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Degradation of MXC by host/guest-type immobilized laccase on magnetic tubular mesoporous silica

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ABSTRACT

In this paper, magnetic host/guest-type immobilized laccase was prepared by co-adsorption of superparamagnetic particles (SPMNPs) and laccase into the pore channels of tubular mesoporous SiO_2 by the "size-matching effect". After immobilization, the thermal stability and repetition usage-ratio of immobilized laccase were improved significantly, the loading amount of enzyme and SPMNPs was determined by Lowry's method and EDS (Energy Dispersive Spectroscopy), respectively. Under the optimal conditions, the removal efficiency of MXC (Methoxychlor) by immobilized laccase reached 69.4% and the removal efficiency still remained 45.4% after six cycles of operations. The studies on degradation kinetics of MXC by free and immobilized laccase were also carried out, respectively. In virtue of GC–MS, ^{1}H NMR and ^{13}C NMR analysis, $(CH_3OC_6H_5)_2C{=}CHCl$ and 1,1-diphenylethylene were identified as intermediate and final degradation products of MXC, respectively, further demonstrating the degradation mechanism of MXC by the immobilized laccase.

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1. Introduction

The tubular mesoporous SiO_2 (SiO_2-NTs) is a new kind of mesoporous silica (MPSs), which can be used as the carrier for enzyme immobilization. The enzyme can not only be adsorbed onto the surface of SiO_2-NTs, but also distributed inside the tubular wall of SiO_2-NTs [1], leading to a high loading amount of enzyme onto SiO_2-NTs.

Laccase (EC 1.10.3.2) belongs to the blue copper oxidase family [2], with copper ions located in the active center, which plays a key role in the catalytic oxidation process. Since the laccase catalyze the oxidation of phenolic and nonphenolic lignin model compounds via one-electron oxidations, the studies on degradation of PCBs (polychorinated biphenyls), DDT (dichlorodiphenyl-trichloroethane), chlorophenol, and polycyclic aromatic carbon by laccase has attracted much attention [3]. However, the free laccases are easily inactivated in environmental conditions, and it also difficult to be separated from the reaction system for reuse, which limits the further industrial application of laccase to some degree.

Immobilization of enzymes in mesoporous materials is an effective method to improve reusability and stability of enzyme, for example, Lei et al. [4] studied the morphological effects of rod-like SBA-15, traditional SBA-15, honeycomb MPSs and flake MPSs on the activity of immobilized lysozyme. Zhang et al. [5] immobilized laccase by using worm-like MPSs with different pore sizes as carrier. They all found that "size-matching effect" between the enzyme molecule size and the pore diameter of MPSs entrances is essential for specific activity and degradation efficiency of immobilized enzyme.

Methoxychlor (MXC) is an organochlorine pesticide currently used as a substitute for DDT. Due to its obvious insecticidal effect and relatively low biological toxicity, MXC is widely used in many countries. However, recent studies have shown that MXC can cause unexpected estrogenic and antiandrogenic activities in terrestrial and aquatic species [6]. Like DDT, MXC has the characteristic of resistance to biodegradation, and is easy to accumulate in environment, thus, seriously influences the health of humans and mammals [7]. Due to the high hydrophobicity, MXC do not dissolve in an aqueous solution [7], hence, the use of organic solvents is inevitable to perform biodegradation of environmental pollutants

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at high concentrations. Michizoe J considered that enzymes do not commonly possess a significant catalytic activity in organic media, while the reversed micellar system [RMs] can afford an appropriate environment for activating enzymes in organic media [8]. So laccase/RMs is able to degrade highly concentrated environmental pollutants directly in an organic solvent [9].

In the development of the technology for degradation of MXC, the magnetic mesoporous materials as supports for enzyme immobilization have shown huge potential in degradation and transformation of organochlorine pesticides, but degradation of MXC in an organic solvent by enzyme immobilized on the magnetic mesoporous materials was rarely reported. In this paper, a magnetic host/guest-type immobilized laccase system was established based on the co-adsorption of SPMNPs and laccase into the pore channels of SiO_2-NTs using cross-linking methods. The MXC was degraded by immobilized laccase, and the factors affecting degradation process were also studied. Besides, the degradation mechanism and the degradation products of MXC were further studied by means of GC–MS, ^{1}H NMR and ^{13}C NMR.

2. Experimental

2.1. Materials

Cetyltrimethylammonium bromize (CTAB), (ethylenedinitrilo) tetraacetic acid disodium salt (Na_2EDTA), dioctylsulfosuccinate sodium (AOT), tetraethoxysilcane (TEOS), calcium chloride, ammonia, oleic acid, oleylamine, diphenyl ether, anhydrous acetic acid, anhydrous sodium acetate, guaiacol, *n*-hexane, absolute ethyl alcohol, 1,2-hexadecane glycol, glutaraldehyde (above reagents, all A.R. grade, Shanghai Lingfeng Chemical Reagent Co., Ltd., Shanghai, China), methoxychlor (A.R. grade), laccase (Reagent grade, Sigma–Aldrich Co.).

2.2. Preparation of magnetic host/guest-type immobilized laccase

The SiO_2-NTs was prepared with soft template method, and the SPMNPs were prepared with thermal decomposition method, which were all described in Supplementary material.

The typical preparation process of host/guest-type immobilized laccase was performed as follows based on the optimal condition shown in the Fig. S1, in the first stage, 0.02 g SPMNPs was added into 0.05 mol × L^{-1} AOT/hexane solution (5 mL) to form reverse microemulsion at a SPMNPs concentration of 4 g × L^{-1}. After several minutes of ultrasonic dispersing, the formed microemulsion was mixed with 0.113 mL of 0.4 mg × mL^{-1} laccase/buffer solution (pH 4.5). Then 0.1 g SiO_2-NTs was thoroughly dispersed in mixed microemulsion solution for co-adsorption of SPMNPs and laccase. The mixture was shaken (250 rpm) at 25 °C for 6 h, and following the sample was dried at 25 °C until the hexane was completely evaporated. Then in the second stage, the sample was further treated with 5 mL glutaraldehyde (6%) for cross-linking, and the mixture was incubated at 25 °C with shaking for 6 h (250 rpm). After centrifugation and washing three times with deionized water, the host/guest-type immobilized laccase was obtained, kept in refrigerator at 4 °C until use. The average dry weight of immobilized laccase was then measured at 117.4 mg in triplicate, while the amount of laccase immobilized on the SiO_2-NTs was calculated by determing the initial and final concentration of protein in laccase solution using Lowry's method [10]. Besides, SPMNPs amount loaded onto the SiO_2-NTs was determined by EDS analysis.

The determination of laccase activity was based on the method reported by Zhang et al. [5]. 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. Guaiacol aqueous solution (4 mmol/L) is used as the substrate, at a pH of 5.0 adjusted by HAc–NaAc buffer solution, the laccase activity is determined at 465 nm. The specific activity of the immobilized laccase (U/g) = the total activity of immobilized enzyme/mass of the dry immobilized laccase.

2.3. Thermal and operating stability

The thermal stability studies on free and immobilized laccase with glutaraldehyde cross-linking were conducted by treating laccase in 5 mL of 4 mmol/L guaiacol merging with 5 mL NaAc–HAc buffer solution (pH 5) for 30 min at different temperature. After reaction, the solution was separated using a high-speed centrifuge at 4000 r/min for 1 min, and 5 mL of the supernatant liquid was taken for the activity measurement immediately.

To demonstrate positive effect of glutaraldehyde cross-linking on the activity of immobilized laccase, the immobilized laccase without cross-linking was prepared according to the procedure described in the first stage of the typical preparation process. The operating stability of the immobilized laccase with and without glutaraldehyde cross-linking was assessed by performing several consecutive operating cycles with 5 mL of 4 mmol/L guaiacol as substrate at 30 °C, respectively. At the end of each cycle, the immobilized laccase was separated by centrifugation, washed three times with NaAc–HAc buffer solution (pH 5), and repeated with a fresh aliquot of substrate.

2.4. Degradation of MXC by laccase immobilized on magnetic microporous silica

In a typical degradation of MXC by immobilized laccase, 0.1 g magnetic host/guest-type immobilized laccase was added into the reaction vessel containing 5 mL of 20 mg/L MXC/hexane solution, following 3 drops of HAc–NaAc buffer solution (pH 4.5) was added. The reaction vessel was then placed in model TQZ-312 platform temperature-controlled shaking incubator and the reaction progressed at 35 °C for 10 h. Finally, the reaction mixture was filtered to separate the immobilized laccase; following the filtrate was collected to concentrate by vacuum rotary evaporator, and then the concentrated residue and 1 mL of 30 mg × L^{-1} octacosane (internal standard)/hexane was all dissolved in hexane to 5 mL, and 3 μL samples were subsequently subjected to GC analysis. By determining the peak-area ratio of A_{MXC}/A_s, the concentration of the tested MXC composition C_{MXC}(mg/L) can be obtained according to the equation, $C_{MXC} = 66.9(A_{MXC}/A_s) + 0.46$ originated from standard curve (Fig. S2). Each set of experiments was carried out in triplicate, and the arithmetic mean values were calculated, with the standard deviations less than 3%.

2.5. Determination of MXC degradation intermediates

In the determination of MXC degradation intermediates, 5 copies of degradation experiment were conducted, the separated solutions were collected and stored, respectively, and the 5 separated immobilized enzymes were regenerated after washing three times. Then with all of 5 regenerated immobilized enzymes, the degradation experiment was repeated twice. Finally, the three times-separated solutions were combined and dried by anhydrous Na_2SO_4. Then they were vacuumly concentrated to 5 mL and subjected to GC/MS analysis.

2.6. Calculation of the degradation rates

In the degradation process of MXC by magnetic host/guest-type immobilized laccase, the SiO_2-NTs was used as carriers, which can directly adsorb MXC due to its large specific surface area. Thus,

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