Chapter 2
An Insight into the Bacterial Biogenesis of Silver Nanoparticles, Industrial Production and Scale-up

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2.1 Introduction

"Nano" refers to any parameter when it is expressed as a measure of 10^{-9} times of SI units. Until recent past, the very existence of nanoparticles and their applications remained undetected. Nanotechnology was first proposed to have applications in the field of electronics for the miniaturization of the electronic devices. In fact, the term "Nanotechnology" has been coined by Norino Taniguchi, a researcher at the University of Tokyo, Japan (Taniguchi 1974). This slowly expanded to various fields. Even when various scientists reported the remediation of various heavy metals by microorganisms, the remediated nanosized zero valent metal crystal remained unnoticed (Mullen et al. 1989). But, when the significances of the nanoparticles were discovered, their applications also increased. When it comes to silver nanoparticles, they are used as antimicrobial agents in most of the public places such as elevators and railway stations in China. Besides, they are used as antimicrobial agents in surgically implanted catheters in order to reduce the infections caused during surgery and are proposed to possess antifungal, antiinflammatory, antiangiogenic and antipermeability activities (Kalishwaralal et al. 2009; Gurunathan et al. 2009a, b; Sheikpranbabu et al. 2009). Primarily, silver nanoparticles are considered as an alternative to silver ions (obtained from silver nitrate), which were used as antimicrobial agents. Silver was used as storage devices during historical periods and silver nitrate solution was directly used for wound healing during Second World War (Chu et al. 1988; Deitch et al. 1987; Margraff and Covey 1977; Silver 2003; Atiyeh et al. 2007; Law et al. 2008). Before the advent of silver nanoparticles, silver was the main component in the various creams for wounds. However, silver ions have the disadvantage of forming complexes and the effect of the ions remained only for a short time. This disadvantage has been overcome by the use of the silver nanoparticles which are in inert form and also exhibit

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antimicrobial function by inducing the production of reactive oxygen species such as hydrogen peroxide. Both the top-down and bottom-up approaches have been followed to synthesize nanoparticles. Here, chemical and biological methods have been successfully applied to synthesize silver nanoparticles.

2.2 Brief History

Silver was known only as a metal till recently and it is only when the nano era came into existence that people started to believe that silver could even be produced at the nanoscale. As previously mentioned, nanoparticles remained unnoticed even when many organisms were used for remediation of various metals. The competent organisms have been used to remove various reactive metal salts from the environment. Both the Gram positive and Gram negative metals have been used for the biosorption of metals such as silver, cadmium and copper. Various organisms have been tested for their efficiency to adsorb these metal ions and these metal ions remained as a colloidal aggregate mostly on the cell surface, occasionally on the cytoplasm. The size of the nanoparticles played no significant role during those times. These capable organisms were in turn termed as the "competent organisms" which could bind large amounts of metal ions (Beveridge and Murray 1976; Mullen et al. 1989; Doyle et al. 1980; Beveridge and Fyfe 1985). Cell walls of Gram positive bacteria such as *Bacillus subtilis* were found to bind with large quantities of metals than the Gram negative bacteria such as *Escherichia coli* (Beveridge and Fyfe 1985). The synthesis methods in the early 1980s described the reduction of metal ion as a two-step procedure; in first step very small particles were synthesized, which were then enlarged to several nanometers. The difference remained in the use of the reducing agent for the synthesis where in the former step a stronger reducing agent was used, and in latter step a weaker reducing agent was used (Sintubin et al. 2009). Chemical methods were used for the size-dependent synthesis of silver nanoparticles, a controlled process mediated by the addition of specific reducing agents at raised temperatures and various pH values. Silver nanoparticles were also synthesized through an array of methods such as spark discharging, electrochemical reduction, solution irradiation and cryochemical synthesis.

The biosynthesis of nanoparticles as an emerging highlight of the intersection of nanotechnology and biotechnology has received increasing attention due to a growing need to develop environmentally benign technologies in material synthesis (Kalishwaralal et al. 2008). The biological synthesis of nanoparticles germinated from the experiments on biosorption of metals with Gram negative and Gram positive bacteria. The synthesized molecules were not identified as nanoparticles but as aggregates (Mullen et al. 1989).

The role of microbial cells in the fate of metals in the environment was not thoroughly examined; however, it was conceived that they represent an important component of metal dynamics. The first evidence of synthesizing silver nanoparticles was established in 1984 using the microorganism *Pseudomonas stutzeri* AG259, a bacterial strain that was originally isolated from silver mine (Haefeli et al. 1984;

Zhang et al. 2005; Nair and Pradeep 2002). Thin sections of bacteria under the transmission electron microscope showed the deposition of the silver on the cell membrane. This opened new avenues towards the preparation of nanostructured materials that incorporate silver-based crystalline particles with defined structural, compositional and morphological properties. Biological methods are gaining impetus because of the use of normal conditions for the synthesis that enable control over the size of the nanoparticles. The formation of nanoparticles has been well elucidated in *Morganella* sp. The organism was grown at different concentrations of $AgNO_3$, but the bacterium was able to grow at 0.5 mM $AgNO_3$. The growth rate decreased with increase in $AgNO_3$ concentration. The bacterium was allowed to grow up to the late exponential phase, and different concentrations of $AgNO_3$ were then added. As a consequence, similar experiments have been performed in the closely related bacterium *E. coli* to understand the role of silver resistance in AgNPs production from *Morganella* sp. (Parikh et al. 2008).

2.3 An Account of Organisms Synthesizing Silver Nanoparticles

Biological methods of silver nanoparticle synthesis require a special ability: "Resistance of the organism to silver ions" (the resistance mechanism will be explained later in this chapter). It is to be noted that those organisms which synthesize silver nanoparticles are also vulnerable to higher concentrations of silver ions. For example, *Bacillus licheniformis* is one such organism used to synthesize silver nanoparticle at 1 mM concentration, i.e., when the concentration of the silver ion in the environment is 1 mM, the organism can synthesize silver nanoparticles without undergoing cell death. But, when the concentration of the silver ions is raised, say 10 mM, the organism undergoes cell death within minutes, i.e., when the concentration crosses the threshold level (Kalimuthu et al. 2008; Pandian et al. 2010). Even though the organism has the resistance to silver ions, it becomes useless at the higher concentration. That is why silver can be rightly called "moiety with two functions" – one is inducing the organism to synthesize nanoparticles at lower concentration, another is the induction of cell death at higher concentration. The following are some of the organisms which had been reported to synthesize silver nanoparticles.

2.3.1 Various Species of Microorganisms Synthesizing Silver Nanoparticles

Bacteria:

S. No	Organism	Size (nm)	Author (year)
1	*Pseudomonas stutzeri AG259*	200	Tanja et al. (1999)
2	*Lactobacillus Strains*	500	Nair and Pradeep (2002)
3	*Bacillus megaterium*	46.9	Fu et al. (1999)
4	*Klebsiella pneumonia* (culture supernatant)	50	Ahmad et al. (2007)

(*continued*)

S. No	Organism	Size (nm)	Author (year)
5	*Bacillus licheniformis*	50	Kalimuthu et al. (2008)
6	*Bacillus licheniformis* (culture supernatant)	50	Kalishwaralal et al. (2008)
7	*Corynebacterium* sp.	10–15	Zhang et al. (2005)
8	*Bacillus subtilis* (culture supernatant)	5–60	Saifuddin et al. (2009)
9	*Geobacter sulfurreducens*	200	Law et al. (2008)
10	*Morganella* sp.	20 ± 5	Parikh et al. (2008)
11	*Bacillus subtilis*	5–60	Saifuddin et al. (2009)
12	*Escherichia coli*	1–100	Gurunathan et al. (2009a, b)
13	*Proteus mirabilis*	10–20	Samadi et al. (2009)
14	*Bacillus* sp.	5–15	Pugazhenthiran et al. (2009)
15	*Bacillus cereus*	4 and 5	Ganesh Babu and Gunasekaran (2009)
16	*Staphylococcus aureus*	1–100	Nanda and Saravanan (2009)
17	Lactic acid bacteria	11.2	Sintubin et al. (2009)
18	*Brevibacterium casei*	50	Kalishwaralal et al. (2010)

Fungi:

S. No	Organism	Size (nm)	Author (year)
1	*Fusarium oxysporum*	5–50	Ahmad et al. (2003)
2	*Aspergillus fumigatus*	5–25	Bhainsa and D'Souza (2006)
3	*Aspergillus niger*	20	Gade et al. (2008)
4	*Phanerochaete chrysosporium*	100	Vigneshwaran et al. (2006)
5	*Aspergillus flavus*	8.92 ± 1.61	Vigneshwaran et al. (2007)
6	*Cladosporium cladosporioides*	10–100	Balaji et al. (2009)
7	*Fusarium semitectum*	10–60	Basavaraja et al. (2008)
8	*Trichoderma asperellum*	13–18	Mukherjee et al. (2008a, b)
9	*Cladosporium cladosporioides*	10–100	Balaji et al. (2009)
10	*Trichoderma viride*	5–40	Fayaz et al. (2010)
11	*Penicillium fellutanum*	1–100	Kathiresan et al. (2009)
12	*Penicillium brevicompactum* WA 2315	23–105	Shaligram et al. (2009)
13	*Verticillium* sp.	25 ± 12	Mukherjee et al. (2001)
14	*Fusarium solani*	5–35	Gade et al. (2009)
15	*Fusarium acuminatum*	5–40	Ingle et al. (2008)
16	*Aspergillus clavatus*	10–25	Verma et al. (2010)

Plants:

S. No	Organism	Size	Author (year)
1	*Azadirachta indica*	50	Shankar et al. (2004)
2	*Cinnamomum camphora* leaf	55–80	Huang et al. (2007)
3	*Glycine max* (soybean) leaf extract	25–100	Vivekanandhan et al. (2009)
4	*Jatropha curcas*	10–20	Bar et al. (2009)
5	*Cinnamomum camphora* Leaf	5–40	Huang et al. (2008)
6	*Phyllanthus amarus*	18–38	Kasthuri et al. (2009)
7	*Carica papaya*	60–80	Mude et al. (2009)
8	*Gliricidia sepium*	10–50	Raut et al. (2009)
9	*Coriandrum sativum* leaf extract	26	Sathyavathi et al. (2010)

When genomic analysis of these organisms has been performed, it may show that one thing which confers resistance to silver ions will be common in most of the above-mentioned organisms.

2.4 Mechanism Involved in Silver Nanoparticle Synthesis

Not all the organisms are found to be competent for the synthesis of silver nanoparticles. As previously mentioned, those organisms which contain the "Silver resistance machinery" can synthesize silver nanoparticles provided that the concentration of the silver ions does not cross the "threshold limit". The resistance mechanism differs with organisms. Extracts from bio-organisms may act both as reducing and capping agents in Ag NPs synthesis. The reduction of Ag^+ ions by combinations of biomolecules found in these extracts such as enzymes/proteins, amino acids, polysaccharides and vitamins is environmentally benign, yet chemically complex. But, the mechanism which is widely accepted for the synthesis of silver nanoparticles is the presence of enzyme "Nitrate reductase" (Anil Kumar et al. 2007; Kalimuthu et al. 2008). Nitrate reductase is an enzyme in the nitrogen cycle responsible for the conversion of nitrate to nitrite (Duran et al. 2005). The reduction mediated by the presence of the enzyme in the organism has been found to be responsible for the synthesis. The use of a specific enzyme α-NADPH-dependent nitrate reductase in the in vitro synthesis of nanoparticles is important because this would do away with the downstream processing required for the use of these nanoparticles in homogeneous catalysis and other applications such as nonlinear optics. During the catalysis, nitrate is converted to nitrite, and an electron will be shuttled to the incoming silver ions. This has been excellently described in the organism *B. licheniformis*. *B. licheniformis* is known to secrete the cofactor NADH and NADH-dependent enzymes, especially nitrate reductase, that might be responsible for the bioreduction of Ag^+ to Ag^0 and the subsequent formation of silver nanoparticles. Figure 2.1 shows that the nitrate reductase present in the bacteria may aid the synthesis of silver nanoparticles (Kalimuthu et al. 2008).

Although all these are speculation, direct evidence was provided by Anil Kumar et al. (2007) who directly used the purified nitrate reductase from the organism *Fusarium oxysporum* for the synthesis of silver nanoparticle in test tube. Their reaction mixture contained only the enzyme nitrate reductase, silver nitrate and NADPH. Slowly, the reaction mixture turned brown with all the characteristics of silver nanoparticles. This is the first direct evidence for the involvement of nitrate reductase in the synthesis of silver nanoparticles.

Although silver nanoparticles synthesis is considered as a "capability" of the organism, it is primarily considered as a defense mechanism by the organisms to the incoming very reactive silver ions. Interesting facts about silver nanoparticle synthesis can be understood when the real mechanism involved in the antimicrobial activity of silver ions is known (Silver et al. 2006). Silver ions are very reactive and are known to bind with various vital components of the cells inducing cell death.

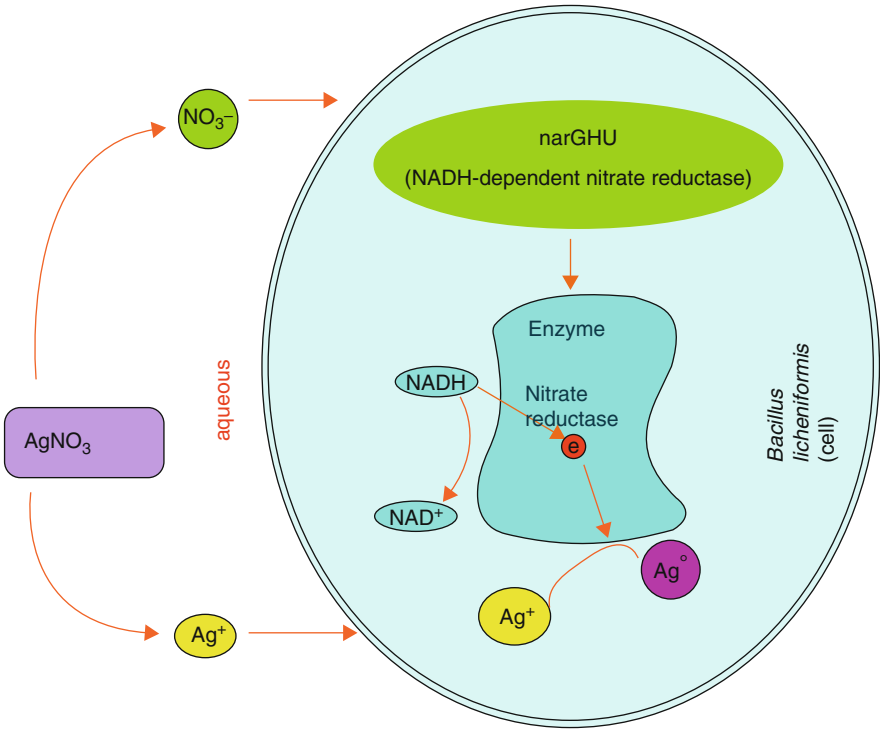

Fig. 2.1 A hypothetic diagram of how silver ions are reduced to silver atom by the enzyme nitrate reductase [figure adopted from Kalimuthu et al. (2008)]

Interestingly, "apoptosis" is a mechanism which is considered to be applicable not only to multicellular organisms but also to unicellular microorganisms. (Engelberg-Kulka et al. 2006). The following are the effects by which silver ions exhibit their antimicrobial functions. (1) Binding of silver ions to the negatively charged DNA (prokaryotes do not contain histones) thereby making the DNA to lose its structure and also inhibiting the replication of DNA. (2) Binding of silver ions with the thiol-containing proteins, thereby inhibiting the function of proteins. (3) Induction of reactive oxygen species synthesis leading to the formation of highly reactive radicals that destroy the cells. Silver ions are known to particularly inhibit enzymes such as NADH dehydrogenase II in the respiratory system, which is implicated as a candidate for the site of production of reactive oxygen species in vivo. The free radicals resulting from H_2O_2 are mainly hydroxyl (OH−) groups that result from the Fenton reaction (Matsumura et al. 2003; Gautam and Sharma 2002; Cabiscol et al. 2000). This mechanism can also be indirectly studied, i.e., catalase is considered as a scavenger of reactive oxygen species in microbes. During experiments with *B. licheniformis* the increase in the silver ions concentration was accompanied by an increase in the catalase synthesis, which is upto the minimal inhibitory concentration, beyond which even catalase did not help the cell to

"survive". This was also evidenced by Matsumura et al. (2003) where the mutated strain of *E. coli* UM1 (katE katG), deficient in catalase, was found to be more sensitive to both silver zeolite and silver nitrate than its parent. This shows that silver ions induce apoptosis in bacteria. Therefore, the bacterial cell tries to protect itself from the incoming silver ions, by converting them to inactive Ag^0 form. Further incoming silver ions were also shuttled with electrons and this leads to the growth of the crystals (Fig. 2.2).

This defense mechanism is applicable to various metals, where the difference occurs only in the respective enzyme. Here, in *B. licheniformis*, the nitrate reductase is found at the cell membrane as respiratory nitrate reductase. A picture of cells treated with silver nitrate shows the particles to be present on the circumference of the cell, i.e., at the cell membrane (Fig. 2.3) (Pandian et al. 2010). Therefore, it can be regarded as that in most of the organisms identified to synthesize silver nanoparticles, nitrate reductase will be a part and parcel of the organism.

Moreover, when the condition of the silver nanoparticle synthesis is alkaline, the synthesis will be faster than in acidic conditions. In other words, synthesis enhances as the pH increases towards alkaline region and reaches the maximum at pH 10 after

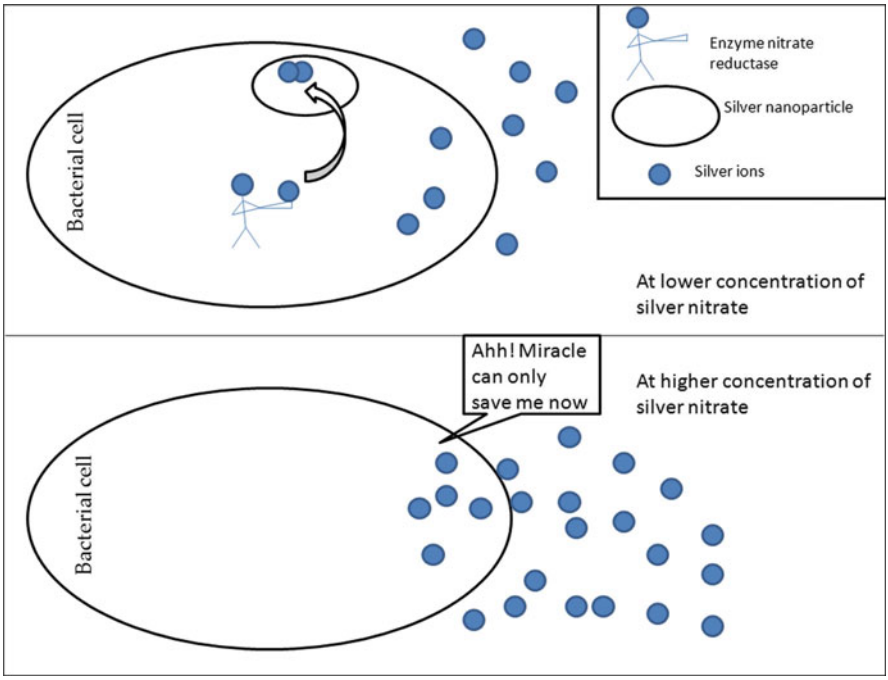

Fig. 2.2 Silver is a bifunctional molecule. At lower concentration the enzyme responsible for nanoparticle synthesis (nitrate reductase) may convert the incoming Ag^+ to Ag^0 and favor the deposition of them as crystals. Whereas at higher concentrations (beyond the threshold limit), the conversion may occur, but more reactive silver ions induces "apoptosis" in bacterial cells by various mechanisms

Fig. 2.3 TEM image of thin section of the bacteria challenged with 1 mM silver nitrate, showing the synthesis of silver nanoparticles along the circumference of the bacteria. Image adopted from Pandian et al. (2010)

which the speed of the nanoparticle synthesis decreases. This shows that the synthesis of silver nanoparticles will be favored by the alkaline environment. At alkaline conditions there is no need of agitating the mixture for the formation of silver nanoparticles and all the silver ions supplied will be converted to silver nanoparticles even within 30 min. The proteins involved in the synthesis may bind with silver at thiol regions (–SH) forming a –S–Ag bond, a clear indication of which aids the conversion of Ag^{+} to Ag^{0}. In addition, the alkaline ion (−OH) is very much required for the reduction of metal ions. It takes 4 days for the production of silver ions in normal conditions whereas it is very much less than an hour when the pH is made alkaline. Moreover, under alkaline conditions the ability of the enzyme responsible (not only nitrate reductase) for the synthesis of silver nanoparticles increases (Sanghi and Verma 2009).

2.4.1 Size Control Over the Biological Synthesis of the Nanoparticles

Although chemical synthesis aids the size control over the synthesis of nanoparticles, size control can also be achieved in biological methods. A report by Gurunathan et al. (2009a, b) showed that by controlling the environment of nanoparticle synthesis, silver nanoparticles of various sizes and shapes could be synthesized. At room temperature, silver nanoparticles of 50 nm are synthesized whereas at 60°C nanoparticles of 15 nm are synthesized. Similarly at acidic pH the size of the nanoparticle ranged 45 nm whereas at pH 10 the size is just 15 nm. Even the size of 2–20 nm silver nanoparticles could be synthesized by organisms such as *Verticillium* sp.

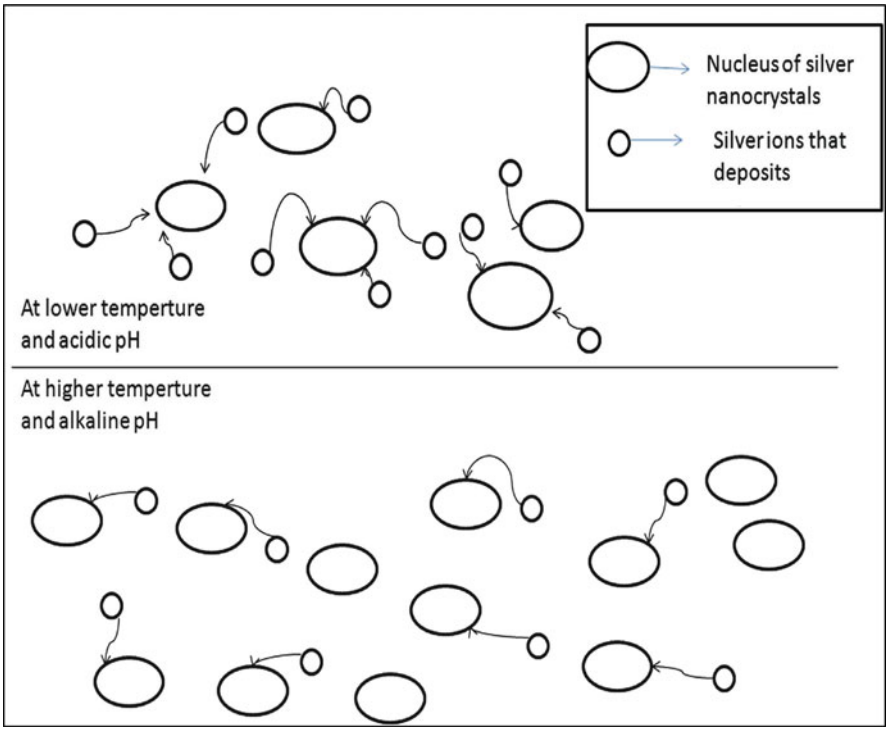

Fig. 2.4 A schematic imaginary diagram that depicts the reason for size control over the synthesis of silver nanoparticles. At lower temperature and pH, less nucleation occurs thereby forming larger particles whereas at higher pH and temperature more nucleation may occur thus forming smaller particles

(Mukherjee et al. 2001) intracellularly. The size-controlled synthesis of silver nanoparticles by controlling the environment is due to the formation of many seed crystals. At acidic pH and lower temperatures there will be less nucleation for silver crystal formation on which new incoming silver atoms deposit to form larger sized particles. But as the pH and temperature increase, the dynamics of the ions increase and more nucleation regions are formed due to the availability of –OH ions and increased temperature. The conversion of Ag^{+} to Ag^{0} increases followed by increase in the kinetics of the deposition of the silver atoms (Fig. 2.4).

2.5 Process Design for Silver Nanoparticulate Synthesis in the Industry and Identification of the Chief Components Involved in Synthesis

The above-mentioned fact is limited only to the biomass, i.e., only to the cells. The problems which may occur during nanoparticle synthesis by the biomass are:

(a) Primarily silver nanoparticles are attached to the cell membrane. So the first step will be to break the cells and to isolate the silver nanoparticles.
(b) To purify the nanoparticles from the other components present in the supernatant.

This may sound tedious for some applications and large-scale production. To overcome the first problem, the particles can be synthesized using a culture supernatant. But when it comes to the supernatant, there are various components which may aid the synthesis of silver nanoparticles. There are also reports of synthesis of silver nanoparticles by the culture supernatant. Although supernatant can contain the enzyme nitrate reductase, it is less likely that NADPH is present in the supernatant. It is here where the question arises, what is the real factor that aids the synthesis of silver nanoparticles by the cell-free culture supernatant? Culture supernatant is found to be the easiest way for the size-controlled synthesis of silver nanoparticles. The environment of the culture supernatant can be easily modified and maintained than the biomass, where the components in the cytoplasm would try to maintain constant environment such as heat shock proteins. Therefore, culture supernatant can be used for the synthesis of silver nanoparticles rather than cells itself. But, synthesis of nanoparticles by culture supernatant as a whole is not specific. Moreover, the culture supernatant possesses the second problem of downstream processing. When synthesis of silver nanoparticles is closely analyzed some components are vital for the synthesis. The components required for the synthesis of silver nanoparticles are:

(a) Silver nitrate
(b) $NADH_2$
(c) Stabilizer
(d) Catalyst that converts silver ion to nanoparticles

Therefore a mixture similar to culture supernatant can be prepared which can be successfully applied for the nanoparticle synthesis. This could overcome the aforesaid problems in the nanoparticle synthesis.

The reasons for using a mixture similar to culture supernatant for the synthesis of silver nanoparticles are given below.

2.5.1 Large Quantity of Synthesis

Since silver ion is toxic to cells, the concentration of silver ions for the synthesis should be lesser than what we say as the “threshold level”, i.e., the conditions beyond which the cells die. So for the synthesis of silver nanoparticles by biomass the optimum concentration that has been patented is 1 mM (Ahamd et al. 2003; Kalimuthu et al. 2008). But higher concentrations of silver ions had been reported for the synthesis of silver nanoparticles by the crude culture supernatant (Gurunathan et al. 2009a, b). When a specific mixture is used, the concentrations can be increased as the amount of enzyme is increased.

2.5.2 Specific Reaction Can be Induced by Adding Various Inducers

Silver nanoparticle synthesis can be made specific, because only the enzyme nitrate reductase will be present in the supernatant (Kalishwaralal et al. 2008). Moreover, the activity of the enzyme can be induced by various inducers of the enzyme. Because ionic silver has the ability to bind with the various components present in the cells, 100% yield of silver nanoparticle synthesis will be attained after a specific period of time which may be due to the impedance created by the cell wall and plasma membrane because of osmotic pressure. These could be overcome by using the enzyme alone.

2.5.3 Easy Control of Size (and shape) by Controlling the Environment

Previously Gurunathan et al. (2009a, b) have reported the size-controlled synthesis of silver nanoparticles by controlling the temperature and pH of the supernatant of *E. coli*. The supernatant was added with 1 mM $AgNO_3$ and incubated at various temperatures and pH. The average size distribution varied according to the environment. When the temperature was increased the size of the nanoparticles reduced up to 60°C, which increased thereafter. This can be explained on the basis of thermal kinetics, i.e., as temperature increases and favors more nucleation the particle size is reduced. But, after a certain temperature deposition of silver was rather more than the nucleation and aggregation may also have been a part of it. In case of pH the average size went on decreasing up to pH 10 after which it also increased.

2.5.4 Immobilization of Enzymes

Biomass once used for the synthesis of nanoparticles cannot be reused, which applies for the enzymes in free form also. But when the enzymes are immobilized using a support the enzymes can be used for a large number of reactions. Essentially the enzymes should be immobilized upon the surface of particle of immobilization to rule out the diffusion limitation (Clark 1994).

2.5.5 Easy Downstream Steps for the Purification of Nanoparticles

Think of a small comparison. Will it be easy to purify the compound with large number of impurities or with very less impurities. Obviously the latter is the

better. Using purified enzymes will make the downstream steps much simpler as the enzyme will mostly be the impurity, but when immobilized enzymes are used this can also be ruled out where the downstream steps can be very much reduced.

2.5.6 *Easy for Scaling-up of the Reactions*

Use of biomass for the large-scale synthesis requires various power-consuming processes such as agitation and harvesting the biomass. In addition, the purification of the nanoparticles is a tedious process. Here using immobilized nitrate reductase will also reduce most of the power consumption processes and downstream processing steps.

2.5.7 *Separation of Nanoparticles by Gel Electrophoresis According to Size and Shape*

The synthesis of nanoparticles results in a wide distribution in the size of the nanoparticles. An easy method for separation of the nanoparticles using gel has been reported (Hanauer et al. 2007). To use size- and/or shape-dependent material properties, such as quantum confinement or plasmon resonances, it is critical to have nanoparticles with the lowest size and shape dispersion possible. An alternative to the high-yield synthesis of nanoparticles with ultranarrow size distribution is the postsynthetic separation of particles similar to cleaning procedures in organic synthesis. Gel electrophoresis is commonly used to separate biomolecules, but this technique is also used in the separation of nanoparticles based on size. The separation is mediated by the number of polymer chains attached and these are characterized by the strong colors induced by plasmon resonance. The strong influence of size and shape on the frequency or wavelength of the plasmon resonance would make it desirable to obtain monodisperse samples for various applications. Compared to the other separation techniques, gel electrophoresis has the advantage of allowing multiple runs in parallel on the same gel, which is a considerable advantage at the stage of understanding mechanisms and optimizing conditions. To stabilize the nanoparticles, we coat them with a layer of polyethylene glycol (PEG, MW 5,000), which is covalently attached at one end to the metal surface via a thiol group (“PEGylation”). The other end of the polymer chain may carry different functional groups, which we exploit for controlling the overall particle charge and mobility. The size and shape of the nanoparticles obtained are used to synthesize particles of different shapes and these have been analyzed using gel electrophoresis.

2.6 Scaling-up of Silver Nanoparticle Synthesis

One of the important challenges in nanomaterials production is scaling up laboratory processes to the industrial scale. Mathematical modeling is an integral component of our research strategy, both for process scale-up and design and for process optimization and control. Large-scale biological synthesis of nanoparticles has always been a big challenge. Here also the synthesis of silver nanoparticles by biomass is a tedious process. Therefore, the second option of nanoparticle synthesis by nitrate reductase will be a better option. Scaling up of silver nanoparticle synthesis biologically should start from the first step of production of nitrate reductase. In a report by Vaidyanathan et al. (2010) this step has been reported where they have optimized the synthesis of nitrate reductase by the organism *B. licheniformis*. The optimization of nitrate reductase led to the enhanced synthesis of silver nanoparticles. The synthesis was found to be dependent on the enzyme activity. Since all the culture supernatants were supplied with same concentration of silver ions, there is no chance of extra silver ions coming into picture. This has revealed that increase in the amount of enzyme increases the rate of reaction.

Although, currently there are no reports on very large quantity of synthesis of silver nanoparticles, some prototypes have been designed in continuous flow reactors similar to chemical methods. The use of plant product from *Cinnamomum camphora* leaf has been used to synthesize silver nanoparticles in a continuous flow tubular microreactor (Huang et al. 2008). Scaled-up production of monodisperse colloidal nanoparticles has become an important research subject in recent years. In this regard, continuous flow reactors are generally favored over batch reactors. Decomposition of organometallic precursors in organic solvents is one of the most commonly used approaches to nanoparticles because the narrow size distribution can be achieved in such reaction systems. Rapid injection of precursors into a heated mixture of solvent, coordinating ligands, and other precursors is often required. A batch process is suitable for those reactions, but often limited to small-scale synthesis due to the low production yield of nanoparticles and the time-consuming nature of the process. A flow reactor, on the other hand, can generate products on a continuous basis once the reaction reaches steady state and is more appropriate for a large-scale production than the batch reactor. Furthermore, targeted reaction temperatures can be achieved in second or even millisecond time scales in a microreactor. The advantage of tubular reactor is that it can be easily scaled up by increasing the length of the reactor (Synthesis of Silver Nanoparticles in a Continuous Flow Tubular Microreactor). Based on the above methods, two kinds of process design can be made for the synthesis of silver nanoparticles by the biomass and the enzyme.

Using the former design the biomass synthesized can be harvested and used for the synthesis of silver nanoparticles (Fig. 2.5). But it may require some more additional downstream steps such as purification of the nanoparticle from the other proteins. But when the immobilized enzymes are used, the downstream

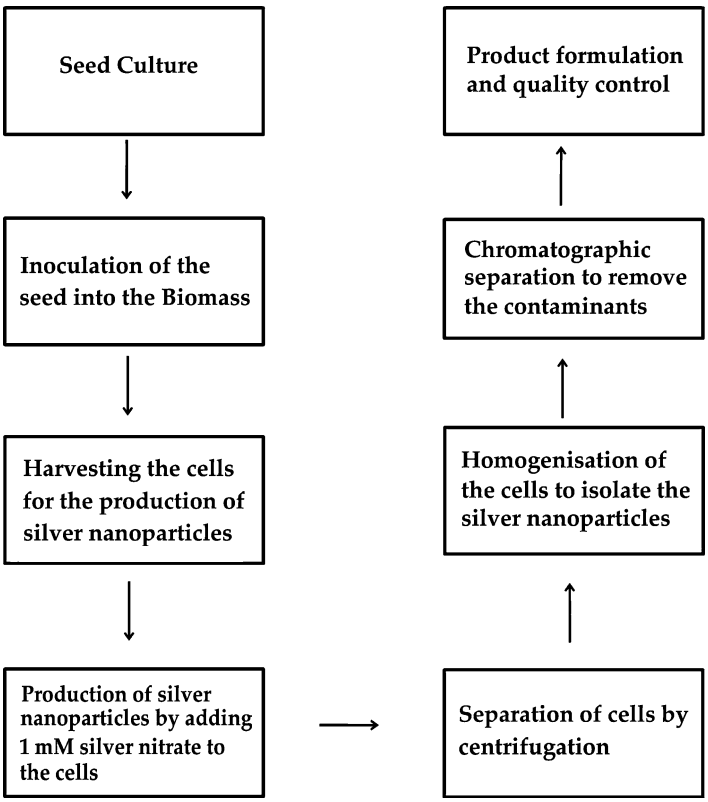

Fig. 2.5 Process design for the industrial scale synthesis of silver nanoparticle synthesis by biomass

steps will be very less and will be advantageous as the nitrate reductase can be reused for the synthesis of silver nanoparticles (Fig. 2.6).

2.7 Conclusion

Reducing the size of a particle has a greater impact on the property of the molecule and the properties also completely differ from the bulk material. But the synthesis of nanomaterials at nanoscale involves tedious phenomena (reducing agents) when synthesized chemically. Moreover, the chemically synthesized nanoparticles require another step for the prevention of aggregation of the particles. But, biological synthesis of silver nanoparticles uses harmless, eco-friendly reducing agents and the nanoparticle structure is stabilized by the proteins present in the environment eliminating the extra step in preventing the aggregation of

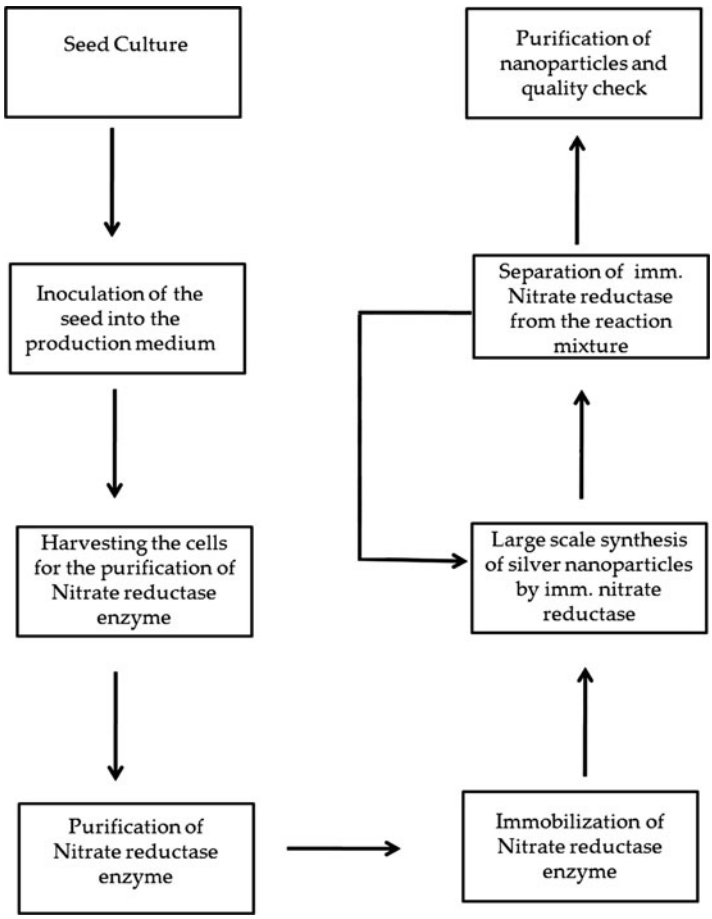

Fig. 2.6 Process design for the industrial scale synthesis of silver nanoparticle by immobilized nitrate reductase

chemically synthesized nanoparticles by stabilizers. Since the biological molecules are eco-friendly, the pollution due to chemicals and byproducts can be prevented from reaching the environment. The size and shape of the particle can also be completely regulated by controlling the environment where the nanocrystal growth occurs (pH and temperature). Moreover, biological methods have the greater advantage of easy bulk synthesis which can be exploited for industrial scale production too.

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