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Effects of nonionic surfactant Triton X-100 on the laccase-catalyzed conversion of bisphenol A

JI Guanglei[1], ZHANG Haibo[2], HUANG Feng[2], HUANG Xirong[1,3,*]

1. School of Environmental Science and Engineering, Shandong University, Jinan 250100, China. E-mail: chm_jigl@ujn.edu.cn
2. State Key Laboratory of Microbial Technology of China, Shandong University, Jinan 250100, China
3. Key Laboratory of Colloid and Interface Chemistry of the Education Ministry of China, Shandong University, Jinan 250100, China

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Abstract

The laccase-catalyzed conversion of bisphenol A (BPA) in aqueous solutions was studied in the absence and presence of nonionic surfactant Triton X-100. It was found that the addition of Triton X-100 into the reaction system increased the conversion of BPA, especially near the critical micelle concentration of Triton X-100. Also it was found that the stability of laccase was greatly improved in the presence of TritonX-100. Studies on the endogenous fluorescence emission of laccase indicated that there existed an interaction between Triton X-100 and laccase, which was beneficial to folding and stabilizating of laccase. The binding of Triton X-100 to the laccase surface also mitigated the inactivation effect caused by the free radicals and polymerization products. Under otherwise identical conditions, a lower dosage of laccase was needed for the higher conversion of BPA in the presence of Triton X-100.

Key words: conversion; laccase; bisphenol A; Triton X-100

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Introduction

Bisphenol A (BPA) is suspected of having estrogenic activity and to be an endocrine disrupting chemicals (EDC). It can disturb the secretion of normal hormone in human body, affect the reproductive function, and lead to the generation of malignant tumors (Krishnan *et al*., 1993; Staples *et al*., 1998; Yin *et al*., 2007). Its damage to environment and organisms has caused an extensive concern. In recent years, various methods have been tried to remove BPA, such as physical process including adsorption (Zhao *et al*., 2008) and ultrafiltration (Dong *et al*., 2008), chemical process including electrochemical oxidation (Kuramitz *et al*., 2001), ozone oxidation (Deborde *et al*., 2008; Irmak *et al*., 2005), sonochemical reaction (Inoue *et al*., 2008), and biodegradation process involving fungi and algae (Kang and Kondo, 2002; Tsutsumi *et al*., 2001). However, it is difficult to completely remove BPA just by physical and/or chemical process. The biodegradation process by fungi and bacteria has received much attention. These microorganisms can secrete a series of oxidative enzymes. Some of them, such as laccase (Kim and Nicell, 2006a, 2006b; Uchida *et al*., 2001), manganese peroxidase (Hirano *et al*., 2000), horseradish peroxidase (Li and Nicell, 2008), and lignin peroxidase (Takamiya *et al*., 2008) have been proved effective in removing BPA from aqueous solutions.

Laccases are multinuclear copper-containing oxidases. They can catalyze the oxidation of polyphenol and polyaminophenol at the expense of molecular oxygen. These ecofriendly enzymes have been widely applied in the conversion of phenolic pollutants (Riva, 2006). When laccase was used for BPA conversion, it was found that laccase could be easily inactivated, due to its interactions with the oxidative polymerization products of BPA even under an optimized reaction condition (Kim and Nicell, 2006b). Similar phenomenon was observed during the degradation of other phenolic pollutants with oxidoreductases. To solve these problems, some additives such as polyethylene glycol (PEG) (Modaressi *et al*., 2005; Tonegawa *et al*., 2003; Wu *et al*., 1998) and surfactants (Kim *et al*., 2007; Sakurai *et al*., 2003; Tonegawa *et al*., 2003) were added to the reaction system. Compare to other additives, the use of nonionic surfactants has some advantages. In addition to improve the solubility of hydrophobic phenolic pollutants, nonionic surfactants have less effect on the conformation of enzyme because of its neutral in charge.

In this study, we examined the degradation of BPA with laccase in the absence and presence of nonionic surfactant TritonX-100 and discussed the related mechanism.

1 Materials and methods

1.1 Reagents

Laccase from *Trametes versicolor*, 2, 6-dime- thoxyphenol (DMP), BPA and Triton X-100 were purchased from

* Corresponding author. E-mail: xrhuang@sdu.edu.cn

Sigma (USA). All other reagents were of analytical purity.

1.2 Laccase activity assay

Laccase activity was determined spectrophotometrically by monitoring the conversion of 1 mmol/L DMP in 100 mmol/L sodium acetate buffer at pH 4.2 and 470 nm (ε_{470} = 49600 L/(mol·cm)). The spectrophotometric assays were performed on a spectrophotometer (Shimadzu UV-2550, Japan) equipped with a water-circulating thermostat. The enzymatic reactions were carried out at 30°C. One unit of the enzyme activity was defined as the amount of laccase that produced 1 μmol of the quinone dimer per minute from DMP.

1.3 Surface tension measurements

The critical micelle concentration (CMC) of Triton X-100 in real systems was determined according to the Wilhelmy plate method on an interfacial tensiometer (Tensiometer K12, Germany). The aqueous solutions with different concentrations of Triton X-100 were prepared with 50 mmol/L sodium acetate buffer (pH 4.2) in the absence and presence of BPA. The temperature for the measurement was set at (25 ± 1)°C.

1.4 Fluorescence spectroscopy

Fluorescence emissions between 290 and 400 nm were recorded by a spectrofluorometer (Perkin Elmer LS55, USA) with an excitation wavelength of 274 nm (bandwidth, 2 nm). The concentration of laccase for fluorescence measurement was 13 mg/L in 50 mmol/L sodium acetate buffer (pH 4.2). The temperature of the samples was kept at (25 ± 1)°C.

1.5 Bioconversion of BPA

Stock solutions of BPA (100 mmol/L) were prepared in methanol. The laccase catalyzed oxidation of BPA in the absence and presence of Triton X-100 was initiated by adding a certain amount of laccase to 3 mL 50 mmol/L sodium acetate buffer (pH 4.2) that contained BPA and Triton X-100 in the desired quantities. After incubating at 25°C for an appropriate period, 100 μL sample was taken from the reaction system for compositional analysis. The residual activity of laccase was measured as described above. The conversion of BPA was measured.

Prior to the measurement of BPA conversion, the sample was acidified with small amount of concentrated acetic acid to reduce pH to approximately 2, thereby halting the enzymatic reaction. The BPA concentrations of the acidified samples were determined using a high performance liquid chromatography (Shimadzu LC-20AT, Japan) fitted with a UV-Vis detector (SPD-20A). The detection wavelength was set at 276 nm. The mobile phase was composed of water and acetonitrile at a volume ratio of 40:60. The flow rate was set at 1.0 mL/min.

2 Results and discussion

2.1 Effect of Triton X-100 on the conversion of BPA

Figure 1 shows the conversion of BPA with laccase as a function of Triton X-100 concentrations. With a small addition of Triton X-100, the conversion of BPA increased rapidly. The conversion ratio of BPA reached 91.8% when the concentration of Triton X-100 rose up to 50 mg/L. After that, the conversion remained almost unchanged within the Triton X-100 concentration range 50–100 mg/L. When the concentration of Triton X-100 exceeded 100 mg/L, the conversions gradually decreased, but it was still higher than that in the absence of Triton X-100 (13.4%). These results indicated that Triton X-100 has a positive effect on the conversion of BPA.

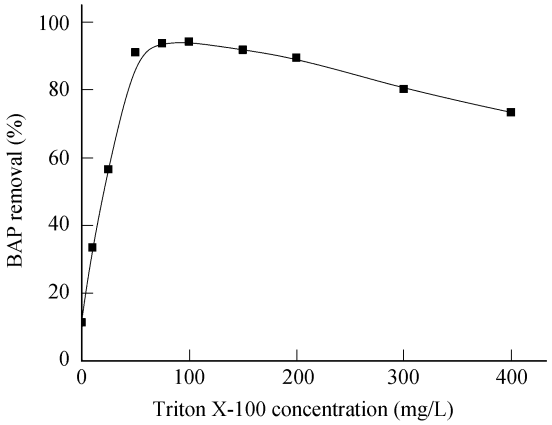

Fig. 1 Effect of the concentration of Triton X-100 on the conversion of BPA. Conditions: BPA 0.3 mmol/L; laccase 0.37 U/mL; sodium acetate buffer 50 mmol/L; pH 4.2, tempetreture 25°C; time 30 min.

2.2 Enhancement of Triton X-100

Triton X-100 is an amphiphilic molecule. At high concentration, Triton X-100 can aggregate to form a micelle. To investigate the mechanism, we determined the CMC of Triton X-100 at 25°C in 50 mmol/L sodium acetate buffer (pH 4.2). Figure 2 shows the variation of the surface tension with the logarithm of the TritonX-100 concentration at 25°C in the absence and presence of BPA. It indicates that the CMC of Triton X-100 was about 150 mg/L in the buffer solution system and that, when BPA was present, it decreased to 100 mg/L (Fig. 2). As shown in Fig. 1, the conversion of BPA was high in the Triton X-100 concentration range 50–100 mg/L. Within this concentration range, Triton X-100 exists in a monomer or pre-micelle (small aggregate). This indicates that the monomer or pre-micelle has a better enhancing effect on the conversion of BPA than micelle. The conversions of BPA decreased when the concentration of Triton X-100 exceeded 100 mg/L, i.e., the micelles formation, is caused by that some BPA molecules were partitioned into the micelle pseudophase due to the hydrophobic properties of BPA, which prevented its contact with laccase, leading to a decrease in the conversion. Further increase in the concentration of Triton X-100 caused an increase in the number of aggregated micelles, which had more BPA molecules partition into the micelles, thereby the conversion decreased gradually.

Triton X-100 had little effect on the reactivity of BPA in the absence of laccase. Therefore, the enhancing effect of Triton X-100 on the enzymatic reaction should be owing

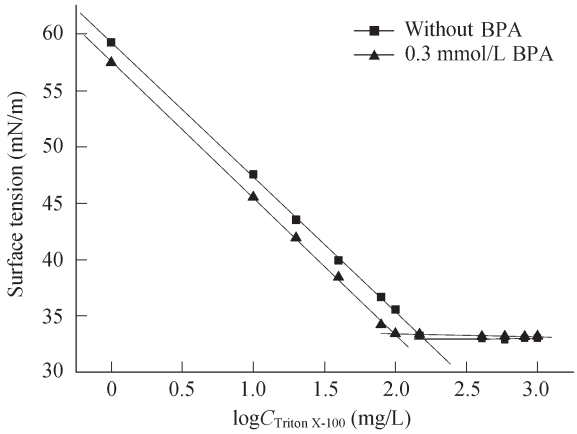

Fig. 2 Variation of surface tension vs. the logarithm of the Triton X-100 concentration (*C*) at 25°C in the absence and presence of BPA. Conditions: sodium acetate buffer 50 mmol/L; pH 4.2.

to the improvement of activity and stability and the results are shown in Fig. 3.

In the BPA conversion system, without Triton X-100 the laccase activity decreased 73% in 3 min, and half hour later, it retained only 13% of the initial activity. When Triton X-100 was present (50 mg/L) in the BPA conversion system, however, laccase could maintain higher catalytic activity in a longer time and, more importantly, the stability was even better than that only in the buffer solution. In sodium acetate buffer only, a 22% decrement of the laccase activity was observed in 10 min although the subsequent descending rate became slow; and one hour later, 70% of the initial activity was retained. In addition, Triton X-100 has some activating effect on laccase at the initial stage. Similar results were observed previously (Gong *et al*., 2003; Srinivasulu and Rao, 1993; Yoon and Robyt, 2005).

The catalytic activity of an enzyme is closely related to its conformation. The above results indicate that Triton X-100 helps laccase to be in the optimal folding state. This conclusion was also supported by the endogenous fluorescence emission of laccase in the present of different concentrations of Triton X-100 (Fig. 4). The maximum fluorescence emission of laccase in the absence of Triton X-100 located at 340 nm with low intensity. With the increase of Triton X-100 concentration, the fluorescence intensity increased greatly and the maximum fluorescence emission was shifted to a shorter wavelength (ca. 300 nm). The results indicated that hydrophobic amino acid residues (Tyr, Trp, etc.) of laccase were obviously exposured to a less polar environment or buried into the inner part of the conformation of laccase. This is beneficial for folding and stabilizing of laccase.

The stabilization of laccase by Triton X-100 also resulted from the low possibility of the attack of intermediate free radicals and the adsorption of oxidative polymerization products on laccase. It is accepted that the enzyme inactivation in the enzyme-catalyzed transformation system was mainly induced by heating-inactivation, intermediate free radical attack, and the adsorption by oxidative polymerization products. It is also known that the laccase-catalyzed oxidation of phenolic compounds is a free-radical-involved oxidation-polymerization-degradation process. For BPA, first, it is oxidized by laccase, generating free radicals; then these radicals form a variety of oligomers by coupling; finally, the formed oligomers are degraded into fragments (Cabana *et al*., 2007; Fukuda *et al*., 2001; Leonowicz *et al*., 2001; Uchida *et al*., 2001). It was demonstrated that the oligomers were the dimer, trimer, tetramer, pentamer, and hexamer of BPA, which are subsequently subjected to the degradation, releasing the fragments of oligomers each with phenol molecules and 4-isopropenylphenol (Fukuda *et al*., 2004). The free radical formed in the reaction system could bind to the active site of laccase via covalent bonds, thereby hindering the catalytic cycle and decreasing the catalytic efficiency of laccase. On the other hand, the polymerization products of BPA may deposit on laccase surface, resulting in the enzyme encapsulation and hindering the access of BPA to the active sites of laccase. The presence of Triton X-100 in the medium

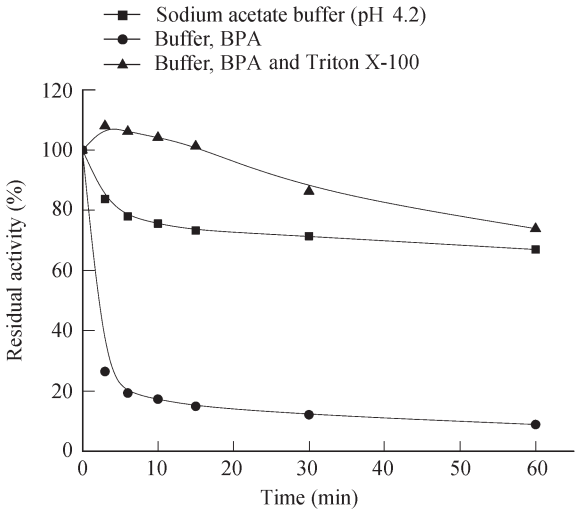

Fig. 3 Stability of laccase in the buffer, buffer with BPA, and buffer with both BPA and Triton X-100. Conditions are the same as that in Fig. 1 except that Triton-100 concentration was 50 mg/L.

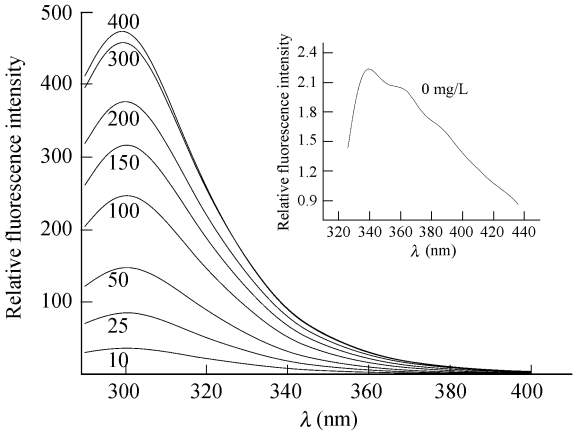

Fig. 4 Fluorescence emission spectra of laccase in the presence of different concentrations (0–400 mg/L) of Triton X-100 at 25°C. Conditions: laccase concentration 13 mg/L; sodium acetate buffer 50 mmol/L; pH 4.2; excitation wavelength 274 nm.

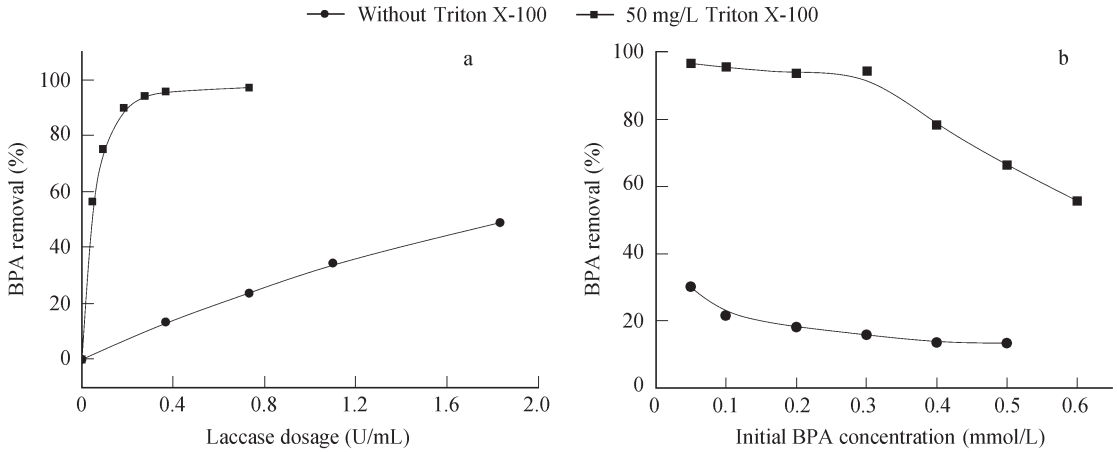

Fig. 5 Changes of the BPA removal with the different laccase dosage (a), and with the different initial BPA concentrations (b) in the absence and presence of Triton X-100. Conditions: BPA concentration: 0.3 mmol/L, laccase concentration: 0.55 U/mL, sodium acetate buffer: 50 mmol/L, pH: 4.2, temperature: 25°C, time: 30 min.

effectively prevented the laccase inactivation induced by the intermediate free radical attack and the adsorption of the polymerization products on laccase.

2.3 Effects of laccase and BPA

Figure 5 shows the relationship between the initial laccase dosage and the BPA removal percentage in the absence or presence of Triton X-100. As shown in Fig. 5a, the conversion of BPA in both cases increased as the laccase dosage increase. For a given laccase dosage, 0.18 U/mL, for example, a 90% conversion of BPA was obtained in the presence of 50 mg/L Triton X-100, whereas, in the absence of Triton X-100 only 4.8% BPA was removed. These results indicated that laccase is more efficient in the presence of Triton X-100 for the BPA removal, which may due to the protecting effects of Triton X-100 mentioned above.

The same result could be seen from Fig. 5b which shows the change of BPA conversion with different initial concentrations in the absence and presence of Triton X-100. Over the BPA concentration range studied, the conversions of BPA in the presence of Triton X-100 were much higher than those in the absence of Triton X-100, although the percentage was gradually decreased as the BPA concentration increase.

3 Conclusions

The nonionic surfactant Triton X-100 has an enhancing effect on the catalytic conversion of BPA by laccase when its concentration is around CMC. This effect is originated from the interaction between the monomers or pre-micelles of Triton X-100 and laccase. In addition to mitigating the inactivation effect on laccase by the free radicals and polymerization products of BPA, the interaction is beneficial to folding and stabilizing of laccase. These favorable effects lead to a higher conversion of BPA with a lower dosage of laccase.

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