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Formation of Dimethyl Sulfide and Methanethiol in Anoxic Freshwater Sediments

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Concentrations of volatile organic sulfur compounds (VOSC) were measured in water and sediment columns of ditches in a minerotrophic peatland in The Netherlands. VOSC, with methanethiol (4 to 40 nM) as the major compound, appeared to be mainly of sediment origin. Both VOSC and hydrogen sulfide concentrations decreased dramatically towards the water surface. High methanethiol and high dimethyl sulfide concentrations in the sediment and just above the sediment surface coincided with high concentrations of hydrogen sulfide (correlation factors, $r = 0.91$ and $r = 0.81$, respectively). Production and degradation of VOSC were studied in 32 sediment slurries collected from various freshwater systems in The Netherlands. Maximal endogenous methanethiol production rates of the sediments tested (up to $1.44 \mu\text{mol per liter of sediment slurry} \cdot \text{day}^{-1}$) were determined after inhibition of methanogenic and sulfate-reducing populations in order to stop VOSC degradation. These experiments showed that the production and degradation of VOSC in sediments are well balanced. Statistical analysis revealed multiple relationships of methanethiol production rates with the combination of methane production rates (indicative of total anaerobic mineralization) and hydrogen sulfide concentrations ($r = 0.90$) or with the combination of methane production rates and the sulfate/iron ratios in the sediment ($r = 0.82$). These findings and the observed stimulation of methanethiol formation in sediment slurry incubations in which the hydrogen sulfide concentrations were artificially increased provided strong evidence that the anaerobic methylation of hydrogen sulfide is the main mechanism for VOSC formation in most freshwater systems. Methoxylated aromatic compounds are likely a major source of methyl groups for this methylation of hydrogen sulfide, since they are important degradation products of the abundant biopolymer lignin. Increased sulfate concentrations in several freshwater ecosystems caused by the inflow of water from the river Rhine into these systems result in higher hydrogen sulfide concentrations. As a consequence, higher fluxes of VOSC towards the atmosphere are conceivable.

The production and degradation of volatile organic sulfur compounds (VOSC) have been studied intensively due to their impacts on global warming and on acid precipitation processes (1–3, 8, 32) and because of their major role in the global sulfur cycle (6, 23). It is generally accepted that dimethyl sulfide (DMS) and, to a lesser extent, methanethiol (MT) are the most abundant VOSC in marine ecosystems. Dimethylsulfoniopropionate (DMSP), an osmolyte of marine algae and phytoplankton (14, 21, 46), is the dominant precursor for DMS formation in marine systems. DMSP degradation results either in DMS and acrylate or in 3-methylpropionate, 3-mercaptopropionate, and MT (11, 29).

Precursors of VOSC formation in freshwater ecosystems are less well documented. Some authors have mentioned the production of VOSC from decaying freshwater algae (5). A few species of freshwater algae do release some DMS after the addition of sodium hydroxide, but it remains unclear whether this release indicates the presence of DMSP or of other sulfur-containing compounds (7, 36). Therefore, the large quantities of VOSC (not only DMS) released during the decay of freshwater algae are unlikely to originate from DMSP. Thus, DMSP is considered irrelevant as a precursor of VOSC formation in freshwater environments. In freshwater environments, the

presence of sulfur-containing amino acids and methoxylated aromatic compounds gives rise to VOSC production. VOSC formation from S-containing amino acids is well described and results mainly in MT and DMS production (16, 22, 39, 41, 48). Methoxylated aromatic compounds are common in nature since they are degradation products of lignin, one of the most abundant biopolymers on earth. A mechanism resulting in anaerobic VOSC production from methoxylated aromatic compounds has been demonstrated (4, 17, 30). According to this mechanism, the methyl group of the methoxylated compound is transferred to sulfide or MT, resulting in MT and DMS, respectively. After this *o*-demethylation, the remaining phenolic derivatives are degraded to acetate (4, 30). VOSC formation from methoxylated aromatic compounds has also been found in *Sphagnum* peat slurries (26).

Degradation of DMS and MT in anaerobic marine environments has been ascribed to both sulfate-reducing and methanogenic bacteria (24, 25, 27, 35). In anaerobic freshwater systems DMS and MT were reported to be degraded by methanogenic bacteria (48, 49). The role of sulfate-reducing bacteria in freshwater sediments remains unclear.

Unlike marine, estuarine, and other saline environments, freshwater ecosystems have hardly been studied with regard to the factors that influence VOSC fluxes from these systems. The present study describes for the first time an extensive survey of freshwater sediments of different origins with respect to the endogenous production of VOSC and the parameters that affect this production.

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MATERIALS AND METHODS

Sampling sites. VOSC concentrations in water and sediment columns were measured in ditches of minerotrophic peatland in De Bruuk, The Netherlands. "Minerotrophic" refers to systems that get their major input of minerals from seepage or groundwater rather than from deposition by rainwater. Sediment samples for slurry incubation were collected in the summer at the following 11 locations in The Netherlands: Tienhoven, Molenpolder, Breukelen, Maarssen, Zegveld, Loosdrechtse Plassen, De Bruuk, De Weerribben, De Meern, Harmelen, and Nieuwkoopse Plassen. At these locations various sites were sampled, resulting in a total of 32 sediment samples.

Water sample collection. Samples of the water column were taken through Teflon tubing in 10-cm intervals. The Teflon tubing was fixed to a polyvinyl chloride (PVC) pipe which was placed at the location 1 or 2 days before sampling (Fig. 1a). The water samples were collected by suction in vacuum serum bottles of 500 ml. Microbial activity was stopped by the addition of 1 ml of HCl (6 M) per bottle.

Sediment pore water was collected by filtration through ceramic lysimeters (Fig. 1a) and was treated as described by Roelofs (38). Water samples were analyzed for VOSC within 6 h after sampling.

The organic top layer (10 cm) of freshwater sediments was collected at various locations (see above). Sediment samples were taken by suction in anaerobic bottles (N_2/CO_2 ratio, 80:20, vol/vol) with minimal disturbance of the sediment layer by using the apparatus shown in Fig. 1b. The sediments were stored in the dark at 15°C and were used within 24 h.

Sediment incubations. After settling for 1 h, the sediment samples were adjusted to give a water/sediment ratio of 1:1 (vol/vol) by removing either water or sediment in an anaerobic cabinet. The adjusted samples were stirred, and aliquots (25 ml) of the homogeneous slurries were dispensed into 60-ml crimp top serum bottles sealed with grey butyl rubber stoppers which did not emit or adsorb VOSC. Bottles were preincubated for 48 h at 15°C after the headspace was flushed with oxygen-free N_2-CO_2 (80:20, vol/vol). Before the experiment was started, the bottles were flushed again. Some bottles served as untreated controls, while to the other bottles either bromoethanesulfonic acid (BES), sodium molybdate, or both compounds were added (triplicate incubations). BES and sodium molybdate were added from neutralized stock solutions to final concentrations of 25 mM and 5 to 10 mM, respectively. The sediment slurries were incubated in the dark without shaking at 15°C, the mean in situ temperature measured during the period of sampling. Sediment slurries heated for 1 h at 70°C served as abiotic controls.

Sulfide added to sediment slurries was taken from a neutralized sulfide solution in order to avoid pH changes. This solution was prepared by neutralizing (pH 7.0) a sodium sulfide solution with pure H_2S . The sulfide concentration was 230 mM. After sulfide addition, the pH of the sediment slurries was checked and, if necessary, adjusted to the value of the original sample (pH 6.85).

Analytical procedures. (i) **Methane.** Methane was analyzed on a Pye Unicam gas chromatograph equipped with a flame ionization detector and a Porapak Q (80/100 mesh) column (20). Ethane was used as an internal standard.

(ii) **VOSC.** VOSC were determined with a Packard 438A gas chromatograph equipped with a flame photometric detector and a Carboxack B HT100 column (40/60 mesh) as described by Derikx et al. (13). VOSC in the headspace of the bottles used for sediment slurry incubations were analyzed by 1-ml gas injections. VOSC in water samples were concentrated on Tenax tubes submerged in liquid N_2 by flushing 100 ml of each water sample with N_2 at 360 ml/min for 15 min (43). The stripping efficiency for VOSC was higher than 95%, as determined with different amounts of pure compounds in water. The Tenax tubes were stored in liquid N_2 to prevent loss of the adsorbed VOSC. The Tenax columns were analyzed for VOSC by insertion into the injection port of a Packard 438A gas chromatograph.

(iii) **Inorganic components.** The dry weight of sediments was determined by drying to constant weight at 80°C. To determine the organic matter content, dried sediments were ashed (for 4 h at 550°C). Dried sediments were analyzed for nitrogen, sulfur, and carbon contents, and pore water was analyzed for HCO_3^- , SO_4^{2-} , Fe^{2+} , PO_4^{3-} , NH_4^+ , NO_3^- , Ca^{2+} , Mg^{2+} , K^+ , Na^+ , Cl^- , and Mn^{2+} , according to the work of Roelofs (38).

Statistical data analysis. Statistical data analysis was performed by using two comprehensive procedures (PROC CORR and PROC REG STEPWISE) of SAS statistic software (40).

RESULTS

H_2S and VOSC concentration profiles. To study the significance of H_2S and VOSC in the flux of sulfur-containing components from the sediments through the water column to the atmosphere, depth profiles were measured in ditches of a minerotrophic peatland (De Bruuk, The Netherlands). Although there was great variation in the H_2S and VOSC concentrations in both space and time, all concentration profiles ($n = 7$) resulted in the same general pattern (Fig. 2). H_2S and VOSC concentrations were highest in and just above the sediment and

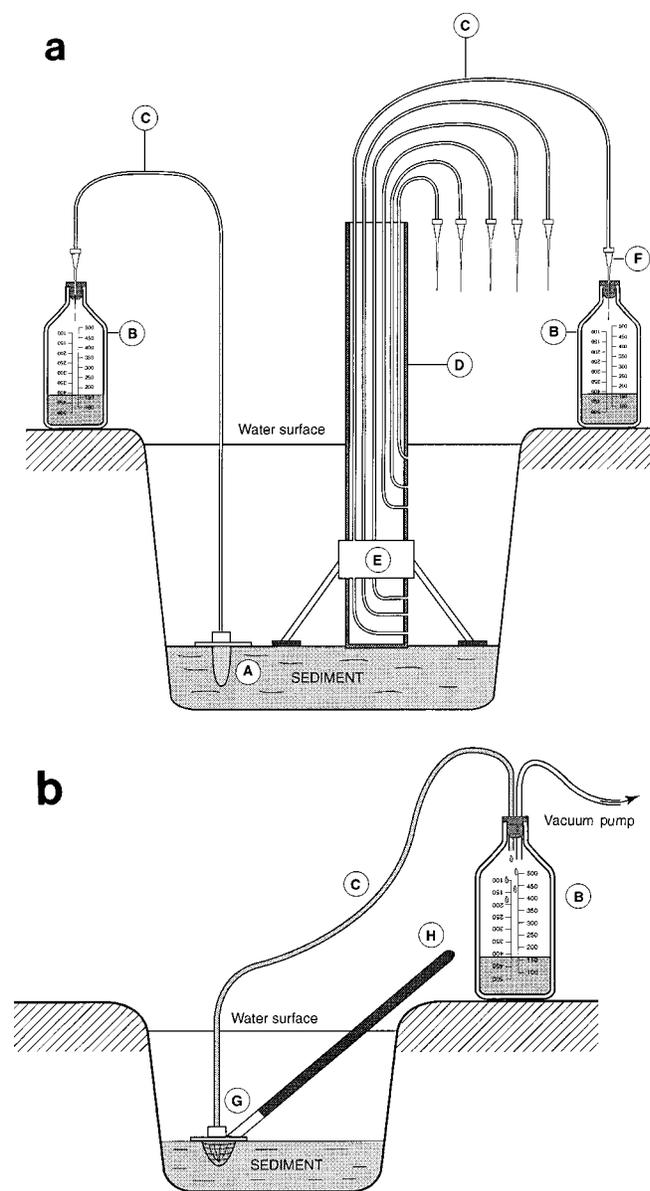


FIG. 1. Schemes of sampling devices for (pore) water and sediment slurries. (a) Sampling devices for (pore) water were placed on sampling sites 1 or 2 days before sampling. Sediment pore water was sucked from the upper 10 cm of the sediment layer, by using porous ceramic cups (A) connected to vacuum serum bottles (B), through Teflon tubing (C). Microbial activity was stopped by the addition of 1 ml of HCl (6 N). Samples collected from the water column were also sucked through Teflon tubing (C) which was fixed to a PVC pipe (D) at different depths. E, tripod for stabilization of PVC pipe; F, injection needle. (b) Sediment slurry samples were taken through suction by using a maze construction (G) (opening, 0.25 cm²) into vacuum serum bottles (B). All bottles were flushed (with N_2-CO_2 , 80:20, vol/vol) before evacuation to guarantee the absence of oxygen. H, handling stick.

decreased towards the water surface. In the sediment, the H_2S concentrations were 2 to 4 orders of magnitude higher than the VOSC concentrations. Since H_2S concentrations decreased more dramatically, the contribution of MT, DMS, and CS_2 to the total amount of volatile sulfur compounds became more significant in the subsurface water (Table 1). In the sediment pore water MT was the dominant organic sulfur compound,

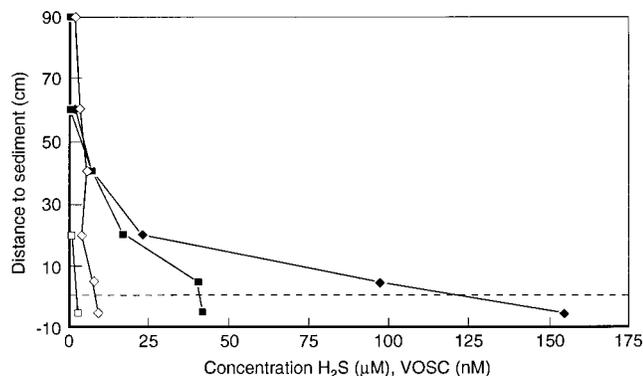


FIG. 2. Typical concentration-depth profiles of H₂S (◆), MT (■), DMS (◇), and DMDS (□) throughout the water column and sediment. Dashed line, sediment-water column boundary line. Datum points are means for duplicate samples. Micromolar concentrations of H₂S and nanomolar concentrations of DMS and DMDS are given. This profile of a freshwater ditch in a minerotrophic peatland (De Bruuk) was analyzed in November 1994.

while at the surface DMS and CS₂ were the major compounds. The CS₂ concentration did not change along the water column. Close to the sediment, small quantities of dimethyl disulfide (DMDS) were found. The presence of DMDS always coincided with high concentrations of MT. This might be caused by chemical oxidation of MT into DMDS during sample collection.

Regression analysis of the data on H₂S and VOSC concentrations in and just above the sediment of the seven profiles (*n* = 31) demonstrated that the MT concentration and, to a lesser extent, the DMS concentration were both correlated to the H₂S concentration (*r* = 0.91 and *r* = 0.81, respectively) (Fig. 3A and B). Consequently, DMS concentrations also correlated with MT concentrations (*r* = 0.83) (Fig. 3C). Thus, MT and DMS concentrations were high in profiles with high H₂S concentrations and low in profiles with low H₂S concentrations. CS₂ and DMDS did not show any correlation with H₂S (Fig. 3D), MT, or DMS.

In vitro sediment incubations. Steady-state concentrations of VOSC depend on the balance between production, degradation, and diffusion from the sediment towards the water surface and the atmosphere. To study which parameters affect the production of VOSC, maximal endogenous production rates of sediments collected at various locations were measured. Some characteristics of the sediments are given in Table 2. Methane production rates were determined from sediments which did not receive any addition, since they are an ideal

TABLE 1. Ranges of volatile sulfur concentrations in the sediment and at the water surface observed during different (*n* = 7) depth profile analyses in De Bruuk

Compound	Concentration ^a	
	Sediment ^b	Surface ^c
H ₂ S	20–651	0.01–0.03
MT	3–76	1.1–7.8
DMS	1–44	0.6–20
CS ₂	2–30	10–23
DMDS	2–3	0.1–0.6

^a Micromolar concentrations of H₂S and nanomolar concentrations of the other compounds are given.

^b Samples taken 5 to 10 cm below to 5 to 10 cm above the sediment surface.

^c Samples taken 10 cm below the water surface layer.

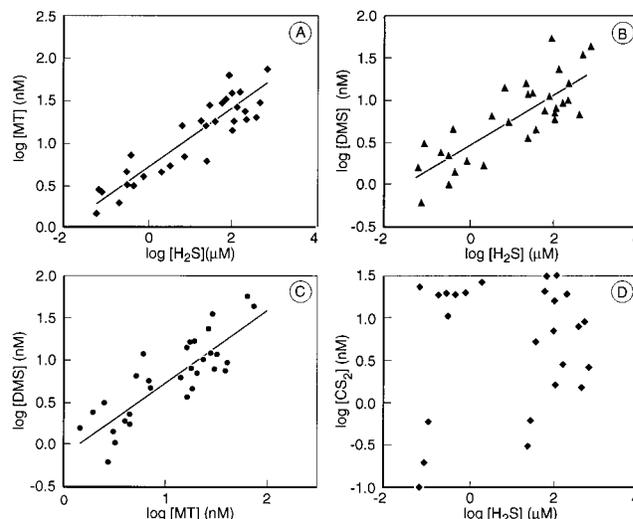


FIG. 3. Relationships between in situ concentrations 5 to 10 cm below and 5 to 10 cm above the sediment surfaces of minerotrophic freshwater ditches in De Bruuk. (A) H₂S versus MT concentration (*n* = 31; *r* = 0.91); (B) H₂S versus DMS concentration (*n* = 31; *r* = 0.81); (C) MT versus DMS concentration (*n* = 31; *r* = 0.83); (D) H₂S versus CS₂ concentration (*n* = 25; *r* = 0.13).

indicator of anaerobic microbial activity (44). VOSC production rates were estimated in sediment slurry incubations without addition or with BES (25 mM), sodium molybdate (5 to 10 mM), or both added. BES and molybdate inhibit methanogenic and sulfate-reducing bacteria, respectively, the most likely candidates for the anaerobic degradation of VOSC. Residual methane formation in BES-inhibited sediments demonstrated that inhibition efficiency was higher than 90%. Production rates in BES- or molybdate-amended sediment slurries therefore approach maximal in situ VOSC values. Although up to 10-times-higher VOSC production rates were measured at 30°C (data not shown), all incubations were carried out at 15°C because this was found to be the mean in situ sediment temperature during the sampling period.

Steady-state concentrations of DMS and MT in incubations

TABLE 2. Sediment pore water and slurry characteristics of sediments collected at various locations

Characteristic	Range
OM ^a	6.9–48.1 mg/ml, 6.4–53.9% dry weight
[H ₂ S].....	0–242 µM
[SO ₄ ²⁻].....	12–2,120 µM
[Fe ²⁺].....	0.3–751 µM
[PO ₄ ³⁻].....	0.1–264 µM
[NH ₄ ⁺].....	2–898 µM
[Na ⁺].....	428–3,528 µM
[K ⁺].....	19.8–980 µM
[Cl ⁻].....	355–4,435 µM
pH.....	6.5–7.8
Alkalinity.....	1.8–15.3 mM
CH ₄ formation rate	
OM.....	0.01–0.77 nmol/mg of OM · h ⁻¹
Slurry.....	2.6–372 µmol/liter of slurry · h ⁻¹
MT formation rate	
OM.....	0–5.02 pmol/mg of OM · h ⁻¹
Slurry.....	0–1.44 µmol/liter of slurry · day ⁻¹

^a OM, organic matter.

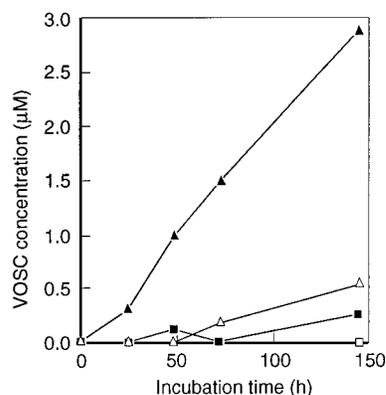


FIG. 4. Typical time courses of MT (closed symbols) and DMS (open symbols) production in an anoxic sediment slurry with nothing added (squares) or inhibited with 25 mM BES (triangles). Datum points are means for triplicate bottles. Velocities of MT accumulation which differed among the various sediments were used in further data analysis.

without additions never exceeded 0.5 μM (Fig. 4). In sediment slurry incubations MT accumulated within a few hours after BES addition, reaching levels of up to 3 to 6 μM after 6 days. The MT formation was of biological origin, since it did not accumulate in heated controls (data not shown). Except for low concentrations of DMS (up to 1 to 1.5 μM) in incubations in which MT concentrations reached high levels (>2 μM), no other VOSC were found (VOSC detection limit ± 0.3 μM , 0.03 $\text{nmol} \cdot \text{ml}^{-1}$ of headspace). VOSC accumulation was not found in molybdate-inhibited sediment slurries. MT accumulation in sediment slurries inhibited with both BES and molybdate was consistently lower than or equal to that in BES-inhibited slurries. Therefore, maximal MT production rates were estimated from slurry incubations in which DMS and MT degradation were inhibited with BES and ranged from 0 to 5 $\text{pmol} \cdot \text{mg}$ of organic matter $^{-1} \cdot \text{h}^{-1}$ (equal to 0 to 62.5 $\text{pmol} \cdot \text{ml}$ of sediment slurry $^{-1} \cdot \text{h}^{-1}$).

These average MT production rates of triplicate incubations were used in a statistical analysis with SAS software (40). In this analysis, MT production rates of the various sediments ($n = 32$) were examined for possible single and multiple correlations with the following parameters: methane production rates; concentrations of H_2S , sulfate, iron, sodium, phosphate, ammonium, nitrate, calcium, magnesium, potassium, chloride, bicarbonate, and manganese in the surface and pore sediment water; and the sulfur, nitrogen, and carbon content of the

TABLE 3. Correlation of several single and multiple relationships of parameters with MT production and H_2S concentration as response variables, determined by SAS data analysis

Response variable	Correlation parameter(s)	Correlation factor (r)
MT	Single correlations	
	Log (SO_4/Fe)	0.72 ($n = 27$; $P < 0.005$)
	Log [H_2S]	0.69 ($n = 32$; $P < 0.005$)
	CH_4 production rate	0.55 ($n = 32$; $P < 0.005$)
	Multiple correlations	
Log [H_2S] and CH_4 production rate	0.90 ($n = 32$; $P < 0.005$)	
Log (SO_4/Fe) and CH_4 production rate	0.82 ($n = 27$; $P < 0.005$)	
H_2S	Log (SO_4/Fe)	0.82 ($n = 27$; $P < 0.005$)

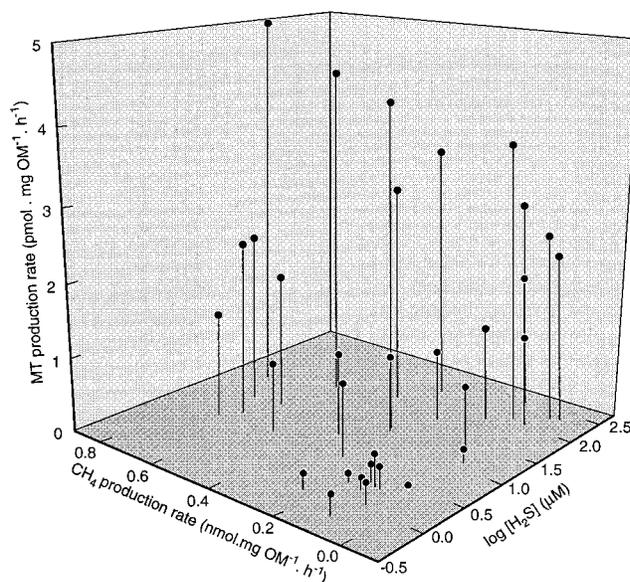


FIG. 5. Three-dimensional plot of multiple correlations between MT production rate, methane production rate, and H_2S concentration as determined with SAS software. Every dot represents one of the sediments tested. The H_2S concentration can be replaced by the ratio of the sulfate concentration to the free-iron concentration in the sediment pore water.

sediments (Table 2). Results of SAS analysis (Table 3) show that MT production rates correlate with the ratio of sulfate and free-iron concentrations of the sediment pore water ($r = 0.72$), the H_2S concentration ($r = 0.69$), and the methane production rate ($r = 0.55$). The MT production rates show strong multiple correlation with the combination of H_2S concentrations and methane production rates ($r = 0.90$). This multiple relationship is visualized in Fig. 5. The mathematical plane presented can be used for predicting an MT production rate (at 15°C) by using the methane production rate (at 15°C) and the H_2S concentration of a freshwater sediment. According to the results of the SAS analysis, the H_2S concentration in this relationship could be replaced by the sulfate/Fe(II) concentration ratio of the pore water (Table 3). None of the other parameters determined, such as sulfate and salt concentrations, alkalinity, or pH, showed any correlations with the MT production rates.

H_2S addition experiments. The correlation found between the H_2S concentration and the MT production rate was further studied by increasing the H_2S concentration of a sediment slurry rich in organic matter and poor in H_2S (the freshwater ditch at De Bruuk). Sulfide added to these sediment slurries was taken from a neutralized solution of sodium sulfide in order to avoid pH changes. Initially, added sulfide precipitated as iron sulfide, forming characteristic black precipitates. To achieve high H_2S concentrations, the free iron present had first to be precipitated as iron sulfide. In this way the final H_2S concentration in several serum bottles could be varied over a range of 1 to 600 μM . MT degradation by methanogenic bacteria was inhibited by the addition of 25 mM BES. As a result of the increased sulfide concentration, the MT production rate increased from 5 to 80 $\text{pmol} \cdot \text{ml}$ of sediment slurry $^{-1} \cdot \text{h}^{-1}$ without a lag period (Fig. 6). Maximum stimulation was found at an H_2S concentration of about 500 μM . An apparent K_s for H_2S could be calculated from the experimental data and was in the range of 200 to 400 μM .

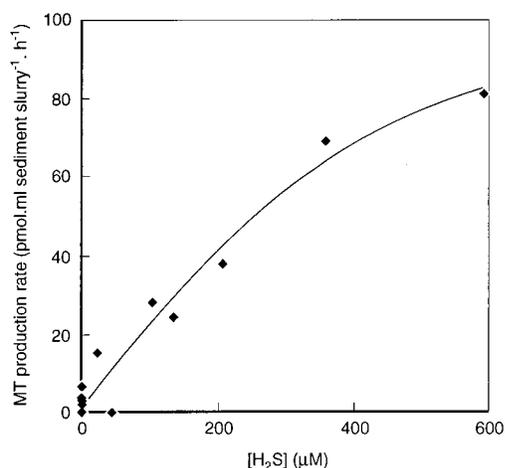


FIG. 6. Stimulation of MT production in sulfide-poor sediment slurries by addition of sulfide.

DISCUSSION

VOSC formation and factors affecting VOSC formation were studied in freshwater systems by in situ measurements of VOSC concentration profiles and in vitro sediment slurry incubations. The in situ profiles, showing steep gradients from the sediment towards the water surface, indicated that the sediment is the principal VOSC-producing freshwater compartment, as has also been mentioned in other studies (12, 18, 19, 36). MT and DMS are the major accumulating intermediates, and their concentrations in the sediment and the surface water are in the same orders of magnitude as are found in other freshwater and saline systems (3, 12, 18, 19, 28, 36, 37, 45, 47). Whereas in the sediment the contribution of VOSC to the total amount of volatile sulfur is low compared to that of H_2S , in the surface water this contribution is in the same order of magnitude. Obviously, this is caused by the more rapid oxidation of H_2S in the water column compared to VOSC. In freshwater systems, VOSC therefore play an important role in the sulfur flux towards the atmosphere, as has been described for high-salinity environments (8, 23).

MT is the dominant VOSC in the sediment, whereas DMS dominates in the subsurface water (Table 1). These changes in relative contribution along the water column, also mentioned by Richards et al. (36), are probably caused by the higher sensitivity of MT to oxidation.

Strong correlations found between in situ sediment concentrations of H_2S and either MT or DMS (Fig. 3) provide evidence for a model in which methylation of H_2S and MT, resulting in MT and DMS, respectively, is the major mechanism for VOSC formation in freshwater sediments. The lack of correlation between the H_2S concentrations and the concentrations of other VOSC (CS_2 and DMDS) further substantiates this model.

The in vitro experiments were performed with a large number of sediment slurries ($n = 32$) of different origins and various compositions (Table 2). Slurries incubated without addition showed MT accumulation up to $0.5 \mu M$. This MT accumulation, which was especially found in H_2S -rich sediments, provides additional evidence that sediments are a net source of VOSC.

The strong accumulation of MT in sediment slurries which were inhibited with BES indicates that endogenous MT production is relatively well balanced with its degradation by

methanogenic bacteria, resulting in low steady-state concentrations. Sulfate-reducing bacteria do not seem to be important in MT degradation in the freshwater sediments tested, since MT accumulation was not enhanced by the addition of molybdate to sediments or BES-inhibited sediments. The relevance of methanogenic bacteria in VOSC degradation in freshwater sediments has also been mentioned by Zinder and Brock (48, 49). In our study BES addition always led to increased MT accumulation in minor or major amounts, indicating VOSC degradation by methanogenic bacteria. This was further supported by additional experiments in which pulsewise-added DMS was stoichiometrically degraded to methane (data not shown). According to Kiene and Hines (26), BES addition resulted in inhibition of VOSC formation. They suggested that the accumulation of acetate inhibited the VOSC-producing microorganisms. Similar inhibitory effects in our experiments would even lead to an underestimation of MT formation.

Results of the multiple-correlation analysis (SAS software) showed that MT production rates are strongly correlated with the combination of the methane production rate and the H_2S concentration of the sediment (Fig. 5; Table 3). In this multiple relationship, H_2S could be replaced by the ratio of the sulfate to the iron concentration of the sediment. In freshwater anaerobic environments, methane production rates commonly reflect the rate of anaerobic mineralization of the organic matter in the sediment, since methane is the most important end product in these systems (44). Thus, the correlation between MT and methane production rates is probably caused by the fact that high mineralization rates of the organic matter in sediments will lead not only to high production of methane but also to high production of precursors for MT formation (e.g., methoxylated aromatic compounds). This is consistent with observations that lake sediments with a high methane production and organic-matter content also showed high levels of VOSC (37). MT formation rates are correlated with H_2S concentrations because H_2S is a precursor of MT formation, since it may act as a methyl acceptor in the degradation of organic matter in sediments. Methoxylated aromatic compounds have been shown to be degraded by this sulfide-mediated *o*-demethylation, resulting in MT and DMS (4, 17, 26). High concentrations of H_2S cause the flow of methyl groups to go to MT or DMS rather than to other possible compounds. This was also supported by the experiment in which sulfide concentration in the sediment slurry was artificially increased. In these sediment slurries MT production rates increased dramatically from 5 to $80 \text{ pmol of MT} \cdot \text{ml of sediment slurry}^{-1} \cdot \text{h}^{-1}$ due to the high sulfide concentrations (Fig. 6). In general, the mineralization of organic matter, producing compounds serving as methyl group donors, and the concentration of H_2S (methyl acceptor) are the main factors which determine the rate of MT formation.

Degradation of organic sulfur compounds (e.g., sulfur-containing amino acids), another source of MT and DMS formation, is unlikely to be stimulated by the addition of sulfide. Therefore, these compounds will be of minor importance in MT and DMS formation in freshwater sediments where sulfide is abundant (H_2S concentrations of >50 to $100 \mu M$) (Fig. 5 and 6). Correlations between H_2S and MT are unlikely to be caused by the enhanced sulfur incorporation and subsequent release to MT and DMS (37) because the stimulation of MT formation after H_2S addition did occur without a lag period. Drotar et al. (15) demonstrated that the widespread occurrence of *S*-adenosylmethionine-dependent thiol methyltransferase activities among aerobic bacteria could be a mechanism of MT production by these bacteria when exposed to H_2S . This pathway, however, will be of minor importance in anaerobic

sediments, since the pathway was not observed in obligately anaerobic bacteria (31).

Two types of pathways were demonstrated for anaerobic MT and DMS formation from methoxylated aromatic compounds (4): (i) a sulfide-dependent *o*-demethylation, as shown for strain SA2 (4), and (ii) a sulfide-independent *o*-demethylation performed by *Holophaga foetida* (30). The latter bacterium prefers sulfide for *o*-demethylation but is capable of carbonylation of the methyl group to form acetate under conditions in which sulfide is absent. The remaining phenolic residues are then degraded to acetate. *Holophaga foetida* thus combines the metabolic activities of demethylating anaerobes, such as *Acetobacterium woodii*, with those of ring-degrading organisms, such as *Pelobacter acidigallici*.

MT and DMS production rates will be lower under H_2S -limited conditions, since methoxylated aromatic compounds will be degraded at lower rates or will be degraded primarily to acetate (30), depending on the dominance of one of these two mechanisms in situ. In conditions of high H_2S concentrations, sulfide-mediated *o*-demethylation of the methyl groups is favored above carbonylation with CO_2 , resulting in high levels of MT and DMS (30). This explains the relationship between MT formation rates and H_2S concentrations mentioned in this study. This impact of sulfide thus results in low methyl group recoveries (1%) of added syringate or 3,4,5-trimethoxybenzoate under H_2S -limited conditions (26) and high methyl group recoveries (50%) at non- H_2S limited conditions (17). Given the high methyl group recoveries of added syringate or 3,4,5-trimethoxybenzoate (17, 31a), sulfide-mediated *o*-demethylation will be a major sink for methyl groups (of methoxylated aromatic compounds) in H_2S -rich freshwater sediments.

The H_2S concentration appeared to be influenced primarily by the sulfate and free-iron concentrations in the sediment pore water (Table 3). In freshwater systems sulfate reduction is generally sulfate limited. As a consequence, sulfate introduction normally increases sulfate reduction in sediments rich in organic matter (9, 38). In these sediments, however, high sulfate reduction rates result in high H_2S concentrations only if the free iron present is depleted. Iron reduction and successive precipitation to iron sulfide will lead to low H_2S concentrations (Fig. 7) (42). Indeed, the H_2S concentration in the sediments correlated with the ratio of the sulfate concentration to the free-iron concentration (Table 3). The correlation of the sulfate/free iron concentration ratio with MT production rates is consistent with observations that iron sulfide precipitation is a major sink for H_2S in iron-rich sediments (10) and is less important in iron-poor sediments (33, 34). The interference of free iron with the sulfate and H_2S relationship can explain the lack of correlation between sulfate concentrations and MT and DMS concentrations in low-sulfate systems mentioned by Richards et al. (36). Similar suggestions were made by Zinder and Brock (48) to explain the lack of H_2S production from cysteine added to sediments. In freshwater sediments DMS and MT concentrations are therefore better correlated to H_2S concentrations or sulfate/iron ratios than to sulfate concentration alone (37). The importance of sulfide methylation in the formation of VOSC can explain the lack of correlation between the sulfate concentration and other VOSC, such as COS, CS_2 , and DMDS, found by Richards et al. (37) and by ourselves (Fig. 3D), since these compounds are not products of sulfide-mediated *o*-demethylation.

In conclusion, this study provides strong evidence for sulfide methylation as a major mechanism in the formation of VOSC in freshwater sediments. On the basis of these results, a model for anaerobic freshwater sediments is proposed (Fig. 7). Given the correlations between MT production rates and the sulfate/

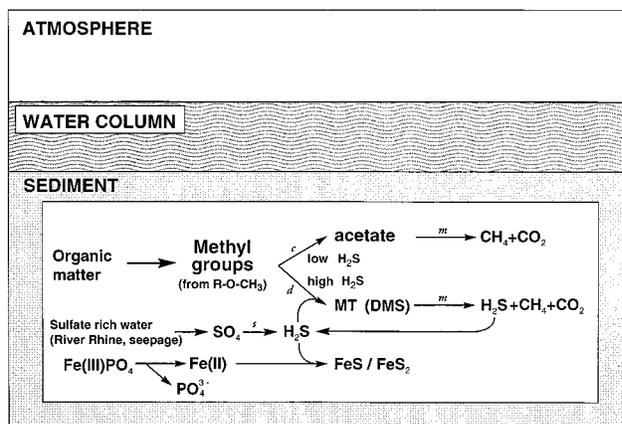


FIG. 7. Scheme of flow of methyl groups from methoxylated (aromatic) compounds ($R-O-CH_3$) to either acetate or MT and DMS as a major mechanism of VOSC formation in anoxic freshwater sediments. The formation of acetate from methyl groups occurs under conditions of low sulfide concentrations through carbonylation (c). The formation of MT and DMS occurs only under conditions of high sulfide concentrations, since sulfide is required as a methyl group acceptor (d). Sulfide acting as a methyl group acceptor to form MT and, subsequently, DMS is generated in the system by sulfate reduction (s), decomposition of organic matter, and degradation of MT and DMS by methanogenic activity (m). Sulfate can be introduced in the system by several processes (inflow of sulfate-rich Rhine water, deposition, and seepage water). MT, DMS, and H_2S can be restored or converted in the sediment or can diffuse to the higher, aerobic-water column and atmosphere.

iron ratios shown in this study, introduction of sulfate and the subsequent increase in the H_2S concentration eventually will enhance VOSC production. In The Netherlands, sulfate concentration in many freshwater systems are increasing as Rhine water (sulfate rich and poor in iron) is introduced in order to avoid aridification (38). Increased VOSC concentrations and consequently MT formation rates in freshwater sediments are therefore conceivable.

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