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# Dye Decolorization using Fungal Laccase

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**ABSTRACT**
*The increasing use of synthetic dyes is alarming and their discharge as textile waste may cause substantial ecological damage. Biological decolorization of dye using microorganisms is an environmentally friendly and cost- competitive alternative to chemical methods. This study involves the application of laccase obtained from Pleurotus ostreatus, a common fungus, in decolorization and degradation of blue HFRL dye. This azo dye is a major constituent present in most of the textile mill effluents. The fungal cultures were inoculated and laccase was produced by solid state fermentation. The obtained enzyme was further used for dye decolorization on solid medium, liquid broth based medium and by using centre composite design. The results suggest the potential dye decolorization capacity of this fungal laccase.*
***Key words:*** *Laccase, Pleurotus ostreatus, Blue HFRL dye, Solid state fermentation, Centre composite design, Dye decolorization*

## INTRODUCTION

Bioremediation is gaining its significance in utilizing the biological activity of microorganisms to degrade toxic chemicals in the environment [1]. The textile industries utilize large volumes of water in their processing operations and generate substantial quantities of wastewater [2]. The presence of dyes even in small amounts affects the quality and properties of water, in particular contributes the major fraction of biochemical oxygen demand. To remove dye from waste water, the physical and chemical methods like adsorption, chemical precipitation, flocculation, photolysis, chemical oxidation and reduction, electro-chemical treatment and ion-pair extraction are extensively used [3-4]. Biotechnological methods adopting fungi and their enzymes in the dye degradation has been well appreciated globally, because of their potential use in detoxification and degradation of dyes [5-8].Biological methods of treatment combined with physical or chemical methods or both for color removal are immensely useful and cost effective.

*Pleurotus ostreatus* is a common edible mushroom. It was first cultivated in Germany as a subsistence measure during the Great War [9] and is now grown commercially around the world for food. Oyster mushrooms can also be used industrially for mycoremediation purposes. The Oyster mushroom may be considered a medicinal mushroom since it contains statins such as lovastatin which is used to reduce cholesterol [10].

Laccases are widely distributed in higher plants and fungi [11] and have also been found in insects and bacteria [12]. They belong to the group of phenol oxidases. These copper containing enzymes are oxidative enzymes detected in many plants and secreted by numerous fungi [13]. Laccases are distributed in Ascomycetes, Deuteromycetes, and Basidiomycetes, being particularly abundant in many white rot fungi that are involved in lignin metabolism [14]. Owing to the higher redox potential of +800mV of fungal laccases compared to plants or bacterial laccases they are implicated in several biotechnological applications especially in the degradation of lignin [15]. Laccase based decolorization treatments are potentially advantageous to bioremediation technologies since the enzyme is produced in larger amounts.

The present study determines the ability of *Pleurotus ostreatus* in the decolorization of the reactive dye from textile mill effluents, using its extracellular enzyme system involving laccase enzyme.

## MATERIALS AND METHODS

### Sample collection:

The azo dye blue HFRL was obtained from the local dye manufacturing unit, Coimbatore, Tamilnadu, India.

### Organism and Inoculum:

The fungal culture *Pleurotus ostreatus* was obtained from the Department of Plant Biology and Plant Biotechnology, Meenakshi college, Chennai and maintained in Potato Dextrose Agar medium (PDA). Mycelial disc (6 mm) from 5-day-old culture of the fungus was grown on PDA plates and was used as inoculum for the experiments. The experiments were conducted in 2011 in the Department of Biochemistry, Meenakshi College.

**Laccase Enzyme production by solid-state fermentation** [16]**:**

Lignocellulosic substrate namely rice bran was moistened (70 %) with Arulmani media [17] and autoclaved. 5-day-old mycelium disc (6 mm) was inoculated and incubated at 30°C. Sterile water was added at every week's interval to maintain the moisture content up to 16$^{th}$ day. The content was extracted with 120 ml sodium acetate buffer (pH 5.8, 0.2 M) and kept overnight at 4°C.The filtrate was centrifuged at 5000rpm for 20 minutes. The supernatant was collected, stored and used as enzyme source of laccase.

**Dye decolorization on solid medium** [18]**:**

The Czapek's dox agar medium was prepared with HFRL dye with six different concentrations such as 50ppm, 100ppm, 150ppm, 200ppm, 250ppm, and 300ppm and autoclaved. Then the medium was sterilized and plated. The 5 days old fungal culture (6mm) was inoculated in it and incubated at 30$^0$ C for 5 days. The dye plates were analyzed for decolorization.

**Dye decolorization in liquid culture** [19]**:**

The Czapek's` dox basal medium (100 ml) was prepared; 50 ppm dye was added to the medium and sterilized. Mycelial discs 6mm from 5-day-old fungal culture were transferred to the medium and incubated at room temperature (30±2°C). The culture was allowed to grow for 5 days. A sterile control, without mycelial discs was maintained and duplicates of cultures were also maintained under same conditions. Culture filtrates were harvested at every 24 h interval and monitored for decolorization for 3 days with varying pH (4-8) of sodium acetate buffer. The culture filtrate was centrifuged at 5000 rpm for 10 minutes and the supernatant was taken for the measurement of decolorization in the spectrophotometer.

**Dye decolorization by direct method by using Response surface methodology (RSM):** Centre composite design **(**CCD) was used to observe the percentage of dye decolorization by varying various factors like pH of sodium acetate buffer, dye concentration; incubation time for 17 runs, and the added enzyme volume was 2 ml. The readings were taken using spectrophotometer and the dye decolorization percentage was calculated as:

Dye decolorization percentage= (Initial absorbance - final absorbance/ initial absorbance) x 100

## RESULTS AND DISCUSSION

The largest group of synthetic colorants is the azo group of dyes which constitute almost a half of all known synthetic dyes. Azo dyes and their pigments are extremely versatile colorants and most of them are released into the environment during dyeing process.

The release of colored compounds from synthetic dyes into the environment may not only affect photosynthesis in aquatic plants, but also seem to be and produce products which are toxic or mutagenic to living organisms [20]. Biological decolorization and degradation is an eco-friendly alternative to chemical decomposition and can be performed at reasonable rates. Several microorganisms, such as bacteria, yeast, and fungi have been investigated for their ability to biodecolorize dyes [21]. Biochemical studies revealed that the enzymes like laccase, peroxidase, and lignin peroxidase from fungi are involved in the decolorization of dyes [22].

In the current study, the enzyme laccase was harvested from the fungal culture, *Pleurotus ostreatus* by solid state fermentation. Out of various dye concentrations used, maximum dye decolorization on solid medium was observed on Czapek`s dox medium agar plates with dye concentration of 100ppm (Figure 1). Dye decolorization percentage when observed in Czapek's Dox broth medium (Figure 2), among the 5 different pH, pH 5 had shown the highest percentage of dye decolorization on all 3 days (Table 1, Figure 3). Dye decolorization percentage was optimized through Centre Composite Design using the Response surface methodology. In the 17 runs, high degree of dye degradation (97.67%) was obtained at pH 5 of sodium acetate buffer with 75ppm of dye concentration for 48 hours of incubation (Table 2, Figure 4).

**Table 1. Dye decolorization in liquid culture**

| pH | On 1st day Dye decolorization % | On 2nd day Dye decolorization % | On 3rd day Dye decolorization % |
|---|---|---|---|
| 4 | 24.34 | 79.22 | 83.41 |
| 5 | 71.04 | 79.64 | 81.99 |
| 6 | -39.10 | -12.81 | 1.83 |
| 7 | -72.57 | -72.89 | -39.91 |
| 8 | -13.65 | -86.67 | -63.43 |

**Table 2. Dye decolorization by Centre composite design**

| CCD Runs | Factor 1: Incubation hours | Factor 2: Dye concentration ppm | Factor 3: pH | Dye decolorization percentage |
|---|---|---|---|---|
| 1 | 24 | 50 | 5 | 94.98 |
| 2 | 72 | 50 | 5 | 96.61 |
| 3 | 24 | 100 | 5 | 90.73 |
| 4 | 72 | 100 | 5 | 91.53 |
| 5 | 24 | 75 | 4.5 | 91.43 |
| 6 | 72 | 75 | 4.5 | 88.67 |
| 7 | 24 | 75 | 5.5 | 91.90 |
| 8 | 72 | 75 | 5.5 | 96.59 |
| 9 | 48 | 50 | 4.5 | 88.44 |
| 10 | 48 | 100 | 4.5 | 77.55 |
| 11 | 48 | 50 | 5.5 | 88.01 |
| 12 | 48 | 100 | 5.5 | 91.50 |
| 13 | 48 | 75 | 5 | 97.24 |
| 14 | 48 | 75 | 5 | 97.67 |
| 15 | 48 | 75 | 5 | 96.59 |
| 6 | 48 | 75 | 5 | 96.25 |
| 17 | 48 | 75 | 5 | 96.97 |

**1**

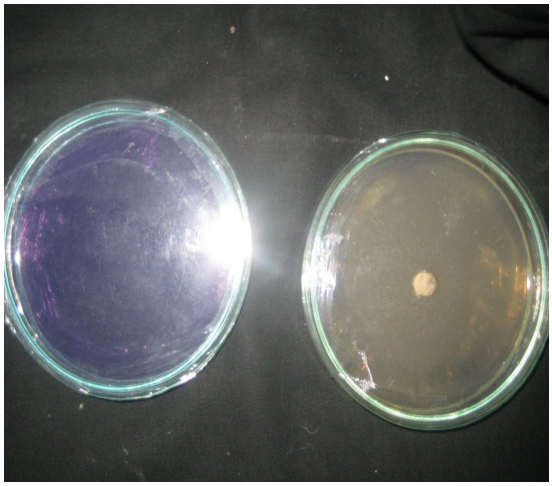

**Fig 1.** Dye decolorization on solid medium

**2**

**Fig 2.** Dye decolorization in liquid medium

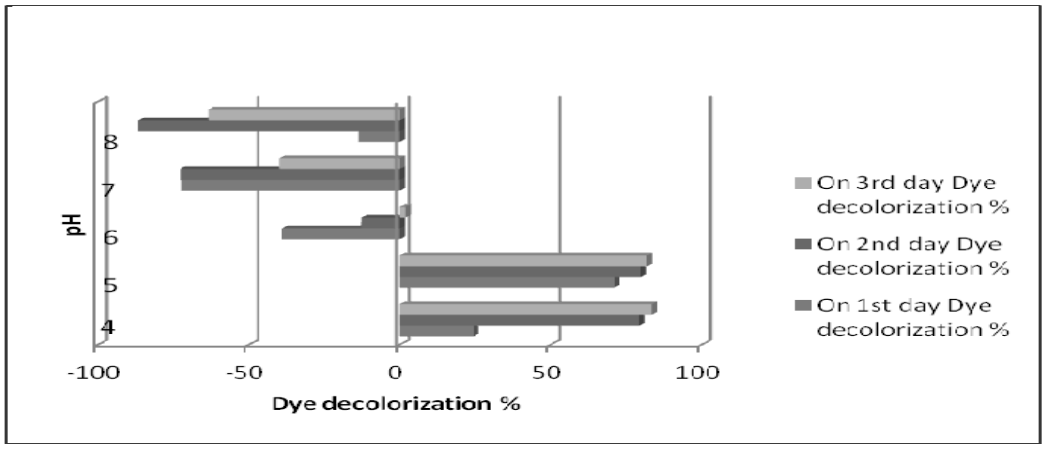


**Fig 3.** Statistical chart: Dye decolorization in liquid medium at different pH

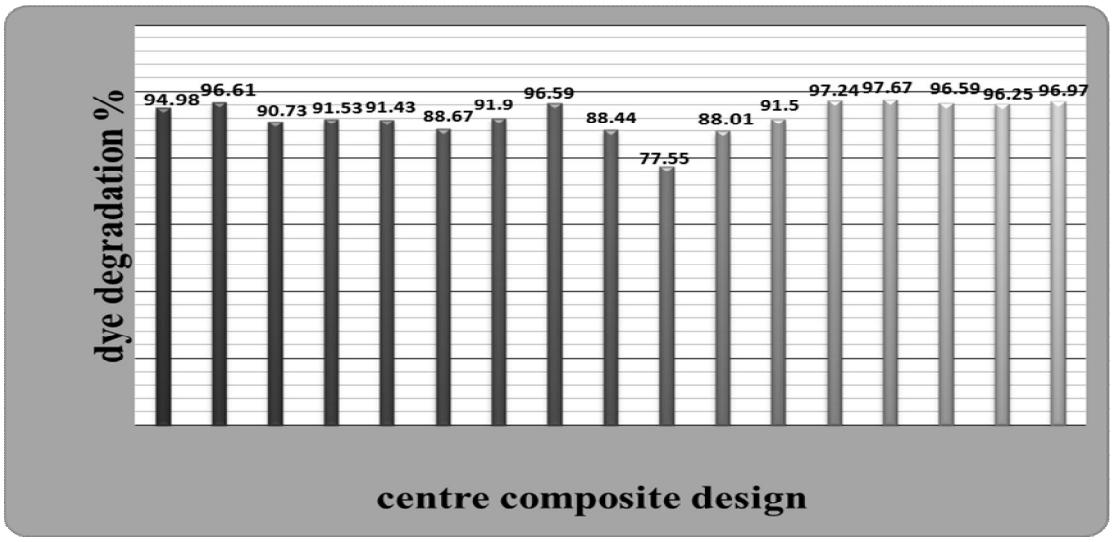


**Fig 4**. Statistical chart: Dye decolorization by Centre composite design

To improve the environmental and industrial safety and to ensure eco-friendly production, enzymes are being used in textile processing. These enzymes have the potential to react with their respective substrates and with synthetic and xenobiotic compounds as well [23-24]. Such reactions can transform a compound from a recalcitrant state to one that is more biodegradable [24]. Extracellular enzymes have also increased the degradation rate of biodegradable substances, such as activated sludge, permitting more efficient treatment processes [25].

Laccase is generally produced during the secondary metabolism of fungi. It catalyzes the oxidation of both phenolic and non-phenolic compounds and thus can act on a wide range of synthetic dyes. This non-specific mechanism of laccase makes it a versatile biocatalyst suitable for several applications such as industrial wastewater treatment, bioleaching and biopulping [26].

This study indicated the application of laccase from the fungal culture of *Pleurotus ostreatus,* in the decolorization of azo dye blue HFRL, a major constituent of textile effluents and facilitates further insight into dye decolorization using fungal enzymes.

## CONCLUSION

Dye discharge from textile industries poses a serious threat to the water resources. Many different and complicated molecular structures of dyes make dye wastewater difficult to be treated by conventional

methods. Therefore, innovative treatment technologies need to be investigated. This study suggests laccase enzyme from the fungus *Pleurotus ostreatus* possesses a significant dye degradation capacity and further can be applied in bioremediation of toxic industrial dyes.

## REFERENCE


1. King, R.B., Long, G.M. & Sheldon, J.K. (1998). Practical Environmental Bioremediation, Field Guide, Boca Raton, Lewis Publishers.
2. Hutton, D.G. (1972). Improved biological wastewater treatment. Dupoint Innovation, 2:6-26.
3. Ansari, A.A. & Thakur, B.D. (2006). Biochemical reactor for treatment of concentrated textile effluent, Colourage, 2:27-31.
4. Zhang, F., Yediler, A., Liang, X. & Kettrup, A. (2002). Ozonation of the purified hydrolyzed azo dye reactive red 120 – CI. Journal of Environmental Science and Health. Part A Toxic/ Hazardous Substances and Environmental Engineering, 37:707 - 780.
5. Ferreira, V.S., Magalhaes, D.B., KIing, S.H., De Silva, J.G. & Bon, E.P. (2000). N-Demethylation of methylene blue by lignin peroxidase from *Phanerochaete chrysosporium*. Stoichiometric relation for H2O2 consumption. Applied Biochemistry and Biotechnology, 84: 255-265.
6. Mielgo, L., Moreira, M.T., Feijoo, G. & Lema, J.M. (2001). A packed bed fungal bioreactor for the continuous decolorization of azo dyes (Orange II). Journal of Biotechnology, 89:99-106.
7. Claus, H., Faber, G. & Konig, H. (2002). Redox mediated decolorization of synthetic dyes by fungal laccases. Applied Microbial Biotechnology, 59:672-678.
8. Assadi, M.M., Maryam, M., Taher, S.N., Noohi, A., Shahamat, M. & Levin, M. (2003). Biosorption of Baftkar textile effluent. Indian Journal of Experimental Biology, 41:900-905
9. Eger, G., Eden, G. & Wissig, E. (1976). *Pleurotus ostreatus* — breeding potential of a new cultivated mushroom. Theoretical and Applied Genetics, 47:155-163.
10. Gunde-Cimerman, N. & Cimerman, A. (1995). *Pleurotus* fruiting bodies contain the inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme a reductase-lovastatin. Exp Mycol, 19(1):1-6.
11. Messerschmidt, A. & Huber, R. (1990). The blue oxidases, ascorbate oxidase, laccase and ceruloplasmin Modelling and structural relationships. European Journal of Biochemistry, 187(2):341-352.
12. Kunamneni, A., Ballesteros, A., Plou, F.J. & Alcalde, M. (2007). Fungal laccase - a versatile enzyme for biotechnological applications. Communicating Current Research and Educational Topics and Trends in Applied Microbiology, A. Mendez-Vilas (Ed.), 233-245.
13. Gianfreda, L., Xu, F. & Bollag, J.M. (1999). Laccases: a useful group of oxidoreductive enzymes. Bioremediation Journal, 3(1):1–25.
14. Bourbonnais, R., Paice, M.G., Reid, I.D., Lanthier, P. & Yagguchi, M. (1995). Lignin oxidation by laccase isoenzymes from *Trametes versicolor* and the role of the mediator 2,2'-Azinobis (3-Ethylbenzthiazoline-6-Sulfonate) in kraft lignin depolymerization. Applied Environmental Microbiology. 619(5):1876-1880.
15. Thurston, C.F. (1994). The structure and function of fungal Laccases. Microbiology, 140(1):19-26.
16. Hegde, S., Kavitha, S., Varadaraj, M.C., Muralikrishna, G. (2006). Degradation of cereal bran polysaccharide-phenolic acid complexes by *Aspergillus niger* CFR1105. Food Chem., 96:9-14.
17. Manavalan Arulmani, Kalaichelvan Aparanjini, Kalyanasundaram Vasanthi, Perumal Arumugam, Manavalan Arivuchelvi & Thangavelu Kalaichelvan, P. (2007). Purification and partial characterization of serine protease from thermostable alkalophilic *Bacillus laterosporus* -AK1. World journal of Microbiology and Biotechnology, 23:475-481
18. Kalaichelvan, P.T. (2003). Production of extracellular lignin peroxidase and laccase by *Ganoderma lucidum* PTK3 on sugarcane bagasse lignin. Indian J. Exp. Biol., 34:1121-1125.
19. Anna jarosz-wilkolazka, Janina Kochmanska-Rdest, Elzbieta malarczyk, Wladyslaw Wardas. (2002). Fungi and their ability to decolorize azo and anthraquinonic dyes. Enzyme and microbial technology, 30:566-572.
20. Weisburger, J.H. (2002). Comments on the history and importance of aromatic and heterocyclic amines in public health. Mutat Res 506-507:9–20.
21. Azmi, W., Sani, R.K., & Banerjee, U.C. (1998). Biodegradation of triphenylmethane dyes. Enzyme and Microbial Technology, 22:185–191.
22. Tekere, M., Zvauya, R., Read, J. (2001). Ligninolytic enzymes production in selected sub-tropical white rot fungi under different culture conditions. J. Basic Microbiol., 41:115-12.
23. Bollag, J.M. (1992). Decontaminating soil with enzymes: An in situ method using phenolic and anilinic compounds. *Environ. Sci. Technol.,* 26(10):1876-1881.
24. Gianfreda, L., & Rao, M.A. (2004). Potential of extra cellular enzymes in remediation: A review. Enzyme Microb Technol., 35:339-354.
25. Whiteley, C.G., Heron, P., Plelschke, B., Rose, P.D., Tshivhunge, S., Van Jaarsveld, F.P., Whinington-Jones, K. (2002). The enzymology of sludge solubilisation utilizing sulphate reducing systems: The properties of protease and phosphatases. Enzyme Microb. Technol., 31: 419-424.
26. Krishnaveni, M. (2011). Characterization and decolorization of dye and textile Effluent by laccase from *Pleurotus florida*- a white-rot fungi. International Journal of Pharma and Bio Sciences, 2:B117-123.