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 (NO₃⁻) there was O₂-dependent production of nitrous oxide (N₂O), which was inhibited by acetylene (C₂H₂) and by nitrapyrin, suggesting that coupled nitrification-denitrification was responsible. Denitrification of added NO₃⁻ was almost as rapid under an aerobic gas phase as under anaerobic conditions. The N₂O that accumulated persisted in the presence of 0.4 atm of C₂H₂, but was gradually reduced by some sediment samples at lower C₂H₂ concentrations. Low rates of C₂H₂ reduction were observed in the dark, were maximal at 0.2 atm of C₂H₂, and were decreased in the presence of O₂, NO₃⁻, or both. High rates of light-dependent C₂H₂ reduction occurred under anaerobic conditions. Predictably, methane (CH₄) production, which occurred only under anaerobiosis, was delayed by added NO₃⁻ and inhibited by C₂H₂. Consumption of added CH₄ occurred only under aerobic conditions and was inhibited by C₂H₂.

The acetylene (C₂H₂) inhibition method for the measurement of denitrification (2, 12, 29) was used for soil (28) and was further validated by ¹⁵N (21) and ¹⁴C (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 C₂H₂ reduction (N₂ fixation) were measured simultaneously (7). However, there is little information on the effects of O₂, nitrate (NO₃⁻), methane (CH₄), and light on sediment processes during a "C₂H₂ inhibition" assay.

The present paper describes the first laboratory studies of the production and reduction of nitrous oxide (N₂O), reduction of C₂H₂, and production and consumption of CH₄ by samples of lake sediment in the presence and absence of C₂H₂, O₂, NO₃⁻, CH₄, 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 NaNO₃ (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 C₂H₂, CH₄, CH₄, and N₂O 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 Mini-nert valve (Precision Sampling Corporation, Baton Rouge, La.) and analyzed for CH₄, C₂H₂, C₂H₆, CO₂, and N₂O 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 O₂ in initially aerobic flasks was determined by gas chromatography (6), and O₂ was added to replace that consumed. The O₂ 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 N₂O per flask.

RESULTS

In the absence of added NO₃⁻, no N₂O was produced except under an initially aerobic gas phase (henceforth referred to as aerobic) in the absence of C₂H₂ (Fig. 1). This production of N₂O was inhibited by C₂H₂, and further information on this phenomenon is presented later. Patterns of N₂O production from added NO₃ were similar under anaerobic and aerobic conditions, although rates were slightly lower in the latter case (Fig. 1). In the absence of C₂H₂, N₂O 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 N₂O with no subsequent reduction, except under anaerobic conditions with 0.1 atm C₂H₂, where some reduction occurred after 12 days. The second peak of N₂O accumulation under aerobic conditions at 16 days is not understood (Fig. 1).

Production of ethylene (C₂H₄) did not occur in the absence of C₂H₂ (Fig. 2). Furthermore, no

<table>
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<tr>
<th>Table 1. Dry weight, loss on ignition, and pH of the Lake St. George sediments used in this study</th>
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<tr>
<td>Water depth (m)</td>
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* Mean pH values of sediment after 21 days of incubation under an initially aerobic gas phase (aerobic) and under He (anaerobic).

![Diagram](http://aem.asm.org)
metabolism of an added 40 nmol of C₂H₄ per g of sediment occurred under either anaerobic or aerobic conditions (data not presented). Most C₂H₄ was produced under anaerobic conditions without NO₃⁻ supplement. No C₂H₄ was produced under aerobic conditions with NO₃⁻, and the other conditions gave intermediate rates (Fig. 2). Slightly greater rates of C₂H₄ production were observed with 0.4 atm than with 0.1 atm C₂H₂, and further data on this aspect will be presented later.

As was expected, release of CH₄ into the gas phase did not occur under aerobic conditions, but did occur under anaerobiosis (Fig. 3). In the absence of NO₃⁻, production of CH₄ occurred with no lag and at a constant rate; however, the addition of NO₃⁻ caused a lag of about 5 to 8 days before production began. Methane production was completely inhibited by C₂H₂ (Fig. 3). The base-line levels of CH₄ observed in the presence of C₂H₂ in aerobic flasks (Fig. 3) probably represent CH₄, already present in the sediment, which equilibrated with the atmosphere, its metabolism being inhibited by C₂H₂. Such endogenous CH₄ would be removed by the evacuation involved in the creation of anaerobic conditions.

The production of N₂O and of C₂H₄ was affected by the concentration of C₂H₂ to which the sediment was exposed (Fig. 4). The initial period of rapid production of N₂O from NO₃⁻ (e.g., at day 1) was not affected by C₂H₂ concentration. The smaller amount of N₂O present after 8 days at C₂H₂ concentrations lower than 0.4 atm represents the somewhat less effective inhibition of subsequent reduction of N₂O at such concentrations. The production of C₂H₄ between 1 and 8 days (Fig. 4) was maximal at 0.2 atm but somewhat submaximal at 0.1 atm of C₂H₂.

In similar studies of other samples of sedi-
ments, patterns of production of \( \text{N}_2\text{O} \), \( \text{C}_2\text{H}_4 \), and \( \text{CH}_4 \) 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 (\( \text{N}_2\text{O} \) production from \( \text{NO}_3^- \) in the presence and absence of \( \text{C}_2\text{H}_2 \)) was somewhat greater in the 5-m-depth sediments. The subsequent reduction of accumulated \( \text{N}_2\text{O} \) in the presence of \( \text{C}_2\text{H}_2 \) was negligible except in the 5-m surface sediment, where it was appreciable. This is also reflected in the maximum amounts of \( \text{N}_2\text{O} \) observed in the presence of 0.1 atm of \( \text{C}_2\text{H}_2 \) (Table 3). Least \( \text{N}_2\text{O} \) accumulated in the 5-m surface sediment and most in the 14-m subsurface samples. This relationship likely depends on the efficacy of the \( \text{C}_2\text{H}_2 \) inhibition of \( \text{N}_2\text{O} \) reduction.

In the absence of a \( \text{NO}_3^- \) supplement, \( \text{N}_2\text{O} \) was produced only in aerobic conditions after a lag of 3 to 5 days (Table 2). Such production of \( \text{N}_2\text{O} \) was completely inhibited by \( \text{C}_2\text{H}_2 \). This \( \text{O}_2 \)-dependent production of \( \text{N}_2\text{O} \) in the absence of added \( \text{NO}_3^- \) was investigated in several further experiments (Table 4). The data were rather variable, but \( \text{N}_2\text{O} \) was generally produced after a lag of 1 to 8 days in the absence (but not in the presence) of \( \text{C}_2\text{H}_2 \). The addition of the nitrification inhibitor, nitrapyrin, delayed the appearance of \( \text{N}_2\text{O} \), but subsequently the rate of \( \text{N}_2\text{O} \) production was not very different from that in the controls.

Rates of production of \( \text{C}_2\text{H}_4 \) from \( \text{C}_2\text{H}_2 \) (Table 2) decreased as water depth and sediment depth increased and, as might be expected, were also reduced by aerobiosis and the addition of \( \text{NO}_3^- \). However, all such rates observed during dark incubation were very low.

Methane was produced only under anaerobic conditions, and addition of \( \text{NO}_3^- \) induced a lag of 4 to 10 days (Table 2). Rates were greater in
the surface sediments and were negligible in the 14-m subsurface samples. In all cases, CH₄ production was inhibited by 0.1 atm of C₂H₂.

Light markedly stimulated the rate of C₂H₂ 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 C₂H₄ production was inhibited by addition of CH₄ under both anaerobic and aerobic conditions (Fig. 5), but this phenomenon was not further investigated. Predictably, added CH₄ (3.6 μmol/g) was not metabolized under anaerobic conditions or in the presence of C₂H₂. It was rapidly oxidized under aerobicism in the absence of C₂H₂ and had completely disappeared within 3 days (Fig. 5).

**DISCUSSION**

The lack of N₂O production by anaerobically incubated sediment in the absence of added NO₃⁻ indicates that there was no endogenous NO₃⁻ present at the time of the experiments. This is the first report of the production of N₂O by sediment under an aerobic gas phase in the

**TABLE 2. Rates of production and subsequent reduction of N₂O and of production of C₂H₄ and CH₄ in the presence and absence of 0.1 atm of C₂H₂ by Lake St. George sediments**

<table>
<thead>
<tr>
<th>Water depth (m)</th>
<th>Sediment depth (cm)</th>
<th>Added NO₃ (2 μmol/g)</th>
<th>Gas phase</th>
<th>N₂O (nmol/g per day)</th>
<th>C₂H₄ production (nmol/g per day)</th>
<th>CH₄ production (nmol/g per day) 0–21 days</th>
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<tr>
<td></td>
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<td></td>
<td></td>
<td>Production 0–2 days</td>
<td>Reduction 2–12 days</td>
<td>0–3 days (C₂H₄)</td>
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<tr>
<td>5</td>
<td>0–5</td>
<td>−</td>
<td>Anaerobic</td>
<td>0 0</td>
<td>− −</td>
<td>2,700</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+</td>
<td>Anaerobic</td>
<td>315 530</td>
<td>320 96</td>
<td>400</td>
</tr>
<tr>
<td>5–10</td>
<td>−</td>
<td>Anaerobic</td>
<td>0 0</td>
<td>2,100</td>
<td>18 4</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>Anaerobic</td>
<td>9 5</td>
<td>300</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5–10</td>
<td>−</td>
<td>Anaerobic</td>
<td>460 500</td>
<td>−8</td>
<td>300</td>
<td>27 10</td>
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<tr>
<td></td>
<td></td>
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<td>250 340</td>
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<td>0</td>
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<tr>
<td>14</td>
<td>0–5</td>
<td>−</td>
<td>Anaerobic</td>
<td>0 0</td>
<td>− −</td>
<td>1,300</td>
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<tr>
<td></td>
<td></td>
<td>+</td>
<td>Anaerobic</td>
<td>8 3 100</td>
<td>400</td>
<td>0</td>
</tr>
<tr>
<td>5–10</td>
<td>−</td>
<td>Anaerobic</td>
<td>220 350</td>
<td>12</td>
<td>320</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>Anaerobic</td>
<td>170 260</td>
<td>12</td>
<td>320</td>
<td>0</td>
</tr>
</tbody>
</table>

*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.*
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$_2$O formation was inhibited by C$_2$H$_2$ and delayed by nitrapyrin is consistent with this interpretation, since C$_2$H$_2$ 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$_2$O 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$_2$O in the absence of C$_2$H$_2$ indicates a high mole fraction of N$_2$O in the denitrification products during the first 2 days and suggests that the N$_2$O-reducing system here is quite sensitive to NO$_3^-$ concentrations in 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$_2$O accumulation in the presence of 0.1 atm of C$_2$H$_2$ probably reflected total denitrification during the first 2 or 3 days, it did not subsequently represent complete conversion of the added N$_2$O. The data suggest that this was partly due to the incomplete inhibition of N$_2$O reduction by C$_2$H$_2$ at concentrations of the order of 0.1 to 0.2 atm. However, it may also reflect some dissipatory or assimilatory reduction of NO$_3^-$ to NH$_4^+$, which has been observed in some sediments (16, 23).

High rates of C$_2$H$_2$ 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$_2$H$_2$ reduction was also reported for concentrations in the range 0.2 to 10 mM NO$_3^-$ (14). Data showed that maximum rates of C$_2$H$_2$ production were observed with 0.2 atm of C$_2$H$_2$. 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$_2$H$_2$ required to inhibit N$_2$O 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$_2$H$_2$ seem to be adequate, particularly in short-term experiments. The high rates of light-dependent C$_2$H$_2$ reduction observed would not significantly deplete the concentration of C$_2$H$_2$ 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
the lack of release of CH₄ by sediment under air could have been due to the greater potential for consumption than for production. Both production and consumption of CH₄ were completely inhibited by 0.1 atm of C₂H₂, as was previously reported (11, 20).

The use of C₂H₂ in assays of denitrification and N₂ fixation thus clearly inhibits activity of any organisms whose growth is supported by CH₄ (9, 11). Denitrifying bacteria supported by CH₄ (10) and by methanol (24) have been reported, and methanotrophic N₂ 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 N₂ fixation activity and high denitrification activity that were observed, however, occurred in the presence of C₂H₂ and therefore could not be attributed to the activity of methanotrophic bacteria.

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LITERATURE CITED


