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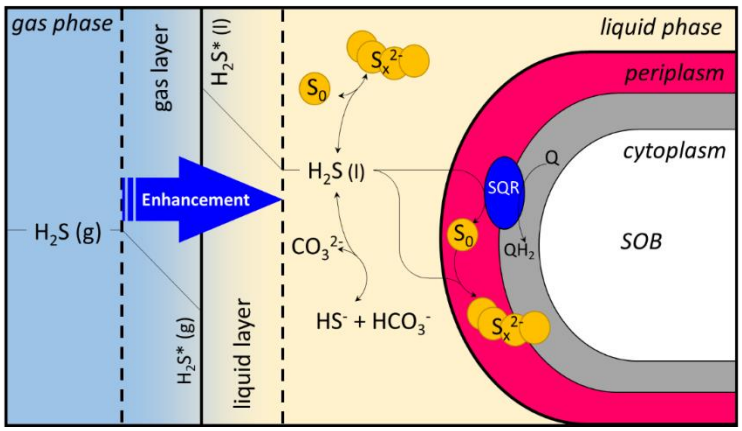

Abstract

We studied a biotechnological desulfurization process for removal of toxic hydrogen sulfide (H_2S) from sour gas. The process consists of two steps: i) Selective absorption of H_2S into a (bi)carbonate solution in the absorber column and ii) conversion of sulfide to sulfur by sulfide oxidizing bacteria (SOB) in the aerated bioreactor. In previous studies, several physico-chemical factors were assessed to explain the observed enhancement of H_2S absorption in the absorber, but a full explanation was not provided. We investigated the relation between the metabolic activity of SOB and the enhancement factor. Two continuous experiments on pilot-scale were performed to determine H_2S absorption efficiencies at different temperatures and biomass concentrations. The absorption efficiency improved at increasing temperatures, i.e. H_2S concentration in the treated gas decreased from 715±265 ppmv at 25.4°C to 69±25 ppmv at 39.4°C. The opposite trend is expected when H_2S absorption is solely determined by physico-chemical factors. Furthermore, increasing biomass concentrations to the absorber also resulted in decreased H_2S concentrations in the treated gas, from approximately 6000 ppmv without biomass to 1664±126 ppmv at 44 mgN/l. From our studies it can be concluded that SOB activity enhances H_2S absorption and leads to increased H_2S removal efficiencies in biotechnological gas desulfurization.

Key words: gas desulfurization, sulfide oxidizing bacteria (SOB), H_2S absorption, biological enhancement factor

Highlights:

- Absorption of H_2S from sour gas in the biodesulfurization process appears not to be purely physico-chemical
- Sulfide oxidizing bacteria enhance the absorption of H_2S from sour gas
- Biologically enhanced H_2S absorption is dependent on temperature and biomass concentration
- SOB are also active in the anaerobic absorber of the biodesulfurization process

1 Introduction

Gas streams, such as natural gas, biogas and several refinery gases arising from processing crude oil and gas, may contain toxic and corrosive hydrogen sulfide gas (H_2S). Therefore, these sour gas streams require treatment before combustion. In the 1990's, a biotechnological gas desulfurization process has been developed as an environmentally friendly and cost effective alternative to conventionally applied chemical-physical desulfurization processes, such amine-Claus, scavengers and liquid iron based technologies [1]. Advantages of the biological process are (i) operation at ambient temperatures and pressures, (ii) no requirement of toxic and expensive chemicals and (iii) formation of re-usable sulfur [2]. The process is widely applied in industry with over 250 installation worldwide in 2017 [3]. To further develop the process, a better understanding of the absorption step is required.

Removal of H_2S gas is achieved in an absorber column by counter-current contact with process solution, see figure 1. For effective removal of H_2S, the process solution is a haloalkaline (high salt, high pH) sodium (bi)carbonate solution of approximately 1M Na^+ and a pH of 7.5-9.0. In the absorber, H_2S gas is dissolved in the solution as bisulfide (HS^-) and polysulfide (S_x^{2-}). The sulfide-rich solution is then routed to a bioreactor. By the controlled addition of air to this bioreactor, all dissolved sulfide is oxidized, mainly to elemental sulfur by sulfide oxidizing bacteria (SOB). The biological produced sulfur has an oxidation state of zero and therefore it is denoted as S^0. In the bulk of the solution, the elemental sulfur mainly exists in the form of rings of 8 S-atoms (S_8) [4]. S^0 formation is preferred over sulfate and thiosulfate formation to minimize caustic use and bleed stream formation. Process solution from the bioreactor, which is recycled to the absorber, contains sodium (bi)carbonate, sulfur particles, SOB and sodium (thio)sulfate. A small stream of the bioreactor solution is routed to the sulfur separation section, which typically consists of a settler and/or decanter centrifuge.

The biological and chemical conversion reactions under haloalkaline, microaerophilic conditions (i.e. the processes occurring in the bioreactor) have been studied extensively to optimize the conversion efficiency

towards S_8 formation [5-13]. The role of microbial activity on the absorption step of the process has never been elucidated, because it is unlikely that SOB, using O_2 as final electron acceptor for sulfide oxidation, are active under the strictly anaerobic conditions that prevail in the absorber column. However, a recent study shows that in a batch experiment SOB are capable of removing HS^- (6.9 µmol/mgN) from solution in the absence of oxygen in approximately 5 minutes [14]. The removal of sulfide by SOB under anaerobic conditions was also observed in a continuously operated biodesulfurization system [8]. If SOB would remove HS^- from the solution in the absorber, this would increase the mass transfer rate of H_2S and thus SOB would contribute to the absorption of H_2S.

In general, the transfer of a compound from the gaseous to the liquid phase occurs when a driving force exists, i.e. an activity difference at the interphase of the gas and liquid phase. According to Fick's law of diffusion, a components mass transfer rate depends on its concentration gradient in the liquid film of the gas-liquid interface, which is often the rate limiting step. A chemical reaction of the respective component in the liquid film, such as protonation, dissociation or conversion, increases the overall mass transfer rate. The increase of mass transfer by a chemical reaction is called 'enhancement' and is quantified by the enhancement factor *E*. Quantification of enhancement factors is not straightforward as it requires knowledge of the reaction paths and rates, which in turn depend on e.g. temperature, irreversible and reversible kinetics, stoichiometry and product diffusion coefficients and concentrations [15].

A number of mechanisms that enhance H_2S absorption (i.e. increase the rate of mass transfer) in biological gas desulfurization are known. After the H_2S has dissolved in the liquid (equation 1), the first enhancement mechanism is the reaction of H_2S with the alkaline process solution, forming HS^- (equation 2) [16]. Under the conditions in the process (i.e. pH in the range of 7.5-9.0 and alkalinity in the range of 0.4-0.9M), the main alkaline component is bicarbonate (HCO_3^-), which is in equilibrium with carbonate (CO_3^{2-}) and hydroxide (OH^-) ions, according to equation 3. Due to the buffer system, the pH is relatively constant. At higher CO_2 and H_2S pressures in the sour gas, a higher buffer capacity (i.e. alkalinity) may be required.

H_2S (g) ⇋ H_2S (aq) eq. 1

H_2S (aq) + OH^- ⇋ HS^- + H^+ (aq) eq. 2

CO_3^{2-} + H_2O ⇋ HCO_3^- + OH^- eq. 3

Equation 2 is referred to as the 'homogeneous reaction'. Hence, the enhancement of H_2S absorption can be increased by increasing the (bi)carbonate concentration (the alkalinity) and the pH of the process solution.

A second mechanism is the autocatalytic reaction of dissolved sulfides with S^0 particles, forming a range of polysulfide species (S_x^{2-}, where $2 \leq x \leq 9$), according to equation 4 [16, 17]. This reaction is referred to as a 'heterogeneous reaction' [16].

HS^- (aq) + (x-1) S^0 (s) ⇋ S_x^{2-} (aq) + H^+ (aq) eq. 4

As for the homogeneous reaction, a higher S^0 concentration results in a higher enhancement factor. However, S^0 is in excess at relatively low concentrations (i.e. 0.03 g/L at an HS^- concentration of 1mM). At excess S^0 concentrations, the maximum enhancement is reached [16]. Kleinjan et al. concluded that enhancement of H_2S however cannot be explained by heterogeneous reaction alone and their findings suggest the existence of an additional factor contributing to the enhanced H_2S absorption [16]. Since it was shown that SOB can remove sulfide from solution under anaerobic conditions [8, 14], our hypothesis is that the metabolic activity of SOB is involved in the absorption of H_2S from sour gas.

In this study, we investigated the relation between the metabolic activity of SOB and H_2S absorption efficiencies from the gas phase into the liquid phase in the absorber column of the biological desulfurization process under haloalkaline conditions. As microbial respiration depends on temperature, and the process operates at mesophilic conditions, firstly the relation between temperature (25°C - 60°C) and sulfide-oxidizing activity of the SOB was investigated. Secondly, the effect of the SOB activity on H_2S

absorption was assessed in a pilot-scale biodesulfurization plant, via (i) varying the temperature of the solution (containing SOB) to the absorber column, and (ii) varying biomass concentration to the solution of the absorber column.

2 Materials and methods

2.1 Biomass respiration tests

To determine the effect of temperature on the respiration activity of SOB, dedicated respiration tests were performed as described by Klok et al. [10, 18]. In these tests, the SOB activity was measured as the oxygen consumption rate upon injection of sulfide. The experiments were performed in 20 mL temperature controlled glass reactors, equipped with magnetic stirrer. The reactors were closed and supplied with a dissolved oxygen (DO) sensor (PSt3, PreSens Precision Sensing GmbH, Regensburg, Germany). First, SOB taken from a lab-scale biodesulfurization reactor [18] were suspended in a buffer solution (containing 0.66M $NaHCO_3$ and 1.34M $KHCO_3$) to a biomass concentration of 2.5 mg N/l. The SOB suspension was aerated for at least 5 minutes until the solution was saturated with oxygen. Subsequently, 20 µL of sulfide stock solution (Na_2S) was injected, resulting in a sulfide concentration of 0.2 mM. Previous research demonstrated that SOB show the highest activity at this sulfide concentration [10]. The decrease in DO concentration was measured with time intervals of 5 seconds. The slope of the oxygen concentration of the first minute after the injection of sulfide, was used as a measure of the oxidation rate. Sulfide oxidation rates were determined for temperatures between 25°C and 60°C, in at least triplicate for each temperature. The time between the addition of SOB and sulfide to the temperature controlled buffer solution varied (ranging from 5 to 30 minutes). Measurements without SOB were performed in the same buffer solution to determine the chemical oxidation rate of sulfide.

2.2 H_2S absorption experiments

To investigate how SOB activity influences H_2S absorption in the absorber of the biological desulfurization process, experiments in a pilot-scale biodesulfurization installation were performed. In this continuously operated installation, the efficiency of the conversion of sulfide to S^0 was studied [8]. For that study, the

pilot was in stable operation for 111 days. Directly after this period, experiments were conducted to assess the effect of SOB activity on H_2S absorption efficiency. This was done by: (i) by varying the temperature of the solution to the absorber, and (ii) by varying the biomass concentration in the solution to the absorber.

2.2.1. Experimental set-up

The pilot-scale biodesulfurization installation consisted of an H_2S absorber, an anaerobic bioreactor (volume of 5.3 l) and an aerated bioreactor (volume 11.4 l) (Figure 1). The H_2S absorber was built of a stainless steel column with an inner diameter of 5 cm and a height of 4 m, containing 2 meter of packed bed. In the column gas and liquid were contacted in counter current mode. The packed bed consisted of glass spheres with a diameter of 1.2cm, resulting in a bed porosity of 43% [19]. Hence, the free volume in the packed bed was 1.7L. Each meter of bed was supported by a perforated stainless steel plate, which acted as liquid redistribution plate in order to minimize wall effects. A synthetic sour gas (stream A in figure 1), consisting of 4.45 vol% H_2S, 50 vol% CO_2 and 45.55 vol% N_2, was fed to the absorber column with a flow rate of 100 Nl/h, using mass flow controllers (Profibus, Brooks instrument, Hatfield, USA). Hence, the total H_2S load was 4.8 mol/day and the CO_2 load was 53.5 mol/day. The synthetic sour gas mostly resembles amine acid gas, but its H_2S/CO_2 ratio is also representative for a biogas or natural gas stream.

During the temperature experiment, the excess liquid from the aerobic bioreactor was directed to a bleed vessel (stream 5) via an overflow weir. For the biomass experiment, the auxiliary vessel (5 L) was included in the set-up (see section 2.2.3). The temperature of the process solution was controlled by warm water from a thermostat bath (Kobold, Germany), which was routed through the water jackets of the anaerobic bioreactor, the aerated bioreactor and the auxiliary vessel (during the biomass experiment). The temperature of the solution was measured in the aerated bioreactor (T 1 in figure 1) and at the inlet of

the absorber (T 2). The lean solution line and the absorber column were covered with insulating material to maintain a constant temperature. Further details of the set-up are provided in the Supporting Information (SI 1).

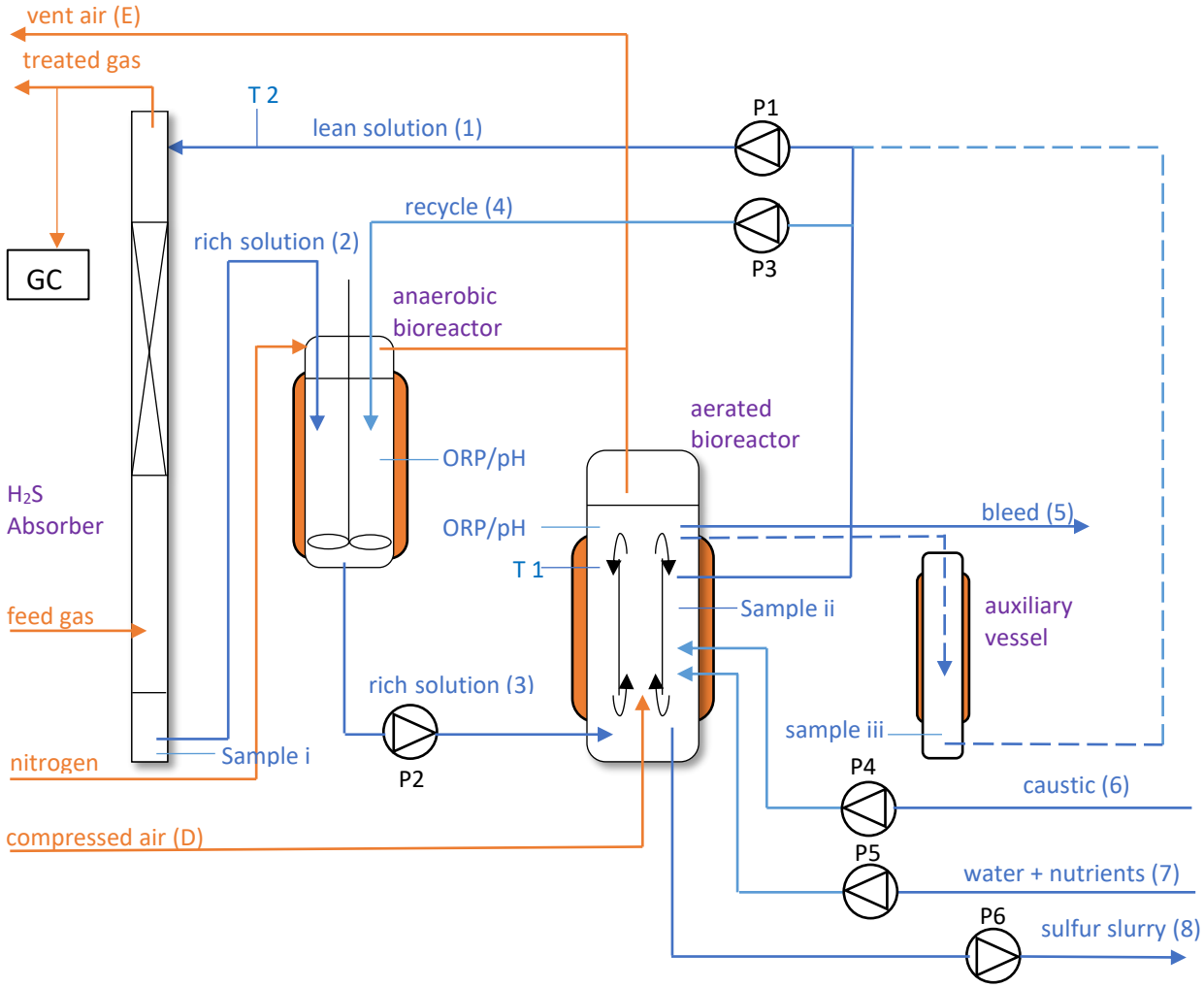

Figure 1: Flow scheme of the experimental set-up. The dashed lines apply to the biomass experiment. During the temperature experiment the auxiliary vessel was not part of the set-up.

2.2.2. Effect of temperature

The effect of temperature on the H_2S absorption efficiency was assessed during continuous operation of the system by varying the temperature stepwise. In this experiment, the lean solution ((bi)carbonate solution, containing SOB and sulfur particles) to the absorber was taken from the aerated bioreactor and the auxiliary vessel was bypassed. The feed gas flow was started after stabilization of the lean solution flow (stream 1) (10 kg/h), temperature (41.2°C in the aerated bioreactor and 37.9 °C at the absorber inlet), and pressure (3.1 bar(g) in the absorber). Then, the temperature (measured at the absorber inlet) was decreased from 38.1 to 25.4°C in four steps of 2-4 °C, by decreasing the temperature setpoint of the water bath. Each temperature setting was maintained for at least 2.5 hours. The treated gas composition was analyzed every 4 minutes, meaning that at least 37 measurements of the treated gas composition were taken during each temperature step. In the last step, the temperature was increased from 25.4 to 39.4°C to determine whether the effect on H_2S absorption is reversible in this temperature range. The standard deviation on the temperature measurement in each temperature step was max 0.3 °C.

During the three days of the experiment, the process solution composition was measured daily by analysis of a sample from the aerated bioreactor (sample ii in figure 1). The liquid circulated through all sections of the system (i.e. absorber, anaerobic bioreactor and aerated bioreactor) at a relatively high flow rate (i.e. 9.1 l/h). Therefore, the HRT in the various process sections was low (7 minutes in the absorber, 17 minutes in the anaerobic bioreactor and 36 minutes in the aerated bioreactor) compared to the HRT of the integrated system (8.9 days). As a result, concentrations of SO_4^{2-}, $S_2O_3^{2-}$, S^0 and bacteria are assumed to be equal throughout the system. On average, the reactor solution contained 0.67 ± 0.01M $NaHCO_3$, 0.068 ± 0.008 M SO_4^{2-} and 0.031 ± 0.001 M $S_2O_3^{2-}$. The average specific conductivity, a measure for the dissolved salt concentration, was 48.7 ± 0.6 mS/cm. The biomass concentration, was 70.7 ± 9.0 mgN/L and the TSS (concentration of suspended solids, mainly S^0) was 1.75 g/L. The pH was 7.74 ± 0.06 in the anaerobic bioreactor and 8.24 ± 0.05 in the aerated bioreactor (average over the complete experiment). The average pressure during the experiment was 3.12 ± 0.02 bar(g). Furthermore, the presence of sulfide

in the aerated bioreactor was assessed daily using lead-acetate paper (H_2S-Test Paper, Tintometer GmbH, Dortmund, Germany). During the complete experiment, no sulfide was detected.

2.2.3 Effect of biomass on H_2S absorption

The effect of biomass concentration on the H_2S absorption process was determined in a dynamic experiment, in which the biomass concentration in the solution to the absorber was increased from 0 to approximately 40 mgN/l within the course of 3.5 hours. To start with a solution without SOB, the auxiliary vessel was filled with a freshly prepared 0.7M $NaHCO_3$ solution. The solution from the auxiliary vessel was directed to the absorber (see dashed lines in figure 1). When solution flow from auxiliary vessel to absorber was started, the aerated bioreactor (containing the process solution with SOB and S^0) immediately started to overflow into the auxiliary vessel. Hence, the biomass concentration in the flow to the absorber started to increase as soon as the solution flow was initiated. The biomass concentration was measured in the bottom section of the auxiliary vessel (sample iii in figure 1) after 0h, 0.5h, 1h and 1.5h after starting the liquid circulation. The time constant of the changing biomass concentration in the flow to the absorber was preliminary determined by HRT of the auxiliary vessel. The HRT of the auxiliary vessel was 55 minutes and each run lasted for 3.5 hours (i.e. >3 x HRT of the auxiliary vessel). To describe the change in biomass concentration in the flow to the absorber, a dynamical model was developed to calculate the biomass concentration in the auxiliary vessel. The model was validated against the measured biomass concentrations.

The chemical composition of the solution in the absorber bottom, anaerobic bioreactor, aerated bioreactor, auxiliary vessel and tubing, was measured immediately before the start of the experiment by taking a sample taken from the aerated bioreactor (sample ii). The alkalinity was 0.69M; SO_4^{2-} was 0.053 M, and $S_2O_3^{2-}$ was 0.044M. The conductivity was 48.6 mS/cm and the biomass concentration was 56.6

mgN/L. The temperature in the reactors (including auxiliary vessel) was controlled at 43.1°C, (measured in the aerated bioreactor). Subsequently, the lean solution flow to the top of the H_2S absorber was started at 6 kg/h. Immediately hereafter, the feed gas flow was started.

The experiment was performed in duplicate. Therefore, after 3.5 hours, the auxiliary vessel was drained and refilled with a 0.70M $NaHCO_3$ solution where after the experiment was repeated. The solution's temperature at the inlet of the absorber was 38.5 ± 2 °C in the first experiment and 38.4 ± 0.4 °C in the second experiment. The average pressure in the absorber during both runs was 3.12 ± 0.003 bar(g). During the complete experiment, no sulfide was detected in the solution to the absorber.

2.3 Analyses

The concentrations of H_2S, CO_2 and N_2 in the treated gas of the absorber were analyzed every four minutes using a gas chromatograph (Elster Encal 3000, Honeywell, USA). CO_2 and N_2 were analyzed using a mol sieve capillary column (10m) coupled to a thermal conductivity detector (TCD), operated at 100°C and 200 kPa. H_2S was analyzed on a PPU column (10m) and another TCD, operated at 60°C and 200 kPa. Carrier gas was helium (flow 472 ml/min). The injector temperature was 100°C and the GC was calibrated weekly.

The alkalinity, total concentration of HCO_3^- and CO_3^{2-}, expressed as concentration HCO_3^-, was measured by titrating with 0.1M HCl, using a titrator (Titralab AT1000, Hach Lange, Germany).

The biomass concentration was measured as the amount of total organic N using the Hach Lange cuvette test LCK138 (Hach Lange, Germany). The difference between the culture's supernatant (centrifuged sample for 10 minutes at 14000 rcf) and non-centrifuged sample indicates the total amount of N present in the biomass. Therefore, the biomass concentration is expressed as mgN/L. Presence of biologically produced sulfur particles did not affect the results, provided that the samples were diluted at least 5

times. Considering the stoichiometric equation of HA-SOB, i.e. $CH_{1.8}O_{0.5}N_{0.2}$ [20], the total N accounts for 10-11 mol% of the total dry weight biomass.

The composition of the microbial community was analyzed once during the experiments by 16S rRNA gene Amplicon Sequencing. The materials and methods and results of this analysis can be found in the Supporting Information SI 4. Experiments were performed on 9 consecutive days and the sample for the microbial community analysis was taken on day 5. The dilution rate of the system (based on the effluent streams) was 0.09 day^{-1}, so we assume that the microbial community throughout all experiments was similar.

The conductivity of the samples was monitored with an offline conductivity sensor (LF 340, WTW, Weilheim, Germany).

Sulfate and COD (a measure for thiosulfate) concentrations were analyzed in duplicate using Hach Lange cuvette tests LCK353 and LCK514 (Hach Lange, Germany) in the sample's supernatant. Cells and S^0 were removed from the solution by centrifugation for 10 minutes at 14000 x g. Sulfate was measured at 800nm and COD at 605nm using a spectrophotometer (Hach Lange, Germany).

The total suspended solids concentration (TSS), mainly consisting of S^0 particles, was analyzed in triplicate. 15 mL sample was filtered over a pre-dried and pre-weighed GF/C Glass microfiber filter (Whatman). After (pre)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.

To determine the sulfide removal from solution by SOB in the absorber, 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 absorber bottom by titration with a solution of 0.1 M $AgNO_3$, 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 S^0 and bacteria. 2 mL of filtered sample was added to 80 mL 4% (w/v) NaOH, with 1 mL of

30% (w/v) NH_4OH to stabilize S_{tot}^{2-}. A comparison between unfiltered and filtered samples did not show significant differences.

2.4 Calculations and models

The specific HS^- removal efficiency in the anaerobic bioreactor (γ, in mg S / mg N) was calculated based on the H_2S load, the liquid flows, the measured HS^- concentration and the biomass concentration, according to eq. 4.

$$\gamma = \frac{\frac{H_2S\ load}{Q_{lean}} - [S_{tot}^{2-}]\ meas}{Xb} \qquad \text{eq. 5}$$

Here, *H_2S load* is the mass loading in the H_2S absorber (mg S h^{-1}), and the Q_{lean} is the lean liquid flow to the absorber (L h^{-1}). $[S_{tot}^{2-}]\ _{meas}$ is the total measured sulfide concentration (mg S L^{-1}) and *Xb* is the biomass concentration (mg N L^{-1}) in the absorber.

A differential equation model was developed in Excel to describe the biomass concentration in the lean solution over time. A detailed description of this model can be found in the Supporting Information (SI 2).

3 Results and discussion

3.1 Effect of temperature on SOB activity

To study the effect of temperature on the rate of biological sulfide oxidation, respiration tests were performed with SOB from a lab-scale biodesulfurization reactor [18], between 25°C and 60°C, see Figure 2.

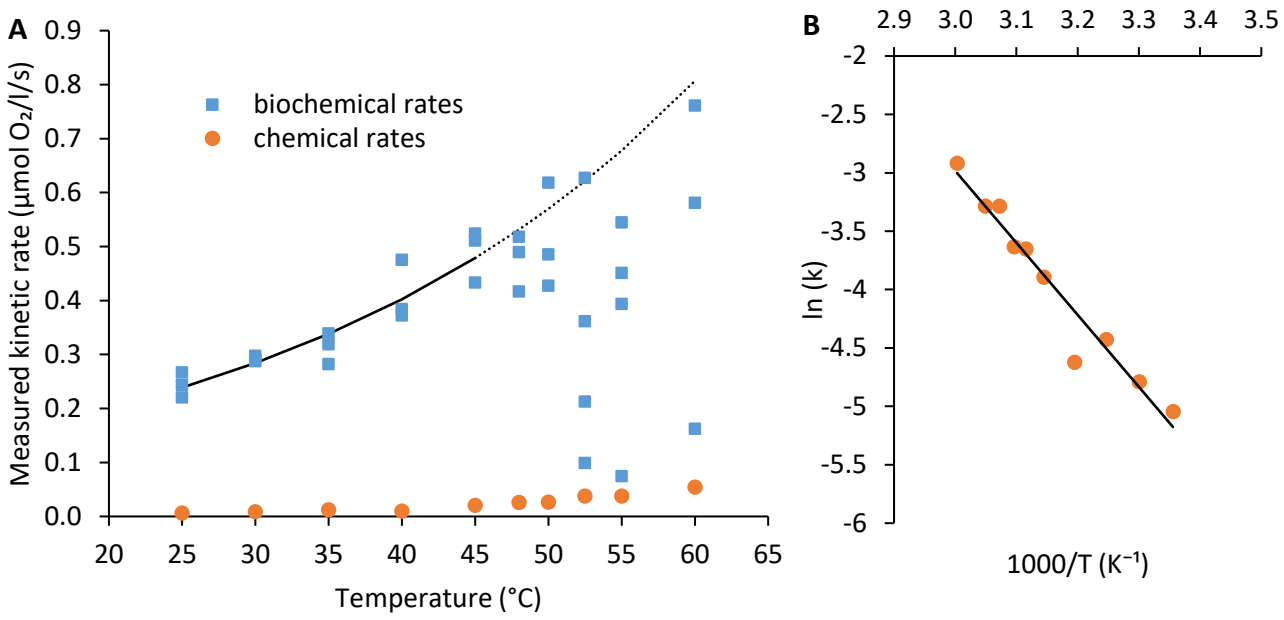

Figure 2A: Results of the respiration tests. The orange dots represent the chemical sulfide oxidation rates (without SOB) and the blue squares the combined biological and chemical rates (with SOB). Between 25°C and 45°C SOB activity increases exponentially with temperature (indicated by the solid line); at higher temperatures, thermal inactivation of SOB takes place and the majority of the measurements are below the theoretical curve (dashed line).

Figure 2B: Arrhenius plot, constructed using the chemical sulfide oxidation rates of figure 2A.

As can be seen in figure 2A, the chemical oxidation rates that were obtained in the absence of SOB, are negligible compared to the combined biological and chemical rates. For temperatures up to 45°C, the replicates show little deviation and an exponential increase in activity is found with increasing temperatures (see solid line in figure 2A). However, at temperatures higher than 45°C, the deviation between the replicates increases and the majority of the measurements are well below the exponential curve. The decreased SOB activity at temperatures above 45°C is the result of thermal inactivation of the bacterial population, while the relatively large differences in activity between the replicates is caused by the wide range of incubation times of SOB at the respective temperature. The activity decline in time of SOB at temperatures of 52.5, 55 and 60 °C is described by Klok [18].

Biological oxidation of sulfide is thus strongly influenced by temperature. Between 25 and 45 °C, biological activity increased with temperature; at temperatures above 45°C, biological activity decreased due to thermal inactivation of SOB. Hence, the temperature window of 25-45°C was chosen for the temperature dependency studies on H_2S absorption in the absorber of the pilot-scale biodesulfurization process.

The experimental data of the chemical oxidation rates were used to estimate parameters in Arrhenius' law (see figure 2B). The observed chemical oxidation rates show a good fit, with E_A = 50.0 kJ mol^{-1}, A = 3.49 mol L^{-1} s^{-1} and T_r 35°C [18]. The calculated activation energy (E_A) of sulfide oxidation is comparable to the activation energy reported for sulfide oxidation in seawater; E_A = 51 kJ mol^{-1} [21].

3.2 Effect of temperature on H_2S absorption

Next, the effect of the temperature on the absorption of H_2S in the pilot-scale biodesulfurization system was investigated. The results are shown in Figure 3. At the lowest temperature (25.4 °C), the H_2S concentration in the treated gas was 714 ± 265 ppmv. At increasing temperatures, the H_2S concentration in the treated gas showed a linear decrease to 69 ± 25 ppmv at 39.4°C. Thus, at higher temperatures,

more H_2S was removed from the gas, indicating that the absorption efficiency was higher. The H_2S concentrations in the treated gas at the higher temperatures are comparable to the normal performance of the pilot-system during continuous operation before the temperature experiment. For example, the average H_2S concentration in the treated gas during a typical day with the same feed gas was 150 ± 68 ppmv. At this day, the temperature at the absorber inlet was 35.2 °C, the pressure was 3.2 bar(g) and the alkalinity 0.68M. The liquid flow was also 10 kg/h (same as during the temperature experiment). The coefficient of variation (CV) of the H_2S concentration measurements (i.e. standard deviation divided by the average) during each temperature step is high, but fairly constant. Four of the temperature levels have a CV of 37%. At temperature 31°C, the CV is 25% and at 38°C it is 48%.

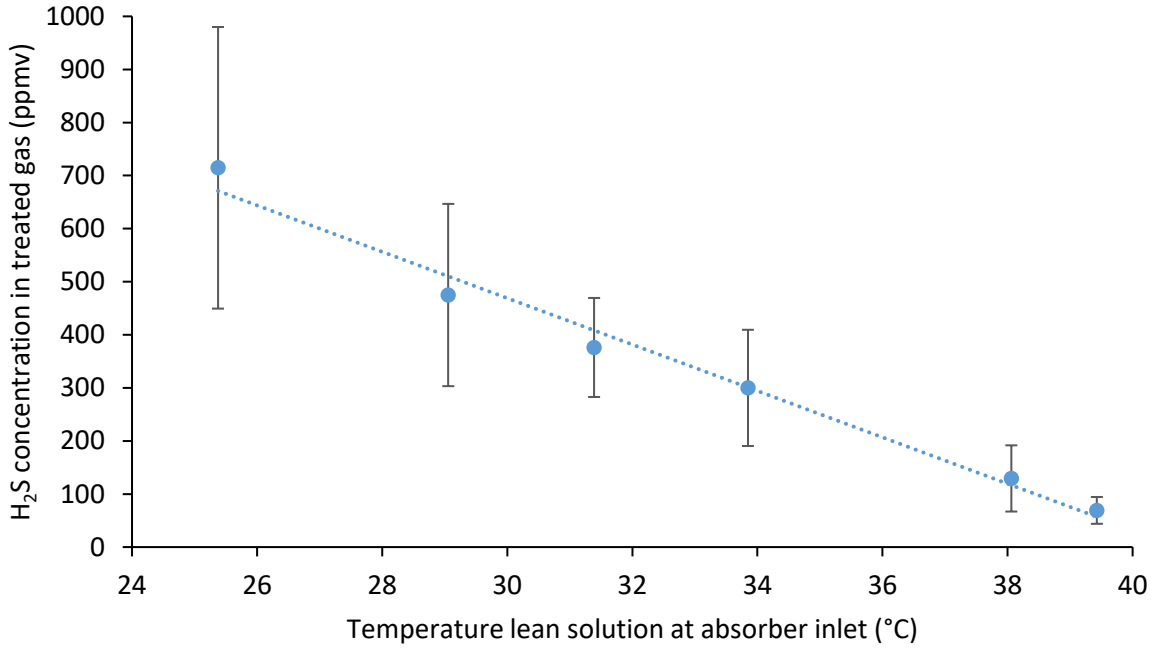

Figure 3: Results of the temperature experiment. The average H_2S concentration in the treated gas at each temperature setting, with standard deviation, is indicated with a blue dot and error bar. The inlet H_2S concentration was constant at 4.45 v% (44500 ppmv). When the lean solution (containing SOB) to the absorber has a higher temperature, the H_2S concentration in the treated gas is lower, which means more H_2S is absorbed from the gas. A dashed linear line is included to guide the eye.

In general, the mass transfer rates of H_2S and CO_2 depend on a combination of temperature effects on reaction kinetics, diffusivities and solubilities. It is known that a lower temperature leads to lower equilibrium partial pressures of H_2S and CO_2 [22, 23] (see also Supporting Information SI 3), which results in an increased H_2S and CO_2 absorption efficiency. Another effect of a lower temperature is the decrease of reaction rates of CO_2 with the (bi)carbonate buffer system. Because dissociation of H_2S is faster than hydrolysis of CO2 [24, 25], more buffer capacity is available for H_2S absorption. Thus, if the H_2S absorption in the biodesulfurization process would be solely based on these physico-chemical laws, at lower temperatures a lower H_2S concentration in the treated gas would be expected [26]. Our results show the opposite trend. We therefore speculate that the increased absorption efficiency at higher temperatures is likely to be caused by the effect of temperature on the SOB activity in the lean solution to the absorber, as will be discussed in more detail below.

In a batch experiment, Ter Heijne et al. found that SOB are able to remove sulfide from a solution under anaerobic conditions and in the absence of external electron acceptors [14]. It was hypothesized that bacteria can oxidize sulfide to sulfur under anaerobic conditions and store the released electrons in the form of reduced electron carriers, such as cytochromes and quinones. In our continuously operated reactor experiments, we observed the same phenomenon. The calculated sulfide concentration in the sulfide rich solution from the absorber would be 21.9 mM based upon the mass balance (i.e. all sulfide levels in gas streams and liquid streams coming into and leaving the absorber column). However, the measured sulfide concentration (the sum of S^{2-}, HS^- and polysulfide-sulfane) in the bottom section of the absorber was 18.0 ± 0.8 mM, indicating that part of the sulfide was removed from the solution by SOB in the absorber. The removal of dissolved sulfide cannot be explained by the presence of external electron acceptors, such as nitrate or dissolved oxygen. Nitrate is not present in the process solution, as it is not

supplied to the process and the dissolved oxygen concentration in the aerated bioreactor is below the detection limit of 100 nM.

3.3 Effect of biomass concentration on H_2S absorption

To further assess the effect of SOB on H_2S absorption, the biomass concentration in the lean solution to the top of the absorber was varied. The results of this experiment, which was performed in duplicate, are shown in Figure 4.

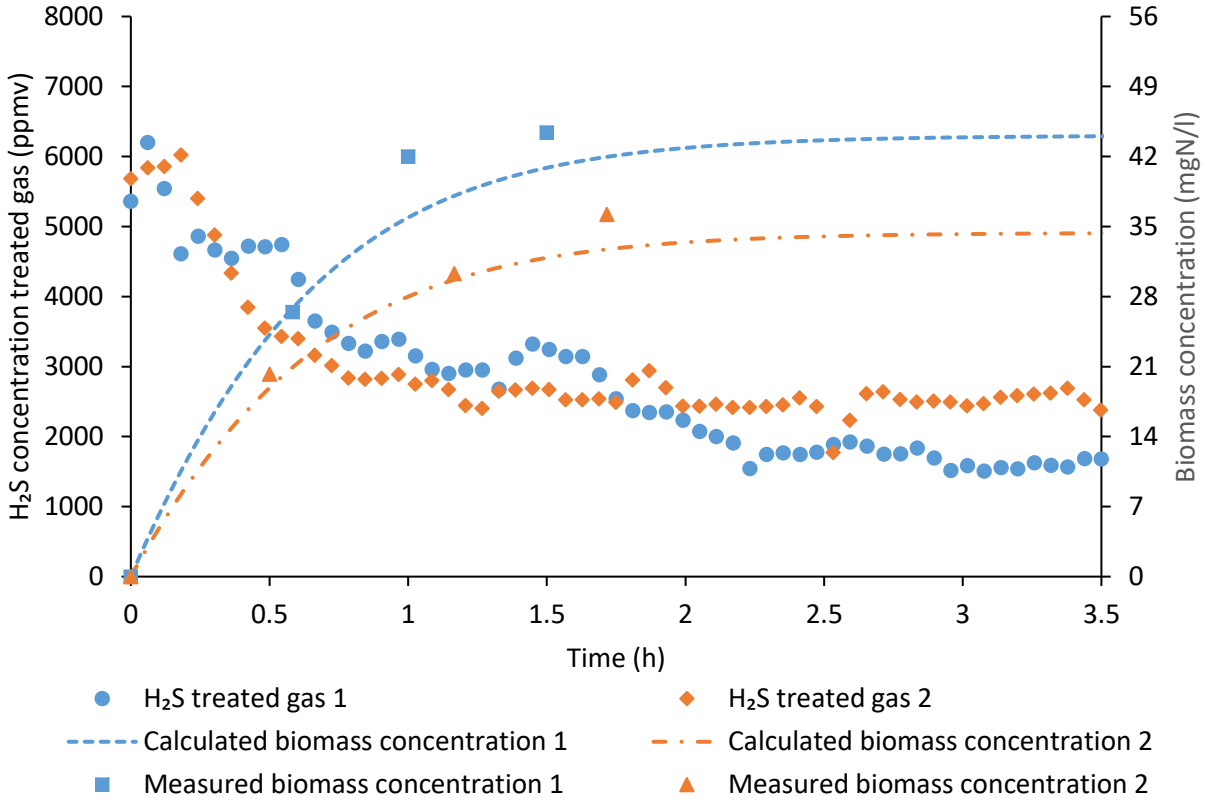

Figure 4: Results of the experiment in which the biomass concentration in the flow to the absorber is increased in time. *The H_2S concentration in the treated gas is represented by the blue dots (run 1) and orange diamonds (run 2). The dashed lines represent the modelled biomass concentration (in mgN/l) in the solution to the absorber. The actual biomass concentration was measured several times (blue squares*

for run 1 and orange triangles for run 2) to verify the model. The inlet H_2S concentration was constant at 4.45 v% (44500 ppmv). The results show that the H_2S concentration in the treated gas decreases with increasing biomass concentrations, indicating that SOB increase the H_2S absorption rate.

In the first run, which is depicted in blue, the biomass concentration in the lean solution to the absorber increased from 0 to 43 mgN/L. The calculated biomass concentrations (see Supporting Information SI 3) were in good agreement with laboratory measurements. The initial H_2S concentration in the treated gas was 6200 ppm. During the initial stage of the run, no SOB were present in the lean solution to the absorber and the obtained H_2S concentration is the result of physical-chemical absorption only. As the biomass concentration in the lean solution increased, the H_2S concentration in the treated gas decreased and stabilized after 2-2.5 hours of operation. The average H_2S concentration in the last hour (2.5 – 3.5h) of the experiment was 1664 ± 126 ppmv. This is higher than in the above described temperature experiment because the solution flow rate to the absorber in the biomass experiment was lower (6 kg/h instead of 10 kg/h). In the second run, the biomass concentration reached a maximum value of 35 mg N/l. A similar trend in H_2S concentration was observed compared to run 1: the H_2S concentration in the treated gas was 6024 ppmv at the start of the run (without SOB in the lean solution), and with increasing biomass concentration, the H_2S concentration in the treated gas decreased. The H_2S concentration in the treated gas stabilized at 2509 ± 104 ppmv, which is higher than in the first experimental run. This is as expected, since the biomass concentration in run 2 was lower, which is the result of 20% biomass removal after replacing the bicarbonate solution in the auxiliary vessel. Based on the work of Kleinjan et al., S^0 is present in excess amounts compared to HS^- [16]. Therefore, it is unlikely that the decreased absorption efficiency of H_2S in run 2 compared to run 1 can be explained by decreased enhancement of polysulfide formation, i.e. the heterogeneous reaction. Hence, this experiment also indicates that SOB increase the absorption efficiency of H_2S.

3.4 Biologically enhanced H_2S absorption

In previous research by Kleinjan et al., two different enhancement factors for H_2S mass transfer were identified: (i) reaction with (bi)carbonate (homogenous reaction) and (ii) reaction with S^0 particles (e.g. the heterogeneous reaction) [16]. The enhancement factor for the homogeneous reaction (at an alkalinity of 0.15M and pH 8.5) varied from 41-51 and the enhancement factor due to sulfur particles reached values up to 2.5 [16]. However, these two mechanisms could not fully explain the total enhancement of H_2S absorption found by measurements. It was hypothesized by Kleinjan et al. that the remaining enhancement could be caused by a shuttle mechanism of large hydrophobic sulfur particles that H_2S can bind to [16]. Such a shuttle mechanism of particles was for example shown for activated carbon particles enhancing CO_2 absorption [27]. However, this hypothesis hasn't been investigated for the biological desulfurization process described in this paper.

The objective of this study was to determine the effect of the SOB activity under oxygen-free conditions, i.e. in the absorber column, on the enhancement of H_2S absorption. The results presented in this paper show that the activity of SOB enhance H_2S absorption by removing sulfide from the process solution. The kinetics of the removal of sulfide by SOB depends on the SOB activity (i.e. temperature) and the SOB concentration. To obtain insight in possible biological mechanisms of the enhanced H_2S absorption, the composition of the microbial community was analyzed using 16S rRNA gene Amplicon Sequencing (Supporting Information SI 4). The two most abundant SOB species in the system were *Thioalkalivibrio sulfidiphilus* (53.5%) and *Alkalilimnicola ehrlichii* (25.7%). Both strains are members of the family *Ectothiorhodospiraceae* (*Gammaproteobacteria*), which are gram-negative bacteria. These bacteria, and especially *Tv. sulfidiphilus*, are often the dominant species in these biodesulfurization installations, both lab-scale and full-scale [12, 28-31]. However, when an anaerobic reactor was added to the line-up of the

biodesulfurization process to suppress biological formation of sulfate, it was found that *Alkalilimnicola ehrlichii* became dominant over *Tv. sulfidiphilus* [8]. At the same time, the selectivity for sulfur formation increased significantly, from approximately 90 to 97%. The analysis of the complete genome of *Tv. sulfidiphilus* [32], a dominant SOB species found in the full scale facility of Industriewater Eerbeek, the Netherlands [28], showed the presence of genes encoding an FCC type of sulfide dehydrogenase, which converts HS^- to S^0. Flavocytochrome c/sulfide dehydrogenase is a membrane-bound enzyme in alkaliphilic autotrophic bacteria and transfers electrons to cytochromes c [33]. Cytochromes *c* are oxidized by cytochrome c oxidase (CcO), using O_2 as final electron acceptor.

Alkalilimnicola ehrlichii is a facultative chemolithoautotroph [34]. *Alkalilimnicola* most probably oxidizes HS^- by use of the membrane-bound sulfide-quinone reductase (SQR), which is another well-known enzyme associated with HS^- oxidation. SQR uses quinones as electron carriers. Reduced quinones, i.e. quinol, can be oxidized by either quinol oxidase (QO), using O_2 as electron acceptor, or by NADH dehydrogenase (DH), forming NADH from NAD^+ [10, 11, 35-37]. Several studies have proposed mechanisms for sulfide oxidation by SQR and the product of SQR is, most probably, (soluble) polysulfide [35, 37-39]. Due to absence of external electron acceptors (i.e. oxygen or nitrate), no oxidation of the electron carriers can occur in the absorber.

Several biological mechanisms could contribute to anaerobic sulfide removal by SOB in the absorber in the absence of external electron acceptors. Upon absorption of H_2S in the process liquid, HS^- and S_x^{2-} are transferred over the outer cell membrane to the periplasm of the gram-negative SOB. We assume that here the sulfide is converted, as the total periplasmic volume would be too small to account for the total amount of sulfide removed . Sulfide could be converted either by (i) forming cell-bound polysulfides upon reaction with internal stored S^0 [20], (ii) oxidation by sulfide oxidizing enzymes (SQR and FCC), thereby reducing its associated electron carriers (quinones, cytochromes and NAD^+) [10], and/or (iii) binding to the active sites of SQR to form a polysulfide chain of 3 or 4 S atoms [39]. As a result of these conversion

reactions, reduction of oxidized molecules takes place in the SOB, i.e. the SOB will reach a lower oxidation state, which is represented by eq. 6. Subsequent oxidation of SOB occurs via reduction of oxygen (eq. 7), in the aerated bioreactor. As the process solution containing the SOB is continuously circulated between absorber and bioreactor, bacteria can shuttle electrons obtained from sulfide oxidation and intracellular binding in the absorber to the bioreactor. We hypothesize that this shuttle mechanisms results in so-called biologically enhanced H_2S absorption.

$$H_2S + SOB^+ \rightarrow SOB^- + S^0 + 2\ H^+ \qquad \text{eq. 6}$$

$$SOB^- + 2\ H^+ + ½\ O_2 \rightarrow SOB^+ + H_2O \qquad \text{eq. 7}$$

3.5 Considerations

This paper shows that SOB in the process solution presumably enhance H_2S absorption and comprise (part of) the remaining enhancement factor. Although some potential mechanisms for biological H_2S enhancement have been discussed, still more research is required to fully understand underlying mechanisms. Biologically enhanced H_2S absorption differs from conventional physico-chemical factors (i.e. the homogeneous and heterogeneous reaction), because it depends on several physiologically based parameters, such as the composition of the microbial community and gene expression levels. These physiological parameters depend mainly on the process conditions. For example, the pilot-scale system, which was used in the experiments described in this paper, has an additional anaerobic bioreactor (dual-reactor system). It was found by Ter Heijne et al. that SOB taken from a dual-reactor system removed more sulfide from the solution than SOB from a system that consisted of a single aerated bioreactor [14]. An increased sulfide removal capacity of SOB can be the result of the anaerobic reactor, as this increases the contact time of SOB with dissolved sulfide in the absence of oxygen [8]. Therefore, the biological enhancement factor is expected to be different for different microbiomes and operational conditions. As

the mechanism of anaerobic sulfide removal is not yet fully understood, it is not possible to quantify the biological enhancement factor based on our experimental data. Further research is required to understand underlying kinetics and reaction pathways. Since the H_2S concentration in the gas phase decreases along the height of the absorber column, i.e. from bottom to top, the conventional driving force for H_2S transfer based on Fick's Law is lowest in the top section. Therefore, we hypothesize that the effect of the biological enhancement factor is most pronounced in the top section of the absorber column. For efficient H_2S absorption, it is important to obtain the required and very low H_2S concentrations in the treated gas whilst minimizing liquid recirculation and column height. Since the absorption step is limited by the rate of mass transfer of H_2S, maximizing biological enhancement by operation at optimal microbial metabolism rates, e.g. temperature and biomass concentration, will contribute to more efficient H_2S removal. Furthermore, when it would be possible to quantify the biological enhancement factor, design and operation of full-scale facilities can be improved.

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5 Supporting Information

1. Details experimental set-up
2. Biomass concentration model

3. Theoretical physico-chemical H_2S and CO_2 absorption at different temperatures

4. NGS analysis microbial community composition.

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Supporting Information

SI 1: Details experimental set-up

The pilot-scale biodesulfurization installation consisted of an H_2S absorber, an anaerobic bioreactor, an aerated bioreactor and auxiliary vessel (Figure 1 in the manuscript). The feed gas supplied to the H_2S absorber (stream A in Fig. 1) was a mixture of H_2S (8.9 vol% H_2S, 91.1 vol% N_2) and CO_2 (99.995 vol%). These gases were supplied as separate streams using mass flow controllers (Profibus, Brooks instrument, Hatfield, USA) to the main feed gas line. The feed gas entered the absorber just below the packed bed. Process solution (stream 1 in Figure 1 of the manuscript) containing no sulfide ('lean solution') was continuously pumped to the absorber top by an eccentric screw pump (P1 in figure 1). The liquid flow was measured and controlled by a flow meter (Endress+Hausser, Reinach, Switzerland). The pressure in the absorber was controlled with a Tescom Europe backpressure regulator (Emerson Electric co., St. Louis, USA) and measured with a pressure meter (Endress+Hausser, Reinach, Switzerland), located at the outlet gas line. A sub stream of the treated gas was routed to a gas chromatograph (GC) (EnCal 3000, Honeywell, USA), by a diaphragm vacuum pump (knf, Freiburg, Germany) for analysis of the treated gas composition.

The liquid which has absorbed the H_2S ('rich solution'), was collected in the absorber bottom (total volume approximately 1 L). The flow of rich solution from the bottom of the absorber to the anaerobic bioreactor (stream 2) was controlled with a valve and driven by the pressure difference between the pressurized absorber and the atmospheric anaerobic bioreactor. A Level indictor (Endress+Hausser, Reinach, Switzerland), acting on the control valve, kept the liquid level on a constant pre-set value. The anaerobic bioreactor solution (liquid volume of 5.3 L) was continuously mixed by an installed mechanical mixer (rzr2020, Heidolph Instruments, Schwabach, Germany). The effluent of the anaerobic bioreactor was

directed to the aerated bioreactor (stream 3) with a peristaltic pump (P2). The aerated bioreactor was a gas-lift bioreactor with a liquid volume of 11.4 L. Compressed air (stream D) was supplied to the aerated bioreactor using a mass flow controller (Profibus, Brooks instrument, Hatfield, USA). The airflow was controlled via the ORP measurement in the aerated bioreactor, using a PID controller in the PLC [40]. The ORP was maintained at -360 mV. Under these conditions, SOB oxidize HS^- mainly to S_8, which regenerates the process solution. In addition, a sensor for measuring dissolved O_2 (PSt 6 Presens, Regensburg, Germany) was positioned in the aerated bioreactor. The produced S_8 was settling in the cone-shaped bottom of the aerated bioreactor. This S_8 slurry was removed with a pump (P6) (101 U/R, Watson Marlow, Wilmington USA) (stream 8). To compensate for the removed slurry, diluted nutrient solution (stream 7) and a 5 w/w% NaOH solution (stream 6) were continuously supplied to the aerated bioreactor with pumps P5 and P4 (both 101 U/R, Watson Marlow, Wilmington, USA). The nutrients contained 28.6 g L^{-1} nitrogen as urea, 20 g L^{-1} potassium as KNO_3, 6.5 g L^{-1} P as H_3PO_4 and trace metals as described by [41]. These components are required for growth of the SOB. Caustic soda (NaOH) was supplied to maintain a constant alkalinity. Furthermore, the flow from the aerated bioreactor to anaerobic bioreactor (stream 4) was set a flow rate of 10 l/h, using pump P3.

SI 2: Dynamic model to describe biomass concentrations in all process vessels

nomenclature and indices

Xb	biomass concentration, in mg N/l
Q	flow solution, in kg/h
t	time, h
ρ	density of the solution, in kg/l
θ	parameter vector
abs	absorber
aux	auxiliary vessel
BR1	bioreactor 1, anaerobic bioreactor
BR2	bioreactor 2, aerated bioreactor
rec	recycle flow from BR2 to BR1
lean	lean solution from auxiliary vessel to top absorber

The model describing the concentrations of biomass in the system (see figure 4) is presented as a set of ordinary differential equations. Assumed is that the volume of the tubing between all vessels is neglectable and all reactor vessels are ideally mixed. The differential equation, describing the dynamic biomass concentration in the absorber is given by Eq. SI 2.1.

$$\frac{dXb_{abs}(t)}{dt} = \frac{Q_{lean}}{\rho \cdot V_{abs}} \cdot \left(Xb_{aux}(t) - Xb_{abs}(t)\right) \qquad \text{Eq. SI 2.1}$$

The differential equation, describing the dynamic biomass concentration in bioreactor 1, the anaerobic bioreactor, is given by Eq. SI 2.2.

$$\frac{dXb_{BR1}(t)}{dt} = \frac{Q_{lean}}{\rho \cdot V_{BR1}} \cdot Xb_{abs}(t) + \frac{Q_{rec}}{\rho \cdot V_{BR1}} \cdot Xb_{BR2}(t) - \frac{Q_{lean}+Q_{rec}}{\rho \cdot V_{BR1}} \cdot Xb_{BR1}(t) \qquad \text{Eq. SI 2.2}$$

The differential equation, describing the dynamic biomass concentration in BR 2, the aerated bioreactor, is given by Eq. SI 2.3.

$$\frac{dXb_{BR2}(t)}{dt} = \frac{Q_{lean}+Q_{rec}}{\rho \cdot V_{BR2}} \cdot \left(Xb_{BR1}(t) - Xb_{BR2}(t)\right)$$ Eq. SI 2.3

The differential equation, describing the dynamic biomass concentration in the auxiliary vessels is given by Eq. SI 2.4.

$$\frac{d(t)}{dt} = \frac{Q_{lean}}{\rho \cdot V_{aux}} \cdot \left(Xb_{BR1}(t) - Xb_{aux}(t)\right)$$ Eq. SI 2.4

Initial conditions and process parameters

The initial conditions for the concentrations of the biomass concentrations are given by the following vector:

$$\begin{pmatrix} Xb_{abs,0} \\ Xb_{BR1,0} \\ Xb_{BR2,0} \\ Xb_{aux,0} \end{pmatrix} = \begin{pmatrix} Xb_0 \\ Xb_0 \\ Xb_0 \\ 0 \end{pmatrix}$$

Where Xb_0 was 56.55 mgN/L in Run 1 and 43.4 mgN/L in Run 2. The parameter vector θ, containing all process parameters, is defines as:

$$\theta = \begin{pmatrix} Q_{lean} \\ Q_{rec} \\ \rho \\ V_{abs} \\ V_{BR1} \\ V_{BR2} \\ V_{aux} \end{pmatrix} = \begin{pmatrix} 6 \\ 10.5 \\ 1.05 \\ 1.0 \\ 5.3 \\ 11.4 \\ 5 \end{pmatrix}$$

SI 3: Theoretical physico-chemical H_2S and CO_2 absorption at different temperatures

The relationship between temperature and liquid/vapour fractions for CO_2 and H_2S was assessed with Aspen Technology software, using the electrolyte non-random two-liquid model. For both gases the model shows that at lower temperatures leads to lower equilibrium partial pressures of H_2S and CO_2. Hence, at lower temperatures, a higher absorption of these gases into the liquid is expected.

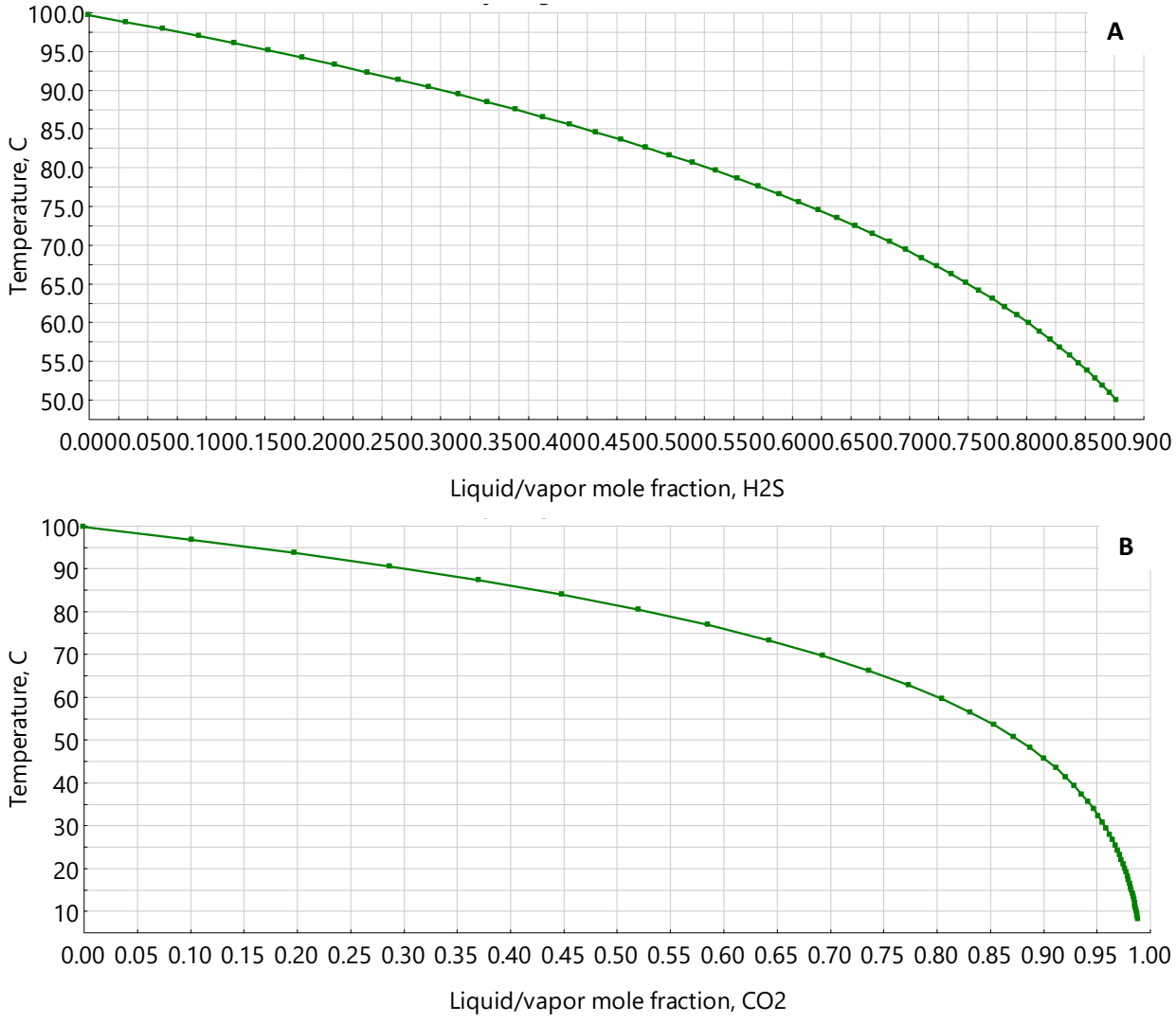

Figure SI 3.1: Model predictions of the liquid/vapour mole fractions of H_2S (A) and CO_2 (B) at different temperatures.

SI 4: NGS analysis microbial community composition

The microbial community composition of the system was analyzed using *by 16S rRNA gene Amplicon Sequencing.* The sample, taken from the aerated bioreactor, was 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 [42], using the Illumina MiSeq sequencer. 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 dataset and no correction on differences in library sized was applied. The reported species name is the species most related to the detected sequence. Results are shown in Figure SI 4.1

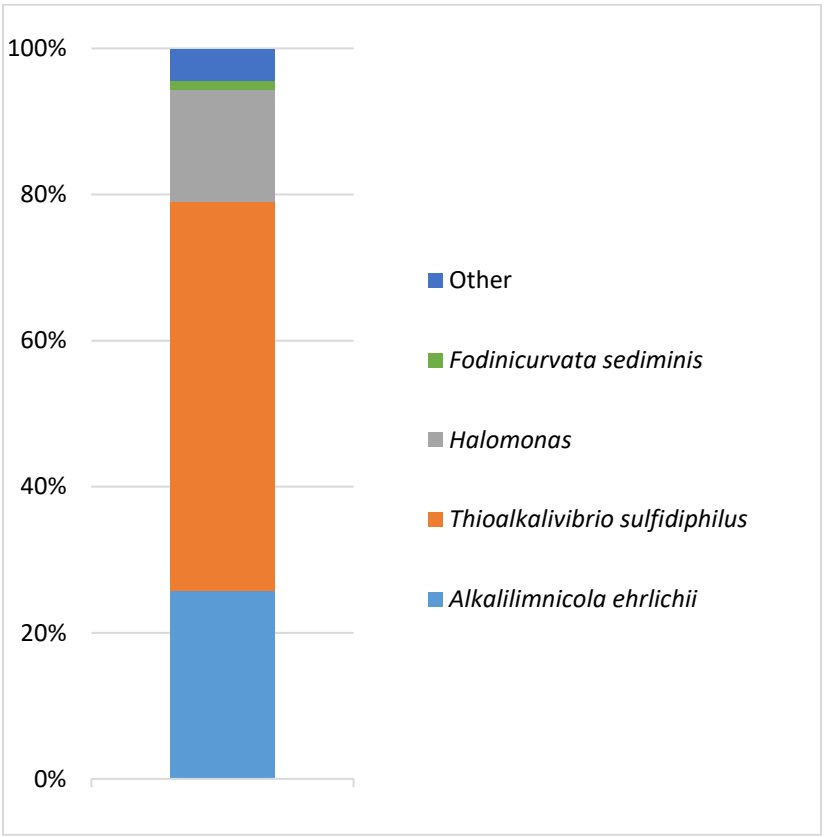

Figure SI 4.1: Mapping of bacterial diversity in the system during the temperature and biomass concentration experiments. Sample taken from aerated reactor.