Journal of Hazardous Materials 176 (2010) 41–47

Contents lists available at ScienceDirect

Journal of Hazardous Materials

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

PAHs biodegradation potential of indigenous consortia from agricultural soil and contaminated soil in two-liquid-phase bioreactor (TLPB)

Congying Wang[a,b], Fang Wang[a], Tao Wang[a], Yongrong Bian[a], Xinglun Yang[a], Xin Jiang[a,*]

[a] State Key Laboratory of Soil and Sustainable Agriculture, Institute of Soil Science, Chinese Academy of Sciences, Nanjing 210008, China
[b] School of Environmental Science and Resources, Shanxi University, Taiyuan 030006, China

ARTICLE INFO

Article history:
Received 28 April 2009
Received in revised form 30 October 2009
Accepted 30 October 2009
Available online 6 November 2009

Keywords:
PAHs biodegradation
Indigenous consortia
Microbial activity
Sequential utilization
Two-liquid-phase bioreactor

ABSTRACT

Estimation of PAHs degradation potential of indigenous consortia is essential for remediation of polluted soils. In this study, the biodegradation of a mixture of 11 PAHs was compared using a long-term PAH-contaminated soil (CS) and an unpolluted agricultural soil (AS) as inocula in a two-liquid-phase bioreactor (TLPB). In the TLPB, silicone oil was used as the organic phase to increase the PAHs bioavailability. The microbial numbers were also determined during the biodegradation. The results demonstrated that naphthalene, fluorene, phenanthrene, anthracene, fluoranthene and pyrene could be completely biodegraded in both soils within 4–50 days. With the exception of dibenzo(a,h)anthrancene, the other PAHs including benzo(a)anthracene, benzo(a)pyrene, benzo(b)fluoranthene and benzo(k)fluoranthene were degraded to different extents in both soils at the end of 170 days. Complete biodegradation of benzo(a)anthracene and benzo(b)fluoranthene only occurred in CS. During the process, microbial growth was highly correlated to the biodegradation of PAHs. Sequential utilization of PAHs showed a competitive-inhibition in the multi-substrate system. The half-life times of PAHs obtained here were much shorter than those reported previously in soils, indicating that indigenous microbes in both soils had high PAHs degradation potential, facilitated by TLPB.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a group of persistent organic pollutants that are widespread in the ecosphere [1]. They are usually produced from natural and anthropogenic sources, such as forest fire, coke and petroleum refining industries, accidental spills and leakages, and incomplete incineration of fossil fuels [2,3]. Some of them have raised public concern due to their potential deleterious effects on human health [4–6].

Microbial biodegradation is a friendly and effective means to remove PAHs from the environment [7,8] and has been extensively used [9–12]. However, poor bioavailability of PAHs is due to both their low solubility in water and their strong sorption to soil or sediment matrix, which limits the bioremediation of sites polluted with PAHs. Therefore, increasing mass transfer from the solid or solid-bound phase to aqueous phase plays an important role in PAHs degradation [13]. Two-liquid-phase system (TLPS) or bioreactor (TLPB) is highly effective in increasing the bioavailability of poorly soluble compounds in aqueous phase. It consists of an aqueous phase and a water immiscible, non-volatile organic phase, which is biocompatible with microbes. The organic phase acts as a carrier for hydrophobic compounds to diffuse from the organic phase to the aqueous phase, then the microbes can metabolize the compounds at the interface of the two phases and/or in the aqueous phase [14]. Additionally, the organic phase usually exhibits a higher affinity for oxygen than pure water, thus larger amounts of oxygen can be transferred to TLPB, which facilitates the aerobic treatment of pollutants [15]. So far the TLPB has been widely employed in biodegradation of organic compounds such as styrene [16,17], chlorobenzenes [18] and PAHs [19–21], and silicone oil is most frequently selected as the organic phase [14,22,23]. Such applications achieved high efficiency in eliminating hydrophobic compounds when suitable microorganisms were introduced [22,24–26].

It has been accepted that in bioremediation, indigenous microorganisms screened from polluted soils are more effective in metabolizing pollutants than organisms obtained from elsewhere [27]. However, clean agricultural soils without prior exposure to pollutants raise a large population of indigenous organisms too, and their ability of utilization of hydrocarbons has been reported in limited literature [28]. It is unknown to what extents the microbes in clean agricultural soils are able to degrade PAHs. Additionally, the biodegradation in TLPB – without limitation of mass transfer – was much different from that in soils. Therefore, it is necessary to investigate the biodegradation of PAHs in clean agricultural soils in order

* Corresponding author. Tel.: +86 25 8688 1195; fax: +86 25 8688 1000.
E-mail address: jiangxin@issas.ac.cn (X. Jiang).

0304-3894/$ – see front matter © 2009 Published by Elsevier B.V.
doi:10.1016/j.jhazmat.2009.10.123

Table 1
Physico-chemical properties of the soils (AS: soil from an agricultural site, CS: soil from a contaminated site).

Soil	TOC (g kg^{-1})	Total N (g kg^{-1})	P_2O_5 (mg kg^{-1})	Total K (g kg^{-1})	CEC (cmol kg^{-1})	Clay (%)	Silt (%)	Sand (%)
AS	23.05	1.32	11.37	15.94	11.80	16.47	52.23	31.30
CS	27.52	1.20	17.68	14.22	14.80	23.82	57.05	19.13

to further comprehend their indigenous decontamination ability. This is beneficial in remedying sites in cases of accidental pollution and also contributes to better assessments of soil environmental risks and bioremediation strategies.

2. Materials and methods

2.1. Soil materials

Soil samples from the suburb of Nanjing, China were used in the experiment. One was a clean agricultural soil without prior PAHs contamination (AS) and the other was a long-term PAH-contaminated soil sampled near a steel-manufacturing factory (CS). They were silt loams, classified as Luvisol according to WRB (World Reference Base for soil resources). Soils were sampled from the upper 20 cm, sieved (<2 mm) and stored at 4 °C. Before starting the laboratory experiments, the soil samples were equilibrated for 1 week at 28 °C with 60% of the water holding capacity. Selected physico-chemical properties of the soils and PAHs concentrations in the soils are listed in Tables 1 and 2, respectively.

2.2. Chemicals and culture medium

The PAHs standards (naphthalene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo(a)anthracene, benzo(a)pyrene, benzo(b)fluoranthene, benzo(k)fluoranthene and dibenzo(a,h)anthrancene) were obtained from Supelco Corporation (USA). Silicone oil: polydimethylsiloxane, fluid type; molecular weight, 2000; viscosity, 50 centistokes; density, 0.96 g/cm^3 was purchased from Sinopharm Chemical Reagent Company in China. All the other chemicals and solvents were of analytical purity and were purchased from Nanjing reagent Company (China).

Mineral medium (MM, per liter) consisted of 8.8 g $Na_2HPO_4{\cdot}2H_2O$, 3.0 g KH_2PO_4, 1.0 g NH_4Cl, 0.5 g NaCl, 1.0 mL 1 M $MgSO_4$, and 2.5 mL of a slightly modified trace element solution ([per liter] 23 mg $MnCl_2{\cdot}2H_2O$, 31 mg H_3BO_3, 36 mg $CoCl_2{\cdot}6H_2O$, 10 mg $CuCl_2{\cdot}2H_2O$, 20 mg $NiCl_2{\cdot}6H_2O$, 30 mg $Na_2MoO_4{\cdot}2H_2O$ and 50 mg $ZnCl_2$) (pH 7) [29].

2.3. Contaminated silicone oil preparation

The purchased silicone oil was treated with activated carbon to get rid of unpleasant smell. To prepare the contaminated silicone oil, certain amounts of each PAH were mixed homogenously with the treated silicone oil by stirring with a Teflon-coated magnetic stir bars in a beaker. After that, the contaminated silicone oil was filtered through a 0.22 μm membrane to eliminate impurities and microorganisms. The final concentrations of PAHs in spiked silicone oil are listed in Table 2.

2.4. Degradation of PAHs in TLPB

The degradation of PAHs was conducted in a simulated TLPB as follows: 5 g equilibrated soil (AS or CS, dry weight equivalent) was transferred into a 250 mL flask containing 95 mL of sterilized MM and then 40 g contaminated silicone oil was added to the flask. The treatments were labeled as T-AS and T-CS, respectively. The abiotic controls for AS (T-ASs) and for CS (T-CSs) were conducted by adding solid $HgCl_2$ to the bioreactor to obtain a final concentration of 1000 mg/L. The blank control T-CK constituted of 100 mL MM and 40 g contaminated silicone oil and $HgCl_2$ (1000 mg/L) but without any soil. All the treatments were performed in triplicates and were incubated at 28 °C on a rotary shaker at 160 rpm in dark. Silicone oil and soil suspension were sampled periodically for PAH determination and cell counting.

2.5. Soil microbes enumeration

The enumeration of microorganisms in TLPB was performed using the dilution plate-counting technique. One mL of soil suspension was sampled and diluted with sterilized MM. Nutrient broth (NB) and PDA agar media were prepared for culturing bacteria and fungi separately. Colony-forming units (CFU) were counted after incubating at 28 °C for 4 days.

2.6. Determination of PAHs concentrations

The PAHs in silicone oil were determined according to the procedure reported by Villemur et al. [30]: 0.5 mL silicone oil sample was vortexed with 1 mL of *N*,*N*-dimethyl formamide (DMF) for 2 min and centrifuged at 1150 × *g* for 1 min to separate the two phases. A volume of 0.5 mL of the upper phase (DMF) was then mixed with 0.5 mL of acetonitrile containing 0.1% acetic acid. The mixture was finally analyzed by a Shimadzu HPLC (LC-20A, Japan) with a fluorescence detector and a Supelco (USA) PAHs special chromatographic column according to the program as described by Yin et al. [31].

2.7. Data analysis

SigmaPlot (Version 10.0) was used for both data analysis and plotting graphs.

3. Results and discussion

3.1. The biodegradation of PAHs in TLPB

The biodegradation of 11 PAHs in TLPB was followed for a period of 170 days (Figs. 1 and 2). The $HgCl_2$-sterilized treatments with and without soils were compared. Apparently the sterile soil did not adsorb the PAHs as the fact that the lack of difference between the two samples in terms of concentrations of the PAHs was observed. Indeed, at the end of the experiment, the concentrations in the soils were measured and found to be less than 10% of those in oil phase (data not shown). This is similar to the ratio at the beginning of the experiment (Table 2).

With the exception of DBahA, all other compounds exhibited significant degradation in T-CS, and most PAHs showed a steep disappearance at the early stage of incubation followed by a slow fall during the later phase. No obvious lag periods were observed in the degradation of NAP, FLR, ANT, PHE, PYR and FLN (Fig. 1). Among these PAHs, NAP was degraded fastest, decreasing from the initial concentration of 7.9 mg L^{-1} to the detection limits in 4 days. It took 10, 13, and 23 days for PHE, FLN and PYR, respectively, to be degraded from approximately 11 mg L^{-1} to their detection limits. FLR and ANT, reaching the detection limits at about the 34th and 52nd day, respectively, showed much slower degradation.

Although degradation was also observed for BaA and larger molecular weight PAHs including BaP, BbF and BkF, they however

Table 2
The aqueous solubility of PAHs and their concentrations in the soils (AS: soil from an agricultural site, CS: soil from a contaminated site) and their concentrations in spiked silicone oil.

PAHs	Abbrev.	Aqueous solubility[a] ($mg\,L^{-1}$)	Concentration in soils ($mg\,kg^{-1}$)		PAHs in 5 g soil in TLPB (μg)		Initial concentrations in spiked silicone oil ($mg\,L^{-1}$)	PAHs in 40 g spiked silicone oil in TLPB (μg)
			AS	CS	AS	CS		
Naphthalene	NAP	34.80	0.029	0.121	0.15	0.61	7.92	316.80
Fluorene	FLU	2.23	0.027	0.039	0.14	0.20	11.61	464.40
Phenanthrene	PHE	1.20	0.007	0.419	0.04	2.10	10.82	432.80
Anthracene	ANT	0.070	0.042	0.101	0.21	0.51	10.55	422.00
Fluoranthene	FLN	0.207	0.006	1.214	0.03	6.07	11.36	454.40
Pyrene	PYR	0.150	0.005	1.115	0.03	5.58	11.05	442.00
Benzo(a)anthracene	BaA	0.013	0.015	0.662	0.08	3.31	11.91	476.40
Benzo(b)fluoranthene	BbF	0.0011	0.005	0.908	0.03	4.54	10.02	400.80
Benzo(k)fluoranthene	BkF	0.0011	0.004	0.397	0.02	1.99	8.91	356.40
Benzo(a)pyrene	BaP	0.0018	0.008	0.812	0.04	4.06	11.37	454.80
Dibenzo(a,h)anthrancene	DBahA	0.00056	0.009	0.129	0.05	0.65	12.94	517.60

[a] The data were obtained from "Handbook of Physical–Chemical Properties and Environmental Fate for Organic Chemicals" [32].

exhibited lag phases (Fig. 2). The lag phases were about 23 and 34 days for BaA and BaP, respectively. For BbF and BkF, the lag period was around 52 days. It seemed that the biodegradation of DBahA was just initiated at about 140 days in T-CS. After the initial lag period, fast biodegradation was initiated and complete disappearance of BaA and BbF was observed. This showed that the biodegradation of the LMW PAHs was easier than that of the HMW PAHs (Figs. 1 and 2). Moreover, the rates and extents of PAHs biodegradation decreased with the condensation of benzene rings [5,33]. As Phale et al. [34] pointed out, the microorganisms would prefer the simple carbohydrates first and leave the complex substrates for latter use.

It was notable that significant biodegradation of most PAHs was also observed in T-AS (Figs. 1 and 2). In addition to complete degradation of PAHs from 2-ring NAP to 4-ring FLN, some HMW PAHs involving BaA, BaP, BbF and BkF, which are generally recalcitrant to microbial attack, showed a significant degradation after a long period of lag phase (Fig. 2). It revealed that the non-polluted soil did contain microbial communities able to degrade both LMW and HMW PAHs. Andreoni et al. [35] succeeded in enriching a phenanthrene-degrading mixed culture from a clean agricultural soil without exposing it to PAHs. Dong et al. [36] isolated six phenol-degrading bacteria from a natural soil without exposing it to phenol, and demonstrated that most of the strains could tolerate the phenol concentration up to 6 mM or more. The results indicated that the microorganisms might contain the PAH-degrading enzymes even if they did not undergo a long-term adaptation. That was the possible reason why no lag phases were observed in the biodegradation of 2-ring NAP to 4-ring FLN in T-AS, as well as in T-CS (Fig. 1). So it could be concluded that the microbes capable of degrading hydrocarbons might be widespread in natural soils [37,38], and that they could be playing an important role in the natural dissipation of the PAHs from the environment. These results are especially important as they indicate that in the case of unexpected incidents of pollution (PAHs) in natural soils e.g. oil spills, it can be expected that such PAHs could be degraded without further remediation. Furthermore, it is beneficial in that it leads to better evaluation of the proper bioremediation potential of the natural soils.

As shown in Figs. 1 and 2, with the exception of the simplest carbon source of NAP, the rates and extents of PAHs biodegradation in T-CS were higher than those in T-AS. The differences between the two treatments were more remarkable with increasing rings of PAHs. This indicated that the indigenous microflora in contaminated soils were more effective in degrading PAHs than microorganisms in the agricultural soil. This phenomenon can easily be explained by the fact that microbes in contaminated sites have been exposed to pollutants (PAHs) for a long period and have consequently adapted to the PAHs-polluted environment. These adapted degraders are therefore more effective and efficient in metabolizing pollutants [27,39].

During the whole incubation period, the sequential utilization of the PAHs was quite apparent in both soils. As showed in Fig. 3A, the initial degradation of 3-ring (PHE, FLR and ANT) and some 4-ring PAHs (PYR and FLN) was observed in the 4th day after the complete disappearance of 2-ring PAHs (NAP) was achieved. Then the degradation of BaA was initiated on the 23rd day and reached a maximum degradation rate when FLN disappeared, followed by the metabolism of total 5-ring PAHs (BbF, BkF and BaP) on the 52nd day. The pattern of sequential utilization of PAHs in contaminated soil T-CS (Fig. 3B) was similar to and more clear than that in T-AS. The sequential utilization of these carbon sources was attributed to the interaction of competitive-inhibition among the substrates during the metabolism, which often occurred in a multi-substrate system [40,41]. Consequently, facile degradation of a more soluble PAH could repress enzymes for HMW PAHs,

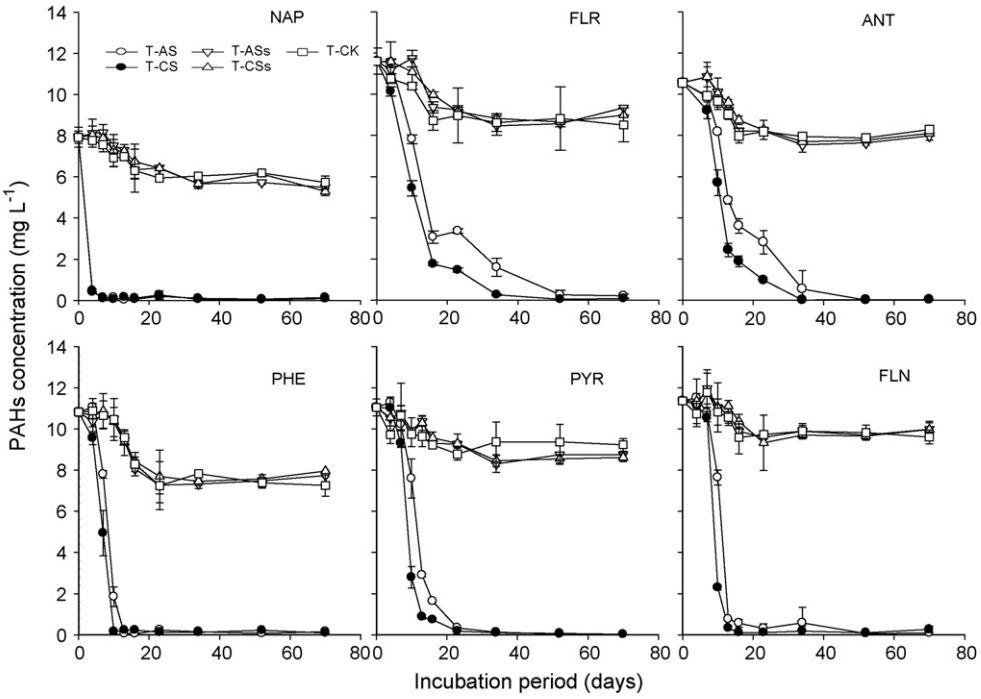

Fig. 1. The biodegradation of naphthalene (NAP), fluorene (FLR), anthracene (ANT), phenanthrene (PHE), pyrene (PYR) and fluoranthene (FLN)in TLPB without lag phase.

giving rise to a lag phase [42]. As aforementioned, different lag periods were observed before the biodegradation of HMW PAHs occurred.

Co-metabolism could have played a dominant role in BaA and 5-ring PAHs degradation, given that no degradation of HMW PAH such as BaA or BbF was observed in TLPB with contaminated soil within the 170 days incubation period, when they were used as sole carbon and energy source separately (data not shown). In contrast, they were degraded in 170 days when they were in a PAHs mixture (Fig. 2). HMW PAHs might be co-metabolized by the enzymes induced through LMW PAHs or some metabolites such as salicylate in the degradation process [43].

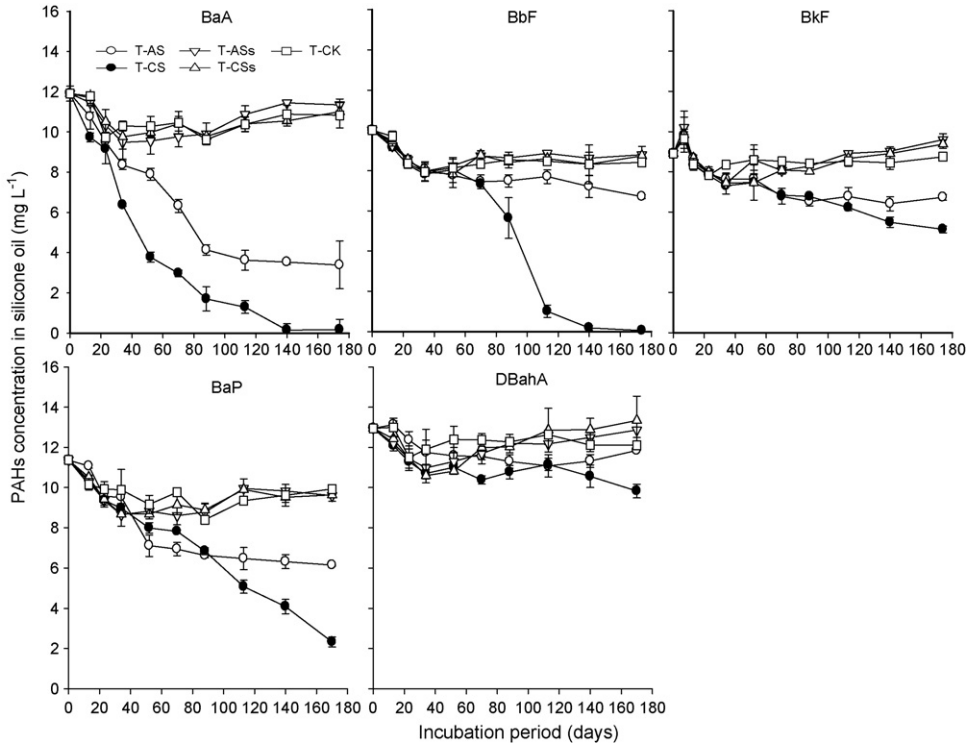

Fig. 2. The biodegradation of benzo(a)anthracene (BaA), benzo(b)fluoranthene (BbF), benzo(k)fluoranthene (BkF), benzo(a)pyrene (BaP) and dibenzo(a,h)anthrancene(DBahA) in TLPB with obvious lag phase.

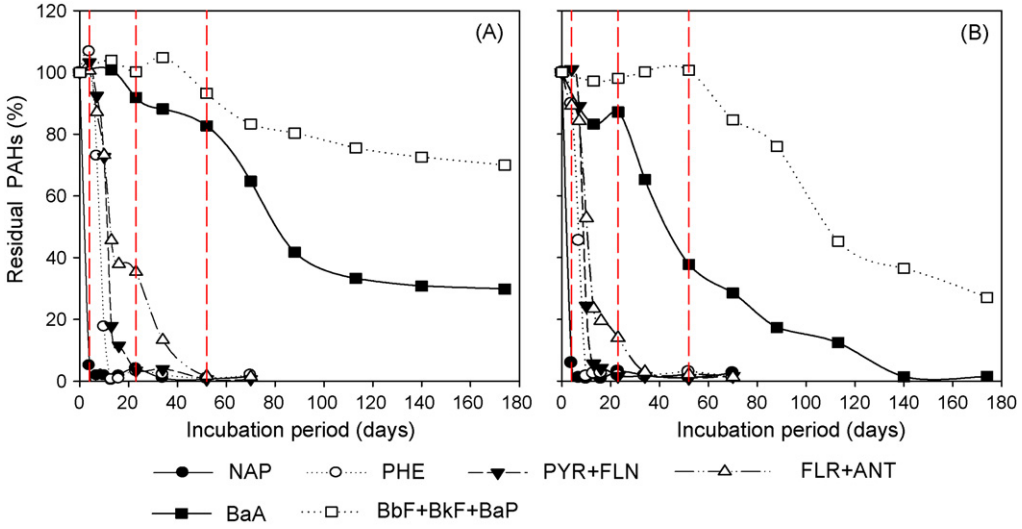

Fig. 3. The sequential utilization of 2- to 5-ring PAHs in TLPB with agricultural soil (A) and contaminated soil (B) during the incubation.

3.2. Microbial growth in TLPB

Microbial biomass is usually used as an index to study the dynamics of pollutants biodegradation. It is determined by the optical density of the liquid culture at a certain wavelength [44] or the protein content [45]. However, the two methods could not be employed easily in the present study because of the soil-water slurry in the TLPB. So the number of microbes including bacteria and fungi was determined. As presented in Fig. 4, the increase in the number of bacteria and fungi in the TLPB showed similar dynamics (Fig. 4A and B). Moreover, the fluctuation of the microbial number in both treatments of T-AS and T-CS corresponded highly to the degradation of PAHs (Fig. 4C and D). At the beginning of the incubation, the number of fungi in T-CS (2.3×10^3 CFU mL^{-1}) was much higher than that in T-AS (9.8×10^2 CFU mL^{-1}). In contrast, the bacterial number in the T-CS (1.4×10^6 CFU mL^{-1}) was lower than that in T-AS (1.8×10^6 CFU mL^{-1}). The number of fungi in both treatments increased quickly accompanied with the rapid utilization of 2-ring NAP at the initial days. It continued to increase and reached the 1st peak of 5.0×10^3 and 3.5×10^3 CFU mL^{-1} in T-AS and T-CS, respectively, in 16 days when 3- to 4-ring PAHs were degraded intensively. Then the number decreased steeply within 20 days and then kept nearly constant till the end of the incubation. Bacterial numbers also showed a fast increase followed by a quick decrease within 30 days of incubation. In contrast to the fungi, the bacterial numbers started to increase again and reached the 2nd peak in 52 days when BaA showed about 20% degradation rate in T-AS, while more than 60% degradation rate was observed in T-CS. Accordingly, the increase of bacterial number in T-AS was smaller than that in T-CS. The 3rd peak of bacterial numbers appeared on days 113 and 140 for T-AS and T-CS, respectively, which contributed to strong microbial utilization of BaA and total 5-ring PAHs (BbF, BkF and BaP). This showed that the biodegradation of 2- to 4-ring PAHs was the result of collaboration of bacteria and fungi, while the metabolism of HMW PAHs could be primarily attributed to bacteria. Although the bacterial numbers in T-AS was always higher than that in T-CS, the degradation in the former was weaker than that in the latter. This indicated that the removal of pollutants depends more on the quality of the organisms rather than on their quantity.

3.3. Half-life of PAHs in TLPB

When PAHs residuals were plotted against incubation time, an exponential decay curve was found. So a first-order reaction model was used to describe the biodegradation behaviour of PAHs in TLPB [46]:

$$C = C_0 e^{-kt}$$

where C is the concentration of PAH, t is time (day) of incubation, C_0 is the initial concentration of PAH, k is the rate constant of the change of PAHs concentrations in TLPB (day^{-1}). Then half-life time ($t_{1/2}$) of PAHs is calculated as below:

$$t_{1/2} = \frac{\ln 2}{k}$$

Table 3
Overview of half-life values of PAHs in our study and previous studies.

PAHs	Regression equation		r^2		$t_{1/2}$ (d)		Half-life values in references			
	AS	CS	AS	CS	AS	CS	a	b	c	d
NAP	$C = 7.92e^{-0.73t}$	$Y = 7.92e^{-0.69t}$	0.99	0.99	1.0	1.0	0.2–110	15	<2	2.1–2.2
FLR	$C = 12.72e^{-0.061t}$	$Y = 12.44e^{-0.089t}$	0.95	0.97	11.4	7.8	1.4–262	28	>3.2	nr
ANT	$C = 11.84e^{-0.059t}$	$Y = 11.53e^{-0.084t}$	0.92	0.90	11.7	8.3	2.1–280	48	7.9	50–134
PHE	$C = 12.67e^{-0.13t}$	$Y = 12.15e^{-0.15t}$	0.83	0.88	5.3	4.6	1.3–255	14	5.7	16–35
PYR	$C = 13.09e^{-0.081t}$	$Y = 12.93e^{-0.11t}$	0.87	0.85	8.6	6.3	25–1840	51	8.5	199–260
FLN	$C = 13.05e^{-0.10t}$	$Y = 12.86e^{-0.14t}$	0.86	0.89	6.9	5.0	8.9–432	16	7.8	268–377
BaA	$C = 11.86e^{-0.0093t}$	$Y = 12.55e^{-0.0204t}$	0.97	0.98	74.5	34.0	40–2450	84	8.1	162–387
BbF	$C = 9.19e^{-0.0020t}$	$Y = 11.01e^{-0.011t}$	0.77	0.82	346.6	63.0	127–845	334	9.0	211–294
BkF	$C = 8.69e^{-0.0023t}$	$Y = 8.89e^{-0.0034t}$	0.71	0.89	301.4	201.9	98–2220	55	8.7	nr
BaP	$C = 11.09e^{-0.0048t}$	$Y = 11.44e^{-0.0072t}$	0.84	0.96	144.4	96.3	163–910	112	8.2	229–309
DBahA	$C = 12.82e^{-0.0010t}$	$Y = 12.14e^{-0.0013t}$	0.37	0.65	693.1	533.2	33–915	nr	nr	361–420

a, weeks [47]; b, days [50]; c, years [51]; d, days [52].

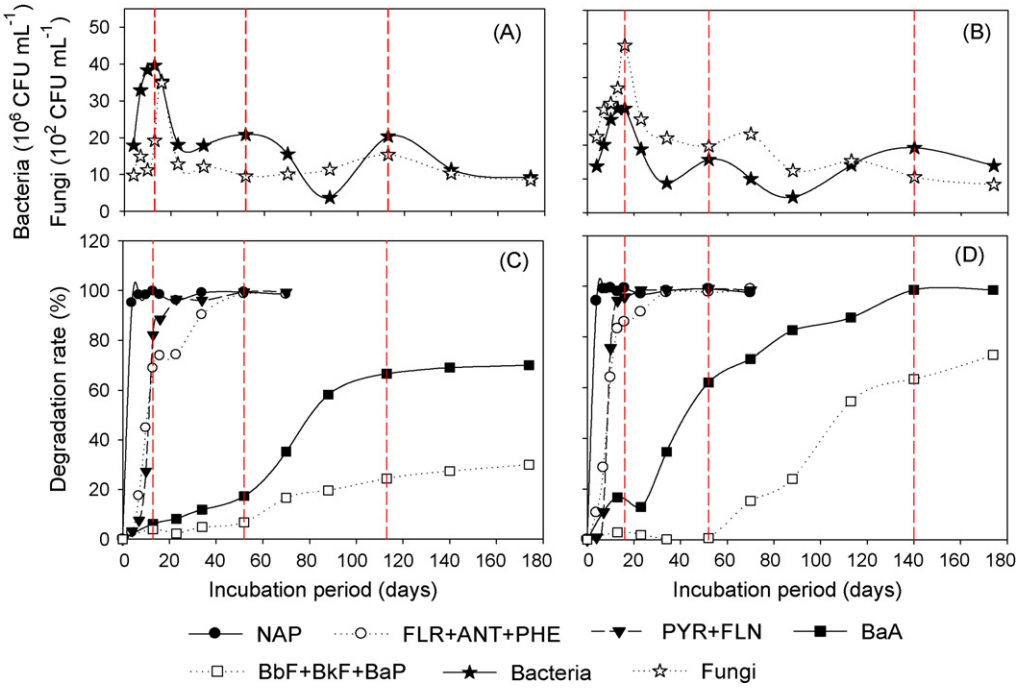

Fig. 4. The population of bacteria and fungi in TLPB with agricultural soil (A) and contaminated soil (B) and their correspondence to the degradation rates of 2- to 5-ring PAHs as function of incubation period in T-AS (TLPB with agricultural soil) (C) and T-CS (TLPB with contaminated soil) (D).

The nonlinear regression equation and the half-life values of PAHs in T-AS and T-CS are shown in Table 3. Generally, a larger value of r^2 suggested better fit for T-CS than T-AS.

The half-lives of PAHs ranged from 1 day for NAP to 693 days, and to 553 days for DBahA, in the treatments of T-AS and T-CS, respectively. However, longer half-life times than predicted by this model could have occurred, given the low values of r^2 for DBahA in both of T-AS and T-CS. The half-life values obtained in the treatment of T-AS were generally larger than those of T-CS, indicating that the biodegradation of PAHs in the T-CS was faster than that in T-AS, as aforementioned. The expected increase in half-life time with increasing molecular weight of PAHs was observed. As shown in Table 3, the half-life values of 5-ring PAHs were 10-fold higher than those of 2- to 4-ring PAHs. The half-lives for the LMW PAHs were in the order of NAP < PHE < FLN < PYR < FLR < ANT, showing that 3-ring FLR and ANT were more persistent than PHE, with 3 aromatic rings, as well as even 4-ring PYR and FLN. This is in line with results of previous studies in soils [47,48]. Thiele-Bruhn and Brümmer [47] found the highest residues to be those of ANT during the biodegradation of a mixture of 15 PAHs in soil. Bossert and Bartha [48] demonstrated that ANT was removed more slowly than PHE in soil due to the lower aqueous solubility of ANT. The relatively slow biodegradation of FLR could be explained by the fact that its molecular structure contains a nonaromatic ring structure, which resulted in slower degradation than in compounds with purely benzoid structures of similar molecular weight [49].

To our knowledge, this is the first report of the half-life values for so many PAHs in TLPB. So the half-lives of the PAHs in the present study were compared with other values in previous studies on soils (Table 3). Most half-life values of PAHs obtained in the TLPB were much lower than those reported in soils, which could be attributed to the improved mass transfer and bioavailability of PAHs by TLPB. It indicated that TLPB could optimize the microenvironment for microbes to make them maintain high levels of activity [20]. Thus, the microbes exhibited their utmost potential in degrading pollutants.

4. Conclusions

The biodegradation of most of the studied PAHs involving 2- to 5-rings occurred in TLPB with both soils, but the rates and extents of biodegradation in the contaminated soil were higher than those in the agricultural soil. The results indicated that the PAH-degrading bacteria might be ubiquitous in the environment and that biodegradability of the adapted microorganisms was better than that of unadapted ones. The sequential utilization of PAHs was observed suggesting a competitive-inhibition in a multi-substrate system, with microbial growth showing high correlation to the biodegradation of PAHs.

The shorter half-lives of PAHs in the TLPB relative to those reported for soils in literature, indicates that the microbial consortia in both contaminated soil and clean agricultural soil have a high potential and efficacy for the removal of PAHs—facilitated by TLPB. The results indicate that the natural capability of agricultural soils for remediation, and biological factors such as biodegradation potential, should be considered in soil pollution risk assessment.

Acknowledgments

This study was financially supported by the National High Technology Research and Development Program of China (No. 2007AA061101), the National Natural Science Foundation of China and Jiangsu (No. 40921061, No. 20707028 and No. BK2007264), and the Innovative project of Chinese Academy of Sciences (No. ISSASIP0714). Special thanks to Fredrick Orori Kengara of Chemistry Department, Maseno University in Kenya for his great help in language revision.

References

[1] M. Kästner, M. Breuer-Jammali, B. Mahro, Impact of inoculation protocols, salinity, and pH on the degradation of polycyclic aromatic hydrocarbons (PAHs) and survival of PAH-degrading bacteria introduced into soil, Appl. Environ. Microbiol. 64 (1998) 359–362.

[2] S.K. Samanta, O.V. Singh, R.K. Jain, Polycyclic aromatic hydrocarbons: environmental pollution and bioremediation, Trends Biotechnol. 20 (2002) 243–248.
[3] S.Y. Yuan, S.H. Wei, B.V. Chang, Biodegradation of polycyclic aromatic hydrocarbons by a mixed culture, Chemosphere 41 (2000) 1463–1468.
[4] M.A. Heitkamp, W. Franklin, C.E. Cerniglia, Microbial metabolism of polycyclic aromatic hydrocarbons: isolation and characterization of a pyrene-degrading bacterium, Appl. Environ. Microbiol. 54 (1988) 2549–2555.
[5] R.A. Kanaly, S. Harayama, Biodegradation of high-molecular-weight polycyclic aromatic hydrocarbons by bacteria, J. Bacteriol. 182 (2000) 2059–2067.
[6] L. Valentin, G. Feijoo, M.T. Moreira, J.M. Lema, Biodegradation of polycyclic aromatic hydrocarbons in forest and salt marsh soils by white-rot fungi, Int. Biodeterior. Biodegrad. 58 (2006) 15–21.
[7] S. Harayama, Polycyclic aromatic hydrocarbon bioremediation design, Curr. Opin. Biotechnol. 8 (1997) 268–273.
[8] J.B. Hughes, D.M. Beckles, S.D. Chandra, C.H. Ward, Utilization of bioremediation processes for the treatment of PAH-contaminated sediments, J. Ind. Microbiol. Biotechnol. 18 (1997) 152–160.
[9] C.E. Cerniglia, Biodegradation of polycyclic aromatic hydrocarbons, Curr. Opin. Biotechnol. 4 (1993) 331–338.
[10] K. Rehmann, H.P. Noll, C.E.W. Steinberg, A.A. Kettrup, Pyrene degradation by *Mycobacterium* sp. strain KR2, Chemosphere 36 (1998) 2977–2992.
[11] C.J. Carlstrom, O.H. Tuovinen, Mineralization of phenanthrene and fluoranthene in yardwaste compost, Environ. Pollut. 124 (2003) 81–91.
[12] M.P. Kolomytseva, D. Randazzo, B.P. Baskunov, A. Scozzafava, F. Briganti, L.A. Golovleva, Role of surfactants in optimizing fluorene assimilation and intermediate formation by Rhodococcus rhodochrous VKM B-2469, Bioresour. Technol. 100 (2009) 839–844.
[13] F. Volkering, A.M. Breure, A. Sterkenburg, J.G. Andel, Microbial degradation of polycyclic aromatic hydrocarbons: effect of substrate availability on bacterial growth kinetics, Appl. Microbiol. Biotechnol. 36 (1992) 548–552.
[14] J. Marcoux, E. Déziel, R. Villemur, F. Lépine, J.-G. Bisaillon, R. Beaudet, Optimization of high-molecular-weight polycyclic aromatic hydrocarbons' degradation in a two-liquid-phase bioreactor, J. Appl. Microbiol. 88 (2000) 655–662.
[15] D.R. Nielsen, A.J. Daugulis, P.J. Mclellan, A novel method of simulating oxygen mass transfer in two-phase partitioning bioreactors, Biotechnol. Bioeng. 83 (2003) 735–742.
[16] S. Aalam, A. Pauss, J.-M. Lebeault, High efficiency styrene biodegradation in a biphasic organic/water continuous reactor, Appl. Microbiol. Biotechnol. 39 (1993) 696–699.
[17] P. Osswald, P. Baveye, J.C. Block, Bacterial influence on partitioning rate during the biodegradation of styrene in a biphasic aqueous-organic system, Biodegradation 7 (1996) 297–302.
[18] M. Ascon-Cabrera, J.-M. Lebeault, Selection of xenobiotic-degrading microorganisms in a biphasic aqueous-organic system, Appl. Environ. Microbiol. 59 (1993) 1717–1724.
[19] E. Déziel, Y. Comeau, R. Villemur, Two-liquid-phase bioreactors for enhanced degradation of hydrophobic/toxic compounds, Biodegradation 10 (1999) 219–233.
[20] B. Guieysse, M.D.D.T.G. Cirne, B. Mattiasson, Microbial degradation of phenanthrene and pyrene in a two-liquid phase-partitioning bioreactor, Appl. Microbiol. Biotechnol. 56 (2001) 796–802.
[21] S. Mutnuri, N. Vasudevan, M. Kaestner, Degradation of anthracene and pyrene supplied by microcrystals and non-aqueous-phase liquids, Appl. Microbiol. Biotechnol. 67 (2005) 569–576.
[22] H. Gardin, J.M. Lebeault, A. Pauss, Biodegradation of xylene and butyl acetate using an aqueous-silicon oil two-phase system, Biodegradation 10 (1999) 193–200.
[23] K.D. Vandermeer, A.J. Daugulis, Enhanced degradation of a mixture of polycyclic aromatic hydrocarbons by a defined microbial consortium in a two-phase partitioning bioreactor, Biodegradation 18 (2007) 211–221.
[24] M.C. Romero, E.H. Reinoso, A.M. Kiernan, M.I. Urrutia, Chlorinated biphenyl degradation by wild yeasts pre-cultured in biphasic systems, Electron. J. Biotechnol. 9 (2006) 241–247.
[25] H. Zilouei, B. Guieysse, B. Mattiasson, Two-phase partitioning bioreactor for the biodegradation of high concentrations of pentachlorophenol using *Sphingobium chlorophenolicum* DSM 8671, Chemosphere 72 (2008) 1788–1794.
[26] M. Rodriguez, K. Thomas Klasson, B. Davison, Enhancement of the conversion of toluene by *Pseudomonas putida* F-1 using organic cosolvents, Appl. Biochem. Biotechnol. 91 (2001) 195–204.
[27] X. Li, P. Li, X. Lin, C. Zhang, Q. Li, Z. Gong, Biodegradation of aged polycyclic aromatic hydrocarbons (PAHs) by microbial consortia in soil and slurry phases, J. Hazard. Mater. 150 (2008) 21–26.
[28] A. Johnsen, U. Karlson, PAH degradation capacity of soil microbial communities—does it depend on PAH exposure? Microb. Ecol. 50 (2005) 488–495.
[29] L. Bastiaens, D. Springael, P. Wattiau, H. Harms, R. Dewachter, H. Verachtert, L. Diels, Isolation of adherent polycyclic aromatic hydrocarbon (PAH)-degrading bacteria using PAH-sorbing carriers, Appl. Environ. Microbiol. 66 (2000) 1834–1843.
[30] R. Villemur, E. Déziel, A. Benachenhou, J. Marcoux, E. Gauthier, F. Lépine, R. Beaudet, Y. Comeau, Two-liquid-phase slurry bioreactors to enhance the degradation of high-molecular-weight polycyclic aromatic hydrocarbons in soil, Biotechnol. Prog. 16 (2000) 966–972.
[31] C.-Q. Yin, X. Jiang, X.-L. Yang, Y.-R. Bian, F. Wang, Polycyclic aromatic hydrocarbons in soils in the vicinity of Nanjing, China, Chemosphere 73 (2008) 389–394.
[32] D. Mackay, W. Ying Shiu, K.-C. Ma, S.C. Lee, Handbook of Physical–Chemical Properties and Environmental Fate for Organic Chemicals, second ed., CRC Press Taylor & Francis Group, Boca Raton, 2006.
[33] A.L. Juhasz, R. Naidu, Bioremediation of high molecular weight polycyclic aromatic hydrocarbons: a review of the microbial degradation of benzo[a]pyrene, Int. Biodeterior. Biodegrad. 45 (2000) 57–88.
[34] P.S. Phale, A. Basu, P.D. Majhi, J. Deveryshetty, C. Vamsee-Krishna, R. Shrivastava, Metabolic diversity in bacterial degradation of aromatic compounds, Omics 11 (2007) 252–279.
[35] V. Andreoni, L. Cavalca, M.A. Rao, G. Nocerino, S. Bernasconi, E. Dell'Amico, M. Colombo, L. Gianfreda, Bacterial communities and enzyme activities of PAHs polluted soils, Chemosphere 57 (2004) 401–412.
[36] X. Dong, Q. Hong, L. He, X. Jiang, S. Li, Characterization of phenol-degrading bacterial strains isolated from natural soil, Int. Biodeterior. Biodegrad. 62 (2008) 257–262.
[37] H. Rehm, I. Reiff, Mechanisms and occurrence of microbial oxidation of long-chain alkanes, in: Reactors and Reactions, Springer-Verlag, Berlin/Heidelberg, 1981, pp. 175–215.
[38] R.M. Atlas, Petroleum biodegradation and oil spill bioremediation, Mar. Pollut. Bull. 31 (1995) 178–182.
[39] F. Wang, S. Grundmann, M. Schmid, U. Dörfler, S. Roherer, J.C. Munch, A. Hartmann, X. Jiang, R. Schroll, Isolation and characterization of 1,2,4-trichlorobenzene mineralizing *Bordetella* sp. and its bioremediation potential in soil, Chemosphere 67 (2007) 896–902.
[40] W. Stringfellow, M. Aitken, Competitive metabolism of naphthalene, methylnaphthalenes, and fluorene by phenanthrene-degrading pseudomonads, Appl. Environ. Microbiol. 61 (1995) 357–362.
[41] A. Desai, R. Autenrieth, P. Dimitriou-Christidis, T. McDonald, Biodegradation kinetics of select polycyclic aromatic hydrocarbon (PAH) mixtures by *Sphingomonas paucimobilis* EPA505, Biodegradation 19 (2008) 223–233.
[42] S.K. Lotfabad, M.R. Gray, Kinetics of biodegradation of mixtures of polycyclic aromatic hydrocarbons, Appl. Microbiol. Biotechnol. 60 (2002) 361–366.
[43] S.-H. Chen, M.D. Aitken, Salicylate stimulates the degradation of high-molecular weight polycyclic aromatic hydrocarbons by *Pseudomonas saccharophila* P15, Environ. Sci. Technol. 33 (1998) 435–439.
[44] K.F. Reardon, D.C. Mosteller, J.B. Rogers, N.M. DuTeau, K.-H. Kim, Biodegradation kinetics of aromatic hydrocarbon mixtures by pure and mixed bacterial cultures, Environ. Health Perspect. 110 (2002) 1005–1011.
[45] C.D. Knightes, C.A. Peters, Multisubstrate biodegradation kinetics for binary and complex mixtures of polycyclic aromatic hydrocarbons, Environ. Toxicol. Chem. 25 (2006) 1746–1756.
[46] P. Sukul, M. Spiteller, Influence of biotic and abiotic factors on dissipating metalaxyl in soil, Chemosphere 45 (2001) 941–947.
[47] S. Thiele-Bruhn, G.W. Brümmer, Kinetics of polycyclic aromatic hydrocarbon (PAH) degradation in long-term polluted soils during bioremediation, Plant Soil 275 (2005) 31–42.
[48] I.D. Bossert, R. Bartha, Structure-biodegradability relationships of polycyclic aromatic hydrocarbons in soil, Bull. Environ. Contam. Toxicol. 37 (1986) 490–495.
[49] C.D. Knightes, C.A. Peters, Aqueous phase biodegradation kinetics of 10 PAH compounds, Environ. Eng. Sci. 20 (2003) 207–218.
[50] S.R. Wild, K.C. Jones, Biological and abiotic losses of polynuclear aromatic hydrocarbons (PAHs) from soils freshly amended with sewage sludge, Environ. Toxicol. Chem. 12 (1993) 5–12.
[51] S.R. Wild, J.P. Obbard, C.I. Munn, M.L. Berrow, K.C. Jones, The long-term persistence of polynuclear aromatic hydrocarbons (PAHs) in an agricultural soil amended with metal-contaminated sewage sludges, Sci. Total Environ. 10 (1991) 235–253.
[52] K.S. Park, R.C. Sims, R.R. Dupont, W.J. Doucette, J.E. Matthews, Fate of PAH compounds in two soil types: influence of volatilization, abiotic loss and biological activity, Environ. Toxicol. Chem. 9 (1990) 187–195.