Enhanced Marine SMFCs for the Biodegradation of a Mixture of Low and High Molecular Weight PAHs

Hamdan Z. Hamdan (hzh05@mail.aub.edu); Darine A. Salam (ds40@aub.edu.lb) (American University of Beirut, Beirut, Lebanon)

ABSTRACT: In this study, the use of marine SMFCs for the simultaneous bioremediation of a complex mixture of PAHs was investigated Cylindrical SMFCs with a volume of 4.4 L were used. The biodegradation of 2-ringed naphthalene, 3-ringed fluorene, 4-ringed pyrene and 5-ringed benzo(a)pyrene (BAP) was monitored under anode reducing conditions in the presence and absence of sulfate reduction inhibition and ferric iron stimulation. Control SMFCs were operated under open circuit condition. Volatilization was the major removal mechanism of low molecular weight PAHs (2 and 3 rings), given the similar removal kinetics compared to the control SMFCs. In the case of the 4 ringed pyrene, higher removal was observed under iron stimulation when sulfate reduction was inhibited (33.9% for closed circuit Fe stimulated SRB inhibited and 33.1% for open circuit Fe stimulated SRB inhibited SMFCs), as compared to the natural attenuation control which showed around 20% removal. In the case of the 5-ringed BAP, similar removal values of 31.5% and 29.6% were observed in the case of iron stimulation under closed and open circuit operation and under sulfate reduction inhibition, respectively, as compared to only 3.6% removal under natural attenuation. Additionally, significant variation in the microbial community composition were observed among the operating conditions, with several electrochemically active microbes being identified in SRB inhibited and iron stimulated SMFCs. The results of this study show that conventional SMFCs approach is not a sustainable system for the bioremediation of high molecular weight PAHs in contaminated marine environments. Findings suggest that a better understanding of the performance of the system is needed when targeting complex combinations of PAHs in order to develop an improved and a more efficient SMFCs bioremediation approach for the treatment of polluted marine areas.

INTRODUCTION

Sediment Microbial Fuel Cells (SMFCs) take advantage of the natural ability of certain indigenous microbes to transfer their intracellularly generated electrons to external insoluble electron acceptors placed within the anaerobic sediment. This, coupled to the ability of these communities to adapt to and degrade organic contaminants, allows for using SMFCs for stimulating degradation of persistent organic pollutants such as polycyclic aromatic hydrocarbons (PAHs) by using an anode embedded in the anaerobic sediments as a favorable terminal electron acceptor (TEA). PAHs bioremediation studies in SMFCs are limited to a couple reporting low molecular weight PAHs (2 and 3 aromatic rings), which are mostly lost by volatilization rather than by biodegradation (Yan et al., 2012). High molecular weight PAHs (4 and more aromatic rings) are much harder to attenuate once settled in the environment. Additionally, SMFCs/PAHs studies are focused on freshwater sediments that are, unlike marine sediments, rich in ferric iron. Ferric iron is important for enrichment of the exoelectrogenic microbial community, which is the major direct utilizer of the anode as a TEA. However, no studies addressed ferric iron stimulation of exoelectrogens in marine SMFCs to aid them competing with the dominant sulfate reducers, allowing a better performance of the anodes. Thus, assessment of marine SMFCs for the bioremediation of high molecular weight PAHs is essential to understand the applicability of the system as a treatment approach to real case scenarios, where contaminated sediments are usually affected by a variety of complex and persistent hydrocarbons.

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 7 meters below the water surface and 30 cm below the water-sediment interface, about 500 meters off the shoreline. Sediment samples were passed through a 2 mm sieve to remove any coarse materials that might interfere with the sediment analysis and the experimental setup. Sediments were then extracted with 100 mL of deionized water to analyze for the physicochemical characteristics using standard methods. Sediments exhibited a pH of 8.23 ± 0.26, sulfates 721 ± 83 mg/kg dry, nitrates 12.22 ± 2.68 mg/kg dry, total nitrogen 21.5 ± 1.23 mg/kg dry, total iron 0.168 ± 0.009 mg/kg dry, Fe^{3+} 0.107 ± 0.009 mg/kg dry, organic content 1.08 ± 0.2%, wet density 2.040 ± 0.007 g/mL, dry density 1.693 ± 0.004 g/mL, total phosphorous 0.32 ± 0.02 mg/kg dry, phosphates 0.41 ± 0.03 mg/kg dry.

Design, Setup and Monitoring of the SMFCs. Laboratory scale SMFCs were constructed and consisted of 4.4 L Plexiglas reactors (height = 25 cm; diameter = 15 cm) (Figure 1). The reactors were filled with sediments to 9 cm height. Seawater was added to form a 12 cm column above the sediments. The electrodes were made of cylindrical carbon fiber brushes twisted around titanium wiring (Mill-Rose Company; Ohio, USA). In each reactor, the anodes (7 cm × 13 cm L x D) was placed vertically in the middle of the sediment column, while the cathode (13 cm × 9 cm L x D) was suspended horizontally in the middle of the water column and perpendicularly to the

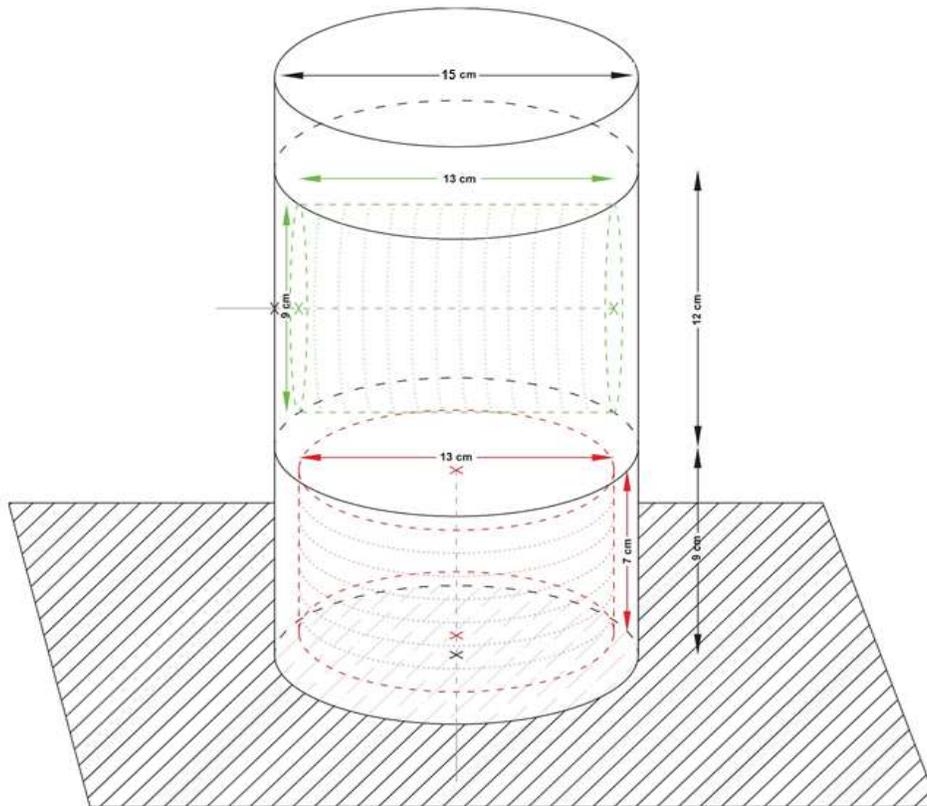*Figure 1. SMFCs design*

anode. Anode and cathode were 10.5 cm apart, center to center. An external load of 10 Ω (resistor) was provided in the closed circuit SMFCs. PAHs Spiking was performed to achieve an initial concentration of 20 mg/kg of dry sediments of each chemical, representing relatively high levels of contamination of marine sediments; except for naphthalene which was spiked at 100 mg/kg of dry sediments to account for its high volatility. After spiking, measured PAHs concentrations were 18.8±5.0, 16.9±0.9, 18.9±0.9, and 16.7±1.0 mg/kg of dry sediments for naphthalene, fluorene, pyrene and BAP, respectively. The sediments were equilibrated with the overlying seawater for 1 day before connecting the anode to the cathode in the closed circuit SMFCs. SMFCs were operated at controlled room temperature (20±1°C). Autoclaved distilled water was added periodically to the seawater to compensate for the water lost through evaporation.

Five quadruplicate sets of SMFCs were operated under the anode reducing/closed-circuit condition, while three quadruplicate sets were operated under the open-circuit condition. A last set was operated with no electrodes. This made a total of 36 SMFC reactors. Ferric iron enrichment and SRB inhibition was induced in several of the conditions. Table 1. Defines the different operating conditions.

Table 1. SMFCs operating conditions

Operating condition	Closed/ Open circuit	Iron enrichment	SRB inhibition	Abbreviation
1	Closed	-	-	C.C.
2	Closed	-	+	SRB inh C.C.
3	Closed (autoclaved)	-	-	Abiotic control
4	Open	-	-	O.C.
5	Closed	+	-	Fe C.C.
6	Closed	+	+	Fe SRB inh C.C.
7	Open	+	-	Fe O.C.
8	Open	+	+	Fe SRB inh O.C.
9	No electrodes	-	-	Natural attenuation

One of the quadruplicates of each of the operating conditions was sacrificed periodically over a period of 26 weeks (week 0, week 1, week 7, week 18, and week 26). At each sampling event, one SMFC from each condition was sacrificed and analyzed for PAHs, sulfates, and iron levels in the sediments, as well as for the microbial communities in both and sediments and anode. Soxhlet extraction by dichloromethane was used to extract PAHs from sediments and anodes (250 mL dichloromethane for 100 g of sediment per extraction). The extracts were then collected and concentrated to a final volume of 25 mL using a rotary evaporator (Buchi R-205 Rotavapor System). PAHs were then determined following the method described by Yan et al. (2012) using High Performance Liquid Chromatography (HPLC) equipped with a Diode Array Detector DAD (Agilent 1100). Sulfates and ferric iron were determined spectrophotometrically using standard HACH methods.. Microbial communities in the sediments and anodes were characterized using 16S rRNA gene pyrosequencing. Triplicate sediment samples from each SMFC were 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 anode carbon fibers which were cut randomly from the anodes of the different SMFCs. The triplicate DNA extracts were then combined and further processed for characterization using 16S rRNA gene pyrosequencing at MRDNA (MR DNA, www.mrdnalab.com, Shallowater, TX).

RESULTS AND DISCUSSION

PAHs Biodegradation. More than 50% of naphthalene was lost during the first week of operation, after which it gradually disappeared to reach less than 10% in all of the SMFCs after 26 weeks (Figure 2). SMFCs with the highest remaining percent of naphthalene were Fe SRB inh C.C., Fe SRB inh O.C. and Fe O.C. at 8.0%, 8.3% and 8.69%, respectively, while all the other SMFCs reached less than 2% remaining naphthalene including the abiotic control SMFCs. This indicates that there was little contribution of actual biodegradation corresponding to the attenuation of naphthalene from the sediments. Abiotic removal of fluorene was significant as observed in the abiotic control SMFCs, with 10.85% of the original concentration being lost after 26 weeks of operation. Given the semi-volatile nature of 3-ringed PAHs, the removal of fluorene is highly driven by volatility, with no apparent trend affecting the decay constants of this PAH.

The 4-ringed pyrene and the 5-ringed BAP showed similar trends in terms of the operating conditions affecting the removal rates of these two PAHs. Abiotic losses were minimal in the case of pyrene (9.8%) and insignificant in the case of BAP (2.5%). Pyrene was previously reported to show some volatility properties under wet settings (TOXNET, 2018). In both cases, highest removal was observed in the Fe SRB inh O.C. SMFCs, reaching 27.71% and 32.23% for pyrene and BAP, respectively. Fe SRB inh C.C. SMFCs showed the second highest removal of 21.96% and 26.07% for pyrene and BAP, respectively. Fe C.C. followed as the third highest removal with 19.1% removal for pyrene and 15.1% removal for BAP. The major contributor to the enhanced biodegradation of the high molecular weight PAHs was found to be the presence of ferric iron, irrespective of the SMFC operation under anode reducing condition (i.e. closed-circuit condition) or not. In addition, SRB inhibition, when combined with Fe amendment, appears to greatly enhance the degradation rates rather than solely applying SRB inhibition even under anode reducing condition (19.1% removal for pyrene and 15.13% removal for BAP in C.C. SMFCs). The same was observed when comparing the SRB inhibition with Fe amendment in both O.C. and C.C. SMFCs to Fe amended ones operated with no SRB-inhibition, which showed only 15.28% pyrene removal and 7.56% BAP removal under open circuit, and 19.10% pyrene removal and 15.13% BAP removal under anode reducing condition.
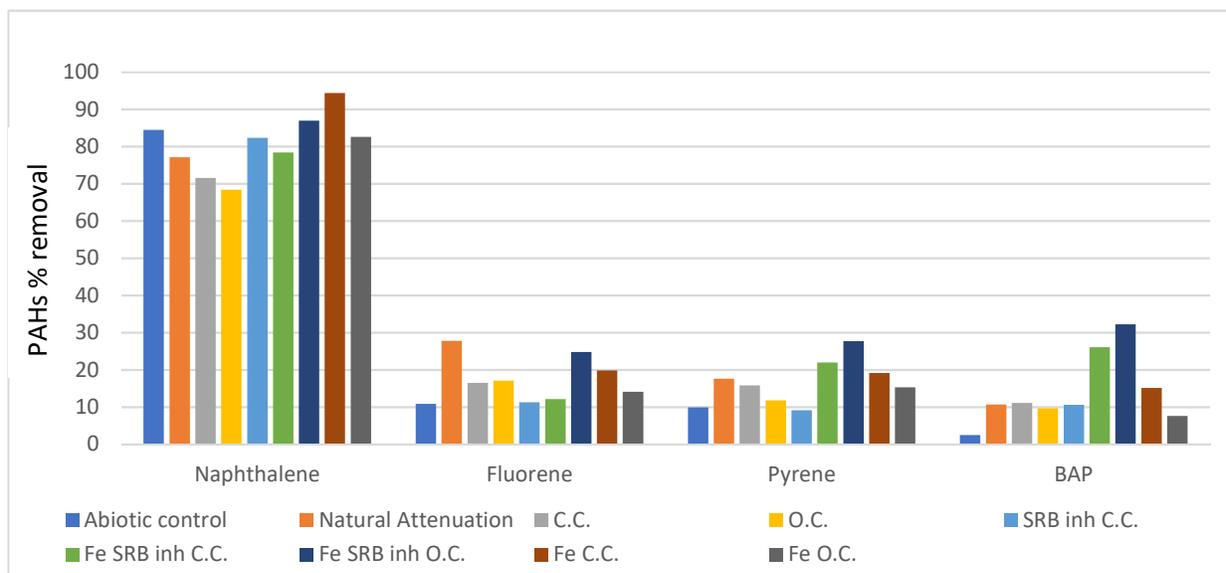

Figure 2. PAHS removal percentage in the SMFCs

TEA UTILIZATION. Highest utilization of ferric iron was observed in the case of Fe C.C. SMFCs (43%). Lowest utilization was observed in SRB inhibited Fe amended SMFCs, both C.C. and O.C., showing 24.01% and 26.10 % utilization, respectively. Finally, Fe O.C. SMFCs showed moderate ferric iron utilization compared to the other ones at 33.76%. Combination of both, the presence of high concentrations of ferric iron and anode reducing condition, stimulated the activity of Ferric iron Reducing Bacteria (FeRB) in the corresponding SMFCs. SRB inhibition did not appear to largely influence the utilization of iron in the SRB inhibited Fe amended SMFCs which showed the lowest utilization of ferric iron. Fe amendment without SRB inhibition also contributed to ferric utilization even under the open circuit operation. Highest utilization of sulfate was observed in Fe C.C. SMFCs (87.99%), which also showed the highest utilization of iron. Similar observation was noticed in the case Fe O.C. SMFCs, showing slightly lower utilization of the available sulfate at 80.33%, while also showing significant ferric reduction. This indicates that even in the presence of an active SRB population, FeRB were able to compete. This means that the presence of ferric iron itself at the used concentrations allowed FeRB to grow efficiently.

CONCLUSIONS

Ferric iron stimulation in marine SMFCs proved to be successful in terms of enriching for electrochemically active microbial populations under anode reducing condition, which was not possible under conventional SMFC setup employing only the anode as an alternative TEA. Such enrichment is heavily dependent on the sediment characteristics including the type of contamination and more importantly the low abundance of iron reducers in marine sediments. SRB inhibition, which limited the dominance of sulfate reducers in the sediments, allowed for a better potential for the SMFCs to evolve toward heavy abundances of known exoelectrogens in the Fe SRB inh C.C. SMFCs. However, the enhancement of the PAHs bioremediation potential of marine SMFCs appears to be better correlated to parameters that are different from simple employment of anode reducing condition. Results from this study suggest that implementation of SMFCs for the removal of persistent mixtures of PAHs could be more effective when stimulating the sediments with ferric iron rather than employment of an anode as a TEA within the sediments. Observations show that better understanding of the ferric iron enrichment in marine settings is needed to better understand its potential for removal of petroleum-related organic pollutants from sediments, suggesting an alternative approach to common bioremediation approaches often utilized in such settings.

REFERENCES

Maslo, A., Panjan, J., Žagar, D., 2014. Large-scale oil spill simulation using the lattice Boltzmann method, validation on the Lebanon oil spill case. Mar. Pollut. Bull. 84, 225–235. doi:10.1016/j.marpolbul.2014.05.008

Shaban, A., Ghoneim, E., Hamze, M., El-Baz, F., 2007. A post-conflict assessment to interpret the distribution of oil spill off-shore Lebanon using remote sensing. Leban. Sci. J. 8, 75–85.

TOXNET [WWW Document], 2018. URL https://toxnet.nlm.nih.gov/cgi-bin/sis/search2/r?dbs+hsdb:@term+@rn+@rel+129-00-0 (accessed 12.24.18).

Yan, Z., Song, N., Cai, H., Tay, J.H., Jiang, H., 2012. Enhanced degradation of phenanthrene and pyrene in freshwater sediments by combined employment of sediment microbial fuel cell and amorphous ferric hydroxide. J. Hazard. Mater. 199–200, 217–225. doi:10.1016/j.jhazmat.2011.10.087