Research article

Ferric iron stimulation in marine SMFCs: Impact on the microbial structure evolution in contaminated sediments with low and high molecular weight PAHs

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ABSTRACT

The impact of ferric iron stimulation on the evolution of microbial structure in marine sediment microbial fuel cells (SMFCs), operated for the bioremediation of a complex mixture of low and high molecular weight PAHs (naphthalene, fluorene, pyrene and benzo(a)pyrene), was assessed. Microbial evolution profiles showed high relative abundances of exoelectrogenic iron-reducing bacteria throughout the biodegradation, namely *Geoalkalibacter*, under ferric iron stimulation and anode reducing conditions, irrespective of sulfate reducing bacteria (SRB) inhibition. Highest PAHs removal was measured in the absence of anode reduction, under Fe stimulation and SRB inhibition, reaching 40.85% for benzo(a)pyrene, the most persistent PAH used in this study. Results suggest that amendment of contaminated sediment with ferric iron could constitute a better bioremediation strategy than using SMFCs. This becomes significant when considering the well-established and dominant indigenous SRB population in marine sediments that usually limits the performance of the anode as a terminal electron acceptor in marine SMFCs.

1. Introduction

Electrochemically active bacteria, also known as exoelectrogens, are capable of using external insoluble terminal electron acceptors (TEA), such as iron, as part of their metabolism (Chen et al., 2019). Bioelectrochemical systems, such as SMFCs, take advantage of the natural ability of such microbial communities, by providing an inexhaustible TEA within the anaerobic sediment to enhance the natural degradation rates of organic contaminants (Li et al., 2018a, b). Dissimilatory iron reducing bacteria (FeRB) belonging to the class *Deltaproteobacteria*, such as *Geobacter*, and the class *Gammaproteobacteria,* such as *Shewanella*, are well known for their involvement as exoelectrogens in bioelectrochemical systems (Logan, 2008). This, coupled to the ability of these communities to adapt to and degrade organic contaminants, allows for using SMFCs for stimulating the degradation of persistent organic pollutants by utilizing the anode as a TEA, at a rate that is higher than their natural attenuation rate (Lu et al., 2014). SMFCs then allow for the transfer of the electrons to a cathode, placed in the overlying oxygen rich water, and connected to the anode, where the electrons can reduce oxygen. In the last step, oxygen, which combines with the

transferred electrons, act as an ultimate TEA that is thermodynamically favorable over the use of other TEAs (Li et al., 2018a; Logan, 2008).

The natural biodegradation of PAHs is usually limited mainly due to minimal adaptation of the microbial communities to utilizing these compounds, especially the highly stable high molecular weight (MW) PAHs. This is due to the evolution of the microbial communities towards generalist hydrocarbon degraders that are capable of degrading simpler compounds rather than more complex hydrocarbons (Abdel-Shafy and Mansour, 2016; Ghosal et al., 2016; Khuman et al., 2018; Yang et al., 2019). Enhanced biodegradability of PAHs through using SMFCs was previously investigated in literature, but was mainly limited to low molecular weight PAHs, which are relatively easier to degrade (Hamdan et al., 2017; Sherafatmand and Ng, 2015; Yan et al., 2012). Information regarding application of SMFCs for the attenuation of high MW PAHs is very limited, especially in marine environments where such contamination is very common. Most studies focused on 2-ringed or 3-ringed low MW PAHs, which are mainly removed by volatilization rather than biodegradation. To our knowledge, only one study evaluated the biodegradation of the 4-ringed PAH pyrene using SMFCs (Yan et al., 2012). Assessment of the use of SMFCs for the bioremediation of the

more persistent high MW PAHs is still lacking.

In SMFCs, the anode biofilm is a critical factor in understanding and optimizing bioelectrochemical processes, such as anode reduction. Thus, better enhancement of the performance of these systems needs careful analysis and understanding of the associated microbes (Jung et al., 2014). However, the microbial transition from reduction of naturally occurring TEAs, such as the extremely abundant sulfate in marine sediments, to anode reduction in SMFCs, is not straightforward. This is mainly due to anode reducing microbes, namely FeRB, not being able to outcompete the existing microbial populations. This is due to the limited abundance of FeRB compared to the highly established sulfate reducing bacteria (SRB). This was observed in a previous study, in which FeRB enrichment, through amending the sediment with ferric iron, failed to shift the sediment microbial community away from sulfate reduction, which was probably due to the low population size of FeRB (Coates et al., 1996). In another study, Yan et al. (2012) demonstrated successful enhancement of SMFCs performance for the removal of low MW PAHs through the addition of ferric iron to sediments. However, this was performed in freshwater SMFCs, in which FeRB are much more common compared to marine sediments. On the other hand, Zhou et al. (2014) reported that amendment of freshwater SMFCs with ferric iron stably enhanced SMFCs voltage outputs with no apparent enhancement in the organic matter removal from the sediments. Hamdan et al. (2017) identified anode respiring bacteria at a high relative abundance in marine SMFCs under SRB inhibition. To further stress the importance of sediment characteristics on the anodic microbial structure, several studies report that freshwater and marine SMFCs show different microbial profiles, with the exoelectrogenic iron reducer *Geobacter* being enriched at the level of the anode in freshwater SMFCs, while *Desulfuromonas* usually dominating in marine SMFCs (Chan and Li, 2014; De Schamphelaire et al., 2008; Jung et al., 2014). Iron reduction is an important biodegradation pathway in freshwater sediments, which is not the case in the sulfate rich marine environments where sulfate reduction predominates.

Most studies usually assess few easily biodegradable compounds rather than a mixture of complex pollutants, which is not representative of field conditions. Furthermore, there is a lack of proper evaluation of the evolution of the sediment and anode microbial communities, namely in marine SMFCs (Abbas et al., 2017; Kronenberg et al., 2017; Sherafatmand and Ng, 2015). Indeed, most studies often report the microbial structure at the end of the operation of the system rather than the evolution of the microbial structure and abundance throughout the bioremediation process (Morris and Jin, 2012; Sherafatmand and Ng, 2015; Viggi et al., 2015; Yan et al., 2012; Zhou et al., 2014). As such, assessing the successive dominance of different microbial genera which develop and evolve in response to changes in the sediment characteristics, is necessary in evaluating the bioremediation potential of marine SMFCs (Neethu et al., 2019; Sun et al., 2018). Moreover, most studies had evaluated the use of SMFCs for PAHs bioremediation in iron rich freshwater sediments with high background abundance of electrochemically active microbes. Limited studies had addressed the use of SMFCs in sulfate rich marine sediments due to limited occurrence of electrochemically active bacteria. Therefore, enrichment of exoelectrogenic microbes in marine sediments, through the addition of iron, is of importance to assess enhancement of PAHs removal in marine SMFCs.

In this study, we assess the impact of FeRB enrichment on the microbial structure in marine SMFCs operated for the purpose of bioremediation of a range of low and high molecular weight PAHs. 2-, 3-, 4-, and 5-ringed PAHs, represented respectively with naphthalene, fluorene, pyrene and benzo(a)pyrene (BAP), were spiked into marine sediments to simulate a complex mixture of PAHs contamination. SMFCs were operated under anode reducing conditions in the presence and absence of microbial sulfate reduction inhibition and ferric iron stimulation. The evolution of microbial populations during operation of the SMFCs was monitored to provide proper understanding of the microbial communities involved in PAHs degradation in marine SMFCs. This

would provide essential information for future optimization of the system for bioremediation approaches.

2. Materials and methods

2.1. Sediments and seawater

Marine sediments were collected near the Jiyeh Power Station in Lebanon, where a significant oil spill has occurred in 2006, releasing more than 15,000 tons of heavy fuel oil into the shoreline (Maslo et al., 2014; Shaban et al., 2007). The site is also prone to regular oil contamination from daily power plant activities such as shipping and loading/unloading of fuel oil. Sediment and seawater samples were collected from 5 points that are 20 m apart from one another, about 200 m off the shore. Details about the sampling location and the sampling points are presented in supplementary information (Figure S1). Grab sediment samples were collected at a depth of around 5–6 m below the water surface, and 30 cm below the sediment/water interface, to ensure collection of anaerobic microbial communities. Five seawater samples were then collected from 1 m above the sediment/water interface from the respective sediment collection points. Samples were transported in closed containers and stored at 4 °C until further use.

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. Aliquots of 4–5 g of sediments were then extracted with 100 mL deionized water and analyzed for their physicochemical characteristics using standard methods (APHA, 2012). Sediments exhibited a pH of 8.23 ± 0.26, sulfates 721 ± 83 mg/kg of dry sediment, 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, ferric iron (Fe^{3+}) 0.107 ± 0.009 mg/kg dry, organic content $1.08 \pm 0.2\%$, 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, and phosphates 0.41 ± 0.03 mg/kg dry. Seawater exhibited a pH of 8.18 ± 0.11, sulfates 2980 ± 228 mg/L, sulfides 0.003 ± 0.001 mg/L, nitrates 0.66 ± 0.2 mg/L, total nitrogen 0.79 ± 0.02 mg/L, and total phosphorous 0.02 ± 0.01 mg/L.

2.2. Experimental setup

SMFCs design

SMFCs were based on a cylindrical design to enhance the homogeneity of the system. Plexiglass reactors with a relatively large volume of 4.4 L (height = 25 cm; diameter = 15 cm) were used. Detailed design of the reactors is presented in Fig. 1. The reactors were filled with a sediment column of 9 cm in depth. Seawater was added to form a 12 cm layer above the sediments. The electrodes were made of cylindrical carbon fiber brushes that were twisted around titanium wiring (Mill-Rose Company; Ohio, USA). Anodes (7 cm × 13 cm L x D) were placed vertically in the middle of the sediment column, while cathodes (13 cm × 9 cm L x D) were suspended horizontally in the middle of the water layer such that the electrodes were perpendicular to each other and 10.5 cm apart (center to center). The vertical placement of the anode in the middle of the sediment compartment permitted an equidistant distribution of the anode carbon fibers from the SMFC wall to ensure an even distribution of the carbon fibers within the sediment. There is no such essential requirement in the cathode compartment, and thus, the horizontal placement of the cathode in the seawater compartment was for ease of manipulation. An external load of 10 Ω (resistor) was provided in the closed-circuit SMFCs. The SMFCs were operated at room temperature.

SMFCs operation:

Several PAHs were utilized in this study, which were selected based on their inclusion among the EPA list of priority pollutants A mixture of
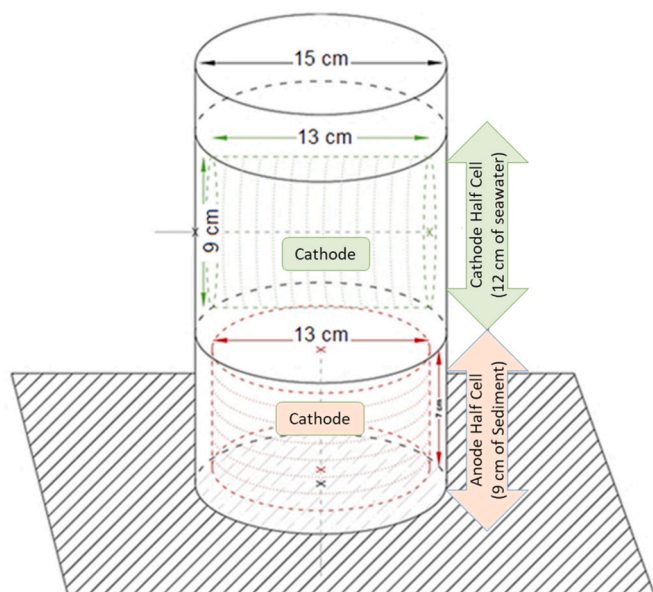
Fig. 1. Schematic of the SMFC design showing the anodic and the cothodic compartments containing the sediment and the seawater layer, respectively.

2-ringed naphthalene, 3-ringed-fluorene, 4-ringed pyrene and 5-ringed BAP, was prepared in dichloromethane and spiked into the collected marine sediments to achieve a concentration of 20 mg/kg of dry sediment of each of the studied PAHs, except for naphthalene, for which a higher concentration of 100 mg/kg dry sediment was initially targeted to compensate for expected losses during the spiking process due to its high volatility. This is representative of a high level of PAHs contamination in marine sediments (Botsou and Hatzianestis, 2012; Linden and Rust, 2008). Wet sediments were used during the spiking process by mechanically mixing the sediments at low speed for 45 min while slowly adding the PAHs solution to achieve a homogeneous distribution of the PAHs. The sediments were then distributed into the different SMFCs to achieve a depth of 9 cm in each reactor. The SMFCs were then left to settle for few hours, after which the water layer settling on top of the sediments was discarded. After that, the cathode compartment was filled with seawater, and the SMFCs were left to equilibrate for one day before closing the circuit in the corresponding closed-circuit SMFCs.

SMFCs were operated either under anode-reducing condition (closed-circuit) or under open-circuit condition. Two main major treatments were tested under each operating condition. The first treatment considered the conventional operation of the SMFCs under naturally occurring marine sediment chemical properties, while the second treatment considered the addition of ferric iron to assess potential enhancement of the SMFCs performance during the PAHs biodegradation process.

Iron amended SMFCs employed both soluble ferric iron (in the form of ammonium ferric citrate, $(NH_4)_5 [Fe(C_6H_4O_7)_2])$ as well as amorphous ferric iron (in the form of amorphous ferric hydroxide, FeO(OH)) to enhance the pore water ferric availability in the sediments for FeRB enrichment. Soluble ammonium ferric citrate provided an easily accessible short-term source of iron in the pore water, compared to the amorphous ferric hydroxide which constituted a long-lasting source of ferric iron during the operation of the SMFCs. In a previous study utilizing freshwater sediments, Yan et al. (2012) amended freshwater SMFCs with 10 g of amorphous ferric hydroxide per Kg sediment. In this study, a total concentration of 15 g/kg of dry sediments of ferric iron was used, which were added as 10 g of amorphous ferric iron per Kg of dry sediment, and an additional 5 g of soluble ferric iron per kg of dry sediment. The high amount of added ferric iron was intended to significantly drive the evolution of the microbial community towards

FeRB, which exist at extremely low abundance in marine sediments. The combination of ferric iron addition and the use of anode as a TEA was aimed at significantly shifting the microbial population on the anode toward FeRB, thus shifting the overall contaminant oxidation towards the thermodynamically faster and more efficient iron/anode reduction pathway.

Furthermore, SMFCs with inhibited SRB activity were prepared to assess the role of FeRB populations in SMFCs in the absence of sulfate reduction. SRB inhibition was performed using molybdate at a molybdate/sulfate molar ratio of 3 for complete inhibition of sulfate reduction (de Jesus et al., 2015; Predicala et al., 2008).

In total, 36 SMFCs were operated as quadruplicates representing 9 operating conditions. Five sets of SMFCs were operated under the anode-reducing/closed-circuit condition, while three sets were operated under the open-circuit condition. A final set was operated under natural attenuation condition with no electrodes embedded in the reactors. Table 1 presents a detailed description of the different treatments and their respective controls, along with the rationale behind the selected operating conditions.

2.3. SMFCs monitoring

The PAHs biodegradation experiments using SMFCs were carried out over a period of 26 weeks with periodic sampling. Continuous monitoring of SMFCs voltages was performed by recording the voltage output across the electrodes once every 15 min using a data acquisition system (2700; Keithley Instruments Inc., United States). The voltage data was averaged daily.

Five SMFCs sampling events were performed at weeks 0, 1, 7, 18 and 26. During the first sampling event (time 0), triplicate sediment samples from each operating condition were used for extraction and quantification of the starting PAHs concentrations. At each sampling event thereafter, one of the quadruplicates of each of the operating conditions was sacrificed for characterization. Sacrificing the reactors required discarding the seawater column as well as the top sediment layer in contact with oxygen (top 1 cm). The remaining sediments were then disassembled from the anode brush, homogenized, and analyzed for PAHs, sulfates and iron in separate triplicates per each of the sacrificed SMFCs. Data from the triplicate extractions was used to calculate the average concentration of the PAHs, sulfates, and iron within the sacrificed SMFCs. Sediments and anode microbial communities were also determined.

Soxhlet extraction was used to extract PAHs from the sediments. Three separate extractions were performed for each of the reactors in order to calculate the average concentration of the PAHs within the corresponding sediments. For each extraction, 100 g of wet sediment were mixed with anhydrous sodium sulfate and then placed in glass fiber thimbles. The mixture was then extracted over 18 h using 250 mL of dichloromethane. Additionally, anodes used in the SMFCs were also extracted for PAHs determination using the same method, by placing the whole carbon brush in the Soxhlet apparatus with a glass wool plug at the bottom of the Soxhlet extraction compartment. 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). 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% deionized water at a flow rate of 1 mL/min. The column temperature was maintained at 30 °C. Naphthalene was best detected at 220 nm, fluorene at 264 nm, pyrene at 238 nm and BAP at 295 nm.

DNA was extracted from the SMFCs sediments and anodes in triplicate samples, using the PowerSoil® DNA Isolation Kit (MO BIO Laboratories) according to the manufacturer's instructions. Anode DNA was extracted from the carbon fibers after being randomly cut from each of

Table 1

Details of the SMFCs' operating conditions showing operation under closed circuit or open circuit, in the presence and absence of iron enrichment, and in the presence and absence of molybdate for SRB inhibition, and the rationale behind each of these conditions.

Operating condition	Closed/ Open circuit	Iron enrichment	SRB inhibition	Abbreviation	Description	Significance
1	Closed	–	–	CC SMFCs	SMFCs operated under closed-circuit condition.	To evaluate the potential of SMFCs in enhancing PAHs biodegradation. This is expected to occur due to the activity of both SRB and exoelectrogenic bacteria associated with the anode.
2	Open	–	–	OC control SMFCs	Control SMFCs operated under open-circuit conditions.	To assess the degradation of PAHs associated with the activity of indigenous SRB in the absence of the anode as a potential TEA. In this case, the anode plays the role of a support medium, allowing for assessment of the presence of the brush itself on the sediment biodegradation capacity
3	Closed	–	–	Abiotic control SMFCs	Autoclaved control SMFCs. Sediments were autoclaved twice for 30 min at 120 °C and amended with sodium azide to ensure complete inactivation of microbes. Closed-circuit condition was applied.	To monitor potential non-biological processes involved in the removal of PAHs from the sediments. These might include volatilization, photolysis, and chemical oxidation.
4	Closed	–	+	CC Mo SMFCs	SMFCs operated under closed-circuit with molybdate addition to inhibit SRB	To assess the efficiency of the anode as the main terminal electron acceptor in the biodegradation of PAHs.
5	Closed	+	–	CC Fe SMFCs	SMFCs operated under closed-circuit condition with iron enrichment	To assess the effect of iron addition on SMFCs performance. It was anticipated that iron addition will lead to a better utilization of the anode as a TEA. PAHs removal will result from the activity of competing SRB and FeRB.
6	Open	+	–	OC Fe SMFCs	Control SMFCs operated under open-circuit conditions with iron enrichment.	To assess the effect of iron addition on the degradation of PAHs in a SRB dominated environment and in the absence of the anode as a potential TEA
7	Closed	+	+	CC Mo Fe SMFCs	SMFCs operated under closed-circuit condition with molybdate addition to inhibit SRB and iron addition.	To assess the effect of iron addition on SMFCs performance in the absence of SRB. This allows evaluation of the extent of the involvement of FeRB and anode utilization as a TEA in PAHs degradation.
8	Open	+	+	OC Mo Fe SMFCs	Control SMFCs operated under open-circuit conditions with iron enrichment and SRB inhibition.	To assess the biodegradation of high MW PAHs under the sole action of FeRB.
9	No electrodes	–	–	Natural attenuation control; NA control SMFCs	Control reactors operated with no brushes.	To assess the sediments bioremediation potential of PAHs under natural conditions

the anodes of the different SMFCs prior to the Soxhlet extraction. The triplicate DNA extracts corresponding to the sediment and the anodes, respectively, were then combined and further processed for characterization using 16 S rRNA gene pyrosequencing at MRDNA (MR DNA, Shallowater, TX, USA). Time variation of the microbial communities under each tested condition was plotted using microbial composition data of both the anode and the sediment for each of the sacrificed SMFC units at a specific sampling time, and statistical variations among the operating conditions were analyzed.

3. Results and discussion

3.1. PAHs biodegradation

The initial measured PAHs concentration at the start of the SMFCs operation varied slightly among treatments due to various losses during the handling and setting up of the reactors, except for the highly volatile

2-ringed naphthalene for which a relatively higher standard deviation in the initial PAHs concentration was measured among the different treatments. It is important to note that the spiked sediments were prepared in batches, with each batch used to prepare quadruplicates of a single operating condition. Naphthalene starting concentration was 18.87 ± 5.37 mg/kg of dry sediment at day 0 of the experiments, which was significantly lower than the spiked concentration (100 mg/kg of dry sediment) due to its high volatility during the sediment mixing process. The initial concentrations of fluorene, pyrene, and BAP were 16.97 ± 0.99, 18.95 ± 1.02, and 16.767 ± 1.16 mg/kg of dry sediment, respectively. The initial concentrations of PAHs in the different individual treatments are presented in table S1 in supplementary information. Fig. 2 shows the PAHs degradation curves during the 26 weeks of SMFCs operation. The removal efficiencies of the different SMFCs operating conditions are presented in Figure S2 in supplementary information.

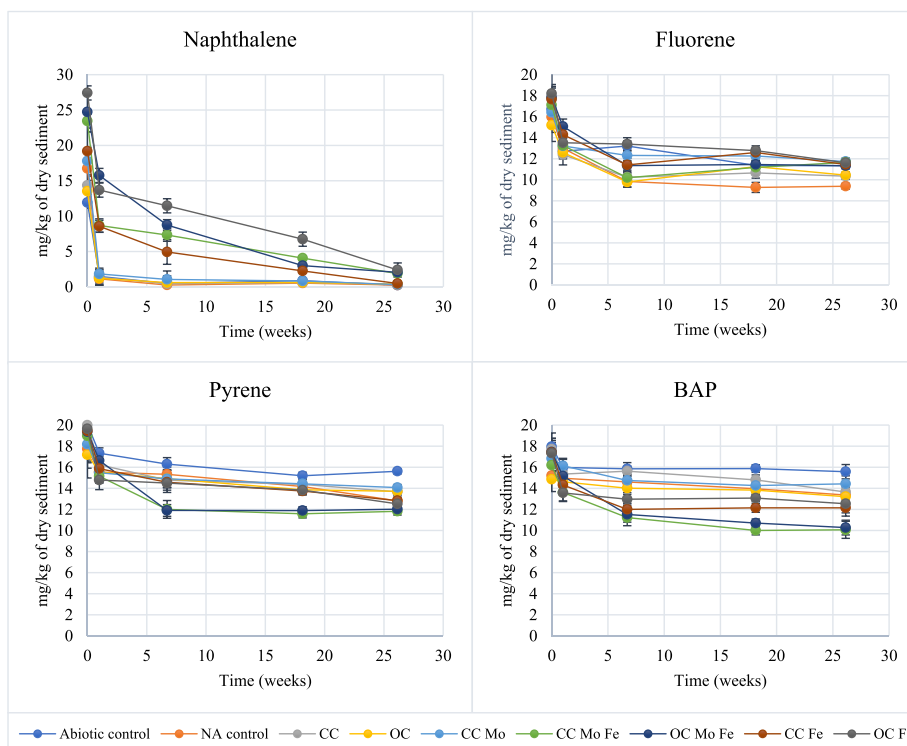The degradation of PAHs was described by the first order kinetics

Fig. 2. Biodegradation curves of PAHs in SMFCs under the different operating conditions showing the change in concentration over the 26 weeks of operation. Each point corresponds to one of the sampling events. Error bars represent the standard deviation of the triplicate extractions from each of the sacrificed SMFCs at each sampling event.

equation $C = C_0 e^{-kt}$ (Li et al., 2010). PAHs decay constants (k) are presented in Table 2. C_0 represents the original PAHs concentration in mg/kg of dry sediment (time zero), C represents the PAHs concentration in mg/kg of dry sediment at time t, and k represents the first order decay constant (d^{-1}).

Naphthalene showed a similar high removal in all operating conditions, including the abiotic control, thus highlighting its high volatility as the major removal pathway rather than biodegradation (Figure S2). More than 50% of naphthalene was removed during the first week of operation, after which it gradually disappeared to reach very low levels in all SMFCs after 26 weeks. SMFCs with the highest removal of naphthalene were CC Mo, NA SMFCs and abiotic control SMFCs at 98.20% (0.3 mg/kg of dry sediment remaining), 98.51% (0.2 mg/kg of sediment remaining) and 98.18% (0.2 mg/kg of dry sediment remaining), respectively, while all other SMFCs reached less than 2.3 mg/kg of dry sediment with more than 91% removal in all of them.

Abiotic removal of fluorene was significant as observed in the abiotic control SMFCs, with 32.12% of the original concentration being removed after 26 weeks of operation, reaching 11.3 mg/kg of dry sediment. Given the semi-volatile nature of 3-ringed PAHs, the removal of fluorene is highly driven by volatility. Highest removal was observed in the CC SMFCs (41.47% removal; reaching 10.35 mg/kg of dry sediment) and the natural attenuation control (40.86% removal; reaching 9.38 mg/kg of dry sediment). OC Mo Fe SMFCs showed a high removal, reaching a concentration of 11.33 mg/kg of dry sediment at the end of the incubation period (35.55% removal). The removal of fluorene in the remaining treatments ranged between 28.12% for CC Mo SMFCs and 34.93% for CC Fe SMFCs. ANOVA test indicated no significant differences in the biodegradation rate constants (k) ($p > 0.05$) among the various treatments, except for the natural attenuation control showing a significant increase in the decay constant compared to abiotic control, CC, CC Mo and CC Fe SMFCs ($p < 0.05$). The high removal of fluorene in the abiotic treatment along with the comparable removals among all other treatments indicates a major involvement of abiotic processes,

Table 2
Biodegradation rate constants of PAHs in SMFCs under different operating conditions. The degradation constants were calculated by fitting the triplicate data points (PAHs concentrations) at each sampling event in SigmaPlot. The error values represent the standard errors of the means. r^2 represents the correlation coefficient of the fitted data.

	Naphthalene				Fluorene				Pyrene				BAP			
	k (d^{-1})			r^2	k (d^{-1})			r2	k (d^{-1})			r^2	k (d^{-1})			r^2
Abiotic Control	0.2990	±	0.0581	0.98	0.0017	±	0.0008	0.58	0.0012	±	0.0005	0.61	0.0005	±	0.0003	0.4
Natural attenuation control	0.3851	±	0.0472	0.99	0.0029	±	0.0012	0.66	0.0014	±	0.0004	0.83	0.0007	±	0.0000	0.98
CC SMFCs	0.3377	±	0.0520	0.99	0.0023	±	0.0015	0.45	0.0016	±	0.0008	0.61	0.0010	±	0.0004	0.7
OC SMFCs	0.3447	±	0.0645	0.99	0.0015	±	0.0011	0.41	0.0011	±	0.0003	0.78	0.0006	±	0.0001	0.91
CC Mo SMFCs	0.3257	±	0.0628	0.99	0.0013	±	0.0008	0.51	0.0010	±	0.0005	0.58	0.0008	±	0.0003	0.72
CC Mo Fe SMFCs	0.1385	±	0.0851	0.74	0.0017	±	0.0013	0.44	0.0024	±	0.0012	0.59	0.0026	±	0.0009	0.74
OC Mo Fe SMFCs	0.0197	±	0.0061	0.93	0.0023	±	0.0011	0.61	0.0026	±	0.0012	0.63	0.0029	±	0.0010	0.77
CC Fe SMFCs	0.1065	±	0.0539	0.87	0.0018	±	0.0011	0.5	0.0019	±	0.0007	0.7	0.0016	±	0.0009	0.51
OC Fe SMFCs	0.0123	±	0.0061	0.78	0.0018	±	0.0009	0.54	0.0018	±	0.0009	0.57	0.0012	±	0.0008	0.42

k, first order decay constant; r2, correlation coefficient.

namely volatilization, in the removal of fluorene.

Abiotic losses reached 21.97% in the case of pyrene and 13.21% in the case of BAP, with 15.61 mg/kg of dry sediment and 15.57 mg/kg of dry sediment remaining at the end of the experiments, respectively. Pyrene has been previously reported to show some volatility properties under wet settings (TOXNET, 2018). For both PAHs, the highest removal was observed in the CC Mo Fe SMFCs for pyrene and OC Mo Fe SMFCs for BAP, reaching 36.87% and 40.85% removal, with final concentrations of 11.82 mg/kg of dry sediment and 10.27 mg/kg of dry sediment for pyrene and BAP, respectively. Relative significant removals of pyrene and BAP were also observed in the case of the OC Mo Fe SMFCs and CC Mo Fe SMFCs, respectively, and measured 36.69% and 37.88%, reaching respective final concentrations of 12.02 and 10.06 mg/kg of dry sediment compared to the controls. Removals of 33.39% for pyrene and 30.13% for BAP were reported in the case of CC Fe SMFCs by the end of the incubation period, with final concentrations of 12.83 and 12.15 mg/kg of dry sediment, respectively, while 36.22% and 27.60% removals were measured in the OC Fe SMFCs, with final concentrations of 12.52 and 12.53 mg/kg of dry sediment, respectively, being attained at the end of the incubation period. Analysis of variance showed significantly enhanced removal ($p < 0.05$) of pyrene in the OC Mo Fe SMFCs as compared to abiotic controls, NA controls, OC controls, and CC Mo SMFCs. This stresses the observation that Fe amendment, in combination with sulfate reduction inhibition, contributed to better removal of pyrene from the sediment. Similarly, a higher removal of the highly stable BAP PAH was observed under iron enrichment conditions. Analysis of variance showed significant difference ($p < 0.05$) in the biodegradation rate constants in all iron amended SMFCs (OC Mo Fe, CC Mo Fe, OC Fe, and CC Fe) as compared to the other treatments, with the SRB inhibited Fe amended SMFCs showing a higher removal. The results thus indicate that Fe amendment was a significant factor in the enhanced removal of BAP compared to the control, with the inhibition of SRB further improving the contaminant removal.

These results suggest that the major contributor to the enhanced biodegradation of the high MW PAHs is the presence of ferric iron, irrespective of the operation under anode reducing condition (i.e. closed-circuit condition). The data also suggests that SRB inhibition, combined with Fe addition, further enhanced the removal of the high MW PAHs.

It is well known that PAHs can be readily biodegraded, especially low MW ones (2-ringed and 3-ringed PAHs), although sometimes a high concentration of these low MW PAHs could inhibit the degradation of high MW PAHs (Haritash and Kaushik, 2009). The 4-ringed pyrene was described to be microbially degraded, with previous reports of enhanced biodegradation in Fe amended sediments (Haritash and Kaushik, 2009; Yan et al., 2012). Some studies reported several microbial groups that can utilize pyrene as the sole carbon source (Hu et al., 2011; Lease et al., 2011). However, 5-ringed and 6-ringed PAHs are very hard to degrade, and although their biodegradation is inducible under certain settings (Haritash and Kaushik, 2009), the process is usually very slow. Wischmann and Steinhart (1997) measured substantial residues (54%) of BAP after 15 weeks of induced PAHs biodegradation in artificially contaminated soil. In one study, macrophyte enhanced freshwater SMFCs were able to induce pyrene and BAP removal from sediments up to 87.18% and 76.4%, respectively, after 367 days of operation (Yan et al., 2015). Such a variation in the reported sediment/soil capabilities of degrading complex mixtures of PAHs indicate that the potential for bioremediation is most probably correlated to the specific characteristics of the sediment/soil in question, namely with respect to the presence of an abundant and highly diverse microbial structure capable of flourishing when PAHs contamination is applied.

Additionally, it is previously reported that the addition of ferric iron into hydrocarbon-contaminated sediments usually enhances the attenuation rates of target pollutants. For example, Zhou et al. (2014) reported an enhanced SMFCs voltage outputs under ferric iron amendment conditions. They also suggested that iron enrichment in SMFCs could

stimulate organic matter oxidation. Kato et al. (2010) reported that the presence of ferrous iron, which could be added externally or produced internally from reduction of ferric iron, could act merely as an electron shuttle between the bacteria and the anode. This could explain why iron amendment could enhance voltage outputs more significantly than enhancing the biodegradation potential of the sediments. Finally, it is important to note that Liu et al. (2011) reported that ferric amendment could hinder anode reduction in microbial fuel cells, due to the tendency of the microbial populations to simply use the available ferric iron as a TEA rather than the anode itself. Such reports further explain the observation that the biodegradation of high MW PAHs in this study was more noticeable in the presence of ferric iron, even in the open-circuit SMFCs, where anode reducing condition was not enforced.

3.2. TEA utilization (ferric iron and sulfate)

Anaerobic microbial populations play an important role in pollutants decomposition using a variety of TEAs as compared to the single TEA (oxygen) utilized by the aerobic microbes in the top layer of the sediments (Li et al., 2009). Given that PAHs tend to accumulate more in deeper sediments, the presence of several anaerobic degradation pathways, and the complex interactions among various microbial groups, play an important role in the recycling of PAHs in contaminated sediments (Li et al., 2009). Fig. 3 shows the temporal variation in the sediment ferric iron and sulfate concentrations.

Ferric iron consumption in Fe amended SMFCs showed a similar trend among the various conditions. Highest utilization of ferric iron was observed in the case of CC Fe SMFCs, reaching 1727 ± 132 mg/kg of dry sediment at the end of the experiment (43.5% utilized), which was significantly different from the other conditions ($p < 0.05$). Lowest utilization was observed in SRB inhibited Fe amended SMFCs, reaching 2314 ± 230 (24.01% utilization) and 2242 ± 153 mg/kg of dry sediment (26.10% utilization) for CC Mo Fe SMFCs and OC Mo Fe SMFCs at the end of the experiments, respectively. No significant difference in the final concentrations of ferric iron ($p > 0.05$) was reported in this case. Finally, OC Fe SMFCs showed moderate ferric iron utilization of 33.76%, with 2031 ± 1 40 mg/kg of dry sediment remaining at the end of the experiments, which was statistically different from the other conditions utilizing ferric iron stimulation. A higher utilization of ferric iron could be mostly correlated to a higher activity of FeRB communities. The results suggest that the iron enrichment under anode reducing condition, favors the activity of FeRB in the corresponding SMFCs.

It is interesting to note the sharp increase in iron utilization in CC Fe SMFCs, after week 18 of incubation. In these SMFCs (CC Fe), sulfate utilization was the highest compared the other operating conditions, reaching 86.6 mg/kg of dry sediment (88% utilization) after 26 weeks of operation. The data suggests that, the major consumption of sulfates after 18 weeks, could have decreased the activity of the SRB in CC Fe SMFCs, leading to the spike in FeRB. Indeed, a rapid increase in the relative abundance of the iron reducing bacterium *Geoalkalibacter* was observed in the middle and late stages of operation of the CC Fe SMFCs (Section 3.5). In this case, *Geoalkalibacter* dominated the microbial community, reaching a relative abundance of 48.4% at the end of experiments compared to much lower relative abundances of other genera. This possibly contributed to the increased utilization of ferric iron during the later stages of operation, which is associated with the utilization of Fe^{3+} as a building block for the cytochromes required for exoelectrogenic processes (Feng et al., 2014; Zhang et al., 2014). A similar observation was noticed in the case of OC Fe SMFCs, showing significant ferric reduction (35.3%) combined with high utilization of the available sulfate at 80.33% (141.8 ± 72 mg/kg of dry sediment remaining at the end of the experiment). In both cases (OC Fe and CC Fe), the final concentration of sulfate was significantly different from all other conditions ($p < 0.05$). 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
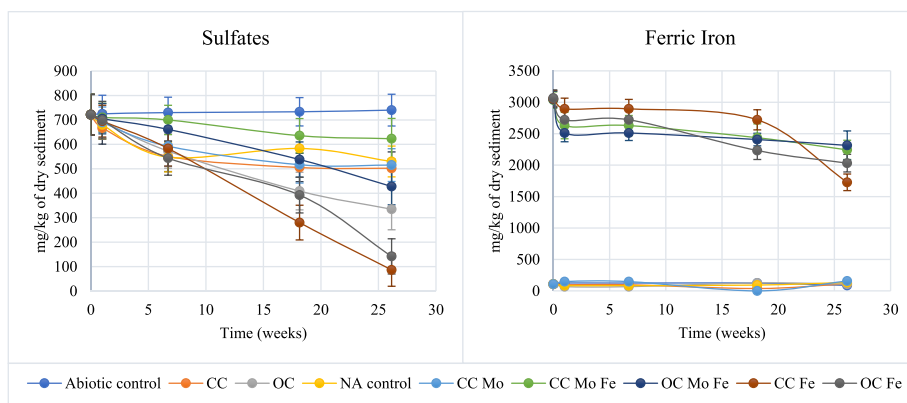
Fig. 3. Sediment TEA utilization curves showing the change in the concentration of sulfate and iron over the 26 weeks of operation. Each point corresponds to one of the sampling events. Error bars represent the standard deviation of the triplicate extractions from each of the sacrificed SMFCs at each sampling event each of the sacrificed SMFCs.

significantly compete, which was more noticeable under anode reducing condition (CC Fe SMFCs).

Temporal variation of sulfates showed no change in the sulfate concentration in the abiotic controls. The lowest sulfate utilization (13.62%) was observed in SMFCs operated under SRB inhibition in the presence of iron and anode reducing conditions (CC Mo Fe SMFCs), with 623 ± 83 mg/kg of dry sediment remaining at the end of the experiments. Significant consumption of sulfate was observed in the OC Mo Fe SMFCS (40.71%) where the highest enhancement of PAHs removal occurred, reaching a final concentration of 427.7 ± 75 mg/kg of dry sediment after 26 weeks of operation. The utilization of sulfate under SRB inhibition conditions (CC Mo Fe and OC Mo Fe SMFCs) indicates that the effect of molybdate was not sustained throughout the incubation period of the experiments. OC control SMFCs utilized 53.60% of the available sulfate over 26 weeks (334.7 ± 84 mg/kg of dry sediment remaining at the end of operation), while natural attenuation control reactors showed less sulfate utilization of 26.52% (530.1 ± 63 mg/kg of dry sediment remaining at the end of operation). The relatively high consumption of sulfates in the OC and NA controls was expected given that sulfate reduction is the dominant process involved in the attenuation of organic contamination from marine sediments. Compared to the natural attenuation controls, the presence of electrodes in the OC controls seems to have stimulated the overall sediment biological activity by providing a high surface area for microbial biofilm attachment on the carbon fiber brush, which was reflected in a higher sulfate consumption.

The highest sulfate utilization was observed under Fe amendment (CC Fe and OC Fe), irrespective of enforcing the anode reducing condition. This could be due to enhanced pore water availability of iron, which can act as an electron mediator or a shuttle for sulfate reduction within the sediment compartment of the SMFCs (Zhang et al., 2015; Zhou et al., 2014). Compared to other conditions, CC SMFCs and CC Mo

SMFCs showed similar moderate consumption of sulfate, with 502.2 ± 67 and 515.2 ± 67 mg/kg of dry sediment remaining at the end of operation of the experiments, respectively (30.38% and 28.58% utilization, respectively). This indicates that the presence of the anode under CC conditions can partially affect SRB through allowing other microbial communities to compete for electron donors, which is further enhanced by selectively inhibiting sulfate reduction (through molybdate addition) and stimulating other populations (through Fe and anode reducing conditions).

3.3. SMFCs voltage profile

The SMFCs voltage profile was recorded throughout the biodegradation experiments, and was used to monitor the microbial activity in the reactors. Fig. 4 presents the SMFCs voltage profiles under different operating conditions.

No voltage was observed across the terminals of the abiotic control SMFCs, indicating successful inhibition of microbial activity in the reactors. All open circuit SMFCs showed a high potential difference across the electrodes. The Fe amended SMFCs (OC Fe SMFCs) showed the highest open circuit potential compared to the control open circuit SMFCs (OC control SMFCs). In both cases, the recorded voltage increased gradually from near 0 mV to reach a maximum after 5 weeks, which was around 700 mV in the Fe amended SMFCs compared to 450 mV in the case of the OC control. The voltage fluctuated around the maximum until week 18, after which the voltage dropped slightly to reach 650 mV and 320 mV at the end of the experiments for the OC Fe SMFCs and the OC control SMFCs, respectively. SRB inhibited SMFCs, which were operated under Fe amendment (OC Mo Fe SMFCs), showed a similar profile compared to the OC control, increasing gradually from zero to reach a maximum of around 450 mV at week 9 of operation;
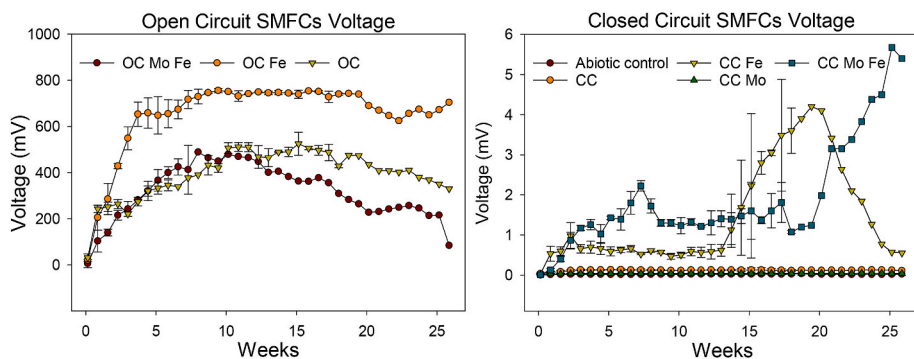
Fig. 4. SMFCs voltage profile over the duration of the experiment. The open-circuit curves show the variation in the potential difference across the electrodes of the open-circuit SMFCs. The closed-circuit curves show the variation in the voltage recorded across the 10 Ω resistor connecting the electrodes of the SMFCs. Points represent the average of the recordings of the replicate SMFCs. The error bars represent the standard deviation among the replicates of each operating condition. The data points after week 18 are single points that correspond to the single remaining SMFC per operating condition after the sacrificing of the other replicates during the previous sampling events.

however, it significantly dropped after that compared to the OC controls to reach around 200 mV after 26 weeks of operation. The observed gradual increase in the SMFCs open circuit potential is mostly related to the changes in the properties of the reactors induced by the establishment of the microbial populations. The slight drop at the end of the operation of the SMFCs could be also due to additional changes in the properties of the SMFCs induced by the gradually diminishing microbial activity upon consumption of the available nutrients and substrates required for microbial metabolism.

Closed circuit SMFCs operated with no Fe amendment (both CC SMFCs and CC Mo SMFCs) showed a slight increase in the voltage from 0 to 0.04 mV and to 0.12 mV, within 2 weeks of operation, respectively, after which the voltage remained almost constant until the termination of the experiments. These observations are not consistent with the results obtained from previous experiments, where SRB inhibited SMFCs showed significantly higher voltages throughout the experiments, indicating a higher microbial activity of the enriched FeRB in the absence of competing SRB (Hamdan et al., 2017). In the current study, the lower voltages recorded under SRB inhibition are probably due to specific characteristics of the sediments used in the SMFCs preparation that could have affected the evolution pathway of the sediment microbial community, which, even under inhibition of SRB community, did not evolve towards anode reducers.

Fe amended SMFCs showed significant enhancement of the voltage output of the corresponding SMFCs compared to those operated with no ferric iron stimulation. CC Fe SMFCs showed an increase from 0 to 1 mV after 2 weeks of operation, after which it dropped slightly to fluctuate around 0.7 mV after week 3. After 13 weeks of operation, the voltage in these SMFCs started to rise again to reach a maximum of 4.2 mV at week 20, dropping afterwards to reach 0.5 mV after 26 weeks of operation. CC Mo Fe SMFCs had a similar trend compared to non-SRB inhibited iron amended C.C. SMFCs, with overall higher recorded voltages. The voltage in these SMFCs reached a peak of 2.2 mV after around 7 weeks of operation, after which it dropped to fluctuate around 1.2 mV from week 9 to week 18, when the voltage started to rise again to a maximum of 5.7 mV after 25 weeks of operation. The voltage started dropping again during the last week of the experiments. These observations indicate that the presence of Fe significantly enhanced the voltage outputs of the

operated SMFCs, similar to what was reported previously by Zhou et al. (2014) in freshwater SMFCs. Additionally, the multiple peaks in the recorded voltages in these SMFCs could be due to oxidation of easily biodegradable materials followed by the gradual adaptation of the microbial community toward oxidation of other complex compounds, as also reported by Zhou et al. (2014). Such activity is directly correlated to the involved microbial biofilm inhabiting the anode and the sediments in the SMFCs and its evolution throughout the biodegradation experiments. However, the enhanced voltages observed in Fe amended SMFCs, compared to the controls, were not translated into a higher removal of the contaminants. This stresses that the presence of iron enhanced the electrical properties of the SMFCs rather than their biodegradation capacity.

3.4. Microbial community analysis

Variation in the overall microbial community

Non-metric Multidimensional Scaling (NMDS) was used to show the similarity among the sediment and anode microbial structure of the operated SMFCs (Fig. 5). Rstudio was used to generate the NMDS (beta diversity) plot using the ampvis2 package. The plot was generated using the amp_ordinate command based on Bray-Curtis distance and using the Hellinger transformation.

NMDS revealed a clear separation between microbial communities of Fe-stimulated and non-Fe-stimulated SMFCs. Microbial populations in the non-Fe-stimulated SMFCs were grouped more closely together, with further clustering of the corresponding anode and sediment populations. On the other hand, Fe-stimulated microbial communities were more spaced out on the NMDS plot, with no clear distinction between anode and sediment microbial structures. These observations indicate that iron amendment had a significant effect on the observed deviation of the microbial communities in two distinct groups as they evolved compared to the background community. The separate clustering of the anode and sediment microbial populations in non-Fe-stimulated SMFCs indicates that specialization took place, with certain microbes being more anode-oriented due to the specific characteristics of the anode compared to the sediments. In Fe-stimulated SMFCs, the anode and sediment microbial
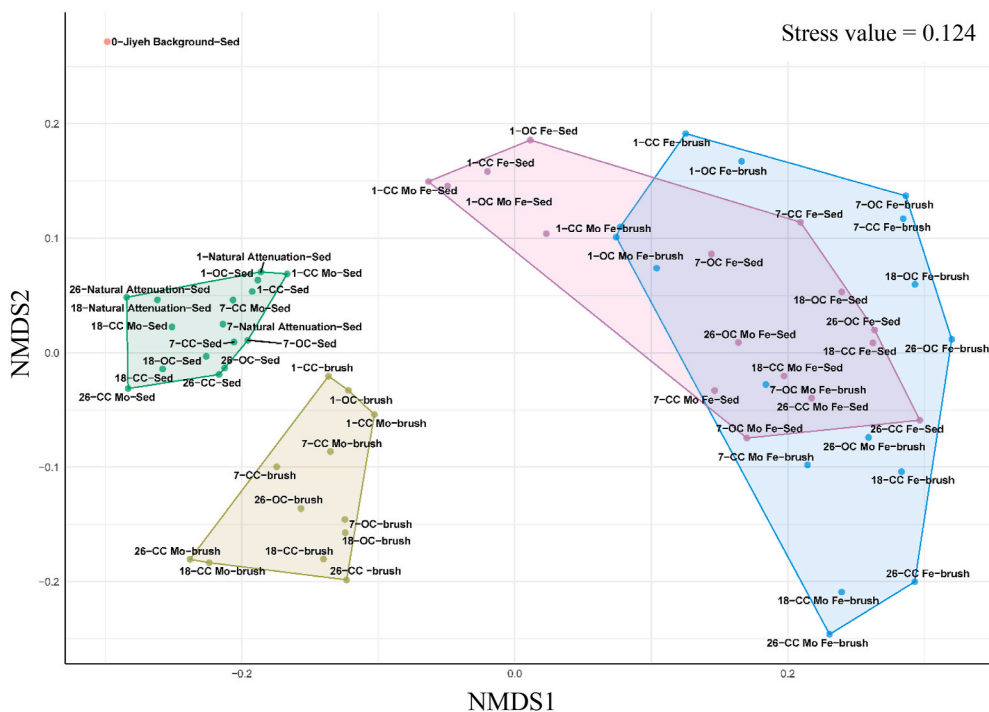
Fig. 5. Nonmetric multidimensional scaling (NMDS) plot based on Bray-Curtis distance showing the relatedness of the microbial community structure. The numbers represent the time (weeks) of the experiments at which the microbial analysis was performed. Green and yellow groups represent sediment and anodic microbial communities of SMFCs operated without ferric iron amendment, respectively. Blue and pink groups represent sediment and anodic microbial communities of SMFCs operated with ferric iron amendment, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

communities in the various applied conditions are overlapping on the NMDS plot, showing no significant effect of anode reducing conditions and SRB inhibition on the overall evolution of the microbial communities, which was mainly driven by the addition of Fe. This was in line with the observed similar performance of SRB-inhibited Fe-stimulated SMFCs (CC Mo Fe SMFCs and OC Mo Fe SMFCs), where no major differences in PAHs removal and ferric iron reduction rates were observed, irrespective of the involvement of the anode as a TEA. Similar performance was also noted in Fe-stimulated SMFCs operated in the absence of SRB inhibition (CC Fe SMFCs and OC Fe SMFCs), further indicating a major impact of ferric iron stimulation on the change of microbial composition of the anodes and the sediments, compared to no Fe stimulation and anode reducing condition. These results indicate that although significant enhancement to the bioremediation potential of SMFCs was observed under Fe stimulation, the major impact of ferric iron amendment was enhanced voltage outputs, an observation noted previously in freshwater SMFCs but not in marine SMFCs (Zhou et al., 2014).

Anode and sediment microbial structure and evolution.

Fig. 6 represents the SMFCs microbial community evolution plots throughout the experiments. Genera comprising the majority of the microbial community were presented to evaluate the microbial evolution under the different SMFCs operating conditions. The evolution plots data were extracted from the heatmaps that show the sediment and the anode genera relative abundance at each sampling event. The data in the evolution plots shows the total SMFC microbial community in each operating condition by taking both the sediment as well as the anode

microbial structure into consideration while generating the heatmaps from the Operational Taxonomic Unit (OTU) table. Heatmaps were generated using Rstudio through the ampvis2 package, using the amp_heatmap command. Detailed heatmaps are presented in supplementary information (Figure S3). To identify the OTUs which were significantly different in abundance among the samples, STAMP software (Statistical Analysis Of Taxonomic And Functional Profiles) was used (Parks et al., 2014). Welch's 2-sided t-test with a confidence interval of 0.95 was used to compare sets of conditions utilizing the total microbial community over the course of the experiments. ANOVA Tukey-Kramer post-hoc test was utilized to compare means of individual genera among the operating conditions over the course of the experiments with a confidence interval of 0.95. Genera below 0.5% abundance were filtered out to enable comparison among the most abundant genera that were observed over the 26 weeks of operation. Detailed explanation about abundance, functions, and dynamics of the microbial structure of the SMFCs corresponding to the most abundant genera that were active during the biodegradation process is included in SI.

Considering the most abundant genera that were dominating the biodegradation process, several operating conditions showed no significant difference in the overall microbial community (p > 0.05). The control natural attenuation SMFCs showed a unique profile that is significantly different from all other operating conditions (p < 0.05), indicating that the applied treatments were able to affect the microbial community evolution during the biodegradation process. OC and CC SMFCs showed no difference in the overall microbial community at the genus level, indicating that the sole presence of the anode as a TEA had
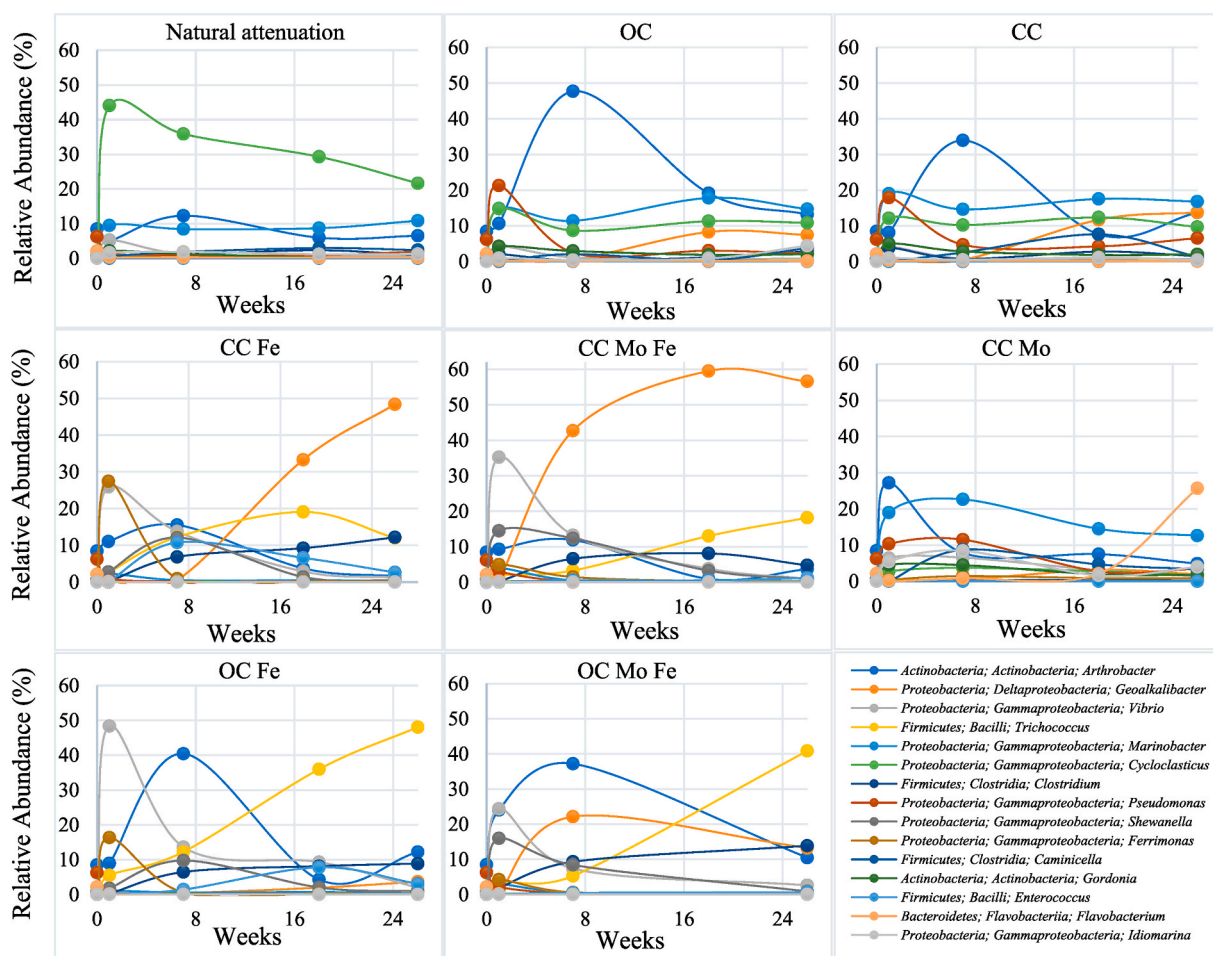

Fig. 6. Microbial community evolution plots representing the variation in relative abundance (%) of the most abundant genera throughout the biodegradation experiments. The microbial composition of each of the operating conditions presents the total average microbial community of both the anodic and the sediment microbial composition at each sampling point.

no significant difference on the microbial community response in the SMFCs, under the tested operating conditions. The iron amended closed-circuit SMFCs (CC Fe and CC Mo Fe) showed identical microbial communities evolution throughout the biodegradation process (p > 0.05), as was the case for their open-circuit counterparts (OC Fe and OC Mo Fe) (p > 0.05). However, the two sets of SMFCs presented a significant difference in microbial evolution between them (p < 0.05). This indicates that in the presence of iron, the anode did have a significant effect on the microbial community response and evolution, with minimal contribution of the sulfate reduction inhibition compared to the impact of iron.

Major differences in the evolution of the microbial communities were also observed between CC Mo SMFCs and CC SMFCs, where *Clostridium* (a sulfate reducer and a hydrocarbon degrader) and *Cycloclasticus* (a hydrocarbon degrader) showed a significant decrease under sulfate reduction inhibition, while *Idiomarina* (oil hydrocarbon degrader) showed a significant increase in the same treatment (p < 0.05). This indicates that in the absence of iron, the presence of molybdate was able to significantly change the microbial community evolution throughout the biodegradation, indicating a stronger impact of iron stimulation compared to minimal impact of sulfate reduction inhibition (Prince et al., 2018; Sallam and Steinbuchel, 2009; Yang et al., 2016).

ANOVA results of the most abundant genera are presented in Fig. 7. *Arthrobacter*, *Ferrimonas*, and *Pseudomonas* were found to be statistically

similar among all of the operating conditions compared to the natural attenuation control, indicating that the applied treatments did not have a significant impact on these genera. *Arthrobacter* is known to host efficient hydrocarbon degraders, of which many are able to grow using PAHs as the sole carbon source (Efroymson and Alexander, 1991; Haleyur et al., 2018; Stevenson, 1967). *Ferrimonas* is a facultative ferric reducing bacterium previously identified in Mediterranean sediments and reported to be abundant in sediments exposed to PAHs (Rosselló-Mora et al., 1995; Zhou et al., 2009). *Pseudomonas* is known to be an important PAHs degrader in marine sediments (Prince et al., 2018). Such observations indicate that these genera could be involved as generalist PAHs degraders in the SMFCs with no correlation to anode reduction, sulfate reduction, and iron stimulation.

Marinobacter, *Caminicella*, and *Gordonia* showed statistical similarity in abundance among the non-Fe amended SMFCs (CC, CC Mo, and OC SMFCs), in which there was an observable significant increase compared to the natural attenuation control SMFCs, namely in the case of *Marinobacter*. On the other hand, these genera were significantly suppressed in the presence of ferric iron irrespective of the application of anode reduction and sulfate reduction inhibition (CC Fe, CC Mo Fe, OC Fe, and OC Mo Fe SMFCs), presenting negligible abundance under these operating conditions. *Marinobacter* is commonly and widely spread in marine environments, and is known for hosting species that are efficient degraders of aromatic and aliphatic hydrocarbons (Duran, 2010; Handley and Lloyd, 2013). *Caminicella* was previously reported to be a part of the
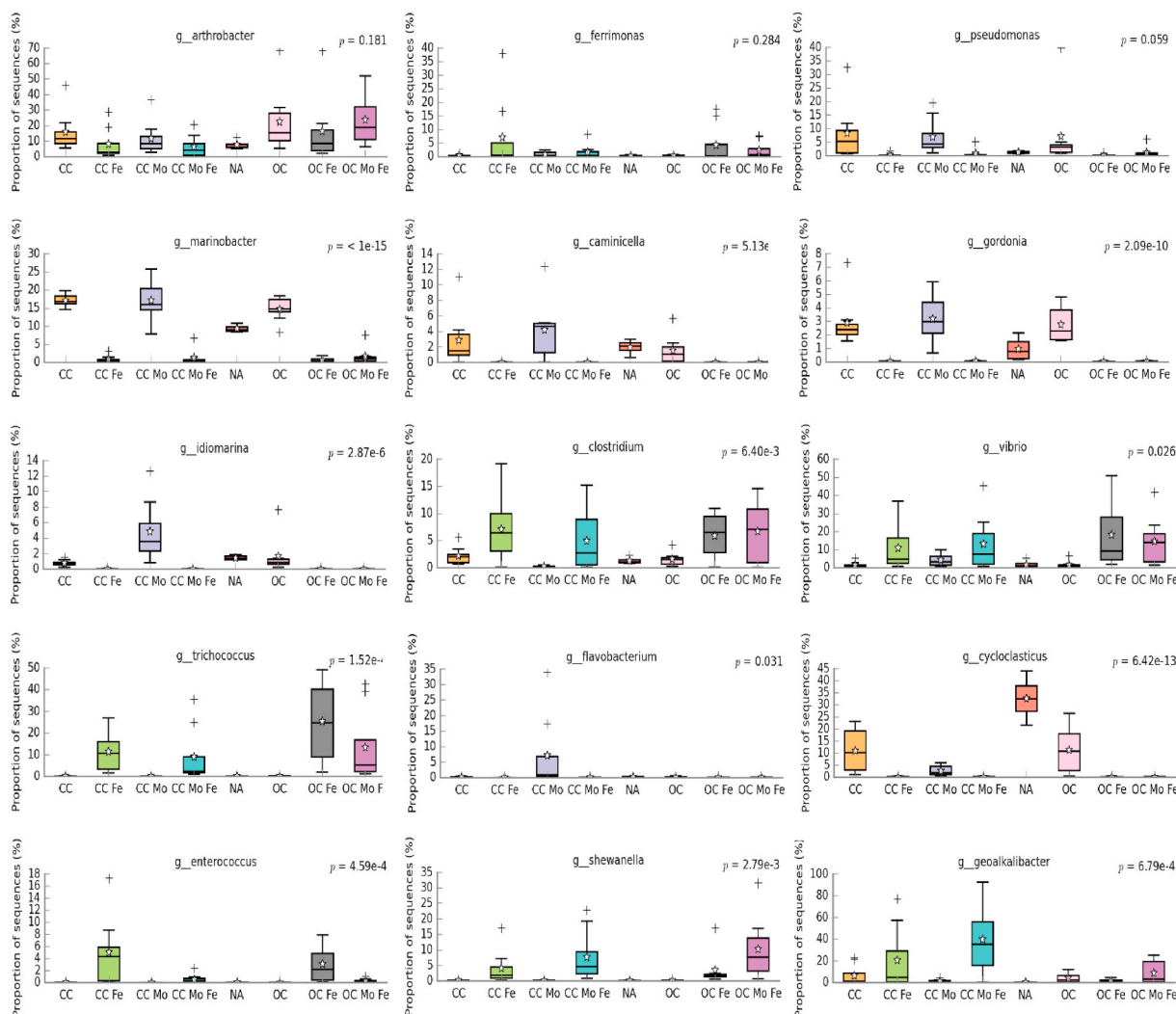

Fig. 7. ANOVA plots showing the statistical variation of the most abundant microbial genera among the operating conditions throughout the biodegradation process.

microflora present in oil reservoirs (Yernazarova et al., 2016). *Gordonia* was reported to be capable of degrading PAHs in different environmental samples (Hamdan and Salam, 2020). The observations indicate that the presence of PAHs most probably contributed to the enrichment of these genera even under sulfate reduction inhibition, indicating that their involvement in the degradation of PAHs does not depend on sulfate reduction in the sediments. The observations also indicate that there was no impact of anode availability as a TEA for these genera during the experiments.

Idiomarina was mostly present in CC Mo SMFCs, showing statistical difference compared to all other SMFCs, and increasing to around 5% compared to around 1.7% in the natural attenuation control. *Idiomarina* was statistically similar in the other non-Fe stimulated SMFCs in which it was negligible (<0.1%). Thus, stimulation of *Idiomarina* in the biodegradation process is mostly related to the decreased competition with other microbial genera, in the absence of both sulfate and iron reducers.

Clostridium, Vibrio, and *Trichococcus* were negligible in the natural attenuation and did not increase significantly in the SMFCs operated in the absence of iron stimulation (CC, CC Mo and OC SMFCs). These three genera were however significantly increased in the presence of iron irrespective of the applied operating conditions (CC Fe, CC Mo Fe, OC Fe and OC Mo Fe SMFCs), and were not significantly different among these operating conditions. *Trichococcus* has been previously reported to be involved in external electron reduction processes in fuel cell anode biofilms (Takahashi et al., 2016). *Clostridium* members were previously reported as hydrocarbon degraders including certain PAHs, and for their involvement in current production in SMFCs (Berdugo-Clavijo et al., 2012; Gieg et al., 2014; Hamdan et al., 2017; Logan, 2009). Sequences of *Vibrio* spp. were identified previously in microbial fuels cells and were reported to be PAH-degrading marine bacteria (Gezginci and Uysal, 2015; Hedlund and Staley, 2001). Such results suggest that *Clostridium, Vibrio* and *Trichococcus* are associated with the presence of iron in SMFCs with no observed effect of the presence of the anode as a TEA on their evolution in the aforementioned SMFCs. Although these genera are associated with electrochemical processes in microbial fuel cells, the similarity among the Fe stimulated SMFCs in this study could be possibly due to the high concentration of iron used for stimulation. This also suggests that the effect of heavy iron stimulation could reduce the impact of anode availability as a TEA and the use of molybdate as a sulfate reduction inhibitor, which also explains the similarity among the biodegradation performance of these SMFCs.

Flavobacterium was negligible in all SMFCs and was significantly enriched to an average of around 7.5% in the CC Mo SMFCs. *Flavobacterium* is known to be a degrader of PAHs in various environmental settings (Chaudhary and Kim, 2018; Hemalatha and VeeraManikandan, 2011). Its enrichment under anode reduction in the absence of sulfate reduction could be simply due to the decreased competition with other dominant sulfate reducing bacteria and could suggest a possible association with the presence of the anode as a TEA.

Cycloclasticus was mainly present in the natural attenuation control SMFCs. Compared to the NA controls, Fe stimulated SMFCs (CC Fe, CC Mo Fe, OC Fe and OC Mo Fe) showed negligible occurrence of *Cycloclasticus*, while SMFCs operated with no Fe stimulation (CC, CC Mo and OC SMFCs) showed moderate similar abundances of this genus. *Cycloclasticus* is a marine microbe specialized in the anaerobic oxidation of a range of PAHs in marine settings, including naphthalenes and pyrenes, which were used in this study (Cui et al., 2014; Staley, 2010). This explains the higher removal rates of PAHs, namely fluorene, observed in the natural attenuation controls and corresponding to the higher abundance of this genus. Additionally, these controls showed better removal rates of pyrene and BAP, compared to several other operating conditions which were originally proposed to enhance the natural decay rates of the introduced PAHs, such as in OC control SMFCs and CC SMFCs in the case of pyrene and BAP, and OC Fe SMFCs and CC Mo SMFCs in the case of pyrene.

Enterococcus was negligible in the natural attenuation control and in the SMFCs operated in the absence of Fe stimulation (CC, CC Mo and OC). Fe stimulated SMFCs operated in the presence of sulfate reduction inhibition showed a slight increase in *Enterococcus,* which was significantly different from the natural attenuation control albeit at a very low average abundance of less than 0.5%. However, *Enterococcus* was heavily stimulated in the CC Fe SMFCs and the OC Fe SMFCs, both showing statistical similarity in the abundance of *Enterococcus* at around 4%. *Enterococcus* was previously reported for degradation of petroleum hydrocarbons (Rahayu et al., 2019; Ravichandran and Sangeetha, 2010), and was also reported for having the ability of extracellular electron transfer (Keogh et al., 2018). This explains the significant enhancement of *Enterococcus* in the Fe stimulated SMFCs, suggesting a possible involvement in the exoelectrogenic processes in the SMFCs. *Enterococcus* is also previously reported to be involved in sulfate reduction (Yan et al., 2018), which could explain the higher relative abundance of this genus in Fe stimulated SMFCs when no sulfate reduction inhibition was applied.

Last but not least, *Shewanella* and *Geoalkalibacter,* which are known exoelectrogens associated with direct electron transfer and are usually highly abundant in freshwater SMFCs but negligible in marine SMFCs (Badalamenti et al., 2013; Hamdan et al., 2017; Li and Nealson, 2015), were successfully enriched in the SMFCs operated under the closed-circuit condition and in the presence of ferric iron, namely when sulfate reduction was inhibited. Both genera were negligible in the original sediment and in the natural attenuation control SMFCs. *Shewanella* was statistically similar among the Fe stimulated SMFCs (CC Fe, CC Fe Mo, OC Fe and OC Mo Fe SMFCs), indicating that the major contributor to the enrichment of *Shewanella* was ferric iron enrichment rather than the application of the anode as a TEA and the inhibition of sulfate reduction.

Geoalkalibacter was however much more enriched in the closed-circuit Fe stimulated SMFCs (CC Fe and CC Mo Fe) compared to the open-circuit Fe stimulated SMFCs (OC Fe and OC Mo Fe). In the case of the open-circuit ones (OC Fe and OC Mo Fe), *Geoalkalibacter* showed a rapid increase to 22.14% after 7 weeks of operation in OC Mo Fe SMFCs, decreasing then slightly to reach 13.2% at the end of the experiments, while negligible abundance was observed in the OC Fe SMFCs. This suggests that the higher PAHs removal rates observed in the case of OC Fe SMFCs are due to the evolution of the microbial community toward high abundances of PAH degraders that are also able to utilize iron as a TEA, without the need for involvement of the anode as a TEA. Additionally, the presence of *Geoalkalibacter* is probably the reason behind the significantly higher open circuit potential observed in the OC Mo Fe SMFCs. In the Fe stimulated SMFCs operated under anode reducing condition, in the presence and absence of SRB inhibition (CC Fe SMFCs and CC Mo Fe SMFCs), *Geoalkalibacter,* was successfully stimulated, increasing drastically from negligible levels to reach 48.4% and 56.6% in CC Fe and CC Mo Fe SMFCs at the end of the experiments, respectively, with no statistical differences being measured. This is the highest abundance of a single genus observed, indicating successful specific stimulation of a direct anode reducer in marine SMFCs. Interestingly, *Geoalkalibacter* comprised almost all of the identified microbial community at the anode level in CC Mo Fe SMFCs (92.5%) and in CC Fe SMFCs (77.1%) at the end of the experiments (Supplementary information; Figure S3), indicating significant involvement of the anode in driving the evolution of the total microbial community towards anode reducers in the presence of ferric iron stimulation, which was further enhanced by inhibiting SRB. *Geoalkalibacter* is known for producing high current densities through direct electron transfer in microbial fuel cells; however, it is not yet clearly correlated to degradation of aromatic hydrocarbons (Badalamenti et al., 2013).

4. Conclusion

Results from this study suggest that iron stimulation could be a better approach to enhance persistent PAHs biodegradation in marine

sediments than the implementation of SMFCs where the anode acts as a TEA. This becomes significant when considering the well-established indigenous SRB population in marine sediments, which is often hard to compete with to harness the full potential of the anode as an inexhaustible TEA. An adequate method for ferric iron enrichment in open marine systems remains essential to optimize this approach and to make it technically and economically valid.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jenvman.2020.111636.

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