Optimization studies on biosynthesis of iron nanoparticles using *Rhizopus stolonifer*

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**Abstract**

Isolates from pure culture of *Rhizopus stolonifer* were aseptically grown in potato dextrose broth to early exponential phase and centrifuged. The biological synthesis of Iron nanoparticles from the fungus was carried out using simple techniques. Filtrates obtained by simple methods were reacted with 1 M Ferric chloride solution. Characterization of the synthesized iron nanoparticles was monitored using Ultraviolet-visible spectrophotometer and Fourier transform infrared spectroscopy (FTIR). An increase in synthesis of the iron nanoparticles by over 200% occurred when culture of selected isolate was agitated. At 325 nm, peaks of absorbance (3.5) were read at pH 4.5 and 6, while maximum production of the iron nanoparticles was reached at 35 ºC. Peaks of transmittance of biosynthesized iron nanoparticles from selected isolate as shown by the FTIR spectrum were located at 750, 1100, 1700, 2500 and 3750 cm⁻¹, representing the CH, C-O, C=O, SH and OH groups respectively. The varying degree of transmittance and pH reported presents the selected *R. stolonifer* isolate as a biological entity for the synthesis of stable iron nanoparticles.

**Keywords**: *Rhizopus stolonifer*, iron nanoparticles, optimization, UV-vis spectrophotometry, FTIR

**1 Introduction**

Nanotechnology, a study of materials at the nanoscale (that is less than 100 nm), is fast becoming one of the most active research fields in Biology, Chemistry, Physics, Mathematics, Technology and Engineering [1]. The study of nanosized particles has attracted increasing attention due to their unique size-dependent properties which has various applications [2, 3]. Industrial demands of these particles continue to increase as the number of potential applications for the materials grows [4]. Over the decades, applications of soft magnetic nanoparticles are being studied because of their impact on the delivery of drugs, magnetic resonance imaging, cell separation,
antimicrobial activities, and the treatment for cancer, [5]. For biochemical applications, there is the need for ecofriendly and nontoxic synthesis of nanoparticles [6]. Iron NPs have been considered more toxicologically desirable because iron is ubiquitous in the body and can be handled by the body even at higher doses than other metals [7]. FeNPs have strong magnetic properties which as a result aid the ease of separation of the particles from the medium after synthesis [8, 9]. Use of chemical and physical methods for the synthesis of nanoparticles has been regarded as not suitable for biochemical applications due to the generation of toxic chemicals [4]. Biological methods on the other hand, are considered as green technology and are being manipulated through the use of bacteria, fungi, algae, actinomycetes, and plants [10]. The use of magnetotactic bacteria has become one of the most well-known biological methods for production of magnetic nanoparticles. Magnetotactic bacteria are known to synthesize magnetite (Fe₃O₄) through the mechanism of direct mineralization. There is however, a need to cheaply mass produce magnetic nanoparticles and fungal synthesis of iron magnetic nanoparticles rather than other microorganisms is preferred due to the ease of handling, low maintenance cost and ease of downstream processing [4, 11]. *Rhizopus oryzae* has been reported as a natural ‘nanofactory’ with a simplified process scale-up and reduced production costs for the synthesis of gold nanoparticles from fungal protein extracts [12]. The aim of this study was to screen and optimize selected parameters for synthesis of nanoparticles from *R. stolonifer* isolated from soil samples.

2 Materials and Methods

2.1 Isolation and identification of pure cultures from soil samples

Soil samples were randomly collected from different locations in Abeokuta in sterile bottles and immediately conveyed to the laboratory. Standard procedures of serial dilution and pour plate method on sterilized Potato Dextrose Agar (PDA) plates acidified with acetic acid were employed for the isolation. Pure forms of filamentous fungi with light black spores were subcultured into PDA plates. Microscopic examination was done by viewing stained slides (x40) under the light microscope. Structures observed under the microscope and compared with reported standards [12]. Identified pure cultures of *R. stolonifer* were stored on PDA slants at 4 °C and sub-cultured bi-monthly.
2.2 Production of fungal biomass

Biomass was prepared by introducing fungal spores ($1 \times 10^6$ spores /ml) into Erlenmeyer flasks containing 100 ml of sterile Potato Dextrose Broth. The conical flasks containing fungal spores were cotton plugged and placed in an Orbital Shaker, incubated at 25 °C and agitated at 100 rpm for 72 h. The biomass 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.

2.3 Mycosynthesis of Iron nanoparticles

Ferric chloride solution (1 mM) was prepared by dissolving 16.22 g of anhydrous ferric chloride into 1000 ml of distilled water. Biomass (10 g) was inoculated into conical flasks containing 100 ml of distilled water and placed in the orbital shaker again at 100 rpm for 72 h. Mixture of the biomass and the distilled water was then centrifuged again after which the resulting cell filtrate (50 ml) was mixed with 50 ml of 1 M Ferric chloride solution. This flask was then finally agitated in the orbital shaker for 72 h. A conical flask containing only Ferric chloride solution without the filtrate was also placed in an orbital shaker to serve as a control sample. The development of color change and stability as compared with the control indicated synthesis of FeNPs. Fungal isolate with the most reactive filtrate was selected for further studies.

2.4 Characterization and Optimization studies of synthesized FeNPs

The synthesized iron nanoparticles were characterized using ultraviolet-visible spectroscopy and Fourier transform infra-red spectroscopy (FTIR). Effects of pH (4.5-6.5), temperature (25-40 °C) and agitation on the biosynthesis of iron nanoparticles by selected fungal isolates were monitored.

3.0 Results and Discussion

3.1 Screening for FeNPs biosynthesis from fungal isolates

Out of twenty (20) fungi isolated from soil samples, ten (10) were identified as *R. stolonifer* as shown on Figure 1. Four isolates showed positive results indicated by a color change from the initial brown color to a black color indicating a production of iron nanoparticles after incubation, while no color change was observed in control conical flask containing only ferric chloride solution. Filtrates from fungal biomass reacting the most with the ferric chloride solution as
indicated with the intensity of color formation within 72 h of incubation, was selected for further studies. The development of the black color is as a result of the extracellular secretion of the fungal enzymes or metabolites which led to decomposition of FeCl₃ to Fe₂O₃. Observation from this study agrees with earlier reports [13] that fungi are able to synthesize high amounts of proteins and other enzymes which are used for bioreduction of metals which triggers the synthesis of biologically compatible nanoparticles, without much sophistication of instruments as used in this study. The reducing enzymes secreted from fungi have been reported to play the vital role in the biosynthesis of nanoparticles. These metabolites are secreted extracellularly into the solution in both a static and agitated cultures [14, 15].

3.2 Ultraviolet-visible spectrophotometry
The ultraviolet-visible spectrophotometry of the solution showed the peak of absorbance (surface plasma resonance absorption spectrum) of the nanoparticles produced by *Rhizopus stolonifer* at 325 nm as shown in Figure 3. This is in agreement with previous studies that the band gap of absorption of FeNPs detected by UV-vis exists between 200-900 nm. The UV-visible spectrophotometry has been described as a reliable technique used in the primary characterization of synthesized metal nanoparticles. The technique has also been used in the monitoring the synthesis and stability of metal nanoparticles, as they have high extinction coefficient and bright color which is visible by naked eye. Results obtained therefore have qualitatively reliable information of the nanoparticles [16].

![Figure 1. Selected isolate of *Rhizopus stolonifer* (X40)](image-url)
3.3 Optimization studies

The effects of pH, temperature and agitation on the synthesis of FeNPs from selected *R. stolonifer* were studied at 325 nm. Synthesis was observed to be optimal at two peaks of pH (4.5 and 6.0) with absorbance values of 3.430 and 4.311 respectively as shown in Figure 4. Study on the effect of temperature as shown on Figure 5 shows that optimum biosynthesis was observed at 35 °C with an absorbance value of 4.311. The biosynthetic process was carried out with and without agitation. There was increase in absorbance values from 1.783 (without agitation) to 3.430 (with agitation) during biosynthesis of FeNPs by selected fungal isolate at optimum pH and temperature studied. This study agrees with previous studies on the effect of physical parameters such as pH and temperature on the activity of membrane bound oxidoreductases in the synthesis of metal nanoparticles [17]. Peak of FeNPs biosynthesis observed at low pH of 4.5 in this study is similar to the previous research on fungal oxidoreductases enzymes which were
observed to be sensitive to pH. The enzyme activity was in alternative manner being inactivated at a high pH while at low pH, get activated [18].

3.4 FTIR analysis of iron nanoparticles produced by *Rhizopus stolonifer*

FTIR spectrum showed peaks located within the region of 617.4 cm\(^{-1}\) to 3908.6 cm\(^{-1}\). The FTIR gives information on surface composition and the ligand banding pattern of compound. It is used to identify and specify the chemical bonds or functional groups in a compound based on their specific unique absorption signatures which measure absorption energy dissipated by the stretch and bending of the chemical bonds through infrared spectroscopy [16]. This pattern is shown in Figure 6. The peaks at 750 cm\(^{-1}\) and 1100 cm\(^{-1}\) are the fingerprint region which representing the CH (alkane and out of plane band) and the C-O (carboxylic) groups respectively. The functional groups responsible for the nanosizing are the carboxylic acid (C=O), mercaptans (S-H) and the free hydroxyl (OH), which were observed at 1700 cm\(^{-1}\), 2500 cm\(^{-1}\) and 3750 cm\(^{-1}\), respectively. The C=OH and S-H groups can be associated with the fungus used and therefore playing the role of capping the nanoparticles after reduction. Green synthesis of metallic nanoparticles is a redox reaction and the presence of the carboxyl, sulfhydryl and mercaptans (phenols and alcohols) as observed in this study, have been reported to be responsible for the transformation of metal ions to nanoparticles earlier studies [19].

![Figure 4](image-url). Effect of pH on biosynthesis of FeNPs from *Rhizopus stolonifer* extract
Figure 5. Effect of temperature on the biosynthesis of FeNPs from *Rhizopus stolonifer* extract

Figure 6. FTIR spectrum of FeNPs produced by *Rhizopus stolonifer* extract
4 Conclusion

The present study presents the potentials of cell extract of *Rhizopus stolonifer* to synthesize FeNPs through simple techniques. Most of reports on the synthesis of FeNPs involved the use of magnetotactic bacteria. Fungi are generally regarded as safe as compared to bacteria for industrial applications and therefore fungal isolates with desirable characteristics as presented in this study should be synthesis of NPs which are increasingly gaining significance in a number of industries. The mild conditions for optimum synthesis of FeNPs from *R. stolonifer*, as observed in this study further presents the isolate as an attractive agent for green synthesis of FeNPs. This study therefore, helps to address the dearth of information on the use of filamentous fungi such as *R. stolonifer* in green synthesis of iron nanoparticles.

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