

Bioremediation of PAHs-Contaminated Marine Sediments Using SMFCs under Different Redox Conditions

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ABSTRACT: In this study, we investigate the use of sediment microbial fuel cells (SMFCs) for the bioremediation of PAHs in contaminated marine sediments where hydrocarbon 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 a major biodegradation pathway, employment of anode as a terminal electron acceptor (TEA) under inhibited sulfate reducing bacteria (SRB) 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 ($93.83 \pm 1.68\%$) was measured in the closed circuit SMFCs with the anode acting as the main TEA and under combined anode and sulfate reduction conditions ($88.51 \pm 1.3\%$). A much lower removal ($40.37 \pm 3.24\%$) 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 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.

INTRODUCTION

PAHs contamination in marine sediments is a significant environmental and health problem owing to their toxicity and recalcitrance to biodegradation. Recent studies addressing this problem have concentrated on the deployment of intrinsic bioremediation practices including monitoring the natural microbial community (e.g., SRB) and quantifying responses to engineering decision such as the addition of exogenous nutrients and electron donors, to simulate remediation of PAHs. These studies demonstrated that the in-situ biodegradation of PAHs in marine sediments is coupled to sulfate reduction as the terminal electron accepting process owing to the fact that sulfate is abundant in seawater (Lei et al., 2005; Townsend et al., 2003). To our knowledge, studies using alternative electron acceptors in the marine environment, in particular electrodes harvesting electricity in SMFCs, which could enrich for microorganisms with a more energetically favorable mechanism for PAHs oxidation than sulfate, are absent. In addition, only two studies had addressed the bioremediation of PAHs using SMFCs in low-sulfate freshwater environments and concluded that SMFCs stimulate the removal of PAHs in contaminated

freshwater sediments under anaerobic pathways including the use of exogenous sulfates, nitrates and insoluble ferric hydroxide (Sherafatmand and Ng, 2015; Yan et al., 2012). The effect of high sulfate concentrations, such as in the marine environment, on the performance of SMFCs used for PAHs bioremediation was not reported previously. Also, analysis of the microbial community associated with the biodegradation of PAHs in marine SMFCs is not addressed in the literature.

MATERIALS AND METHODS

Collection of Sediment and Seawater Samples. Anaerobic sediments were collected from a previously contaminated site next to the Jiyeh thermal power station in Lebanon, where more than 15000 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). Grab sediment samples were collected at a depth of about 7m below the water surface and 30 cm below the water-sediment interface, about 500 meters 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 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 exhibited pH 7.87 ± 0.01 , sulfates 4800 ± 141 mg/L, sulfides 0.003 ± 0.001 mg/L, phosphates 0.015 ± 0.007 mg/L, total nitrogen 2.15 ± 0.21 mg/L, total phosphorous 0.025 ± 0.007 mg/L. Nitrates were below the detection limit in the seawater samples.

Background levels of PAHs in the sediments were determined following the method described by Yan et al. (2012). 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). These PAHs were hence used for further testing in the SMFCs.

Design, Setup and Monitoring of the SMFCs. Laboratory scale SMFCs were constructed and consisted of 0.9 L Plexiglas reactors (10L x 6W x 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 hour 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-methylnaphthalene 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 x 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. The setup of the SMFCs is presented in the schematic in Figure 1. The sediments were equilibrated with the overlying seawater for 1 day before connecting the anode to the cathode via a fixed resistor of 10 ohms in the closed circuit

SMFCs. SMFCs were operated at controlled room temperature ($20\pm 1^\circ\text{C}$) and in the dark to prevent photolysis. An autoclaved phosphate buffer and nutrient solution 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 TEA 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 in addition to the voltage across the electrodes were monitored throughout the biodegradation experiments which lasted for 28 weeks.

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 which were cut randomly from the anodes of the different SMFCs. DNA samples were then amplified via PCR using the HotStarTaq Plus Master Mix (QIAGEN, Valencia, CA), 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.

RESULTS AND DISCUSSION

PAHs Biodegradation. Biodegradation of the PAHs was monitored through the temporal variations of the concentration of the contaminants in the sediments. Concentration profiles of naphthalene, 2-methylnaphthalene, and phenanthrene are shown in Figure 2.

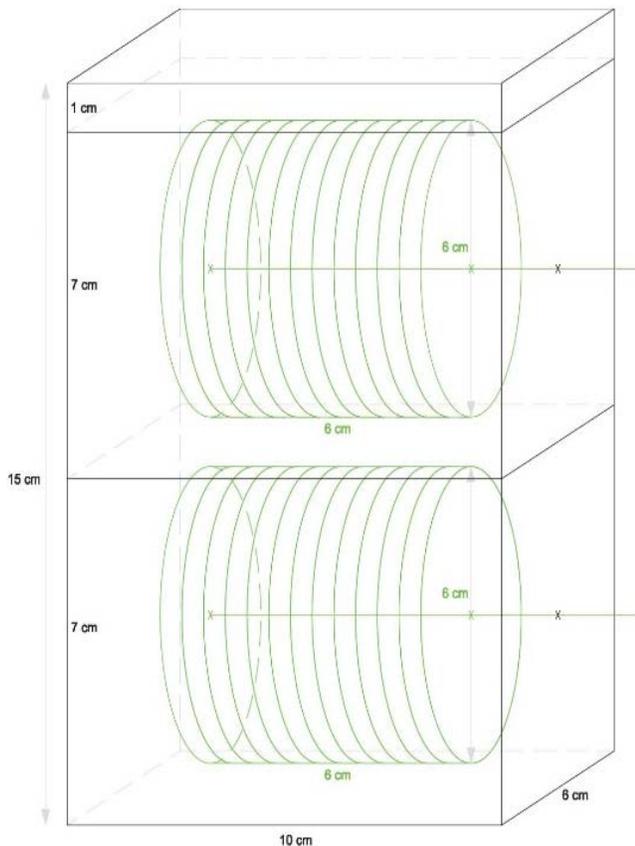


FIGURE 1. SMFCs schematic diagram.

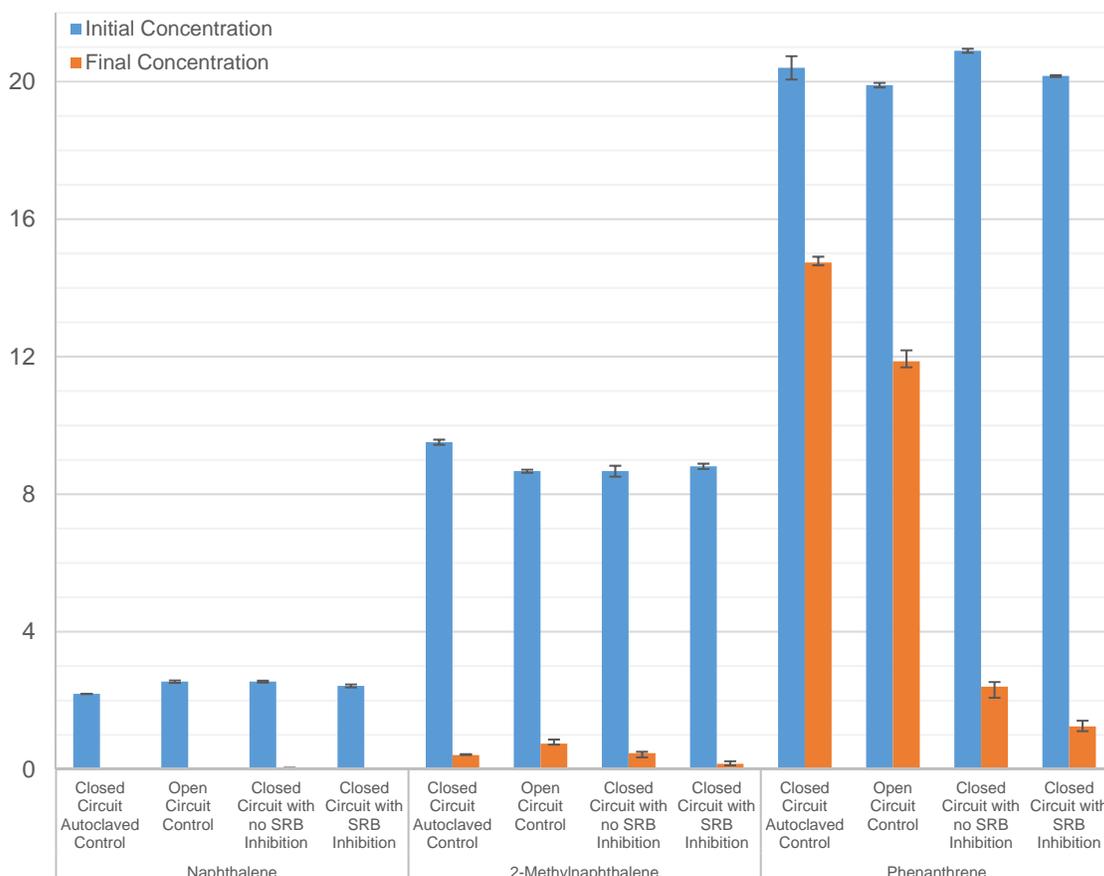


Figure 2. Concentration profile of PAHs in the SMFCs (mg/kg of dry sediment).

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, with a fast drop during the first week of incubation followed by a slower removal rate over the remaining 27 weeks of incubation, reaching less than 0.1 mg/kg of dry sediment and less than 0.7 mg/kg of dry sediment for naphthalene and 2-methylnaphthalene at the end of the biodegradation experiment, respectively. This 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 (Wick et al., 2011; Mohan et al., 2006).

Minimal removal of phenanthrene 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 was observed with the concentration of phenanthrene reaching only 12 mg/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. 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).

Sulfate Consumption. A significant sulfate consumption was observed in the absence of anode reduction (i.e. open circuit control), decreasing 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, reaching 860 mg/kg of dry sediment, could be attributed to sulfate diffusion to the overlying water.

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 SRB activity. 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 of the closed circuit SMFCs with no SRB inhibition.

Voltage Profile. 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 ± 0.11 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 ± 0.08 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 competing 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.

Microbial Community Analysis. 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. *Alphaproteobacteria* and *Deltaproteobacteria* were also present at a noticeable percentage, composing respectively 14.29% and 9.74% of the overall microbial diversity.

Interestingly, the microbial community composition of SMFCs under inhibited SRB conditions was different from the microbial community composition of SMFCs without molybdate addition. *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. These two genera are known for their exoelectrogenic ability (Logan, 2009). *Desulfuromonas* is commonly enriched on anodes of marine SMFCs (Chan and Li, 2014; Jung et al., 2014; De Schamphelaire et al., 2008). Exoelectrogenic bacteria are able 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.

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 microbial fuel cells (Logan, 2009), and was detected at a relatively higher abundance in the sediments of the closed circuit SMFCs.

CONCLUSIONS

Our findings showed a higher removal efficiency of the PAHs 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 contaminants. Significant differences in the microbial community composition were observed among the different treatments at the levels of the sediments and the anodes, 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. Findings from this study demonstrate the role of the anode in enhancing PAHs biodegradation in contaminated marine sediments and suggest a higher system efficiency when SRB are inhibited, namely due to the anode enrichment with exoelectrogenic bacteria which are more efficient at degrading PAHs than the naturally occurring microbial community. These results 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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