Role in Food Processing Industries

Lakshmishri Roy, Debabrata Bera, and Sunita Adak

Abstract Green synthesis of nanoparticles (NPs) is an evolving branch of nanotechnology. The use of fungi for the synthesis of NPs is referred to as mycosynthesis of metal NPs. Fungal endophytes have been recognized as important sources of a variety of structurally novel active secondary metabolites with anticancer, antimicrobial, and other biological activities. This mode of synthesis of metal nanoparticles is gaining more importance owing to its simplicity, rapid rate of synthesis of NP of attractive and diverse morphologies, and elimination of elaborate maintenance of cell cultures and eco-friendliness. Presently, the researchers are looking into the development of cost-effective procedures for producing reproducible, stable, and biocompatible metal NPs using fungal cultures. The present chapter is an exhaustive overview that assesses the role of fungi in the synthesis of nanoparticles, the mechanism involved in the synthesis, the effect of different factors on the reduction of metal ions in developing low-cost techniques for the synthesis, and recovery of nanoparticles. Finally, the application of nanoparticles in food processing industries, i.e., antimicrobial mechanisms, etc., has also been discussed.

Keywords Antimicrobial · Food processing · Nanoparticles · Mycosynthesis · Cost-effective

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R. Prasad et al. (eds.), *Fungal Nanobionics: Principles and Applications*,
https://doi.org/10.1007/978-981-10-8666-3_12

12.1 Introduction

Myconanotechnology is the interface between "mycology" and "nanotechnology" and has considerable potential, partly due to the wide range and diversity of the fungi (Rai et al. 2009a). Mycofabrication can be defined as the synthesis of metal nanoparticles using fungi. The fungal system in recent times has emerged as "bionanofactories" synthesizing nanoparticles of silver, gold, platinum, cadmium, etc. Mycosynthesized nanoparticles have been observed to be of good monodispersity and dimensions. Physicochemical and biological mechanisms of accumulation of metal ions by fungi include extracellular binding by metabolites and polymers, binding to specific polypeptides, and metabolism-dependent accumulation (Volesky and Holan 1995; Prasad 2016, 2017).

Fungi have attracted more attention for research pertaining to biological production of metallic nanoparticles due to their metal toleration and bioaccumulation capability (Sastry et al. 2003); ease for scale-up studies for nanoparticle synthesis (e.g., utilizing a thin solid substrate fermentation technique); feasibility of fungal enzymes (Castro et al. 2012) that results into higher yield of nanoparticles (Sastry et al. 2003; Mandal et al. 2006; Gade et al. 2010); economic livability and facility of employing fungal biomass; short incubation and growth period required by a number of fungal species, thereby making their culturing and maintenance in laboratory very simple (Castro et al. 2011; Prasad et al. 2016); their high wall-binding and intracellular metal uptake capacities (Volesky and Holan 1995); ability to produce metal nanostructure via reducing enzyme intracellularly or extracellularly; and the procedure of biomimetic mineralization (Ahmad et al. 2003a; Duran et al. 2005; Prasad 2016, 2017).

In a nutshell the potential advantages of fungi as bionanofactories may be depicted as given in Figs. 12.1 and 12.2.

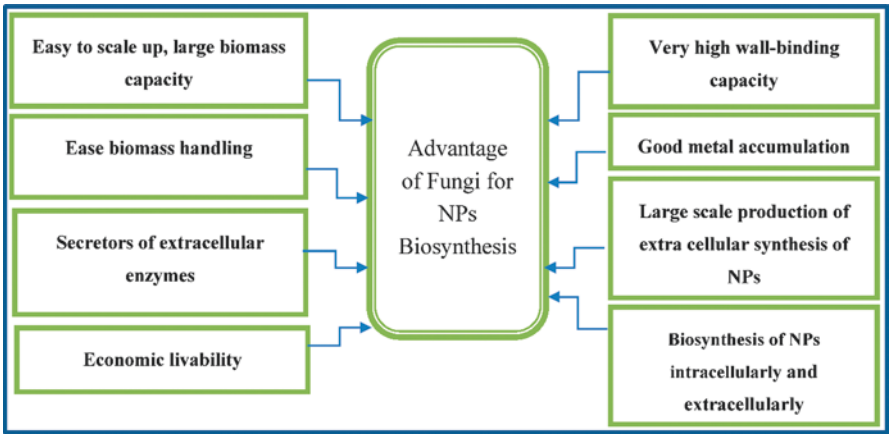

Fig. 12.1 Advantages of fungi to be used as biofactories for NP production. Thus, using these dissimilatory properties of fungi, it could be extensively used for the rapid and eco-friendly biosynthesis of metal nanoparticles

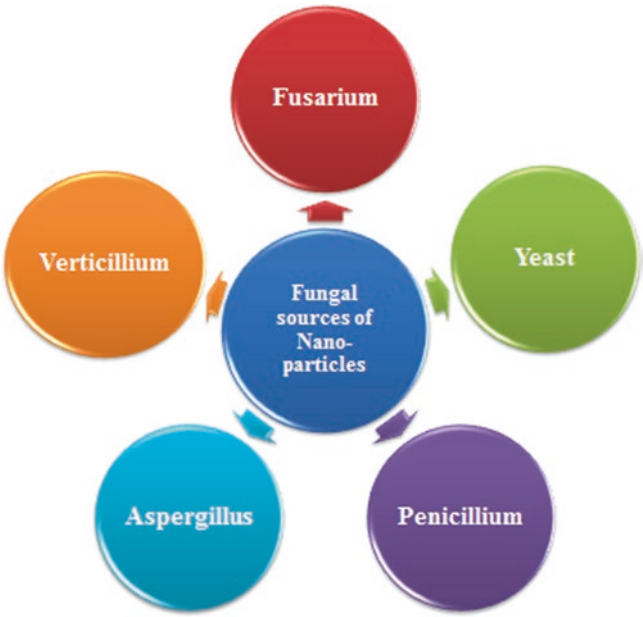

Fig. 12.2 Types of fungal sources for nanoparticle synthesis

12.2 Mycosynthesis of Nanoparticles (NPs)

Microbial synthesis of NPs is a green chemistry approach. Reports on fungal and other microbe-mediated biosynthesis of gold, silver, gold-silver alloy, selenium, tellurium, platinum, palladium, silica, titania, zirconia, quantum dots (QDs), magnetite, and uraninite are available (Narayanan and Sakthivel 2010). These nanoparticles are biocompatible, less toxic, and of multifarious utilities in numerous fields. The exact mechanism of biosynthesis has not been clearly elucidated. Microbial interactions with nanomaterials may in certain instances produce nanostructured materials and enhance microbial growth (Kumar et al. 2007). This is because of their multiple attributes that include excellent performance, selective adsorption of metal ions, operation over a broad range of ecological conditions (pH, ionic strength, temperature), low cost, free availability, regeneration, high biosorption capacity, etc. (Mandal et al. 2006).

Mycosynthesis of nanoparticles is of considerable industrial significance because of the following distinctive features of fungal systems:

Are capable of growing on readily available and inexpensive substrates.
Produce stable nanoparticles which do not aggregate even upon prolonged storage and are thus characterized by longevity.
Enhanced tolerance toward a higher concentration of metal nanoparticles which are also well dispersed in the medium offering large advantage.

Mycelial and mesh-like characteristics of fungal species aid them to withstand flow pressure and other conditions in bioreactors or other chambers as compared to other microbes and plant materials (Narayanan and Sakthivel 2010).
Secrete a number of enzymes (Mohanpuria et al. 2007).
Easy to grow and maintain (Mukherjee et al. 2008a, b).
No stringent requirements for special equipments.
Biomass concentration remains much higher than bacteria.
Possess inherent ability of producing extracellular metabolites that serve as defensive agents supporting their own survival when exposed to different environmental stresses like toxic materials (such as metallic ions), predators, and temperature variations.
Can be effectively immobilized and employed.
Synthesis of metal nanoparticles.

The process becomes cost-effective and safe without requirement of any specific instruments (Mohanpuria et al. 2007) and offers distinct advantage for large-scale production, extraction, and recovery unlike the bacterial strains reported (Mukherjee et al. 2008a, b; Gaikwad et al. 2013; Prasad et al. 2016).

The fungal system shows the capability of both intracellular and extracellular synthesis of nanoparticles (Mandal et al. 2006; Riddin et al. 2006; Ingle et al. 2008). In the recent past, research work using the fungal system has been carried out using both aspects for synthesis of nanoparticles of gold, CdS, silver, silica, titania, zirconia, etc. (Mukherjee et al. 2001a, b; Ahmad et al. 2002; Chen et al. 2003; Bansal et al. 2004, 2005). Reports on a number of fungal species like *Verticillium*, *Phoma* sp., *Fusarium oxysporum*, *Aspergillus fumigatus*, *Trichoderma asperellum*, and *Mucor hiemalis* for myconanosynthesis have been explored (Mukherjee et al. 2001a, b, 2008a, b; Chen et al. 2003; Bansal et al. 2005; Aziz et al. 2016).

12.3 Mechanistic Aspects of Mycosynthesis of Nanoparticles

Mechanism of nanoparticle formation, in all microorganisms and plants, is still an open question, even though much research has been attempted (Meyer 2008; Kathiresan et al. 2009). Biogenic synthesis of metal nanoparticles involves bioreduction of metal salts to elemental metal which may be stabilized by organic molecules present in the microbes such as fungi and bacteria; and the alternative way could be biosorption where metal ions in the aqueous medium are bonded to the surface of the cell wall of the organisms.

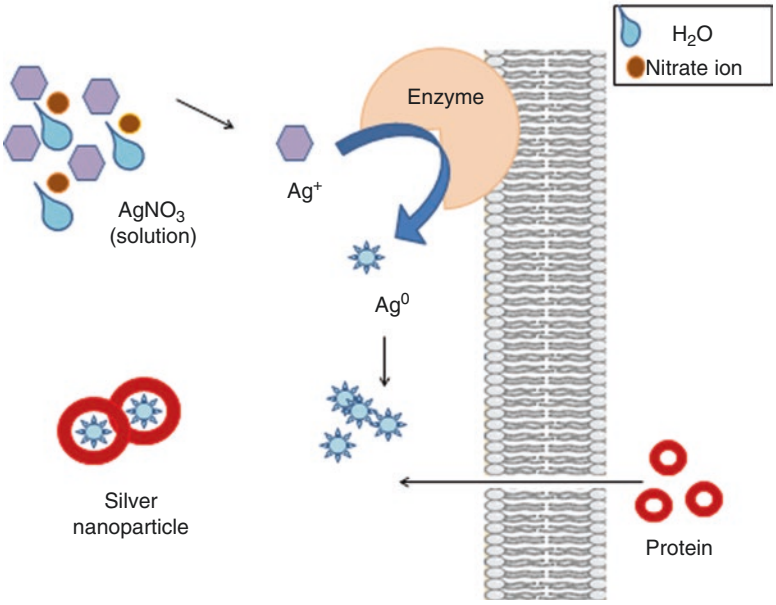

Fig. 12.3 Mechanism of extracellular biosynthesis of silver nanoparticles using fungi (Juibari et al. 2011)

12.3.1 Extracellular Synthesis

Several hypotheses for explanation of the mechanism of extracellular-mediated nanoparticles (Fig. 12.3) include:

Nitrate reductase was suggested to initiate nanoparticle formation by many fungi including *Penicillium* species, along with several enzymes like NADPH-dependent reductases, nitrate-dependent reductases, etc. In case of extracellular synthesis of silver nanoparticles due to the presence of these enzymes in fungal cytoplasm, reduction of silver metal ions into silver nanoparticles takes place. This finally leads to the formation of highly stable silver nanoparticles.

Jain et al. (2011) reported that AgNP synthesis for *Aspergillus flavus* occurs initially by a "33 kDa" protein followed by a protein (cysteine and free amine groups), which stabilizes the NPs by forming a capping agent (Jha and Prasad 2010). A number of researchers supported nitrate reductase for extracellular synthesis of NPs (Jain et al. 2011; Juibari et al. 2011; Bansal et al. 2004). Kumar et al. (2007) stated that the process of formation of silver nanoparticles requires the reduction of -NADPH to -NADP and the hydroxyquinoline probably acts as an electron shuttle transferring the electron generated during the reduction of nitrate to Ag+ ions converting them to Ag0. They further reported that the action of hydroxyquinoline is similar to that of quinones in the electron transport taking place in the mitochondria or the chloroplast.

Duran et al. (2005) had driven out the mechanism of biosynthesis of silver nanoparticles and stated that the synthesis of silver nanoparticles occurred in the presence of anthraquinone and NADPH-nitrate reductase. In this case, the electron required to fulfill the deficiency of aqueous silver ions (Ag^{+}) and convert it into Ag neutral (Ag0) was donated by both quinone and NADPH.

Edible mushroom *Volvariella volvacea* was also used for the synthesis of Au, Ag, and Au-Ag alloy nanoparticles (Chertok et al. 2008). The morphology of these nanoparticles was greatly influenced by temperature and pH. All the nanoparticles synthesized by using this fungus were highly crystalline and photoluminescent when observed their XRD patterns. It has been suggested that the polysaccharides and oligosaccharides present in the broth might be responsible for the reduction of metal ions to metal nanoparticles.

Mukherjee et al. (2008a, b) also suggested Michaelis-Menten type of mechanism for the synthesis of nanoparticles, where the reaction initially exhibits pseudo-zero-order kinetics and then follows higher-order kinetics. Thus, at initial phase when the concentration of silver nitrate is higher, the reaction is rather slow, and as the reaction proceeds, the concentration of silver nitrate lowers down considerably. The authors proposed that bioreduction of metal nanoparticles was brought about by protein extract containing amino acid with -SH bonds. Most likely cysteine undergoes dehydrogenation on reaction with silver nitrate to produce silver nanoparticles, while the free amino acid groups possibly serve as a capping for silver nanoparticles. The involvement of polypeptides/proteins in the bioreduction of metal ions was also reported by Das et al. (2009). In this study, FTIR spectra of fungal culture containing $AuCl_4^{-}$ (auric chloride) revealed the presence of amide I, II, and III groups and the disappearance of carbonyl groups present in the mycelia. The shifting of peaks from 1034 to 1075 cm^{-1} illustrated the role of phosphate bonds in the reduction process. Thus, the authors hypothesized that the surface-bound protein molecules acted as reducing and stabilizing agent. Silver nanoparticles synthesized by *Coriolus versicolor* also showed the reduction of silver ions by amide I and amide II groups (Sanghi and Verma 2009). The stabilization of nanoparticles was attained by fungal protein.

Jain et al. (2011) reported a two-step hypothetical mechanism for synthesis of silver nanoparticles. In the first step, reduction of bulk silver ions to silver nanoparticles takes place by a 32 kDa protein, which might be a reductase secreted by *A. flavus*. In the second step, silver nanoparticles were capped by a 35 kDa protein that binds with the nanoparticles and confers stability. Similar results were reported with *F. oxysporum* showing the presence of two extracellular proteins with molecular weight of 24 and 28 kDa responsible for the synthesis of zirconium oxide nanoparticles (Bansal et al. 2004; Duran et al. 2005).

Chen et al. (2003) exploited three different macro-fungi (*Pycnoporus sanguineus*, *Schizophyllum commune*, and *Lentinus sajor-caju*) for synthesis of silver nanoparticles. The authors supposed that the reduction of silver ions was possibly due to the presence of diketone compound, which was also confirmed by GC-MS analysis.

12.3.2 Intracellular Mechanism

Intracellular synthesis method involves a specific ion transportation system in the microbial cell. In this the cell wall of the microorganism plays an important role in biosynthesis of metallic nanoparticles. The hypothetical mechanism involves the electrostatic interaction forces found between the opposite charges, i.e., the negatively charged cell wall of microorganism and the positively charged metal ions. The enzymes present in the cell wall of microorganisms reduce these metal ions to nanoparticles which subsequently get diffused off through the cell wall (Fig. 12.4).

During the intracellular synthesis of gold nanoparticles, the gold metal ions firstly bind on the fungal cell surface. After that absorbed metal ions were reduced by enzymes present in the cell wall of fungi. These enzymes contain positively charged groups which lead to the aggregation of nano-shaped structures and finally formation of metal nanoparticles.

Other possibility is the migration of ions to cytoplasmic membrane to get reduced subsequently. The synthesis of silver nanoparticles requires the reduction of NADPH to NADP. Here, hydroxyquinone acts as an electron shuttle transferring the electrons generated during the reduction of nitrate to Ag ions converting them to Ag0.

The actual mechanism of mycosynthesis of nanoparticles, however, is still not fully understood. According to Mukherjee et al. (2001a, b), in intracellular synthesis, metal nanoparticles are synthesized below the cell surface, which is possibly due to the reduction of metal ions by enzymes present in the cell membrane.

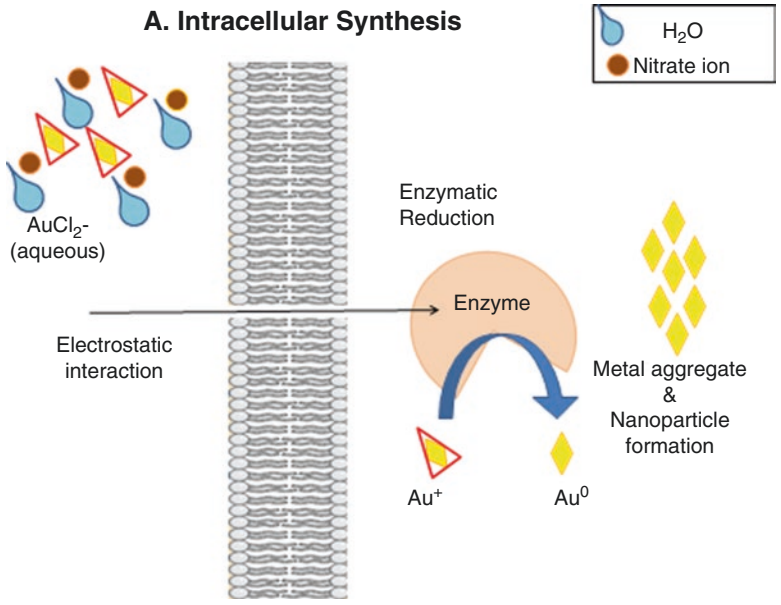

Fig. 12.4 A mechanism of intracellular synthesis of nanoparticles through fungi (Sarkar et al. 2010)

Synthesis proceeds firstly by the entrapment of metal ions on the surface of fungal cell, which occurs due to the electrostatic interaction between lysine residues and metal ions (Riddin et al. 2006). The second step in the synthesis is the enzymatic reduction of metal ions, which leads to aggregation and formation of nanoparticles. The cell-wall sugars also play a major role in the reduction of metal ions (Mukherjee et al. 2001a, b). Although the mechanisms for the intracellular synthesis of other metals are not available, the reduction of other metals may occur in a similar pattern as described for silver and gold nanoparticles.

12.4 Production of Myconanoparticles

The mycosynthesis of nanoparticles involves a series of steps that have been described in brief below:

12.4.1 Techniques of Isolation and Screening of Fungi Synthesizing Nanoparticles

12.4.1.1 Microbial Cultures Externally Synthesizing Nanoparticles

Different microbial sources are collected, milled, suspended in sterile normal saline, and subjected to screening process. Prepared Sabouraud dextrose agar (SDA)/potato dextrose agar (PDA) culture medium is supplemented with solution of metal ion (10 g/l) to final concentration equal to 100 mg/l of metal ion. One ml of the suspensions prepared from the collected samples are ten times diluted and spread on the surface of the metal ion-supplemented SDA/PDA plates (100 mg/l). The plates are incubated aerobically at 30 °C, and after 1 week, all colored colonies observed on the ion-supplemented media plates are picked up and transferred to metal ion-free PD or SD broth medium.

In the next step, all broth media are further incubated for 7–10 days at 25–30 °C in an incubator (150 rpm). During incubation periods several samples (0.5 ml) are aseptically withdrawn from culture flasks, centrifuged at 4000 × g for 20 min, and added to 4.5 ml metal ion solution (100 mg/l). After 60 min, the formation of specific colored colloid is checked in all reaction vessels. Red is the color of generated elemental SeNPs while blue for copper, brown for silver, yellow for gold, etc., and thus serves as a provisional marker to identify a culture supernatant of an isolate capable to form metal NPs.

12.4.1.2 Isolation of Microbial Cultures Intracellularly Synthesizing Metal Nanoparticles

The culture is grown in suitable liquid media incubated on shaker at optimal temperature. After incubation the flask is kept at static condition to allow the biomass to settle following which the supernatant is discarded and water is added for washing the cells. The flask is kept steady for 30 min to settle the biomass, post which the supernatant is again discarded. The biomass is then separated from the sterile distilled water by centrifugation for 10 min. The wet biomass is exposed to 50 ml of sterilized aqueous solution of metals at various dilutions and incubated on shaker at suitable temperature till visual color change is observed.

12.4.2 Identification of the Microbial Isolate

The identification of the isolate is carried out by 28S ribosomal deoxyribonucleic acid (rDNA) sequence analysis. Genomic DNA is obtained from fungal cells harvested from 72 h culture mediums and washed three times with sterile distilled water. The cells are disrupted by grinding with liquid nitrogen and the slurry subjected to phenol-chloroform DNA extraction procedure. DNA materials obtained are subjected to 28S rDNA polymerase chain reaction amplification for automated sequencing using the primers. Sequence similarity searches are done with the BLAST database (National Center for Biotechnology Information) and the sequence submitted to NCBI GenBank Nucleotide Sequence Database (accession number KC145152).

12.4.3 Culture Techniques and Optimal Conditions for Mycosynthesis of Nanoparticles

Biological synthesis approaches are novel routes for the production of nanoparticles and development of natural factories. Culture techniques and media vary depending upon the requirements of the fungal isolate involved; however the general procedure is more or less the same. Most of the important features of process conditions for synthesis of nanoparticles are constantly being searched by the researchers to get nanoparticles of desirable shape and size. The following parameters play an important role in biosynthesis of nanoparticles.

12.4.3.1 Sources for Production of Nanoparticles

For the synthesis of nanoparticles, it is important to select the best source on the basis of their intrinsic properties such as growth rate, enzyme production, and metabolic pathways which must be taken into account. Some of the microorganisms like bacteria, viruses, fungi, yeasts, and algae are known for the biosynthesis of metallic nanoparticles (Prasad et al. 2016).

12.4.3.2 Biomolecules Responsible for Biosynthesis

Biomolecules such as enzymes and proteins act as reducing and stabilizing agent for the biosynthesis of nanoparticles. Whole cells of microorganisms, crude enzymes which can be either in crude form or in purified form obtained from the microorganisms, are more often utilized. Nanoparticles synthesized mainly involve bioreduction process which also needs some coenzymes such as NADH, NADPH, FAD, etc. It is found that nanoparticle synthesis with the help of whole cell of fungi is much cheaper as compared to purified enzymes from the same fungus strain.

12.4.3.3 Optimal Reaction Conditions

Harvesting of microbial biomass is an important process subsequent to biosynthesis of nanoparticles. To avoid complications during synthesis process, it is important to avoid unwanted residual nutrients and metabolites. At industrial level, scale-up of production rate and yield is more important and needs to be optimized (including their exposure time, pH, temperature, etc.). Optimization of these factors can aid in controlling morphology and properties of desirable nanoparticles. Hence nowadays researchers have focused their attention to find optimal reaction conditions and actual mechanics that have been involved in the bioreduction process.

12.4.3.4 Favorable Conditions for Inoculum Growth

Growth conditions of microorganisms are important criteria while synthesizing nanoparticles. Enzyme production and growth of inoculum are the factors. So, the nutrients, pH, temperature, etc., should be optimized. Likewise when we are using whole cells and crude enzymes, harvesting time is also an important parameter so that it is necessary to monitor the enzyme activities during the time course of inoculum growth.

12.4.4 Factors Affecting Biosynthesis of Metal Nanoparticles

Major parameters affect the synthesis of nanoparticles including their size, shape, and monodispersity. Each of these depends on the physical and chemical parameters that mainly include the temperature, pH, presence of specific enzymes, type of biomass, exposure time to substrate and the substrate concentration, etc.

12.4.4.1 pH

pH is an important factor having efficient effect on the synthesis of metal nanoparticles. Gericke and Pinches (2006) carried out a research in which they demonstrated the change in shape of nanoparticles with variation in pH. They also found that *Verticillium luteoalbum* synthesizes nanoparticles of spherical shapes of size (<10.0 nm) at pH 3, but when pH is increased to 5, the shape obtained are hexagonal, triangular, and rodlike. Further increases in pH, that is, 7–9 nanoparticles with irregular and undefined shapes, were obtained. Similar work was carried out by Sanghi and Verma (2009); they studied the effect of pH on the fabrication of nanoparticles synthesized by *Coriolus versicolor*. The obtained results also suggested that reduction of metal ions was highly sensitive to pH.

12.4.4.2 Temperature

Temperature is an important physical parameter that also influences the synthesis of metallic nanoparticles. Dhillon et al. (2012) reported that the movement of ions and activity of microbial biomass were controlled by variation in temperature. It could be also suggested that temperature plays an important role on the growth of fungus as well as on metal uptake by the surrounding environment. Gericke and Pinches (2006) revealed that the formation of nanoparticles was directly affected by temperature. They found that at low temperature variety of nanoparticles formed after 1 h exposure to gold metal ion solution of spherical shapes. An increase in incubation time up to 24 h is likely to decrease in the number of smaller nanoparticles, whereas the large particles show well-defined shapes of bigger sizes. This is due to the segregation of smaller nanoparticles at high temperature. When temperature is increased up to 50 °C, there will be no difference observed in the shape and size of nanoparticles synthesized after 1 and 24 h exposure to metal ion solution. Further study on the effect of temperature on biosynthesis of metal nanoparticles suggested that the size of nanoparticles can be controlled by operating the reaction mixture at low temperature; however it would allow formation of nanoparticles at slower rate (Punjabi et al. 2015).

12.4.4.3 Concentration of Metal Ions

The concentration of metal ions also influences the synthesis of metal nanoparticles. Earlier reports have been suggested that the high concentration of fungus *Penicillium fellutanum* would inhibit the synthesis of nanoparticles. Kathiresan et al. (2009) demonstrated that at high concentration of silver ions, the size, shape, and monodispersity of the nanoparticles vary from the desired nano-size. As in the chemical reactions, the concentration of reactants decides the rate of reaction and also affects the size and shape of the synthesized particles. According to the study carried out by Gericke and Pinches (2006), they were synthesizing gold nanoparticles using *Verticillium luteoalbum*. The obtained results also suggested that when the concentration of $AuCl^{4-}$ was below 500 mg/L, the size of the gold nanoparticles was slightly narrow and uniform size ranges (>20 nm). The size of the synthesized nanoparticles increases with increase in the concentration $AuCl^{4-}$. In addition to this at high concentration of metal ions, the aggregation of smaller particles occursokay.

12.4.4.4 Exposure Time to Substrate

Synthesis of nanoparticles at different time intervals and their influence on synthesis process were also studied. It was found that as the incubation time increases, the shape and size of nanoparticles also vary with variation in the incubation period of reaction mixture. It has been also found that with increase in the incubation time, the synthesis of nanoparticles also increases. In one of the study, Gade et al. (2010) conducted a study in which they found that the majority of nanoparticles are synthesized after 1 h incubation to metal-containing solution. When incubation time increases up to 24 h, it shows the synthesis of nanoparticles of bigger sizes, and this is due to the clump formation or segregation of smaller-size nanoparticles. It has been also seen that when time increases up to 2–4 days, the synthesized nanoparticles show the alteration in their actual shape and size.

12.4.4.5 Type of Enzyme Used

The use of different types of enzymes for the bioreduction of metal ions into metal nanoparticles is greatly influenced by the type of enzymes secreted by the microorganisms. The main advantage to use these enzymes for the biosynthesis purpose is for the in vitro synthesis of nanoparticles by using the fungal mycelia; it also eliminates the need of optimization and harvesting process during the intracellular synthesis of nanoparticles. In case of fungi, it has been found that the enzymes secreted by them are also used for the synthesis of different metallic nanoparticles of various chemical composition, shapes, and sizes. In a study which was carried out by Ahmed et al. (Shakibaie et al. 2010), they found that the synthesis of silver nanoparticles does not take place in the presence of *Fusarium moniliforme* but it synthesizes when the reaction mixture is containing *Fusarium oxysporum* strain. The detailed

study of these two fungal metabolites and their protein assay suggested that the specific reductase enzyme (NADH-dependent reductase) was only produced by the fungal strain of *F. oxysporum*, while the other strain *F. moniliforme* does not produce this specific reductase enzyme. Obtained results also indicate that the synthesis of metal nanoparticles through *F. oxysporum* is due to the presence of specific nitrate reductase enzymes present in the cytoplasm or cell membrane. In another study Bansal et al. (2005) reported that *F. oxysporum* also synthesizes silica nanoparticles. The fungus *F. oxysporum* bioleached the silicates into silicic acid present in the zircon sand and then finally into silica nanoparticles.

12.4.5 Optimization

Vast emerging applications of nanoparticles (NPs) in distinct fields have led to an impending increase in demand for NPs. This ultimately leads to an enhanced demand, hence necessitating increased yield, for which optimization of the process is essential. The synthesis of NPs at nanoscale is still a challenge. Optimization of conditions during microbial culture production and post-induction becomes imperative in order to increase the shelf life of NPs with minimum investment. Statistical tools may also be employed for determining the optimal conditions.

12.4.5.1 Optimization of Process Conditions Pertaining to Culture Media is Carried Out for the Optimal Fungal Growth and Nanosynthesis

Different media compositions, namely, malt glucose yeast peptone broth, potato dextrose broth, protease production media, lipase assay medium, sucrose peptone yeast broth, and Sabouraud broth, have varied effects on the yield and quality of nanoparticles synthesized. Hence all the media are screened for the optimum and stable nanoparticle synthesis. The cell filtrate is used for protein estimation and synthesis of NPs.

It is well known that in different culture media conditions and compositions, microbial cell responds differently and secretes different metabolites and different kinds of proteins. Variation in pH and temperature on the nanoparticle synthesis is also significant. Also, it is known that the biological syntheses of several nanoparticles are enzyme-catalyzed reaction (Xie et al. 2007). More often enzymes, i.e., proteins, have multiple effects on the dispersion, including potential screening of the surface charges that helps to maintain the repulsion between the particles and bridging-type interactions.

OH^- ions are nucleophiles which play crucial role in maintaining the stability of certain nanoparticles by adsorbing on it and in synthesis of smaller size particles by providing electrons for reduction in metal ions. More nucleation regions are formed due to the availability of OH^- ions which helps in preventing the aggregates that are

formed through adsorbing on nanocrystals and maintains the smaller size (Gurunathan et al. 2009). At alkaline pH silver nanoparticles are stable and aggregates formed at lower pH. It indicates that, by controlling the pH of certain nanoparticle synthesis, it is easy to control the size.

It is hypothesized that the proton concentration affects conformational changes in the nitrate reducing enzymes present in the fungal filtrate, which may alter the morphology and size of the AgNPs. When the condition of the nanoparticle fabrication is alkaline, the synthesis will be faster than in acidic conditions.

With increase in temperature, the kinetic energy of the nanoparticles in the solution also increases; as a result, the collision frequency between the particles also rises, and this leads to the higher rate of agglomeration. This is because the surface potential of nanoparticles is inversely proportional to temperature which leads to the formation of aggregates as the particle demonstrates growth rate over a range of ionic strengths. Reaction temperatures cause variation in the interaction of particles via electrostatic and van der Waals forces.

Likewise the exact mechanism of light-mediated synthesis is not established. There may be photosensitization of aromatic amino acids (photosensitizer) of filtrate protein that may absorb the light energy and transfer to the reactants, while itself doesn't undergo any changes. In case of high-intensity light, heat generates, which may also accelerate the rate of synthesis of SNPs or photolysis. Cultural (culture medium, quantity of biomass, filtrate volume, and salt concentration) and physical conditions (pH, temperature, and light intensity) also are found to affect the maximum yield, rate of synthesis, and size of nanoparticles. Thus optimization of the process parameters ultimately leads to the rapid and large-scale production of NPs at industrial level.

12.4.5.2 Optimization of Post-induction Conditions, i.e., pH and Temperature, is Determined for Enhancing the Shelf Life

For large-scale synthesis and stability of fungal synthesized nanoparticles, studies have been conducted by suspending the fungal biomass in distilled water having different pH maintained by buffering system. Effect of temperature on the rate of synthesis of SNPs is studied by transferring fungal biomass into distilled water and incubated at its optimum temperature overnight, and later they are exposed to different temperatures.

12.5 Factors Controlling the Size and Shape of Biologically Synthesized Metallic Nanoparticles

The shape and size of nanoparticles depend on the volume of fungal biomass extract used (Philip 2009):

(i) Different shapes for the NPs can be obtained by varying the content of the extract in the reaction medium. In case of gold nanoparticle synthesis, lower

extract contents yield spheres and triangles, while higher extract contents yield flower-like NPs.

(ii) By tuning the experimental parameters: like the reaction time, the concentration of $HAuCl_4$, and the pH (Das et al. 2010). Interestingly, it is possible to favor one shape at the expense of another by just a slight tuning of the above-mentioned parameters.

(iii) Nature of the biomass used and on the species for a given microorganism. There is a variety of oxide- and carbonate-based NP biosynthesis such as those made, for instance, of zinc oxide (ZnO) (Azizi et al. 2014), copper oxide, magnetite (Fe_3O_4) (Mahdavi et al. 2013), silica (SiO_2) and titania (TiO_2) (Bansal et al. 2005), zirconia (ZrO_2) (Bansal et al. 2005), and calcium carbonate ($CaCO_3$) (Li et al. 2011). From a chemical composition point of view, the biosynthesis of iron oxide is more diverse compared to the previously mentioned oxides (Bharde et al. 2006).

(iv) By challenging the biomass with various conditions: the biosynthesis of quantum dots (QDs) of CdTe can be achieved by fungi. It is possible to tailor the optical properties of CdSe QDs, produced by challenging the biomass of the yeast *Saccharomyces cerevisiae* by aqueous solutions of $NaSe_2O_2$ and $CdCl_2$, through the screening of the following experimental parameters: time of addition, concentration and inoculating duration of $NaSe_2O_2$, and concentration and inoculating duration of $CdCl_2$. For instance, by varying the latter-inoculating duration of $CdCl_2$ from 14 to 44 h, the color spans from green to red, yellow being the one of the sample obtained at the in-between inoculating duration. Similar studies have been reported by several coworkers (Mao et al. 2006).

12.6 Recovery Methods

12.6.1 Extracellular

Fermented broth is incubated at optimal temperature until precipitate is observed at the bottom. After removal of the biomass, the solution is then centrifuged at 10,000 rpm for 20–30 min in non-refrigerated centrifuge. Supernatant is discarded and pellet composed of the nanoparticles is collected and dried.

12.6.2 Intracellular

Fermented broth incubated at optimal temperature is taken and the biomass is separated. Biomass is then subjected to cell disruption techniques, and the requisite separation techniques are employed for the recovery of nanoparticles.

12.6.3 Separation Techniques

Several separation techniques can be used for the nanoparticles (da Silva et al. 2011; Farre et al. 2011; Pycke et al. 2011; Bandyopadhyay et al. 2012). Techniques, including capillary electrophoresis (CE), chromatography, and field-flow fractionation (FFF), among others (Magnuson et al. 2011), are employed for separation of nanoparticles. The use of high-performance liquid chromatography (HPLC), ultra-performance liquid chromatography, and CE with FFF has been reported as efficient separation techniques for nanomaterials from several kinds of samples (Magnuson et al. (2011)).

12.6.3.1 Chromatography

In chromatography, compounds can be separated based on their charge (weak/strong cation or ion-exchange chromatography [IEC]), molecular mass (size-exclusion chromatography[SEC]), hydrophobicity/polarity (reversed-phase HPLC, hydrophobic interaction chromatography), and specific characteristics (affinity chromatography), depending on the type of materials in the stationary phase (Williams et al. 2002; Lead and Wilkinson 2006; Tiede et al. 2008). Luykx et al. (2008) reported the use of SEC and IEC to measure nanomaterials in several different matrices.

HPLC allows the separation of several different types of components (Luykx et al. 2008; Magnuson et al. 2011). Hydrodynamic chromatography is also a very efficient technique to separate NPs in samples based on their hydrodynamic radius (Tiede et al. 2008).

12.6.3.2 Field-Flow Fractionation (FFF)

FFF is a technique similar to HPLC and can be used to separate nanomaterials based on thermal or hydraulic gradients, electrical forces, and sedimentation (Hassellov et al. 2008; Luykx et al. 2008; Bolea et al. 2010). The general principles of the FFF technique are described in details (Schimpf et al. 2000). It is a flexible elution technique where simultaneous separation and measurement can be done across a broad macromolecular colloidal particulate, ranging from about 1 nm to more than 100 nm (Giddings 1993). A major advantage of this method is the lack of a stationary phase, thus restraining the interaction between the sample and the equipment surfaces (Giddings 1993; Schimpf et al. 2000). FFF can be coupled with fluorescence, MS, and light-scattering techniques for the quantitative detection of nanomaterials in complex systems (Hassellov et al. 2008).

Sedimentation FFF (SdFFF) is suitable for separation and characterization of emulsions. It is an elution-based analytical technique, which provides higher solution separation of nanomaterials in gentle, low-shear conditions.

Asymmetric flow FFF (AF4) is another technique for nanomaterial characterization. Bouby et al. (2004) reported the characterization of Fe_3O_4/hydroxide colloids by using a combined AF4 and laser-induced breakdown technique with trace detection limit of 1 mg/L. This combination can be ideal for measuring NMs in samples.

12.7 Techniques for Characterization of Nanoparticles

The availability of advanced instrumentation techniques for the characterization of metal nanoparticles has proven to be very useful for gaining an insight into various morphological and structural features. The stability of the particles can also be ascertained by these techniques. Characterization is an important step for the identification of the nanoparticles by their size, shape, chemical composition, surface area, and dispersity. Thorough characterization studies of the nanoparticles aid in lucid understanding and control of the synthesis and applications of the nanoparticles. Different techniques available for the characterization of nanomaterials have been represented in the Fig. 12.5. The methods may be:

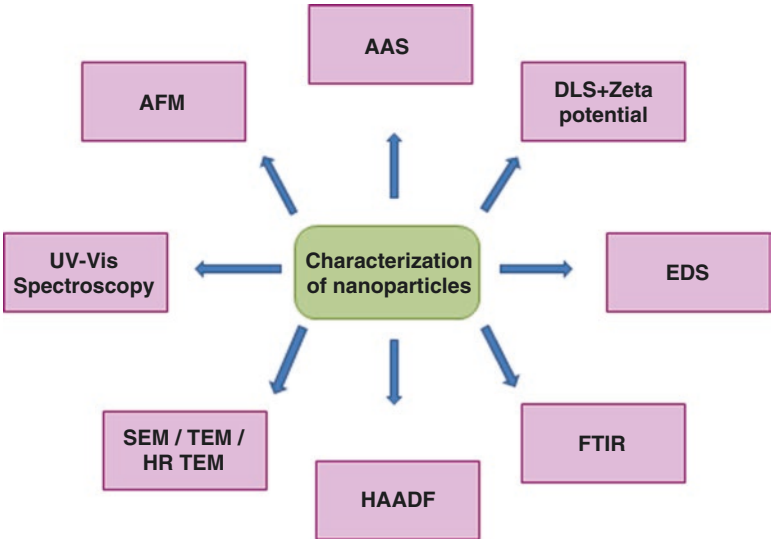

Fig. 12.5 Techniques for characterization of nanoparticles

12.7.1 For Determination of the Size, Shape, and Conformity of the Nanoparticles Synthesized

12.7.1.1 X-ray Diffraction (XRD)

X-ray diffraction data provides information about crystallinity, crystallite size, orientation of the crystallites, and phase composition and aids in molecular modeling to determine the structure of the material (Joshi et al. 2008). XRD is mainly used for the crystal analysis and phase identification of the synthesized nanomaterials. It also determines the overall oxidation state of the particle as a function of time (Hergt and Dutz 2007).

Advantages: Simplicity of sample preparation, rapidity of measurement, analyzes mixed phases, and determines sample purity.

Limitations: Requirement of homogenous and powdered material, peak overlays lead to unclear data.

12.7.1.2 Electron Microscopy

Electron microscopy is used for the surface and morphology characterization of the molecule. Similarly scanning electron microscope and transmission electron microscope are also used for the morphological characterization at nanometer to micrometer scale.

12.7.1.3 Scanning Electron Microscope (SEM)

The SEM images the sample surface by scanning it with a high-energy beam of electrons. When the beam of electrons strikes the surface of the specimen and interacts with atoms of sample, signals in the form of secondary electrons, backscattered electrons, and characteristic X-rays are generated that contain information about sample's surface topography, composition, etc. (Joshi et al. 2008).

Advantages: SEM is its two-dimensional imaging, ease of sample preparation, and provision of digital data forms.

Limitations: Improper sample preparation can lead to confusion between artifacts and actual data. Obvious limitations are the size, cost, and maintenance.

12.7.1.4 Transmission Electron Microscope (TEM)

In TEM the crystalline sample interacts with electron beam mostly by diffraction rather than by absorption. The intensity of diffraction depends on orientation of planes of atoms in a crystal. This produces a variation in the electron intensity that reveals information of the crystal structure. Along with distribution and dispersion,

exfoliation, intercalation, and orientation of nanoparticles can also be visualized using a TEM micrograph (Joshi et al. 2008).

It is found that the TEM has much higher resolution as compared to the SEM. SEM gives the information of the morphological characteristics of the molecules at submicron level and the elemental information at the micron level. Due to the high resolution, TEM is widely used for the identification of the exact shape and size of the nanoparticles. Advantages of SEM and TEM include giving two-dimensional imaging, easy to sample preparation, and data in digital forms.

Advantages: High-quality, detailed, and powerful magnification of element and compound structures.

Limitations: Laborious sample preparation, artifacts from sample preparation, and definitely large and expensive.

12.7.1.5 High-Resolution Transmission Electron Microscope (HRTEM)

It is an imaging mode of TEM that allows imaging of crystallographic structure of samples at an atomic scale. In HRTEM electron wave after interacting with sample undergoes phase change and interacts with image wave in the imaging plane. Thus, individual atoms and crystalline defects can be imaged clearly using HRTEM (Joshi et al. 2008).

12.7.1.6 Atomic Force Microscopy (AFM)

AFM is ideally used for the qualitative estimation of surface roughness and also gives the complete visualization of the surface of synthesized nanoparticles. It gives very high three-dimensional spatial imaging resolution of the synthesized nanomaterials. The surface of the sample is scanned using a probe, and the oscillation amplitude is used to measure the surface characteristics of the sample (Mukherjee et al. 2001a, b; Joshi et al. 2008).

Advantages: AFM provides higher resolution than SEM. It gives true atomic resolution compared with scanning tunneling microscopy and transmission electron microscopy.

Limitations: AFM cannot scan images as fast as SEM and image artifacts, and it gives a single-scan image size. All these technologies are very reliable and useful for the complete analysis and detailed characterization of synthesized nanoparticles.

12.7.1.7 Zeta Potential Measurement

It is used for the analysis of the stability of the synthesized nanoparticles. The value of zeta potential is as high as the nanoparticles are more stabilized.

12.7.1.8 Dynamic Light Scattering (DLS)

It is mainly used for the qualitative detection of nanoparticles and also characterizes the surface charge and size of the nanoparticles. It is a well-established technique for measuring the size of molecules and particles. The fluctuation in the intensity of the scattered light from laser-illuminated particles is size dependent, and hence, the size of particles can be analyzed. Thus, the size and size distribution of particles can be studied by DLS (Joshi et al. 2008). With the help of this, we can also analyze the polydispersity index of the synthesized nanoparticles (Mukherjee et al. 2002).

Advantages: Offers measurement of particle sizes of 1 nm, precision of ±1%, repeatable analysis, no sample preparation, and liquid sample.

Limitations: Offers low resolution of polydisperse samples and multiple light scattering.

12.7.2 For Functional Group Identification of Synthesized Nanoparticles

UV-visible spectroscopic analysis, EDX analysis, and FTIR analysis techniques are different techniques employed for the evaluation of nanoparticles.

12.7.2.1 UV-Visible Spectrophotometer

Detection of the formation and presence of nanoparticles in the fungal medium can be done using UV-vis spectroscopy. It is also used for the identification and characterization of metallic nanoparticles. This is the most widely applicable technique because of its simplicity and reliability. Two hundred to eight hundred nanometer light wavelength is generally used for the identification of nanomaterials of size ranges 2–100 nm. It is well known that, for monodispersed NPs, only one plasma band is obtained. The increase in its intensity is an indication of the advanced degree of reaction with increase in the number of particles. Metal nanoparticles scatter optical light because of collective resonance of the conduction electrons in the metal known as surface plasmon resonance (SPR). This SPR peak is shown in UV absorption spectra by these nanoparticles. The magnitude of peak, wavelength, and spectral bandwidth associated with nanoparticles are dependent on size, shape, and material composition (Joshi et al. 2008).

Advantages: UV-vis spectroscopy is a rapid means of analysis. It provides very high precision and accuracy. It is useful for a wide variety of chemicals and can be used both quantitatively and qualitatively.

Limitation: It is nonselective for compounds that absorb at the same wavelength (Waghmare et al. 2011).

12.7.2.2 Fourier-Transform Infrared Spectroscopy (FTIR)

FTIR gives data of proteins and other compounds present in the mixture that interact with metal ions. The identification of functional groups leads to determination of the reducing agent and the capping agent responsible for synthesis and stability of nanoparticles (Jeevan et al. 2012). FTIR is mainly used for the detection of the organic functional groups attached to the surface of nanoparticles. It is also useful for the analysis of surface chemistry of the synthesized nanomaterials.

Advantages: Identifying and detecting changes in protein secondary structures, can resolve between similar components.
Limitations: Overlapping peaks make it difficult to distinguish, difficult to quantify, better results with solid components.

12.7.2.3 Energy-Dispersive Spectroscopy (EDS)

EDS is used for the analysis of elemental composition of the metal nanoparticles. It gives the complete information regarding elemental knowledge of the nanomaterials. This technique is used in conjugation with SEM. The characteristic X-rays are used to identify composition of sample by a technique known as energy-dispersive X-ray (EDX), thus giving an overall mapping of sample (Joshi et al. 2008).

Advantages: It improves quality control and helps in process optimization and identification of contaminant and gives higher production yield.
Limitations: Quantitative analysis requires standards of known composition and that fluorescence of emitted x-rays limits the precision.

12.8 Applications of Mycosynthesis of Nanoparticles in Food Processing Industries

The developing field of nanotechnology has opened up new avenues of progress in various technologies. Nanoparticles or nanomaterials are finding their application in different areas like polymer, paints, nutraceuticals, pharmaceuticals, cosmetics, food and beverage, agriculture, surface coatings, etc. (Bhattacharya and Gupta 2005; Ingale and Chaudhari 2013). Nanoparticles' multifaceted application is due to their unusual physicochemical and optico-electrical properties. These special properties arise due to confinement of electrons in particles of smaller dimensions compared to bulk electron delocalization, which is known as quantum confinement (Gade et al. 2010). Same properties have been also reported in nanoparticles derived from biological entities like bacteria, fungus, yeast, and extracts of microbes and plants (Prasad et al. 2016; Prasad 2014).

Metal nanoparticles are reported to have antimicrobial activity against many pathogenic organisms. Silver nanoparticles are found to be the forerunner in this aspect with wide antimicrobial activity against methicillin-resistant *Staphylococcus*

aureus, *Enterobacter cloacae*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Escherichia coli*, *Acinetobacter baumannii*, and *Proteus vulgaris*. Impregnating silver nanoparticles in textile fabrics and wound dressings has been successful in controlling infection by pathogens (Rai et al. 2009a, b; Aziz et al. 2016). In similar line of research reported, active role of silver nanoparticles externally synthesized using fungus, in increasing antifungal activity of fluconazole and susceptibility of multidrug-resistant bacteria against antibiotics, respectively (Gajbhiye et al. 2009; Gopinath et al. 2015; Aziz et al. (2016). Growth of many microbes like *E. coli*, *Salmonella choleraesuis*, *Staphylococcus aureus*, and *Saccharomyces cerevisiae* has been reported to be inhibited by copper nanoparticles as well (Gade et al. 2010; Yadav et al. 2017). Apart from antibacterial textiles, ultrahydrophobic fabrics, suits against biohazards, face masks, etc., have been developed using silver nanoparticles (Ramaratnam et al. 2008; Aziz et al. 2015). Nanoparticle-based delivery systems for drugs and genes prove better than their conventional counterpart. Metal nanoparticles as drug delivery system showed high stability, carrier capacity, and variable route of administration (Gelperina et al. 2005).

Gold nanoparticles with unique optical properties are promising candidates for development of sensors. Nanoparticles based on iron oxides have been used in different imaging techniques for in vivo diagnostic studies (Glomm 2005; Gao et al. 2008). For solar energy applications, magnetotactic bacteria which produced magnetite and greigite are used in optical coatings (Joerger et al. 1999).

As bioremediation tools also, nanoparticles can be used in wastewater treatment (Das et al. 2009). Potent activity of the S-layer of *Bacillus sphaericus* JG-A12 has been reported in bioremediation of radioactive wastes (Duran et al. 2007). Application of nanoparticles in food can be found at different stages, involving processing, packaging, and safety. Even at the agricultural level, nanoparticles are used in form of nanopesticides and nanoherbicides (Prasad et al. 2014, 2017). They are also being used as nanosupplements and nano-delivery systems to enhance nutritional aspects.

12.8.1 Food Processing

Food processing involves conversion of raw materials into consumable and marketable form having longer shelf life. Preservation, toxin and pathogen removal, and nutritional value as well as shelf-life improvement are some of the targeted aspects covered in food processing. Antimicrobial activity of nanoparticles is employed for disinfection process. Silver-based nanoparticles or nanocomposites like silver-containing zeolites have been approved by US FDA to be used as food contact disinfectant. Subsequent formation of reactive oxygen species (ROS) causes oxidative stress and subsequent cell damage (He and Hwang 2016).

On contrary less reactive nanomaterials have been developed to act as antioxidants. SiO_2-gallic acid nanoparticles show scavenging capacity of 2,2-diphenyl-1-picrylhydrazyl radicals (Deligiannakis et al. 2012). Such antioxidant-based nanoparticles used as edible coating have been reported to control browning of

fresh-cut fruits. One such example is ZnO-coated active packaging which improved shelf life of "Fuji" apples (Li et al. 2011).

Development of nanocoating as carrier of functional ingredients such as antioxidants, enzymes, flavors, and anti-browning agents has been done employing chitosan-based nano-silver or silver zeolite-incorporated edible film (Berekaa 2015). Immobilization of porcine triacylglycerol lipase on nanoscale SiO_2 improved the hydrolytic efficiency of olive oil as well as enzyme stability (Bai et al. 2006). Thus, nanoparticles can be successfully used for enzyme immobilization. Several inorganic nanoparticles based on silver, iron, calcium, magnesium, selenium, and silica have been used as preservatives and additives to improve taste and flavor of food. TiO_2 and SiO_2 have been approved by FDA as food color additives (He and Hwang 2016).

Yet another application could be on microbe-based food processing like fermentation, as many nanoparticles were found to enhance the microbial reaction rates (Zhang et al. 2011).

12.8.2 Food Packaging

Food packaging is one of the earliest commercial applications of nanotechnology. This helps to preserve the food along with making it marketable. Nanocomposites designed based on nanoclays, carbon nanoparticles, nano-metals, and oxides can improve various properties like mechanical strength, heat resistance, and barrier against ultraviolet radiation, oxygen, carbon dioxide, moisture, etc., of food package materials. Various edible nanocoatings have been developed with better moisture, lipid, and gas barrier properties that could be used for coating of fruits, vegetables, chocolate, bakery items, meats, etc. (Wesley et al. 2014).

Silver, copper, titanium oxide, and carbon nanotube-based packaging can provide antibacterial property and prevent the growth of pathogens. Chitosan-based nanocomposite films of silver showed potential antimicrobial effect (Rhim et al. 2006).

Another mode of active packaging employs entrapping enzyme in between polymer films which act as oxygen scavenger. Further, nanotechnology-based release of preservatives present in food package is being designed by researchers in the Netherlands (Sekhon 2010). The use of carbon nanotubes in packaging to pump out carbon dioxide or absorb undesirable flavors is also being developed (Sinha et al. 2006). Nano-based tracking technologies are being designed which often include an ingestible BioSilicon which could be placed in foods for monitoring purposes and pathogen detection (Wesley et al. 2014).

12.8.3 Food Safety

Another potential use of nanotechnology deals with development of nanosensors for detection of contaminant and pathogens in food system. Various gold, silver, silicon, magnetic, and carbon nanotube-based nanosensors have been developed to detect *E. coli*, *Salmonella*, *Listeria monocytogenes*, *Mycobacterium avium*, etc. The

detection techniques based on optical or electrical properties employ voltammetry, spectroscopy, epifluorescence microscopy, and amperometry having a detection range of 15 CFU/ml to 1×10^9 CFU/ml (Inbaraj and Chen 2016). A disposable immune-sensing strip may be employed to detect the presence of *E. coli* O157:H7 in milk. These strips worked better in the presence of gold NPs imparting high sensitivity to the technique (Lin et al. 2008).

Microbial toxins can be also successfully detected using gold, zinc oxide, magnetic, and carbon nanotube-based nanosensors. Staphylococcal enterotoxin B, aflatoxin, and mycotoxin can be sensed using chemiluminescence (CL) immunosensors, immunoelectrode, and indium-tin-oxide glass plate detector (Sonawane et al. 2014). Tang et al. (2011) developed a sensitive electrochemical immunosensor by immobilizing BTX-BeBSA conjugate on GNP-decorated amine-terminated polyamidoamine dendrimers (GNPePAADs) for rapid screening of brevetoxin B (BTX-B) produced by *Karenia brevis* in food samples.

Different food contaminants like melamine, carbofuran, etc. can be detected using nanosensors based on colorimetric or electrochemical probes (Sonawane et al. 2014). Nanosensors can also track any adulteration in food, such as pork adulteration in meat products. Mostly gold NPs have been successfully used for this purpose. In this case the detection is based on color change following incubation of gold NP-DNA conjugates in the meat samples. Aggregation of gold NPs in the presence of pork DNA-containing sample leads to color change from red to purple gray, making it a simple detection tool (Ali et al. 2012).

12.9 Current Status and Recent Advancements of Mycosynthesis of Nanoparticles

The fungi are now known to be efficient tool for synthesis of nanoparticles by both intra- and extracellular methods. The fungal system has shown its compatibility over other groups of organisms as the handling of fungal biomass and its downstream processing is much simpler. A number of metallic nanoparticles including silver, gold, titanium, silica, zirconium, and platinum have been successfully synthesized using the fungal system. The fungal-derived nanoparticles have depicted a wide range of applications in different fields of science including medicines, pharmaceutical industry, agriculture, electronics, etc. But there are certain areas which need to be worked out before exploring the complete potential. An exact mechanism of synthesis of nanoparticles is yet to be discovered. Understanding the exact mechanism involved in the synthesis of nanoparticles and the effect of different factors on the reduction of metal ions will help in developing low-cost techniques for the synthesis and recovery of nanoparticles. Thus, sketching different practicalities and reducing agent involved in the synthesis of nanoparticles would help in understanding the fungal system as one of the most efficient systems for harnessing nanoparticles.

12.10 Conclusion

The last decade has witnessed tremendous developments in the field of microorganism-produced nanoparticles and their applications. However, more efforts are essential to improve the production efficiency and control particle size and morphology. The following are the key areas identified that need to be further addressed:

Reduction of synthesis time: Microbe-mediated synthesis of nanoparticles is quite a slow process (several hours and even a few days) in comparison to physical and chemical approaches. Minimization of synthesis time will make this biosynthesis route much more enticing.

Size and monodispersity: Evaluation of nanoparticle synthesis is determined by particle size and monodispersity.

Stability of nanoparticles: Degradation of microbially synthesized nanoparticles after a certain period of time has been reported. Thus, the stability of nanoparticles deserves further enhanced study (Xiang et al. 2007; Hergt et al. 2005; Hergt and Dutz 2007).

The control of particle shape in chemical and physical synthesis of nanoparticles is still an ongoing area of research. Hence, biological processes with the ability to strictly control particle morphology are perceived to be advantageous over the other processes. Variation of parameters, like microorganism type, growth stage (phase) of microbial cells, growth medium, synthesis conditions, pH, substrate concentrations, source compound of target nanoparticle, temperature, reaction time, and addition of nontarget ions, could be plausible strategies to obtain sufficient control of particle size and monodispersity. Biosynthesis methods are advantageous also because nanoparticles are sometimes coated with a lipid layer that confers physiological solubility and stability, which is critical for biomedical applications and is the bottleneck of other synthetic methods. Research is currently carried out manipulating cells at the genomic and proteomic levels. With a better understanding of the synthesis mechanism on a cellular and molecular level, including isolation and identification of the compounds responsible for the reduction of nanoparticles, it is expected that short reaction time and high synthesis efficiency can be obtained.

Biosynthesis of nanoparticles by microbes is thought to come under the purview of “green chemistry” procedures. Microbe employment for nanoparticle synthesis can be classified into intracellular and extracellular synthesis according to the location where they are formed. Rate of intracellular particle formation and control of the size of the nanoparticles could, to an extent, be manipulated by controlling parameters such as pH, temperature, substrate concentration, and exposure time to substrate. Research is currently executed by manipulating microorganisms at the genomic and proteomic levels. With the recent progress and the ongoing efforts in improving particle synthesis efficiency and exploring their biomedical applications, it is hopeful that the implementation of these approaches on a large scale and their commercial applications in medicine and healthcare are likely to open up more vistas in the coming years.

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