Anode potential for sulfide removal in oil spill contaminated marine sediments

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Abstract

Bioelectrochemical techniques have been recently evolving as an alternative in-situ low cost method for oil spills bioremediation. Hydrocarbons bioremediation involves toxic sulfide accumulation due to the sulfate reducing microorganisms activity. Determining the optimal anodic potential for efficient electrobioremediation and simultaneous removal of toxicants becomes a challenge. In this experiment the (bio)electrochemical removal of sulfide was tested at different anodic potentials (i.e. -205 mV, +195 mV and +300 mV *vs* Ag/AgCl) with the addition of a pure culture of *Desulfobulbus propionicus*. Current production, sulfide concentration and sulfate concentration were measured over time. At the end of the experiment sulfur deposition on the electrodes was measured by SEM-EDS and the microbial communities were characterized by next generation sequencing of the 16S rRNA gene. Current production is linked to sulfide removal and sulfate was formed after inoculation. The highest electron recovery was obtained at -205 mV anode polarization.

Keywords: sulfide oxidation; bioelectrochemical systems; *Desulfobulbus propionicus.*

1. Introduction

An oil spill occurrence in the ocean creates a domino effect of tar balls formation, which precipitate on the sea floor and incorporate into sediments with subsequent ecotoxicological effects on the marine environment. When the oil has sunk to the bottom, the water surface is clean again; this gives the illusion that the oil has been removed (Annunciado *et al*. 2005). However, hydrocarbons are recalcitrant compounds that persist in marine sediments due to the absence of thermodynamically favourable electron acceptors below the oxic zone. Microbes in the anoxic zone choose first for the most electropositive electron acceptors, due to electron acceptor deficiency, i.e. nitrate (NO^{3-}), manganese (Mn^{4+}), iron (Fe^{3+}), sulfate (SO_4^{2-}) and lastly CO_2 (the most electronegative electron acceptor) usually for methanogenesis. This sequential selection of electron acceptors is linked to the stratification of the anoxic region of the sediment, from top to bottom. Sulfate reduction generates hydrogen sulfide (H_2S), a toxic gas, sometimes in very high concentration in marine sediments, leading to a characteristic foul smell similar to that of rotten eggs. Unless removed, sulfide can disperse into the oxic water zone and poison the respiratory enzymes of oxygen-respiring cells (Boetius *et al*. 2000). The anode of bioelectrochemical systems (BES) serve as an alternative electron acceptor and thus can facilitate oil spill bioremediation by enrichment of native species (Cruz Viggi *et al*. 2015, Morris and Jin 2012, Zhang *et al*. 2010). Sulfide produced by sulfate reducers during hydrocarbon biodegradation in BES can act as an electron shuttle and being oxidized to elemental sulfur, which can be reduced again to sulfide or back oxidized to sulfate (Daghio *et al*. 2016, Daghio *et al*. 2017). Acknowledging the role of sulfur, either toxic or required for effective *in-situ* oil spill bioremediation, the investigation of the ideal potential for sulfide oxidation and power production becomes an appealing question. A multi-electrode reactor containing anodes poised at different potentials was set up. Objectives of this study included: 1) stimulation of microbial sulfide to sulfate oxidation with electron generation delivered to the anode electrode, 2) identification of the potential with the highest current production, 3) characterization of the microbial communities enriched on the electrodes surface.

2. Materials and Methods

2.1. Reactor construction and operation

A multi-electrode custom-made bottle-type glass reactor (500mL) was used to immerse eight untreated rough graphite working electrodes (WEs) (2 × 2 × 0.5 cm) used as anodes. WE 1-8 were positioned geometrically identical relative to the cathode in the reactor (Fig. 1). A stainless steel mesh (7 × 4.5 cm) was used as a counter electrode (CE) placed in a 50 mL chamber, separated with a cation exchange membrane (CEM, CMI-7000, Membranes International, Ringwood, USA). An Ag/AgCl (MF-2052, Basil) electrode served as reference (RE) (all the potentials are referred *vs* Ag/AgCl). The reactor was filled with synthetic seawater containing sulfide (Aquarium Systems Instant Ocean Aquarium Salt, 33.4 g/L), sulfide rich sediment from Grevelingen (the Netherlands) and was spiked with a stock solution ($Na_2S•H_2O$) to replenish sulfide in the reactor. The separated chamber, which contained the membrane and the stainless steel mesh CE, was filled with 50 mL four times diluted instant ocean

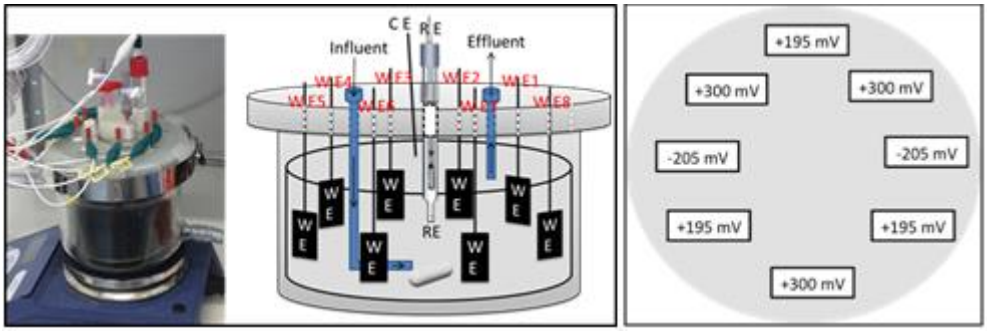

Figure 1. Multi-electrode reactor set-up photo, lay-out and anodes (WE: working electrodes) poised potentials of vs. Ag/AgCl reference electrode (RE). Sediment source: Grevelingen, Royal Netherlands Institute for Sea Research (NIOZ).

water to prevent from corrosion of the stainless steel mesh. The reactor was incubated at 20℃ under stirring and covered from light. The WE were poised at +300, +195 or -205 mV in replicates (Fig. 1) using a CHI 1000C Multi-Potentiostat (CH Instruments, Austin, TX, USA). Sulfide and sulfate profiles were measured as previously reported (Daghio *et al*. 2016). Current generation was monitored by chronoamperometry. At the end of the experiment, microbial characterization was performed.

2.2. Characterization of the microbial communities

The microbial biofilm was aseptically removed from the anodes and total bacterial DNA was extracted using the FastDNA Spin for Soil kit (MP Biomedicals, Solon, OH, USA). DNA was also extracted from the bulk anolyte collected from the reactor and from the sediment used for the initial inoculation of the reactor. The V5-V6 hypervariable regions of the 16S rRNA gene were PCR-amplified using the 783F and 1046R primers (Ferrentino *et al*. 2016). Amplicons were purified with the Wizard® SV Gel and PCR Clean-up System (Promega Corporation, Madison, WI, USA) and quantified using Qubit® (Life Technologies, Carlsbad, CA, USA). Sequencing was performed at Parco Tecnologico Padano (Lodi, Italy) by MiSeq Illumina (Illumina, Inc., San Diego, CA, USA). Reads from sequencing were demultiplexed according to the internal barcodes. The first 200 bp of R1 and 150 bp of R2 were used for the following elaborations. The Uparse pipeline (Edgar 2013) was used for the bioinformatics elaborations as previously reported (Daghio *et al*. 2016). Classification of the sequences representative of each OTU was done using the RDP classifier (≥ 80% confidence) (Wang *et al*. 2007).

3. Results and Discussion

After an initial decrease of the current production current peaks were observed when sulfide was further added in the reactor (Fig. 2). Sulfide was rapidly removed from the medium after each spiking (Fig. 3A) likely due to oxidation to elemental sulfur on the anodic surface. However, back oxidation of elemental sulfur to sulfate was not observed during the first days of operation. Sulfate concentration decreased rapidly from more than 2000 mg/L, probably due to polysulfide formation, and remained constant at about 200 mg/L. Previous studies have already showed that (bio)electrochemical sulfide oxidation is an elective process to remove sulfide from wastewater (Dutta *et al*. 2009). However when sulfide is oxidized to elemental sulfur, it precipitates on the anode, reducing thus the electron transfer in long term operation (Dutta *et al*. 2008). On day 16, a pure culture of *Desulfobulbus propionicus* DSM 2032 (107 cells/mL) was inoculated in the reactor. This strain was chosen due to its ability to promote back oxidation of elemental sulfur to sulfate in bioelectrochemical systems (Zhang *et al*. 2014). On day 20 sulfate formation increased again up to about 2200 mg/L suggesting that *D. propionicus* was able to stimulate the removal of elemental sulfur from the electrodes surface. The maximum current reached was 0.2 ± 0.1 mA/cm^2 (day 87), 1.5 ± 0.7 mA/cm^2 (day 91) and 1.5 ± 1.0 mA/cm^2 (day 91) for the electrodes polarized at -205 mV, +195 mV and +300 mV respectively. The current peak was reached after the last addition of sulfide in the reactor (day 80), but no data about sulfide removal are available. The cumulative charge calculated for the electrodes polarized at -205 mV was about 1200 C while about 1300 C and 950 C were calculated for the electrodes polarized at +195 mV and +300 mV respectively (Fig. 2D). The higher electron transfer observed at -205 mV suggests that the lower potential applied was the most effective in the stimulation of sulfide removal. The classification (80% confidence) showed that the most abundant order in the initial sediment was the order *Campylobacterales*, which was also enriched in the bulk after the incubation (Fig. 4A). The order *Desulfobacterales* was the most abundant on the electrodes at all the tested potentials and ranged between 49% ± 4% (-205 mV) and 57% ± 9% (+ 195 mV). High enrichment of microorganisms of the families *Desulfobulbaceae* and *Desulfobacteraceae*, both members of the *Desulfobacterales*, was observed in the communities selected on the anodes (polarized at 0 mV and +300 mV) of bioelectrochemical reactors for toluene removal in marine environments (about 2 g/L of sulfate) (Daghio *et al*. 2016). The authors suggested the possible role of these families in hydrocarbon degradation and in oxidation of biologically produced sulfide (Daghio *et al*. 2016). Microorganisms of the order *Desulfuromonadales* were also detected on the electrodes. The abundance of the order *Desulfuromonadales* on the electrodes poised at +195 mV and +300 mV varied between 1.4% ± 0.3% and 2% ± 1% respectively and was comparable to the abundance observed in the initial sediment (2%) and in the bulk of the reactor (1%). Conversely, a low enrichment of the order *Desulfuromonadales* was observed on the anode poised at -205 mV (5% ± 2%). *Desulfuromonas acetoxidans*, a member of the order *Desulfuromonadales*, is able to use both elemental sulfur and anodes as electron acceptors (Bond *et al*. 2002). The presence of this order may thus be

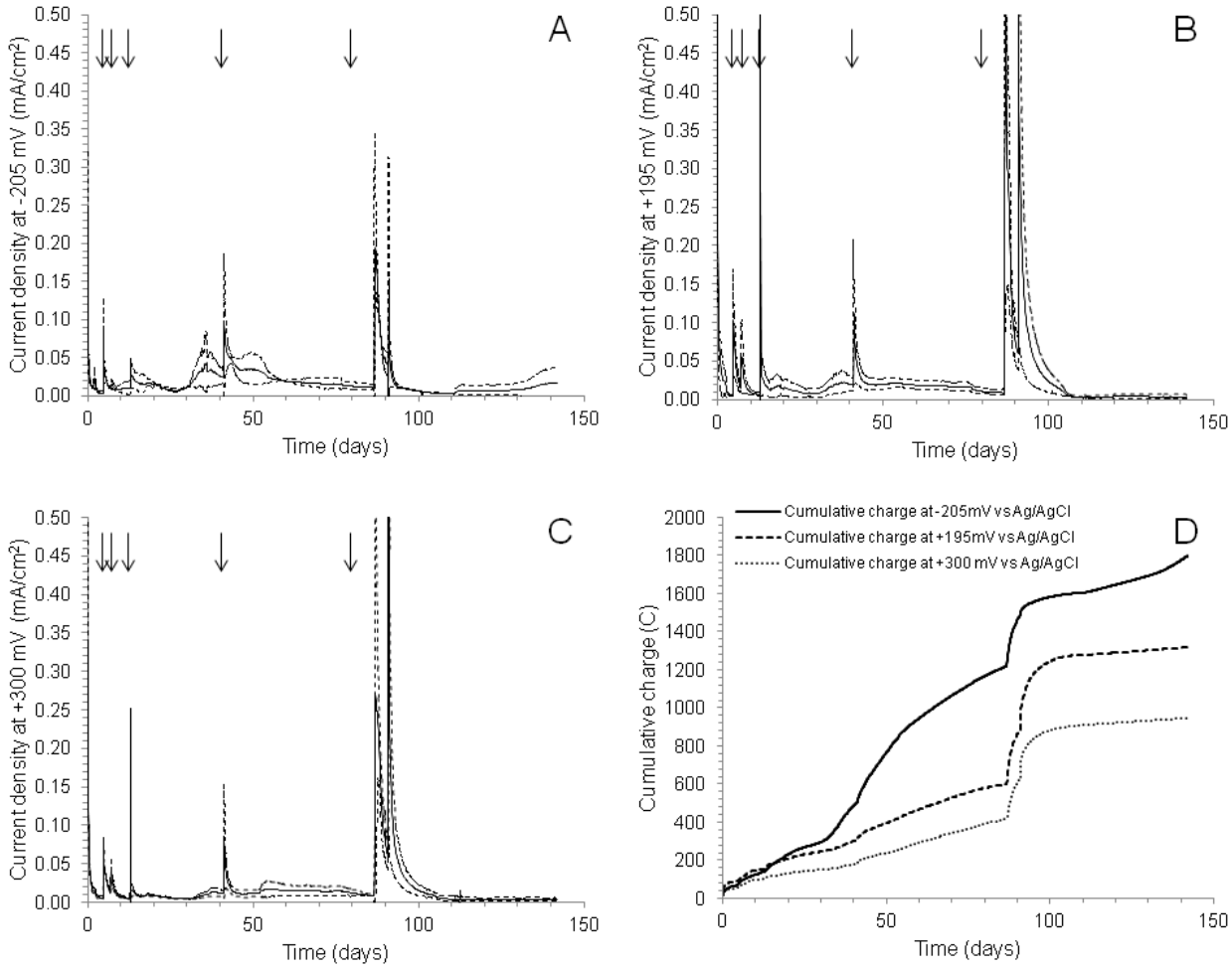

Figure 2. Current density measured for the electrodes polarized at -205 mV (A), +195 mV (B) and +300 mV (C) during the experiment. Sulfide spiking is indicated with an arrow. The maximum current values reported in the graphs are lower than the maximum recorded at +195 mV and +300 mV to highlight the differences in the conditions. (D) Cumulative charge calculated for the tested potentials.

linked in the reduction of elemental sulfur producing sulfide. Within the order *Desulfobacterales* the most abundant OTUs were OTU_1 and OTU_3 (Fig. 4B).OTU_1 was highly selected in the microbial communities enriched at +195 mV and +300 mV in which accounted for more than 90% of the order *Desulfobacterales*. The higher abundance of OTU_3 was observed in the samples collected from the anodes polarized at -205 mV (44% ± 15% of the order *Desulfobacterales*). The sequences of both OTU_1 and OTU_3 was used to determine the best match to sequences in the RDP database. OTU_1 was close to *Desulfocapsa sulfexigens* (seqmatch score 0.986) a microorganism that is able to perform disproportionation of elemental sulfur (Finster *et al*. 2013). OTU_3 showed similarity to *D. propionicus* (seqmatch score 0.644), the same microorganism inoculated in the reactor at day 16. Despite the sequence alignment of OTU_3 against *D. propionicus* was low these data suggest that the pure culture added in the reactor was able to colonize the electrodes polarized at -205 mV facilitating sulfur removal and its back oxidation to sulfate. Indeed sulfur deposition was lower on the anode polarized at the lower potential (7% ± 4%) compared to the anodes polarized at +195 mV and +300 mV (16% ± 6% and 15% ± 7% respectively) (Fig. 3B).

4. Conclusions

The best performances in terms of electrons recovery were observed when -205 mV were applied. The inoculation with a pure culture of *D. propionicus* successfully led to the back oxidation of elemental sulfur to sulfate.

Acknowledgements

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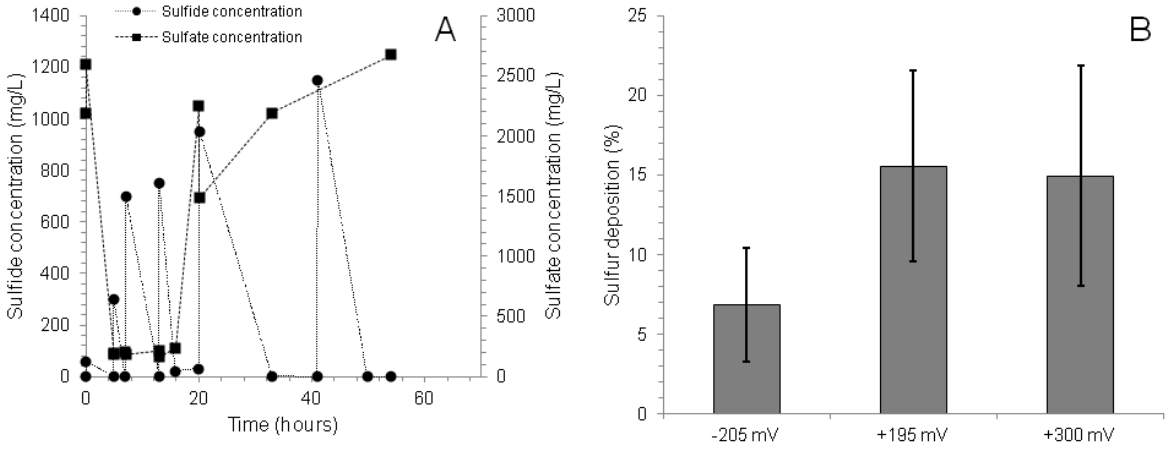

Figure 3. Sulfide concentration and sulfate concentration measured in the reactor during the experiment (A). Sulfur deposition on the electrodes after the experiment detected by SEM-EDS (B).

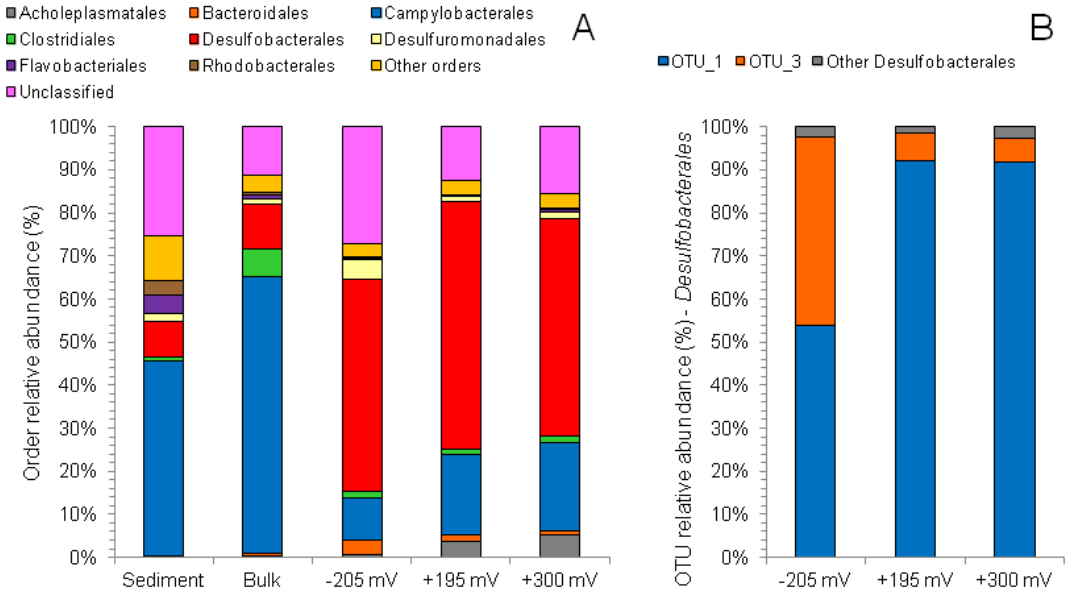

Figure 4. Taxonomic composition of the microbial communities at the order level (A). OTU relative abundance within the order *Desulfobacterales* on the electrodes (B). Average abundances are reported for the electrodes.

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