Ammonium Production in Sediments Inhibited with Molybdate: Implications for the Sources of Ammonium in Anoxic Marine Sediments†

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Ammonium production in the presence of specific inhibitors of sulfate reduction and methanogenesis was investigated in six marine sediments which differed in bulk properties and organic matter input. In all cases, little effect of the inhibitors on ammonium production was observed, although sulfate reduction was suppressed by molybdate. This gives evidence that the processes of fermentation and hydrolysis are of primary importance in ammonium generation at the sites studied. Although sulfate reduction rates may appear to be coupled to ammonium production rates, sulfate reduction does not necessarily contribute directly to generation of ammonium in marine environments.

Ammonium is generated by a number of processes during organic matter decomposition in anoxic marine sediments. These include hydrolytic deamination of amino acids and oligopeptides, degradation of nucleotides, and metabolism of methylamines by methanogens (10). The relative contribution of these different reactions to the generation of ammonium during decomposition of nitrogenous materials in anoxic sediments has not been assessed in previous studies. Sulfate reduction is often summarized in a geochemically formulated equation, where NH_4^+ production and SO_4^{2-} reduction are stoichiometrically related (1, 3, 4, 6, 7, 15, 17). However, this stoichiometric relationship does not necessarily have any direct mechanistic basis. While sulfate-respiring bacteria have been correlated with ammonium production (2, 7, 19), this correlation may be due to release of ammonium during fermentation and hydrolysis of complex organic compounds to simple compounds that are subsequently used by sulfate reducers. There is some evidence in the literature that sulfate reducers use amino acids as substrates (22, 23), but the amount of ammonium derived in sediment from these sources has not been assessed.

Work done in sewage and rumen digestors indicates that deamination of complex organic substrates occurs during fermentation and precedes decarboxylation (8, 14). It is sometimes assumed that deamination precedes decarboxylation of organic compounds in marine sediments (e.g., see reference 3), although this assumption has not been directly tested.

Methanogenesis may also contribute to ammonium generation in sediments. Trimethylamine (TMA) is a fermentative breakdown product of glycine betaine and can be decomposed to CO_2 and NH_4^+ by methanogens (9, 10). TMA is known to occur in salt marshes associated with *Spartina* stands (9, 16). However, it is not clear whether the magnitude of glycine betaine production and decomposition is

sufficient to have a measurable impact on total dissolved ammonium production rates in marsh sediments.

Specific inhibitors have been used to examine various aspects of organic matter decomposition. While the approach does have limitations since no inhibitor appears to be absolutely specific in effect, sodium molybdate and bromoethanesulfonic acid (BES) have been found to be effective inhibitors of sulfate reduction and methanogenesis, respectively (R. S. Oremland and D. G. Capone, Adv. Microb. Ecol., in press). In this study, we evaluated the relative importance of different pathways of ammonium generation during organic matter decomposition in six distinct marine sediments by using specific inhibitors.

MATERIALS AND METHODS

Study sites. The sites used in this study (Fig. 1) were chosen for their distinct biogeochemical characteristics. They included a temperate salt marsh (Flax Pond, N.Y.), an estuarine environment with organic matter-rich muds (Carmans River, N.Y.), a sandy sediment in Great South Bay (Roe Avenue, N.Y.), and three tropical carbonate sediment sites (two in Australia and one in the Bahama Islands).

The salinity of water in Flax Pond is ~26 to 28.5‰ and does not vary seasonally. Organic inputs to the sediments include marsh detritus (primarily *Spartina alterniflora*), terrigenous matter, and plankton debris. The specific area sampled for this study was a mudflat with loss-on-ignition and organic carbon values ranging from 8 to 10 and 2 to 3% by weight, respectively (unpublished data). The sediment was anoxic below the upper few millimeters.

A muddy site near the mouth of the Carmans River estuary was also studied. The total organic matter content of these sediments was ~13% by weight. The salinity of the water at the time of sampling was ~7‰.

Sandy sediments were collected at a site in Great South Bay at Roe Avenue. These sediments have a low organic matter content (~0.5%; 13). The site was in about 1 m of water; salinity ranged between ~22 and 26‰.

Carbonate sediments were collected at two sites on the Great Barrier Reef, Australia, and at a site in the Bahama Islands. Bowl Reef is located on the outer edge of the Great Barrier Reef. Samples were collected in the relatively calm
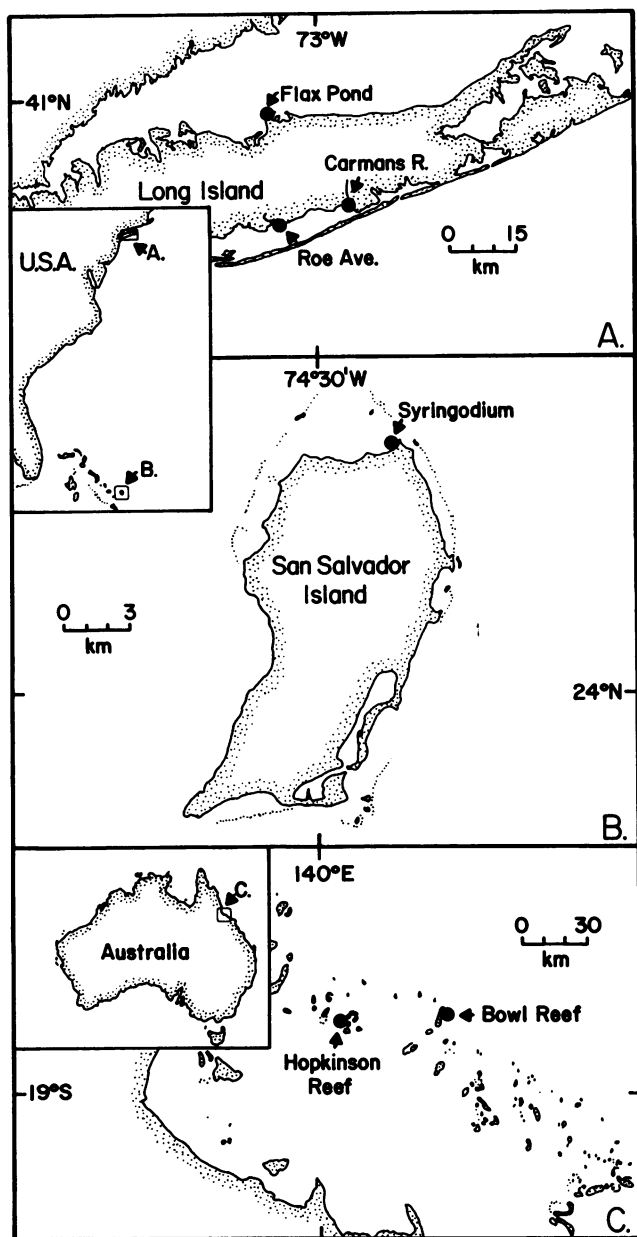

FIG. 1. Site locations for this study.

waters of the reef flat (10-m depth). Numerous *Callianassa* mounds dominated the sediments. The sediments at this site were fine-grained sands with an organic matter content of about 0.2%. The organic matter content of Hopkinson Reef sediments was similar to that of Bowl Reef sediments.

Sediments were collected from a stand of the tropical seagrass *Syringodium filiforme* at a site on the northern tip of San Salvador Island, The Bahamas. The sampling site was in a protected cove in Graham's Harbor, about 2 km north of the Center of the College of the Finger Lakes field station.

Sediment sampling and inhibitor additions. Sediments from the Flax Pond, Carmans River, and Roe Avenue sites were collected during the spring of 1985 with either hand-held acrylic box corers (165-cm^2 cross-sectional area) or 7.6-cm (outside diameter) butyrate core liners. The upper 6 cm of sediment was removed, thoroughly homogenized, and sep-

arated into different subsamples for subsequent treatment in a glove bag under a nitrogen atmosphere. Treatments consisted of either sodium molybdate (20 mM final concentration; Flax Pond, Roe Avenue, Carmans River incubations) or sodium molybdate plus BES (20 mM final concentration for both inhibitors; Flax Pond incubations only). Controls received only distilled water. The additions of distilled water and the inhibitors corresponded to 0.2 ml for every 10 ml of wet sediment. After being mixed, the sediment from each treatment was added to a separate 50-ml plastic centrifuge tube. One set of replicate tubes (two to four replicates) for each treatment was centrifuged at room temperature ($1,800 \times g$ for 15 min) immediately to separate pore waters and sediment solids for analyses. The remaining tubes were incubated at 25°C in the dark under nitrogen. After 0.5 to 20 days, replicate tubes corresponding to each treatment were removed, and pore water was extracted by centrifugation. All pore water samples were filtered through 0.22-μm-pore-size Millipore type SG filters.

For the depth profiles of ammonium production in carbonate sands, triplicate samples were collected in 60-cm^3 Plastipak syringes with plungers in place but from which the end had been removed to allow for coring. Cores were taken to a depth of at least 8 cm. The open end was capped in the field with a no. 6 black rubber stopper. Upon return to the laboratory, the plunger was carefully removed by breaking the seal with a needle. Approximately 10 ml of ambient seawater was gently added to provide an overlying water phase. For sediments receiving molybdate, 300 μl of a 2 M solution was injected through the center of the core by using a 500-μl Hamilton microsyringe. Also, 100 μl of the molybdate solution was injected into the overlying water. The plungers were then carefully replaced on all cores. Triplicate cores for both control and molybdate treatments were sampled at zero time and at various time intervals to establish the rate of ammonium production. At sampling times, the plunger was gently removed by breaking the seal with a needle. Overlying water was removed by syringe, and the samples were filtered through Whatman GF/C filters and refrigerated until analyzed for ammonium.

The plunger was then replaced and used to extrude and section the core from the bottom up, in 8- to 6-, 6- to 4-, 4- to 2-, and 2- to 0-cm segments. Each segment was placed in a filtration tower of a Hoffer 10-position vacuum filtration manifold, and the pore waters were expressed through GF/C filters into scintillation vials in the manifold. For the initial samples (e.g., zero time and 6 h), replicates for each horizon had to be combined to obtain sufficient volume (2 to 3 ml) for analysis. At subsequent time points, individual replicates were analyzed separately since they required dilution.

Iron spike experiments. Fermentation reactions may be inhibited by buildup of reaction end products, which include low-molecular-weight organic acids, alcohols, hydrogen, and hydrogen sulfide (11, 21). Ambient hydrogen sulfide can be found to be as high as 8 mM in Flax Pond during the summer and may be present at even higher levels in incubation experiments (K. T. Swider, J. E. Mackin, and M. E. Jacobson, manuscript in preparation). Hydrogen sulfide should build up in control tubes of the incubation experiments described previously but not in molybdate-amended tubes because of cessation of sulfide production in the tubes containing molybdate. Therefore, to determine the potential effects of hydrogen sulfide buildup on ammonium accumulation, experiments were performed in which Fe (III) was added to sediments to sequester the hydrogen sulfide produced.
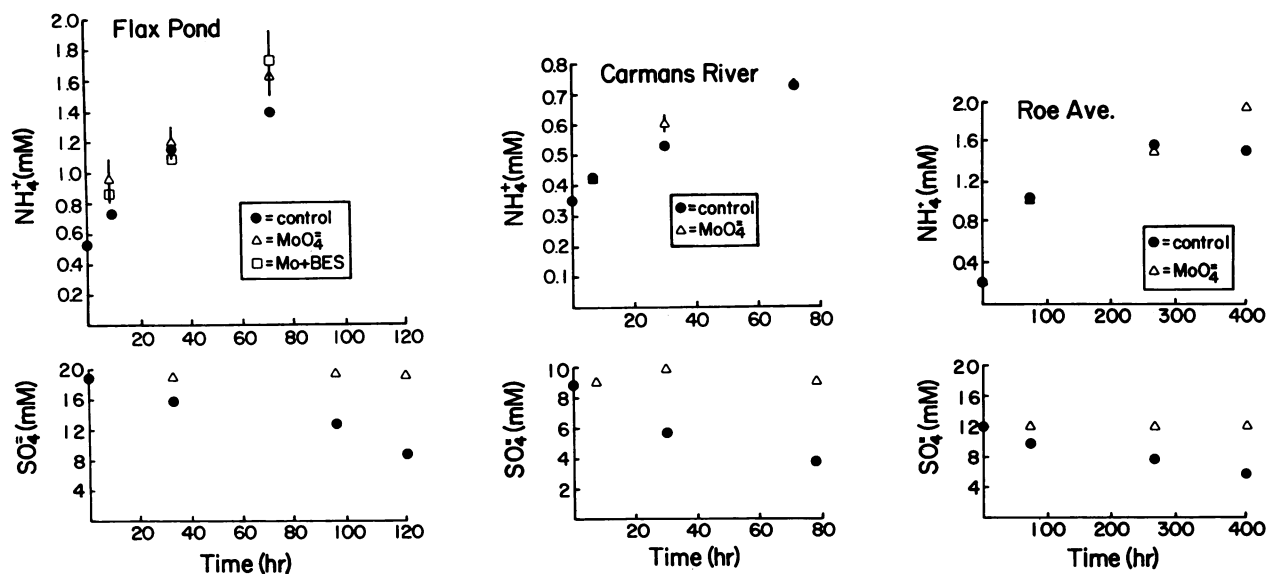
FIG. 2. Dissolved ammonium and sulfate concentrations as a function of time in incubations of sediment from the Flax Pond, Roe Avenue, and Carmans River sites.

The iron oxyhydroxide used in our experiments was prepared by the method of Rickard (18), which produces predominantly goethite. After the iron was prepared, it was ground and sieved through a 200-μm (pore size) sieve to ensure some uniformity in particle sizes. Sediments from Flax Pond (the 0- to 4-cm interval) were mixed under nitrogen before additions and divided, and the preweighed iron oxyhydroxide (400 mM iron, final concentration) was then mixed into a measured sediment sample. In these experiments, ammonium production, sulfate reduction, hydrogen sulfide production, and dissolved iron production were compared with those that occurred in subsamples of the same sediment but without iron oxyhydroxide.

Analytical methods: solute concentrations. Sediment pore waters were analyzed for dissolved ammonium by the phenol hypochlorite method (20), which was adapted for use on an autoanalyzer for the San Salvador samples (22). The precision of the method is ~2 to 3%. Sulfate was determined gravimetrically after addition of $BaCl_2$ to acidified samples. The precision of these analyses was ~0.25 mM. Hydrogen sulfide was determined in some of the samples by a colorimetric method (5) modified by Swider et al. (in preparation). The precision of the method is 5%. Dissolved iron was measured by use of the o-phenanthroline hydrochloride method (13).

RESULTS

Incubation experiments. Figure 2 shows dissolved sulfate and ammonium concentrations as a function of incubation time in sediments from Flax Pond, the Carmans River, and Roe Avenue. In all cases, although there was evidence of substantial sulfate consumption (i.e., reduction) in control samples (no molybdate or BES added), there was no obvious change in sulfate concentrations with time in samples amended with sodium molybdate. Ammonium production was similar in control and molybdate-amended (no BES) samples in most of the incubation experiments. Flax Pond sediments incubated with both BES and molybdate also showed no major differences in ammonium accumulation. At

the nonvegetated carbonate sediment sites (Bowl and Hopkinson Reefs), apparent ammonium production by the end of the experiment was somewhat higher in molybdate-inhibited cores (Fig. 3). No significant difference in ammonium production between treatments was seen at the *Syringodium* site.

Ammonium production and sulfate reduction rates were calculated from the slopes of best-fit lines of ammonium and sulfate versus time, corrected for adsorption as described below (Table 1). For sulfate, only control samples were used for these calculations since there was no significant sulfate reduction in molybdate-inhibited samples. The dissolved ammonium production rates determined at the terrigenous sediment sites were corrected for adsorption by using an assumed average equilibrium ion-exchange adsorption coefficient of $K = 1.3$ (12). In separate experiments, we measured $K = 1.1 \pm 0.2$ for Flax Pond sediments (unpublished data), which does not differ significantly from the assumed average value. For the carbonate sediments, $K = 0$ was assumed (12).

Figure 4 compares dissolved sulfate, dissolved iron, ammonium, and hydrogen sulfide concentrations during incubations of sediments with and without solid iron oxyhydroxides. Hydrogen sulfide accumulation was apparent in control incubations, but no hydrogen sulfide accumulated in the samples which contained the iron addition. Sulfate decreased in a similar fashion in both control and iron spike treatments, implying that iron addition did not substantially alter organic matter decomposition pathways. Dissolved iron began to appear between 20 and 30 days into the iron addition experiments. This phenomenon corresponded approximately to the time when sulfate concentrations approached zero. Despite large discrepancies in hydrogen sulfide buildup between the control and iron-spiked sediments, the ammonium generation patterns were similar.

DISCUSSION

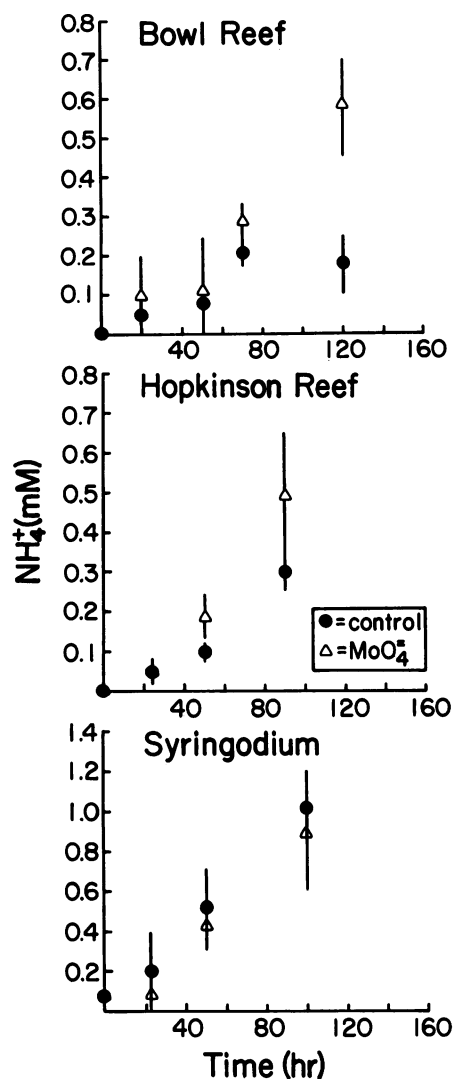**Ammonium production.** There was a wide variation in ammonium production and sulfate reduction between the

FIG. 3. Dissolved-ammonium concentrations as a function of time in incubations of sediments from Bowl Reef, Hopkinson Reef, and *Syringodium* beds from the Bahama Islands. The points represent average values from depths of 0 to 2, 2 to 4, 4 to 6, and 6 to 8 cm. Error bars indicate the standard error of the mean.

TABLE 1. Ammonium production and sulfate reduction rates[a]

Site	Ammonium production (μM/h)		Sulfate reduction (μM/h)	Ammonium/ sulfate ratio
	Control	Molybdate inhibition		
Flax Pond	63.75	65.69	73	0.89
Carmans River	29.90	19.55	59	0.42
Roe Avenue	8.34	6.65	14	0.54
Hopkins	3.56	6.12	ND[b]	ND
Bowl Reef	1.66	4.71	4	0.80
Syringodium beds	10.75	11.37	9	1.23

[a] Calculated from the line of best fit through appropriate points in Fig. 2 and 3. Sulfate reduction rates for Bowl Reef and *Syringodium* beds are from M. E. Hines (personal communication).

[b] ND, Not determined.

tion rates observed between the iron-spiked and control sediments can be accounted for by simple dilution of sedimentary organic matter by the addition of iron. Although it has been noted that exogenous additions of amorphous Fe(III) inhibit methanogenesis and sulfate reduction in some sediments that are dominated by these processes (D. R. Lovley and R. C. Aller, personal communication), this was not observed in Flax Pond. The uncertainty of the relative importance of inorganic and bacteriologically mediated iron reduction in this system makes interpretation of these results ambiguous.

The sum of all of the experiments indicates that molybdate does not significantly affect the microbial populations responsible for ammonium production in sediments of widely varying characteristics. The data strongly imply that the dominant organic matter deamination reactions in sediments occur during fermentation and hydrolysis.

It has been hypothesized that 35 to 61% of methane production can be attributed to TMA turnover in some intertidal sediments (10), with perhaps another 20% of TMA turnover due to sulfate reduction. The lack of a significant effect of BES on ammonium production in the salt marsh sediments of Flax Pond indicates that, for this particular site, TMA decomposition during methanogenesis does not con-

areas tested. Ammonium accumulation in incubations of sediment with molybdate was at least equal to that of controls at all sites, although sulfate reduction was eliminated in the molybdate-amended samples. Figure 5 illustrates the differences in ammonium production among the sites tested and the overall agreement between inhibited and noninhibited samples. The ratio of ammonium produced to sulfate reduced showed no consistent pattern (Table 1). Differences in the ratio of ammonium produced to sulfate reduced may indicate area-specific variations in overall C:N stoichiometry of utilizable organic matter present for reduction in the system.

Iron addition experiments done at Flax Pond indicate that, for the time frame of the molybdate inhibition experiments, hydrogen sulfide had no adverse effect on ammonium generation. This potential complication can therefore be ignored in interpretations of the inhibitor experiments. The 5% difference in the sulfate reduction and ammonium produc-
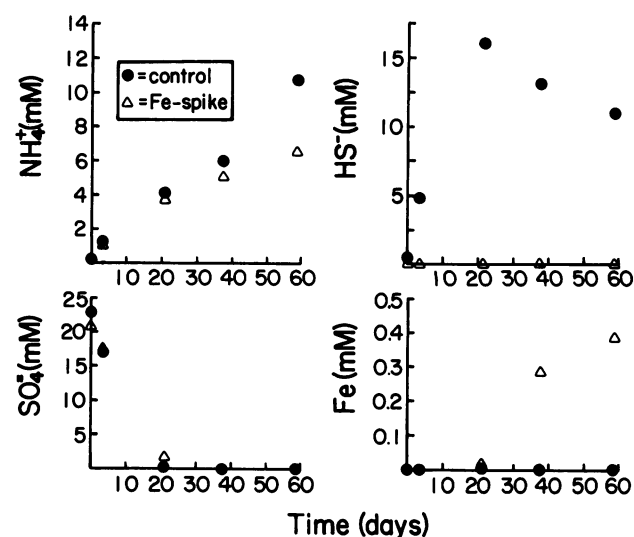

FIG. 4. Concentration of dissolved ammonium, hydrogen sulfide, sulfate, and dissolved iron as a function of time in incubations of sediments from Flax Pond.
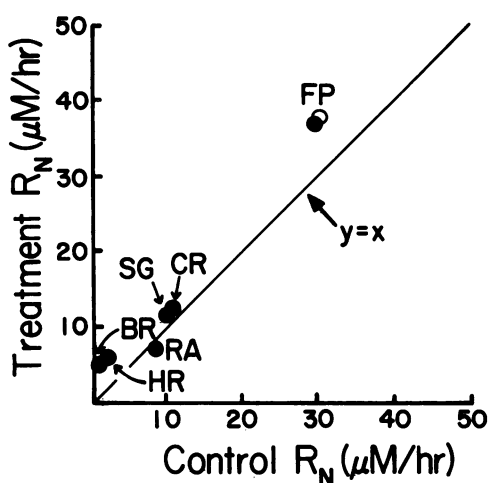
FIG. 5. A comparison of ammonium production from incubation experiments with and without inhibitors. All sites are included in this figure. Symbols: ●, comparison of molybdate treatment with control; ○, comparison of molybdate plus BES with control. Abbreviations: FP, Flax Pond; CR, Carmans River; RA, Roe Avenue; SG, *Syringodium* beds; BR, Bowl Reef; HR, Hopkinson Reef; R_N, rate of ammonium production.

tribute significantly to ammonium production. The predominant production of ammonium in Flax Pond seems to occur through fermentative and hydrolytic pathways. The data also indicate that this inhibitor, like molybdate, does not substantially alter fermentative pathways in these sediments. We conclude that ammonium generation is not directly linked to sulfate reduction or methanogenesis but is indirectly and closely coupled via fermentation and hydrolysis reactions. Deamination can result in release of long-chain acids which may be further fermented before they are used by sulfate-reducing bacteria.

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