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Volatile Fatty Acids and Hydrogen as Substrates for Sulfate-Reducing Bacteria in Anaerobic Marine Sediment

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The addition of 20 mM MoO_4^{2-} (molybdate) to a reduced marine sediment completely inhibited the SO_4^{2-} reduction activity by about 50 nmol g^{-1} h^{-1} (wet sediment). Acetate accumulated at a constant rate of about 25 nmol g^{-1} h^{-1} immediately after MoO_4^{2-} addition and gave a measure of the preceding utilization rate of acetate by the SO_4^{2-}-reducing bacteria. Similarly, propionate and butyrate (including isobutyrate) accumulated at constant rates of 3 to 7 and 2 to 4 nmol g^{-1} h^{-1}, respectively. The rate of H_2 accumulation was variable, and a range of 0 to 16 nmol g^{-1} h^{-1} was recorded. An immediate increase of the methanogenic activity by 2 to 3 nmol g^{-1} h^{-1} was apparently due to a release of the competition for H_2 by the absence of SO_4^{2-} reduction. If propionate and butyrate were completely oxidized by the SO_4^{2-}-reducing bacteria, the stoichiometry of the reactions would indicate that H_2, acetate, propionate, and butyrate account for 5 to 10, 40 to 50, 10 to 20, and 10 to 20%, respectively, of the electron donors for the SO_4^{2-}-reducing bacteria. If the oxidations were incomplete, however, the contributions by propionate and butyrate would only be 5 to 10% each, and the acetate could account for as much as two-thirds of the SO_4^{2-} reduction. The presence of MoO_4^{2-} seemed not to affect the fermentative and methanogenic activities; an MoO_4^{2-} inhibition technique seems promising in the search for the natural substrates of SO_4^{2-} reduction in sediments.

In coastal marine sediments, the SO_4^{2-} respiration may account for a major part of the anaerobic oxidation of organic matter (10), but the relative importance of the natural substrates for the process has not been measured.

Earlier work on SO_4^{2-} reduction in a lake sediment indicated an important role for lactate as a natural substrate for the SO_4^{2-}-reducing bacteria (6, 7). Lactate and a limited number of other fermentation products are used as substrates by the SO_4^{2-}-reducing bacteria of the genus *Desulfovibrio* (14), but only a partial oxidation of the substrates is accomplished due to the lack of a complete tricarboxylic acid cycle in these bacteria. Acetate is produced during the lactate oxidation, and a mutualistic relationship between the SO_4^{2-}-reducing and methanogenic bacteria was postulated on the basis of an acetate transfer between the two types of bacteria (6, 7). In addition, an H_2 transfer may take place in media low in SO_4^{2-} (5).

Other SO_4^{2-}-reducing bacteria have now been isolated, however, which oxidize acetate (18) and other important fermentation products: C_3 to C_{18} fatty acids, keto acids, alcohols, and aromatic compounds (F. Widdel, Ph.D. thesis, University of Göttingen, Göttingen, West Germany, 1980). These results have important ecological implications. Thus, a complete anaerobic oxidation of the organic matter by the SO_4^{2-}-reducing bacteria should be possible, and a competitive, rather than a stimulatory, relationship may exist between the SO_4^{2-}-reducing and methanogenic bacteria in the sediments.

Much attention is now being paid to the competitive interactions between SO_4^{2-}-reducing bacteria and methanogens for acetate and H_2 in the sediments. It was shown that the presence of SO_4^{2-} inhibited methanogenesis in a lake sediment, apparently due to the competition by the SO_4^{2-}-reducing bacteria; the inhibition was overcome only by addition of excess acetate or H_2 (19). The competition for acetate and H_2 was also the subject of several studies on marine sediments (1, 2, 15, 16).

The use of ^{14}C-labeled lactate and acetate has elucidated the pathways of degradation and the turnover rates of these compounds in the sediments (3, 7, 15, 20), but the data are not implicitly related to the activity of SO_4^{2-} reduction. Alternatively, fluoroacetate (CH_2FCOO^-) has been used to inhibit acetate-dependent reactions in the sediments (6, 7, 13), but these applications should be performed with caution since the in-

hibitory pattern may be complicated. Thus, the actual mechanism of acetate oxidation by the SO_4^{2-}-reducing bacteria has not yet been studied in detail. Use of molybdate (MoO_4^{2-}), an analog of SO_4^{2-}, to inhibit the activity of SO_4^{2-} reduction in a marine sediment was presented by Oremland and Taylor (16) in a study on the competition for H_2 between the SO_4^{2-}-reducing bacteria and the methanogens. In the present study, we used MoO_4^{2-} to arrest SO_4^{2-} reduction in a marine sediment and measure the subsequent accumulation of substrates such as short-chain fatty acids and H_2 in the environment. We propose that such accumulations of the substrates may be used as a quantitative measure of their rates of oxidation by SO_4^{2-} reduction in the sediments.

MATERIALS AND METHODS

Sulfide-rich sediment was collected from a shallow, coastal lagoon and stored in the dark in a cold room (2°C). A batch of this material was sieved through a 1-mm screen and diluted fivefold with seawater (25 mM SO_4^{2-}) to obtain a homogeneous slurry with a water content of about 95% (wt/wt). In a control experiment, SO_4^{2-}-free, artificial seawater was used for dilution to exclude the SO_4^{2-} reduction activity. The slurries were then incubated under N_2 in 1.3-liter, rubber-stoppered glass bottles which had an outlet at the bottom for subsampling of the sediment. At the top of the bottle a rubber septum in the stopper served for sampling from the gaseous headspace. A slight but constant N_2 pressure was applied to the bottle through a Pasteur pipette in the stopper. The pipette was partially submerged into the slurry to prevent a backflow of the biogenic gases. The slurries were preincubated under constant mixing for at least 3 days in the dark and at room temperature to establish constant rates of SO_4^{2-} reduction and CH_4 production.

The SO_4^{2-} reduction rate was measured in the SO_4^{2-}-containing sediment by a ^{35}S tracer technique. This assay was initiated by injecting 2 μl of carrier-free $^{35}SO_4^{2-}$ (about 3×10^6 dpm) into the slurry. At intervals, subsamples of about 5 g were then taken directly into 5 ml of a Zn acetate solution to trap any ^{35}S-labeled sulfide produced. The acid-labile sulfide was then released from the fixed sample by acidification under N_2 and transferred to a Zn acetate trap by the distillation procedure of Jørgensen (9). The analysis of $^{35}SO_4^{2-}$ activity in the acidified and sulfide-free sediment was performed on a 1-ml sample of the supernatant after centrifugation. Samples were made up to 5 ml with distilled water before counting. The ^{35}S activities in the two sets of samples were measured in a liquid scintillation counter (Intertechnique SL 30) after addition of 5 ml of Insta-Gel (Packard Instrument Co.) to the vials. Background and quench corrections were performed (external standard channels ratio). A gravimetric assay of the SO_4^{2-} concentration in the sediment was performed on filter-sterilized water samples obtained by pressure filtration (0.45-μm membrane filters [Millipore Corp.] at 3 atm [101.325 kPa] N_2). The rate of SO_4^{2-} reduction in the sediment was then calculated as described by Jørgensen (9).

In both the SO_4^{2-}-free and the SO_4^{2-}-containing sediments, the short-chain fatty acids (acetate, propionate, and butyrate) were determined at intervals in the supernatant of subsamples that were centrifuged at 2,000 × *g* for 5 min. A vacuum distillation procedure was used to extract the volatile fatty acids (VFAs) from the filter-sterilized water. The filter-sterilized water was first acidified (pH 1) and heated under vacuum, and the VFAs were transferred to a cold trap. The cold trap was then detached, and the distillate was analyzed by a gas chromatographic procedure with flame ionization detection (Packard model 419) at 200°C. To obtain a better sensitivity (10-fold) for the analysis of the butyrate fractions, a 2-ml filter-sterilized water sample was lyophilized at high pH. The solids were then acidified, and a 0.2-ml sample was distilled as described above. The acids were separated at 160°C on a Porapak QS column (1 m long, 6-mm outer diameter, glass), and a source of formic acid vapor was inserted in the N_2 carrier flow (30 ml min^{-1}) to facilitate the desorption of the VFAs from the column (3).

The degradation of propionate was studied by adding about 2 μCi of carrier-free [2-^{14}C]propionate (Amersham, England) into 0.5 liter of a SO_4^{2-}-containing sediment. At intervals, subsamples were taken into 1 ml of a 1 N NaOH solution to stop further activity and prevent loss of volatile ^{14}C-labeled compounds. The $^{14}CO_2$ was liberated from the subsamples by acidification and transferred into vials with scintillation fluid by purging with air (3). After $^{14}CO_2$ extraction, the ^{14}C-labeled VFA pool was vacuum distilled from water samples obtained by centrifugation. The ^{14}C activity in the acetate and propionate fractions of the VFA distillate was collected in scintillation fluid at the flame ionization detector outlet. The ^{14}C activities were then measured in the scintillation counter. Details of the VFA and ^{14}C procedures will be given elsewhere (D. Christensen, manuscript in preparation).

Gas samples (0.2 ml) from the head space of the bottles were analyzed for CH_4 by the same gas chromatographic procedure as described for the VFAs. Separate gas samples were analyzed for H_2 on a Packard model 417 gas chromatograph with thermal conductivity detection at 80°C. A Porapak Q column (1.8 m long, 3.2-mm outer diameter, stainless steel) operated at 50°C was used with a N_2 carrier flow of 15 ml min^{-1}. Corrections were made for the solubility of CH_4 and H_2 and for the volume changes in the bottles.

After the endogenous rate of SO_4^{2-} reduction was measured in the SO_4^{2-}-containing sediment, further reduction was stopped by addition of a 2 M Na_2MoO_4 solution to give a concentration of 20 mM MoO_4^{2-} in the slurry. A modification of the sulfide distillation procedure proved to be necessary for the MoO_4^{2-}-containing subsamples. The MoO_4^{2-} prevents a release of the sulfide from the Zn acetate traps during acid treatment. Five washings of the Zn sulfide precipitate were therefore first performed with 50-ml samples of seawater to remove the MoO_4^{2-}. The Zn sulfide precipitate was then treated by acid distillation as described above. The assays for the VFAs and for H_2 and CH_4 were continued with regular sampling intervals after the application of MoO_4^{2-}. The inhibitoir did not inter-

fere with the procedure for VFA analysis. A similar addition of MoO_4^{2-} was made to the SO_4^{2-}-free sediment to measure any effects of the inhibitor on microbial processes other than the SO_4^{2-} reduction.

RESULTS

Sulfate-free sediment. The SO_4^{2-}-free control was preincubated for 1 week to obtain a complete absence of SO_4^{2-}. In subsamples of these slurries, a rapid exhaustion of added $^{35}SO_4^{2-}$ (a few minutes) indicated the absence of SO_4^{2-} before initiation of the control experiment (data not shown). The absence of SO_4^{2-} reduction resulted in significant accumulations of CH_4 and of fermentation products such as acetate and propionate. The concentrations of acetate and propionate were 2 mM and 0.5 mM, respectively, after this preincubation period. In the SO_4^{2-}-free sediment, the carbon flow apparently became limited by the terminal processes of acid degradation; however, production of CH_4 was significant during the preincubation period, and a final pressure of 10^{-2} atm was measured. The H_2 pressure was below the detection limit of 5×10^{-5} atm.

The addition of 20 mM MoO_4^{2-} after 24 h had no apparent effect on the acid accumulations (Fig. 1). During the 2 days of measurements, the H_2 pressure remained very low (at the detection limit), which indicated that a rapid utilization of H_2 took place by the methanogenic bacteria in the sediment. A rate of CH_4 production of 25 nmol g^{-1} h^{-1} was measured during a period of 6 h before the addition of the MoO_4^{2-}. A similar rate was also observed after the addition of MoO_4^{2-}; methanogenesis was apparently not affected by the inhibitor.

Sulfate-containing sediment. The SO_4^{2-}-containing sediment was preincubated for about 1 week; the SO_4^{2-} concentration was 10 to 20 mM after this period. The acetate, propionate and butyrate concentrations remained low in the sediment, about 5 μM for acetate and 1 μM for the others. The endogenous H_2 pressure was at the detection limit of 5×10^{-5} atm in this sediment, and the CH_4 pressure was about 10^{-4} atm.

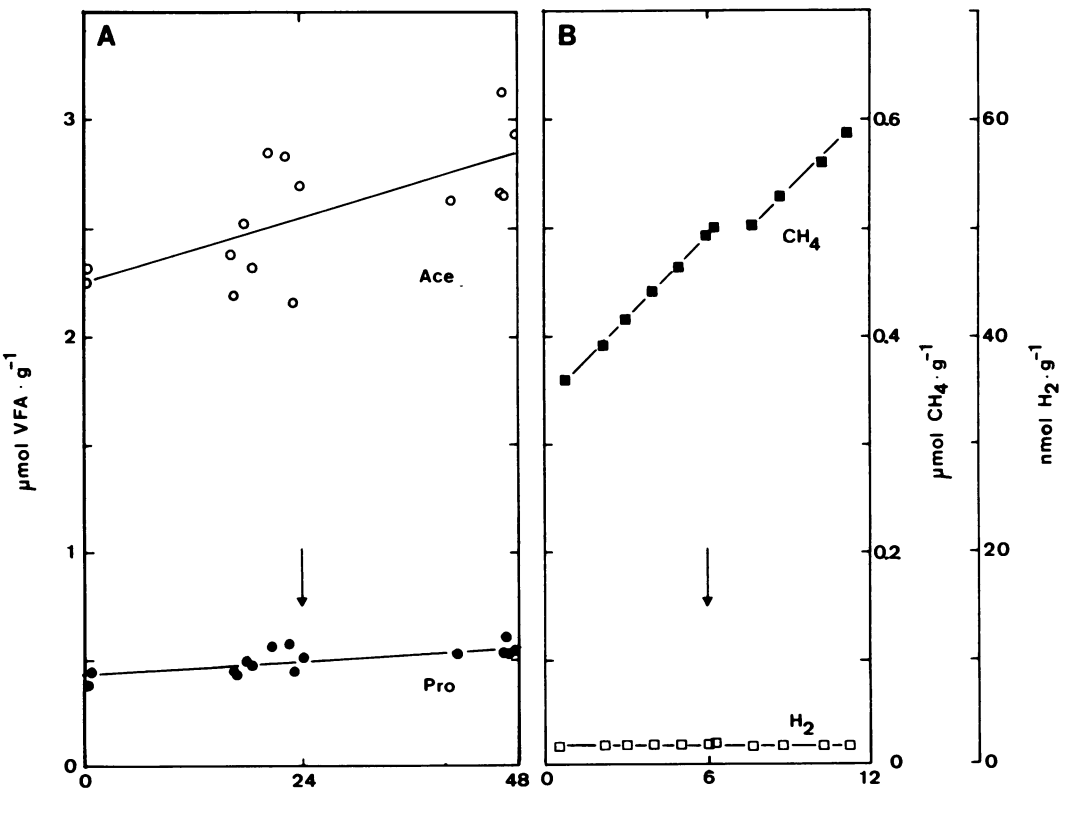

FIG. 1. *Sulfate-free sediment. Acetate* (○) *and propionate* (●) *concentrations in micromoles per gram of wet sediment (A), and* H_2 (□) *and* CH_4 (■) *concentrations in micromoles per gram of wet sediment (B). The addition of 20 mM* MoO_4^{2-} *is indicated by the arrow.*

During a period of 2 h before the addition of the MoO_4^{2-} inhibitor, the SO_4^{2-} reduction activity was measured by the ^{35}S tracer technique, and a linear regression analysis of the data gave a rate of 50 nmol g^{-1} h^{-1} in the experiment referred to below. A complete inhibition of the SO_4^{2-} reduction activity was obtained by the addition of MoO_4^{2-} as shown by the absence of further ^{35}S-labeled sulfide production (data not shown). The application of MoO_4^{2-} resulted in a spontaneous and linear accumulation of acetate, propionate, butyrate, and isobutyrate in the slurry (Fig. 2), and calculations of the slopes gave accumulation rates of 22, 3.3, 0.3, and 1.6 nmol g^{-1} h^{-1} for acetate, propionate, butyrate, and isobutyrate, respectively. A production of CH_4 of 0.3 nmol g^{-1} h^{-1} was noted before the addition of MoO_4^{2-}, and the presence of the inhibitor stimulated the CH_4 production to a constant rate of 3.0 nmol g^{-1} h^{-1} in the sediment. An accumulation rate for H_2 of about 16 nmol g^{-1} h^{-1} was also observed, and the H_2 pressure increased to about 3×10^{-4} atm in the bottle during the 4 h of incubation with MoO_4^{2-}.

In Table 1 are shown the data from this experiment (no. 1) and from three other, similar experiments (no. 2, 3, and 4). The data sets show similar SO_4^{2-} reduction activity. Also, the accumulation rates for acetate show little variation. One of the experiments (no. 4) showed no accumulation of H_2 after the application of MoO_4^{2-}; it seemed that this was associated with elevated accumulation rates for the reduced acids. The butyrate and isobutyrate contributions were possibly higher in the absence of H_2 formation, and it was apparent that the propionate contribution to the SO_4^{2-} reduction activity was markedly higher in this experiment.

Addition of [2-^{14}C]propionate to SO_4^{2-}-containing sediment gave rise to a spontaneous production of both ^{14}C-labeled acetate and $^{14}CO_2$ (Table 2). After 3 min, 50% of the added ^{14}C activity was converted to $^{14}CO_2$, and the rest was apparently accounted for by the volatile fatty acids. A 2:1 ratio of ^{14}C activity in the acetate and propionate pools was observed at this time. When MoO_4^{2-} was added after 3.5 min, both acetate and propionate oxidation stopped immediately, as shown by the absence of further $^{14}CO_2$ production and [^{14}C]VFA utilization. The 2:1 ratio of ^{14}C activity in the acetate and propionate remained constant until the experiment

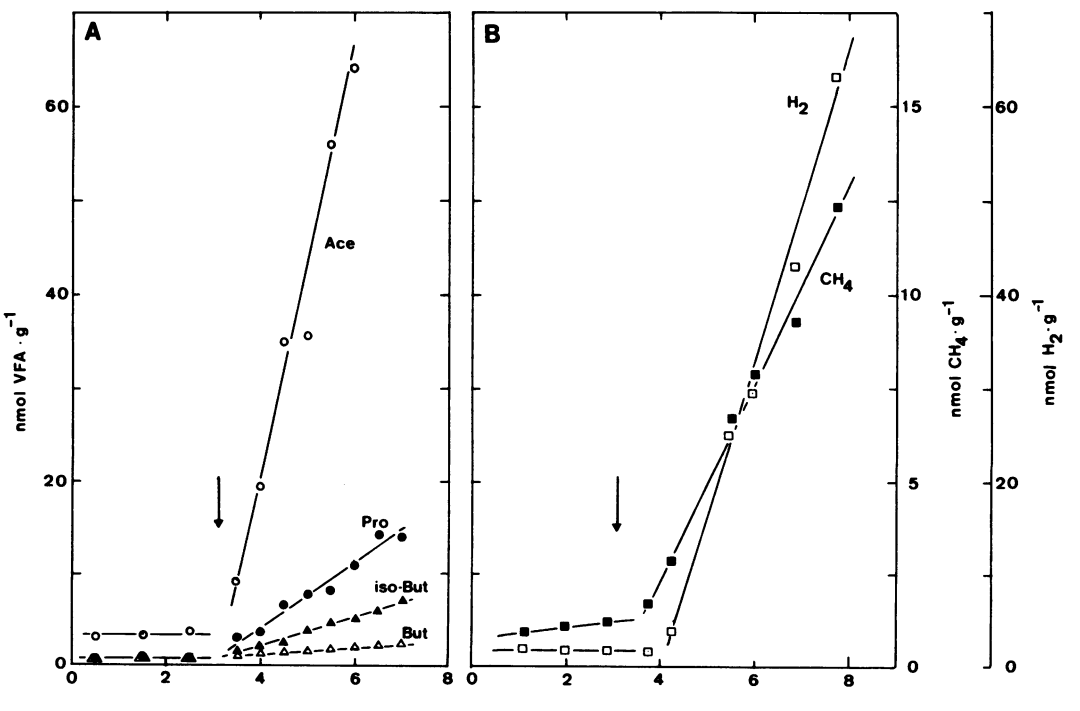

FIG. 2. *Sulfate-containing sediment. Acetate (○), propionate (●), butyrate (△) and iso-butyrate (▲) concentrations in nanomoles per gram of wet sediment (A), and H_2 (□) and CH_4 (■) concentrations in nanomoles per gram of wet sediment (B). The addition of 20 mM MoO_4^{2-} is indicated by the arrow.*

TABLE 1. *Comparison of activity of SO_4^{2-} reduction and MoO_4^{2-}-induced accumulations of substrates*

Expt	Endogenous activity[a] (nmol g^{-1} h^{-1})		MoO_4^{2-}-induced accumulation[b] (nmol g^{-1} h^{-1})					
			Gas accumulation		VFA accumulation			
	SO_4^{2-} reduction	CH_4 production	H_2	CH_4	Acetate	Propionate	Butyrate	Isobutyrate
1	50	0.3	16 (13)[c]	2.7[d]	22 (44)	3.3 (12)	0.3 (2)	1.6 (8)
2	47	0.2	8 (8)[c]	1.7[d]	20 (43)	3.5 (13)	1.0 (4)	1.5 (7)
3	41	ND[e]	ND	ND	21 (51)	3.0 (13)	1.2 (6)	1.2 (6)
4	54	1	0 (4)[c]	2.0[d]	27 (50)	7.0 (23)	1.6 (6)	2.4 (12)

[a] Activity 0 to 2 h before addition of 20 mM MoO_4^{2-}.
[b] Activity 0 to 4 h after addition of 20 mM MoO_4^{2-}. Numbers in parentheses indicate percent contribution as electron donor for SO_4^{2-} reduction, if complete substrate oxidation is assumed (see text).
[c] Including H_2 equivalents in CH_4 accumulation (see text).
[d] Accumulation of CH_4 in excess of endogenous activity.
[e] ND, Not determined.

TABLE 2. *Degradation of [2-^{14}C]propionate*

Time[a]	^{14}C activity[b]		^{14}C activity ratio (acetate/propionate)
	CO_2	VFA	
0.3	1.1	9.3	0.4
1.3	3.0	7.9	1.5
2	3.7	7.3	1.9
3	5.2	6.3	1.7
4*	5.1	5.6	1.9
6*	4.5	5.5	2.0
8*	5.0	5.1	2.0
13*	5.0	5.2	2.0
76*	4.7	4.9	2.0
195*	4.5	5.2	1.9

[a] Minutes after addition of [2-^{14}C]propionate. An asterisk indicates presence of 20 mM MoO_4^{2-} added after 3.5 min.
[b] Thousands of counts per minute per gram.

was terminated (195 min). The spontaneous inhibition by MoO_4^{2-} of the VFA oxidations indicated that SO_4^{2-} reduction was responsible for both acetate and propionate oxidation in these sediments. The results also indicated that part of the propionate oxidation was an incomplete reaction with acetate as an intermediate product.

DISCUSSION

Sulfate-free sediment. We anticipated that SO_4^{2-}-free sediments would be useful to indicate MoO_4^{2-}-induced perturbations of the fermentative and methanogenic reactions either as direct effects or as secondary effects due to increasing substrate concentrations in the environment.

In particular, the regulatory role of the H_2 pressure on some fermentative reactions (see Bryant [4] and Thauer et al. [17] for recent reviews) was a matter of concern. An H_2 accumulation could potentially inhibit some of the fermentative reactions and stimulate methanogenesis. However, the measured H_2 pressure in the control experiment with a SO_4^{2-}-free slurry remained at the detection limit of 5×10^{-5} atm, which is similar to or lower than H_2 concentrations measured in other SO_4^{2-}-free, methanogenic systems (8, 11). Further evidence for the absence of MoO_4^{2-}-induced perturbations of the control sediment was provided by the VFA accumulations. The H_2 and acetate formation from propionate is, for thermodynamic reasons, most sensitive to H_2 inhibition as compared with similar reactions involving, e.g., butyrate and ethanol (12). In a digestor, an H_2 pressure below 10^{-4} atm was maintained by a rapid transfer to methanogenic bacteria which allowed the oxidation of propionate to take place. When H_2 was added at a concentration corresponding to 1.5×10^{-2} atm, an increased propionate accumulation was observed (11). It seems plausible that the accumulation of propionate relative to that of acetate could indicate H_2 perturbations of the fermentative reactions in the sediments. However, the constant ratio of carbon accumulating as propionate and as acetate (0.29 ± 0.03), estimated from the concentration data in the control experiment, was not affected by the addition of MoO_4^{2-}. Finally, the nonaffected activity of methanogenesis supported the evidence for a lack of secondary MoO_4^{2-} effects.

Sulfate-containing sediment. The absence of $^{14}CO_2$ production from [U-^{14}C]acetate in MoO_4^{2-}-inhibited sediment (data not shown) showed that the CH_4 production from acetate according to

$$CH_3COO^- + H_2O \rightarrow HCO_3^- + CH_4$$

did not occur in the sediment. The absence of $^{14}CO_2$ production also showed that respiratory processes other than SO_4^{2-} reduction were absent. Thus, H_2 was the most likely precursor of the stimulated CH_4 formation in the sediment (Fig. 2), and the accumulation of H_2 in the

presence of MoO_4^{2-} was due to the limited capacity for H_2 removal by the methanogenic bacteria. The relative increment of CH_4 production in the presence of MoO_4^{2-} was 2.7 nmol g^{-1} h^{-1} and should represent an equivalent H_2 production of 10.8 nmol g^{-1} h^{-1} available for the SO_4^{2-}-reducing bacteria before the inhibition by MoO_4^{2-}. This calculation was made according to the stoichiometry for methanogenesis from H_2 and CO_2

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$$

The measured rate of H_2 accumulation in the sediment was 16 nmol g^{-1} h^{-1}, and we obtained a total rate of accumulation of H_2 equivalent to 26.8 nmol g^{-1} h^{-1}. The acetate accumulation was 22 nmol g^{-1} h^{-1}, and according to the stoichiometry for H_2 and acetate utilization by the SO_4^{2-} reducers

$$4H_2 + SO_4^{2-} \rightarrow HS^- + OH^- + 3H_2O$$

and

$$CH_3COO^- + SO_4^{2-} \rightarrow HS^- + 2HCO_3^-$$

these substrates should account for fractions of the SO_4^{2-} reduction of 7 and 22 nmol g^{-1} h^{-1}, respectively. The measured rate of SO_4^{2-} reduction in the sediment was 50 nmol g^{-1} h^{-1}, and the H_2 and acetate should thus contribute by 13 and 44%, respectively.

The propionate accumulation was also spontaneous after the addition of MoO_4^{2-} and took place at a rate of 3.3 nmol g^{-1} h^{-1} in the sediment. The accumulation was linear, like that of acetate, during the 4 h before the experiment was terminated. The ratio of carbon accumulating as propionate and as acetate was thus constant (0.30) during this period. The results indicated that propionate was also a substrate for the SO_4^{2-}-reducing activity in the sediment and that the propionate accumulation was not affected by the increasing H_2 tension. Evidence to support that SO_4^{2-} reduction was responsible for both propionate and acetate oxidation in the sediment was provided by the spontaneous inhibition by MoO_4^{2-} of [2-^{14}C]propionate degradation (Table 2) and [*U*-^{14}C]acetate degradation (data not shown). It is possible that the H_2-sensitive VFA oxidations performed by the "acetogenic" bacteria are of minor importance or absent in the sediments where SO_4^{2-} reduction may be responsible for the reactions. If we assume a complete oxidation of the propionate by the SO_4^{2-}-reducing bacteria according to

$$4C_2H_5COO^- + 7SO_4^{2-} \rightarrow 7HS^- + H^+ + 12HCO_3^-$$

then the rate of accumulation of 3.3 nmol g^{-1} h^{-1} should correspond to about 12% of the SO_4^{2-}-reducing activity. Similarly, the observed accumulations of butyrate and isobutyrate were spontaneous and linear at rates of 0.3 and 1.6 nmol g^{-1} h^{-1}, respectively. If we assume a complete oxidation of these compounds according to

$$2C_3H_7COO^- + 5SO_4^{2-} \rightarrow 5HS^- + H^+ + 8HCO_3^-$$

then their oxidation should correspond to a total of about 10% of the SO_4^{2-} reduction activity. Alternatively, if the propionate and butyrate oxidations were incomplete and acetate was produced according to

$$4C_2H_5COO^- + 3SO_4^{2-} \rightarrow 3HS^- + H^+ + 4HCO_3^- + 4CH_3COO^-$$

and

$$2C_3H_7COO^- + 3SO_4^{2-} \rightarrow 3HS^- + H^+ + 4HCO_3^- + 2CH_3COO^-$$

then we would overestimate the importance of the propionate and butyrate and, accordingly, underestimate the importance of acetate.

On the basis of the results reported in Table 1, we drew the following conclusions from the present study. Acetate is a major substrate for the SO_4^{2-}-reducing bacteria in the sediment and may account for 50% of the electron donors for the process. The acetate seems unimportant as a substrate for methanogenesis in the sediment. Hydrogen may account for 5 to 10% of the SO_4^{2-}-reducing activity and seems to be the major substrate for methanogenesis in the sediment. If propionate and butyrate plus isobutyrate were oxidized completely by the SO_4^{2-}-reducing bacteria, these substrates would each account for 10 to 20% of the process. If the oxidations were incomplete, however, half of these contributions should be assigned to the acetate which may then account for two-thirds of the SO_4^{2-} reduction under such conditions.

More work is needed on the oxidations of reduced fermentation products like propionate and butyrate to confirm their quantitative role for the SO_4^{2-} reduction. In the present study, we have not looked for the possible contributions by formate, ethanol, and lactate. The residual electron donor demand, not accounted for by H_2, acetate, propionate, butyrate, and isobutyrate, may also be searched for among such potential substrates as the long-chain fatty acids and the aromatic compounds. A combination of the present inhibitor technique with tracer studies of the oxidation of the reduced fermentation products may lead to a complete recovery and quantitation of the in situ substrates for SO_4^{2-} reduction in the sediments.

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