



Fungal Decolorization Of Dyes

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ABSTRACT

Dyes released by the textile industries pose a threat to the environmental safety. Recently, dye decolourization through biological means has gained momentum as these are cheap and can be applied to wide range of dyes. This review paper focuses on the decolourization of dye wastewaters through fungi via two processes (biosorption and bioaccumulation) and discusses the effect of various process parameters like pH, temperature, dye concentration etc. on the dye removing efficiency of different fungi. Various enzymes involved in the degradation of the dyes and the metabolites thus formed have been compiled. Genetic manipulations of microorganisms for production of more efficient biological agents, various bioreactor configurations and the application of purified enzymes for decolourization, which constitute some of the recent advances in this field, have also been reviewed. The studies discussed in this paper indicate fungal decolourization has a great potential to be developed further as a decentralized wastewater treatment technology for small textile or dyeing units. However, further research work is required to study the toxicity of the metabolites of dye degradation and the possible fate of the utilized biomass in order to ensure the development of an eco-friendly technology.

Keywords: fungi, decolorization, dyes, biosorption, enzymes, bioreactor, treatment

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I. INTRODUCTION

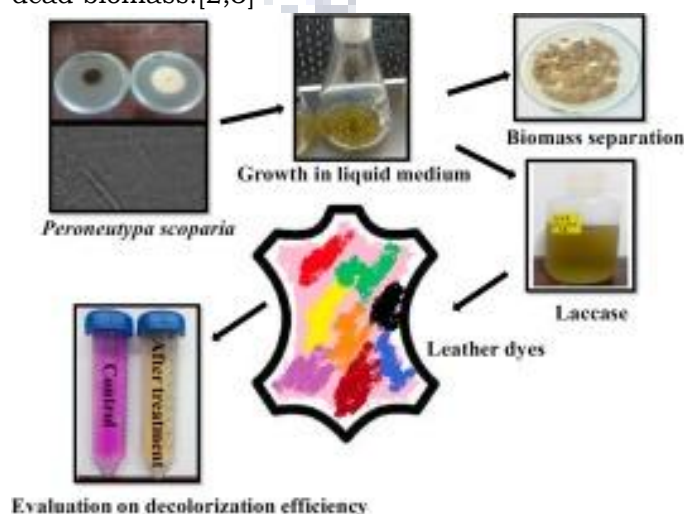
Decolorization of synthetic dyes was performed using cultures of white-rot fungi producing ligninolytic enzymes and radical-generating reactions that could be involved in the mechanism of fungal decolorization. Among the white-rot fungi tested, *Pleurotus ostreatus* exhibited the highest decolorization rates, and also the highest production of laccase and Mn-peroxidase. *P. ostreatus* strain f6 gave 69% decolorization of Eosin Yellowish, 96% of Evans Blue, 75% of Phenol Red (all at 1 mM) and 88% of Poly B-411 (20 ppm) during a 14-day treatment. Treatment with Cu/succinic acid/H₂O₂ resulted in 96% decolorization of Evans Blue and Poly B-411 within 24 h. However, only 48% and 2% decolorization was achieved with Phenol Red and Eosin Yellowish, respectively. Similar decolorization rates were also obtained when Cu was replaced with Co. The results show that treatment of dye-containing

solutions with both fungal cultures and biomimetic catalytic reactions results in decolorization.[1]

Currently much attention is focused on fungal decolorization processes. Fungal biomass is used as a sorbent and/or producer of enzymes involved in biodegradation/biotransformation. The process of biosorption is rapid, efficient and adaptable to diverse types of textile effluents. The results of different experiments emphasize that fungal processes are mostly associated with biotransformation but not biosorption. Biosorption is observed mostly for non-ligninolytic fungi, such as *Aspergillus niger*, of which (dead) biomass may be used as an adsorbent. Among these, the most widely researched are white rot fungi, such as *Phanerochaete*

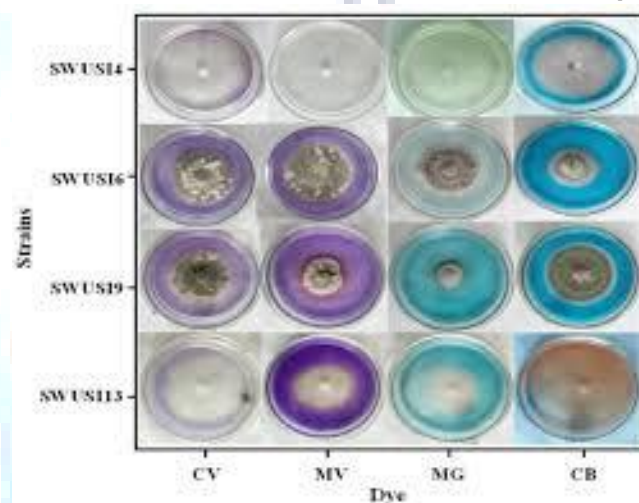
chrysosporium, *Bjerkandera* sp., *Trametes versicolor*, *Irpex lacteus*, and *Pleurotus ostreatus*, which produce enzymes, such as lignin peroxidase, manganese peroxidase and laccase. They are able to degrade many aromatic compounds due to their non-specific enzymatic activity. As described

previously, white-rot fungi are capable of decolorizing dyes significantly, and in most cases, this is due to the activities of lignin peroxidase (LiP) and Mn-dependent peroxidase (MnP). Some studies have demonstrated laccase (Lac)-mediated dye decolorization. It was also reported that non-ligninolytic enzymes may play a role in the decomposition of dyes, such as triphenylmethane crystal violet. Process conditions have a significant influence on biotransformation effectiveness. The best results of dye removal by fungi were obtained in more aerated shaken samples. Living biomass of tested strains removed dyes more effectively than dead biomass.[2,3]



Decolorization effectiveness depends mainly on the strain used in the process as well as on the specific structure of the dye and composition of the dye effluents. It was demonstrated that strains isolated from polluted sites had a greater decolorization potential than others. Additionally, the form and composition of the culture medium play an important role in decolorization processes. The most important factors are the sources and concentrations of carbon and nitrogen, which have a significant influence on production of ligninolytic enzymes. The influence of different carbon sources on decolorization effectiveness has been extensively studied. It should be mentioned that the effectiveness of dye removal depends also on the way biomass is used. Fungal free-cell treatment shows some drawbacks since the mycelium may be more exposed to environmental stresses. Therefore, a good alternative might involve the immobilization of biomass on different supports. Immobilization protects the biomass and improves fungal activity. It has been reported that immobilization of fungal cells may stably maintain the production of various enzymes at levels higher than those achieved with suspended or pellet forms. Moreover, the

immobilization of fungal biomass increases fungal resistance to environmental stresses, such as the presence of toxic molecules at high concentrations. Immobilization improves decolorization efficiency of biomass due to less dense fiber packing in comparison with the free fungal biomass. This is because the microorganism has a larger surface area available for dye adsorption. The increase in the surface area of fungal biomass tends to reduce the mass transfer limitations, which in turn increases access to pollutant degradation. Immobilization may allow the use of the system repeatedly, allowing easier liquid-solid separation and avoiding clogging phenomena. [4,5]



Decolorization process

Researchers demonstrated that white rot fungi pellets may be used for effective decolorization of textile dyes. It was also possible to induce dye decolorization activity of *Funalia troglia* by carefully selecting the optimal culture conditions. Pellets could be used several times and still maintain high decolorization activity. Using pellets would allow treatment of effluents with varying dye compositions and in high concentrations of dyes, which are normally toxic at low concentrations.⁵ The aim of the present study was to evaluate the influence of the solid support used for biomass immobilization on the decolorization efficacy. Different solid supports were used in the experiment to obtain intense growth of fungal biomass and to assist in the process of dye removal. After the environmental safeties of the solutions after the decolorization processes were assessed, the zoo- and phytotoxicity were evaluated.[6,7]

II. DISCUSSION

The textile dyes used in industry are formed by complex structures with high coloration and are often difficult to treat for decolorization. These dyes, present in high concentration, have a toxic

effect on the ecosystem and bioremediation methods using microorganisms cause less toxicity impact on the environment. In this work, four distinct *Aspergillus* spp. strains, capable of decolorizing dyes used in the textile industry, congo red and indigo carmine, diluted in Normal Decolorization Medium, were used. The screening of the best fungus was performed to evaluate the decolorization capacity in liquid medium. The best decolorization results were chosen to carry out the ecotoxicity assays and characterization of the biotreated dye parameters. The four strains evaluated showed a percentage of up to 96.10% of decolorization of the tested dyes. Phytotoxicity tests were carried out, which, in comparison with the negative control group, showed significant and promising results, with cucumber seed germination and root growth. The characterization of the dye demonstrated biological oxygen demand (BOD), chemical oxygen demand (COD), turbidity and pH according to established parameters. Therefore, the removal of dyes was achieved through decolorization carried out by fungal microorganisms, and this corresponded to a decrease in the phytotoxicity of congo red.[8,9]

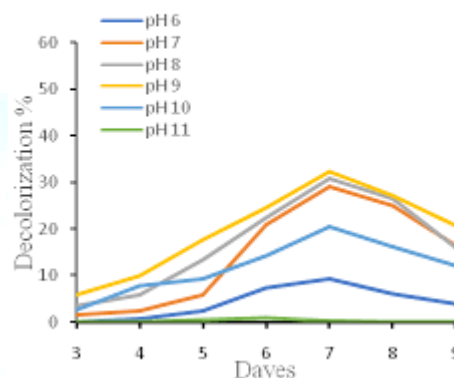
Locally isolated marine-derived fungal strains exhibited dye decolorization activities of synthetic dyes, specifically, crystal violet and Congo red, in solid and liquid culture media. Three marine-derived fungal strains, *Phialophora* sp. (MF 6), *Penicillium* sp. (MF 49), and *Cladosporium* sp. (EMF 14) showed complete decolorization of 0.01% Congo red and up to 91 % decolorization of 0.01% crystal violet. Aeration/agitation does not seem to be a factor for the dye decolorization activities of the test fungi. Enzyme production and/ or biosorption were inferred as possible mechanisms responsible for dye decolorization of these fungi.[10,11]

III. RESULTS

Bio-decolourization of lignin-containing pulp and paper wastewater using white rot fungi *P.chrysosporium* and *Tictoporia* sp. Due to high oxidative potential of many of the enzymes associated with white rot fungi, e.g. ligninase, laccase, Mn-peroxidase. Several other dye decolorizing fungal species have been reported, which include *Aspergillus niveus* & *Fusarium moniliforme*. It is thus not surprising those efforts to isolate from nature microorganisms utilizing azo dyes as carbon sources were unsuccessful. However, adaptation experiments in chemostats and carefully adjusted selective pressure led to

bacterial cultures which mineralised the carboxylated azo dyes. As for dye colour removal, review described the ability of *Rhodococcus*, *Bacillus cereus* and *Plasmimonas/Achromobacter* to degrade soluble dyes, acid red dye and five azo-dyes, respectively. On the other hand, textile dyes were found strongly adsorbed and held by wastewater treatment plant sludge that was land filled. This suggests that adsorption may play another key role in bio-decolourization.[12,13]

B) Reactive Red (4BL) dye



Decolorization at different pH

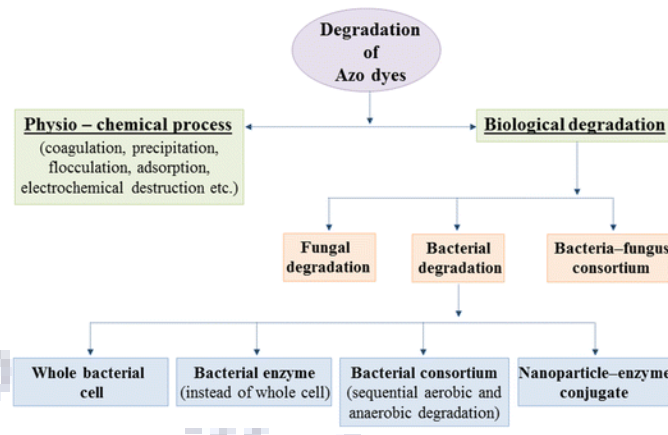
Fungi and bacteria, both are the principal degraders of organic matters, but fungi are better known for the purpose due to their superiority in the enzyme production. Traditionally, fungi have been classified as white-, brown-, or soft-rot fungi on the basis of technical decay and descriptions, regardless of their taxonomic position. Because the enzyme systems and metabolic pathways involved in the breakdown of carbohydrates and lignins probably are truly distinct in these fungi, rather than just modified in one or a few specific enzyme activities, decay type is of significant taxonomic importance. One important physiological characteristic of decay fungi in culture is the production of extracellular enzymes phenoloxidases and peroxidases. Certain fungi produce brown diffusion zones in agar plates when supplemented with 0.5%(w/v) Gallic or tannic acid, as a result of oxidation of the respective phenolic acid by extra- or intra-cellular phenoloxidases. It was suggested that the presence of phenoloxidases in white-rot fungi is directly correlated with the abilities of these fungi to the decomposition of lignin. Researchers were the first to extend this method to 210 species of wood decaying fungi. Of all tested white-rot fungi, 96% were positive on gallic acid agar, tannic acid agar, or on the both media. Scientists tested 173 wood-decaying species on the plates supplemented with 28 substances and found wide variations in

the reactions of given strains to specific phenolic compounds. The basidiomycetous fungus *Phanerochaete chrysosporium* has an unusual degradative capability and termed as “white rot fungus” because of its ability to degrade lignin, a randomly linked phenylpropane-based polymeric component of wood. The fungus possesses great potential for its commercial use in bioremediation of dyes and lignin-cellulosic materials present in textile effluents. The fungus has become a model example for its uses in mycoremediation and for its biotechnological application in biodegradation. Scientists screened 170 strains of white-, brown-, and soft-rot decay fungi and non-decaying xylophilophilous fungi for phenoloxidase activity with the polymeric dye Poly-478, to relate dye decolourization to know ligninolytic activity and the presence of phenoloxidase and peroxydases.[14,15]

IV. CONCLUSION

Scientific developments are considered as key factors for the progress of both developed and developing countries, but unfortunately, most of the industries do not have proper waste treatment facilities or arrangement, and releasing a large quantity of effluents.

A definitive solution for colourants problem of textile effluents would provide a marked advantage for the industrial sector. The success of a microbial process for colour removal from the effluent depends on the utilization of microorganisms that effectively decolourize synthetic dyes of different chemical structures. The fungal degradation/decolourization of textile dyes has been demonstrated mainly in the laboratory studies. The fungal utilization in dye decolourization is still under investigation to assess the information's on process implementation. The results obtained mainly from the laboratory tests depend on specific growth medium and optimization (addition of co-substrate, nutrients, mediator molecules, physical parameters optimization) and a good handling of fungal strains or biomasses. Therefore, essential works on the topic are still in the laboratories or less at commercial scale to solve the problem of colourants in effluents through mycoremediation.



The degradation/decolourization and mineralization of recalcitrant dyes and organo-chlorinated compounds are effective by certain fungi. With regard to fungi, which contribute to effluents degradation either by dead mycelia or with enzymes, there is need to design them for the purpose. Commonly dyes removal treatments do not adequately eliminate the azo dyes from the effluent waters of textile mills and dyestuff industries. In order to develop suitable technology to degrade/decolourize synthetic dyes discharged in the effluent and to convert them into beneficial products simultaneously, a well-planned scientifically acceptable technology is needed. The waste fungal biomass from fermentation industry is also a good absorbent that used as bio-sorption for biological effluent treatment in dyes and textile sectors. Enzymatic processes are particularly sought for the treatment of dye-containing effluents, mainly because of their specificity and relatively ease of engineering towards improved robustness; enzymes only “attack” the dye molecules, while valuable dyeing additives or fibers are kept intact and can potentially be re-used. Likewise, new recycling technologies will allow a huge reduction in water consumption in the textile finishing industry. One of the most important factors, which have a great impact on the setting of a proper bioremediation plant for textile wastewater, is the effluent characteristics. Majority of the researches concerning the fungi for mycoremediation of textile wastewaters have been focused the potentiality of fungi in dyes degradation/decolourization .[16,17]

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