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## Aerobic conversion of dimethyl sulfide and hydrogen sulfide by Methylophaga sulfidovorans: implications for modeling DMS conversion in a microbial mat

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#### Abstract

Methylophaga sulfidovorans is an obligately methylotrophic, DMS-oxidizing organism, isolated from microbial mat sediment. DMS and  $H_2S$ , both present in marine microbial mats, can be used as energy sources by this organism. In batch cultures of M. sulfidovorans, sequential  $H_2S$  and DMS utilization occurred. In energy-limited continuous cultures, with DMS, methanol and  $H_2S$  as substrates, mixotrophic growth of M. sulfidovorans was observed, showing that at low concentrations these substrates can be used simultaneously. Oxygen and  $H_2S$  uptake experiments showed that the critical concentration at which sulfide inhibition of DMS oxidation occurred was between 15 and 40  $\mu$ mol  $l^{-1}$ . Also in crude enrichments of DMS oxidizers a decrease of 50% in DMS-oxidizing capacity for about 200  $\mu$ mol  $l^{-1}$   $H_2S$  was observed. The new physiological data obtained with the pure cultures of M. sulfidovorans were incorporated in a compartment model of a microbial mat and gave improved predictions of DMS profiles and DMS emissions from the mat, both when phototrophic activity is present (day) and when it is absent (night).

Keywords: Microbial mat; Dimethyl sulfide oxidation; Hydrogen sulfide oxidation; Compartment model; Methylophaga sulfidovorans

### 1. Introduction

In marine microbial mats, different functional groups of microorganisms form a cooperative community [1]. The quantitatively most important functional groups are well-described, and consist of phototrophs, colorless sulfur bacteria, heterotrophs and sulfate-reducing bacteria. A quantitatively less important group is the population of bacteria that

convert organic sulfur compounds. The different groups of microorganisms produce and consume several sulfur compounds, of which  $H_2S$  and DMS are important representatives.  $H_2S$  is often produced in large amounts by sulfate-reducing bacteria. A sulfate-reducing activity of 10  $\mu$ mol ml<sup>-1</sup> day<sup>-1</sup> was measured in a *Microcoleus chthonoplastes*-dominated microbial mat [2]. These high production rates may result in sulfide concentrations as high as 200–500  $\mu$ mol  $1^{-1}$  in the lower, anoxic layers of a mat [3].  $H_2S$  is oxidized to sulfate by the (aerobic) colorless and (anoxygenic) phototrophic sulfur bacteria. The resulting concentration gradient leads to upward dif-

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fusion of H<sub>2</sub>S in the mat. DMS occurs in much smaller amounts than sulfide. In marine sediments, the main source of DMS is biological degradation of dimethyl sulfoniopropionate (DMSP) [4], an osmolyte present in many phototrophs [5]. The production rate of DMS during a bloom period of a marine microbial mat was estimated to be  $\pm 20$  nmol ml<sup>-1</sup> day<sup>-1</sup> (a factor 500 lower than sulfide production) and coupled to photosynthesis [6]. DMS degradation in sediments can take place under oxic and anoxic conditions [7-9]. Since it is formed in the upper, generally oxic, layer of the mat, it is assumed that DMS will be converted primarily by aerobic bacteria. As already found, H<sub>2</sub>S and DMS coexist near the oxic/anoxic interface of the mat [8]. H<sub>2</sub>S is considered to be an intermediate in the aerobic DMS metabolism of known DMS-oxidizing bacteria [9-13], and can be used as a supplementary energy source for growth. Little is known, however, of possible diauxic or inhibitory effects when DMS and H2S are supplied together, or whether the presence of H<sub>2</sub>S affects DMS degradation. These factors may have a considerable effect on the prediction of the release of DMS from a microbial mat into the atmosphere.

Using the highest dilution to extinction method, a specialist in aerobic DMS oxidation has been isolated: Methylophaga sulfidovorans [9]. This bacterium is an obligate methylotroph that can only use methanol, methylamine, dimethyl amine or DMS as energy and carbon sources. Hydrogen sulfide can be used as an energy source. Thiosulfate rather than sulfate is the end product of sulfide oxidation. As M. sulfidovorans is considered to be a representative bacterium for aerobic DMS oxidation in marine microbial mats, its kinetic properties could be used to predict the effect of H<sub>2</sub>S on DMS degradation in situ, using a previously published (mathematical) compartment model [9].

In this paper we report on the extension of the model with physiological data on H<sub>2</sub>S inhibition on DMS oxidation. The breakdown of DMS in the presence of hydrogen sulfide was studied in batch and continuous cultures of *M. sulfidovorans*. The inhibitory effect of H<sub>2</sub>S on DMS oxidation in the pure culture was compared with observations obtained with crude enrichments of fresh microbial mat samples. These results of the crude enrichment cultures

were used as a validation for the assumption that kinetic and physiological data of *M. sulfidovorans* can be used in simulations during a diurnal cycle.

#### 2. Materials and methods

#### 2.1. Bacterial culture and natural samples

Microbial mat sediment samples were obtained in May 1996 from the Oosterschelde, an estuarine intertidal region on the south-west coast of the Netherlands. *Methylophaga sulfidovorans* (LMD 95.210) had been previously isolated from microbial mat sediment samples obtained from the same site [9].

#### 2.2. Culture media

Mineral medium contained per liter: 15 g NaCl, 0.5 g (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 0.33 g CaCl<sub>2</sub>·6H<sub>2</sub>O, 0.2 g KCl, 1 g MgSO<sub>4</sub>·7H<sub>2</sub>O, 0.02 g KH<sub>2</sub>PO<sub>4</sub>, 2 g Na<sub>2</sub>CO<sub>3</sub>, 1 mg FeSO<sub>4</sub>·7H<sub>2</sub>O, 1 ml trace element solution [9], 1 ml vitamin solution [9]. pH was maintained at 7.5 (±0.3) with 1 N HCl. Liquid medium containing DMS was kept in glass bottles, which were sealed with butyl rubber stoppers to prevent DMS loss.

The purity of the cultures was routinely checked by streaking on brain heart infusion (BHI) plates supplemented with 1.5% NaCl.

### 2.3. Analytical techniques

DMS from the headspace of the cultures was measured with a gas chromatograph with a sulfur-specific, flame photometric detector, equipped with a Hayesep R column [9]. The detection limit was 0.5 ppm in the gas phase. This detection method also served for semi-quantitative  $H_2S$  measurements in serum bottles with *M. sulfidovorans* and with sediment samples. The detection limit was 100  $\mu$ mol  $l^{-1}$  in the liquid phase at pH = 7.

Oxygen consumption rates by bacterial suspensions were measured with a polarographic Clarktype electrode in a well-mixed Biological Oxygen Monitor at 28°C. The oxygen concentration in the headspace of serum bottles filled with sediment samples was measured with a Fisons Instruments (8000)

series) gas chromatograph equipped with a molecular sieve 5A packed column.

Thiosulfate was determined according to Sörbo [14]. Sulfide was determined by iodometric titration for sulfide concentrations higher than 1 mmol  $1^{-1}$  (detection limit 0.1 mmol  $1^{-1}$ ) [15], and by the methylene blue method for sulfide concentrations lower than 1 mmol  $1^{-1}$  (detection limit 1  $\mu$ mol  $1^{-1}$ ) [16].

Biomass was determined as organic carbon using total organic carbon analysis with a Tocamaster type 815-B. The carbon content of *M. sulfidovorans* was 50% (w) [9]. Biomass was also measured by dry weight determinations [9].

At the pH of the experiments, sulfide will be present as HS<sup>-</sup> and H<sub>2</sub>S. These two forms will be referred to as 'sulfide' or 'H<sub>2</sub>S'. Sulfide was always added to media as a solution of Na<sub>2</sub>S·8H<sub>2</sub>O.

#### 2.4. Cultivation of M. sulfidovorans

M. sulfidovorans was cultivated in batch and chemostat cultures. Batch cultures in mineral medium supplemented with 10 mmol l<sup>-1</sup> methanol were made at 25°C at a pH of 7.5 ± 0.5 in flasks on a rotary shaker at 100 rpm. Batch cultures with DMS and H<sub>2</sub>S were made in closed serum bottles with a butyl rubber septum. The bottles were filled with 20:80% medium:air. At the concentrations of DMS and H<sub>2</sub>S used, less than 30% of the available oxygen would be required for their complete oxidation. The cell suspension used for these batch experiments contained only  $\sim 4$  mg l<sup>-1</sup> (dry weight) to ensure low but detectable oxidation rates that would make measurements at different time intervals accurate. These bottles were kept stationary in the dark at 25°C at a pH of  $7.5 \pm 0.5$ . Fermentors for continuous cultivation were made of glass and polycarbonate, instead of the more conventional glass and stainless steel. This experimental setup was essential for the prevention of severe corrosion problems. Continuous cultures with mineral medium and growth-limiting methanol (10 mmol  $l^{-1}$ ) or with growth-limiting mixtures of methanol (10 mmol 1<sup>-1</sup>), sulfide (10 mmol 1<sup>-1</sup>) and dimethyl sulfide (4 mmol 1<sup>-1</sup>) as substrates were grown at 28°C, a pO<sub>2</sub> of 50% air saturation, pH 7.6 and a dilution rate of 0.03 h<sup>-1</sup>. For the cultures grown mixotrophically on methanol, sulfide and DMS, it was found that the

optical density (OD, 430 nm) was correlated linearly with the dry weight (DW) and the organic carbon analysis (TOC) of these cultures. The correlation was: DW (mg  $1^{-1}$ ) = 194×OD+14 (r = 0.96; n = 15).

# 2.5. Oxygen and H<sub>2</sub>S consumption by M. sulfidovorans with and without DMS

Cells used for oxygen and H<sub>2</sub>S uptake experiments were cultivated in continuous cultures with mineral medium with DMS (2-3 mmol l<sup>-1</sup>) as the sole substrate. H<sub>2</sub>S uptake was determined in vials filled with 5 ml cell suspension and sealed with butyl rubber septa. Cells were centrifuged  $(10000 \times g)$  and resuspended in 25:5 mmol l<sup>-1</sup> carbonate:HEPES buffer in 15 g  $l^{-1}$  NaCl. DMS (100  $\mu$ mol  $l^{-1}$ ) was added with a syringe. At time=0, 25 µmol 1<sup>-1</sup> Na<sub>2</sub>S·8H<sub>2</sub>O (pH = 8) was added. This low concentration of sulfide was chosen to prevent chemical oxidation (see Section 2.6). The disappearance rate of sulfide was then measured by stopping the reaction with zinc acetate (in acetic acid) solution at different time intervals. The oxygen uptake rate of cell suspension taken directly from the chemostat was measured in a biological oxygen monitor, using H2S concentrations ranging from 0 to 130 µmol l<sup>-1</sup>, with or without DMS (260  $\mu$ mol l<sup>-1</sup>).

# 2.6. Measurement of DMS oxidation capacity of crude enrichments at different H<sub>2</sub>S concentrations

Sediment samples were diluted 125-fold in mineral medium. The (diluted) slurry (30 ml) was kept stationary in the dark in 100 ml serum bottles, sealed with butyl rubber septa. In order to obtain a crude enrichment of DMS utilizing bacteria, the diluted sediment suspensions were first supplied with 0.5-1.0 mmol  $1^{-1}$  DMS. One sample was heated to 90°C for ~2 min. This sample showed no DMS disappearance, confirming its biological nature. After DMS had been consumed in the unheated bottles with an average rate (determined over the whole period of incubation) of  $\sim 5 \, \mu \text{mol } 1^{-1} \, \text{ml slurry}^{-1}$ day<sup>-1</sup>, the samples were aerated for 2 min. Carbonate buffer ( $\sim 10 \text{ mmol } l^{-1}$ ) was added and the pH was adjusted to 7.5 with 1 N HCl. DMS was added to the bottles ( $\sim 0.5$  mmol  $l^{-1}$ ) and within 24 h, DMS oxidation had resumed. Only after 24 h was Na<sub>2</sub>S (0–2 mmol l<sup>-1</sup>) added in order to ensure low oxygen tension in the medium. These samples were all incubated at 25°C. DMS removal was quantitatively determined with gas chromatography. H<sub>2</sub>S could only be determined qualitatively with gas chromatography, since the proportion of H<sub>2</sub>S in the gas phase was dependent on the changing pH as thiosulfate was produced from DMS and H<sub>2</sub>S.

The rate of chemical oxidation of sulfide in mineral medium was measured with a (biological) oxygen monitor. It was found that both for sulfide concentrations < 100  $\mu$ mol l<sup>-1</sup> and for pO<sub>2</sub> < 50% air saturation, chemical oxidation was insignificant (<0.1  $\mu$ mol O<sub>2</sub> l<sup>-1</sup> min<sup>-1</sup>). In the stationary batch cultures with crude enrichments or with pure cultures of *M. sulfidovorans* with ~0.5 mmol l<sup>-1</sup> DMS, the oxygen concentration, measured after 24 h of incubation, was sufficiently low to exclude significant chemical oxidation.

### 2.7. Compartment model of microbial mat

The compartment model for a microbial mat has been described in detail elsewhere [6], and is therefore only described in general terms here. The mathematical model is based on biological production and consumption of organic carbon, dimethyl sulfide, hydrogen sulfide and oxygen. These components are transported between the different compartments of the mat by diffusion. The pH in the layers of the mat is dominated by a bicarbonate buffer, acid

Table 1
List of the functional groups of micro-organisms with their metabolic reactions, used in the mathematical model

	·	
Functional group	Metabolic reaction	
Oxygenic phototrophs	Light+H <sub>2</sub> O+CO <sub>2</sub> →	
	$CH_2O+O_2+(CH_3)_2S^a$	
Chemolithoautotrophs	$H_2S+2O_2 \rightarrow H_2SO_4$	
Methylotrophs	$(CH_3)_2S+5O_2 \rightarrow 2CO_2+H_2SO_4+2H_2O$	
	$H_2S+2O_2 \rightarrow H_2SO_4^b$	
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 (a) 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 [6]. In the present model (b) the methylotrophs can convert both DMS and  $H_2S$ .

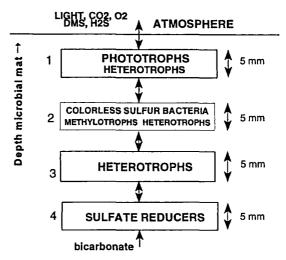


Fig. 1. Schematic representation of a microbial mat, adapted from [6]. Heterotrophs are situated in the first, second and third compartments, instead of only the third compartment.

and base production by the microorganisms, and diffusion into the different compartments. The model is used for simulation of concentration gradients and fluxes of these components. In the model, the five functional groups of microorganisms of the cooperative community are considered to be distributed over different compartments, carrying out the reactions listed in Table 1. These five groups are phototrophs, methylotrophs (i.e. DMS-oxidizing bacteria), colorless sulfur bacteria, heterotrophs and, in the lowest compartment, sulfate-reducing bacteria. The emphasis of the model was on DMS emissions and fluxes. The bacterium Methylophaga sulfidovorans (provisionally named Methylopila sulfovorans in a previous publication on the model) was (and is) used as a model organism for the DMS-oxidizing community. The metabolic activity of the microorganisms in the mat is driven by photosynthesis in the daylight which serves as the sole source of organic carbon (Table 1). The minimum amount of biomass required for stationary growth was used as initial biomass values for the simulations. For the colorless sulfur bacteria, methylotrophs and sulfate-reducing bacteria, these carbon-based concentrations were 10, 0.02 and 25 mmol C 1<sup>-1</sup>, respectively. 1 C-mol biomass was defined as  $CH_{1.8}O_{0.5}N_{0.2}\approx 24$  g [6]. The concentration of heterotrophs was estimated to be 15 mmol C l<sup>-1</sup>. The pH in the mat rises sharply during the light

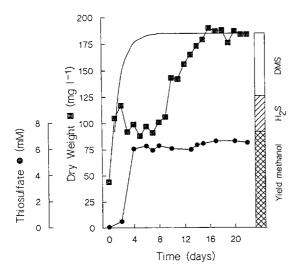


Fig. 2. Results of continuous cultivation of M. sulfidovorans grown in mineral medium with methanol  $(10\pm0.1 \text{ mmol } 1^{-1})$ , DMS  $(4.5\pm1 \text{ mmol } 1^{-1})$  and  $H_2S$   $(9\pm0.5 \text{ mmol } 1^{-1})$ . On day 0 and day 1 the dilution rates were 0.01 and 0.02  $h^{-1}$ , respectively. From day 3 onwards, the dilution rate was 0.03  $h^{-1}$ . The dry weight was 'constant' for the first 8 days, and was near the value of biomass that would be expected if only methanol was used as sole energy and carbon source. The product of DMS and  $H_2S$ , thiosulfate was present in the culture after 2 days. The expected biomass yields on the three substrates, for these conditions, are indicated by the three bars (mg dry weight  $1^{-1}$ ), based on the concentrations of DMS and sulfide in the medium supply. The theoretical growth curve is indicated by the drawn line.

period [6], because of the depletion of the natural carbonate buffer (i.e. due to carbon dioxide fixation), and slowly decreases during the night, due to diffusion of acid (H<sup>+</sup>) and base (OH<sup>-</sup>).

Four major adjustments to the earlier model were made in order to make the simulations in light and dark periods realistic. Firstly, the heterotrophic community, previously situated in the third layer of the mat, has now been situated in the first, second and third layers (Fig. 1), with initial biomass concentrations of 2.5, 2.5 and 5 mmol C  $1^{-1}$ , respectively. Compartment 2 now contains three functional groups: the methylotrophs, heterotrophs and colorless sulfur bacteria. The presence of oxygen-consuming heterotrophs proved essential in the model to allow the oxygen concentration in the lower parts of the mat to decrease to practically zero (i.e.  $< 0.5 \ \mu mol \ 1^{-1}$ ). Secondly, the specific growth rates for oxygen are now simulated according to a satura-

tion curve (Monod-like, with affinity constants of 1  $\mu$ mol l<sup>-1</sup> O<sub>2</sub> and maximum oxidation rates in the same range as the values for the substrate uptake kinetics). In the earlier version of the model a linear relation between the oxygen concentration and maximum specific uptake rate was assumed. Thirdly, pH dependence of substrate consumption rates of the colorless sulfur bacteria, the heterotrophs and the sulfate-reducing bacteria was modeled with a second-order function; the optimum pH was 7.5 and a decrease or increase of 2.5 pH units reduced these rates to zero. The pH dependence of the phototrophic activity and DMS conversion was already implemented in the first model. Finally, H<sub>2</sub>S oxidation by both the colorless sulfur bacteria and the methylotrophs in compartment 2 is now recognized (Fig. 1, Table 1), whereas in the previous model, sulfide was oxidized solely by the colorless sulfur bacteria. The affinity constant used for the colorless sulfur bacteria ( $K_s$ , sulfide) was 1  $\mu$ mol l<sup>-1</sup> sulfide [6]. For the methylotrophs, it was assumed to be identical to the affinity constant of Methylophaga sulfidovorans, i.e. 5  $\mu$ mol  $l^{-1}$  sulfide (unpublished results). The mathematical model was developed using the programming meta-language PSI, based on the language C [6] and is available on floppy disk for interested readers.

# 2.8. Oxygen consumption during dark periods (modeling considerations)

The simulations, reported earlier [9], were made for a 12 h light period. The simulations have now been run for 36 h, in 12 h light and dark periods, instead of only a 12 h light period. This necessitated a closer look into the metabolic activity of the functional groups in the dark, when there is no oxygen production. The sulfate-reducing bacteria produce H<sub>2</sub>S independently of the light or dark [1]. DMS is excreted by the phototrophs in the top layers of the mat. The oxygen shortage at night (= substrate excess) gives rise to the following two questions. (1) How is the available oxygen divided over the three functional groups, the heterotrophs, colorless sulfur bacteria and methylotrophs? (2) Which substrate, H<sub>2</sub>S or DMS, will be preferred under O<sub>2</sub> limitation by the methylotrophs? With respect to the first question, the affinities for oxygen of the different func-

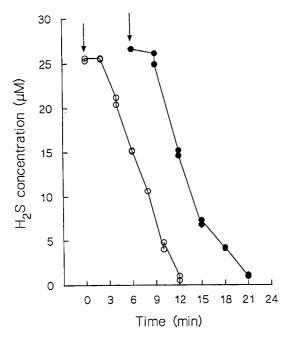


Fig. 3.  $H_2S$  uptake experiments in the presence ( $\bigcirc$ ) or absence ( $\bigcirc$ ) of 100  $\mu$ mol 1<sup>-1</sup> DMS. Washed cell suspension of 40 mg 1<sup>-1</sup> M. sulfidovorans from a DMS-limited culture was used. The activity of the cells in buffer was  $\sim$ 70 nmol  $H_2S$  min<sup>-1</sup> mg biomass<sup>-1</sup>. The arrow indicates the time when sulfide was added.

tional groups need to be known. In general, oxygen consumption rates are described by Monod kinetics (the same as for substrate uptake, see [6]). The oxygen uptake rate is described by the affinity constant for oxygen, the maximum oxygen uptake rate and the population size. In the model, the value for the affinity constant for oxygen is assumed to be similar for all three groups (1  $\mu$ mol l<sup>-1</sup> O<sub>2</sub>). The maximum oxygen uptake rates of the different functional groups are assumed to be in the same range as the maximum substrate uptake rates. In the present model, sulfide (major substrate) can also be oxidized in addition to DMS (minor substrate) by the methylotrophs. The ratio of the colorless sulfur bacteria: methylotrophs will be approximately 1:1. The actual concentration of both groups in the new model was 5 mmol  $1^{-1}$  C. The total heterotrophic population (compartments 1, 2 and 3) was 10 mmol  $1^{-1}$  C. It can be concluded that oxygen will be used by all three groups in the model as the parameters for the oxygen uptake rate are all of the same order of magnitude.

The second question was which substrate will be preferred by the methylotrophs under oxygen limitation (DMS and/or H<sub>2</sub>S). Factors that determine this are the affinity constant for the substrate, the maximum specific growth rate and the energy generation per electron (the  $K_s$ ,  $\mu_{\rm MAX}$  and  $\Delta G_e^{\circ}$ ). A low affinity constant, a high specific growth rate and a high energy (per electron) generation cause a substrate to be favored. The kinetic parameters of Methylophaga sulfidovorans for DMS are 1  $\mu$ mol 1<sup>-1</sup> ( $K_s$ ) and  $0.055 \text{ h}^{-1}$  ( $\mu_{MAX}$ ). For  $H_2S$  the affinity constant is 5  $\mu$ mol l<sup>-1</sup> ( $K_s$ ). The apparent maximum specific growth rate was estimated from the H2S consumption rate of  $\sim 112$  nmol mg biomass<sup>-1</sup> min<sup>-1</sup>, observed in batch cultures and the increase of ~4 g biomass mol<sup>-1</sup> H<sub>2</sub>S observed in continuous cultures with methanol and H<sub>2</sub>S [6]. This would translate into a maximum specific growth rate of 0.026 h<sup>-1</sup> ( $\mu_{MAX}$ ). The energy generation per electron for sulfide when it is combusted fully to sulfate is 21 kJ e-mol<sup>-1</sup>. For formaldehyde (intermediate in DMS oxidation) this is 45 kJ e-mol<sup>-1</sup> [17]. Considering these three factors it may, therefore, be expected that DMS conversion would be preferred over H<sub>2</sub>S conversion.

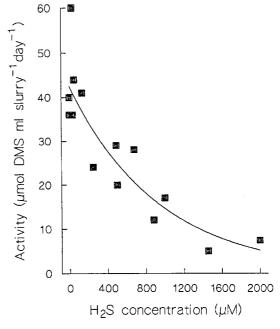


Fig. 4. DMS oxidation rate in slurries of microbial mat sediment, against different amounts of H<sub>2</sub>S added under oxic conditions.

#### 2.9. DMS production (modeling considerations)

It is not yet clear whether DMS production is directly coupled to photosynthesis. For DMS production and consumption during the day and night in the model, therefore, three different situations (A, B and C) were considered. In situation A, DMS production is fully coupled to photosynthesis (absent at night). In situation B, DMS production is independent of day or night, and the average of the production in situation A. In the third situation, DMS production is still independent of day or night (as in B), but sulfide inhibition (see Section 3) is taken into account.

### 3. Results

# 3.1. Batch growth of M. sulfidovorans on DMS and $H_2S$

Batch cultures with both substrates showed sequential use of  $H_2S$  and DMS (diauxy). Only after  $H_2S$  had been completely oxidized (i.e. < 100  $\mu$ mol  $I^{-1}$ ) was DMS consumed. This occurred at all  $H_2S$  concentrations tested (0.5–6 mmol  $I^{-1}$ ). The  $H_2S$  oxidation rate for all samples was  $112\pm13$  nmol min<sup>-1</sup> mg biomass<sup>-1</sup>. DMS was oxidized at a rate of  $100\pm20$  nmol min<sup>-1</sup> mg biomass<sup>-1</sup>.

# 3.2. Continuous cultivation of M. sulfidovorans on DMS, methanol and $H_2S$

Chemostat cultivation with a growth-limiting mixture of methanol, DMS and H<sub>2</sub>S in the medium supply eventually resulted in stable, multiple substrate-limited cultures (Fig. 2). On day 0, continuous cultivation of a culture pregrown in batch on methanol started with all three substrates in the medium supply at a dilution rate of 0.01 h<sup>-1</sup>. On day 1, the dilution rate was increased to 0.02 h<sup>-1</sup> and then to 0.03 h<sup>-1</sup> from the third day onwards. Over a period of 22 days, the optical density (430 nm), total organic carbon, dry weight, thiosulfate concentration in the cell suspension, sulfide concentration in the medium supply and cell suspension, and the DMS concentration in the cell suspension were determined at regular intervals. After addition of DMS and H<sub>2</sub>S,

the thiosulfate concentration rose and became constant after 2 days. The thiosulfate concentration was approximately 6 mmol  $l^{-1}$ . This agrees well with the expected 6–7 mmol  $l^{-1}$  for complete oxidation of DMS and  $H_2S$ . A large increase in the biomass concentration could be observed after 8 days (5.8 volume changes).

# 3.3. Oxygen and H<sub>2</sub>S consumption with and without DMS

The sulfide uptake experiments were carried out with cells from a continuous culture with DMS as a growth-limiting substrate (Fig. 3). The  $H_2S$  uptake rate was not influenced by the presence of DMS. This experiment was essential for the interpretation of the DMS-dependent oxygen uptake experiments with  $H_2S$ , i.e. that oxygen uptake at 'high' sulfide concentration could be (fully) attributed to the oxidation of sulfide. It was found that DMS-dependent oxygen uptake was fully inhibited for  $H_2S$  concentrations exceeding 40  $\mu$ mol  $1^{-1}$  (Table 2).

# 3.4. Effect of H<sub>2</sub>S concentration on DMS degradation in crude sediment enrichments

The relationship between the DMS disappearance rate in crude enrichments of DMS-consuming sedi-

Table 2 Results of oxygen uptake experiments with increasing  $H_2S$  amounts, with or without DMS

H <sub>2</sub> S (µmol l <sup>-1</sup> )	$qO_2$ (+DMS)	$qO_2$ (-DMS)	Ratio
0	216 ± 13	0	_
8	690 ± 51	$390 \pm 30$	1.8
15	$621 \pm 88$	$427 \pm 3$	1.5
28	$559 \pm 130$	$450 \pm 15$	1.2
36	382 ± 99	345 ± 5	1.1
40	$342 \pm 82$	$250 \pm 5$	1.4
64	257 ± 5	$245 \pm 24$	1.0
128	$240 \pm 13$	$250 \pm 5$	1.0

The cells were obtained from a DMS-limited continuous culture. Cell suspensions were washed in (iron-free) mineral medium. The concentration of M. sulfidovorans was  $30\pm2$  mg  $l^{-1}$ . The DMS concentration was 260  $\mu$ mol  $l^{-1}$ . All data points for the oxygen uptake with  $H_2S$  and DMS were measured in triplicate.  $H_2S$  measurements were done in duplicate.  $qO_2$  stands for specific oxygen uptake rate (nmol min<sup>-1</sup> mg biomass<sup>-1</sup>). The ratio of oxygen uptake is defined as  $qO_2$  (+DMS)/ $qO_2$  (-DMS). For rates > 1, DMS is (partly) oxidized next to full oxidation of sulfide.

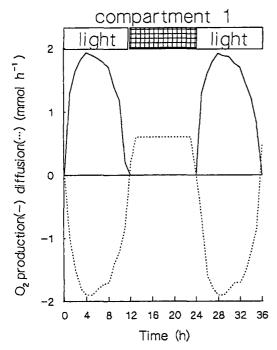


Fig. 5. Simulation of oxygen production (solid line) in and diffusion (dotted line) in (positive values) and out of (negative values) compartment 1 during a 3×12 h period (light/dark/light).

ment samples and the added H<sub>2</sub>S concentration are shown in Fig. 4. A maximum activity of 60 µmol DMS ml slurry $^{-1}$  day $^{-1}$  was found when the sulfide concentration was zero. The DMS oxidation rate was reduced to approximately half the maximum rate at sulfide concentrations of 200-500  $\mu$ mol l<sup>-1</sup>. In the liquid phase of the crude enrichments, anaerobic sites may have occurred. The inhibition value found is thus an average of sulfide inhibition of anaerobic and aerobic DMS oxidation. This experiment therefore serves merely as a validation that sulfide inhibition is a general phenomenon for DMS oxidation. Sufficient oxygen had been available for the complete oxidation of DMS since at the end of the experiment the samples containing 0, 490 and 1490  $\mu$ mol 1<sup>-1</sup> H<sub>2</sub>S still had oxygen tensions of 54, 50 and 64% in their headspaces, respectively.

### 3.5. Predictions from the compartment model

A simulation run of 36 h of alternating light, dark and light periods (12 h each) shows the oxygen pro-

duction within compartment 1 and diffusion of oxygen out of (negative values) and into (positive values) compartment 1 (Fig. 5). Oxygen is produced during photosynthesis, according to the following (simplified) reaction:  $H_2O+CO_2+light \rightarrow CH_2O+O_2$ . The oxygen produced diffuses partly to the upper boundary (10%) and partly to compartment 2 (about 90%), depending on the oxygen gradients between compartment 1 and its upper boundary (surface) and its lower boundary (compartment 2). During the dark period, when oxygen is not produced, oxygen diffuses into compartment 1 from the upper boundary. Only 25% of the oxygen that diffuses into compartment 2 during the day is now available for oxidation reactions of heterotrophs, colorless sulfur bacteria and methylotrophs. The oxygen concentration in compartment 2 in this simulation was  $\sim 15$  $\mu$ mol l<sup>-1</sup> O<sub>2</sub> in the light period and  $\sim 0.1 \mu$ mol l<sup>-1</sup> O<sub>2</sub> in the dark period. This implies that during the dark period oxygen, rather than sulfide (see also Fig. 7), was the rate-limiting compound in oxidation reactions in compartments 2 and 3.

Simulations were made of the pH and sulfide pro-

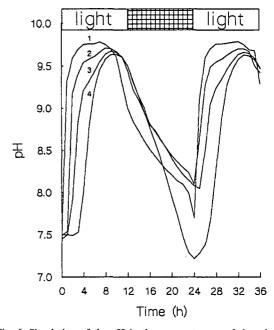


Fig. 6. Simulation of the pH in the compartments of the microbial mat during a  $3\times12$  h, alternating light/dark/light period. The numbered curves represent the four compartments (layers) of the model.

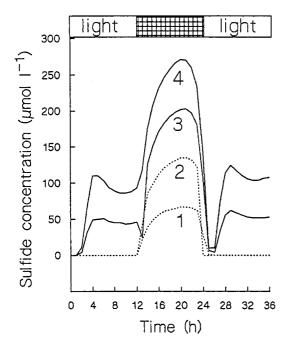


Fig. 7. Simulation of sulfide concentrations in all four compartments of the microbial mat during a  $3\times12$  h, alternating light/dark/light period.

files in the four compartments (Figs. 6 and 7). During the light period the pH increases due to photosynthesis. The decrease in pH in compartment 4 at the start of a light period is caused by sulfate reduction. This decrease is overruled within 2 h by the large increase in pH (i.e. OH- production) by photosynthesis. During the night, the pH in all layers of the mat drops to the initial value of  $\sim 7.5$ , due to diffusion. The sulfide concentration increases from almost zero to 75 and 125 µmol l<sup>-1</sup> in compartments 1 and 2, respectively, during the night. This can be explained by the 95% decrease in the specific growth rate of the colorless sulfur bacteria during oxygen limitation (i.e. the dark period). In compartments 3 and 4, H<sub>2</sub>S is not oxidized. The profiles here are fully determined by production and diffusion of H2S. During the night, the concentrations of H<sub>2</sub>S will increase in compartments 3 and 4, as the concentration gradient (the driving force of the diffusion process) is less than during the day. At the end of the dark period, the sulfide production in compartment 4 decreases, due to the depletion of organic carbon, which leads to a decrease in the sulfide concentrations in all compartments. When the light period starts again, organic carbon is again replenished by photosynthesis.

Three different situations of DMS production in a mat were considered (see Section 2.9). For all three cases DMS is initially completely oxidized, during a light period. However, already after a few hours, DMS oxidation stops due to the pH rise (shown in detail in [6]), and the concentration in compartment 2 increases. At night, in the first case (A, Fig. 8), the DMS concentration is nil because DMS is not produced. In the second case (B, Fig. 8), DMS conversion takes place after the pH in the mat has settled below 8.5 (after 15 h), during the dark period. This leads to a decrease in the DMS concentration. Full oxidation of DMS does not take place due to the limited availability of oxygen. In the third simulation (Fig. 8C) the presence of sulfide in compartment 2 (Fig. 7) results in the inhibition of DMS oxidation, and thus leads to a significant concentration of DMS during the dark period. The DMS concentration in compartment 2 is directly related to the emission of DMS from the sediment to the atmosphere as diffu-

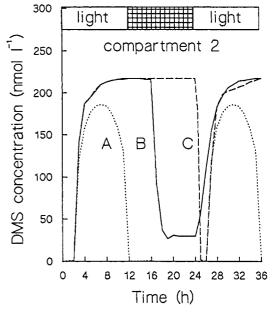


Fig. 8. Simulation of DMS concentration in compartment 2. A: DMS production is coupled to photosynthetic activity; B: without the inhibition effect of  $H_2S$  on DMS oxidation; C: with this effect.

sion is the only DMS-related process, taking place in compartment 1.

These simulations, based on experimental work with *Methylophaga sulfidovorans*, show that sulfide profiles in the sediment are strongly related to DMS emissions from the sediment.

#### 4. Discussion

The batch experiments with M. sulfidovorans show the sequential use of H2S and DMS indicating that above 100 µmol 1<sup>-1</sup> H<sub>2</sub>S diauxy occurs. In continuous culture, simultaneous oxidation of methanol, DMS and H<sub>2</sub>S occurs. This shows that at low concentrations of sulfide and DMS, mixed substrate utilization is possible. However, a delayed increase in biomass concentration occurred. DMS and H<sub>2</sub>S were both completely oxidized to thiosulfate and methanol was fully converted from the first day of the experiment. The biomass increase observed after six volume changes followed the theoretical growth curve assuming that all cells present would be capable of obtaining energy from the oxidation of DMS and H<sub>2</sub>S. This excluded the possibility of a strain mutation, since, in that case, the biomass increase would have been less steep. The morphology and physiological characteristics of the cells (oxygen uptake kinetics for DMS) remained the same. This phenomenon also occurred for mixed substrate continuous cultures on methanol and DMS and/or H<sub>2</sub>S under the standard environmental conditions used. Apparently, this is due to uncoupling of energy transduction and biomass growth. There is, as yet, no satisfactory explanation for this. The observation had no implications for the model described in this paper, as DMS was consumed without a lag time. The relevant observation here is that DMS and H<sub>2</sub>S can be used simultaneously at low H2S concentra-

In cultures of M. sulfidovorans, sulfide oxidation was not affected by the presence of DMS (Fig. 3). In contrast, DMS oxidation was inhibited by sulfide. DMS oxidation was 100% inhibited at sulfide > 40  $\mu$ mol 1<sup>-1</sup>. Experiments with 10 different crude enrichments indicated that this inhibition is apparently common, although at different levels. On average, 50% inhibition was found for  $H_2S$  concentrations

of  $\sim 200~\mu\text{mol l}^{-1}$ . The sulfide inhibition constant of the methyl mercaptan oxidase of *Hyphomicrobium* EG was 90  $\mu$ mol l<sup>-1</sup>, and thus in the same range [11] as observed in the sediment and the cultures of *M. sulfidovorans*. The concentration of 40  $\mu$ mol l<sup>-1</sup> sulfide, found to fully inhibit DMS oxidation by *M. sulfidovorans*, was used in the simulations.

In the 36 h simulation of the physical and biological processes in a mat, the fact that there is less oxygen available at night (Fig. 5) results in an increase in H<sub>2</sub>S in all layers of the mat, with the effect being most pronounced in compartments 1 and 2. This is of interest, since the concentration rises from almost zero to significant values (70-130 μmol l<sup>-1</sup>) that may inhibit DMS oxidation. An important outcome of the modeling was that this inhibition did indeed have a significant effect on the predicted DMS emissions from the mat. If continuous DMS production during day and night is considered, the DMS concentration remains high if H<sub>2</sub>S inhibition of DMS oxidation is taken into account. The DMS emission from the mat, corresponding with these 'high' DMS concentration, is  $\sim 5 \mu mol$  $m^{-2}$   $h^{-1}$ , whereas it is  $\sim 2 \mu mol m^{-2} h^{-1}$  if this inhibition is not taken into account. The inhibition of DMS oxidation found in crude enrichments occurs at higher sulfide concentrations, but would still result in an increased DMS emission.

There is little published information on day/night variations in sulfur emissions from mats that can be used for validation of the predictions made with this model. It has, however, been shown that H<sub>2</sub>S emissions from a Danish estuary were related to the day/ night cycle with detectable emissions during the night [18]. DMS emission varied strongly, with an average of 1 µmol m<sup>-2</sup> day<sup>-1</sup>, with peaks of 335 µmol m<sup>-2</sup> day<sup>-1</sup> (= 14  $\mu$ mol DMS m<sup>-2</sup> h<sup>-1</sup>), which is in the range of the predicted emissions. It was found that DMS emission took place during the day and the early night. There is currently no clear relationship between DMS production from DMSP and the diurnal cycle [19-21]. On the basis of the measured [18] and predicted emissions it might be hypothesized that DMS production will be coupled to photosynthetic activity and will, thus, be absent at night. This would lead to the observed absence of DMS emissions at night.

The model was developed to serve as an explana-

tory tool for the observations made from a marine microbial mat. This work tries to bridge the gap between physiology and modeling by collecting physiological data of a representative of the aerobic DMS-oxidizing bacteria and the implementation of that in a mathematical model. By using this kind of general data, extrapolations may be made about DMS emissions from a mat. In general, the model leads to a better insight into the relevant processes in an ecosystem and additionally may serve as a helpful tool in formulating relevant (experimental) research questions. Unfortunately, only a limited set of data (kinetics, physiology) is presently available, and the assumptions that have to be made in the model are considerable. The further development of this kind of model (for any ecosystem) will, therefore, be dependent on the ample availability of kinetic and physiological data of representatives of the different functional groups in the ecosystem.

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