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ENVIRONMENTAL BIOTECHNOLOGY

Inhibition of microbiological sulfide oxidation by methanethiol and dimethyl polysulfides at natron-alkaline conditions

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Abstract To avoid problems related to the discharge of sulfidic spent caustics, a biotechnological process is developed for the treatment of gases containing both hydrogen sulfide and methanethiol. The process operates at natron-alkaline conditions (>1 mol L^{-1} of sodium- and potassium carbonates and a pH of 8.5–10) to enable the treatment of gases with a high partial CO_2 pressure. In the process, methanethiol reacts with biologically produced sulfur particles to form a complex mixture predominantly consisting of inorganic polysulfides, dimethyl disulfide (DMDS), and dimethyl trisulfide (DMTS). The effect of these organic sulfur compounds on the biological oxidation of sulfide to elemental sulfur was studied with natron-alkaliphilic bacteria belonging to the genus *Thioalkalivibrio*. Biological oxidation rates were reduced by 50% at 0.05 mM methanethiol, while for DMDS and DMTS, this was estimated to occur at 1.5 and 1.0 mM, respectively. The inhibiting effect of methanethiol on biological sulfide oxidation diminished due to its reaction with biologically produced sulfur particles. This reaction increases the feasibility of biotechnological treatment of gases containing both hydrogen sulfide and methanethiol at natron-alkaline conditions.

Keywords Methanethiol · Polysulfide · Sulfide oxidation · Spent caustics · *Thioalkalivibrio*

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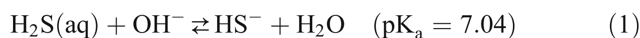
Introduction

Biogas and gases produced in oil refining and chemical industries often contain volatile sulfur compounds of which hydrogen sulfide (H_2S) is the most common. Besides H_2S, these gases may also contain volatile organic sulfur compounds (VOSCs) such as methanethiol (MT), ethanethiol, propanethiol, carbonyl sulfide (CS_2), dimethyl sulfide (DMS), and dimethyl disulfide (DMDS). Examples of various gas and refinery streams containing sulfide and VOSCs are given in Table 1.

To prevent emission of sulfur dioxide and problems related to odor, toxicity, and corrosivity, these sulfur compounds need to be removed from the gas before combustion or discharge. For the removal of H_2S from sour gases produced in oil refining and chemical industries, caustic scrubbing is often applied. In this process, H_2S is absorbed into an aqueous alkaline solution in an absorber, under the formation of hydrosulfide (HS^-, referred to as "sulfide"):

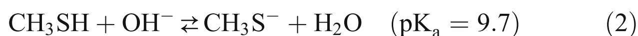


$$H_2S(aq) + OH^- \rightleftarrows HS^- + H_2O \quad (pK_a = 7.04) \qquad (1)$$

Table 1 Examples of gas and refinery streams contaminated with sulfide and VOSCs

Source	Sulfur compound	Concentration (ppmv)	Reference
Aerobic brewery waste water treatment plant	H_2S	4	(Smet and Langenhove 1998)
	DMS	30	
Anaerobic wastewater sludge digester	H_2S	100–200	(Iranpour et al. 2005)
	MT	2–4	
Landfill gas	H_2S	Up to 2300	(Börjesson 2001; Kim et al. 2005)
	MT	Up to 40	
	DMS	Up to 9	
	CS_2	Up to 6	
Kraft paper production process	H_2S	19	(Karnofski 1975; Smet and Langenhove 1998)
	MT	94	
	DMS	17	
	DMDS	22	
Liquefied petroleum gas (LPG)	H_2S	60 ppmw	(Manieh and Ghorayeb 1981)
	MT	432 ppmw	
	DMS	217 ppmw	
Refinery sulfidic spent caustics	Sulfide	31–67 ppmw	(Sipma et al. 2004)
	MT	0.6–20 ppmw	
	DMS	0–0.63 ppmw	

Methanethiol is one of the most common VOSCs in sour gases (Labouri et al. 2001; Andersson et al. 2004; Carlsson and Rajani 2005) and can also be absorbed in the alkaline scrubbing solution:



$$CH_3SH + OH^- \rightleftarrows CH_3S^- + H_2O \quad (pK_a = 9.7) \tag{2}$$

The sulfide-loaded alkaline solution, also referred to as “sulfidic spent caustics,” is often directed to a wastewater treatment plant. This may cause several problems such as corrosion, release of odors, and safety hazards (Ellis 1998; Altas and Büyükgüngör 2008). Negative impacts of sulfide on the performance of wastewater treatment facilities have also been observed, e.g., problems related to nitrification (Meza-Escalante et al. 2008) and the formation of bulking sludge due to the growth of filamentous sulfur-oxidizing bacteria such as *Thiothrix* and *Beggiota* species (Williams and Unz 1985).

To overcome these problems, a three-step biotechnological process has been developed for the removal of H_2S from sour gases with high CO_2 partial pressures (Buisman et al. 2003). The first step consists of absorption of H_2S into an alkaline scrubbing solution as described above. In the second step, the sulfide-loaded alkaline solution is sent to a bioreactor where sulfide is biologically oxidized to elemental sulfur (S^0, referred to as “biosulfur”). The overall reaction for the conversion of H_2S to biosulfur is shown in Eq. 3. To maximize the recovery of biosulfur and to reduce the consumption of caustic and make-up water, the complete oxidation of H_2S to sulfate (SO_4^{2-}, Eq. 4) is unwanted (Janssen et al. 1995). It was shown previously that sulfate formation can be prevented by operating the bioreactor at oxygen-limiting conditions (Van den Bosch et al. 2007).

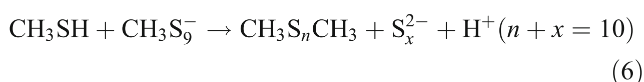
$$H_2S + 0.5\ O_2 \rightarrow S^0 + H_2O \tag{3}$$

$$H_2S + 2\ O_2 \rightarrow SO_4^{2-} + 2\ H^+ \tag{4}$$

In a third step, biosulfur particles are separated from the reactor liquid by sedimentation. To treat sour gases with a high partial CO_2 pressure and to maximize the H_2S loading capacity of the alkaline solution, the process has to be operated at high concentrations of (bi)carbonate. With sodium (Na^+) and potassium (K^+) as the counter ions for (bi)carbonate, the process operates at natron-alkaline conditions (e.g., 2 mol L^{-1} $Na^+ + K^+$, pH 9). Therefore, specialized sulfur-oxidizing bacteria (SOB) are applied in the process. The feasibility of sulfide oxidation to elemental sulfur at natron-alkaline conditions was previously tested in lab-scale bioreactors, using sediments from hypersaline soda lakes as inoculum (Van den Bosch et al. 2007). Microbiological analysis of the successfully operating bioreactors showed a domination of obligately chemolithoautotrophic and extremely halo-alkaliphilic SOB belonging to the genus *Thioalkalivibrio* (Sorokin et al. 2008). Based on 16S-rRNA gene sequencing, dominant isolates obtained at pH 9.5 clustered around the core group of the genus, which includes the species *Thioalkalivibrio versutus*, *Thioalkalivibrio thiocyanoxidans*, *Thioalkalivibrio jannaschii*, and *Thioalkalivibrio nitratis*. Another group of isolates obtained at pH 8.8 belonged to the cluster of the facultative alkaliphilic halophile *Thioalkalivibrio halophilus*.

To avoid the discharge of sulfidic spent caustics in a wastewater treatment plant (WWTP), the above-mentioned biotechnological process is further developed for the treatment of gases containing both H_2S and MT. Previous research on sulfidic spent caustics suggests that biological oxidation of MT to sulfate at neutral pH conditions occurs via intermediary DMDS (Sipma et al. 2004). The effect of MT on the biological conversion of H_2S to elemental sulfur at natron-alkaline conditions is however not yet known. Being a strong nucleophile, MT can react with sulfur particles in an aqueous solution, initially forming methyl polysulfide by opening of the sulfur ring (Eq. 5) (Steudel 2002). In subsequent spontaneous reaction steps, shorter dimethyl polysulfides are formed along with inorganic (poly)sulfides (Eq. 6).

$$CH_3SH + S_8 \rightarrow CH_3S_9^- + H^+ \quad (5)$$



$$CH_3SH + CH_3S_9^- \rightarrow CH_3S_nCH_3 + S_x^{2-} + H^+ (n + x = 10) \quad (6)$$

In a previous study, we have shown that these reactions indeed take place between MT and biosulfur particles (Van Leerdam 2008). The main end products of MT in addition to an excess of biosulfur at pH 8.7 are polysulfide, sulfide, and (di)methyl polysulfides, consisting predominantly of DMDS and dimethyl trisulfide (DMTS). In the presence of oxygen, DMDS also can be formed by rapid auto-oxidation of MT (Jocelyn 1972):

$$2\ CH_3SH + 0.5\ O_2 \rightarrow CH_3SSCH_3 + H_2O \quad (7)$$

This paper focuses on the inhibitory effects of MT and the products of its reaction with biosulfur on the biological oxidation of sulfide and polysulfide by natron-alkaliphilic SOB. Knowledge on the potential toxic effects of these compounds is required to further develop a biotechnological process for removal of H_2S and MT from sour gases as alternative to treatment of sulfidic spent caustics in a WWTP.

Materials and methods

Respiration tests

Respiration tests were performed in a thermostated 7.5 mL glass chamber mounted on a magnetic stirrer and fitted with a piston holding a dissolved oxygen (DO) electrode (Yellow Springs Instr., OH, USA). This piston contained a small opening to allow air to be removed from the chamber and to add reactants. A schematic representation of the setup is shown in Fig. 1. Cell suspensions (70–100 μL) were added to a carbonate buffer solution (pH 9.0) to a final concentration of 14 to 21 mg N L^{-1}. The solution was saturated with oxygen by bubbling with air for at least 5 min. Experiments were started by injection of 20 to 320 μL of sulfur substrate stock solutions (sulfide, polysulfide, MT, DMDS, DMTS, and a mixture of MT and biosulfur). The decrease of the DO concentration was measured in time, and the initial slope ($d[O_2]/dt_0$) was used as a measure of the oxidation rate. Oxidation rates were measured in the absence (chemical oxidation rates) and in presence of cells. Biological oxidation rates were calculated by subtracting the chemical oxidation rate from the rate in presence of cells (combined chemical and biological oxidation). At the end of each respiration experiment, samples were taken for analysis of the residual total sulfide (S_{tot}^{2-}) concentration to enable the calculation of the molar O_2/S_{tot}^{2-} consumption ratio. All experiments were performed in duplicate. Biological controls were performed with autoclaved cells (20 min, 121°C).

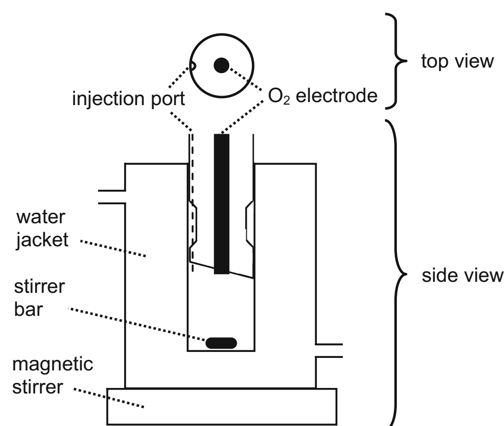


Fig. 1 Schematic representation of the setup used for respiration tests

Biomass source

Natronophilic SOB were obtained from a lab-scale gas-lift bioreactor inoculated with a mixture of hypersaline soda lake sediments from Mongolia, southwestern Siberia, and Kenya (Sorokin and Kuenen 2005). The reactor was operated at natron-alkaline, sulfur-producing conditions (2 mol L^{-1} Na^+ + K^+, pH 9.0±0.2, [S_{tot}^{2-}]=0.2–0.3 mM, DO<0.1% saturation, H_2S supply of 2.2 mM h^{-1}, T=35°C). A detailed description of the reactor setup is given elsewhere (Van den Bosch et al. 2007). Microbiological analysis of the bioreactors revealed a domination of obligately chemolithoautotrophic and extremely natron-alkaliphilic sulfur-oxidizing bacteria belonging to the genus *Thioalkalivibrio*, as described before (Sorokin et al. 2008). Bacterial cells were separated from extracellular sulfur by several successive steps of low-speed centrifugation (500 rpm), washed and resuspended in a (bi)

carbonate buffer (pH 9.0, 1.67 mol L^{-1} K^+, 0.33 mol L^{-1} Na^+), resulting in cell suspensions with a final concentration of 1,100–1,700 mg N L^{-1}.

In a dedicated experiment, MT (1.5–3.3 μM h^{-1}) and H_2S (2.2 mM h^{-1}) were continuously supplied to the bioreactor (pH 9.1±0.1). With this approach, the natronalkaliphilic biomass was exposed to (di)methyl sulfur compounds for a prolonged period. The DO concentration was controlled at >5% saturation, and the S_{tot}^{2-} concentration was kept below 0.01 mM to prevent limitation. After 27 days of operation, cells were harvested from the bioreactor as described above and used in respiration tests.

Analytical procedures

The maximum oxygen solubility of the alkaline buffer solution was determined in an air-tight thermostated vessel (400 mL). After oxygen saturation by bubbling with air (DO=100% saturation), the vessel was equipped with a DO electrode. A 50-mM sodium sulfite solution (Merck, Darmstadt, Germany) was added stepwise to the vessel in the presence of copper sulfate to act as a catalyst. Based on the stoichiometry of the oxidation of sulfite to sulfate, combined with the decrease of the DO concentration, the maximum oxygen solubility of the buffer was found to be 0.15 mmol L^{-1} at 35°C (data not shown).

Total sulfide (S_{tot}^{2-}) concentrations were measured on the basis of a modified methylene blue method as described previously (Van den Bosch et al. 2007). At the experimental pH of 9.0, the main sulfide species are HS^- and S_x^{2-}. Therefore, the total sulfide concentration can be described as:

$$\left[S_{tot}^{2-}\right] = \left[HS^-\right] + \left[S_x^{2-}\right] \quad (8)$$

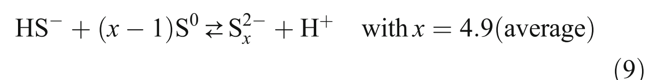
Polysulfide anion concentrations were determined spectrophotometrically as described elsewhere (Kleinjan et al. 2005a; Van den Bosch et al. 2007), at a wavelength of 285 nm (Perkin-Elmer, Lambda 2, Norwalk, CT, USA). With this method, which can only be used in the absence of VOSCs, the total concentration of zerovalent sulfur atoms in polysulfide (S_x^{2-}–S^0) is determined. Biomass concentrations were measured as the amount of total nitrogen, as described previously (Van den Bosch et al. 2007).

Chemicals used

Carbonate buffer was prepared by mixing bicarbonate (pH 8.3) and carbonate (pH 12.3) buffers to a final pH of 9.0. Both buffers contained 0.67 mol L^{-1} Na^+ and 1.33 mol L^{-1} K^+ as (bi)carbonates (Merck, Darmstadt, Germany).

Sodium sulfide stock solutions (20 to 30 mM) were freshly prepared by dissolution of $Na_2S{\cdot}9H_2O$ crystals (Merck, Darmstadt, Germany) in de-aerated ultrapure (Milli-Q) water. Before dissolution, the oxidized surface of the crystals was removed by flushing with de-aerated water. The exact sulfide concentration of the stock solutions was determined afterwards.

Polysulfide stock solutions were prepared by reaction of excess biosulfur (250 mM) with a 30 mM sulfide solution at 50°C, as described elsewhere (Kleinjan et al. 2005b). Polysulfide solutions prepared in this way consist of a mixture of polysulfide anions (S_x^{2-}) and sulfide (HS^-), of which the equilibrium is defined by Eq. 10 (Kleinjan et al. 2005a). The pH of the polysulfide stock solutions was 10.3. At this pH, 93% of the S_{tot}^{2-} concentration is present as polysulfide, which was confirmed by analysis of S_{tot}^{2-} and polysulfide concentrations.



$$HS^- + (x-1)S^0 \rightleftarrows S_x^{2-} + H^+ \quad \text{with}\, x = 4.9(\text{average}) \quad (9)$$

$$K_x = \frac{\left[S_x^{2-}\right]\left[H^+\right]}{\left[HS^-\right]} \quad \text{with}\, pK_x = 9.17 \quad (10)$$

A sodium methylmercaptide ($NaCH_3S$) solution (2.5 mol L^{-1}) was supplied by Arkema Group (Rotterdam, the Netherlands). Stock solutions of 20 mM MT were prepared by dilution with oxygen-free ultrapure (Milli-Q) water and kept at a slight nitrogen gas overpressure to prevent oxidation by air.

Biosulfur was obtained from a full-scale biogas treatment facility (Eerbeek, the Netherlands) and dialyzed in demineralized water to remove salts to a conductivity below 40 μS cm^{-1}. The mixture of MT and biosulfur (further referred to as "MT–S^0 mixture") was prepared by addition of 20 mM MT and 400 mM biosulfur to oxygen-free carbonate buffer (pH 9.0). The MT–S^0 mixture was incubated overnight at 30°C. After incubation, the S_{tot}^{2-} concentration in the MT–S^0 mixture was 9.6 mM, which is close to the expected value of 10 mM, based on the reaction stoichiometry according to Eqs. 5 and 6. The exact composition of the MT–S^0 mixtures was not determined, but a more detailed description of the composition of similar MT–S^0 mixtures (30°C, pH 8.7) is given elsewhere (Van Leerdam 2008). Before use, remaining biosulfur particles were allowed to settle so that no biosulfur was introduced to the respiration chamber.

Stock solutions of DMDS and DMTS (20 mM) were prepared from pure solutions (Merck, Darmstadt, Germany) by dilution in water (DMDS) or HPLC-grade methanol (DMTS).

Results

Sulfide and polysulfide oxidation rates in the absence of methyl sulfur compounds

Oxidation rates of sulfide and polysulfide ions were initially determined in the absence of (di)methyl sulfur compounds. Upon addition of biomass (15 mg N L^{-1}) to oxygen-saturated buffer, the DO concentration decreased at a rate of up to 4.8 mM O_2 h^{-1}, even without addition of substrate (data not shown). This phenomenon was also previously observed and is assumed to be attributed to the oxidation of membrane-bound polysulfur compounds (Banciu et al. 2004; Van den Bosch et al. 2008). After re-aeration for at least 5 min, only endogenic oxygen consumption was observed, and experiments were started. Oxidation rates were determined with sulfide (Fig. 2a) and polysulfide (Fig. 2b) as substrates (0.05 to 0.5 mM S_{tot}^{2-}) in the absence and in presence of cells. In the absence of cells (chemical oxidation), oxidation rates increased slightly with increasing sulfide concentrations. For polysulfide, chemical oxidation rates increased proportionally with the concentration, indicating first-order reaction kinetics as reported elsewhere (Kleinjan et al. 2005b). For polysulfide, the chemical oxidation rate was approximately twice as high as that of sulfide. Controls with autoclaved cells revealed similar oxidation rates as without the presence of cells (results not shown).

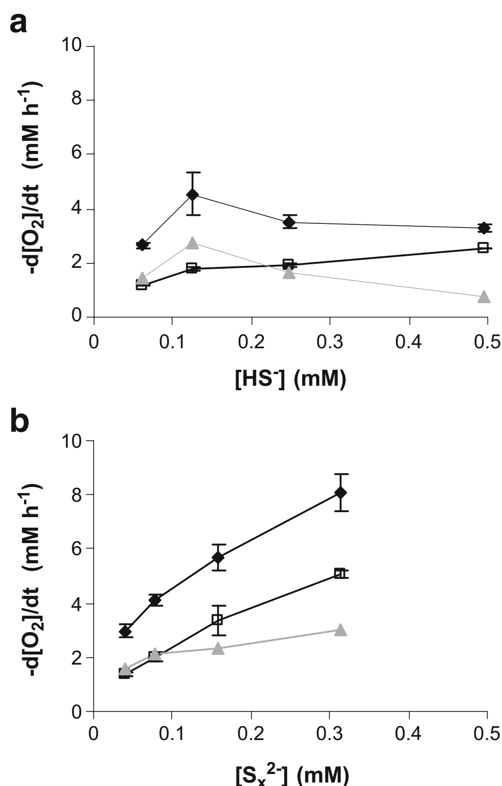


Fig. 2 Oxidation rates with different concentrations of HS^- (**a**) and S_x^{2-} (**b**). Both Figures show rates in the absence of cells (*open squares*); in the presence of cells (*closed diamonds*), and rates as a result of biological oxidation only (*gray triangles*). Biomass concentration=15 mg N L^{-1}; pH=9.0; total salt=2 mol L^{-1} Na^+ + K^+ as carbonates

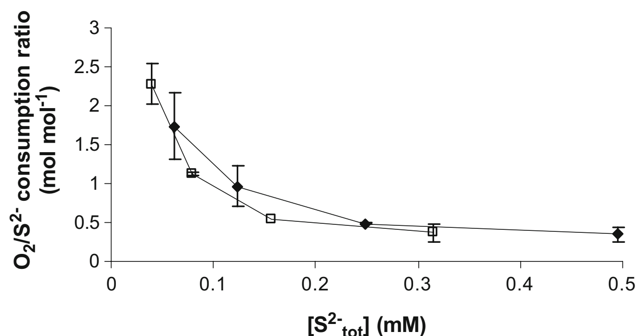


Fig. 3 Molar O_2/S_{tot}^{2-} consumption ratio as a result of biological oxidation of sulfide (*closed diamonds*) and polysulfide (*open squares*). Biomass concentration=15 mg N L^{-1}; pH=9.0; total salt=2 mol L^{-1} Na^+ + K^+ as carbonates

Biological oxidation of sulfide showed a maximum rate of 2.7±0.3 mM O_2 h^{-1} (0.18±0.2 mM O_2 mg N^{-1} h^{-1}) at a sulfide concentration of 0.12 mM, whereas at higher sulfide concentrations, the oxidation rate decreased. For biological polysulfide oxidation, a similar maximum oxidation rate of 3.0±0.1 mM O_2 h^{-1} (0.20±0.1 mM O_2 mg N^{-1} h^{-1}) was observed at the highest polysulfide concentration tested (0.31 mM S_{tot}^{2-}). By comparison of the molar O_2/S_{tot}^{2-} consumption ratio in the absence and in presence of cells, the stoichiometry of biological substrate oxidation was calculated (Fig. 3). For both sulfide and polysulfide bio-oxidation, the molar O_2/S_{tot}^{2-} consumption ratio was around 2 at a S_{tot}^{2-} concentration of around 0.05 mM. At increasing S_{tot}^{2-} concentrations, the molar O_2/S_{tot}^{2-} consumption ratio gradually decreased to a value of around 0.5 at S_{tot}^{2-} concentrations of 0.20–0.25 mM and above. Unfortunately, the oxidation products could not be measured due to the high salt concentration in combination with low product concentrations.

Inhibition by methanethiol and (di)methyl polysulfides

The effect of MT, DMDS, and DMTS on sulfide oxidation was studied at various VOSC concentrations. As elemental sulfur is the preferred end product of sulfide oxidation, a S_{tot}^{2-} concentration of 0.25 mM was applied. It was found previously that at a S_{tot}^{2-} concentration of 0.25 mM and above, no sulfate is produced (Van den Bosch et al. 2007). In the current study, this was confirmed by the molar O_2/S_{tot}^{2-} consumption ratio of 0.5 at S_{tot}^{2-} concentrations of 0.20–0.25 mM and above. Oxygen consumption rates with only MT, DMDS, and DMTS were negligible both in

the absence and in presence of cells (<0.1 mM h^{-1} at 0.4 mM). Chemical oxidation rates of sulfide were only slightly affected (data not shown). Biological sulfide oxidation rates on the other hand were strongly affected by MT (Fig. 4). A 50% decrease of the oxidation rate (IC_{50}) was already observed at a MT concentration of 0.05 mM, while at concentrations above 0.65 mM, biological sulfide oxidation was completely inhibited. Inhibition by DMDS and DMTS was less severe. At the highest concentration applied (0.85 mM), the oxidation rate of sulfide in the presence of DMDS or DMTS decreased to 55–60%. Based on a log-linear fit, it was estimated that for DMDS and DMTS, the IC_{50} value was 1.5 and 1.0 mM, respectively.

Oxidation rates of the MT–S^0 mixture were also tested. Chemical oxidation rates increased proportionally to the amount of MT–S^0 mixture added (Fig. 5), and the relation between oxidation rate and $S_{tot}{}^{2-}$ concentration was comparable to that of polysulfide (Fig. 2a). Biological oxidation rates obtained with the MT–S^0 mixture increased with increasing $S_{tot}{}^{2-}$ concentrations, reaching a maximum rate of 2.7±0.3 mM O_2 h^{-1} (0.18±0.2 mM O_2 mg N^{-1} h^{-1}) at 0.1 mM $S_{tot}{}^{2-}$. The measured rates were comparable to those of biological polysulfide oxidation (Fig. 2b). The relation between the $S_{tot}{}^{2-}$ concentration and the molar $O_2/S_{tot}{}^{2-}$ consumption ratio as a result of biological oxidation showed a similar pattern as observed with only sulfide and polysulfide as substrates (Fig. 3). According to the stoichiometry of the reaction between MT and biosulfur (Eqs. 7 and 8), the formed dimethyl polysulfide concentration is equal to the formed inorganic polysulfide concentration. As the maximum (inorganic) polysulfide concentration applied in the respiration tests was 0.2 mM, this implies that at least up to the same concentration of 0.2 mM, dimethyl

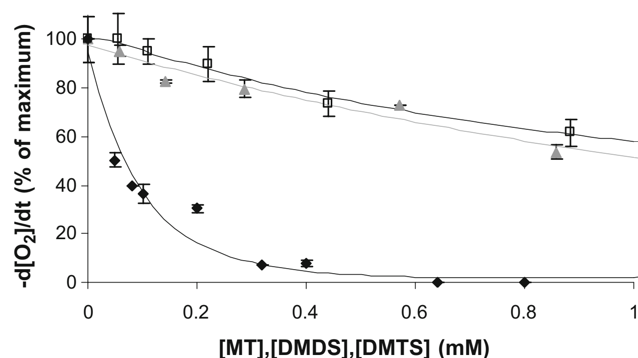


Fig. 4 Relative biological oxidation rates during sulfide oxidation (0.25 mM HS^-) in the presence of varying concentrations of MT (*closed diamonds*), DMDS (*open squares*), and DMTS (*gray triangles*). Biomass concentration, 20.5 mg N L^{-1}; specific biological oxidiation rate with sulfide only (100%), 0.18±0.02 mmol O_2 mg N^{-1} h^{-1}. pH=9.0; total salt=2 mol L^{-1} $Na^+ + K^+$ as carbonates. *Solid lines* represent the results of a log-linear regression model fitted to the results

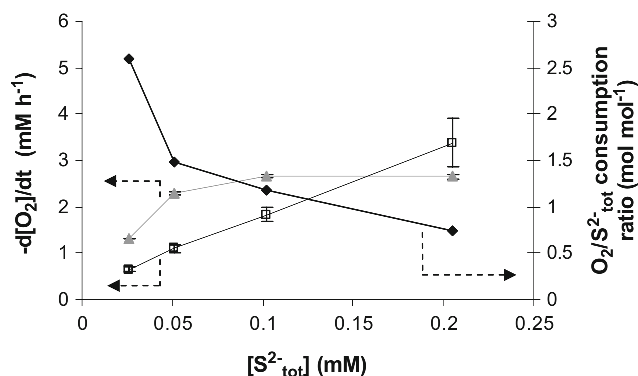


Fig. 5 Oxidation rates with the MT–S^0 mixture. The *x-axis* shows the initial $S_{tot}{}^{2-}$ concentration after addition of the mixture, being equal to the sum of concentrations of (di)methyl polysulfides, according to the stoichiometry of the reaction between MT and biosulfur (Eqs. 3 and 4). The *primary y-axis* shows oxidation rates in the absence of cells (*open squares*) and as a result of biological oxidation only (*gray triangles*). The *secondary y-axis* shows the molar $O_2/S_{tot}{}^{2-}$ consumption ratio as a result of biological oxidation only (*closed diamonds*). Biomass concentration=18 mg N L^{-1}; pH=9.0; total salt=2 mol L^{-1} $Na^+ + K^+$ as carbonates

polysulfides (DMDS and DMTS) did not inhibit biological sulfide oxidation.

Respiration rates after exposure to MT

In the respiration experiments discussed so far, the biomass had not been exposed to MT, DMDS, or DMTS prior to the respiration experiments. To test whether adaptation of biological sulfide oxidation to MT occurs after long-term exposure to (di)methyl sulfur compounds, biomass was pre-exposed to MT and the products formed from its reaction with biosulfur in a H_2S-oxidizing bioreactor operating without O_2 limitation. The results of the bioreactor runs are discussed elsewhere (Van den Bosch et al. 2009). At a continuous MT supply of 1.5–3.3 μM h^{-1}, all H_2S supplied to the reactor (2.2 mM h^{-1}) was converted to biosulfur, while little if any accumulation of sulfate, sulfide, or thiosulfate was found. This indicates that the biological activity was not severely inhibited by MT. After 27 days of adaptation, a comparison was made between pre-exposed and not pre-exposed biomass. For both types of cells, comparable sulfide oxidation rates were found at a sulfide concentration of 0.16 mM (Fig. 6). Oxidation rates with only MT (0.09 mM) were slightly higher for pre-exposed cells (0.07±0.02 mM mg N^{-1} h^{-1}) compared to the not pre-exposed cells (0.04±0.00) mM h^{-1}). Although the oxidation rate with MT was somewhat higher in the presence of cells compared to abiotic control experiments, it was not clear if any biological MT degradation occurred as concentrations of MT and DMDS could not be measured in the high salt medium. When both sulfide (0.16 mM) and MT (0.09 mM) were added, respiration rates with pre-

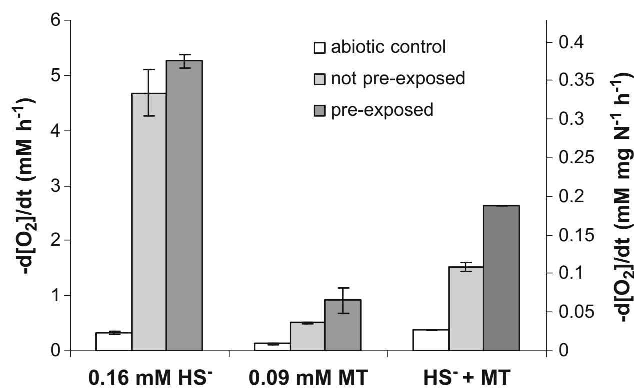


Fig. 6 Oxidation rates with HS^- (0.16 mM), MT (0.09 mM), and a mixture of HS^- (0.16 mM) and MT (0.09 mM) for respiration experiments without biomass (abiotic control), biomass that has not been pre-exposed to MT (*light gray bars*), and biomass that was pre-exposed to MT (*dark gray bars*) in a H_2S-oxidizing bioreactor. The *primary y-axis* shows volumetric oxygen consumption rates. The *secondary y-axis* shows the specific biological oxygen consumption rates, corrected for abiotic oxidation. Biomass concentration=14 mg N L^{-1}; pH=9.0; total salt=2 mol L^{-1} $Na^+ + K^+$ as carbonates

exposed cells (0.19±0.01 mM mg N^{-1} h^{-1}) were significantly higher compared to rates with not pre-exposed cells (0.11±0.01 mM mg N^{-1} h^{-1}).

Discussion

Inhibition by methylated sulfur compounds

This study shows that sulfide oxidation to elemental sulfur by natron-alkaliphilic SOB is severely inhibited in the presence of MT, already at low concentrations (IC_{50}= 0.05 mM MT). As an intermediate in the methionine metabolism, MT is reported to be responsible for inhibition of cytochrome *c* oxidase activity (Finkelstein and Benevenga 1986). It was suggested that inhibition by MT is caused by steric hindrance and ionic or hydrophobic interactions. As alkaliphilic SOB have a high cytochrome *c* content and a very high cytochrome *c* oxidase activity (Sorokin et al. 2001; Banciu et al. 2008), it is likely that inhibition by MT is caused by inhibition of cytochrome *c* oxidase activity. Being a strong nucleophile, MT can also break S–S bonds in proteins and thereby render enzymes inactive (Singh and Whitesides 1993). If the nucleophilicity of MT indeed plays a role in the inhibiting effect, a high pH is expected to result in more severe inhibition compared to low pH values, as the deprotonated form of MT (CH_3S^-) is a stronger nucleophile than molecular MT. Most information about the inhibiting effects of MT on microorganisms originates from experiments in anaerobic environments. Reported IC_{50} values for methanogenic granular sludge are 6–8 mM (with acetate), 10 mM (with methanol), and 7 mM (with hydrogen) (Sipma et al. 2003; Van Leerdam et al. 2006). Under aerobic conditions, much lower substrate inhibition values for MT are reported: 8 μM for *Thiobacillus thioparus* (Smith and Kelly 1988) and 14 μM for *Hyphomicrobium* species (Suylen et al. 1987). The IC_{50} value for MT on sulfide oxidation at natron-alkaline conditions found in our tests is thus comparable to values reported for aerobic neutrophilic SOB.

It was shown that compared to MT, DMDS and DMTS exhibit a less severe inhibiting effect on sulfide oxidation by natron-alkaliphilic SOB. This may be explained by the lower nucleophilicy of DMDS and DMTS compared to MT. Apparently, hydrophobic interactions do not play a major role in the inhibition of sulfide oxidation by these VOSCs, as DMDS and DMTS are more hydrophobic compared to MT. As polysulfide, DMDS, and DMTS are the main products from the reaction between MT and biosulfur, this reaction effectively results in a partial detoxification of MT. This was confirmed by bio-oxidation experiments performed with MT–S^0 mixtures as the oxidation rates with these mixtures were similar to those using only polysulfide as a substrate (Figs. 2b and 5). Formation of DMDS has been previously proposed to play a role in detoxification of MT in the aerobic treatment of sulfidic spent caustics (Sipma et al. 2004). It was hypothesized that biological oxidation of MT proceeds in two steps. First, MT is chemically oxidized to DMDS, where-after DMDS is biologically oxidized to sulfate. Biological oxidation of DMDS was not observed during the short duration of our respiration experiments (max. 15 min.), but it may take place after prolonged incubation periods.

Our results indicate that adaptation of the natronophilic biomass to MT takes place after prolonged exposure to low concentrations of MT. It is not known if this was the result of adaptation of the cells or a change in the composition of the mixed bacterial population. Aerobic growth on organic sulfur compounds was shown for the methylotrophic bacterium *Hyphomicrobium* VS (Pol et al. 1994). After growth in presence of low concentrations of dimethyl sulfide, this bacterium was able to able to respire MT, DMDS, and DMTS. Also, *Thiobacillus* species have been shown to grow aerobically on organic sulfur compounds. Oxidation of DMS, MT, DMDS, and H_2S from contaminated air was demonstrated by *T. thioparus* (Kanagawa and Mikami 1989; Cho et al. 1991). A high resistance to DMDS was found for a *Pseudomonas fluorescens* strain, able to utilize DMDS as a sulfur source up to a concentration of 9 mM (Ito et al. 2007). Little is known about the effect of DMTS on microorganisms. *Pseudonocardia asacharolytica* has been reported to oxidize 0.5 mM DMTS as sole carbon and energy source (Rappert and Müller 2005). It was hypothesized that DMTS was first converted into DMDS and subsequently to sulfate and CO_2.

Influence of the S_{tot}^{2-} concentration

This study shows that the biological molar O_2/S_{tot}^{2-} consumption ratio varies with the S_{tot}^{2-} concentration. At S_{tot}^{2-} concentrations around 0.05 mM, the molar O_2/S_{tot}^{2-} consumption ratio was around 2, while at $[S_{tot}^{2-}]>0.2$–0.25 mM, the molar O_2/S_{tot}^{2-} consumption ratio was around 0.5 (Fig. 3). The explanation for these results is that, at S_{tot}^{2-} concentrations around 0.05 mM, (poly)sulfide is completely oxidized to sulfate, with a theoretical molar O_2/S_{tot}^{2-} consumption ratio of 2 (Eq. 6). At $[S_{tot}^{2-}]>0.2$–0.25 mM, (poly)sulfide is biologically converted to elemental sulfur, with a theoretical molar O_2/S_{tot}^{2-} consumption ratio of 0.5 (Eq. 3). Intermediate molar O_2/S_{tot}^{2-} consumption ratios can be explained by a combined sulfate and sulfur formation, with the selectivity shifting towards sulfur formation with increasing S_{tot}^{2-} concentrations. This relation was found for all substrates used (sulfide, polysulfide, and the MT–S^0 mixture, Fig. 5). The reaction stoichiometry could not be confirmed by analysis of the oxidation products (sulfate, thiosulfate, and biosulfur), as the concentrations were too low to be detected in the carbonate buffer. The same relation between S_{tot}^{2-} concentration and the molar O_2/S_{tot}^{2-} consumption ratio was observed in bioreactor studies described previously (Van den Bosch et al. 2007). In these bioreactor studies, the products of (poly)sulfide oxidation could be analyzed, confirming the stoichiometry of sulfate and sulfur formation according to Eqs. 3 and 4. While the DO concentration in the bioreactor study was always below the detection limit of 0.1% saturation, the respiration experiments in the current study were performed at saturated DO conditions. This indicates that the S_{tot}^{2-} concentration and not the DO concentration determines the selectivity for the various products (i.e., sulfur or sulfate) from biological oxidation of (poly)sulfide.

Although chemical and biological oxidation rates of sulfide and polysulfide observed in the respiration experiments were comparable (Figs. 2a, b and 5), biological oxidation of these substrates can outcompete chemical oxidation in a bioreactor. Chemical oxidation rates of sulfide and polysulfide are higher at increased DO concentrations (O'Brien and Birkner 1977; Kleinjan et al. 2005b). Consequently, at the low DO concentration (<0.1% saturation) prevailing in a sulfur-producing bioreactor (Janssen et al. 1995; Van den Bosch et al. 2007), chemical oxidation rates are much lower compared to the rates found in the respiration experiments, which were performed at saturated DO concentrations.

Gas treatment considerations

Application of the newly developed process for treatment of gases containing both H_2S and MT at natron-alkaline conditions will mainly depend on the concentrations of MT, DMDS, and DMTS prevailing in the bioreactor. Usually, the MT concentration in sour gases is much lower than the H_2S concentration (Labouri et al. 2001; Carlsson and Rajani 2005). Moreover, as a result of the reaction between MT and biosulfur particles, MT is converted in the absorber column into the far less toxic DMDS and DMTS. Also, auto-oxidation of MT to DMDS may contribute to this apparent detoxification of MT. The rate of these reactions determines if the MT concentration in the bioreactor remains below values that severely inhibit biological sulfide oxidation (<0.05 mM). Another prerequisite for biotechnological treatment of H_2S- and MT-containing gases is the degradation of the dimethyl polysulfides (mainly DMDS and DMTS) that are produced from the reaction between MT and biosulfur. If these compounds are degraded at a sufficient rate, no accumulation will occur, preventing inhibitory concentrations (1–1.5 mM). Degradation of DMDS and DMTS may proceed by biological oxidation, although this was not observed in the respiration tests presented in this study. Further study on MT, DMDS, and DMTS in a H_2S-oxidizing bioreactor operating at natron-alkaline conditions is therefore essential to give more insight in the feasibility of treatment of gases containing both H_2S and MT.

In our experiments, only MT and its derived compounds produced from the reaction with biosulfur particles were studied for their inhibitory effects. However, besides MT, also higher organic sulfur compounds like ethanethiol and propanethiol may be present in sour gases (Holub and Sheilan 2003). Like MT, also these higher thiols can react with biosulfur particles (Van Leerdam 2008). Possibly, this reaction has the same detoxifying effect as observed with MT, so that treatment of sour gases containing these higher thiols may also be feasible.

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