

Green Synthesis of Nanoparticles

Bacteria and Fungi

Abstract

Nanoparticles are the spearheads of the rapidly expanding field of nanotechnology. An array of physical and chemical methods is used for the synthesis of nanoparticles. The development of impeccable protocols for the synthesis of highly monodisperse nanoparticles of various sizes, geometries and chemical composition is one of the most challenging obstacles in the field of nanotechnology. Ultraviolet irradiation, aerosol technologies, lithography, laser ablation, ultrasonic fields, and photochemical reduction techniques have been used successfully for nanoparticle synthesis, but they continue to remain expensive and involve use of hazardous chemicals. There is growing concern to develop eco-friendly and economically viable methods for synthesis of nanoparticles. Biological systems, masters of ambient condition chemistry offer an environment-friendly alternative way to produce nanoparticles than the currently used protocols. Microbial synthesis of nanoparticles is a green chemistry approach that interconnects the fields of nanotechnology and microbial biotechnology. Biological synthesis of gold, silver, gold-silver alloy, platinum, palladium, selenium, tellurium, silica, titania, zirconia, magnetite and uraninite particles by bacteria, actinomycetes, fungi and yeasts have been reported worldwide. In spite of the stability of nanoparticles synthesized this way, the method faces several challenging obstacles like low monodispersity and production rates and higher production costs. Detailed study of cellular, biochemical and molecular mechanisms that govern the growth of the nanoparticle in biological systems is required to overcome these obstacles. In this paper, we describe the current status of nanoparticle production by microorganisms.

Introduction

Nanotechnology has attracted a great deal of attention over the recent years due to applications in various fields such as energy, medicine, electronic and space industries. There are two strategies for nanoparticle synthesis: top-down and bottom-up. In top-down approach, bulk material is broken down into small pieces gradually and in bottom-up approach, atoms and molecules are brought together to synthesize nano-sized particles. Bottom-up approach is generally used for biological synthesis of nanoparticles.

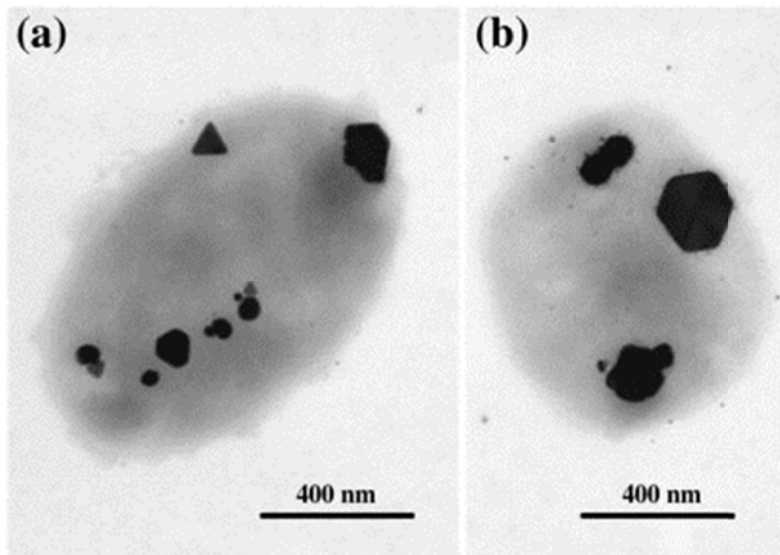
Due to their small size and high surface area nanoparticles have characteristic physical, chemical, mechanical, electronic, electrical, optical, magnetic, thermal, dielectric and biological properties not possessed by their larger sized counterparts. Optoelectronic, physiochemical and all other properties of nanoparticles are determined by the shape, size and monodispersity of the particle. These characteristics depend upon the method of synthesis of nanoparticles. Physical and Chemical methods used now for production of nanoparticles though lead to monodisperse nanoparticles, but they are less stable and various toxic chemicals are used. The use of toxic chemicals and non-polar solvents in synthesis leads to the inability to use nanoparticles in clinical fields. Therefore, development of clean, non-toxic, biocompatible and eco-friendly method for synthesis of nanoparticles deserves recognition. Even though biological synthesis of nanoparticles is considered cost effective, safe, environment-friendly and sustainable, it has various drawbacks. The culturing of microorganisms is time-consuming and it is difficult to have fine control over shape, size and crystallinity. The particles are not monodisperse and the rate of production is slow. These are the various problems which have vexed the biological synthesis of nanoparticles. But optimization of factors involved like pH, temperature, metal ion

concentration, and the strain of the microbe used has given hope for large scale application of biological synthesis. Moreover genetically engineered strains which express the reducing agent maximally can be used in the future which will provide better control over the shape and size of nanoparticles. Interaction between microbes and metals has been known for long and is used in bioremediation, biomineralization, bioleaching and biocorrosion but its use in the synthesis of nanoparticles is a recent discovery and lot of study is required before it can be put to practical use.

Nanoparticle synthesis by Bacteria

Microorganisms often produce inorganic materials of nano-size either extracellularly or intracellularly. Microbial systems are able to detoxify heavy metals by virtue of their ability to reduce the metal ions or precipitate the soluble toxic ions into insoluble non-toxic metal nanoparticles. A great deal of study has been carried out on synthesis of nanoparticles by prokaryotic bacteria since they are the easiest organisms to handle and can be manipulated most easily. Bacteria are able to form nanoparticles both intracellularly via bioaccumulation and extracellularly on the cell wall using its enzymes. Intracellular nanoparticles are of a fixed size with less monodispersity than extracellular particles. Hence, extracellular production has more commercial applications in various fields. Since monodispersity is the major factor in usefulness of nanoparticles, biological processes must be designed in such a way to ensure maximum monodispersity.

To obtain intracellular particles from bacteria requires further processing steps like ultrasound treatment or reaction with suitable detergents. This property can be exploited for extraction of precious metals from mine wastes and the metal nanoparticles can also be used as catalysts. When cell wall reductive enzymes or secreted enzymes are involved in the reduction of metal ions then it is logical to find the metal nanoparticles outside the cell. The extracellular nanoparticles have wider applications in the field of optoelectronics, bioimaging and sensor technology than intracellular particles.



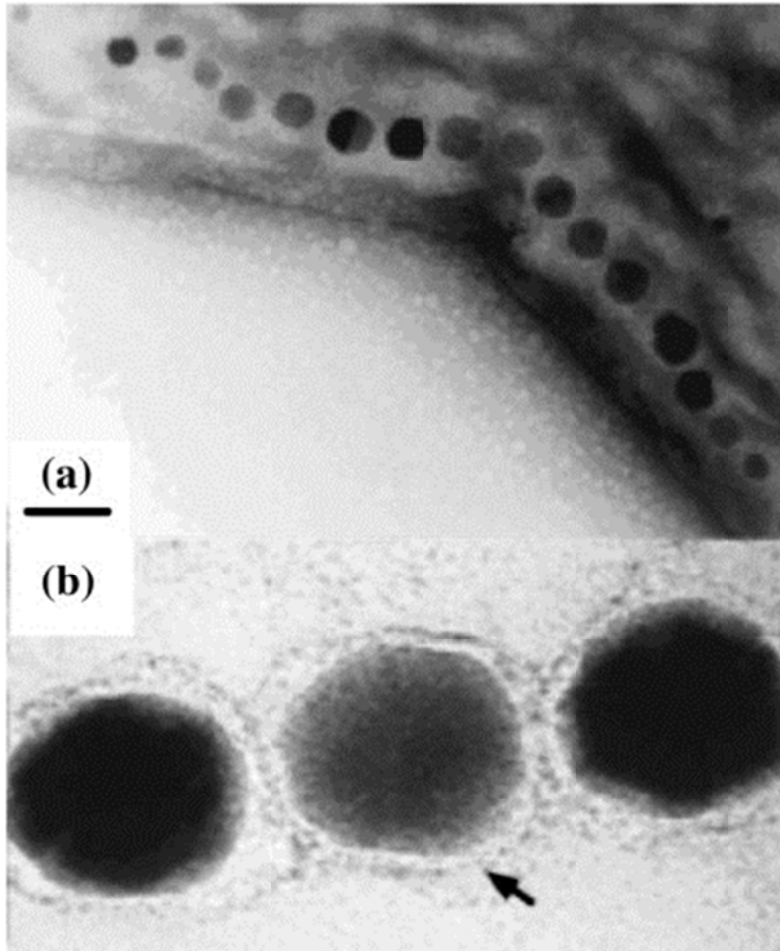
In one of the earliest study in this field, it was found that a silver-resistant bacterial strain isolated from silver mines, *Pseudomonas Stutzeri* AG259 was able to accumulate silver nanoparticles in its periplasmic space with the size ranging between 36-45 nanometers along with some silver sulfide. It was also noted that when this bacteria is placed in a concentrated aqueous solution of silver nitrate larger nanoparticles of up to 200 nanometers with defined morphology were formed. Cell growth and metal incubation conditions may be the reason for disparity in the size. The exact mechanism of formation of nanoparticles by this species of bacteria is yet to be understood. The ability of microorganisms to resist high concentration of toxic metal ions may result from specific reaction mechanisms. These may include efflux systems, extracellular precipitation by secreted/cell wall enzymes, alteration of solubility and toxicity y changing the oxidation state of the metal or absence of certain transport systems. Nanocrystalline silver can be recovered from the bacteria by thermally treating the bacteria to yield a carbonaceous nanomaterial. This material is composed of five percent by weight silver nanoparticles and rest dry biomass and has found applications in thin-film coating materials. *Bacillus subtilis* is able to reduce gold to form octahedral gold nanoparticles of size between 5 to 25 nanometers when incubated along with gold chloride solution. These are examples of generation of nanoparticles by bacteria in their natural settings. Some bacteria are able to produce nanoparticles in presence of concentrated metal solution and appropriate incubation settings even when naturally they do not face such conditions and do not produce any nanoparticles naturally. The exposure of some *Lactobacillus* strains (found in buttermilk), like *Lactobacillus sp.A09*, to silver and gold solution

lead to the formation of nanoparticles. It can also be used for the production of gold-silver alloy. Similarly, dried cell mass of *Corynebacterium sp. SH09* was able to produce silver nanoparticles with silver diamine complex at 60 degree centigrade after 72 hours. It is believed that organic matrix of the cell provides peptides that contain amino acid moieties that serve as a nucleation point for the start of nanoparticle formation. Silver precipitating peptides were found to have capable of reducing aqueous silver face centered cubic structured silver crystals. The exact mechanism of the formation is not yet known and further research needs to be carried out.

A prokaryotic bacterium *Rhodopseudomonas capsulata*, was found to deposit gold nanoparticles of 10-20 nanometers at 7 pH and room temperature extracellularly. As the pH of the solution was changed various nanoparticles with different sizes and geometries (like triangular and spherical at 4.0 pH) were formed. It was found experimentally that cell free extract of *Rhodopseudomonas Capsulata* can also be used for production of gold nanoparticles. SDS-PAGE analysis of the extract demonstrated the involvement of one or more proteins (14-98 kDa) in the reduction of gold and capping of gold nanoparticles. Similarly, silver nanoparticles can be produced extracellularly using *Enterobacter* culture supernatant. These bacteria secrete enzymes in their culture solutions which are able to reduce silver and assist in the formation silver nanoparticles. UV-visible spectroscopy and Transmission Electron Microscopy of the solution estimates the size of particles between 28-122 nanometers with the average size of 52.5 nanometers.

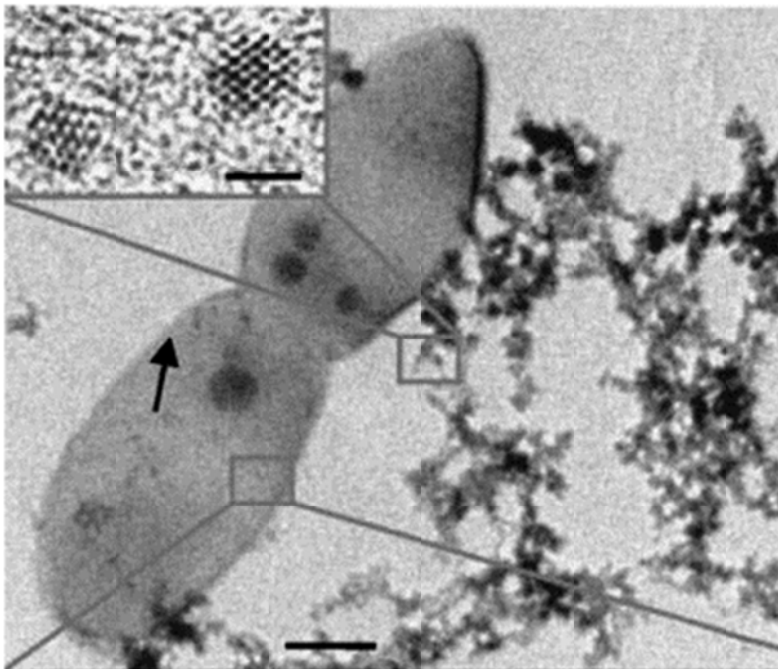
In addition to gold and silver much attention has been focused on synthesis protocols of semiconductors (quantum dots) like cadmium sulfide, zinc sulfide and lead sulfide. These luminescent quantum dots are emerging as a new set of materials with important applications in cell imaging and biosensing, based on the conjugation between biorecognition molecules and quantum dots. On conjugation these can be visualized easily because of their luminescence. *Clostridium thermoaceticum* was found to deposit CdS nanoparticles on the cell surface as well as in the solution in presence of cadmium chloride and cysteine hydrochloride. Possibly, cysteine hydrochloride acts as the source of sulfur. When *Klebsiella aerogenes* is exposed cadmium ions in the growth medium it forms cadmium sulfide nanoparticles of 20-200 nanometers deposited on the cell surface. *Escherichia coli* when incubated with cadmium chloride and sodium sulfide forms intracellular cadmium sulfide nanoparticles in wurtzite crystal phase. Experiments show that the growth phase of the cells affect the formation rate of nanoparticles and is 20 times more I

stationary phase than in late logarithmic phase. Zinc sulfide nanoparticles are formed by the family Desulfobacteriaceae in their natural settings by a complex mechanism. This can be used to bring down the zinc concentration in drinking water to below acceptable levels. Magnetic iron sulfide nanoparticles can also be produced by sulfite reducing bacteria.



Studies have shown that a microaerophilic bacteria *Aquaspirillum magnetotacticum* was able to form single domain magnetite nanoparticles with octahedral geometry. Marine magnetotactic bacterium MV-1 isolated from sulfide rich sediments was also able to form magnetite nanoparticles (parallelepiped) of dimensions 40*40*60 nanometers. A thermophilic fermentative bacterial strain TOR-39 was also able to form single domain (<12 nanometers) magnetite octahedral nanoparticles exclusively outside the cell. Another bacteria *Magnetospirillum magnetotacticum* was also able to form single domain nanoparticles which are subsequently assembled into folded chain and flux closure assemblies. Their 2-D arrangement is responsible for the head-tail assembly. Magnetization studies have shown that the magnetite nanoparticles

are not superparamagnetic. All magnetotactic bacteria producing magnetic particles intracellularly contain another organelle called magnetosomes which are comprised iron mineral crystals protected by a membrane vesicle. This membrane is most likely the structure that holds the crystal at a particular location in the cell as well as serves as the starting point (nucleation point) for nanoparticle formation. Possibly, biological systems exert control over growth of the crystals using the same magnetosomal membrane. The bacteria producing the magnetic nanoparticles can be separated from the culture using micro electromagnets. After they are separated from the culture they can be subjected to lysis to leave the crystals at desired locations. Magnetite nanoparticles have also been produced by non magnetotactic bacteria *Geobacter metallireducens* GS-15 isolated from the sediments of Potomac River. The amorphous ferric oxide acts as a terminal electron acceptor during organic matter oxidation and magnetite crystals are formed.



Stenotrophomonas maltophilia SELTE02, a strain isolated from soil near selenium accumulator legume was found capable of transforming selenite to elemental selenium and accumulate it inside the cells as well as deposit it extracellularly. A facultative anaerobe *Enterobacter cloacea* SLD1a-1 and *Desulfovibrio desulfuricans* have also been found capable to reduce selenite to selenium. *Desulfovibrio desulfuricans* NCIMB 8307 was able to generate palladium and platinum nanoparticles.

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