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# Biological Synthesis of Nanoparticles Using Bacteria and Their Applications

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## ABSTRACT

Green synthesis of nanoparticles is eye catching area of nanoscience and nanotechnology. It involve development of clean, biocompatible, non-toxic and eco-friendly methods for nanoparticles synthesis as compared to conventional method like physical and chemical which are often toxic. In the present scenario variety of nanoparticles with well-defined chemical compositions, sizes and morphology have been synthesized using different microorganisms and their applications in various cutting-edge technological areas have been explored. This review highlights the recent developments of the biosynthesis mechanisms of different types of nanoparticles using bacteria. Nanoparticles have been used in diagnosis, treatment, drug delivery, medical device coating, wound dressings, medical textiles, contraceptive devices, anti-fungal, anti-inflammatory etc. Future prospects for synthesis of nanoparticles using bacteria have also been discussed.

**Keywords**: Nanotechnology, Nanoparticles, Microorganisms, Drug delivery, Diagnosis.

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## INTRODUCTION

The primary concept of nanotechnology was presented by Richard Feynman in 1959. The term nanotechnology was introduced by Tokyo Science University Professor Norio Taniguchi[1]. 'Nano' is a Greek word synonymous to dwarf meaning extremely small. Nanoparticles are usually 0.1 to 1000 nm in each spatial dimension and are commonly synthesized using two strategies: top-down and bottom-up[2]. One involves molding and etching materials into smaller components and the other involves the assembling structures into useful devices. Nanotechnology is currently a frontier of research due to wide applications of nanomaterials in biomedical, agricultural, catalysis, optical and electronic fields[3-5]. Recently inorganic nanoparticles (NP) have invoked a lot of interest owing to their distinct physical, chemical and biological properties as compared to the respective bulk materials[6]. The nanoparticles are synthesized by physical, chemical, biological and other hybrid methods. The physical and chemical methods are very useful for production of monodispersed nanoparticles. These methods are harmful in one or the other way, as the chemicals used are toxic, flammable and do not dispose-off in the environment easily[7]. These methods lead to presence of toxic chemicals adsorbed on the surface of nanoparticles that may have adverse effect in the medical applications[8]. Therefore, development of clean, biocompatible, non-toxic and eco-friendly methods for nanoparticles synthesis deserves merit. Although biological methods are regarded as safe, cost-effective, sustainable and environmental friendly processes. Green approaches for the synthesis of nanomaterials utilize of biological components, primarily prokaryotes and eukaryotes. Microbes play direct or indirect roles in several biological activities. **"**Green chemistry**"** explores the biological pathway and biological resource like plant, plant extract, fungi, bacteria, virus for bio-production of nanoparticles. Green chemistry more demanding approach for bio-production of nanoparticles via highly stable, eco-friendly process with no toxic chemical and large scale production.

## PHYSICAL AND CHEMICAL METHODS

A Variety of chemical and physical procedures could be used for synthesis of nanoparticles. Various physical and chemical processes have been exploited in the synthesis of several inorganic metal nanoparticles by wet and dry approaches viz., ultraviolet irradiation, aerosol technologies, lithography, laser ablation, ultrasonic fields, and photochemical reduction techniques. However, these methods are burdened with various problems including use of harmful chemical agents, production of hazardous commodities, expensive chemicals and high vigour consumption. Though numerous chemical methods prevailed for nanoparticle production, numerous problems are often experienced with stability of product, control of the crystal growth and aggregation of particles on long term exposure. The use of toxic chemicals and organic solvents during nanoparticles synthesis and their occurrence on the surface of nanoparticles limit their applications. Accordingly, there is a necessary need to extend for environmentally benign procedures for synthesis of nanoparticles. A promising move towards to reach this objective is to develop the array of biological resources in nature[9]. The high energy requirement in physical methods of nanoparticle synthesis and the waste disposal problems in chemical synthesis, due to the heavy use of organic solvents, toxic reducing agents and capping agents, both methods are costly and generate toxic by product are major demerits of the conventional nanoparticle synthesis[10-12]. Such drawbacks necessitate the development of clean, biocompatible, nonhazardous, inexpensive, energy-efficient, and eco-friendly methods for nanoparticles synthesis. Consequently, biological systems have been focused on and exploited for the synthesis of nanoparticles[13] providing a safer alternative to physical and chemical methods. Indeed, over the past several years, the biological method for the synthesis of nanoparticles employs use of biological agents like plants, algae, fungi, actinomycetes, yeast, bacteria, and viruses have been used for production of nanoparticles[14-18]. The rate of reduction of metal ions using biological agents is found to be much faster and also at ambient temperature and pressure conditions. It is well known that microbes such as bacteria[19, 20] yeast[21], fungi[22, 23] and alga[24, 25] are capable of adsorbing and accumulating metals. The biological agents secrete a large amount of enzymes, which are capable of hydrolyzing metals and thus bring about enzymatic reduction of metals ions.

**Why the Biological Method used for Nanoparticles Synthesis**

Biological approaches to nanoparticle and nanocrystal synthesis have been extended to intact biological particles. The biological process is more acceptable green route and is not energy intensive and is also ecofriendly. The most abundant organisms in our biosphere is bacteria. This biogenic approach is greatly indented with bacteria by providing ambient conditions such as temperature, pH, pressure and etc. The nanoparticles synthesized by biological process have higher catalytic reactivity, greater specific surface area and also improves the enzyme and metal salt[26]. The main interest is production of nanoparticles using a biological method from a cheap resource and uniform production of nanoparticles. Utilizing a biological source gives an easy approach, easy multiplication, and easy increase of biomass and size uniformity. Though numerous chemical methods prevailed for nanoparticle production, numerous problems are often experienced with stability of product, control of the crystal growth and aggregation of particles on long term exposure[27]. Application of microorganisms is one of the most conspicuous methods through various bio-methods of nanoparticle production[28-31]. Bacterial cells live under difficult situations and this survival ability is one of the most important factors. Their ability to growing in presence of high concentration of metals may be because of specific tolerance mechanisms. These mechanisms include efflux system, solubility variation by changing redox potential of metal ions, extra-cellular combination and precipitation, and failure in transportation of special metal system[32, 33]. Bacterial strains that do not tolerate high concentrations of metal can also be used as convenient microorganisms[34, 35]. Nanoparticles are synthesized by microbes and have biological applications in the fields of bioremediation, bio-mineralization, bioleaching, and bio-corrosion.

**Mechanism Used by Bacteria**

The most versatile location of biosynthesis of nanoparticles is biological cellular entitles and their cell membrane[29]. Because biological entitles and inorganic materials have constant touch with each other ever since inception of life on the earth. Biosynthesis is the phenomena which take place by means of biological or enzymatic reaction. Microbes produce inorganic materials either intra or extra cellular route often in nanoscale dimension with exquisite morphology[36]. Microorganism can survive and grow in high concentration of toxic metals due to their chemical detoxification as well as due to their energy –dependent efflux from the cell by membrane protein that function either as ATPase or as chemo-osmotic or proton anti-transporters[37-39]. The exact mechanism for synthesis of nanoparticles using biological agents has not been invented yet as different biological agent used different mechanism with different metals and also there are different biomolecules response for synthesis of nanoparticles. According to Beveridge,1997 the mechanisms which are considered for the biosynthesis of nanoparticles included efflux system, alteration of solubility and toxicity via reduction or oxidation, bio-absorption, bioaccumulation, extracellular complexion or precipitation of metals, and lack of specific metal transportation system[40]. The extracellular production of nanoparticles has more commercial as compare to intracellular process. List of bacterial species involve in intra and extra cellular synthesis of nanoparticles are given in table 1. Here below scheme show one of the reduction methods of nanoparticles synthesis. (Figure 1)

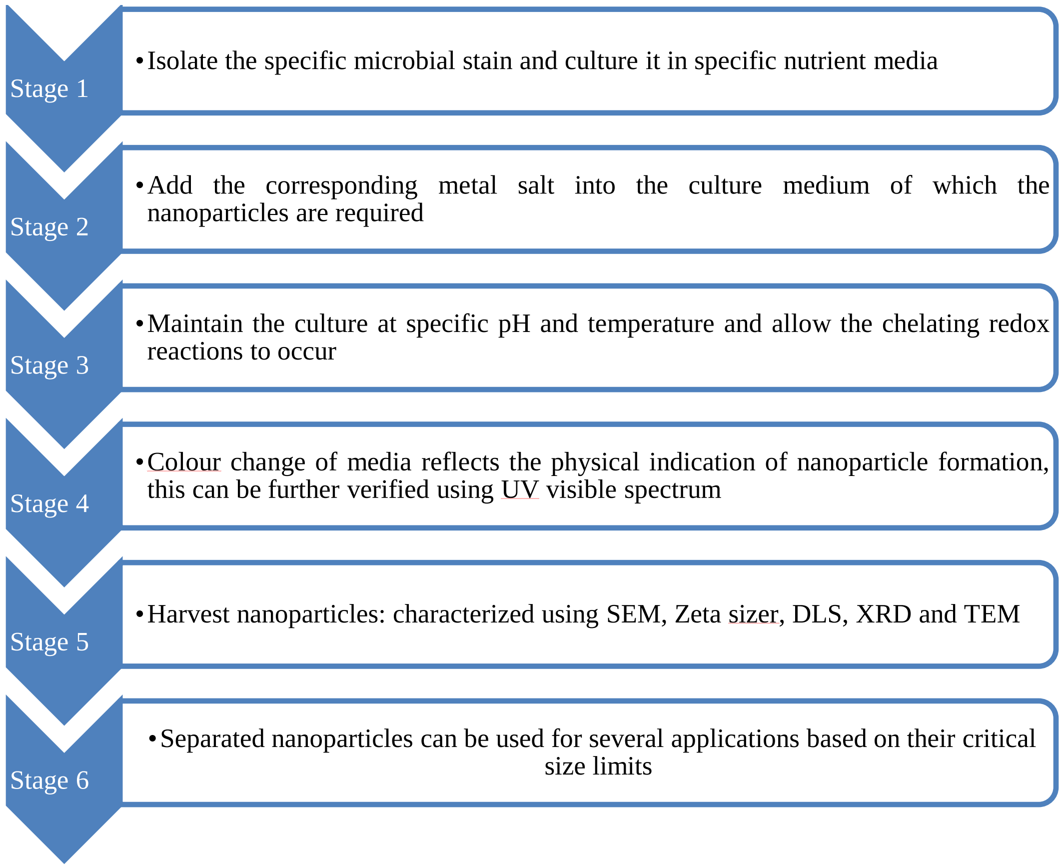


**Figure 1: Generalized Flow Chart for Biosynthesis of Nanoparticles using microorganisms**

**Table 1 Nanoparticles synthesis by bacteria either intra and extracellular**

| S,No, | Name of bacteria | Type | Size | Reference |
|---|---|---|---|---|
| 1 | *Rhodopseudomonas sps.* | Ag | 6-10nm | 41 |
| 2 | *halococcus salifodinae BK6* | Ag | | 42 |
| 3 | *Bacillus megaterium* | Ag, Pb, Cd | 10-20 | 43 |
| 4 | *Thermophilic Bacillus* sp | Ag | Unusal shape and size | 44 |
| 5 | *Pseudomonas fluorescens* | Au | 50-70nm | 27 |
| 6 | *Lactobacillus spp* | Ag | 2-20nm | 45 |
| 7 | *Streptomyces sp. HBUM171191.* | Mn,Zn | 10-20nm | 46 |
| 8. | *Bacillus cereus* | Ag | 10-30nm | 47 |
| 9 | *Pseudomonas fluorescens* | Ag | 85.46nm | 48 |
| 10 | *Bacillus sp. NJYRK* | Ag | 10-30nm | 49 |
| 11 | *Planomicrobium sp* | $TiO_2$ | 8.89nm | 50 |
| 12 | *Lactobacillus acidophilus, Lactobacillus casei, Bifidobacterium sp., Klebsiella pneumonia* | Se | 50-500,50-500,400-500,100-550 | 51 |
| 13 | *Lactobacillus sporogens* | $ZnO_2$ | 145.7nm | 31 |
| 14 | *Lactobacillus* | $TiO_2$ | 150nm | 52 |
| 15 | *Marinobacter Pelagius* | Au | 10nm | 53 |
| 16 | *Vibrio alginolyticus* | Ag | 50-100nm | 9 |
| 17 | *Pseudomonas aeruginosa* | Ag | 20-100nm | 54 |
| 18. | *Idiomarina sp. PR58-8* | Ag | 26nm | 55 |

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|---|---|---|---|---|
| 19. | *Pseudomonas aeruginosa* | Ag | | 56 |
| 20. | *Bacillus sp.* | Ag | 65-70nm | 57 |
| 21 | *Bacillus strain CS 11* | Ag | 42-92nm | 58 |
| 22 | *Lactobacillus crispatus* | AG | 70.98nm | 59 |
| 23 | *Stenotrophomonas malophilia* | Ag | ~40nm | 60 |
| 24 | *Bacillus cereus* | Cds | 30-100nm | 61 |
| 25 | *Baccilus sps* | Au | 20-50nm | 62 |
| 26 | *Exiguobacterium mexicanum PR 10.6.* | Ag | 5-40nm | 63 |
| 27. | *Acinetobacter sp. SW 30* | Au | 20±10nm | 64 |
| 28 | *Alteromonas macleodii* | Ag | 70nm | 65 |
| 29 | *Pseudomonas aeruginosa* | Au | 15-30 | 66 |
| 30 | *Pseudomonas stutzeri* | Ag | Up to 200nm | 67,33 |
| 31 | *Rhodococcus sp.(Actinomycete)* | Au | 5-15nm | 68 |

## Sliver Nanoparticles

Among the NPs exploited in nanomedicine, silver NPs (AgNPs) are very promising because of their unique properties. A number of bacterial species have been reported for the synthesis of silver nanoparticles. They exhibit high antimicrobial activity against Gram positive and negative bacteria, fungi, protozoa, and certain viruses. Recently, the antitumor activity of AgNPs against various cancerous cell lines was also reported[69]. Various new biomedical applications of AgNPs have been found, including diagnostic applicative biological tags and biosensors, as well as antibacterial agents in apparel, anti-fungal, anti-inflammatory, anti-angiogenic and anti permeability activity. It has also been used in cosmetic [70-72] footwear, wound dressings and healing,[35, 73-77] paints, and plastics to thermal, electrical, and optical applications, water filter apparatus[78].

## Gold Nanoparticles

Gold nanoparticles have enormous applications in cancer treatment, drug delivery and nano-biosensor due to their biocompatibility. The synthesis of gold nanoparticles has received considerable attention and has been a focus of research due to their high chemical and thermal stability, fascinating optical, electronic properties[79], and promising applications such as nanoelectronics, biomedicine[80], sensing[81, 82] and catalysis[83] biosensor and DNA label[84]. The gold nanoparticles have multidisciplinary application found such as heavy metal detection by colorimetric technique[85].

**Table 2 Nanoparticles synthesis using bacteria reported**

| S.No. | Microorganism | Nanoparticle | Localization/morphology | Size (nm) | Reference |
|---|---|---|---|---|---|
| | **Bacterium-intracellular** | | | | |
| 1 | *Bacillus subtilis 168* | Au | Octahedral inside cell wall | 5-25 | 19 |
| 2 | Sulfate-reducingbacteria | Au | Cell envelope | <10 | 109 |
| 3 | *Shewanella algae* | Au | Periplasmic space,<br>bacterial envelope | 10-20<br>15-200 | 117 |
| 4 | *Plectonemaboryanum UTEX485* | Au | Membrane vesicles/Cubic | 10 | 111-112 |
| 5 | *Escherichia coli DH5α* | Au | Cell surface/Spherical | ND | 113 |
| 6 | *Rhodobactercapsulatus* | Au | Plasma membrane | ND | 114 |
| 7 | *Pseudomonas stutzeri AG259* | Ag, Ag2S | Periplasmic space | <200 | 67,33 |
| 8 | *Bacillus sp.* | Ag | Periplasmic space | 5-15 | 115 |
| 9 | *Lactobacillus sp.* | Au, Ag, Au–Ag | Hexagonal/Contour | 20–50 | 32 |
| 10 | *P. aeruginosa SNT1* | Se | Spherical/Contour | ND | 116 |
| 11 | *S. algae* | Pt | ND | ~5 | 110 |
| 12 | *Desulfovibrio desulfuricans NCIMB 8307* | Pd | Cell surface | ~50 | 118 |
| 13 | *S. oneidensis MR–1* | Pd | Periplasmic space | ND | 119 |
| 14 | *Aquaspirillum Magnetotacticum* | $Fe_3O_4$ | Octahedral prism | 40–50 nm | 120 |
| 15 | *Magnetotactic bacterium MV-1* | $Fe_3O_4$ | Cell inside/Parallelepiped | 40×40×60 | 121 |
| 16 | *Magnetotactic Bacterium* | $Fe_3S_4$, $FeS_2$ | Octahedral/Cubo-octahedral | 7.5 | 122 |
| 17 | Sulfate-reducing Bacteria | FeS | Cell surface | 2 | 123 |
| 18 | *Magnetospirillum* Magnetotacticum | $Fe_3O_4$ | Membrane bound/ Cubo-octohedrons | 47.1 | 124 |
| 19 | *M. magnetotacticum (MS-1)* | $Fe_3O_4$ | Inside the cell/Cuboctahedral | ~50 | 125 |
| 20 | *Desulfosporosinus sp.* | $UO_2$ | Cell surface | 1.5–2.5 | 126 |
| 21 | *Clostridium Thermoaceticum* | CdS | Cell surface | ND | 127 |

| | | | | | |
|---|---|---|---|---|---|
| 22 | *Klebsiella pneumonia* | CdS | Cell surface | 5–200 | 128 |
| 23 | *Escherichia coli* | CdS | Spherical, elliptical | 2–5 | 129 |
| 24 | *Desulfobacteriaceae* | ZnS | Spherical | 2–5 | 130 |
| 25 | *Plectonema boryanum (Cyanobacteria)* | Ag | Intracellular | 1–10 | 111 |
| 26 | *Shewanella algae* | Au | Intracellular ph -7 | 10-20 | 131 |
| 27 | *Rhodococcus sp.* | Au | Intracellular | 5-15 | 68 |
| 28 | *Lactobacillus sp.* | Au | Intracellular | >100 | 32 |
| 29 | *Shewanella algae* ATCC 51181 | Au | Intracellular | 10-20 | 132 |
| 30 | *Escherichia coli* | Au | Intracellular | <10 | 131 |
| 31 | *Stenotrophomonas maltophilia* | Au | Intracellular | 40 | 60 |
| | **Bacterium-extracellular** | | | | |
| 1 | *Rhodopseudomonas capsulate* | Au | Spherical<br>Triangular nanoplates<br>Spherical nanowires | 10–20<br>50–400<br>50–60 | 133 |
| 2 | *Pseudomonas Aeruginosa* | Au | ND | 15-30 | 66 |
| 3 | *B. megatherium D01* | Au | Spherical | 1.9±0.8 | 134 |
| 4 | *Aeromonas sp SH10* | Ag | ND | 6.4 | 135 |
| 5 | *Enterobacter cloacae, Klebsiella pneumonia, E. coli* | Ag | ND | 52.5 | 136 |
| 6 | *B. licheniformis* | Ag | ND | ~50 | 137 |
| 7 | *Acetobacter xylinum* | Ag | Cellulose fibre | ND | 138 |
| 8 | *Morganella sp.* | Ag | Spherical | 20±5 | 139 |
| 9 | *Sulfurospirillum barnesii, B. selenitireducens, Selenihalanaerobacter shriftii* | Se | Nanospheres | ~300 | 140 |
| 10 | *B. selenitireducens* | Te | Nanorods | ~10 | 141 |
| 11 | *Sulfurospirillum barnesii* | Te | Irregular Nanospheres | <50 | 141 |
| 12 | *Lactobacillus sp.* | Ti | Spherical | 40–60 | 105 |
| 13 | *Geobacter metallireducens* | Magnetite | ND | 10-15 | 114 |

| | | | | | |
|---|---|---|---|---|---|
| | *GS-15* | | | | |
| 14 | *Thermoanaerobacter ethanolicus (TOR-39)* | Co, Cr, Ni-substituted | Octahedral | ND | 142 |
| 15 | *Actinobacter sp.* | Magnetite | Quasi-spherical | 10–40 | 100 |
| 16 | *S. oneidensis MR-1* | UO2 | Extracellular (UO2-EPS) | 1–5 | 143 |
| 17 | *Klebsiella aerogenes* | CdS | Spherical on cell wall | 20–200 | 144 |
| 18 | *Rhodopseudomonas palustris* | CdS | Spherical | 8.01±0.25 | 145 |
| 19 | *Gluconoacetobacter xylinus* | CdS | Cellulose fibre | 30 | 146 |
| 20 | *Rhodobacter sphaeroides* | ZnS | Spherical | 8 | 147 |
| 21 | *R. sphaeroides* | PbS | Spherical | 10.5±0.15 | 148 |
| 22 | *Brevibacterium casei* | Co3O4 | ND | 5-7 | 149 |
| 23 | *Thermomonospora sp.* | Au | Extracellular | 30-60 | 150 |
| 24 | *Klebsiella pneumonia* | Ag | Extracellular | 5-32 | 151 |
| 25 | Shewanella oneidensis | Uranium (iv) | Extracellular | ND | 152 |
| 26 | *Escherichia coli* | Au | Extracellular | 20-50 | 153 |

## Other Nanoparticles

Microorganisms produce different type of nanoparticles. The microorganism produce Ti, Co, Cr, Ni, Cd, Fe, Se, Zn, Pd, Pt and Ur nanoparticles. Different nanoparticles have different importance in different – different field, like Copper nanoparticles have wide applications as heat transfer systems, antimicrobial materials,[86,87] super strong materials, [88] sensors, [89,90] and catalysts[91]. A significant amount of interest is focused on the synthesis of magnetite nanoparticles (MNPs) coated with water soluble polymers targeted for biomedical applications such as magnetic resonance imaging (MRI)[92], drug delivery systems[93-95], enzyme and protein immobilization[96,97], ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) purification[97,98], and gene therapy[99] environmental remediation[100,101]. Titanium dioxide (TiO2) is a material of great significance in many fields, e.g., photo catalysis, solar cell devices, gas sensors, and biomaterials[102]. In biomedical sciences such as bone tissue engineering as well as in pharmaceutical industries[103]. TiO2 is efficient photocatalysts for the degradation and inhibition of numerous toxic environmental contaminants such as air and water cleaning and surface cleaning [104]. In medical applications the titanium pins are due to because of their non-reactive nature when contacting bone and flesh[105]. Cadmium sulfide has been expansively considered due to its budding technological applications in field effect transistors, solar cells, photovoltaic, light emitting diodes, photo catalysis, photoluminescence, infrared photo detector, environmental sensors and biological sensors [106-108].

### Application of Nanoparticles

Nanotechnology has a wide variety of applications in various fields like optics, electronics, catalysis, bio-medicine, magnetics, mechanics, energy science, etc. Nanobiotechnology is a multidisciplinary field involving research and development of technology in different fields of science like biotechnology, nanotechnology, physics, chemistry, and material science[154, 155]. It deals with bio-fabrication of nano-objects or bi-functional macromolecules usable as tools to construct or manipulate nano-objects. Since, microbial cells offer many advantages like wide physiological diversity, small size, genetic manipulability and controlled cultivability; they are thus regarded as ideal producers for the synthesis of diversity of nanostructures, materials and instruments for nanosciences[156]. The characteristic features of nanoparticles such as their high volume/surface ratio, surface tail or ability, improved solubility and multi functionality open many new possibilities for biomedicine[157]. The optical, electronic and electrical properties of nanoparticles are size-dependent and various novel methods for the size controlled synthesis of silver nanoparticles are being developed[158]. Nano- sliver particles have been widely used for diagnosis[159], treatment[160], drug delivery[161], medical device coating[162], wound dressings[163], medical textiles[164] and contraceptive devices[165].

## CONCLUSION

The present study is concentrate on nanoparticles synthesis by bacteria and their application. In this study have been tremendous developments in the field of microorganism-produced nanoparticles and their applications over the last decade. However, much work is needed to improve the synthesis efficiency and the control of particle size and morphology.

## FUTURE PROSPECTS

It is known that synthesis of nanoparticles using microorganisms is a slow process (several hours to a few days) compared to physical and chemical approaches. Reduction of synthesis time will make this biosynthesis route much more attractive. Particle size and monodispersity are two important issues in the evaluation of nanoparticle synthesis. Therefore, effective control of the particle size and monodispersity must be extensively investigated. Since the control of particle shape in chemical and physical synthesis of nanoparticles is still an ongoing area of research, biological processes with the ability to strictly control particle morphology would therefore offer considerable advantage. By varying 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 non-target ions, it might be possible to obtain sufficient control of particle size and monodispersity. 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.

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