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# Bacteria as an Electron Shuttle for Sulfide Oxidation

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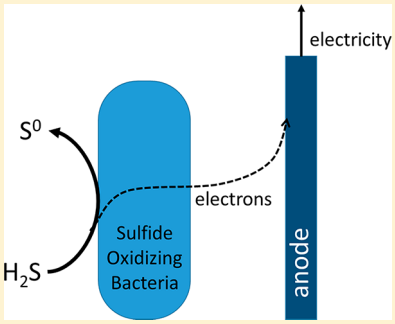
Supporting Information

**ABSTRACT:** Biological desulfurization under haloalkaliphilic conditions is a widely applied process, in which haloalkalophilic sulfide-oxidizing bacteria (SOB) oxidize dissolved sulfide with oxygen as the final electron acceptor. We show that these SOB can shuttle electrons from sulfide to an electrode, producing electricity. Reactor solutions from two different biodesulfurization installations were used, containing different SOB communities; 0.2 mM sulfide was added to the reactor solutions with SOB in absence of oxygen, and sulfide was removed from the solution. Subsequently, the reactor solutions with SOB, and the centrifuged reactor solutions without SOB, were transferred to an electrochemical cell, where they were contacted with an anode. Charge recovery was studied at different anode potentials. At an anode potential of +0.1 V versus Ag/AgCl, average current densities of 0.48 and 0.24 A/m$^2$ were measured for the two reactor solutions with SOB. Current was negligible for reactor solutions without SOB. We postulate that these differences in current are related to differences in microbial community composition. Potential mechanisms for charge storage in SOB are proposed. The ability of SOB to shuttle electrons from sulfide to an electrode offers new opportunities for developing a more sustainable desulfurization process.




## 1. INTRODUCTION

Dihydrogen sulfide ($H_2S$) is a toxic, odorous, and corrosive component present in, for example, natural gas and biogas. If present in gas streams, it oxidizes into $SO_2$ upon combustion, resulting in air pollution, acid rain, and smog. Therefore, sour gas stream desulfurization is required before use. The biological desulfurization process under haloalkaline conditions is one of the processes for removing $H_2S$ from gas streams.[1] In this process, halkaliphilic sulfide-oxidizing bacteria (HA-SOB) convert dissolved bisulfide ($HS^-$) and dissolved oxygen ($O_2$) into elemental sulfur crystals ($S_8$), described by the overall reaction

$$HS^- + \frac{1}{2}O_2 \rightarrow \frac{1}{8}S_8 + OH^- \quad (1)$$

The traditional process consists of two steps: absorption of $H_2S$ in an absorber column and oxidation into $S_8$ in an aerated bioreactor. Operating the process at high salt concentrations (halophilic) and alkaline conditions leads to enhanced $H_2S$ absorption and therefore a robust absorption process.[1] The haloalkaliphilic biodesulfurization process is widely applied in the food, paper, mining, and oil and gas industries.[2]

Sulfide oxidation is a process that also occurs in sediments. It has been shown that cable bacteria present in sediments can oxidize sulfide in anaerobic layers and transport electrons over distances of several centimeters.[3] They thus act as an electron shuttle between anaerobic sediment and higher aerobic layers. Sulfide has also been studied as an electron donor in the field of bioelectrochemical systems (BESs), where electrodes are used for the treatment of sulfide- and sulfate-containing wastewater. Typically, two processes occur in the anode compartment of such microbial fuel cells. (1) Sulfate is reduced into sulfide by sulfate-reducing bacteria, and (2) sulfide is oxidized into elemental sulfur at the anode of microbial fuel cells, either electrochemically or bioelectrochemically, resulting in the production of electricity.[4−7] Major challenges observed in these sulfide-oxidizing BESs are (i) formation of sulfur deposits at the surface of the electrode, leading to inactivation of electrodes and affecting process stability, and (ii) formation of different products besides $S_8$, like thiosulfate and sulfate, as a result of both chemical and biological reactions.

In this work, we show that HA-SOB remove sulfide from a haloalkaline solution in the absence of oxygen. Subsequently, when the planktonic HA-SOB are transferred to an electrochemical cell, they release electrons at an anode. We demonstrate that HA-SOB can act as an electron shuttle

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between two systems, enabling the recovery of electricity in an electrochemical cell.

## 2. MATERIALS AND METHODS

**2.1. Microorganisms and Solution Composition.** Two types of sludges (reactor solutions containing SOB) from different biodesulfurization installations were used: (i) sludge of the full-scale installation in Eerbeek (Industriewater Eerbeek BV, The Netherlands), consisting of an absorber column and an aerated bioreactor, fed with $H_2S$-containing biogas, and (ii) sludge of a pilot-scale installation, consisting of an absorber column, an anaerobic bioreactor and an aerated bioreactor, fed with a synthetic gas containing $H_2S$, $CO_2$, and $N_2$.[8] Sludge of the full-scale installation in Eerbeek will be termed a single-reactor (SR) solution, and sludge of the pilot-scale installation will be termed a dual-reactor (DR) solution.

Reactor solutions were harvested from the aerated bioreactor, operated under primarily $S_8$ forming conditions, for both installations. The DR solution had an alkalinity (expressed as the concentration of $HCO_3^-$) of 0.68 M, a pH of 8.22, and a conductivity of 43.2 mS/cm. The SR solution had an alkalinity of 0.88 M, a pH of 8.5, and a conductivity of 56 mS/cm. The alkalinity was determined by titration with 0.1 M HCl with the Titrino plus instrument (Metrohm, Herisau, Switzerland). pH and conductivity were measured with a HQ440d multi instrument (Hach Lange). In addition to sodium carbonate/bicarbonate, the reactor solutions contained a mixed culture of mainly SOB, elemental $S_8$ crystals, and sodium sulfate and thiosulfate. The concentration of bacteria in the reactor solution was 72.4 mg of N/L for the SR-SOB and 29.2 mg of N/L for the DR-SOB, measured as the difference between total N and dissolved N (supernatant of a sample centrifuged for 10 min at 10000 rpm) with test LCK138 (Hach Lange).

**2.2. Solution Pretreatment.** To test the ability of SOB to remove sulfide from solution under anaerobic conditions, a three-step pretreatment procedure was followed.

First, the reactor solution with SOB was aerated overnight to allow complete oxidation of the SOB. Oxidation was thought to be complete when the solution remained saturated with oxygen, as measured with a DO sensor (ProSense, Oosterhout, The Netherlands).

Second, the reactor solution was flushed with $N_2$ until all oxygen was removed (i.e., the DO was below detection limit of 26 nmol/L). After flushing, the pH had increased to 9.61 for the DR solution and 9.43 for the SR solution due to $CO_2$ stripping. Furthermore, 6−8% of water was evaporated, leading to slightly higher conductivity and alkalinity at the start of the sulfide uptake and discharge experiments. From this step onward, pH, conductivity, and alkalinity stayed constant during all experiments.

Third, 0.2 mM sulfide was added as $Na_2S{\cdot}{\sim}3H_2O$ (Analar NORMAPUR, VWR, analytical grade).

**2.3. Discharge Experiments.** The pretreated reactor solution was transferred to an electrochemical cell with a total liquid volume of 50 mL (Figure S1) and a headspace of 30 mL. The anode was a graphite rod electrode (3 mm × 3 mm × 80 mm) with a submerged external surface area of 3.1 $cm^2$. The cathode was a Pt foil (2.8 $cm^2$) connected to the outside of the cell via a Pt wire. The reference electrode [Ag/AgCl, 3 M KCl (+0.205 V vs SHE)] was inserted into the solution via a capillary. The solution was stirred with a magnetic stirrer. Before each experiment, the electrochemical cell was cleaned with demineralized water, and the anode was cleaned using sandpaper (Silicon Carbine 1200/4000, P2000 grit, Gauss Union Co.). Before injection of the sample into the electrochemical cell, the cell was flushed with $N_2$ to remove oxygen. Experiments were performed with (i) a pretreated reactor solution with SOB and (ii) a pretreated reactor solution without SOB. To obtain a reactor solution without SOB, the solution was centrifuged after the sulfide uptake experiment (10 min, 10000 rpm) and the supernatant was transferred to the electrochemical cell. Reactor solutions with and without SOB were tested at least twice; replicates were performed with new reactor solutions after pretreatment. The number of replicates is indicated with $n$, and averages and standard errors are reported. Experiments were performed in a temperature-controlled cabinet at 25 °C.

**2.4. Sulfide Uptake Measurements.** To verify whether sulfide was removed from solution, sulfide uptake experiments were performed. Reactor solutions with and without SOB were subjected to the pretreatment described in section 2.2 and tested in duplicate. Five minutes after 0.2 mM sulfide was added, the final sulfide concentration was measured using a Hach Lange test LCK-635, after filtering over a 0.45 $\mu$m filter. In case no free sulfide was measured, an additional test was performed with lead(II) acetate paper (Merck, Darmstadt, Germany). This lead(II) acetate paper shows color when trace sulfide levels (less than parts per billion) are present.

**2.5. Electrochemical Control and Measurements.** A potentiostat (Ivium n-stat with IviumSoft version 2.594, Eindhoven, The Netherlands) was used to control the anode potential in a three-electrode setup, with the anode as the working electrode, Ag/AgCl as the reference electrode, and the cathode as the counter electrode. Two methods were used: chronoamperometry and linear sweep. During chronoamperometry, the anode potential was controlled at −0.1, 0, and +0.1 V versus the reference electrode for a defined time period (usually 600 s). During linear sweep, the anode potential was changed from −0.6 to +0.4 V versus Ag/AgCl with a scan rate of 1 mV/s (performed once for each solution).

**2.6. Chemical Analysis and Calculations.** Sulfide was added from an anaerobic stock solution, of which the concentration was verified by titration with a solution of 0.1 M $AgNO_3$, using a Titrino Plus Titrator (Metrohm, Herisau, Switzerland). One milliliter of the stock solution was added to 80 mL of 4% (w/v) NaOH, with 1 mL of 30% (w/v) $NH_4OH$ to stabilize sulfide. The sulfide concentration (0.2 mM) was very low compared to the concentrations of sulfur, sulfate, and thiosulfate. Hence, it was impossible to accurately determine the end product of sulfide conversion, which is required to set up sulfur mass balances in these experiments.

The anode Coulombic efficiency $\eta_C$ (%) was calculated according to the equation $\eta_C = [(\int_0^t I\,dt)/([S^{2-}]_0 V \times 2F)] \times 100\%$, where $I$ is the current (amperes), $t$ is the time (seconds), $[S^{2-}]_0$ is the sulfide concentration at the start of the experiment, $V$ is the liquid volume (50 mL), 2 is the number of electrons transferred (assuming two-electron oxidation of sulfide to $S_8$ as the installation was performed under primarily $S_8$ forming conditions), and $F$ is the Faraday constant (96485 C/mol).

## 3. RESULTS AND DISCUSSION

**3.1. Electricity Was Recovered from Sulfide-Oxidizing Bacteria.** After pretreatment of reactor solutions (overnight aeration, flushing with $N_2$, and addition of 0.2 mM sulfide),



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SOB were tested for their ability to use the electrode as an electron acceptor. Current was measured in the electrochemical cell at an anode potential of +0.1 V versus Ag/AgCl. The total charge recovered in the first 10 min from reactor solutions with and without SOB is shown in Figure 1 (based

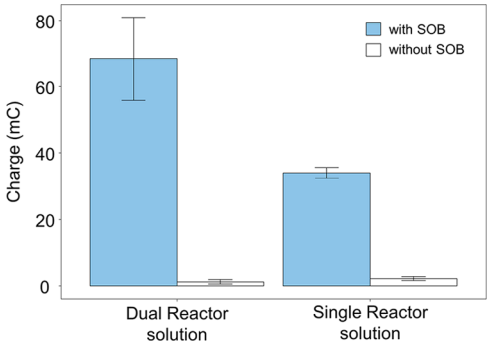


**Figure 1.** Charge was recovered from SOB for dual-reactor ($n$ = 4) and single-reactor ($n$ = 2) solutions, including the standard error. Reactor solutions without SOB resulted in negligible charge. The total charge was higher for DR-SOB than for SR-SOB, even though the biomass concentration was lower (29.2 mg of N/L) than for the SR-SOB (72.4 mg of N/L). The total charge was measured during the first 600 s at an anode potential of +0.1 V vs Ag/AgCl.

on current profiles as shown in Figure S2). The total charge recovered was 68 ± 12 mC for DR-SOB ($n$ = 4) and 34 ± 2 mC for SR-SOB ($n$ = 2), while the charge recovered from a centrifuged reactor solution without bacteria was negligible (1.2 ± 0.7 and 2.2 ± 0.6 mC, respectively). This shows that that electrons were extracted from suspended SOB and not from other components present in the reactor solution, e.g., traces of sulfide. DR-SOB produced an average current density of 0.48 A/m$^2$ (averaged over 600 s), whereas SR-SOB produced an average current density of 0.24 A/m$^2$. SOB in both types of installations can thus use the anode as an electron acceptor.

The higher charge for DR-SOB compared to that of SR-SOB could be a result of the nature of the process in which the SOB were grown. In the single-reactor process, oxidation of sulfide and reduction of oxygen can take place simultaneously. In the dual-reactor process, SOB are exposed to a regime of alternating reducing conditions (anaerobic reactor and abundance of sulfide) and oxidizing conditions (aerobic reactor and low sulfide). As a result, DR-SOB are exposed to a regime in which oxidation of sulfide and reduction of oxygen are taking place in successive steps. We hypothesize that this intermittent exposure to sulfide and oxygen may stimulate electron storage; potential mechanisms will be discussed below.

Additional experiments were performed at different anode potentials of −0.1, 0, and +0.1 V versus Ag/AgCl, for both types of SOB, to study the effect of anode potential on charge recovery. The charge was normalized to biomass concentration and expressed as millicoulombs per milligram of N (Figure 2).

At a +0.1 V anode potential, the highest current and total charge were measured, as this is the situation with the highest overpotential (driving force). The current and total charge decreased with a more negative anode potential.

To exclude the possibility that the produced current originated from the $H_2$ gas formed in the single-chamber electrochemical cell, an additional experiment was performed

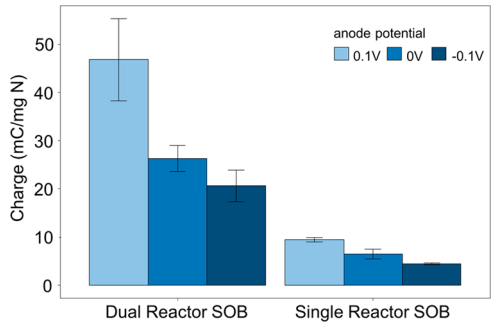


**Figure 2.** Charge, normalized to the amount of biomass, was highest at a +0.1 V anode potential and decreased for lower anode potentials. DR-SOB showed current densities higher than those of SR-SOB. All experiments were at least two replicates, and the standard error is shown.

in which the cathode was placed in a separate bottle, connected to the anode chamber via a 3 M KCl salt bridge. Similar currents were observed, showing that the effect of $H_2$ on current production was negligible.

The 10 min discharge period did not allow for complete discharge of HA-SOB. To determine the Coulombic efficiency, which represents the portion of the electrons from sulfide that end up as electric current, the experiments were also performed for a longer time period of 24 h with DR-SOB. Assuming $S_8$ as the product (as observed in the DR installation), 13−35% of the charge in sulfide was recovered as electricity. These values are in the same range as those reported for sulfide oxidation at bioanodes.[7]

**3.2. Sulfide Was Removed by SOB under Anaerobic Conditions.** Sulfide uptake experiments were performed in duplicate to confirm that sulfide was removed from solution by SOB under anaerobic conditions. Reactor solutions with and without SOB were exposed to 0.2 mM sulfide. For the DR-SOB, no free sulfide was detected after exposure for 5 min, which was further confirmed with lead acetate paper. For the SR-SOB, sulfide concentrations decreased. Control tests on a centrifuged reactor solution without SOB also showed a decrease in sulfide concentration (see Table S1). To prove that removal of sulfide by SOB was significant, an unpaired one-sided equal variance $t$ test based on the two measured replicates for each situation was performed. This $t$ test showed that sulfide levels for both DR-SOB and SR-SOB were lower ($p$ < 0.001 for DR-SOB, and $p$ = 0.023 for SR-SOB) than sulfide levels for the centrifuged solutions without SOB. The specific sulfide uptake in these experiments was 0.22 ± 0 mg of S/mg of N for DR-SOB and 0.063 ± 0.002 mg of S/mg of N for SR-SOB.

**3.3. Linear Sweep.** Linear sweep measurements were performed for both types of SOB to study the dynamic response of SOB and the reactor solution to a change in anode potential (Figure 3). At more positive anode potentials, a higher current was measured for the DR-SOB than for the SR-SOB, even though the biomass concentration was 2.5 times lower. To analyze background effects, both reactor solutions were tested without SOB. Minor current production was observed, presumably related to the capacitance of the electrode and to oxidation of trace concentrations of sulfide. An additional measurement was performed on the centrifuged reactor solution without SOB amended with 0.2 mM sulfide, showing that sulfide is electrochemically oxidized in a potential range similar to that of SOB.



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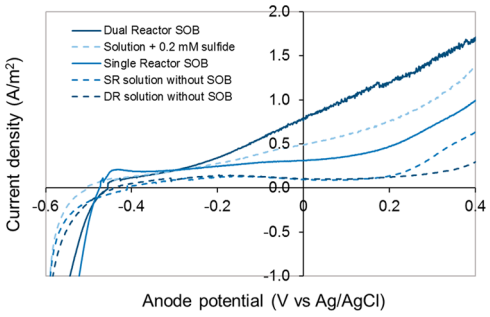


**Figure 3.** Linear sweep reveals current profiles for SOB from both reactors that increase with an increase in anode potential. The current in the presence of SOB is higher than in absence of SOB.

**3.4. Storage Mechanisms.** Analysis of the microbial communities revealed bacteria from the genus *Thioalkalivibrio* as the dominant SOB in the single-reactor plant, whereas bacteria from the genus *Alkalilimnicola* were the dominant SOB in the dual-reactor plant (see Figure S2). Two types of enzymatic routes for oxidation of biological sulfide to elemental sulfur are known: flavocytochrome *c* sulfide dehydrogenase (FCC) and sulfide quinone reductase (SQR).[9] In *Thioalkalivibrio sulfidophilus*, the electrons from sulfide oxidation enter the respiration chain via FCC.[10] These electrons are transferred to cytochromes *c* and are finally transferred to oxygen by cytochrome *c* oxidase. For *Alkalilimnicola ehrlichii*, it has been reported that sulfide is oxidized using the SQR enzyme, which is bound to the plasma membrane,[9,11] thereby reducing the electron carrier quinone to its reduced equivalent quinol. Quinol can be oxidized in several ways, for example, by oxygen using quinol oxidase. Because both oxidation routes are a cascade of electron transfer reactions, sulfide and oxygen are not simultaneously converted. On the basis of this knowledge of sulfide conversion mechanisms of SOB in haloalkaliphilic systems and electron transfer mechanisms in electro-active biofilms,[12,13] we postulate that SOB can store electrons in their electron carriers, such as cytochromes and quinones. Electrodes, in combination with other techniques, will offer new opportunities to study the role of cytochromes, quinones, and storage mechanisms in the electron transport chain of sulfide-oxidizing bacteria.

**3.5. Outlook.** Whereas sulfide oxidation at a bioanode has been described before,[4−7] the novelty of this study lies in the charge shuttling capacity of SOB. In this two-stage process, planktonic SOB from biodesulfurization systems take up sulfide under anaerobic conditions and produce current using an anode as an electrode acceptor.

Compared to the established sulfide removal processes, electrodes have the advantage that sulfide can be used as an energy source. In combination with an oxygen-reducing cathode, the main product would be electricity, or when this is coupled to a hydrogen-producing cathode, electricity can be converted into hydrogen. Both processes result in a more positive energy balance compared to electricity use for aeration of the bioreactor. In addition, the absence of oxygen might lead to higher selectivity toward $S_8$, as the use of electrodes provides a way to decouple sulfide oxidation from mixing, which is coupled to aeration in the traditional process.

Compared to MFCs for sulfide removal, sulfur deposition on the anode may be prevented, leading to a more stable process, as the SOB take up sulfide outside the electrochemical cell. In addition, the high bicarbonate concentration results in a low risk of anode acidification, a problem commonly encountered in MFCs,[14] and it results in low ohmic losses due to the high ionic conductivity of the reactor solutions, being 45−55 mS/cm, ∼10 times higher than conductivity in wastewaters used in MFCs.

The use of electrodes in the biological desulfurization process is a new application, and many questions remain unanswered. Aspects that need to be addressed are, among others, the mechanisms for charge storage in SOB, the conditions under which HA-SOB can sustain growth using the electrode as an electron acceptor, product formation, the effect of free sulfide in interaction with the electrode, and optimization of the system and process conditions. Insights into all these factors may result in a new process in which electrodes are integrated into the biodesulfurization process, further increasing its sustainability.

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.estlett.8b00319.

Scheme of the electrochemical cell, figure with chronamperometry results, table with measured sulfide concentrations in the presence and absence of SOB, and NGS methodology and results (PDF)

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### Notes

The authors declare no competing financial interest.

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