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Triclosan removal by laccase immobilized on mesoporous nanofibers: Strong adsorption and efficient degradation

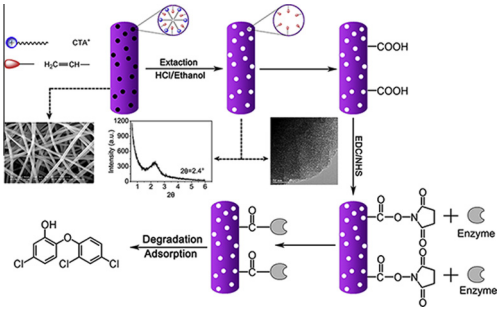
Ran Xu *, Yifang Si, Xiaotao Wu, Fengting Li, Bingru Zhang

State Key Laboratory of Pollution Control and Resource Reuse, College of Environmental Science and Engineering, Tongji University, Shanghai 200092, China

HIGHLIGHTS

- Mesoporous nanofibers were used as enzyme supports.
- Mesostructures were beneficial for adsorption and biodegradation of pollutants.
- Laccase immobilized on nanofibers exhibited high activity and stabilities.
- Triclosan were efficiently removed from water by laccase-nanofibers system.

GRAPHICAL ABSTRACT



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ABSTRACT

Triclosan is difficult to remove or degrade in natural aquatic environment due to its stable chemical structure and low concentration. This study aimed to enhance its removal rate from water through combining the biocatalytic activity of laccase with high adsorption capacity of mesoporous materials. Vinyl-modified poly(acrylic acid)/SiO_2 nanofibrous membranes prepared in this work possessed mesoporous structure (pore size 1.73–3.54 nm, pore volume 0.379 cm^3/g) and high specific surface area (542.91 m^2/g). Laccase was immobilized on the membranes through covalent crosslinking and the enzyme loading was about 417 mg/g. The physical, chemical, biochemical properties of the immobilized laccase and its application in triclosan removal were comprehensively investigated. The immobilized laccase showed better storage stability and higher tolerance to the changes in pH and temperature compared with free laccase. It also exhibited a better performance (65% removal, 2 h) in triclosan removal than free laccase (29.2% removal, 2 h) under the optimum conditions (pH = 4, 30 °C). The results demonstrated that the mesostructure of nanofibers was beneficial for the adsorption and degradation of triclosan. It may provide a new idea for removal of organic pollutants from water environment using enzyme and adsorption technology.

1. Introduction

Degradation of pollutants catalyzed by enzyme is considered as an economical, environmentally friendly, and highly efficient way of water treatment [1]. Enzymes are known as highly selective catalysts and accelerate both the rate and specificity of pollutant degradation under mild conditions [2]. At the same time, because enzymes area type of protein, their application is limited by low stability, short lifetime, and high price [3]. The aforementioned problems can be solved by immobilizing enzymes in/on various organic/inorganic supports [4].

Recently nanomaterials such as nanofibrous membranes and mesoporous nanoscale materials have been used for the treatment of wastewater [5,6] and for the immobilization of enzyme [7] since they can provide a large surface area or high pore volume for the

* Corresponding author. Tel.: +86 2165980567; fax: +86 2165986313.
E-mail address: carol.xuran@tongji.edu.cn (R. Xu).

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enzyme immobilization to increase enzyme loadings and enhance enzyme performance, such as certain robust and adaptable biocatalysts reported [3,4,8]. At the meanwhile, the carrier itself can be used in distributing and absorbing contaminants. For example, mesoporous materials have been widely applied in adsorbing heavy metal ions [9], dyes [10], organic [11], and inorganic pollutants [12] during the process of water treatment. However, most studies focus on the nanomaterials and mesoporous materials in the form of nanoparticles that are difficult to separate after immobilizing enzymes in/on them.

Electrospun mesoporous nanofibrous membranes have numerous superior qualities over the traditional nanomaterials and mesoporous materials because the former combines the advantages of the latter two, such as nanometer scale, mesoporous structures, and high specific surface area [13–15]. As a result, if the electrospun membranes are utilized as the enzyme carriers, the immobilized enzymes can be easily recycled and reused during their application in water treatment. In addition, the combination of cross-linking and adsorption during enzyme immobilization and stabilization could efficiently prevent the leaching of enzymes in larger mesoporous pores and improve the properties for repeated use.

Triclosan, 5-chloro-2-(2,4-dichlorophenoxy)phenol (TCS), is a type of Pharmaceutical and Personal Care Products (PPCPs) that is difficult to remove or degrade in natural aquatic environment because of its stable chemical structure and low concentration. Now TCS has been detected in surface water, sediment, biosolids, soils, aquatic species, and humans [16–18]. More attention is paid to TCS because of concerns that the product might be harmful to the human health and the environment [19,20]. Laccase is an oxidoreductase that contains Cu^{2+} in its catalytic center and is widely applied in the field of environmental protection. Numerous non-biodegradable compounds, such as polycyclic aromatic hydrocarbon (PAHs), chlorophenols, pesticides, and dyes, can be transformed by laccase [21]. Reasonable exploitation and application of laccase show potential to reduce the use of chemicals and the cost of wastewater treatment.

This study aims to immobilize laccase on mesoporous vinyl-modified PAA/SiO_2 nanofibers and use the immobilized laccase to catalyze the degradation of TCS. This study represents the first time that TCS were removed from water by combining the biocatalytic activity of laccase with the high adsorption capacity of mesoporous fibers. The physical, chemical, and biochemical properties of the immobilized laccase were comprehensively studied. In addition, the factors that influenced the TCS removal by the immobilized laccase were investigated.

2. Materials and methods

2.1. Materials

Laccase from white-rot fungi was purchased from Pangtong Bio-tech Ltd., Co. (Nanning, China). 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), poly (acrylic acid) (PAA, high molecular weight, M_w = 450,000), N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride crystalline, 1-ethyl-3-(3-dimethylamino-propyl) carbodiimide (EDC), N-hydroxysuccinimide (NHS), triclosan (Irgasan), Brilliant Blue G, and methanol for High Performance Liquid Chromatography (HPLC) analysis were purchased from Sigma–Aldrich. Cetyltrimethylammonium bromide (CTAB), ethanol, disodium hydrogen phosphate, citric acid and tetraethyl orthosilicate (TEOS), and triethoxyvinylsilane (VTES)) were purchased from Sinopharm Chemical Reagent Co., China. Deionized water was utilized throughout this study.

2.2. Preparation of mesoporous vinyl-modified PAA/SiO_2 nanofibers

Vinyl-modified PAA/SiO_2 gel for electrospinning was prepared by following these steps. First, 2.19 g of CTAB was dissolved in 5.53 g of ethanol and stirred at 40 °C for 10 min to obtain a homogenous solution. Then, 6.84 g of deionized water, 1.44 g of VTES, and 6.64 g of TEOS were added into the solution and stirred for 1 h at 40 °C. Finally, 5 g of 10 wt.% PAA was added into the solution and stirred for 1 more hour at 40 °C to obtain a homogeneous electrospinning gel of vinyl-modified PAA/SiO_2. The electrospinning solution of non-modified PAA/SiO_2 was prepared by replacing VTES with TEOS and following the other aforementioned steps.

The aforementioned modified PAA/SiO_2 gel was added into a 10 mL plastic syringe equipped with a stainless steel needle with an inner diameter of 1.2 mm and connected to a high-voltage generator. The solution was pumped at the speed of 1 mL/h under the voltage of 16 kV, and the nanofibrous membranes were gathered using an aluminum-foil paper collector.

The membranes were placed in ethanol/HCl (molar ratio of 20:1) for 24 h at 60 °C to remove CTAB and then dried for 12 h at 60 °C in an oven.

2.3. Characterization of nanofibrous membranes

Scanning electron microscope (SEM) images were taken on a field emission XL-30 SEM at 30 kV. Transmission electron microscope (TEM) images were recorded with a CM200FEG electron microscope operating at 200 kV. N_2 adsorption–desorption isotherm measurements were taken on Micromeritics ASAP 2020 accelerated surface area and porosimetry analyzer. The immobilization amount of laccase and residual activity of the free and immobilized laccase were measured by Shimadzu UV-1700 spectrophotometer. The functional groups of nanofibers and enzyme-immobilized nanofiber samples were obtained through Fourier transform infrared (FTIR) attenuated total reflectance spectroscopy. Wide-angle X-ray diffraction patterns were characterized on a diffractometer, Rigaku D/MAX-IIA, with a scan scope of 10–80° at a step of 0.02°. Small-angle X-ray diffraction patterns were conducted using a diffractometer, Rigaku D/MAX-rB (40 kV, 100 mA, Cu Kα, $2\theta°$ = 0.6–6°).

2.4. Laccase immobilization

EDC and NHS were utilized in cross-linking nanofibrous membranes with enzymes. Dosage of EDC·HCl/NHS (10–50 mg), pH level (2–8), and time (1–6 h) for laccase immobilization were optimized. The optimized molar ratio was EDC·HCl/NHS = 1.67:1. First, 30 mg EDC·HCl and 30 mg NHS were dissolved in 15 mL of sodium dihydrogen phosphate/citric acid buffer solution (CPBS) at pH = 4, and 0.1 g of laccase powder was added into 100 mL of CPBS solution to obtain 1 mg/mL of laccase solution. Then, a piece of PAA/SiO_2 membrane was added into the solution. The membrane was activated by EDC/NHS at 4 °C for 2 h. Thereafter, the activated membrane was added into 1 mg/mL of laccase solution and cross-linked for 24 h in the rocking device at 25 °C. Finally, the immobilized laccase was obtained. Concentration of laccase on membranes was determined by Coomassie Brilliant Blue G-250.

2.5. Activity and stability assays

2.5.1. Laccase activity assay

The laccase activity was measured by monitoring the absorbance change of ABTS during its oxidation with a UV–visible spectrophotometer at 420 nm.