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Full Length Research Paper

Decolorization of textile dyes and their effluents using white rot fungi

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Reactive dyes are important chemical pollutants from textile industries .The two species of white rot fungi were evaluated for their ability to decolorize Blue CA, Black B133, Corazol Violet SR. *Trametes hirsuta* and *Pleurotus florida* displayed the greatest extent of decolorization. Laccase is the ligneolytic enzyme from these fungi. The laccase activity was measured using both solid and aqueous state assays. The dye absorption ability of the mycelium was studied using appropriate medium containing dyes at the concentration of 75 mg/l. The effective decolorization of Blue CA and Corazol Violet SR dyes by both microorganisms were observed in the fifth day of incubation. Further decolorization activity was verified using various concentrations of dyes such as 25, 50 and 75 mg/l. Maximum decolorization was observed in Blue CA and Corazol Violet SR dyes. The effluent from the dye house was treated using both organisms with different concentration of glucose (1 and 2%). Effective decolourization was found to be more by the *Pleurotus florida* in 2% glucose.

Key words: Reactive dyes, dye house effluent, *Pleurotus florida*, *Trametes hirsuta*, laccase.

INTRODUCTION

Due to rapid industrialization and urbanization, a lot of chemicals including dyes are manufactured and used in day-to-day life. Dyes are synthetic and aromatic molecular structural compounds. According to their dissociation in an aqueous solution, dyes can be classified as acid, direct reactive dyes (anionic), basic dyes (cationic) and disperse dyes (nonionic). They are used on several substrates in food, cosmetics, paper, plastic and textile industries. Solutions retain them by physical adsorption by making compounds with metals and salts using covalent bonds. Many chemical dyes have been used increasingly in textile and dyeing industries because of their ease and cost effectiveness in synthesis, firmness and variety in color compared to that of natural dyes.

About 100,000 commercial dyes are manufactured including several varieties of dyes such as acidic, basic, reactive, azo, diazo, anthraquinone based meta complex dyes. Over 10,000 dyes with an annual production of over 7 X 10^5 metric tons are commercially available (Campos et al., 2001). Approximately 50% of the dyes are released in the industrial effluents (Zollinger, 1991).

Colored industrial effluents from the dyeing industries represent major environmental problems. Unbound reactive dyes undergo hydrolysis due to temperature and pH values during the dyeing processes. The strong color of discharged dyes even at very small concentrations has a huge impact on the aquatic environment caused by its turbidity and high pollution strength; in addition toxic degradation products can be formed.

Dye wastewater discharged from textile and dyestuff industries have to be treated due to their impact on water bodies and growing public concern over their toxicity and carcinogenicity. Many different and complicated molecular structures of dyes make dye wastewater difficult to be treated by conventional biological and physico-chemical process. Therefore, innovative treatment technologies need to be investigated. Decolorization of dye wastewa-

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ter by fungal metabolic activities is the subject of many studies.

Fungi from the Basidiomycetes group, known as white rot fungi are a hetereogenous group of microorganisms but have in common the capacity to degrade lignin as well as other wood components (Kirk and Farrell, 1987). The white rot fungi are by far the most efficient liginiolytic microorganisms. They are able to degrade a wide variety of recalcitrant pollutants including various types of dyes. Most information on the biodegradation of synthetic dyes by ligninolytic fungi has been obtained with *Phanerochaete chyrsosporium* (Cripps et al., 1990; Paszczynski and Crawford 1995). White rot fungus showed some capacities to remove dyes from industrial effluents. The fungus has been studied for their ability to degrade recalcitrant organopollutants such as polyaromatic hydrocarbons, chlorophenols and polychlorinated biphenyl. The decolorization of phenol red, methylene blue, coomassive blue, dextran blue etc., has been used to indicate ligninolytic activity.

Laccase based decolorization treatments are potentially advantageous to bioremediation technologies since the enzyme is produced in larger amounts. Laccases belong to the group of phenoloxidases. These copper containing enzymes are oxidative enzymes detected in many plants and secreted by numerous fungi (Gian Freda et al., 1999; Rodriguez et al., 1999). In the present study is to determine the ability of *Trametes hirsuta* and *P. florida* in the decolorization of the reactive dyes using their extracellular enzyme system. The solid and aqueous state dye decolorization assay and identification of laccase enzyme were carried out.

MATERIALS AND METHODS

Culture

The white rot fungi *Trametes hirsuta* (MTCC-136) was procured from institute of Microbial Type Culture Collection, Chandigarh, India and *Pleurotus florida* from the University of Madras. *T. hirsuta* was maintained Yeast Extract Agar (YEA) media and *P. florida* was cultured in Potato Dextrose Agar (PDA) media and stored at 4˚C.

Medium composition

The growth medium of *T. hirsuta* consists of (g/l) glucose10 g, yeast extract 5 g, agar 15 g and pH 6 - 6.2. *P. florida* was cultured on the medium containing (g/l) potato extract (extracted from 200 g potato), dextrose 15 g, agar 15 g and pH 6 - 6.2.

Dyes

Reactive dyes are the dyes, which are mostly used in the textile industries. The following dyes were selected for use in this study; Blue CA, Black B133, Corazol Violet SR.

Solid substrate enzyme production

Laccases, which are extracellular secretion of white rot fungus, were able to oxidize different substrates such as guiacol, syringoldazine, and non-phenolic compounds. The oxidase enzyme system of *T. hirsuta* and *P. florida* was checked based on Trejo Hernandez et al. (2001). The laccase production media containing wheat bran flakes 4.5%, yeast extract 1.5%, glucose 1%, NH_4Cl 0.25%, thiamine dichloride 0.05%, KH_2PO_4 0.2%, $MgSO_4.7H_2O$ 0.05%, $CaCl_2$ 0.01%, KCl 0.05% and pH 5.0 was prepared and the guiacol (50 µg/100 ml) was added as a substrate for laccase screening. In this study, a disc of 6 day-old culture was placed at the centre of the plate. The plates were incubated at the dark place and observed for reddish brown zone in the medium which will be formed as a result of laccase oxidative polymerization with guiacol.

Solid state decolorization studies

Screening test was carried out based on the Cenek Novotny et al. (2001). The YEA media for *T. hirsuta* and PDA for *P. florida* was prepared. Dye solution of 75 mg/l concentration was prepared for all the dyes. After sterilization of media in sterile condition, 1 ml of dye solution for 50 ml of media was added using nylon syringe filter. For each plate, a disc of inoculum was placed at the center, and uninoculated medium was maintained as control. These plates were incubated at room temperature and observed for decolorization. The experiments were performed in duplicate for each culture.

Aqueous batch dye decolorization studies

When a particular concentration of sucrose or glucose provided the decolorization or color removal process by white rot fungus was enhanced (Srinivasan and Murthy, 2000). It was carried out after observing the decolorization efficiency of the dyes in the screening assay. The dyes such as Blue CA, Black B133, Corazol Violet SR were selected for this assay. Three different concentrations of the dyes 25, 50 and 75 mg/lwere prepared for all the three dyes.

Glucose 0.5% and 50 ml distilled water were added in saline bottles and sterilized. After sterilization in sterile condition dye solution was added in each bottles. For each concentration of a dye a control, *T. hirsuta* and *P. florida* inoculated bottles were maintained. Inoculum of 5 discs per bottle was kept and decolorization rate was observed by UV-Visible spectrophotometer (Beckman DU-40).

Aqueous batch effluent decolorization studies

The effluent treatment was carried out based on work done by Srinivasan and Murthy (2000).The effluent was collected from AAR PEE Colour House, Thirupur, India, which is mostly using the reactive dyes. The pH of the effluent was checked (pH 11) and 1 and 2% glucose was added, and for each a control, *T. hirsuta* and *P.florida* inoculated bottles were maintained. Likewise for the adjusted pH (pH 6), effluent was also taken and 1 and 2%glucose was added. These bottles were kept at room temperature and observed for decolorization.

Laccase confirmation using inducers

Laccase enzyme production was enhanced when inducers such as guiacol and gallic acid were added. Nutrient salt medium containing (g/l) glucose 10 g, $(NH_4)_2PO_4$ 0.2 g, KH_2PO_4 1.0 g, $MgSO_4.7H_2O$ 0.5 g, KCl 0.5 g, $FeSO_4.7H_2O$ 0.005 g, thiamine hydrochloride 0.001 g and pH adjusted to 6.0 was prepared and inoculated with 5 discs of *P. florida*. On the third day guiacol 5 µl/50 ml of media was added and gallic acid 0.05 g/100 µl was prepared and added in 50

ml of media. Each of the mediums along with the microorganism but without inducer was maintained as control and observed for color change in the medium.

Laccase enzyme activity assay

For this assay *P. florida* alone was taken. Nutrient salt media was prepared in 250 ml Erlenmeyer flasks. After sterilization the inoculums of 5 discs of *P. florida* was inoculated. After 3 days Blue CA, and Corazol Violet SR dye solution of 25 mg/l was prepared and added to the culture media using nylon syringe filter. The uninoculated medium with dye was considered as control.

Estimation of protein

The protein was estimated according to the method of Bradford (1976). The culture filtrate of Blue CA dye amended was centrifuged at 8000 rpm for 15 min, from which 0.1 ml of supernatant was collected and made up to 1ml with water. 5 ml of CBB G-250 was added, and incubated for 15-20 min and read at 595 nm in a Beckman DU-40 spectrophotometer. Reagent with water served as blank. The amount of protein was calculated using Bovine Serum Albumin (BSA) as standard.

Laccase enzyme assay

Extracellur laccase activity was assayed spectrophotometically as described by Wolfenden and Wilson (1982) with ABTS (2,2'-azino bis 3-ethyl-benzothiazoline-6-sulphonate) as substrate. The reaction mixture contain 1 mM ABTS in 0.1 ml of enzyme. The oxidation reaction was monitored by measuring the change in A_{436} for 3 min. One unit enzyme activity was defined as 1 μmol of ABTS oxidized per minute at 25°C. $E_{436}=29300M^{-1}CM^{-1}$. The activities were expressed in U/1.

RESULTS

White rot fungus *T. hirsuta* and *P. florida* were tested for their dye decolorizing ability. Their activity was tested against most common reactive dyes such as Blue CA, Black B133, and Corazol Violet SR of various concentrations. The various parameters such as solid-state decolorization, aqueous state decolorization, extra cellular protein, and laccase enzyme assay were analysed.

Screening of fungi for extracellular enzyme production

White rot fungi are capable of producing extracelluar enzymes such as lignin peroxidase, manganese peroxidase and laccase. Laccase is mainly responsible for the decolorization of aromatic compounds. It is able to oxidize substrates such as ABTS, guiacol. In this test dark reddish brown zones appeared on both the culture plates. In *P. florida* inoculated plate, the appearance of dark reddish brown color takes place within 24 h and the complete color change was observed in fourth day. In *T. hirsuta* inoculated plate, the color change starts at the first day but active change starts only from the third day.

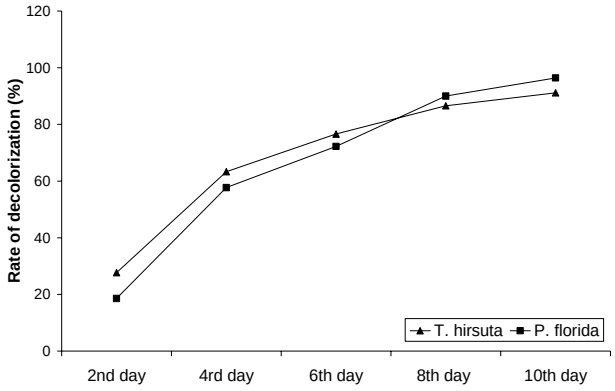

Figure 1. Solid state decolorization of Blue CA by white rot fungi.

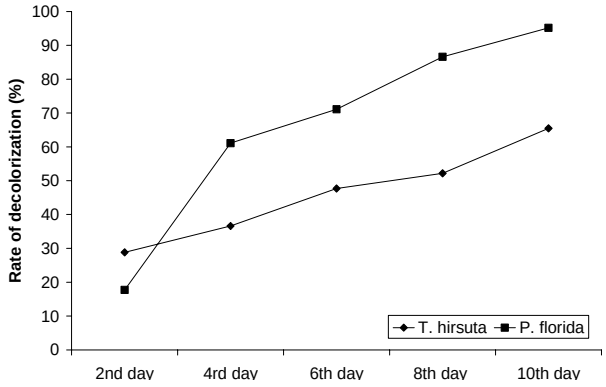

Figure 2. Soild state decolorization of Black 133 by white rot fungi.

Solid state decolorization assay

In this solid state decolorization, the mycelial growth of the microorganism starts at the first day. But decolorization starts from the third day onwards.

Blue CA

The decolorization of dye by *P. florida* was observed to be faster than the decolorization by *T. hirsuta*. About 96.4% decolorization by *P. florida* was observed on the 10^{th} day of incubation and in *T. hirsuta* 91.1% of decolorization was observed (Figure 1).

Black B133

At the concentration of 75 mg/l P. *florida* decolorized the dye by 95.2% on the 10^{th} day of incubation and 65.5% of decolorization by *T. hirsuta* was observed (Figure 2).

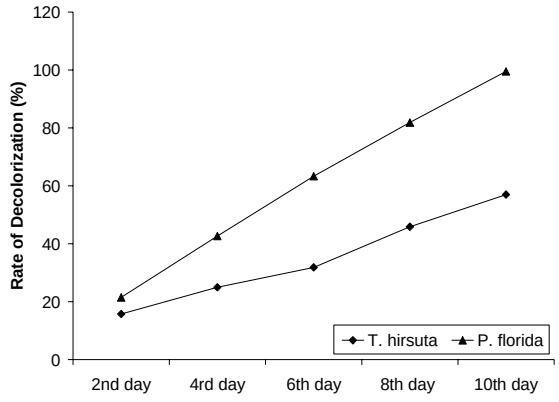

Figure 3. Solid state decolorization of Violet by white rot fungi.

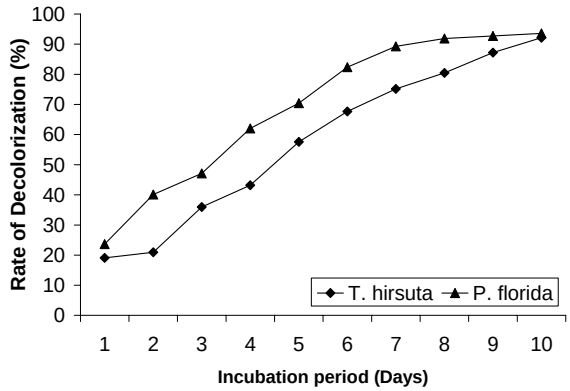

Figure 4. Aqueous state of decolorization of Blue CA (25mg/l) bywhite rot fungi.

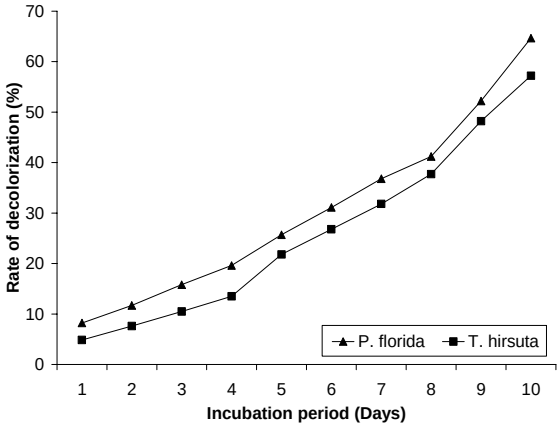

Figure 5. Aqueous state of decolorization of Black 133 (25 mg/l) by white rot fungi.

Corazol violet SR

Violet color decolorization was effective by *P. florida* on the 10th day, it was found to be 99.5% and 56.98% by *T. hirsuta* (Figure 3).

Aqueous state decolorization assay

Decolorization of Blue CA, Black B133 and Corazol violet SR in 0.5% glucose medium was observed at 580, 590 and 530 nm respectively in Beckman DU-40 spectrophotometer for 10 days at everyday interval.

Blue CA

At lower concentration of 25 mg/l visual decolorization by *P. florida* and *T. hirsuta* was observed within 24 h. Maximum decolorization was found to be 93.54% *P. florida* on the 10th day of incubation and 92.17% were found to be in *T. hirsuta* (Figure 4). At 50 mg/l, decolorization rate gradually increased to 61.27% for *P. florida* on the 10th day and 56.64% for *T. hirsuta*. 52.42% decolorization by *P. florida* and 39.45% by *T. hirsuta* was found to be the maximum at a concentration of 75 mg/l during the incubation period.

Black B133

Decolorization of Black B133 was less when compare with other dyes. At 25 mg/l concentration the maximum decolorization by *P. florida* and *T. hirsuta* were 64.67% and 57.21%, respectively (Figure 5). At 50 mg/l concentration, the visual color change was not observed. But absorbance value change was noticed. The maximum decolorization was found to be 33.94% and 29.97% for *P. florida,* and *T. hirsuta*, respectively. Decolorization rate was observed very low in Black B133 at 75 mg/l. The maximum decolorization by *P. florida* was 28.57% and 24.04% by *T. hirsuta*.

Corazol violet SR

P. florida and *T. hirsuta* showed effective decolorization on Corazol violet SR. 83.70% decolorization was found at the concentration of 25 mg/l by *P. florida* and 62.02% by *T. hirsute* was observed. At 50 mg/l concentration dye, the maximum decolorization of 64.67% by *P. florida* and 57.21% by *T. hirsuta* were observed. *P. florida* and *T. hirsuta* decolorized 75 mg/l concentration of the dye at the maximum rate of 58.04% and 43.48%, respectively.

Effluent treatment

The rate of decolorization was observed at 560 nm for incubation period of 5 days at everyday interval. Effluent of unadjusted pH (11) was decolorized by *P. florida* and *T. hirsuta*; 37.87%, and 28.78%, respectively. In 1% glu-

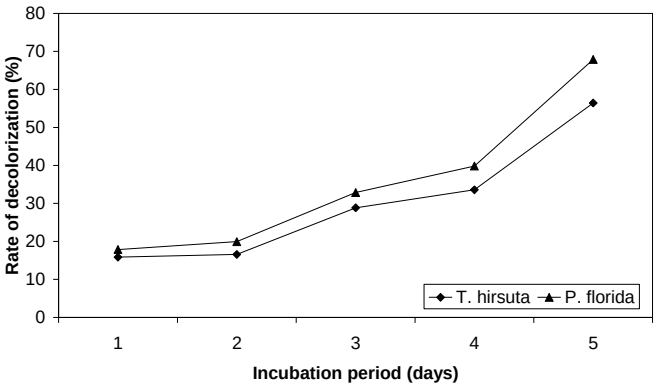

Figure 6. Decolorization of effluent (2% glucose concentration) by white rot fungi.

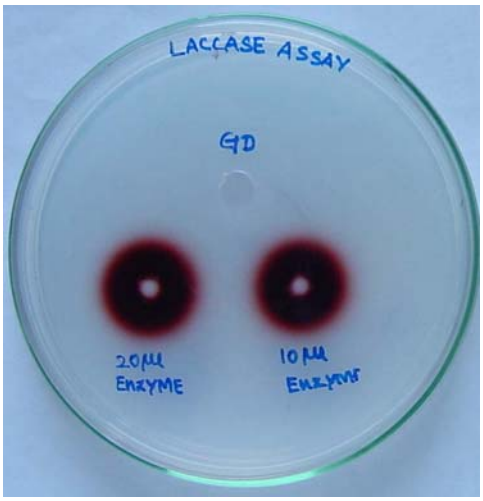

Figure 7. Laccase assay-Dark brown colors indicates laccase activity.

cose medium, 61.98% and 45.98% decolorization occured for *P. florida* and *T. hirsuta*, respectively. In 2% glucose medium, highest decolorization of 64.09 and 52.78% by *P. florida* and *T. hirsuta*, respectively, was observed. The pH adjusted-effluent was decolorized by both *P. florida* and *T hirsuta* at a maximum of 45.62% and 42.07%, respectively. When the concentration of 1% glucose was added the decolorization, efficiency was increased up to the maximum of 56.86% and 52.57% by *P. florida* and *T. hirsuta*, respectively. At 2% glucose amended-effluent the highest decolorization of 67.86% and 64.1% by *P. florida* and *T. hirsuta*, respectively, observed (Figure 6).

Confirmation of laccase production using inducers

In nutrient salt medium, the mycelial growth of *P. florida* starts from the first day. When inducer guiacol and gallic acid was added on the third day of pre grown culture, color change of the medium occur within 24 h, which confirmed that laccase was present on the medium.

Dye decolorization in nutrient salt medium

In nutrient salt medium, the mycelial growth of *P. florida* starts from the first day. When Blue CA and Corazol violet SR was added on the third day to pre grown culture the decolorization of Blue CA by *P. florida* within 24 h was observed. Corazol violet SR decolorization starts at the first day and is completely decolorized within 48 h.

Protein estimation

The amount of protein present in the culture filtrate was 80 µg/l using BSA standard.

Laccase enzyme assay

The laccase enzyme plate assay showed the presence of laccase enzyme in the culture filtrate. The laccase enzyme activity of the culture filtrate was 0.125 U/ml (Figure 7).

DISCUSSION

In the present study the dye decolorization ability of *T. hirsuta* and *P. florida* was studied using their extracellular laccase enzyme system. Both the microorganisms have the oxidase activity and decolorized the Blue CA, Black B133 and Corazol Violet SR. High percentage of Blue CA, Black B133, Corazol Violet SR decolorization was observed in the plate assay. Compared with Black B133, Blue CA and Corazol Violet SR are highly decolorized and these two were taken for laccase assay. However, the extent of color removal is not consistent with all the dyes. Decolorization depends upon on the laccase production, media and dyes. The similar observation regarding the dye degradation by the white rot fungus *P. chrysosporium* has been observed by Cripps et al. *(*1990) and Spardaro et al. (1992).

Rapid dye decolorization by the white rot fungus *P. florida* was observed within 24 h, when the dye was added with 3 days pre grown culture. This was due to the extracellular laccase enzyme production by white rot fungus. It takes 48 h for the decolorization of violet dyes. It may be due to complexity in structure, the enzyme secretion starts at the third day of culture, indicating that the dye decolorization is only due to laccase enzyme. Although both the microorganisms are having the ability to decolorize the dyes, *P. florida* alone was assessed for laccase assay, because of its fast growing character-

ristics. It grows faster compared with the *T. hirsuta*. In the effluent treatment also, when 2% glucose is added, effective color removal was observed. It is similar in both pH-adjusted and unadjusted effluent.

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