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Decolourization of Synthetic Textile Dyes using the Edible Mushroom Fungi *Pleurotus*

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Abstract: The ability of three *Pleurotus* species (*P. florida* LCJ 65, *P. ostreatus* LCJ 183 *and P. sajorcaju* LCJ 184) was compared for the decolourization of bromophenol blue, brilliant green and methylred using by solid and liquid medium. All three *Pleurotus* species were effective in decolourizing the dyes on potato dextrose agar plate. During quantitative decolourization experiments, the absorption spectrum of the dye solution showed a steady decrease in decolourization with the increase in the days of incubation. The decolourization efficiency varied for species to species and it was found that *P. sajorcaju* LCJ 184 effectively decolourized the selected dyes by 85-98%. In present study, different factors (dye concentration, inoculums size, pH, static and shaking culture conditions) influencing the ability of *Pleurotus* species to decolourize three different dyes is documented and the result proposes *P. florida* LCJ 65 and *P. sajorcaju* LCJ 184 as potential strains for decolourization of bromophenol blue, brilliant green and methylred dye.

Key words: Fungi, decolourization, textile dyes, bioremediation, *Pleurotus* species

INTRODUCTION

Pleurotus is a white rot fungus belonging to the family pleurotaceae. The ligninolytic enzyme system of the genus *Pleurotus* has been extensively investigated for its numerous applications, such as conversion of agro-wastes into edible biomass, which could be used as food, animal feed or as a source of drugs and for the biodegradation of organic pollutants, xenobiotics and different industrial contaminants (Cohen *et al*., 2002). Among its various applications, *Pleurotus* species plays a significant role in textile dye degradation. Worldwide, annually 700, 000 tonnes of dyes are required for the production of 30 million tones of textile products (Talarposhti *et al*., 2001). The excess amount of effluent released from these textile industries causes serious negative impact to the environment.

Removal of dye containing effluents can be carried out by conventional method (physical and chemical methods) but the major drawbacks of these physic-chemical methods are limited flexibility, expensive, slow process and sludge generation (Bansal and Goyal, 2005; Kim *et al*., 2005; Huang *et al*., 2007; Kumar *et al*., 1998). However, biological methods are regarded as economically feasible and eco-friendly for dye degradation. Biodegradation of textile dyes can be done using several microorganisms which includes bacteria and fungi. Among these, the most efficient group in breaking down textile dyes are the white rot fungi which have the ability to degrade a wide range of recalcitrant organic compounds that includes several dyes (Wesenberg *et al*., 2003). In this context, three different *Pleurotus* sp. (*P. florida* LCJ 65, *P. ostreatus* LCJ 183 and *P. sajorcaju* LCJ 184) were selected for the present investigation to compare the efficacy of *Pleurotus* sp. in decolourization of bromophenol blue, brilliant green and methyl red which are commonly used textile dyes.

MATERIALS AND METHODS

The dye decolourization study was carried out with three strains of *Pleurotus* sp. (*P. florida* LCJ 65, *P. ostreatus* LCJ 183 *and P. sajorcaju* LCJ 184). The cultures were maintained on Potato Dextrose Agar (PDA) slants.

Qualitative assay for dye decolourization: PDA plates were supplemented with Bromophenol blue, Brilliant green and Methyl red at 0.05% (w/v) respectively. Then, the dye containing PDA plates were inoculated by placing mycelial discs obtained from actively growing culture. The decolourization of dyes was observed by the formation of decolourization zones under and around developing mycelia.

Quantitative assay for dye decolourization: Shake flask cultures for the decolourization study were carried out in 250 mL Erlenmeyer flasks containing 100 mL of basal

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medium Jonathan and Fasidi, 2001 (g L^{-1}): Glucose-10, KH_2PO_4-1, $MgSO_4$-0.5, $CaCl_2$-0.14, Yeast extract-1, Thiamine-0.0025) containing 0.05% of the respective dyes. The basal liquid medium containing the respective dyes were autoclaved at 121°C for 15 min. Then, three fungal mycelium discs were inoculated into the conical flask, under sterile condition and incubated in the rotary shaker at 120 rpm at room temperature. The OD of each of the sample was taken at the respective absorption maxima of each of the dye at different time intervals. The percentage of decolourization was calculated as per the following equation:

$$\text{Decolorization (\%)} = \frac{\text{Initial absorbance-final absorbance}}{\text{Initial absorbance}} \times 100$$

The effect of dye concentration, inoculum size, pH and agitation on the decolourization of the dyes was examined in basal medium containing, respective dyes with actively grown fungal biomass. The dye decolourization was monitored at different time intervals (1 to 6 h) and results were calculated as per the dye decolourization formula.

RESULT AND DISCUSSION

Dye decolourization on agar plate: Decolourization ability of the selected white rot fungi (*P. florida* LCJ 65, *P. ostreatus* LCJ 183 and *P. sajorcaju* LCJ 184) were compared to investigate their potential to decolourize the three textile dyes (Bromophenol blue, Brilliant green and Methyl red) by cultivation on a solid medium. The selected *Pleurotus* sp. was able to completely decolourize all three dyes within 10-14 days of incubation (Table 1). Among the three dyes tested, brilliant green and methyl red were rapidly decolourized, followed by bromophenol blue. Among the three fungi tested *P. sajorcaju* was able to completely decolourize all three dyes within 10 days. A number of studies have reported that white-rot fungi have the ability to degrade synthetic dyes (Harazono and Nakamura, 2005; Lee *et al*., 2007). Our data obtained with *Pleurotus* sp. support the observations of Eichlerova *et al*. (2006) who reported that *Pleurotus* sp. were able to decolourize orange G and remazol brilliant blue R on agar plates after 12-18 days of incubation. Knapp and Newby (1995) reported that *P. ostreatus* was able to decolourize various azo, anthraquinone, Cu-phthalocyamine and triphenylmethane dyes, but on the contrary the report by Swamy and Ramsay (1999) highlighted that *P. ostreatus* was not able to decolourize azo and Cu-phthalocyanine dyes on agar plates. *P. sajorcaju* was able to decolourize amaranth, new coccine and orange G on agar plates within eight days of incubation (Chagas and Durrant, 2001). Few other studies also emphasize that *P. sajorcaju* decolourize indigo blue dye on agar plates within four days of incubation (Balan and Monteiro, 2001). The present result on dye decolourization on agar plate clearly indicates that although the fungi belongs to the same genus, the dye decolourization ability varies from species to species.

Dye decolourization in liquid medium: White rot fungi are the most intensively studied dye decolourizing microorganisms. Studies on the decolourization of dyes (bromophenol blue, brilliant green and methylred) by *Pleurotus* sp. are few. However, the current study has significantly vitalized the role of such fungus. The decolourization studies were carried out in basal medium containing the respective dyes. All the three selected *Pleurotus* sp. efficiently decolourized bromophenol blue, brilliant green and methylred. The decolourization percentage clearly showed extensive removal of bromophenol blue by *Pleurotus* sp. (*P. florida* LCJ 65, *P. ostreatus* LCJ 183 and *P. sajorcaju* LCJ 184) as evidenced by the decrease in the absorbance of the culture medium. *P. sajorcaju* LCJ 184 showed 85% of decolourization (Fig. 1) whereas; in the case of *P. florida* LCJ 65 and *P. ostreatus* LCJ 183 the decolourization percentage was 81 and 82% respectively (Fig. 1, 2). In brilliant green, *P. sajorcaju* LCJ 184 showed 94% of decolourization (Fig. 3), followed by *P. florida* LCJ 65 and *P. ostreatus* LCJ 183 (Fig. 1, 2). Three selected

Table 1: Qualitative decolourization of dyes by *Pleurotus* sp.

	Day 2	Day 4	Day 6	Day 8	Day 10	Day 12	Day 14
Bromophenol blue (decolourized zone in cm)							
P. florida LCJ65	0.0	0.0	1.4	2.2	4.0	4.8	6.0
P. ostreatus LCJ183	0.0	0.0	0.4	0.7	2.5	2.5	4.0
P. sajorcaju LCJ184	0.0	0.4	1.0	2.4	4.5	5.0	5.9
Brilliant green (decolourized zone in cm)							
P. florida LCJ65	0.0	0.3	2.0	2.6	4.8	5.1	6.0
P. ostreatus LCJ183	0.0	0.0	0.5	1.0	2.0	3.5	4.0
P. sajorcaju LCJ184	0.0	0.5	2.0	4.0	4.5	5.1	6.0
Methyl red (decolourized zone in cm)							
P. florida LCJ65	0.0	0.0	2.0	2.9	4.5	5.5	6.0
P. ostreatus LCJ183	0.0	0.0	1.0	2.0	3.5	5.0	6.0
P. sajorcaju LCJ184	0.0	1.0	3.0	3.6	4.0	4.9	6.0

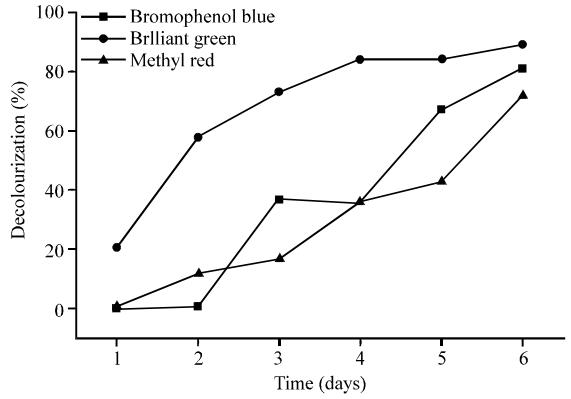

Fig. 1: Decolourization of dyes using *P. florida* LCJ 65

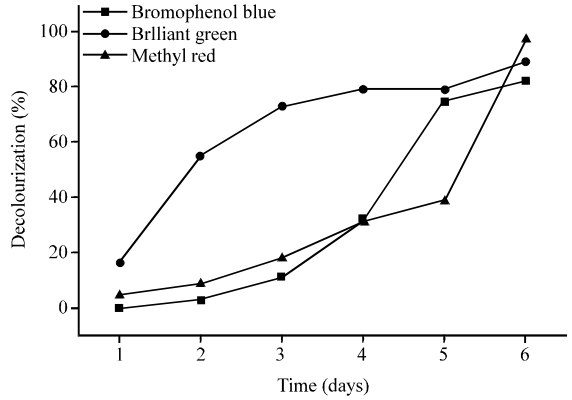

Fig. 2: Decolourization of dyes using *P. ostreatus* LCJ 183

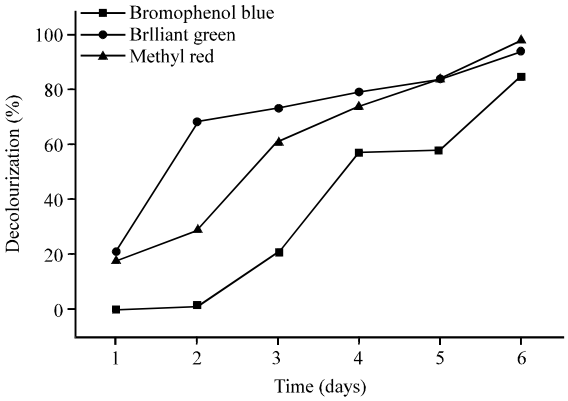

Fig. 3: Decolourization of dyes using *P. sajorcaju* LCJ 184

Pleurotus sp. showed maximum decolourization of bromophenol blue and it was clearly summarized in Fig. 1-3. Dye removal in the present study was seen merely due to biosorption or bioadsorption. Likewise, few other studies have also clearly mentioned biosorption and bioadsorption of certain white rot fungi. Biosorption is reported to be the primary dye removal process in wood rotting basidiomycetes (Balan and Monteiro, 2001; Fu and Viraraghavan, 2000; Knapp and Newby, 1995). Similar, observation was evident in the present study, however, the fungal biomass gradually turned colourless after dyes bioadsorption. These observations are in line with the findings of Balan and Monteiro, 2001, where they reported the removal of Indigo dye by fungal adsorption and extracellular degradation. Bioadsorption, in particular, has been linked to electrostatic pull between the negatively charged dyes and the positively charged cell wall components (Aksu and Tezer, 2000; Aksu *et al*., 1999). Yesilada *et al*. (2003) reported that *P. ostreatus* shows 97, 89 and 84% decolourization of azo dyes. Another report by Kalmis *et al*. (2008) reported that wild type of two *P. ostreatus* strains showed maximum decolourization of Benazol Black ZN textile dye. Kalmis *et al*. (2007) also found that *Pleurotus* sp. effectively decolourize five different textile dyestuffs namely Indanthren yellow F3GC Collosiol, Blue CC Dranix, Indanthren blue CLF Collosiol, Remazol Brilliant blue BB and Levafix Brilliant blue E-B. Another reason for dye decolourization might be due to the production of extracellular enzymes by *Pleurotus* sp., during the biodegradation of tested dyes. Decolourization of dyes by using lignin modifying enzymes were studied extensively using laccase from *Trametes hirsute*, *T. modesta*, *Sclerotium roysii* (Nyanhongo *et al*., 2002) *Laccaria fraternal, P. ostreatus* (Balaraju *et al*., 2007), LiP from *Phanerochaete chrysosporium* (Chivukula and Renganathan, 1995) and MnP from *P. eryngii* (Heinfling *et al*., 1998) and *P. ostreatus* (Shrivastava *et al*., 2005).

Factors influencing dye decolourization: Various factors like concentration of dyes, biomass concentration and pH, static and shaking conditions greatly affect the decolourization process. In the present study, the decolourization of bromophenol blue, brilliant green and methylred was investigated with *Pleurotus* sp. (*P. florida* LCJ 65, *P. ostreatus* LCJ 183 and *P. sajorcaju* LCJ 184) using different concentrations of the dyes (10 to 80 mg L^{-1}). At lower concentration of selected dyes (10 μg mL^{-1}), detectable high decolourization percentage was observed in *P. florida* LCJ 65 (16-34%), *P. ostreatus* LCJ 183 (13-41%) and *P. sajorcaju* LCJ 184 (17-48%), respectively. Thus, the percentage of removal of dyes decreased with an increase in dye concentration (Table 2). Chen *et al*. (2003) reported that higher concentration of azo dye inhibits nucleic acid biosynthesis and cell Growth. Similarly, Pearce *et al*. (2003) suggested that the dye concentration influenced the efficiency of dye removal through a combination of factors, including the toxicity of the dye at higher

Table 2: Effect of different factors on dye decolourization using *Pleurotus* sp. on 6th h of incubation

	Decolourization (%)								
	P. florida LCJ65			*P. ostreatus* LCJ183			*P. sajorcaju* LCJ184		
Experiments	BG	BB	MR	BG	BB	MR	BG	BB	MR
Concentration of dye (mg L^{-1})									
10	16	43	34	13	32	41	17	32	48
20	11	40	20	18	23	33	12	21	24
40	11	40	20	10	23	39	09	21	06
60	11	20	19	05	20	35	10	16	07
80	11	19	16	06	16	28	02	07	04
Inoculum size (g L^{-1})									
0.25	12	42	41	00	24	13	12	11	05
0.50	23	46	49	03	31	17	20	12	12
1.00	30	49	61	04	32	19	23	49	14
1.50	32	54	68	06	40	20	25	61	20
2.00	49	82	89	12	56	21	27	69	33
pH									
pH 4	04	56	18	03	18	03	05	47	29
pH 5	27	70	16	22	48	13	29	60	38
pH 6	25	64	29	19	37	15	36	69	63
pH 7	05	52	16	03	18	06	08	19	38
pH 8	08	45	16	03	13	09	08	17	11
Static	07	39	15	18	52	03	08	52	14
Shaking	80	53	21	59	60	65	29	59	50

(BB: Brilliant green, BB: Bromophenol blue, MR: Methyl red)

concentrations. Khehra *et al.* (2005) also suggested that the decrease in decolorization efficiency might be due to the toxic effect of dyes. In a study by Yesilada *et al.* (2002) to research the decolorization ability of Astrazon FBL culture medium containing *Funalia trogii* pellets, it was found out that the decolorization percentage increased in parallel with increase in the dye concentration to 66 mg L^{-1} and that it started to decrease when the dye concentration exceeded 66 mg L^{-1}. Another study by Yesilada *et al.* (2003) reported that *P. ostreatus* shows 97, 89 and 84% decolorization for azo dyes (264 mg L^{-1}), namely, astrazone red, astrazone blue and astrazone black, respectively. Kalmis *et al.* (2008) reported that wild type of two *P. ostreatus* strains (MCC020 and MCC007) showed 83.6 and 81.8% decolorization for Benazol Black ZN textile dye (1000 mg L^{-1}).

Table 2 shows the effect of inoculums size (0.25 to 2 g of actively grown biomass) and time on decolourization of bromophenol blue, brilliant green and methylred at 10 μg mL^{-1} initial concentration. It is clear from the table that percentage removal of dye increased with an increase in time for all concentration of inoculums. After 6 h the percentage removal of dyes using *P. florida* LCJ 65, *P. ostreatus* LCJ 183 and *P. sajorcaju* LCJ 184 was found to be 0 to 96. In present study, *P. ostreatus* LCJ 183 (0-56%) exhibited minimal colour decolourization when compared to *P. florida* LCJ 65 (12-89%) and *P. sajorcaju* LCJ 184 (5-79%). Thus, the present result clearly emphasizes that when the quantity of biomass increased the dye decolourization percentage also increased. This was consistent with the study by Assadi *et al.* (2001). Yesilada *et al.* (2002) reported that when *F. trogii* pellets were incubated in culture medium containing Astrazon FBL; with high inoculums concentration the decolorization percentages increased as well. Benito *et al.* (1997) in their study using *T. versicolor* determined that by increasing the inoculums concentration the decolorization rate increases accordingly.

Effect of pH (4 to 8) on decolourization in various time intervals using the dyes (bromophenol blue, brilliant green and methylred) at 10 μg mL^{-1} initial concentration of dye is tabulated in Table 2. The table clearly shows that the percentage removal of dye increased with increase in time irrespective of pH. The maximum removal (60-70%) of selected dyes was found at pH 5 and 6 after 6 h of incubation period. Further increase in pH beyond 6 and decrease in pH below 5 resulted in decreased percentage removal of dye. The optimum pH was found to be between 5 and 6 for maximum removal of dyes. Chen *et al.* (1999) reported that pH has a major effect on the efficiency of dye decolorization and the optimal pH for colour removal is often between 6.0 and 10.0 for most of the dyes. Pearce *et al.* (2003), reported that the optimum pH for colour removal by white-rot fungi was often at a neutral or slightly alkaline pH and the rate of colour removal tended to decrease rapidly under strongly acid or strongly alkaline conditions, without any relationship to dye structure. Few studies by Young and Yu (1997) showed that an azo-based dye was more efficiently degraded by white-rot fungi under acidic conditions.

Cultural stability proved to have an important role in fungal growth and is associated with dyes removal and this is observed in the present study. Decolourization of dyes using *Pleurotus* sp. (*P. florida* LCJ 65, *P. ostreatus* LCJ 183 and *P. sajorcaju* LCJ 184) was significantly increased when shifted from static to shaking treatment (Table 2). Under shaking conditions *Pleurotus* sp. (*P. florida* LCJ 65, *P. ostreatus* LCJ 183 and *P. sajorcaju* LCJ 184) exhibited higher removal of bromophenol blue, brilliant green and methylred (50-80%). On the contrary, the same organisms showed lower removal of dyes in static condition (3-52%). Knapp *et al.* (1997) reported that there was only 45% of decolourization of Orange II after 23 h incubation in static conditions whereas in shaking conditions 97.5% decolourization occurred. Fungi being aerobic organisms normally show better dyes degradation activities under facilitated agitated condition (Faison and Kirk, 1985; Ge *et al*., 2004). Shaking increases the mass (substrate) and oxygen transfer from the culture medium to the cells, thereby, it facilitates optimum fungal growth and enzyme (oxidase) production (Swamy and Ramsay, 1999).

CONCLUSION

As a conclusion, the present studies indicate that *Pleurotus* sp. is capable of efficient decolourization of a wide range of chemically different dyes. Among *Pleurotus* sp. tested, *P. florida* and *P. sajacaju* was found to be more effective than *P. ostreatus* in decolourizing bromophenol blue, brilliant green and methylred. Therefore *Pleurotus* sp. is one of the promising eco-friendly tools for the dye containing effluent treatment of textile industries. So, the colour removal mechanism of textile dyes by ligninolytic enzymes of *Pleurotus* sp. needs further research.

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