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Enhanced degradation of phenanthrene and pyrene in freshwater sediments by combined employment of sediment microbial fuel cell and amorphous ferric hydroxide

Zaisheng Yan [a], Na Song [a], Haiyuan Cai [a], Joo-Hwa Tay [b], Helong Jiang [a,*]

[a] State Key Laboratory of Lake Science and Environment, Nanjing Institute of Geography and Limnology, Chinese Academy of Sciences, Nanjing 210008, China
[b] Department of Environmental Science & Engineering, Fudan University, Shanghai 200433, China

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ABSTRACT

The degradation of phenanthrene and pyrene in freshwater sediment was investigated under three kinds of treatments (addition of amorphous ferric hydroxide to sediments, employment of sediment microbial fuel cell (SMFC), and the combination of ferric addition and SMFC employment). After 240 days of experiments, it was found that the combined treatment led to the highest removal efficiencies of phenanthrene (99.47 ± 0.15%) and pyrene (94.79 ± 0.63%), while the employment of SMFC could obtain higher removal efficiencies than Fe(III) addition. The combined approach improved potentials of phenanthrene and pyrene biodegradation in sediments under anaerobic pathways except methanogenic condition, and also stimulated humification of organic matters in sediments. At the end of experiments, ratios of humic acid to fulvic acid in sedimentary organic matters reached to 2.967 ± 0.240 in the combined treatment, and were only around 1.404–1.506 in the other treatments. Thus, organic matters in sediments in the combined treatment could adsorb tightly residual PAHs with less bioavailability. Considering both enhanced biodegradation and final sequestration of PAHs in sediments, the combined application of Fe(III) addition and SMFC employment offered a new promising remediation technology for contaminated sediments.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are widespread environmental pollutants which are mainly from incomplete combustion of fossil fuels and organic compounds [1,2]. These compounds are of major public concern due to their toxicity to organisms in carcinogenic and mutagenic potential. Once in sediments, PAHs tend to adsorb on and accumulate in sediments, and undergo various degradation, transformations, and sequestration [3,4]. Biodegradation under aerobic or anaerobic condition is a major process for PAHs removal [5,6]. Nevertheless, natural attenuation cannot appreciably remove pollutants, and a lack of suitable electron acceptors is one of major factors limiting biodegradation of PAHs in sediments. Therefore, biostimulation by introducing oxygen and/or other electron acceptors could improve the native microbiological activity within sediments [7–9].

Compared with anaerobic biodegradation, aerobic degradation of PAHs would provide higher degradation rates. However, aerobic bioremediation might not be cost-effective because of the introduction of oxygen is very difficult and limited due to the low solubility and high volatility of oxygen [10]. Furthermore, aeration will cause the re-suspension of sediments and the release of excess nutrients from sediments [11]. In fact, hydrocarbon-contaminated sediments usually become anoxic with a redox gradient along water–sediment interface, and microorganisms can anaerobically degrade PAHs in polluted aquatic sediments with alternative electron acceptors, such as nitrate, sulfate, or Fe(III) oxides [10,12–14].

Nitrate or sulfate as a terminal electron acceptor in enhancing biodegradation of PAHs has been reported previously [10,12]. However, the addition of nitrate and sulfate as electron acceptors in open sedimentary environments is problematic, as these chemicals are soluble and will diffuse away from the point of application under hydrodynamic influences [10]. In comparison, the addition of insoluble Fe(III) oxides into sediments became an alternative option to stimulate degradation of PAHs [13,15]. However, the effect of Fe(III) addition on PAH degradation in sediments was sometimes vague, and it was reported that addition of amorphous ferric hydroxide did not have any significant effect on the biodegradation of PAHs in mangrove sediments [16].

Recently, it was proposed that sediment microbial fuel cell (SMFC) technology could be applied to enhance the removal of organic matter and aromatic hydrocarbons in contaminated

* Corresponding author at: Nanjing Institute of Geography and Limnology, Chinese Academy of Sciences, 73 East Beijing Road, Nanjing 210008, China.
Tel.: +86 25 8688 2208; fax: +86 25 8688 2208.
E-mail addresses: hljiang@niglas.ac.cn, helong1971@hotmail.com (H. Jiang).

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sediments [8,17]. Anode in SMFC can act as a permanent, high potential electron acceptor with low-cost and continuous sink for electrons [8,18]. The microbial degradation of contaminants in sedimentary environments was not restricted by available electron acceptor after application of SMFC. Both electrodes in SMFCs and Fe(III) oxides represent insoluble and extracellular electron acceptors. However, effects of SMFC employment and Fe(III) addition on biodegradation of PAHs in freshwater sediments are unknown. In addition, it was not clear whether these two methods could be combined for sediment bioremediation.

In this study, phenanthrene and pyrene were selected as the target compounds as the two PAHs were often detected in surface sediments with high concentrations [19]. Degradation of phenanthrene and pyrene in freshwater sediments was investigated under addition of amorphous ferric hydroxide, employment of electrode in SMFC, and combined application of the two approaches as electron acceptors. Combined application of ferric oxide and SMFC was found to lead to higher removal rates of phenanthrene and pyrene in sediments compared to application of ferric addition or SMFC employment alone.

2. Materials and methods

2.1. Sediments and chemicals

Bulk samples of sediments were collected from East Taihu Lake (31°10′N, 120°24′E), a large shallow lake in China, sieved at 2 mm, and homogenized. The physical and chemical properties of the sediments were measured as follows: pH 7.8, moisture content 44.9%, total nitrogen amount 2.5030 g kg^{-1} dry sediment, and total phosphate amount 11.0827 g kg^{-1} dry sediment. Original concentrations of phenanthrene and pyrene in sediments were 0.0954 and 0.0316 mg kg^{-1} dry sediment, respectively.

To spike PAHs into sediments, the solutions of PAH mixture, containing phenanthrene and pyrene (98% purify, Alfa Acsar Co., UK), was firstly prepared in methanol, and then added drop-wise to wet sediments followed by mixing mechanically at low speed for 2 h [20,21]. The amounts of phenanthrene and pyrene to sediment were 10 and 5 mg kg^{-1} dry sediment, respectively. Prior to experiments, the spiked sediment samples were stored in the dark for 12 days for partial aging.

2.2. Setup of sediment column bioreactors

Five plexiglass columns with approximately 4-L volume (12 cm × 35 cm, diameter × height) were used as sediment column bioreactors (SCBs) to perform the biodegradation experiment in a dark environment at 25 °C. Each bioreactor contained 1600 g wet sediment and 1 L overlying water. The compositions of the mineral salts medium in the overlying water was (g L^{-1}): $K_2HPO_4{\cdot}3H_2O$, 0.0001; KH_2PO_4, 0.0002; NH_4Cl, 0.0115; $MgCl_2{\cdot}6H_2O$, 0.1; $CaCl_2{\cdot}2H_2O$, 0.1; and $FeCl_2{\cdot}4H_2O$, 0.02. SCB0 served as sterile control in which sediments were autoclaved twice at 121 °C for 30 min. SCB1 was applied to mimic the natural attenuation without addition of electron acceptor. In SCB2, amorphous ferric hydroxide with a 16 g of wet weight, prepared according to the method described elsewhere [22], was mixed and homogenized with sediments to test effect of iron amendment. SCB3 and SCB4 were operated with an electrode serving as the electron acceptor through deployment of SMFCs as described below. In addition, amorphous ferric hydroxide with the same amount as that amended to sediments in SCB2 was added to sediments in SCB4.

Two set of SMFCs were installed in SCB4 and SCB5 according to detailed description in previous study [23]. The anode, composed of two stainless steel cylinders (80 mesh, 1 mm thickness) was buried 2 cm below the sediment surface. The space between external stainless steel cylinder (9.6 cm × 10 cm, diameter × height) and internal cylinder (4.8 cm × 10 cm, diameter × height) was about 2.4 cm. The cathode, composed of a stainless steel cylinder (9.6 cm × 4 cm, diameter × height), was placed 6 cm above the sediment. The voltage signal between the anode and cathode across an external load of 100 Ω was measured using a multimeter (model 2700, Keithley Instruments, Cleveland, OH, USA).

2.3. Measurement of PAH degradation potential in sediments under various anaerobic redox conditions

At the end of experiments, sediment samples with a 0.5 g wet weight were transferred from the SCBs into 100 mL serum bottles in an anaerobic chamber filled with ultra-high purity nitrogen in order to measure PAH degradation potential of sediments under various anaerobic redox conditions. Test bottles were then filled with 50 mL medium with a compositions consisting of (g L^{-1}): K_2HPO_4, 0.27; KH_2PO_4, 0.35; NH_4Cl, 2.7; $MgCl_2{\cdot}6H_2O$, 0.1; $CaCl_2{\cdot}2H_2O$, 0.1; and $FeCl_2{\cdot}4H_2O$, 0.02.

The concentrated phenanthrene and pyrene solution, which were prepared through firstly dissolved in methanol and then mixed with deionized water, was added to test bottles with initial phenanthrene and pyrene concentrations in serum bottles of 0.5 mg L^{-1}, respectively. Four different anaerobic redox conditions (nitrate-reducing, iron-reducing, sulfate-reducing, and methanogenic) were maintained through adding four different electron acceptors (20 mM sodium nitrate, 50 mM ferric citrate, 20 mM sodium sulfate, and 20 mM sodium hydrogen carbonate) to serum bottles, respectively. The pH of liquid medium in bottles was adjusted to 7.0. After capped with butyl rubber stoppers, serum bottles were incubated at 25 °C in the dark without shaking for anaerobic treatments. Sterile controls were autoclaved twice at 121 °C for 30 min. Those test bottles were sampled daily with a syringe for determination of residual PAHs concentrations. All above operations were carried out under anaerobic chamber, and experiments were performed in duplicate.

2.4. The potential Fe(III)-reducing microbial activity in sediments

At the end of experiments, sediment samples (2 g) taken from each SCB were added to 100 mL serum bottles, followed by supplement of 60 mL of freshwater medium containing 12 mM glucose and 4 mM ferric citrate as electron donor and acceptor, respectively. Serum bottles were sealed with butyl rubber stopper and incubated at 25 °C in the dark. Sampling was done using syringes and needles. All manipulations of culture samples were carried out under strictly anoxic conditions. The reduction of Fe(III) was measured as the production of Fe(II) in HCl extracts using the colorimetric reagent ferrozine under strictly anoxic conditions [24]. Reduction rates were determined by the linear least square regression of the Fe(II) concentration versus time.

2.5. Fractionation and characterization of sediment organic matter

At the end of experiments, sediments from SCBs were sampled. Fulvic acid (FA), humic acid (HA) and biopolymer (BP) were isolated from sediment organic matter (SOM) samples using NaOH and HCl extraction procedure [25]. Organic carbon contents in three SOM fractions were quantified as total organic carbon (TOC) concentrations, which were measured using a Fisons CHN analyzer (Model EA1108, UK). The molecular structures and functional groups of FA and HA from SOM were further analyzed by Fourier transform infrared (FTIR) spectroscopy (Model NEXUS870, USA).

2.6. DNA extraction, PCR, and cloning of the 16S rRNA gene

After 240 days incubation, electrodes were removed from sediments, and the surface of electrodes was rinsed free of visible debris with a stream of sterile freshwater medium. 1 mm biomass on the stainless steel electrode was scraped vigorously with a sterile razor blade into 1.5 mL Tris-EDTA (TE) buffer. The commercial UltraClean Soil DNA Isolation Kit (Mo Bio Laboratories, Inc, USA) was used to extract genomic DNA of biofilms on the anode surfaces in SCB4 and SCB5. 16S rRNA genes were amplified by PCR using a touchdown program with forward primer Eubac27F and reverse primer Universal 1492R, as described previously [26].

PCR products recovered from the two anode-biofilm samples were cloned using the TOPO TA cloning kit (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. A total of 126 clones from the 2 libraries were picked for sequence analysis. Clones were sequenced using Bigdye terminator chemistry and an ABI 3100 Genetic Analyzer (Applied Biosystems, USA). All sequences obtained were carefully checked for chimeric artifacts using the BLAST (blastn) program (http://www.ncbi.nlm.nih.gov/BLAST), and chimera sequences were excluded. A neighbour-joining analysis was used to reconstruct phylogenetic trees using the MEGA software (Molecular Evolutionary Genetics Analysis, Version 5.05) with the 16S rRNA sequences together with their closest homologues in GenBank detected by BLAST. A bootstrap analysis (1000 replicates) was performed with a Kimura 2-parameter model to evaluate the topology of the phylogenetic tree. The 16S rRNA gene sequences obtained in this study have been deposited in GenBank under accession numbers JN805603–JN805730.

2.7. Analytical methodology

Phenanthrene and pyrene in sediment samples was extracted according to the procedure described in the previous study [21]. To extract phenanthrene and pyrene in water samples, aliquots of the samples (1 mL) were placed in a grass centrifuge tube, and 1 mL of methanol was added. After shaking for 90 min, the tubes were centrifuged at 1500 × *g* for 10 min. The supernatant liquid was filtrated through 0.22 μm filter units, and then analyzed with a high-performance liquid chromatograph (HPLC) (Agilent 1200, USA) fitted with a 4.6 mm × 150 mm reverse phase C_{18} column using methanol–water (90:10) as the mobile phase at a flow rate of 1 mL min^{-1}. Chromatography was performed at 30 °C. Phenanthrene and pyrene were detected at 254 and 238 nm. All data were subject to strict quality control procedures. The recoveries of the extraction method for phenanthrene were 90.2 ± 4.3% for water, and 89.7 ± 3.6% for sediment. For pyrene, the recoveries of the extraction were 86.2 ± 2.3% for water, and 90.3 ± 6.5% for sediment. The method detection limits for phenanthrene and pyrene in all samples were 0.1 μg kg^{-1} dry sediment.

Concentrations of three different electron acceptors (nitrate, Fe(III), and sulfate) in sediments were measured as previously described [14,16]. Nitrate and sulfate were extracted by Milli-Q water and analyzed by ion chromatography (dionex ICs-2000). The amount of hydroxylamine-reducible Fe(III) was calculated as the difference between the Fe(II) measured in the hydroxylamine and HCl-extractable Fe(II) concentration. HCl-extractable Fe(II) was determined as described previously [22].

Concentration of low-molecular-weight organic acids (including acetate, lactate, and propionate) in pore water of sediments were quantified by HPLC (Agilent 1200, USA) fitted with a reverse phase C_{18} column (EclipseXDB-C_{18}, 4.6 mm × 150 mm, 5 μm) using 0.2% H_3PO_4–acetonitrile (5:95, v/v) as the mobile phase at a flow rate of 1 mL min^{-1}. The detection wavelength was 215 nm and the column temperature was 25 °C.

2.8. Biodegradation kinetics and removal efficiency calculation

The biodegradation of PAHs was described by the first order kinetics Eq. (1) [16],

$$C = C_0 e^{-kt} \quad (1)$$

where C_0 is the concentration of PAH at time zero, C is the concentration of PAH after subtracting the abiotic loss at time t, and k is the first-order rate constant (biodegradation rate) of the reaction. The half life for each PAH compound, $t_{1/2}$, was calculated by the formula (2):

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

2.9. Statistical analysis

Statistical significance of differences was determined by one-way analysis of variance using the Origin software (OriginPro 7.5, OriginLab, USA). A $P < 0.05$ was considered significant.

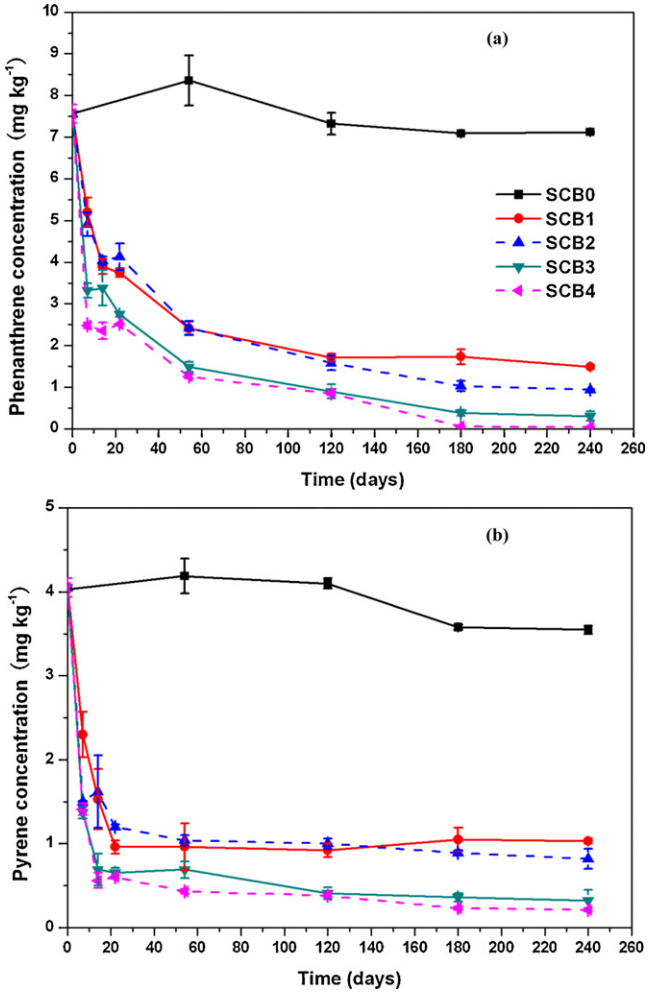

Fig. 1. The concentrations of phenanthrene (a) and pyrene (b) in sediments in different sediment column bioreactors during 240 days.

Table 1
The first-order rate constant (k, d^{-1}) of PAH-degradation, half-lives ($t^{1/2}$, day) and correlation coefficient (r^2) in sediment column bioreactors (SCB1–4).

	Phenanthrene			Pyrene		
	k (d^{-1})	r^2	$t_{1/2}$ (d)	k (d^{-1})	r^2	$t_{1/2}$ (d)
First phase (0–22 days)						
SCB1 (natural attenuation)	0.0375	0.923	18.5	0.0699	0.990	9.9
SCB2 (FeOOH)	0.0332	0.827	20.9	0.0725	0.822	9.6
SCB3 (SMFC)	0.0573	0.859	12.1	0.1307	0.969	5.3
SCB4 (FeOOH + SMFC)	0.0836	0.880	8.3	0.1363	0.970	5.1
Second phase (22–240 days)						
SCB1 (natural attenuation)	0.0047	0.811	147.5	No fit[a]	No fit	–[b]
SCB2 (FeOOH)	0.0086	0.934	80.6	0.0016	0.935	433.2
SCB3 (SMFC)	0.0125	0.969	55.5	0.0039	0.893	177.7
SCB4 (FeOOH + SMFC)	0.0155	0.956	44.7	0.0050	0.940	138.6

[a] 'No fit' means that no convergence is obtained in the model.
[b] '–' indicates that no data are available.

3. Results

3.1. PAH degradation in sediment column bioreactors (SCBs)

Phenanthrene and pyrene biodegradation in SCBs under various treatments were shown in Fig. 1. The abiotic losses of phenanthrene and pyrene in SCB0 were relatively low, with 5.8% and 12.3% reduction after 240 days experiments, respectively. The PAHs biodegradation in SCB1-4 showed similar biphasic curves. The degradation rate was considered as two ranges of fast and slow degradation. Reduction of PAH concentrations occurred mainly within the first 22 days of experiments. Therefore, the degradation rate was calculated separately rather than over the entire experimental period. The biodegradation rates and the half-lives of phenanthrene and pyrene in the first phase and second phase were shown in Table 1. Biodegradation rates of phenanthrene in SCB2 with Fe(III) addition was almost same as that in SCB1 as natural attenuation in the first phase, but higher than that in SCB1 in the second phase. In the two phases, biodegradation rates of phenanthrene in SCB4 with Fe(III) addition and SMFC employment were higher than those in SCB3 with only employment of SMFC, and biodegradation rates of phenanthrene in SCB3 were higher than those in SCB2.

For pyrene, biodegradation rates in SCB2 in the two phases were less than those in SCB3 and SCB4. Although there was almost no difference between biodegradation rates of pyrene in SCB3 and SCB4 in the first phase, the biodegradation rate of pyrene in SCB4 in the second phase was higher than that in SCB3. Meanwhile, implementation of both Fe(III) addition and SMFC employment significantly reduced half-lives of PAHs biodegradation during the whole experiments due to high degradation rates. Removal efficiencies of PAHs increased from SCB1 to SCB4 after 240 days of experiments (Table 2). These results indicated that the deployment of electrode as electron acceptor in SMFC was better than Fe(III) amendment for PAH biodegradation. In addition, application of both Fe (III) and electrode together could further result in a much enhancement of PAHs biodegradation.

3.2. Change in electron acceptors concentrations

The initial concentrations of nitrate, sulfate, and Fe(III) in sediment samples were 20.99 ± 0.92 mg kg^{-1}, 311.99 ± 12.1 mg kg^{-1} and 681.1 ± 13.9 mg kg^{-1}, respectively (Fig. 2). While sulfate reduction did not happen until 125th day, nitrate and iron-reducing reactions occurred immediately after experiments, confirming that the electron pathways (nitrate-reducing and iron-reducing) appeared to be more thermodynamically favorable than using sulfate-reducing. Nitrate concentrations in SCB1–4 all showed a gradual decrease with experiments. However, sulfate concentrations in SCB3 showed a rapid increase in the initial 120 days, and then decreased gradually. In SCB2 and SCB4 with addition of Fe(III) oxide, Fe(III) concentrations were gradually consumed with ferric concentrations in SCB4 less than those in SCB2.

3.3. Voltage signal from sediment MFCs

The voltage produced in SMFCs in SCB3 and SCB4 were illustrated in Fig. 3. The fluctuation of voltage output in each SMFC was observed throughout the measurement period. The average

Table 2
PAH concentration and removal efficiency, organic carbon content of sediment organic matter (SOM), and potential Fe(III) reduction rate in sediment column bioreactors (SCB1–4). Data are means ± standard deviation.

	Sediment column bioreactors			
	SCB1	SCB2	SCB3	SCB4
Phenanthrene				
Residual concentration (mg kg^{-1})	1.49 ± 0.05	0.94 ± 0.01	0.30 ± 0.11	0.04 ± 0.01
Removal efficiency (%)	80.29 ± 0.09	87.56 ± 0.23	96.14 ± 1.28	99.47 ± 0.15
Pyrene				
Residual concentration (mg kg^{-1})	1.03 ± 0.03	0.82 ± 0.12	0.32 ± 0.13	0.21 ± 0.02
Removal efficiency (%)	74.50 ± 1.40	79.91 ± 2.32	92.13 ± 3.29	94.79 ± 0.63
Organic carbon content of SOM[a]				
HA (mg-C g^{-1})	1.539 ± 0.169	1.413 ± 0.167	1.392 ± 0.175	0.892 ± 0.023
FA (mg-C g^{-1})	1.128 ± 0.208	1.024 ± 0.087	0.935 ± 0.057	0.303 ± 0.032
BP (mg-C g^{-1})	0.110 ± 0.005	0.135 ± 0.018	0.143 ± 0.007	0.015 ± 0.001
Ratio of HA/FA	1.441 ± 0.415	1.404 ± 0.282	1.506 ± 0.280	2.967 ± 0.240
Ratio of HA/BP	13.988 ± 0.906	10.471 ± 0.178	9.817 ± 1.692	58.386 ± 2.596
Potential Fe(III) reduction rate (mM Fe^{2+} g^{-1} sediment d^{-1})	0.0330 ± 0.0005	0.0384 ± 0.0012	0.0622 ± 0.0010	0.0982 ± 0.0007

[a] HA, humic acid; FA, fulvic acid; BP, biopolymer.

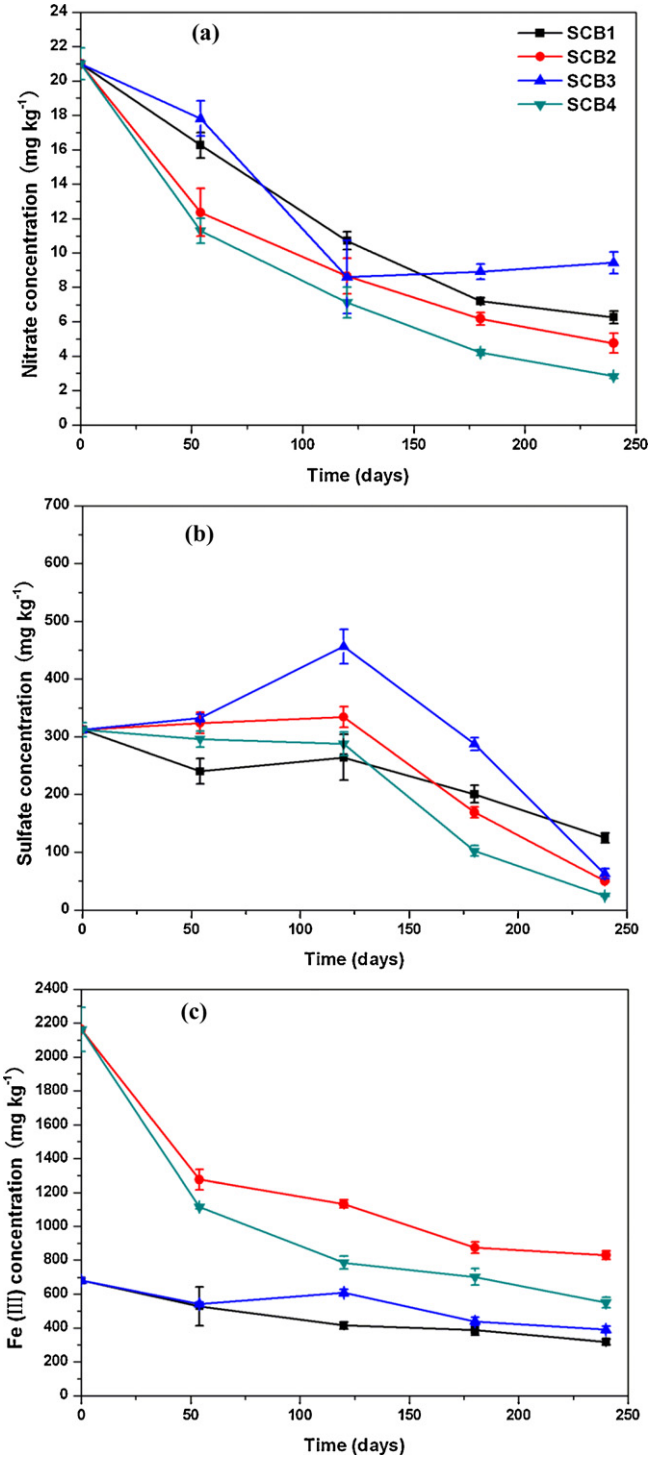

Fig. 2. The change in concentrations of nitrate (a), sulfate (b), and Fe(III) (c) in sediments in sediment column bioreactors.

voltages from SMFCs in SCB3 and SCB4 were 16.5 ± 8.9 mV and 17.1 ± 3.8 mV, respectively.

3.4. Organic acid concentrations in sediments

Concentration of acetate, lactate, and propionate in sediments were showed in Fig. 4. During the whole experimental period, acetate was only detected on day 14 for all SCBs, and acetate concentrations in SCB3 and SCB4 reached to 6.0 and 4.9 mg L^{-1}, respectively, while acetate concentrations in SCB2 and SCB3

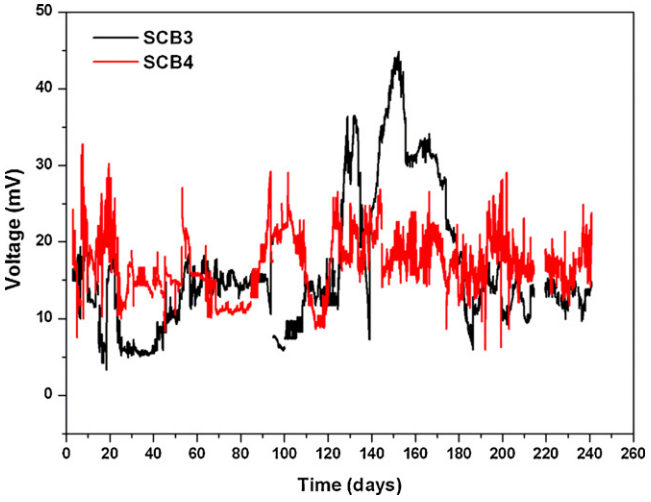

Fig. 3. Voltage outputs of SMFCs during the 240 days operation. The fixed external resistance was 100 Ω.

were only around 2.5–2.6 mg L^{-1}. Compared to the maximum acetate concentrations, lactate and propionate concentrations in the entire period were relatively low. Lactate with concentrations of 0.35–0.37 mg L^{-1} accumulated in the sediment pore water at the beginning, and then was consumed gradually with experiments for SCB1–4. Profiles of propionate concentrations in SCB1 and SCB2 followed similar pattern, while the change of propionate concentrations in SCB3 was almost same as that in SCB4.

3.5. Potential activities of PAH degradation under various redox conditions

After 240 days of experiments, sediment samples from SCB1–4 were taken to test potential activities of PAH biodegradation under various anaerobic redox conditions. As shown in Table 3, Fe(III) addition in SCB2 improved potential activities of phenanthrene biodegradation under ferric and sulfate reduction conditions, and inhibited potential activities of phenanthrene biodegradation under nitrate reduction and methanogenic conditions to some extent, but potential activities of pyrene under the four different anaerobic redox conditions were not affected. Compared to Fe(III) amendment, deployment of SMFC increased potential activities of phenanthrene biodegradation under ferric reduction, and decreased the potential activities of phenanthrene and pyrene biodegradation under sulfate reduction. Combined application of ferric addition and SMFC enhanced potential activities of phenanthrene and pyrene biodegradation under nitrate reduction, ferric reduction, and sulfate reduction conditions. However, potential activities of phenanthrene and pyrene biodegradation under methanogenic condition were inhibited as compared to the control treatment.

3.6. Potential of microbial ferric iron reduction in sediments

At the end of experiments, sediment from SCB1–4 was sampled for measurement of potential of the microbial Fe(III)-reducing activity. As shown in Table 2, potential of microbial Fe(III) reduction rates for sediments taken from SCB1 to SCB4 were increased with the highest value of 0.0982 mM d^{-1} in SCB4.

3.7. Changes in properties of sediment organic matter

SOM in sediments at the end of experiments were measured as shown in Table 2. While HA, FA, and PA in sediments

Table 3
Removal efficiencies of PAH mixture after 6 days of incubation under various redox conditions for sediment samples taken from sediment column bioreactors (SCBs). (Initial phenanthrene concentration: 500 μg L^{-1}; initial pyrene concentration: 500 μg L^{-1}.) Data are mean ± standard deviation.

Redox condition[a]	PAH							
	Phenanthrene removal efficiency (%)				Pyrene removal efficiency (%)			
	SCB1	SCB2	SCB3	SCB4	SCB1	SCB2	SCB3	SCB4
NRC	94.74 ± 0.46	89.78 ± 0.32	91.44 ± 0.72	96.56 ± 0.32	84.68 ± 2.62	85.32 ± 2.18	86.38 ± 0.76	92.78 ± 0.28
FRC	82.46 ± 2.1	87.24 ± 1.5	92.66 ± 3.04	94.94 ± 0.64	79.84 ± 2.02	80.28 ± 0.72	84.46 ± 1.84	89.74 ± 0.84
SRC	53.14 ± 2.42	61.36 ± 2.06	39.88 ± 1.38	60.82 ± 4.06	57.84 ± 1.04	58.80 ± 3.86	53.88 ± 4.02	59.92 ± 0.76
MC	59.14 ± 1.9	51.62 ± 3.86	50.26 ± 4.42	52.30 ± 2.62	49.16 ± 2.24	45.00 ± 3.16	48.18 ± 5.78	47.54 ± 6.42

[a] NRC, nitrate reduction condition; FRC, ferric iron reduction condition; SRC, sulfate reduction condition; MC, methanogenic condition.

in SCB1–3 did not show significant difference, any of three parts of SOM in sediments in SCB4 were obviously less than those in SCB1–3. In addition, the ratios of HA/FA and HA/PA in sediments in SCB4 were much higher than those in SCB1–3.

Information on chemical property in HA fractions of SOM was obtained from FTIR spectral analysis as presented in Fig. 5. It was clear that the composition of HA in sediments varied significantly. The transmittance peaks for the biotic control was around 3400 cm^{-1}, corresponding to O–H stretching due to alcoholic,

Table 4
Summary of 16S rRNA gene sequence types obtained from clone libraries of anode biofilms in SCB3 and SCB4.

Cluster	No. in library (frequencies)			Closest organism		
	SCB3	SCB4	Accession no. of 16S rRNA gene	Name	Percent homology	Description
Betaproteobacteria						
Methylotenera	2	7	CP001672	*Methylotenera mobilis*	98%	Obligate methylamine utilizer
Gallionella	3		EU266812	Uncultured Gallionellaceae bacterium clone	97%	Isolated from sediments
Sideroxydans		1	DQ386264	*Sideroxydans lithotrophicus* ES-1	98%	Oxygen-dependent ferrous iron-oxidizing bacteria
Ralstonia		3	CP001645	*Ralstonia pickettii*	99%	Resistant to exceptionally high concentrations of copper as well as zinc, cadmium, and nickel
Dechloromonas	1		AY032611	*Dechloromonas* sp. JJ	99%	Anaerobic benzene oxidation coupled to nitrate reduction
Deltaproteobacteria						
Desulfobacterium	3	3	FR695868	Uncultured Desulfobacterium sp.	97%	PAH-degrading sulfate-reducing culture N47
Geobacter		2	CP000148	*Geobacter metallireducens*	96%	Dissimilatory Fe(III)-reducing bacteria
Syntrophobacter	3	4	AY651787	*Syntrophobacter sulfatireducens*	96%	Sulfate and thiosulfate both served aselectron acceptors for propionate degradation
Desulfovirga	1	1	NR_036764	*Desulfovirga adipica*	98%	Adipate-degrading, sulfate-reducing bacterium
Gammaproteobacteria	1	2	JN038698	Uncultured Gamma proteobacterium	99%	Isolated from tidal wetland
Nitrospirae						
Nitrospira	17	5	Y14644	*Nitrospira* sp. GC86	99%	Prepared from a nitrite oxidizing sequencing batch reactor
Acidobacteria	2	8	DQ811916	Uncultured Acidobacteria bacterium	99%	Isolated from mangrove title
Chlorobi	3	2	AY693833	Uncultured Chlorobi bacterium	98%	Isolated from anaerobic sludge
Chloroflexi	12	6	HQ397029	Uncultured Chloroflexi bacterium	98%	Isolated from haloalkaline soil
Firmicutes						
Clostridium		2	CP002416	*Clostridium thermocellum* DSM 1313	95%	Cellulolytic anaerobic thermophile bacteria
Verrucomicrobia	2	2	DQ676379	Uncultured Verrucomicrobia bacterium	97%	Isolated from suboxic freshwater pond
Bacteroidetes	3	1	CU926896	Uncultured Bacteroidetes bacterium	98%	Isolated from sludge
Planctomycetes	2	5	GU230455	Uncultured Planctomyces sp. clone	98%	Isolated from Atlantic Ocean
Unknown						
Cluster I		2	FJ269099	Iron-reducing bacterium enrichment culture colne	98%	Potential iron reducer
Cluster II	1		EF515473	Uncultured bacterium clone 24h02	100%	Isolated from electricigen enrichment in a MFC

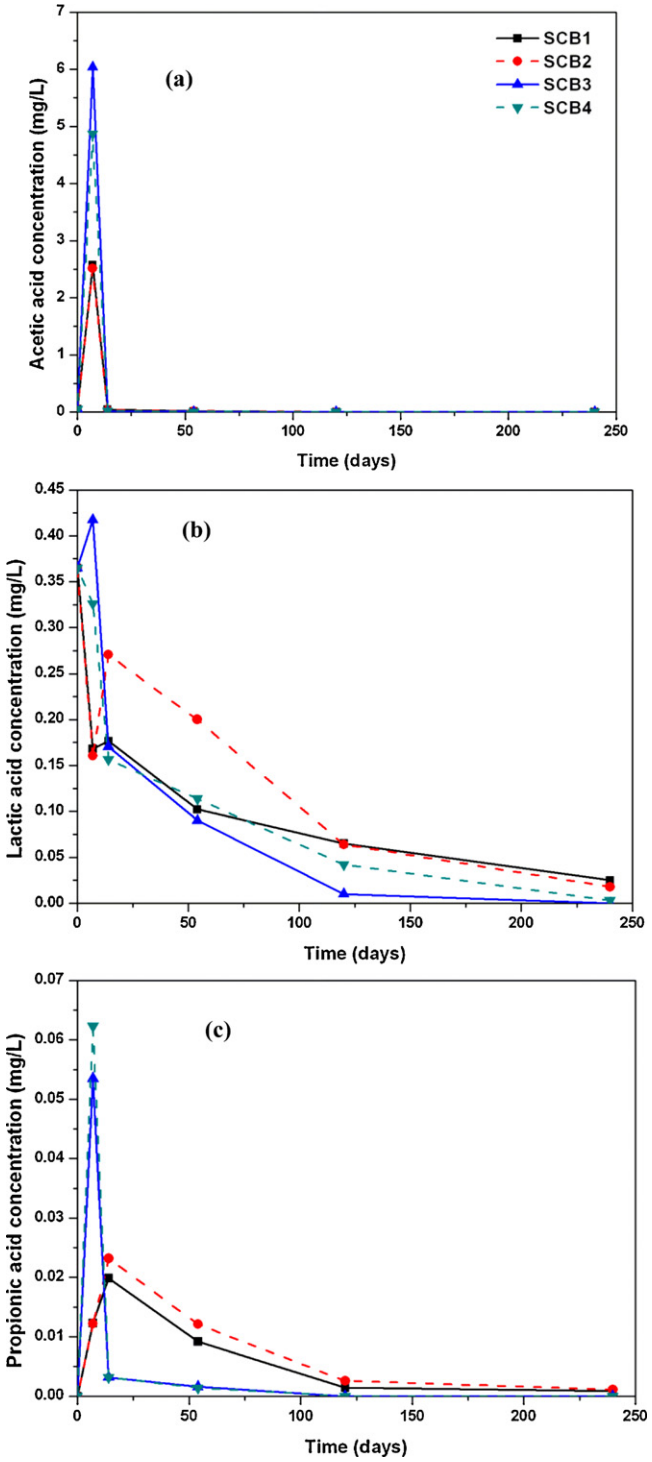

Fig. 4. The concentrations of acetate (a), lactate (b), and propionate (c) in pore water of sediments under various treatments.

phenolic, acid groups, and associated hydrogen bonds, was more pronounced than those for Fe(III) addition and/or SMFC employment. The peaks between 1700 and 900 cm^{-1}, ascribed to carboxyl C=O stretching, aromatic C=C stretching, O–H stretching for alcohols and carboxyl acids, and C–O stretching for carboxylic and arylethers, were stronger for SCB4 than for SCB1–3, suggesting relatively higher molecular weight of HA in SCB4. It should be noted that the peak between 1650 and 1600 cm^{-1}, which is assigned to C=C bonds of aromatic structures, became more pronounced in SCB4 compared to SCB1–3.

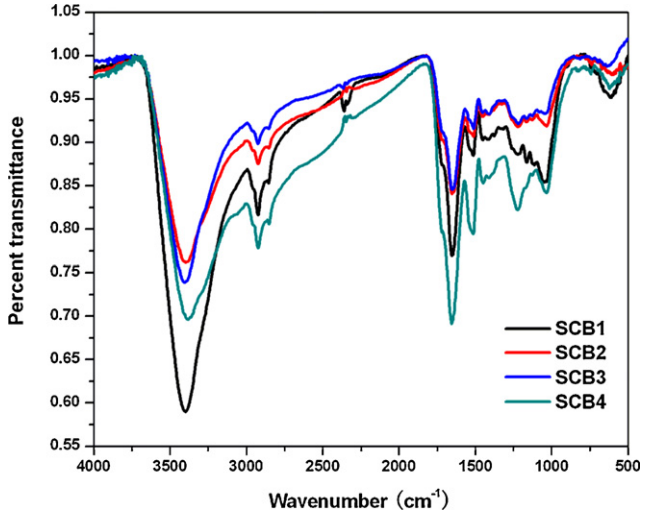

Fig. 5. Comparison of infrared spectra for humic acid fractions of SOM in sediment column bioreactors.

3.8. Microbial community analysis of anode in SMFC

Microbial communities of anode biofilms in SMFCs in SCB3 and SCB4 analyzed through construction of clone libraries as shown in Table 4. And phylogenetic relationship with closely related bacterial strains in SMFCs in SCB3 and SCB4 was shown in Fig. S1 (Supporting information). Significant differences in bacterial community composition were observed between clone libraries. Phylogenetic analysis indicated that clones retrieved from anode biofilm in SMFC in SCB4 were dominated by *Beta-* and *Delta-proteobacteria* with frequencies of 19.6% and 17.8%, respectively. However, anode biofilm in SCB3 was dominated by *Nitrospira* and *Chloroflexi* with frequencies of 30.3% and 21.4%, respectively. Clones affiliated with organisms in genus *Geobacter*, which were capable of dissimilatory iron reduction and/or electrode-reducing, were only found in the anode biofilm library in SCB4.

4. Discussion

This study demonstrated that the employment of SMFC could obtain higher removal efficiencies of PAHs in freshwater sediments than Fe(III) addition. More importantly, the combination of SMFC employment and Fe(III) addition was able to further improve removal of PAHs in sediments. SMFC employment and Fe(III) addition caused difference influences on sediment environments. Employment of SMFC meant addition of one new anaerobic respiration pathway with electrode as electron acceptor namely electrode-reduction in sediments [27]. Anodes in SMFCs could act as a stable, long-term permanent electron acceptor, and then the oxidation of the organic compounds within sediments is no longer limited by the availability of electron acceptors within the sediment.

In addition, SMFC is characterized with the spatial separation of the oxidative, electron-generating half-reaction at the anode and the electron-consuming half-reaction with oxygen at the cathode [28]. In this way, application of SMFCs technology might be similar to replenish oxygen into sediment. Thus, a relatively high value of an anodic redox potential could be obtained, and the anode as electron acceptor appeared to be more thermodynamically favorable than using ferric as electron acceptor. For the same reason, SMFC could suppress sulfate-reducing process and lead to a substantial decrease in methanogenic activity in sediments [28].

As shown in Fig. 2, sulfate reduction in SCB3 during the initial 125 days was completely suppressed through the employment of

an electron-capturing anode. During this period, sulfate concentrations within sediments in SCB3 did not decrease but increase; this might be due to the microbial oxidation of sulfide/sulfur to sulfate stimulated in MFC [28]. However, such effect on sulfate reduction did not happen in SCB4 with combination of Fe(III) addition and electrode employment. In addition, this combined treatment led to the highest potential iron-reducing activity for sediments in SCB4 at the end of experiments. In fact, improvement of iron-reducing activity in SCB4 was not surprising, considering that many of the electrode-reducing microorganisms enriched in SMFCs were dissimilatory metal-reducing bacteria [29].

PAHs were found to be anaerobically biodegraded under nitrate-reducing, iron-reducing, sulfate-reducing, and methanogenic conditions [11], thus it seems likely that PAHs would also be directly oxidized under electrode-reducing condition [8,30]. According to the change in three types of electron acceptor concentrations during the entire experiment, it seemed clear that several metabolic pathways had been involved in the PAH biodegradation process. In SCB1 as the natural attenuation control, a 78.3% of PAHs removal indicated that indigenous microbial population really possessed ability to metabolize PAHs in sediments. Table 1 showed relatively higher potential activities of PAHs degradation under nitrate-reducing, ferric-reducing, and sulfate-reducing condition for sediments in SCB4 compared to sediments in SCB1, which suggested that enhanced PAHs removal in SCB4 was attributed to not only electrode-reducing process but also other anaerobic redox reactions. From this point, electrode reduction and other anaerobic pathways could coexist in sediments, and were not necessarily mutually exclusive.

Like other PAHs bioremediation studies [31–33], similar biphasic degradation curves with an initial phase of rapid PAH mixture degradation followed by a phase of much slower transformation, have been observed in all SCB1–4 in this study. The initial rapid phase of degradation was primarily due to microbial transformation of the most readily available and rapidly desorbed fraction of PAHs [31,34]. The slow degradation rate in the second phase seemed not the result from the shortage of electron acceptors as ion concentrations of electron acceptors were still high after the first phase, and generated electron could be flowed to electrode in the whole phase in SCB3 and SCB4. A possible mechanism of the slow rate of PAHs in the second phase was the lack of readily degradable carbon source like acetate, which otherwise could act as co-substrate to stimulate PAH degradation.

Actually, the second degradation phase was probably governed by slow desorption of PAH compounds from SOM for the microbial degraders [35]. It was recognized that the affinities of PAHs to different fractions of SOM were extraordinarily different, and PAHs adsorbed to HA became more easily sequestrated with non-bioavailable than FA. Redox conditions mediated by indigenous soil microorganisms always altered the physical and chemical properties of the SOM substantially [25,36]. With the experimental duration extended, PAHs adsorbed to less humificated SOM (like FA) would be accessible to microbial degradation with decomposition of these SOMs, or gradually translocated from FA to HA and finally to the humic fractions [3]. The humification was found to be more stimulated under combined treatment conditions, confirmed by higher HA fraction, larger molecule size, and higher aromaticity, and less oxidized functional groups. Therefore, residual PAHs in combined treatment possessed less bioavailable possibility than those in the other treatments, or even were stabilized with reduced environmental risks.

5. Conclusions

Microorganisms in freshwater sediments preferred to use the electrodes in SMFCs over amorphous ferric hydroxide added as an electron acceptor for degradation of PAHs. Application of iron and electrode as electron acceptor could work together with synergy for PAHs biodegradation with removal efficiencies of phenanthrene and pyrene reaching to $99.47 \pm 0.15\%$ and $94.79 \pm 0.63\%$ respectively after 240 days of experiments. In addition, the combination strategy of SMFC employment and Fe(III) addition improved the potentials of PAHs biodegradation within sediments under anaerobic pathways except methanogenic condition, and stimulated humification of SOM in sediments. Considering both enhanced biodegradation and final stabilization of PAHs in sediments, the combined application of Fe(III) addition and SMFC employment offered a new promising remediation technology for contaminated sediments.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jhazmat.2011.10.087.

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