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METABOLISM OF FERMENTATION INTERMEDIATES IN LAKE SEDIMENTS

By

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ABSTRACT

METABOLISM OF FERMENTATION INTERMEDIATES IN LAKE SEDIMENTS

By

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The metabolism of fermentation intermediates in anaerobic lake sediments was examined. ^{14}C-labelled compounds and inhibitor techniques were used to determine the rates of production and fates of fermentation intermediates in the surface sediments of a eutrophic lake. Acetate and hydrogen were the primary fermentation intermediates with lesser production of propionate, butyrates, valerates, and lactate. The fermentation pattern was similar to those previously described for anaerobic wastes and the rumen but differed markedly from the results previously reported for other lake sediments. Propionate and lactate were metabolized to acetate. The metabolism of propionate, butyrates, and valerates was dependent upon the maintenance of low hydrogen partial pressures by methanogens which were the primary consumers of hydrogen and acetate.

The competition between methanogens and sulfate reducers for hydrogen and acetate was studied using kinetic analyses of the uptake of electron donors, inhibitor experiments, and simulation modelling. When sulfate was not limiting sulfate reducers had a higher affinity for hydrogen uptake which permitted them to maintain the hydrogen partial pressure below threshold levels necessary for methanogenesis. However, methanogens outcompeted sulfate reducers for hydrogen in eutrophic lake sediments since the maximum potential for sulfate reduction was limited by sulfate availability to a value that was much

lower than the rate of hydrogen production. In oligotrophic lake sediments sulfate reducers were able to compete effectively with methanogens as evidenced by an increase in methane production from both hydrogen and acetate following the inhibition of sulfate reduction. Sulfate reducers had a higher affinity for acetate at *in situ* sulfate concentrations. A comparison of the partitioning of terminal carbon and electron flow between sulfate reducers and methanogens in the sediments of lakes of different trophic status as well as simulation modelling of the competition between sulfate reducers and methanogens demonstrated that the relative importance of methane production and sulfate reduction in consuming hydrogen and acetate is primarily controlled by the rate of organic matter decomposition in the sediments.

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CHAPTER I

Literature Review and Experimental Rationale

The decomposition of sedimented organic carbon in freshwater lakes proceeds primarily through anaerobic metabolism. This is evidenced by benthic respiratory quotients (RQ = CO_2 released/O_2 taken up) that exceed unity in most lakes that have been examined (34-36). Potential electron acceptors other than oxygen such as nitrate and sulfate are often depleted from the interstitial water of sediments (30, 47, 48) and methanogenesis becomes the predominate terminal process in the sediments. A compilation of data on five North American lakes has demonstrated that methane is the ultimate fate of 36-58 percent (mean = 47) of the carbon input to eutrophic lakes (21). The estimate of the total amount of carbon proceeding through methanogenesis in these sediments approaches or exceeds the carbon input estimates since the rate of carbon dioxide production should approximate the rate of methane production if the redox state of the initial substrate is near zero.

The Theoretical Methane Fermentation:

A theoretical framework for the metabolism of organic carbon in methanogenic environments has been developed based upon results from studies on the rumen, anaerobic waste digestors, enrichments, and mixed and pure cultures. The decomposition of complex organic matter proceeds through three major phases: 1) the hydrolysis and

fermentation of initial substrates; 2) the metabolism of fatty acids produced from fermentation and lipid hydrolysis to acetate and hydrogen; and 3) the metabolism of acetate and hydrogen to methane (6, 24, 52). Although fermentative bacteria in pure culture produce a number of fermentation products such as succinate, ethanol and lactate these products are formed in only small amounts in natural systems containing methanogenic bacteria (16, 17, 49, 50). In methanogenic ecosystems acetate, hydrogen and carbon dioxide are the primary fermentation products with lesser amounts of propionate, butyrates, and valerate being formed. Acetate and hydrogen are the ecologically significant fermentation products in methanogenic environments because the maintenance of low hydrogen partial pressures by methanogenic bacteria makes the reoxidation of reduced nicotinamide nucleotides with the formation of hydrogen thermodynamically favorable (49). Thus fermentative bacteria can metabolize pyruvate to acetate rather than oxidizing NADH with the reduction of pyruvate, acetyl-coA, or other fermentation intermediates to form reduced fermentation end products. The ability of methanogenic bacteria to alter the fermentation pattern of fermentative bacteria in this manner has been demonstrated many times (13, 14, 18, 23, 43).

Fatty acids of longer chain length than acetate and aromatic compounds are metabolized to acetate and hydrogen by proton-reducing acetogenic bacteria (4, 6, 15, 28, 29). The metabolism of fatty acids with hydrogen production is thermodynamically favorable only at low hydrogen partial pressures and is thus dependent upon hydrogen metabolism by methanogenic bacteria (6, 20, 28, 29). Fatty acids with even chain lengths are metabolized to acetate and hydrogen whereas

those with odd chain lengths are metabolized to acetate and propionate (27-29). A bacterium that decomposes fatty acids in this manner, Syntrophomonas wolfei has been isolated in coculture with hydrogen-consuming methanogens or sulfate reducers (29). Propionate is degraded by a different bacterium which metabolizes propionate to acetate, hydrogen and carbon dioxide (6, 20, 27, 41). A bacterium that metabolizes propionate to acetate, Syntrophobacter wolinii, has also been obtained in coculture with hydrogen consuming bacteria (4).

In the third stage of decomposition methanogenic bacteria consume the hydrogen and acetate produced during fermentation and acetogenesis and produce methane. In anaerobic wastes acetate is the precursor of approximately seventy percent of the methane with hydrogen metabolism presumably accounting for most of the balance of methane production (27, 37).

Methanogenesis and fermentation in sediments:

Despite the acceptance of the decomposition scheme described above as the general pattern for non-gastrointestinal methanogenic systems there has been little data to indicate whether such a pattern is applicable to lake sediments in which methanogenesis dominates carbon flow. Previous studies have focused primarily on the precursors and rates of methanogenesis in sediments. Both hydrogen and acetate are converted to methane (3, 12, 26, 42, 46-48). The reported relative importance of the two precursors varies widely. Acetate turnover rates of 16 (48) and 18 (42) μmoles per liter of sediment per hour and 33 μmoles per 100 grams of wet sediment (12) have been estimated from the metabolism of [^{14}C] acetate and hydrogen turnover has been estimated from kinetic uptake parameters (42).

However the relevance of the reported rates to in situ rates is uncertain since the methods of sediment collection, storage and incubation in these studies did not mimic in situ conditions.

The pathways for the metabolism of initial substrates to methanogenic precursors in sediments has only been examined superficially. The effect of addition of various potential fermentation intermediates on methane production has been examined (26, 42, 46). However, such studies are inconclusive since a stimulation of methane production does not mean that the intermediate is important in situ and a lack of stimulation may indicate that the turnover of that compound is near saturation in situ rather than that it is an unimportant intermediate. However, it has been convincingly demonstrated that the methanogenic bacteria in lake sediments have a high affinity for hydrogen and are capable of maintaining low hydrogen partial pressures in sediments (42, 46). This evidence suggests that a pattern of carbon flow similar to that proposed for other anaerobic ecosystems is possible in sediments.

The only studies on the turnover of fermentation intermediates other than acetate and hydrogen have indicated that carbon flow in anaerobic lake sediments is quite different than that in other anaerobic ecosystems. In a series of reports on the anaerobic metabolism in the sediments of Lake Vecten, Cappenberg and co-workers (7-12) reported that lactate is an important intermediate in carbon flow with a turnover rate 10-fold greater than acetate. As outlined previously, lactate production would be expected to be minimal in environments with low hydrogen partial pressures. Since the acetate turnover rate is only a fraction of the lactate turnover rate in Lake

Vechten sediments, most of the carbon proceeding through the lactate pool must not enter the acetate pool. This is contrary to the expected metabolism of lactate to acetate and hydrogen under low hydrogen partial pressure (13).

Sulfate reduction in freshwater sediments:

Although methanogenesis has been reported to be the predominate terminal process in freshwater sediments in which the overlying water is oxygen-depleted sulfate reduction also occurs (19, 38, 39, 51). Carbon budget calculations indicate that sulfate reduction may be the terminal process for about twenty-five percent of the carbon metabolism in sediments (19, 38). However, these calculations have not been based on simultaneous measurements of methane production and sulfate reduction.

Pure cultures of sulfate reducing bacteria can use short chain fatty acids and hydrogen as electron donors (5, 22, 44, 45) and the ability of sulfate reducing bacteria to use these substrates in marine sediments has been demonstrated (2, 33, 40). Sulfate reducers prevent methane production from hydrogen and acetate in marine sediments. Although the mechanisms have not been demonstrated it has been suggested that sulfate reducers outcompete methanogens for these substrates (1, 2, 25, 33, 40, 47).

Sulfate reducers in freshwater sediments have the ability to outcompete methanogens for hydrogen and acetate when sulfate is added to the sediments (47). However, most evidence suggests that sulfate reducers lack the ability to compete effectively with methanogens at freshwater sulfate concentrations. Additions of hydrogen stimulates sulfate reduction (19, 39). However, the partial pressures of

hydrogen that were added were approximately 100,000 times greater than the in situ partial pressure. When sulfate reduction is inhibited in freshwater sediments there is no stimulation of methane production (39) suggesting that sulfate reducers are not metabolizing a significant fraction of hydrogen production in situ. In the same study, inhibition of sulfate reduction partially inhibited acetate oxidation. Since no corresponding increases in methane production were observed the significance of acetate metabolism by sulfate reducer in freshwater sediments is uncertain.

Previous studies on sulfate reduction have focused on eutrophic lake sediments which have a high electron acceptor demand and may rapidly become sulfate depleted when samples are removed from the sulfate input from the overlying water. A significant portion of the metabolism in sediments of less productive lakes is also anaerobic (35) and the metabolism in these sediments should also be examined.

Experimental rationale:

The purpose of the studies reported here was to determine the pathways and controls of carbon and electron flow involved in the production of fermentation products and terminal metabolism in anaerobic freshwater sediments. The production rates of fermentation intermediates were estimated from the turnover rate of ^{14}C-labelled compounds injected into sediments maintained under conditions approximating those in situ and from the accumulation of fermentation products when the metabolism of fermentation products was inhibited. The finding that acetate and hydrogen were the primary fermentation intermediates in eutrophic sediments led to an examination of the factors that affect the fate of these intermediates in sediments.

Since sulfate reducers and methanogens were expected to be the primary consumers of acetate and hydrogen in anaerobic, nitrate-depleted sediment, the factors controlling the competition between sulfate reducers and methanogens for hydrogen and acetate were studied.

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. Abram, J. W., and D. B. Nedwell. 1978. Hydrogen as a substrate for methanogenesis and sulphate reduction in anaerobic saltmarsh sediments. Arch. Microbiol. 117:93-97.

3. Belyaev, S. S., Z. I. Finkelstein, and M. V. Ivanov. 1975. Intensity of bacterial methane formation in ooze deposits of certain lakes. Microbiology 44:272-275.

4. Boone, D. R., and M. P. Bryant. 1980. Propionate-degrading bacterium, Syntrophobacter wolinii sp. nov. gen. nov. from methanogenic ecosystems. Appl. Environ. Microbiol. 40:626-632.

5. Brandis, A., and R. Thauer. 1981. Growth of Desulfovibrio species on hydrogen and sulfate as sole energy source. J. Gen. Microbiol. 126:249-252.

6. Bryant, M. P. 1979. Microbial methane production-theoretical aspects. J. Anim. Sci. 48:193-201.

7. Cappenberg, T. E. 1975. Relationship between sulfate-reducing and methane-producing bacteria. Plant and Soil 43:125-139.

8. Cappenberg, T. E. 1976. Methanogenesis in the bottom deposits of a small stratifying lake. pp. 125-134. In Schlegel, H. G., B. Gottschalk and N. Pfenning (eds.) Microbial production and utilization of gases (H_2, CH_4, CO). Goltze-Verlag, Gottingen.

9. Cappenberg, T. E. 1979. Kinetics of breakdown processes of organic matter in freshwater sediments. Arch. Hydrobiol. Beih. Ergebn. Limnol. 12:91-94.

10. Cappenberg, T. E., and E. Jongejan. 1978. Microenvironments for sulfate reduction and methane production in freshwater sediments. pp. 129-138. In W. E. Krumbein (ed.) Environmental biogeochemistry and geomicrobiology. Vol. 1. Ann Arbor Science Publications, Ann Arbor.

11. Cappenberg, T. E., E. Jongejan, and J. Kaper. 1978. Anaerobic breakdown processes of organic matter in freshwater sediments. pp. 91-99. In M. W. Loutit and J.A.R. Miles (eds.) Microbial Ecology. Springer, Berlin.

12. Cappenberg, T. E., and R. A. Prins. 1974. Interrelations between sulfate-reducing and methan producing bacteria in bottom deposits of a freshwater lake. III. Experiments with ^{14}C-labelled substrates. Ant. van Leeuwenhoek J. Microbiol. Serol. 40:457-469.

13. Chen, M., and M. J. Wolin. 1977. Influence of CH_4 production by Methanobacterium ruminantium on the fermentation of glucose and lactate by Selenonmonas ruminantium. Appl. Environ. Microbiol. 34:756-759.

14. Chung, K. 1976. Inhibitory effects of H_2 on growth of Clostridium cellobioparum. Appl. Environ. Microbiol. 31:342-348.

15. Ferry, J. G., and R. S. Wolfe. 1976. anaerobic degradation of benzoate to methane by a microbial consortium. Arch. Microbiol. 107:33-40.

16. Hungate, R. E. 1975. The rumen microbial ecosystem. Annu. Rev. Ecol. Syst. 6:39-66.

17. Hobson, P. N., S. Bousfield, and R. Summers. 1974. Anaerobic digestion of organic matter. Critical Reviews in Environmental Control 4:131-191.

18. Iannotti, E. L., D. Kafikewitz, M. J. Woilin, and M. P. Bryant. 1973. Glucose fermentation products of Ruminococcus albus grown in continuous culture with Vibro succinogenes: changes caused by interspecies transfer of hydrogen. J. Bacteriol. 114:1231-1240.

19. 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.

20. Kaspar, H. F., and K. Whurmann. 1978. Product inhibition in sludge digestion. Microbial Ecol. 4:241-248.

21. Kelley, C. A., and D. P. Chynoweth. 1981. The contribution of temperature and organic input in controlling rates of sediment methanogenesis. Limnol. Oceanogr. 26:891-897.

22. Laanbroek, H. J., and N. Pfennig. 1981. Oxidation of short-chain fatty acids by sulfate-reducing bacteria in freshwater and marine sediments. Arch. Microbiol. 128:330-335.

23. Latham, J. J., and M. J. Wolin. 1977. Fermentation of cellulose by Ruminococcus flavefaciens in the presence and absence of Methanobacterium ruminantium. Appl. Environ. Microbiol. 34:297-301.

24. Mah, R. A.; D. M. Ward, L. Baresi, and T. L. Glass. 1977. Biogenesis of methane. Ann. Rev. Microbiol. 31:309-341.

25. Martens, C. S., and R. A. Berner. 1977. Interstitial water chemistry of anoxic Long Island Sound sediments. 1. Dissolved gases. Limnol. Oceanogr. 22:10-25.

26. Mallard, G. E., and J. I. Frea. 1972. Methane production in Lake Erie sediments: temperature and substrate effects. Proc. 15th Conf. Great Lakes Res. 1972:87-93.

27. McCarty, P. L. 1964. The methane fermentation. pp. 314-343. In H. Heukelekian and N. C. Dondero (eds.) Principles and Applications in Aquatic Microbiology. John Wiley & Sons, New York.

28. McInerney, M. J., M. P. Bryant, and N. Pfennig. 1979. Anaerobic bacterium that degrades fatty acids in syntrophic association with methanogens. Arch. Microbiol. 122:129-135.

29. McInerney, M. J., M. P. Bryant, R. B. Hespell, and J. W. Costerton. 1981. Syntrophmonas wolfei gen. nov. sp. nov., an anaerobic, syntrophic fatty acid-oxidizing bacterium. Appl. Environ. Microbiol. 41:1029-1039.

30. Molongoski, J. J., and M. J. Klug. 1980. Anaerobic metabolism of particulate organic matter in the sediments of a hypereutrophic lake. Freshwater Biol. 10:507-518.

31. Mountfort, D. O., and R. A. Asher. 1981. Role of sulfate reduction versus methanogenesis in terminal carbon flow in polluted intertidal sediments of Waimea Inlet, Nelson, New Zealand. Appl. Environ. Microbiol. 42:252-258.

32. Mountfort, D. O., R. A. Asher, E. L. Mays and J. M. Tiedje. 1980. Carbon and electron flow in mud and sandflat intertidal sediments and Delaware Inlet, Nelson, New Zealand. Appl. Environ. Microbiol. 39:686-694.

33. Oremland, R. S., and B. F. Taylor. 1978. Sulfate reduction and methanogenesis in marine sediments. Geochim. Cosmochim. Acta 42:209-214.

34. Rich, P. H. 1979. Differential CO_2 and O_2 benthic community metabolism in a soft-water lake. J. Fish. Res. Board. Can. 36:1377-1389.

35. Rich, P. H., and A. H. Devol. 1978. Analysis of five North American Lake ecosystems. Verh. Int. Verein. Limnol. 20:1377-1389.

36. Rich, P. H., and R. G. Wetzel. 1978. Detritus in the lake ecosystem. Amer. Natural. 112:57-71.

37. Smith, P. H., and R. A. Mah. 1966. Kinetics of acetate metabolism during sludge digestion. Appl. Microbiol. 14:368-371.

38. Smith, R. L., and M. J. Klug. 1981. Reduction of sulfur compounds in the sediments of a eutrophic lake basin. Appl. Environ. Microbiol. 41:1230-1237.

39. 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.

40. Sørensen, J., D. Christensen, and B. B. Jørgensen. 1981. Volatile fatty acids and hydrogen as substrates for sulfate-reducing bacteria in anaerobic marine sediment. Appl. Environ. Microbiol. 42:5-11.

41. Stadtman, T. C., and H. A. Barker. Studies on the methane fermentation. VIII. Tracer experiments on fatty acid oxidation by methane bacteria. J. Bacteriol. 61:67-80.

42. 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.

43. Weimer, P. J., and J. G. Zeikus. 1977. Fermentation of cellulose and cellobiose by Clostridium thermocellum in the absence and presence of Methanobacterium thermoautotrophicum. Appl. Environ. Microbiol. 33:289-297.

44. Widdel, F., and N. Pfennig. 1977. A new anaerobic, sporing, acetate-oxidizing, sulfate reducing bacterium, Desulfotomaculum (amend.) acetoxidans. Arch. Microbiol. 112:119-122.

45. Widdel, F., and N. Pfennig. 1981. Studies on dissimilatory sulfate-reducing bacteria that decompose fatty acids. I. Isolation of a new sulfate-reducing bacteria enriched with acetate from saline environments. Description of Desulfobacter postgatei gen. nov. sp. nov. Arch. Microbiol. 129:395-400.

46. Winfrey, M. R., R. R. Nelson, S. C. Krevickis, and J. G. Zeikus. 1977. Association of hydrogen metabolism with methanogenesis in Lake Mendota sediments. Appl. Environ. Microbiol. 33:312-318.

47. 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.

48. Winfrey, M. R., and J. G. Zeikus. 1979. Anaerobic metabolism of immediate methane precursors in Lake Mendota. Appl. Environ. Microbiol. 37:244-253.

49. Wolin, M. J. 1974. Metabolic interaction among intestinal microorganisms. Am. J. Clin. Nutr. 27:1320-1328.

50. Wolin, M. J. 1979. The rumen fermentation: a model for microbial interactions in anaerobic ecosystems. Adv. Microbial. Ecol. 3:49-77.

51. Zaiss, U. 1981. The sediments of the new artificial lake Bostalsee (Saarland, Germany) with particular reference to microbial activity. Arch. Hydrobiol. 92:346-358.

52. Zehnder, A.J.B. 1978. Ecology of methane formation. pp. 349-376. In R. Mitchell (ed.) Water pollution microbiology. Vol. 2. John Wiley and Sons, New York.

CHAPTER II

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

† Article no. 10149 of the Michigan Agricultural Experiment Station and no. 458 of the Kellogg Biological Station.

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

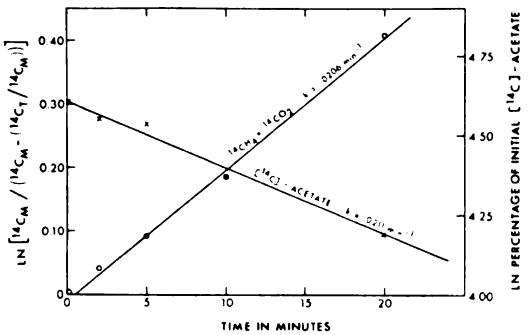

FIG. 1. Disappearance of [2-^{14}C]acetate and production of $^{14}CH_4$ and $^{14}CO_2$ in summer surface sediments (0- to 2-cm depth) collected from gravity cores.

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.

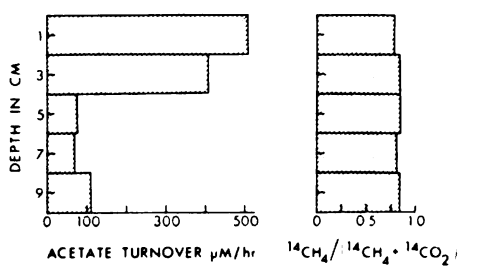

FIG. 2. Acetate turnover rates and ratio of $^{14}CH_4$ production to total $^{14}CH_4$ and $^{14}CO_2$ production with depth in summer sediments sampled from gravity cores.

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).

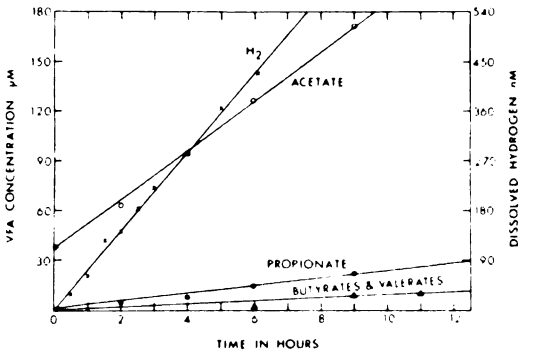

FIG. 3. Accumulation of volatile fatty acids and hydrogen after the inhibition of methanogenesis in summer surface sediments collected with an Eckman dredge.

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-

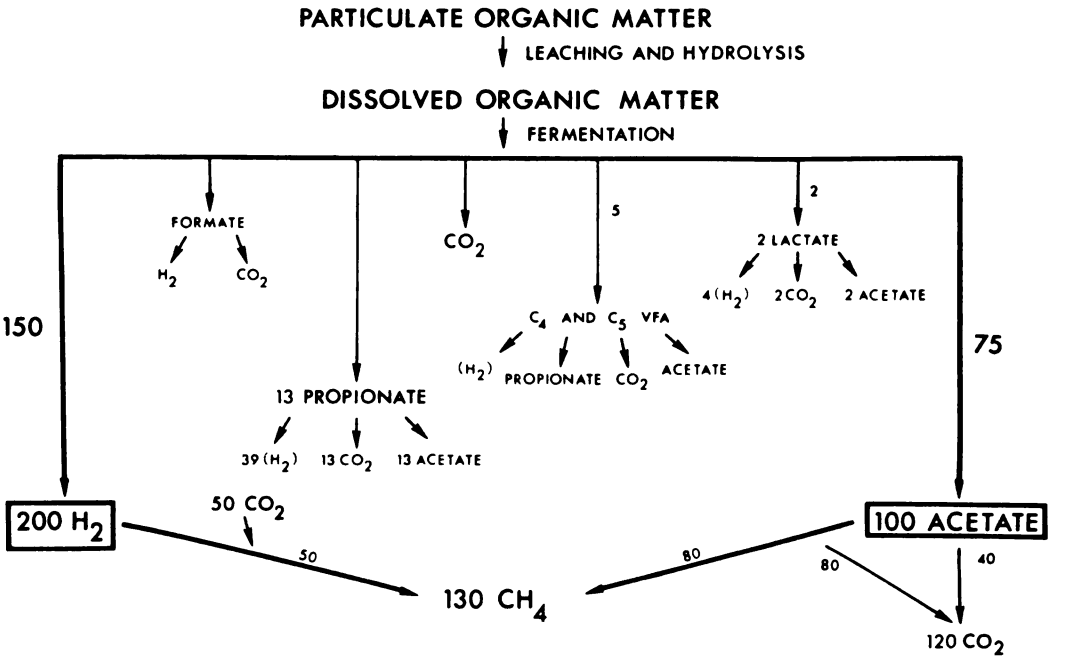

FIG. 4. Model of carbon and electron flow in the profundal surface sediments of Wintergreen Lake. The numbers adjacent to compounds represent the molar amounts of each compound produced or metabolized for every 100 mol of the total acetate pool that is metabolized. The numbers adjacent to arrows represent 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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LITERATURE CITED

1. **Bethge, P. O., and K. Lindstrom.** 1974. Determination of organic acids of low relative molecular mass (C_1 to C_4) in dilute aqueous solution. Analyst (London) **99**:137–142.
2. **Boone, D. R., and M. P. Bryant.** 1980. Propionate-degrading bacterium, *Syntrophobacter wolinii* sp. nov. gen. nov., from methanogenic ecosystems. Appl. Environ. Microbiol. **40**:626–632.
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**:201–204.
4. **Bryant, M. P.** 1976. The microbiology of anaerobic degradation and methanogenesis with special reference to sewage, p. 107–118. *In* A. G. Schlegel and J. Barnea (ed.), Microbial energy conversion. Verlag Erich Goltze KG, Gottingen.
5. **Bryant, M. P.** 1979. Microbial methane production—theoretical aspects. J. Anim. Sci. **48**:193–201.
6. **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.
7. **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-labelled substrates. Antonie van Leeuwenhoek J. Microbiol. Serol. **40**:457–469.
8. **Chynoweth, D. P., and R. A. Mah.** 1971. Volatile acid formation in sludge digestion. Adv. Chem. Ser. **105**:41–54.
9. **Counotte, G. H. M., and R. A. Prins.** 1978/1979. Regulation of rumen lactate metabolism and the role of lactic acid

in nutritional disorders of ruminants. Vet. Sci. Commun. **2**:277–303.

10. **Demeyer, D. I., and C. J. Van Nevel.** 1975. Methanogenesis, an integrated part of carbohydrate fermentation and its control, p. 366–382. *In* E. W. McDonald and A. C. T. Warner (ed.), Digestion and metabolism in the ruminant. University of New England, Armidale, Australia.
11. **Hobson, P. N., S. Bousfield and R. Summers.** 1974. Anaerobic digestion of organic matter. Crit. Rev. Environ. Control **4**:131–191.
12. **Hungate, R. E.** 1975. The rumen microbial ecosystem. Annu. Rev. Ecol. Syst. **6**:39–66.
13. **Hungate, R. E., J. Reichland, and R. Prins.** 1971. Parameters of rumen fermentation in a continuously fed sheep: evidence of a microbial rumination pool. Appl. Microbiol. **22**:1104–1113.
14. **Jayasuriya, G. C. N. and R. E. Hungate.** 1959. Lactate conversions in the bovine rumen. Arch. Biochem. Biophys. **82**:274–287.
15. **Kaspar, H. F., and K. Wuhrmann.** 1978. Kinetic parameters and relative turnovers of some important catabolic reactions in digesting sludge. Appl. Environ. Microbiol. **36**:1–7.
16. **Kaspar, H. F., and K. Whurmann.** 1978. Product inhibition in sludge digestion. Microb. Ecol. **4**:241–248.
17. **Lawrence, A. W.** 1971. Application of process kinetics to design of anaerobic processes. Adv. Chem. Ser. **105**:163–189.
18. **Mackie, R. I., and M. P. Bryant.** 1981. Metabolic activity of fatty acid-oxidizing bacteria and the contribution of acetate, propionate, butyrate and CO_2 to methanogenesis in cattle waste at 40 and 60°C. Appl. Environ. Microbiol. **41**:1363–1373.
19. **McInerney, J. J., M. P. Bryant, R. B. Hespell, and J. W. Costerton.** 1981. *Syntrophomonas wolfei* gen. nov. sp. nov., an anaerobic, syntrophic fatty acid-oxidizing bacterium. Appl. Environ. Microbiol. **41**:1029–1039.
20. **McInerney, M. J., M. P. Bryant, and H. Pfennig.** 1979. Anaerobic bacterium that degrades fatty acids in syntrophic association with methanogens. Arch. Microbiol. **122**:129–135.
21. **Molongoski, J. J., and M. J. Klug.** 1980. Quantification and characterization of sedimenting particulate organic matter in a shallow hypereutrophic lake. Freshwater Biol. **10**:497–506.
22. **Molongoski, J. J., and M. J. Klug.** 1980. Anaerobic metabolism of particulate organic matter in the sediments of a hypereutrophic lake. Freshwater Biol. **10**:507–518.
23. **Monokova, S. V.** 1975. Volatile fatty acids in bottom sediments of the Rybinsk Reservoir. Hydrobiol. J. **11**:45–48.
24. **Robertson, K. C.** 1979. Quantitative comparison of the significance of methane in the carbon cycle of two small lakes. Arch. Hydrobiol. Beih. Ergebn. Limnol. **12**:123–135.
25. **Rufener, W. H., and M. J. Wolin.** 1968. Effect of CCl_4 on CH_4 and volatile acid production in continuous cultures of rumen organisms and in a sheep rumen. Appl. Microbiol. **16**:1955–1956.
26. **Smith, R. L., and M. J. Klug.** 1981. Reduction of sulfur compounds in the sediments of a eutrophic lake basin. Appl. Environ. Microbiol. **41**:1230–1237.
27. **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.
28. **Sorensen, J., D. Christensen, and B. B. Jorgensen.** 1981. Volatile fatty acids and hydrogen as substrates for sulfate-reducing bacteria in anaerobic marine sediment. Appl. Environ. Microbiol. **42**:5–11.
29. **Strayer, R. F., and J. M. Tiedje.** 1978. *In situ* methane production in a small hypereutrophic hard-water lake: loss of methane from sediments by vertical diffusion and ebullition. Limnol. Oceanogr. **23**:1201–1206.
30. **Strayer, R. F. and T. 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. **Takai, Y.** 1970. The mechanism of methane fermentation in flooded paddy soil. Soil Sci. Plant Nutr. **16**:238–244.
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 J. G. Zeikus.** 1977. Effect of sulfate on carbon and electron flow during microbial methanogenesis in freshwater sediments. Appl. Environ. Microbiol. **33**:275–281.
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. **Wolin, M. J.** 1979. The rumen fermentation: A model for microbial interactions in anaerobic ecosystems. Adv. Microb. Ecol. **3**:49–77.
36. **Zehnder, A. J. B., and T. D. Brock.** 1979. Methane formation and methane oxidation by methanogenic bacteria. J. Bacteriol. **137**:420–432.
37. **Zehnder, A. J. B., B. Huser, and T. D. Brock.** 1979. Measuring radioactive methane with the liquid scintillation counter. Appl. Environ. Microbiol. **37**:897–899.

CHAPTER III

Kinetic Analysis of Competition Between Sulfate Reducers and Methanogens for Hydrogen in Sediments

Introduction:

It is generally considered that sulfate reducing bacteria (SRB) can inhibit the activity of methanogenic bacteria (MB) when millimolar quantities of sulfate are present. Thermodynamic calculations can be used to predict the exclusion of methane production in sulfate-containing sediments (8,14,29). However, it is invalid to argue that a reaction that is more thermodynamically favorable will exclude another reaction that is also thermodynamically favorable (15). Therefore, MB must be inhibited by toxic metabolites, the lack of methane precursors, or required growth factors in the presence of sulfate. The prevalent conclusion is that SRB inhibit MB by outcompeting them for hydrogen and acetate (1,2,6,14,18,21,29), but the mechanism(s) for this have not been elucidated. MB are frequently present in sulfate-containing sediments and have the potential to consume methane precursors as evidenced by methane production when sulfate reduction is inhibited or when hydrogen or acetate is added to the sediments (2, 21, 26, 29). Our working hypothesis was that SRB have a higher affinity for hydrogen and acetate than MB, which enables SRB to maintain the pool of these substrates at concentrations too low for MB to effectively utilize when sulfate is not limiting to SRB. The studies reported here concentrated on the competition for hydrogen

since acetate-utilizing MB are generally absent in natural sediments in which SRB effectively outcompete MB (8,19,26) and the ultimate competition is thus for hydrogen.

Materials and Methods:

Measurements of In Situ Rates:

Sediments were collected during summer stratification from two sites in Wintergreen Lake, a eutrophic lake located in southwestern Michigan. During summer stratification the sediments at the profundal site, site A, lie below an anaerobic, sulfate-depleted hypolimnion (sulfate concentration range 30-160 μM) whereas those at the depth of the themocline, site B, have oxygen (range 1-4 mg oxygen per liter) and sulfate (180 μM) in the overlying water (16,17).

Sulfate reduction rates were measured by the direct injection method of Jørgenson (10) as described in detail by King and Klug (11). Briefly, 10 μl of carrier free $^{35}SO_4^{=}$ (1 μCi) was injected into sediment cores incubated at in situ temperatures. The incubation was stopped by quick freezing. The $^{35}S^{2-}$ produced was distilled, trapped, and quantified by liquid scintillation counting. Sulfate reduction rates were calculated by multiplying the rate of conversion of $^{35}SO_4^{2-}$ to $^{35}S^{2-}$ by the in situ sulfate pool. Interstitial water was collected with dialysis samplers (17) and analyzed for sulfate turbidimetrically (28).

Methane production was measured on 5 ml subcores taken through ports in cores (7 cm inner diameter) collected using SCUBA. The subcores were extruded into pressure tubes (Bellco Glass) or 20 ml serum bottles (Wheaton Scientific) under an atmosphere of 93 % nitrogen and 7 % carbon dioxide. The vessels were stoppered with

butyl rubber stoppers (Bellco Glass), sealed with an aluminum crimp, and incubated at in situ temperatures. The rate of increase in methane concentration in the headspace was measured at intervals over a 20-30 hour incubation period. The tubes were shaken before each methane analysis to equilibrate the dissolved gases with the headspace. Methane was analyzed on a Varian 600D gas chromatograph as described below.

Laboratory studies:

Sediments for laboratory studies were collected from the A and B site with an Eckman dredge. Depending on the experiment 500, 700, or 800 ml of sediment was transferred under anaerobic conditions to 1 liter reagent bottles (Wheaton Scientific) and sealed with a rubber stopper. A final concentration of either 10 or 20 mM ferrous sulfate (sulfate-amended sediments) or ferrous chloride (control sediments) was added to the sediments. Ferrous salts were used to prevent the accumulation of free sulfide, which is toxic to methanogens at high concentrations (7,29). Ferrous chloride was added to control flasks to eliminate any potential differential effects of excess iron on hydrogen uptake or production. The sediments were incubated at 20 $\pm$ 2°C in the dark without mixing or were placed on a cell production bottle roller (Bellco) and slowly turned. Molydate was added to the sediments as a nitrogen-flushed 0.5 M solution of sodium molybdate to give a final concentration of 5 mM. Molybdate is regarded as an effective and specific inhibitor of sulfate reduction in sediments (20,21,25,26).

Carbon dioxide and methane in the headspace of the bottles were analyzed on a Carle basic gas chromatograph equipped with a

microthermistor detector. The gases were separated on a 1 m column of Poropak N (Waters Associates) with a helium carrier at a flow rate of 20 ml/min and an oven temperature of 60°C. When greater sensitivity for methane was desired, a Varian 600D gas chromatograph with a flame ionization detector was used. Gases were separated with a helium carrier on a 1 m column of Poropak N at 50°C. Hydrogen was analyzed on a Varian 3700 gas chromatograph with a thermal conductivity detector. The gases were separated on a 3 m column of Poropak N with nitrogen as the carrier at 15 ml per min and an oven temperature of 35°C. The detection limit was 0.04 pascals. One pascal is approximately equivalent to 9.9×10^{-6} atmospheres and a dissolved hydrogen concentration of 8 nM. The bottles were shaken vigorously before sampling to equilibrate the dissolved gases with the headspace.

Interstitial water for sulfate analysis was collected by centrifugation and analyzed by high pressure liquid chromatography. Ions were separated at room temperature on a 5 x 0.46 cm Vydac column (Anspec) with a solvent of 1 mM pthallic acid (pH 5.5) at a flow rate of 2 ml/min. Sulfate was detected with a Wescan conductivity detector (Anspec).

For the kinetic analysis of hydrogen uptake 4 or 6 ml samples of sediments were dispensed into 25 x 142 mm roll tubes (Bellco). The tubes were flushed with oxygen-free nitrogen before and during the transfer. In experiments where chloroform was added to sediments, a 50-75 ml sample of sediment was first transferred to a 120 ml serum bottle. Chloroform was added directly (final concentration, 0.003% V/V). The sediments were mixed and dispensed into tubes as above. The tubes were incubated with slow rolling on a tube roller to create

a thin film of sediment (27). Hydrogen was added and headspace samples were withdrawn over time and analyzed for hydrogen or methane or both.

Two experimental approaches were used to ensure that chloroform did not alter the potential of SRB to take up hydrogen. In the first experiment, sediments were amended with 550 μM (final concentration) of sulfate to saturate SRB for sulfate. The sediments were incubated under saturating hydrogen (50 kPa) on the tube roller, and the rates of sulfate depletion over a 2 h incubation period in sediments treated with chloroform and control sediments were compared. In the second experiment, sediments that had been adapted to 20 mM sulfate were incubated on the tube roller with an initial hydrogen concentration of 1 kPa. The initial rate of hydrogen uptake was measured in untreated sediments, sediments treated with chloroform and sediments treated with molybdate. If chloroform did not inhibit hydrogen uptake by SRB, then the sum of hydrogen uptake in sediments treated with chloroform and sediments treated with molybdate would equal the hydrogen uptake in untreated sediments.

Kinetic Analysis:

Hydrogen uptake in sediments has previously been shown to follow Michaelis-Menten kinetics (27).

$$V = \frac{V_M \cdot S}{K + S} \qquad (1)$$

where V is the velocity of uptake, V_M is the maximum potential uptake velocity, S is the substrate concentration and K is the substrate concentration at which $V = 0.5\ V_M$. Kinetic parameters were estimated

from progress curves of hydrogen consumption over time. A linearlized expression of an integrated form of the Michaelis-Menten expression can be derived (23).

$$\frac{\ln S_o/S_t}{t} = \frac{-1}{K} \cdot \frac{S_o - S_t}{t} + \frac{V_M}{K} \qquad (2)$$

where S_o is the initial substrate concentration and S_t is the substrate concentration at time, t. This method gives kinetic parameters for hydrogen uptake in sediments comparable to those estimated from initial velocity studies (27) and has the added advantage that variability between sediment samples for a particular kinetic analysis can be eliminated since all the substrate concentrations are in effect tested on the same sediment sample.

Sediments containing hydrogen-consuming MB and SRB populations can be expected to have a total hydrogen uptake described by a two-term Michaelis-Menten equation.

$$V_T = \frac{V_{MSRB} \cdot S}{K_{SRB} + S} + \frac{V_{MMB} \cdot S}{K_{MB} + S} \qquad (3)$$

where V_T is the total rate of hydrogen uptake, V_{MSRB} and K_{SRB} are the V_M and K of the SRB population, and V_{MMB} and K_{MB} are the V_M and K for the MB. This two-term equation was used in the analysis of hydrogen uptake in sulfate-containing sediments that had both MB and SRB populations. Kinetic parameters for the two populations were entered into a program which calculated total hydrogen uptake over time.

RESULTS:

Concurrent methane production and sulfate reduction were observed in the surface sediments (0-2 cm) of both site A and site B (Table 1).

Table 1: Relative importance of methane production and sulfate reduction in the surface sediments (0-2 cm) of Wintergreen Lake during summer stratification.

Sediment Site	Sulfate Concentration (μM)	Methane[a] Production	Sulfate[a] Reduction	Sulfate Reduction[b] (% of Total)
A[c]	71	40 ± 10	6.2 ± 1.7	13
B	59	26 ± 12	4.0 ± 1.3	13

[a] μmoles per liter of sediment per hour; mean ± standard error of 3 or more rate measurements.

[b] Sulfate reduction rate divided by total of sulfate reduction rate and methane produciton rate.

[c] Sulfate concentration and reduction rate for A site from King and Klug (11).

Methane production was the dominant process and comprised about the same proportion of the total of methane production and sulfate reduction at both sites.

Methane production in sediments from both sites was completely inhibited within 2-5 days at 20°C by the addition of 10 or 20 mM sulfate. Active sulfate reduction in the sulfate-amended sediments was evidenced by the loss of dissolved sulfate and the appearance of black ferrous sulfide over time. There was also an increase in carbon dioxide production in sulfate-amended sediments over that in control sediments.

Sulfate-amended sediments in which methane production was inhibited had significantly lower hydrogen partial pressures than $FeCl_2$ controls and untreated sediments (Table 2). Monitoring over time demonstrated that the inhibition of methane production and the decrease in hydrogen were concurrent (Figure 1). Both control and sulfate-amended sediments had high initial rates of methane production and elevated hydrogen partial pressures; presumably due to disturbances in carbon flow resulting from the initial manipulations with the sediment. The hydrogen partial pressure stabilized in control ($FeCl_2$-amended) sediments at approximately 1 Pa while methane production continued at lower rates. However, in sulfate-amended sediments the methane production rate and hydrogen partial pressure dropped sharply until methane production was no longer detectable. The hydrogen partial pressure continued to slowly decline after methane production had ceased.

Addition of 5mM (final concentration) sodium molybdate to inhibit sulfate reduction in the sulfate-amended sediments resulted in the

Table 2. Hydrogen partial pressure in sediments with and without added sulfate.

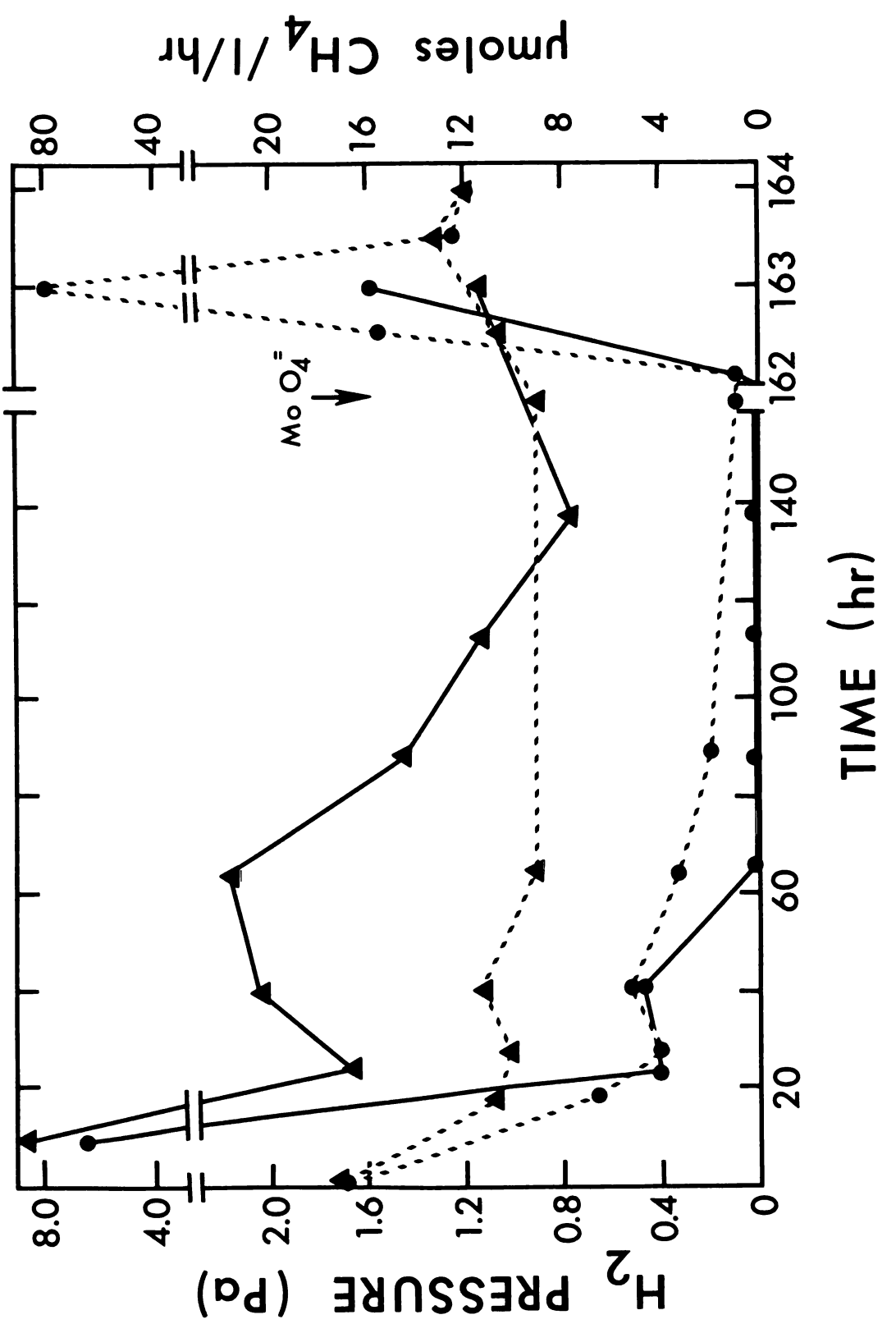
Sediment Treatment	Methane[a] Production	Hydrogen Partial Pressure[b] (Pascals)
No additions	+	1.11 ± 0.16
Plus $FeCl_2$[c]	+	1.09 ± 0.14
Plus $FeSO_4$[c]	-	0.17 ± 0.16

[a] + Indicates detectable methane production; - indicates methane production was not detectable

[b] Mean ± standard error of 5 observations

[c] Incubated at least 5 days but less than 5 weeks with added $FeCl_2$ or $FeSO_4$

Figure 1. Methane production rates and hydrogen partial pressures over time in sulfate-amended and control sediments collected from the A site and incubated at 20° C on a bottle roller. Arrow designates addition of molybdate to sulfate-amended sediments. Values are means of duplicate bottles of each treatment and are representative of the results obtained in several similar experiments. Symbols ●—● and ▲—▲, methane production rates in sulfate-amended and control sediments; ●···● and ▲···▲, hydrogen partial pressure in sulfate-amended and control sediments.


H_2 PRESSURE (Pa)
8.0
4.0
2.0
1.6
1.2
0.8
0.4
0
20
60
100
140
162
163
164
TIME (hr)
$Mo O_4^=$
pmoles CH_4 /l/hr
80
40
20
16
12
8
4
0

resumption of methanogenesis at a rate comparable to that in control sediments (Figure 1). This corresponded with an increase in the hydrogen partial pressure which, after an initial accumulation, stabilized at partial pressures similar to those in control sediments. Molybdate had no effect on the hydrogen partial pressure in control sediments (data not shown).

Since MB maintained their potential to metabolize hydrogen in sulfate-amended sediments, a suitable inhibitor that would prevent MB from taking up added hydrogen but would not inhibit hydrogen uptake by SRB had to be found before kinetic analysis of hydrogen uptake by SRB could be made. Chloroform (0.003% V/V) inhibited methane production but had no significant effect on the potential of SRB to metabolize hydrogen, as measured by the rate of sulfate reduction or the rate of hydrogen uptake (Table 3).

Sulfate-amended sediments had a higher potential for hydrogen uptake than control sediments (Figure 2, Table 4). The addition of chloroform to the control sediments resulted in the accumulation of hydrogen as previously shown (12), but in sulfate-amended sediments a significant potential for hydrogen uptake remained (Figure 2, Table 4). The V_M of the population that was inhibited by chloroform in the sulfate-amended sediments can be calculated as the difference between the V_M in the sulfate-amended sediments with and without added chloroform. The value obtained, 0.8 mmol H_2 per liter of sediment per h, was equivalent to the V_M of the control sediments. This indicates that the hydrogen-uptake potential of the MB population was not changed in the sulfate-amended sediment, but that there had been an increase in a hydrogen-consuming potential that was not inhibited by chloroform.

Table 3. Effect of chloroform on the hydrogen uptake potential of methanogens and sulfate reducers.

SRB Parameter Measured	Percent Inhibition by Chloroform[a]	
	Methane Production[b]	Sulfate Reduction
Sulfate Reduction	> 94	0.7 (8.9)[b]
Hydrogen Uptake	> 96	6.1 (9.8)[c]

[a] Mean with standard error in parentheses; n=3 for each treatment.

b Percent inhibition equals [1 - (rate in sediments treated with Chloroform X rate in control sediments^{-1})] X 100. A minimum estimate for methane inhibition is shown since there could have been methane production at rates lower than what could be detected during the incubation period.

c Percent inhibition equals [1 - (sum of the rate of hydrogen uptake in sediments treated with chloroform and sediments treated with molybdate X uptake rate in controls^{-1})] X 100.

Half-saturation constants, K, for hydrogen uptake were lower in sulfate-amended sediments than in control sediments (Table 4). When the MB in sulfate-amended sediments were inhibited with chloroform, the resultant K was 3-fold lower than the K in control sediments. When the results of kinetic analyses on sediments collected throughout the summer of 1981 from both the A and B site were compiled, the overall mean K values and 95% confidence intervals for hydrogen uptake not inibited by chloroform was 141 $\pm$ 33 Pa (n=8). This compared with the K for MB in control sediments of 597 $\pm$ 186 Pa hydrogen (n=8).

The theoretical progress curves of hydrogen uptake in sulfate-amended sediments that were calculated from the two-term Michaelis-Menten expression (equation 3) closely corresponded with those observed experimentally (Figure 2). For these calculations V_{MSRB} and K_{SRB} were taken as the mean values from the chloroform-treated, sulfate-amended sediment. It was assumed that V_{MMB} was equal to 0.8 mmoles H_2 per liter per hour, as calculated above, and that K_{MB} was equal to the K in control sediments.

DISCUSSION:

The fact that the inhibition of sulfate reduction in sulfate-amended sediments resulted in an increase in the hydrogen partial pressure and methane production rates to levels found in methanogenic sediments demonstrated that when sulfate concentrations were not limiting SRB inhibited methane production by lowering the hydrogen partial pressure below a threshold level necessary for hydrogen utilization by MB. The inhibition of methane production was not due to the toxic presence of sulfate or sulfide, as previously demonstrated (1,2,6,14,29), nor to the depletion of some factor other

Figure 2. Typical hydrogen uptake progress curves. Symbols: O, sulfate-amended sediments; X, sulfate-amended sediments treated with chloroform; ●, control sediments. T represents expected hydrogen partial pressure in sulfate-amended sediments calculated from equation 3 and the appropriate kinetic parameters as described in the text.

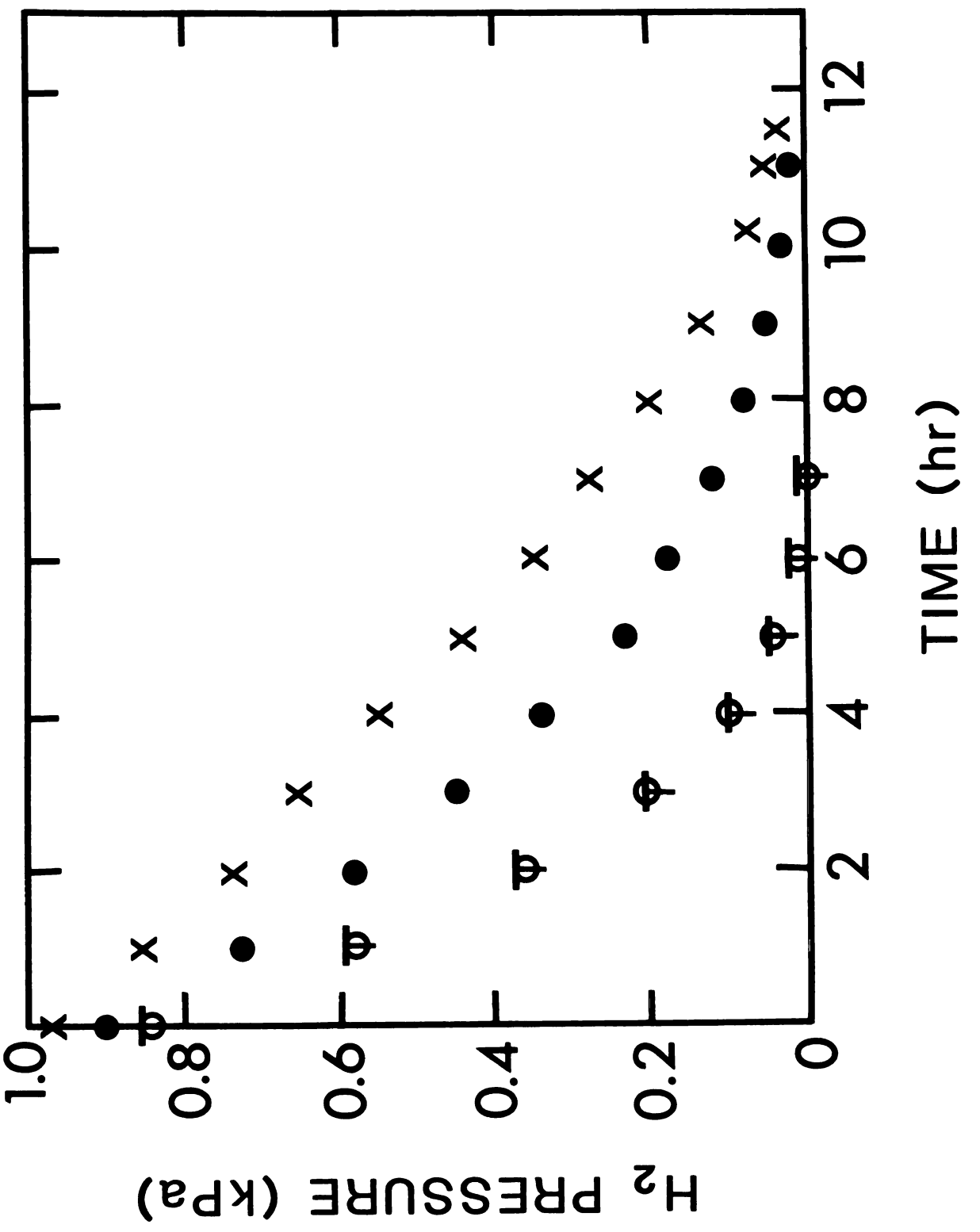

H_2 PRESSURE (kPa)
1.0
0.8
0.6
0.4
0.2
0
2
4
6
8
10
12
TIME (hr)

Table 4: Kinetic parameters for hydrogen uptake in sediments collected from the A site. Progress curves were run concurrently with those shown in Figure 2.

Sediment Type	Kinetic Parameters[a]	
	V_M (mM H_2 per hr)[b]	K (pascals)
Control Sediments	0.8 ± 0.1	588 ± 70
Sulfate-Amended	1.2 ± 0.2	455 ± 111
Sulfate-Amended Treated with Chloroform	0.4 ± 0.04	175 ± 45

[a]Mean and standard error of values from triplicate progress curves for each treatment.

[b]Millimoles of hydrogen per liter of sediment per hr.

than the electron donors necessary for methanogenesis. This conclusion was further supported by the comparable V_M for hydrogen uptake by MB in control and sulfate-amended sediments. Thus, the inhibition of methane production by added sulfate differs from the inhibition by oxygen (31) or nitrogen oxides (3) where the added electron acceptor or a product of its metabolism directly inhibits MB.

The inhibition of methane production at low hydrogen partial pressures was probably due to the decreased energy yield from methane production. The available free energy for methane production from hydrogen was calculated from the standard free energy of -139.23 kJ (30) and the methane, hydrogen, and carbon dioxide partial pressures to be -16.3 to -16.8 kJ per mole of methane produced in the control sediments shown in Fig. 1. The calculated free energy was approximately -6.7 kJ per mol of methane produced in the sulfate-amended sediments during the initial days of the inhibition of methane production and +4.7 kJ at 6-7 days after the sulfate addition. Though care must be taken in extrapolating from bulk phase pool sizes to those actually experienced by the bacteria, it is clear that the hydrogen partial pressure in the sulfate-amended sediment were sufficiently lowered to significantly reduce the energy available for methane production from hydrogen.

The lower hydrogen pool in the sulfate-amended sediments was associated with the lower overall K for hydrogen uptake and, specifically, with the low K for hydrogen uptake by the bacterial population that was not inhibited by chloroform. The K for hydrogen uptake in chloroform-treated, sulfate-amended sediments is considered to represent the K for the SRB population because: (1) there was no

detectable hydrogen uptake in the presence of chloroform in sediments not amended with sulfate; (ii) chloroform did not affect hydrogen uptake by SRB; and (iii) molybdate inhibited the hydrogen uptake in sulfate-amended sediments that chloroform did not inhibit. The K for the MB reported here is within the range estimated independently for methanogenic sediments and other methanogenic environments, such as sludge digestors and the rumen (J. A. Robinson and J. M. Tiedje, submitted for publication). Though there was a possibility of hydrogen uptake by bacteria fermenting hydrogen and carbon dioxide to acetate, the importance of these bacteria in methanogenic environments is low relative to methanogens (5,12). The conclusion that MB and SRB were the only two important hydrogen-consuming populations is further supported by the observation that the total hydrogen uptake in the sulfate-amended sediments could be predicted by using the K for the sulfate-depleted control sediment as the K for the population inhibited by chloroform.

Under steady state conditions in environments, such as sediments, where there is negligible physical removal or dilution of the microbial population the substrate pool size can be described by:

$$S = \frac{K}{(V_M \cdot y/k) - 1} \qquad (4)$$

where y and k are yield and mortality constants and K and V_M are expressed on a per cell basis (4,15). Thus, the hydrogen partial pressure should be dependent solely upon the physiological characteristics of the hydrogen-consuming populations. In the sulfate-amended sediments, the lower SRB K for hydrogen uptake (and possibly a higher yield and V_M per cell) resulted in a lower hydrogen pool. Some of the inhibition of methane production in sulfate-amended

sediments may be attributed to the metabolism of substrates by SRB rather than proton-reducing bacteria and the subsequent lower rates of hydrogen production (6). However, the maintenance of a lower hydrogen partial pressure by SRB that consumed hydrogen was the ultimate cause of the complete inhibition of methane production since the hydrogen partial pressure was independent of the rate of hydrogen production.

The maximum potential rate of substrate uptake is equally important as the affinity for substrate in determining the outcome of competition (9). The slow inhibition of methane production in Wintergreen Lake sediments amended with 20 mM sulfate can be explained by the small initial potential for hydrogen uptake of SRB. In freshly collected sediments incubated with saturating hydrogen, the turnover time for 1 mM sulfate (a saturating sulfate concentration) is 204 hours (25). Assuming that all the sulfate reduction was due to hydrogen uptake this yields a maximal V_M estimate for the SRB population of 19.6 μmol of hydrogen per liter of sediment per h. With the estimate that hydrogen is the precursor for approximately 40% of the methane production in these sediments (12), the rate of hydrogen production can be calculated from the methane production rate (Table 1) as 64 μmol per liter of sediment per h or 3-fold higher than the SRB V_M for uptake. Using the V_M and K for the MB, the K for the SRB, and the hydrogen partial pressure determined in the present study, it can be calculated from equation 3 that at saturating sulfate concentrations SRB would initially be able to use at most only 10 percent of the total hydrogen consumed by the two populations. Since the in situ sulfate concentration in these sediments is typically at

or below the SRB K for sulfate reduction (24), the limitation of SRB by sulfate can be expected to lower the SRB maximum potential for hydrogen uptake (22) and result in an in situ hydrogen uptake by SRB that is much less than 10 percent of the total hydrogen turnover. This result calculated from kinetic parameters agrees well with previous conclusions derived from experimental results (12).

MB are able to compete successfully with SRB in Wintergreen Lake sediments despite the lower SRB K for hydrogen uptake because the maximal potential for hydrogen uptake by SRB is limited by sulfate availability. The competition between SRB and MB for acetate is expected to have similar mechanisms as those for hydrogen competition. MB and SRB should coexist in other anaerobic sulfate-containing environments in which the rate of sulfate supply supports a potential for hydrogen and acetate uptake by SRB that is lower than the rate of hydrogen and acetate production.

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. Abram, J. W., and D. B. Nedwell. 1978. Hydrogen as a substrate for methanogenesis and sulphate reduction in anaerobic saltmarsh sediments. Arch. Microbiol. 117:93-97.

3. Balderston, W. L., and W. J. Payne. 1976. Inhibition of methanogenesis in saltmarsh sediments and whole-cell suspensions of methanogenic bacteria by nitrogen oxides. Appl. Environ. Microbiol. 32:264-269.

4. Billen, G., C. Joiris, J. Wijnant, and G. Gillain. 1980. Concentration and microbiological utilization of small organic molecules in the Scheldt Estuary, the Belgian coastal zone of the North Sea and the English Channel. Estuarine Coastal Mar. Sci. 11:279-294.

5. Braun, M., S. Schoberth, and G. Gottschalk. 1979. Enumeration of bacteria forming acetate from H_2 and CO_2 in anaerobic habitats. Arch. Microbiol. 120:201-204.

6. Bryant, M. P., L. L. Campbell, C. A. Reddy, and M. R. Crabill. 1977. Growth of Desulfovibrio in lactate or ethanol media low in sulfate in association with H_2-utilizing methanogenic bacteria. Appl. Environ. Microbiol. 33:1162-1169.

7. Cappenberg, Th. E. 1975. A study of mixed continuous cultures of sulfate-reducing and methane-producing bacteria. Microb. Ecol. 2:60-72.

8. Claypool, G. E., and I. R. Kaplan. 1974. The origin and distribution of methane in marine sediments, p. 99-139. In I. R. Kaplan (ed), Natural Gases in Marine Sediments. Plenum Publishing Corp., New York.

9. Healey, F. P. 1980. Slope of the Monod equation as an indicator of advantage in nutrients competition. Microb. Ecol. 5:281-286.

10. Jørgensen, B. B. 1978. A comparison for methods for the quantification of bacterial sulfate reduction in coastal marine sediments. I. Measurements with radiotracer techniques. Geomicrobiol. J. 1:11-27.

11. King, G. M., and M. J. Klug. 1982. Comparative aspects of sulfur mineralization in sediments of a eutrophic lake basin. Appl. Environ. Microbiol. 43:

12. 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.

13. Mackie, R. I., and M. P. Bryant. 1981. Metabolic activity of fatty acid-oxidizing bacteria and the contribution of acetate, propionate, butyrate and CO_2 to methanogenesis in cattle waste at 40 and 60°C. Appl. Environ. Microbiol. 41:1363-1373.

14. Martens, C. S., and R. A. Berner. 1977. Interstitial water chemistry of anoxic Long Island Sound sediments. 1. Dissolved gases. Limnol. Oceanogr. 22:10-25.

15. McCarty, P. L. 1972. Energetics of organic matter degradation, p. 91-118. In R. Mitchell (ed.), Water pollution microbiology. John Wiley and Sons, Inc., New York.

16. Molongoski, J. J., and M. J. Klug. 1980. Quantification and characterization of sedimenting particulate organic matter in a shallow hypereutrophic lake. Freshwater Biol. 10:497-506.

17. Molongoski, J. J., and M J. Klug. 1980. Anaerobic metabolism of particulate organic matter in the sediments of a hypereutrophic lake. Freshwater Biol. 10:507-518.

18. Mountfort, D. O., and R. A. Asher. 1981. Role of sulfate reduction versus methanogensis in terminal carbon flow in polluted intertidal sediments of Waimea Inlet, Nelson, New Zealand. Appl. Environ. Microbiol. 42:252-258.

19. Mountfort, D. O., R. A. Asher, E. L. Mays, and J. M Tiedje. 1980. Carbon and electron flow in mud and sand flat intertidal sediments at Delaware Inlet, Nelson, New Zealand. Appl. Environ. Microbiol. 39:686-694.

20. Oremland R. S., and M. P. Silverman. 1979. Microbial sulfate reduction measured by an automated electrical impedance technique. Geomicrobiol. J. 1:355-372.

21. Oremland, R. S., and B. F. Taylor. 1978. Sulfate reduction and methanogenesis in marine sediments. Geochim. Cosmochim. Acta 42:209-214.

22. Ramm, A. E., and D. A. Bella. 1974. Sulfide production in anaerobic microcosms. Limnol. Oceanogr. 19:110-118.

23. Segel, I. H. 1975. Enzyme kinetics. John Wiley and Sons, Inc., New York.

24. Smith, R. L., and M. J. Klug. 1981. Reduction of sulfur compounds in the sediments of a eutrophic lake basin. Appl. Environ. Microbiol. 41:1230-1237.

25. Smith, R. L., and M. J. Klug. 1981. Electron donors utilized by sulfate reducing bacteria in eutrophic lake sediments. Appl. Environ. Microbiology 42:116-121.

26. Sørensen, J., D. Christensen, and B. B. Jørgensen. 1981. Volatile fatty acids and hydrogen as substrates for sulfate-reducing bacteria in anaerobic marine sediment. Appl. Environ. Microbiol. 42:5-11.

27. 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. 35:330-340.

28. Tabatabai, M. A. 1974. Determination of sulfate in water samples. Sulfur Inst. J. 10:11-13.

29. 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.

30. Zehnder, A. J. B. 1978. Ecology of methane formation. p. 349-376. In R. Mitchell (ed.) Water pollution microbiology, vol. 2. John Wiley and Sons, Inc., New York.

31. Zehnder, A. J. B., and K. Wuhrmann. 1977. Physiology of a Methanobacterium strain A. Z. Arch. Microbiol. 111:199-205.

CHAPTER IV

Factors Controlling the Relative Importance of Sulfate Reduction and Methanogenesis in Lakes of Varying Tropic Levels

Introduction

The factors controlling the relative distribution of methanogenesis and sulfate reduction in freshwater sediments are poorly understood. In nitrate-depleted, anaerobic sediments hydrogen and acetate are the primary fermentation intermediates (12). Thus, the proportion of carbon and electron flow that proceeds through methanogenesis and sulfate reduction can be expected to be primarily dependent on the partitioning of these two resources between the methanogenic bacteria (MB) and the sulfate reducing bacteria (SRB).

It has been demonstrated that freshwater SRB can utilize hydrogen and acetate and can outcompete MB for these substrates when sulfate is increased to millimolar concentrations (13, 28). Studies in freshwater sediments not supplemented with sulfate have indicated that SRB are not competitive with MB at <u>in situ</u> freshwater sulfate concentrations. In eutrophic lake sediments SRB utilize less than 10% of the hydrogen production and less than 15% of acetate production (12, 13, 24). Inhibition of sulfate reduction does not result in a stimulation of methane production (24) or the accumulation of acetate and hydrogen (12) which further indicates that SRB are consuming an insignificant fraction of the total hydrogen and acetate production. Sulfate reduction rates in eutrophic sediments are less than 15% of

the methane production rates (13, 31). Others have suggested that SRB are net producers of acetate and hydrogen in freshwater sediments (11, 28).

The restriction of previous studies to eutrophic lake sediments may have underestimated the ability of SRB to compete with MB in freshwater. The sulfate gradient into eutrophic lake sediments is very steep (18, 23, 38) and the most active zone of sulfate reduction is probably confined to the most superficial sediments (8). Present methods are not adequate for sampling this zone and maintaining in situ sulfate concentrations.

If SRB can effectively compete with MB at freshwater sulfate concentrations then the relative importance of sulfate reduction and methanogenesis as terminal processes in sediment metabolism should be related to the proportion of the zone of actively metabolizing sediment in which sulfate is available to SRB. The depth that sulfate penetrates should in turn be related to the rate of sulfate flux into the sediment and the rate it is consumed within the sediment.

Materials and Methods

Sediment Sampling and Incubation:

Lawrence Lake is a small hardwater lake located in southwestern Michigan. The physical, chemical and biological characteristics of the lake have been described in detail (26). Profundal sediments were sampled from within the 12 meter depth contour. Oxygen was undetectable in the overlying water with the Winkler technique during the sampling period (R. G. Wetzel personal communication). Sulfate concentrations in the interstitial water with depth were determined from dialysis samplers (5) with ports spaced at 1 cm intervals. For

measurements of methane production with depth sediments were collected with a gravity corer as previously described (12). Subcores were taken at 2 cm intervals with cut-off 3 ml plastic syringes which were then sealed with a rubber stopper and incubated underwater at the in situ sediment temperature during transport. The sediments were extruded into 10 ml Vacutainers (Becton-Dickenson) and treated as outlined below. In all subsequent experiments sediments were sampled with an Eckman dredge. Care was taken to collect only the floculent surface sediments and associated water. The sediments were transported on ice to slow sulfate depletion in sealed containers with no gas headspace.

Sediments were transferred under a nitrogen atmosphere to anaerobic pressure tubes (Bellco Glass) or 10 ml Vacutainers (Becton-Dickenson). Incubations were at 10°C in the dark. Methane production was measured as the rate of accumulation of methane over time. The tubes were vigorously shaken to equilibrate dissolved methane with the headspace before samples were taken. Sulfate reduction rates were estimated from the rate of sulfate depletion in the sediment over a 3.5 hour incubation period. Replicate samples were sacrificed over time and centrifuged to collect the interstial water which was analyzed for sulfate. Solutions of molybdate, acetate and sulfate were flushed with nitrogen and added to the sediments with a syringe and needle. The added volume was less than one percent of the total sediment volume. Molybdate was added to the sediment as the sodium salt to a final concentration of 1 or 2 mM.

For hydrogen uptake experiments 100 ml of sediment were placed in 120 ml serum bottle (Wheaton Scientific). Additions of chloroform

(0.003% V/V) or molybdate were made prior to transferring 5 ml aliquots into pressure tubes. The sediments were incubated with slow rolling on a tube roller to create a thin film of sediment. The sediments were allowed to equilibrate 1-3 hours. Hydrogen was added to the headspace to give an initial partial pressure of approximately 450 pascals. The rate of hydrogen uptake was measured over a 15 or 30 minute time period.

Radioactive tracer studies:

Solutions of radioactive compounds were pre-flushed with nitrogen. The final volume of the added solution was five percent or less of the sediment volume. The concentration of the added compounds was less than one percent of the *in situ* pool size. [2-^{14}C] acetate (54 mCi mmol^{-1}) and [^{14}C]-sodium bicarbonate (0.1 mCi mmol^{-1}) were purchased from New England Nuclear.

The kinetics of acetate uptake were determined using the method of Wright and Hobbie (30). Various final concentrations of acetate were added to sediments along with [2-^{14}C] acetate. The data were analyzed according to the equation of Hobbie and Crawford (7):

$$\frac{t}{f} = \frac{(K + S_n)}{V} + \frac{A}{V} \qquad (1)$$

where t is the incubation time, f is the fraction of the added activity converted to product, S_n is the *in situ* substrate concentration, A is the amount of substrate added, V is the maximum uptake velocity and K is the substrate concentration that gives half-maximal velocity of uptake. A plot of t/f versus A yields a straight line with an x-intercept of - K+Sn if uptake follows Michaelis-Menten kinetics.

Analytical Techniques:

Methane concentrations were measured on a Varian 600 D gas chromatograph with a flame ionization detector. Gases were separated with a helium carrier on a one meter column of Poropack N (Waters Associates) at 50°C. Hydrogen was analyzed on a Varian 3700 gas chromatograph with a thermal conductivity detector. The gases were separated at 35°C on a 3 meter column of Poropak N with nitrogen as the carrier at 15 ml per minute. $^{14}CH_4$ and $^{14}CO_2$ were separated on the same column but with helium as the carrier gas and an oven temperature of 45°C. The effluent from the detector was passed through a gas proportional counter for quantification of the radioactivity. Total $^{14}CO_2$ in the tubes was corrected for dissolved ^{14}C-inorganic carbon with an empirical factor that was determined from the distribution of $H^{14}CO_3^-$ added to sediment.

Sulfate was analyzed by high pressure liquid chromatography. Ions were separated on a 5 x 0.46 cm Vydac column (Anspec) with a solvent of 1 mM pthallic acid (pH 5.5) at a flow rate of 2 ml per minute at room temperature. Sulfate was detected with a Wescan conductivity detector (Anspec).

Modelling of Competition:

A computer model, called ACETATE, was developed to simulate competition between SRB and MB for acetate in sediments. Uptake of acetate by MB was described by the Michaleis-Menten equation:

$$v = \frac{V \times A \times X}{K_A + A}$$

where v is the velocity of acetate uptake, V is the maximum rate of

acetate uptake per unit biomass, X is the biomass, A is the acetate concentration and K_A is the acetate concentration that gives an uptake of acetate one half of V. The uptake of acetate by SRB was modelled with a multiplicative model (22) and was computed as

$$v = \frac{V \times A \times X}{K_A + A} \times \frac{SU}{K_{SU} + SU}$$

where SU is the sulfate concentration and K_{SU} is the half-saturation constant for sulfate uptake. Growth was computed as:

$$\Delta X = (v \times \Delta t \times Y) - (b \times X)$$

where ΔX is the increase in biomass over the time interval Δt, Y is the yield of biomass per quantity of acetate taken up and b is the mortality coefficient. The parameters used in the model are listed in Table (1). The K_A estimates were from the kinetic analysis of acetate uptake by MB and SRB in Lawrence Lake sediments. The K_{SU} was from the sulfate half-saturation constant for freshwater sediments determined previously (23). The mortality coefficient was estimated from the decline of the maximum potential for methanogenesis in freshwater sediments in which methane production was inhibited with sulfate (unpublished data). Theoretical yields were calculated from thermodynamic considerations using the method of McCarty (15, 16). The maximum rates of acetate uptake per unit biomass were calculated from the above parameters and the expected relationship between substrate pool size and growth parameters at steady state (1, 16).

Sulfate flux from the overlying water was assumed to be controlled by diffusion.

$$F = -D \frac{\Delta C}{\Delta Z}$$

Table 1. Growth parameters for the simulation model of methanogen and sulfate reducer competition for acetate in sediments.[a]

Parameter	Methanogens	Sulfate Reducers
K_A (μM)	50	3
K_{SU} (μM)	-	68
V (μmoles x mg cells^{-1} x min^{-1})	0.0109	0.0069
Y (mg cells x mmol acetate^{-1})	2.136	3.84
b (min^{-1})	7×10^{-6}	7×10^{-6}

[a] The basis for the parameters is outline in the text.

where F is the rate of flux and ΔC is the change in sulfate concentration over the depth interval ΔZ. D, the diffusion coefficient, was assumed to equal 1.5×10^{-5} $cm^2 \times sec^{-1}$ which is the middle of the range found by Hessleian (6) for lake sediments in summer at 20°C.

Acetate uptake by MB and SRB with depth was modelled by assuming an active depth of acetate production of 3 cm which approximates the depth concluded to be the most active in sediment decomposition (9,12). The rate of acetate production was assumed to be uniform with depth. The sulfate concentration in the overlying water was assumed to remain constant. The sediment was divided into 1 mm layers and individual layers were assumed to be homogeneous. Sulfate flux into each layer from the overlying layer, the sulfate flux out of the layer to the next deeper layer, the amount of acetate produced, the acetate and sulfate uptake and the growth of MB and SRB were calculated at one minute time intervals. The model was generally run for a time equivalent to greater than five years. This time was sufficient to approach as steady state as indicated by insignificant changes in the rate, concentration and biomass parameters over a modelled time period of approximately one year. Therefore the results of the model were not a true steady-state solution but were considered to be a close approximation and sufficient for the purposes of this study in view of the fact that the sediments being modelled would never be expected to be at steady-state.

Results

In Situ H_2 and Acetate Utilization by SRB:

Methane production rates were highly variable in subcores of Lawrence Lake profundal sediments taken at the same depth and time (Figure 1). A high day to day variability was also noted especially at the lower depths examined. Sulfate concentrations decreased rapidly with depth indicating active sulfate reduction in the surface sediments (Figure 1). To obtain sediments with the highest sulfate concentrations and to reduce variability within a sediment sample for a given date subsequent studies were performed with well-mixed samples of surface sediments.

The addition of molybdate to the sediment completely inhibited sulfate depletion and stimulated methane production (Table 2). The extent of molybdate stimulation of methane production was variable with increasing stimulation on successive dates. Molybdate had no significant effect on methane production in sediments that were preincubated to deplete the sulfate pool (Table 2). The addition of sulfate inhibited methanogenesis suggesting that the potential of SRB to compete with MB was limited by sulfate availability.

The increase in the number of moles of methane produced in molybdate-treated sediments was 96% of the number of moles of sulfate reduced in untreated sediments (Table 3). Since the electron equivalents required for the production of methane and the reduction of sulfate are equal (25) this indicates that carbon and electron flow that was blocked from going through sulfate reduction proceeded through methanogenesis. Additions of molybdate increased the production of $^{14}CH_4$ from both [2-^{14}C] acetate and $^{14}CO_2$ (Table 4).

Figure 1. Methane production mean and range of standard error on 12 August ●, and 13 August ■ and sulfate concentrations with depth on 24 August at the 12.5 m depth site of Lawrence Lake.

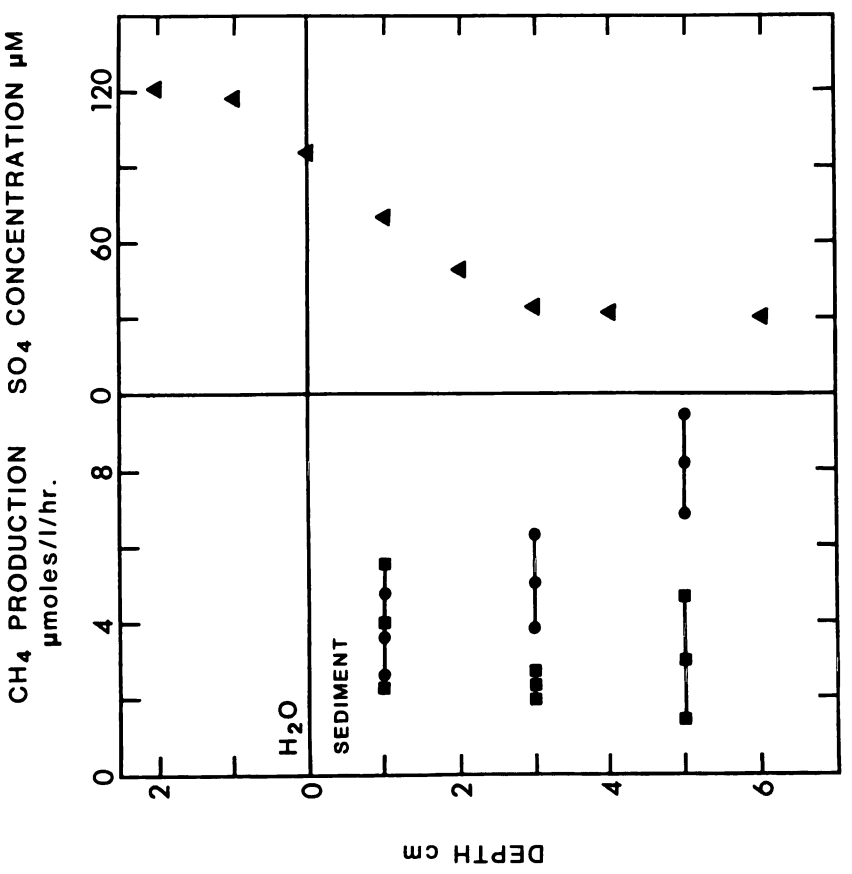

CH4 PRODUCTION
µmoles/l/hr.
SO4 CONCENTRATION µM
0
4
8
0
60
120
H2O
SEDIMENT
2
0
2
4
6
DEPTH cm

Table 2. Effect of molybdate and sulfate additions on methane production in Lawrence Lake surface sediments.

Date	Incubation Period (hr)	Initial Sulfate Concentration (μM)	Number of Replicates Per Treatment	Methane Production Rate in Untreated Sediment (μmoles/l/hr)	Methane Production Rate Ratio[a]	
					with $MoO_4^=$	with SO_4[b]
9/7	25	ND[c]	4	2.48 $\pm$ .61	1.42	ND
9/23	21.5	85	5	5.85 $\pm$ 1.96	1.45	.50 (125 μM) .40 (2.5 mM)
9/26	8.25	72	4	3.00 $\pm$ 1.11	1.54	.75 (2.5 mM)
10/5	20	105	5	0.97 $\pm$ 0.09	3.91	ND
10/9	25	ND	3	1.44 $\pm$ 0.03	5.35	ND
Sulfate[d] Depleted	22.75	<2	5	2.29 $\pm$ 0.35	1.06	ND
Sulfate[d] Depleted	4.75	<2	5	3.16 $\pm$ 0.77	1.02	ND

[a] (Methane production in treated sediment) x (Methane production in untreated sediments)$^{-1}$.

[b] Concentration of the added sulfate is in parentheses.

[c] Not determined.

[d] Sediment incubated in the dark to deplete the sulfate pool.

Table 3. Methane production and sulfate reduction in control and molybdate-treated Lawrence Lake sediments over a 20 hour incubation period.

	Methane Produced (μmoles l^{-1})[a]	Sulfate Reduced (μmoles l^{-1})
Control	19.4 (1.8)	58.9 (2.9)
Molybdate-treated[b]	75.7 (8.2)	0
Difference[c]	-56.3	58.9

[a] Mean and standard error in parentheses; n = 5 for methane production; n = 4 for sulfate reduction.

[b] Molybdate final concentration 1 mM.

[c] (Control sediments)-(Molybdate-treated sediments).

Table 4. Effect of molybdate addition on the conversion of [2-^{14}C] acetate and $^{14}CO_2$ to $^{14}CH_4$.

Percentage Increase with Molybdate Addition[a]		
Total Methane Production	$^{14}CH_4$ from [2-^{14}C]-acetate	$^{14}CH_4$ from $^{14}CO_2$
48 (.28)[b]	74 (.30)	ND[c]
435 (.09)	ND	565

[a] Total methane production determined over a three hour incubation period and $^{14}CH_4$ production over a 1 hour incubation period for experiment with [2-^{14}C]-acetate. Total methane and $^{14}CH_4$ production determined over 20 hour period for experiment with $^{14}CO_2$. The sediments for the two experiments were collected on separate dates in October. Percentage increase equals ($MoO_4^=$ treated sediments-control sediments) x control^{-1} x 100.

[b] Coefficient of variation for difference between molybdate-treated and untreated sediments in parentheses.

[c] Not determined.

From the information that the increase in methane production in molybdate-treated sediments corresponded with the rate of sulfate reduction in untreated sediments it could be calculated from the data in Table 2 that sulfate reduction comprised from 30-81% (mean = 50) of the total of the methane production and sulfate reduction rates. Direct simultaneous measurements of sulfate reduction and methane production in early October (Table 6) indicated that sulfate reduction comprised 72% of the total of both rates. The percentage of [2-^{14}C]-acetate metabolized to $^{14}CO_2$ was 86% and ranged from 40-97% (mean = 74%) throughout the study period.

The uptake of added hydrogen was inhibited by chloroform or molybdate alone and the effect of the inhibitors was additive (Table 5). However, even when methane production and sulfate reduction were completely inhibited there was still a significant rate of hydrogen uptake. Hydrogen uptake in the presence of the inhibitors continued even when the sediments were preincubated in the dark to deplete sulfate (less than 2 μM) and presumably other more thermodynamically favorable electron acceptors such as nitrogen oxides and iron (Table 5).

Kinetic analysis of [2-^{14}C] acetate turnover in freshly collected sediments with a sulfate concentration of 56 μM indicated that the K + Sn for $^{14}CO_2$ production, 5 μM, was significantly lower than the K + Sn of 33 μM for $^{14}CH_4$ production (Figure 2). Since prior experiments indicated that the addition of molybdate resulted in a 90% inhibition of the rate of $^{14}CO_2$ production from [2-^{14}C]-acetate, the kinetics of acetate metabolism to $^{14}CO_2$ primarily represented the kinetics of the SRB. To further ensure that the kinetics for $^{14}CO_2$ production were due to sulfate reduction, sediments were preincubated in the dark for

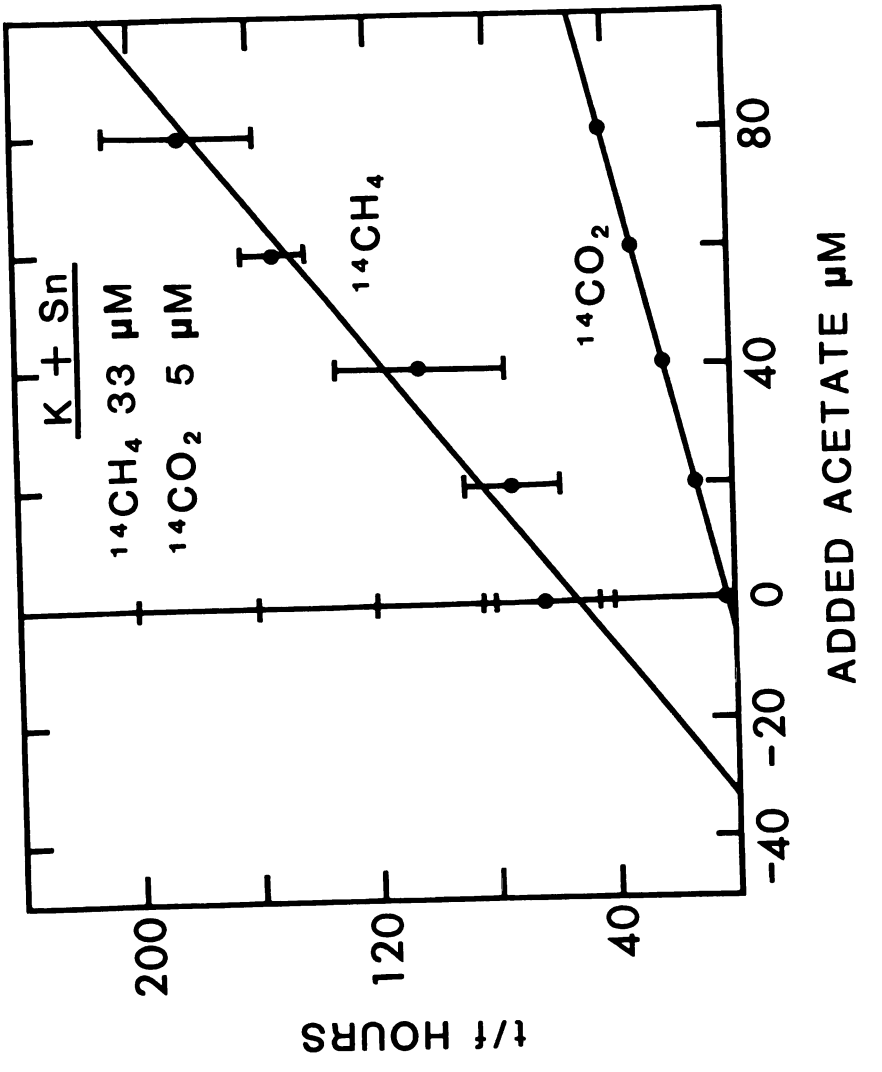
Table 5. Effect of inhibitors on hydrogen uptake in freshly collected and sulfate-depleted sediments.

Sediment Type	Additions		k[a]	Percent Inhibition
	$CHCl_3$	MoO_4		
Fresh Sediment	0	0	.74 ± .14	-
Fresh Sediment	+	0	.55 ± .02	26
Fresh Sediment	0	+	.55 ± .05	26
Fresh Sediment	+	+	.39 ± .06	47
SO_4-Depleted Sediment[b]	0	0	.84 ± .12	-
SO_4-Depleted Sediment[b]	+	+	.65 ± .08	23

[a] k is the first order rate constant of hydrogen where $k = \left(\frac{So-Sf}{t}\right) \times \left(\frac{So+Sf}{2}\right)^{-1}$ and So and Sf are the hydrogen concentrations at the initial time point and at time t. Mean and standard deviation of triplicate determinations given.

[b] SO_4-depleted sediment was a subsample of the fresh sediment that was stored in the dark for 5 days and had a SO_4 concentration less than 2 μM. Sediments preincubated in the dark for 4 weeks showed similar results.

Figure 2. Metabolism of [2-^{14}C] acetate in Lawrence Lake surface sediments to $^{14}CH_4$ and $^{14}CO_2$ at different concentrations of added acetate. Error bars represent standard error of the mean. Standard error for $^{14}CO_2$ is contained within the area of the symbols.


K + Sn
$^{14}CH_4$ 33 µM
$^{14}CO_2$ 5 µM
$^{14}CH_4$
$^{14}CO_2$
t/f HOURS
200
120
40
-40
-20
0
40
80
ADDED ACETATE µM

approximately two weeks to deplete electron acceptors for acetate oxidation. The sediments were then amended with 500 μM (final concentration) calcium sulfate three days prior to kinetic analysis. The sulfate concentration on the day of the analysis was 350 μM. The K + Sn for $^{14}CH_4$ and $^{14}CO_2$ production were 65 μM and 3 μM respectively.

Effect of total sediment metabolism on competition:

Comparison of initial rates of sulfate reduction and methane production and the fate of [2-^{14}C] acetate that were measured simultaneously in Lawrence Lake sediments with data from other lake sediments indicated that sulfate reduction was of greater relative importance in the terminal metabolism of Lawrence Lake sediments than in sediments that had higher total rates of methane production and sulfate reduction (Table 6). To examine whether this difference could be explained on the basis of resource competition between SRB and MB and the overall rate of organic matter decomposition in the sediments, the simulation model, ACETATE, was developed and simulations of the activity of SRB and MB within sediments with different rates of carbon metabolism and concentrations of sulfate in the overlying water were run.

The model indicated that partitioning of total terminal metabolism between sulfate reduction and methanogenesis was related to the depth in the sediment that sulfate remained above the minimal threshold concentration necessary to support sulfate reduction. As can be seen by comparing the two profiles in Figure 3, sulfate remained above threshold levels at deeper depths in sediments with lower rates of acetate production which permitted SRB to compete with

Table 6. Methane production, sulfate reduction and related parameters in the sediments of several lakes during summer stratification.

Lake	Depth Internal (cm)	Methane Production[a]	Sulfate Reduction[a]	% Acetate Metabolized to Methane[b]	SO_4 (μM)[c]	
					Water	Sediment
Lawrence[d]	Surface grab	1.8	4.6	14	110-130	50-110
Wintergreen[e] Site A	0-2	40	6.2	80	30-280	30-80
Wintergreen[e] Site B	0-2	26	4.0	67	180-290	40-80
Bostalsee[f]	0-10	10.0	1.0	NA[i]	130-200	50-70
Mendota[g]	0-2	NA	NA	61	200	ND[j]
Vechten[h]	0-5	34.0	NA	76	0-100	ND

[a] Rate expressed in μmoles per liter of sediment per hour.

[b] Based on % of $^{14}CH_4$ of total $^{14}CH_4$ and $^{14}CO_2$ produced from [2-^{14}C] acetate.

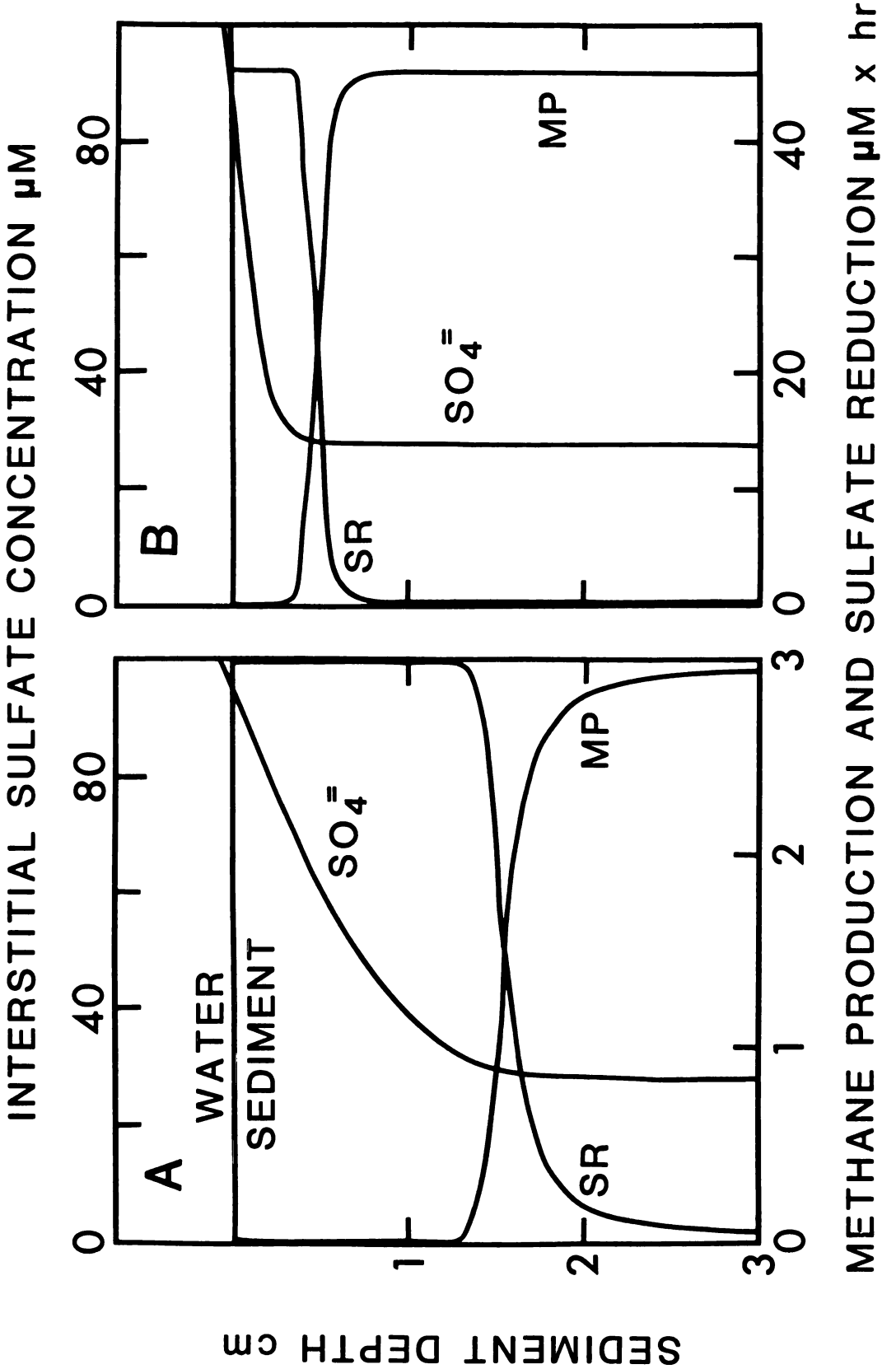
[c] Sulfate in water overlying the sediment and in surface sediments.
[d] Initial rates 0-3.5 hrs in sediment collected in early October.
[e] Data from (12, 13, 23) and unpublished data of this laboratory.
[f] Mean rates and concentration ranges from (31).
[g] Data from Fig. 4 of Winfrey and Ziekus (29).
[h] Data from Cappenberg (2)
[i] Data not available; NA.
[j] Sulfate not detectable with method employed; ND.

MB over a greater proportion of the total zone of active decomposition. The total rate of sulfate reduction in sediments with higher rates of acetate production was greater than the rate in sediments with the same sulfate concentration in the overlying water and lower rates of acetate production. However, the relative importance of sulfate reduction to total terminal metabolism was greater in the sediments with lower acetate production.

More comprehensive results of the model are presented in Figure 4. As the rate of total terminal metabolism was increased there was a sharp initial increase in the relative importance of methane production followed by a more gradual increase (Figure 4). The exact shape and position of the curve depended on the sulfate concentration in the overlying water; the lower the sulfate concentration the more abrupt the initial increase and the higher the proportion of total terminal metabolism proceeding through methanogenesis. When depth intervals other than 0-2 cm were considered the relationship between methane production and total terminal metabolism was similar to that shown in the figure except that for given values of total terminal metabolism and sulfate in the overlying water the relative importance of methane production increased as the depth of the sediment interval was increased.

The relative importance of methane production to total terminal metabolism proceeding through methane production and sulfate reduction in Lawrence and Wintergreen Lakes followed a pattern similar to that predicted with the simulation model (Figure 4). The Lake Bostalsee data did not appear to fit the general trend but this was probably due to the fact that the rate measurements were made on mixed sediments

Figure 3. Examples of methane production, sulfate reduction and sulfate concentration profiles from simulation model. The simulations depicted in both panels had 100 μM sulfate in the overlying water. The acetate production rates were 3 and 46 μmoles per liter per hour for the sediments in panels A and B respectively.


INTERSTITIAL SULFATE CONCENTRATION µM
METHANE PRODUCTION AND SULFATE REDUCTION µM x hr⁻¹
SEDIMENT DEPTH cm
A
B
WATER
SEDIMENT
SO4=
SR
MP
0
40
80
1
2
3
20

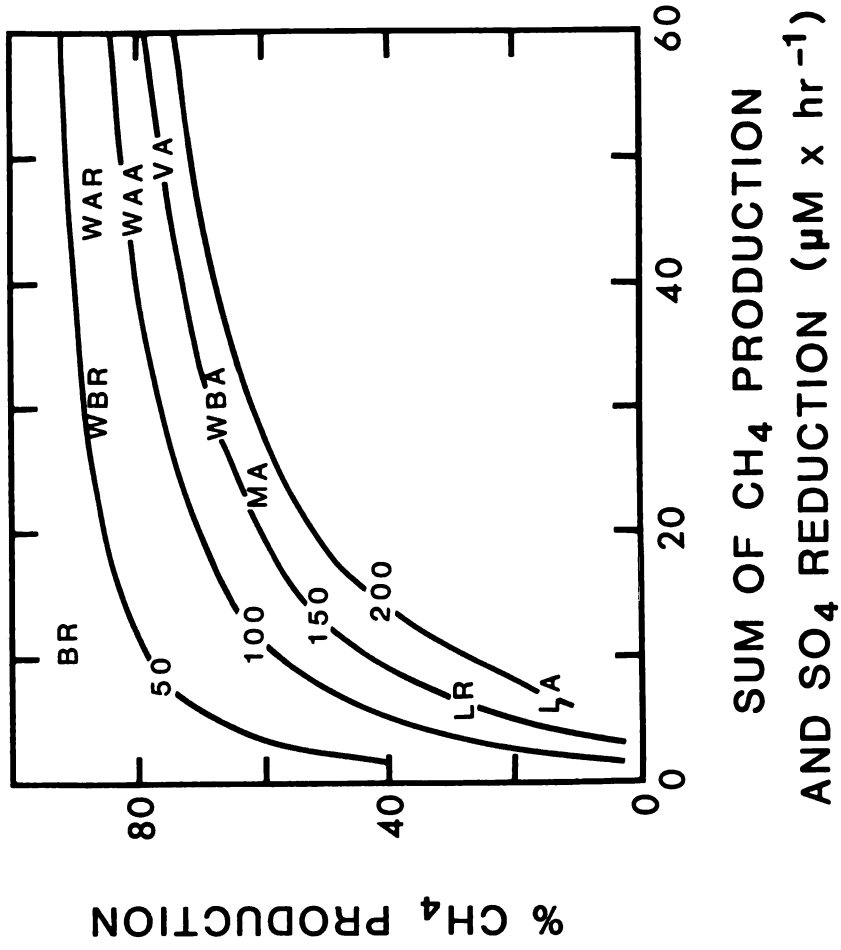
from 0 to 10-cm depth. The proportion of $^{14}CH_4$ produced from [2-^{14}C] acetate metabolism to $^{14}CH_4$ and $^{14}CO_2$ also had a similar relationship to total methane production and sulfate reduction (Figure 4). Since data on total methane production and sulfate reduction were not available for Lake Mendota and Lake Vechten, the total was estimated from acetate turnover data. A stoichiometry of two moles of hydrogen metabolized per mole of acetate metabolized was assumed (12). Given that one mole of acetate or four moles of hydrogen reduce one mole of sulfate or produce one mole of methane (25) hydrogen should be the precursor for one third of the total methane production and sulfate reduction from hydrogen and acetate. An estimate of total sulfate reduction and methane production was therefore calculated as 1.5 times the acetate turnover rate.

Discussion

The studies with Lawrence Lake surface sediments demonstrated that SRB can compete effectively with MB for hydrogen and acetate at freshwater sulfate concentrations. SRB had a higher affinity than MB for acetate in Lawrence Lake sediments even though they were sulfate limited. A comparison of the data of Lawrence and McCarty (14) and Middleton and Lawrence (17) on the acetate growth kinetics of MB and SRB respectively support the conclusion that SRB have a higher affinity for acetate than MB and also suggests that SRB have a higher maximum rate of acetate utilization per unit biomass and a higher growth yield. These factors should further increase the advantage of SRB in competing with MB for acetate.

Previous studies have demonstrated that freshwater SRB have a lower K for hydrogen than MB when sulfate is not limiting (13).

Figure 4. The percent methane production of the total of methane production and sulfate reduction in sediments. Curves represent the results from simulation modelling for the 0-2 cm depth interval with sulfate concentrations in the overlying water of 200, 150, 100 and 50 μM. Symbols for percentage methane production of total calculated from rate measurements: LR, Lawrence Lake; BR, Lake Bostalsee; WBR, Wintergreen Lake site B; WAR, Wintergreen Lake site A. Symbols for percentage methane production from [2-^{14}C] acetate: LA, Lawrence Lake; MA, Lake Mendota; WBA, Wintergreen Lake site B; WAA, Wintergreen Lake site A; VA, Lake Vechten.


% CH4 PRODUCTION
80
40
0
0
20
40
60
SUM OF CH4 PRODUCTION
AND SO4 REDUCTION (μM x hr-1)
BR
WBR
WAR
WAA
VA
WBA
MA
LR
LA
50
100
150
200

However, the previously employed approach of using molybdate and chloroform as selective inhibitors for sulfate reduction and methane production could not be used in Lawrence Lake sediments since there was a potential hydrogen-consuming population(s) other than SRB and MB. Preliminary evidence indicated that nitrogen oxides were not available as alternate electron acceptors for hydrogen uptake. Ferric iron seems an unlikely hydrogen acceptor since ferric iron should have been depleted from sediments that were preincubated before analysis, but hydrogen uptake continued in the presence of the inhibitors in these sediments. Metabolism of hydrogen with the reduction of carbon dioxide to acetate should have been inhibited by chloroform (21), though this was not evaluated. Although fatty acids may function as hydrogen acceptors when hydrogen uptake by methanogens is disrupted, fatty acids would not be expected to be important hydrogen acceptors at in situ hydrogen partial pressures (3,4). Additional study is required to identify the electron acceptor(s) and to determine whether this process competes with methanogenesis and sulfate reduction at in situ hydrogen partial pressures. The stimulation of methane production from hydrogen following the addition of molybdate did however indicate that SRB could effectively compete with MB for hydrogen at the low sulfate concentrations available in freshwater.

The molybdate stimualtion of methane production in Lawrence Lake sediments contrasts with the lack of an effect of molybdate on methane production in eutrophic Wintergreen Lake sediments (24). SRB use only a small portion of the total acetate and hydrogen production in Wintergreen Lake surface sediments (12, 13, 24). The difference in the relative distribution of the two terminal processes is not due to

differences in the sulfate concentrations in the water overlying the sediments since the sulfate concentrations overlying Wintergreen Lake sediments (particularly the B site) are as high or higher than those at the profundal site of Lawrence Lake. Rather, the enhanced ability of SRB to compete with MB in Lawrence Lake sediments is related to the lower rates of organic input to the sediments (33 mgC per m^2 per year (24) versus more than 200 mgC per m^2 per year for eutrophic Wintergreen Lake (18)) and the subsequent lower rate of organic matter decomposition.

The results of the simulation modelling as well as the compilation of field results support the conclusion that the rate of organic matter decomposition in the sediments can control the relative importance of sulfate reduction and methanogenesis as terminal processes in freshwater sediments. It must be stressed that the sole purpose of the simulation model was to give an indication of the nature of the relationship that could be expected if the distribution of methane production and sulfate reduction was controlled solely by competition for electron donors and the diffusive flux of sulfate from the overlying water. Competition for acetate was considered to be a suitable model for the overall competition between MB and SRB because acetate and hydrogen are the primary fermentation products in anaerobic sediments (12) and the mechanisms governing the competition for hydrogen are apparently the same as those for acetate. The agreement between the actual values of the model and field measurements was somewhat unexpected since the values of many of the parameters were derived from literature estimates or theoretical considerations. More experimental data and sophisticated simulation

approaches are necessary before fully predictive models of sediment metabolism can be constructed. Although actual measurements of overall rates of carbon metabolism were not available for field studies, the sum of sulfate reduction and methane production should be directly proportional to the total rate of organic matter decomposition. This is true because with the exception of site B in Wintergreen Lake oxygen was not detectable in the overlying water and nitrate and nitrite were depleted from those sediments for which values were available. When oxygen and nitrogen oxides are not available sulfate reduction and methane production should be the predominate terminal processes and the sum of their rates proportional to the total rate of fermentation.

Methane production increases in relative importance with increasing rates of sediment metabolism because of the different sources of the electron acceptors involved in sulfate reduction and methane production. Sulfate must be generated external to the zone of sediment decomposition since the primary supply of sulfate to freshwater sediments is diffusion from the overlying water (23). Thus, there is a maximum potential for sulfate reduction which is controlled by the maximum rate of diffusive flux of sulfate into the sediments. A comparison of the sulfate profiles in eutrophic sediments (19, 23, 28) with the sulfate profile in Lawrence Lake, as well as the results of the simulation model, indicate that the faster the rate of organic matter turnover, the shallower the depth at which sulfate concentrations are depleted to minimum levels. Thus, the proportion of the actively metabolizing sediment in which sulfate reduction can be a terminal process decreases with increasing total

organic matter metabolism. The electron acceptors for fermentation (organic compounds and protons), acetogenic fatty acid metabolism (protons), and methanogenesis (carbon dioxide) can be termed internal electron acceptors since they are generated through the decomposition processes within the sediment. Therefore, the maximum potential for methane production is not limited by the flux of electron acceptors from the overlying water and MB have the potential to increase their metabolism as the rate of fermentation increases at all depths of active decomposition.

The sulfate concentration in the overlying water is an important factor controlling the maximum rate of sulfate flux into the sediment and thus the relative distribution of methanogenesis and sulfate reduction. However, with the exception of the hypolimnia of Wintergreen Lake and Lake Vechten late in summer stratification, the range of sulfate concentrations in the overlying water reported for the various lakes was relatively narrow (Table 6). In contrast, the estimates of total rates of terminal metabolism varied over seven-fold. Thus the rate of organic matter turnover appears to be a more dynamic variable in the control of the relative importance of sulfate reduction and methanogenesis. The more rapid depletion of sulfate from the hypolimnia of stratified lakes with higher rates of organic matter turnover in the sediment should accentuate the increase in the relative importance of methane production in sediments with higher rates of metabolism.

The rates of organic matter decomposition in sediments is a function of the input of organic carbon into the sediments and a direct relationship between rates of organic sedimentation and methane

production in sediments has previously been demonstrated (10). Increased organic inputs to marine intertidal sediments have been shown to increase the relative importance of methanogenesis as a terminal process (20). Little is known about the sediment metabolism of lakes with low rates of organic input to the sediments. However the factors controlling the relative importance of sulfate reduction might apply to other respiratory processes that rely on electron acceptors that are generated external to the zone of anaerobic decomposition such as oxygen and nitrate. If so, then sediment metabolism in eutrophic sediments would be expected to be primarily dependent upon internal electron acceptors regardless of the availability of external electron acceptors in the overlying water. Along a gradient from eutrophic to oligotrophic lakes, a shift in sediment metabolism to processes requiring external electron acceptors would be expected. The partitioning of carbon and electron flow among the various processes would primarily be a function of the rate of organic matter decomposition in the sediments.

LITERATURE CITED

1. Billen, G., C. J. Joiris, J. Wijnant, and G. Gillain. 1980. Concentration and microbiological utilization of small organic molecules in the Scheldt Estuary, the Belgian Coastal Zone of the North Sea and the English Channel. Estuarine Coastal Mar. Sci. 11:279-294.

2. Cappenberg, T. E. 1976. Methanogenesis in the bottom deposits of a small stratifying lake. p. 125-134. In: H. G. Schlegel, N. Pfenning, and G. Gottschalk (Eds.) Microbial production and utilization of gases (H_2, CH_4, CO). Goltze-Verlag, Gottingen.

3. Chynoweth, D. P., and R. A. Mah. 1971. Volatile acid formation in sludge digestion. Adv. Chem. Ser. 105:41-54.

4. Henderson, C. 1980. The influence of extracellular hydrogen on the metabolism of Bacteroides ruminicola, Anaerovibrio lipolytica and Selenomonas ruminantium. J. Gen. Microbiol. 119:485-491.

5. Hesslein, R. G. 1976. An in situ sampler for close interval pore water studies. Limnol. Oceanogr. 21:912-914.

6. Hesslein, R. H. 1980. In situ measurements of pore water diffusion coefficients using tritiated water. Can. J. Fish. Aquat. Sci. 37:545-551.

7. Hobbie, J. E., and C. C. Crawford. 1969. Respiration corrections for bacterial uptake of dissolved organic compounds in natural waters. Limnol. Oceanogr. 14:528-532.

8. 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.

9. Kelly, C. A., and D. P. Chynoweth. 1980. Comparison of in situ and in vitro rates of methane release in freshwater sediments. Appl. Environ. Microbiol. 40:287-293.

10. Kelley, C. A., and D. P. Chynoweth. 1981. The contribution of temperature and organic input in controlling rates of sediment methanogenesis. Limnol. Oceanogr. 26:891-897.

11. Laanbroek, H. J., and N. Pfennig. 1981. Oxidation of short-chain fatty acids by sulfate-reducing bacteria in freshwater and marine sediments. Arch. Microbiol. 128:330-335.

12. 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.

13. Lovley, D. R., D. F. Dwyer, and M. J. Klug. 1982. Kinetic analysis of competition between sulfate reducers and methanogens in sediments. Appl. Environ. Microbiol. 43:1373-1379.

14. Lawrence, A. W., and P. L. McCarty. 1969. Kinetics of methane fermentation in anaerobic treatment. J. Water Pollut. Control Fed. 41:R1-R17.

15. McCarty, P. L. 1971. Energetics and kinetics of anaerobic treatment. Adv. Chem. Ser. 105:91-107.

16. McCarty, P. L. 1972. Energetics of organic matter degradation. p. 91-118. In: R. Mitchell (ed.) Water pollution microbiology. John Wiley and Sons, Inc., New York.

17. Middleton, A. C., and A. W. Lawrence. 1977. Kinetics of microbial sulfate reduction. J. Water Pollut. Control Fed. 49:1659-1670.

18. Molongoski, J. J., and M. J. Klug. 1980. Quantification and characterization of sedimenting particulate organic matter in a shallow hypereutrophic lake. Freshwater Biol. 10:497-506.

19. Molongoski, J. J., and M. J. Klug. 1980. Anaerobic metabolism of particulate organic matter in the sediments of a hypereutrophic lake. Freshwater Biol. 10:507-518.

20. Mountfort, D. O., and R. A. Asher. 1981. Role of sulfate reduction versus methanogenesis in terminal carbon flow in polluted intertidal sediments of Waimea Inlet, Nelson, New Zealand. Appl. Environ. Microbiol. 42:252-258.

21. Prins, R. A., and A. Lankhorst. 1977. Synthesis of acetate from CO_2 in the cecum of rodents. FEMS Microbiol. Lett. 1:255-258.

22. Ramm, A. E., and D. A. Bella. 1974. Sulfide production in anaerobic microcosms. Limnol. Oceanogr. 19:110-118.

23. Smith, R. L., and M. J. Klug. 1981. Reduction of sulfur compounds in the sediments of a eutrophic lake basin. Appl. Environ. Microbiol. 41:1230-1237.

24. 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.

25. Thauer, R. K., K. Jongermann, and K. Decker. 1977. Energy conservation in chemotrophic anaerobic bacteria. Bacteriol. Rev. 41:100-180.

26. Wetzel, R. G. 1975. Limnology. W. B. Saunders Co., Philadelphia.

27. Wetzel, R. G., P. H. Rich, M. C. Miller, and H. L. Allen. 1972. Metabolism of dissolved and particulate detrital carbon in a temperate hard-water lake. Mem. Ist. Ital. Idrobiol. 29(Suppl.):185-243.

28. 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.

29. Winfrey, M. R., and J. G. Zeikus. 1979. Anaerobic metabolism of immediate methane precursors in Lake Mendota. Appl. Environ. Microbiol. 37:244-253.

30. Wright, R. T., and J. E. Hobbie. 1966. Use of glucose and acetate by bacteria and algae in aquatic ecosystems. Ecology 47:447-464.

31. Zaiss, U. 1981. The sediments of the new artificial Lake Bostalsee (Saarland, Germany) with particular reference to microbial activity. Arch. Hydrobiol. 92:346-358.

CHAPTER V

Summary

The results presented in the preceding chapters can be used to construct an initial outline of the mechanisms involved in the metabolism of fermentation intermediates in freshwater sediments. In eutrophic sediments initial fermentation substrates, polymers and their constituent monomers, are fermented primarily to acetate and hydrogen. Acetate and hydrogen are consumed almost exclusively by methanogens. Propionate, the fatty acid fermentation product of secondary importance, is metabolized to acetate with the production of hydrogen. Butyrates, valerates, and lactate are produced in lesser amounts and are also eventually metabolized to hydrogen and acetate. The metabolism of C_3-C_5 volatile fatty acids is dependent upon the maintenance of low hydrogen partial pressures by the methanogens.

There is a potential for competition between methanogens and sulfate reducers for the primary fermentation products, acetate and hydrogen, in freshwater sediments. Sulfate reducers have a higher affinity for acetate and hydrogen than methanogens which permits sulfate reducers to maintain the hydrogen and acetate pools below minimum concentrations necessary for the uptake of these substrates by methanogens when sulfate is not limiting. However, sulfate reducers were sulfate limited in the freshwater sediments examined and

methanogens and sulfate reducers coexisted in the actively metabolizing surface sediments.

Sulfate reducers compete more effectively with methanogens in oligotrophic sediments than in eutrophic sediments. The higher rates of acetate and hydrogen production in eutrophic sediments results in a depletion of sulfate to minimal levels within the superficial sediments. Sulfate is not consumed as rapidly in the superficial sediments of oligotrophic lakes and sulfate is available for sulfate reduction throughout a greater portion of the zone of actively metabolizing sediment.

These results suggest that the primary steps in carbon and electron flow in anaerobic freshwater sediments are the metabolism of particulate organic matter to hydrogen and acetate and the metabolism of hydrogen and acetate by methanogens and sulfate reducers. Therefore, factors which control the rate and extent of anaerobic decomposition in sediments probably act at either the level of initial fermentation or terminal processes. Although factors controlling the rates of methanogenesis and sulfate reduction have received considerable attention, little is known about the controls on the initial fermentation of particulate organic matter in sediments. The latter subject warrants further study.