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An Introduction to Microbial Metal Nanoparticle Preparation Method

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Abstract
Reduction of metal is an important defense mechanism in microorganisms as a way to manage metal toxicity. The inherent, clean, nontoxic and environmentally friendly ability of eukaryotic and prokaryotic microorganisms to form the metal nanoparticles is particularly important in the development of nanobiotechnology. New methods have been developed to control disparity, chemical composition, the size, and the shape to get the best particles which can be well applied in different fields of science. However, there is a growing need to understand the basics of this technique to facilitate application of the new methodology to laboratory and industrial needs. This review contains a brief outlook of the processes and classification of metal nanoparticles biosynthesis by different microorganisms.

Introduction

Nanotechnology refers to the study of compounds of 100 nanometers or smaller in one dimension. Nanotechnology has a variety of applications in fields such as optics, electronics, bio-medicine, magnetics, mechanics, catalysis, energy science, etc. Thus, developing different branches of nanotechnology confidently results in developing the related sciences, and is a consequential goal of scientific word. (Mohanpuria et al. 2008; Bhattacharya and Gupta 2005).

Metal nanoparticles are very fine and strong particles which have many applications in different fields like medical imaging (Lee et al. 2008), drug delivery (Horcajada et al. 2008), electronics (Lipovskii et al. 2008), nanocomposites (Ting et al. 2007), biolabeling (Tan et al. 2006; Parak et al. 2005), biocide or antimicrobial agents (Pallab et al. 2008; Kirchner et al. 2004), filters (Boskovic et al. 2008), sensors (Jiang et al. 2008), non-linear optics (Ebothe et al. 2006), hyperthermia of tumors (Pissuwan et al. 2006), intercalation materials for electrical batteries (Joerger et al. 2001), optical receptors (Dahan et al. 2003), catalysis in chemical reactions (Kralik et al. 2000), and etc (Nam and Lead 2008; Bhattacharya and Mukherjee 2008; Parak et al. 2003; Pellegrino et al. 2005). The wide range of applications of nanoparticles is due to their unique optical, thermal, electrical, chemical, and physical properties that are due to a combination of the large proportion of high-energy surface atoms compared to the bulk solid (Panigrahi et al. 2004). Of course the application of every particle depends on its structure. Metal nanoparticles are characterized by different chemical composition, shape, size, and monodispersity. To alter these characteristics, three main methods of synthesis techniques have been developed: chemical synthesis (Masala and Seshadri 2004), Physical synthesis (Swihar 2003) and biological synthesis (Yashhiro et al. 2006). Here, the biological synthesis of metal nanoparticles methods and their biosynthesis routes is briefly reviewed.

Biosynthesis

Biological syntheses or biosyntheses refers to the phenomena which takes place by means of biological processes or enzymatic reactions. These eco-friendly processes, referred to as green technology, can be used to obtain better metal nanoparticles from microbial cells (Mandal et al. 2006). In fact microorganisms can survive and grow in high metal ion concentration due to their ability to fight the stress. The mechanisms include: efflux systems; alteration of solubility and toxicity via reduction or oxidation; bioabsorption; bioaccumulation; extracellular complexation or precipitation of metals; and lack of specific metal transport systems (Bruins et al. 2000; Beveridge et al. 2007). One mechanism used to create metal nanoparticles by microorganisms is bioreduction. Here, the microbial cell reduces the metal ions by use of specific reducing enzymes like NADH-dependent reductase or nitrate-dependent reductase (Mandal et al. 2006). Microbial resistance to high metal concentration is being considered in fields like bioleaching (Rohwerder et al. 2004; Olson et al. 2004) of ores, bioremediation of waters (Satinder et al. 2006; Head et al. 2003) and so on.

Biosynthesis is considered better than chemical and physical synthesis because: 1.Biological nanoparticle synthesis would have greater commercial viability and large savings in reductant and energy costs and high production rate in comparison with conventional methods (Mukherjee et al. 2004). 2. Large-scale production by chemical and physical methods usually results in particles larger than several micrometers while the biological synthesis can be successfully used for production of small nanoparticles in large-scales operations (Klaus et al. 1999).3. It is a clean, nontoxic and eco-friendly method (Senapati et al. 2005).4. Physical methods need high temperature and chemical methods need high pressure which is a harder situation to provide. (Bansal et al. 2004).

Processes

Basic steps for metal nanoparticle biosynthesis are the same. First, a metal ion supplemented culture medium should be prepared. Conventional laboratory culture media supplemented with sub-inhibitory concentration of target metals can be used for this purpose. For example for gold, $HAuCl_{4,}$ or for silver, $AgNO_3$ must be added to culture as metal resource. Second, the prepared culture media should be inoculated with the selected microorganisms for nanoparticle bioreduction. During the different phases of microbial growth, the metal reduction process may take place by intercellular or extracellular bioreductant ingredients (Marcato et al. 2005).

The reaction condition can be optimized by changing experimental factors such as pH, incubation time, presence of light source, temperature, the composition of the culture medium, etc. This optimization will improve the chemical composition, shape and size, and monodispersity of the particles synthesized (Klaus et al. 1999). For example, in synthesizing gold nanoparticles by mesophilic bacterium *Shewanella algae* at pH=7, gold nanoparticles of 10-20 nm were synthesized in the periplasmic space of *S. algae* cells. When the solution pH was decreased to 1, gold nanoparticles of 50-500 nm were precipitated outside of cells. So here, by controlling and optimization of pH, the size of biogenic gold particles and the location of its deposition can be controlled (Yashuiro 2006). In biosynthesis of gold nanoparticles by *Rhodopseudomonas capsulata,* variation of pH affects the morphology that the spherical gold nanoparticles in the range of 10–20 nm can be seen at pH=7, whereas a number of nanoplates were observed at pH=4 (He et al. 2007). In preparation of silver nanoparticles by *Plectonema boryanum* the range of particles' size can be controlled by the variation of temperature (Lengke et al. 2007).

Classification

Metal nanoparticle biosynthesis techniques are classified as either intracellular or extracellular biosynthesis. In extracellular biosynthesis, Two different preparation methods are used: rapid synthesis and slow synthesis. The former can be done in a few minutes, while the latter requires several hours or even days. As an example, forming silver nanoparticles in 5 minutes by culture supernatant of *Klebsiella pneumonia* is classified as a rapid synthesis (Shahverdi et al. 2007), while its formation in 24 hours by mycelia mat of *Phaenerochaete chrysosporiom* has been classified as a slow synthesis (Vigneshwaran et al. 2006). Intracellular biosynthesis requires in-vivo synthesis in cells, which is a time limiting factor. Detoxification process of hazardous materials which are mediated by some enzymatic reactions may be involved in bioreduction of metals and their deposition inside of cells. In this mode, nanoparticles should be separated from the cells after getting synthesized by designed method (Ahmad et al. 2003). In extracellular biosynthesis of metal nanoparticles, the reduction does not take place in microbial growth phases. In this approach, biological reagents required for the bioreduction are presented in bioliquids (Kalishwaralal et al. 2008). Any of the following options can be used instead of culture as a matrix solution: 1.the supernatant of cultures which are prepared from centrifuging the microbial culture after its growth (Shahverdi et al. 2007).2.Steriled supernatant which comes from sterilizing the supernatant by filter which makes it completely free from microbes. This is very important when long time incubation is meant to be used without microbial growth in culture. Because if microbial growth happens, the mode changes to intracellular (Husseiny et al. 2007).3. Water containing cell biomass (Durán et al. 2005).4.Water which has kept biomass for a day. In this mode, the biological materials are released from the biomass into the water, and this water can be used as a reductant for reducing the metal ions and creating the metal nanoparticles (Ahmad et al. 2007).

Extracellular biosynthesis, in comparison, has two main advantages. First, since the nanoparticles are formed inside the biomass in the intracellular fashion, there exists an additional step of processing to release the nanoparticles from the biomass by ultrasound treatment or by reaction with suitable detergents. In extracellular biosynthesis however, this step is no longer necessary. Second, the extracellular biosynthesis is a cheaper and simpler downstream processing. Because of these advantages - and also because sometimes, achieving nanoparticles in the biomass is not feasible in the intracellular method - much focus has been given to the development of an extracellular process for biosynthesis of metal nanoparticles (Durán et al. 2005).

Reduction

Protein assays indicate that an NADH-dependent reductase, which is a well-known enzyme, is the main responsible factor of biosynthesis processes. This reductase gains electrons from NADH and oxidizes it to NAD^+. The enzyme is then oxidized by the simultaneous reduction of metal ions (Senapati et al. 2005). One other important enzyme that is responsible for this reduction in some microorganisms is nitrate-dependent reductase. In *Fusarium oxysporum,* this enzyme is conjugated with an electron donor (quinine), reduces the metal ion, and changes it to elemental form (Durán et al. 2005). In the case of rapid extracellular synthesis, because the reduction happens in very few minutes, complex electron shuttle materials may be involved in the biosynthesis process. In almost all chemical nanoparticle synthesis methods, a stabilizer is necessary to prevent the aggregation of fine particles (Raveendran et al. 2005). Conversely, in TEM images of nanoparticle samples synthesized with biological methods, it is clear that even aggregated nanoparticles don't have direct contact with one another. This is due to the fact that nanoparticles are stabilized in solution by capping proteins, which are secreted from microorganisms. One important enzyme that may be responsible for this is Cytochrome C. (Mukherjee et al. 2004).

Microorganisms and Nanoparticles

Bacteria (such as *Pseudomonas aeruginosa* (Husseiny et al. 2007) and *Escherichia coli* (Du et al. 2007)), fungi (such as *Fusarium oxysporum* (Ahmad et al. 2003) and *Aspergillus fumigatus* (Bhainsa and D'Souza 2006)), alga (such as *Sargassum wightii* (Singaravelu et al. 2007)) and in fact, most microorganisms can be used in this technique. Furthermore, fungi have been reported as particularly prominent bio-manufacturing units by some authors, because they are easier to handle compared to other classes of microorganisms (Sastry et al. 2003). However, fungi have a disadvantage; Some macromolecules such as proteins which is present in the fungal mycelial mat may be contaminated metal nanoparticle colloids (Basavaraja et al. 2008).

It must be noted that the resulted particles are not always in the elemental form. Different microorganisms may produce metal based particles in three forms: Elemental form, like gold and silver nanoparticles (Mukherjee et al. 2001; Kalimuthu et al. 2008), oxide form, like titanium and iron nanoparticles (Moon et al. 2007; Bansal et al. 2005) and sulfide form, like zinc and cadmium nanoparticles(Flenniken et al. 2004; Bai et al. 2006).

Mechanism

Besides the need method optimization, many things about the biochemical and molecular mechanism of these processes remain unknown and should be revealed. In fact, the biochemical mechanisms referred to finding materials like enzymes, which may mediate the biosynthesis mechanism. The studies of the enzyme structure and the genes which code these enzymes may help improve our understanding of how metal nanoparticle synthesis is performed. Improvements in chemical composition, size and shape and dispersity of generated nanoparticles could allow the use of nanobiotechnology in a variety of other applications (Bharde et al. 2007; Bharde et al. 2006; Roh et al. 2006).

Discussion and Conclusion

Today, nano metal particles, such as silver and gold, have drawn the attention of scientists because of their extensive application to new technologies in chemistry, electronics, medicine, and biotechnology. Beside many physical and chemical methods which have been developed for preparing metal nanoparticles, nanobiotechnology also serves as an important method in the development of clean, nontoxic, and environmentally friendly procedures for the synthesis and assembly of metal nanoparticles. This new biotechnological method has important advantages in comparison to conventional methods. For instance, it is an easier and cheaper procedure. From the two different classes of this method, the intracellular and the extracellular, the latter is under much more attention because of its own advantages, like having an easier downstream processing. To be utilized in different scientific fields, biological synthesis still requires the optimization of reaction conditions, and an understanding of the biochemical and molecular mechanisms of the reaction for obtaining better chemical composition, shape, size, and monodispersity. As said before, almost all kinds of microorganisms can be used for various metal base nanoparticle production in this method, and in regard with its considerable advantages, it could be the leading large-scale production method for nanoparticles in future.

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References
Ahmad, A et al. (2003) Intracellular synthesis of gold nanoparticles by a novel alkalotolerant actinomycete, Rhodococcus species. Nanotechnology 14: 824-828.

Ahmad, A et al. (2003) Extracellular biosynthesis of silver nanoparticles using the fungus Fusarium oxysporum. Colloids and Surfaces B: Biointerfaces 28: 313-/318.

Bai, HJ et al. (2006) Biological synthesis of semiconductor zinc sulfide nanoparticles by immobilized Rhodobacter sphaeroides. Biotechnology Letters 28: 1135–1139.

Bansal, V et al. (2004) Biosynthesis of zirconia nanoparticles using the fungus Fusarium oxysporum. Journal of Materials Chemistry 14: 3303 – 3305.

Bansal, V et al. (2005) Fungus-mediated biosynthesis of silica and titania particles. Journal of Material Chemistary 15: 2583 – 2589.

Basavaraja, S et al. (2008) Extracellular biosynthesis of silver nanoparticles using the fungus Fusarium semitectum. Materials Research Bulletin 43(5): 1164-1170.

Beveridge, TJ et al. (1997) Metal–microbe interactions: contemporary approaches. Advances in Microbial Physiology 38: 177–243.

Bhainsa, KC and S.F. D'Souza (2006) Extracellular biosynthesis of silver nanoparticles using the fungus Aspergillus fumigatus. Colloids and Surfaces B: Biointerfaces 47: 160–164.

Bharde, A et al. (2006) Extracellular Biosynthesis of Magnetite using Fungi. Small 2(1): 135 – 141.

Bharde, A et al. (2007) Bacterial Enzyme Mediated Biosynthesis of Gold Nanoparticles. Journal of Nanoscience and Nanotechnology 7: 1–9.

Bhattacharya, D and R.K. Gupta (2005) Nanotechnology and Potential of Microorganisms. Critical Reviews in Biotechnology 25: 199–204.

Bhattacharya, R and P. Mukherjee (2008) Biological properties of "naked" metal nanoparticles. Advanced Drug Delivery Reviews 60(11):1289-1306.

Boskovic, L et al. (2008) Filter efficiency as a function of nanoparticle velocity and shape. Journal of Aerosol Science 39(7): 635-644.

Bruins, RM et al. (2000) Microbial resistance to metal in the environment. Ecotoxicology and Environmental Safety 45: 98–207.

Dahan, M et al. (2003) Diffusion Dynamics of Glycine Receptors Revealed by Single-Quantum Dot Trackin. Science 302(5644): 442 – 445.

Du, L et al. (2007) Biosynthesis of gold nanoparticles assisted by Escherichia coli DH5a and its application on direct electrochemistry of hemoglobin. Electrochemistry Communications 9: 1165–1170.

Durán, N et al. (2005) Mechanistic aspects of biosynthesis of silver nanoparticles by several Fusarium oxysporum strains. Journal of Nanobiotechnolog 3: 8.

Ebothe, J et al. (2006) Pd nanoparticles as new materials for acoustically induced non-linear optics. Physica E: Low-dimensional Systems and Nanostructures 35(1): 121-125.

Flenniken, M et al. (2004) Microbe Manufacturers of Semiconductors. Chemistry & Biology 11(11): 1478 – 1480.

He, S et al. (2007) Biosynthesis of gold nanoparticles using the bacteria Rhodopseudomonas capsulata. Materials Letters 61: 3984–3987.

Head, Ian et al. <u>Bioremediation: A Critical Review.</u> Newcastle: Horizon Scientific Press, 2003.

Horcajada, P et al. (2008) Flexible Porous Metal-Organic Frameworks for a Controlled Drug Delivery. Journal-American Chemical Society 130(21): 6774–6780.

Husseiny, MI et al. (2007) Biosynthesis of gold nanoparticles using Pseudomonas aeruginosa. Spectrochimica Acta Part A 67: 1003–1006.

Jiang, K et al. (2008) Manipulation of ZnO nanostructures using dielectrophoretic effect. Sensors and Actuators B: Chemical 134(1): 79-88.

Joerger, TK et al. (2001) Bacteria as workers in the living factory: metal-accumulating bacteria and their potential for materials science. Trends in Biotechnology 19(1): 15-20.

Kalimuthu, K et al. (2008) Biosynthesis of silver nanocrystals by Bacillus licheniformis. Colloids and Surfaces B: Biointerfaces 65(1) 150-153.

Kalishwaralal, K et al. (2008) Extracellular biosynthesis of silver nanoparticles by the culture supernatant of Bacillus licheniformis. Materials Letters 62(29): 4411-4413.

Kirchner, C et al. (2004) Cytotoxicity of Colloidal CdSe and CdSe/ZnS Nanoparticles. Nano Letters 5(2): 331-338.

Klaus, T et al. (1999) Silver-based crystalline nanoparticles, microbially fabricated. The Proceedings of the National Academy of Sciences Online (US) 96(24): 13611–13614.

Kralik, M et al. (2000) Catalysis by Metal Nanoparticles Supported on Functionalized Polymers. chemistary papers 54(6b): 254-264.

Lee, HY et al. (2008) PET/MRI dual-modality tumor imaging using arginine-glycine-aspartic (RGD)-conjugated radiolabeled iron oxide nanoparticles. Journal of Nuclear Medicine 49(8): 1371-1379.

Lengke, MF et al. (2007) Biosynthesis of Silver Nanoparticles by Filamentous Cyanobacteria from a Silver(I) Nitrate Complex. Langmuir 23: 2694-2699.

Lipovskii, AA et al. (2008) Electric field imprinting of sub-micron patterns in glass–metal nanocomposites. Nanotechnology 19: 415304-415309.

Mandal, D et al. (2006) The use of microorganisms for the formation of metal nanoparticles and their application. Applied Microbial Biotechnology 69: 485–492.

Marcato, PD et al. Antibacterial Activity of Silver Nanoparticles Synthesized by Fusarium oxysporum Strain. 2nd Mercosur Congress on Chemical Engineering and 4th Mercosur Congress on Process Systems Engineering; 2005 Aug 14-18; Village Rio das Pedras, Club Med, Rio de Janeiro.

Masala, O and R. Seshadri (2004) Synthesis Routes for Large Volumes of Nanoparticles. Annual Review of Materials Research 34: 41-81.

Mohanpuria, P et al. (2008) Biosynthesis of nanoparticles: technological concepts and future applications. Journal of Nanoparticle Research 10(3): 507-517.

Moon, JW et al. (2007) Microbial preparation of metal-substituted magnetite nanoparticles. Journal of Microbiological Methods 70: 150–158.

Mukherjee, P et al. (2001) Bioreduction of $AuCl_4^-$ Ions by the Fungus, Verticillium sp. and Surface Trapping of the Gold Nanoparticles Formed. Angewandte Chemie International Edition 40(19): 3585 – 3588.

Mukherjee, P et al. (2004) Extracellular Synthesis of Gold Nanoparticles by the Fungus Fusarium oxysporum. ChemBioChem 3(5): 461 – 463.

Nam, YJ and J.R. Lead. (2008) Manufactured nanoparticles: An overview of their chemistry, interactions and potential environmental implications. Science of the Total Environment 400(1-3): 396-414.

Olson, GJ et al. (2004) Bioleaching review part B: Progress in bioleaching: applications of microbial processes by the minerals industries. Applied Microbiology and Biotechnology 63(3): 249-257.

Panigrahi, S et al. (2004) General method of synthesis for metal nanoparticles. Journal of Nanoparticle Research 6(4): 411-414.

Parak, WJ et al.(2003) Biological applications of colloidal nanocrystals. Nanotechnology 14: R15–R27.

Parak, WJ et al. (2005) Labelling of cells with quantum dots. Nanotechnology 16: R9–R25.

Pellegrino, T et al. (2005) On the Development of Colloidal Nanoparticles towards Multifunctional

Structures and their Possible Use for Biological Applications. Small 1(1): 48-63.

Pissuwan, D et al. (2006) Therapeutic possibilities of plasmonically heated gold nanoparticles. TRENDS in Biotechnology 24(1): 62-67.

Raveendran, P et al. (2005) A simple and ‘‘green’’ method for the synthesis of Au, Ag, and Au–Ag alloy nanoparticles. Green Chemistry 8: 34–38.

Roh, Y et al.. (2006) Extracellular Synthesis of Magnetite and Metal-Substituted Magnetite Nanoparticles. Journal of Nanoscience and Nanotechnology 6: 3517–3520.

Rohwerder, T et al. (2004) Bioleaching review part A: Progress in bioleaching: fundamentals and mechanisms of bacterial metal sulfide oxidation. Applied Microbiology and Biotechnology 63(3): 239-248.

Sanpui, P et al. (2008) The antibacterial properties of a novel chitosan–Ag-nanoparticle composite. International Journal of Food Microbiology 124(2): 142-146.

Sastry, M et al. (2003) Biosynthesis of metal nanoparticles using fungi and actinomycete. Current Science 85(2): 162-170.

Satinder, K et al. (2006) Bioremediation of Hazardous Wastes—A Review. Practice Periodical of Hazardous, Toxic, and Radioactive Waste Management 10(2): 59-72.

Senapati, S et al. (2005) Extracellular Biosynthesis of Bimetallic Au-Ag Alloy Nanoparticles. Small 1(5): 517 – 520.

Shahverdi, AR et al. (2007) Rapid synthesis of silver nanoparticles using culture supernatants of Enterobacteria: A novel biological approach. Process Biochemistry 42: 919–923.

Singaravelu, G et al. (2007) novel extracellular synthesis of monodisperse gold nanoparticles using marine alga, Sargassum wightii Greville. Colloids and Surfaces B: Biointerfaces 57: 97–101.

Swihar, MT (2003) Vapor-phase synthesis of nanoparticles. Current Opinion in Colloid & Interface Science 8(1): 127-133.

Tan, M et al. (2006) Synthesis and characterization of titania-based monodisperse fluorescent europium nanoparticles for biolabeling. Journal of Luminescence 117(1): 20-28.

Ting, PK et al. (2007) Synthesis and characterization of silica–titania nanocomposite via a combination of sol–gel and mechanochemical process. Journal of Alloys and Compounds 446(1-2): 304-307.

Vigneshwaran, N et al. (2006) Biomimetics of silver nanoparticles by white rot fungus, Phaenerochaete chrysosporium. Colloids and Surfaces B: Biointerfaces 53: 55–59.

Yashhiro, K (2006) Microbial Synthesis of Noble Metal Nanoparticles Using Metal-Reducing Bacteria. Journal of the Society of Powder Technology 43(7): 515-521.