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Competition for Dimethyl Sulfide and Hydrogen Sulfide by *Methylophaga sulfidovorans* and *Thiobacillus thioparus* T5 in Continuous Cultures

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Pure and mixed cultures of *Methylophaga sulfidovorans* and *Thiobacillus thioparus* T5 were grown in continuous cultures on either dimethyl sulfide, dimethyl sulfide and H_2S, or H_2S and methanol. In pure cultures, *M. sulfidovorans* showed a lower affinity for sulfide than *T. thioparus* T5. Mixed cultures, grown on dimethyl sulfide, showed coexistence of both species. *M. sulfidovorans* fully converted dimethyl sulfide to thiosulfate, which was subsequently further oxidized to sulfate by *T. thioparus* T5. Mixed cultures supplied with sulfide and methanol showed that nearly all the sulfide was used by *T. thioparus* T5, as expected on the basis of the affinities for sulfide. The sulfide in mixed cultures supplied with dimethyl sulfide and H_2S, however, was used by both bacteria. This result may be explained by the fact that the H_2S-oxidizing capacity of *M. sulfidovorans* remains fully induced by intracellular H_2S originating from dimethyl sulfide metabolism.

Hydrogen sulfide (H_2S) and dimethyl sulfide (DMS) are two compounds generally found in marine sediments covered by microbial mats. A microbial mat can be characterized as a laminated ecosystem with only a few functional groups of microorganisms that cooperate to form a stable ecosystem (8, 10). These groups are oxygenic and anoxygenic phototrophs, colorless sulfur bacteria, aerobic and anaerobic heterotrophs, and sulfate-reducing bacteria and the bacteria that convert DMS. H_2S is produced by sulfate-reducing bacteria using organic material (or degradation products thereof) produced in photosynthesis. It is reoxidized by anoxygenic phototrophs and colorless sulfur bacteria. DMS is produced as a degradation product of dimethylsulfoniopropionate, a presumed osmolyte, present in some phototrophs (4). DMS is not a quantitatively important compound in microbial mats, but it is of interest as microbial mat sediments and estuarine sediments are important sources of DMS emission into the atmosphere. DMS, in the atmosphere, influences the radiation balance. Enhanced DMS emissions might lead to a cooling effect on the climate (1). Emissions of DMS from a microbial mat were determined by the DMS concentration in the upper layer of the mat, and hence it is important to understand the factors controlling the microbial breakdown of DMS.

DMS in microbial mats might be metabolized by different functional groups. *Thiobacillus thioparus* T5 and *Methylophaga sulfidovorans* are two isolates from microbial mat sediment that belong to different functional groups but which have a partly similar substrate range. *T. thioparus* T5 was isolated with thiosulfate as the sole substrate (11). It is a facultative chemolithoautotrophic bacterium capable of fast growth on thiosulfate or sulfide. It has also been reported to oxidize DMS to sulfate and carbon dioxide. However, its rate of DMS oxidation is only marginal as its affinity for DMS is low. *T. thioparus* T5 may be considered a representative of the functional group of the colorless sulfur bacteria. *M. sulfidovorans* was isolated from marine sediment on DMS from a 10^5 dilution (2). This obligate methylotroph can grow not only on DMS (maximum specific growth rate [μ_{max}] = $\pm 0.08\ h^{-1}$) but also on methanol ($\mu_{max} = 0.3\ h^{-1}$) by using the ribulose monophosphate route for formaldehyde fixation. Sulfide can be used as a supplementary energy source during growth on C_1 compounds. The reduced sulfur in both DMS and H_2S can be converted stoichiometrically to thiosulfate. *M. sulfidovorans* may be considered a representative of methylotrophs in microbial mats. The relevant reactions performed by *M. sulfidovorans* and *T. thioparus* T5 are summarized in Table 1.

As representatives of both groups of bacteria can be expected to coexist in microbial mats, competition for either or both DMS and H_2S may occur. In marine sediments, *Thiobacillus* spp. are numerous (up to $2 \times 10^9\ ml^{-1}$ [11]), as sulfide is a major substrate. Counts of DMS oxidizers from similar sediment were about $10^5\ ml^{-1}$ (11). It can be concluded that only a minor fraction of the thiobacilli counted were capable of DMS oxidation, indicating that other groups of bacteria may also be involved in DMS oxidation in this environment. In this study, competition for DMS and the sulfide formed as an intermediate in DMS oxidation and for externally supplied sulfide was examined with *T. thioparus* T5 and *M. sulfidovorans* to obtain insight into the respective roles of these functional groups in the turnover of sulfur compounds in their natural environment. To this end, continuous culture experiments with DMS, methanol-H_2S, and DMS-H_2S were carried out with both pure and mixed cultures.

MATERIALS AND METHODS

Bacterial cultures. *M. sulfidovorans* (lmd 95.210) was isolated from microbial mat sediment in 1994 (2). *T. thioparus* T5 was isolated from microbial mat sediment in 1991 (11) and was a generous gift from F. van den Ende of the University of Groningen, Groningen, The Netherlands.

Culture medium. The medium used contained the following per liter: 15 g of NaCl, 0.5 g of $(NH_4)_2SO_4$, 0.33 g of $CaCl_2 \cdot 6H_2O$, 0.2 g of KCl, 1 g of $MgSO_4 \cdot 7H_2O$, 0.02 g of KH_2PO_4, 2 g of Na_2CO_3, 1 mg of $FeSO_4 \cdot 7H_2O$, 1 ml of trace element solution, and 1 ml of vitamin solution. pH was set at 7.5 (±0.3) with 1 N HCl. The trace element solution and vitamin solution were described earlier (2). DMS, sulfide, thiosulfate, and methanol were added to the medium as required. Liquid medium containing DMS was kept in glass bottles sealed with butyl rubber or teflon stoppers to avoid DMS loss. Sulfide was always added to

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TABLE 1. Stoichiometry of dissimilatory reactions by *M. sulfidovorans* (2) and *T. thioparus* T5 (11) on dimethyl sulfide, sulfide, thiosulfate, and methanol

Organism	Dissimilatory reaction
M. sulfidovorans	$(CH_3)_2S + 4O_2 \rightarrow 2CO_2 + H^+ + 1/2S_2O_3^{2-} + 2\ 1/2H_2O$
	$H_2S + O_2 \rightarrow 1/2H_2S_2O_3 + 1/2H_2O$
	$CH_3OH + 11/2O_2 \rightarrow CO_2 + 2H_2O$
T. thioparus T5	$(CH_3)_2S\ 5O_2 \rightarrow 2CO_2 + H_2SO_4 + 2H_2O$
	$H_2S + 2O_2 \rightarrow H_2SO_4$
	$H_2S_2O_3 + 2O_2 + H_2O \rightarrow 2H_2SO_4$

the medium as a solution of $Na_2S \cdot 8H_2O$. At the pH used for the experiments, sulfide is present as HS^- and H_2S. These two forms are both referred to herein as sulfide or H_2S. Culture purity was checked with 2% brain heart infusion plates.

Analytical techniques. DMS from the headspace of the cultures was measured with both a gas chromatograph equipped with a Hayesep R column and with a sulfur-specific, flame photometric detector (2). Thiosulfate concentrations were determined according to the method described by Sörbo (6). Sulfide concentrations were determined by iodometric titration for concentrations higher than 1 mmol liter^{-1} (5) and by the methylene blue method for concentrations lower than 1 mmol liter^{-1} (7). Biomass was measured by dry weight determination. Although considerable scatter in dry weight determination was observed (due to the low biomass concentration), the increase or decrease in biomass concentration was confirmed by optical density measurements at 430 nm.

Viable counts. Viable cell numbers of *T. thioparus* T5 were determined in samples from chemostat cultures of *T. thioparus* T5 and from mixed cultures of *T. thioparus* T5 and *M. sulfidovorans*. A 10^4 dilution of the culture was made in mineral medium (pH = 7.5), and 10 μl was streaked on 2% agar plates in mineral medium with 10 mmol of thiosulfate liter^{-1}. After 7 days, the colonies on the plates (±100 to 400) were counted. Viability was 75% ± 15%.

Cultivation of *M. sulfidovorans* and *T. thioparus* T5. Batch cultures with mineral medium supplemented with 10 mmol of methanol liter^{-1} (*M. sulfidovorans*) or 10 mmol of sodium thiosulfate liter^{-1} (*T. thioparus* T5), used for inoculation of continuous cultures, were grown at 25°C at a pH of 7.5 ± 0.5 in shake flasks in a rotary shaker at 100 rpm. Batch cultures with mineral medium and DMS were made in 75-ml serum bottles sealed with butyl rubber stoppers. A total of 80% of the volume was kept headspace to ensure that sufficient oxygen was available for complete DMS oxidation. DMS (0.5 to 1.0 mmol liter^{-1}) was added with a syringe, and the breakdown of DMS was monitored by gas chromatography.

Continuous cultures were grown in a temperature-, oxygen-, and pH-controlled polycarbonate and glass fermentor in mineral medium at 27°C, pO_2 of 50%, pH of 7.6, a dilution rate of 0.03 h^{-1}, and an oxygen tension of 50% air saturation (9).

Mixed cultures on DMS and DMS-H_2S were obtained by adding 500 ml of *T. thioparus* T5 grown in continuous culture on 10 mmol of thiosulfate liter^{-1} to a fermentor with a 500-ml cell suspension of *M. sulfidovorans* grown with 3 mmol of DMS liter^{-1}. After the cultures were mixed, the dilution rate was decreased to 0.01 h^{-1}. After 48 h, the dilution rate was adjusted to 0.03 h^{-1}. After 3 volume changes, a constant dry weight and optical density of the culture were observed. H_2S (2.5 mmol liter^{-1}) was added to the medium after 6 volume changes, and a new steady state (i.e., constant dry weight and constant optical density) was obtained. A similar procedure was followed for the mixed cultures on methanol and H_2S.

Maximum specific oxygen uptake. Oxygen uptake rates of cell suspensions were determined in a 5-ml vessel at 28°C, equipped with a polarographic oxygen electrode. Cell suspensions were obtained from steady-state continuous cultures, and the maximum oxygen uptake rate ($V_{O,max}$ [micromoles of O_2 liter^{-1} min^{-1}]) was tested with methanol (0.5 mmol liter^{-1}), thiosulfate (0.5 mmol liter^{-1}), DMS (0.25 mmol liter^{-1}), and sulfide (50 μmol liter^{-1}). As a first approximation it was assumed that all of the oxygen consumed was used for dissimilatory substrate conversion. In that case the $V_{O,max}$ is stoichiometrically related to the $V_{S,max}$ (maximum substrate uptake rate) as follows: $V_O = V_{S,max} \cdot \alpha$, where α equals the moles of oxygen needed for the oxidation of 1 mole of substrate (see Table 1).

Sulfide affinity. The affinity for sulfide was tested for both bacteria and served as a predictive tool of the fate of sulfide in mixed cultures. It is customary to define the affinity for one substrate as the slope of the Monod curve when the substrate concentration approaches zero, i.e., $\mu_{max} \cdot K_s^{-1}$. However, in the presence of more than one substrate (as in these experiments) the biomass of the culture increases and the description of the affinity must include the biomass yield (on both substrates). Substrate affinity is, thus, defined by $V_{max} \cdot C_x^{-1} \cdot K_s^{-1}$ ($= q_{max} \cdot K_s^{-1} = \mu_{max} \cdot Y_{sx}^{-1} \cdot K_s^{-1}$) in which C_x represents the biomass concentration (milligrams of biomass liter^{-1}), K_s is the affinity constant (micromoles of substrate liter^{-1}), q_{max} is the maximum specific substrate uptake rate (millimoles of substrate milligram of biomass^{-1} h^{-1}), μ_{max} is the maximum specific growth rate (h^{-1}), and Y_{sx} is the biomass yield on substrate (milligrams of biomass millimoles of substrate^{-1}]. For growth on two substrates μ_{max} and Y_{sx} do not have the conventional physiological meaning, and these parameters were not used in that case.

Distinction between *M. sulfidovorans* and *T. thioparus* T5 in mixed cultures. In order to study mixed cultures of *T. thioparus* T5 and *M. sulfidovorans* the composition of a mixed culture needs to be quantified. The methods for doing so are limited by the fact that *M. sulfidovorans* is not capable of growth on plates (2). Counts on agar plates with thiosulfate (see "Analytical techniques" above) were used to determine the number of *T. thioparus* T5. Total microscopic counts (of both species in the mixed culture), in combination with the plate counts, were too inaccurate for quantitative determination of the number of both bacteria in a mixed culture. To accomplish a clear distinction, the maximum substrate uptake rate (V_{max}) (moles of substrate liter^{-1} h^{-1}) for species-specific substrates (i.e., compounds that can only be converted by either one of the bacteria) was used. In the described experiments, thiosulfate and DMS are the species-specific substrates (see Results). The V_{max} values on species-specific substrates and their relations to substrate supply rates (obtained in pure cultures) serve, then, as a calibration for the V_{max} values (and the corresponding biomass) observed in the mixed culture.

RESULTS

Continuous cultures with *M. sulfidovorans* on DMS and methanol-H_2S. *M. sulfidovorans* was grown to steady state in DMS-limited continuous culture at a dilution rate of 0.03 h^{-1} with an increasing DMS concentration in the influent (1.1 to 3.0 mmol liter^{-1}). The steady-state biomass increased linearly with the increasing DMS concentration. The observed biomass yield was 13 ± 2 g of biomass mol^{-1} of DMS. Unexpectedly, the $V_{O,max}$ for DMS of the culture did not increase but stayed relatively constant at 6.5 ± 2.5 μmol of O_2 liter^{-1} min^{-1}. This corresponds to a DMS consumption rate of 1.6 ± 0.6 μmol liter^{-1} min^{-1}. The affinity constant, K_s, determined for a continuous cultivation with 3 mmol of DMS liter^{-1} in the medium, was 0.76 ± 0.15 μmol of DMS liter^{-1}. Sulfide and methanol could also be oxidized by cells cultivated on DMS, as described earlier (2). The maximum oxygen uptake rates for sulfide and DMS were similar. The maximum specific growth rate for DMS was roughly estimated from a $V_{DMS,max}$ of 1.6 ± 0.6 μmol liter^{-1} min^{-1}, determined with 1 mmol of DMS liter^{-1} in the medium. On the basis of these data a μ_{max} on DMS of 0.08 ± 0.03 h^{-1} was calculated (see Materials and Methods).

The $V_{O,max}$s for DMS and sulfide of *M. sulfidovorans*, cultivated in continuous cultures on methanol and sulfide, showed a roughly linear increase with an increasing sulfide concentration in the medium (Table 2). The growth yield on methanol was 9 ± 1 g of biomass mol^{-1} of methanol (2). H_2S was used as a supplementary energy source and yielded on additional biomass of 2.8 ± 0.4 g mol^{-1} of H_2S. The K_ss were 2.4 ± 0.4 μmol liter^{-1} for methanol and 4 ± 2 μmol liter^{-1} for H_2S.

Continuous and batch cultures of *T. thioparus* T5 on thiosulfate, thiosulfate-DMS, and sulfide. The maximum oxygen uptake rates of *T. thioparus* T5, cultivated in continuous cul-

TABLE 2. Results of continuous culture experiments with *M. sulfidovorans* grown on methanol (M) and sulfide at $D = 0.03$ h^{-1}[a]

Substrate (methanol/sulfide [mM/mM])	Product (mM)	$V_{O,max}$ (μmol of O_2 liter^{-1} min^{-1})		
		M	DMS	Sulfide
10/1.2 ± 0.1	0.9 ± 0.1	13.1 ± 0.5	1.0 ± 0.2	1.3 ± 0.3
10/2.7 ± 0.1	1.2 ± 0.1	12.3	3.4	4.7
10/5.4 ± 0.2	2.6 ± 0.1	12.4 ± 0.1	5.4 ± 0.4	5.7 ± 0.4
10/7.5 ± 0.3	3.4 ± 0.2	12.0 ± 0.1	8.8 ± 0.7	9.8 ± 0.9
10/9.7 ± 0.3	4.8 ± 0.1	12.0 ± 1.0	11.1 ± 1.1	9.7 ± 0.7

[a] The thiosulfate concentration in the fermentor and the maximum specific oxygen uptake rates on methanol, DMS, and sulfide were determined in steady state in triplicate. Sulfide in the medium supply was measured at least in triplicate. Values are means ± standard deviation.

TABLE 3. Results of continuous culture experiments with *T. thioparus* T5 grown on thiosulfate at $D = 0.03\ h^{-1}$[a]

Concn of thiosulfate substrate (mM)	$V_{O,max}$ for $S_2O_3^{2-}$ (μmol of O_2 liter^{-1} min^{-1})	Cell counts (10^8 ml^{-1})
2.1 ± 0.1	13.8 ± 0.3	
2.6 ± 0.1	16.4 ± 2.0	0.79 ± 0.07
4.8 ± 0.1	25.8 ± 1.6	1.28 ± 0.13
8.5	86.0 ± 3.3	2.24 ± 0.22
10.4 ± 1.3	160.0 ± 4.6[b]	3.86 ± 0.30

[a] The maximum oxygen uptake rates on thiosulfate were determined in steady state in triplicate. Thiosulfate in the medium supply and cell counts on agar plates were measured at least in triplicate. Values are means ± standard deviations.

[b] $V_{O,max}$ values for sulfide equaled those for thiosulfate. This value was not used for linear regression.

TABLE 5. Results of mixed cultures of *M. sulfidovorans* and *T. thioparus* T5 grown on different mixtures of DMS, H_2S, and methanol (M) as substrates at $D = 0.04\ h^{-1}$[a]

Expt	Substrate concn (mM)			$V_{O,max}$ (μmol of O_2 liter^{-1} min^{-1})				Cell counts (10^8 ml^{-1})
	DMS	H_2S	M	DMS	S^{2-}	M	$S_2O_3^{2-}$	
1	3	0	0	9.7 ± 0.3	18 ± 5		14 ± 0.7	0.5 ± 0.1
2	3	2.5	0	8.3 ± 0.8	23 ± 1		21.6 ± 0.5	0.6 ± 0.1
3	0	9.3	10	1.3	81 ± 2	10.3 ± 0.3	57 ± 3	2.8 ± 0.5

[a] The maximum specific oxygen uptake rates on DMS, sulfide, and thiosulfate were determined in steady state in triplicate. Sulfide in the medium supply and cell counts on agar plates were measured at least in triplicate. Values are means ± standard deviations.

tures with different concentrations of thiosulfate (Table 3) or sulfide (Table 4), increased with an increase of the concentration of these compounds in the medium (i.e., the maximum specific oxygen uptake rate per unit of biomass remained approximately constant). The $V_{sulfide,max}$ equaled the $V_{thio,max}$ and is not additionally listed in Table 3. Cell yield increase or oxygen uptake on DMS was not observed for cells cultivated in continuous culture on 10 mmol of thiosulfate liter^{-1} and 3 mmol of DMS liter^{-1}. The observed loss of DMS in the culture medium could be accounted for by air stripping. Also in batch cultures, the available strain of *T. thioparus* T5 could not degrade DMS, whereas DMS was readily degraded by *M. sulfidovorans* under similar conditions. The growth yields of *T. thioparus* T5 on thiosulfate and sulfide were 4.5 ± 1.2 g mol^{-1} and 3.1 ± 0.1 g mol^{-1}, respectively. With the $V_{O,max}$ found for sulfide and thiosulfate (Table 4), the yield data, and the reaction stoichiometry (Table 1), μ_{max} was estimated to be 0.30 ± 0.03 h^{-1} for both substrates. The K_s was 6.2 ± 0.6 μmol of thiosulfate liter^{-1} and 10 ± 2 for μmol of sulfide liter^{-1}. Oxygen uptake experiments showed that the enzymes for sulfide degradation were present in cells grown with thiosulfate and vice versa.

The results of the experiments with pure cultures indicated that DMS can only be degraded by *M. sulfidovorans*. $V_{DMS,max}$ values found in mixed culture will always be correlated with the biomass concentration of *M. sulfidovorans*. A similar conclusion can be drawn for thiosulfate consumption by *T. thioparus* T5.

Mixed cultures of *M. sulfidovorans* and *T. thioparus* T5. Mixed cultures experiments were carried out with DMS (experiment 1), DMS and H_2S (experiment 2), and methanol and sulfide (experiment 3). A mixed culture on DMS was started from a pure culture of *M. sulfidovorans*, grown on 3 mmol of DMS liter^{-1}. The dry weight of this culture of *M. sulfidovorans* was 37 ± 8 mg liter^{-1}. The culture contained 1.5 mmol of thiosulfate liter^{-1}. After addition of *T. thioparus* T5 to this culture, the dry weight increased to 45 ± 8 mg liter^{-1}, and the thiosulfate concentration in the fermentor fell below the detection level (<10 μmol liter^{-1}), confirming that *T. thioparus* T5 used thiosulfate. Although the scatter in the dry weight determination was considerable, the biomass increase was confirmed by measurements of the optical density. The results of the maximum oxygen uptake experiments and cell counts are listed in Table 5 (experiment 1). The quantitative interpretation of these data will be discussed below. In experiment 2, 2.5 mmol of sulfide liter^{-1} was supplemented to the inflowing DMS medium, and again $V_{O,max}$ values and cell counts were determined. The results show (Table 5) that the $V_{O,max}$ for DMS remained roughly constant and that the $V_{O,max}$ for both sulfide and thiosulfate increased.

A mixed culture on methanol and sulfide was started with a pure culture of *M. sulfidovorans*, grown on 10 mmol of methanol liter^{-1} and 9.3 mmol of sulfide liter^{-1}. The dry weight of this culture was 118 ± 9 mg liter^{-1}. The culture contained 4.9 mmol of thiosulfate liter^{-1}. After addition of *T. thioparus* T5, the dry weight remained within the range of 120 mg liter^{-1}. The thiosulfate concentration in the fermentor, again, decreased below the detection level (<10 μmol liter^{-1}). The results of the oxygen uptake experiments and cell counts are listed in Table 5 (experiment 3). The quantitative implications of these results are explained in the Discussion section.

TABLE 4. Results of continuous culture experiments with *T. thioparus* T5 grown on sulfide at $D = 0.03\ h^{-1}$[a]

Concn of sulfide substrate (mM)	$V_{O,max}$ (μmol of O_2 liter^{-1} min^{-1})		Cell counts (10^8 ml^{-1})
	S^{2-}	$S_2O_3^{2-}$	
2.6 ± 0.1	33.3 ± 0.4	25.2 ± 0.6	0.8 ± 0.2
3.0	44.0	32.0	
5.1 ± 0.4	50.4 ± 2.1	44.1 ± 1.3	1.7 ± 0.3
7.5 ± 0.1	81.6 ± 4.6	56.0 ± 9.0	2.6 ± 0.6
9.5 ± 0.1	95.4 ± 1.6	81.7 ± 1.8	3.5 ± 0.2

[a] The maximum specific oxygen uptake rates on sulfide and thiosulfate were determined in steady state in triplicate. Sulfide in the medium supply and cell counts on agar plates were measured at least in triplicate. Values are means ± standard deviations.

DISCUSSION

DMS and H_2S in microbial mats may be metabolized aerobically by bacteria belonging to different functional groups i.e., the colorless sulfur bacteria and the methylotrophs. The first group may be considered to be specialized in the oxidation of inorganic sulfur compounds, whereas the second group is considered to be specialized in the oxidation of methylated compounds, among which are the organic sulfur compounds. The combination of substrate affinity ($q_{max} \cdot K_s^{-1}$; see Materials and Methods) and biomass concentration of each functional group is decisive for which group outcompetes the other. *T. thioparus* T5 and *M. sulfidovorans* were isolated from marine sediment and were considered to be representatives of the colorless sulfur bacteria and the methylotrophs, respectively. Both bacteria were reported to oxidize DMS and H_2S. Mixed cultures of both bacteria are expected to coexist or compete for DMS and/or H_2S. Coexistence occurs when all DMS and/or sulfide is converted to thiosulfate by *M. sulfidovorans* and the

thiosulfate is converted to sulfate by *T. thioparus* T5. Competition occurs if both bacteria actually use the supplied DMS and/or H_2S. For purposes of clarity and convenience of comparisons, some of the (extensive) calculations are listed in Appendix. These calculations are referred to in the text by numbered letters (a_1 to a_6) and can be found in Appendix.

Experiments with pure cultures of *M. sulfidovorans* showed a constant $V_{DMS,max}$ with an increasing supply of DMS, i.e., a decreasing $q_{DMS,max}$. Full conversion of 3 mmol of DMS liter^{-1} would need a $V_{DMS,max}$ of 1.5 μmol of DMS liter^{-1} min^{-1} (see "a_1"). The observed $V_{DMS,max}$ (±1.6 μmol of DMS liter^{-1} min^{-1}), thus, was sufficient for complete oxidation of the DMS concentrations chosen in the experiments. Nevertheless, it had been expected that increasing the supply of DMS in the medium would result in an increasing $V_{DMS,max}$ for DMS. The $V_{DMS,max}$ seems to be limited; at concentrations above 3 mmol of DMS liter^{-1} in the medium, oxidation was incomplete and the culture washed out, independent of the concentration of biomass in the fermentor. This inhibitory effect was not observed in the potential DMS-oxidizing capacity of cultures of *M. sulfidovorans* cultivated on methanol and H_2S. (Table 2). In these cultures, $V_{O(DMS),max}$ rose to 11.1 μmol of O_2 liter^{-1} min^{-1}, which is equivalent to 2.8 μmol of DMS liter^{-1} min^{-1} for 9.3 mmol of sulfide liter^{-1}. This $V_{DMS,max}$ is larger than what was observed in DMS-limited cultures ($V_{DMS,max}$ = 1.6 μmol of DMS liter^{-1} min^{-1}). This suggests that the inhibition observed might be caused by a toxic intermediate formed in the degradation of DMS to H_2S. As expected, the $V_{O,max}$ for methanol (M), $V_{O(M),max}$ (Table 2), stayed constant for the cultures grown on methanol and sulfide, since the methanol supply rate was not altered during the experiments.

Continuous cultures with *T. thioparus* T5 showed a linear relationship between the supplied thiosulfate or sulfide concentration in the medium and the $V_{O,max}$ on thiosulfate and sulfide (Tables 3 and 4). Also, the cell counts increased linearly with an increasing concentration of substrate. In contrast to what was observed by others (11), even marginal DMS oxidation could not be demonstrated in continuous cultures or in batch cultures of the available strain of *T. thioparus* T5.

In order to interpret the data of the mixed cultures it was assumed that the overall conditions in the mixed cultures were the same as in the pure cultures and that under these conditions the fluxes of substrates through each organism would be reflected in the same enzyme levels as observed in the pure cultures. Hence, from the $V_{O,max}$ values on thiosulfate, DMS, and sulfide in the mixed cultures, the amount of substrate used by either one of the bacteria was estimated. The data listed in Tables 2, 3, and 4 were linearly interpolated to analyze the observed $V_{O,max}$ data of the mixed cultures. It should be noted that the cell counts in mixed cultures always refer to counts of *T. thioparus* T5.

Three experiments were carried out with mixed cultures of *M. sulfidovorans* and *T. thioparus* T5 (Table 5). The mixed culture experiment on DMS (experiment 1) showed a $V_{O,max}$ of 9.7 μmol of O_2 liter^{-1} min^{-1} (=2.4 μmol of DMS liter^{-1} min^{-1}), which is consistent with the fact that DMS is only oxidized by *M. sulfidovorans*. Theoretically, *T. thioparus* T5 in the mixed culture is capable of using both thiosulfate, i.e., the end product of DMS oxidation, and sulfide, an intermediate in the DMS oxidation. The latter option presupposes that the intracellular sulfide, produced in DMS degradation by *M. sulfidovorans*, would be transported out of the cell and used by *T. thioparus* T5. If *T. thioparus* T5 only used 1.5 mmol of thiosulfate liter^{-1} (the end product of the conversion of a 3-mmol liter^{-1} concentration of DMS), the $V_{O,max}$ on thiosulfate and the cell counts would be approximately 10 μmol liter^{-1} min^{-1} and 0.35 10^8 ml^{-1}, respectively (derived from Table 3). If 3 mmol of H_2S liter^{-1} would have been converted by *T. thioparus* T5, the $V_{O,max}$ on thiosulfate and sulfide would have been 32 and 44 μmol of O_2 liter^{-1} min^{-1} for thiosulfate and sulfide oxidation, respectively, and the cell counts would have been at least 0.8 × 10^8 ml^{-1} (Table 4). The observed $V_{O,max}$s on thiosulfate and sulfide in the mixed culture were 14 and 18 μmol of O_2 liter^{-1} min^{-1}, respectively, and the cell counts were 0.5 × 10^8 ml^{-1}. These results show that based on the $V_{O,max}$ on thiosulfate at least 82% [(32-14)/(32-10)] × 100 of the H_2S originating from DMS is converted first to thiosulfate by *M. sulfidovorans* and then to sulfate by *T. thioparus* T5. As we have shown that this strain of *T. thioparus* T5 cannot use DMS, it is concluded that all of the H_2S originating from DMS was converted by *M. sulfidovorans*. The predicted increase of biomass after the addition of *T. thioparus* T5 to the *M. sulfidovorans* culture was approximately 7 mg liter^{-1}, if only thiosulfate would have been used by *T. thioparus* T5. This is consistent with the observed increase from 37 to 45 mg liter^{-1}. Similar calculations for the explanation of further experiments are listed in Appendix.

In experiment 2 (Table 5), sulfide was added as an additional energy source to the mixed culture. The sulfide can be used by either one or both of the species in the mixed culture. For *M. sulfidovorans* the affinity for sulfide, when grown on 3 mmol of DMS liter^{-1}, was 0.044 liter mg of biomass^{-1} min^{-1} (see "a_2"). For *T. thioparus* T5 the affinity for sulfide was 0.054 liter mg of biomass^{-1} min^{-1} (see "a_3"). These affinities are in the same order of magnitude, and it might be predicted that 2.5 mmol of sulfide liter^{-1}, added to the DMS-limited culture, will be used by both bacteria. From the two theoretical extremes (i) *M. sulfidovorans* uses all of the externally supplied sulfide or (ii) *T. thioparus* T5 uses all of the external sulfide, the ratio of sulfide going to either one of the species can be calculated (see "a_4"). It was concluded that 38% ± 5% of the sulfide is used by *T. thioparus* and 62% ± 5% is used by *M. sulfidovorans*. The cell counts of the mixed culture were too low for either one of the extreme situations: (0.6 ± 0.1) × 10^8 cells ml^{-1} were observed and either 0.8 × 10^8 (i) or 1.15 × 10^8 (ii) cells ml^{-1} would have been expected. Therefore neither theoretical extreme was taken into consideration. The only slight increase in *T. thioparus* T5 counts observed when H_2S was added to the inflowing medium supports the assumption that the sulfide is largely used by *M. sulfidovorans*, since in such case only 1.25 mmol of thiosulfate liter^{-1} would have become available to *T. thioparus* T5, explaining the small increase in its cell count.

In experiment 3 competition for sulfide was examined. For *T. thioparus* T5, grown on 10 mmol of sulfide liter^{-1}, the affinity for sulfide was 0.16 liter mg of biomass^{-1} min^{-1} (see "a_5"). For *M. sulfidovorans*, the affinity for sulfide under these conditions was 0.02 liter mg of biomass^{-1} min^{-1} (see "a_6"). As the affinity of *M. sulfidovorans* for sulfide is significantly lower than that of *T. thioparus* T5, it might be expected that the sulfide in the mixed culture would be used by the latter. The results of the mixed culture show that the $V_{O,max}$s on sulfide and thiosulfate have values similar to that found for *T. thioparus* T5 grown on 7.5 mmol of sulfide liter^{-1} (Table 4), indicating that the majority (i.e., 7.5 mmol liter^{-1}) of the 9.3 mmol of sulfide liter^{-1} in the mixed culture was used by *T. thioparus* T5. The cell count was 2.8 × 10^8 cells ml^{-1}, which is within range of the value observed for growth on 7.5 mmol of sulfide liter^{-1}. The observed $V_{O,max}$ on DMS (Table 5) in the mixed culture was low, indicating that only a very low proportion of the sulfide was used by *M. sulfidovorans* (≈0.8 mmol of H_2S liter^{-1}). In other words, *T. thioparus* T5 is capable of reducing the level of sulfide in the mixed culture to a point where it no

longer induces the sulfide-oxidizing enzymes to the level found in pure cultures. The results indicated that 8 to 20% of the sulfide is used by *M. sulfidovorans.*

In summary, *M. sulfidovorans* and *T. thioparus* T5 can coexist if they are cultivated solely on DMS. DMS is fully converted to thiosulfate by *M. sulfidovorans*, and thiosulfate is subsequently converted by *T. thioparus* T5. The intracellular sulfide in *M. sulfidovorans* is not oxidized by the *Thiobacillus*, even though the affinity for exogenous sulfide of the latter is significantly higher than that of *M. sulfidovorans*. In methanol-H_2S-limited mixed cultures, *T. thioparus* T5 used the majority of the sulfide. It can be predicted (3), nevertheless, that when the biomass concentration of *M. sulfidovorans* is high, compared to that of *T. thioparus* T5, more effective competition by *M. sulfidovorans* for sulfide may occur. This would be accomplished by an increasing methanol/sulfide ratio. Competition for sulfide improved when *M. sulfidovorans* was supplied with DMS. Our interpretation is that the intracellular sulfide formed from DMS degradation leads to the presence of a relatively high concentration of sulfide-oxidizing enzymes in *M. sulfidovorans*. If only exogenous H_2S is supplied to the mixed culture, the enzymes of *M. sulfidovorans* are not induced to the same level as that observed during DMS utilization. This implies that under these conditions *M. sulfidovorans* would only marginally use sulfide. It can be concluded that as long as DMS is available, H_2S can represent a substantial portion of the menu for *M. sulfidovorans.*

APPENDIX

The calculations referred to in the text are listed here.

a_1. DMS oxidation rate for 3 mmol of DMS liter^{-1} supplied to the medium and a dilution rate of 0.03 h^{-1}. $V_{DMS} = (3.0 \times 10^3) \times 0.03/60 = 1.5$ μmol liter^{-1} min^{-1}.

a_2. Sulfide affinity of *M. sulfidovorans* (*M.s.*) grown on 3 mmol of DMS liter^{-1}. Sulfide affinity = $V_{M.s.,sulfide,max} \cdot C_{M.s.}{}^{-1} \cdot K_{M.s.,sulfide}{}^{-1}$. $V_{M.s.,sulfide,max} = 6.5 \pm 2.5$ μmol of S^{2-} liter^{-1} min^{-1}, $C_{M.s.} = 37 \pm 8$ mg of biomass liter^{-1}, and $K_{M.s.,sulfide} = 4$ μmol of sulfide liter^{-1}. Sulfide affinity = 0.044 liter mg of biomass^{-1} min^{-1}.

a_3. Sulfide affinity of *T. thioparus* T5 (*Tt*T5) grown in mixed culture on 3 mmol of DMS liter^{-1}. Sulfide affinity = $V_{TtT5,sulfide,max} \cdot C_{TtT5}{}^{-1}$. $V_{TtT5,O(sulfide),max} = 7.5$ μmol of O_2 liter^{-1} min^{-1} = 3.8 μmol of S^{2-} liter^{-1} min^{-1}, $C_{TtT5} = 7$ mg of biomass liter^{-1} (1.5 mmol of thiosulfate liter^{-1} × 4.5 mg of biomass mmol^{-1} of thiosulfate), and $K_{TtT5,sulfide} = 10$ μmol of sulfide liter^{-1}. Sulfide affinity = 0.054 liter mg of biomass^{-1} min^{-1}.

a_4. $V_{O,max}$ values corresponding with the two extremes *M. sulfidovorans* uses all external sulfide (i) and *T. thioparus* T5 uses all external sulfide (ii). (i) All thiosulfate consumed by *T. thioparus* T5 = 2.75 mmol liter^{-1} (3 mmol of DMS liter^{-1} → 1.5 mmol of thiosulfate liter^{-1}; 2.5 mmol of H_2S liter^{-1} → 1.25 mmol of thiosulfate liter^{-1}), resulting in a $V_{O(thio),max} = V_{O(sulfide),max}$ of ± 18 μmol of O_2 liter^{-1} min^{-1}. (ii) $V_{O,max}$s on sulfide and thiosulfate = 33 and 25 μmol of O_2 liter^{-1} min^{-1}, respectively. In the mixed culture, $V_{O(sulfide),max}$ and $V_{O(thiosulfate),max} = 23$ and 21 μmol of O_2 liter^{-1} min^{-1}, respectively. Thus, the observed increase in $V_{O(thio),max}$ is 3 (21-18). Relative to the maximum possible increase of 7 (25-18), this is 43% [(3/7) × 100]. Hence, 43% of the externally supplied sulfide has been consumed by *T. thioparus* T5. Based on the $V_{O(sulfide),max}$, *T. thioparus* T5 uses 33% [(23-18)/(33-18) × 100] of the sulfide. On average, *T. thioparus* T5 used 38% ± 5% of the sulfide supplied.

a_5. Sulfide affinity of *T. thioparus* T5 grown in mixed culture on 10 mmol of sulfide liter^{-1}. Sulfide affinity = $V_{TtT5,sulfide,max} \cdot C_{TtT5}{}^{-1} \cdot K_{TtT5,sulfide}{}^{-1}$. $V_{TtT5,O(sulfide),max} = 103$ μmol of O_2 liter^{-1} min^{-1} (Table 4, linear extrapolation) = 51 μmol of S^{2-} liter^{-1} min^{-1}. $C_{TtT5} = 31$ mg of biomass liter^{-1} (10 mmol liter^{-1}, $Y_{sulfide,TtT5} = 3.1$ mg of biomass mmol of sulfide^{-1}) and $K_{TtT5,sulfide} = 10$ μmol of sulfide liter^{-1}. Substrate affinity = 0.16 liter mg of biomass^{-1} min^{-1}.

a_6. Sulfide affinity of *M. sulfidovorans* grown on 10 mmol of sulfide liter^{-1} and 10 mmol of methanol liter^{-1}. Sulfide affinity = $V_{M.s.,sulfide,max} \cdot C_{M.s.}{}^{-1} \cdot K_{M.s.sulfide}{}^{-1}$. $V_{M.s.,O(sulfide),max} = 10$ μmol of O_2 liter^{-1} min^{-1} = 10 μmol of S^{2-} liter^{-1} min^{-1} (Table 2). $C_{M.s.} = 118$ mg of biomass liter^{-1} and $K_{M.s.,sulfide} = 4$ μmol of sulfide liter^{-1}. Substrate affinity = 0.02 liter mg of biomass^{-1} min^{-1}.

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