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Selection and application of sulfide oxidizing microorganisms able to withstand thiols in gas biodesulfurization systems

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Abstract

After the first commercial applications of a new biological process to remove hydrogen sulfide (H_2S) from low pressure biogas, the need arose to broaden the operating window to enable the removal of organosulfur compounds from high pressure sour gases. In this study we selected microorganisms, from a full-scale biodesulfurization system, that are capable of withstanding the presence of thiols. This full-scale unit has been in stable operation for more than 10 years. We investigated the microbial community by using next-generation sequencing of 16S rRNA gene amplicons which showed that methanethiol gave a competitive advantage to bacteria belonging to the genera *Thioalkalibacter* (*Halothiobacillaceae* family) and *Alkalilimnicola* (*Ectothiorhosdospiraceae* family). The sulfide-oxidizing potential of the acclimatized population was investigated under elevated thiol loading rates (4.5 - 9.1 mM d^{-1}), consisting of a combination of methanethiol, ethanethiol, and propanethiol. With this biomass, it was possible to reach a stable bioreactor operation at which 80% of the supplied H_2S (up to 61 mM d^{-1}) was oxidized to elemental sulfur. Moreover, it was found that a conventionally applied method to control oxygen supply to the bioreactor, i.e. by maintaining a redox potential set-point value, appeared to be ineffective in the presence of thiols.

1. Introduction

Thiols are analogues of alcohols with a general formula of RSH, where R represents an aliphatic chain. These organosulfur compounds can be present in many sour gas streams such as landfill gas, liquefied petroleum gas and natural gas.[1–3] The presence of thiols in gas streams is associated with the occurrence of hydrogen sulfide (H_2S). Both are corrosive and acidifying compounds that contribute to air pollution and are toxic to humans. Hence, H_2S and thiols need to be removed from sour gas streams prior to consumption by applying either physicochemical or biological desulfurization processes.[4]

We have developed a family of gas biodesulfurization processes that operate under haloalkaline conditions, i.e. at elevated pH values (>8.5) and carbonate concentrations (~ 1 M) to ensure high H_2S absorption rates from the reaction with hydroxyl and (bi)carbonate ions.[5,6] It is known that haloalkaliphilic sulfur-oxidizing bacteria (SOB) are suitable for sulfide oxidation because of their high sulfide-oxidizing capacity at elevated salt and pH levels.[7,8]

Short chain thiols such as methanethiol (MT), ethanethiol (ET), and n-propanethiol (PT) have pK_a values around 10.4.[9,10] Consequently, these compounds are removed from gas streams together with H_2S under alkaline conditions (Eq. 1). However, in contrast to H_2S, thiols inhibit SOB already at ~0.6 µM.[11,12] Dissociated thiols (RS^-) are strong nucleophiles and therefore very reactive.[13] For example, a reaction between thiol and biologically produced sulfur globules results in the rapid formation of diorgano polysulfanes (Eq. 2).[14] The first reaction product is diorgano pentasulfide, which immediately undergoes interconversion reactions to form a mixture of organosulfur compounds (Eq. 3).

Moreover, thiols can be easily oxidized by oxygen to diorgano disulfides (Eq. 4), where the oxidation rate of thiols decreases with increasing molecular weight.[15]

$$RSH + OH^- \rightarrow RS^- + H_2O \quad (1)$$

$$2\ RS^- + S_8 \rightarrow RS_nR + S_x^{2-}, \text{ with } n+x = 10 \quad (2)$$

$$2\ RS_nR \leftrightarrow RS_{n-1}R + RS_{n+1}R, \text{ with } n>3 \quad (3)$$

$$2\ RSH + 0.5\ O_2 \rightarrow RS_2R + H_2O \quad (4)$$

Literature about the effect of thiols and diorgano polysulfanes on biodesulfurization processes is scarce and there is a growing need to expand the application window of existing biodesulfurization systems for thiol removal which necessitates more research. This paper describes the effect of high loading rates (4.5 - 9.1 mM d^{-1}) of the most common volatile thiols (MT, ET, and PT) on the overall performance of gas biodesulfurization processes. According to our knowledge, the effect of higher thiols (ET and PT) on biological H_2S removal process has not been extensively studied yet. In order to find an appropriate inoculum for a bench-scale bioreactor, the biomass from a full-scale installation treating a natural gas condensate stream containing H_2S and thiols was used. A detailed description of this desulfurization plant is provided in the Supplementary Information (S1). The acclimatization to and the effect of MT, ET, and PT on the microbial population was monitored by using next-generation sequencing of 16S rRNA gene amplicons.

2. Materials and methods

2.1. Experimental setup

The experimental setup consisted of a falling film gas absorber and a fed-batch air-lift bioreactor (Fig. 1). A falling-film absorber was chosen to avoid any

sulfur plugging issues, as previously encountered with a packed column. Table 1 shows the dimensions and process conditions of the gas absorber. The liquid volume of the bioreactor was 2.2 L and the volume of the gas absorber was 0.2 L. The total liquid volume remained constant throughout each experimental run. Oxygen gas (99.995 vol.%) was supplied to the bioreactor by a mass flow controller (type EL-FLOW, model F-201DV-AGD-33-K/E, 0-30 mL min^{-1}, Bronkhorst, the Netherlands) to control the oxidation reduction potential (ORP) value. The same type of mass flow controller was used to feed H_2S (99.8 vol.%, 0-17 mL min^{-1}), MT, ET, and PT (1 vol.% in N_2, 0-30 mL min^{-1}), and N_2 gas (99.995 vol.%, 0-350 mL min^{-1}) to the gas absorber and the bioreactor. Carbon dioxide (99.99 vol.%) was fed to the inlet of the gas absorber with a solenoid valve (125318, Burkert, Germany) when the pH of the bioreactor medium was above its set-point value. The oxygen and carbon dioxide supply rates were controlled with a multiparameter transmitter (Liquiline CM442; Endress+Hauser, the Netherlands) based on real-time signals from the redox potential electrode (Ag/AgCl reference electrode, Orbisint CPS12D; Endress+Hauser) and a pH sensor (Orbisint CPS11D; Endress+Hauser), respectively. A gear pump (EW-74014-40, Metrohm Applikon, Schiedam, the Netherlands) recycled the liquid between the bioreactor and the gas absorber. The gas phase from the bioreactor headspace was continuously sent to the bottom of the bioreactor (34 L min^{-1}) with a gas compressor (N-840, KNF, Germany). The bioreactor and the gas absorber were kept at 35 ℃ with a thermostatted bath (DC10-P5/U, Haake, Germany). Gaseous samples were collected from sampling points placed at the inlet and outlet of the gas absorber and in the bioreactor headspace (Fig. 1). Liquid samples were collected from a sampling point located in the middle section of the bioreactor (Fig. 1).

2.1.1. Medium composition

The reactor medium included a carbonate/bicarbonate buffer of 0.1 M Na_2CO_3 and 0.8 M $NaHCO_3$. Furthermore, the medium contained 1.0 g K_2HPO_4, 0.2 g $MgCl_2 \times 6\ H_2O$, and 0.6 g urea per 1 L Milli-Q ultrapure water. A trace elements solution was added (1 mL L^{-1}) as described elsewhere.[16] The final pH of the medium was kept constant at 9.00 ± 0.01 at 35 ℃.

2.1.2. Inoculum

The bioreactor was inoculated with cells obtained by centrifugation (30 min at 16,000 x *g*) of a 2-L culture collected from a full-scale installation used to desulfurize a natural-gas condensate containing low concentrations of thiols (as described in S1, Supporting Information).

2.2. Experimental design

Unless stated otherwise, all experiments were performed in the experimental setup as described in section 2.1. Firstly, we acclimatized the biomass originating from the full-scale installation (described in S1, Supporting Information) to MT, which is the most common and toxic of all thiol species.[17,18] This process was performed under a gradually increasing MT loading rate ranging from 0.1 to 7.6 mM d^{-1} over a period of 63 days (see Section 3.1). During the selection period there was no N_2 flow over the bioreactor (Fig. 1).

Secondly, we studied the potential application of the acclimatized biomass to remove H_2S in the presence of MT, ET, and PT. These experiments were conducted under various flow rates of N_2 gas (0-18 L h^{-1}) over the bioreactor to assess the effect

of the volatile organic sulfur compounds (VOSCs) stripping on the system performance. An overview of the operating conditions for these experiments is given in Table 2. The total MT and ET loading rate of the gas absorber was 9.1 mM d^{-1}, while the PT loading rate was 4.5 mM d^{-1} for experiments in which single thiols were supplied to the system. All experimental runs were conducted under sulfur-producing conditions (ORP = -390 ± 5 mV). The H_2S loading rate was kept constant at 61.3 mM d^{-1}. Before the onset of each experiment, the ORP was kept constant within ± 5 mV. Each experiment lasted for 24 h, during which four gas and liquid samples were taken at regular time intervals. For experiments described in Table 2, the ionic charge balance was established by comparing equivalent amounts of cations and anions (see Section 2.3). The difference was 4 ± 3 % indicating that there was no significant gap in the sulfur balance.

2.3. Analytical techniques

Liquid sample preparation and chemical analysis of sulfur compounds (sulfur, sulfate, and thiosulfate) and biomass concentration were performed as described previously.[19] In addition to sulfur containing anions, we also analyzed Na^+ and K^+ with ion chromatography as described for the anions[19] except that a Metrohm Metrosep C4 - 150/4.0 mm column was used with a mobile phase of 0.9 mL 3 mM HNO_3 min^{-1}.

To close the ionic charge balance, the carbonate and bicarbonate ion concentrations were calculated using the Henderson-Hasselbalch equation[20] on the basis of inorganic carbon determined using high temperature catalytic oxidation at 680 ºC in a TOC-VCPH/CPN analyzer (Shimadzu, the Netherlands). Before starting

the analyses, all solids were removed by filtration over a 0.22 µm syringe filter (Millex G5 filter unit; Millipore). The samples were subsequently stored at 4 ℃.

Concentrations of gaseous compounds (H_2S, O_2, N_2, and VOSCs; i.e. MT, ET, PT, and their diorgano polysulfanes) were analyzed using gas chromatography with a flame photometric detector as described previously.[19] To identify VOSCs, we used gas chromatography (6890N, Agilent, the Netherlands) coupled with a triple quadrupole mass spectrometer (5975, Agilent, the Netherlands), equipped with an Agilent column (HP-5MS, 30 m x 0.25 mm x 0.25 µm, Agilent, the Netherlands). Initially, the oven temperature was 50 ℃. After 2 min, a gradient of 12.5 ℃ min^{-1} was applied to reach 200 ℃. We operated the mass spectrometer in SIM mode with a filament voltage of 70 eV and an electron multiplier voltage of 1200 to 2800 V. Helium was the carrier gas, with a flow rate of 1.3 mL min^{-1}. The injection volume was 1 mL.

2.4. DNA extraction and 16S rRNA gene sequencing

Biomass samples were collected for microbial community analysis of respectively (1) the inoculum; (2) samples collected during the selection process for MT-tolerant biomass; and (3) at the beginning and the end of each experimental run (Table 2). The samples were washed twice with a buffer of pH 9 and 0.5 M Na^+ to prevent the occurrence of an osmotic shock. The washing was performed by (1) centrifuging the samples at 20,000 x *g* for 5 min; (2) removal of the supernatant; and (3) addition of fresh buffer and mixing with a vortex to re-suspend the pellet. Afterwards, Total Genomic DNA was extracted from the washed biomass using the PowerBiofilm™ DNA Isolation Kit (MoBio, USA) following the manufacturer's instructions. All the above procedures were performed in duplicate for each sample.

The 16S rRNA gene profiling for the samples collected during the selection process was performed as described previously.[17] For biomass samples taken during the experimental runs with different thiols, partial 16S rRNA genes were amplified using primers 341F and 805R[21] following a modified PCR protocol by Hugerth et al. (2014).[22] Dual-index multiplexed library construction and sequencing was carried out at Science for Life Laboratory, Sweden (www.scilifelab.se) on the Illumina MiSeq platform as 2 × 301 pair-ends according.[23] Sequence data were processed using the UPARSE pipeline[24] and annotated against the SINA/SILVA database (SILVA 119)[25] before analysis in Explicet 2.10.5.[26] The number of clustered operational taxonomic units (OTUs) and additional sequence data are given in Supporting Information (S2). The EMBL-EBI accession number for presented 16S rRNA sequences is PRJEB14146.

2.5. Effect of diorgano polysulfanes on the redox potential

We investigated the effect of dimethyl-, diethyl-, and dipropyl polysulfanes on the ORP by using a setup consisting of a glass mini-reactor (60 mL) equipped with a magnetic stirrer as described elsewhere.[27] The reactor was closed with a Teflon piston. The ORP was measured with a redox potential electrode (Ag/AgCl reference electrode, Orbisint CPS12D; Endress+Hauser). A multiparameter transmitter (Liquiline CM442; Endress+Hauser, the Netherlands) was used to record the signals from the ORP sensor. All the experiments were performed at 35 ℃ (DC10-P5/U thermostat bath, Haake, Germany).

Solutions of dimethyl-, diethyl-, and dipropyl polysulfanes were prepared by addition of 6 g L^{-1} biosulfur to 1.2 mM MT, ET, or PT solutions and incubated on a shaker for 24 hours at room temperature to allow for complete reaction between thiol

and biosulfur (Eq. 2). The conversion efficiency of thiols was verified using GC analysis. Solutions of dimethyl and diethyl polysulfanes were injected separately to the mini-reactor filled with the medium using a glass syringe with the injection volume between 50-150 µL. Each concentration was analyzed in triplicate.

3. Results and discussion

3.1. Selection for the MT-tolerant biomass originating from the full-scale system

To select microorganisms capable of withstanding MT, we exposed biomass obtained from the full-scale bioreactor to gradually increasing MT concentrations for a period of 63 days. The laboratory reactor conditions (pH, ORP, and salinity) were kept the similar to those in the full-scale bioreactor (Supporting Information S1). Bioreactor performance and microbial population dynamics were followed during the selection period (Fig. 2).

During the first 20 days of operation, the MT loading rate was negatively correlated with biotic production of sulfur due to inhibition of the originally dominating SOB. This resulted in increased abiotic production of thiosulfate (Fig. 2) through polysulfide anions.[28] From day 20 to 40, sulfur production was between 60 and 75% under increased MT loading range (2 - 6 mM d^{-1}). After day 50, we found that the selectivity for biological sulfur formation was higher (70 *v.* 50%) and chemical production of thiosulfate was lower (20 *v.* 46%) compared to the initial phase of the selection process (day 17 *v.* 60; Fig. 2). A clear shift in the original microbial community in the laboratory bioreactor occurred during first the 20 days of incubation with MT (Fig. 2). The relative abundance of bacteria belonging to the

Ectothiorhosdospiraceae family decreased from 28% to 3%, whilst the relative abundance of *Bacillaceae* family doubled in the same period. For the inoculum and sample collected on day 20, the most abundant genus belonging to *Ectothiorhosdospiraceae* family was found to be *Thioalkalivibrio*, which is commonly found in full-scale Thiopaq™ reactors.[29] A decrease in the abundance of *Ectothiorhosdospiraceae* indicated that bacteria from the genus *Thioalkalivibrio* are highly vulnerable to MT, which is in line with our previous observations.[19]

At the end of the selection period (day 61), we observed a complete change of the microbial population compared to the inoculum (Fig. 2). Bacteria from *Halothiobacillaceae* family became the dominant phylogenetic group (67%), while these bacteria were not detectable in the full-scale plant (Fig. 2). Within this family, the dominant group belonged to the genus *Thioalkalibacter* that were also identified in samples from soda lakes.[30] Moreover, we observed a recovery of the relative abundance of *Ectothiorhosdospiraceae* family from 3 to 21% compared to day 20. However, within this family, the dominant SOB genus shifted from obligatory autotrophic *Thioalkalivibrio* to facultative autotrophic *Alkalilimnicola*.[31,32] The most probable cause of this shift was the relatively high MT loading rate (up to 7.6 mM d^{-1}, Fig. 2), which is almost 20 fold higher than in the full-scale installation as described in the Supporting Information (S1). Moreover, thiols present in the full-scale feed-gas mainly consisted of higher thiols (PT and buthanethiol) which show less inhibition to SOB than MT.[17] These factors might have given a competitive advantage to the SOB from *Thioalkalibacter* and *Alkalilimnicola* genus dominating after the shift. Additionally, we observed a decrease in abundance of heterotrophic genus *Halomonas* from 55 to 11% relative abundance. The reason for this can be a

decreased amount of organic compounds originating from the full-scale sludge. The selected biomass was then used in the follow up experiments.

3.2. Application of the highly MT-tolerant population for H_2S removal in the presence of thiols

3.2.1. Performance of the lab-scale biodesulfurization system

To assess the overall performance of the integrated laboratory bioreactor it is necessary to firstly measure the scrubbing efficiency of H_2S and thiols in the gas absorber (Fig. 1). In all experiments (Table 2), H_2S was almost completely absorbed from the inlet gas stream (99.829 ± 0.007%) while the scrubbing efficiency of MT, ET, and PT was around 69 ± 4%, 65 ± 2%, and 44 ± 2%, respectively. The difference in scrubbing efficiencies between H_2S and thiols was the result from a higher solubility of H_2S, resulting from a lower pK_a value than that of thiols (7.0 *v.* ~10.4) and a higher Henry's law constant (0.41 *v.* ~0.15; values for water at 20 °C).[33]

Nitrogen gas was continuously added to the bioreactor suspension (as presented in Fig. 1) to assess the effect of thiols stripping on the bioreactor performance and to mimic open full-scale reactors. In such reactors, air is used for both oxygen supply and mixing of the bioreactor suspension, where the excess gas is discharged to the atmosphere after passing a compost filter.[34] The VOSC concentrations in the bioreactor decreased with increased flow rate of N_2 (Fig. 3A-C) and showed that thiols can be effectively stripped by applying a low volumetric flow rate of an inert gas. Moreover, it can be observed that the sum of VOSCs in the bioreactor headspace decreased with increasing thiol hydrophobicity (Fig. 3A-C).[33]

A lower selectivity for sulfur production was found at higher VOSC concentrations. Probably this resulted from the inhibitory effects of VOSCs on the

overall biological sulfide oxidation rate[12,19] which in turn, leads to an increasing abiotic formation of polysulfide anions causing spontaneous thiosulfate formation.[28,35] However, it was demonstrated that stripping of VOSCs resulted in a significant increase in the selectivity for sulfur formation when the inlet gas stream was supplemented with MT (Fig. 3A). At the highest VOSCs concentration, the selectivity for biologically produced sulfur was the lowest (56 mol%) and more thiosulfate (38 mol%) was formed. However, at decreasing VOSCs concentration as a result of stripping by N_2, the selectivity for sulfur formation increased to about 74 mol% (Fig. 3A), which is relatively close to the uninhibited process.[36]

Contrary to experiments with MT, supplementation of the feed gas with ET (Experiments 5-8, Table 2) resulted in an increase in the selectivity for biological sulfate production with increasing VOSC concentrations, while the thiosulfate selectivity remained constant at around 18 mol% (Fig. 3B). We hypothesize that this unexpected change from sulfur to sulfate production was caused by diethyl polysulfanes in the medium, formed from the reaction between ET and biosulfur particles (Eq. 2-3), on the ORP. Our previous research has shown that the ORP is primarily determined by the sulfide concentration.[37] However, in the present study we found that in addition to sulfide, diethyl polysulfanes also lower the ORP resulting in an excessive supply of oxygen to the bioreactor in order to maintain the desired set point value of e.g. -390 mV. This could be seen in an increased concentration of oxygen in the bioreactor (Fig. 3A *v.* Fig. 3B) and a doubling of the O_2/H_2S supply ratio (from 0.75 to 1.4 mol mol^{-1}) compared to experiments with MT and other studies.[5,11] This ratio is related to the total sulfide concentration[38] and the stoichiometry of the simplified bio-oxidation reactions:[37]

$$HS^- + 0.5\ O_2 \rightarrow 1/8\ S_8 + OH^-\ \text{(biotic)} \qquad (7)$$

316 $HS^- + 2\ O_2 \rightarrow SO_4^{2-} + H^+$ (biotic) (8)

317 We therefore conclude that the selectivity from sulfur to sulfate can be shifted by
318 increasing the O_2/H_2S supply ratio.[39,40] In experiments with only MT, any clear
319 change in product selectivity was not seen. An explanation for this observation is that
320 diethyl polysulfanes lower the ORP by 36 ± 4 mV more than dimethyl polysulfanes
321 that are formed from the abiotic oxidation of MT (Fig. 4). Moreover, diethyl
322 polysulfanes are also less volatile than dimethyl polysulfanes,[41] which resulted in a
323 lower stripping rates from the bioreactor and thus in higher concentrations.

324 The results show that a commonly used method to control the bioreactor by
325 the ORP is insufficient as the ORP was no longer solely determined by the sulfide
326 concentration. Hence, it is necessary to develop a new method to control the O_2
327 supply to biodesulfurization reactors. Such a method should not rely on indirect
328 parameters such as the ORP but should be based on the direct and on-line analyses
329 of the sulfide and VOSC concentration, e.g. spectrophotometric methods.

330 In experiments with PT, the loading rate was lowered from 9.1 to 4.5 mM d^{-1},
331 as the bioreactor appeared to be unstable under the higher loading rate. Unstable
332 operation of the bioreactor occurs when it is not possible to maintain a constant ORP
333 value by dosing O_2 to the gas recycling loop (Fig. 1). The reason for instability of the
334 bioreactor was the presence of dipropyl disulfides in the medium, which are more
335 reducing compounds than dimethyl disulfides (Fig. 4). Another contributing factor
336 could be the higher hydrophobicity and lower volatility of dipropyl polysulfanes
337 compared to dimethyl and diethyl polysulfanes[41] resulting in higher concentrations in
338 the bioreactor. After lowering the PT loading rate to 4.5 mM d^{-1}, no significant effects
339 were observed on the product selectivity (Fig. 3C). The sulfur selectivity remained

constant at about 80 mol% which was also found for a sulfide oxidation system that was not impacted by thiols.[36]

Next, the effect of combined supply of three of thiols (MT, ET, and PT) on the bioreactor performance was investigated under N_2-flow rates varying between 0 and 18 L h^{-1}. The loading rate of each thiol was 2.5 mM d^{-1} whilst the H_2S loading rate was kept constant at 61.3 mM d^{-1}. However, no other effect than those described above was observed.

3.2.2. Effect of thiols on the biomass composition

At the end of each experimental run (Section 3.2.1) biomass samples were collected to investigate the effect of thiols on the microbial community composition (Fig. 5). The presence of thiols appeared to provide a competitive advantage to various populations when compared to previous studies with sulfide-oxidizing bioreactors at haloalkaline conditions.[29] When MT and ET were supplied at elevated concentrations, the fraction of populations belonging to the family *Halothiobacillaceae* significantly increased which was also observed in the above described thiol acclimation experiments. The only described haloalkaliphilic autotrophic sulfide oxidizing species in this family is *Thioalkalibacter halophilus*.[30] The sequence identity analysis with nucleotide Basic Local Alignment Search Tool (BLAST)[42] was used to identify close relatives in the NCBI taxonomy database and revealed that all sequences in the *Halothiobacillaceae* family had 97-99% identity to *Thioalkalibacter*. This genus of SOB was not detectable in samples from full-scale biodesulfurization plants treating gas without thiols where species belonging to the genus *Thioalkalivibrio* (family *Ectothiorhodospiraceae*) are dominant.[29] Hardly any literature information is available to explain why *Thioalkalibacter* became more abundant in the

presence of MT and ET. It might be that these bacteria have a higher tolerance towards thiols than other haloalkaliphilic SOB. In contrast, in the presence of PT the population decreased to below values that were present in the inoculum. The fact that PT was supplied in lower concentrations compared to MT and ET might explain the difference as other bacteria might still be tolerant to thiols and thus take away the selective advantage of *Thioalkalibacter*. As there is no any information available on the relation between the identified SOB and thiols, more studies are necessary on the influence of thiols on pure cultures.

After exposure to elevated thiol concentrations another shift was observed in the composition within the family *Ectothiorhodospiraceae* (Fig. 5), whereby the originally dominating obligatory autotrophic *Thioalkalivibrio* was outcompeted by a facultative autotrophic haloalkaliphilic sulfide oxidizer belonging to the genus *Alkalilimnicola* (98-100% identity).

It was also observed that the relative abundance of the haloalkaliphilic anaerobic genus *Tindallia* (97-99% identity) belonging to the *Clostridiales* increased in all experiments with thiols (e.g. from 0.7 to 14% in the presence of PT). *Tindallia* species have been isolated from soda lakes where thiosulfate and sulfur can act as electron acceptors.[43] Recently, they were also identified as a dominant sulfidogenic bacterium in anaerobic bioreactors operated at haloalkaline conditions.[44] This might indicate that in such reactors a full dissimilatory sulfur cycle can occur at oxygen limitations (i.e. $HS^- \rightarrow S_8 \rightarrow HS^- \leftarrow S_2O_3^{2-} \leftarrow HS^-$).

In addition to the already known haloalkaliphilic SOB species, we observed an increased abundance of obligate haloalkalitolerant heterotrophic bacteria belonging to the genus *Aliidiomarina*.[45] To the best of our knowledge, there is no information available about their possible sulfide oxidizing capacity or use of VOSCs. Although

the reason for increased presence of these bacteria in bioreactor fed with ET and PT is yet unknown it is possible that growth occurs on dead cells. This is because they are able to hydrolyse DNA and proteins.[46]

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References

(1) Wang, W.; Turn, S. Q.; Keffer, V.; Douette, A. Study of process data in autothermal reforming of LPG using multivariate data analysis. *Chem. Eng. J.* **2007**, *129* (1), 11–19.

(2) Kim, K.-H.; Choi, Y.; Jeon, E.; Sunwoo, Y. Characterization of malodorous sulfur compounds in landfill gas. *Atmos. Environ.* **2005**, *39* (6), 1103–1112.

(3) Fredericks, E.; Harlow, G. Determination of mercaptans in sour natural gases by gas liquid chromatography and microcoulometric titration. *Anal. Chem.* **1964**, *36* (2), 263–266.

(4) Song, C. An overview of new approaches to deep desulfurization for ultra-clean gasoline, diesel fuel and jet fuel. *Catal. Today* **2003**, *86* (1), 211–263.
(5) Van den Bosch, P. L. F.; van Beusekom, O. C.; Buisman, C. J. N.; Janssen, A. J. H. Sulfide oxidation at halo-alkaline conditions in a fed-batch bioreactor. *Biotechnol. Bioeng.* **2007**, *97* (5), 1053–1063.
(6) Buisman, C. J. N.; Geraats, B. G.; IJspeert, P.; Lettinga, G. Optimization of sulphur production in a biotechnological sulphide-removing reactor. *Biotechnol. Bioeng.* **1990**, *35* (1), 50–56.
(7) Sorokin, D. Y.; Banciu, H.; Robertson, L. A.; Kuenen, J. G.; Muntyan, M. S.; Muyzer, G. Halophilic and haloalkaliphilic sulfur-oxidizing bacteria. In *The Prokaryotes*; Rosenberg E. et al., Ed.; Springer-Verlag: Berlin-Heidelberg, 2013; pp 529–554.
(8) Sercu, B.; Nunez, D.; Van Langenhove, H.; Aroca, G.; Verstraete, W. Operational and microbiological aspects of a bioaugmented two-stage biotrickling filter removing hydrogen sulfide and dimethyl sulfide. *Biotechnol. Bioeng.* **2005**, *90* (2), 259–269.
(9) Weast, R. C. *CRC handbook of chemistry and physics*; CRC Press, Boca Raton, 1986.
(10) Crampton, M. *The Chemistry of the Thiol Group: Part 1*; Patai, S., Ed.; New York, NY: John Wiley & Sons, 1974; pp 379–415.
(11) Roman, P.; Bijmans, M. F.; Janssen, A. J. Influence of methanethiol on biological sulphide oxidation in gas treatment system. *Environ. Tech.* **2016**, *37* (13), 1693–1703.
(12) Van den Bosch, P. L. F.; de Graaff, M.; Fortuny-Picornell, M.; van Leerdam, R. C.; Janssen, A. J. H. Inhibition of microbiological sulfide oxidation by methanethiol and dimethyl polysulfides at natron-alkaline conditions. *Appl. Microbiol. Biotechnol.* **2009**, *83* (3), 579–587.
(13) Steudel, R. The chemistry of organic polysulfanes RS (n)-R (n> 2). *Chem. Rev.* **2002**, *102* (11), 3905–3946.
(14) Van Leerdam, R. C.; Bosch, P. L. F.; Lens, P. N. L.; Janssen, A. J. H. Reactions between methanethiol and biologically produced sulfur. *Environ. Sci. Technol.* **2011**, *45* (4), 1320–1326.
(15) Jocelyn, P. C. *Biochemistry of the SH Group*; Academic Press London, 1972; Vol. 270.
(16) Pfennig, N.; Lippert, K. D. Über das vitamin B_{12}-bedürfnis phototropher Schwefelbakterien. *Arch. Microbiol.* **1966**, *55* (3), 245–256.
(17) Roman, P.; Lipińska, J.; Bijmans, M. F.; Sorokin, D. Y.; Keesman, K. J.; Janssen, A. J. Inhibition of a biological sulfide oxidation under haloalkaline conditions by thiols and diorgano polysulfanes. *Water Res.* **2016**, *101* (15), 448—456.
(18) Lee, S.; Xu, Q.; Booth, M.; Townsend, T. G.; Chadik, P.; Bitton, G. Reduced sulfur compounds in gas from construction and demolition debris landfills. *Waste Manage.* **2006**, *26* (5), 526–533.
(19) Roman, P.; Veltman, R.; Bijmans, M. F. M.; Keesman, K.; Janssen, A. J. Effect of methanethiol concentration on sulfur production in biological desulfurization systems under haloalkaline conditions. *Environ. Sci. Technol.* **2015**, *49*, 9212–9221.
(20) Po, H. N.; Senozan, N. The Henderson-Hasselbalch equation: its history and limitations. *J. Chem. Educ.* **2001**, *78* (11), 1499.

(21) Herlemann, D. P.; Labrenz, M.; Jürgens, K.; Bertilsson, S.; Waniek, J. J.; Andersson, A. F. Transitions in bacterial communities along the 2000 km salinity gradient of the Baltic Sea. *ISME J.* **2011**, *5* (10), 1571–1579.
(22) Hugerth, L. W.; Wefer, H. A.; Lundin, S.; Jakobsson, H. E.; Lindberg, M.; Rodin, S.; Engstrand, L.; Andersson, A. F. DegePrime, a program for degenerate primer design for broad-taxonomic-range PCR in microbial ecology studies. *Appl. Environ. Microbiol.* **2014**, *80* (16), 5116–5123.
(23) Lindh, M. V.; Figueroa, D.; Sjöstedt, J.; Baltar, F.; Lundin, D.; Andersson, A.; Legrand, C.; Pinhassi, J. Transplant experiments uncover Baltic Sea basin-specific responses in bacterioplankton community composition and metabolic activities. *Front. Microbiol.* **2015**, *6*.
(24) Edgar, R. C. UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nat. Methods* **2013**, *10* (10), 996–998.
(25) Quast, C.; Pruesse, E.; Yilmaz, P.; Gerken, J.; Schweer, T.; Yarza, P.; Peplies, J.; Glöckner, F. O. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic. Acids. Res. 41* **2012**, D590–596.
(26) Robertson, C. E.; Harris, J. K.; Wagner, B. D.; Granger, D.; Browne, K.; Tatem, B.; Feazel, L. M.; Park, K.; Pace, N. R.; Frank, D. N. Explicet: graphical user interface software for metadata-driven management, analysis, and visualization of microbiome data. *Bioinformatics 29* **2013**, 3100–3101.
(27) Kleinjan, W. E.; Keizer, A. de; Janssen, A. J. Kinetics of the chemical oxidation of polysulfide anions in aqueous solution. *Water Res.* **2005**, *39* (17), 4093–4100.
(28) Chen, K. Y.; Morris, J. C. Kinetics of oxidation of aqueous sulfide by oxygen. *Environ. Sci. Technol.* **1972**, *6* (6), 529–537.
(29) Sorokin, D. Y.; Muntyan, M. S.; Panteleeva, A. N.; Muyzer, G. *Thioalkalivibrio sulfidiphilus* sp. nov., a haloalkaliphilic, sulfur-oxidizing gammaproteobacterium from alkaline habitats. *Int. J. Syst. Evol. Microbiol.* **2012**, *62* (Pt 8), 1884–1889.
(30) Banciu, H. L.; Sorokin, D. Y.; Tourova, T. P.; Galinski, E. A.; Muntyan, M. S.; Kuenen, J. G.; Muyzer, G. Influence of salts and pH on growth and activity of a novel facultatively alkaliphilic, extremely salt-tolerant, obligately chemolithoautotrophic sufur-oxidizing Gammaproteobacterium *Thioalkalibacter halophilus* gen. nov., sp. nov. from South-Western Siberian soda lakes. *Extremophiles* **2008**, *12* (3), 391–404.
(31) Sorokin, D. Y.; Zhilina, T. N.; Lysenko, A. M.; Tourova, T. P.; Spiridonova, E. M. Metabolic versatility of haloalkaliphilic bacteria from soda lakes belonging to the *Alkalispirillum-Alkalilimnicola* group. *Extremophiles* **2006**, *10* (3), 213–220.
(32) Oremland, R. S.; Hoeft, S. E.; Santini, J. M.; Bano, N.; Hollibaugh, R. A.; Hollibaugh, J. T. Anaerobic oxidation of arsenite in Mono Lake water and by a facultative, arsenite-oxidizing chemoautotroph, strain MLHE-1. *Appl. Environ. Microbiol.* **2002**, *68* (10), 4795–4802.
(33) Matsis, V.; Georgantas, D.; Grigoropoulou, H. Removal of n-butyl mercaptan using stripping with an inert gas: A nonequilibrium approach via mass balances. *Ind. Eng. Chem. Res.* **2006**, *45* (5), 1766–1773.
(34) Cline, C.; Hoksberg, A.; Abry, R.; Janssen, A. J. H. Biological Process for H_2S Removal from Gas Streams: The Shell-Paques/THIOPAQ™ Gas Desulfurization Process. In *Proceedings of the Laurance Reid Gas Conditioning Conference*; 2003; pp 1–18.

(35) Janssen, A. J. H.; Sleyster, R.; Van der Kaa, C.; Jochemsen, A.; Bontsema, J.; Lettinga, G. Biological sulphide oxidation in a fed-batch reactor. *Biotechnol. Bioeng.* **1995**, *47* (3), 327–333.
(36) Van den Bosch, P. L. F.; Fortuny-Picornell, M.; Janssen, A. J. Effects of methanethiol on the biological oxidation of sulfide at natron-alkaline conditions. *Environ. Sci. Technol.* **2009**, *43* (2), 453–459.
(37) Janssen, A. J. H.; Meijer, S.; Bontsema, J.; Lettinga, G. Application of the redox potential for controling a sulfide oxidizing bioreactor. *Biotechnol. Bioeng.* **1998**, *60* (2), 147–155.
(38) Roman, P.; Bijmans, M. F.; Janssen, A. J. Quantification of individual polysulfides in lab-scale and full-scale desulfurisation bioreactors. *Environ. Chem.* **2014**, *11* (6), 702–708.
(39) Klok, J. B. M.; van den Bosch, P. L. F.; Buisman, C. J. N.; Stams, A. J. M.; Keesman, K. J.; Janssen, A. J. H. Pathways of sulfide oxidation by haloalkaliphilic bacteria in limited-oxygen gas lift bioreactors. *Environ. Sci. Technol.* **2012**, *46* (14), 7581–7586.
(40) Alcántara, S.; Velasco, A.; Muñoz, A.; Cid, J.; Revah, S.; Razo-Flores, E. Hydrogen sulfide oxidation by a microbial consortium in a recirculation reactor system: sulfur formation under oxygen limitation and removal of phenols. *Environ. Sci. Technol.* **2004**, *38* (3), 918–923.
(41) Przyjazny, A.; Janicki, W.; Chrzanowski, W.; Staszewski, R. Headspace gas chromatographic determination of distribution coefficients of selected organosulphur compounds and their dependence on some parameters. *J. Chromatogr. A* **1983**, *280*, 249–260.
(42) Altschul, S. F.; Gish, W.; Miller, W.; Myers, E. W.; Lipman, D. J. Basic local alignment search tool. *J. Mol. Biol.* **1990**, *215* (3), 403–410.
(43) Sorokin, D. Y.; Detkova, E. N.; Muyzer, G. Sulfur-dependent respiration under extremely haloalkaline conditions in soda lake “acetogens” and the description of *Natroniella sulfidigena* sp. nov. *FEMS Microbiol. Lett.* **2011**, *319* (1), 88–95.
(44) Sousa, J. A.; Sorokin, D. Y.; Bijmans, M. F.; Plugge, C. M.; Stams, A. J. Ecology and application of haloalkaliphilic anaerobic microbial communities. *Appl. Microbiol. Biotechnol.* **2015**, *99* (22), 9331–9336.
(45) Chiu, H.-H.; Rogozin, D. Y.; Huang, S.-P.; Degermendzhy, A. G.; Shieh, W. Y.; Tang, S.-L. *Aliidiomarina shirensis* sp. nov., a halophilic bacterium isolated from Shira Lake in Khakasia, southern Siberia, and a proposal to transfer Idiomarina maris to the genus Aliidiomarina. *Int. J. Syst. Evol. Microbiol.* **2014**, *64* (Pt 4), 1334–1339.
(46) Huang, S.-P.; Chang, H.-Y.; Chen, J.-S.; Jean, W. D.; Shieh, W. Y. *Aliidiomarina taiwanensis* gen. nov., sp. nov., isolated from shallow coastal water. *Int J Syst. Evol. Microbiol.* **2012**, *62* (1), 155–161.

559 **TABLES**

560 **Table 1.** Dimensions and process conditions of the gas absorber for H_2S and thiols

561 removal.

Dimensions and process conditions	
Column diameter [m]	0.011
Column height [m]	0.8
Total gas flow [$Nm^3\ s^{-1}$]	2.8×10^{-6}
Empty bed retention time [s]	27
H_2S loading [$Nm^3\ s^{-1}$]	4.2×10^{-8}
Thiols loading [$Nm^3\ s^{-1}$]	$1.7 – 6.2 \times 10^{-9}$
Liquid flow [$Nm^3\ s^{-1}$]	2.8×10^{-6}

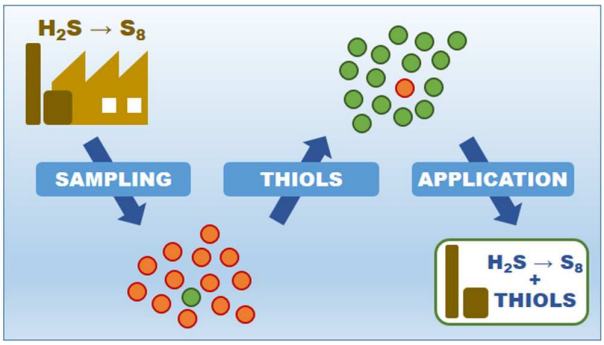
562

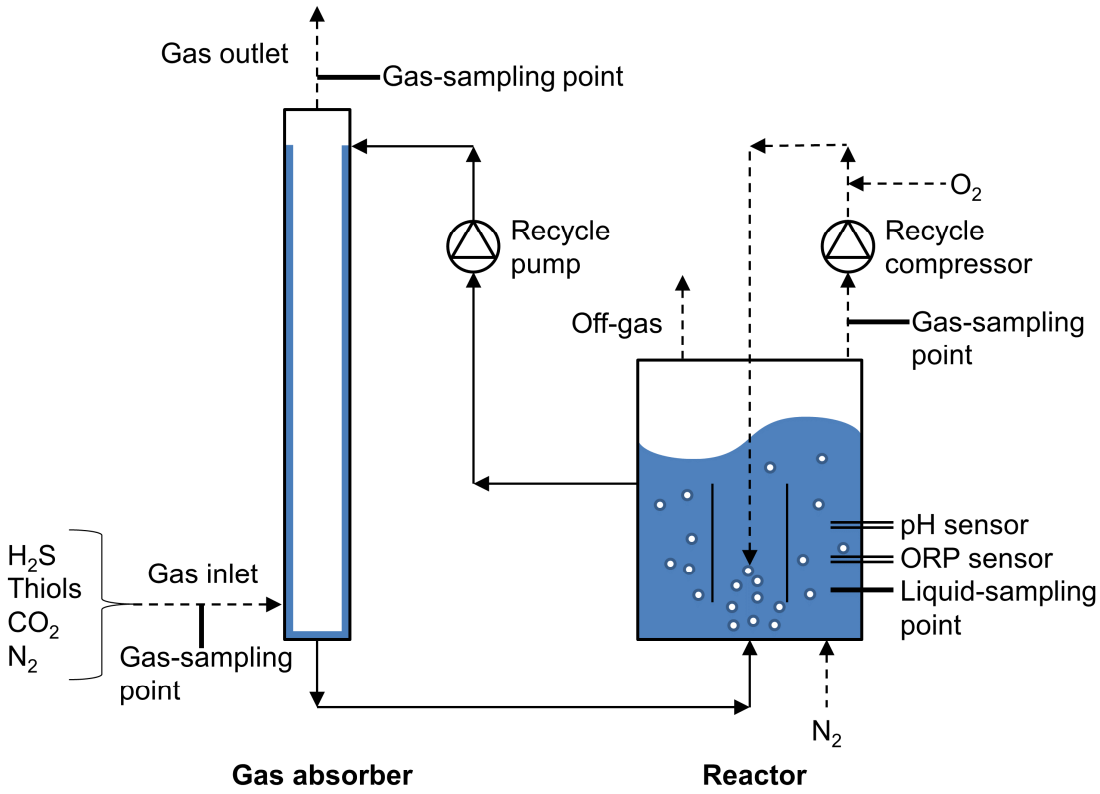
563 **Table 2.** Loading rate of thiols to the experimental setup for each experiment
564 operated under constant H_2S loading rate (61.3 mM d^{-1}) and reduction-oxidation
565 potential (-390 mV).

Experiment number	Supplied thiol to the gas absorber	Thiol loading rate [mM d^{-1}]	N_2 flow over the bioreactor [NL h^{-1}]
1	Methanethiol	9.1	0 (gas-tight reactor)
2			6
3			12
4			18
5	Ethanethiol	9.1	0 (gas-tight reactor)
6			6
7			12
8			18
9	Propanethiol	4.5	0 (gas-tight reactor)
10			6
11			12
12			18

FIGURES

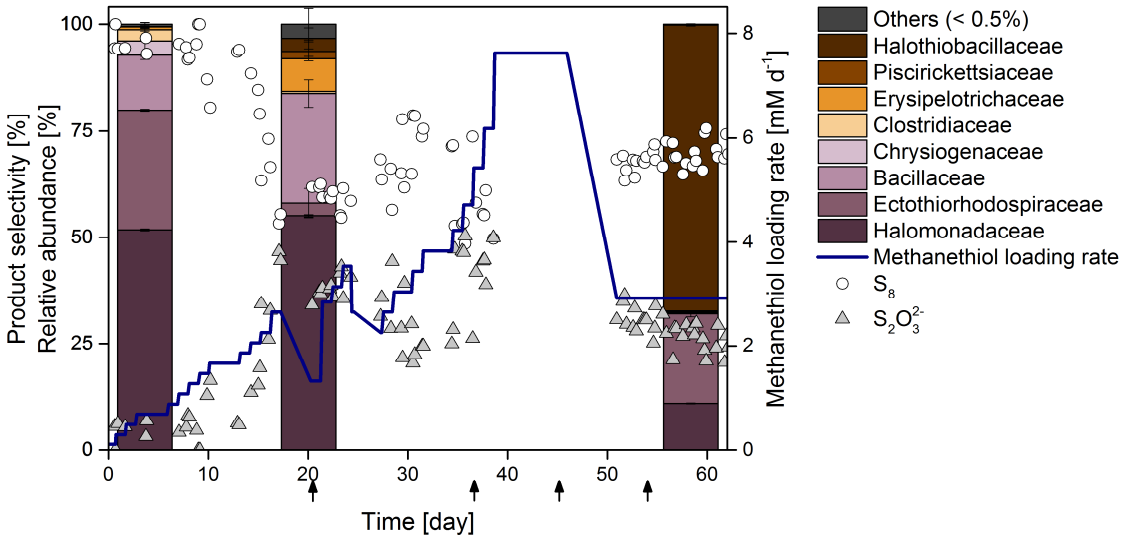
Graphical abstract:





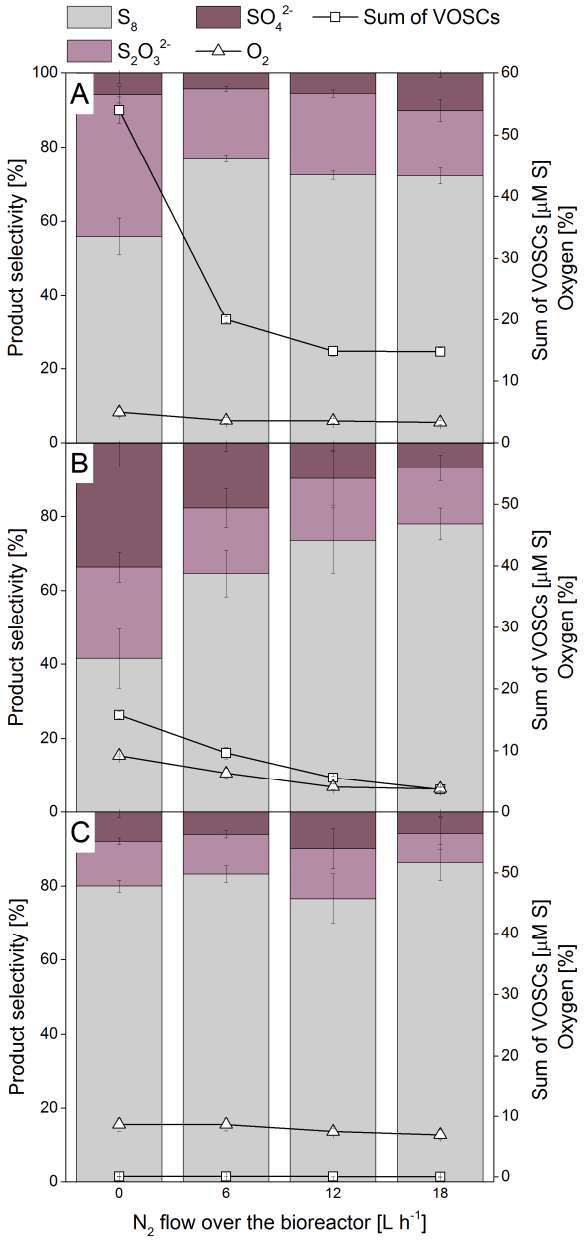
569

570 **Figure 1.** Flow scheme of experimental setup used for fed-batch experiments.



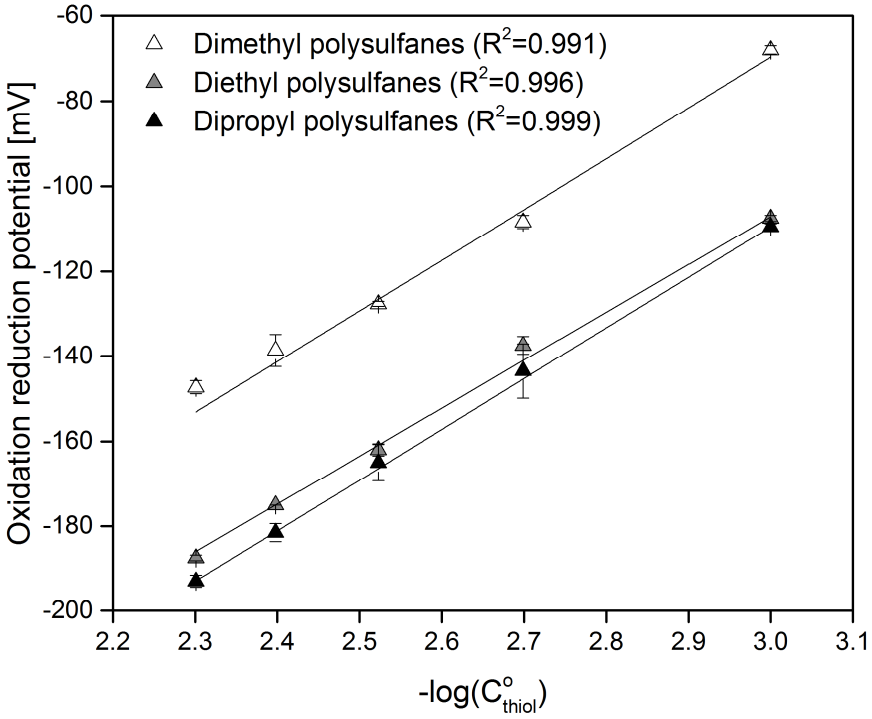
571

572 **Figure 2.** Performance of the laboratory bioreactor during acclimatization of the
573 biomass to methanethiol and the relative abundance of microbial composition based
574 on the 16S rRNA gene. DNA was extracted and sequenced from biomass at day: 0
575 (inoculum from Thiopaq™ full-scale plant in Southern Illinois), 20, and 61, during
576 which the reactor was exposed to methanethiol (0.1 - 7.6 mM d^{-1}). Only bacteria with
577 a relative abundance higher than 0.5% are listed (remaining bacteria are grouped
578 into “Others”). The results represent the average value between two replicates and
579 the error bars represent the standard deviation. The laboratory reactor was operated
580 at oxidation reduction potential of -390 mV, pH = 9 and the H_2S loading rate was
581 61.3 mM d^{-1}.



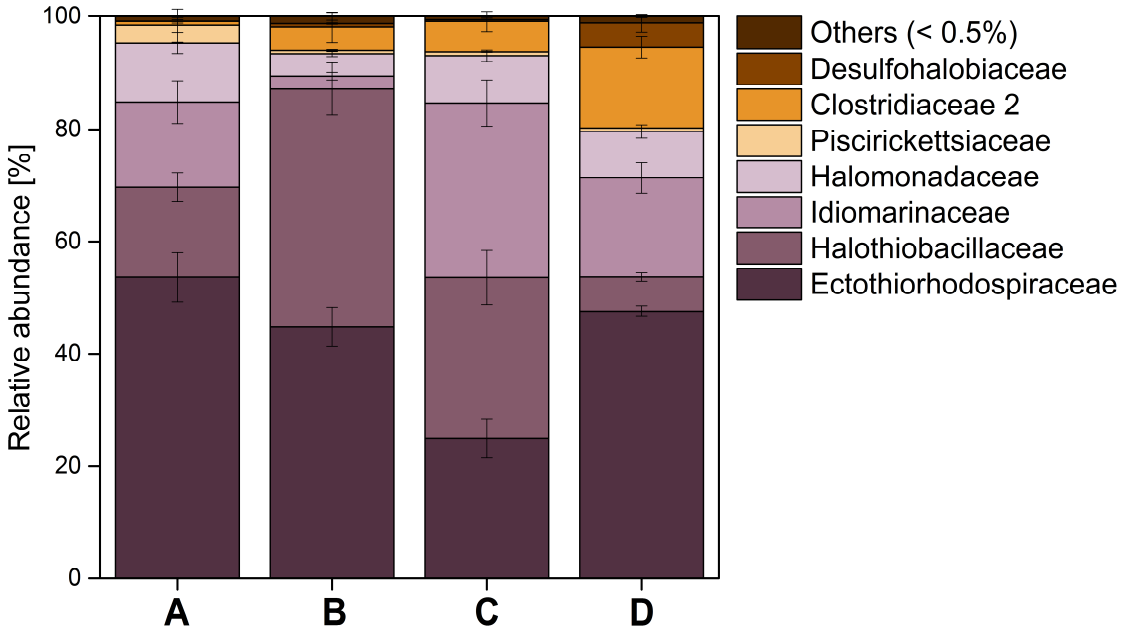
582

583 **Figure 3.** Performance of the gas biodesulfurization system fed with H_2S
584 (61.3 mM d^{-1}) plus: **A.** methanethiol; loading rate was 9.1 mM d^{-1}. **B.** ethanethiol;
585 loading rate was 9.1 mM d^{-1}. **C.** propanethiol; loading rate was 4.5 mM d^{-1}. The
586 reactor system was operated at an ORP of -390 mV, pH = 9 and multiple flows of
587 nitrogen over the bioreactor. The error bars represent the standard deviation from
588 quadruple samples.



589

590 **Figure 4.** Relationship between the oxidation reduction potential and the initial thiol
591 concentration (C°_{thiol}, mM) in a reaction between thiol and biosulfur leading to
592 formation of diorgano polysulfanes. All points were measured in triplicated and the
593 error bars represent the standard deviation. Measurements were performed in a
594 medium with [$Na^+ + K^+$] = 1 M, pH = 9 and T = 35°C.



595

596 **Figure 5.** Relative abundance of the microbial composition based on partial 16S
597 rRNA gene sequences. DNA was extracted and sequenced from the inoculum (A)
598 and biomass taken at the end of MT (B), ET (C), and PT (D) experiments. The
599 laboratory reactor was operated at oxidation reduction potential of -390 mV, pH = 9
600 and the H_2S loading rate was 61.3 mM d^{-1}. Only bacteria with a relative abundance
601 higher than 0.5% are listed (remaining bacteria are grouped into “Others”). The
602 results represent the average value between two replicates and the error bars
603 represents the standard deviation.

604