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Decolorization of leather dyeing wastewater by laccase of the white rot fungus *Pycnoporus* sp. Y1

Xinli lu[1], Chun Hai Zhao[3*], Aijun Zhao[2], Yuqi Zhang[1], Yichun Wu[3], Suliang Chen

[1]Hebei Center for Desease Prevention and Control, Shijiazhuang 050021, P. R., (CHINA)

[2]University Of Business and Economics in Hebei, Shijiazhuang 050051, P. R., (CHINA)

[3]Binzhou Polytechnic Shan dongbinzhou 256603 P. R., (CHINA)

Email: zch79214@163.com

ABSTRACT

In this study, laccase produced by the white rot fungus strain *Pycnoporus* sp. Y1 was used for the leather dyeing wastewater decolorization. The mediators including veratryl alcohol (VA), 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), hydroxybenzoic acid (HA), hydroxybenzotrizole (HOBT), and temperature, pH values, laccase concentration, leather dyeing wastewater dilution ratio were all investigated in the experiments, and then four factors (mediators, temperatures, pH values, dilution ratios) were optimized by orthogonal experimental design, and the optimal conditions for wastewater decolorization were obtained as follows: temperature of 50 °C, pH 3.0, laccase concentration 4 U/ml, and the dilution ratio of 1 fold, the decolorization ratio could reach 58.42% in 10 min under the optimized conditions at last.

KEYWORDS

Leather dyeing wastewater; Decolorization; Optimization; Laccase; *Pycnoporus* sp.

INTRODUCTION

Synthetic dyes are widely used in the leather and textile industries, and these materials are mainly aromatic molecular structural chemicals which can cause serious environmental problems [1]. So the colored effluents discharged from the leather and textile industries have to be treated due to their toxicity and carcinogenicity, which might be very harmful to the environments, particularly soil and water bodies. There are many different complicated structures of chemicals in the dyes which were very difficult degraded by physical or chemical ways, such as volatilization, chemical oxidation, photo-oxidation, bioaccumulation, and organic reagents extraction[2,3]. However, these methods have some disadvantages and limitations during the practical applications at present. For example, the photo-degradation and chemical degradaion would introduce other organic reagents to the water or soil, and furthermore, these methods also need some critical factors such as lights, media, and conditions of water or soil of the environment, or even lead to secondary pollution by the organic reagents and other chemicals.

Biological treatment could degrade many dyes and environmental friendly, so it has been attracted a lot of attention throughout the world[4,5]. Laccase (EC 1.10.3.2, benzenediol: oxygen oxidoreductases) is a kind of polyphenol oxidase belonging to the multinuclear copper-containing oxidase which could catalyze the oxidation of many kinds of substrates (lignin, phenolic compounds)[6], and has been used mainly for pulp wastewater or textile effluent treatment. It has been reported that laccase usually produced by white rot fungi belonging to the Basidiomycetes group, which were known have ability to degrade lignin[7,8]. In the wastewater treatment, laccase is also a kind of promising enzyme in recent years. However, there was few reports on leather wastewater treatment by laccase at present. In this study, laccase produced by *Pycnoporus* sp. Y1 was used for leather dyeing wastewater, and the conditions for color degradation were optimized. Based on the results of the experiments, laccase was considered of great potential for leather wastwater decolorization.

MATERIALS AND METHODS

Leather dyeing wastewater was collected from a leather dyeing factory in Xinji City, Hebei Province. The samples with blue color were scanned from 300-800 nm by a spectrophotometer (752 ultraviolet-visible spectrophotometer, Shanghai Optical Instrument Factory) and the OD_{max} was at 521 nm.

Microorganism

Pycnoporus sp. Y1, stored at 4 ^{o}C on the potato dextrose agar (PDA) slant containing potato extract 20% (w/v), glucose 20 g/l, agar 15 g/l.

Laccase production

Mycelia or spores of the strain *Pycnoporus* sp. Y1 were transferred and incubated on the PDA plates in 30 ^{o}C for 7 d, and then the mycelia or spores were cultured in a 250 ml Erlenmeyer flask containing 50 ml seed culture (potato dextrose medium, potato extract 20% (w/v), glucose 20 g/l) and 6-7 glass beads (diameter of 2-3 mm) to prevent the mycelial pellet formed. The seed was incubated in 30 ^{o}C for 24 h on a reciprocal shaker at 200 rpm. The seed culture was then inoculated to the fermentation medium (containing 50 g/l glucose, 10 g/l soybean meals, wheatbran 60 g/l, $NH_4H_2PO_4$ 1 g/l, KH_2PO_4 2 g/l, $MgSO_4$ 0.5g/l, $CuSO_4 \cdot 5H_2O$ 2 mmol/l) at 5% (v/v) inoculation volume, incubated in 30 ^{o}C at 200 rpm for 10 d.

Crude laccase solution preparation

The fermentation broth was collected with a flask, and the mycelia and other insoluble substrates were removed by a refrigerated centrifuge at 10,000 *g* for 10 min in 4 ^{o}C, the yellow supernatant was used as crude laccase for later processes.

LACCASE ACTIVITY ANALYSIS

Laccase activity was assayed at room temperature using 2, 6-dimethoxyphenol (DMP) as substrate. The assay mixture contained of 0.5 ml 10mmol/l DMP and 1.9 ml of 0.1 mol/l sodium acetate buffer (pH 3.0). 0.1 ml of crude laccase solution was added and the oxidation of DMP was followed by absorbance increase at 470 nm (ε=49600$M^{-1}\cdot cm^{-1}$). One unit (U) of laccase activity was defined as the amount of enzyme that catalyzed the formation of 1.0 μ mol of product per minute[9]. All assays were performed in duplicate, with an average sample mean deviation of less than 10%.

RESULTS AND DISCUSSION

Laccase production by the strain *Pycnoporus* sp. Y1

In this study, laccase was produced by the strain *Pycnoporus* sp. Y1, and the enzyme activity was determined every day. It could be found that the laccase activity reached over 19 U/ml after 10 days of fementation, and then decreased (Figure 1), and the laccase activity was found only 6.8 U/ml in the end of the fermentation (15 days). So the fermentation period was 10 days for laccase production in the experiment.

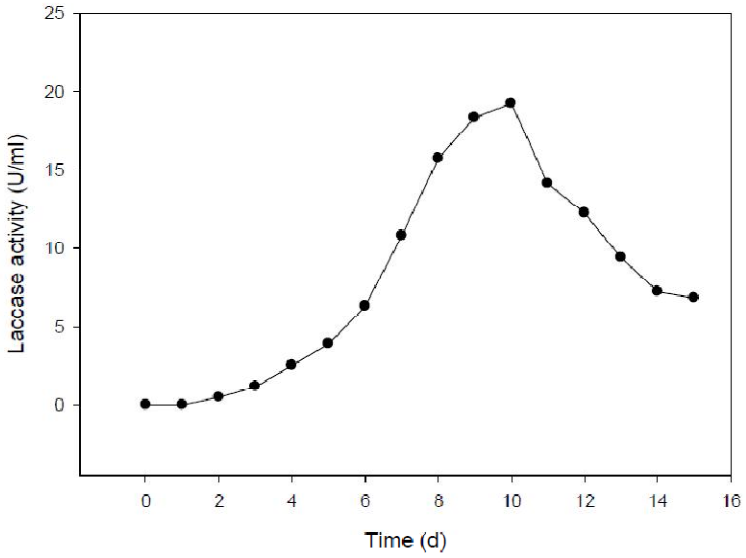
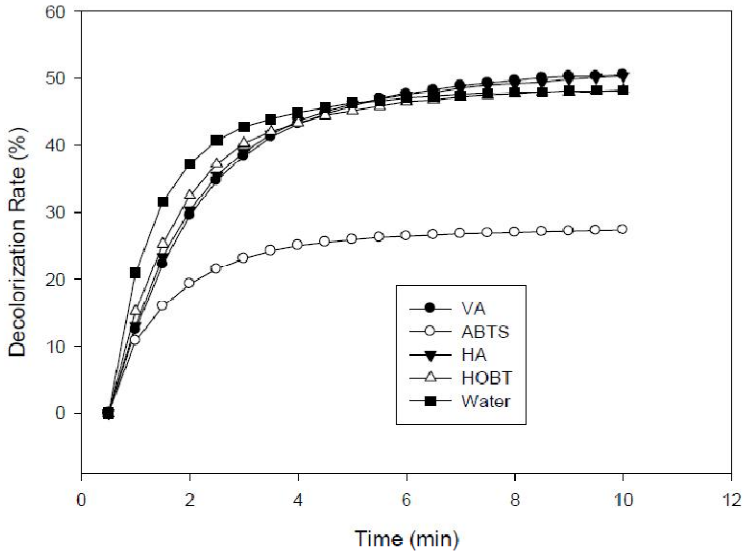


Figure 1: Time course of laccase production by the strain *Pycnoporus* sp. Y1

Effect of different mediators on the leather dyeing wastewater decolorization

Mediators could promote the wastewater decolorization processes by laccase. However, in this study, the selected mediators (including veratryl alcohol (VA), 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS), hydroxybenzoic acid (HA) and hydroxybenzotrizole (HOBT)) were not showed significant influence on the leather dyeing wastewater decolorization. And ABTS even showed negative effect on the wastewater decolorization, which might because of its own color affect the reaction system. Furthermore, other mediators and water showed similar results and the decolorization rates were all above 50%. When no mediators were added, water even showed more rapid decolorization rate, which is shown in Figure 2. According to the results, water should be suitable for leather dyeing wastewater decolorization and it was used for further experiments.



(VA : veratryl alcohol; ABTS: 2, 2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid; HA: hydroxybenzoic acid; HOBT: hydroxybenzotrizole)

Figure 2 Effects of the mediators on the wastewater decolorization rate

Effects of different temperature on the leather dyeing wastewater decolorization

The results of the effect of different temperature on the leather wastewater decolorization are shown in Figure 3. It could be found that the decolorization rate reached their peaks in the first 10 min, and the highest decolorization rate was appeared at 80^{o}C, the reason might be high temperature destroyed the structure of some substants in the wastewater. However, the temperature gradients investigated showed similar decolorization rate in the experiment, and 50 ^{o}C was selected considering the energy consumption.

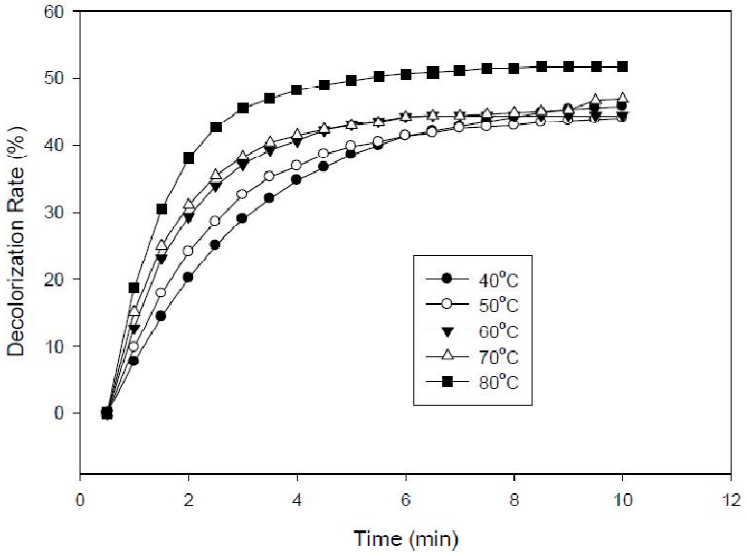


Figure 3: Effects of different temperature on the wastewater decolorization rate

Effect of different pH values on the leather dyeing wastewater decolorization

pH values showed significant effect ($P<0.05$) on the leather wastewater decolorization in this study. When the pH value was 3.0 or 4.0, the decolorization rates were both over 50% in 10 min; and when the pH values increasing, the wastewater decoloriaztion rate decreased significantly. Laccase activity was significantly affected by pH values in this study, and the decolorization rates were very low

when pH values were great than or equal to 6.0, which might because that the laccase activity effected significantly by pH values, and showed higher activity when pH valus below 6.0, and the optimal pH value for the wastewater decolorization was 4.0 or 3.0.

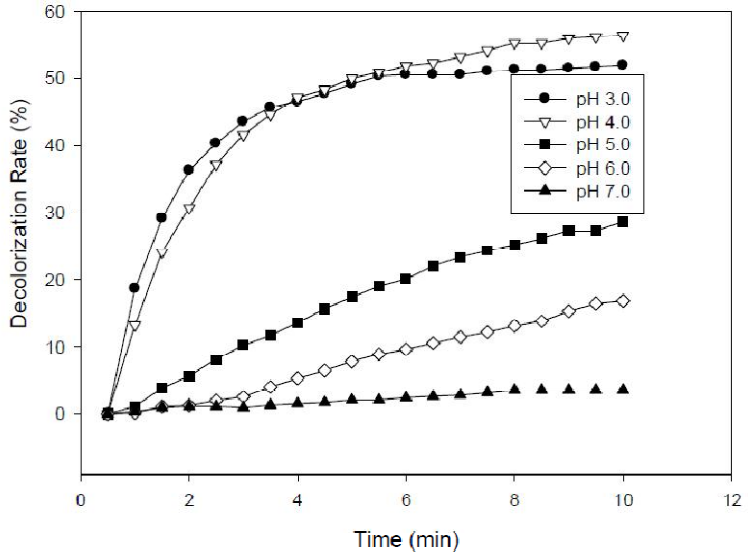


Figure 4: Effect of pH value on the wastewater decolorization rate

Effects of laccase concentrations on the leather dyeing wastewater decolorization

The laccase concentrations had a certain effect on the wastewater decolorization, as is shown in Figure 5, the dicolorization rates increased when the laccase concentrations from 0.4 U/ml to 20 U/ml, and the decolorization rates of 10 U/ml and 20 U/ml laccase were close to each other, both above 50% decolorization rate, so the optimal laccase concentration was 10 U/ml in the experiment.

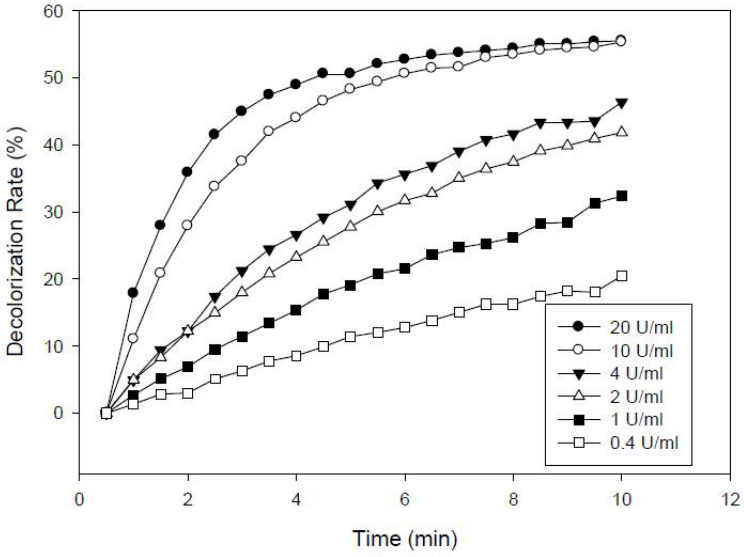


Figure 5: Effects of laccase concentrations on the wastewater decolorization rate

Effect of dilution ratios on the leather dyeing wastewater decolorization

Different dilution folds (1 fold, 1.3 folds, 2 folds, 4 folds, 8 folds, 16 folds) was investigated in this study and the results are shown in Figure 6. It could be concluded that when the wastewater was diluted to 8 folds, the decolorization rates were decreased, and when 1-4 folds of the wastewater not showed significant changes on the wastewater decolorization.

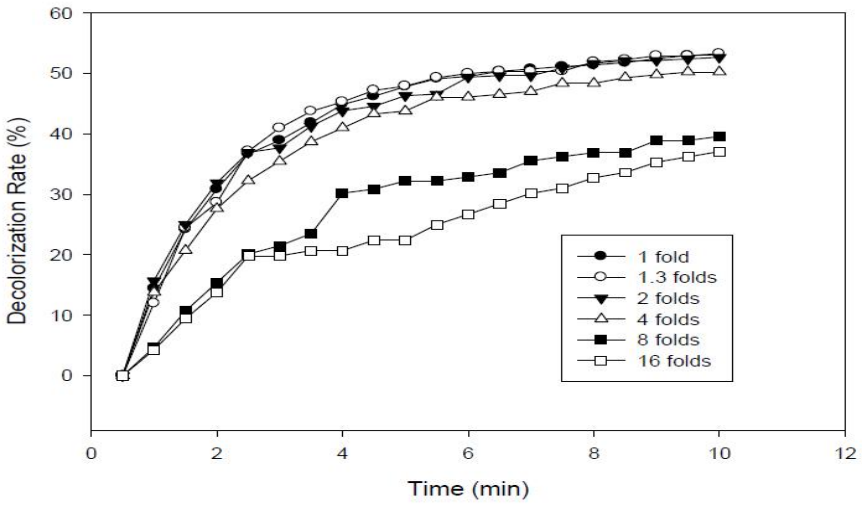


Figure 6: Effect of dilution ratios on the wastewater decolorization rate

Optimization of the decolorization conditions with orthogonal experimental design

Based on the data of the experiments, four factors such as temperature, pH value, laccase concentration and the dilution ratios were selected for further optimization by orthogonal experimental design, and the factors and levels are listed in TABLE 1, and analysis of the tests are shown in TABLE 2.

TABLE 1: L_9 (3^4) orthogonal experimental design

Tests	A: Temperature (^oC)	B: pH	C: Laccase/(U/ml)	D: Dilution ratios/(folds)
1	30	3.0	10	1
2	40	4.0	4	2
3	50	5.0	2	4

TABLE 2: Results of the orthogonal testIt could be found that the optimized

Run no.	A: Temperature (^oC)	B: pH	C: Laccase/(U/ml)	D: Dilution ratios/(folds)	Decolorization rates
1	1(30)	1(3)	1(10)	1(1)	53.73%
2	1(30)	2(4)	2(4)	2(2)	37.63%
3	1(30)	3(5)	3(2)	3(4)	18.63%
4	2(40)	1(3)	2(4)	3(4)	53.05%
5	2(40)	2(4)	3(2)	1(1)	35.18%
6	2(40)	3(5)	1(10)	2(2)	44.43%
7	3(50)	1(3)	3(2)	2(2)	47.00%
8	3(50)	2(4)	1(10)	3(4)	35.74%
9	3(50)	3(5)	2(4)	1(1)	51.84%
k_1	36.66%	51.26%	44.63%	46.92%	_
k_2	44.22%	36.18%	47.51%	43.02%	_
k_3	44.86%	38.30%	33.60%	35.81%	_
R	8.20%	15.08%	23.91%	11.11%	_
Optimal Level	A3	B1	C2	D1	_

It could be found that the optimized conditions for the leather wastewater decolorization were A3B1C2D1, temperature 50 ^oC, pH value 3.0, laccase concentration 4 U/ml, and dilution ratio was 1 fold. Under this condition, the decolorization rate reached 58.42% after three times of replicates.

CONCLUSIONS

In this study, laccase produced by the strain *Pycnoporus* sp. Y1 was used for leather wastewater decolorization. And the optimal techniques of the wastewater decolorization was established and the conditions were: temperature 50 ^{o}C, pH value 3.0, laccase concentration 4 U/ml, and dilution ratio was 1 fold, and it was found that the decolorization rate could reach 58.42% under the optimum conditions in 10 min. The results could provide useful information for the leather wastewater decolorization by laccase produced by white rot fungi.

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