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Effect of Fall Turnover on Terminal Carbon Metabolism in Lake Mendota Sediments

T. J. PHELPS[1] AND J. G. ZEIKUS[2]*

Department of Bacteriology, University of Wisconsin-Madison, Madison, Wisconsin 53706,[1] and Michigan Biotechnology Institute, Departments of Biochemistry and Microbiology, Michigan State University, East Lansing, Michigan 48824[2]

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The carbon and electron flow pathways and the bacterial populations responsible for the transformation of H_2-CO_2, formate, methanol, methylamine, acetate, ethanol, and lactate were examined in eutrophic sediments collected during summer stratification and fall turnover. The rate of methane formation averaged 1,130 μmol of CH_4 per liter of sediment per day during late-summer stratification versus 433 μmol of CH_4 per liter of sediment per day during the early portion of fall turnover, whereas the rate of sulfate reduction was 280 μmol of sulfate per liter of sediment per day versus 1,840 μmol of sulfate per liter of sediment per day during the same time periods, respectively. The sulfate-reducing population remained constant while the methanogenic population decreased by one to two orders of magnitude during turnover. The acetate concentration increased from 32 to 81 μmol per liter of sediment while the acetate transformation rate constant decreased from 3.22 to 0.70 per h, respectively, during stratification versus turnover. Acetate accounted for nearly 100% of total sedimentary methanogenesis during turnover versus 70% during stratification. The fraction of $^{14}CO_2$ produced from all ^{14}C-labeled substrates examined was 10 to 40% higher during fall turnover than during stratification. The addition of sulfate, thiosulfate, or sulfur to stratified sediments mimicked fall turnover in that more CO_2 and CH_4 were produced. The addition of *Desulfovibrio vulgaris* to sulfate-amended sediments greatly enhanced the amount of CO_2 produced from either [^{14}C]methanol or [2-^{14}C]acetate, suggesting that H_2 consumption by sulfate reducers can alter methanol or acetate transformation by sedimentary methanogens. These data imply that turnover dynamically altered carbon transformation in eutrophic sediments such that sulfate reduction dominated over methanogenesis principally as a consequence of altering hydrogen metabolism.

The consumption of hydrogen and simple carbon metabolites is a very important biochemical reaction performed by terminal bacterial trophic groups in anoxic environments (38, 39). Three diverse bacterial groups have been recognized which can consume H_2, one-carbon substrates (e.g., formate or methanol), and two-carbon substrates (e.g., acetate or ethanol) as energy sources; these groups are the methanogens, sulfidogens, and acetogens. It is generally known that low temperatures, oxygen, and high sulfate concentrations inhibit methanogenesis in sedimentary ecosystems.

A tremendous amount of environmental research has focused on understanding the roles of sulfate-reducing and methane-producing bacteria during anaerobic decomposition processes in freshwater and marine sediments (1, 3, 4, 6–8, 11, 14, 16, 17, 21, 24–26, 28–37). The results of these studies support the concept that sulfate reducers dominate in sediments where sulfate is in excess by outcompeting methanogens for common energy sources. It has also been suggested (12, 14, 19) that sulfate reducers can outcompete methanogens for hydrogen because of better hydrogen metabolism kinetics (i.e., lower K_m and higher V_{max}).

In eutrophic Lake Mendota, sulfate is depleted from the hypolimnion during late-summer stratification, whereas during fall turnover, sulfate and oxygen are replenished in the bottom waters (5, 6). The addition of sulfate has been suggested to inhibit methanogenesis in Lake Mendota sediments as a consequence of competition between sulfate reducers and methanogens for common energy sources (36). The present study tested the hypothesis that fall turnover dynamically alters carbon and electron flow pathways in Lake Mendota sediments and provides selective advantages for sulfate-reducing bacteria.

* Corresponding author.

MATERIALS AND METHODS

Description of experimental site and sampling procedures. Lake Mendota is a 3,900 ha, neutral pH, eutrophic, hard-water lake of glacial origin located in Madison, Wis. It has a maximum depth of 24 m, is anaerobic below 10.5 m during summer stratification, and has a maximum sediment temperature of 14°C. All sediment samples were collected by anaerobic techniques (37) and were obtained from the 24-m depth with an Eckman dredge. Previous studies demonstrated that replicate surface (0 to 2 cm) sediment samples taken from this site on a given date during stratification displayed equivalent methanogenic and sulfidogenic rates within 95% confidence levels (7, 37). Sediment was placed into N_2-flushed, 300-ml glass bottles, sealed with butyl rubber stoppers, stored in ice chests, and transported to the laboratory. All analyses and experiments used fresh sediment (0- to 2-cm depth) collected from 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 Science Co., Skokie, Ill.) to remove oxygen. All chemicals used were of reagent grade obtained from Mallinckrodt, Inc., Paris, Ky., or Sigma Chemical Co., St. Louis, Mo. Radioactive [2-^{14}C]acetate (56 mCi/mmol) was obtained from Amersham Corp., Arlington Heights, Ill., and dissolved in water. Radioactive [^{14}C]methylamine (4 mCi/mmol) dissolved in ethanol and [^{14}C]methanol (50 mCi/mmol) dissovled in water were purchased from ICN Pharmaceuticals Inc., Irvine, Calif. Radioactive [^{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, [3-^{14}C]lactate (21.5 mCi/mmol) in H_2O, and $^{35}SO_4$ (739 mCi/mmol) in aqueous solution were obtained from New England Nuclear Corp., Boston, Mass.

Quantification of sediment metabolites. Dissolved methane, oxygen, carbon dioxide, sulfate, and sulfide were measured as described previously (37). The limits of detection for sulfate and oxygen were 20 μmol/liter and 0.01 μmol/liter, respectively. Soluble organic metabolites were measured in interstitial water obtained from centrifuged sediments. Methanol and ethanol were measured by gas chromatographic methods (10) with detection limits of 20 μM. Formate levels were determined by enzymatic methods (10) with detection limits of 2 μM. Samples for acetate and lactate analysis were concentrated as described elsewhere (37) and quantified by gas chromatography with limits of detection of 10 μM. Methylamine was quantified in concentrated samples by high-pressure liquid chromatography (HPLC) with a Perkin-Elmer series 3 liquid chromatograph equipped with a Whatman Partisil PXS-10/25 0DS-3 column eluted with degassed 5.0 mM tetrabutylammonium phosphate buffer (pH 7.5). The detector used was a Refracto Monitor 1107 (Laboratory Data Control) with a sensitivity limit of 50 μM.

General studies. All manipulations in anaerobic test tubes included syringe techniques for the transfer of anoxic sediments, additions of anoxic sterile solutions, or the removal of samples for analysis. Unless otherwise stated elsewhere in the text, all individual experiments were performed in triplicate sets of tubes that each contained 5 ml of sediment and an N_2 gas phase and which were sealed with black butyl rubber bungs and incubated at 14°C, the maximum in situ temperature.

Radioisotopic studies. All solutions (1.0 to 5.0 μCi) were transferred with gas tight syringes (The Hamilton Co., Reno, Nev.) equipped with Teflon plungers, and transfers of gases were performed with Pressure-Lok syringes (Precision Sampling Co., Baton Rouge, La.). Radioactive $^{14}CO_2$ and $^{14}CH_4$ were quantified by gas chromatograph-gas proportion counting methods (22). Total CO_2 was determined from acidified controls. Carbon transformation parameters were calculated as previously described (26). The amount of $^{35}SO_4^{2-}$ transformed into $^{35}S^{2-}$ was determined by techniques described elsewhere (7). Radioactive [^{14}C]acetate was separated either by Dowex Agl anion exchange chromatography of HPLC methods, and then radioactivity was measured by liquid scintillation procedures (18). At each point in a time course, carbon or sulfur transformation was terminated in triplicate sets of tubes and then analyzed. Transformation in experimental tubes containing ^{14}C-labeled radioisotopes was terminated by adding 0.1 ml of a 40% Formalin solution, while transformation in each of those tubes containing ^{35}S-labeled radioisotopes was terminated with 0.1 ml of a 5.0 N NaOH solution. The rate of sulfate consumption was determined by multiplying the turnover rate constant for $^{35}SO_4^{2-}$ by the pool size for sulfate times 24 h.

Enumeration studies. Three-tube, most-probable-number (MPN) analyses were performed for both methanogens and sulfate reducers. A carbonate-buffered medium and a phosphate-buffered medium (20) were used for the enumeration of methanogens. Additions to the media included 0.05% yeast extract, a 20 mM energy source or an H_2-CO_2 (80:20) headspace, and 0.5 μCi of the indicated ^{14}C-labeled carbon source. Sulfate reducers were enumerated in a medium described previously (2) plus 2 mM ferrous sulfate. The tubes were incubated at 30°C for 1 month. Positive results were recorded if greater than 1% of the tracer was converted to $^{14}CO_2$ (total heterotrophs) or $^{14}CH_4$ (methanogens) or if a black ferrous sulfide precipitate (sulfate reducers) was formed. The first dilution tube was not used for enumeration calculations.

Studies requiring exogenous additions. *Desulfovibrio vulgaris* was grown in the phosphate-buffered medium described previously (2) plus 0.05% yeast extract with H_2 (2.0 atm [1 atm = 101.29 kPa]) serving as the electron donor. The cells were cultured in 158-ml serum vials (Wheaton Industries, Millville, N.J.) containing 75 ml of medium. When growth reached 75% of maximum (optical density at 660 nm, 0.4) the vials were centrifuged and inverted, and the medium was expelled through venting needles. The cells were suspended in the basal medium and washed two times. Cell suspensions were prepared so that 0.05 to 0.2 ml was added per tube of sediment.

Exogenous electron donors, electron acceptors, or inhibitors (±0.15 ml) were added via syringe to experimental tubes as anaerobic solutions.

RESULTS

Initial experiments were designed to compare the endogenous rates of methanogenesis and sulfate reduction during summer stratification and fall turnover. During summer stratification, the average rates for methanogenesis and sulfate reduction were 0.963 mmol of CH_4 per liter of sediment per 24 h and 0.28 mmol of sulfate consumed per liter of sediment per 24 h, whereas during fall turnover, average values of 0.463 CH_4 per liter of sediment per 24 h and 1.84 mmol of sulfate consumed per liter of sediment per 24 h were observed.

The seasonal variation of temperature, fraction of CO_2 from [2-^{14}C]acetate, sulfate concentration, and abundance of oxygen in bottom waters are illustrated in Fig. 1. During stratification (i.e., July through September), the sediment temperature was 8 to 14°C. The sulfate concentration was lowered to 20 μM, oxygen was not present in the bottom waters, and the lowest gas ratio values from [2-^{14}C]acetate transformation were observed. The rate of methanogenesis exceeded that of sulfate reduction only during the warmest period of summer statification when sulfate was depleted from the hypolimnion (data not shown).

Pool sizes and microbial populations. Experiments were designed to qualitatively determine which terminal intermediary metabolites and bacterial trophic groups were significant in carbon cycling during summer stratification and fall turnover in Lake Mendota sediments. The results (Table 1) represent the average values obtained from multiple experiments performed on sediment samples collected over a 3-year sampling period.

The approximate pool sizes of microbial metabolites detected in sediment interstitial water are compared in Table 1. As expected, the sediments were devoid of oxygen and low in sulfate concentration during summer stratification. The concentrations of methane, carbon dioxide, acetate, ethanol, and lactate were typical of values reported in other eutrophic freshwater sediments (4, 17, 34, 37).

These results led to enumeration studies aimed at comparing the approximate populations of methanogens and sulfate-reducing bacteria during lake turnover and stratification. Specific populations were enumerated on substrates shown to be consumed as energy sources for growth of methanogens and sulfate reducers in pure cultures (39). The sulfate-reducing and heterotrophic populations were nearly equivalent, while the methanogenic populations were lower

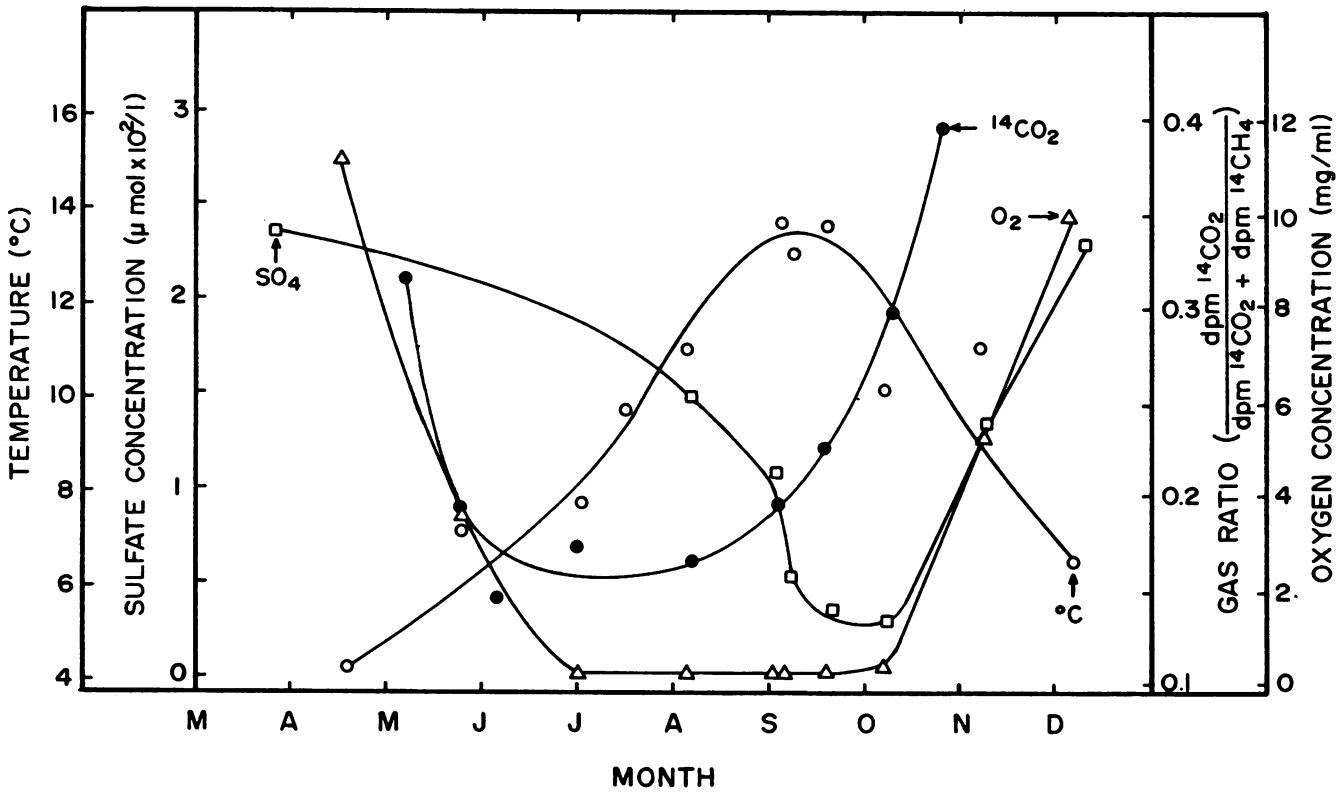

FIG. 1. Influence of seasonal variation on temperature, sulfate and O_2 concentrations, and the fraction of CO_2 produced from [2-^{14}C]acetate in Lake Mendota sediments. The data obtained were averaged from 13 separate samples collected over a 3-year period. The gas ratio is represented as $^{14}CO_2$ on the plot.

(Table 2). The population of sulfate reducers was larger than the population of methanogens capable of consuming H_2 or formate but not acetate. During fall turnover, total heterotrophic and sulfate-reducing populations remained constant while the numbers of methanogens decreased by at least one order of magnitude on all substrates examined.

Carbon substrate transformation parameters. The importance of the detected intermediary metabolites in methanogenesis was assessed by comparing their carbon mineralization parameters. In these experiments, the substrate transformation rate constant was based on the rate of total $^{14}CO_2$ or $^{14}CH_4$ production or both. The results of these experiments, which represent the average value of multiple determinations made during a 3-year period, are presented in Table 3. Carbon dioxide (i.e., total inorganic carbon) turned over slowly as a consequence of its large pool size, corresponding to transformation times of 142 and 220 h under stratified versus unstratified conditions. Formate displayed the highest transformation rate constant and the fastest transformation rate, but similar to other freshwater environments (17), it was a minor immediate methane precursor. Acetate was rapidly transformed during summer stratification (transformation time, 23 min) and, as expected (17, 37), was the major methanogenic precursor. During fall turnover, acetate was transformed considerably more slowly (transformation time, 86 min), yet more CO_2 was produced from C_2 acetate. Labeled methanol and methylamine were also transformed to labeled methane and CO_2 but were judged to be insignificant to total methanogenesis because their combined in situ concentrations (at the detection limits of the analytical methods used) suggested that their contribution was less than 5% of the total methane produced. Nonetheless, the CO_2 gas ratio from methanol and methylamine increased during fall turnover. Also during fall turnover, the substrate transformation rate of lactate plus that of ethanol equaled the transformation rate of acetate, suggesting that lactate and ethanol were major precursors of acetate during unstratified periods. HPLC analyses of sediment time course experiments containing ^{14}C-labeled metabolites demonstrated that

TABLE 1. Qualitative concentrations of intermediary metabolites in sediment interstitial waters from Lake Mendota during late summer stratification and fall turnover[a]

Metabolites	Mean metabolite pool sizes [μmol/liter (SD)] during:	
	Summer stratification	Fall turnover
Oxygen	ND[b]	0.45 (0.1)
Methane	2,814 (1,060)	1,793 (436)
Sulfide	960 (200)	1,084 (240)
Sulfate	25 (10)	186 (49)
Carbon dioxide	7,500 (1,500)	7,800 (1,800)
Formate	6.1 (9.6)	8.6 (12.3)
Acetate	32 (8)	81 (42)
Ethanol	43 (40)	30 (40)
Lactate	ND	77 (26)

[a] Metabolites that were not detected (limits of detection were <5 to 100 μmol): methanol, methylamine, thiosulfate, and sulfur. Each metabolite was measured in 3 to 13 separate samples collected during summer stratification and fall turnover.

[b] ND, Not detectable.

TABLE 2. Relation of seasonal variation to qualitative abundance of methanogenic and sulfate-reducing bacteria in Lake Mendota sediments[a]

Electron donor	Population levels (MPN/g [dry weight] of sediment) of indicated bacterial types during:					
	Summer stratification			Fall turnover		
	Total	Methanogens	Sulfate reducing	Total	Methanogens	Sulfate reducing
Glucose	10^7		10^6	10^7		10^6
Lactate	10^6		10^6	10^6		10^6
H_2		10^5	10^6		10^3	10^6
Formate	10^6	10^4	10^6	10^6	10^3	10^6
Methylmercaptan	10^4	10^4		10^3	10^3	
Methylamine	10^4	10^4		10^3	10^3	
Methanol	10^5	10^5	ND	10^5	10^3	ND
Acetate	10^4	10^4	ND	10^3	10^3	ND
Ethanol	10^5		10^5	10^5		10^5

[a] Experimental conditions: MPN determinations used triplicate tubes and were based on detecting ferrous sulfide for sulfate reducers, $^{14}CH_4$ for methanogens, or $^{14}CO_2$ for a total population index control. MPN values reported are from 13 separate determinations over a 3-year sampling period from June 1981 to 1983 and are averaged to the nearest exponent. ND, Not detectable.

acetate was the major immediate product of lactate and ethanol decomposition in Lake Mendota sediments (data not shown).

Additional analyses were performed to evaluate the importance of the major immediate methane precursors (i.e., acetate and H_2-CO_2) during summer stratification and fall turnover. Acetate accounted for 70% of the total observed methane formation rate during summer stratification while it accounted for all of the methane formed during fall turnover (Table 4). The amount of methane formed from H_2-CO_2 during fall turnover was one-fourth the amount formed during summer stratification, indicating that fall turnover led to alterations in the pathway of carbon and electron equivalents flowing to methanogenesis.

Influence of exogenous additions on carbon metabolism in stratified sediments. The fraction of $^{14}CO_2$ produced from [^{14}C]labeled methane precursors was compared in the presence or absence of 5.0 mM sulfate to ascertain whether the addition of sulfate to stratified sediments led to increased carbon oxidation simiilar to the increase observed during fall turnover. The specific CO_2 gas ratios were significantly increased upon the addition of exogenous sulfate to stratified sediments (Table 5). Thiosulfate and sulfur, which are used as electron acceptors by sulfate-reducing bacteria, also led to increased $^{14}CO_2$ evolution from [2-^{14}C]acetate, [^{14}C]methanol, or [3-^{14}C]lactate (Fig. 2).

Studies were initiated to explain how higher environmental sulfate concentrations increased $^{14}CO_2$ production from [2-^{14}C]acetate or [^{14}C]methanol when sulfate-reducing populations capable of consuming these substrates were not detectable. These experiments examined the hypothesis that hydrogen-consuming bacteria alter the metabolism of methanogens that transform acetate or methanol in lake sediments. Therefore, the influence of adding exogenous amounts of a sulfate-reducing species, which consumes hydrogen but not acetate or methanol as an electron donor,

TABLE 3. Relation of seasonal variation to carbon metabolite biodegradation parameters in Lake Mendota sediments[a]

Substrate	Substrate transformation h^{-1} (±SD)	Substrate transformation (μmol of substrate per liter of sediment per h)	Produced gas ratio (dpm of $^{14}CO_2$ + $^{14}CH_4$)	Methane production (μmol/liter of sediment per h)
Summer stratification				
Carbon dioxide	0.0065 (±0.0014)	49		9.8
Formate	2.95 (±1.87)	18	0.98	0.57
Acetate (C_2)	2.55 (±0.78)	82	0.23	33.1
Methylamine	0.043 (±0.014)	ND	0.29	ND
Methanol	0.135 (±0.06)	ND	0.24	ND
Lactate (C_3)	0.4 (±0.22)	ND	0.30	ND
Ethanol (C_2)	0.54 (±0.09)	23	0.33	3.0
Fall turnover				
Carbon dioxide	0.0045 (±0.0026)	33.9		2.44
Formate	2.98 (±1.87)	25.4	0.98	0.17
Acetate (C_2)	0.70 (±0.42)	56.3	0.37	18.9
Methylamine	0.041 (±0.018)	ND	0.56	ND
Methanol	0.27 (±0.12)	ND	0.35	ND
Lactate (C_3)	0.39 (±0.09)	29	0.40	6.3
Ethanol (C_2)	0.89 (±0.13)	26.7	0.42	4.6

[a] Experimental conditions: triplicate anaerobic tubes contained 5 ml of sediment and 5 μCi of [^{14}C]HCO_3 or 1 μCi of the ^{14}C-labeled organic substrate indicated and were incubated at 14°C. ND, Not determined because the metabolite pool size was below the limits of detection. Controls treated with Formalin did not produce $^{14}CO_2$ or $^{14}CH_4$. Values represent averages of experiments conducted three or more times during a 3-year period from 1981 to 1983.

TABLE 4. Relationship of seasonal variation to methanogenesis from acetate versus carbon dioxide in Lake Mendota sediment

Methane value	Methane production [μmol/liter of sediment per day (±SD; % contribution to methane)[a]] during:	
	Summer stratification	Fall turnover
Total methane[b]	1,130 (±295)	437 (±211)
[2-^{14}C]acetate-dependent methanogenesis	795 (±160; 70)	453 (±270; 103)
$^{14}CO_2$-dependent methanogenesis	235 (±47; 21)	58 (±32; 13)

[a] % Contribution to methane = methane production rate from precursor (see Table 3)/total methane production rate.
[b] Total methane, Average production rate over the 3-year study.

TABLE 5. Influence of exogenous sulfate addition on gas production in Lake Mendota sediments collected during summer stratification

^{14}C-labeled precursor	Produced gas ratio [dpm of $^{14}CO_2$/(dpm of $^{14}CO_2$ + $^{14}CH_4$)] (±SD)	
	Minus sulfate	Plus sulfate
Methylamine	0.29 (±0.05)	0.39 (±0.13)
Methanol	0.24 (±0.05)	0.42 (±0.05)
Acetate (C_2)	0.23 (±0.07)	0.53 (±0.1)
Ethanol (C_2)	0.33 (±0.03)	0.72 (±0.04)
Lactate (C_3)	0.30 (±0.07)	0.47 (±0.13)

[a] Experimental conditions: as described in footnote *a* of Table 3, except 50 mM sodium sulfate (0.1 ml) was added where indicated.

on the CO_2 gas ratio was determined (Table 6). The addition of sulfate and *D. vulgaris* to stratified sediment increased the fraction of CO_2 produced from [^{14}C]methanol or [2-^{14}C]acetate (Table 6). The fraction of CO_2 produced increased when higher levels of *D. vulgaris* were added. When an inhibitor of sulfate reduction, 2 mM sodium molybdate, was added to tubes containing *D. vulgaris* and sulfate, the fraction of CO_2 decreased nearly to control levels. In separate control experiments, chloroform (100 μm) was added to inhibit methanogenesis in sediments with or without exogenous sulfate. In both cases, total methane and CO_2 production was inhibited by 90%, implying that the consumption of these substrates from acetate or methanol was dependent on methanogens.

DISCUSSION

These results demonstrated that in eutrophic Lake Mendota sediments, seasonal variation dramatically altered the rate and direction of terminal carbon and electron flow during organic decomposition processes. These results provided the first well documented evidence that in situ chemical changes alter the relationship of methanogenesis and sulfate reduction and verified the importance of specific methanogenic substrates in eutrophic freshwater sediments. The microbial community apparently adjusted to the physical-chemical changes inherent with fall turnover by increasing sulfate-reducing activities at the expense of methanogenesis. In general, fall turnover and the increased sulfate concentration in the surface sediments were qualitatively associated with (i) decreased pool sizes and methane and enhanced rates of sulfate reduction, (ii) decreased rates of methanogenesis and smaller populations of methanogens, and (iii) increased levels of CO_2 produced from simple, reduced-carbon metabolites.

During fall turnover, larger pool sizes of acetate were associated with slower rates of decomposition and lower numbers of methanogens. Lactate, a substrate known to be used by sulfate reducers, exhibited transformation rates during fall turnover which were greater than or equal to those during summer stratification, while the sulfate-reducing population remained nearly constant. Sulfate reduction rates and sulfide production rates also increased with the advent of fall turnover. The stiumulation of sulfate reducers over methanogens during fall turnover may be attributed to more sulfate, which would enable them to successfully compete with methanogens for electron donors (12, 14, 17), and their ability to survive in oxygenated waters (9), allowing them to maintain a metabolically active community in the sediments.

During fall turnover, competition between sulfate reducers and methanogens for H_2 was evidenced by a fivefold decrease in the amount of methane formed from H_2-CO_2. The addition of exogenous sulfate to sediments obtained during summer stratification resulted in higher levels of $^{14}CO_2$ production from all ^{14}C-labeled metabolites examined. Previously, the sulfate-dependent increase in $^{14}CO_2$ production from [2-^{14}C]acetate in lake sediments was interpreted (37) to represent competition between sulfate reducers and methanogens for acetate. Based on the experiments here in which *D. vulgaris* was added to sediments, it is proposed that the sulfate-dependent increase in the fraction of $^{14}CO_2$ formed from [2-^{14}C]acetate or [^{14}C]methanol is in part due to H_2 consumption by sulfate reducers. In other words, sulfate reducers may not necessarily compete with methanogens for acetate, methylamine, or methanol in freshwater sediments but may compete for reducing equivalents generated from these substrates (i.e., transferred to the sulfate reducer via interspecies H_2 transfer with the methanogen serving as the H_2 donating species).

Physiological studies (13, 27) support the suggestion that the increased fraction of CO_2 produced from ^{14}C-labeled substrates in sediments collected during fall turnover or during stratification in the presence of exogenous sulfate is caused by competition for H_2 between methanogens and

TABLE 6. Influence of *Desulfovibrio vulgaris* and sulfate additions on [2-^{14}C]acetate and [^{14}C]methanol metabolism in stratified Lake Mendota sediment[a]

Additions	Produced gas ratio (dpm $^{14}CO_2$/dpm $^{14}CO_2$ + $^{14}CH_4$)	
	[^{14}C]acetate	[^{14}C]methanol
Control conditions		
None	0.24 ± 0.06	0.37 ± 0.11
Sulfate[a]	0.29 ± 0.08	0.43 ± 0.12
D. vulgaris[b]	0.23 ± 0.03	0.44 ± 0.10
Experimental conditions		
Sulfate and 0.001× *D. vulgaris*	0.36 ± 0.07	0.49 ± 0.06
Sulfate and 0.03× *D. vulgaris*	0.39 ± 0.09	0.53 ± 0.07
Sulfate and 1× *D. vulgaris*	0.52 ± 0.04	0.58 ± 0.06
Sulfate and 1× *D. vulgaris* and Na_2MoO_4	0.33 ± 0.18	0.38 ± 0.09

[a] Experimental conditions were as follows. Two series of triplicate tubes containing 5.0 ml of sediment, an N_2 gas phase, and 1.0 μCi of the ^{14}C-tracer were incubated at 14°C for 2 h. Additions, where indicated, were sulfate, 0.5 mM; substrate, 0.5 mM; sodium molybdate, 2.0 mM; and *D. vulgaris* (1×), 0.65 mg (dry wt) per tube.

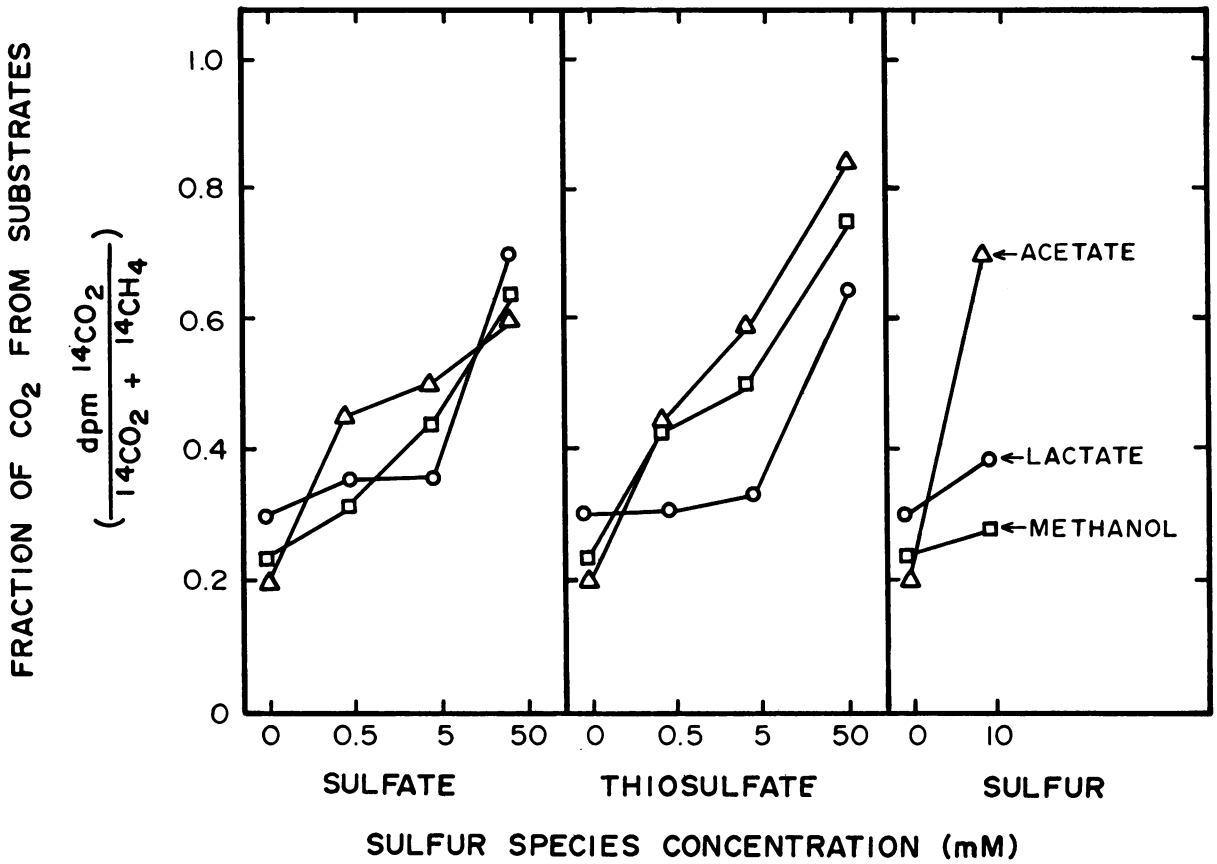

FIG. 2. Influence of exogenous sulfur-containing electron acceptors on the produced gas ratio from [^{14}C]methanol, [2-^{14}C]acetate and [3-^{14}C]lactate. Experimental conditions were as follows. Anaerobic tubes contained an N_2 gas phase, 5 ml of sediment, 2×10^6 dpm of the ^{14}C-labeled substrate indicated, and the appropriate S-containing electron acceptor (±0.15 ml addition) and were incubated for 24 h at 14°C. Data are averages from three or more sample dates during summer stratification.

sulfate reducers. It is of interest that trace amounts of H_2 are produced in pure cultures of *Methanosarcina* species when grown on organic substrates (15, 23, 27). Furthermore, we have shown (27) that cocultures of *D. vulgaris* and *M. barkeri* can be grown on methanol or acetate plus sulfate and that hydrogen consumption by the sulfate reducer decreases methanogenesis as a consequence of increasing the fraction of CO_2 produced from these electron donors.

In a subsequent paper (R. Conrad, T. Phelps, and J. G. Zeikus, submitted for publication), we provide evidence that the main reason the addition of sulfate inhibited methanogenesis in sediments collected during stratification is not kinetic competition for H_2 but alteration in the metabolism of sulfate reducers from production to consumption of H_2. This could also explain why sulfate-reducing populations are nearly constant during lake stratification or turnover.

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