Optimum removal conditions of aniline compounds in simulated wastewater by laccase from white-rot fungi

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Abstract

Background Aniline compounds are widely applied as important chemical raw materials. However, they are so toxic and harmful to humans and environment that they need to be removed by an effective and economic approach, such as enzymatic reaction, which is in line with contemporary green development concepts.

Methods The effects of major factors, such as temperature, reaction time, concentration of laccase and the initial concentration of substrate on the removal of substrate were investigated by OFAT approach. After simulated wastewater is treated with enzymes, aniline concentration was determined by N-(1-Naphthyl)ethylene-diamine dihydrochloride spectrophotometric method. Concentration of o-phenylenediamine was determined by ferric ammonium alum spectrophotometric method.

Results For the removal of aniline, the optimum conditions were as follows: 50 °C, initial aniline concentration of 80 mg/L and laccase concentration of 1 g/L. In this case, the total removal of aniline reached 97.1% after 8 h, this also involves the volatilization of aniline itself. The optimum conditions of o-phenylenediamine were as follows: 50 °C, initial concentration of 100 mg/L and laccase concentration of 1 g/L. Under the above condition, the o-phenylenediamine could be removed completely after 60 min.

Conclusion The results show that the removal of aniline compounds by laccase from white-rot fungi has good effect and potential application prospect.

Keywords Aniline · Laccase · o-phenylenediamine · Removal · Wastewater

Introduction

Aniline compounds are important organic chemical raw materials which are widely used in printing and dyeing, pesticide, pharmaceutical industry, and so on. Because of their toxicity and carcinogenicity to human health, they have been included in the black list of priority pollutants by many countries in the world. So the removal of aniline compounds from wastewater is particularly important for human health and environmental protection. At present, the conventional methods of removing aniline compounds include adsorption [1, 2], biological degradation [3–5], catalytic oxidation process [6, 7], electrochemical techniques [8] and other methods [9].

With the continuous improvement of environmental protection requirements, some traditional methods may not be suitable for the treatment of aniline wastewater. For example, physical-chemical methods are simple, efficient and time-saving, but the pollutant cannot be removed completely, the cost is high and the conditions are harsh. In contrast, the advantages of biological treatment technology are low cost, high efficiency, mild reaction conditions, and without secondary pollution. For example, enzyme catalysis processes can improve the wastewater treatment efficiency, save treatment time and reduce infrastructure investment, belonging to the “environment-friendly” approach. It’s necessary to explore the optimal conditions of enzymatic reaction for dealing with aniline compounds-containing wastewater.

Laccase from white-rot fungi has excellent properties compared with other enzymes. It’s a kind of copper-containing polyphenol oxidase which exists widely in plant, fungi [10], and bacteria [11]. Laccases can catalyze a broad range of recalcitrant pollutants, including hydroxylated polychlorinated

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biphenyls [12], chlorophenols [13], polycyclic aromatic hydrocarbons [14], and dyes [15] by a one-electron transfer mechanism. Furthermore, it was demonstrated that laccase is effective in removing the diphenylamine [16]. Related research showed that the immobilized laccase has a broader range of pH and temperature compared with free laccase [17]. There are many reports on the application of the purified laccases or laccase-producing organisms for removal of toxic organic matters in recent years [18, 19].

In this study, the influencing factors for removing aniline and *o*-phenylenediamine by laccase were systematically investigated by OFAT approach based on our previous research, including temperature, reaction time, the initial concentration of substrate and the concentration of laccase. And the best catalytic conditions were inferred considering the actual situation.

Methods

Enzyme and chemicals

Laccase from white-rot fungi (Ivory and ecru particles, specific activity 28,000 U/g) was purchased from Shanghai Denychem Chemical Technology Co., Ltd.

Aniline, *o*-phenylenediamine, ammonium sulfamate, *N*-(1-Naphthyl)ethylene, diaminedihydrochloride, sodium nitrite, ferric ammonium alum were of analytical reagent.

Analytical methods

Aniline concentration was determined by *N*-(1-Naphthyl)ethylene-diamine dihydrochloride spectrophotometric method. Concentration of *o*-phenylenediamine was determined by ferric ammonium alum spectrophotometric method [20].

Experimental procedure

Since aniline contains amino basic groups, it is more susceptible to catalytic oxidation under acidic conditions. Based on our previous studies and literature investigations, the optimum pH of laccase from white-rot fungi is 6. So the laccase was dissolved in pH of 6.0 buffer solution, the effect of pH on the removal of substrate was not discussed in this paper. Experiments of aniline, concerning time and temperature, were conducted both in the presence and absence of laccase under the same conditions.

In order to investigate the effect of temperature on the removal of substrate and determine the optimum temperature, the flasks were incubated at different temperatures ranging from 30 °C to 80 °C. The initial concentration of

substrate was 100 mg/L, and laccase concentration was 1 g/L. The reaction system was a 250 mL Erlenmeyer flask containing 100 mg/L of substrate and 1 g/L of laccase which was put into a shaking table at optimum temperature (50 °C). The reaction time was 1, 2, 4, 6, 8, and 10 h, respectively. The absorbance value of the reaction mixture was analysed to calculate the removal.

The effect of the initial substrate concentration on the removal was investigated at temperature of 50 °C, laccase concentration of 1 g/L. Initial concentrations of aniline in the reactors were 20 mg/L, 40 mg/L, 60 mg/L, 80 mg/L, 100 mg/L, 120 mg/L, 140 mg/L, and 160 mg/L. The experiments on the removal of aniline were run both in the presence and absence of laccase. Initial concentrations of *o*-phenylenediamine in the reactors were 50 mg/L, 100 mg/L, 150 mg/L, 200 mg/L, 250 mg/L, 300 mg/L, 350 mg/L, and 400 mg/L. The reaction time of aniline and *o*-phenylenediamine was 8 h and 60 min, respectively.

The effect of laccase concentration on the removal of substrate was determined at temperature of 50 °C. The initial concentration of aniline and *o*-phenylenediamine was 80 mg/L and 100 mg/L, respectively. The reaction time was 8 h and 60 min, respectively. Laccase concentrations for aniline removal were 0.2 g/L, 0.5 g/L, 1 g/L, 2 g/L, and 3 g/L, while for *o*-phenylenediamine were 0.025 g/L, 0.05 g/L, 0.1 g/L, 0.2 g/L, 0.5 g/L, 1.0 g/L, and 2 g/L.

Results and discussion

Removal mechanism of aniline compounds by laccase

The active site of laccase includes four copper ions, one is T1 (type 1) copper ions, one is T2 (type 2) copper ions, others are T3 (type 3) copper ions. And T2 and T3 copper ions are arranged in a triangular core [21]. Take aniline as example to discuss reaction mechanism. According to relevant research from Jiangnan University, T1 copper ions oxidize four aniline molecules to produce four aniline radicals, due to reduction of T1 and T2 copper ions and four single-electron oxidations (Fig. 1). The free radicals formed are easily polymerized so that convert to solid substance, which can be removed by filter [22]. In addition to the catalysis of laccase, the volatility of aniline had a certain influence on the experimental results.

When the reaction system has only single substrate, generally polymers are formed. While there are two or more substrates in the reaction system, cross-coupling will occur between the substrates after catalyzed oxidation by laccase to form complex cross-linked products. Therefore, aniline is almost converted to polymers in the experiments.

Fig. 1 Catalytic removal of aniline by laccase

Determination of the optimum reaction conditions of aniline by laccase

Effect of temperature

Laccase is a kind of copper-containing monomeric glycoprotein, which has the highest activity at its optimum temperature. Effect of temperature on the removal of aniline was shown in Fig. 2, and control experiments (absence of laccase) were carried out under the same conditions.

Figure 2 shows that the removal of aniline was gradually increasing with the temperature rising at temperature below 50 °C. At 50 °C, the highest total removal of aniline reached 96.2%. When temperatures is above 50 °C, the removal of aniline remains essentially unchanged. Since aniline is a low-boiling substance, it can volatilize with water vapor.

Fig. 2 Effect of temperature on the removal of aniline

The volatilization of aniline was gradually increasing with the temperature rising. Therefore, the total removal of aniline involves not only catalytic reaction by laccase, but also by volatilization of aniline itself. It can be seen from Fig. 2 the volatilization of aniline itself accounts for 46.1% of the total removal at 50 °C. Therefore, the following experiments were carried out on the basis of optimum temperature (50 °C). Due to the higher volatilization of aniline it needs to be collected or in a closed system when it was heated,. In general, most laccases derived from fungi have better enzymatic activity at 50~70 °C [23], the experimental results are consistent with it.

Effect of reaction time

Effect of time on the removal of aniline was shown in Fig. 3.

The removal of aniline was slowly increasing with the extension of time, and the total removal reached 96.2% after 8 h.

Fig. 3 Effect of time on the removal of aniline

When enzymatic reaction was stopped, the volatilization was also almost stopped. As shown in Fig. 3, the volatilization of aniline itself also accounts for a certain proportion of the removal of aniline in the presence of laccase. After a certain period of time, the effect of time on removal is no longer significant, so the optimum time is 8 h.

Effect of the initial concentration of aniline

The removal efficiency of substrate is related to its molecular structure, including the type, the position, and number of substituents. The removal of aniline by laccase is mainly achieved by polymerizing of substituents to form a low-soluble polymer. The initial concentration of aniline also affects its removal, as shown in Fig. 4.

It can be seen from Fig. 4 that the total removal of aniline gradually increased with the rise of its initial concentration, and reached the highest efficiency (97.2%) at a concentration of 80 mg/L. Under the condition of the initial concentration of aniline is 80 mg/L, copper ions in laccase are fully utilized to convert aniline into free radicals. When the initial concentration of aniline is above 80 mg/L, the active center of laccase was fully occupied by aniline so that laccase cannot exhibit more activity. Of course, the volatilization of aniline itself also has a certain effect on the removal. In actual operation, attention should be paid to the concentration of aniline in the wastewater.

Effect of the concentration of laccase

Similarly, the concentration of laccase has a great influence on the removal efficiency of aniline. When the concentrations of laccase were 0.2 g/L, 0.5 g/L, 1 g/L, 2 g/L, and 3 g/L separately, the removal of aniline was shown in Fig. 5.

Figure 5 represented that the removal of aniline was significantly enhanced with the increasing of laccase concentration from 0.2 g/L to 1 g/L, there was no increase of total removal when laccase concentration exceeded 1 g/L. The reason is that laccase was relatively excessive when it exceeded 1 g/L, the enzymatic reaction cannot be promoted. So the optimum

Fig. 4 Effect of initial concentration of aniline on the removal of aniline

Fig. 5 Effect of concentration of laccase on the removal of aniline

concentration of laccase was 1 g/L, under this condition the removal of aniline reached 97.1% after 8 h.

Determination of the optimum reaction conditions of *o*-phenylenediamine by laccase

Effect of temperature and reaction time

Effect of temperature and reaction time on the removal of *o*-phenylenediamine was shown in Fig. 6. Since *o*-phenylenediamine isn't volatile, the removal of *o*-phenylenediamine was completely depends on the enzymatic reaction by laccase.

Laccase has a good catalytic activity to *o*-phenylenediamine, it was almost completely removed after 60 min at 50 °C. As the temperature increased in the system, the velocity of the molecular motion was accelerated, the molecular energy and the percentage of activated molecules were increased. Thus the reaction rate is accelerated and the removal of the substrate is increased. At the relatively low temperature, the removal of *o*-phenylenediamine increased with the temperature rising, since the laccase activity was enhanced. When the temperature exceeded the optimum value, the laccase activity was inhibited to a certain extent, and the rate of enzyme-catalyzed reaction fell slightly. As far as possible to reduce energy consumption, the optimum temperature was 50 °C; the optimum reaction time was 60 min.

Fig. 6 Effect of temperature and reaction time on the removal of *o*-phenylenediamine

Fig. 7 Effect of initial concentration on the removal of *o*-phenylenediamine

Effect of the initial concentration of *o*-phenylenediamine

The influence of the initial concentration of *o*-phenylenediamine on the removal of *o*-phenylenediamine was shown in Fig. 7.

When the initial concentration of *o*-phenylenediamine was below 100 mg/L, it could be removed completely after 60 min. The removal gradually decreased with the increase of *o*-phenylenediamine concentration when it exceeded 100 mg/L. Since the amount of *o*-phenylenediamine in the system was relatively excessive at this time, and the ability of laccase-catalyzed was inhibited. The active center of laccase is occupied, and the substrate cannot be oxidized in time to generate free radicals. This is similar to the situation of the removal of aniline by laccase. The optimum initial concentration of *o*-phenylenediamine was 100 mg/L.

Effect of the concentration of laccase

The removal of *o*-phenylenediamine with different laccase concentration was shown in Fig. 8.

The removal of *o*-phenylenediamine by laccase increased gradually with the increase of laccase. When the concentration of laccase was more than 1 g/L, the *o*-phenylenediamine could be removed completely. Then the laccase concentration was further increased, the removal was almost unchanged. The reason is that the laccase is relatively excessive when it exceeded 1 g/L. The removal is based on the amount of *o*-phenylenediamine, excessive laccase couldn't improve the

Fig. 8 Effect of concentration of laccase on the removal of *o*-phenylenediamine

removal efficiency. The optimum laccase concentration for the removal of *o*-phenylenediamine is 1 g/L.

Conclusions

The removal effect for aniline and *o*-phenylenediamine in the presence and absence of laccase was evaluated in the paper. The study has shown that laccase secreted from white-rot fungi is capable of removing aniline compounds. Aniline compounds are oxidized to free radicals by laccase and free radicals form polymers, which are solid substances and can be removed easily.

The removal of aniline relies on its volatility and laccase-catalyzed reaction. Under the optimum condition (temperature: 50 °C, initial aniline concentration: 80 mg/L and laccase concentration: 1 g/L), the total removal of aniline reached 97.1%. Due to the non-volatility of *o*-phenylenediamine, the removal of *o*-phenylenediamine depends on the laccase-catalyzed reaction. Under the optimum condition (temperature: 50 °C, initial aniline concentration: 100 mg/L and laccase concentration: 1 g/L), it was almost removed completely by laccase catalytic reaction. The catalytic effect of laccase on *o*-phenylenediamine is better than on aniline. The study demonstrates the potential of using enzymatic treatment to remove aniline compounds from wastewater in industry.

The experiment results could provide a scientific basis for the removal of aniline compounds by laccase from white-rot fungi. It is of great significance to the green development of industries.

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Authors' contributions Bo Yang put forward the idea and participated in the whole experiments. Removal studies for aniline compounds were performed by Yacheng Wang and Zhiguo Liu. Jun Liu wrote of the part of manuscript. M Jiaming Cai involved in purchasing of required materials and instruments, designing of removal experiments, analyzing of data and reviewing of the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Competing interests The authors declare that they have no competing interests.

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