Mycofabrication of Iron Nanoparticles: Applications and Future Prospectus

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Abstract: This review article is concerned with the environmentally benign manufacturing of nanoparticles using microorganisms, particularly fungi. To create nanoparticles, scientists and researchers have Used a variety of physical and chemical processes and procedures. However, these types of nanoparticles are not environmentally friendly and frequently allow for the release of hazardous substances, which could have a negative impact on both humans and the environment. As a result, it is crucial to develop affordable and eco-friendly nanoparticle synthesis techniques and adopt green syntheses by utilizing natural microorganisms like bacteria, fungi, algae, etc. Fungi are the main focus for shaping these materials into nanoparticles since they can help cut processing time and manufacture nanoparticles in the proper size and form. They are the favored biological materials as the synthesized nanoparticles are non-toxic, thus energy efficiency is improved along with reduced environmental pollution. This is also the main justification for using fungal microorganisms in the synthesis of nanoparticles to produce ideal and non-toxic materials, which can assist in lessening the effects on the environment, improving energy efficiency, and reducing environmental pollution. The biological mechanism by which fungi synthesize iron nanoparticles, the development of diverse forms of iron nanoparticles by fungi, and the use of novel nanoparticles in the currently emerging industry are the main focus of this review.

Keywords: Mycofabrication; Iron; Nanoparticles; Applications; Future; Prospectuses.

INTRODUCTION

Nanotechnology is the manipulation or self-assembly of single atoms, molecules, or clusters of molecules to create structures that give rise to novel and fundamentally different kinds of objects and systems. The "Nano" prefix typically designates a size scale with at least one dimension between 1 nanometer (nm) and 100 nm (Mansoori, *et al.*, 2005) Due to its special properties, nanotechnology offers the necessary tools and subsequently the technology for learning more about the biological significance of nutrient availability and designing cutting-edge devices, as well as for applying this knowledge in a variety of fields, including physics, chemistry, biology, and engineering (Pietro-Souza Wde *et al.*, 2020). The emergence of nanotechnology in the field of particle assembly has not only advanced the applications of such particles but also a cost-effective synthesis of particles retaining the desired physical properties. Previous studies have mentioned that

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gold and silver nanoparticles are extensively used in cosmeceutical and medical products (Mittapally *et al.*, 2019). More stable nanoparticles are being devised through constant research in the particular area. Besides silver and gold other metals are also used as synthesizing base materials such as zinc, cadmium, iron, and platinum (Marinescu *et al.*, 2020). Regardless of nanotechnological-led revolutionary changes in the area of agriculture, environment, medicine, pharma cosmetics, and food products/containers, the conventional method of synthesis poses severe toxic threats to the environment. A study by Canu *et al.,* (2018) assessed the adverse effects caused by the synthesis and improper disposal of nanoparticles on the health of both animals and humans. Thus, researchers are looking for an alternative to the conventional chemical synthesis of nanoparticles. In such trials, a novel approach of using plants and microbes to synthesize nanoparticles is gaining popularity. The advantages of this technology over conventional techniques are cost-effectiveness, energy efficiency, and non-toxic end products (Nasrollahzadeh *et al.*, 2019). It also provides a significant advantage in regulating the shape, morphology, and size of the particle, thus, a high recovery of desired particle properties is achieved (Müller and Pyo, 2019).

There are various reports of green synthesis of nanoparticles using fungi (mycofabrication/ mycosynthesis). Mycology is the study of fungi including yeast and mushrooms, while nanotechnology deals with the creation, synthesis, and use of tiny, strategically placed particles for various technological applications (Pietro-Souza Wde *et al.*, 2020). Mycofabrication in this context refers to the development and production of metal nanoparticles using fungus. The fungal system has recently been found to be a significant bio-nano factory for the manufacture of nanoparticles like gold, silver, cadmium, platinum, etc. Fungi produce nanoparticles as a product of defense against contaminants, more specifically heavy metals. These heavy metals when encountered are reduced by the proteins and cell membrane-bound molecules into nanoparticles. Engineering these products to achieve desired particles is also called "myconanotechnology" (Jain *et al.*, 2011, Hanafy, 2018). Myconanotechnology has a lot of potential, in part because of the diversity and vast range of fungi (Khande & Shahi, 2018). Crucial nanoparticles with appropriate size and monodispersity have been created in fungal bio-nano factories (Guilger-Casagrande & R. D Lima, 2019). A study by Fouda *et al.,* (2021) reported that maghemite nanoparticles fabricated by the fungus *Penicillium expansum* showed excellent properties of decolourizing and extraction of heavy metals from textile effluents. Likewise, another fungus *Fusarium oxyporum* has been used to synthesize platinum nanoparticles (Syed and Ahmad, 2012).

Fungi's capacity to be swiftly scaled up, especially in thin solid substrate fermentation processes, is a key benefit of employing them to produce nanoparticles. Additionally, fungi produce extracellular enzymes on a massive scale, making them ideal secretors. Another benefit of utilizing a green technique to make metallic nanoparticles via fungi is the ease of using biomass. Since different fungal species are widespread, it is easy to produce and maintain them in a controlled environment of the laboratory (Castro-Longoria *et al.*, 2012). Furthermore, downstream biomass handling and processing are aided by extracellular enzyme release. Fungi are also favored for nanobiotechnological applications because of their high intracellular and wall-binding metal absorption rates (Dorcheh, & Vahabi 2016). Fungi are also simple to isolate and cultivate. The production of nanoparticles using microorganisms is a desirable green nanotechnology option, and using biological systems, such as fungi, has only recently become a novel approach. (Castro-Longoria *et al.*, 2012, Dorcheh & Vahabi 2016, Elegbede *et al.*, 2021)

According to reports, fungi create enormous amounts of enzymes, which are crucial for the large-scale manufacturing of nanoparticles. Metal ions are hydrolyzed by the majority of fungal proteins. Further, fungal nanoparticles containing polysaccharides, enzymes, proteins, and other macromolecules come in a variety of sizes and are primarily released extracellularly. Due to the crucial role that fungi play in preventing environmental contamination, the extraction and purification of fungal enzymes are less complicated than techniques that are required to produce chemical-based synthetic nano-materials.

METHODS

The research and review articles, textbooks, and patents that make up this review article's scientific information have all been thoroughly analyzed utilizing electronic search engines including ScienceDirect, PubMed, Elsevier, Google Scholar, Sci-Finder, ACS, Medline Plus, and Web of Science.

Identification	•Records identified through PUBMED (n=827) •Records through Google scholar (n=1,500)
Screening	•Title /abstarct screening (n=78) •After manual screening duplicates excluded (n=13)
Eligibility	•Records after duplicates removed (n=65) •Record screened (n=60) (records excluded n=5)
Inclusion	•Full text assesed for eligibility (n=60)

Table 1. Inclusion and exclusion criteria for article selection.

DATA SELECTION PROCESS

Initially, 78 records were counted and distributed as follows in the different databases. After eliminating those that were duplicated or otherwise, the following stages were established: Stage 1. The reviewers analyzed all the articles and focused on which languages they were written in to eliminate those that were not in English. Stage 2. After that, the titles and abstracts were looked at to exclude those that did not correspond to the research. Stage 3. The different eligibility criteria were applied, i.e., whether it corresponds to the methodology we are looking for, the participants the scope and the study. All this is reflected in the above flowchart (Table 1).

MECHANISM OF SYNTHESIS

Nanoparticle formation using fungi is processed through multiple steps including immobilization, complexation, bio-coupling, precipitation, biosorption, purification, and ion modification. Filamentous fungi are widely used as reducing agents and have tolerance to heavy metals. The mycelial mass of fungi is also tolerant to agitation and pressure, which are commonly used in industrial-level production. Biomolecules have a tendency to interact with metal ions for example during conversion of NADPH/NADH to NADP/NAD. Thus, NADH and dependent nitrate reductase enzymes are extremely important for the biogenic synthesis of metal nanoparticles through fungal culture (Baymiller *et al.*, 2017). To achieve desirable properties of nanoparticles such as monodispersity, stability and compatibility one has to check the fungi employed to synthesize the nanoparticles as well as the processing conditions such as agitation, temperature, light, and period of synthesis. Previous studies have found that changes in temperature, metal precursor, pH, media and amount of biomass can be used to achieve the desired properties of metal-nanoparticle.

Biogenic synthesis of metal nanoparticles through the reduction of metal ion and their precipitation either extracellularly or intracellularly is shown in Fig. 1. The diagram represents intracellular absorption of molecules by fungi and reduction and coating by intracellular molecules. Similarly, the extracellular method involves the release of molecules extracellularly and reducing the metal ions to produce metal nanoparticles.

IRON NANOPARTICLE

Iron nanoparticles have remarkable chemical, magnetic and optical properties that are unique in many comparisons with other metal nanoparticles. According to nano-engineers, the best property of iron nanoparticles is its significantly higher surface area. Not only the higher surface area but its high ability to oxidize and reduce multiple pollutant matters makes it a perfect remediating agent for water, soil and sediments. Above all, iron nanoparticles

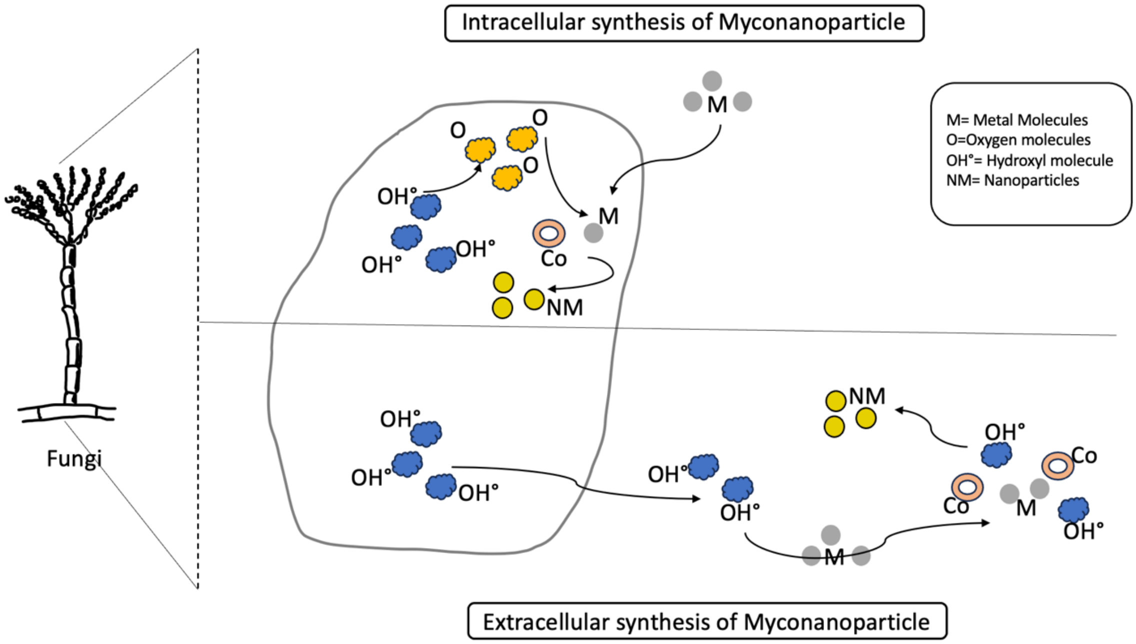

Figure 1. Intracellular and extracellular synthesis of nanoparticles through fungi.

are non-toxic. Likewise, the coating of these particles does not lessen, providing high coercivity at very low temperatures. Further, they have excellent catalytic and reductive properties for wastewater treatment, among other nanomaterials. Most of the time, these are used to eliminate pollutants like heavy metals, dyes, antibiotics, etc., these nanoparticles have low toxicity and are helpful as support for bioactive materials such as peptides, enzymes, antibodies, and nucleic acids. Due to their magnetic nature, it is possible to recover and repurpose these nanoparticles as they can be isolated from reaction mixtures using magnets. Moreover, noble metals can be ideal elements for coating iron particles resisting the oxidizing agent and oxygen. Its high reactivity with air gives it a unique chemical identity. It can be used as a catalyst due to its oxidizing ability in ambient conditions. Imagine manufacturing these extremely valuable nanoparticles through nanotechnology, it will not only reduce the cost but also be more sustainable. In the following sections, we will discuss the biological synthesis of iron nanoparticles and its wide applications.

BIOLOGICAL SYNTHESIS OF IRON NANOPARTICLES

The process of creating iron nanoparticles from biological sources, such as bacteria, fungi, plants, and algae, is known as iron nanoparticle biosynthesis. In contrast to chemical processes, which demand toxic ingredients and result in hazardous waste, such synthesis is thought to be more cost-efficient, sustainable, and environmentally benign. Iron nanoparticles can also be bio-synthesized either intracellularly or extracellularly. Biomolecules released by microorganisms or plants provide a reducing environment to the iron ions during extracellular production. The reduction and stabilization of iron ions during intracellular production are catalyzed by the biomolecules found inside the cell, such as enzymes. Due to their potential uses in environmental remediation, particularly in the removal of organic contaminants from wastewater, iron nanoparticles produced by fungus have attracted attention lately. Through the use of an extracellular or intracellular reducing enzyme and a bioinspired mineralization process, fungi can produce metal nanoparticles and nanostructures (Bourzama & Ouled-Haddar *et al.*, 2021; Mughal & Zaidi 2021; Siddiqi *et al.*, 2016).

Inorganic nanoparticles such as gold, silver, calcium, silicon, iron, gypsum, and lead are biosynthesized by microorganisms such as bacteria, cyanobacteria, actinomycetes, yeast, and fungus. They can produce extracellular and intracellular nanoparticles (Durán *et al.*, 2005). However, retrieving the nanoparticles produced during intracellular biosynthesis is difficult because of additional processing such as ultrasonication and therapy with

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the appropriate detergents (Asmathunisha *et al.,* 2013). It is therefore; crucial to screen the bacteria that produce nanoparticles extracellularly (Sharma & Sahi *et al.,* 2007). Currently, a small number of metal sulphides, metal chlorides, and very few oxides are biosynthesized as nanomaterials by microorganisms.

Eukaryotic saprophytic fungi are widespread in domestic environments. Only 70,000 of the estimated 1.5 million different species of fungi on Earth have been identified. According to a recent study, sequencing techniques indicate that there are approximately 5.1 million fungal species globally (Blackwell *et al.,* 2011) that can digest extracellular food and release certain enzymes to hydrolyze complicated components into more comprehensible molecules, which are then absorbed and utilized as an energy source (Ahmad *et al.,* 2003). The study of fungi's involvement in nanobiotechnology is deemed essential. Due to their tolerance and ability to accumulate metals, fungi have drawn increased attention in studies on the biological synthesis of metallic nanoparticles.

A relatively new and developing field is the use of fungi in the biological synthesis of iron nanoparticles. Different extracellular and intracellular enzymes and metabolites produced by fungi are capable of reducing metal ions to the corresponding nanoparticles. Ecologically sound, economical, and effective methods are used in the biological manufacture of iron nanoparticles, Involving fungi in the manufacture of nanoparticles is advantageous because of how easily they can be scaled up (for example, using a thin solid substrate fermentation approach). It is possible to produce large quantities of enzymes because fungi are particularly efficient extracellular enzyme secretors (Castro-Longoria & Vilchis-Nestor *et al.,* 2011). Another advantage of using this method to synthesize metallic nanoparticles is its economic viability and ease of using biomass. Additionally, a few species have a rapid rate of growth, making laboratory maintenance and rearing fairly straightforward (Vigneshwaran *et al.,* 2006). The majority of fungi have strong intracellular metal absorption and wall-binding capacities (Volesky *et al.,* 1995). Fungi can produce metal nanoparticles/meso and nanostructure by employing reducing enzymes, either intracellularly or extracellularly, as well as the biomimetic mineralization process (Ahmad *et al.,* 2003).

Fungus-like *Fusarium oxysporum* and *Verticillium* sp. when mixed with ferric and ferrous salts at room temperature, different sizes of magnetic particles can be produced extracellularly. Fungi-produced cationic proteins hydrolyze extracellularly the anionic iron complexes. This results in the creation of crystalline magnetite particles with very little spontaneous magnetism at low temperatures (Bharde *et al.,* 2006). Kaul *et al.,* (2012) investigated the formation of iron nanoparticles in two bacteria, *A. faecalis*, and five different fungal species, including *Pochonia chlamydosporium*, *Aspergillus fumigatus*, *Aspergillus wentii*, *Curvularia lunata*, and *Chaetomium globosum* (Kaul *et al.,* 2012). The *Alternaria alternata* fungus, which has been investigated using several spectroscopic techniques, was used by another team of researchers to produce iron nanoparticles.

One of the most promising applications of iron nanoparticles synthesized from fungi is decolorization of wastewater xenobiotic dyes. Dye pollution is a significant environmental concern due to its adverse effects on aquatic ecosystems, human health, and soil contamination. Iron nanoparticles synthesized from fungi have effectively removed a wide range of dyes from wastewater, including reactive and cationic dyes, acid dyes, and basic dyes (Mohamed *et al.,* 2015). The high surface area of iron nanoparticles generated from fungus, which offers a lot of active sites for dye adsorption, is thought to be the cause of their decolorization abilities. Additionally, the nanoparticles' small size allows for efficient diffusion and adsorption of the dyes, leading to their rapid removal from wastewater and nearby land areas.

Alternatives to chemical and physical processes, such as the bio-synthesis of iron nanoparticles from fungi, are environmentally friendly and economical (Fig.2). Additional study is necessary to optimize the production and boost the effectiveness of these nanoparticles for large-scale applications, Researchers have used biological extracts in the creation of metallic nanoparticles. They adhered to simple procedures like those for decreasing metal ions. They did this by using extracellular or intracellular reductants made from biomass extracts (Chaudhary *et al.,* 2015).

They are all restricted to the microbial life found in earthy sources. Microorganisms are used in culture settings to determine the biological synthesis of nanoparticles, hence it is vital to standardize these conditions to generate nanoparticles on a large scale. Despite rigorous examination of the form, size, and mix of the particles, it is known that many

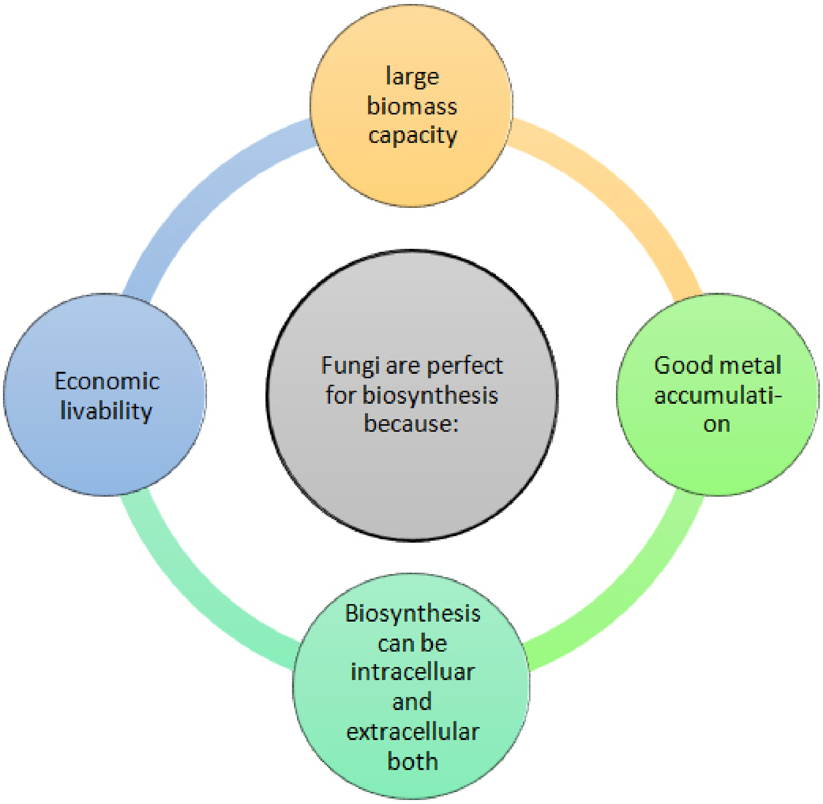

Figure 2. Distinct advantages of fungi when used in Nanoparticle synthesis

microorganisms may produce metallic nanoparticles with specific features similar to nanomaterials that are synthesized chemically (Ali *et al.*, 2012). It is expected that other metal oxides can be formed as a result of the microbes' hydrolytic activity. In conclusion, microbes can create nanoscale materials at modest pressures and temperatures. Moreover, using a microbial process to create nanomaterials is affordable, simple, efficient, energy-saving, and environmentally beneficial (Saravanakumar *et al.*, 2019).

Source	Primary salt used	Reference
Pleurotus florida	Ferric chloride	Manikandanand & Ramasubbu (2021)
Rhizopus stolonifera	Ferric chloride	Adeleye *et al.*, (2020)
STS10 (similar to *Trichoderma asperellum)*	$FeCl_3$	Mahanty *et al.*, (2019)
Ageratum conyzoides	$FeCl_3{\cdot}6H_2O$	Madivoli *et al.*, (2019)
Aspergillus oryzae TFR9	$FeCl_3$	Tarafdar *et al.*, (2013)
Penicillium oxalicum	$FeSO_4$	Mathur *et al.*, (2021)
Fusarium oxysporum	$FeSO_4$	Abdeen *et al.*, (2013)
Aspergillus flavus	$(FeSO_4)$	Sidkey (2020).
Aspergillus tamarii isolate AV11	$FeSO_4$	Sayed *et al.*, (2021)
Chaetomium globosum	Fe_2O_3	Kaul *et al.*, (2012)
Alternaria alternata	Iron(III)oxide	Mohamed *et al.*, (2015)
Aspergillus niger YESM 1	$FeSO_4$, $FeCl_3$	Abdeen *et al.*, (2016)
F. oxysporum	$K_3[Fe(CN)_6]$	Bharde *et al.*, (2006)
Verticillium sp.	$K_4[Fe(CN)_6]$	Bharde *et al.*, (2006)

Table 2. Fungal sources and type of salt used in Nanoparticle formation.

The enzymes that are produced by the fungus can transform iron ions into iron nanoparticles as shown in Table 2. The type of fungus utilized and the particular conditions of the biosynthesis process may have an impact on the shape, size, and characteristics of the synthesized nanoparticles (Fig. 3).

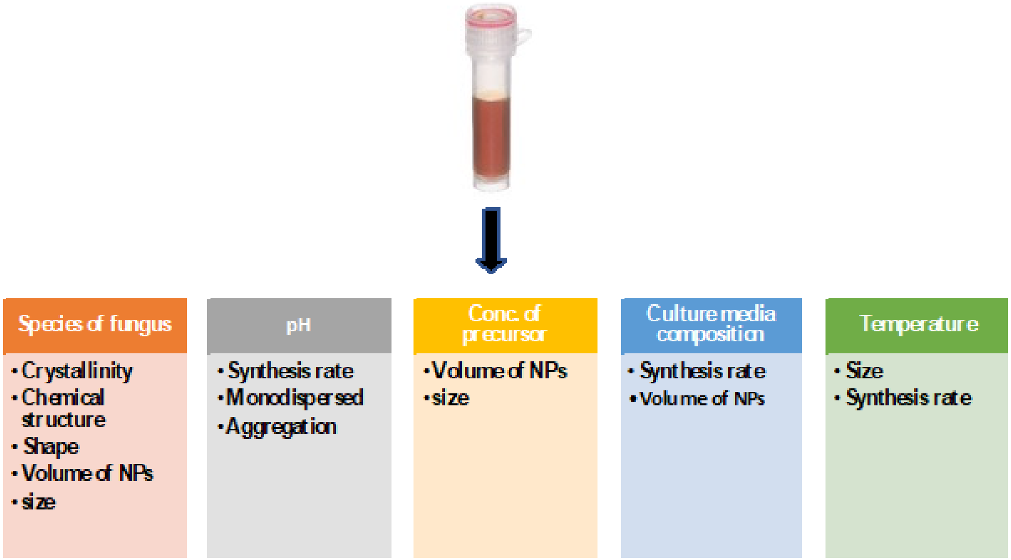

Figure 3. Factors related to synthesis and characterization of nanoparticles

APPLICATIONS

Nanoparticles synthesized using fungi have huge potential in healthcare, agriculture, and pest control. Although nanoparticles can be synthesized by different sources such as fungi, bacteria, or plants, however, fungi have a greater production range compared to other sources. Myconanoparticles have various applications in the health sector involving the control of infection by germs, as this increases the production of reactive oxygen species (ROS) (Gudikandula *et al.*, 2017). Similarly, in the area of agriculture, it can be applied to increase productivity. Many studies have shown the regulation of phytopathogenic fungi and pests in agriculture (Elgorban *et al.*, 2016; Gherbawy *et al.*, 2013; Sundaravadivelan and Padmanabhan, 2014). It appears to have a concentration-dependent effect on the anti-pest, anti-fungal activities. Biogenic nanoparticles can restrict the hatching of parasitic eggs by almost 91% which performed better than triclabendazole (Gherbawy *et al.*, 2013).

Iron nanoparticles have a wide range of applications in various areas including magnetic and electrical applications, catalytic applications, and biomedical applications. In the following section, this review will discuss literature that provides trends and applications of iron nanoparticles synthesized from fungi.

Environmental Remediation

Iron nanoparticles can be utilized to clean up polluted water and soil. They are efficient in cleaning up contaminated water and soil of pollutants like heavy metals and organic compounds (Pattanayak *et al.*, 2021).

Biomedical Applications

Iron nanoparticles have potential applications in medicine, including drug delivery, magnetic resonance imaging (MRI), and cancer therapy. Iron nanoparticles can be functionalized with drugs, peptides, and other molecules for targeted drug delivery. They can also be used as contrast agents in MRI due to their magnetic properties (Montiel Schneider *et al.*, 2022).

Wastewater Treatment

Iron nanoparticles can be used in wastewater treatment to remove organic compounds, dyes, and other pollutants. They are effective in removing pollutants such as phenols, chlorinated hydrocarbons, and pesticides (Chaudhary *et al.*, 2023).

Energy Applications

Iron nanoparticles have potential applications in energy storage and conversion. They can be used in the production of batteries, fuel cells, and solar cells due to their unique magnetic and electronic properties (Wang *et al.,* 2015).

Catalysis

Iron nanoparticles have potential applications in catalysis, including hydrogenation, oxidation, and reduction reactions. They can be used as catalysts for the production of chemicals such as ammonia and methanol (Zaera, 2013).

Magnetic Fluids

Iron nanoparticles can be used to produce magnetic fluids that have potential applications in data storage, sensors, and biomedicine. Magnetic fluids can be used in targeted drug delivery, hyperthermia, and imaging (Wang *et al.*, 2019).

Food packaging

When nanoparticles are utilized in food packaging, the penetration characteristics change, the barrier properties improve, the thermal resistance increases, and there is noticeable antibacterial activity. (Keshk *et al.*, 2019).

The enormous surface area and high surface energy of nanoparticles define them. As a result, the nanoparticles and polymer bonds interact strongly at the interface. The characteristics of the biopolymers used for packaging are greatly improved as a result (Jafarzadeh *et al.*, 2019). Microorganisms spoiling food during storage is the main issue. To reduce the influence of microorganisms on food during processing or storage, utilize iron nanoparticles and nanocomposites with antibacterial properties. They increase food safety and lengthen shelf life in this way (Mary *et al.*, 2022). Iron nanoparticles can be utilized to prevent food oxidation by applying antimicrobial coatings to the interior surface of the packaging. Such a coating either gradually releases antimicrobial compounds or immobilizes them on the functional surface of the packaging (Puspasari *et al.,* 2022). The decolourization properties of iron nanoparticles synthesized from fungi are attributed to their high surface area, which provides a large number of active sites for the adsorption of dyes. Additionally, the nanoparticles' small size allows for efficient diffusion and adsorption of the dyes, leading to their rapid removal from wastewater.

According to El Messaoudi *et al.*, (2022), textile wastewater discharge is the industrial byproduct that has the most detrimental environmental effects. These are released in significant amounts into streams and rivers. Given that they are large contaminants, and have the potential to be poisonous, carcinogenic, allergenic, and resistant to degradation, textile dyes pose a serious hazard to the environment (El Messaoudi *et al.,* 2022). Textile dyes can be categorized in a variety of ways, including acidic, dispersive, basic, and direct. Wastewater resources that contain dye should be properly treated using environmentally friendly technology to prevent adverse effects on both human and environmental health. Since they are safer, simpler to use, generate less sludge, and provide non-toxic byproducts, biological approaches are more promising.

Finally, iron nanoparticles made from fungus have demonstrated excellent results in the decolourization of wastewater colors. The environmentally friendly and economically advantageous biological production of iron nanoparticles from fungi replaces traditional chemical and physical processes. To optimize the production procedure and boost the effectiveness of these nanoparticles for large-scale applications, additional study is necessary.

FUTURE PERSPECTIVES AND CONCLUSIONS

For some particular applications, such as in biomedicine, where fungi may not only generate and maintain the nano-particles but also provide additional biomolecules with significant beneficial effects, the choice of species may be of great interest. Numerous fungus species can synthesize the biomolecules required for the creation of diverse iron-containing NPs, according to studies that have been reported to date. However, to scale up to commercial manufacturing, these procedures will need to be optimized. Future research may examine these types of interactions between the organic capping and the inorganic core.

Differing synthesis conditions could lead to various nanoparticle properties as well as synthesis success or failure. On the other hand, the effects of the numerous parameters are uncertain, demanding additional thorough examinations for each fungus

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utilized. Determining the necessary physicochemical characteristics of the NPs is also essential to determine the synthesis's variables, including temperature, pH, and time. The quick manufacturing of significant amounts of NPs should be possible thanks to the optimization of synthesis methods. It now becomes possible to use nanomaterials to combat issues like bacterial antibiotic resistance and phytopathogens that impair agricultural production.

A more in-depth understanding of the mechanisms involved in the synthesis of iron nanoparticles, which cover the entire process from nucleation with the help of enzymes or based on bio templates, is necessary to advance our understanding of fungal synthesis.

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