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ORIGINAL PAPER

Degradation of fluoranthene by a newly isolated strain of *Herbaspirillum chlorophenolicum* from activated sludge

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Abstract A fluoranthene-degrading bacterial strain FA1 was isolated from activated sludge and identified as *Herbaspirillum chlorophenolicum*, a newfound bacterial species that can grow well on fluoranthene as sole carbon and energy source. The kinetic characteristic of strain FA1 was tested in the aqueous model system (AMS) and the effects of nonionic surfactants on fluoranthene biodegradation in the AMS were then investigated. Tween 80 exhibited the best solubilization capacity for fluoranthene among three surfactants and its bioavailability decreased with an increase in its concentration and its degradation kinetics fit well with the first-order of power index model. The biotransformation of fluoranthene was greatly improved by Tween 80, and 58.5% fluoranthene degradation was obtained as Tween 80 was 100 mg/l. However, the bioavailability of fluoranthene decreased gradually with the increase of Tween 80 concentration. Bioremediation tests for fluoranthene in soil–water system were designed further to examine the degrading ability of strain FA1 with the presence of indigenous flora or not. The measurements showed that in the presence of indigenous flora, the optimum 30-day fluoranthene degradation in soil–water system reached 77.4%. Evidently, strain FA1 seems both efficient and high-effective and deserves further exploration on the enhanced bioremediation technologies for the treatment of fluoranthene-polluted soil.

Keywords Fluoranthene · *Herbaspirillum chlorophenolicum* · Biodegradation · Surfactants · Soil remediation

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Introduction

Fluoranthene is one of the US Environmental Protection Agency's 16 priority pollutant polycyclic aromatic hydrocarbons (PAHs), and can be found in many combustion products, along with other PAHs. Due to the clear or potential carcinogenic, teratogenic and mutagenic effects on animal and human cells (Rybicki et al. 2006; Brody et al. 2007), the fate of PAHs is of great scientific interest and has become a pressing global issue. Fluoranthene has a five-membered ring in its molecular structure which is similar to dioxin and dibenzofuran. Therefore, it has often been taken as a model compound for research on the bioremediation of high-molecular-weight PAHs (HMW PAHs) (Weissenfels et al. 1990; Willumsen and Arvin 1999; Gordon and Dobson 2001; Fuchedzhieva et al. 2008).

PAHs have the characteristics of low aqueous solubility and high affinity to soil, which result in slow intrinsic degradation and continuous accumulation of PAHs in nature. These importunate characteristics of PAHs cause persistent pollution in the environments. Meanwhile, they reduce the efficiency of the application of bioremediation technology (Mackay and Cherry 1989). Degradation of PAHs has been proved to be enhanced by inoculation with PAHs-degrading bacterial strains (TrzesickaMlynarz and Ward 1996; Juhasz and Naidu 2000; Pathak et al. 2009), especially when the site has been polluted by poorly degradable HMW PAHs at high concentrations or the site has only recently been contaminated. To improve the bioavailability of PAHs, several kinds of surfactants have been applied in the biotreatment to investigate their potential ability in facilitating the PAHs biodegradation in recent years (Kim et al. 2001; Collina et al. 2007; Jin et al. 2007; Seo and Bishop 2007). Some studies showed that the surfactants could significantly enhance the apparent solubility of PAHs via micelle solubilization, and then improve the bioavailability of PAHs into microorganisms (Edwards et al. 1994; Sun et al. 1995; Yu et al. 2007), while others reported that excessive addition of surfactants might inhibit the biodegradation of PAHs (Doong and Lei 2003; Jin et al. 2007; Li and Chen 2009). Most of earlier researches focus on the distribution of surfactants and PAHs in soil or water systems, and the biodegradation of PAHs with the presence of surfactants, etc. However, during the application of surfactant enhanced bioremediation (SEBR) technology, the biodegradability of surfactants should be taken into consideration in addition to their solubilization capacity and effects on PAHs degradation. Moreover, with proper degradation rates of surfactants in the remediation system, the substrate could be released gradually to the aqueous phase and became more available to the strains, which in turn enhanced the biodegradability of substrate (Li and Chen 2009).

The in situ bioremediation of soil environment is a multi-phase environment system including soil matrix and water, while the predation in natural environment seems to inhibit the effects and efficiency of introduced PAHs degrading bacteria (Harms and Bosma 1997). Hence, when the preliminary information is acquired on the characterization of isolated PAHs degrading strains, further degradation tests for PAHs should be performed to evaluate the degradation behavior and efficiency of the strains in soil–water system with the presence of indigenous bacteria.

In this paper, a pure fluoranthene degrading strain FA1 was isolated from the activated sludge of wastewater treatment plant, and identified through 16S rDNA sequence analysis. A 30-day degradation kinetics test was designed to preliminarily investigate the degrading potential of strain FA1 and a series of batch experiments were carried out to investigate the effects of three nonionic surfactants on the biodegradation of fluoranthene in the AMS. And then, bioremediation tests for fluoranthene in soil–water system were designed and the optimum conditions were determined by orthogonal test. The experimental results in this study should be the basis of further exploration on the treatment of fluoranthene-polluted soil using enhanced bioremediation technologies.

Materials and methods

Medium

The modified mineral salt medium (MMSM) used for the growth of the microorganisms and degradation contained (l^{-1}) (100 ml phosphate buffer (pH 7.0), 0.0675 g $MgSO_4$, 0.0364 g $CaCl_2$, 2.5 $\times$ 10^{-4} g $FeCl_3$, 1.0 ml microelement solution [Lei et al. 2004) (Mn_2SO_4 39.9 mg, $ZnSO_4{\cdot}H_2O$ 42.8 mg, $(NH_4)Mo_7O_{24}{\cdot}4H_2O$ 34.7 mg), per liter] and the pH was adjusted to 7.0. Agar was added at 2.0% when necessary.

Sampling and isolation of fluoranthene-degrading bacterial strains

The potential fluoranthene-degrading strain used in this paper was isolated from the activated sludge of wastewater treatment plant, Yangzi petrochemical company in Nanjing, China. The samples were acclimated in the MMSM with the fluoranthene concentration of 10 mg/l and incubated under shaking condition at 120 rpm at 30°C for a week. Then the spread-plate method was applied to select pure strains. Repetitive transfers were performed with fresh medium containing fluoranthene until homogenous colonies were observed on the plate. A pure culture of strain FA1 was observed after 21 days of incubation. Purity was confirmed by microscopic examination of the colonies and morphological

characterization test was done. Strain FA1 was preserved in enriched solid medium at 4°C or in aqueous MMSM containing 20% sterilized glycerol at −80°C. The inocula for degradation experiment were prepared with a cell density of 7.2×10^9 CFU/ml for further use (Willumsen and Karlson 1998).

The degradation capability of strain FA1 for fluoranthene at different temperature or pH was tested. As a result, 30°C and pH 8.0 were proved to be high-effective and used in the following biodegradation tests. And then the kinetics of fluoranthene degradation in the AMS was investigated using batch systems. The experiment was carried out in 25-ml tubes enclosed with eight sheets of sterile gauze, each of which contained 9.0 ml of MMSM and 1.0 ml bacteria inocula with an initial fluoranthene concentration of 20 mg/l. Samples were then agitated in the rotary shaker at 120 rpm and harvested at periodic intervals to analyze the residual fluoranthene.

16S rDNA sequence analysis of fluoranthene-degrading strain

Genomic DNA was extracted from the strain culture (Lu et al. 2003) and 1 μl of which was used as template in PCR reaction (50 μl), which consisted of 5 μl 10× PCR buffer, 4 μl 2.5 mM of dNTP, 3 μl 25 mM $MgCl_2$, 0.25 μl rTaq polymerase and 0.5 μl of each specific primers 27F and 1492R, and 35.75 μl sterile deionized water. Amplification cycles (30 cycles in total) consisting of initial denaturation step at 94°C for 5 min, followed by a 30 s denaturation step at 94°C, a 45 s annealing step at 55°C, and 1 min elongation step at 72°C followed by a final extension step at 72°C for 10 min were performed (Biorad Pt-200, USA). The obtained 16S rDNA sequence was aligned to sequences in GenBank using the BLAST program (Version 2.2.22) (Zhang et al. 2000). The phylogenetic tree was produced using MEGA (Version 4.1). The accession number of the 16S rDNA in GenBank is HM107777.

Effects of nonionic surfactants on fluoranthene biodegradation in the AMS

Solubilization test

The characteristics of nonionic surfactants (Chen et al. 2006) selected in this solubilization test, Tween 20, Tween 80 and Triton X-100, are given in Table 1.

Table 1 Properties of nonionic surfactants

Surfactants	Molecular formula	Molecular weights (g/mol)	HLB	CMC (mg/l)
Tween 20	$C_{58}H_{124}O_{26}$	1,226	16.7	60
Tween 80	$C_{64}H_{124}O_{26}$	1,310	15.0	13–15
Triton X-100	$C_{33}H_{60}O_{10.5}$	625	18.7	144

HLB hydrophile-lipophile balance

The apparent solubility experiments in aqueous environment with the presence of a series of surfactants solutions (100, 250, 500, 1,000 and 2,000 mg/l) were carried out according to the method of Yang et al. (2003). Surfactant with the best solubilization ability was chosen to investigate its biodegradability and effects on fluoranthene degradation.

Biodegradation of Tween 80

Biodegradation of different concentrations (100, 250, 600 mg/l) of Tween 80 by strain FA1 were performed in 500 ml Erlenmeyer flasks containing 200 ml of MMSM. Inocula of strain FA1 were amended into the mineral solution to have a cell density of 7.2×10^8 CFU/ml. Subsequently, the flasks were cultured in rotary shaker at 120 rpm at 30°C. Samples were periodically taken from the flasks to determine the surfactant concentrations.

Effects of Tween 80 on the fluoranthene degradation

Effects of Tween 80 (100, 250, 600 mg/l) on fluoranthene degradation in the AMS were inspected using continuously batch experiments. The cultures were carried out in 25-ml centrifuge tubes by adding different amount of Tween 80 to the MMSM as stated above, with fluoranthene concentration equal to 20 mg/l. Strain FA1 was inoculated to the solution to have a cell density of 7.2×10^8 CFU/ml. The samples were then agitated on the rotary shaker at 120 rpm at 30°C, and periodically picked up for the HPLC analysis of residual fluoranthene.

Bioremediation tests for fluoranthene in soil–water system

Soil preparation

Surface soil samples (5–20 cm) were collected from the campus of Nanjing University and contained no

detectable 16 PAHs (US EPA). After natural drying, soils were crushed and sieved through a 1 mm pore size sieve for further use. The soil contained 0.011% nitrogen, 0.042% phosphate and a total organic carbon content of 0.12%. The pH of soil was 7.7 and the moisture content was 2.7% (dry weight).

The sterilized soil was prepared by autoclaving at 121°C for 1 h, and no microbial growth was detected after incubating the soil in LB medium. The artificial contaminated soil was prepared by adding fluoranthene–methanol solution with appropriate concentrations, and uniformly mixed. The soil was aged in dark at 4°C for 1 week before bioremediation experiment and then placed in the fume hood until methanol completely volatilized.

Biodegradation of fluoranthene in soil–water system

The fluoranthene bioremediation experiments in soil–water system were performed in Erlenmeyer flasks. 5.0 g soil with an initial fluoranthene concentration of 20 mg/kg soil was added into the flasks and inoculated with strain FA1 to have a cell density of 7.2×10^7 CFU/g soil. MMSM were then added to obtain a soil–water ratio of 1:3 (Doick and Semple 2003). The final pH of the mixture was adjusted to 8.0. Three different kinds of soil–water system were investigated: (1) soil without sterilization was amended with fluoranthene, and no inocula were added; (2) soil was sterilized before adding target pollutant and inoculated with strain FA1; (3) soil without sterilization was added with fluoranthene and inoculated with strain FA1. The control samples were prepared by adding fluoranthene to sterile soil and using sterile distilled water instead of inocula. Samples were thereafter incubated at constant temperature of 30°C, thoroughly mixed and watered every other day to maintain a constant water level. There were two replicates for each sample. And the fluoranthene concentrations were determined periodically.

Optimization of fluoranthene degradation conditions

Orthogonal test of six factors with three levels was used to investigate the effects of biotic and abiotic factors on fluoranthene bioremediation in the soil–water environment with the presence of indigenous bacteria. The selected six factors were initial fluoranthene concentration, soil–water ratio, salinity (total amount of nitrogen and phosphate), inocula, surfactant concentration and nitrogen–phosphorus ratio, respectively. The levels of the factors are shown in Table 2. The test was performed according to the design (Table 3), and a total number of 18 experimental combinations were carried out at 30°C and pH 8.0. Samples were mixed uniformly and watered every 2 days to keep a constant water level. The fluoranthene degradation was measured after 1 month of culture.

The optimal levels of the factors were determined by analysis of the orthogonal experiment results, and were then tested for validation of the orthogonal design.

Analytical procedures

Fluoranthene extract and analysis

Samples were acidified with hydrochloric acid, and extracted by ultrasonic for 20 min with dichloromethane as the solvent for three successive extractions (Ping et al. 2007). For fluoranthene in soil–water system, acidified samples amended with dichloromethane were placed in a rotary shaker at 150 rpm for 1 h after standing for 2 h and then extracted for 1 h by ultrasonic. The extracted solution was collected by centrifugation at 2,770×*g* for 20 min. The extract was dried by anhydrous sodium sulfate, evaporated in vacuum at 30°C, and then diluted with methanol to 10 ml and filtered through 0.22 μm syringe filter for HPLC analysis. The HPLC conditions were HPEclipse XDB-C8 column (5 μm, 4.6 × 150 mm), methanol–water (80:20, v/v), flow rate of 1.0 ml/min,

Table 2 Levels of factors tested in the orthogonal experiment

Levels	Fluoranthene (mg/kg soil) A	Soil–water ratio B	Salinity (%) C	Inocula (ml/kg soil) D	Tween 80 (mg/kg soil) E	N:P F
1	10	1:1	8	1	250	5:1
2	5	1:3	5	3	0	10:1
3	20	1:5	2	2	100	2:1

Table 3 Orthogonal experiment results

Run	Factors and levels						Fluoranthene degradation[a] (%)
	Initial fluoranthene concentration (A)	Soil–water ratio (B)	Salinity (C)	Inocula (D)	Tween 80 concentration (E)	N:P(F)	
1	1	1	1	1	1	1	61.9 ± 1.03
2	1	2	2	2	2	2	65.4 ± 1.16
3	1	3	3	3	3	3	67.0 ± 1.49
4	2	1	1	2	2	3	65.8 ± 1.53
5	2	2	2	3	3	1	63.9 ± 1.42
6	2	3	3	1	1	2	74.1 ± 0.25
7	3	1	2	1	3	2	67.8 ± 0.67
8	3	2	3	2	1	3	59.9 ± 1.02
9	3	3	1	3	2	1	52.8 ± 0.63
10	1	1	3	3	2	2	66.5 ± 1.58
11	1	2	1	1	3	3	61.8 ± 0.18
12	1	3	2	2	1	1	61.5 ± 0.02
13	2	1	2	3	1	3	63.2 ± 1.00
14	2	2	3	1	2	1	75.3 ± 0.69
15	2	3	1	2	3	2	63.2 ± 0.06
16	3	1	3	2	3	1	70.2 ± 0.15
17	3	2	1	3	1	2	61.3 ± 0.25
18	3	3	2	1	2	3	54.6 ± 0.80
Mean 1	0.616	0.635	0.611	0.659	0.637	0.643	
Mean 2	0.676	0.646	0.627	0.644	0.610	0.640	
Mean 3	0.611	0.622	0.664	0.601	0.657	0.621	
Range	0.065	0.024	0.053	0.058	0.047	0.022	

[a] Significance analysis between every two of the 18 Runs has been done ($p \leq 0.05$)

detection wavelength of 235 nm, and column temperature of 40°C.

When calculating the degradation, the fluoranthene removal due to biosorption could be neglected in that fluoranthene adsorbed to biomass could be extracted during the ultrasonic extraction process (Chan et al. 2006; Chen et al. 2010). The mean values of extraction efficiency for fluoranthene in AMS and soil–water system were (94.80 ± 1.08) and (84.60 ± 1.81)%, respectively.

TOC measurement of bacteria and surfactant concentration

A total organic carbon (TOC) analyzer (IL 550, Lachat, USA) was used to determine the concentrations of Tween 80 and biomass in the AMS. Samples were centrifugated at 11,672×*g* for 15 min to remove bacteria cells, and then analyzed by TOC. The biomass concentration was calculated by subtracting the TOC of Tween 80 from the TOC of the uncentrifugated sample containing both Tween 80 and bacteria.

Statistical analysis

Data was statistically analyzed by analysis of variance (ANOVA) and the mean differences were compared by Tukey's multiple-comparison test at $p \leq 0.05$ (SPSS 13.0, Chicago, IL, USA).

Results

Isolation of fluoranthene-degrading strain FA1

Strain FA1, capable of using fluoranthene as sole carbon source, was obtained after enrichment of

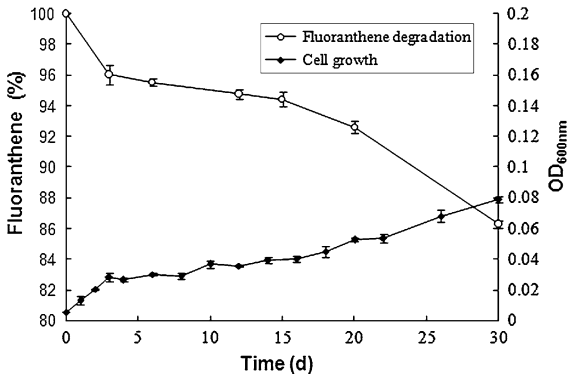

Fig. 1 Kinetics of fluoranthene degradation by strain FA1

activate sludge samples. Morphological characterization test showed the selected strain was Gram negative and rod shaped. The 30-day degradation kinetics for fluoranthene (20 mg/l) in the AMS by strain FA1 at 30°C and pH 8.0 was shown in Fig. 1. In the first days fluoranthene was degraded rapidly. Five days afterwards, the fluoranthene degradation kept almost constant, and until 15 days, the fluoranthene degradation became fast again. However, the degradation velocity in the later period was a bit lower than that in the beginning period.

16S rDNA sequence analysis of strain FA1

According to the BLAST analysis of complete 16S rDNA sequence of strain FA1, FA1 clustered in a phylogenetic branch that contained *Herbaspirillum* species, which was the first discovery that this bacterial species can degrade fluoranthene. Strain FA1 showed a high degree of similarity (99%) to the complete sequence of *Herbaspirillum chlorophenolicum* strain CPW301 16S ribosomal RNA (NCBI Accession No. NR024804.1). A phylogenetic tree based on related *Herbaspirillum* species is shown in Fig. 2. The new fluoranthene-degrading strain FA1 was then referred to as *Herbaspirillum chlorophenolicum* strain FA1 (*H. chlorophenolicum* FA1).

Solubilization of nonionic surfactants for fluoranthene

The solubilization test showed that, the apparent solubility of fluoranthene was linearly proportional to the concentration of surfactants in the range of 0.1–2.0 g/l and the effect of solubilization enhancement of three nonionic surfactants was Tween

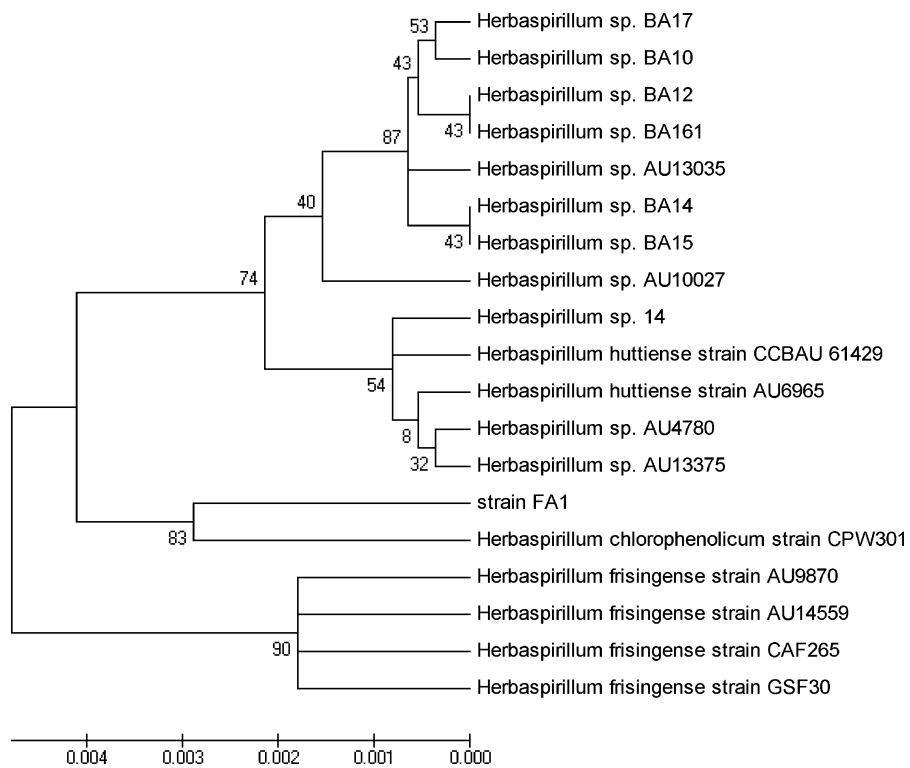

Fig. 2 Phylogenetic tree derived from 16S rDNA sequence of strain FA1 (The tree was evaluated by bootstrap analysis of the neighbor-joining method with 1,000 resamplings using MEGA 4.1)

80 > Tween 20 > Triton X-100. Surfactants of lower hydrophile-lipophile balance (HLB) (Tween 80 < Tween 20 < Triton X-100) (Table 1) resulted in higher solubility of fluoranthene, which implied that the solubility of HOCs by surfactants were related to the molecular structure of micelle formed above the critical micelle concentration (CMC). And Tween 80 was selected for further study for its great solubilization potential for fluoranthene.

Effects of nonionic surfactants Tween 80 on the degradation of fluoranthene

Biodegradation of Tween 80

With the increasing environmental interest, biodegradable surfactants are preferred in bioremediation of PAHs. The biodegradability of Tween 80, ranging from 100 to 600 mg/l, was investigated before being used to stimulate the biodegradation of fluoranthene. The experiment was conducted with Tween 80 being the sole carbon source available to *H. chlorophenolicum* FA1. The degradation kinetics and cell growth are presented in Fig. 3. During the degradation, cell density of *H. chlorophenolicum* FA1 increased with an increase of initial Tween 80 concentration. It was observed that the degradation data of Tween 80 fit well to the exponential first-order reaction kinetics model (Shen 2002):

$$-\frac{dS}{dt} = kS^1 = kS \quad (1)$$

where S is the concentration of substrate in TOC (mg carbon/l); k is the first-order reaction constant (h^{-1}).

It is noteworthy to point out that, the application of Eq. 1 is based on the assumption that the biodegradation of substrate with long-chain molecules could be described without regard to microbial growth (Shen 2002).

Effect of Tween 80 on fluoranthene biodegradation in AMS

The effects of different concentrations of Tween 80 on fluoranthene (20 mg/l) biodegradation in the AMS were tested. As shown in Fig. 4, the fluoranthene degradation was greatly promoted by Tween 80 in the range of 100–600 mg/l due to the enhanced apparent solubility, and the 30-day degradation increased to 58.5% when Tween 80 was equal to 100 mg/l. However, the degradation of fluoranthene decreased with a further rise in Tween 80 concentration.

Furthermore, the fluoranthene degradation increased dramatically during the period of 12–15 days when the concentration of surfactant initially present was

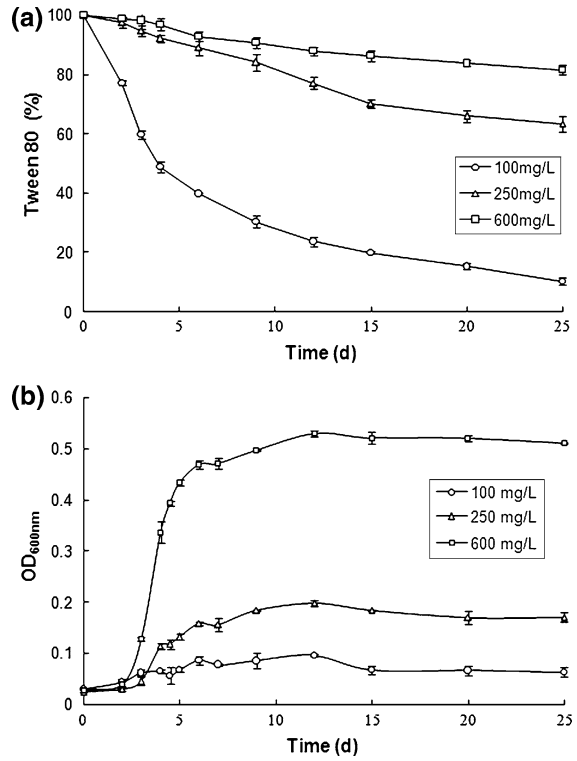

Fig. 3 Effect of Tween 80 concentrations on its biodegradation kinetics and bacterial growth

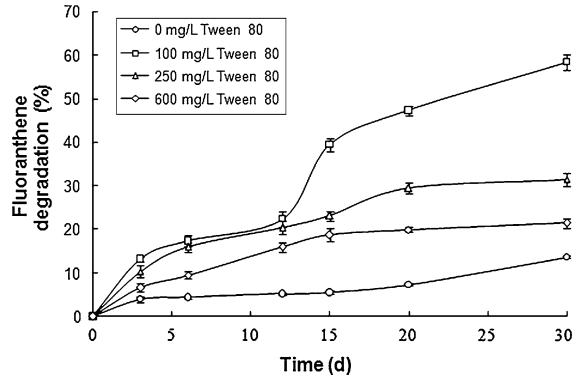

Fig. 4 Effect of different Tween 80 concentrations on biodegradation of fluoranthene

100 mg/l. This particular period was often referred as the structure-breaking point by Li and Chen (2009). During this period, Tween 80 molecules were broken down to small moieties before mineralization, and the bioavailability of micelle-phase dissolved fluoranthene increased, which resulted significant increase in the degradation.

Fluoranthene degradation by *Herbaspirillum chlorophenolicum* strain FA1 in soil–water system

Fluoranthene degradation in soil–water system

The application of bioremediation technology usually involves two-phase medium of soil/sediment–water. Thus, the remediation of fluoranthene by *H. chlorophenolicum* FA1 in soil–water systems was simulated in vitro to estimate its growth and degradation potential with the presence of indigenous flora. As shown in Fig. 5, in soil–water systems with nonsterile soil and without inocula (set 1), 9.34% of fluoranthene was removed at 15 days, which demonstrated that the indigenous microflora could degrade fluoranthene to a certain degree after adapting to the environment. In systems with sterile soil, where bacterium FA1 acted as the dominant species, the fluoranthene degradation was higher than the other two systems during the whole period. While in set 3, the 15-day degradation was decreased by nearly 10.0% comparing to set 2.

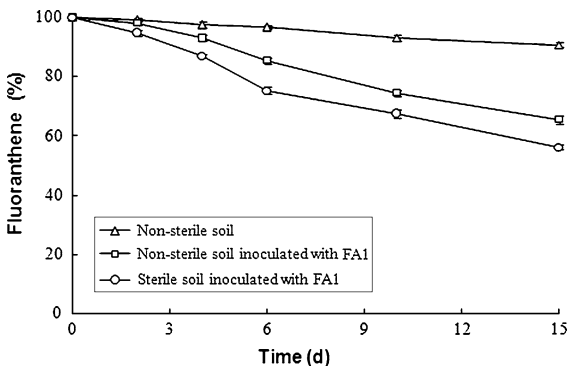

Fig. 5 Fluoranthene degradation by *Herbaspirillum chlorophenolicum* strain FA1 in different simulated soil–water system

Optimization of fluoranthene bioremediation in soil–water systems

The laboratory biodegradation experiment is usually conducted under abundant organic carbon and nutrient source, and proper pH conditions, the inocula are in dominant control. While in in situ remediation, there would be biological and non-biological disadvantages for the inoculated bacteria to overcome to maintain their enzyme activity and biodegradation ability. Hence, an orthogonal test of six factors with three levels was performed to analyze their effects on fluoranthene degradation in soil–water system.

The results of orthogonal test are presented in Table 3. Statistical analysis of every two of the 18 runs has been performed. The results showed no significant differences between every two degradation data among Group (61.9, 61.8, 61.5, 61.3) or (65.4, 67.0, 65.8, 66.5). And differences of all the other every two of the 18 runs were significant ($p \leq 0.05$). The influence of factors on fluoranthene degradation was determined by range analysis, and it was established that fluoranthene concentration (A) was the most significant factor, following by inocula (D), salinity (C), surfactant concentration (E), soil–water ratio (B) and nitrogen–phosphate ratio (F). According to the results calculated by direct analysis, the optimum level combination was A2D1C3E3B2F1.

To validate the efficient of orthogonal design, the fluoranthene bioremediation in soil–water system was carried out with the optimized levels: fluoranthene concentration of 5 mg/kg soil, soil–water ratio of 1:3, salinity of 2%, inocula of 1 ml/kg soil, Tween 80 concentration of 100 mg/kg soil and N:P of 5:1. An enhanced fluoranthene degradation of 77.4% was obtained after being cultured for 30 days, which demonstrated the validity of the orthogonal design.

Discussion

The concentration and distribution of PAHs in the environments has been studied extensively. It was reported by Wilcke (2000) that the concentration of fluoranthene in temperate topsoils ranged from 3.0 to 14,200 μg/kg. Ma et al. (2009) studied the distribution of the 16 EPA priority PAHs in the green-belt soils in Shanghai, China. They found that the concentration of fluoranthene was (19–1,940) μg/kg,

and the 4–6 ring PAHs including fluoranthene accounted for about 90% of the total PAHs. Up to now, several types of bacteria have been reported to be able to degrade fluoranthene, such as *Bacillus cereus* (Fuchedzhieva et al. 2008), *Pseudomonas alcaligenes* PA-10 (Hickey et al. 2007), *Sphingomonas paucimobilis* (Willumsen and Arvin 1999) and *Alcaligenes denitrificans* (Weissenfels et al. 1990). In the present research, *H. chlorophenolicum* strain FA1, a new fluoranthene-degrading bacterial strain was isolated and identified. The 30-day degradation of fluoranthene (20 mg/l) by strain FA1 in the AMS was 13.7%, and it increased to 58.5% when Tween 80 was equal to 100 mg/l. This preliminarily implies the degradation potential of strain *H. chlorophenolicum* FA1.

Surfactants have been proved efficient in increasing the bioavailability of HOCs (Kim et al. 2001; Yu et al. 2007). The solubilization of HOCs by surfactants in AMS is determined by the micelle formation and the corresponding partition coefficients (Edwards et al. 1994; Sun et al. 1995). When the surfactant concentration was above the CMC, the micelle formed with the hydrophilic polar groups towards outside and the hydrophobic non-polar interiors. The HOCs was then solubilized in the hydrophobic moieties and the apparent solubility increased linearly as the micelle concentration increased. Apart from economical reason, nonionic surfactants were applied in this study for their effectiveness of solubilizing PAHs, low toxicity to the PAHs-degrading strains, and successful applications in previous studies (Willumsen and Karlson 1998; Willumsen and Arvin 1999; Prak and Pritchard 2002; Doong and Lei 2003). Tween 80 showed the greatest solubilization capacity on fluoranthene among three nonionic surfactants. As the concentration was 250 mg/l, the biodegradability of Tween 80 decreased significantly. This indicates that the increase concentration of surfactant may also inhibit the biodegradation. The retardation may be not caused by the toxicity of surfactant because the bacteria could survive in solutions with surfactant concentration up to 2.0 g/l (data not shown). Figure 3b shows that the bacterial growth was not inhibited within the concentration range of Tween 80. The inhibition may be attributed to the more compact molecular structure of micelle at high concentration which reduces the contact between micelle and bacteria. Similar inhibition effect of high concentration on the biodegradability of surfactant was also reported by Li and Chen (2009).

For PAHs like fluoranthene, there are two patterns of cells utilizing the substrate: (1) direct contact with the substrate; (2) utilization of substrate in dissolved phase, including molecules in both the aqueous phase and micelle phase. The latter has been reported to be the major way in terms of solid organic compounds (Maier et al. 2004). The more packed and constructed micelle formed at high surfactant concentration would limit the material transfer and utilization of micelle-phase substrate, and consequently reduce the degradation of substrate (Jin et al. 2007; Li and Chen 2009). It was also well verified in this work by the change in fluoranthene biodegradability in the AMS with addition of Tween 80 (Fig. 4). Guha and Jaffe (1996) reported that the only a fraction of the phenanthrene partitioned into the micelle of Triton N101 was directly bioavailable, and the bioavailable fraction f ($0 < f < 1$) decreased as the concentration of micelles increased. Moreover, the most common initial transform of PAHs degradation is aromatic ring hydroxylation, and *cis*-dihydrodiol is known to be the initial metabolite of fluoranthene (Maier et al. 2004). The initial metabolites are still relatively hydrophobic and might also be solubilized in the surfactant micelle. The bioavailability of initial metabolites dissolved in micelle phase would decrease at high surfactant concentration and contribute to the inhibition (Willumsen and Arvin 1999).

There has been considerable debate over the efficiency of bioaugmentation (Jorgensen et al. 2000; Li et al. 2005; Pathak et al. 2009). In system with the presence of both inocula and indigenous bacteria, there would be competition for carbon source and nitrogen source like nitrogen and phosphate, etc., and the activity of inocula might also be inhibited because of phagocytosis. The simulated bioremediation of fluoranthene in soil–water system (Fig. 5) manifested the colonizing and degradation capacity of *H. chlorophenolicum* FA1 along with competition with indigenous flora, which was comparable to some other bacterial isolates (TrzesickaMlynarz and Ward 1996; Tam and Wong 2008; Wang et al. 2010). Therefore, the isolated strain FA1 could be applied for further exploration.

According to the results of orthogonal test, the initial fluoranthene concentration (A) in the soil–water system was the most significant factor. By comparison, salinity (C) and inocula (D) had minor

effects on fluoranthene degradation and their optimum values were the lowest of the designed three levels. In the present study, the optimum cell density was 3.6×10^7 CFU/kg soil, while the inocula size in previous studies ranged from 2.1×10^6 to 2×10^9 CFU/ml (Kelley and Cerniglia 1991; Willumsen and Karlson 1998; Kim and Weber 2005; Tam and Wong 2008). Generally, the cell density in system after inoculation should achieve 10^6 CFU/ml or 10^6 CFU/g to maintain a certain amount of inoculated bacteria during the degradation process (Shen 2002). With the presence of Tween 80, the apparent solubility of fluoranthene increased and the original adsorbed fluoranthene was released into the solution, and thereby increased its bioavailability. However, PAHs degradation will be inhibited at high concentration of surfactant in that surfactant is adsorbed to soil and unavailable to bacteria (Guha and Jaffe 1996), and the PAHs dissolved in which is thereby unavailable either. In addition, the more structured and packed micelles will also inhibit the PAHs degradation. Soil–water ratio and N:P had the minimum effect on fluoranthene degradation, whose optimum levels were determined to be 1:3 and 5:1, respectively. Doick and Semple (2003) reported that the mineralization rate of phenanthrene reached its most when the soil water ratio was between 1:2 and 1:5. The optimum N:P ratio was close to its value in cell, which showed that the nutrient supply should be in accordance with the composition of cell elements.

In recent years, the soil remediation for PAHs has become an issue of global concern, including fluoranthene. Most significant for this study, a bacterial strain FA1, belong to *H. chlorophenolicum*, was firstly isolated and discovered to be capable of growing well on fluoranthene as sole carbon and energy source. With the presence of surfactant Tween 80 and the optimized degradation conditions, strain FA1 revealed both efficient and high-effective degradation ability for fluoranthene. To some degree, this work offered a feasibility prediction for the in situ bioremediation of fluoranthene-polluted soil by the newly isolated bacterial strain FA1. Next, the practical and applied study and exploration should be necessary as FA1 performed well in the soil tested, *H. chlorophenolicum* FA1 needs to be tested in other soils, in the presence of mixtures of PAHs and under conditions found at contaminated sites.

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