APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Feb. 1977, p. 275-281
Vol. 33, No. 2

Printed in U.S.A.

Effect of Sulfate on Carbon and Electron Flow During Microbial Methanogenesis in Freshwater Sediments

M. R. WINFREY AND J. G. ZEIKUS*

Department of Bacteriology, University of Wisconsin, Madison, Wisconsin 53706

Received for publication 14 June 1976

The effect of sulfate on methane production in Lake Mendota sediments was investigated to clarify the mechanism of sulfate inhibition of methanogenesis. Methanogenesis was shown to be inhibited by the addition of as little as 0.2 mM sulfate. Sulfate inhibition was reversed by the addition of either H_2 or acetate. Methane evolved when inhibition was reversed by H_2 additions was derived from $^{14}CO_2$. Conversely, when acetate was added to overcome sulfate inhibition, the evolved methane was derived from [2-^{14}C]acetate. A competition for available H_2 and acetate was proposed as the mechanism by which sulfate inhibited methanogenesis. Acetate was shown to be metabolized even in the absence of methanogenic activity. In the presence of sulfate, the methyl position of acetate was converted to CO_2. The addition of sulfate to sediments did not result in the accumulation of significant amounts of sulfide in the pore water. Sulfate additions did not inhibit methanogenesis unless greater than 100 μg of free sulfide per ml was present in the pore water. These results indicate that carbon and electron flow are altered when sulfate is added to sediments. Sulfate-reducing organisms appear to assume the role of methanogenic bacteria in sulfate-containing sediments by utilizing methanogenic precursors.

Several investigators (2, 10) have shown that sulfate inhibits methanogenesis in freshwater sediments. However, the basis for sulfate inhibition of methanogenesis is unknown. MacGregor and Keeney (10) demonstrated that 150 μg of sulfate per ml inhibited methanogenesis in Lake Mendota sediments. These investigators proposed that the inhibition was due to an increase in sediment Eh. Cappenberg (2) demonstrated a near complete inhibition of methanogenesis by 0.1% sulfate in Lake Vechten. He suggested that the sulfide produced by sulfate-reducing bacteria inhibited sediment methanogens.

Martens and Berner (11) have presented evidence that methanogenesis in marine sediments does not occur until sulfate is depleted. They speculated that the inhibition of methanogenesis by sulfate was due to a competition for available hydrogen or related to the relative free energy yields of carbonate and sulfate reduction. This theory has also been proposed by Claypool and Kaplan (6).

The metabolism of methane precursors in anaerobic environments is ill defined. Koyama (9) reported that 60% of the methane produced in rice paddy soils was derived from the methyl position of acetate. Cappenberg (3, 5) reported that the acetate accounted for 70% of the methane formed in Lake Vechten. Reduction of CO_2 by hydrogen is generally believed to account for the balance of methane formed in these environments. Recently, Winfrey et al. (20) demonstrated that carbonate reduction by hydrogen was important in sediment methanogenesis; however, methane production was limited by the availability of hydrogen.

We report here the effect of sulfate on methane formation and the metabolism of methane precursors in Lake Mendota sediments.

MATERIALS AND METHODS

Sampling procedures. Sediment samples were collected from Lake Mendota throughout the year (March 1975-April 1976) at a site under 18 m of water, as described previously (23). Sediment pore water was sampled (see Fig. 1) with a specially designed pore water sampler (R. Hesslein, Limnol. Oceanogr., in press). The sampler contained 19 rows of four chambers milled in 1-inch (2.54-cm) Plexiglas at 2-cm intervals (Fig. 1B). The chambers (4.5 ml, total volume) were filled with deionized, degassed water and sealed with a dialysis membrane (Union Carbide, no. 3¼ DM). The membrane was held in place by a 0.19-inch (ca. 0.48-cm) Plexiglas retaining plate with holes over each chamber and secured with plastic screws. The sampler was placed vertically in the sediment by scuba divers such that 7 rows of chambers were above the sediment water interface and 12 rows of chambers were below. The sampler was attached to a buoy on the surface. A 2-week incubation period was used to allow complete

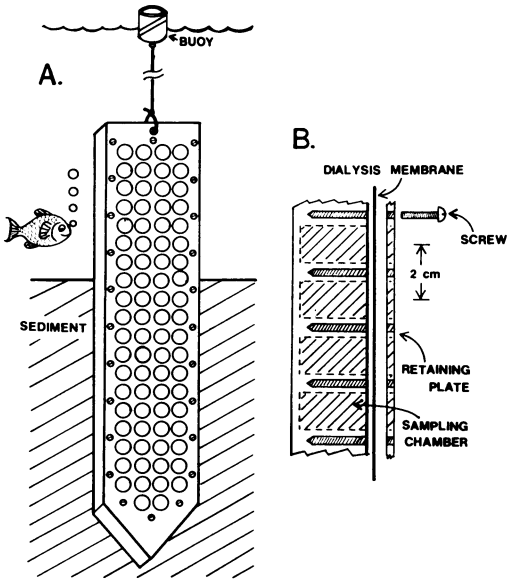

FIG. 1. *Interstitial water sampler: (A) schematic diagram showing sampler in place; (B) cross section showing assembly.*

equilibration with the pore water. Upon retrieval, the samples were immediately removed for analysis.

Interstitial water was obtained from sediment incubated in the lab as follows. A sealed anaerobic tube containing 10 ml of sediment was centrifuged for 15 min at 700 × *g* on a Sorvall type NSE angle centrifuge. The supernatant was then removed with a syringe and filtered through a 0.45-μm membrane filter. The filtrate was then used for analysis.

Experimental procedures. Experiments were performed in duplicate in anaerobic tubes (18 by 142 mm), as described by Winfrey et al. (20), and repeated a minimum of two times with different sediment samples. All additions were made anaerobically from solutions stored under N_2. In isotopic tracer experiments, sulfate was added 20 h before the addition of radioactive compounds to insure complete diffusion of sulfate throughout the sediments. Hydrogen was added by gassing tubes with a mixture of 15% H_2-85% N_2 (88 μmol of H_2 per tube). All tubes were vortexed for 1 min to ensure complete mixing of additions, sealed with a no. 2 neoprene stopper, and incubated at 30°C. Thirty degrees was chosen as the incubation temperature as this temperature was nearer the optimum for methanogenesis in Lake Mendota (23). Also, it was previously demonstrated (23) that less variance occurred at 30°C than at in situ incubation temperatures, when endogenous rates of methanogenesis were quantified. Although 30°C was considerably above the in situ temperatures (4 to 12°C), representative experiments performed at 10°C demonstrated the same phenomona reported here. However, the rates of methanogenesis were considerably lower.

Detection of gaseous end products. Gases were detected by gas chromatography and gas proportional counting as previously described (12). Dissolved methane in pore water was analyzed by the syringe stripping method of Rudd et al. (15).

Analysis of sulfide and sulfate in pore water. Sulfide concentrations in sediment pore water were measured by the method of Pachmayr as modified by Caldwell and Tiedje (1). Samples were removed from the pore water sampler chambers with a syringe and injected into a 10-ml serum vial that contained 0.5 ml of 0.2% zinc acetate in 0.2% acetic acid. Analysis was performed within 24 h after collection on a Gilford model 240 spectrophotometer. Sulfide concentrations in pore water after the addition of sulfate and sulfide to sediment were determined as follows. A 2-ml aliquot of filtered sediment supernatant was added to a vial containing 1.0 ml of zinc acetate reagent. Samples were assayed as described above.

Sulfate concentrations in pore water were determined as follows: 9 ml of pore water was placed into acid-washed test tubes (18 by 142 mm) and assayed by the turbidometric method of Tabatabi (19) on a Gilford model 240 spectrophotometer at 420 nm.

Chemicals and radioactive compounds. All chemicals used were reagent grade. Stock solutions (1 M) of Na_2SO_4, $NaCH_3COO$, and Na_2S were prepared for sediment additions and stored in N_2 gassed serum vials. Sulfide solutions were adjusted to pH 8 with HCl. The following radioactive chemicals (Amersham/Searle) were used: $NaH^{14}CO_3$, specific activity 60 mCi/mmol; and sodium [2-^{14}C]acetate, specific activity 56 mCi/mmol.

RESULTS

Analysis of sediment pore water. Water overlying the sediment and sediment interstitial water was analyzed to determine the in situ concentrations of sulfide and methane. Figure 2 shows a profile during fall turnover. The horizontal line indicates the proximity of the sediment water interface, although this region is ill defined. Sulfate was present in high concentrations in the water column, decreased at the sediment-water interface, and was depleted in the sediment. (Sediment values were not above background values for the assay.)

Methane and sulfide were not detected in the oxygenated water (6 cm above the interface) above the sediment. Methane concentrations increased at the sediment interface and reached a maximum value of 2,400 μmol/liter in the sediment. The maximum free sulfide concentration (1.35 μg/ml) occurred at the interface and decreased with increasing sediment depth.

The concentration of free sulfide detected in collected sediment samples was considerably less than that measured in situ (Table 1). This was attributed to loss of volatile sulfide during sampling and extraction of pore water.

Effect of sulfate addition. Concentrations (0.2 to 10 mM) of sulfate were added to sediments to determine the concentrations required

for inhibition of methanogenesis (Fig. 3). As little as 0.2 mM sulfate inhibited methanogenesis for 10 h. Ten millimolar sulfate caused near complete inhibition for 100 h, whereas intermediate concentrations of sulfate inhibited methanogenesis for shorter periods of time. For all sulfate concentrations tested, the rate of methanogenesis after inhibition ceased paralleled the endogenous rate. In future inhibition experiments 10 mM sulfate was used, as this concentration caused inhibition of methane production throughout the time course of the experiment. Low rates of methanogenesis (4 to 5% of the control) were observed even with the addition of 10 mM sulfate.

To study the path of methane precursors in sediment containing sulfate, $H^{14}CO_3^-$ and [2-^{14}C]acetate were used as tracers in sediment tubes containing 10 mM sulfate or no added sulfate. Evolved gases were monitored for 50 h. In the absence of added sulfate (Fig. 4A), $H^{14}CO_3^-$ was slowly converted to $^{14}CH_4$. [2-^{14}C]acetate was rapidly converted mainly to $^{14}CH_4$, although significant amounts of $^{14}CO_2$ were also produced. In sediment containing added SO_4^{2-} (Fig. 4B), only traces of $^{14}CH_4$ were produced from $H^{14}CO_3^-$ or [2-^{14}C]acetate. A fourfold increase in $^{14}CO_2$ evolution was observed from [2-^{14}C]acetate in the presence of added sulfate. To test for possible methane oxidation in Lake Mendota sediment, $^{14}CH_4$ was added to sediment that contained 10 mM sulfate and sediment without added sulfate. No decrease in $^{14}CH_4$ or production of $^{14}CO_2$ was observed for 120 h in the presence or absence of added sulfate.

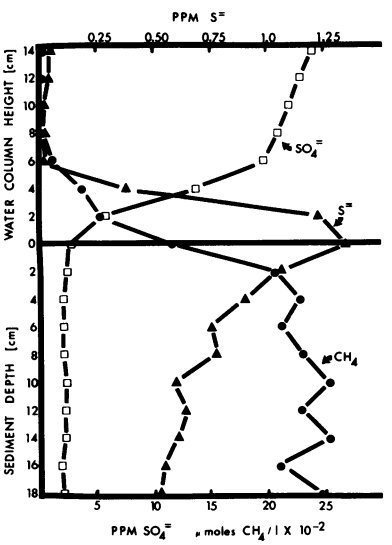

FIG. 2. *Profile showing SO_4^{2-}, S^{2-}, and CH_4 concentrations in Lake Mendota sediment. Symbols: ppm of SO_4 (micrograms per milliliter) (□); ppm of sulfide (▲); micromoles of CH_4 per liter (●). Samples were taken during lake turnover when O_2 was present throughout the water column. Values for sulfide in the sediment were below the sensitivity limit of the assay and are interpreted as essentially zero.*

Reversal of sulfate inhibition. Experiments were initiated to test the hypothesis that sulfate inhibition was a result of competition for available hydrogen. Methanogenesis and hydrogen depletion were monitored in sediment tubes that contained 88 μmol of H_2 and 10 mM sulfate. This was compared with controls without added sulfate or added H_2. Figure 5A shows that H_2 reversed sulfate inhibition of methanogenesis. When H_2 was depleted from the gas

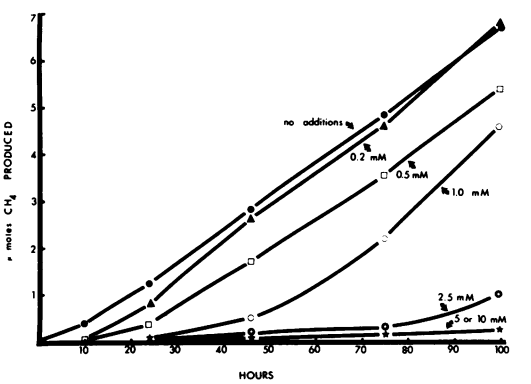

FIG. 3. *Effect of sulfate additions on methanogenesis in Lake Mendota sediments. Symbols: no additions (●); 0.2 mM SO_4 (▲); 0.5 mM SO_4^{2-} (□); 1.0 mM SO_4^{2-} (○); 2.5 mM SO_4^{2-} (✪); 5.0 or 10.0 mM SO_4^{2-} (★).*

TABLE 1. *Effect of sulfide additions on methanogenesis in Lake Mendota sediments*

Concn of added sulfide (mM)	Rate of methanogenesis (nmol/h per tube ± % S)[a]	Sulfide concn (μg/ml) after:	
		1 h of incubation	50 h of incubation
0.0	74 ± 5	0.11	0.18
0.5, 1.0, 2.5 (16, 32, 80 μg/ml)	76 ± 6	0.38	0.02
5 (160 μg/ml)	112 ± 10	9.9	1.1
10 (320 μg/ml)	56 ± 10	142	102
25 (800 μg/ml)	15 ± 6	621	610

[a] % S, Percent standard deviation.

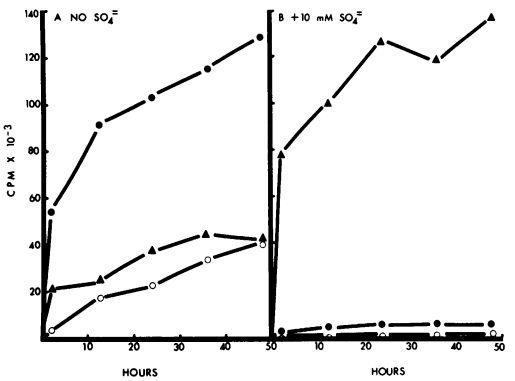

FIG. 4. *Effect of sulfate on evolution of radioactive gases from methane precursors. (A) Sediment without added sulfate; (B) sediment with 10 mM SO_4 added Symbols: $^{14}CH_4$ from 5.7 × 10^5 cpm of [2-^{14}C]-acetate (●); $^{14}CH_4$ from 10^6 cpm of $H^{14}CO_3^-$ (○); $^{14}CO_2$ from 5.7 × 10^5 cpm of [2-^{14}C]acetate (▲).*

phase, methanogenesis from sediment that contained sulfate ceased.

As large amounts of $^{14}CO_2$ are released from [2-^{14}C]acetate in sediment that contained sulfate (Fig. 4B), and because acetate appears to limit sediment methanogenesis (20), it seemed likely that competition for acetate may also be an explanation for sulfate inhibition of methanogenesis. To test this hypothesis, 1 mM acetate was added to sediment in the presence and absence of 10 mM SO_4. Rates of methanogenesis were compared with control tubes that contained no additions and sulfate alone (Fig. 5B). Addition of 1 mM acetate reversed inhibition of methanogenesis by sulfate. After 20 h the rate of methanogenesis from sediment that contained sulfate and acetate additions decreased sharply and paralleled the rate with sulfate alone, presumably due to depletion of added acetate. Similarly, the rate of methanogenesis in control tubes with acetate alone returned to the endogenous level after about 20 h.

To determine the origin of the methane produced when sulfate inhibition of methanogenesis was reversed by H_2 or acetate, ^{14}C tracer experiments were initiated. $H^{14}CO_3^-$ and [2-^{14}C]acetate were added to sediments that contained sulfate and 88 μmol of H_2 or 1 mM acetate. Figure 6A shows that $^{14}CO_2$ was converted to methane in sulfate-containing sediment when inhibition was reversed by H_2 addition. No $^{14}CO_2$ was converted to methane, however, when inhibition was reversed by acetate.

Figure 6B demonstrates that the methyl position of acetate was converted to methane when 1 mM acetate was added to reverse sulfate inhibition of methanogenesis. Small amounts of $^{14}CH_4$ were also evolved from [2-^{14}C]acetate when H_2 was added to sediment that contained sulfate. There was, however, a rapid evolution of $^{14}CO_2$ from the methyl position of acetate, which decreased until H_2 was depleted from the tubes. The rate of $^{14}CH_4$ evolution also decreased when H_2 was depleted. As $^{14}CO_2$ was converted to methane in sediments with added sulfate when H_2 was present (Fig. 6A), the $^{14}CH_4$ evolved in this experiment appears to be derived from the $^{14}CO_2$ and not the intact methyl position of acetate.

Effect of sulfide addition to sediment. Sulfide was added to sediments to test the hypothesis that sulfide produced from sulfate reduction inhibited methanogenesis. Rates of methanogenesis and sulfide concentrations in the interstitial water were monitored for 50 h. These results are shown in Table 1. The addition of up to 2.5 mM sulfide had no effect on methanogenesis, and sulfide concentrations in the pore water were not above in situ level. The addition of 5 mM sulfide caused stimulation of methanogenesis, and sulfide concentrations in the pore

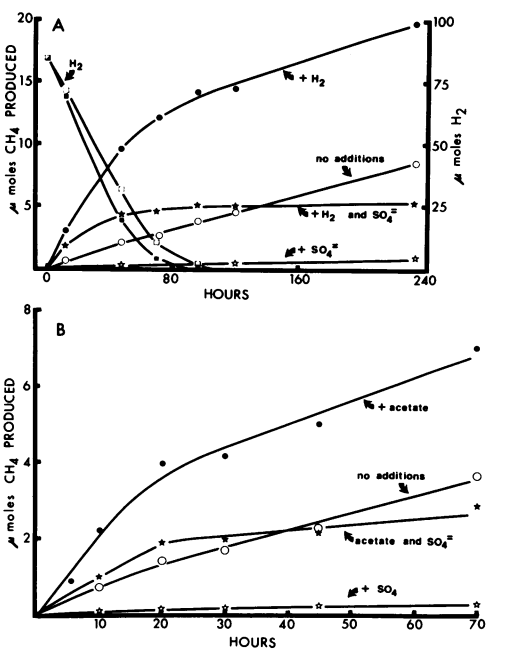

FIG. 5. *Effect of H_2 and acetate additions on sulfate inhibition of methanogenesis in Lake Mendota sediments. (A) Addition of H_2. Symbols: CH_4 produced with no additions (○); addition of 15% H_2 (●); addition of 10 mM SO_4^{2-} (☆); addition of 10 mM SO_4^{2-} and 15% H_2 (★); hydrogen depletion from sediment without additions (■); H_2 depletion from sediment containing 10 mM SO_4^{2-} (□). (B) Addition of 1.0 mM acetate. Symbols: CH_4 from sediment with no additions (○); with 1.0 mM acetate added (●); 10 mM SO_4^{2-} added (☆); 10 mM SO_4^{2-} and 1.0 mM acetate added (★).*

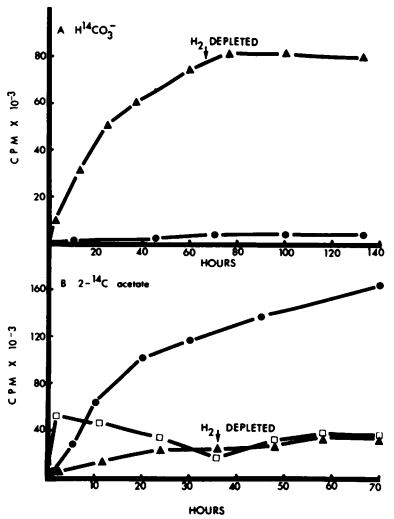

FIG. 6. *Path of carbon precursors in sediment containing 10 mM sulfate and 1.0 mM acetate or 15% H_2 additions. (A) Conversion of $H^{14}CO_3^-$ (10^6 cpm) to $^{14}CH_4$. (B) Conversion of [2-^{14}C]acetate (5.7×10^5 cpm) to $^{14}CH_4$ and $^{14}CO_2$. Symbols: $^{14}CH_4$ produced with added H_2 (▲); $^{14}CH_4$ produced with added acetate (●); $^{14}CO_2$ evolved with added H_2 (B) only (□).*

water ranged from 9.9 to 1.1 μg/ml (0.28 to 0.03 mM) throughout the course of the experiment. Addition of 10 mM sulfide caused inhibition of methanogenesis, and sulfide concentrations in pore water ranged from 142 to 102 μg/ml (4.4 to 3.2 mM). Maximum inhibition (20% of control) was obtained by the addition of 25 mM sulfide, and pore water concentrations ranged from 621 to 610 μg/ml (19.4 to 19.1 mM).

To examine the possible accumulation of sulfide in sediment pore water, 10 mM SO_4 was added to sediment and the sulfide concentration in the interstitial water was monitored for 250 h. Figure 7 shows that slight accumulation of sulfide occurs, reaching a maximum concentration of 0.83 μg/ml (0.03 mM). It is important to note that some sulfide was lost during the processing of these samples, and should not be compared with the values measured in situ. These values do, however, show relative changes in the sulfide concentration in pore water after additions.

DISCUSSION

These data indicate that sulfate inhibits methanogenesis in Lake Mendota sediments by altering normal carbon and electron flow during anaerobic mineralization. As little as 0.2 mM (19 μg/ml) SO_4^{2-} was able to effect near complete inhibition of methanogenesis (for 10 h) whereas larger concentrations inhibited for longer periods of time. Sulfate was depleted in Lake Mendota sediments, however, and would therefore not affect methanogenesis in situ.

Methane precursors, CO_2 and acetate, were not converted to methane in the presence of 10 mM sulfate. The methyl position of acetate was converted to CO_2 instead of CH_4. It appears likely that in situ hydrogen, used to reduce CO_2 to CH_4 in unaltered sediment, reduced sulfate when 10 mM SO_4^{2-} was added.

Winfrey et al. (20) have shown that H_2 and acetate are factors limiting methanogenesis in Lake Mendota sediments. The addition of either of these substrates to sediment containing sulfate was able to reverse inhibition of methanogenesis. When H_2 was added to sulfate-inhibited sediment, methane was produced from CO_2 but not from acetate. Conversely, when acetate was added, the evolved CH_4 was derived from acetate but not from CO_2. Thus, competition for both acetate and H_2 appears to be responsible for inhibition of methane production by sulfate.

This mechanism of sulfate inhibition of methanogenesis is partially compatible with the thermodynamic argument proposed by Martens and Berner (11) and Claypool and Kaplan (6). The reduction of CO_2 by H_2 to methane yields 135.1 J (32.3 kcal), whereas the reduction of sulfate to sulfide by H_2 yields 154.0 J (36.8 kcal) (7). Thus, sulfate reducers, if present in high enough numbers, should be able to outcompete methanogens for available H_2.

These results give evidence that competition for acetate is also a factor in inhibition of methanogenesis by sulfate. This observation is also supported by thermodynamic considerations. The conversion of acetate to CO_2 and CH_4 yields 28.5 J (6.8 kcal) (7). The utilization of acetate in the presence of sulfate via the reaction $SO_4^{2-} + CH_3COO^- + H^+ \rightarrow H_2S + 2HCO_3^-$ yields 47.3 J (11.3 kcal) (7). Therefore, utilization of acetate by this reaction could occur preferentially over the conversion of acetate to meth-

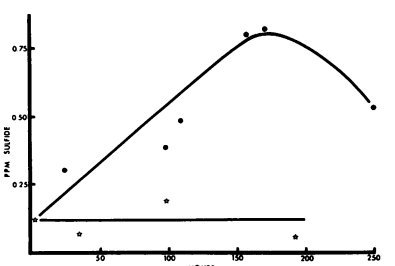

FIG. 7. *Effect of the addition of 10 mM SO_4 on sulfide concentration in sediment pore water. Symbols: ppm of sulfide (micrograms per milliliter) with no additions (☆); ppm of sulfide with 10 mM SO_4^{2-} added (●).*

ane. It is important to note that the thermodynamics of these reactions may vary with in situ or intracellular concentrations. Thus, although valuable in determining the feasibility of sediment reactions, energy yields may vary under in situ conditions. Also, the competition observed may well be the result of more efficient use of H_2 and acetate by sulfate reducers than methanogens and have a kinetic rather than a thermodynamic basis.

The discovery that acetate is utilized in the absence of methanogenic activity may have important implications concerning the anaerobic metabolism of acetate. $^{14}CO_2$, as well as $^{14}CH_4$, has previously been shown to arise from methyl-labeled acetate in lake sediments (5, 20). In the presence of sulfate, however, there is a fourfold increase in $^{14}CO_2$ and no $^{14}CH_4$ is evolved from [2-^{14}C]-acetate, indicating that acetate is being metabolized by nonmethanogenic organisms, which appear to be stimulated by SO_4. These organisms may also be responsible for the conversion of the methyl of acetate to CO_2 in the presence of methanogenic activity. Russian investigators (14, 18) have demonstrated that *Desulfovibrio* species can grow on H_2, acetate, and CO_2. Sorokin (17, 18) reported that acetate was not respired but was used for cell carbon synthesis. Species of sulfate-reducing bacteria have not been reported to oxidize acetate to CO_2. The results presented here indicate that acetate-respiring organisms may exist in the anaerobic sediment ecosystem. Recently, Pfennig and Biebl (Arch. Microbiol., in press) have described an anaerobic sulfur-reducing, acetate-oxidizing organism. It is possible that this or similar organisms are responsible for the oxidation of acetate to CO_2.

Cappenberg and Prins (5) have suggested that $^{14}CO_2$ derived from [2-^{14}C]acetate in Lake Vechten sediments was formed from CH_4 oxidation by sulfate-reducing bacteria. However, data reported here demonstrate that $^{14}CH_4$ was not oxidized to $^{14}CO_2$ in the presence or absence of added sulfate. Thus, it appears that $^{14}CO_2$ produced from [2-^{14}C]acetate in Lake Mendota sediments is a result of acetate respiration and not methane oxidation.

The proposed mechanism of sulfate inhibition of methanogenesis is not compatible with previous theories. A rise in Eh, as proposed by MacGregor and Keeney (10), seems unlikely, since the addition of H_2 or acetate in the presence of high concentrations of sulfate (10 mM) allowed methanogenesis. Furthermore, very small concentrations of sulfate (0.2 mM), which would be unable to change the Eh of the sediment, were able to inhibit methanogenesis. Cappenberg's hypothesis (2) that methanogenesis is inhibited by sulfide produced from sulfate reduction is also an unlikely explanation. Sulfide produced from sulfate reduction appears to be rapidly precipitated in Lake Mendota sediments. Free sulfide concentrations in pore water were far less than would be expected from the amount of sulfate reduced. This is likely due to the precipitation of sulfide by metals present in the sediment. Furthermore, no free sulfide was detected after the addition of as much as 2.5 mM (80 μg/ml) sulfide, and only small amounts (1 to 10 μg/ml) were detectable after the addition of 5 mM (160 μg/ml) sulfide. This concentration of sulfide in the pore water, in fact, stimulated methanogenesis, and greater than 100 μg of free sulfide per ml in the pore water was required to effect inhibition. As a maximum of 0.83 μg of sulfide per ml was detected in sediment after the addition of 10 mM sulfate, sulfide inhibition cannot be a cause of inhibition of methanogenesis. These results do, however, indicate that extremely high (greater than 100 μg/ml) concentrations of sulfide are inhibitory to sediment methanogenesis and that the concentration of sulfide in sediment may be suboptimal for methanogenesis. The exact mechanism of inhibition of methane production by high concentrations of sulfide is not known. It is possible that metal precipitation or other secondary effects may be responsible for the effect of sulfide on methanogenesis.

The utilization of acetate by sulfate-reducing organisms may have important implications in marine sediment environments containing high amounts of sulfate. Methane bacteria are terminal organisms in anaerobic food chains. They are believed to play a key role in anaerobic degradation by using fermentation end products and allowing thermodynamically unfavorable reactions to proceed (21). It seems likely that sulfate-reducing bacteria, by using acetate and H_2, may assume this same role in marine environments where methanogenesis in inhibited. This would allow anaerobic degradation to function efficiently in the absence of methanogenesis.

Oremland (13) recently reported the production of small amounts of methane (1.81 to 1.86 μmol/m^2 per h) in shallow marine sediments. The highest rates of methane production were from sea grass beds that were rich in organic matter. The results presented here may explain this observation, as more acetate and H_2 would be produced from anaerobic fermentations in organic-rich areas than in marine sediments low in organic matter. Thus, there would be less competition for these methanogenic substrates by sulfate reducers, and low rates of methanogenesis could occur.

Sulfate-reducing bacteria have been reported

to be present in high numbers in freshwater sediments, although sulfate was depleted (2). However, little is known about their metabolic activities in these environments. Cappenberg (4) has presented evidence of a commensal association between sulfate-reducing and methanogenic bacteria. He suggests that acetate produced by the sulfate reducers is metabolized by the methanogens. Bryant (Abstr. 158th Meet. Am. Chem. Soc., p. 18, 1969) has presented evidence of a symbiotic relationship between these organisms. He was able to grow strains of *Desulfovibrio* on lactate in the absence of sulfate, if a methanogen was present in the culture. Bryant concluded that the sulfate reducer transferred H_2 to the methanogen as an alternative electron sink in the absence of sulfate. Therefore, in sulfate-depleted Lake Mendota sediments, it is possible that sulfate reducers may be metabolically active by living in association with methanogenic bacteria instead of reducing sulfate.

ACKNOWLEDGMENTS

We thank D. W. Schindler and Ray Hesslein of the Experimental Lakes Area, Ontario, Canada, for describing the pore water sampling device. We also thank Pete Thomas and Bob Fallon for placing the device in the sediments using scuba equipment, and Steve Klevickis for help in collecting samples.

This research was supported by the College of Agricultural and Life Sciences, University of Wisconsin, Madison, and by grant DEB 73-01511-A01 from the National Science Foundation.

LITERATURE CITED

1. **Caldwell, D. F., and J. M. Tiedje.** 1975. The structure of anaerobic bacterial communities in the hypolimnia of several Michigan lakes. Can. J. Microbiol. **21**:377-385.
2. **Cappenberg, T. E.** 1974. Interrelations between sulfate-reducing and methane-producing bacteria in bottom deposits of a fresh water lake. I. Field observations. Antonie van Leeuwenhoek J. Microbiol. Serol. **40**:285-295.
3. **Cappenberg, T. E.** 1974. Interrelations between sulfate-reducing and methane-producing bacteria in bottom deposits of a fresh water lake. II. Inhibition experiments. Antonie van Leeuwenhoek J. Microbiol. Serol. **40**:297-306.
4. **Cappenberg, T. E.** 1975. A study of mixed continuous culture of sulfate-reducing and methane-producing bacteria. Microbl. Ecol. **2**:60-72.
5. **Cappenberg, T. E., and R. A. Prins.** 1974. Interrelations between sulfate-reducing and methane-producing bacteria in bottom deposits of a fresh water lake. III. Experiments with ^{14}C-labeled substrates. Antonie van Leeuwenhoek J. Microbiol. Serol. **40**:457-469.
6. **Claypool, G. E., and I. R. Kaplan.** 1974. The origin and distribution of methane in marine sediments, p. 99-139. *In* I. R. Kaplan (ed.), Natural gases in marine sediments. Plenum Press, New York.
7. **Decker, K., K. Jungermann, and R. K. Thauer.** 1970. Energy production in anaerobic organisms. Angew. Chem. Int. Ed. Engl. **9**:138-158.
8. **Jeris, J. S., and P. L. McCarty.** 1965. The biochemistry of the methane fermentation using C^{14} tracers. J. Water Pollut. Control Fed. **37**:178-192.
9. **Koyama, T.** 1963. Gaseous metabolism in lake sediments and paddy soils and the production of atmospheric methane and hydrogen. J. Geophys. Res. **68**:3971-3973.
10. **MacGregor, A. N., and D. R. Keeney.** 1973. Methane formation by lake sediments during in vitro incubation. Water Res. Bull. **9**:1153-1158.
11. **Martens, C. S., and R. A. Berner.** 1974. Methane production in the interstitial waters of sulfate-depleted marine sediments. Science **185**:1167-1169.
12. **Nelson, D. R., and J. G. Zeikus.** 1974. Rapid method for the radioisotopic analysis of gaseous end products of anaerobic metabolism. Appl. Microbiol. **28**:258-261.
13. **Oremland, R. S.** 1975. Methane production in shallow water, tropical marine sediments. J. Appl. Microbiol. **30**:602-608.
14. **Rozanova, E. P., and A. I. Khudiakova.** 1974. A new non-sporulating thermophilic organism *Desulfovibrio thermophilus* nov. sp. reducing sulfate. Mikrobiologiya **43**:1069-1075.
15. **Rudd, J. W., R. D. Hamilton, and N. E. R. Campbell.** 1974. Measurement of microbial oxidation of methane in lake water. Limnol. Oceanogr. **19**:519-524.
16. **Smith, P. H., and R. A. Mah.** 1966. Kinetics of acetate metabolism during sludge metabolism. Appl. Microbiol. **14**:368-371.
17. **Sorokin, Y. I.** 1966. Investigation of the structural metabolism of sulfate reducing bacteria with C^{14}. Microbiology (USSR) **35**:806-814.
18. **Sorokin, Y. I.** 1966. Sources of energy and carbon for biosynthesis in sulfate-reducing bacteria. Microbiology (USSR) **35**:643-647.
19. **Tabatabi, M. A.** 1974. Determination of sulfate in water samples. Sulphur Inst. J. **10**:11-13.
20. **Winfrey, M. R., D. R. Nelson, S. C. Klevickis, and J. G. Zeikus.** 1976. Association of hydrogen metabolism with methanogenesis in Lake Mendota sediments. Appl. Environ. Microbiol. **33**:312-318.
21. **Wolfe, R. S.** 1970. Microbial formation of methane. Adv. Microbiol. Physiol. **6**:107-145.
22. **Zeikus, J. G., P. J. Weimer, D. R. Nelson, and L. Daniels.** 1975. Bacterial methanogenesis: acetate as a methane precursor in pure culture. Arch. Microbiol. **104**:129-134.
23. **Zeikus, J. G., and M. R. Winfrey.** 1976. Temperature limitation of methanogenesis in aquatic sediments. Appl. Environ. Microbiol. **31**:99-107.