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Intermediary Metabolism of Organic Matter in the Sediments of a Eutrophic Lake†

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The rates, products, and controls of the metabolism of fermentation intermediates in the sediments of a eutrophic lake were examined. ^{14}C-fatty acids were directly injected into sediment subcores for turnover rate measurements. The highest rates of acetate turnover were in surface sediments (0- to 2-cm depth). Methane was the dominant product of acetate metabolism at all depths. Simultaneous measurements of acetate, propionate, and lactate turnover in surface sediments gave turnover rates of 159, 20, and 3 μM/h, respectively. [2-^{14}C]propionate and [*U*-^{14}C]lactate were metabolized to [^{14}C]acetate, $^{14}CO_2$, and $^{14}CH_4$. [^{14}C]formate was completely converted to $^{14}CO_2$ in less than 1 min. Inhibition of methanogenesis with chloroform resulted in an immediate accumulation of volatile fatty acids and hydrogen. Hydrogen inhibited the metabolism of C_3-C_5 volatile fatty acids. The rates of fatty acid production were estimated from the rates of fatty acid accumulation in the presence of chloroform or hydrogen. The mean molar rates of production were acetate, 82%; propionate, 13%; butyrates, 2%; and valerates, 3%. A working model for carbon and electron flow is presented which illustrates that fermentation and methanogenesis are the predominate steps in carbon flow and that there is a close interaction between fermentative bacteria, acetogenic hydrogen-producing bacteria, and methanogens.

Although terminal microbial processes in anaerobic lake sediments have been the subject of many studies (6, 7, 22, 24, 26, 30, 32-34), the intermediate pathways for carbon and electron flow from initial substrates to terminal processes have not been intensively studied. In anaerobic environments such as sludge (11, 15, 17) and the rumen (12, 35) acetate is the dominant fermentation intermediate. However, Cappenberg and Prins (7) report that in the sediments of Lake Vechten, lactate, a fermentation product of minor importance in other anaerobic ecosystems, has a turnover rate that is 10-fold greater than the acetate turnover rate. Propionate, butyrates, valerates, and formate have also been detected in freshwater sediments (22, 23, 31), but their importance as intermediates in in situ carbon metabolism has not been determined. The accumulation of C_3-C_5 volatile fatty acids (VFA) in sediments in the presence of added hydrogen or when methanogenesis is inhibited (J. J. Molongoski, Ph.D. thesis, Michigan State University, 1978) suggests that these intermediates may be metabolized to acetate with the production of hydrogen as in other non-gastrointestinal systems (2, 4, 5, 15, 16, 20). Formate may be a direct methane precursor in sediments (30) or converted to hydrogen and carbon dioxide (32).

The reported difference in the relative importance of lactate and acetate and the lack of data on the role of other short-chain fatty acids (SCFA) in sediment metabolism led to the present study. Our purpose was to determine the rates of production and fates of the fermentation intermediates in the profundal sediments of a eutrophic lake to identify the central intermediates in carbon metabolism. In view of the importance of terminal processes in controlling carbon flow in other anaerobic ecosystems (4, 5, 16), we also examined the relative importance of sulfate reduction and methanogenesis in controlling the turnover of fermentation intermediates.

MATERIALS AND METHODS

Sediment sampling. Sediments were collected from within the 6-m depth contour of Wintergreen Lake, a shallow (maximum depth, 6.5 m), eutrophic lake located in southwestern Michigan. Seasonal changes in input of particulate organic matter (21), metabolite pool size (22), rate of methane production (22, 29), and sulfate reduction (26) have been previously described. Surface sediments were sampled with an Eckman dredge or a gravity corer. Only the unconsolidated surface sediments were collected from the Eckman dredge samples. The water content of these sediments was greater than 90%. The coring apparatus was

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designed to allow unrestricted movement of water through the core tube as it was lowered into the sediment with a winch. The unconsolidated surface sediments remained intact, and the sediment cores were identical to cores taken by hand using SCUBA. The 7-cm-diameter plastic core tube had holes spaced at 2-cm intervals which permitted subcoring over the depth of the core. For direct injection studies subcores of sediment were taken with a 5-ml plastic syringe with the needle end cut off. A subcore of approximately 6 ml of sediment was taken, and the syringe was sealed with a serum bottle stopper (Wheaton Scientific) while sediment was extruded to a final volume of 5 ml. Surface sediments (0- to 2-cm depth) for incubations in tubes were collected from gravity cores with a 5-ml plastic syringe through a 16-gauge needle. The needle was pushed into a rubber stopper as sediment was slowly extruded to seal the syringe for storage. Subcores or syringes filled with sediment were stored under water in the dark at in situ temperature.

Measurement of SCFA turnover with ^{14}C-tracers. Turnover experiments were initiated within 3 h of collecting the sediment. A 50-μl sample of the appropriate ^{14}C-labeled substrate that had been preflushed with oxygen-free nitrogen was directly injected into the sediment subcores with a 250-μl Hamilton syringe. The concentration of [^{14}C]SCFA in the tracer solutions was maintained as high as possible to obtain maximal radioactivity without exceeding the expected in situ concentration of the SCFA in the interstitial water. The syringe needle was inserted through the serum stopper, and the tracer was injected as the needle was withdrawn. The subcores were incubated at in situ sediment temperature for appropriate time intervals, and the incubation was stopped by immersing the subcores in an ethanol-dry ice bath. The frozen sediments were extruded into anaerobic pressure tubes (Bellco Glass), stoppered, sealed with an aluminum crimp, and stored at −10°C until further analysis. Tubes were placed in boiling water for 15 min to stop activity and were promptly analyzed for $^{14}CH_4$, $^{14}CO_2$, and [^{14}C]SCFA (see below).

First-order turnover rate constants, k, of each tracer substrate were determined from triplicate replications of at least three time points. The in situ turnover rates were calculated by multiplying k by the in situ pool size. Acetate turnover rate constants were estimated from the first-order loss of [^{14}C]acetate over time. The slope of a plot of the natural logarithm of [^{14}C]acetate versus time with [^{14}C]acetate expressed as total counts or a percentage of the initial counts is the negative of the first-order rate constant, k. Alternatively, k was estimated from plots of the natural logarithm of $\{^{14}C_M/[^{14}C_M-(^{14}C_T/^{14}C_M)]\}$ versus time, where C_M equals the total amount of $^{14}CH_4$ and $^{14}CO_2$ produced from [^{14}C]acetate at time points when $^{14}CH_4$ and $^{14}CO_2$ production had reached a maximum, and C_T equals $^{14}CH_4$ and $^{14}CO_2$ present at time t. The slope of this line equals k. Propionate turnover rate constants were calculated from the slopes of plots of the natural logarithm of [^{14}C]propionate versus time. Sufficient [^{14}C]lactate to detect with the radiochromatography technique could not be injected into subcores without injecting a solution that had a lactate concentration that was significantly higher than the in situ pool. Since [^{14}C]lactate was converted solely to [^{14}C]acetate, $^{14}CO_2$, and $^{14}CH_4$, (see below), the lactate turnover rate constant (k = fraction of available ^{14}C evolved per unit time) was estimated from the linear rate of $^{14}CO_2$ evolution during incubation periods of 10 min or less, before the [^{14}C]acetate that was produced was further metabolized. The amount of label available for $^{14}CO_2$ production during this time period was therefore equivalent to the ^{14}C disintegrations per minute in the carboxyl carbon of the [^{14}C]lactate.

Sediments (5 ml) were incubated in pressure tubes or 10-ml Vacutainer tubes (Beckton, Dickinson & Co.) for preliminary measurements of acetate turnover, for determining the fate of [U-^{14}C]lactate, and for measuring the fraction of total methane produced from hydrogen and carbon dioxide. Before and during the transfer of sediments, tubes were flushed with a 93% N_2–7% CO_2 gas mixture that had been passed through a heated column of reduced copper. Solutions were added to the tubes through the stopper with a syringe and needle and mixed on a Vortex mixer for 10 to 15 s. The sediments were incubated without any further shaking, and activity was stopped by freezing as above. The fraction of methane that was produced from hydrogen and carbon dioxide was estimated by adding [^{14}C]sodium bicarbonate and comparing the specific activity of the methane produced after 16 h of incubation with the specific activity of the carbon dioxide in the headspace. The latter did not change significantly over the incubation period.

The following radiochemicals were used: [2-^{14}C]acetate (54 mCi/mmol; New England Nuclear Corp.), [2-^{14}C]propionate (55.7 mCi/mol; International Chemical and Nuclear), [^{14}C]formate (56 mCi/mmol; International Chemical and Nuclear), [1-^{14}C]butyrate (14 mCi/mmol; New England Nuclear), [^{14}C]sodium bicarbonate (0.1 mCi/mmol; New England Nuclear), and [U-^{14}C]lactate (138.6 mCi/mmol; New England Nuclear).

Measurement of VFA production with H_2, $CHCl_3$, and Na_2MoO_4 inhibition. The method of Kaspar and Whurmann (15) was adapted to measure the production of VFA that are metabolized with hydrogen production. To determine whether hydrogen inhibited the metabolism of C_3-C_5 VFA, approximately 80 ml of sediment was transferred to a 100-ml serum bottle (Wheaton Scientific). The bottle was stoppered with a butyl rubber stopper (Bellco Glass, Inc.) and shaken by hand. Samples (4 ml) from the serum bottle were placed in pressure tubes containing a 93% N_2–7% CO_2 atmosphere. One milliliter of an N_2-flushed solution of the VFA under study was added to the sediment remaining in the serum bottle and, the sediment was shaken. Samples (4 ml) of the amended sediment were added to tubes as above. A 4-ml sample was also taken to measure the initial VFA concentration. Hydrogen (30 ml) was added with a syringe and needle to half of the tubes containing unamended sediment and to half of the tubes containing amended sediment to provide an initial hydrogen partial pressure of approximately 1 atm (100 kPa). All of the tubes were incubated overnight in a horizontal position on a tube roller to enhance hydrogen diffusion into the sediments.

A short-term time course was performed to estimate the rates of production of C_3-C_5 VFA. Hydrogen was added to the headspace of tubes of sediments incubated on the tube roller as above. Replicate tubes were sacrificed at 2- or 3-h intervals over

a 9-h incubation period for VFA determinations.

Rates of acetate production were estimated by an adaption of the method of Chynoweth and Mah (8). Chloroform and sodium molybdate were added to 80 ml of sediment to give final concentrations of 100 μM and 2 mM, respectively. The chloroform did not inhibit sulfate reduction, and the molybdate did not affect methanogenesis (27; unpublished data). The chloroform and molybdate inhibited acetate uptake by methanogens and sulfate reducers, respectively. Sediments were incubated in the serum bottles or added in 4-ml samples to pressure tubes as above. Subsamples were removed from serum bottles or tubes were sacrificed in triplicate for acetate determinations.

The role of sulfate reduction and methanogenesis in controlling the metabolism of fermentation intermediates was determined by treating sediments with either chloroform or sodium molybdate as above. Subsamples were removed for measurement of VFA and hydrogen over time. To determine whether sulfate reducers could compete for hydrogen at in situ concentrations, sulfate reduction was saturated for sulfate by adding sodium sulfate to sediments in serum bottles to a final concentration of 2 mM. Control sediments received no added sulfate. Methane production was measured over a 17.5-h incubation period. Lower rates of methane production in sediments with added sulfate would indicate increased hydrogen or acetate uptake (or both) by sulfate reducers.

Analytical techniques. Interstitial water was collected with dialysis samplers (22) or by centrifuging sediment. Samples obtained with both methods gave comparable results. SCFA were converted to their benzyl esters by a modification of the method of Bethge and Lindstrom (1). Tetraethylammonium hydroxide was added as the counterion, and heptanoate was used as an internal standard. The samples were evaporated to dryness at 70°C under a stream of air that was passed through a trap containing NaOH to remove volatile organic compounds. Benzyl bromide in acetone (typically 1:400, vol/vol) was added to the dried sample and allowed to react for at least 2 h at room temperature. The benzyl esters were separated on a 2-mm (inner diameter) by 2-m glass column packed with 10% Dexsil 300 on 100/120 mesh Supelcoport, (Supelco, Inc.). Helium was used as carrier gas at a flow rate of 30 ml/min. The injector and detector temperatures were 240° and 270°C, respectively. The oven temperature was programmed at 120°C for 4 min, increased to 230°C at a rate of 20°C/min and held at 230°C for 8 min. A 1/8-in. (ca. 3.1-mm; outer diameter) by 2-m stainless steel column of 10% butane diol succinate on Supelcoport (100/120 mesh) was frequently used to confirm the results obtained with the Dexsil column. Nonesterified fatty acids and ethanol were also analyzed on a glass column packed with 10% SP 1220–1% H_3PO_4 on Chromsorb W (Supelco). Operating temperatures were: injector, 160°C; detector, 180°C; oven, 120°C for VFA and 60°C for ethanol. When necessary VFA were concentrated by making the sample basic with NaOH and evaporating the sample as above. Free acids were acidified with H_3PO_4 before analysis. Quantitative analysis of VFA as free acids or as their benzyl derivatives gave comparable results. A Varian 3700 (Varian Instruments) with dual flame ionization detectors was used throughout. A Finnigan model E_1-C_1 gas chromatograph-mass spectrometer was used to further confirm the structure of some VFA. VFA were separated on Carbopack C–3% 20 M Carbowax–0.1% H_3PO_4 (Supelco Inc.) for this analysis.

[^{14}C]SCFA were detected by a modification of the radiochromatography procedure of Zehnder and Brock (36). [^{14}C]SCFA were chromatographed as outlined above. The flame ionization detector of the chromatograph was capped with the flow-measuring assembly that was provided with the instrument. The effluent from the detector was passed through a heated stainless steel line to two scintillation vials that were connected in series. The vials contained 3 ml of scintillation-grade ethanolamine (Eastman Kodak Co.) and 4 ml of methanol as a CO_2-trapping solution. An additional 5 ml of methanol and 7 ml of scintillation cocktail (15 g of 2,5-diphenyloxazole and 1 g of *p*-bis-(*o*-methylstyrl)-benzene in 1 liter of scintillation grade toluene) were added to the vials for liquid scintillation counting in a Beckman LS 8500. Trapping efficiencies as determined with [^{14}C]SCFA were consistently greater than 95%. Counting efficiencies were determined with an internal standard of [^{14}C]toluene (4.5×10^5 dpm/ml; New England Nuclear Corp.).

Specific activities of $^{14}CH_4$ and $^{14}CO_2$ were analyzed with a Varian 3700 gas chromatograph with a thermal conductivity detector that was connected in series with a gas proportional counter. The proportional counter was constructed in the laboratory and operated on the same principles as commercially available proportional counters. Gases were separated at 45°C on a 3-m column of Porapak N (100/120 mesh, Waters Associates) with a helium flow rate of 20 ml/min. The injector and detector temperatures were 120 and 160°C, respectively. Hydrogen was determined on the same column, but with nitrogen as the carrier gas. $^{14}CO_2$ that was produced from direct injection studies with [^{14}C]lactate was too low to be readily measured with the proportional counter. Samples (1 ml) of headspace gas were injected through a septum and cap (37) into a scintillation vial that contained the CO_2-trapping solution described above. The vials were shaken to trap all of the $^{14}CO_2$ and counted as above. The total $^{14}CO_2$ in tubes was corrected for dissolved ^{14}C-inorganic carbon with an empirical factor that was determined by injecting $H^{14}CO_3^-$ into sediment cores (direct injection experiments) or tubes containing sediments (tube incubations).

RESULTS

Maximum concentrations of SCFA in Wintergreen Lake sediments were within the 0 to 4-cm depth interval. Acetate and propionate concentrations at this depth ranged from 30 to 340 and 10 to 90 μM, respectively, during the 1980 summer stratification. Formate, isobutyrate, butyrate, isovalerate, and valerate were occasionally detected at concentrations of 2 μM or less. Lactate was generally undetectable and was never detected at concentrations greater than 4 μM. Ethanol was never detected.

In preliminary acetate turnover experiments comparable first-order rate constants were obtained by measuring [2-^{14}C]acetate disappearance or $^{14}CH_4$ and $^{14}CO_2$ production (Fig. 1). During summer stratification the rates of acetate

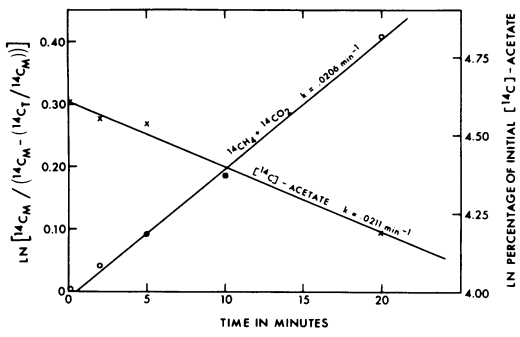

FIG. 1. Disappearance of [2-^{14}C]acetate and production of $^{14}CH_4$ and $^{14}CO_2$ in summer surface sediments (0- to 2-cm depth) collected from gravity cores.

turnover to CH_4 and CO_2 were highest in the surface sediments (0 to 2 cm) and decreased rapidly below the 2- to 4-cm depth interval (Fig. 2). The high proportion of [2-^{14}C]acetate that was metabolized to $^{14}CH_4$ demonstrated that methanogenesis was the predominant terminal process at all depths. $^{14}CH_4$ accounted for approximately 80% of the total $^{14}CH_4$ and $^{14}CO_2$ produced from [2-^{14}C]acetate in surface sediments throughout the stratified period.

An exponential first-order loss of [2-^{14}C]propionate was observed in surface sediments. Radioactivity that was lost from the propionate pool after the zero-time samples could be completely accounted for as [^{14}C]acetate, $^{14}CO_2$ and $^{14}CH_4$ (Table 1). $^{14}CO_2$ production from [*U*-^{14}C]lactate was linear over the 0- to 10-min incubation period as previously shown (27). Surface sediments that were incubated with [*U*-^{14}C]lactate for 10 min had 32% of the added ^{14}C remaining in the lactate pool, 51% as [^{14}C]acetate, 23% as $^{14}CO_2$, and less than 1% as $^{14}CH_4$. No other [^{14}C]SCFA were detected.

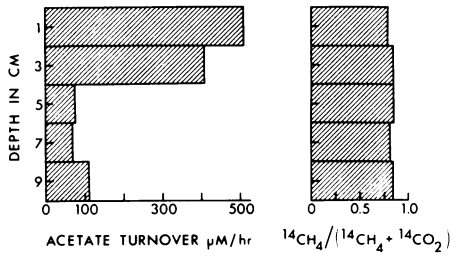

FIG. 2. Acetate turnover rates and ratio of $^{14}CH_4$ production to total $^{14}CH_4$ and $^{14}CO_2$ production with depth in summer sediments sampled from gravity cores.

Turnover rates of SCFA were measured simultaneously on two dates during summer stratification. On 27 June 1980 acetate and propionate were the only SCFA that were detected in surface sediments. Acetate had a turnover rate constant of 3.11/h and a pool size of 110 μM yielding a turnover rate of 342 μM/h. The rate constant, pool size, and turnover rate for propionate were 1.86/h, 90 μM, and 167 μM/h, respectively.

On 4 September 1980 SCFA other than acetate and propionate were detected, and a more complete examination of carbon flow in surface sediments was conducted (Table 2). Acetate had the highest turnover rate of the SCFA examined. Propionate turnover was less than 20% of acetate turnover on a molar basis, and lactate production was minor. The formate turnover rate could not be measured accurately, as [^{14}C]formate was completely converted to $^{14}CO_2$ in less than 1 min. The isobutyrate and butyrate concentrations were 0.3 and 1 μM, respectively. These concentrations were too low to conduct tracer studies with the available specific activities of the ^{14}C-labeled compounds and the radiochromatography technique. Isovalerate and valerate were not detectable. Thirty-seven percent (standard error, 4) of the total methane production was derived from reduction of carbon dioxide on this date.

Methanogenesis but not sulfate reduction was found to be necessary for the metabolism and maintenance of low pool sizes of VFA and hydrogen. The inhibition of methanogenesis with chloroform resulted in an immediate accumulation of hydrogen and VFA (Fig. 3). Acetate accumulated most rapidly, with lower accumulation rates of propionate, butyrates, and valerates. Inhibition of sulfate reduction with 2 mM sodium molybdate did not result in an accumulation of VFA or hydrogen even after a 24-h incubation. Increasing the sulfate pool by 2 M had no effect on methane production. The mean and 95% confidence intervals (n = 12) for methane production in sediment that received sulfate

TABLE 1. Percent distribution over time of ^{14}C-labeled compounds produced from [2-^{14}C]propionate in surface sediments (0- to 2-cm depth) collected from gravity cores

Time (min)	[^{14}C]propionate	[^{14}C]acetate	Other [^{14}C]SCFA	$^{14}CH_4$	$^{14}CO_2$	Total recovery
0	100	0	0	0	0	
10	80	9	0	2	6	97
30	48	25	0	10	24	107

TABLE 2. Pool sizes, turnover rates, and fates of fermentation intermediates in surface sediments (0- to 2-cm depth) collected from gravity cores on 4 September 1980

Intermediate	Pool size (μM)	Rate constant (per h)	Turnover rate (μM/h)	Fate			
				Acetate	CO_2	CH_4	Other SCFA
Acetate	100	1.59	159		+	+	0
Propionate	14	1.44	20	+	+	+	0
Lactate	1	2.76	3	+	+	+	0
Formate	2	NM[a]	NM	0	+	0	0

[a] [^{14}C]formate was completely converted to $^{14}CO_2$ in less than 1 min, a rate constant was not measurable (NM).

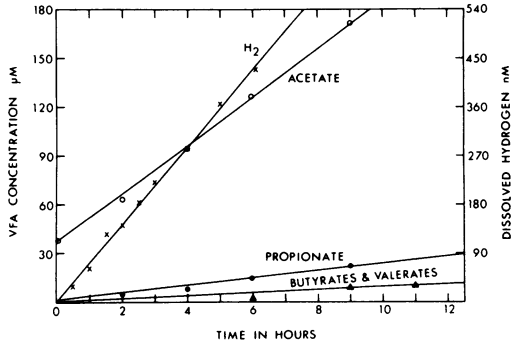

FIG. 3. Accumulation of volatile fatty acids and hydrogen after the inhibition of methanogenesis in summer surface sediments collected with an Eckman dredge.

was 48.1 ± 7.1 μmol/liter per h compared with a rate of 51.7 ± 6.4 μmol/liter per h measured for sediments that did not receive sulfate.

The effect of hydrogen partial pressure of approximately 1 atm on the metabolism of volatile fatty acids is illustrated in Table 3. Propionate, isobutyrate, isovalerate, and valerate accumulated in sediments which were exposed to increased hydrogen partial pressures. Added hydrogen completely inhibited the metabolism of propionate, isobutyrate, isovalerate, and valerate that was added to sediment, whereas over 90% of the added VFA were metabolized in controls that received no hydrogen. Butyrate metabolism was only partially inhibited by added hydrogen. However, a compound accumulated that cochromatographed with isobutyrate. Gas chromatography-mass spectrometry confirmed that the cochromatographing compound was isobutyrate. The sum of butyrate and isobutyrate concentrations indicated nearly complete inhibition of the mineralization of butyrate carbon (Table 3). Incubations of sediment with [1-^{14}C]butyrate and added hydrogen resulted in the inhibition of butyrate carbon mineralization and the conversion of approximately 50% of the added [^{14}C]butyrate to [^{14}C]isobutyrate. In similar experiments hydrogen only partially inhibited the metabolism of added lactate (data not shown).

The rate of accumulation of C_3-C_5 VFA in the

TABLE 3. Effect of hydrogen on the metabolism of volatile fatty acids in winter surface sediments collected with an Eckman dredge

VFA added	Concentration (μM)[a]			Mean % inhibition[b]
	H_2, final	H_2 + VFA		
		Initial	Final	
Propionate	16.7 (2.7)[c]	15.4	34.3 (2.5)	114
Isobutyrate	1.6 (0.2)	5.1	6.4 (0.5)	97
Butyrate[d]	<0.5	26.9	11.6 (0.9)	43
Butyrate[e]	<0.5	34.5	31.2 (1.2)	92
Isovalerate	2.2 (0.3)	2.0	4.2 (.07)	100
Valerate	0.4 (0.3)	20.8	23.4 (0.9)	110

[a] Sediment additions were: none, only H_2, only VFA, and VFA and H_2. Over 90% of all added VFA in VFA alone treatment was metabolized during the overnight incubation period. Initial concentrations of C_3-C_5 VFA in sediments that were not amended with VFA were less than 0.5 μM.

[b] Mean percent inhibition calculated as (mean final concentration of VFA in sediments with added H_2 and VFA − mean final concentration in sediment with only H_2 added)/(mean initial concentration of VFA in sediments with VFA added) × 100.

[c] Standard error of mean within parentheses.

[d] Butyrate concentration alone used in calculations.

[e] Sum of butyrate and isobutyrate concentrations used in calculations.

TABLE 4. Production rates of VFAs in winter surface sediments (collected with an Eckman dredge) as determined by inhibitor experiments

Fatty acid	Rate of production (μM/h) and molar % production[a]						Mean molar % production
	Sediment A		Sediment B		Sediment C		
	Rate	%	Rate	%	Rate	%	
Acetate	6.5	81	7.1	88	4.1	85	85
Propionate	1.2	15	0.7	9	0.3	6	10
Butyrates	0.3	3	0.1	1	0.1	2	2
Valerates	ND[b]	0	0.2	2	0.3	6	3

[a] Percentage of total number of moles of fatty acids produced.
[b] No detectable increase over the 9-h incubation period.

presence of increased hydrogen partial pressures and the rate of acetate accumulation in the presence of 100 μM $CHCl_3$ and 2 mM sodium molybdate in sediments that were collected at three separate times in midwinter are shown in Table 4. Although there was considerable variability in the absolute rates of VFA production on different dates, the relative pattern of production was similar, i.e., acetate > propionate > butyrates and valerates. The ratio of acetate to propionate production was similar to that measured in summer sediments, although the rates of acetate and propionate turnover in winter sediments were less than 10% of summer rates.

DISCUSSION

The ^{14}C tracer and inhibitor studies both demonstrated that acetate is the dominant carbon fermentation intermediate in profundal sediments of Wintergreen Lake. Rate measurements obtained from ^{14}C tracer studies are considered to closely represent in situ activities since the sampling method and subsequent manipulations provide a relatively undisturbed sediment sample. The rates of VFA production that were measured in the inhibitor experiments do not represent in situ rates, since sediments were mixed and not incubated at the in situ temperature. The inhibitor approach is also considered to have underestimated the actual acetate production rates and overestimated the production of C_3-C_5 SCFA, since increased hydrogen concentration resulting from the inhibition of methanogens or hydrogen addition can be expected to shift carbon flow toward the production of more reduced fermentation products (8, 10, 25). The inhibitor experiments, however, provided an independent measure of the relationship between propionate and acetate turnover rates and enabled the estimate of the turnover of butyrates and valerates in sediments where low pool sizes precluded the use of tracer studies due to the low specific activities of the available ^{14}C-labeled compounds.

The high rate of acetate turnover relative to the turnover rates of the other fermentation intermediates indicates that most of the acetate was produced in the initial fermentation of substrates rather than indirectly through other SCFA. Although some acetate may also be formed by bacteria fermenting hydrogen and carbon dioxide to acetate, the number of these acetogenic bacteria in sediments is low (3), and only a small percentage of the acetate in other anaerobic environments is derived from carbon dioxide (18). The overall pattern of SCFA production in Wintergreen Lake sediments is similar to that in the rumen and anaerobic sludge (Table 5). Although differences exist in the quality and quantity of the carbon inputs to these systems, these similarities in carbon flow suggest that the steps and controls in initial carbon metabolism are the same in all three systems. Hydrogen partial pressures are low in all three systems (11, 12, 15, 30, 32), and acetate and hydrogen are expected to be the primary fermentation products in systems with low partial pressures of hydrogen (12, 35).

The noted exception to this general pattern of carbon flow in anaerobic ecosystems is in the sediments of Lake Vechten (Table 5). Although the rate constants for lactate turnover in Wintergreen Lake and Lake Vechten are comparable, 2.74 and 2.37/h, respectively, the lactate pool in Lake Vechten sediments is 12.2 μg/g of wet sediment compared with less than 0.35 μg/g of wet sediment in Wintergreen Lake sediments. The lactate pool is higher than the acetate pool in Lake Vechten, which is unique since lactate is generally not detectable in anaerobic sewage sludge (11) and in the rumen, where acetate pool sizes are generally greater than 60 mM, the lactate pool size is much less than 1 mM unless the animal is suddenly shifted to a high-energy diet (9).

The inhibition of the turnover of propionate, butyrates, and valerates by increased hydrogen partial pressures in the sediments of Wintergreen Lake suggests that these compounds are metabolized with the evolution of molecular hydrogen. Hydrogen inhibits the metabolism of propionate to acetate in sludge (15, 16; P. H.

TABLE 5. Relative production of fermentation intermediates in various anaerobic ecosystems

Ecosystem	Production rates (molar %)[a]				
	Acetate	Propionate	Butyrates	Valerates	Lactate
WGL sediments (summer)[b]	86	13	ND[c]	ND	2
WGL sediments (winter)	85	10	2	3	ND
Anaerobic sludge[b,d]	80	14	6	<3	<6
Rumen[e]	72	18	8	2	<1
Lake Vechten[f]	9	ND	ND	ND	91

[a] Percentage of total number of moles of fatty acids produced.

[b] Contribution to acetate from propionate, butyrate, and lactate turnover subtracted from acetate turnover to estimate acetate coming from initial substrates. WGL, Wintergreen Lake.

[c] ND, Not determined.

[d] Acetate, propionate, and butyrate from basal mesophilic rates of Mackie and Bryant (18). Lactate and valerates from the scheme of Kaspar and Wuhrmann (15).

[e] From the data of Hungate et al. (13) and Jayasuriya and Hungate (14).

[f] From the data of Cappenberg and Prins (7).

Smith, F. M. Bordeaux, and P. S. Shuba, Abstracts of papers of the 159th National Meeting of the American Chemical Society, WATR 49, 1970) and the degradation of butyrate to acetate in cocultures (19). The production of [^{14}C]acetate as the only [^{14}C]SCFA from [^{14}C]lactate suggests that lactate is also metabolized to acetate and hydrogen. Although the in situ fates of C_4-C_5 VFA could not be determined, it seems likely they are metabolized to acetate either directly or indirectly through the propionate pool as proposed for anaerobic sludge. Whether the metabolism of butyrate to isobutyrate as observed in the hydrogen addition experiments represents a step in in situ metabolism of butyrate has yet to be determined.

The immediate accumulation of VFA and hydrogen after the inhibition of methanogenesis demonstrates the importance of methanogenesis in controlling carbon and electron flow in Wintergreen Lake sediments. Although approximately 20% of the acetate in surface sediments is completely metabolized to carbon dioxide, primarily by sulfate reducers (27), sulfate reducers are not considered to be essential in the metabolism of VFA since there was no build-up of VFA when sulfate reduction was inhibited. The accumulation of C_3-C_5 VFA when methanogenesis is inhibited is probably the result of product inhibition by hydrogen and possibly acetate since no known methanogens are capable of directly utilizing C_3-C_5 VFA. Sorensen and co-workers (28) have noted an accumulation of VFA and hydrogen in marine sediments when sulfate reduction is inhibited which is similar to the accumulation in Wintergreen Lake sediments when methanogenesis is inhibited. They attribute the accumulation of propionate and butyrates to the inhibition of the direct metabolism of these compounds by sulfate reducers. However, they did not examine the possibility that the hydrogen or acetate (or both) that accumulated might inhibit the metabolism of propionate and butyrate by acetogenic hydrogen-producing bacteria. If the latter were the case the sulfate reducers and methanogens would serve the same function in control of the metabolism of VFA in the two respective habitats.

A working model for the metabolism of sedimented organic matter in the profundal surface sediments of Wintergreen Lake has been developed (Fig. 4). The hydrogen production rate is based on the assumption that methanogens are the principal hydrogen consumers, and acetate is the only methane precursor other than hydrogen and carbon dioxide. Although there is a potential for hydrogen uptake by sulfate reducers (27), they are not considered to be significant in in situ hydrogen uptake since additions of sulfate concentrations that are saturating for sulfate reduction do not initially alter the rates of methanogenesis in these sediments. Furthermore, the inhibition of sulfate reduction does not stimulate methanogenesis (27). Approximately 40% of the total methane production is derived from hydrogen and carbon dioxide, whereas 80% of the acetate turnover is through methanogenesis. Thus, for each 100 mol of methane produced, [0.6 (fraction of total methane production from acetate) × 100]/0.8 (fraction of total acetate turnover that goes to methane) = 75 mol of acetate and 0.4 (fraction of methane from hydrogen) × 4 (moles of H_2 per CH_4) × 100 = 160 mol of hydrogen are metabolized. The resultant hydrogen/acetate production ratio is 2.1, which is close to the hydrogen/acetate production ratio of 2 that is expected from the fermentation of an initial substrate with a redox state of 0.

The model emphasizes that the controlling steps in carbon flow in profundal sediments of Wintergreen Lake are likely to be the initial fermentation of substrate to acetate, hydrogen, and carbon dioxide and the subsequent metabo-

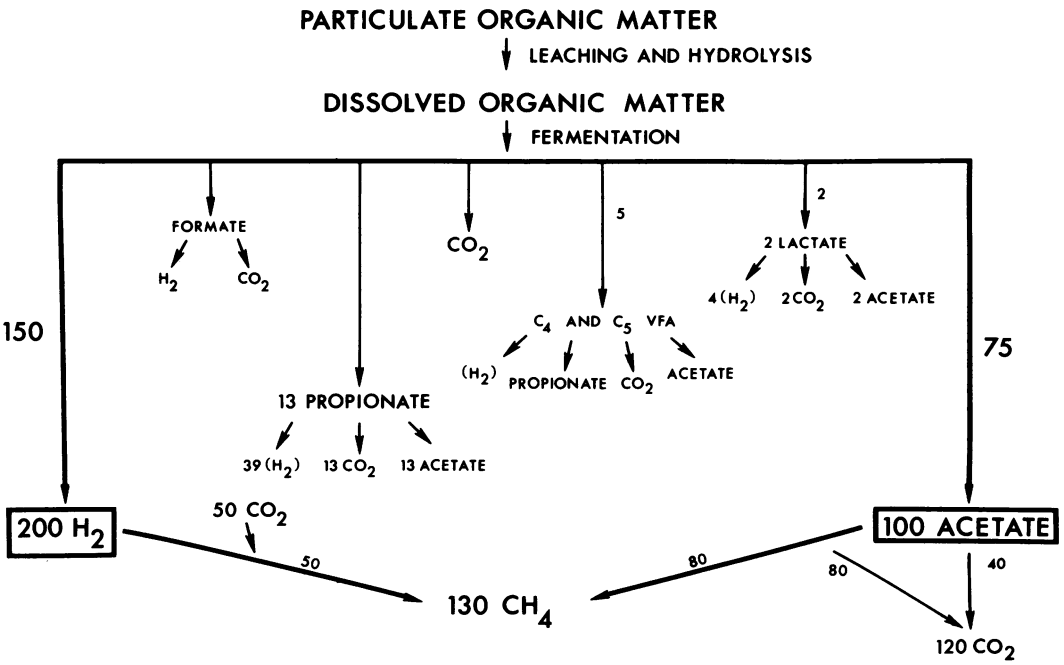

FIG. 4. Model of carbon and electron flow in the profundal surface sediments of Wintergreen Lake. The numbers adjacent to compounds represent the molar amounts of each compound produced or metabolized for every 100 mol of the total acetate pool that is metabolized. The numbers adjacent to arrows represent the molar contribution from substrate to product. All acetate and hydrogen produced from initial fermentation and the metabolism of SCFA enter common pools as designated by the boxes. (H_2) represents possible reduction of sulfate as well as molecular hydrogen production.

lism of these compounds by methanogens. The formation of acetate as the major SCFA intermediate, the flux of more reduced SCFA through the acetate pool, and the maintenance of low pool sizes of SCFA indicate a close coupling between fermentative bacteria, acetogenic hydrogen-producing bacteria, and methanogens. Based on the pool sizes of SCFA and dissolved inorganic carbon in the sediment, the concentration of dissolved hydrogen must be 30 nM or less to make the oxidation of propionate to acetate thermodynamically favorable ($\Delta G \leq 0$). We conclude that acetate and hydrogen are the central intermediates in carbon and electron flow in the profundal sediments of Wintergreen Lake and that carbon metabolism is dependent upon the maintenance of low hydrogen concentrations by methanogenic bacteria. Studies on carbon flow in the sediments of less productive lakes are in progress to determine whether this pattern of anaerobic carbon flow can be generalized to other lake sediments where sulfate reduction rather than methanogenesis is the dominant terminal process.

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