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AEROBIC DMS DEGRADATION IN MICROBIAL MATS

The Use of a Newly Isolated Species *Methylophaga sulfidovorans* in the Mathematical Description of Sulfur Fluxes in Mats

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SUMMARY

A new dimethyl sulfide (DMS)-degrading bacterium, *Methylophaga sulfidovorans,* was isolated from a Dutch intertidal mud flat. This bacterium was obligately methylotrophic, and able to use sulfide as an additional electron donor. Experiments with *M. sulfidovorans* were carried out in continuous and batch cultures in order to measure DMS oxidation under the types of conditions in a microbial mat. As *M. sulfidovorans* appears to be representative of the aerobic DMS oxidizing community in such a mat, its (kinetic) characteristics were used for the mathematical modelling of sulfur fluxes in a microbial mat. The predicted fluxes from a microbial mat sediment to the atmosphere in a 12 hour light period were within the range observed by direct measurements (derived from literature) in the field.

INTRODUCTION

Dimethyl sulfide (DMS) metabolism has been described for a small number of aerobic and anaerobic bacteria from freshwater and marine systems. Interest in freshwater DMS oxidizing bacteria originates primarily from their potential use in biological waste gas treatment systems for gases that contain (organic) sulfur compounds (9, 18, 20, 29). DMS in waste gases arises either as a product of the chemical lignin degradation in the paper industry, or from food manufacturing where DMS is mainly formed from the degradation of sulfur containing amino acids (11). The main precursor of DMS in marine environments is dimethylsulfoniopropionate (DMSP), an osmolyte present in many marine phototrophic organisms (10). Production of DMSP can be estimated at 4.10^{15} g S (DMSP) y^{-1} (15). Bacterial degradation of oceanic DMS is estimated to be about 90% of the total DMS

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(26). Furthermore, measurements have shown a DMS-uptake capacity in sediments, indicating the presence of a microbial DMS oxidizing population (13).

This paper describes the isolation and characterisation of an aerobic, DMS-oxidizing bacterium from a marine microbial mat. The physiological characteristics of the isolate are used in a mathematical model describing sulfur fluxes in a microbial mat. In this general model, both biological activity and transport processes are considered in order to predict DMS release into the atmosphere.

MATERIALS AND METHODS

Bacterial Culture and Natural Samples

Microbial mat sediment samples were obtained from an estuarine intertidal region at the south-west coast of the Netherlands (51° 26.3′ N, 4° 7.5′ E). Samples were obtained in 1993 and 1994. *Methylophaga sulfidovorans* was isolated from these mat samples (7).

Culture Media

Medium contained per liter: 15 or 25 g NaCl, 0.5 g $(NH_4)_2SO_4$, 0.33 g $CaCl_2.6H_2O$, 0.2 g KCl, 1 g $MgSO_4.7H_2O$, 0.02 g KH_2PO_4, 2 g Na_2CO_3, 1 mg $FeSO_4.7H_2O$, 1 ml trace solution, 1 ml vitamin solution. pH was set at 7.5 (± 0.3) with 1 N HCl. The trace element solution was as described by Widdel and Pfennig (28), except for the addition of 0.6 μM $Na_2SeO_4.5H_2O$ (final concentration). The vitamin solution contained per liter: biotin (20 mg), nicotinic acid (200 mg), thiamine (100 mg), *p*-aminobenzoic acid (100 mg), pantothenate (50 mg), pyridoxine.HCl (500 mg), riboflavine (10 mg) and vitamin B_{12} (10 mg). Medium used for purification of strains was supplemented with HEPES buffer (1mM).

Artificial sea water, used for suspension of sediment samples, contained 22.5 g l^{-1} NaCl, 24.6 g l^{-1} $MgSO_4.7H_2O$, 1.5 g l^{-1} KCl and 2.2 g l^{-1} $CaCl_2$. pH was set at 7.5.

Liquid medium containing DMS was kept in glass bottles, which were sealed with butyl rubber or teflon stoppers to avoid DMS loss.

The purity of the cultures was checked by streaking on Brain Heart Infusion plates supplemented with 1.5% NaCl.

Analytical Techniques

DMS from the headspace of the cultures was measured with a gas chromatograph with a sulfur-specific flame photometric detector (20), equipped with a Hayesep R column. Measurements of DMS were accurate (less than 5% variance) down to 0.5 μM in the gas phase. The equilibrium constant for DMS at room temperature was estimated at 13.5 (19).

Oxygen consumption rates of bacterial suspensions were measured with a polarographic Clark-type electrode in a temperature-controlled Biological Oxygen Monitor. Oxygen concentrations in the gas phase were determined by gas chromatography, using a molecular sieve packed column and hard wire detector.

Culture density was determined using a Total Organic Carbon analyzer (Tocamaster 815B). The biomass obtained from continuous cultures was analyzed for its carbon, hydrogen and nitrogen content with a Carlo Erba CHNS-O elemental analyzer. The formula for biomass composition was found to be $CH_{1.75}O_{0.46}N_{0.19}$ for *M. sulfidovorans* which shows that about 50% of the biomass is carbon. Biomass was also measured by dry weight determination.

produced (15). Marine DMS oxidizing bacteria therefore play a significant role in the regulation of fluxes from the oceans to the atmosphere, and thus in climate regulation processes based on DMS fluxes (1).

DMS degradation under anoxic conditions has been studied with several methanogenic bacteria from marine systems (12, 16, 17). It has been found that sulfate-reducing bacteria play a role in DMS oxidation in marine sediments (13), but so far only a freshwater-sulfate reducing bacteria able to use DMS as a substrate has been isolated (22).

Aerobic DMS degradation has only been described for a small number of marine bacteria (24, 27). A large part of the research on DMS oxidation in marine systems has concentrated on DMS transformations in salt marsh sediments and microbial mats (13, 14, 25, 30). The physiological characteristics of aerobic DMS-oxidizing bacteria must therefore be examined in greater detail, in order to provide understanding of the role of these bacteria in the oxidation of DMS. In the current study, microbial mats are used as model systems to describe aerobic DMS oxidation.

Microbial Communities in a Microbial Mat

A microbial mat is a laminated microbial ecosystem a few millimetres thick that develops on solid surfaces (23). The driving force behind this ecosystem is photosynthesis in the top layer. Photosynthesis is, in most cases, the only source of organic carbon in mats (2). A microbial mat can, therefore, be regarded as a semi-closed system, with only input of light energy, carbon dioxide and sulfate. Only a few groups of microorganisms with different metabolic activities are therefore theoretically necessary to explain the interactions and activities within such a mat. In our model, five functional groups of microorganisms are considered; oxygenic phototrophs, sulfide-oxidizing bacteria, aerobic heterotrophs, aerobic DMS oxidizing bacteria and sulfate-reducing bacteria. These groups form a commensal relationship in substrate production and consumption. Carbon dioxide is photosynthetically fixed. The organic carbon is then used by the heterotrophic community in the aerobic zone of the mat, and by the sulfate-reducing bacteria in the anaerobic zone of the mat. The sulfide produced by the sulfate-reducers is oxidized with oxygen (produced by the oxygenic phototrophs) by colorless sulfur bacteria in the aerobic zone of the mat. The top layer of the mat contains phototrophs that may contain DMSP (25) from which DMS is produced. A population of DMS-oxidizing bacteria will, therefore, probably be present in the top layer of the mat where most of the DMS is produced. As our interest lies in the role of these organisms in the control of DMS fluxes, the DMS-metabolizing bacteria represent a separate functional group of organisms in our model.

The metabolic processes in a microbial mat are strongly dependent on the day/night cycle, during which pH values, oxygen and sulfide concentrations can reach extreme values. The pH in the top of the microbial mat can rise to above 10 due to the depletion of bicarbonate, which is the natural buffer in marine systems (24). During the night, the pH falls to 7 or 8 because of carbon dioxide production from the dissimilatory processes of the microbial community, and diffusion of carbon dioxide and bicarbonate throughout the mat. The oxygen produced during photosynthesis during the day can result in supersaturation (2 to 3 times the air saturation value) of oxygen (500 μM O_2) at the *in situ* temperature (4). Concentrations of 500 μM H_2S can be found in microbial mats (26). During the dark period the oxygen may only diffuse slowly from the atmosphere into the mat, and not all of the sulfide will be oxidized. Sulfide can then diffuse upwards and may reach the surface resulting in hydrogen sulfide emissions from the mat.

DMS concentration profiles have been determined in microbial mat sediments, ranging from 250 $\mu mol\ l^{-1}$ at the top 5 mm to almost 0 at 25 mm depth in a specific sediment

DMS Consumption in Microbial Mat Sediment Samples

DMS oxidation rates in natural samples were determined in 100 ml serum bottles filled with 20 ml sediment (1 part) and artificial seawater (4 parts), leaving the larger part as headspace. Aerobic and anaerobic headspaces were created by flushing with air or N_2, respectively. The bottles were sealed with butyl rubber septa and teflon stoppers, in order to prevent inward oxygen diffusion and outward DMS diffusion. DMS was added to the samples (< 1 mM DMS) with a syringe from a stock solution (27 mM DMS). As the bottles were not shaken during incubation, it must be assumed that even with an air-flushed headspace, part of the slurry in the flask rapidly became anoxic. The total DMS removal was therefore the sum of the aerobic and anaerobic breakdown of DMS. When the headspace was flushed with nitrogen, only anaerobic DMS degradation could occur.

Most Probable Number (MPN) Counts of Aerobic DMS-Oxidizing Bacteria in Sediment Samples and Isolation Procedure

Dilution series were made from natural samples using bicarbonate buffered mineral medium, and incubated at 25°C. The tubes containing the highest positive dilution were used for enrichment and strain purification. Enrichments were carried out in the same liquid medium. The flasks were inoculated in a desiccator with DMS in the headspace at a concentration that would not exceed 1 mM DMS in the liquid phase. One enrichment culture was selected for further study on the basis of its DMS-oxidizing capacity. As this culture did not grow on agar plates with DMS in the headspace, the aerobic DMS-oxidizing bacterium was isolated using several dilutions to extinction. Culture purity was checked with BHI agar plates and microscopy, and confirmed when the DNA of the culture gave only one visible 16 S rDNA band after PCR amplification.

Physiology and Taxonomy of the Isolate

The substrate range of the isolate was tested in batch cultures at 25°C and 100 rpm. Aerobic growth was tested for the following substrates: DMS, methane, methanol, formate, thiosulfate, hydrogen sulfide, methylamine, dimethylsulfoxide, acrylate, acetate, ethanol, propanol, lactate, galactose, glucose and fructose. Furthermore, denitrification capacity with methanol as electrondonor and nitrate as electronacceptor was tested in a nitrogen flushed chemostat at 25°C.

To determine the kinetics of DMS utilization by the isolate, it was grown in a methanol-limited continuous culture under standard conditions: temperature = 27°C, pH = 7.6, oxygen tension = 50% air saturation and dilution rate = 0.05 h^{-1}. After a steady state on methanol had been reached, the medium supply to the continuous culture was stopped, and the aeration of the chemostat was changed to headspace recirculation, in order to prevent loss of DMS through the outgoing airflow. A pulse of DMS was added to the chemostat culture, resulting in a DMS concentration of 0.8 mM, and the rate of DMS consumption was followed by gas chromatography. The Michaelis–Menten parameters, v_{max} and k_s, were then determined in a Biological Oxygen Monitor and directly estimated from the rate of DMS disappearance in the chemostat.

Biomass yields on DMS were determined in shaking batch cultures, incubated at 25°C at 100 rpm. The flasks were sealed with butyl rubber stoppers to prevent DMS loss. DMS was added with a syringe and did not exceed 0.5 mM in order to prevent DMS toxicity and to prevent oxygen exhaustion. Biomass formation was measured by total organic carbon analysis.

For further identification of the isolate the full (1500 base pairs) 16 S rDNA sequence was determined and compared with known eubacterial sequences at the Deutsche Sammlung für Microorganismen.

Cultivation of *M. sulfidovorans* at Various pH Values, H_2S and O_2 Concentrations

The influence of pH and oxygen tension on the Michaelis–Menten parameters (the maximum oxidation rate v_{max} and the half saturation constant k_s) for DMS-oxidation by *M. sulfidovorans* was determined with chemostat grown cells. After a steady state under standard conditions on methanol had been reached a pulse of about 0.8 mM DMS was added to the chemostat culture in order to adapt the culture to DMS as a substrate. After the DMS had dissappeared from the chemostat (partly through the outgoing airflow and partly because of consumption), a particular variable (pH or oxygen tension) was changed. One hour later, DMS was added to the chemostat (now operated as a batch fermenter with a recirculating headspace) and its disappearance from the culture was followed in time.

To test the effect of hydrogen sulfide on the kinetics of DMS oxidation, *M. sulfidovorans* was cultivated in batch cultures in serum bottles sealed with butyl rubber or teflon septa. The serum bottles (75 ml) were filled with 20 ml mineral medium with $Na_2S.9H_2O$ to final concentrations ranging from 0 to 6 mM. The bottles were inoculated with about 4 mg l^{-1} biomass from a chemostat culture grown under standard conditions. DMS solution was added to give a concentration of 0.5 mM DMS, in order to avoid toxic and inhibitory effects and maintain an aerobic headspace during the experiment. The bottles were incubated at 25 °C. DMS removal rates were determined with gas chromatography. The rate of DMS removal was low, due to the small inoculum, to ensure that the medium remained oxic. The v_{max} was calculated from the initial DMS oxidation. The affinity constant was calculated from the slower DMS oxidation rate obtained with lower DMS concentrations.

During these experiments purity was carefully checked with BHI agar plates and microscopy.

Mathematical Modelling of Fluxes in a Mat

To investigate the interrelationships between microorganisms in a microbial mat a mathematical model has been developed. This model is used to design relevant experiments with *M. sulfidovorans* and to predict the fluxes of DMS to the atmosphere. The model is based on simple Michaelis Menten kinetics and diffusion processes through sediment.

In the model, five functional groups of microorganisms are considered (23). These groups of organisms are listed in table 1 in order of (vertical) appearance in the mat, with the most significant biological reactions carried out by each group (figure 1). It should be

Table 1. List of the functional groups of microorganisms with their metabolic reactions, used in the mathematical model

Functional group	Metabolic reaction*
Oxygenic phototrophs	$Light + H_2O + CO_2 \rightarrow CH_2O + O_2 + (CH_3)_2S$
Chemolithoautotrophs	$H_2S + 2O_2 \rightarrow H_2SO_4$
Methylotrophs	$(CH_3)_2S + 5O_2 \rightarrow 2CO_2 + H_2SO_4 + 2H_2O$
Heterotrophs	$CH_2O + O_2 \rightarrow CO_2 + H_2O$
Sulfate reducers	$H_2SO_4 + 2CH_2O \rightarrow H_2S + 2CO_2 + 2H_2O$

(*)The stoichiometric relations of the reactions are presented, except for the DMS production by the phototrophic bacteria. The DMS formation rate is assumed to be coupled to the average DMSP content of photosynthetic bacteria and the photosynthetic rate, as explained in detail in the text. The functional groups are listed in order of vertical appearance in the model (see also figure 1).

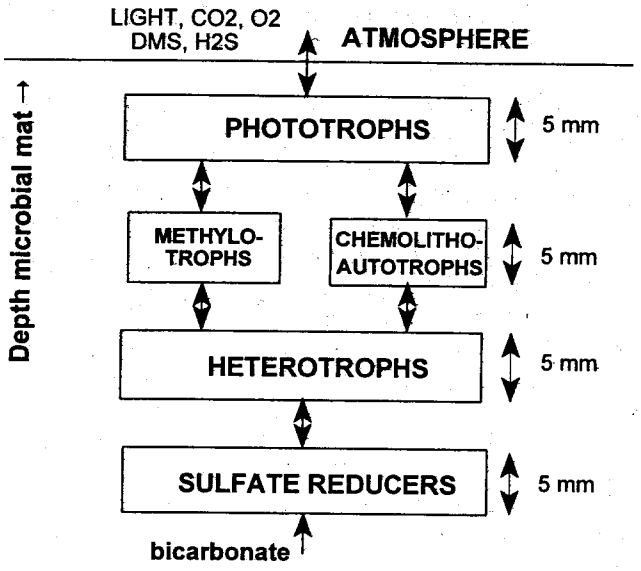

Figure 1. Schematic, simplified representation of a microbial mat. In the model, 5 groups of organisms are assumed to be in separate compartments, situated in different layers of the mat.

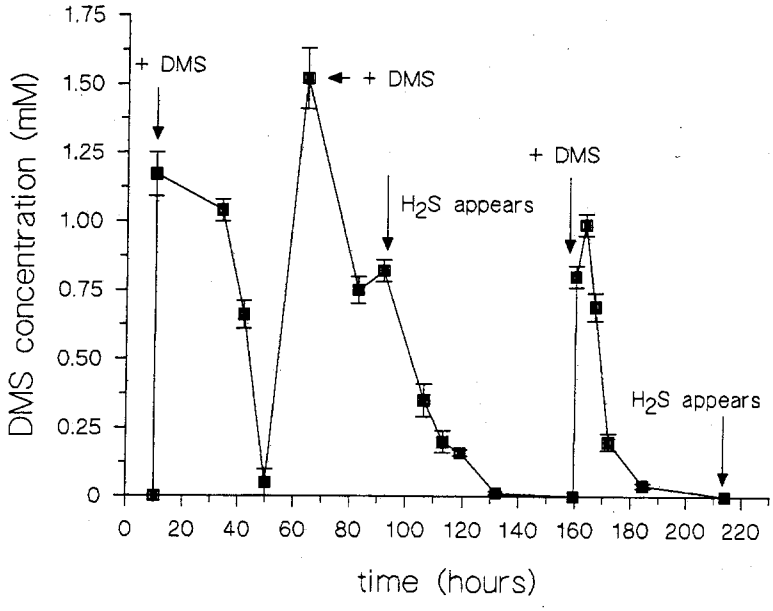

Figure 2. Aerobic and anaerobic DMS consumption in microbial mat sediment. The "aerobic" experiment had a gas phase containing air, but parts of the sediment in the bottle were anaerobic, due to low oxygen diffusion from the headspace to the sediment. This experiment therefore represents a sum of aerobic and anaerobic DMS conversion. In the anoxic experiment, DMS was added to the sediment after the headspace was flushed with N_2.

noted that the anoxygenic phototrophs, for simplicity reasons, have not been included in this model. Such a group could be accommodated in an additional compartment below the compartments of the colorless sulfur bacteria and the DMS-oxidizing bacteria, where the oxygen tension will be low. It can be seen from the five reactions shown in table 1, that all products can be consumed. The reactions take place in separate compartments (figure 1), in different layers, of the mat. These five metabolic reactions are the basic, and, when considering control of DMS production and metabolism, quantitatively the most important biological reactions. Metabolic activity is described with Michaelis–Menten kinetics (v_{max} and k_s). Transport to and from the compartments was described with simple transport equations based on diffusion. A general mass balance was set up in the model for each component in each compartment, and the changes of different components with time were described. Details of this model have been published elsewhere (6).

Calculations over 12-Hour Periods

The model was used to describe changes that take place in a microbial mat during a 12-hour light period. The driving force in the microbial mat is then photosynthesis, during which the pH in the mat will rise because of the depletion of the bicarbonate that buffers the mat. The effect of increasing pH on the Michaelis–Menten parameters for DMS oxidation was determined for *Methylophaga sulfidovorans,* and incorporated in the model. The influence of pH on DMS fluxes from the mat to the atmosphere was simulated using the model.

RESULTS

DMS Consumption in Microbial Mat Sediments. MPN Enumerations with Sediment Samples

Different samples from an intertidal coastal area were tested for their DMS- degrading capabilities under aerobic and anaerobic conditions. Most of the fresh samples (which had been stored in the dark) produced DMS. After 1 to 3 days, the production of DMS ceased, and DMS uptake rates under aerobic and anaerobic conditions could be determined, as shown for one sample in figure 2. As long as oxygen could be detected in the gas phase, the experiment was labelled "aerobic". When the oxygen was depleted (at the end of the aerobic experiment, or during the anaerobic experiments), hydrogen sulfide was detected. Most

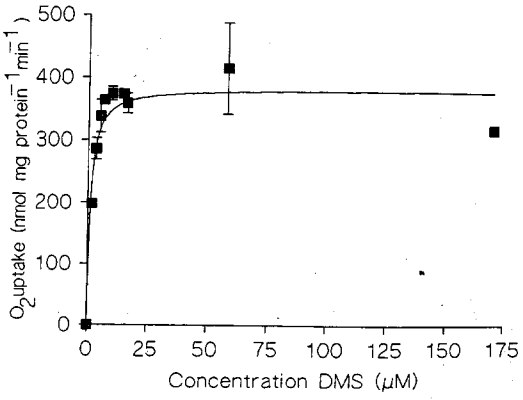

Figure 3. Michaelis Menten-type saturation curve for *Methylophaga sulfidovorans,* of the specific oxygen consumption rate versus the DMS concentration. From this curve the kinetic parameters v_{max} and k_s were estimated with a Line Weaver–Burk Plot: $v_{max} = 383 \pm 16$ nmol O_2 mg^{-1} biomass min^{-1} and $K_s = 1.0 \pm 0.32$ μmol l^{-1} DMS.

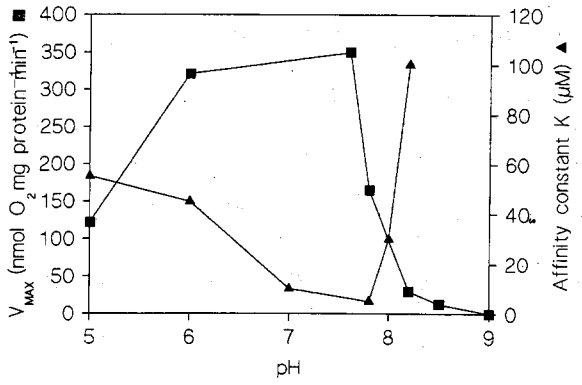

Figure 4. The effect of pH on the kinetic parameters (v_{max} and k_s) for DMS oxidation of *M. sulfidovorans*.

probably the hydrogen sulfide produced in the anoxic part of the sediment had been directly oxidized with oxygen by chemolitho (auto)trophic bacteria present in these sediments. The aerobic DMS-oxidation rate was estimated at 1 (± 0.5) μmol ml^{-1} d^{-1} for the different samples, by subtracting the DMS oxidation rate found under *anoxic* conditions from the rate found under "aerobic" conditions.

The same samples (to which no DMS was added) were used for MPN enumerations using a bicarbonate-buffered mineral medium with DMS in the headspace. The counts obtained ranged from 10^4 to 10^5 cells ml^{-1} sediment.

Physiology and Taxonomy of Isolate

As evident from its limited substrate range, the isolate appeared to be an obligately methylotrophic organism, capable of growth on DMS, methanol, methylamine and dimethylamine. Hydrogen sulfide could be used as additional energy source, but autotrophic growth on H_2S was not possible.

From experiments with cells grown in a methanol-limited chemostat to which DMS was added, the maximum oxidation rate of DMS (v_{max}) was found to be 400 (± 40) nmol O_2 min^{-1} mg^{-1} biomass, and the affinity constant (k_s) was 1.5 (± 0.5) μM DMS (figure 3).

The yield of the isolate was found to be 10.36 (± 1.44) g biomass mol^{-1} DMS (n =15). Standard tests for identification (e.g. API 20 and 20 NE) are partly based on substrate range. All of these tests were negative for the isolate, confirming its limited range of metabolic possibilities. Sequence analysis of the 16 S rRNA showed that the isolate has the highest similarity with the members of the gamma subclass of the Proteobacteria. The maximum similarity found was 86.1% with *Methylomonas methanica*. This indicates that the isolate belongs to a new genus. It has therefore provisionally been named *Methylophaga sulfidovorans*.

Cultivation of *M. sulfidovorans* at Various pH Values and O_2 Concentrations

The pH was found to significantly affect DMS oxidation by *M. sulfidovorans*. Different oxygen tensions had no significant effect on DMS oxidation in the chemostat (between 10 and 100% air saturation). Growth did not occur under anoxic conditions with nitrate as alternative electron acceptor.

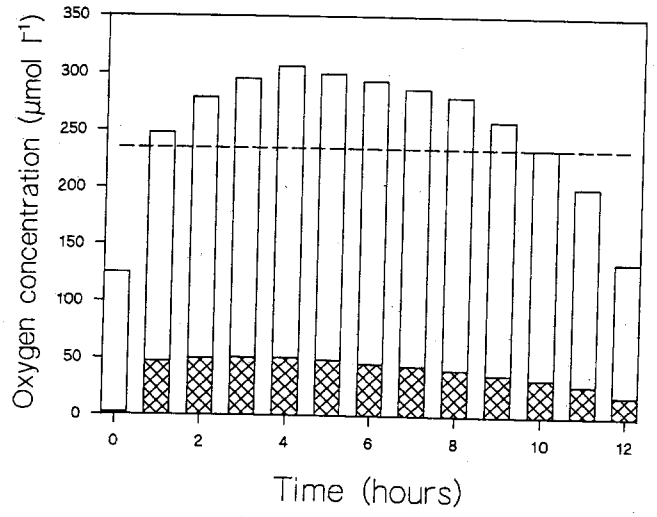

Figure 5. Simulated O_2 concentration in the phototrophic compartment (open bars) and in the deeper layers of the mat (closed bars) during a 12-hour light period.

The maximum oxidation rate of DMS rapidly decreased at pH-values higher than 8 (figure 4). For low pH values (4-7) the activity was relatively high. The pH value in marine microbial mats varies from 7 to 10 (24), and aerobic DMS metabolism would thus come to a halt at the high pH values, observed at the end of the light period. The affinity constant responded in a similar fashion, as shown in figure 4. The value steeply increased with higher pH values, and less steeply increased with low pH values.

In batch cultures in mineral medium with sulfide and DMS, diauxic use of sulfide and DMS was observed. Sulfide was first oxidized at a low rate (estimated at 100 (± 30) nmol H_2S mg^{-1} biomass min^{-1}), with no detectable biomass production, after which the DMS was oxidized. The v_{max} and k_s were not influenced by the concentration of sulfide (up to 6 mM H_2S) present in the batch cultures. The rates of conversion of DMS were independent of the amount of H_2S utilized before DMS consumption began.

In some of the batch and continuous culture experiments, a low level of a persistent satellite population, *Stenotrophomonas maltophilia* (identified by the Delft Culture Collection) was detected (< 5%). This infection could not use DMS (or the methanol, used as substrate in the continuous culture experiments) and did not affect the observed kinetic parameters for DMS oxidation.

Model Description of a Microbial Mat Ecosystem

The model was used to calculate the effects of microbial activity and transport processes on the concentration of compounds in the microbial compartments over a 12 hour light period. All parameters used in the model (e.g. the saturation constants, yields and maximum oxidation rate constants for the different organisms, buffer concentration, mass transfer coefficients, etc.) were tested for parameter sensitivity. From this analysis, it appeared that biological and physical constants significantly affected both reference variables. The most influential biological parameters were the k_s for DMS oxidation and the size of the DMS-oxidizing population. The most important physical constants were the mass

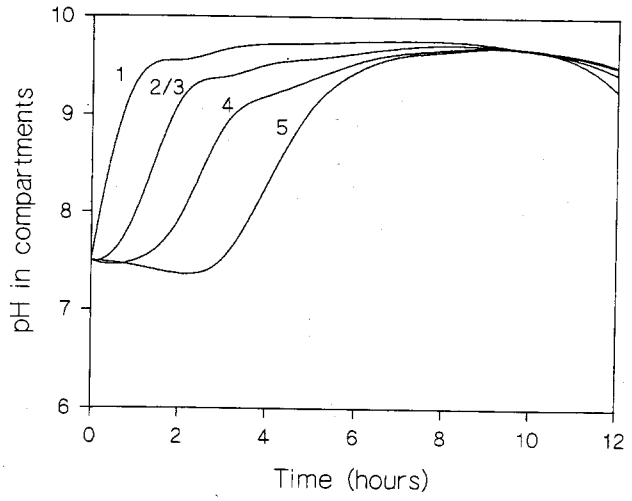

Figure 6. Simulation of pH changes in compartments 1 to 5 during a 12-hour light period. 1 to 5 represents compartments of the phototrophs, methylotrophs, chemolithoautotrophs, heterotrophs and the sulfate-reducing bacteria, respectively.

transfer coefficients of bicarbonate, H^+ and OH^- in the sediment. These values were obtained from the literature.

Figure 5 shows a simulation of the oxygen distribution through the mat for a light period of 12 hours. As can been seen, the oxygen concentration in the top layer of the mat rises above the air saturation of seawater (235 μmol O_2 l^{-1} at 25 °C). If the mass transfer of oxygen to the atmosphere was more effective (a higher mass transfer coefficient for oxygen), this would give a lower oxygen tension in the top layer of the mat.

The simulation of pH change in different layers of the mat (because of photosynthesis and settling, or evening out, of the pH) is shown in figure 6. It is assumed here that photosynthetic activity was maximum at neutral pH, with a linear decrease in activity for higher pH values (adjusted from (3)). The extent of the time delay before adjustment of the

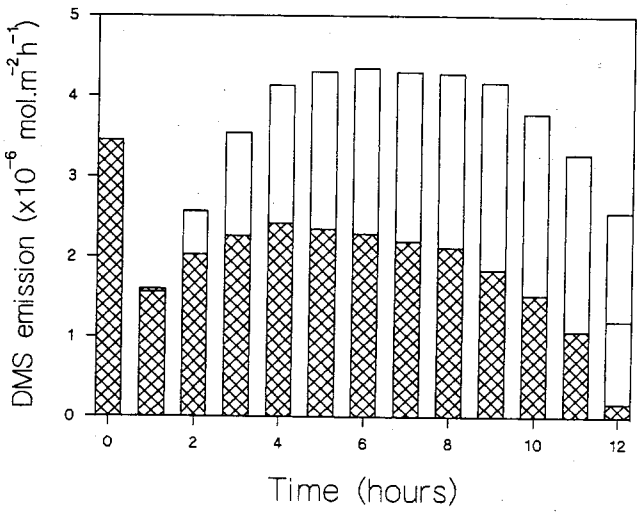

Figure 7. DMS emission from the phototrophic compartment during a 12-hour light period. The open bars represent the simulation results when the pH effect on DMS oxidation is incorporated. The closed bars represent the simulation results if this effect is neglected.

pH was mostly dependent on the value of the mass transfer coefficient, but also on the rate of photosynthesis, DMS oxidation (e.g. H_2SO_4 production), sulfate reduction, H_2S production etc. After 6 hours, the pH in the entire system reached the predicted value of 9 or higher.

The quantitative effect of a high pH value on the microbial activity of the DMS-oxidizing community was assumed to be similar to that of *M. sulfidovorans* (figure 4). A quantitative relation of the Michaelis–Menten parameters for DMS oxidation with pH was obtained and incorporated in the model.

Figure 7 shows the simulated DMS emission from the mat by diffusion of DMS from the photosynthetic compartment into the atmosphere. Simulations with and without the effect of pH on DMS-oxidation were made. Ignoring the increase in the pH gave relatively low DMS emission rates. The amount emitted was strongly related to the affinity (v_{max}/k_s) of the DMS oxidizing-community for DMS. If the effect of increasing pH on DMS oxidation was taken into account, the affinity for the substrate became very low. Emission was then higher, since at higher pH values (reached after 2-3 hours only (figure 6)) the microbial oxidation of DMS was negligible, leading to an increased DMS concentration in the compartments. Obviously, a higher concentration in the mat will give a higher emission rate.

DISCUSSION

DMS Oxidation in Sediments

Sediment samples from an intertidal region on the South-West coast of the Netherlands proved to be active in both DMS production and consumption. Figure 2 shows that aerobic and anaerobic consumption of DMS took place, and that a community of aerobic DMS oxidizing bacteria must therefore have been present. The aerobic DMS oxidation rate was estimated to be 1 μmol DMS g^{-1} d^{-1}. The value for anaerobic DMS removal agrees well with rates found by Kiene and Capone (14), who reported removal of about 100 μmoles DMS in 12 days by 25 ml sediment slurry. Recalculated for these experiments, this is about 0.33 μmol DMS ml^{-1} slurry d^{-1}.

The most probable number counts of aerobic DMS oxidizing bacteria in these sediment samples gave results that were similar to results obtained by Visscher et al. (24). They found that approximately 10^5 DMS-oxidizing cells cm^{-3} were present in a marine microbial mat on the Island of Texel (The Netherlands). Both of these counts were carried out with mineral medium, and clearly underestimate the aerobic DMS oxidizing bacteria in the sediment samples. If $5x10^4$ bacteria $gram^{-1}$ sediment are responsible for the removal of 1 μmol DMS in 24 hours, assuming that 1 bacterial cell weighs 10^{-12} g, and that the yield of bacteria on DMS is ± 10 g biomass mol^{-1} DMS, the growth rate on DMS would have to be ± 8.5 h^{-1} (a division time of 5 min). Growth rates of known DMS-oxidizing bacteria, (5) have had maximum specific growth rates (μ_{max}) of around 0.05 h^{-1}. This indicates that enumeration underestimates the aerobic DMS oxidizing community present in the sediment.

Physiology and Taxonomy of Isolate

The new DMS-metabolizing marine isolate has a 16 S rRNA sequence that differs by at least 14% from known sequences of eubacteria (DSM analysis, sequence was compared with 4000 different sequences). In view of its methylotrophic and sulfide-oxidizing properties this proteobacterium has been provisionally named *Methylophaga sulfidovorans*. Thus far, *Hyphomicrobium spp.* (18, 20, 29) and *Thiobacillus spp.* (9, 11) have generally been found to be responsible for aerobic DMS degradation in (fresh water) waste water systems. From marine systems, *Thiobacillus spp.* capable of aerobic DMS degradation have been

isolated(24, 27). *M. sulfidovorans* is an obligately methylotrophic bacterium that can degrade DMS aerobically. It represents a new group of bacteria capable of degrading DMS and methyl mercaptans. Kiene and Bates (15) have already concluded from inhibition experiments in sea water that mainly methylotrophic organisms (known to be inhibited by chloroform) are responsible for DMS degradation.

The DMS uptake characteristics of *M. sulfidovorans* have affinity constants and maximum growth rates comparable with other known DMS-utilizing bacteria. However, the biomass yield on DMS is comparatively low, about 10 g biomass mol^{-1} DMS. *Hyphomicrobium spp.* gave a yield of ± 18 g biomass mol^{-1} DMS, which is in the same range as the yield found for *Thiobacillus thioparus* T5.

Cultivation of *M. sulfidovorans* at Various pH Values, H_2S and O_2 Concentrations

The variation in the kinetics of DMS uptake as a function of pH indicated that at pH values above 8, the DMS-oxidizing capacity of *M. sulfidovorans* rapidly decreased. This has significance in microbial mats, since the pH during the day time may reach values above 10. This rapid decrease in DMS oxidation for pH values higher than 8 was also found in all natural samples tested.

The presence of relatively high concentrations of hydrogen sulfide and DMS in *M. sulfidovorans* cultures resulted in diauxic consumption of H_2S and DMS. Maximum hydrogen sulfide concentrations in mats vary from 0.1 to 0.5 mM H_2S (26), but are detected well below the oxic zone. At the interface of oxygen and hydrogen sulfide, the concentration of hydrogen sulfide is usually very low (< 50 μM) (26)), which makes simultaneous use of sulfide and DMS possible. *M. sulfidovorans* should therefore have its niche in a zone in the microbial mat, where during light periods, the H_2S concentration is sufficiently low.

Model Description of a Microbial Mat Ecosystem

The results obtained (figure 5) with the simulation experiment agreed well with reported observations of oxygen super-saturation during a light period in a microbial mat (4). Figure 5 indicates that a (stepwise) gradient from high oxygen tensions at the top to low oxygen concentrations at the bottom of the mat occur. The amount of oxygen in the different layers depends on the mass transfer of oxygen in the sediment, and the oxygen consumption rates of the colorless sulfur bacteria and the DMS-oxidizing bacteria.

The simulated rise in pH in the top layer (figure 6) was in accordance with experimental measurements of pH in the top layers of a mat (4). This rise can be explained by the depletion of bicarbonate by the phototrophs. The production of sulfuric acid by the colourless sulfur bacteria does not compensate for the rise in OH^- ions.

The simulated emission from the mat of 4 μmol DMS m^{-2} h^{-1}, fits with the observed maximum DMS emission rates of 335 μmol m^{-2} d^{-1} which were detected above a sediment from a brackish estuary (8). Assuming a constant emission of DMS during the day, this rate equals 14 μmol m^{-2} h^{-1} for the brackish estuary, which is in the same order of magnitude as predicted here. Furthermore, Jørgensen and Okholm–Hansen (8) found that DMS was emitted in the late afternoon. The model predicts this effect (figure 7), since pH rises in the mat during the day, which results in a decreased microbial activity for DMS removal.

The model presented here gives a simplistic view of a complex ecosystem. Relatively important sinks for hydrogen sulfide, including anoxygenic photosynthesis (23), were not taken into account. Furthermore, anoxygenic photosynthetic bacteria can use DMS as an electron donor which provides an additional sink for DMS under anaerobic conditions. As

DMS is produced in the top layer of the mat, the anaerobic conversions of DMS that would occur in the lower layers of such mats (14, 24) were not taken into account. It should be emphasized that this model design is of a very general nature, and can easily be extended with additional compartments without the need for new fundamental assumptions (6).

In spite of its simplicity, the model gives satisfactory predictions of, for example, oxygen distribution, pH-settling and DMS emissions. The model, together with the new isolate *M. sulfidovorans*, will help in quantitative understanding of processes in microbial mats, and will indicate the processes that must be examined in more detail in order to understand the regulation of the carbon and sulfur fluxes in a microbial mat.

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