Effects of pH, temperature and agitation on the biosynthesis of iron nanoparticles produced by *Trichoderma* species

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**Abstract**

There is an increasing global search for commercially and environmentally clean synthesis of metallic nanoparticles from bioresources. However, there is insufficient reported information on the production of iron nanoparticles (FeNPs) from fungi such as *Trichoderma* species which has potentials for greater for yield of FeNPs as compared with bacterial sources. Filtrates obtained from biomass of pure cultures grown (72 h) in Potato Dextrose Broth were reacted with 1 M ferric chloride solution. A change in coloration monitored by ultraviolet-visible spectrophotometer (200-600 nm) as compared with control (ferric chloride solution) indicated a positive result. Nanoparticle synthesized in the reactive filtrate was characterized using ultraviolet-visible spectroscopy and Fourier transform infrared spectroscopy (FTIR). The effects of some physical parameters such as agitation, pH and temperature were monitored. The UV-vis spectrum revealed the peak of absorbance of synthesized nanoparticle by selected *Trichoderma* species at 275 nm. Optimum conditions for nanoparticle biosynthesis were observed at pH 4.5, 35 °C and when cells were agitated. Peaks of transmittance were observed at 950, 1800, 2250, 3000 and 3500 cm⁻¹. These peaks represent the C-H, C=O, C≡N, C=H and the OH functional groups respectively. The presence of the alkene, carboxyl and phenol groups suggests a capping of the NPs by the organism after redox reaction. The properties of extracellular iron nanoparticles synthesized by *Trichoderma* species from this study, presents the fungi as a bioresource for synthesis of stable NPs.

1 Introduction

Nanotechnology is a multidisciplinary branch of science encompassing numerous disciplines in science and technology [1]. Nanoparticles have become important due to their size compatibility with cells (10-100 nm), viruses (20-450 nm), proteins (5-50 nm) and genes (2 nm wide by 10-
100 nm long). They can therefore, move within the body without interruption of normal functions and also access places that are rather unreachable by other materials [2]. Magnetic nanoparticles (MNPs) are one of the most commonly used materials within the nanoscale which possess a type of core/shell structure that consists of a magnetic core trapped within an organic polymeric coating [1]. MNPs naturally possess a hydrophobic surface which has a large surface-to-volume ratio and an agglomerating potential [3]. MNPs, such as iron nanoparticles (FeNPs) which exhibit special magnetic properties different from the bulk of other nanomaterials, are beginning to attract significant interests in applications, such as a storage medium in magnetic memory devices to probes and vectors in the biomedical sciences [4]. The large surface-to-volume ratio of MNPs makes available in abundance, a chemical site of activity for biomolecules to bind, as a result allowing for precision in their engineering and design to suit specific functions, such as prolonged circulation within the blood-stream, target specificity to tissues, ease of optical detection and drug delivery [4, 5]. In addition, superparamagnetic FeNPs have the ability to escape mononuclear phagocytes of the immune system [1].

Currently, the biological approach to the synthesis of nanoparticles is gaining more interest over the physico-chemical methods, which have numerous disadvantages [6]. Biological systems have unique features for the synthesis of nanomaterials with well-defined and predetermined properties. Certain microorganisms such as filamentous fungi play an important role in bioremediation through the reduction of toxic metals. These fungal isolates have become the favorites for the nanotechnologist due to the wide variety of advantages they offer over bacteria, yeast, actinomycetes, plants, and other physico-chemical properties they possess [7, 8]. These features include ease of handling, simple nutrient requirements, high wall-binding capacity and an intracellular metal uptake capability. There are reports on the use of Trichoderma species for the synthesis of nanoparticles [9, 10]. However, there is paucity of information on the synthesis of iron nanoparticles from this group of organisms. This research focuses on biological synthesis of FeNPs from Trichoderma species isolated from soil samples. We also report the effects of some physical parameters on the synthesis of FeNPs and characterization of the synthesized material using simple techniques.
2 Materials and Methods

2.1 Sample collection

Soil samples were collected from farm area of the Federal University of Agriculture, Abeokuta, into sterile polyethylene bags and taken to the Microbiology laboratory.

2.2. Isolation and identification of pure cultures

Serial dilution of the soil sample was done and aliquot (1 ml) of appropriate diluent was inoculated on acidified Potato Dextrose Agar (PDA) plates using pour plate method. Plates were incubated for 3 days at 25 °C. Colonial and microscopic examination characteristics of isolates were compared with reported standards [11]. Identified pure cultures of *Trichoderma* species were stored on PDA slants at 4 ºC and sub-cultured bi-monthly.

2.3 Production of fungal biomass

Biomass was prepared by introducing spores of *Trichoderma* species (1 x 10⁷ spores /ml) into 250 ml flasks containing 100 ml of sterile Potato Dextrose Broth. The flasks were cotton-plugged and incubated at 25 °C and agitated at 120 rpm for 72 h. The biomass of each isolate was harvested and then centrifuged at 5000 rpm for 20 min. The filtrates obtained were discarded and the biomass (residue) was washed twice with sterile distilled water.

2.4 Iron nanoparticle biosynthesis

Harvested biomass was washed with after 24 h incubation in sterile distilled water. Cell filtrates (50 ml) obtained were reacted with 50 ml of 1M Ferric chloride solution. The flasks were incubated on an orbital shaker at for 72 h [12]. A conical flask containing only Ferric chloride solution without the filtrates was set up as a control experiment. The flasks were retrieved from the orbital shaker and observed for presence and intensity of color changes. Synthesis and formation of iron nanoparticle in the reaction mixture was monitored using UV-visible spectrophotometry between the wavelengths of 200-600 nm.

2.5 Characterization of synthesized FeNPs

The synthesized iron nanoparticles were characterized using ultraviolet-visible spectroscopy and Fourier transform infra-red spectroscopy (FTIR).

2.6 Effects of pH, temperature and agitation on synthesis of FeNPs

The effects of parameters such as pH (4.5-6.5) and temperature (25-40 °C) on the synthesis of the nanoparticle by the selected *Trichoderma* species were monitored. Effect of agitation at optimum pH and temperature on synthesis was also studied.
3 Results and Discussion

3.1 Screening for FeNPs production in isolated *Trichoderma* species

About five (5) fungal isolates identified as *Trichoderma* species were obtained from soil sample. Isolate with the most reactive filtrate was selected. Colonial characteristic of the isolate is shown on Figure 1. Synthesis of FeNPs indicated by development of brownish coloration as compared with the control in the reaction mixture of the filtrates with Ferric chloride is shown on Figure 2. As observed in this study, fungi such as *Trichoderma* have been reported to extracellularly produce reducing enzymes in simple culture medium a medium [13,14] Nitrate-dependent reductases have been identified as the main mediators of bioreduction progression along with a shuttle quinine extracellular process leading to the reduction of FeCl₃ to Fe₂O₃ [15].

![Figure 1](image)

*Figure 1.* Plate showing growth of selected *Trichoderma* species on PDA
3.2 Characterization of FeNPs using ultraviolet-visible spectrophotometry
The absorption spectrum of the nanoparticle synthesized by the selected Trichoderma species was observed to peak at 275 nm as shown on Figure 3. This result is in the range reported in previous study on the synthesis of iron nanoparticles from Pleurotus species with absorbance at 265 nm wavelength [16]. Absorbance at the range has been explained to be due to electronic excitation in tryptophan and tyrosine residue in the protein present [17]. It has also been reported that solutions of metals nanoparticles tend to have distinct color characteristics and corresponding peaks. Further analysis of these peaks helps to differentiate the nanomaterials synthesized by their bond structures and functional groups in a given spectrum [18].

3.3 Optimization studies
The effects of pH, temperature and agitation on the synthesis of FeNPs from selected Trichoderma species studied at 275 nm were observed to be optimal at pH 4.5, temperature of 35 °C with agitation of the cells as shown on Figures 4, 5 and 6 respectively. A further increase of both pH and temperature led to decrease in biosynthesis. This reduction is similar to reports on inactivation of activity at increased physical conditions such as pH and temperature [19]. Characterization of physical parameters has been considered important and required along with others in vitro and in vivo studies [20].
Figure 3. Effect of change in pH on iron nanoparticles produced by *Trichoderma* sp. at 275 nm

Figure 4. Effect of change in temperature on iron nanoparticles produced by *Trichoderma* sp. at 275 nm

Figure 5. Effect of agitation on iron nanoparticles produced by *Trichoderma* sp. at 275 nm
3.4. FTIR analysis of iron nanoparticles produced by *Trichoderma* species

Characterization of synthesized FeNPs using FTIR revealed the percentage transmittance of the biosynthesized iron nanoparticle showing peaks in the range of 950-500 cm\(^{-1}\) and is represented on Figure 6. The result reveals the surface composition and the ligand banding pattern of nanosized compound. It identifies specific chemical bonds or functional groups present based on their absorption signatures which is measured by absorption energy dissipated by the stretch and bending of the chemical bonds through infrared spectroscopy [14]. The peak occurring at 950 cm\(^{-1}\) is the fingerprint region which represents the CH (aromatic and out of plane band). The functional groups responsible for the nanosizing are found in the 1800, 2200, 3000 and 3500 cm\(^{-1}\) wavelengths representing the C=O (carboxylic acid), C≡N (nitrile), C=H (alkene) and the OH (phenol) groups respectively. C-H and OH groups can be associated with capping of the nanoparticle after reduction. Biological synthesis of Fe nanoparticles is a redox reaction a which have been reported to be significantly influenced by the presence of functional groups such as the carboxyl, and mercaptans (phenols and alcohols) as observed to be present in this study [21]. The Fourier transform infrared spectroscopy provides information on the binding properties of biosynthesized NPs.

![FTIR spectrum of iron nanoparticles produced by *Trichoderma* sp.](image)

**Figure 6.** FTIR spectrum of iron nanoparticles produced by *Trichoderma* sp.
4 Conclusion
The results of this study showed the potential of synthesis of FeNPs from *Trichoderma* species using simple techniques. Furthermore, mild conditions of pH and temperature for optimum synthesis of the nanoparticle by the fungus, as a better alternative to chemical synthesis which is usually characterized by harsh conditions. The study also confirms the significant role of agitation during the green synthesis of nanoparticles. The study moreover, had addressed the paucity of information reported on the synthesis of FeNPs from filamentous fungi such as *Trichoderma* species.

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