**Short Communication**

# Biosorption of textile dyes and effluents by *Pleurotus florida* and *Trametes hirsuta* with evaluation of their laccase activity

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***Abstract***

The rate and efficiency of decolorization of dyes like Blue CA, Black B133, and Corazol Violet SR were tested to evaluate white rot fungal strains. *Trametes hirsuta* and *Pleurotus florida* showed the greatest extent of decolorization on nutrient salt media. Maximum decolorization of 200 mg/l of Blue 133 was obtained by 4 days old incubated *Pleurotus florida* followed by *Trametes hirsuta* after 6 days. An attempt was made to improve the decolorization activity of both organisms with different concentrations of glucose 1 and 2% (w/v). The decolorization activity may be due to the laccase enzyme of white rot fungi. The production of this enzyme was estimated using solid state fermentation with rice bran as a substrate. It was found that *P. florida* exhibited 0.175 U/ml of laccase activity followed 0.126U/ml by *T. hirsute*, respectively. Decolourization was found to be more effective with *P. florida* in the presence of 2% (w/v) glucose. Crude extract containing the laccase enzyme was isolated and confirmed by SDS PAGE.

***Keywords*:** Dye house effluent; laccase; *Pleurotus florida;* reactive textile dyes; *Trametes hirsuta.*

Dyes are extensively used for several industrial applications and approximately 5% of them end up in effluents. Unfortunately, conventional wastewater treatments are ineffectual at removing dyes and involve high costs, formation of hazardous by-products and intensive energy requirements (Stolz, 2001). Approximately, one lakh commercial dyes are manufactured which include several varieties of dyes such as acidic, basic, reactive, azo, diazo and anthraquinone based meta complex dyes. Over 10,000 dyes with an annual production of over $7 \times 10^5$ metric tonnes are commercially available (Campos *et al*., 2001).

Fungal laccase as part of the ligninolytic enzyme system is produced by almost all wood and litter transforming Basidiomycetes. This group of N-glycosylated extracellular blue oxidases with molecular masses of 60-390 kDa (Call and Mücke, 1997) contain four copper atoms in the active site (as $Cu^{2+}$ in the resting enzyme) which are distributed among different binding sites (McGuirl and Dooley, 1999).

Laccases have been reported to oxidize many recalcitrant substances, such as chlorophenols (Fahr *et al*., 1999), and polycyclic aromatic hydrocarbons lignin-related structures (Bourbonnais *et al.,* 1996), organophosphorous compounds (Amitai *et al*., 1998), nonphenolic lignin model compounds (Majcherczyk *et al*., 1999), phenols, and aromatic dyes (Abadulla *et al*., 2000). Laccases are able to oxidize polyphenols, methoxy substituted polyphenols, diamines, and considerable range of other compounds. Besides reduction of environmental pollution, enzymatic decolorization of dyeing effluents has recently been shown to enable reuse of the treated water in the dyeing process (Abadulla *et al*., 2000).

The present study is to investigate the ability of *Trametes hirsuta* and *Pleurotus florida* to carry out biosorption of the reactive dyes at three different concentrations. The effect of glucose in improving the production of laccase enzyme system was also studied.

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Pure cultures of *T. hirsuta (*MTCC-136) and *P. florida* were obtained from the Microbial Type Culture Collection (MTCC), Chandigarh and University of Madras, India, respectively. *T. hirsuta* was maintained in Yeast Extract Agar Media (YEA) and *P. florida* was cultured in Potato Dextrose Agar media (PDA) and stored at 4°C. Laccases, which are extracellular secretion of white rot fungus, were able to oxidize different substrates such as guiacol, syringoldazine, and non-phenolic compounds. Oxidase enzyme system of *T. hirsuta* and *P. florida* was checked based on Trejo-Hernandez *et al*. (2001).

Three different concentrations of the dyes (Blue CA, Black B133, and Corazol Violet SR) at 25, 50, and 75 mg/l were prepared with 0.5% (w/v) glucose as a carbon source. The effluent was collected from the Thirupur dye house, Tamil nadu, India, which mostly uses the reactive dyes. Glucose (1 and 2% (w/v) was added to both the raw effluent (pH 11) and the pH adjusted effluent (pH 6) which were inoculated with *T. hirsuta* and *P. florida* and control was maintained. They were kept at room temperature and observed for decolorization. Kirk's nutrient salt medium was prepared and inoculated with 5 discs of *P. florida* for the determination of laccase activity. On the third day 5 µl of guiacol was added to 50 ml of medium and 0.05 g/100 µl of gallic acid was prepared and added to 50 ml of the medium. The same media was used for the decolorization of these three dyes at the concentration of 200 mg/l. The rate of dye decolorization was estimated by the following formula:

Rate of decolorization (%) =

$$100 - \left( \frac{\text{Absorbance of treated dye solution}}{\text{Absorbance of control dye solution}} \times 100 \right)$$

Solid state fermentation was carried out in 500 ml Erlenmeyer flasks with rice bran as substrate for laccase production. Extracellular enzyme was extracted from solid cultures, which was obtained by means of soaking rice bran substrate with mycelia in 10 mM sodium acetate buffer (pH 6.0) for an hour, followed by filtration using a 0.45 µ nylon membrane filter. The filtrate was dialyzed and subsequently concentrated in a lyophilizer. Extracellular laccase activity was assayed spectrophotometrically as described by Wolfenden and Wilson (1982) with 2, 2′- azino bis 3-ethyl-benzothiazoline-6-sulphonate ABTS as substrate. One unit of enzyme activity was defined as 1 µmol of ABTS oxidized per minute at 25ºC ($\varepsilon_{436}$= 29300 $M^{-1}CM^{-1}$). The protein concentration was estimated according to the method of Bradford (1976). SDS-PAGE [12% (w/v)] was performed according to the protocol of Laemmli (1970) and samples were treated with 1% SDS, β-mercaptorthanol and boiled for 5 min. Proteins were visualized by staining with silver nitrate. Laccase is mainly responsible for the decolorization of aromatic compounds and can oxidize substrates such as ABTS and guiacol. The dark reddish brown zones appeared on both the culture plates in the laccase assay.

Decolorization of Blue CA, Black B133, and Corazol violet SR in 0.5% (w/v) glucose medium was observed at 580, 590, and 530 nm, respectively using a Beckman DU-40 spectrophotometer, up to 10 days at an interval of two days. The visual decolorization was observed within 24 h at a concentration of 25 mg/l by both white rot fungi. Maximum decolorization was found to be 93.54% and 92.17% on the 10th day of incubation by *P. florida* and *T. hirsuta,* respectively (Figure 1). The decolorization rate for 50 mg/l of dye

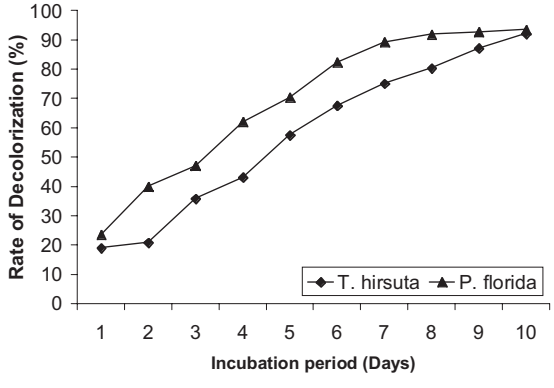


**Figure 1.** Aqueous state decolorization of Blue CA (25mg/l) by white rot fungi.

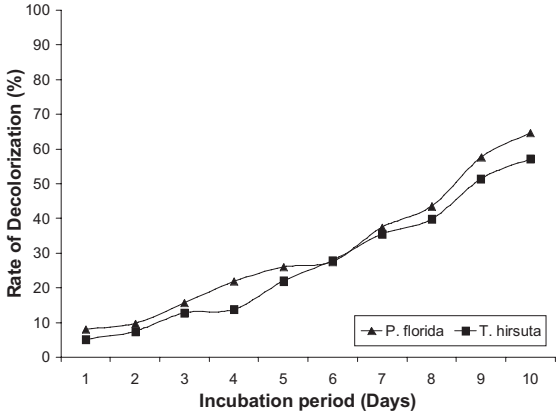


**Figure 2.** Aqueous state decolorization of Black 133 (25mg/l) by white rot fungi.

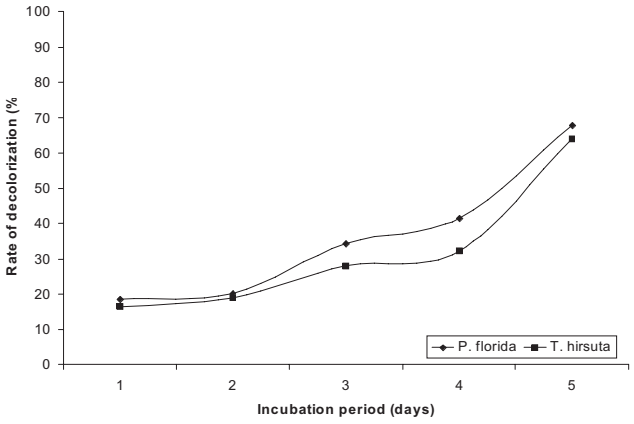


**Figure 3.** Decolorization of effluent (2% w/v) glucose concentration) by white rot fungi.

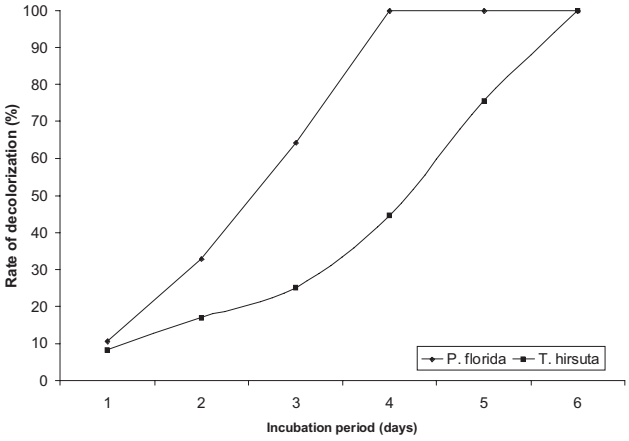


**Figure 4.** Decolorization of Blue CA (200 mg/l) by white rot fungi.

was found to be 61.27% by *P. florida and* 56.64% by *T. hirsuta* on the 10th day of incubation. At a concentration of 75 mg/l this decolorization was found to be 52.42% and 39.45% by *P. florida* and *T. hirsuta,* respectively.

Decolorization of Black B133 was lower than the other two dyes. At the given concentration of 25 mg/l, maximum decolorization was 64.67% by *P. florida* and 57.21% by *T. hirsuta* (Figure 2). However, at a concentration of 50 mg/ml the maximum decolorization was recorded as 33.94% and 29.97% by *P. florida* and *T. hirsuta,* respectively. Very low decolorization of 75 mg/l of Black B133 by both the organisms on the 10$^{th}$ day was found to be 28.57% by *P. florida* and 24.04% by *T. hirsuta*.

An effective decolorization of 25 mg/l corazol violet SR was observed as indicated by 83.70% in the presence of *P. florida* and 62.02% the presence of *T. hirsuta*. The decolorization activity was found to be 69.12% by *P. florida* and 62.13% by *T. hirsuta* when 50 mg/l of corazol violet SR was used. At the 75 mg/l concentration, the maximum decolorization rate was 58.04% and 43.48% by *P. florida* and *T. hirsuta,* respectively. However, the extent of color removal is not consistent with all the dyes and it depends upon laccase activation by the dyes. This may be due to the structural and chemical composition of dyes. Similar observations regarding dye degradation by the white rot fungus *P. chrysosporium* has been observed by Cripps *et al*. (1990).

The rate of effluent decolorization was observed at 560 nm, on a daily basis up to five days of the incubation period. The pH adjusted (pH 6) effluent was decolorized by *P. florida* and *T. hirsuta* by up to 45.62% and 42.07%, respectively. In the presence of 1% (w/v) glucose, the decolorization efficiency was increased up to 56.86% and 52.57% by *P. florida* and *T. hirsuta,* respectively. By the addition of 2% (w/v) of glucose, the highest decolorization efficiency was found as 67.86% and 64.1% by *P. florida* and *T. hirsuta,* respectively (Figure 3).

The mycelial growth of *P. florida* started from the first day of inoculation in nutrient salt medium. On third day, inducer guiacol and gallic acid were added and the resulting mixture color change after 24 h indicates that laccase present in the medium. The results of time course studies regarding decolorization of Blue CA are shown in Figure 4. The *P. florida* culture produced approximately 100% decolorization after 4 days of incubation and complete color removal was observed after 6 days of incubation by *T. hirsuta*. In comparison to the other two dyes, on the 6$^{th}$ day of incubation, poor decolorization of Black 133 was observed with 90% by *P. florida* and 70% in the presence of *T. hirsuta* (Figure 5). In case of Corazol violet SR, *P. florida* showed 95% decolorization and *T. hirsuta* exhibited only 90% decolorization during 6 days of incubation (Figure 6).

The maximum amount of protein was observed (from *P. florida* and *T. hirsuta* amended Blue CA culture filtrate) to be 110 µg/l and 85 µg/l, respectively. The laccase enzyme plate assay showed the presence of laccase in the culture filtrate (Figure 7). The laccase enzyme activity of *P. florida* and *T. hirsuta* culture filtrate were obtained as 0.175 U/ml and 0.126 U/ml, respectively.

The molecular profile of the enzyme laccase is shown in Figure 8. Presence of the intense band with

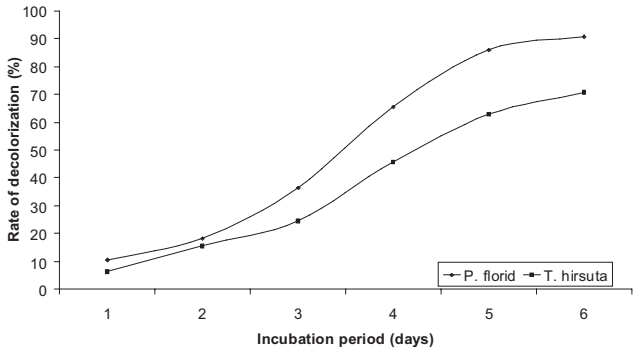


**Figure 5.** Decolorization of Black 133 (200 mg/l) by white rot fungi.

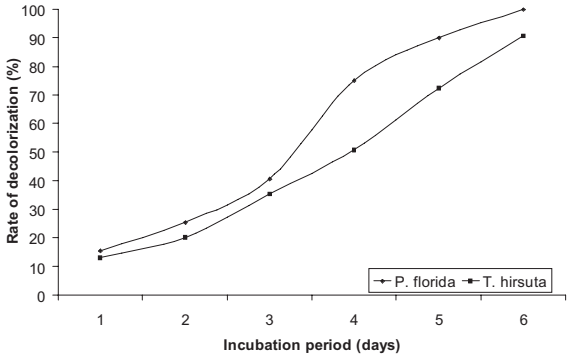


**Figure 6.** Decolorization of Corazol violet SR (200 mg/l) by white rot fungi.

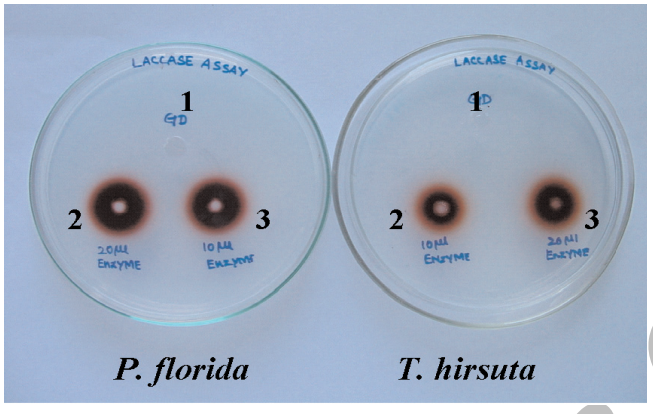


**Figure 7.** Dark brown color indicates laccase activity of culture filtrate (well-1 Glass distilled water; well-2 20 µl of crude enzyme and well-3 30 µl crude enzyme).

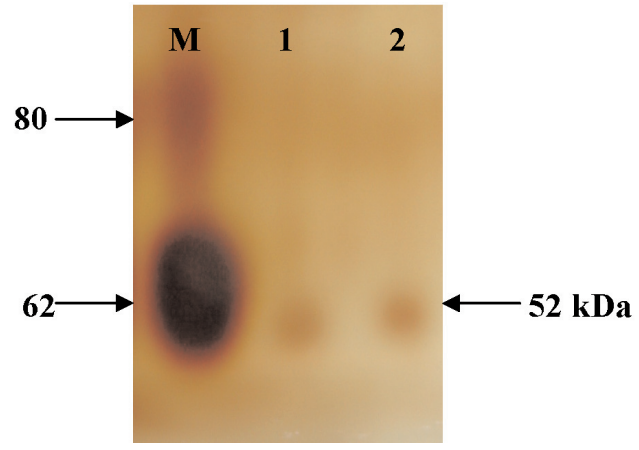


**Figure 8.** SDS-PAGE-Molecular profile of laccase enzyme. M-Standard marker; Lane-1 laccase of *P. florida*; Lane-2 laccase of *T. hirsuta*.

reference to 62 kDa of standard protein marker suggests the molecular weight of the partially purified laccase. In conclusion, *P. florida* was found to be more effective than *T. hirsuta* in decolorizing the different reactive dyes like CA, Black B133, Corazol Violet SR, and the dye house effluent. Addition of glucose as a carbon source increased the dye decolorization efficiency. Furthermore, both the test organisms produced the laccase enzyme in the media which was confirmed by suitable assays. Therefore, this biological system may be applied to treatment of dye house effluents in order to avoid environmental pollution.

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