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Assessment of the performance of SMFCs in the bioremediation of PAHs in contaminated marine sediments under different redox conditions and analysis of the associated microbial communities

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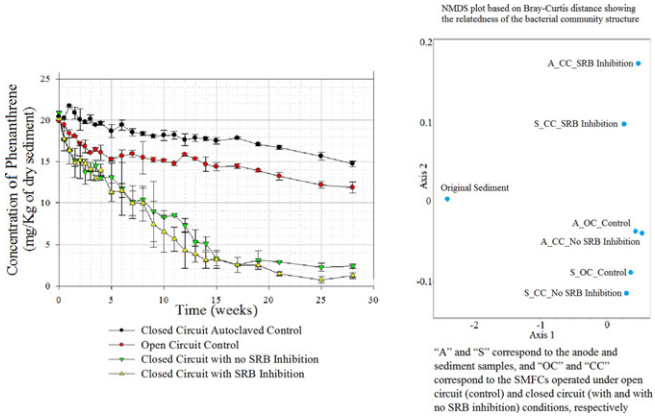
HIGHLIGHTS

- Successful remediation of PAHs in contaminated marine sediments using SMFCs
- High removal of PAHs was achieved when anodes acted as sink for electrons.
- Significant variation in microbial composition among the operating conditions
- Enrichment of exoelectrogenic bacteria in closed circuit SRB inhibited SMFCs.

GRAPHICAL ABSTRACT



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ABSTRACT

The biodegradation of naphthalene, 2-methylnaphthalene and phenanthrene was evaluated in marine sediment microbial fuel cells (SMFCs) under different biodegradation conditions, including sulfate reduction as a major biodegradation pathway, employment of anode as terminal electron acceptor (TEA) under inhibited sulfate reducing bacteria activity, and combined sulfate and anode usage as electron acceptors. A significant removal of naphthalene and 2-methylnaphthalene was observed at early stages of incubation in all treatments and was attributed to their high volatility. In the case of phenanthrene, a significant removal (93.83 ± 1.68%) was measured in the closed circuit SMFCs with the anode acting as the main TEA and under combined anode and sulfate reduction conditions (88.51 ± 1.3%). A much lower removal (40.37 ± 3.24%) was achieved in the open circuit SMFCs operating with sulfate reduction as a major biodegradation pathway. Analysis of the anodic bacterial community using 16S rRNA gene pyrosequencing revealed the enrichment of genera with potential exoelectrogenic capability, namely *Geoalkalibacter* and *Desulfuromonas*, on the anode of the closed circuit SMFCs under inhibited SRB activity, while they were not detected on the anode of open circuit SMFCs. These results demonstrate the role of the anode in enhancing PAHs biodegradation in contaminated marine sediments and suggest a higher system

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efficiency in the absence of competition between microbial redox processes (under SRB inhibition), namely due to the anode enrichment with exoelectrogenic bacteria, which is a more energetically favorable mechanism for PAHs oxidation than sulfate.

1. Introduction

Among all pollutants that get adsorbed to the marine sediments, a special concern is given to Polycyclic Aromatic Hydrocarbons (PAHs) due to their mutagenic, carcinogenic and teratogenic potentials to humans, fauna and flora (Botsou and Hatzianestis, 2012; Man et al., 2013; Thavamani et al., 2012). PAHs are organic pollutants composed of two or more fused aromatic benzene rings that are mainly present in petroleum and petroleum derived products such as asphalt and tar. These pollutants tend to be ubiquitous in the environment and they enter through natural and anthropogenic pathways. PAHs tend to adsorb readily to sediments and soils due to their hydrophobic chemical structure and low water solubility, which renders them stable and persistent in the environment. They also tend to accumulate in fat tissues of food chains such as fish and other aquatic organism, thus extending their effect to human consumers of polluted food (Ene et al., 2012; Mahmoudi et al., 2013).

The natural degradation of PAHs involves several processes, including volatilization, photo-oxidation, chemical oxidation, bioaccumulation, adsorption, and biodegradation (Balachandran et al., 2012). Low molecular weight PAHs have been reported to degrade readily by bacteria and fungi (Amezcua-Allieri et al., 2012) and even by algae (Mahmoudi et al., 2013). Taking advantage of such natural bioprocesses to enhance the degradation of PAHs is a potential inexpensive method for increasing the rate of removal of these contaminants in polluted environments without producing toxic byproducts (Amezcua-Allieri et al., 2012). Yet, the non-polar nature of PAHs, their high affinity for organic matter and their low water solubility make them poorly available to microorganisms, especially when they go through the process of aging by partitioning onto sediments or soil particles and diffusing into micropores (Mahmoudi et al., 2013).

Several studies have investigated the biodegradation of PAHs in sediments under anaerobic conditions using sulfate, nitrate or iron (III) as terminal electron acceptors (TEAs) (Chang et al., 2002; Langenhoff et al., 1996; Lei et al., 2005; Townsend et al., 2003). Due to its abundance in marine sediments, sulfate was found to be the predominant anaerobic TEA used in the biodegradation of PAHs (Townsend et al., 2003), and high populations of sulfate reducers were associated with contaminated sediments in most instances (Lei et al., 2005). However, in heavily contaminated marine sediments, the biodegradation process is limited by the availability of indigenous sulfate that may be depleted rapidly and needs to be frequently replenished (Rothermich et al., 2002). Moreover, aerobic biodegradation of PAHs is limited by the low availability of oxygen in the marine sediments (Yan et al., 2012).

In 2001, a new system increasing the rate of the anaerobic biodegradation of organic matter in aquatic sediments was introduced and called sediment microbial fuel cells (SMFCs) (Logan, 2008; Reimers et al., 2001). It consists of an anode buried in the anaerobic marine sediments and connected to a cathode suspended in the overlying oxygen-rich water. Indigenous exoelectrogenic bacteria can grow attached to or in the vicinity of the anode to which they transfer the electrons generated from the oxidation of organic compounds. The electrons then migrate to the cathode producing an electric current (Logan, 2008). The high ionic strength of seawater-sediment medium allows cations (H^+) produced during organic matter biodegradation to migrate towards the cathode where they combine with oxygen and electrons to form H_2O (Hong et al., 2010).

The main factors that affect the viability and efficiency of a SMFC are the electrode materials and oxygen availability at the cathode. Thus, the selection of an appropriate combination of materials may dictate the success or failure of the SMFC to achieve its intended objective. The electrode materials in microbial fuel cells (MFCs) must be highly conductive, non-corrosive, non-toxic to microorganisms, and inexpensive (Rezaei et al., 2007). Electrode materials with high specific surface area (area per unit volume) and an open structure (high porosity) are deemed the most important, as the former provides a large area for microorganisms to grow and thus enhances removal efficiencies, and the latter prevents biofouling. Typical electrode materials used in SMFCs are carbon felt, carbon cloth, graphite plates, and stainless steel (Liu et al., 2016; Logan et al., 2007; Najafgholi and Rahimnejad, 2016; Sherafatmand and Ng, 2015; Yan et al., 2012; Zhou et al., 2016). In this study, carbon fiber brushes were used as electrodes in the SMFCs. Compared to the commonly reported carbon-based electrodes in PAHs bioremediation, these electrodes offer a significantly higher surface area available for anodic microbial growth, and an increased interaction of cathodic electrons with oxygen, contributing to the enhancement of the biodegradation process.

Reported literature on the use of SMFCs for the bioremediation of PAHs in aquatic sediments taking advantage of the intrinsic microbial communities is limited and namely focused on contaminated freshwater sediments. In one recent study, the bioremediation of PAHs in a lake sediments were investigated under aerobic and anaerobic conditions provided in the cathodic chamber and the authors reported removal efficiencies of 41.7%, 31.4% and 36.2% PAHs in aerobic environment and 76.9%, 52.5% and 36.8% in anaerobic environment for naphthalene, acenaphthene and phenanthrene, respectively (Sherafatmand and Ng, 2015). In another study conducted by Yan et al. (2012), the degradation of phenanthrene and pyrene in freshwater sediment was investigated under three types of treatments including the addition of amorphous ferric hydroxide to sediments, employment of 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 ($99.47 \pm 0.15\%$) and pyrene ($94.79 \pm 0.63\%$), while the employment of SMFC could obtain higher removal efficiencies than Fe(III) addition.

While iron reduction prevails in low-sulfate freshwater systems, sulfate reduction constitutes the major terminal electron accepting process in sulfate-rich marine ecosystems (Laufer et al., 2016). This has significant implications in determining the microbial community structure in SMFCs in each aquatic environment and consequently the fate and removal kinetics of organic pollutants in contaminated sediments. In addition, the high salinity in seawaters plays an important role in determining many aspects of the water chemistry and biological processes within it. The halophilic sulfate-reducing *Desulfuromonas* species are usually enriched on the anodes of marine SMFCs in addition to *Desulfobulbus* and *Desulfocapsa*, while *Geobacter* species predominate in freshwater SMFCs (Chan and Li, 2014; De Schamphelaire et al., 2008; Jung et al., 2014).

In this study, we investigate the use of SMFCs for the bioremediation of PAHs in contaminated marine sediments where hydrocarbons degradation is mainly coupled to sulfate reduction, and we assess the system performance and degradative organisms' diversity in the presence and absence of competition between microbial redox processes. For this aim, the degradation of naphthalene, 2-methylnaphthalene and phenanthrene was investigated in marine SMFCs under different biodegradation conditions including sulfate reduction as major biodegradation pathway, employment of anode as TEA under inhibited sulfate reducing bacteria (SRB) activity, and combined sulfate and anode usage as

electron acceptors. Carbon fiber brushes were used as electrodes to enhance the biodegradation process in the SMFCs. The microbial communities associated with the PAHs biodegradation under the different adopted treatment schemes were determined using 16S rRNA gene pyrosequencing. Naphthalene, 2-methylnaphthalene and phenanthrene were tested for being the major lingering PAHs detected in the contaminated marine sediments used in this study.

2. Materials and methods

2.1. Sediments and seawater collection and characterization

Anaerobic sediments were collected from a previously contaminated site next to the Jiyeh thermal power station in Lebanon, where > 15,000 tons of heavy fuel oil were released from the plant's storage tanks into the eastern Mediterranean Sea polluting 160 Km of coastline in 2006 (Maslo et al., 2014; Shaban et al., 2007). The sampling location is presented in Fig. S1 in Supplementary information (SI).

Grab sediment samples were collected at a depth of about 7 m below the water surface and 30 cm below the water-sediment interface, about 500 m off the shoreline. The top layer of the sediments was discarded for potential exposure to oxygen and the sediments were then sieved through a 2 mm sieve in an anaerobic glove box. Sediment samples were extracted with 50 mL of deionized water, analyzed, and exhibited pH 8.33 ± 0.014, sulfates 1130.2 ± 14.1 mg/kg of dry sediment, nitrates 6.6 ± 0.6 mg/kg of dry sediment, total nitrogen 12.2 ± 0.4 mg/kg of dry sediment, iron 4.3 ± 0.3 mg/kg of dry sediment, organic content 2.7 ± 0.1% of dry sediment, wet density 2.02 ± 0.01 g/mL, and dry density 1.57 ± 0.02 g/mL. Sulfides, nitrites and phosphates were below detection limits in the sediments. In addition, seawater samples collected at 1 m above the water-sediment interface were analyzed for the same parameters and the results are presented in SI (Table S1).

Background levels of PAHs in the sediments were determined following the method described by Yan et al. (2012). PAHs were extracted with methanol and analyzed by High Performance Liquid Chromatography (HPLC) equipped with a Diode Array Detector DAD (Agilent 1100). Chromatographic analysis was performed using a C18 column (DISCOVERY HS C18, 25 cm, 4.6 mm, 5 μm). An isocratic elution was carried out using 90% methanol and 10% water at a flow rate of 1 mL/min. The column temperature was maintained at 30 °C. Background PAHs prevailing in the marine sediments consisted of naphthalene (0.20 ± 0.06 mg/kg of dry sediment), 2-methylnaphthalene (0.049 ± 0.008 mg/kg of dry sediment), and phenanthrene (0.063 ± 0.004 mg/kg of dry sediment), which were best detected at 220 nm (naphthalene and 2-methylnaphthalene) and 251 nm (phenanthrene). These PAHs were hence used for further testing in the SMFCs.

2.2. SMFCs construction and operation

Laboratory scale SMFCs were constructed and consisted of 0.9 L Plexiglas reactors (10L × 6W × 15H cm) filled with composite samples of 1:1 (v/v) sediments/seawater. Marine sediments used in the SMFCs were spiked with a solution of naphthalene, 2-methylnaphthalene, and phenanthrene prepared in methanol. The sediments were spiked under anaerobic conditions while being mechanically mixed at low speed for 1 h to achieve a homogeneous distribution of the PAHs. Spiking was performed to achieve an initial concentration of 20 mg/kg of dry sediments of each of the PAHs, representing relatively high levels of contamination of marine sediments. However, due to their high volatility enhanced by the mixing process, achieved initial concentrations of naphthalene and 2-methylnapthalene were only 2.5 and 9 mg/kg of dry sediments, respectively.

The SMFCs were filled with spiked marine sediments to a depth of 7 cm. Seawater was then added to form a 7 cm layer on the top of the sediments. In each SMFC, an anode and a cathode consisting of carbon fiber brushes (6L × 6D cm) twisted around a titanium wire (Mill-Rose Company; Ohio, USA) were placed at 3.5 cm below and above the water-sediment interface, respectively. This ensured the placement of the anode under complete anaerobic conditions and a complete submersion of the cathode in the water column, which was continuously aerated to maintain a dissolved oxygen concentration above 2 mg/L at the cathode level. A schematic diagram of the SMFCs is presented in Fig. S2 in SI. The sediments were equilibrated with the overlying seawater for 1 day before connecting the anode to the cathode via a fixed resistor of 10 Ω in the closed circuit SMFCs. SMFCs were operated at controlled room temperature (20 ± 1 °C) and in the dark to prevent photolysis. An autoclaved phosphate buffer and nutrient solution ($NaH_2PO_4 \cdot H_2O$, 0.75 g/L; NH_4Cl, 0.31 g/L) was added periodically to the seawater to provide N and P source for bacterial growth and to compensate for the water lost through evaporation.

Four sets of duplicate SMFCs were prepared. Two sets were operated under closed circuit condition to evaluate the role of the anode as a final electron acceptor in the biodegradation of PAHs. Molybdate, a SRB inhibitor, was added to one of the sets to assess the efficiency of the anode as the main TEA in PAHs biodegradation. The other two sets constituted control SMFCs. One control set was operated under open circuit condition to assess PAHs degradation associated with the activity of indigenous SRB in the absence of the anode as a potential TEA (natural attenuation). The other control set was operated under closed circuit condition using autoclaved sediments and seawater to evaluate the role of abiotic removal of PAHs, namely through volatilization.

PAHs and sulfates concentrations in the sediments were monitored throughout the biodegradation experiments which lasted for 28 weeks. The voltage was recorded every 15 min across a fixed load (10 Ω) using a multimeter with a data acquisition system (Model 2700; Keithley Instruments Inc., United States), and the obtained data were averaged on a daily basis.

2.3. Microbial community analysis

At the end of the experiments, all SMFCs were sacrificed and the microbial communities in the sediments and anodes were characterized using 16S rRNA gene pyrosequencing. The top half centimeter layer of the sediments in contact with oxygen in the overlying water column was discarded and the remaining anaerobic sediments in each SMFC were mixed for homogenization. Triplicate sediment samples from each SMFC were then used for DNA extraction using the PowerSoil® DNA Isolation Kit (MO BIO Laboratories) according to the manufacturer's instructions. Similarly, DNA was extracted from the carbon fibers cut randomly from the anodes of the different SMFCs. For each extracted DNA sample (sediment and anode), triplicate PCR reactions were performed in a 25 μL reaction volume using the HotStarTaq Plus Master Mix (QIAGEN, Valencia, CA), 0.25 μM of primer and 20 ng of template DNA. Bacterial 16S rRNA genes were amplified using the bacteria-specific forward primer 341F (5′-Adaptor A-Barcode-CA Linker- CCTACGGGNGGCWGCAG-3′) and reverse primer 805R (5′-Adaptor B-TC Linker-GACTACHVGGGTATCTAATCC-3′) (Klindworth et al., 2013). These primers targeted the V4 region of the bacterial 16S rRNA gene. PCR was performed using Life Technologies Veritus Thermal Cycler with the following PCR conditions: 94 °C for 3 min, followed by 28 cycles at 94 °C for 30 s each; 53 °C for 40 s and 72 °C for 1 min; after which a final elongation step at 72 °C for 5 min was performed. Following PCR, all amplicon products from different samples were mixed in equal concentrations, purified using Agencourt Ampure beads (Agencourt Bioscience Corporation, MA, USA), and pyrosequenced on the Roche 454 FLX Titanium genome sequencer (Roche, Indianapolis, IN) according to the manufacturer's instructions.

The 16S rRNA gene sequences were processed using a proprietary analysis pipeline (www.mrdnalab.com, MR DNA, Shallowater, TX). Raw reads were first demultiplexed to trim the barcodes and primers and then low quality sequence reads outside the bounds of 200 and 1000 bp, sequences containing >6 ambiguous base or 6 homopolymers,

and sequences with quality score <25, were removed. Sequences were then denoised and chimeras removed. Sequences were clustered into operational taxonomic units (OTUs) after removal of singletons with a 97% sequence identity threshold. A representative sequence from each OTU was phylogenetically assigned to a taxonomic identity using BLASTn against a curated GreenGenes database (DeSantis et al., 2006). Nonmetric multidimensional scaling (NMDS) was used to compare the similarity between the different samples based on OTUs using the Community Analysis Package 4.0 (PISCES Conservation Ltd., UK). The NMDS plot was generated based on Bray–Curtis dissimilarity index (Bray and Curtis, 1957) after square root transformation of the relative abundance of OTUs.

3. Results and discussion

3.1. SMFCs experiments

3.1.1. Degradation of PAHs in the SMFCs

Biodegradation of the PAHs was monitored through the temporal variations of the concentration of the contaminants in the sediments. Biodegradation curves of naphthalene, 2-methylnaphthalene, and phenanthrene are shown in Fig. 1.

Naphthalene and 2-methylnaphthalene exhibited similar pattern of removal from the sediments in all SMFCs, with no significant ($p > 0.05$) difference in the achieved PAHs removal being observed among the different treatments in each case (Fig. 1a, b). Within less than a week, most of the naphthalene disappeared and its concentration dropped significantly from 2.5 to <0.75 mg/kg of dry sediment even under autoclaved conditions (Fig. 1a). A slower rate of removal of the PAH was then observed with the concentration of the contaminant decreasing to about 0.2 mg/kg of dry sediment after four weeks and reaching <0.1 mg/kg of dry sediment after 28 weeks. In the case of 2-methylnaphthalene (Fig. 1b), the contaminant level dropped from 9 mg/kg to <6 mg/kg of dry sediment in all SMFCs after the first week of incubation, followed by a slower degradation rate of the contaminant which concentration decreased to about 4 mg/kg of dry sediment after 4 weeks of incubation and reached <0.7 mg/kg of dry sediment at the end of the biodegradation experiments.

The first order decay rates for naphthalene and 2-methylnaphthalene, calculated excluding the first week of SMFCs operation associated with the fast loss of the contaminants, are shown in Table 1.

The decay rates for the two PAHs were almost the same in the autoclaved SMFCs and under natural attenuation conditions, and slightly higher under closed circuit SMFCs. The similar pattern in the drop in the concentration of naphthalene and 2-methylnaphthalene under the different treatments suggests that their removal is associated with abiotic processes, namely volatilization, rather than with biodegradation. Volatilization has been reported to be the major process involved in the removal of low molecular weight PAHs from contaminated sediments and soils (Mohan et al., 2006; Wick et al., 2011). The slightly higher decay constants observed under closed circuit conditions (with or without SRB inhibition) is due to the simultaneous effect of volatilization and the anode induced microbial degradation.

The effect of the different SMFCs treatments on the degradation of PAHs was better observed with phenanthrene (Fig. 1c), a relatively stable semi-volatile 3-ring PAH (Zaugg et al., 2006). Minimal removal of the PAH was observed in the autoclaved SMFCs and was associated with abiotic losses. In the case of the SMFCs operating under natural attenuation conditions, a slow rate of PAH removal ($k = 0.0021\ d^{-1}$) was observed with the concentration of phenanthrene reaching only 12 mg/

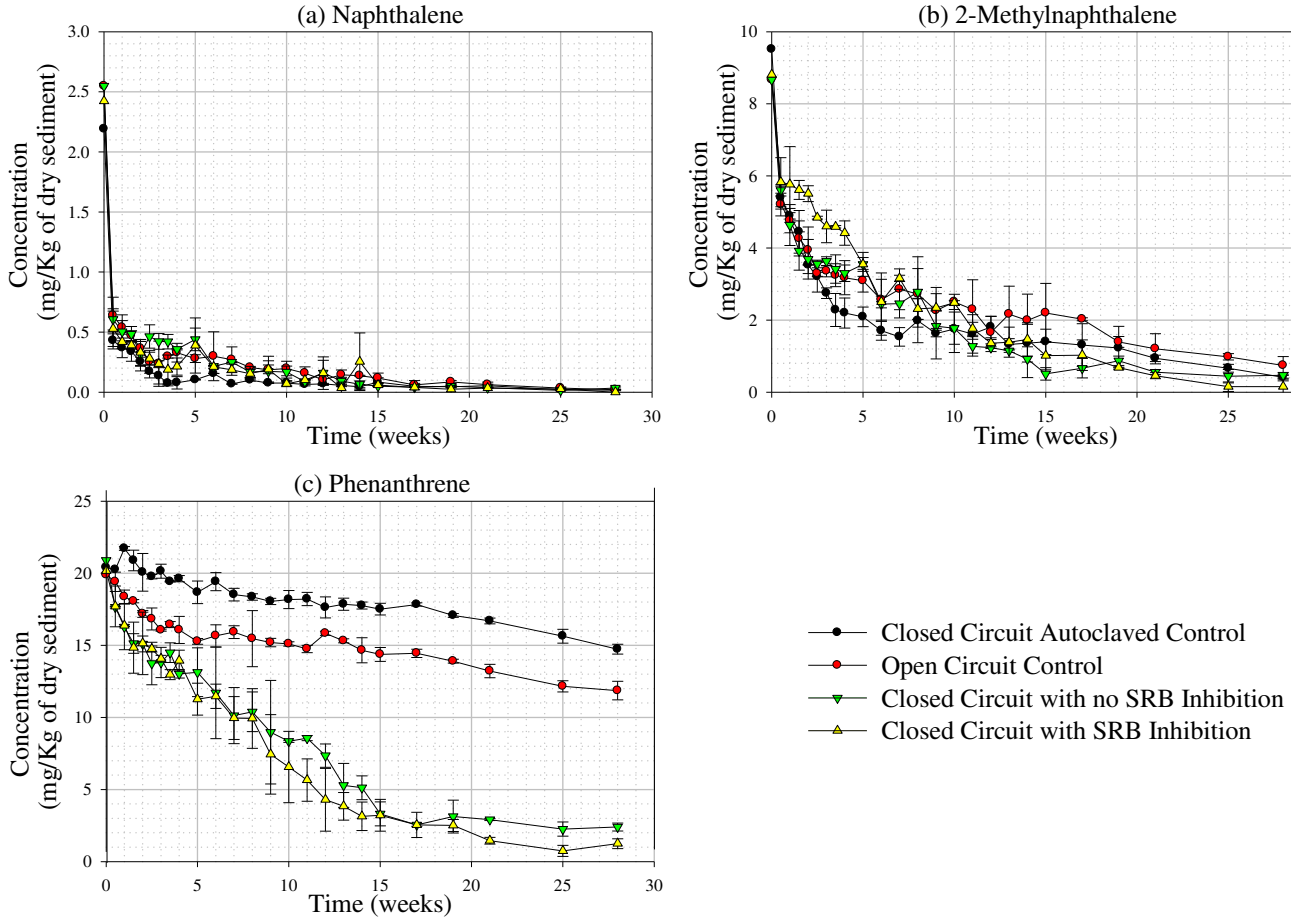

Fig. 1. Concentration profile of PAHs in the SMFCs during 28 weeks of operation.

Table 1
Decay constants of studied PAHs in the SMFCs.

SMFCs treatments	Naphthalene		2-Methylnaphthalene		Phenanthrene	
	k	r^2	*k*	r^2	*k*	r^2
Closed circuit autoclaved control	0.0124 ± 0.0007	0.73	0.0099 ± 0.0011	0.87	0.0016 ± 0.00007	0.92
Open circuit control	0.0145 ± 0.0018	0.92	0.0084 ± 0.0009	0.93	0.0021 ± 0.0001	0.86
Closed circuit with no SRB inhibition	0.0190 ± 0.0016	0.95	0.0141 ± 0.0026	0.91	0.0124 ± 0.0010	0.93
Closed circuit with SRB inhibition	0.0170 ± 0.0026	0.87	0.0204 ± 0.0067	0.97	0.0168 ± 0.0021	0.96

k, first order decay constant of PAH degradation ± standard error between duplicate SMFCs; r^2, correlation coefficient.

kg of dry sediment after 28 weeks. Removal of phenanthrene significantly increased in the closed circuit SMFCs operating with and without SRB inhibition, with the PAH concentration reaching 1.2 and 2.4 mg/kg of dry sediment at the end of the biodegradation experiments, respectively (Fig. 1c). A relatively steep drop in the contaminant concentration was observed up to week 17 of incubation and leveled off thereafter. This suggests that in the case of closed circuit conditions, the anode behaved as the major TEA enhancing the biodegradation of phenanthrene. The initial steep drop in the PAH concentration followed by a slower rate of degradation indicates an initial loss of the loosely adsorbed molecules to the sediment and organic matter followed by the gradual loss of the strongly adsorbed molecules as suggested by Mohan et al. (2006). The slightly higher decay constant observed under SRB inhibition (Table 1) suggests that sulfate reduction was not a major sink of electrons from phenanthrene degradation.

3.1.2. Sulfate concentration profile in the SMFCs

The temporal variation in sulfate concentration in the different SMFCs is shown in Fig. 2. Sulfate is reported to be an important TEA in PAHs biodegradation and was initially measured at a considerably higher concentration in the marine sediments compared to other available TEAs (NO_3^{2-}, Fe^{3+}).

A significant sulfate consumption was observed in the absence of anode reduction (i.e. open circuit control). In this case, sulfate concentration decreased from 1130 to 368 mg/kg of dry sediment after 28 weeks of incubation. Higher sulfate concentrations were measured in the closed circuit SMFCs reaching 475 mg/kg of dry sediment under the combined action of anode and SRB, and 590 mg/kg of dry sediment under sulfate reduction inhibition. This suggests that the anode provides a more suitable TEA for PAHs biodegradation. The relatively slight drop in sulfate concentration in the autoclaved SMFCs could be attributed to sulfate diffusion to the overlying water.

It is worth mentioning that the drop in sulfate concentrations under inhibited sulfate reduction conditions could be partly attributed to a partial inhibition of the sulfate reducing bacteria under the extended SMFCs incubation period, allowing for the use of sulfate as TEA with the progress of the biodegradation experiments. Indeed, different authors had reported a temporary inhibition of SRB by molybdate after which sulfate reduction is resumed (de Jesus et al., 2015; Predicala et al., 2008). SRB may actually acclimatize to the presence of molybdate with time as reported by Isa and Anderson (2005). Nevertheless, although a complete SRB inhibition might not have been achieved throughout the experiments duration in the closed circuit SMFCs with added molybdate, a significantly lower sulfate consumption was observed in these microcosms and is associated with a lower activity of sulfate reducing bacteria. The effect of the inhibition of SRB under closed circuit SMFCs conditions was reflected in the bacterial composition associated with this treatment and which was significantly different from the microbial structure observed in the closed circuit SMFCs with no SRB inhibition as later discussed.

3.1.3. Voltage profile in the SMFCs

The variation in the voltage measured across the electrodes of the SMFCs is shown in Fig. 3.

In the autoclaved closed circuit control SMFCs, no voltage was measured at any time during the experiments due to the absence of any microbial activity. In the closed circuit SMFCs with combined anode and sulfate reduction the voltage initially increased rapidly from 0 mV to a maximum value of 0.33 mV after about 4 weeks of operation, then decreased gradually to reach almost zero at the end of the experiments. Higher voltages were attained in the SRB inhibited SMFCs and reached a maximum value of 0.57 mV after about 4 weeks of incubation. This is most likely due to an enhancement of the activity of the anode respiring bacteria in the absence of SRB competition for substrate. In the open circuit SMFCs, the potential difference across the electrodes increased after the launching of the experiments from 100 mV to reach an average value of 450 mV after 35 days. This gradual increase in the potential difference is most probably associated with the change in the SMFCs properties induced by the establishment of microbial communities.

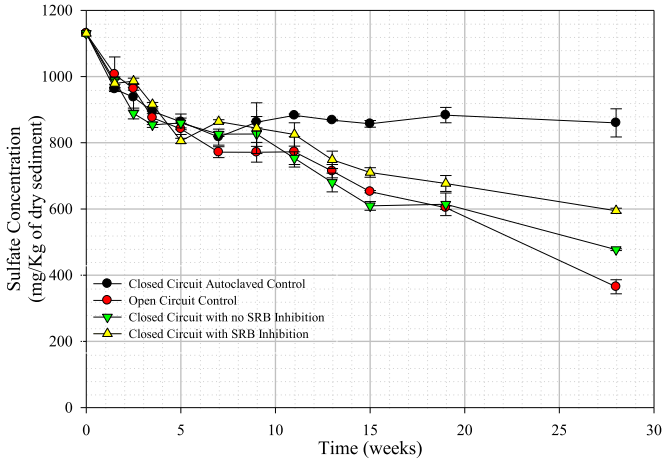

Fig. 2. Sediment sulfate concentration profile in the SMFCs during 28 weeks of operation.

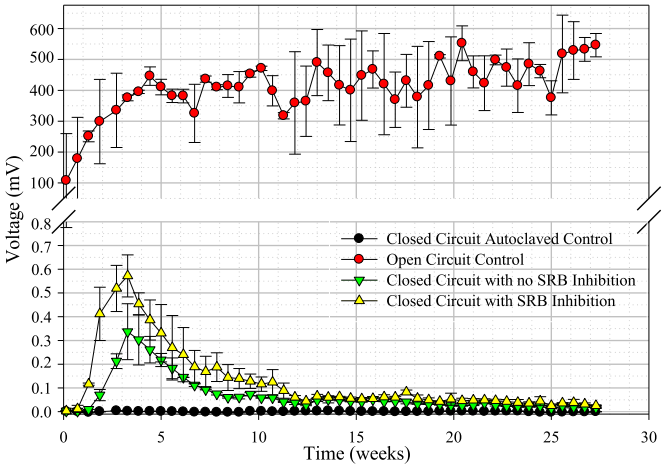

Fig. 3. Voltage profile of the SMFCs over 28 weeks of operation.

3.2. Microbial community analysis

Microbial community analysis was performed on the original collected sediments and on the SMFCs sediments and anodes under the adopted different treatment schemes. A total of 133,823 high-quality reads (Average sequence length ~425 bp) were generated for the samples after denoising, quality filtering and removal of chimeric sequences. The sequences were clustered into 2249 OTUs at a 97% sequence identity threshold. Fig. 4 shows the relative abundance of the identified microbial classes and genera in the original marine sediments and in the sacrificed SMFCs sediments and anodes at the end of the biodegradation experiments.

The original sediments showed the existence of two major bacterial classes, *Gammaproteobacteria* and *Actinobacteria*, composing 32.13% and 28.15% of the total sequence reads, respectively. *Alphaprotebacteria* and *Deltaproteobacteria* were also present at a noticeable percentage, composing respectively 14.29% and 9.74% of the overall microbial diversity. Several other classes were identified in the original sediments at a lower percentage, including *Flavobacteria* (4.93%), *Acidobacteria* (2.51%), *Cyanobacteria* (1.81%) and *Bacilli* (1.08%) (Fig. 4a). Further identification at the genus level showed the existence of *Streptomyces* (15.28%) and *Aciditerrimonas* (8.60%) belonging to the class of *Actinobacteria*, and *Pseudomonas* (7.50%) and *Thioprofundum* (4.82%) belonging to the class of *Gammaproteobacteria* (Fig. 4b). *Streptomyces*, *Aciditerrimonas*, and *Pseudomonas* are commonly reported to be involved in aerobic degradation of aliphatic and aromatic hydrocarbons (Balachandran et al., 2012; Lin et al., 2011; Lv et al., 2014), and their presence in the tested sediments is well correlated to the continuous exposure of the marine shoreline nearby the power plant to petroleum based contamination. In addition, sulfate reducing bacteria (*Desulfosarcina*; 2.29%) and iron reducing bacteria (*Geothermobacter*; 1.41%) were identified under the class of *Deltaproteobacteria*. Aerobic

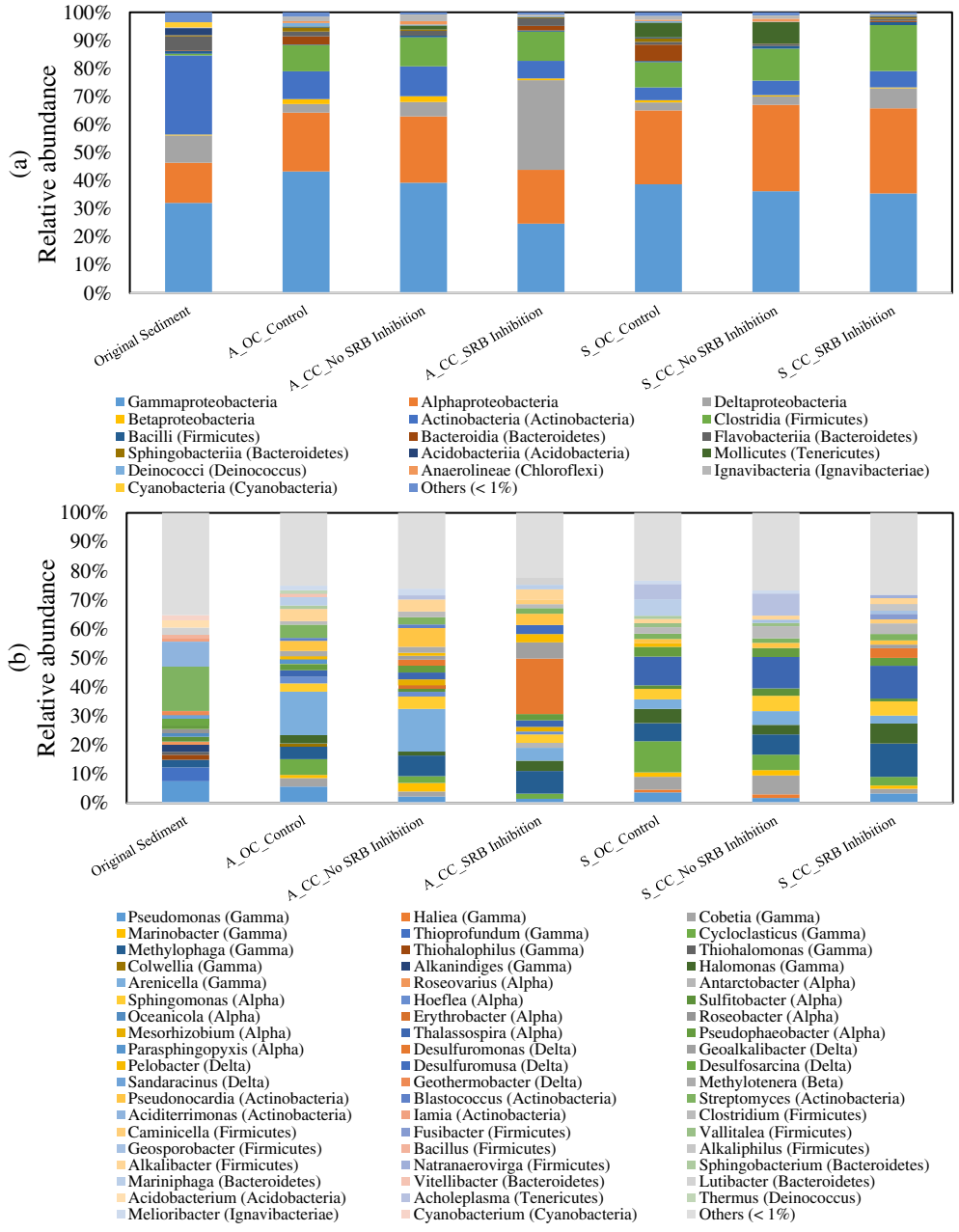

Fig. 4. Relative abundance of bacterial reads retrieved from the anode and sediment samples of the different SMFCs classified at the (a) class level and (b) genus level. "A" and "S" correspond to the anode and sediment samples, and "OC" and "CC" correspond to the SMFCs operated under open circuit (control) and closed circuit (with and with no SRB inhibition) conditions, respectively. The corresponding phyla and proteobacterial classes is presented in parentheses.

genera including *Oceanicola* (1.35%) and *Rosebacter* (1.26%) were also identified under the class of *Alphaproteobacteria*.

Interestingly, the microbial community composition of SMFCs under inhibited SRB conditions was different from the microbial community composition of SMFCs without molybdate addition (Fig. 4). This was also evidenced in the NMDS analysis based on OTUs where the bacterial communities in the SMFCs under inhibited SRB conditions clustered separately from the samples of the open circuit and closed circuit SMFCs without molybdate addition (Fig. 5).

Deltaproteobacteria was significantly higher (31.92%) at the anode of the closed circuit SMFC under inhibited SRB conditions compared to the anode of the open circuit (3.04%) and closed circuit SMFC without molybdate addition (5.14%). In particular, two genera (*Desulfuromonas* and *Geoalkalibacter*) were enriched at the anode of the closed circuit SMFC under inhibited SRB conditions (Fig. 4b).

Desulfuromonas and *Geoalkalibacter* are included under two different families within the class *Deltaproteobacteria*, which are *Desulfuromonadaceae* and *Geobacteraceae*, respectively. Most of the microorganisms within *Geobacteraceae* were isolated from anoxic sedimentary environments including rivers and are known for forming biofilms (Greene et al., 2009; Holmes et al., 2004). All members of *Geobacteraceae* are capable of utilizing iron(III) as a TEA and some of them have the ability of using sulfur as well. *Desulfuromonas* is known to be a sulfur reducing bacterium that is usually infrequent in freshwater environments but is rather very common in marine sediments. Additionally, both *Desulfuromonas* and *Geoalkalibacter* are capable of oxidizing acetate and other multi-carbon organic substrates and are sometimes included under the same family *Geobacteraceae* (Holmes et al., 2004). A phylogenetic tree was constructed for both *Geoalkalibacter* and *Desulfuromonas* and is presented in Fig. S3 in SI.

These two genera are known for their exoelectrogenic ability (Logan, 2009). *Desulfuromonas* is commonly enriched on anodes of marine SMFCs (Chan and Li, 2014; De Schamphelaire et al., 2008; Jung et al., 2014). Exoelectrogenic bacteria have the ability to transfer electrons extracellularly to an external electron acceptor. In this case, the anode was acting as a sink for electrons. The relatively higher voltages measured in the corresponding SMFCs along with the faster degradation of PAHs (namely phenanthrene) are most probably associated with the presence of these exoelectrogens and demonstrate the enhancement of the PAHs biodegradation through the direct use of the anode as a TEA in the absence of SRB competition.

Furthermore, an increase in the abundance of *Alphaproteobacteria* was measured in all SMFCs and was significant in the sediment samples (up to 30.79%). Several genera emerged under this class including *Sphingomonas*, *Hoflea* and *Mesorhizobium*. It was notable that *Mesorhizobium* existed only at the level of the anodes in the closed circuit SMFCs (1.63% and 2.07% in closed circuit SRB inhibited and non-inhibited SMFCs, respectively), which suggests its involvement in anode reduction.

Newly identified genera (*Arenicella* and *Cycloclasticus*) under the class *Gammaproteobacteria* were detected in the SMFCs. *Arenicella* predominated at the level of the anodes of the open circuit SMFCs (14.95%) and non-inhibited SRB closed circuit SMFCs (14.72%), while *Cycloclasticus* predominated in the sediments (10.72%) of the open circuit SMFCs. *Arenicella* is reported in marine sediments (Romanenko et al., 2010), and *Cycloclasticus* is known to grow by using a limited number of organic compounds including naphthalene and phenanthrene, as sole carbon sources (Geiselbrecht et al., 1998).

The class *Actinobacteria* was significantly lower in all SMFCs sediment and corresponding anode samples compared to the original sediment samples, with a noticeable decrease in the *Streptomyces* genus, mainly due to the strict anaerobic conditions in the SMFCs sediments. However, other genera emerged under this class and include *Pseudonocardia*, which is known for its ability to degrade PAHs in the environment (Lin et al., 2011).

Furthermore, new microbial classes emerged in all SMFCs of which the class *Clostridia* was noticeable, with a higher percentage (16.35%) being measured in the sediments of closed circuit SMFCs with SRB inhibition. Indeed, genera under this class are mostly anaerobic and commonly reported in PAHs contaminated soil and sediments (Zhang et al., 2010). Among the genera identified under *Clostridia*, *Clostridium* have been reported to be involved in current production in MFCs (Logan, 2009), and was detected at a relatively higher abundance in the sediments of the closed circuit SMFCs.

4. Conclusion

This study showed a significant removal of the low molecular weight PAHs naphthalene and 2-methylnaphthalene in SMFCs associated with their high volatility, while biodegradation constituted the major pathway for the removal of phenanthrene, a relatively stable semi-volatile 3-ring PAH. A higher removal efficiency of phenanthrene was measured in the case of the employment of the anode as the sole TEA as compared to the combined sulfate and anode usage as electron acceptors. Both treatments achieved a higher PAHs removal efficiency than in the case of natural attenuation of the contaminant. Significant differences in the microbial community composition were observed among the

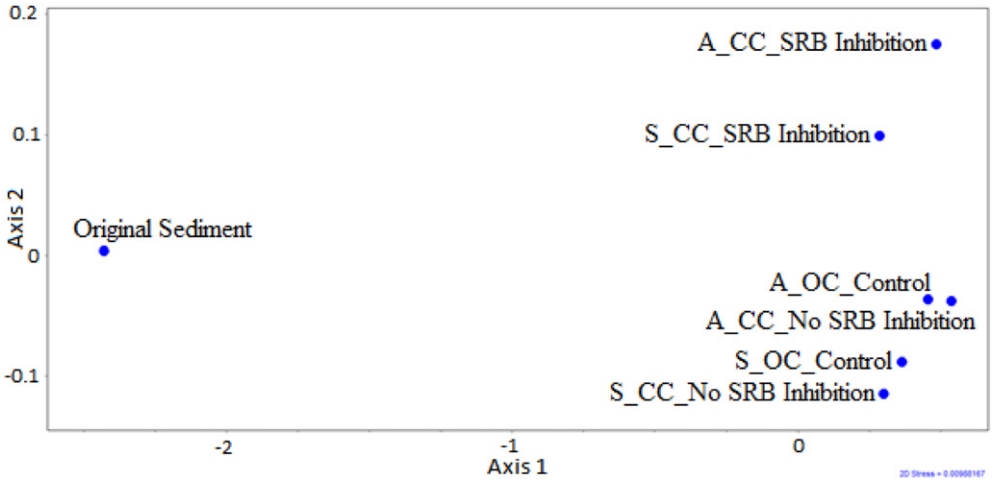

Fig. 5. Nonmetric multidimensional scaling (NMDS) plot based on Bray-Curtis distance showing the relatedness of the bacterial community structure of samples collected from the anodes and sediments of the different SMFCs. "A" and "S" correspond to the anode and sediment samples, and "OC" and "CC" correspond to the SMFCs operated under open circuit (control) and closed circuit (with and with no SRB inhibition) conditions, respectively.

different treatments at the levels of the sediments and the anodes. In particular, two genera with potential exoelectrogenic capability, namely *Geoalkalibacter* and *Desulfuromonas*, were enriched on the anode of the closed circuit SMFCs under inhibited SRB activity and were associated with the higher removal efficiency observed in these SMFCs, being more efficient at degrading PAHs than the natural occurring microbial community. These results demonstrate the role of the anode as an efficient TEA for PAHs biodegradation and have a significant impact on the bioremediation community suggesting an alternative approach for the bioremediation of PAHs in polluted marine environments.

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Appendix A. Supplementary data

Details on the collection of sediments and seawater, seawater analysis, design of the sediment microbial fuel cells, and phylogenetic tree of *Geoalkalibacter* and *Desulfuromonas*, are available in supplementary information. Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.scitotenv.2016.09.232.

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