VALORIZATION AND BIODECOLORIZATION OF DYE ADSORBED ON LIGNOCELLULOSICS USING WHITE ROT FUNGI

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Biosorption of dyes by lignocelluloses may be an effective method for removing dyes from textile effluents. However, the resulting dye-adsorbed lignocellulosic materials may constitute another pollution problem. An integrated method can solve this problem. Here, various lignocelluloses were tested for their Astrazon Black and Astrazon Blue dyes removal activities. The dye adsorbed after 30 min contact time was 90% (45 mg/L), 70% (35 mg/L), and 98% (49 mg/L) for wheat bran, pine cone, and cotton stalk, respectively. These dye-adsorbed lignocellulosic wastes then were used as solid substrates to produce laccase enzyme with *Funalia trogii* and *Trametes versicolor* under solid state fermentation (SSF). Among the lignocellulosic substrates, the dye-adsorbed wheat bran served as the best solid substrate for laccase production under SSF. Therefore, it was also tested as a solid source for laccase production under submerged fermentation. During solid state fermentation, these two fungi were able to highly decolorize these dyes. While *F. trogii* decolorized 80% of Astrazon Black dye adsorbed onto wheat bran, *T. versicolor* decolorized 86%. On the other hand, the decolorization values for Astrazon Blue dye were 69% and 84%, respectively.

Keywords: Biomaterials; Biosorption; Decolorization; Fermentation; Laccase; Lignocellulosics; Textile dye; White rot fungi

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INTRODUCTION

Textile wastewaters contain dyes, which have negative impacts on ecosystem (Hu and Wu 2001). These wastewaters are usually discharged into water bodies. When dyes are discharged to the environment they can show their toxic effect on organisms (Birhanli and Ozmen 2005). Dyes in rivers or lakes reduce the sunlight penetration, thereby reducing the photosynthetic activity and dissolved oxygen concentration. Therefore, discharge of dyes into water bodies needs to be prevented. Many different physical and chemical methods are used for elimination of dyes from wastewaters. However, these methods have some disadvantages. Therefore, new alternative environmentally friendly methods must be developed.

Biosorption (adsorption) by lignocelluloses may be an alternative method for removing dyes from effluents. There are studies on biosorption potential of various species of lignocellulosic biomass (Kahraman and Yalcin 2005; Tunc et al. 2009; Wang

and Chen 2009; Sen et al. 2011). Because dye-adsorbed lignocelluloses create another source of pollution, adsorption of dyes onto lignocelluloses does not completely solve this problem. In light of this situation, a two-step method including the combination of biosorption and solid state fermentation (SSF) may be an alternative and effective solution.

Lignocellulosic substrates could be utilized as solid substrate during solid state fermentation (SSF) (Moldes et al. 2003; Rodriguez-Couto and Sanroman 2005; Shah et al. 2005; Stajic et al. 2006; Pant and Adholeya 2007; Boran and Yesilada 2011). A lot of studies for enzyme production with SSF have been reported. Laccase, which is an important enzyme, can also be produced with this method. This enzyme has a great potential for textile dyes decolorization, xenobiotic removal and degradation, production of organic materials, and preparation of biosensors, etc. (Strong and Claus 2011). White rot fungi, a main class of laccase producer organisms, can be cultured on solid substrates.

Therefore, these two methods, biosorption and biodecolorization by solid state fermentation, could be integrated for effective biodecolorization. This two-step method has potential to be a suitable process for dye removal by lignocelluloses and for laccase enzyme production by white rot fungi for biodecolorization of dye-adsorbed lignocelluloses. Although there are lots of studies on dye decolorization activity of white rot fungi during liquid fermentation; however there have been limited studies on dye biodecolorization and laccase production by this kind of two-step method (Nigam et al. 2000; Robinson and Nigam 2008; Rodriguez-Couto et al. 2009; Kadam et al. 2011). Our previous work showed that *Funalia trogii* ATCC 200800 and *Trametes versicolor* ATCC200801 are effective dye decolorizers under liquid fermentation conditions (Yesilada et al. 2003). But, there has been no study on dye decolorization activity of these strains during solid state fermentation. Moreover, there has been no study on laccase production using Astrazon dye-adsorbed wheat bran and on decolorization of Astrazon dye-adsorbed wheat bran by white rot fungi. Therefore, the aim of this study was to remove some textile dyes by using various types of lignocelluloses, use dye-adsorbed lignocelluloses as a solid substrate to produce laccase enzyme, and also to decolorize dye-adsorbed lignocellulose by white rot fungi.

EXPERIMENTAL

Materials

Organisms

Two white rot fungi, *Funalia trogii* (ATCC 200800) and *Trametes versicolor* (ATCC 200801), sub-culturing every 2 to 3 weeks on Sabouraud Dextrose Agar (SDA) plates, were used.

Dyes

Astrazon Black and Astrazon Blue dyes obtained from a local textile factory, Malatya, Turkey were used. These are basic textile dyes. Astrazon Black is a mixture of C. I Basic Blue 159, Basic Blue 3, Basic Red 46, and Basic Yellow 28 dyes. Astrazon Blue dye is mixture of C. I Basic Blue 159 and Basic Blue 3 dyes (Yesilada et al. 2010).

Adsorbent

Cotton stalk, pine cone, and wheat bran were used as adsorbents. The adsorbents were ground and sieved to obtain a size fraction of about 0.20 to 0.40 mm.

Methods

Adsorption of dyes

Dye stock solutions of 50 mg/L were prepared with distilled water. The adsorbents (1 g) were added separately into 250 mL flasks containing 100 mL of dye stock solution and incubated at 30 °C and 150 rpm for 30 min. At the end of the incubation period the suspensions were centrifuged and then the supernatant solutions were analyzed. The adsorption of dyes on adsorbents was measured by monitoring the absorbances of these solutions at their wavelengths of maximum absorbance, and adsorption was expressed in terms of percentage compared with control.

Preparation of inoculum

Fungi were cultured at 30 °C in tubes on Sabouraud-dextrose agar. After 1 week, mycelium suspensions of each culture (*F. trogii* and *T. versicolor*) were prepared by adding 10 mL of sterilized distilled water to the SDA slant culture and rubbing the mycelia with sterilized loop. Then, 5 mL of suspension was transferred into a 250 mL flask containing 100 mL Sabouraud Dextrose Broth and incubated at 30 °C, 150 rpm for 5 days. Then, these cultures were homogenized and used as inocula.

Solid State Fermentation, Sampling and Extraction Studies

For solid state fermentation (SSF) studies, Erlenmeyer flasks (250 mL) containing 1.0 or 1.5 g dye-adsorbed lignocelluloses (1.0 g for cotton stalk and pine cone, and 1.5 g for wheat bran) were moistened with 5 mL, for cotton stalk and pine cone, and 10 mL for wheat bran, of Stock Basal Medium (SBM) as moistening agent and autoclaved at 121 °C for 20 min. SBM consisted of (g/L): KH_2PO_4 0.2; $CaCl_2.2H_2O$ 0.1; $MgSO_4.7H_2O$ 0.05; $NH_4H_2PO_4$ 0.5; $FeSO_4.7H_2O$ 0.035; Glucose 2; and Yeast extract 1 (Yesilada et al. 2010). After autoclaving, the flasks were inoculated separately with 2 mL of each inoculum and incubated at 30 °C under static conditions for 20 days with enzyme activities measured at 5 d intervals. For the laccase activity assay, 15 mL of distilled water was added to solid cultures and they were agitated on rotary shaker for 2h at 30 °C. Then, the cultures were filtrated and centrifuged at 4000 rpm for 15 min. After that, the supernatants were used for the laccase activity assay.

Liquid State Fermentation Studies

For LSF studies, 5 mL of each inoculum which was prepared as stated above was transferred into 50 mL of growth medium containing 1 g of dye-adsorbed wheat bran. All cultures were incubated at 30 °C and 150 rpm.

Laccase activity assay

Laccase (E.C. 1.10.3.2) activity was determined spectrophotometrically (Shimadzu-UV-1601, UV/Visible spectrophotometer) by monitoring the increase in absorbance at 420 nm. One unit was defined as the amount of enzyme that oxidized 1

µmol of ABTS [2,2'-Azino-bis (3-ethylbenzthiazoline-6-sulfonic acid)] per minute (Roy-Arcand and Archibald 1991).

Decolorization of dye-adsorbed wheat bran

Dye decolorization was measured by monitoring the absorbance at the maximum absorbance wavelength for each dye solution after 10 days of fungal cultivation, as stated in the Solid State Fermentation, Sampling and Extraction Studies subsection. To calculate the decolorized dye amount from dye-adsorbed wheat bran, a modified method for dye desorption from pellets was used (Yesilada et al. 2003). Here, methanol was added to dye-adsorbed solid cultures as desorption agent, and the mixture was heated. After centrifugation the supernatant was used to measure the dye decolorization. Dye-adsorbed wheat bran without fungal inoculation was used as control.

All values are the mean of at least three replicates, with standard deviation of the mean shown as ± values.

RESULTS AND DISCUSSION

Adsorption of Dyes to Lignocellulosics

Dye adsorption abilities of various types of low-cost, ecofriendly lignocelluloses were tested. The dye adsorbed after 30 min contact time was 90% (45 mg/L), 70% (35 mg/L), and 98% (49 mg/L) for wheat bran, pine cone, and cotton stalk, respectively. The adsorption value obtained with wheat bran was similar to the value reported for Malachite Green removal capacity of wheat bran (Papinutti et al. 2006). Kadam et al. 2011 used rice bran as a cheap adsorbent for Reactive Navy Blue HE2R removal and reported about 90% removal. Although there have been many studies on dye removal by biosorption methods, there are limited studies on valorization and biodecolorization of dye-adsorbed lignocellulosic biomass. Therefore, these dye-adsorbed lignocellulose substrates were used as a growth medium for laccase production by two white rot fungi.

Laccase Production under Solid State Fermentation Condition

It was previously reported that dye-adsorbed lignocellulose substrates could be used for fungal growth and laccase production (Nigam et al. 2000; Robinson and Nigam 2008; Rodriguez-Couto et al. 2009). Therefore, after biosorption studies, the dye-adsorbed lignocellulose substrates were used for laccase production during solid substrate fermentation. *F. trogii* and *T. versicolor*, which have high laccase production capacities, were utilized as laccase producer organisms. *F. trogii* produced 2.78 U/mL laccase enzyme on day 10 in Astrazon Black dye-adsorbed wheat bran medium, and the obtained laccase activity decreased after this day (Fig. 1). When Astrazon Black dye-adsorbed wheat bran medium was used, *T. versicolor* produced 2.74 U/mL laccase enzyme on day 10, and the highest value was detected on day 20 as 3.17 U/mL (Fig. 2). In Astrazon Blue dye-adsorbed wheat bran medium, the highest values were 3.23 and 3.27 U/mL for *F. trogii* and *T. versicolor*, respectively (Figs. 1 and 2). It was reported that Malachite Green-adsorbed wheat bran could be used as a solid substrate to produce lignin peroxidase enzyme with *Fomes sclerodermeus* (Papinutti et al. 2006).

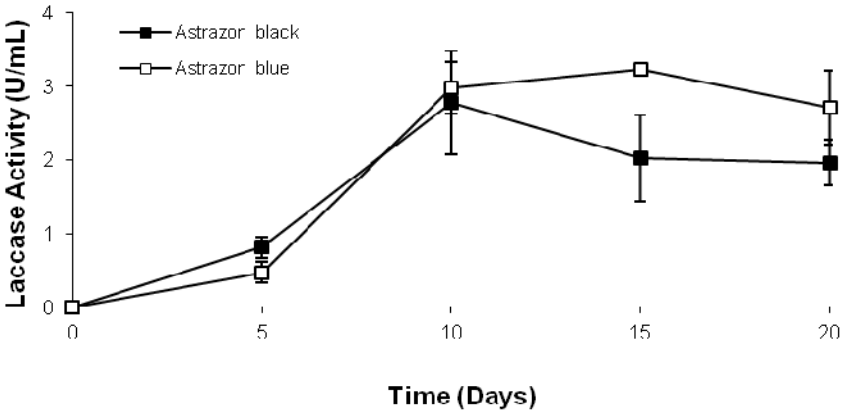

Fig. 1. Laccase production by *F. trogii* in dye-adsorbed wheat bran media

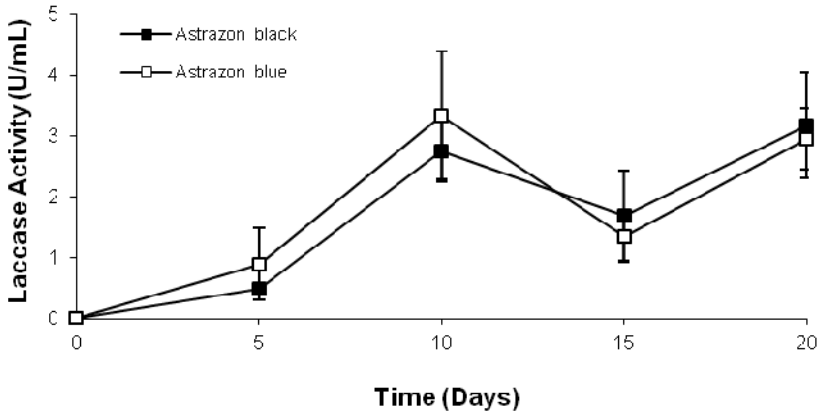

Fig. 2. Laccase production by *T.versicolor* in dye-adsorbed wheat bran media

The laccase activities obtained in Astrazon Black and Astrazon Blue dye-adsorbed cotton stalk media were lower than the activities obtained in dye-adsorbed wheat bran media. With Astrazon Black, the values obtained after 10 days of incubation were only 0.67 U/mL and 0.03 U/mL for *F. trogii* and *T. versicolor*, respectively. These values obtained in Astrazon Blue dye-adsorbed media were detected as 0.43 U/mL and 0.02 U/mL for *F. trogii* and *T. versicolor*, respectively. Laccase activity of 0.34 U/mL was reported with consortium of *Pseudomonas* sp. SUK1 and *Aspergillus acheaceus* NCIM-1146 by solid state fermentation (Kadam et al. 2011). On the other hand, about 7 U/mL laccase activity was reported with *Trametes pubescens* by semi solid substrate fermentation of Reactive Black 5 dye-adsorbed sunflower seed shells (Rodriguez-Couto et al. 2009). It was also reported that addition of inducers, such as copper, induces the laccase production. In the current study, the laccase activity values were obtained without any additional mediators or inducers.

The dye-adsorbed waste pine cone for laccase activity production with these two fungi was also tested. Laccase activity values detected in dye-adsorbed pine cone powder media on day 10 did not exceed 1.0 U/mL for both fungi (Fig. 3).

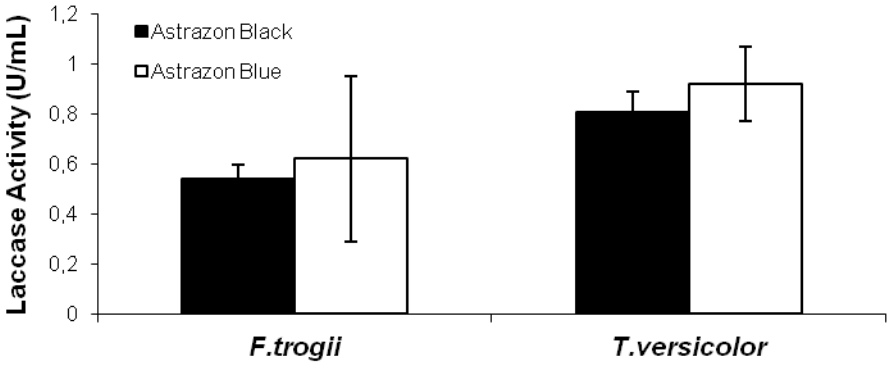

Fig. 3. Laccase production by *F. trogii* and *T. versicolor* in dye-adsorbed pine cone media

This result showed that it was possible to use all these dye-adsorbed lignocellulose substrates for production of laccase during solid state fermentation. However, laccase production efficiency is strain- and substrate-dependent (Tychanowicz et al. 2004). Among three substrates tested, dye-adsorbed wheat bran was determined to be the best solid substrate for laccase production during solid state fermentation. Because wheat bran contains a high amount of carbohydrates that can be utilized as the carbon source, it is a good substrate for fungal growth (Papinutti et al. 2006; Sahakeri et al. 2007). The other two have high lignin contents (Akpınar et al. 2007; Lamsal et al. 2011; Sahin and Arslan 2011). Therefore, they are poor substrates for fungal growth and laccase production. Based on the obtained laccase activities, dye-adsorbed wheat bran was selected for further study to test its effect on laccase production during liquid fermentation.

Laccase Production under Liquid State Fermentation Condition

Solid substrates such as wheat bran can be used as an additional substrate and immobilization matrix for liquid state fermentation (Sahakeri et al. 2007). Three different media were used for liquid state fermentation study: SBM medium without solid substrate, SBM medium with Astrazon Black dye-adsorbed wheat bran, and SBM medium with Astrazon Blue dye-adsorbed wheat bran. The supplemention of dye-adsorbed wheat bran enhanced laccase production. *F. trogii* and *T. versicolor* produced about 1.0-2.0 U/mL of laccase enzyme on day 10 when dye-adsorbed wheat bran was used (Table 1). Laccase activity obtained from *F. trogii* was 0.68 U/mL in liquid cultures without dye-adsorbed wheat bran. But, these values were detected as 1.89 U/mL and 1.96 U/mL in Astrazon Black and Astrazon Blue dye-adsorbed wheat bran media, respectively. Therefore, dye-adsorbed solid substrate significantly stimulated the laccase activity of these fungi in comparison with cultures without dye-adsorbed substrate.

Table 1. Laccase Production by *F. trogii* and *T. versicolor* after 10 days of Incubation

	Laccase Activity (U/mL)	
Culture Media	*F. trogii*	*T. versicolor*
SBM medium without solid substrate	0.68 ± 0.26	0.46 ± 0.09
SBM medium with Astrazon Black dye-adsorbed wheat bran	1.89 ± 0.17	1.61 ± 0.29
SBM medium with Astrazon Blue dye-adsorbed wheat bran	1.96 ± 0.25	1.11 ± 0.22

Decolorization of Dye-Adsorbed Wheat Bran

Because dye-adsorbed lignocelluloses creates pollution problems, white rot fungi could be used to decolorize dye-adsorbed lignocelluloses under solid or semi solid state fermentation. Papinutti et al. (2006) reported that *Fomes sclerodermeus* could decolorize Malachite Green-adsorbed wheat bran during solid state fermentation.

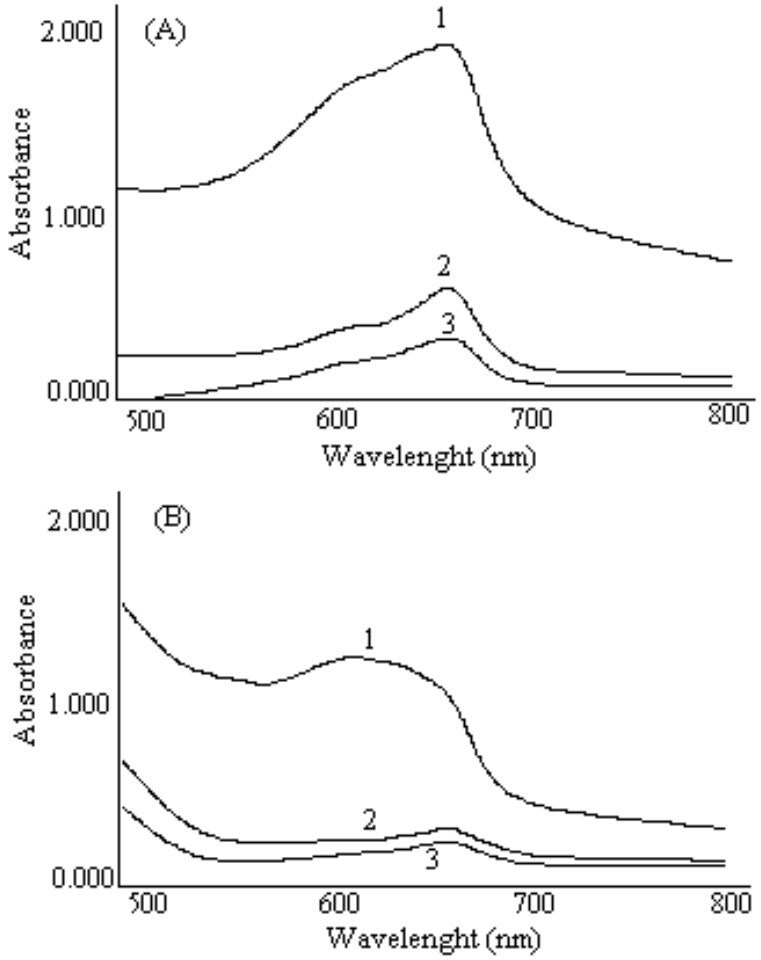

Fig. 4. Adsorption spectra of desorption media from *F. trogii* and *T. versicolor* incubated on Astrazon Blue dye-adsorbed wheat bran (A) and Astrazon Black dye-adsorbed wheat bran (B). Control (1), desorption medium from *F. trogii* (2) and *T. versicolor* (3)

It was also found that decolorization degrees between 49 and 94% could be obtained under semi-solid-state fermentation of sunflower seed shells by *Trametes pubescens* (Rodriguez-Couto et al. 2009). This method can be a suitable process for dye removal from dye-adsorbed lignocelluloses, and for laccase enzyme production by white rot fungi. Therefore, dye decolorization activities of these two fungi in dye-adsorbed wheat bran media were investigated with solid state fermentation. During solid state fermentation, these fungi were also able to decolorize the dyes adsorbed onto solid substrate. Figure 4 shows the absorption spectra of desorption media from fungal cultures. As shown in this figure, the spectra of the dyes were highly diminished after incubation the dye-adsorbed wheat bran with fungi. While *F. trogii* decolorized 69% of Astrazon Blue dye adsorbed onto wheat bran, *T. versicolor* decolorized 84%. On the other hand, the decolorization values for Astrazon Black dye were 80% and 86%, respectively.

Kadam et al. (2011) tested the performance of microbial consortium for Reactive Navy Blue HE2R-adsorbed rice bran, wheat bran, maize bran, and gram bran decolorization activity and reported 92, 52, 60, and 28% decolorization values, respectively. On the other hand, this consortium could not decolorize dye adsorbed on wood husk due to the lesser nutrient content in this lignocellulose. It was reported that mixed dyes adsorbed on barley husks could be decolorized by 14% and 53% at days 15 and 21, respectively, with *Bjerkandera adusta*, and this fermented material could be used as a soil conditioner due to its richness in lignolytic enzymes and fungal biomass (Robinson and Nigam 2008). Results of the current study also showed that these two fungi could decolorize the dyes adsorbed on solid substrate.

CONCLUSIONS

1. Cotton stalk, pine cone, and wheat bran could be used as adsorbent for dye adsorption.
2. *F. trogii* and *T. versicolor* were able to grow on dye-adsorbed wheat bran. Therefore, Astrazon dye-adsorbed lignocellulose could be used as a substrate for fungal growth and laccase production. Laccase production by this method is easy.
3. These two fungi were also able to decolorize dyes adsorbed on wheat bran. Therefore, this integrated method, biosorption and enzyme production during decolorization, could be used to reduce the amount of color in textile wastewater and to produce laccase enzyme during decolorization of dyes adsorbed on lignocellulosic wastes.

ACKNOWLEDGMENTS

The authors are grateful for the support of the Inonu University research fund.

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Article submitted: November 6, 2011; Peer review completed: January 8, 2012; Revised version received and accepted: February 12, 2012; Published: February 15, 2012.