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Denitrification, Acetylene Reduction, and Methane Metabolism in Lake Sediment Exposed to Acetylene

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Samples of sediment from Lake St. George, Ontario, Canada, were incubated in the laboratory under an initially aerobic gas phase and under anaerobic conditions. In the absence of added nitrate (NO_3^-) there was O_2-dependent production of nitrous oxide (N_2O), which was inhibited by acetylene (C_2H_2) and by nitrapyrin, suggesting that coupled nitrification-denitrification was responsible. Denitrification of added NO_3^- was almost as rapid under an aerobic gas phase as under anaerobic conditions. The N_2O that accumulated persisted in the presence of 0.4 atm of C_2H_2, but was gradually reduced by some sediment samples at lower C_2H_2 concentrations. Low rates of C_2H_2 reduction were observed in the dark, were maximal at 0.2 atm of C_2H_2, and were decreased in the presence of O_2, NO_3^-, or both. High rates of light-dependent C_2H_2 reduction occurred under anaerobic conditions. Predictably, methane (CH_4) production, which occurred only under anaerobiosis, was delayed by added NO_3^- and inhibited by C_2H_2. Consumption of added CH_4 occurred only under aerobic conditions and was inhibited by C_2H_2.

The acetylene (C_2H_2) inhibition method for the measurement of denitrification (2, 12, 29) was used for soil (28) and was further validated by ^{13}N (21) and ^{15}N (19) experiments. It has also been applied to sediments (15, 22), and subsequently an in situ field method for sediment-water systems was described in which denitrification and C_2H_2 reduction (N_2 fixation) were measured simultaneously (7). However, there is little information on the effects of O_2, nitrate (NO_3^-), methane (CH_4), and light on sediment processes during a "C_2H_2 inhibition" assay.

The present paper describes the first laboratory studies of the production and reduction of nitrous oxide (N_2O), reduction of C_2H_2, and production and consumption of CH_4 by samples of lake sediment in the presence and absence of C_2H_2, O_2, NO_3^-, CH_4, and light.

MATERIALS AND METHODS

Sediments were collected in June 1977 from 5- and 14-m depths in the eastern basin of Lake St. George, a small eutrophic lake in Richmond Hill County, north of Toronto, Ontario, Canada. The areas studied were free from macrophytes. From each of the areas sampled, 15 48-mm-diameter cores were taken using a Kajak-Brinkhurst core sampler. Sediment was gently extruded, and all the 0- to 5-cm and all the 5- to 10-cm depths were combined in two separate containers. For some experiments, 0- to 10-cm depths were combined. The sediments were gently mixed without introduction of air and stored at about 4°C. Physical characteristics of the sediments were as shown in Table 1.

Ten-gram portions of fresh sediment were placed in 50-ml Erlenmeyer flasks. Solutions of $NaNO_3$ (0.5 ml to give 2 μmol/g of sediment) and nitrapyrin (0.5 ml to give 20 μg/g of sediment) were added as required. The flasks were closed with serum stoppers (Suba-Seal, England), and the atmospheres were either left air-filled (i.e., initially aerobic) or made anaerobic by evacuating and refilling to 1 atm three times with He. Appropriate amounts of C_2H_2, C_2H_4, CH_4, and N_2O were added by means of a syringe through the stoppers after removing an equivalent volume of the gas phase. Flasks were incubated statically in the dark or, if so indicated, under approximately 500 lx from fluorescent lights at 20°C for up to 21 days.

At desired intervals, 0.2-ml samples of the gas phase were removed by means of a 1-ml syringe with Mininert valve (Precision Sampling Corporation, Baton Rouge, La.) and analyzed for CH_4, C_2H_2, C_2H_4, CO_2, and N_2O by single injection into a split-column gas chromatographic system with flame ionization and thermal conductivity detectors (17). After 8 days of incubation, the concentration of O_2 in initially aerobic flasks was determined by gas chromatography (6), and O_2 was added to replace that consumed. The O_2 was found to be rarely depleted below 0.1 atm. Subsequent reference to such conditions as aerobic or initially aerobic does not imply that the whole of the sediment sample was aerobic, merely that the sample was incubated under an oxygen-containing atmosphere.

Pure gases were obtained from Matheson (Canada) Ltd., and nitrapyrin (N-Serve) was obtained from Dow Chemical Co., Sarnia, Ontario. Data are the means of triplicate flasks and are expressed as micromoles per gram of sediment (fresh weight basis). Nitrous oxide data are corrected for solubility in the liquid phase

and for leakage as determined by using similarly treated flasks without sediment and supplemented with 10 μmol of N_2O per flask.

RESULTS

In the absence of added NO_3^-, no N_2O was produced except under an initially aerobic gas phase (henceforth referred to as aerobic) in the absence of C_2H_2 (Fig. 1). This production of N_2O was inhibited by C_2H_2, and further information on this phenomenon is presented later. Patterns of N_2O production from added NO_3^- were similar under anaerobic and aerobic conditions, although rates were slightly lower in the latter case (Fig. 1). In the absence of C_2H_2, N_2O peaked transiently at 2 to 3 days and then disappeared at 5 to 8 days. Acetylene (0.1 or 0.4 atm) caused rapid accumulation of N_2O with no subsequent reduction, except under anaerobic conditions with 0.1 atm C_2H_2, where some reduction occurred after 12 days. The second peak of N_2O accumulation under aerobic conditions at 16 days is not understood (Fig. 1).

Production of ethylene (C_2H_4) did not occur in the absence of C_2H_2 (Fig. 2). Furthermore, no

TABLE 1. *Dry weight, loss on ignition, and pH of the Lake St. George sediments used in this study*

Water depth (m)	Sediment depth (cm)	Dry wt (% fresh wt)	Loss on ignition (% dry wt)	pH[a]	
				Aerobic gas phase	Anaerobic gas phase
5	0–5	5.4	30	6.79	7.39
	5–10	6.7	25	7.19	7.77
14	0–5	8.6	16	6.61	7.41
	5–10	11.2	18	6.68	7.30

[a] Mean pH values of sediment after 21 days of incubation under an initially aerobic gas phase (aerobic) and under He (anaerobic).

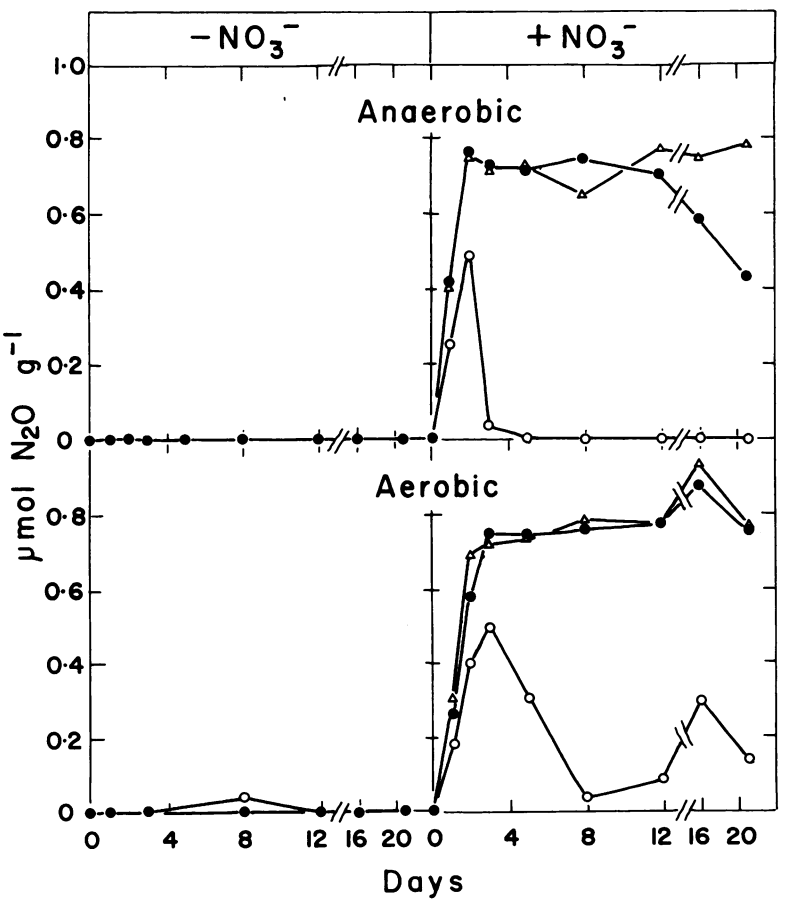

FIG. 1. *Production of N_2O by Lake St. George sediment from 14-m water depth, 0- to 5-cm sediment depth. Samples were incubated statically under He (upper) or air (lower), in the absence (left) and in the presence (right) of 2 μmol of NO_3^- per g of sediment. The gas phase contained no* (○), *0.1 atm* (●), *or 0.4 atm of C_2H_2* (△).

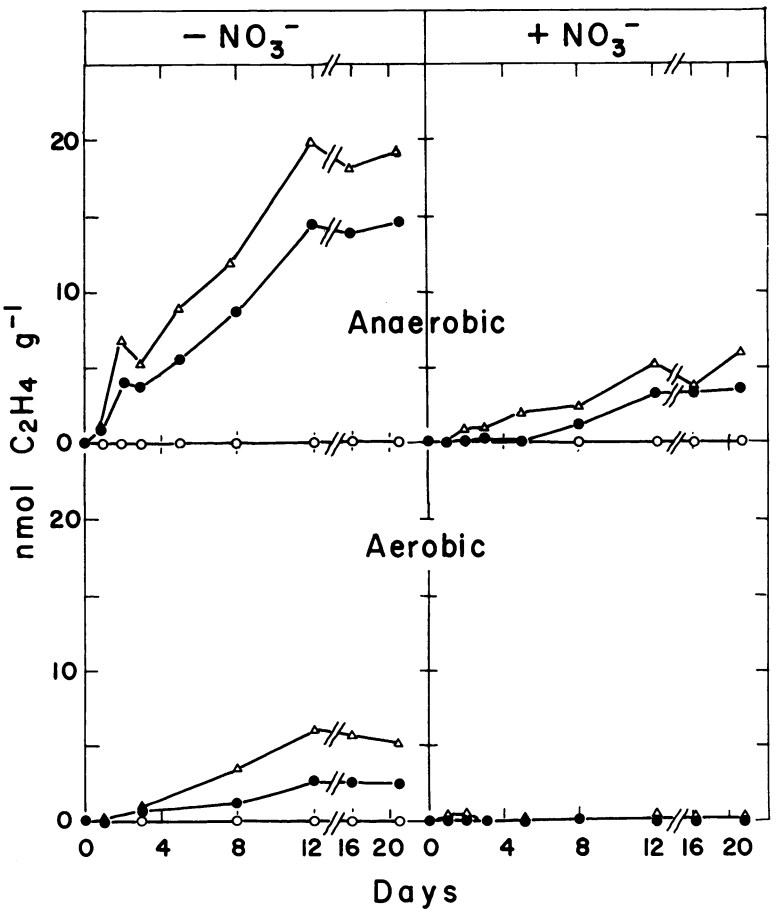

FIG. 2. *Production of C_2H_4 by Lake St. George sediment from 14-m water depth, 0- to 5-cm sediment depth. Conditions and symbols as in Fig. 1.*

metabolism of an added 40 nmol of C_2H_4 per g of sediment occurred under either anaerobic or aerobic conditions (data not presented). Most C_2H_4 was produced under anaerobic conditions without NO_3^- supplement. No C_2H_4 was produced under aerobic conditions with NO_3^-, and the other conditions gave intermediate rates (Fig. 2). Slightly greater rates of C_2H_4 production were observed with 0.4 atm than with 0.1 atm C_2H_2, and further data on this aspect will be presented later.

As was expected, release of CH_4 into the gas phase did not occur under aerobic conditions, but did occur under anaerobiosis (Fig. 3). In the absence of NO_3^-, production of CH_4 occurred with no lag and at a constant rate; however, the addition of NO_3^- caused a lag of about 5 to 8 days before production began. Methane production was completely inhibited by C_2H_2 (Fig. 3). The base-line levels of CH_4 observed in the presence of C_2H_2 in aerobic flasks (Fig. 3) probably represent CH_4, already present in the sediment, which equilibrated with the atmosphere, its metabolism being inhibited by C_2H_2. Such endogenous CH_4 would be removed by the evacuation involved in the creation of anaerobic conditions.

The production of N_2O and of C_2H_4 was affected by the concentration of C_2H_2 to which the sediment was exposed (Fig. 4). The initial period of rapid production of N_2O from NO_3^- (e.g., at day 1) was not affected by C_2H_2 concentration. The smaller amount of N_2O present after 8 days at C_2H_2 concentrations lower than 0.4 atm represents the somewhat less effective inhibition of subsequent reduction of N_2O at such concentrations. The production of C_2H_4 between 1 and 8 days (Fig. 4) was maximal at 0.2 atm but somewhat submaximal at 0.1 atm of C_2H_2.

In similar studies of other samples of sedi-

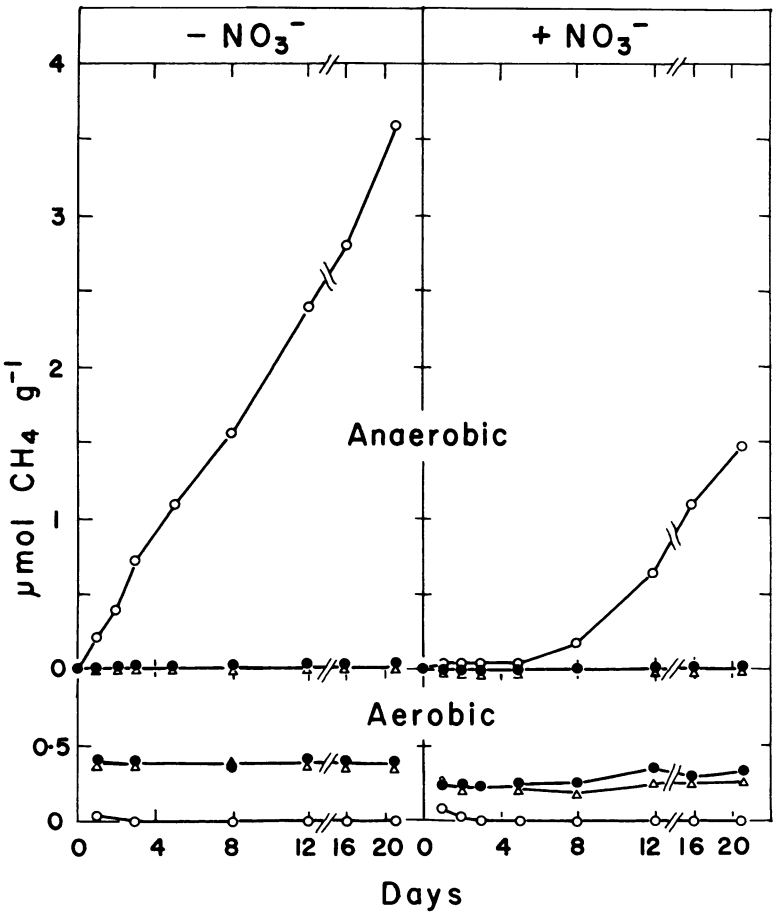

FIG. 3. *Production of CH_4 by Lake St. George sediment from 14-m water depth, 0- to 5-cm sediment depth. Conditions and symbols as in Fig. 1.*

ments, patterns of production of N_2O, C_2H_4, and CH_4 were similar to those shown in Fig. 1 to 3. Rates calculated from the straight-line parts of such curves are summarized in Table 2. Denitrification potential (N_2O production from NO_3^- in the presence and absence of C_2H_2) was somewhat greater in the 5-m-depth sediments. The subsequent reduction of accumulated N_2O in the presence of C_2H_2 was negligible except in the 5-m surface sediment, where it was appreciable. This is also reflected in the maximum amounts of N_2O observed in the presence of 0.1 atm of C_2H_2 (Table 3). Least N_2O accumulated in the 5-m surface sediment and most in the 14-m subsurface samples. This relationship likely depends on the efficacy of the C_2H_2 inhibition of N_2O reduction.

In the absence of a NO_3^- supplement, N_2O was produced only in aerobic conditions after a lag of 3 to 5 days (Table 2). Such production of N_2O was completely inhibited by C_2H_2. This O_2-dependent production of N_2O in the absence of added NO_3^- was investigated in several further experiments (Table 4). The data were rather variable, but N_2O was generally produced after a lag of 1 to 8 days in the absence (but not in the presence) of C_2H_2. The addition of the nitrification inhibitor, nitrapyrin, delayed the appearance of N_2O, but subsequently the rate of N_2O production was not very different from that in the controls.

Rates of production of C_2H_4 from C_2H_2 (Table 2) decreased as water depth and sediment depth increased and, as might be expected, were also reduced by aerobiosis and the addition of NO_3^-. However, all such rates observed during dark incubation were very low.

Methane was produced only under anaerobic conditions, and addition of NO_3^- induced a lag of 4 to 10 days (Table 2). Rates were greater in

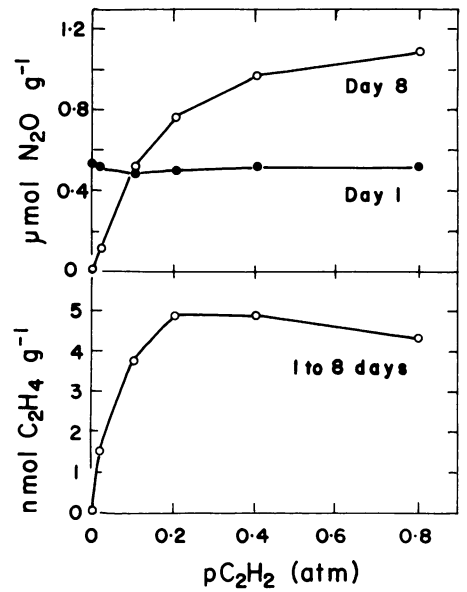

FIG. 4. *Nitrous oxide present after 1 and 8 days of incubation and C_2H_4 produced between 1 and 8 days of incubation by Lake St. George sediment from 5-m water depth, 0- to 10-cm sediment depth. Samples were supplemented at zero time with 2 μmol of NO_3^- per g of sediment and incubated under He with the indicated concentrations of C_2H_2.*

the surface sediments and were negligible in the 14-m subsurface samples. In all cases, CH_4 production was inhibited by 0.1 atm of C_2H_2.

Light markedly stimulated the rate of C_2H_2 reduction in 5-m surface sediment (Fig. 5), but this effect was seen only under anaerobiosis. Microscopic examination of such enriched samples revealed cells which appeared to be unicellular cyanobacteria. No purple or green photosynthetic bacteria were seen, although the former occur in the water column of this lake during late stratification (D. R. S. Lean, personal communication). Dark C_2H_4 production was inhibited by addition of CH_4 under both anaerobic and aerobic conditions (Fig. 5), but this phenomenon was not further investigated. Predictably, added CH_4 (3.6 μmol/g) was not metabolized under anaerobic conditions or in the presence of C_2H_2. It was rapidly oxidized under aerobiosis in the absence of C_2H_2 and had completely disappeared within 3 days (Fig. 5).

DISCUSSION

The lack of N_2O production by anaerobically incubated sediment in the absence of added NO_3^- indicates that there was no endogenous NO_3^- present at the time of the experiments. This is the first report of the production of N_2O by sediment under an aerobic gas phase in the

TABLE 2. *Rates of production and subsequent reduction of N_2O and of production of C_2H_4 and CH_4 in the presence and absence of 0.1 atm of C_2H_2 by Lake St. George sediments*[a]

Water depth (m)	Sediment depth (cm)	Added NO_3 (2 μmol/g)	Gas phase	N_2O (nmol/g per day)				C_2H_4 production (pmol/g per day) 0–3 days ($+C_2H_2$)	CH_4 production (nmol/g per day) 0–21 days	
				Production 0–2 days		Reduction 2–12 days				
				$-C_2H_2$	$+C_2H_2$	$-C_2H_2$	$+C_2H_2$		$-C_2H_2$	$+C_2H_2$
5	0–5	−	Anaerobic	0	0	—	—	2,700	130	0
			Aerobic	0	0	—	—	400	0	0
		+	Anaerobic	315	530	320	96	400	244 (6)	0
			Aerobic	260	390	51	0	0	0	0
	5–10	−	Anaerobic	0	0	—	—	2,100	18 (4)	0
			Aerobic	9 (5)	0	0	—	70	0	0
		+	Anaerobic	460	500	−8	0	300	27 (10)	0
			Aerobic	250	340	0	0	0	0	0
14	0–5	−	Anaerobic	0	0	—	—	1,300	210	0
			Aerobic	8 (3)	0	100	—	400	0	0
		+	Anaerobic	220	350	430	4	200	105 (6)	0
			Aerobic	170	260	84	−1	0	0	0
	5–10	−	Anaerobic	0	0	—	—	700	3	0
			Aerobic	16 (3)	0	12	—	320	0	0
		+	Anaerobic	360	370	180	0	0	0	0
			Aerobic	240	280	107	0	0	0	0

[a] Data are reported on a sediment fresh weight basis. —, Not determined or not applicable. Figures in parentheses indicate the number of days of lag before initiation of the activity reported.

TABLE 3. *Maximum amounts of N_2O observed up to 21 days after addition of 2 μmol of NO_3^- per g of sediment in the presence of 0.1 atm of C_2H_2*

Water depth (m)	Sediment depth (cm)	N_2O (μmol/g)	
		Anaerobic gas phase	Aerobic gas phase
5	0–5	0.59	0.68
	5–10	0.65	0.69
14	0–5	0.78	0.94
	5–10	0.84	1.03

TABLE 4. *Rates of production of N_2O by Lake St. George sediments incubated in air in the presence and absence of nitrapyrin and C_2H_2*[a]

Water depth (m)	Sediment depth (cm)	N_2O (nmol/g per day)		
		Control	+Nitrapyrin (20 μg/g)	+C_2H_2 (0.1 atm)
5	0–10	73 (8)	62 (12)	0
		30 (1–7)	37 (12)	0
	0–5	0	0	0
		17 (5)	—	0
	5–10	9 (5)	9 (8)	0
14	0–5	8 (3)	41 (8)	0
		0	—	0
	5–10	16 (3)	62 (8)	0

[a] Data are reported on a sediment fresh weight basis. No NO_3^- was added to any of these samples. —, Not determined. Figures in parentheses indicate the number of days of lag before initiation of the reported activity.

absence of added NO_3^-. It suggests that coupled nitrification and denitrification occurred due to the existence of an aerobic-anaerobic interface within the sediment. The fact that this N_2O formation was inhibited by C_2H_2 and delayed by nitrapyrin is consistent with this interpretation, since C_2H_2 inhibits nitrification of NH_4^+ by *Nitrosomonas europaea* (13, 27), as does nitrapyrin (3). The latter compound, however, is difficult to apply uniformly in experimental work (5) and is reported to lose effect after about 7 days in sediments (26). This possibly explains the formation of N_2O after at least 8 days with nitrapyrin under the present conditions.

The denitrification of added NO_3^- occurred almost as rapidly under an initially aerobic gas phase as under anaerobic conditions, in agreement with the report that O_2 up to 10 mg/liter in the water column did not greatly inhibit the process in sediment (26). Thus, providing lake bottom water contains NO_3^-, sediment denitrification in anaerobic microenvironments and especially below the aerobic-anaerobic interface is likely to occur rapidly regardless of the dissolved O_2 concentration in the overlying water. The marked but transient accumulation of N_2O in the absence of C_2H_2 indicates a high mole fraction of N_2O in the denitrification products during the first 2 days and suggests that the N_2O-reducing system here is quite sensitive to NO_3^- concentrations of the order of 1 μmol/g of fresh sediment, as has been shown for other systems (4, 18). In many of the present experiments (especially with shallow water and surface sediments), although the N_2O accumulation in the presence of 0.1 atm of C_2H_2 probably reflected total denitrification during the first 2 or 3 days, it did not subsequently represent complete conversion of the added NO_3^-. The data suggest that this was partly due to the incomplete inhibition of N_2O reduction by C_2H_2 at concentrations of the order of 0.1 to 0.2 atm. However, it may also reflect some dissimilatory or assimilatory reduction of NO_3^- to NH_4^+, which has been observed in some sediments (16, 23).

High rates of C_2H_2 reduction were seen in anaerobically incubated shallow-water surface sediment in the light. This activity was attributed to unicellular cyanobacteria which would not be inhibited by the O_2 they produce at the low illumination employed (25). Acetylene reduction rates in the dark were highest in shallow water and in surface sediments under anaerobic conditions. As was expected, activity was inhibited somewhat by an aerobic atmosphere and by the addition of 2 μmol of NO_3^- per g. Partial to complete inhibition of sediment C_2H_2 reduction was also reported for concentrations in the range 0.2 to 10 mM NO_3^- (14). Data showed that maximum rates of C_2H_4 production were observed with 0.2 atm of C_2H_2. Much higher concentrations were reported to be necessary for maximum rates in other sediments (e.g., R. Sylvester-Bradley, Ph.D. thesis, University of Edinburgh, Edinburgh, Scotland, 1976).

The concentrations of C_2H_2 required to inhibit N_2O production completely and to saturate the nitrogen-fixing system appear to depend on the nature of the sediment. Concentrations in equilibrium with a gas phase containing 0.1 to 0.2 atm of C_2H_2 seem to be adequate, particularly in short-term experiments. The high rates of light-dependent C_2H_2 reduction observed would not significantly deplete the concentration of C_2H_2 introduced for a denitrification assay.

As might be expected, methane was produced with little or no lag under anaerobic conditions by all except the deep-water subsurface sediments. The addition of NO_3^- imposed a 6- to 10-day lag, as has been observed by others (1, 8). Added CH_4 was metabolized more rapidly under aerobic conditions than this gas was produced under anaerobic conditions. This indicates that

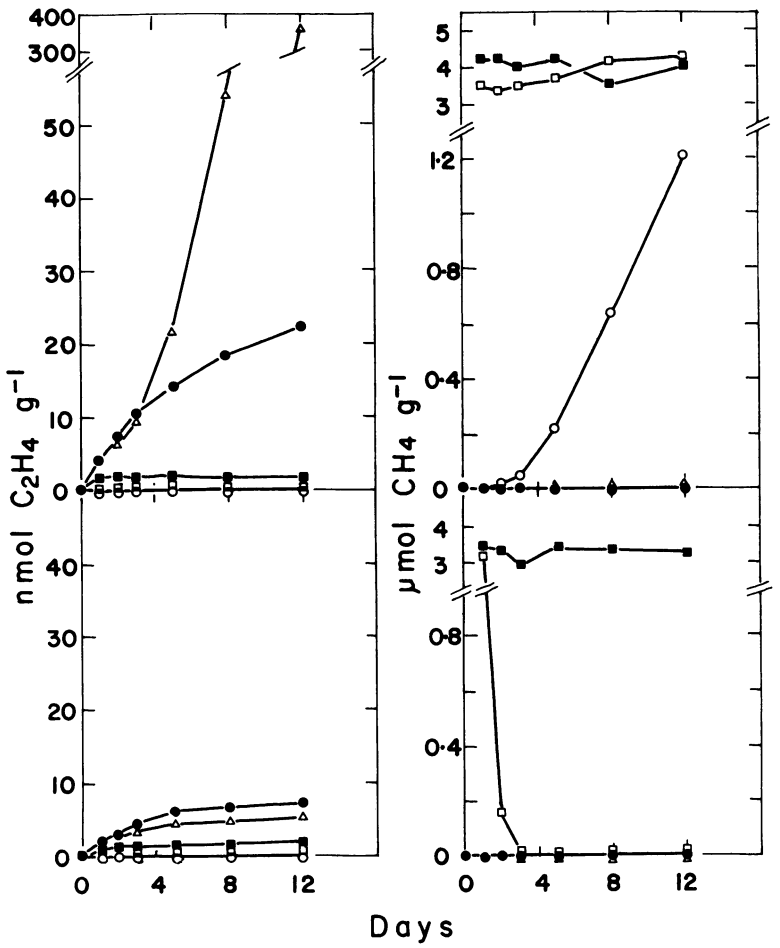

FIG. 5. *Production of C_2H_4 from C_2H_2 (left) and metabolism of CH_4 (right) by Lake St. George sediment from 5-m water depth, 0- to 5-cm sediment depth. Samples did not receive NO_3^- and were incubated under He (upper) or air (lower). Other conditions were: no C_2H_2 (○), 0.1 atm of C_2H_2 (●), 3.6 μmol of CH_4 per g of sediment (□), C_2H_2 + CH_4 (■), and C_2H_2 + light (△).*

the lack of release of CH_4 by sediment under air could have been due to the greater potential for consumption than for production. Both production and consumption of CH_4 were completely inhibited by 0.1 atm of C_2H_2, as was previously reported (11, 20).

The use of C_2H_2 in assays of denitrification and N_2 fixation thus clearly inhibits activity of any organisms whose growth is supported by CH_4 (9, 11). Denitrifying bacteria supported by CH_4 (10) and by methanol (24) have been reported, and methanotrophic N_2 fixers have been studied (9, 11). There is little information on the contributions of such organisms in sediments, and the present data do not permit an estimation of the possible methane-supported activity. The N_2 fixation activity and high denitrification activity that were observed, however, occurred in the presence of C_2H_2 and therefore could not be attributed to the activity of methanotrophic bacteria.

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