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Sedimentation and Anaerobic Metabolism of Particulate Organic Matter in the Sediments of a Hypereutrophic Lake

presented by

John J. Molongoski

has been accepted towards fulfillment of the requirements for

Ph.D. degree in Microbiology

Major professor

Date October 6, 1978

SEDIMENTATION AND ANAEROBIC METABOLISM OF PARTICULATE ORGANIC MATTER IN THE SEDIMENTS OF A HYPEREUTROPHIC LAKE

By

John J. Molongoski

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Microbiology and Public Health

1978

ABSTRACT

SEDIMENTATION AND ANAEROBIC METABOLISM OF PARTICULATE ORGANIC MATTER IN THE SEDIMENTS OF A HYPEREUTROPHIC LAKE

by

John J. Molongoski

Sedimenting particulate organic matter (POM) was collected from May through October during 1976 and 1977 in sedimentation traps anchored 0.5 meters above the pelagic sediments of hypereutrophic Wintergreen Lake. Sedimentation rates ranged from 2.7 - 19.3 $g \cdot m^{-2} \cdot day^{-1}$ in 1976, and from 2.6 - 8.5 $g \cdot m^{-2} \cdot day^{-1}$ in 1977. Because of the shallow nature of Wintergreen Lake (maximum depth 6.5 meters), the quantity and quality of sedimenting POM was closely linked to the production dynamics of the phytoplankton. Sedimenting POM measured in 1977 was 40% less than that measured in 1976, reflecting the reduced rate of primary production in the lake during the second year. Chemical analysis indicated that sedimenting POM was dominated by protein and was planktonic in origin. Short sedimentation distances coupled with the close proximity of the anaerobic hypolimnion to the photic zone insured that the majority of sedimenting POM reached the sediments in a relatively undegraded form.

Decomposition of sedimented POM occurred rapidly as shown by increased production and release of ammonia, hydrogen sulfide, volatile fatty acids (acetate and

propionate), and methane from the sediments 2-3 weeks after large inputs of organic matter. Maximum concentrations of each metabolite were found at the sediment-water interface indicating that the initial anaerobic degradation of freshly deposited POM occurred at this site.

Carbon output as methane was measured by quantifying methane lost from the sediments by ebullition and by estimating soluble methane lost to the water column by diffusion. Total methane release accounted for 34% of the particulate organic carbon input to the sediments in 1976, and for 44% of the input carbon in 1977. Methane release was directly related to the rate of sedimentation of POM. However, methane production was temporarily inhibited following high rates of sedimentation in 1976, suggesting that the rate of organic loading is an important factor controlling anaerobic decomposition in these sediments.

Laboratory studies were conducted to examine more closely some of the factors controlling anaerobic digestion in Wintergreen Lake pelagic sediments. Substrate quality was shown to be an important determinant of the rate of methanogenesis in these sediments. Alterations in the rate of methanogenesis and in volatile acid concentrations caused by addition of alternate electron acceptors (nitrate and sulfate), as well as by the addition of inhibitors of methane production, indicated that anaerobic digestion in Wintergreen Lake sediments is normally a tightly coupled process between fermentative and methanogenic stages of

metabolism. Coupling of initial and terminal stages of decomposition was also susceptible to disruption as a result of increased organic loading to the sediments. The accumulation of high concentrations of volatile fatty acids in sediments perturbed by increased partial pressures of hydrogen indicated that interspecies hydrogen transfer may also be of great importance in regulating anaerobic digestion in eutrophic lake sediments.

To Lizzie

ACKNOWLEDGEMENTS

I am deeply grateful to Dr. Michael Klug for his advice, encouragement, and understanding patience during the course of this investigation. Special thanks are also extended to Dr. James Tiedje and Dr. Robert Wetzel for their helpful advice and encouragement, and for allowing me use of their laboratory facilities.

I am greatly indebted to Richard Greening and Karen Hogg for their generous assistance and conscientious dedication to the "Wintergreen Lake Project". A special thanks is due Pamela Salvas whose willingness to dive in Wintergreen Lake during all seasons of the year greatly expedited the present investigation.

I would also like to acknowledge the assistance of my fellow graduate students, John Dacey, Richard Smith, and Wilson Cunningham, whose discussions and advice on a variety of topics greatly facilitated my work.

My appreciation is extended to Stephen Weiss who is responsible for the computer graphic presentation of much of the data in Chapters II and III, and to George Spengler and Gordon Godschalk for their photographic expertise.

A special note of appreciation is extended to Mr. Arthur Weist for his invaluable and unfailing assistance in the construction of equipment utilized in this investigation.

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CHAPTER I

INTRODUCTION

Nature of particulate organic matter. Decomposition of particulate organic matter (POM) is an important feature of the carbon cycle of productive lakes. In general, the ratio of dissolved organic carbon (DOC) to particulate organic carbon (POC) remains rather constant at about 10:1 in most unproductive to moderately productive lakes (Wetzel, 1975). As lake productivity increases, however, the ratio of DOC:POC fluctuates greatly both seasonally and spatially with depth. An excellent example is hypereutrophic Wintergreen Lake, where the annual average ratio of DOC:POC is approximately 5:1. During periods of intense algal production in the trophogenic zone and bacterial production in the metalimnion and hypolimnion, this ratio may approach 1:1 or less (Wetzel, 1975).

POM is present in lakes in the form of plant and animal biomass, and as precipitated DOM. Autochthonous primary production by planktonic and littoral flora are the major contributors of POM to most lake systems. Sources of POM shift in their relative contribution to the particulate matter pool as the lake system progresses through increasing stages of fertility (Wetzel, 1975). In general, during stratified periods, the spatial and temporal distribution of POM in the pelagic zone of lakes of varying productivity closely follows the production and biomass distribution of the phytoplankton (Wetzel, 1975; White and Wetzel, 1975; Gasith, 1976; Kimmel and Goldman, 1977).

Sedimentation and metabolism of POM. In lake ecosystems, biochemical transformations of POM are of fundamental importance to the dynamics of nutrient cycling and energy flux. Decomposition of particulate matter in lakes occurs both in the water column and in the sediments. The extent of degradation of organic matter which occurs in the water column is governed by a number of physical, chemical, and morphometric conditions in relation to the magnitude of the input (Kerr, et al., 1973; Wetzel, 1975). The rate at which POM settles out of the water column is dependent both on its mass and on the turbulence of the water. The amounts of organic input to the water mass of oligotrophic lakes are generally small. Sedimentation occurs through large volumes of aerobic water for long distances (and thus greater time). Degradation of sedimenting organic matter in the water column is relatively complete in these lakes and little organic matter reaches the sediments. During stratified periods in lakes of moderate depth, 75 to greater than 99 per cent of POM synthesized in the trophogenic zone may be decomposed in the water column before it reaches the sediments (Kuznetsov, 1975; Wetzel, 1975; Kimmel and Goldman, 1977). However, as lakes become more shallow and productive, a greater proportion of the synthesized organic matter is shifted to the sediments for benthic metabolism. Massive inputs of organic matter in eutrophic lakes result in rapid sedimentation over shorter distances, less volume of aerobic water, and accelerated deposition of organic compounds in anoxic hypolimnia and

sediments (Wetzel, 1975; Gasith, 1976; Bloesch, 1977; Kajak and Lawacz, 1977).

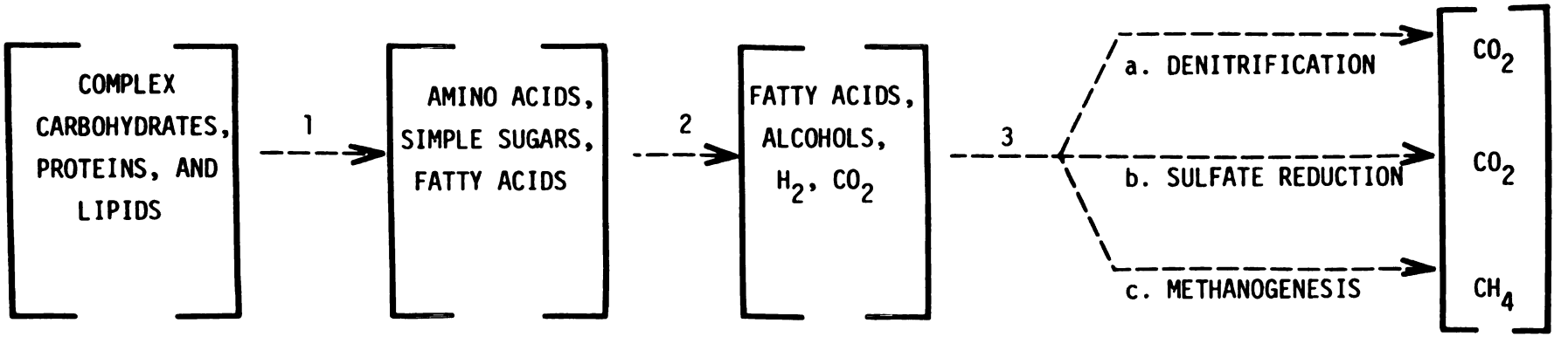
The chemical composition of the organic matter reaching pelagic lake sediments also differs over a spectrum from deep oligotrophic lakes to more shallow, eutrophic basins. As noted above, greater than 90 per cent of sedimenting POM may be metabolized in deep oligotrophic lakes (Wetzel, 1975). Only the most resistant carbon compounds will reach the sediments in such lakes due to extensive metabolism in the water column. In shallower, eutrophic lakes, non-lignified phytoplankton generally dominates the POM of the pelagic zone. Dramatic increases in particulate matter often occur in such lakes from recurrent blooms of algae. As nutrients and/or light becomes limiting, these blooms rapidly "crash" and quickly descend through the anoxic hypolimnia present in most productive lakes, reaching the sediments in a largely undegraded form. The occurrence of such "algal rains" has been documented in studies conducted on eutrophic Lake Erie, where sedimentation traps and photographic techniques demonstrated the primary input of organic matter to the sediments to be largely unmetabolized phytoplankton (Braidech, et al, 1972).

The chemical and species composition of the phytoplankton also affect the qualitative nature of the POM reaching lake sediments. This relationship is particularly true in eutrophic lakes where the phytoplankton is often dominated in the summer months by dense blooms of green and blue-green

algae (Manny, 1971; Molongoski and Klug, 1976). On an average dry weight basis, blue-green algae consist of approximately 50% protein, 30% carbohydrate, 5% lipid, and 15% ash (Fogg, 1973). Gunnison and Alexander (1975) determined that the cell wall fraction of the blue-green alga Cylindrospermum sp. contained 16.5% protein. Thus, blue-green algae are characterized by a higher protein content than any other group of algae. Although green algae contain less protein than blue-green algae, they may nevertheless also contain up to 30% protein on a dry weight basis (Fogg, et al., 1973). Compositional differences in algal species influence the rate of POM degradation as well. Particulate matter derived from the walls of blue-green algae was shown to decompose at a faster rate than that derived from green algae or diatoms; desmid algal POM was particularly resistant to decomposition (Gunnison and Alexander, 1975; Wetzel, 1975).

Nature of anaerobic metabolism in pelagic lake sediments. The generally low light intensities that reach the pelagic sediments of lakes, and the sedimentation of dead POM insure that benthic metabolism is primarily heterotrophic and detrital (Wetzel, 1975). The limited diffusion of oxygen into sediments and its rapid utilization there results in sedimentary metabolism being primarily anaerobic even when extensive hypolimnetic oxygen depletion does not occur (Hutchinson, 1941; Mortimer, 1941 and 1942). The anaerobic dissimilation of POC is a multi-stage process (Figure 1). Anaerobic digestion is characterized by Wolfe (1971) as an "anaerobic

Figure 1. Generalized scheme of the anaerobic metabolism of particulate organic matter.



1. HYDROLYSIS STAGE
2. FERMENTATIONS STAGE
3. TERMINAL ELECTRON ACCEPTING STAGE

microbial food chain". Particulate organic matter is first degraded to low molecular weight compounds (volatile fatty acids, H_2, CO_2) by fermentative bacteria, followed by further metabolism of these compounds by a second group of bacteria to final decomposition products (CO_2 and CH_4). A third phase of metabolism may also exist, consisting of "acetogenic" bacteria which convert short chain organic acids and alcohols to acetate and H_2 (Toerien, et al., 1971; Bryant, 1976). In order for complete anaerobic digestion of organic matter to occur, members of each group of organisms must be present.

Fermentative bacteria are capable of reducing complex organic compounds to lower molecular weight forms which are subsequently used by physiologically diverse groups of microorganisms. The latter bacteria mediate the terminal electron accepting stages of the digestion process. Electrons derived from the oxidation of organic compounds are transferred to the most energetically favorable inorganic acceptor. In anaerobic habitats, the most common order of acceptance is NO_3, SO_4, and CO_2 respectively (Figure 1). A succession of physiologically distinctive bacteria (nitrate-reducing, sulfate-reducing, and methanogenic bacteria, respectively) accompanies the depletion of available electron acceptors.

The percentage of carbon processed through each stage will depend on the availability of the appropriate electron acceptor. The availability of electron acceptors is, in turn, dependent on the particular sediment type examined. Littoral sediments of freshwater lakes, for instance, would be

expected to exhibit greater concentrations of nitrate and sulfate relative to deeper pelagic sediments. Increased mixing in the littoral zone will periodically replenish littoral sediments with these compounds. Fermentation and methanogenesis, however, are generally responsible for the majority of the carbon processing which occurs in the pelagic sediments of eutrophic lakes, primarily because of the low concentrations and rapid utilization of nitrate and sulfate in these sediments (Robinson, 1978; Strayer and Tiedje, 1978a). However, periodic intrusions of nitrate and sulfate to pelagic sediments caused by increased turbulence during stratified periods (Robinson, 1978), as well as re-introduction of these electron acceptors to pelagic sediments during lake turnover, make nitrate and sulfate reduction quantitatively important in these sediments at particular times of the year. Moreover, evidence has recently appeared indicating that interactions between methanogens and sulfate-reducing bacteria can occur, both in the presence or absence of sulfate (Cappenberg, 1974, 1975; Reeburgh, 1976; Bryant, et al., 1977; Winfrey and Zeikus, 1977; Abram and Nedwell, 1978a, 1978b; Oremland and Taylor, 1978).

As noted, methanogenesis likely represents the terminal stage in the anaerobic decomposition of POM in most eutrophic lake sediments (Winfrey, et al., 1977; Robinson, 1978; Strayer and Tiedje, 1978a). Investigations in other anaerobic habitats, primarily the rumen, have indicated that anaerobic digestion is a tightly coupled process which is greatly

dependent on metabolic interactions between fermentative and methanogenic bacteria (Wolin, 1974; Chung, 1976; Weimer and Zeikus, 1977). Hungate (1967) postulated that in the rumen, electrons derived from the oxidation of fermentation substrates by carbohydrate-fermenting organisms are shunted away from the reduction of fermentation intermediate products such as pyruvate, and are available for use by H_2-utilizing methanogenic bacteria. Laboratory studies involving mixed cultures of methanogens and H_2-producing, fermentative bacteria have substantiated Hungate's hypothesis by demonstrating that such interspecies hydrogen transfer affects both quantitatively and qualitatively the types of organic end products produced in the mixed cultures (Reddy, et al., 1972; Iannotti, et al., 1973; Wolin, 1974). Fermentation changes caused by interspecies hydrogen transfer result in a decrease in reduced fermentation end products and a corresponding increase in oxidized end products, an increase in hydrogen produced as methane, an increase in energy production, and possibly in substrate utilization as well (Reddy, et al., 1972).

Strayer and Tiedje (1978b) have demonstrated a high affinity of eutrophic lake sediments for hydrogen, indicating that hydrogen-utilizing reactions are capable of maintaining low partial pressures of hydrogen in these sediments. If hydrogen transfer is operative in these sediments, anaerobic decomposition of particulate organic matter in this habitat would be highly reliant on coupled metabolism as has been

demonstrated in the rumen (Wolin, 1974).

As indicated above, large amounts of undegraded POM may reach eutrophic lake sediments during the rapid decline of algal blooms (Braidech, et al., 1972). Large increases in the input of metabolizable substrate may have important effects on the nature of anaerobic digestion in these sediments. Robinson (1978) has noted the temporary inhibition of methane production in lake sediments after a large increase in sedimentation. Inhibition of methane production in sewage sludge digesters has also been observed after the addition of large quantities of fermentable substrate (Schulze, 1958; Andrews and Pearson, 1965; Hobson, et al., 1974). Such inhibition of methanogenesis may result from increased acid production, in turn leading to a decline in pH and alkalinity. Methanogenic bacteria have been shown to be extremely sensitive to alterations in these parameters (Hobson, et al., 1974). An example of an ecosystem in which excessive organic loading has permanently altered anaerobic metabolism is a bog. High acidity in the latter habitat contributes to a slowing or complete inhibition of anaerobic digestion (Wetzel, 1975).

The preceding discussion is not intended to be an exhaustive survey of the literature concerning sedimentation and anaerobic digestion in lake ecosystems, but rather is meant to provide an overview and rationale for the research described in the subsequent chapters. Experimental objectives as well as additional discussion of applicable literature are contained in the individual chapter introductions.

LITERATURE CITED

1. Abram, J.W. and E.B. Nedwell. 1978a. Inhibition of methanogenesis by sulfate reducing bacteria competing for transferred hydrogen. Arch. Microbiol. 117: 89-92.

2. Abram, J.W. and E.B. Nedwell. 1978b. Hydrogen as a substrate for methanogenesis and sulfate reduction in anaerobic saltmarsh sediment. Arch. Microbiol. 117: 439-461.

3. Andrews, J.F. and E.A. Pearson. 1965. Kinetics and characteristics of volatile acid production in anaerobic fermentation processes. Int. J. Air Wat. Poll. 9: 439-461.

4. Bloesch, J. 1977. Sedimentation rates and sediment cores in two Swiss Lakes of different trophic state. In: Interactions Between Sediments and Freshwater. H.L. Golterman, editor. Dr. W. Junk B.V. Publishers. The Hague. 65pp.

5. Braidech, T., P. Gehring, and C. Kleveno. 1972. Biological studies related to oxygen depletion and nutrient regeneration processes in the Lake Erie central basin. Project Hypo. Canada Centre Inland Waters Paper No. 6 U.S. E.P.A. Tech. Report TS-05-71-208-24. 51pp.

6. Bryant, M.P. 1976. The microbiology of anaerobic digestion and methanogenesis with special reference to sewage. In: Microbial Energy Conversion. H.L. Schlegel and J. Barnea (Ed). Goltze, Gottingen. 107pp.

7. 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.

8. Cappenberg, T.A. 1974. Interrelations between sulfate-reducing and methane-producing bacteria in bottom deposits of a freshwater lake. I. Field observations. Antonie van Leeuwenhoek 40: 285-295.

9. Cappenberg, T.A. 1975. A study of mixed continuous cultures of sulfate-reducing and methane-producing bacteria. Microbial Ecol. 2: 60-72.

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

11. Fogg, G.E., W.D.P. Stewart, P. Fay, and A.E. Walsby. 1973. The Blue-Green Algae. Academic Press. London. 78pp.

12. Gasith, A. 1976. Seston dynamics and tripton sedimentation in the pelagic zone of a shallow eutrophic lake. Hydrobiologia 51: 225-231.

13. Gunnison, D. and M. Alexander. 1975. Basis for the susceptibility of several algae to microbial decomposition. Can. J. Microbiol. 21: 619-628.

14. Hobson, P.N., S. Bousfield, and R. Summers. 1974. Anaerobic digestion of organic matter. Crit. Revs. Environ. Control 4: 131-191.

15. Hungate, R.E. 1967. Hydrogen as an intermediate in the rumen fermentation. Arch. Mikrobiol. 59: 158-164.

16. Hutchinson, G.E. 1941. Limnological studies in Connecticut. IV. The mechanism of intermediary metabolism in stratified lakes. Ecol. Monogr. 11: 21-60.

17. Iannotti, E.L., D. Kafkewitz, M.J. Wolin, and M.P. Bryant. 1973. Glucose fermentation products of Ruminococcus albus grown in continuous culture with Vibrio succinogenes: changes caused by interspecies transfer of H_2. J. Bacteriol. 114: 1231-1240.

18. Kajak, Z. and W. Lawacz. 1977. Comparison of tripton sedimentation in four small lakes. In: Interactions Between Sediments and Freshwater. H.L. Golterman, editor. Dr. W. Junk. B.V. Publishers, The Hague. 72pp.

19. Kerr, P.C., D.L. Brockway, D.F. Paris, and S.E. Craven. 1973. Carbon cycle in sediment-water systems. J. Environ. Qual. 2: 46-53.

20. Kimmel, B.L. and C.R. Goldman. 1977. Production, sedimentation, and accumulation of particulate carbon and nitrogen in a sheltered subalpine lake. In: Interactions Between Sediments and Freshwater. H.L. Golterman, editor, Dr. W. Junk B.V. Publishers, The Hague. 148pp.

21. Kuznetsov, S.I. 1975. The role of microorganisms in the formation of lake bottom deposits and their diagenesis. Soil Sci. 119: 81-88.

22. Manny, B.A. 1971. Interactions of dissolved and particulate nitrogen in lake metabolism. Ph.D. Dissertation. Michigan State University. 67pp.

23. Molongoski, J.J. and M.J. Klug. 1976. Characterization of anaerobic heterotrophic bacteria isolated from freshwater lake sediments. Appl. Environ. Microbiol. 31: 83-90.

24. Mortimer, C.E. 1941. The exchange of dissolved substances between mud and water in lakes. I and II. J. Ecol. 29: 380-329.

25. Mortimer, C.E. 1942. The exchange of dissolved substances between mud and water in lakes. III and IV. J. Ecol. 30: 147-201.

26. Oremland, R.S. and B.F. Taylor. 1978. Sulfate reduction and methanogenesis in marine sediments. Geochimica et Cosmochimica Acta. 42: 209-214.

27. Reddy, C.A., M.P. Bryant, and M.J. Wolin. 1972. Characteristics of S organism isolated from Methanobacillus omelianskii. J. Bacteriol. 109: 539-545.

28. Reeburgh, W.S. 1976. Methane consumption in Cariaco Trench waters and sediments. Earth Planetary Sci. Letters 28: 337-344.

29. Robinson, C.K. 1978. Quantitative comparison of the significance of methane in the carbon cycle of two small lakes. Arch. Hydrobiol. (In press).

30. Schulze, K.L. 1958. Studies on sludge digestion and methane fermentation. I. Sludge digestion at increased solids concentrations. Sewage and Industrial Wastes. 30: 28-45.

31. Strayer, R.F. and J.M. Tiedje. 1978a. In situ methane production in a small, hypereutrophic, hard-water lake: loss of methane from sediments by diffusion and ebullition. Limnol. Oceanogr. (In press).

32. Strayer, R.F. and J.M. Tiedje. 1978b. Kinetic parameters of the conversion of methane precursors to methane in a hypereutrophic lake sediment. Appl. Environ. Microbiol. 36: 330-340.

33. Toerien, D.F., P.G. Thiel, and W.A. Pretorius. 1971. Substrate flow in anaerobic digestion. Water Research II-29. 1-7.

34. 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.

35. Wetzel, R.G. 1975. Limnology. W.B. Saunders, Co. Philadelphia. 743pp.

36. White, W.S. and R.G. Wetzel. 1975. Nitrogen, phosphorus, particulate and colloidal carbon content of sedimenting seston of a hard-water lake. Verh. Internat. Verein Limnol. 19: 330-339.

37. 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.

38. 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.

39. Wolfe, R.S. 1971. Microbial formation of methane. Adv. Microbiol. Physiol. 6: 107-145.

40. Wolin, M.J. 1974. Metabolic interactions among intestinal microorganisms. Am. J. Clin. Nutrition. 27: 1320-1328.

CHAPTER II

QUANTIFICATION AND CHARACTERIZATION OF SEDIMENTING PARTICULATE ORGANIC MATTER IN A SHALLOW HYPEREUTROPHIC LAKE

INTRODUCTION

Increasing lake eutrophication results in greater sedimentation of particulate organic matter (POM) from the water column to pelagic sediments. Since the rate and extent of benthic metabolism is dependent on the availability of metabolizable organic substrates, it is important to know the nature and amount of POM reaching lake sediments. Numerous studies have determined the input of POM to pelagic lake sediments (Pennington, 1974; White and Wetzel, 1975; Jones, 1976; Lastein, 1976; Kimmel and Goldman, 1977). Most of these studies, however, have been made on relatively deep and/or unproductive lakes. In these lakes large volumes of aerobic water and increased sedimentation distances allow considerable decomposition of POM to occur in the water column. Few studies have examined sedimentation rates in more productive shallow lakes where a greater percentage of sedimenting POM would be expected to reach the sediments in an undegraded form. Gasith (1976) reported that 55% of the annual phytoplankton production was lost by sedimentation in shallow Lake Wingra, Wisconsin. The qualitative nature of the sedimenting POM was not determined. However, the chemical composition of the POM reaching the sediments of shallow eutrophic lakes would be expected to differ from that reported in sedimentation studies conducted on deeper

lakes (Lastein, 1976). Brehm (1967) reported that during autolysis of blue-green and green algae, individual amino acids are released or decomposed at specific rates; aspartic and glutamic acids were liberated rapidly whereas leucine, valine, alanine, and proline are lost at comparably slower rates, leading to a relative enrichment of these amino acids in sedimenting dead plankton. Thus, the plankton reaching the sediments of a shallow lake where sedimentation time is greatly reduced might have a much different chemical composition than that reaching the sediments of a much deeper lake where sedimentation distance and time is greatly increased.

The present study determined the quantity and quality of POM reaching the sediments of a shallow eutrophic lake, and provided data to be utilized in an investigation of the rate and extent of anaerobic processing in pelagic lake sediments.

MATERIALS AND METHODS

Investigations were conducted on Wintergreen Lake, a small hardwater basin located within the W.K. Kellogg Bird Sanctuary, Augusta, Michigan. The lake is shallow with a maximum depth of 6.5 m and a mean depth of 3.0 m. Annual mean primary productivity values identify the lake as hypereutrophic, the latter designation based on compression of the trophogenic zone to a point where light rather than available nutrients often limit productivity (Wetzel, 1975). The pelagic zone of Wintergreen Lake is characterized by significant autochthonous organic input, the annual succession

of phytoplankton being punctuated in the summer by dense blooms of blue-green algae. The hypolimnion of the lake is anaerobic for nearly 7 months of the year, with anoxic conditions extending upwards to 3 m during summer stratification. Samples were taken in 1976 and 1977 at 3 stations located within the 6-meter contour of the pelagic zone (Figure 1).

Sedimenting matter (seston) was collected weekly in 1976 and 1977 from the onset of thermal stratification until autumn overturn. Sedimentation traps were anchored 0.5 meters above the sediment surface at each sampling station. The traps were suspended from a subsurface buoy and anchored in the sediment for stability. The traps, modifications of those described by White and Wetzel (1975) consisted of 6 plexiglass tubes 7.5 cm in diameter and 40 cm in length. The seston tubes were arranged in a circular array and were threaded into a PVC base for ease of removal of each tube. Three replicate lower tubes were utilized to permit correction for living and attached material among the sedimenting matter. As a result of the anoxic water in the hypolimnion during both 1976 and 1977, attachment of algae to the traps was insignificant. Attachment of photosynthetic bacteria was also not observed. Each trap was raised to the surface for sampling. Resuspension of the sedimented material in the tubes did not occur as verified by SCUBA. The sedimentation tubes were returned to the laboratory and the contents of each tube, disturbed during transport, were allowed to

Figure 1. Bathymetric map of Wintergreen Lake showing pelagic sampling stations 1-3 used in the study. The three stations triangulated an area of approximately 2000 m^2 (map taken from Manny, 1974).

WINTERGREEN LAKE

KALAMAZOO COUNTY, MICHIGAN

R.9W., T.1N. Sec.8

ELEVATION 271m, AREA 15.8 ha

0 50 100 150
METERS

CONTOUR INTERVALS IN METERS

resettle in the dark until a discrete layer formed at the bottom. The overlying water, containing suspended photosynthetic bacteria, was removed by aspiration. The seston in each tube was removed by rinsing with double distilled water and dried at 50°C. After weighing, the seston was ground to a fine powder with a mortar and pestle and utilized in subsequent chemical analyses.

Triplicate subsamples of seston were analyzed for total carbon and nitrogen by combustion in a Carlo Erba model 1104 elemental analyzer. The organic content of the seston was determined by weight loss after combustion of triplicate subsamples for 18 hours at 550°C, followed by combustion at 950°C to determine inorganic carbon content. Protein determination was by alkaline hydrolysis (Hirs, 1967) of the seston, and subsequent color development with hydrindantin-ninhydrin (Moore and Stein, 1954). Carbohydrate analysis was by the modified phenol-sulfuric acid procedure (Gerchakov and Hatcher, 1972: Liu, Wong, and Dutka, 1973). Organic content, inorganic carbon, carbohydrate, and protein were determined weekly in 1976 and biweekly in 1977.

Water column samples were collected at weekly invervals at station 3 (Figure 1) with a Van Dorn bottle (3 liter, PVC). Dissolved oxygen was determined using the Winkler technique (Standard Methods, 1970). Samples for particulate organic carbon (POC) and nitrogen (PON) analysis were obtained by filtration of water samples through 400-micron nitex netting to remove large zooplankton, and then through 13-mm

precombusted (525°C) glass fiber filters (Reeve-Angel 934AH filters were used in 1976 and Whatman GF/F filters in 1977). After filtration, triplicate filters were dried and analyzed for POC and PON in a Carlo Erba model 1104 elemental analyzer.

RESULTS

The total dry weight of seston and of the organic matter fraction of the seston collected at each sampling period during 1976 and 1977 are shown in Figure 2. During 1976, the sedimentation rate of total seston ranged from 2.7 to 19.3 $g \cdot m^{-2} \cdot day^{-1}$ while organic seston ranged from 1.2 to 8.3 $g \cdot m^{-2} \cdot day^{-1}$. The sedimentation rate of total and organic seston during 1977 ranged from 2.6 to 9.5 and 1.5 to 3.5 $g \cdot m^{-2} \cdot day^{-1}$, respectively. The sedimentation rate was lowest in the spring in both years. In 1976, the sedimentation rate increased throughout the summer, reaching a maximum value on November 2, three weeks after fall turnover. High sedimentation rates were also measured on June 8, August 10, and September 28.

The sedimentation rate increased sharply in June and July in 1977, but unlike 1976, declined to near spring levels in August and September. Sedimentation then peaked again during fall circulation in October of 1977 (Figure 2).

The total carbon and nitrogen content of the sedimenting material showed similar seasonal patterns as did total seston in both years sampled (Figure 3). Maxima in all three parameters usually occurred on the same dates. The carbon/nitrogen ratio of the seston ranged from approximately 5.5

Figure 2. Dry weight of total and of organic fraction of seston collected in 1976 and 1977. Each point is the mean value ± SD of total and organic seston collected at three sites.

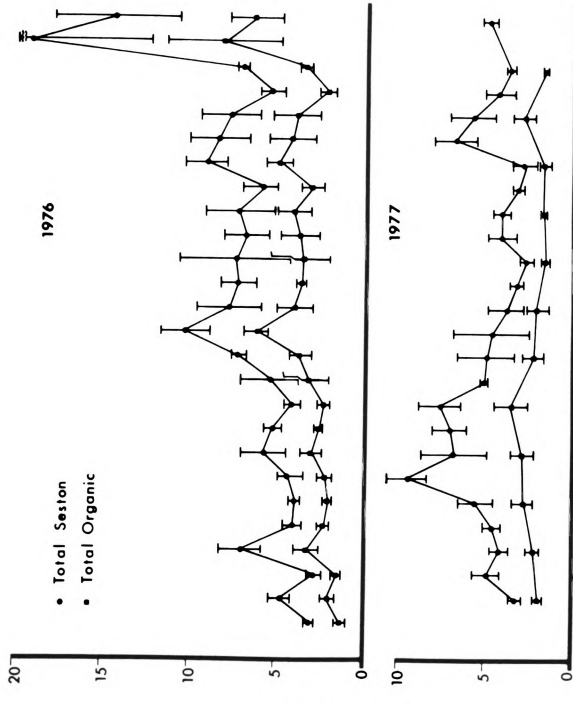
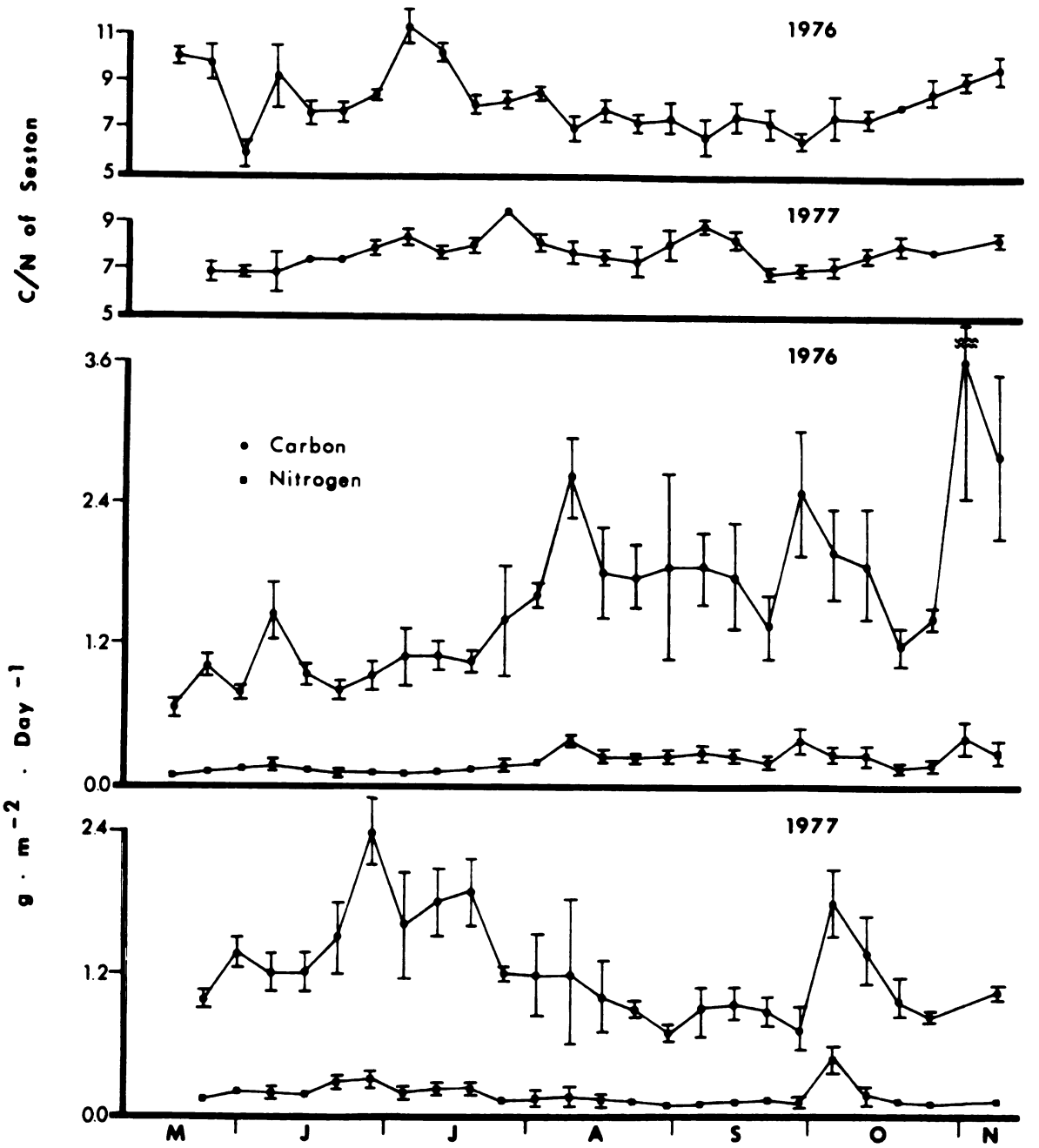

1976
1977
• Total Seston
▪ Total Organic
g · m⁻² · Day⁻¹
20
15
10
5
0
M
J
J
A
S
O
N

Figure 3. Total carbon and nitrogen content, and C/N ratio of seston collected in 1976 and 1977. Each point is the mean value ± SD of three sampling sites.


1976
1977
C/N of Seston
11
9
7
5
3.6
2.4
1.2
0.0
• Carbon
• Nitrogen
g · m⁻² · Day⁻¹
M
J
J
A
S
O
N

to 11 during 1976, and from 6.8 to 9 during 1977 (Figure 3). In 1976, the C/N ratio was highest in the spring and early summer, declined from July through September, and increased again at fall turnover. The C/N ratio of the seston was more constant in 1977, but generally followed the same pattern as in 1976.

The protein and carbohydrate content of the seston also closely followed changes in total seston in both years sampled (Figure 4). Protein and carbohydrate content of the organic fraction of the seston ranged from 16-38% and 5-15% respectively in both 1976 and 1977. The carbohydrate content of the seston relative to the protein content was greater in 1977 than in 1976, as evidenced by the higher carbohydrate/ protein ratio of the seston found in 1977 (Figure 4).

The depth-time distribution of particulate organic carbon (POC) and particulate organic nitrogen (PON) present in the water column varied greatly in the two years examined (Figure 5). Epilimnetic POC and PON values were much greater and showed less dramatic fluctuations in 1977 as compared to 1976. During both years, epilimnetic POC and PON values were lowest in the spring, increased during summer, and reached maximum values in early November after fall turnover had occurred. The C/N ratio of the POM was low, ranging from 4-6 during both years sampled.

Dissolved oxygen was rapidly exhausted from the hypolimnion shortly after spring turnover in both 1976 and 1977 (Figure 6). During mid-summer stratification, anoxic

Figure 4. Carbohydrate and protein content, and carbohydrate/protein ratio of seston collected in 1976 and 1977. Each point is the mean value ± SD of three sampling sites.

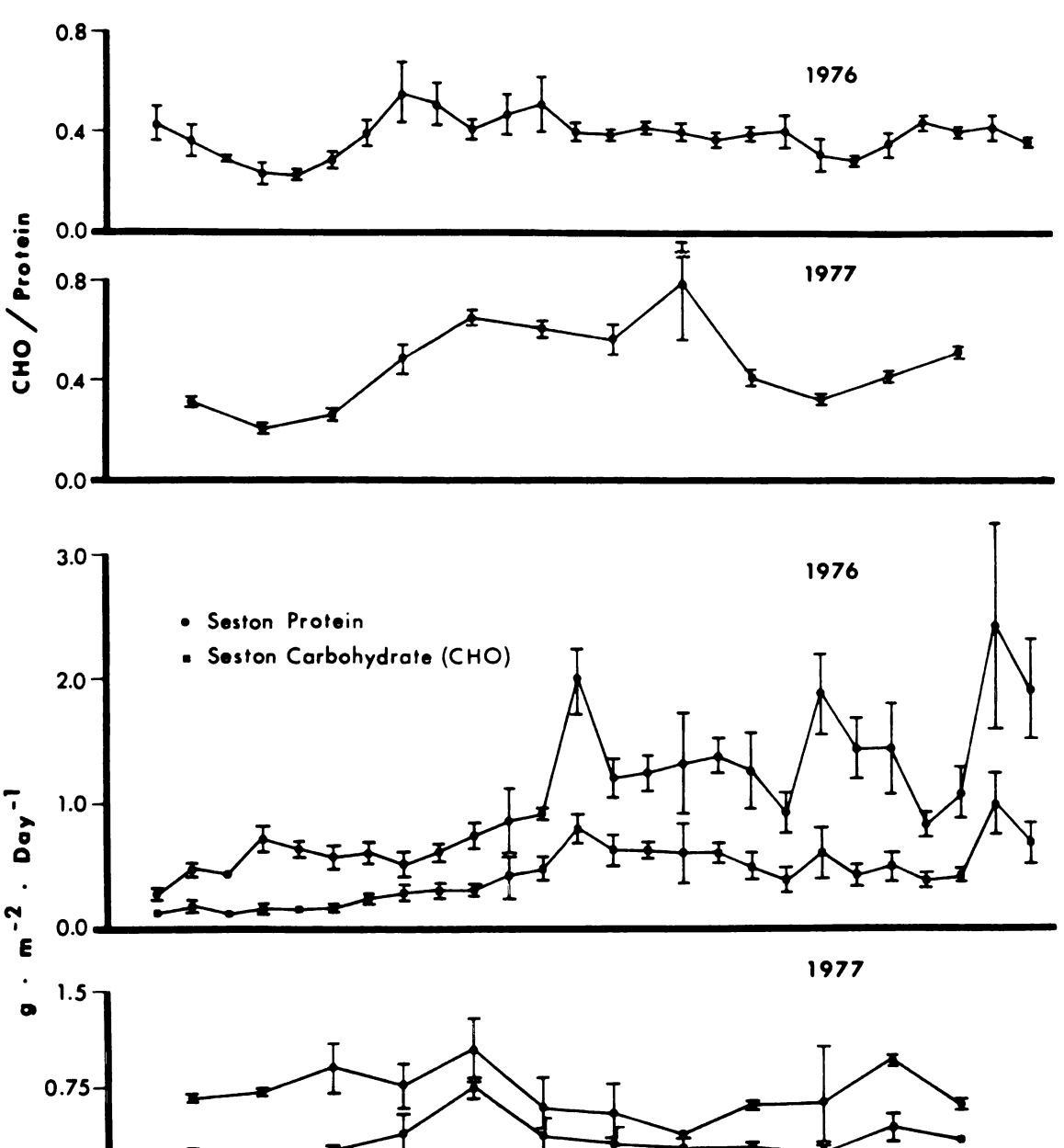

0.8
0.4
0.0
1976
CHO / Protein
0.8
0.4
0.0
1977
3.0
2.0
1.0
0.0
1976
• Seston Protein
▪ Seston Carbohydrate (CHO)
g · m-2 · Day-1
1.5
0.75
0.0
1977
M
J
J
A
S
O
N

Figure 5. Depth-time distribution of POC and PON in the water column of Wintergreen Lake at sampling station 3 during 1976 and 1977. The hypolimnion has been placed distal to the viewer so that the distribution of POC and PON throughout the water column can be more clearly visualized.

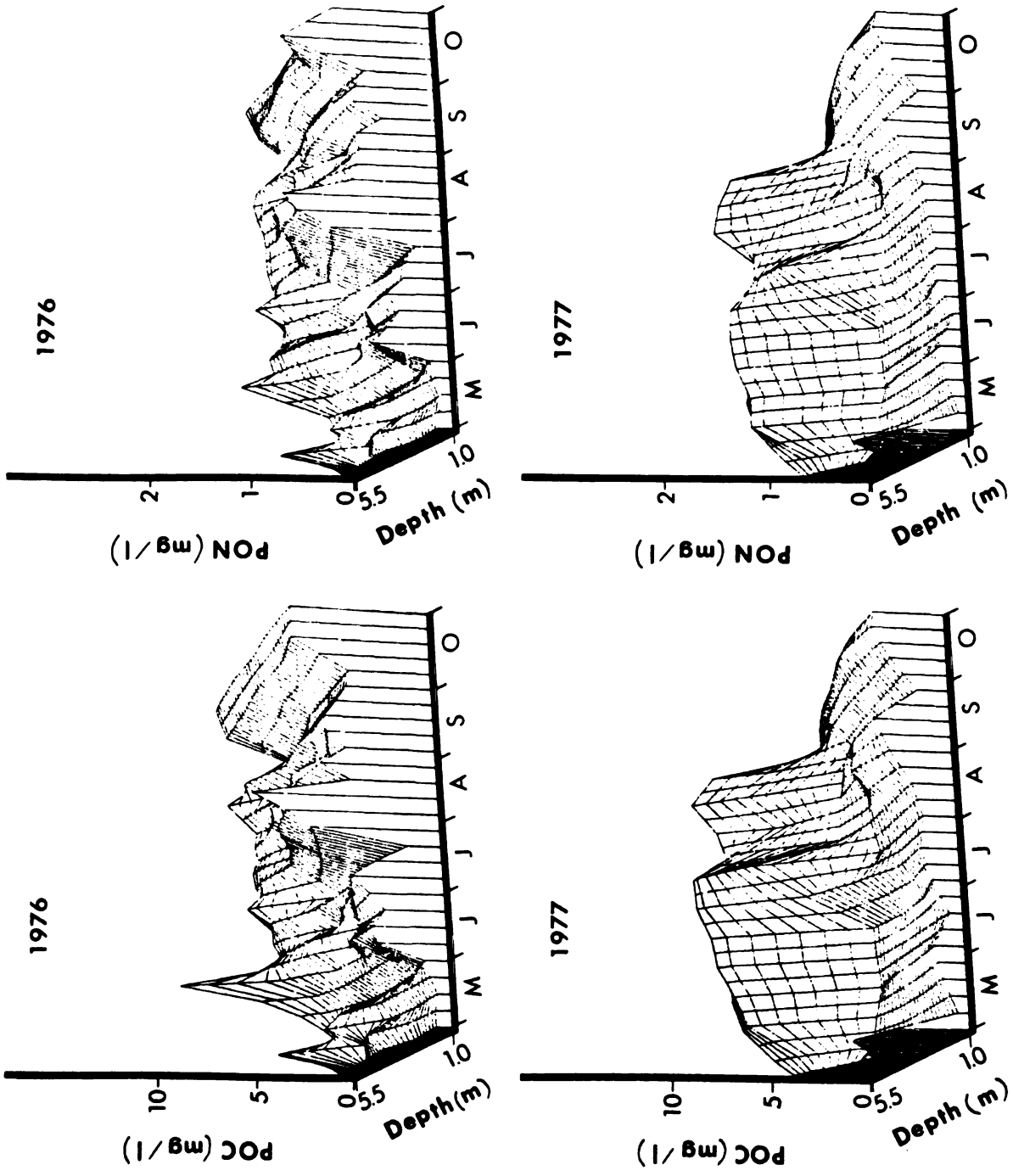
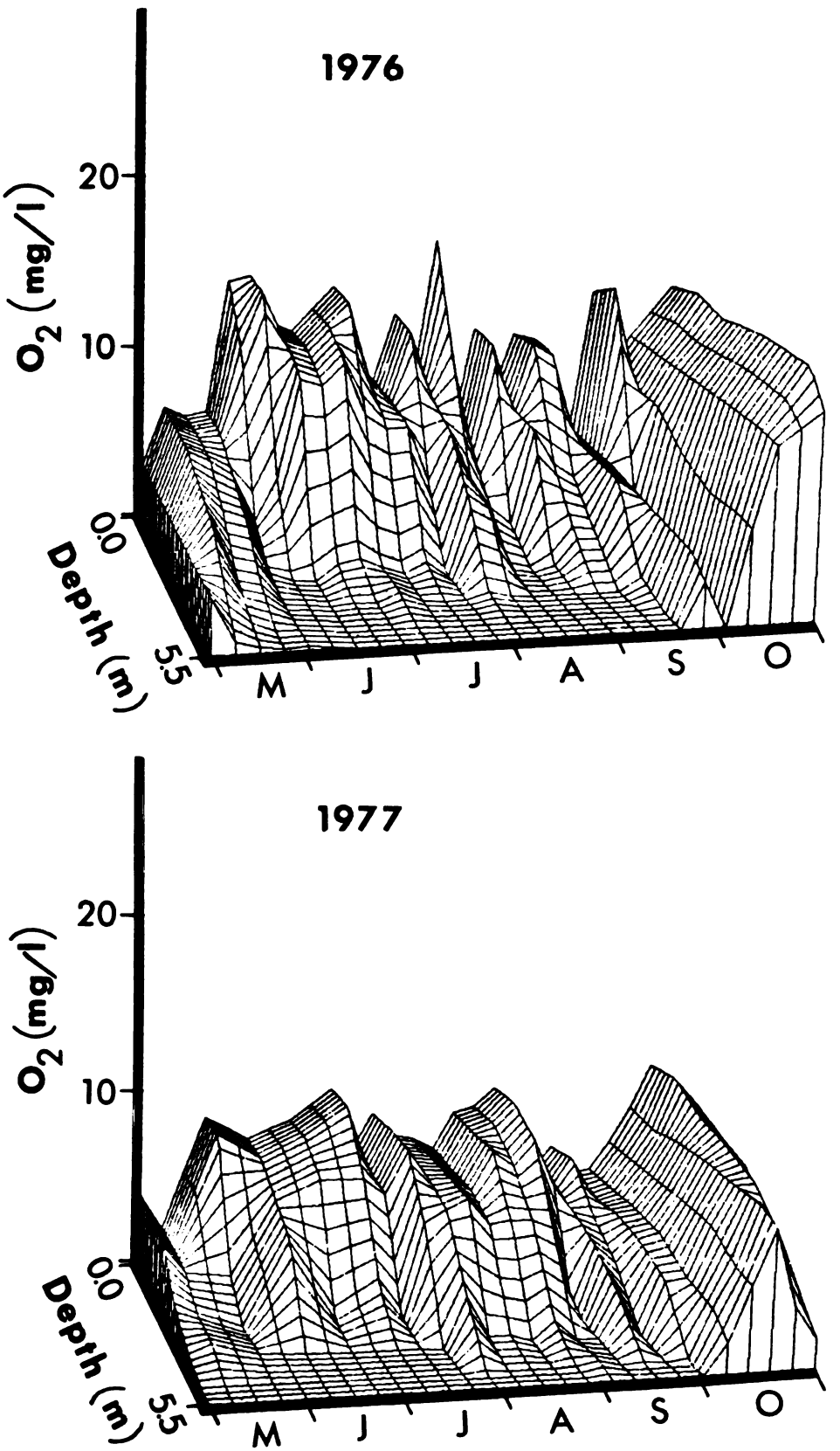

1976
1977
PON (mg/l)
POC (mg/l)
Depth (m)
0
5.5
1.0
M
J
J
A
S
O
1
2
5
10

Figure 6. Depth-time distribution of dissolved oxygen in Wintergreen Lake at sampling site 3 during 1976 and 1977.


1976
20
10
0.0
O_2 (mg/l)
Depth (m)
5.5
M
J
J
A
S
O
1977
20
10
0.0
O_2 (mg/l)
Depth (m)
5.5
M
J
J
A
S
O

conditions extended upward to nearly 3 meters during both years. Epilimnetic oxygen concentrations fluctuated widely during the summer of 1976, but were lower and more uniform during the same period in 1977.

DISCUSSION

The importance of sedimentation as a mechanism for the removal of particulate organic matter from the pelagic zone of Wintergreen Lake is summarized in Table 1. The sedimentation rate of total seston ranged from 2.7 - 19.3 $g \cdot m^{-2} \cdot day^{-1}$ in 1976 and from 2.6 - 8.5 $g \cdot m^{-2} \cdot day^{-1}$ in 1977. These rates are comparable to those reported for other eutrophic lakes (Gasith, 1976, and references therein). There was, however, a striking difference in the sedimentation rate measured in Wintergreen Lake in 1977 relative to 1976. Measured values of total seston, organic seston, and total carbon and nitrogen reaching the sediments in 1977 were only 60% of those measured in 1976 (Table 1). The much greater range of sedimentation and the greater mean sedimentation rate (7.0 $g \cdot m^{-2} \cdot day^{-1}$ vs. 4.9 $g \cdot m^{-2} \cdot day^{-1}$) observed in 1976 compared to 1977 are due primarily to the higher rates of sedimentation measured in August, October, and early November during 1976 (Figure 2). In 1977, major peaks in sedimentation rate were measured in late June and early July, and again in early October. In general, sedimentation rates were more constant in 1977 than in 1976 (Figure 2). Significantly, sedimentation rates declined in August of 1977 in contrast to the high sedimentation rates measured in August

Table 1. Particulate matter sedimentation in the pelagic zone of Wintergreen Lake from May through October, 1976 and 1977.

Year	Total Seston ($g \cdot m^{-2}$)	Total Organic Seston ($g \cdot m^{-2}$)	Total Carbon ($g \cdot m^{-2}$)	Total Nitrogen ($g \cdot m^{-2}$)
1976	1282	640	320	37
1977	785	394	196	25
% of 1976 Value	61	62	61	66

of 1976.

Although primary productivity measurements were not made during the study, it was evident that the reduction in total seston observed in 1977 relative to 1976 was the result of reduced primary productivity in the trophogenic zone during 1977. Epilimnetic POC and PON (Figure 5) concentrations were greatly reduced in 1977 compared to 1976 indicating a reduction in planktonic biomass during the second year. Similarly, epilimnetic dissolved oxygen concentrations (Figure 6) were greatly reduced and much more constant in 1977 relative to 1976, also indicating a reduced rate of photosynthesis during 1977. This reduction in algal biomass was further reflected in the increased transparency (Secchi depth) measurements ($\bar{x}$ value 2.1 meters vs. 1.5 meters) obtained during the stratified period in 1977 relative to 1976.

An interesting consequence of the greater light penetration observed in Wintergreen Lake in 1977 was the large increase in hypolimnetic POC and PON values measured in 1977 compared to 1976 (Figure 5). These increases resulted from the increased proliferation of photosynthetic bacterial populations in the hypolimnion in 1977 in response to the greater availablity of light.

The per cent organic fraction of the seston collected in Wintergreen Lake remained relatively constant throughout the sampling period during both 1976 and 1977, except for small decreases in organic content of the seston which

occurred in early November after fall turnover (Figure 2). This uniformity in organic content implies that resuspension of bottom deposits into the sediment traps was not of consequence during periods of thermal stratification in Wintergreen Lake. Similarly, resuspension of bottom sediments was found to be minimal during stratified periods in both eutrophic Frains Lake, Michigan (Davis, 1968; Robinson, 1978), and Lake Esrom, Denmark (Lastein, 1976). Wetzel, et al. (1972) and White and Wetzel (1975) found that the $CaCO_3$ content of seston in Lawrence Lake consistently exhibited vernal and autumnal maxima, corresponding to periods of circulation, and reflecting resuspension of $CaCO_3$ from bottom sediments. In contrast, the $CaCO_3$ content of seston collected during stratified periods was low, suggesting that resuspension from bottom sediments was not significant during stratified periods in Lawrence Lake (White and Wetzel, 1975).

The relatively low variance between the quantities of seston collected at each of the sampling sites in Wintergreen Lake (Figure 2) suggests that the particulate input to the sediments was uniform across the pelagic zone. The relatively low variance found in the qualitative nature of the seston collected at each of the 3 sampling sites (Figures 3 and 4) also support the uniformity of the input throughout the pelagic zone. The chemical composition of the material collected in the sedimentation traps reflects the planktonic origin of the seston. The high protein content of the

seston (Figure 4) correlates closely with the presence of large populations of blue-green algae, particularly Anabaena and Microcystis, in the water column (Manny, 1971; Molongoski and Klug, 1976). This relationship is further substantiated by the low C/N ratio (4-6) and high protein content (50-60%) of the water column POM during the study, and by the gradually decreasing C/N ratio of the seston observed during the summer of both 1976 and 1977 (Figure 3). The carbohydrate/protein ratio of the seston was higher and showed greater fluctuation in 1977 compared to 1976, reflecting the observed reduced abundance of algal biomass in the lake during the second year.

Analysis of the seston carbohydrate in 1976 revealed that it consisted primarily of glucose (19.2%), other soluble monomers (12.4%), and soluble polysaccharides (43.4%). Cellulosic polysaccharides represented only 7.2% of the total carbohydrates (P.L. Salvas, unpublished results). The high protein content of Wintergreen Lake seston relative to carbohydrate content is in contrast to results reported by other workers. Lastein (1976) reported approximately equal percentages of carbohydrate and protein in seston from Lake Esrom, while Matsuyama (1973) reported that proteinaceous materials were rapidly eliminated in the early stage of settling in Lake Suigetsu. Similarly, Brehm (1967), in a study of German lakes, found that primary degradation of plankton occurred in the epilimnion of the lakes investigated.

In contrast to the deeper lakes studied by Brehm (1967), Matsuyama (1973), and Lastein (1976), Wintergreen Lake is quite shallow. In the latter, the relatively short distance which POM must fall to reach the sediments, and the relatively low turbulence during stratification (Figure 6) insure a rapid rate of sedimentation. This rapid rate of sedimentation was reflected in the POC and PON levels found in the water column. Concentrations of both POC and PON were high throughout the study (Figure 5), and were localized in the epilimnion and hypolimnion. The lowest values in both POC and PON were consistently found in the metalimnion between 3 and 4 meters (Figure 5), at the interface of the trophogenic and tropholytic zones. High concentrations of POM in the hypolimnion were attributable to the rapid sedimentation of material from the epilimnion, as well as to large blooms of photosynthetic bacteria which occurred in the hypolimnion during the summer (Caldwell and Tiedje, 1975).

As a result of rapid sedimentation through the anoxic hypolimnion (Figure 6), the majority of the sedimenting organic matter in Wintergreen Lake reaches the sediments in a largely undegraded form during stratified periods. Microscopic examination of the seston generally revealed the presence of relatively intact and identifiable algal cells. Braidech, et al. (1972), utilizing sedimentation traps and underwater photography, also demonstrated that the primary input of organic matter to Lake Erie sediments consisted of largely undegraded planktonic algae. Similarly, surface

sediments taken from Wintergreen Lake during the summer frequently included a lawn of relatively intact sedimented algal cells.

The rapid sedimentation of algal biomass and the lack of appreciable decomposition of this biomass in the anoxic hypolimnia has been noted by additional investigators. Kimmel and Goldman (1977) found that in Castle Lake, California, mineralization of sedimenting material occurred rapidly in aerobic regions of the hypolimnion, but that decomposition was reduced substantially once anaerobic waters were reached. Fallon (1978) reported that approximately 5% of the algal production in Lake Mendota sedimented out daily. Rudd and Hamilton (1977) also monitored the sedimentation of ^{14}C-labeled POM from the epilimnion to the sediments of experimental Lake 227. The spike of ^{14}C activity descended approximately 8 meters to the sediments in 35 days. The ^{14}C label remained in an approximate ratio of 80:20 (particulate/$^{14}CO_2$) as it sedimented through the water column. There was no detectable ^{14}C-methane production as the labeled material descended through the anaerobic hypolimnion. ^{14}C-methane was detected, however, once the material reached the sediment surface (Rudd and Hamilton, 1977).

The data presented in this study emphasize the importance of sedimentation as a mechanism for the transfer of POM from the water column to the sediments in shallow eutrophic lakes. The relatively short distance which POM must fall to reach the sediments in Wintergreen Lake, coupled with

the close proximity of the anaerobic hypolimnion to the photic zone, greatly increase the quantity of organic matter reaching the sediments. The rapid sedimentation of POM in Wintergreen Lake also greatly influences the qualitative nature of the sedimenting material. Unlike deeper lakes where labile proteinaceous components are largely degraded in the water column (Brehm, 1967; Matsuyama, 1973; Lastein, 1976), appreciable amounts of undegraded protein reach the sediments of Wintergreen Lake.

Because of the shallow and highly productive nature of Wintergreen Lake, the amount of organic matter reaching the sediments is very closely linked to the production dynamics of the phytoplankton. This relationship is evidenced by the 40% decrease in sedimentation measured in the lake in 1977 compared to 1976, resulting from reduced primary production during the second year. Gasith (1976) similarly found sedimentation rates in shallow Lake Wingra to be closely coupled to the dynamics of the planktonic community. Fallon (1978) has made similar observations in Lake Mendota. The quantitative and qualitative nature of the organic matter reaching Wintergreen Lake pelagic sediments has been shown to have important consequences on the nature and extent of anaerobic carbon processing which occurs in these sediments (Chapter III).

LITERATURE CITED

1. Braidech, T., P. Gehring, and C. Kleveno. 1972. Biological studies related to oxygen depletion and nutrient regeneration processes in the Lake Erie central basin. Project Hypo. Canada Centre Inland Waters Paper No. 6 U.S. E.P.A. Technical Report TS-05-71-208-24.

2. Brehm, J. 1967. Untersuchungen uber den Aminosaure - Haushalt holsteinischet Gewasser, insbesondere des Pluss - Sees. Arch. Hydrobiol. Suppl. 32; 3: 313-435.

3. Caldwell, D.E. and J.M. Tiedje. 1975. The structure of anaerobic bacterial communities in the hypolimnia of several Michigan lakes. Can. J. Microbiol. 21: 377-385.

4. Davis, M.B. 1968. Pollen grains in lake sediments: redeposition caused by seasonal water circulation. Science 162: 796-799.

5. Fallon, R.D. and T.D. Brock. 1978. Sedimentation of blue-green algae in Lake Mendota, Wisconsin. Abstracts 41st Ann. Meeting of Amer. Soc. Limnol. Oceanogr.

6. Gasith, A. 1976. Seston dynamics and tripton sedimentation in the pelagic zone of a shallow eutrophic lake. Hydrobiologia 51: 225-231.

7. Gerchakov, S.M. and P.G. Hatcher. 1972. Improved technique for analysis of carbohydrates in sediments. Limnol. Oceanogr. 14: 938-943.

8. Hargrave, B.T. and N.J. Prouse. 1978. Assessment of sediment trap collection efficiency. Abstracts 41st Ann. Meeting Amer. Soc. Limnol. Oceanogr.

9. Hirs, C.H.W. 1967. Detection of peptides by chemical methods. In: Methods of Enzymology. Vol. II. Enzyme Structure. New York, Academic Press. 325pp.

10. Jones, J.G. 1976. The microbiology and decomposition of seston in open water and experimental enclosures in a productive lake. J. Ecol. 64: 241-278.

11. Kimmel, B.L. and C.R. Goldman. 1977. Production, sedimentation, and accumulation of particulate carbon and nitrogen in a sheltered subalpine lake. In: Interactions Between Sediments and Freshwater. H.L. Golterman, Editor. Dr. W. Junk. B.V. Publishers, The Hague. 148pp.

12. Lastein, E. 1976. Recent sedimentation and resuspension of organic matter in eutrophic Lake Esrom, Denmark. Oikos 27: 44-49.

13. Liu, D., P.T.S. Wong and B.J. Dutka. 1973. Determination of carbohydrate in lake sediment by a modified phenol-sulfuric acid method. Wat. Res. 7: 741-746.

14. Manny, B.A. 1971. Interactions of dissolved and particulate nitrogen in lake metabolism. Ph.D. Dissertation. Michigan State University 67pp.

15. Matsuyama, M. 1973. Organic substances in sediment and settling matter during spring in meromictic Lake Suigetsu. J. Oceanogr. Soc. Japan. 29: 53-60.

16. Molongoski, J.J. and M.J. Klug. 1976. Characterization of anaerobic heterotrophic bacteria isolated from freshwater lake sediments. Appl. Environ. Microbiol. 31: 93-90.

17. Moore, S. and W.H. Stein. 1954. A modified ninhydrin reagent for the photometric determination of amino acids and related compounds. J. Biol. Chem. 211: 907-913.

18. Pennington, W. 1974. Seston and sediment formation in five lake district lakes. J. Ecol. 62: 215-251.

19. Robinson, C.K. 1978. Quantitative comparison of the significance of methane in the carbon cycle of two small lakes. Arch. Hydrobiol. (In press).

20. Rudd, J.W.M. and R.D. Hamilton. 1977. Methane cycling in a eutrophic shield lake and its effects on carbon cycling and whole lake metabolism. Limnol. Oceanogr. (In press).

21. Standard Methods for the Examination of Water and Wastewater. 1970. American Public Health Assoc. 13th Edition. Washington, D.C. 270pp.

22. Wetzel, R.G. 1975. Limnology. W.B. Saunders, Co. Philadelphia 743pp.

23. Wetzel, R.B., 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.

24. White, W.S. and R.G. Wetzel. 1975. Nitrogen, phosphorus, particulate and colloidal carbon content of sedimenting seston of a hard-water lake. Verh. Internat. Verein. Limnol. 19: 330-339.

CHAPTER III

ANAEROBIC METABOLISM OF PARTICULATE ORGANIC MATTER IN THE SEDIMENTS OF A HYPEREUTROPHIC LAKE

INTRODUCTION

In shallow, productive lakes, benthic decomposition and nutrient regeneration become of vital importance to the functioning of the entire lake ecosystem. Little is known, however, of the rate and extent of decomposition of sedimenting organic matter in these lakes. Based on the difference between the organic fraction of sedimenting particulate organic matter (POM) and that of surface sediments, Gasith (1976) estimated that 70% of the POM reaching the sediments of shallow Lake Wingra, Wisconsin is decomposed annually. Kimmel and Goldman (1977) estimated the extent of decomposition of organic matter at the sediment surface of Castle Lake, California by comparing the average carbon and nitrogen content of the uppermost 3 cm of sediment with that of sedimented material collected from the hypolimnion of the lake. They determined that 49% of the carbon and 68% of the nitrogen reaching the sediment surface is mineralized between the time of actual deposition and permanent burial.

While useful for gaining an estimate of the extent of degradation, such methods provide no information concerning the mechanisms of decomposition. Additional studies (Hargrave, 1972; Hartwig, 1976; Jones, 1976) utilized oxygen respirometric techniques to estimate the extent of carbon

mineralization in sediments. Values obtained by these methods must be considered underestimates of carbon turnover, however, because they do not include the amount of material which is decomposed anaerobically. The poor diffusion of oxygen into sediments and its rapid utilization results in the majority of benthic decomposition occurring anaerobically, even when hypolimnetic oxygen depletion does not occur (Wetzel, 1975). Rich (1975) reported that, during stratification, *in situ* RQ values (CO_2/O_2) in Durham Pond, Connecticut varied inversely with the availability of oxygen, ranging from less than 1 after spring circulation and oxygen renewal to nearly 3 under anaerobic conditions in the summer. Moreover, the production and release of methane from lake sediments of various trophic levels attest to the occurrence of anaerobic digestion in these sediments (Howard, *et al.*, 1971; Strayer, 1973; Wetzel, 1975; Robinson, 1978).

Anaerobic digestion has generally been thought to occur in two distinct stages (Toerien and Hattingh, 1969). In an initial heterotrophic stage, a heterogeneous group of facultative and obligately anaerobic bacteria convert carbohydrates, proteins, and lipids to an array of soluble and gaseous products, including volatile fatty acids, alcohols, amines, ammonia, hydrogen sulfide, carbon dioxide, and hydrogen. A subsequent methanogenic phase converts short chain fatty acids, carbon dioxide, and hydrogen to methane.

Recent studies in other anaerobic habitats, particularly the rumen, have indicated that anaerobic digestion is a tightly coupled process which is greatly dependent on metabolic interactions which occur between microorganisms involved in the initial and terminal stages of electron transfer (Reddy, et al., 1972; Wolin, 1974; Scheifinger, et al., 1975; Weimer and Zeikus, 1977). If operative in lake sediments, such coupled metabolic reactions would be of extreme functional importance in that they would control the rate, extent and end products of decomposition, as well as the rate of nutrient regeneration in these sediments.

This paper presents initial studies of the extent and mechanisms of anaerobic processing of particulate organic matter in eutrophic lake sediments.

MATERIALS AND METHODS

Investigations were conducted on Wintergreen Lake, a shallow hardwater basin located within the W.K. Kellogg Bird Sanctuary in Augusta, Michigan (Molongoski and Klug, 1976). Samples were taken at three sampling stations located within the 6-meter contour of the pelagic zone (Figure 1 of Chapter II).

Water column samples were collected at weekly intervals at station 3 with a Van Dorn sampler (3 liter, PVC). pH was measured on the collected water samples immediately upon return to the laboratory and equilibration of the sample bottle to room temperature. Samples for the analysis of soluble constituents were filtered through an all Pyrex

filtration apparatus fitted with 47 mm precombusted glass fiber filters (Reeve-Angel, 934 AH used in 1976; Whatman GF/F used in 1977). Filtrates were stored at minus 60°C until analysis. Dissolved nitrate and nitrite were analyzed by the method of Wood, et al. (1967). Ammonia was determined colorimetrically by the method of Harwood and Kuhn (1970). Dissolved sulfate was measured turbidometrically as barium sulfate, the latter held in suspension by addition of 0.3% gelatin (Tabatabai, 1974).

Samples for hydrogen sulfide analysis were collected at 0.5 meter intervals with a Van Dorn sampler. Twenty-ml samples were taken by syringe from a septated port on the Van Dorn bottle and immediately transferred to 50 ml evacuated serum bottles containing 2 ml of 0.2% zinc acetate in 0.2% acetic acid (Caldwell and Tiedje, 1975). Analysis of sulfide was by the method of Cline (1969).

Dissolved methane concentrations were determined at 0.5 meter intervals. At each depth, duplicate 60-ml serum bottles were flushed with sample water and filled to the top. Ten ml were then removed from each bottle by pipette in order to create an air headspace. Each bottle was sealed with a rubber serum stopper. The bottles were equilibrated at 23°C for 2 hours, shook vigorously to insure an equilibrium concentration of gases between the aqueous and vapor phases, and analyzed for methane in the headspace. Methane was analyzed on a Varian model 600D gas chromatograph equipped with a flame ionization detector.

Analysis was made on a coiled stainless steel column (2 m by 0.3 cm OD) packed with Porapak N (80/100 mesh, Waters Associates, Framingham, Mass.). Helium was the carrier gas at a flow rate of 20 ml/min. Chromatographic operating conditions were: inlet temperature 140°C; oven temperature 50°C; detector temperature 140°C.

Dissolved methane in the original water sample was calculated from the headspace methane concentration using an equilibrium constant that described the distribution of methane between the aqueous and vapor phases under the conditions of analysis. This constant was verified experimentally using degassed lake water and standard concentrations of methane and was found to be reproducible over the in situ range of methane concentration (Strayer and Tiedje, 1978).

Methane escaping from the sediment as bubbles was collected in gas traps similar to those of Strayer and Tiedje (1978). Each trap consisted of four inverted polypropylene funnels 28 cm in diameter. An enclosed, graduated plastic cylinder was placed over each funnel stem and gas bubbles were collected by displacement of water from the cylinders. One trap was placed approximately 0.5 meter above the sediment surface at each of 3 pelagic sampling stations (Figure 1 of chapter II). Each trap was sampled weekly by raising it to the surface and recording the volume of gas collected in each tube. Replicate gas samples for methane analysis were taken by syringe through a serum stopper at the top of each graduated cylinder. Methane was

analyzed as previously described.

Concentrations of short chain volatile fatty acids in the surface sediments (0-3 cm) of Wintergreen Lake pelagic sediments were determined at approximately biweekly intervals from May through October, 1976. Sediment cores were collected with a gravity corer, and interstitial water obtained from the 0-3 cm strata with a sediment squeezer (Reeburgh, 1967). Interstitial water from 3 cores was pooled and the volatile fatty acids were converted to tetrabutylammonium salts by the addition of 0.7M tetrabutylammonium hydroxide (Bethge and Lindstrom, 1974). The samples were freeze-dried, reconstituted in a minimum amount of acetone, and converted to their benzyl esters (Bethge and Lindstrom, 1974). The latter were analyzed on a Packard model 409 gas chromatograph equipped with a hydrogen flame ionization detector. Esters were separated on a coiled stainless steel column (2 m by 0.3 cm OD) packed with 3% butane 1,4-diol succinate on Supelcoport (100/120 mesh; Supelco Inc., Bellefonte, Pa.). Helium was used as carrier gas at a flow rate of 40 ml/min. Chromatographic operating conditions were: inlet temperature 150°C; detector temperature, 200°C; oven temperature 120°C for 7 minutes followed by programing to 180°C at a rate of 20°/minute. Quantitative and qualitative analysis was made relative to a known concentration of n-hexanoic acid which was added to the original aqueous samples as an internal standard. Recovery of volatile fatty acids from the original samples by this procedure was 90% or greater as

determined using ^{14}C-acetic acid.

Chemical analysis of the sediment pore water was conducted at weekly intervals in Wintergreen Lake during 1977 utilizing interstitial water samplers similar to those described by Hesslein (1976a) and by Winfrey and Zeikus (1977). Sampling ports were filled with N_2-sparged distilled water and overlain with a 0.22 um pore size polycarbonate membrane (Nucleopore Corporation). The samplers were inserted into the sediment by SCUBA and adjusted so that approximately 5 cm of the sampler remained above the sediment surface. Each sampler was allowed to equilibrate for 2 weeks before being retrieved by SCUBA. Interstitial water obtained in this manner was analyzed for ammonia, sulfate, sulfide, and methane as described previously. Short chain volatile fatty acids were converted to their tetrabutylammonium salts as described above, but after concentration by freeze-drying, were converted to free acids by rehydration in 4.4N H_3PO_4, and analyzed by gas chromatography. The acids were separated on a coiled glass column (2 m by 0.2 mm ID) packed with 10% SP-1000/1% H_3PO_4 on Supelcoport (100/120 mesh; Supelco, Inc., Bellefonte, Pa.). Analysis was made using a flame ionization detector. Chromatographic operating conditions were: inlet temperature 200°C; detector temperature 250°C; column temperature 140°C. Helium carrier gas flow rate was 30 ml/min.

RESULTS

pH values were generally similar in the lake during both years studied (Figure 1). Epilimnetic pH gradually increased through the summer while hypolimnetic pH declined until fall turnover when the pH became the same throughout the water column. The most significant difference in pH noted was the greater fluctuation in epilimnetic pH observed in 1976 compared to 1977.

Dissolved nitrate was rapidly depleted from the water column by early June in 1976 and was not replenished until fall turnover (Figure 2). Nitrate concentrations in 1977 were similar to those measured in 1976.

Dissolved sulfate (Figure 3) was depleted from the water column at a much slower rate than was nitrate (Figure 2). Hypolimnetic sulfate depletion increased markedly beginning in early August during both 1976 and 1977. During 1976, sulfate levels remained low (a minimum value of 3.3 mg/l SO_4-S was measured on September 7) until fall turnover restored higher levels of sulfate throughout the water column. During 1977, sulfate levels were also quite low in late summer except for a brief period of mixing in early September which resulted in a brief intrusion of sulfate into the hypolimnion (Figure 3).

Hypolimnetic concentrations of ammonia were very high during the summer, with values exceeding 15000 ug NH_4-N/l measured in both 1976 and 1977 (Figure 4). Maximum values were recorded in mid-August in 1976 and in late July in 1977.

Figure 1. Depth-time diagram of pH in the pelagic zone of Wintergreen Lake.

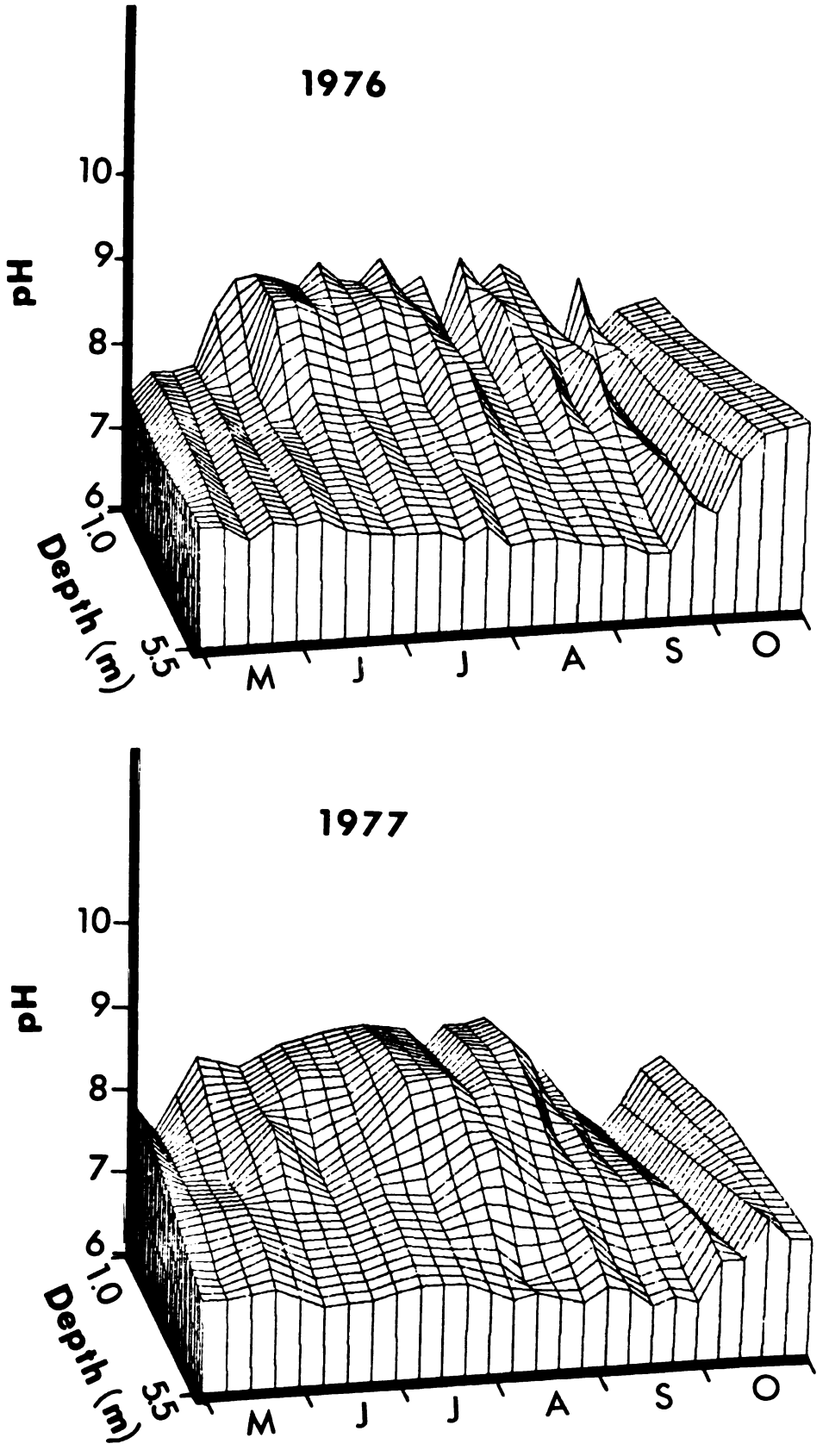

1976
pH
10
9
8
7
6
1.0
Depth (m)
5.5
M
J
J
A
S
O
1977
pH
10
9
8
7
6
1.0
Depth (m)
5.5
M
J
J
A
S
O

Figure 2. Depth-time distribution of dissolved nitrate in pelagic zone of Wintergreen Lake during 1976.

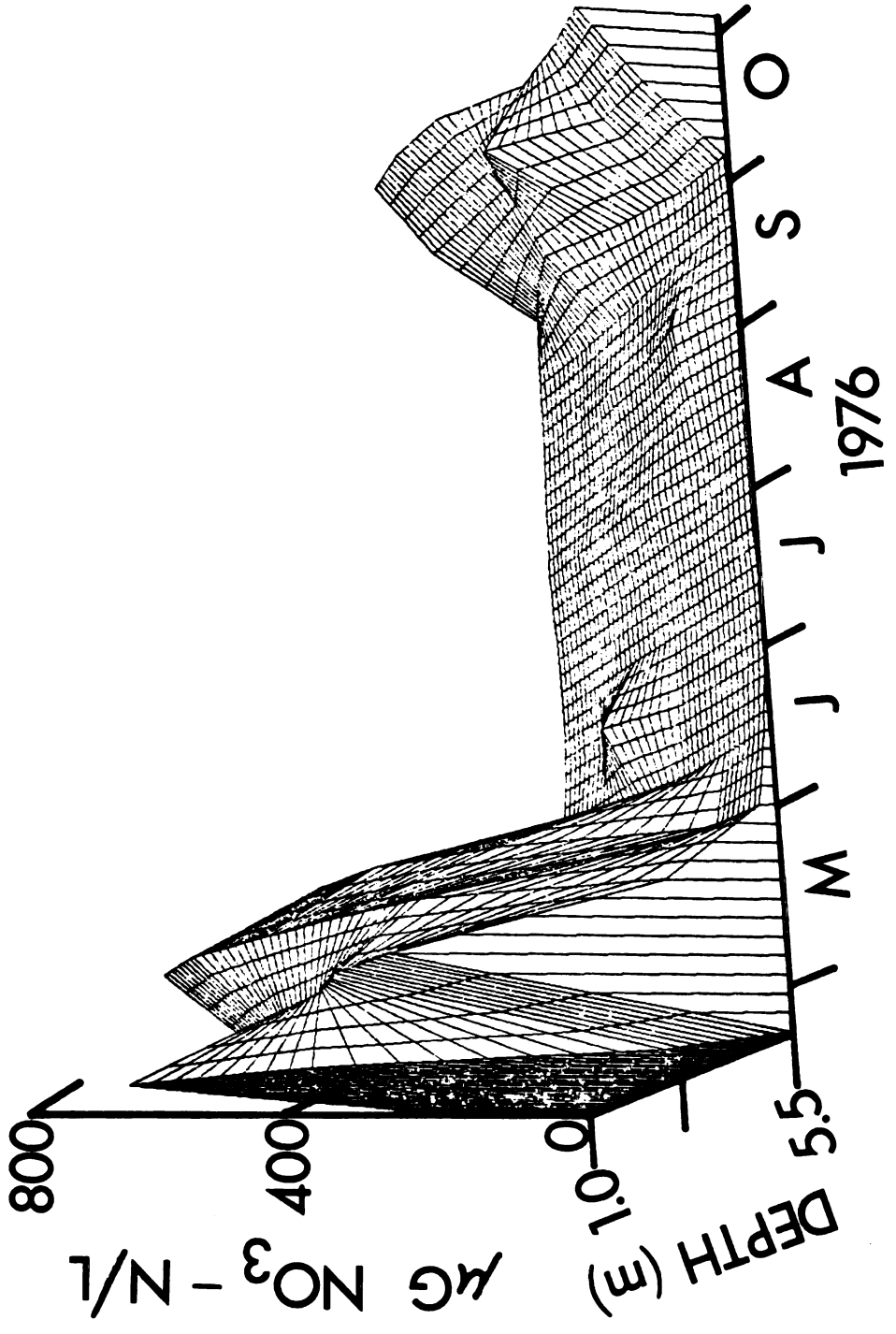

800
400
0
μG NO3 - N/L
1.0
5.5
DEPTH (m)
M J J A S O
1976

Figure 3. Depth-time distribution of dissolved sulfate in the pelagic zone of Wintergreen Lake.

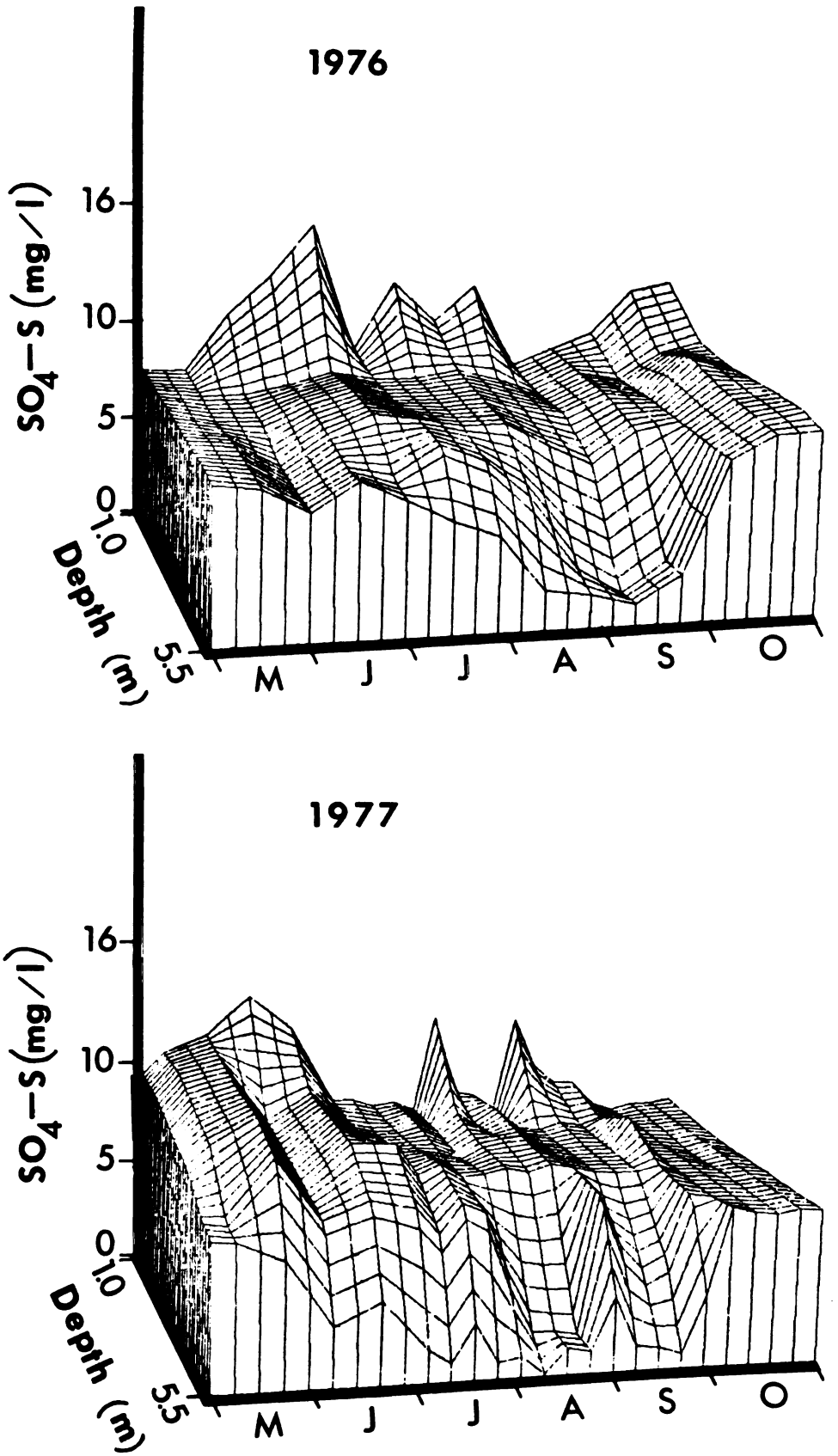
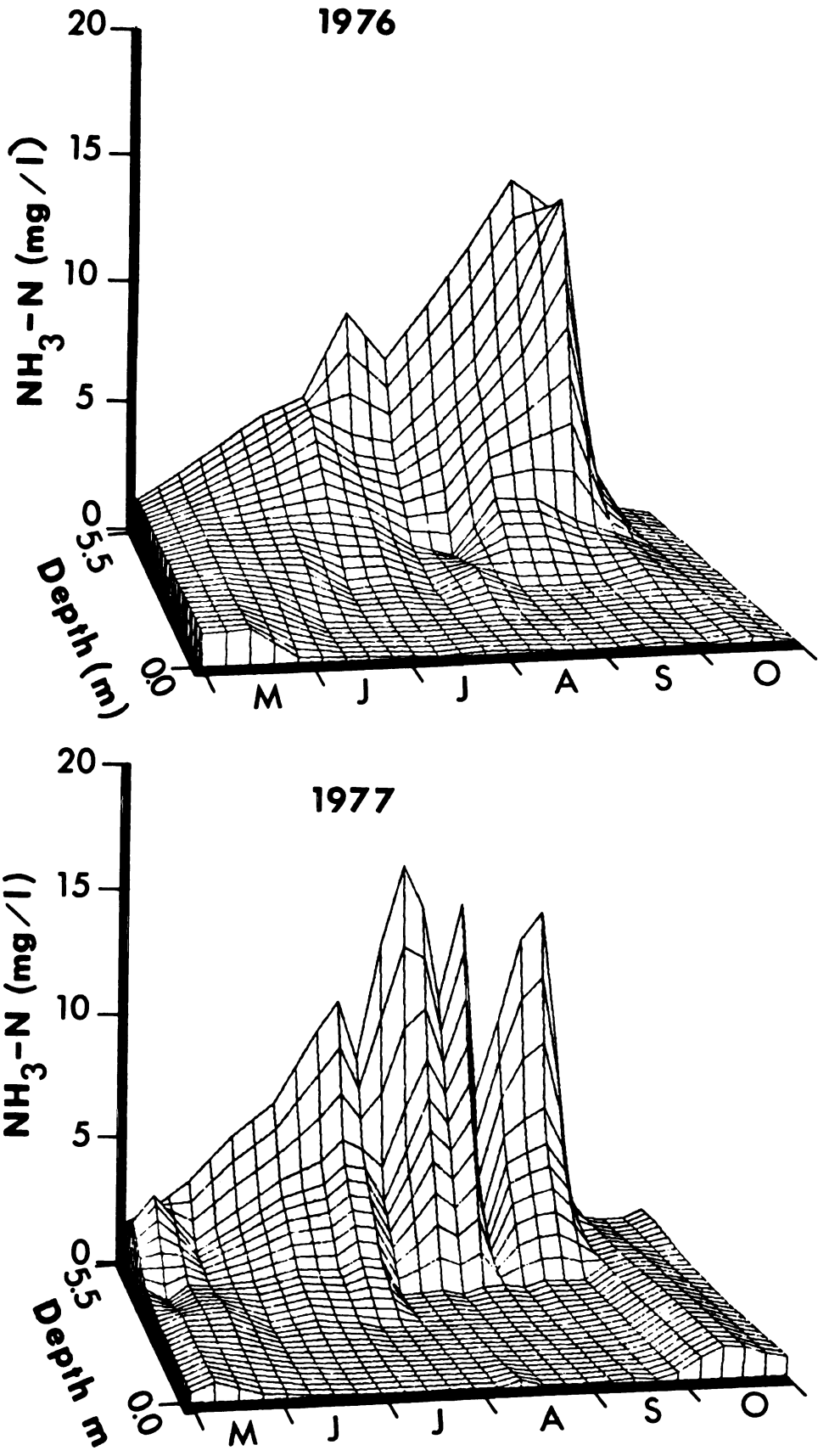

1976
SO_4–S (mg/l)
16
10
5
0
0.0
5.5
Depth (m)
M
J
J
A
S
O
1977
SO_4–S (mg/l)
16
10
5
0
0.0
5.5
Depth (m)
M
J
J
A
S
O

Figure 4. Depth-time distribution of dissolved ammonia in the pelagic zone of Wintergreen Lake. The hypolimnion has been placed distal to the viewer so that the distribution of ammonia throughout the water column can be more clearly visualized.


1976
20
15
10
5
0
NH_3-N (mg/l)
5.5
0.0
Depth (m)
M
J
J
A
S
O
1977
20
15
10
5
0
NH_3-N (mg/l)
5.5
0.0
Depth m
M
J
J
A
S
O

Hypolimnetic ammonia values fluctuated to a greater extent in 1977 than they did in 1976. Epilimnetic values of ammonia were low relative to hypolimnetic values during both years.

Similarly, dissolved sulfide concentrations (Figure 5) were highest in the hypolimnion. Peak sulfide values were measured in late August in 1976, and in late July in 1977. As with ammonia, sulfide values fluctuated greatly in 1977 relative to 1976.

The daily rate at which methane was released from Wintergreen Lake pelagic sediments as bubbles is illustrated in Figure 6. In 1976, the rate of ebullition increased from a low value of 1.9 mmoles $\cdot$ m^{-2} $\cdot$ day^{-1} in the spring to a maximum value of 68 mmoles $\cdot$ m^{-2} $\cdot$ day^{-1} in mid-July. The July maximum was followed by a precipitous decrease in the rate in early August to values approaching those observed in the spring. Rates of methane ebullition then increased once again, reaching a second maximum of 65 mmoles $\cdot$ m^{-2} $\cdot$ day^{-1} in mid-September before decreasing to zero at fall turnover (Figure 6).

In contrast to 1976, methane ebullition from the sediments occurred at a much more gradual rate in 1977, slowly increasing from the low values recorded in the spring to a maximum value of approximately 40 mmoles $\cdot$ m^{-2} $\cdot$ day^{-1} in late September (Figure 6).

The distribution of dissolved methane in the water column (Figure 7) exhibited the same pattern as that observed for methane ebullition in both 1976 and 1977. In 1976,

Figure 5. Depth-time distribution of dissolved sulfide in the pelagic zone of Wintergreen Lake. The hypolimnion has been placed distal to the viewer so that the distribution of sulfide throughout the water column can be more clearly visualized.

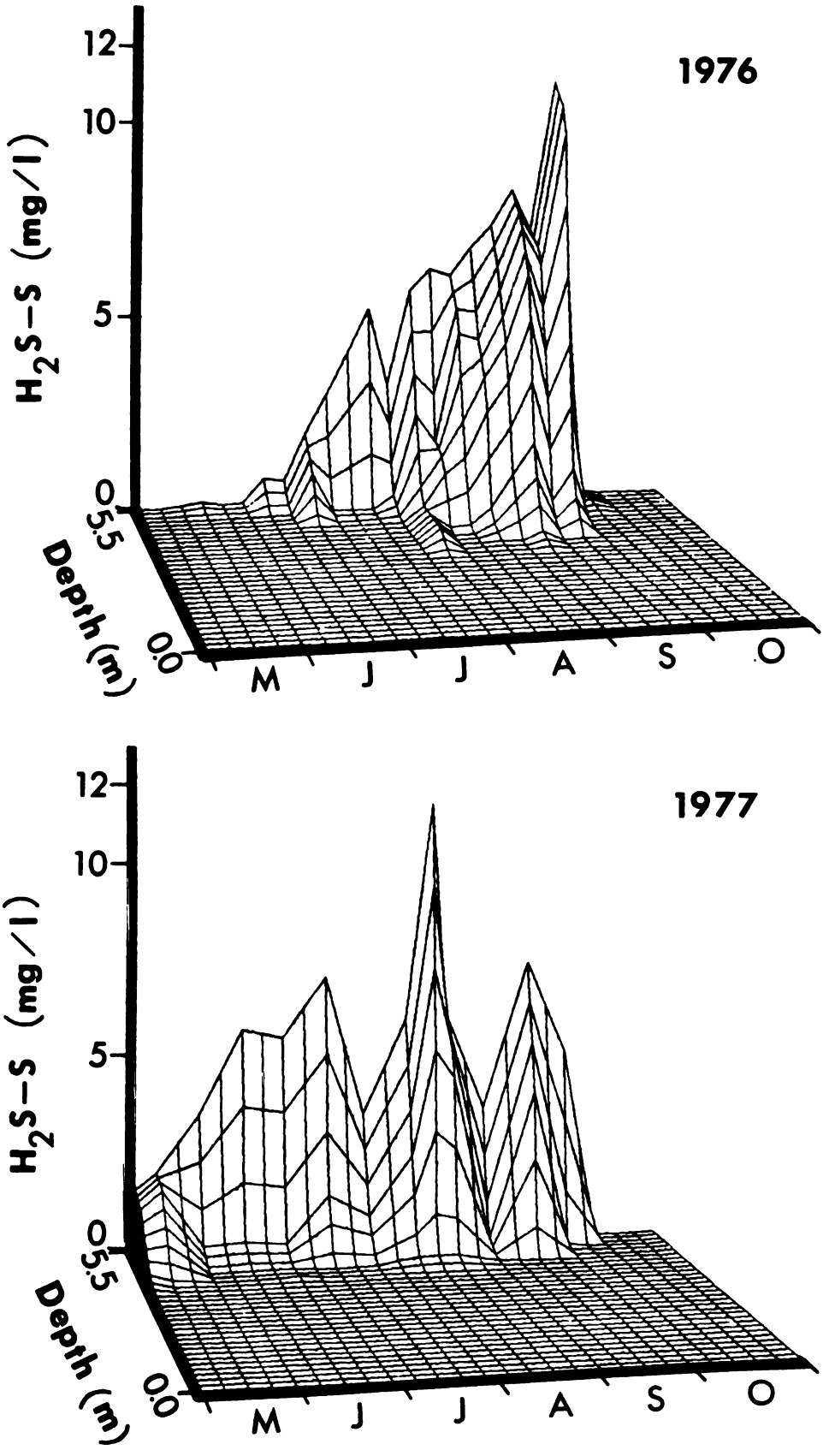

1976
1977
H_2S-S (mg/l)
12
10
5
0
Depth (m)
0.5
0.0
M
J
J
A
S
O

Figure 6. Rate of methane ebullition from Wintergreen Lake pelagic sediments during summer stratification in 1976 and 1977. Each point is the mean value ± SD of the methane gas collected at 3 sampling sites.

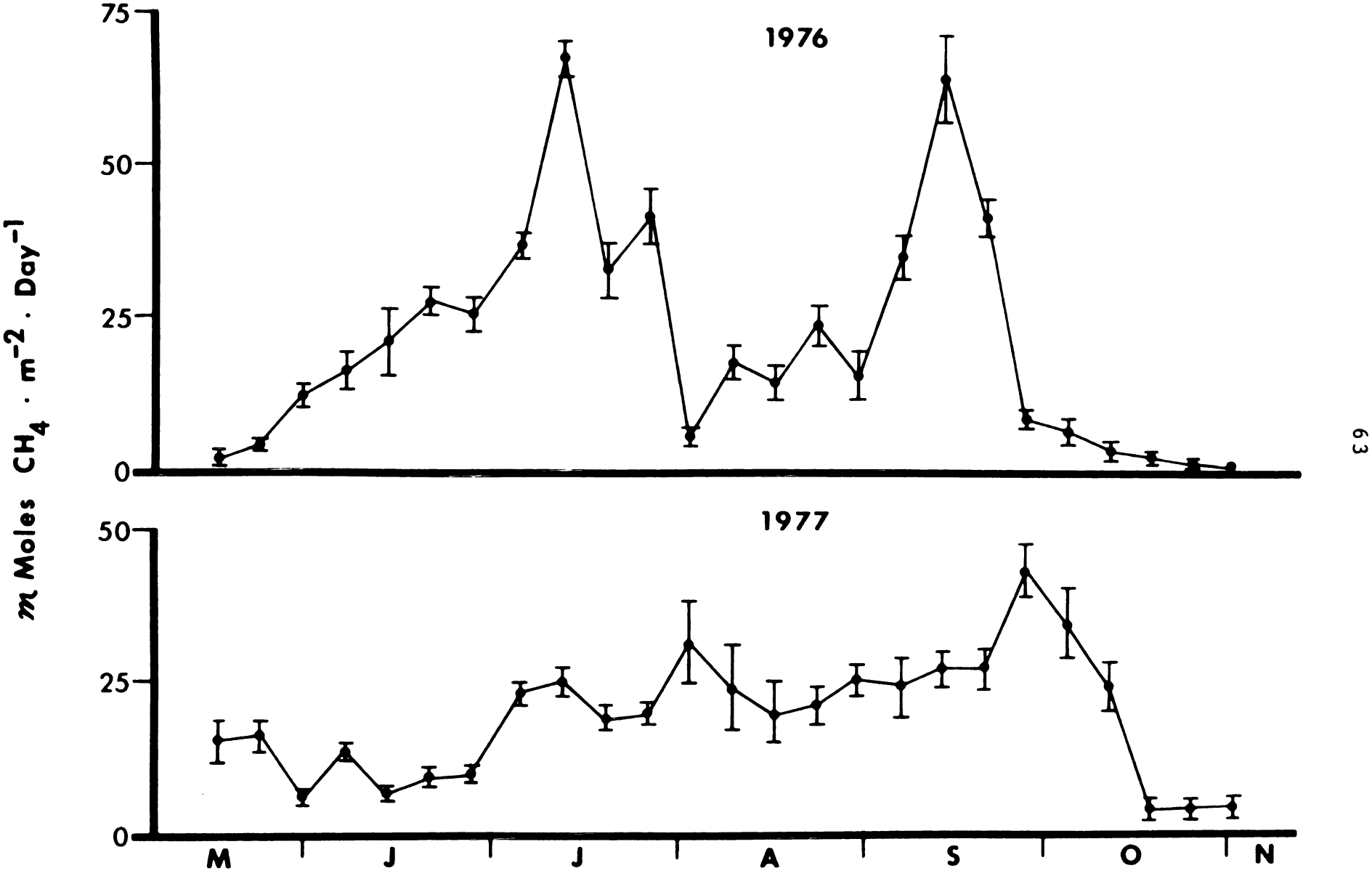
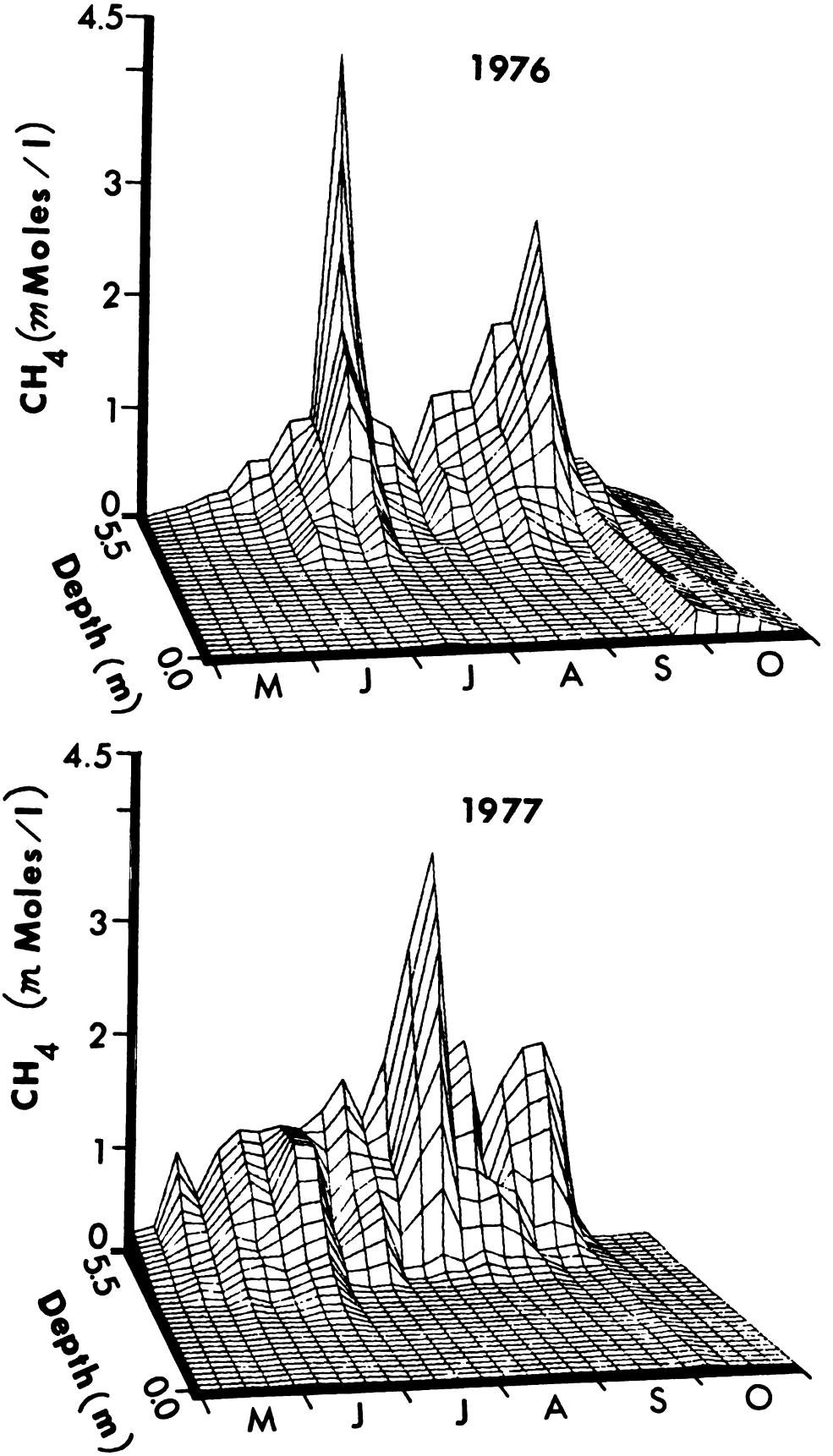

1976
1977
75
50
25
0
50
25
0
M
J
J
A
S
O
N
m Moles $CH_4 \cdot m^{-2} \cdot Day^{-1}$

Figure 7. Depth-time distribution of dissolved methane in the pelagic zone of Wintergreen Lake. The hypolimnion has been placed distal to the viewer so that the distribution of dissolved methane can be more clearly visualized.


1976
4.5
3
2
1
0
CH_4 (m Moles / l)
0.5
0.0
Depth (m)
M
J
J
A
S
O
1977
4.5
3
2
1
0
CH_4 (m Moles / l)
0.5
0.0
Depth (m)
M
J
J
A
S
O

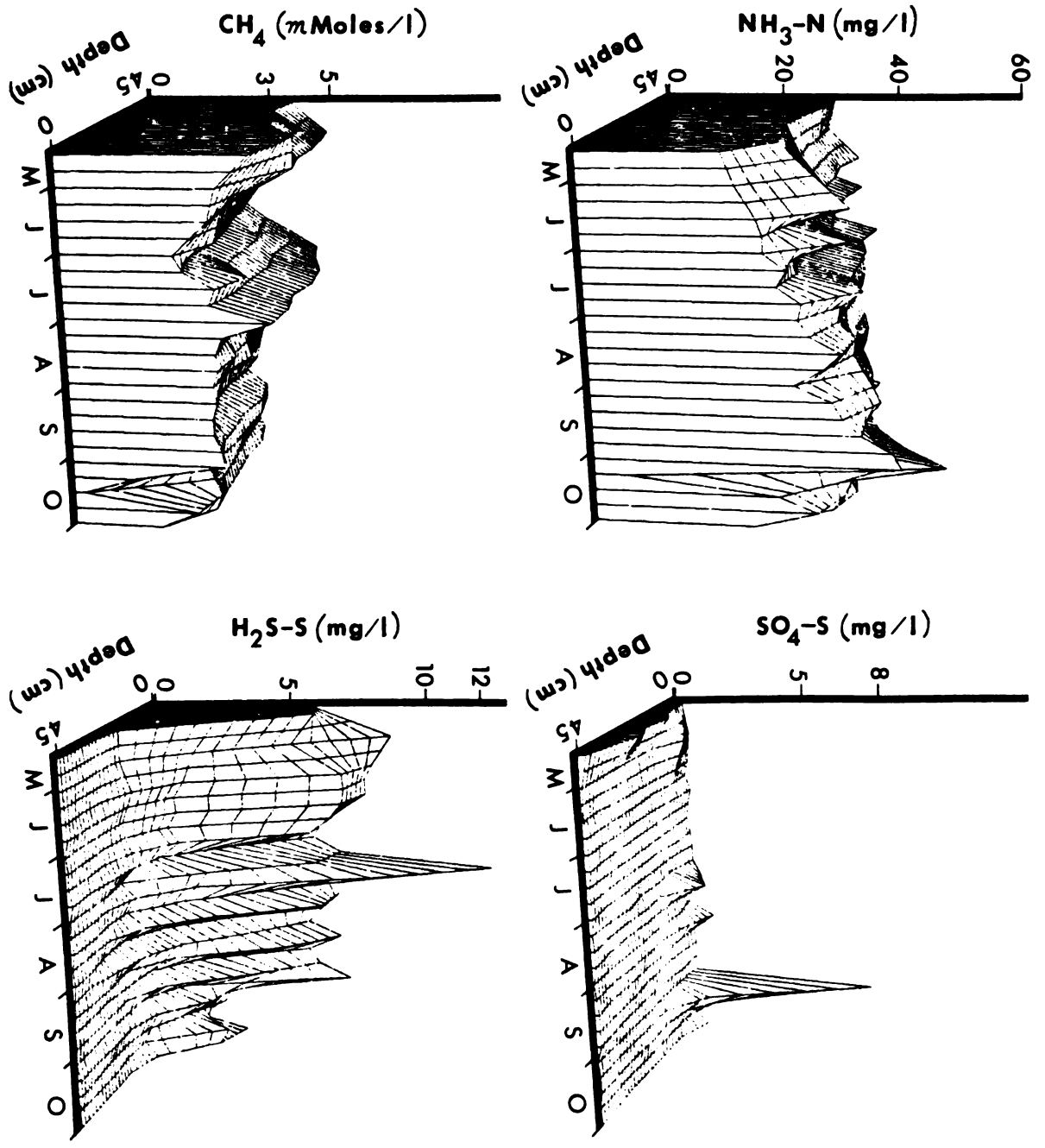
dissolved methane showed two distinct maxima, one in mid-July and a second in mid-September. In 1977, dissolved methane concentrations increased throughout the summer, maximum concentrations being measured in mid-August (Figure 7). Dissolved methane concentrations declined after fall turnover in both years examined.

The results of chemical analysis of the interstitial water of Wintergreen Lake pelagic sediments during 1977 are shown in Figure 8. Interstitial water ammonia concentrations remained high from the sediment surface to a depth of 45 cm into the sediments. Maximum values were, however, always found at the sediment-water interface. Ammonia concentrations generally increased at the sediment surface throughout the summer, maximum values being found in early October.

Similarly, methane concentrations, although generally highest at the sediment-water interface, remained high to a depth of 45 cm in the sediment (Figure 8). Pore water methane concentrations were high in late May, declined abruptly in June, and then increased again in July and August. Methane concentrations at the sediment-water interface declined sharply at fall turnover in October, but increased again subsequent to lake mixing (Figure 8).

Pore water sulfide values (Figure 8) were also highest at the sediment-water interface, but unlike ammonia and methane concentrations, declined rapidly with depth into the sediment. Sulfate was not detectable in the interstitial water, except for a brief period after fall turnover, and for

Figure 8. Depth-time distribution of ammonia, methane, sulfate, and hydrogen sulfide in the interstitial water of Wintergreen Lake pelagic sediments during 1977. In the ammonia and methane plots, the sediment surface has been placed proximal to the viewer, while in the sulfate and sulfide plots the sediment surface is distal to the viewer.


CH_4 (mMoles/l)
Depth (cm)
0
45
0
3
5
M
J
J
A
S
O
NH_3-N (mg/l)
0
20
40
60
H_2S-S (mg/l)
0
5
10
12
SO_4-S (mg/l)
0
5
8

two occasions in August when turbulence in the water column resulted in brief intrusions of sulfate to the surface sediments (Figure 8).

The volatile fatty acids detectable in the interstitial water of Wintergreen Lake pelagic sediments in 1976 and 1977 are presented in Figures 9 and 10 respectively. In 1976, acetate was the only volatile fatty acid consistently detectable in the surface sediments (0-3 cm). Acetate concentrations ranged from 20-80 umoles/l, with maximum concentrations detected in early June (Figure 9).

During 1977, volatile fatty acids were sampled at approximately 2-cm intervals utilizing the interstitial water samplers described previously. Acetate was the predominant acid detected followed by lesser amounts of propionate. Maximum concentrations of each acid were present at or just above the sediment-water interface. Concentrations of both acetate and propionate declined sharply with depth into the sediment and were undetectable below 6-8 cm (Figure 10). Although trace concentrations of butyrate and iso-valerate (less than 2 umoles/l) were occasionally analyzed in the surface sediments during 1977, these acids were not generally detectable in these sediments by the analytical procedures employed.

During 1977, acetate and propionate concentrations were highest during July and August, but gradually declined through September and October. Volatile acid levels were higher in 1977 compared to 1976 (Figures 9 and 10).

Figure 9. Concentrations of acetate in surface sediments (0-3 cm) of Wintergreen Lake pelagic zone during 1976.

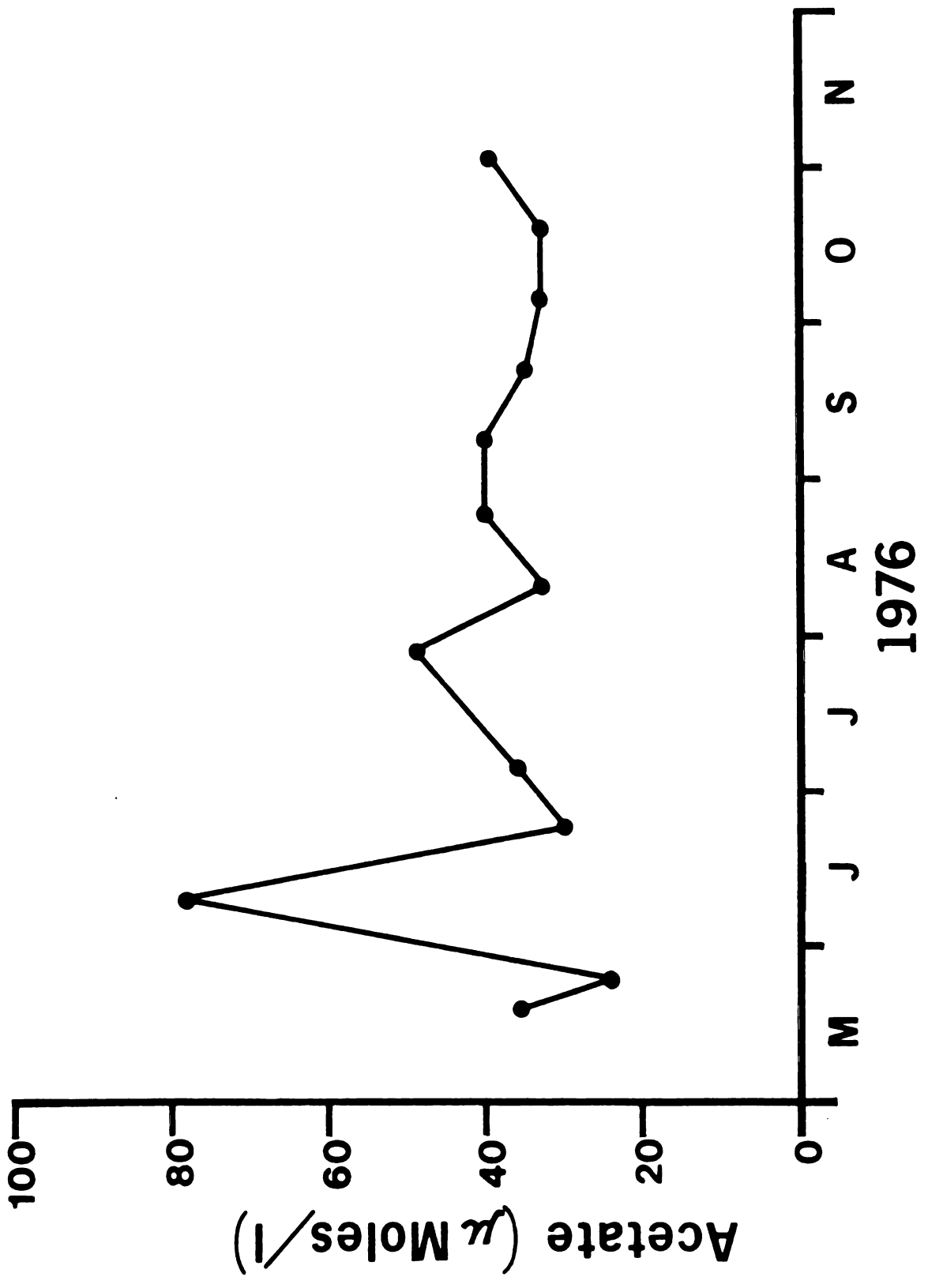
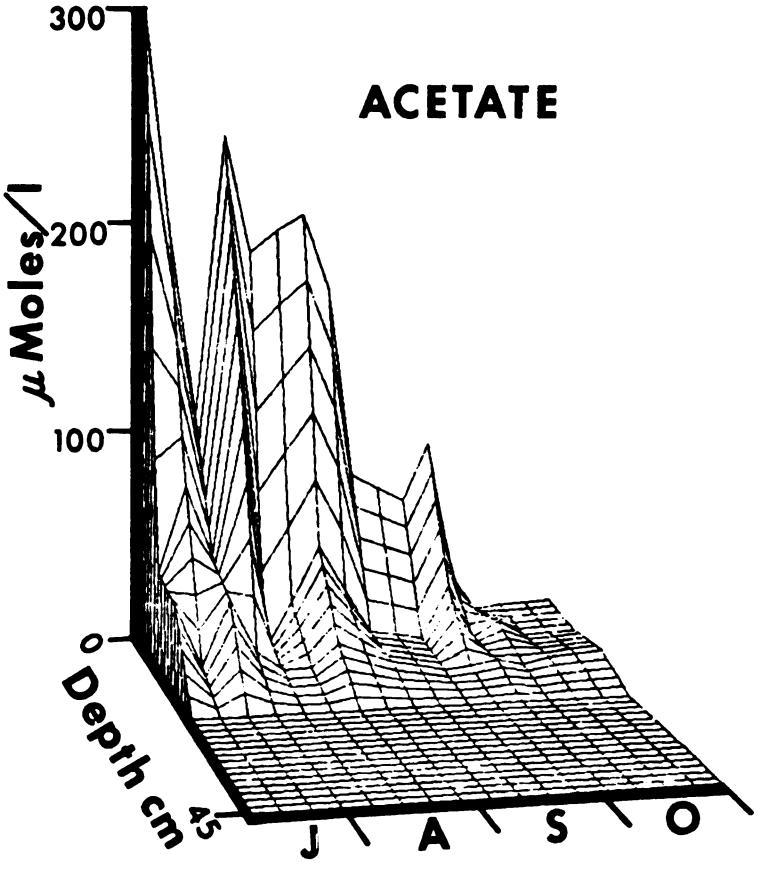
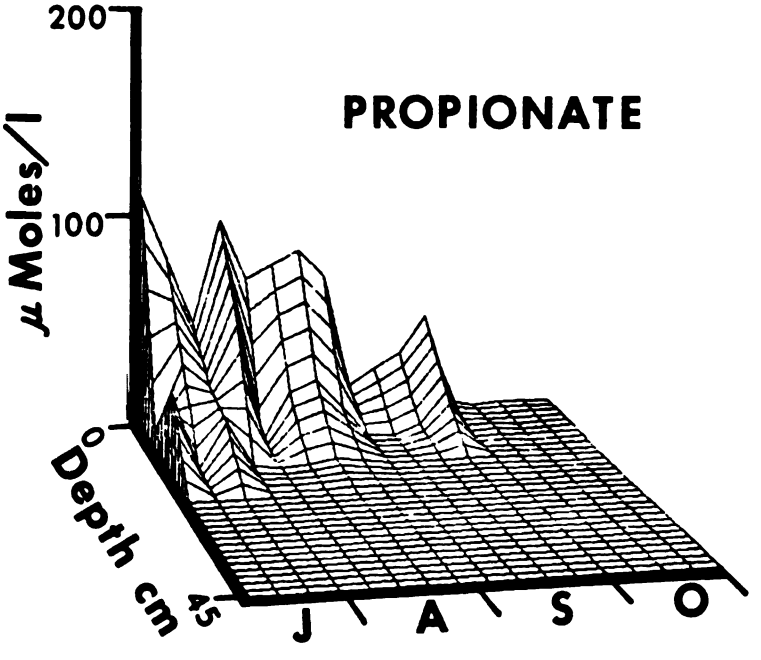

Acetate (μ Moles/l)
100
80
60
40
20
0
M
J
J
A
S
O
N
1976

Figure 10. Concentrations of acetate and propionate in the interstitial water of Wintergreen Lake pelagic sediments during 1977.


ACETATE
300
200
100
0
μ Moles/l
Depth cm
45
J
A
S
O


PROPIONATE
200
100
0
μ Moles/l
Depth cm
45
J
A
S
O

DISCUSSION

The rapid sedimentation of POM in the pelagic zone of Wintergreen Lake results in the majority of sedimenting organic matter reaching the sediments in a largely undegraded form (Chapter II). Hypolimnetic concentrations of ammonia (Figure 4), hydrogen sulfide (Figure 5), and methane (Figure 7) increased greatly in the lake following maxima in sedimentation rate (Chapter II), indicating that degradation of sedimenting POM begins rapidly. The initial anaerobic decomposition of freshly deposited organic matter appears to occur at the sediment-water interface. During 1977, maximum concentrations in ammonia, methane, and hydrogen sulfide (exceeding those measured in the hypolimnion) were found at the sediment-water interface (Figure 8). Large increases in methane, ammonia, and sulfide at the sediment surface occurred 1-2 weeks after large increases in sedimentation rate (Chapter II). Hesslein (1976b), in a study of methane and ammonia flux from sediments, also found highest concentrations of each at the mud-water interface.

Methane production rates were determined periodically in Wintergreen Lake sediments in 1977 by incubating sections of sediment cores under strict anaerobic conditions and monitoring the change in methane concentration with time. Results showed that the rate of methane production during stratified periods was highest in the upper 3 cm of sediment, although methane production was detectable 30 cm into the sediment (Molongoski, unpublished results). Rudd and

Hamilton (1977) similarly demonstrated ^{14}C-methane production 9 days after sedimenting ^{14}C-labeled POM reached the sediment surface. The activity of $^{14}CH_4$ increased most rapidly at the sediment-water interface, indicating that most of the methane production was occurring at this site.

In 1977, maximum concentrations of both acetate and propionate were found at or just above the sediment-water interface, providing additional support that the surface sediments are an important site for the anaerobic decomposition of sedimenting organic matter. These results agree with those of Monokoria (1975) and of Hollis and Rodriguez-Kabana (1967) who reported acetate and propionate to be the major volatile fatty acids present in the bottom sediments of Rybinsk Reservoir and in rice field soils respectively.

In 1977, acetate and propionate concentrations in Wintergreen Lake sediments were highest in July and August (Figure 10) following the high sedimentation rates measured during June and July (Chapter II).

The presence of high levels of acetate and propionate in the water directly overlying the surface sediments suggests that a portion of the volatile fatty acids produced at the sediment-water interface may be lost from the site of production by diffusion rather than being metabolized further in the sediments. Alternatively, these acids could result from metabolism of POM by suspended heterotrophic bacteria

directly overlying the sediments. Caldwell and Tiedje (1975) have described a colorless community of bacteria occurring in a layer 0.1 to 0.7 meter thick immediately above the sediment surface of Wintergreen Lake. They concluded that this bacterial community is primarily a sediment community in that its nutrition is obtained from organic matter derived from the sediment-water interface. The rapid decline in volatile acid concentration with increasing depth in the sediment reflects the decreasing availability of labile, freshly-deposited substrate.

Volatile fatty acid concentrations measured in 1976 were lower and more constant than those found in 1977, in spite of the fact that the rate of organic input to the sediments was greater in 1976 compared to 1977 (Chapter II). This discrepancy is likely due to the different sampling techniques used in 1976 and 1977. During the first year, interstitial water for volatile acid analysis was obtained by squeezing the 0-3 cm sediment strata while in 1977 interstitial water was obtained using dialysis samplers. Utilization of the latter samplers resulted in higher recovery of volatile acids and improved resolution of the distribution of volatile acids with depth. Concentrations of ammonia, methane, and hydrogen sulfide have also been shown to be greater in interstitial water obtained with dialysis samplers compared to water obtained by squeezing sediments (Klug, et al., unpublished results).

Although maximum anaerobic decomposition appears to

occur at the sediment-water interface, concentrations of ammonia and methane remained relatively high to a depth of 45 cm in the sediment, indicating that methane and ammonia production extend over greater depths in the sediment. The absence of appreciable levels of nitrate in the water column (Figure 2) and the high protein content of the seston reaching these sediments (Chapter II) suggest that ammonia is produced primarily through microbial deamination of proteinaceous inputs. Previous studies have shown that 70-75% of the anaerobic bacteria isolatable from these sediments are proteolytic (Molongoski and Klug, 1976).

The amount of hydrogen sulfide which is produced in Wintergreen Lake sediments through dissimilatory sulfate reduction as compared to the decomposition of organic sulfur-containing compounds has not been determined. Sulfate levels in the hypolimnion decreased at a gradual rate during the summer in both 1976 and 1977, then declined rapidly in late August (Figure 3). Pool sizes of SO_4-S in interstitial water obtained during 1976 by squeezing 0-3 cm sediments were examined periodically during the summer and were always found to be less than 1 mg/l, suggesting a rapid turnover of sulfate in these sediments. Unlike ammonia and methane concentrations, hydrogen sulfide concentrations declined precipitously below 4 cm depth in the sediments in samples collected in 1977 (Figure 8). Moreover, sulfate was usually undetectable in the interstitial water during 1977 except at fall turnover and during occasional periods in the

summer when turbulence introduced sulfate to the sediment-water interface (Figure 8). Seventy per cent of the anaerobic heterotrophic bacteria isolatable from these sediments produced hydrogen sulfide from organic sulfur-containing compounds, indicating that a portion of this hydrogen sulfide is derived from the degradation of sulfur-containing organic compounds (Molongoski and Klug, 1976).

Dunnette (1973) compared the production of hydrogen sulfide by putrefaction and by sulfate reduction in two eutrophic lakes in Southeastern Michigan. His results showed that H_2S production from cysteine decomposition (putrefaction) varied from 5.1 to 53% with a mean of approximately 20%. Both sulfate reduction and cysteine decomposition displayed definite seasonal variations, and were subject to relatively abrupt fluctuations of several hundred per cent per month (Dunnette, 1973). Such fluctuations would result from changing rates of input of organic matter to the sediments as occur in Wintergreen Lake (Chapter II), and would stimulate putrefaction and/or sulfate reduction in these sediments.

Robinson (1978), in a study comparing two lakes of differing trophic level, concluded that the rate of supply of organic matter to the sediments was an important factor in determining the rate of methanogenesis. The correlation between increased rates of sedimentation (Chapter II) and the increasing release of dissolved methane (Figure 7) as well as methane bubbles (Figure 6) from the pelagic

sediments of Wintergreen Lake support this relationship. During both 1976 and 1977, maxima in methane ebullition (Figure 6) occurred 2-4 weeks after major increases in sedimentation rate (Chapter II). Maximum rates of methane ebullition in 1977 were only one half of those measured in 1976. The dramatic increase in methane production observed in 1976 compared to 1977 reflect the greater and more sustained rate of sedimentation to the sediments in 1976 relative to 1977 (Chapter II).

Methane leaving the sediments as bubbles in 1976 showed a bimodal seasonal pattern, maxima occurring in mid-July and mid-September, with a precipitous decline to near spring levels occurring in early August (Figure 6). The same bimodal pattern was observed in dissolved methane (Figure 7). A bimodal pattern in methane concentration was also noted in the study of Robinson (1978). These pronounced declines in methane release from Wintergreen Lake sediments suggest that methane production was severely reduced or inhibited during early August of 1976. The minimum observed in dissolved methane (Figure 7) in early August of 1976 may in part be due to methane oxidation caused by oxygen intrusion into the hypolimnion to a depth of 4.5 meters at approximately the same period (Chapter II). However, inhibition of methane production appears to be a more likely explanation for the observed decline in methane.

The exact cause of the interruption of methanogenesis observed in Wintergreen Lake during 1976 is not known.

However, this disruption in methane production was preceded by several large increases in sedimentation rate in June and in early August (Chapter II), suggesting that the temporary inhibition of methanogenesis may be related to excessive organic loading to the sediments. Inhibition of methane production has been observed in sewage digesters after the addition of large quantities of fermentable material and/or an increase in the turnover rate of substrate (Hobson, et al, 1974). Such digester failure is usually accompanied by an increase in volatile fatty acid concentration, continued acid accumulation leading to a decline in pH and in alkalinity which are responsible for the buffering of the sediment at pH 7.2-7.4. Although interstitial water volatile fatty acid levels were not observed to increase during this time period, the steady decline in hypolimnetic pH during 1976 is evidence of the intensity of fermentative activity occurring in these sediments (Figure 1).

Resumption of methane production in Wintergreen Lake in 1976 (Figures 6 and 7) followed a reduction in the loading rate to the sediments (Chapter II). Natural recovery has also been observed in anaerobic sludge digesters upon reduction in the rate of substrate loading (Hobson, et al., 1974).

Table 1 summarizes the seston input and methane output for Wintergreen Lake pelagic sediments during the study period in 1976 and 1977. In order to estimate the total methane leaving the sediments, the amount of dissolved

Table 1. Carbon budget for Wintergreen Lake pelagic sediments, 1976 and 1977.

INPUT

Year	Total Seston ($g \cdot m^{-2}$)	Total Carbon ($g \cdot m^{-2}$)
1976	1282	320
1977	785	196
% of 1976 value	61	62

OUTPUT

Year	CH_4 Bubbles ($g\ C \cdot m^{-2}$)	Dissolved CH_4 ($g\ C \cdot m^{-2}$)	Total CH_4 ($g\ C \cdot m^{-2}$)
1976	45	63	108
1977	39	47	86
% of 1976 value	85	74	79

Year	% input C converted to CH_4 - C	% input C lost from lake as CH_4 - C
1976	34	14
1977	44	20

methane in the entire meter square water column above 5.5 meters was calculated for each sampling date by solving the integral of the methane concentration with depth (Strayer and Tiedje, 1978). The change in dissolved methane in the water column between sampling dates must be due to methane diffusing through the 5.5 meter plane. The change in methane concentration was calculated in 1976 for the periods between early August and mid-September. These two values were summed to obtain a total for the period May through October, 1976. Those periods in which the dissolved methane content of the water column markedly declined (Figure 7) were omitted from the calculation since, as noted previously, methane production was believed to have stopped during these periods. The estimate of methane lost from the sediment through diffusion is an underestimate as the values presented in Table 1 have not been corrected for loss due to methane oxidation.

The total methane leaving Wintergreen Lake pelagic sediments during May through October, 1976 was calculated to be 108 g C $\cdot$ m^{-2} or 34% of the input carbon (Table 1). Forty two per cent of the methane (45 g C $\cdot$ m^{-2}), representing 14% of the input carbon left the sediment as bubbles and was lost from the lake ecosystem. Fifty eight per cent (63 g C $\cdot$ m^{-2}) of the methane produced in the sediments diffused into the water column as soluble methane.

The total methane leaving the sediments for the same period in 1977 was 86 g C $\cdot$ m^{-2} or 44% of the input carbon.

Thus, although the input of seston in 1977 was only 61% of that measured in 1976, the per cent conversion of seston carbon to methane carbon was 10% greater in 1977 compared to 1976. Approximately 45% of the total methane (39 g C · m^{-2}) representing 20% of the input carbon left the sediments as bubbles and was lost from the lake ecosystem (Table 1). Fifty five per cent (47 g C · m^{-2}) of the methane produced in the sediments in 1977 diffused into the overlying water column.

On an annual basis, the percentage of input carbon converted to methane is even greater as methanogenesis proceeded at a reduced rate during winter. The mean values of dissolved methane and methane ebullition at 5.5 meters during February, 1977 were 842 umoles/l and 1.39 mmoles · m^{-2} · day^{-1} respectively.

The results presented in this study demonstrate that a high percentage of POM is decomposed through anaerobic benthic metabolism in eutrophic lake sediments. Foree and McCarty (1970) have also demonstrated efficient anaerobic processing of algal material in laboratory cultures where fermentative and methanogenic activities resulted in 75% stabilization (reduction in chemical oxygen demand) of added substrate after 240 days. The latter investigators found the rate and extent of degradation to be similar to those found by other investigators under aerobic conditions. The temporary inhibition of methanogenesis observed in Wintergreen Lake in 1976 suggests, however, that anaerobic

processing in these sediments may be related to the rate of organic loading, and is dependent upon a tight coupling of fermentative and methanogenic phases of anaerobic processing.

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. The Analyst 99: 137-142.

2. Caldwell, D.E. and J.M. Tiedje. 1975. The structure of anaerobic bacterial communities in the hypolimnia of several Michigan lakes. Can. J. Microbiol. 21: 377-385.

3. Cline, J.D. 1969. Spectrophotometric determination of hydrogen sulfide in natural waters. Limnol. Oceanogr. 14: 454-458.

4. Dunnette, D. 1973. Chamical ecology of hydrogen sulfide production in freshwater lake sediment. Ph.D. Dissertation. University of Michigan.

5. Foree, E.G. and P.L. McCarty. 1970. Anaerobic decomposition of algae. Environ. Sci. Technol. 4: 842-849.

6. Gasith, A. 1976. Seston dynamics and tripton sedimentation in the pelagic zone of a shallow eutrophic lake. Hydrobiologia 51: 225-231.

7. Hargrave, B.T. 1972. Aerobic decomposition of sediment and detritus as a function of particle surface area and organic content. Limnol. Oceanogr. 17: 583-596.

8. Hartwig, E.O. 1976. Nutrient cycling between the water column and a marine sediment. I. Organic carbon. Mar. Biol. 34: 285-295.

9. Harwood, J.E. and A.L. Kuhn. 1970. A colorimetric method for ammonia in natural waters. Water Res. 4: 805-811.

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

11. Hesslein, R.G. 1976b. The fluxes of CH_4, total CO_2, and NH_3-N from sediments and their consequent distribution in a small lake. Ph.D. Dissertation. Columbia Univ. New York.

12. Hobson, P.N., S. Bousfield, and R. Summers. 1974. Anaerobic digestion of organic matter. Crit. Revs. Environ. Control 4: 131-191.

13. Hollis, J.P. and R. Rodriguez-Kabana. 1967. Fatty acids in Louisiana rice fields. Phytopathology 57: 841-847.

14. Howard, D.L., J.I. Frea and R.M. Pfister. 1971. The potential for methane-carbon cycling in Lake Erie. Proc. Ann. Conf. Great Lakes Res. 14: 236-240.

15. Jones, J.G. 1976. The microbiology and decomposition of seston in open water and experimental enclosures in a productive lake. J. Ecol. 64: 241-278.

16. Kimmel, B.L. and C.R. Goldman. 1977. Production, sedimentation, and accumulation of particulate carbon and nitrogen in a sheltered subalpine lake. In: Interactions Between Sediments and Freshwater. H.L. Golterman, Editor. Dr. W. Junk, B.V. Publishers, The Hague. 148pp.

17. Molongoski, J.J. and M.J. Klug. 1976. Characterization of anaerobic heterotrophic bacteria isolated from freshwater lake sediments. Appl. Environ. Microbiol. 31: 83-90.

18. Monokova, S.V. 1975. Volatile fatty acids in bottom sediments of the Rybinsk Reservoir. Hydrobiological Journal 11: 45-48.

19. Reddy, C.A., M.P. Bryant, and M.J. Wolin. 1972. Characteristics of S organism isolated from Methanobacillus omelianskii. J. Bacteriol. 109: 539-545.

20. Reeburgh, W.F. 1967. An improved interstitial water sampler. Limnol. Oceanogr. 12: 163-165.

21. Rich, P.H. 1975. Benthic metabolism of a soft-water lake. Verh. Internat. Verein. Limnol. 19: 1023-1028.

22. Robinson, C.K. 1978. Quantitative comparison of the significance of methane in the carbon cycle of two small lakes. Arch. Hydrobiol. (In press.)

23. Rudd, J.W.M. and R.D. Hamilton. 1977. Methane cycling in a eutrophic shield lake and its effects on carbon cycling and whole lake metabolism. Limnol. Oceanogr. (In press).

24. Scheifinger, C.C., B. Linehan, and M.J. Wolin. 1975. H_2 production by Selenomonas ruminantium in the absence and presence of methanogenic bacteria. Appl. Microbiol. 29: 480-483.

25. 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 diffusion and ebullition. Limnol. Oceanogr. (In press.)

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

27. Toerien, D.F. and W.H.J. Hattingh. 1969. Anaerobic digestion. I. The microbiology of anaerobic digestion. Water Res. 3: 385-416.

28. 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.

29. Wetzel, R.G. 1975. Limnology. W.B. Saunders, Co. Philadelphia. 743pp.

30. Winfrey, M.R. and J.G. Zeikus. 1977. The effect of sulfate on carbon and electron flow during microbial methanogenesis in freshwater sediments. Appl. Environ. Microbiol. 33: 275-281.

31. Wolin, M.J. 1974. Metabolic interactions among intestinal microorganisms. Am. J. Clin. Nutrition. 27: 1320-1328.

32. Wood, E.D., F.A.J. Armstrong and F.A. Richards. 1967. Determination of nitrate in sea water by cadmium-copper reduction to nitrite. J. Mar. Biol. Assoc. U.K. 47: 23-31.

CHAPTER IV

METABOLISM OF SESTON IN ANAEROBIC LAKE SEDIMENTS

INTRODUCTION

Studies of the sedimentation and subsequent metabolism of particulate organic matter (POM) in eutrophic lake sediments have demonstrated that considerable anaerobic processing occurs in these habitats (Robinson, 1978; Strayer and Tiedje, 1978a; Chapters II and III of this volume). Anaerobic processing in these sediments appears to be directly related to the amount and nature of available organic substrate (Chapter III). However, little is presently known of the factors which control the stages of anaerobic metabolism in eutrophic lake sediments, and of how these factors affect the rate, extent, and end products of decomposition of POM.

The rate of organic loading may be a critical factor in controlling anaerobic metabolism in anoxic lake sediments. Dramatic declines in methane release from the sediments of Wintergreen Lake were observed to follow sharp increases in organic input to the sediments, suggesting a temporary overloading of the system (Chapter III). Methane production resumed in these sediments only after the rate of loading had diminished. Robinson (1978) observed a similar decline in methane production following peak sedimentation rates in Frains Lake, Michigan.

In addition to the rate of organic loading, the quality of organic matter reaching lake sediments is important in determining the rate and nature of anaerobic metabolism occurring in these habitats (Chapters II and III). Highest rates of methane production and release from Wintergreen Lake pelagic sediments have been linked to degradation of freshly deposited algal material (Chapter III). Seston reaching the pelagic sediments of Wintergreen Lake has been shown to contain a relatively high amount of protein, likely in the form of easily metabolizable amino acids and peptides. Moreover, the seston present in the pelagic zone of this lake is enriched in simple sugars and monomers relative to structural carbohydrates, further increasing its quality in terms of ease of metabolism (Chapter II).

Strayer and Tiedje (1978b) have demonstrated a high affinity of Wintergreen Lake sediment microbial communities for hydrogen, suggesting that hydrogen-utilizing reactions are capable of maintaining hydrogen concentrations at very low levels in these sediments. Their results suggest that the prerequisites for the occurrence of interspecies hydrogen transfer (Wolin, 1974) are present in Wintergreen Lake sediments, and that substrate utilization in this habitat may be highly reliant on coupled metabolism. If interspecies hydrogen transfer occurs in Wintergreen sediments a perturbation affecting hydrogen or methane production should alter the rate and extent of substrate utilization, as well as the quantitative and qualitative nature of

fermentation end products. Thus, increasing the hydrogen concentration experimentally should cause methanogenesis to continue at the same or faster rate (due to hydrogen being limiting) and fermentation end products should accumulate. Addition of chloromethanes, which inhibit methanogenesis with some degree of selectivity (Bauchop, 1967; Thiel, 1969; Sykes and Kirsch, 1972) should also affect H_2 transfer and volatile metabolite pool sizes in these sediments.

Periodic intrusions of alternative electron acceptors (nitrate and sulfate) to the anoxic sediments of eutrophic lakes, both during stratified periods and during lake turnover, have also been shown to cause temporary reduction or inhibition of methane production in these sediments (Chapter III; Robinson, 1978). However, the effect of such nitrate and sulfate intrusions on the quantitative and qualitative nature of the volatile fatty acids formed, and the rate and extent of substrate utilization as a result of uncoupling from the preferred electron acceptor (CO_2) have not been examined.

The objectives of the present laboratory study were to compare the rate, extent, and end products of anaerobic degradation of algal material in Wintergreen Lake pelagic sediments with those determined *in situ*, and to study in greater detail some of the critical factors controlling anaerobic metabolism in these sediments.

MATERIALS AND METHODS

Sampling. Sediment samples for laboratory experiments were collected from the pelagic zone of Wintergreen Lake (Chapter II) using an Ekman dredge. Mason jars were filled to overflowing with surface sediment and the lids tightly screwed in place. The sealed jars were immediately returned to the laboratory and transferred into an anaerobic glove bag (Coy Manufacturing Co., Ann Arbor, Michigan) containing an atmosphere of $N_2/H_2/CO_2$ (85/10/5, v/v)

Seston experiments. Freshly collected surface sediment was transferred to a 4-liter beaker in the anaerobic glove bag and mixed well. Thirty-ml samples of the homogenized sediment were dispensed by syringe into 120-ml Wheaton serum bottles (Scientific Products, Detroit, Michigan) containing 30 mg each of freeze-dried algal substrate (seston). Large volumes of Wintergreen Lake seston were previously collected by pumping and filtration of epilimnetic water through 30-um mesh nets. The seston, which consisted primarily of the blue-green alga Anabaena, was lyophilized for use in metabolic experiments. Serum bottles containing the lyophilized seston were placed in the anaerobic glove bag at least 24 hours before initiation of experiments to allow the substrate to pre-reduce. Amounts of seston added were chosen to simulate the mean sedimentation rate observed in Wintergreen Lake during 1976 (Chapter II).

The serum bottles were divided into five sets. One set received no further addition and the bottles were sealed

with butyl rubber stoppers (Catalog no. 2048-11800; Bellco Glass Co., Vineland, N.J.). A second and third set were amended with pre-reduced $NaNO_3$ and Na_2SO_4 to give a final concentration of 10 mM of each per bottle. An additional set of bottles was amended with N_2-sparged chloroform to give a final concentration of 0.03%. All bottles were sealed with butyl stoppers and removed from the glove bag. A fifth set of bottles was pressurized to a final pressure of 1.3 atmospheres with O_2-scrubbed, 100% H_2, utilizing a pressurizing manifold described by Balch and Wolfe (1976). The H_2-treated bottles were repressurized daily during the course of the experiment to maintain the H_2 partial pressure at approximately 1.3 atmospheres. The remaining four sets of bottles were flushed with O_2-free 100% N_2 using the Hungate technique (Hungate, 1950) to reduce the headspace H_2 concentration to 0.01% or less. Appropriate sediment control bottles were included for each of the treatments described.

The bottles were incubated with shaking at 15°C to allow thorough wetting and mixing of the added seston and to allow re-equilibration of dissolved sediment gases with the bottle headspace. Initial duplicate samples were analyzed after 1 hour for pressure, methane concentration, and volatile fatty acid concentration. Duplicate bottles of each treatment were sacrificed at intervals over a 22 day period and analyzed for pressure, methane concentration, and volatile fatty acid concentration.

Organic loading experiments. Thirty-ml of freshly-collected Wintergreen Lake pelagic sediment were added to 120-ml serum bottles in the anaerobic glove bag as described above. Appropriate sets of bottles were amended with 5, 10, 20, and 40 mg each of pre-reduced casein hydrolysate. Sediment control bottles received no addition. The bottles were sealed with butyl stoppers, removed from the glove bag, and flushed with 100% N_2 as described above. Incubation and sampling of the bottles were as described for the seston experiments.

Leachate experiments. Freeze-dried seston was allowed to leach in distilled water for 24 hours at 4°C, and the resulting leachate freeze-dried. The remaining leached seston was thoroughly washed and also freeze-dried. Equal weights (30 mg) of unleached seston, leachate, and leached seston were each pre-reduced and rehydrated with Wintergreen Lake surface sediment. Sample preparation, incubation, and sampling were as described for the seston experiments.

Pressure and gas analysis. Pressure in each serum bottle was measured by piercing the butyl rubber stopper with a 25 g Vacutainer needle connected to a mercury manometer. Methane was analyzed on a Varian model 600D gas chromatograph equipped with a flame ionization detector. Analysis was made using a coiled stainless steel column (2 m by 0.3 cm OD) packed with Porapak N (80/100 mesh; Waters Associates, Framingham, Mass.). Helium was the carrier gas at a flow rate of 20 ml/min. Chromatographic

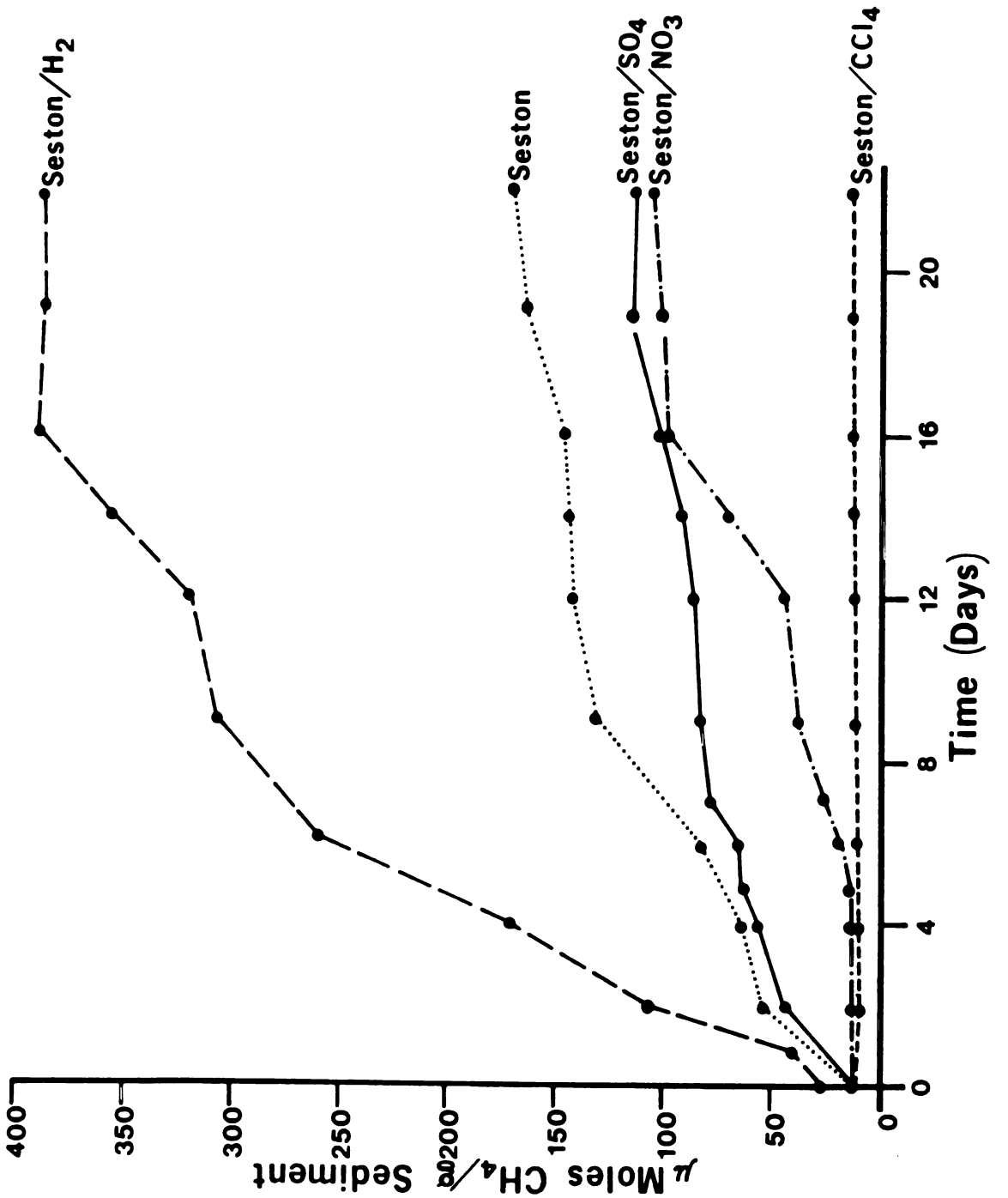
operating conditions were: inlet temperature, 140°C; oven temperature, 50°C; detector temperature, 140°C.

Volatile fatty acid analysis. Interstitial water was obtained from sediment samples by centrifugation. Volatile fatty acids in the interstitial water were converted to their tetrabutylammonium salts by addition of 300 ul of 0.4 M tetrabutylammonium hydrogen sulfate in 0.5 N NaOH to 15 ml of sample water (Bethge and Lindstrom, 1974). The samples were concentrated by freeze-drying, rehydrated in 0.5 ml of 4.4 N H_3PO_4, and analyzed as free fatty acids by gas chromatography. The acids were separated on a coiled glass column (2 m by 0.2 mm ID) packed with 10% SP-1000/1% H_3PO_4 on Supelcoport (100/120 mesh; Supelco, Inc., Bellefonte, Pa.). Analysis was made on either a Packard model 409, or Varian model 2440 gas chromatograph equipped with a hydrogen flame ionization detector. Helium was used as carrier gas at a flow rate of 20 ml/min. Operating temperatures were: column temperature, 140°C; detector temperature, 250°C; injector temperature, 200°C. Quantitative and qualitative analysis was made relative to a known concentration of n-hexanoic acid which was added to the original aqueous sample as an internal standard.

RESULTS

Seston experiments. Figure 1 shows the production of methane from Wintergreen Lake sediments amended with seston, or with a combination of seston and nitrate, sulfate,

Figure 1. Amount of methane produced by Wintergreen Lake sediments amended with seston, or with seston/nitrate, seston/sulfate, seston/chloroform, and seston/hydrogen. Each point is the mean value of duplicate determinations.

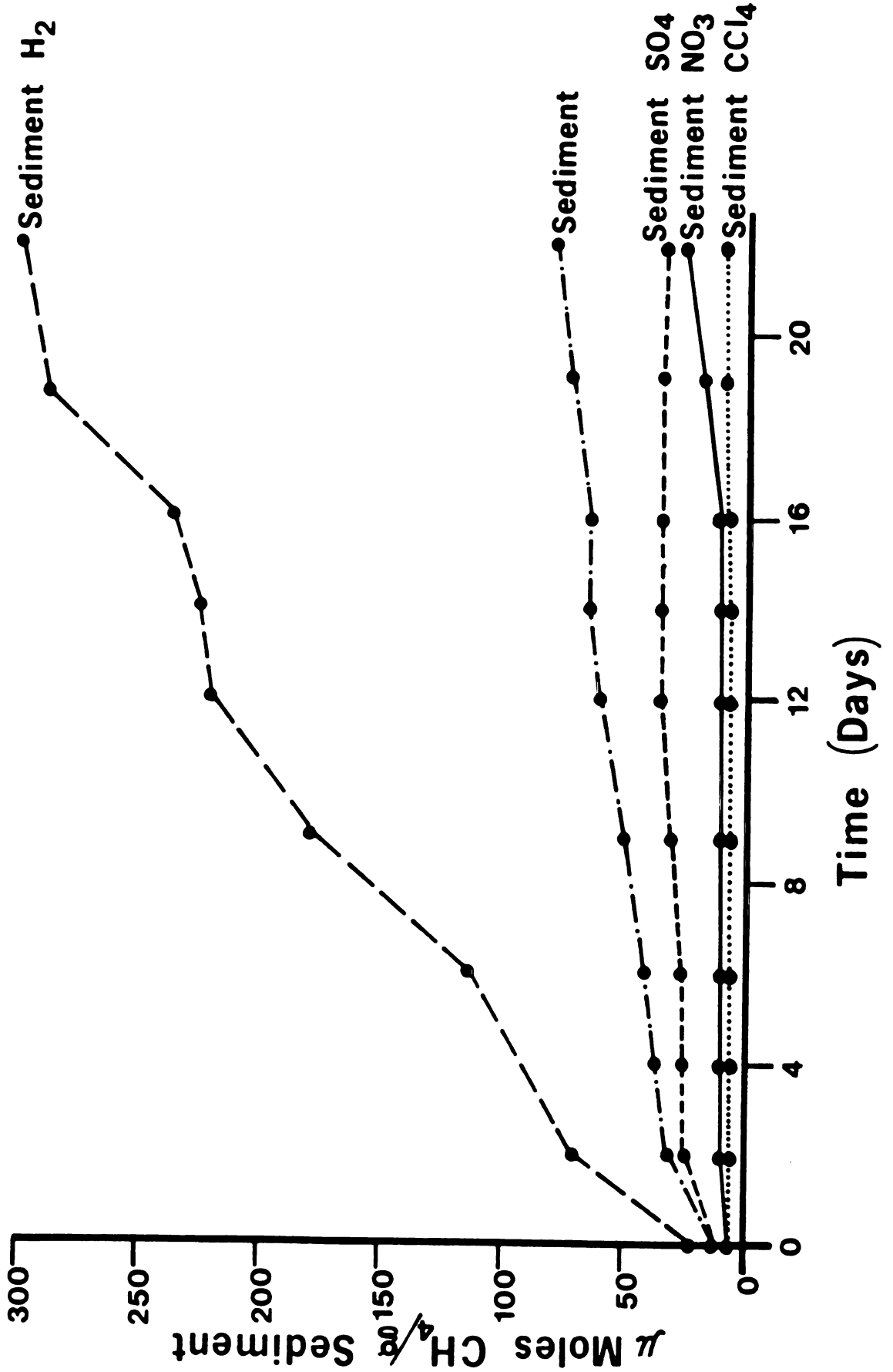

Seston/H_2
Seston
Seston/SO_4
Seston/NO_3
Seston/CCl_4
μMoles CH_4/gm Sediment
Time (Days)
0
50
100
150
200
250
300
350
400
4
8
12
16
20

chloroform, or hydrogen respectively. Figure 2 shows the rate of methane production from unamended sediment, and from sediment amended with nitrate, sulfate, chloroform, and hydrogen respectively. Seston-amended sediments showed an increased rate of methane production relative to the sediment control for 9 days, before the rate began to approach that of the control. The addition of sulfate reduced the rate of methane production in both the sediment samples and in those containing seston. In the sediment/SO_4 treatment, methane production ceased after 12 days while in the seston/SO_4 samples, methane production continued for 19 days before it ceased.

Nitrate completely inhibited methane production in both the sediment and seston treatments during the initial portion of the experiment. However, methane production resumed in both samples after denitrification had exhausted the added nitrate. The resumption of methane production occurred much sooner (day 5) in the seston/NO_3 samples than in the sediment/NO_3 samples (day 16).

The continuous addition of hydrogen initially increased methane production markedly in both the sediment and seston samples, the rate of methanogenesis being much greater in the seston/hydrogen treatment compared to the sediment/hydrogen samples. Methanogenesis continued in the sediment/hydrogen samples throughout the course of the experiment (Figure 2), while methane production ceased in the seston/hydrogen samples after 16 days (Figure 1),

Figure 2. Amount of methane produced by Wintergreen Lake sediments amended with nitrate, sulfate, chloroform, or hydrogen. Each point is the mean value of duplicate determinations.


Sediment H_2
Sediment
Sediment SO_4
Sediment NO_3
Sediment CCl_4
μ Moles CH_4/g Sediment
Time (Days)
300
250
200
150
100
50
0
0
4
8
12
16
20

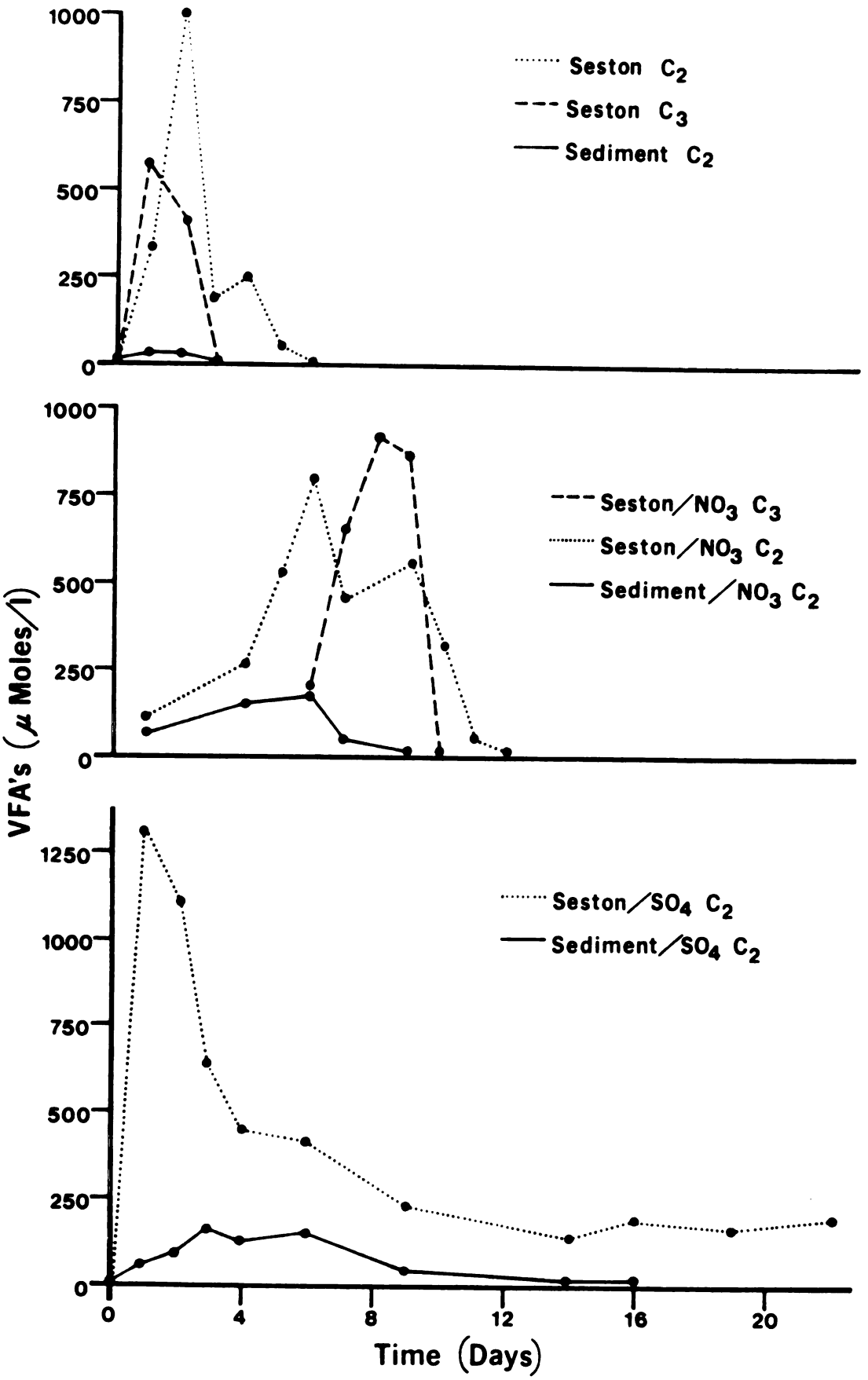
presumably the result of the accumulation of high concentrations of volatile fatty acids in these treatments (Figure 7). The addition of chloroform irreversibly inhibited methane production in both sediment and seston samples (Figures 1 and 2).

The time course of volatile fatty acid concentrations in samples amended with seston, or with seston and nitrate or sulfate respectively, are presented in Figure 3. Acetate and propionate were the major volatile acids produced in the seston-amended sediments. Peak concentrations of acetate occurred in the first 2 days before levels declined to undetectable concentrations after 6 days. Propionate concentrations were highest after 1 day and similarly declined to undetectable levels after 3 days. Acetate concentrations in sediment control samples rose briefly during the initial 2 days of the experiment, then also declined to undetectable levels (Figure 3).

Acetate and propionate were also the predominant acids detected in the seston/NO_3 treatments. Accumulation of each acid was delayed relative to the seston samples, maximum values of acetate and propionate not occurring until day 6 and day 8 respectively. Concentrations of each acid then steadily declined to undetectable levels after approximately 12 days.

Initial acetate levels were higher in the seston/SO_4 treatments relative to the seston samples (Figure 3). Concentrations of acetate also declined much more slowly in

Figure 3. Volatile fatty acid concentrations in Wintergreen Lake pelagic sediments amended with seston, seston/nitrate, or seston/sulfate. (C_2 = acetate; C_3 = propionate).


1000
750
500
250
0
Seston C_2
Seston C_3
Sediment C_2
1000
750
500
250
0
Seston/NO_3 C_3
Seston/NO_3 C_2
Sediment/NO_3 C_2
VFA's (μ Moles/l)
1250
1000
750
500
250
0
Seston/SO_4 C_2
Sediment/SO_4 C_2
0
4
8
12
16
20
Time (Days)

the seston/SO_4 samples relative to the seston treatments, acetate remaining detectable over the entire course of the experiment (22 days). No propionate was determined in the seston/SO_4 treatments (Figure 3).

The addition of chloroform resulted in the accumulation of large quantities of volatile acids in both the sediment/CCl_4 and seston/CCl_4 samples (Figures 4 and 5). In both instances, volatile acids accumulated at a relatively moderate rate until day 5 when the rate of acid accumulation increased sharply. Volatile acids accumulated in the same general order in all chloroform-treated samples, acetate concentrations being the highest followed by lesser amounts of propionate, butyrate, valerate or iso-valerate, or iso-butyrate. Iso-caproate was detected in the seston/chloroform samples, but not in the sediment/chloroform samples. The quantity of each individual acid determined was greater in the seston/chloroform samples relative to the sediment/chloroform treatments.

The continuous addition of hydrogen to Wintergreen sediments and to sediments amended with seston also resulted in the accumulation of large quantities of volatile fatty acids (Figures 6 and 7). As in the case of chloroform-amended samples, acetate accumulated in the greatest amounts followed by propionate, butyrate, iso-valerate, valerate, iso-butyrate, and either caproate or iso-caproate. Greater concentrations of each acid were found in the seston/hydrogen samples. Levels of acetate and propionate began to increase

Figure 4. Volatile fatty acid concentrations in Wintergreen Lake pelagic sediments amended with chloroform. (C_2 = acetate; C_3 = propionate; i-C_4 = iso-butyrate; C_4 = butyrate; i-C_5 = iso-valerate; C_5 = valerate).

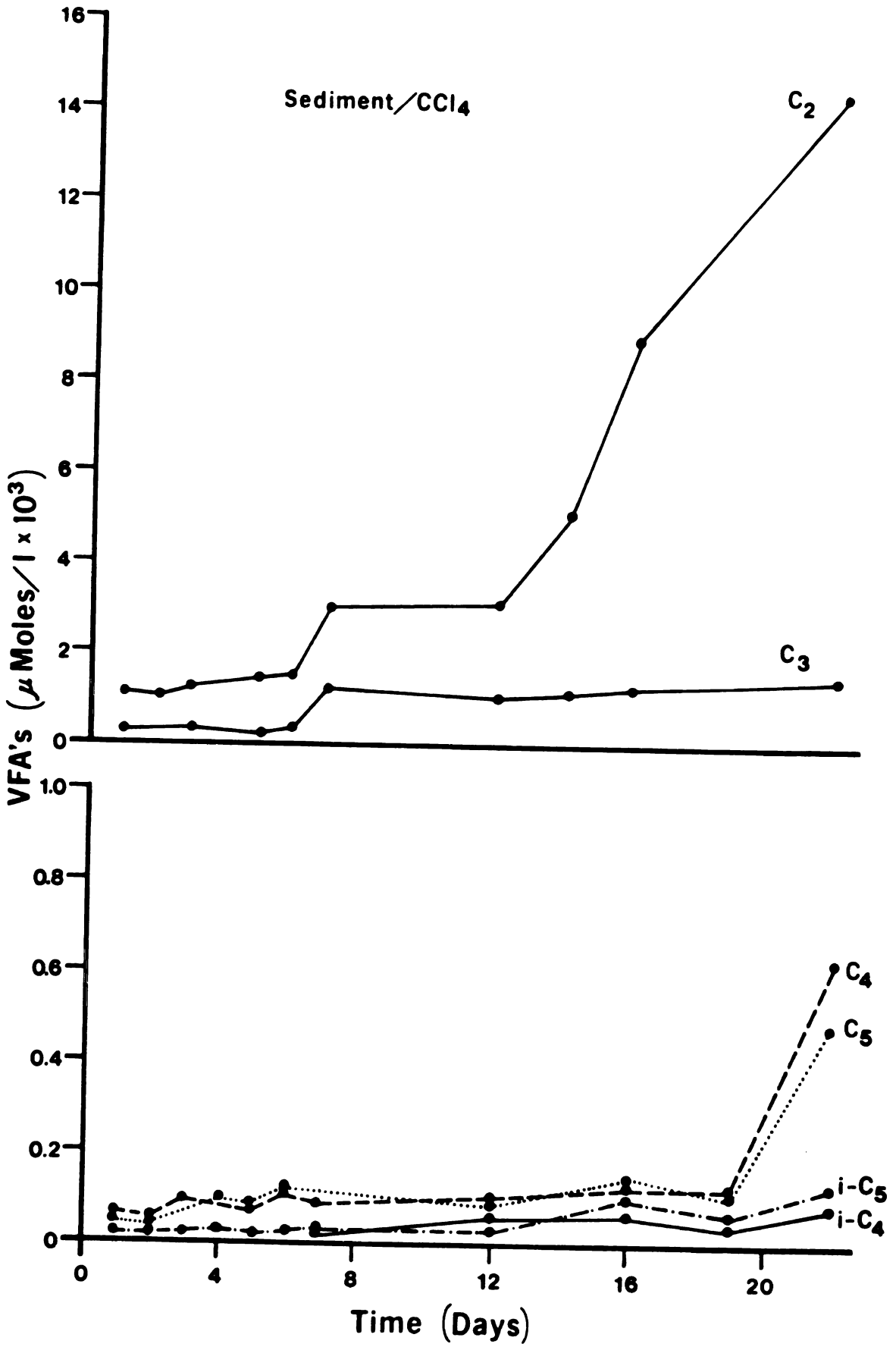

Sediment/CCl_4
C_2
C_3
C_4
C_5
i-C_5
i-C_4
VFA's (μMoles/l x 10^3)
Time (Days)
0
2
4
6
8
10
12
14
16
0.2
0.4
0.6
0.8
1.0
4
8
12
16
20

Figure 5. Volatile fatty acid concentrations in Wintergreen Lake pelagic sediments amended with seston and chloroform. (C_2 = acetate; C_3 = propionate; i-C_4 = iso-butyrate; C_4 = butyrate; i-C_5 = iso-valerate; C_5 = valerate; i-C_6 = iso-caproate).

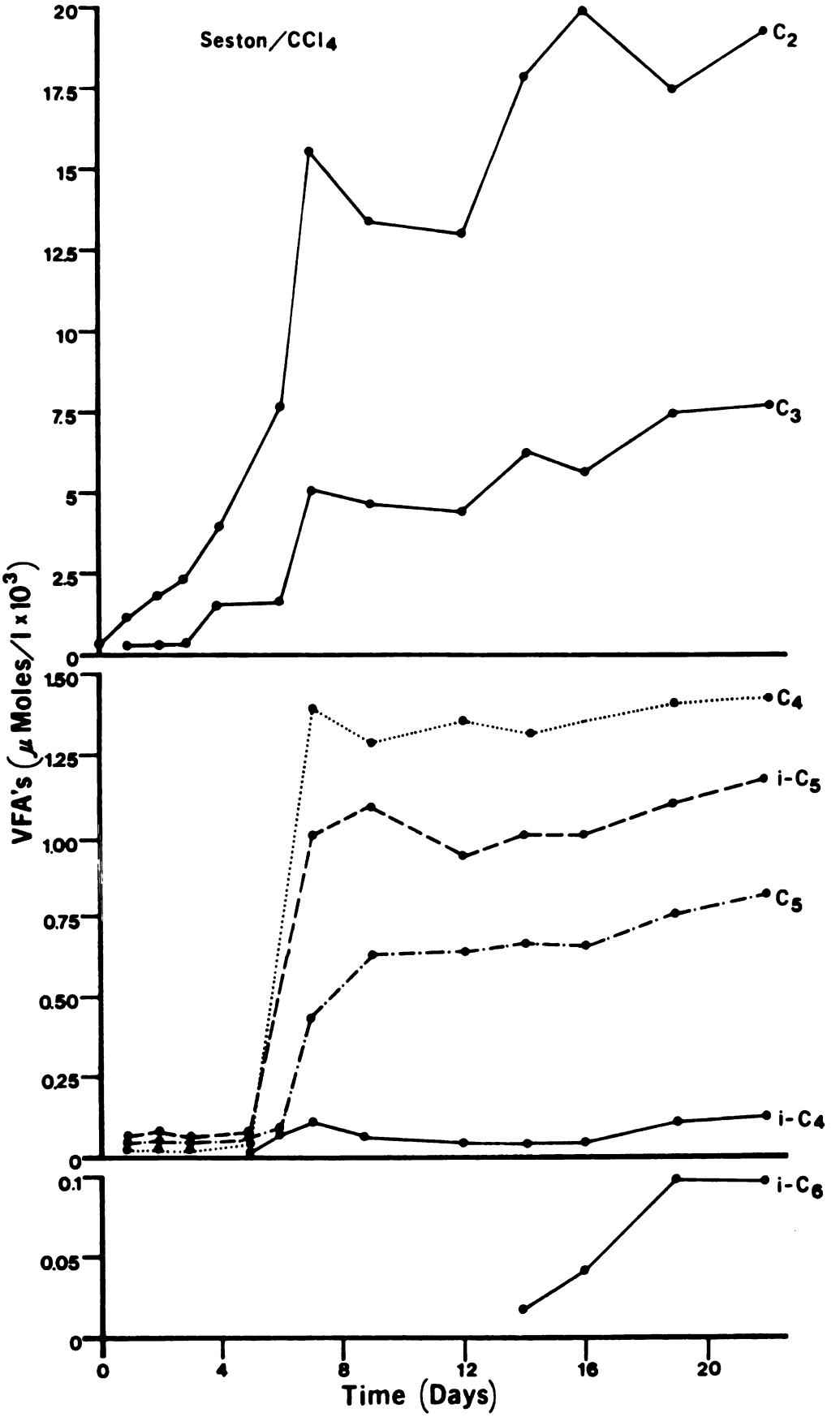

Seston/CCl4
C2
C3
C4
i-C5
C5
i-C4
i-C6
VFA's (μ Moles/l x 10^3)
Time (Days)
20
17.5
15
12.5
10
7.5
5
2.5
0
1.50
1.25
1.00
0.75
0.50
0.25
0
0.1
0.05
0
0
4
8
12
16
20

Figure 6. Volatile fatty acid concentrations in Wintergreen Lake pelagic sediments amended with hydrogen (C_2 = acetate; C_3 = propionate; i-C_4 = iso-butyrate; C_4 = butyrate; i-C_5 = iso-valerate; C_5 = valerate; i-C_6 = iso-caproate; C_6 = caproate).

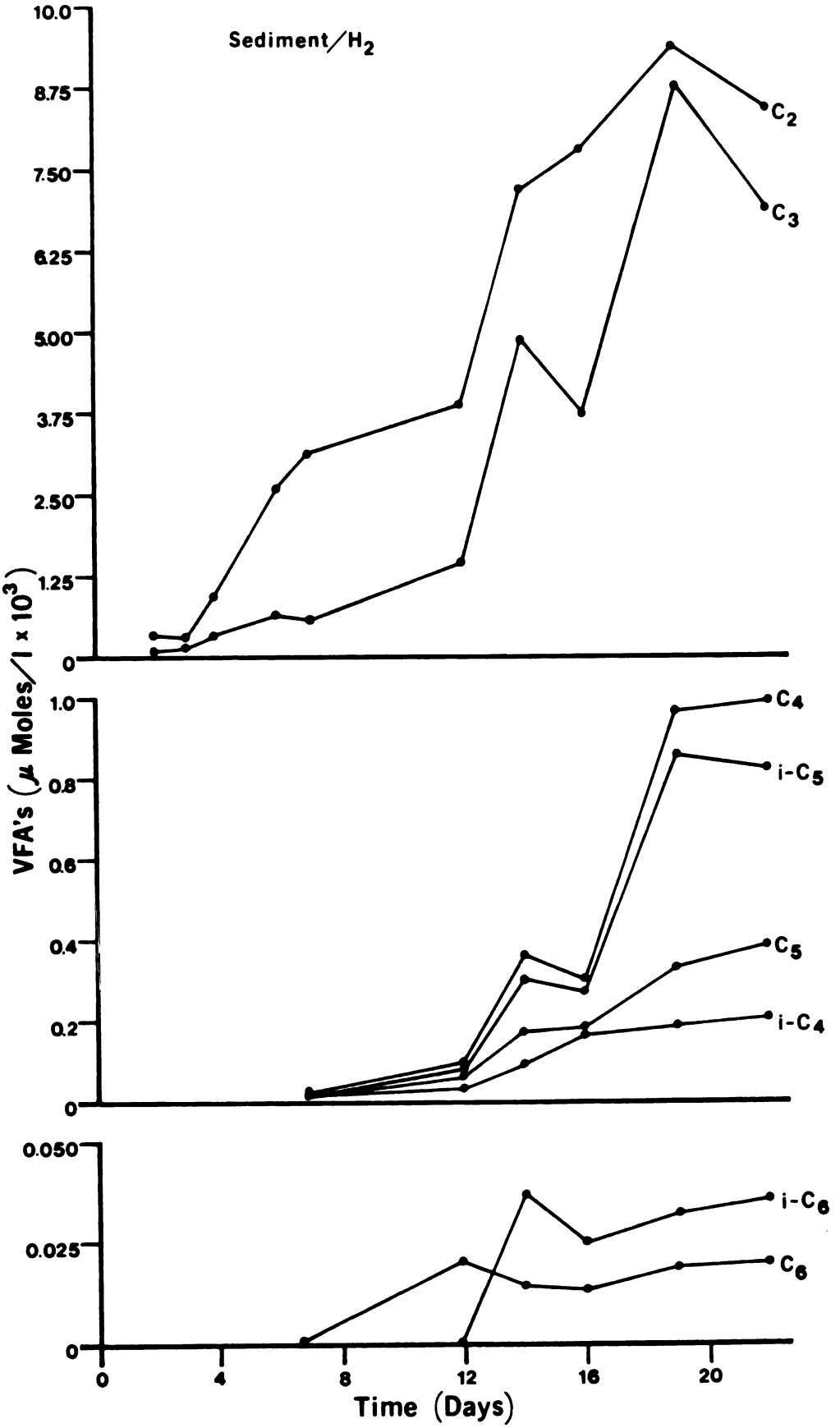
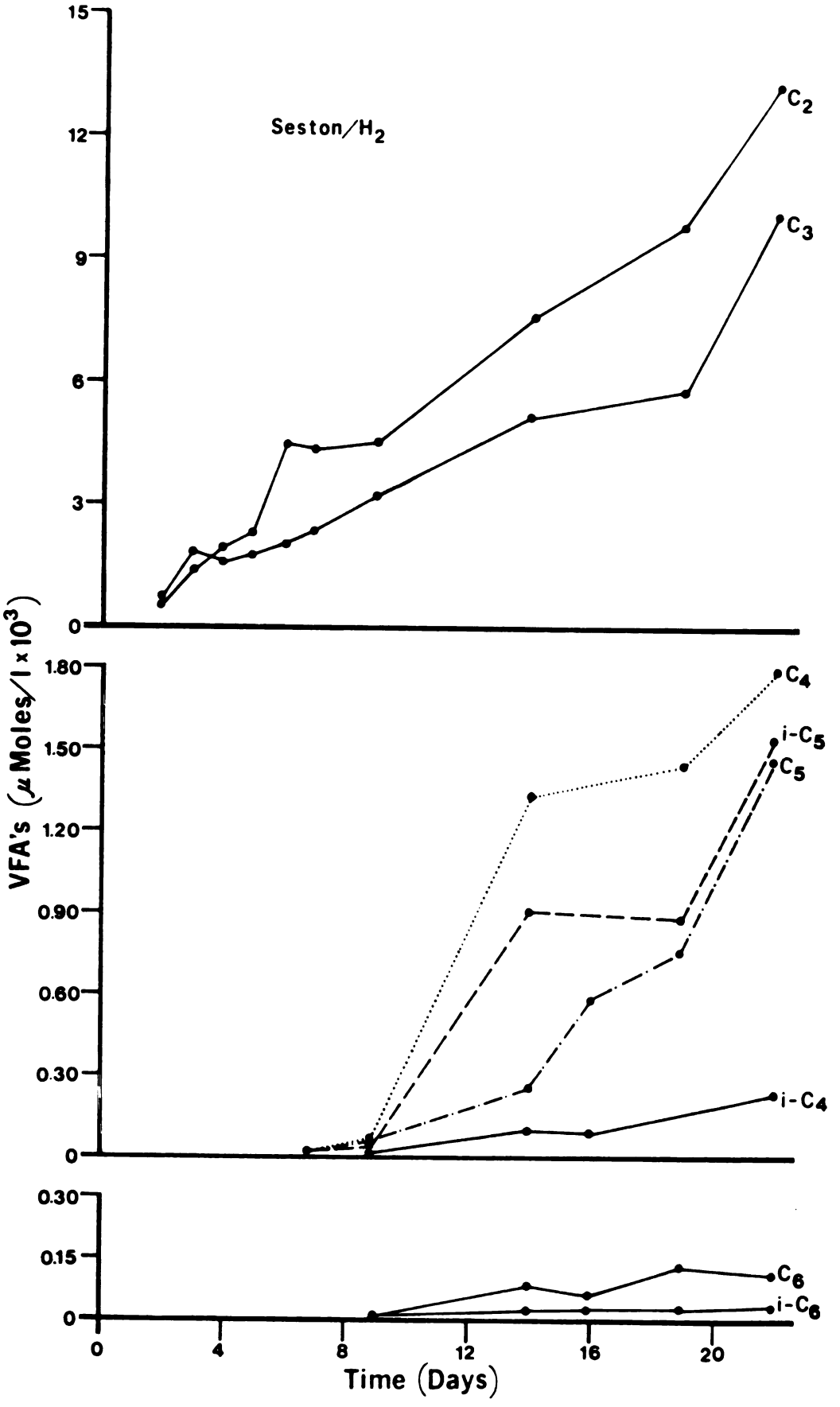

Sediment/H_2
C_2
C_3
C_4
i-C_5
C_5
i-C_4
i-C_6
C_6
VFA's (μ Moles/1 x 10^3)
Time (Days)
10.0
8.75
7.50
6.25
5.00
3.75
2.50
1.25
0
1.0
0.8
0.6
0.4
0.2
0.050
0.025
4
8
12
16
20

Figure 7. Volatile fatty acid concentrations in Wintergreen Lake pelagic sediments amended with seston and hydrogen.


Seston/H_2
C_2
C_3
C_4
i-C_5
C_5
i-C_4
C_6
i-C_6
VFA's (μMoles/l x 10^3)
Time (Days)
15
12
9
6
3
0
1.80
1.50
1.20
0.90
0.60
0.30
0
0.30
0.15
0
0
4
8
12
16
20

within 2 days, while the remaining volatile acids did not reach detectable levels until after 7 days. The propionate/acetate ratio was also found to be greater in the hydrogen-treated samples (Figures 6 and 7) relative to the chloroform-treated samples (Figures 4 and 5).

Leachate experiments. Figure 8 shows the rates of methane production from sediments amended with equal amounts of seston, leachate, and leached seston respectively. Initial methane production rates were 35% greater in samples to which unleached seston or leachate were added relative to sediment amended with leached seston. After 4 days methane production rates were the same in all three samples, remaining linear until day 6, when all three began to approach the same rate as the sediment control (Figure 8). Figure 9 shows the differences in volatile acid concentrations detected when equal weights of leached seston and leachate were added to sediments. Volatile acid concentrations were much higher in leachate-amended sediment compared to sediments amended with leached seston. Qualitatively, acetate predominated in leachate samples followed by decreasing amounts of propionate, valerate, butyrate, and iso-valerate. Leached seston, in contrast, supported lesser amounts of acetate and propionate. Additional volatile acids were not detected (Figure 9).

Organic loading experiments. Addition of increasing amounts of casein hydrolysate to Wintergreen Lake pelagic

Figure 8. Amount of methane produced by Wintergreen Lake pelagic sediments amended with seston, leached seston, and leachate.

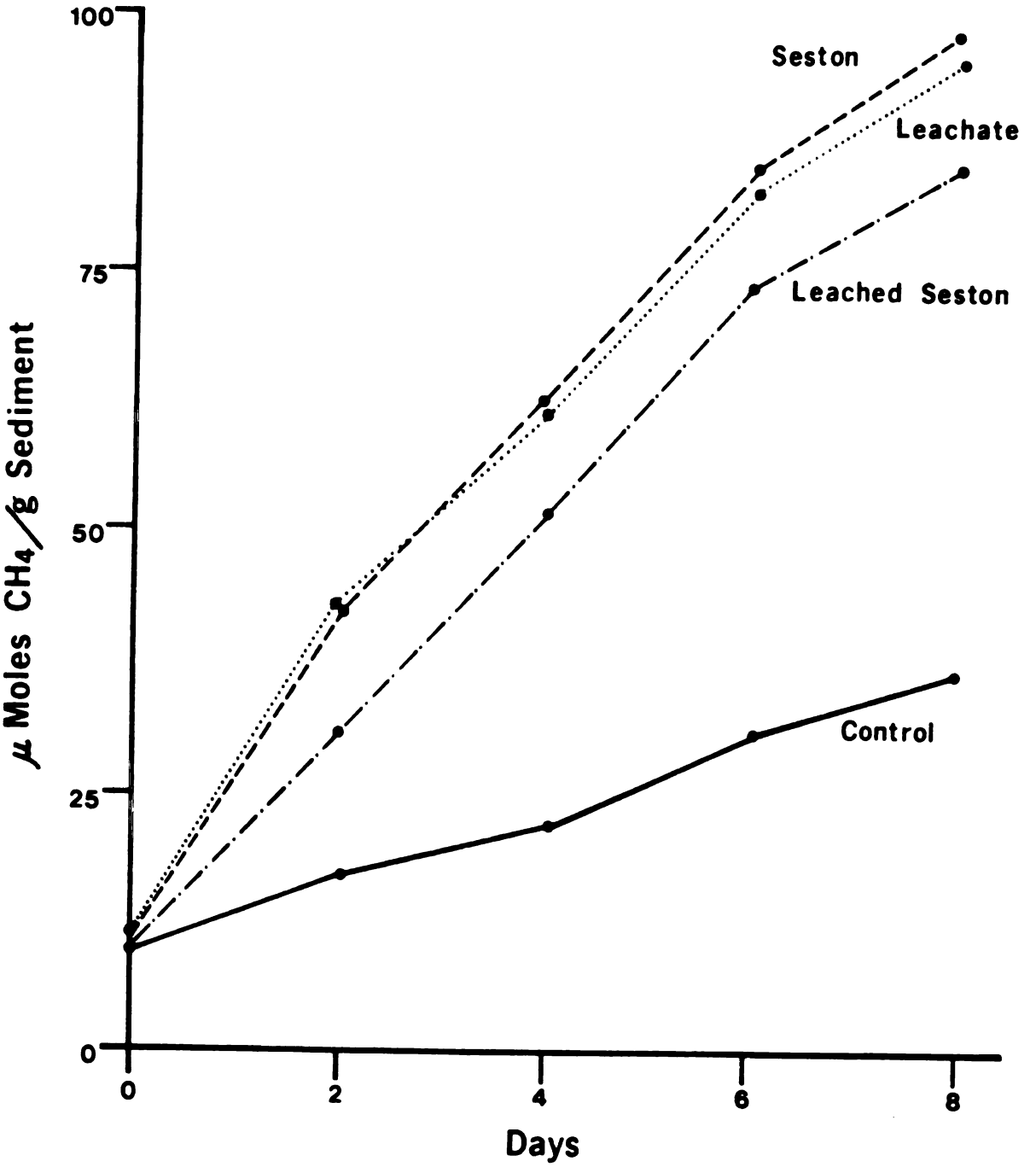
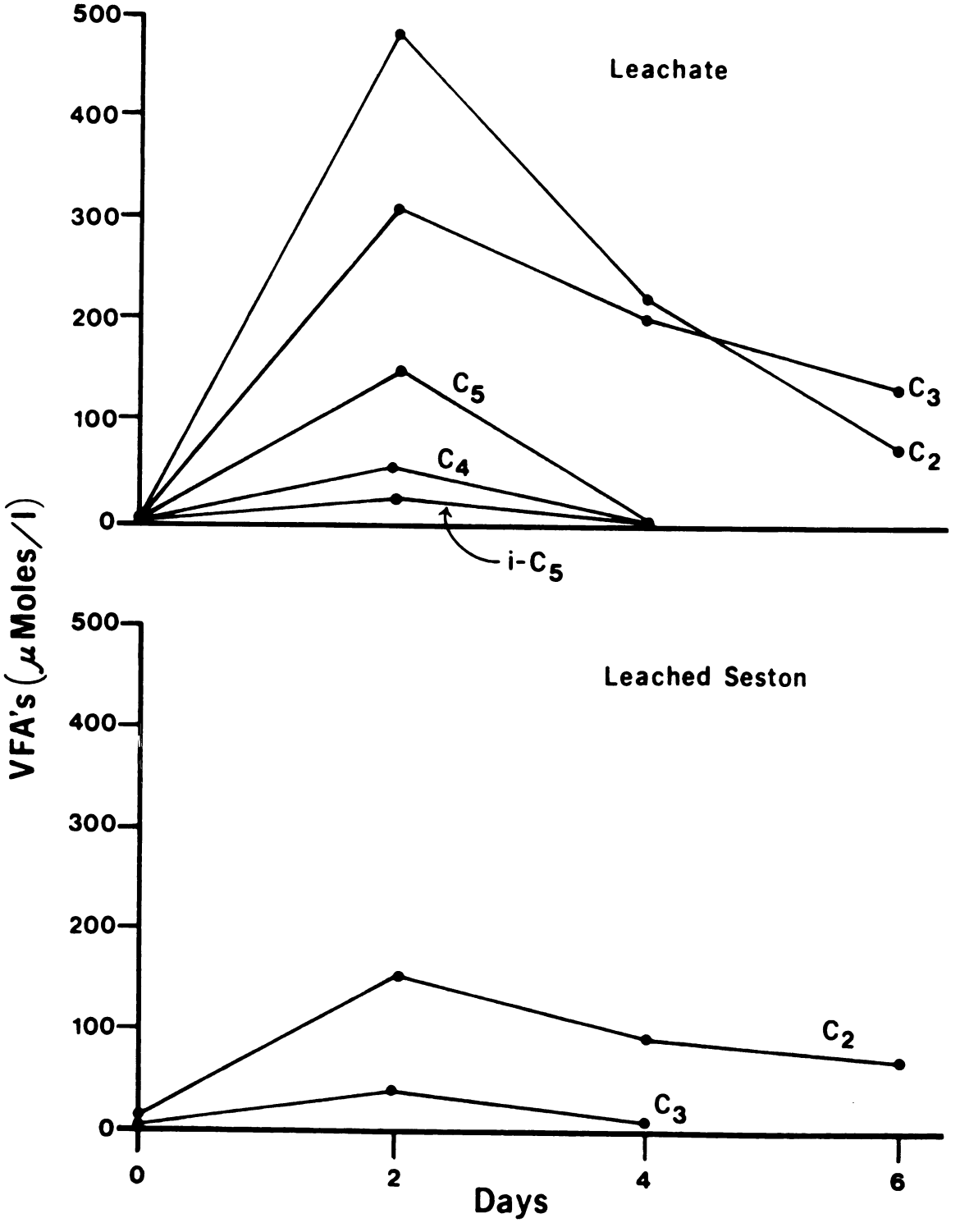

100
75
50
25
0
μ Moles CH_4/g Sediment
Seston
Leachate
Leached Seston
Control
0
2
4
6
8
Days

Figure 9. Volatile fatty acid concentrations in Wintergreen Lake pelagic sediments amended with leachate and with leached seston.


Leachate
500
400
300
200
100
0
C_5
C_4
C_3
C_2
i-C_5
Leached Seston
500
400
300
200
100
0
C_2
C_3
0
2
4
6
Days
VFA's (μMoles/l)

sediments resulted in increased rates of methanogenesis relative to the unamended control (Figure 10). During the first 24 hours, rates of methane production were directly related to the amount of substrate added, up to 10 mg casein hydrolysate. Addition of greater amounts of casein (20 and 40 mg) resulted in no further increase in the rate of methanogenesis during the initial 24 hours (Figure 10). Rates of methane production remained similar in sediment samples amended with 10, 20, and 40 mg respectively of casein for 48 hours, after which methane production was directly related to substrate concentration in all amended samples. The highest rates occurred in sediment amended with 40 mg of casein hydrolysate.

Acetate and propionate were the major volatile fatty acids detected in sediment amended with 5 mg of casein. Maximum concentrations of each acid occurred within 2 days before levels rapidly declined after 4 days (Figure 11). Acetate also predominated in the 10 mg treatments followed by decreasing quantities of propionate, iso-butyrate, iso-valerate, and valerate (Figure 12). Concentrations of propionate exceeded those of acetate after 2 days in both the 20 and 40 mg treatments. Increased amounts of iso-valerate, iso-butyrate, butyrate, valerate, and iso-caproate were also determined in these samples (Figures 13 and 14). Concentrations of each individual volatile acid detected increased with increased concentration of casein hydrolysate added; the ratio of propionate/acetate detected also

Figure 10. Amount of methane produced by Wintergreen Lake pelagic sediments amended with increasing amounts of casein hydrolysate.

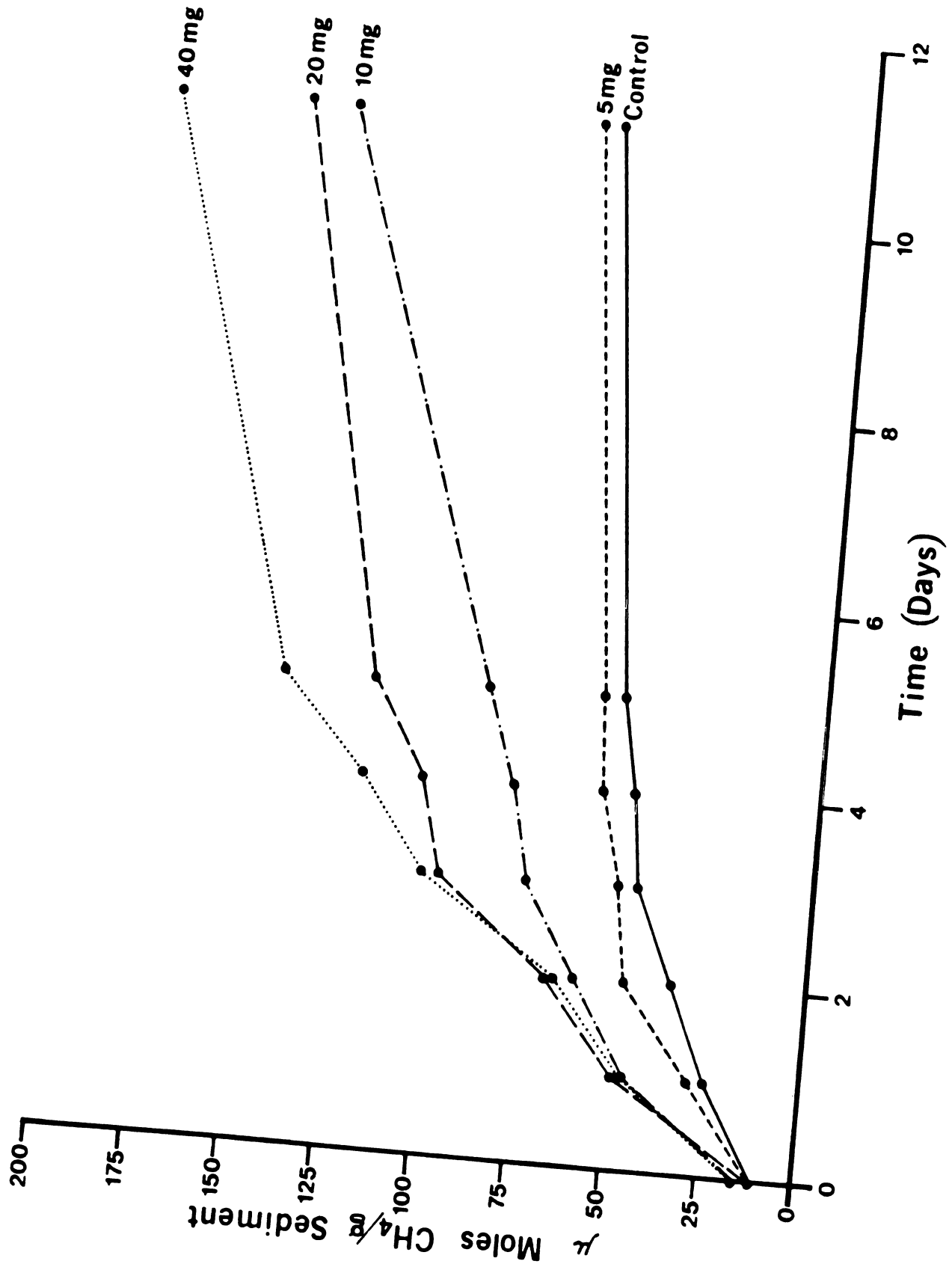

40 mg
20 mg
10 mg
5 mg
Control
Time (Days)
0
2
4
6
8
10
12
Moles CH4/g Sediment
0
25
50
75
100
125
150
175
200

Figure 11. Volatile fatty acid concentrations in Wintergreen Lake pelagic sediments amended with 5 mg of casein hydrolysate. Sediment volume was 30 ml.

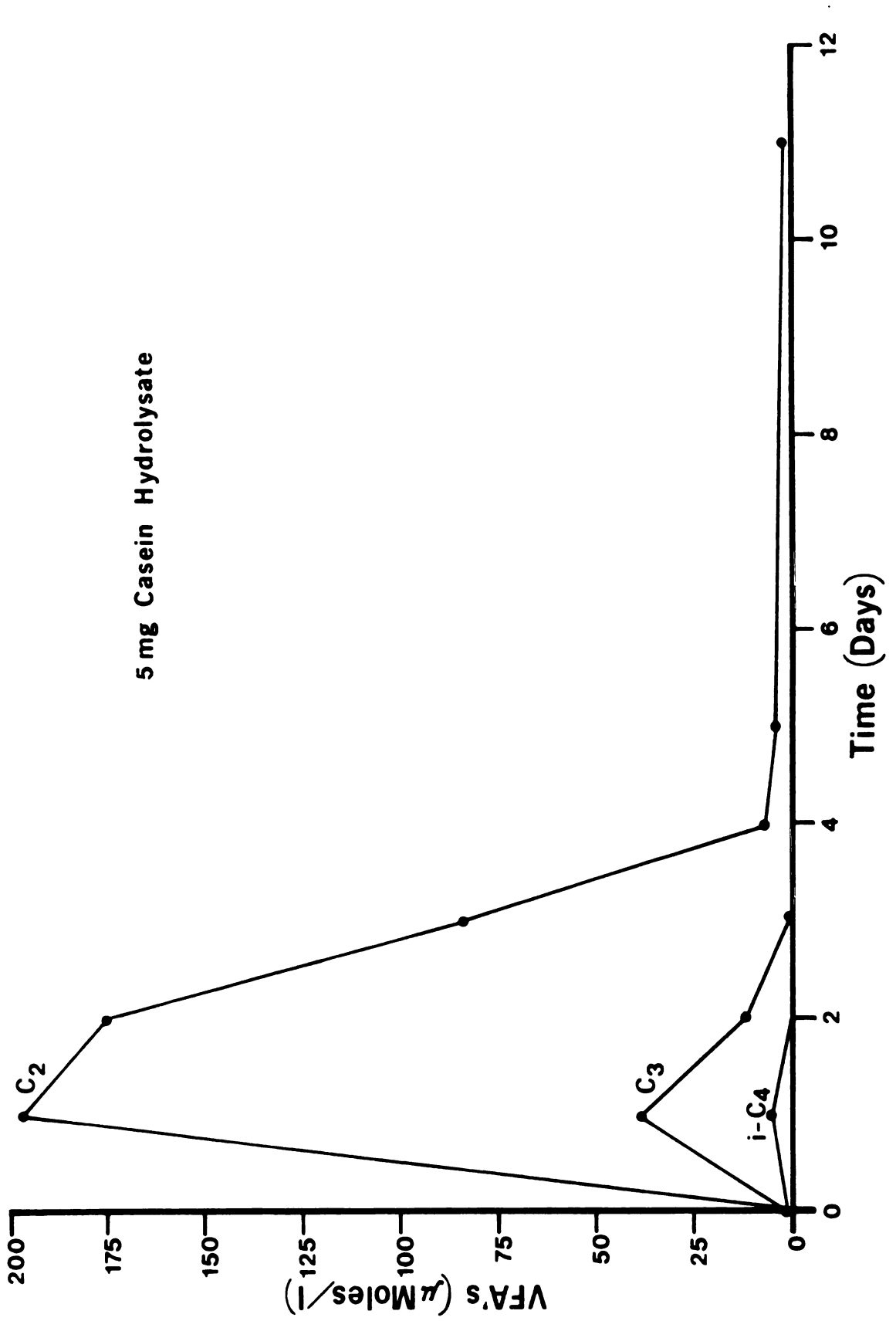

5 mg Casein Hydrolysate
VFA's (μMoles/l)
200
175
150
125
100
75
50
25
0
0
2
4
6
8
10
12
Time (Days)
C2
C3
i-C4

Figure 12. Volatile fatty acid concentrations in Wintergreen Lake pelagic sediments amended with 10 mg of casein hydrolysate. Sediment volume was 30 ml.

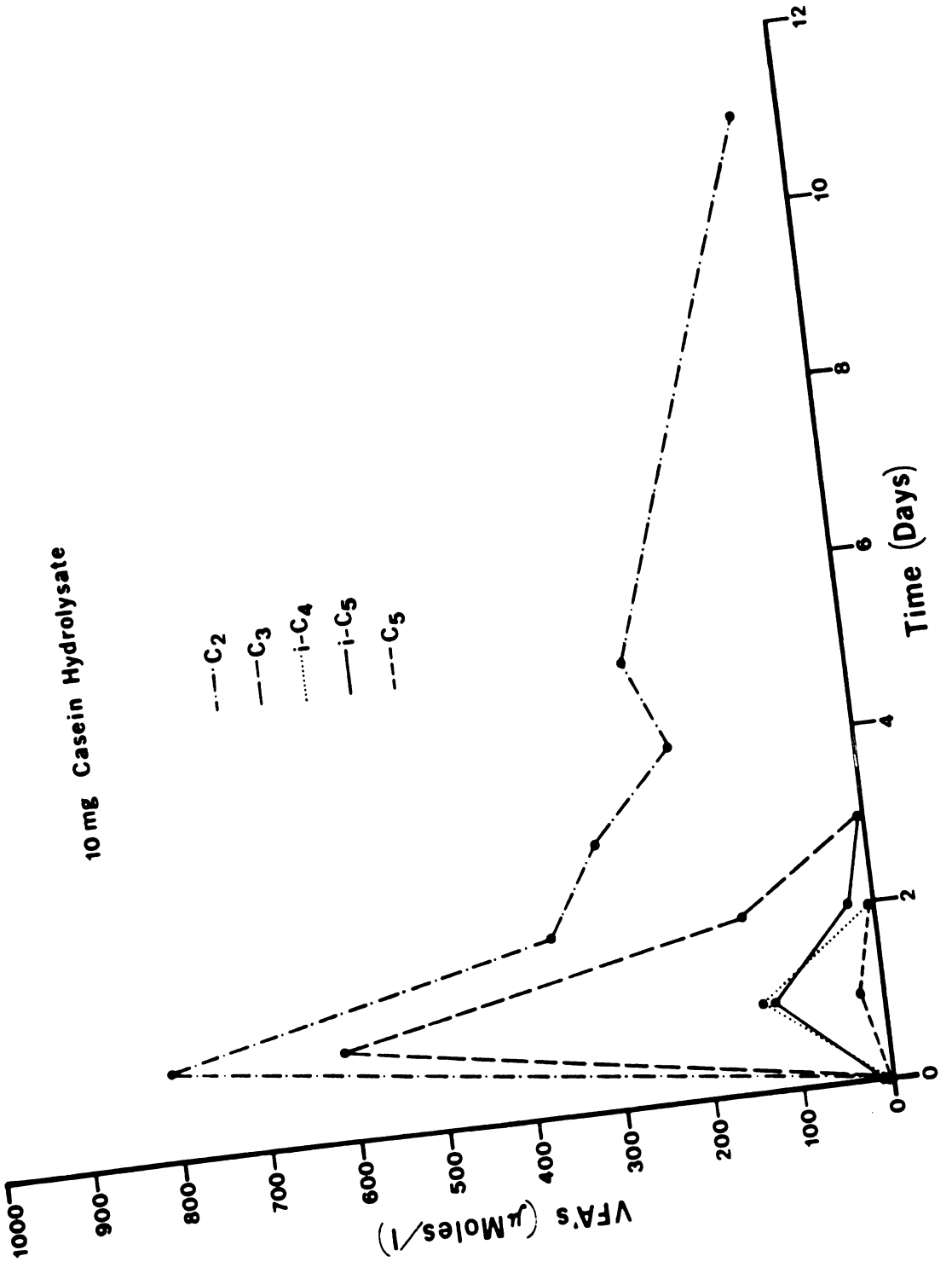

10 mg Casein Hydrolysate
C2
C3
i-C4
i-C5
C5
VFA's (μMoles/l)
Time (Days)
0
100
200
300
400
500
600
700
800
900
1000
0
2
4
6
8
10
12

Figure 13. Volatile fatty acid concentrations in Wintergreen Lake pelagic sediments amended with 20 mg of casein hydrolysate. Sediment volume was 30 ml.

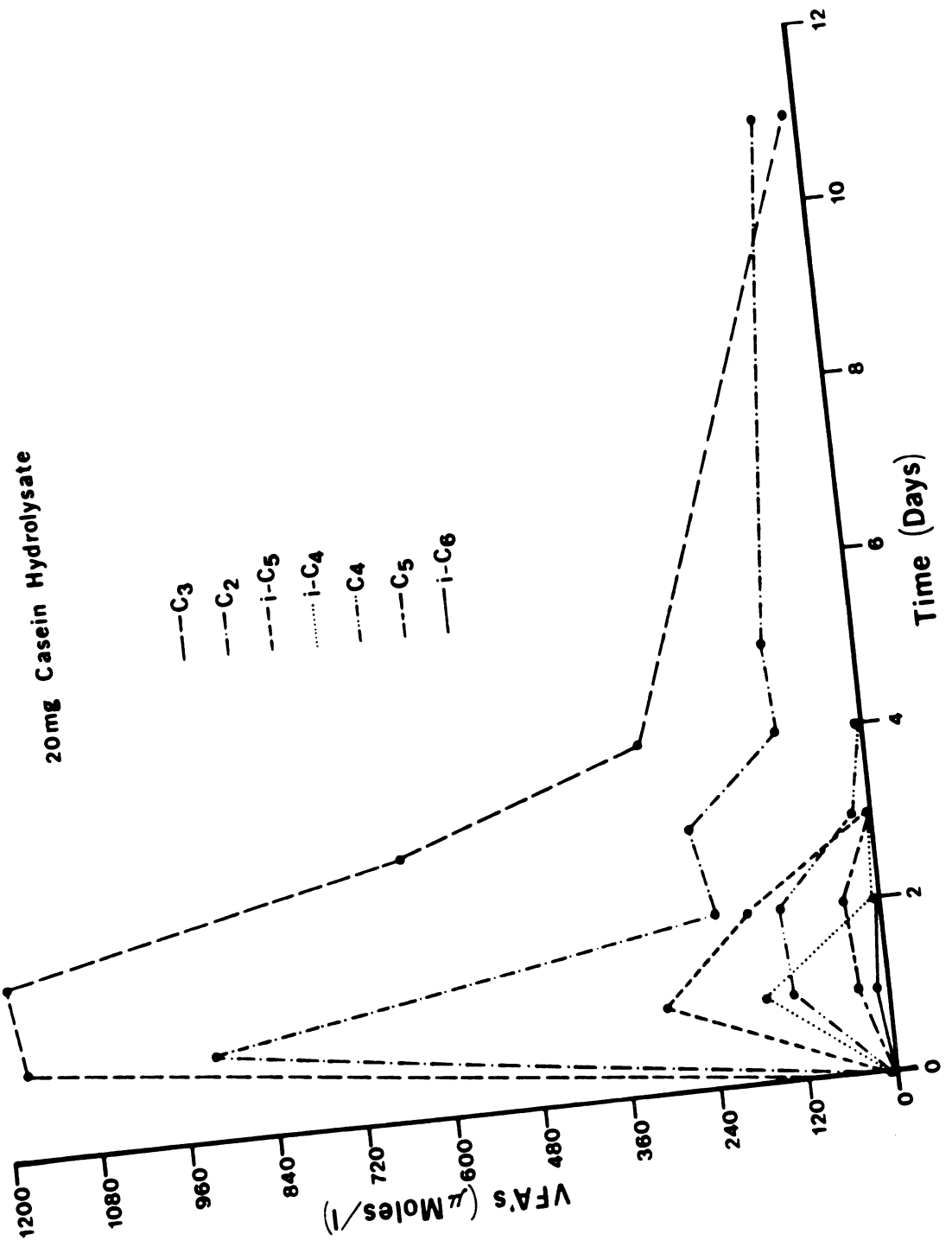
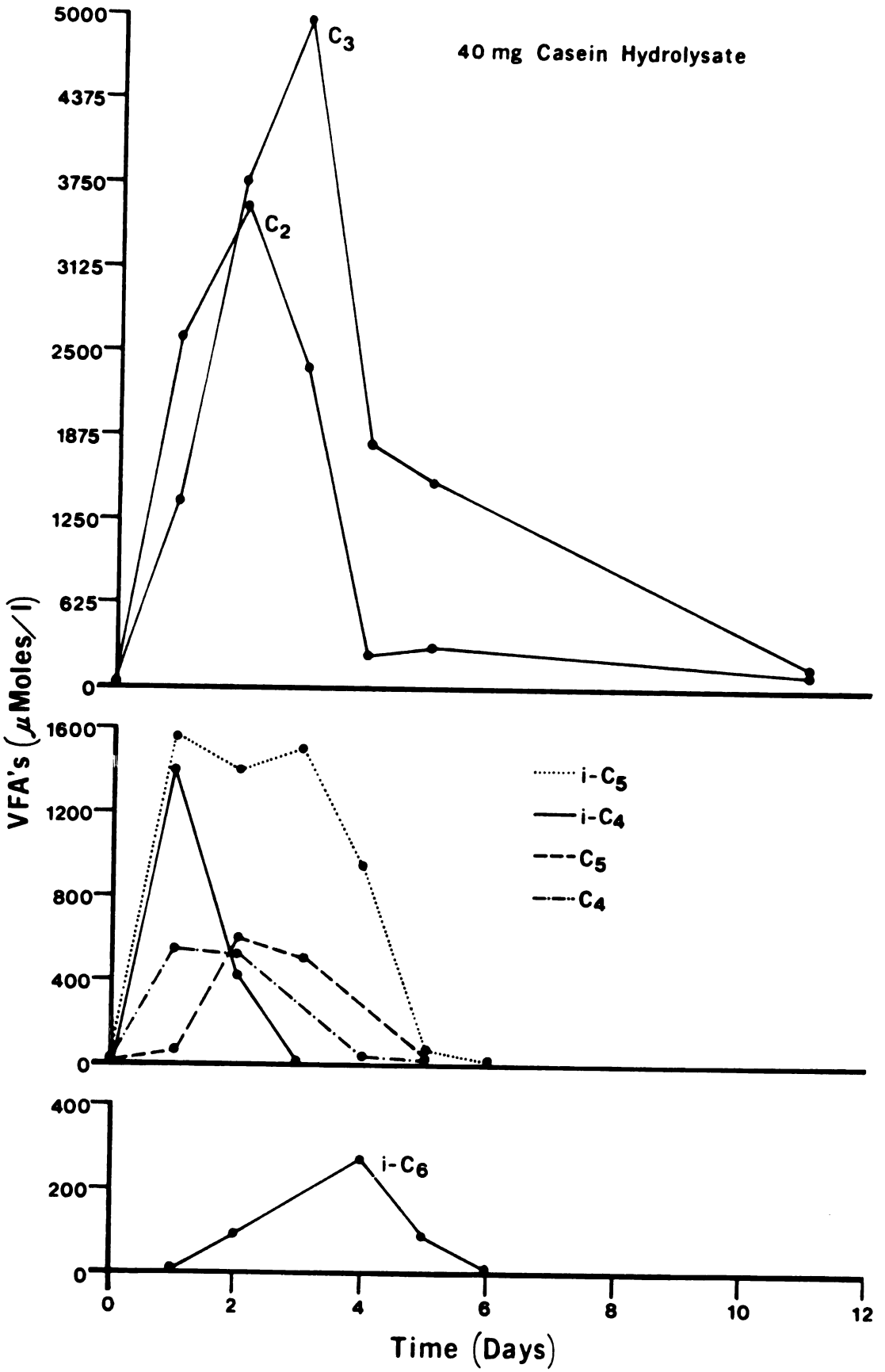

20mg Casein Hydrolysate
—C3
-·-C2
--i-C5
·····i-C4
-··-C4
---C5
——i-C6
VFA's (μMoles/l)
1200
1080
960
840
720
600
480
360
240
120
0
0
2
4
6
8
10
12
Time (Days)

Figure 14. Volatile fatty acid concentrations in Wintergreen Lake pelagic sediments amended with 40 mg of casein hydrolysate. Sediment volume was 30 ml.


40 mg Casein Hydrolysate
C3
C2
i-C5
i-C4
C5
C4
i-C6
VFA's (μMoles/l)
Time (Days)
5000
4375
3750
3125
2500
1875
1250
625
0
1600
1200
800
400
0
400
200
0
0
2
4
6
8
10
12

increased with increasing amount of casein added.

DISCUSSION

Metabolism of seston. The addition of algal material (organic seston) to Wintergreen Lake pelagic sediments resulted in rapid initial increases in the concentrations of volatile fatty acids (Figure 3) and methane (Figure 1). Only acetate and propionate were detected during decomposition of the added algal substrate. These latter results aggree with those of Foree and McCarty (1971) who reported acetate and propionate to be the major volatile acids produced during decomposition of a variety of algal species grown in laboratory culture and then allowed to senesce. Acetate and propionate have also been found to be the primary volatile acids detectable in the pelagic sediments of Wintergreen Lake (Chapter III).

The production and turnover of acetate and propionate in seston-amended sediments occurred rapidly. Peak concentrations of each acid were detected 24-48 hours after addition of seston to the sediment; however, concentrations of each returned to undetectable levels within 6 days (Figure 3). Similar rapid turnover of volatile acids has been noted in sewage sludge digesters after the addition of organic substrate (Chynoweth and Mah, 1971). Methane production in seston-amended sediments continued at an accelerated rate relative to the control for 9-10 days before the rate began to decline (Figures 1 and 2). Of the

13.5 mg of seston-carbon added to the sediment at initiation of the experiment, 30% was recovered as CH_4 in 22 days. These results indicate that the conversion of seston-carbon to methane occurs rapidly in these sediments. Kudryavtsev (1974), in laboratory experiments, similarly reported algal decomposition rates to be highest during the first 8-12 days before the decomposition rate decreased greatly.

Much of the initial methane production observed in seston-amended sediments appears to result from the rapid metabolism of soluble or labile constituents of the seston. Initial methane production rates were 35% greater in sediment to which unleached seston or leachate were added, relative to sediments amended with leached seston (Figure 8). The leachate fraction contains water-soluble constituents of the seston which would be expected to be more easily metabolized by the sediment microflora than the more complex constituents of the leached seston. This is further substantiated by the quantitative and qualitative differences in volatile fatty acids detected when equal weights of leached seston and leachate were added to sediments (Figure 9). Leachate-amended sediment contained higher levels of acetate and propionate, as well as detectable amounts of valerate, butyrate, and iso-valerate. Leached seston, in contrast, supported the production of diminished quantities of acetate and propionate relative to both leachate and to non-leached seston (Figure 9). These results are particularly applicable to the pelagic zone of Wintergreen Lake.

Rapid sedimentation through the anaerobic hypolimnion of the lake results in the deposition of large quantities of nearly intact algal cells in these sediments during the summer (Chapter II). On a weight basis, 50-60% of this algal biomass is present as water soluble constituents. Rapid metabolism of this organic substrate is reflected in the large increases in methane production observed to follow large increases in sedimentation rate in Wintergreen Lake (Chapters II and III).

Effect of organic loading. Addition of increasing concentrations of casein hydrolysate to Wintergreen Lake sediments initially resulted in no further increase in the rate of methanogenesis (Figure 10), and in increased accumulation of volatile acids in the 20 and 40 mg samples compared to the 5 and 10 mg treatments (Figures 11 through 14). These results indicate that the rate of organic loading is an important factor in controlling the stages of anaerobic digestion in these sediments. Normally, anaerobic digestion is a balanced process where the rate of acid formation is equal to or below the rate of methanogenesis. As noted previously, volatile acid levels increased rapidly after addition of seston to Wintergreen Lake sediments, but quickly declined, with little net accumulation of substrate carbon found in the volatile acid pool (Figure 3). Studies in anaerobic sludge digesters have similarly shown volatile acid formation to be closely associated with the feed cycle, sharp increases in volatile acid concentrations occurring

immediately after feeding, followed by an equally rapid decline in acid levels during succeeding days (Schulze, 1958).

Increased rates of organic loading can, however, lead to unbalanced conditions between fermentative and methanogenic stages of anaerobic digestion, resulting in a reduced rate of methanogenesis and in increased volatile acid accumulation. The large increases in volatile acid concentrations, particularly in the amount of propionate relative to acetate, determined in sediments amended with increasing amounts of casein hydrolysate (Figures 11 through 14) suggest that accelerated organic loading to Wintergreen sediments has temporarily disrupted the balance between fermentative and methanogenic processes which is normally operative in these sediments. Similar accumulations of acetate and propionate occur in sewage sludge digesters which have become unbalanced, or which have failed completely as a result of excessive organic loading (McCarty, et al., 1974). Reduction of the rate of organic loading, however, allows re-establishment of balanced conditions between fermentative and methanogenic stages in Wintergreen Lake sediments, as evidenced by the gradual decline in volatile acid concentrations and by increased methanogenesis in the casein-amended samples after 12 days (Figures 10 through 14). A similar overloading of the system may have occurred in situ in the pelagic sediments of Wintergreen Lake in 1976 (Chapter III). As in the laboratory experiments described

above, a subsequent reduction in the rate of organic input to the sediments was followed by a rapid resumption in methanogenesis (Chapter III).

Addition of alternate electron acceptors. Nitrate addition to the sediments immediately stopped methane production (Figures 1 and 2). Similar nitrate inhibition of methanogenesis has been reported previously in soils and salt marshes respectively (Laskowski and Moraghan, 1967; Bollag and Czlonkowski, 1973; Balderston and Payne, 1977). The addition of nitrate also resulted in an alteration in the volatile fatty acid concentrations detectable in the sediment (Figure 3). The reason(s) for the more gradual accumulation of acetate and propionate in seston/NO_3-amended sediments compared to seston-amended sediments is not clear (Figure 3). Inhibition of methanogenesis by nitrate should allow fermentative activities to continue, resulting in the rapid accumulation of organic acids. Volatile acid accumulation did not occur in seston/NO_3-amended sediment, however, until day 6, when methanogenesis had resumed. Organic acid levels then rapidly declined as the rate of methanogenesis increased (Figures 1 and 3). The observed alterations in volatile acid concentrations in nitrate-amended sediments may be the result of competition for substrate between fermentative and denitrifying microorganisms. Denitrifying bacteria may be more successful competitors for added seston carbon, diverting carbon and energy sources from use by fermentative anaerobes when nitrate is present. This

explanation is supported by the fact that methanogenesis resumed much more rapidly in seston/NO_3-amended samples than in sediment/NO_3-amended samples (Figures 1 and 2). This difference may be the result of an increased rate of denitrification due to utilization of added seston carbon by denitrifying bacteria.

Alternatively, acetate, propionate, and additional volatile acids may still be produced during the initial 5 days of the experiment, but do not accumulate due to their rapid utilization by denitrifying bacteria. The latter organisms can utilize a wide array of carbohydrates, organic acids, and other organic compounds as carbon and energy sources. Substrate spectrum becomes restrictive for some denitrifiers under anaerobic conditions compared to aerobic conditions, but is still broad under anaerobic conditions for other denitrifiers (Delwiche and Bryan, 1976; Brezonik, 1977).

Seston/sulfate-amended sediments also showed an altered volatile acid pattern compared to seston-amended sediments (Figure 3). Acetate was the only volatile acid detected in these samples; propionate was not observed. Foree and McCarty (1971) also reported acetate to be the only volatile acid detected during the decomposition of algae by sulfate reduction. As in the case of nitrate, the exact reasons for alterations in the volatile acid patterns observed are unclear. One possible explanation is that propionate production does not occur in sulfate-amended

sediments. This explanation seems unlikely, however, as fermentative activities should continue in the presence of sulfate. Alternatively, propionate may be produced and subsequently utilized in another metabolic reaction. The reported range of substrates which sulfate-reducing bacteria can utilize as electron donors is generally held to be limited, lactate, pyruvate, ethanol, and formate being preferred electron donors (Thauer, et al., 1977). However, propionate and additional volatile acids might also serve as possible electron donors for sulfate reducing bacteria in Wintergreen Lake sediments. If these compounds are utilizable as electron donors, the absence of propionate in seston/SO_4-amended sediments might be explained by its utilization by sulfate-reducing bacteria.

The absence of propionate in seston/sulfate-amended sediments may also be explained by increased competition for hydrogen between methanogenic and sulfate-reducing bacteria. Recent evidence (Abram and Nedwell, 1978a, 1978b) indicates that methanogens and sulfate-reducers may compete for hydrogen, the latter organisms oxidizing hydrogen at the expense of sulfate. Increased competition for hydrogen may thus maintain a low partial pressure of hydrogen such that electron flow is diverted away from propionate and towards more oxidized end products.

Chloroform perturbation of methanogenesis. Complete inhibition of methanogenesis by chloroform resulted in the accumulation of high concentrations of volatile acids in

both sediment and seston samples (Figures 4 and 5). These results suggest that, under normal conditions, fermentative and methanogenic phases of digestion are closely coupled in these sediments, such that organic substrates are converted to carbon dioxide and methane, with little net accumulation of volatile acids occurring. As in the case of nitrate inhibition, there was an unexpected lag in chloroform-treated samples before acid accumulation began. The lag period was noticeably shorter in sediment/CCl_4-amended samples than in seston/CCl_4-amended samples. This lag in acid accumulation may be due to the inhibitory effect of chloroform on fermentative microorganisms as well as on methanogens. Although shown to be a potent inhibitor of methanogenesis (Bauchop, 1967; Sykes and Kirsch, 1972), chloroform may inhibit the activities of some fermentative bacteria as well.

The qualitative nature of the accumulated volatile acids reflected the high protein content of the algal seston (Chapter II). Acetate and propionate were the predominant acids obtained as in the case of sediment samples amended with seston. Appreciable amounts of butyrate, valerate, iso-valerate, iso-butyrate and iso-caproate were also detected in seston/CCl_4-treated samples. These longer chain acids, and the iso-acids in particular, are highly characteristic of the metabolism of proteinaceous substrates (Doelle, 1977; Molongoski and Klug, 1976; Moore, et al., 1966). These latter acids were likely produced in

uninhibited sediments as well, but because of their lower concentration and rapid turnover time, remained undetected.

Hydrogen perturbation of sediments. Strayer and Tiedje (1978b) have reported kinetic evidence that hydrogen is rate-limiting for methanogenesis in the pelagic sediments of Wintergreen Lake, and could thus serve as a potential coupling factor in anaerobic digestion in these sediments. The concept of interspecies hydrogen transfer is based on maintenance of low partial pressures of hydrogen by hydrogen-consuming microorganisms such as the methanogenic bacteria (Wolin, 1974). Laboratory studies involving mixed cultures of methanogens and hydrogen-producing heterotrophs have demonstrated that interspecies hydrogen transfer quantitatively and qualitatively affects the fermentation end products formed in addition to hydrogen by the fermenting species (Reddy, et al., 1972; Iannotti, et al., 1973; Wolin, 1974). The hydrogen perturbation reported here provides additional evidence for the occurrence of hydrogen transfer in Wintergreen Lake sediments. Continuous addition of hydrogen to these sediments resulted in an increased rate of methanogenesis in both sediment/H_2 and seston/H_2 samples (Figures 6 and 7). As reduced end products accumulated in seston/H_2-amended sediments (Figure 7), the production of methane ceased completely (Figure 1) and volatile acid levels increased further indicating that a complete uncoupling of the fermentative and methanogenic phases of digestion had likely occurred.

Particularly notable was the large amount of propionate

produced in the hydrogen-perturbed samples relative to acetate produced. Similar increases in propionate/acetate ratio were also seen in sediments amended with increasing amounts of casein (Figures 11 through 14), but not in chloroform-treated samples (Figures 4 and 5). One of the consequences of uncoupling fermentation and methanogenesis in other anaerobic systems such as the rumen has been an increase in reduced fermentation products (Reddy, et al., 1972; Wolin, 1974). Kasper and Wuhrmann (1978a, 1978b) have demonstrated an increase in propionate relative to acetate in sludge exposed to increased hydrogen concentrations, and have shown that the partial pressure of hydrogen is the ecologically dominating factor. They demonstrated that interruption of hydrogen consumption by methanogens results in an increase in hydrogen partial pressure and an immediate inhibition of propionate degradation. Boone and Smith (1978) and McInerney, et al., (1978) have also presented recent evidence demonstrating that hydrogen partial pressure is an important factor controlling the metabolism of longer chain volatile fatty acids.

In light of these observations, the relatively low ratio of propionate to acetate determined in chloroform-treated sediments compared to casein-amended or hydrogen-perturbed sediments is surprising. Since hydrogen concentrations were not measured in these experiments, it is not known whether hydrogen accumulation occurred in chloroform-treated samples. Bauchop (1967) and Thiel (1969) found that hydrogen accumulated in sludge and in the rumen respectively when chloroform was added. Chynoweth and Mah (1971), in contrast, reported

no accumulation of molecular hydrogen in sludge when methanogenesis was inhibited by chloroform . They found instead, that acetate was converted to formate and butyrate at faster rates in inhibited that in uninhibited sludge, indicating that secondary reactions were probably serving to remove hydrogen produced during fermentation. The non-methanogenic use of hydrogen in sediments has been noted by several investigators (Winfrey, et al., 1977; Strayer and Tiedje, 1978b). Numerous microorganisms have also recently been reported which can synthesize acetate from carbon dioxide and hydrogen (Ohwaki and Hungate, 1977; Prins and Lankhorst, 1977; Schoberth, 1977). Similar microorganisms may be active in Wintergreen Lake sediments as well.

SUMMARY

The results reported in this investigation emphasize the similarity of anaerobic metabolism in Wintergreen Lake pelagic sediments to that found in other anoxic habitats, particularly anaerobic sludge digesters. Both systems receive a continuous input of high quality organic substrate. The alterations in rates of methanogenesis and in concentrations of volatile metabolites caused by addition of alternate electron acceptors (nitrate and sulfate) suggest that, as in sludge digesters, anaerobic digestion in these sediments is a tightly coupled process between fermentative and methanogenic phases of metabolism. As in sludge digesters, the balance between initial and terminal stages

of digestion in sediments is also susceptible to disruption due to excess loading of organic substrate (Chapter III). In both instances, disruption is characterized by an inhibition or reduction in the rate of methanogenesis and an accumulation of reduced end products. Unlike sludge digesters, however, anaerobic digestion in lake sediments may exhibit an increased capacity for subsequent recovery. One likely reason for more rapid recovery of coupled metabolism in sediments is the removal of toxic or inhibitory compounds by diffusion from the sediments to the overlying water. As in sludge digesters, interspecies hydrogen transfer may be of great importance in regulating anaerobic digestion processes in eutrophic lake sediments.

LITERATURE CITED

1. Abram, J.W. and D.B. Nedwell. 1978a. Inhibition of methanogenesis by sulfate reducing bacteria competing for transferred hydrogen. Arch. Microbiol. 117: 89-92.

2. Abram, J.W. and D.B. Nedwell. 1978b. Hydrogen as substrate for methanogenesis and sulfate reduction in anaerobic saltmarsh sediment. Arch. Microbiol. 117: 93-97.

3. Andrews, J.F. and E.A. Pearson. 1965. Kinetics and characteristics of volatile acid production in anaerobic fermentation processes. Int. J. Air Wat. Poll. 9: 439-461.

4. Balch, W.E. and R.S. Wolfe. 1976. New approach to the cultivation of methanogenic bacteria: 2-mercapto-ethanesulfonic acid (HS-CoM)-dependent growth of Methanobacterium ruminantium in a pressurized atmosphere. Appl. Environ. Microbiol. 32: 781-791.

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

6. Bauchop, R. 1967. Inhibition of rumen methanogenesis by methane analogues. J. Bacteriol. 94: 171-175.

7. 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. The Analyst 99: 171-175.

8. Bollag, J.M. and S.T. Czlonkowski. 1973. Inhibition of methane formation in soil by various nitrogen-containing compounds. Soil Biol. Biochem. 5: 673-678.

9. Boone, D.R. and P.H. Smith. 1978. Hydrogen formation from volatile acids by methanogenic enrichments. Abstracts, 78th Annual Meeting. Amer. Soc. Microbiol. p. 200.

10. Brezonik, P.L. 1977. Denitrification in natural waters. Prog. Wat. Tech. 8: 373-392.

11. Chynoweth, D.P. and R.A. Mah. 1971. Volatile acid formation in sludge digestion. pp. 41-54. In: Anaerobic Biological Treatment Processes. Adv. Chem. Ser. 105 F.G. Pohland (Editor) Amer. Chem. Soc. Washington

12. Delwich, C.C. and B.A. Bryan. 1976. Denitrification. Ann. Rev. Microbiol. 30: 241-262.

13. Doelle, H.W. 1977. Bacterial Metabolism. Academic Press. New York.

14. Foree, E.G. and P.L. McCarty. 1970. Anaerobic decomposition of algae. Environ. Sci. Technol. 4: 842-849.

15. Hungate, R.E. 1950. The anaerobic mesophilic cellulolytic bacteria. Bacteriol. Rev. 14: 1-49.

16. Ianotti, E.L., D. Kafkewitz, M.J. Wolin, and M.P. Bryant. 1976. Glucose fermentation products of Ruminococcus albus grown in continuous culture with Vibrio succinogenes: changes caused by interspecies transfer of H_2. J. Bacteriol. 114: 1231-1240.

17. Kaspar, H.F. and K. Wuhrmann. 1978a. Product inhibition in sludge digestion. Submitted to Microbial Ecol.

18. Kaspar, H.F. and K. Wuhrmann. 1978b. Kinetic parameters and relative turnovers of some important catabolic reactions in digesting sludge. Appl. Environ. Microbiol. 36: 1-7.

19. Kudryavtsev, V.M. 1974. Dynamics of the decomposition of tagged algae by bacteria. Mikrobiologiya 43: 903-907.

20. Laskowski, D. and J.T. Moraghan. 1967. The effect of nitrate and nitrous oxide on hydrogen and methane accumulation in anaerobically incubated soils. Plant Soil 27: 357-368.

21. McCarty, P.L., J.S. Jeris, and W. Murdoch. 1963. Individual volatile acids in anaerobic treatment. J. Water Poll. Control Fed. 35: 1501-1515.

22. McInerney, M.J., M.P. Bryant, and N. Pfennig. 1978. Anaerobic bacterium that oxidizes fatty acids in syntrophic association with H_2-utilizing bacteria. Abstracts, 78th Ann. Meeting Amer. Soc. Microbiol. p. 168.

23. Molongoski, J.J. and M.J. Klug. 1976. Characterization of anaerobic heterotrophic bacteria isolated from freshwater lake sediments. Appl. Environ. Microbiol. 31: 83-90.

24. Moore, W.E.C., E.P. Cato, and L.V. Holdeman. 1966. Fermentation patterns of some Clostridium species. Int. J. Syst. Bacteriol. 16: 383-415.

25. Ohwaki, K. and R.E. Hungate. 1977. Hydrogen utilization by clostridia in sewage sludge. Appl. Environ. Microbiol. 33: 1270-1274.

26. Prins, R.A. and A. Lankhorst. 1977. Sunthesis of acetate from CO_2 in the cecum of some rodents. FEMS Microbiology Letters. 1: 255-258.

27. Reddy, C.A., M.P. Bryant, and M.J. Wolin. 1972. Characteristics of S organism isolated from Methanobacillus omelianskii. J. Bacteriol. 109: 539-545.

28. Robinson, C.K. 1978. Quantitative comparison of the significance of methane in the carbon cycle of two small lakes. Arch. Hydrobiol. (In press.)

29. Schoberth, S. 1977. Acetic acid from H_2 and CO_2. Arch. Microbiol. 114: 143-148.

30. Schulze, K.L. 1958. Studies on sludge digestion and methane fermentation. I. Sludge digestion at increased solids concentrations. Sewage and Industrial wastes 30: 28-45.

31. Strayer, R.F. and J.M. Tiedje. 1978a. In situ methane production in a small, hypereutrophic, hard-water lake: loss of methane from sediments by diffusion and ebullition. Limnol. Oceanogr. (In press).

32. Strayer, R.F. and J.M. Tiedje. 1978b. Kinetic parameters of the conversion of methane precursors to methane in a hypereutrophic lake sediment. Appl. Environ. Microbiol. 36: 330-340.

33. Sykes, R.M. and E.J. Kirsch. 1972. Accumulation of methanogenic substrates in CCl_4-inhibited anaerobic sewage sludge digester cultures. Water Res. 6: 41-55.

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

35. Thiel, P.G. 1969. The effect of methane analogues on methanogenesis in anaerobic digestion. Water Res. 3: 215-223.

36. 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.

37. 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.

38. Wolin, M.J. 1974. Metabolic interactions among intestinal microorganisms. Am. J. Clin. Nutrition 27: 1320-1328.