APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Feb. 1979, p. 213-221
0099-2240/79/02-0213/09$02.00/0
Vol. 37, No. 2

Microbial Methanogenesis and Acetate Metabolism in a Meromictic Lake

M. R. WINFREY AND J. G. ZEIKUS*
Department of Bacteriology, University of Wisconsin, Madison, Wisconsin 53706

Received for publication 17 November 1978

Methanogenesis and the anaerobic metabolism of acetate were examined in the sediment and water column of Knaack Lake, a small biogenic meromictic lake located in central Wisconsin. The lake was sharply stratified during the summer and was anaerobic below a depth of 3 m. Large concentrations (4,000 μmol/liter) of dissolved methane were detected in the bottom waters. A methane concentration maximum occurred at 4 m above the sediment. The production of $^{14}CH_4$ from ^{14}C-labeled HCOOH, HCO_3^-, and CH_3OH and [2-^{14}C]acetate demonstrated microbial methanogenesis in the water column of the lake. The maximum rate of methanogenesis calculated from reduction of $H^{14}CO_3^-$ by endogenous electron donors in the surface sediment (depth, 22 m) was 7.6 nmol/h per 10 ml and in the water column (depth, 21 m) was 0.6 nmol/h per 10 ml. The methyl group of acetate was simultaneously metabolized to CH_4 and CO_2 in the anaerobic portions of the lake. Acetate oxidation was greatest in surface waters and decreased with water depth. Acetate was metabolized primarily to methane in the sediments and water immediately above the sediment. Sulfide inhibition studies and temperature activity profiles demonstrated that acetate metabolism was performed by several microbial populations. Sulfide additions (less than 5 μg/ml) to water from 21.5 m stimulated methanogenesis from acetate, but inhibited CO_2 production. Sulfate addition (1 mM) had no significant effect on acetate metabolism in water from 21.5 m, whereas nitrate additions (10 to 14,000 μg/liter) completely inhibited methanogenesis and stimulated CO_2 formation.

The microbial production of methane in anaerobic lake sediments is a common phenomenon and has recently been studied in detail. Methane is largely produced as a result of the microbial metabolism of acetate or H_2 and CO_2, which are produced from the anaerobic degradation of organic matter. Acetate has been shown by several investigators (3, 8) to account for the majority of the methane formed in lake sediments. Recently, evidence has been provided which demonstrates that acetate and H_2 are also metabolized by non-methanogenic organisms in lake sediments (24, 25).

Several chemical parameters of environmental significance have been shown to influence methanogenic activity in nature. Nitrate has long been known to be a potent inhibitor of methanogenesis in lake (9) and estuarine sediments (1) and in anaerobic soils (2, 4). Sulfate has been shown to inhibit methane production in lake sediments (6, 13, 25) and to stimulate the oxidation of acetate to CO_2 (7, 25). Rates of methane formation have also been shown to be influenced by the addition of sulfide. Sediment methanogenesis in Lake Mendota was stimulated by sulfide concentrations up to 0.28 mM (9.9 μg/ml), whereas inhibition did not occur until sulfide concentrations in the pore water reached 3.2 mM (102 μg/ml) (25).

Previous studies on methanogenesis in lakes have been restricted to holomictic lakes which undergo annual or semiannual periods of turnover. Meromictic lakes do not undergo periods of complete mixing and thus provide stable anaerobic environments in the bottom waters. Primary production and photosynthetic bacteria have been studied in several meromictic lakes (10, 20, 22), although little research has been conducted on the microbial activities in the anoxic waters. Matsuyama and Saio (15) have investigated the decomposition of organic matter in meromictic Lake Suigetsu. Active sulfate reduction in the anaerobic portion of the lake was suggested to be the major mechanism of organic decomposition.

Methanogenesis in meromictic lakes has not been studied in the past. Weimer and Lee (23) detected large amounts of dissolved methane in Lake Mary. The distribution of methane in the lake indicated that production occurred primarily in the sediments. Richards et al. (18) have quantified products of anaerobic decomposition

in Lake Nitinat, an anoxic fjord in British Columbia. From the distribution of methane in the water column, these workers suggested that methane may be produced in the water column itself. They did not, however, directly measure the production of methane in the water.

Recently, the chemical and physical properties of Knaack Lake, a meromictic lake located in central Wisconsin, have been studied. We report here on the production of methane in the water column of Knaack Lake and how various environmental parameters influence the anaerobic metabolism of acetate in the lake.

MATERIALS AND METHODS

Description of the lake. Knaack Lake is a small (1.1-hectare) meromictic lake located 8 miles (ca. 1.6 km) south of the town of Marion in central Wisconsin and has a maximum depth of 22 m. Detailed studies on the chemical and physical limnology of the lake have been completed (T. Parkin, M. Winfrey, and T. D. Brock, manuscript in preparation). These workers showed that the lake was anaerobic below the thermocline (3 m) in the summer, mixed to a depth of 12 to 14 m during the fall, and was anaerobic to the ice layer during the winter. No significant mixing occurred after the ice melted in the spring. The small area of the lake, sheltering characteristics of the surrounding landscape, and biogenic production of solutes in the bottom waters were believed to maintain meromixis.

Sampling procedures. Water was collected at a site over the deepest region of the lake (22 m) with a peristaltic pump (Horizon Ecology Co.). The pump was attached to thick-walled 3/16-inch (ca. 4.8-mm) inside diameter amber latex tubing (Sargent Welch Co.) which was affixed to a weighted chain. Sampling depth was regulated by lowering the chain to the desired depth in the lake. Water from the outlet tube of the pump was then collected in sample bottles or syringes as described below. The use of this system minimized exposure of anaerobic water to air and permitted sampling at narrow intervals without disturbing the water. Sediment was collected with an Eckman dredge as previously described (27).

Chemical and physical analysis of lake water. Measurement of temperature and oxygen were made in situ with a Yellow Springs Instruments model 51B combination temperature-oxygen meter. Sulfate and sulfide were quantified as previously described (25). Dissolved methane and dissolved inorganic carbon (dissolved inorganic carbon = CO_2 + HCO_3^- + $H_2CO_3^{2-}$) were determined by injecting 5 ml of lake water into He-gassed anaerobic tubes which contained 0.5 ml of 6 N HCl. The acid converted all HCO_3^- and H_2CO_3 to CO_2 and simplified calculations. Tubes were mixed for 1 min to strip dissolved gasses into the gas phase, and a 0.4-ml sample was removed for gas chromatographic analysis. Dissolved inorganic carbon values were corrected for dissolved CO_2 by using Bunsen absorption coefficients.

Experimental procedures. Initially experiments were performed in anaerobic tubes (18 by 142 mm; Bellco Glass Co.) containing 10 ml of lake water. Water for these experiments was collected from the outlet hose of the pump as follows. The plunger was removed from a 10-ml glass syringe (Glaspak), and the syringe was inserted into the outlet tube of the pump. The syringe was held upright and allowed to overflow with anaerobic water for 30 s. The plunger was then inserted, using caution to prevent trapping air bubbles in the syringe. Water (10 ml) was then injected into N_2-gassed anaerobic tubes fitted with butyl rubber stoppers. Tubes were placed on ice and returned to the laboratory (ca. 4 h) where additions were made by the Hungate technique (5). All tube experiments were incubated at 10°C in the dark. At desired intervals 0.4 ml of the headspace was removed with a 1-ml pressure lock syringe for gas analysis.

Tube experiments were disadvantageous in that gas was stripped from the water into the headspace and sulfide was readily adsorbed to the rubber stoppers. Within 24 h 90% of the endogenous sulfide (0.6 to 1.0 μg/liter) was shown to be removed from the lake water incubated in anaerobic tubes. To avoid these disadvantages, experiments were repeated in 300-ml biological oxygen demand (BOD) bottles completely filled with lake water. The outlet tube of the pump was inserted into a bottle, which was filled and completely flushed with water three times. Additions were made with a 1-ml syringe from anaerobic solutions stored in N_2-gassed serum vials. Ground-glass stoppers were inserted, and the bottles were incubated in the lake at the same depth that the water was collected.

At the termination of the experiment, bottles were removed from the lake, placed on ice, and transported back to the laboratory. Water from each bottle was then analyzed for sulfide, $^{14}CH_4$, $^{14}CO_2$, ^{14}C incorporated into cell carbon, and ^{14}C remaining in the water. A small bubble (<1 ml) was formed from degassing BOD bottles filled with water collected from below 21 m. However, because less than 7% of the dissolved $^{14}CH_4$ and 1% of the $^{14}CO_2$ is lost through degassing, this procedure did not significantly affect the results obtained. $^{14}CO_2$ and $^{14}CH_4$ were removed from water by the syringe stripping technique of Rudd et al. (19). ^{14}C uptake into cell carbon was determined by filtering 5 ml of water from each BOD bottle through a 0.45-μm membrane filter (Millipore Inc.), which was then washed three times with 2 ml of distilled water and dried overnight in HCl fumes. The HCl removed any precipitated $^{14}CO_3^{2-}$ that might be present on the filters (12). Filters were then counted in 10 ml of toluene scintillation cocktail in a Packard Tri-Carb scintillation counter. The filtrate was mixed under a stream of CO_2 for 30 s to remove any $^{14}CH_4$ or $^{14}CO_2$ dissolved in the water. This technique removed more than 99.9% of a known amount of $H^{14}CO_3^-$ which had been added to distilled water. A 0.5-ml sample of the filtrate was then counted in 10 ml of Triton X-100 scintillation cocktail to determine the amount of radioactive carbon remaining in the water. Experiments performed in anaerobic tubes yielded results similar to identical experiments in BOD bottles. However, experiments with BOD bottles gave more reproducible results and displayed less variation between replicate determinations.

Analysis of gaseous end products. Gas samples from experimental tubes or from gas stripped from

lake water were analyzed for CH_4, CO_2, $^{14}CH_4$, and $^{14}CO_2$ by using the gas chromatography-gas proportional counting system described by Nelson and Zeikus (16). CO_2 values were corrected for bicarbonate equilibrium and CO_2 solubility. When used below, the term CO_2 is assumed to represent total CO_2, HCO_3^-, and H_2CO_3.

To readily determine the relative amounts of acetate converted to methane and respired to CO_2, a respiratory index (RI value) was used: RI value = $^{14}CO_2/(^{14}CO_2 + ^{14}CH_4)$, where $^{14}CO_2$ and $^{14}CH_2$ were produced from [2-^{14}C]acetate. RI values ranged from 0 to 1.0; a value of 1.0 indicated oxidation of acetate only to CO_2, and decreasing RI values indicated greater methane production.

Chemicals and radioactive compounds. All chemicals used were reagent grade. Stock solutions were prepared with degassed distilled water and stored in N_2-gassed serum vials. The following radioactive compounds (Amersham/Searle) were used: $NaH^{14}CO_3$, with a specific activity of 60 mCi/mmol; [2-^{14}C]acetate, with a specific activity of 56 mCi/mmol; ^{14}C-methanol, with a specific activity of 60 mCi/mmol; and sodium [^{14}C]formate, with a specific activity of 51 mCi/mmol.

RESULTS

Water chemistry. Various chemical parameters were measured throughout the water column of the lake during a 2-year period. A typical midsummer profile is shown in Fig. 1. A sharp thermocline was observed between 1 and 3 m, where the temperature dropped from 26 to 4°C. The temperature remained at 4°C to a depth of 16 m, where it began to rise, reaching a maximum of 5.5°C at the bottom. This increase in temperature was observed throughout the year and was probably due to an increase in density of bottom waters. Oxygen (8 μg/ml) was detected at the surface, was rapidly depleted at the thermocline, and was absent below 3 m. Sulfide was absent in the oxygenated surface waters, but was detected at 0.6 μg/ml throughout the anaerobic portion of the lake. Sulfate was not detected in the lake. Nitrate was detected periodically in the surface waters, but was never detected below the thermocline.

Figure 1B shows profiles of conductivity and dissolved methane in the water column. Conductivity was 70 μmHO at the surface and was relatively constant to a depth of 12 m. Below this depth conductivity increased, reaching 300 μmHO above the sediment (21.5 m). Methane was absent in the oxygenated surface waters and was first detected at 3 m. Below the thermocline, dissolved methane concentrations gradually increased with depth, reaching a maximum at 18 m, and were relatively constant to the bottom. Similar profiles were observed throughout the year. The detection of maximum methane concentrations several meters above the sediment suggests that methanogenesis occurs in the water column of the lake.

Location and rate of methanogenesis. It was not possible to measure directly rates of methanogenesis in the water column because rates were low and high endogenous concentrations of methane masked production. Therefore, radioisotopic tracer experiments were used to demonstrate methanogenesis. Table 1 shows

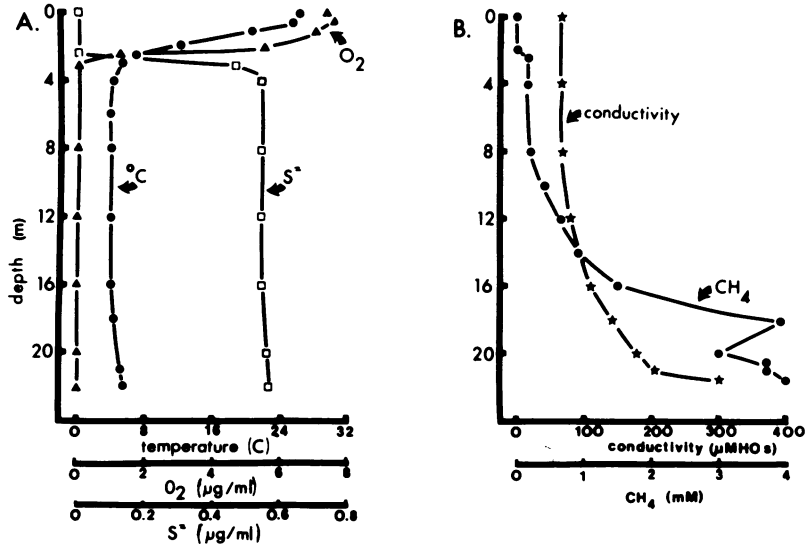

FIG. 1. *Knaack Lake water chemistry. (A) Temperature, oxygen, and sulfide profiles. (B) Conductivity and dissolved methane profiles. The analyses were done on 27 May 1977.*

that the immediate methane carbon precursors CO_2, methanol, formate, and the methyl group of acetate were all converted to methane in water from 21.5 m. The small amount of $H^{14}CO_3^-$ converted to methane was due to dilution of the isotope in the large CO_2 pool (6 to 10 mmol/liter) and the absence of detectable H_2 in the water. ^{14}C-formate was converted primarily to $^{14}CO_2$. Small amounts of $^{14}CH_4$ were formed from the addition of labeled formate or carbonate, probably as the result of dilution with the endogenous CO_2 pool. ^{14}C-methanol and [2-^{14}C]acetate were converted primarily to methane, although significant amounts of $^{14}CO_2$ were also produced.

The rate of methanogenesis in the water column was estimated from the amount of $H^{14}CO_3^-$ converted to $^{14}CH_4$. Because of the large pool size of CO_2 in the monimolimnion, large amounts of $H^{14}CO_3^-$ could be added without affecting the in situ pool size. The amount of methane produced during a given incubation period was calculated by assuming that 35% of the methane produced was derived from CO_2. Water from 16, 18, 21, and 21.5 m was collected in BOD bottles, and 5.5×10^7 dpm of $H^{14}CO_3^-$ was added to each bottle. These bottles were then incubated in situ for 30 days, after which time the specific activity of CO_2 in counts per minute per nanomole and the dissolved $^{14}CH_4$ concentrations were determined. Sediment and surface sediment samples were incubated in anaerobic tubes at in situ temperatures, and rates of methane formation were determined as previously described (24). Methanogenesis was highest in the surface sediments (Table 2). Methane production was considerably lower in the deeper sediments and lowest in the water column. Methane production from $H^{14}CO_3^-$ was not detected in water from 16 m.

To test whether significant anaerobic oxidation of methane occurred in the bottom waters, 2.2×10^6 dpm of $^{14}CH_4$ was added to BOD bottles filled with water from 21.5, 21, 18, and 16 m. No $^{14}CO_2$ was detected after a 30-day in situ incubation.

TABLE 1. *Metabolism of immediate methane precursors in Knaack Lake*[a]

Precursor	Total radioactivity (dpm × 10^{-3}) in:	
	CH_4	CO_2
$H^{14}CO_3$	224	—
$^{14}CH_3OH$	9,760	3,630
$H^{14}COOH$	231	26,600
$^{14}CH_3COOH$	6,640	1,740

[a] Samples were collected from 21.5 m on 13 December 1977; in situ temperature was 5.2°C. Water was incubated in situ with 5.5×10^7 dpm of $H^{14}CO_3$ and $H^{14}COOH$ or 2.2×10^7 dpm of the other isotopes for 30 days in 300-ml BOD bottles before analysis.

TABLE 2. *Rates of methanogenesis in Knaack Lake*

Sample depth (m)[a]	Rate of methanogenesis (nmol of CH_4/h per 10 ml)
16	0.0
18	0.5
21	0.3
21.5	0.6
Surface sediments (22)[b]	7.6
Sediment[c]	1.2

[a] Rate measurements in the water column were estimated by measuring the amount of $^{14}CH_4$ produced from $H^{14}CO_3^-$ added to lake water. Assuming that 35% of the methane produced was derived from CO_2, the specific activity of $^{14}CH_4$ should be 35% of the specific activity of $^{14}CO_2$. The amount of methane produced was calculated from the following formula: nanomoles of CH_4 = disintegrations per minute of $^{14}CH_4/(0.35 \times$ specific activity of $CO_2)$.

[b] Surface sediments were obtained by lowering the pump tubing into the uppermost layer of sediment.

[c] Sediment was collected with an Eckman dredge and was thoroughly mixed.

Acetate metabolism in the water column. Acetate metabolism is an important event in anaerobic decomposition in anoxic habitats. As acetate was both respired to CO_2 and converted to methane in the anaerobic waters of Knaack Lake (Table 1), experiments were designed to examine how various environmental parameters affected acetate metabolism. [2-^{14}C]acetate (1.25×10^6 dpm) was added to water collected from 21.5 m to determine the temperature optimum for acetate conversion to CH_4 and CO_2. Water was incubated in anaerobic tubes at temperatures ranging from 10 to 55°C. Although measurements were made at several time points, the results presented are from an early time point (19 h); use of data from this time eliminates problems due to selection of thermotolerant populations. Figure 2 demonstrates that the temperature optimum for both methanogenesis from acetate and acetate respiration was about 37°C. However, the temperature profile for acetate respiration was not as sharp as that observed for methanogenesis from acetate. At 55°C complete inhibition of methanogenesis was observed whereas acetate respiration was only inhibited by 50%. Similarly, less inhibition of acetate respiration was noted at lower temperatures.

Experiments were designed to determine whether acetate metabolism varied with depth in the water column. [2-^{14}C]acetate (2.2×10^7 dpm) was added to BOD bottles filled with water collected from various depths throughout the water column and incubated in situ. Acetate

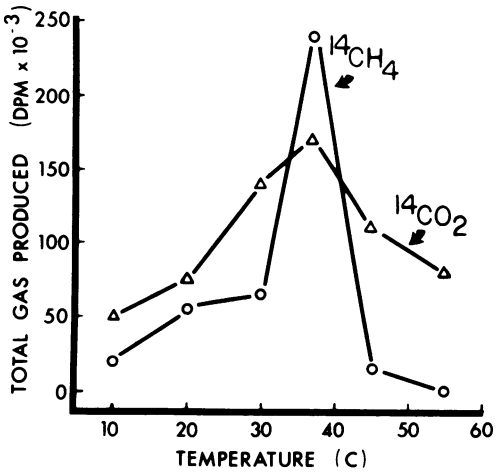

FIG. 2. *Temperature optimum for [2-^{14}C]acetate metabolism in Knaack Lake. Water from 21.5 m was collected on 15 March 1978; in situ temperature was 5.0°C. $^{14}CH_4$ and $^{14}CO_2$ were analyzed after a 19-h incubation.*

(Fig. 3) was respired exclusively to CO_2 in the aerobic surface waters of the lake. Small amounts of methane were produced below the thermocline (4 m). As indicated by the RI value profile, more $^{14}CH_4$ and less $^{14}CO_2$ were formed from methyl-labeled acetate with depth, methanogenesis being greatest at 21 m. The peak of acetate respiration shown at 10 m is not significant and was not observed in replicate experiments. RI values ranged from 1.0 at the surface to a minimum of 0.20 at 21 m. In a separate experiment, sediment was collected from the 22-m sampling site and dispensed (10 ml) into N_2-gassed anaerobic tubes. [2-^{14}C]acetate (1.25×10^6 dpm) was then added, and the tubes were incubated at 6°C in the dark. After a 68-h incubation, an RI value of 0.29 was observed.

Further experiments were initiated to investigate the effect of sulfide additions on the metabolism of acetate in monimolimnetic waters. Water was collected from 21 m in anaerobic tubes, and various concentrations of sulfide were added. HCl was added at 1.25 times the molar quantity of sulfide added to prevent a change in pH after the addition of sulfide. [2-^{14}C]acetate (1.25×10^6 dpm) was then added, and the tubes were incubated at 10°C. The results of this experiment are shown in Fig. 4. The addition of up to 5 μg of sulfide per ml stimulated methanogenesis from acetate, whereas the addition of higher concentrations inhibited methanogenesis. A 94% inhibition of methanogenesis from acetate was observed with the addition of 25 μg of sulfide per ml. Acetate respiration was inhibited by the addition of as little as 1.0 μg of sulfide per ml. The addition of increasing amounts of sulfide resulted in greater inhibition of acetate respiration, and a 90% inhibition was observed with 10 μg of added sulfide per ml.

Sulfate and nitrate were added to water from 21.5 m to determine how these electron acceptors affected the metabolism of acetate to CO_2 and CH_4. Water was collected in BOD bottles, and 1 mM nitrate N (14,000 μg/liter) or 1 mM sulfate S (32,000 μg/liter) was added. After mixing, 2.2×10^7 dpm of [2-^{14}C]acetate was added to each bottle, and the bottles were then incubated in the lake for 24 days. The results of this

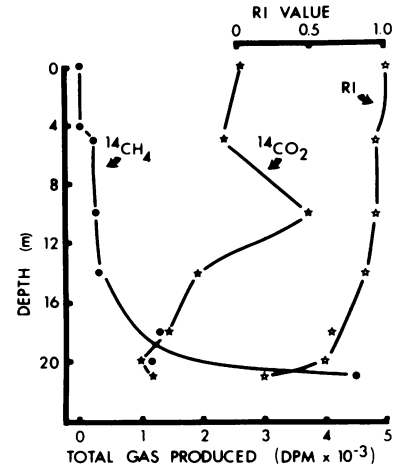

FIG. 3. *Metabolism of [2-^{14}C]acetate in Knaack Lake water. Samples were collected on 8 November 1977. The thermocline was located at 4.5 m, and water was anaerobic below this depth. $^{14}CH_4$, $^{14}CO_2$, and the RI values were determined after a 24-day in situ incubation.*

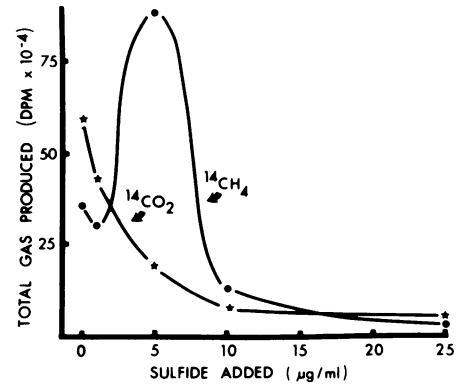

FIG. 4. *Effect of sulfide additions on [2-^{14}C]acetate metabolism in Knaack Lake water from 21 m. Samples were collected on 20 August 1976; in situ temperature was 5.0°C. $^{14}CH_4$ and $^{14}CO_2$ produced were determined after a 7-day incubation.*

TABLE 3. *Effect of electron acceptors on [2-^{14}C]acetate metabolism in Knaack Lake water*[a]

Addition	Total gas produced (dpm × 10^{-4})		RI value	Total ^{14}C in solution (dpm × 10^{-4})	Total ^{14}C in cells (dpm × 10^{-4})
	$^{14}CH_4$	$^{14}CO_2$			
None	441 ± 44	115 ± 12	0.21 ± 0.04	153 ± 2	164 ± 14
1 mM SO_4^{2-}	326 ± 100	115 ± 15	0.27 ± 0.09	164 ± 17	139 ± 4
1 mM NO_3	0	174 ± 50	1.00 ± 0.0	200 ± 18	697 ± 27

[a] Water from 21 m was collected on 8 November 1977; in situ temperature was 5.0°C. All analysis were performed after a 24-day in situ incubation. Values reported are the mean of two determinations ± 1 standard deviation.

experiment are shown in Table 3. The addition of sulfate did not significantly inhibit the production of methane from acetate or increase acetate respiration to CO_2 (RI value, 0.27). The addition of nitrate, however, completely inhibited methanogenesis and slightly stimulated total $^{14}CO_2$ production from the methyl position of acetate (RI value, 1.0). The addition of sulfate had little effect on the amount of radioactivity remaining in the water or the amount of ^{14}C incorporated into cell carbon. However, nitrate addition resulted in a slight increase in ^{14}C remaining in solution and greatly stimulated the uptake of ^{14}C into cell carbon.

Additional experiments were done to determine the minimum concentration of nitrate which was required to inhibit methanogenesis. Varying concentrations of nitrate (0 to 200 μg of nitrate N per liter) were added to bottles filled with water from 21.5 m. After mixing, 2.2×10^7 dpm of [2-^{14}C]acetate was added to each bottle, and all bottles were incubated in situ. Figure 5A shows that as little as 10 μg of added nitrate N per liter completely inhibited methane production from acetate throughout the 4-day incubation. Only slight increases in $^{14}CO_2$ evolution from [2-^{14}C]acetate were observed with increasing nitrate. The endogenous level of sulfide was observed to decrease with increasing nitrate. Sulfide was not detected in water which contained 200 μg of added nitrate per liter. Nitrate and nitrite were not detectable in any of the experimental bottles at the termination of the experiment. The amount of ^{14}C remaining in the water and incorporated into cell carbon at the termination of this experiment is shown in Fig. 5B. In the control bottles without added nitrate, very little isotope remained in the water (7.0×10^5 dpm), and only 9.2×10^5 dpm was incorporated into cell carbon. With the addition of larger amounts of nitrate, the amount of ^{14}C remaining in the water decreased, whereas an increase in the amount of ^{14}C in cell carbon was observed.

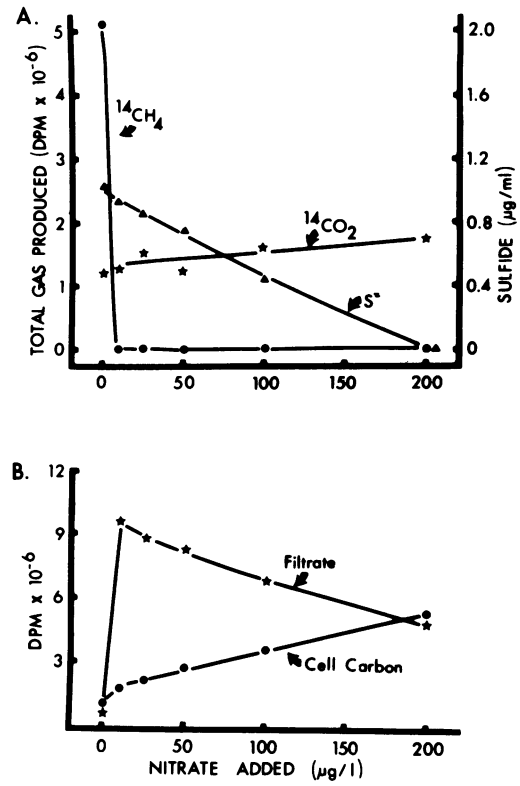

FIG. 5. *Effect of nitrate additions on [2-^{14}C]acetate metabolism in Knaack Lake water from 21 m. (A) Dissolved $^{14}CH_4$, $^{14}CO_2$, and sulfide concentrations. (B) ^{14}C incorporated into cell carbon and ^{14}C remaining in solution. Samples were collected on 27 February 1978; in situ temperature was 5.3°C. All analyses were performed after a 4-day in situ incubation.*

DISCUSSION

These results extend the known habitat of methanogenic bacteria in aquatic ecosystems. Methane production in the water column of Knaack Lake was indicated by the observation that the dissolved methane maximum occurred several meters above the sediment-water interface. This suggests production of methane in the water column because a continually increasing profile to the bottom would be expected if diffusion from the sediments were the only source of dissolved methane in the monimolimnetic waters. Methanogenesis was directly shown to

occur in the anaerobic portions of the lake by the addition of ^{14}C-labeled substrates to water samples. The conversion of $H^{14}CO_3^-$ to $^{14}CH_4$ demonstrated that methane production in the water was unaltered by substrate addition and that electron donors for CO_2 reduction to methane came from the activity of the in situ microbial population. The specific activity of the $H^{14}CO_3^-$ was high enough (56 mCi/mmol) that the added $H^{14}CO_3^-$ did not significantly affect the bicarbonate pool size. Conversion of $^{14}CH_3OH$, ^{14}C-formate, and [2-^{14}C]acetate to $^{14}CH_4$ demonstrates that these substrates are metabolized to methane in Knaack Lake. Methane produced in the water column was shown to be of biological origin, as no $^{14}CH_4$ was produced from ^{14}C-labeled substrates in control experiments that contained formaldehyde. In addition, a temperature optimum of about 37°C was observed for methanogenesis, which is characteristic of biological activity in nonthermophilic environments (27). The occurrence of methanogenesis in the water column implies that the microbial population possesses specialized physiological properties to maintain buoyancy. Methanogenic species have been described (14, 26) that are either motile or gas vacuolated. The methanogenic population of Knaack Lake is currently under investigation.

Rates of methane formation in Knaack Lake were greatest in the surface sediments, decreased in deeper sediments, and were lowest in the water above the sediments. Methanogenesis in holomictic lakes has also been shown to be greatest in the surface sediments (7). This is probably due to the accumulation of readily utilizable sedimented organic matter at the sediment-water interface. Decreased rates of methanogenesis in the water column of Knaack Lake are probably a result of lower levels of readily degradable organic matter than are present in the sediments. Nevertheless, the observed rate of methanogenesis in the water column indicates that methane production in the water column is responsible for the mineralization of significant amounts of carbon in Knaack Lake. This is apparent when the volume of surface sediment and that of the monimolimnetic waters are compared.

The methyl position of acetate was simultaneously metabolized to methane and CO_2 throughout the anaerobic portions of the lake. Acetate oxidation to CO_2 was predominant throughout most of the water column, although acetate was converted primarily to methane in the bottom waters and sediment. The production of CO_2 from the methyl position of acetate has also been reported previously in lake sediments (8, 24, 25). Acetate has only been demonstrated to be respired to CO_2 in the presence of an external electron acceptor (21); thus, an electron acceptor is probably present in the anaerobic water of Knaack Lake. Oxygen, nitrate, and sulfate were not detectable in the anaerobic waters. Thus, the identity of the electron acceptor is not known. Elemental sulfur may be the electron acceptor in Knaack Lake, allowing acetate respiration by organisms metabolically similar to *Desulfuromonas acetooxidans* (17). High populations of photosynthetic bacteria are present in the upper anaerobic waters of this lake (T. Parkin, personal communication), and production of sulfur or other electron acceptors by these organisms may account for the observed oxidation of the methyl position of acetate.

Acetate metabolism was shown to be affected by fluctuations in several chemical parameters of environmental significance. Methane formation from acetate was stimulated by the addition of low levels of sulfide (up to 5 μg/ml) and inhibited by higher concentrations. A similar effect of sulfide on methanogenesis has been reported in Lake Mendota sediments (25), although higher sulfide concentrations were required to cause inhibition. The addition of as little as 1 μg of sulfide per ml was shown to inhibit acetate oxidation in Knaack Lake. This differential effect of sulfide on acetate metabolism suggests that acetate oxidation and methanogenesis are performed by two distinct populations. This is also supported by the different temperature activity profiles observed for these two processes.

The differential effect of low sulfide concentrations on acetate respiration and fermentation may be a result of end product inhibition by HS^- on acetate-respiring organisms. In bacteria like *D. acetooxidans* which employ a tricarboxylic acid cycle (17), the dehydrogenation reactions (for CO_2/acetate, redox potential [E_0'] = −290 mV) of catabolism are coupled to hydrogenation reactions (for $S°/HS^-$, redox potential = −270 mV), and HS^- accumulation makes acetate respiration less favorable thermodynamically (21). However, low concentrations of sulfide would not affect acetate-fermenting methanogens in a similar manner because their energy-yielding metabolism is not linked to HS^- formation. High concentrations of HS^- may inhibit both processes by altering the reduction-oxidation potential of the system.

The addition of 1 mM sulfate, although it is an inhibitor of methanogenesis in lake sediments (6, 13, 25), had little effect on acetate metabolism in Knaack Lake. Sulfate was not detected in the lake, and thus the sulfate-reducing bacterial population may be small. However, the addition of nitrate completely inhibited methanogenesis

from the methyl position of acetate, stimulated acetate oxidation, and stimulated the uptake of acetate into cell carbon. Nitrate was consumed very rapidly in Knaack Lake water, and the 10 μg of added nitrate per liter was likely depleted long before the termination of the experiments. The exact mechanism by which nitrate inhibits methanogenesis is not known. Recent work by Balderson and Payne (1) demonstrated that 1 mM nitrate (14,000 μg of nitrate N per liter) inhibited methane formation in salt marsh soils and in a culture of *Methanobacterium thermoautotrophicum*. The results reported here, that as little as 10 μg of added nitrate per liter completely inhibited methanogenesis from acetate, support the conclusions of Balderson and Payne (1) that some factor(s) other than a change in reduction-oxidation potential or substrate competition accounted for the inhibitory effect of nitrate on methanogenesis. The stimulation of acetate respiration and acetate incorporation into cell carbon observed when nitrate was added to Knaack Lake water is probably a result of the presence of facultative denitrifying microorganisms.

It is interesting to speculate on why methanogenesis occurs in the water column of a meromictic lake and on the importance of this methane formation in the carbon cycle of the lake. Because Knaack Lake never mixes completely, a permanently anaerobic habitat is maintained in the monimolimnetic waters. This absence of periodic mixing probably enables the methanogenic bacteria to establish themselves in the anaerobic waters and actively produce methane. Because of their extreme oxygen sensitivity, methanogens are restricted to the sediments in holomictic lakes, although low amounts of methanogenic activity were noticed in anaerobic waters immediately above Lake Mendota sediments during periods of stratification (Winfrey and Zeikus, unpublished data). The water chemistry in meromictic lakes is also an important factor in determining whether methanogenesis occurs in the water column. Many meromictic lakes remain permanently stratified as a result of highly saline water in the monimolimnion. Sulfate and nitrate are often present in high concentrations in these lakes and may inhibit methanogenic activity. With the absence of electron acceptors (e.g., sulfate or nitrate) in bottom waters, biogenic meromictic lakes such as Knaack Lake produce methane in the anaerobic water column. In the bottom waters and sediment of Knaack Lake and other biogenic meromictic lakes, fermentation and methanogenesis are likely the major mechanism of anaerobic organic mineralization. However, it has been suggested that, in saline meromictic lakes such as Lake Suigetsu (15), anaerobic respiration by sulfate reducers is the major means of organic decomposition.

ACKNOWLEDGMENTS

This research was supported by the College of Agriculture and Life Sciences, University of Wisconsin, and by grant DEB 7824071 from the National Science Foundation. M.R.W. 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.

We thank Karl Knaack for allowing us to cross his land to sample Knaack Lake. We also thank Tim Parkin and Steve Klevickis for help in sampling.

LITERATURE CITED

1. **Balderson, W. L., and W. J. Payne.** 1976. Inhibition of methanogenesis in salt marsh sediments and whole-cell suspensions of methanogenic bacteria by nitrogen oxides. Appl. Environ. Microbiol. **32**:264-269.
2. **Bell, R. G.** 1969. Studies on the decomposition of organic matter in flooded soils. Soil Biol. Biochem. **1**:105-116.
3. **Belyaev, S. S., Z. I. Finkelshtein, and M. V. Ivanov.** 1975. Intensity of bacterial methane formation in ooze deposits of certain lakes. Microbiology **44**:272-275.
4. **Bollag, J. M., and S. T. Czlonkowski.** 1974. Inhibition of methane formation in soil by various nitrogen containing compounds. Soil Biol. Biochem. **5**:673-678.
5. **Bryant, M. P.** 1972. Commentary on the Hungate technique for culture of anaerobic bacteria. Am. J. Clin. Nutr. **25**:1324-1328.
6. **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.
7. **Cappenberg, T. E.** 1976. Methanogenesis in the bottom deposits of a small stratified lake, p. 125-134. *In* H. G. Schlegel, G. Gottschalk, and N. Pfennig (ed.), Microbial production and utilization of gases. Akademie der Wissenschaften zu Gottingen, Gottingen.
8. **Cappenberg, T., and H. 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**:457-460.
9. **Chen, R. L., D. R. Keeney, J. G. Konrad, A. J. Holding, and D. A. Graetz.** 1972. Gas production in sediments of Lake Mendota. J. Environ. Qual. **1**:155-157.
10. **Culver, D. A., and G. J. Brunskill.** 1969. Fayetteville Green Lake, New York. V. Studies of primary production and zooplankton in a meromictic marl lake. Limnol. Oceanogr. **14**:867-873.
11. **Daniels, L., and J. G. Zeikus.** 1978. One-carbon metabolism in methanogenetic bacteria: analysis of short term fixation products of $^{14}CO_2$ and $^{14}CH_3OH$ incorporated into whole cells. J. Bacteriol. **136**:75-84.
12. **Knopka, A., T. D. Brock, and A. E. Walsby.** 1978. Buoyancy regulation by planktonic blue-green algae in Lake Mendota, Wisconsin. Arch. Hydrobiol. **83**: 524-537.
13. **MacGregor, A. N., and D. R. Keeney.** 1973. Methane formation by lake sediments during in vitro incubation. Water Res. Bull. **9**:1153-1158.
14. **Mah, R. A., D. M. Ward, L. Baresi, and T. L. Glass.** 1977. Biogenesis of methane. Annu. Rev. Microbiol. **31**: 309-341.
15. **Matsuyama, M., and Y. Saio.** 1971. Studies on the biological metabolism in a meromictic Lake Suigetsu. J. Oceanogr. Soc. Jpn. **27**:197-206.
16. **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.

17. **Pfennig, N., and H. Biebl.** 1976. *Desulfuromonas acetooxidans* gen. nov. and sp. nov., a new anaerobic, sulfur-reducing, acetate-oxidizing bacterium. Arch. Microbiol. **110**:3-12.
18. **Richards, F. A., J. D. Cline, W. A. Bnenkow, and L. P. Atkinson.** 1965. Some consequences of the decomposition of organic matter in Lake Nitinat, an anoxic fjord. Limnol. Oceanogr. **10**:185-201.
19. **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.
20. **Takahashi, M., and S. Ichimura.** 1968. Vertical distribution and organic matter production by photosynthetic sulfur bacteria in Japanese lakes. Limnol. Oceanogr. **13**:644-655.
21. **Thauer, R. K., K. Jungermann, and K. Decker.** 1977. Energy conservation in chemotrophic anaerobic bacteria. Bacteriol. Rev. **41**:100-180.
22. **Truper, H. G., and S. Genovese.** 1968. Characterization of photosynthetic sulfur bacteria causing red water in Lake Faro (Messina, Sicily). Limnol. Oceanogr. **13**: 225-232.
23. **Weimer, W. C., and G. F. Lee.** 1973. Some considerations of the chemical limnology of meromictic Lake Mary. Limnol. Oceanogr. **18**:414-425.
24. **Winfrey, M. R., D. R. Nelson, S. C. Klevickis, and J. G. Zeikus.** 1977. Association of hydrogen metabolism with methanogenesis in Lake Mendota sediments. Appl. Environ. Microbiol. **33**:312-318.
25. **Winfrey, M. R., and J. G. Zeikus.** 1977. Effect of sulfate on carbon and electron flow during microbial methanogenesis in freshwater sediments. Appl. Environ. Microbiol. **33**:275-281.
26. **Zeikus, J. G.** 1977. The biology of methanogenic bacteria. Bacteriol. Rev. **41**:514-541.
27. **Zeikus, J. G., and M. Winfrey.** 1976. Temperature limitation of methanogenesis in aquatic sediments. Appl. Environ. Microbiol. **31**:99-107.