Pilot scale ex-situ bioremediation of heavily PAHs-contaminated soil by indigenous microorganisms and bioaugmentation by a PAHs-degrading and bioemulsifier-producing strain

Guang-Dong Sun a, b, Yang Xu a, Jing-Hua Jin c, Zhi-Ping Zhong a, Ying Liu a, Mu Luo c, Zhi-Pei Liu a, * a State Key Laboratory of Microbial Resources, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, PR China b School of Environment Tsinghua University, Beijing 100084, PR China c Environmental Protection Research Institute of Light Industry, Beijing 100089, PR China

HIGHLIGHTS

▸ Pilot scale remediation of aged soil polluted by blend HMW-PAHs.
▸ Stimulation of indigenous microbes was effective only for removal of <3 ring-PAHs.
▸ Bioaugmentation with a degrading strain was also efficient for removal of HMW-PAHs.
▸ Inoculation of efficient strains was significant for remediation of heavily contaminated soil.

ARTICLE INFO

Article history:
Received 27 February 2012
Received in revised form 4 June 2012
Accepted 27 June 2012
Available online 4 July 2012

Keywords:
Aged contaminated soil
PAHs
Strain Em1
Bioaugmentation

ABSTRACT

This study aims at the remediation of heavily PAH-contaminated soil containing 375 mg of total PAHs per kilogram dry soil. Pilot scale bioremediation experiments were carried out by three approaches with contaminated soil from abandoned sites of Beijing Coking Plant using outdoor pot trials. The first approach was bioaugmentation with a bacterial strain which degrades PAH and produces bioemulsifier, the second approach comprised of biostimulation of indigenous microorganisms with supplementing nutrients and the last approach involved the combination of both biostimulation and bioaugmentation. An on-site land farming group was set as a control in which the total PAHs and 4–6 ring-PAHs were reduced by 23.4% and 10.1%, respectively after 175 days. Meanwhile, in the first approach group, the total PAHs and 4–6 ring-PAHs were reduced by 26.82% and 35.36%, respectively; in the second approach group both percentages were 33.9% and 11.0%, respectively; while in the third approach group, these pollutants were reduced by 43.9% and 55.0%, respectively. The results obtained suggested that biostimulation and bioaugmentation combined could significantly enhance the removal of PAHs in the contaminated soil.

© 2012 Elsevier B.V. All rights reserved.

1. Introduction

Soil-contaminating PAHs are produced in many processes including the burning of fossil fuels, manufacture of gas and coal tar, wood processing, automobile gasoline exhausts, fuel-burning kitchen stove and incineration of waste [1,2]. PAHs are thus amongst the most widespread organic contaminants in soils, water and wastewater [3]. The accidental release of PAHs causes serious damage to ecosystems when improperly managed and they may persist in soil for a long time [4,5]. Microbiological decontamination of soil contaminated with PAHs is claimed to be an efficient, economic and versatile alternative to physicochemical treatment [6]. Degradation of PAHs-based pollutants can be enhanced by the provision of nutrients to stimulate the activity of indigenous microorganisms [7,8] and by the inoculation of microbial consortia or single isolates known to be able to degrade hydrocarbons [9]. However, soil characteristics, such as moisture content, aeration condition, nutrients, co-substrates, the redox potential, dry-wetting sunlight, soil organic matter, pH, Na+, Cl−, CO32−, and SO42− content, particle and pore size distribution, bioavailability, bioaccessibility and toxicity are all known to affect the removal of PAHs from soil [10].

It is well known that the low biodegradability of high molecular weight PAHs (HMW-PAHs) with 4 and more rings is due to their low aqueous solubility and high sorption to soil particles [11,12] thus preventing their elimination from the contaminated soil. A promising means to enhance the bioavailability of PAHs is the application of surfactants. Addition of surfactant increased the solubilization
Table 1
Principal properties of the contaminated soil sample.a

<table>
<thead>
<tr>
<th>Properties of the soil sample</th>
<th>Quantification of the properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>TOC (%)</td>
<td>1.8 ± 0.6</td>
</tr>
<tr>
<td>Water content (%)</td>
<td>19.38 ± 1.2</td>
</tr>
<tr>
<td>pH</td>
<td>6.8 ± 1.5</td>
</tr>
<tr>
<td>Total PAHs (mg kg⁻¹)</td>
<td>364.276</td>
</tr>
<tr>
<td>4-6 ring PAHs (mg kg⁻¹)</td>
<td>51.143</td>
</tr>
<tr>
<td>Total nitrogen (mg kg⁻¹)</td>
<td>50.1 ± 1.05</td>
</tr>
<tr>
<td>Total phosphorus (mg kg⁻¹)</td>
<td>0.58 ± 0.12</td>
</tr>
</tbody>
</table>

a The errors were expressed as standard deviation of the triplicates.

Table 2
Experimental setup for ex situ remediation of the contaminated soil.

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Bioremediation techniques used</th>
<th>Type of strain</th>
<th>Nutrienta</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>On-site land farming (control)</td>
<td>Present</td>
<td>–</td>
</tr>
<tr>
<td>II</td>
<td>Bioaugmentation</td>
<td>Present</td>
<td>–</td>
</tr>
<tr>
<td>III</td>
<td>Biostimulation</td>
<td>Present</td>
<td>+</td>
</tr>
<tr>
<td>IV</td>
<td>Biostimulation</td>
<td>present</td>
<td>+</td>
</tr>
</tbody>
</table>

a – no addition, + addition.

and biodegradation of PAHs [13]. Synthetic surfactants as well as biosurfactants produced by microorganisms can enhance the solubilization and desorption of PAHs. For example, dirhamnolipid and monorhamnolipid improved the biodegradation of PAHs in aqueous systems [14]. Biosurfactants from Acinetobacter sp. increased the apparent solubility and biodegradation of PAHs [15]. They are biodegradable, less toxic and cheap in comparison to chemical surfactants [16–19]. Although the ability of some species of microorganisms to enhance PAH degradation and produce biosurfactants in soils has well been documented, a few successful examples in field-scale bioaugmentation were reported. To the best of our knowledge, the pilot scale bioremediation of heavily PAHs-contaminated soil containing high concentration of blend HMW-PAHs has not been described till date.

In our laboratory, a bioemulsifier-producing bacterial strain capable of degrading some alkanes and PAH compounds was isolated from petroleum-contaminated soil and identified as Rhodococcus ruber Em1 [20–22]. It had been successfully used for the treatment of refinery wastewater [22]. However, nothing is known about the effect of bioaugmentation by Rhodococcus ruber Em1 on the removal of PAHs and microbial activities in PAH-contaminated soil. This needs to be studied as the soil microorganisms are very sensitive to any ecosystem function shifts because their activity and diversity are rapidly altered by perturbation [23]. In this study, pilot scale bioremediation experiments were conducted using three approaches including bioaugmentation by inoculating Rhodococcus ruber Em1, biostimulation of indigenous microorganisms by supplementing with nutrients and the combination of biostimulation and bioaugmentation, in order to evaluate the performance of the Rhodococcus ruber Em1 strain on the removal of PAHs from an aged contaminated soil and to investigate the possibility of biostimulation of indigenous microorganisms for the bioremediation.

2. Materials and methods

2.1. Contaminated soil

Contaminated soil was obtained from the abandoned sites of Beijing Coking Plant. It had been continuously poisoned by coking chemicals for more than 50 years. Some principal properties and concentration of PAHs in the soil were summarized in Table 1. A high content of PAHs was noted, especially the HMW-PAHs were present indicating a heavy pollution by these compounds, according to the Canadian Environmental Quality Guidelines released by the Canadian Council of Ministers of the Environment (CCME, 2004) [24]. Particle size distribution with sieve analysis was thus impossible due to a lot of cinders, stones and some of the rubber present. Therefore, the samples were homogenized under the natural condition. The dry particle density was 2.3 g cm⁻³ and the pH was about 6.8. The total organic carbon content of the soil was about 1.8% (dry weight) and total N and P were negligible.

2.2. Set up of ex situ remediation experiments and sampling

Pilot scale ex situ remediation experiments were carried out under outdoor conditions as per the experimental set-up detailed in Table 2, to investigate the PAH degradation through bioremediation. All contaminated soil samples were periodically tilled to improve aeration and to promote soil homogeneity for biological degradation. Soil conditions were controlled by monitoring the moisture and nutrient content. In order to follow the PAH degradation, five soil samples were taken once every two weeks from each experimental group for a period of 175 days. The samples were collected from the center of the field and four places 30 cm to the corner. All samples were collected from 10 cm beneath the surface and then they were mixed thoroughly as one sample.

2.3. Bacterial strain, growth condition and preparation of cell suspension

Bacterial strain used in this study was Rhodococcus ruber Em1. It could degrade n-alkanes and PAHs, and produce bioemulsifier resulting in decrease of the surface tension of distilled water from 72 mN/m to 30 mN/m [21,22]. Strain Em1 was grown in LB medium for 2 days. The cells were then harvested by centrifuging at 12,000 rpm for 30 min, washed twice with and resuspended in sterile saline solution to give OD₅₆₀ = 1.0.

2.4. Polycyclic aromatic hydrocarbon (PAHs) standards

Polycyclic aromatic hydrocarbons (PAHs) standards were purchased from AccuStandard, Inc (USA) including naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benz[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, dibenz[a,h]anthracene, benzo[g,h,i]perylene and indeno[1,2,3-cd]pyrene.

2.5. Extraction of PAHs from soil samples

PAHs in the soil samples were extracted by ultrasonic extraction method as recommended by US EPA method 3550C [25]. One gram of contaminated soil was homogenized with 3 g anhydrous Na₂SO₄ (activated at 150 °C for 3 h) and placed into a cotton wool thimble, pre-extracted in Soxhlet with CH₂Cl₂ for 24 h. The thimble was placed into a 100 ml beaker and 30 ml of CH₂Cl₂ was added. The beaker was covered with aluminum foil.
Table 3
Concentration of PAHs in different experimental groups (mg kg⁻¹).

<table>
<thead>
<tr>
<th>Component</th>
<th>Experimental groups</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naphthalene</td>
<td></td>
<td>1.041</td>
<td>0.624</td>
<td>0.775</td>
<td>0.605</td>
</tr>
<tr>
<td>Acenaphthylene</td>
<td></td>
<td>8.947</td>
<td>8.259</td>
<td>6.047</td>
<td>5.624</td>
</tr>
<tr>
<td>Acenaphthene</td>
<td></td>
<td>180.017</td>
<td>120.000</td>
<td>123.010</td>
<td>103.064</td>
</tr>
<tr>
<td>Fluorene</td>
<td></td>
<td>90.016</td>
<td>76.972</td>
<td>79.430</td>
<td>61.44</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td></td>
<td>27.803</td>
<td>21.992</td>
<td>19.191</td>
<td>19.604</td>
</tr>
<tr>
<td>Anthracene</td>
<td></td>
<td>5.309</td>
<td>5.125</td>
<td>5.075</td>
<td>4.713</td>
</tr>
<tr>
<td>Fluoranthene</td>
<td></td>
<td>18.338</td>
<td>15.084</td>
<td>11.584</td>
<td>15.356</td>
</tr>
<tr>
<td>Pyrene</td>
<td></td>
<td>10.048</td>
<td>9.585</td>
<td>7.373</td>
<td>9.533</td>
</tr>
<tr>
<td>Chrysene</td>
<td></td>
<td>4.819</td>
<td>4.545</td>
<td>4.081</td>
<td>4.193</td>
</tr>
<tr>
<td>Benzo[b,k]fluoranthene</td>
<td></td>
<td>3.715</td>
<td>2.835</td>
<td>2.125</td>
<td>2.932</td>
</tr>
<tr>
<td>Benzo[a]pyrene</td>
<td></td>
<td>3.233</td>
<td>3.221</td>
<td>1.951</td>
<td>3.204</td>
</tr>
<tr>
<td>Indeno[1,2,3-cd]pyrene</td>
<td></td>
<td>1.964</td>
<td>1.979</td>
<td>0.736</td>
<td>1.981</td>
</tr>
<tr>
<td>Benzo[g,h,i]perylene</td>
<td></td>
<td>1.995</td>
<td>1.845</td>
<td>1.329</td>
<td>1.605</td>
</tr>
<tr>
<td>Total PAHs</td>
<td></td>
<td>364.276</td>
<td>278.947</td>
<td>266.575</td>
<td>240.639</td>
</tr>
<tr>
<td>4–6 ring PAHs</td>
<td></td>
<td>51.143</td>
<td>45.975</td>
<td>33.057</td>
<td>45.589</td>
</tr>
<tr>
<td>2–3 ring PAHs</td>
<td></td>
<td>313.133</td>
<td>232.972</td>
<td>233.518</td>
<td>195.05</td>
</tr>
</tbody>
</table>

and placed into an ultrasonic bath. The sample was sonicated for 15 min, the extract was collected and its volume was measured. The extraction was repeated three additional times. The CH₂Cl₂ extracts were combined together, homogenized and the volume was recorded. An aliquot of this extract was fractionated through silica gel.

2.6. Analysis methods

Moisture was determined by placing pre-weighed contaminated soil samples in an oven at 105 °C for 24 h. Total organic carbon (TOC) was determined according to Walkley–Black method [26]. Total phosphorus (TP) and total nitrogen (TN) were analyzed according to standard methods [27].

For the analysis of PAHs, a capillary GC method was employed according to Karamalidis and Voudrias [28] and a 16-PAHs priority pollutant standard was used as recommended by US EPA to construct a 5-point calibration curve, which resulted in R² > 0.989 for all compounds. The quantification was based on 4 internal standards including 1-phenylhexane, 2,3-dimethylnaphthalene, 3,6-dimethylphenanthrene and 2,2-binaphthyl. In this study, we reported data in two ways: (1) individual PAH and (2) 16 PAHs in total. All the results, including those from the diluted contaminated soil samples, were expressed as mg kg⁻¹ dry weight of contaminated soil. The analyses were performed on an Agilent 6890N gas chromatograph equipped with a flame-ionization detector and split/splitless injector. For the analyses, a HP-5 (5% phenyl-95% methylpolysiloxane) (30 m × 0.32 mm i.d. 0.25 μm film thickness) column was used. Samples were injected in splitless mode (splitless time: 0.80 min, flow: 20 ml min⁻¹). The oven was programmed to 70 °C for 1 min, 150 °C at a rate of 15 °C min⁻¹, finally to 300 °C at a rate of 6 °C min⁻¹ and held at this temperature for 22 min. Inlet and detector temperature was set at 300 °C and 290 °C, respectively. Helium was used as the carrier gas at a flow rate of 1.2 ml min⁻¹ (linear velocity 23 cm s⁻¹). System control and data acquisition were calculated with the Agilent ChemStation (G2070AA ver. A.10.02).

2.7. Data analysis

All the data obtained in the study were subjected to statistical analysis of two way ANOVA, and post hoc Turkey test with SPSS Version 16.0.

3. Results

3.1. The removal of PAHs in the experimental group I

Indigenous microorganisms can remove PAHs from soil as revealed by previous studies [29–31]. In this study, experimental group I (Table 2) served as control. The results showed that there was a continuous decrease of PAHs in the contaminated soil, as shown in Table 3 and Fig. 1. The degradation percentage of total PAHs reached 23.42% (from 364.276 mg kg⁻¹ to 278.947 mg kg⁻¹) after 175 days (P < 0.05), whereas this value for 4–6 ring-PAHs was 10.1% (P < 0.05), from 51.143 mg kg⁻¹ to 45.975 mg kg⁻¹ (Table 3). Low molecular weight PAHs (LMW-PAHs) were removed preferentially (Table 3). Naphthalene (40.0%) and acenaphthene (33.3%) were degraded to the greatest extent, while 14.49–20.9% decrease in the concentration of fluorene, fluoranthene and phenanthrene was observed after 175 days. No significant changes in the concentration of HMW-PAHs including chrysene, pyrene, benzo[a]anthracene, benzo[a]pyrene, indeno[1,2,3-cd]pyrene and dibenz[a,h]anthracene were observed, except benzo[b,k]fluoranthene (23.68%). This result suggested that most LMW-PAHs can be degraded by indigenous microbes, however, the course of action might be very slow. This was in agreement with previous reports [23,32]. It was also inferred that it is very difficult
for HMW-PAHs to be eliminated by the indigenous microorganisms alone.

3.2. The removal of PAHs in the experiment group II

The indigenous microorganisms in aged contaminated soil could degrade LMW-PAHs (Fig. 1) but bioremediation of contaminated soils can be improved by bioaugmentation with exogenous microorganisms or genetically engineered microorganisms (GEMs) [33,34]. In this study, bioaugmentation was conducted in the experimental group II (Table 2) in order to investigate the performance of the bacterial strain Em1 in the removal of PAHs. The results in Fig. 2 and Table 3 showed that the removal of total PAHs was from 364.276 mg kg$^{-1}$ to 266.575 mg kg$^{-1}$, after treatment for 175 days, the percentage of removal being 26.82% ($P < 0.05$). The removal of 4–6 ring-PAHs was from 51.143 mg kg$^{-1}$ to 33.057 mg kg$^{-1}$, percentage removal being 35.36% ($P < 0.05$). The removal percentage of 4–6 ring-PAHs was much higher than that in the experimental group I. However, LMW-PAHs were not significantly removed in comparison to the experimental group I (Table 3). Naphthalene (25.0%), acenaphthylene (32.4%) and acenaphthene (31.7%) were degraded to the greatest extent, while 22.6–42.7% decrease in the concentration of pyrene, benzo[a]anthracene, benzo[g,h,i]perylene, benzo[a]pyrene and benzo[b,k]fluoranthene was observed after 175 days. It was however noted that the decrease of indeno[1,2,3-cd]pyrene reached 62.5%. This result suggested that although the indigenous microorganisms could degrade LMW-PAHs, the introduction of the strain Em1, as a bioaugmentation strategy, significantly enhanced the degradation of HMW-PAHs in the contaminated soil.

3.3. The removal of PAHs in the experiment group III

Bioaugmentation, i.e., the addition of substrates, nutrients, oxygen, water or surfactants, was commonly used for increasing the removal of pollutants. The indigenous microorganisms in aged contaminated soils are able to metabolize LMW-PAHs but the in situ environmental conditions like nutrient, water content, temperature and aeration might limit degradation of PAHs. Hence, the experimental group III used in this study served to elucidate the effect of bioaugmentation by addition of nitrogen and phosphorous nutrients.

Table 3 and Fig. 3 indicated the change in concentration of PAHs in the contaminated soil by adding nutrients. The concentration of total PAHs decreased by about 33.9%; however, the concentrations of 4–6 ring-PAHs decreased by only 10.85%. The degradation of total PAHs in the experimental group III was higher than that in experimental group I (23.42%) and experimental group II (26.82%), mainly contributed by the decrease of LMW-PAHs. These results suggested that the addition of nutrients to the soil stimulated the removal of LMW-PAHs by the indigenous microorganisms (Table 3). The concentration of naphthalene decreased by 41.8%, and a decrease in range of 29.5–42.7% was seen foracenaphthene, fluorene and phenanthrene. A slight reduction in the concentration of pyrene (5.1%) was also observed. However, no significant decrease in the concentration of the HMW-PAHs (four- and five-rings) occurred suggesting that they were very difficult to be removed by just stimulating the indigenous microorganisms.

3.4. The removal of PAHs in experiment group IV

Reduction of PAHs in contaminated soil occurred in both experimental groups II and III. Hence bioaugmentation and biostimulation are viable methods for improving biodegradation of PAHs in soil as has also been reported earlier that they are strongly linked to the soil microbial capacity to dissipate PAHs [31,32]. Table 3 and Fig. 4 showed the changes in concentration of total PAHs and 4–6 ring-PAHs in experimental group IV after treatment for 175 days. Total PAHs was removed by 43.9% which ranged from 23.4% to 33.9% in other experimental groups. Total 4–6 ring-PAHs was removed by 55.0%, which was about 20–40% higher than that in other experimental groups. The degradation percentages of

Fig. 2. Changes of PAHs in contaminated soil augmented with Rhodococcus ruber Em1 (Experiment group II). (■), total PAHs; (▲), 4–6 ring-PAHs.

Fig. 3. Changes of PAHs in contaminated soil added with nutrients (Experiment group III). (■), total PAHs; (▲), 4–6 ring-PAHs.

Fig. 4. Changes of PAHs in contaminated soil added with nutrients and augmented with Rhodococcus ruber Em1 (Experiment group IV). (■), total PAHs; (▲), 4–6 ring-PAHs.
three-ring, four-ring, five-ring and six-ring PAHs in total reached from 27.7 to 80.6. In contrast to other experimental groups, the degradation of HMW-PAHs, including five-six ring compounds: benzo[a]pyrene indeno[1,2,3-cd]pyrene and benzo[g,h,i]perylene, ranged from 53.1% to 80.6%, respectively.

4. Discussion

It is known that indigenous microorganisms in aged contaminated soils are able to metabolize LMW-PAHs and some species of microorganisms are able to enhance PAH degradation in soils [7,8,35–39]. However, a few successful examples in field-scale bioaugmentation were reported, because of the catabolic properties and survival ability of introduced microorganisms in the target environments [40,41]. In this study, the indigenous microbially dominated population (Group 1) had only a small capacity to degrade the LMW-PAHs. This was improved by the addition of NH₄Cl and KH₂PO₄ as nutritional supplements, affirming that the indigenous microbes with limited metabolic capacity could be stimulated by nutrients (Group 3). The limited ability of indigenous microflora to degrade only the LMW-PAHs in contaminated soil is not an uncommon problem and has been reported in previous studies [38,39]. Due to the hydrophobic nature of PAHs, some biostimulating strategies, including addition of surfactants or nutrients and tilling of contaminated soil were employed to improve the desorption of PAHs and microbial biomass [38,42–44]. In this assay, on-site land farming, addition of nutrients and augmentation with the bacterial strain Em1 were used so as to improve the practicality of bioremediation. In land farming, contaminated soil was periodically tilled to improve aeration and to promote soil homogeneity for biological degradation. Soil conditions were controlled by monitoring the moisture and nutrient content, frequency of aeration and soil pH. In order to optimize the rate of contaminant degradation, contaminated soil was spread over with waste material or mixed with soil amendments such as bulking agents and nutrients to incorporate better degradation and oxidation process by existing microbial population. A study conducted by Wang et al. [44] incorporated fertilization with 10 mg of urea and 4.3 mg of superphosphate per cm² area, liming with 55 mg of powdered agricultural limestone per cm² area and weekly tilling to a 15 cm depth with a hand shovel. Under these conditions, the total hydrocarbon degradation increased considerably, with almost complete elimination of LMW-PAHs in 12 weeks compared to untreated soil. However, quite a large amount of HMW-PAHs was still present. In another study [45], contaminated soil from a manufactured gas plant (MGP) site was excavated to a prepared bed land treatment unit approximately 30 cm deep. The biological land treatment for half to one year revealed that the LMW-PAHs were removed to approximately 90%, whereas there was no reduction of 5- and 6-ring PAHs. The addition of nutrients with inorganic nitrogen and phosphorous was a common bioremediation strategy, as revealed by many reports [7,8,44,45], and was especially efficient for the contaminated soil with high C/N/P ratio, such as the soil used in our experiments (Table 1). In this study, LMW-PAHs were removed by 33.9% in experiment group 3 (added nutrients), much higher than that (23%) in experimental group 1 (as control), after treatment for 175 days; however, there was not much reduction of 4–6 ring PAHs in both groups. These results were broadly in agreement with those reported previously [44,45]. Although the land farming process is a simple technique which is cheap and requires slight maintenance with almost no cleanup liabilities and minimal monitoring efforts, the extent of biodegradation of HMW-PAHs was not effective [44]. The biodegradation of LMW-PAHs occurred much more rapidly and extensively than that of HMW-PAHs [46,47]. All these results suggested that the elimination of HMW-PAHs was not easily performed by indigenous microorganisms, even if they were stimulated, mainly due to the low bioavailability and high sorption to soil particles [11,12].

In this study, the degradation of HMW-PAHs was higher than that of LMW-PAHs in the experimental groups II and IV. For example, the initial concentration of acenaphthene was 180.017 mg kg⁻¹, its degradation percentages were 33.3% and 42.7% in experimental groups I and II (no inoculation of strain Em1), respectively. However, the initial concentration of benzo[g, h, i]perylene (1.995 mg kg⁻¹), indeno[1,2,3-cd]pyrene (1.964 mg kg⁻¹), benzo[a]pyrene (3.233 mg kg⁻¹), benzo[b,k]fluoranthene (3.715 mg kg⁻¹) and chrysene (4.819 mg kg⁻¹) was lower than acenaphthene (180.017 mg kg⁻¹) and other LMW-PAHs, the degradation percentages for those compounds were higher than that of LMW-PAHs in the experimental groups II and IV. This might be due to the inoculation of strain Em1, a biosurfactant-producing bacterium [21]. The biosurfactants produced by strain Em1 might greatly enhance the solubilization and desorption of HMW-PAHs from soil particles thus increasing their bioavailability and degradation. These results were consistent with that of addition of either inorganic surfactants [13,14] or biosurfactants [17,19,41,54]. In experimental groups II and IV, out of all the HMW-PAHs, the highest degradation percentage was obtained for benzo[a]pyrene, this might be due to its highest concentration in the contaminated soil. This result was consistent with previous ones by Li et al., where the authors stated that the initial amount of individual PAHs, higher the degradation percentage that was obtained [48]. This could be explained by the fact that PAH concentration gradients influenced the mass transfer rate of PAHs to microorganisms and lower the toxicity for aged contaminants [49]. Our results suggested that the inoculation of efficient microorganisms might be one of the reasonable methods for the elimination of HMW-PAHs from aged contaminated soil.

The processes by which organic compounds become increasingly desorption-resistant in soil result from sequestration, which originate from the slow diffusion of organic compounds within solid organic matter components, the entrapment within nanopores in soil aggregates and the formation of strong bonds between organic compounds and soil [50]. PAHs are preferentially sequestered in a separable, low-density fraction at levels not predictable by the equilibrium partitioning theory. Furthermore, the low-density fraction apparently controls release of whole-sediment PAHs [51]. Mineralization of PAHs in aged soils appears to be controlled by mass transfer rather than the biodegradation rate [52]. In this study in Fig. 1, there was no change in the concentration of total PAHs from 0 to 40 days compared to the initial concentration. This agreed well with Wang et al. who presented that higher solution-phase concentrations of pyrene and phenanthrene were maintained for the aged soil than that in freshly spiked soil before the 18th day [53].

In this study, there was no significant difference in concentrations of fluorene, acenaphthene and phenanthrene in all experiments. Further study needs to be done to explain the reason. However, this might occur because it was generally assumed that there were microbes which could use some PAHs as a sole source of carbon for growth in aged contaminated soil, and the amounts of nutrients might be the limitation on the degradation of PAHs in the native environment [5]. When the nutrients were added, microorganisms would grow quickly, resulting in improving the degradation of the PAHs. However, the nutrients would be used very rapidly for cell growth, followed by deficiency of some nutrients especially nitrogen which always had a positive effect on biomineralization. The deficiency of nitrogen was probably remedied by that derived from the humic substances resulting in some bound residues remobilized [54].

This study demonstrated the efficacy of bioaugmentation of PAH-contaminated soil with nutrients and bacteria. The ability of
strain Em1 to survive in the soil matrix, to degrade PAHs, produce bioemulsifier and reduce both low and high molecular weight PAHs, are all desirable characteristics for successful bioremediation. However, it was recognized that the degradative performance of any inoculum will depend on soil types and other environmental conditions that may not be easily controlled in the field.

5. Conclusions

This work showed that biostimulation and bioaugmentation may be used to enhance the degradation of PAHs soil. Addition of strain Em1 to aged contaminated soil that enhanced degradation of PAHs may be of much greater utility under less favorable conditions, such as those in landfills or at sites with fewer native degraders, or in remediating environments contaminated with PAHs. The results showed that the effectiveness in degrading both total PAHs and 4- and 6-ring-PAHs followed the order: combination of biostimulation of indigenous microbes and bioaugmentation by inoculation of strain Em1 > bioaugmentation by inoculation of strain Em1 > bioaugmentation of indigenous microbes > control experiment. These results suggested that inoculation of efficient strains and supplementation of some nutrients might be greatly effective in the bioremediation of contaminated soil.

The presence of particular microbial community structures might be highly significant in determining whether in situ bioremediation strategies would be successful at a practical level, Therefore it is very important to define the microbial community effective in PAH degradation in contaminated soil in the future studies.

Acknowledgement

This work was supported by the Knowledge Innovation Program of the Chinese Academy of Sciences (No. KJ2X-YW-L08) and program of Beijing Academy of Science Technology (No. IE01200610019-1).

References


