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REVIEW ON EMPHASIS OF FUNGAL BIOREMEDIATION OF CONTAMINATED WASTEWATER

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ABSTRACT

Aquatic ecosystems are constantly exposed to a considerable load of harmful chemicals that exceeds the purifying capacities, posing a serious threat to animal and human population. The non-biodegradability and bio-magnifications of inorganic pollutants in aquatic ecosystem causes considerable harm to the environment. To combat this persistent issue, bioremediation offers a conventional clean-up technology due to its cost effectiveness and comparatively low harmful impact on the environment. Among the various biological systems, fungi are often used for bioremediation because of their ease of handling and high tolerance of metals. The application of remediation using fungi can be greatly improved by the inclusion of nano-biotechnology. Metallic nanoparticles from fungus have gained attention in remediation of aquatic ecosystem because of their properties such as synthetic dye quenching, metal sensing, biocompatibility, and superparamagnetism. Though, the knowledge of the susceptibility to biodegradation of some contaminants is still lacking, this technology can be used for reducing or eliminating the pollutants in the atmosphere, water and soil environments. This review would particularly provide an overview of current research and the importance of bioremediation for removing the toxic components from wastewater. Furthermore, the bioremediation performance of enzymes and nanoparticles from fungi in wastewater will also be highlighted.

KEYWORDS: Bioremediation, Wastewater, Fungi, Enzymes, Nanoparticles

INTRODUCTION

Environmental pollution is a growing concern due to the indiscriminate and frequently release of hazardous, harmful chemicals from various sources. The pollution of soil and water by industrial chemicals and petroleum hydrocarbons is a serious problem worldwide. Industrialization leads to the generation of enormous quantities of industrial effluents which eventually put tremendous pressure on water utilization. The explosive development of chemical industries has produced a large number of chemical compounds that include pesticides, fuels,

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solvents, alkanes, polycyclic aromatic hydrocarbons (PAHs), explosives, dyes and so on [1]. Therefore, there is a dire need to develop remediation technologies that can remove the contaminants from the environment to protect human health and restore them for productive uses in agriculture, recreation, housing, and industry. Now a days, a great emphasis is placed on environmental biotechnology and attaining sustainable development: in particular, biological techniques can be applied effectively in the remediation of both soil and water contaminated with organic pollutants from a variety of sources.

For the sustainable development of companies and the environment, the industrial effluents must be treated in a precise and economical manner. Diverse electrochemical, oxidation processes, and valorisation techniques have been already reported to lessen the toxicity of wastewater effluents and to make their use more sustainable [2]. However, bioremediation offers an attractive and more conventional clean-up technology due to its comparatively low cost and environmental impact. This technique involves the use of biological organisms such as bacteria, fungi, yeast, algae, etc., and their products to reduce the concentration of hazardous or nontoxic contaminants in the environment. There are several conventional techniques such as chelation method, chemical oxidation, precipitation, solidification, bioventing, bio-augmentation etc. for the bioremediation of both soil and water [3]. However, they have certain limitations such as, weak or lack of binding capacity to the pollutants, high energy supply, formation of secondary contaminants etc. Also, biological organisms are capable of removing heavy metals by oxidizing or reducing the transition metals. Therefore, use of biological components for the process of remediation is the possible way to re-establish the natural condition of water as well as soil. There are different types of mechanisms of bioremediation including bioaccumulation, biosorption, bioleaching biotransformation. Bioremediation is broadly classified into two categories viz. In situ and Ex situ. In situ remediation is the application on subsurface or in saturated groundwater and soil, while, ex situ applies to resources accessible aboveground. The process of bioremediation may be aerobic or anaerobic depending upon the type of organisms used, it may be engineered (man-made) or intrinsic (natural).

The advancement of nanotechnology has made the process of bioremediation more efficient and offers various innovative ways to treat water pollution. The smaller size, high surface area to volume ratio, and higher chemical characteristics of the nanotechnological routes make them more effective than any other conventional counterparts. A path towards the environmentally friendly remediation of contaminants has been created by the green synthesis of nanomaterials from microbes and other organisms. For the removal of contaminants from wastewater and other aquatic ecosystems, many nanomaterials, including metal and their oxides, carbon-base nanotubes have been used. The environmental potential of metal nanoparticles (NPs) can be divided into four categories: green remediation, sensing and detection of pollutants, element sequestration, and the control of contamination. Groundwater, wastewater, and soil are the three main areas where metal NPs can be used for green remediation. According to where NPs are generated, the synthesis of NPs are broadly classified into two methods: intracellular and extracellular. The intracellular technique involves transferring the metal ions inside the organism's cell in the presence of enzymes to produce NPs. Extracellular NPs synthesis includes trapping metal ions on the cell surface and reducing ions in the presence of enzymes. Extracellular biosynthesis of NPs has gained a lot of interest because of its cost effectiveness and lack of downstream processing needs. NPs are considered as a better adsorbing material than any other conventional treatment technologies because of their enormous surface area and catalytic potential. The conventional method to produce NPs are costly, toxic and causes various hazardous compounds. Therefore, there is a need for alternative, environment friendly approach for the green synthesis of NPs. Green synthesis of NPs uses biological natural sources such as algae, plants, bacteria, fungus, diatoms, etc. Among these, fungi are ideal for the synthesis of NPs because they are easy to culture, and have strong wall-binding and intracellular metal absorption capacities. The green nanotechnology involves myco-nanotechnology, where fungi are being explored for the synthesis of various NPs with desired dimensions. The mycosynthesis of fungal NPs for bioremediation of both soil and aquatic systems have recently gained interest. The diversity of fungus has been identified as an important biological component for the biosynthesis of NPs. Synthesis of NPs from fungi offers many advantages compares to other organisms such as, easy downstream processing, easy to handle, eco-friendly, economically feasible. Fungi have the potential to generate NPs both extracellularly and intracellularly, which are used in a variety of applications spanning from textiles to food preservation, medicine, and clinical microbiology, etc.

Despite the fact that much research has been done in the field of bioremediation, several aspects, such as mechanism of uptake and degradation pathways, etc. remain unsolved. Many articles have been published based on the synthesis of NPs from different biological sources. Still, few literatures are available on the distinct applications of the NPs, particularly derived from fungi, on bioremediation of aquatic ecosystem. In this review, we intend to discuss about the distinct potential of several fungi and fungal enzymes from various habitats in bioremediation of different harmful and resistant substances, including pesticides, textile dyes, petroleum, polyaromatic hydrocarbons, medicines, and detergents, etc. This review also aims at highlighting the broad-spectrum bioremediation potential of fungal NPs in aquatic ecosystem.

1.1 Bioremediation potential of Fungi

Fungi plays an important role as decomposers and symbionts in the ecosystem because of their robust morphology and wide metabolic capabilities. Fungi are ideal for bioremediation of soil as well as water due to its biosorbent and metal uptake capacity. The ability of fungal species in producing fruiting bodies from various wastes lies in their efficiency to degrade waste by secreting various hydrolysing and oxidizing enzymes [4]. Another advantage of using a green method led by fungi to create metallic NPs is its economic viability and ease of using biomass. Additionally, because some species develop quickly, it is easy to cultivate and maintain them in a lab setting. The majority of fungi have high wall-binding and intracellular metal absorption capabilities. Fungi can thrive in a various habitat, with complex soil matrix which serves as the most common location for fungal colonisation, as well as freshwater and marine habitats, where fungi colonise in a stable manner. They are also reported to survive in industrial waste effluent treatment plants that process a variety of wastewaters. Though, the availability of fungal species, the accessibility of pollutants, and the presence of a conductive environment are all key elements in the success of remediation using fungi. As a result, understanding the physiology and ecology of fungal species, as well as the characteristics of the polluted locations, are critical elements in choosing an appropriate bioremediation procedure.

There are many reports on bioremediation potential of fungal species, that can convert toxic compounds from industrial effluents, wastewater and degrades intractable xenobiotics. For instance, degradation of xenobiotic compounds was studied by using *Aspergillus flavus*, *Penicillium spiculisporus*, and *P. verruculosum* [5]. Aquatic fungi, like, *Mucor hiemalis* was also investigated for removal of drugs like acetaminophen and diclofenac from contaminated effluents of pharmaceutical industries [6]. It was observed that the extracellular ligninolytic enzymes and intracellular cytochrome 450 were mainly responsible for the degradation of such drugs from pharmaceutical effluents. Edible fungi, like *Pleurotus ostreatus* was also reported to degrade oxo-biodegradable polymers like, plastics [7]. Basic and acid dyes are the most harmful to

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aquatic life and have a propensity to move up the food chain before entering the human body, where they can cause various physiological problems. White-rot fungi are extensively studied for its dye degradative capacities. By co-culturing *Aspergillus versicolor* and *Rhizopus arrhizus*, it was possible to remove 89.4% of Reactive Remazol Blue at pH 6 and 69.23% at pH 3 at 100 mg/L dye concentration in 6 days using dodecyl trimethyl ammonium bromide [8]. Decolourization of dye from various industries, such as, textile, sugar, leather, etc. was also investigated using white rot fungi, *Aspergillus* sp., and *Penicillium* sp., indicating that these fungi have diverse substrate preferences [9]. Marine fungi, *Trichoderma harzianum* was studied for biotransformation of high concentration of organic pollutant, such as, pentachlorophenol [10]. Similarly, degradation of an organophosphate pesticide, such as, chloropyriphos and its metabolite 3,5,6-trichloro-2-pyridinol (TCP) was studied using *Aspergillus niger* JAS1 from contaminated area [11]. Many white rot fungi have been reported to reduce the concentration of phenolics (>60%) from wastewater of olive mill [12]. Fungal isolates of *Aspergillus terreus*, *A. niger*, *Rhizopus nigricans*, and *Cunninghamella* sp. was efficiently studied for its remediation properties [13]. Results revealed that *A. terreus* was effective for removal of nitrate and biological oxygen demand (BOD), and *A. niger* was effective for removal of phosphate and Chemical oxygen demand (COD) from sewage wastewater sample. For water soluble crude oil fractions, marine-derived fungus such as, *Mucor* sp., *Aspergillus* sp., *Penicillium* sp., and slime mould showed bioremediation capability between 0.01 and 0.25 mg/mL [14].

Macrofungi possess tolerance for heavy metals due to presence of some functional groups such as, ether, amide, oxalic acid, etc. [2]. *Termitomyces microcarpus*, *Boletus griseus*, *Tylopilus ballouii*, *Agaricus macrosporus*, etc. were studied for accumulation of heavy metals such as, zinc, copper, lead, cadmium, etc. [15]. Among the studied species, *B. griseus* and *T. microcarpus* has the highest accumulation capacity for cadmium and lead, respectively. Similarly, *Cryptococcus* sp., a deep-sea psychrophilic fungus, was reported to show tolerance and grow in the presence of heavy metals such as, lead, cadmium, copper, and zinc, which may provide insight on their manner of adaptability in such circumstances [16]. *Aspergillus* sp. was studied for removal of chromium from waste water [17]. *Coprinopsis* sp. was investigated for its bioaccumulating capacity of cadmium and lead [18]. Bioaccumulation of copper and nickel was studied using adapted and non-adapted cells of *Candida* sp. [19]. The maximum accumulation capacity of metal ions was observed in wastewaters at pH 4. The oxidized form of selenate [Se(VI)] and selenite [Se(IV)] cause a significant threat to the aquatic ecosystem and human. The selenium removal capacity from industrial and municipal wastewaters was studied using *Alternaria alternata* [20]. The bioremediation potential of various fungi is given in Table 1.

Table 1. Bioremediation potential of fungi

Fungi	Pollutant	References
Aspergillus flavus, Penicillium spiculisporus, and *P. verruculosum*	Xenobiotic compounds	[5]
Mucor hiemalis	Drugs such as, acetaminophen and diclofenac	[6]
Pleurotus ostreatus	Plastics	[7]
Aspergillus versicolor and *Rhizopus arrhizus*	Reactive Remazol Blue	[8]
Aspergillus sp. and *Penicillium* sp.	Dyes	[9]
Trichoderma harzianum	Organic pollutant such as, pentachlorophenol	[10]
Aspergillus niger JAS1	Organophosphate pesticide such as, Chloropyriphos and 3, 5,6-trichloro-2-pyridinol	[11]

Aspergillus terreus and *A. niger*	Nitrate and BOD, Phosphate and COD, respectively	[13]
Mucor, *Aspergillus*, *Penicillium*	Crude oil	[14]
Termitomyces microcarpus, *Boletus griseus*, *Tylopilus ballouii*, and *Agaricus macrosporus*	Heavy metals such as, zinc, copper, lead, cadmium	[15]
Cryptococcus sp.	Heavy metals such as, lead, cadmium, copper, and zinc	[16]
Coprinopsis sp.	Heavy metal such as, cadmium and lead	[18]
Candida sp.	Copper and nickel	[19]
Alternaria alternata	Selenium	[20]
Trichoderma viride Pers NFCCI-2745	Phenolics	[21]

1.2 Bioremediation potential of Fungal enzymes

For many years, fungal enzymes have been extensively utilized for industrial uses; nevertheless, they also show potential in medicinal and bioremediation applications. They can breakdown the organic substances and colonize both biotic and abiotic surfaces with ease. Fungi has the ability to produce extracellular enzymes such as, cellulases, amylases, lipases, xylanases, catalases, proteases, peroxidases, laccases, etc., which have industrial importance and can find potential applications in degrading the organic wastes. Particularly, white-rot fungi produce various ligninolytic enzymes that can be used in bioremediation studies because they are capable of degrading various xenobiotic compounds, including dyes. This depends on the species and environmental factors. The ability of ligninolytic enzymes has becoming a conventional strategy for removing pollutants particularly, PAHs from contaminated sites. Laccases and several other fungal peroxidases from basidiomycetes have known to degrade persistent organic pollutants. Many researchers have documented the importance of fungal enzymes produced from marine fungi in relation to their biotechnological applications. Marine fungi can even withstand an elevated concentrations of heavy metals like lead and copper, and their interactions with metal ions in marine environments can be utilized to synthesize metal NPs with desirable properties [22]. These fungi have a biological advantage over terrestrial fungi because of their capacity to adapt to strong alkaline and acidic environments. The effectiveness of marine microbes in the removal of metal ions suggests the potential of extremophilic organisms in both bioremediation and nanotechnology.

Several reports are already available on the application of fungal enzymes for bioremediation of aquatic ecosystem and wastewater. Majority of the studies have reported that the bioremediation potential of fungi is due to ligninolytic enzymes such as, laccases and peroxidases. Lignin peroxidase and manganese peroxidase from white-rot and basidiomycetes fungi are mainly documented for breakdown of hazardous substances. By encouraging microbial activity with the use of a bio purification system, the ligninolytic enzymes from white-rot fungi have been used to transform a variety of organic contaminants, including pesticides, from contaminated wastewaters [23]. Laccase from *Trametes versicolor* was reported to degrade drugs, like, diclofenac (100%), trimethoprim (95%), carbamazepine (85%), and sulfamethoxazole (56%) from pharmaceutical effluents [24]. Degradation of chloramphenicol was studied using laccase from *Trametes hirsute* [25]. Similarly, oxidizing enzymes from mycelia of an edible fungi, *Lentinula edodes*, was studied for degradation of anti-inflammatory drug like, piroxicam, an endocrine disruptor like, 17α-ethyl-estradiol [26], [27]. Laccase from *T. versicolor* was also investigated for removal of phenolics from olive mill wastewater in the

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presence of 1-hydroxybenzotriazole as a mediator, which increases the oxidation of phenolic compounds [28]. Given the importance of these characteristics in bioremediation, efforts have been made to increase the laccase production in white-rot fungi *T. versicolor* and *Pleurotus ostreatus* through solid state fermentation on orange peels, followed by further testing of its capability for bioremediation of PAHs like, phenanthrene and pyrene [29]. Though, *P. ostreatus* generated 2700 U/L laccase and *T. versicolor* cultures produced 3000 U/L laccase, *P. ostreatus* showed better results in removing phenanthrene and pyrene. Lignolytic enzymes from *Aspergillus glaucus* was also found to degrade fipronil, a phenylpyrazole insecticide and its metabolites from aqueous medium [30]. Extracellular ligninolytic enzymes from various species of *Pleurotus* showed degradation or decolourization capacity for dyes like, Direct Blue 14 [31]. These enzymes also alter the azo dyes structure by destroying chromophoric assemblies, which produce phenoxyl radicals in the reaction [8]. Furthermore, enzymes extract of *Trametes maxima* was studied for degradation of herbicide, like, atrazine [32]. In the example of *Trichoderma viride* Pers NFCCI-2745, which was isolated from an estuary polluted with phenolics, the ability of marine fungi to produce laccase resilient to high salinity and phenolics was effectively exploited [21]. A list of fungal enzymes and its role in degradation of various pollutants is given in Table 2.

Table 2. Fungal enzymes and their application on degradation of various pollutants

Fungi	Enzymes	Pollutants	References
Dentipellis sp., Phanerochaete chrysosporium, Pleurotus ostreatus, Trichoderma harzianum	Cytochrome P-450 monooxygenase, glutathione transferase, dioxygenase, dehydrogenases	PAHs	[33, 34]
Aspergillus flavus, Phlebia acerina, Bjerkandera adusta, Russula virescens	Laccase, lignin peroxidase, manganese peroxidase,	Congo red dye	[4, 35]
Fusarium oxysporum, Penicllium brocae, Purpureocillium lilacinum	Cutinase, estarase	Dihexyl phthalate, dipropyl phthalate	[36, 37]
Funneliformis geosporum, Irpex lacteus, Phlebia radiata, Trametes versicolor, Pleurotus ostreatus, Trichoderma ghanense, Candida sp., Coprinopsis sp., *Aspergillus sp., Penicillium rubens*	Ligninolytic enzymes	Heavy metals such as, arsenic, zinc, chromium, lead, cadmium, copper, nickel	[17, 19, 38]
Pleurotus ostreatus, Lentinula edodes, Irpex lacteus, Mucor hiemalis	Laccases, manganese peroxidases, lignin peroxidase	Drugs, such as, naproxen, ciprofloxacin, norfloxacin ketoprofen, acetaminophen, piroxicam, testosterone and 17α-ethyl-estradiol	[6, 26. 27, 39, 40, 41]
Trametes versicolor	Laccases	Drugs such as, diclofenac, trimethoprim, carbamazepine, and sulfamethoxazole	[24]
Trametes hirsute	Laccases	Antibiotic chloramphenicol	[25]
Aspergillus tamarii, Botryosphaeria laricina, Pleurotus ostreatus, Trametes pavonia, Penicillium verruculosum, Aspergillus glaucus, Trametes maxima	Laccase, hydrolase, protease, cellulase, manganese peroxidase	Insecticides, pesticides, and herbicides, such as, Endosulfan, aldrin, dieldrin, DDT, fipronil, atrazine	[11, 30, 32, 42, 43]
Aspergillus niger, A. fumigatus, Streptoverticillium sp., *Rhizopus* sp., *Geotricum candidum, Cladosporium cladosporioides*	Protease, amylase	Anionic surfactants, household detergents	[44, 45]

1.2.1 Bioremediation using immobilized enzyme

For using stabilizing enzymes, immobilization is one of the most efficient approaches. It limits the mobility of the enzymes with a simultaneous preservation of their viability and catalytic

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functions. Three of the most common method of immobilization are adsorption, entrapment, and cross-linking or covalently binding to a support. Immobilization technique of enzymes enhances the functional and thermal stability, pH tolerance, and do not alter the structural stability of the enzyme. The effective immobilization of enzyme depends on the correct choice of carrier and suitable immobilization technique. Researchers are also developing new strategy of immobilization of enzyme using NPs so that the enzyme can be easily recover and can be recycled.

Fungal enzymes are frequently immobilized on a variety of conventional and nanoscale substrates to increase their stability and catalytic capacity. The extracellular hydrolytic and ligninolytic enzymes from various fungal species are widely used in biotechnological processes. Though, the application of these fungal based enzymes as free enzymes is very limited because of lack of reusability and their instability. Therefore, for stabilizing these enzymes, immobilization is desired. Several reports are published on the various techniques of immobilization and the application of immobilized fungal enzymes for bioremediation of aquatic ecosystem and wastewater as well. Immobilization of laccase, derived from *Pycnoporus sanguineus*, with calcium and chitosan beads was investigated for treatment of wastewater [46]. The immobilized enzyme was studied for the removal of estrogen drug, 17a-ethinylestradiol. Though, the best result was obtained for immobilization with calcium beads without the addition of buffer. Similarly, immobilized laccase from *T. versicolor* and *Myceliophthora thermophila* with polymeric IB-EC1 beads, polyacrylic and carboxylic acids, and ceramic membranes was studied for removal of estrogen drugs in real wastewater [47]. The estrogenicity was removed about 91% with immobilized enzyme. Immobilized enzyme from *Fusarium* sp. was also reported to degrade an organophosphate insecticide, chlorpyrifos [48]. The free enzyme was embedded with polyvinyl alcohol and sodium alginate. The results showed that the degradation of chlorpyrifos was attempted with a degradation rate of 86.7% after 60 minutes. Furthermore, immobilized lignin peroxidase from *Ganoderma lucidum* IBL-05 has been used for the bioremediation of dye-based textile effluents [49]. Immobilization of fungal enzymes was done on non-ionic surfactant-modified clay and was studied for degradation of PAHs such as, naphthalene and phenanthrene [50]. The structural and molecular interactions of the non-ionic surfactant with the clay and the fungal enzyme explained the physical adsorption of non-ionic surfactants onto clay, which is a crucial step in PAH breakdown by fungal laccase. For *in situ* PAHs bioremediation, a system where laccase is immobilized upon TX-100-monomer-modified clay is a good potential bioactive material. Additionally, the catalytic potential of magnetically-separable cross-linked enzyme aggregates (MCLEAs) was studied using laccase enzyme from *T. versicolor* [51]. MCLEAs was used to convert drugs like, acetaminophen and numerous non-phenolic pharmaceutical active compounds, including mefenamic acid, fenofibrate, and indomethacin from biologically treated wastewater effluent with equivalent or even better efficiency than free laccase. Magnetic mesoporous silica microbeads were used to produce MCLEAs. The best result showed in MCLEAs with enzyme load of about 1.53 U laccase/mg MCLEAs. Though, the stability of MCLEAs depends on the pH, the presence of chemical denaturants, and wastewater matrix. Immobilized laccase from *Aspergillus oryzae* was also studied for degradation of micropollutants from sewage and wastewater [52]. Granular activated carbon (GAC) was used for the immobilization technique. The immobilized laccase demonstrated high residual activity over a wide pH and temperature range as compared to the free enzyme. The micropollutants, including sulfamethoxazole, carbamazepine, diclofenac, and bisphenol A, were efficiently eliminated by the GAC-bound laccase. Results revealed that due to improved electron transport between laccase and substrate molecules adhered onto the GAC surface, immobilized laccase was able to

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degrade micropollutants more effectively than free laccase. Similarly, laccase from *T. versicolor* was used for immobilization with nano biochar [53]. The impact of oxidizing nano biochar, a carbonaceous product of biomass pyrolysis, with hydrochloric acid, sulphuric acid, nitric acid, and their combinations on the immobilization of laccase was studied. An anticonvulsant drug, carbamazepine was degraded using the immobilized laccase, and the clearance rates in spiking water and secondary effluents were 83% and 86%, respectively. Also, laccase from *T. versicolor* and *Myceliophthora thermophila* was immobilized onto fumed silica NPs [54]. Immobilized enzymes showed greater long-term stability than free laccases when incubated in a secondary effluent from a municipal wastewater treatment plant. The application of about 8000 U/L of co-immobilized laccase resulted in an almost full elimination of ^{14}C-bisphenol A (BPA) from secondary effluents. Both the microbiological source and the condition of the biocatalyst had a significant impact on the catalytic efficiency. Furthermore, laccase from *Echinodontium taxodii* was also immobilized on concanavalin A-activated Fe_3O_4 NPs [55]. The immobilized enzyme was applied for removal of sulfonamide antibiotics. Along with having stronger thermal and operational stabilities than free laccase, orientated immobilized laccase showed a higher affinity for substrates. The findings revealed that laccase facilitated the transition of sulfonamide antibiotics and S-type compounds, resulting in cross-coupled formation. For the elimination of various micropollutants, such as the plasticizer bisphenol A (BPA), an anti-inflammatory drug diclofenac, and the steroidal hormone 17-ethinylestradiol, laccases from white rot fungi were shown to be attractive. In a two-step adsorption-crosslinking procedure, laccase from *Coriolopsis gallica* was immobilized on mesoporous silica spheres [56]. The biocatalyst could degrade more than 85% of BPA and 17-ethinylestradiol together with 30% of diclofenac when tested in a combination for more than 80 hours in wastewater condition (pH 7.8), which illustrates the potential of biocatalyst for the treatment of aquatic micropollutants. A list of immobilized fungal enzymes and their potential in removing the industrial effluents and wastewater contaminants is given in Table 3.

Table 3. Bioremediation potential of immobilized fungal enzymes

Fungi	Enzyme	Immobilization method	Pollutant	References
Pycnoporus sanguineus	Laccase	Immobilized with Calcium and chitosan beads	Estrogen drug, 17a-ethinylestradiol	[46]
Trametes versicolor and *Myceliophthora thermophila*	Laccase	Polymeric IB-EC1 beads, polyacrylic and carboxylic acids	Hormones and endocrine disrupting compounds	[47]
Fusarium sp.	Free enzymes mixture	Immobilized with sodium alginate	Organophosphate insecticide such as, chlorpyrifos	[48]
Ganoderma lucidum IBL-05	Lignin peroxidase	Immobilized with calcium and sodium alginate beads	Sandal reactive dyes	[49]
Trametes versicolor	Laccase	Immobilized on non-ionic surfactants such as, TX-100 (octylphenol polyethoxylate) and Brij 35 (polyethoxylate lauryl ether)	PAHs such as, naphthalene and phenanthrene	[50]
Trametes versicolor	Laccase	Cross-linked enzyme aggregates	Drugs such as, mefenamic acid, fenofibrate, and indomethacin	[51]
Aspergillus oryzae	Laccase	Immobilized on granular activated	Micropollutants, such as, sulfamethoxazole,	[52]

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		carbon	carbamazepine, diclofenac and BPA	
Trametes versicolor	Laccase	Immobilized on nanobiochar	Anticonvulsant drug, Carbamazepine	[53]
Myceliophthora thermophila, Trametes versicolor	Laccase	Immobilized on fumed silica NPs	Micropollutant, BPA	[54]
Echinodontium taxodii	Laccase	Immobilized on concanavalin A-activated Fe3O4 NPs	Antibiotic, sulfamethazine	[55]
Coriolopsis gallica	Laccase	Immobilized on mesoporous silica spheres	Micropollutants such as, plasticizer BPA, anti-inflammatory drug diclofenac, and steroidal hormone 17-α-ethinylestradiol	[56]

1.3. Application of Fungal nanoparticles for Bioremediation of wastewater

Aside from the techniques mentioned above, a new technology can be used to remediate contaminants and trace elements i.e., Nanotechnology. Through the process of biomimetic mineralization and the use of reducing enzymes either intracellularly or extracellularly, fungi are able to manufacture metal NPs. Fungal cultures have many advantages over other biological organisms, such as strong biomass generation and the lack of additional procedures needed to collect the filtrate. The fungal mycelial mass is resistant to agitation and pressure also, which makes it more appropriate for large-scale production of NPs. Additionally, it is feasible to manage the metabolism of fungus to produce NPs with the required properties, such as a particular size and form, by modifying culture variables including time, temperature, pH, and amount of biomass, etc. Mycosynthesis of various NPs, such as, silver, gold, iron oxide, selenium, magnetite, etc. has been reported by various researchers.

1.3.1. Biosynthesis of Nanoparticles from fungi

Fungal cell walls and cytoplasm contain enzymes that convert metal ions into NPs. Fungi are more productive and more tolerant of metals as it has high cell wall binding capability of metal ions with biomass [57]. By using either the internal or extracellular synthesis pathways, fungal cells can be employed for the manufacture of NPs. If the NPs are formed intracellularly, they are created and localised in the cytoplasm, cell membrane, or cell wall. When metal ions contact with oppositely charged cell surface moieties, both of them are simultaneously reduced and can either diffuse into the cytoplasm or cell membrane or remain bonded to the cell surface. In order to survive, the fungal mycelium when exposed to metal ions, they produce enzymes and metabolites. During this process, metal ions undergo catalytic reduction to produce non-toxic forms of solid NPs.

Several macrofungal species including, *Ganoderma* sp., *Pleurotus* sp., *Oudemansiella* sp., *Trametes* sp., *Agaricus* sp., *Coprinopsis* sp., *Cyathus* sp., etc., and microfungal species including, *Aspergillus* sp., *Mucor* sp., *Trichoderma* sp., *Sporotrichum* sp., *Rhizopus* sp., *Thermoascus* sp., *Penicillium* sp., etc., were reported to be a promising source of metal NPs such as, silver, zinc oxide, gold, titanium, zirconium, magnetite, copper etc. Some thermophilic filamentous fungal strains were investigated for the synthesis of gold NPs using autolysate, extracellular fraction, and the cell free intracellular extract of the fungi [58]. The synthesis of copper and gold NPs was reported using *Penicillum aurantiogriseum*, *P. waksmanii*, and *P. citrinum* [59]. Furthermore, *Rhizopus solonifer* was studied for synthesis of silver NPs using phytochelatin and purified NADPH-dependent nitrate reductase [60]. The extracellular synthesis of CuNPs was studied using *Trichoderma koningiopsis* [61]. Synthesis of FeNPs was investigated from *Fusarium oxyporum* for nano-bioremediation of municipal wastewater [62]. Similarly, green synthesis of AuNPs was produced from *Rhizopus oryzae* to investigate the adsorption capacities of NPs towards various organophosphorous pesticides [63]. Furthermore, *Lentinus edodes* was used to synthesize AgNPs for its water disinfectant properties [64]. Synthesis of AgNPs was also studied from *Penciillium Citreonigum Dierck* and *Scopulaiopsos brumptii Salvanet-Duval* for remediation of water [65]. *Penicillium pimiteouiense* was isolated from Indian Sundarbans to study the synthesis of iron oxide NPs (IO-NPs) for the treatment of heavy metals from wastewater [66]. Similarly, superparamagnetic IO-NPs was also synthesized from mangrove fungus *Aspergillus niger* BSC-1 to detect the removal capacity of heavy metal from wastewater [57]. A list of metallic NPs produced from various fungi has been shown in Table 4.

Generally, under similar experimental parameters, a variety of fungal species can be used to make diverse NPs with different shape and sizes. The condensation of biomolecules, the incubation environment, the precursor, etc. can all contribute to variances in the NPs that are formed. Consequently, NPs were created by modifying key growth variables such as metal ion concentration, solution pH, and reaction time. Despite the fact that stable NPs can be produced by selecting suitable fungal species and optimizing circumstances, the mechanism behind biologically synthesised NPs is not well understood.

Table 4. Biosynthesis of metallic nanoparticles using fungi

Fungi	Nanoparticles	Size (nm)	References
Aspergillus niger BSC-1	Iron oxide	20-40	[57]
Rhizomucor pusillus, Sporotrichum thermophile, Thermoascus thermophilus, Thermomyces lanuginosus	Gold	6-12	[58]
Penicillium aurantiogriseum, *P. citrinum, P.* waksmanii	Gold	153.3, 172, 160.1 respectively	[59]
Rhizopus solonifer	Silver	10-25	[60]
Trichoderma koningiopsis	Copper	87.5	[61]
Fusarium oxyporum	Iron	0.7-3	[62]
Rhizopus oryzae	Gold	10	[63]
Lentinus edodes	Silver	50-100	[64]
Penciillium Citreonigum Dierck and *Scopulaiopsos brumptii Salvanet-Duval*	Silver	6-26 and 4.24-23.2	[65]
Penicillium pimiteouiense	Iron oxide	2-16	[66]
Pycnoporus sanguineus and *Schizophyllum commune*	Silver	52.8-70.2 and 53.9-103.3	[67]
Stereum hirsutum	Copper	5-20	[68]
Phaenerochaete chrysosporium	Selenium	35-400	[69]
Aspergillus niger	Silver	5-30	[70]
Aspergillus fumigatus	Silver	15-45	[71]
Aspergillus flavus	Titanium dioxide	62-74	[72]
Aspergillus oryzae	Silver	10-24.6	[73]
Aspergillus sydowii	Silver	1-24	[74]
Trichoderma longibrachiatum	Silver	10	[75]
Aspergillus foetidus	Silver	35	[76]
	Iron	26.78	[77]

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Fusarium oxysporum			

1.3.2. Role of Fungal Nanoparticles for Bioremediation of wastewater

Many researchers have investigated the application of fungal NPs in bioremediation of wastewater and other aquatic ecosystems. For instance, silicon dioxide NPs have been studied with *Cladosporium sphaerospermum* and *Fusarium oxysporum* for bioremediation of wastewater contaminated with cadmium [1]. The silicon NPs of *Cladosporium sphaerospermum* and *Fusarium oxysporum* almost removed 65.73% and 54.30% of cadmium, respectively. Arsenic contamination of water causes serious health problems such as hyperpigmentation, skin cancer, cardiovascular disease, central nervous system deformities, gangrene of limbs, etc. The biofabricated AgNPs from the biomass of *Aspergillus foetidus* MTCC8876 was studied for diminution of arsenic from contaminated water [76]. The biofabricated AgNPs adhered to the carbonized fungal cell was efficient to remove more than 93% of arsenic from the aqueous environment within 3.5 hours because of its porosity. The interaction of AgNPs to the mesoporous carbonized cell of the fungal strain proved to be an exceptional arsenic removal performance along with simple and eco-friendly biosynthesis process. The extracellular biosynthesis of CuNPs from dead biomass of *Trichoderma koningiopsis* was studied for bioremediation of wastewater [61]. The dead biomass of *T. koningiopsis* was selected instead of live biomass because of its higher capacity to adsorb copper ions. Synthesis of IO-NPs from manglicolous fungi, *Penicillium pimiteouiense*, isolated from Indian Sundarbans, was studied for removal of chromium from synthetic waste water [66]. The mycosynthesized IO-NPs removed chromium from waste water through chemisorption in nature, with an adsorption capacity of 4.623 mg/g. Similarly, synthesis of superparamagnetic IO-NPs was fabricated using *Aspergillus niger* BSC-1 to study the removal capacity of hexavalent chromium from aqueous solution [57]. The results showed that the detoxification of chromium required both adsorption and a redox reaction, according to X-ray photoelectron spectroscopy. The mycosynthesized IO-NPs could therefore be used to remove heavy metals from contaminated wastewater. Also, magnetite NPs were synthesized from *Fusarium oxysporum* for remediation of wastewater [77]. By using a magnetic adsorption procedure, these magnetite NPs were employed to remove the arsenic from the water. The removal effectiveness was determined to be 96%. Furthermore, culture filtrate of *Fusarium solani* YMM20 was used for synthesis of chitosan NPs [78]. The synthesized NPs were used as an adsorbent for the degradation of heavy metals such as, copper, lead, cobalt, nickel, cadmium, separately. For the majority of metals, the fungal culture filtrate method offered the maximum metal removal capacity. The mycosynthesized NPs showed highest removal capacity for lead and lowest for cobalt.

Municipal wastewaters mainly contain two main hazardous substances, heavy metals and pathogenic microorganisms. The mycogenic synthesis of FeNPs from *Fusarium oxysporum* was investigated for its water remediation and antibacterial properties [62]. The mycosynthesized FeNPs was used as an antibacterial agent against various environmental pathogens with concentration about 20 µg/ml. FeNPs were also capable of treating such effluent to almost 95 % for lead and 20–50 % for other heavy metals such as cadmium, zinc, chromium, nickel. The antibacterial effect of AgNPs from *Fusarium oxysporum* was studied against *Staphylococcus aureus* in textile industry effluent [79]. Similarly, biosynthetic AgNPs from *Lentinus edodes* was studied for its potential application in remediation of water [64]. The mycosynthesized AgNPs were used as an effective antimicrobial agent, anti-fouling agent. Similarly, AgNPs from two filamentous fungi, *Penciillium Citreonigum Dierck* and *Scopulaniopsos brumptii Salvanet-Duval* was examined for the removal of pathogenic bacteria from contaminated water [65].

Phenol and phenolic compounds are also being released in the aquatic systems because of industrial effluents. *Trametes* sp. was studied for the removal of phenol from aqueous solution over various concentrations [80]. Though, the uptake capacity of phenol depends on various parameters, such as, pH, size of the particle, biosorbent dosage, etc. The optimum particle size for the maximum removal of phenol was reported to be 150-300 µm at pH 6.0. The green synthesis of gold NPs from *Trichoderma viride* was studied as a heterogeneous catalyst which degraded the para-nitro phenol into amino phenol [81]. Researchers are also working on the use of high-tech nanotechnology to increase the potability of water. The unique characteristics of AuNPs to obtain potable water was studied using *Rhizopus oryzae* [63]. Furthermore, the pesticide adsorption capacity and antibacterial property of bioconjugate AuNPs was also investigated for different organophosphorus pesticides (such as, malathion, parathion, dimethoate, and chlorpyrifos) to facilitate its easy removal from contaminated water. Results revealed that the pesticide adsorption increased with increased concentration of gold chloride. Primary dyes, including rhodamine B, methylene blue, and malachite green are also detected in the wastewater. The combination of Ultraviolet light and titanium dioxide NPs with polyvinylidene difluoride (PVDF) membrane, degrade the dye rhodamine B up to 95% [82]. The bioremediation potential of various fungal NPs is given in Table 5.

Table 5. Bioremediation potential of Fungal NPs

Fungi	Nanoparticles	Wastewater pollutant	References
Cladosporium sphaerospermum and *Fusarium oxysporum*	Silicon dioxide	Cadmium	[1]
Aspergillus niger BSC-1	Iron oxide	Chromium	[57]
Trichoderma koningiopsis	Copper	Copper	[61]
Fusarium oxysporum	Iron	Cadmium, zinc, chromium, nickel	[62]
Rhizopus oryzae	Gold	Organophosphorous pesticides (malathion, parathion, dimethoate, and chlorpyrifos)	[63]
Lentinus edodes	Silver	Antimicrobial agent, anti-fouling agent	[64]
Penciillium sp. and *Scopulaniopsos brumptii*	Silver	Antibacterial activity	[65]
Penicillium pimiteouiense	Iron oxide	Chromium	[66]
Aspergillus foetidus	Silver	Arsenic	[76]
Fusarium oxyporum	Iron	Arsenic	[77]
Fusarium solani YMM20	Chitosan	Heavy metals such as, copper, lead, cobalt, nickel, cadmium	[78]
Fusarium oxysporum	Silver	Antibacterial agent	[79]
Trichoderma viride	Gold	Para-nitro phenol	[81]

CONCLUSION

Over the years, many researches have looked into the effectiveness of various methods for removing different types of contaminants from wastewater and other aquatic ecosystems. For remediation of contaminants in wastewaters, treatment systems based on biological organisms, particularly fungi and their associated hydrolytic and ligninolytic enzyme systems present a

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promising and sustainable option. Fungi are viable candidates for bioremediation at many sites due to their diverse habitats and capacity to secrete a wide range of enzymes. The whole-cell fungi, crude or purified enzymes, and mycosynthesized NPs may effectively eliminate a wide range of contaminants from wastewater. Recent improvements in fungal enzyme production and their immobilization techniques offers practical and long-term solutions for ameliorating the environmental and commercial outcomes. Immobilized techniques of fungal enzymes, including, laccases, tyrosinases, manganese peroxidases, cellulase, amylase, etc.) are capable of reduction or degradation of several hazardous compounds such as, phenolic derivatives, pesticides, estrogen, dyes, bisphenols, etc. from aquatic ecosystem. Although, immobilization can improve stability and facilitate reusability, the majority of immobilization techniques have substantial disadvantages, such being extremely expensive, losing enzyme activity, and having issues with regeneration. Therefore, further research is still required for optimization of immobilized technique of fungal enzymes and their application as a biocatalysts for the removal of various pollutants.

The biogenic synthesis of NPs from biological sources have promising potential in the field of nanobioremediation. The "green" synthesis is quickly replacing the traditional chemical syntheses of NPs due to its eco-friendliness, economic viability, feasibility, and wide variety of applications in various sectors. Biological NPs have a favourable impact on adsorption capacity because of their larger surface area. Fungal NPs have gained much interest in the field of bioremediation because of their flexibility, tolerance, and metal accumulation capabilities. In the field of wastewater treatment, NPs derived from fungi are used to remediate dyes, heavy metals, microbiological pollutants, pharmaceutical drugs, plastics, pesticides, detergents, etc. The importance of fungal NPs is due to its cost effectiveness, nano-size, non-hazardous, crystalline structure, environment friendly nature. It can be concluded that biological resources if utilized successfully and efficiently, can be a solution for different environmental adversities. To develop a dependable and effective approach for the degradation of wastewater pollutants, more research is needed to assess the technical, economic, and environmental aspects of various process combinations.

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Consent for publication:

I hereby provide consent for the publication of the above manuscript, including accompanying data within the manuscript. I declare that this manuscript has not been published previously in any journals.

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