

BIODEGRADATION OF MERCAPTANS UNDER AEROBIC AND ANOXIC CONDITIONS

OBJECTIVE

To determine the ability of *Thiobacillus thioparus* and / or naturally occurring microbes to biodegrade n-propyl mercaptan, isopropyl mercaptan and tertiary butyl mercaptan in soil / water microcosms under aerobic and anoxic conditions when supplied with the proper nutrients under stimulated aquifer conditions.

METHODS AND MATERIALS

Experimental Approach

Microcosms

Anoxic microcosms were prepared by placing 10 grams of aquifer material and 20 milliliters of groundwater into 40 mL EPA VOA vials to minimize biotic losses of mercaptans. Nitrate was used as the final electron acceptor for this portion of the study through the addition of 2.0 grams per liter potassium nitrate. Ammonium nitrogen and ortho-phosphate were supplied by the addition of diammonium phosphate at 1 gram per liter. Mercaptans were added to a final concentration of 10 mg/L each. The microcosms were mixed daily and allowed to incubate statically at room temperature.

Aerobic microcosms consisted of 20 grams of soil and 40 mL of groundwater placed into 160 mL crimp sealed serum bottles. Diammonium phosphate was added at 1 gram per liter. No nitrate was added to these microcosms. Mercaptans were added to a final concentration of 10 mg/L each. The 100 mL headspace contained sufficient oxygen for aerobic degradation to occur while mixing at 100 rpm on a rotary shaker incubator at room temperature.

A stock solution of each mercaptan was prepared by adding one milliliter of each of the three mercaptans to one liter of distilled water and mixing until dissolved. The stock solution contained one milligram of each mercaptan per milliliter. The stock solution was added at 0.3 mL to the 40 mL VOA vial microcosms and at 0.6 mL to the 160 mL serum bottles. Therefore, the 40 mL vials received approximately 0.3 mg / 30 mL (soil + groundwater) or 10 mg/L and the 160 mL serum bottles received 0.6 mg / 60 mL (soil + groundwater) to achieve the same concentration. The experimental matrix is given in Table 1 and a list of the mercaptans studied is given in Table 2.

Table 1. Experimental microcosm matrix for the study of the biodegradation of mercaptans.

ANOXIC	AEROBIC
Distilled Water, Mercaptans	Distilled Water , Mercaptans
Soil, Groundwater, Mercaptans	Soil, Groundwater, Mercaptans
Soil, Groundwater, Nutrients, Mercaptans	Soil, Groundwater, Nutrients, Mercaptans
Soil, Groundwater, Nutrients, Mercaptans, <i>Thiobacillus thioparus</i>	Soil, Groundwater, Nutrients, Mercaptans, <i>Thiobacillus thioparus</i>

Table 2. Mercaptans used in biodegradation study.

Compound	Formula	Molecular Weight
1-Propanethiol (propyl mercaptan)	CH ₃ CH ₂ CH ₂ SH	76.16
2-Propanethiol (isopropyl mercaptan)	(CH ₃) ₂ CHSH	76.16
1-Butanethiol (butyl mercaptan)	CH ₃ (CH ₂) ₃ SH	90.19

Growth Studies

Aerobic and anaerobic growth studies were performed to determine the ability of *Thiobacillus thioparus* to metabolize mercaptans in groundwater from the test site.

Growth of the micro-organisms on the mercaptans was measured by absorbance at 525 nm on a spectrophotometer. This technique is a classical microbiological method that is straightforward, but provides good information with regards to the growth rates of the bacteria on any given substrate. An increase in bacteria concentration is directly proportional to an increase in absorbance. That is, more light is blocked from passing through a cell pathway when more bacteria exist in solution.

The aerobic study was performed in 250mL gas sampling bulbs. Each of three bulbs contained 100mL of site groundwater, diammonium phosphate (1 g/L) and mercaptans. The 150mL headspace provided adequate oxygen for aerobic growth to occur. The mercaptans were dosed at 60, 150 and 300 mg/L into the three gas sampling bulbs, respectively. Each concentration level consisted of one third of each mercaptan under investigation. The gas bulbs were mixed on a rotary shaker at 100 rpm to provide aeration.

The anoxic study was prepared in 10mL glass test tube with Teflon lined caps and Teflon wrapped cap threads. Each tube was completely filled with groundwater containing diammonium phosphate (1 g/L), potassium nitrate (2 g/L) and mercaptans. Mercaptans were dosed at 71, 175 and 350 mg/L and capped with no headspace. Each concentration level consisted of one third of each mercaptan under investigation. The tubes were prepared in triplicate at each concentration level and reported as average.

Analytical

A Hewlett Packard 5890 Series II Plus gas chromatograph equipped with a flame ionization detector and a 30 m x 0.53 mm HP 624 volatiles column was used for the analysis of mercaptans. The column was operated at 50 for 15.5 minutes increased at 10 C/minute to 150 C and held for three minutes.

Primary standards were prepared under a laboratory hood by directly weighing each of the mercaptans into a 100mL volumetric containing xylene on an analytical balance.

The headspace mercaptans in the microcosms were monitored during the biodegradation study by direct injection of 50 uL of headspace gases with a 100 uL gas tight syringe. Liquid phase mercaptans were monitored by 1 uL injections when the headspace indicated that little mercaptan remained. The total mass of mercaptans was determined for each microcosm at the beginning and end of each microcosm study period.

Culture Preparation

Thiobacillus thioparus – the inoculum for the mercaptan biodegradation study was obtained from the ATCC (American Type Culture Collection). The growth medium contained the constituents as given in Table 3.

Table 3. *Thioparus thiobacillus* growth media.

Compound	g/L
Na ₂ HPO ₄	1.2
KH ₂ PO ₄	1.8
MgSO ₄ ·H ₂ O	0.1
(NH ₄) ₂ SO ₄	0.1
CaCl ₂	0.03
FeCl ₃	0.02
MnSO ₄	0.02
Na ₂ S ₂ O ₃	10.0

RESULTS

Analytical

Xylene proved to be a suitable solvent for standard preparations since it eluted after the mercaptan peaks of interest. Headspace and liquid phase analysis were generally always clean, but had detection limits of approximately 0.5 ng mass across the detector.

Microcosms

Microcosm studies demonstrated that the mercaptans were not detectable by gas chromatograph analysis after 48 hours. This was true for headspace analysis as well as a liquid/liquid extraction of the entire microcosm contents with xylene which provided a 10X concentration factor of the analytes. This could be due part to the loss of the mercaptans due to penetration of the septa with syringes during the course of the study or physical/chemical properties of the mercaptans allow them to bind to the silt present in the sandy aquifer material. In any case it did not seem feasible to further pursue this approach in a time efficient manner.

Growth Studies

Growth studies of mercaptan in groundwater demonstrated that *Thiobacillus thioparus* could utilize mercaptans either aerobically or anaerobically as previously suggested by the literature. The groundwater was taken from an uncontaminated well and was analyzed for several parameters. The data is presented below.

Table 4. Aerobic growth of *Thiobacillus* on mercaptans as demonstrated by absorbance at 525 nm.

Time (hrs)	mg/L mercaptans		
	60	150	350
0	0.032	0.042	0.045
16.25	0.072	0.070	0.134
19.25	0.073	0.105	0.160
25.25	0.089	0.076	0.138
41.25	0.102	0.067	0.103

Table 5. Anoxic growth of *Thiobacillus thioparus* on mercaptans as demonstrated by absorbance at 525 nm.

Time (hrs)	mg/L mercaptans		
	70	175	350
0	0.018	0.018	0.019
16.25	0.037	0.044	0.093
19.25	0.025	0.048	0.101
25.25	0.022	0.037	0.102

Table 6. Growth rate constants for aerobic and anoxic studies at 350 mg/L.

Parameter	K (doublings per hour)	1/K (number of hrs to double)
Aerobic	0.095	10.53
Anoxic	0.125	7.98