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Microbial Formation of Dimethyl Sulfide in Anoxic *Sphagnum* Peat†

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Peat bogs dominated by *Sphagnum* spp. have relatively high areal rates of dimethyl sulfide (DMS) emission to the atmosphere. DMS was produced in anoxic slurries of *Sphagnum* peat with a linear time course and with an average rate of 40.4 (range, 22.0 to 68.6) nmol per liter of slurry · day^{-1} observed in nine batches of slurry. Methanethiol (MeSH) was produced at roughly similar rates over the typical 4- to 8-day incubations. DMS and MeSH production in these acidic (pH 4.2 to 4.6) peats were biological, as they were stopped completely by autoclaving and inhibited strongly by addition of antibiotics and 500 μM chloroform. Endogenous DMS production may be due to the degradation of *S*-methyl-methionine, dimethyl sulfoxide, or methoxyaromatic compounds (e.g., syringic acid), each of which stimulated DMS formation when added at 5 to 10 μM concentrations. However, on the basis of the high rates of thiol (MeSH and ethanethiol) methylation activity that we observed and the availability of endogenous MeSH, we suggest that methylation of MeSH is the major pathway leading to DMS formation in anaerobic peat. Solid-phase adsorption of MeSH plays a key role in its availability for biomethylation reactions. Additions of acetate (1.5 mM) or compounds which could cause acetate to accumulate (e.g., glucose, alanine, and 2-bromoethanesulfonate) suppressed DMS formation. It is likely that acetogenic bacteria are involved in DMS formation, but our data are insufficient to allow firm conclusions about the metabolic pathways or organisms involved. Our observations are the first which point to the methylation of MeSH as the major mechanism for endogenous DMS production in any environment. The rates of net DMS production observed are sufficient to explain the relatively high fluxes of DMS emitted to the atmosphere from *Sphagnum* sp.-dominated wetlands.

Sphagnum sp.-dominated wetlands, including bogs and fens, are common in northern latitudes (>50°N) (22). These ecosystems are generally poor in inorganic sulfur (sulfate concentrations of <50 μM), yet they have relatively high areal emissions of volatile sulfur compounds, primarily in the form of dimethyl sulfide (DMS) (2, 8, 12). Sulfur deposition to and emission from peatlands are important aspects of the biogeochemistry of these low-sulfur-concentration systems (26), yet relatively little is known about the cycling of volatile sulfur compounds in peats. In particular, the microbiological processes responsible for DMS production are presently unknown. Most of the existing literature on sulfur in peatlands has focused on inorganic sulfur cycling, i.e., sulfate reduction, or on the incorporation of sulfur into organic matter within the peat (4, 27, 34, 35).

A limited number of studies on the distribution of organosulfur gases in freshwater lakes (5, 11, 25, 28) or in peat bogs (7, 8, 26) exist. Fewer still have focused on the production and consumption of these gases in freshwater environments. Zinder et al. (39) observed that methanethiol (MeSH) and DMS were formed during degradation of natural cyanobacterial mats. In addition, Zinder and Brock observed that methionine was biologically degraded to both MeSH and DMS in anoxic sediments from Lake Mendota (37, 38). The exact mechanism behind DMS formation from methionine was not clear from these early studies. More recent studies by Finster et al. (10) showed that when sulfide was present the methoxy groups of naturally occurring compounds such as syringic acid were efficiently converted to DMS and MeSH in marine and freshwater anoxic sediments. The observed methyl transfer reactions to inorganic sulfide and MeSH were probably carried out by homoacetogenic bacteria (*Pelobacter* type) which were isolated from these habitats (3). Methylation of MeSH to DMS in anoxic salt marsh sediments (21) and in cultures of a variety of aerobic bacteria (9) had previously been observed. The implication of these studies is that thiol methylation reactions may be important with respect to DMS formation. However, no quantitative assessment of this pathway has been made for natural systems. Most of the existing studies of DMS formation in freshwaters used concentrations of substrate(s) which were higher than natural levels, and they did not report endogenous production rates or dynamics. To our knowledge, there are no published studies of organic sulfur gas production in *Sphagnum* sp. peats. In this study, we examined the endogenous production dynamics of DMS in slurries of *Sphagnum* sp. peat and we explored potential mechanisms which could be responsible for DMS production.

MATERIALS AND METHODS

Sample collection and slurry preparation. All peat samples were collected in July 1993 from the central, oligotrophic region of Sallie's Fen, a 1.7-ha fen located in Barrington, New Hampshire (43°12′N, 71°04′W). The study site has been described previously (7). During the sampling period, the pH of the bog water at the sampling site was 4.6 to 4.7. The central region of the bog near where our samples were collected generally had lower pH's than fringing areas which tended to receive more surface water input and hence would be classified as minerotrophic (see reference 7). Vegetation at the collection site was dominated by *Sphagnum* sp. with a few herbaceous plants intermixed. A boardwalk extending into the center of the bog minimized disturbances to the sampling area.

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Brown peat from below the surface was collected by hand and placed immediately into 1-liter jars which were filled to the top and sealed with airtight lids. Some peat samples were used immediately to prepare slurries, while some samples were stored in a refrigerator (4°C) for up to 1 week before use. Storage, even for extended periods (6 months), does not appear to change DMS production patterns in the peat (data not shown). Before slurry preparation, the peat was sorted and any green top sections of *Sphagnum* sp. were discarded, as were most of the woody plant roots. The peat was then placed in a kitchen blender, and an equal volume of bog water (stored in jars with the peat) was added. The mixture was then blended and homogenized. The resulting slurry was poured into 120-ml crimp-top serum bottles through a funnel. For each batch of slurry, an equal volume, which varied between 70 and 90 ml for different batches, was added to the bottles. After being filled, the bottles were sealed with Teflon-faced butyl rubber septa (Wheaton product number 224-100-175) and the headspaces were flushed with N_2 for 1 min. The slurries were incubated in the dark without shaking at 22 to 24°C, a temperature similar to that in the field during July. All slurries were preincubated for 12 to 24 h before experimental additions were made.

In order to test whether DMS production or consumption patterns were different for the top, bright green layers of *Sphagnum* sp. (often found above the water table), we prepared one batch of slurry with the green top sections of the plants. For this batch, the vials were sealed under air or N_2 and additions of DMS were made to one set of each.

In some experiments, several bottles of slurry were autoclaved (20 min at 120°C; 15 psi/g) to serve as sterile controls. In one experiment, we used only bog water without peat.

Sampling for sulfur gas analysis. Subsampling of the slurries for dissolved gas analysis was done by removing 1 ml of the slurry through the stopper with a plastic tuberculin syringe and an 18-gauge needle. The subsample was injected into a sealed 14-ml serum bottle which was immediately vortexed at high speed for 5 s to equilibrate the dissolved gases with the headspace. The headspace of the vial was subsequently swept into a cryotrap with a helium flow of 100 ml/min by means of needle connections (see reference 18 for details on headspace sweeping). The sweeping time was 3 min, during which 77% of the DMS and 78% of the MeSH contained in the vial were removed. The amounts removed were very consistent, and a precision of 5% was typically obtained for replicate analyses of slurries. The detection limits for sulfur gases (DMS and MeSH) were about 1 nM. The concentrations of sulfur gases in the experimental slurries are presented as nanomoles · liter of slurry^{-1} and are not corrected for the amount of gas in the headspace because with the liquid/headspace ratios used, this was negligible (typically 2.5 to 4% of the total in the bottles).

Analysis of sulfur gases. The cryotrapping system was similar to that previously described (19, 24) and consisted of a Rheodyne six-port Teflon valve with a Teflon loop. In the trapping mode, the loop was immersed in liquid N_2 while in the inject mode, the liquid N_2 was quickly replaced with warm water to volatilize sulfur gases and introduce them into the gas chromatograph (GC). All tubing of this system was of Teflon. The GC was a Shimadzu GC-9A equipped with a flame photometric detector and sulfur-selective filter. The column used was a Carbopak B (60/80) (2 m by 3 mm; Teflon) with 1.5% XE-60–1% H_3PO_4. The oven temperature was maintained at 50°C, and the injector and detector were kept at 175°C. The carrier gas used was He at a flow rate of 40 ml/min. MeSH and DMS eluted with retention times of 0.6 and 1.0 min, respectively. CS_2 eluted just after DMS and was adequately separated. CS_2 accumulated in slurries, but this accumulation was mostly likely due to leaching of this gas from the rubber septa. Unpierced, Teflon-coated septa did not leach out significant amounts of CS_2, but as the septa were repeatedly pierced for subsampling, CS_2 began to leach out. CS_2 leaching was greater with other types of stoppers such as unfaced gray butyl and black butyl rubber than with the Teflon-faced variety.

Standardization. Permeation tubes were used to generate a stream of gas with a known concentration of sulfur gases. A sealed empty serum bottle was flushed with the effluent from the permeation tube. A standard curve was generated by withdrawing different volumes (0.1 to 2 ml) of the standard gas in the serum bottle with a plastic syringe and injecting this into an empty vial connected to the sweeping cryotrapping system. As for the slurry samples, the standard was trapped for 3 min before introduction to the GC. Ethylmethyl sulfide (EMS) standards were prepared gravimetrically in distilled water and used immediately.

Experimental procedures. From each batch of slurry, 12 to 16 bottles were obtained. Two to four of these were reserved as untreated controls, while the others received treatments of some kind (in duplicate). Treatments were always made after a 12- to 24-h preincubation period during which any oxygen in the slurries was consumed and sulfide began to accumulate. Sulfide was detectable during the GC runs, but peak areas were not consistent and were often off scale, so they were not quantified. The sulfide concentration was too low to detect via the methylene blue method (6) during the first few days of slurry incubation; only after about a week of incubation was it near the detection limit of this method (~3 to 5 μM). Thus, we were not able to quantify sulfide content during these experiments.

Most experimental additions were made from stock solutions prepared in distilled water. These additions included $Na_2SO_4 \cdot 10H_2O$, $Na_2S \cdot 9H_2O$, dimethylsulfoniopropionate · HCl (DMSP), *S*-methyl-methionine (SMM) sulfonium chloride, L-methionine, dimethyl sulfoxide (DMSO), DMS, methanol, syringic acid, trimethoxybenzoic acid, gallic acid, pectin, glucose, sodium acetate, alanine, sodium tungstate, and sodium 2-bromoethanesulfonate (BES). The highest addition level for any of the compounds was 2 mM. We tested whether an equivalent amount of salt (2 mM NaCl) had any effects on DMS production. This level of NaCl inhibited DMS production slightly over the first 24 h, but thereafter rates of DMS production were the same as those in untreated controls. We thus concluded that the ionic or osmotic effects of additions were minimal. In addition, all compound amendments were tested for effects on slurry pH. With the exception of $NaWO_4$ none were found to change the pH by more than 0.15 U. The addition of 2 mM tungstate caused the pH to rise from 4.6 to 6.1.

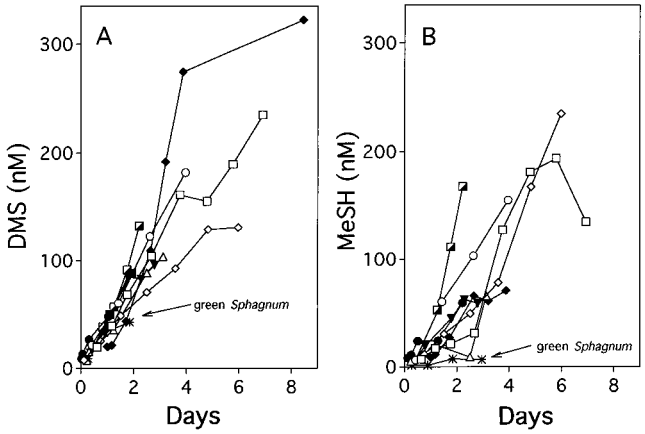

FIG. 1. Time courses of DMS (A) and MeSH (B) in anoxic slurries of *Sphagnum* peat. Data are for the untreated control bottles obtained from 10 separate batches of slurry prepared over a month-long period during July 1993. Each symbol represents a different batch of slurry. All slurries were prepared with brown *Sphagnum* peat except for a batch (*) which used only the green, top parts of the *Sphagnum* plants. The results are the means for duplicate bottles, but for clarity, the ranges are not plotted.

The aromatic acids (syringic, trimethoxybenzoic, and gallic acids) were brought into solution by slowly neutralizing the aqueous stock to pH 7 with NaOH. In some cases, syringic acid and trimethoxybenzoic acid were weighed as solids directly into the bottles before the slurry was added, thereby avoiding the neutralization step and the possibility of hydrolyzing the methyl groups. Both forms of syringic acid yielded similar results. We did not measure the final pH of these slurries, nor do we know how much of the solid acids dissolved during the incubation. The acetate solution was made with a mixture of sodium acetate and acetic acid to bring the final pH close to that of the bog water (4.7). Chloroform was used as an inhibitor of methyl transfer reactions and was added as a pure liquid to a final concentration of 500 μM. The antibiotics chloramphenicol and tetracycline (each at 25 mg · liter of slurry^{-1}) were added from ethanolic stocks. MeSH and ethanethiol (ESH) were added as gases by means of Hamilton gas-tight syringes. The slurries were shaken vigorously after these additions to equilibrate gases with the liquid phase. Tungstate was used as an inhibitor of sulfate reduction and was chosen over molybdate because of its weaker interaction with free thiols (14). The final concentrations of various additions are given in the Results section describing each experiment. In experiments with added DMS precursors, the percentage of the added methyl groups in the substrates, which was recovered as DMS during the experiment, was calculated as follows: % recovery = [(moles of DMS produced in treatment − moles of DMS produced in controls)/moles of methyl groups in added substrate] × 100.

Chemicals. All chemicals were obtained commercially from either Aldrich, Sigma, Fisher Scientific, or Eastman Kodak and were of the highest purity available. The pectin used (Sigma) was derived from apples. MeSH for additions to slurries was obtained from a permeation tube (VCI Metronics) which was punctured and subsequently sealed in a serum bottle with a Teflon septum. A small 10- to 25-μl portion of the vapor phase of this bottle was withdrawn and added to sealed slurry bottles.

RESULTS

DMS production in untreated slurries. DMS was produced by all untreated peat slurries. The time courses of DMS production in untreated slurries from 10 different batches prepared over a month-long period are shown in Fig. 1A. DMS production rates of brown peat slurries varied over a threefold range from 22.0 to 68.6 nmol · liter^{-1} · day^{-1}. The mean production rate was 40.4 nmol · liter^{-1} · day^{-1}. Most time courses

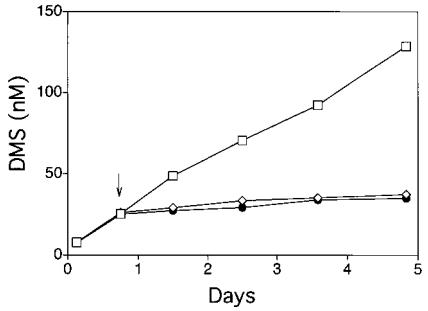

FIG. 2. The effects of 500 μM chloroform (◇) and antibiotics (●) (chloramphenicol plus tetracycline, 25 mg · ml of slurry) on DMS production in anoxic *Sphagnum* peat. Control samples (□) received no additions. Datum points indicate the means for duplicate bottles. The lack of visible error bars indicates that the range fell within the symbol. The time of addition is indicated by the arrow.

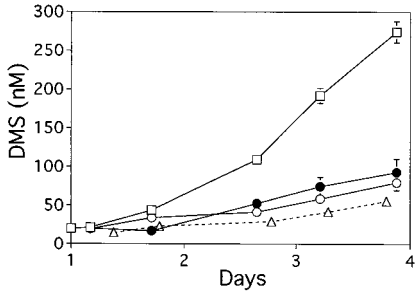

FIG. 4. The effects of 1 mM glucose (●), 2 mM alanine (○), and 1.5 mM acetate (△) on DMS production in anoxic *Sphagnum* peat. Control samples (□) received no additions. The results for acetate are drawn with a dotted line because they were for a separate batch of slurry in which the controls produced DMS at a rate similar to that shown in this figure. Datum points indicate the means for duplicate bottles, with the error bars indicating the range. A lack of visible error bars indicates that the range fell within the symbol.

showed nearly linear production for periods of 2 to 6 days. The results for samples from experiment 6 did not follow this pattern and instead displayed a lag followed by an exponential increase in DMS production and subsequent declining production. When green top sections of the *Sphagnum* plants were used to make a slurry and were incubated under N_2 or air, the rate of DMS production was 20.2 nmol · liter^{-1} · day^{-1}, slightly lower than the lowest rate observed for brown peat slurries (Fig. 1A). Bog water without peat produced DMS more slowly (~6 nmol · liter^{-1} · day^{-1}) than the peat slurries (data not shown). No net consumption of DMS was noted during any of the incubations with these low-pH slurries.

Inhibition of DMS formation. The production of DMS in slurries was inhibited by the addition of 500 μM $CHCl_3$ or a mixture of chloramphenicol and tetracycline (Fig. 2). The effects were immediate and very strong; only small increases in DMS were observed for the inhibited samples. Autoclaved slurries incubated under air or N_2 produced no DMS (data not shown). The addition of 2 mM BES, a known inhibitor of methanogenesis, strongly inhibited DMS formation (62% lower than the control level; $P < 0.04$ by two-tailed t test) (Fig. 3). Lower concentrations of BES (0.1 and 1 mM) were less inhibitory (data not shown). Sodium tungstate (2 mM), an inhibitor of sulfate reduction, also inhibited DMS formation (41% lower than the control level; $P < 0.14$), but the effect was much less than that observed with BES (Fig. 3). The addition of 100 μM Na_2SO_4 yielded production levels of DMS 24% lower than control levels ($P < 0.22$) over 48 h of incubation. In a separate experiment, addition of 10 μM $Na_2S \cdot 9H_2O$ stimulated DMS production slightly (11% above control level) after 2.5 days (data not shown).

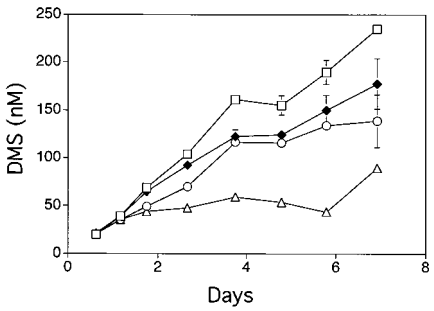

FIG. 3. The effects of 100 μM sulfate (◆), 2 mM tungstate (○), and BES (△) on DMS production in anoxic *Sphagnum* peat. Control samples (□) received no additions. Datum points indicate the means for duplicate bottles, with the error bars indicating the range. A lack of visible error bars indicates that the range fell within the symbol.

Several low-molecular-weight organic compounds were tested for effects on DMS production (Fig. 4). Addition of 1.5 mM acetate strongly inhibited DMS production; 0.15 mM acetate was not inhibitory (data not shown). Similarly, glucose (1 mM) and alanine (2 mM) were both strongly inhibitory toward DMS production (Fig. 4).

Potential precursors of DMS. In order to investigate the pathways leading to formation of DMS, several potential precursors were added to slurries. SMM at 10 μM immediately stimulated DMS production well in excess of that of controls (Fig. 5). Despite the substantial stimulation of DMS production caused by SMM, only 2.7% of the methyl groups in SMM were recovered in the excess DMS after 2 days of incubation. In contrast to the results with SMM, DMSP at the same concentration (10 μM) yielded little DMS above control levels. Addition of DMSO (1 μM) resulted in rapid and quantitative conversion of this substrate to DMS in less than 6 h (Fig. 5).

The addition of the methoxylated compound syringic acid (5 μM, equivalent to 10 μM methyl groups) increased DMS formation by about 40% after several days (Fig. 6). A synthetic

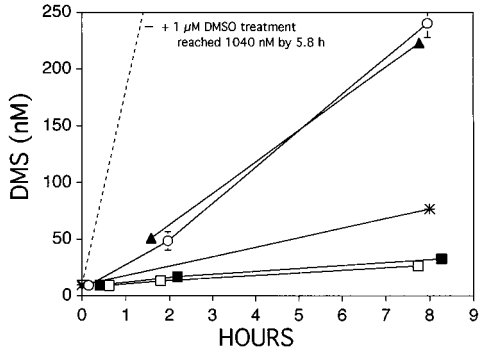

FIG. 5. Short-term time course of DMS production in anoxic *Sphagnum* peat after treatment with no addition (□), 10 μM SMM (○), 10 μM SMM plus antibiotics (▲), 10 μM SMM plus autoclaving (✳), and 10 μM DMSP (■). The results for DMSO are plotted as dotted lines because they were obtained in a separate experiment (controls were similar in both experiments, so comparisons should be valid). The first time point in the DMSO treatment indicated nearly complete conversion of the DMSP to DMS by 5.8 h. Subsequent sampling revealed that the DMS concentration increased only slowly after 5.8 h. The datum points show the means for duplicate bottles, with the error bars indicating the range. A lack of visible error bars indicates that the range fell within the symbol.

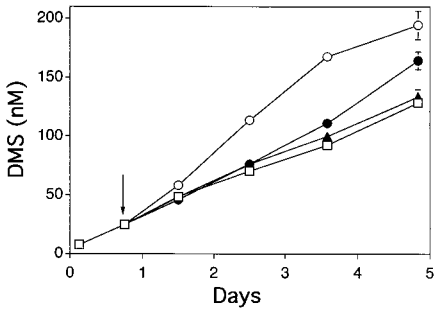

FIG. 6. The effects of 5 μM additions of the methoxyaromatic compounds syringic acid (○) and trimethoxybenzoic acid (●) as well as a nonmethylated analog, gallic acid (△), on DMS production in *Sphagnum* peat. Control samples (□) received no additions. Datum points indicate the means for duplicate bottles, with the error bars indicating the range. A lack of visible error bars indicates that the range fell within the symbol. The time of addition is indicated by the arrow.

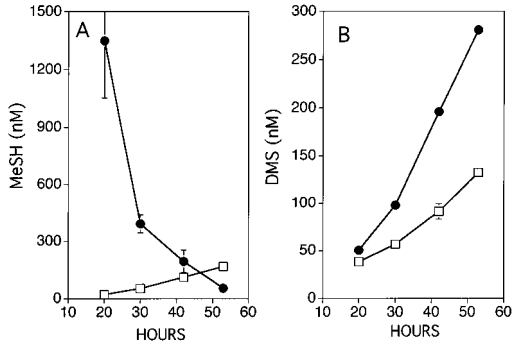

FIG. 8. Time courses of MeSH (A) and DMS (B) in anoxic *Sphagnum* peat slurries after the addition of 1.3 μM MeSH (●). Control samples (□) received no additions. Datum points indicate the means for duplicate bottles, with the error bars indicating the range. A lack of visible error bars indicates that the range fell within the symbol.

analog, trimethoxybenzoic acid, also stimulated DMS formation but to a lesser degree. For both compounds, the yield of DMS in terms of the methyl groups added was very low (<1%). A nonmethylated analog of these compounds, gallic acid, had no effect on DMS production (Fig. 6). Similar stimulatory effects were obtained when these methoxy compounds were added in crystalline form directly to the slurries (data not shown), suggesting that the neutralization of the stock solutions (to solubilize the compounds) was not responsible for the effects on DMS. Addition of the carboxymethyl compound, pectin, at 2 to 40 mg · liter of slurry^{-1} did not stimulate DMS production and instead reduced DMS production by about 20% at the higher concentrations (data not shown).

The role of MeSH in DMS formation. Untreated peat slurries produced MeSH at rates which were roughly similar to those for DMS (mean rate of 39.6 compared with 40.4 nmol · liter^{-1} · day^{-1} for DMS) (Fig. 1B). Time courses of controls for MeSH varied more than those for DMS, with rates ranging from 22 to 107 nmol · liter^{-1} · day^{-1}. Some of the variability in MeSH accumulation may have been due to the chemical adsorption of MeSH to solid-phase materials (as discussed below).

Addition of 10 μM methionine to anoxic slurries greatly stimulated not only MeSH production but also DMS production (Fig. 7). At the maximum in MeSH, 6.5% of the added methionine-sulfur could be accounted for as MeSH. For DMS, the maximum difference between controls and the methionine treatment was observed at the last time point (8.5 days), with the extra DMS accounting for 3.5% of the added methionine-sulfur.

Since methionine cannot degrade directly to DMS, but is known to be degraded directly to MeSH (31, 33), we tested whether the MeSH itself had any effects on DMS production. Additions of MeSH greatly stimulated DMS production (Fig. 8). MeSH levels declined rapidly after addition, and this was found to occur in sterile autoclaved samples as well (Fig. 9). However, DMS formation was noted only in biologically active slurries. Thus, methylation of MeSH was biologically catalyzed.

To further verify that methylation activity was responsible for formation of DMS from MeSH, we added ESH at the relatively high concentrations of 5 and 10 μM and observed linear production of EMS over 15 h (Fig. 10). Both additions of ESH yielded similar rates of EMS formation over the first 15 h of incubation, possibly suggesting saturation of the methylation activity at these initial concentrations. After 15 h, the rate of EMS formation slowed in the lower-concentration ESH treatment. This result was most likely due to the disappearance of ESH from these bottles (primarily due to adsorptive losses similar to those observed with MeSH), which was observable in GC runs. We could not quantify the ESH because it eluted too close to DMS. We could, however, distinguish the presence of ESH by a slight shift in retention time of the DMS peak. By 15 h, the DMS plus ESH peak for the lower-concentration ESH

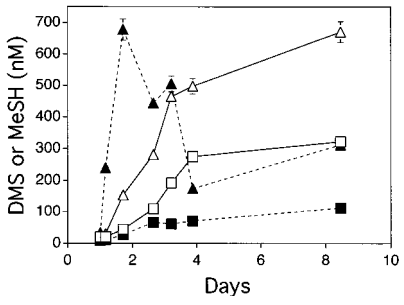

FIG. 7. The effects of 10 μM methionine addition on DMS and MeSH time courses in anoxic *Sphagnum* peat. Dotted lines (and solid symbols) represent MeSH data, while solid lines (and open symbols) represent DMS data. Symbols: □ and ■, untreated controls; △ and ▲, addition of 10 μM L-methionine. Datum points indicate the means for duplicate bottles, with the error bars indicating the range. A lack of visible error bars indicates that the range fell within the symbol.

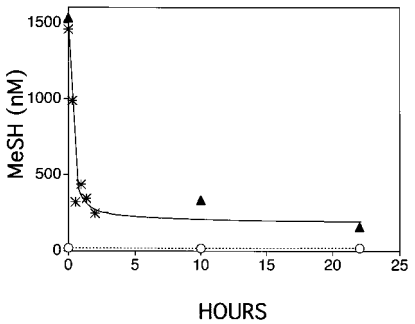

FIG. 9. Disappearance of added dissolved MeSH in autoclaved *Sphagnum* peat slurries. Because of the relatively long sampling interval in the first trial (▲), the rapid change in MeSH concentration was missed. A second trial with close-interval sampling (*) was therefore carried out. The curve was fit manually. Also shown are the DMS concentrations in autoclaved samples from the first trial (○). DMS concentrations were 15 nM throughout the incubations. The results shown are for single bottles.

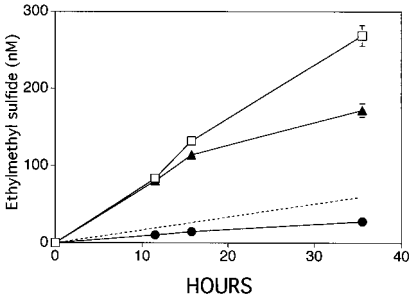

FIG. 10 Time course of EMS production in anoxic *Sphagnum* peat after the addition of 5 μM ESH (△), 10 μM ESH (□), or 10 μM ESH plus $CHCl_3$ (●). The dotted line represents the mean rate of endogenous DMS production observed in nine batches of slurry used during this study. Datum points indicate the means for duplicate bottles, with the error bars indicating the range. A lack of visible error bars indicates that the range fell within the symbol.

treatment had decreased to the level of those of DMS in untreated bottles. In contrast, substantial ESH remained for the higher-concentration treatment, as evidenced by a large DMS plus ESH peak, and EMS levels continued to increase. Inclusion of chloroform in the ESH samples strongly inhibited formation of EMS (Fig. 10). The initial rates of EMS formation (173 to 200 nmol · liter^{-1} · day^{-1}) exceeded the average rate of DMS formation (40 nmol · liter^{-1} · day^{-1}) for all experiments and in fact were higher than even the maximal DMS production rate observed.

DISCUSSION

Formation of DMS in low-pH *Sphagnum* peat appears to be more closely tied to carbon metabolism than to inorganic sulfur cycling. Amendments of sulfate or sulfide had little or no effect on DMS production over week-long incubations, whereas additions of organosulfur compounds or other organic compounds (acetate, glucose, and syringic acid, etc.) had substantial effects on DMS production. We identified several potential pathways for the generation of DMS in *Sphagnum* peat. These included degradation of SMM, reduction of DMSO, degradation of methoxyaromatic compounds, and methylation of endogenous MeSH.

The stimulation of DMS production by 10 μM SMM was immediate (Fig. 5), but the overall percent recovery of methyl groups as DMS over a 2-day incubation was relatively low (2.7%). This low percent recovery could have been due to incomplete utilization of SMM, adsorption of produced DMS, or degradation of SMM to other nonvolatile products. Relatively little is known about the distribution or degradation of SMM in the environment. SMM was probably converted to DMS by a lyase enzyme similar to that described in reference 23. Our evidence supports a primarily biological conversion, since autoclaving and $CHCl_3$ greatly inhibited DMS formation from added SMM. Antibiotics did not slow this conversion when they were added immediately prior to the addition of SMM (Fig. 5), but they were inhibitory when added 24 h before the addition of SMM (data not shown). It is therefore likely that bacteria played a role in degrading SMM and that the enzyme system responsible was constitutive in the peat. Contrasting results were obtained with DMSP, which produced much less DMS than did SMM. DMSP is commonly found in the marine environment, but is not thought to be prevalent in terrestrial or freshwater systems. It is therefore not surprising that degradation of this compound to DMS was minimal in the freshwater bog. In marine sediments, where DMSP is commonly found, it undergoes rapid degradation to DMS and other sulfur products (17, 20, 21, 33).

Our results showed that 1 μM DMSO was rapidly and completely reduced to DMS in just 6 h (Fig. 5). DMSO was most likely utilized by microorganisms as a terminal electron acceptor for anaerobic growth (36). Biological reduction of DMSO to DMS in anoxic salt marsh sediments has been observed previously (17). Abiotic reduction of DMSO by hydrogen sulfide is possible but unlikely to be significant in these circumstances, since sulfide levels were below 5 μM. DMSO is present at concentrations of 1 to 200 nM in most natural waters (1, 18), but its concentrations in the bog are presently unknown.

Because we do not have adequate data on the concentrations of either SMM or DMSO in the bog, we cannot estimate their importance to natural DMS formation. Although we cannot rule out the possibility that DMS is formed from these compounds in the bog, several lines of evidence lead us to believe that these precursors are of relatively minor importance in anoxic peat. If a large pool of a labile precursor like SMM were present in *Sphagnum* plants, it would be expected to be more abundant in the green, growing parts of the plants than in the brown, partially degraded sections below. A large precursor pool would be expected to degrade rapidly to DMS in the slurry. This phenomenon was not observed, as slurries prepared from the green parts of the *Sphagnum* plants did not produce more DMS (or MeSH) than brown peat (Fig. 1). Further evidence against SMM being a major precursor in the bog is the fact that antibiotics had no immediate effect on DMS production resulting from added SMM (Fig. 5) while antibiotics greatly inhibited DMS production in otherwise untreated slurries immediately after being added (Fig. 2).

Because DMSO was converted to DMS so rapidly (Fig. 5), it is not likely that this compound was responsible for the slow, steady production of DMS observed with anoxic slurries. Any preexisting DMSO should have been reduced rapidly, and little or no regeneration of DMSO would be expected in anoxic samples held in the dark (16).

Methylation activity and production of DMS. Methionine addition resulted in very rapid accumulation of MeSH, which caused DMS production to be stimulated (Fig. 7). Methionine was probably not converted directly to DMS but rather was converted to MeSH which was subsequently methylated to DMS. With added 10 μM methionine, a transient maximum MeSH concentration of about 600 nM was reached, indicating that probably >90% of the MeSH was bound in some way (assuming that all the methionine was demethiolated). This finding is consistent with the results for autoclaved slurries which showed that >80% of the MeSH was lost from solution in just a few hours (Fig. 9). Zinder and Brock (37) reported substantial sediment binding for methionine degradation products and MeSH in lake sediments, and this was also observed with anoxic marine sediments (14). Despite significant abiological losses, direct addition of MeSH immediately stimulated DMS formation (Fig. 8). As the concentration of added MeSH decreased, the rate of DMS formation became more like those of the controls. This may be taken as evidence that the MeSH must be in the free form to participate in the methylation process. The results of ESH additions and EMS production (Fig. 10) also suggest that only the free form of the thiol is methylated. This is an important observation and bears further investigation. With our experimental additions of 5 and 10 μM ESH, we appeared to saturate methylation activity for about 15 h, after which time the lower concentration of ESH had decreased (perhaps as a result of abiological binding) and EMS formation slowed. During the maximal production of EMS, the

rate of methylation from endogenous methyl donors was 173 to 200 nmol · liter^{-1} · day^{-1}. This rate can be compared with the DMS production rates for untreated slurries, which ranged from 22 to 69 nmol · liter^{-1} · day^{-1}. The high level of thiol methylation activity observed for peats and the availability of endogenous MeSH (Fig. 1B) suggest that this may be the major pathway by which DMS is formed in these anaerobic peats.

With the probable significance of the role of MeSH in the formation of DMS, it becomes necessary to consider the sources and sinks for MeSH. MeSH could arise from degradation of methionine as has been shown here and in other studies (21, 37). It could also arise from methylation of inorganic sulfide (9, 10). The study by Finster et al. (10) demonstrated that the methyl groups of methoxylated aromatic compounds were sequentially converted to MeSH and then DMS in both marine and freshwater sediments. Acetogenic bacteria which carried out this reaction were isolated and found to utilize sulfide in the growth media for the formation of MeSH and DMS (3). The reactions proposed by Finster et al. for the enzymatic methylation of sulfide and MeSH are as follows: R-O-CH_3 + H_2S→R-OH + CH_3SH and R-O-CH_3 + CH_3SH→R-OH + CH_3SCH_3, where R- is an aromatic ring.

It is not clear at present whether the same enzyme system is responsible for methylating both H_2S and MeSH or whether there is any difference in the affinities of the two compounds. Very high yields of MeSH and DMS (>50%) were found in sediment experiments by Finster et al. (10), though differences in the relative amounts of MeSH and DMS formed were noted for different environments. In the pure-culture studies of Bak et al. (3) the organisms produced DMS almost exclusively and with a high conversion efficiency. Though we did observe stimulation of DMS production by syringic acid and trimethoxybenzoic acid in peat, the excess DMS produced accounted for less than 1% of the added methyl groups present in the methoxy compounds. The reasons for the different results may include little sulfide availability in the bog (H_2S concentration of <5 μM) and the fact that Finster et al. used 100 to 1,000 μM additions of the methoxy compounds while we used 5 μM concentrations. At the highest addition levels, intense MeSH production may saturate binding sites, thereby allowing MeSH to accumulate and making it available for further methylation to DMS. At the lowest substrate concentrations, the majority of the MeSH produced may bind and not be available for conversion to DMS. In pure cultures, little binding of MeSH would be expected and therefore efficient conversion of MeSH to DMS can take place.

We suggest that the binding of MeSH plays a critical role in the formation of DMS in anaerobic peats by affecting the availability of MeSH for biomethylation. Such a phenomenon might help to explain why sulfate additions had minimal effects on DMS formation in our experiments (Fig. 3) and why 10 μM Na_2S stimulated DMS production by only 11% (data not shown). Elevation of H_2S concentrations, either directly by addition or indirectly via SO_4^{2-} amendment, may result in most of the methyl groups being diverted to H_2S rather than to MeSH. Accumulation of free MeSH would be damped by solid-phase binding, and little DMS would accumulate. This scenario suggests that the relative availability of H_2S or MeSH as methyl receptors might be very important with respect to DMS formation. Furthermore, it underscores the need to work with natural levels of substrates to avoid artifacts associated with availability of the substrates or intermediate reaction products.

As with the other potential DMS precursors, we do not know the concentrations of methoxylated substrates such as syringic acid in the peat. Syringic acid moieties are found in lignin, which is probably not abundant in *Sphagnum* spp. but could be derived from a number of herbaceous plants growing in the peat or from leaf litter which falls from the surrounding canopy. *Sphagnum* spp. are known to produce large amounts of pectin-like compounds (23a). Pectin contains methyl groups esterified on the carboxyl group of galacturonic acid monomers. We did not observe any stimulation of DMS or MeSH production by pectin, even when it was added at relatively high levels (2 to 40 mg · liter of slurry^{-1}). This result indicates a possible distinction between carboxymethyl groups and aromatic-ring methoxy groups (like those found in syringic acid). The latter compounds may undergo O demethylation, while pectin may undergo hydrolysis to yield methanol (29). Interestingly, we found that methanol had no effects on DMS formation when added at 10 μM (data not shown). The homoacetogenic bacteria isolated by Bak et al. (3) formed DMS from syringate and trimethoxybenzoate but did not use methanol, nor did they produce methanol from methoxyaromatic compounds. The homoacetogenic metabolism of *Acetobacterium woodii* and *Clostridium thermoaceticum*, which grow on methoxylated aromatic compounds, involves a methyl transferase which catalyzes O demethylation of the methoxy groups and not a methanol-yielding hydrolysis. The fact that some acetogens are known to carry out O demethylations and to form DMS (3) suggests that this group of microorganisms may be involved in DMS production in anaerobic peats.

Although acetogens are likely to be involved in DMS formation, their role or that of any other group is not proved by our data. Our data do suggest that the concentration of acetate, the end product of acetogenic metabolism, may play an important role in DMS formation. Relatively high concentrations (1.5 mM) of acetate strongly inhibited DMS formation (Fig. 4). Likewise, compounds like glucose and alanine which could be degraded to acetate were also highly inhibitory. We speculate that high concentrations of acetate resulting from these additions interfered with methyl group metabolism (either by acetogens or other groups) and thereby affected DMS production via the methylation pathway. When methanogenesis was inhibited with BES, we also found that DMS formation was inhibited. We cannot rule out a direct role of methanogens in forming DMS, but the inhibitory effect may also have been due to accumulation of acetate, for which methanogenesis is probably a major sink (32). Evidence against there being a direct role of methanogens in DMS formation comes from the fact that methanogenesis was only slightly inhibited by 1.5 mM acetate and it was stimulated by 1 mM glucose and 1.5 mM alanine (data not shown). Each of these treatments resulted in strong inhibition of DMS formation (Fig. 4). It is less clear why tungstate was inhibitory to DMS formation, since it is not known whether sulfate-reducing bacteria in bogs contribute significantly to acetate consumption or whether they directly methylate sulfur compounds. The moderate inhibitory effects of tungstate (Fig. 3) also may have been due to a significant pH increase (4.6 to 6.1) or to chemical complex formation between WO_4^{2-} and MeSH, effectively lowering the availability of MeSH for methylation (16a). It will be necessary to isolate pure cultures or organisms from these acidic peats to determine which kinds of organisms are involved in DMS formation.

A comparison between nonhomogenized plant material and slurried peat samples incubated under N_2 yielded similar DMS production rates and similar distribution patterns (18a). The slurries thus appear to allow reasonable estimates of DMS production for the anaerobic peats. Assuming an average DMS production rate of 40 nmol · liter^{-1} · day^{-1}, and integrating

this over 15 cm of depth, we obtain an areal estimate for DMS production of 6 $\mu mol \cdot m^2 \cdot day^{-1}$. This production rate is about three times higher than the daily emission rates observed for DMS at this site during the summer (7). Thus, the production of DMS in the top layers of peat is sufficient to explain the emission fluxes. Furthermore, it appears that a relatively large fraction (~30%) of the DMS produced at this low-pH site may escape to the atmosphere. This is uncharacteristic of many trace gases, for which only a small percent of the production typically escapes the aquatic environment (13, 15, 30). Our conclusion is consistent with observations that DMS consumption in peat from the central, low-pH region of Sallie's Fen is slow or absent (16a). We have learned since conducting our study that peat from the more minerotrophic (pH of >4.7) areas of Sallie's Fen shows substantial DMS consumption as well as production. We are currently investigating the reasons for these site differences.

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REFERENCES

1. **Andreae, M. O.** 1980. Dimethylsulfoxide in marine and freshwaters. Limnol. Oceanogr. **25:**1054–1063.
2. **Andreae, M. O.** 1990. Ocean-atmosphere interactions in the global biogeochemical sulfur cycle. Mar. Chem. **30:**1–29.
3. **Bak, F., K. Finster, and F. Rothfuss.** 1992. Formation of dimethylsulfide and methanethiol from methoxylated aromatic compounds and inorganic sulfide by newly isolated anaerobic bacteria. Arch. Microbiol. **157:**529–534.
4. **Brown, K. A.** 1986. Formation of organic sulphur in anaerobic peat. Soil Biol. Biochem. **18:**131–140.
5. **Caron, F., and J. R. Kramer.** 1989. Gas chromatographic determination of volatile sulfides at trace levels in natural freshwaters. Anal. Chem. **61:**114–118.
6. **Cline, J. D.** 1969. Spectrophotometric determination of hydrogen sulfide in natural waters. Limnol. Oceanogr. **14:**454–458.
7. **de Mello, W. Z., and M. E. Hines.** 1994. Application of static and dynamic enclosures for determining dimethylsulfide and carbonyl sulfide exchange in *Sphagnum* peatlands. J. Geophys. Res. **99:**14.601–14.607.
8. **de Mello, W. Z., and M. E. Hines.** Emissions of dimethylsulfide from northern *Sphagnum*-dominated wetlands: controls on production and flux. Submitted for publication.
9. **Drotar, A., G. A. Burton, Jr., J. E. Tavernier, and R. Fall.** 1987. Widespread occurrence of bacterial thiol methyltransferases and the biogenic emission of methylated sulfur gases. Appl. Environ. Microbiol. **53:**1626–1631.
10. **Finster, K., G. M. King, and F. Bak.** 1990. Formation of methylmercaptan and dimethylsulfide from methoxylated aromatic compounds in anoxic marine and fresh water sediments. FEMS Microbiol. Ecol. **74:**295–302.
11. **Henatsch, J. J., and F. Juttner.** 1990. Occurrence and distribution of methane thiol and other volatile organic sulphur compounds in a stratified lake with anoxic hypolimnion. Arch. Hydrobiol. **119:**315–323.
12. **Hines, M. E.** Emissions of sulfur gases from wetlands. *In* D. Adams, P. Crill, and S. Seitzinger (ed.), Cycling of reduced gases in the hydrosphere, in press. E. Schweitzerbart'sche Verlagsbuchhandlungen, Stuttgart, Germany.
13. **Kiene, R. P.** 1991. Production and consumption of methane in aquatic systems, p. 111–146. *In* J. E. Rodgers and W. B. Whitman (ed.), Microbial production and consumption of greenhouse gases: methane, nitrogen oxides, and halomethanes. American Society for Microbiology, Washington, D.C.
14. **Kiene, R. P.** 1991. Evidence for the biological turnover of thiols in anoxic marine sediments. Biogeochemistry **13:**117–135.
15. **Kiene, R. P.** 1993. Microbial sources and sinks for methylated sulfur compounds in the marine environment, p. 15–33. *In* D. P. Kelly and J. C. Murrell (ed.), Microbial growth on C_1 compounds. Intercept Ltd., London.
16. **Kiene, R. P.** Microbial cycling of organosulfur gases in marine and freshwater environments. *In* D. Adams, P. Crill, and S. Seitzinger (ed.), Cycling of reduced gases in the hydrosphere, in press. E. Schweitzerbart'sche Verlagsbuchhandlungen, Stuttgart, Germany.

16a. **Kiene, R. P.** Unpublished data.

17. **Kiene, R. P., and D. G. Capone.** 1988. Microbial transformations of methylated sulfur compounds in anoxic salt marsh sediments. Microb. Ecol. **15:**275–291.
18. **Kiene, R. P., and G. Gerard.** 1994. Determination of trace levels of dimethylsulfoxide (DMSO) in sea water and rainwater. Mar. Chem. **47:**1–12.

18a. **Kiene, R. P., and M. E. Hines.** Unpublished data.

19. **Kiene, R. P., and S. K. Service.** 1991. Decomposition of dissolved DMSP and DMS in estuarine waters: dependence on temperature and substrate concentration. Mar. Ecol. Prog. Ser. **76:**1–11.
20. **Kiene, R. P., and B. F. Taylor.** 1988. Biotransformations of organosulphur compounds in sediments via 3-mercaptopropionate. Nature (London) **332:**148–150.
21. **Kiene, R. P., and P. T. Visscher.** 1987. Production and fate of methylated sulfur compounds from methionine and dimethylsulfoniopropionate in salt marsh sediments. Appl. Environ. Microbiol. **53:**2426–2434.
22. **Matthews, E., and I. Fung.** 1987. Methane emission from natural wetlands: global distribution, area and environmental characteristics of sources. Global Biogeochem. Cycles **1:**61–86.
23. **Mazelis, M., B. Levin, and N. Mallinson.** 1965. Decomposition of methyl methionine sulfonium salts by a bacterial enzyme. Biochim. Biophys. Acta **105:**106–114.

23a. **Mitch, W. J., and J. G. Gosselink.** 1993. Wetlands, 2nd ed. Van Nostrand Reinhold, New York.

24. **Morrison, M. C., and M. E. Hines.** 1990. The variability of biogenic sulfur flux from a temperate salt marsh on short time and space scales. Atmos. Environ. **24A:**1771–1779.
25. **Nriagu, J. O., and D. A. Holdway.** 1989. Production and release of dimethyl sulfide from the Great Lakes. Tellus **41B:**161–169.
26. **Nriagu, J. O., D. A. Holdway, and R. D. Coker.** 1987. Biogenic sulfur and the acidity of rainfall in remote areas of Canada. Science **237:**1189–1192.
27. **Peterson, J. C., S. M. Bennett, and L. L. Kramer.** 1991. Sulfur mineralization in *Sphagnum* peat. Commun. Soil Sci. Plant Anal. **22:**225–232.
28. **Richards, S. R., C. A. Kelly, and J. W. M. Rudd.** 1991. Organic volatile sulfur in lakes of the Canadian Shield and its loss to the atmosphere. Limnol. Oceanogr. **36:**468–482.
29. **Schink, B., and J. G. Zeikus.** 1982. Microbial ecology of pectin decomposition in anoxic lake sediments. J. Gen. Microbiol. **128:**393–404.
30. **Schutz, H., W. Seiler, and R. Conrad.** 1989. Processes involved in formation and emission of methane in rice paddies. Biogeochemistry **7:**33–53.
31. **Segal, W., and R. L. Starkey.** 1969. Microbial decomposition of methionine and identity of the resulting sulfur products. J. Bacteriol. **98:**908–913.
32. **Svensson, B. H.** 1984. Different temperature optima for methane formation when enrichments from acid peat are supplemented with acetate or hydrogen. Appl. Environ. Microbiol. **48:**389–394.
33. **Taylor, B. F.** 1993. Bacterial transformations of organic sulfur compounds in marine environments, p. 745–781. *In* R. Oremland (ed.), Biogeochemistry of global change—radiatively active trace gases. Chapman and Hall, New York.
34. **Wieder, R. K., and G. E. Lang.** 1988. Cycling of inorganic and organic sulfur in peat from Big Run Bog, West Virginia. Biogeochemistry **5:**221–242.
35. **Wieder, R. K., G. E. Lang, and V. A. Granus.** 1987. Sulphur transformations in *Sphagnum*-derived peat during incubation. Soil Biol. Biochem. **19:**101–106.
36. **Zinder, S. H., and T. D. Brock.** 1978. Dimethyl sulfoxide as an electron acceptor for anaerobic growth. Arch. Microbiol. **116:**35–40.
37. **Zinder, S. H., and T. D. Brock.** 1978. Methane, carbon dioxide, and hydrogen sulfide production from the terminal methiol group of methionine by anaerobic lake sediments. Appl. Environ. Microbiol. **35:**344–352.
38. **Zinder, S. H., and T. D. Brock.** 1978. Production of methane and carbon dioxide from methane thiol and dimethyl sulfide by anaerobic lake sediments. Nature (London) **273:**226–228.
39. **Zinder, S. H., W. N. Doemel, and T. D. Brock.** 1977. Production of volatile sulfur compounds during the decomposition of algal mats. Appl. Environ. Microbiol. **34:**859–860.