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Methanobacterium enables high rate electricity-driven autotrophic sulfate reduction

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The autotrophic reduction of sulfate can be sustained with a cathode as the only electron donor in bioelectrochemical systems (BES). This work studies the effect of inoculum source on autotrophic sulfate reduction start-up and performance of autotrophic sulfide production rates using a biocathode in fed-batch operation mode. After 180 days, low electron and sulfate consumption was observed using BES controlled at -0.9 V vs SHE and inoculated with mixed microbial consortia from sewer biofilm reactors, anaerobic sludge and mangrove sediments. However, when an enriched electroactive consortium capable of cathodic CO_2 reduction to acetate was used as biocatalyst in combination with the above inocula, the maximal cathodic current increased to -3.4 A m^{-2} within 55 days at the same applied potential. High-throughput microbial community sequencing revealed that enhanced performance was likely caused by the enrichment of hydrogen-producing *Methanobacterium* (26% relative abundance). The biofilm and planktonic cells also contained the autotrophic hydrogen and sulfate consumer *Desulfovibrio* at 2.8% relative abundance. The resulting microbial community demonstrated sulfate and electron consumption rates of 0.115 ± 0.009 mol SO_4^{2-}-S m^{-2} d^{-1} and 1.5 ± 0.7 mol m^{-2} d^{-1} (39 times higher sulfate reduction rate and 186-fold cathodic electron consumption rate than control reactors with same configuration but lacking the enriched electroactive consortia). Cyclic voltammetry furthermore revealed a positive shift of the cathodic onset current by ~0.2 V, which points to the electrocatalytic role of the biocatalyst.

Introduction

Biological sulfate reduction is rapidly becoming a key treatment process for many sulfate rich waste streams including wastewater, off-gas and solid waste[1, 2]. While sulfate can be readily reduced to sulfide under anaerobic conditions through biological processes, the latter require specific sources of electron donors[3], such as fermentation products (e.g. acetate, propionate, etc.) or hydrogen[4, 5]. However, such electron donors are commonly missing in the industrial scenarios where sulfate-rich waste streams are produced (e.g. acid mine drainage in mining)[6]. There have certainly been attempts to bring in or generate suitable electron donors to such sites in order to reduce sulfate discharge, yet the costs of transportation and production of these donors remain a barrier for widespread implementation[7].

Recent studies have suggested that biological sulfate reduction can also be driven by electricity as the sole electron source by using a potentiostatically-controlled bioelectrochemical system (BES) and applying a suitable potential for direct sulfate reduction (Eq. 1)[8, 9]

$$SO_4^{2-} + 9H^+ + 8e^- \rightarrow HS^- + 4H_2O \qquad E^{0\prime}=-0.213 \text{ V vs. SHE at pH=7} \quad \text{(Eq.1)}$$

Su *et al.* [8] reported on microbially-catalysed sulfate reduction with polarised electrode at the theoretical potential (−0.2 V vs. SHE) as the sole electron donor without electron shuttles or hydrogen production. Another study by Coma and colleagues[9] observed sulfate reduction when the cathodic potential was poised at -0.26V vs. SHE. However, these studies did not back the claimed electrode reactions with sulfur balances, nor were electrochemical or microbial characterisations undertaken. Furthermore, Luo *et al.*[10] compared the sulfate removal efficiency of autotrophic biocathodes at different cathode potentials. They suggested that hydrogenotrophic sulfate reduction was the main mechanism for sulfate reduction (Eq. 2, 3). Nevertheless, there has been no detailed investigation on this assumption.

$$8H^+ + 8e^- \rightarrow 4H_2 \qquad E^{0\prime}= -0.420 \text{ V vs. SHE at pH=7} \qquad \text{(Eq.2)}$$

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$4H_2 + SO_4^{2-} + H^+ \rightarrow HS^- + 4H_2O$ (Eq.3)

Although these systems claimed the removal of sulfate, there was no study of the effect of microbial community on the start-up time and sulfide production rates.

In this work, we aim to prove that autotrophic sulfate reduction to sulfide can be achieved with a cathode as the sole electron donor in bioelectrochemical systems without organic compounds or hydrogen supply. Furthermore, this study aims to link start-up time and sulfate reduction rates to microbial community composition.

2 Material and methods

2.1 Source of sulfate-reducing microorganisms

Two inocula were investigated: (1) Non-acclimated mixed microbial inoculum (NAI) and (2) Electroactive inoculum (EI) from a CO_2-reducing biocathode. The non-acclimated inoculum was a combination of microbial samples collected from sewer biofilm reactors [11], anaerobic sludge from the Luggage Point Waste Water Treatment Plant digester (Brisbane, Australia) and samples of mangrove sediments located at the Brisbane Botanic Gardens (latitude -27.478520, longitude 153.03014) in a 1:1:1 weight ratio. The electroactive inoculum was a mixture of the aforementioned non-electroactive inoculum (after 180 days of operation in BES) and planktonic microbial consortia from autotrophic acetate-producing bioelectrochemical reactors [12] in a 1:1 weight ratio.

2.2 BES operation

Reactor setup and operation

Four three-electrode bioelectrochemical systems (Figure 1) were operated in fed-batch mode at room temperature (20 ± 3^{0}C) and dark conditions under constant stirring, with a further identical but abiotic control reactor operated in the same conditions for one month. A custom-made borosilicate bottle (liquid volume 0.5 L) hosted the cathode working electrode, while the counter electrode (anode) was confined in a glass tube inserted into the bottle and separated from the latter by a cation exchange membrane (CEM) (Ultrex CMI-7000, Membranes International Inc., USA). The cathode consisted of two pieces of plain carbon cloth (Fuel Cell Store, USA) cut in a rectangle of 85 × 25 × 1 mm. The effective surface area of the cathode was therefore 85 cm^2 (taking into account that there were two pieces of cloth and both sides were exposed) for a specific surface area of 17 m^2 m^{-3}.

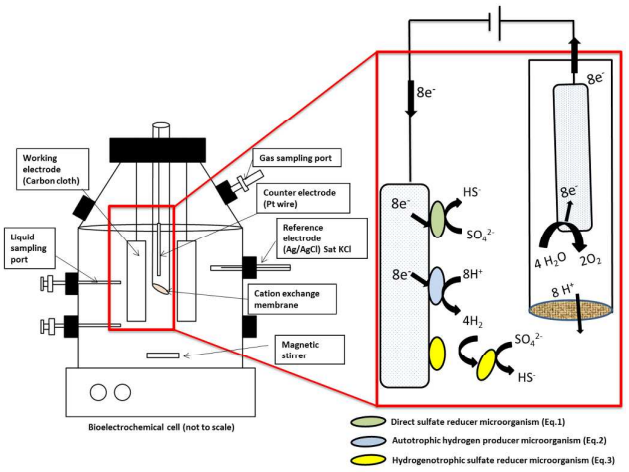

Figure 1. Autotrophic bioelectrochemical sulfate reduction reactor design and suggested mechanisms for current-driven autotrophic sulfate reduction.

As a pre-treatment, the electrodes were cleaned by immersion in RO water: isopropanol 1:1 solution 3 times, rinsed in deionized water and pre-treated in N_2 plasma for 15 minutes to remove organic and metal contamination[13]. Ti mesh connected to the carbon cloth was used as current collector. The cathode chamber was initially sparged with nitrogen to ensure anaerobic conditions. The anode chamber consisted of a 16 cm long glass tube (1cm diameter) inserted through the top (14 cm^3), with platinum wire as counter electrode (purity 99.95%, 0.50 mm diameter x 50 mm long, Advent Research Materials Ltd, UK). A KCl saturated Ag/AgCl reference electrode (+197 mV vs. SHE) was inserted in proximity of the cathode. All potentials are reported versus Standard Hydrogen Electrode (SHE).

Experiments were carried out on two different multichannel potentiostats, a CHI1000B (CH Instruments, USA) and a VMP-3 (Bio-Logic SAS, France). The catholyte was modified M9 medium containing 6 g L^{-1} Na_2HPO_4, 3 g L^{-1} KH_2PO_4, 0.1 g L^{-1} NH_4Cl, 0.5 g L^{-1} NaCl, 0.04 g L^{-1} $MgCl_2{\cdot}6H_2O$, 0.015 g L^{-1} $CaCl_2$, and 1 mL L^{-1} trace elements solution as previously described[14]. A final concentration of 0.12-1.5 g L^{-1} $NaHCO_3$ and 2.2 g L^{-1} Na_2SO_4 were added every 3-5 days as sole carbon and sulfate sources. The medium was prepared under anaerobic conditions (flushed with 100% N_2). The anolyte composition was 0.05 g L^{-1} Na_2HPO_4 and 0.025 mg L^{-1} KH_2PO_4. Two ports were equipped with butyl-rubber septa to take samples from both the liquid phase and the headspace. A gas bag (SKC standard Flexfoil, Air-Met Scientific Pty Ltd, Australia), specified for collection of CO/CO_2, H_2, H_2S and CH_4 was connected to the reactor to measure gas composition and avoid overpressure within the cathode chamber. Two BESs were inoculated with 10 ml of NAI and another two with 10 ml of EI to a final concentration of 200 mg COD L^{-1} in each BES. During the experiment, the catholyte medium was replaced every 3-5 days with 400 ml of fresh medium to prevent the impact

of salinity and sulfide build-up on the performance of sulfate reducing reactors. The catholyte pH was adjusted to 7.3 at the same time. The reactors performance was evaluated for each different inoculum applied by sampling of the liquid phases and online current measurements. The frequency of sampling was every 10 days for reactor with NAI and every 1-2 days for reactors inoculated with EI. BESs were controlled chronoamperometrically at -0.9 V vs SHE. Additionally, their electrochemical performance was assessed and characterized using cyclic voltammetry (CV) for the identification of any catalytic effects at day 19 for electroactive inoculum and day 180 for non-electroactive mixed microbial inoculum. The scan range of the CV was from -0.1 to -1.2 V vs SHE at a low scan rate of 1 mV s^{-1} to minimise the interference from capacitive currents [15]. Statistical analyses were performed using the R software. After verifying t-test assumptions, a Welch's two sample t-test was conducted between duplicates and different inocula. Significant differences of means were detected at the 5% significance level. Direct autotrophic sulfate reduction is catalyzed via two distinct suggested mechanisms, which involve direct electron transfer by a suggested sulfate reducer microorganism (green cell in Figure 1) or indirect (H_2-mediated) catalysed by an autotrophic hydrogen producer microorganism (blue cell in Figure 1), which allow hydrogenotrophic sulfate reduction (yellow cell in Figure 1).

2.3 Analytical methods

All samples for chemical analysis were filtered through a 0.22 µm filter (Millipore, USA). A special sample collection and preservation protocol was used to minimize oxidation and stripping of sulfide, with liquid samples immediately preserved in sulfide anti-oxidant buffer solution (SAOB) [16]. The SAOB solution was also used to dilute the samples where necessary. The dissolved sulfide (HS^--S and S^{2-}-S), sulfate (SO_4^{2-}-S), thiosulfate ($S_2O_3^-$-S) and sulfite (SO_3^{2-}-S) concentrations were measured with ion chromatography (IC) using a Dionex ICS-2000 system. The IC was equipped with an AG18 column (Dionex, USA). A UV detector was used for sulfide measurement while a suppressed conductivity detector was used for sulfate, thiosulfate and sulfite. The mobile phase was KOH at a flow rate of 1.0 mL min^{-1}.

2.4 Microbial community analysis

DNA extraction

Genomic DNA was extracted from 50 mg of raw sample using an initial bead beating step followed by extraction using the Maxwell® 16 Research Instrument (Promega, USA) according to the manufacturer's protocol with the Maxwell 16 Tissue DNA Kit (Promega). Four samples were analysed: non-acclimated initial inoculum (referred to as NAI), non-electroactive inoculum after 180 days of operation (hereafter NEI-180), electroactive inoculum after 55 days of operation as planktonic cells (AI-P) and in the biofilm (AI-B). DNA concentration was measured using a Qubit assay (Life Technologies, Carlsbad, CA) and standardized to 5 ng μl^{-1}.

Amplicon sequencing and data analysis

The 4 DNA samples were then provided to Australian Centre for Ecogenomics (ACE) for 16S rRNA Amplicon paired-end sequencing by MiSeq Sequencing System (Illumina) using the 926F (5'-AAACTYAAAKGAATTGRCGG-3') and 1392R (5'-ACGGGCGGTGWGTRC-3') primers set [17]. Prior to analysing the sequencing data, a pre-processing approach was adopted to ensure that adapter sequences would not be incorporated into the newly assembled genome, according to the procedure used by Bolger *et al.* [18]. Raw paired reads were first trimmed by trimmomatic to remove short (less than 190bp) and low quality reads (lower than Phred-33 of 20). The trimmed paired reads were then assembled by Pandaseq with default parameters[19]. Once the paired reads were assembled, the adapter sequences were removed using FASTQ Clipper of the FASTX-Toolkit [20]. High quality joined sequences were analysed using QIIME v1.8.0 [21]. An open-reference OTU picking approach was applied at 3% phylogenetic distance and taxonomic identification was assigned by uclust [22] against the greengenes database [23, 24]. OTUs with only one read through all samples were filtered from the OTUs table by command filter_otus_from_otu_table.py in QIIME. Filtered OTUs table was normalised by cumulative-sum scaling method using metagenomeSeq [25] in R (version 3.2.1; R core team, 2015). Normalised OTUs table were imported to Galaxy[26] for gene copy number correction and generation of the final OTUs table by CopyRighter [27].

Statistical analyses of pyrosequencing data

The final OTUs table was imported into R v3.2.1 for statistical analysis. A principle component analysis (PCA) was generated using Euclidean distance with the rda function in the package "vegan" [28] on Hellinger transformation datasets [29]. A heat map was subsequently generated using the top 20 OTUs with high standard deviation among samples with function heatmap.2 in the "gplots" package [30].

3. Results and discussion

3.1 Establishing an autotrophic sulfate-reducing BES

The cumulative electron and sulfate consumption of duplicate reactors is plotted in Figure 2. The rate is calculated as the slope of this curve at each

interval time. After 180 days of chronoamperometry test using non-electroactive mixed microbial inoculum, a low electron consumption of 0.008 ± 0.005 mol m^{-2} d^{-1} was recorded, while sulfate was consumed at the rate of 0.0029 ± 0.001 mol SO_4^{2-}-S m^{-2} d^{-1} (see Figure 2A), with no significant differences for electron and sulfate rates consumption between duplicates.

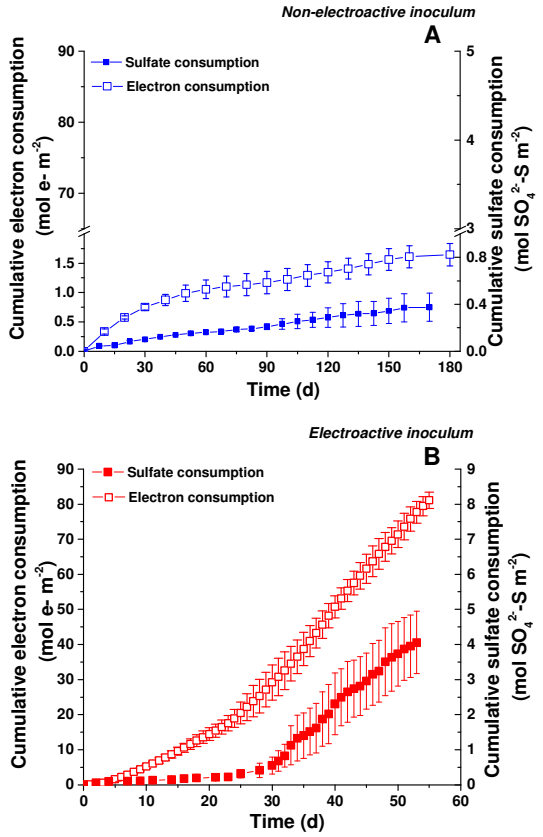

Figure 2. Cumulative electron and sulfate consumption in fed-batch BES (A) Non-electroactive mixed microbial inoculum and (B) Electroactive mixed microbial consortia.

A 186-fold increase in cathodic electron consumption rate to 1.5 ± 0.7 mol m^{-2} d^{-1} was attained 55 days after inoculation when using an electroactive inoculum in duplicate reactors using an identical configuration and conditions. This electroactive inoculum also exhibited a 39-times higher sulfate consumption rate of 0.115 ± 0.009 mol SO_4^{2-}-S m^{-2} d^{-1}, which corresponds to 188 ± 14 g SO_4^{2-} m^{-3} day^{-1} or 11.1 ± 0.9 g SO_4^{2-} m^{-2} day^{-1} (normalization to reactor volume and surface area, respectively). It is important to mention that these levels of sulfate reduction were achieved in fed-batch mode and at room temperature (20 ± 3^{0}C). In comparison, Luo *et al.* [10] reported a maximum volumetric sulfate reducing activity of 16.3 g SO_4^{2-} m^{-3} day^{-1} also in fed-batch but with a cathode potential of -0.6 V vs SHE, whilst Su and colleagues[8] attained 14.59 g SO_4^{2-} m^{-3} day^{-1} at -0.2 V vs SHE and Coma *et al.* [9] achieved up to 60 g SO_4^{2-} m^{-3} day^{-1} at -0.26 V vs SHE but using a continuous-flow system completely filled with granular graphite (thus increasing significantly the working surface-to-volume ratio).

From cyclic voltammetry, no redox activity was detected in abiotic conditions between -0.1 to -1.1 V vs SHE at pH 7.3. The flat CV profile of the abiotic reactor stood in contrast with the reduction wave seen in the non-electroactive reactor after 180 days of operation as shown in Figure 3. However, the most remarkable shift of the reductive wave onset was attained with the electroactive inoculum, almost 0.2 V higher than the reactors with non-electroactive inoculum (Figure 3).

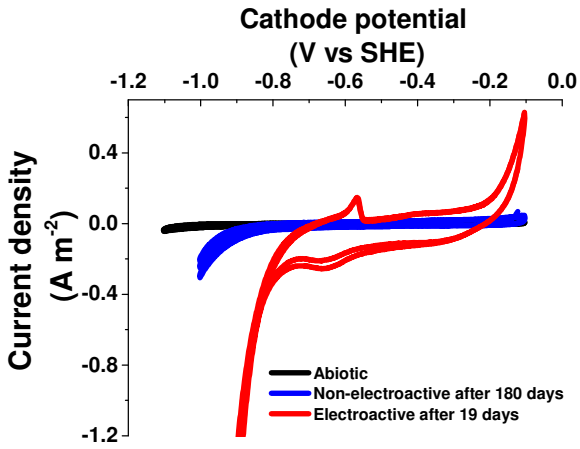

Figure 3. Cyclic voltammetry on abiotic (black trace), non-electroactive mixed microbial inoculum after 180 days of operation (blue trace), and electroactive mixed microbial consortia after 19 days of operation (red trace). Scan rate of 1mV s^{-1}, three cycles.

This reductive wave shift is consistent with CVs obtained from BESs with live biocathodes producing hydrogen, acetate and methane[14, 31]. Accordingly, a much higher cathodic current of about -1.2 A m^{-2} was achieved at – 0.9 V vs SHE after 19 days of operation while current consumption using a non-acclimated inoculum remained at -0.07 A m^{-2} at the same potential and pH. These results are in agreement with the findings of Rozendal *et al.*[31] and Jourdin *et al.*[14] which showed similar trends in autotrophic hydrogen-producing biocathodes.

The sulfide production rate increased alongside current and sulfate consumption as described in Figure 4. The current density increased for the first 20 days after inoculation from -0.54 ± 0.03 to -1.60 ± 0.06 A m^{-2}, however there was no sign of sulfate reduction until day 25. It can be observed that after this lag phase (25 d), sulfide as end-product of sulfate reduction began to be produced in both replicate BESs. After repeated catholyte medium replacements, the current density picked up to -3.4 A m^{-2} and sulfide production increased to 0.112 ± 0.009 mol HS^- m^{-2} day^{-1}.

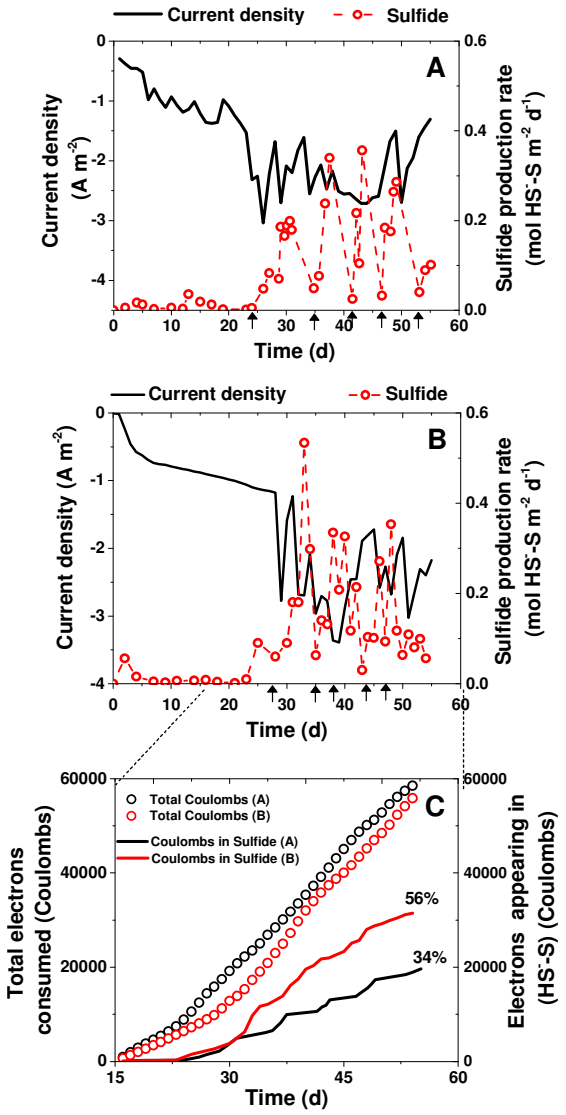

Figure 4. Current density and sulfide (HS^--S) production rate for duplicate reactors (A and B) using electroactive inoculum at applied cathode potential of -0.9 V vs SHE. Coulombic efficiencies to sulfide in duplicate reactors (C). The arrows show the day of medium exchanges where sulfate was added and pH adjusted to 7.2.

Due to the high demand of protons (8) for sulfate reduction process, the pH increased to over 8 (data not shown) at the cathode side, and as a result a decrease of current can be observed in Fig. 4. After sulfate depletion, the media was replaced with fresh catholyte and the pH was reduced to 7.2 as indicated by the arrows. Coproduction of acetic acid and methane (data not shown) was not observed throughout this study when sulfate was present as electron acceptor. To date, three papers reported that autotrophic sulfate reduction could be attained in BESs[8-10], but only two of them reported sulfide production rate as volumetric unit. Unfortunately, there has been no detailed information about surface area of electrodes as a way of comparison. In this study, the maximum sulfide production rate was 0.53 mol HS^--S m^{-2} d^{-1} equivalent to 288 g HS^--S m^{-3} d^{-1}. The electron recovery or coulombic efficiency in sulfide was 56% in one reactor at -0.9 V vs SHE. This volumetric rate is 137 times higher than of the BESs driven by a mixed community at -0.6 V vs SHE in fed-batch operation developed by Luo *et al.* [10]. The same authors also reported sulfide production rates of 2.6 g HS^--S m^{-3} d^{-1} using continuous operation, which is 110 times lower than the rate reached in this study in fed-batch operation mode. Su *et al.*[8] found that sulfide production reached a maximum of 4.8 g HS^--S m^{-3} d^{-1} in BES with polarized electrodes (−0.2 V vs. SHE) as the sole electron donor, 60 times lower than the rate achieved in this work.

3.2 Microbial diversity and links to autotrophic sulfate reduction

Illumina 16S rRNA amplicon sequencing recovered a total of 157,487 high quality reads with average 39,372 ± 4,149 (standard error). Similar microbial community distribution can be revealed in principal component analysis (PCA) showing the differentiation along PC1 (66% differences explained) between non-acclimated inoculum (NAI) and other groups due to high abundance of *Methanosaeta* and *unknown Bacteroidales* in NAI only (Figure 5). Although the non-electroactive inoculum at day 180 (NEI-180) shared the microbial community with electroactive inoculum (EI) to a certain extent, they are separated along the PC2 axis (with 27% differences explained) mainly driven by the emergence of *Methanobacterium* following weaker effects from *unknown Ignavibacteriaceae* and *Desulfovibrio* in both electroactive biofilm (EI-B) and planktonic (EI-P) respectively. This suggests that *Methanobacterium* could be the most likely cause of electroactivity in these samples.

To date, several studies have reported the ability of various methanogens of direct electron uptake using an electrode as current supplier[32-34]. Furthermore, pure culture studies have shown that *Methanobacterium*-like archaeon strain IM1 can utilize electrons from carbon based electrodes without artificial electrons mediators for highly selective production of CH_4 from CO_2 at a set potential of -0.4 V vs SHE [35]. In that work, methane production gave way to hydrogen production at lower potentials, indicating that this strain is able to catalyse cathodic hydrogen production. It is possible, therefore, that the faster start-up of the electroactive BESs was caused by hydrogen-producing strains, putatively *Methanobacterium,* which would be responsible for the H_2 supply driving the autotrophic sulfate reduction. This species was not found in BESs with non-electroactive inoculum despite the same operational conditions/configuration. Previous studies evaluating how methanogenic microorganisms conserve energy observed hydrogen production as a protective mechanism to deal with excess electron supply[36]. Armstrong and Hirst[37] concluded that the

interconversion of H_2 and H^+ is crucial in the metabolism of microorganisms that use hydrogenases to catalyse hydrogen production from the excess of electrons and relieve negative charge close to the microbial cell.

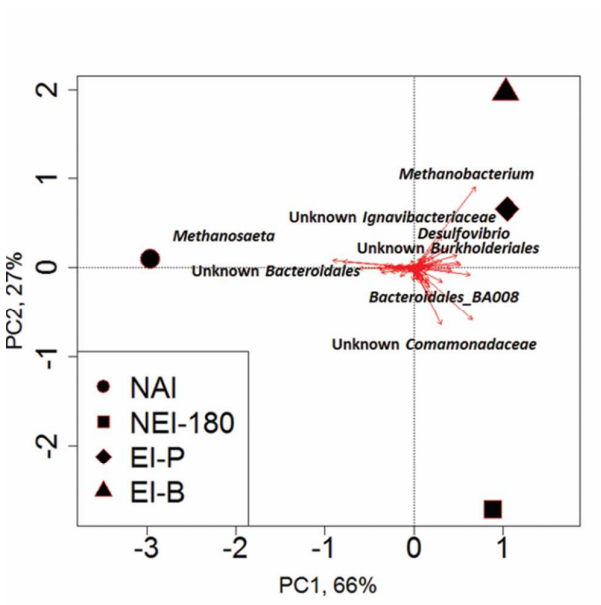

Figure 5. Principle component analysis on microbial community of non-acclimated initial (NAI round), non-electroactive inoculum after 180 days of operation (NEI-180 square), (electroactive inoculum after 55 days of operation as planktonic cells (EI-P diamond) and in the biofilm (EI-B triangle). Top OTUs drive the differentiations among samples were labelled with corresponding identifications.

The heat map in Figure 6 represents 50 (NAI and NEI-180) to 70% (EI-P and EI-B) of the total community. The NAI as initial inoculum collected from natural and engineered environments was dominated by the genus *Methanosaeta* (18.4%) and OTUs affiliated with the order *Bacteroidales* (23.7%, referred to as Unknown *Bacteroidales*). The microbial community was replaced by *Comamonadaceae* (15.5%, referred to as *unknown Comamonadaceae*), *Bacteroidales*_BA008 (9.4%) and *Thiobacillus* (3.7%) after 180 days of operation (NEI-180), which suggests that the initial dominant microbes are not involved in the major biological activities in these BES. Lower relative abundance of sulfate reducers putatively *Desulfovibrio spp.* (0.5%) *and Desulfomonile spp.* (0.8%) could be responsible for the observed sulfate reduction in reactors with NEI-180.

The electroactive inoculum (EI) (combination of NEI-180 with pre-acclimated biomass [38]) was inoculated in new BESs with the same reactor configuration at -0.9 V. After 55 days of operation, pyrosequencing analysis in the cathodic biofilm (EI-B) and surrounding planktonic cells (EI-P) showed high abundance of the same OTUs found in NEI-180 including *Thiobacillus* (6%), unknown *Betaproteobacteria* (5.5%) and *Bacteroidales*_BA008 (8.4%), indicating that these may not be the major drivers of the electron transfer mechanism. However, other microbial groups which were not found in NEI-180 including OTUs affiliated to *Methanobacterium* (26%) and *Ignavibacteriaceae* (3.4%), which was enriched in the cathodic biofilm (EI-B) and in planktonic form (EI-P).

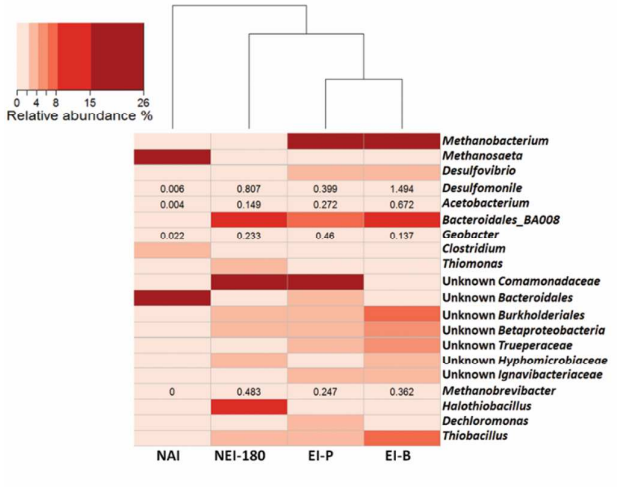

Figure 6. Heat map summarizing the percent relative abundances microbial community of non-acclimated initial inoculum (NAI), non-electroactive inoculum after 180 days of operation (NEI-180), electroactive inoculum after 55 days of operation as planktonic cells (EI-P) and in the biofilm (EI-B).

Higher relative abundance of sulfate reducers (putatively *Desulfovibrio spp.*) was found in EI-B (2.8%) and EI-P (2.7%) than the abundance of NAI (0.03%) and NEI-180 (0.5%) respectively. Importantly however, sequences derived from *Desulfovibrio spp.* were at <3% relative abundance in all samples, but still sulfate reduction occurred in the electroactive BES. A decrease of electron acceptor SO_4^{2-} in batch operation systems could have led to shifts in the microbial community, and as a consequence could have lowered the abundance of sulfate reducers. In a recent study [39], the sulfate reducing microbial community responded to changes in electron acceptor in membrane biofilms reactors using hydrogen as electron donor. In that work, the authors found that the relative abundance of sulfate reducers in the microbial community was strongly affected by the depletion of sulfate – an expected phenomenon in fed-batch operation as used in this study.

4. Conclusions

Our results demonstrate that by choosing an adequate electroactive inoculum the autotrophic electrode-driven reduction of sulfate to sulfide is possible without addition of organic compounds or hydrogen. The autotrophic reduction rate reached in this study is at least 60 times higher than what has been reported to date. The findings of this study suggest that an important enrichment of *Methanobacterium* (26% of relative abundance) in the cathodic biofilm could be responsible of higher electroactivity in BESs, taking into account that this enrichment was not found in non-electroactive systems. To the best of our knowledge, this is the highest sulfate reduction

rate achieved using bioelectrochemical systems. It is likely that the autotrophic sulfate reduction rates observed in this study could be further improved with either continuous mode operation or through electrodes with higher specific surface areas. These findings open a path towards sustainable and economical treatment of sulfate-bearing, organic-carbon-free waste streams such as acid mine drainage.

References

1. P. Lens and L. H. Pol, *Environmental Technologies to Treat Sulfur Pollution: Principles and Engineering*, IWA, 2000.
2. P. Lens, M. Vallerol, G. Esposito and M. Zandvoort, *Re/Views in Environmental Science and Bio/Technology*, 2002, **1**, 311-325.
3. G. Muyzer and A. J. Stams, *Nature reviews. Microbiology*, 2008, **6**, 441-454.
4. P. M. Matias, I. A. Pereira, C. M. Soares and M. A. Carrondo, *Prog Biophys Mol Biol*, 2005, **89**, 292-329.
5. J. A. B. Sousa, C. M. Plugge, A. J. M. Stams and M. F. M. Bijmans, *Water Research*, 2015, **68**, 67-76.
6. W. Liamleam and A. P. Annachhatre, *Biotechnology advances*, 2007, **25**, 452-463.
7. S. Papirio, D. K. Villa-Gomez, G. Esposito, F. Pirozzi and P. N. L. Lens, *Critical Reviews in Environmental Science and Technology*, 2012, **43**, 2545-2580.
8. W. Su, L. Zhang, Y. Tao, G. Zhan, D. Li and D. Li, *Electrochemistry Communications*, 2012, **22**, 37-40.
9. M. Coma, S. Puig, N. Pous, M. D. Balaguer and J. Colprim, *Bioresource Technology*, 2013, **130**, 218-223.
10. H. Luo, S. Fu, G. Liu, R. Zhang, Y. Bai and X. Luo, *Bioresource Technology*, 2014, **167**, 462-468.
11. G. Jiang, K. R. Sharma, A. Guisasola, J. Keller and Z. Yuan, *Water Research*, 2009, **43**, 4430-4440.
12. L. Jourdin, T. Grieger, J. Monetti, V. Flexer, S. Freguia, Y. Lu, J. Chen, M. Romano, G. G. Wallace and J. Keller, *Submitted*.
13. V. Flexer, M. Marque, B. C. Donose, B. Virdis and J. Keller, *Electrochimica Acta*, 2013, **108**, 566-574.
14. L. Jourdin, S. Freguia, B. C. Donose and J. Keller, *Bioelectrochemistry*, 2015, **102**, 56-63.
15. F. Harnisch and S. Freguia, *Chemistry – An Asian Journal*, 2012, **7**, 466-475.
16. B. Keller-Lehmann, S. Corrie, R. Ravn, Z. Yuan and J. Keller, 2nd international IWA Conference on Sewer Operation and Maintenance, Vienna, Austria, 2006.
17. A. Engelbrektson, V. Kunin, K. C. Wrighton, N. Zvenigorodsky, F. Chen, H. Ochman and P. Hugenholtz, *The ISME journal*, 2010, **4**, 642-647.
18. A. M. Bolger, M. Lohse and B. Usadel, *Bioinformatics*, 2014, **30**, 2114-2120.
19. A. P. Masella, A. K. Bartram, J. M. Truszkowski, D. G. Brown and J. D. Neufeld, *BMC bioinformatics*, 2012, **13**, 31.
20. W. R. Pearson, T. Wood, Z. Zhang and W. Miller, *Genomics*, 1997, **46**, 24-36.
21. J. G. Caporaso, J. Kuczynski, J. Stombaugh, K. Bittinger, F. D. Bushman, E. K. Costello, N. Fierer, A. G. Pena, J. K. Goodrich, J. I. Gordon, G. A. Huttley, S. T. Kelley, D. Knights, J. E. Koenig, R. E. Ley, C. A. Lozupone, D. McDonald, B. D. Muegge, M. Pirrung, J. Reeder, J. R. Sevinsky, P. J. Turnbaugh, W. A. Walters, J. Widmann, T. Yatsunenko, J. Zaneveld and R. Knight, *Nature methods*, 2010, **7**, 335-336.
22. R. C. Edgar, *Bioinformatics*, 2010, **26**, 2460-2461.
23. D. McDonald, M. N. Price, J. Goodrich, E. P. Nawrocki, T. Z. DeSantis, A. Probst, G. L. Andersen, R. Knight and P. Hugenholtz, *The ISME journal*, 2012, **6**, 610-618.
24. J. J. Werner, O. Koren, P. Hugenholtz, T. Z. DeSantis, W. A. Walters, J. G. Caporaso, L. T. Angenent, R. Knight and R. E. Ley, *The ISME journal*, 2012, **6**, 94-103.
25. J. N. Paulson, O. C. Stine, H. C. Bravo and M. Pop, *Nat Meth*, 2013, **10**, 1200-1202.
26. B. Giardine, C. Riemer, R. C. Hardison, R. Burhans, L. Elnitski, P. Shah, Y. Zhang, D. Blankenberg, I. Albert, J. Taylor, W. Miller, W. J. Kent and A. Nekrutenko, *Genome research*, 2005, **15**, 1451-1455.
27. F. E. Angly, P. G. Dennis, A. Skarshewski, I. Vanwonterghem, P. Hugenholtz and G. W. Tyson, *Microbiome*, 2014, **2**, 11.
28. J. Oksanen, F. Blanchet, R. Kindt, P. Legendre, P. Minchin, R. ÓHara, G. Simpson, P. Solymos, M. Stevens and H. Wagner, Vegan: Community Ecology Package. R. Package Version 2.0 e7, http://CRAN.R-project.org/package=vegan).
29. P. Legendre and E. Gallagher, *Oecologia*, 2001, **129**, 271-280.
30. G. R. Warnes, B. Bolker, L. Bonebakker, R. Gentleman, W. Huber, A. Liaw, T. Lumley, M. Maechler, A. Magnusson and S. Moeller, *R package version*, 2009, **2**.
31. R. A. Rozendal, A. W. Jeremiasse, H. V. Hamelers and C. J. Buisman, *Environ Sci Technol*, 2008, **42**, 629-634.
32. M. Villano, F. Aulenta, C. Ciucci, T. Ferri, A. Giuliano and M. Majone, *Bioresource Technology*, 2010, **101**, 3085-3090.
33. C. W. Marshall, D. E. Ross, E. B. Fichot, R. S. Norman and H. D. May, *Applied and environmental microbiology*, 2012, **78**, 8412-8420.
34. J. S. Deutzmann, M. Sahin and A. M. Spormann, *mBio*, 2015, **6**.
35. P. F. Beese-Vasbender, J.-P. Grote, J. Garrelfs, M. Stratmann and K. J. J. Mayrhofer, *Bioelectrochemistry*, 2015, **102**, 50-55.
36. U. Deppenmeier and V. Müller, in *Bioenergetics*, eds. G. Schäfer and H. Penefsky, Springer Berlin Heidelberg, 2008, vol. 45, ch. 26, pp. 123-152.
37. F. A. Armstrong and J. Hirst, *Proceedings of the National Academy of Sciences*, 2011, **108**, 14049-14054.
38. L. Jourdin, S. Freguia, B. C. Donose, J. Chen, G. G. Wallace, J. Keller and V. Flexer, *Journal of Materials Chemistry A*, 2014, **2**, 13093-13102.
39. A. Ontiveros-Valencia, Y. Tang, H.-P. Zhao, D. Friese, R. Overstreet, J. Smith, P. Evans, B. E. Rittmann and R. Krajmalnik-Brown, *Environmental Science & Technology*, 2014, **48**, 7511-7518.