Preliminary evaluation of microbial fuel cells applicability to bioremediate marine sediments contaminated by polycyclic aromatic hydrocarbons

Mılıa S.[1,*], Camedda C.[2], Erby G.[1], Mascıa M.[3], Vırdıs B.[4] And Carucci A.[2]

[1]National Research Council – Institute of Environmental Geology and Geoengineering (IGAG-CNR). Via Marengo 2, 09123 Cagliari, Italy

[2]University of Cagliari – Department of Civil-Environmental Engineering and Architecture (DICAAR). Via Marengo 2, 09123 Cagliari, Italy

[3] University of Cagliari – Department of Mechanical, Chemical and Material Engineering (DIMCM). Via Marengo 2, 09123 Cagliari, Italy

[4] The University of Queensland, Advanced Water Management Centre (AMWC), Level 4 Gehrmann Building (60) Brisbane, Qld, 4072, Australia

*corresponding author: smilia@unica.it

Abstract. This study reports a preliminary evaluation of applicability of sediment microbial fuel cells (SMFCs) to bioremediate marine sediments contaminated by phenanthrene, a polycyclic aromatic hydrocarbon (PAH). The anodic compartments of two SMFCs were batch-fed with a slurry (5% w/w real dry sediment in artificial seawater) contaminated with phenanthrene (200 mg/kg_{dw}), while the corresponding cathodic compartments were filled with 0.5 M $K_3[Fe(CN)_6]$. Both anode and cathode consisted of a piece of conductive graphite felt (4 cm^2). A cation-permeable membrane was used to separate the compartments. SMFC-1 was operated in static conditions, whereas mechanical stirring was applied in SMFC-2. Good phenanthrene removals were achieved in SMFC-1 (61%) and SMFC-2 (88.5%) after 20 days of operation; mechanical stirring played a role in accelerating phenanthrene degradation. Biocatalytic activity was characterized by linear sweep voltammetries: maximum power densities and optimal current densities in SMFC-1 and -2 were 9.2 and 38.4 μW/cm^2, and 26.2 and 142.7 μA/cm^2, respectively. Such promising results are putatively associated to the capability of electroactive microorganisms to promote phenanthrene degradation at the anodes. The use of electrodes as electron sink in electrochemical remediation of contaminated slurries deserves further investigation, since it represents a cost-effective alternative to conventional treatments requiring energy-consuming aeration.

Keywords: bioremediation, marine sediments, polycyclic aromatic hydrocarbons, phenanthrene, microbial fuel cells

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are mainly produced by anthropogenic activities and can easily accumulate in sediments, due to their hydrophobicity, low water solubility and affinity to organic matter (Bamforth and Singleton, 2005; Biella *et al.*, 2013). Moreover, most PAHs exert toxic effects and have mutagenic and carcinogenic properties, thus representing a potential hazard for both aquatic ecosystems and human health. For these reasons, 16 PAHs have been included in the US EPA pollutants priority list (U.S. EPA, 2014). Although biological degradation of PAHs is achievable under aerobic and, to a lesser extent, anaerobic conditions (Coates *et al.*,1997), conventional remediation systems still suffer from a number ofdrawbacks. For instance, process implementation and monitoring of *in situ* treatments are difficult, and operating costs are usually high (*e.g.*, for amendments and chemicals supply). On the other hand, *ex situ* treatments such as sediment slurry sequencing batch reactors (SS-SBRs) require higher costs for aeration, pre-treatments and chemical supply (Pisciotta and Dolceamore, 2016). Such drawbacks can potentially be overcome in microbial electrochemical technologies (METs), which couple biological and electrochemical processes to remove organic pollutants and simultaneously generate electricity in microbial fuel cells (MFCs), or produce hydrogen or other chemicals of interest, in microbial electrolysis and electrosynthesis cells. In MFCs the chemical energy contained in soluble organic molecules can be directly recovered as electric energy to power electrical devices. Among METs, MFCs usually consist of two chambers containing the electrodes (the anode and the cathode), separated by an ion-selective membrane and electrically connected via an external electric circuit containing an electrical load. Bacteria oxidize organic matter at the anode (under anaerobic conditions), thereby liberating electrons and protons. Electrons are transferred to the anode and through the electric circuit towards the cathode. Instead, protons migrate to the cathode compartment through the electrolyte and the ion-selective membrane. At the cathode, protons and electrons combine to reduce a terminal electron acceptor at a high electrochemical reduction potential, usually oxygen to generate water (Logan *et al.*, 2006).

Recent studies have shown that MFC treating contaminated sediments (therefore named sediment microbial fuel cells, SMFCs) can accelerate bioremediation rates in anoxic environments while providing a stable permanent electron acceptor (Pisciotta and Dolceamore, 2016). So far, only few studies investigated the ability of SMFC to remediate sediments contaminated by complex compounds such as PAHs: an SMFC was proven to enhance the removal efficiency of phenanthrene and pyrene in freshwater sediments (Yan *et al.*, 2012); Sherafatmand and Ng (2015) investigated degradation of naphthalene, acenaphthene and phenanthrene in lake sediments. Recently, Hamdan *et al.* (2017) carried out the first attempt to assess the performance of a SMFC treating PAH-contaminated marine sediments. In this study, two SMFCs with a tubular cathode compartment were tested in different operating conditions, and their applicability to the bioremediation of marine sediments contaminated by phenanthrene (Phe) was investigated.

2. Materials and methods

2.1 Marine sediments

Sediments were collected from the port of Cagliari (Sardinia, Italy), sieved in order to eliminate particles greater than 2 mm in diameter, and stored at 4°C. Main sediment characteristics are reported in Table 1.

2.2 SMFC configuration

Both SMFCs consisted of a modified 500 mL Duran-glass bottle (Figure 1). The tubular cathode compartment consisted of a polyvinyl chloride (PVC) tube (thickness, 1 mm; length, 10 cm; diameter, 3 cm), inserted in the bottle cap and sealed with an o-ring and lid. A cation exchange membrane (CMI 7000S, Membrane International Inc., USA) was glued at the bottom of the PVC tube to separate the cathode and anode compartments. Both anode and cathode consisted of a conductive graphite felt (thickness, 0.5 cm; area, 4 cm^2); titanium wires (diameter, 0.75 mm) were used to connect both anode and cathode to a voltmeter and data acquisition system (PicoLog 1012, Pico Technology). The anode chamber was equipped with a saturated calomel reference electrode (SCE, 0.2412 V *vs* the standard hydrogen electrode, Amel, 303/SCG/12). All potentials herein are reported with respect to this reference electrode. The cells were operated at ambient temperature (20°C).

Table 1. Main characteristics of sediments from the port of Cagliari

Parameter	Value
Silt – Clay [%]	65
Sand [%]	35
Total organic carbon (TOC) [% w/w]	3.3
Total extractable carbon (TEC) [% w/w]	1.3
Humic and fulvic acids (HA+FA) [% w/w]	0.4
Total solids (TS) [% w/w]	72.1
Volatile solids (VS) [% w/w]	8.3

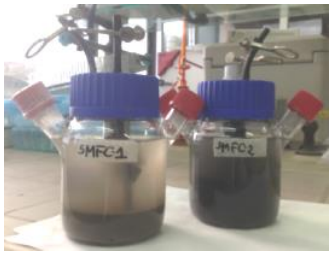

Figure 1. SMFCs used in this study

2.3 Phenanthrene degradation assessment

SMFC-1 was operated in static conditions (anode suspended in the sediment supernatant), whereas mechanical stirring was applied in SMFC-2.

Both SMFCs were operated in batch mode. In Phase 1 (20 days), they were started-up with phenanthrene spiked sediments (50 mg_{Phe}/kg_{dw}, 5% w/w real dry sediment in artificial seawater) and inoculated with 30 mL of slurry drawn from a lab-scale SS-SBR treating PAHs in our laboratories. The anolyte solution had the following composition: K_2HPO_4, 0.35 g/L; KH_2PO_4, 0.27 g/L; NH_4Cl, 0.1 g/L; $MgSO_4 \cdot 7H_2O$, 0.1 g/L. Trace elements (1 mL/L) were prepared according to Rabaey *et al.* (2005). In order to sustain the development of an electrochemically active biomass, 0.27 g/L of readily degradable sodium acetate were initially fed to the anodic compartment. The composition of the catholyte solution was: $Na_2HPO_4 \cdot 12H_2O$, 1.89 g/L; KH_2PO_4, 0.375 g/L; $K_3(Fe(CN)_6)$, 41.2g/L (0.5 M). The external resistance was initially set to 400 Ω, and progressively reduced to 200Ω using a variable potentiometer.

In the second run (Phase 2), half of the slurry in both SMFCs was replaced by fresh spiked sediment (200 mg_{Phe}/kg_{dw}, 5% w/w real dry sediment in artificial seawater), and sodium acetate was not added to the anolyte solution. The external resistance was set again at 400 Ω, and gradually reduced to 250 Ω. Phenanthrene removal was monitored for 20 days. In order to better evaluate the extent of Phe degradation in SMFC-1, as well as to assess biocatalytic activity in both SMFC-1 and -2, Phase 2 was prolonged until day 90.

A Control test (200 mg_{Phe}/kg_{dw}, 5% w/w real dry sediment in artificial seawater) was performed under open circuit conditions in order to quantify the extent of Phe removal not associated to the bioelectrochemical process. In addition, an abiotic control was run under open circuit conditions using a pre-heated sediment (560°C, 24 hours) spiked with phenanthrene (200 mg_{Phe}/kg_{dw}, 5% w/w real dry sediment in artificial seawater), in order to evaluate its adsorption on uncolonized graphite felt electrode.

2.4 Analytical methods

For phenanthrene determination, samples were periodically collected from the anode compartment, and centrifuged at 4,000 rpm for 15 minutes; the solid phase was dried by adding diatomaceous earth (1:1 w/w) and exposed to accelerated solvent extraction (Dionex, ASE 150); the liquid extracts were stored in sealed vials and analyzed by GC-MS (Agilent 6890N, 5975C) equipped with an autosampler (Agilent, 7863B) and a capillary column

(Agilent, VF-5ms, 30 m x 0.25 mm x 0.25 µm). Total organic carbon (TOC, % w/w), total extractable carbon (TEC, % w/w), humic and fulvic acids (HA+FA, % w/w) were determined according to published protocols (ANPA, 2001). Biocatalytic activity was assessed by linear sweep voltammetries (LSV) using an Autolab potentiostat-galvanostat (PGSTAT302N), whereby the cell voltage was ramped linearly between the open circuit voltages (OCV) (recorded over a period of 5.4 min for SMFC-1 and 6 min for SMFC-2) and 0, at the scan rate of 2 mV/s. Current I (A) was calculated using the Ohm's law (R= V/I), where R is the external resistance (Ω) and V is the measured voltage (V). Power (P) was calculated as P=V·I (W). Current density (A/m^2) and power density (W/m^2) were calculated dividing I and P by the nominal anodic surface area (4 cm^2), in order to allow comparison between different systems. Coulombic Efficiency (CE) was calculated as described by Logan *et al*. (2006).

Total solids (TS, % w/w) and volatile solids (VS,% w/w) were determined according to published protocol (IRSA-CNR, 2005).

3. Results and discussion

3.1 Degradation of phenanthrene in SMFCs, voltage and current profiles

The biodegradation of phenanthrene was monitored for 20 days during each Phase. At the end of Phase 1 (start-up), phenanthrene removal efficiency in SMFC-1 and -2 was high (89.6% and 96.8%, respectively), indicating the successful development and maintenance of phenanthrene degrading biomass in the system. Although SMFC-2 showed higher phenanthrene removal efficiencies, degradation was initially slower than in SMFC-1 (Fig. 2): such result suggests a contradictory role of mechanical shear stress, which might have hindered the attachment and formation of the electrochemically active biofilm on the anode in the early start-up, but then promoted phenanthrene degradation rates as soon as biomass colonized the anode.

In Phase 2, readily degradable sodium acetate was not added to the anolyte, and phenanthrene concentration was raised to 200 mg_{Phe}/kg_{dw}. Despite the lower phenanthrene removal efficiencies observed at the end of Phase 2 (61% and 88.5% in SMFC-1 and -2, respectively), the results suggest that phenanthrene degradation can be carried out even without the supply of an external carbon source, thus reducing the operating costs significantly. Moreover, phenanthrene degradation in SMFC-2 was higher than in SMFC-1 (Fig. 2), confirming that mechanical stirring accelerates phenanthrene removal if a well active biofilm is attached to the anode, by promoting the contact between biomass and substrate.

Such conclusion was supported by the profiles of current density *vs* time, reported in Fig.3, measured in SMFC-1 and -2 during the second part of Phase 2,

with an external resistance of 350 and 250 Ω (in the first part of Phase 2 the external resistance was 400 Ω, data not shown). As to SMFC-1, current density was fairly stable, reaching a maximum of 38.5 $\mu A/cm^2$ and a net voltage production of 38.5 mV, under an external resistance of 250 Ω. Such values are higher of those reported in previous studies (Sheraftmand and Ng, 2015, and Yan *et al.*, 2012), in achieved static conditions.

A completely different electrical behavior was observed in SMFC-2, compared with SMFC-1, as the external resistance was reduced to 250 Ω: current density increased up to 144.2 $\mu A/cm^2$ (day 19) (the corresponding net voltage production was 144.2 mV).

The current densities achieved in this study, as expected, are lower than those obtained when much more readily degradable substrates were used (Liu and Logan, 2004). Also the observed CE was low (< 1%) on both SMFCs. Such behavior may be ascribed to the incomplete (or very slow) mineralization of Phe, as previously reported by Coates *et al.* (1997). However, the constant presence of an electrical activity in both SMFCs suggested that an electroactive biomass had developed at the anode. Moreover, the improvement of SMFC-2 electrical performance was coherent with the acceleration in phenanthrene degradation observed during Phase 2, suggesting that electroactive biomass played an important role in its removal.

In order to evaluate the extent of achievable phenanthrene degradation in SMFC-1, the duration of Phase 2 was extended: as expected, phenanthrene degradation proceeded slowly, and complete removal was achieved on day 60, in agreement with other studies reported in literature (Hamdan *et al.*, 2017; Sheraftmand and Ng, 2015; Yan *et al.*, 2012).

To better estimate the role of bioelectrochemical removal, a Control test was performed under open

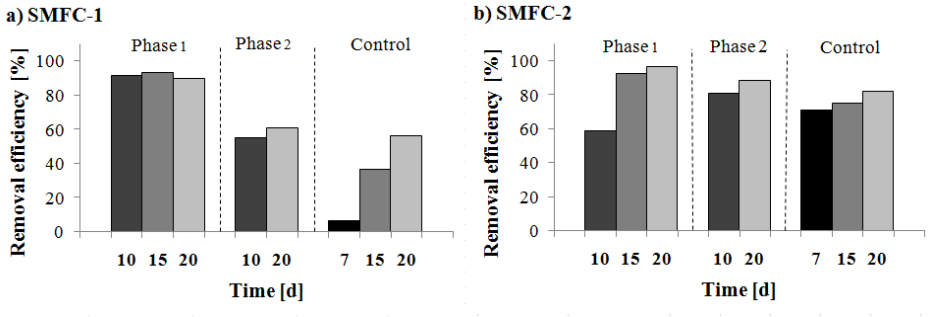

Figure 2. Removal efficiencies of Phe in a) SMFC-1 and b) SMFC-2 during each working cycle, compared to the Control test with open circuit

circuit conditions (*i.e.*, without an electrical load applied between anode and cathode), and using the same Phe concentration as in Phase 2. After 20 days of operation, the Phe removal efficiency was 56.4% and 81.8% in SMFC-1 and -2, respectively, that is lower than in the closed circuit systems (Fig. 2). In agreement with previous considerations, phenanthrene degradation was remarkably slower in SMFC-1 (static conditions). Furthermore, phenanthrene removal under anaerobic conditions (*i.e.*, excluding the bioelectrochemical effect) was slower than in Phase 2, although the removal achieved after 20 days was similar. Such results are in agreement with those reported by Yan *et al.* (2012).

The degradation of PAHs observed under open circuit conditions was probably related to the presence of alternative electron acceptors, or to the activity of anaerobic microbial populations diverse than biofilm attached to the anode, as previously demonstrated by Hamdan *et al.* (2017) and Sheraftmand and Ng (2015). The same interferences in Phe degradation should be taken into account in closed circuit conditions, requiring further investigations in order to assess the actual extent of the bioelectrochemical process. Moreover, the presence of alternative electron acceptors in the sediments could be another possible explanation for the previously described low CE achieved in both SMFCs.

The additional abiotic control showed no removal of phenanthrene in the absence of microbial communities, nor the adsorption on uncolonized graphite felt.

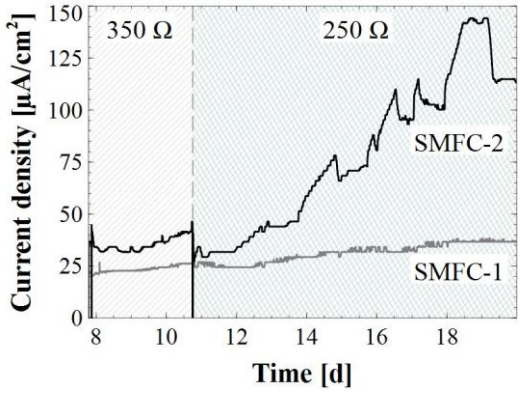

Figure 3. SMFC-1 and -2 current density profiles during Phase 2, with an external resistance of 350 and 250 Ω

a. Biocatalytic activity assessments

In order to characterize the microbial electrocatalysis, linear sweep voltammetries of the SMFCs and of the half-cells (anodic compartments, data not shown) were recorded. Results of polarization and power curves generated by the SMFCs are presented in Figure 4.

Maximum power densities and optimal current densities achieved in SMFC-1 and -2 were 9.2 and 38.4 μW/cm^2, and 26.2 and 142.7 μA/cm^2, respectively. Maximum open circuit voltages (OCVs) were 0.62 and 0.69 V; anodic potential at OCV recorded in half cells polarization curves were -0.31 and -0.47 V, respectively. Anodic potentials showed an increased catalysys associated with SMFC-2, in accordance with higher removal efficiencies obtained in that cell. At the same time, SMFC-2 polarization curve shape showed higher activation losses than SMFC-1: higher overpotentials were required to start the electron transfer in SMFC-2. As expected, the internal resistance *(i.e.*,the slope of the polarization curvesin the "ohmic polarization region", where voltage decreases linearly with current) of SMFC-2 was lower than SMFC-1 (0.5 and 3.1 mΩ, respectively), confirming the better overall performances under stirred conditions, and the absence of mass transfer limitations under this range of currents.

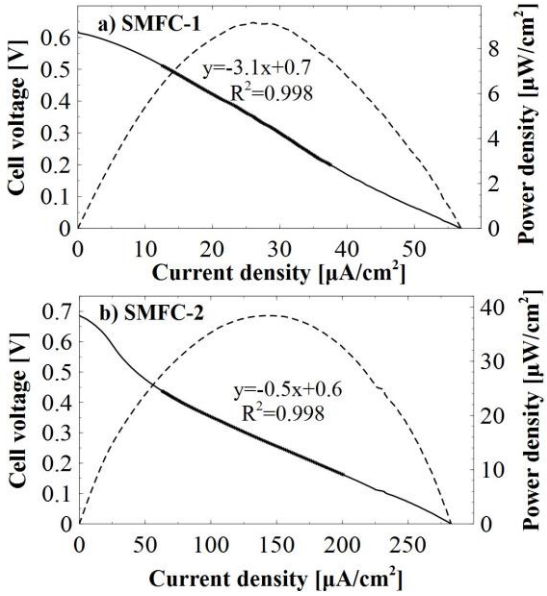

Figure 4. Polarization curves (solid lines) and power curves (dashed lines) of a) SMFC-1 and b) SMFC-2. Reported straight linear equations indicate the slope of polarization curves

4. Conclusions

The results achieved in this preliminary study suggest that SMFCs can support the development of a bio-electroactive, Phe-degrading biofilm at the anode, accelerating Phe degradation with fairly good electrical performances. The peculiar geometrical configuration of these SMFCs makes them particularly suitable for their integration in on-site slurry bioreactors. In particular, mechanical stirring (SMFC-2) was proved to hinder the attachment and development of an electrochemically active biofilm on the anode in the early start-up, and to enhance phenanthrene degradation rates as soon as biomass colonizes the anode.

With such premises, the use of electrodes as electron sink in electrochemical remediation of contaminated slurries might represent a cost-effective alternative to conventional treatments requiring energy-intensive aeration; removal rates of compounds in both aerobic treatment and SMFCs should be examined and compared. However, such preliminary results are promising, and worthy of further investigation, already planned: *e.g.*, SMFCs performance will be evaluated after inhibiting the microbial population not attached to the anode, and after having considered the possible influence of alternative electron acceptors, in order to assess the actual extent of bioelectrochemical contribution to Phe degradation; a detailed microbial characterization will be carried out, in order to characterize which organisms are involved in Phe degradation; marine sediments will be spiked with a mixture of PAHs, in order

to evaluate SMFCs behavior with more complex substrates; new static SMFCs configurations will be tested, in order to assess process performance for *in situ* applications.

References

ANPA (2001), Metodi di Analisi del compost, Manuali e Linee guida, **3**, 55-70.

Bamforth S.M. and Singleton I. (2005), Review: Bioremediation on polycyclic aromatic hydrocarbons: current knowledge and future directions, *Journal of Chemical Technology and Biotechnology*, **80**, 723-736.

Biella R., Bullita E., Cappai G., Erby G., Ruggeri C., Sergi S. Carucci A., and Tamburini E. (2013), Comparison of PAH degradation by autochthonous microbial communities in sediments of different Mediterranean port areas, *Rem Tech 2013*, DEA Edizioni, 85-92.

Coates J.D., Woodward J., Allen J., Philp P. and Lovley D.R. (1997), Anaerobic degradation of Polycyclic Aromatic Hydrocarbons and Alkanes in Petroleum-Contaminated Marine Harbor Sediments, *Applied and Environmental Microbiology*, Sept. 1997, 3589-3593.

Hamdan H.Z., Salam D.A., Hari A.R., Semerjian L. and Saikaly P. (2017), Assessment of the performance of SMFCs in the bioremediation of PAHs in contaminated marine sediments under different redox conditions and analysis of the associated microbial communities, *Science of the Total Environment*, **575**, 1453-1561.

IRSA-CNR (2005), Gli ecosistemi e i sedimenti: Caratterizzazione dei Sedimenti, 91-98.

Liu H. and Logan B.E. (2004), Electricity Generation Using an Air-Cathode Single Chamber Microbial Fuel Cell in the Presence and Absence of a Proton Exchange Membrane, *Environmental Science & Technology*, **38**, 4040-4046.

Logan B. E., Hamelers B., Rozendal R., Schröder U., Keller J., Freguia S., Aelterman P., Verstraete W. and Rabaey K. (2006). Microbial fuel cells: methodology and technology, *Environmental Science & Technology*, **40**(17), 5181–5192.

Pisciotta J.M. and Dolceamore J.J. (2016), Review: Bioelectrochemical and Conventional Bioremediation of Environmental Pollutants, *Journal of Microbial & Biochemical Technology*, **8**(4), 327-343.

Rabaey K., Ossieur W., Verhaege M. and Verstraete W. (2005), Continuous microbial fuel cells convert carbohydrates to electricity, *Water Science and Technology*, **52** (1–2), 515–523.

Sherafatmand M. and Ng H.Y. (2015), Using sediment microbial fuel cells (SMFCs) for bioremediation of polycyclic aromatic hydrocarbons (PAHs), *Bioresource Technology*, **195**, 122-130.

U.S. EPA (2014), Priority Pollutant List. In https://www.epa.gov/sites/production/files/2015-09/documents/priority-pollutant-list-epa.pdf

Yan Z., Song N., Cai H., Tay J. and Jiang H. (2012), Enhanced degradation of phenanthrene and pyrene in freshwater sediments by combined employment of sediment microbial fuel cell and amorphous ferric hydroxide, *Journal of Hazardous Materials*, **199-200**, 217-225.