APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Dec. 1984, p. 1088–1095
0099-2240/84/121088-08$02.00/0
Copyright © 1984, American Society for Microbiology
Vol. 48, No. 6

Influence of pH on Terminal Carbon Metabolism in Anoxic Sediments from a Mildly Acidic Lake

T. J. PHELPS AND J. G. ZEIKUS*

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

Received 5 June 1984/Accepted 13 August 1984

The carbon and electron flow pathways and the bacterial populations responsible for transformation of H_2-CO_2, formate, methanol, methylamine, acetate, glycine, ethanol, and lactate were examined in sediments collected from Knaack Lake, Wis. The sediments were 60% organic matter (pH 6.2) and did not display detectable sulfate-reducing activity, but they contained the following average concentration (in micromoles per liter of sediment) of metabolites and end products: sulfide, 10; methane, 1,540; CO_2, 3,950; formate, 25; acetate, 157; ethanol, 174; and lactate, 138. Methane was produced predominately from acetate, and only 4% of the total CH_4 was derived from CO_2. Methanogenesis was limited by low environmental temperature and sulfide levels and more importantly by low pH. Increasing in vitro pH to neutral values enhanced total methane production rates and the percentage of CO_2 transformed to methane but did not alter the amount of $^{14}CO_2$ produced from [2-^{14}C]acetate (~24%). Analysis of both carbon transformation parameters with ^{14}C-labeled tracers and bacterial trophic group enumerations indicated that methanogenesis from acetate and both heterolactic- and acetic acid-producing fermentations were important to the anaerobic digestion process.

The transformation of hydrogen and various one- and two-carbon metabolites during organic decomposition in anoxic environments is performed by terminal bacterial trophic groups (37). The metabolic activity of these terminal trophic groups is vital in anaerobic digestion processes because H_2, ethanol, and acetic, formic, and lactic acids become toxic if they accumulate, and then the decomposition of organic matter is inhibited (39). At present, physiologists recognize (38) three diverse bacterial groups that can consume H_2, one-carbon substrates (e.g., formate or methanol), and two-carbon substrates (e.g., acetate or ethanol) as energy sources: the methanogens, the sulfate reducers, and the homo- or H_2-consuming acetogens. How low pH specifically influences anoxic carbon decomposition processes has not been well characterized. Nonetheless, it is generally known that the souring of anaerobic digestors by low pH causes inhibition of organic waste treatment and methane production (11).

Considerable research has focused on understanding how sulfate-reducing bacteria and methane-producing bacteria function during anaerobic digestion processes in freshwater and marine sediments (1, 2, 4–8, 10, 15–19, 21–23, 25–36). In general, the results of these studies can support a unified concept that, depending on the environmental organic input, sulfate-reducing bacteria dominate in sediments when sulfate is in excess by outcompeting methanogens for common energy sources (i.e., primarily H_2 and acetate). Recently, physiological studies have suggested (12, 14, 18a, 25) that sulfate reducers can outcompete methanogens for hydrogen because they possess a hydrogen metabolism activity with more favorable kinetic properties (i.e., lower K_m and higher V_{max}).

The purpose of the present paper was to test the hypothesis that pH (i.e., proton activity) in mildly acidic lake sediments can account for environmental inhibition of methanogenesis, alteration of carbon and electron flow pathways, and selective advantages for particular bacterial trophic groups. In this ecosystem, low pH appears to alter the sedimentary microbial processes involved in terminal carbon mineralization even more dynamically than perturbations caused by temperature (40) or sulfate (36) in other environments.

(These results were presented in preliminary form elsewhere [T. J. Phelps, Abstr. Annu. Meet. Am. Soc. Microbiol. 1983, I163, p. 166].)

MATERIALS AND METHODS

Description of experimental site and sampling procedures. Knaack Lake is a small (1.1-ha) meromictic lake located 8 mi (ca. 13 km) south of Marion, Wis., whose bottom waters and sediments are permanently anoxic, devoid of detectable sulfate, and remain at a temperature of 4°C (24). All sediment samples were collected by anaerobic techniques (35) and were obtained from the maximum depth (22 m) with an Eckman dredge. Sediment was placed into N_2-flushed 300-ml glass bottles which were sealed with butyl rubber stoppers, stored in ice chests, and transported to the laboratory. All analyses and experiments employed fresh sediments (i.e., less than 8 h after procurement) collected during fall 1979 to 1983.

Gases, chemicals, and isotopes. Nitrogen, N_2-CO_2 (95:5%), H_2, and H_2-CO_2 (80:20%) were greater than 99.9% pure (Matheson Gas Co., Joliet, Ill.) and were passed over copper-filled Vycor furnaces (Sargent-Welch Scientific Co., Skokie, Ill.) to remove oxygen. All chemicals used were of reagent grade and were obtained from Mallinckrodt (Paris, Ky.) or Sigma Chemical Co. (St. Louis, Mo.). [2-^{14}C]acetate (56 mCi/mmol) was obtained from Amersham Corp. (Arlington Heights, Ill.) and suspended in water. [^{14}C]methylamine (4 mCi/mmol) suspended in ethanol and [^{14}C]methanol (50 mCi/mmol) suspended in water were purchased from ICN Pharmaceuticals Inc. (Irvine, Calif.). [^{14}C]formate (55 mCi/mmol) in ethanol-water (7:3), [2-^{14}C]ethanol (50 mCi/mmol) in water, $^{14}HCO_3^-$ (46 mCi/mmol) in alkaline solution, [2-^{14}C]glycine (60 mCi/mmol) suspended in 0.01 N HCl, [3-^{14}C]lactate (21.5 mCi/mmol) in water, and $^{35}SO_4^{2-}$ (739 mCi/mmol) in aqueous solution were obtained from New England Nuclear Corp. (Boston, Mass.).

* Corresponding author.

Quantification of sediment metabolites. Organic matter was determined as ash-free dry weight after combustion at 600°C for 8 h. Dissolved methane, carbon dioxide, sulfate, and sulfide were measured as described previously (35). Soluble organic metabolites were measured in interstitial water obtained from centrifugation of sediments. Methanol and ethanol were measured by gas chromatographic methods (9) with detection limits of 20 μM. Formate was determined by enzymatic methods (9) with detection limits of 2 μM. Samples for acetate and lactate analysis were concentrated as described elsewhere (35) and quantified by gas chromatography with detection limits of 10 μM. Glycine and methylamine were quantified in concentrated samples by using a Perkin-Elmer series 3 high-pressure liquid chromatograph equipped with a Whatman Partisil PXS-10/25 ODS-3 column eluted with degassed 5.0 mM tetrabutylammonium phosphate buffer (pH 7.5). The detector used was an LDC Refracto Monitor 1107 with 50 μM sensitivity limits.

General studies. All manipulations in anaerobic test tubes employed syringe techniques for transfer of anoxic sediments, additions of anoxic sterile solutions, or removal of samples for analysis. Unless stated elsewhere in the text, all individual experiments were performed in triplicate sets of tubes which contained 5 ml of sediment and a 19-ml N_2 headspace and which were sealed with black butyl rubber bungs and incubated at in situ temperature (4°C).

In experiments designed to examine the influence of pH on methanogenesis, sediments were adjusted to the indicated pH by the addition of HCl or NaOH, allowed to equilibrate, and flushed with N_2, and then methanogenesis was monitored during a 16-h time course of incubation at 30°C. Under these conditions, the adjusted pH values remained constant during the experiments.

Radioisotopic studies. All solutions (1.0 to 5.0 μCi) were transferred with gastight syringes (Hamilton Co., Reno, Nev.) equipped with Teflon plungers, and transfers of gases employed Pressure-Lok syringes (Precision Sampling Co., Baton Rouge, La.). $^{14}CO_2$ and $^{14}CH_4$ were quantified by gas chromatograph-gas proportion counting methods (20). The amount of $^{35}SO_4^{2-}$ transformed to $^{35}S^{2-}$ was determined by techniques described elsewhere (7). [^{14}C]acetate was separated either by Dowex Ag1 anion-exchange chromatography or high-pressure liquid chromatography, and the radioactivity was measured by liquid scintillation procedures (14). Total liquid addition to any tube was ≤0.15 ml. At each point in a time course, carbon or sulfur transformation was terminated in triplicate sets of tubes and then analyzed. Experimental tubes containing ^{14}C-radioisotopes were terminated by adding 0.1 ml of a 40% Formalin solution, whereas those containing ^{35}S-radioisotopes were terminated with 0.1 ml of a 5.0 N NaOH solution.

Enumeration studies. Three-tube most-probable-number (MPN) analyses were performed with both a carbonate- and a phosphate-buffered medium (18a) which contained 0.05% yeast extract, 20 mM of an organic energy source or an H_2-CO_2 (80:20) headspace, and 0.5 μCi of the indicated ^{14}C-labeled carbon source. The tubes were incubated at 30°C for 1 month. Positive results were recorded if more than 1% of the tracer was converted to $^{14}CO_2$, $^{14}CH_4$, or [^{14}C]acetate.

Determination of substrate transformation parameters. The substrate transformation rate constant is a determination which is based on the production of total $^{14}CO_2$ and $^{14}CH_4$ from a given ^{14}C-radiotracer. When both the substrate transformation rate constant and the turnover rate constant which is based on the disappearance of a uniformly ^{14}C-labeled metabolite (i.e., for acetate, lactate, glucose, or cellulose) were determined in sewage sludge or in lake sediments, both constants had similar values (Phelps and Zeikus, manuscript in preparation). The substrate transformation rate constants were used here because of the ease and practicality of their determination. To determine the substrate transformation rate constants, total $^{14}CO_2$ and $^{14}CH_4$ production from ^{14}C-labeled metabolites was monitored over time course experiments, and the maximum value achieved for total $^{14}CO_2$ and $^{14}CH_4$ production was assumed to be 100% of the radioactivity available to the measured reaction. The percentage of the radioactivity still available to the reaction at each previous time point was calculated and plotted on a semi-log scale (i.e., 100% minus the percentage of radioactivity recovered as gaseous products at that time point). The resulting slope of this line corresponds to the type of measurement for determining a turnover rate constant (i.e., slope/0.4343 = substrate transformation rate constant). The substrate transformation rate is the amount of the substrate flux per hour, and it was calculated by multiplying the substrate transformation rate constant times the pool size of the substrate. The CO_2 production rate corresponds to the amount of $^{14}CO_2$ produced from a single position in the ^{14}C-labeled compound tested per hour and was determined as follows:

$$\text{CO}_2\text{ production rate} = \text{substrate transformation rate} \times \frac{^{14}CO_2}{^{14}CO_2 + ^{14}CH_4} \times \frac{\text{maximum dpm of } ^{14}CO_2 + ^{14}CH_4}{\text{total dpm of } ^{14}\text{C added}}$$

where the last term represents the fraction of the substrate pool available to the gas-producing reaction (typically 55 to 70%). The CH_4 production rate corresponds to the amount of $^{14}CH_4$ produced from a single position in the ^{14}C-labeled compound tested and was calculated as follows:

$$\text{CH}_4\text{ production rate} = \text{substrate transformation rate} \times \left(1 - \frac{^{14}CO_2}{^{14}CO_2 + ^{14}CH_4}\right) \times \frac{\text{maximum dpm of } ^{14}CO_2 + ^{14}CH_4}{\text{total dpm of } ^{14}\text{C added}}$$

RESULTS

General analysis of anaerobic metabolism. Our laboratory previously documented (35) that Knaack Lake sediments are actively methanogenic, but the addition of 1 mM exogenous sulfate surprisingly did not increase the amount of $^{14}CO_2$ or $^{14}CH_4$ derived from [2-^{14}C]acetate. In the present studies, experiments were initiated to compare the endogenous rates of methanogenesis and sulfate reduction as a function of sediment incubation temperature. Table 1 shows that methanogenesis was active and that increasing in vitro incubation temperature from 4°C (in situ) to 30°C resulted in the expected enhancement of methanogenesis rates (40). Sulfide formation was not detectable by measurement of sulfide production rates at incubation temperatures of 4 to 30°C, whereas sulfate reduction was not detectable at in situ temperature but was observed at very low rates when sediments were incubated with exogenous sulfate at 14 or 30°C. This finding supports the conclusion that dissimilatory sulfate reduction is not a significant process in Knaack Lake sediments because of low in situ sulfate concentrations (24, 35).

The ecological importance of this result was further investigated by comparing the effects of exogenous sulfide addition on total and acetate-derived methanogenesis in Knaack Lake sediments. Sulfide concentration values greater than that in situ (10 μmol/liter of sediment) but less than or equal

TABLE 1. Comparison of methane production and sulfate reduction rates in Knaack Lake sediments[a]

Incubation temp (°C)	Methanogenesis		Sulfate reduction	
	μmol of CH_4/g of organic matter per day	μmol of CH_4 per liter of sediment per day	μmol of sulfate consumed per liter of sediment per day[b]	μmol of sulfide produced per liter of sediment per day
4	2.2	56 ± 7.4	ND	ND
14	4.4	130 ± 9.8	0.0008 ± 0.0001	ND
30	7.8	210 ± 25.7	0.013 ± 0.001	ND

[a] Sediments (5 ml) were incubated in triplicate for a 48-h time course during which time CH_4 and sulfide values were quantified. ND, Not detectable by the methods used.

[b] The rate of sulfate consumption was determined in tubes as above but 0.4 mM sodium sulfate plus 1 μCi of $^{35}SO_4^{2-}$ was added and the rate was calculated as the turnover rate constant for $^{35}SO_4^{2-}$ times the pool size of SO_4^{2-} times 24 h.

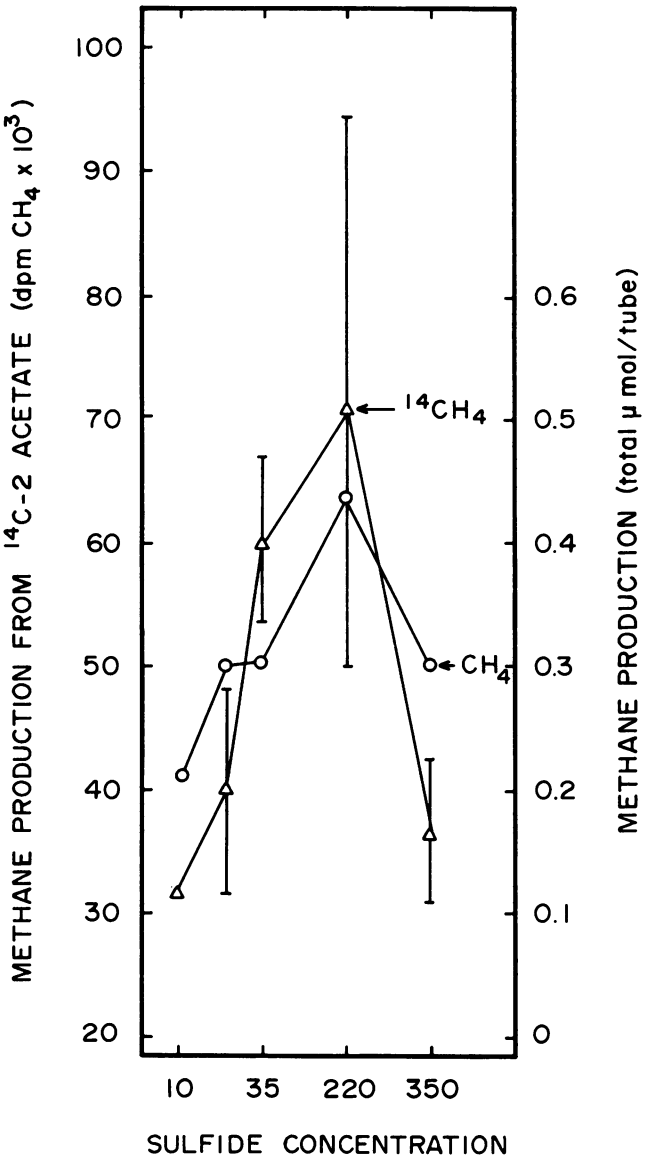

FIG. 1. Influence of exogenous sulfide addition on acetate transformation to methane in Knaack Lake sediments. Anoxic test tubes contained 5 ml of sediment, an initial N_2 headspace, 3×10^5 dpm of [2-^{14}C]acetate, and the amount of soluble sulfide indicated (micromoles per liter). The tubes were incubated at 4°C, and the amount of methane was determined after 16 h.

to 220 μmol/liter of sediment greatly stimulated methanogenesis (Fig. 1). This finding implies that low dissimilatory sulfate-reducing activity may actually limit methanogenic activity in natural aquatic ecosystems.

Pool sizes and microbial populations. Pool size and microbial population experiments were designed to determine which metabolites and which terminal bacterial groups were of significance in the biodegradation of organic matter in Knaack Lake sediments. The results represent the average values obtained from multiple experiments performed over a 3-year sampling period.

Table 2 compares the approximate pool size of microbial metabolites detected in sediment interstitial water. The sedimentary pH of 6.2 remained constant during the study period. As expected (35), the concentrations of hydrogen, sulfate, and sulfide were low. The concentrations of formate, CO_2, ethanol, acetate, and lactate were higher, however, suggesting that heterolactic acid fermentations were taking place. The concentrations of these carbon metabolites are higher in Knaack Lake than the values reported in freshwater sediments that display significant sulfate-reducing activity such as in Lake Vechten (4, 5), Wintergreen Lake (15, 16, 18, 29), and Lake Mendota (6, 32, 34, 36; Phelps and Zeikus, manuscript in preparation).

These results led to enumeration studies aimed at estimating the approximate sedimentary populations of anaerobes that could consume glucose, lactate, and various one- and two-carbon metabolites. These experiments employed ^{14}C-tracer techniques to identify positive end-dilution tubes by detection of ^{14}C-substrate conversion to either $^{14}CO_2$ (con-

TABLE 2. Concentrations of metabolites and end products in anoxic sediment interstitial waters from Knaack Lake[a]

Metabolites	Mean pool size (μmol/liter)	SD
Sulfide	10	5
Hydrogen[b]	0.041	0.029
Methane	1,540	170
Carbon dioxide	3,950	700
Formate	25	12
Acetate	157	40
Ethanol	174	93
Lactate	138	104

[a] Gases were determined by gas stripping and gas chromatographic techniques, whereas soluble metabolites were determined as described in the text. The following metabolites were not detected (limits of detection sensitivity were ≤5 to 50 μmol/liter): sulfate, methanol, methylamine, and glycine. Values represent the average from a 3-year study period (June 1982 to 1984).

[b] Data from Conrad et al. (in preparation).

TABLE 3. Estimated abundance of selected anaerobic populations in Knaack Lake sediments

Energy source	Population level (MPN/g [dry wt] of sediment)[a]		
	Heterotrophic	Acetogenic	Methanogenic
Lactate	10^5	10^5	
Glucose	10^7	10^6	
H_2-CO_2		10^5	10^3
Formate	10^4	10^4	10^2
Methanol	10^5	10^5	10^3
Methylamine	10^3		10^3
Glycine	10^5	10^5	
Acetate	10^4		10^4
Ethanol	10^4	10^4	

[a] MPN determinations were with triplicate tubes and were based on detecting a specific ^{14}C-labeled end product (i.e., [^{14}C]acetate, $^{14}CH_4$, or $^{14}CO_2$ for acetogens, methanogens, or a total population index control, respectively) from the ^{14}C-labeled carbon sources used. The values reported are the MPN values for multiple determinations for a 3-year sampling period from June 1981 to 1983 and are averaged to the nearest exponent.

trol for total heterotrophs), [^{14}C]acetate (total acetate producers), or $^{14}CH_4$ (total methanogens). Enumeration of acetogenic and methanogenic bacteria were only calculated for substrates shown to be consumed as energy sources for growth in pure cultures (38) of homoacetogens and methanogens. Table 3 shows that higher MPN values were obtained for one- and two-carbon-metabolizing acetogenic populations than methanogenic populations and that the acetate-producing population had MPN values as high on all energy sources tested (except for glucose) as the control for total heterotrophic anaerobes.

Substrate transformation parameters. To test the validity that the intermediary metabolites detected were important precursors of sedimentary methanogenesis and carbon mineralization, we initiated experiments to compare their contribution with the total rate of methane and CO_2 production. In these experiments, the specific substrate transformation rate constant (dissimilar from a turnover rate constant) was based on total $^{14}CO_2$ or $^{14}CH_4$ or both produced from a specifically ^{14}C-labeled carbon substrate.

Table 4 shows the results of these experiments which represent the average values of multiple determinations made during a 3-year sampling period. Carbon dioxide (i.e., total inorganic carbon) turned over slowly as a consequence of its large pool size and accounted for 4% of the total measured methane production. Formate displayed the highest substrate transformation rate constant and the fastest transformation rate but it was a minor immediate methane precursor. Acetate was rapidly transformed and contributed to greater than 95% of the total methane produced. During the time course of these experiments, the amount of total methane produced averaged 62 μmol of CH_4 per liter of sediment per day, whereas the calculated contribution of acetate and CO_2 to methane averaged 62 and 2.6 μmol of CH_4 per liter of sediment per day, respectively. Labeled methanol and methylamine were also transformed to labeled methane and CO_2, but due to their small pool size, their combined contribution was less than 4% of the total methane produced. Lactate, ethanol, and glycine were also degraded to methane and CO_2.

Based on specific activity analysis of gas after 24 h of incubation, 49.6 ± 5.2 μmol of methane was formed from the C-2 of acetate, whereas only 1.5 ± 0.3 μmol was formed from CO_2. In general, the low value observed for methanogenesis from CO_2 reduction is atypical of anaerobic digestion processes in environments with a neutral pH in which about 30% of the methane is derived by this mechanism (38, 39). Thus, experiments were initiated to assess the contribution of CO_2 to acetogenesis. $^{14}CO_2$ was transformed to [^{14}C]acetate in sediments (Fig. 2). The rate of acetate production from CO_2 was 2.8 ± 0.2 μmol/liter of sediment per day or 2% of the acetate transformation rate. In other experiments, the addition of a 5% hydrogen headspace was shown to increase by threefold the total amount of [^{14}C]acetate produced at 10 days. These results suggest that homoacetogenic bacterial populations are active in the sediment, but the absolute amount of H_2-dependent CO_2 reduction cannot be calculated by this approach because H_2-consuming acetogens also readily exchange CO_2 into acetate when they metabolize multicarbon substrates (38).

The C-3 position of lactate was readily transformed into acetate by sediments and accounted for ~14% of the total CH_4 formed (Fig. 3). Similarly, other results showed that the C-2 position of ethanol was an important acetate precursor in Knaack Lake sediments (B. S. Schink, T. J. Phelps, and J. G. Zeikus, manuscript in preparation). In other experiments, [^{14}C]acetate was produced from formate, glycine, or methanol transformation in sediments (data not shown).

Influence of pH on biodegradation. We previously demonstrated that the in vitro transformation rate constant for

TABLE 4. Comparison of terminal carbon metabolite biodegradation parameters in Knaack Lake sediments[a]

Substrate	Substrate transformation rate constant (h^{-1})[b]	Substrate transformation rate (μmol of substrate per liter of sediment per h)	Carbon dioxide production rate (μmol/liter of sediment per h)	Methane production rate (μmol/liter of sediment per h)
$^{14}CO_2$	0.0005 ± 0.0002	1.86		0.11
[^{14}C]formate	3.28 ± 0.44	11.0	6.98	0.07
[2-^{14}C]acetate	0.038 ± 0.010	5.90	0.81	2.60
[^{14}C]methylamine	0.012 ± 0.004	ND	ND	ND
[^{14}C]methanol	0.021 ± 0.003	ND	ND	ND
[3-^{14}C]lactate	0.018 ± 0.0015	1.37	0.18	0.37
[2-^{14}C]ethanol	0.032 ± 0.008	5.57	0.35	0.65
[2-^{14}C]glycine	0.020 ± 0.003	ND	ND	ND

[a] Triplicate anoxic tubes for each time point analyzed contained 5 ml of sediment and 5 μCi of $^{14}HCO_3$ or 1 μCi of the ^{14}C-labeled organic metabolite indicated. All tubes were incubated at 4°C, and gas samples were analyzed during a 20-day time course. ND, Not determined because the metabolite pool size was below the detection sensitivity limits of analysis. Controls treated with Formalin to inhibit biological activity did not produce CO_2 or CH_4. Results represent the average value obtained from repeated experiments during 1981 to 1983. All measurements reflect only transformation of the position of the carbon labeled.

[b] This constant was determined as described in the text.

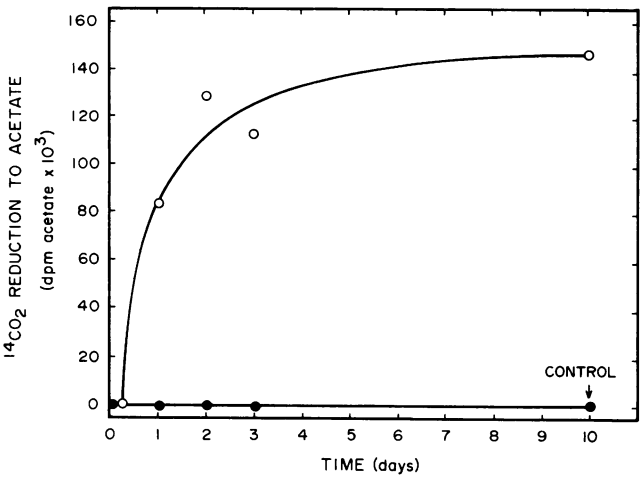

FIG. 2. $^{14}CO_2$ transformation to [^{14}C]acetate in Knaack Lake sediments. Anoxic test tubes contained 5 ml of sediment, an initial N_2 headspace, and 10^7 dpm of $Na_2{}^{14}CO_3$ and were incubated at 4°C. The amount of [^{14}C]acetate was determined by high-pressure liquid chromatography at the times indicated. Controls which contained Formalin to inhibit biological activity did not show CO_2 transformation into acetate.

pectin degradation to methane at in situ temperatures is considerably smaller in the surface sediments of Knaack Lake than in those of Lake Mendota (27). Consequently, experiments were organized to examine whether low in situ pH limits or alters methanogenesis in sediments.

The rate of endogenous sedimentary methanogenesis was significantly altered by changes in pH, and the rate of methanogenesis was optimal at neutral pH values and not at in situ or more acidic pH values (Table 5). Nonetheless, methanogenesis still continued linearly with time at in situ

TABLE 5. Influence of various pH values on methanogenesis in Knaack Lake sediments

pH	Methane production rate[a] (total μmol/liter of sediment per day)
5.6	74 ± 14
6.2	86 ± 5
6.7	90 ± 5
7.0	97 ± 1.9
7.3	131 ± 2
7.8	77 ± 4.9

[a] Sediment (5 ml) was placed into anoxic tubes and the pH was adjusted to the value indicated by the addition of HCl or NaOH. The rate of methane production was determined during a 16-h time course at 4°C.

(pH 6.2) or more acidic pH values (pH 5.6) but at lower rates.

Figure 4 compares the influence of various sedimentary pH values on acetate biodegradation to CO_2 and CH_4. No $^{14}CH_4$ or $^{14}CO_2$ was produced in controls that contained Formalin to inhibit biological activity. The optimal amounts of methane and CO_2 produced from acetate increased from acidic to neutral pH values and then declined at more alkaline pH values. Altering sediment pH did not change the percentage of $^{14}CO_2$ produced from the methyl group of [^{14}C]acetate. It should be noted that ca. 24% of the methyl group was oxidized at in situ pH values. In parallel experiments with [1-^{14}C]acetate, 7.3 ± 3.1% of the carboxyl of acetate was transformed to $^{14}CH_4$.

Additional experiments were performed to test if pH altered sedimentary electron flow by determining the influence of pH on $^{14}CO_2$ reduction to $^{14}CH_4$. The contribution of CO_2 to total methane formed increased linearly as in vitro pH was raised from acidic to neutral values (Fig. 5). In

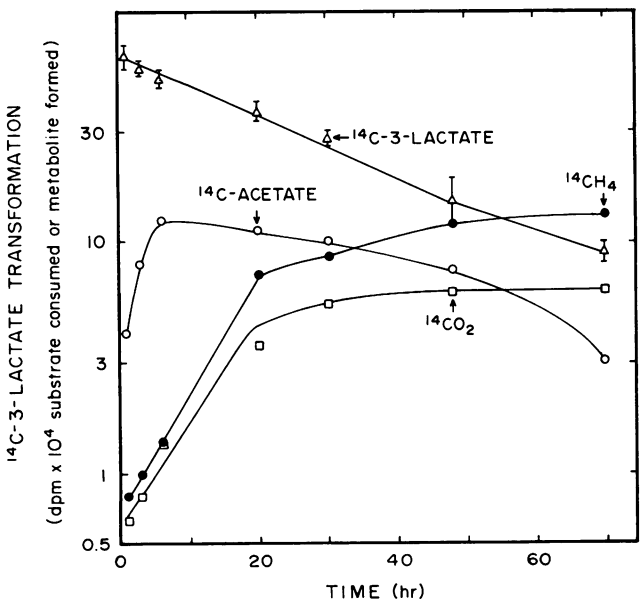

FIG. 3. Relationship between lactate degradation, acetate metabolism, and methanogenesis in Knaack Lake sediments. Anoxic test tubes contained 5 ml of sediment, an initial N_2 headspace, and 6 × 10^5 dpm of [3-^{14}C]lactate and were incubated at 4°C. Substrate transformation was monitored as described in the text.

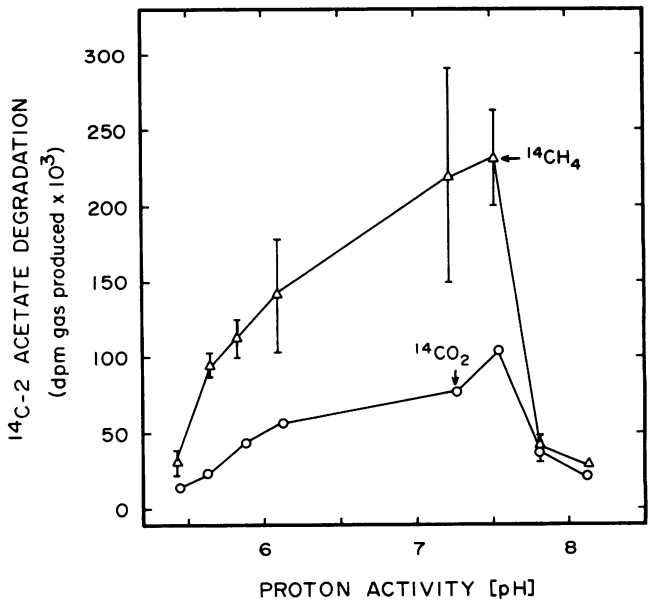

FIG. 4. Influence of in vitro pH on the degradation of [^{14}C]acetate into $^{14}CO_2$ and $^{14}CH_4$ in Knaack Lake sediments. Anoxic test tubes contained an initial N_2 headspace and 5 ml of sediment which was adjusted to the pH indicated before the addition of 2.2 × 10^6 dpm of [2-^{14}C]acetate. $^{14}CO_2$ and $^{14}CH_4$ were quantified by gas chromatographic-gas proportion counting techniques after 1 day of incubation at 4°C.

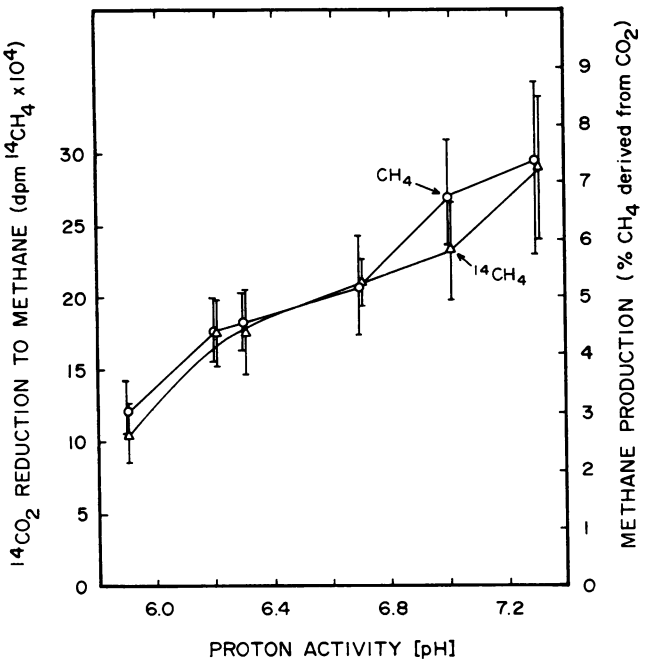

FIG. 5. Influence of in vitro pH on CO_2 reduction to methane in Knaack Lake sediments. Anoxic test tubes contained an initial N_2 headspace and 5 ml of sediment which was adjusted to the pH indicated before the addition of 10^7 dpm of $Na_2{}^{14}CO_3$. $^{14}CH_4$ and CH_4 were quantified by gas chromatographic-gas proportion counting procedures after 16 h of incubation at 4°C.

replicate experiments which contained excess exogenous hydrogen (i.e., incubations were performed under an H_2-N_2 [5:95%] headspace versus 100% N_2), CO_2 reduction to methane accounted for 14 to 20% of the total methane produced regardless of the mildly acidic to mildly alkaline in vitro pH values used. Thus, the methanogenic population appears limited by pH and available H_2 in Knaack Lake sediments.

DISCUSSION

These results demonstrated that in mildly acidic lake sediments devoid of significant sulfate reduction, pH (i.e., proton activity) dynamically alters the rate and direction of carbon and electron flow during the biodegradation of complex organic matter. The microbial population appears limited by the in situ pH, but it is nevertheless adapted to carbon mineralization at lower rates and with higher pool sizes of acidic metabolites. These results provide the first well-documented evidence that methanogenesis occurs in nature at acid pH values although as a consequence of metabolic alterations. In addition, these results show that Knaack Lake sediments differ dramatically from well-characterized neutral sediments of freshwater and marine environments because hydrogen metabolism does not appear to be dominated by sulfate reduction or methanogenesis.

The organic content (in grams of organic matter per gram [dry weight] of sediment) of Knaack Lake (60 ± 6%) is higher than that of eutrophic Lake Mendota (27 ± 4%). Nonetheless, the specific methane production rate constant (in micromolars per hour) reported for Knaack Lake (6.4 ± 1.2) is closer to the values in an oligotrophic lake (2.75) than in Lake Mendota (34.2 ± 0.1) (Conrad, Lupton, and Zeikus, manuscript in preparation; Conrad, Goodwin, and Zeikus, manuscript in preparation). The present data suggest that low pH (i.e., high proton activity) in Knaack Lake sediments inhibits the overall methane production rates as well as the specific rates of intermediary metabolite degradation (e.g., lactate, acetate, or H_2-CO_2 transformation to methane) and the previously reported rate of pectin hydrolysis (27). In addition, the specific substrate transformation rates for various one- and two-carbon metabolites in Knaack Lake are lower than those reported in neutral Lake Mendota sediments (Phelps and Zeikus, in preparation).

The high pool sizes and the specific carbon biodegradation patterns for formate, acetate, lactate, and ethanol, as well as the documentation of low levels of CO_2 transformation to acetate, support the general conclusion that the direction of carbon and electron flow in these sediments is dominated by anaerobic bacteria which produce organic acids. Lactate, ethanol, formate, and acetate, which are products of heterolactic acid fermentations, may accumulate at high levels in Knaack Lake sediments because the terminal trophic groups are inhibited by low pH values and sulfate-reducing activity is not significant. Nonetheless, formate, ethanol, and lactate were further transformed into acetate which itself accounted for nearly all of the methane produced.

One point concerning anaerobic degradation of acetate in Knaack Lake is particularly interesting. Namely, high levels (24%) of acetate methyl group oxidation to CO_2 are observed in the absence of sulfate reduction. The levels of $^{14}CO_2$ production from [2-^{14}C]acetate in Knaack Lake are higher than those recently reported by Krzycki et al. (13) during exponential growth of *Methanosarcina barkeri* on acetate. This methanogen was shown to have two mechanisms of acetate transformation and to produce 86% of the total methane by reduction of the methyl group to methane and to form 14% of the methane from coupling the oxidation of the methyl group to CO_2 with reduction of the carboxyl group to methane. The prevalent methanogen identified in Knaack Lake sediments is physiologically similar to *M. barkeri* (Phelps and Zeikus, in preparation). Thus, it is suggested here that previous environmental interpretations (36) of the significance of acetate transformation via methanogenesis versus sulfidogenesis which are based on calculation of a "respiratory index" (i.e., the amount of $^{14}CO_2/[^{14}CO_2 + {}^{14}CH_4]$ produced from [2-^{14}C]acetate) be tendered with extreme caution in light of these new environmental and physiological results.

The present data suggest that alteration of carbon and electron flow by low pH in Knaack Lake sediments is of selective advantage to homoacetogenic bacteria, and this enables them to compete with methanogens and obligate H_2-producing acetogens for common substrates (i.e., H_2, lactate, ethanol, formate, methanol). This supposition is supported by trophic group population estimates and analysis of the prevalent bacteria in end-dilution tubes. Among the common one-carbon substrates (i.e., H_2-CO_2, CH_3OH, HCOOH) whose metabolism can support energy conservation for either methanogens or homoacetogens (38), higher numbers of homoacetogens were found in Knaack Lake sediments. The prevalent methanol-degrading anaerobe isolated from end-dilution tubes was similar to *Butyribacterium methylotrophicum* (Phelps and Zeikus, in preparation). In a subsequent paper (Schink, Phelps, and Zeikus, submitted for publication) which compares the exact carbon flow pathways for ethanol transformation in different ecosystems, it is concluded that ethanol is metabolized in Knaack Lake predominantly via acetogenesis and that *Clostridium aceticum* is the prevalent ethanol-degrading species, whereas in

sewage sludge obligate syntrophic species degraded ethanol into acetate and H_2.

A final point which has not yet been substantiated is worth mentioning because the high numbers of H_2-consuming acetogens in Knaack Lake is atypical of their relative number in different ecosystems (3, 38). Namely, homoacetogens may be well adapted to maintaining low partial pressures of hydrogen in acidic environments but not necessarily because of consuming H_2-CO_2 alone. Rather, this may be a consequence of not producing excess hydrogen during the metabolism of multicarbon substrates such as glucose, lactate, and ethanol. In a subsequent study (Conrad et al., in preparation), analysis of hydrogen metabolism in Knaack Lake sediments indicates that the amount of dissolved hydrogen available and turned over by the endogenous anaerobes present is extremely low but is enough to account for the low (i.e., 4%) amount of methane which was observed from CO_2 reduction. Furthermore, these sediments contain *Methanospirillum hungatii* (Schink et al., submitted for publication), an H_2-consuming, non-acetate-degrading methanogen. Thus, methanogens appear to compete with homoacetogens for hydrogen.

ADDENDUM IN PROOF

After this paper was submitted for publication, R. T. Williams and R. L. Crawford (Appl. Environ. Microbiol. **47**:1266–1271) demonstrated that methanogenesis occurs at acidic pH values in peats.

LITERATURE CITED

1. **Abram, J. W., and D. B. Nedwell.** 1978. Inhibition of methanogenesis by sulphate reducing bacteria competing for transferred hydrogen. Arch. Microbiol. **117**:89–92.
2. **Belyaev, S. S., Z. I. Finkelstein, and M. V. Ivanov.** 1975. Intensity of bacterial methane formation in ooze deposits of certain lakes. Microbiology **41**:272–275.
3. **Braun, M., S. Schoberth, and G. Gottschalk.** 1979. Enumeration of bacteria forming acetate from H_2 and CO_2 in anaerobic habitats. Arch. Microbiol. **120**:201–214.
4. **Cappenberg, T. E.** 1974. Interrelations between sulfate-reducing and methane-producing bacteria in bottom deposits of a freshwater lake. I. Field observations. Antonie van Leeuwenhoek J. Microbiol. Serol. **40**:285–295.
5. **Cappenberg, T. E., and R. A. Prins.** 1974. Interrelations between sulfate-reducing and methane-producing bacteria in bottom deposits of a freshwater lake. III. Experiments with ^{14}C-labeled substrates. Antonie van Leeuwenhoek J. Microbiol. Serol. **40**:457–469.
6. **Ingvorsen, K., and T. D. Brock.** 1982. Electron flow via sulfate reduction and methanogenesis in the anaerobic hypolimnion of Lake Mendota. Limnol. Oceanogr. **27**:559–564.
7. **Ingvorsen, K., J. G. Zeikus, and T. D. Brock.** 1981. Dynamics of bacterial sulfate reduction in a eutrophic lake. Appl. Environ. Microbiol. **42**:1029–1036.
8. **Jones, J. G., B. M. Simon, and S. Gardener.** 1982. Factors affecting methanogenesis and associated anaerobic processes in the sediment of a stratified lake. J. Gen. Microbiol. **128**:1–11.
9. **Kerby, R., W. Niemczura, and J. G. Zeikus.** 1983. Single-carbon catabolism in acetogens: analysis of carbon flow in *Acetobacterium woodii* and *Butyribacterium methylotrophicum* by fermentation and ^{13}C nuclear magnetic resonance measurement. J. Bacteriol. **155**:1208–1218.
10. **King, G. M., M. J. Klug, and D. R. Lovley.** 1983. Metabolism of acetate, methanol, and methylated amines in intertidal sediments of Lowes Cove, Maine. Appl. Environ. Microbiol. **45**:1848–1853.
11. **Kirsch, E. J., and R. M. Sykes.** 1971. Anaerobic digestion in biological waste treatment. Prog. Ind. Microbiol. **9**:155–239.
12. **Kristjansson, J. K., P. Schonheit, and R. K. Thauer.** 1982. Different K_s values for hydrogen of methanogenic bacteria and sulfate reducing bacteria: an explanation for the apparent inhibition of methanogenesis by sulfate. Arch. Microbiol. **131**:278–282.
13. **Krzycki, J. A., R. H. Wolkin, and J. G. Zeikus.** 1982. Comparison of unitrophic and mixotrophic substrate metabolism by an acetate-adapted strain of *Methanosarcina barkeri*. J. Bacteriol. **149**:247–254.
14. **Lovitt, R. W., R. Longin, and J. G. Zeikus.** 1984. Ethanol production by thermophilic bacteria: physiological comparison of solvent effects on parent and alcohol-tolerant strains of *C. thermohydrosulfuricum*. Appl. Environ. Microbiol. **48**:171–177.
15. **Lovley, D. R., D. R. Dwyer, and M. J. Klug.** 1982. Kinetic analysis of competition between sulfate reducers and methanogens for hydrogen in sediments. Appl. Environ. Microbiol. **43**:1373–1379.
16. **Lovley, D. R., and M. J. Klug.** 1983. Methanogenesis from methanol and methylamines and acetogenesis from hydrogen and carbon dioxide in the sediments of a eutrophic lake. Appl. Environ. Microbiol. **45**:1310–1315.
17. **Lovley, D. R., and M. J. Klug.** 1983. Sulfate reducers can outcompete methanogens at freshwater sulfate concentrations. Appl. Environ. Microbiol. **45**:187–192.
18. **Lovley, D. R., and M. J. Klug.** 1982. Intermediary metabolism of organic matter in the sediments of a eutrophic lake. Appl. Environ. Microbiol. **43**:552–560.

18a. **Lupton, F. S., and J. G. Zeikus.** 1984. Physiological basis for sulfate dependent hydrogen competition between sulfidogens and methanogens. Curr. Microbiol. **11**:7–12.

19. **Martens, C. S., and R. A. Berner.** 1977. Interstitial water chemistry of anoxic Long Island Sound sediments. I. Dissolved gases. Limnol. Oceanogr. **22**:10–25.
20. **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.
21. **Oremland, R. S., L. M. Marsh, and S. Polcin.** 1982. Methane production and simultaneous sulphate reduction in anoxic, salt marsh sediment. Nature (London) **296**:143–145.
22. **Oremland, R. S., and S. Polcin.** 1982. Methanogenesis and sulfate reduction: competitive and noncompetitive substrates in estuarine sediments. Appl. Environ. Microbiol. **44**:1270–1276.
23. **Oremland, R. S., and B. F. Taylor.** 1978. Sulfate reduction and methanogenesis in marine sediment. Geochim. Cosmochim. Acta **42**:209–214.
24. **Parkin, T. B., M. R. Winfrey, and T. D. Brock.** 1980. The physical and chemical limnology of a Wisconsin meromictic lake. Wis. Acad. Sci. Arts Lett. **68**:111–125.
25. **Robinson, J. A., and J. M. Tiedje.** 1984. Competition between sulfate-reducing and methanogenic bacteria for H_2 under resting and growing conditions. Arch. Microbiol. **137**:26–32.
26. **Sansone, F. J., and C. S. Martens.** 1981. Methane production from acetate and associated methane fluxes from anoxic coastal sediments. Science **211**:707–709.
27. **Schink, B. S., and J. G. Zeikus.** 1982. Microbial ecology of pectin decomposition in anoxic lake sediments. J. Gen. Microbiol. **128**:393–404.
28. **Senior, E., E. B. Lindström, I. M. Banat, and D. B. Nedwell.** 1982. Sulfate reduction and methanogenesis in the sediment of a saltmarsh on the East Coast of the United Kingdom. Appl. Environ. Microbiol. **43**:987–996.
29. **Smith, R. L., and M. J. Klug.** 1981. Electron donors utilized by sulfate-reducing bacteria in eutrophic lake sediments. Appl. Environ. Microbiol. **42**:116–121.
30. **Strayer, R. F., and J. M. Tiedje.** 1978. Kinetic parameters of the conversion of methane precursors to methane in a hypereutrophic lake sediment. Appl. Environ. Microbiol. **36**:330–340.
31. **Wellinger, A., and K. Wuhrmann.** 1977. Influence of sulfide compounds on the metabolism of *Methanobacterium* strain AZ. Arch. Microbiol. **115**:13–17.
32. **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.

33. **Winfrey, M. R., and D. M. Ward.** 1983. Substrates for sulfate reduction and methane production in intertidal sediments. Appl. Environ. Microbiol. **45**:193–199.
34. **Winfrey, M. R., and J. G. Zeikus.** 1979. Anaerobic metabolism of immediate methane precursors in Lake Mendota. Appl. Environ. Microbiol. **37**:244–253.
35. **Winfrey, M. R., and J. G. Zeikus.** 1979. Microbial methanogenesis and acetate metabolism in a meromictic lake. Appl. Environ. Microbiol. **37**:213–221.
36. **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.
37. **Zeikus, J. G.** 1983. Metabolic communication in nature, p. 423–462. *In* J. H. Slater, R. Whittenbury, and J. W. T. Wimpenny (ed.), Microbes in their natural environment. Cambridge University Press, Cambridge, United Kingdom.
38. **Zeikus, J. G.** 1983. Metabolism of one carbon compounds by chemotrophic anaerobes. Adv. Microbial Physiol. **24**:215–299.
39. **Zeikus, J. G.** 1980. Microbial populations in digestors, p. 61–87. *In* D. A. Stafford et al. (ed.), Anaerobic digestion. Applied Science Publishers, Ltd., London.
40. **Zeikus, J. G., and M. R. Winfrey.** 1976. Temperature limitation of methanogenesis in aquatic sediments. Appl. Environ. Microbiol. **31**:99–107.