Journal of Environmental Sciences Vol. 15, No. 1, pp. 5—8, 2003

ISSN 1001—0742
CN11—2629/X

Article ID: 1001-0742(2003)01-0005-04 **CLC number**: Q55; X788 **Document code**: A

Production of laccase by *Coriolus versicolor* and its application in decolorization of dyestuffs: (Ⅱ) Decolorization of dyes by laccase containing fermentation broth with or without self-immobilized mycelia

LIN Jian-ping, LIAN Wei, XIA Li-ming, CEN Pei-lin*

(Department of Chemical Engineering and Bioengineering, Zhejiang University, Hangzhou 310027, China. E-mail: cenpl@cmsce.zju.edu.cn)

Abstract: The capability of decolorization for commercial dyes by *Coriolus versicolor* fermentation broth containing laccase with or without immobilized mycelium was evaluated. With cell-free fermentation broth containing laccase, high decolorization ratio was achieved for acid orange 7, but not for the other dyes concerned. The immobilized mycelium was proved to be more efficient than the cell-free system. All the four dyestuffs studied were found being decolourized with certain extent by immobilized mycelium. The repeated-batch decolorization was carried out with satisfactory results. The experimental data showed that the continuous decolorization of wastewater from a printing and dyeing industry was possible by using the self-immobilized *C. Versicolor*.

Keywords: laccase; textile dyes; immobilized mycelium; decolorization; *Coriolus versicolor*

Introduction

Wastewater from textile printing and dyeing industry is one of the main pollution sources in Eastern China, where the country's most textile industry are stationed. Thousands kinds of dyes with different structures are being consumed and large amount of colorful wastewater containing dyes is being produced. Color removal from the colorful wastewater becomes an urgent task for many companies. Most of these dyes are synthetic polyaromatic compounds and are difficult to be degraded in environment and are not uniformly susceptible to decomposition by active sludge in conventional aerobic process. Some azo dyes have the potential to form carcinogenic breakdown products in environment(Chung, 1992). As an important lignioolytic enzyme, laccase provided the possibility to decolourize different synthetic textile dyes (Chivukula, 1995; Kirby, 2000; Wong, 1999). Their research works were focused the decolorization of dyes by extracted free or immobilized laccase.

In the previous work, the production of laccase was studied. The purposes of this work were to study the possibility to directly use fermentation broth with or without *C. versicolor* mycelium pellets for decolorizing several commercially used textile dyes.

1 Materials and methods

1.1 Microorganisms

The strain of *Coriolus versicolor* was maitained at 4℃ on YMS agar slant (glucose 20 g/L, malt extract 20 ml/L, peptone 1 g/L, and agar 20 g/L).

1.2 Chemicals

All dyes used in this work were commercial one produced by local factories.

1.3 Enzyme assays

The fermentation broth was filtered to remove mycelia, the filtrate was then diluted to reach proper enzyme concentration for analysis. Both sample and ABTS solution were preheated to 30℃ for 5 min, then mixing 1 ml diluted sample with 1 ml ABTS solution in a 1 cm cuvette to start reaction. The reaction was continued for 5 min and the OD_{420} values were recorded on-line by the HP 8452A spectrometer. The linear region of OD_{420} was used to determine the enzymatic activity of laccase. One unit of activity was defined as

Foundation item: The National Natural Science Foundation of China (No. 29976038); * Corresponding author

the amount of enzyme causing 1 unit change of OD_{420} in 1 min.

1.4 Bioreactor

The bioreactor used for continuous decolorization of dyes was a 1.3L three-phase fluidized-bed reactor.

1.5 Analytical methods

Decolorization ratio of dyes was measured by the absorption decline detected by HP8452A spectrometer at the characteristic absorption peak.

2 Results and discussion

2.1 The decolorization of dyes by fermentation broth without mycelia

The advantage of laccase to degrade lignin or polyaromatic compound is that the reaction does not need H_2O_2 as co-substrate. So the laccase harvested from *C. versicolor* fermentation broth can be used to decolourize dye solution directly. The decolorization of dyes, such as acid orange 7, carplon red, weak acid scarlet and light-fast direct red by fermentation broth without mycelia was studied. Expect acid orange 7, no evidence of decolorization for the other three dyes were found. In the fermentation broth, laccase activity is about 5 U/ml. Possibly due to the existence of protease in the broth, the laccase activity in the fermentation broth without mycelia will decrease fast, which will certainly affect the decolorization of dye solution.

Generally speaking, the enzyme-catalyzed reaction is dependent on the temperature and pH value. The effects of temperature on the decolorization of acid orange 7 solution are shown in Fig.1. It is observed that the decolorization rate will increase with the temperature and reaches the highest at 60℃. After then, the rate as well as the effectiveness of decolorization will decrease. So the optimal temperature should be 50—60℃. The optimal pH for decolorization by fermentation broth is about pH 5 as shown in Fig.2.

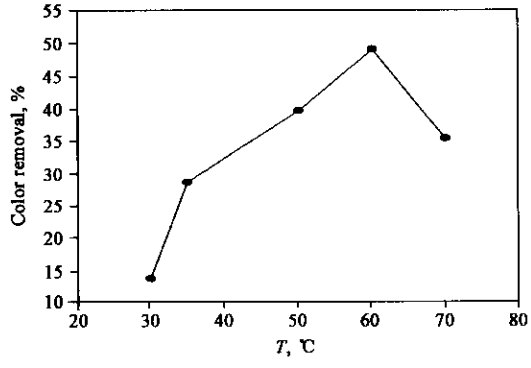

Fig.1 Effect of temperature on the decolorization of acid orange 7 with fermentation broth

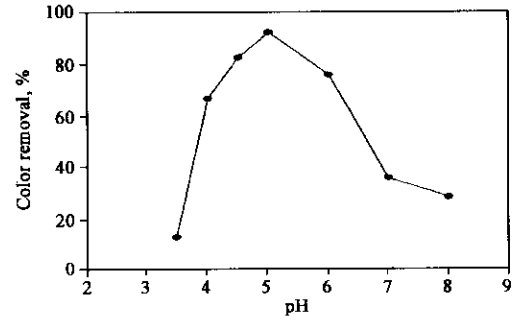

Fig.2 Effect of pH value on the decolorization of acid orange 7

The effects of initial laccase activity on the decolorization of acid orange 7 are shown in Fig.3. It is obvious that with the increasing in laccase activity, the color removal rate as well as decolorization ratio will be higher. Fig.4 shows the effects of acid orange 7 concentration on the color removal. The results indicated that high concentration of acid orange 7 did not inhibit the decolorization reaction severely.

2.2 The decolorization of dyes with self-immobilized mycelia

Because of existence of active *Coriolus versicolor* mycelia, the laccase can be produced continuously, thus the deactivation of laccase by protease or other factors will not affect the decolorization of dyes. *Coriolus versicolor* mycelia can be used continuously or repeatedly. Therefore, The decolorization of dyes with self-immobilized mycelia should be preferred.

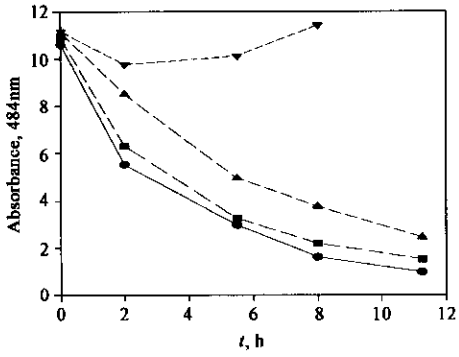

Fig.3 Effect of initial laccase activity on the decolorization of acid orange 7
● 3.41 U/ml; ■ 2.06 U/ml; ▲ 0.99 U/ml; ▼ 0.20 U/ml

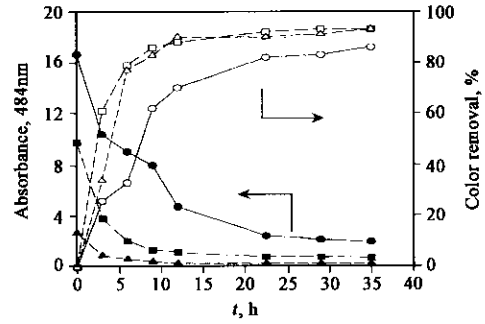

Fig.4 Effect of initial acid orange 7 on the decolorization
Initial absorbance at 484 nm: ● 16.8; ■ 9.5; ▲ 3.0; initial laccase activity: 2.78 U/ml; pH 4.5, 30℃

The experimental results of decolorization of acid orange 7 by self-immobilized *C. versicolor* in repeated-batch operation are shown in Table 1. The time period for each cycle is 24 hours and 2 g/L of glucose was added in each cycle for keeping mycelia active except first batch. It can be noticed that the decolorization ratio in each batch is very stable and excesses 97%. During each cycle, when the broth was replaced by the fresh solution containing dye and glucose, it can be observed that mycelium pellets turned to bright yellow because the mycelium pellets adsorbed the dye firstly. With the time going, the yellowish of the pellets disappeared gradually. Finally, the mycelium pellets as well as broth recovered to white and colorless, respectively. At the end of each batch, the laccase activity was kept high as shown in Table 1.

Table 1 Experimental results of acid orange 7 decolorization in repeated-batch process with mycelium pellets

Batch sequence	1	2	3	4	5	6	7	8	9	10
Original absorbance at 480 nm	10.2	9.7	11.7	11.9	9.6	9.3	9.1	9.0	13.8	14.1
Final absorbance at 480 nm	0.21	0.18	0.19	0.21	0.20	0.22	0.20	0.22	0.29	0.28
Decolorization ratio, %	97.9	98.2	98.4	98.2	97.9	97.6	97.8	97.6	97.9	98.0
Final laccase activity, U/ml	2.83	2.92	2.38	2.85	6.95	2.75	4.90	3.08	3.24	2.78

Similarly, the decolorization of carplon red, weak acid scarlet and light-fast direct red were also examined. The results are listed in Table 2. For light-fast direct red, the decolorization ratio in the first batch was relative low, but increased to more than 80%. The possible reason is that the dye is harmful for *C. versicolor* mycelia growth and laccase production at first, then changes in intracellular enzymes or mutation will take place to develop a resistance on the dye inhibition. For weak acid scarlet and carplon red, the decolorization capability of mycelium pellets became weaker and weaker with the increase in cycle number. It explained that *C. versicolor* could not develop the resistance for these two kinds of dyes.

Table 2 Experimental results of carplon red, weak acid scarlet and light-fast direct red decolorization in repeated-batch process with mycelium pellets

Batch sequence		1	2	3	4	5	6
Light-fast direct red	Decolorization ratio, %	54.9	79.3	88.8	88.5	80.5	83.9
	Laccase activity, U/ml	1.41	1.08	1.90	2.85	1.45	1.02
Weak acid scarlet	Decolorization ratio, %	88.0	44.4	76.9	71.0	50.5	
	Laccase activity, U/ml	0.70	1.28	2.62	0.74	0.31	
Carplon red	Decolorization ratio, %	87.9	66.0	38.0			
	Laccase activity, U/ml	2.11	1.67	1.49			

2.3 Continuous decolorization of acid orange 7 with self immobilized mycelia

The experiment was carried out in a three-phase fluidized bed reactor in which the mycelium pellets were fluidized in the reactor because of aeration. The volume of bioreactor is 1.4L and the aeration rate is 1 vvm. The wastewater was continuously fed into the bioreactor. The results of continuous decolorization of acid orange 7 with self-immobilized *Coriolus versicolor* pellets at different dilution rate are shown in Table 3. In continuous process, laccase activity will be lower because of the lost in effluent, therefore, the dilution rate should be kept very low for high decolorization ratio.

Table 3 Experimental results of continuous decolorization of acid orange 7 with self-immobilized *Coriolus versicolor* pellets

Dilution rate, h^{-1}	0.0056	0.014	0.026
Decolorization ratio, %	97.0	70.0	53.0

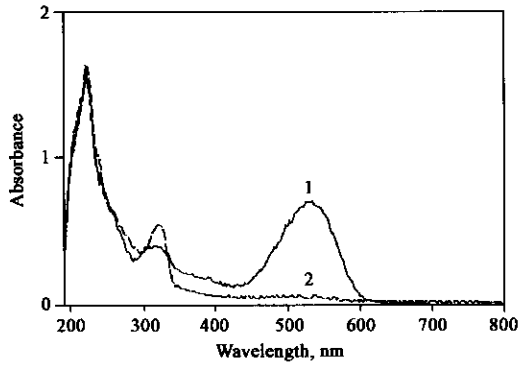

Fig.5 Comparison of absorbance photographs between inlet and outlet wastewater(1 inlet; 2 outlet)

2.4 Decolorization of wastewater from printing and dyeing industry

The wastewater in deep red color from a local printing and dyeing company was also used in continuous decolorization by self-immobilized mycelium pellets. The initial absorbance of wastewater in 526 nm is 0.679. Only 2 g/L of glucose were added into the wastewater as carbon source. When dilution rate was set at 0.014 h^{-1}, the absorbance at effluent was lowered to 0.045, which means a color-removing ratio of 93.5%. The effluent looks colorless and very clear. The comparison of absorbances between wastewater in inlet and effluent indicated the disappearing of absorbance peak at 526 nm as shown in Fig.5. The operation was lasted more than 10 days.

3 Conclusions

The decolorization of dye solution can be achieved by *C. versicolor* fermentation broth with or without self-immobilized mycelium pellets. The fermentation broth with self-immobilized mycelium was more efficient in decolorization than the fermentation broth alone. The laccase containing broth can only be used to decolour acid orange 7 with the optimal temperature and pH is 60℃ and pH 5.0 respectively. No decolorization of carplon red, weak acid scarlet and light-fast direct red by fermentaion broth alone was observed, whereas all four kinds of dyes studied can be decolorized by fermentation broth with self-immobilized mycelia. The decolorization of dyes can be performed either by repeated batch or continuous operation. The self-immobilized *Coriolus versicolor* mycelium pellets can also continuously decolorize wastewater from a local printing and dyeing company.

References:

Chung K-T, Cernilia C E, 1992. Mutagenicity of azo dyes: structure-activity relationships[J]. Mutat Res, 77: 201—220.

Chivukula M, Rengannathan V, 1995. Phenolic azo dye oxidation by laccase from pyricularia oryzae[J]. Appl Environ Microbiol, 61: 4373—4377.

Kirby N, Marchant R, McMullan G, 2000. Decolourization of synthetic textile dyes by *phlebia tremellosa*[J]. FEMS Microbiolgy Letters, 188: 93—96.

Wong Y, Yu J, 1999. Laccase-catalyzed decolourisation of synthrtic dyes[J]. Wat Res, 33(16): 3512—3520.

(Received for review October 8, 2001. Accepted December 29, 2001)