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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].

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

where K_p is the partition coefficient, L g^{-1}; K_d stands for the affinity coefficient, μg L^{-1}; q_e is equilibrium sorption amount, μg g^{-1}; C_e is equilibrium solution phase concentration, μg L^{-1}; Q_0 is sorption capacity, μg g^{-1}.

2.7. PAH degradation

Three pieces of LCEFMs (1.5 cm × 1.5 cm, total wt. 60–70 mg, with GA treatment) loaded with about 2.5 mg of laccase were added into 50 mL of four PAHs mixture solution at the concentration of 50 μg L^{-1}. The mixtures were incubated with stirring (100 rpm) for 24 h and sampled periodically. At specific time point, the laccase catalysis reaction was terminated by adding 50 μL of sodium azide (20 mmol L^{-1}) before sampling. The control experiments for free laccase were carried out in the same reactor using an equivalent amount of laccase. All experiments were carried out at 25 ± 1 °C and pH 6.5. The PAH concentration was measured by HPLC as well and was the sum of PAHs in aqueous phase and on/in the electrospun fibers (washed by acetonitrile). All samples were produced in triplicates including control.

3. Results and discussion

3.1. Morphology characterization of LCEFMs

The morphologies of LCEFMs were characterized by SEM, and the images demonstrate the beads-in-string structural fibers in all the four type of LCEFMs (see Fig. 1). As shown in Fig. 1, the beads of P(LA/CL) LCEFM are generally 2–4 μm in length and 1–2 μm in diameter, and the diameter of fibers are much smaller, varying from hundreds of nanometers to tens of nanometers. By contrast, the fibrous diameters of PDLLA, PDLGA and MPEG–PLGA LCEFMs are similar to those of P(LA/CL) LCEFMs, but the beads of the three LCEFMs have a broader size distribution, ranging from 3 to 6 μm in length and from 2 to 4 μm in diameter. Moreover, the surface morphologies of these beads show significant differences. The pores with the diameters of tens of nanometers are distributed all over the beads and fibers of P(LA/CL) and PDLLA LCEFMs (see Fig. 1a and b, inset), and the pores on the beads and fibers of PDLGA LCEFM are much less (see Fig. 1c, inset). However, the pores on the beads and fibers of MPEG–PLGA LCEFM are difficult to be seen in the SEM images (see Fig. 1d, inset), and the determination of the pore volume has proved the existence of pores (shown in Table 1). Furthermore, the bead surface of P(LA/CL) LCEFM is much smoother than those of PDLLA, PDLGA and MPEG–PLGA LCEFMs, and there are many folds especially on the beads of PDLLA and PDLGA LCEFMs.

Beads-in-string structural fibers have been observed in many researches, and the formation of beads was considered to be closely related to the surface tension and instability of the jet of electrospinning solution during ordinary electrospinning [28–30]. However, the mechanism becomes much more complicated during emulsion electrospinning because of the simultaneous existence of the water phase and oil phase. During the electrospinning process of a water-in-oil (W/O) emulsion, phase separation of the aqueous and organic components might occur due to a combination of solvent evaporation and shear forces [23,31]. This extensive separation of the emulsion components led to the evolution of coaxial phases, including an external phase rich in polymer and an internal phase dominated by the aqueous components [30,32]. Moreover, since the surface tension of the aqueous components is higher than that of the methylene dichloride; when the electrostatic force is not enough to overcome the surface tension of the aqueous components, the aqueous components are difficult to be split and most of them may exist in the fibers in the form of droplets. A fact that

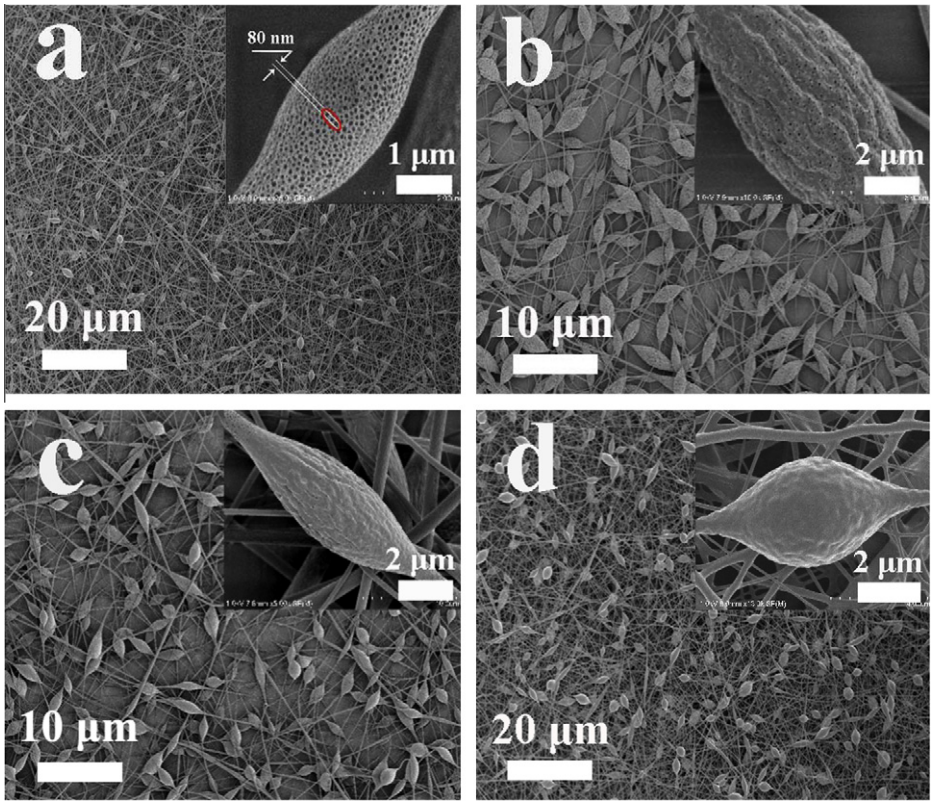

Fig. 1. Morphology and structure of the four LCEFMs: (a) P(LA/CL); (b) PDLLA; (c) PDLGA; (d) MPEG–PLGA; the inserted image shows the high resolution image of beads and the pores on the surface of beads.

Table 1
Selected properties of four LCEFMs.

LCEFMs	Retained activity[a] (%)	Retained activity[b] (%)	A_{surf}[c] ($m^2 g^{-1}$)	V_{total}[d] ($cm^3 g^{-1}$)	Contact angle[e] (°)
PDLLA	72.9	71.1	3.43	0.0123	95.1
P(LA/CL)	80.5	78.3	5.26	0.0198	86.6
PDLGA	76.8	75.6	2.82	0.0105	70.3
MPEG–PLGA	83.7	82.8	2.17	0.0086	57.0

[a] The activity of LCEFM without GA treatment relative to that of free laccase.
[b] The activity of LCEFM with GA treatment relative to that of free laccase.
[c] Surface area, calculated from the adsorption–desorption isotherm of N_2 at 77 K by multi-point BET.
[d] Single point adsorption total pore volume.
[e] Contact angle of polymer.

should be noted is that, the organic solvent evaporates much faster than the water. The outer layer of the fiber (polymer solution) solidifies quickly and the inner layer (aqueous droplets) barely has time to volatilize, so the fiber interior swells and ultimately forms beads. This is a likely mechanism of beads-in-string structural fibers formation in our experiments. Furthermore, the electrospinning parameters may also affect the formation and morphology of the beads-in-string structural fibers [1,28].

The formation of pores on the surface of beads and fibers is also considered to be a dominant phase separation mechanism [1,2]. But this phase separation occurs between the oil phase (polymer solution) of the emulsion and the air phase. Since the polymer solution may distribute non-uniform at the outer layer of the fibers or beads under the influence of strong electric fields, and there may be some polymer-rich and polymer-poor regions. During the evaporation of the organic solvent, the air phase may occupy the polymer-poor region, resulting in the formation of the pores. The distinctions in beads morphologies of these LCEFMs may be mainly attributed to the different hydrophilic–hydrophobic properties of four polymers in the present study. Furthermore, the formation of pores is also related to the ambient humidity, the types of organic solvents and polymers used [1].

Beads on electrospun fibers were generally considered as undesirable "by-products" or defects, as their presence greatly reduces the large surface area, which is an important property for ultrafine or nanoscale fibers [2,15]. Interestingly, in our experiments, these beads-in-string structural fibers are desirable. As discussed above, the aqueous components, i.e. laccase solution can be encapsulated into the core domain of the beads during the emulsion electrospinning process, which was verified by the LCSM characterization of FITC-labeled laccase. As seen from Fig. 2, the beads-in-string structural fibers of the P(LA/CL) LCEFM emits green fluorescence under excitation light; especially the beads emits brighter fluorescence,

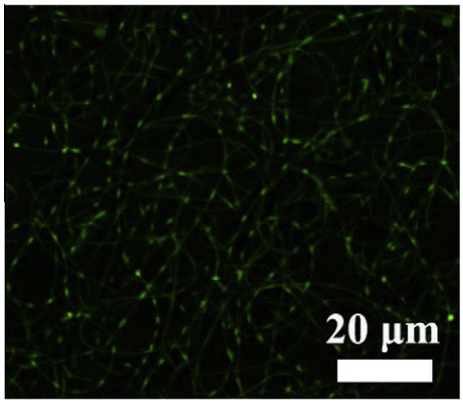

Fig. 2. Laser confocal microphotograph of the P(LA/CL) LCEFM. The laccase is labeled by FITC, and the excitation and emission wavelengths are 488 and 535 nm, respectively.

which indicated that most of laccase was encapsulated into the beads. Since the higher concentration of laccase can provide more active sites and then increase the probability of laccase catalytic reactions with substrates, the beads-in-string structural LCEFMs may be available for efficient degradation of PAHs in water.

3.2. Laccase activity and stability analysis

The retained activities of the four types of LCEFMs (GA treatment and without GA treatment) relative to free laccase are summarized in Table 1. Although the same type of LCEFMs with GA treatment lose slightly more activities than those without GA treatment, the activities of all LCEFMs account for more than 70% of that of free laccase in the present study, and this is a higher activity as compared with other types of immobilization laccase [33,34]. In many cases, crosslinking reaction destroys the active sites of enzyme and affects its activity [35,36]. In our studies, the higher retained activity of these LCEFMs may be attributed to their special structures. Since laccase is encapsulated into the beads and fibers, just a little bit of GA vapor can enter into the fibers through the pores on the fibrous surface, resulting in little activity loss of these LCEFMs with GA treatment. Moreover, the outer layer of beads and fibers consists of polymers with good biocompatibilities, which not only have little influence on enzyme activity themselves, but also can protect laccase from complex external surroundings. However, there are also some distinctions among LCEFMs prepared from different polymers. The retained activity of MPEG–PLGA LCEFM is the highest (reached 83.7%), which may ascribe to the hydrophility of MPEG–PLGA (see Table 1). The most hydrophobic PDLLA results in the lowest activity retention of PDLLA LCEFM. Since the undesired change on the conformation of enzyme tended to occur in the hydrophobic microenvironment [27]. Interestingly, the hydrophobicity of P(LA/CL) is higher than that of PDLGA, but the retained activity of P(LA/CL) LCEFM (80.5%) is also higher than that of PDLGA LCEFM. This may be mainly attributed to the relatively larger pores on the surface of P(LA/CL) beads and fibers, which is much easier for the substrates to diffuse to/off the laccase and results in higher enzyme activity. Due to the larger pores, the activity losses of P(LA/CL) and PDLLA LCEFMs with GA treatment are slightly higher than those of PDLGA and MPEG–PLGA LCEFMs.

In addition to the activity retention of immobilized laccase, the storage and operational stabilities are also the important factors to evaluate the performance of LCEFMs. Fig. 3 shows the storage stabilities of free laccase and four types of LCEFMs (GA treatment and without GA treatment) for 60 days. The results indicate that the stabilities of LCEFMs, especially those with GA treatment, are significantly improved, compared to that of the free one. The free laccase lose more than 70% of its initial activity in 14 days, and after 60 days in storage, the relative activity of free laccase is less than 10%; whereas the P(LA/CL) and PDLLA LCEFMs with GA treatment retain over 70% of its initial activity. Moreover, the operational

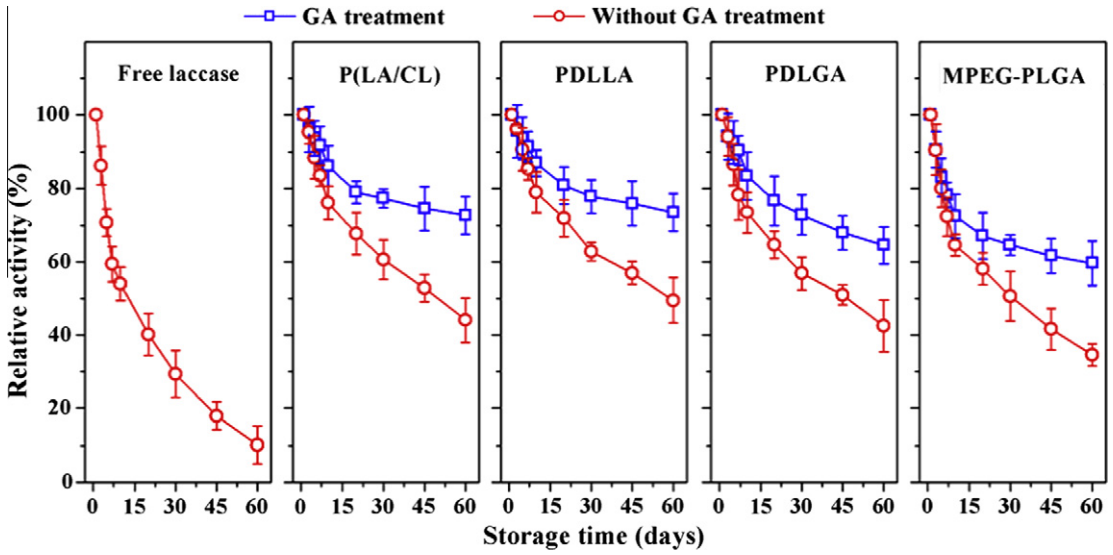

Fig. 3. Storage stabilities of free laccase and four LCEFMs in phosphate buffer (pH 3.5) at 4 °C.

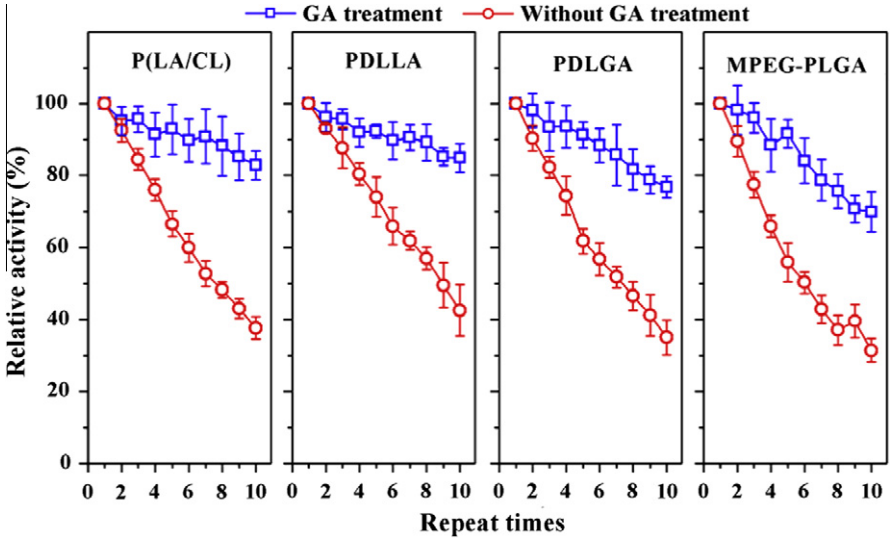

Fig. 4. Operational stabilities of four LCEFMs for oxidation of ABTS.

stabilities of these LCEFMs were tested by using the same LCEFMs for oxidation of ABTS repeatedly. As shown in Fig. 4, after GA treatment, the P(LA/CL) and PDLLA LCEFMs retain over 80% of their initial activity after oxidizing 10 batches of 0.5 mM ABTS, and PDLGA and MPEG–PLGA LCEFMs can also retained more than 70% of their initial activity. Compared to these LCEFMs, the relative activities of the LCEFMs without GA treatment, ranging between 30% and 50%, are obviously lower. This may be due to the fact that the GA vapor reacts with the primary amine groups of the enzyme forming intermolecular cross-linking and larger enzyme aggregates, which would be less likely to leach out from the fibers or the pores on the fibrous surface [35]. Furthermore, the good storage and operational stabilities of these LCEFMs may be also related to the core-shell structure of beads and fibers, which can keep laccase in the core domain of fibers and from external influences.

The storage and operational stabilities of four LCEFMs are ranked in the order of PDLLA > P(LA/CL) > PDLGA > MPEG–PLGA LCEFMs, which is inversely related to the hydrophility of the polymer. Owing to the best hydrophility of MPEG–PLGA, the initial retained activity of MPEG–PLGA LCEFM is higher than those of other three LCEFMs, but both its storage and operational stabilities are the worst. After the MPEG–PLGA LCEFM is immersed in aqueous medium for a long time, its hydrophility may lead to swell and disintegrate of fibers, resulting in enzyme leakage and activity loss. Therefore, the hydrophilic–hydrophobic property of the polymer influences not only the retained activity, but also the storage and operational stabilities of LCEFMs.

3.3. Sorption properties of LCEFMs for PAHs

Electrospun fibrous membrane is not only an excellent support for immobilization of laccase [24], but also a good sorbent for concentration and removal of PAHs in water [22]. The sorption capacities of the deactivated LCEFMs are shown in Fig. 5 and Table S2. The results show that the sorption capacities of deactivated P(LA/CL) LCEFM for phenanthrene, benz[*a*]anthracene and benzo[*a*]pyrene are relatively higher than those of other LCEFMs, which reach 2238.8 ± 15.9, 1339.1 ± 8.5 and 872.5 ± 3.4 μg g^{-1}, respectively. This may be mainly attributed to the highest specific surface area of P(LA/CL) LCEFM. However, the sorption capacity of deactivated

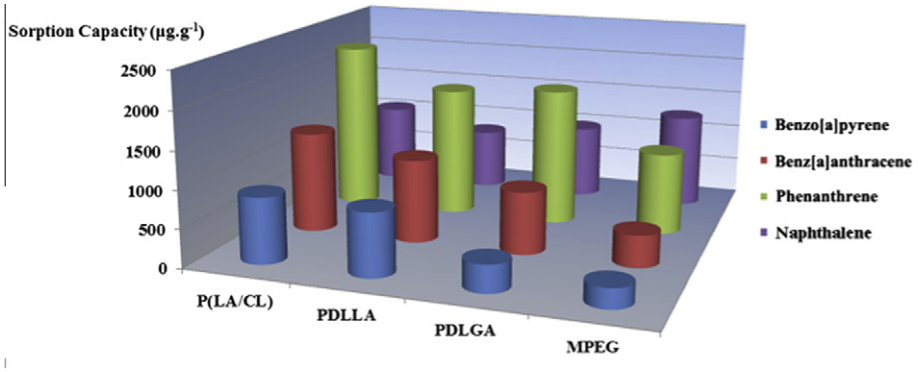

Fig. 5. Sorption capacities of four deactivated LCEFMs for PAHs.

P(LA/CL) LCEFM for naphthalene is lower than that of MPEG–PLGA LCEFM, demonstrating that the surface area is an important factor but not the only factor to govern the sorption capacities of LCEFMs. Since the water solubility of naphthalene is higher than other PAHs and the P(LA/CL) is more hydrophobic than MPEG–PLGA, naphthalene is easier to distribute on the surface of MPEG–PLGA LCEFM, indicating that the hydrophilic–hydrophobic properties of the polymers affect the sorption capacities of LCEFMs for PAHs in water.

Although the beads on the fibers have adversely effect on the surface areas of the LCEFMs, the nanoscale fibers exhibit excellent sorption performances. Under a fluorescence microscope, the rapid adsorption process was observed apparently. As seen from Fig. S1 in Supporting information, the benz[*a*]anthracene in aqueous solution are almost completely adsorbed onto the beads and fibers of the deactivated P(LA/CL) LCEFM within 10 min. The solution was quickly clear and the concentrations of PAHs in the aqueous phase were low. The rapid sorption processes were also verified by the sorption experiments. Almost all the PAHs could be adsorbed by the four types of deactivated LCEFMs and the sorption processes reached dynamic equilibrium in 3 h.

However, in the four PAH mixture system, the sorption rates of these deactivated LCEFMs for PAHs are different, as shown in Fig. 6. Except for benzo[*a*]pyrene, the sorption rates of the deactivated P(LA/CL) LCEFM for the other three PAHs are the highest, which

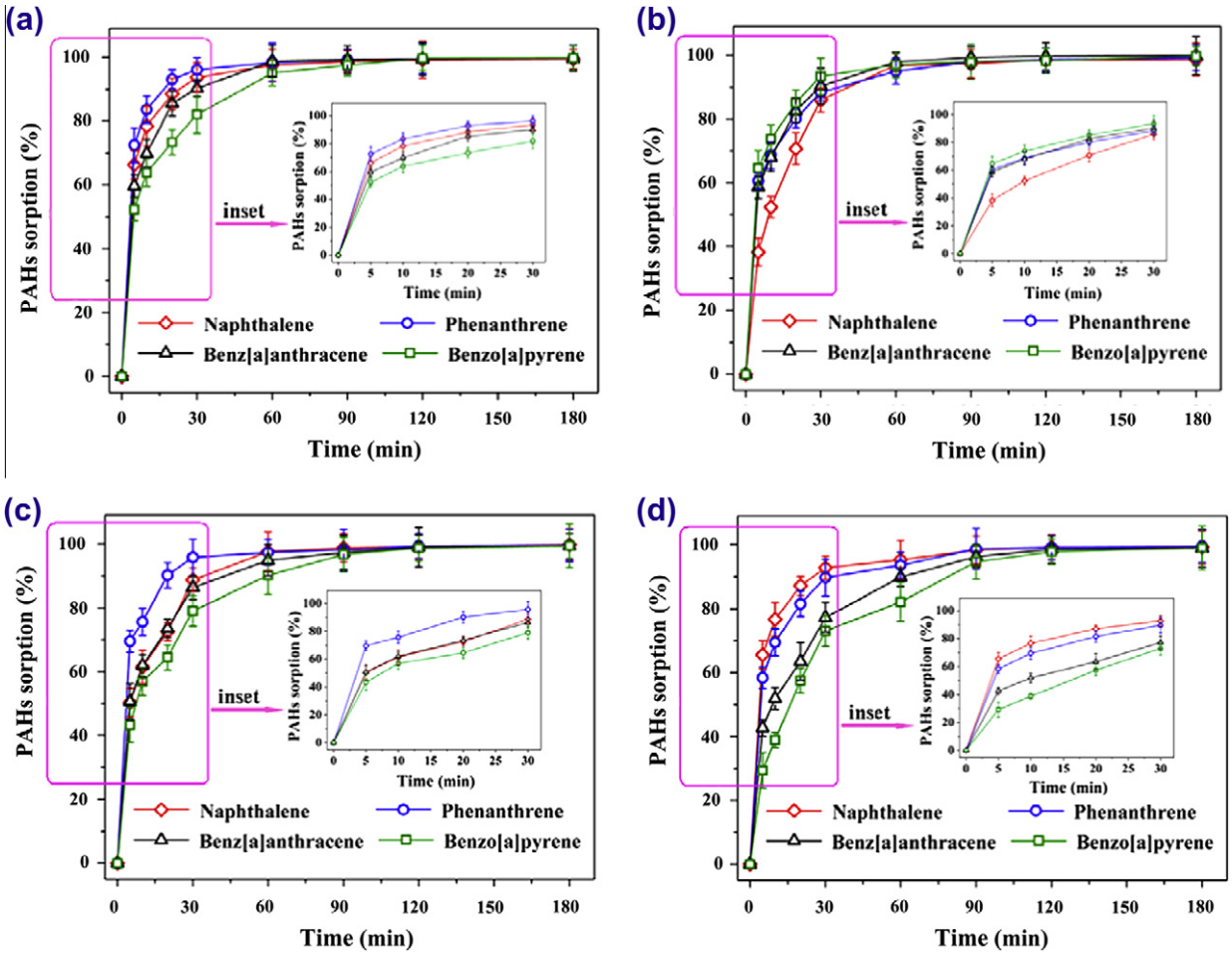

Fig. 6. Sorption rates of four deactivated LCEFMs for PAHs: (a) P(LA/CL); (b) PDLLA; (c) PDLGA; (d) MPEG–PLGA.

may be also ascribed to its highest specific surface area. The deactivated P(LA/CL) LCEFM can provide more adsorption sites for PAH molecules, resulting in the emergence of PAH concentration difference in a relatively shorter time, which can improve the diffusive rates of PAH molecules and increase the sorption rates of LCEFM. The deactivated PDLLA LCEFM, with the largest hydrophobicity, shows the fastest sorption rate for benzo[*a*]pyrene. Since the $\log K_{ow}$ value of benzo[*a*]pyrene (shown in *Table* S1) is higher than those of other PAHs, it is easier to distribute on the surface of the deactivated PDLLA LCEFM through hydrophobic interaction. Moreover, the sorption rate of the deactivated MPEG–PLGA LCEFM for naphthalene is much higher than those of the deactivated PDLLA and PDLGA LCEFMs. These results indicate that the hydrophilic–hydrophobic property of the polymers may also influence the sorption rates of LCEFMs for PAHs. Therefore, the specific surface area of the LCEFM and the hydrophilic–hydrophobic property of the polymer are the important factors to determine the sorption capacity and rate of the LCEFM for PAHs.

3.4. PAH degradation by LCEFMs

Since PAHs could be almost completely adsorbed by the LCEFMs quickly, the removal efficiencies of PAHs reached nearly 100%. However, in our experiments, we are interested in not only the sorption removal of PAHs by LCEFMs, but also the *in situ* degradation of PAHs by laccase encapsulated in the EFMs. Therefore, the degradation efficiencies of PAHs are calculated by subtracting the PAHs in aqueous phase and on/in the LCEFMs (washed by acetonitrile) from total PAHs. Fig. 7 shows the degradation efficiencies of PAHs by free laccase and the four types of LCEFMs. The results illustrate that all the degradation efficiencies of PAHs by the four LCEFMs are obviously higher than those by free laccase. Especially for benzo[*a*]pyrene, its degradation efficiency by free laccase is less than 25%, whereas that by the four types of LCEFMs exceed 70%. Because the initial activity of immobilized laccase is lower than that of free laccase in our experiments, the higher degradation efficiencies of PAHs by the LCEFMs are ascribed to the sorption of PAHs on the membrane. For a given PAH, its degradation efficiencies by the P(LA/CL), PDLLA, PDLGA and MPEG–PLGA LCEFMs show a little difference. For phenanthrene, its degradation efficiencies by the four types of LCEFMs are 93.8%, 86.6%, 90.3% and 89.7%, respectively; for naphthalene, the degradation efficiencies are 88.4%, 80.2%, 88.7% and 91.8%, respectively. These orders of degradation efficiencies are positively correlated with neither the retained activities nor the stabilities of the LCEFMs, indicating that the activities of the four types of LCEFMs are not the sole determinant for PAH degradation in this study, and the PAH sorption on the LCEFMs also play an important role in its degradation by laccase.

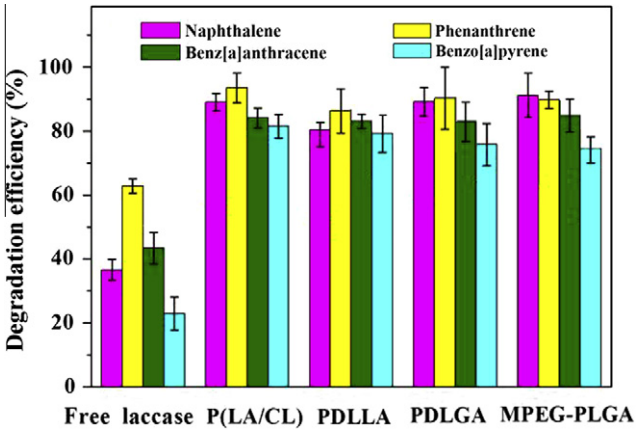

Fig. 7. Degradation efficiencies of PAHs by free laccase and four LCEFMs.

In a diffusion controlled degradation process, the degradation rates by free enzyme for low-concentrated substrates are usually slow, since the enzyme molecules have fewer chances to react with substrates and the substrates also need some time to migrate to the catalytic active sites of enzymes [37]. After exposure in aqueous solution for a long time, enzymes may lose their activity, which result in the low degradation efficiency. However, in our experimental system, the PAHs can be adsorbed on the surface of LCEFM and concentrated around the active sites of laccase firstly. Owing to the elimination of external diffusion, the apparent reaction rates of PAH degradation is enhanced. Thus, the sorption of PAHs on the LCEFMs is advantageous for enhancing degradation efficiencies of PAHs.

Considering the superior sorption performances and laccase activity of these LCEFMs, a mechanism of PAH degradation promoted by sorption is proposed. In the reaction systems, PAH molecules diffuse from the bulk solution to the external surfaces of the LCEFMs and are adsorbed onto the surface of the fibers and beads at first. This external diffusion process is very fast, which is mainly due to the hydrophobic interactions between the LCEFM and PAHs. Because of the adsorption–desorption effect, the concentrated PAHs may form a PAH layer surrounding the beads and fibers. Then, the pores on the surface of beads and fibers may play an important role in mass transfer. Presumably, the large area of the porous wall can increase the mass-transfer rate of the PAHs to laccase active sites. Under such circumstance, PAHs will reach the interior of the beads rapidly. After the PAHs transfer into the beads, they will gain access to the active sites of laccase and be degraded.

4. Conclusions

Four types of LCEFMs with beads-in-string structural fibers are prepared through emulsion electrospinning, and the laccase can be encapsulated into the core of the beads and fibers. This special structure makes these LCEFMs possess high laccase-catalytic activity, good storage and operational stabilities and adsorptivity. The hydrophilic–hydrophobic property of the polymer and the specific surface area are the important factors influencing the retained activity, stabilities, sorption capacities and rates of the LCEFMs. Furthermore, the rapid adsorption of PAHs onto the LCEFMs can obviously promote their degradation efficiencies by laccase. The environmental friendly LCEFMs are expected to be used as the ideal enzymatic membrane-bioreactor for removal of organic pollutants from water.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.seppur.2012.11.013.

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