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Full Length Research Paper

Colour removal of textile dyes by culture extracts obtained from white rot fungi

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In this study, the decolourizations of 36 different textile dyestuffs were studied with 6 different white rot fungi by solid state fermentation. Extra cellular enzymes secreted by fungi and total protein analyses were carried out in culture extracts obtained, according to the enzyme analyses, the highest activity was found in *Laetiporus sulphurous* and *Coriolus versicolor*. In our experiments involving dyes, it was found that 2 different dyestuffs were removed with culture extracts of various fungi but the most rapid and efficient dye remove occurred with *L. sulphurous* and *C. versicolor* for Levafix blue CA and Procion dark blue H-EXL dyestuffs. The optimum conditions for the above mentioned process were pH 4.0 for all media and temperature was 65°C for both fungi culture extracts with Levafix blue CA and 55°C for Procion dark blue H-EXL. The optimum enzyme amount was found to be 35 and 25 l for *L. sulphureus* using Levafix blue CA and Procion dark blue H-EXL respectively. It was found as 45 and 60 l for *C. versicolor.* The results show that the enzyme catalyzing the reactions of dye remove of Levafix blue CA and Procion dark blue H-EXL was laccase, when comparing with the information in literature.

Key words: Colour removal, textile dyes, white rot fungi, *Laetiporus sulphurous, Coriolus versicolor,* solid state fermentation.

INTRODUCTION

The wastewaters discharged from textile industries are the most pollutant wastes both as volume and waste composition. The dangerous wastes cause the biggest ecological problems (Robinson et al., 2001). Colour is the first contaminant to be recognized in wastewater and has to be removed before discharging into waterways or land (Ozyurt and Atacag, 2003). Recent fundamental works have shown the existence of many micro organisms capable of decolourizing wide range of dyes. Various and same micro organisms have been used in our previous works for this purpose (Atacag et al., 2004; Ünyayar et al., 2005; Mazmanci and Ünyayar, 2005; Atacag and Ozyurt, 2005; Ozyurt et al., 2005, Ozsoy et al., 2005; Erkurt et al., 2007) The biological methods for the decolourization of dyes are aerobic and anaerobic biodegradation, biological oxidation, reduction, adsorption on biological surfaces and enzymatic remediation (Kargı, 1992). In the world, each year about 10000 various dyes and pigments are produced and 5 - 10% of these are discharged into industrial wastes (O'Neill et al., 2000). Schliphake and Lonergan (1996) have used laccase obtained from *Pycoporus cinnabarinus* in colour removal Remazol brilliant blue R used in textile industry. Peroxidases also catalyze various reactions but H_2O_2 is necessary for the activation of them (Breen and Singleton, 1999).

Peroxidase enzymes are involved in the first step of biodegradation process for azo dyestuffs (Perez et al., 1998). *Trametes versicolor, Phanerochaete chrysosporium* and *Phlebia radiate* are the best biodegrading micro organisms (Majcherczyk et al., 1998). Solid state fermentations are used for the production of enzymes (Podgornik et al., 1999; Unyayar et al., 2005). For instance, Kaluskar et al. (1999) have found that *T. versicolor, P. chrysosporium* and *Bjerkandera adusta* could break down all the dyestuffs.

The objective of our study was to determine the effect

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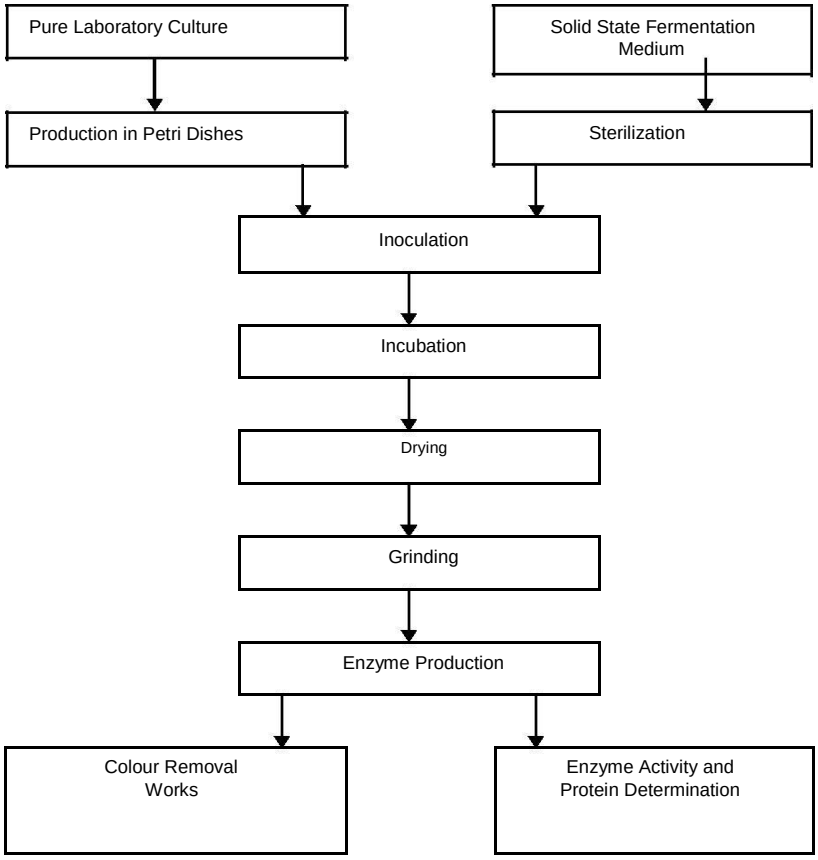

Figure 1. The experiment flow diagram.

MATERIALS AND METHODS

The fungi used in our study have been provided from Environmental Biotechnology Laboratory of Mersin University. The fungi used are *Coriolus versicolor, Laetiporus sulphurous, Pleurotus ostreatus, P. chrysosporium, Pleurotus eryngii* and *Funalia trogii*. They were subcultured each every 20 days on Potato dextrose agar and kept at +4°C after incubated at 30°C for 5 days (Sanyo 500 B). Solid state fermentation technique was used for the production of microorganisms. Wheat bran and soybean hull were used as substrate in solid state fermentation medium. This medium was damped 70 pct in mass.

The solid-substrate fermentation (SSF) medium used for producing the fungal biomass consisted of wheat bran 36 g and soybean flour 4 g. The substrate was humidified with a 0.1 M pH 6.0 sodium phosphate buffer added at 60% v/w. The humidified medium was placed in 1 l Erlenmeyer flasks and autoclaved (120°C, 60 min). The autoclaved medium was inoculated with the fungal stock cultures that had been grown on PDA. The flasks were incubated for 10 days at 30°C. After this, the contents of the flasks were dried (Sanyo MIR 152 incubator) for 24 h at 40°C. The dried material was grind in a coffee grinder for 2 min (Ünyayar et al., 2005).

The culture medium in powder form was kept at pH 6 in KPO_4 buffer for 15 min. The extra cellular enzymes were taken into liquid medium by this process. It was centrifuged at 6000 rpm for 15 min to remove suspended solids and then filtered through what man No.1 filter paper. The culture extract was prepared for enzyme and protein determinations by these processes. Laccase activity was measured using Guaikol (Sigma) as substrate. The specific colour was determined at 436 nm in spectrophotometer (Hack DR 2010). 0.2 M, pH 4.5 sodium phosphate buffer containing 333 M Guaikol was prepared and 2.8 ml of this 0.2 ml enzyme source was added and incubated at 30°C for 30 min and mixed each 10 min. Laccase activity was defined as colorimetric unit (CU) for 0.1 unit increase/min at optic density of incubation mixture (Tanaka et al., 1998).

Peroxidase activity was measured in a similar way with laccase activity. The difference is the preparation of reaction mixture as containing 167 M H_2O_2. Total protein was determined by Lowry method (Soares et al., 2002). Temperature and pH optimizations were made for colour removal studies. The experiment at flow diagram is shown in Figure 1.

RESULTS AND DISCUSSION

The present results have shown that enzymatic methods can be used as an alternative system for colour removal of textile dyes. Many studies (Schlosser et al., 1997; Smith et al., 1998; Garzillo et al., 1998; Koroljova et al., 1999) have also shown that ligninolytic enzyme activities synthesized from white rot fungi by solid state fermentation depending on incubation temperature and pH. The maximum values of ligninolytic activity were pH 6 and 30°C. The laccase activity synthesized from white rot fungi by solid state fermentation was given at Table 1.

The maximum activity was found as 4.70 CU the 10^{th}

Table 1. Laccase activities synthesized from white rot fungi grown by solid state fermentation.

Fungus species	Laccase activity (CU)	Total protein (g/l)	Spesific activity (CU/mg protein)
C. versicolor	4.35	6.26	3.500
L. sulphureus	4.70	3.93	6.000
P. ostreatus	0.13	0.10	6.500
P. chrysosporium	0.02	7.90	0.015
P. eryngii	0.05	4.00	0.065
F. trogii	1.55	6.22	1.250

Table 2. Peroxidase activities synthesized from white rot fungi grown by solid state fermentation.

Fungus species	Peroxidase activity (CU)	Total protein (g/l)	Specific activity (CU/mg protein)
C. versicolor	3.24	6.26	2.60
L. sulphureus	3.35	3.93	4.25
P. ostreatus	0.26	0.10	13.05
P. chrysosporium	0.01	7.90	0.01
P. eryngii	0.03	4.00	0.04
F. trogii	1.07	6.22	0.86

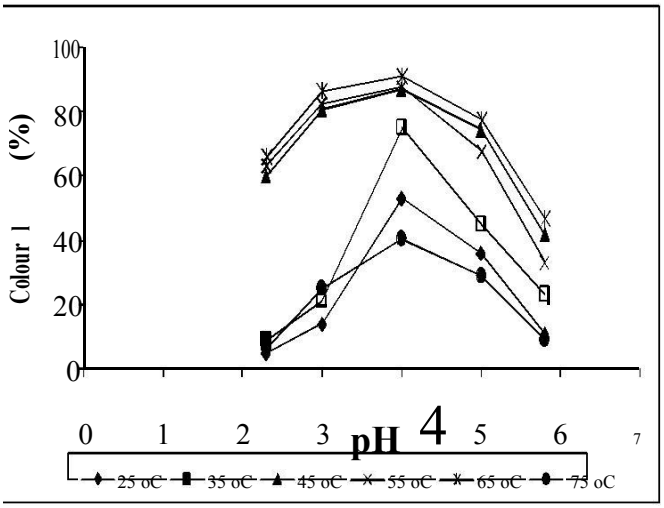

Figure 2. Effect of temperature and pH on colour removal of Levafix Blue CA with culture extract obtained from *Laetiporus sulphurous* (C_0=100 mg/L, culture extract quantity 25 L, incubation time 3 min).

day of incubation for *L. sulphurous*. The peroxidase activity is also given at Table 2.

The maximum peroxidase synthesis has been found at 3.35 CU and temperature of 30°C and 10th day of incubation for *L. sulphurous.* It was 0.38 CU for mangan peroxidase with *L. sulphurous* and 0.38 for lignin peroxidase with *F. trogii.* As would be seen in Figure 2, the temperature and pH where the maximum colour removal of Levafix blue CA was for 65°C temperature and for pH 4. Also with Procion dark Blue H-EXL was 55°C and pH 4 (Figure 3). Our results have also found that colour removal increased with increasing dyestuff concentration. This result can be seen in Figure 4. Figure 5 shows the effect of temperature and pH on colour removal of Levafix blue CA with culture extract obtained from *C. versicolor.* The maximum colour removal was found at 65°C and pH 4. Figure 6 also shows the effect of temperature and pH on colour removal of Procion dark blue H-EXL with culture extract obtained from *C. versicolor.* The maximum colour removal was found at 55°C and pH 4. In Figure 7, the effect of dyestuff concentration on colour removal with the culture extract obtained from *C. versicolor* with Levafix blue CA is seen. Similar result was reported by Sun et al. (2009). Among the 6 various white rot fungi tested during our work, *L. sulphurous* and *C. versicolor* have showed the highest activities with Levafix blue CA and Procion dark blue H-EXL at the end of enzyme analyses. The optimum enzyme quantity obtained with *L. sulphurous* was found to be 35 l for Levafix blue CA and 25 l with Procion dark blue H-EXL. With *C. versicolor*, 45 l for Levafix blue CA and 60 l for Procion dark blueH-EXL. In optimum conditions, in case of increasing the con-centration of dyestuff, the colour removal also increased. In the work carried out with *L. sulphurous* , the colour removal was in balance for Levafix Blue CA and Procion dark blue H-EXL in the concentrations of 300 mg/l and over, in sequence about 207 and 215 mg/l. For *C. versicolor*, the colour removal was in balance for Levafix blue CA and Procion dark blue H-EXL in concentrations

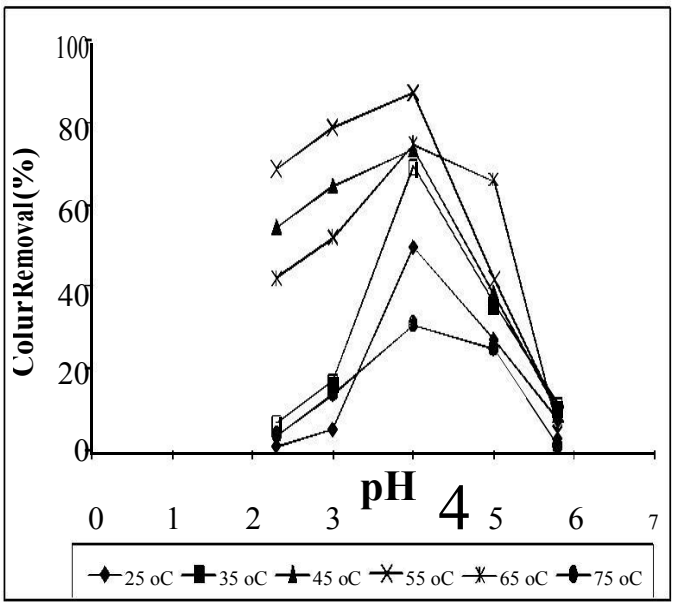

Figure 3. Effect of temperature and pH on colour removal of Procion Dark Blue H-EXL with culture extract obtained from *Laetiporus sulphurous* (C_o=100 mg/L, culture extract quantity 25 L, incubation time 3 min).

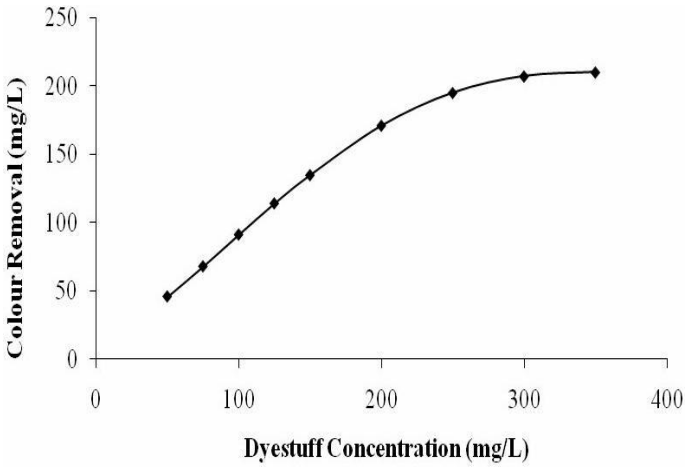

Figure 4. Effect of dyestuff concentration on colour removal of Levafix Blue CA with culture extract obtained from *Laetiporus sulphurous* (pH 4.0, temp. 65°C, culture extract quantity 35 L, incubation time 3 min).

of 150 mg/l and over and in sequence about 79 and 90 mg/l.

We used reaction mixture containing crude filtrate and dye solution but no H_2O_2, mediators and decolourisation reactions were carried out at temperatures between 30 - 60°C (data not shown). Thus, observation of decolourisation made us think that this reaction was not due to peroxidases. This result was supported by earlier studies (Deveci et al., 2004; Unyayar et al., 2005).

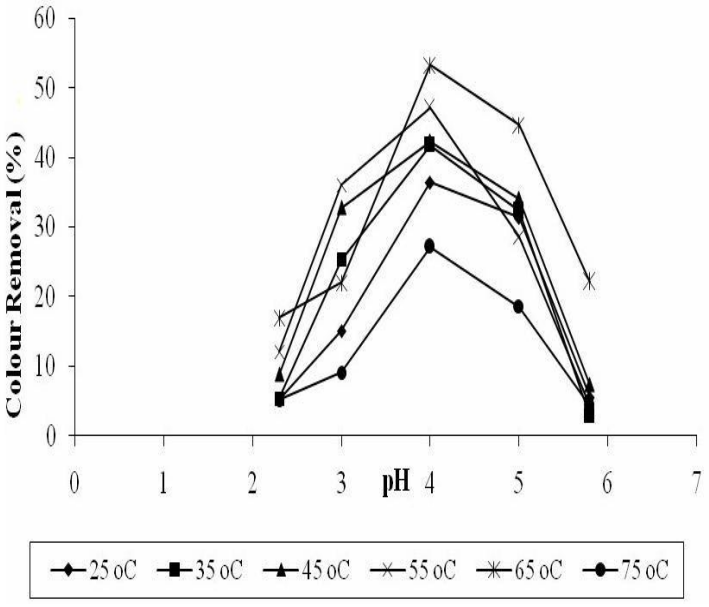

Figure 5. Effect of temperature and pH on colour removal of Levafix Blue CA with culture extract obtained from *Coriolus versicolor* (C_o=100 mg/L, culture extract quantity 25 L, incubation time 3 min)

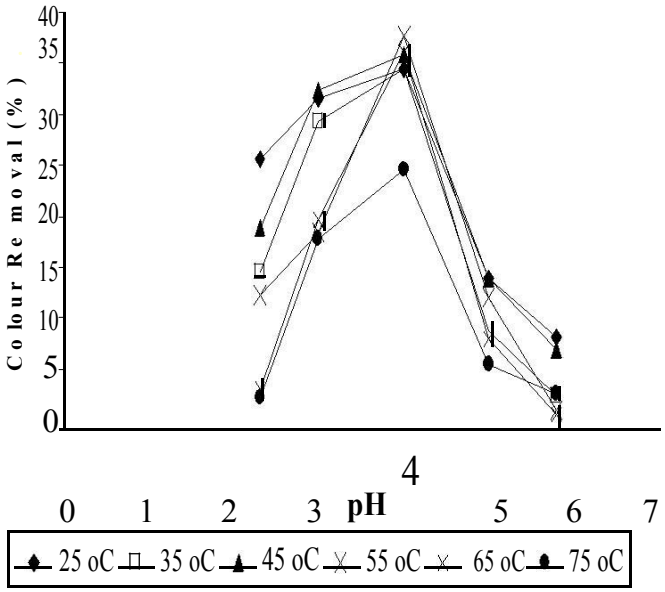

Figure 6. Effect of temperature and pH on colour removal of Procion Dark Blue H-EXL with culture extract obtained from *Coriolus versicolor* (C_o=100 mg/L, culture extract quantity 25 L, incubation time 3 min).

Conclusion

The work for the colour removal of textile dyestuffs has shown that this process should be realized with white rot fungi efficiently. Laccase would be possibly the catalyzing

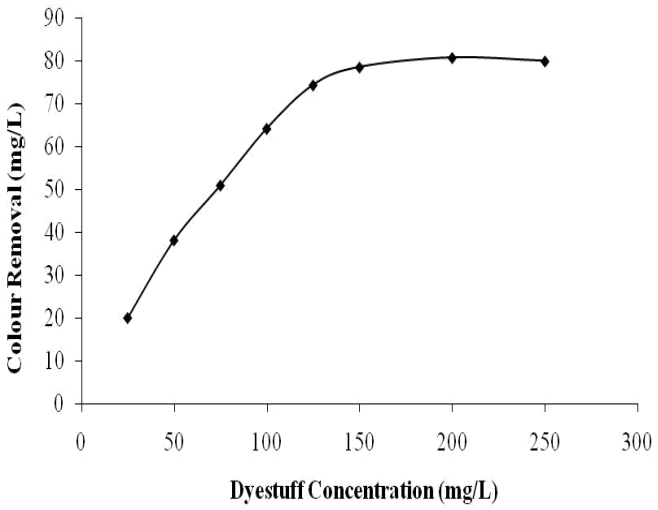

Figure 7. Effect of dyestuff concentration on colour removal of Levafix Blue CA with culture extract obtained from *Coriolus versicolor* (pH 4.0, temp. 65° C, culture extract quantity 45 L, incubation time 3 min).

enzyme for the colour removal reactions of Levafix blue CA and Procion dark blue H-EXL. The enzymes would play a significant role for the treatment processes working in a reactor instead of treatment units. As a result, the mechanical equipments will be reduced and also pre-investment expenses will drop. The biotechnological methods presented in this work would be expected to reduce the operational cost.

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