Bioelectrochemical sulphate reduction on batch reactors: Effect of inoculum-type and applied potential on sulphate consumption and pH

Manuel A. Gacitúa a,b,⁎, Enyelbert Muñoz a, Bernardo González a

⁎ Laboratory de Bioingeniería, Facultad de Ingeniería y Ciencias, Universidad Adolfo Ibáñez, Center of Applied Ecology and Sustainability (CAPES), Chile

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ABSTRACT

Microbial electrolysis batch reactor systems were studied employing different conditions, paying attention on the effect that biocathode potential has on pH and system performance, with the overall aim to distinguish sulphate reduction from H2 evolution. Inocula from pure strains (Desulfovibrio paquesii and Desulfovibrio halotolerans) were compared to a natural source conditioned inoculum. The natural inoculum possess the potential for sulphate reduction on serum bottles experiments due to the activity of mutualistic bacteria (Sedimentibacter sp. and Bacteroides sp.) that assist sulphate-reducing bacterial cells (Desulfovibrio sp.) present in the consortium. Electrochemical batch reactors were monitored at two different potentials (graphite-bar cathodes poised at −900 and −400 mV versus standard hydrogen electrode) in an attempt to isolate bioelectrochemical sulphate reduction from hydrogen evolution. At −900 mV all inocula were able to reduce sulphate with the consortium demonstrating superior performance (SO42− consumption: 25.71 g m−2 day−1), despite the high alkalinisation of the media. At −400 mV only the pure Desulfovibrio halotolerans inoculated system was able to reduce sulphate (SO42− consumption: 17.47 g m−2 day−1) and, in this potential condition, pH elevation was less for all systems, confirming direct (or at least preferential) bioelectrochemical reduction of sulphate over H2 production.

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1. Introduction

Worldwide, mining represents a significant economic activity, with reported revenues near US $400 billion during 2015 from the top 40 mining companies [1]. Mining activity has inherent environmental consequences, since mineral processing produces several types of industrial waste, with acid mine drainage (AMD) among the most concerning [2]. AMD has multiple sources from mining activities (mineral extraction from deposits, leaching activity for metal purifying, smelter wastes, etc.) and its composition varies depending on its source. Some reports indicate that a typical AMD process in Chilean mining activities [3] may contain high contents of metals including aluminium (ca. 1100 ppm), copper (ca. 2300 ppm), magnesium (ca. 630 ppm), iron (ca. 620 ppm); anions from acid-treatment, such as sulphate (ca. 14,400 ppm); and pH values as low as 2.5 [4]. Therefore, AMD discharges may provoke catastrophic environmental consequences, such as heavy metal release and acidification of water bodies, [5] with health consequences to communities nearby. Therefore, treatment of such anthropogenic waste before it is released into the environment is critical for sustainable development [6].

Interest in microbial electrochemical reactors (MER) has grown in the last several decades for the related purpose of wastewater treatment [7]. MER is used for hazardous waste remediation and combines the principles of microbiological augmentation [8–12] and electrochemical catalysis [13–17]. Briefly, these systems consist of an electrolysis reactor that harnesses bacterial metabolism to accelerate a particular reaction relevant to wastewater treatment. This technology could remediate AMD if sulphate-reducing bacteria (SRB), a set of bacteria and archaea capable of performing anaerobic respiration using sulphate instead of oxygen [18–21], were considered in the design. SRB have been used in electrochemical set-ups with diverse scopes and varied designs. There are some reports describing the use of SRB in the design of microbial fuel cells (MFC) for energy harvesting [22–25], but most recent research on the subject has been devoted to the design of microbial electrolysis cells (MEC) with different purposes such as H2 production [13,26–28], wastewater treatment [29–31] and treatment of sulphate-polluted water [32–40]. The latter, sulphate reduction, has been widely applied to the design of AMD remediation systems. However, several conditions must be considered to ensure system efficiency. For instance, sulphate itself should be directly reduced, not through external redox mediators, but by the biocathode (i.e. direct electron transfer) in order to avoid energy waste during the process. Among all parameters, however, ensuring exclusive or at least preferential direct sulphate reduction is essential, so cathode potential selection must be...
carefully considered [32]. Previous studies have established that sulphate reduction would occur separately from H2 evolution, if the cathode were poised around −200 mV vs. standard hydrogen electrode potential [32]. However, this value may vary depending on the electro-chemical set-up and inoculum composition. Another important aspect to control is pH [36]. Failure to achieve effective potential during fixed-potential experiment results in H2 evolution, prompting sudden shifts in pH shifts that produce toxic detrimental conditions for bacterial growth and activity and compromise system efficiency in long-term operations.

A key common aspect in most reports on this subject is the use of consortia instead of pure strains. SRB consortia show advantages over pure SRB strains, mainly due to complementary features and mutualism among SRB individuals with the other members of the consortium. In this context, species from the genus Desulfovibrio, Desulfobulbus, Desulfolomicrobium, and/or Desulfobacter are typically abundant in different sulphate reducing electrochemical systems [41]. Su et al. (2012) [32] demonstrated that when a wastewater plant consortium was fed with H2, Pseudomonas, Clostridium, and Desulfobulbus species were abundant, while connection to a power source prompted direct sulphate reduction and shifts to Geobacter species dominance. Wang et al. (2017) [39] recently reported that a sulphate reducing system inoculated with a culture from a sewage sludge factory varied in bacterial genus members’ dominance depending on the electrochemical conditions; the most efficient systems showed dominance of members from Proteocella (36%), Dysgonomonas (22%), and Desulfobacter (18%) genera, while the least efficient system (with higher impedance) showed abundance of members from Desulfovibrio (44%), Pantoaea (13%), and Enterobacter (10%) genera. Therefore, despite finding that SRB community compositions may depend on the electrochemical conditions, there is no clear trend or expected outcome from a phylogenetic standpoint. Moreover, few groups have sought to explain the specific role of key members of the bacterial communities in these systems.

Here, we contrast the sulphate consuming potential of single species and a SRB consortium in a MEC system. The novelty of this work lies in determining the effect that controlling potential has on pH and system performance, with the overall aim to distinguish sulphate reduction from H2 evolution.

2. Materials and methods

2.1. Strains, medium and culture conditions

SRB pure commercial strains Desulfovibrio paucisii (DP) and Desulfobacter halotolerans (DH) were obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig, Germany). A SRB consortium (SRBc) was formed using sediment samples from Juncalito thermal springs (Diego de Almagro, Atacama, Chile; 26°30′ 41.5″ S 68°49′10.9″ W). Culture media preparation, growing conditions and inoculation procedures were performed as previously described [13], with some minor variations. Briefly, all strains and the consortium were pre-grown at 30 °C in 500 mL (total volume) sealed serum bottles containing 400 mL of anaerobic medium supplemented with lactate (15 mM or 1340 ppm) and sulphate (21 mM or 2000 ppm), under a pure CO2 atmosphere. Sulphate and bacterial concentration were monitored during growth to estimate the most efficient culture conditions. Stationary-phase (14 day-old) cultures were used as inoculum for the bioelectrochemical experiments. The liquid at the cathode compartment was replaced (maintaining anaerobic condition) with the same volume of the corresponding culture.

2.2. Bioelectrochemical cell

All bioelectrochemical experiments were carried out in MEC consisting of a two-compartment electrochemical cell comprised of two gas-tight borosilicate bottles (250 mL) separated by a 3-cm2 AMI-70015 anion exchange membrane (Scheme 1).

The working electrode (cathode) was a spectroscopic grade 6 mm graphite rod (Aldrich®) covered with an epoxy resin until 2 cm before the rod end, yielding a calculated geometrical surface area of 3.5 cm2. The cathode surface was intentionally limited in order to ensure a more reproducible performance of sulphate reduction rates and to enable transformation of “current” outputs on values of “current-density”, which is a more correct way to compare the results with those from other authors. The counter electrode was made of the same material without the epoxy resin in order to ensure a bigger area: 19.3 cm2 calculated by taking into account only the part of the electrode that was immersed in the liquid phase. The cathode and anode were inserted through holes in the glass-bottles plastic caps, and the separation between them was 12 cm. A KCl saturated Ag/AgCl reference electrode (+199 mV vs. standard hydrogen electrode, SHE) (CH Instruments, Inc.) was also placed in the cathode chamber, assisted by a Luggin capillary. The electrolyte solution, both at the cathode and at the anode, consisted of an anaerobic medium supplemented with 2000 ppm of sulphate. The target cathode reaction was SO4−2 reduction, while the anode reaction was water oxidation. Additional information regarding the experimental setup and reactor geometry is provided in previous publications [13,27]. All potentials were recalculated and reported vs. SHE.

2.3. Bioelectrochemical experiments

All electrochemical measurements and experiments were carried out using a MultiEmStat 4 (Palm Sens®) multichannel potentiostat. For chronoamperometric tests, the working electrode (i.e. cathode) was polarized at −400 mV and −900 mV in order to favour direct sulphate reduction over hydrogen evolution, respectively. Both abiotic (control) and biotic chronoamperometric tests were conducted and monitored for two weeks. For the biotic tests, the cathode compartment contained either 200 mL of DP, DH, or SRBc cultures. For the controls, the cathode compartment contained 200 mL of anaerobic medium supplemented with SO4−2 (2000 ppm). As sulphate removal from the medium was the key parameter to assess system performance, sulphate consumption was monitored in liquid samples taken every week. All results represented average values of sulphate consumption from three replicates. Analysis of variance (ANOVA) performed on results showed that there were effective statistical differences (95% confidence) between treatments. During tests, the cell was maintained at room temperature (25 °C) under vigorous magnetic stirring to ensure that current generation was not substantially affected by mass transfer phenomena. For cyclic voltammetry experiments, the electrode potential was varied in the range −1000 mV to +700 mV, at a scan rate of 10 mV s−1.

2.4. Consortium community structure determination

A 500 mL active SRBc inoculum (three weeks old with about 2.0 × 107 cells mL−1) was firstly centrifuged at 7250 × g in order to separate solids (metallic sulphides and biomass) from the supernatant. The pellet was washed with PBS buffer, followed by centrifugation, in order to eliminate any possible DNA polymerase inhibitor; this step was repeated three times. Total DNA was extracted using a FastDNA kit for Soil DNA Extraction (MPB), following manufacturer’s instructions. DNA was quantified and its purity was checked using a NanoQuant (Infinite M200 PRO Tecan®) spectrophotometer.

After DNA extraction and purity confirmation, the 16S rRNA gene sequences were amplified using 27F-(5′-AGA GTT TGA TCC TGG CTC AG-3′) and 1492R-(5′-CGG CTA CCT TGT TAC GAC TT-3′) bacterial primer pairs [42]. Each polymerase chain reaction (PCR) mix consisted of 1.25 μL Taq polymerase (GIBCO-BRL®), 0.2 μM of each primer, 0.2 μM of each deoxynucleotide phosphate, 50 ng μL−1 of bovine serum albumin, 3.0 mM of MgCl2 and 2.0 μL of DNA. The reactions were carried out using a thermal
cycler 2720 (Applied Biosystems®). PCR conditions were as follows: 94 °C for 5 min; 30 cycles of 94 °C per 45 s, 56 °C of annealing temperature per 1 min, 72 °C of elongation temperature per 2 min and a final step of elongation for 7 min. PCR products with 1.5 kb were analysed using 1.5% agarose gel electrophoresis in TAE 1× buffer, dyed with gel red BIOTUM® (0.02% v/v), and ran at 90 mV for 45 min. Visualization was accomplished using an UV trans illuminator.

Amplicons were purified using the Wizard (Promega®) cleaning kit and cloned using the TOPO-TA (Invitrogen®) cloning kit, following the manufacturer’s instructions. Plasmids from a total of 45 colonies were randomly selected and incubated at 95 °C for 15 min for DNA extraction. The presence of recombinant plasmids was verified by PCR amplification with M13F and M13R primer pairs. PCR conditions were the same as described above.

For preliminary grouping of clones by ribotype, PCR products were digested with the restriction enzyme MspI. Digested PCR products were checked using 3.0% agarose gel electrophoresis in TAE 1× buffer, dyed with gel red BIOTUM® (0.02% v/v), and ran at 90 mV for 90 min. Visualization was accomplished using a UV trans illuminator. Identical patterns were considered to belong to same genus. A DNA sample of each putatively different ribotype was analysed by 16S rRNA gene sequencing (Macrogen®, South Korea), employing M13 primer pairs. The Basic Local Alignment Search Tool from NCBI® was used to identify each sequence against the ten best results found in the GenBank®.

2.5. Analytical methods

Sulphate was determined using a Sulphate Assay Kit (Aldrich®) following the manufacturer’s instructions. Sulphate concentration was always measured at the cathode and anode. Therefore, sulphate levels (%) correspond to the percentage variation (starting from SO\(_4^{2-}\) 2000 ppm) of total sulphate, SO\(_4^{2-}\) \text{TOTAL}, through time:

\[ \text{SO}_4^{2-} \text{TOTAL} = \frac{\text{SO}_4^{2-} \text{anode} \times V_{\text{cathode}} + \text{SO}_4^{2-} \text{cathode} \times V_{\text{anode}}}{V_{\text{cathode}} + V_{\text{anode}}} \]  

(B1)

Bacterial concentration was directly monitored by counting the number of cells, using a Neubauer chamber of 0.1 mm depth and an optical microscope with phase contrast filter.

Biofilm formation was estimated through the use of crystal violet dye, a staining reagent that specifically colours plasmatic membranes and negatively charged exopolysaccharides. The method was adapted from Merrit et al. (2005) [43] for the quantification of biofilms formed on abiotic surfaces, to estimate biofilm formation over cathodes. Abiotic control cathode and an inoculated-system cathode were treated with the following steps: rinsing by immersion on clean distilled water for three times; staining by immersion on 0.1% crystal violet (Aldrich®) solution for 10 min; drying on air for 1 h; dissolution of dye on 5.0 mL of absolute ethanol (Merck®) for 1 h; and measuring absorbance of crystal violet using a spectrophotometer at a \( \lambda = 570 \) nm wavelength. Absorbance values were directly interpreted as biofilm estimations.

3. Results and discussion

3.1. Bacterial growth in sulphate amended cultures

In order to acclimate the different strains and consortium to sulphate-reducing conditions and to estimate overall sulphate consumption performance, experiments were carried out in serum-bottles. The remediation capacity of the sulphate reducing pure strains and the consortium (SRBc) was estimated by measuring their sulphate consumption and bacterial growth (Fig. 1). The SRBc was conditioned to sulphate reducing conditions by growing sequential culture generations on a sulphate rich medium.

The bacterial consortium consumed sulphate more efficiently than pure strains. DP and DH consumed ca. 50% of sulphate during the first 14 d and essentially stopped consuming sulphate in subsequent weeks. In contrast, the SRBc culture accomplished almost full depletion of sulphate during the four weeks’ test. Taken together, these results indicate the different sulphate removal capabilities of pure-strains and versus the SRBc. It should be noted that evolution of bacterial cell numbers was similar in all cases (Fig. 1), indicating that the bacterial consortium was more efficient removing sulphate.

The superior SRBc performance motivated further inquiry into its bacterial community composition. Clonal analysis of the 16S rRNA gene sequences indicated the presence of three main bacterial members (Table 1).

It is quite clear that the sulphate reducing capability of this bacterial consortium is due to the predominance of Desulfovibrio sp. members (91.1% of abundance, Table 1), since the other two members are, in principle, not able to perform any sulphate reduction related activity. Desulfovibrio species have been found in different systems designed for sulphate consumption using bacterial consortia from wastewater and sewage sludge, among other systems [36–39]. Sedimentibacter (6.6% abundance) is not directly related to sulphate reduction [44], although other reports have indicated the presence of Sedimentibacter in a naturally enriched consortium with dechlorinating abilities. In particular, van Doesburg et al. (2005) [45] found Sedimentibacter sp. in an
They proposed that role of this bacterium was to stimulate the growth of the protagonist bacteria in the consortium. Finally, _Bacteroides_ sp. were found at 2.3% abundance. These are anaerobic bacteria typically found in gastrointestinal microbiota of mammals [46,47], but have been also detected in humic-substances anaerobic degrading consortium, which employed sulphate reduction as electron sink for water remediation [48]. Interestingly, members of this genus have been found to cooperate with SRB to degrade organic forms of sulphur [47,48]. _Bacteroides_ sp. may synthesize sulphatase [49], an enzyme that participates in intermediate reactions of the sulphur cycle (from SO$_4^{2-}$ to S$^{2-}$) catalysing the hydrolysis of sulphated esters. Therefore, the functional implication of the SRBc corresponds to, at least, one SRB strain, which may be assisted by two other species to more efficiently perform sulphate reduction. Taken together, these observations explain the superior performance of the SRBc compared to the pure strains.

### 3.2. Bioelectrochemical systems

The three bacterial cultures were then tested on a batch electrochemical reactor to assess their electro-catalytical capabilities as a biocathode for sulphate reduction. To do so, the working solution of the cathode compartment was replaced with an active culture (14 days of growth, ca. 1.3 $\times$ 10$^7$ cells mL$^{-1}$) and the system was maintained polarized for two weeks at two different potentials (−900 and −400 mV vs SHE). Regardless of the inoculum-type or the applied potential, cyclic voltammetry (CV) profiles presented similar characteristics in all experiments (Fig. 2).

When the profiles of the inoculated systems and the control were compared, there were important differences in shape and intensity. At day 0, two intense irreversible reduction (−0.42 and −0.85 V), and one oxidation (0.15 V) processes could be distinguished (Fig. 2a). The most intense cathodic reduction should correspond, based on its position and shape, to hydrogen evolution from water [27], reaching a maximum of current density of ca. −1.0 mA cm$^{-2}$. The anodic process is similar to shape and position reported by Yu et al. (2011) [50] when they studied the hydrogen evolution and oxidation process of a biocathode formed by _Desulfuvibrio caledoniensis_ over pyrolytic graphite electrodes; the anodic process at 0.15 V was attributed to H$_2$ oxidation but was not analysed further since this was a biocathode design study. The reduction at −0.42 V could be assumed to represent a direct sulphate-reduction process catalysed by bacteria, as other authors reported [33]. Based on the Pourbaix diagram for sulphur [51], sulphide (H$_2$S/HS$^-$) is the stable species at $E_C$ below −0.4 V and around pH 7.0. After maintaining the system polarized for 14 days (Fig. 2b), the current became more intense and only two peaks were observed: a sharp

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**Table 1**

<table>
<thead>
<tr>
<th>Clone #</th>
<th>Abundance %</th>
<th>Related organism</th>
<th>Access N°</th>
<th>Identity %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>91.1</td>
<td><em>Desulfovibrio gigas</em></td>
<td>NR_043856.1</td>
<td>99</td>
</tr>
<tr>
<td>2</td>
<td>6.6</td>
<td><em>Sedimentibacter saalensis</em></td>
<td>NR_025498.1</td>
<td>97</td>
</tr>
<tr>
<td>3</td>
<td>2.3</td>
<td><em>Bacteroides xylanolyticus</em></td>
<td>NR_104899.1</td>
<td>93</td>
</tr>
</tbody>
</table>

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**Fig. 2.** Cyclic Voltammetry profiles of control (−) and biocathode after inoculation at day 0 (···) and day 14 (−) during chronoamperometric runs. Interface: Graphite | mineral medium with 2000 ppm SO$_4^{2-}$ at the beginning of the test. Scan rate: 10 mV s$^{-1}$. [29]
Reduction process at −0.75 V and an oxidation process at 0.10 V. The reduction in this case increased in current reaching −4.0 mA cm⁻², almost four times higher than day 0. This process also corresponded to H₂ evolution, but since several days of set-potential had passed, bacteria would have modified the electrode surface (presumably by colonization or biofilm formation) [50]. As a consequence of an electrochemically active biofilm, the same processes require less energy (less potential difference) and the reactions over the biocathode may take place faster (current increment); i.e., thanks to long fixed-potential times the process electro-catalysed itself. Biofilm estimation results revealed an absorbance of crystal violet dye (570 nm) of 1.391 when the cathode was immersed for 14 days on inoculated medium and of 0.692 on abiotic conditions, strongly suggesting that biofilm effectively modified the cathode surface, establishing a biocathode. A similar process has been reported for determination of total biomass attached to gold microelectrodes on an instrumental-type paper for quantification of biofilms from pathogens formed over electrode surfaces and corroborated by impedance measurements [52].

The performance of the electrochemical systems was additionally assessed by comparing the sulphate consumption abilities using the different cultures while the reactors where maintained polarized at −900 and −400 mV vs. SHE (Fig. 3). On a report published by Villano et al. (2010) [15] authors probe that, when using same electrochemical set-up, but with a methanogenic culture, biocathodes cannot produce H₂ if set potentials aren’t as low as −750 mV vs SHE. Also, from same research group (Aulenta et al. 2012) [13] results on D. Paquesi inoculated batch system did not produced any current/nor hydrogen if cathodes were polarized to values less than −900 mV vs SHE. Therefore, is reasonable to think that on present study, there it should be, at the very least, a big difference between H₂ production when biocathodes are poised at −400 and −900 mV vs SHE.

SO₄²⁻ was effectively reduced to H₂S since black particles were formed on the medium (presumably sulphide-metals) and the characteristic rotten eggs smell was detected after opening the reactors. Sulphate consumption was different depending on the electrochemical potential set up. Abiotic controls did not consume any sulphate during testing (data not shown). When the biocathode was poised at −900 mV, all cultures were capable of consuming sulphate. The overall performance was similar for the three systems with DH < DP < SRBc with ca. 20, 25 and 30% of sulphate consumed after 14 days, respectively. According to the CV results, hydrogen evolution took place at this potential. H₂ is a useful energy source for bacteria [14,27] and, hence, all tested cultures likely used it and thus continued consuming sulphate as their oxygen sink. Nevertheless, as a consequence of water reduction, culture medium alkalinized; two weeks of continuous chronoamperometric run elevated pH about 1.2 units (Fig. 3). In a batch-type reactor, this may threaten bacterial normal metabolism and consequently stability of the systems. In this context, Zhen et al. (2014) [36] reported that by reaching pH values as high as 8.5, sulphate consumption may decrease at least a 30% with respect to the neutral condition.

On the other hand, tests at −400 mV showed interesting results. At this potential, D. halotolerans was the only culture capable of considerable sulphate reduction. After two weeks, the DH biocathode consumed about 20% (corresponding to 400 ppm) of sulphate. Su et al. (2012) [32] reported a similar batch system, with carbon-felt electrodes (16 cm²) poised at −200 mV vs. SHE, reaching 80% (corresponding to 190 ppm) sulphate removal after 10 d of operation. Using graphite electrodes poised at −400 mV, hydrogen production should be less important, meaning that this culture was likely able to catalyse direct electrochemical reduction of sulphate, at least under the tested experimental conditions, pH also elevated (about 0.4 units) with set-potential of −400 mV. However, compared with that for −900 mV experiments, this alkalisation represented only a slight increase. This result confirms the premise that pH [36] along with stable sulphate reduction can be effectively controlled by selection of an appropriate potential and suitable electroactive microorganisms. Bacterial survival was also monitored during electrochemical runs, and as observed in serum-bot-tles experiments, behaved similarly for each inoculum-type and was independent of the applied potential: starting (day 0) with ca. 1.3 × 10⁷ cells mL⁻¹, then went to 1.7 × 10⁵ (day 7) and ended with 9.4 × 10³ (day 14). Therefore, batch operation kills planktonic bacteria since no carbon-based energy source remains, while sulphate is probably consumed by and limited to the biocathode surface. The different pH evolution through time on each condition accounts for the different performance observed between treatments, as pointed out in literature [36, 53]. The overall performance of the different systems expressed as sulphate reduction rates (in grams of SO₄²⁻ per biocathode-square-meter per day) is presented in Table 2.

![Fig. 3. Sulphate consumption (bars) and pH (lines) evolution of biocathodes at two different potential conditions. Desulfovibrio paquesii (DP), Desulfobacter halotolerans (DH) and the SRB consortium (SRBc).](image)

<table>
<thead>
<tr>
<th>Culture</th>
<th>Rate (g SO₄²⁻ m⁻² day⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>−900 mV</td>
</tr>
<tr>
<td>Desulfovibrio paquesii</td>
<td>22.1</td>
</tr>
<tr>
<td>Desulfobacter halotolerans</td>
<td>14.4</td>
</tr>
<tr>
<td>SRBc</td>
<td>25.7</td>
</tr>
</tbody>
</table>

Systems working at −900 mV were capable of reducing sulphate faster, with the SRBc inoculated system performing best with a rate of 25.7 g SO₄²⁻ m⁻² day⁻¹. Nevertheless, when poised potential was set
to $-400 \text{ mV}$ only the *D. halotolerans* inoculated system was able to perform substantial sulphate reduction, at a rate of $17.5 \text{ g SO}_4^{2-} \text{ m}^{-2} \text{ day}^{-1}$. In a similar system, Su et al. (2012) [32] reported removal rates of $0.00192 \text{ g SO}_4^{2-} \text{ cm}^{-2} \text{ day}^{-1}$ when poising the biocathode to $-200 \text{ mV}$ vs. SHE. In turn, Blazquez et al. (2016) [37] reported consumption rates of $0.4 \text{ g SO}_4^{2-} \text{ L}^{-1} \text{ day}^{-1}$ on batch reactors using graphite-brush electrodes and an inoculum from a sulphate-rich wastewater. More recently, Luo et al. (2017) [53] claimed a considerable sulphate reduction rate of $0.057 \text{ g SO}_4^{2-} \text{ L}^{-1} \text{ day}^{-1}$ on an autotrophic continuous bioelectrochemical reactor when poising their biocathode to $-700 \text{ mV}$ vs. SHE, and fed with a sulphate-rich medium. Therefore, considering the essential differences between systems, the reported results herein represent a comparatively high performance of bioelectrochemical sulphate reduction by *D. halotolerans* with appropriate potential control. Another important fact is that, according to Brandt and Ingversen (1997) [54], *D. halotolerans* is known for being unable to grow when fed with H$_2$ as electron source. Therefore, its performance is similar regardless of the applied potential, and the differences observed (higher SO$_4^{2-}$ decomposition reaction rate at $-400 \text{ mV}$) may account for the different pH shifts observed.

To compare charge accumulation on each condition, current density generation over time during chronoamperometric runs was recorded. Since DH performed best on sulphate consumption at $-400 \text{ mV}$, current generation for this experiment is shown and compared to $-900 \text{ mV}$ and an abiotic control (Fig. 4). Current density production values over time for the other inocula are available in Supplementary Material for the treating potentials (Figs. S1 and S2). Again, DH exceeded on current generation at $-400 \text{ mV}$ compared to the other strains, while at $-900 \text{ mV}$ no clear trend is observed.

Fig. 4 shows that current development during long-period potentiostatic tests was different for each potential condition. Abiotic controls did not produce any current development during testing. However, when the system was inoculated with DH and potential was settled at $-900 \text{ mV}$, there was a more pronounced current increase reaching a maximum of $-175 \text{ mA cm}^{-2}$, which was maintained for three days and then stabilized to $-130 \text{ mA cm}^{-2}$ until the end of the experiment (day 14). On the other hand, at $-400 \text{ mV}$ there was a discrete but considerable current increase reaching stabilization during the first days of functioning at $-48 \text{ mA cm}^{-2}$, which was maintained during the rest of the experiment. In the analogous system reported by Su et al. (2012) [32], maximum current outputs around $-0.023 \text{ mA cm}^{-2}$ while operating their batch system using $-200 \text{ mV}$ vs. SHE on their cathodes, were found. Recently, Luo et al. (2017) [53] reported current ($2.0 \text{ mA}$) over large ($3 \text{ cm diameter and 3 cm length}$) graphite-brush cathodes poised to $-700 \text{ mV}$ vs. SHE. Therefore, keeping in mind the key differences between reviewed systems on literature, current outputs reported here reveal a comparable electrochemically active system. Nevertheless, despite the considerable charge development when poising at $-900 \text{ mV}$, sulphate reduction did not increase. As such, it is possible that at this potential much of the current is directed to H$_2$ evolution, which elevated pH and reduced system performance in terms of sulphate reduction. In fact, it was estimated that in a system at $-400 \text{ mV}$, the ratio between sulphate consumption and accumulated charge is at least three times higher than at $-900 \text{ mV}$. Therefore, potential control is critical for accurate selection of reaction (sulphate reduction over H$_2$ evolution) while avoiding electrical energy waste.

4. Conclusions

An enriched consortium, SRBC, exhibited efficient sulphate reduction capacity in serum bottles experiments. This observation was likely due to the presence of *Sedimentibacter* and *Bacteroides* sp., that help the more prominent SRB *Desulfovibrio* member. In electrolysis reactor systems operated at potential $-900 \text{ mV}$, SRBc presented the best performance for sulphate reduction compared to pure strains. In contrast, at $-400 \text{ mV}$, only *D. halotolerans* inoculated systems were capable of performing sulphate reduction, whereas sulphate consumption for systems at $-900$ and $-400 \text{ mV}$ was essentially the same for this culture. Medium alkalinisation seems to be the main challenge for further improving performance of this batch system. Despite the observation that current output during potentiostatic experiments was much higher when poising the system at $-900 \text{ mV}$, such a system was less efficient, as it required three times the current in order to consume equivalent amounts of sulphate when compared to the performance at $-400 \text{ mV}$. Bioelectrochemical systems and inocula require further testing and conditioning, respectively, in order to achieve sulphate consumption on AMD-like conditions, with the aim of producing a potential tool for wastewater treatment from mining activities.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.bioelechem.2017.08.006.