STUDIES INTO THE EFFECTS OF LOW pH ON THE METHANOGENIC FERMENTATION IN ANAEROBIC FRESHWATER LAKE SEDIMENTS

by

Gregor Martin Awang

A Thesis Submitted to
The Faculty of Graduate Studies
The University of Manitoba
In Partial Fulfillment of the
Requirements for the Degree
of

Master of Science

Department of Microbiology
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To Mom and Dad

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ABSTRACT

The effect of low pH on methanogenic fermentation in anoxic freshwater sediments was studied using two approaches. In vitro systems in which sediments were directly acidified, and core systems in which the water overlying the sediments was acidified.

Methane production in directly acidified sediments was partially inhibited at about pH 5.5 and completely inhibited at pH 4.0-4.5. This inhibition was temperature and time dependent. Increasing the incubation temperature from $8^{o}C$ to $28^{o}C$ partially relieved the inhibition. The inhibition of $^{14}CH_4$ production from ^{14}C-labelled Chlorella at about pH 5.5 decreased after 10 days incubation in long term (27 days) in vitro experiments. The ratio of $^{14}CO_2$:$^{14}CH_4$ produced increased with a decrease in sediment pH in the in vitro experiments utilizing ^{14}C-labelled substrates. This pH dependent shift in the relative amounts of CO_2:CH_4 produced was further evidenced by the decreases in the maximum velocity of reaction (V) for $^{14}CO_2$ and $^{14}CH_4$ production from ^{14}C-labelled acetate: V for methane production decreased ten times more than V for carbon dioxide production between pH 6.2 and 4.0.

Methane production in undisturbed cores (in which the water overlying the sediments was acidified) was not inhibited because acid added to the water column did not invade the underlying sediments: $^{14}CH_4$ production from ^{14}C-labelled Chlorella in cores was partially inhibited at pH 6.2-5.8 because the ^{14}C-labelled Chlorella was added at the sediment surface in the cores and was exposed to acidic water. The ratio of CO_2:CH_4 increased slightly, and the percentage return of carbon as methane and carbon dioxide to the water column decreased,

with a decrease in the pH of the overlying water. $^{14}CH_4$ production from ^{14}C-labelled acetate in sediment sections from acidified cores was not inhibited until the pH of the overlying water was adjusted to below 3, because the sediments involved were not in direct proximity to the acidified water. The CO_2:CH_4 ratio produced from ^{14}C-labelled sodium acetate was lower than those in all other experiments, and an unknown anoxic carbon dioxide producing microbial process was postulated as being responsible for the additional carbon dioxide produced in the other experiments.

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INTRODUCTION

Bacterial decomposition of organic matter of biogenic origin is an important means of nutrient recycling in natural ecosystems. In the freshwater environment, much of this decomposition occurs in anoxic sediments, in which complex organic compounds (eg. cellulose, lignins, lipids, and proteins) are fermented predominantly to methane and carbon dioxide by a consortium of three metabolic types (Zeikus, 1977; Mah and Smith, 1981; Wuhrmann, 1982): 1) organisms which ferment the complex organic compounds to organic acids, hydrogen, and carbon dioxide; 2) organisms which oxidize organic acids to acetate; 3) methanogenic bacteria which utilize acetate to produce methane and carbon dioxide. Since methane production is the predominant terminal process of anaerobic fermentation in freshwater sediments, it can be used as a measure of heterotrophic activity in these sediments, and thus as an indicator of the effects of sediment perturbations on this activity (Robertson, 1979; Kelly and Chynoweth, 1979).

Acid precipitation has been implicated in the acidification of several lake regions in Europe and North America (Wright et al, 1980). The effects of lake acidification on aquatic biota have been documented (Wright et al, 1976; Almer et al, 1978; Schindler, 1980; Schindler and Turner, 1982), however very little of the reported research addressed the effect of acidification on bacterial decomposition processes.

There has been a small amount of research which indicated that the decomposition of organic matter in sediments was inhibited by

lake acidification (Hendry et al, 1976; Laake, 1976; Andersson, 1978; Gahnstrom, 1980; Walichnowski and Lawrence, 1982), however only Walichnowski and Lawrence (1982) used methane production as a measure of heterotrophic activity under acidic conditions. The latter authors reported that *in vitro* methanogenesis in freshwater sediments was completely inhibited below pH 5.5, but *in situ* methanogenesis was only partially inhibited when lakewater above the sediments was adjusted to pH 4.8.

In light of the results of Walichnowski and Lawrence (1982), this project was undertaken with the following purposes: 1) to compare the effects of low pH on methanogenesis in an *in vitro* system and a core system similar to the *in situ* situation; 2) to determine if the entire fermentative consortium and the methanogenic bacteria specifically, are sensitive to low pH in the experimental systems used. *In vitro* experiments utilized directly acidified sediments and were designed to determine a critical pH at which the methanogenic fermentation was inhibited. The core system maintained an intact sediment-water interface, and was designed to determine if acidification of water overlying the sediments (as would occur in whole lake acidification) affected methanogenesis in the sediments. The production of ^{14}C-labelled methane ($^{14}CH_4$) from ^{14}C-labelled *Chlorella* cells (a source of particulate organic carbon) was used to determine the effect of low pH on fermentation of complex organic substrates. The production of $^{14}CH_4$ from [1,2-^{14}C] labelled sodium acetate (a direct precursor for bacterial methane production) was used to determine the effect of low pH on methanogenic bacteria.

HISTORICAL REVIEW

Methanogenic Bacteria

The biological production of methane is carried out in nature by a small group of strictly anaerobic bacteria known as methanogens or methanogenic bacteria. These organisms remained a poorly studied group for many decades because they were difficult to isolate and cultivate (Wolfe, 1979). The pioneering laboratory in the study of methanogens was the Delft School of Microbiology in the Netherlands. R.E. Hungate of the Delft School was the first to develop a reliable method of isolating, enumerating, and cultivating methanogens (Hungate, 1950; 1969) in prereduced media. Numerous refinements of the Hungate method (Wolfe, 1971; Macy et al, 1972; Edwards and McBride, 1975; Balch and Wolfe, 1976; Zeikus, 1977; Balch and Wolfe, 1979a; Mah and Smith, 1981) over the years have contributed to intense research on methanogenesis.

The nutritional requirements of methanogenic bacteria range from simple to complex, therefore isolation media usually include a wide range of nutrients to maximize the probability of obtaining an isolate (Mah and Smith, 1981). Ammonium ion (NH_4^+) satisfies the nitrogen requirement in all methanogens (Bryant et al, 1971; Zeikus, 1977; Mah and Smith, 1981). Sulfide is the required sulfur source in most methanogens (Zeikus, 1977). The carbon requirement is mainly satisfied by acetate or carbon dioxide. Though formate, methanol, and methylamines may be used by certain species (Mah and Smith, 1981; Wolfe, 1982).

The methanogenic bacteria are taxonomically diverse, but metabolically unique. Cell morphologies include sarcina, rods, spheres, and spirals. There is considerable variation in cell

dimension and organization (Zeikus, 1977; Balch et al, 1979; Mah and Smith, 1981). A major revision in the taxonomy of these bacteria based on comparative studies of their 16S rRNA oligonucleotide sequences, cell walls, and lipids has been done recently (Fox et al, 1977; Woese and Fox, 1977; Woese, 1977; Balch et al, 1979; Wolfe, 1979; Mah and Smith, 1981). These studies indicate that methanogens belong to a distinct phylogenetic group of prokaryotes designated Archaebacteria, which also includes thermoacidophilic bacteria and extremely halophilic bacteria. It has been suggested that an "ancestral" archaebacterium gave rise to the three groups, with halophiles evolving from ancient methanogens and thermoacidophiles evolving separately (Fox et al, 1980).

Balch et al (1979) proposed a taxonomic scheme dividing methanogens into seven genera containing thirteen species (Table 1). A new species Methanococcus thermolithotrophicus, and a new genus Methanococcoides were subsequently described (Huber et al, 1982; Sowers and Ferry, 1983). Recently a cheap and rapid identification and classification method based on immunological techniques has been reported (Conway de Macario et al, 1981; 1982), which yields results analogous to the more elaborate methods previously cited. The unique metabolic properties of methanogens are as follows: A) They produce methane and carbon dioxide. B) Methane is produced from a narrow range of substrates. C) Methane is produced at a reducing potential near that of the hydrogen electrode (-330 mV). D) Seven unique cofactors (CoM, F_{420}, F_{342}, F_{430}, B, CDR, M. mobile factor) have been found in methanogens. E) The sequences of 16S and

Table 1. Methanogenic species in pure culture.

Order	Family	Genus	Species
Methanobacteriales	Methanobacteriaceae	Methanobacterium	formicicum
			bryantii
			thermoautotrophicum
		Methanobrevibacter	ruminantium
			smithii
			arboriphilus
Methanomicrobiales	Methanomicrobiaceae	Methanomicrobium	mobile
		Methanogenium	cariaci
			marisnigri
		Methanospirillum	hungatei
		Methanococcoides	methylutens
	Methanosarcinaceae	Methanosarcina	barkeri
	Methanococcaceae	Methanococcus	vannielii
			voltae
			thermolithotrophicus

23S RNA of their ribosomes are only distantly related to the corresponding RNA in both eukaryotes and typical prokaryotes. F) The tRNA of methanogens does not contain a common arm sequence found in all other prokaryotes (Wolfe, 1979). G) The cell walls lack muramic acid and D-amino acids (Kandler and König, 1978). H) The lipids are mostly nonsaponifiable (Makula and Singer, 1978; Tornabene and Langworthy, 1978; Tornabene et al, 1978) and may be a previously unrecognized source of multi-branched hydrocarbons found in sediments and petroleum (Wolfe, 1979). I) Methanogens do not contain cytochromes and quinones (Wolfe, 1979). J) The carbon dioxide fixation pathway is unique.

The Ecological Significance of Methanogenesis

Methanogenic bacteria are distributed ubiquitously in anoxic environments where organic matter undergoes decomposition (Wolfe, 1971; Mah et al, 1977; Zeikus, 1977; Balch et al, 1979; Mah and Smith, 1981; Wuhrmann, 1982). These methanogenic habitats include the intestinal tracts of man and animals (Miller and Wolin, 1982; 1983; Hungate, 1982), various sewage digesters and landfills (Zeikus, 1977; Mah and Smith, 1981), the heartwood of living trees (Zeikus and Ward, 1974), decomposing algal mats of hot springs (Zeikus, 1977; Ward, 1978; Zeikus et al, 1980), oil bearing sedimentary rocks (Belyaev et al, 1983), and aquatic sediments (Mah and Smith, 1981).

In natural habitats methane producing bacteria are the terminal organisms in the anaerobic fermentation of organic matter. The present rate of global methane production in terrestrial and aquatic systems as well as in animal intestines has been estimated to be

$5.5 - 11 \times 10^{14}$ gm per year (Erhalt, 1976). Methane is a desirable end product of fermentation in anaerobic ecosystems because it is poorly soluble, non-toxic, and readily escapes the site of production (Zeikus, 1977; Bryant, 1979; Rudd and Taylor, 1980). It may play an important part in the carbon cycle of certain ecosystems (such as freshwater lakes) in which it is recycled to carbon dioxide by methane oxidizing bacteria (Rudd and Taylor, 1980).

In nature, methane is produced by the hydrogenation of carbon dioxide (Hydrogenotrophy) or by the protonation of the methyl group in acetate (Acetotrophy) according to the reactions:

1) $CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$ $\quad \Delta G^{\circ} = -139.1$ KJ/reaction

2) $CH_3COO^- + H_2O \rightarrow CH_4 + HCO_3^-$ $\quad \Delta G^{\circ} = -31$ KJ/reaction.

Hydrogenotrophic and acetotrophic methanogens tend to segregate into different habitats: hydrogenotrophic species are common in thermophilic environments with a pH around seven, while acetotrophic species prefer psychrophilic and mesophilic environments. There has been only one reported isolation of an acetotroph from a thermophilic environment (Zinder and Mah, 1979). The dominant acetotrophic organism found in most natural environments is Methanothrix söhngenii (Zehnder et al, 1980; Wuhrmann, 1982) which has a temperature optimum of 37-40°C and a required pH for growth of 7.4-7.8. In general, all methanogenic bacteria require a pH range of 7-8 for optimum growth (Mah and Smith, 1981; Wuhrmann, 1982) though methane production in acidic peat bogs at pH 4 has been reported (king et al, 1981).

There are two broad groups of methanogenic ecosystems in nature (Wuhrmann, 1982): group I encompasses ecosystems

utilizing carbon dioxide and hydrogen from geochemical sources (terrestrial, sublacustrian, submarine thermal springs) to produce methane and ATP by chemolithotrophy according to equation (1). Group II systems utilize acetate, carbon dioxide and hydrogen produced by the fermentation of organic matter of biogenic origin. The functionally simple chemolithotrophic group I ecosystems have not been extensively studied. Methane production in Lake Kiwu, an African rift lake (Deuser et al, 1973) and in thermal springs in Yellowstone Park (Zeikus and Wolfe, 1972) is typical of group I ecosystems. The rest of this section will deal with the more common and extensively studied group II ecosystems.

Group II can be divided into two ecotypes: 1. the rumen of cloven-footed animals, and 2. natural or technical systems for anaerobic degradation of organic matter (eg. sediments, soils, sludge digesters). The primary sources of carbon in group II systems are terrestrial or aquatic plants containing various percentage compositions of cellulose and other polymeric carbohydrates, lignin, proteins, and lipids. Three groups of bacteria are required for producing methane from organic matter in nature: 1) organisms for the fermentative oxidation of the primary organic materials to organic acids, hydrogen, and carbon dioxide; 2) organisms which oxidize organic acids to acetate; 3) methanogenic bacteria. The overall rate of decomposition may be limited by the rate of decomposition of the primary organic materials, most of which are insoluble polymers. In addition, certain pollutants such as ethylene glycol and its polymers and phenolic compounds have been reported to be degradable in bacterial methanogenic

systems (Dwyer and Tiedje, 1983; Boyd et al, 1983).

The pathways of liquefaction and fermentation of substrates in group II systems are largely unknown except for the rumen, where extensive investigations of species composition and inter-relationships exist (Hungate, 1966; 1982). Numerous studies of group II systems (other than the rumen) in which co-cultures of methanogenic bacteria and other bacteria or enrichment cultures degraded substrates to methane and carbon dioxide have been reported (Khan and Trottier, 1978; Khan et al, 1979; McInerney and Bryant, 1981; Mountfort et al, 1982; Schink and Zeikus, 1982, Koch et al, 1983; Bouwer and McCarty, 1983), but knowledge from these experiments should be viewed with caution because such experiments may favor organisms and pathways not predominant in natural systems. In the last decade or so, the use of radiotracer methods to study methanogenesis in "natural" samples has become widespread, particularly in the study of the sediments of freshwater bodies (Cappenberg and Jonejan, 1978; Strayer and Tiedje, 1978; Winfrey and Zeikus, 1979; 1979a; Cappenberg and Vergouw, 1982; King and Klug, 1982; Lovley and Klug, 1982; 1983). Most of the latter research has been concentrated specifically on methanogenic bacteria and has ignored the two groups of bacteria that supply the methanogens with carbon sources, with the exception of two studies. Cappenberg and Verdouw (1982 studied the breakdown of kinetics of carbon-14 labelled algal (Chlorella sp.) cell walls in freshwater sediment and reported that the initial fermentative oxidation step was rate limiting to anaerobic mineralization. King and Klug (1982) studied the mass flow from carbon-14 labelled

glucose through fermentation to lactate, propionate, acetate and ultimately methane in freshwater sediments, and estimated that glucose catabolism could account for thirty-six percent of total methane formation in the ecosystem. Wuhrmann (1982) lays out a working hypothesis regarding the pathways of acetate formation. In rumen, a favorable fermentation environment is maintained by the absorption of potentially toxic organic acids and alcohols through the rumen wall, and by the respiration of hydrogen to methane due to Methanobrevibacter ruminantium. In other group II systems the accumulation of organic acids lowers the pH and stops fermentation. The reactions leading to efficient breakdown of organic acids to acetate and hydrogen only become feasible thero-dynamically at low partial pressures of hydrogen (10^{-5} atm). The chemolithotrophic methanogens remove hydrogen by carbon dioxide reduction and thus alter electron flow in favor of hydrogen and acetate production. Acetate is then degraded to methane and carbon dioxide by acetotrophic methanogens.

To this point, the methanogenic systems described have been low in sulfate and nitrate ions. In ecosystems containing high levels of nitrate or sulfate naturally (eg. marine sediments) or by addition (eg. due to acid deposition), a shift in electron flow from methane production to sulfate or nitrate reduction occurs (Balderton and Payne, 1976; Bryant et al, 1977; Winfrey and Zeiksus, 1977; Knowles, 1979; Mountfort et al, 1980; Kelly et al, 1982; Senior et al, 1982). While methane production and sulfate reduction are mutually exclusive in anaerobic systems (Martens and Berner, 1974; Mountfort et al, 1980; Winfrey et al, 1981), methanogenic

bacteria are frequently present in sulfate containing sediments and will produce methane when methanogenic precursors are added (Winfrey and Zeikus, 1977; Abram and Nedwell, 1978; Oremland and Taylor, 1978; Sørenson et al, 1981). The generally accepted idea is that sulfate reducing bacteria outcompete methanogenic bacteria for hydrogen and acetate (Bryant et al, 1977; Martens and Berner, 1977; Winfrey and Zeikus, 1977; Oremland and Taylor, 1978; Mountfort and Asher, 1981). Lovley et al (1982) have suggested that: "sulfate reducing bacteria have a higher affinity for hydrogen and acetate than methanogenic bacteria, which enables sulfate reducing bacteria to maintain the pool of these substrates at concentrations too low for methanogenic bacteria to effectively utilize when sulfate is not limiting to sulfate reduction." Nitrate reducing bacteria similarly inhibit methanogenic bacteria by substrate competition (Bollag and Czankowski, 1973; MacGregor and Keeney, 1973; Balderton and Payne, 1976; Zeikus, 1977; Knowles, 1979). Anoxic methane oxidation may compete with methane production in marine and freshwater sediment (Cappenberg 1972; Martens and Berner, 1977) though it is usually of negligible importance in freshwater systems (Zehnder and Brock, 1980).

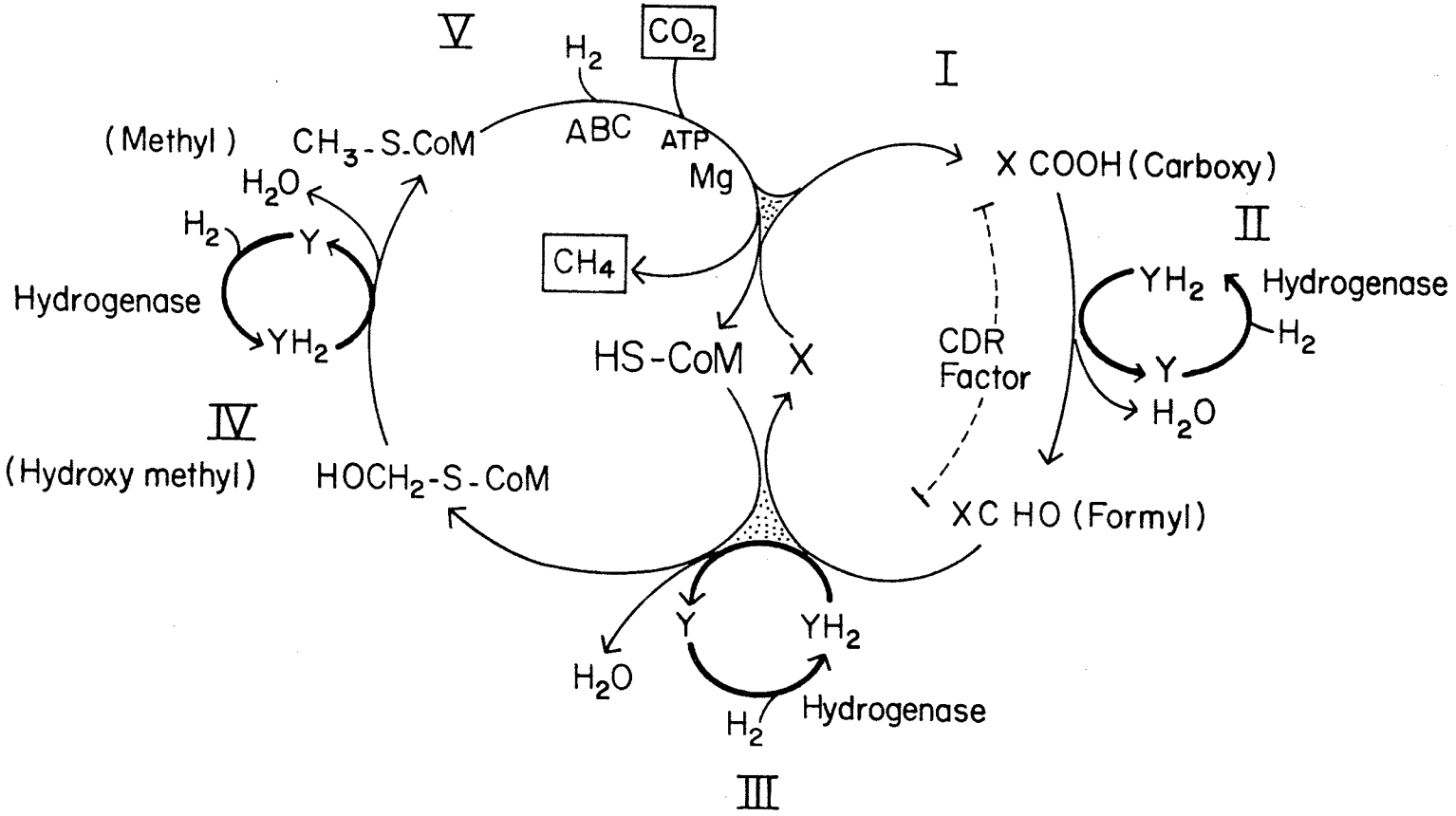
Methane production has been measured in freshwater lakes (Koyama, 1976; Rudd and Hamilton, 1978; Kelly and Chynoweth, 1980; Fallon et al, 1980; Cappenberg and Verdouw, 1982) and was proposed as a measure of chemoorganotrophic activity in anaerobic lake sediments (Kelly and Chynoweth, 1979). Methane is produced in freshwater lakes from organic plant matter which accumulates in the sediments. Kelly and Chynoweth (1981) reported that methane release

accounted for 37-58% of organic carbon input into sediments in five lakes of varying trophic status. Rudd and Hamilton (1978) reported that methane release accounted for 55% of total carbon input in a small eutrophic freshwater lake. Cappenberg and Verdouw (1982) studied the breakdown kinetics of organic matter to methane in Lake Vechten, The Netherlands, including the distribution of "algae cell wall-splitting bacteria" and methanogens in the sediments. Kelly et al (1982) discussed the potential importance of the relative levels of methanogenesis, nitrate reduction, and sulfate reduction in regulating the rate of acidification of lakes.

Biochemistry of Methanogenic Bacteria

The present state of knowledge about the biochemistry of methane production has been due primarily to the work of R.S. Wolfe. In reviews, Wolfe (1979, 1982) outlines the historical evolution of knowledge, the presently accepted pathways, and unanswered questions concerning the biochemistry of methanogens. The pathway of methane formation from carbon dioxide and hydrogen has been more intensively studied than the pathway from acetate because hydrogenotrophic methanogens yield well to mass culture, whereas the only acetotrophic methanogen in pure culture (Methanosarcina) is extremely fastidious and gives poor cell yield (Wolfe, 1982). Wolfe (1979) proposed a cyclic pathway of methane formation from carbon dioxide and hydrogen (Figure 1). The whole system is based on a methyl carrier molecule coenzyme M(CoM) discovered by McBride and Wolfe (1974) which has the structure 2-mercaptoethanesulfonic acid (Taylor and Wolfe, 1974). Carbon dioxide activation in step I is linked to step V (methyl reductase) and a carbon dioxide reduction cofactor (CDR). The

Figure 1. Methane formation from CO_2 and H_2 (after Wolfe, 1979).


V
H2
CO2
I
(Methyl)
CH3-S-CoM
ABC
ATP
Mg
X COOH (Carboxy)
H2O
H2
Y
II
CH4
YH2
Hydrogenase
H2
Hydrogenase
HS-CoM
X
CDR
Factor
Y
H2O
IV
(Hydroxy methyl)
HOCH2-S-CoM
XC HO (Formyl)
Y
YH2
H2O
H2
Hydrogenase
III

carrier X is of unknown structure. The electron donors for the hydrogenase are unknown, though a coenzyme F_{420} may be involved (Eirich et al, 1978). Step V requires an enzyme, methyl-CoM reductase (ABC). ATP acts as an activator in step V, fifteen moles of methane being formed per mole of ATP added (Wolfe, 1982).

Although this tentative pathway for methane production from carbon dioxide is emerging, much work remains to be done. The pathway of carbon dioxide assimilation into cell carbon is totally unknown. The ATP generation pathway in acetotrophic methanogens, as well as the conversion of acetate to methane are unknown. Wolfe (1982) summarized progress in the study of the biochemistry of methanogens as follows: "it would appear that we have only scratched the biochemical surface of those interesting organisms. For example, at the present time not a single mutant or phage has been isolated." One year later, a single plasmid was isolated from a methanogen (Thomm et al, 1983).

Acid Precipitation

The effects of acid precipitation on the environment have been intensively studied within the last decade. There have been eleven major international conferences documenting progress in acid precipitation research from 1975 to the present (Cowling, 1982). Many of the present concepts concerning acid precipitation problems were outlined in the proceedings of the international conference on the ecological impact of acid precipitation (Drabløs and Tollan, 1980). A historical review documenting growth of awareness and understanding of acid precipitation was published by Cowling (1982). Whelpdale and Barrie (1982) described the state of atmospheric

monitoring network operations in Canada and pinpointed areas of impaction of wet acid deposition.

Freshwater lakes in regions underlain by non-calcarious granitic bedrock (eg. The Canadian Shield) have little buffering capacity against acid precipitation (Likens et al, 1979; Scheider et al, 1979; Henriksen, 1980). Wright et al (1980) chronicled susceptible lake regions of Europe and North America which have been subject to acidification. There have been numerous investigations of the effects of acidification on aquatic biota reviewed by Wright et al (1976) and Almer et al (1978). The effects of artificial acidification on several trophic levels of small Canadian Shield lakes were described by Schindler (1980), and Schindler and Turner (1982). Studies have been carried out on the effects of acid precipitation on fishes (Schofield, 1976; Daye, 1980; Rosseland et al, 1980; Spry et al, 1981); zooplankton (Almer et al, 1974; Sprules, 1975; Hendry et al, 1976; Raddum et al, 1980; Malley et al, 1982), periphyton (Hendry et al, 1976; Müller, 1980), and phytoplankton (Hendry et al 1976; Yan and Stokes, 1978; Findlay and Saesura, 1980; Raddum et al, 1980).

There has been a small amount of research on the effects of lake acidification on bacteria. The decomposition of organic detritus in sediments was found to be decreased by the imposition of acidic conditions (Hendry et al, 1976; Traaen, 1980). Traaen (1980) also suggested that a reduction in numbers of planktonic bacteria due to acidity could be ameliorated by high concentrations of soluble organic matter. Laake (1976) showed that heterotrophic activity by bacteria at the surface of sediments was inhibited by low pH. Gahnstrom et al (1980) found that the decomposition rate of organic

matter in littoral sediments of an acidified lake was lower than that in an unacidified lake, whereas the decomposition rates in profundal sediments were similar in both lakes. Usually only the uppermost 2-4 cm of profundal sediment is affected by overlying acidic water (Andersson, 1978; Gahnstrom, 1980), which would account for the reported lack of inhibition of decomposition. Walichnowski and Lawrence (1982) reported that methane production in anoxic sediments of a eutrophic lake was inhibited below pH of 5.5 in laboratory experiments, but only partially inhibited at lakewater pH 4.8 in field studies. Kelly et al (1982) discussed the potential importance of alkalinity production from nitrate reduction and sulfate reduction in regulating lake acidification.

MATERIALS AND METHODS

Study Site

The field work and some of the laboratory work were conducted at the Experimental Lakes Area (ELA) located 52 kilometres east southeast of Kenora, Ontario, at $93^{o}30'$ - $94^{o}00W$, $49^{o}30'$ - $49^{o}45'N$ (Brunskill and Schindler, 1971).

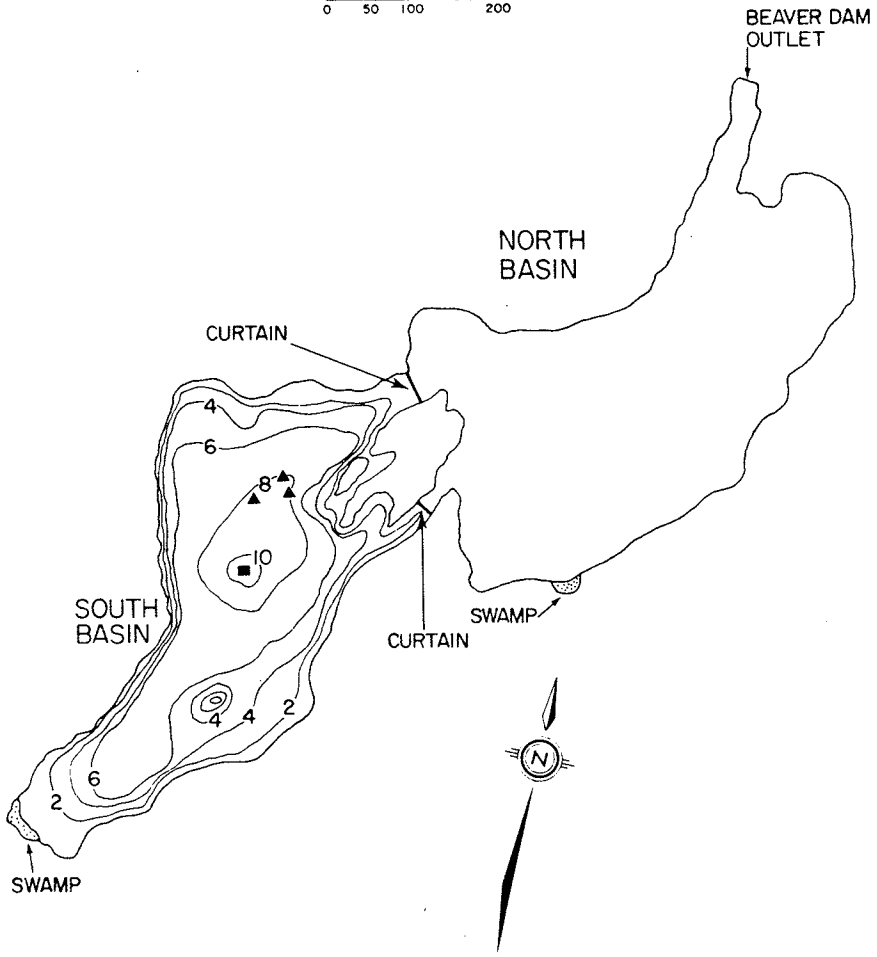
Lake 302, the main study lake, has two basins of roughly equal size separated by a shallow narrows which is blocked by an island and vinyl impregnated nylon curtains (Schindler et al, 1980). Lake 302 has a total surface area of 23.7 hectares and a maximum depth of 13.8 metres (Cleugh and Hauser, 1971). The hypolimnion of the north basin was fertilized from 1972 to 1976 with 0.54 gm of phosphorous (as phosphoric acid), 2.79 gm of nitrogen (as ammonium chloride), and 3.73 gm carbon (as sucrose) per metre squared per year (Schindler et al, 1980). The epilimnion of the north basin was acidified with nitric acid (HNO_3) during summer stratification in 1982 (3,424.4 kg HNO_3) and 1983 (3,690.5 kg HNO_3) (Cruikshank, 1984, in press). The epilimnion of the south basin was acidifed with sulfuric acid (H_2SO_4) during summer stratification in 1982 (2,211.3 kg H_2SO_4) and 1983 (1,993.9 kg H_2SO_4) (Cruikshank, 1984 in press). Studies were restricted to the south basin (Lake 302S) which has a surface area of 10.9 hectares and a maximum depth of 10.6 m (Figure 2).

Sedimentation Trap Experiments

The rate of sedimentation of particulate organic carbon (POC) into the hypolimnion of Lake 302S during summer stratification was estimated using sedimentation traps. However, preliminary experiments designed to determine if cylindrical traps were more efficient POC

Figure 2. Map of Lake 302 showing the morphometry of the south basin only, and the sites of sediment trap installation (▲) and sediment sampling (■).

LAKE 302

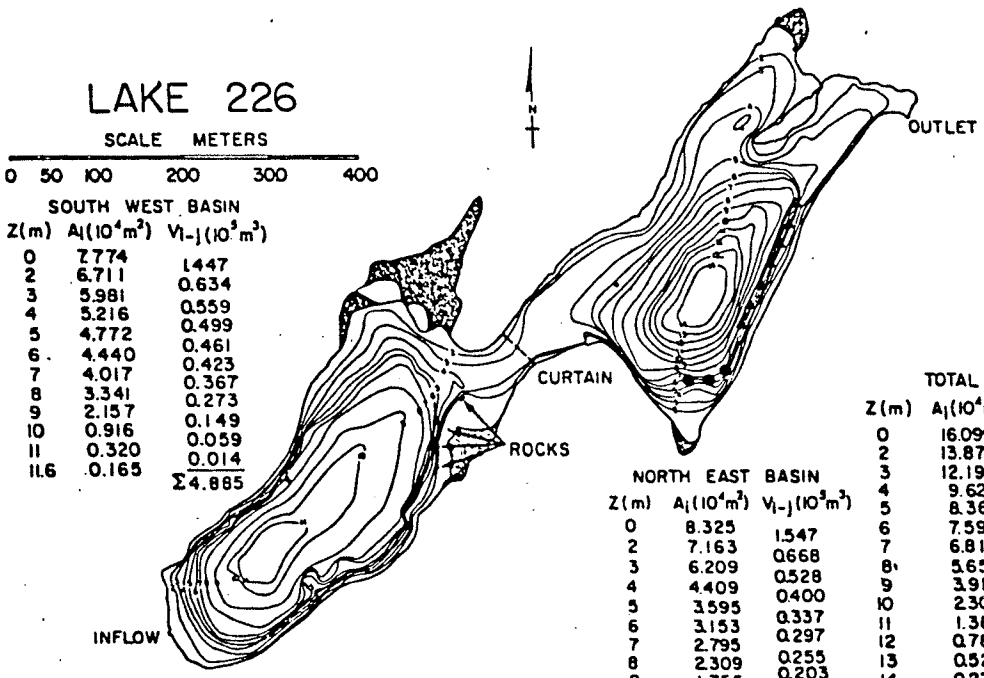

SCALE METERS
0 50 100 200
BEAVER DAM
OUTLET
NORTH
BASIN
CURTAIN
4
6
8
10
SOUTH
BASIN
CURTAIN
SWAMP
4
4
2
6
2
SWAMP
N

collectors than standard ELA bag traps (Hesslein et al, 1980), and if sodium chloride (NaCl) prevented microbial degradation of particulate matter in traps, were conducted in the northeast basin of Lake 226 (Figure 3). The northeast basin is slightly eutrophic, having been fertilized with 0.6 gm PO_4-phosphorous, 3.2 gm NH_3-nitrogen, and 6.1 gm sucrose-carbon per metre squared per year from 1973 to 1979 (Schindler and Fee, 1974). This lake basin was used to test the sedimentation trap methodology because its eutrophic nature ensured that ample particulate matter was trapped in all treatments.

Cylindrical sedimentation traps were constructed of plexiglass cylinders of internal diameter 11.7 cm and length 50 cm, closed at one end. Each trap unit consisted of two cylinders secured in an aluminum harness and suspended in the water column as shown in Figure 4. Prior to the installation of cylindrical traps in the lake, 50 gm of table salt and one litre of cold ($\sim 5^{\circ}C$) hypolimnetic water was added to one cylinder of each unit to obtain a 5% NaCl solution. The "unsalted" cylinder served as the unit's control. Installation was achieved by lowering the trap unit into the water column without disturbing the salt solution. The units were left in the lake for one to two weeks and then retrieved into a boat by slowly hauling in the string, being careful not to resuspend the particulate matter collected at the bottom of the cylinders. The cylinders were removed from the harness and most of the water was decanted without loss of particulate matter. The remaining water and the particulate matter were mixed by vigorous shaking and poured into half-gallon wide-mouth plastic bottles (Nalgene). The samples

Figure 3. A morphometric map of Lake 226 showing the sites of installation of cylindrical trap units (▲) and bag traps (●).

LAKE 226



SOUTH WEST BASIN

Z(m)	$A_i(10^4 m^2)$	$V_{i-j}(10^3 m^3)$
0	7.774	1.447
2	6.711	0.634
3	5.981	0.559
4	5.216	0.499
5	4.772	0.461
6	4.440	0.423
7	4.017	0.367
8	3.341	0.273
9	2.157	0.149
10	0.916	0.059
11	0.320	0.014
11.6	0.165	Σ 4.885

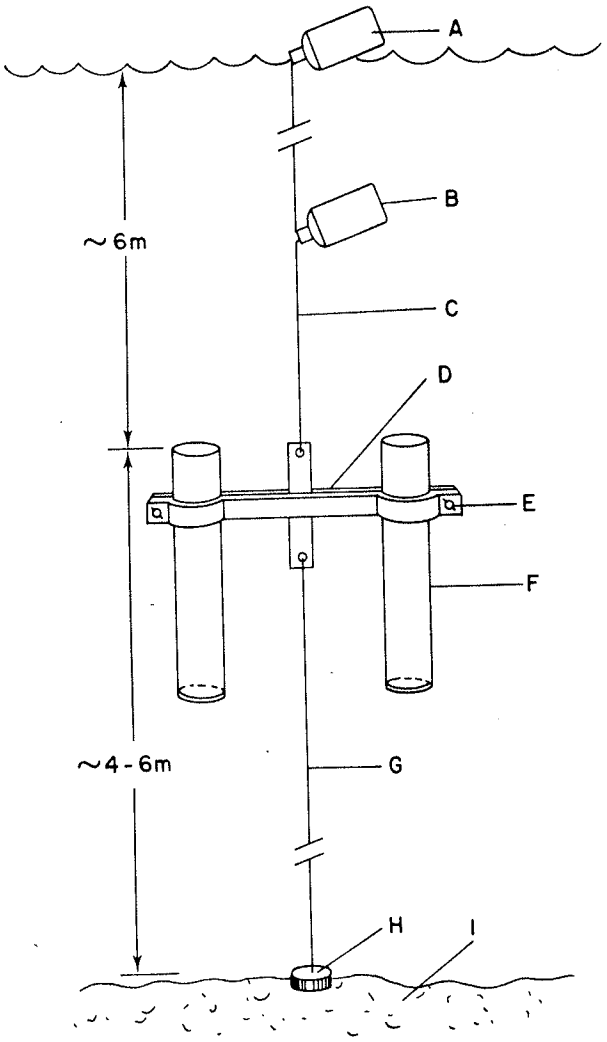
NORTH EAST BASIN

Z(m)	$A_i(10^4 m^2)$	$V_{i-j}(10^3 m^3)$
0	8.325	1.547
2	7.163	0.668
3	6.209	0.528
4	4.409	0.400
5	3.595	0.337
6	3.153	0.297
7	2.795	0.255
8	2.309	0.203
9	1.755	0.157
10	1.387	0.122
11	1.065	0.092
12	0.788	0.065
13	0.521	0.039
14	0.270	0.013
14.7	0.115	Σ 4.723

TOTAL LAKE

Z(m)	$A_i(10^4 m^2)$	$V_{i-j}(10^3 m^3)$
0	16.099	2.994
2	13.874	1.302
3	12.190	1.087
4	9.625	0.899
5	8.367	0.798
6	7.593	0.720
7	6.812	0.622
8	5.650	0.476
9	3.912	0.306
10	2.303	0.181
11	1.385	0.106
12	0.788	0.065
13	0.521	0.039
14	0.270	0.013
14.7	0.115	Σ 9.608

Figure 4. Cylindrical sedimentation trap unit (after C. Kelly, pers. comm.). Legend: A - marker float; B - submerged float; C - string; D - aluminum harness; E - Wing nut and screw; F - plexiglass cylinder; G - anchor rope; H - anchoring weight; I - lake bottom.

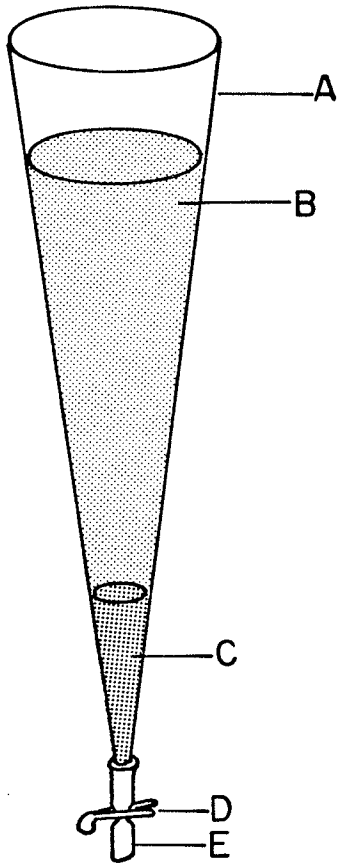

A
B
~6m
C
D
E
F
~4-6m
G
H
I

were returned to the lab for processing.

Sedimentation traps of the bag-type were constructed of polyethylene bags of diameter 22 cm and length 50 cm. The design, and the method of installation and retrieval have been previously described (Hesslein et al, 1980). The entire contents of the bag traps were placed into polyethylene bags after retrieval and returned to the laboratory for processing. Seven cylindrical trap units and three bag traps were utilized in the Lake 226 experiment.

Samples were killed in the laboratory by the addition of 1 ml of saturated $HgCl_2$ solution. The samples were stirred vigorously to resuspend the particulate matter and were poured into cone-shaped settling chambers (Sybron/Nalge) of internal diameter 10 cm and depth 45 cm (Figure 5). Particulate matter was allowed to collect at the bottom of the chamber for approximately twenty-four hours and was then removed through the chamber outlet into preweighed plastic dishes. The particulate matter was dried by placing the dishes into a $60^{o}C$ oven overnight and then into a partially evacuated dessicator for fifteen minutes. The dishes containing dry particulate matter were weighed immediately after dessication on a Mettler precision balance (Model H10T) and the weight of the particulates was determined by difference. The samples were pulverized to a fine powder and stored in labelled glass vials. The pulverized samples were analyzed for particulate organic carbon (POC), nitrogen (PON), and phosphorous (POP) by the Analytical Chemistry laboratory of the Freshwater Institute (FWI) using standard methods outlined in Stainton et al (1977). The total amount of carbon, nitrogen, or phosphorous collected in a trap during an

Figure 5. Cone-shaped settling chamber for concentrating particulate matter in a water sample. Legend: A - plastic cone; B - water; C - particulate matter; D - outlet clamp; E - outlet.


A
B
C
D
E

experimental run was calculated as follows:

Total C, N, or P = mg C, N, or P/gm particulate matter x weight of particulate matter collected (gm).

The POC sedimentation rate was calculated as follows:

$$\text{POC sedimentation rate} = \frac{\frac{\text{mg POC collected}}{\text{mouth area of trap } (cm^2)} \times 10{,}000}{\text{collection time (days)}}$$

$$= mgCm^{-2}\ day^{-1}.$$

The average rate of seven replicates for each cylindrical trap treatment, and three replicates for bag traps was calculated for each collection interval.

Cylindrical traps were utilized to estimate the POC sedimentation rates in Lake 302S during summer stratification in 1981 and 1982. Three trap units were placed at a depth of 7 m, 1 m off the lake bottom (Figure 2). Both cylinders of each trap unit were "salted" (5% NaCl). Installation, retrieval and sampling of traps were identical to the Lake 226 experiment, but laboratory processing of the samples was different.

The volume of each sample was determined by pouring it into a one litre volumetric cylinder. The sample was thoroughly resuspended by shaking and a subsample of recorded volume was removed using a 10 ml disposable glass synringe (Plaspak). The subsample was vacuum filtered through a preweighed, precombusted glass fibre filter (Whatman GF/C, diameter 4.7 cm). The filter was dried for 8-12 hours in a 60°C oven, placed in a partially evacuated dessicator for 15 minutes, then weighed. The total weight of particulate matter collected in a trap was calculated as follows:

$$\text{Total weight collected} = \frac{\text{total sample volume (ml)}}{\text{subsample volume (ml)}} \times \text{subsample weight}$$

The content of POC, PON, POP, and the POC sedimentation rates for each sample were determined as previously described.

In situ Methane and Carbon Dioxide Production

Water samples for methane concentration were taken in 1980 and 1981 using a portable peristaltic pump (Masterflex) and a weighted plastic tubing (Tygon, internal diameter = 9 mm) sampling line marked off at one metre intervals. Samples were taken in 1982 using a portable peristltic pump and a vertical point sampler (Rudd and Hamilton, 1975) permanently stationed at the centre of the basin. This apparatus allowed sampling at precise points in the water column with time, irrespective of the lake water level and without disturbing the methane gradient in the lake (Rudd and Hamilton, 1975). Samples were taken at one metre intervals from the maximum depth to a depth of five metres in the three years monitored.

Twenty-five millitre samples, followed by 25 cc of air, were drawn into a 50 cc disposable plastic synringe (Plastipak) which was then sealed with a 26.G needle, the tip of which was imbedded in a rubber bung. The syringe was shaken vigorously for one minute to strip methane into the gas phase, then the gas phase was injected into a 10 ml serum vial (for storage) according to the method of Rudd et al (1974). Two-tenths of a millilitre of the serum vial gas sample was injected into a gas chromatograph (Pye 104 in 1980, Varian 3700 in 1981 and 1982) equipped with a flame ionization detector and a Poropak Q column. Methane concentration

was calculated according to the method of Stainton et al (1977). Methane concentration profiles of the water column were taken at least once every month during summer stratification.

The rate of release of dissolved methane from anoxic sediments during summer stratification was estimated by measuring the change in methane content with time of a specified volume of the hypolimnion, according to the method of Kelly and Chynoweth (1979). The volume of the lake below 9 m depth was utilized to avoid the complication of results by possible methane oxidation at lesser depths containing oxygen. This volume was divided into two depth intervals, 9 to 10 metres, and 10 to 10.6 metres. The concentration of dissolved methane at the upper depth limit of each interval was multiplied by the volume of the interval to yield a mass of methane. The masses of methane in the two intervals were summed and divided by the basin area at 9 metres depth to yield a mass per unit area of methane. The change in mass per unit area with time represented an estimate of the release of dissolved methane from the sediments. Similarly the rate of release of total carbon dioxide (dissolved inorganic carbon) below 9 metres was calculated using concentration profiles obtained from the ELA chemistry laboratory.

The amount of carbon dioxide generated by methanogenesis during summer stratification was estimated using the method of Kelly et al (1982): the amounts of carbon dioxide produced by the bacterial reduction of O_2, SO_4^{2-}, NO_3^-, Fe^{3+}, and Mn were calculated from the measured amount of electron acceptor reduced and the theoretical amount of carbon dioxide produced by the stoichiometry of the oxidation of organic material of the Redfield composition

$((CH_2O)_{106}(NH_3)_{16}(H_3PO_4))$. Reduction rates of oxygen, sulfate and nitrate were assumed to be equal to total loss of each chemical species from the hypolimnetic water column during summer stratification. "Reduction of iron and manganese was estimated as the increase of total dissolved iron and manganese in the water column." The amounts of carbon dioxide produced from these reactions were summed, and subtracted from the total carbon dioxide produced to yield carbon dioxide of methanogenic origin. The data concerning the amounts of the chemical species O_2, SO_4^{2-}, NO_3^-, Fe^{3+} and Mn were obtained from the ELA chemistry laboratory.

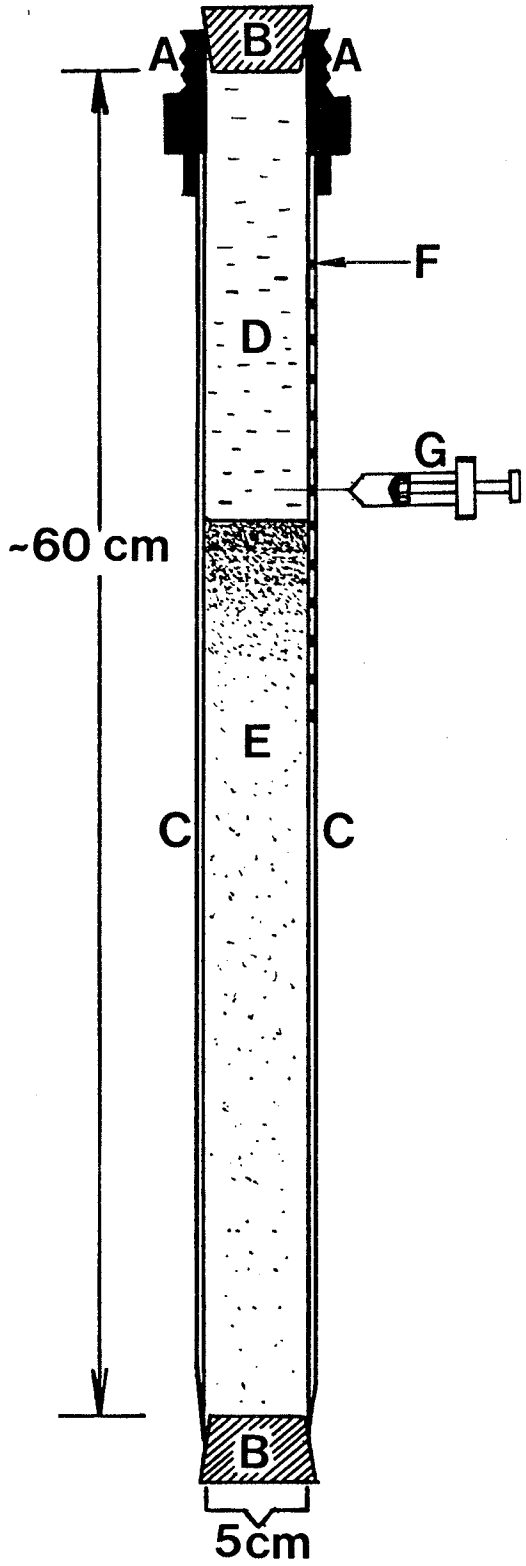
Sampling Methods for Sediment

Sediment samples were collected at the centre of Lake 302S using either a modified Ekman type grab (Burton and Flannagan, 1973) or a gravity cover (K-B corer).

Grab samples represented sediment from the top 10 cm of the lake bottom. The sediments from successive grabs were stored in a 20 litre thick-walled polyethylene carboy (Nalgene) equipped with a screw cap. The carboy was filled completely to minimize exposure of the sediment to oxygen. On returning to the field laboratory, sediment was transferred to acid washed 2.5 litre glass bottles which were sealed with silicone stoppers. These sediments were used immediately or stored at 4^oC.

Sediment cores were taken in acrylic plastic core liners (Figure 6), capped immediately with rubber stoppers to maintain anaerobiosis, and transported to the laboratory. Small holes drilled at 1 cm intervals on the core liner and filled with silicone sealant (Canadian General Electric) permitted removal of

Figure 6. Core liner. Legend: A - PVC male connector (Scepter Manufacturing Co. Ltd. Winnipeg) for attaching the liner to the K-B corer; B - black rubber stopper, no. 11; C - acrylic plastic tubing; D - water; E - sediment; F - small holes filled with silicone sealant; G - sampling or inoculation syringe.


A
B
A
F
D
G
~60 cm
E
C
C
B
5cm

sediment samples, and additions of chemicals via syringes equipped with 18.G needles. When core sections or sediment samples larger than 5 ml were required, cores were extruded and sectioned in a nitrogen filled glove box using the method of Kelly and Chynoweth (1979).

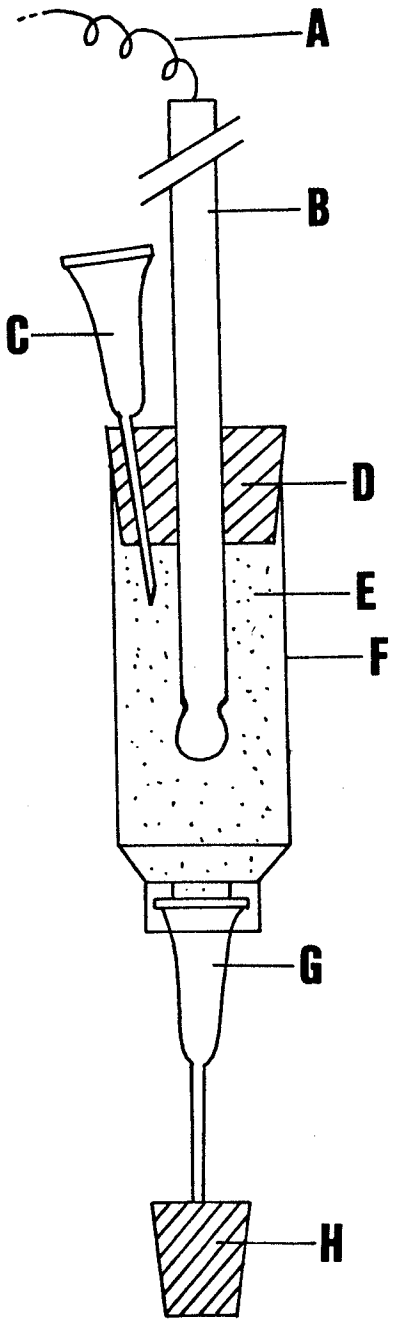
Acidification and pH Measurement of Samples

Sediment and water samples were acidified using 0.1 N hydrochloric acid (HCl) unless stated otherwise. The appropriate volumes of HCl required to adjust the pH of samples to specific values were estimated by titrating the acid against subsamples of known volume prior to commencement of an experiment. The actual pH imposed in a sample was measured by removing a 2.5 ml subsample and dispensing it into a specially designed (C. Kelly and J. Rudd, pers. comm.) pH measurement chamber (Figure 7). This chamber allowed the measurement of pH in an airtight environment, eliminating the possibility of pH fluctuations during measurement due to the equilibration of carbon dioxide between the air and the sample.

Test Tube and Vial Experiments

In vitro methanogenesis in sediments under several conditions was assayed using either 120 ml serum vials or 15x130 mm Hungate-type screw capped test tubes as incubation vessels. The vials were sealed with serum stoppers (13x18 mm, A.H. Thomas Co.) and the Hungate tubes were sealed with flange-type butyl rubber stoppers held in place by a Hungate screw cap (Bellco). Serum vials were utilized in experiments requiring the incubation of whole core sections (~39 ml) or the use of enrichment media. All other

Figure 7. pH measurement chamber. Legend: A - cord connecting the pH electrode to the pH meter; B - pH measurement electrode; C - 18G 1½ inch needle; D - silicone stopper; E - sediment sample; F - barrel of 5 cc plastic syringe (Plastipak) cut at the 3 cc graduation; G - 18G 1½ inch needle, and H - rubber stopper used as a seal of the chamber.


A
B
C
D
E
F
G
H

experiments were done in Hungate tubes. The sediments used were taken from intact cores and were added to the vessels in a nitrogen filled glove-box, unless otherwise stated.

After removal of the sealed incubation vessels from the glove-box, they were shaken vigorously for one minute to strip methane from the sediment into the gas phase. The gas phase was then flushed with oxygen-free nitrogen for ten minutes so that methane in the system was removed at "time zero" of the experiment. The methane concentration at various times during incubation was monitored by shaking the vessels for one minute and injecting 0.2 ml of the gas phase into a gas chromatograph. Methane production per gram wet weight of sediment could be calculated by assuming that shaking stripped all of the methane into the gas phase, since the gas phase volume and the wet weight of sediment in the vessel were known. The accumulation of methane with time was used as a measure of methanogenic activity. All experimental treatments were done in triplicate with each replicate containing sediment from a different core.

The enrichment medium used was modified LPBM basal medium (Zeikus, 1977) (Table 2). Sixty millilitres of medium were dispensed into each serum vial. Sediment extract was used instead of distilled water as the solvent in some cultures to provide "growth factors" for fastidious methanogens. The gas phase was flushed with sterile oxygen-free nitrogen gas until the color of the medium changed from bright red to colorless due to the reduction of resazurin, an oxidation-reduction indicator. Sediment inocula of volume 0.5 ml were removed from cores in the field using a

Table 2. Modified LPBM basal medium for isolation and growth of methogenic bacteria.

Carbon source[a]	1% (w/v)
KH_2PO_4	0.75 gm
$K_2HPO_4 \cdot 3H_2O$	1.45 gm
NH_4Cl	0.90 gm
$MgCl_2 \cdot H_2O$	0.20 gm
$Na_2S \cdot 9H_2O$[b]	0.30 gm
Cysteine-HCl[b]	0.30 gm
Trace minerals[c]	9.00 ml
Vitamin solution[d]	5.00 ml
Resazurin	0.0001% (w/v)
33% filtered lakewater or sediment extract[e]	1000 ml
pH to 6.80 with KOH	

a: sodium acetate, glucose, or cellulose.
b: The reducing agents $Na_2S \cdot 9H_2O$ and Cysteine·HCl were prepared together in distilled water, stored under N_2, and added to the medium after autoclaving.
c: Trace mineral solution contained in gms per litre of distilled water (pH = 7 KOH):Nitrilotriacetic and, 4.50; $FeCl_2 \cdot 4H_2O$, 0.40; $MnCl_2 \cdot 4H_2O$, 0.10; $CoCl_2 \cdot 6H_2O$, 0.17; $ZnCl_2$, 0.10; $CaCl_2$, 0.02; H_3BO_3, 0.02; and sodium molybdate, 0.01.
d: Vitamin solution contained in mg per 100 ml: thiamine·HCl, 20.0; calcium-d-panththenate, 20.0; nicotinamide, 20.0; riboflavin, 20.0; pyridoxine HCl, 20.0; biotin, 0.5; folic acid, 0.3; vitamin B_{12}, 0.2.
e: Sediment extract was made by boiling a mixture of 15-20 gm wet weight of sediment and one litre of distilled water for 10 minutes and then filtering to remove the solid matter.

nitrogen-flushed 1-cc glass syringe and were injected through the serum stoppers into vials. The enrichment cultures were incubated at 37°C in the dark for 35-50 days.

In vitro experiments to determine the effect of low pH on methane production were conducted. Five millilitre aliquots of sediment were added to Hungate tubes. The sediment pH was adjusted by injecting HCl through the cap of the tubes. These tubes were incubated for 2-5 days, depending on the amount of methane produced. The actual pH imposed in each tube was measured after incubation was complete as previously described.

^{14}C-general Methods

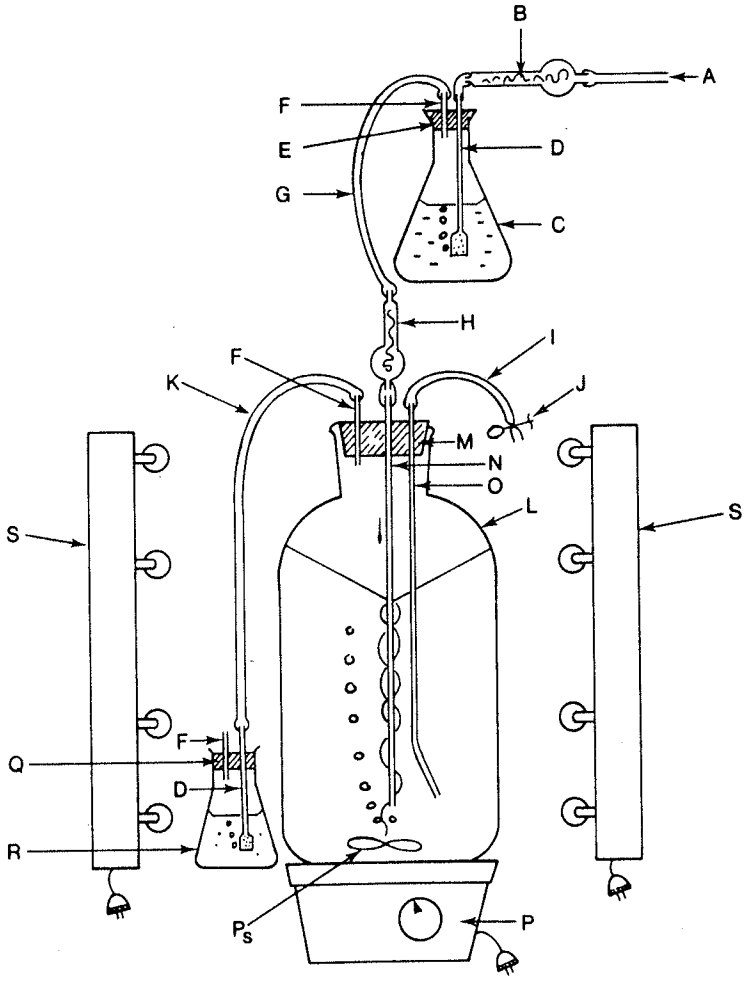
Activity in samples due to ^{14}Carbon (^{14}C) was measured by liquid scintillation counting (LSC) using a 1215 Rackbeta liquid scintillation counter (LKB Wallac). This counter gave results corrected to DPM. Aqueous samples were counted in 10 ml of Formula-947 water accepting cocktail (New England Nuclear). ^{14}C-labelled methane ($^{14}CH_4$) produced during incubations was oxidized to ^{14}C-labelled carbon dioxide ($^{14}CO_2$), trapped in β-phenethylamine, and was counted in a toluene fluor (Rudd et al, 1974). All of the samples for LSC were contained in glass vials.

A modification of the method of Rudd et al (1974) was used to trap $^{14}CH_4$ and $^{14}CO_2$ contained in sediment or water samples: a twenty-five millilitre sample contained in a 50 cc syringe was adjusted to a pH of 11 (using 3 N KOH) so that $^{14}CO_2$ would be retained in solution during subsequent manipulations. Twenty-five millilitres of air were drawn into the syringe, which was sealed, and shaken for one minute to strip $^{14}CH_4$ into the gas phase. The

entire gas phase was then injected into the $^{14}CH_4$ oxidation loop of Rudd et al (1974) in which $^{14}CH_4$ was oxidized to $^{14}CO_2$ (by passage over hot copper oxides); and in which the $^{14}CO_2$ was trapped in β-phenethylamine. The pH of the sediment or water sample was adjusted to 3 (using 3 N HCL) to ensure that $^{14}CO_2$ would be lost from solution, and the above procedure was repeated to trap $^{14}CO_2$ in β-phenethylamine.

The labelled substrates for methanogenesis utilized were [1,2-^{14}C]-sodium acetate and ^{14}C-labelled Chlorella pyrenoidosa cells. The stock [1,2-^{14}C]-sodium acetate (New England Nuclear) had a specific activity of 55.0 mCi $mMol^{-1}$ and a concentration of 100 μCi ml^{-1} ethanol. The stock solution was diluted ten-fold to make the working solution utilized in experiments. ^{14}C-labelled Chlorella pyrenoidosa cells were obtained by incubating batch cultures of the alga in the presence of ^{14}C-labelled sodium bicarbonate which was fixed into cell carbon during photosynthesis. The Chlorella culture was obtained from Dr. G. Robinson of the Department of Botany, University of Manitoba. The alga was "maintained" in 200 ml flask cultures using Bold's basal medium (Stein, 1975). These cultures were incubated in a 28°C illuminated chamber with moderate agitation. Growth was monitored by the increase in cell concentration with time using a Hemacytometer counting chamber method (Stein, 1975). Subcultures were made every 8-9 days. ^{14}C-labelling of Chlorella was carried out in a 10-litre batch culture system (Figure 8). Before labelling experiments were attempted, the growth characteristics of Chlorella pyrenoidosa in the 10-litre system were illucidated. Growth was measured as in

Figure 8. Ten-litre batch culture system for growing ^{14}C-labelled algae. Legend: A - air inlet; B - cotton filled filter for removing particles from the air supply; C - 1000 ml flask containing distilled water for hydration of the air supply; D - fritted glass sparger (Pyrex); E - rubber stopper; F - glass air outlet (I.D. = 5 mm); G - plastic tubing (Tygon, I.D. = 6 mm); H - sterile cotton filled bacterial filter; I - plastic sampling tube (Tygon, I.D. - 6 mm); J - sampling tube clamp; K - air outlet tubing (Tygon, I.D. = 6 mm); L - 15-litre glass vessel (Pyrex) containing 10-litre sterile Bold's medium; M - rubber stopper, no. 12; N - glass air inlet (I. D. - 5 mm); O - glass sampling tube (I.D. = 5 mm); P - Bell-Stir heavy duty magnetic stirrer (Bellco Co.); P_s - stirring magnet; Q - rubber stopper; R - 250 ml flask containing 10 N KOH for trapping $^{14}CO_2$ in the outlet gas from the culture; S - battery of 4 cool white fluorescent lamps (General Electric Co.).


B
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Q
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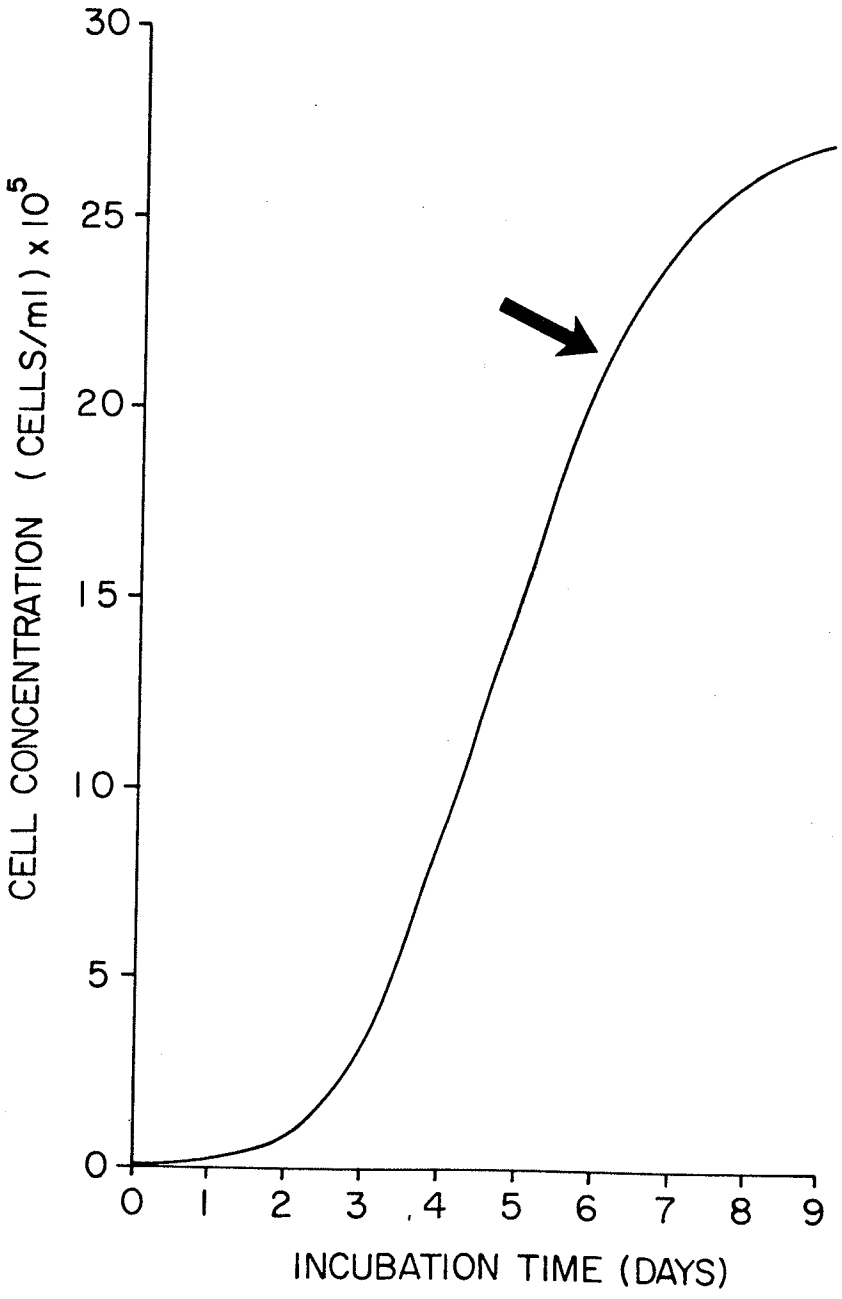
R
P_S
P

the maintenance cultures and incubation wat at 28°C. These 10-litre cultures were inoculated with 10 ml of 3-day old ($\sim 10^6$ cells/ml) maintenance cultures. Subsamples of 5 ml for cell concentration were taken daily by clamping the air outlet and opening the sampling tube according to the method of Rodgers and Depinto (1981). An average growth curve was constructed from the data of three consecutive growth experiments (Figure 9). Thirteen millicuries of ^{14}C-labelled sodium bicarbonate was added to two such mass cultures of the alga on the sixth day of incubation (beginning of the declining growth phase) to minimize loss of label due to excretion. Greater than 95% of the label was fixed into particulate carbon within one day. It was assumed that the ^{14}C-label was uniformly incorporated into cell components. The cultures were harvested on the ninth day of incubation by continuous centrifugation (Heraus-Christ junior 15000 continuous centrifuge) at 5000 RPM, washed twice in 2% HCl and distilled water, and dried overnight at 60°C in aluminum dishes. The dried cells were pulverized to a fine powder and were stored in glass vials at -10°C. The specific activity of the labelled cells was estimated by mixing 5 mg of dried cells, 3 ml of NCS (tissue solubilizer), and 10 ml of Formula-947 and counting the activity by LSC. The specific activity obtained was 3.17×10^5 DPM/mg of cells.

Figure 9. Average growth curve of Chlorella pyrenoidosa in 10 litre batch culture at 28°C. (The arrow indicates the time of addition of ^{14}C-labelled sodium bicarbonate.)


30
25
20
15
10
5
0
CELL CONCENTRATION (CELLS/ml) x 10^5
0 1 2 3 4 5 6 7 8 9
INCUBATION TIME (DAYS)

In Vitro Experiments Utilizing ^{14}C-labelled Substrates

Either ^{14}C-labelled *Chlorella* cells or [1,2-^{14}C]-sodium acetate was added as a methanogenic substrate to acidified sediment. The sediment utilized in these experiments was collected shortly after the onset of summer stratification using an Ekman type grab. An incubation temperature of 28°C was employed to stimulate the methanogenic rate above *in situ* levels. All chemicals used in these experiments were nitrogen flushed, and stored under nitrogen.

Experiments in which ^{14}C-labelled *Chlorella* was the substrate were done in 125 ml Erlenmeyer flasks. Fifty-five ml aliquots of sediment were dispensed under nitrogen into these flasks, which were then sealed with serum-type stoppers (Subaseal, no. 49) and flushed with nitrogen for thirty minutes. The sediments were acidified to the desired pH (duplicate flasks at each pH) by injecting 3 N HCl through the serum stoppers. The pH of the sediment was measured at the beginning and the end of the experiment by removing 5 ml of sediment through the serum stopper and dispensing these subsamples into the pH measurement chamber previously described. The average pH over the course of incubation was calculated. The flasks containing acidified sediments were acclimated overnight at 28°C in the dark on a rotary shaker (150 RPM), then 1-ml of ^{14}C-labelled *Chlorella* cell suspension (0.02 gms cells, 6.34×10^6 DPM) was injected into each flask and incubation was begun under acclimation conditions. Two samples of 0.5 ml were removed from the gas phase of each flask using a 0.5 cc glass syringe (Glaspak) every five days and were injected into a $^{14}CH_4$ oxidation loop to assay for $^{14}CH_4$ and $^{14}CO_2$. During injection of the first sample, the $^{14}CH_4$ oxidizing

column was switched off so that any $^{14}CO_2$ trapped originated from $^{14}CO_2$ in the sample (and not from oxidation of $^{14}CH_4$ to $^{14}CO_2$). During injection of the second sample the $^{14}CH_4$ oxidizing column was switched on, thus the activity trapped was due to $^{14}CO_2$ originally present in the sample as well as $^{14}CO_2$ produced by $^{14}CH_4$ oxidation in the loop apparatus. The activity due to $^{14}CH_4$ was calculated by subtracting the activity of the first sample from the activity of the second sample. The activity of $^{14}CO_2$ in the 0.5 ml gas samples was corrected for the total gas phase volume (VOL_{gas}) in the flasks to yield the total activity in the gas phase (DPM_{gas}). The activity in the sediment phase of the flasks (DPM_{sed}) was calculated as follows:

$$DPM_{sed} = \left(1 + \alpha' \frac{VOL_{sed}}{VOL_{gas}}\right) \times DPM_{gas}$$

where α' = the apparent solubility of CO_2 at 28°C in an aqueous solution, corrected for pH,

and VOL_{sed} = volume of sediment in the flask.

This formula was adapted from a method for calculating dissolved methane in Stainton et al (1977). The apparent solubility α' was calculated as follows:

$$\frac{\alpha'}{\alpha} = [\text{antilog}(pH - pk'_{28^oC})] + 1 \quad \text{(Umbreit, 1957)}$$

where α = solubility of CO_2 in distilled water at 28°C

and pk'_{28^oC} = The log of the apparent concentration dissociation constant of carbonic acid at 28°C.

The total activity due to $^{14}CO_2$ in the flasks was expressed as DPM

gm^{-1} dry weight of sediment and was calculated as follows:

$$DPM\ gm^{-1}\ \text{dry wt} = \frac{DPM_{gas} + DPM_{sed}}{\text{dry wt of sediment}} .$$

The change in activity due to $^{14}CO_2$ production during incubation was used as a relative measurement of anoxic CO_2 production at various pH. The activity due to $^{14}CH_4$ was calculated in a similar manner except that the effect of pH on solubility of CH_4 was ignored, thus α was used to calculate DPM_{sed}.

Experiments in which [1,2-^{14}C]-sodium acetate was the substrate were done in acid washed 50 cc all glass syringes (London Luer). Sediment aliquots of 25 ml were dispensed under nitrogen into the syringes, sterile unlabelled sodium acetate was added to a concentration of 80 µM, and the syringes were sealed and acclimated at 28°C for one hour. After acclimation, duplicate syringes were acidified to specific pH, 100 µl of [1,2,-^{14}C]-sodium acetate (1 µCi) solution was added to each, a small amount of nitrogen was drawn in to allow mixing of the contents, and they were sealed and incubated at 28°C for 2 hours. The incubation was stopped by adjusting the pH to 11 with 3 N KOH. The activity of $^{14}CO_2$ and $^{14}CH_4$ produced was determined as previously described for syringe samples. Activity was expressed as DPM gm^{-1} dry weight of sediment to yield estimates of the amounts of $^{14}CO_2$ and $^{14}CH_4$ produced in 2 hours at different pH.

The method of Wright and Hobbie (1966) for determining the kinetics of conversion of acetate to methane and carbon dioxide was employed to compare methanogenic efficiency in acidified (pH 4) and unacidified (pH 6.2) sediment. Unlabelled sodium acetate was added to sediments contained in 50 cc syringes to final added concentrations

of 0 μM, 20 μM, 40 μM, 60 μM, and 80 μM. One hundred μl of [1,2,-^{14}C]-sodium acetate (1 μCi) was added to each syringe and they were then incubated at 28°C for 2 hours. The activity due to $^{14}CO_2$ and $^{14}CH_4$ were determined after incubation was complete. A modified Lineweaver-Burke plot was constructed with y axis: $\frac{C\mu t}{c}$, and x axis: concentration of "cold" sodium acetate added, where:

Cμ = activity (DPM) of sodium acetate added

t = incubation time (hrs)

c = activity (DPM) of $^{14}CO_2$ or $^{14}CH_4$ produced.

Extrapolation of a linear regression of this data to the x-axis gave a value $(K + S_n)$, a growth constant equivalent to the Michaelis constant K_m which is the substrate concentration at which half of the maximum velocity of an enzymatic reaction can occur. The maximum velocity (V) was calculated using the equation of Wright and Hobbie (1966):

$$\frac{Cut}{c} = \frac{(K+Sn)}{V} + \frac{A}{V},$$

where A = the amount of "cold" acetate added. At A=0, the equation became:

$$V = \frac{(K+Sn)}{Cut/c},$$

where $\frac{Cut}{c}$ was the y-axis intercept of the modified Lineweaver-Burke plot.

Intact Core Experiments

Experiments utilizing ^{14}C-labelled Chlorella or [1,2-^{14}C]-sodium acetate as substrates were done in intact cores to find out which stages of the overall fermentation were inhibited by low pH in an intact sediment-water system. The water overlying the sediments in the cores was the site of acidification. The cores were incubated at near in situ temperature (8°C).

Experiments utilizing ^{14}C-labelled Chorella cells were performed

as follows: five cores were collected from the centre of Lake 302S. The appropriate volumes of HCl were injected through holes in the core-liner to acidify the water overlying the sediments in the cores. The pH was measured after one, eleven, and twenty-five days incubation and an average pH for the incubation period was calculated. One day after acidification, 10 mg of ^{14}C-labelled Chlorella cells (3.17×10^6 DPM in 1 ml of distilled water) was injected into each core just above the sediment-water interface. The cores were incubated in the dark for 26 days. After incubation was complete, a 25 ml water subsample was taken from each core using a 50 cc disposable syringe and was assayed for $^{14}CH_4$ and $^{14}CO_2$. The activity (DPM) in each subsample was corrected for the total volume of the water phase in each core to yield the total amount of $^{14}CH_4$ or $^{14}CO_2$ released from the sediment during incubation. This total was divided by the area of the core liner and the incubation time to yield a rate of release of $^{14}CH_4$ or $^{14}CO_2$ in DPM cm^{-2} day^{-1}. The amount of ^{14}C-labelled dissolved organic carbon (^{14}C-DOC) produced in the upper sediments was determined as follows: the upper 5 cm of sediment in each core was sliced off, dispensed into 250 ml plastic centrifuge bottles (Nalgene), and centrifuged at 8000 RPM for 20 minutes in a Sorvall RC-5 high speed centrifuge. After centrifugation 10 ml of the supernatant was decanted into a glass vial, acidified to pH 2 with 1 N HCl, and bubbled for 3 minutes with nitrogen to remove dissolved $^{14}CO_2$. A 100 µl subsample of this acidified supernatant was assayed for radioactivity by LSC.

Six cores were utilized for experiments in which [1,2-^{14}C]-sodium acetate was the substrate. Duplicate cores were acidified to

various pH. The cores were acclimated for five hours at 8°C in the dark, the water phases were acidified, and then the cores were incubated overnight. After incubation the upper 5 cm section of sediment in each core was removed under nitrogen in a glove box. Sediment samples of 25 ml from each core section were drawn into 50 cc all glass syringes, 100 µl of [1,2-^{14}C]-sodium acetate (1 µCi) solution was added, and the syringe subsamples were incubated in the dark for 2 hours. Five ml sediment samples from core sections were also drawn into 10 ml plastic syringes (Plastipak) for later pH analysis. After incubation the amounts of $^{14}CO_2$ and $^{14}CH_4$ produced were assayed using the method described previously for syringe samples. The activities (DPM) obtained were corrected for the total volume of the sediment section removed from cores, the area of the core liner, and the incubation time to yield a rate of release of either $^{14}CH_4$ or $^{14}CO_2$ in DPM cm^{-2} day^{-1}.

RESULTS

POC Sedimentation Rates

The mean sedimentation rates of POC which were estimated using cylindrical traps containing 5% sodium chloride (NaCl) were usually higher than the rates which were estimated using either cylindrical control traps or bag traps (Figure 10). The corresponding numerical values of these rates and their standard deviations are listed in Table 3. During week 4 the bag trap rate was higher than the cylindrical control trap rate, and during week 6 the cylindrical control trap rate rate was higher than the rate from cylindrical traps which contained 5% NaCl. The carbon:nitrogen:phosphorous ratios (C:N:P) of the particulate matter which was collected in the traps were not affected by the three trap treatments tested (Table 4).

Qualitative changes in the appearance of particulate matter collected in sedimentation traps which contained 5% NaCl were observed. The particulate matter was brown in contrast to the dark green color observed in "unsalted" traps. Also there were less microscopically observable motile microorganisms in the particulate matter contained in "salted" traps.

The mean sedimentation rates of POC into the hypolimnion of Lake 302S were estimated during summer stratification in 1981 and 1982 (Figure 11). The corresponding numerical values of these rates and the standard deviations are listed in Table 5. The 1981 data did not span enough time to show trends in the change of POC sedimentation rate. During 1982 the POC sedimentation rate increased from 81 $mgC\ m^{-2}\ day^{-1}$ in late May, to 298 $mgC\ m^{-2}\ day^{-1}$ in

Figure 10. The mean POC sedimentation rates at 6 m depth in Lake 226NE (during summer stratification) estimated using cylindrical sedimentation traps which contained 5% NaCl (A), cylindrical sedimentation traps not containing 5% NaCl (B), and bag traps (C).

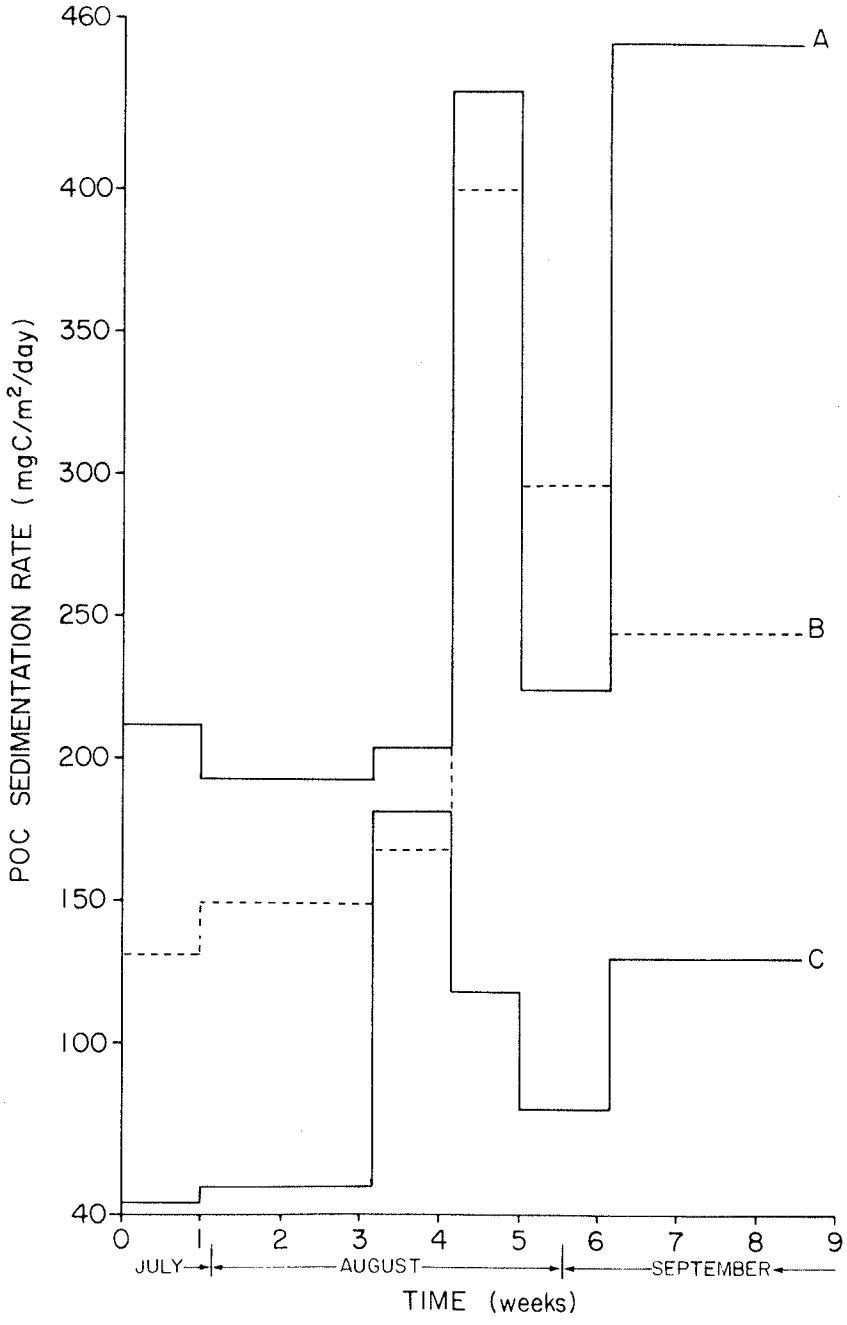

460
400
350
300
250
200
150
100
40
POC SEDIMENTATION RATE (mgC/m²/day)
A
B
C
0
1
2
3
4
5
6
7
8
9
JULY
AUGUST
SEPTEMBER
TIME (weeks)

Table 3. The mean POC sedimentation rates ($mgC\ m^{-2}\ day^{-1}$) and standard deviations (S.D.) measured during consecutive time intervals in Lake 226NE using cylindrical traps containing 5% NaCl, cylindrical traps not containing 5% NaCl, and bag-type traps.

Time Interval	POC sedimentation rate ($mgC\ m^{-2}\ day^{-1}$) ± S.D.		
	Cylindrical + 5% NaCl	Cylindrical	Bag-Type
Jul. 23-30	211.53 ± 56.68	131.48 ± 45.96	44.37 ± 0.76
Jul. 31- Aug. 14	193.19 ± 36.66	148.70 ± 12.58	50.36 ± 16.34
Aug. 15-21	204.06 ± 64.51	167.64 ± 43.70	182.00 ± 9.90
Aug. 22-27	435.46 ± 99.74	400.21 ± 61.03	117.60 ± 85.38
Aug. 28-Sept. 4	223.68 ± 59.47	296.11 ± 101.41	77.29 ± 51.51
Sept. 5-21	451.04 ± 235.26	243.75 ± 64.78	129.62[a]

a: only one sample available.

Table 4. The C:N:P ratios in particulate organic matter collected in Lake 226NE, using cylindrical sedimentation traps containing 5% NaCl (Cyl + 5% NaCl), control cylindrical traps (Cyl), and bag-type traps (Bag).

Trap Type	C:N:P ratios during consecutive trapping intervals					
	Jul. 23-30	Jul.31-Aug.14	Aug. 15-21	Aug. 22-27	Aug.28-Sept.4	Sept. 5-21
Cyl + 5% NaCl	83:8:1	66:7:1	63:7:1	60:5:1	66:6:1	61:5:1
Cyl	71:11:1	68:11:1	73:10:1	76:8:1	75:9:1	52:9:1
Bag	73:14:1	59:10:1	76:10:1	75:9:1	67:7:1	53:8:1

Figure 11. Mean POC sedimentation rates into the hypolimnion of Lake 302S during summer stratification in 1981 (---) and 1982 (——).

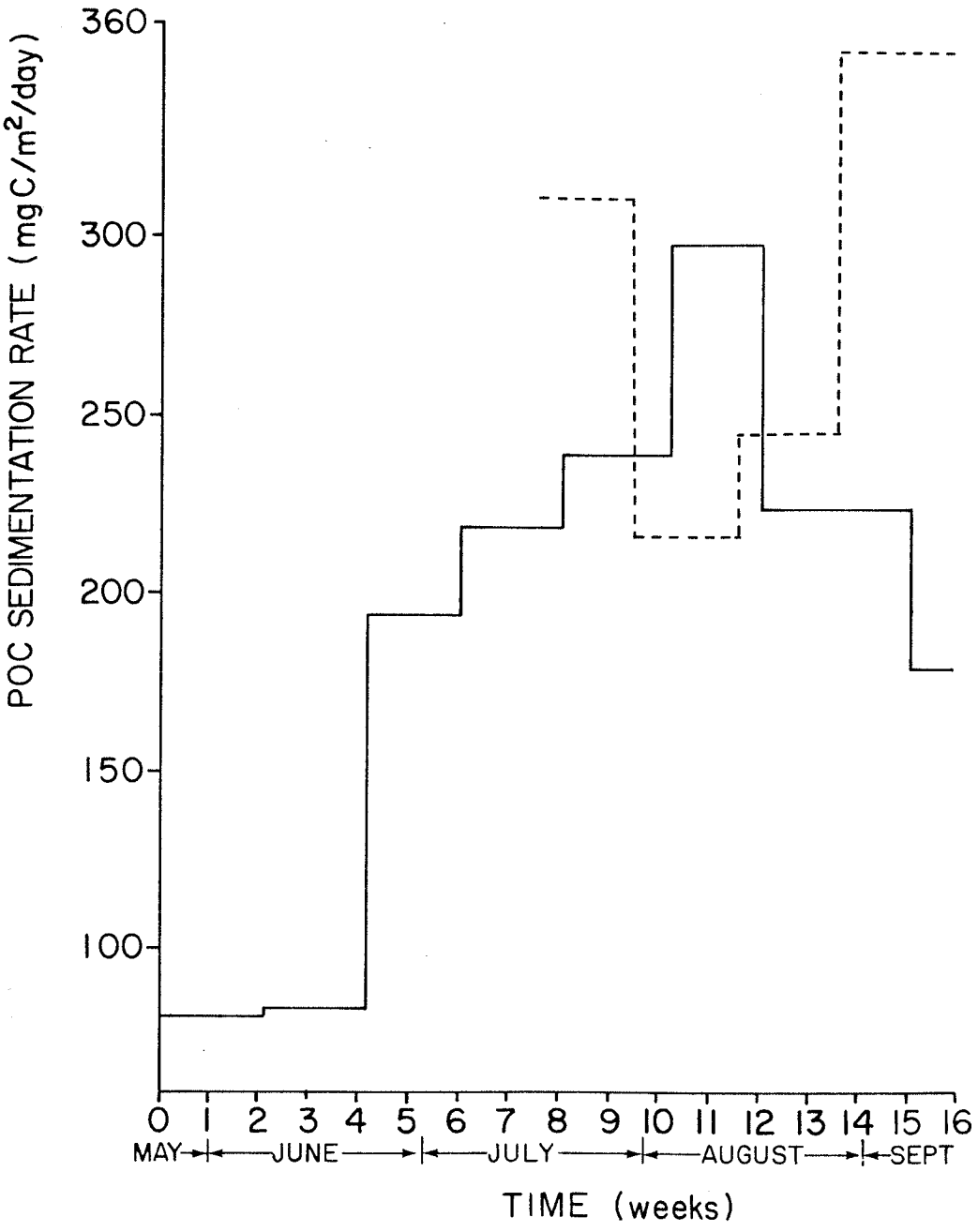

POC SEDIMENTATION RATE (mg C/m²/day)
360
300
250
200
150
100
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
MAY
JUNE
JULY
AUGUST
SEPT
TIME (weeks)

Table 5. The mean POC sedimentation rates (mgC m^{-2} day^{-1}) and standard deviations (S.D.) in Lake 302S during summer stratification in 1981 and 1982.

Year	Time Interval	POC sedimentation rate (mgC m^{-2} day^{-1}) ± S.D.
1981		
	Jul. 16-29	311.27 ± 40.04
	Jul. 30-Aug. 13	215.71 ± 21.30
	Aug. 14-27	244.54 ± 25.73
	Aug. 28-Sept. 10	351.89 ± 22.28
1982		
	May 24-Jun. 8	80.77 ± 9.40
	Jun. 9-22	83.16 ± 10.68
	Jun. 23-Jul. 5	193.93 ± 48.45
	Jul. 6-19	218.58 ± 50.15
	Jul. 20-Aug. 3	239.01 ± 55.68
	Aug. 4-16	297.61 ± 37.50
	Aug. 17-Sept. 6	223.62 ± 36.02
	Sept. 7-12	179.05 ± 52.12

early August, then decreased to 179 mgC m^{-2} day^{-1} in early September. A comparison of the average POC sedimentation rates during analogous time periods in 1981 (July 16-September 10) and 1982 (July 20-September 12) yielded rates of 280.9 mgC m^{-2} day^{-1} and 234.8 mgC m^{-2} day^{-1} respectively. The C:N:P ratio of particulate matter (which was collected in the sedimentation traps) increased during summer stratification in 1981 and 1982 (Table 6). A comparison of the average C:N:P ratio in particulate matter which was collected during the analogous time periods in 1981 and 1982 previously mentioned, yielded ratios of 47:5:1 and 87:9:1 respectively.

In situ Methane and Carbon Dioxide Production

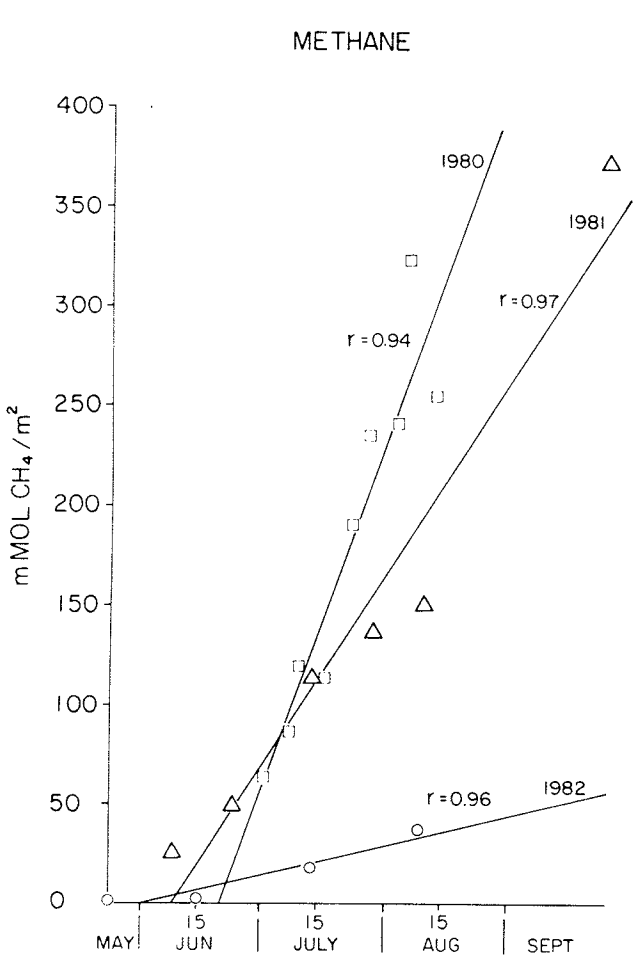
The increase in the mass of methane per unit area below 9 m in Lake 302S during summer stratification was linear in the 3 years monitored (Figure 12). The corresponding methane production rates (calculated from the rate of change of mass per unit area with time) for the 3 years were 5.61 mMoles m^{-2} day^{-1} in 1980, 3.10 mMoles m^{-2} day^{-1} in 1981, and 0.48 mMoles m^{-2} day^{-1} in 1982.

The increase in the mass of carbon dioxide per unit area below 9 m in Lake 302S was linear during 1982 only (Figure 12). The r values of the linear regressions of the carbon dioxide data were low in 1980 (r=0.77) and 1981 (4=0.59), but high in 1982 (r=0.94). The corresponding carbon dioxide production rates for the 3 years were 7.0 mMoles m^{-2} day^{-1} in 1980, 1.30 mMoles m^{-2} day^{-1} in 1981, and 0.94 mMoles m^{-2} day^{-1} in 1982. The ratios of methanogenic carbon dioxide to methane, and anoxic carbon dioxide to methane produced below 9 m during summer stratification were calculated (Appendix 1) for 1980, 1981, and 1982 (Table 7).

Table 6. The C:N:P ratios of particulate matter collected in sedimentation traps in Lake 302S during summer stratification in 1981 and 1982.

Year	Trapping Interval	C:N:P
1981		
	Jul. 16-29	29:5:1
	Jul. 30-Aug. 13	38:4:1
	Aug. 14-27	47:5:1
	Aug. 28-Sept. 10	73:7:1
1982		
	May 24-Jun. 8	56:4:1
	Jun. 9-22	50:7:1
	Jun. 23-Jul. 5	55:7:1
	Jul. 6-19	63:7:1
	Jul. 20-Aug. 3	58:7:1
	Aug. 4-16	83:8:1
	Aug. 17-Sept. 6	103:10:1
	Sept. 7-12	105:11:1

Figure 12. The mass of methane and carbon dioxide per unit area below 9 m depth in Lake 302S during summer stratification in 1980 (□), 1981 (△), and 1982 (○).


METHANE
400
350
300
250
200
150
100
50
0
m MOL CH_4 / m^2
1980
1981
r = 0.94
r = 0.97
r = 0.96
1982
MAY
15 JUN
15 JULY
15 AUG
SEPT

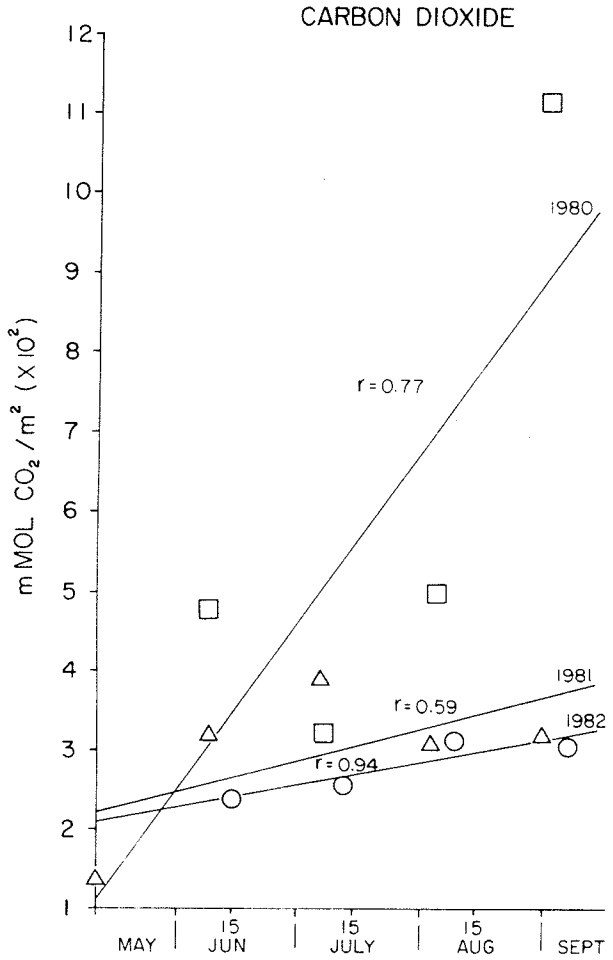

CARBON DIOXIDE
12
11
10
9
8
7
6
5
4
3
2
1
m MOL CO_2 / m^2 (X 10^2)
1980
r = 0.77
1981
r = 0.59
1982
r = 0.94
MAY
15 JUN
15 JULY
15 AUG
SEPT

Table 7. The ratios of methanogenic CO_2:CH_4 and anoxic CO_2:CH_4 produced below 9 m depth in Lake 302S during summer stratification in 1980, 1981, and 1982.

Year	Anoxic CO_2:CH_4	Methanogenic CO_2:CH_4
1980	1.25	--[a]
1981	0.42	0.40
1982	1.73	1.65

[a]The chemical data for calculating the contribution of various reduction processes to CO_2 production were not available for 9 m depth in 1980.

The percentage returns of carbon as methane and carbon dioxide relative to POC input into the hypolimnetic sediments of Lake 302S were calculated for 1981 and 1982 (Table 8). Though the POC inputs were similar in both years, the percentage returns were lower in 1982.

In vitro Test-tube and Vial Experiments

Incubation of sediment core sections representing sediment from the 0-6 cm depth interval indicated that most of the methanogenic activity occurred below 2 cm depth (Figure 13). An enrichment culture experiment (utilizing acetate as the carbon source) yielded maximum activity in the 4-5 cm depth core section (Figure 14). Subcultures made from the above enrichment cultures did not produce methane under the incubation conditions used. An enrichment culture experiment in which various carbon sources were used, also failed to yield methane producing subcultures: the methane concentration in the cultures containing sediment extract increased rapidly within the first 5 days of incubation, remained constant for 10 days then decreased (Figure 15). The amount of methane produced and the subsequent decrease in methane concentration were greater in cultures to which cellulose or acetate was the carbon source added, than in cultures to which glucose was added. A set of cultures which contained acetate as the carbon source but did not contain sediment extract, yielded a linear increase in methane concentration after a fifteen day lag period, but methane concentration did not decrease during the incubation.

In vitro methane production in sediment (which was incubated at *in situ* temperature of $8^{o}C$) was inhibited by decreasing the

Table 8. The percentage returns of carbon as methane and carbon dioxide from hypolimnetic sediments relative to POC input in Lake 302S during July 16-September 10 in 1981, and July 20-September 12 in 1982.

Year	Amount of POC input ($gmC\ m^{-2}$)	Amount of gas production ($gm\text{-}atoms\ C\ m^{-2}$)		Gas production as a % of POC input	
		CH_4	CO_2	CH_4	CO_2
1981	15.94	2.12	0.89	13.30	5.58
1982	13.22	0.31	0.54	2.34	4.08

Figure 13. *In vitro* methane production in sediment core sections incubated at 20°C. Legend: (0) 0-2 cm depth, (□) 2-4 cm depth, (△) 4-6 cm depth.

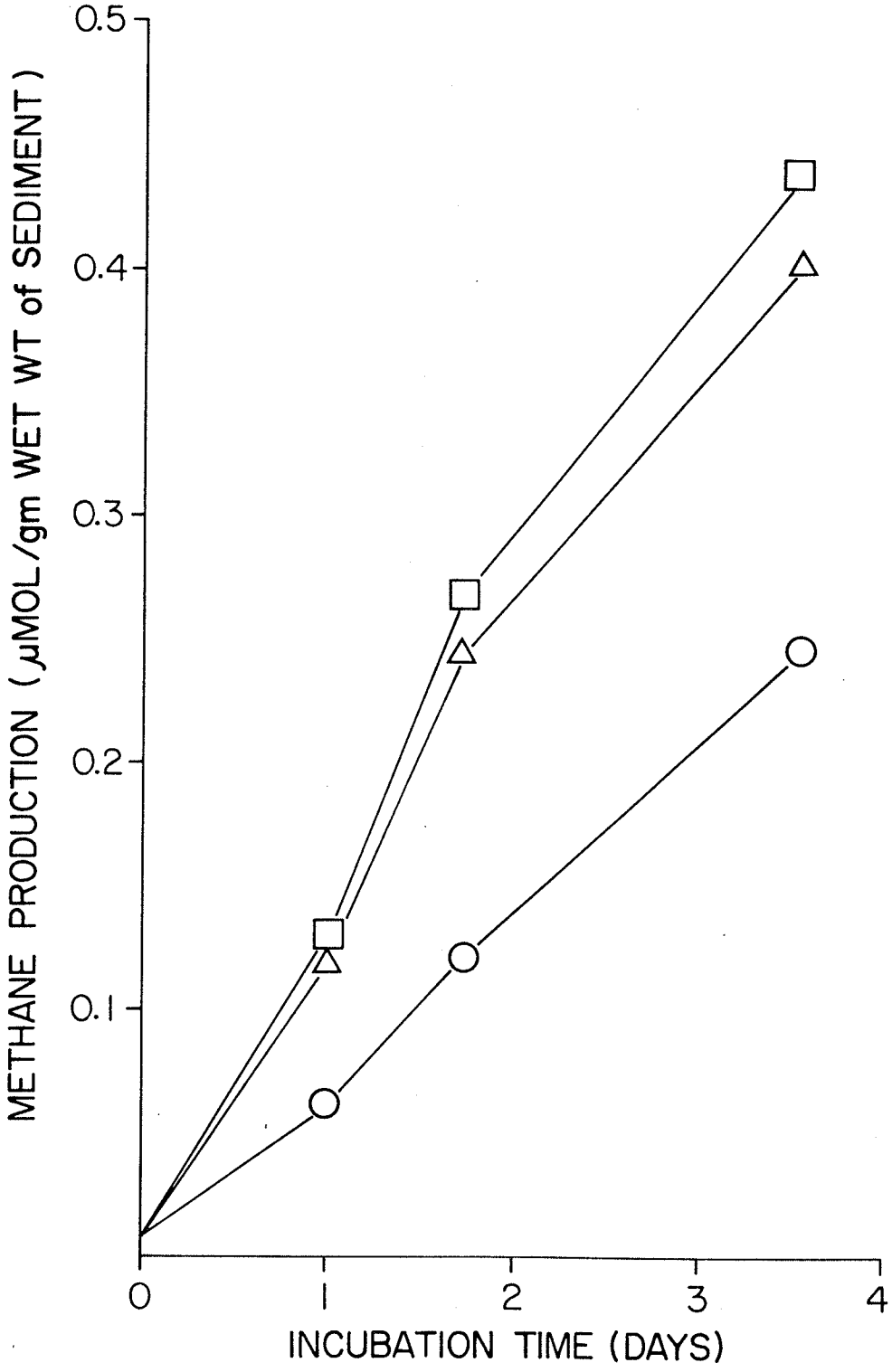

METHANE PRODUCTION (μMOL/gm WET WT of SEDIMENT)
0.5
0.4
0.3
0.2
0.1
0
1
2
3
4
INCUBATION TIME (DAYS)

Figure 14. Methane production in enrichment cultures which were inoculated with sediment from various depth intervals in cores and were incubated at 37°C. Legend: (□) 0-2 cm depth; (0) 2-3 cm depth; (△) 3-4 cm depth; (●) 4-5 cm depth.

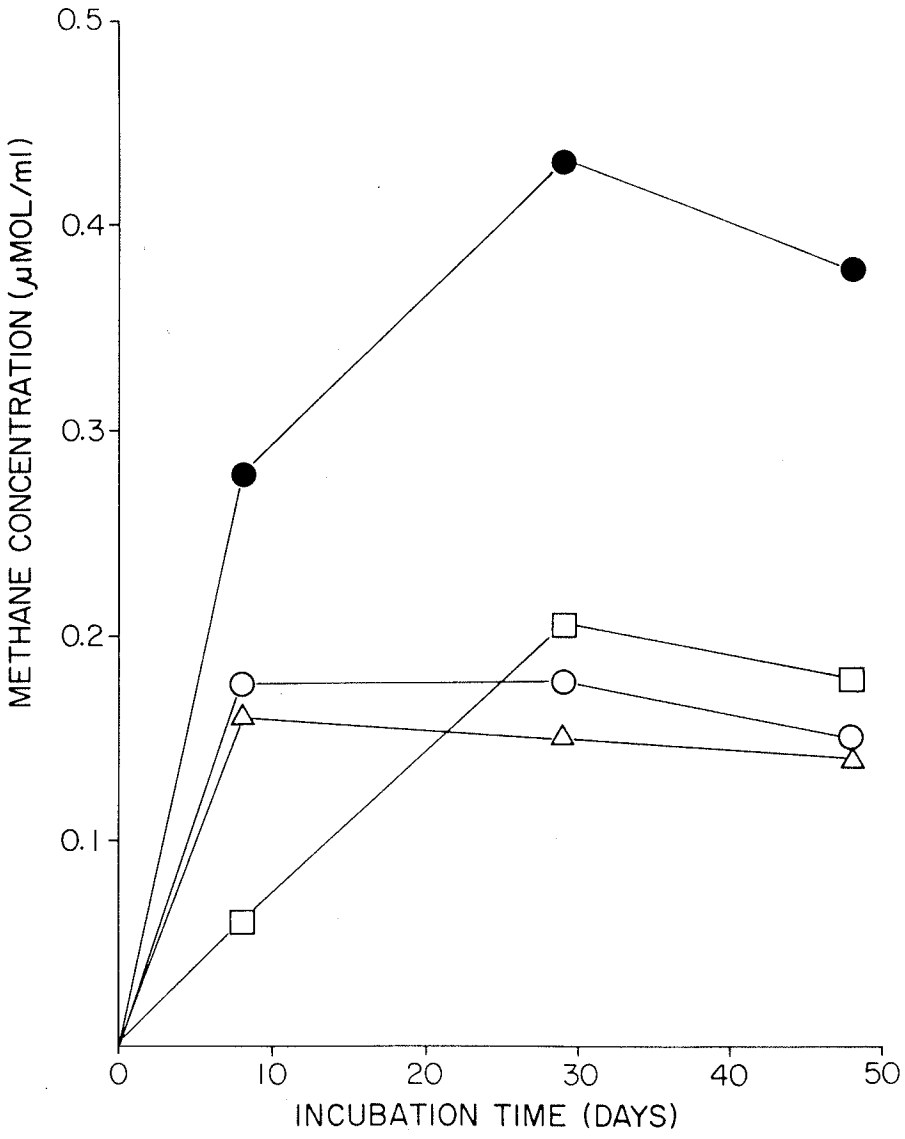
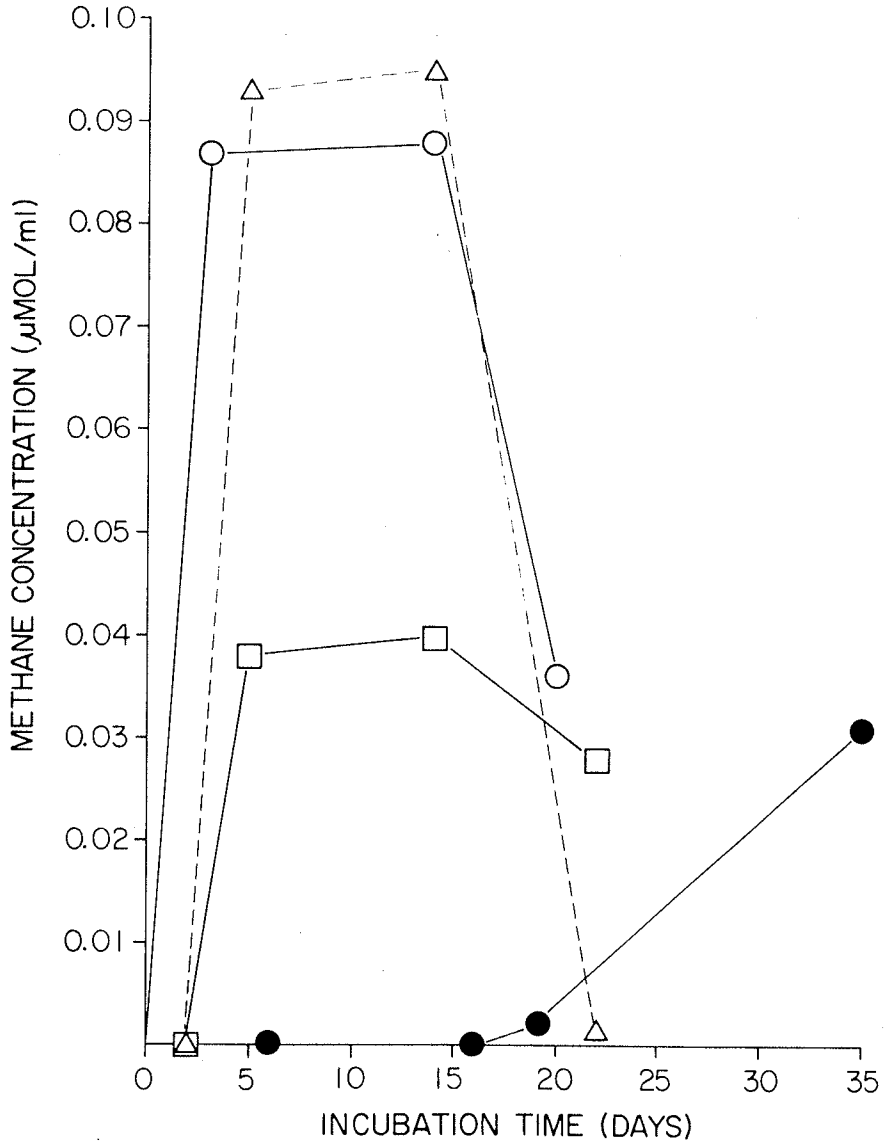

0.5
0.4
0.3
0.2
0.1
METHANE CONCENTRATION (μMOL/ml)
0
10
20
30
40
50
INCUBATION TIME (DAYS)

Figure 15. Methane concentration in enrichment cultures containing different carbon sources +/- sediment extract. Legend: (□) Glucose + sediment extract; (0) Acetate + sediment extract; (△) Cellulose + sediment extract; (●) Acetate.


0.10
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
METHANE CONCENTRATION (μMOL/ml)
0
5
10
15
20
25
30
35
INCUBATION TIME (DAYS)

sediment pH below ambient (6.4) (Figure 16A). Partial inhibition occurred at pH 6.0 and complete inhibition occurred at pH 5.5 and lower. Increasing the incubation temperature to 28°C yielded detectable methane production at low pH (Figure 16B).

In Vitro ^{14}C-labelled Substrate Experiments

The production of $^{14}CH_4$ and $^{14}CO_2$ from ^{14}C-labelled Chlorella mixed into acidified sediment was inhibited at low pH (Figure 17). There was an initial lag phase in $^{14}CH_4$ production at pH 5.3, then the production increased to yield a final level of $^{14}CH_4$ similar to the level attained in sediment at ambient pH 6.3. Methane production was low at pH 4.4 and absent at pH 3.6. The production of carbon dioxide in these same sediments showed a similar pattern, except that the initial lag phase in production at pH 5.3 was not pronounced and a small amount of $^{14}CO_2$ was produced at pH 3.6. The average ratio of $^{14}CO_2$:$^{14}CH_4$ produced during incubation at each pH is listed in Table 9.

The production of $^{14}CH_4$ and $^{14}CO_2$ from [1,2-^{14}C] sodium acetate mixed into acidified sediment was inhibited at low pH (Figure 18). $^{14}CH_4$ production was completely inhibited at pH 4 and $^{14}CO_2$ production was completely inhibited at pH 3. The ratio of $^{14}CO_2$:$^{14}CH_4$ produced at each pH is listed in Table 10.

The kinetics of production of methane and carbon dioxide from acetate in sediments at pH 4 and 6.2 were compared (Figure 19). The $(K+S_n)$ values and the maximum velocities (V) for methane and carbon dioxide production at sediment pH of 6.2 and 4.0 are listed in Table 11.

Figure 16. *In vitro* methane production in sediment incubated at 8°C (A) and 28°C (B) and various pH. Legend: (□) pH = 6.4; (■) pH = 6.3; (▲) pH = 6.0; (●) pH= 5.5; (△) pH = 4.5; (○) pH = 3.6.

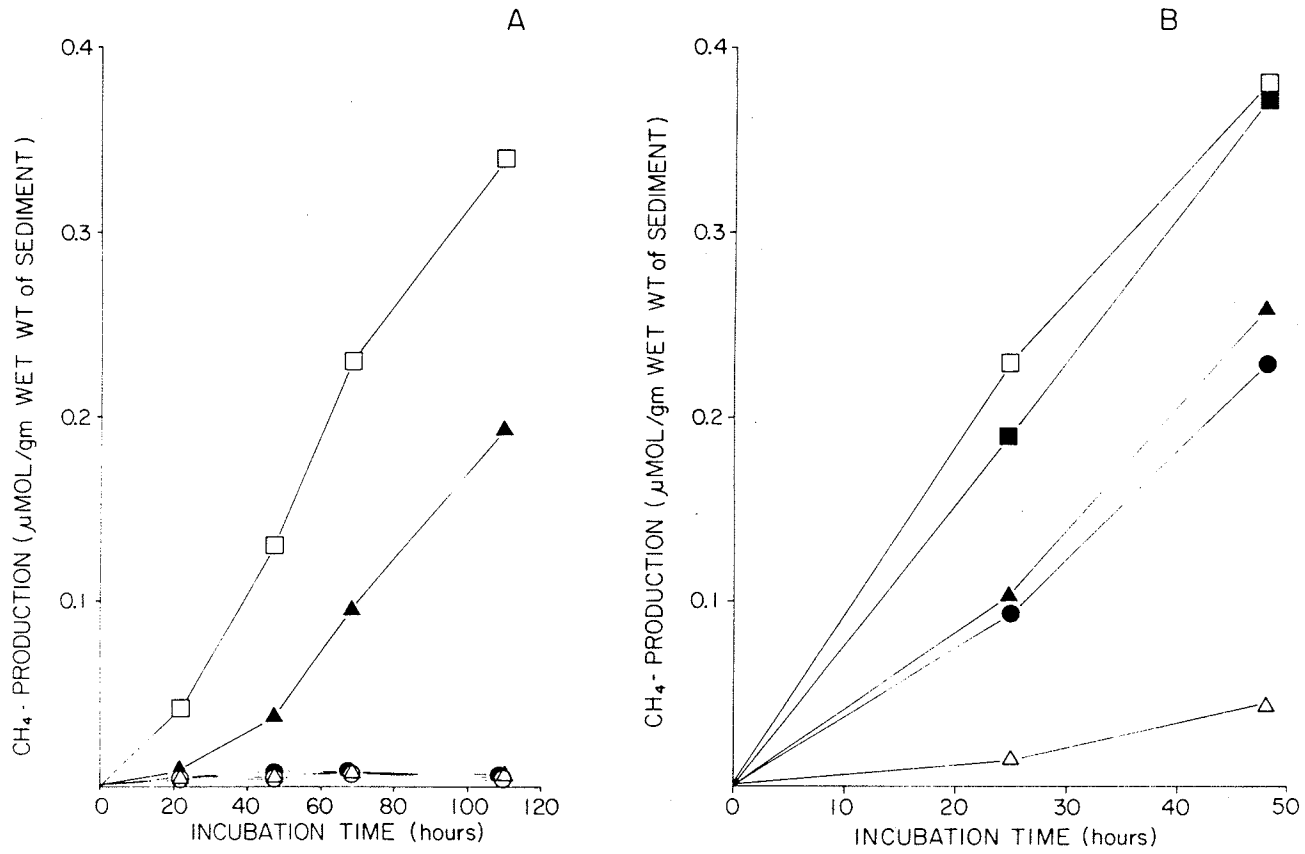
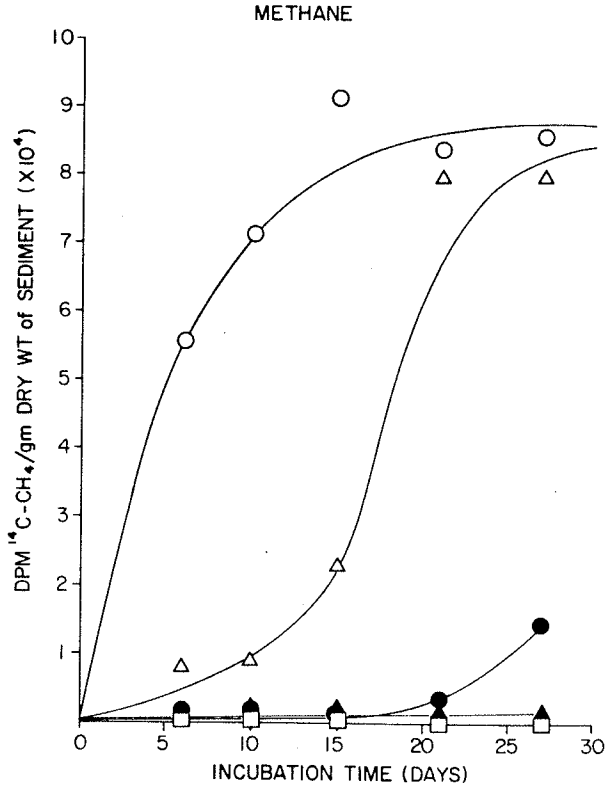

A
B
0.4
0.3
0.2
0.1
CH_4 - PRODUCTION (μMOL/gm WET WT of SEDIMENT)
0
20
40
60
80
100
120
10
30
50
INCUBATION TIME (hours)

Figure 17. *In vitro* $^{14}CH_4$ and $^{14}CO_2$ production from ^{14}C-labelled *Chlorella* in acidified sediments at 28°C. Legend: (□) $HgCl_2$ killed control; (○) pH = 6.3; (△) pH = 5.3; (●) pH = 4.4; (▲) pH = 3.6.


METHANE
DPM ^{14}C-CH_4/gm DRY WT of SEDIMENT (X 10^4)
INCUBATION TIME (DAYS)

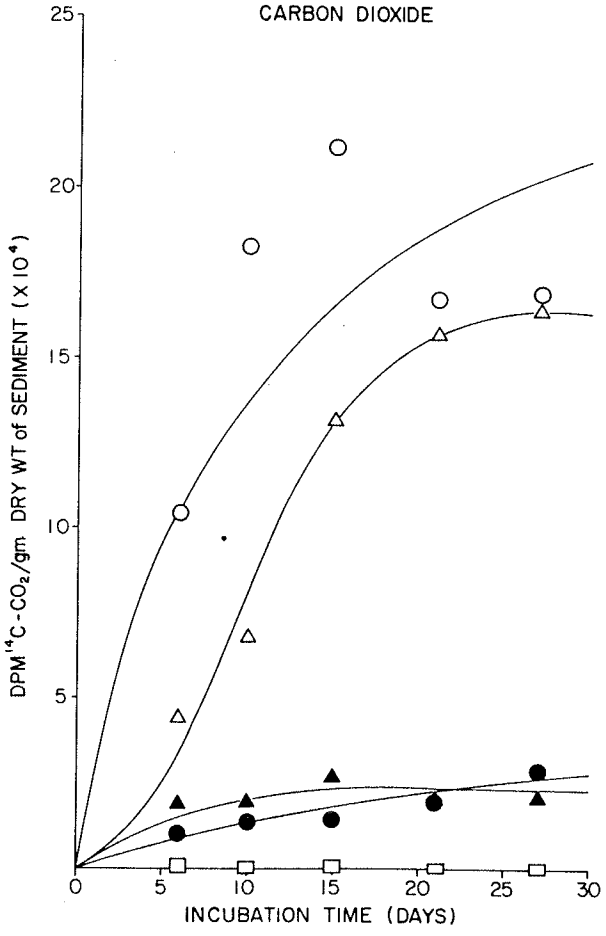

CARBON DIOXIDE
DPM ^{14}C-CO_2/gm DRY WT of SEDIMENT (X 10^4)
INCUBATION TIME (DAYS)

Table 9. The average ratio of $^{14}CO_2$:$^{14}CH_4$ produced from ^{14}C-labelled *Chlorella* during the *in vitro* incubation of acidified sediment at 28°C.

Sediment pH	$^{14}CO_2$:$^{14}CH_4$
6.3	2.14
5.3	4.81
4.4	7.76
3.6	∞

Figure 18. In vitro production of $^{14}CH_4$ (●) and $^{14}CO_2$ (△) from [1,2-^{14}C]sodium acetate in acidified sediments at 28°C.

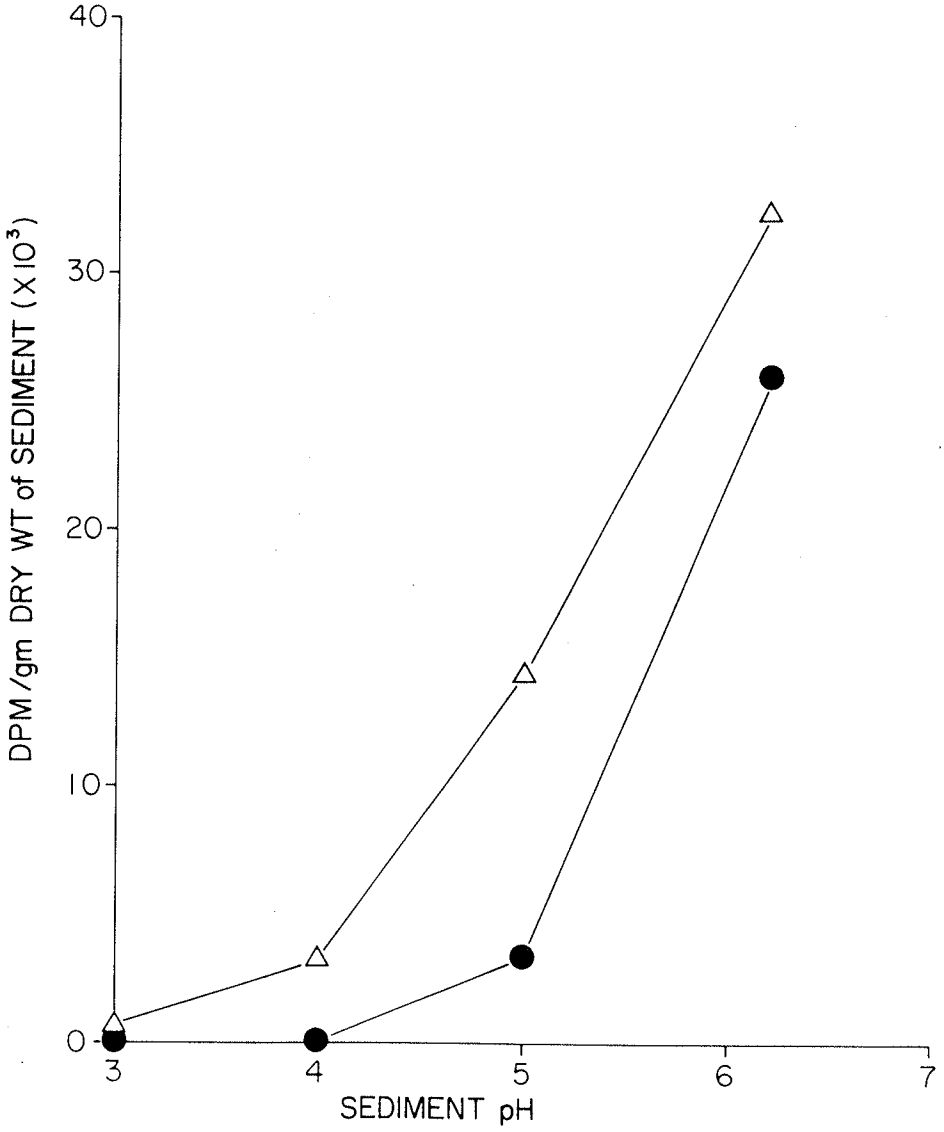
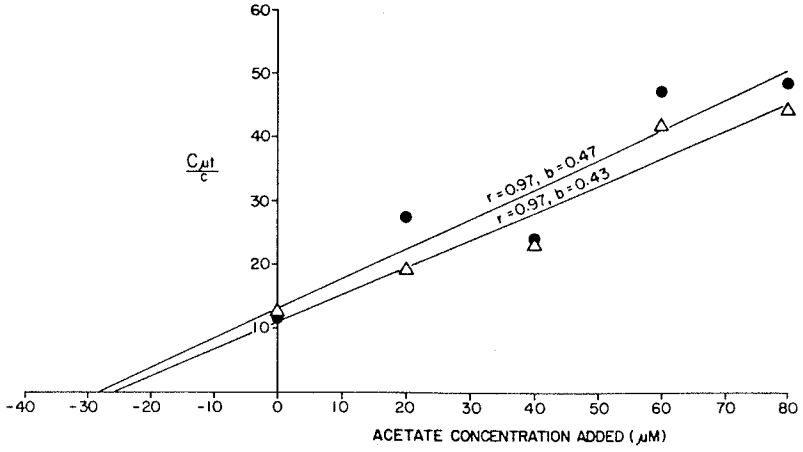

40
30
20
10
0
DPM/gm DRY WT of SEDIMENT (X10³)
3
4
5
6
7
SEDIMENT pH

Table 10. The ratio of $^{14}CO_2$:$^{14}CH_4$ produced from [1,2,-^{14}C]sodium acetate during the *in vitro* incubation of acidified sediment at 28°C.

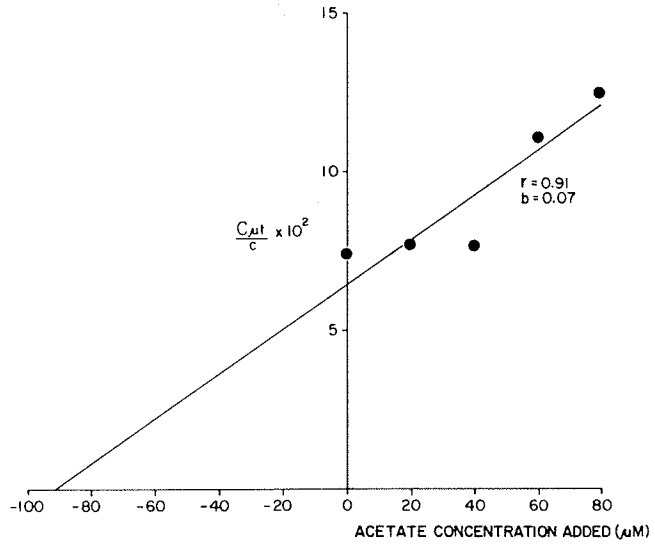
Sediment pH	$^{14}CO_2$:$^{14}CH_4$
6.2	1.2
5.0	4.3
4.0	∞
3.0	∞

Figure 19. Kinetics of the breakdown of [1,2-^{14}C] sodium acetate to $^{14}CH_4$ (●) and $^{14}CO_2$ (△) in sediments at pH 6.2 and 4.0. Incubation temperature was 28°C.

METHANE AND CARBON DIOXIDE - pH = 6.2



METHANE - pH = 4.0



CARBON DIOXIDE - pH = 4.0

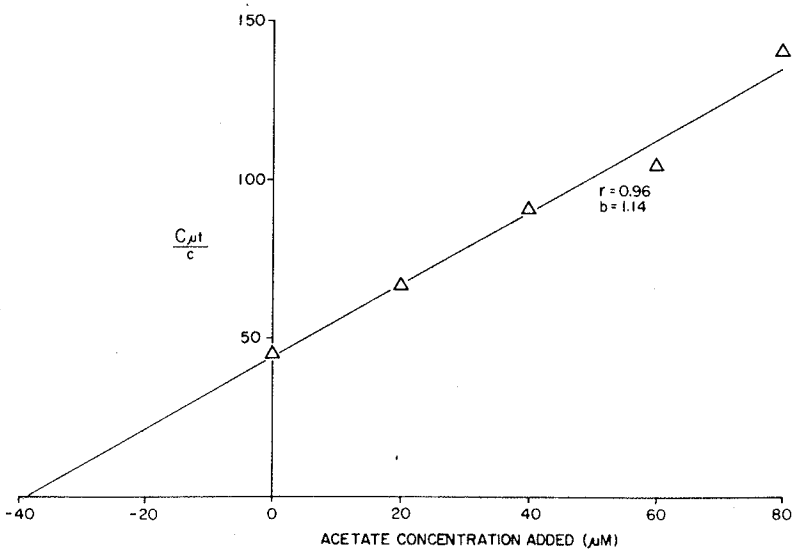


Table 11. The kinetics of production of methane and carbon dioxide from acetate in sediments at pH 4 and 6.2

Product	sediment pH 6.2		sediment pH 4.0	
	$(K+S_n)$ (μm)	V (μmoles l^{-1} hr^{-1})	$(K+S_n)$ (μm)	V (μmoles l^{-1} hr^{-1})
CH_4	27.60	2.36	92.00	0.001
CO_2	25.30	2.04	38.50	0.022

Core Experiments

The average C:N:P in the upper 5 cm of unconsolidated sediments in the cores was 119:11:1.

Acidification of the water overlying sediment in the cores did not result in a correspondingly sharp decrease in sediment pH (Table 12). In the extreme case where the water was acidified to pH 2.37, the sediment pH decreased from 6.38 to 6.02.

The rates of production of $^{14}CH_4$ and $^{14}CO_2$ from ^{14}C-labelled Chlorella added to the water-sediment interface of cores were inhibited by acidifying the water phase of these cores (Figure 20). The $^{14}CH_4$ production rate decreased considerably between pH 6.20 and 5.78, and the carbon dioxide production rate decreased considerably between pH 5.78 and 5.58. There was no further inhibition of either the methane or carbon dioxide production rate between pH 5.58 and 4.67. The production rate of ^{14}C-labelled dissolved organic carbon did not change appreciably at any pH. At low pH, the ratio of $^{14}CO_2$:$^{14}CH_4$ produced was increased, and the percentage return of carbon as $^{14}CH_4$ and $^{14}CO_2$ (relative to input of ^{14}C-labelled Chlorella) was decreased (Table 13).

The rates of production of $^{14}CH_4$ and $^{14}CO_2$ from [1,2-^{14}C] sodium acetate in sediment sections from acidified cores (in which the water overlying the sediments was acidified) were not much inhibited until below pH 3 (Figure 21). Neither $^{14}CH_4$ nor $^{14}CO_2$ production were completely inhibited in the above core sections. Rates of production of 4×10^5 DPM cm^{-2} day^{-1} and 6×10^5 DPM cm^{-2} day^{-1} were obtained for methane and carbon dioxide respectively at pH 2.37. The ratio of $^{14}CO_2$:$^{14}CH_4$ produced in the core sections did not change greatly at any pH (Table 14).

Table 12. Typical pH of the sediments (0-5 cm depth) in cores in which the water overlying the sediments was acidified.

Core #	pH of the overlying water	Sediment pH
1	6.49	6.38
2	6.35	6.29
3	3.40	6.22
4	3.36	6.24
5	2.37	6.02

Figure 20. The rates of production (DPM cm^{-2} day^{-1}) of $^{14}CH_4$ (O), carbon dioxide (Δ), and dissolved organic carbon (□) from ^{14}C-labelled Chlorella in acidified cores which were incubated at in situ temperature (8°C) for 27 days.

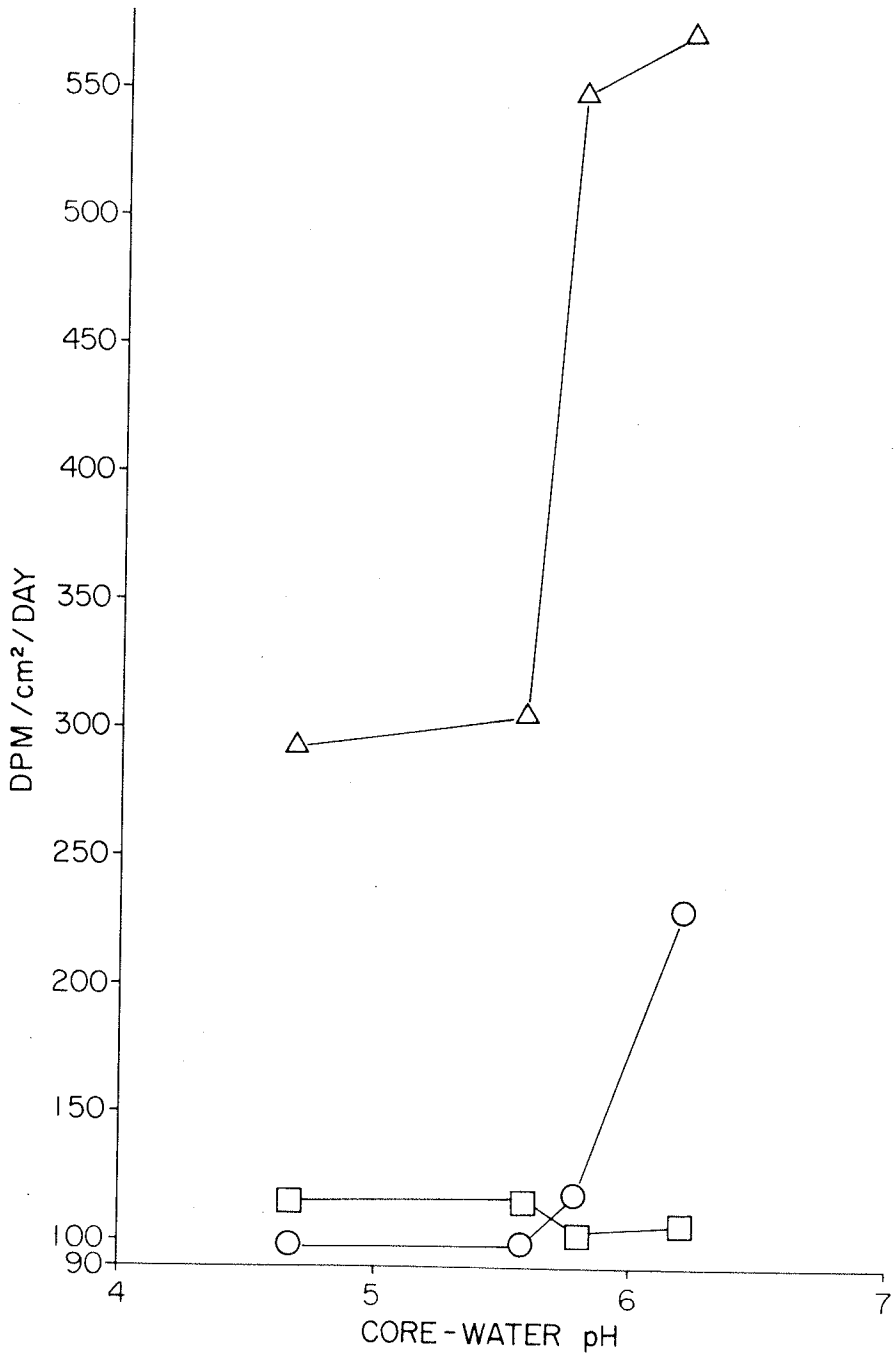

550
500
450
400
350
300
250
200
150
100
90
DPM / cm² / DAY
4
5
6
7
CORE - WATER pH

Table 13. The ratio of $^{14}CO_2$:$^{14}CH_4$ produced and the % return of carbon to the water column as $^{14}CH_4$ and $^{14}CO_2$ in acidified cores to which ^{14}C-labelled Chlorella was added.

pH of the overlying water	$^{14}CO_2$:$^{14}CH_4$	% return of carbon	
		CH_4	CO_2
6.20	2.49	3.82	9.53
5.78	4.65	1.97	9.16
5.58	3.10	1.65	5.11
4.67	3.00	1.63	4.89

Figure 21. The rates of production (DPM cm^{-2} day^{-1}) of $^{14}CH_4$ (O) and $^{14}CO_2$ (Δ) from [1,2-^{14}C]sodium acetate in sediment sections from acidified cores which were incubated at 8°C.

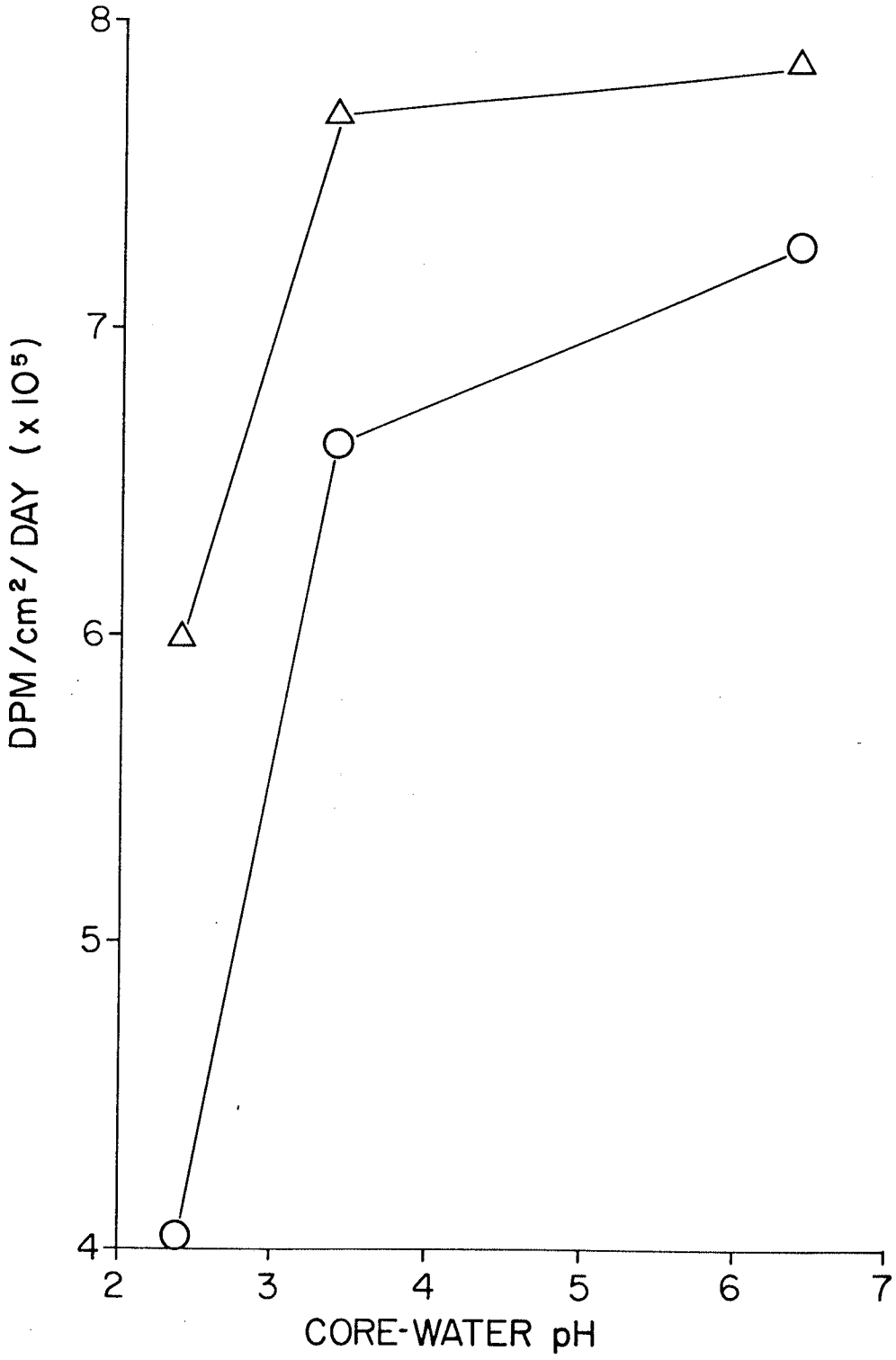

8
7
6
5
4
DPM/cm²/DAY (x 10⁵)
2
3
4
5
6
7
CORE-WATER pH

Table 14. The ratio of $^{14}CO_2$:$^{14}CH_4$ produced from [1,2-^{14}C] sodium acetate in core sections from acidified cores at 8°C.

pH of overlying H_2O	$^{14}CO_2$:$^{14}CH_4$
6.42	1.13
3.38	1.16
2.37	1.49

DISCUSSION

POC Sedimentation Rates

The addition of 5% NaCl to cylindrical sedimentation traps increased the amount of trapped POC either by inhibiting mineralization of collected particulate organic matter (POM) or by establishing a turbulence-decreasing NaCl density gradient within the traps. Although less motile bacteria were observed in traps containing 5% NaCl, the C:N:P ratios of matter collected in both cylindrical trap treatments during each trapping interval (Table 4) were only slightly different. The C:N:P ratio of matter collected in traps not containing 5% NaCl would be orders of magnitude higher (due to microbial decomposition of readily degradable nitrogeneous and phosphorogenous compounds) if mineralization had a significant effect on the amount of POC trapped. The 5% NaCl solution probably established a density gradient within the traps which enhanced the retention of trapped particulate matter (Rigler et al, 1974; Kirchner, 1975). The use of chemicals to inhibit the mineralization of POM in sedimentation traps has been reviewed in Bloesch and Burns (1980). The low oxygen concentrations at 7 m depth in Lake 226NE (Appendix II, Table 16) during the collection period probably contributed to low POM mineralization rates in the sedimentation traps. The bag-type traps, which were roughly cone shaped, yielded lower POC sedimentation rates than the corresponding cylindrical trap rates (Figure 10). Hargrave and Burns (1979), and Bloesch and Burns (1980), reported that cone-shaped traps were susceptible to the loss of collected POM due to water turbulence *in situ* and during sampling. The most widely accepted trap design is a cylinder with an aspect ratio (height/diameter) greater than 5 (Bloesch and Burns, 1980; Gardner,

1980) which has a turbulence-free zone at the bottom. The cylindrical traps used in this study had an aspect ratio of 4.3, a compromise between trapping efficiency and the ease of manipulation in the field. Traps with a smaller diameter (but larger aspect ratio) were not considered because they reportedly overestimate POM sedimentation rates (Hargrave and Burns, 1979).

The average POC sedimentation rate in Lake 302S from the middle of July to the middle of September was similar in 1981 (280.85 mgC m^{-2} day^{-1}) and 1982 (234.82 mgC m^{-2} day^{-1}), but insufficient 1981 data limited a comparative discussion of the fluctuations in the rate from May to September in both years. The peak in the POC sedimentation rate in August 1982 (Figure 11) was probably caused by a peak in primary production in late July. Malongoski and Klug (1980) reported that sedimentation rate maxima usually followed 1-3 weeks after increases in primary production and/or algal biomass. The C:N:P ratio in POM collected in Lake 302S in 1981 and 1982 (Table 6) illustrated that more carbon relative to nitrogen and phosphorous was collected in 1982 (where comparison is possible). The latter phenomenon may have been caused by higher POM mineralization rates in 1982, because the oxygen concentration at 7 m depth (where traps were suspended) was higher throughout summer stratification in 1982 (Appendix III, Figure 22).

Horizontal "patchiness" in the sedimentation of POC was probably the cause of the higher standard deviations from the mean POC sedimentation rates in the Lake 226NE experiment (Table 3) compared to those in the Lake 302S experiment (Table 5): the trapping sites in Lake 226NE were dispersed over a larger area than the sites in

Lake 302S.

Reynolds (1979) suggested caution in the quantitative evaluation of sediment trap data unless traps were used in conditions of minimal turbulence and low oxygen concentration.

In Situ Methane and Carbon Dioxide Production

The methane production rate in the hypolimnetic sediments was constant during summer stratification within each year monitored, as evidenced by the linear accumulation of methane in the water column below 9 m depth (Figure 12). These constant rates are caused by the year round uniformity of temperature of the bottom waters of lakes (Rudd and Hamilton, 1978), methane production being temperature limited (Zeikus and Winfrey, 1976; Kelly and Chynoweth, 1981). The low rate of methane accumulation in 1982 (0.48 mMoles m^{-2} day^{-1}), compared to 1980 (5.61 mMoles m^{-2} day^{-1}) and 1981 (3.10 mMoles m^{-2} day^{-1}), was caused by the persistence of significant oxygen concentrations in the hypolimnion in 1982 (Appendix III, Figure 22) which could allow increased methane oxidation rates. The oxygen concentration in lakewater below 9 m depth was within the reported optimum range for methane oxidation of 0.1-1.0 mg O_2 $litre^{-1}$ (Rudd and Hamilton, 1975a) during most of stratification in 1982. In addition, the ratio of anoxic CO_2:CH_4 was high in 1980 and 1982 (Table 7) (years in which oxygen persisted for a longer period of time than in 1981), possibly as a consequence of methane oxidation to carbon dioxide. Further, evidence of anaerobic methane utilization was obtained from enrichment cultures which were inoculated with Lake 302 sediment (Figure 15). The quantitative significance of this process *in situ* was not investigated further. The average pH

of the lakewater just above the anoxic sediments (Appendix III, Table 18) did not decrease sufficiently in 1982 (due to artificial acidification of the epilimnion) to inhibit methane accumulation in the hypolimnion.

The poor linear fit of carbon dioxide accumulation data for 1980 and 1981 (Figure 12) may have been due to the photosynthetic utilization of the carbon dioxide by algae in the hypolimnion. Fee (1976) reported peaks of algal biomass in the hypolimnion of ELA lakes.

The potential contributions of the microbial reduction of Fe^{2+}, Mn^{2+}, SO_4^{2-}, NO_3^-, and O_2 to carbon dioxide production in the hypolimnion in 1981 and 1982 were small, since the differences between the ratio of anoxic CO_2:CH_4 and methanogenic CO_2:CH_4 were small (0.02-0.08). Kelly et al (1982) reported larger differences (0.33-0.80) between two ratios in three other ELA lakes.

The percentage return of sedimented POC to the water column as methane (13.30% in 1981, 2.39% in 1982) was considerably lower than most reported returns in other lakes. Rudd and Hamilton (1978), Strayer and Tiedje (1978), Robertson (1979), Fallon (1980), and Jones and Simon (1981) reported returns of 55%, 24-37%, 36-59%, 54%, and 10-20% respectively. However, most of the lakes cited in these studies were eutrophic and probably maintained anoxic hypolimnia (essential for the production and accumulation of methane) longer than the less productive Lake 302S. While the latter possibility partially explains the low percentage returns of carbon as methane, the estimates of these returns would have been higher if methane release rates could have been corrected for methane oxidation losses.

In vitro Experiments

The incubation of sediment core sections and enrichment cultures inoculated with sediment from cores (Figures 13 and 14) pinpointed the most methanogenically active sediments to be between 4-6 cm below the sediment-water interface, in agreement with the reported location of 0-6 cm cited in the literature (Koyama, 1976; Zeikus and Winfrey, 1976; Kelly and Chynoweth, 1981; Cappenberg and Verdouw, 1982). Attempts to isolate methanogenic cultures from the 0-5 cm sediment section of cores did not yield active sub-cultures, though methane was produced in initial enrichment cultures (Figures 14 and 15). The addition of sediment extract to these cultures (as a source of unknown growth factors) stimulated methane production, but also stimulated the growth of a methane utilizing organism (possibly an aerobic methane oxidizing organism) which removed most of the generated methane.

In vitro methane production at 8°C (in situ temperature) from "natural" POC in sediments was partially inhibited at pH 6.0 (57% of ambient CH production) and totally inhibited at pH 5.5 and lower (Figure 16A). This inhibition of methanogenesis by low pH was temperature dependent, since raising the incubation temperature to 28°C allowed methane production at pH 5.5 (60% of ambient CH_4 production) and 4.5 (12% of ambient CH_4 production).

In a similar manner to the above experiments, $^{14}CH_4$ production from ^{14}C-labelled substrates in acidified sediments was partially inhibited at pH 5.0-5.3 and completely inhibited at pH 3.6-4.0. The inhibition of $^{14}CH_4$ production from ^{14}C-labelled Chlorella at pH 5.3 in sediments was time dependent, because the $^{14}CH_4$ content

in vials began to increase after 10 days incubation (Figure 17) to yield an amount which was 92% of the amount produced at ambient pH 6.3. The inhibition became less time dependent at pH below 5.0. The production of $^{14}CH_4$ from [1,2-^{14}C] sodium acetate was also sensitive to low pH: the amount of $^{14}CH_4$ produced at pH 5.0 was 13% of the amount produced at ambient pH 6.2 (Figure 18). However the latter inhibition may have been temporary, since an incubation time of two hours was used, and since the inhibition of $^{14}CH_4$ production at low pH was shown to be time dependent in earlier experiments (utilizing ^{14}C-labelled Chlorella as substrate) which had an incubation time of twenty-seven days. A small amount of $^{14}CO_2$ production from ^{14}C-labelled substrates (added to acidified sediments) occurred at pH where $^{14}CH_4$ production was zero (Figures 17 and 18). Further, this persistence of $^{14}CO_2$ production to $^{14}CH_4$ production was evidenced by increases in the ratio of $^{14}CO_2$:$^{14}CH_4$ produced at low pH (Tables 9 and 10). The maximum velocities (V) for carbon dioxide and methane production from acetate in sediments at pH 6.2 and 4.0 were compared (Table 11): while the V for both methane and carbon dioxide were similar at pH 6.2, the decrease in V at pH 4.0 was one order of magnitude larger for methane production than for carbon dioxide production. The latter results suggested that anaerobic decomposition shifted from methane production to a less efficient carbon dioxide producing fermentation at low pH. The $^{14}CH_4$ production from ^{14}C-labelled Chlorella cells at pH 5.3 may be due to either a time dependent recovery of acetotrophic methanogenesis, utilization of alternate methanogenic substrates (such as fatty acids, organic acids, alcohols, or carbon dioxide)

generated from the fermentation of ^{14}C-labelled Chlorella cells, or the utilization of both acetate and alternate substrates.

Core Experiments

The pH of the sediments in cores was not appreciably decreased by acidifying the overlying water (Table 12). Either the flux of hydrogen ions into the sediment was too slow to obtain an effect on pH within the incubation time, or the sediments were buffering against acidification. Gahnstrom et al (1980) reported that buffering capacity by sediments was responsible for the small effect of lakewater acidification on decomposition in profundal sediments, and that only the uppermost 0-4 cm of sediment was affected by overlying acidic water.

The production of $^{14}CH_4$ and $^{14}CO_2$ from ^{14}C-labelled Chlorella cells on the surface of core sediment was partially inhibited between pH 6.2 and 5.58 (Figure 20). The lack of further inhibition below pH 5.58 may have been caused by $^{14}CH_4$ and $^{14}CO_2$ production from dissolved products of ^{14}C-labelled Chlorella mineralization which diffused into the sediment below the zone affected by the acidity of the water (0-4 cm). The CO_2:CH_4 of 2.50 obtained in the cores at ambient pH (6.20) was larger than the ratio of 2.14 obtained in in vitro experiments utilizing ^{14}C-labelled Chlorella, and the ratio of 1.73 produced in situ in Lake 302S during summer stratification in 1982 (the year during which sediments were collected). The discrepancies between these ratios were probably caused by the experimental manipulation of sediments and suggested caution in the extrapolation of results from in vitro and core incubations to the in situ situation. The CO_2:CH_4 from ^{14}C-labelled Chlorella

in cores increased at low pH as in the *in vitro* experiments. The percentage return of carbon (from ^{14}C-labelled *Chlorella*) as $^{14}CH_4$ to the water column in cores (at ambient pH) (Table 13) was 1.6 times larger than the *in situ* value. The percentage return of carbon as carbon dioxide was 2.3 times larger than the *in situ* value. The higher returns in the core experiments were due to the availability of more readily degradable organic compounds in *Chlorella* cells than in the *in situ* sediments. The *Chlorella* cells contained a larger proportion of readily degradable nitrogenous and phosphorogenous compounds (C:N:P = 26:4:1) than the sediment (C:N:P = 119:11:1). The decrease in percentage return at low pH (Table 13) could result in a build-up of detrital matter in sediments if it occurred in a lake. Traaen (1980) reported that such a build-up of "coarse detritus" in a lake was symptomatic of acidified lakes. The breakdown of detrital carbon (such as *Chlorella* cells) in sediments is a particularly sensitive part of anaerobic fermentation, since it is the rate-limiting step of the pathway (Cappenberg and Verdouw, 1982).

The production of $^{14}CH_4$ and $^{14}CO_2$ from [1,2-^{14}C]-labelled acetate in acidified cores was not inhibited until a pH of 2.37 was imposed in the overlying water (Figure 21), and then not completely. Cores in which the water phase was acidified to 2.37 had a sediment pH of 6.02 (Table 12), a pH at which partial inhibition of *in vitro* methanogenesis in directly acidified sediments occurred (Figures 15 and 18). The absence of inhibition at pH 6.42 and 2.37 was probably due to the lack of substantial decrease in the sediment pH of cores until a pH of below 3 was imposed in

the water phase (Table 12), and due to the location of maximum methanogenic activity at a depth of 4-6 cm into the sediments where hydrogen ions did not penetrate. The ratio of $^{14}CO_2$:$^{14}CH_4$ of 1.13 produced from [1,2-^{14}C] sodium acetate in the cores at ambient pH was similar to that of 1.20 obtained in *in vitro* experiments utilizing the same labelled substrate. The latter ratios were both lower than the *in situ* CO_2:CH_4 ratio of 1.73 and the average ratio of 2.83 from experiments utilizing ^{14}C-labelled *Chlorella* (*in vitro* and cores). An unknown anoxic carbon dioxide producing microbial process probably accounted for the additional carbon dioxide (relative to methane production) *in situ* and in experiments utilizing ^{14}C-labelled *Chlorella*.

In conclusion, it is apparent that the methanogenic fermentation consortium in anoxic sediment is partially inhibited at about pH 5.5 and completely inhibited at pH 4.0-4.5. Further, the partial inhibition at about pH 5.5 decreased with time. The increase in the ratio of $^{14}CO_2$:$^{14}CH_4$ produced from ^{14}C-labelled substrates in acidified sediments suggested that anoxic fermentation shifted to a less efficient CO_2 producing pathway under acidic conditions. It is also apparent that in a system containing an intact sediment-water interface (cores or lakes) overlain by acidic water, the methanogenic bacteria are not inhibited because they are located below the zone of penetration of the hydrogen ions into sediments. The breakdown of newly-settled organic carbon (represented by ^{14}C-labelled *Chlorella* in these studies) to methane in such intact systems is sensitive to low pH because new carbon accumulates at the sediment-water interface and is thus exposed to acidic water.

Methane production in an acidified lake would probably continue until organic carbon in the lower sediment strata was used up or until the buffering capacity of the sediments was overwhelmed and the sediments were acidified. Even if the sediments eventually became acidified, methane production would not be completely inhibited until a pH below 4.5 occurred. At pH 3, all anoxic fermentation to methane or carbon dioxide would cease.

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APPENDIX I

Calculation of *in situ* anoxic CO_2:CH_4 and methanogenic CO_2:CH_4

The rates of change in the amounts of certain chemical species below 9 m depth in Lake 302S were calculated for summer stratification in 1981 and 1982 (Table 14) (years for which all the necessary background data was available): the rates of change of Fe^{2+}, Mn^{2+}, SO_4^{2-}, NO_3^-, and O_2 were assumed to be due to bacterial reduction during the oxidation of organic material. The amount of CO_2 produced by methanogenesis was estimated by subtracting the sum of the amounts of CO_2 produced by the reduction of the above chemical species from the measured total amount of CO_2 (DIC) produced below 9 m depth (Table 15). The ratios of anoxic CO_2:CH_4 and methanogenic CO_2:CH_4 were then calculated using the appropriate mass per unit area values listed in Table 15.

Table 15. Rates of change of chemical species below 9 m depth in Lake 302S during summer stratification in 1981 and 1982 and the corresponding amounts of CO_2 produced.

Year	Species	Interval (no. of days)	Δ (mMoles m^{-2} day^{-1})	CO_2 production (mMoles m^{-2})
1981				
	Fe^{2+}	Jun.9-Sept.1(85)	-0.01	0.00
	MN^{2+}	Jun.9-Sept.1(85)	+0.05	+1.91
	SO_4^{2-}	Jun.9-Sept.1(85)	-0.02	+3.40
	NO_3^-	Jun.9-Sept.1(85)	0.00	0.00
	O_2	Jun.9-Sept.1(85)	+0.002	-0.17
				Σ 5.14
	DIC	Jun.9-Sept.1(85)	+1.30	+110.50
	CH_4	Jun.9-Sept.1(85)	+3.10	-
1982				
	Fe^{2+}	Jun.15-Sept.7(85)	-0.07	0.00
	Mn^{2+}	Jun.15-Sept.7(85)	+0.08	+3.06
	SO_4^{2-}	Jun.15-Sept.7(85)	-0.001	+0.17
	NO_3^-	Jun.15-Jul.28(44)	-0.004	+0.22
	NO_3^-	Jul.29-Sept.7(41)	0.00	0.00
	O_2	Jun.15-Sept.7(85)	-0.14	9.16
				Σ 12.61
	DIC	Jun.15-Sept.7(85)	+0.94	79.90
	CH_4	Jun.15-Sept.7(85)	+0.48	-

Table 16. The amounts of CH_4, anoxic CO_2, methanogenic CO_2, and the ratios of anoxic CO_2:CH_4 and methanogenic CO_2:CH_4 produced below 9 m depth in Lake 302S during summer stratification in 1981 and 1982.

	Year	
	1981	1982
CH_4 (mMoles m^{-2})	263.50	40.80
Anoxic CO_2 (mMoles m^{-2})	110.50	79.90
Methanogenic CO_2 (mMoles m^{-2})	105.36	67.29
Anoxic CO_2:CH_4	0.42	1.73
Methanogenic CO_2:CH_4	0.40	1.65

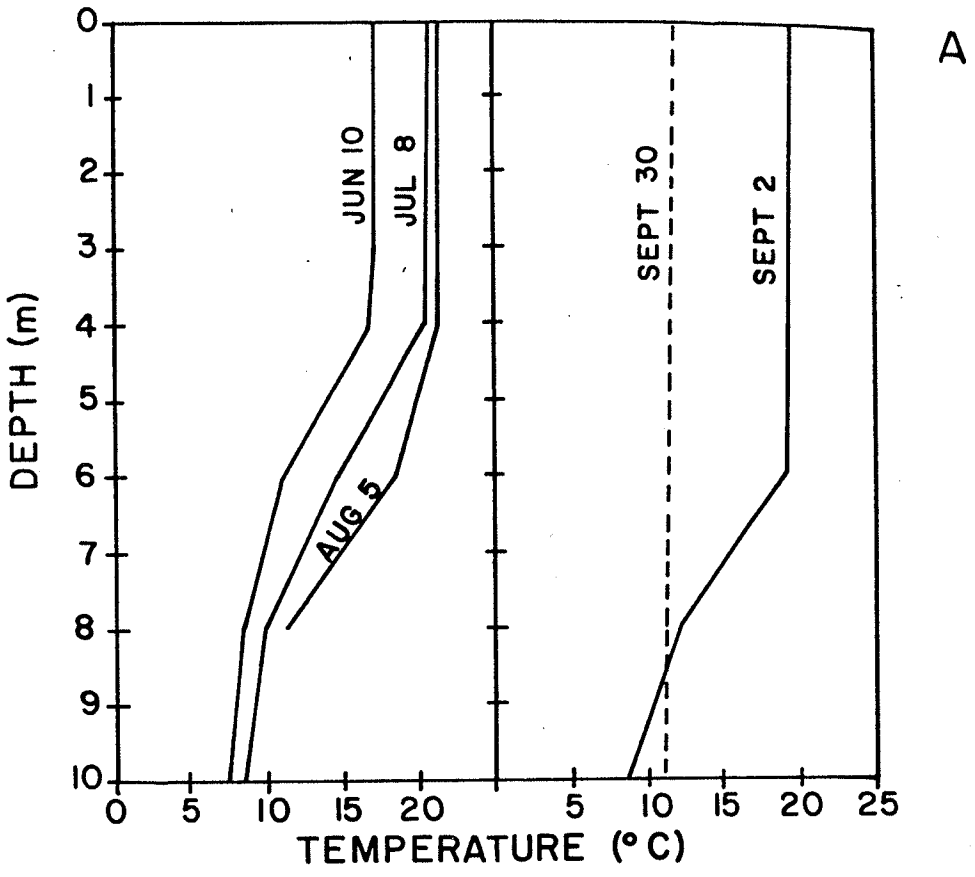
APPENDIX II

Table 17. Oxygen concentration ($[O_2]$) at a depth of 7 m in the water column of Lake 226 northeast basin on selected dates during summer stratification in 1980.

Sampling date	$[O_2]$ mg O_2/litre
Jun. 11	0.88
Jul. 9	0.79
Aug. 6	0.01
Sept. 3	≤0.01
Oct. 29	≤10.80

APPENDIX III

Figure 22. Water column profiles of temperature and oxygen concentration in Lake 302S during summer stratification in 1980(A), 1981(B), and 1982(C).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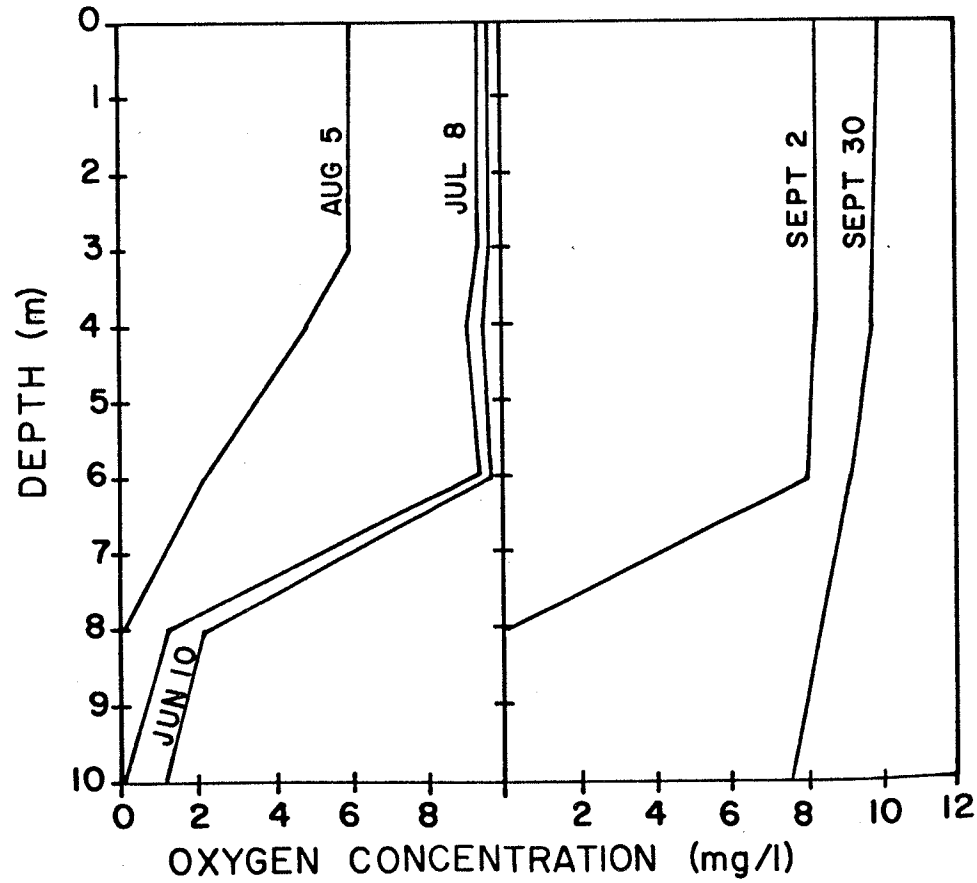
TEMPERATURE (°C)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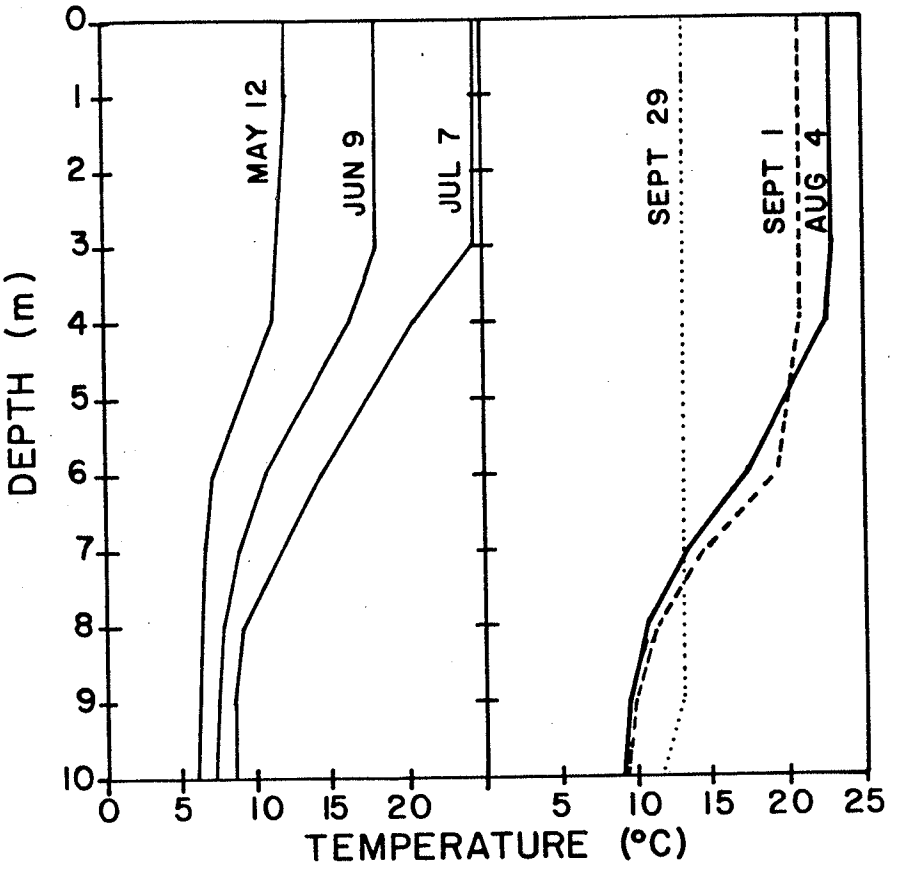
OXYGEN CONCENTRATION (mg/l)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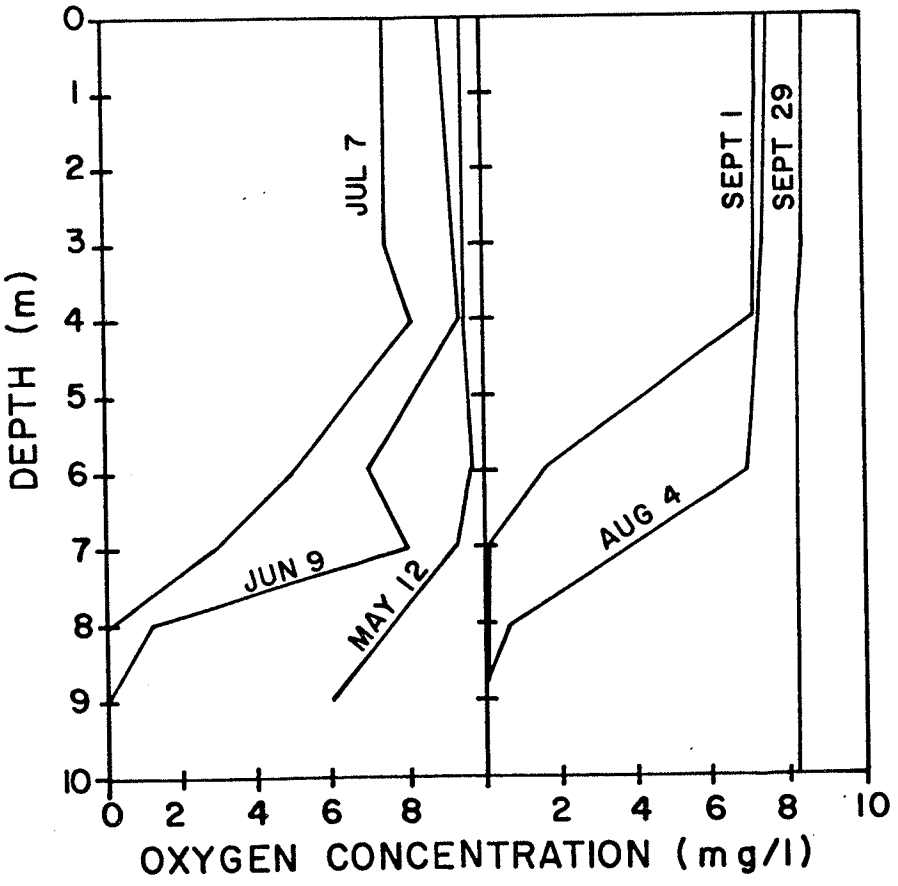
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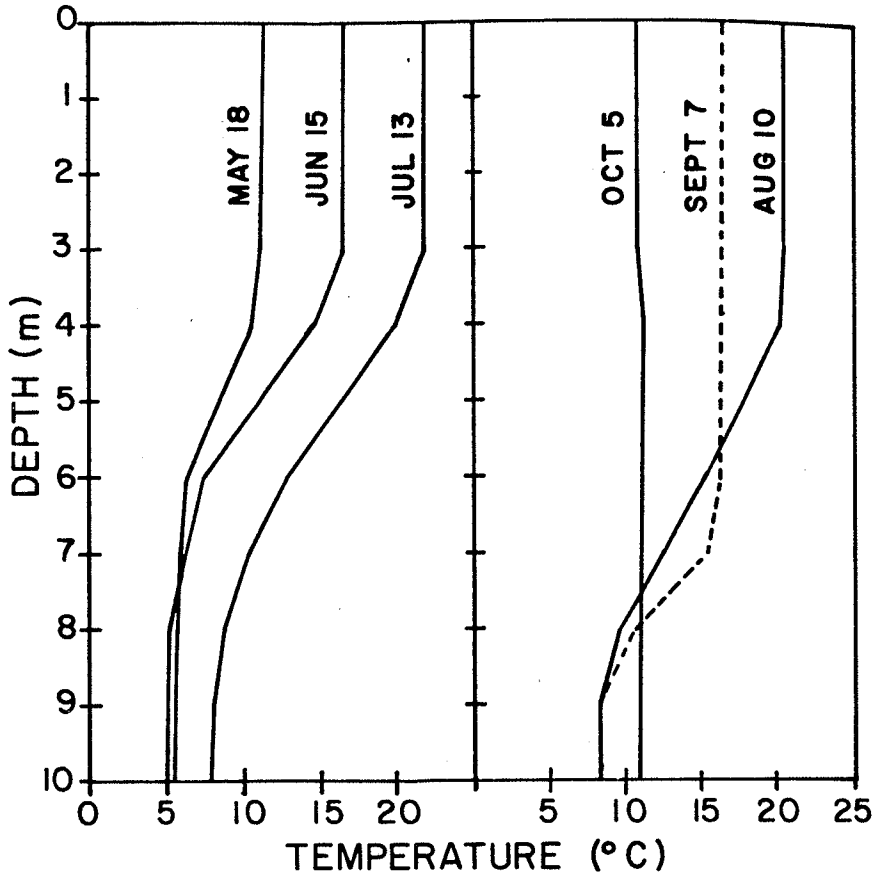
OXYGEN CONCENTRATION (mg/l)

C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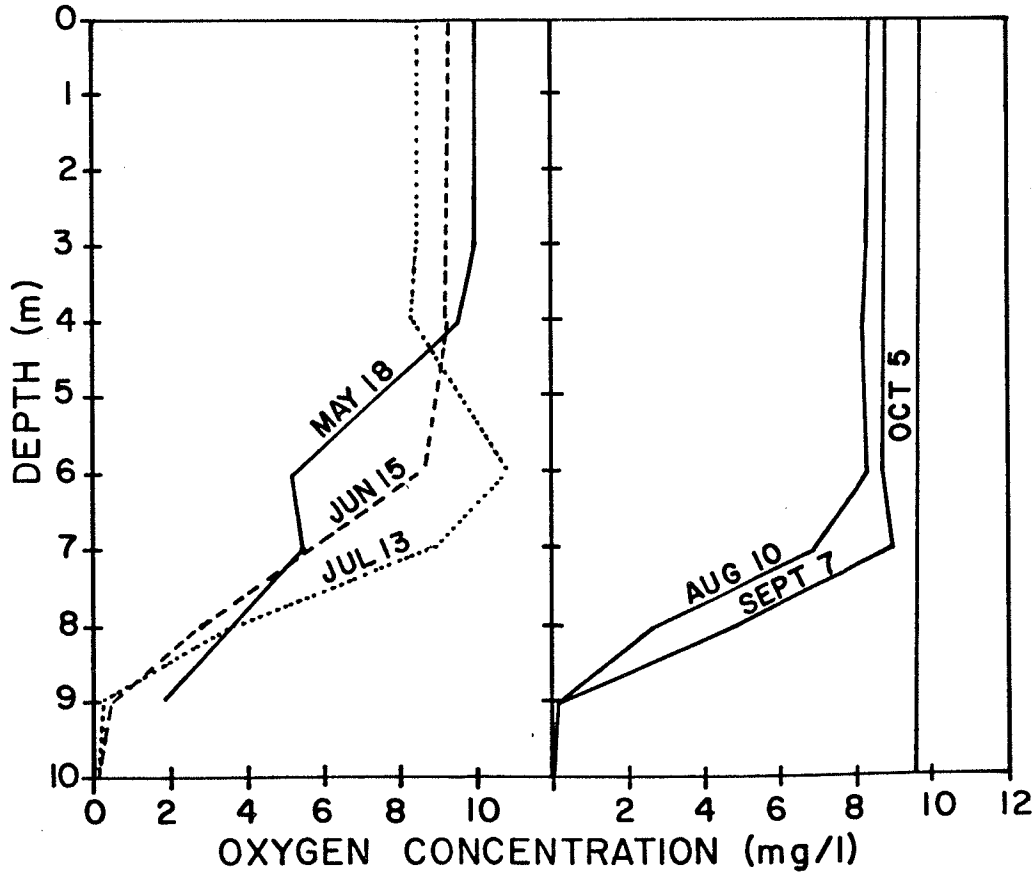
TEMPERATURE (°C)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OXYGEN CONCENTRATION (mg/l)

Table 18. The average pH of the lakewater above the anoxic sediments (depth = 10 m) in Lake 302S during summer stratification in 1980, 1981, and 1982.

	1980	1981	1982
Lakewater pH	6.39	6.10	6.04