Decolorization of Textile Wastewater by Immobilized *Coriolus versicolor* RC3 in Repeated-Batch System with the Effect of Sugar Addition

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ABSTRACT

Synthetic textile wastewater and real wastewater from Batik dyeing process were decolorized by immobilized white-rot fungus Coriolus versicolor RC3 in repeated-batch system. Synthetic wastewater containing 150 ppm of commercial textile dye and 0.5 vvm of aeration was used with 10-liter air-bubble bioreactor. Immobilized cells of the fungus were prepared in potato dextrose broth with 1.5 cm^3 polyurethane foam, incubated at 37°C on 120 rpm orbital shaker for 4 days and used as inoculum. It was found that three cycles of repeated-batch decolorization were obtained with more than 90% decolorization in 24 hours when a half of wastewater was removed and replaced with new fresh dye. Addition of various carbon sources such as glucose, sucrose and molasses in repeated-batch decolorization was also investigated and it was found that 3 g/l of sucrose was selected as the suitable carbon source and 14 cycles of repeated-batch decolorization could be achieved. Decolorization of Batik wastewater obtained from the factory in Lamphun province was also investigated in the same manner. The immobilized fungal cells decolorized up to 80% and 67% of COD value was reduced.

Key words: Decolorization, *Coriolus versicolor*, Laccase, Immobilized cells, Textile wastewater

INTRODUCTION

Wastewater from textile industries constitute a threat to the environment in large parts of the world, as the degradation products of textile dyes are often carcinogenic. In addition, light absorption hindered by textile dyes creates problems to photosynthetic aquatic plants and algae. The main important pollutants in textile effluent are recalcitrant organic compounds, color, toxicant and inhibitory compounds, surfactants and chlorinated compounds. During processing, 5-20% of the used dyestuffs are released into the process water (Wong and Yu, 1999; Soares et al., 2001) and dye is the most difficult constituent to treat by conventional biological wastewater treatment. In addition to their visual effect and their adverse impact in terms of chemical oxygen demand, many synthetic dyes are toxic, mutagenic and carcinogenic

(Chung et al., 1992). The current existing techniques for the treatment of wastewater containing dyes have high cost, formation of hazardous by-products or intensive energy requirement (Stolz, 2001). Ligninolytic enzymes produced by white-rot fungi are substrate- nonspecific; therefore, can degrade a wide variety of recalcitrant compounds, especially aromatic polluted complex. Extracellular enzyme system enables white-rot fungi to tolerate high concentration of pollutants (Kapdan et al., 2000) that offers significant advantages for decomposition of recalcitrant compounds by this group of fungi. Immobilized microbial cell has received increasing interest in the field of wastewater treatment because of various advantages, including long retention time of biomass in the system, protection from high concentration of recalcitrant organics that are toxic to free cells, high potential to degrade toxic chemicals faster than conventional wastewater treatment systems, ease of use in a continuous reactor and their ability for scale up (Christopher et al., 2002).

In this paper, a thermotolerant white-rot fungus *Coriolus versicolor* RC3, isolated from Chiang Mai, Thailand (Khanongnuch et al., 2004) was immobilized on polyurethane foam. Decolorization of synthetic wastewater containing commercial dye by immobilized *C. versicolor* RC3 in air bubble bioreactor with repeated-batch was determined. In addition, real textile wastewater from Batik dyeing process was also investigated.

MATERIALS AND METHODS

Microorganism and wastewater

White-rot fungus *Coriolus versicolor* RC3 isolated from Chiang Mai, was used in this experiment. One-hundred-and-fifty ppm of commercial dye and textile wastewater from Batik factory in Lamphun were used as synthetic and real wastewater, respectively.

Preparation of immobilized *C. versicolor* RC3, immobilized support and medium

Polyurethane foam (PUF) was cut into 1.5 cm^3 pieces and pretreated by boiling for 10 minutes, washing 2 times with distilled water. After drying overnight at room temperature, pretreated PUF was used as an immobilized support. Potato dextrose agar (PDA) was used to maintain the fungal strain. Immobilized *C. versicolor* RC3 was prepared by transferring 1 g of 1.5 cm^3 of PUF into 50 ml potato dextrose broth (PDB) in 250 ml Erlenmeyer flask and inoculated with one agar plug (diameter, 1 cm) of the fungal strain. The culture was incubated in a rotating shaker at 120 rpm and 37°C for 4 days. The colonized fungal cells on PUF were used as the immobilized cells and inoculum.

Decolorization of synthetic wastewater by immobilized cells in air-bubble bioreactor and effect of sugar addition

Prepared 7.5 liters of dye and 0.5 liter of inoculum in 10-liter air-bubble bioreactor and supplemented with 0.5 vvm of aeration rate by aquarium pump. Sampling and determination of decolorization (%), pH, laccase activity and total sugar were carried out until decolorization reached more than 90%. Drained 50% of dye volume from the reactor and added 50% volume of fresh dye and repeated the previous step again until decolorization was found to be lower than 90% in 24 h. If the total sugar was lower than 0.03 g/l, various sugars such as glucose, sucrose or molasses were added in the reactor.

Decolorization of real wastewater by immobilized cells in 10-liter air-bubble bioreactor

Before the decolorization experiment, real wastewater was characterized for absorbance spectrum at 200-800 nm by spectrophotometer along with pH, COD, total suspended solid, total plate count and color determination. Decolorization was conducted using 7.5 liters of real wastewater and 0.5 liter of inoculum in 10-liter air-bubble bioreactor and supplemented with 0.5 vvm of aeration rate by aquarium pump. With 6 h-interval sampling, dye solution was centrifuged at 10,000 rpm for 10 min and measured the absorbance at 500 nm. Laccase activity and pH were also determined.

Enzyme assay

Laccase activity was determined by oxidation of 2,6-Dimethoxyphynol (DMP), and was determined for an increase in absorbance at 470 nm as described by Khanongnuch et al., (2004). One unit of laccase activity was defined as the amount of enzyme that oxidized 1 μmol of DMP per minute.

RESULTS AND DISCUSSION

Decolorization of synthetic wastewater by immobilized *C. versicolor* RC3 on PUF and effect of sugar addition

Decolorization of synthetic wastewater by immobilized *C. versicolor* RC3 on PUF without carbon source addition is shown in Figure 1. The repeated-batch was started when decolorization was more than 90%. In the first cycle, decolorization reached 94.15% in 22 h while in the second and third cycle, it reached 91.45% and 90.38% in 7 and 14 h, respectively. However, in the fourth cycle, decolorization came up with more than 90% but it took more than 24 h. These results showed that the decolorization capacity was stable only for 3 cycles of decolorization and gave more than 90% of decolorization in 24 h. The total volume of dye removal was 12 litres (150 ppm) in 43 h. However, the laccase activity, which was the main catalyst for dye decolorization activity, was found through out the experiment but the decolorization yield did not keep along with the experiment. This may be due to the reduction of metabolites produced from fungal cells including laccase mediator, a necessary molecule for enzymatic oxidation by laccase (Zille et al., 2005).

The effect of sugar addition with repeated-batch in 10-liter air-bubble bioreactor is shown in Table 1 and Figure 2. It was found that more than 10 cycles of decolorization could be obtained when glucose and sucrose were added. The highest cycle number of decolorization was found with 3 g/l of glucose. Effect of sugar addition on the catalytic activity of laccase still was not mechanically identified, but Galhaup et al., (2002) reported that addition of glucose 10-40g/l was found to increase the laccase activity 5 folds in the cultivation of *Trametes pubescens*. This might be caused from the increase of fungal metabolism and the generation of the redox mediator which involved in the catalytic activity of laccase that caused the increase in dye decolorization. Although addition of 3 g/l glucose showed the best activity of dye decolorization, but addition of 3 g/l sucrose was selected as the suitable concentration in this experiment according to an economic reason. Addition of molasses, the cheapest carbon source, gave only 4 cycles of decolorization at 3 g/l (w/v) addition. Utilization of molasses higher than 3 g/l (w/v) was limited due to the brown color of molasses which strongly interfered with the absorbance measurement.

Table 1. Maximum cycle number of decolorization of synthetic wastewater with sugar.

Sugar addition	Cycle number of decolorization at various concentrations			
	20 (g/l)	5 (g/l)	3(g/l)	1 (g/l)
Glucose	11	15	16	13
Sucrose	10	14	14	11
Molasses	ND	ND	4	2

ND: Not detected

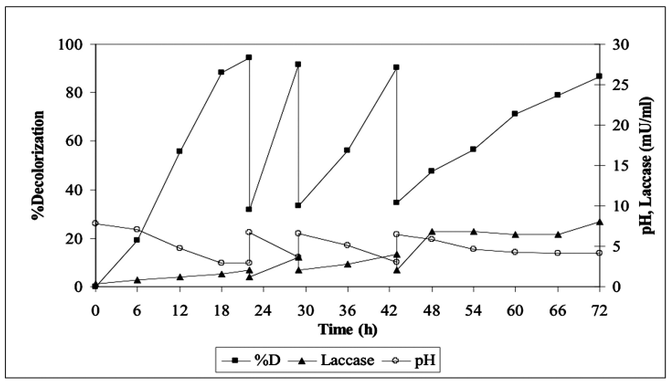

Figure 1. Profile of synthetic dye decolorization using immobilized *C. versicolor* RC3 on PUF in 10-liter air-bubble bioreactor.

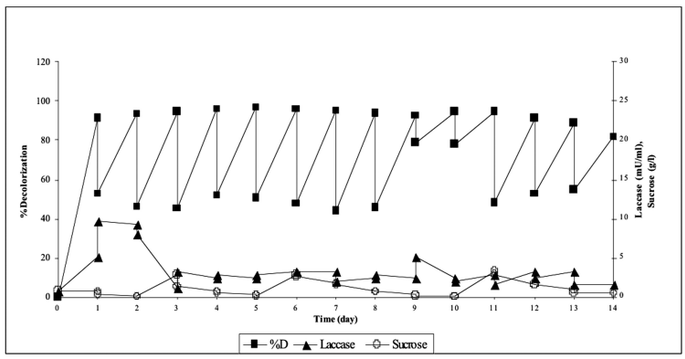

Figure 2. Decolorization of synthetic wastewater with repeated-batch with addition of 3 g/l sucrose.

Decolorization of real wastewater by immobilized *C. versicolor* RC3 on PUF with repeated-batch in 10-liter air-bubble bioreactor

Figure 3 shows the wavelength scan of real wastewater before and after decolorization. The peak at 500 nm was found in wastewater before decolorization and disappeared after decolorization. The parameters of real wastewater before and after decolorization in

10-liter air-bubble bioreactor are shown in Table 2. It was found that *C. versicolor* RC3 is still active in real wastewater and the reduction of pH from 8.22 to 3.91 was observed. The amount of bacterial count was decreased from $2.1x10^5$ to $4x10^3$ cfu which suggested that it was caused from the result of bacterial growth suppression by pH reduction from 8.22 to 3.91. After decolorization, it was found that 80% and 67% of color and COD removal were achieved within 48 h, respectively and 14.1 mU/ml of laccase degrading color structures in wastewater was also found. Both the characters of real wastewater from Batik factory and the wavelength scan data demonstrated the disappearance of dye.

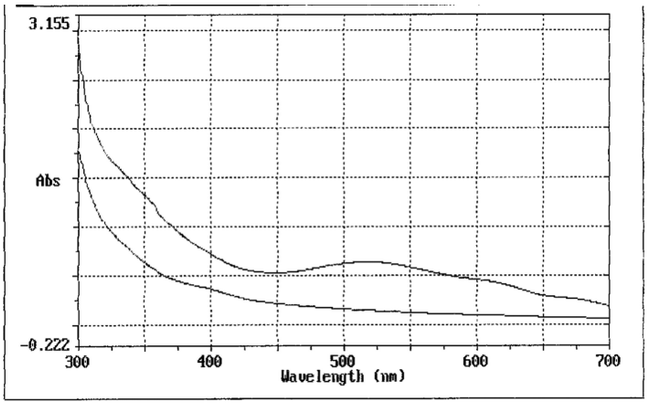

Figure 3. Wavelength scanning of real wastewater from Batik factory before (A) and after decolorization (B).

Table 2. Parameters of real wastewater before and after decolorization in 48 h.

Parameters	Influent	Effluent	%Removal
Peak in WL scan (nm)	500	-	-
pH	8.22	3.91	-
COD (mg/L)	3680	1200	67
TSS (g/L)	0.067	0.05	-
Total plate count (cfu)	$2.1x10^5$	$4x10^3$	81
OD_{500}	0.49	0.097	80
Color	Purple	Colorless	-

CONCLUSION

Polyurethane foam was found to be the suitable support for commercial dye decolorization by *C. versicolor* RC3. Immobilized cells were used for dye decolorization in large scale because most of the cells were fixed on PUF. The repeated-batch system could be repeated 3 times when no sugar was added in the system. Fourteen cycles of repeated batch could be achieved when supplemented with 3 g/l of sugar. For real textile wastewater, 80% and 67% of color and COD removal were achieved in 48 h, respectively.

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REFERENCES

Christopher, J., P.W. Owen, and S. Ajay. 2002. Biodegradation of dimethylphthalate with high removal rates in a packed-bed reactor. World Journal of Microbiology Biotechnolology 18: 7-10.

Chung, K.T., S.E. Stevens, and C.E. Cerniglia. 1992. The reduction of azo dyes by the intestinal microflora. Critical Review of Microbiology 18: 175-190.

Galhaup, C., H. Wagner, B. Hinterstoisser, and D. Haltrich. 2002. Increased production of laccase by the wood-degrading basidiomycetes Trametes pubescens. Enzyme and Microbial Technology 30: 529-536.

Kapdan, I.K., F. Kargi, G. McMullan, and R. Marchant. 2000. Effect of environmental conditions on biological decolorization of textile dyestuff by C. versicolor. Enzyme and Microbial Technology 26: 381-387.

Khanongnuch, C., N. Wanphrut, S. Lumyong, and T. Watanebe. 2004. Thermotorelant wood rotting fungi isolated from northern Thailand and their potential uses in lignin degrading application. Fungal Diversity 15: 187-196.

O'Neill, C., F.R. Hawkes, D.L. Hawkes, N.D. Lourenco, H.M. Pinherio, and W. Delee. 1999. Colour in textile effluents-sources, measurement, discharge consents and simulation: a review. Journal of Chemical Technology, and Biotechnology 74: 1009-1018.

Soares, G.M.B., M.C. Ferreira, and M.T.P. de Amorim. 2001. Decolorization of an anthraquinone-type dye using a laccase formulation. Bioresource Technology 79: 171-177.

Stolz, A. 2001. Basic and applied aspects in the microbial degradation of azo dyes. Applied Microbiology and Biotechnology 56: 69-80.

Wong, Y., and J.Yu. 1999. Laccase catalyzed decolorization of synthetic dyes. Water Research. 33: 3512-3520.

Zille, A., F.D. Munteanu, G.M. Gübitz, and A. Cavaco-Paulo. 2005. Laccase kinetics of degradation and coupling reactions. Journal of Molecular Catalysis B: Enzymatic 33: 23-28.