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Full Length Research Paper

Comparative study on decolorization of reactive dye 222 by white rot fungi *Pleurotus ostreatus* IBL-02 and *Phanerochaete chrysosporium* IBL-03

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White rot basidiomycetes have unique ability to decolorize synthetic industrial dyes. Initial experiment was performed with five locally isolated indigenous white rot species fungi *S. commune* IBL-01 (SC), *P. ostreatus* IBL-02 (PO) *P. chrysosporium* IBL-03 (PC), *T. versicolor* IBL-04 (TV) and *G. lucidum* IBL-05 (GL), for the selection of white rot fungal cultures based on their maximal decolorization potential. Based on the screening experiment, two white rot fungi *P. ostreatus* IBL-02 and *P. chrysosporium* IBL-03 (PC) showing maximum decolorization of dye under study were selected. A comparative study was conducted for the selected white rot fungi to get the maximum decolorization of synthetic azo dye. Different fermentation conditions and nutritional factors were optimized to enhance the efficiency of white rot fungal cultures for dye decolorization. Both cultures produced all the three major ligninolytic enzymes including lignin peroxidase (LiP), manganase peroxidase (MnP) and laccase which are responsible for decolorization process. Under optimum conditions fermentation conditions, *P. ostreatus* IBL-02 (PO) and *P. chrysosporium* IBL-03 (PC) decolorized the azo dye by 92.7 and 85.9%, respectively.

Key words: Dye decolorization, white rot fungi, ligninolytic enzymes, process optimization.

INTRODUCTION

Presence of color and causative compounds has always been undesirable in water used for either industrial or domestic needs. More than 10,000 chemically different types of dyes are currently manufactured and the biggest consumers of these dyes are the textile, paper, pulp, tannery and Kraft bleaching industries which are probably the most potential contributors as far as color pollution is concerned (Zhang et al., 2003).

A dye house effluent typically contains 0.6 to 0.8 dye g/L, hence color and dye removal, in particular, has recently become an area of major scientific interest as indicated by the multitude of research reports. Waste water treatment using physical, chemical and biological or combinations of these methods are well established for color removal (Gahr et al., 1994). Biodegradation of widely used synthetic dyes in textile industry especially azo dyes by *P. ostreatus* and *P.. chrysosporium* has been investigated previously (Cripps et al., 1990; Goszezynski et al., 1994; Beydilli et al., 1995; Naidu et al., 2003).

The lignin degrading system of white rot fungi (WRF) consists of various extracellular enzymes such as laccases, peroxidases and oxidases making them capable of degrading a wide range of organic pollutants for example, phenolic compounds and synthetic dyes (Leatham et al., 1993; Minussi, 2001; Shah and Nerud, 2002). Biodecolorization of lignin containing pulp and paper wastewater measured by the decrease in color absorption using fungi like *P. chrysosporium* and *Tinctoporia* sp. was reported in early 1980's and the mechanism of color removal involved a lignin peroxidase and manganese peroxidase or laccase enzyme (Michael and Lewis, 1985). Reports have shown that veratryl

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alcohol stimulates the ligninase activity, which seems to be linked to decolorization (Knapp et al., 1995). Several other wood-rotting fungi capable of decolorizing a wide range of structurally different dyes were also reported to be more effective (Kirk and Farrell, 1987). Lignin peroxidase (LiP) is a glycoprotein secreted during secondary metabolism in a response to nitrogen limitation (Tien and Kirk, 1984; Shrivastava et al., 2005). LiP, MnP and Laccase based decolorization treatments are potentially advantageous to bioremediation technologies since the enzymes are produced in larger amounts. These oxidative enzymes have been detected in many plants and are secreted by numerous fungi (Gianfreda et al., 1999; Rodriguez et al., 2008).

The use of biotechnology in the processing of fibers and textiles is rapidly gaining wider recognition because of its non-toxic and eco-friendly characteristics. Among the low cost and viable alternatives available for textile dye degradation, biological processes are recognized due to their extra specificity. Several microorganisms, including fungi, bacteria, yeasts and algae, can decolorize and even completely mineralize azo dyes under certain environmental conditions. In recent years, the possible utilization of the biodegradative abilities of white rot fungi has shown promise. The study was carried out to determine the ability of *P. ostreatus* IBL-02 (PO) and *P. chrysosporium* IBL-03 (PC) in the decolorization of Reactive dye 222. The ligninolytic enzymes produced during dye decolorization by the fungi were also studied.

MATERIALS AND METHODS

Microorganisms and dye sample

Pure cultures of five indigenous white rot fungi *S. commune* IBL-01 (SC), *P. ostreatus* IBL-02 (PO), *P. chrysosporium* IBL-03 (PC), *T. versicolor* IBL-04 (TV) and *G. lucidum* IBL-05 (GL) were obtained from Industrial Biotechnology Laboratory (IBL), University of agriculture, Faisalabad and grown on potato dextrose agar (PDA) slants for 6 to 7 days at pH 4.5 and 30°C. The slants were preserved at 4°C. All the fungal cultures were revived periodically during the study period. Reactive dye 222 (C.I.No. Reactive blue 222) provided by Sandal Dyestuff, Pakistan, was used for decolorization studies.

Inoculum

Inoculum was prepared by growing the fungi in Kirk's basal nutrient medium (Tien and Kirk, 1988). Basal salt medium contained (g/L): ammonium tartarate, 0.22; potassium dihydrogen phosphate, 0.2; magnesium sulphate, 0.05; calcium chloride, 0.01; thiamine, 1 mg/L; 10% between 80 solution, 10 ml/L; 100 mM veratryl alcohol, 1 ml/L and trace element solution, 10 ml/L. The flasks containing inoculums media for individual fungi were adjusted at pH 4.5 with 1.0 M NaOH/1.0 M HCl and autoclaved at 121°C for 15 min. After cooling at room temperature, the loopful spores from individual cultures were inoculated and incubated at 30°C in shaking incubator (150 rpm) for the period of 7 days to get homogenous spore suspension (1×10^6 spores/ml).

Experimental protocol

The inoculated flasks (0.01 g dye/100 ml of inoculum) were incubated at 120 rpm for a period of 10 days. Supernatants were collected after 2, 4, 6, 8 and 10 days of growth. The pH was measured and samples were used for the determination of decolorization (%) and enzyme activities. Controls consisting of uninoculated flasks were also processed along with the experimental flasks.

Optimization of biodecolorization process

The process of biological decolorization was optimized for selected two white rot fungi best suited for decolorization of Reactive dye 222 on the basis of screening experiment. Decolorization experiments were run under various growth conditions by varying one at a time while keeping others constant. All experiments were performed in triplicate.

Effect of pH

The percentage decolorization of the Reactive dye 222 using *P. ostreatus* IBL-02 (PO) and *P. chrysosporium* IBL-03 (PC) at various initial pH conditions ranging 3.5, 4.0, 4.5, 5.0 and 5.5 were studied.

Effect of temperature

In order to study the effect of temperature on decolorization of the Reactive dye 222 using *P. ostreatus* IBL-02 (PO) and *P. chrysosporium* IBL-03 (PC), studies were carried out at different incubation temperatures ranging from 25, 30, 35 and 40°C.

Effect of inoculum size

In order to study the effect of inoculum size on percentage decolorization of the Reactive dye 222 using *P. ostreatus* IBL-02 (PO) and *P. chrysosporium* IBL-03 (PC), studies were carried out at different inoculum sizes ranging from 1 to 5 ml.

Effect of additives

The activity of fungi can be increased by using varying concentrations of additional nutrients like carbon, nitrogen, mediators and metal ions.

Effect of carbon and nitrogen additives

Carbon is a vital source of energy for microorganisms. The potential of white rot fungi for decolorization of dye was checked by the addition of carbon and nitrogen sources. Effect of various carbon sources (glucose, starch , glycerol, rice bran and wheat bran) and nitrogen sources (corn steep liquor, yeast extracts, maize gluten 30%, maize gluten 60% and ammonium oxalate) were used to determine their effects on growth and extent of dye decolorizing ability of two screened white rot fungi i.e. *P. ostreatus* IBL-02 (PO) and *P. chrysosporium* IBL-03 (PC).

Effect of low molecular mass mediators

Mediators have a profound effect on microbial growth, ligninolytic enzymes which decolorize different dyes. Effect of various

mediators like veretryl alcohol, $MnSO_4$, glycerol, ethanol, 2,2'-Azinobis (3-ethylbenzathiazoline)-6-sulphonic acid (ABTS), oxalic acid and H_2O_2 on decolorization of Reactive dye 222 by *P. ostreatus* IBL-02 (PO) and *P. chrysosporium* IBL-03 (PC) was taken in to consideration.

Effect of metal ions

Metal ions play the role of cofactors and enhance accumulative effect of various additives particularly low molecular mass mediators. Effect of various metal ions like cadmium nitrate, calcium chloride, zinc sulphate, ferrous sulphate and copper sulphate on decolorization of Reactive dye 222 by *P. ostreatus* IBL-02 (PO) and *P. chrysosporium* IBL-03 (PC) was studied.

Enzyme activities

Lignin peroxidase was assayed by the method of Tien and Kirk (1984). The oxidation rate of veratryl alcohol to veratraldehyde was determined at 310 nm in 50 mM sodium acetate buffer (pH 3) in the presence of H_2O_2. MnP was assayed by the method of Wariishi et al. (1991). The assay mixture contained 1 ml of 1 mM $MnSO_4$, 1 ml of 50 mM sodium malonate buffer of pH 4.5 and 100 µL of culture supernatant. 500 µL of 0.1 mM H_2O_2 was added as an oxidizing agent.). One unit of laccase activity was defined as the amount of enzyme that oxidizes 1µmol ABTS in 1 minute. Laccase activity was measured by monitoring the oxidation of 2, 2 azinobis (3-ethylbenzthiazoline)-6 sulphonate (ABTS) by culture supernatants at 436 nm (Wolfendon and Wilson, 1982).

Decolorization assay via UV-Vis spectroscopy

Samples with drawn at regular intervals of 24 h were centrifuged at 12000 rpm and analyzed for decolorization for a visible spectrum of dye using UV-visible spectrophotometer at 615 nm. The uninoculated dye sample was used as standard. All assays were performed in triplicate. The decolorization efficiency of fungal isolates was expressed using the following equation:

Decolorization (%) = I – F/I X 100

Where, I = Absorbance of standard
F = Absorbance of decolorized medium

RESULTS AND DISCUSSION

Screening of fungi

Pure cultures of five indigenous white rot fungi *S. commune* IBL-01 (SC), *P. ostreatus* IBL-02 (PO), *P. chrysosporium* IBL-03 (PC), *T. versicolor* IBL-04 (TV) and *G. lucidum* IBL-05 (GL) were studied to evaluate their degradation potential for Reactive dye 222**.** The fungal cultures showed varying decolorization potential for Reactive dye 222. *P. ostreatus* IBL-02 (PO) and *P. chrysosporium* IBL-03 (PC) showed maximum dye decolorization of 92.7 and 85.9%, respectively, on the sixth day of incubation (Figure 1a). Enzyme activities of all the cultures were also studied. Manganese Peroxidase (MnP) by PO and Lignin peroxidase (LiP) by PC turned out to be the major enzymes than other fungal strains (Figure 1b). Cetin and Donmez (2006) reported that *P. chrysosporium* decolorized Reactive Red RR (94.9%), Reactive Black RB (91.0%) and Remazol Blue (63.6%). Abdullah and Zafar (1999) found that the presence of LiP and/or MnP in addition to Laccase, increased decolorization of Reactive blue up to 85%.

Optimization of decolourization process

Based on the results of screening experiment, *P. ostreatus* IBL-02 (PO) and *P. chrysosporium* IBL-03 (PC) showing maximum decolorization of Reactive dye 222 were selected for subsequent optimization studies. Different parameters were optimized for maximum decolorization of Reactive dye 222 by *P.* ostreatus IBL-02 (PO) and *P. chrysosporium* IBL-03 (PC).

Optimization of pH

The percent decolorization went on increasing with the rise in pH of the medium, with the maximum decolorization, 92.92% by *P. ostreatus* IBL-02 (PO) (Figure 2a) and 85.69% by *P. chrysosporium* IBL-03 (PC) (Figure 2b), for the dye studied in the present work was observed at pH 4.5. The major production of MnP (103.70 U/ml) by *P. ostreatus* IBL-02 (Figure 2a) and LiP (105.38 U/ml) by *P. chrysosporium* IBL-03 (PC) (Figure 2b), were found at the optimized pH.

The decrease in percent decolorization with the further increase in pH (> 4.5) might be due to the fact that change in pH may alter the three dimensional structure of the enzymes, as the ligninolytic enzymes work under optimal pH, a change in pH may affect the enzyme activities (Knapp and Newby, 1995). The effective decolorization of Reactive Red 190 by isolated *Citrobactor* sp. CK3 in strongly acidic condi-tions having pH 4 has been reported (Wang et al., 2009). Cripps et al. (1990) performed enzymatic oxidation of azo dyes by crude lignin peroxidase at pH 4.5. Although pH 4.5 to 5 is within the physiological pH range that is near optimum value for growth of lignin peroxidase, a value that agrees well published pH profile for veratryl alcohol (Aksu and Donmez. 2003; Cetin and Donmez, 2006).

Optimization of inoculum size

Experimental results on the estimation of optimum concentration of inoculum size necessary to achieve maximum decolorization, showed that the maximum decolorization (%) shown by *P. ostreatus* IBL-02 (PO) and *P. chrysosporium* IBL-03 (PC) at an inoculum size of 4 ml, were 91.19% (Figure 3a) and 85.42%, respectively (Figure 3b). The high activity of MnP (101.32 U/ml) by

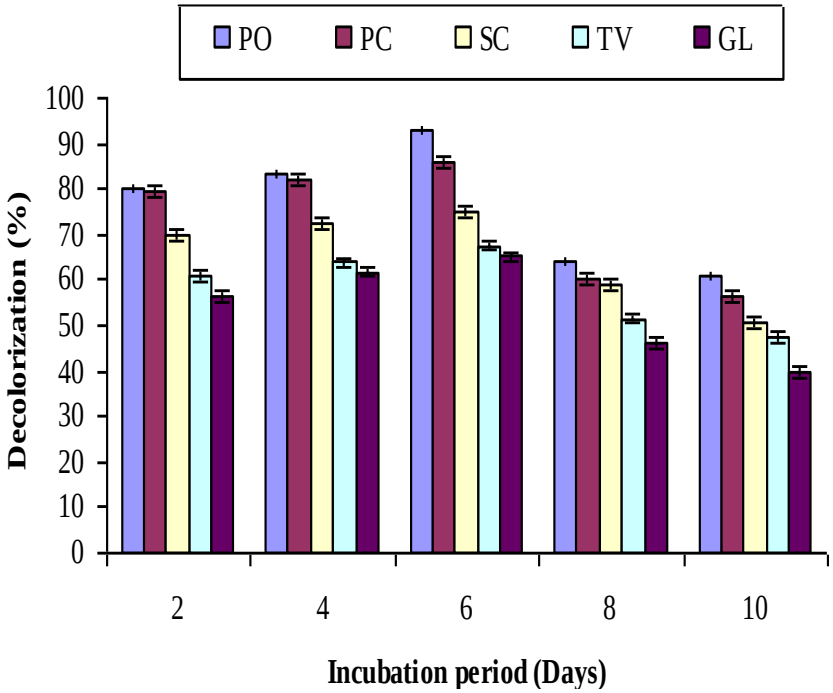

Figure 1a. Decolorization of reactive dye 222 by five white rot fungi.

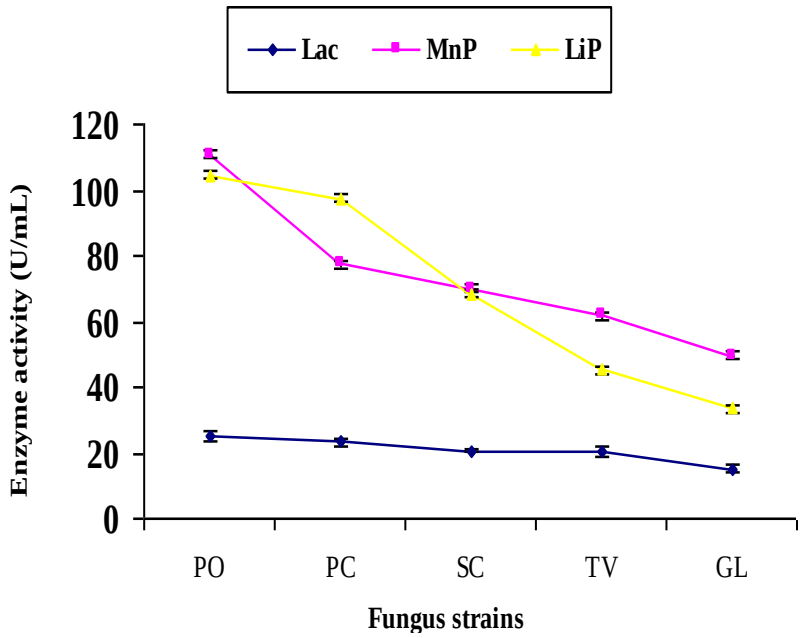

Figure 1b. Comparision of ligninolytic enzymes produced by five white rot fungi during decolorization of Reactive dye 222.

was detected, at optimized level of inoculum (4 ml). Usually lower inoculum size requires longer time for the cells to multiply to sufficient number to utilize the substrate and produce enzymes. An increase in the inoculum size would ensure a rapid proliferation and biomass synthesis. However, after a certain limit, enzymes production could decrease because of depletion of nutrients which would decrease in metabolic activity (Gao et al., 2008). From a previous study of experimental PO (Figure 3a) and LiP (118.28 U/ml) by PC (Figure 3b)

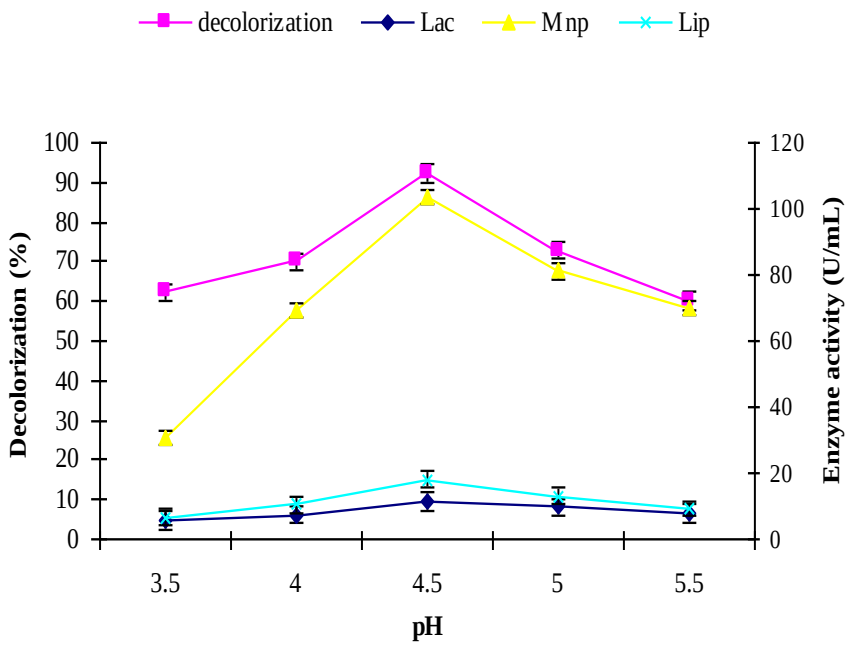

Figure 2a. Effect of pH on activities of ligninolytic enzymes of *P. ostreatus* IBL-02 and decolorization of Reactive dye 222.

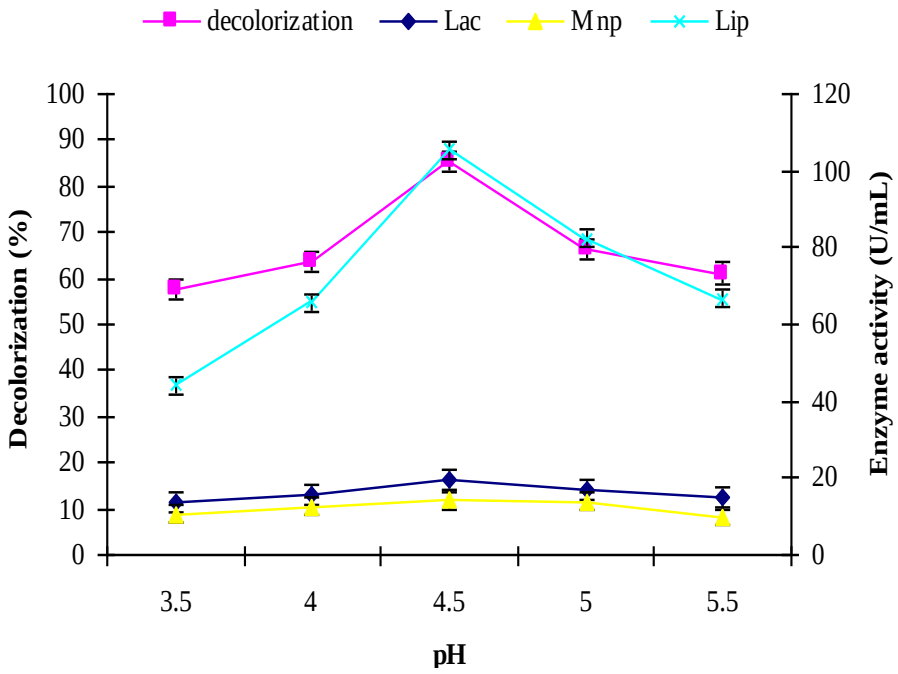

Figure 2b. Effect of pH on activities of ligninolytic enzymes of *P. chrysosporium* IBL-03 and decolorization of Reactive dye 222.

findings, a correlation was made that a lower fungal inoculum size may not be sufficient to initiate the growth, whereas a higher inoculum level may cause competitive inhibition (Sabu et al., 2005).

Optimization of incubation temperature

The maximum percent decolorization values shown by by *P. ostreatus* IBL-02 (PO) and *P. chrysosporium* IBL-03

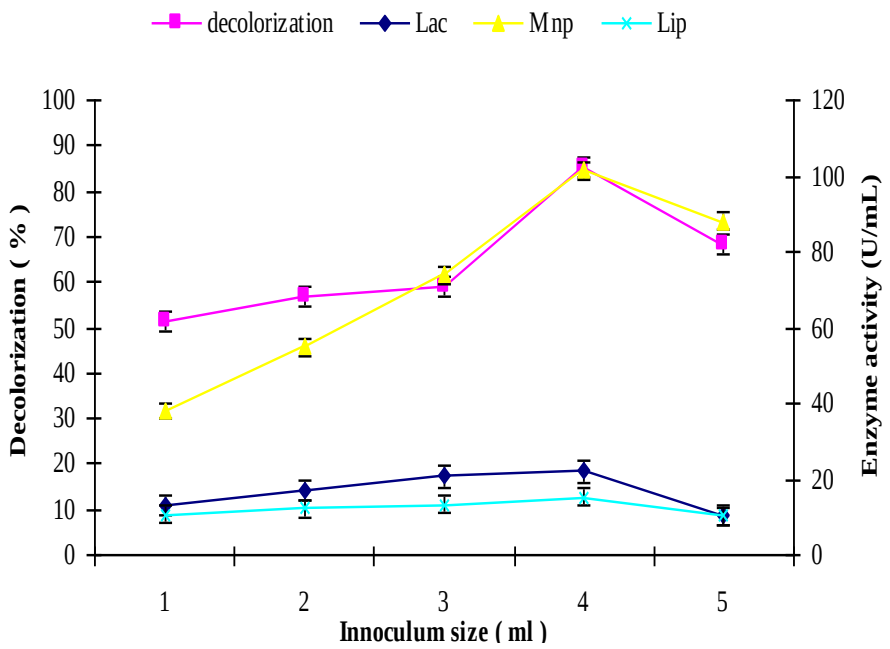

Figure 3a. Effect of inoculum size on activities of ligninolytic enzymes of *P. ostreatus* IBL-02 and decolorization of Reactive dye 222.

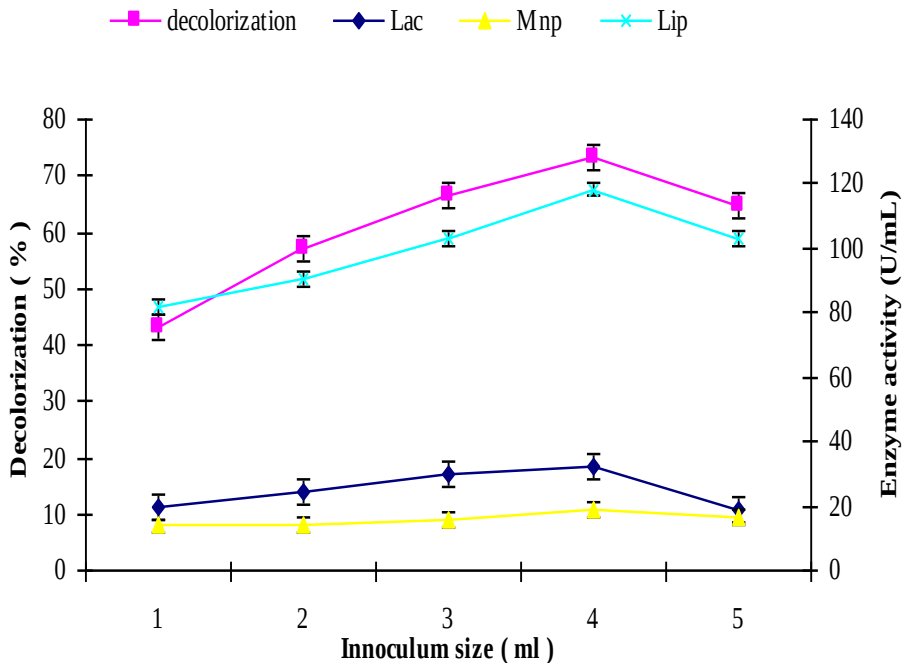

Figure 3b. Effect of inoculum size on activities of ligninolytic enzymes of *P. chrysosporium* IBL-03 and decolorization of Reactive dye 222.

(PC) at incubation temperature of 30°C were 92.14 and 85.71%, respectively (Figure 4a and 4b). The enzyme profiles revealed the major production of MnP (102.5 U/ml) by PO (Figure 4a) and LiP (104.32 U/ml) by PC (Figure 4b), at the optimized incubation temperature (30°C). The temperatures higher than 30°C, the decolorization ability of both fungi get reduced indicating that either the fungus is not able to produce the

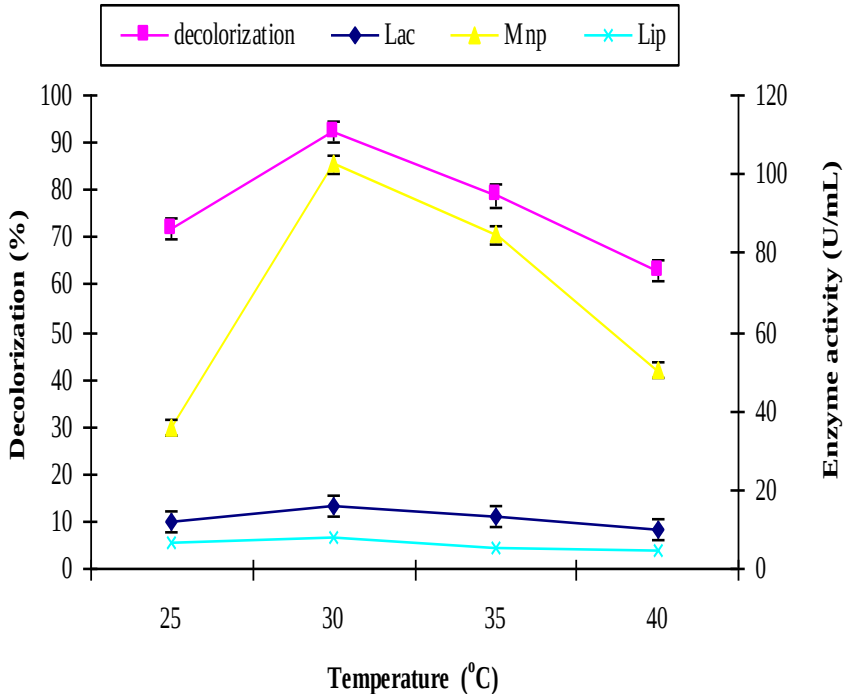

Figure 4a. Effect of temperature on activities of ligninolytic enzymes of *P. ostreatus* IBL-02 and decolorization of Reactive dye 222.

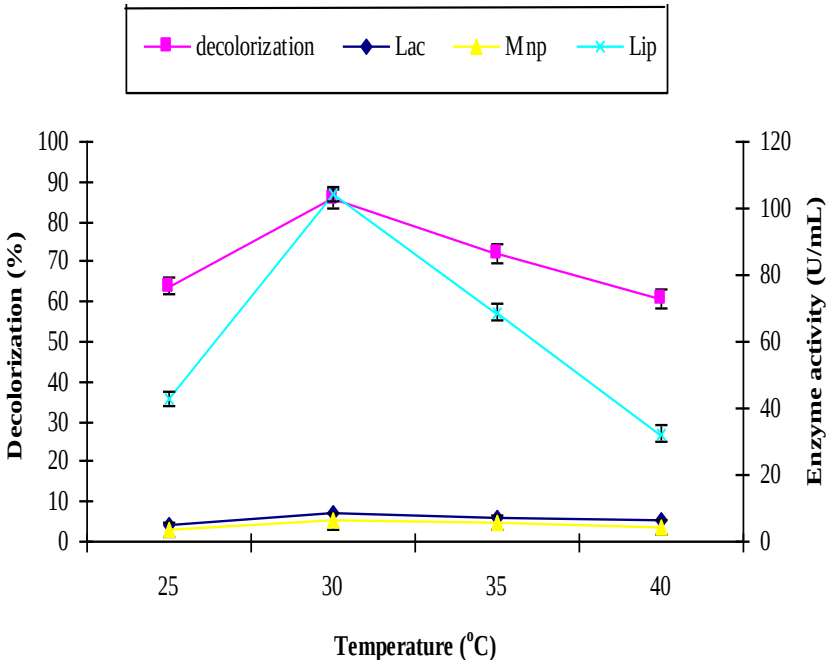

Figure 4b. Effect of temperature on activities of ligninolytic enzymes of *P. chrysosporium* IBL-03 and decolorization of Reactive dye 222.

peroxidases for decolorization or they get denatured (Baldrian and Snajdr, 2006; Niladevi et al., 2007). The optimum temperature for substrate oxidation by white rot fungi in the presence of MnP as a major enzyme by PO along with other ligninocellulosic enzymes has been consistently reported as 30 to 35°C. These results are in

Table 1a. Effect of additional carbon sources on activities of ligninolytic enzymes of *P. ostreatus* IBL-02 and decolorization of Reactive dye 222.

Carbon sources (2%)	Decolorization (% ± S.E)	Enzyme activity (U/ml)		
		Lac	MnP	LiP
Starch	56.68 ± 0.56	13.89 ± 0.48	73.34 ± 0.50	14.63 ± 0.47
Glycerol	57.46 ± 0.50	16.67 ± 0.45	75.07 ± 0.49	16.63 ± 0.46
Glucose	65.83 ± 0.45	17.36 ± 0.48	92.32 ± 0.53	18.92 ± 0.48
Rice bran	94.34 ± 0.51	18.25 ± 0.45	125.1 ± 0.52	20.81 ± 0.43
Wheat bran	93.52 ± 0.50	16.20 ± 0.48	98.24 ± 0.51	11.62 ± 0.42

Table 1b. Effect of additional carbon sources on activities of ligninolytic enzymes of *P. chrysosporium* IBL-03 and decolorization of Reactive dye 222.

Carbon sources (2%)	Decolorization (% ± S.E)	Enzyme activity (U/ml)		
		Lac	MnP	LiP
Starch	56.68 ± 0.56	13.89 ± 0.48	11.34 ± 0.50	44.63 ± 0.47
Glycerol	57.46 ± 0.50	16.67 ± 0.45	18.07 ± 0.49	74.63 ± 0.46
Glucose	65.83 ± 0.45	17.36 ± 0.48	19.32 ± 0.53	98.92 ± 0.48
Rice bran	92.11 ± 0.51	18.25 ± 0.45	20.1 ± 0.52	122.81 ± 0.43
Wheat bran	90.21 ± 0.50	16.20 ± 0.48	8.24 ± 0.51	101.62 ± 0.42

consistent with findings of various researchers (Tan et al., 2000; Nyanhango et al., 2002; Masud and Anantharaman, 2006) who explained that fungal growth was supported in a limited temperature range with dye removal. Other researchers reported that color reduction increases with temperature up to 30 to 35°C (Chen et al., 2002).

Optimization of additional carbon sources

The maximum decolorization (%) values shown by *P. ostreatus* IBL-02 (PO) and *P. chrysosporium* IBL-03 (PC) in the presence of rice bran as additional C-source were 96.92% (Table 1a) and 94.9%, respectively (Table 1b). The enzyme profiles revealed the major production of MnP (125.11 U/ml) by PO (Table 1a) and LiP (122.81 U/ml) by PC (Table 1b), in the presence of rice bran as additional C-source. A number of researchers have reported an enhanced transformation of dyes by using supplemental carbon sources and found their role in enzymes production (Chen et al., 2002). Others reported that in start metabolism of additional carbon like rice bran might cause an increased multiplication of fungal cells, hence increased production of ligninolytic enzymes which resulted in enhanced decolorization of dye under study (Kumarasamy et al., 2009). Many previous studies that both the nature and concentration of additional carbon sources other than dye itself are powerful nutritional factors regulating ligninolytic enzymes production by wood-rotting basidiomycetes (Galhaup et al., 2002). Variability among the earlier reported studies and forgoing research might be due to difference in microbial characteristic and enzymes responsible for color removal. It can be concluded that in metabolism of additional carbon sources glucose or rice bran might caused an increased production of nucleotides leading to increase in decolorization efficiency (Khehra et al., 2005).

Optimization of nitrogen additives

The maximum decolorization (%) values shown by *P. ostreatus* IBL-02 (PO) and *P. chrysosporium* IBL-03 (PC) in the presence of ammonium oxalate as additional nitrogen source were 82.12% (Table 2a) and 75.3%, respectively, relative to other nitrogen sources (Table 2b). The enzyme profiles revealed the major production of MnP (103.49U/ml) by PO (Table 2a) and LiP (105.47 U/ml) by PC (Table 2b), in the presence of ammonium oxalate as additional nitrogen source. Moreover, enzymes production also decreased in the presence of supplemental nitrogen (as it is evident from the Tables 2a and 2b). Since less decolorization (%) and enzyme values were obtained in the presence of ammonium oxalate as an additional nitrogen source as compared to decolorization (%) and enzyme values in the presence of additional carbon sources by both PO and PC which were 96.92 and 94.9%, respectively. It might be due to the fact that fungi require no additional nitrogen source and it may utilize the nitrogen of dye effectively for maximum percent decolorization (Steffensen and

Table 2a. Effect of additional nitrogen sources on activities of ligninolytic enzymes of *P. ostreatus* IBL-02 and decolorization of Reactive dye 222.

Nitrogen sources (0.1%)	Decolorization (% ± S.E)	Enzyme activity (U/ml)		
		Lac	MnP	LiP
Corn steep liquor	59.5 ± 0.28	15.97 ± 0.49	31.93 ± 0.51	10.7 ± 0.44
Yeast extract	64.2 ± 0.28	21.03 ± 0.47	37.10 ± 0.52	15.8 ± 0.42
Maize gluten 60%	79.4 ± 0.30	31.8 ± 0.46	53.49 ± 0.54	24.4 ± 0.41
Ammonium oxalate	82.12 ± 0.26	35.1 ± 0.46	103.49 ± 0.50	29.5 ± 0.43
Maize gluten 30%	80.6 ± 0.28	13.2 ± 0.46	69.61 ± 0.50	13.0 ± 0.45

Table 2b. Effect of additional nitrogen sources on activities of ligninolytic enzymes of *P. chrysosporium* IBL-03 and decolorization of Reactive dye 222.

Nitrogen sources (0.1%)	Decolorization (%± S.E)	Enzyme activity (U/ml)		
		Lac	MnP	LiP
Corn steep liquor	59.2±0.38	15.0±0.52	13.73±0.42	30.1±0.52
Yeast extract	65.5±0.36	17.36±0.52	14.65±0.41	33.3±0.53
Maize gluten 60%	66.8±0.30	18.4±0.53	15.99±0.40	50.3±0.54
Ammonium oxalate	75.3±0.35	20.0±0.51	21.77±0.42	105.4±0.5
Maize gluten 30%	71.9±0.36	13.47±0.5	15.30±0.45	88.5±0.56

Table 3a. Effect of redox mediators on activities of ligninolytic enzymes of *P. ostreatus* IBL-02 and decolorization of Reactive dye 222.

Mediators (1 mM)	Decolorization (% ± S.E)	Enzyme activity (U/ml)		
		Lac	MnP	LiP
Ethanol	59.9 ± 0.48	8.6 ± 0.48	28 ± 0.66	15.0 ± 0.57
Glycerol	61.6 ± 0.42	10.7 ± 0.50	36 ± 0.62	16.10 ± 0.5
ABTS	69.5 ± 0.42	11.3 ± 0.52	70 ± 0.64	18.1 ± 0.56
Oxalic acid	70.8 ± 0.43	13.6 ± 0.51	93.5 ± 0.61	19 ± 0.59
$MnSO_4$	93.82 ± 0.42	21.2 ± 0.54	153 ± 0.63	20 ± 0.57
H_2O_2	75.9 ± 0.44	19.6 ± 0.49	85 ± 0.64	13.2 ± 0.56
V.Alcohol	70.2 ± 0.48	14.2 ± 0.50	79 ± 0.62	11.8 ± 0.58

Alexander, 1995). Literature survey have shown that the addition of nitrogen sources seemed to be less effective in promoting decolorization, probably due to the preference of the cells in assimilating the dye as the nitrogen source (Saratale et al., 2009).

Effect of redox mediators

Maximum decolorization (93.82 and 91.11%) was achieved in the presence of $MnSO_4$ as a redox mediator by both *P. ostreatus* IBL-02 (PO) and *P. chrysosporium* IBL-03 (PC), respectively (Tables 3a and 3b). The enzyme profiles revealed the major production of MnP (153 U/ml) by PO and LiP (150 U/ml) by PC in the presence of $MnSO_4$ as a redox mediator. Enhancement in the anaerobic reduction of azo dyes by *Escherichia coli* was observed in the presence of $MnSO_4$ as redox mediator, and this is because of the induction in the azoreductases activity reported earlier (Lorenco et al., 2000; Giardina et al., 2000; Rau et al., 2002; Kamitsuji et al., 2004; Liu et al., 2009).

Other researchers also reported the color removal from textile wastewater in the presence of redox mediators like ABTS, $MnSO_4$ and oxalic acid, suggesting the enhancement of decolorization performance of azo dyes at a concentration of 0.1 to 0.2mM by *Escherichia coli* JM 109. Our results are in consistent with these findings (Sauriasari et al., 2007).

Table 3b. Effect of redox mediators on activities of ligninolytic enzymes of *P. chrysosporium* IBL-03 and decolorization of Reactive dye 222.

Mediators (1 mM)	Decolorization (% ± S.E)	Enzyme activity (U/ml)		
		Lac	MnP	LiP
Ethanol	56.8 ± 0.42	7.6 ± 0.41	10 ± 0.47	35.0 ± 0.57
Glycerol	57.1 ± 0.50	11.7 ± 0.44	15 ± 0.51	58.1 ± 0.56
ABTS	62.8 ± 0.45	13.5 ± 0.38	16 ± 0.48	105 ± 0.56
Oxalic acid	86.9 ± 0.45	17.5 ± 0.44	18.1 ± 0.50	110 ± 0.59
$MnSO_4$	91.1 ± 0.45	22.8 ± 0.39	19 ± 0.48	150 ± 0.57
H_2O_2	91.2 ± 0.45	13.5 ± 0.42	13 ± 0.46	130 ± 0.56
V. Alcohol	90.9 ± 0.51	11.1 ± 0.43	10.2 ± 0.51	118 ± 0.58

Table 4a. Effect of metal ions on activities of ligninolytic enzymes of *P. ostreatus* IBL-02 and decolorization of Reactive dye 222.

Metal ions (1 mM)	Decolorization (% ± S.E)	Enzyme activity (U/ml)		
		Lac	MnP	LiP
Cd $(NO_3)_2$	76.58 ± 0.28	17.8 ± 0.55	48.5 ± 0.60	17.3 ± 0.54
$CaCl_2$	78.67 ± 0.28	22.5 ± 0.53	63.5 ± 0.61	20.95 ± 0.55.
$ZnSO_4$	83.87 ± 0.30	33.4 ± 0.51	73.5 ± 0.57	33.32 ± 0.54
$CuSO_4$	96.16 ± 0.26	38.2 ± 0.54	161 ± 0.57	37.88 ± 0.55
$FeSO_4$	86.83 ± 0.28	23.9 ± 0.52	78 ± 0.58	20.23 ± 0.58

Table 4b. Effect of metal ions on activities of ligninolytic enzymes of *P. chrysosporium* IBL-03 and decolorization of Reactive dye 222.

Metal ions (1 mM)	Decolorization (% ± S.E)	Enzyme activity (U/ml)		
		Lac	MnP	LiP
Cd $(NO_3)_2$	76.8 ± 0.55	16 ± 0.43	21 ± 0.60	118.3 ± 0.54
$CaCl_2$	77.7 ± 0.49	24.78 ±0.4	22 ± 0.62	134.95 ± 0.55.
$ZnSO_4$	80.8 ± 0.52	34.2 ± 0.41	34.5 ± 0.58	149.32 ± 0.54
$CuSO_4$	95.56 ± 0.42	37.5 ± 0.41	39.5 ± 0.61	158.1 ± 0.55
$FeSO_4$	87.4 ± 0.52	21.5 ± 0.45	20.11 ± 0.59	103.23 ± 0.58

Effect of metal ions

The maximum decolorization (%) values shown by *P. ostreatus* IBL-02 (PO) and *P. chrysosporium* IBL-03 (PC) in the presence of $CuSO_4$ as metal ion source were 96.16 and 95.56% (Tables 4a and 4b), respectively. The enzyme profiles revealed the major production of MnP (161 U/ml) by PO (Table 4a) and LiP (158.1 U/ml) by PC (Table 4b) in the presence of $CuSO_4$ as metal ion source. The metal ions are general potent inhibitors of enzyme reactions but they might play a role of co-factors and enhance the activity of enzymes up to 1 mM. However, increasing the metal ion concentration decreased the reactivity of ligninolytic enzymes. These results are in harmony with those reported many researchers (Georgiou et al., 2004). It is well reported that addition of metal ions apparently induces the reduction of azo bonds effectively (Bras et al., 2001). In another study, decolorization of Reactive orange 16 by *Bacillus sp*. ADR in the presence of $CuSO_4$ as metal ion source was greatly enhanced up to 97% (Van der Zee et al., 2001; Pearce et al., 2003; Telke et al., 2009).

Conclusion

The *Pleurotus ostreatus* IBL-02 comparatively showed better decolorization potential for Reactive dye 222 as compared to *Phanerochaete chrysosporium* IBL-03. Their ligninolytic enzymes are responsible for the decolorization of Reactive dye 222, that work efficiently under optimal growth conditions. The results showed that both fungal strains could be successfully used for efficient decolorization of reactive dyes.

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