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Enhanced anoxic bioremediation of PAHs-contaminated sediment

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ABSTRACT

In this study, the biodegradation of 16 polycyclic aromatic hydrocarbons (PAHs) in marine sediment was investigated under three different anoxic conditions, i.e. sulfate-only, nitrate-only and mixed nitrate/sulfate as electron acceptors. All two-, three- and four-ring PAHs showed significant biodegradation with the removal efficiencies ranging from 42% to 77%, while five- and six-ring PAHs showed little degradation. The results illustrated that two- to three-ring PAHs could be degraded at a rate of 4.01×10^{-2}–6.42×10^{-2} d^{-1} under nitrate-reducing condition, faster than that of under sulfate-reducing condition. Biodegradation of two- and three-ring PAHs followed first-order model well with the rate constants of 1.62×10^{-2}–6.42×10^{-2} d^{-1}. The biodegradation of four ring PAHs followed the zero-order kinetic model with the rate constants of 1.26×10^{-2}–2.22×10^{-2} mg/kg/d. Molecular analysis indicated that *nah*Ac gene increased by two orders of magnitude during the biodegradation and served as a good indicator of PAHs-degrading bacterial population and biodegradation process.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are composed of a large group of organic contaminants which derived from natural sources, such as forest fires, and anthropogenic sources, such as combustion of fossil fuels and oil spills (Bamforth and Singleton, 2005). 16 PAHs, listed as priority pollutants by the United States Environmental Protection Agency (US-EPA), are a major concern for their toxicity, mutagenicity, carcinogenicity and environmental persistence (Keith and Telliard, 1979). As demonstrated by studies on their occurrence, transportation, adsorption and biodegradation, their concentrations in the nature environment can be detected in a wide range of 1 μg/kg to 300 g/kg (Gan et al., 2009; Kanaly and Harayama, 2000; Li et al., 2010b; Liu et al., 2011; Smith and Harrison, 1996). High concentrations of PAHs in soils and sediment caused significant hazards to organisms directly and to human health indirectly (MacRae and Hall, 1998; Vanrooij et al., 1993). Therefore, degradation of PAHs and remediation of contaminated environments have drawn much more attentions recently (Li et al., 2010a; Lu et al., 2011b; Mahanty et al., 2008; Maletic et al., 2009).

Aerobic biodegradation seems not cost-effective to implement due to the limitation of oxygen delivered to the subsurface for a couple of reasons, especially for sediment. Firstly, aeration will cause the re-suspension of sediment and the release of excess nutrient from sediment. Secondly, pure oxygen is expensive and low solubility. However, anoxic biodegradation is considered to be a practicable, inexpensive remediation technique for soil and sediment contaminated by PAHs. Several studies have demonstrated that low molecular weight (LMW) PAHs, such as naphthalene and phenanthrene, could be biodegraded under denitrifying conditions (Coates et al., 1996; Eriksson et al., 2003; Yuan and Chang, 2007). Some researchers found that high molecular weight (HMW) PAHs tended to be more easily biodegraded under sulfate-reducing conditions (Rothermich et al., 2002). However, there is no systematic comparison of the degradation of PAHs using different electron acceptors. Although PAHs can be biodegraded by a variety of bacteria and fungi as the sole carbon and energy source (Arun and Eyini, 2011; Dean-Ross et al., 2001; Zhang et al., 2009a), successful biodegradation of PAHs is mainly dependent on many environmental factors, including temperature, pH, nitrate concentration, surfactant effect and co-substrates (Chen et al., 2008; Desai et al., 2008; Kanaly and Harayama, 2000; Yuan and Chang, 2007; Zhao et al., 2011). In addition, quite a few studies argued that the limited bioavailability of PAHs to microorganisms was the main problem for anaerobic or anoxic biodegradation (Lei et al., 2005). Some of the above factors have been studied in the previous published papers (Lu et al., 2011a), but not in a combined way. Previous kinetic studies were mostly conducted on a single compound and by pure cultures (Chen et al., 2008; Ortiz et al., 2003). However, the biodegradation of 16 PAHs by a mixed culture was not well studied.

The aim of the study was to compare the removal efficiencies of 16 PAHs in marine sediment using three different electron acceptors (nitrate, sulfate, and a mixture of nitrate and sulfate). Biodegradation kinetics of 16 PAHs in marine sediment was also studied. In addition, quantitative polymerase chain reaction (qPCR) was

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Table 1
Physical and chemical properties of 16 PAHs [8,25].

PAHs	No. of benzene rings	Solubility (μg/l)	Carc.[a,b]	log K_{ow}	$t_{1/2}$[c]	LD_{50} (ng/g) to mice
NAP	2	31,700	0	3.37	2.1–30.8	533–710
ACY	3	3800	0	3.48	N.A[d].	N.A.
ACE	3	16,100	0	3.92	N.A.	N.A.
FLU	3	1900	0	3.77	N.A.	N.A.
PHE	3	1290	0	3.24	16–216	750
ANT	3	73	0	4.54	N.A.	N.A.
FLT	4	260	+	5.22	137–377	100
PYR	4	135	0	5.18	19.4–630	514
BaA	4	14.0	+	5.91	270–323	N.A.
CHR	4	2.0	+	5.61	693	N.A.
B[b]F	5	1.5	++	5.8–6.1	N.A.	N.A.
B[k]F	5	0.8	++	6.0–6.8	N.A.	N.A.
BaP	5	4.0	+++	6.04	211–1400	232
DahA	5	0.6	+	6.5	N.A.	N.A.
INPY	6	N.A.	+	6.58	N.A.	N.A.
BghiP	6	0.3	+	6.5	N.A.	N.A.

[a] Carcinogenicity.
[b] 0: No carcinogenicity; +: low carcinogenicity (<33%); ++: high carcinogenicity; (>33%); +++ extreme high carcinogenicity.
[c] First-order kinetics (d).
[d] Not applicable.

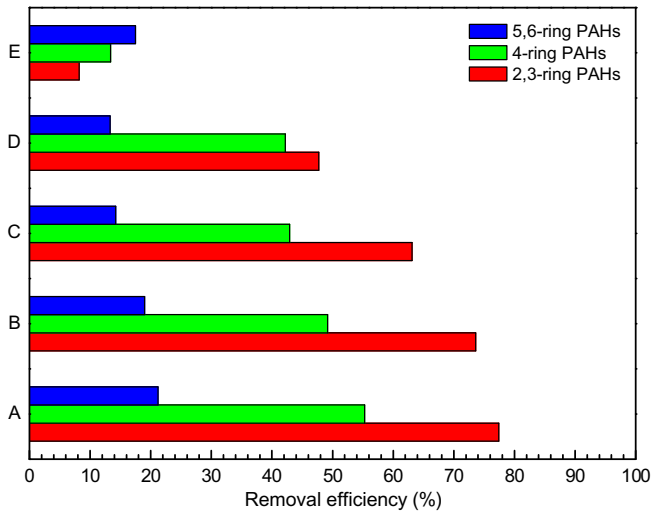

Fig. 1. Removal efficiencies of PAHs under different conditions. (a) Nitrate as the sole electron acceptor with enhancement measures; (b) nitrate and sulfate as electron acceptors with enhancement measures; (c) sulfate as the sole electron acceptor with enhancement measures; (d) As-is and (e): abiotic control.

employed to quantify the change of PAHs-degrading population during the degradation of PAHs using *nah*Ac gene as the biomarker.

2. Methods

2.1. Sediment samples

The sediment used in this study was collected from Kai Tak Approach Channel, Hong Kong, at 0–4 m depth from water/sediment interface and then transported to the laboratory and stored at 4 °C before use. The characteristics of the sediment, including COD (chemical oxygen demand), TOC (total organic carbon), VS (volatile solids), TS (total solid), water content and density had been reported in our previous study (Zhang et al., 2009b). The overlaying seawater with a salinity of 3.6% was taken from Shek O, Hong Kong and used to prepare calcium nitrate solution after filtering through a 0.45 μm membrane (Gelman Science, Ann Arbor, MI). The seawater had a pH of 7.8–8.0, the sulfate concentration of 2400 mg/l and the nitrate concentration below the detection limit of 1 mg/l.

2.2. Chemicals and sample preparation

Target 16 PAHs, including naphthalene (NAP), acenaphthylene (ACY), acenaphthene (ACE), fluorene (FLU), phenanthrene (PHE), anthracene (ANT), fluoranthene (FLT), pyrene (PYR), benzo[a]anthracene (BaA), chrysene (CHR), benzo[b]-fluoranthene (B[b]F), benzo[k]fluoranthene (B[k]F), benzo-[a]pyrene (BaP), dibenz[a,h]-anthracene (DahA), indeno[1,2,3-c,d]pyrene (INPY) and benzo[g,h,i]perylene (BghiP), were purchased from the Dr. Ehrenstorfer Co. (Germany). The physico-chemical properties were summarized in Table 1 (Cerniglia, 1993; MacRae and Hall, 1998). Hexane (HPLC grade >95%), and dichloromethane (HPLC grade >95%) were purchased from Sigma–Aldrich (US) and used without further purification. Laboratory-spiked sediment samples were prepared by adding 2 ml mixed 16 PAHs (concentration of individual PAH: 1 g/l) in cyclohexane solution into 100 ml serum bottles with 20 g sediment for each treatment by slurry spiking.

2.3. Optimization of culture dosage

The culture dosage in the following batch experiments was optimized through pre-experiment. Biodegradation of naphthalene (30 mg/l) in aqeuous solution by enriched culture was investigated under nitrate-reducing conditions for 10 d with various additions of enriched mixed culture (0.1–0.4 mg VSS/mg naphthalene). The seeding dosage of 0.3 mg VSS/mg was finally chosen for the following batch, since the removal efficiency achieved the plateau at this critical point.

2.4. Batch experiment design

A series of batch experiments was conducted with PAHs-spiked sediment under various treatment using "As-is" treatment as the control. Each batch experiment was carried out using different electron acceptors (i.e. nitrate, sulfate, and a mixture of nitrate/sulfate). In addition, abiotic control (0.1% NaN_3) was set up to investigate the abiotic loss of PAHs (Li and Zhang, 2010). In the batch test, four serum bottles of 150 ml with 20 g sediment and 100 ml feed solution were run simultaneously at 25 °C for 30 d. All the serum bottles were placed in a closed chamber to avoid possible photolysis. All the experiments were conducted at pH 7.0 and temperature 25 °C. Samples were taken from the bottles at 0, 12, 22, 26, 30 d for PAHs measurement using GC/MS. The differences on the