Colloids and Surfaces B: Biointerfaces 97 (2012) 7–12

Contents lists available at SciVerse ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

Effect of Triton X-100 on the removal of aqueous phenol by laccase analyzed with a combined approach of experiments and molecular docking

Yu Zhang[a,b], Zhuotong Zeng[c], Guangming Zeng[a,b,*], Xuanming Liu[d,**], Zhifeng Liu[a,b], Ming Chen[a,b], Lifeng Liu[a,b], Jianbing Li[e], Gengxin Xie[a,b]

[a] College of Environmental Science and Engineering, Hunan University, Changsha 410082, PR China
[b] Key Laboratory of Environmental Biology and Pollution Control (Hunan University), Ministry of Education, Changsha 410082, PR China
[c] State Key Laboratory of Medical Genetics, Central South University, Changsha 410078, PR China
[d] College of Biology, State Key Laboratory for Chemo/Biosensing and Chemometrics, Hunan University, Changsha 410082, PR China
[e] Environmental Science and Engineering Program, University of Northern British Columbia, 3333 University Way, Prince George, BC V2N 4Z9, Canada

ARTICLE INFO

Article history:
Received 17 November 2011
Received in revised form 25 March 2012
Accepted 2 April 2012

Keywords:
Phenol
Surfactant
Triton X-100
Laccase
Molecular docking

ABSTRACT

Effects of Triton X-100 on the removal of aqueous phenol catalyzed by laccase were studied. The optimal concentration of Triton X-100 was 155 μM to improve phenol removal when the concentrations of phenol and laccase were 50 mg/L and 0.05 mg/mL, respectively. Laccase activity was increased with Triton X-100 at concentrations from 31 to 930 μM and the highest increase was about 17% by 930 μM Triton X-100. The removal efficiencies of phenol with 155 μM Triton X-100 were 1.2, 1.6, 3.4, 4.5, and 5.7 fold those of the control after 6 h when the initial concentrations of phenol were 50, 100, 200, 400 and 600 mg/L, respectively. Molecular docking method was used to analyze the interactions between laccase and substrates. Docking results showed that phenol formed hydrogen bonds and hydrophobic interactions with laccase, whereas Triton X-100 formed hydrophobic interactions with laccase, which may increase the laccase activity and enhance phenol removal. The reaction of phenol removal was also characterized using UV spectra. The results indicated that the presence of low concentrations of Triton X-100 for phenol removal catalyzed by enzymes may be an alternative to the present phenol removal processes in water treatment or remediation.

1. Introduction

Phenol and its derivatives are important raw materials and widely used in many industries including petrochemical, chemical, pharmaceutical, pulp, paper, tannery and coal refining industries [1]. Phenolic contaminants can also be introduced to the environment via pesticide applications and as a result of partial degradation of some aromatic organic contaminants, such as polycyclic aromatic hydrocarbons, polychlorinated biphenyls and surfactants [2]. Once released into the environment, they may accumulate in ground water, surface water and soil [3,4]. Phenolic compounds and their derivatives are priority pollutants in the US EPS list since they are toxic, carcinogenic, mutagenic and teratogenic [4]. Thus, the removal of phenolic compounds from water and soils is significant.

Laccase (EC 1.10.3.2) can catalyze the oxidation of various aromatic compounds, especially phenolic substrates, coupled to the reduction of molecular oxygen to water [5–7]. This method has specific properties such as high effectiveness and low toxic residues compared with the conventional technologies for phenol removal such as extraction, adsorption on activated carbon, steam distillation, chemical oxidation, electrochemical techniques, irradiation and bacterial degradation [3,4]. The advent of new technology for the production, isolation and purification of laccase has made the use more competitive, leading to the development of new commercial and environmental applications of laccase [8].

The effects of surfactants on enzymes have been studied previously. It has been reported that the presence of surfactant can reduce the interactions between enzymes and the oxidative polymerization products and protect them from inactivation [9]. In addition, the interactions between enzyme and surfactant may induce a change in the conformation and/or active site of the enzyme, thereby affecting the enzyme's activity and stability [10,11]. However, to our knowledge, there is a lack of investigation on the mechanism of interactions between enzyme and surfactant. Molecular docking is a method able to predict protein–ligand interactions [12]. For example, recently, Chen et al. [13] analyzed the

* Corresponding author at: College of Environmental Science and Engineering, Hunan University, Changsha 410082, PR China. Tel.: +86 731 88822754; fax: +86 731 88823701.
** Corresponding author.
E-mail addresses: zgming@hnu.edu.cn (G. Zeng), xml05@126.com (X. Liu).

0927-7765/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.colsurfb.2012.04.001

integration of ligninolytic enzymes with lignin (a lignin derivate was selected as lignin model substrate) using molecular docking. Ji et al. [14] found that Triton X-100 has positive effect on the removal of bisphenol A catalyzed by laccase. In this study, the non-ionic surfactant Triton X-100 was used as additive to study its effects on the removal of phenol which has quite different characteristic from bisphenol A, such as solubility. Bisphenol A is a kind of phenolics with little solubility while phenol is appreciably soluble in water with about 8.3 g dissolving in 100 mL [15]. Molecular docking was also employed to explore the binding modes and interactional profiles between laccase and phenol or Triton X-100.

2. Materials and methods

2.1. Materials and reagents

Laccase (23.1 U/mg) produced by *Trametes versicolor* (*T. versicolor*) was from Fluka (Steinheim, Germany). Triton X-100 (scintillation grade, purity > 99%) was from BDH Chemicals (Poole, England). Phenol (purity > 99%) was obtained from Tianjin University Chemical Experimental Factory (Tianjin, China). Other reagents were of analytical grade. Britton-Robinson buffer consisting of 0.1 M phosphoric acid, 0.1 M boric acid, 0.1 M acetic acid was used during the experiments; its pH was adjusted to desired values by adding 2.0 M sodium hydroxide [16]. Stock solutions of laccase at 10 mg/mL were stored at 4 °C and allowed to equilibrate to 25 °C prior to use in experiments.

2.2. Experimental protocol

The experiments were performed with the modified methods of Kurniawati and Nicell [17]. All batch experiments were carried out in 40 mL open borosilicate glass vials with 5.0 mL mixture. Before reaction, the buffer containing phenol and Triton X-100 was saturated with oxygen by vigorously stirring the mixture for 15 min. The reaction was started by the addition of laccase at the initial concentration of 0.05 mg/mL (1.155 U/mL). The vials were incubated without stirring in a water bath set at the desired temperature. Blank medium without laccase was placed under the same condition to evaluate the volatilization and spontaneous removal of phenol, which could be neglected according to the test results. Medium without surfactant was carried out as the control experiment. All the experiments were performed at least in triplicate, and the standard deviation was lower than 5%. Phenol concentration was analyzed by high performance liquid chromatography (HPLC) as described by Liu et al. [18]. Laccase activity was analyzed with 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonate) (ABTS) as a color-generating substrate according to the method described by Liu et al. [18]. UV–visible spectra of the reaction solutions were measured with a UV–visible spectrophotometer (model UV-2550; Shimadzu company, Tokyo, Japan).

2.3. Molecular docking

The three dimensional (3D) structure of laccase from *T. versicolor* (PDB ID: 1GYC) [19] was obtained from the Protein Data Bank (PDB) (http://www.pdb.org/pdb/home/home.do; [20]), whereas the 3D structures of phenol (CID: 996) and Triton X-100 (CID: 5590) were downloaded from PubChem (http://pubchem.ncbi.nlm.nih.gov/; [21]). Molegro Virtual Docker (MVD) was used to dock phenol or Triton X-100 into the binding sites of laccase [12]. The parameters were set as described by Chen et al. [13] with some modification. Docking was performed with a minimum of 20 runs and a max number of 10 poses returned. The best pose was selected for further interactional profile analysis using LigPlus which is the graphic interface of LigPlot [22]. It must be noted that LigPlot can only identify hydrophobic interactions and hydrogen bonds.

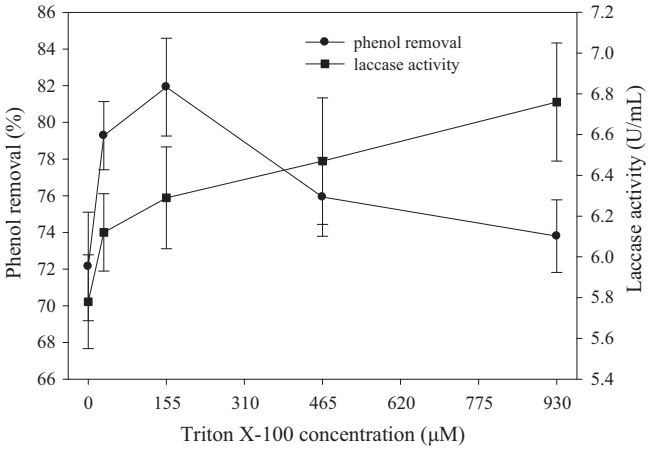

Fig. 1. Effect of Triton X-100 on phenol removal and laccase activity as a function of surfactant concentration. Conditions for phenol removal: 50 mg/L phenol, 0.05 mg/mL (1.155 U/mL) laccase and surfactant in Britton-Robinson buffer at pH 6 and 25 °C after 6 h reaction. Conditions for laccase activity: 2.0 mM ABTS, 0.25 mg/L laccase and surfactant in Britton-Robinson buffer at pH 4.5 and 25 °C. The results are the means of three determinations ± standard deviation.

3. Results and discussion

3.1. Effect of Triton X-100 concentration on phenol removal and laccase activity

In aqueous solutions, surfactants can exist as monomer (below the CMC) or as micelles (above the CMC), thus the effects promoted by the surfactants on the behavior of enzymes could depend on their organization forms [23]. Triton X-100 was added to the reaction solution at various concentrations below or above its CMC (about 310 μM [24]) to study the influence of surfactant concentration on phenol removal. As shown in Fig. 1, Triton X-100 at concentrations from 31 to 930 μM improved phenol removal, and the optimal concentration was 155 μM. Similar phenomena also occurred when laccase catalyzed the oxidation of bisphenol A in the presence of Triton X-100 [14]. Sakurai et al. [9] also reported that the presence of Triton X-100 at the concentrations below its CMC could improve the removal efficiency of phenol catalyzed by *Coprinus cinereus* peroxidase. When the concentration of Triton X-100 was above its CMC, the removal efficiency of phenol decreased with increasing Triton X-100 concentration (Fig. 1). The results suggest that the effect of Triton X-100 on the phenol removal related closely to its concentration. Surfactants in aqueous solutions can affect the enzymatic reactions either below or above the CMC. It can be a consequence of two factors, namely: (i) the interactions between enzyme and surfactant; (ii) the partitioning of substrate between the micelles and the external medium [25]. These interactions between enzyme and surfactant can involve the free surfactant molecules and/or the micelles leading to conformational changes which could modify the catalytic rate constant and/or the enzyme–substrate binding constant. Phenol, the low-molecular weight polarizable compound having an aromatic ring, adsorbs initially to the micelle interface and then enters deeper into the hydrophobic core [26,27]. In addition, the aggregation process of surfactant molecule was a stepwise process [28]. The hydrophobic tails of surfactant molecules collected together and formed aggregates. The large and incompact micelle aggregates turned into the small and compact micelles with increasing surfactant concentration. Therefore, surfactant molecules at higher concentrations adsorbed more phenol to partition it into the micelles

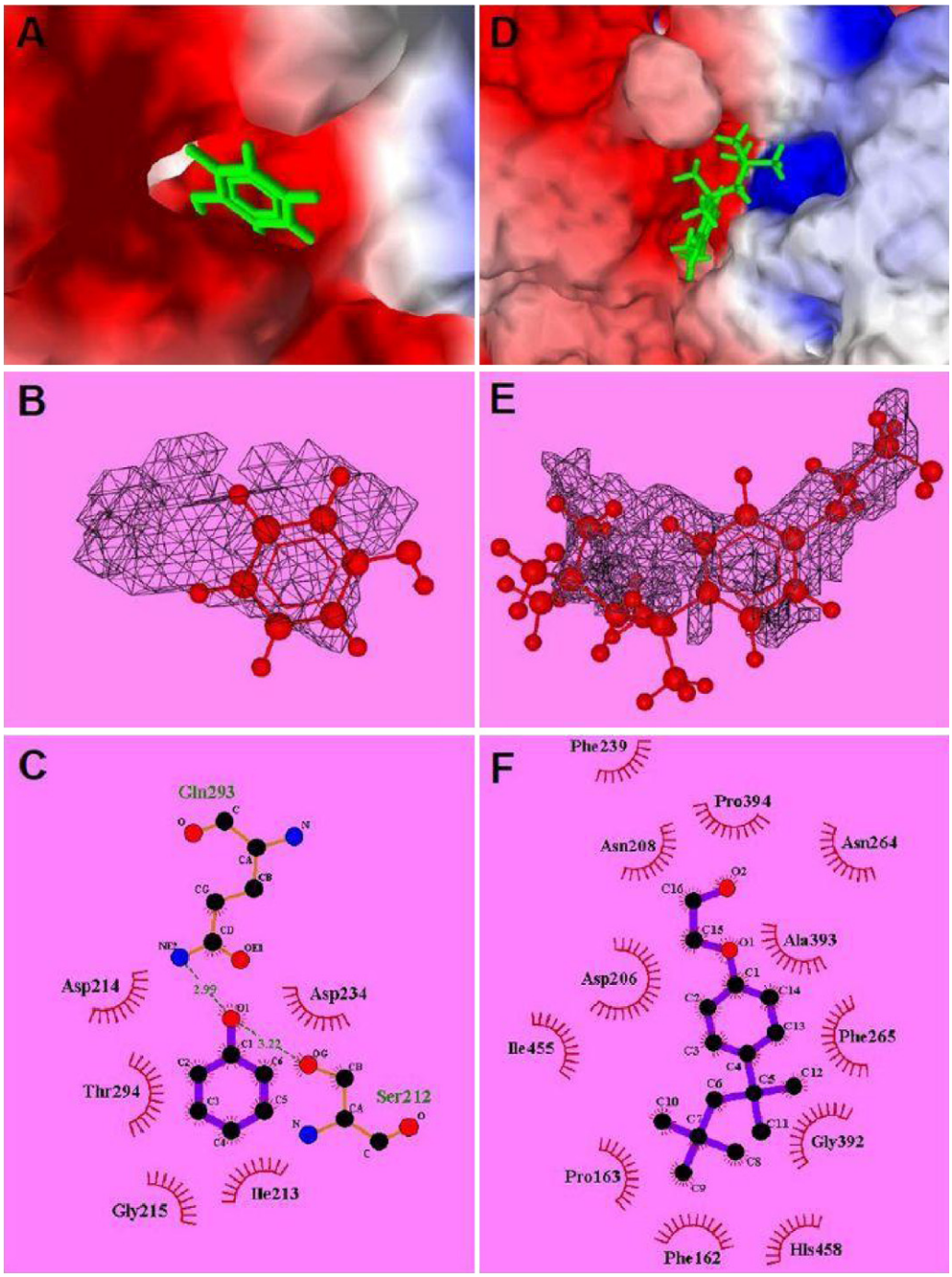

Fig. 2. Binding modes between laccase and phenol or Triton X-100. Panels A and D show the close-up view of the active sites of laccase with phenol and Triton X-100, respectively. The laccases are shown in the form of molecular surface colored according to the electrostatic properties. The electropositive sections are colored in red, while the electronegative sections in blue. Phenol and Triton X-100 are shown in green stick models. Panels B and E show the optimum poses of phenol and Triton X-100 in the active pockets of laccases, respectively. The black meshed girds represent the active pockets. The red ball-and-stick models are the phenol and Triton X-100, respectively. Panels C and F show the detailed interactions of the laccase-phenol complex and laccase-Triton X-100 complex, respectively. The black, red and blue solid circles indicate atoms. The blue bold bonds belong to phenol or Triton X-100, and the orange thin bonds belong to laccase. The green dashed lines refer to the hydrogen bonds between laccase and phenol, and the numbers on the lines show the length (in Å) of the hydrogen bonds. The red spoked arcs pointing to phenol or Triton X-100 represent the hydrophobic contacts. The letters beside the atoms of phenol or Triton X-100 show the type of the atoms. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

which reduced the free phenol concentration [29]. This might be an explanation for the phenomenon that the removal efficiency of phenol decreased with increasing Triton X-100 above the CMC as shown in Fig. 1. The effect of Triton X-100 concentration on laccase activity was studied to further investigate the influence mechanisms during phenol removal. Triton X-100 had positive effect on the activity of laccase (Fig. 1). When Triton X-100 was at 3.0 CMC, laccase activity was increased by approximately 17%. Li [30] also found that Triton X-100 at 5 mg/L significantly increased planarian cholinesterase activity, while Sakurai et al. [9] found Triton X-100 at 30 mg/L slightly influenced the total activity of *C. cinereus* peroxidase.

Table 1
Effects of Triton X-100 on phenol removal. Conditions: 50–600 mg/L phenol and 0.05 mg/mL (1.155 U/mL) laccase in Britton-Robinson buffer at pH 6 and 25 °C after various reaction time with or without 155 μM Triton X-100.

Initial phenol concentration (mg/L)	Time (h)	Control		Trion X-100		
		Phenol removal ± SD (%)	Phenol removal rate ± SD (mg/(L h))	Phenol removal ± SD (%)	Ratio[a]	Phenol removal rate ± SD (mg/(L h))
50	2	42.8 ± 1.2	10.7 ± 0.3	52.6 ± 1.7	1.2	13.2 ± 0.6
	6	72.1 ± 3.0	6.0 ± 0.2	85.4 ± 3.7	1.2	7.1 ± 0.12
	10	79.6 ± 1.1	4.0 ± 0.1	94.2 ± 0.3	1.2	4.7 ± 0.0
	24	89.6 ± 1.3	1.9 ± 0.0	98.4 ± 0.0	1.1	2.1 ± 0.0
100	2	31.5 ± 1.4	15.7 ± 0.7	50.1 ± 2.4	1.6	25.1 ± 0.8
	6	50.2 ± 2.3	8.4 ± 0.4	81.9 ± 1.5	1.6	13.7 ± 0.6
	10	58.1 ± 1.6	5.8 ± 0.2	93.6 ± 2.3	1.6	9.4 ± 0.0
	24	66.7 ± 3.2	2.8 ± 0.1	99.0 ± 0.0	1.5	4.1 ± 0.0
200	2	13.6 ± 0.5	13.6 ± 0.3	42.4 ± 1.6	3.1	42.4 ± 1.6
	6	23.7 ± 1.0	7.9 ± 0.0	79.9 ± 3.5	3.4	26.6 ± 1.2
	10	28.3 ± 0.6	5.7 ± 0.2	92.1 ± 0.2	3.3	18.4 ± 0.5
	24	31.0 ± 0.6	2.6 ± 0.1	98.4 ± 0.1	3.2	8.2 ± 0.0
400	2	6.2 ± 0.1	12.4 ± 0.2	34.9 ± 1.4	5.6	69.8 ± 2.9
	6	11.0 ± 0.4	7.3 ± 0.3	50.0 ± 1.5	4.5	33.3 ± 1.0
	10	13.9 ± 0.6	5.6 ± 0.3	59.6 ± 2.8	4.3	23.8 ± 1.1
	24	15.2 ± 0.5	2.5 ± 0.1	63.7 ± 2.9	4.2	10.6 ± 0.5
600	2	2.7 ± 0.1	8.2 ± 0.2	22.6 ± 1.0	8.4	67.9 ± 3.0
	6	6.2 ± 0.2	6.2 ± 0.2	35.6 ± 1.2	5.7	35.6 ± 1.2
	10	9.2 ± 0.2	5.5 ± 0.1	42.4 ± 1.5	4.6	25.4 ± 0.9
	24	10.3 ± 0.4	2.6 ± 0.1	44.5 ± 1.2	4.3	11.1 ± 0.3

[a] The ratio of phenol removal efficiency in the presence of Triton X-100 to that of control by their means after the same reaction time.

3.2. Binding mode

Laccase is a well-known kind of enzyme for phenol oxidation. The interaction between phenol and laccase was the first step of the reaction. The binding mode of phenol and laccase was shown in Fig. 2. Docking results showed phenol was in the binding pocket of laccase, and formed hydrogen bonds and hydrophobic interactions with laccase. Two residues Gln293 and Ser212 participated in the formation of hydrogen bonds with phenol, whereas five residues Asp214, Asp234, Thr294, Gly215 and Ile213 had hydrophobic interactions with phenol (Fig. 2).

Interaction profile between Triton X-100 and laccase was shown in Fig. 2. The results showed the residues Phe239, Asn208, Pro394, Asn264, Asp206, Ala393, Ile455, Phe265, Pro163, Gly392, Phe162 and His458 played a crucial role in binding to Triton X-100 in the form of hydrophobic interactions. No other interactions were detected by LigPlus. The interactions between Triton X-100 and laccase may cause a conformational change to a more active form and/or stabilized its native folded structure [29]. Ji et al. [14] found that the presence of Triton X-100 induced the hydrophobic amino acid residues (Tyr, Trp, etc.) of laccase obviously exposured to a less polar environment or buried into the inner part of the conformation of laccase.

3.3. Various phenol concentrations and reaction times

As shown in Table 1, the removal of phenol at concentrations ranging from 50 to 600 mg/L in the absence or presence of 155 μM Triton X-100 was studied. The presence of Triton X-100 increased the removal efficiency of phenol catalyzed by laccase. In addition, the higher the initial phenol concentration was, the more obviously the effect of the surfactant exhibited. For example, the removal efficiencies of phenol with Triton X-100 were 1.2, 1.6, 3.4, 4.5, and 5.7 fold those of the control after 6 h when the initial concentration of phenol increased from 50 to 600 mg/L. Phenols are strong inhibitors of the activity of enzyme, such as cellulases, β-glucosidases and hemicellulases [31]. In the control, the maximum phenol removal rate appeared when the initial concentration of phenol was 100 mg/L. For example, the removal rates were 6.0, 8.4, 7.9, 7.3 and 6.2 mg/(L h) after 6 h when initial phenol concentration increased from 50 to 600 mg/L. It is possible that, though the rate of phenol transformation increased with phenol concentration, so did the rate of inactivation of the enzyme; overall, the combination of these two competing phenomena could result in better transformation; however, as the reaction progressed at elevated phenol concentration, the degree of inactivation became more significant and the high reaction rates were lost. The removal rate increased with phenol concentration in the presence of Triton X-100 after the same reaction time. For example, the removal rates with Triton X-100 were 6.8, 14.2, 26.6, 33.3 and 35.6 mg/(L h) after 6 h when the initial concentration of phenol increased from 50 to 600 mg/L. One important reason may be that the presence of Triton X-100 could increase the activity of laccase to enhance the phenol removal.

3.4. UV–visible spectra

The UV–visible spectra of the reaction solutions were shown in Fig. 3. The shape and strength of peaks on UV–visible spectra changed greatly, suggesting that phenol was effectively oxidized in the enzyme treatment. Some dark brown precipitates were produced in the reaction system, which related closely to the result that the absorbance of reaction solution with laccase was usually higher than that without laccase (Fig. 3a). Peaks at wavelengths of 220 and 270 nm disappeared. A new peak at 400 nm appeared in the absence or presence of Triton X-100 after 6 h, indicating the presence of a quinone system. With reaction processing, this new peak also disappeared, indicating that most of quinone disappeared and transformed into precipitates. The average absorbance with Triton X-100 was higher than that without it, as shown in Fig. 3a. The results indicated that Triton X-100 improved the oxidation reaction and more products were produced. As shown in Table 1, phenol was not completely oxidized in the reaction solution, especially in the system with laccase after 6 h when the removal efficiency was 72.1%. However, the UV spectra did not show the specific absorbance of phenol (Fig. 3a). One of the prime reasons may be that the absorbance of the dark brown precipitates suspended in solution shielded that of phenol. The mixtures of Triton X-100 and

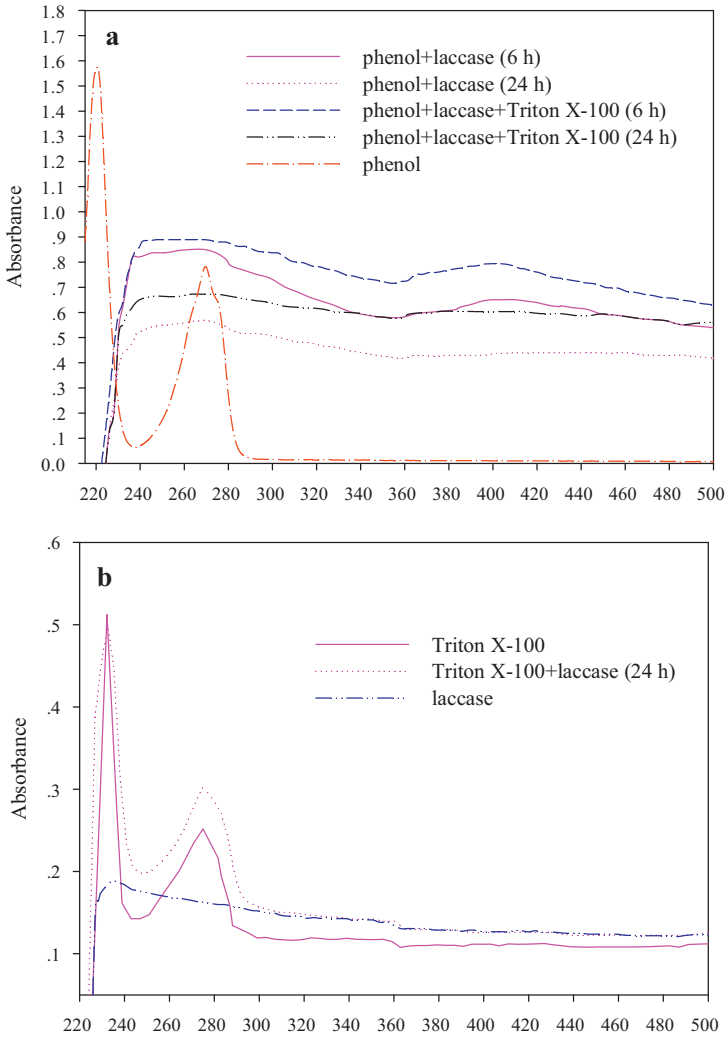

Fig. 3. UV spectra of various reaction solutions. Conditions: 50 mg/L phenol, 0.05 mg/mL (1.155 U/mL) laccase, and 155 μM Triton X-100 in Britton-Robinson buffer at pH 6 and 25 °C.

laccase after 24 h were also determined using UV–visible spectrophotometer, as shown in Fig. 3b. Peaks at 230 and 270 nm were attributed to the aromatic structure of Triton X-100. The UV spectra of Triton X-100 with or without laccase changed slightly. This suggests that Triton X-100 was not oxidized in the laccase treatment.

4. Conclusions

The effects of Triton X-100 on the removal of aqueous phenol catalyzed by laccase were studied. Triton X-100 at concentrations from 31 to 930 μM could improve the efficiency of phenol removal. The optimal concentration of Triton X-100 was 155 μM to improve phenol removal when the concentrations of phenol and laccase were 50 mg/L and 0.05 mg/mL, respectively. The enhancement on phenol removal may be due to the increased laccase activity by Triton X-100, which was demonstrated in this study. Molecular docking results showed that phenol formed hydrogen bonds and hydrophobic interactions with laccase before it was oxidized, while Triton X-100 formed hydrophobic interactions with laccase, which may be one reason for the increased laccase activity. The reaction of phenol removal was also characterized using UV spectra. Typically, phenol removal catalyzed by laccase was enhanced in the presence of low concentrations of Triton X-100. It is concluded that the presence of low concentrations of Triton X-100 for phenol removal catalyzed by enzymes may be an alternative to the present phenol removal processes in water treatment or remediation. However, the problems of the stability of laccase cannot be overlooked and more in-depth investigation is needed.

Acknowledgments

The authors sincerely thank the editor and anonymous reviewers for suggestions on improving this paper. The study was financially supported by the National Natural Science Foundation of China (50908081, 50978088, 51039001), the Environmental Protection Technology Research Program of Hunan (2007185), the Hunan Key Scientific Research Project (2009FJ1010), the Hunan Provincial Natural Science Foundation of China (10JJ7005), the Program for New Century Excellent Talents in University (NCET-08-0181), the Research Fund for the Doctoral Program of Higher Education of China (20100161110012), the Hunan University Graduate Education Innovation Project (531107011019), and the Hunan Provincial Innovation Foundation for Postgraduate (CX2009B078, CX2009B080, CX2010B157).

References

[1] Z.-F. Liu, G.-M. Zeng, J. Wang, H. Zhong, Y. Ding, X.-Z. Yuan, Process Biochem. 45 (2010) 805–809.
[2] Q. Huang, J. Tang, W.J. Weber Jr., Water Res. 39 (2005) 3021–3027.

[3] L. Gianfreda, F. Sannino, M.A. Rao, J.M. Bollag, Water Res. 37 (2003) 3205–3215.
[4] M.S. Coniglio, V.D. Busto, P.S. González, M.I. Medina, S. Milrad, E. Agostini, Chemosphere 72 (2008) 1035–1042.
[5] A.M. McMahon, E.M. Doyle, S. Brooks, K.E. O'Connor, Enzyme Microb. Technol. 40 (2007) 1435–1441.
[6] A.I. Cañas, M. Alcalde, F. Plou, M.J. Martínez, A.T. Martínez, S. Camarero, Environ. Sci. Technol. 41 (2007) 2964–2971.
[7] L. Tang, G.M. Zeng, J.X. Liu, X.M. Xu, Y. Zhang, G.L. Shen, Y.P. Li, C. Liu, Anal. Bioanal. Chem. 391 (2008) 679–685.
[8] K. Modaressi, K.E. Taylor, J.K. Bewtra, N. Biswas, Water Res. 39 (2005) 4309–4316.
[9] A. Sakurai, M. Masuda, M. Sakakibara, J. Chem. Technol. Biotechnol. 78 (2003) 952–958.
[10] F. Karbassi, K. Haghbeen, A.A. Saboury, B. Ranjbar, A.A. Moosavi-Movahedi, Colloids Surf. B 32 (2003) 137–143.
[11] Z. Yang, J. Deng, L.F. Chen, J. Mol. Catal. B: Enzym. 47 (2007) 79–85.
[12] R. Thomsen, M.H. Christensen, J. Med. Chem. 49 (2006) 3315–3321.
[13] M. Chen, G. Zeng, Z. Tan, M. Jiang, H. Li, L. Liu, Y. Zhu, Z. Yu, Z. Wei, Y. Liu, G. Xie, PLos ONE 6 (2011) e25647.
[14] G. Ji, H. Zhang, F. Huang, X. Huang, J. Environ. Sci. 21 (11) (2009) 1486–1490.
[15] A.J. Stewart, R.F. Stewart, Encyclopedia of Ecology, Elsevier, Amsterdam, 2008.
[16] F. Xu, Effects of redox potential and hydroxide inhibition on the pH activity profile of fungal laccases, J. Biol. Chem. 272 (1997) 924–928.
[17] S. Kurniawati, J.A. Nicell, Bioresour. Technol. 99 (2008) 7825–7834.
[18] Z.-F. Liu, G.-M. Zeng, H. Zhong, X.-Z. Yuan, H.-Y. Fu, M.-F. Zhou, X.-L. Ma, H. Li, J.-B. Li, World J. Microbiol. Biotechnol. 28 (2012) 175–181.
[19] K. Piontek, M. Antorini, T. Choinowski, J. Biol. Chem. 277 (2002) 37663–37669.
[20] P.W. Rose, B. Beran, C. Bi, W.F. Bluhm, D. Dimitropoulos, D.S. Goodsell, A. Prlic, M. Quesada, G.B. Quinn, J.D. Westbrook, J. Young, B. Yukich, C. Zardecki, H.M. Berman, P.E. Bourne, Nucleic Acids Res. 39 (2011) D392.
[21] Y. Wang, J. Xiao, T.O. Suzek, J. Zhang, J. Wang, S.H. Bryant, Nucleic Acids Res. 37 (2009) W623–W633.
[22] A.C. Wallace, R.A. Laskowski, J.M. Thornton, Protein Eng. 8 (1995) 27–34.
[23] M.A. Biasutti, E.B. Abuin, J.J. Silber, N.M. Correa, E.A. Lissi, Adv. Colloid Interface Sci. 136 (2008) 1–24.
[24] X. Yuan, F. Ren, G. Zeng, H. Zhong, H. Fu, J. Liu, X. Xu, Appl. Microbiol. Biotechnol. 76 (2007) 1189–1198.
[25] E. Abuin, E. Lissi, R. Duarte, J. Colloid Interface Sci. 283 (2005) 539–543.
[26] G.-M. Zeng, K. Xu, J.-H. Huang, X. Li, Y.-Y. Fang, Y.-H. Qu, J. Membr. Sci. 310 (2008) 149–160.
[27] H. Adamczak, K. Materna, R. Urbanski, J. Szymanowski, J. Colloid Interface Sci. 218 (1999) 359–368.
[28] Y.-Y. Fang, G.-M. Zeng, J.-H. Huang, J.-X. Liu, X.-M. Xu, K. Xu, Y.-H. Qu, J. Membr. Sci. 320 (2008) 514–519.
[29] G. Savelli, N. Spreti, P.D. Profio, Curr. Opin. Colloid Interface Sci. 5 (2000) 111–117.
[30] M.H. Li, Chemosphere 70 (2008) 1796–1803.
[31] W.F. Anderson, D.E. Akin, J. Ind. Microbiol. Biotechnol. 35 (2008) 355–366.