Immobilization of Laccase on 2D Mesoporous SiO_2 and Its Use in Chlorophenol Degradation

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Abstract. In this paper, 2D hexagonal mesoporous silica with large specific surface area was used to immobilize laccase by the glutaraldehyde cross-linking method, the optimum enzyme immobilization process was screened out. Compared with the free laccase, the reusability of immobilized laccase for chlorophenol degradation is significantly improved.

1 Introduction

Due to be degraded difficultly, chlorphenol (2, 4-DCP) can exist and accumlate in the environment for a long term, which can change characteristics of biological tissue. Because immobilized laccase is more stable and has a higher efficiency than free laccase, immobilized laccase has a good application prospect in pesticide degradation. The two dimensional (2D) mesoporous silica carrier can also enhance the thermal, pH, operational and storage stabilities of the immobilized laccase significantly, their ability of degradation and usability all have been improved [1-4]. In this paper, laccase was immobilized on the 2D mesoporous silica via glutaraldehyde cross-linking method, the properties and 2, 4-DCP degradation of the immobilized enzyme were studied respectively.

2 Experimental

2.1 Immobilization of laccase

The 2D mesoporous silica was prepared by using CTAB templating, and amino functionalized mesoporous silica, named M-SiO_2-NH_2, was prepared by modification of mesoporous silica by APTES in toluene solvent at reflux temperature.

In a typical immobilized laccase system, 0.1 g M-NH_2-SiO_2 and 10 ml 4% glutaraldehyde were mixed for 8 h of cross-coupling reaction. Following the mixture was centrifugalized to wash away gutaraldehyde unreacted. After that, the cross-linked carriers

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and 10 mL 0.2 g/L laccase solution were shaken in 5 mL of buffer solution (the pH is 5.4) for 6 h. The obtained immobilized laccase was centrifugalized and washed by deionized water and its activity was calculated according to the definition that a unit of laccase activity (U) is defined as the quantity of laccase needed to increase the absorbency of 0.001 per mmol substrate per minute under the specified condition of 30°C. The specific activity of the immobilized laccase (U/g) = the total activity of immobilized enzyme/mass of the dry immobilized laccase.

2.2 Degradation of 2, 4-DCP by immobilized laccase

In the process of 2, 4-DCP degradation, carrier mesoporous silica can absorb 2, 4-DCP because of its large specific surface area. The influence of adsorption of 2, 4-DCP by the carrier should be taken into account when degradability was calculated. The removal ratio, the adsorption and degradation rates for 2, 4-DCP were calculated by the following equations:

$$\text{The removal ratio of 2, 4-DCP} = (C_0\text{-}C_d)/C_0 \times 100\% \quad (1)$$

$$\text{The adsorption rate of 2, 4-DCP} = (C_0\text{-}C_a)/C_0 \times 100\% \quad (2)$$

$$\text{The degradation rate of 2, 4-DCP} = \text{The removal ratio} - \text{The absorption rate} \quad (3)$$

Where, C_0 is the original 2, 4-DCP concentration, C_a is the 2, 4-DCP concentration after adsorption, and C_d is the 2, 4-DCP concentration after degradation.

2.3 The factors that influence immobilized laccase degrading 2, 4-DCP

In a typical experiment on 2, 4-DCP degradation, 10 mL of 50 mg/L 2, 4-DCP solution was added into a vessel, following 10 mL NaAc-HAc buffer solution and 0.2 g immobilized laccase was added in proper order. Next, the reactor was placed in a constant shaking incubator and oscillated at the desired temperature for 6 h. Finally, the reaction mixture was filtered to separate the immobilized laccase; and the filtrate was collected to determine the concentration of 2, 4-DCP after degradation. In order to study the effects of 2, 4-DCP concentration, pH and temperature on degradation of 2, 4-DCP, the experiments were carried out by manually adjusting one parameter while the other two of them were kept changed. The curves of removal rate, adsorption rate and degradation rate vs. the concentrations of 2, 4-DCP, pH values, and temperatures were plotted respectively. The repetition use of immobilized laccase was also determined.

3 Results and Discussion

3.1 Characterization of modified mesoporous silica

2D Mesoporous silica was modified using post-grafting method to obtain amino functional groups. The nitrogen content of sample was measured by elemental analysis and found to be 2.96%, following the content of amino was calculated to be 2.19 mmol/g SiO_2.

Using N_2 adsorption method and XRD analysis method, it can be found that the specific surface area of the amino-modified sample decreases from 1259.48 to 566.36 cm^2/g, and pore diameter changes from 2.64 nm to 2.30 nm, indicating the modified product was still mesoporous material, as shown in Fig. 1.

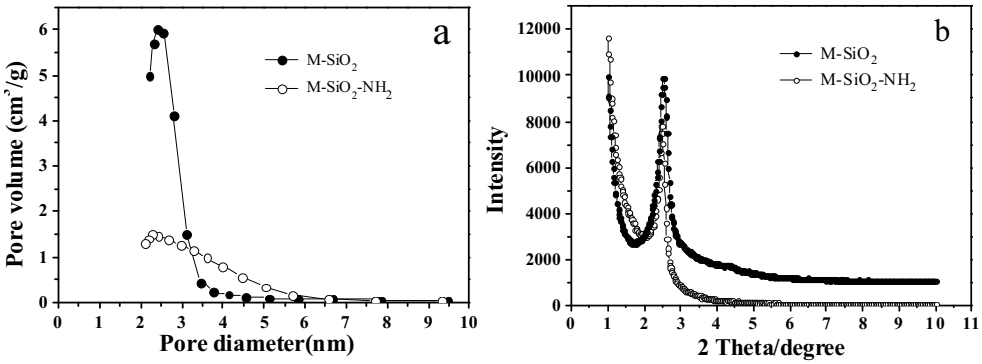

Figure 1: Characterization of modified mesoporous silica: (a) Pore size distribution of unmodified and modified mesoporous silica; (b) The XRD patterns of unmodified and modified mesoporous silica

3.2 Immobilization of laccase and its properties

3.2.1 Optimal conditions of laccase's immobilization

Immobilized enzyme is a new technology originating from the 1950's, with this technology, the enzyme can be recovered and re-used for a long time. Liu synthesized immobilized laccase on large-sized macroporous silica to biodegradate 2-chlorophenol, and found that a maximum of 96.4% of 2-CP could be removed after 5 h, indicating their ability of degradation and usability all have been improved [5,6]. Therefore, the effects of preparation conditions on 2D Mesoporous silica immobilized enzyme activity were studied in this paper, and the results can be seen in the Fig. 2.

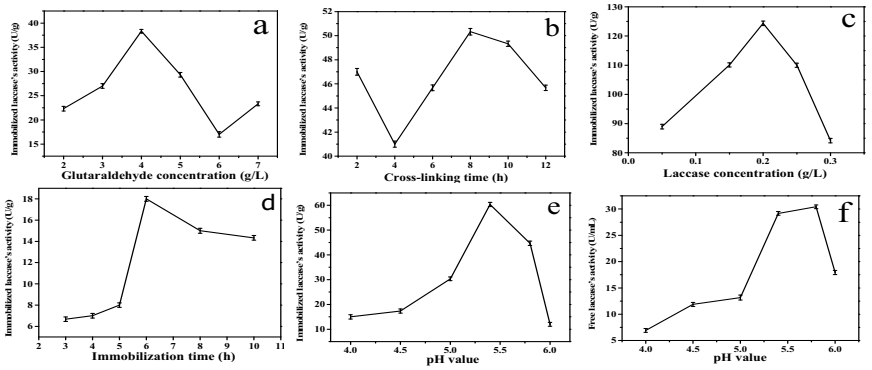

Figure 2: The different conditions of laccase's immobilization. (a) Effect of glutaraldehyde concentration; (b) Effect of cross-linking time; (c) Effect of laccase concentration; (d) Effect of immobilization time; (e), (f) Effect of pH value

As shown in Fig. 2 a~f, when concentration of glutaraldehyde reached 4 g/L (Fig. 2 a), cross-linking time was 8 h, laccase concentration was 0.2 g/L and immobilization time was

6 h, specific activity of immobilized laccase reached the maximum value. Because the excessive glutaraldehyde can prevented covalent immobilization of laccase on carrier, the more cross-linking time can lead to excessive aldehyde groups, which have a toxic effect on laccase. Also too much laccase molecules begin to closely gather and overlap, leading to orientation changes of laccase molecules, and make it difficult for them to be combined with substrate. Moreover, long immobilization time would cause spatial hindrance increase, affecting the effective contact between laccase and substrate. When pH of solution was 5.4, the mesoporous silica and laccase had maximum electrostatic interaction between them, leading to maximum amount of immobilized laccase on carrier. In contrast to immobilized laccase, the optimum pH value of free laccase was 5.8, indicating mesoporous silica carrier with IEP value less than 3 led to movement of working pH of laccase to low pH value.

3.2.2 The properties of immobilized laccase

As shown in Fig. 3, M-SiO_2-NH_2 sample exhibited an irregular platy shape, with the average length 4.25 μm and average width 3.41 μm. There were obvious and large amount of gaps between platy shaped particles, probably inducing M-SiO_2-NH_2 sample have high porosity. The surface of glutaraldehyde cross-linked carrier without immobilizing laccase presented irregular flocculent shape and had small amount gaps between the particles. This may due to carrier becoming flocculent shape after being cross-linked. After the carrier was immobilized with laccase, the mesoporous silica surface was covered with laccase to form large blocks and no any other gaps were observed.

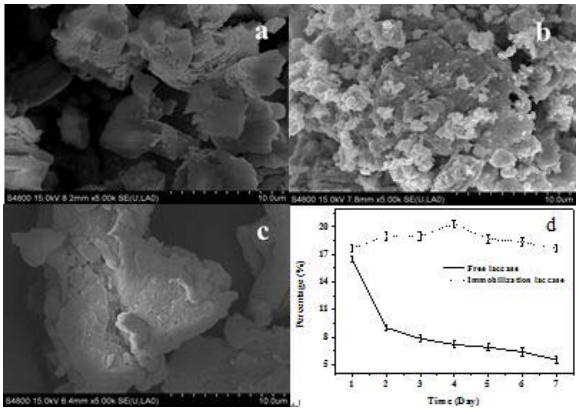

Figure 3: HRSEM images of three sample: (a) HRSEM image of M-SiO_2-NH_2; (b) HRSEM image of M-SiO_2-NH_2 crosslinked by glutaraldehyde; (c) HRSEM image of immobilized laccase. And (d) The stability of free laccase and immobilized laccase, the standard deviations less than 1%

3.2.3 Degradation of 2, 4-DCP with immobilized laccase

In this paper, laccase was immobilized on the 2D mesoporous silica via carrier-bonding using glutaraldehyde as cross-linking reagent. The 2, 4-DCP was degraded by immobilized laccase, and the conditions of enzyme immobilization and the properties of the immobilized enzyme were studied respectively. At the same time, the factors affecting degradation process were also studied, with the results shown in the Fig. 4.

As shown in Fig. 4 a~c, when concentration of the 2, 4-DCP reached 50 mg/L, the degradation rate got the maximum value, while concentration of the 2, 4-DCP continuted to increase to 100 mg/L, the removal rate and degradation rate all decreased. The degradation

rate and removal rate of 2, 4-DCP all reached maximum when the pH value of the solution was 5.5, because the optimum pH 5.5 of immobilized laccase moves towards alkalinity end as compared with the optimum pH (5.0 [7]) of free laccase. The change of the optimum pH is determined by the electrostatic charge of the carrier.

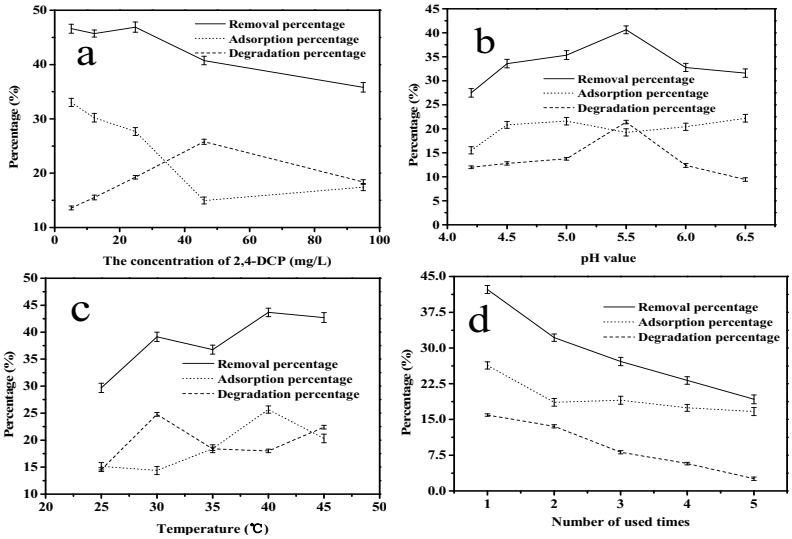

Figure 4: Degradation of 2, 4-DCP with immobilized laccase. (a) Effect of 2, 4-DCP concentration; (b) Effect of pH value; (c) Effect of reaction temperature; (d) The stability of immobilized laccase. All the standard deviations less than 1%

The experimental results found that the removal rate and degradation rate of 2, 4-DCP by immobilized laccase were comparatively high within the temperature range 30~45 °C, demonstrating that immobilized laccase can degrade 2, 4-DCP in a relatively wide temperature range. This is because laccase's spatial structure is influenced by carrier and the effect of temperature on laccase spatial structure becomes weak, so the immobilized laccase becomes less sensitive to temperature. It can be also obtained from Figure 4d that removal rate and degradation rate were 42.28% and 15.93% respectively under optimum condition. When the regenerated immobilized laccase was second re-used, the degradation rate decreased slightly, but the removal rate decreased obviously with a marked decrease of adsorption rate. With the number of cycles of the degradation, removal rate and degradation rate of 2, 4-DCP all decreased significantly, but the adsorption rate almost kept at the value of 17.5%. When the number of cycles reached 5, the rates of degradation and removal of 2, 4-DCP still retained over 19.23% and 2.57%, respectively. In contrast to the free enzyme, the reusability of immobilized laccase was improved significantly. It can be seen the immobilized laccase is much more reusable.

4 Summary

In this paper, 2D mesoporous silica with large specific surface area was used to immobilize laccase, the best enzyme immobilization process was carried out for 6 hours at 25 °C and pH of 5.4 in the presence of glutaraldehyde (4%) and laccase solution (0.2 g/L). After that, the removal and degradation rate of 2, 4-dichlorphenol under different conditions were also studied. The optimum condition for immobilized laccase to degrade 2, 4-DCP is described as follows: pH of the solution is 5.5, temperature range is 30~45 °C, and degradation time is 6 h at 2, 4-DCP concentration of 50 mg/L. The removal ratio of 2,

4-DCP was 19.86% after immobilized laccase was used continuously to degrade 2, 4-DCP four times under the best conditions. Compared with the free laccase, the immobilized laccase is much more reusable.

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