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BIOLOGICAL SYNTHESIS OF NANOPARTICLES USING BACTERIA

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Abstract:

Several ways such as physical, chemical and biological methods were used to synthesize metal nanoparticles. It has been confirmed that some microorganisms have the ability to produce nanoparticles. Numerous bacteria, fungi and plants have shown the potential to synthesise metallic nanoparticles. Bioreduction is one of the mechanisms to biosynthesize metal nanoparticles by microorganisms. Nanoparticles are formed on the surface of cell walls, and trapping of the metal ions on this surface is considered the first step in bioreduction.

For example The bioreduction of metal ions and stabilization of the silver NPs occured due to an enzymatic process and It is suggested that nitrate reductase is one of the enzymes responsible in the bioreduction of ionic gold.

The formation of nanoparticles with a particular size and morphology is ultimately determined by the interaction and biochemical processing activities of a specific microorganism and the influence of environmental factors such as pH and temperature

Key words:

Nanoparticles, Biosynthesize, Environmental factors

Nanoscience

There has been a great interest on Nanoscience and nanotechnology due to its substantial impact on many scientific areas such as energy, medicine, pharmaceutical industries, electronics, and space industries. Nanoparticles (NPs), on the other hand, have many applications in various fields such as medical imaging, nanocomposites, filters, drug delivery, and hyperthermia of tumors (1-4).

In recent years the topic of nanoparticles has been in the center of a wide range of fields. The term “nano” derives from the Greek word “nanos” meaning dwarf and is a sign of a measurement on the scale of one-billionth of a metere in size(5,6).

Several ways such as physical, chemical and biological methods were used to synthesize metal nanoparticles. Although, the production of physical and chemical nanoparticles need non-living conditions, animated conditions should be provided for biosynthesis of nanoparticles. It has been confirmed that some microorganisms have the ability to produce nanoparticles. This production of nanoparticles has been performed by reduction of metal ions. This method, relatively, has the advantage of being environmentally friendly. Microbial cells neutralize the metal ions by their reduction into insoluble metal either inside or outside of the cells and thus produce nanoparticles. The accumulation site of nanoparticles depend on localization of the reductive enzymes. Nanoparticles are produced within the cells, If reductive enzymes secreted by the cells bioreduction occure out of the cells and if reductive enzymes remain and act inside of the cells (7, 71,72).

Biosynthesis of nanoparticles

Biosynthetic methods that employ either biological microorganisms or plant extracts, have appeared as a simple and viable alternative to chemical synthetic procedures and physical methods (8-15).
Numerous bacteria, fungi and plants have shown the potential to synthesise metallic nanoparticles and, of course, all have their own advantages and disadvantages (16-18).
For the synthesis of NPs, one of the options is to use natural processes such as employing of enzymes, microbial enzymes, vitamins, polysaccharides, biodegradable polymers, microorganisms, and biological systems(19-21).
Bioreduction is one of the mechanisms to biosynthesize metal nanoparticles by microorganisms (22). In microbial bioreduction procedure, myriads of proteins, carbohydrates and biomembranes are involved (23). Nanoparticles are formed on the surface of cell walls, and trapping of the metal ions on this surface is considered the first step in bioreduction. This process probably occurs as a result of the electrostatic interaction between the metal ions and positively charged groups in enzymes present at the cell wall. This is followed by enzymatic reduction of the metal ions, resulting in their aggregation and thus the formation of nanoparticles (24). The microbial cell causes metal ions to reduce by employing specific reducing enzymes like NADH-dependent reductase or nitrate dependent reductase (25). Formation of nanoparticles can be either extracellular or intracellular depending on the microorganismas (26-30).

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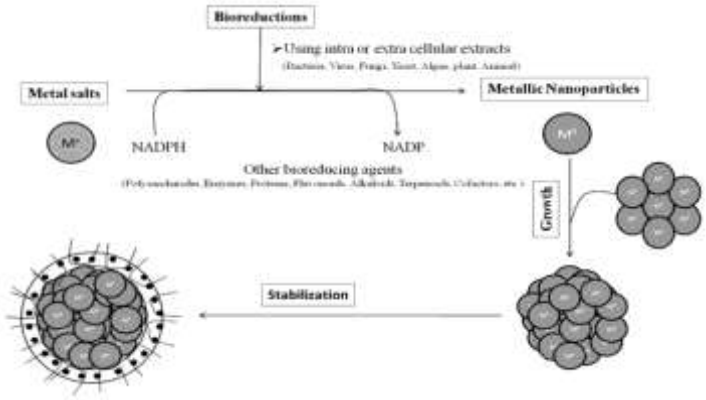
Nevertheless, the need for toxic solvents and the contamination resulted from chemicals used in nanoparticle production, limit their potential use in biomedical applications (31). Therefore, to allow a “green”, non-toxic way of synthesizing metallic nanoparticles, is needed to be used in a wider range of industries. This is a potential way to achieve, by using biological methods.

There are two categories to synthesize “natural” biogenic metallic nanoparticle. Synthesis can be divided into two categories. The first is through bioreduction, in which metal ions are chemically reduced into more stable forms biologically. Many organisms are able to utilise dissimilatory metal reduction. In this way, reduction of a metal ion is coupled with the oxidation of an enzyme (32).

When producing nanoparticles by employing the intracellular and an extracellular extract of organisms, the extract is simply mixed with a solution of the metal salt at room temperature. Within a few minutes, the reaction is complete. Nanoparticles of silver, gold and other metals have been produced previously (33). In Fig.1 a picture of various organisms that are used for the biosynthesis of nanoparticles is shown. It is known that the nature of the living extract, its concentration, the concentration of the metal salt, the pH, temperature and contact time affect the rate of production of nanoparticles, their quantity and other characteristics.

Fig. 1

Mechanisms of nanoparticle synthesis



Bacteria in Nanoparticle Synthesis

One approach depicting immense potential is based on the biosynthesis of NPs using bacteria. Thus, in green nanotechnology numerous bacterial species have been used to research alternative methods to synthesize NPs. Bacteria are considered as a potential biofactory for the synthesis of NPs like gold, silver, platinum, palladium, titanium, titanium dioxide, magnetite, cadmium sulphide, and so forth. Magnetotactic bacteria and S-layer bacteria are some well-known examples of bacteria synthesizing inorganic materials (34–37). For instance, nanoparticles such as silver and gold have shown to be effective in inhibiting growth of both Gram-positive and Gram-negative bacteria (38-40).

Bacteria possess considerable ability to reduce heavy metal ions and are one of the best candidates for synthesizing nanoparticles. Some bacterial species, for instance have developed the ability to resort to specific defense mechanisms to quell stresses like toxicity of heavy metal ions or metals. It has been observed that some of them could survive and grow even at high metal ion concentrations (41,42).

Bacterial cells were capable of binding large quantities of metallic cations. In addition, some of these bacteria were able to synthesize inorganic materials like the magnetotactic bacteria, which synthesize intracellular magnetite NPs (43).

The first report of bacteria synthesizing silver nanoparticles dates back to 1984 when Haefeli reported Pseudomonas stutzeri AG259, a bacterial strain originally isolated from silver mine capable of synthesizing silver nanoparticles (44).

When growing on elemental sulfur as an energy source, Thiobacillusferrooxidans, T. thiooxidans, and Sulfolobusacidocaldarius were able to reduce ferric ion to the ferrous state (45). T.thiooxidans was aerobically able to reduce ferric iron at low pH medium. The ferrous iron formed was stable to autoxidation and T. thiooxidans was unable to oxidize ferrous iron, but because of the rapid bacterial reoxidation of the ferrous iron in the presence of oxygen the bioreduction of ferric iron using T. ferrooxidans was not aerobic (45).

Samples of nanoparticles

Silver NPs

A novel approach in combinational synthesis for green biosynthesis of silver NPs is using a combination of culture supernatant of Bacillus subtilis and microwave irradiation in water (46).

Supernatants of B.subtilis are used in the extracellular biosynthesis of monodispersed silver NPs, but in order to increase the rate of reaction and reduce the aggregation of the produced NPs, microwave radiation is used which might provide uniform heating around the NPs and could assist the digestive ripening of particles with no aggregation (46).

Extracellular synthesis of silver NPs by bioreduction of aqueous Ag^+ ions with the culture supernatant of Bacilluslicheniformis was reported (47). In addition, B. licheniformis were used to synthesize well-dispersed silver nanocrystals (48). In case of Bacillus flexus, silver NPs with spherical and triangular shape were successfully biosynthesized. Efficacy of NPs on antibacterial property against clinically isolated multidrug resistant (MDR) microorganisms were observed (49). Antibacterial activity resulted from produced NPs were shown against pathogenic bacteria like E. coli, Pseudomonasaeruginosa, Salmonella typhi, and Klebsiella pneumoniae.

The bioreduction of metal ions and stabilization of the silver NPs occured due to an enzymatic process. On the surface of the cytoplasmic cell membrane, silver NPs were formed inside the cytoplasm and outside of the cells, that possibly is due to the bioreduction of the metal ions by enzymes present on the cytoplasmic membrane and within the cytoplasm. Nitroreductase enzymes might be responsible for bioreduction of silver ions (50). There are important links between the way nanoparticles are synthesised and their potential uses. Silver nanoparticles (AgNPs) have been shown in numerous studies to display antibacterial properties (51,52).

Gold NPs

Gold NPs are biosynthesized both intracellularly and extracellularly. The nucleation of gold NPs occurres on the cell surface by sugars and enzymes in the cell wall, and then the metal nuclei are transported into the cell where they aggregate to larger-sized particles. The solution pH is an important factor in controlling the morphology of biogenic gold NPs. It is also significant in the location of gold deposition (53). The shape of gold NPs is controlled by pH (54).

The $AuCl_4^-$ ions can bind to the biomass through the main groups of secreted enzymes. These enzymes show a significant role in reduction of AuCl ions. Bioreduction of Au (3^+) to Au (0) and formation of gold NPs might be due to NADH dependent enzymes which are secreted by *R. capsulate*. The mechanism of reduction seems to be initiated by electron transfer from NADH by NADH dependent reductase as the electron carrier (55). Extracellular synthesis of gold NPs is determined by incubation of the plant growth-promoting culture supernatant with gold (III) chloridetrihydrate ($HAuCl_4 \cdot 3H_2O$). It is suggested that nitrate reductase is one of the enzymes responsible in the bioreduction of ionic gold (56).

Sulfate and sulfit reductase

Soluble sulfate enters into immobilized beads via diffusion to form ZnS nanoparticles, and later is carried to the interior membrane cell facilitated by sulfate permease. Then, ATP sulfurylase and phosphoadenosine phosphosulfate reductase cause sulfate to be reduced to sulfite, and next sulfite is reduced to sulfide by sulfite reductase. The sulfide reacts with O-acetyl serine to synthesize cysteine via O-acetylserinethiolyase (57), and then in presence of zinc, cysteine produces S^{2-} by a cysteine desulfhydrase. After this process, S^{2-} reacts with the soluble zinc salt and thus ZnS nanoparticles are synthesized (57). Finally, ZnS nanoparticles are discharged from immobilized cells to the solution (58).

Control of size, morphology, shape and monodispersity of nanoparticles

The formation of nanoparticles with a particular size and morphology is ultimately determined by the interaction and biochemical processing activities of a specific microorganism and the influence of environmental factors such as pH and temperature (59,60).

The size control on the nanoparticle synthesis is significantly important. The control of size and monodispersity is a major challenge of the biosynthesis. The studies have revealed that some parameters such as the type of microorganism, growth medium, and synthesis conditions can result in the size and monodispersity control of nanoparticles, (61-63).

Each species of each type of microorganisms could form different size and shape of the same composition particles. In the meantime, microbial growth medium and cultivation condition also affect the particle size and monodispersity of nanoparticles (64).

The direct control of factors, such as pH, substrate concentrations, source compound of target nanoparticle, temperature, reaction time, irradiation and agitation, has shown to facilitate the nanoparticle synthesis process (65).

The results showed that at pH 3, the particle size below 10 nm was predominant. While with the increase of pH from 3 to 5, 7 and 9, the main size of particles grew up until 30 nm with various shapes and some aggregates under the same synthesis system (66). Therefore, particle size and monodispersity can be controlled by the reaction time (67). The results showed that the smaller size particles and good monodispersity were observed in synthesis with shorter cells exposing time to ion solution (1h) than the synthesis with longer cells exposing time to the ion solution (24h). It indicates that the particle size and monodispersity can be controlled by manipulating the reaction time of biosynthesis (68).

Temperature

Temperature is one of the significant physical parameters for synthesis of nanoparticles. Synthesis of nanoparticles increases while increasing the reaction temperature. The higher rate of reduction occurs at higher temperature due to the consumption of metal ions in the formation of nuclei whereas, the secondary reduction stops on the surface preformed nuclei. The broadening peak obtained at low temperature shows formation of large sized nanoparticles and the narrow peak obtained at high temperature, indicates the nanoparticles synthesized are smaller in size. Thus, it can be established that higher temperature is optimum for nanoparticles synthesis (69).

Time

In a study, synthesis of nanoparticles at various time intervals was studied after reaction for 1 hour. The Ag-NPs obtained showed a UV-vis absorption peak, and the intensity of the peak increased as the reaction time increased, which indicated the continued reduction of the silver ions. The increase of the absorbance with the reaction time indicates that the concentration of AgNPs is increased. When the reaction time reached 3 hours the absorbance increased, and the λmax value was slightly shifted. This phenomenon continued for reaction times of 6 and 24 hours, indicating that the size of particles was decreased. At the end of the reaction i.e. 48 hours the absorbance was considerably increased and there was no significant change in λmax (430 nm), compared with the 24 hour reaction time. The Transmission electron microscopy (TEM) results indicate that the samples obtained over a longer time period retained a narrower particle size distribution. The average size of all prepared AgNPs was 20nm (70).

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