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Article

Polysulfide Concentration and Chain Length in the Biological Desulfurization Process: Effect of Biomass Concentration and the Sulfide Loading Rate

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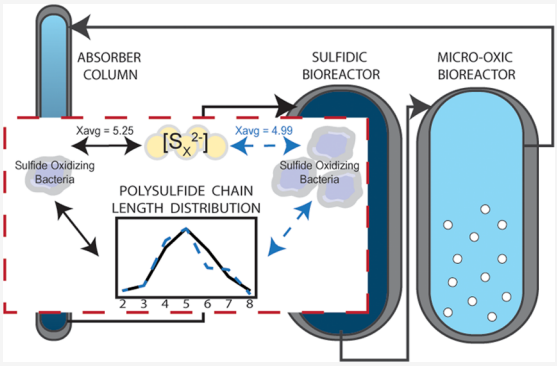
ABSTRACT: Removal of hydrogen sulfide (H_2S) can be achieved using the sustainable biological desulfurization process, where H_2S is converted to elemental sulfur using sulfide-oxidizing bacteria (SOB). A dual-bioreactor process was recently developed where an anaerobic (sulfidic) bioreactor was used between the absorber column and micro-oxic bioreactor. In the absorber column and sulfidic bioreactor, polysulfides (S_x^{2-}) are formed due to the chemical equilibrium between H_2S and sulfur (S_8). S_x^{2-} is thought to be the intermediate for SOB to produce sulfur via H_2S oxidation. In this study, we quantify S_x^{2-}, determine their chain-length distribution under high H_2S loading rates, and elucidate the relationship between biomass and the observed biological removal of sulfides under anaerobic conditions. A linear relationship was observed between S_x^{2-} concentration and H_2S loading rates at a constant biomass concentration. Increasing biomass concentrations resulted in a lower measured S_x^{2-} concentration at similar H_2S loading rates in the sulfidic bioreactor. S_x^{2-} of chain length 6 (S_6^{2-}) showed a substantial decrease at higher biomass concentrations. Identifying S_x^{2-} concentrations and their chain lengths as a function of biomass concentration and the sulfide loading rate is key in understanding and controlling sulfide uptake by the SOB. This knowledge will contribute to a better understanding of how to reach and maintain a high selectivity for S_8 formation in the dual-reactor biological desulfurization process.



KEYWORDS: *biotechnology, desulfurization, polysulfides, sulfide-oxidizing-bacteria, sulfur*

INTRODUCTION

Biological gas desulfurization under haloalkaline conditions was developed in the 1990s[1,2] and has been commercially applied worldwide.[3,4] This process removes hydrogen sulfide (H_2S) from various sour gas streams, such as natural gas and biogas, and predominantly oxidizes it to elemental sulfur (S_8). Unlike other physical and chemical processes, the biological desulfurization process operates at ambient temperature and pressure, making it a more sustainable and cost-effective technology.[5]

The removal and conversion of H_2S to S_8 is achieved through a multistep process configuration that conventionally uses an absorber column and a micro-oxic bioreactor. The H_2S-containing gas (sour gas) is fed upward through an absorber column, where it is counter-currently contacted with a (bi)carbonate process solution. H_2S is absorbed into the haloalkaline process solution, and subsequently, the majority of the dissolved H_2S is converted into a mixture of bisulfide (HS^-) and polysulfides (S_x^{2-}). S_x^{2-} is formed via the chemical equilibrium reaction between HS^- and S_8 present in the solution.[6]

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

Due to chemical equilibrium reactions, S_x^{2-} of chain length x exists in solution and is distributed over the range of 2−9. This mechanism can be generally expressed via the following chemical reaction (eq 2).[7]

$$2S_x^{2-} \rightleftarrows S_{x-1}^{2-} + S_{x+1}^{2-}, \quad x \geq 2 \quad (2)$$

The process solution containing (poly)sulfides leaves the absorber column and is fed to a micro-oxic bioreactor where sulfide-oxidizing bacteria (SOB) convert the (poly)sulfides in solution to S_8, forming a solid fraction.[8] The end-product S_8 can be removed from the process via, for example,

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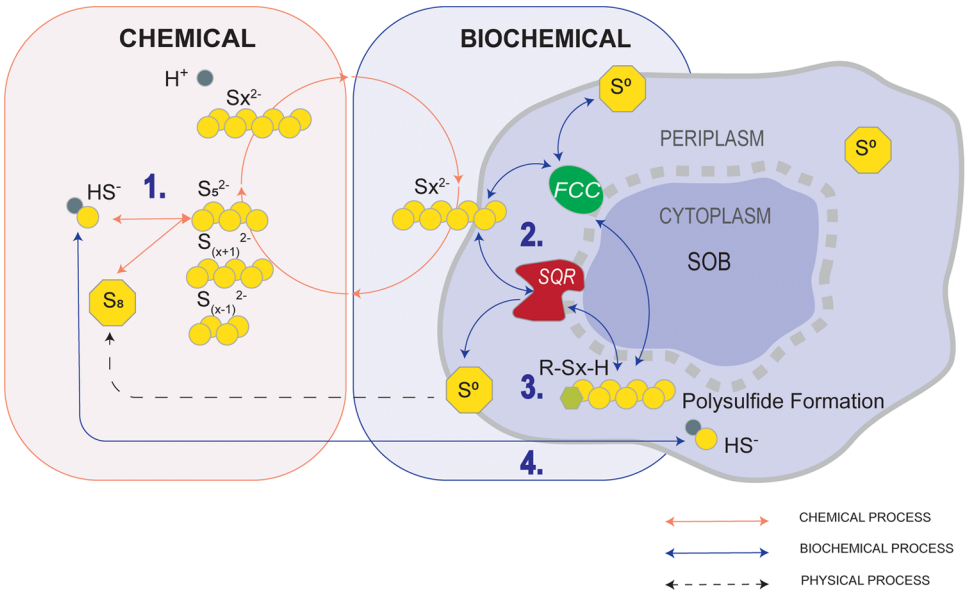

Figure 1. Summary of the current knowledge on the multiple pathways between chemical S_x^{2-} formation outside of the cell and the biochemical formation and breakdown of S_x^{2-} in and around the sulfide-oxidizing bacteria (SOB). Illustrated above, (1) chemically, sulfide and sulfur react in solution to form S_x^{2-}. In the biochemical processes, current knowledge illustrates (2) the potential use of S_x^{2-} by the enzyme system SQR or FCC,[20,26,27] followed by (3) the formation of sulfur and potentially S_x^{2-};[28] (4) sulfide is known to be able to cross-cell membranes.[29]

centrifugation. After removal and dewatering, S_8 can be used in agricultural applications.[9]

In the biological desulfurization process, sulfate (SO_4^{2-}) and thiosulfate ($S_2O_3^{2-}$) are produced as unwanted acidifying by-products. Sulfate is produced via the biological oxidation of HS^- and $S_2O_3^{2-}$,[10] and $S_2O_3^{2-}$ is produced through the chemical oxidation of HS^- and S_x^{2-}.[11] The formation of both by-products has been shown to be highly influenced by the operational process parameters.[12−14] By-product formation results in the need for additional caustic and makeup water, a bleed stream, and greater usage of oxygen and nutrients, all of which increase operational costs.

In recent years, a new process configuration has been introduced that uses an anaerobic (sulfidic) bioreactor, i.e., nonaerated and at high sulfide concentrations, located in the flow scheme between the absorber column and the micro-oxic bioreactor.[15] This dual-bioreactor process scheme has been proven to decrease the formation of both SO_4^{2-} and $S_2O_3^{2-}$. The formation for SO_4^{2-} decreased because the sulfidic conditions in the sulfidic bioreactor inhibited a key enzymatic pathway. The formation for $S_2O_3^{2-}$ decreased because part of (poly)sulfide was removed from the solution in the sulfidic bioreactor, resulting in a lower concentration entering the micro-oxic bioreactor.[15,16]

In addition to limiting by-product formation, the new biological desulfurization configuration has also provided insight into the abilities of the SOB. It has been observed that the SOB possesses an electron shuttling capacity, i.e., the bacteria catalyze the redox reactions and act as both an electron acceptor and an electron donor. SOB can partially remove (poly)sulfide in the sulfidic bioreactor and later reduce oxygen within the micro-oxic bioreactor.[17] The main biological and chemical reactions of (poly)sulfide removal, especially within the solution, have been studied, and recent experiments have found the removal to be more efficient at higher pH and higher sulfide loads.[16,18] However, the underlying mechanisms of (poly)sulfide removal within and near the SOB remain to be determined.

Previous research has determined that SOB can remove S_x^{2-} in solution and biologically oxidize it to elemental sulfur.[19,20] S_x^{2-} can potentially be consumed and/or produced by the enzymes within the SOB (Figure 1). Within the SOB, sulfide oxidation to elemental sulfur can be performed by either flavocytochrome sulfide dehydrogenase (FCC) or sulfide:quinone reductase (SQR).[21,22] The FCC route is shown to be suppressed when exposed to high sulfide concentrations leading SQR to become dominant.[10,15,23] Previous studies have shown that SQR produces soluble S_x^{2-} as the primary product[24] and that S_x^{2-} can be present in the cells of SOB[25] or used as the intermediate in the production of sulfate.[20,26] Therefore, SOB exposed to (poly)sulfides may be able to store and further oxidize them to elemental sulfur.

In addition to biological interactions, chemical S_x^{2-} equilibrium and distribution have been studied extensively without biology.[6,30,31] Calculated thermodynamic constants (pK) are reported between 9.18 and 14.43 for S_x^{2-} of chain lengths 2−8,[31] and for biologically produced sulfur, a pK value of 9.17 has been reported.[31,32] In addition to pK values, the concentration of S_x^{2-} has been shown to increase with pH in excess of HS^- and S^0, the distribution of S_x^{2-} does not change from pH of ∼7 to 12, and equilibrium between S_x^{2-} species occurs rapidly in the order of 10 s.[33] However, the rate at which equilibrium is reached depends on HS^- concentration, pH, temperature, and the state of elemental sulfur, making measurements necessary to understand S_x^{2-} in solution.[34−36]

S_x^{2-} concentrations and their chain lengths were previously measured by Roman et al. within a full-scale biological desulfurization process at the outlet of the absorber column, but with the single reactor configuration. Additionally, the samples were stabilized after 2 h of transportation time, which permitted (bio)chemical reactions to take place. In follow-up studies, S_x^{2-} concentrations were measured from samples that were stabilized immediately in controlled lab-scale sulfidic

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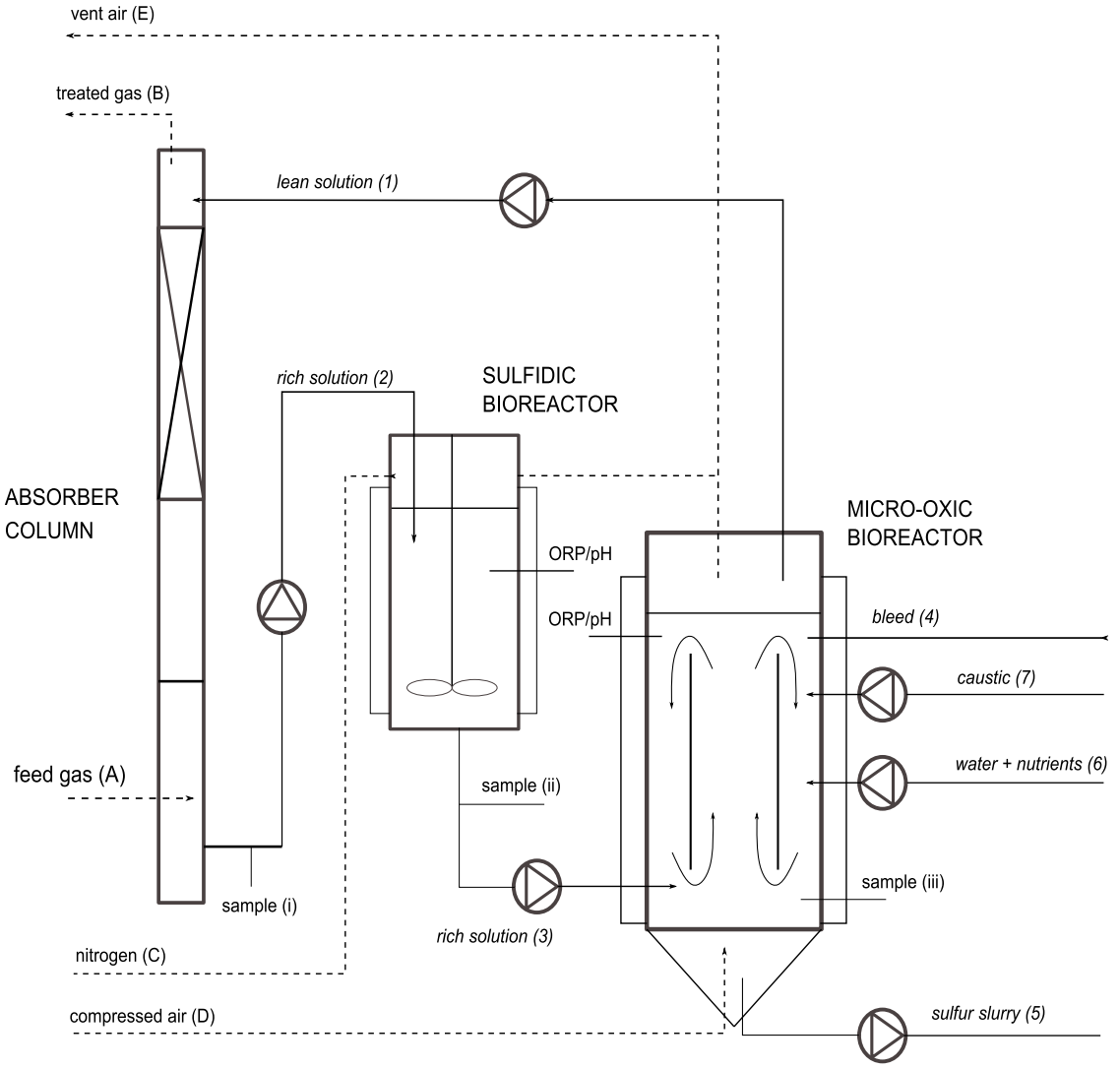

Figure 2. Schematic overview of the pilot system with the gas (dashed line) and liquid (solid line) flows. More details of the setup can be found elsewhere.[15]

bioreactors. These experiments, however, focused on the removal of thiols and were performed under low volumetric H_2S loading rates typically not seen in practice.[37,38] Considering these factors, S_x^{2-} has yet to be measured under controlled conditions and at sulfide concentrations like those seen in industrial applications. Therefore, the role of all and specific species of S_x^{2-} in the biological desulfurization process remains unknown despite it being pivotal to understanding how to design the sulfidic bioreactor for the best S_8 selectivity. Improving the S_8 selectivity can improve the efficiency and stability of the biological desulfurization process, which can increase its implementation over other desulfurization technologies.

The aim of this study was to assess S_x^{2-} concentrations and their chain lengths under industrially relevant conditions utilizing a pilot-scale biological desulfurization setup with a sulfidic bioreactor. Additionally, the study aimed to elucidate a relationship between S_x^{2-} concentrations and chain length and the biomass concentration in the sulfidic bioreactor.

MATERIALS AND METHODS

Experimental Setup. The experimental setup used a pilot-scale biological desulfurization installation, which included a pressurized absorber column maintained between 2.7 and 3.0 bar (g), sulfidic bioreactor, micro-oxic bioreactor, and decanter centrifuge (Figure 2). A mixture of nitrogen (N_2), carbon dioxide (CO_2), and H_2S gases entered below the packing material in the absorber column. Each gas was supplied and controlled separately through mass flow controllers (Profibus, Brooks instruments, Hatfield, PA). The sump of the absorber column (where the sulfidic solution was collected) had a total liquid volume of 1.0 L. The liquid volumes were set at 2.4 and 11.4 L in the sulfidic bioreactor and micro-oxic bioreactor, respectively. The sulfidic bioreactor was equipped with a mechanical mixer (rzr2020, Heidolph Instruments, Schwabach, Germany), and N_2 was injected into the headspace to ensure anaerobic conditions. Both bioreactors were equipped with water jackets connected to a thermostat bath (Kobold, Germany) to keep the bioreactor liquid temperatures constant at ~35 °C.

Pumps continuously circulated the process liquid containing buffered medium, sulfur particles, dissolved sulfur species, and

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SOB over the entire system. The solution leaving the absorber column contained dissolved sulfide (i.e., H_2S, HS^-, S_x^{2-}, and S^{2-} "rich" solution), whereas dissolved sulfide could not be detected in the solution entering the absorber column from the micro-oxic bioreactor ("lean" solution). Nutrients, caustic solution, and makeup water (~150 mL day^{-1}) were continuously supplied to the bioreactor. The same nutrient solution used by de Rink et al. was supplied for the growth of the bacteria. Caustic was dosed to maintain a constant alkalinity of 0.5 M HCO_3^-, and makeup water was supplied to maintain a constant conductivity (i.e., salinity) of 55 mS-cm^{-1}. Solution left the system at an average flow of 1.2 kg day^{-1} over 3 months through an overflow from the micro-oxic bioreactor, giving the system a total hydraulic retention time (HRT) of ~14 days.

During the experiments, the oxidation−reduction potential (ORP) and pH were continuously measured with a probe (SE552/2 Inducon ORP/pH sensor) connected to a Stratos Pro Transmitter (Knick, Berlin, Germany). An integrated Ag/AgCl electrode was a reference for both ORP and pH.

Experimental Operation. The bioreactor was inoculated with a bleed solution from the dual-reactor pilot unit that had been stored at 4 °C. The total experiment duration was one month, in which the H_2S loading rate and biomass concentration were varied. During this period, the composition of the SOB microbial community was monitored weekly using next-generation sequencing (NGS). Detailed sample preparation, analyses, and bioinformatics information can be found in Supporting Information 1.

The setup was continuously operated with 10 kg h^{-1} of liquid circulating throughout the system. At this circulation rate, the HRT was approximately 5 min for the absorber column, 15 min for the sulfidic bioreactor, and 45 min for the micro-oxic bioreactor. The pressure in the absorber column was kept between 2.7 and 3.0 bar (g). A constant stream of 100 Ln h^{-1} (normal liters) of N_2 flowed to the absorber column. The CO_2 flow varied between 15 and 40 Ln h^{-1} as it fluctuated to maintain the system's pH. The pH of the micro-oxic bioreactor was between 8.3 and 8.7, with an average of 8.48 ± 0.10, while the pH of the sulfidic bioreactor varied between 7.7 and 8.0. The solution's alkalinity was, on average, 0.54 M ± 0.12 M (total concentration of $NaHCO_3$ and Na_2CO_3 and expressed as the concentration of Na^+). The H_2S flow varied between 0.8 and 4.4 Ln h^{-1}, corresponding to ~30 to 150 g-S day^{-1}. The lowest H_2S flow rate had a volumetric loading rate of 1.92 g-S $L^{-1}day^{-1}$, more than 3 times higher than previous volumetric H_2S loads in lab-scale bioreactors.[37,38] Compressed air was supplied to the micro-oxic bioreactor with the flow rate controlled by a PI controller to maintain the ORP value set-point at −350 mV, with the average being −349 ± 21 mV throughout the experiment.

Liquid samples were taken from the absorber column and the sulfidic bioreactor. The first sampling port was located at the bottom of the absorber column before the sulfidic bioreactor, and the second sampling port was located in a recirculation line of the sulfidic bioreactor (Figure 2). The H_2S load was increased incrementally (~10 g-S day^{-1} each time) over the duration of multiple weeks. Samples were taken at H_2S loading rates of 27, 38, 47, and 58 g-S day^{-1} over the entire system volume of 14.3 L at two different biomass concentrations, 24 and 90 mg-N L^{-1} to study the influence of biomass on the S_x^{2-} concentration and chain-length distribution. Full-scale units typically operate at biomass concentrations between 50 and 100 mg-N L^{-1}. Therefore, 24 mg-N L^{-1} is considered "low" as it is half of the typical biomass concentration.[15] To achieve the higher biomass concentration, the nutrient dosing rate was increased in the micro-oxic bioreactor.

Changing the H_2S inflow to the absorber caused fluctuations in the ORP of the micro-oxic bioreactor. Therefore, samples were taken only after a minimum 1 h wait time—15 min for the ORP to stabilize again plus a minimum of 45 min (3 HRTs) for the sulfidic bioreactor to reach a steady state.

Batch Bottle Control Experiments. Abiotic batch bottle experiments were conducted to determine the S_x^{2-} concentration and distribution under abiotic conditions. Batch bottles were chosen, as continuous operation of the pilot desulfurization system is unfeasible without the presence of SOB, as hydrogen sulfide would build up over time. During the experiment, only sulfide, biologically produced sulfur, and carbonate medium were added to the batch bottles. Biologically produced sulfur was washed with Milli-Q water and centrifuged 3 times. With each centrifuging step, biomass was scraped from the surface of the pellet and removed. The resulting biosulfur was analyzed using a scanning electron microscope (SEM) to ensure biomass was no longer attached. Sodium carbonate medium was created using 77 g-L^{-1} of $NaHCO_3$, 4.8 g-L^{-1} of Na_2CO_3, 1 g-L^{-1} of K_2HPO_4, and 0.2 g-L^{-1} of $MgCl_2{\cdot}6H_2O$. The resulting solution had a Na^+ concentration of 1 M and a pH of ~8.5. Micronutrients were not added to the medium, as trace metals are known to have a catalytic effect on S_x^{2-}.

A 100 mM-S sulfide stock solution was made using $NaHS{\cdot}9H_2O$. This solution was added in different volumes to create initial sulfide concentrations similar to those found in the pilot with 3.9, 5.2, 6.5, and 7.9 mM-S initial sulfide concentrations and 4.7, 6.3, 7.9, and 9.4 mM-S sulfur concentrations. Sulfur was always in excess, with 20% more sulfur than the highest sulfide concentration.

The bottles were left to mix overnight on a shake table at 25 °C. Samples were taken from the bottles the next day within an anoxic environment, and the same analysis method was used to determine S_x^{2-} and their chain lengths. Sample analysis was performed in triplicate. All sample volume was taken from the same sampling syringe.

Analyses. *Biomass Analysis.* Biomass concentration was quantified spectrophotometrically based on the total amount of organic nitrogen (N) oxidized to nitrate by ammonium persulfate (LCK138, Hach Lange, Tiel, The Netherlands). Biomass-N is an indicator of the total biomass in the system, which includes SOB based on NGS data (Supporting Information 1). A sample was taken from the micro-oxic bioreactor and split into two separate parts. One part was centrifuged for 10 min at 15 000 rpm, while the other part was left uncentrifuged. The total concentration of biomass in terms of nitrogen was determined by subtracting the supernatant total N from the total N of the uncentrifuged samples. Biologically produced sulfur has been found to not interfere with the results if the samples were 5 times diluted.[14,15] The haloalkaline SOB has the generic stoichiometric chemical equation $CH_{1.8}O_{0.5}N_{0.2}$ where N is 10% mole of the total dry-weight biomass, allowing for indirect characterization of the biomass.[20] Since the biological desulfurization system is a continuous process in a steady state, it can be assumed that biomass concentrations are uniform throughout the system.

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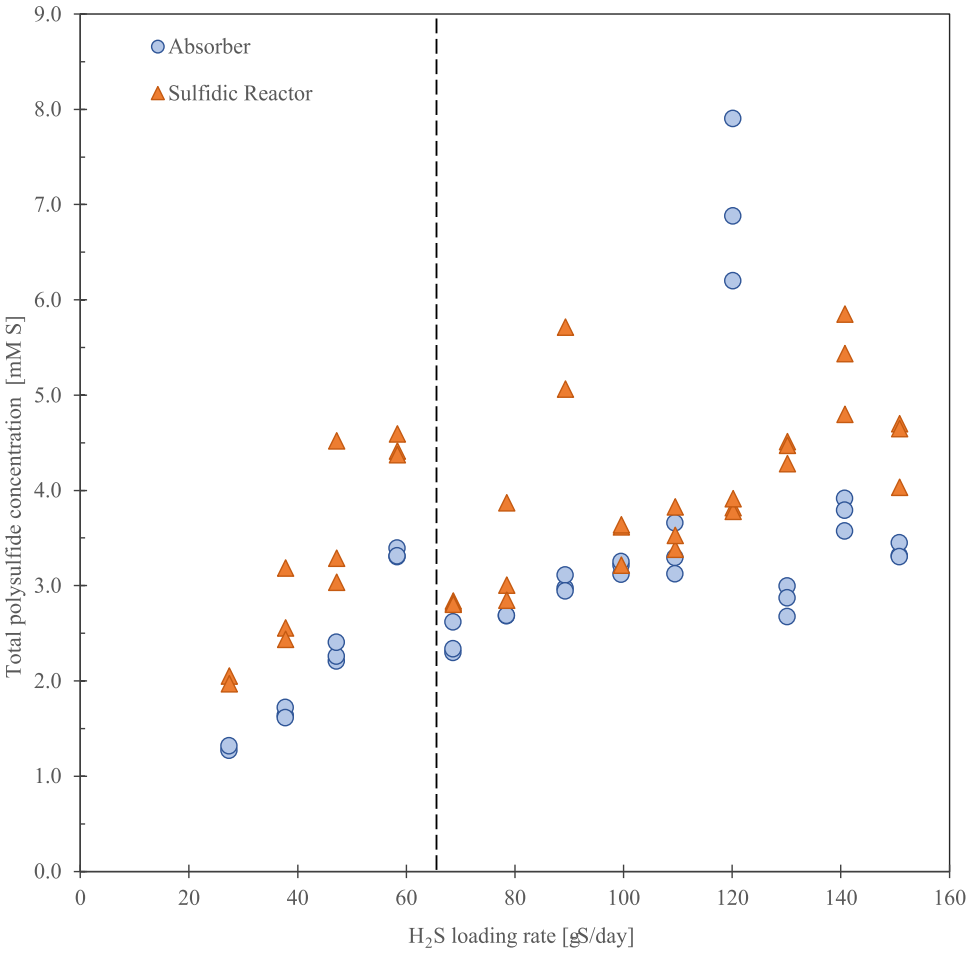

Figure 3. Total S_x^{2-} concentration (mM-S) at increasing sulfide loading rates (g-S day^{-1}) in the rich solution of the absorber column and sulfidic bioreactor. The dashed vertical line indicates when the nutrient dosing was increased to grow more biomass to handle the increased H_2S supply to the system.

Sample Preparation for Polysulfide Analysis. S_x^{2-} samples were stabilized by using the procedure described in Roman et al.[37] (Supporting Information 2). The pH for each sample was determined by using a pH probe prior to stabilizing the samples with methyl triflate (≥98% pure, Sigma-Aldrich, The Netherlands) to form more stable dimethyl polysulfanes (eq 3).[7]

$$S_x^{2-} + 2CF_3SO_3Me \rightarrow Me_2S_x + 2CF_3SO_3^- \quad (3)$$

After methylation and the addition of the internal standard (dibenzo-a, h-anthracene, Supelco Analytical) dissolved in benzene (Sigma-Aldrich, The Netherlands), samples were stored at 4 °C for no more than 4 days. Samples were centrifuged at 3300*g* for 10 min to ensure any remaining particles had settled out of the solution before analysis.

Polysulfide Analysis. The samples were analyzed using an ultra-high-performance liquid chromatograph (uHPLC) with a UV detector (Dionex UtliMate 3000RS) to determine the separate fractions of different chain lengths of S_x^{2-} in solution. The uHPLC was equipped with an Agilent column (Zorbax Extend-C18 18 μm, 2.1 × 50 mm^2) operated at 20 °C, and the UV detector was set to 210 nm. For the uHPLC analysis, the flow rate was 0.371 mL min^{-1} with 1.25 μL injection volume. First, a mobile phase consisting of a methanol (15% vol) and water (85% by vol) mixture entered the column. After 0.72 min, a convex gradient developed until methanol reached 85% vol after 10 min had passed. For the next 10 min, the system was isocratic. In the following 5 min, methanol decreased to 15% vol. The last 5 min of the uHPLC run were isocratic again.

The concentration of individual S_x^{2-} chain lengths (2−8 sulfur atoms) was determined using the peak areas from the uHPLC column, internal standard, and response factors (RF) from Roman et al. Total S_x^{2-} concentration is the sum of all of the chain lengths, and the average chain length was calculated based on the S_x^{2-} fraction of each chain length present in the samples. All samples were analyzed in at least triplicate from the same sampling syringe.

RESULTS AND DISCUSSION

Total Polysulfide Concentration Varies With Increased Sulfide Loading Rates. The pilot-scale biological desulfurization system was operated for 24 days during which the sulfide loading rate was incrementally increased from 28 to 151 g-S day^{-1}. S_x^{2-} concentrations and their chain lengths (x = 2−8) were determined in the rich solution in the absorber column sump and the sulfidic bioreactor.

The total S_x^{2-} concentration ranged from 1.3 to 7.9 mM-S in the absorber column and 2.0−5.9 mM-S in the sulfidic bioreactor (Figure 3). Excluding one loading rate (121 g-S day^{-1}), the sulfidic bioreactor contained, on average, 45% more S_x^{2-} than the absorber column. A strong linear correlation was

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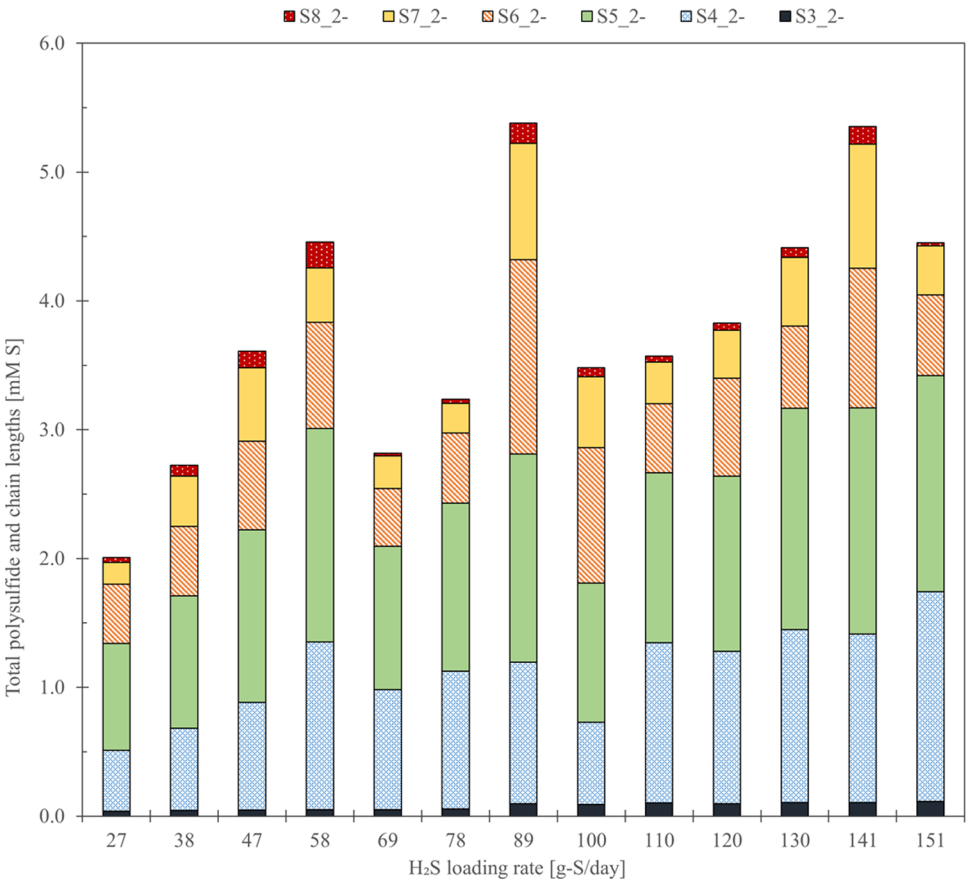

Figure 4. Distribution of S_x^{2-} chain lengths was determined over increasing sulfide loading rates. S_2^{2-} was present at values below 0.3% and was excluded from the graph as its values were negligible in comparison with the other chain-length percentages (more than a factor of 10 lower than the next lowest chain length). Raw data can be found in Supporting Information 4.

observed between total S_x^{2-} concentration and H_2S loading rates of 28−69 g-S day^{-1}. However, total S_x^{2-} concentration varied once the H_2S loading rate increased beyond 69 g-S day^{-1} (Figure 3). Other operational process parameters, such as the pH and ORP, remained constant throughout the entire experiment. The most likely explanation for this abrupt decrease in total S_x^{2-} concentration would be a change in biomass concentration as more bacteria enabled higher cell membrane permeation of S_x^{2-} into the cells.[29,39] Over the course of the experiment, the biomass concentration increased 68% from ∼24 to 90 mg-N L^{-1} (Supporting Information 3).

Even though the chemical conditions are similar for both solutions in the absorber column and sulfidic bioreactor, a difference in S_x^{2-} concentration was observed. The average concentrations of S_x^{2-} were found to be in the range of 2.69 ± 1.10 mM-S (with the outlier at 15.7 mM-S removed) in the absorber column and 3.80 ± 1.17 mM-S in the sulfidic bioreactor (Figure 3). The difference in total S_x^{2-} concentration was expected due to the difference in HRTs between the two reactor sections (i.e., 5 min for the absorber column and 20 min in total (5 + 15) for the sulfidic bioreactor), which may not be sufficient time to reach equilibrium, as described in a later section.

In addition to the total S_x^{2-} concentration, individual S_x^{2-} anions were determined. Results showed that pentasulfide (S_5^{2-}) was the predominant species for all conditions, which is in agreement with previous results[37] (see also eqs 4 and 5). The formation of S_5^{2-} leads to the formation of tetrasulfide (S_4^{2-}), hexasulfide (S_6^{2-}), trisulfide (S_3^{2-}), heptasulfide (S_7^{2-}), disulfide (S_2^{2-}), and octasulfide (S_8^{2-}) (Figure 4).

$$S_8^0 + HS^- \leftrightarrow S_9^{2-} + H^+ \quad (4)$$

$$S_9^{2-} + HS^- \leftrightarrow 2S_5^{2-} + H^+ \quad (5)$$

During this experiment, S_8^{2-} was present at all loading rates even though S_8^{2-} had not been detected in prior experiments using either lab setups or a full-scale installation.[37,38] The absence of S_8^{2-} in previous experiments can most likely be attributed to three reasons: (1) lower sulfide concentrations and sulfide:sulfur ratios, (2) the time between sampling and sample derivatization, and (3) the sulfidic retention time. First, previous lab-scale experiments were conducted at much lower sulfide levels (between 0.16 and 0.24 mM-S), leading to lower overall S_x^{2-} concentrations (eq 1).[37,38] Greater concentrations of S_x^{2-} in solution can lead to larger chain lengths, such as S_8^{2-} being present at quantifiable concentrations. Second, immediate stabilization was not possible for S_x^{2-} from the prior full-scale installation experiments. Before S_x^{2-} was derivatized, samples had to be transported to the laboratory, which took around 2 h. Therefore, the chemical equilibrium in the solution likely changed due to the presence of active biomass and potential oxidation. Finally, S_x^{2-} formation is highly influenced by the sulfidic retention time, which was limited in both previous studies due to experimental setup constraints.

Impact of Biomass Concentration on the Concentration and Chain-Length of Individual Polysulfide

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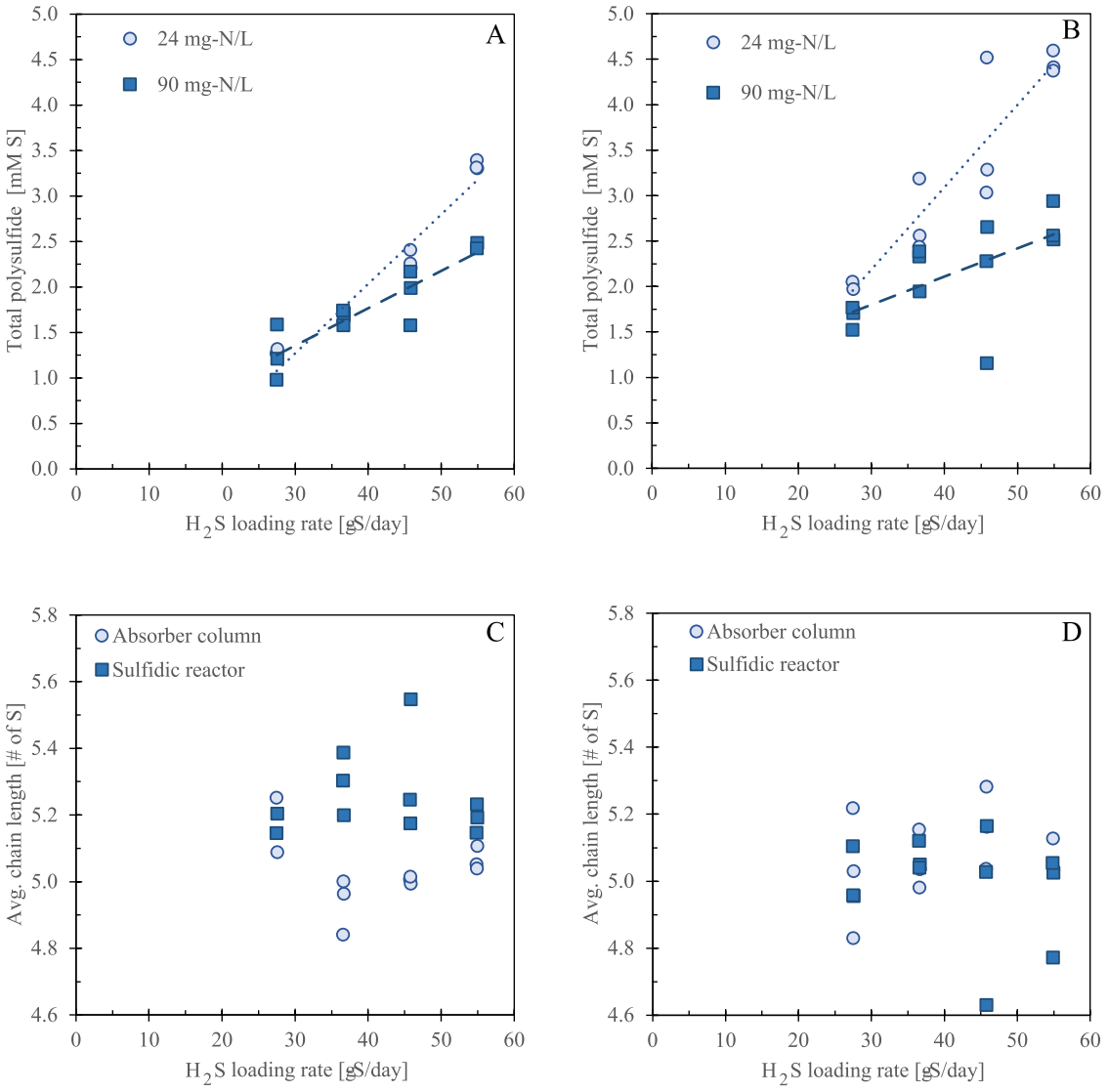

Figure 5. Total S_x^{2-} within the system at a pH set-point of 8.5 versus the H_2S loading rate under low (24 mg-N L^{-1}) and high (90 mg-N L^{-1}) biomass concentrations in both the (A) absorber column and (B) sulfidic bioreactor. The dashed lines on the graph are placed as a guide for the eye. The average chain length of S_x^{2-} was determined within the system at a pH set-point of 8.5 versus the concentration of sulfide within the absorber column and sulfidic bioreactor during the operations with (C) low (24 mg-N L^{-1}) and (D) high (90 mg-N L^{-1}) biomass concentrations.

Anions in the Process Solution. After the initial experiment where only the H_2S loading on the system increased, the first four H_2S loading rates (27, 38, 47, and 58 g-S day^{-1}) were repeated using a higher biomass concentration. The amount of biomass increased from 24 to 90 mg-N L^{-1} by increasing the nutrient dosing rate.

At the initial H_2S loading rate of 27 g-S day^{-1} (theoretical concentration of 3.93 mM-S of sulfide), the total S_x^{2-} concentration in the absorber was 1.29 ± 0.04 mM-S at low biomass and 1.25 ± 0.10 mM-S at high biomass, while the total S_x^{2-} concentration in the sulfidic bioreactor was 2.01 ± 0.06 mM-S at low biomass and 1.67 ± 0.07 mM-S at high biomass. These starting values are similar to each other despite the fact that biomass concentration increased by a factor of ∼4. Once the dissolved sulfide concentration was increased, total S_x^{2-} increased for both biomass concentrations and in both the absorber column and the sulfidic bioreactor (Figure 5A,B). The total S_x^{2-} concentration was lower at high biomass concentrations (90 mg-N L^{-1}) than at low biomass concentrations (24 mg-N L^{-1}). The greatest difference in total S_x^{2-} concentration between the low and high biomass concentrations was at the loading rate of 58 g-S day^{-1} (7.85 mM-S of sulfide). At the 58 g-S day^{-1} loading rate, a difference of 0.88 ± 0.08 mM-S in the absorber and 1.79 ± 0.14 mM-S in the sulfidic bioreactor was observed.

As previously mentioned, the differences between the absorber column and sulfidic bioreactor can most likely be attributed to different HRTs. The absorber column had a 5 min HRT, whereas the sulfidic bioreactor had a 15 min HRT on top of the 5 min already spent in the absorber column. This lower total S_x^{2-} concentration in the absorber suggests that HS^- and S_8 had not yet reached equilibrium with S_x^{2-} in the absorber column. Previous studies show that the rate at which chemical S_x^{2-} equilibrium occurs is dependent on process conditions such as HS^- concentration, pH, temperature, and the state of the elemental sulfur.[34−36] Since the conditions in the system were similar, most likely, the SOB influenced the equilibrium of S_x^{2-} in solution since the concentration of biomass increased by a factor of almost 4. Thus, similar observations in the absorber column are made for low and high

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biomass, as the HRT was not long enough for the SOB to influence the concentration of total S_x^{2-}.

To further understand the results obtained in the pilot experiment, batch bottles were used to obtain data on S_x^{2-} in solution without biomass. When the solution is undersaturated with respect to sulfur, overnight mixing is sufficient for chemical equilibrium to be reached.[40] If, however, the solution is supersaturated with regards to sulfur, the kinetics will change and equilibrium may take much longer.[41] Total S_x^{2-} concentrations resulted in a linear relationship with a resulting slope similar to that from the data obtained from the lower biomass concentration experiment, but with a smaller intercept (1 mM-S at sulfide concentration of 4 mM-S). This similar slope in the line indicates that experiments with a low biomass concentration and with no biomass have similar equilibria in solution. With the addition of more biomass, there is a shift in equilibrium, as shown by the decrease in slope. It is hypothesized that as the ratio of sulfide to biomass is higher, the chemical reactions dominate the solution, as sulfur is always in excess.

When the biomass concentration was increased, the total biomass (which included the SOB) was in excess and able to grow to a concentration that is unachievable at the low H_2S loading rates. Our hypothesis is that the excessive SOB caused a shift in S_x^{2-} equilibrium by increasing the amount of SOB–S_x^{2-} interactions. Biologically, S_x^{2-} can cross over the cell membrane due to their lipophilicity and can interact with the sulfur-producing enzymes within the SOB.[29,42] For example, the SOB with the SQR enzyme has been shown to store S_x^{2-} in their periplasm in the form of organic S_x^{2-} and make less S_x^{2-} available in solution to form more S_x^{2-}.[43] With an excessive amount of biomass, more S_x^{2-} could be stored within the SOB because their main form of substrate is limited, forcing them into "starvation" and increasing storage. Thus, less S_x^{2-} could be present in the bulk solution as these SOB are no longer "fully active" due to the lack of a substrate.

The average chain length for both high and low biomass operations was determined by calculating the weighted average of S_x^{2-} with 2–8 sulfur atoms (Figure 5C,D). At low biomass, the average chain length for the four H_2S loading rates was 5.05 ± 0.02 for the absorber and 5.25 ± 0.03 for the sulfidic bioreactor. At higher biomass levels, the average chain length for the four sulfide loading rates was 5.08 ± 0.04 for the absorber and 4.99 ± 0.06 for the sulfidic bioreactor. When comparing the measurements from the absorber column and the sulfidic bioreactor, S_x^{2-} in the absorber column were, on average, 0.2 sulfur atoms shorter than in the sulfidic bioreactor and had a lower average chain length at 75% of the time (Figure 5C). As for the higher biomass concentration, no distinct difference in the S_x^{2-} chain length between the absorber column and sulfidic bioreactor could be observed (Figure 5D).

Since the system operational parameters remained constant throughout the experiment, it is presumed that the equilibrium of the bulk solution did not significantly impact the average S_x^{2-} chain length at the excess biomass concentration. We hypothesize that the higher average S_x^{2-} chain length in the sulfidic bioreactor compared to the absorber column at the lower biomass concentration could be explained due to the limited 5 min HRT of the absorber column. As the system is assumed to be at a steady state, the SOB could shift the system equilibrium if they are at a high enough concentration and utilize S_x^{2-} as a substrate. At a lower biomass concentration, we hypothesize that this "fully active" biomass could assist in the production of longer S_x^{2-} chains. Previous research has shown S_x^{2-} to be microbiologically produced, and could be excreted by the SOB or react with sulfur globules inside the cell.[25,33]

In addition to the average chain length, the chain-length distribution was compared between both the low and high biomass concentrations (Figure 6). With no biology present, a

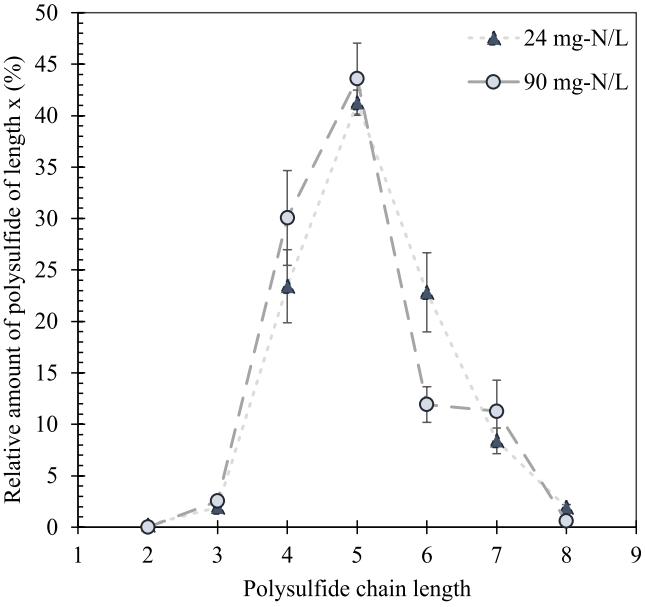

Figure 6. Profile of the distribution of S_x^{2-} chain lengths for the 27 g-S day^{-1} loading rate in the sulfidic bioreactor for low and high biomass concentrations.

distribution based on eq 2 forms. Pentasulfide (S_5^{2-}) is the first to form and continues to form mixtures of S_x^{2-} ions, leading to the formation of other chain lengths.[37] A close to the normal distribution is expected based on this chemical equilibrium with S_5^{2-} as the most dominant chain length. At the lower biomass concentration (i.e., 24 mg-N L^{-1}), S_5^{2-} is the dominant chain length with the other chain lengths, following the "normal" distribution pattern. However, at the higher biomass concentration (90 mg-N L^{-1}), a different distribution is seen with S_5^{2-} still being the most prevalent chain length, but an increase in S_4^{2-} and S_7^{2-} and a substantial decrease in S_6^{2-} can be observed. This pattern occurs in both the absorber column and sulfidic bioreactor for all of the repeated sulfide loading rates (Supporting Information 5).

During the high biomass experiment, the SOB could be considered "in excess," making the biochemical reactions that utilize S_x^{2-} dominant in comparison to the chemical reactions. Therefore, the distribution at high biomass was disturbed due to the excess amount of SOB in solution, causing S_x^{2-} with chain lengths of 6 and 8 to decrease, while chain lengths of 4 and 7 increased. We hypothesize that the SOB influences S_x^{2-} through one or only a few chain lengths to disrupt the typical distribution. Even though S_x^{2-} is lipophilic, potentially, the charge over the entire S_x^{2-} molecule influences what can pass through the periplasmic membrane of the SOB, i.e., the larger the chain length, the less relative charge the entire S_x^{2-} molecule has. Additionally, the enzymes within the SOB could prefer certain chain lengths over others due to adapted binding sites, suggesting a preference for S_6^{2-} and S_8^{2-}. However, more research is needed to understand the complex

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relationship between the SOB and S_x^{2-} of specific chain lengths.

CONSIDERATIONS

In this study, S_x^{2-} of chain lengths between 3 and 8 were detected and quantified in the biological desulfurization process. Up until this work, S_8^{2-} had not been detected in the biological desulfurization system, as previous work only found chain lengths of 2−7. The concentration of S_x^{2-} in the absorber column was consistently less than the concentration in the sulfidic bioreactor. This difference in concentration can be attributed to the difference in HRTs between the absorber column (5 min) and sulfidic bioreactor (5 + 15 min).

The biological desulfurization system is complex due to the multiple physical and chemical properties that can affect S_x^{2-} concentration and chain length. Therefore, to elucidate the relationship between S_x^{2-}, H_2S loading rate, and biomass, only the H_2S loading rates and biomass concentration were varied. This study found that the presence and concentration of biomass influence the concentration of S_x^{2-} in solution and the chain-length distribution. When the biomass concentration increased, the total concentration of S_x^{2-} decreased at the four different H_2S loading rates. The lower biomass concentration resulted in a difference in the average S_x^{2-} chain length between the absorber column and sulfidic bioreactor, where the average S_x^{2-} chain length was lower for the absorber column. We hypothesize that the difference in chain lengths was most likely due to longer chains of S_x^{2-} being taken up by the SOB or that the SOB facilitates the production of S_x^{2-} when given a longer time exposed to sulfidic conditions. Additionally, changes in relative concentration were observed between S_x^{2-} chain lengths where S_6^{2-} was found to decrease, while S_4^{2-} and S_7^{2-} increased at high biomass concentrations. More research is needed to determine if the SOB prefers certain chain lengths and if intracellular S_x^{2-} accumulates. However, it seems apparent that the SOB uses S_x^{2-} as an intermediate in the sulfidic bioreactor, which is a major step forward in understanding reaction mechanisms in the biological desulfurization process.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.3c03017.

(1) Detailed NGS materials, methods, and results; (2) detailed information on the polysulfide sample preparation; (3) biomass concentration results during the experiment; (4) detailed polysulfide chain-length distribution results; (5) summary of average polysulfide chain lengths results, and (6) results of chain-length profiles of additional H_2S loading rates (PDF)

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Notes

The authors declare no competing financial interest.

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