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Green Synthesis and Characterization of Nano-Iron using *Termitomyces* spp.

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Abstract: *Recently, nano-science is one of the most important fields. Production of nanoparticles by using microbes is a new area of interest. Therefore, this study aimed to synthesize nano-iron using Termitomyces spp. by cell-free water extract method followed by confirmation and character- ization of nano-iron by UV-visible spectrophotometry and electron microscopy. Reduction of iron ions to iron atoms was visualized through the change in the color of the mixture from pink to dark brown color with a peak at 226 nm corresponding to the Surface Plasmonic Resonance (SPR) of nano-iron by UV-Vis Spectroscopy. Transmission Electron Microscopy (TEM) showed spherical shaped nano-iron with a size range of 2.5 to 20 nm. The formation of a typical crystalline structure of iron atoms was confirmed by Selected Area Electron Diffraction (SAED). In conclusion, Termitomyces spp. is an excellent bio-factory replacing the conventional chemical and physical techniques.*

Keywords: Biosynthesis; *Termitomyces spp.*; Nano-iron; Nanotechnology

I. INTRODUCTION

Nanotechnology is a field that focuses on the study and manipulation of materials at the nanoscale, typically less than 100 nm in size. Nanoparticles (NPs) can be synthesized using two fundamental approaches: the top-down method, where bulk materials are broken down into nanosized particles, and the bottom-up method, where nanoparticles are assembled from atomic or molecular units. Nanomaterials exhibit superior properties compared to their bulk counterparts, making them highly valuable for various applications due to their distinct physical and chemical characteristics. Additionally, their unique optical, electrochemical, and electronic properties have been widely reported.

Traditional physicochemical methods used for nanoparticle synthesis often present challenges such as short-term stability and safety concerns. To overcome these limitations, biological synthesis methods using microorganisms have been explored. Various organisms are capable of producing either intracellular or extracellular inorganic nanoparticles, offering an eco-friendly and sustainable alternative to chemical synthesis. For instance, certain bacteria are known to produce magnetite nanomaterials, while fungi have been found to play a significant role in nanoparticle biosynthesis due to their ability to secrete large quantities of enzymes that facilitate the process.

Advancements in nanotechnology require the development of innovative and efficient synthesis techniques that allow for precise control over nanoparticle size, shape, and composition. Green nanotechnology focuses on the use of biological systems, including bacteria, fungi, yeast, and plant extracts, to produce nanoparticles in an environmentally friendly manner. While conventional physical and chemical methods, such as mechanical milling, sodium borohydride reduction, solvo-thermal synthesis, and carbo-thermal methods, have been widely used for nano-iron synthesis, these approaches often lead to rapid nanoparticle agglomeration due to interparticle Van der Waals and magnetic forces. Additionally, nano-iron particles synthesized through these methods are prone to oxidation in the presence of environmental oxidants, which can limit their reactivity.

Biological synthesis offers an alternative approach to overcome these challenges. Various microorganisms, including *Pleurotus* species and *Sargassum muticum*, as well as plant extracts such as *Passiflora tripartite mollissima* fruit extract, have demonstrated the ability to synthesize nano-iron. The potential applications of nano-iron in environmental

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remediation have been widely studied, particularly in areas such as water purification, catalysis, filtration, and antimicrobial activity. Among the different metallic nanoparticles, nano-iron (FeNPs) has shown significant promise in addressing environmental pollution due to its adsorptive and catalytic properties.

This study aims to synthesize and characterize nano-iron particles using *Termitomyces spp.*, highlighting its potential as an efficient biological system for nanoparticle production.

II. MATERIALS AND METHODS

Culturing of *Termitomyces spp.*

The fungal strain *Termitomyces spp.* was obtained from Dr. Laxman Rathod , Botany Department, Faculty of Science, MPASC College panvel , and evaluated for its ability to synthesize nano-iron. The fungus was cultured on potato dextrose agar (PDA) medium, which consisted of potato extract (200 mL), glucose (20 g), and agar (20 g) in 1 L of distilled water. The culture was incubated at 25°C for five days to promote sufficient fungal growth. Pure fungal cultures were preserved at 4°C on PDA slants for future use.

Biosynthesis of Nano-Iron by *Termitomyces spp.*

To obtain fungal biomass, 3–5 fungal agar discs were inoculated into MGYP broth medium containing malt extract (3 g), glucose (10 g), yeast extract (3 g), and peptone (5 g) in 1 L of distilled water. The medium was sterilized in an autoclave at 121±1°C for 15 minutes. After incubation at 28°C with continuous shaking at 180 rpm, the fungal biomass was harvested by filtration and thoroughly washed with sterile distilled water three times to eliminate any residual media components.

For biosynthesis, 20 g of fresh fungal biomass was immersed in 100 mL of sterile deionized water and incubated under the same conditions (28°C, 180 rpm) for 24 hours. The cell-free aqueous extract was then collected by filtration through Whatman No. 1 filter paper. To this extract, 1 mM of carefully weighed $FeCl_3$ was added, and the mixture was incubated in the dark at 28°C with continuous shaking at 180 rpm to prevent photo-oxidation of iron ions. A control sample, containing only the fungal extract without iron ions, was prepared for comparison.

Characterization of Iron Nanoparticles (Fe-NPs)

i. UV-Visible Spectroscopy

The initial detection of nano-iron synthesis was assessed by observing a color change in the reaction mixture. The sample was analyzed using a UV-visible spectrophotometer (Unicam UV-VIS) to confirm nanoparticle formation based on its surface plasmon resonance (SPR).

ii. Transmission Electron Microscopy (TEM)

To determine the morphology and size distribution of synthesized iron nanoparticles, TEM analysis was conducted using a JEOL JEM-2100 microscope (U.S.A). Due to its high magnification capacity (up to 1.5 million times), TEM provided detailed structural insights. A colloidal solution of nanoparticles was drop-coated onto a carbon-coated grid (Type G 200, 3.05 µm diameter, TAAP, U.S.A) for imaging.

iii. Selected Area Diffraction Pattern (SADP) Analysis

Selected Area Diffraction Pattern (SADP) analysis was performed within the TEM setup to examine the crystalline nature of synthesized nanoparticles. A thin section (~100 nm) of the sample was exposed to a high-energy electron beam (100–400 keV), allowing the electrons to interact with the atomic structure of the material. The resulting diffraction pattern appeared as bright spots, confirming the crystallinity of the synthesized nano-iron. This method is highly suitable for characterizing nanoparticle structures at the atomic level.

III. RESULTS

Biosynthesis of Nano-Iron by *Termitomyces spp.*

The ability of *Termitomyces spp.* to synthesize iron nanoparticles was investigated. When cultured on PDA medium, the fungus exhibited a characteristic pinkish-white color on its aerial hyphae after seven days of incubation at 25°C.

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After three days of incubation in MGYP broth at 28°C and 180 rpm, the fungal biomass displayed a noticeable reddish pigmentation (Figure 1). Upon mixing the cell-free aqueous extract with $FeCl_3$, iron nanoparticle formation was visually indicated by a distinct color change from pink to brown. This transformation is attributed to the gradual reduction of iron ions to their zero-valent state, confirming successful nanoparticle synthesis (Figure 2).

IV. DISCUSSION

Nanoparticles are ultra-small materials typically ranging between 10 and 100 nm in size. Among the various synthesis techniques, biological approaches have gained significant attention due to their cost-effectiveness, non-toxic nature, and eco-friendly characteristics. These methods allow controlled nanoparticle synthesis while minimizing environmental impact.

In the present study, *Termitomyces spp.* successfully synthesized nano-iron through the reduction of aqueous $FeCl_3$ (1 mM) under dark conditions. The formation of nanoparticles was initially indicated by a visible color change to brown, which was further confirmed by UV-visible spectrophotometry, showing an absorbance peak at 226 nm. These findings are in agreement with previous studies, where iron oxide nanoparticles exhibited a peak at 222 nm, and iron nanoparticles synthesized by *Pleurotus* sp. showed peaks at 226 and 276 nm. Conversely, nano-iron synthesized using *Sargassum muticum* extract exhibited absorption peaks at 402 nm and 415 nm, suggesting that variations in peak wavelengths may be influenced by particle size and composition.

Transmission Electron Microscopy (TEM) analysis revealed that the synthesized nano-iron particles were primarily spherical, with a size range of 2.5–20 nm. Similar results were observed in previous research, where nano-iron synthesized using *Sargassum muticum* extract (which contains sulfated polysaccharides acting as a reducing agent) had an average size of 18±4 nm with a cubic morphology. Additionally, iron oxide nanoparticles produced using an aqueous extract of *Passiflora tripartita mollissima* fruit were reported to have a mean spherical particle size of 22.3±3 nm.

V. CONCLUSION

In summary, the findings of this study demonstrate that *Termitomyces spp.* is an effective biological agent for the synthesis of nano-iron particles, offering an environmentally sustainable alternative to conventional chemical methods.

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