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Experiments and modelling of phenanthrene biodegradation in the aqueous phase by a mixed culture

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Abstract: Pollution by polycyclic aromatic hydrocarbons(PAHs) is widespread due to unsuitable disposal of industrial waste. They are mostly defined as priority pollutants by environmental protection authorities worldwide. Phenanthrene, a typical PAH, was selected as the target in this paper. The PAH-degrading mixed culture, named ZM, was collected from a petroleum contaminated river bed. This culture was injected into phenanthrene solutions at different concentrations to quantify the biodegradation process. Results show near-complete removal of phenanthrene in three days of biodegradation if the initial phenanthrene concentration is low. When the initial concentration is high, the removal rate is increased but 20%—40% of the phenanthrene remains at the end of the experiment. The biomass shows a peak on the third day due to the combined effects of microbial growth and decay. Another peak is evident for cases with a high initial concentration, possibly due to production of an intermediate metabolite. The pH generally decreased during biodegradation because of the production of organic acid. Two phenomenological models were designed to simulate the phenanthrene biodegradation and biomass growth. A relatively simple model that does not consider the intermediate metabolite and its inhibition of phenanthrene biodegradation cannot fit the observed data. A modified Monod model that considered an intermediate metabolite (organic acid) and its inhibiting reversal effect reasonably depicts the experimental results.

Keywords: biodegradation model; phenanthrene; microbial growth and decay; Monod growth model

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are composed of two or more fused aromatic rings. They are known to possess genotoxic, mutagenic and carcinogenic properties. The U. S. Environmental Protection Agency(USEPA) has defined 16 typical PAHs as priority pollutants (Kanaly and Harayama, 2000). Many industrialised areas have been highly polluted by PAHs, which are frequently produced by combustion of organic matter, the petroleum industry, coal refining and motor vehicle exhaust fumes.

Phenanthrene, a three-ring PAH, is moderately toxic (Wodzinski and Coyle, 1974). It can enter the body by ingestion, inhalation or skin absorption. It is known to be a human skin photosensitiser and mild allergen, and is mutagenic to the human microbial system under specialised conditions. It has also been found to be an inducer of sister chromatid exchanges and a potential inhibitor of gap-junctional intercellular communication. To protect freshwater aquatic life, the permissible concentration of phenanthrene in water is only 0.28 mg/L due to its toxicity. As phenanthrene contains bay-regions and K-regions (Fig.1), it is also used as a model substrate for the studies on the metabolism of bay-and K-regioncontaining carcinogenic PAHs such as benzo[a]pyrene, benzo[a]anthracene and chrysene.

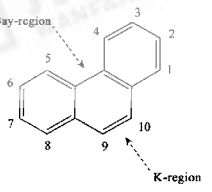

Fig.1 Bay-region and K-region of phenanthrene

PAHs in contaminated soils must be treated to avoid any possible hazard to human health. Chemical, physical and biological methods have been utilized to remediate PAH-contaminated soils. The principle processes for their successful removal are thought to be microbial transformation and degradation (Gibson *et al.*, 1975). Bioremediation has become an intensive area for research and, as a result, rapid progress has been made in developing effective microbial bioremediation techniques. Different features such as microbial composition, nutrient concentration, temperature, surfactant addition, agitation, etc. have been

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employed to enhance the bioremediation process.

The viability of PAH-degrading microbes requires a specific set of factors, including the contaminant concentration, pH, temperature and the presence of essential elements (Joshi and Lee, 1995). Since the pH can significantly affect the activity and diversity of microorganisms, a better understanding of its effect could lead to improved process control of bioremediation systems.

In this study, phenanthrene was selected as a target compound. There has been considerable research effort on PAH biodegradation in soil systems (Mulder *et al.*, 2001; Lotfabad and Gray, 2002; Woo *et al.*, 2004) with a focus on mass-transfer behaviour and biodegradation. In these studies the biodegradation rate is thought to be limited by the mass transfer rate from the pure phenanthrene to the aqueous phase. Here, we specifically look into the biodegradation of phenanthrene in the aqueous phase where we remove the mass transfer limitation in order to study the biodegradation process. The biodegradation of solutions with up to 1000 mg/L phenanthrene by a mixed microbial consortium were studied. The variation of degradation rate with initial substrate concentration was investigated systematically. A phenomenological modelling approach was also explored to investigate the main processes influencing phenanthrene biodegradation.

1 Materials and methods

1.1 Chemicals and medium

Phenanthrene (98%) was obtained from Acros Organics, Geel, Belgium. Its aqueous solubility at 20℃ is 1.4 mg/L (Mackay *et al.*, 1992). Other chemicals were purchased from Beijing Chemicals, China. Deionized water from a Milli-Q purification system(Millipore, USA) with resistivity>18.2 MΩ cm was used in preparing samples.

Biodegradation experiments were conducted in a rotary shaker(150 r/min) at 30℃ with autoclaved mineral salts medium (MSM) consisting of 800 mg K_2HPO_4, 200 mg KH_2PO_4, 800 mg NH_4NO_3, 250 mg $MgSO_4$, 90 mg $FeSO_4 \cdot 7H_2O$ and 32 mg $CaCl_2$ per litre of deionised water.

1.2 Enrichment of mixed culture from petroleum contaminated soil

Oil-contaminated soil was collected from the Zihe River sediment in Shandong Province, China. This site has been contaminated by petroleum for more than 30 years(Zhu *et al.*, 2000). The soil samples were stored at 4℃ until use. A 5-g contaminated soil sample was inoculated into 300 ml autoclaved MSM and then shaken at 175 r/min on a rotary shaker set at 30℃ for 4 h to transfer the microorganisms into water. After shaking, a 50 ml culture was reinoculated into 250 ml autoclaved MSM with 100 mg/L of phenanthrene and then shaken for more than 3 d until more than half of the phenanthrene crystals in the shaking bottle were dissolved. At this stage the liquid turns light yellow in colour. The enrichment process was repeated at least three times. Samples were then inoculated using an aqueous-silicon oil two-phase system (Gardin *et al.*, 1999) with phenanthrene as the sole carbon source.

The aqueous-silicon oil two-phase system is an efficient biomass growth system. Phenanthrene was pre-dissolved in the silicon oil phase to attenuate its hydrophobicity, thereby permitting a supply of phenanthrene to the microorganisms in the aqueous phase based on equilibrium considerations and the real time demand of the microorganisms. This system is thought to enhance the biomass growth rate(Dé ziel *et al.*, 1999; Guieysse and Mattiasson, 2001). This procedure yields a phenanthrene concentration of 1000 mg/L. After inoculation for 3 d the bacteria count of the mixed culture was approximately $\times 10^{11}$ cell/ml. It was then diluted using autoclaved MSM to give a stock culture with cell populations of approximately 8×10^8 cell/ml.

Morphologically, the majority of cells were rod-shaped. The length of the cells was about 1 μm.

1.3 Preparation of phenanthrene solutions

Most PAHs have low solubility in water. Indeed, the solubility of phenanthrene in water is only 1.29 mg/L at 20℃. As mentioned already, previous research has shown biodegradation might be inhibited by the limited mass-transfer rate (Mulder *et al.*, 2001; Woo *et al.*, 2004). Therefore, we used three concentrations of phenanthrene solution in our experiment, i. e., 100, 500 and 1000 mg/L. In order to get such high concentrations of phenanthrene in solution, phenanthrene was first extracted with hexane (extraction>95%). The extractant was then analysed using a SHIMADZU LC-10AD HPLC. A 20 μl injection of the extract was separated on a 250 ×4.8 Aichrom C18 column and determined spectrophotometrically at 254 nm using a SHIMADZU SPD-10AV. The volumetric flow rate of the mobile phase, 85% methanol, was 1.0 ml/min. The minimum detectable phenanthrene concentration was 0.1 mg/L. Phenan threne stock solution was prepared in dimethylsulfoxide (DMSO). Prior to use, phenanthrene was added to MSM to give the final concentration(Yuan *et al.*, 2000).

The above preparation procedure produces

microscopic phenanthrene particles distributed throughout the aqueous phase. Thus, the microbes are able to utilize phenanthrene in higher concentrations than its equilibrium aqueous solubility (Makkar and Rockne, 2003), in which case biodegradation will not limited by the mass transfer process. This is verified in the experiment and simulation results later.

1.4 Biodegradation of phenanthrene

The biodegradation experiments of phenanthrene were performed in 500-ml flasks on a rotary shaker (150 r/min, 30℃). The flasks contained 250 ml autoclaved MSM. Phenanthrene dissolved in DMSO was placed in each flask to generate different concentrations (100, 500 and 1000 mg/L as well as a 100 mg/L control). The solutions were immediately supplemented with 5 ml of phenanthrene-degrading microorganism culture. The 100 mg/L control was prepared by adding 5 ml of 10 g/L NaN_3 instead of microbial culture. The reacted solution was sampled at set time intervals and the phenanthrene in those samples was analysed using HPLC.

At each sampling event, samples were removed for pH measurement using an IQ150 pH meter, while soluble protein was analysed as an indicator for microbial growth using the Lowry method (Lowry *et al.*, 1951).

2 Results and discussion

The relative concentrations of phenanthrene, the protein and pH changes in the culture containing different concentrations of phenanthrene at 100, 500 and 1000 mg/L are shown in Fig.2.

Fig.2a shows that after about a 1— 2 d lag phase, phenanthrene in different initial concentrations all decreased rapidly. There was 100% removal for the flask containing 100 mg/L phenanthrene. Phenanthrene was degraded completely within 3 d. But only 88.3% and 64.0% removal occurred at the flasks with 500 and 1000 mg/L phenanthrene, respectively. Therefore, higher concentrations of phenanthrene show lower removal percentage. However, the average degradation rates of phenanthrene show an opposite trend, which are 0.54 mg/h for 100 mg/L phenanthrene; 1.70 mg/h for 500 mg/L phenanthrene and 2.88 mg/h for 1000 mg/L phenanthrene. A higher concentration of phenanthrene results in a higher amount of phenanthrene removal. It is because the low concentration of phenanthrene has limited the microbial growth(Wong *et al.*, 2002).

Protein changes shown in Fig.2b indicate the microbial growth and decay. The results show that protein in the flasks all reached peaks at around 3—4 d for the 3 cases, followed by a slight decrease. The higher concentration of phenanthrene could result in the higher protein content since the phenanthrene acts as the carbon source for the microbes. In other words, the higher concentration of phenanthrene is beneficial to the growth of the mixed culture. The difference between the case of 100 mg/L and the cases of 500 and 1000 mg/L is that, after the peak, the protein content keeps decreasing until the end of the experiment for the case of 100 mg/L while for the other cases the protein contents decreased for the first 5 d then increased for the rest of the experiment. These results suggest that phenanthrene at 100 mg/L is all degraded and thus that the intermediate metabolite is relatively low. The lower concentration of intermediate metabolite is insufficient to be a noticeable carbon source for microbial growth in which case the biomass decreases. On the other hand, for the cases of 500 and 1000 mg/L, high concentrations of intermediate metabolite are produced, which then provides carbon for continued biomass growth.

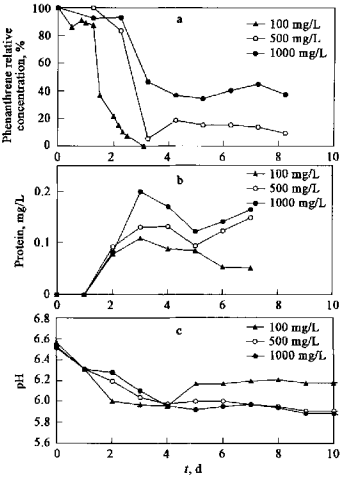

Fig.2 Effect of different initial phenanthrene concentrations on (a) removal rate; (b) microbial growth; (c) pH

Fig.2c shows that the degradation of phenanthrene results in a decrease of pH, especially in the first 4 d, with a lower pH for the biodegradation of the higher phenanthrene concentrations. The decrease in pH can be explained by the release of 1-hydroxy-2-naphthoic acid, an early intermediate of phenanthrene metabolism(Boldrin *et al.*, 1993; Tiehm, 1994; Wong

et al., 2002).

3 Simulation of phenanthrene biodegradation

3.1 Model I: Degradation of phenanthrene and growth of biomass

The mathematical description of biodegradation kinetics and associated processes is an effective tool for better understanding biological activity and for engineering design purposes. Barry *et al.* (2002) reported a versatile approach to biological and biogeochemical modelling for use at the laboratory or field scales. Lotfabad and Gray (2002) applied a Michaelis-Menten rate expression to the kinetic biodegradation of PAHs, while accounting for inhibition as well as diffusion from a non-aqueous phase liquid. Mulder *et al.* (2001) and Woo *et al.* (2004) also predicted PAH biodegradation in soil/slurry systems using mechanistic models, concluding that the degradation is mainly mass-transfer limited.

The Monod growth model was used to describe the PAH biodegradation/microbial growth rate. Per unit of biomass, the microbial growth rate increases with the PAH concentration first but plateaus later(Eq. (1)). The total biomass variation depends not only on the microbial growth but also on its decay (Eq. (2)). The biodegradation rate is associated with the microbial growth by a stoichiometric factor, it is also proportional to the quantity of biomass (Eq. (3)). The model is given by:

$$\mu_1 = \frac{\mu_{\max,1} C_1}{k_{s1} + C_1} \tag{1}$$

$$\frac{dX}{dt} = \mu_1 X - K_d X \tag{2}$$

$$\frac{dC_1}{dt} = -\frac{\mu_1}{Y_1} X \tag{3}$$

Where: X, C_1 are the concentration of microbes and phenanthrene (mol/L), respectively; $\mu_{\max,1}$ is the maximum microbial population growth rate during phenanthrene biodegradation (T^{-1}); k_{s1} is the half-saturation constant for phenanthrene degradation (mol/L); Y_1 represents the mass of phenanthrene degradation per unit mass of microbe generation while K_d is the first-order biomass decay coefficient(T^{-1}).

The process of phenanthrene biodegradation and associated microbe growth and decay was simulated using MATLAB ODE solvers. In order to calibrate parameters, MATLAB's FMINSEARCH function was also applied, where the objective function is:

$$\min[(\omega_{PAH} \sum_{i=1}^{n_{PAH}} (S_{PAH,i} - E_{PAH,i})^2 + \omega_{BAC} \sum_{i=1}^{n_{BAC}} (S_{BAC,i} - E_{BAC,i})^2)] \tag{4}$$

Where: n_{PAH} and n_{BAC} are the total number of measured samples; S_{PAH} and E_{PAH} are the simulated and observed concentrations of phenanthrene(mol/L); S_{BAC} and E_{BAC} are the simulated and observed concentration of microbes; ω_{PAH} and ω_{BAC} represent the weighting of the error for phenanthrene and microbes, respectively.

Comparison of simulation and experiment results is shown in Fig.3, with the calibrated parameters listed in the figure caption. For the low concentration case where the initial phenanthrene concentration equals 100 mg/L, the simulation result is in good agreement with the experimental data. It shows that the phenanthrene will degrade slowly in the first day, during which the microbes adjust. It then degrades quickly in the following 2 d until it vanishes after 3 d. The microbial growth rate is larger than the decay rate in the first 3 d which results in an increase of the microbial concentration. Then the growth rate decreases and the decay results in a decrease in microbial concentration. But, for the higher initial phenanthrene concentration cases, the experiments show steady phenanthrene concentrations after 3 d while the simulations erroneously show the phenanthrene to be fully degraded. As a result, the simulations show higher microbe concentration peaks.

That the phenanthrene was not totally degraded for higher initial concentrations indicates the presence of an inhibition factor affecting microbial growth. Organic acid (1-hydroxy-2-naphthoic acid) generated during the phenanthrene biodegradation is postulated to influence the biodegradation rate. In that case, a more complex model, which includes the participation of an intermediate metabolite (organic acid), is introduced below.

3.2 Model II: Inclusion of organic acid

The generation of organic acid and its consumption are considered in this model, where the degradation process is divided into two stages. In stage one, the microbes are assumed to degrade phenanthrene to organic acid while in stage two the organic acid is degraded into inorganic carbonate. The growth rate of the microbial community as a result of phenanthrene biodegradation was assumed to be inhibited by the presence of organic acid. At the same time, the microbial growth rate due to consumption of organic acid was also assumed to be inhibited by the present of phenanthrene. This leads to the following model:

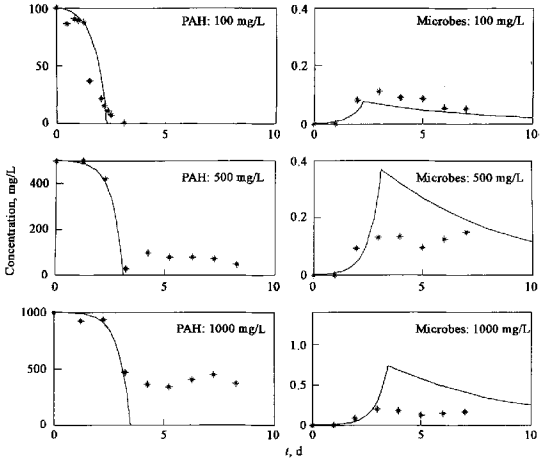

Fig.3 Comparison of experiment and simulation results of PAHs biodegradation and microbial growth and decay using Model I
The calibrated parameters are $Y_1 = 0.008$, $K_{s1} = 0.1282$, $\mu_{max1} = 2.0516$, $K_d = 0.1673$

$$\mu_1 = \frac{\mu_{max,1} C_1}{k_{s1} + C_1 + b_1 \exp(K_1 C_2)} \quad (5)$$

$$\mu_2 = \frac{\mu_{ax,2} C_2}{k_{s2} + C_2 + b_2 \exp(K_2 C_1)} \quad (6)$$

$$\frac{dX}{dt} = \mu_1 X + \mu_2 X - K_d X \quad (7)$$

$$\frac{dC_1}{dt} = -\frac{\mu_1}{Y_1} X \quad (8)$$

$$\frac{dC_2}{dt} = \frac{\mu_1}{Y_{21}} X - \frac{\mu_2}{Y_{22}} X \quad (9)$$

where: C_2 is the concentration of organic acid which is the intermediate product (mol/L); $\mu_{max,2}$ is the maximum microbial population growth rate during organic acid biodegradation (T^{-1}), b_1, b_2, K_1 and K_2 are coefficients used for quantifying the inhibition of biodegradation by phenanthrene or the organic acid; k_{s2} is the half-saturation constant for organic acid biodegradation (mol/L); Y_{21} represents the mass of the organic acid production per unit mass production of phenanthrene biodegrading microbes, and Y_{22} is the mass of the organic acid degradation for per unit mass production of organic acid biodegrading microbes.

The same solver and optimisation tools in MATLAB were used for simulation and parameter calibration. Organic acid is also included during the optimisation, which uses the following objective function:

$$\min[\omega_{PAH} \sum_{i=1}^{n_{PAH}} (S_{PAH,i} - E_{PAH,i})^2 + \omega_{BAC} \sum_{i=1}^{n_{BAC}} (S_{BAC,i} - E_{BAC,i})^2 + \omega_{OA} \sum_{i=1}^{n_{OA}} (S_{OA,i} - E_{OA,i})^2] \quad (10)$$

Where: S_{OA} and E_{OA} are the simulated and observed concentration of organic acid (mol/L); ω_{OA} represents the weighting of the error for organic acid.

Fig.4 shows the simulation for this model compared with the experiment results. It shows the phenanthrene biodegradation is in good agreement with the experiment data. In particular, it simulates the incomplete removal in the 500 mg/L and 1000 mg/L concentration cases. The organic acid is indicated by the relative concentration based on pH measurements. The organic acid predictions are in reasonable agreement with the experimental data. Although the experimental data for the low phenanthrene concentration case is slightly higher than the predicted result, they both show a sharp increase in hydrogen concentration in the first several days with little change later. Predicted biomass variations show the same trend as the experimental results, suggesting that the biomass increases in the first stage and then peaks appear during the experimental period. The difference between these two sets of data might be because of the interpretation of experimental measurements, as we assume protein is proportional to the biomass. Although our model is still a simplified approach for

describing the phenanthrene biodegradation, it is commensurate with the experimental data and could be refined or extended given more detailed experimental measurements.

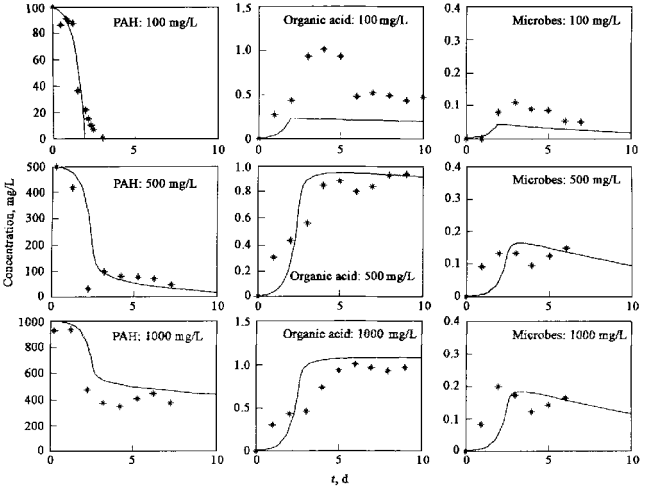

Fig.4 Comparison of experiment and simulation result of PAHs biodegradation and microbial growth and decay using Model II
The calibrated parameters are: $Y_1 = 0.00045$, $Y_{21} = 0.1957$, $Y_{22} = 0.0005$, $K_{s1} = 0.0114$, $K_{s2} = 0.0835$, $K_1 = 15.3682$, $K_2 = 0.5127$, $\mu_{max1} = 2.0493$, $\mu_{max2} = 0.0001$, $K_d = 0.0115$, $b_1 = 0.0014$, $b_2 = 0$

4 Conclusions

A microcosm experiment and accompanying simulations were carried out to study the biodegradation of phenanthrene in solution by cultures sampled from a contaminated river sediment. The experimental data show that: (1) the phenanthrene is biodegradable by this particular native culture, with the highest biodegradation rate in the first 3 d; (2) a higher initial phenanthrene concentration shows a higher biodegradation rate in the first several days. However, the experiments leave residual phenanthrene in solution. We have hypothesised that this is caused by the inhibition effect due to an intermediate metabolite, i.e., organic acid; (3) a higher initial phenanthrene concentration results in a larger pH decrease. In keeping with the above hypothesis, this is due to the increased production of organic acid; (4) a peak in the protein concentration appears at the end of 3 d, due to the initially rapid microbial growth rate followed by decay. Another peak is observed in the cases with higher initial phenanthrene concentrations which is assumed to be the result of increased microbial growth on the organic acid produced during the biodegradation.

Numerical models were designed based on the Monod-growth model, first without considering the generation of organic acid and its inhibition effect on phenanthrene biodegradation. The experimental data were not well simulated with this model. In consequence, the model was modified to include the inhibition effect of organic acid, which shows a better fit.

Despite the agreement between the modelling results and experimental data, based on this study we suggest that there are areas where future experimental design could benefit. For example, we used pH to indicate the generation of organic acid. In fact the intermediate metabolite we assumed is likely a group of compounds, suggesting that we should identify the reaction route, chemical species and their particular concentrations during the experiment. The biomass monitoring technique could be improved as well. The use of protein to indicate biomass quantity makes it difficult to classify microbial groups and their activity.

References:

Barry D A, Prommer H, Miller C T *et al.*, 2002. Modelling the fate of oxidisable organic contaminants in groundwater [J]. Advances in Water Resources, 25: 945—983.

Boldrin B, Tiehm A, Fritzsche C, 1993. Degradation of phenanthrene,

fluorene, fluoranthene, and pyrene by a Mycobacterium sp. [J]. Applied and Environmental Microbiology, 59: 1927—1930.

Déziel E, Comeau Y, Villemur R, 1999. Two-phase bioreactors for enhanced degradation of hydrophobic/toxic compounds [J]. Biodegradation, 10: 219—233.

Gardin H, Lebeault J M, Pauss A, 1999. Biodegradation of xylene and butyl acetate using an aqueous-silicon oil two-phase system [J]. Biodegradation, 10: 193—200.

Gibson D T, Mahadevan V, Jerina D M *et al*., 1975. Oxidation of the carcinogens benzo[a]pyrene and dibenz[a,h]anthracene to dihydrodiols by a bacterium[J]. Science, 189: 295-297.

Guieysse B, Mattiasson C B, 2001. Microbial degradation of phenanthrene and pyrene in a two-liquid phase-partitioning bioreactor [J]. Applied Microbiology and Biotechnology, 56: 796—802.

Joshi M M, Lee S, 1995. Biological remediation of polynuclear aromatic hydrocarbon contaminated soils using *Acinetobacter* sp. [J]. Energy Sources, 18: 167—176.

Kanaly R A, Harayama S, 2000. Biodegradation of high molecular weight polycyclic aromatic hydrocarbons by bacteria [J]. Journal of Bacteriology, 182: 2059—2067.

Lotfabad S K, Gray M R, 2002. Kinetics of biodegradation of mixtures of polycyclic aromatic hydrocarbons [J]. Applied Microbiology and Biotechnology, 60: 361—365.

Lowry O H, Rosebrough N J, Farr A L *et al*., 1951. Protein measurement with the Folin phenol reagent [J]. Journal of Biological Chemistry, 193: 265—275.

Mackay D, Shiu W Y, Ma K C, 1992. Illustrated handbook of physical chemical properties and environmental fate for organic chemicals, Vol. I and II [M]. Boca Raton, FL, USA: Lewis Publishers.

Makkar R S, Rockne K J, 2003. Comparison of synthetic surfactants and biosufactants in enhancing biodegradation of polycyclic aromatic hydrocarbons [J]. Environmental Toxicology and Chemistry, 22: 2280—2292.

Mulder H, Breure A M, Rulkens W H, 2001. Prediction of complete bioremediation periods for PAH soil pollutants in different physical states by mechanistic models [J]. Chemosphere, 43: 1085—1094.

Tiehm A, 1994. Degradation of polycyclic aromatic hydrocarbons in the presence of synthetic surfactants [J]. Applied and Environmental Microbiology, 60: 258—263.

Wodzinski R S, Coyle J E, 1974. Physical state of phenanthrene for utilization by bacteria [J]. Applied Microbiology, 27: 1081—1084.

Wong J W C, Lai K M, Wan C K *et al*., 2002. Isolation and optimization of PAH-degradative bacteria from contaminated soil for PAHs bioremediation [J]. Water Air and Soil Pollution, 139: 1—13.

Woo S H, Lee M W, Park J M, 2004. Biodegradation of phenanthrene in soil-slurry systems with different mass transfer regimes and soil contents[J]. Journal of Biotechnology, 110: 235—250.

Yang J, Liu X, Long T *et al*., 2003. Influence of non-ionic surfactant on solubilization and bioremediation of phenanthrene [J]. Journal of Environmental Sciences, 15(6): 859—862.

Yuan S Y, Wei S H, Chang B V, 2000. Biodegradation of polycyclic aromatic hydrocarbons by a mixed culture [J]. Chemosphere, 41: 1463—1468.

Zhu X, Liu J, Zhu J *et al*., 2000. Characteristics of distribution and transport of petroleum contaminants in fracture-karst water in Zibo Area, Shandong Province, China [J]. Science in China, Series D: Earth Sciences, 43: 141—150.

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