Partitioning Effects during Terminal Carbon and Electron Flow in Sediments of a Low-Salinity Meltwater Pond near Bratina Island, McMurdo Ice Shelf, Antarctica

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A study of anaerobic sediments below cyanobacterial mats of a low-salinity meltwater pond called Orange Pond on the McMurdo Ice Shelf at temperatures simulating those in the summer season (<5°C) revealed that both sulfate reduction and methane production were important terminal anaerobic processes. Addition of [2-14C]acetate to sediment samples resulted in the passage of label mainly to CO₂. Acetate addition (0 to 27 mM) had little effect on methanogenesis (a 1.1-fold increase), and while the rate of acetate dissimilation was greater than the rate of methane production (6.4 nmol cm⁻³ h⁻¹ compared to 2.5 to 6 nmol cm⁻³ h⁻¹), the portion of methane production attributed to acetate cleavage was <2%. Substantial increases in the methane production rate were observed with H₂ (2.4-fold), and H₂ uptake was totally accounted for by methane production under physiological conditions. Formate also stimulated methane production (twofold), presumably through H₂ release mediated through hydrogen lyase. Addition of sulfate up to 50-fold the natural levels in the sediment (interstitial concentration, ~0.3 mM) did not substantially inhibit methanogenesis, but the process was inhibited by 50-fold chloride (36 mM). No net rate of methane oxidation was observed when sediments were incubated anaerobically, and denitrification rates were substantially lower than rates for sulfate reduction and methanogenesis. The results indicate that carbon flow from acetate is coupled mainly to sulfate reduction and that methane is largely generated from H₂ and CO₂ where chloride, but not sulfate, has a modulating role. Rates of methanogenesis at in situ temperatures were four- to fivefold less than maximal rates found at 20°C.

The McMurdo Ice Shelf is in the northwestern corner of the Ross Ice Shelf, between Ross Island and Brown Peninsula. An area of about 1,500 km² is known as Dirty Ice, an ablation zone covered by gravel. A large portion of this gravel originates from marine sediment, and much of the shelf ice is frozen seawater (7). During the summer melt, the area is covered by ponds of a wide size range between hummocks with a vertical profile as high as 20 m (12). Ponds form and disappear again over decades, leading to a wave-like cycling of the shelf surface through ponds and hummocks (2). Freezing, thawing, and evaporation often lead to pronounced solute gradients and water column stratification (9a).

The bottoms of these ponds are covered with thick mats consisting of cyanobacteria, diatoms, and green algae (11, 12), below which is a layer of anaerobic sediment. Variations in chemical and physical conditions between the ponds lead to community differentiation within and between the mats and to differences in mat morphology, thus creating in a small area a variety of modern unlithified stromatolites unique on Earth (31). The mats harbor a small population of grazers, mainly the rotifer Philodinia gregaria, but their activity does not appear to have a key function in the ecosystem. Apart from the discrete ponds on the ice shelf, there are also ponds in estuaries. These ponds are connected by seawater at high tide and also contain diverse assemblages of algae and cyanobacteria with primary production characteristics similar to those for discrete ponds (8).

Previous studies have shown that ponds may vary in conductivity, from highly saline to almost freshwater (12). While considerable knowledge has been gained about the photosynthetic activity and carbon flux attributed to the algal mats of these ponds, together with physical and chemical characteristics (2, 11), no studies on the processes occurring in the underlying pond sediments have been published.

In this paper we describe some of the major heterotrophic processes occurring in the sediments of a low-salinity meltwater pond, Orange Pond, near Bratina Island. The rates of terminal anaerobic processes are given together with the contributions of these processes to terminal carbon and electron flow, and we discuss their significance for carbon flux in the mat-sediment ecoupl.

MATERIALS AND METHODS

Location of sampling area and study pond. The study area was immediately south of Bratina Island (78°00'S, 165°35'E). The area was surveyed in January 1991 by B. R. George (New Zealand Department of Survey and Land Information, K 191, plan 37/165A). The study pond was Orange Pond, the water chemistry and algal mats of which have been described previously (11, 12, 29). The pond is oval, covers an area of approximately 27 m², and has a maximum water depth of 1 m. Underlying the cyanobacterial mat at the bottom of the pond was a layer of anaerobic sediment at a depth of about 18 to 20 cm.

Sampling procedure. Sediments were sampled by using 60-ml syringes from which the tips had been cut off. Cores were taken of the entire thawed portion of sediment. For depth profile studies, cores were cut into 2-cm segments after removal of the algal mat. Segments of identical depth were pooled, mixed, and then transferred to containers, which were sealed and frozen for chemical studies or maintained at <5°C for biological studies. Samplings along a transect were carried out at sites ranging from 2.2 m landwards from the water’s edge (51 cm above the pond level) to 1.5 m into the pond (28 cm deep). For kinetic studies of anaerobic processes, unless stated otherwise, sediment samples taken at a water depth of 10 to 20 cm, from 0 to 5 cm below the cyanobacterial mat, were pooled and stored at <5°C in sealed, near-filled containers.
Incubation techniques. For studies without radiolabel, sediment was transferred to 70-ml serum bottles (10 cm3) or 26.5-ml Balch tubes (5 cm3) under a gas stream of 70% N₂–30% CO₂. Degassed pond water was added in a ratio of 1 part of water to 2 parts of sediment by volume. Two tubes were sealed with septum stoppers secured with aluminum closures and were incubated at 2 to 4°C in a cold room for 3 days to 6 weeks. The effects of electron acceptors and short-chain fatty acids (SCFA) on methanogenesis were tested by the addition of an electron acceptor (nitrate or sulfate) or SCFA to incubation mixtures in the range of 0 to 20 mM (final added concentration) in the interstitial water. The effect of hydrogen on methanogenesis was tested by the addition of the gas to incubation mixtures in tubes in the range of 0 to 30 kPa. Tubes were incubated by using a radial shaker as previously described (16) to minimize the slow diffusion of the gas from the gas phase to the sediment. Methanogenesis was determined by the addition of methanol to septum-stoppered serum bottles (initial concentration in an air-gas phase, 1% [vol/vol]) containing sediment taken from 0 to 1 cm below the cyanobacterial mat. Denitrification was determined by incubating sediments axenically in the presence of nitrate (0.7 μg of atomic N cm⁻²). Incubations investigating the partitioning of acetate to methane and CO₂ were carried out on sediments taken at 2-cm depth intervals at a water depth of 20 cm. [²⁵⁴C]Acetate (0.2 ml; 51 mCi mmol⁻¹; 25 μCi ml⁻¹) was added via syringe to butyl septum-stoppered 70-ml serum bottles, each containing 10 ml of sediment (taken from 2-cm depth intervals) diluted with 5 ml of degassed pond water under a gas mixture of 70% N₂–30% CO₂. Shuffles were incubated at 2 to 4°C. Bottles also contained a glass center tube for CO₂ capture by NaOH. Incubation vial and counted in a toluene-based scintillant. Polypore H column was used; acetate in the eluate was collected in a scintillation vials sealed with butyl septum stoppers and counting in 20 ml of toluene-methanol scintillant (15). Analysis of radiolabelled samples was carried out on sediments taken at 2-cm depth intervals at a water depth of 4 to 18 cm.

 physical and chemical characteristics of sediment along pond transect. The chemical composition of Orange Pond sediments along the transect is summarized in Table 1. Sulfate levels were highest above the waterline (i.e., on the land) at 0- to 2-cm depths and decreased with increasing depth. Lower levels of sulfate were present in sediments taken at and below the waterline (i.e., at the pond’s edge and in the pond). No clear relationship existed between depth and sodium concentrations in sediments at different points along the transect. SRP levels were highest in the 0- to 2-cm depth zone below the waterline. The highest levels of Kjeldahl nitrogen were found in the 0- to 4-cm depth zone above the waterline, in the 4- to 8-cm depth zone at the waterline, and at depths of >8 cm below the waterline. Trends for NH₄ levels were similar, but below the waterline there was no relationship between depth and concentration. The highest pH values were in the 0- to 2-cm depth range at all points along the transect.

The dry weight as a percentage of the wet weight of the sediment was 80% above the waterline, 75 to 80% at the
and measurements of hydrogen utilized revealed that methane accounted for >82 to <40% of the hydrogen utilized at initial H₂ levels ranging from 3 to 28 kPa (Table 4). Transformation of the results in Table 4 (plotting 1/percent H₂ utilized for CH₄ versus initial H₂ in kilopascals [Fig. 2b]) predicts that under physiological conditions (H₂ < 5 Pa), methanogenesis would account for all of the hydrogen (1 intercept >95%).

Effects of added short-chain volatile fatty acids on rates of methane production. Addition of SCFA to sediments (final added concentrations, 0 to 25 mM in the interstitial water) stimulated methanogenesis in decreasing order as follows: formate (2-fold) > butyrate (1.25-fold) > acetate (1.1-fold) (Fig. 3a).

Effect of nitrate and effect of sulfate and NaCl on methanogenesis. Addition of sodium sulfate at levels in the range of 0 to 15.3 mM inhibited methanogenesis slightly, whereas addition of nitrate in the same concentration range inhibited the process substantially (Fig. 3b). Addition of NaCl at low levels (12 mM) did not inhibit methanogenesis, but >50% inhibition occurred when the salt was present at higher levels (36 mM).

Sulfate reduction rate versus methanogenesis and acetate dissimilation rates. Determinations of the sulfate reduction, methanogenesis, and acetate dissimilation rates for sediments are shown in Table 5. Rates of acetate dissimilation were determined from the turnover rate constants obtained from the slopes of the plots in Fig. 4 multiplied by the pool size. Rates of sulfate reduction exceeded methanogenesis rates by a ratio of 3. Based on the value of pox for acetate degradation and rates of acetate dissimilation together with the rates of the two terminal processes, acetate was calculated to contribute to 2%

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sulfate reduced per cubic centimeter per hour

3 sampling substantially inhibited methanogenesis, indicating the potential

of methane production and 70% of sulfate reduction. On the other hand, H₂ was nearly all utilized for methanogenesis (Fig. 2b).

Determination of methane oxidation and denitrification rates in sediments. Rates of methane oxidation in surface sediments under aerobic conditions almost matched the rates of methanogenesis found in the deeper anaerobic sediments (Tables 5 and 6). No net rate of methane oxidation was observed for the same sediment incubated under anaerobic conditions. Sediments taken from both the shallow and deeper profiles showed a capacity for denitrification, but the rates were substantially lower than those for methanogenesis and sulfate reduction.

Optimal temperature for methanogenesis. The highest rate of methanogenesis was at 20°C (Fig. 5). Rates at 4°C were about 20% of the maximum.

DISCUSSION

Previous to this work anaerobic microbial processes have been described for antarctic ecosystems including dry valley and coastal lakes (3–5, 14). However there has been no previous documentation of the processes occurring beneath the mats of McMurdo Ice Shelf ponds. In this communication we describe the terminal anaerobic processes occurring in the sediments beneath the cyanobacterial mats of Orange Pond, a low-salinity meltwater pond. Both methanogenesis and sulfate reduction were found to be important terminal events in which the rate ratio (sulfate reduction to methanogenesis) was about 3.

In our previous studies of sulfate reduction and methanogenesis in temperate coastal sediments where active methanogenesis occurred, the p_H₂ was >0.5, indicating that a substantial amount of methyl carbon from acetate was converted to methane (16, 17). Furthermore, sulfate addition to such sediments substantially inhibited methanogenesis, indicating the potential for sulfate-reducing organisms to compete for the methanogenic substrates. In a totally methanogenic system, the methyl group of acetate is stoichiometrically converted to methane (30). While Orange Pond sediments were actively methanogenic, the passage of carbon from acetate was mainly to CO₂. Acetate did not stimulate methanogenesis, which is unusual in a typical methanogenic environment unless levels of acetate are already saturating. These findings indicate that the Orange Pond sediments were unusual in the context of known anaerobic systems.

Clarification of the results on acetate metabolism was obtained through studies on hydrogen addition, from which we calculated that under physiological conditions, methane could be wholly accounted for by this precursor. The hydrogen addition studies were also consistent with the results on stimulation of methanogenesis by formate, in which the acid was most likely cleaved by formate hydrogen lyase to release CO₂ and hydrogen for methanogenesis. Hydrogen as the major precursor of methane is not unusual in the context of Antarctic ecosystems. Ellis-Evans (3) demonstrated from studies with 14CO₂ that in sediments of Antarctic lakes H₂ and CO₂ were the major precursors of methane, and Smith et al. (24) showed that in incubations of sediments from Lake Fryxell the rate of methanogenesis from H₂ and CO₂ was approximately four times that of acetate cleavage to methane.

Estimates of the proportions of sulfate reduction and methane production contributed by acetate and hydrogen in Orange Pond sediments are shown in Fig. 6. They are based on rate data (Table 5), together with the data on p_H₂ and hydrogen utilization. The diagram also gives the proportions of carbon flow associated with acetate and hydrogen metabolism in methanogenic and sulfate-reducing ecosystems based on known stoichiometric conversions. In Orange Pond sediments the passage of carbon flow is not that of a typically methanogenic or sulfate-reducing ecosystem in that sulfate reduction accounts for most of the acetate metabolized and methane accounts for most of the hydrogen. The rates of the two precursors are therefore partitioned between the two processes. To our knowledge this is the first detailed report of such an event occurring in an anaerobic ecosystem.

There is some precedent for believing that changes in the Gibbs free energy of key processes, differential sensitivities of populations to temperature, and temperature-dependent substrate affinities are key factors in influencing microbial community function at low temperatures and that they contributed to the results we observed. Anaerobic communities consisting of different methanogenic populations utilizing either acetate or hydrogen and with different temperature optima have been described for subarctic peat (26). In anoxic paddy soil, reduc-

<table>
<thead>
<tr>
<th>Yr of sampling</th>
<th>Sulfate reduction rate (nmol · cm⁻³ · h⁻¹)</th>
<th>Methane production rate (nmol · cm⁻³ · h⁻¹)</th>
<th>Acetate dissimilation rate (nmol · cm⁻³ · h⁻¹)</th>
<th>p_H₂</th>
<th>% CH₄ from acetate</th>
<th>% Sulfate reduction from acetate oxidation</th>
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</thead>
<tbody>
<tr>
<td>1994</td>
<td>10.99 ± 1.87</td>
<td>3.32 ± 1.21</td>
<td>7.2</td>
<td>0.99</td>
<td>2.00</td>
<td>65.0</td>
</tr>
<tr>
<td>1998</td>
<td>6.83 ± 0.82</td>
<td>1.78 ± 0.23</td>
<td>5.6</td>
<td>0.99</td>
<td>3.10</td>
<td>81.2</td>
</tr>
</tbody>
</table>

a Sediments were taken in two separate summer seasons.

b Values are means of at least duplicate determinations ± 1 standard deviation.

c Determined from the slopes of the plots (Fig. 4) × 2.303 × acetate pool size.

d Obtained from Table 3.

Based on the equation CH₃COO⁻ + H⁺ → CH₄ + CO₃⁻ and determined from (nanomoles of acetate per cubic centimeter per hour × (1 - p_H₂)/nanomoles of CH₄ per cubic centimeter per hour) × 100.

Based on the equation CH₃COO⁻ + SO₄²⁻ → 2HCO₃⁻ + HS⁻ and determined from (nanomoles of acetate per cubic centimeter per hour × p_H₂/nanomoles of sulfate reduced per cubic centimeter per hour) × 100.
ing the temperature decreased turnover and the Gibbs free energy of \( \text{H}_2 \)-mediated methanogenesis, resulting in decreases in the contribution of \( \text{H}_2 \)-utilizing methanogens to overall methanogenesis (1). A detailed study by Nedwell and Rutter (19) demonstrated that temperature affected growth rate and substrate affinity in two psychrotolerant Antarctic bacteria and that the two organisms responded differently to temperature changes. The net outcome of one or several of the above temperature-dependent factors may be critical to the degree of carbon and electron partitioning between members of an anaerobic community. In Orange Pond sediments this has fa-

vored sulfate reducers for the utilization of acetate and \( \text{H}_2 \)-utilizing methanogens for the production of methane.

Also contributing to the processes we observed are the physical and biological characteristics of the ponds, e.g., the flux of inorganic ions through the pond system and the decay of the overlying cyanobacterial mat, contributing organic matter to the sediment. Levels of sulfate and, to a lesser extent, \( \text{Na}^+ \) in the sediment were low compared to those in soil adjacent to the pond (Table 1), where mirabilite (\( \text{Na}_2\text{SO}_4 \cdot 10\text{H}_2\text{O} \)) is de-

posited (2). It is likely that the latter acts as a reservoir contrib-

uting sulfate to the pond sediment via meltwater. However, reoxidation of sulfide at the aerobic-anaerobic interface be-

tween microbial mat and sediment may be the major source of sulfate for the most active sediment immediately underneath the microbial mat. The mat is the only significant source of organic carbon in the pond ecosystem (9), and its importance to anaerobic processes is reflected in the substantial stimulation of methanogenesis upon its addition to sediment (18a). The very low levels of \( \text{NO}_3^- \) (Table 1) would have precluded denitrification as a significant process. The rates (Table 6) obtained by enzyme assay reflect the capacity of the sediment for denitrification, which is typically several orders of magnitude higher than in situ rates found in long-term anaerobic incubations (13). The potential for denitrification can also ex-

<table>
<thead>
<tr>
<th>Process</th>
<th>Conditions</th>
<th>Depth below cyanobacterial mat (cm)</th>
<th>Rate (nmol of ( \text{N}_2 ) or g of atomic ( \text{N} \cdot \text{cm}^{-2} \cdot \text{h}^{-1} ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methane oxidation</td>
<td>Anaerobic</td>
<td>0–1</td>
<td>0 (^a)</td>
</tr>
<tr>
<td></td>
<td>Aerobic</td>
<td>0–1</td>
<td>3.0 ± 0.6</td>
</tr>
<tr>
<td>Denitrification</td>
<td>Anaerobic</td>
<td>0–1</td>
<td>0.83 ± 0.24</td>
</tr>
<tr>
<td></td>
<td>Anaerobic</td>
<td>0–5</td>
<td>1.11 ± 0.34</td>
</tr>
</tbody>
</table>

\(^a\) Values are means of at least duplicate determinations ± 1 standard deviation and are for sediments taken at water depths between 10 and 20 cm and incubated at 4°C.

\(^b\) Sediments showed net production of methane.
plain the substantial inhibition of methanogenesis upon the addition of nitrate to the sediment (Fig. 3b), as it is the preferred electron acceptor in H2-consuming reactions (27). Among other processes that could have contributed to methane production is the reductive demethylation of dimethyl sulfide (DMS). DeMora and colleagues have detected DMS in the water column of the pond systems (2). However, while sediments have the capability of producing DMS from dimethylsulfiniopropionate, DMS was not detected in incubation mixtures degrading the cyanobacterial mat, nor did its addition to sediments stimulate methanogenesis, with or without hydrogen addition (18a). Oxidation of methane occurred in surface sediment under aerobic conditions at rates almost matching rates of methane production from the deeper anaerobic sediments (Tables 5 and 6), but the same sediments under anaerobic conditions produced methane. Except for the first few millimeters below the mat, the absence of oxygen (9) limits methane oxidation. However, the measured methane oxidation rates and the methane gradients in Table 2 suggest that most of the anaerobically generated methane does not leave the mat-sediment system but is re-oxidized.

Inhibition of methanogenesis by the addition of salt was most likely due to the effects of osmotic stress action on the wider anaerobic bacterial community. It is unlikely that salt directly inhibited methanogenesis, as the process is dependent on sodium (23). The effects of salinity on the anaerobic processes in sediments of ponds of varying salinity will be described in a future paper (18b).

Of all the factors, temperature, via the mechanisms already detailed, is likely to have been the major factor limiting anaerobic degradation in Orange Pond. During the summer the temperature of thawed Orange Pond sediments ranged between 7.1 and −1.5°C. Rates of degradation as determined by methanogenesis in this temperature range were substantially lower than those determined for the same sediments incubated at higher temperatures (Fig. 5). The pond temperatures were also below the growth temperature optima of many psychrotolerant and psychrophilic organisms (10, 22).

The partitioning of carbon and electron flow between methanogenesis and sulfate reduction in sediments of Orange Pond ensures that CO2 production is maximized via acetate oxidation for uptake by the cyanobacterial community for photosynthesis, in a process occurring at relatively low sulfate concentrations. Our results suggest, however, that any methane that is produced is likely to be oxidized to CO2 in an aerobic sediment zone immediately below the cyanobacterial mat. The net effect of the sediment processes is total conversion of CO2 to CH4 by a combination of anaerobic degradation and methane oxidation, the result of which is a tight coupling between photosynthetic and heterotrophic processes via CO2, the lack of which would limit primary production (9).

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