

Degradation of methanethiol in an UASB reactor: reactor performance and salt tolerance

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ABSTRACT. Because of their potential negative effects on the environment, organosulfur compounds have to be removed from petroleum and fossil fuels. Alkaline solutions (*e.g.* Na₂CO₃) are used to extract hydrogen sulfide and acidic organic sulfur compounds, such as thiols (R-SH) from natural gas or LPG. A caustic wastewater is formed containing these sulfur compounds. The objective of this study was to investigate if methanethiol could be biologically degraded in an Upflow Anaerobic Sludge Bed (UASB) reactor. A screening with several anaerobic sludges was carried out for the anaerobic degradation of methanethiol (MT), ethanethiol (ET) and propanethiol (PT). Only degradation of MT was found possible with some sludge types. A UASB reactor (1.6 L), inoculated with a positively screened sludge, was fed with MT (2-6 mM) and operated at 30°C and pH 7.2-7.5. Volatile organic sulfur compounds (VOSC) and sulfide were monitored during UASB reactor operation. The removal efficiency of MT was more than 90% for almost the entire experiment, while 70-85% of the organic sulfur was recovered as sulfide in the effluent. In separate batch tests it was found that sludge, which had been exposed to MT for 126 days in the reactor, degraded MT 3-7 times faster compared to the sludge at time zero. Addition of 25 g/L NaCl or 29 g/L NaHCO₃ caused a 50% reduction in the MT degradation rate compared to low salt conditions.

1 INTRODUCTION

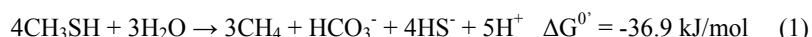
Because of their potential negative effects on the environment, organosulfur compounds present in petroleum and fossil fuels are receiving considerable attention. The sulfur content of petroleum from different sources ranges from 0.025 to more than 5 wt %. In addition to the inorganic sulfur species (sulfide, sulfate, sulfite, thiosulfate) more than 200 sulfur containing organic compounds have been identified in crude oils. These include sulfides, thiols, thiophenes, substituted benzo- and dibenzothiophenes and many more complex molecules (Londry and Suflita, 1998; Monticello and Finnerty, 1985).

Alkaline solutions (*e.g.* Na₂CO₃) are used to extract hydrogen sulfide and acidic organic sulfur compounds, such as thiols (R-SH) from natural gas and LPG. This results in a caustic wastewater, containing volatile organic sulfur compounds (*e.g.* up to 60 mM methanethiol) and a sodium concentration of at least 1 M.

Currently, thiol containing LPG is treated by the Merox Process. Thiols are extracted from LPG and in an oxidizer catalytically oxidized to water insoluble disulfide oil (UOP,

2003). Anaerobic treatment of the thiol containing extraction water might be an alternative, because it converts the thiols into sulfide, which can be oxidized to elemental sulfur, and thus avoids the production of disulfide oils.

Not much is known about the anaerobic treatment of these types of wastewaters. Several methylotrophic methanogens capable of growth on methanethiol (CH₃SH, MT) as sole source of carbon and energy in marine and freshwater sediments have been described (Finster *et al.* 1992; Lomans, 2001). These methanogens are also present in (digested) wastewater sludge (Zinder and Brock, 1978; Sipma *et al.* 2002). Finster *et al.* (1992) proposed the following stoichiometry for the anaerobic degradation of MT, similar to the methanol metabolism of *Methanosarcina barkeri* (eq 1):



The autooxidation product of MT is dimethyldisulfide (DMDS). It is assumed to be degraded anaerobically via two MT molecules. Thus, anaerobic treatment could also be used to remove DMDS from wastewaters.

This paper reports results from the screening of anaerobic sludges for the capability of anaerobic degradation of MT, ET (ethanethiol) and PT (propanethiol). Results of a continuous reactor run are presented for the anaerobic degradation of MT as well. Separate batch tests were done to investigate the adaptation of the reactor sludge to MT and to examine the effect of the sodium concentration on the MT degradation rate. The objective of this study was to get a first insight in the feasibility of anaerobic treatment of thiol containing wastewaters.

2 MATERIALS AND METHODS

2.1 Biodegradability tests

Tests for assessing the anaerobic biodegradability of MT, ET and PT were performed in duplicate at 30°C for seven (anaerobic) sludge samples (Table 1).

The samples were incubated in 120 ml serum bottles filled with 50 ml bicarbonate buffered medium, sealed with viton stoppers. The headspace contained a N₂/CO₂ mixture (80:20) at 1.7 bars. The medium contained 4000 mg/L NaHCO₃, 408 mg/L KH₂PO₄, 534 mg/L Na₂HPO₄·2H₂O, 218 mg/L NH₄Cl, 218 mg/L NaCl, 73 mg/L MgCl₂·6H₂O, 73 mg/L CaCl₂·2H₂O, 0.5 mg/L resazurin (redox indicator), 240 mg/L Na₂S·9H₂O (reductant), vitamins and micronutrients according to De Bok (2002). The final pH of the medium was 7.2. MT, ET and PT were added (100, 50 and 50 μmol per batch respectively) from stock solutions in every bottle. Degradation of the thiols was monitored by measuring the thiol concentrations in the headspace using a Hewlett Packard CP9000 gas chromatograph equipped with a CP-Porabond Q column (25 m x 0.53 mm). To test if lower mercaptans could be co-metabolized with alcohols, the corresponding alcohols (5 mM) or lactate (1 mM) were added in a separate series of batches.

Table 1. Origin of sludge samples from full-scale wastewater treatment facilities.

Type of industry	Type of wastewater/sludge
Zinc factory (ZF)	Sulfate reducing sludge
Chemical industry (CI)	Anaerobic sludge
Industrial wastewater treatment plant (IWP1)	Sulfate reducing plant where ethanol is dosed
Industrial wastewater treatment plant (IWP2)	Anaerobic plant treating methanol and glycol
Industrial wastewater treatment plant (IWP3)	Sulfide oxidation plant
Sewage water treatment plant (SWT)	Digested, dewatered sludge
Chemical sulfur industry (CSI)	Aerobic sludge from pilot-plant treating ethylthiourea

2.2 Continuous reactor

For the continuous reactor experiment, a single stage laboratory-scale Upflow Anaerobic Sludge Bed (UASB) reactor with a volume of 1.6 L was used. The reactor was fed with an oxygen free synthetic influent of pH 12 containing 50-100 mM MT (stock solution) and with dilution water of pH 4, which contained the following nutrients: 500 mg/L NH_4Cl , 150 mg/L K_2HPO_4 , 60 mg/L $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 120 mg/L KCl , 200 mg/L $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ and 15 mg/L yeast extract. Micronutrients (1 ml/L) were added according to Paulo (2002). The MT stock solution was stored in a closed glass bottle in which the consumed liquid volume was replaced by nitrogen gas. An influent containing 2-6 mM MT was produced by mixing the MT stock solution with dilution water near the inlet of the reactor.

The reactor was inoculated with 96 g VSS (dry weight) of granular sludge originating from a full-scale anaerobic reactor treating paper mill wastewater (Eerbeek, The Netherlands, sampling date: January 16th 2003). This sludge has the capability to degrade methanethiol (Sipma, 2003). The reactor was operated at 30° C using a thermostat bath. The pH of the reactor liquid was measured with a sulfide resistant Flushtrode pH electrode (Hamilton Flushtrode, Hilkomij B.V., Rijswijk, The Netherlands) and controlled with two changeable set points to correct the pH by adding 0.1 M sodium hydroxide or hydrochloric acid. The pH of the reactor was maintained between 7.2 and 7.5 throughout the experiment.

The superficial liquid up-flow velocity in the reactor was maintained at 1.0 m/h for the first 100 days and was then increased to 1.5 m/h to improve mixing. This was done by applying internal circulation of the reactor liquid. The influent and circulation flows were 4.2-4.8 L/d and 188-283 L/d, respectively. The hydraulic retention time (HRT) was 8-9 h.

The removal efficiency of the reactor was calculated as follows: (eq. 2 based on VOSC in the effluent, eq. 3 based on sulfide in the effluent)

$$[1 - (\text{VOSC}_{\text{effl}} / \text{VOSC}_{\text{infl}})] * 100\% \quad (2)$$

$$(\text{HS}^-_{\text{effl}} / \text{VOSC}_{\text{infl}}) * 100\% \quad (3)$$

where $\text{VOSC}_{\text{infl}}$ is the VOSC concentration in the influent (80-100% MT, 0-20 % DMDS), $\text{VOSC}_{\text{effl}}$ is the sum of VOSC in the effluent (MT, ET, DMS, DMDS) and $\text{HS}^-_{\text{effl}}$ is the sulfide concentration in the effluent.

2.3 Effect of adaptation and salt concentration

Additional batch tests were performed with the granular sludge used to inoculate the reactor (test 1, sludge sampling date at full scale plant: January 16th, 2003) and with adapted sludge from the lab scale reactor (test 2, sampling at day 126). The incubation procedure was similar to the biodegradability tests (see 2.1). Tests were carried out in

duplicate at 30°C. All bottles of test 1 were incubated with 3 mM MT and all bottles of test 2 with 2 mM MT. Bottles 1 and 2 were autoclaved for 20 minutes at 120°C and served as (killed) controls to check for abiotic conversions (Table 2). The degradation of methanethiol was monitored by measuring the sulfide concentration in the liquid phase.

Table 2. Experimental design for batch tests 1 and 2.

Number	Test 1		Test 2	
	VSS (g)	Characteristic	VSS (g)	Characteristic
1a/b	0	Autoclaved	-	-
2 a/b	0.39	Autoclaved	0.23	Autoclaved
3 a/b	0.39	No extra addition	0.23	No extra addition
4 a/b	0.38	+ 10 g/l NaCl	0.22	+ 10 g/l NaCl
5 a/b	0.39	+ 25 g/l NaCl	0.24	+ 25 g/l NaCl
6 a/b	0.39	+ 10 g/l NaHCO ₃	0.22	+ 10 g/l NaHCO ₃
7 a/b	0.39	+ 25 g/l NaHCO ₃	0.22	+ 25 g/l NaHCO ₃

2.4 Analytical methods

All chemicals used were of analytical grade and supplied by Merck (Darmstadt, Germany). A concentrated MT solution (3 M) of analytical grade was supplied by Atofina (Rotterdam, The Netherlands).

Volatile organic sulfur compounds were analyzed by high pressure liquid chromatography (HPLC) (Separations, H.I. Ambracht, The Netherlands), using a Chrompack (Bergen op Zoom, The Netherlands) C-18 column with a length of 20 cm. The oven temperature was 30° C. The composition of the eluent was 35% acetonitrile and 65% water and the flowrate was 0.6 mL/min. The injection volume of the samples was 20µL and an UV detector (Gynotek Germering, Germany) was used to monitor the VOSC concentration at a wavelength of 210 nm. Total and volatile suspended solids were analyzed according to Standard Methods (1985). Sulfide was measured photometrically as described by Trüper and Schlegel (1964). Biogas composition was analyzed on a gas chromatograph (model 8340, Fisons, Interscience, Breda, The Netherlands), equipped with a stainless steel column packed with Chrompack Molecular Sieve 5Å according to Van der Maas *et al.* (2004). Polysulfide was measured spectrophotometrically at 285 nm and the concentration was calculated using the Lambert-Beer Law (Kleinjan, 2005).

3 RESULTS AND DISCUSSION

3.1 Anaerobic biodegradability of thiols

Methanogens capable of converting MT were present in several sludges obtained from full scale anaerobic wastewater treatment facilities. (Sipma *et al.*, 2003). In the present screening sludges were also tested on their capability of degrading ET and PT. In the test with sludge from ZF and CSI no disappearance of MT and hardly any production of methane was observed. In the tests with IWP1, IWP2, IWP3 and SWT methane formation was found after 42-47 days incubation, which suggests the presence of methylotrophic methanogens in these sludges. In the tests with IWP1 and IWP2, methanethiol was degraded completely after prolonged periods of incubation (105

days). Addition of alcohols or lactate shortened the lag phase to 42-80 days. In the test with SWT, MT was degraded completely within 42 days in all batches (with or without extra substrate). In none of the batch experiments, any conversion of ET and PT was observed.

3.2 Methanethiol degradation in a continuously operated UASB-reactor

The continuous reactor experiment was started with fresh granular sludge from a full-scale anaerobic reactor treating paper mill wastewater. The MT influent concentration was 2 mM at the start of the experiment. After 8 days, sulfide production started (Figure 1). The influent MT concentration was increased to 4 mM at day 16 and to 6 mM at day 39. The latter corresponded to a volumetric loading rate of $15 \text{ mmol MT} \cdot \text{L}^{-1} \cdot \text{dag}^{-1}$. A maximum of 20% of MT in the stock influent solution was present as DMDS, due to autooxidation.

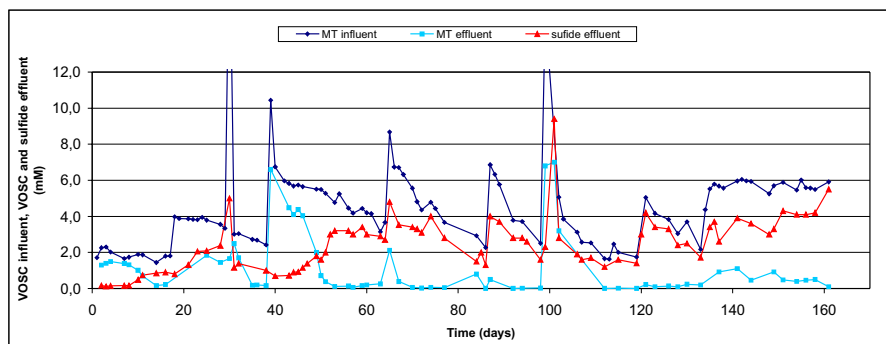


Figure 1. Degradation of methanethiol in the reactor into sulfide.

At day 30 a shock load of 20 mM was applied to the reactor for 20 hours. The effluent sulfide concentration increased to 5 mM (Figure 1) indicating that degradation of MT and DMDS continued. The effect of the shock load appeared not to be very severe, since the reactor performance recovered within a few days after the lower MT influent was restored.

At day 39, an increase in influent concentration from 2.5 mM to 10 mM did not cause an instantaneous increase in sulfide concentration in the effluent. The effluent MT concentration increased and the removal efficiency of the reactor deteriorated (Figure 2). The influent concentration was reduced to 6 mM MT on day 40. It took 10 days before the MT concentration in the effluent was below 1 mM again (Figure 1).

At day 100, a combination of a shock concentration of 15 mM during 20 hours and increase in MT loading to $28 \text{ mmol MT} \cdot \text{L}^{-1} \cdot \text{day}^{-1}$ caused an instantaneous increase in sulfide concentration in the effluent to 9.4 mM sulfide. These shortly increased MT and sulfide concentrations did not seem to affect the reactor performance during the continuation of the experiment.

Except during shock loadings, the total VOSC concentration in the effluent never exceeded 0.5 mM S-organic, from day 50 on. Besides MT and DMDS, also DMS ($< 0.1 \text{ mM}$) and ET ($< 0.05 \text{ mM}$) were detected in the effluent. They were probably formed by the methanogens in biochemical side reactions according to equations 4 and 5, where one of the two MT molecules is the methyl-donating and the other one is the methyl-accepting compound. Note that sulfide is released during the DMS and ET formation

via this route. Sipma *et al.* (2002) already reported the formation of DMS from MT in an anaerobic bioreactor treating MT.



The anaerobic degradation of DMS (eq. 2) is assumed to proceed via MT as intermediate (Lomans *et al.*, 1997).

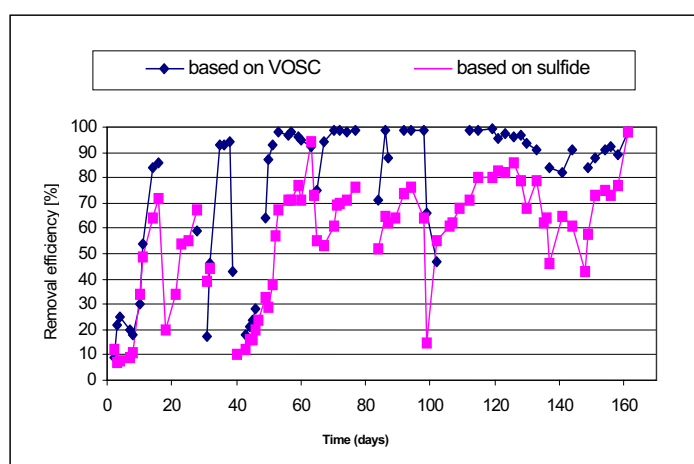
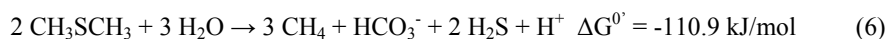


Figure 2. MT removal efficiency of the UASB reactor.

Based on the total VOSC concentration in the influent and the effluent, the removal efficiency was >90% almost the entire period after day 50 (Figure 2). In general, only after increasing the MT load and after MT shock loadings, the removal efficiency decreased temporarily. Only 70% till 85% of the sulfur from MT/DMDS was recovered as sulfide in the effluent during stable reactor performance. This difference could be attributed to:

- Formation of other (volatile) sulfur compounds, which were not identified on the HPLC.
- Formation of elemental sulfur on the top of the reactor, due to partial oxidation of sulfide with residual oxygen in the headspace of the reactor.
- Polysulfide formation due to the reaction of elemental sulfur with sulfide. In the effluent concentrations of around 0.2 mM S_1^0 were found as polysulfide (data not shown).
- Volatilization of H_2S (and methanethiol) into the biogas. Calculations of Sipma *et al.* (2003) showed that H_2S is maximal 1.2% of the biogas composition under comparable conditions. However, in the present investigation no H_2S was detected in the biogas.

At day 64, the biogas consisted of 60% CH₄ and 40 % N₂ whereas no CO₂ and H₂S were detected. The biogas production was low at the beginning of the experiment. During the first half of the experiment, the average gas production was 50 mL/day. This increased till an average of 235 mL/day during the last two weeks of the experiment. Part of the produced methane dissolved and left the reactor via the effluent. This was verified qualitatively by gas chromatography where a soluble methane peak was identified in effluent samples. From different sources (Lide *et al.*, 1995; Dean, 1992, Kavanaugh and Trussel, 1980; Wilhelm and Battino 1977) the dimensionless Henry coefficient ($m = C_g/C_l$) was calculated for methane at 30°C and the average value from these sources was $m = 32$. If 60% of the biogas is methane, it can be calculated using the Ideal Gas Law and the distribution coefficient, that the dissolved methane concentration was 0.7 mM. For a total effluent flow of 4 L/d, this was equivalent to a extra biogas stream of 74 mL/day at 30°C.

3.3 Effect of Adaptation and Salt Concentration

Additional batch tests were carried out to examine salt effects on MT degradation rate and to compare the sludge activity on MT before and after exposure to MT in the above described reactor experiment. The lag phases were quite long for the inoculum Eerbeek sludge, which had not been exposed to MT before (Table 3). Degradation of MT with sludge withdrawn from the lab reactor after 126 days started instantaneously, except for the tests with an additional 25 g/L NaHCO₃.

Table 3. Lag phases (days) of the batch tests for “fresh sludge” and “adapted sludge” .

Additional salt (g/L)	Sludge not exposed to MT before	Sludge after 126 days in the reactor
0	36	0
0	36	0
10, NaCl	36-50	0
10, NaCl	36-58	0
25, NaCl	58	0
25, NaCl	58-78	0
10, NaHCO ₃	36	0
10, NaHCO ₃	50	0
25, NaHCO ₃	50-78	7
25, NaHCO ₃	58-78	7

Figure 4 shows a decrease in MT degradation rate with increasing salt concentration. In all tests a standard amount of 4 g/L NaHCO₃ was added as a pH buffer. Addition of 10 g/L NaCl or NaHCO₃ showed only a minimal decrease. However, a decrease in degradation rate of around 50% was found at the highest salt concentrations tested for both the inoculum and the adapted sludge.

The sulfide production rate of the adapted sludge was 3-7 times higher than corresponding rate of the inoculum sludge (Figure 4). This clearly indicates that the microbiological community adapted to MT degradation during the UASB reactor operation.

4 CONCLUSIONS

Several sludges from wastewater treatment plants were capable of degrading MT, but none of them was able to convert ET or PT. A UASB reactor (30°C, pH 7) degraded MT to H₂S, CH₄ and CO₂, the maximal MT loading rate with more than 90% degradation during the reactor run was 15 mmol MT·L⁻¹·day⁻¹.

Adaptation of the sludge to MT in the reactor increased the sulfide production rate by a factor 3-7. The presence of 25 g/L NaCl or 29 g/L NaHCO₃ caused a 50% reduction of

the MT degradation rate, compared to a situation without extra salt addition. The results reported in this paper show that anaerobic treatment of MT containing wastewaters might become an interesting alternative for the currently used methods.

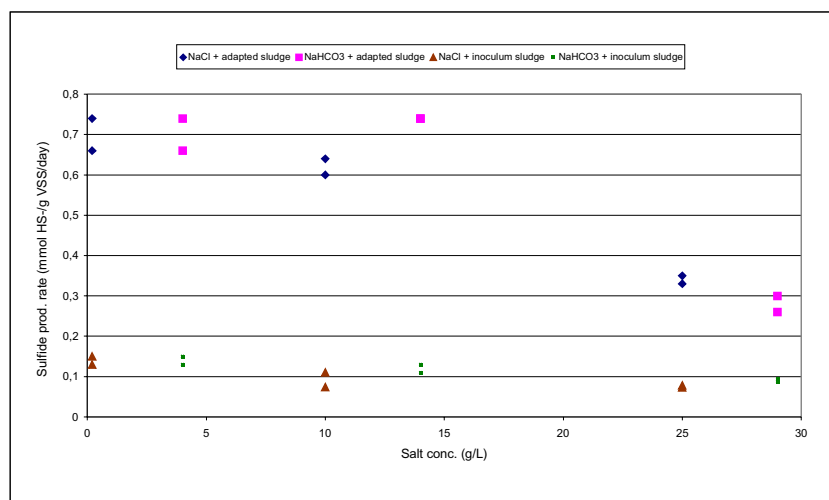


Figure 4. Effect of the salt concentration on the sulfide production rate.

5 ACKNOWLEDGEMENTS

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