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Effect of process conditions on the performance of a dual-reactor biodesulfurization process

Rieks de Rink [a,b], Suyash Gupta [c,d], Flavia Piccioli de Carolis [a], Dandan Liu [b], Annemiek ter Heijne [a,*,1], Johannes B.M. Klok [a,b,c], Cees J.N. Buisman [a,c]

[a] Environmental Technology, Wageningen University & Reseach, P.O. Box 17, 6700 AA Wageningen, The Netherlands
[b] Paqell B.V., Reactorweg 301, 3542 AD Utrecht, The Netherlands
[c] Wetsus, European Centre of Excellence for Sustainable Water Technology, Oostergoweg 9, Leeuwarden, The Netherlands
[d] Microbial Systems Ecology, Department of Freshwater and Marine Ecology, Institute for Biodiversity and Ecosystem Dynamics, University Amsterdam, P.O. Box 94240, 1090 GE Amsterdam, The Netherlands

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ABSTRACT

The biotechnological gas desulfurization process under haloalkaline conditions is widely applied for removal of toxic H_2S from sour gas streams. In this process H_2S is biologically oxidized into elemental sulfur. Recently, the process has been extended with an anaerobic process step (dual-reactor line-up), increasing the selectivity for elemental sulfur (S_8) from ~85–97% and decreasing the formation of (thio)sulfate. It was also found that biological sulfide uptake took place in the anaerobic bioreactor. In order to apply this process in industry, more insight is needed of the effect of the process conditions on the process performance. The effect of the process conditions HRT and sulfide concentration in the anaerobic bioreactor and pH on the overall product selectivities and on biological sulfide uptake in the anaerobic bioreactor were investigated. 7 experiments were performed in a pilot-scale biodesulfurization set-up. In all experiments, high selectivities (>95%) for S_8 formation were obtained, except when the pH in the aerated bioreactor was increased from 8.5 to 9.1 (selectivity of 88%). Furthermore, biological sulfide uptake in the anaerobic bioreactor increased at higher sulfide concentrations and at higher pH. We hypothesize the biological sulfide uptake under anaerobic conditions is related to polysulfide formation. Our results increase the understanding how to control biological sulfide conversion in the dual-reactor biodesulfurization process.

1. Introduction

In the 1990s, a biological gas desulfurization process was developed for the removal and conversion of toxic hydrogen sulfide gas (H_2S) from biogas [1,2]. In this process, 'sour' gas is counter-currently contacted with process solution in an absorber column, whereby H_2S is removed from the gas stream and absorbed into the process solution. For optimal and biocompatible removal of H_2S, the process solution is a haloalkaline (bi)carbonate solution. When absorbed, H_2S is converted into both soluble bisulfide (HS^-) and, due to the presence of elemental sulfur, into soluble polysulfide (S_x^{2-}). This 'sulfide rich' solution from the absorber is directed to an aerated bioreactor where the dissolved sulfides (i.e. HS^- and S_x^{2-}) are oxidized by haloalkaliphilic sulfide oxidizing bacteria (SOB) into predominantly elemental sulfur (S_8). The solution from the aerated bioreactor is circulated over the sulfur recovery section to remove S_8 from the process solution. In this way, the sulfur content of the bioreactor solution is controlled, and typically contains around 0.5 wt% solids. The sulfur recovery section often consists of a gravity settler and/or decanter centrifuge. The produced sulfur can be reused for e.g. agricultural purposes [3,4]. Nowadays, the technology is applied globally for desulfurization of various types of sour gas streams, such as natural gas and several refinery gases [5,6].

In general, about 10–20% of the incoming H_2S is oxidized into the byproducts sulfate (SO_4^{2-}) and thiosulfate ($S_2O_3^{2-}$) [7,8]. SO_4^{2-} formation occurs biologically when too much O_2 is supplied. In addition, $S_2O_3^{2-}$ is formed due to a chemical reaction between sulfide and O_2. Therefore, levels of O_2 and H_2S are minimized in the aerated bioreactor. The formation of the aforementioned side products is unwanted as its

* Corresponding author.
E-mail address: annemiek.terheijne@wur.nl (A. ter Heijne).
[1] Visiting address: Bornse Weilanden 9, 6708 WG Wageningen, Building Axis z, building nr. 118.

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formation i) requires NaOH addition to compensate proton formation, ii) requires removal via a bleed stream to maintain salinity levels, iii) reduces recovery of elemental sulfur, and iv) requires more O_2 and therefore more energy [9]. Thus, decreasing side product formation will considerably contribute to the reduction in operational costs and carbon footprint of the process. Although the use of an extra bioreactor increases the investment costs, this decrease of operational costs makes the process economically more attractive [6,9,10].

Recently, the process has been extended with an anaerobic bioreactor, placed in between the absorber and aerated bioreactor (dual reactor line-up) [9]. With the incorporation of this anaerobic bioreactor, a selectivity for S_8 of 97% was achieved. Selectivities for SO_4^{2-} and $S_2O_3^{2-}$ were 2% and 1% respectively. The sulfidic conditions in the anaerobic reactor suppressed biological SO_4^{2-} formation, because sulfide reversibly blocks enzymes in the pathway for SO_4^{2-} formation [9]. In addition, a change in the microbial community was observed. It was hypothesized that the adopted population had a lower tendency for SO_4^{2-} formation as it was suggested that the bacteria that became dominant (*Alkalilimnicola*) were unable to form SO_4^{2-}, i.e. could only oxidize sulfide to S_8 [11,12]. Furthermore, it was found that the measured sulfide concentration in the anaerobic bioreactor was lower than the sulfide concentration as calculated based on the mass balance of H_2S and liquid circulation flow rate. This indicates sulfide was biologically removed from solution in the anaerobic bioreactor. Since a lower sulfide concentration in the rich solution leads to lower chemical formation rates of $S_2O_3^{2-}$, sulfide uptake in the anaerobic bioreactor also contributed to the increased selectivity for S_8 formation.

In the previous study by de Rink et al., [9] the performance of the dual reactor process was assessed at fixed process conditions. Therefore, it is unknown how different process conditions affect the process performance of the dual reactor desulfurization process. In full scale application, several process factors, such as the sulfide concentration in the rich solution and the pH of the process solution, are dependent by the specification of the gas stream that needs to be treated. Furthermore, the hydraulic retention time (HRT) in the anaerobic bioreactor is determined by its designed size.

The aim of this study is to investigate the effect of the process factors sulfide concentration and HRT in the anaerobic bioreactor and pH on product formation and biological sulfide uptake. We hypothesize that a higher sulfide concentration and a longer retention time in the anaerobic bioreactor increase the inhibitory effect on SO_4^{2-} formation. Furthermore, we want to gain better understanding of biological sulfide uptake in the anaerobic bioreactor, since this may influence the selectivity as well. To investigate the effect of the sulfide concentration and HRT in the anaerobic bioreactor and the pH, we operated a pilot-scale dual-reactor biodesulfurization installation under different conditions, i.e. varying the gas flow rates of H_2S and CO_2, liquid circulation rate and volume in the anaerobic reactor. We analyzed product selectivity of the process and sulfide uptake in the anaerobic bioreactor as the main process performance parameters. Since the microbial community composition also influences process performance [13], we also analyzed the microbial community compositions of the inoculum and at the end of each experiment.

2. Materials and methods

2.1. Experimental set-up

Experiments were performed in a pilot-scale biodesulfurization installation, consisting of an absorber column, anaerobic bioreactor and aerated bioreactor, as described elsewhere [9]. The details of the experimental set-up can be found in supplementary material A.

2.2. Experimental operation

To study the effect of the sulfide concentration and HRT in the anaerobic bioreactor and the effect of pH on process performance, 7 experiments were performed in which the conditions were varied (see Table 1). The operational conditions were set by varying the H_2S flow rate (i.e. the S-load), the CO_2 flow rate (this determines the pH), the solution circulation flow rate (flow from aerated bioreactor to absorber to anaerobic bioreactor to aerated bioreactor) and the volume of the anaerobic bioreactor (this determines the HRT). A large stock of inoculum was prepared by mixing effluent from a full-scale desulfurization installation treating amine acid gas [14] and effluent from previous experiments in the same installation [9] in ratio of approximately 1:1. The inoculum was stored at 4 °C, and each experiment was started with the inoculum from this inoculum stock. For each experiment, the system was filled with a 50/50 mixture of a 0.8 M $NaHCO_3$ solution and inoculum.

After filling the system, the flow of N_2 (100 NL h^{-1}, i.e. 100 L h^{-1} under standard pressure and temperature) and CO_2 was started to pressurize the absorber to 3 bar(g). Then the solution circulation was started, see Table 1 for the circulation flow rates in each experiment. When the temperature had increased to 37 °C, the flow of H_2S was initiated. When the ORP in the aerated bioreactor reached −370 mV, the air flow was started in manual mode. After the ORP stabilized, the air flow was controlled at −370 mV automatically by a PI controller. At the same time, the caustic and nutrient dosing were started. The caustic was a 5% (w/w) NaOH solution and was dosed to maintain the alkalinity of the process solution, which is lost due to formation of SO_4^{2-} and $S_2O_3^{2-}$ and via the bleed. The nutrients are required for growth/maintenance of the bacteria. Nutrients solution consisted of macro nutrient as described by de Rink et al. [9] and 1 mL L^{-1} trace element mix as described by Pfennig and Lippert [15]. The nutrient dosing rate was set in such way that the total residual nitrogen concentration in the supernatant was < 15 mg-N L^{-1}. In this way, overdosing of nutrients is prevented and the bacteria consume all nutrients. The decanter was started after several days of operation to remove elemental sulfur from the process solution in order to maintain the TSS around 5 g L^{-1}.

Each experiment was performed for at least 1 system HRT, which was typically around 30 days. The system HRT is calculated as the total system volume divided by the bleed flow. Experiment 5 was performed for 2.9 HRT. Experiment 7 was performed for 4.1 system HRT. In this experiment, the highest S-load (170 g day^{-1}) was applied and therefore required a longer startup time in order to grow enough bacteria to handle the high amount of sulfide.

2.3. Analyses

All analyses were performed on samples of the aerobic bioreactor, which were taken every weekday. Samples to analyze the sulfide concentration were taken 2–3 times per week from the anaerobic bioreactor. The alkalinity and concentrations of SO_4^{2-}, $S_2O_3^{2-}$, S_8 and bacteria were assumed homogenous throughout all process sections, due to the solution circulation through the process sections (absorber, anaerobic- and aerated bioreactor) [9]. pH and conductivity were analyzed offline using a HQ440d multi analyzer (Hach, Germany). Alkalinity, expressed as the concentration HCO_3^-, was measured with an automated TitrinoPlus titrator (Metrohm) by titrating to pH 4.3 using a 0.1 M HCl solution. SO_4^{2-} and $S_2O_3^{2-}$ concentrations were determined on the samples supernatant (after centrifuging for 10 min at 14,000 g) using a Dionex ICS-2100 Ion Chromatograph (ThermoScientific) with a Thermo Fisher Scientific IonPac AG17 Guard (Thermo Fisher Scientific, Waltham, MA, USA) and Thermo Fisher Scientific IonPac AS17 column (Thermo Fisher Scientific) at 30 °C. The eluent was KOH at a flowrate of 1.0 mL min^{-1}. The sample injection volume was 10 µl. The biomass concentration was measured as the amount of total nitrogen based on the absorbance of nitrophenol, using the Dr. Lange cuvette test LCK138 (Hach Lange, Germany), as described by de Rink et al. [9]. The difference between the supernatant (i.e., a sample centrifuged for 10 min at 14,000 g) and a non-centrifuged sample indicated the total amount of N present in the (suspended) biomass. To exclude interference by salts and biologically produced S_8,

the samples were diluted at least 5 times. N accounts for approximately 10% of the dry weight biomass [16].

To determine the sulfide uptake 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 $AgNO_3$, using a Titrino Plus Titrator (Metrohm, Switzerland). Before titration, the sample was filtered over a 0.45 μm cellulose acetate membrane filter to remove S_8 and bacteria. The 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-}.

The microbial community of the inoculum and the process solution at the end of each experiment (taken from the aerated bioreactor) was analyzed using 16 S rRNA gene based amplicon sequencing. Details of the analysis can be found in supplementary material B. The EMBL-EBI accession number for the presented 16 S rRNA sequencing set is PRJEB44162.

2.4. Calculations

As the formed S_8 particles tend to attach to the reactor wall, it was not possible to calculate the S_8 production rate from the analyses. As no products other than S_8, SO_4^{2-} and $S_2O_3^{2-}$ were measured in the reactor [8], the production rate of S_8 was calculated from the mass balance as shown in Eq. (1):

$$P_{S_8-S} = I_{H_2S} - P_{SO_4^{2-}-S} - P_{S_2O_3^{2-}-S} \quad (1)$$

Here, P_{S_8-S}, $P_{SO_4^{2-}-S}$ and $P_{S_2O_3^{2-}-S}$ are the production rates of S_8, SO_4^{2-}, and $S_2O_3^{2-}$, respectively, in mol-S day^{-1} and I_{H_2S} is the volumetric H_2S influent in mol day^{-1}. The production rates of both SO_4^{2-}-S (Eq. (2)) and $S_2O_3^{2-}$-S (Eq. (3)) were calculated as follows:

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

$$P_{S_2O_3^{2-}-S} = \frac{effluent \bullet \overline{[S_2O_3^{2-} - S]} + V \bullet \Delta[S_2O_3^{2-} - S]}{\Delta t} \quad (3)$$

Here, *effluent* is the total effluent of the system (L) in time interval Δt (days) (i.e. sample volumes and bleed), $\overline{[SO_4^{2-} - S]}$ and $\overline{[S_2O_3^{2-} - S]}$ the average concentration (mol-S L^{-1}) over time interval Δt, V the total liquid volume of the system and $[SO_4^{2-} - S]$ and $[S_2O_3^{2-} - S]$ the concentration changes (mol-S L^{-1}) over time interval Δt.

The selectivities were calculated according to Eqs. (4), (5) and (6):

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

$$S_{S_2O_3^{2-}} = \frac{P_{S_2O_3^{2-}-S}}{I_{H_2S}} \quad (5)$$

$$S_{S_8} = 1 - \frac{P_{SO_4^{2-}-S} - P_{S_2O_3^{2-}-S}}{I_{H_2S}} \quad (6)$$

Since these values are calculated from the measured concentrations of SO_4^{2-} and $S_2O_3^{2-}$ and the measured bleed rate, there is some scatter on the daily data points. Therefore, the results also show the moving average (average over 5 consecutive days).

The specific sulfide uptake efficiency in the anaerobic bioreactor was calculated based on the H_2S load, the liquid flows and the measured sulfide concentration, according to Eq. (7).

$$sulfide\ \ uptake\ \ anaerobic\ \ bioreactor = \frac{H_2S\ \ load}{solution\ \ flow} - measured\ \ [S_{tot}^{2-}] \quad (7)$$

The specific sulfide uptake is calculated by dividing the sulfide uptake by the biomass concentration. Since the biomass concentration was measured as the total organic N [8,9], the specific sulfide uptake is expressed as mg-S mg-N^{-1}.

3. Results and discussion

3.1. Representative experiment

The performance of the dual-reactor desulfurization process in a representative experimental run (experiment 2) is shown in Fig. 1. Fig. 1A shows the measured concentrations of SO_4^{2-}-S, $S_2O_3^{2-}$-S, alkalinity and conductivity of the process solution. At start-up (day 0), the concentration of SO_4^{2-}-S was 0.10 M, $S_2O_3^{2-}$-S was 0.01 M, alkalinity was 0.65 M and the conductivity was 45.3 mS cm^{-1} . Throughout the experiment, the conductivity, which is a measure for the salinity, was more or less constant (49.1 ± 1.5 mS cm^{-1} on average). The measured SO_4^{2-} and $S_2O_3^{2-}$ concentrations changed based on their net rate of formation and the bleed rate. The SO_4^{2-}-S concentration showed a slight increase during the experiment and increased from 0.10 M to 0.17 M at the end of the experiment. This means that, overall, the formation rate of SO_4^{2-} was higher than its removal rate via the bleed stream. The $S_2O_3^{2-}$-S concentration initially increased and reached its highest value at day 13 (0.03 M). This means that, until day 13, the formation rate of $S_2O_3^{2-}$ was higher than its removal rate via the bleed stream. After day 13, the concentration started to decrease, reaching 0 (i.e. below the detection limit of 0.2 mM) at day 22 and remained 0 until the end of the experiment (day 29). The alkalinity of the process solution decreases because of the formation of SO_4^{2-} and $S_2O_3^{2-}$ (both resulting in proton production) and loss via the bleed. Alkalinity was controlled by the caustic dosing rate, as caustic addition increases the alkalinity. During the experiment, the caustic dosing rate was adjusted in order the maintain the alkalinity as constant as possible. The average alkalinity during the experiment was 0.58 ± 0.05 M and shows a slight decreasing trend. At the end of the experimental run, it was 0.48 M.

Fig. 1B shows the product selectivities (daily values and 5-day moving average). In the initial 7 days of operation, the average selectivity for S_8 formation was 97.6 ± 7.1% and selectivities for SO_4^{2-} and $S_2O_3^{2-}$ were 0.7 ± 6.2 and 1.7 ± 2.1%. During the experiment, the selectivity for S_8 formation decreased gradually and was 91.5 ± 4.3% and the end of the experiment (day 29). This was mainly the result from an increase in the selectivity for SO_4^{2-} during the experiment, to 8.5 ± 4.3% (average of the last 5 days). The selectivity for $S_2O_3^{2-}$ formation (moving average) was around 2% up to day 13, then started to decrease, and from day 20 onwards, the moving average was < 0%. This means that the formation rate of $S_2O_3^{2-}$ was lower than the conversion rate of $S_2O_3^{2-}$. The $S_2O_3^{2-}$-S concentration from day 25 onwards was 0 (i.e. below the detection limit of 0.2 mM), meaning that all $S_2O_3^{2-}$ which was formed, was converted. $S_2O_3^{2-}$ can be used by SOB as substrate [17,18].

Fig. 1C shows the concentration of biomass-N in the process solution and the sulfide uptake in the anaerobic bioreactor, which is the difference between the calculated sulfide concentration and the measured concentration. The initial biomass concentration was 32 mg-N L^{-1} and, due to growth of the bacteria, increased to 62 mg-N L^{-1} at the end of the experiment. The biomass concentration depends on the nutrient dosing rate, biomass growth rate and the removal of biomass via the bleed stream and sulfur cake. The increase in biomass concentration during the experiment indicates the growth rate of the bacteria was higher than removal via bleed and sulfur cake. The sulfide concentration in the rich solution (i.e. the solution in the anaerobic bioreactor) in case no biological removal would take place, was 220 mg-S L^{-1} , which is calculated based on the mass balance of the H_2S dosing rate and the liquid circulation flow rate. The measurement of the sulfide concentration in the anaerobic bioreactor, however, showed that the actual sulfide concentration was lower than calculated, due to the sulfide uptake by bacteria [9,19,20]. On the 2nd day of operation, the measured sulfide concentration was 158 mg-S L^{-1} , i.e. the sulfide uptake was 62 mg-S L^{-1} . On day 20, the sulfide uptake had increased to 98 mg-S L^{-1} . In this period,

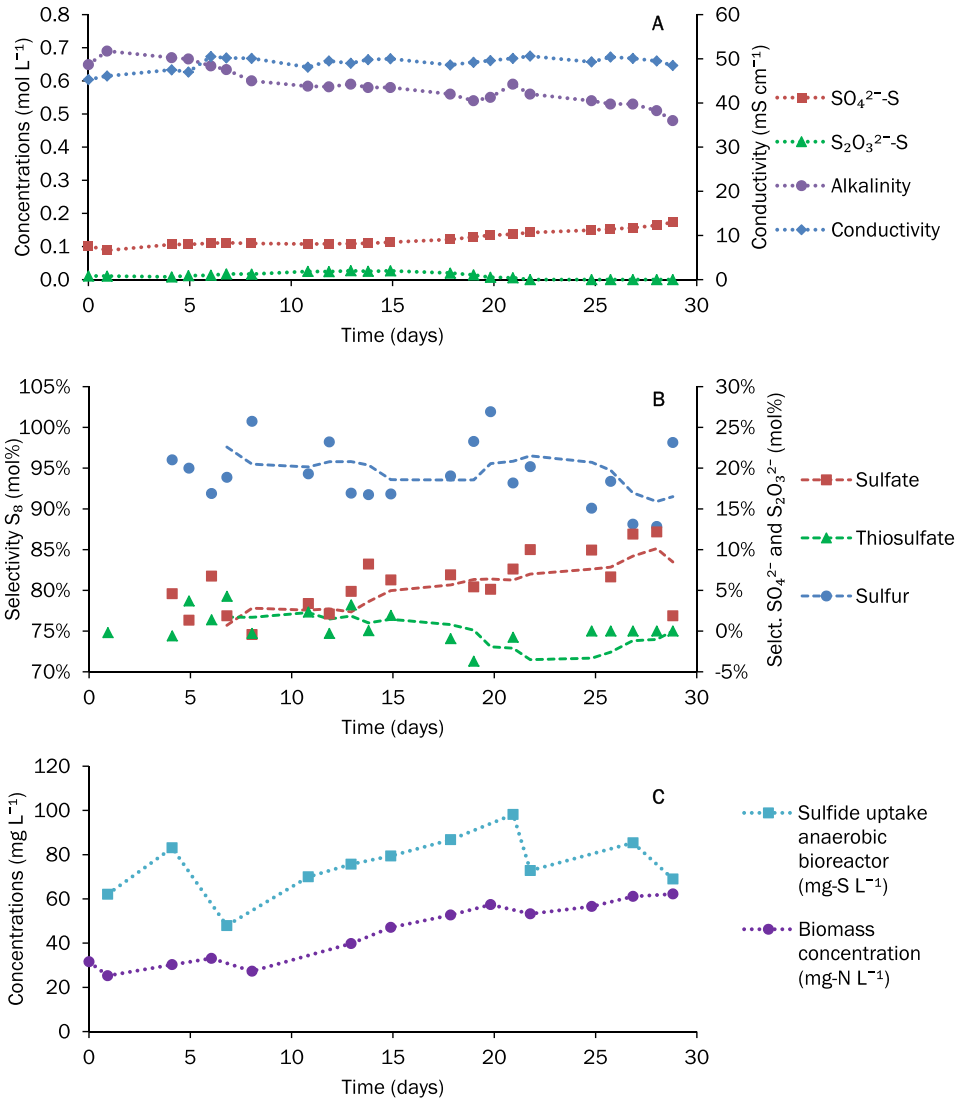

Fig. 1. Experimental results of experiment 2. Fig A shows the concentrations of the SO_4^{2-}, $S_2O_3^{2-}$, the alkalinity and the conductivity. Fig B shows the calculated product selectivities. The dots are the daily measurements and the dashed line indicates the moving average (average value of 5 consecutive measurements). Fig C shows the biomass concentration in the process solution and the sulfide uptake by the bacteria in the anaerobic bioreactor.

the increase in sulfide uptake was proportional to the increase in biomass concentration, and the specific uptake was 1.5 – 2.0 mg-S mg-N^{-1} From day 20–39, the sulfide uptake slightly decreased, although biomass concentration further increased, and on day 29, the specific uptake was 1.1 mg-S mg-N^{-1} . The average sulfide uptake during the whole experiment was 75.5 ± 13.5 mg-S L^{-1} and the average specific uptake was 1.86 ± 0.52 mg-S mg-N^{-1} .

3.2. Effect of process conditions

To study the effect of the conditions in the anaerobic reactor on the process performance, 7 long-term experiments were performed in which the conditions of the anaerobic bioreactor (i.e. HRT and sulfide concentration) were varied. Furthermore, the effect of pH was investigated. An overview of the experimental settings, conditions and results is provided in Table 1. Graphs with results of all experimental runs can be found in supplementary material C.

In none of the experiments, free sulfide was detected in the aerated bioreactor (except during process upsets), meaning that during normal operation all sulfide was converted. Based on the volume of the aerated bioreactor, the sulfide conversion rates were 5.7 g L^{-1} day^{-1} for experiments 1–3, 10.5 g L^{-1} day^{-1} for experiments 4–6 and 14.9 g L^{-1} day^{-1} for experiment 7. To the best of our knowledge, 14.9 g L^{-1} day^{-1} is the highest sulfide conversion rate described in literature.

Statistical analysis with the One-way ANOVA test showed that the pH had a significant effect on the selectivity for S_8 formation (p-value <0.01) in our experiments. Experiments 5 and 6 had the same sulfide concentration and HRT in anaerobic bioreactor (0.48 g L^{-1} and 20 min), but the pH in the aerated bioreactor in experiment 5 was 8.5 and in experiment 6 9.1. The selectivity for S_8 formation decreases from 94.6 ± 3.5% in experiment 5–88.4 ± 6.3% in experiment 6. The decreased selectivity for S_8 formation in experiment 6 was the results of a higher selectivity for SO_4^{2-} formation: 4.7 ± 3.2% in experiment 5 and 12.1 ± 6.3% in experiment 6. The selectivities for $S_2O_3^{2-}$ formation were similar (0.8 ± 2.1% in experiment 5 and −0.5 ± 4.7% in experiment 6). Previous research in a system without anaerobic bioreactor showed that the selectivity for S_8 formation decreased at higher pH and that $S_2O_3^{2-}$ formation increased at higher pH [21]. Based on our experiment it can be concluded that also in the dual reactor line-up, increasing pH results in lower selectivity for S_8.

To assess the effect of sulfide concentration and HRT in the anaerobic bioreactor, experiments 1–5 and 7 were compared. The original hypothesis was that a higher sulfide concentration and a higher HRT in the anaerobic bioreactor increase the inhibition of SO_4^{2-} formation and thus lead to a higher selectivity for S_8 formation. Significant differences were

Table 1
Overview of experimental settings and results. Each experiment was started with the same inoculum, except for experiment 6, which was a continuation of experiment 5.

	Exp 1	Exp 2	Exp 3	Exp 4	Exp 5	Exp 6	Exp 7
S-load (g-S day^{-1})	65	65	65	120	120	120	170
CO_2 flow rate (NL h^{-1})	50	50	50	50	50	5	50
Circulation flow rate (L h^{-1})	12.4	12.4	12.4	10.5	10.5	10.5	8.4
Volume anaerobic reactor (L)	2.2	4.3	6.0	1.7	3.5	3.5	1.5
Theoretical sulfide concentration anaerobic reactor (g L^{-1})	0.22	0.22	0.22	0.48	0.48	0.48	0.85
HRT anaerobic reactor (min)	10	20	30	10	20	20	10
Duration (days)	30	29	31	28	43	17	47
Number of system HRT's	1.5	1.0	1.4	1.5	2.9	1.2	4.1
Average pH anaerobic reactor (-)	7.6 ± 0.1	7.7 ± 0.1	7.7 ± 0.1	7.7 ± 0.2	7.5 ± 0.1	8.8 ± 0.3	7.8 ± 0.1
Average ORP anaerobic reactor (mV)	-420 ± 3	-423 ± 3	-421 ± 2	-428 ± 4	-431 ± 4	-463 ± 5	-441 ± 7
Average pH aerated reactor (-)	8.4 ± 0.1	8.4 ± 0.1	8.4 ± 0.1	8.5 ± 0.1	8.5 ± 0.1	9.1 ± 0.2	8.7 ± 0.2
Average conductivity (mS cm^{-1})	51.8 ± 3.7	49.1 ± 1.5	61.1 ± 2.1	49.0 ± 2.3	51.0 ± 3.2	60.0 ± 2.7	48.5 ± 3.9
Average alkalinity (M)	0.55 ± 0.10	0.58 ± 0.06	0.67 ± 0.06	0.58 ± 0.08	0.63 ± 0.04	0.48 ± 0.06	0.61 ± 0.11
Average biomass concentration (start – end concentration) (mg-N L^{-1})	45 (20 – 89)	44 (32 – 62)	43 (26 – 74)	51 (24 – 82)	48 (29 – 57)	74 (57 – 104)	62 (22 – 114)
Sulfide conversion rate aerated bioreactor (g L^{-1} day^{-1})	5.7	5.7	5.7	10.5	10.5	10.5	14.9
Average selectivity S_8 (%)	91.5 ± 8.4	95.1 ± 5.2	94.2 ± 4.5	96.5 ± 6.6	94.6 ± 3.5	88.4 ± 6.3	94.9 ± 3.5
Average selectivity SO_4^{2-} (%)	7.5 ± 9.3	5.0 ± 5.0	5.6 ± 5.5	3.0 ± 3.4	4.7 ± 3.2	12.1 ± 9.0	3.9 ± 3.2
Average selectivity $S_2O_3^{2-}$ (%)	1.1 ± 3.6	-0.1 ± 5.0	0.2 ± 2.7	0.5 ± 6.5	0.8 ± 2.1	-0.5 ± 4.7	1.2 ± 1.4
Absolute sulfide uptake (mg L^{-1})	54 ± 18	76 ± 14	57 ± 12	113 ± 36	60 ± 43	237 ± 9	377 ± 6
Specific sulfide uptake (mg- S mg-N^{-1})	1.18 ± 0.51	1.86 ± 0.52	1.37 ± 0.55	2.27 ± 0.69	1.25 ± 1.07	4.11 ± 0.46	3.99 ± 0.82
Part of sulfide removed in anaerobic bioreactor (%)	25	35	26	24	13	49	44

found between experiment 1 and 4 (p-value of 0.043) and experiments 1 and 7 (p-value 0.048). In experiments 1, 4 and 7, the HRT in the anaerobic bioreactor was 10 min and sulfide concentrations were 0.22, 0.48 and 0.85 g L^{-1} respectively. These sulfide concentrations appeared not be inhibiting the overall process. In experiment 1, the average selectivity for S_8 formation was 91.5 ± 8.4%. In experiment 4 and 7, selectivities for S_8 formation of 96.5 ± 6.6% and 94.9 ± 3.5% were obtained. This means that with an HRT of 10 min, the selectivity for S_8 was lower at a sulfide concentration of 0.22 g L^{-1} compared to sulfide concentrations of 0.48 and 0.85 g L^{-1} .

Except for experiments 1 and 6, in all experiments, a very high selectivity for S_8 was achieved (>94%). To achieve a selectivity for S_8 formation > 94% in the dual reactor line-up, a certain combination of sulfide concentration and HRT in the anaerobic bioreactor should be met. When the sulfide concentration in the rich solution is 0.22 g L^{-1} , the HRT in the anaerobic reactor should be at least 20 min. When the sulfide concentration is 0.48 g L^{-1} or higher, an HRT of 10 min in the anaerobic bioreactor is sufficient. The highest average selectivity for S_8 formation was achieved in experiment 4 (96.5 ± 6.6%). In a previous study in the same set-up, a selectivity for S_8 formation of 96.9 ± 2.4% was achieved (average over the last 5 days of the experimental run) [9]. In this experiment, the sulfide concentration and HRT in the anaerobic bioreactor were 0.45 g L^{-1} and 20 min, and the pH in the aerated bioreactor was 8.3, i.e., the conditions were most similar to experiment 5 in this study.

The most dominant by-product in all experiments, was SO_4^{2-}, with selectivities ranging from 3.0 ± 3.4% in experiment 4, to 12.1 ± 9.0% in experiment 6. The highest average selectivity for $S_2O_3^{2-}$ formation was observed in experiment 7, which had the highest sulfide loading rate (1.2 ± 1.4%). The lowest average selectivity for $S_2O_3^{2-}$ formation was observed in experiment 6 (the experiment with higher pH): − 0.5 ± 4.7%. The average selectivities for $S_2O_3^{2-}$ formation for all experiments were lower than the average selectivities for SO_4^{2-} formation, meaning that SO_4^{2-} was the main by-product. For experiments 2 and 6, the average selectivity for $S_2O_3^{2-}$ formation was < 0%. As it is known that at least some $S_2O_3^{2-}$ is being produced, and the formation rate increases at higher pH [21], we conclude that the biological oxidation of $S_2O_3^{2-}$ to SO_4^{2-} plays a significant role in the process. This should be further confirmed by biological respiration tests.

The rates of the biological reactions in the biological gas desulfurization process (i.e. formation of S_8 and SO_4^{2-}) are dependent on the O_2/H_2S supply ratio [7,8,21,22]. At higher O_2/H_2S supply ratios, the selectivity for SO_4^{2-} will increase, whereas selectivity for S_8 formation will increase at lower O_2/H_2S supply ratios. In our set-up, the redox potential, which is a measure for the O_2/H_2S supply ratio [8,23,24], was used to control the air flow to the aerated bioreactor. The O_2/H_2S ratio itself was not measured here. In all experiments, the ORP in the aerated bioreactor was controlled at −370 mV by automatic adjustment of the airflow rate, except for a small period in experiment 7, where it was controlled at −380 mV. −370 mV was found to be a convenient ORP [9], meaning that high selectivity for S_8 was achieved with stable operation (i.e. no sulfide in the bulk of the solution in the aerated bioreactor). In case the ORP is too high (i.e. more O_2 dosed), more SO_4^{2-} is formed; in case ORP is too low, sulfide may accumulate, leading to process failure [8]. Furthermore, previous studies found that limitations in the biological activity (e.g. by addition of toxic organo-sulfur compounds), selectivity for $S_2O_3^{2-}$ formation increases whilst selectivity of SO_4^{2-} decreases [13,17]. Potentially, higher selectivities for S_8 formation (i.e. lower selectivities for SO_4^{2-} formation) could have been achieved with a lower ORP set-point and lower biomass concentrations [13], although the drawback is that this will result in a less stable process.

The average pH values, conductivity, alkalinity and redox potential in the anaerobic reactor are shown in Table 1 as well. The average conductivity (a measure for total salinity) and alkalinity (buffer capacity) values in all experiments were in the range of 48–61 mS cm^{-1} and 0.48–0.67 M. In addition, the average pH in the aerated bioreactor in experiments 1–3, all with an S-load of 65 g day^{-1} , was 8.4. The pH in the biological desulfurization process is mainly determined by the absorption of CO_2 in the absorber and the stripping of CO_2 in the aerated bioreactor. Due to the high buffer capacity (alkalinity), fluctuations in pH were limited. In experiments 4 and 5, the pH in the aerated bioreactor was 8.5. Since the S-load in these experiments was higher (120 g day^{-1}), more air was required for the conversion of sulfide. Hence, more CO_2 is stripped, leading to a slightly higher pH. In experiment 7, the pH in the aerated bioreactor was 8.7 because the S-load was 170 g day^{-1}. The average pH values of the rich solution, measured in the anaerobic bioreactor, were 7.6 – 7.8. In experiment 6, however, the pH in both bioreactors was higher due to the lower partial pressure CO_2 in the feed gas. pH values in the aerated and anaerobic bioreactor were 9.1 and 8.8 respectively. In the anaerobic reactor, the ORP is mainly dependent on the sulfide concentration, with more negative ORP at higher sulfide concentrations. For experiments 1–3 (theoretical sulfide concentration of 0.22 g L^{-1}), the average ORP was −420 to −423 mV. In experiments 4 and 5 (0.48 g L^{-1} sulfide), the average ORP was −429 to −431 mV and in experiment 7

(0.85 g L^{-1} sulfide) −437 mV. In experiment 6 (0.48 g L^{-1}sulfide) and a higher pH, the ORP in the anaerobic reactor was −463 mV.

3.3. Microbial community analysis

To determine the microbial community composition and the changes thereof, microbial community analysis based on 16S rRNA gene sequencing of the inoculum and at the end of each experiment has been performed. The results show various known and potential sulfur-oxidizing bacteria (SOB) from the class *Gammaproteobacteria*, such as *Alkalilimnicola*, *Thioalkalivibrio*, *Thioalkalimicrobium* and *Thioalkalispirila* (Fig. 2). Members of the genera *Alkalilimnicola* (relative abundance of 13–67%) and *Thioalkalivibrio* (7–27%) were in particular highly abundant in all experimental runs. These results are comparable to the results obtained in previous experiments in a set-up with an incorporated anaerobic bioreactor, in which *Thioalkalivibrio* appeared as the most abundant genus at the start of the reactor run and was outnumbered by *Alkalilimnicola* by the end of the experiment [9]. *Alkalilimnicola* can grow chemo-autotrophically under anaerobic conditions and therefore has an advantage over *Thioalkalivibrio* which can only grow aerobically [25,26]. The highest relative abundance of *Alkalilimnicola* was observed in experiment 3 (67%), which had the highest HRT in the anaerobic bioreactor. In experiment 7, with the highest sulfide concentration (0.85 g L^{-1}) and low HRT (10 min), the relative abundances of *Alkalilimnicola* and *Thioalkalivibrio* were similar (around 27%), suggesting less competition between these two bacteria species due to availability of more substrate. The other SOB *Thioalkalimicrobium* and *Thioalkalispira* were less abundant (<10%) at the end of all the experiments. Apart from the above-mentioned SOB, members of family *Rhodobacteracea* were found to be highly abundant in all the experiments (11–29%), but not in the inoculum (2%). Known members of this family are *Roseinatronbacter* and *Rhodobaca*, which can oxidize sulfide during organotrophic growth [27–30]. Hence, the high relative abundance of members of the *Rhodobacteracea* can be explained by their anoxygenic growth. Another family member is *Stappia*, which has potential to oxidize $S_2O_3^{2-}$ to SO_4^{2-} and has been detected in sulfide removing bioreactors [31–33]. Both *Alkalilimnicola* and *Thioalkalivibrio* were less abundant at the end of experiments 5 and 6. Members of the family *Rhodospirillacaea* were abundant in these runs and were more relatively abundant at a high pH. The members of *Rhodospirillacaea*, *Rhodospira* and *Rhodopseudomonas sulfidophila* can actively oxidize sulfide at microaerophilic conditions, while using organic compounds as substrates [34,35]. As no organic carbon is added in the system, the potential source of organic compounds could be the dead biomass. The abundance of proteolytic bacteria *Wenzhouxiangella* also indicates the presence of dead biomass. The lowest selectivity for S_8 formation in experiments 6 can therefore be associated with a decrease in the number of chemoautotrophs and increase in heterotrophic bacteria.

The microbial composition analysis clearly revealed the great diversity and high abundance of both autotrophic and heterotrophic SOB. However, to better understand their role, it is essential to know which of them were potentially active. Additionally, to have more insights into the biological processes occurring in the system, it is relevant to understand if other process parameters could influence microbial community composition or vice versa.

3.4. Biological sulfide uptake in the anaerobic bioreactor

To investigate the effect of the sulfide concentration in the anaerobic bioreactor on the specific sulfide uptake, experiments 1, 4 and 7 were compared. In all these experiments, the HRT in the anaerobic reactor was 10 min. The results are shown in Fig. 3A. For experiments 1 and 4, the average specific uptake over the entire run is shown. For experiment 7, the average uptake for days 36–46 is shown, as in this period the sulfide concentration in the anaerobic reactor (i.e. S-load) was at the anticipated level. It was found that an increasing sulfide concentration resulted in a higher specific sulfide uptake.

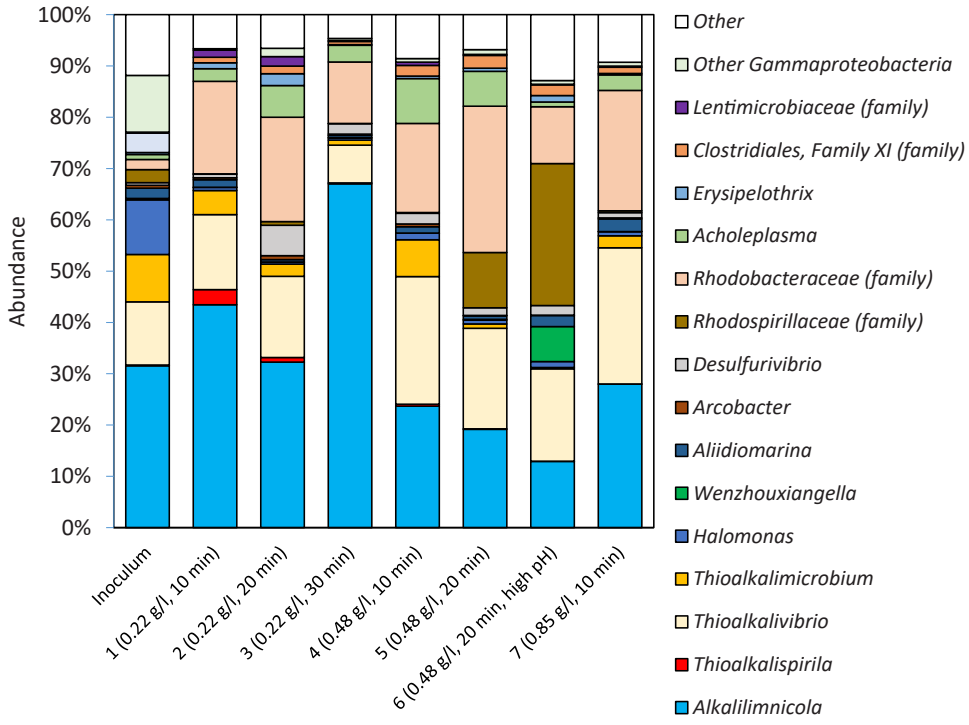

Fig. 2. Taxa bar plot showing the relative abundances of microbial taxa in the aerated bioreactor at the end of the experiments. All species (or families) with a relative abundance of at least 5% in one of the samples are shown.

Secondly, it was noticed that small variations of the pH in the anaerobic bioreactor within a run had effect on the specific sulfide uptake. In Fig. 3B the specific sulfide uptake in experiments 3, 4 and 7 is plotted against the calculated [OH^-]. This shows that the pH in the anaerobic bioreactor and specific sulfide uptake are positively correlated. The specific sulfide uptake for experiments 4, 5 and 6 was also compared, see Fig. 3C. In these experiments, the theoretical sulfide concentration in the anaerobic reactor was 0.48 g L^{-1} . It was found that the average specific sulfide uptake increased with increasing pH. In experiment 6, the experiment with increased pH, the highest specific sulfide uptake was found.

Our results show that more sulfide uptake from the process solution in the anaerobic bioreactor takes place when: i) the sulfide concentration is higher, and ii) the pH in the anaerobic bioreactor is higher. Whereas H_2S can freely pass the cell membrane [36–38], most ions require active transport [39]. At higher pH, more sulfide is present in the ionic form (i.e. as HS^-). The fact that H_2S can freely pass the cell membrane whilst HS^- probably requires active transport seems contradictory with the observation that more sulfide is removed from solution at higher pH. However, it is also known that at higher pH, more sulfide is present as polysulfides (S_x^{2-}), which is formed due to an equilibrium reaction between HS^- and elemental sulfur (S_8), see Eq. (8) [40,41]. As S_8 is present in excess, the concentration of S_x^{2-} in the experiments increases with higher HS^- concentration (i.e. higher S-load) and at higher pH [42].

$$HS^-(aq) + \frac{x-1}{8}S_8(s) \leftrightharpoons S_x^{2-}(aq) + H^+(aq) \quad (8)$$

The permeability of membranes for solutes depends on their partition coefficient, i.e. the ratio in solubility in the membranes and water [43], which suggests that S_x^{2-} is easier to transport than HS^-. Hence, we hypothesize that mainly S_x^{2-} is removed from solution in the anaerobic bioreactor. The method we have used measured the sum of all forms of sulfide (i.e. S^{2-}, HS^- and polysulfide-sulfane (S_x^{2-})). We haven't experimentally quantified the concentration of S_x^{2-} separate from the concentration of HS^-. Further experimental work dedicated on S_x^{2-} is required to confirm the role of S_x^{2-} in the observed biological sulfide removal in the anaerobic bioreactor.

Based on this observation we hypothesize the following sulfide conversion routes in the biodesulfurization process are taking place (see Fig. 4). In the 'sulfidic zone', consisting of the absorber and anaerobic bioreactor, H_2S is initially chemically absorbed from the gas phase to the liquid phase. In the alkaline solution it is deprotonated into bisulfide (HS^-). Due to the equilibrium reaction with elemental sulfur (see Eq. (8)), polysulfide (S_x^{2-}) is formed. Based on our results, we hypothesize that S_x^{2-} is taken up by the bacteria. Part of it is stored in the cell ("bound-S_x^{2-}") and part is converted to S_8, e.g. by reducing electron carriers, which stores electrons ("e^--shuttling") [9,19,20]. This means that the bacteria are becoming reduced in the sulfidic zone. In the 'microaerophilic zone', i.e. the aerated bioreactor, dissolved O_2 from the compressed air is used as final electron acceptor. In this zone the bacteria are transferred into the oxidized form. Since there is no free sulfide in the effluent of the aerated bioreactor, all residual sulfide is converted. The suppression of enzymes in the pathway for SO_4^{2-} formation, by sulfide in the sulfidic zone, results in an increased overall system performance in terms of selectivity for S_8 formation.

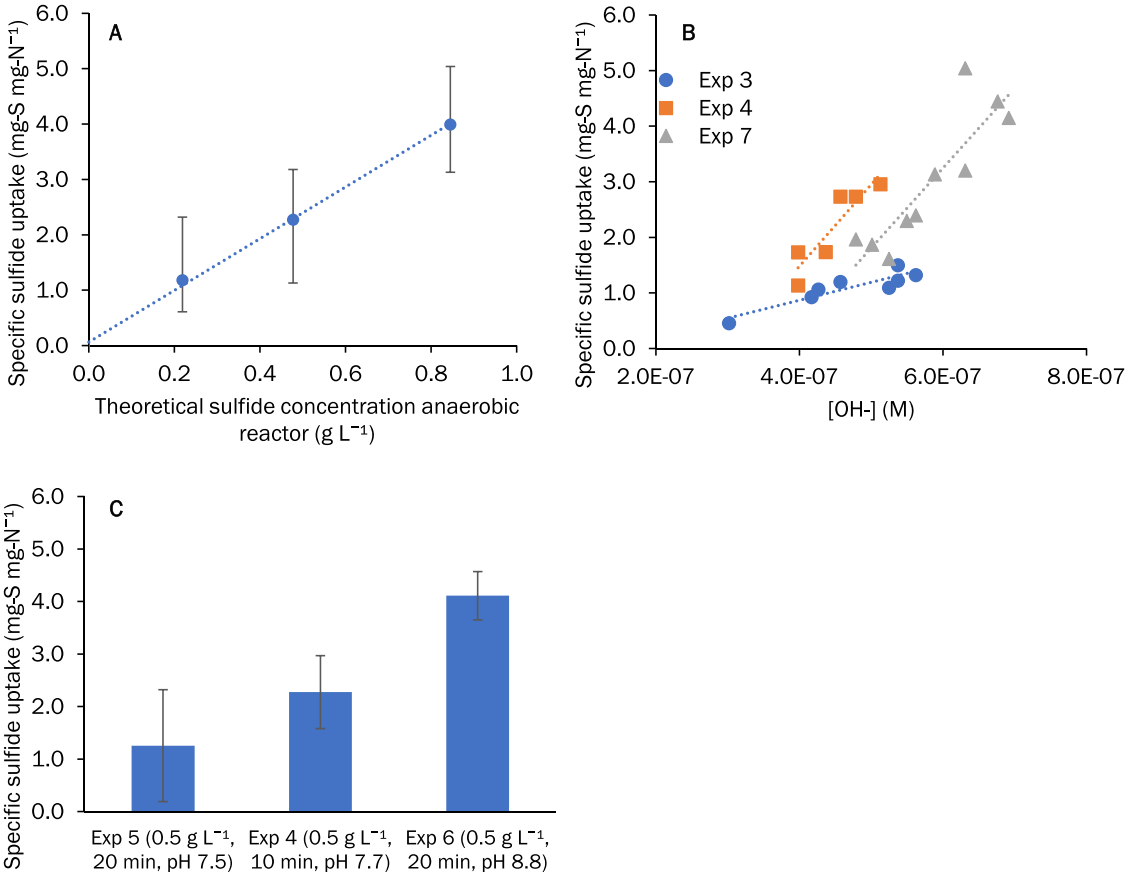

Fig. 3. Effect of process conditions on the specific sulfide uptake in the anaerobic bioreactor. Fig. A shows the effect of sulfide concentration in the anaerobic reactor. The specific sulfide uptake increases with sulfide concentration. Average values with the minimum and maximum measured values are shown. A trendline is included to guide the eye. Figs. B and C show the effect of pH in the anaerobic bioreactor on specific sulfide uptake. Figure B shows the daily measurements of specific sulfide uptake plotted against the calculated OH^- concentration for several experiments (3, 4 and 7). Linear dashed lines are included to 'guide the eye'. Figure C shows the average specific sulfide uptake for the experiments with a theoretical sulfide concentration in the anaerobic bioreactor of 0.48 g L^{-1} .

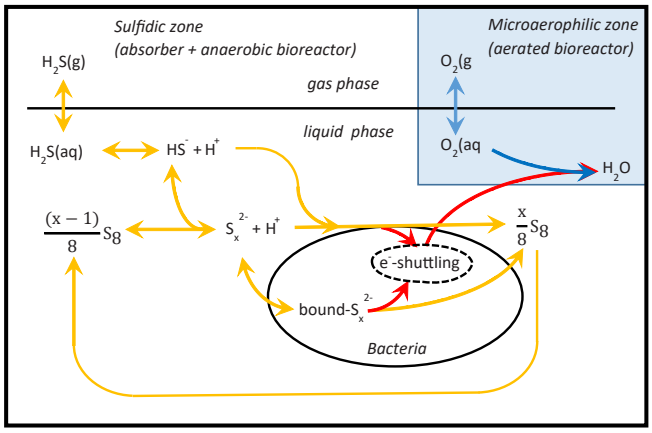

Fig. 4. Schematic overview of hypothesized sulfide conversion route in the biological gas desulfurization process. The process is separated in the 'sulfidic zone' (in white), consisting of the absorber and anaerobic bioreactor, and the microaerophilic zone (in blue), consisting of the aerated bioreactor. In the sulfidic zone, sulfide is present and O_2 is absent; in the microaerophilic zone, O_2 is available. In the sulfide zone, polysulfide is taken up from the solution by the bacteria and is either stored (bound-S_x^{2-}), or converted to S_8 and the electrons are being stored. In the microaerophilic zone O_2 is used as final electron acceptor and all sulfide is converted. Conversion of sulfur compounds is indicated with yellow arrows; electron shuttling is indicated with red arrows and reactions with O_2 are indicated with blue arrows.

4. Conclusion

We investigated the effect of the process factors sulfide concentration and HRT in the anaerobic bioreactor and pH on product formation and biological sulfide uptake in the dual reactor biodesulfurization process. For the experiments with a pH of 8.4 – 8.7 in the aerated bioreactor, the selectivities for S_8 were 94 – 96 mol% (average over an experiment), provided the HRT in the anaerobic bioreactor was higher than 10 min or the sulfide concentration in the anaerobic bioreactor was higher than 0.2 g L^{-1}. An increase in pH in the aerated bioreactor to 9.1 resulted in higher SO_4^{2-} formation and therefore lead to a lower selectivity for S_8 formation (88 mol%). Furthermore, biological sulfide uptake in the anaerobic reactor increased at higher sulfide concentration in the anaerobic reactor and higher pH, suggesting the biological uptake of sulfide in the anaerobic bioreactor is related to polysulfide formation. Although a higher pH results in higher sulfide uptake in the anaerobic bioreactor, this leads to a lower selectivity for S_8 formation of the overall process.

CRediT authorship contribution statement

Rieks de Rink planned and performed the experiments, analyzed the results and wrote the manuscript. Suyash Gupta analyzed the microbial community data and wrote the section on the microbial communities. Flavia Piccioli de Carolis and Dandan Liu supported experimental work and data analysis. Jan Klok, Annemiek ter Heijne and Cees Buisman supported study organization and data analysis and revised the manuscript. All authors read and approved the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jece.2021.106450.

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