

Increasing the Selectivity for Sulfur Formation in Biological Gas Desulfurization

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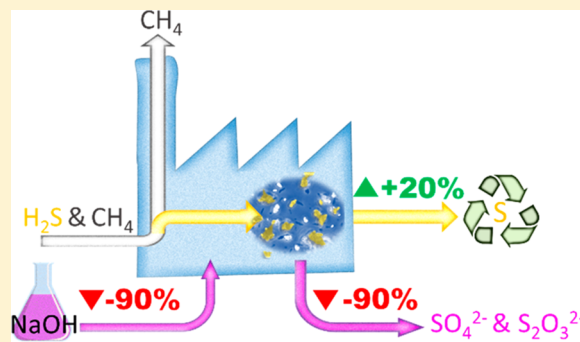
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Supporting Information

ABSTRACT: In the biotechnological desulfurization process under haloalkaline conditions, dihydrogen sulfide (H_2S) is removed from sour gas and oxidized to elemental sulfur (S_8) by sulfide-oxidizing bacteria. Besides S_8 , the byproducts sulfate (SO_4^{2-}) and thiosulfate ($\text{S}_2\text{O}_3^{2-}$) are formed, which consume caustic and form a waste stream. The aim of this study was to increase selectivity toward S_8 by a new process line-up for biological gas desulfurization, applying two bioreactors with different substrate conditions (i.e., sulfidic and microaerophilic), instead of one (i.e., microaerophilic). A 111-day continuous test, mimicking full scale operation, demonstrated that S_8 formation was 96.6% on a molar H_2S supply basis; selectivity for SO_4^{2-} and $\text{S}_2\text{O}_3^{2-}$ were 1.4 and 2.0% respectively. The selectivity for S_8 formation in a control experiment with the conventional 1-bioreactor line-up was 75.6 mol %. At start-up, the new process line-up immediately achieved lower SO_4^{2-} and $\text{S}_2\text{O}_3^{2-}$ formations compared to the 1-bioreactor line-up. When the microbial community adapted over time, it was observed that SO_4^{2-} formation further decreased. In addition, chemical formation of $\text{S}_2\text{O}_3^{2-}$ was reduced due to biologically mediated removal of sulfide from the process solution in the anaerobic bioreactor. The increased selectivity for S_8 formation will result in 90% reduction in caustic consumption and waste stream formation compared to the 1-bioreactor line-up.



1. INTRODUCTION

Since its introduction in 1993, biotechnological desulfurization under haloalkaline conditions has proven to be effective in removal and conversion of H_2S from various types of gas streams.¹ It offers significant advantages over conventional physicochemical processes. Key advantages are that biotechnological desulfurization operates at ambient temperatures and pressures without the use of complex and toxic chemicals.

The first step of the biotechnological desulfurization process is the selective absorption of dihydrogen sulfide (H_2S) from a sour gas into the process solution in an absorber column. To optimally remove the H_2S from the gas stream, the sour gas and process solution are counter-currently contacted. Due to the mildly alkaline washing solution, the majority of the dissolved sulfide is chemically converted to soluble bisulfide

(HS^-). To recover sulfur (S_8), the HS^- -rich solution is subsequently routed to an aerated microaerophilic bioreactor. Here, chemolithoautotrophic haloalkaliphilic sulfide-oxidizing bacteria (SOB) oxidize HS^- to solid S_8 particles at oxygen-limited conditions (i.e., dissolved oxygen concentration <100 nM). The solid sulfur particles are harvested from the solution by gravity settling and/or centrifugation and can be reused.² The technology is applied on commercial scale for a wide range of different sour gases, such as biogas, natural gas and amine acid gas (>250 installations worldwide in 2017).^{3,4}

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Although operating conditions can be optimized for S_8 formation, part of the HS^- is oxidized to sulfate (SO_4^{2-}) and thiosulfate ($S_2O_3^{2-}$). In current systems, a maximum of 80–90 mol % conversion to S_8 can be obtained.^{5–7} SO_4^{2-} and $S_2O_3^{2-}$ formation are accompanied by proton formation and therefore require the addition of caustic soda (NaOH) (see Reactions 1–4 in Table 1). Moreover, as SO_4^{2-} and $S_2O_3^{2-}$ accumulate, the salinity increases and a bleed stream is required to avoid biological failure. When chemical consumption (caustic) and waste stream formation (bleed stream) can be reduced, the biodesulfurization process becomes more attractive.

Table 1. Major Biological and Chemical Conversions in the Biotechnological Desulfurization Process under Haloalkaline Conditions^{1,6–8}

number	reaction equation	description of the reaction
1	$HS^- + \frac{1}{2} O_2 \rightarrow \frac{1}{8} S_8 + OH^-$	biological sulfide oxidation
2	$\frac{1}{8} S_8 + 1\frac{1}{2} O_2 + H_2O \rightarrow SO_4^{2-} + 2 H^+$	biological sulfur oxidation
3	$HS^- + O_2 \rightarrow \frac{1}{2} S_2O_3^{2-} + \frac{1}{2} H_2O$	chemical sulfide oxidation
4	$\frac{1}{2} S_2O_3^{2-} + O_2 + \frac{1}{2} H_2O \rightarrow SO_4^{2-} + H^+$	biological thiosulfate oxidation
5	$HS^- + S_{x-1} \rightleftharpoons S_x^{2-} + H^+$	chemical polysulfide formation

A key aspect in optimizing biological systems is the (active) microbiological community. The biological desulfurization process under haloalkaline conditions is dominated by haloalkaliphilic SOB belonging to the genus of *Thioalkalivibrio*.^{9,10} The analysis of the complete genome of *Thioalkalivibrio sulfidiphilus*,¹¹ showed that HS^- oxidation to S_8 is catalyzed by flavocytochrome *c* sulfide dehydrogenase (Fcc). Subsequently, S_8 can be oxidized by a reversed dissimilatory sulfite reductase (rDSR) system to sulfite (SO_3^{2-}) and further to SO_4^{2-} . Hence, in current full-scale biodesulfurization systems, the formation of SO_4^{2-} is inevitable due to the dominance of SO_4^{2-} producing SOB, such as *Tv. sulfidiphilus*.

Next to the biological oxidation of HS^- to SO_4^{2-} , the efficiency of S_8 formation is hampered by the chemical oxidation of HS^- . When dissolved oxygen (O_2) and HS^- are both present in the process solution, HS^- is chemically oxidized to $S_2O_3^{2-}$.¹² In addition, the presence of reactive polysulfides (S_x^{2-}), which are formed rapidly with S_8 and HS^- (reaction 5 in Table 1), also enhances the formation of $S_2O_3^{2-}$.⁸ As the HS^- -rich process solution coming from the absorber is directly injected in an aerated bioreactor in the current process, formation of $S_2O_3^{2-}$ cannot be prevented.

In order to increase the selectivity for S_8 formation, production of the byproducts SO_4^{2-} and $S_2O_3^{2-}$ should be minimized. Key in the enzymatic formation of SO_4^{2-} via rDSR is the oxidized electron carrier cytochrome *c*. The oxidation of cytochrome *c* occurs via reduction of oxygen by cytochrome *c* oxidase (CcO), which is reversibly inhibited by HS^- .¹³ Hence, when exposing SOB, such as *Tv. sulfidiphilus*, to elevated HS^- levels, the oxidation route from HS^- to SO_4^{2-} is severely hampered due to suppression of CcO.¹⁴ As a side effect of this inhibition, other enzyme systems using oxidized cytochromes, such as Fcc, are suppressed as well. When the main route of HS^- oxidation to S_8 is suppressed, it is expected that when the

system is continuously operated under the CcO suppressing conditions, another HS^- oxidizing enzyme system eventually becomes dominant, which is not subjected to HS^- inhibition: sulfide-quinone reductase (SQR).

To expose SOB to elevated HS^- levels, it is suggested to include an extra bioreactor in the biodesulfurization process line-up.¹⁵ This bioreactor, placed in between the absorber and aerated bioreactor, imposes extra retention time for the SOB in the HS^- rich process solution from the H_2S absorber. Recently, it has been shown that SOB taken from biodesulfurization systems, are capable of HS^- uptake in the absence of oxygen.¹⁶ Hence, it can be hypothesized that when SOB are exposed to elevated HS^- levels in the anaerobic bioreactor, HS^- concentration will be decreased and the HS^- concentration in the influent of the aerobic bioreactor is lower. As a result, chemical oxidation rates in the aerated bioreactor will decrease and $S_2O_3^{2-}$ formation will be lower. Therefore, it is hypothesized that retention time at elevated HS^- levels in the anaerobic reactor (i) imposes enzymatic suppression of SO_4^{2-} formation, (ii) imposes a change in dominating HS^- oxidation route (i.e., from Fcc to SQR) and (iii) reduces the chemical oxidation rate of HS^- to $S_2O_3^{2-}$ by stimulating HS^- uptake by SOB.

The aim of this research is to study how the addition of an anaerobic bioreactor to the process line-up will further optimize the selectivity for S_8 formation in the biological gas desulfurization process under haloalkaline conditions. The new dual bioreactor line-up was tested in continuous mode for 111 days at pilot scale (20 L), mimicking operation of full scale conditions. During the experiment, the process performance (i.e., selectivity for S_8 , SO_4^{2-} , and $S_2O_3^{2-}$ formation) was analyzed. In addition, HS^- levels in the anaerobic reactor were monitored to test the hypothesis of biological HS^- uptake. Moreover, Next Generation Sequencing (NGS) was used to assess changes in the microbial community. The results were compared with an experiment in the same setup without using the anaerobic bioreactor (i.e., the 1-bioreactor line-up).

2. MATERIALS AND METHODS

2.1. Experimental Setup. The experiments were performed in a continuous reactor system, consisting of an H_2S absorber and two bioreactors: an anaerobic bioreactor and an aerated bioreactor (Figure 1).

The feed gas supplied to the H_2S absorber (stream A in Figure 1) was a mixture of H_2S (8.9 vol% H_2S , 91.1 vol% N_2), N_2 (99.995 vol%) and CO_2 (99.995 vol%). These gases were supplied as separate streams using mass flow controllers (Profibus, Brooks instrument, Hatfield, PA) to the main feed gas line. Washing solution (stream 1) with no sulfide (“lean” solution) from the aerated bioreactor was circulated over the H_2S absorber by an eccentric screw pump (P1 in Figure 1). The HS^- -containing solution (“rich” solution) was collected at the bottom of the H_2S absorber (approximately 1 L). The flow of rich solution from the bottom of the H_2S absorber to the anaerobic bioreactor (stream 2) (or to the aerated bioreactor directly in the control experiment) was driven by the pressure difference between the pressurized H_2S absorber and the atmospheric anaerobic bioreactor, and was controlled with a valve. The effluent of the anaerobic bioreactor was directed to the aerated bioreactor (stream 3) with a peristaltic pump (P2). More information about the H_2S absorber, liquid flows, pumps and pump controls is supplied in the Supporting Information (SI 1). The total liquid volume of the anaerobic bioreactor (5.3

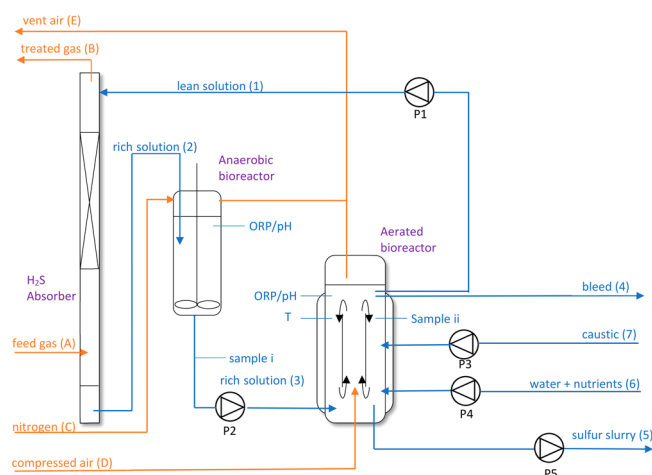


Figure 1. Schematic representation of the experimental setup used for the continuous experiments. The orange lines represent gaseous flows and the blue lines liquid flows. During the control experiment, the flow from the absorber bottom was routed directly to the aerated bioreactor.

L) was continuously mixed by an installed mechanical mixer (rzr2020, Heidolph Instruments, Schwabach, Germany). In both bioreactors, the pH and oxidation/reduction potential (ORP) were measured with a combined SE552/2 Inducon ORP/pH sensor, connected to a Stratos Pro Transmitter (Knick, Berlin, Germany). An integrated Ag/AgCl electrode was used as reference for ORP and pH. To avoid air leakage into the anaerobic bioreactor, a small flow of N_2 (stream C) was supplied to its headspace.

The aerated bioreactor was a gas-lift bioreactor with a wet volume of 11.4 L with dimensions 690/150 mm (H/D). Both the anaerobic bioreactor and the aerated bioreactor were kept at a constant temperature using warm water from a thermostat bath (Kobold, Germany), which was routed through the water jackets of both reactors. The temperature in the aerated bioreactor during the experiments was 36.9 ± 1.0 °C. The temperature in the anaerobic bioreactor was slightly lower (35.9 ± 1.0 °C). This temperature is similar to the operating temperature of full-scale systems, which is between 30 and 40 °C. The ORP in the aerated bioreactor was maintained at a constant preset value by aeration. Compressed air (stream D) was supplied with a mass flow controller (Profibus, Brooks Instruments). In addition, a sensor for measuring dissolved O_2 (PSt 6 Presens, Regensburg, Germany) was positioned in the aerated bioreactor. This reactor was overflowing into a bleed vessel (stream 4). The bottom of the aerated bioreactor was cone-shaped, to secure the removal of settling S_8 . This S_8 slurry was removed with a pump (P5) (101 U/R, Watson Marlow, Wilmington DE) (stream 5). To compensate for the removed slurry, diluted nutrients solution via pump P3 (101 U/R, Watson Marlow) (stream 6) and a diluted 5% (w/w) caustic solution via pump P4 (101 U/R, Watson Marlow) (stream 7) were continuously supplied. The presence of dissolved HS^- and polysulfide in the aerated bioreactor was regularly checked with lead acetate paper (H_2S -Test Paper, Tintometer GmbH, Dortmund, Germany). To determine the liquid influent and effluent streams, the vessels for storing and collecting nutrients, caustic, bleed water and sulfur slurry solutions were weighed each time a sample was taken, using a balance (PM11-K, Mettler-Toledo, Greifensee, Switzerland).

Liquid samples for analyses were taken from two sampling points located at (i) the sample port at the bottom of the anaerobic bioreactor, and (ii) the sample port of the lean solution from the aerated bioreactor.

A schematic overview of the dual reactor and the 1-bioreactor line-up is given in the Supporting Information (SI 5).

2.2. Experimental Operation. The system (i.e., H_2S absorber bottom, anaerobic bioreactor, aerated bioreactor and tubing) was filled with a mixture of bioreactor solutions from a full-scale system, described by (Roman et al., 2016).¹⁷ The N_2 flow to the H_2S absorber was started at 100 nL/h to pressurize the column to 3 bar(g). Subsequently, the lean solution flow to the top of the H_2S absorber was started at 8 kg/h. After stabilization of the solution temperature at 37 °C, the addition of carbon dioxide and hydrogen sulfide was initiated. The total H_2S load was kept constant at 4.78 molS/day and the CO_2 ranged from 6.43–53.54 mol/day. At the same time, the nutrient and caustic supply were started. The nutrients contained 2.86 mg L^{-1} nitrogen as urea, 2 mg L^{-1} potassium as KNO_3 , 0.65 mg L^{-1} P as H_2PO_4 and trace metals as described by (Pfennig and Lippert, 1966)¹⁸ and were supplied together with the makeup water. The nutrients are required for growth of the bacteria and the nutrient dosing rate was used to control the biomass concentration. Caustic soda (NaOH) is required to maintain the alkalinity, which is lost by SO_4^{2-} and $S_2O_3^{2-}$ formation and by the effluent streams. After 1 day operation, the sulfur discharge pump (P5) was started to continuously remove the sulfur slurry from the bottom of the aerated bioreactor.

Upon introduction of H_2S , the air supply rate to the aerated bioreactor was controlled by the ORP measurement in the aerated bioreactor, using a PID controller in the PLC.¹⁹ At start-up (i.e., first 5 days), the ORP setpoint was −325 mV. Then the setpoint was decreased to −365 mV, which was maintained through the rest of the experiment. The air supply was in the range of 40–100 nL/h throughout the complete experiment. The CO_2 supply was manually set to control the pH in the aerated bioreactor; at increasing CO_2 supply rates, the pH decreased. The measured pH values in the anaerobic bioreactor were 7.5–8.7, and 8.4–9.0 in the aerated bioreactor. The pH in the aerated bioreactor during the control experiment was between 8.53 and 8.93 and the ORP setpoint was −350 mV.

2.3. Analysis. The reagents were of analytical grade unless stated otherwise. All analyses were performed on samples taken from the aerated bioreactor unless stated otherwise. The liquid was circulated through all sections of the system (i.e., H_2S absorber, anaerobic bioreactor and aerated bioreactor) with a flow of 8 kg/h (streams 1, 2, and 3 in Figure 1). Hence, the HRT's in the different sections of the process were: 4 min in the absorber bottom, 44 min in the anaerobic bioreactor and 94 min in the aerated bioreactor. Therefore, the alkalinity and concentrations of SO_4^{2-} , $S_2O_3^{2-}$, S_8 and bacteria were equal throughout the complete system (which was verified by measurements) and the concentrations measured in samples of the aerated bioreactor were also representative for the liquid in the H_2S absorber and anaerobic bioreactor.

The composition of the treated gas of the H_2S absorber (sampled every 3 min) was analyzed for H_2S , CO_2 and N_2 , using a gas chromatograph (GC) (EnCal 3000, Honeywell, Morristown, NJ). The GC was equipped with a molsieve and a ppu column (both 10m), using helium as carrier gas. The

molsieve column was operated at a pressure of 200 kpa and 100 °C. The GC was calibrated weekly.

To determine the HS[−] removal in the anaerobic bioreactor, the total sulfide concentration (S_{tot}^{2-}), which is the sum of S^{2-} , HS[−] and polysulfide-sulfane (S_x^{2-}), was measured in a sample of the anaerobic reactor by titration with a solution of 0.1 M AgNO₃, using a Titrino Plus Titrator (Metrohm, Herisau, Switzerland). Before titration, the tested sample was filtered over a 0.45 μm cellulose acetate membrane filter to remove S₈ and bacteria. Two mL of filtered sample was added to 80 mL 4% (w/v) NaOH, with 1 mL of 30% (w/v) NH₄OH to stabilize S_{tot}^{2-} . A comparison between unfiltered and filtered samples did not show significant differences.

The specific HS[−] removal efficiency in the anaerobic bioreactor (mg S/mg N) was calculated based on the H₂S load, the liquid flows, the measured HS[−] concentration and the biomass concentration, according to eq 1.

$$\text{HS}^- \text{ removal} = \frac{\frac{H_2S \text{ load}}{\text{solution flow}} - [S_{\text{tot}}^{2-}]_{\text{meas}}}{X_b} \quad (1)$$

Here, H₂S load is the mass loading in the H₂S absorber (mg S h^{−1}), and the solution flow is the liquid flow to the anaerobic reactor (L h^{−1}). $[S_{\text{tot}}^{2-}]_{\text{meas}}$ is the total measured sulfide concentration (mg S L^{−1}) and X_b is the biomass concentration (mg N L^{−1}).

The biomass concentration was measured as the amount of total organic N using the Dr. Lange cuvette test LCK138 (Hach Lange, Germany). The difference between the supernatant (i.e., a sample centrifuged for 10 min at 14 000g) and a noncentrifuged sample indicated the total amount of N present in the biomass. It was confirmed that the presence of biologically produced S₈ did not affect the N analyses, provided that the samples were at least 5 times diluted. Considering the generic stoichiometric chemical equation for HA-SOB, that is, CH_{1.8}O_{0.5}N_{0.2},²⁰ the total N amount accounts for 10 mol % of the total dry weight biomass.

The specific conductivity of the samples was monitored using an offline conductivity sensor (LF 340, WTW, Weilheim, Germany). The alkalinity was measured with titration with 0.1 M HCL to pH 4.3, using a titrator (Titralab AT1000, Hach Lange, Germany). The method was verified with an analysis in accordance with WAC/III/A/006.

After removal of cells and S₈ by centrifugation for 10 min at 14 000g, the supernatant samples were analyzed for COD—in duplicate, (Lange cuvette test LCK514, Hach Lange Germany) at 605 nm, and using a spectrophotometer (Hach Lange, Germany)—to determine total dissolved S₂O₃^{2−}. In addition, the sample was analyzed for SO₄^{2−}, by using a Hach Lange cuvette test LCK353 and spectrophotometer at 800 nm. These methods were verified with an ion chromatography method, in accordance with ISO 10304-1.

The concentration of total suspended solids (TSS), consisting of mainly S₈, was analyzed in triplicate. A 5–15 mL sample (depending on the TSS concentration) was filtered over a predried (24h, 60 °C) and preweighed GF/C Glass microfiber filter (Whatman). After drying (60 °C for at least 24h), the filters were weighed again. The TSS was determined as the difference between the final weight and initial weight, divided by the sample weight.

As the formed S₈ particles have a tendency to attach to the reactor wall, it was not possible to calculate the S₈ production rate from the analyses. As no products other than S₈, SO₄^{2−}

and S₂O₃^{2−} were measured in the reactor,⁷ the production rate of S₈ could be calculated from the following mass balance:

$$P_{S_8} = I_{H_2S} - P_{SO_4^{2-}} - P_{S_2O_3^{2-}} \quad (2)$$

Here, P_{S_8} , $P_{SO_4^{2-}}$ and $P_{S_2O_3^{2-}}$ are the production rates of S₈, SO₄^{2−}, and S₂O₃^{2−}, respectively, in mol S-product day^{−1} and I_{H_2S} is the volumetric H₂S influent in mol S day^{−1}. The production rates of both SO₄^{2−} and S₂O₃^{2−} (not shown) are calculated as follows:

$$P_{SO_4^{2-}} = \frac{\text{effluent} \cdot [\overline{SO_4^{2-}}] + V \cdot \Delta[SO_4^{2-}]}{\Delta t} \quad (3)$$

The selectivities were calculated according to eq 4:

$$S_{SO_4^{2-}} = \frac{P_{SO_4^{2-}}}{I_{H_2S}} \quad (4)$$

Here, effluent is the total effluent of the system (L) in time interval Δt (days) (i.e., sample volumes, S₈ slurry, and bleed), $[\overline{SO_4^{2-}}]$ the average concentration (mol S L^{−1}) over time interval Δt, V the total liquid volume of the system (18.7 L) and Δ[SO₄^{2−}] the concentration changes (mol S L^{−1}) over time interval Δt. The mathematical equation to determine the caustic use and bleed flow of a full scale system based on the product selectivities can be found in the [Supporting Information \(SI 4\)](#).

The samples for microbial community analysis were conserved immediately after sampling by addition of ethanol up to 50% (v/v). DNA was extracted with the MPbio FastDNA SPIN Kit for Soil. Subsequently, PCR was used to amplify the V3 and V4 region of the 16S rRNA gene of bacteria giving a 400bp product. The library prep, sequencing and data analysis was performed via the 16S BioProphyler method,²¹ using the Illumina PE300 platform and MiSeq sequencer. Within the BioProphyler method all primer sequences and low quality reads were deleted from the raw data sets. The obtained sequences were compared with the online nt database with the aid of the BLAST algorithm. Low abundance reads were not removed from the data set and no correction on differences in library size was applied. The reported species name is the species which is most related to the detected sequence. The [Supporting Information](#) contains the accession numbers of the sequences with the respective number of reads. The EMBL-EBI accession number for presented 16S rRNA sequencing set is PRJEB31478.

3. RESULTS AND DISCUSSION

3.1. Operational Results. The tests in the new dual-bioreactor line-up were performed over a period of 111 days (i.e., 8.5 times HRT) and the control experiment was performed over a period of 57 days (i.e., 3 times HRT). An overview of the control experiments is shown in the [Supporting Information \(SI 3\)](#) and the performance of the new process line-up in the 111 days experimental run is shown in [Figure 2](#). During the control, the liquid composition was stable from day 35–56 (i.e., the last HRT). The data during this period were used to determine the performance of the 1-bioreactor line-up. The alkalinity and conductivity during this period were 0.38 ± 0.12 M and 74.0 ± 1.8 mS/cm. The SO₄^{2−}-S and S₂O₃^{2−}-S concentrations were 0.56 ± 0.06 M and 0.32 ± 0.02 M respectively. The selectivity for S₈ formation in

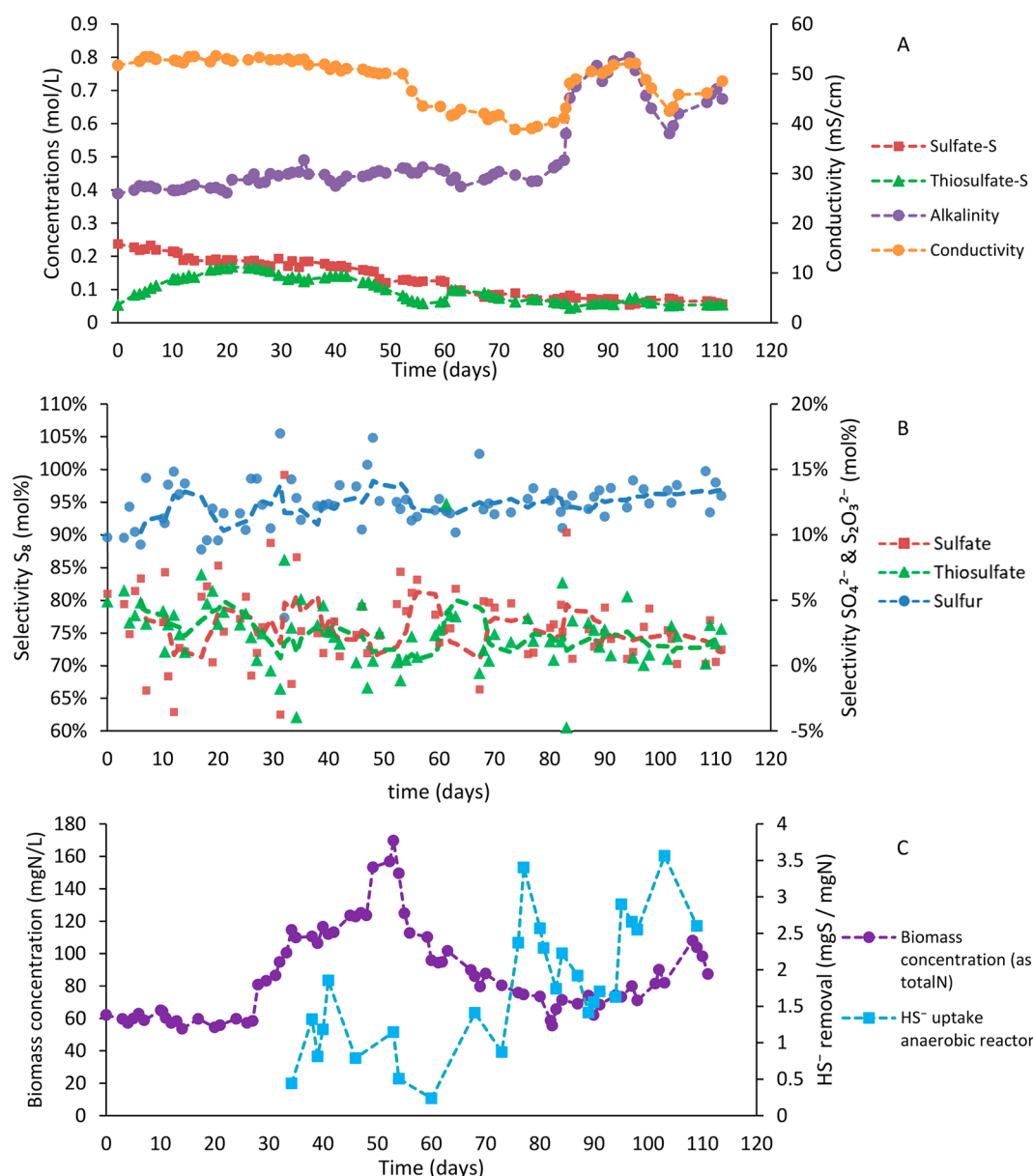


Figure 2. Results of the continuous experiment with anaerobic bioreactor. Panel A shows the measured concentrations of SO_4^{2-} and $\text{S}_2\text{O}_3^{2-}$, the measured conductivity (a measure for the total salinity) and the measured alkalinity in the effluent of the aerated bioreactor. In Panel B the calculated product selectivities are shown. The markers indicate the calculated daily analyses and dotted lines the moving averages (based on a 5-day period). Panel C displays the measured concentrations of biomass (as total N) and specific sulfide uptake (HS^- and polysulfide) by the biomass in the anaerobic bioreactor.

the control experiment was 75.6 ± 1.3 mol %; selectivities for SO_4^{2-} and $\text{S}_2\text{O}_3^{2-}$ were 14.9 ± 0.2 and 9.5 ± 1.5 mol %. These product selectivities are similar as described in literature for systems without anaerobic bioreactor.^{5–7}

Figure 2A shows the concentrations of the dominant anions in process solution of the aerated bioreactor of the new-line up experiment. After inoculation, the medium contained 0.15 mol-S as SO_4^{2-} and 0.033 mol-S as $\text{S}_2\text{O}_3^{2-}$. From the start of the run, the SO_4^{2-} levels in the process liquid decreased up to day 80, which is an indication that the rate of SO_4^{2-} formation was lower than its removal via the bleed stream. From day 80 onward, the SO_4^{2-} concentration was stable at 0.07 ± 0.01 mol-S L^{-1} . For $\text{S}_2\text{O}_3^{2-}$, a different pattern was observed; during the first 24 days of operation, the levels of $\text{S}_2\text{O}_3^{2-}$ increased, indicating chemical oxidation of HS^- took place. This could be

due to limited biological activity at start-up, which is often observed in biological desulfurization systems. Thereafter, up to day 60, a decrease was observed, and from day 80 onward the $\text{S}_2\text{O}_3^{2-}$ concentration stabilized at 0.57 ± 0.01 mol-S L^{-1} .

The measured conductivity and alkalinity at the start were 51.7 mS/cm and 0.4 M respectively. The conductivity was controlled via the caustic dosing, and is dependent on the different salts in solution (Na_2SO_4 , $\text{Na}_2\text{S}_2\text{O}_3$, NaHCO_3 and Na_2CO_3). Around day 50, both SO_4^{2-} and $\text{S}_2\text{O}_3^{2-}$ concentrations decreased quickly, which caused a decrease in conductivity as well. To restore the conductivity to ~50 mS/cm, the caustic dosing was increased. This caused a rapid increase in alkalinity from 0.45 M to 0.80 M. The alkalinity (total concentration of NaHCO_3 and Na_2CO_3 and expressed as concentration NaHCO_3) is important for efficient H_2S

removal in the H_2S absorber and has to be in the range of 0.30–0.90 M. The main reason for the difference in conductivity between the control and the experiment with anaerobic reactor is the higher sulfate concentration in the control experiment.

The average H_2S removal efficiency in the H_2S absorber, calculated as $1 - ([\text{H}_2\text{S}_{\text{out}}]/[\text{H}_2\text{S}_{\text{in}}])$, was $99.75\% \pm 0.83\%$. This means that the H_2S absorber efficiently removed H_2S from the fed sour gas, that is, only a negligible amount of H_2S was not absorbed. During the entire experimental run, no dissolved HS^- (or S_x^{2-}) was detected in the aerated bioreactor, meaning that all H_2S fed to the aerated bioreactor was converted. Furthermore, all dissolved O_2 concentration measurements in the aerated bioreactor indicated values below detection limit of 1 ppb, meaning that the environment in the aerated bioreactor was microaerophilic.

Figure 2B shows the calculated selectivities for SO_4^{2-} , $\text{S}_2\text{O}_3^{2-}$ and S_8 formation from H_2S . These calculations are based on concentrations of SO_4^{2-} and $\text{S}_2\text{O}_3^{2-}$ in the effluent of the aerated bioreactor and on the bleed and sulfur slurry flows as indicated by eqs 2, 3, and 4. As the calculated numbers are based on a sum of measurements with corresponding variance, the reported numbers scatter significantly up to day 60 of the experimental run. Therefore, the moving averages (dotted lines) are shown as well, which is the average value of five consecutive calculated selectivities.

In the first week of operation, the selectivity for S_8 formation was around 90 mol % (based on a 5-day average), with selectivity for SO_4^{2-} and $\text{S}_2\text{O}_3^{2-}$ formation around 5 mol %. In the first 60 days of operation, large fluctuations in calculated selectivity were observed. In this period, the moving average varied between 90.5 and 98.3 mol % for S_8 formation (with average $94.2 \pm 4.7\%$); average selectivities for SO_4^{2-} and $\text{S}_2\text{O}_3^{2-}$ formation were $3.2 \pm 3.7\%$ and $2.6 \pm 2.4\%$. While the experiment proceeded, fluctuations in the calculated selectivities decreased and a new steady state was reached. The calculated selectivity for S_8 formation (5-day average) increased to 96.9 mol % and the calculated selectivities for SO_4^{2-} and $\text{S}_2\text{O}_3^{2-}$ were 1.1% and 2.0 mol % respectively by the end of the run (day 111).

It can be noticed that the calculated product selectivities show large fluctuations at the beginning of the experiment. The fluctuations decreased as the experiment progressed. From day 70 onward, no fluctuations were observed anymore; instead, a gradual increase was found for the selectivity for S_8 formation. An explanation can be found in the change of the microbiological community. The establishment of a new biological equilibrium is a relatively slow process and might explain the pattern in the process selectivity calculations. The microbiological composition will be discussed in the next section.

The initial biomass concentration was 60 mg N L^{-1} (see Figure 2C). During operation it was found to be difficult to obtain a stable concentration of bacteria in the system. Some bacteria adhered to the produced S_8 particles and were therefore removed with the sulfur slurry/bleed. For example, it was found that a liquid sample taken from the aerated bioreactor contained $54 \pm 1.0 \text{ mg N L}^{-1}$ ($\text{TSS} = 2.0 \pm 0.3 \text{ g L}^{-1}$) while the sulfur slurry contained around $300 \pm 50 \text{ mg N L}^{-1}$ ($\text{TSS} = 59 \pm 0.5 \text{ g L}^{-1}$). The adhesion of biomass to S_8 fluctuated over time, which could be due to variation in pH.^{22,23} Moreover, the fluctuation in biomass concentration was related to some variation in the bleed flow. Hence, the

concentration of bacteria in the system fluctuated between 55.5 and 108 mg N L^{-1} . The biomass concentration in full-scale systems is typically in the range of 50–150 mg N L^{-1} , dependent on the specific conditions in the system.

The total HS^- concentration in the effluent stream of the anaerobic bioreactor (stream 3) was measured on a regular basis. In the first 33 days of the experiment, the HS^- concentration was analyzed using a spectrophotometric method based on methylene blue. This method was found to be inconvenient for the HS^- concentrations in this experiment. Due to the low measurement range of this method, the measured values showed large deviations. Therefore, these results are not shown in Figure 2C. From day 34 onward, a potentiometric titration with AgNO_3 was used to measure the HS^- concentrations, as described in the materials and methods. The measured HS^- concentration was consistently lower than the calculated values, assuming that no oxidation of dissolved total HS^- occurred in the H_2S absorber. In the anaerobic bioreactor, 9 to 54% of the total absorbed dissolved HS^- was removed. If this decrease was caused by chemical oxidation of HS^- to $\text{S}_2\text{O}_3^{2-}$, selectivity toward biological S_8 formation could not become >90%, as S_8 cannot be formed chemically under the described operational conditions, that is, in the absence of oxygen.²⁴ Therefore, the SOB seem to remove HS^- in the anaerobic reactor, that is, in the absence of oxygen. Based on the difference between calculated and measured HS^- concentrations, the specific HS^- uptake in the anaerobic bioreactor was calculated (i.e., mg HS^- per mg N of biomass (see Figure 2C). The specific HS^- uptake in the anaerobic bioreactor was between 0.24 and 3.4 mg S/mg N , whereas ter Heijne¹⁶ observed an HS^- uptake of 0.22 mg S/mg N under anaerobic conditions in a batch experiment. The HS^- uptake will be further discussed in Section 3.3.

An overview of performance of the 1-bioreactor line-up and the new dual bioreactor line-up is shown in Table 2. Based on the results, we conclude that two main reasons can be hypothesized for the increased selectivity for sulfur formation: (i) the reduction of biologically mediated formation of SO_4^{2-} (ii) the reduction of HS^- in the anaerobic reactor, resulting in a decreased chemical formation of $\text{S}_2\text{O}_3^{2-}$ in the aerobic reactor. In the next sections, both hypotheses will be discussed in more detail.

3.2. Reduction of Sulfate Formation. The initial selectivity for SO_4^{2-} formation (average of first 5 days of operation) in the new process line up was $5.0 \pm 1.6 \text{ mol } \%$, which is considerably lower than in the control experiment without anaerobic reactor ($14.9 \pm 0.2 \text{ mol } \%$). This short-term effect cannot be explained by population dynamics or sudden changes in operation conditions of the aerated bioreactor. It is more likely that this effect is caused by instantaneous inhibition of enzymatic routes, which results in a decrease of the formation rate of SO_4^{2-} . As SO_4^{2-} formation is a biotic reaction and as such the microbial community could be influenced by this inhibition, the composition of the bacterial community was analyzed. The composition of the bacterial community was analyzed at day 3, day 19 and day 73 (see Figure 3).

The initial microbial community composition (day 3) showed that the population was dominated by *Tv. sulfidiphilus*, with a relative abundance of 54%, see Figure 3. This is in line with observations of Sorokin et al.^{9,10} This composition mostly resembled the microbial community found at the biogas desulfurization facility in Eerbeek (Industriewater Eerbeek

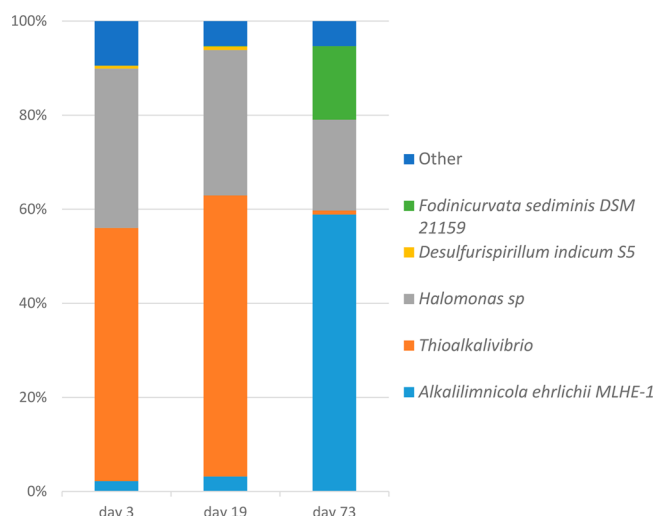


Figure 3. Mapping of bacterial diversity in the system by 16S rRNA gene amplicon sequencing. Samples were obtained from the aerated bioreactor. The analysis was performed with the 16S BioProphyler method,²¹ using the Illumina MiSeq sequencer. The obtained sequences were compared with an online database, with the aid of the BLAST algorithm. The reported species name is the species most related to the detected sequence. The inoculum was obtained from a biodesulfurization system without anaerobic bioreactor.

B.V.)^{10,17} and microbiological community of the seed SOB for the control experiment, both without anaerobic bioreactor (no NGS data were obtained throughout the control experiment). Due to the presence of *Fcc*, *rDSR*, and *CcO* genes in the analysis of the genome of *Tv. sulfidophilus*,^{10,11} the high relative abundance presence of *Tv. sulfidophilus* at day 3 indicates that the system has a potential for SO_4^{2-} formation.

On day 19 (after approximately 1.5 the total system's HRT), the community was more or less similar to the community at day 3, indicating no major influence on microbial community composition of the anaerobic bioreactor in the process line-up. From day 19 to day 73 (after approximately 5.5 x HRT) a clear shift occurred in the microbiological community. The abundance of *Tv. sulfidophilus* was reduced to less than 1% and the community became dominated by (16S rRNA gene sequences related to) *Alkalilimnicola ehrlichii* MLHE-1 (relative abundance of 59%), which is a haloalkaliphilic member of the family *Ectothiorhodospiraceae* (*Gammaproteobacteria*).²⁵ The representatives of two closely related genera—*Alkalilimnicola* and *Alkalispirillum*—are haloalkaliphilic facultative chemolithoautotrophs, with the ability to grow by either aerobic or nitrate-dependent oxidation of HS^- . As the N-source was urea, growth of *Alkalilimnicola* and *Alkalispirillum* can only be explained by oxidation of HS^- using O_2 as final electron acceptor. As it was shown that these facultative SOB species oxidize HS^- exclusively to the state of elemental sulfur,^{26,27} the SO_4^{2-} formation potential of the overall microbiological population present in the process solution was decreased.

In the conventional 1-bioreactor biodesulfurization process, it is expected that *Tv. sulfidophilus* will be dominating.⁹ However, for the dual bioreactor line-up we postulate that due to the suppression of the *CcO* by the elevated levels of HS^- in the anaerobic bioreactor, growth of *Tv. sulfidophilus* is suppressed. In contrast to *Tv. sulfidophilus*, *Alkalilimnicola* expresses one or more genes for the membrane-bound sulfide-

quinone reductase (SQR), which is another well-known enzyme associated with HS^- oxidation.^{26,28} Since HS^- oxidation by SQR does not depend on the activity of *CcO*, *Alkalilimnicola* has a new niche to grow. This needs to be further confirmed by genomic and enzymological analyses in future work.

By day 73 the SO_4^{2-} formation was further decreased to ~2 mol %. This reduction might be explained by the shift in community composition, as the S_8 -forming *Alkalilimnicola* became dominant over the SO_4^{2-} -forming *Tv. sulfidophilus*. In the dual-bioreactor process line up 90.7 ± 2.8 mol % of all fed HS^- was oxidized to S_8 on day 19. This continuously increased: 95.5 ± 1.4 mol % on day 73 and 96.9 ± 2.4 mol % on day 111, see Figure 2B. While formation of S_8 increased, SO_4^{2-} formation decreased to 1.4 ± 1.3 mol % at the end of the experiment. Hence, these results support the hypothesis that introducing an anaerobic reactor in the line-up suppresses the formation of SO_4^{2-} .

From the results we conclude that there is a short-term and a long-term effect of the dual-reactor line-up on SO_4^{2-} formation. On short-term, SO_4^{2-} formation is lower compared to the 1-bioreactor line-up (5.0 ± 1.6 mol % vs 14.9 ± 0.2 mol %) due to instantaneous inhibition of the SO_4^{2-} forming enzymatic routes.

On the longer term, the microbial community showed a decrease in the potential to form SO_4^{2-} due to the dominance of *Alkalilimnicola*, which is limited to S_8 formation. This resulted in a further decrease of selectivity for SO_4^{2-} formation to 1.4 ± 1.3 mol %.

3.3. Reduction of Thiosulfate formation. While the nature of SO_4^{2-} formation is biological, $\text{S}_2\text{O}_3^{2-}$ is formed chemically when both dissolved oxygen and HS^- and/or S_x^{2-} are present in the process solution. When comparing the control experiment (1-bioreactor line-up) with the start-up of the dual bioreactor line-up, the dual-bioreactor line-up showed a lower $\text{S}_2\text{O}_3^{2-}$ formation (4.5 ± 1.0 mol %) than the 1-bioreactor line-up (9.5 ± 1.5 mol %).

From day 34 onward, the HS^- levels in the anaerobic reactor effluent were analyzed and found to be significantly lower than expected based on the mass balance of gas and liquid flows, as discussed in Section 3.1. For example, on day 34, the HS^- concentration was expected to be 19.6 mM based on the mass balance of gas and liquid flows. However, a HS^- concentration of 18.4 mM was measured. Ter Heijne et al.¹⁶ showed that SOB remove HS^- from solution in the absence of oxygen in a batch experiment. The results obtained in this study indicate that the SOB also remove HS^- in the anaerobic reactor (i.e., in the absence of oxygen) in a continuous reactor system. At day 34, the calculated HS^- uptake was 0.40 mg S/mg N, which is slightly higher than measured by ter Heijne et al, i.e. 0.22 mg S/mg N.¹⁶ Over the period of day 34 up to day 72, an average uptake rate of 1.1 mg S/mg N was found.

After day 72, an increase in removal efficiency of HS^- was observed (Figure 2C). The increase in HS^- uptake over time indicates that the bacterial population acquires the ability of (higher) HS^- uptake under anaerobic conditions. While at day 34, 9% of all the fed sulfide was removed, at day 111 54% of all fed HS^- was removed from the process solution (i.e., a HS^- concentration of 9.3 mM had been measured, while the mass balance dictates 20.6 mM). As a result of the lower HS^- levels, chemical oxidation of HS^- in the aerobic reactor are reduced. While the average $\text{S}_2\text{O}_3^{2-}$ selectivity over the first 5 days was 4.5 ± 1.0 mol %, in the last 5 days of the experiment a

selectivity for $\text{S}_2\text{O}_3^{2-}$ formation of 2.0 ± 1.2 mol % was found. Since the influent of the aerated bioreactor contains 50% less HS^- , also selectivity of $\text{S}_2\text{O}_3^{2-}$ formation decreased with 50%.

Yet, the mechanisms behind the HS^- uptake under anaerobic conditions are not very well understood. It has been shown that H_2S can be taken up by bacteria as it can freely diffuse over a cell membrane into the periplasm.^{29,30} In the periplasm the H_2S or HS^- can either react with internally stored sulfur particles forming polysulfide or it can undergo biological conversion.²⁴ For SQR enzymes, which became the dominant HS^- oxidizing enzyme system during the experiment, it has been shown that it can bind up to four S-atoms without the presence of oxygen.³¹ Furthermore, oxidized electron carriers, such as quinones and cytochromes, might be used as (intermediate) electron acceptor, enabling conversion of (part of) the HS^- by SQR to elemental sulfur.³¹

The reduction of $\text{S}_2\text{O}_3^{2-}$ formation in the dual-bioreactor line-up is the result of biological HS^- uptake in the anaerobic bioreactor. At the start of the experiment, only 10% of HS^- was taken up in the anaerobic bioreactor and the selectivity for $\text{S}_2\text{O}_3^{2-}$ was 4.5 ± 1.0 mol %. As the microbial population developed over time, HS^- uptake eventually increased to over 50% of the dosed H_2S . This resulted in a decrease in selectivity for $\text{S}_2\text{O}_3^{2-}$ formation of 2.0 ± 1.2 mol % at the end of the experiment.

3.4. Implications for Full-Scale Systems. We show in this work that incorporation of an anaerobic bioreactor in the line-up of the biological gas desulfurization process under haloalkaline conditions improves the selectivity for sulfur formation due to (i) the reduction of biological SO_4^{2-} formation by suppression of SO_4^{2-} forming enzymatic route and change of the microbial community composition and (ii) reduction of chemical HS^- oxidation to $\text{S}_2\text{O}_3^{2-}$, which is the result of HS^- uptake in the anaerobic reactor, thereby lowering the HS^- concentration in the rich solution to the aerated bioreactor.

Two main factors that determine the operational costs of full-scale biodesulfurization systems are consumption of caustic and production of the bleed stream. Both are associated with the production of SO_4^{2-} and $\text{S}_2\text{O}_3^{2-}$. The selectivity for S_8 formation in the 1-bioreactor line-up (H_2S absorber and aerated bioreactor) was 75.6 ± 1.3 mol %. Selectivities for SO_4^{2-} and $\text{S}_2\text{O}_3^{2-}$ formation were 14.9 ± 0.2 and 9.5 ± 1.5 mol %. The results presented in this paper show that the introduction of an anaerobic bioreactor between the H_2S absorber and the aerated bioreactor reduces the selectivity for SO_4^{2-} formation to 1.4 ± 1.3 mol % and for $\text{S}_2\text{O}_3^{2-}$ to 2.0 ± 1.2 mol %.

In full scale facilities, sulfur is usually removed by a decanter centrifuge, resulting in the formation of a sulfur cake of 60–70 weight% S. Therefore, the bleed of a full scale system is dictated by the formation of the production of SO_4^{2-} and $\text{S}_2\text{O}_3^{2-}$. Based on the product selectivities of both experiments (with and without anaerobic reactor) the specific caustic consumption and bleed formation were calculated (Table 2). Detailed calculation is provided in the Supporting Information (SI 4).

The specific caustic consumption for the 1-bioreactor line-up is 1.25 kg NaOH/kg S and the bleed formation 24.1 L/kg S. With the new dual bioreactor line-up, the caustic consumption and bleed formation are 0.1 kg NaOH/kg S and 2.4 L/kg S. This means a reduction of 90% for both caustic use and bleed stream formation. These results show that the new dual-

Table 2. Comparison of the Conventional 1-Bioreactor and the New Dual-Bioreactor Line-up Biological Desulfurization Processes, In Terms of Product Selectivity, Caustic Use, and Bleed Stream Formation^a

	conventional process (1-bioreactor line-up)	new process (dual- bioreactor line-up)
selectivity for S_8 formation (%)	75.6	96.6
selectivity for SO_4^{2-} formation (%)	14.9	1.4
selectivity for $\text{S}_2\text{O}_3^{2-}$ formation (%)	9.5	2.0
caustic use (kg/kgS)	1.25	0.12
bleed (L/kgS)	24.1	2.4

^aProduct selectivities are based on the average performance during the last HRT in both experiments. Based on these product selectivities, the caustic use and bleed stream formation for full-scale systems have been calculated, assuming an alkalinity of 0.8M, a total sodium concentration of 1.3M, and 40% water content in the sulfur cake.

bioreactor line-up strongly improves the competitive edge of biological desulfurization processes. For industrial applications, there is a trade-off between cost savings for caustic consumption and bleed water production, and costs for an additional bioreactor. Especially at higher sulfur loads, the dual reactor system will become economically favorable. In addition, other site specific conditions, such as logistics for supplies and disposal, have to be taken into account.

■ ASSOCIATED CONTENT

§ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.8b06749.

- (1) Detailed information on experimental setup (H_2S absorber, liquid flows, pumps and pump controls),
- (2) Detailed information on NGS results (accession numbers of the sequences with the respective number of reads),
- (3) Results control experiment without anaerobic bioreactor,
- (4) Calculations caustic use and bleed flow based on product selectivities,
- (5) Scheme old and new process line-up (PDF)

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Notes

The authors declare no competing financial interest.

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