Treatment of high-strength sulfate wastewater using an autotrophic biocathode in view of elemental sulfur recovery

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A R T I C L E   I N F O
Article history:
Received 17 June 2016
Received in revised form 8 September 2016
Accepted 8 September 2016
Available online 10 September 2016

Keywords:
Bioelectrochemical systems (BESs)
Elemental sulfur
Recovery
Sulfate-reducing bacteria (SRB)
Sulfide-oxidizing bacteria (SOB).

A B S T R A C T
Treatment of high-strength sulfate wastewaters is becoming a research issue not only for its optimal management but also for the possibility of recovering elemental sulfur. Moreover, sulfate-rich wastewater production is expected to grow due to the increased SO2 emission contained in flue gases which are treated by chemical absorption in water. Bioelectrochemical systems (BESs) are a promising alternative for sulfate reduction with a lack of electron donor, since hydrogen can be generated in situ from electricity. However, complete sulfate reduction leads to hydrogen sulfide as final sulfur compound. This work is the first to demonstrate that, in addition to an efficient sulfate-rich wastewater treatment, elemental sulfur could be recovered in a biocathode of a BES under oxygen limiting conditions. The key of the process is the biological oxidation of sulfide to elemental sulfur simultaneously to the sulfate reduction in the cathode using the oxygen produced in the anode that diffuses through the membrane. High sulfate reduction rates (up to 388 mg S-SO4/ L·d−1) were observed linked to a low production of sulfide. Accumulation of elemental sulfur over graphite fibers of the biocathode was demonstrated by energy dispersive spectrometry, discarding the presence of metal sulfides. Microbial community analysis of the cathode biofilm demonstrated the presence of sulfate-reducing bacteria (mainly Desulfovibrio sp.) and sulfide-oxidizing bacteria (mainly Sulfuricurvum sp.). Hence, this biocathode allows simultaneous biological sulfate reduction and biological sulfide oxidation to elemental sulfur, opening up a novel process for recovering sulfur from sulfate-rich wastewaters.

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1. Introduction

High-sulfate content wastewaters are generated in many processes such as pulp and paper industry, food processing, animal husbandry, dye and detergent manufacture (Lens and Pol, 2015). High concentrations of sulfate are also found in acid mine drainage wastewaters, which also present high content of metals (Kaksonen et al., 1991). Sulfate is not a very harmful pollutant, but the discharge of excessive loads of sulfate can affect public water supplies and human health. The upper concentration limit of sulfate in water intended for human consumption is recommended at 250 mg L−1 (Clair et al., 2003). Therefore, sulfate-rich wastewaters require treatment before being discharged to the environment.

The biological treatment of such effluents is restricted to anaerobic reactors where biological sulfate reduction is demonstrated as an efficient process for removing sulfate from wastewaters with either H2 (Eq. (1)) or organic matter (Eq. (2)) as electron donor. Sulfate-reducing bacteria (SRB) are anaerobic microorganisms that use sulfate as a terminal electron acceptor resulting in the production of sulfide (Muyzer and Stams, 2008), that may lead to significant issues such as corrosion, bad odors and toxic issues on human health (Pol et al., 1998). Under microaerophilic conditions, sulfide can be partially oxidized (Eq. (3)) by sulfide-oxidizing bacteria (SOB) to elemental sulfur. Therefore, oxygen limiting conditions are needed to avoid this sulfide and sulfate production.

\[
\text{SO}_4^{2-} + 4\text{H}_2 + \text{H}^+ \rightarrow \text{HS}^- + 4\text{H}_2\text{O} \quad \Delta G^0 = -151.9 \text{ KJ mol}^{-1}
\]

(1)
SO$_4^{2-}$ + CH$_3$COO$^-$ → HS$^-$ + 2HCO$_3^-$  $\Delta G^o = -47.6$ KJ mol$^{-1}$  

(2)

HS$^-$ + 0.5O$_2$ → S$^0$ + OH$^-$  $\Delta G^o = -169$ KJ mol$^{-1}$  

(3)

Sulfate-rich wastewaters are usually deficient in electron donors and hence an external supply is necessary (Liamleam and Annachhatre, 2007). Hydrogen is commonly used as electron donor for sulfate reduction because, according to thermodynamics, hydrogenotrophic sulfurification is more favorable than methanogenesis (Weijma et al., 2002) since SRB are generally more efficient in hydrogen utilization than methanogenic bacteria (Davidova and Stams, 1996). In wastewater treatment systems, hydrogen can be supplied to the reactor or generated from other electron donors.

Recent studies have suggested that biological sulfate reduction can also be driven by electricity as the sole electron source by using bioelectrochemical systems (BESs). BESs are a novel process where the oxidation reaction occurs in an anodic compartment and a reduction reaction occurs in a cathodic compartment, while one or both reactions are catalyzed by bacteria. However, depending on the semireactions occurring, the process may not be thermodynamically spontaneous and, then, an additional voltage is required. Sulfate reduction to hydrogen sulfide in a biologically catalyzed cathode has been reported by several authors (Coma et al., 2013; Luo et al., 2014; Pozo et al., 2015; Su et al., 2012; Teng et al., 2016). These works operated a two-chamber BES to study the sulfate removal efficiency in the biocathodic compartment and to evaluate both the current output and electron recovery efficiency. Even so, these studies are based on the sulfide reductive process and, as such, they have not studied the possibility to recover sulfur in the same device.

Hence, we propose a novel process for elemental sulfur recovery from high-strength sulfate wastewaters using a BES which integrates both the sulfate reduction to sulfide and a sequential partial oxidation of sulfide to elemental sulfur in the same reactor. The principle of the system is illustrated in Fig. 1. Sulfate is biologically reduced to sulfide in the biocathode (using hydrogen as an intermediate rather than direct electron transfer) while sulfide is partially oxidized to elemental sulfur using part of the oxygen produced in the anodic water electrolysis. Consequently, elemental sulfur is produced in the cathode. Hydrogen would be bioelectrochemically generated without the need of external organic fermentable compounds or external hydrogen gas supply. Therefore, we need a reductive process to drive sulfate reduction to sulfide and an oxidative process to obtain elemental sulfur from sulfide. The difficulty of the system lays in providing these two scenarios in the same single-chamber.

Thus, the aim of this work is to show the technical feasibility of this novel process for the treatment of high-strength sulfate wastewaters without external donor dosage and i) to obtain high-rate autotrophic sulfate reduction by SRB with hydrogen as the sole electron donor, ii) to recover elemental sulfur in the same compartment under oxygen limiting conditions and iii) to study the microbial communities of the system.

2. Material and methods

2.1. Hydrogenotrophic and autotrophic SRB enrichment

The inoculum for our BES systems needed to be highly enriched in hydrogenotrophic autotrophic SRB (AutH$_2$-SRB). AutH$_2$-SRB were selected in a reactor of 1.0 L and a headspace of 0.6 L operated in batch mode and connected to a 1 L gas sampling bag with a twist-type valve (Cali-5-Bond, Ritter). This reactor was inoculated with biomass from a lab-scale sewer system (Auguet et al., 2015). The reactor was periodically sparged with CO$_2$ that served as: i) carbon source, ii) pH buffer, and iii) agent for sulfide stripping, thus preventing possible inhibitions. After CO$_2$ sparging with the reactor open, the reactor was closed and a gas sampling bag was filled with a mixture of H$_2$ and CO$_2$ and connected to the reactor so that both were continuously absorbed in the liquid phase following gas-liquid equilibrium. Hydrogen was the sole electron donor available. The gas phase consisted of approximately 60% of H$_2$ and 40% of CO$_2$. Every 3–4 days, 125 mL of sludge were purged and the volume was replaced with new mineral medium. Sulfate content was ensured by periodically adding 10 mL of a concentrated pulse of MgSO$_4$ to reach a concentration of 500 mg S-SO$_4^{2-}$/L. The reactor was operated at room temperature ($T = 24 \pm 2$ °C) and pH was in the range 6.5–7.

The mineral medium used for the SRB growth was a modification of that used in Coma et al. (2013). The medium was prepared with tap water and contained (mg L$^{-1}$): 2311 MgSO$_4$·7H$_2$O, 146.6 NaHCO$_3$, 9.2 NH$_4$Cl, 327 Na$_2$HPO$_4$·2H$_2$O, 144 KH$_2$PO$_4$·2H$_2$O, 1.7 CaCl$_2$·2H$_2$O, 1.6 KCl and 1 mL of microelements solution. The microelements solution was described by Montpart et al. (2014) and contained (mg L$^{-1}$): 1000 EDTA, 164 CoCl$_2$·6H$_2$O, 228 CaCl$_2$·2H$_2$O, 20 H$_3$BO$_3$, 40 Na$_2$MoO$_4$·2H$_2$O, 2 Na$_2$SeO$_3$, 20 Na$_2$WO$_4$·2H$_2$O, 40 NiCl$_2$·6H$_2$O, 2320 MgCl$_2$, 1180 MnCl$_2$·4H$_2$O, 100 ZnCl$_2$, 20 CuSO$_4$·5H$_2$O and 20 AlK(SO$_4$)$_2$·12H$_2$O.

2.2. BES description and operation

The BES for elemental sulfur recovery consisted of two-chamber systems with anode and cathode separated by an anion-exchange membrane (AEM). Two configurations at different scale were used: i) two parent cube shaped BES (C-BES) with 35 mL of working volume in the cathodic compartment (Fig. 2a) and ii) H shaped BES (H-BES) with 350 mL of working volume in the cathodic compartment (Fig. 2b). The results presented under the C-BES configuration come from both parent cells.

The C-BES consisted of two 28 mL methacrylate vessels separated by the membrane inserted in a lateral aperture of 3.8 cm in diameter. The cathode compartment had a glass cylinder on top (with a total cathode compartment volume of 40 mL), tightly sealed with PTFE rubber cap that enabled gas diffusion to the catholyte using a gas-tight bag (0.1 L, Cali-5-Bond, Ritter) connected through the rubber cap to the glass cylinder. The cathode consisted of a

![Fig. 1. Schematic of the proposed BES for elemental sulfur recovery.](image-url)
titanium wire connected to a graphite fiber brush (20 mm diameter x 30 mm length) made with fibers of 7.2 μm in diameter (type PANEX33 160 K, ZOLTEK).

The H-BES comprised two 400 mL glass vessels separated by a membrane with a lateral 7 cm diameter aperture. The cathode consisted of a graphite fiber brush (70 mm diameter x 70 mm length) made with the same fibers of 7.2 μm in diameter. The cathode compartment was stirred and connected to a gas bag (0.5 L, Cali-5-bond, Ritter).

The graphite brushes of the cathodes were thermally treated at 450 °C for 30 min to enhance biomass adhesion. The anodes of both cells were a titanium sheet (Ti plus 50 g m⁻² Pt, Magneto, The Netherlands). Both electrodes were connected to a power source (HQ Power, PS-23023) applying a potential between 2.7 and 3.0 V to obtain a cathode potential of -1 V vs Ag/AgCl (or -0.8 V vs SHE). In both BES configurations, a reference electrode (Ag/AgCl NaCl 3 M, model RE-1B, BAS Inc., +197 mV vs SHE) was used to measure the cathode potential. An AEM was used in both configurations (AMI-7001CR, Membranes International INC). Before their use, membranes were pretreated to allow membrane hydration and expansion by soaking them overnight in a 5 wt% sodium chloride solution at 37 °C according to the supplier indications.

Cathodes of both BESs were inoculated with the AutH₂-SRB-enriched sludge. After the inoculation, cycles of 3–7 days were completed using fresh mineral medium in order to acclimate biomass. The fresh medium was prepared with distilled water and contained (mg L⁻¹): 444-4438 Na₂SO₄, 1000 NaHCO₃, 300 NH₄Cl, 3484 K₂HPO₄·2H₂O, 2722 KH₂PO₄·2H₂O, 85 MgCl₂·2H₂O, 100 KCl and 1 mL of microelements solution. The anodic medium contained 2 g NaCl L⁻¹ dissolved in distilled water. The whole experimental operation was divided into three different periods according to the initial sulfate concentrations of the batch experiments (Table 1). A first period to enrich the microbial community (period I), a second period to study the activity at a moderate initial substrate concentration (period II, 500 mg S-SO₄²⁻ L⁻¹) and a third period of high initial sulfate concentration (period III, 1000 mg S-SO₄²⁻ L⁻¹) to increase the biological activity. Moreover, in order to determine the sulfate diffusion through the membrane in the worst-case scenario, the anolyte was replaced for a fresh one in the last five cycles of period III in the H-BES and the sulfate concentration of both compartments was measured.

During the operation of the H-BES, the pH in the cathode was monitored with a pH probe (Hach pH electrode Crison 5233) connected to a pH meter (Hach MultiMeter Crison 44), and was automatically controlled at 7.0 through the addition of HCl (1 M) with a dispensing burette (Multi-Burette 2S-D, Crison Instruments). The pH of the C-BES was also measured but not automatically controlled. Both BESs were operated at room temperature (T = 24 ± 2 °C).

An abiotic experiment was also carried out using another H-BES to characterize the oxygen transfer from the anode to the cathode through the membrane with a dissolved oxygen (DO) probe (CellOx 325, WTW) in the cathode. Cathodic DO evolution was monitored in two scenarios: i) with a cathode potential set at -0.8 V vs SHE and ii) without potential applied and pure oxygen sparged into the anodic compartment.

### Table 1
Operational conditions of both BES for each experimental period.

<table>
<thead>
<tr>
<th>Period</th>
<th>Days</th>
<th>Initial sulfate concentration (mg S-SO₄²⁻ L⁻¹)</th>
<th>Duration of cycles (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0–178</td>
<td>100–500</td>
<td>3–7</td>
</tr>
<tr>
<td>II</td>
<td>178–230</td>
<td>500</td>
<td>3–4</td>
</tr>
<tr>
<td>III</td>
<td>230–330</td>
<td>1000</td>
<td>3–4</td>
</tr>
</tbody>
</table>

2.3. Analytical methods

All samples were filtered at 0.22 μm (Millipore, USA). Sulfate, sulfite and thiosulfate concentrations were analyzed by ion chromatography with conductivity detection using a Dionex ICS-2000 equipment with an Ultimate 3000 Autosampler Column Compartment, an IonPac AS18 column and an IonPac AG18 pre-

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Fig. 2. a) Cube shaped C-BES set-up and b) H shaped H-BES set-up.
column (ThermoScientific, USA). Total dissolved sulfate concentration was measured off-line with a sulfide selective electrode (VWR International Eurolab, SL). Samples were previously diluted with sulfide anti-oxidant buffer solution in order to minimize oxidation and stripping of sulfide.

2.4. Calculations

The observed sulfate reduction rate (SRR, Eq. (4)) and sulfide production rate (SPR, Eq. (5)) in the cathode were calculated as follows:

\[
SRR = \frac{C_{S^2} - C_{S^2}t - C_{S^2}f}{t}
\]

(4)

\[
SPR = \frac{C_{S^2} - C_{S^2}f - C_{S^2}t}{t}
\]

(5)

where \(C_{S^2}t\) and \(C_{S^2}f\) are the concentrations of sulfate at the beginning and at the end of cycle, \(C_{S^2}t\) and \(C_{S^2}f\), are the concentration of total dissolved sulfate at the end and at the beginning of the cycle and \(t\) is the number of days of the cycle.

The observed SRR and SPR calculations did not consider the possible sulfate diffusion through the membrane, which was evaluated in Section 3.2 for the worst-case scenario.

2.5. DNA extraction

The cathodic biofilm of H-BES was collected in a sterile Epden-dorf from the graphite brush with a sterile spatula and then centrifuged at 10,000 g (Thermo Scientific Hareus Pico17, USA). The supernatant was eliminated to remove residues from the growth medium. DNA was extracted using a PowerSoil DNA Isolation Kit (MoBio Laboratories, Inc., Carlsbad, CA) according to the manufacturer instructions. The quality and quantity of the DNA was measured using a NanoDrop spectrophotometer (ThermoScientific, USA). DNA was visualized under UV in a 0.7% gel electrophoresis with TBE 0.5 × (Tris–borate 50 mM; EDTA 0.1 mM; pH 7.5–8).

2.6. High-throughput 16S rRNA gene pyrosequencing

Tag-encoded FLX amplicon pyrosequencing (bTEFAP) was performed in a 454 Titanium FLX system at the Research and Testing Laboratory (RTL; Lubbock, TX) based upon RTL protocols from a cathode DNA sample (20 ng μL⁻¹, quality ratio of 1.8). The DNA sample was analyzed (average of 3000 reads/assay) with 28F-388R primers, comprising the V3–V5 regions of the bacterial 16S rRNA gene. Sequences were checked using Dechipher (Database Enabled Code for ideal Probe Hybridization Employing R) with Decipher’s Find Chimeras web tool to uncover short-length sequence (less than 1000 nucleotides) chimeras (http://dechipher.cee.wisc.edu/FindChimeras.html, Wright et al., 2012). Sorting and trimming were performed using the Pipeline Initial Process at the Ribosomal Database Project (RDP) Pyrosequencing Pipeline (http://rdp.cme.msu.edu/index.jsp) (Cole et al., 2009) with default settings. The RDP Classifier was used to assign 16S rRNA gene sequences to a taxonomical hierarchy with a confidence threshold of 95%, since DNA sequences were <250 bp (Claesson et al., 2009). The relative abundance of each phylogenetic group was calculated as the number of sequences associated with that group divided by the total number of sequences per sample. Because of the large coverage of the bTEFAP, relative abundance thresholds of OTUs identified were set at 1%, which corresponded to a minimum of 340 reads per sequence.

2.7. Scanning electron microscopy

Samples of graphite fiber brush (cathode) were collected, fixed with a solution of 3% glutaraldehyde, and processed according to conventional electron microscopy methods as previously described (Julían et al., 2010). Samples were treated with osmium tetroxide, dehydrated with ethanol and dried at critical point with carbon dioxide (BAL-TEC CPD030; BalTec). Then, the samples were coated with few nanometers of Au–C (E5000 Sputter Coater) to increase signal detection and visualized on a Scanning Electron Microscope (SEM, Zeiss EVO® MA 10). Elemental sulfur deposition over the biocathode was further determined by an energy dispersive spectrometer (EDS, Oxford INCA) connected to the SEM.

2.8. Solid phase characterization

The solid phase, accumulated from reactor purges, was characterized following the procedure developed by Montebello et al. (2014). Solid samples were centrifuged at 7000 rpm during 10 min to separate the liquid phase and graphite leftovers from elemental sulfur and metal sulfides. Graphite fibers were easily removed from the solid centrifuged because of the density differences. Samples were lyophilized and homogenized. A thermogravimetric analysis (simultaneous differential scanning calorimetry and differential thermal analysis system, NETZSCH -STA 449 F1 Jupiter) was carried out with pure oxygen atmosphere to determine if the temperature of volatilization of solid samples corresponded to the oxidation of elemental sulfur to sulfur dioxide. In addition, 50 mg of the solid were combusted in an adiabatic bomb calorimeter at 1200 °C with pure oxygen (CHNS analyzer, Thermo Scientific Flash 2000) to quantify the total sulfur concentration. After SO₂ absorption, the sulfate formed was analyzed with a high-performance liquid chromatograph (HPLC Alliance, Waters 2695, Waters) and a conductivity detector (Waters 432, Waters). The total sulfur concentration was quantified from the sulfate concentration. Finally, analysis of metals was performed from 100 mg of solid samples previously digested with aqua regia (HCl:HNO₃ = 3:1 (v/v)) in a microwave at 190 °C (Ethos Plus, Milestone Laboratory System) during 25 min. Then, the digested solution was filtered through a free-ash filter and the volume was made up to 100 mL. Metals analysis was conducted by inductively coupled plasma mass spectrometry (ICP-MS) (7500ce, Agilent).

3. Results and discussion

This work shows a novel concept to treat high-strength sulfate wastewaters without the need of an external electron donor using an autotrophic biocathode and with the possibility of recovering part of the sulfur as elemental sulfur. According to the processes involved in our S-recovery system (Fig. 1), four different aspects were considered relevant in order to assess the feasibility of this novel concept:

i) A high biological SRR in the cathode coupled to low SPR and low thiosulfate production rate (TPR), thus indicating that most of the sulfate supplied is reduced to sulfide and finally oxidized to elemental sulfur.

ii) Oxygen diffusion from the anode to the cathode and oxygen depletion in the cathode, since oxygen limiting conditions are required for elemental sulfur production while excess of oxygen would lead sulfide to be further oxidized to sulfate.

iii) Elemental sulfur presence in the cathode after a long-term operation.
iv) Presence of a mixed SRB and SOB community in the cathode able to conduct both sulfate reduction and sulfide oxidation steps.

3.1. Autotrophic sulfate removal

Two BESs (C-BES and H-BES) were inoculated with an enriched AutH₂-SRB community in the cathode, which was set at −0.8 V vs. SHE. Both BESs were operated in batch mode during 330 days showing promising results. Fig. 3 shows the results obtained during the whole experimental period, which was divided into three different periods (Table 1).

In the first period, the H-BES showed higher observed SRR and observed SPR than the C-BES. Note that we use the term observed SRR and observed SPR since, as discussed below, part of these ions could be transferred through the AEM. During period II, the observed SRR increased and finally, in period III, the C-BES showed an observed SRR up to 280 mg S-SO₄²⁻ L⁻¹ d⁻¹. The SRR in the H-BES for period III were similar to those in period II showing a maximum SRR up to 150 mg S-SO₄²⁻ L⁻¹ d⁻¹. On the other hand, SPR was always much lower than SRR and hence the amount of sulfate removed did not match the sulfide produced, indicating that other S species should be playing an important role.

Table 2 shows the average SRR and SPR per cell and per period. The lowest SRR and SPR were found in the start-up period. In period II, the H-BES showed higher SRR and SPR than the C-BES. However, in period III, the SRR of the C-BES increased due to a higher initial sulfate concentration while the SRR of H-BES decreased because of a H₂ limitation due to a reduction of cell intensity from 7.2 mA to 3.5 mA. Table 2 also reflects the important imbalance between SRR and SPR previously observed in Fig. 3. Considering all the experimental results, SRR was 10 times higher than SPR.

Six different cycles of three days each from periods II and III for each BES configuration were daily monitored. Fig. 4 shows the average results of sulfate, sulfide and thiosulfate evolution along the cycles. The maximum rates observed during Period II were 266 mg S-SO₄²⁻ L⁻¹ d⁻¹ in the C-BES (Fig. 4a) and 231 mg S-SO₄²⁻ L⁻¹ d⁻¹ in the H-BES (Fig. 4b) and both corresponded to the first day of the cycle. Thiosulfate concentrations were mainly below 5 mg S-S₂O₃²⁻ L⁻¹ d⁻¹ in both configurations and sulfite was not detected. Thus, TPR was considered negligible. The average sulfate concentration measured at the end of the cycles was 37 ± 9 mg S-SO₄²⁻ L⁻¹ for the C-BES and 37 ± 14 mg S-SO₄²⁻ L⁻¹ for the H-BES, much lower than the initial sulfate concentration. Thus, such sulfur imbalance was hypothesized to indicate elemental sulfur production or sulfate/sulfide losses as discussed below.

The maximum rates observed the first day of cycle in selected cycles of period III were 388 mg S-SO₄²⁻ L⁻¹ d⁻¹ in the C-BES (Fig. 4c) and 256 mg S-SO₄²⁻ L⁻¹ d⁻¹ in the H-BES (Fig. 4d), which corresponded to a 45% and 11% increase, respectively, compared to these in period II. Similarly, thiosulfate concentrations were also mainly below 5 mg S-S₂O₃²⁻ L⁻¹ d⁻¹ and sulfite was not detected in both configurations. The average sulfate concentration measured at the end of the selected cycles was 36 ± 13 mg S-SO₄²⁻ L⁻¹ for the C-BES and 25 ± 15 mg S-S₂O₃²⁻ L⁻¹ for the H-BES, which indicated, again, a large sulfur imbalance, explained in Section 3.2.

Fig. 3. Average sulfate removal rate (SRR) and average sulfide production rate (SPR) observed during the long-term operation of a) the C-BES and b) the H-BES.
controlled at 7). Thus, we observed higher maximum SRR and SPR along the first day in C-BES (Fig. 4a) than in H-BES (Fig. 4b). However, the SRR was progressively reduced as pH increased thereafter. On the other hand, the pH-controlled H-BES showed more constant SRR. In this way, the maximum rates observed the first day were always higher in C-BES than in H-BES.

\[2H^+ + 2e^- \rightarrow H_2 \quad \Delta G^0 = 0 \text{ KJ mol}^{-1}\]  

(6)

The SRRs obtained in this work are one order of magnitude higher than most found in bioelectrochemical systems in literature. Su et al. (2012) and Coma et al. (2013) operated continuous-flow systems and achieved, respectively, SRR of 14.6 mg S-SO\textsubscript{4}\textsuperscript{2-} L\textsuperscript{-1} d\textsuperscript{-1} (at −0.2 V vs. SHE) and around 60 mg S-SO\textsubscript{4}\textsuperscript{2-} L\textsuperscript{-1} d\textsuperscript{-1} (at −0.26 V vs. SHE). Moreover, no sulfur imbalance was detailed. Luo et al. (2014) attained a maximum SRR of 16.3 mg S-SO\textsubscript{4}\textsuperscript{2-} L\textsuperscript{-1} d\textsuperscript{-1} in fed-batch operation at 0.8 V of fixed cell voltage, obtaining a cathode potential of −0.76 V vs. SHE. Interestingly, they recovered only 5% of sulfate as sulfide. Teng et al. (2016) worked in fed-batch experiments obtaining SRR of 32 mg S-SO\textsubscript{4}\textsuperscript{2-} L\textsuperscript{-1} d\textsuperscript{-1} under acidophilic conditions (pH = 3) and at 0.7 V of fixed cell voltage. In this case authors assumed that all sulfate was reduced to sulfide and then precipitated as ZnS because of the supply of Zn\textsuperscript{2+}. Pozo et al. (2015) obtained the maximum SRR reported until now in a BES biocathode, which corresponds to a SRR of 188 mg S-SO\textsubscript{4}\textsuperscript{2-} L\textsuperscript{-1} d\textsuperscript{-1} at a fixed cathode potential of −0.9 V vs. SHE. In spite of a higher cathodic potential and, thus, a lower hydrogen production, our system provided higher SRR of 388 mg S-SO\textsubscript{4}\textsuperscript{2-} L\textsuperscript{-1} d\textsuperscript{-1} at −0.8 V vs. SHE. These are outstanding results in terms of sulfate reduction capacity considering that the BES presented herein has not been optimized and further improvements may lead to increased SRRs. These SRRs obtained are far from those reported in high-rate expanded granular sludge blanket reactors using H\textsubscript{2} (10 g S-SO\textsubscript{4}\textsuperscript{2-} L\textsuperscript{-1} d\textsuperscript{-1}, van Houten et al., 1994). However, BES offer important advantages in terms of H\textsubscript{2} production, gas-liquid transfer, efficiency and H\textsubscript{2} supply costs to make BES a competitive technology for treatment of high-strength sulfate wastewaters.

### 3.2. Sulfur imbalance: elemental sulfur formation

Regarding the sulfur imbalance, 13.8 ± 4.1% of the S-SO\textsubscript{4}\textsuperscript{2-} removed was accounted for as S-S\textsuperscript{2-} in the C-BES and 11.4 ± 7.9% in the H-BES in period II. Despite the higher initial sulfate concentrations in period III, very similar percentages were obtained compared with period II. Only 13.3 ± 12.1% of the S-SO\textsubscript{4}\textsuperscript{2-} removed was accounted for as S-S\textsuperscript{2-} in the C-BES and 12.0 ± 7.5% in the H-BES. We verified that such sulfur imbalance was caused by elemental sulfur production rather than to sulfate/sulfide losses

### Table 2

Average of sulfate removal rate (SRR) and sulfide production rate (SPR) in both BES configurations during the three periods of operation.

<table>
<thead>
<tr>
<th>Period</th>
<th>C-BES SRR (mg S-SO\textsubscript{4}\textsuperscript{2-} L\textsuperscript{-1} d\textsuperscript{-1})</th>
<th>H-BES SRR (mg S-SO\textsubscript{4}\textsuperscript{2-} L\textsuperscript{-1} d\textsuperscript{-1})</th>
<th>C-BES SPR (mg S-S\textsuperscript{2-} L\textsuperscript{-1} d\textsuperscript{-1})</th>
<th>H-BES SPR (mg S-S\textsuperscript{2-} L\textsuperscript{-1} d\textsuperscript{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>32 ± 19</td>
<td>64 ± 32</td>
<td>2.4 ± 2.1</td>
<td>4.1 ± 4.8</td>
</tr>
<tr>
<td>II</td>
<td>73 ± 29</td>
<td>112 ± 22</td>
<td>9.8 ± 4.6</td>
<td>12.0 ± 7.4</td>
</tr>
<tr>
<td>III</td>
<td>121 ± 66</td>
<td>97 ± 25</td>
<td>12.9 ± 7.4</td>
<td>12.3 ± 8.8</td>
</tr>
</tbody>
</table>

Fig. 4. Sulfate, sulfide and thiosulfate concentrations averaged of six cycles at 500 mg S-SO\textsubscript{4}\textsuperscript{2-} L\textsuperscript{-1} of initial concentration (Period II): a) C-BES and b) H-BES and in six cycles at 1000 mg S-SO\textsubscript{4}\textsuperscript{2-} L\textsuperscript{-1} of initial concentration (Period III): c) C-BES and d) H-BES.
(i.e. through the membrane or precipitation). Sulfate, thiosulfate and total dissolved sulfide were also analyzed in the anodic compartment of the H-BES during several experiments of period III to quantify the possible sulfate/sulfide losses through the membrane (Table S1). A fresh anolyte was used to observe the possible diffusion through the membrane in the worst-case scenario. The average diffusion obtained was of 4.7 ± 2.3% per day of sulfate. Neither sulfide nor thiosulfate were detected in the anodic compartment. Thus, almost 5% of the sulfate present in the cathode was transferred to the anode every day the first cycles with fresh anolyte. Otherwise, after some cycles, the diffusion was reduced because the anolyte increased its sulfate concentration, decreasing the gradient of concentration between both compartments, which is the driving force for the diffusion. Also, the gas bag was analyzed in order to assess the concentration of H2S in the gas phase, which was found negligible. However, such sulfate migration through the membrane cannot explain the observed sulfur imbalance. Thus, elemental sulfur formation was identified as the reason for most of this sulfur imbalance.

3.3. Oxygen limiting conditions in the biocathode

An electron acceptor is needed to drive sulfide oxidation to elemental sulfur. This work proposes a limited supply of oxygen so that sulfide oxidation ends up in elemental sulfur as final product. Providing oxygen through an aeration device directly to the cathode would add complexity and increased costs to the process. The oxygen produced in the anode due to water hydrolysis that partially diffused through the membrane to the cathode was used instead. Hence, controlled oxygen diffusion was required. The extent of DO diffusion through the membrane was assessed using an abiotic anolyte. Fig. 5 shows the DO concentration in the cathodic compartment when the anodic compartment was saturated in oxygen (sparging pure O2 in order to obtain a similar DO in the anodic compartment as when some potential is applied) in two different scenarios: i) without applied potential (Fig. 5a) and ii) when the cathode was set at −0.8 V vs. SHE (Fig. 5b). A DO increase at a slow rate of 0.84 mg O2 L−1 h−1 was observed without applied potential due to DO transport through the membrane in agreement with Mariam et al. (2015), who showed the low oxygen permeability of the membrane used (AMI-7001). In contrast, DO was consumed in the cathode at an applied potential of −0.8 V vs. SHE and no oxygen was observed. However, DO concentrations about 20 mg O2 L−1 or higher (reached in the anode during the experiments due to oxygen production as a result of water electrolysis) increased the DO gradient and, concomitantly, the oxygen diffusion through the membrane. It should also be noted that poisoning the cathode triggers off the competition between SOB and the cathode, which is able to reduce DO to water (Eq. (7)) likewise for a microbial fuel cell (Rabaey and Verstraete, 2005). Such DO scavenging also helps to obtain the limiting oxygen conditions required for partial sulfide oxidation.

\[
O_2 + 2H_2 \rightarrow 2H_2O \quad \Delta G^0 = -237.1 \text{ KJ mol}^{-1} \quad (7)
\]

3.4. Uncovering elemental sulfur

Elemental sulfur production in the cathode was assessed after approximately 200 days of operation. Samples of graphite fiber brush were collected from the biocathode for SEM analysis (Fig. 6). The growth of biofilm (Fig. 6a) and a solid deposition (Fig. 6b) over the cathode surface can be observed. The circled part of the solid deposition was analyzed by EDS and the spectra obtained (Fig. 6c) indicated that the main element found in this solid was S. Also C and O could be observed, probably because of the presence of graphite and the biofilm, but at a lower level. No metals were detected by EDS, discarding sulfide precipitation and indicating that the main component of the solid depositions on the surface of biocathode was elemental sulfur. Other solid depositions were randomly analyzed by EDS and no metals were detected in none of them, being sulfur almost the sole component. These results confirmed that the sulfur imbalance produced during the experiments was due to elemental sulfur production. Even then, precipitates of salts of Mg, P and O were also detected in some few cases (Fig. S1). Probably, Mg3(PO4)2 was formed due to the abundance of magnesium and phosphate in the mineral medium.

The solid phase accumulated from the purges of batch experiments were collected and characterized by thermogravimetry (Fig. S2), CHNS analysis and ICP-MS (Table 3). Thermogravimetry measures the mass loss through the increase of temperature and the release of enthalpy. The curve of the solid sample analyzed shows a high mass loss and a peak of enthalpy release at 232 °C, that corresponds to the autoignition temperature of sulfur (Pohanish, 2008). Since thermogravimetry was performed in the presence of O2, the peak detected corresponded to the oxidation of elemental sulfur to sulfur dioxide.

Almost 50% of the solid recovered from the cell was elemental sulfur according to the results of the CHNS analysis (Table 3). Carbon had also a high predominance due to the graphite fibers of the cathode. However, a large difference was found between sulfur results of the CHNS analysis and that of ICP-MS, which was attributed to a partial digestion of the solid during the pretreatment of the sample for the ICP-MS analysis. Even so, at least 14.2% ± 0.5 of the solid recovered, detected by ICP-MS,
corresponded to sulfur, and no metals were detected. In this analysis, also Mg and P were detected, corroborating the precipitation of magnesium and phosphate salts.

3.5. Microbial community analysis

The microbial community of the H-BES biocathode was analyzed after 2 months of operation (Fig. 7). Results obtained were in close agreement with experimental observations since the biocathode community was mainly composed of SRB (17.2%) and SOB (18.9%). Results showed a large percentage (47%) of unclassified species at different levels. Quality check data (data not shown) demonstrated the complexity to assign identity probably due to the large diversity of the community, to the limited coverage of the sequence database and to the amplicons length (360 bp on average).

*Desulfovibrio* sp. was the main SRB genus detected, which is also the most studied genus of SRB. *Desulfovibrio* sp. has one of the highest affinities for hydrogen among SRB (Laanbroek et al., 1984) and has been also detected in bioelectrochemical systems (Rago et al., 2015) as an electroactive Deltaproteobacteria for sulfate reduction (Cordas et al., 2008; Teng et al., 2016; Yu et al., 2011). Moreover, *Desulfovibrio* sp. is generally considered strictly anaerobic, even if some species of this genus are aerotolerant at the expense of having a limited growth (Sigalevich et al., 2000). Also *Paludibacter* sp. has been found to have a good removal capacity of sulfate under acidic conditions (Liang et al., 2013). A small fraction of *Sulfurospirillum* sp., a SRB able to reduce sulfur under microaerophilic conditions (Finster et al., 1997), was also detected. Other SRB included *Desulfomicrobium* sp., *Desulfobulbus* sp., *Desulforpalus* sp. and *Geobacter* sp., which belong to Deltaproteobacteria class. Despite the low percentage of some species in the microbial community, results evidenced the large diversity of SRB in the biocathode.

Regarding SOB, *Sulfuricurvum* sp. was the main genus detected, which is capable of growing under microaerophilic and anaerobic conditions (Kodama and Watanabe, 2004). Moreover, *Sulfuricurvum* sp. has been described as an autotrophic genus capable to oxidize sulfide, elemental sulfur, polysulfide, sulfite, thiosulfate and also hydrogen and to accumulate elemental sulfur extracellularly (Handley et al., 2014). Also, *Hyphomicrobium* sp., which was found in a small percentage, has also been described to be able to oxidize hydrogen sulfide (Zhang et al., 1991).

Moreover, homoacetogenic bacteria were not observed in the analysis of the biocathode community, indicating that, in the presence of sulfate and hydrogen as sole electron donor, homoacetogens were outcompeted by SRB. This fact is a key aspect to further scale-up by ensuring an effective, high-throughput use of the electron donor (H₂) for sulfate reduction purposes only.

According to bTEFAP results, SRB and SOB, dominated basically

---

**Table 3**

<table>
<thead>
<tr>
<th>Compound</th>
<th>CHNS analysis</th>
<th>ICP-MS</th>
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<tbody>
<tr>
<td>C (mg g⁻¹)</td>
<td>104 ± 28</td>
<td>142 ± 5</td>
</tr>
<tr>
<td>S (mg g⁻¹)</td>
<td>446 ± 39</td>
<td>77 ± 27</td>
</tr>
<tr>
<td>P (mg g⁻¹)</td>
<td>43 ± 10</td>
<td>&lt;10</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Me (mg g⁻¹)</th>
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<tbody>
<tr>
<td>Mg</td>
<td></td>
</tr>
<tr>
<td>Fe</td>
<td></td>
</tr>
<tr>
<td>Cu</td>
<td></td>
</tr>
<tr>
<td>Co</td>
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<tr>
<th>Amount of all metal ions detected.</th>
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<tr>
<td>After the digestion of all samples a solid fraction remained that was not possible to be analyzed.</td>
</tr>
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by *Desulfovibrio* sp. and *Sulfuricurvum* sp., played a key role in the sulfate reduction and elemental sulfur recovery (Eq. (1) and Eq. (3)) in this system.

### 3.6. Practical implications

The results presented in this work show a novel methodology for the treatment of high-strength sulfate wastewaters using an autotrophic biocathode and without the need of external electron donor. Hydrogen is produced in-situ in the cathode and its production rate can be controlled with an adequate selection of the applied voltage. This methodology presents significant economical savings and practical advantages with respect to the current use of an external chemical electron donor combined with microaeration. Moreover, this work shows how sulfur is not only reduced to sulfide but it can be recovered as elemental sulfur in the cell.

A worldwide stable market demand for elemental sulfur of about 70 million tons annually exists (Cope, 2012) and global elemental sulfur demand is increasing because of its use in the fertilizer market. Elemental sulfur is also used in producing inorganic pigments, such as biomass percentage is not important. In practice, filtration would be sufficient in the latter case. However, other processes requiring purer S would need further downstream processing. In any case, we understand that further research is still needed in order to efficiently extract this elemental sulfur as well as to provide robust separation methods.

Regarding the process economics, the process is currently not economically advantageous if the sole objective is to recover and sell this elemental sulfur. Using thermodynamics and assuming 100% efficiency processes, 32 kWh/kgH2 would be required to produce hydrogen in the cathode from water, which could lead to a minimum requirement of 4 kWh/kgS. Assuming a favorable price of electricity (e.g. 0.15 €/kWh), a price of 600 €/ton of S would be needed just to cover electricity in a 100% ideal system. Hence, the benefits of the presented process should not be only focused on elemental S recovery but also on the more efficient treatment of sulfate-rich wastewaters. However, one should not forget on the current advances on renewable energy sources. In this sense, the BES process could be fairly competitive if electricity can be produced from renewable energies such as solar energy, which are adequate for systems that require a low and constant voltage applied.

Nowadays, no economically viable industrial physical-chemical processes exist for recovering elemental sulfur from sulfate-rich wastewaters. Instead, several biological processes have been proposed offering large advantages over physical-chemical treatments in terms of reduced costs and wastes generation (Gasiorek, 1994; Jiang et al., 2013; Philip and Deshusses, 2003; Qian et al., 2015). Such processes are mostly based on combining the activity of SRB and SOB to obtain elemental sulfur and require the external addition of electron donors for sulfate reduction and a sequential sulfide oxidation with an external electron acceptor such as oxygen or nitrate. Since wastewaters with high sulfur load are usually deficient in electron donors (Liamleam and Annachhatre, 2007), expensive carbon sources or H2 are required for sulfate reduction. In all cases, a separate reactor is needed for sulfide oxidation, which increases investment costs.

The work presented opens up a new alternative for elemental sulfur recovery from high-strength sulfate wastewaters using bi-electrochemical systems. The BES concept developed herein...
eliminates i) the addition of an organic carbon source or the addition of external H₂; ii) the addition of an external electron acceptor; iii) the use of different bioreactors for both sulfate reduction and sulfide oxidation. An additional advantage over common heterotrophic processes for sulfate reduction is a reduced production of biological sludge.

4. Conclusions

This study shows for the first time the treatment of high-strength sulfate wastewater using bioelectrochemical systems with the possibility to recover elemental sulfur and without any external electron donor dosage. The process is characterized by microaerophilic conditions in the biocathode compartment due to oxygen diffusion through the membrane during water electrolysis in the anode. A mixture of SRB (17%, mainly *Desulfovibrio* sp.) and SOB (19%, mainly *Sulfuricurvum* sp.) able to reduce sulfite to sulfide and partially oxidize sulfide to elemental sulfur in an autotrophic biocathode was detected. In addition, homoacetogenic bacteria, a potential hydrogen scavenger, were not detected in our system.

The autotrophic biofilm grown in the BES was able to remove sulfate with hydrogen as the sole electron donor at a much higher SRR (up to 388 mg S-SO₄⁻² /L /d⁻¹) than those previously reported, even under a low applied potential for hydrogen production (≈0.8 V vs. SHE). Moreover, the sulfur imbalance detected in our BES, in spite of sulfate diffusion through the membrane (up to 5% per day in the worst-case scenario), was due to the production of elemental sulfur over the cathode surface as was detected by energy dispersive spectrometry and CHNS and ICP-MS analyses.

Acknowledgements

This work was supported by the Spanish Government with a grant “Explora Ciencia y Explora Tecnología” (CTM2014-62179-EXP). The authors are members of the GENOCOV group (Grup de Recerca Consolidat de la Generalitat de Catalunya, 2014SGR 1255). Dr. Oriol Gutierrez (Institut Català de Recerca de l’Aigua) is thanked for providing the inoculum used in this study. Dr. Mabel Mora and Dr. Laura Rago are also thanked for their contribution to this work. The authors want to thank the ideas and suggestions of an anonymous reviewer which were used to upgrade the discussion of the results.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.watres.2016.09.014.

References


