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The effects of sediment slurrying on microbial processes, and the role of amino acids as substrates for sulfate reduction in anoxic marine sediments

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Abstract. In sediment slurry experiments with anoxic marine sediments collected in Cape Lookout Bight, NC, and a site in mid-Chesapeake Bay, the rates of sulfate reduction and ammonium production decrease with increasing dilution of sediment with oxygen-free seawater. The effect of sediment dilution on the rates of these processes can be described by a simple mathematical relationship, and when these rates are corrected for sediment dilution they yield values which agree well with direct measurements of these processes.

In sediment slurry studies of amino acid utilization in Cape Lookout Bight sediments, the fermentative decarboxylation of glutamic acid (to γ-aminobutyric acid) or aspartic acid (to alanine or β-alanine) did not occur when either of these amino acids were added to Cape Lookout Bight slurries. The addition of glutamic acid did however lead to a small ($\sim 1\%$) transient build-up of β-aminoglutaric acid. Measured rates of glutamic acid uptake in these slurries also decreased with increasing sediment dilution.

Molybdate inhibition experiments demonstrated that dissolved free amino acids represent 1-3% of the carbon sources/electron donors used for sulfate reduction in Cape Lookout Bight sediments. The direct oxidation of amino acids by sulfate reducing bacteria also accounts for 13-20% of the total ammonium produced. Glutamic acid, alanine, β-aminoglutaric acid, aspartic acid and asparagine are the major amino acids oxidized by sulfate reducing bacteria in Cape Lookout Bight sediments.

Introduction

Recent studies have shown that the remineralization of sedimentary (i.e., acid hydrolyzable) amino acids accounts for significant fractions of the total organic carbon and nitrogen that are regenerated in anoxic marine sediments (Henrichs & Farrington 1987; Burdige & Martens 1988). While these sedimentary amino acids are ultimately transformed to NH_3, CO_2 and/or CH_4 with either sulfate reduction or methanogenesis the "terminal" microbial processes, the exact mechanism(s) of their overall decomposition are poorly understood. Because sulfate reducers and methanogens are only able to utilize a limited number of substrates, the complete anoxic remineralization of sedimentary organic matter requires that fermentative organisms initially degrade sedimentary organic matter, eventually producing waste products which are then utilized by either sulfate reducers or methanogens (e.g., Laanbroek & Veldkamp 1982).

The anaerobic fermentation of amino acids can produce end products such as H_2 and volatile fatty acids (VFAs) like acetate (Gottshalk 1979; Barker 1981; Stams & Hansen 1984), which previous studies have shown are important substrates for sulfate reduction and methanogenesis in anoxic marine sediments (e.g., Banat et al. 1981; Laanbroek & Pfennig 1981; Sørensen et al. 1981; Christensen 1984; Sansone & Martens 1984; Sansone 1986). However, these studies of amino acid fermentation have generally been performed with pure bacterial cultures (i.e., single strains) and the relative proportions of fermentation end products often change with the growth conditions of the organisms (Gottshalk 1979). There have also been reports of the direct utilization of amino acids by sulfate reducing bacteria (Coleman 1960; Skyring et al. 1977; Smith & Klug 1981; Stams et al. 1985; Stams & Hansen 1986; Burdige 1988). Taken together, these observations suggests that only studies with natural, mixed microbial populations will yield accurate information regarding the *in situ* pathways of amino acid cycling in anoxic sediments.

One way to examine the transformations of amino acids in sediments involves the use of sediment "slurries" (experiments in which natural sediments are diluted with sterile O_2-free seawater). The use of sediment slurries has the advantage that good, reproducible time course experiments can be performed, since sediment heterogeneity is reduced. In previous studies of carbon and nitrogen dynamics in anoxic sediments, slurry techniques have been used to examine the metabolism of VFAs (Laanbroek & Pfennig 1981; Sørensen et al. 1981; Sansone 1986) and of methylated amines (Oremland & Polcin 1982; King 1984). Although slurrying decreases the measured rates of microbial processes (Jørgensen 1978; Alperin & Reeburgh 1985), studies to date suggest that the overall patterns (or pathways) of microbial remineralization are not affected by the slurrying procedure (Sørensen et al. 1981; Christensen 1984; Alperin & Reeburgh 1985).

In this paper, the technique of sediment slurrying will be further examined, with specific reference to its effects on biogeochemical processes in anoxic marine sediments. Results will also be presented from sediment slurry experiments in which molybdate is added as a specific inhibitor of bacterial sulfate reduction (Oremland & Taylor 1978; Smith & Klug 1981; Sørensen et al. 1981). The results of these experiments indicate that in the anoxic marine sediments of Cape Lookout Bight, NC, between 1–3% of the measured sulfate reduction occurs using dissolved free amino acids (DFAAs) as carbon substrates/electron donors.

Materials and methods

Study sites and sediment sample collection

Surface (0–5 cm) sediments from Cape Lookout Bight, NC (CLB) were collected by divers for use in these studies. Sediments were stored refrigerated in Mason jars (filled to the top) until used in these experiments. Cape Lookout Bight is a small ($\sim 2\,km^2$) coastal lagoon located behind a barrier island cuspate foreland on the Outer Banks of North Carolina, USA (see map in Chanton et al. 1983). The sediments of Cape Lookout Bight are fine grained silts and clays (Chanton et al. 1983), organic rich ($\sim$ 5% organic carbon in the surface sediments) and highly reducing just below the sediment-water interface. Organic matter remineralization in these sediments is dominated by sulfate reduction and methanogenesis (Martens & Klump 1984). The use of Cape Lookout Bight sediments in this study allows for a direct comparison of the results obtained here with previous studies of these sediments (e.g. Marens & Klump 1984; Sansone & Martens 1984; Crill & Martens 1987; Burdige & Martens 1988; Klump & Martens 1989).

Experiments were also carried out using anoxic sediments collected from a site in mid-Chesapeake Bay (CB). This site is located at 38° 56′ N, 76° 23′ W east and slightly south of Annapolis, MD. The water depth in this portion of the Bay is approx. 31 m and the bottom water salinities range from 16–20‰. Sediments in this portion of the bay are fine grained silts and clays (J. Halka, pers. comm.), and the organic carbon content of the surface sediments at this particular site is 3–4% (G. Cutter, pers. comm.). Sulfate reduction also appears to be the predominant process by which organic matter is remineralized in the upper portion of these Chesapeake Bay sediments. Complete depletion of pore water sulfate occurs in the upper 10–20 cm of sediments with the concomitant buildup of ΣCO_2 and inorganic nutrients such as ammonium and phosphate (data not shown; also see Matisoff et al. [1975] for similar data from nearby sites). These mid-Chesapeake Bay sediments have 100's of μM to mM pore water sulfide concentrations just below the sediment-water interface, with concentrations increasing with depth in the sediments (San Diego-McGlone and Cutter, 1988). The sediments also contain 1.5–7 mg S/gr_{sed} as acid volatile sulfides and 6–7 mg S/gr_{sed} as pyrite (Kluckhohn & Cutter, unpublished data).

Surface sediments from this mid-Chesapeake Bay site were collected using an acrylic soft sediment box corer (Fabrau, Ramona, CA) and again were stored refrigerated in Mason jars until used in experiments.

Sediment slurry experiments

Sediment slurries were prepared by diluting sediments with either Cape Lookout Bight surface seawater or mid-Chesapeake Bay bottom water (as appropriate) which had been filtered through a 0.22 μm membrane filter and degassed with N_2 (after this treatment the pH of the CLB seawater was 8.1 while that of the CB bottom water was 8.0). The sediment:seawater ratios of slurries (wt:wt) ranged from 1:2 to 1:4, depending on the specific experiments.

CLB slurries were incubated in 500 ml Ehrlenmeyer flasks sealed with specially designed two-hole butyl rubber stoppers and black electrical tape. Stir bars were also placed in each flask. The rubber stoppers contained two glass tubes, each of which had a 3-way plastic stopcock attached with epoxy cement to the end of the glass tube outside of the flask. Inside the flask, one of these tubes extended down into the sediment slurry while the other tube ended in the small headspace overlying the slurry. This sample vessel maintained anaerobic conditions during the course of these experiments, and allowed samples to be removed from the flask with no apparent oxygen contamination.

Once the sediment slurries were prepared and sealed in the flasks, they were degassed for 15 minutes by opening both stopcocks and bubbling nitrogen through the "longer" glass tube. This removed any oxygen which entered the flasks while the experiment was set up. After this degassing, both 3-way stopcocks were closed and the flasks were either placed on stirring plates inside of a temperature controlled incubator ($T = 19 \pm 1°C$; CLB sediment dilution experiments) or placed on a shaker table in the lab ($T = 22 \pm 2°C$; CLB molybdate inhibition experiments). The speed of the stirring plates or the shaker table were set to just keep the slurries mixed.

Over the course of these experiments, the flasks were sampled by attaching a plastic syringe to the 3-way stopcock on the "longer" glass tube, opening the stopcock and then removing a 5–10 ml aliquot of the slurry. The flask was then degassed as described above for 3 minutes. Sulfide was always detected by smell during this degassing, indicating that anaerobic conditions were maintained in the flasks during the course of these experiments.

Slurries of mid-Chesapeake Bay sediments were prepared as described above and incubated in 500 ml Ehrlenmeyer flasks sealed with black rubber stoppers that were placed inside of GasPak anaerobic jars (BBL). These jars were placed on stirring plates inside of a constant temperature incubator ($T = 25 \pm 2°C$). Anaerobic conditions were maintained in these experiments by opening and closing the jars and sampling the flasks inside of a Coy Anaerobic Chamber (Coy Laboratory Products, Ann Arbor, MI) containing N_2 and 0.5–1% H_2. In conjunction with Pd catalyst pellets placed inside the chamber this hydrogen acts to chemically scrub any oxygen which might leak or diffuse into the chamber. Oxygen and hydrogen concentrations inside of the chamber were monitored with a Coy Laboratory Products

Model 10 Gas Analyzer, while oxygen concentrations in the anaerobic jars were monitored with methylene blue indicator strips (BBL).

Slurry aliquots removed by either of these sampling procedures were centrifuged, and the resulting supernatant filtered through a 0.45 μm filter. A portion of this sample was acidified to pH < 2, and stored refrigerated. On these acidified sub-samples dissolved sulfate was measured as $BaSO_4$, gravimetrically in the CLB experiments (Crill & Martens 1987) and turbidimetrically in the CB experiments (Tabatabai 1974). Ammonium was determined colorimetrically using a modification of the procedure described by Solorzano (1969). Dissolved free amino acids were separated and quantified using an HPLC technique (Lindroth and Mopper 1979; Burdige & Martens 1988) on a second unacidified portion of selected samples that were stored frozen until analyzed.

Sediment dilution experiments

Sediments were diluted approx. 1:2, 1:3 and 1:4 (wt:wt) with surface seawater as described above. For the first 4 days (96 h) of the CLB experiment, the flasks were periodically sampled for dissolved sulfate and ammonium. At the end of this four day period, 5 ml of a 2 mM glutamic acid solution (prepared in filtered Cape Lookout Bight seawater) was added to three of the four flasks (flasks I, II, and III) giving initial glutamic acid concentrations of 20–50 μM. These additions were made through the 3-way valves and the flasks were then degassed as described above. Flask IV served as a control flask and received no glutamic acid addition. Over the next 24 hours all four flasks were sampled (as described above) for dissolved free amino acids.

CB sediments were also diluted approx. 1:2 to 1:4 (wt:wt) as described above. These experiments were sampled for dissolved sulfate and ammonium over seven days.

Molybdate inhibition experiments

CLB sediments were diluted approx. 1:4 (wt:wt) with surface seawater as described above. For each experiment, two parallel flasks were set up and sampled for dissolved sulfate, ammonium and amino acids over the first four days of the experiments. At the end of this period, 10 ml of a 1 M molybdate solution ($Na_2MoO_4 \cdot 2H_2O$, prepared in filtered Cape Lookout Bight seawater) was added to one of the flasks, giving an initial molybdate concentration of 20 mM. The second flask in each experiment served as a control flask and received no molybdate addition. Over the next 24 hours these flasks were sampled for dissolved free amino acids.

Results

Sediment dilution experiments

As can be seen in Figs. 1 and 2, in both sets of sediment slurry experiments sulfate decreases with time (indicative of bacterial sulfate reduction) while ammonium concentrations increase (as a result of the remineralization of organic nitrogen compounds). These changes are consistent with the known biogeochemical processes occuring in anoxic marine sediments such as these (Berner 1980; Martens & Klump 1984; Klump & Martens 1989). Linear least squares fitting of the data was used to determine the sulfate reduction

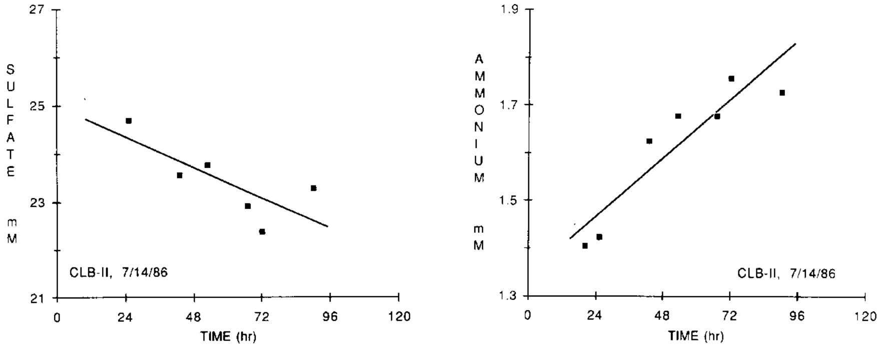

Fig. 1. Sulfate and ammonium concentrations versus time in Flask II from the Cape Lookout Bight sediment dilution experiment. The slopes of these lines are listed in Table 1.

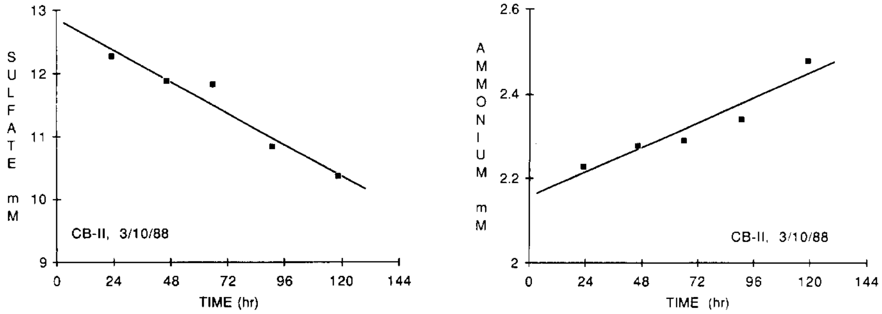

Fig. 2. Sulfate and ammonium concentrations versus time in Flask II from the mid-Chesapeake Bay sediment dilution experiment. The slopes of these lines are listed in Table 2.

Table 1. Results from the Cape Lookout Bight sediment dilution experiments.

Flask	I	II	III	IV
Sediment:seawater ratio (approx.)	1:2	1:4	1:3	1:3
DF (L/g_s)[a]	1.67×10^{-3}	3.58×10^{-3}	2.96×10^{-3}	2.71×10^{-3}
Sulfate reduction rate (SRR, mM/h)	-0.046 ± 0.016	-0.027 ± 0.011	-0.040 ± 0.009	-0.024 ± 0.009
dC/dt, Ammonium (mM/h)	0.0053 ± 0.0039	0.0051 ± 0.0010	0.0044 ± 0.0021	0.0069 ± 0.0018
K, Ammonium adsorption coefficient[b]	0.40	0.19	0.22	0.24
Ammonium production rate, (APR, mM/h)[c]	0.0075 ± 0.0054	0.0061 ± 0.0012	0.0054 ± 0.0026	0.0086 ± 0.0022
Glutamic acid uptake rate (GUR, μM/h)	-4.94 ± 0.29	-3.29 ± 0.85	-2.80 ± 0.36	
SRR/APR	-4.89	-4.38	-7.36	-2.77
Average SRR/APR[d]	-4.85 ± 1.90			
R_{srr} (Sulfate reduction)[e]	$-8.84 \pm 1.98 \times 10^{-5}$ mmol/h/g_s			
R_{apr} (Ammonium production)[e]	$1.83 \pm 0.44 \times 10^{-5}$ mmol/h/g_s			
R_{gur} (Glutamic acid uptake)[e]	$-9.45 \pm 1.66 \times 10^{-3}$ μmol/h/g_s			

[a] DF = Dilution Factor, calculated using eq. (3)

[b] Calculated using eq. (2)

[c] Calculated using eq. (1)

[d] Based on the four values listed above

[e] These R values are defined by eq. (4)

Table 2. Results from the mid-Chesapeake bay sediment dilution experiments.

Flask	I	II	III	IV
Sediment:seawater ratio (approx.)	1:4	1:2	1:1	1:2
DF (L/g_s)[a]	4.47×10^{-3}	2.56×10^{-3}	1.88×10^{-3}	2.77×10^{-3}
Sulfate reduction rate (SRR, mM/h)	-0.010 ± 0.004	-0.021 ± 0.003	-0.024 ± 0.002	-0.015 ± 0.003
dC/dt, Ammonium (mM/h)	0.0014 ± 0.0006	0.0024 ± 0.0004	-0.0028 ± 0.0008	0.0020 ± 0.0006
K, Ammonium adsorption coefficient[b]	0.10	0.15	0.20	0.14
Ammonium production rate (APR, mM/h)[c]	0.0016 ± 0.0007	0.0028 ± 0.0005	0.0033 ± 0.0010	0.0023 ± 0.0007
SRR/APR	−6.03	−7.40	−6.57	−7.03
Average SRR/APR[d]	-6.76 ± 0.59			
R_{srr} (Sulfate reduction)[e]	$-4.56 \pm 0.45 \times 10^{-5}$ mmol/h/g_s			
R_{apr} (Ammonium production)[e]	$0.67 \pm 0.04 \times 10^{-5}$ mmol/h/g_s			

[a] DF = Dilution Factor, calculated using eq. (3)

[b] Calculated using eq. (2)

[c] Calculated using eq. (1)

[d] Based on the four values listed above

[e] These R values are defined by eq. (4)

and ammonia production rates listed in Tables 1 and 2. The validity of using this approach is discussed in the next section. For ammonium the slopes of these lines (dC/dt) were corrected for ammonium adsorption to sediment particles to determine the true ammonium production rate (Rosenfeld 1979; Klump & Martens 1988),

$$\mathrm{APR} = (1 + \mathrm{K})(\mathrm{dC/dt}) \tag{1}$$

where APR is the ammonium production rate (mM/hr). K, the dimensionless ammonium adsorption coefficient, is a function of sediment porosity (ϕ; $\mathrm{cm^3_{pw}/cm^3_s}$) and the measured linear adsorption coefficient (K*), and is given by (Berner 1980):

$$\mathrm{K} = \rho_{\mathrm{ds}}[(1 - \phi)/\phi]\mathrm{K}^* \tag{2}$$

where ρ_{ds} is the dry sediment density. The measured value of K* for Cape Lookout Bight sediments is $2.30\,\mathrm{cm^3_{pw}/g_{ds}}$ (Klump & Martens 1989), while a K* value of 1.84 was used for the mid-Chesapeake Bay sediments (this value is based on data reported by Mackin & Aller 1984). For both sediments the value of ρ_{ds} was taken to be $2.5\,\mathrm{g/cm^3_{ds}}$. Because the adsorption of sulfate is negligible in these types of sediments (Berner 1980) a similar correction is not required for the sulfate reduction rates.[1]

Both sulfate reduction and ammonium production rates decrease with increasing dilution of the wet sediment with filtered seawater (Figs. 3 and 4; Tables 1 and 2). The "Dilution Factor" (DF; units of $\mathrm{L/g_s}$) for each flask is defined as the total volume of water in the slurry divided by the weight of wet sediment initially added to the slurry, and is given by:

$$\mathrm{DF} = [(\phi \mathrm{W_s}/\rho_s) + \mathrm{V_{sw}}]/[1000\,\mathrm{W_s}] \tag{3}$$

where $\mathrm{W_s}$ is the initial weight of wet sediment, $\mathrm{V_{sw}}$ is the initial volume of filtered seawater and ρ_s is the wet sediment density, calculated from the dry sediment density and the porosity of the wet sediment (ϕ). The measured porosity of the CLB sediment used in these experiments was 0.86, giving a wet sediment density of $1.21\,\mathrm{g/cm^3_s}$, while for the CB sediments the respective values were 0.91 and $1.13\,\mathrm{g/cm^3_s}$. In Figs. 3 and 4 rates of sulfate reduction and ammonium production are plotted against the inverse of DF; the reasons for this will be discussed in the next section.

Glutamic acid added to CLB sediment slurries was rapidly removed from solution (see Fig. 5 and Table 1), and the rates also decreased with increasing sediment dilution (see Fig. 3 and Table 1). In all three flasks the uptake of

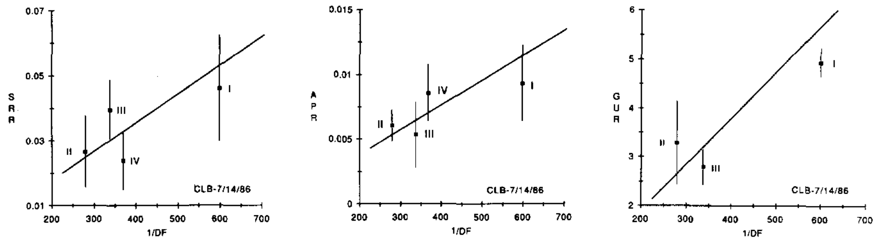

Fig. 3. Sulfate reduction rates (SRR; mM/h), ammonium production rates (APR; mM/h), glutamic acid uptake rates (GUR; μM/h): all versus 1/DF for the flasks from the Cape Lookout Bight sediment dilution experiment. DF (the Dilution Factor) is defined by eq. (3). The best fit straight lines through these data are given by eq. (5) and the slopes of these line (R_{srr}, R_{apr} and R_{gur}) are listed in Table 1.

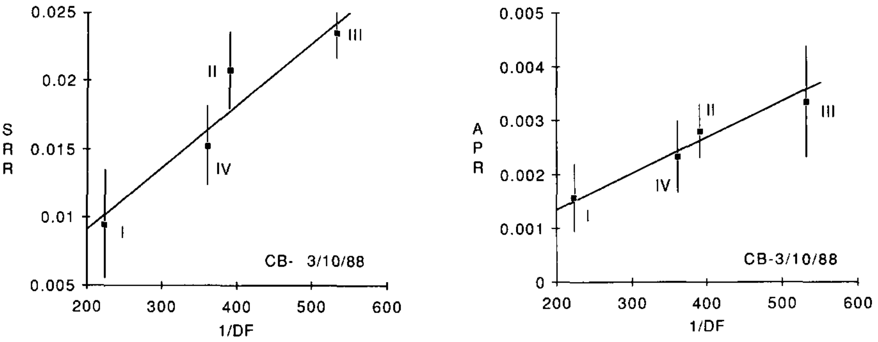

Fig. 4. Sulfate reduction rates (SRR; mM/h) and ammonium production rates (APR; mM/h) versus 1/DF for the flasks from the mid-Chesapeake Bay sediment dilution experiment. DF (the Dilution Factor) is defined by eq. (3). The best fit straight lines through these data are given by eq. (5) and the slopes of these line (R_{srr} and R_{apr}) are listed in Table 2.

glutamic acid was not accompanied by increases in the concentration of γ-aminobutyric acid (γ-aba). A similar experiment in which Cape Lookout Bight sediment slurries were amended with 20–30 μM aspartic acid resulted in the rapid removal of aspartic acid at rates similar to those listed in Table 1, with no production of either alanine or β-alanine (data not shown). These results indicate that the fermentative decarboxylation of either aspartic or glutamic acid (Barker 1979; Gottshalk 1981) did not occur in these experiments.

In Flask I however, there was a transient build-up of β-aminoglutaric acid (β-aga) following the addition of glutamic acid (see Fig. 5). β-aminoglutaric acid is an isomer of glutamic acid that has been found to be a major constituent of the dissolved free amino acids in the pore waters of many marine sediments (e.g., Henrichs & Farrington 1979, 1987; Henrichs et al.

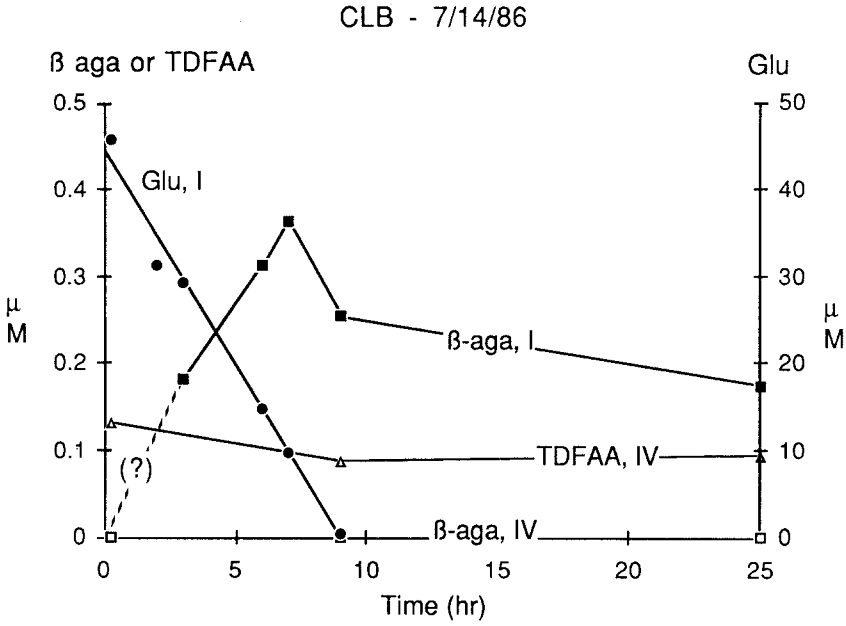

Fig. 5. Glutamic acid (Glu) in Flask I (filled circles); β-aminoglutaric acid (β-aga) in Flask I (filled squares) and Flask IV (open squares); total dissolved free amino acids (TDFAA) in Flask IV (open triangles), all versus time, in the CLB sediment dilution experiment. Flask I received an addition of glutamic acid at t = 0 while Flask IV was a control flask and received no addition of glutamic acid. The slope of the best fit straight line through the glutamic acid data from Flask I is listed in Table 1. Note that t = 0 h in this figure represents t = 96 h in Fig. 1.

1984; Burdige & Martens 1984 and unpublished data), although its source(s) to pore waters are not well understood. The maximum concentration of β-aga observed in Flask I (0.36 μM) occurred 9 hours after the addition of the glutamic acid (see Fig. 5) and accounted for less than 1% of the initial glutamic acid added to this flask. However this was significantly greater than the concentrations of either β-aminoglutaric acid or total dissolved free amino acids in the control flask IV (which received no glutamic acid additions), suggesting that this transient build-up of β-aga was indeed related to glutamic acid utilization. Samples from Flasks II and III were not analyzed for β-aga.

Molybdate inhibition experiments

The addition of 20 mM molybdate to CLB sediment slurries resulted in an increase in the concentration of total and individual dissolved free amino acids at rates significantly greater than those in the control flasks which had

Table 3. Results from the Cape Lookout Bight molybdate inhibition experiments.

	2/27/85 Expt.		1/4/85 Expt.	
Flask	I	II	I	II
Sulfate reduction rate (SRR, mM/h)	−0.026 ±0.005	−0.030 ± 0.004[a]	−0.015 ± 0.020	−0.017 ± 0.009[a]
dC/dt, Ammonium (mM/h)	−0.0033 ± 0.0011	0.0048 ± 0.0021[a]	n.d.[d]	n.d.[d]
K, Ammonium adsorption coefficient[b]	0.17	0.17	-	-
Ammonium production rate (APR, mM/h)[c]	0.0038 ± 0.013	0.0055 ± 0.0025[a]	-	-
TDFAA accumulation rate (μM, h) (in the absence of molybdate)	0.01 ± 0.02	-	0.01 ± 0.01	-
TDFAA accumulation rate (μM/h) (after the addition of molybdate, MAA)	-	0.73 ± 0.05 (t < 7h) 0.79 ± 0.06 (t > 7h)	-	0.13 ± 0.02
MAA/SRR[a]		2.4 – 2.8% (t < 7h)		0.8 – 0.9%
MAA/APR[e]		13.3 – 19.2%		

[a] These rates were determined prior to the addition of molybdate to these flasks (i.e., −96 < t 0h in Figs. 6 and 7).

[b] Calculated using eq. (2)

[c] Calculated using eq. (1).

[d] n.d. = not determined

[e] Assuming a 1:1 stoichiometry between sulfate reduced (or ammonium produced) and amino acids oxidized. The reported ranges are based on normalizing the MAA values from Flask II of each experiment to the measured rates of sulfate reduction or ammonium production from both flasks.

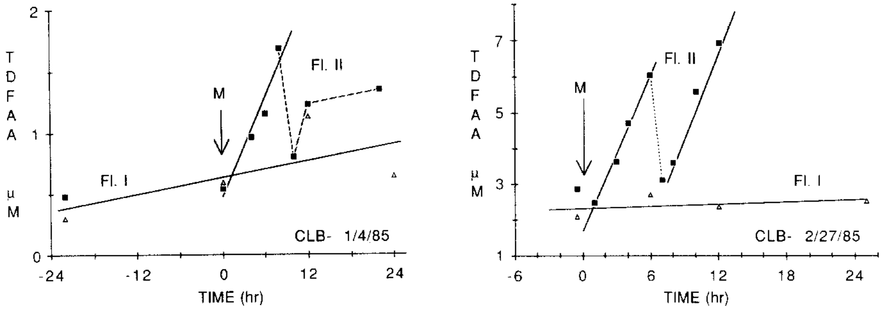

Fig. 6. Total dissolved free amino acids (TDFAA) versus time in the flasks of the 1/4/85 and 2/27/85 CLB experiments. Samples from Flask I are indicated by open triangles while those of Flask II are indicated by filled squares. In both experiments molybdate was added to Flask II at $t = 0$ (indicated by the arrow) while Flask I received no molybdate additions. The slopes of the best fit lines through the data are listed in Table 3 (for Flask II of the 1/4/85 experiment only the data for $0 < t < 7$ h were fit). Accumulation rates of individual amino acids in both Flask IIs after the addition of molybdate are listed in Table 4. Note the differences in the scales of the concentration axes of the two figures.

no molybdate additions (see Fig. 6 and Table 3). This increase was dominated by increases in the concentrations of aspartic acid, glutamic acid, β-aminoglutaric acid, asparagine, and alanine (Table 4). In both experiments the concentrations of dissolved amino acids increased for approximately 6–8 hours, followed by a rapid decrease in concentration and a second concentration increase at rates comparable to the initial rate of increase. This second increase is more clearly defined in the 2/27/85 experiment (due to the frequency of sampling), and quantitative discussions of this phenomena will be limited to the results of this experiment.

Discussion

The effect of sediment slurrying on biogeochemical processes in anoxic marine sediments

Studies to date have indicated that the overall patterns (or pathways) of the microbial remineralization of organic matter do not appear to be affected by the sediment slurrying procedure (Sørrensen et al. 1981; Christensen 1984; Alperin & Reeburgh 1985). The results from the sediment dilution experiments described here can be used to further examine this topic.

At sulfate concentrations greater than about 3–4 mM, the rate of bacterial sulfate reduction has been shown to be independent of sulfate concentration (Boudreau & Westrich 1984), and processes such a sulfate reduction or

Table 4. Rates of individual and total dissolved free amino acids accumulation in the Cape Lookout Bight molybdate inhibition experiments.*

	2/27/85 Expt.				1/4/85 Expt.	
	Rate (a) (t < 7 h)	% of TDFAA rate	Rate (a) (t > 7 h)	% of TDFAA rate	Rate (a) (t < 8 h)	% of TDFAA rate
Cys	0.01	1.4%	0.01	1.3%	(b)	
Asp	0.05 ± 0.01	6.8%	0.01	1.3%	0.01	7.7%
Glu	0.15 ± 0.01	20.5%	0.13 ± 0.01	16.5%	0.03	23.1%
β-aga	0.05 ± 0.01	6.8%	0.08 ± 0.01	10.3%	0.02	15.4%
Asn	0.05 ± 0.02	6.8%	0.04 ± 0.01	5.1%	0.01	7.7%
Ser	0.03 ± 0.01	4.1%	(b)		0.00	
Gln	0.03 ± 0.01	4.1%	0.03 ± 0.01	3.8%	(b)	
His	0.03	3.1%	0.03	3.8%	(b)	
Gly	0.03 ± 0.01	4.1%	0.04 ± 0.01	5.1%	(b)	
Thr	0.02	2.7%	0.02 ± 0.01	2.5%	(b)	
Arg	0.01	1.4%	0.01	1.3%	(b)	
β-ala	0.02 ± 0.01	2.7%	0.03 ± 0.01	3.8%	(b)	
Taur	(b)		0.01	1.3%	0.00	
Ala	0.17 ± 0.02	23.4%	0.23 ± 0.02	29.3%	0.05 ± 0.02	38.4%
γ-aba	0.01 (d)	1.4%	0.03 (d)	3.8%	(b)	
Tyr	0.01 ± 0.01	1.4%	(b)		(b)	
δ-amv	(b)		0.02	2.5%	0.00	
α-aba	0.01	1.4%	0.01	1.3%	(b)	
Met	0.00		0.00		(b)	
Try	0.00		0.00		0.00	
Val	0.03	3.5%	0.02	2.5%	(b)	
Phe	(c)		0.01	1.3%	0.00	
Iso	0.02	3.1%	0.01	1.3%	(c)	
Leu	0.01	1.4%	0.01	1.3%	0.00	
TDFAA	0.73 ± 0.05		0.79 ± 0.06		0.13 ± 0.02	

* All amino acid abbreviations are standard except: Cys = Cysteic Acid; β-aga = β-aminoglutaric acid; β-ala = β-alanine; γ-aba = γ-aminobutyric acid; δ-amv = δ-aminovaleric acid; α-aba = α-aminobutyric acid; TDFAA = total dissolved free amino acids.

(a) μM/h; for rates that do not have errors listed, the errors are < 0.01 μM/h. (b) < 0.01 μM/h (c) The rate actually was less than zero. (d) These rates are for t < and t > 8 h.

ammonium production have generally been modelled as being first order with respect to the amount of metabolizable organic carbon (or nitrogen) present in the sediments (e.g. Berner 1980). If the amount of metabolizable organic carbon and nitrogen remains roughly constant over the time course of an experiment such those described here, then one would predict zero order kinetics (i.e. linear concentration changes with time) for processes such as sulfate reduction and ammonium production. In previous whole sediment incubation and sediment slurry experiments, these processes (as well as methanogenesis, phosphate and ΣCO_2 production) have been shown to be linear with time, on time scales similar to those of the experiments described here (i.e., several hours to several days; see Aller & Yingst 1980; Sørensen et al. 1981; Crill & Martens 1983; King 1984; McNichol 1986; Klump & Martens 1989; and other references cited therein). For these reasons the data from these experiments were analyzed using linear least squares fitting. As can be seen in Figs. 1 and 2, this appears to be a valid fitting procedure for this data.

Based on the previous discussion, in the experiments described here slurrying of the sediments should affect sulfate reduction and ammonium production only by decreasing their rates as a result of the dilution of the wet sediment (and the organic matter therein) by seawater. Mathematically, the measured rates of these processes and the dilution factors in each of the flasks can then be related by the following expression:

$$(\text{SRR or ARP}) \times \text{DF} = R_{srr} \text{ or } R_{apr} \tag{4}$$

or

$$(\text{SRR or APR}) = (R_{srr} \text{ or } R_{apr}) \times (1/\text{DF}) \tag{5}$$

where R_{srr} and R_{apr} are the rates in undiluted sediments (having units of mmole/hr/g_s). The results of least squares fitting of the data from these experiment to eq. (5) are listed in Tables 1 and 2, and illustrated in Figs. 3 and 4.

In undiluted sediments DF (as defined by eqn. (3)) equals $\phi/1000\rho_s$ (7.1×10^{-4} L/g_s in CLB sediments and 8.0×10^{-4} L/g_s in CB sediments). DF now acts to convert the units of R_{srr} and R_{apr} from mmol/g_s/h to mM/h. As can be seen in Table 5, there is good agreement between the rates calculated using the results of these experiments (using eq. (4) and the values of R_{srr} and R_{apr} in Tables 1 and 2) and measured rates of sulfate reduction and ammonium production in undisturbed CLB and CB sediments. This suggests that slurrying of sediments affects processes such as sulfate reduction and ammonium production only by decreasing their measured rate as

Table 5. Rates of sulfate reduction and ammonium production in Cape Lookout Bight and mid-Chesapeake Bay sediments.

	Sulfate reduction*	Ammonium production*	SRR/APR**
Cape Lookout Bight sediments			
Rates in undiluted sediments based on the results of the sediment dilution experiments[a]	2.98 ± 0.67	0.62 ± 0.15	4.85 ± 1.90
Direct measurements of rates in undiluted sediments[b]	0.21 – 3.14	0.15 – 0.55	3.39 ± 1.22
Mid-Cheaspapeake Bay sediments			
Rates in undiluted sediments based on the results of the sediment dilution experiments[a]	1.36 ± 0.13	0.20 ± 0.01	6.76 ± 0.59
Direct measurements of rates in undiluted sediments[c]	1 to 2	–	–

* mM/day

**SRR = sulfate reduction rate; APR = ammonium production rate (corrected for ammonium adsorption).

[a] R_{srr} and R_{apr} converted from units of mmol/h/g_s to mM/day using eq. (4) (see text).

[b] These are the range of summer rates in CLB sediments from Crill & Martens (1987) and Klump & Martens (1989). Comparisons are made to summer rates since the sediments used in this experiment were collected in July and incubated at temperatures (19 ± 1° C) comparable to summer temperatures in Cape Lookout Bight (Martens & Klump 1984).

[c] These sulfate reduction rates are from Roden & Tuttle (1987) for the upper 1 to 2 cm of reducing sediments in the mesohaline region of Chesapeake Bay. To the best of my knowledge there are no reported direct measurements of ammonium production rates in the sediments of this portion of the Chesapeake Bay.

a result of sediment dilution, and that this "dilution effect" can be described in a very straight-forward manner (i.e., with eq. (4)). Further evidence of this is the observation that the ratio SRR/APR in the CLB experiments (4.85 ± 1.90) is similar to that determined by Klump and Martens (1989) in whole sediment incubations of Cape Lookout Bight sediments (3.39 ± 1.22).

These results differs from those of Jørgensen (1978), who found that measured rates of sulfate reduction could not be corrected for sediment dilution in the manner described above. In his study, corrected rates of sulfate reduction (per unit volume of undiluted sediment) decreased with increasing sediment dilution, and he attributed this to the effects of the handling of sediments during the mixing or slurrying procedure. However his results were obtained with 8 hour incubations while the experiments presented here were longer in duration (96–168 hours; see Figs. 1 and 2). This suggests that if there is a "handling" artifact in these types of experiments, this effect (at least for these two anoxic sediments) may be a short lived phenomenon.

The uptake of glutamic acid by sediment slurries

In addition to the biological utilization of amino acids as carbon substrates by anaerobic microorganisms, amino acids may also be removed from solution by abiotic processes such as adsorption onto organic or inorganic constituents of the solid phase of the sediments (e.g., Doyle & Henrichs 1986; Hedges & Hare 1987; Henrichs & Farrington 1987; Burdige 1988 and unpublished data). Although it is not possible to quantitatively estimate the importance of abiotic versus biotic processes in the glutamic acid uptake observed in these experiments (see Figs. 4 and 5), the results of these previous studies would suggest that the majority of the glutamic acid uptake observed in the CLB experiments is most likely due to the microbial remineralization of the glutamic acid.

The measured rates of glutamic acid uptake in these experiments also follow eq. (4) (see Fig. 5), suggesting that the process(es) responsible for glutamic acid uptake are also affected by slurrying only through sediment dilution. It is not possible to compare R_{gur} to other measured quantities in Cape Lookout Bight sediments since the various processes responsible for glutamic acid uptake in CLB sediments have not been as carefully quantified as have sulfate reduction and ammonium production.

The results of this study suggest that the occurrence of β-aminoglutaric acid in anoxic marine pore waters may in part involve a reaction in which glutamic acid is the precursor (see Fig. 4), as originally suggested by Henrichs & Farrington (1979). The lack of production of γ-aminobutyric acid from glutamic acid (and of β-alanine or alanine from aspartic acid) indicates that the fermentative decarboxylation of these acidic amino acids is not an important process in the anoxic sediments of Cape Lookout Bight. This finding is consistent with the low levels of γ-aba and β-ala found in most anoxic marine sediments (e.g., Henrich et al. 1984; Henrichs & Farrington 1987) including Cape Lookout Bight (Burdige & Martens 1988 and unpublished data). In comparison, abyssal (oxic) marine sediments contain much higher levels (up to 50 mole % or greater) of γ-aba and β-ala in their sedimentary amino acids (e.g., Degens et al. 1964; Whelan 1977).

Processes affecting dissolved free amino acids in molybdate inhibited sediment slurries

As will be discussed in the next section, the initial increase in DFAAs is taken to be the rate at which these compounds were utilized as electron donors in sulfate reduction prior to the addition of molybdate (Sørensen et al. 1981). The rapid decrease and subsequent second increase in dissolved free amino acids in these experiments (see Fig. 6) may be related to the microbial populations adjusting to a situation in which sulfate reduction is no longer the terminal process in organic matter remineralization and therefore is presumed to be an artifact of the experimental manipulations (i.e., molybdate addition).

A careful examination of the data from the 2/27/85 experiment (see Table 4) strongly suggests that these two increases are indeed related to different phenomena, in spite of the fact that both have similar rates (0.73 ± 0.05 and 0.79 ± 0.06 μM/hr). For certain amino aicds (aspartic acid, serine, isoleucine) the second rate of increase ($t > 7$ hr) is less than the first rate of increase ($t < 7$ hr) while for other amino acids (β-aga, alanine, γ-aba, phenylalanine, δ-aminovaleric acid) the opposite is true. For both phenylalanine and δ-aminovaleric acid there is actually no increase in their concentrations until this second concentration increase occurs.

The role of amino acids as substrates for bacterial sulfate reduction

As an analog to sulfate, molybdate (at the 20 mM concentration level) has been shown to be an effective inhibitor of bacterial sulfate reduction (e.g., Oremland & Taylor 1978; Smith & Klug 1981; Sørensen et al. 1981; Christensen 1984). When molybdate is added to a sediment (or sediment slurry) the short term (i.e., several hour) accumulation of organic compounds (or H_2) has been taken to represent the rate at which these compounds were oxidized by sulfate reducing bacteria prior to the molybdate inhibition (Sørensen et al. 1981). Based on this assumption, amino acids account for about 1–3% of carbon sources/electron donors for sulfate reduction in Cape Lookout Bight sediments (Table 4). Utilization of amino acids by sulfate reducing bacteria also accounts for 13–20% of the observed ammonium production in these experiments (see Table 4). These calculations assume a 1:1 stoichiometry between moles of amino acids oxidized and moles of sulfate reduced (or ammonium produced). However, the incomplete oxidation of certain amino acids to acetate during sulfate reduction (Stams et al. 1984) will lead to stoichiometries less than 1:1, while the complete oxidation of 1 mole of glutamic acid (or β-amioglutaric acid) to CO_2 by sulfate reducing bacteria will reduce 2.25 moles of SO_4^{2-}. With the present data I cannot determine whether these amino acids are completely oxidized to CO_2 by sulfate reduction, or only partially oxidized (to perhaps acetate). In addition, these calculations most likely underestimate the role of amino acids in these processes, due to adsorption of amino acids to sediment particles (Rosenfeld 1979; Doyle & Henrichs 1986; Hedges & Hare 1987;

Henrichs & Farrington 1987). When molybdate is added, a certain portion of the amino acids that would be utilized by sulfate reducers may also adsorb to sediment particles as well as accumulate in solution.

While the direct utilization of dissolved free amino acids by sulfate reducing bacteria in these sediments is only a small fraction of the total sulfate reduction and ammonium production (Table 4), previous studies (Burdige & Martens 1988) have shown that the remineralization of sedimentary amino acids in Cape Lookout Bight sediments accounts for ~25% of the organic carbon regeneration and 80–90% of the total nitrogen regeneration. These observations are not inconsistent, but imply that most sedimentary amino acids in CLB sediments are fermented (and deaminated) rather than directly utilized by sulfate reducing bacteria. The products of these processes may then be VFAs such as acetate, and H_2 (Gottshalk 1979; Barker 1981; Stams & Hansen 1984), since previous studies have shown that these are the most important substrates for sulfate reduction in anoxic marine and estuarine sediments such as Cape Lookout Bight (Banat et al. 1981; Laanbroek & Pfennig 1981; Sørensen et al. 1981; Christensen 1984; Sansone & Martens 1984; Sansone 1986).

The results of these studies indicate that several amino acids are utilized by sulfate reducing bacteria in Cape Lookout Bight sediments (see Fig. 6 and Table 4). The individual amino acids that account for the majority of this utilization (glutamic acid, alanine, β-aminoglutaric acid, aspartic acid and asparagine) are also the major dissolved free and sedimentary (i.e., acid hydrolyzable) amino acids in Cape Lookout Bight and other anoxic, marine sediments (e.g., Henrichs & Farrington 1979, 1987; Burdige & Martens 1984, 1988). Sediment slurry experiments using ^{14}C labelled glutamic acid and alanine also indicate that these two amino acids are utilized by sulfate reducing bacteria in both thse mid-Chesapeake Bay sediments and sediments from Bordenstake Bay, an anoxic tidal flat on Virginia's Eastern Shore (Burdige 1988 and unpublished data).

The observation that these particular amino acids constitute the predominant amino acid oxidized by sulfate reducing bacteria in CLB sediments is interesting from several additional standpoints. Previous reports of the utilization of individual amino acids by pure cultures of sulfate reducing bacteria have indicated that growth was obtained with only certain amino acids, such as alanine, serine and glycine and aspartic acid (Coleman 1960; Stams et al. 1985; Stams & Hansen 1986). Stams et al. (1985) also noted that asparagine and glutamic acid supported little growth by the two marine strains of *Desulfovibrio* they examined. Similarly, Stams & Hansen (1984) reported that they were unable to isolate glutamic acid utilizing sulfate reducing bacteria from anoxic estuarine sediments. As can be seen in Table 4, glutamic acid and asparagine constituted significant fractions (~20% and ~7%, respectively) of the total amino acid oxidation by sulfate reduction in Cape Lookout Bight sediments.

The utilization of β-aminoglutaric acid by sulfate reducing bacteria in Cape Lookout Bight sediments is also intriguing given the fact that in most anoxic marine sediments this amino acid becomes the dominant dissolved free amino acid with depth (Henrichs & Farrington 1979, 1987; Burdige & Martens 1984). This behavior has been ascribed to β-aga being more refractory than other amino acids (Henrichs & Farrington 1979), although the results of these experiments would suggest otherwise. The accumulation of β-aminoglutaric acid with depth in certain anoxic sediments could be due to the fact that it cannot be metabolized by methanogenic bacteria. In sediments such as those of Cape Lookout Bight complete sulfate reduction occurs in the upper sediments (10–30 cm) and a zone of methanogenesis exists below the zone of sulfate reduction (Martens & Klump 1984). However, other explanations must account for the accumulation of β-aga in anoxic, yet non-methanogenic marine sediments (e.g., Peru upwelling region sediments (Henrichs et al. 1984); Buzzards Bay (Henrichs & Farrington 1987)).

Conclusions

In long term (several day) sediment slurry experiments with anoxic marine sediments collected in Cape Lookout Bight, NC, and at a site in mid-Chesapeake Bay, the rates of sulfate reduction and ammonium production decrease with increasing amounts of dilution of wet sediment with oxygen-free seawater. A simple relationship can be used to describe the effects of sediment slurrying on the rates of these processes. Using this equation to "correct" slurry rates for sediment dilution yields sulfate reduction and ammonium production rates which agree well with direct measurements of these processes.

The addition of glutamic acid to Cape Lookout Bight sediment slurries led to a rapid uptake of this amino acid with no production of γ-aminobutyric acid (which can be produced by the fermentative decarboxylation of glutamic acid). However there was a small (~1%) transient build-up of β-aminoglutaric acid following the addition of glutamic acid. The measured rates of glutamic acid uptake also decreased with increasing sediment dilution. In a similar experiment aspartic acid was also rapidly taken up by sediment slurries with no production of either alanine or β-alanine, indicating that the fermentative decarboxylation of aspartic acid also did not occur in these sediments.

Molybdate inhibition experiments demonstrated that dissolved free amino acids represented between 1–3% of the carbon soruces/electron donors for sulfate reduction in these sediments. This direct oxidation of amino acids by sulfate reducing bacteria also accounted for 13–20% of the total ammonium produced. Glutamic acid, alanine, β-aminoglutaric acid, aspartic acid and asparagine were the major amino acids oxidized by sulfate reducing bacteria in Cape Lookout Bight sediments. These results however, do not indicate whether these amino acids were completely oxidized to CO_2 by sulfate reducers or only partially oxidized (to perhaps acetate).

Taken together, the results of these experiment suggest the usefulness of future, more sophisticated slurry experiments (using radiolabelled amino acids) in delineating the pathways by which amino acids are utilized in anoxic sediments.

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Notes

1. Throughout this paper subscripts on units of length, area, volume and weight signify the following: pw = pore water, s = bulk or whole wet sediment; ds = dry sediment.

References

Aller RC & Yingst JC (1980) Relationships between microbial distributions and the anaerobic decomposition of organic matter in surface sediments of Long Island Sound, USA. Mar. Biol. 56: 29–42

Alperin MJ & Reebuirgh WS (1985) Inhibition experiments on anaerobic methane oxidation. Appl. Environ. Microbiol. 50: 940–945

Banat IM, Lindstrom EB, Nedwell DB & Balba MT (1981) Evidence for the existence of two distinct functional groups of sulfate-reducing bacteria in salt marsh sediments. Appl. Environ. Microbiol. 42: 985–992

Barker HA (1981) Amino acid degradation by anaerobic bacteria. Ann. Rev. Biochem. 50: 23–40

Berner RA (1980) Early Diagenesis, A theoretical approach. Princeton University Press: Princeton, N.J.

Boudreau BP & Westrich JT (1984) The dependence of bacterial sulfate reduction on sulfate concentrations in marine sediments. Geochim. Cosmochim. Acta 48: 2503–2516

Burdige DJ (1988) The microbial utilization of alanine and glutamic acid in anoxic marine sediments. EOS 69: 1122

Burdige DJ & Martens CS (1984) Amino acid cycling in an organic-rich sediment. EOS 65: 960

Burdige DJ & Martens CS (1988) Biogeochemical cycling in an organic-rich marine basin – 10. The role of amino acids in sedimentary carbon and nitrogen cycling. Geochim. Cosmochim. Acta 52: 1571–1584

Chanton JP, Martens CS & Kipphut GW (1983) Lead-210 sediment geochronology in a changing coastal environment. Geochim. Cosmochim. Acta 47: 1791–1804

Christensen D (1984) Determination of substrates oxidized by sulfate reduction in intact cores of marine sediments. Limnol. Oceanogr. 29: 189–192

Coleman GS (1960) A sulfate reducing bacterium from the sheep rumen. J. Gen. Microbiol. 22: 423–436

Crill PM & Martens CS (1983) Spatial and temporal fluctuations of methane production in anoxic coastal marine sediments. Limnol. Oceanogr. 28: 1117–1130

Crill PM & Martens CS (1987) Biogeochemical cycling in an organic-rich marine basin – 6. Temporal and spatial variations in sulfate reduction rates. Geochim. Cosmochim. Acta. 51: 1175–1186

Degens ET, Reuter JH & Shaw KNF (1964) Biochemical compounds in offshore California sediments and sea waters. Geochim. Cosmochim. Acta 28: 45–66

Doyle AP & Henrichs SM (1986) Decomposition of amino acids in marine sediments. EOS 67: 1043

Gottschalk G (1979) Microbial Metabolism. Springer-Verlag

Hedges JI & Hare PE (1987) Amino acid adsorption by clay minerals in distilled water. Geochim. Cosmochim. Acta 51: 255–259

Henrichs SM & Farrington JW (1979) Amino acids in interstitial waters of marine sediments. Nature 279: 319–322

Henrichs SM & Farrington JW (1987) Early diagenesis of amino acids and organic matter in two coastal marine sediments. Geochim. Cosmochim. Acta 51: 1–15

Henrichs SM, Farrington JW & Lee C (1984) Peru upwelling region sediments near 15° S. 2. Dissolved free and total hydrolyzable amino acids. Limnol. Oceanogr. 29: 20–34

Jørgensen BB (1978) A comparison of methods for the quantification of bacterial sulfate reduction in coastal marine sediments. 1. Measurements with radiotracer techniques. Geomicrobiol. J. 1: 11–27

King GM (1984) Metabolism of trimethylamine, choline, and glycine betaine by sulfate-reducing and methanogenic bacteria in marine sediments. Appl. Environ. Microbiol. 48: 719–725

Klump JV & Martens CS (1989) The seasonality of nutrient regeneration in an organic rich coastal sediment: Kinetic modelling of changing pore water nutrient and sulfate distributions. Limnol Oceanogr. In press

Laanbroek HJ & Veldkamp H (1982) Microbial interactions in sediment communities. Phil. Trans. Royal Soc. London B 297: 533–550

Lindroth P & Mopper K (1979) HPLC determination of subpicomole amounts of amino acids by precolumn fluorescence derivitization with o-phthaldialdehyde. Anal. Chem. 51: 1667–1674

Mackin JE & Aller RC (1984) Ammonium adsorption in marine sediments. Limnol. Oceanogr. 29: 250–257

Martens CS & Klump JV (1984) Biogeochemical cycling in an organic-rich marine basin – 4. An organic carbon budget for sediments dominated by sulfate reduction and methanogenesis. Geochim. Cosmochim. Acta 48: 1987–2004

Matisoff G, Bricker OP III, Holdren GR Jr & Kaerk P (1975) Spatial and temporal variations in the interstitial water chemistry of Chesapeake Bay sediments. In: Church TM (Ed) Marine Chemistry in the Coastal Environment (pp 343–363). ACS Symposium ser. 18

McNichol AP (1986) A study of the remineralization of organic carbon in nearshore sediments using carbon isotopes. PhD thesis MIT/WHOI

Oremland RS & Taylor BF (1978) Sulfate reduction and methanogenesis in marine sediments. Geochim. Cosmochim. Acta 42: 209–214

Roden EE & Tuttle JH (1987) Regional variations in sulfur cycling in Chesapeake Bay sediments. EOS 68: 1757

Rosenfeld JK (1979) Ammonium adsorption in nearshore anoxic sediments. Limnol. Oceanogr. 24: 356–364

San Diego-McGlone, M.L. & Cutter GA (1988) Processes affecting redox sensitive elements in Chesapeake Bay. EOS 69: 375

Sansone FJ (1986) Depth distribution of short-chain organic acid turnover in Cape Lookout Bight sediments. Geochim. Cosmochim. Acta 50: 99–105

Sansone FJ & Martens CS (1982) Volatile fatty acid cycling in organic-rich marine sediments. Geochim. Cosmochim. Acta 45: 101–121

Skyring GW, Jones HE & Goodchild D (1977) The taxonomy of some new isolates of dissimilatory sulfate-reducing bacteria. Canad. J. Microbiol. 23: 1415–1425

Smith RL & Klug MJ (1981) Electron donors utilized by sulfate-reducing bacteria in eutrophic lake sediments. Appl. Environ. Microbiol. 42: 116–121

Solorzano L (1969) Determination of ammonia in natural waters by the phenol-hypochlorite method. Limnol. Oceanogr. 14: 799–801

Sørensen J, Christensen D & Jørgensen BB (1981) Volatile fatty acids and hydrogen as substrates for sulfate-reducing bacteria in anaerobic marine sediments. Appl. Environ. Microbiol. 42: 5–11

Stams AJM & Hansen TA (1984) Fermentation of glutamate and other compounds by *Acidaminobacter hydrogenoformans* gen. nov. sp. nov., an obligate anaerobe isolated from black mud. Studies with pure cultures and mixed cultures with sulfate-reducing and methanogenic bacteria. Arch. Microbiol. 137: 329–337

Stams AJM & Hansen TA (1986) Metabolism of L-alanine in *Desulfotomaculum ruminis* and two marine *Desulfovibrio* strains. Arch. Microbiol. 145: 277–279

Stams AJM, Hansen TA & Skyring GW (1985) Utilization of amino acids as energy substrates by two marine *Desulfovibrio* strains. FEMS Microbiol. Lett. 31: 11–15

Tabatabai MA (1974) A rapid method for determination of sulfate in water samples. Environ. Letter 7: 237–243

Whelan JK (1977) Amino acids in a surface sediment core of the Atlantic abyssal plain. Geochim. Cosmochim. Acta 41: 803–810