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Laccase-Carrying Electrospun Fibrous Membranes for Adsorption and Degradation of PAHs in Shoal Soils

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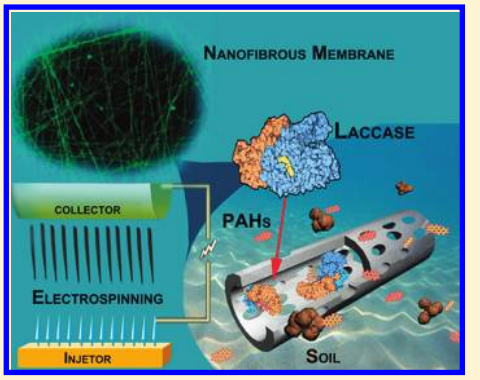
Supporting Information

ABSTRACT: The removal of polycyclic aromatic hydrocarbons (PAHs) from soil is costly and time-consuming. The high hydrophobicity of PAHs makes PAH diffusion from soil particles by hydraulic flow difficult. The phase transfer of PAHs from soil to another available mediator is crucial for PAH removal. This study focuses on the remediation of PAH-contaminated shoal soil, located in Yangtze, China, using three types of laccase-carrying electrospun fibrous membranes (LCEFMs) fabricated via emulsion electrospinning. These LCEFMs were composed of core−shell structural nanofibers (for PAH adsorption), with laccase in the core (for PAH degradation) and pores on the shell (for mass transfer). The LCEFMs with strong adsorptivity extracted the PAHs from the soil particles, resulting in an obvious enhancement of PAH degradation. The removal efficiencies in 6 h for phenanthrene, fluoranthene, benz[*a*]anthracene and benzo[*a*]pyrene were greater than 95.1%, 93.2%, 79.1%, and 72.5%, respectively. The removal half-lives were 0.003−1.52 h, much shorter than those by free laccase (17.9−67.9 h) or membrane adsorption (1.25−12.50 h). The third-order reaction kinetics suggested that the superficial adsorption and internal diffusion were the rate-limiting steps of the overall reaction. A synergistic effect between adsorption and degradation was also proposed on the basis of the triple phase distribution and kinetics analyses.



INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are a class of potentially carcinogenic and ubiquitous global pollutants consisting of two or more fused benzene rings. PAHs can be emitted to the environment as a result of natural combustion processes and human activities.[1,2] Due to their hydrophobic nature, PAHs tend to bind to particles or dust, and they eventually enter soils through direct deposition or rainwash. Soils act as a natural repository for PAHs, resulting in increasingly serious soil environmental pollution.[3−5] These PAHs exert adverse effects on both the edaphon and crops. In some instances, PAH may also cause serious health problems in humans and/or cause genetic alterations, through uptake and accumulation in food chains.[6,7]

PAHs are strongly hydrophobic and are difficult to dissolve in water. This property makes it easy for PAHs to adsorb onto soil particles but difficult for them to dissociate from these particles.[8] Therefore, PAHs in soils are of low bioavailability, have poor degradability, and are difficult to be removed from the soils.[9,10] For this reason, the phase transfer of PAHs (from soils to another available mediator) is crucial for the purification of soils.[11,12] Much effort has been made to extract PAHs from soil particles. PAHs could be washed off from the soil particles by surfactant solubilization.[13] However, it is difficult for researchers to find an environmentally friendly surfactant with low cost and high efficiency. Other approaches, including extraction methods by supercritical liquid,[14] organic solvent,[15] hot water,[16] and steam,[17] could also transfer PAHs from soil particles to a liquid or gaseous phase. Nevertheless, the feasibility and practicality of these techniques need to be improved economically. In addition, the extracted PAH requires further treatment. Therefore, a feasible method for PAH to interphase transfer is urgently needed for the removal of PAHs from soils. Moreover, a subsequent degradation procedure of minimized environmental interference and high removal efficiency is indispensable for rapid purification.

In our previous study, the strong adsorption of PAHs on electrospun fibrous membranes (EFMs) was evidenced based on the micro and macro characterizations.[18] Emulsion electrospinning, a feasible method to obtain the core−shell nanofibers, has also been developed for enzyme immobilization in EFMs in the degradation of crystal violet (triphenylmethane dye).[19] These findings inspired us to combine the two approaches for the adsorption and degradation of PAHs in soils via a phase transfer procedure. Herein, laccase was employed as the biocatalyst for PAH degradation. Laccase (*p*-diphenol:dioxygen oxidoreductases, EC 1.10.3.2) is a copper-containing oxidase. Most of the 16 priority PAHs listed by the United States Environmental Protection Agency (USEPA) can be transformed by laccase, and produce some intermediate products, including dihydroxyphenanthrene, dihydrodiol, diphenic acid, anthraquinone, anthrahydroquinone, benzo[*a*]pyrene

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quinones, hydroxybenzo[*a*]pyrene, anthraquinone, etc.[20−23] Wu et al. revealed the remediation of PAH contaminated soils by free laccase and found significant dissimilation of benzo[*a*]pyrene and anthracene after 14 days of treatment.[24] Nevertheless, free laccase is expendable and easily loses its activity when exposed to the actual environment. For practical application, the immobilization of laccase has been considered to enhance stabilities, adjust reaction, and improve recyclability of laccase. The improved performance of laccase-carrying electrospun fibrous membranes (LCEFMs) has been evidenced in wastewater treatment.[19] On the other hand, considering the strong hydrophobicity of PAHs, we speculated that the polymer fibers of LCEFMs might extract PAHs from soil particles in the aqueous solution. However, there is still some doubt about whether the LCEFMs can remove PAHs from soil efficiently. In this study, three types of polymers, including poly(D,L-lactide) (PDLLA), poly(D,L-lactide-*co*-glycolide) (PDLGA), and methoxypolyethylene glycol−poly(lactide-*co*-glycolide) (MPEG−PLGA), were utilized as the support materials for the immobilization of laccase. All the selected polymers have excellent biocompatibility, mechanical properties, spinnability, and biodegradability. Emulsion electrospinning was introduced to fabricate LCEFMs for the immobilization of laccase. The LCEFMs were then used for the removal of phenanthrene, fluoranthene, benz[*a*]anthracene, and benzo[*a*]pyrene from soil in the aqueous solution. The cooperation of membrane-adsorption and laccase-degradation was investigated on the basis of the phase distribution and reaction kinetic analyses. The removal mechanisms and the possible interactions between the LCEFMs and the PAHs in the soil are also discussed.

EXPERIMENTAL SECTION

Materials. PDLLA, PDLGA, and MPEG−PLGA (MW 100 000, for each) were provided by Daigang Biomaterials (Jinan, China). A triblock copolymer PEO−PPO−PEO (F108) was supplied by BASF. Laccase (*p*-diphenol:dioxygen oxidoreductases, EC 1.10.3.2) from *Trametes versicolor* with a 23 U mg^{-1} solid activity and a substrate of 2,2-azinobis-3-ethylbenzothiazoline-6-sulfonate (ABTS, 99%) were obtained from Sigma-Aldrich. Fluorescein isothiocyanate (FITC), phenanthrene (99.7%), fluoranthene (99.5%), benz[*a*]anthracene (99.7%), and benzo[*a*]pyrene (99.0%) were obtained from J&K, and some of their properties are summarized in Table S1 (see the Supporting Information).

The soil samples were collected from Baisha Shoal, which is located near to the Yangtze River, China. The soil samples were cleared off large pieces of debris (e.g., stones, bricks, and plant residue) and then they were freeze-dried and passed through a 250 μm sieve. The concentrations of the four typical PAHs in the soil were 312.7 ± 0.5, 228.5 ± 1.3, 292.6 ± 2.1, and 186.3 ± 0.9 $\mu g\ kg^{-1}$, respectively. The soil was stored at −18 °C prior to analysis and use. The physicochemical properties of the shoal soil are listed in Table S2 (Supporting Information).

Preparation of Emulsions. The polymer was first dissolved in methylene dichloride with stirring for 3 h to form a homogeneous solution. The polymer concentrations of PDLLA, PDLGA, and MPEG−PLGA were 15, 10, and 12 wt % in methylene dichloride, respectively. Each polymer solution concentration was determined upon formation of stable jets and bead-free fibers under stable electrospinning. To obtain the stable W/O emulsions, 10 wt % (relative to polymers) of F108 was used as an emulsifier in the polymer/methylene dichloride solution. Then, 0.5 mL of 20 mg mL^{-1} laccase solution was mixed with the polymer/F108/methylene dichloride solution to obtain the uniform emulsions. To verify that the laccase was or was not encapsulated in the electrospun fibers, the same amount of FITC-labeled laccase was used in the emulsions for laser confocal scanning microscopy (LCSM, LSM-510, Zeiss, Germany) observation. The laccase was labeled by FITC, as described in detail elsewhere.[19] Moreover, three types of pure polymer solutions were prepared without F108 and laccase.

Electrospinning. Electrospinning was conducted on a self-made multiend electrospinning apparatus (see Figure S1, Supporting Information). In a typical procedure, the emulsion was first loaded into a 10 mL spinning solution cartridge with 12 30-gauge needles (0.5 mm inner diameter) attached. A syringe pump (RWD) was set to inject the emulsion at a flow rate of 0.5 mL min^{-1}. Electrospinning was conducted at a voltage of 12 kV, and the distance between the tip of the needle and the collector was 15 cm. The electrospun fibers were collected on a barrel covered with aluminum foil. It usually took a few minutes to obtain acceptably thick and integrated LCEFMs. These LCEFMs were then kept in glutaraldehyde vapor for 30 min. The vapor was obtained from a vacuum vessel containing 10 mL of glutaraldehyde aqueous solution (25 wt %) under 0.5 bar at 30 °C. These LCEFMs were then stored at 4 °C before application. The deactivated LCEFMs and pure electrospun fibrous membranes (PEFMs) were prepared with inactivated laccase (boiling for 10 min) and pure polymer solutions, respectively. Using the multiend electrospinning, the LCEFMs could be obtained immediately, minimizing the adverse effects of solvent, electric field, and dehydration on the activity of laccase.

Characterization. The morphology of the LCEFMs was characterized using a field emission scanning electron microscope (FESEM S-4800, Hitachi). The fibers were collected on microscope glass slides during electrospinning and observed under LCSM. The excitation and emission wavelengths were 488 and 535 nm, respectively. The specific surface area and pore volume of the LCEFMs were measured using an ASAP 2020 analyzer (Micromeritics). The contact angle was measured on a Dataphysics OCA20 angle measuring instrument.

Activity and Stability Assays. The activity of the laccase was determined by measuring the change of absorbance during the catalytic oxidation of ABTS by laccase, with a UV−vis spectrophotometer (Cary 50, Varian) at 420 nm. The detailed measurement and calculation methods of laccase activity have been described elsewhere.[19]

The residual activities were measured for one month to evaluate the storage stability of the free laccase and the LCEFMs. Between each measurement, the samples were stored in a phosphate buffer (pH 3.5) at 4 °C. To assess the operational stability, the LCEFMs were separated from the reaction system after each run in an assay. These LCEFMs were washed three times with the phosphate buffer and then transferred to the fresh ABTS solution. This operation was repeated 10 times.

Purification of PAH Contaminated Soil. All PAH removal experiments were carried out at 25 ± 1 °C and pH 6.5. First, 5 g (dry weight) of PAH-contaminated soil was added into 25 mL of deionized water, and the suspension was shaken at 150 rpm in the dark for 24 h (on KS4000i, IKA) to reach the adsorption−desorption equilibrium. Then, five LCEFMs (2 cm ×2 cm, total weight 195−200 mg) loaded with 5 mg of laccase were added. The reaction mixtures were then stirred for 24 h and sampled periodically. For each sampling, 50 μL of sodium azide (20 mmol L^{-1}) was added to terminate the laccase catalysis. Control experiments

with the equivalent amount of free laccase, deactivated LCEFMs, PEFMs, and PEFMs/free laccase were carried out in the same reactor. Moreover, blank experiments without membranes and laccase were also performed. All samples were produced in triplicate, including the controls.

Analytical Methods. Water samples were extracted with 25 mL of dichloromethane/methanol mixture (1:1, v/v), and then the aqueous extracts were reduced to 2 mL in a rotary evaporator (RV 05 basic, IKA, Germany), and re-extracted with 5 mL of methanol again. PAHs were extracted from soil samples through accelerated solvent extraction apparatus (ASE300, Dionex) with 40 mL of hexane/acetone mixture (1:1, v/v). The extracts were concentrated to 2 mL and purified by using open glass columns containing 2 g of 5% deactivated silica gel (100−200 mesh, from Qingdao Ocean Chemical Plant, China) and 1 g of anhydrous sodium sulfate. The columns were eluted with 10 mL of hexane/methylene dichloride mixture (1:1, v/v). The concentration and solvent replacement procedures of the eluents were similar to water samples. The extractions of PAHs from the LCEFMs were carried out in an ultrasonic bath (KQ-502B, Kunshan Ultrasonic Instruments, China) for 30 min by using acetonitrile as the solvent. Quantification analysis of the PAHs was performed on a high-performance liquid chromatograph (HPLC, Dionex U3000) equipped with a ChromSep C_{18} column (250 × 4.6 mm, 5 μm, Varian) and a fluorescence detector.

For three types of LCEFMs, both degradation (degraded by laccase) and removal (adsorbed by electrospun fibrous membrane and degraded by laccase) efficiencies were introduced to evaluate the PAH removal from soils. The adsorption efficiency was defined as the PAHs adsorbed by the deactivated LCEFMs. The degradation efficiency and removal efficiency were expressed as follows

$$\text{degradation efficiency } (\%) = 1 - (A_1 + A_2 + A_3 + B) \quad (1)$$

$$\text{removal efficiency } (\%) = 1 - (A_3 + B) \quad (2)$$

where A_1, A_2, and A_3 are the mass ratio of PAHs in the aqueous solution, on the membrane, and in the soil particle to initial total PAHs, respectively, and B is the mass ratio of the experimental loss to initial total PAHs.

RESULTS AND DISCUSSION

Morphology and Structure of LCEFMs. The SEM images and diameter distribution of the three types of LCEFMs are shown in Figure S2 (Supporting Information). All three membranes consist of continuous porous fibers with an average diameter of 100−500 nm. The three kinds of fibers were covered with nanoscale pores. The formation of pores on the surface of the fibers was considered as a dominant phase separation mechanism between the polymer and the air. Under the stress of strong electric fields, the polymer in the solution may be distributed randomly, resulting in the formation of polymer-rich and polymer-poor regions on the surface of the fibers. Probably the pores are formed from bubbles in the polymer-poor regions during the evaporation of the methylene dichloride.[25] The formation of pores was also affected by the ambient humidity, the organic solvent, and the polymer.[26,27] The porous structure might act not only as an arena for the adsorption but also as the access for mass transfer during the reaction.[19,28]

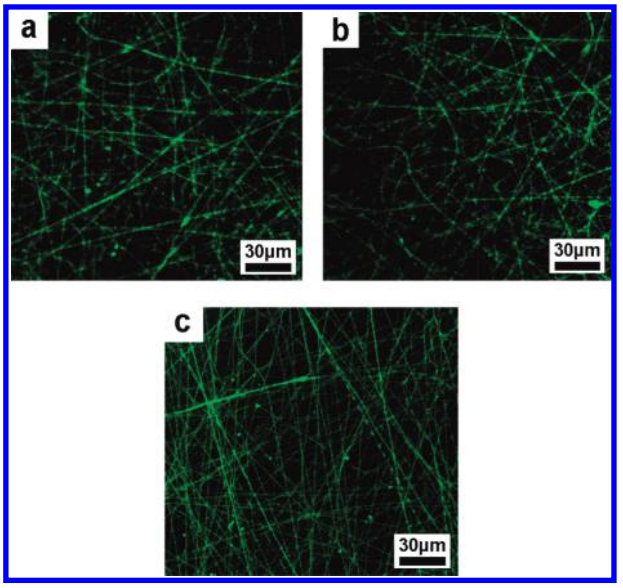

Figure 1. Laser scanning confocal microscope (LSCM) images of PDLLA (a), PDLGA (b), and MPEG−PLGA (c) laccase-carrying electrospun fibrous membranes (laccase was labeled by fluorescein isothiocyanate).

Emulsion electrospinning has been used to prepare ultrafine fibers with core−shell structures, in which the water-soluble enzyme or drug could be encapsulated into a hydrophobic or amphiphilic polymer.[29,30] In our case, all three fibers emitted green fluorescence, which indicated that the laccase had been successfully encapsulated into them and also verified their core−shell structures (Figure 1). The formation mechanisms of the core−shell structured fibers during emulsion electrospinning were illustrated in our previous work.[19]

Activity and Stability Assays. The retained activities of the three types of LCEFMs relative to free laccase were all >75% (see Table S3, Supporting Information). Here, the biocompatible polymer shell both maintained the activity of the enzyme and protected the laccase from the influence of the external surroundings. Meanwhile, the porous structure on the surface of the fibers may facilitate the diffusion of substrate to/off the laccase, resulting in increased laccase activity. Furthermore, these special structures improved the storage and operational stabilities of the LCEFMs. As shown in Figure 2a, the free laccase lost more than 50% of its initial activity in 2 weeks. After a month of storage, the relative activity of the free laccase was less than 30%, whereas the LCEFMs still retained more than 70% of their initial activities. Figure 2b illustrates that the PDLLA LCEFMs retained 85% of its initial activity after oxidizing 10 batches of 0.5 mM ABTS, and the PDLGA and MPEG−PLGA LCEFMs also retained more than 70% of their initial activities. In addition to the special structures of LCEFMs, the glutaraldehyde vapor treatment can also improve their stability. Glutaraldehyde may react with the primary amine groups of the enzymes, forming intermolecular cross-links and larger enzyme aggregates, which would be less likely to leak out of the fibers.[31]

However, there are also some differences among the LCEFMs prepared from the different polymers. The storage and operational stabilities of the three LCEFMs follow the order of PDLLA > PDLGA > MPEG−PLGA, which might be due to the differences in the hydrophilicity−hydrophobicity of the polymers

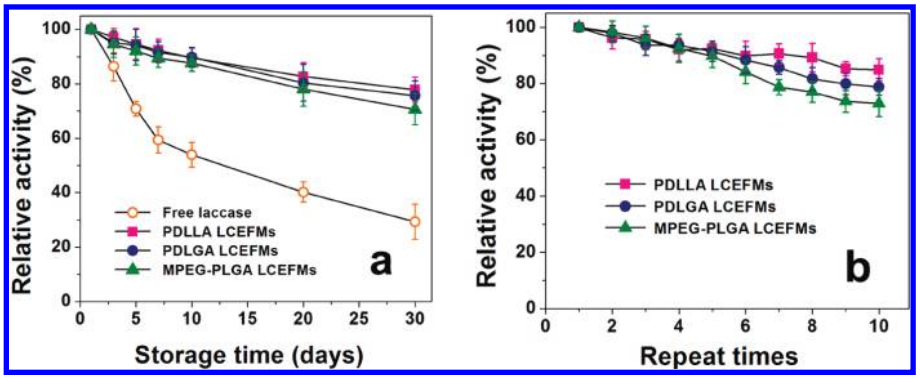

Figure 2. Storage stability (in phosphate buffer at 4 °C) and reusability of laccase-carrying electrospun fibrous membranes.

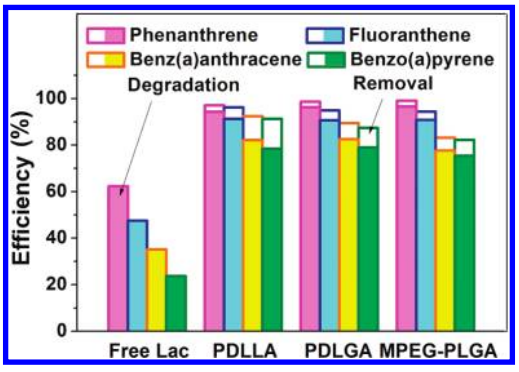

Figure 3. The degradation and removal efficiencies of phenanthrene, fluoranthene, benz[*a*]anthracene, and benzo[*a*]pyrene by free laccase, PDLLA, PDLGA, and MPEG−PLGA laccase-carrying electrospun fibrous membranes. The solid columns represent degradation efficiency, and the addition of solid columns and hollow columns represent removal efficiency.

Figure 4. The schema of the PAH phase transfer and degradation process by laccase-carrying electrospun fibrous membrane.

(see Table S3, Supporting Information). The most hydrophobic PDLLA might undermine the conformation stability of the enzyme, resulting in the lowest activity retention. Both the highest retained activity and the worst stability of the MPEG−PLGA LCEFMs were due to the hydrophilicity. If the MPEG−PLGA LCEFMs are immersed in an aqueous medium for a long time, their hydrophility may lead to the swelling and disintegration of the fibers, causing enzyme leakage and activity loss. In comparison, the most hydrophobic PDLLA LCEFMs maintained the best storage and operational stability.

PAH Removal by Free Laccase and LCEFMs. In our previous study, it was demonstrated that EFMs adsorbed a large amount of PAHs.[18] Figure 3 shows the degradation and removal efficiencies of PAHs by free laccase and the three types of LCEFMs. The degradation efficiencies for the four PAHs were ranked in the order of phenanthrene > fluoranthene > benz[*a*]anthracene > benzo[*a*]pyrene, which was inversely related to the variation of log K_{ow} (shown in Table S1, Supporting Information). However, the degradation efficiencies of the PAHs by all three LCEFMs were much higher than those by free laccase, especially for benzo[*a*]pyrene, whose degradation efficiency by free laccase was less than 30%, whereas those by the three LCEFMs exceeded 70%. Generally, it is accepted that the activity of immobilized laccase should be lower than that of free laccase. The unexpected higher degradation efficiencies might be due to the adsorption of PAHs on the membranes. In the soil suspension, some PAHs in the soils were released into the aqueous solution, but the concentrations of PAHs in the aqueous solution were low. The degradation rate by free laccase for low concentrations of substrate is usually low because of the low probability of collision and the diffusion limitation. Moreover, free laccase can easily lose its activity after exposure to an aqueous solution for a long time, which results in the low degradation efficiency. However, for LCEFMs, PAHs in the aqueous solution can be adsorbed on the surface of the LCEFMs and concentrated around the active sites of laccase; thus, the degradation rates of PAHs were significantly enhanced. Therefore, the higher degradation efficiencies of PAHs by the LCEFMs than those by free laccase may be mainly due to the preconcentration of PAHs on the LCEFMs. For the three types of LCEFMs, their removal efficiencies for PAHs were higher than the degradation efficiencies because some PAHs adsorbed on the membranes had not been completely degraded by the laccase at 24 h. The difference in the removal efficiencies of the three LCEFMs may be due to the different adsorptivities and laccase activities of the LCEFMs for the four PAHs.

The preconcentration process of PAHs on the LCEFMs can be explained as shown in Figure 4. To achieve equilibrium distribution between soil surface and aqueous phase, the PAHs were released into the water from the soil particles. The process is very slow due to the hydrophobicity of the PAHs. However, in the presence of the LCEFMs, the mass transfer of PAHs was accelerated since the PAHs in the water could be adsorbed onto the membrane. Benefitting from the strong adsorptivity of the membrane, little PAH was left in the aqueous phase and more PAH was released from the soil particles under the stress of equilibrium. The adsorbed PAHs were strong enough to maintain a stable PAH "atmosphere" around the fibers, meanwhile, they would migrate into the core through the porous shell and react with

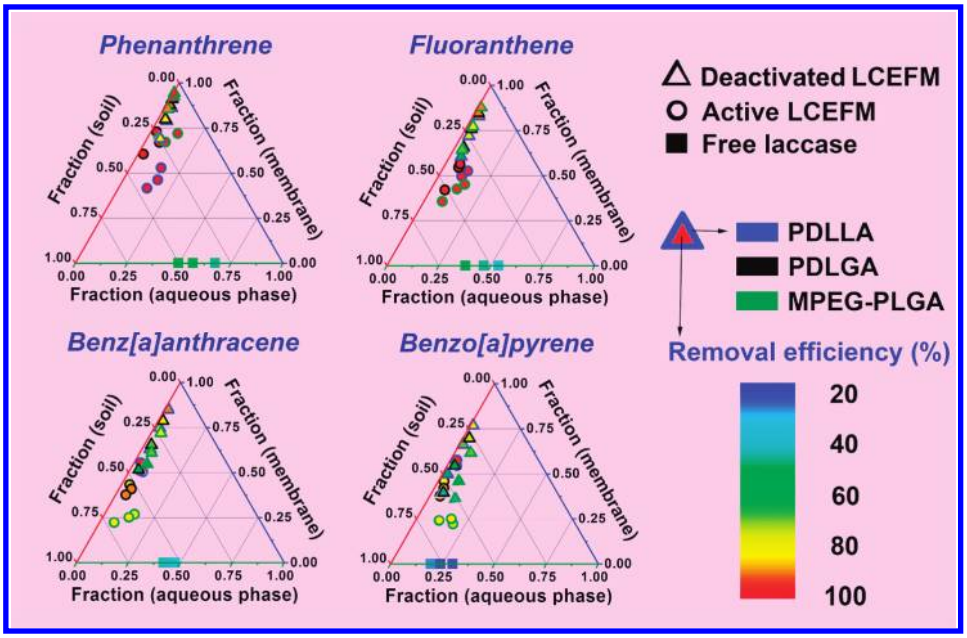

Figure 5. The triphase (soil, membrane, and aqueous phase) distribution diagram of phenanthrene, fluoranthene, benz[*a*]anthracene, and benzo[*a*]pyrene in the presence/absence of laccase degradation. The data points for each laccase-carrying electrospun fibrous membrane were drawn from the samples at 6, 12, and 24 h.

laccase immobilized in the core. As shown in Figure S2 (Supporting Information), the diameter of the pores on the fibers was tens of nanometers, which was sufficiently wide for the mass transfer of the PAHs and their degradation products. Therefore, the porous shell of the fibers may not only shelter the laccase from environmental interference but may also provide access for the exchange of substance. With the migration of PAHs from the surface into the interior of the fibers, there would be more sites for PAH adsorption, facilitating the phase transfer of PAHs from the soil to the membrane. These functional structures of the LCEFMs were advantageous for enhancing the degradation efficiencies of the PAHs in the soils.

The triphase distribution diagram (Figure 5) shows the relationship between adsorption and degradation. Obviously, little PAH could be removed by free laccase under the limitation of external diffusion, and the degradation efficiencies for most of the PAHs at 24 h were lower than 50%. However, the four PAHs were rapidly adsorbed on the deactivated LCEFMs. More than 90% of the low ring PAHs, including phenanthrene and fluoranthene, were transferred from the soil onto the deactivated LCEFMs. Benz[*a*]anthracene and benzo[*a*]pyrene were so hydrophobic that only 60−80% of them could be transferred. As time proceeded, the distribution ratios of the PAHs on the membranes increased, while those on the soils decreased. The adsorptivities of the three deactivated LCEFMs for the different PAHs mainly depended on the hydrophilic−hydrophobic properties of the polymers and the log K_{ow} of the PAHs, which have been discussed in the previous study.[18]

As a result of the preconcentration of PAHs, the degradation efficiencies of the active LCEFM were improved to more than 90% (for phenanthrene and fluoranthene) and 80% (for benz[*a*]anthracene and benzo[*a*]pyrene). By comparison, the distribution ratios of PAHs on the soils in the active LCEFM systems were higher than those in the deactivated LCEFM systems, whereas the distribution ratios of the PAHs on membranes were much less, as a result of the reduction of total PAH amount after the laccase degradation. In addition, the distribution ratios of the PAHs in the aqueous solution were low because they could be rapidly adsorbed onto the active LCEFMs and degraded by laccase. The PAHs were transferred from the soils to the aqueous solution and then distributed onto the active LCEFMs. Theoretically, this process continued until all PAHs were are removed from the entire system.

The synergistic effect between membrane adsorption and laccase degradation need to be further evidenced by kinetic analysis. The degradation/adsorption kinetic order can be determined by the corresponding parameters of the regression equation. The kinetic rate constant (k) and the reaction half-life ($t_{1/2}$) can be calculated by eqs 3−5 (IUPAC, *Compendium of Chemical Terminology*, 2nd ed., 1997). The first-order reaction for PAH degradation by free laccase (eq 3), second-order reaction for PAH adsorption by the deactivated LCEFMs (eq 4), and third-order reaction (eq 5) for PAH degradation and removal by the active LCEFMs are expressed as listed

$$\ln(C_0/C_A) = kt; \quad t_{1/2} = \ln 2/k \tag{3}$$

$$C_A/[C_0(1 - C_A)] = kt; \quad t_{1/2} = 1/(kC_0) \tag{4}$$

$$(1/{C_A}^2 - 1/{C_0}^2) = 2kt; \quad t_{1/2} = 3/(2k{C_0}^2) \tag{5}$$

where C_0 and C_A are the initial and instantaneous concentration, respectively. The detailed kinetic parameters are listed in Table 1.

The kinetic parameters for the catalytic degradation of PAHs by free and immobilized laccase can be well-regressed. For example, the degradation rate constant (k_{deg}) of the free laccase for phenanthrene, fluoranthene, benz[*a*]anthracene, and benzo[*a*]pyrene were 0.0387, 0.0258, 0.0172, and 0.0102 h^{-1}, respectively, whereas their degradation rate constants (k_{deg}) by the active PDLLA LCEFM were 3.12, 0.51, 0.12, and 0.09 $dm^6\,mmol^{-1}\,h^{-1}$, respectively. Correspondingly, the degradation half-lives of the PAHs were dramatically shortened from 17.9−67.9 to 0.115−1.875 h.

Table 1. Degradation/Adsorption/Removal Half-Life ($t_{1/2}$) and Reaction Rate Constant (k) by Free Laccase and Three Laccase-Carrying Electrospun Fibrous Membranes

		kinetics parameter	Phe[a]	Flu[a]	BaA[a]	BaP[a]
free laccase	degradation	$t_{1/2}$ (h)	17.9	26.8	40.3	67.9
		k_{deg} (h^{-1})	0.0387	0.0258	0.0172	0.0102
PDLLA	adsorption	$t_{1/2}$ (h)	2.82	4.12	3.76	6.71
		k_{ads} (dm^3 $mmol^{-1}$ h^{-1})	3.50	2.52	2.71	1.57
	degradation	$t_{1/2}$ (h)	0.115	0.307	1.525	1.875
		k_{deg} (dm^6 $mmol^{-1}$ h^{-1})	1.32	0.57	0.12	0.08
	removal	$t_{1/2}$ (h)	0.029	0.052	0.214	0.301
		k_{rem} (dm^6 $mmol^{-1}$ h^{-1})	5.11	2.96	0.72	0.52
PDLGA	adsorption	$t_{1/2}$ (h)	2.07	3.73	5.56	9.09
		k_{ads} (dm^3 $mmol^{-1}$ h^{-1})	5.07	2.74	1.83	1.13
	degradation	$t_{1/2}$ (h)	0.048	0.317	1.525	1.666
		k_{deg} (dm^6 $mmol^{-1}$ h^{-1})	3.12	0.51	0.12	0.09
	removal	$t_{1/2}$ (h)	0.007	0.094	0.375	0.751
		k_{rem} (dm^6 $mmol^{-1}$ h^{-1})	21.60	1.60	0.40	0.20
MPEG−PLGA	adsorption	$t_{1/2}$ (h)	1.25	2.78	7.69	12.50
		k_{ads} (dm^3 $mmol^{-1}$ h^{-1})	8.07	3.62	1.33	0.86
	degradation	$t_{1/2}$ (h)	0.428	0.306	1.875	2.509
		k_{deg} (dm^6 $mmol^{-1}$ h^{-1})	3.52	0.52	0.08	0.06
	removal	$t_{1/2}$ (h)	0.003	0.0125	1.52	1.45
		k_{rem} (dm^6 $mmol^{-1}$ h^{-1})	45.40	1.20	0.13	0.09

[a] Phe, phenanthrene; Flu, fluoranthene; BaA, benz[a]anthracene; BaP, benzo[a]pyrene.

Generally, the activity of the immobilized enzyme is lower than that of the free enzyme under the same conditions.[32,33] To the best of our knowledge, the significantly enhanced activity of immobilized laccase for PAHs has been rarely reported. More interestingly, the PAH degradation by the active LCEFMs was a third-order reaction. It is generally accepted that an enzyme reaction should be a first-order reaction under the low concentration of the substrate or a zero-order reaction under the high concentration of the substrate in terms of the Lineweaver−Burk equation.[34] The change of the reaction order might be attributed to the multiple control steps. During the PAH degradation by the LCEFMs, the reaction might be affected by the nature of soil particles, the surface of fibers, the porous structure, and the immobilized laccase. In addition, the PAH diffusions from the soil particle, in the solution, on the aqueous film, on the surface of fibers, in the pores, and near the immobilized laccase also influenced the kinetics of the overall reaction. However, it is difficult to determine which step was the rate-limiting step. As shown in Table 1, the reaction rates of PAH degradation by the LCEFMs were much faster than those of the membrane adsorption. Therefore, the adsorption and diffusion might be the rate-limiting steps of the entire process. PAH adsorption onto the membrane (second-order kinetics) can be viewed as a link of the overall reaction. Other than the adsorption, the mass transfer from the fibrous surface to the active sites of laccase was another possible rate-limiting step related to the PAH concentration. Hence, the third-order reaction might be attributed to the synergy of superficial adsorption and internal diffusion. However, the specific kinetics for this complex system requires further investigation.

The results showed that the PAH degradation reactions by LCEFMs were much faster than those by free laccase. The exact laccase-catalysis reaction rate was difficult to measure. However, the ideal reaction rates on laccase in the LCEFMs, at least, were faster than the apparent removal rates by LCEFMs, because the adsorption and diffusion were the rate-limiting steps. The absolute enhancement could be explained by the strong interactions between the concentrated PAHs and the active sites of the laccase. Therefore, the additivity of the reaction orders conveyed the synergistic effect of the membrane adsorption and laccase degradation.

However, differences in the performance of the different LCEFMs were also observed on the basis of the kinetic analysis. As shown in Table 1, the removal rate of phenanthrene by the MPEG−PLGA and the PDLGA LCEFMs was much faster than that by the PDLLA LCEFMs, whereas the removal rate of benzo[a]pyrene by the MPEG−PLGA and PDLGA LCEFMs was lower than that by PDLLA LCEFMs. The log K_{ow} of the PAHs determined the difference in the removal rates. Moreover, the membrane adsorption enhanced the differences in the laccase degradation.

The strong adsorption of the membrane, the mass transfer via the pores, and the high activity of the laccase enabled the LCEFMs to efficiently remove the PAHs from soils. In the PAH removal process, the PAHs were first released into the water under the adsorption−desorption equilibrium of the soil−water. The distribution partition shifted immediately when the LCEFMs were introduced into the PAH solution. The PAHs were rapidly extracted by the LCEFMs, transferred into the fibers via the pores, and subsequently degraded by the laccase. Obviously, the preconcentration of PAHs enhanced the degradation efficiency of the laccase. The functional activity and stability of the LCEFMs were much higher than those of free laccase during the removal of PAHs from contaminated soil. This finding might be helpful for the new environmental application of enzymes and raise a new approach to the use of nanomaterials for the removal of pollutants from soils.

■ ASSOCIATED CONTENT

Supporting Information. Physicochemical properties of chemicals and samples and additional experimental results. This material is available free of charge via the Internet at http://pubs.acs.org.

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