Title: Anode potential selection for sulfide removal in contaminated marine sediments

Authors: Matteo Daghio, Eleni Vaiopoulou, Federico Aulenta, Angela Sherry, Ian Head, Andrea Franzetti, Korneel Rabaey

PII: S0304-3894(18)30694-0
DOI: https://doi.org/10.1016/j.jhazmat.2018.08.016
Reference: HAZMAT 19640

To appear in: *Journal of Hazardous Materials*

Received date: 12-4-2018
Revised date: 13-7-2018
Accepted date: 6-8-2018

Please cite this article as: Daghio M, Vaiopoulou E, Aulenta F, Sherry A, Head I, Franzetti A, Rabaey K, Anode potential selection for sulfide removal in contaminated marine sediments, *Journal of Hazardous Materials* (2018), https://doi.org/10.1016/j.jhazmat.2018.08.016

Anode potential selection for sulfide removal in contaminated marine sediments

Matteo Daghio[1], Eleni Vaiopoulou[2,3*], Federico Aulenta[4], Angela Sherry[5], Ian Head [5], Andrea Franzetti[1], Korneel Rabaey[2]

[1] Department of Earth and Environmental Sciences – University of Milano-Bicocca, Piazza della Scienza 1, 20126 Milan, Italy

[2] Center for Microbial Ecology and Technology (CMET), Ghent University, Coupure Links 653, B-9000 Ghent, Belgium

[3] Concawe, Environmental Science for the European Refining Industry, Boulevard du Souverain 165, B-1160 Brussels, Belgium

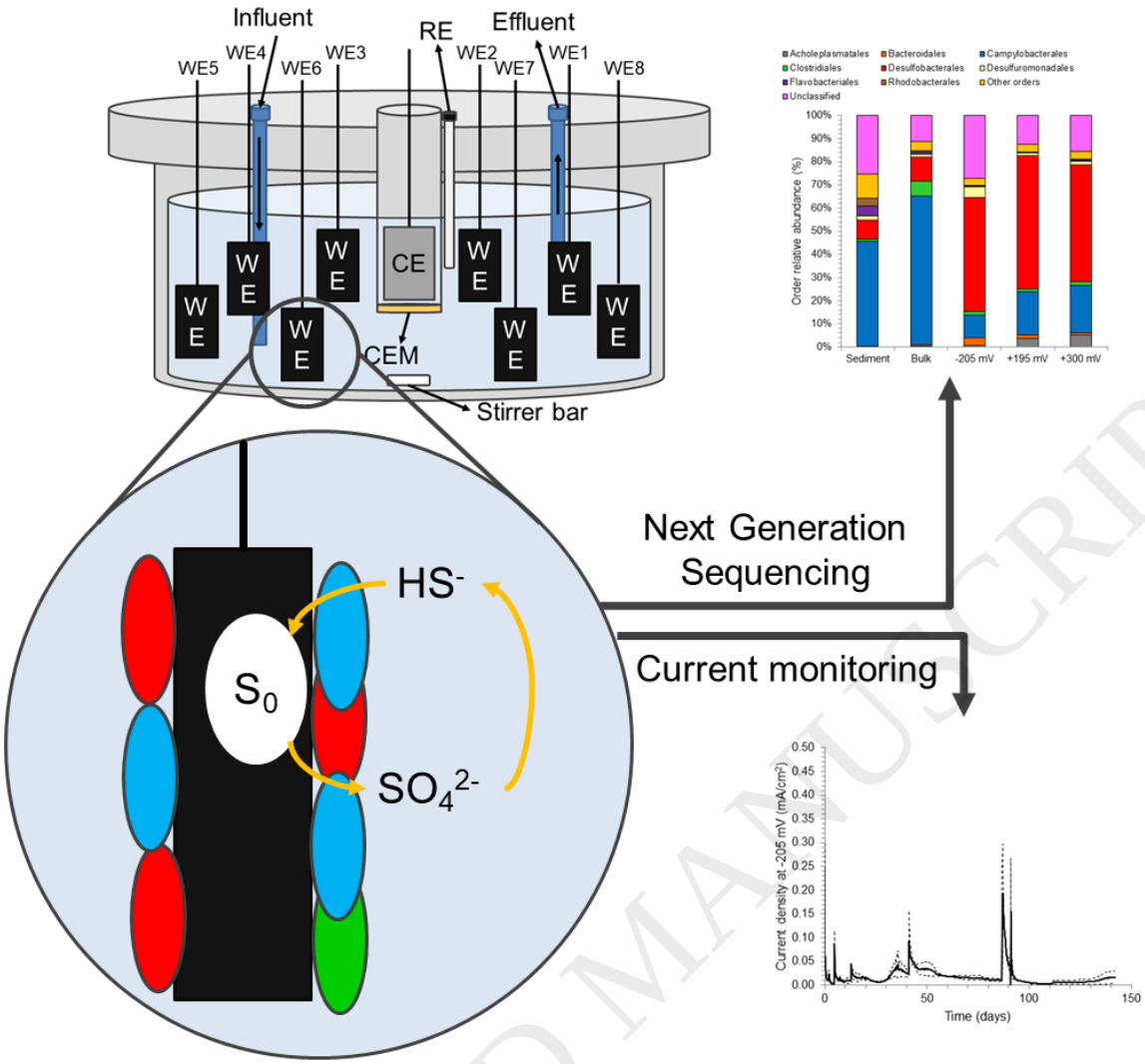
[4] Water Research Institute (IRSA), National Research Council (CNR), Via Salaria km 29,300, 00015 Monterotondo (RM), Italy

[5] School of Natural and Environmental Sciences 3rd Floor Devonshire Building Newcastle University Newcastle upon Tyne NE1 7RU UK

*corresponding author:

e-mail: vaiopoulou@yahoo.com

Graphical.abstract



Highlights

- (Bio)electrochemical removal of sulfide was tested at different anodic potentials
- Potentials of -205 mV, +195 mV and +300 mV (*vs* Ag/AgCl) were tested
- Current production linked to sulfide removal was observed
- *Desulfobulbus propionicus* promoted back oxidation of elemental sulfur to sulfate
- Highest electron recovery at +195 mV and lowest sulfur deposition at -205 mV

Abstract

Sulfate reducing microorganisms are typically involved in hydrocarbon biodegradation in the sea sediment, with their metabolism resulting in the by-production of toxic sulfide. In this context, it is of utmost importance identifying the optimal value for anodic potential which ensures efficient

toxic sulfide removal. Along this line, in this study the (bio)electrochemical removal of sulfide was tested at anodic potentials of -205 mV, +195 mV and +300 mV *vs* Ag/AgCl), also in the presence of a pure culture of the sulfur-oxidizing bacterium *Desulfobulbus propionicus*. Current production, sulfide concentration and sulfate concentration were monitored over time. At the end of the experiment sulfur deposition on the electrodes and the microbial communities were characterized by SEM-EDS and by next generation sequencing of the 16S rRNA gene respectively. Results confirmed that current production is linked to sulfide removal and *D. propionicus* promoted back oxidation of deposited sulfur to sulfate. The highest electron recovery was observed at +195 mV *vs* Ag/AgCl, and the lowest sulfur deposition was obtained at -205 mV *vs* Ag/AgCl 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 [1]. When the oil has sunk to the bottom, the water surface is clean again; this gives the illusion that the oil has been removed [2]. However, hydrocarbons are recalcitrant compounds that persist in marine sediments due to the absence of thermodynamically favorable electron acceptors below the oxic zone. In the anoxic zone the most electropositive electron acceptors are used first. The order in which the electron acceptors are consumed in the anoxic zone is: 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 [3]. This sequential consumption 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 diffuse into the oxic water zone and poison the respiratory enzymes of oxygen-respiring cells [4]. The anode of bioelectrochemical systems (BES) serves as an alternative electron acceptor and thus can facilitate oil spill bioremediation by enrichment of native bacteria [3,5–7]. Sulfide produced by sulfate reducers during hydrocarbon biodegradation in BES can act as an electron shuttle, being oxidized to elemental sulfur (Equation 1), which can be reduced again to sulfide [8–10].

Equation 1 $HS^- \rightarrow S_0 + H^+ + 2e^-$

Another process that has been observed is the anodic back oxidation of the elemental sulfur to sulfate (Equation 2), in a process mediated by microorganisms of the families *Desulfobulbaceae* and *Desulfuromonadaceae* [3,10].

Equation 2 $S_0 + 4H_2O \rightarrow SO_4^{2-} + 8H^+ + 6e^-$

Acknowledging the role of sulfur, either toxic or required for effective *in-situ* oil spill bioremediation, the investigation of the ideal anode potential for sulfide oxidation and power production becomes an appealing question. To specifically address this issue, a multi-electrode reactor containing anodes poised at different potentials was set up. Objectives of this study included: 1) stimulation of (bio)electrochemical sulfide to sulfate oxidation with the electrode serving as terminal electron acceptors, 2) identification of the potential with the highest electric current generation, 3) the correlation between the microbial communities and the performance.

2. Materials and Methods

2.1. Reactor construction and operation

A multi-electrode custom-made bottle-type glass reactor (500 mL) was used to immerse eight untreated rough graphite working electrodes (WEs) (2 × 2 × 0.5 cm) used as anodes [11]. WEs 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 (3 M KCl, all the potentials within the manuscript are referred *vs* Ag/AgCl, unless otherwise stated) electrode served as reference (RE) (all the potentials are referred *vs* Ag/AgCl). The reactor was filled with synthetic seawater (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 sediment was anaerobic and its organic content was 3.40% ± 0.04% (n=23). The indigenous population of the sediment served as the initial inoculation of the reactor. Synthetic seawater, used to simulate seawater, was free of organic matter. Target concentration of sulfide in the bulk solution was 200 mg/L and spiking was taking place when the current signal was declining close to zero. Bulk solution was slightly alkaline but sulfide spiking introduced temporarily pH drops. The separated chamber, which contained the membrane and the stainless steel mesh CE, was filled with 50 mL four times diluted artificial ocean water to

prevent from corrosion of the stainless steel mesh. The reactor was incubated at 20℃ under stirring and covered from light. The WEs were poised at +300, +195 or -205 mV in replicates (Fig. 1) using a CHI 1000C Multi-Potentiostat (CH Instruments, Austin, TX, USA).

2.2. Desulfobulbus propionicus cultivation

The strain *Desulfobulbus propionicus* (DSMZ 2023) was obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ, Germany). *D. propionicus* strain was cultivated in the DSMZ recommended growth medium (193. *Desulfobacter* Postgatei Medium; 194. *Desulfobulbus* Medium) with the adaptations indicated in Table 1. Inoculation was performed using the same inlet for sulfide spiking and the injection was instant.

2.3. Chemical and microscopy analysis

Current generation was monitored by chronoamperometry. Sulfide and sulfate profiles were measured as previously reported [9]. In order to monitor sulfate concentration, samples were filtered and stored at 4°C until the analysis using an ion chromatograph (Metrohm 761 Compact IC). Sulfide was measured immediately after sampling with the Nanocolor sulfide standard test (Macherey-Nagel, Düren, Germany). Scanning electron microscopy-energy dispersive spectrometry (SEM-EDS) analysis of the electrode surface was performed according to Daghio et al. 2016 [9].

2.4. Characterization of the microbial communities

At the end of the experiment, samples to characterize the microbial communities were collected and stored at -20°C until further processed. The microbial biofilm was aseptically scraped from the surface of each anode 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 [12]. 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 [13] was used for the bioinformatics elaborations as previously reported [9]. Classification of the sequences representative of each OTU was done using the RDP classifier (≥ 80% confidence) [14]. A hierarchical cluster analysis based on Hellinger transformed Operational Taxonomic Units (OTUs) relative abundance was performed with the HCLUST procedure in R 3.4.2 [15].

3. Results and Discussion

3.1. Electric current, sulfide and sulfate concentration profiles

During the initial 7 days, the reactor was operated without external addition of sulfide and the electric current resulting from the oxidation of organic and inorganic compounds already present in the bulk liquid and in the sediment was monitored. During this phase, regardless the set anode potential, the electric current rapidly dropped below 0.01 mA/cm^2 within few days. This means that there was no current production in the absence of an electron donor, i.e. sulfide. After this initial decrease of the current production, current peaks were only observed when sulfide was further added in the reactor (Fig. 2). Sulfide was rapidly removed from the medium after each spiking (Fig. 3A) most likely due to oxidation to elemental sulfur on the anodic surface. This is supported by previous studies that showed that (bio)electrochemical sulfide oxidation is an elective process to remove sulfide from wastewater [16]. When sulfide is oxidized to elemental sulfur, it precipitates on the anode surface, thereby reducing the electron transfer capabilities of the electrode over long term operation [16]. Under naturally occurring conditions, sulfide production is the result of sulfate-reducing activity, fueled by organic substances and contaminants, such as petroleum hydrocarbons,

occurring in the sediment. Therefore, a subsequent step to sulfide oxidation to elemental sulfur would be a microbially-driven back oxidation of elemental sulfur to sulfate, in order the replenish the electron acceptor availability. However, this step was not observed during the first days of operation (Fig. 3A). Indeed, sulfate concentration decreased rapidly from more than 2,000 mg/L to about 200 mg/L and was probably linked to the oxidation of biodegradable organic substances in the sediment used to inoculate the rector at the beginning of the experiment. To encourage the back oxidation of elemental sulfur to sulfate, a pure culture of *D. propionicus* DSMZ 2032 (10^7 cells/mL) was inoculated on day 16 in the reactor. On day 20, sulfate formation increased again up to about 2,200 mg/L. Previous studies have already demonstrated that *D. propionicus* is able to promote the back oxidation of elemental sulfur to sulfate in bioelectrochemical systems by electron transfer to the electrode [17,18]. Sulfate formation on day 20 provided, thus, a clear indication that *D. propionicus* stimulated the back oxidation of elemental sulfur previously deposited over the electrodes surface. Even though it appears likely that sulfur oxidation is directly coupled to the electrode, we cannot fully exclude a possible disproportionation reaction in which microorganisms can use sulfur as electron donor and acceptor, producing sulfide and sulfate [19]. The instant re-oxidation of the sulfide at the anode makes excluding this option highly complex. The maximum current was reached after the addition of *D. propionicus* and was 0.19 ± 0.06 mA/cm^2 (day 87), 1.5 ± 0.4 mA/cm^2 (day 91) and 1.5 ± 0.6 mA/cm^2 (day 91) for the electrodes polarized at -205 mV, +195 mV and +300 mV respectively. The aforementioned current peak values were reached after the last addition of sulfide in the reactor (day 80), but no data about sulfide removal are available. The cumulative charge calculated was 889 ± 166 C, 1,306 ± 365 C and 936 ± 147 C for the electrodes polarized at -205 mV, +195 mV and +300 mV respectively (Fig. 2D). Furthermore, the electron transfer was not constant during the experiment. The main contribution to the cumulative charge for the electrodes polarized at -205 mV was observed between the addition of *D. propionicus* (day 16) and the last addition of sulfide (day 80), while at higher potentials the charge transferred to the electrode was higher from day 0 to day 16, and after day 80, when the current

peak was observed (Table 2). This suggests that the highest potentials (i.e. +195 mV and +300 mV) were the most effective to possibly encourage sulfide oxidation, and there is an apparently enhanced *D. propionicus* activity at the potential of -205 mV.

The above assumptions are further supported by the SEM-EDS results (Fig 3B), which clearly indicated that at the end of the experimental period sulfur deposition was higher ($p < 0.05$) on the anodes polarized at +195 mV and +300 mV (16% ± 2% and 15% ± 2% respectively) compared to the anodes polarized at the lowest potential, i.e. -205 mV, (7% ± 1%).

3.2 Microbiological analysis of potentiostatically controlled bioanodes

The cluster analysis clearly indicated that the microbial community enriched on the electrodes poised at -205 mV was different compared to the communities enriched at the higher potentials (i.e +195 mV and +300 mV), in the bulk reactor, and in the marine sediment used to inoculate the reactor (Fig. 4). The classification (80% confidence) showed a selective shift in the communities from the initial inoculum to the biofilm formed on the electrodes. The most abundant order in the initial sediment was the order *Campylobacterales*, which was also found enriched in the bulk after the end of the experiment (Fig. 5A and Supplementary material). The order *Desulfobacterales* was the most abundant on the electrodes at all the tested potentials and ranged between 49% ± 3% (-205 mV), 50% ± 8% (+300 mV) and 57% ± 5% (+195 mV). High enrichment of microorganisms of the families *Desulfobulbaceae* and *Desulfobacteraceae*, both members of the *Desulfobacterales*, was observed previously in 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) [9]. There is an indication of these families contributing in hydrocarbon degradation and in oxidation of biologically produced sulfide [9]. 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% ± 0.2% and 1.5% ± 0.4% respectively and was comparable to the abundance observed in the initial sediment (2%) and in the bulk of the

reactor (1%). Conversely, a slight enrichment ($p < 0.05$) of the order *Desulfuromonadales* was observed on the anode poised at -205 mV (5% ± 1%). *Desulfuromonas acetoxidans*, a member of the order *Desulfuromonadales*, is able to use both elemental sulfur and anodes as electron acceptors [20]. The presence of this order may thus be linked to the reduction of elemental sulfur producing sulfide. Within the order *Desulfobacterales* the most abundant OTUs were OTU_1 and OTU_3 (Fig. 5B). 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 ($p < 0.05$) was observed in the samples collected from the anodes polarized at -205 mV (44% ± 11% of the order *Desulfobacterales*) compared to the samples collected at more positive potentials (6.48% ± 0.07% at +195 mV, 5.5% ± 0.9% at +300 mV). The sequences of both OTU_1 and OTU_3 was used to determine the best match to sequences in the RDP database (Supplementary material). OTU_1 was close to *Desulfocapsa sulfexigens* (seqmatch score 0.986) a microorganism that is able to perform disproportionation of elemental sulfur [21], and may have had a role in sulfur disproportionation on the electrodes in this study. 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. However, the lower amount of sulfur detected on the anodes at -205 mV, may also be due to a lower sulfide oxidation rate at this potential, as suggested both by the current profile and the cumulative charge.

4. Conclusions

Sulfide oxidation coupled to electrons delivery to the anode in BES was tested at three anode potentials (i.e. -205 mV, +195 mV and +300 mV *vs* Ag/AgCl). Sulfide removal was most likely coupled to sulfur formation and deposition on the electrode surface. The best performances in terms of electrons recovery were observed when the anode was poised at +195 mV. As both high electron

recovery and similar sulfur deposition were recorded at the positive polarization potentials (+195 and +300 mV), these findings might imply a polarization threshold for achieving sulfide oxidation with a non-critical electrode poisoning. The inoculation with a pure culture of *D. propionicus* successfully led to the back oxidation of elemental sulfur to sulfate. Microorganisms phylogenetically close to *D. propionicus* were enriched on the anode surface at the lowest applied potential (i.e. -205 mV), therefore confirming the role of this microorganism during sulfide (bio)electrochemical scavenging and suggesting that this microorganism can be advantaged when a potential of -205 mV is applied. However, also possible sulfur disproportionation may have occurred due to the presence of a close relative to *D. sulfexigens*. Further studies are thus needed to provide useful information for the characterization of the microbial activity during sulfur removal from electrodes. Our results provide a further indication that the use of BES in petroleum contaminated environments is a powerful approach, not only to directly stimulate the microbial degradation of hydrocarbons, but also to scavenge toxic metabolites (i.e. sulfide) and replenish the metabolic electron acceptor (i.e. sulfate), when microorganisms able to remove elemental sulfur from the anode surface are enriched. Furthermore, the presence of microorganisms able to remove elemental sulfur from the surface of the anodes could increase the lifetime of the electrodes, decreasing the need of their replacement during long term operations.

Overall, the combined effect of sulfide scavenging and sulfur back oxidation to sulfate driven by a polarized anode may represent a viable strategy to stimulate the metabolic activity of sulfate-reducing communities involved in the anaerobic degradation of hydrocarbons. Clearly, further work is needed to verify the scalability and actual viability of this approach under fully representative environmental conditions. In this context, the relatively high electrical conductivity of seawater (typically >40 mS/cm) which would in turn result in low Ohmic resistances combined with the low current densities typically required in electrobioremediation processes (in the range of a few A/m^2) point to a relatively straightforward scalability of the proposed technology.

Acknowledgements

This work was financially supported by the European Commission within the Seventh Framework Programme under Grant Agreement No. 312139, "Kill-Spill: Integrated biotechnological solutions for combating marine oil spills". MD, AF, and FA are supported by Fondazione Cariplo in the framework of the project BEvERAGE - BioElEctrochemical RemediAtion of Groundwater plumes (2015-0195). Authors wish to thank Royal Netherlands Institute for Sea Research (NIOZ) for supplying sediment material. Kim Suetens is acknowledged for her assistance in lab work.

References

[1] A.M. Warnock, S.C. Hagen, D.L. Passeri, Marine tar residues: a review, Water. Air. Soil Pollut. 226 (2015) 68. doi:10.1007/s11270-015-2298-5.

[2] T.R. Annunciado, T.H.D. Sydenstricker, S.C. Amico, Experimental investigation of various vegetable fibers as sorbent materials for oil spills., Mar. Pollut. Bull. 50 (2005) 1340–1346. doi:10.1016/j.marpolbul.2005.04.043.

[3] M. Daghio, F. Aulenta, E. Vaiopoulou, A. Franzetti, J.B.A. Arends, A. Sherry, A. Suárez-Suárez, I.M. Head, G. Bestetti, K. Rabaey, Electrobioremediation of oil spills, Water Res. 114 (2017) 351–370. doi:10.1016/j.watres.2017.02.030.

[4] A. Boetius, K. Ravenschlag, C.J. Schubert, D. Rickert, F. Widdel, A. Gieseke, R. Amann, B.B. Jørgensen, U. Witte, O. Pfannkuche, A marine microbial consortium apparently mediating anaerobic oxidation of methane., Nature. 407 (2000) 623–626. doi:10.1038/35036572.

[5] C. Cruz Viggi, E. Presta, M. Bellagamba, S. Kaciulis, S.K. Balijepalli, G. Zanaroli, M. Petrangeli Papini, S. Rossetti, F. Aulenta, The "Oil-Spill Snorkel": an innovative bioelectrochemical approach to accelerate hydrocarbons biodegradation in marine sediments, Front. Microbiol. 6 (2015) 881. doi:10.3389/fmicb.2015.00881.

[6] J.M. Morris, S. Jin, Enhanced biodegradation of hydrocarbon-contaminated sediments using

microbial fuel cells., J. Hazard. Mater. 213–214 (2012) 474–477. doi:10.1016/j.jhazmat.2012.02.029.

[7] T. Zhang, S.M. Gannon, K.P. Nevin, A.E. Franks, D.R. Lovley, Stimulating the anaerobic degradation of aromatic hydrocarbons in contaminated sediments by providing an electrode as the electron acceptor., Environ. Microbiol. 12 (2010) 1011–1020. doi:10.1111/j.1462-2920.2009.02145.x.

[8] B. Matturro, C. Cruz Viggi, F. Aulenta, S. Rossetti, Cable bacteria and the bioelectrochemical snorkel: the natural and engineered facets playing a role in hydrocarbons degradation in marine sediments, Front. Microbiol. 8 (2017) 952. doi:10.3389/fmicb.2017.00952.

[9] M. Daghio, E. Vaiopoulou, S.A. Patil, A. Suárez-Suárez, I.M. Head, A. Franzetti, K. Rabaey, Anodes stimulate anaerobic toluene degradation via sulfur cycling in marine sediments, Appl. Environ. Microbiol. 82 (2016) 297–307. doi:10.1128/AEM.02250-15.

[10] M. Daghio, A. Espinoza Tofalos, B. Leoni, P. Cristiani, M. Papacchini, E. Jalilnejad, G. Bestetti, A. Franzetti, Bioelectrochemical BTEX removal at different voltages: assessment of the degradation and characterization of the microbial communities, J. Hazard. Mater. 341 (2018) 120–127. doi:10.1016/j.jhazmat.2017.07.054.

[11] K. Guo, B.C. Donose, A.H. Soeriyadi, A. Prévoteau, S.A. Patil, S. Freguia, J.J. Gooding, K. Rabaey, Flame oxidation of stainless steel felt enhances anodic biofilm formation and current output in bioelectrochemical systems, Environ. Sci. Technol. 48 (2014) 7151–7156. doi:10.1021/es500720g.

[12] R. Ferrentino, M. Langone, I. Gandolfi, V. Bertolini, A. Franzetti, G. Andreottola, Shift in microbial community structure of anaerobic side-stream reactor in response to changes to anaerobic solid retention time and sludge interchange ratio, Bioresour. Technol. 221 (2016) 588–597. doi:10.1016/j.biortech.2016.09.077.

[13] R.C. Edgar, UPARSE: highly accurate OTU sequences from microbial amplicon reads., Nat.

Methods. 10 (2013) 996–998. doi:10.1038/nmeth.2604.

[14] Q. Wang, G.M. Garrity, J.M. Tiedje, J.R. Cole, Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy., Appl. Environ. Microbiol. 73 (2007) 5261–5267. doi:10.1128/AEM.00062-07.

[15] R Core Team, R: A Language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. (2013). http://www.r-project.org/.

[16] P.K. Dutta, J. Keller, Z. Yuan, R.A. Rozendal, K. Rabaey, Role of sulfur during acetate oxidation in biological anodes, Environ. Sci. Technol. 43 (2009) 3839–3845. doi:10.1021/es803682k.

[17] D.E. Holmes, D.R. Bond, D.R. Lovley, Electron transfer by *Desulfobulbus propionicus* to Fe(III) and graphite electrodes, Appl. Environ. Microbiol. 70 (2004) 1234–1237. doi:10.1128/AEM.70.2.1234.

[18] T. Zhang, T.S. Bain, M.A. Barlett, S.A. Dar, O.L. Snoeyenbos-West, K.P. Nevin, D.R. Lovley, Sulfur oxidation to sulfate coupled with electron transfer to electrodes by *Desulfuromonas* strain TZ1., Microbiology. 160 (2014) 123–129. doi:10.1099/mic.0.069930-0.

[19] K. Finster, Microbiological disproportionation of inorganic sulfur compounds, J. Sulfur Chem. 29 (2008) 281–292. doi:10.1080/17415990802105770.

[20] D.R. Bond, D.E. Holmes, L.M. Tender, D.R. Lovley, Electrode-reducing microorganisms that harvest energy from marine sediments., Science. 295 (2002) 483–485. doi:10.1126/science.1066771.

[21] K.W. Finster, K.U. Kjeldsen, M. Kube, R. Reinhardt, M. Mussmann, R. Amann, L. Schreiber, Complete genome sequence of *Desulfocapsa sulfexigens*, a marine deltaproteobacterium specialized in disproportionating inorganic sulfur compounds., Stand. Genomic Sci. 8 (2013) 58–68. doi:10.4056/sigs.3777412.

Figure legends

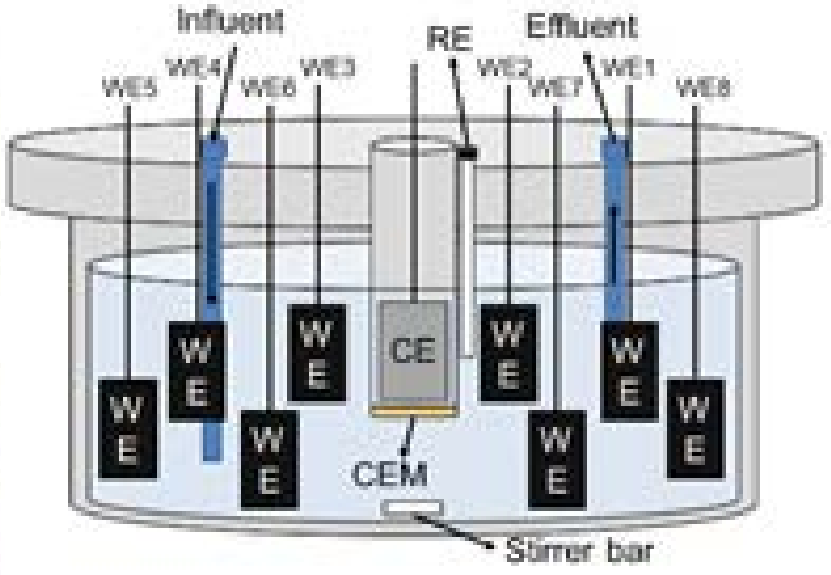
Fig. 1 - Multi-electrode reactor set-up photo, lay-out and anodes (WE: working electrode) poised potentials *vs* Ag/AgCl reference electrode (RE). Location of the counter electrode (CE) and of the Cation Exchange Membrane (CEM) is reported. Sediment source: Grevelingen, Royal Netherlands Institute for Sea Research.

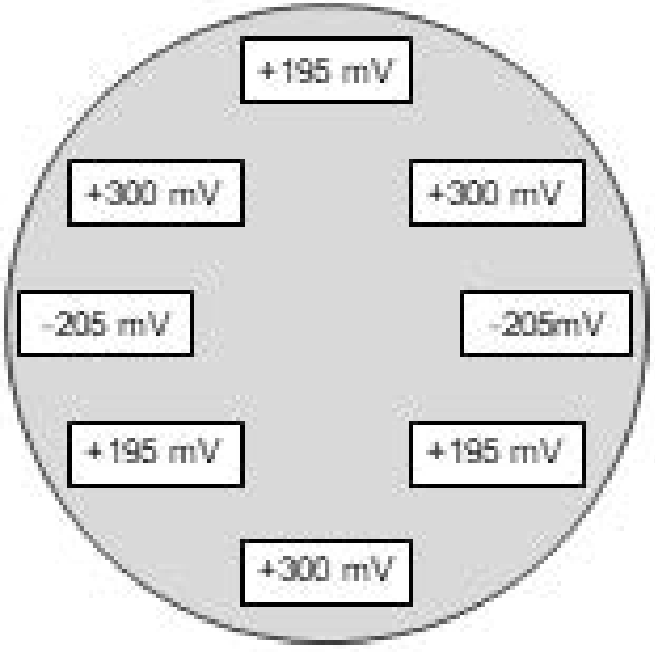
Fig. 2 - Current density measured for the electrodes polarized at -205 mV (A), +195 mV (B) and +300 mV (C) during the experiment. Sulfide spiking is indicated with an arrow. Solid line depicts average current value ± standard error (dotted lines) per electrode potential tested. The maximum current values 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.

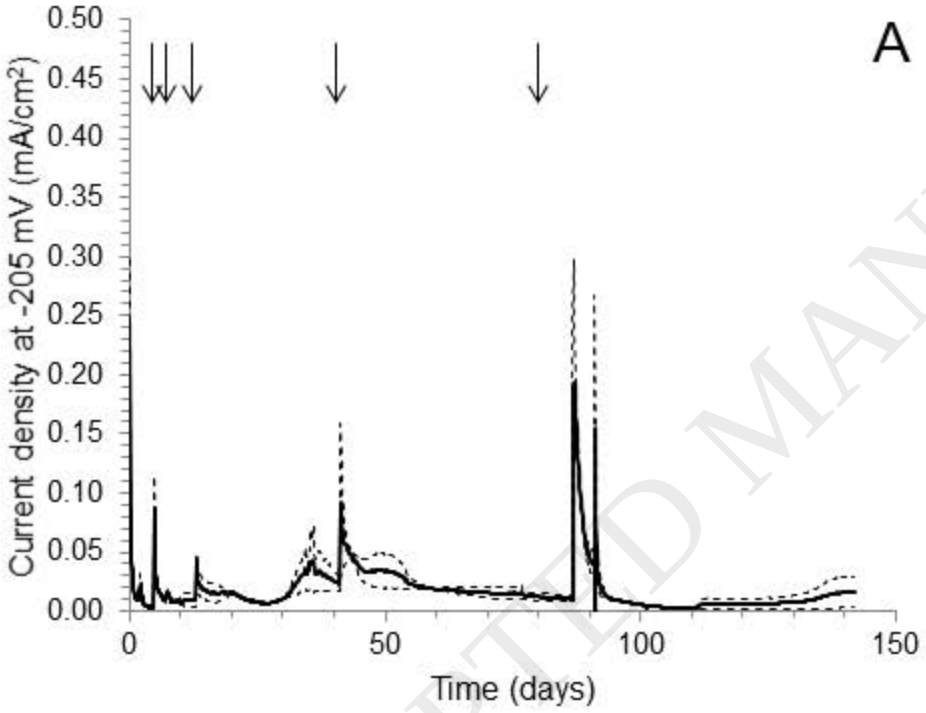
Fig. 3 - Sulfide concentration and sulfate concentration measured in the reactor during the experiment (A). Sulfur deposition (average ± standard error) on the electrodes after the experiment detected by SEM-EDS (B).

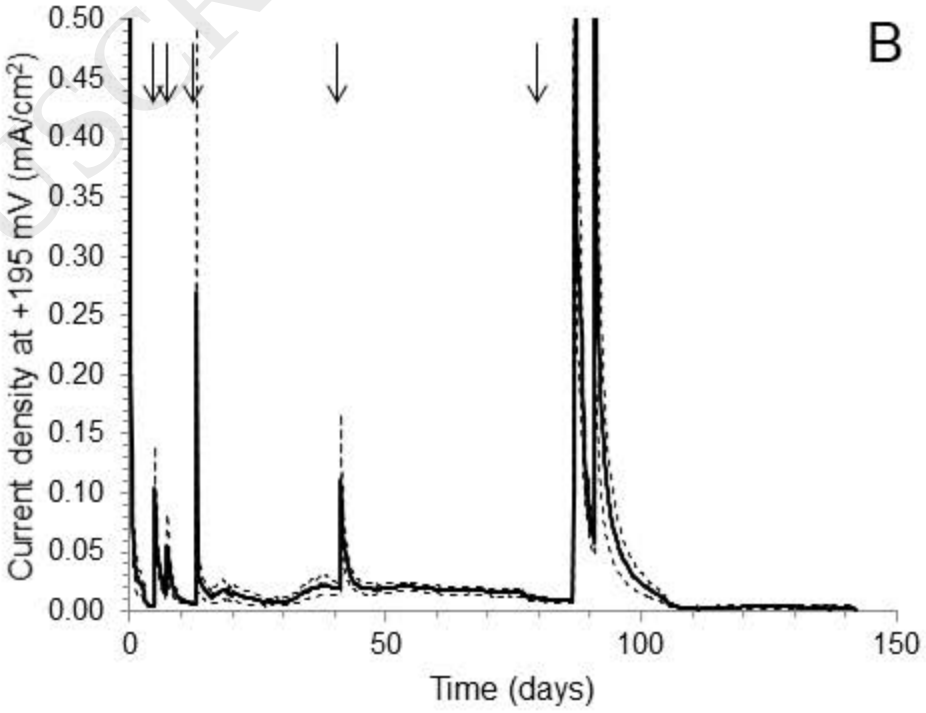
Fig. 4 - Hierarchical cluster analysis performed on Hellinger transformed Operational Taxonomic Units (OTUs) relative abundance. The main clusters are highlighted with different colors.

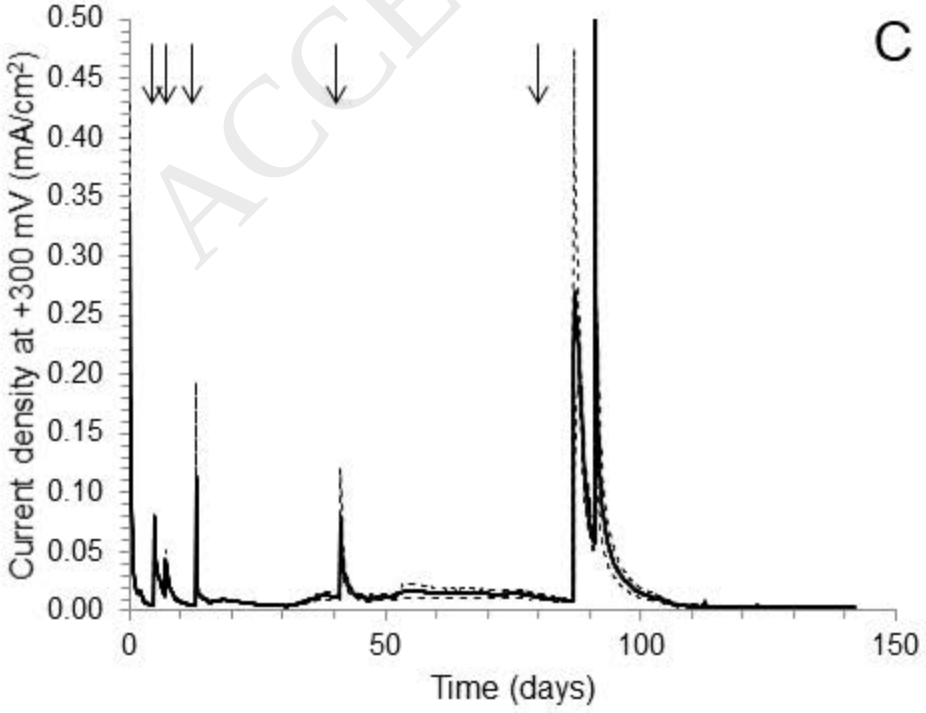
Fig. 5 - Taxonomic composition of the microbial communities at the order level (A). OTU relative abundance within the order *Desulfobacterales* on the electrodes (B). Average abundances are reported for each tested potential.

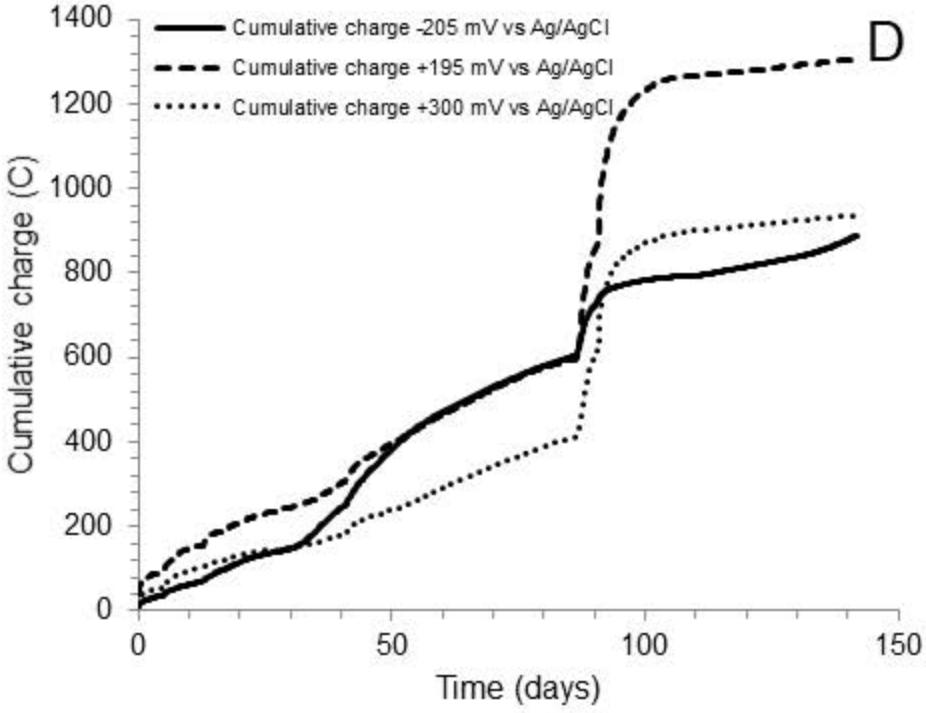

Influent
Effluent
RE
WE5
WE4
WE6
WE3
WE2
WE7
WE1
WE8
WE
CE
CEM
Stirrer bar

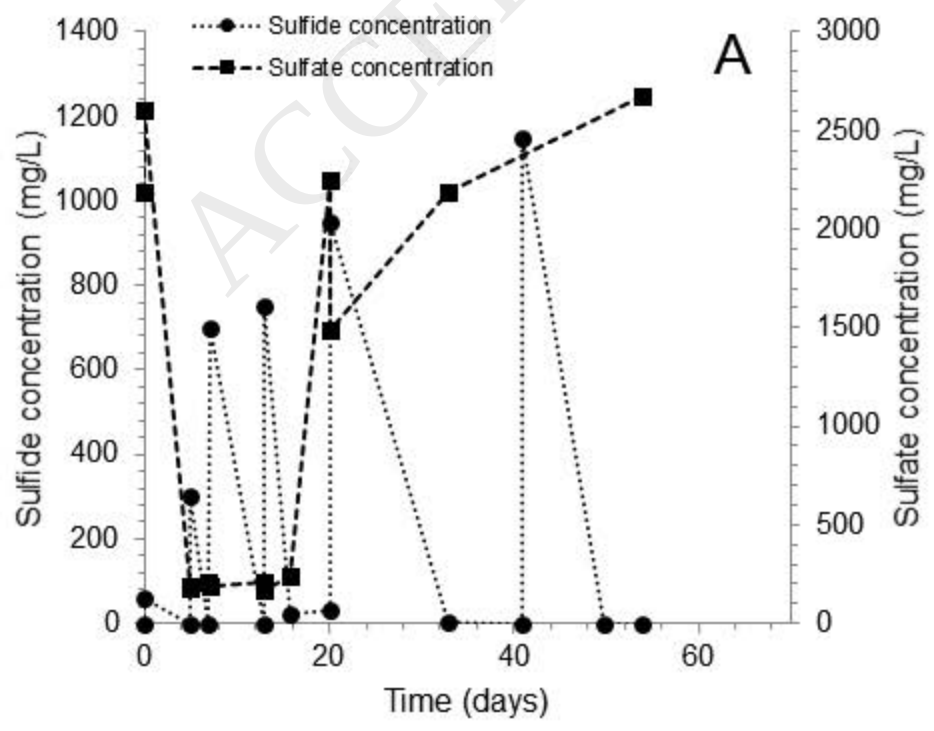

+195 mV
+300 mV
+300 mV
-205 mV
-205mV
+195 mV
+195 mV
+300 mV

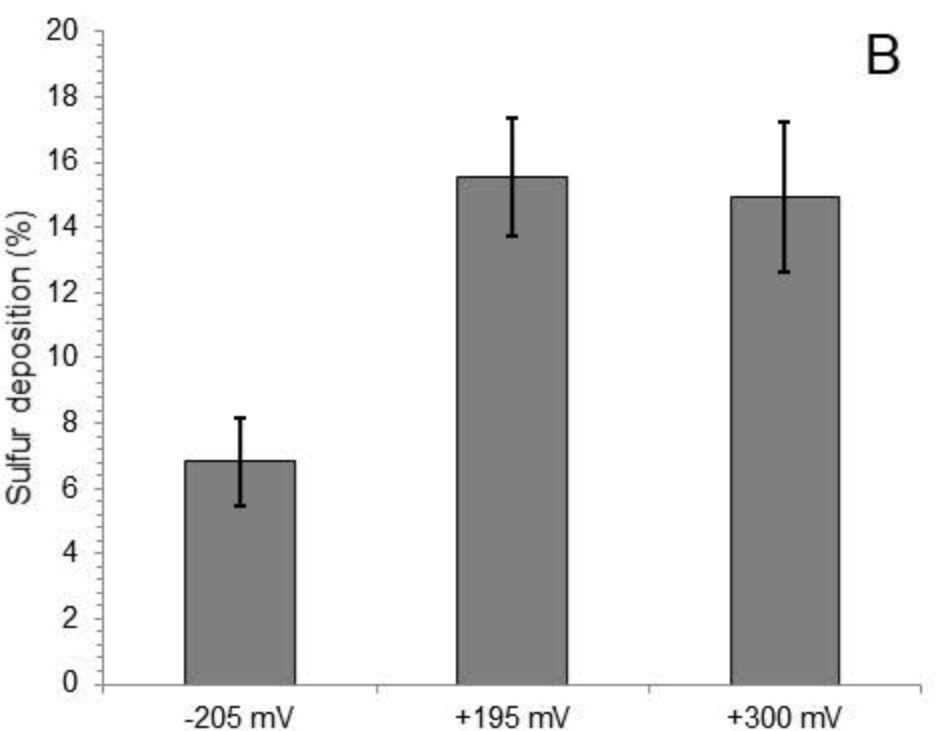


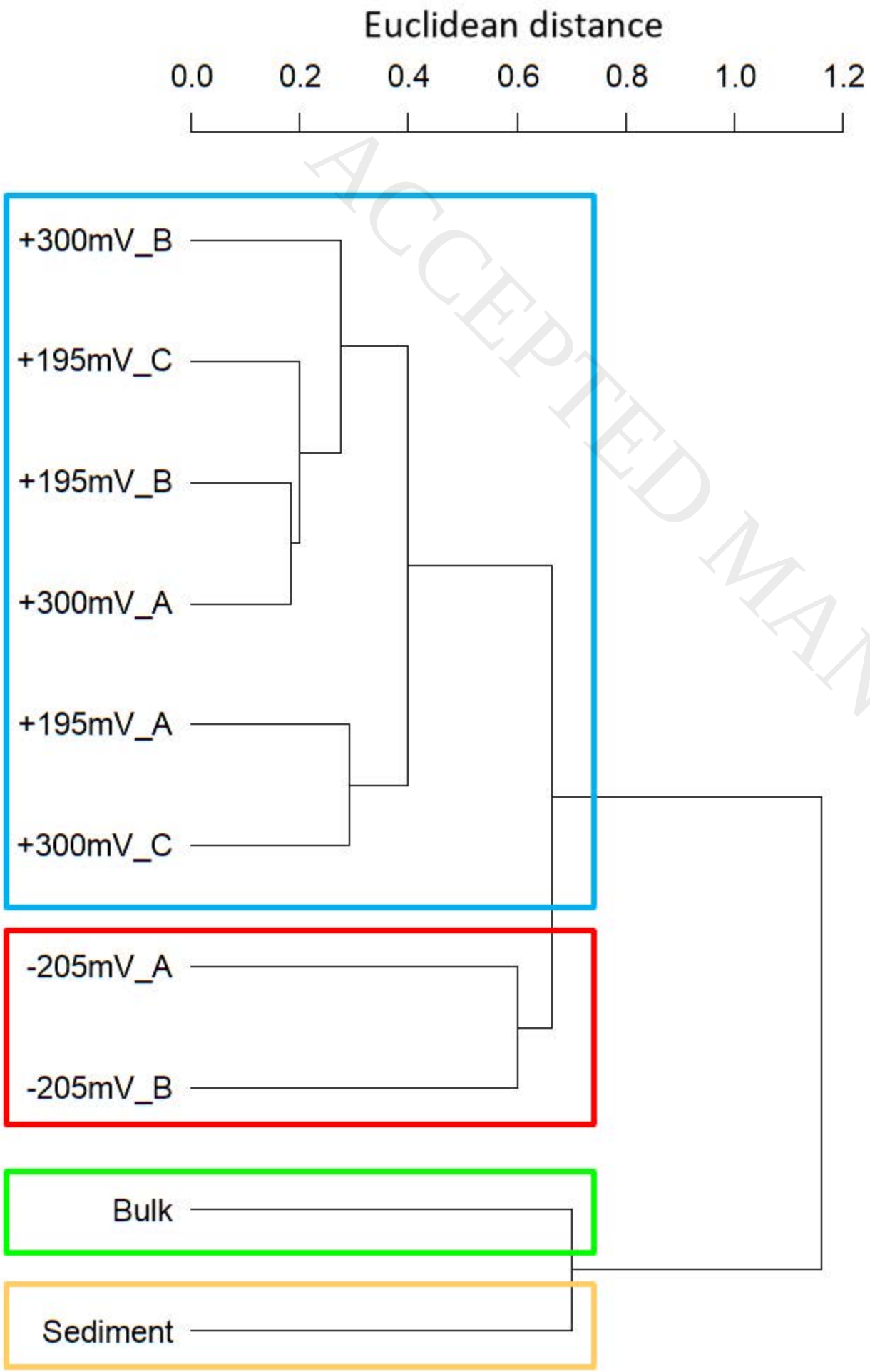


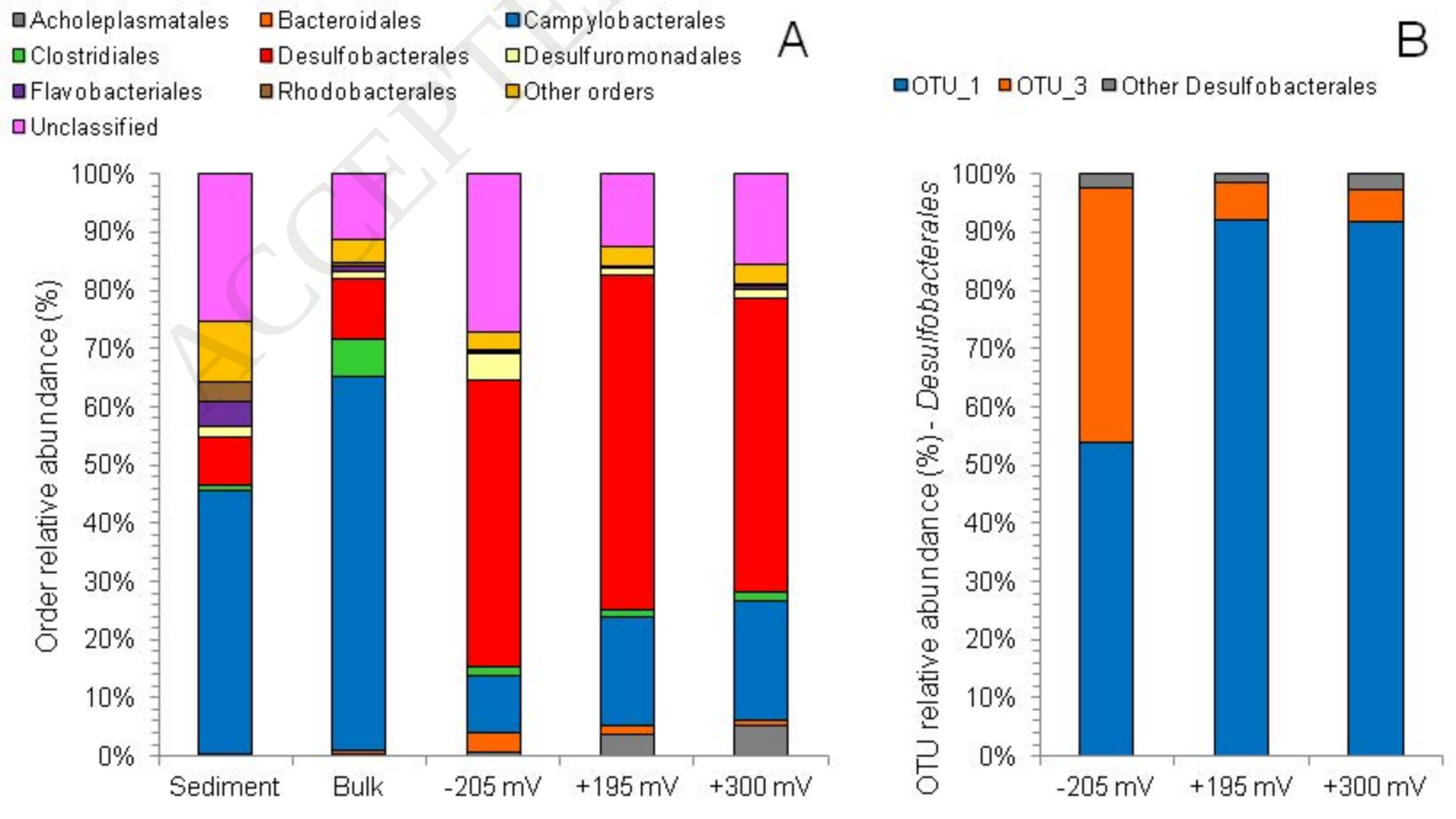





Sulfide concentration
Sulfate concentration
A
Sulfide concentration (mg/L)
Sulfate concentration (mg/L)
Time (days)
0
200
400
600
800
1000
1200
1400
500
1000
1500
2000
2500
3000
0
20
40
60


B
Sulfur deposition (%)
0
2
4
6
8
10
12
14
16
18
20
-205 mV
+195 mV
+300 mV




Acholeplasmatales
Bacteroidales
Campylobacterales
Clostridiales
Desulfobacterales
Desulfuromonadales
Flavobacterales
Rhodobacterales
Other orders
Unclassified
A
Order relative abundance (%)
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Sediment
Bulk
-205 mV
+195 mV
+300 mV
B
OTU_1
OTU_3
Other Desulfobacterales
OTU relative abundance (%) - Desulfobacterales
-205 mV
+195 mV
+300 mV

Tables

Table 1 - Adapted growth medium of the DSMZ for *D. propionicus*

Changes to original DSMZ medium
Solution A: 1.00 g NaCl
Solution A: 0.4 g $MgCl_2$ x 6 H_2O
Solution C: $NaHCO_3$ + cysteine HCl 5%
Solution D: 1.5 g sodium propionate

Table 2 - Cumulative charge calculated during the experiment (0-16d: before *D. propionicus* addition; current peaks attributed mainly to sulfide electro-oxidation, 16-80d: after *D. propionicus* addition; additional e-transfer due to S removal, 80-142d: post *D. propionicus* addition; related both to sulfide electro-oxidation and S removal, before maximum current production). Data are reported as average ± standard error

Days	**Cumulative charge -205 mV (C)**	**Cumulative charge +195 mV (C)**	**Cumulative charge +300 (C)**
0-16	**94 ± 6**	**188 ± 70**	**119 ± 9**
16-80	**486 ± 69**	**389± 95**	**271 ± 54**
80-142	**309 ± 102**	**729 ± 244**	**547 ± 122**