Hindawi Publishing Corporation
Journal of Nanoparticles
Volume 2013, Article ID 141274, 4 pages
http://dx.doi.org/10.1155/2013/141274

Research Article

Rapid, Low-Cost, and Ecofriendly Approach for Iron Nanoparticle Synthesis Using *Aspergillus oryzae* TFR9

Jagadish Chandra Tarafdar and Ramesh Raliya

Central Arid Zone Research Institute, Jodhpur 342003, India

Correspondence should be addressed to Jagadish Chandra Tarafdar; jctarafdar@yahoo.in

Received 31 January 2013; Accepted 28 February 2013

Academic Editor: Mohammad H. Maneshian

Development of reliable and ecofriendly green approach for synthesis of metallic nanoparticles biologically is an important step in the field of application of nanoscience and nanotechnology. The present paper reports the green approach for iron nanoparticle synthesis using *Aspergillus oryzae* TFR9 using $FeCl_3$ as a precursor metal salt. Valid characterization techniques employed for biosynthesized iron nanoparticles including dynamic light scattering (DLS), transmission electron microscopy (TEM), and high resolution-transmission electron microscopy (HR-TEM) for morphological study. X-ray energy dispersive spectroscopy (EDS) spectrum confirmed the presence of elemental iron signal in high percentage. Apart from ecofriendliness and easy availability, low-cost biomass production will be more advantageous when compared to other chemical methods. Biosynthesis of iron nanoparticles using fungus has greater commercial viability that it may be used in agriculture, biomedicals and engineering sector.

1. Introduction

Nanotechnology is the manipulation or self-assembly of individual atoms, molecules, or molecular clusters into structures to create material and devices with new and vastly different properties. "Nano" suffix usually refers to a size scale between 1 nanometer (nm) and 100 nm at least at one dimension [1]. Owing to its inimitable properties, nanotechnology provides the basic tools and subsequently the technology for gathering information and designing innovative devices to probe questions related to biological importance of nutritional availability, and the application of this knowledge in diverse sector including physics, chemistry, biology, and engineering [2].

Conventional methods such as sol-gel, aerosol, template assisted, sonochemical, laser exposer, wet-chemical synthesis, thermal decomposition, plasma synthesis, and hydrothermal synthesis often required several processing steps, controlled pH, temperature, pressure, much expensive equipment, and toxic chemicals. Furthermore, such techniques also generate several byproducts which are toxic to ecosystems. So, there is a need to develop a low-cost, ecofriendly method for agriculturally important nanoparticles [3, 4]. Therefore, microbial systems are now being increasingly explored as safer alternatives for production of nanoparticles [5, 6]. Shahi and Patra [7] produced nanoparticles of usnic acid with an ascomycetes fungus. Lee et al. [8] produced superparamagnetic nanoparticles by *Shewanella* sp. Yadav et al. [9] produced selenium containing nanostructures using *Pesudomonas aeruginosa* while Sadowski and Maliszewska [10] produced nanoparticles of silver using *Pesudomonas stutzeri*. Senapati et al. [11] produced bimetallic alloy of Au-Ag using microorganisms.

Synthesis of nanoparticles using fungi (eukaryotic organism) has several advantages over prokaryotic mediated approach regarding reproducibility of nanosized materials, and these also include ease to multiplication, grow, handling, and rest of downstream process for this top-down approach of nanobiosynthesis through nanofactories [3].

During the recent years, there has been a great deal of interest in iron nanoparticles due to technological applications such as high-density magnetic recording media, biosensors, ferrofluids, magnetic resonance imaging, nutritions and biomedicine [12]. Iron is the metal used at the active site of many important redox enzymes dealing with cellular

respiration and oxidation reduction in plants and animals. Iron also acts as cofactor and structural component for various enzymes.

In the present investigation attempt was made for rapid, low cost and ecofriendly iron nanoparticles synthesis using the fungi *Aspergillus oryzae* TFR9. This study includes morphological and elemental characterization of the biosynthesized iron nanoparticles.

2. Experimental

2.1. Isolation of Fungi. The fungus, *Aspergillus oryzae* TFR9 (NCBI GenBank accession no. JQ675292), was isolated from agricultural research farm (26°18′N 73°01′E) of Central Arid Zone Research Institute, Jodhpur, India. The pure culture of fungi was isolated by plating the primary inoculum on Martin's-rose Bengal agar medium (HiMedia, India, pH 7.2) after serial dilutions of collective soil sample. Antibiotic chloramphenicol (Sigma-Aldrich, St. Louis, MI, USA) was added at a concentration of 10 μg mL^{-1} after autoclaving to prevent bacterial contamination. Inoculated plates were incubated at 28°C for 72 h in biological oxygen demand (BOD) incubator. Individual fungal colonies were picked and further purified by subculturing on potato dextrose agar (PDA) media (HiMedia, India). Preliminary identification of fungal isolates was performed on the basis of morphological characteristics, results not shown here.

2.2. Molecular Identification of Used Fungus. The molecular level identification in which partial sequencing of 18S and 28S rRNA and complete sequence of internal transcribed sequence 1 (ITS-1), internal transcribed sequence 2 (ITS-2), and 5.8S rRNA gene (complex of 18S-ITS1-5.8S-ITS2-28S-) was made using universal primer ITS1 (5′-TCCGTAGGTGAACCTGCG-3′) and ITS 4 (5′-TCCTCCGCTTATTGATATGC-3′). The genomic DNA was isolated by cetyltrimethylammonium bromide (CTAB) extraction method suggested by Sambrook et al. [13]. The rRNA (ribonucleic acid) sequence was submitted to the GenBank of National Centre for Biotechnological Information (NCBI).

2.3. Biosynthesis of Iron Nanoparticles. The fungi, *Aspergillus oryzae* TFR9 (NCBI GenBank accession no. JQ675292), was grown up in 150 mL Erlenmeyer flask containing 50 mL potato dextrose broth medium. After adjusting, the pH of the medium to 5.8, the culture was grown with continuous shaking on a rotary shaker (150 rpm) at 28°C for 72 h. After complete incubation, fungal mycelia were separated from the culture broth by filtration process using Whatman filter paper no. 1 (Whatman, England) under biosafety cabinet (iMSet, Surana Scientific, India) followed by washed thrice with sterile double distilled water. The harvested fungal mycelia were resuspended in 50 mL sterile Milli-Q-water in 150 mL Erlenmeyer flask and again kept on a rotary shaker (150 rpm) at 28°C for 12 h. After incubation, the cell-free filtrate was obtained by separating the fungal biomass using 0.45 μ size membrane filter (Whatman, England). Using cell-free filtrate, salt solution of $FeCl_3$ (Sigma, USA) was prepared with final concentration of 10^{-3} M in Erlenmeyer flasks, which was found to be optimum precursor salt concentration for the synthesis of iron nanoparticles. The entire mixture was kept on rotary shaker at 28°C at 150 rpm. The reaction was allowed to carry out for a period of 12 h. The biotransformed product was collected periodically for characterization of particle size on the basis of dynamic light scattering method.

Isolation of Fungi

Fungus was isolated (Martin's rose Bengal agar medium, pH 7.2, 28°C for 72 h)

↓

Further subculturing on potato dextrose agar media

↓

Molecular identification

↓

Biosynthesis of iron nanoparticles

Biosynthesis of Iron Nanoparticles

Fungus was grown up in 150 mL Erlenmeyer flask containing 50 mL potato dextrose broth

medium (pH 5.8, 28°C for 72 h at 150 rpm on shaker)

↓

Mycelia separated from the culture broth by filtration using Whatman filter paper no. 1

↓

Harvested fungal mycelia resuspended in 50 mL sterile Milli-Q water in 150 mL Erlenmeyer

flask and kept on a shaker (150 rpm) at 28°C for 12 h.

↓

Cell-free filtrate obtained by separating the fungal biomass using 0.45 μ size membrane filter

↓

Salt solution of $FeCl_3$ prepared using cell-free filtrate (10^{-3} M)

↓

Entire mixture kept on shaker (150 rpm) at 28°C for 12 h.

↓

Iron nanoparticle synthesized

2.4. Characterization of Iron Nanoparticles

2.4.1. DLS Analysis. The particle size of iron nanoparticles was monitored using dynamic light scattering (DLS) measurements which determines particle size by measuring the rate of fluctuations in the laser light intensity scattered by particles as they diffuses through solvent. Particle size analyzer (Beckman DelsaNano C, USA) was used for size measurement and confirmation of nanoparticles size distribution.

2.4.2. TEM and HR-TEM Analysis. For the confirmation of biosynthesized iron nanoparticle size and shape, transmission electron microscope (TEM) measurements were carried out using drop coating method in which a drop of solution containing nanoparticles was placed on the carbon coated copper grids and kept under vacuum desiccation for overnight before loading them onto a specimen holder. TEM and high-resolution transmission electron microscope (HR-TEM) micrographs of the sample were taken using the JEM-2100F TEM instrument. The instrument was operated at an accelerating voltage of 200 kV.

2.4.3. EDS Analysis. X-ray energy dispersive spectroscopy (EDS) was used for elemental composition analysis, and samples were prepared on a carbon coated copper grids and kept under vacuum desiccation for three hours before loading them onto a specimen holder. Elemental analysis on single particles was carried out using Thermo-Noran EDS attachment equipped with TEM (JEM-2100F). It was performed for determination of the elemental composition and purity of the sample by atom percentage of metal.

3. Results and Discussion

3.1. Identification of Fungi. Identification of fungi *Aspergillus oryzae* TFR9 (NCBI GenBank accession no. JQ675292) was made on the basis of molecular characterization of fungal isolate which was performed by partial sequencing of 18S and 28S rRNA and complete sequence of internal transcribed sequence 1 (ITS-1), internal transcribed sequence 2 (ITS-2), and 5.8S rRNA gene (complex of -18S-ITS1-5.8S-ITS2-28S) and has been submitted in the NCBI GenBank (Accession no. JQ675292). The sequence was compared using Basic Local Alignment Search Tool (BLAST) of NCBI and submitted sequence is available on a public domain http://www.ncbi.nlm.nih.gov.

3.2. Biosynthesis of Iron Nanoparticles. The biosynthesis of iron nanoparticles was carried out by exposure of a precursor salt aqueous $FeCl_3$ solution of 10^{-3} M concentration to fungal cell-free filtrate obtained by incubating the fungus *Aspergillus oryzae* TFR9 in an aqueous solution. The reaction was carried out for 12 h at 28°C.

3.3. Characterization of Iron Nanoparticles. Particle size of biosynthesized iron nanoparticles was analyzed using particle size analyzer (Figure 1). Histogram clearly shows particle size in ranges between 10 nm and 24.6 nm. The polydispersity index (PDI) was 0.258 which shows high monodisperse nature of the particles. PDI reflects the uniformity in synthesized nanoparticles and measured by DLS. The size and uniformity in the biosynthesized iron nanoparticles were further validated by TEM study.

TEM measurements were used to study the morphological confirmation of iron nanoparticles. A TEM micrograph (Figure 2) showed well distribution of spherical iron nanoparticles. Furthermore it can be seen in the HR-TEM

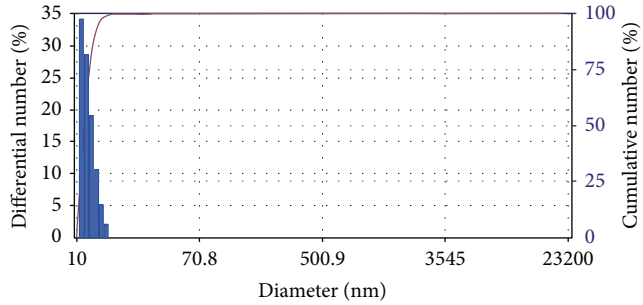

Figure 1: DLS histogram of iron nanoparticles for particle size analysis.

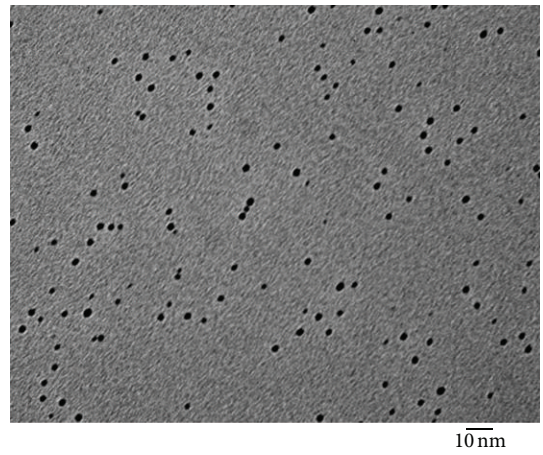

Figure 2: TEM micrograph of biologically synthesized iron nanoparticles.

micrograph (Figure 3), which supports also the crystalline nature of biosynthesized iron nanoparticle.

EDS analysis of the particles revealed a strong signal for iron nanoparticles at 6.4 keV, characteristic of iron metal. The EDS spectrum of drop coated biosynthesized iron nanoparticles was shown in Figure 4. Quantitative measuring results obtained from EDS analysis reflect that 84% atom particles were of iron metal which confirms the purity of iron metal in the biotransformed product. The other peaks of chlorine (12.4% atom) and oxygen (3.6% atom) are owing to used precursor salt for biosynthesis of iron nanoparticles.

Microorganisms are well known to defense themselves from metal stress either by segregating the ions intracellular or by secreting them into the external medium [14]. This defensive behavior can be exploited for the biosynthesis of advanced functional iron nanomaterials which has definite edge over chemical synthesis methods. Hazardous organic solvents and surfactants which are often employed in chemical synthesis can be avoided through biosynthesis. Further bio-based synthesis of iron nanoparticles can be reproducible and the resulting nanoparticles were further stabilized by the proteins and reducing agents secreted by the fungus [15]. Extracellular secretion of enzymes offers the advantage to obtain pure, monodisperse nanoparticles, which are free

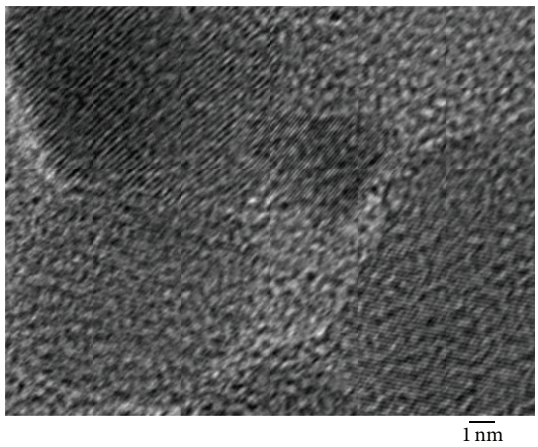

Figure 3: HR-TEM micrograph of single iron nanoparticles.

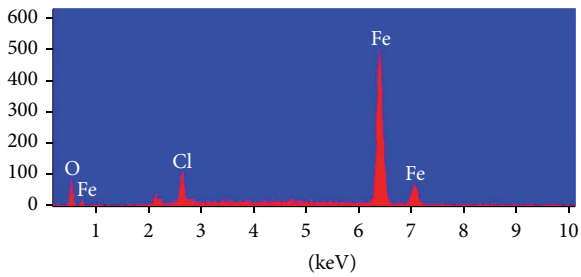

Figure 4: EDS spectrum of biosynthesized iron nanoparticles.

from cellular components, associated with organisms and easy downstream processing.

4. Conclusions

In conclusion, the present research of economically efficient, ecofriendly green approach was made to synthesize iron nanoparticles using the fungi *Aspergillus oryzae* TFR9. These useful features of the biosynthesized iron nanoparticles may benefit in agriculture, biomedical, and engineering sector.

Conflict of Interests

The authors declare that they have no conflict of interests.

Acknowledgment

The authors are grateful to ICAR for funding the NAIP project under which the present work has been carried out.

References

[1] J. C. Tarafdar and R. Raliya, *Nanotechnology*, Scientific Publishers, Jodhpur, India, 2012.

[2] N. Jain, A. Bhargava, S. Majumdar, J. C. Tarafdar, and J. Panwar, "Extracellular biosynthesis and characterization of silver nanoparticles using *Aspergillus flavus* NJP08: a mechanism perspective," *Nanoscale*, vol. 3, no. 2, pp. 635–641, 2011.

[3] N. Jain, A. Bhargava, J. C. Tarafdar, S. K. Singh, and J. Panwar, "A biomimetic approach towards synthesis of zinc oxide nanoparticles," *Applied Microbiology and Biotechnology*, vol. 97, no. 2, pp. 859–869, 2013.

[4] J. C. Tarafdar, A. Agrawal, R. Raliya, P. Kumar, U. Burman, and R. K. Kaul, "ZnO Nano-particles induced synthesis of polysaccharides and phosphatases by *Aspergillus* fungi," *Journal of Advance Science, Engineering and Medicine*, vol. 4, pp. 324–328, 2012.

[5] R. Raliya and J. C. Tarafdar, "Novel approach for silver nanoparticle synthesis using *Aspergillus terreus* CZR-1: mechanism perspective," *Journal of Bionanoscience*, vol. 6, pp. 12–16, 2012.

[6] R. K. Kaul, P. Kumar, U. Burman et al., "Magnesium and iron nanoparticles production using microorganism and varying salts," *Materials Science-Poland*, vol. 30, no. 3, pp. 254–258, 2012.

[7] S. K. Shahi and M. Patra, "Microbially synthesized bioactive nanoparticles and their formulation active against human pathogenic fungi," *Reviews on Advanced Materials Science*, vol. 5, no. 5, pp. 501–509, 2003.

[8] J. H. Lee, Y. Roh, and H. G. Hur, "Microbial production and characterization of superparamagnetic magnetite nanoparticles by Shewanella sp. HN-41," *Journal of Microbiology and Biotechnology*, vol. 18, no. 9, pp. 1572–1577, 2008.

[9] V. Yadav, N. Sharma, R. Prakash, K. K. Raina, L. M. Bharadwaj, and N. T. Prakash, "Generation of selenium containing nanostructures by soil bacterium, *Pseudomonas aeruginosa*," *Biotechnology*, vol. 7, no. 2, pp. 299–304, 2008.

[10] Z. Sadowski and I. H. Maliszewska, "Synthesis of silver nanoparticles using microorganisms," *Materials Science-Poland*, vol. 26, pp. 419–442, 2008.

[11] S. Senapati, A. Ahmad, M. I. Khan, M. Sastry, and R. Kumar, "Extracellular biosynthesis of bimetallic Au-Ag alloy nanoparticles," *Small*, vol. 1, no. 5, pp. 517–520, 2005.

[12] I. Koh, X. Wang, B. Varughese, L. Isaacs, S. H. Ehrman, and D. S. English, "Magnetic iron oxide nanoparticles for biorecognition: evaluation of surface coverage and activity," *Journal of Physical Chemistry B*, vol. 110, no. 4, pp. 1553–1558, 2006.

[13] J. Sambrook, E. F. Fritsch, and T. Maniatis, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbour Laboratory Press, New York, NY, USA, 1989.

[14] K. B. Narayanan and N. Sakthivel, "Biological synthesis of metal nanoparticles by microbes," *Advances in Colloid and Interface Science*, vol. 156, pp. 1–13, 2010.

[15] S. Anil Kumar and M. I. Khan, "Heterofunctional nanomaterials: fabrication, properties and applications in nanobiotechnology," *Journal of Nanoscience and Nanotechnology*, vol. 10, no. 7, pp. 4124–4134, 2010.