APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Feb. 1979, p. 244-253
0099-2240/79/02-0244/10$02.00/0

Vol. 37, No. 2

Anaerobic Metabolism of Immediate Methane Precursors in Lake Mendota

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Received for publication 13 November 1978

Lake Mendota sediments and the immediate overlying water column were studied to better understand the metabolism of the methanogenic precursors H_2/CO_2 and acetate in nature. The pool size of acetate (3.5 μM) was very small, and the acetate turnover time (0.22 h) was very rapid. The dissolved inorganic carbon pool was shown to be large (6.4 to 8.3 mM), and the turnover time was slow (111 h). CO_2 was shown to account for 41 ± 5.5% of the methane produced in sediment. Acetate and H_2/CO_2 were simultaneously converted to CH_4. The addition of H_2 to sediments resulted in an increased specific activity of CH_4 from $H^{14}CO_3^-$ and a decrease in specific activity of CH_4 from [2-^{14}C]acetate. Acetate addition resulted in a decrease in specific activity of CH_4 from $H^{14}CO_3^-$. The metabolism of $H^{14}CO_3^-$ or [2-^{14}C]acetate to $^{14}CH_4$ was not inhibited by addition of acetate or H_2. After greater than 99% of added [2-^{14}C]acetate had been turned over, 42% of the label was recovered as $^{14}CH_4$, 20% was recovered as $^{14}CO_2$, and 38% was incorporated into sediment. Inhibitor studies of [2-^{14}C]acetate metabolism in sediments demonstrated that $CHCl_3$ completely inhibited CH_4 formation, but not CO_2 production. Air and nitrate addition inhibited CH_4 formation and stimulated CO_2 production, whereas fluoroacetate addition totally inhibited acetate metabolism. The oxidation of [2-^{14}C]acetate to $^{14}CO_2$ was shown to decrease with time when sediment was incubated before the addition of label, suggesting depletion of low levels of an endogenous sediment electron acceptor. Acetate metabolism varied seasonally and was related to the concentration of sulfate in the lake and interstitial water. Methanogenesis occurred in the sediment and in the water immediately overlying the sediment during periods of lake stratification and several centimeters below the sediment-water interface during lake turnovers. These data indicate that methanogenesis in Lake Mendota sediments was limited by "immediate" methane precursor availability (i.e., acetate and H_2), by competition for these substrates by nonmethanogens, and by seasonal variations which altered sediment and water chemistry.

In anaerobic environments, organic matter is degraded via several trophic levels. Methanogenic bacteria are terminal organisms in the anaerobic microbial food chain present in freshwater sediments. Methanogens alter thermodynamically unfavorable decomposition reactions by metabolizing H_2 and allow for complete mineralization of organic matter by converting acetate to CH_4 (18, 34).

Acetate and H_2/CO_2 have been shown to be the major immediate precursors for methanogenesis in anaerobic ecosystems (18, 34). The relative amounts of methane derived from each of these substrates vary in different environments. Acetate has been shown to account for approximately 70% of the methane produced in anaerobic digestors (10, 26). Koyama (13) has demonstrated that 60% of the methane produced in paddy soils was derived from acetate. In Lake Vechten sediments, Cappenberg (5, 8) reported that 70% of the evolved methane was produced from the methyl position of acetate. Belyaev et al. (1) have calculated that 36 to 98% of the methane produced in several Russian lakes was derived from CO_2.

Numerous factors have been shown to influence methanogenic activity in sediments. Mallard and Frea (19) demonstrated that the production of methane in Lake Erie sediments was enhanced by the addition of methanol, acetate, ethanol, propanol, butyrate, and hydrogen gas. Similarly, Cappenberg (4) reported that methane production in Lake Vechten sediments was stimulated by lactate, acetate, and formate. Oremland (22) has shown that methanogenesis in marine sediments was stimulated by the addition of 70% H_2. The addition of nitrate (15) and sulfate (4, 33) have been shown to inhibit meth-

ane production in anoxic sediments. Oremland and Taylor (23), however, have noted that slow rates of methanogenesis occurred in marine sediments even in the presence of high concentrations of sulfate.

The methyl position of acetate has been shown to be oxidized to CO_2 in anaerobic sediments. Cappenberg (8) reported that 13% of the methyl position of acetate was converted to CO_2 in Lake Vechten sediments. Furthermore, acetate oxidation was shown to increase with the addition of sulfate to sediments (6) and to decrease with depth in a sediment core (7).

A detailed investigation was initiated in 1972 to help understand the environmental parameters that regulate methanogenesis in Lake Mendota, Wis. Methanogenesis was found to be severely limited by temperature, and the observed rates of methanogenesis varied seasonally (36). The increased rates of methanogenesis that were associated with seasonal changes correlated with increased numbers of methanogens and increased rates of metabolic activity when sediment temperatures more closely approximated the optimum temperature for methanogenesis (37 to 42°C). Radioisotopic studies (32) demonstrated that known energy sources for methanogenic bacteria were readily metabolized in sediments and that methane formation via CO_2 reduction was greatly limited by low in situ H_2 concentrations. The addition of H_2, glucose, formate, and ethanol stimulated sediment methanogenesis and CO_2 reduction. Winfrey and Zeikus (33) showed that sulfate inhibited sediment methanogenesis by altering normal carbon and electron flow during anaerobic mineralization. It was suggested that sulfate-reducing and methane-producing bacteria compete for H_2 and acetate when sulfate is present in sediments. In the present paper, we report on precursor pool sizes and turnover times, the amounts of methane derived from the carbon precursors CO_2 and acetate, and the effect of environmental parameters on the anaerobic metabolism of acetate in Lake Mendota.

MATERIALS AND METHODS

Sampling procedures. Sediment grab samples were collected from Lake Mendota throughout the year at a site under 18 m of water as previously described (32, 36). In situ sediment temperatures varied from 3 to 12°C throughout the sampling period. Sediment cores were taken with a Jenkins core sampler (36). Cores were sectioned in the laboratory as follows. The water overlying the sediment was removed by siphoning while being gassed with N_2. A plunger was inserted into the tube, the core was inverted, and sections were extruded through the bottom. Sections were transferred to N_2-gassed glass bottles, sealed with a no. 6 neopreme stopper, and stored at in situ temperature. It is important to note that considerable compression of cores (approximately 30% in cores taken by scuba divers) occurred during collection. Therefore, sediment water chemistry determined from the pore water "peeper" described below may not correlate exactly with core sections. Water above the sediment was sampled with a peristaltic pump (Horizon Ecology Co.). Water was collected from the outlet tube of the pump in 10-ml glass syringes (Glaspak), and 10 ml was injected into N_2-gassed anaerobic tubes (18 by 240 mm; Bellco Glass Co.). Tubes were stored on ice until returned to the lab where isotopic additions were made.

Sediment pore water was sampled with the Plexiglas pore water peeper (9) as previously described (33). Similar results were obtained from chemical assays done on water collected by either the pump or the pore water peeper.

Experimental procedures. The pool size of dissolved inorganic carbon ($CO_3^{2-} + HCO_3^- + H_2CO_3$) in Lake Mendota was determined as described by Stainton (27) with the modification that sealed anaerobic tubes were used instead of syringes.

Due to the extremely small pool size of acetate in Lake Mendota sediment, sediment pore water was concentrated before analysis. Freshly collected sediment was transferred to 250-ml centrifuge bottles and centrifuged at 4°C for 15 min at 4,000 × *g* on a Sorvall RC-5 centrifuge. The supernatant was filtered through a 0.45-μm membrane filter (Millipore) into a sterile flask. The filtrate (200 ml) was then transferred to a 1,000-ml round-bottomed vacuum flask, and the pH was adjusted to 10 by the addition of 3 N NaOH. The flask was fitted to a roto-evaporator (Brinkmann Instruments) and evaporated to dryness. The residue was redissolved in a known volume of water and was adjusted to pH 1.0 with HCl. A 4-μl sample was then removed for gas chromatographic analysis.

The turnover rate constant (*k*) of acetate in sediments was determined by following the decrease of [2-^{14}C]acetate in sediments. Formaldehyde (1%) was added to duplicate tubes that contained [2-^{14}C]acetate (10^5 cpm) and 10 ml of sediment at 10-min intervals for 1 h. The tubes were then centrifuged for 15 min at 750 × *g*, and the supernatant was filtered through a 0.45-μm membrane filter. The filtrate was mixed for 1 min under a stream of 100% CO_2 to remove the dissolved $H^{14}CO_3^-/{}^{14}CO_2$ produced from [2-^{14}C]acetate. This treatment removed greater than 99% of the radioactivity when a known amount of $H^{14}CO_3^-$ was added to distilled water. Sediment pore water collected 1 h after the addition of [2-^{14}C]acetate was added to a Dowex AG-1 X-10 formate column (0.7 by 15 cm) to determine whether acetate was the only radioactive compound present. The column was eluted with an increasing formic acid gradient, and 1-ml fractions were collected. Acetate was the only radioactive peak, and 95% of the added radioactivity was recovered in this peak. A 0.5-ml sample of the filtrate from each time point was counted in 10 ml of Triton X-100 scintillation cocktail on a Packard Tri-Carb scintillation counter. The Triton X-100 cocktail contained the following constituents dissolved in 3 liters of toluene: 1 liter of Triton X-100, 16 g of PPO (2,5-diphenyloxazole), and 1 g of dimethyl POPOP [1,4-bis-(5-phenyl-

oxazolyl)benzene]. The efficiency of counting obtained with this cocktail was 75 to 80%. Sediment k values were then calculated by plotting the natural log counts per minute versus time and determining the slope of the line.

Isotope tracer experiments were performed in duplicate as previously described (32). All experiments were carried out in anaerobic tubes (18 by 142 m) which contained 10 ml of sediment or lake water. Sediment grab samples were used for all experiments unless stated otherwise. All experiments were incubated at 10°C in the dark. Each experiment was done with duplicate tubes and repeated a minimum of two times to ensure reproducibility.

Gas chromatographic procedures. Evolved gases were quantified with the gas chromatography-gas proportional counting system described by Nelson and Zeikus (20). Gas samples (0.4 cm^3) were removed with a 1-cm^3 glass syringe fitted with a miniert pressure-lock syringe valve (Supelco). The syringe was flushed with O_2-free N_2 before removal of gas samples to prevent addition of O_2 to experimental tubes. Dissolved methane was determined by the gas stripping technique of Rudd et al. (25) with the modification that sealed anaerobic tubes were used instead of syringes. Measured $^{14}CO_2$ values were corrected for bicarbonate equilibrium and dissolved $^{14}CO_2$ with Bunsen absorption coefficients (27). The validity of these calculations was tested by adding a known amount of $H^{14}CO_3^-$ to sediment and measuring the amount of $^{14}CO_2$ in the headspace. By using these calculations, greater than 90% of the added $H^{14}CO_3^-$ was accounted for.

Acetate was quantified with a Packard 419 gas chromatograph with a flame ionization detector. A Teflon column (6 feet by 1/8 inch [ca. 183 by 0.32-cm ID]) packed with 3% Carbowax-20 M 0.5% H_3PO_4 on 60/80 Carbopack B (Supelco) was used for the separation. The following conditions were used: He carrier gas, 60 cm^3/min; H_2, 30 cm^3/min; air, 300 cm^3/min; oven temperature, 170°C; injector temperature, 200°C; detector temperature, 200°C.

Analysis of sulfate and sulfide. Sulfide was assayed by the method of Pachmayr as described by Caldwell and Tiedje (3). Sulfate was quantified by the turbidometric method of Tabatabai (29). Optical density was measured on a Gilford model 240 spectrophotometer.

Gases, chemicals, and radioactive compounds. All gases used were passed through heated copper filings before use to remove any traces of O_2. Mixtures of H_2 and N_2 were made with a gas proportioner (Matheson Gas Products). Aqueous stock solutions of reagent grade sodium acetate (1 M), sodium nitrate (1 M), $CHCl_3$ (1 mM), and fluoroacetate (100 mM) were prepared and stored in N_2-gassed serum vials sealed with black rubber bungs (Bellco). The following radioactive chemicals (Amersham/Searle) were used: $NaH^{14}CO_3$, specific activity 60 mCi/mmol; sodium [2-^{14}C]acetate, specific activity 56 mCi/mmol.

RESULTS

Pools and turnover times of methanogenic precursors. To determine the availability of methanogenic precursors in sediment, the pool sizes and turnover rate constants (k values) of acetate and dissolved inorganic carbon were measured. The results of these determinations are shown in Table 1. The pool size of acetate was very low (2.7 to 4.5 μM), which was near the detection limit of the assay method. A turnover rate constant of 4.51 h^{-1} was calculated for acetate in sediments, giving a turnover time of 0.22 h. The concentration of dissolved inorganic carbon ranged from 6.4 to 8.3 mM in sediments. Turnover calculations indicated that the carbonate pool was being turned over very slowly in sediments (k = 0.009 h^{-1}) and had a turnover time of 111 h. From these determinations, the turnover rate of acetate was calculated to be 15.7 μmol/liter·h, and the turnover rate of CO_2 was 66.6 μmol/liter·h.

Effect of precursor pool sizes on the amounts of methane derived from acetate and H_2/CO_2. Experiments were initiated to determine the amount of methane produced from acetate and H_2/CO_2. Because the pool size of acetate was extremely small and near the limit of detection, it was not possible to accurately determine the percentage of methane produced from acetate. However, the percentage of methane derived from CO_2 was determined as follows. $H^{14}CO_3^-$ (2.4×10^6 dpm) was added to tubes containing 10 ml of sediment and an N_2 gas phase. Experimental tubes were mixed under a stream of N_2 to remove dissolved methane in the sediment and incubated at 10°C. After 13 h of incubation, the specific activity (SA) of CO_2 and CH_4 was determined. The SA of CO_2 did not change significantly during this period (Table 2). From the ratio of the SA of CH_4 to the SA of

TABLE 1. *Turnover times and pool sizes of methanogenic precursors in Lake Mendota sediments*[a]

Methanogenic precursor	Pool sizes (mM)		Turnover rate constant (h^{-1})	Turnover time (h)	Turnover rate (μmol/liter of sediment·h)
	Range	Mean			
Acetate	2.7×10^{-3}–4.5×10^{-3}	3.5×10^{-3}	4.5	0.22	16
DIC[b]	6.3–8.4	7.4	0.009	111	67
H_2[c]	ND–3×10^{-3}				

[a] Sediment was collected on various dates throughout 2 sampling years.
[b] DIC, Dissolved inorganic carbon.
[c] Data previously reported (32). ND, Not detectable.

TABLE 2. *Percentage of methane derived from CO_2 in Lake Mendota sediments*[a]

Experiment no.	Methane analysis[b]			CO_2 analysis[b]			% CH_4 from CO_2
	μmol	dpm ($\times 10^{-3}$)	SA (dpm/nmol)	μmol	dpm ($\times 10^{-3}$)	SA (dpm/nmol)	
1	0.86	86.0	100	2.46	540	220	46
2	0.91	91.6	100	2.27	500	220	46
3	0.91	86.0	92.8	1.99	500	251	37
4	0.72	62.0	84.8	1.09	408	242	35
$\bar{x} \pm S$	0.85 ± 0.09	81 ± 12.8	94.8 ± 6.8	2.1 ± 0.34	484 ± 56	233 ± 15.6	41 ± 5.8

[a] Sediment was collected on 15 February 1978; in situ temperature was 3°C.
[b] Total gas present per tube.

CO_2, the percentage of methane derived from CO_2 was calculated. An average of 41% (±5.5%) of the methane produced in Lake Mendota sediment was found to arise from CO_2 reduction. Comparable results were obtained for sediments examined on various dates throughout the sampling year.

Experiments were designed to determine how acetate or H_2 additions affected the amount of methane arising from acetate fermentation or CO_2 reduction. Sediment tubes were gassed with varying concentrations of H_2 (0 to 50%) and mixed to equilibrate sediment pore water with the H_2 and to remove endogenous methane in the sediment. N_2 accounted for the remainder of the gas phase. $H^{14}CO_3^-$ (1.25×10^6 dpm) or [2-^{14}C]acetate (1.25×10^6 dpm) was added to each tube, and the tubes were mixed and incubated at 10°C. With the addition of hydrogen, the SA of methane from $H^{14}CO_3^-$ increased from 9.0 to 18 to 21 (Fig. 1A). The SA of methane produced from [2-^{14}C]acetate decreased eightfold with the addition of hydrogen. The addition of large amounts of H_2 (greater than 50%) did not further change the SA of methane from $H^{14}CO_3^-$ or [2-^{14}C]acetate. The partial pressures of added hydrogen did not significantly decrease within the time course of this experiment. Although the addition of hydrogen decreased the specific activity of methane derived from acetate, it is important to note that N_2 additions did not significantly affect the amount of $^{14}CH_4$ produced from [2-^{14}C]acetate (Fig. 1B).

To test the effects of increased acetate pools on the conversion of $H^{14}CO_3^-$ to methane, increasing concentrations of sodium acetate (0 to 5 mM) were added to anaerobic tubes containing 10 ml of sediment. After acetate additions were thoroughly mixed, $H^{14}CO_3$ (1.25×10^6 dpm) was added, and the tubes were incubated at 10°C. The SA of methane evolved from $H^{14}CO_3^-$ decreased approximately threefold with increasing concentrations of added acetate (Fig. 2A). The effect of acetate additions on the SA of methane derived from [2-^{14}C]acetate was not examined because the added acetate would dilute the isotope in the acetate pool. Changes in the SA of the evolved methane would be a result of a change in the SA of the acetate and not due to a change in the amount of methane produced from acetate. Figure 2B shows that acetate additions did not significantly affect the conversion of $H^{14}CO_3^-$ to $^{14}CH_4$.

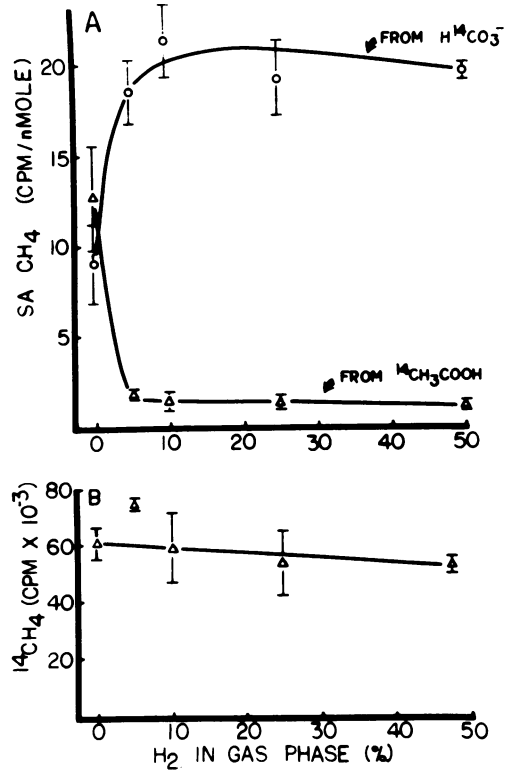

FIG. 1. *Effect of H_2 additions on the conversion of [2-^{14}C]acetate and $H^{14}CO_3^-$ to methane in Lake Mendota sediments. Sediment was collected on 22 October 1975; in situ temperature was 5°C. (A) SA of CH_4 derived from $H^{14}CO_3^-$ (at 13 h) and [2-^{14}C]acetate (at 1 h). (B) $^{14}CH_4$ produced at 1 h from [2-^{14}C]acetate. Error bars represent ±1 standard deviation.*

Anaerobic oxidation of acetate. Experiments were initiated to determine the extent

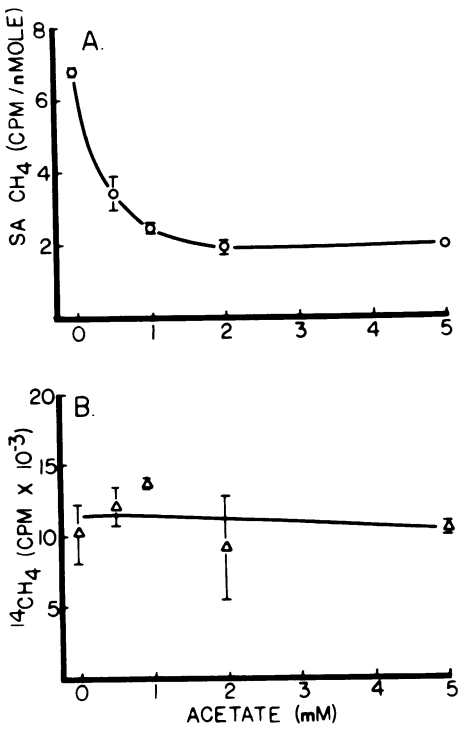

FIG. 2. *Effect of acetate additions on the conversion of $H^{14}CO_3^-$ to methane in Lake Mendota sediment. Sediment was collected on 12 April 1976; in situ temperature was 5°C. (A) SA of CH_4 derived from $H^{14}CO_3^-$ (at 150 h). (B) $^{14}CH_4$ produced at 150 h from $H^{14}CO_3^-$. Error bars represent ±1 standard deviation.*

of the oxidation of the methyl group of acetate to CO_2 in sediments and the factors that influenced its oxidation. To determine how much of the methyl of acetate was converted to CH_4 and CO_2, 1.3×10^6 dpm of [2-^{14}C]acetate was added to sediment, and the amount of $^{14}CH_4$ and $^{14}CO_2$ produced was measured after nine turnovers (i.e., 2 h) had elapsed. This allowed for removal of greater than 99% of the [2-^{14}C]acetate from the acetate pool. Table 3 shows that 42% of the isotope was recovered as $^{14}CH_4$, 20% was recovered as $^{14}CO_2$, and 38% was incorporated into sediment. To show the degree of acetate oxidation to CO_2, a respiratory index (RI value) was used. The RI value is defined as follows: RI value = $^{14}CO_2/(^{14}CO_2 + {}^{14}CH_4)$, where $^{14}CO_2$ and $^{14}CH_4$ are produced from [2-^{14}C]acetate. An RI value of 1.0 indicates oxidation of acetate only to CO_2, whereas increasing lower values indicate increasing amounts of methane formed from the methyl position of acetate. The RI value for acetate metabolism in sediments was 0.32. However, considerable variation (±50%) in this value was observed with sediment grab samples collected on different dates.

TABLE 3. *Amount of CO_2 and CH_4 derived from the methyl position of acetate in Lake Mendota sediment*[a]

Experiment no.	$^{14}CH_4$[b] (dpm × 10^{-5})	$^{14}CO_2$[c] (dpm × 10^{-5})	RI value
1	5.20	1.43	0.21
2	5.83	3.52	0.38
3	5.48	2.48	0.31
4	5.30	2.96	0.36
$\bar{x} \pm S$	5.48 ± 0.25	2.60 ± 0.83	0.08±

[a] Sediment was collected on 15 February 1978; in situ temperature was 3°C.
[b] Recovery of acetate as CH_4 was 42%.
[c] Recovery of acetate as CO_2 was 20%.

To test whether the CO_2 produced from the methyl position of acetate in sediments was due to methane-producing bacteria, [2-^{14}C]acetate (1.25×10^6 dpm) was added to sediment that contained various inhibitors (Table 4). Inhibitors were added 8 h before the addition of [2-^{14}C]acetate. $CHCl_3$, an inhibitor of methane production, completely inhibited acetate conversion to methane, but caused only slight inhibition of acetate oxidation to CO_2. Both air and nitrate completely inhibited methanogenesis from acetate, but stimulated acetate oxidation. Fluoroacetate inhibited both acetate conversion to methane and oxidation to CO_2.

Experiments were designed to determine the basis for the large variance in the amount of the methyl of acetate oxidized to CO_2 in sediment samples collected on different dates. To test whether electron acceptors were present which allowed acetate respiration to CO_2, sediment was incubated in the lab to allow consumption of endogenous electron acceptors. At various time intervals, [2-^{14}C]acetate (1.25×10^6 dpm) was added to the sediment, the amounts of evolved $^{14}CH_4$ and $^{14}CO_2$ were monitored with time, and the RI value was calculated. The RI values shown (Fig. 3) were determined 30 min after the addition of the [2-^{14}C]acetate. As the incubation time increased, more $^{14}CH_4$ and less $^{14}CO_2$ were produced from methyl-labeled acetate, and the RI value decreased from 0.49 to 0.15. Identical results were obtained with sediments collected when the lake was stratified.

Effect of sediment depth and seasonal variation on [2-^{14}C]acetate metabolism. Sediment cores and water overlying the sediment were collected to examine acetate metabolism at different depths in the core and in the water immediately above the sediment. [2-^{14}C]acetate (1.25×10^6 dpm) was added to anaerobic tubes containing 10 ml of lake water or sediment, and RI values were determined. Sulfate and sulfide concentrations were also monitored at each sampling depth. Experiments were

TABLE 4. *Effect of inhibitors on [2-^{14}C]acetate metabolism in Lake Mendota sediments*

Inhibitor	Gas produced (dpm × 10^{-3})		RI value
	$^{14}CH_4$	$^{14}CO_2$	
None[a]	157	44.8	0.22
100 μM $CHCl_3$[a]	0.0	37.0	1.00
0.1 mM fluoroacetate[a]	0.0	0.0	
100% Air[b]	0.0	85.7	1.00
5 mM NO_3^-[b]	0.0	97.7	1.00

[a] Sediment was collected in November, 1976; in situ temperature was 5°C. Values were determined after 1.5 h of incubation.

[b] Sediment was collected on 21 February 1977; in situ temperature was 3°C. Values were determined after 1.0 h of incubation. Air was added by mixing a tube open to the atmosphere for 1 min. These tubes were mixed again in air (1 min) when the isotope was added.

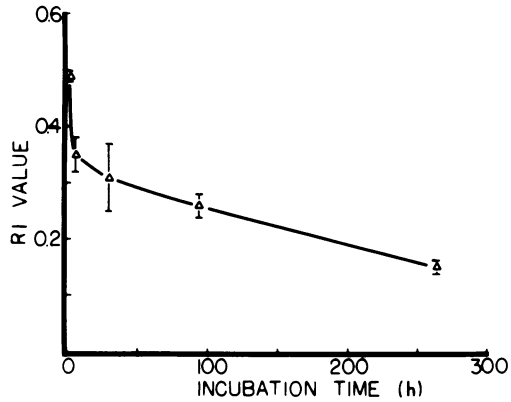

FIG. 3. *Effect of sediment incubation time before isotope addition on [2-^{14}C]acetate metabolism in Lake Mendota sediment. Sediment was collected on 16 May 1977; in situ temperature was 9°C. Error bars represent ±1 standard deviation.*

repeated at various times of the year to test the effect of stratification and lake turnovers on acetate metabolism. Figure 4A shows the concentration of sulfide and sulfate in Lake Mendota sediment and overlying water during summer stratification. Sulfide was present in the water above the sediment at a concentration of about 1 μg/ml and about 3 μg/ml in the sediment. Sulfate was present in water 1 m above the sediment at 8 μg of SO_4^{2-}-S per ml and decreased gradually to 6.6 μg/ml at the interface. Sulfate was not detected below the sediment-water interface. An RI value of 1.0 was calculated for water collected between 10 and 100 cm above the interface (Fig. 4B). The RI value began to decrease at 5 cm above the interface, indicating the production of methane from acetate in the water column above the sediment. RI values of 0.3 to 0.4 were calculated in the sediment, indicating acetate was being metabolized primarily to methane. During periods of ice cover, Lake Mendota undergoes reverse stratification, and oxygen is again depleted in the water above the sediment. Experiments with cores and water samples collected during February, 1978, revealed that methanogenesis again occurred in the water above the sediment. An RI value of 0.75 was calculated at 5 cm above the sediment-water interface, whereas samples above this depth had RI values of 1.0. RI values between 0.3 and 0.5 were measured in the sediment core.

Figure 5 shows the results of an experiment performed during the fall turnover when oxygen was mixed throughout the water column of the lake. Sulfide (Fig. 5A) was absent in the aerobic waters above the sediment, increased to a maximum of 1.0 μg/ml immediately below the sediment, and decreased in deeper regions of the core. Sulfate was present at 8 μg of SO_4-S per ml in the water overlying the sediment, but was not detectable in the sediments. Figure 5B shows that acetate is metabolized only to CO_2 in the water above the sediment and even below the interface. Methane production from acetate was first detected at 5 cm below the interface. RI values of 0.5 to 0.6 were calculated for sediment core sections. Experiments performed during the spring turnover revealed results similar to those obtained during the fall turnover.

DISCUSSION

These results demonstrate that methanogenesis in Lake Mendota is limited by methanogenic precursor availability. The pool sizes of acetate reported here and of H_2 (32) were very minute and turned over rapidly in sediments. CO_2, however, was abundant in sediments, and the CO_2 pool turned over more slowly. Thus, methanogenesis in this aquatic ecosystem differs significantly from man-made anaerobic sewage digestors where anaerobic decomposition has been reported to be limited by the rate at which methanogenic bacteria ferment acetate (12, 34). In Lake Mendota sediments the rate-limiting step in methanogenesis appears to be the rate at which H_2 and acetate are supplied to the small methane precursor pool. This fact may in itself be a reflection of the rate at which microorganisms catabolize particulate matter (i.e., biopolymers) into immediate methane precursors. Strayer and Tiedje (28) have also shown H_2 limitation of methanogenesis in Lake Wintergreen sediments. However, in this sediment environment, methanogenesis was not stimulated by acetate addition, suggesting that acetate metabolism to CH_4 was occurring at or near the

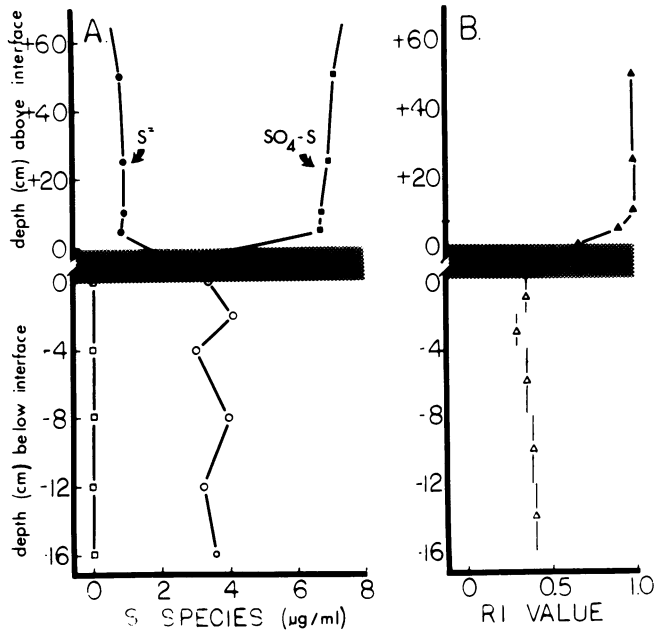

FIG. 4. *Relation between acetate metabolism and sulfur water chemistry in Lake Mendota during summer stratification. (A) Lake and interstitial water chemistry. Samples were collected on 29 June 1977. Symbols: S^{2-} (µg/ml), samples collected by pump (●); S^{2-} (µg/ml), samples collected from pore water peeper (○); SO_4-S (µg/ml), samples collected by pump (■); SO_4-S (µg/ml), samples collected from pore water peeper (□). (B) RI values in sediment and bottom waters. Water and sediment core were collected on 2 July 1977; in situ temperature was 11.2°C. Symbols: RI value in water column (▲); bars indicate RI value in sections of sediment core. The length of the bar designates the thickness of core section used for sediment samples. The shaded area in the figure indicates the region of the sediment-water interface.*

maximal rate. It is important to note that both H_2/CO_2 and acetate were simultaneously converted to methane in sediments, even in the presence of excess H_2 or acetate. This indicates that metabolism of these substrates is not mutually exclusive in nature and suggests that acetate fermentation by methanogens does not require low H_2 partial pressures as has been suggested for decomposition of higher fatty acids (2). Van den Berg et al. (30) showed that H_2 addition had no effect on acetate catabolism by an enrichment culture. Kaspar and Wuhrmann (11) reported that acetate turnover in sewage sludge was not affected by an increase in the H_2 partial pressure. Acetate has been shown to be metabolized by pure cultures of *Methanosarcina* in the presence or absence of H_2 (30a, 35). This organism has been isolated from Lake Mendota sediments (30b, 36). The significance of acetate metabolism by *Methanosarcina* in nature has not been established. The turnover rate of acetate in laboratory cultures (30a) under optimal conditions (37°C in complex medium) by *M. barkeri* isolated from Lake Mendota sediments is approximately 50 times greater in the presence or absence of added H_2 than the rate observed here in sediments. Thus, organisms with metabolic properties similar to *M. burkeri* (16, 17, 30a, 30b) may be of importance to sediment methanogenesis.

The amount of methane formed from acetate-carbon and CO_2-carbon has been shown to vary significantly in different lake sediments (1, 8). In Lake Mendota sediments 41% (±5.5%) of the methane was derived from HCO_3^--C. It was not possible to derive a statistically significant value for the amount of methane formed from acetate-C because of the variation in pool size, rapid turnover rate, and metabolism of [2-^{14}C]acetate to both $^{14}CO_2$ and $^{14}CH_4$. Based on the assumption that CO_2 and acetate alone are the methane-carbon precursors in nature (18), 59% of the methane would be formed from acetate in Lake Mendota sediments. However, CH_3OH, CH_3NH_2 (30b), and other undescribed substrates may also be of significance to total methane formation.

The differences in values observed for the origin of methane in various lake sediments (1, 8) may be a result of variations in interstitial water chemistry and microbial activities. The relative concentrations of H_2 and acetate in sediments were shown to determine what the major

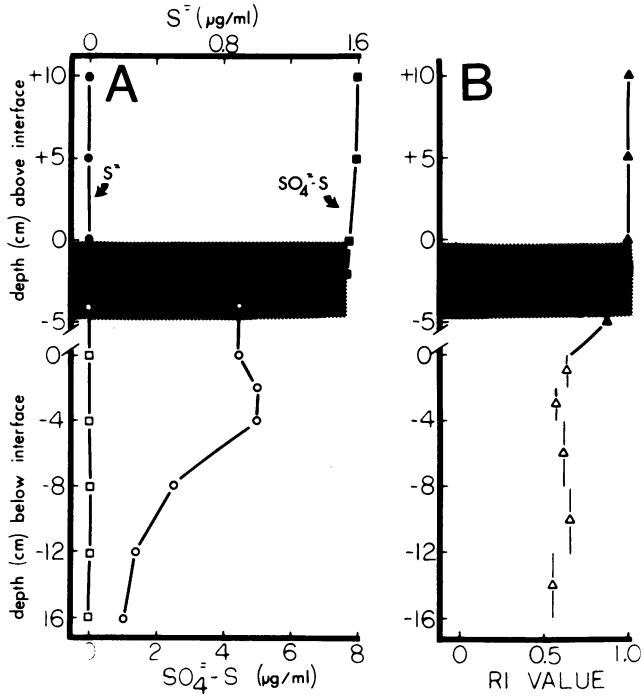

FIG. 5. *Relation between acetate metabolism and sulfur water chemistry in Lake Mendota during fall turnover. (A) Lake and interstitial water chemistry. Samples were collected on 3 November 1977. Symbols are the same as in Fig. 4. (B) RI values in sediment and bottom waters. Water and sediment collected on 3 November 1977; in situ temperature was 10.4°C. Symbols are the same as in Fig. 4. Surface sediments (2 and 5 cm below interface) were collected by lowering the end of the pump tubing the designated distance below the sediment-water interface. The sediment-water interface (shaded area) is considerably wider than in Fig. 4 because of the inclusion of RI values calculated from surface sediments collected with the pump.*

methane-carbon precursor will be. In the presence of excess H_2, CO_2 was the major methane-carbon precursor, whereas acetate was the major precursor when excess acetate was added to sediments. These results support the hypothesis of Zeikus et al. (35) that acetate may be the major methane precursor under conditions of limiting H_2. The simultaneous conversion of the methyl position of acetate to CO_2 and CH_4 in Lake Mendota sediments and the variances of RI values observed in different sediment samples are also significant in evaluating the amount of methane derived from acetate. Oxidation of significant amounts of acetate would further limit the amount of acetate available for methanogenesis. In addition, acetate and H_2 (32) consumption by nonmethanogens (33) indicate that other sediment anaerobes share the role of methane-producing bacteria as terminal organisms in anaerobic microbial food chains.

The anaerobic oxidation of the methyl of acetate to CO_2 reported here has previously been observed in the sediments of Lake Mendota (32, 33) and Lake Vechten (6–8). Equal amounts of CO_2 and CH_4 were produced from the methyl of acetate in freshly collected (1 h) sediment. The amount of C_2-acetate converted to CO_2 in situ may be even greater than demonstrated in Lake Mendota sediment as the amount of oxidation to CO_2 rapidly decreased with older sediment samples. The low levels of C_2-acetate metabolism to CO_2 observed in the presumed absence of endogenous electron acceptors (e.g., S°, SO_4^{2-}) may be a result of methanogenic bacteria. In the presence of methanol or methylamine significant amounts of C_2-acetate are converted to CO_2 by *M. barkeri* (30a); smaller amounts of CO_2 are produced by methanogens (30a, 35) in the presence of H_2/CO_2.

The oxidation of acetate in the presence of $CHCl_3$, an inhibitor of methanogenesis, demonstrated that nonmethanogens were largely responsible for this process in freshly collected sediments. Complete oxidation of acetate to CO_2 has only been demonstrated in organisms which use the tricarboxylic acid cycle and transfer electrons to an external electron acceptor. Total inhibition of acetate metabolism by fluoroacetate suggests that tricarboxylic acid cycle enzymes are involved in acetate oxidation. Fluo-

roacetate has been shown to result in the formation of fluorocitrate a potent inhibitor of aconitase (14). Lack of activity in this enzyme would result in an inactive tricarboxylic acid cycle. The addition of sulfate (33), air, and nitrate stimulated oxidation of the methyl of acetate, although these electron acceptors were not detected in Lake Mendota sediment. Thus, there is some question as to what the electron acceptor used for acetate oxidation is in Lake Mendota sediment. Pfennig and Biebl (24) have recently isolated *Desulfuromonas acetoxidans*, which is able to anaerobically oxidize acetate to CO_2 using elemental sulfur as a terminal electron acceptor. Sulfur may well be the endogenous electron acceptor, because Nriagu (21) has measured between 13 and 36 μg of elemental sulfur per ml in Lake Mendota sediments. Thus, *D. acetoxidans* or metabolically similar organisms may be responsible for acetate oxidation in Lake Mendota sediments.

In surface sediments and the overlying anaerobic waters, sulfate is likely the major electron acceptor responsible for acetate oxidation. In the anaerobic sulfate-containing waters overlying Lake Mendota sediments, acetate was converted primarily to CO_2. The amount of acetate oxidation sharply decreased, however, when sulfate became depleted immediately below the sediment-water interface. These data suggest that both methanogenesis and sulfate reduction are important processes in carbon mineralization in Lake Mendota. Widdel and Pfennig (31) have demonstrated that *Desulfotomaculum acetoxidans* grows by acetate oxidation using sulfate as a terminal electron acceptor. This species or other metabolically similar bacteria may be responsible for anaerobic oxidation in waters overlying Lake Mendota sediment and could compete with methanogens for acetate as an energy source.

The results presented here demonstrate the dynamic nature of methanogenesis in Lake Mendota. Methanogenic rates have been shown in several lakes to be highest in surface sediments (18, 34), although the water chemistry and microbial activities in this region drastically change with seasonal turnovers. Methanogenic activity in Lake Mendota clearly reflects these seasonal changes. During periods of stratification, methanogenesis was observed in the water overlying the sediment, but was first detected several centimeters below the sediment-water interface during periods of turnover. During turnovers, oxygenated water is mixed throughout the water column, and oxygen-sensitive methanogens appear to be inhibited. Mixing of increased amounts of sulfate from overlying waters with surface sediments may further inhibit methanogenesis after oxygen depletion. Due to the extremely small pool size of acetate in Lake Mendota, competition for this energy source between methanogenic and acetate-oxidizing organisms may greatly affect methanogenesis. Nutrient limitation and the rapid metabolism of endogenous substrates with time are important considerations in sampling and designing experiments to assess microbial activities in sediments. Because of these considerations, it is also important to recognize that laboratory experiments may not accurately demonstrate actual in situ activities. The data presented here further demonstrate differences in various anaerobic ecosystems and emphasize that care should be taken when comparing Lake Mendota sediments to other methanogenic environments.

ACKNOWLEDGMENTS

This research was supported by the College of Agriculture and Life Sciences, University of Wisconsin, and by grant DEB 73-01511-A01 from the National Science Foundation. M. R. Winfrey was supported by a Cellular and Molecular Biology Training Grant from the National Institutes of Health and by National Science Foundation grant DEB 73-01511-A01 and DEB 782-4071.

We thank Bob Fallon for placing the pore water peeper in place by scuba diving and Steve Klevickis for help in sampling.

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