Nature Environment and Pollution Technology
An International Quarterly Scientific Journal

ISSN: 0972-6268	Vol. 14	No. 4	pp. 881-884	2015

Original Research Paper

Decolorization of Leather Dyeing Wastewater by Laccase of the White Rot Fungus *Pycnoporus* sp. Y1

Xinli Lu*, Chun Hai Zhao, Aijun Zhao***, Yuqi Zhang*, Yichun Wu**, Suliang Chen* and Xiaohui Liang****†**

*Hebei Center for Disease Prevention and Control, Shijiazhuang 050021, P. R. China
**Binzhou Polytechnic Shan dongbinzhou 256603, P. R. China
***University of Business and Economics in Hebei, Shijiazhuang 050051, P. R. China
****Energy Research Institute, Shandong Academy of Sciences, Jinan, China
†Corresponding author: XiaoHui Liang; Xinli Lu and Chun Hai Zhao: Co-first authors; All these authors contributed equally to this work

Nat. Env. & Poll. Tech.
Website: www.neptjournal.com
Received: 08-12-2014
Accepted: 19-01-2015

Key Words:
Leather dyeing wastewater
Decolorization
Laccase
Pycnoporus sp.

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, and 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. 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.

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 (Young & Yu 1997). So the coloured 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 environment, particularly soil and water bodies. There are many different complicated structures of chemicals in the dyes, which are very difficult to degrade by physical or chemical ways, such as volatilization, chemical oxidation, photo-oxidation, bioaccumulation and organic reagents extraction (Kokol et al. 2007, Li et al. 2007). However, at present, these methods have some disadvantages and limitations during the practical applications. For example, the photo-degradation and chemical degradation would introduce other organic reagents to water or soil, and furthermore, these methods need some critical factors such as light, media, and specific 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 is environment friendly, so it has attracted a lot of attention throughout the world (Alcantara et al. 2008, Asgher et al. 2013). Laccase (EC 1.10.3.2, benzenediol: oxygen oxidoreductases) is a kind of polyphenol oxidase belonging to the multinuclear copper-containing oxidase which could catalyse the oxidation of many kinds of substrates (lignin, phenolic compounds) and has been used mainly for pulp wastewater or textile effluent treatment. It has been reported that laccase is usually produced by white rot fungi belonging to the Basidiomycetes group, which were known to have the ability to degrade lignin (Wang et al. 2013, Manavalan et al. 2013). In the wastewater treatment, laccase is also a kind of promising enzyme in recent years. However, there were few reports on leather wastewater treatment by laccase at present. In this study, laccase produced by *Pycnoporus* sp. Y1, was used in the leather dyeing wastewater, and the conditions for colour degradation were optimized. Based on the results of the experiments, laccase was considered of great potential for leather wastewater decolorization.

MATERIALS AND METHODS

Leather dyeing wastewater was collected from a leather dyeing factory in Xinji City, Hebei province. Microorganism: *Pycnoporus* sp. Y1, stored at 4°C on a 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 at 30°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, 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 rpm for 10 min at 4°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 of 10 mmol/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 an 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 catalysed the formation of 1.0 μ mol of product per minute (Wang et al. 2010). 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. It could be found that the laccase activity reached over 19 U/mL after 10 days of fermentation, and then decreased (Fig. 1), and the laccase activity was found only 6.8 U/mL at the end of the fermentation (15 days).

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)) did not show significant influence on the leather dyeing wastewater decolorization. When no mediators were added, water even showed more rapid decolorization rate, which is shown in Fig. 2. According to the results, water should be suitable for leather dyeing wastewater decolorization and it was used for further experiments.

Effects of different temperatures on the leather dyeing wastewater decolorization: The results of the effect of different temperatures on the leather wastewater decolorization are shown in Fig. 3. The highest decolorization rate appeared at 80°C. However, the temperature gradients investigated, showed a similar decolorization rate in the experiment, and 50°C was selected considering the energy consumption.

Table 1: $L_9(3^4)$, orthogonal experimental design.

Tests	A: Temperature(°C)	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 test.

Run no.	A: Temperature(°C)	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	-

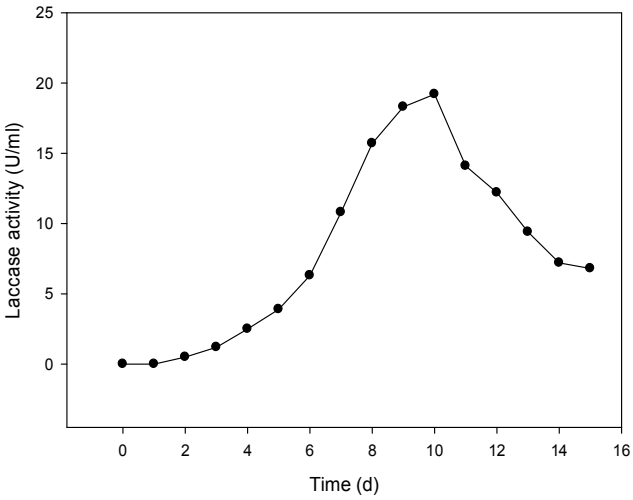

Fig. 1: Time course of laccase production by the strain *Pycnoporus* sp. Y1.

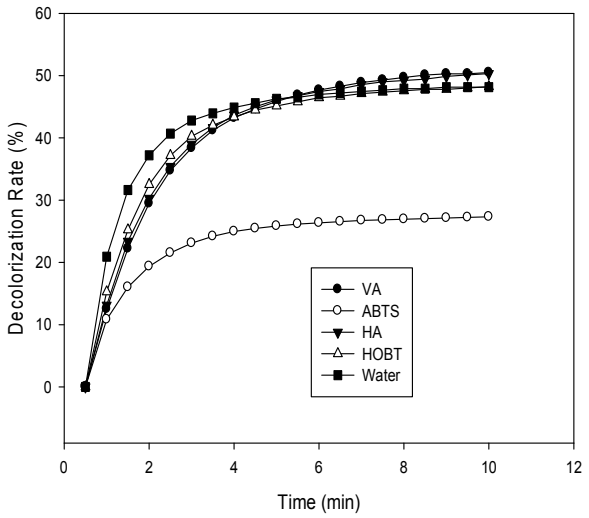

Fig. 2: Effects of the mediators on the wastewater decolorization rate.

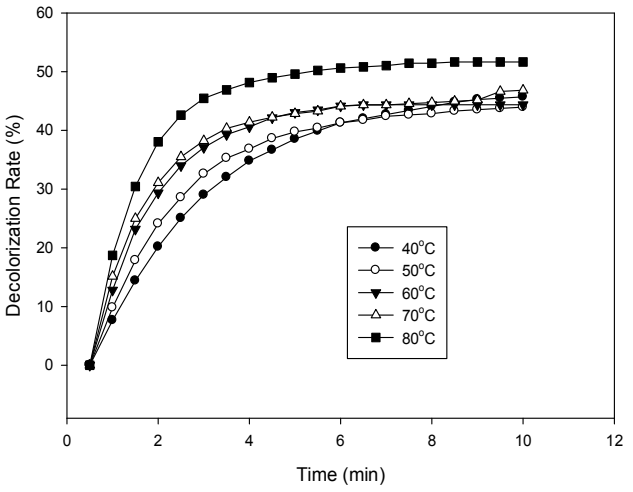

Fig. 3: Effects of different temperatures on the wastewater decolorization rate.

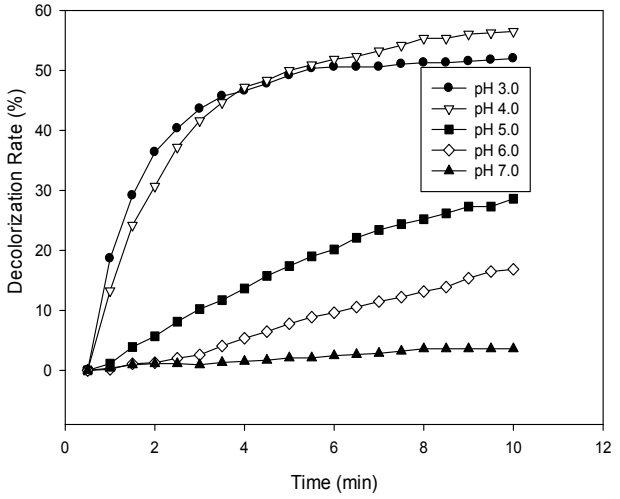

Fig. 4: Effect of pH value on the wastewater decolorization rate.

Effect of different pH values on the leather dyeing wastewater decolorization: pH values showed a significant effect ($P<0.05$) on the leather wastewater decolorization in this study. The pH values below 6.0 showed higher activity, and the optimal pH value for the wastewater decolorization was 4.0 or 3.0.

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 in Table 2.

It could be found that the optimized conditions for the leather wastewater decolorization were A3B1C2D1, temperature 50°C, pH value 3.0, laccase concentration 4 U/mL, and dilution ratio 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 were established and the conditions were: temperature 50°C, pH value 3.0, laccase concentration 4 U/mL, and dilution ratio 1-fold. It was found that the decolorization rate could reach

58.42% under the optimum conditions in 10 min. The results can provide useful information for the leather wastewater decolorization by laccase produced by white rot fungi.

ACKNOWLEDGMENT

This study was funded by the Key Project on Medical Science of Hebei Provence (No. 20120256). This research is supported by Grant BS2012SW001 from National Natural Science Foundation of Shan Dong Province and the J14LE58 Project of Shandong Province Higher Educational Science and Technology Program from Shandong Provincial Education Department, 2013ZC2002, 2014ZC0301 from Bin zhou Science and Technology Bureau, and 2015 Visiting scholar project from Shandong Provincial Education Department .

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