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Laccase-carrying electrospun fibrous membrane for the removal of polycyclic aromatic hydrocarbons from contaminated water

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ABSTRACT

Four types of laccase-carrying electrospun fibrous membranes (LCEFMs), with high laccase-catalytic activity and sorption capacity, were fabricated by emulsion electrospinning. These LCEFMs were composed of beads-in-string structural fibers, with nanoscale pores distributed on the surface and active laccase encapsulated inside. This obtained structure could protect laccase from external disturbance, resulting in that all of the four LCEFMs retained more than 70% of activity relative to free laccase, and after glutaraldehyde treatment, their storage and operational stabilities were definitely improved. The retained activities and stabilities of the LCEFMs were closely related to the hydrophilic–hydrophobic property of the polymer. Moreover, these LCEFMs possessed high adsorptivity for polycyclic aromatic hydrocarbons (PAHs), and the sorption capacities and rates were mainly influenced by the specific surface area of the LCEFMs and the hydrophilic–hydrophobic property of the polymer. The sorption of PAHs on the LCEFMs could significantly enhance their degradation efficiencies by laccase, which was obviously higher than those by free laccase. A mechanism of PAH degradation promoted by sorption was proposed.

1. Introduction

Electrospinning is considered as a versatile and potential high-throughput method to continuously process ultrafine fibers [1,2]. It has attracted considerable attention recently in many applications, such as drug and protein carriers in controlled release [3,4], scaffolds in tissue engineering [5,6], filtration [7–9], biosensors [10,11] and food manufacturing [12]. Electrospinning could therefore become a simple yet powerful means of preparing desirable fibrous membrane if it could be performed in a reliable and predictable way.

Especially, electrospun fibrous membranes (EFMs) are considered as excellent supports for enzyme immobilization benefiting from their variable spinning materials, high porosity and interconnectivity [13–15]. However, in most cases, EFMs are used as supports directly or after surface modification, and enzymes are immobilized on the surface of EFMs by adsorption or chemical crosslinking [16–18], which usually leads to the poor stability and the lower loading capacity and retained activity of enzyme. Furthermore, the immobilization of enzyme on the surface of the EFMs hinders other usage of their surface.

Recently, EFMs are used as sorbents in environmental remediation and sample pretreatment possess [19–21], owing to their high specific surface area, porous structures and resultant superior mechanical properties. EFMs prepared from different polymers have been applied for direct extraction of trace organic pollutants from environmental water and effective removal of heavy metal ions and various organic compounds in waters, such as phenolphthalein, polycyclic aromatic hydrocarbons (PAHs), humic acid and oil [19,20,22]. Although these results suggested the excellent adsorption properties of EFMs for organic pollutants in water, especially for the hydrophobic organics, the subsequent treatment for the adsorbed pollutants is still a huge dilemma.

Herein, we employed the emulsion electrospinning to prepare the laccase-carrying EFMs (LCEFMs). Emulsion electrospinning is an *in situ* enzyme immobilization technology to prepare the core-shell structural ultrafine fibers with water-in-oil (W/O) emulsion [4,23–25]. By this means, laccase can be directly encapsulated into the core of the electrospun fibers. Therefore, the LCEFMs can work as both supporter and sorbent, and have functions of adsorption, biocatalysis and separation simultaneously. Four types of polymers with different structures and properties, including poly(D,L-lactide) (PDLLA), poly(lactide-co-caprolactone) (P(LA/CL)), poly(D,L-lactide-co-glycolide) (PDLGA) and methoxy polyethylene glycol–poly (lactide-co-glycolide) (MPEG–PLGA) were chosen as electrospinning materials. The morphology, structure and enzymatic properties of different LCEFMs were investigated. Moreover, the LCEFMs were employed as the enzymatic membrane-bioreactor for removal of polycyclic aromatic hydrocarbons (PAHs, typical persistent toxic contaminants with hydrophobicity) from water. Their sorption properties and catalytic degradation performances for PAHs in

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water, including naphthalene, phenanthrene, benz[*a*]anthracene and benzo[*a*]pyrene, were studied. It is noteworthy that both the catalytic activity of an immobilized enzyme and the adsorptivity of a sorbent are closely related to the properties and morphologies of supports or sorbents [26,27]. Consequently, it is essential to explore (1) how the properties of polymer and the morphologies of fibers influence the enzymatic-catalysis and sorption performances of LCEFMs, and (2) how the PAH sorption on the LCEFMs affects their catalytic degradation by laccase in the membrane.

2. Experimental

2.1. Reagents and materials

PDLLA, P(LA/CL), PDLGA and MPEG–PLGA were purchased from Jinan Daigang biomaterials Co., Ltd. (Shandong, China). The molecular weight of each polymer was approximately 100,000. Triblock copolymer PEO–PPO–PEO (F108) was supplied by BASF (Germany). Laccase (*p*-diphenol: dioxygen oxidoreductases, EC 1.10.3.2) from *Trametes Versicolor* with the 23 U/mg solid activity and its substrate 2,2-azinobis-3-ethylbenzothiazoline-6-sulfonate (ABTS, 99%), and fluorescein isothiocynate (FITC) were obtained commercially from Sigma–Aldrich (USA). Methylene dichloride, acetonitrile and methanol (HPLC, 99.9%) were purchased from JTBaker (USA). Naphthalene (99.0%), phenanthrene (99.5%), benz[*a*]anthracene (99.7%), benzo[*a*]pyrene (99.0%) were provided by Sigma–Aldrich (USA). Some of their properties are summarized in Table S1. All other reagents and solvents were analytical grade and used without further purification. All solutions were prepared using high-purity water obtained from a Milli-Q Plus/Millipore purification system (USA).

2.2. Preparation of emulsions

A certain amount of polymer was dissolved in methylene dichloride with gentle stirring for 3.0 h at ambient temperature (25 ± 1 °C). The polymer concentrations of PDLLA, P(LA/CL), PDLGA and MPEG–PLGA were maintained at 9, 4, 7 and 8 wt% in methylene dichloride, respectively. In order to obtain stable and homogeneous W/O emulsions, 10 wt% (relative to polymers) of F108 was used as a surfactant in the polymer/methylene dichloride solution. A volume of 1 mL of 20 mg mL^{-1} laccase solution was then added to the polymer/F108/methylene dichloride solution and mixed fully via vortexing to obtain uniform emulsions. To confirm that the laccase could be encapsulated into electrospun fibers, laccase in the emulsion was replaced by the same amount of FITC-labeled one for laser confocal scanning microscopy (LCSM, LSM510, ZEISS, Germany) observation. The method for preparation of FITC-labeled laccase has been demonstrated in our previous work [24]. For sorption experiments, the laccase solution was boiled for 30 min before use to prepare deactivated LCEFMs.

2.3. Electrospinning

Electrospinning was carried out on a self-made multi-end electrospinning apparatus in our laboratory. In a typical procedure, the emulsion was firstly loaded into a 10 mL spinning solution cartridge with twelve 30-gauge needles attached. The emulsion was injected using a syringe pump at a rate of 0.6 mL min^{-1}. The distance between the tip of the needle and the collector was about 12 cm, with an applied voltage of 10 kV. The polymer fibers were collected on an aluminum foil covered collecting barrel. It usually took 1–2 h to obtain sufficiently thick and integrated LCEFMs. To explore the effects of crosslinking on enzyme activity, some LCEFMs were kept for 30 min in glutaraldehyde (GA) vapor surrounding, obtained from a vacuum vessel containing 10 mL of GA aqueous solution (25 wt%) under 0.5 bar at 30 ± 1 °C. All the LCEFMs were stored at 4 °C before usage. All experiments were conducted at room temperature (25 ± 1 °C) and a relative humidity of about 20 ± 2%. By using the multi-end electrospinning, the LCEFMs could be obtained more rapidly than that by traditional electrospinning devices, which could also minimize the adverse effects of solvent, electric field, and dehydration on the activity of laccase.

2.4. Characterization

The morphologies of LCEFMs were observed with a field emission scanning electron microscope (FESEM S-4800, HITACHI, Japan). The presence and distribution of laccase in the electrospun fibers were observed on LCSM. The excitation and emission wavelengths were 488 and 535 nm, respectively. The specific surface area and pore volume of the LCEFMs were determined using a full-automatic specific surface area analyzer (ASAP 2020, Micromeritics, USA). The hydrophilic–hydrophobic properties of the polymer were tested on a contact angle measuring system (OCA20, Dataphysics, Germany).

2.5. Activity and storage stability assays

The activity of laccase was determined spectrophotometrically using ABTS as the substrate. The absorbance of the solution was measured in a UV–vis spectrophotometer (Cray 50, VARIAN, USA) at a wavelength of 420 nm. The detailed measurement and calculation methods of laccase activity and the initial retained activity of LCEFM were described in detail elsewhere [24].

The residual activities were measured over the course of 60 days for testing the storage stability of free laccase and LCEFMs (GA treatment and without GA treatment). Between activity measurements, the samples were stored in phosphate buffer (pH 3.5) at 4 °C. For assessment of the operational stability, the LCEFMs were separated from the reaction mixture after one assay. These LCEFMs were washed three times with phosphate buffer and then transferred to the fresh ABTS solution. This operation was repeated 10 times. The relative activity at each data point was calculated from the ratio of residual activity to initial activity. All samples were produced in triplicates including control.

2.6. Sorption of PAHs

All sorption experiments were carried out using a batch equilibration technique at 25 ± 1 °C and pH 6.5. For each of the four PAHs, three pieces of deactivated LCEFMs (1.5 cm × 1.5 cm, total wt. 60–70 mg, with GA treatment) and 100 mL of PAH aqueous solution was mixed together. Initial concentrations of PAHs ranged from 1 to 500 μg L^{-1}. The solution was kept in a shaking table (100 rpm) until the adsorption–desorption balance reached after 3 h. To explore the sorption kinetics, the deactivated LCEFMs were added into the 50 mL mixture solution containing four PAHs at the concentration of 50 μg L^{-1}. Periodically, a volume of 0.5 mL sample was taken from reaction system for high-performance liquid chromatography (HPLC, Dionex U3000, USA) analysis. All experiments were conducted three times, and the average value was adopted. The PAH sorption process was also observed with a fluorescence microscope (CX41-32RFL, Olympus, Japan). A few drops of four PAHs mixture solution (100 μg L^{-1}) were added onto the P(LA/CL) LCEFM, and this system was observed under excitation of light at λ 288 nm. The partition adsorption equation was used to analyze the adsorption/desorption of PAHs on/from the deactivated LCEFMs [22].

$$q_e = K_p C_e + Q_0 C_e/(K_d + C_e) \quad (1)$$