Effect of dimethyl disulfide on the sulfur formation and microbial community composition during the biological H₂S removal from sour gas streams

Karine Kiragosyan[a,b,*], Magali Picard[a,c], Dimitry Y. Sorokin[a,d,e], Jelmer Dijkstra[a], Johannes B.M. Klok[a,b,f], Pawel Roman[a], Albert J.H. Janssen[b,g]

[a] *Wetsus, European Centre of Excellence for Sustainable Water Technology, Oostergoweg 9, 8911 MA Leeuwarden, The Netherlands*
[b] *Environmental Technology, Wageningen University, P.O. Box 17, 6700 AA Wageningen, The Netherlands*
[c] *Eurofins Agroscience Services Chem SAS 75, chemin de Sommières 30310, Vergèze, France*
[d] *Winogradsky Institute of Microbiology, Research Centre of Biotechnology, Russian Academy of Sciences, Prospect 60-let Oktyabrya 7/2, Moscow, Russian Federation*
[e] *Department of Biotechnology, Delft University of Technology, Van der Maasweg 9, 2629 HZ Delft, The Netherlands*
[f] *Paqell B.V., Reactorweg 301, 3542 AD Utrecht, The Netherlands*
[g] *Shell, Oostduinlaan 2, 2596 JM the Hague, The Netherlands*

GRAPHICAL ABSTRACT
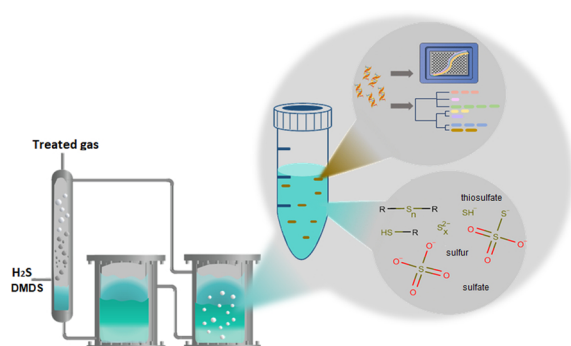
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ABSTRACT

Removal of organic and inorganic sulfur compounds from sour gases is required because of their toxicity and atmospheric pollution. The most common are hydrogen sulfide (H₂S) and methanethiol (MT). Under oxygen-limiting conditions about 92 mol% of sulfide is oxidized to sulfur by haloalkaliphilic sulfur-oxidizing bacteria (SOB), whilst the remainder is oxidized either biologically to sulfate or chemically to thiosulfate. MT is spontaneously oxidized to dimethyl disulfide (DMDS), which was found to inhibit the oxidation of sulfide to sulfate. Hence, we assessed the effect of DMDS on product formation in a lab-scale biodesulfurization setup. DMDS was quantified using a newly, in-house developed analytical method. Subsequently, a chemical reaction mechanism was proposed for the formation of methanethiol and dimethyl trisulfide from the reaction between sulfide and DMDS. Addition of DMDS resulted in significant inhibition of sulfate formation, leading to 96 mol% of sulfur formation. In addition, a reduction in the dominating haloalkaliphilic SOB species, *Thioalkalivibrio sulfidiphilus*, was observed in favor of *Thioalkaibacter halophilus* as a more DMDS-tolerant with the 50 % inhibition coefficient at 2.37 mM DMDS.

1. Introduction

Natural gas and other sour gas streams often contain (organo)sulfur compounds, such as hydrogen sulfide (H_2S) and thiols (Smet et al., 1998). Sulfide and volatile organosulfur compounds (VOSCs) are highly toxic, corrosive, and malodorous compounds, which have adverse effects on animal and human health as well as the environment (Kelly, 2010). Hence, the removal of these pollutants is needed before the gas streams can be utilized further. This triggered a number of technological developments for efficient H_2S and VOSC removal (De Angelis, 2012). Now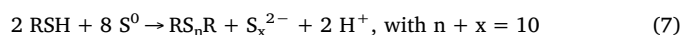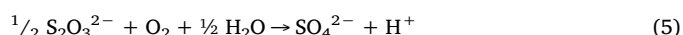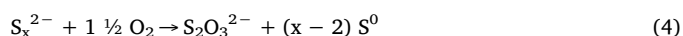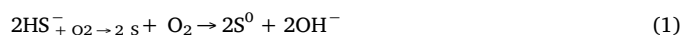adays, a variety of desulfurization technologies is commercially available for the removal and conversion of sulfurous compounds among which the biological gas desulfurization technology described in this paper that has been developed by our group in collaboration with industry (Janssen et al., 2007).

The technology studied relies on naturally occurring haloalkaliphilic sulfur-oxidizing bacteria (SOB). The process was first developed for the removal of H_2S from biogas generated by anaerobic digestion processes in wastewater treatment plants and landfills (Driessen et al., 2011). Subsequently, the process was further upgraded to treat high-pressure natural gas streams and refinery gas streams, often containing a number of other toxic compounds such as BTEX hydrocarbons, hydrogen cyanide, ammonia (Janssen et al., 2013). This process, also known as Thiopaq O&G, consists of an alkaline absorber column to remove H_2S from the gas, a microaerophilic bioreactor for dissolved sulfide oxidation to insoluble sulfur, and a gravity settler for the removal of the formed biosulfur particles (Van Den Bosch et al., 2007). Recently, the line-up was improved by the addition of an anaerobic bioreactor for achieving higher sulfide-to-sulfur bioconversion efficiencies (De Rink et al., 2019).

In the biodesulfurization process, H_2S and thiols are counter-currently absorbed from gas streams into an alkaline aqueous solution (~ 1 M sodium bicarbonate) at a pH between 8–9 (Roman et al., 2016a; Van Den Bosch et al., 2009b). The loaded liquid containing bisulfide (HS^-) and thiols (RS^-) is subsequently directed to an anaerobic bioreactor to allow the microbial enzyme systems to reach a fully reduced redox state and to dissolve any small sulfur particles into polysulfides (Kleinjan et al., 2005). From our previous studies, it follows that the addition of an anaerobic reactor increases the overall sulfide conversion efficiency to elemental sulfur (Roman et al., 2016a). The dominating biological reactions are the conversions of HS^- into respectively elemental sulfur (S^0) and sulfate (SO_4^{2-}) under oxygen-limiting conditions

(Eq. (1) and (2)) (Roman et al., 2014). Simultaneously, a number of chemical oxidation reactions occur mainly resulting in the formation of thiosulfate ($S_2O_3^{2-}$) either directly from the oxidation of sulfide or indirectly via autooxidation of polysulfide anions (S_x^{2-}) (Eqs. (3) and (4)) (Roman et al., 2016a). S_x^{2-} anions are chemically stable at high pH values in the absence of O_2, but in the presence of O_2 they are rapidly oxidized chemically to thiosulfate or by SOB to sulfur and sulfate (Eq. (5)) (Kleinjan et al., 2005). It was also found that formation rates of sulfate and sulfur depend on the SOB community composition and their activity status and the prevailing process conditions in the bioreactors (Roman et al., 2016b). When thiols are present, a rapid reaction with O_2 will take place leading to the formation of diorgano polysulfanes (DOPS) (Eq. (6)) (Van Leerdam et al., 2011). In addition, diorgano polysulfanes ($n > 3$) will be formed from the reaction between thiols and biosulfur (Eq. (7)). Subsequently, DOPS and diorgano polysulfanes will react to meta-stable intermediates that will quickly decompose to stable di- and trisulfides (Eq. (8)) (Roman et al., 2016a, 2015).

$$2HS^-_{+\,O2\,\rightarrow\,2\ s} +\ O_2 \rightarrow 2S^0 + 2OH^- \tag{1}$$

$$HS^- + 2\ O_2 \rightarrow SO_4^{2-} + H^+ \tag{2}$$

$$HS^- + 2\ O_2 \rightarrow \tfrac{1}{2}\ S_2O_3^{2-} + \tfrac{1}{2}\ H_2O \tag{3}$$

$$S_x^{2-} + 1\,\tfrac{1}{2}\ O_2 \rightarrow S_2O_3^{2-} + (x-2)\ S^0 \tag{4}$$

$$\tfrac{1}{2}\ S_2O_3^{2-} + O_2 + \tfrac{1}{2}\ H_2O \rightarrow SO_4^{2-} + H^+ \tag{5}$$

$$2\ RSH + \tfrac{1}{2}\ O_2 \rightarrow RS_2R + H_2O \tag{6}$$

$$2\ RSH + 8\ S^0 \rightarrow RS_nR + S_x^{2-} + 2\ H^+, \text{with } n + x = 10 \tag{7}$$

$$2\ RS_nR \leftrightarrow RS_{n-1}R + RS_{n+1}R, \text{with } n > 3 \tag{8}$$
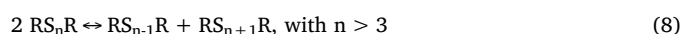
Several studies showed that the toxic effect of thiols, and especially methanethiol, on SOB result in a decrease of the sulfur formation rates (Roman et al., 2016a; Van Den Bosch et al., 2009a). Roman et al. unveiled the toxic effects of various thiols and DOPS on the activity of haloalkaliphilic SOB (Roman et al., 2016c). From this study, it follows that formation of sulfate as end-product of biological oxidation of sulfide is mostly affected by DOPS (dimethyl disulfide, diethyl disulfide, and dipropyl disulfide), while formation of sulfur as end-product is mainly affected by thiols (Roman et al., 2016c). In this paper, we studied the most commonly present DOPS, i.e. dimethyl disulfide (DMDS), in full-scale biodesulfurization reactors (Roman et al., 2016b). Moreover, DMDS is relatively stable and has little affinity for the produced
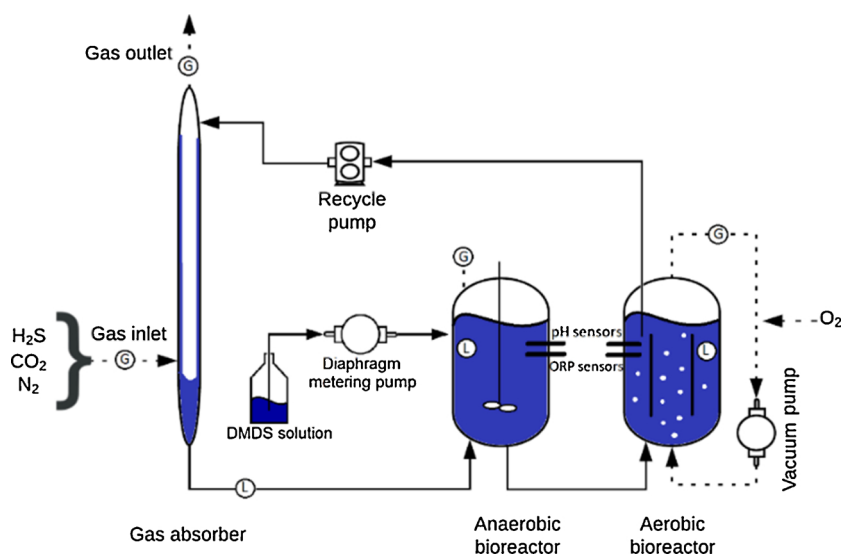

Fig. 1. Schematic representation of the experimental setup used for the experiments. G – gas sampling point, L – liquid sampling point, DMDS – dimethyl disulfide. Blue area indicates liquid. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

biosulfur particles (Roman et al., 2016b). Therefore, this study aims to evaluate the effect of DMDS on both the reactor performance and SOB community composition with the objective to minimize sulfate formation and possibly maximize sulfur formation.

2. Materials and methods

2.1. Experimental setup and experimental design

The laboratory setup consisted of a counter-current falling film gas absorber and two bioreactors in series; the first one is an anaerobic reactor for reducing the bacterial cytochromes followed by an aerobic reactor for sulfide oxidation (Fig. 1). The composition of the feed-gas was controlled using mass flow controllers (type EL-FLOW, model F-201DV-AGD-33-K/E, Bronkhorst, the Netherlands). For each type of gas, a dedicated mass flow controller was selected based on the gas supply rates. For hydrogen sulfide a range of $0 - 17\,mL\,min^{-1}$ was used; for nitrogen gas, the selected range was $0 - 350\,mL\,min^{-1}$; for O_2 $0 - 30\,mL\,min^{-1}$ and carbon dioxide $0 - 40\,mL\,min^{-1}$. Hydrogen sulfide and nitrogen gas were continuously supplied, whereas O_2 and carbon dioxide dosing rates were pulse-wise controlled with a multiparameter transmitter (Liquiline CM442-1102/0, Endress + Hauser, Germany). Supply of O_2 to the aerobic bioreactor was regulated based on a feedback controller (PID) receiving input signals from a redox sensor equipped with an internal Ag/AgCl reference electrode (Orbisint 12D-7PA41; Endress + Hauser, Germany). CO_2 was supplied through the gas inlet, but the dosing rate was regulated based on signals from a pH sensor located in aerobic bioreactor (Orbisint 11D-7AA41; Endress + Hauser, Germany). Dimethyl disulfide (Sigma-Aldrich, the Netherlands) was supplied to the anaerobic bioreactor with a diaphragm metering pump (Simdos 10, KNF Lab, the Netherlands). The concentration of the DMDS was stepwise increased, starting from 0.15 up to 0.6 mM day^{-1} (Table 1). The oxidation-reduction potential (ORP) set-point was chosen at $-390\,mV$ to suppress sulfate formation (Roman et al., 2016b).

A digital gear pump was used to assure liquid recirculation between the aerobic bioreactor and the gas absorber (EW-75211-30, Cole-Palmer, USA) at a constant flow of $10\,L\,h^{-1}$. A gas compressor (N 820 FT.18, KNF Laboport, USA) was used to continuously recycle gas ($20\,L\,min^{-1}$ at atmospheric pressure) over the aerobic bioreactor. The anaerobic bioreactor was equipped with a stirrer to assure mixing. The gas absorber and the bioreactors temperatures were controlled at 35 °C by a thermostat bath (DC10, Thermo Haake, Germany). Both gas and liquid samples were taken from the experimental system. Liquid samples were taken in triplicate from two sampling points located at the bottom section of the absorber and in the bioreactor (Fig. 1). Single gas phase samples were taken from three locations: gas inlet, bioreactor headspace, and absorber outlet.

2.2. Medium composition

The haloalkaline medium was buffered with 0.045 M Na_2CO_3 and 0.91 M $NaHCO_3$. The fresh medium contained 1.0 g K_2HPO_4, 0.20 g $MgCl_2 \times 6H_2O$ and 0.60 g urea, per 1 L of ultrapure water (Millipore, ISO 3696) and a trace element solution as described in Pfenning and Lippert (Pfennig and Lippert, 1966). The pH of the medium was controlled at 8.5 ± 0.05 at 35 °C.

2.3. Inoculum

The bioreactor inoculum consisted of a mix of different biomass sources, originating from four different biodesulfurization installations: Oilfield - 1, Oilfield - 2, Landfill and Pilot plant (Kiragosyan et al., 2019a, 2019b). By preparing a mix of inoculum we enabled a higher microbial diversity and increased the chances for the best suitable organisms to become the dominating species. The inocula were mixed in the following volumetric ratio: 2:1:1:2. After mixing, the cells were concentrated by centrifugation (15 min at 16,000 g). Hereafter the collected pellets

containing the bacteria (and also a small fraction of sulfur) were used to inoculate 5 L experimental system. The description of the biomasses reflects the various industries where the installations are located. Oilfield - 1 full-scale installation treats associated gas from an oil production site containing low concentrations of thiols $50 - 200\,ppm$ and 1–5 % of H_2S, whereas Oilfield - 2 treats acid gas from an amine installation, containing 10–20 % of H_2S and $20 - 500\,ppm(v)$ thiols (Kiragosyan et al., 2019b). The landfill installation treats landfill gas containing 0.3 % of H_2S whilst the pilot plant treats pure (100 %) H_2S gas (De Rink et al., 2019).

2.4. Respiration tests

Respiration tests, also known as biological oxygen monitoring or activity tests, were performed to assess reaction rates of biological sulfide oxidation in an air-saturated carbonate/bicarbonate buffer. The setup and detailed test performance are described in Kiragosyan et al. (2019a). In the current study, we measured the effect of DMDS on both sulfide and thiosulfate oxidation in thermostated batch reactors. The experiments were first carried out at a sulfide concentration of 0.12 mM because in our previous studies the maximum reaction rate was reached at this concentration (Kiragosyan et al., 2019a; Roman et al., 2015). Then the experiments were repeated at thiosulfate of 0.12 mM and finally in the presence of both sulfide and thiosulfate at a total concentration of 0.12 mM. Although this concentration was below the normally measured values in the bioreactor, the experiments allowed us to estimate whether any thiosulfate oxidation would takes place as the affinity constant for thiosulfate oxidation, K_s, is significantly lower, i.e. $6 \pm 3\,\mu M$ (Banciu et al., 2008). All stock solutions were freshly prepared and before usage the sulfide concentration was confirmed using the methylene blue test (LCK653, Hach Lange, Tiel, the Netherlands). Respiration tests were immediately performed after completion of the lab-scale bioreactor experiments using SOB cell pellets.

In addition, biological sulfide oxidation kinetics (in the presence and absence of DMDS) were studied with a pure culture of *Thioalkalibacter halophilus*, as this species proliferated in the presence of DMDS in our lab-scale bioreactor experiments. Based on the obtained data, we calculated IC_{50} values for DMDS. The obligate chemolithoautotrophic haloalkaliphilic SOB *Thioalkalibacter halophilus* ALCO 1 strain was obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ).

2.5. Analytical techniques

Biomass quantification was based on the amount of organic nitrogen that was oxidized to nitrate by ammonium persulphate (LCK238 and LCK338, Hach Lange, Tiel, the Netherlands). Sample (1 mL) for biomass quantification contained both elemental sulfur particles and biomass. After centrifugation at $20\,238 \times g$, we washed and resuspended the pellet with 0.5 M sodium carbonate solution to separate sulfur and any dissolved N-containing salts from the sample. In the washing procedure, we mainly picked up biomass pellets and minor amounts of sulfur particles with a pipette and transferred them to another Eppendorf tube where the sample was resuspended and then allowed to settle by centrifugation. As biomass has a lower density than sulfur, it settles on top

Table 1
An overview of the process conditions in the experimental setup.

Parameter	Value
H_2S loading rate, mM S day^{-1}	58.15
DMDS, mM S day^{-1}	0.15–0.6
Salinity, M Na^+	1.0
Carbonate alkalinity, M	1.0
pH set-point	8.5 ± 0.05
Temperature, ºC	35 ± 1
ORP set-point, mV	390

of the heavier sulfur particles and forms a separate pellet. After three cycles, sulfur particles were almost absent from the biomass sample. By using the before mentioned protocol, no sulfur was observed. Furthermore, we also would like to state that proteins attached to the sulfur are also considered biomass, even though they are extracellular. Moreover, data from our experiments on biomass concentration are compatible with results from our colleagues (Rink et al., 2020; Roman et al., 2016b). Sulfate and thiosulfate were measured by ion chromatography (Compact IC 761, Metrohm Nederland, Barendrecht, the Netherlands) with an anion column (Metrohm Metrosep A Supp 5, 150/4.0 mm, Metrohm Nederland, Barendrecht, the Netherlands) equipped with a pre-column (Metrohm Metrosep A Supp 4/5 Guard, Metrohm Nederland, Barendrecht, the Netherlands) to eliminates any particles. Immediately after sampling all solids were removed by filtration over a 0.45 μm membrane syringe filter (HPF Millex, Merck, Amsterdam, the Netherlands) and mixed with 0.2 M zinc acetate in a 1:1 ratio to prevent chemical sulfide oxidation. The biological sulfur concentration was calculated from the sulfur mass balance based on the cumulative amount of supplied sulfide and the actual sulfate and thiosulfate concentrations, according to:

$$[S^0]_t = (\Delta t \,(H_2S \text{ supplied})/V_{liquid}) - [SO_4^{2-}]_t - 2*[S_2O_3^{2-}]_t - x*[S_x^{2-}]_t$$

The initial sulfur concentration is assumed to be zero. This is a general method to establish the concentration of accumulated sulfur per time interval (Δt) (De Rink et al., 2019; Klok et al., 2012; Roman et al., 2015; Van Den Bosch et al., 2009b). Concentrations of dissolved sulfide, polysulfides, and possible volatile organosulfur compounds were not taken into account, as their combined contribution to the total concentration of sulfur species is negligible (Van Den Bosch et al., 2009b). We also assume pseudo 'steady-state' conditions of the system, which was confirmed by consecutive liquid and gas samples (Kiragosyan et al., 2019a; Roman et al., 2016b; Van Den Bosch et al., 2008).

Sulfide and bisulfide were measured as total sulfide (S_{tot}^{2-}) using the methylene blue method with a commercially available method (LCK653, Hach Lange, Tiel, the Netherlands). Total sulfide quantification was carried out immediately after sampling and samples were diluted in oxygen-free Milli-Q water (sparged with N_2 gas for 30 min) to exclude any chemical sulfide oxidation (Roman et al., 2016c).

In addition to sulfur-containing anions, sodium and potassium concentrations were measured with ion chromatography as described earlier (Roman et al., 2015). A Metrohm Metrosep C4−, 150/4.0 mm column, was used with 3 mM HNO_3 as the eluent at 0.9 mL min^{-1}.

To close the electron balance as described by (Roman et al., 2016b), carbonate and bicarbonate ion concentrations were established using the Henderson-Hasselbalch equation (Po and Senozan, 2001). For that, liquid samples were analyzed for total inorganic carbon using high-temperature (680 °C) catalytic oxidation with a TOC-L CPH analyzer (Shimadzu Benelux, 's-Hertogenbosch, the Netherlands).

In total, two types of liquid samples were prepared: (1) filtrated and precipitated with zinc acetate for anions measurements and (2) non-filtrate for biomass quantification and TOC analysis. All liquid samples were stored at 4 °C before being analyzed (about three days).

The various gas phases, i.e. absorber inlet and outlet and bioreactor gas recycle, were analyzed for H_2S, N_2, CO_2, and O_2) with a gas chromatograph (CP4900 Micro GC, Varian, Middelburg, the Netherlands) equipped with two separate column modules, namely a 10-m-long Mol Sieve 5A PLOT (MS5) and a 10-m-long PoraPlot U (PPU).

2.6. Analysis of DMDS using gas chromatography with flame photometric detector (GC-FPD)

2.6.1. GC-FPD system and calibration and gas samples analysis

The Thermo Scientific Trace GC Ultra GC-FPD system consisted of a gas sampling valve (GSV) mounted in a valve oven, a split/splitless injector with a purge-and-trap adaptor as an interface for the GSV, a programmed temperature oven and an FPD detector (all supplied by Interscience, Breda, the Netherlands). An Agilent Technologies HP-5MS analytical column (Agilent Technologies, Amstelveen, the Netherlands) was used to separate the mixture of sulfur compounds with a constant helium flow of 1.3 mL min^{-1}.

The GC-FPD system was calibrated using a two-channel gas mixing device, the Alytech GasMix Aiolos II (Da Vinci Laboratory Solutions, Rotterdam, the Netherlands). Hydrogen sulfide was connected to channel 1 (volumetric flow range 1–50 N mL min^{-1}), and nitrogen was connected to channel 2 (volumetric flow range 30–2000 N mL min^{-1}). Calibration standards of 30, 50, 100, 150, 200, 300, 400 and 600 ppm mol H_2S were prepared and injected in triplicate to create the quadratic calibration curve, which is characteristic for FPD detectors. Limits of detection (LOD) and quantification (LOQ) were determined as described by (Vial et al., 2003), using a target RSD of 20 % for LOQ; LOD and LOQ were found to be 2.25 and 7.50 ppm mol of H_2S, respectively. It should be noted that an FPD detector has equimolar sensitivity for sulfur atoms, which allowed us to use H_2S as a calibration standard for DMDS and, if needed, all other sulfur species. All stainless-steel gas lines going in and coming out of the GasMix as well as all gas lines in the GC-FPD system were Sulfinert treated by Restek (Bellefonte, USA) to prevent sulfide adsorption to the bare steel.

For identification of other VOSCs, the GC method parameters, as well as the analytical column, were transferred to an Agilent Technologies GC–MS system, consisting of a 6890 N GC and a 5975 inert XL mass spectrometer (Agilent Technologies, Amstelveen, the Netherlands). The mass spectrometer allowed identification of all peaks based on their mass spectrum, which was compared with a mass spectrum library (NIST MS Search version 2.0d, 2005).

2.6.2. Liquid samples analysis

All reagents were of analytical grade unless stated otherwise. Thioanisole (TAS), methanethiol, and DMDS were supplied by Sigma Aldrich (Zwijndrecht, the Netherlands), while *n*-hexane was purchased from VWR International (Amsterdam, the Netherlands). Hydrogen sulfide gas (2500 ppm mol; balanced by nitrogen), nitrogen 5.0 and helium 5.0 were obtained from Linde Gas Benelux (Schiedam, the Netherlands). Colloidal particles of biologically produced sulfur were obtained and purified as described elsewhere (Roman et al., 2016a). Matrix composition of all samples described in this section is the same as the reactor medium (Section 2.2) unless stated otherwise.

2.6.3. Liquid–liquid extraction and sample injection

An in-house developed liquid–liquid extraction of sulfuric compounds was used because other analytical techniques such as GC analyses will suffer from the high content of sulfur particles and salts present in the bioreactor samples. The extraction was performed as follows: 500 μL of the liquid sample was added to 500 μL of thioanisole (388 mg L^{-1}) in hexane, which is equivalent to 0.1 g S L^{-1}, in a closed silanized glass vial using a glass syringe (Hamilton model 750, VWR International, Amsterdam, the Netherlands). Then, the vial was placed in a shaker for 30 min at 600 rpm. After removing the sample from the shaker, it was left to stand for at least 5 min to allow for phase separation to complete. During the process of method optimization, the efficiency of the extraction was found dependent on the extraction time (Appendix A).

Subsequently, 0.80 μL of the top hexane layer was injected into the GC-FPD system with a 10 μL Hamilton 1800 series gastight glass microsyringe (VWR International, Amsterdam, the Netherlands) for sulfur species quantification. During method optimization, an optimum injection volume was found to achieve a complete recovery (100 %) and a small relative standard deviation (1 %) (Appendix A). The 'sandwich' injection technique was employed: (a) first 1 μL of air was drawn into the syringe, followed by (b) 2 μL of extraction solvent from the sample vial. The next step was (c) to closely monitor the meniscus of the

hexane inside the barrel of the syringe while the syringe needle still resided in the sample vial until meniscus was stagnant – indicating the gaseous headspace above the hexane in the barrel had been saturated with hexane vapors. Afterward, (d) the plunger was returned to the 1 μL mark, thereby expelling all liquid hexane from the syringe barrel, next (e) 0.8 μL of hexane was drawn into the syringe. Finally (f), the needle of the syringe was raised above the liquid level inside the sampling vial, and 1 μL of air was drawn into the syringe, resulting in a small hexane column sandwiched between two columns of gas. The syringe was then removed from the sample vial and transferred to the GC inlet, where it was quickly injected into the hot inlet. Because the sandwich technique was used, there was no direct contact of hexane with the hot inlet when penetrating the inlet septum with the needle, thus minimizing injection volume losses. Injection volume losses were further compensated by using thioanisole (TAS) as injection volume correction standard: the response factor (RF) of the pure extraction solvent containing $388 \, mg \, L^{-1}$ TAS was determined from the average of 10 replicate injections. Subsequently, this average RF was used to correct the results for all compounds of every injection of extraction liquid.

To close the mass balance in a gas-liquid system, we converted the detected gas concentrations (ppm mole H_2S) into mM S and vice versa by multiplying the ppm mole with the conversion factor as described in Appendix B.

2.7. DNA isolation and purification

Samples for genomic DNA extraction were taken at two-time points: inoculum and end of the process operation with DMDS. Taken 100 ml of the process medium from the bioreactor was centrifuged to obtain bacterial cells and washed with $0.5 \, M \, Na^+$ solution to prevent osmotic shock. Washed and concentrated cell pallet was divided into three equal aliquots to obtain representative data. These triplicates are highly dependent as they originate from the same system. Thus, they are technical and not biological replicates.

Genomic DNA was extracted using the DNeasy PowerLyzer PowerSoil Kit (Qiagen) following the manufacturer's instructions. Extracted DNA was quantified using QuantiFluor dsDNA systems and a Quantus™ fluorometer (Promega, The Netherlands). DNA integrity was evaluated with gel electrophoresis.

2.8. 16S amplicon sequencing and qPCR

16S rRNA gene amplicon sequence libraries were sequenced on an Illumina MiSeq using the V3 chemistry to generate 300 bp paired-end reads with 515f (5′-GTGCCAGCMGCCGCGGTAA-3′) (Caporaso et al., 2012) and 926r (5′-CCGYCAATTYMTTTRAGTTT-3′) (Quince et al., 2011)

primer-set at MrDNA Molecular Research LP, Shallowater, TX, USA (Chiodini et al., 2015). Sample identifier barcode sequences were extracted from forward and reverse reads in QIIME (Caporaso et al., 2011). We then used the bioinformatics toolkit implemented in QIIME2 (version 2018.2 and 2018.11) to perform quality control and filtering of sequence data (Bolyen et al., 2019). Briefly, samples were first demultiplexed, and primer sequences were then trimmed from sequence reads using cutadapt (Martin, 2011). Subsequently, a feature table and a list of representative sequences for each unique amplicon sequence variant (ASV) were constructed after quality control of paired-end reads (i.e., denoising, error-correction, and chimera removal) using DADA2 version 2018.2.0 (Callahan et al., 2016). Quality control warranted a minimum quality Phred score of 30 by removal of the first ten bases of all reads and trimming of forward and reverse reads at 210 and 240 bases, respectively. Representative sequences for each ASV were de novo aligned using MAFFT (Katoh and Standley, 2013). The alignment was subsequently filtered to construct a phylogenetic tree using Fasttree2 (Price et al., 2010). Taxonomy assignment was performed on representative sequences using the scikit-learn naive Bayesian classifier (Pedregosa et al., 2011) trained on full 16S rRNA sequences from the SILVA database version 132 (Quast et al., 2013). The pre-trained classifier is publicly available from qiime2.org/2018.11/data-resources. Data analysis was performed using phyloseq version 1.22.3 (Mcmurdie and Holmes, 2013) in R statistical software version 3.5.0 (R Core Team, 2018). QIIME and R scripts are available as supplementary information. The EMBL-EBI accession number for presented 16S rRNA sequencing set is PRJEB31230.

For absolute quantification of three species of interest, qPCR was used with designed species-specific primers. Detailed method description can be found in Kiragosyan et al. (2019b).

3. Results and discussion

3.1. Effect of DMDS on biological sulfide oxidation and product formation

In order to develop a performance baseline, the first experimental run only contained H_2S in the feed gas. Hereafter the effect of DMDS was studied under similar conditions. The H_2S experimental run lasted for 15 days, and the calculated average selectivities for sulfur, sulfate and thiosulfate formation were $91.5 \pm 1.2 \, mol\%$, $6.7 \pm 1.1 \, mol\%$ and $1.8 \pm 0.3 \, mol\%$, respectively (Fig. 2A). Then, DMDS was supplied for six days at a rate of $0.2–0.6 \, mM \, S \, day^{-1}$. From day 1–4, the formation of thiosulfate was $8 \pm 1 \, mol\%$ while no sulfate (i.e. $0 \, mol\%$) was formed (Fig. 2B). This shows an immediate inhibition of sulfate formation by DMDS. On day five and six, the DMDS supply rate was at its maximum value of $0.6 \, mM \, S \, day^{-1}$, the thiosulfate formation was $9 \pm 0.5 \, mol\%$, and sulfate formation was about $1.0 \, mol\%$. The root-
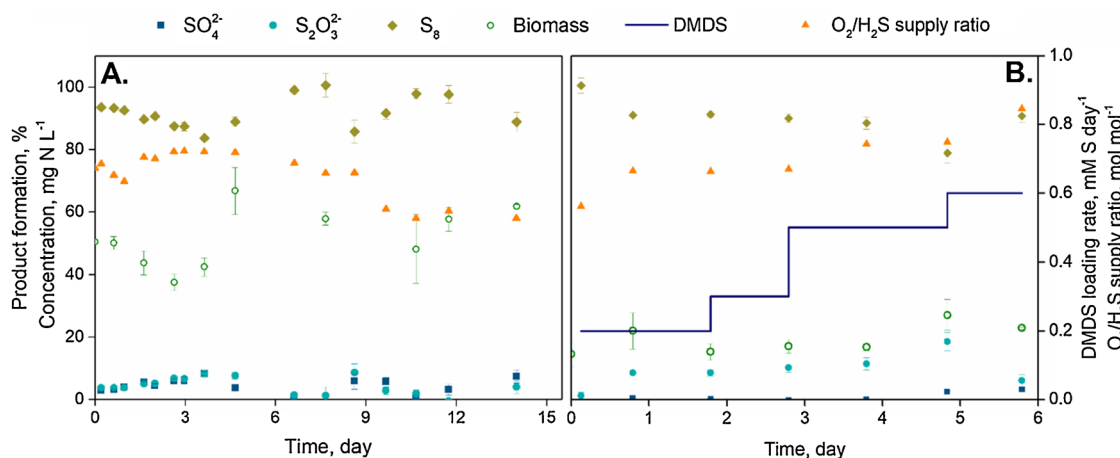

Fig. 2. Performance of the laboratory bioreactor during (A) experiment 1 - the addition of H_2S and (B) experiment 2 - the addition of H_2S + DMDS addition. The system was operated at ORP setpoint of $-390 \, mV$, pH setpoint of 8.5, T setpoint of 35 °C and the H_2S loading rate was $58.15 \, mM \, S \, day^{-1}$.
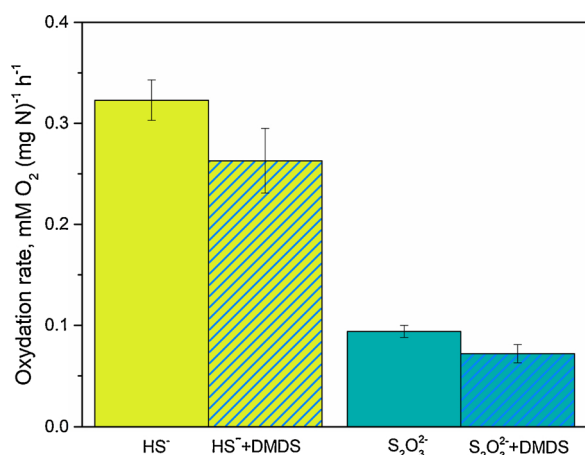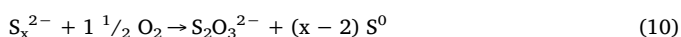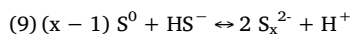
Fig. 3. Average reaction rates for sulfide (0.12 mM) and thiosulfate (0.12 mM) oxidation by the developed SOB biomass in the presence and absence of 0.30 mM of DMDS. The biomass was adapted to DMDS. The respiration tests vessel was operated at T = 35 °C and with carbonate/bicarbonate buffered medium. The pH was 8.5. Error bars indicate the standard deviation between measured triplicates.

cause for sulfate formation can be found in the ORP probe. Previous studies show that for sour gas streams that only contain H_2S, the measured ORP is governed by the dissolved sulfide concentration (Janssen et al., 1998). However, in the presence of DOPS, the measured ORP is affected by the DOPS (Roman et al., 2016b). Hence, we have tested the effect of dipropyl disulfide on the measured ORP, and our results show a decrease of 20 mV. Consequently, the supply of O_2 to the system increased in order to reach the ORP setpoint value, which resulted in increasing O_2/H_2S supply ratios from 0.60 mol mol^{-1} on day 1 to 0.85 mol mol^{-1} on day six. Because significantly more oxygen is available, a part of the sulfide will be oxidized to sulfate as observed in many other studies (Janssen et al., 1997; Roman et al., 2016a; Van Den Bosch et al., 2007). From previous studies we know that a reduction of the ORP set-point value to -450 mV does not lead to the desired O_2/H_2S supply ratio because the probe becomes somewhat insensitive, which is similar to controlling pH at very low or very high values. Clearly, in future studies an alternative oxygen supply strategy should be developed to ensure the formation of elemental sulfur in the presence of organic sulfur compounds.

After six days of operation with DMDS in the feed stream, an average of 90.0 ± 0.5 mol% of sulfur formation was achieved with 9.0 ± 0.5 mol% of thiosulfate and 1.0 ± 0.2 mol% of sulfate. Hence, the addition of DMDS decreased sulfate formation, which was accompanied by increased rates of thiosulfate formation, resulting in a net-zero increase in sulfur formation. Increased thiosulfate formation was also observed in previous studies with organic sulfur compounds, which were found to severely inhibit SOB and ultimately lead to a complete drop-in biological sulfide-oxidizing activity (Roman et al., 2016c, 2016a, 2015). Generally, thiosulfate accumulation is commonly found in both full-scale and lab-scale biodesulfurization installations due to the chemical oxidation of (poly)sulfides (Van Den Bosch et al., 2008), according to (Eq. (9) and (10)) (Steudel, 1996; Steudel et al., 1986):

(9) $(x - 1) \, S^0 + HS^- \leftrightarrow 2 \, S_x^{2-} + H^+$

$S_x^{2-} + 1\,^{1}/_{2} \, O_2 \rightarrow S_2O_3^{2-} + (x - 2) \, S^0$ \hfill (10)

Two reasons can be found to explain the production and accumulation of thiosulfate: Firstly, inhibition of biological thiosulfate oxidation to sulfate, according to Eq. (5) (Schreiber and Pavlostathis, 1998). Secondly, inhibition of biological sulfide oxidation leading to enhanced abiotic oxidation rates of (poly)sulfides to thiosulfate (Roman et al., 2016a). To study the prevailing mechanism, we performed respiration tests with sulfide and thiosulfate as the sole substrates and SOB cells

grown in the presence and absence of DMDS. Results from respiration tests show that the maximum specific biological sulfide oxidation capacity of biomass grown in the experiment with DMDS addition was almost a factor of three lower than for biomass grown in the experiment with H_2S only, i.e. 0.32 ± 0.02 mM O_2 (mg N h)$^{-1}$ and 0.86 ± 0.04 mM O_2 (mg N h)$^{-1}$ at 0.12 mM of sulfide (Appendix C, Fig. C1). Due to this decreased capacity for sulfide oxidation rate, more thiosulfate formation occurred in the presence of DMDS. This means that full scale systems treating sour gas streams containing both H_2S and DMDS have to be larger to accommodate for the reduced oxidation capacity, or alternatively, should be operated at higher biomass concentrations. In addition, to the observed differences in biological sulfide oxidation rates, specific loading rates also varied. In the experiment with DMDS addition the observed specific loading rates for sulfide oxidation are almost a factor three higher than during H_2S addition only, 2.54 mM H_2S (mg N)$^{-1}$ h^{-1} vs. 0.94 mM H_2S (mg N)$^{-1}$ h^{-1}. The difference in specific loading rates is a result of different biomass concentrations that can be noted in the Fig. 2A and B. In addition, performed respiration tests indicate a slight decrease in the oxidation rates of both sulfide and thiosulfate in the presence of DMDS, i.e., a decrease of 19 % and 23 %, respectively (Fig. 3). In addition, we studied the simultaneous oxidation of sulfide and thiosulfate to identify sequence of reactions. From the recorded O_2 consumption profiles it appears that the initial maximum rates found for the simultaneous oxidation of thiosulfate and sulfide were in a similar order of magnitude as for HS- oxidation only (Appendix C, Fig. C2). Thus, sulfide oxidation is the prefered oxidation reaction by SOB (Steffers, 1993). This is in accordance with Ang et al. (Ang et al., 2017), which reported that thiosulfate oxidation not occurred in a number of SOB as long as dissolved sulfide is present in solution. When analyzing the overall measurements of the simultaneous oxidation of sulfide:thiosulfate, it can be found that when all sulfide was consumed, similar oxidation rates were found for biological thiosulfate oxidation. The ability of bacteria to utilize two substrates is known as diauxy (Gottschal et al., 1981). The order of substrate consumption depends on several factors such as amount of energy gained, toxicity of the compound (Marangoni, 2003), and by the ORP potential in case of sulfide/thiosulfate pair. Furthermore, lab- and full-scale biodesulfurization process operation is performed at a relatively low ORP value (i.e. -390 mV) at which SOB are induced for sulfide oxidation. Hence, in case of a combined sulfide/thiosulfate substrate, sulfide would be preferentially oxidized, and only in its absence, thiosulfate proceeds. This corresponds with our findings, i.e. the oxidation rates for sulfide are three times higher than those of thiosulfate as follows from the O_2 consumption profiles in Appendix C.

We found that both the aforementioned reasons can contribute to thiosulfate accumulation. However, based on the established order of sulfide and thiosulfate oxidation and reaction rates, we conclude that the primary reason for thiosulfate accumulation is chemical (poly)sulfide oxidation. In order to increase the biological conversion rates at the expense of the chemical oxidation rate of (poly)sulfide we increased the biomass concentration (from 23 ± 1 mg N L^{-1} to 51 ± 3 mg N L^{-1}) and started another experiment with H_2S and DMDS as feed compounds. At increasing biomass concentrations, no thiosulfate formation (i.e. 0 mol%) was found, while the selectivity for sulfur formation was increased to 96 ± 1 mol% at 0.6 mM S day^{-1} supplied DMDS (Appendix D, Fig. D1). Moreover, we found that only 4 ± 1 mol% of sulfate was formed.

The composition of the liquid in both bioreactors was analyzed for the presence of diorganic polysulfides using our newly developed GC-FPD method. No significant differences were found between the anaerobic and aerobic bioreactor, assuming that no conversion of DMDS taking place. Next, to DMDS, dimethyl trisulfide and MT were detected in the process solution (Fig. 4A and B). In Fig. 4, only the results of the anaerobic bioreactor are presented, since concentrations of DMDS, DMTS, and MT were identical in both reactors. The DMDS concentration in the liquid of anaerobic bioreactor was on average 0.65 ± 0.03 mM S and, in the headspace, 0.41 ± 0.01 mM S, regardless

of an increase in the DMDS supply. This not a full absorption of DMDS can be explained by the saturation of the alkaline process medium.

In Fig. 4A and B, it can be seen that during startup of the experiments, DMDS and DMTS were present in the process solution. The measured concentrations at the onset of the experimental run were two times higher than expected from the influent concentrations. To explain this phenomenon, we first reconfirmed the concentration in the DMDS stock solution to assure that no experimental errors were made. We also checked if the stock solution would contain any DMTS. As this was not the case, a likely explanation for the higher DMDS concentration is the presence of minor amounts of DMDS and DMTS attached on sulfur particles surface, which remained from previous experiments as diorgano polysulfanes have a high affinity for sulfur. For instance, Roman et al. tested the ability of dimethyl polysulfides (dimethyl di-, tri- and tetrasulfide) to adsorb onto the surface of biosulfur particles (Roman et al., 2016a). They found desorption of DOPS from sulfur particles into the vial headspace. From the result of gaseous DMDS, DMTS and MT (Fig. 4A), it follows that our experimental system was in equilibrium as the DMDS, DMTS and MT concentrations in the headspace of aerobic bioreactor and absorber outlet were identical.

The DMDS and DMTS concentrations were almost similar, i.e., about 0.5 mM (Fig. 4A and B). Typically, both compounds are formed during the chemical oxidation of methanethiol (MT) (Eqs. (6)–(8)). Because no MT was added to the lab-scale biodesulfurization system, and biological degradation of DMDS was not feasible as electron donor was lacking for the reaction (Fig. 4C). Hence, a different reaction occurred, leading to DMTS formation. In the lab-scale biodesulfurization setup, significant levels of sulfide and DMDS were present in the anaerobic bioreactor. It is known that sulfide is a strong nucleophile that can open sulfur-sulfur bonds, e.g., in S_8 rings (Steudel, 2003). In addition, sulfide can react with diorgano polysulfanes to form persulfides (Eq. (10)). We found that in the presence of trace amounts of O_2 and at alkaline conditions, this reaction will lead to the formation of multiple products. For example, MT, dimethyl trisulfide, dimethyl tetrasulfide, and sulfite anions were formed when DMDS was exposed to sulfide (Appendix F). Therefore, it is proposed that this reaction can proceed with Bunte salts (Milligan et al., 1963) as an intermediate product in the following steps:
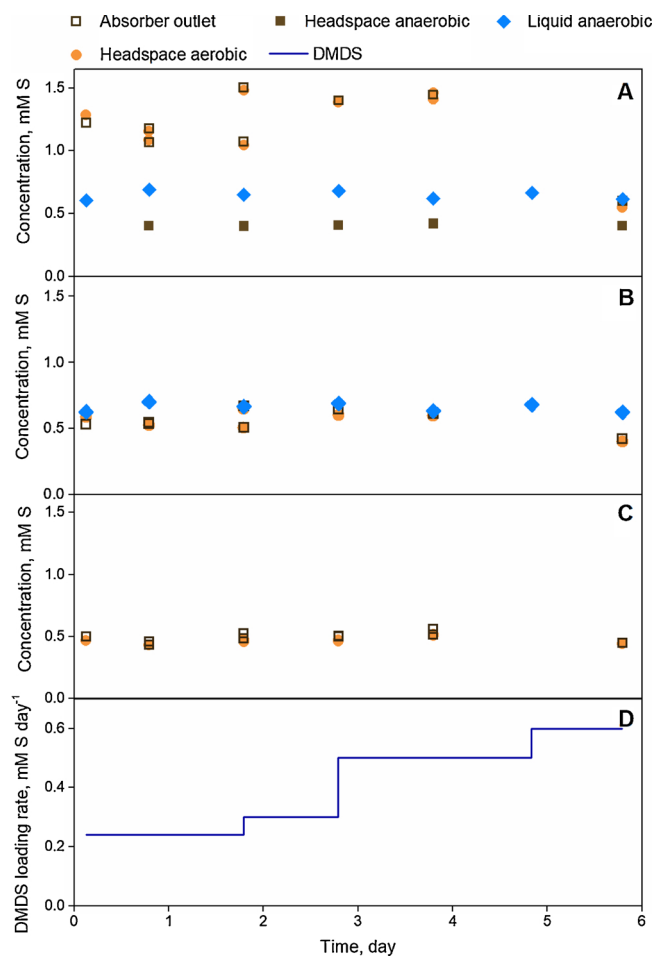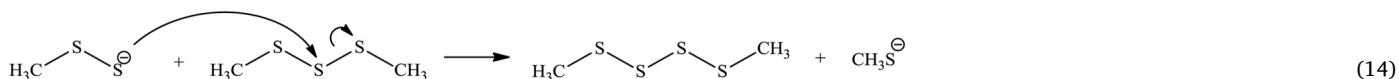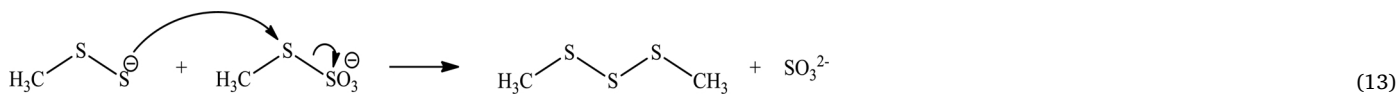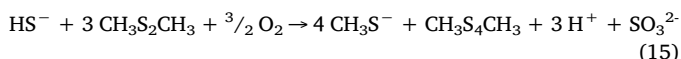

Fig. 4. Concentrations of **A.** dimethyl disulfide (DMDS), **B.** dimethyl trisulfide (DMTS) **C.** methanethiol (MT) in liquid and gas samples and **D.** loading rate of DMDS. Concentration is expressed per the molecule of sulfur (mM S) to enable comparison.

$$(11)$$

$$(12)$$

$$(13)$$

$$(14)$$

where the overall equation can be written as follows:

$$HS^- + 3\,CH_3S_2CH_3 + {}^3/_2\,O_2 \rightarrow 4\,CH_3S^- + CH_3S_4CH_3 + 3\,H^+ + SO_3{}^{2-} \quad (15)$$

However, formed sulfite ($SO_3{}^{2-}$) will fast react with oxygen and form sulfate (Eq. (16)), and will react with formed biosulfur particles to thiosulfate (Eq. (17)):

$$O_2 + 2\,SO_3{}^{2-} \rightarrow 2\,SO_4{}^{2-} \quad (16)$$

$$S^0 + SO_3{}^{2-} \leftrightarrow S_2O_3{}^{2-} \quad (17)$$

The obtained results did not confirm the formation of dimethyl tetrasulfide, probably due to a low concentration. However, we found that methanethiol, dimethyl trisulfide, and dimethyl tetrasulfide formed from sulfide and DMDS in the batch test (Appendix F). For the Eq. (15), the free energy change at standard conditions ($\Delta G_R°$) is -341 kJ reaction^{-1}, indicating a spontaneous forward reaction. $\Delta G_R°$ value decreases linearly (Hofmann, 2018) with an increasing number of sulfur atoms in diorgano polysulfanes reaching -738 kJ reaction^{-1} for the reaction between sulfide and dimethyl octasulfide. This could indicate that sulfide will react with longer diorgano polysulfanes more readily.

As a result of the reaction between sulfide and DMDS, less volatile and more hydrophobic diorgano polysulfanes are formed that can be better removed from the bioreactor suspension by adsorption onto the surfaces of biosulfur particles (Roman et al., 2016b).

3.2. Effect of DMDS on the microbial community composition

To elucidate any long-term effects of DMDS addition on the composition of the SOB community in the biodesulfurization setup, we collected biomass samples before and after DMDS addition (0.6 mM S day^{-1}). We performed 16S rRNA gene amplicon sequencing to establish the microbial composition and additionally performed qPCR to establish absolute counts of the three haloalkaliphilic SOB key-players. From the results of amplicon sequencing, it appears that the presence of DMDS provided a competitive advantage to the *Thioalkalibacter* genus. The only described species in this genus is a moderately halophilic and facultatively alkaliphilic obligate chemolithoautotrophic *Thioalkalibacter halophilus* (Banciu et al., 2008), which was also found among the dominant SOB in the previous study with thiol addition (Roman et al., 2016b). This SOB species has a relatively low growth yield but relatively high growth rate (μ_{max} 0.09–0.1 h^{-1}) over a

broad range of pH and salinity (Banciu et al., 2008). Fig. 5 shows that the gammaproteobacterial genera *Thioalkalivibrio, Thioalkaimicrobium, Alkalilimnicola,* and *Halomonas,* were abundant in the inoculum, but their numbers decreased after the DMDS addition. The genera *Thioalkalivibrio* and *Thioalkalimicrobium* (currently reclassified as *Thiomicrospira*) are obligate chemolithoautotrophic haloalkaliphilic SOB dominating in soda lakes and desulfurization bioreactors operating at haloalkaline conditions (Ahn et al., 2017; Sorokin and Kuenen, 2005), which have different growth strategies. *Thioalkalimicrobium* species are characterized by low growth yield but high growth rates and extremely high sulfide and thiosulfate oxidizing activity, whereas *Thioalkalivibrio* species are relatively slowly growing organisms with at least two times higher specific growth yield on sulfide or thiosulfate (Sorokin et al., 2003; Sorokin and Kuenen, 2005). Based on its highly specialized sulfide oxidation activity, *Thioalkalivibrio sulfidiphilus* is the dominant member of *Thioalkalivibrio* genus that was consistently found to dominate in the biogas desulfurization installations operating at low red-ox potential and haloalkaline conditions (Sorokin et al., 2012, 2011). In previous studies, it was also found that *Tv. sulfidiphilus* was abundant in sulfide removing bioreactors in lab- and full-scale gas biodesulfurization systems in the absence of any organic sulfur compounds (De Rink et al., 2019; Kiragosyan et al., 2019a, 2019b; Roman et al., 2016a; Sorokin et al., 2008). Other two genera, *Halomonas,* and *Alkalilimnicola,* that were identified in the inoculum and end samples, are commonly found in the haloalkaline environments containing both organic and reduced sulfur compounds (Wang et al., 2007). *Alkalilimnicola* species, particularly *Alkalilimnicola ehrlichii*, are haloalkaliphilic facultative chemolithoautotrophic SOB, some of which can utilize sulfide, CO and formate as the electron donors and O_2 or nitrate as *e*-acceptor (Hoeft et al., 2007). *Alk. ehrlichii* was previously found in gas biodesulfurization systems (De Rink et al., 2019; Roman et al., 2016b). *Halomonas* species are aerobic or facultative anaerobic chemoorganotrophic halo- and haloalkaliphilic gammaproteobacteria that utilize a wide range of organic substrates, whilst inorganic sulfur compounds, particularly thiosulfate, are incompletely oxidized to tetrathionate (García et al., 2005; Sorokin, 2003).

The most important finding from the community profiling is that proliferation of *Thb. halophilus* occurred already after a short-term (i.e. 6 days) exposure to DMDS. This suggests the importance of *Thb. halophilus* in the gas biodesulfurization process in the presence of VOSCs. This conclusion was confirmed in a different study in which a qPCR protocol with target-specific primers was used (Kiragosyan et al., 2019b). In addition, we have analyzed the absolute abundance of *Tv.*
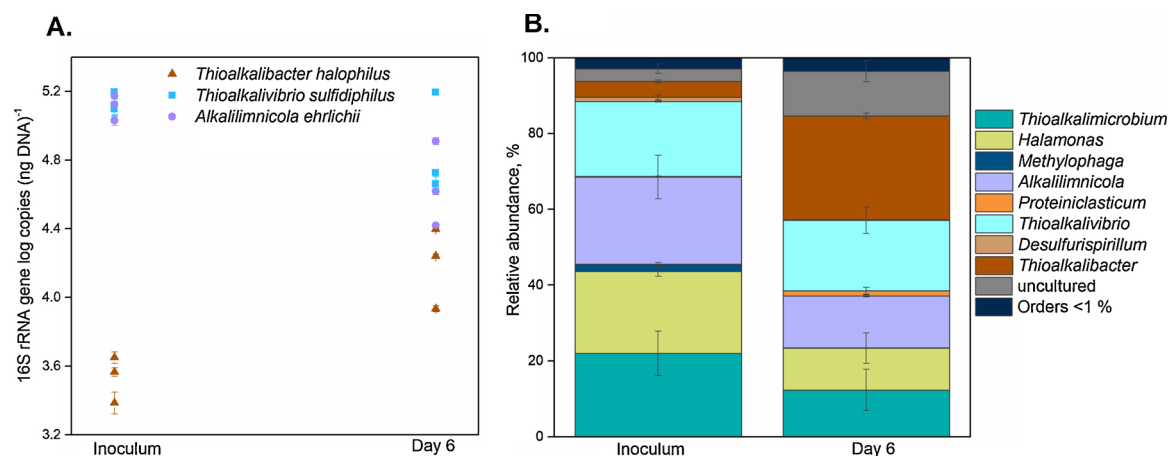

Fig. 5. A. Quantified 16S rRNA gene copies of *Thioalkalivibrio sulfidiphilus*, *Thioalkalibacter halophilus* and *Alkalilimnicola ehrlichii* before and after the addition of 0.60 mM S day^{-1} DMDS to the lab-scale gas biodesulfurization system. Presented data points are average values of measured triplicates, whereas each data point is a technical replicate at each time point. Error bars indicate the standard deviation between duplicates. **B.** The relative abundance of the microbial composition at the inoculum stage and after addition of 0.60 mM S day^{-1} of DMDS (End) based on partial 16S rRNA gene amplicon could. The lab-scale gas biodesulfurization bioreactor system was operated at a low oxidation-reduction potential of -390 mV (against an Ag/AgCl reference), pH 8.5, and H$_2$S loading rate was 58.12 mM S day^{-1}. Only bacteria with a relative abundance higher than 0.5 % are listed (remaining species are clustered into "Others"). Results represent the average value between three pseudo-replicates, and the error bar represents the standard deviation.

sulfidiphilus and *Alk. ehrlichii* in the samples, as they are known to be key players in the biodesulfurization process as well (Roman et al., 2016b, 2016a; Sorokin et al., 2008).

Results of the qPCR showed a significant increase of one log-scale of 16S rRNA gene copies (ng DNA)$^{-1}$ of *Thb. halophilus* after exposure to DMDS (Fig. 5A). This indicates a preferential development of this SOB species in the presence of DMDS. Moreover, the results allow us to explain the findings in previous studies, where the increased relative abundance of *Thb. halophilus* was associated with thiol addition (Roman et al., 2016b). As a result, we have established the effect of DMDS on the microbial community. Thus, it can be concluded, that in the previous study formed DMDS from the oxidation of MT gave advantage to *Thb. halophilus*. Results for *Tv. sulfidiphilus* and *Alk. ehrlichii* showed a slight reduction in the 16S rRNA gene copies (Fig. 5A), which corresponds with an observed decrease in relative abundance. Several studies have been performed on DMDS elimination in a biotrickling filter, including microbial community analyses. For example, Arellano-Garcia et al. found Thioalkalivibrio sulfidiphilus as a dominant species (44.2 %) in the alkaline biotrickling filter that was used to simultaneously treat DMDS and H$_2$S (Arellano-García et al., 2018). Other studies did not observe proliferation or a high abundance of either Thioalkalivibrio sulfidiphilus nor presence of *Thb. halophilus* and *Alk. ehrlichii* (Chen et al., 2016; Tu et al., 2019).

To confirm the capacity of the pure culture of *Thb. halophilus* for sulfide oxidation in the presence of DMDS, we performed a respiration test at different DMDS concentrations. The obtained data were used to calculate an IC$_{50}$ value, indicating the DMDS concentration at which 50 % inhibition occurs at a given sulfide concentration (Roman et al., 2016c). Results showed that *Thb. halophilus* maximum sulfide oxidation rate was reached at 0.12 mM sulfide, and the IC$_{50}$ value was reached at 2.37 ± 0.1 mM of DMDS (Appendix G, Fig. G1). In comparison, the obtained IC$_{50}$ value for *Thb. halophilus* was almost two times higher than for *Tv. sulfidiphilus* (1.4 ± 0.1 mM) (Roman et al., 2016c). This finding prove the ability of *Thb. halophilus* to withstand high DMDS concentrations, which is relevant for industrial applications.

To understand the underlying cause for the inhibitory effects of DMDS on biological sulfide oxidation, we looked into the respiratory oxidases involved in the electron transport chain of the SOB species present in our lab-scale reactor system. For instance, *Thioalkalivibrio* species are known to only contain cytochrome *c* oxidases of the heme-copper superfamily, mostly of the *cbb$_3$* type (Muntyan et al., 2015; Muyzer et al., 2011; Sorokin et al., 2001). Also enzymology studies of *Alkalilimnicola* spp. showed the presence of cytochrome *c* oxidase, which may be of the *cbb$_3$* type as well (Sorokin et al., 2010). The same conclusion can be drawn from the analysis of publically available genome of *Alkalilimnicola ehrlichii*. In contrast, the genome of *Thioalkalibacter halophilus* strain ALCO1 encodes a quinol oxidase of the *bd* type (CydBAD) in addition to the cytochrome *c* oxidase *cbb$_3$* (Coo-NOQPHG) (our unpublished data). It is known that organic sulfur compounds inhibit cytochrome *c* oxidases (Roman et al., 2016b), while the quinol oxidases, particularly of the *bd* type, are less prone to the commonly known cytochrome *c* oxidase inhibitors, such as cyanide and CO (Quesada et al., 2007). For instance, *Thioalkalibacter* halophilus ALCO1 has been enriched over the commonly dominating *Thioalkalivibrio* in high salt alkaline medium from soda lakes in the presence of 50 % CO in the gas phase (Banciu et al., 2008). This could explain why *Thb. halophilus* is more resistant to the presence of DMDS.

Finally, we performed a serum bottle experiment to assess the ability of *Thb. halophilus* to biodegrade DMDS under both anaerobic and aerobic conditions. We measured methanethiol production from the reduction of DMDS. This is a known reaction studied by (Kiene et al., 1986; Liang et al., 2015; Lomans et al., 2002). It is an oxygen-independent and reversible reaction where a molecule of DMDS is split by DMDS reductase to produces 2 mol of MT (Smith and Kelly, 2009). The results of the tests were negative, which clearly indicated the inability of *Thb. halophilus* and developed biomass to directly degrade DMDS,

while still being active in sulfide oxidation in its presence.

Overall, the qPCR, amplicon sequencing, and respiration tests conclude that *Thb. halophilus* preferentially developed in the lab-scale gas biodesulfurization system in the presence of DMDS and most probably due to the presence of an alternative (quinol) oxidase of the *bd* type.

4. Conclusion

A critical success factor for the further development of the ascribed process for sour gas desulfurization is the quantification of DMDS, together with the formed degradation products, like methanethiol, dimethyl tri- and tetrasulfide. Hence, a new GC-FPD method was developed to gain more insights into the biochemistry of the prevailing gas biodesulfurization process. Moreover, the newly developed method can also be used in oil and gas operating sites to identify the presence of liquid DOPS in, e.g. produced water and gas condensates. Our studies show that DOPS will inhibit the sulfate formation rates and increase sulfur selectivity to 96 mol% at high biomass concentration (51 ± 3 mg N L^{-1}). Hence, organic sulfur compounds could be supplied to an oxidizing sulfide bioreactor to increase the yield of sulfur formation. A disadvantage is that DMDS decreases the rate of biological sulfide oxidation, what subsequently triggers an increase in thiosulfate formation. This means that full scale systems that treat sour gas streams containing both H$_2$S and DMDS will have to be larger to accommodate for the reduced oxidation capacity, or alternatively, should be operated at higher biomass concentrations. Addition of DMDS not only affected the product formation but also triggered changes in the microbial community. *Thioalkalibacter halophilus* proliferated as we found that it was highly resistant to elevated DMDS concentrations (2.37 ± 0.10 mM). In addition, a reduction of the dominating species *Tv. sulfidiphilus* and *Alkalilimnicola ehrlichii* was observed. In conclusion, we recommend that at the start-up of full-scale gas biodesulfurization installations the inoculum is abundant in *Thioalkalibacter halophilus*, to achieve a stable process operation and minimize the chemical consumption and formation of a diluted bleed stream.

Author contribution

Karine Kiragosyan conceived and designed the analyzes, performed the analysis, collected the data and wrote the paper. Magali Picard performed the analysis and wrote the paper. Dimitry Sorokin contributed with writing. Jelmer Dijkstra co-developed GC-FPD method and guided through analysis and data interpretation. Johannes B.M. Klok guided through the experimental process. Pawel Roman supported with the experimental design, co-developed GC-FPD method and worked on the mechanism of the new reaction. In addition, he guided through analysis and data interpretation and contributed to writing. Albert J.H. Janssen contributed with writing and guidance.

Appendix B. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jhazmat.2019.121916.

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