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# Fungi as important agents in water bioremediation

Currently, human life expectancy has increased considerably compared to that before the 19th century, however, the large world population and the enormous industrial and technological development have caused a serious problem of environmental pollution. The presence of large quantities of xenobiotic and recalcitrant compounds in the air, soil and water produce serious damage to the ecosystems and cause various diseases in humans such as neuronal damage, gastro-intestinal problems, partial paralysis, blindness and certain cancers of the skin, lung, liver, kidney and prostate, etc. (Mustafa and Najmaldin, 2024). For this reason, intense efforts have been made by the researchers worldwide to remediate the environment. Various physical, chemical and biological techniques have been developed for cleaning the environment and widely researched.

Biological technique commonly termed as 'Bioremediation' involves the use of plants, animals and microorganisms that serve as bio-accumulators and effectively aid in the bioremediation of polluted environment. In bioremediation processes, algae, bacteria and fungi are mainly used, taking advantage of their metabolic capacities or enzymes produced by these organisms under controlled conditions, where in both cases the degradation or elimination of contaminants is sought to avoid the toxic or harmful effects on living beings(Bibbins-Martínez *et al*., 2023).

Hydrosphere forms the major realm of the planet earth. Despite occupying 70% of the earth, only 2% is fresh water, and 0.036% is available since the rest is found in the glaciers at the poles and in inaccessible places such as subterranean rivers. Water pollution is a serious problem worldwide that needs urgent attention as well as effective solution. Anthropogenic activities are one of the major causes of environmental pollution. Regular and uncontrolled discharge of toxic compounds like pesticides, detergents, heavy metals, phenolic compounds, hydrocarbons, textile and food dyes, pharmaceutical and cosmetic wastes, etc., are contaminating the aquatic ecosystems.

Fungi are efficient bio-remediators as well as bio-accumulators of toxic compounds present in the water bodies. Their remediation mechanisms involve biosorption, bioaccumulation and biodegradation (Legorreta-Castañeda *et al*., 2020; Bibbins-Martínez *et al*., 2023). Biosorption by fungi (whether alive or dead) is a process where contaminants have an affinity for one of the components of the cell wall such as glucans, chitins and mannans and functional groups such as phosphate, carboxyl, amino and thiol; the affinity can be physical and/or chemical, ion exchange, complexation, chelation and microprecipitation (Sadhasivam *et al*., 2009; Bibbins-Martínez *et al*., 2023). Bioaccumulation is based on active metabolic transport where contaminating compounds pass through the membrane into the cell, accumulating and, in the best of cases, gets metabolized (Volesky, 2007; Bibbins-Martínez *et al*., 2023). Biodegradation occurs mainly by enzymatic pathways, either by extracellular enzymes or by those involved in the fungal metabolism (Bibbins-Martínez *et al*., 2023). It is important to mention that fungi have been classified based on their capacity to degrade wood, like soft-rot fungi (degradation of starches, pectins, etc), brown-rot fungi (degrading even cellulose and hemicellulose) and white-rot fungi, which are capable of degrading cellulose, hemicellulose and lignin, since in addition producing a wide range of carbohydrases they also produce oxidases such as laccases and various peroxidases, which are among the most important enzymes due to their potential application in bioremediation processes.

In view of the above and concerned about contributing to the solution of water bioremediation, I and my team have been working for last two decades in search of fungal strains, in particular *Pleurotus* genus that belong to the group of white-rot fungi and produces laccase enzymes, as well as establishing the cultivation conditions to increase their production. Laccases are enzymes that have been categorized in the group of multicopper blue oxidases, they are glycosylated and use oxygen as the final electron acceptor forming water. They eliminate hydrogen from the hydroxyl of methoxy-substituted monophenols, ortho- and para-diphenols, but they can also oxidize aromatic amines and non-phenolic compounds forming free radicals. These enzymes have a potential use in

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various bioremediation processes that include the elimination or removal of phenolic compounds and decolorization of dyes present in the industrial wastewater. They are used in several processes like bleaching of paper pulp, delignification, wine clarification, etc (Diaz-Godinez, 2011). Initially, we evaluated the laccase activity in ten strains of *Pleurotus* genus using three substrates (2,6-dimethoxyphenol, p-anisidine and o-tolidine), finding that the *Pleurotus ostreatus* ATCC 32783 strain was the one that presented the highest laccase activity both at intracellular and extracellular level (Tellez-Tellez et al., 2005). All the subsequent studies performed with this strain, cultured in both solid-state fermentation and submerged fermentation showed differences in their activity levels and number of isoenzymes depending on the culture system (Tlecuitl-Beristain et al., 2008; Téllez-Téllez et al., 2008; Díaz-Godínez *et al.,* 2017). Studies on the effect of the pH of the culture medium on the activity of laccases have shown different patterns of production of laccase isoenzymes, with maximum activity at pH 4.5 (Díaz *et al*., 2011, 2013). In another study, the best culture temperature of the fungus for laccase production was observed at 25 $^{0}$C (Montalvo *et al*., 2020).

The presence of dyes in wastewater from textile industries represents one of the main sources of environmental pollution, so it is urgent to find solutions to this serious problem. In this sense, fungi have the potential capacity to discolor and degrade this type of compounds (Martinez-Berra *et al*., 2018). Based on the knowledge about the production of laccases by the ATCC 32783 strain, we separately evaluated the decolorization of textile dye, Remazol Brilliant Blue R and the degradation of the phenolic compound 2,6-dimethoxyphenol, on the submerged fermentation medium of *Pleurotus ostreatus*. At the same time, the activities of laccases, manganese peroxidase and veratryl alcohol oxidase, which are enzymes of the ligninolytic system of the fungus, were evaluated. It was observed that both the dye and the phenolic compound were eliminated from the culture medium due to the enzymatic action of *Pleurotus ostreatus*, which are promising results for application in bioremediation processes of water contaminated with these types of compounds (Grandes-Blanco *et al*., 2013). Subsequently, in an another study, Remazol Brilliant Blue R and Acetyl Yellow G dyes were added to the submerged fermentation medium of *Pleurotus ostreatus*, in order to determine their decolorization potential and capacity to induce laccase isoenzymes; their showed increased enzymatic activity, with Remazol Brilliant Blue R being the one to increase the laccase activity and the number of isoenzymes, concluding that the response of laccase production by *Pleurotus ostreatus* is different depending on the dye evaluated (Garrido-Bazán *et al.*, 2016).

In search of other fungi capable of decolorizing textile dyes, we isolated six strains from a textile industry effluent. There were three strains of *Emmia latemarginata* producing laccases and three strains of *Mucor circinelloides* producing tyrosinases; all the strains produced versatile peroxidase, manganese peroxidase and lignin peroxidase. The ability of the strains to decolorize the dyes Indigo, Remazol Yellow 145, Remazol Red 3B, Remazol Brilliant Blue R, Disperse Black 1, Disperse Yellow 3, Disperse Red 19 and Acetyl Yellow G was evaluated in Petri dishes with agar. All strains were able to decolorize all dyes at different levels. In submerged fermentation, two Remazol dyes (red and yellow) were less susceptible to decolorization, but total decolorization of the dyes Indigo and Remazol Brilliant Blue R was achieved by a strain of *Emmia latemargina* (Juárez-Hernández *et al.*, 2021).

To illustrate decolorization of textile dyes by fungi in the laboratory, Fig. 1 shows the four main stages of bioprocess using a basidiomycete. The first stage involves selection of the fungal strain based on its habitat, metabolic capacity and enzymatic activity, followed by isolation, identification and physiological and biochemical characterization thereafter decolorization studies are carried out looking for the conditions of maximum decolorization.

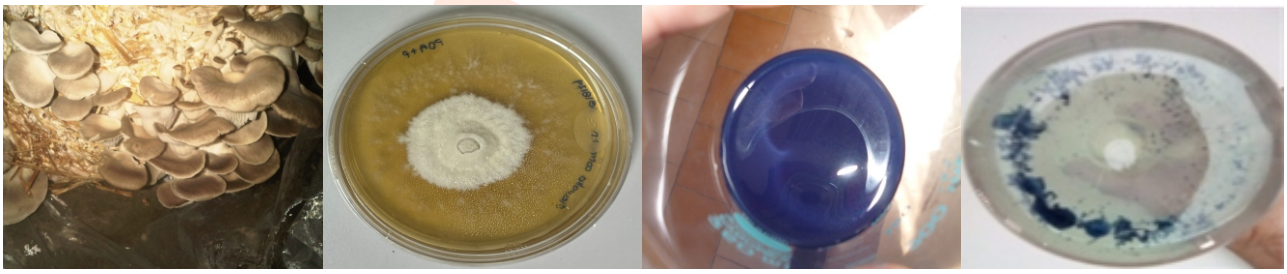

**Fig. 1:** General stages of the study of textile dye discoloration by a basidiomycete at laboratory level. (a) Fruiting body of the fungus, (b) isolated mycelium, (c) submerged fermentation system with textile dye, (d) discoloration of textile dye. Photographs by (a) González-Palma I., (b) Díaz-Godínez G. and (c and d) Martínez-Berra C.

The use of fungi and their enzymes in bioremediation process is a viable alternative, but despite the results obtained so far, there are still many challenges, including the scaling and design of processes that industries can adopt so that their discharges have less

pollutants and consequently, the harmful effect on the environment is reduced for the benefit of ecosystems and humans. The great interest and effort of the scientific community seeking techniques for the recovery of the environment must be accompanied by mindful actions and fruitful activities, that is why it is emphasized that a healthy planet will not be one that is cleaned more or where bioremediation is more efficient, but one where it is less polluted, so society from different sectors must become aware and act to emit less pollutants.

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