Journal of Hazardous Materials 248–249 (2013) 254–260

Contents lists available at SciVerse ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Adsorption and transformation of PAHs from water by a laccase-loading spider-type reactor

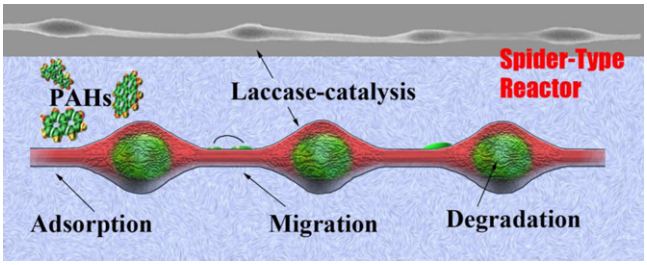
Junfeng Niu*, Yunrong Dai, Huiyuan Guo, Jiangjie Xu, Zhenyao Shen

State Key Laboratory of Water Environment Simulation, School of Environment, Beijing Normal University, Beijing 100875, PR China

HIGHLIGHTS

- Laccase-loading spider-type reactor (LSTR) is got by emulsion electrospinning.
- LSTR consists of beads-in-string fibers with more laccase and higher activity.
- LSTR can achieve the rapid and efficient removal of PAHs from water.
- Aquatic environmental factors have little influence on the PAH removal by LSTR.
- A synergetic mechanism includes adsorption, directional migration and degradation.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 8 October 2012
Received in revised form
12 December 2012
Accepted 9 January 2013
Available online 16 January 2013

Keywords:
PAHs
Enzyme-reactor
Laccase
Adsorption
Biodegradation

ABSTRACT

The remediation of polycyclic aromatic hydrocarbons (PAHs) polluted waters has become a concern as a result of the widespread use of PAHs and their adverse impacts on water ecosystems and human health. To remove PAHs rapidly and efficiently *in situ*, an active fibrous membrane, laccase-loading spider-type reactor (LSTR) was fabricated by electrospinning a poly(D,L-lactide-co-glycolide) (PDLGA)/laccase emulsion. The LSTR is composed of beads-in-string structural core–shell fibers, with active laccase encapsulated inside the beads and nanoscale pores on the surface of the beads. This structure can load more laccase and retains higher activity than do linear structural core–shell fibers. The LSTR achieves the efficient removal/degradation of PAHs in water, which is attributed to not only the protection of the laccase activity by the core–shell structure but also the pre-concentration (adsorption) of PAHs on the surface of the LSTR and the concentration of laccase in the beads. Moreover, the effects of pH, temperature and dissolved organic matter (DOM) concentration on the removal of PAHs by the LSTR, in comparison with that by free laccase, have been taken into account. A synergetic mechanism including adsorption, directional migration and degradation for PAH removal is proposed.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are widespread environmental contaminants in aquatic environments, which mainly originate from fossil fuel combustion and the release of petroleum and petroleum products [1–3]. Some PAHs with four or more benzene rings, such as benzo[*a*]anthracene, chrysene and benzo[*a*]pyrene, can generate covalent DNA adducts and oxidative DNA lesions, resulting in mutagenic and carcinogenic effects [4,5]. Moreover, PAHs are persistent in the environment and can cause long-term adverse effects [6,7]. Thus, 16 PAHs have been listed as priority pollutants by the U.S. Environmental Protection Agency (US EPA). However, PAH emissions in many countries have continuously increased owing to the rapid growth in energy consumption

* Corresponding author. Tel.: +86 10 5880 7612; fax: +86 10 5880 7612.
E-mail addresses: junfengn@bnu.edu.cn (J. Niu), daiyunrong@mail.bnu.edu.cn (Y. Dai), hyguo0216@163.com (H. Guo), 1993120hb@163.com (J. Xu), zyshen@bnu.edu.cn (Z. Shen).

0304-3894/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jhazmat.2013.01.017

and development of traffic and shipping [8–10]. Recently, accidents have released millions of barrels of oil containing massive amounts of PAHs into bodies of water, *e.g.*, in the Gulf of Mexico [11] and the Port of Dalian in China [12], causing perceptible damage to marine and freshwater ecosystems. This has inspired environmental researchers to seek and develop effective technology for the removal of PAHs from contaminated water.

Efforts have been made to reduce PAHs in the water environment. However, *ex situ* treatments are usually laborious and time-consuming for aquatic environments. Traditional *ex situ* techniques, including biodegradation [13], ozone oxidation [14], electro-oxidation [15], and photocatalysis [16] are incapable of the *in situ* removal of massive amounts of PAHs at low concentrations in polluted waters. Sorption treatment might be a feasible method for the *in situ* removal of PAHs in open waters [17,18]. However, the recycling of sorbents and the subsequent treatment of PAHs are difficult, and this can present a risk of secondary pollution. Therefore, more simple, rapid, and efficient *in situ* techniques for PAH removal are urgently needed. Moreover, to minimize disturbances to the environment, an ideal remediation system should be environmentally friendly, practical and recoverable.

Electrospinning is a suitable technique for the preparation of environmentally friendly and recoverable fibrous membranes [19,20]. Electrospun fibrous membrane (EFM) possesses many extraordinary properties, including high specific area, porous structures, and resultant superior mechanical properties [21]. Thus EFM has attracted considerable attentions and has been used in environmental remediation and sample pretreatment, which indicated that it possessed excellent sorption property and exhibited significant potential for the enrichment of organic pollutants in water [22,23]. Moreover, EFMs are also excellent carriers for enzyme immobilization [24,25]. The encapsulation of enzymes in electrospun fibers can be achieved by emulsion electrospinning, an *in situ* enzyme immobilization technology that was developed in recent years; and the encapsulated enzymes usually retain high activities [25,26]. In our previous study, we developed a highly efficient system for laccase-carrying electrospun fibrous membrane (LCEFM) preparation by emulsion electrospinning for the removal of PAHs in shoal soil [27]. The LCEFM is composed of core–shell structural fibers with active laccase in the core part of the fibers. It has been proposed that the degradation efficiency of PAHs by LCEFM is obviously enhanced by the sorption of PAHs on the membrane. However, the structural properties of LCEFM have not been well described, and the environmental effects on the applied potential of this technique are still in question.

Interestingly, the LCEFM may be potentially enhanced on its enzyme loading, activity and efficiency for removal of PAHs from water through its structural improvement. Recently, a study on the directional adsorption and migration of water on the surface of spider silk was published on *Nature* by Zheng et al. [28]. They found that the beads-in-string structure results in a surface energy gradient between the spindle-knots (beads) to achieve continuous condensation and directional collection of water molecular. This finding inspired us to improve LCEFM by fabricating beads-in-string structural fibers, with more laccase encapsulated in the beads. Here, we assume that the PAHs in contaminated water could be adsorbed on to the fibers rapidly, and then transfer to the beads and *in situ* degraded by laccase catalysis ultimately. Thus, the improved LCEFM with beads-in-string structural fibers can be called as laccase-loading spider-type reactor (LSTR), like spider capturing preys. Furthermore, we also expect that the LSTR would collect and treat the PAHs more efficiently than the core–shell structural LCEFM. Sequentially, the synergetic mechanism of PAH adsorption, migration and degradation was need to be discussed. If it is feasible, the PAH pollution in open waters by accidents may be controlled and eliminated promptly by the follow-up techniques. To investigate the applied potential of this material, the environmental effects of the LSTR were also analyzed.

2. Experimental

2.1. Reagents and materials

Poly(D,L-lactide-co-glycolide) (PDLGA) was obtained from Daigang Biomaterials Co. (China). Its molecular weight was *ca.* 100,000, and its intrinsic viscosity was 1.9 dl g^{-1}. Triblock copolymer F108 ($(PEO)_{133}$ $(PPO)_{50}$ $(PEO)_{133}$) was supplied by BASF (Germany). Laccase (p-diphenol: dioxygen oxidoreductases, EC 1.10.3.2) from *Trametes versicolor* with an activity of 23.0 U mg^{-1} protein and the substrate 2,2-azinobis-3-ethylbenzothiazoline-6-sulfonate (ABTS, 99%) were purchased from Sigma–Aldrich (USA). Seven types of PAHs, including naphthalene (99.0%), phenanthrene (99.5%), anthracene (99%), benz[*a*]anthracene (99.7%), chrysene (99.5%), benzo[*b*]fluoranthene (99%) and benzo[*a*]pyrene (99.0%) were also provided by Sigma–Aldrich (USA). Some of their properties are listed in Table S1 (see the Supporting Information). Methylene dichloride, acetonitrile and methanol (HPLC, 99.9%) were obtained from JT Baker (USA). All other chemicals were of analytical grade and used without further purification. Ultrapure water (TOC < 15 ppb) obtained from a Milli-Q Plus/Millipore purification system (USA) was used to prepare the reaction solutions.

2.2. Preparation of LSTR

The procedure used for the emulsion electrospinning of LSTR was as follows: 2 g of PDLGA was dissolved in methylene dichloride (10.0 wt%) at 25 ± 1 °C with vigorous stirring for 3 h. F108 (10.0 wt%, *vs.* PDLGA) was used as a surfactant to obtain a stable and homogeneous W/O emulsion. Then, 1.5 mL of 20.0 mg mL^{-1} laccase solution was added to the PDLGA/F108/methylene dichloride solution and mixed fully by vortexing to obtain a uniform emulsion. The emulsion was then loaded into a 10 mL spinning solution cartridge attached to twelve 30-gauge needles and then injected by a peristaltic pump at 1.0 mL min^{-1}. Electrospinning was performed at 10 kV with a distance of 15 cm between the tip of the needle and the collector. The fibers were collected on an aluminum foil-covered collecting barrel. All experiments were conducted at 25 ± 1 °C and a relative humidity of 40 ± 2%. Finally, the LSTR was kept for 30 min in glutaraldehyde vapor obtained from a vacuum vessel containing 10 mL of glutaraldehyde aqueous solution (25 wt%) under 0.5 bar at 30 ± 1 °C. The LSTRs were stored at 4 °C before use. As a control, deactivated LSTR (DA-LSTR) was prepared by replacing the laccase solution with an inactivated solution (boiled for 10 min).

2.3. Morphology characterization and activity assays

The fiber morphology of the LSTR was examined using a field emission scanning electron microscope (FESEM S-4800, HITACHI, Japan) after gold sputtering. To explore whether the laccase could be encapsulated into beads of LSTR, the laccase in the emulsion was replaced by the same amount of FITC-labeled one and observed under laser confocal scanning microscopy (LCSM, LSM510, ZEISS, Germany). The specific surface area and pore volume of the LSTR were measured with a full-automatic specific surface area analyzer (ASAP 2020, Micromeritics, USA). The hydrophilic–hydrophobic property of the PDLGA was tested on a contact angle measuring system (OCA20, DataPhysics, Germany). The detailed methods for the measurement and calculation of LSTR activity have been described elsewhere [25]. To investigate the enzyme loading of the LSTR, it was washed three times with phosphate buffer (pH 3.5), and the protein concentrations of the washes were determined using

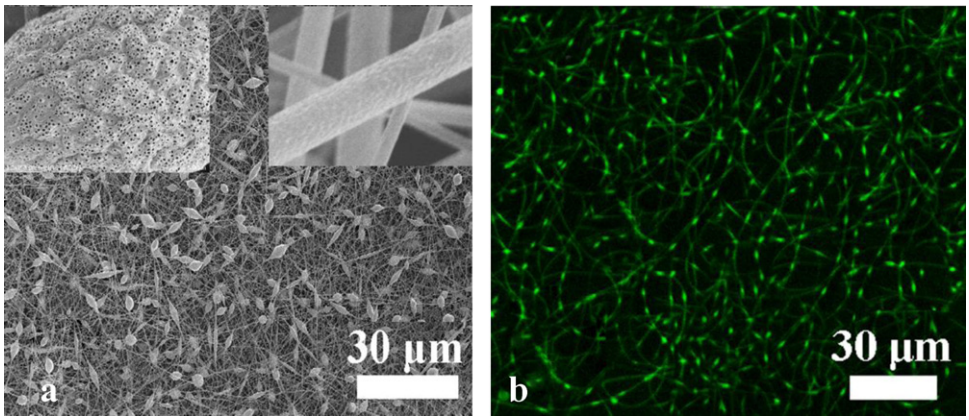


Fig. 1. (a) A scanning electron microscopy (SEM) image of the laccase-loading spider-type reactor (LSTR), the inserted images show the high-resolution details of the beads and fibers; (b) a laser confocal scanning microscopy (LCSM) image of the LSTR. The laccase was labeled by FITC, and the excitation and emission wavelengths were 488 and 535 nm, respectively.

Coomassie Brilliant Blue reagent following the Bradford method. All samples were tested in triplicate.

2.4. PAH removal experiments

Five pieces of LSTR (15 mm × 15 mm, total weight 150–160 mg) loaded with approximately 6.0 mg of laccase were added into 50 mL of a solution containing a mixture of seven PAHs at 10.0 μg L^{-1} each. The mixtures were kept in sealed brown conical flasks and incubated in a horizontal shaker at 150 rpm for 24 h in darkness. Experimental uncertainties evaluated in conical flasks without LSTR were less than 5% of the initial concentrations, except for naphthalene, which reached about 8–10%. The laccase catalysis reaction was terminated by adding 50 μL of sodium azide (20 mol L^{-1}) before sampling. Three groups of control experiments including equivalent amounts of free laccase, DA-LSTR or DA-LSTR/free laccase were carried out in the same reactor. All experiments were performed in triplicate. The PAH concentration in the aqueous phase and on/in the LSTR (washed by acetonitrile) was measured by high-performance liquid chromatography (HPLC, DIONEX U3000, USA). The recovery rates of PAHs in the PDLGA DA-LSTR adsorption experiment were determined as follows:

$$\text{Recovery rate}\,(\%) = \frac{W_{aq} + W_{LSTR}}{W_{total}} \quad (1)$$

where W_{aq} is the mass of each PAH retained in the aqueous solution, W_{LSTR} is the mass of each PAH adsorbed on/in the PDLGA DA-LSTR (*i.e.*, the mass of each PAH in the acetonitrile eluent), and W_{total} is the total mass of each PAH in the reaction solution.

2.5. The effects of environmental parameters on the removal of PAHs

The effect of pH was examined at 25 ± 1 °C by assaying the PAH removal/degradation efficiency of the LSTR at pH values of 1.0, 3.0, 5.0, 7.0 and 9.0, adjusted using NaOH and H_3PO_4 solutions. The dependence of the PAH removal/degradation efficiency on temperature was assessed at pH 5.0 and temperatures ranging from 5 to 45 °C. To investigate the effect of dissolved organic matter (DOM) on the PAH removal/degradation efficiency, four real environmental water samples were collected from the Yangtze Estuary, China. In the present study, DOM was defined as the portion of organic matter in water that passes through a 0.45 μm membrane filter, and the concentration of DOM was given in mg L^{-1} dissolved organic carbon (DOC) in water [29,30], measured by high-temperature catalytic combustion with a TOC analyzer (SHIMADZU, TOC-5000, Japan). The pH values of these samples were between 5.5 and 6.3, and the concentrations of DOM were 6.5, 12.3, 20.4, and 36.7 mg L^{-1}, respectively. After filtration through a 0.45 μm membrane, a certain amount of the seven PAH solution (dissolved in methanol) was added to the treated water samples to prepare an aqueous PAH solution (10.0 μg L^{-1} for each PAH). The total concentrations of the 16 PAHs in the four samples were 68.3–136.5 ng L^{-1}, much lower than the experimental concentrations. To facilitate the calculation of experimental results, the PAH concentrations of the real samples were ignored. The PAH removal experiments were performed using the methods described above, and all experiments were carried out in triplicate.

3. Results and discussion

3.1. LSTR morphology and structure

The morphology of PDLGA LSTR is shown in Fig. 1a, indicating that the LSTR consists of beads-in-string structural fibers. The beads are *ca.* 3–5 μm in length and 1–3 μm in diameter, and the diameters of the fibers range from tens to hundreds of nanometers. Moreover, there are many wrinkles on the beads, and pores with diameters of tens of nanometers are distributed all over the beads (see Fig. 1a). By contrast, the fibers are much smoother and less porous.

Beads-in-string structural fibers have been observed widely [21,31]. The formation of beads in ordinary electrospinning is mainly related to the viscosity and surface tension of the solution, and net charge density carried by the jet, but it becomes much more complicated in emulsion electrospinning [31]. Generally, when electrospinning a water-in-oil (W/O) emulsion with a small amount of aqueous solution, it is easy to harvest the core–shell structural linear fibers due to the occurrence of the phase separation between the aqueous and organic components, and the aqueous phase could be evenly encapsulated in the core of fibers [25,27]. In the condition of the fixed surfactant, the distribution of aqueous phase in the inner of fibers became heterogeneous with the increase of aqueous solution, since some aqueous droplets in the emulsion tended to bind together. When the combined droplet was large enough and its surface tension exceeded the electrostatic field force, the polymer with large aqueous droplet could not be split and the droplet formed the core domain of the bead; while the polymer with small aqueous droplet was easy to be stretched to linear fibers. This is a possible formation mechanism of the beads-in-string structural fibers in emulsion electrospinning. Therefore, the beads-in-string morphology of LSTR could be achieved by adjusting the addition amount of the enzyme aqueous solution. In our experiments, 1.5 mL

of laccase solution (20.0 mg mL^{-1}) was near the largest amount for preparing the beads-in-string structural fibers. If exceeding this value, the LSTR consisted of many droplets, rather than fibers, and it could not be detached from the collector.

Moreover, many pores formed on the beads of LSTR during the electrospinning process, which was attributed to the phase separation between the oil phase (polymer solution) of the emulsion and the air phase [21,27]. Under the influence of strong electric fields, the polymer solution may distribute non-uniform, leading to the occurrence of some polymer-rich and polymer-poor regions at the outer layers of the fibers or beads. When the methylene dichloride evaporates, the air phase may occupy the polymer-poor region, resulting in the formation of the pores. Due to the existence of aqueous droplets, uneven polymer regions are much easier to form on the outer layers of the beads. Thus, there are more pores on the beads than those on the fibers. Furthermore, many folds were observed on the surface of the beads. This is mainly because that the polymer at the outer layers of the beads shrank and solidified rapidly after the evaporation of methylene dichloride.

Although PDLGA LSTR with a beads-in-string morphology was successfully obtained, it was unclear whether the laccase was really encapsulated into the core domain of the beads. The LCSM characterization of the PDLGA LSTR verifies this conjecture. As shown in Fig. 1b, the beads-in-string structural fibers exhibit a core–shell structure and emit green fluorescence under excitation, and the beads emit brighter fluorescence, indicating that most of FITC-labeled laccase was encapsulated into the beads.

3.2. Activity and stability analysis

Experimental results showed that less than 5% of the protein (relative to the total amount of laccase added) was present in the washes, indicating most of the laccase was encapsulated into the beads or fibers of the PDLGA LSTR, and the enzyme loading of LSTR was about three times greater than that of the equivalent PDLGA LCEFM. Moreover, the activity of the PDLGA LSTR accounted for more than 85.3% of that of free laccase, which was also higher than that of the PDLGA LCEFM (79.8%) [27]. Not only benefitting from the protection of the special core–shell structure, the higher concentration of laccase in the beads of LSTR may also provide more active sites and thus increase the probability of laccase catalysis of its substrates, resulting in the higher retained activity.

In addition to the enzyme loading and retained activity, the storage and operational stabilities of the PDLGA LSTR were also improved. As observed in Fig. 2, the free laccase lost nearly 50% of its initial activity within 10 days, and after 60 days in storage, the relative activity of free laccase was less than 10%; in contrast, the PDLGA LSTR retained over 70% of its initial activity. Moreover, the PDLGA LSTR retained approximately 83% of its initial activity after oxidizing 10 batches of 0.5 mM ABTS, performing better than the PDLGA LCEFM [27]. This may be because the laccase encapsulated in the beads of LSTR can form intermolecular cross-links and much larger enzyme aggregates when the glutaraldehyde vapor reacts with the primary amine groups of the enzyme. These aggregates would be less likely to leach from the pores on the surface of beads [32].

Because the morphostructure and enzymatic performance of the PDLGA LSTR were good, the material was used to purify PAH-contaminated water. In the DA-LSTR adsorption control experiments, the recovery rates of the seven PAHs ranged from 93% to 98%. Both degradation (degraded by free laccase or LSTR) and removal (adsorbed by DA-LSTR, or the sum of the PAHs adsorbed by DA-LSTR and degraded by free laccase, or the sum of the PAHs adsorbed by LSTR and degraded by LSTR) efficiencies were calculated to evaluate the PAH removal from water.

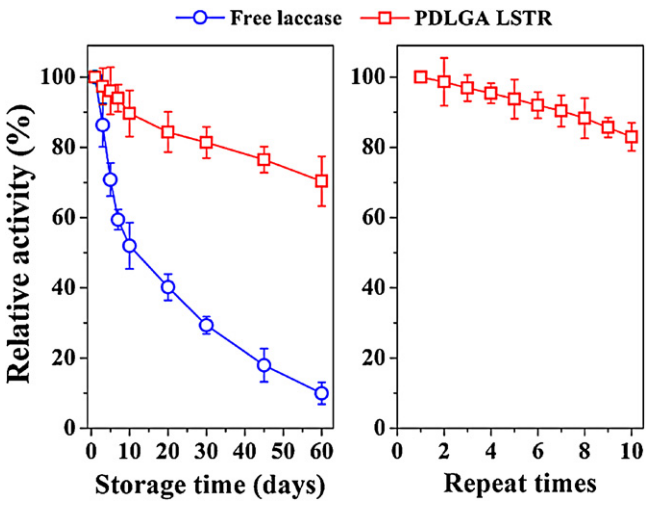


Fig. 2. The storage stability (left) of laccase-loading spider-type reactor (LSTR) and free laccase in phosphate buffer (pH 3.5) at 4 °C and the operational stability (right) of laccase-loading spider-type reactor (LSTR) for the oxidation of ABTS.

3.3. PAH removal by LSTR: effect of pH

The degradation and removal efficiencies of PAHs reacting for 24 h at different pH and 25 ± 1 °C were determined. The results are described in Fig. 3. On the whole, under the same pH values, the low-ring PAHs (*i.e.*, naphthalene, phenanthrene and anthracene) are catalytically degraded by laccase much more easily than the high-ring PAHs (*i.e.*, benz[*a*]anthracene, chrysene, benzo[*b*]fluoranthene and benzo[*a*]pyrene), which is consistent with the results of other studies [33–35]. The pH value of the aqueous solution has a relatively greater influence on the degradation efficiency of free laccase than on that of the PDLGA LSTR. As observed in Fig. 3, most PAHs were degraded most efficiently by free laccase at pH 5.0. However, for PDLGA LSTR, the highest degradation efficiencies were observed for a pH range of 3–7, and the difference in degradation efficiency between these pH values was negligible. Under different pH conditions (from 1.0 to 9.0), the maximum difference in degradation efficiency for each PAH by free

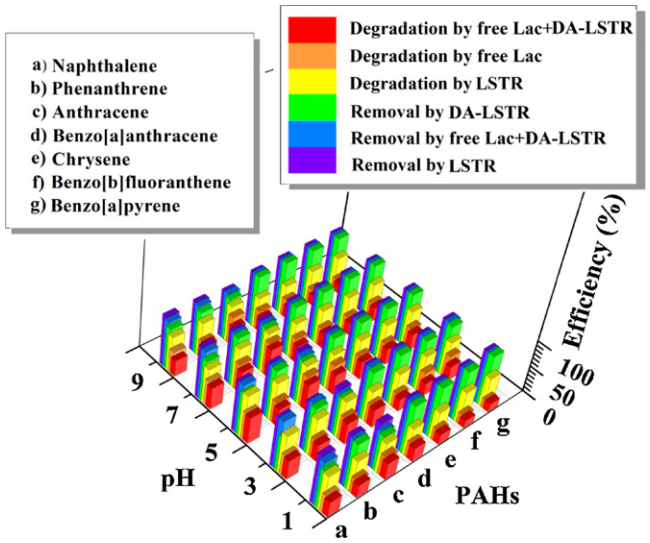


Fig. 3. The effects of pH value on the degradation and removal efficiencies of seven PAHs (naphthalene, phenanthrene, anthracene, benz[*a*]anthracene, chrysene, benzo[*b*]fluoranthene and benzo[*a*]pyrene) by free laccase, laccase-loading spider-type reactor (LSTR), deactivated LSTR (DA-LSTR) and free laccase/DA-LSTR over 24 h.

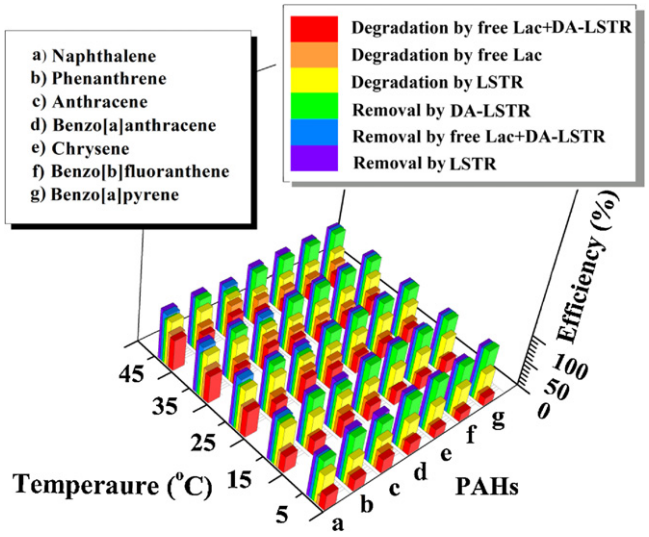


Fig. 4. The effects of temperature on the degradation and removal efficiencies of seven PAHs (naphthalene, phenanthrene, anthracene, benz[*a*]anthracene, chrysene, benzo[*b*]fluoranthene and benzo[*a*]pyrene) by free laccase, laccase-loading spider-type reactor (LSTR), deactivated LSTR (DA-LSTR) and free laccase/DA-LSTR over 24 h.

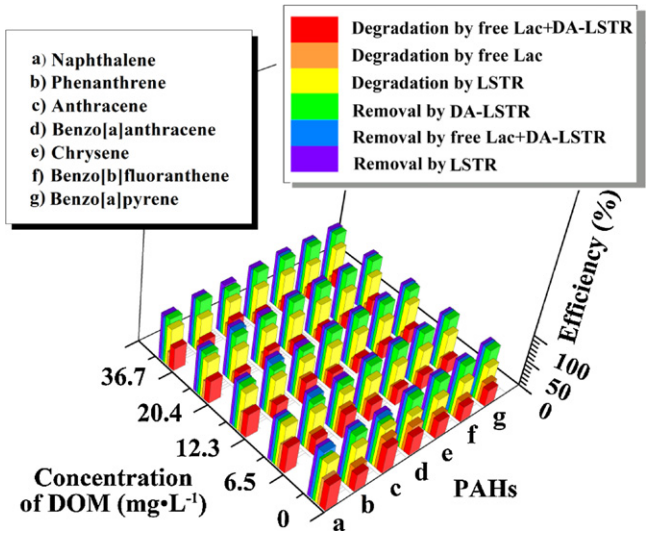


Fig. 5. The effects of DOM concentration on the degradation and removal efficiencies of seven PAHs (naphthalene, phenanthrene, anthracene, benz[*a*]anthracene, chrysene, benzo[*b*]fluoranthene and benzo[*a*]pyrene) by free laccase, laccase-loading spider-type reactor (LSTR), deactivated LSTR (DA-LSTR) and free laccase/DA-LSTR over 24 h.

laccase ranged from 16.4% to 28.1%, while the maximum difference for PDLGA LSTR was between 9.3% and 16.3% (detailed data are shown in Table S3 in the Supporting Information), indicating that the PDLGA LSTR shows a broader pH range for the efficient degradation of PAHs. This is mainly because the core–shell structure of the beads can protect laccase from external influences.

Furthermore, the degradation efficiencies of PDLGA LSTR were 13.6–41.1% higher than those of free laccase at the same pH, which may be attributed to the pre-concentration (adsorption) of the PAHs on the LSTR, as discussed in our previous study [27]. The adsorption of the DA-LSTR lowers the concentration of the PAHs retained in the aqueous solution, which decreases the probability that free laccase will react with PAHs, resulting in the lower degradation efficiencies of PAHs in the DA-LSTR/free laccase experiments than in the free laccase experiments. Because adsorption plays an important role in the degradation of PAHs, the effect of pH on the adsorption by DA-LSTR and LSTR were also analyzed. As shown in Fig. 3, the high-ring PAHs were more easily adsorbed onto the surface of the PDLGA LSTR due to their relatively higher log K_{ow}. More than 95.3% and 97.6% of these compounds could be adsorbed and removed from water by the DA-LSTR and LSTR at different pH values, respectively. The effects of the pH value of the aqueous solution on the adsorption or removal of these PAHs can be considered negligible because they approach the leveling effect. Effects of pH value on the removal efficiencies of low-ring PAHs by the DA-LSTR or LSTR were observed, but the maximum differences in removal efficiency of each low-ring PAH by the DA-LSTR and LSTR were less than 12.3% and 6.9%, respectively, demonstrating that the adsorption of PAHs by the DA-LSTR and LSTR depended slightly on the pH value of the aqueous solution.

In conclusion, the pH value of the aqueous solution has less influence on the degradation and removal efficiencies of PAHs by the PDLGA LSTR, compared with those by free laccase. The LSTR can efficiently degrade and remove PAHs from water under a wider pH range.

3.4. PAH removal by LSTR: effect of temperature

Enzyme-catalyzed reaction starts at a faster rate with increasing temperature up to a point, where the enzyme activity begins to decrease due to heat denaturation [36]. As observed in Fig. 4, the degradation efficiency of free laccase was obviously enhanced with increased temperature from 5 °C to 45 °C, and the increasing degradation efficiencies for different PAHs were distributed between 25.9% and 46% (detailed data are shown in Table S4). At the low temperature, the laccase activity is inhibited, resulting in the relatively lower degradation efficiency for PAHs [37]. The highest degradation efficiency for free laccase occurred at approximately 45 °C. It was observed that the volatilization amounts of most PAHs at 45 °C were less than 5%, except naphthalene, which reached approximately 10%. Thus, the loss of PAHs in aqueous solution is indeed the result of catalytic degradation by laccase. In comparison, the increase in degradation efficiency for each PAH by the PDLGA LSTR with increasing temperature ranged from 11.2% to 20.9%, which is much smaller than the difference for free laccase, indicating that temperature has less influence on the degradation efficiency of the PDLGA LSTR than on that of free laccase. The temperature-efficiency profiles of the PDLGA LSTR illustrate that the PDLGA LSTR could achieve relatively high PAH degradation efficiencies at the range of 25–45 °C. Even at the low temperature (5 °C in our experiments), the PAH degradation efficiencies of the PDLGA LSTR were much higher than those of free laccase. In addition to the PAH adsorption by the PDLGA LSTR, the protection of the core–shell structure also plays an important role in enhancing the degradation efficiencies of PAHs, which minimizes or prevents the effects of temperature variation on enzyme activity. Furthermore, the effects of temperature on the adsorption and removal efficiencies of PAHs by the PDLGA DA-LSTR and LSTR showed little distinction, and the maximum differences in the adsorption and removal efficiencies of each PAH at different temperatures were less than 4.9% and 9.7%, respectively. Therefore, the PDLGA LSTR is resistant to temperature effects, to some degree, and can be used to remove PAHs from water under different temperature conditions.

3.5. PAH removal by LSTR: effect of DOM

In natural environments, DOM in water can interact with organic contaminants through various binding and adsorption interactions, and these interactions have a considerable effect on the migration and transformation of hydrophobic organic pollutants [29,30,38,39]. Consequently, it is essential to analyze the

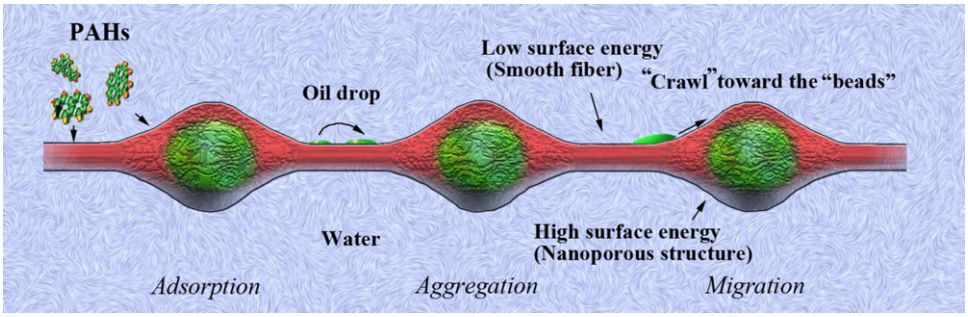


Fig. 6. The predicted reaction mechanism for the PAH adsorption and aggregation from the bulk water onto the surface of LSTR, and directional migration into the beads for final degradation by laccase.

effects of DOM on the degradation and removal of PAHs in aqueous solution.

Fig. 5 shows the degradation and removal efficiencies of PAHs from four real water samples with different concentrations of DOM. The degradation efficiencies of PAHs by free laccase clearly decrease with increased DOM concentration. Compared to those of the sample prepared with ultrapure water (the concentration of DOM was regarded as $\sim 0\,mg\,L^{-1}$), the PAH degradation efficiencies of the real sample with the highest concentration of DOM ($36.7\,mg\,L^{-1}$) decreased by 22.7%–34.1% (detailed data are shown in Table S5). This may be in part because PAHs can associate with DOM, which reduces the concentration of freely dissolved PAH in the solution [38]. Furthermore, it has been reported that humic acid can inhibit the activity of laccase from *T. versicolor* [40]. However, the decreases in PAH degradation efficiencies by DA-LSTR/free laccase with the increase in DOM concentration were relatively smaller than those for free laccase. That is because DOM can also be adsorbed onto the surface of the PDLGA DA-LSTR due to its hydrophobicity, which may reduce the adsorption of PAHs and the inhibition of laccase activity by DOM.

For the PDLGA LSTR, its degradation efficiency decreased slightly with the increase of DOM concentration. Due to the competitive sorption of DOM and PAHs, the PAHs adsorbed on the LSTR reduce, resulting in the observed decrease in the degradation efficiency. Moreover, the PAHs adsorbed on the LSTR might associate with DOM and form larger aggregates that are too large to enter into the core through pores and react with laccase. Nonetheless, the protection of laccase by the polymer shell and the adsorption of DOM, which can minimize the inhibition of DOM to laccase activity, allow the PDLGA LSTR to achieve much higher PAH degradation efficiencies than free laccase and DA-LSTR/free laccase at different DOM concentrations. Furthermore, the influences of DOM on the removal of PAHs by the PDLGA LSTR were not obvious. The maximum differences in removal efficiencies of PAHs were less than 9%, demonstrating that the PDLGA LSTR is suitable for use in removing PAHs from water containing DOM.

3.6. PAH removal mechanism

The highly efficient removal of PAH should be attributed to the adsorption and laccase-catalytic degradation, and the enhancement of such process might be due to the directional migration of PAHs on the beads-in-string structural LSTR. Herein, the synergetic mechanism including adsorption, directional migration and degradation for PAH removal was hypothesized on the basis of experimental and theoretical analysis (Fig. 6). During the reaction, PAH molecules was diffused from the bulk water and adsorbed onto the external surfaces of the LSTR. Benefitting from the hydrophobicity of PDLGA (see Support Information), this external adsorption was achieved rapidly. To reach the adsorption–desorption dynamic equilibrium, a PAH atmosphere layer might be constructed surrounding the beads and fibers. On the other hand, the pores on the shells of the beads could provide accesses for mass transfer. Therefore, the PAHs adsorbed on the surface of the beads would reach the interior through the pores and react with laccase.

However, most of PAHs were adsorbed onto the surface of the fibers rather than the beads since the larger specific surface area of fibers. How can the mass of PAHs reach the beads to react with the laccase? In our opinion, the PAHs adsorbed on the fibers tend to aggregate with each other due to their distinct hydrophobicity, especially for the high-ring PAHs. The aggregated PAHs might form some PAH drops and the drops might grow gradually because of the surface tension between PAHs and water [28]. That is to say, the surface tension was inflating such the PAH drops and turn many little drops into lesser big drops. It is important to be noted that the surfaces of the beads are rough and porous, while the fibers as the joints of the beads are relatively smooth. Therefore, the surface structural anisotropy results in a surface energy gradient [28], which drives PAH drops to move from the lower surface energy (fibers) to the high surface energy region (beads), as the scheme (Fig. 6) suggests. Ultimately, the PAHs were enriched on the surface of beads, aggregated into big drops, and finally degraded by laccase.

This process could also be roughly observed with a fluorescence microscope (CX41-32RFL, Olympus, Japan). As shown in Fig. S1, the PAHs could be rapidly adsorbed onto the surface of the fibers and beads in a few seconds, and both the fibers and beads emit green fluorescence. About 20 min later, the green fluorescence on the fibers becomes weaker and weaker, while that on the beads gets much stronger, which may indicate the directional migration of PAHs from the fibers to the beads. After this, the green fluorescence on the beads gradually becomes weaker over time, which may be because of the degradation of PAHs by laccase in the beads. In such sense, the rapid concentration and directional migration of PAHs might, in our case, dramatically improve the degradation process. However, the detail of the process needs to be investigated and verified further, experimentally and theoretically.

4. Conclusions

PDLGA LSTR, an active fibrous membrane, was fabricated by emulsion electrospinning and used to remove PAHs from water. The LSTR is composed of beads-in-string structural core–shell fibers, with more laccase encapsulated in the beads and fibers and higher activity than the linear structural core–shell fibers. Furthermore, the LSTR can collect and treat the PAHs more efficiently than the core–shell structural LCEFM, and it achieves the rapid and efficient removal/degradation of PAHs in water under the condition of variational pH, temperature and DOM concentration. The highly efficient removal of PAH should be due to the adsorption and laccase-catalytic degradation, and the enhancement of such

process might be attributed to the directional migration of PAHs on the beads-in-string structural LSTR.

The removal of PAHs from water by the PDLGA LSTR likes a process of spider preying, in that the spider (laccase in the beads) rapidly captures its "prey" (PAHs) in the "web" (interlaced beads-in-string fibers), and then consumes the prey leisurely. The PDLGA LSTR consists of nanoscale polymer fibers for PAH sorption, a porous structure for mass transfer, and the laccase-containing beads for PAH degradation. By virtue of the protective porous shell, this special system shows enhanced efficiency, durability and recoverability compared to the free laccase system, making it an ideal candidate for the *in situ* remediation of PAHs in water.

Acknowledgements

This work was supported by the National Basic Research Program of China (973 Program, 2010CB429003), the Fund for Creative Research Groups of the National Natural Science Foundation of China (Grant No. 51121003), and the Program of the Co-Construction with Beijing Municipal Commission of Education of China.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jhazmat.2013.01.017.

References

[1] K.P. Timoney, P. Lee, Polycyclic aromatic hydrocarbons increase in Athabasca river delta sediment: temporal trends and environmental correlates, Environ. Sci. Technol. 45 (2011) 4278–4284.
[2] J.E. Jung, D.S. Lee, S.J. Kim, D.W. Kim, S.K. Kim, J.G. Kim, Proximity of field distribution of polycyclic aromatic hydrocarbons to chemical equilibria among air, water, soil, and sediment and its implications to the coherence criteria of environmental quality objectives, Environ. Sci. Technol. 44 (2010) 8056–8061.
[3] F. Tian, J. Chen, X. Qiao, Z. Wang, P. Yang, D. Wang, L. Ge, Sources and seasonal variation of atmospheric polycyclic aromatic hydrocarbons in Dalian, China: factor analysis with non-negative constraints combined with local source fingerprints, Atmos. Environ. 43 (2009) 2747–2753.
[4] H.P.H. Arp, N.A. Azzolina, G. Cornelissen, S.B. Hawthorne, Predicting pore water EPA-34 PAH concentrations and toxicity in pyrogenic-impacted sediments using pyrene content, Environ. Sci. Technol. 45 (2011) 5139–5146.
[5] Z. Cao, J. Liu, Y. Luan, Y. Li, M. Ma, J. Xu, S. Han, Distribution and ecosystem risk assessment of polycyclic aromatic hydrocarbons in the Luan River, China, Ecotoxicology 19 (2010) 827–837.
[6] L. Patrolecco, N. Ademollo, S. Capri, R. Pagnotta, S. Polesello, Occurrence of priority hazardous PAHs in water, suspended particulate matter, sediment and common eels (Anguilla Anguilla) in the urban stretch of the River Tiber (Italy), Chemosphere 81 (2010) 1386–1392.
[7] L. Wang, Z. Yang, J. Niu, J. Wang, Characterization, ecological risk assessment and source diagnostics of polycyclic aromatic hydrocarbons in water column of the Yellow River Delta, one of the most plenty biodiversity zones in the world, J. Hazard. Mater. 169 (2009) 460–465.
[8] A. Malik, P. Verma, A.K. Singh, K.P. Singh, Distribution of polycyclic aromatic hydrocarbons in water and bed sediments of the Gomti River, India, Environ. Monit. Assess. 172 (2011) 529–545.
[9] J. Guo, F. Wu, X. Luo, Z. Liang, H. Liao, R. Zhang, W. Li, X. Zhao, S. Chen, B. Mai, Anthropogenic input of polycyclic aromatic hydrocarbons into five lakes in Western China, Environ. Pollut. 158 (2010) 2175–2180.
[10] C. Liu, F. Tian, J. Chen, X. Li, X. Qiao, A comparative study on source apportionment of polycyclic aromatic hydrocarbons in sediments of the Daliao River, China: positive matrix factorization and factor analysis with non-negative constraints, Chin. Sci. Bull. 55 (2010) 915–920.
[11] T.J. Crone, M. Tolstoy, Magnitude of the 2010 Gulf of Mexico oil leak, Science 330 (2010) 634.
[12] W. Guo, Y. Wang, M. Me, Y. Cui, Modeling oil spill trajectory in coastal waters based on fractional Brownian motion, Mar. Pollut. Bull. 58 (2009) 1339–1346.
[13] A.K. Haritash, C.P. Kaushik, Biodegradation aspects of polycyclic aromatic hydrocarbons (PAHs): a review, J. Hazard. Mater. 169 (2009) 1–15.
[14] S.N. Chu, S. Sands, M.R. Tomasik, P.S. Lee, V.F. McNeill, Ozone oxidation of surface-adsorbed polycyclic aromatic hydrocarbons: role of PAH–surface interaction, J. Am. Chem. Soc. 132 (2010) 15968–15975.
[15] X. Zheng, J.F. Blais, G. Mercier, M. Bergeron, P. Drogui, PAH removal from spiked municipal wastewater sewage sludge using biological, chemical and electrochemical treatments, Chemosphere 68 (2007) 1143–1152.
[16] J. Sabate, J.M. Bayona, A.M. Solanas, Photolysis of PAHs in aqueous phase by UV irradiation, Chemosphere 44 (2001) 119–124.
[17] Y. Dai, J. Niu, L. Yin, J. Xu, Y. Xi, Sorption of polycyclic aromatic hydrocarbons on electrospun nanofibrous membranes: sorption kinetics and mechanism, J. Hazard. Mater. 192 (2011) 1409–1417.
[18] R.J. Krupadam, M.S. Khan, S.R. Wate, Removal of probable human carcinogenic polycyclic aromatic hydrocarbons from contaminated water using molecularly imprinted polymer, Water Res. 44 (2010) 681–688.
[19] N. Bhardwaj, S.C. Kundu, Electrospinning: a fascinating fiber fabrication technique, Biotechnol. Adv. 28 (2010) 325–347.
[20] D. Li, Y. Xia, Electrospinning of nanofibers: reinventing the wheel? Adv. Mater. 16 (2004) 1151–1170.
[21] A. Greiner, J. Wendorff, Electrospinning: a fascinating method for the preparation of ultrathin fibers, Angew. Chem. Int. Ed. 46 (2007) 5670–5703.
[22] D. Qi, X. Kang, L. Chen, Y. Zhang, H. Wei, Z. Gu, Electrospun polymer nanofibers as a solid-phase extraction sorbent for the determination of trace pollutants in environmental water, Anal. Bioanal. Chem. 390 (2008) 929–938.
[23] V. Thavasi, G. Singh, S. Ramakrishna, Electrospun nanofibers in energy and environmental applications, Energy Environ. Sci. 1 (2008) 205–221.
[24] Z. Wang, L. Wan, Z. Liu, X. Huang, Z. Xu, Enzyme immobilization on electrospun polymer nanofibers: an overview, J. Mol. Catal. B: Enzym. 56 (2009) 189–195.
[25] Y. Dai, J. Niu, J. Liu, L. Yin, J. Xu, In situ encapsulation of laccase in microfibers by emulsion electrospinning: preparation, characterization, and application, Bioresour. Technol. 101 (2010) 8942–8947.
[26] Y. Yang, X. Li, M. Qi, S. Zhou, J. Weng, Release pattern and structural integrity of lysozyme encapsulated in core-sheath structured poly(DL-lactide) ultrafine fibers prepared by emulsion electrospinning, Eur. J. Pharm. Biopharm. 69 (2008) 106–116.
[27] Y. Dai, L. Yin, J. Niu, Laccase-carrying electrospun fibrous membranes for adsorption and degradation of PAHs in shoal soils, Environ. Sci. Technol. 45 (2011) 10611–10618.
[28] Y. Zheng, H. Bai, Z. Huang, X. Tian, F. Nie, Y. Zhao, J. Zhai, L. Jiang, Directional water collection on wetted spider silk, Nature 463 (2010) 640–643.
[29] J. Akkanen, A. Tuikka, J.V.K. Kukkonen, On the borderline of dissolved and particulate organic matter: partitioning and bioavailability of polycyclic aromatic hydrocarbons, Ecotoxicol. Environ. Safety 78 (2012) 91–98.
[30] M. Haitzer, S. Hoss, W. Traunspurger, C. Steinberg, Relationship between concentration of dissolved organic matter (DOM) and the effect of DOM on the bioconcentration of benzo a pyrene, Aquat. Toxicol. 45 (1999) 147–158.
[31] H. Fong, I. Chun, D.H. Reneker, Beaded nanofibers formed during electrospinning, Polymer 40 (1999) 4585–4592.
[32] A.S.K.B. Thurston, E. Herricks, Direct fabrication of enzyme-carrying polymer nanofibers by electrospinning, J. Mater. Chem. 15 (2005) 3241–3245.
[33] K.T. Steffen, S. Schubert, M. Tuomela, A. Hatakka, M. Hofrichter, Enhancement of bioconversion of high-molecular mass polycyclic aromatic hydrocarbons in contaminated non-sterile soil by litter-decomposing fungi, Biodegradation 18 (2007) 359–369.
[34] A. Majcherczyk, C. Johannes, A. Huttermann, Oxidation of polycyclic aromatic hydrocarbons (PAH) by laccase of *Trametes versicolor*, Enzyme Microb. Technol. 22 (1998) 335–341.
[35] L. Bezalel, Y. Hadar, C.E. Cerniglia, Mineralization of polycyclic aromatic hydrocarbons by the white rot fungus *Pleurotus ostreatus*, Appl. Environ. Microb. 62 (1996) 292–295.
[36] D.E. Dodor, H.M. Hwang, S.I.N. Ekunwe, Oxidation of anthracene and benzo[a]pyrene by immobilized laccase from *Trametes versicolor*, Enzyme Microb. Technol. 35 (2004) 210–217.
[37] J. Zhang, X. Liu, Z. Xu, H. Chen, Y. Yang, Degradation of chlorophenols catalyzed by laccase, Int. Biodeter. Biodegr. 61 (2008) 351–356.
[38] J.W. Moon, M.N. Goltz, K.H. Ahn, J.W. Park, Dissolved organic matter effects on the performance of a barrier to polycyclic aromatic hydrocarbon transport by groundwater, J. Contam. Hydrol. 60 (2003) 307–326.
[39] J. Poerschmann, F.D. Kopinke, Sorption of very hydrophobic organic compounds (VHOCs) on dissolved humic organic matter (DOM). 2. Measurement of sorption and application of a Flory–Huggins concept to interpret the data, Environ. Sci. Technol. 35 (2001) 1142–1148.
[40] Y.S. Keum, Q. Li, Copper dissociation as a mechanism of fungal laccase denaturation by humic acid, Appl. Microbiol. Biotechnol. 64 (2004) 588–592.