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Chemical and biological mobilization of Fe(III) in marsh sediments

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Abstract. Iron reduction in marine sulfitic environments may occur via a mechanism involving direct bacterial reduction with the use of hydrogen as an electron donor, direct bacterial reduction involving carbon turnover, or by indirect reduction where sulfide acts to reduce iron. In the presented experiments, the relative importance of direct and indirect mechanisms of iron reduction, and the contribution of these two mechanisms to overall carbon turnover has been evaluated in two marsh environments. Sediments collected from two Northeastern US salt marshes each having different Fe (III) histories were incubated with the addition of reactive iron (as amorphous oxyhydroxide). These sediments were either incubated alone or in conjunction with sodium molybdate. Production of both inorganic and organic pore water constituents and a calculation of net carbon production were used as measures to compare the relative importance of direct bacterial reduction and indirect bacterial reduction. Results indicate that in the environments tested, the majority of the reduced iron found results from indirect reduction mediated by hydrogen sulfide, a result of dissolution and precipitation phenomena, or is a result of direct bacterial reduction using hydrogen as an electron donor. Direct iron reduction plays a minor role in carbon turnover in these environments.

Introduction

Reduction of Fe (III) in anaerobic aquatic environments may involve both microbial dissimilatory reduction and chemical reduction (Froelich et al. 1979; Berner 1980; Sorensen 1982; Hines et al. 1984; Aller et al. 1986; Jacobson et al. 1987; Lovley 1991). In marine sulfitic environments, direct enzymatic reduction of Fe (III) is associated with carbon degradation (equation 1A) or the use of hydrogen as an electron donor resulting in reoxidation of NADH and no increase in molar growth (Balashova & Zavarzin 1980; Jones et al. 1984; Coleman et al. 1993) (equation 1B). Iron reduction may represent a significant pathway for organic matter mineralization (equation 1A).

$$CH_2O + Fe(OH)_3 + H_2CO_3 \leftrightarrows Fe^{2+} + 2HCO_3^- + H_2O + 3H^+ \quad (1A)$$

$$4H_2 + SO_4^{2-} \leftrightarrows S^{2-} + 4H_2O \quad (1B)$$

In the process of sulfate reduction indirect (or chemical) reduction of iron may occur. This process is one step removed from the biogeochemical degradation of organic matter. Sulfate reduction results in the production of hydrogen sulfide which may then react with Fe (III) (equation 2a). This is referred to as indirect Fe-S-C coupling (Aller et al. 1986).

$$2CH_2O + SO_4^{2-} \leftrightarrows H_2S + 2HCO_3^- \quad (2A)$$

In addition sulfide oxidation may be coupled with iron reduction (Aller et al. 1986).

$$2FeOOH + 4H^+ + H_2S \leftrightarrows 2Fe^{2+} + S^0 + 4H_2O \quad (2B)$$

$$6FeOOH + 10H^+ + S^0 \leftrightarrows 6Fe^{2+} + SO_4^{2-} + 8H_2O \quad (2C)$$

When sulfide oxidation occurs, in addition to Fe (III) reduction, the same overall reaction will produce Fe (II) without any apparent sulfate reduction (see eq. 2B, 2C). Both direct and indirect reduction pathways contribute to the process of organic matter oxidation. The experiments in this paper were designed to address the quantitative importance of different Fe (III) reduction pathways in marine sulfidogenic environments.

When direct enzymatic reductions occur, the carbon substrate used is most often acetate (Coleman et al. 1993; Lovley 1991). This fermentation acid may be oxidized by iron reducing bacteria to produce CO_2 and Fe (II). This is evidenced by the fact that there is not a large accumulation of fermentation acids in iron reducing environments (Lovley & Phillips 1987), implying use of these acids for the production of Fe (II). These fermentation acids are also considered important carbon substrates for use by sulfate reducing bacteria and methanogens. Since these fermentation products are preferred substrates for bacterial groups mentioned above, there should be competition for these substrates between sulfate reducers and iron reducers.

The biologically mediated oxidation of organic matter by Fe (III) is generally favored energetically over sulfate reduction (Berner 1980; Lovley & Phillips 1986, 1987). This thermodynamic advantage which can be computed using standard free energy values (Froelich et al. 1979) permits iron reducing bacteria to dominate iron reduction processes in some environments (Lovley 1986) while in other environments there may be limitations to bacterial metabolism brought on by inorganic interactions of the electron acceptor Fe (III). In many systems dissolved iron is found in sediment pore waters concurrently with active sulfide production (Hines et al. 1984; Canfield 1989; Canfield & Berner 1986; Giblin & Howarth 1984; Sorensen 1982; Jacobson et al. 1987). Dissolved iron found in these environments may be produced either by direct or indirect iron (III) reduction.

The importance of specific processes leading to iron reduction are difficult to asses. Both direct and indirect reduction mechanisms have been

described in the literature (Sorensen 1982; Jones et al. 1983, 1984; Canfield & Berner 1986; Lovley & Phillips 1986, 1987, 1988; Bell et al. 1987). The relative importance of chemical and bacterial Fe (III) reduction has been inferred (Aller et al. 1986, Canfield 1989) but not been adequately evaluated in any environment. In this paper I quantified pathways of both bacterial and inorganic Fe (III) reduction. Organic and inorganic parameters associated with iron reduction were measured. A mass balance approach was used to determine the relative importance of the two mechanisms to the dynamics of iron cycling.

Methods

Approach

The utilization of sediment fatty acids by the natural bacterial consortium was measured in the presence of amorphous FeOOH and sodium molybdate. Marine sediments were incubated at either 20° or 4 °C. The 4 °C incubations were done in an attempt to accentuate inorganic processes. Pore waters were sampled at selected times to determine Fe (II) concentrations, net fatty acid production, alkalinity, and other associated pore water parameters.

Study site

The primary study site was Flax Pond N.Y. This natural history and biogeochemistry of this intertidal mud flat has been described previously (Woodwell & Pecan 1973; Mackin 1986; Jacobson et al. 1987; Swider & Mackin 1989). The salinity of the water in Flax Pond is 26–28 ppt and does not vary significantly with season. The sediments are anoxic below the upper 2 mm with integrated sulfate reduction rates (0–3 cm zone) usually high (>2mM d^{-1}) and seasonal variations dependent mainly on temperature (Swider & Kackin 1989). Pyrite is the major reduced sulfur pool. Dissolved Fe (II) production is only observed near the sediment-water interface (0–0.3 cm) and at low temperatures (4 °C) with pyrite (chromium reduced sulfur) ranging from 75–255 μmoles S/gdw-sed. Acid volatile sulfur is roughly an order of magnitude less abundant (Swider & Mackin 1989).

A second site used in this study (Great Bay, New Hampshire) is described by Hines et al. (1984). This site is also estuarine but unlike the Flax Pond site, total extractable iron in the sediments averages 2–5% by weight of the solid phase (Hines, pers. comm.)

Sediment sampling

Sediments from Flax Pond site B (Mackin 1986) were collected in April and June (1986–1988) with hand held acrylic box cores (165 cm^2). Site B is an

intertidal mudflat sited adjacent to *Spartina* stands. After collection all subsequent sediment handling was done under a nitrogen atmosphere in a glove bag. Sediments were sectioned and the 2–4 cm depth fraction was selected and mixed to insure uniformity. Treatments included: iron addition, molybdate addition, iron and molybdate addition, and a control (no addition). After treatments, sediment subsamples were packed into 5–8 centrifuge tubes (50 cc), which were either sampled immediately or stored under nitrogen in the dark at the appropriate temperature (4 °C or 20 °C) for later sampling over a time period of two months. At the end of the selected incubation periods sediment was separated from pore waters by a combination of centrifugation and filtration. After centrifugation (1800 × g for 15 minutes), samples were returned to a nitrogen atmosphere where the pore water was decanted into syringes and expressed through 0.4 μm pore-size Nuclepore membrane filters. For experiments run at 4 °C, sediment was kept on ice packs during preparations to prevent warming.

Iron – addition experiments

Iron-oxyhydroxide preparation

A synthetic iron-oxyhydroxide was used in all iron addition experiments. The iron-oxyhydroxide was prepared as described by Rickard (1974) by addition of KOH to $FeCl_3$. Careful attention was given to pH control since only the poorly crystalline form of Fe (III) has been shown to be available to bacteria for reduction. Analysis by x-ray diffraction (General Electric Powder Diffractometer) confirmed the prepared sample to be an amorphous compound. After the iron oxyhydroxide was prepared, it was ground and sieved through a 125 μm sieve to ensure uniformity in particle size.

Reactive iron availability

A time-course experiment was initiated to examine the production of reduced iron in anoxic marine sulfitic sediments (Jacobson et al. 1987). Sediment taken from the upper 4 cm of Flax Pond on 10 June (1986) was homogenized, and amorphous Fe-oxyhydroxide (0.062 g/ml wet sediment) was added to a split of the sediment under nitrogen, and sediment was incubated at 20 °C. The Fe-oxyhydroxide addition raised the total extractable iron content of the sediment 5% by dry weight. In all experiments, homogenizing the sediment probably initially destroyed pore water microdistributions and had some effect on localized microzones associated with iron reduction. However, the addition of iron and subsequent incubations created new iron rich microzones in the sediment which were intended to facilitate the development of a numerically dominant iron reducer population.

Iron and molybdate additions

In a second set of experiments, deoxygenated sodium molybdate (20mM final concentration), in addition to Fe-oxyhydroxide were added to sediment from

both Flax Pond and Great Bay New Hampshire. Sodium molybdate, a specific inhibitor of sulfate reduction (Oremland & Capone 1988), was used in this experiment to facilitate the measurement of fermentation and hydrolysis products which would otherwise be metabolized by sulfate and/or iron reducers. In each case the homogenized sediment (0–4 cm) was divided into four subsamples which received either (1) no addition (control), (2) Fe (III), (3) molybdate or (4) Fe (III) and molybdate. In the sediment where molybdate was not added, an appropriate amount of deoxygenated distilled water was added to compensate for slight dilution effects that resulted from the additions to other samples. Dilutions were no more than 5% of the total sediment wet weight. Flax Pond experiments were run at a high (20 °C, 25 June, 1987) and at a low (4 °C, 27 April, 1988) temperature. The Great Bay experiment was run at 20 °C. The amounts of iron oxyhydroxide added in different experiments ranged from 0.035–0.070 grams/cm^3 wet sediment. The additions raised the total iron content of the sediments to between 4%–10%.

Analytical methods

Pore water constituents measured in incubation experiments included: SO_4^{2-} (by direct gravimetric $BaSO_4$ method, Presley 1971), HS^- (Cline 1969), NH_4^+ (Solorzano 1969), and titration alkalinity (Edmond 1970). Average precision was <1% for alkalinity, and <3% for the ammonium, sulfate and sulfide. Sediment pH was measured by direct insertion of a semimicro glass combination electrode into both initial sediment samples and at each incubation time point (precision is 0.02 pH units). The ferrozine method of Stookey (1970) was used to analyze for dissolved iron. Average precision of the iron analysis is <1%.

The concentration of acetate, propionate, and butyrate as well as fatty acids up to C-8 were measured by gas chromatography after salts were removed from pore waters using a modification of the vacuum microdistillation method of Christensen & Blackburn (1982). Samples (200 µl) were acidified with 40 µl of 0.5 M H_3PO_4 to volatilize the fatty acids. A sample vial was connected by a U-tube to a collection vial and the apparatus was evacuated to 500 µtorr. Distillation was completed by immersing the collection end of the sealed apparatus in an acetone ice bath (–8 to –20 °C) while the sample end was heated with an infrared lamp for 15 minutes. The distillation efficiency, determined using standards in seawater, was 87–96%. Instrumental analysis was done using a HP 5890 A gas chromatograph fitted with a 15 m Nukol fused silica capillary column (Supelco) and flame ionization detector. The sample size was 1 µl. Helium was the carrier gas (flow rate = 17.7 ml min^{-1}), and a temperature program facilitated the appearance of fatty acids (through valeric) within 8 minutes of the initial injection. The temperature program was: 93 °C for 5 min, 93–95 °C at 2°/min and hold for 3 min, 95–110 °C at 5 °C/min and hold for 3 min, and return to 93 °C at 10°/min. The injection temperature was 130 °C and the detector temperature was 175 °C. Some of the early fatty acid analyses were done using a 0.3 cm i.d. column packed

with 0.5 FFAP coated Chromosorb 101 (Supelco). A 1% formic acid solution (40 µl) was added to samples and 1.0 and 0.1% formic acid solutions were injected between samples to reduce ghosting effects. In this case the column temperature was kept at 135 °C and the injector and detector temperatures were both 175 °C. The carrier was nitrogen (40 ml min^{-1}). All peak areas were integrated using an HP 3390A integrator. The concentration of the fatty acids was calculated from a calibration using aqueous fatty acid standards which were processed by the same method as the samples. Separate tests were done to ensure that the salt effects did not alter the fatty acid calibration. The detection limit for the packed column was approximately 5 µM for all of the fatty acids. The detection limit for the capillary column was 3 µM for acetate and lower for the other fatty acids measured (C1–C8).

A calculation of total Metabolized Carbon (TMC) was used to compare carbon metabolism between all treatments. The TMC should be equal in all treatments from an experimental set, making a deficit in net carbon indicative of an unmeasured source of carbon. Should the later occur it would indicate a deviation from the mass balance assumption of this experiment.

Total dissolved inorganic carbon concentrations (ΣCO_2) were calculated based upon alkalinity and pH measurements. Carbonate alkalinity was corrected for contribution of HS^-, S^{-2}, charged fatty acids and molybdate as well as borate, phosphate, and silicate by considering the milliequivalent contribution of these species at the pH of the samples. The contribution of molybdate to titration alkalinity in molybdate amended sediments was assumed to be constant, and equal to the difference between the alkalinities of the initial amended and unamended sediments. Finally, ΣCO_2 was calculated from alkalinity and pH using thermodynamic constants for appropriate salinities and temperatures (Millero 1986). Total metabolized carbon (TMC) was taken to be:

$$TMC = (\Sigma CO_2) + \sum_{i=1}^{n} N_i(C_i(0) + (1 + K_i)\ (\Delta C_i))$$

where:

C_i = fatty acid concentration
K_i = linear adsorption coefficient
$C_i(0)$ = the initial fatty acid concentration (in most cases undetectable by methods used)
(ΔC_i) = the change in fatty acid concentration from t = 0 to t – time of incubation
N_i = number of carbon atoms/molecule

Fatty acid production rate estimates were calculated from the slopes of linear regressions of solute concentrations against time. Adsorption correction was made for acetate (the most abundant fatty acid present). The measured value of K = 0.66 (s.d. 0.02) was used (Michelson et al. 1989). For other

fatty acids, K_i was assumed to be zero because direct measurements were not available. In addition, fatty acids other than acetate represent a relatively small fraction of the total metabolized carbon, and therefore our assumption that $K_i = 0$ has only a minor impact on the calculation of TMC. This was confirmed by a calculation made using literature adsorption values from other environments (Sansone et al. 1987) (the difference between calculations using the latter values and those assuming a $K_i = 0$ was 4% on average). Since no adsorption data was available for the New Hampshire sediments, the linear adsorption coefficient for acetate was taken to be $K_i = 0.66$ as measured for Flax Pond. Similar measurements were reported by Sansone et al. (1987) in Cape Lookout Bight.

Results

Effect of Iron (III) addition

The addition of iron oxyhydroxide to Flax Pond sediments (20 °C experiment) resulted in an immediate 5-fold decrease in sulfide concentration found in sediments relative to a control (Fig. 1b). No additional bydrogen sulfide

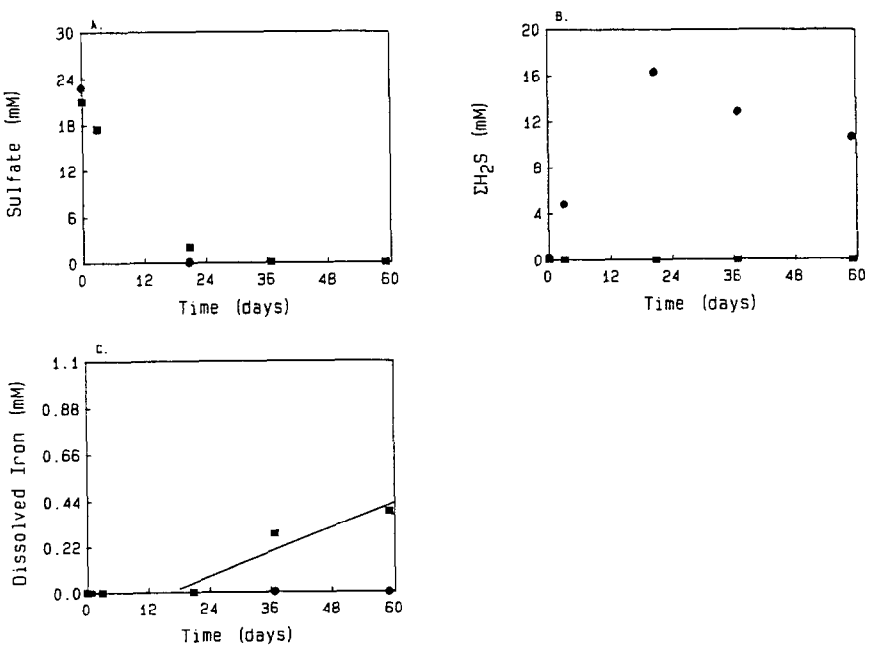

Fig. 1. Changes in concentration of sulfate (a), total sulfide (ΣH_2S) (b), and dissolved Fe (II) (c), in incubated sediments from Flax Pond where Fe (III) was added. ● = control (no addition), ■ = addition of iron oxyhydroxide. A line representative of the linear regression is draw through the iron data ($r^2 = 0.94$).

production was measured in iron-oxyhydroxide amended sediments. The decrease in sulfide after 36 days in control sediments reflects sulfate depletion in the pore water at that time as well as sequestering of HS^- by any Fe (III) or Fe (II) found free in the environment (Fig. 1c). Dissolved iron began to appear in the pore waters (Fig. 1c) only when sulfate was almost completely depleted (Fig. 1a) and only in the Fe-oxyhydroxide amended sample. Sulfate reduction rates were similar in both control and iron amended sediments (Fig. 1a). The dissolved Fe (II) generated in this experiment could be the result of either a reaction of solid phase iron with HS^- and/or direct bacterial production of dissolved iron by reducing bacteria. Further experiments were therefore necessary to separate these processes.

Effect of iron (III) and molybdate additions

Flax Pond sediments were amended with iron and molybdate and incubated at 20 °C. Sulfate reduction rates were again similar in control and iron (III) treated sediments (Fig. 2a), and dissolved iron appeared in iron-amended sediments only after sulfate was depleted (on day 27). Addition of molybdate to samples affected both sulfate reduction and subsequent dissolved sulfide production (Figs. 2a, 2b). Immediate production of dissolved Fe (II) was observed in the molybdate and Fe (III) amended samples (Table 1, Fig. 2c). Consumption (or decrease in production) of dissolved iron occurred in later samples. The rate of dissolved iron production in the samples where Fe (III) was added alone, was lower than in samples where Fe (III) and molybdate was added. There was no difference in initial rates of production of acetate and propionate in either molybdate or molybdate and iron treated sediments (Fig. 2e, Table 2d, 2e). Butyrate production decreased after 19 days of incubation in samples where both iron and molybdate were added. Total metabolized carbon production rates did not differ between any treatments (Fig. 2g).

The results of the 4 °C Flax Pond experiment differed from those observed in the 20 °C Flax Pond experiment (Fig. 3). First, the sulfate reduction rates were lower overall. Secondly, at 4 °C dissolved iron production was higher when iron (III) was added alone than when it was added in conjunction with molybdate (compare Fig. 2c and Fig. 3c). As in the 20 °C experiment, significant net production of sulfide was found only in controls. No difference was seen in fatty acid production in samples where molybdate alone was added when compared to samples where molybdate and Fe (III) were added (Figs. 3d, 3e, 3f). Total metabolized carbon production rates were similar in all treatments (Fig. 3g).

For experiments involving sediments collected from Great Bay New Hampshire, hydrogen sulfide was below detection in all of the samples. Sulfate reduction rates were comparable in the control and in sediment receiving an iron addition (Fig. 4a, Table 1). Dissolved iron was present in all samples.

The pattern of fatty acid production in Great Bay, New Hampshire differed

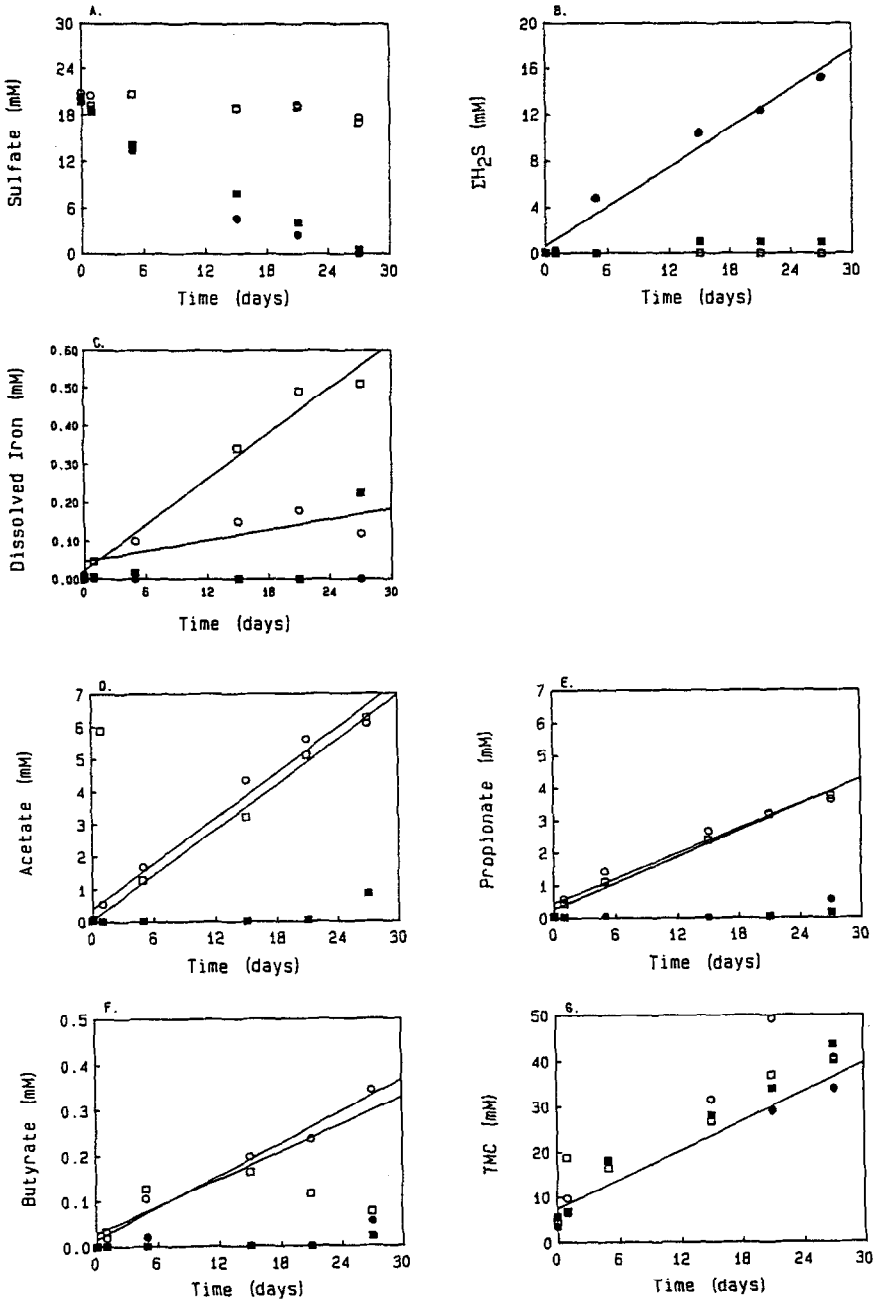

Fig. 2. Changes in concentration of sulfate (a), hydrogen sulfide (b), dissolved Fe (II) (c), acetate (d), propionate (e), butyrate (f), and total metabolizable carbon (TMC) (g) concentration in Flax Pond Sediments incubated at 20 °C. ● = control, ■ = Fe (III) addition, ○ = molybdate addition, □ = Fe (III) + Molybdate addition. In the case of TMC the regression is for control points. Slopes of the other regressions do not differ from the control ($r^2 > 0.97$ in all cases).

Table 1. Production rates for inorganic variables measured in sediment pore waters of both Flax Pond and Great Bay. In these experiments Fe (III) was added either alone or in conjunction with molybdate. The control sediments were incubated with no additions.

Experiment type	Treatment	Sulfate reduction (mM/D)	Sulfide production (mM/D)	Iron (II) production (μM/D)	Incubation temperature (Degrees C)
Flax Pond Fe (III) Addition	Control	0.945	0.732	–	20
	Fe (III) Addition	0.891	0.002	9.67	20
Fe (III) + Molybdate	Control	0.810	0.566	–	20
	Fe (III) Addition	0.696	0.044–0.07	1.13	20
	Molybdate Addition	–	–	–	20
	Iron + Molybdate Addition	–	–	13.05*	20
Fe (III) + Molybdate	Control	0.145	0.128	–	4
	Fe (III) Addition	0.056	0.0001	80.5	4
	Molybdate Addition	0.030	0.002	–	4
	Iron + Molybdate Addition	0.020	–	6.99	4
Great Bay Fe (III) + Molybdate Addition	Control	0.36–0.41	–	2.60	4
	Fe (III) Addition	0.25–0.30	–	7.86	4
	Molybdate Addition	–	–	4.59–7.89	4
	Iron + Molybdate Addition	–	–	4.99–10.25	4

* = corrected for molybdate interference as outlined in text.

Table 2. Fatty acid production rates measured in sediment pore waters of both Flax Pond and Great Bay. In these experiments Fe (III) was added alone or in combination with molybdate. Control sediments received no additions.

Experiment type	Treatment	Acetate production (μM/D)	Propionate production (μM/D)	Butyrate production (μM/D)	Incubation temperature (Degrees C)
Flax Pond Fe (III) + Molybdate	Control	–	7.24	4.3	20
	Fe (III) Addition	1.24	0.89	–	20
	Molybdate Addition	231	128	11.75	20
	Iron + Molybdate Addition	231	134	1.6–10	20
Fe (III) + Molybdate	Control	–	–	–	4
	Fe (III) Addition	–	–	–	4
	Molybdate Addition	12	18	4	4
	Iron + Molybdate Addition	9	22	2	4
Great Bay Fe (III) + Molybdate	Control	–	–	–	20
	Fe (III) Addition	–	–	–	20
	Molybdate Addition	85	35	1.4	20
	Iron + Molybdate Addition	41	26	1.5	20

from that in Flax Pond. The amount of acetate generated was higher in molybdate-inhibited sediments where no iron was added than in sediments where iron and molybdate were added together (Figs. 4c–4e). There was no significant difference in other fatty acid production rates between Fe (III) amended and unamended molybdate treatments. There were also no significant differences between total metabolized carbon in any of the sample treatments.

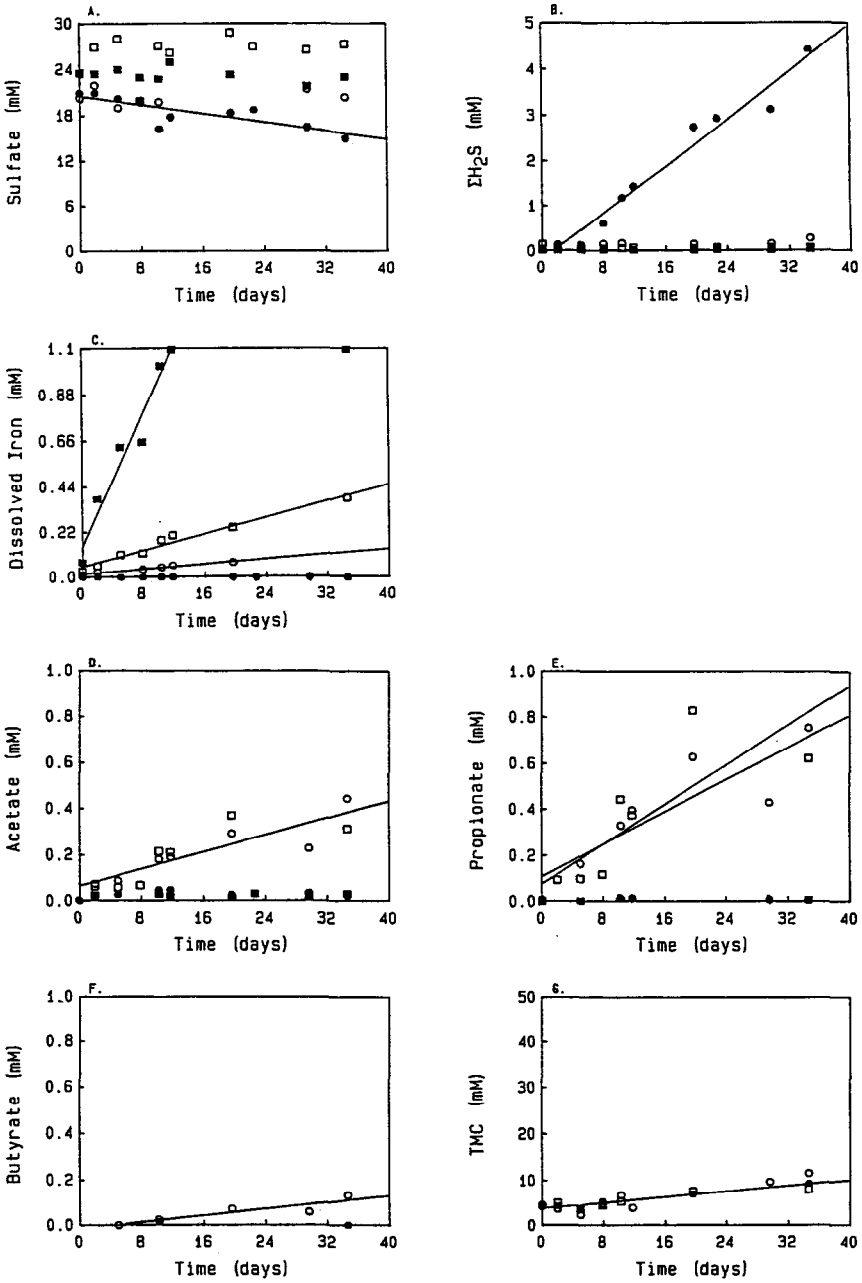

Fig. 3. Changes in concentration of sulfate (a), hydrogen sulfide (b), dissolved Fe (II) (c), acetate (d), propionate (e), butyrate (f), and total metabolizable carbon (TMC) (g) concentration in Flax Pond Sediments incubated at 4 °C. ● = control, ■ = Fe (III) addition, ○ = molybdate addition, □ = Fe (III) + Molybdate addition. In the case of TMC the regression is for control points. Slopes of the other regressions do not differ from the control (r^2 values: SO_4^{2-} = 0.66, ΣH_2S = 0.97, Fe (II) = 0.97, Acetate = 0.96, Propionate = 0.86, TMC = 0.97).

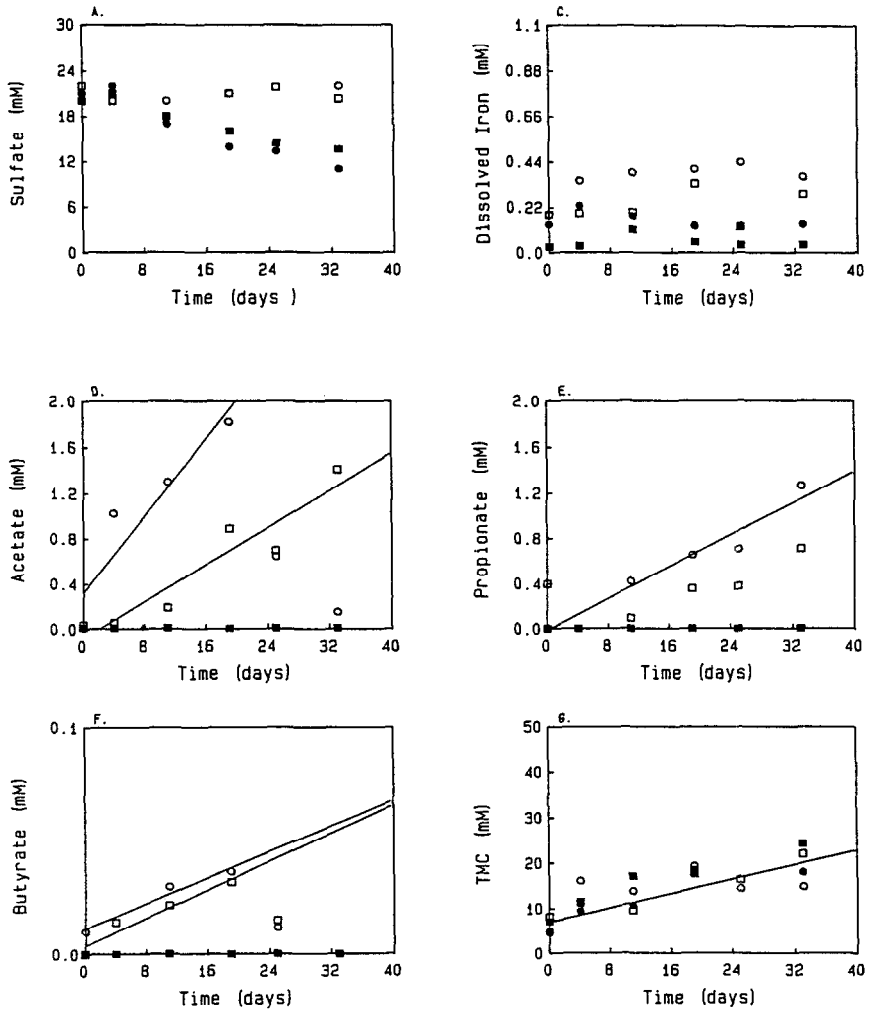

Fig. 4. Changes in concentration of sulfate (a), dissolved Fe (II) (c), acetate (d), propionate (e), butyrate (f), and total metabolizable carbon (TMC) (g) concentration in Great Sediments incubated at 20 °C. ● = control, ■ = Fe (III) addition, ○ = molybdate addition, □ = Fe (III) + Molybdate addition. In the case of TMC the regression is for control points. Slopes of the other regressions do not differ from the control ($r^2 > 0.95$ in all cases).

Net production rates of naturally occurring fatty acid carbon measured in molybdate-inhibited cores are compared to total carbon produced as CO_2 in control sediments. The measured rates of fatty acid production are sufficient to account for the total CO_2 production found in the controls. There are therefore, no other biologically significant unmeasured sources of reduced carbon in these systems.

Discussion

Appraisal of iron reduction associated with organic matter decomposition and direct bacterial reduction

Iron (III) reduction occurred in the two environments tested. In all sediments, sulfate reduction rates were sufficient to account for all of the Fe (II) produced. In flax Pond sediments the addition of iron did not inhibit sulfate reduction, however, addition of iron did decrease the net dissolved sulfide production. This sulfide is most likely sequestered in solid phase iron-sulfides. Net dissolved Fe (II) production in flax Pond sediments was highest in molybdate-inhibited sediments where iron was added. The iron production considered in the above discussion was not associated with organic matter decomposition. The results do not indicate significant bacterial use of fatty acids associated with iron reduction therefore, in this environment there is no direct evidence for bacterial iron reduction.

In sediments of Great Bay, New Hampshire, there appears to be organic matter decomposition associated with iron reduction. Acetate and propionate accumulation rates decreased in sediments where iron was added. The differences in rates of net acetate production between molybdate treatments, with and without iron indicate consumption of fatty acids by iron reducing bacteria in these sediments. A potential of approximately 40% of the acetate production in this environment may be used for iron reduction. Based on pure culture work (Lovley & Phillips 1989), eight moles of Fe (III) may be reduced per mole of acetate produced. The potential for bacterial iron reduction as calculated from the acetate production data can be up to 0.352 mM/d. The rate of reduction due to hydrogen sulfide has a range of 0.25–0.41 mM/d (as calculated from data in Table 1). Therefore bacterial iron reduction and inorganic reductions have comparable potential rates. However, the measured differences in fatty acid production are not manifested in significant differences in total carbon metabolism. Acetate shows the greatest difference in net production rates but the higher carbon content of the other fatty acids collectively influence the TMC calculation decreasing the importance of iron reduction to total carbon turnover in this environment. In addition, one may not rule out fatty acid consumption associated with chelation of Fe (III) as suggested by Luther et al. (1992).

Volatile fatty acids have been used in short term enrichment cultures of iron reducing bacteria (Tugel et al. 1986) with successful development of iron reduction capacity and concurrent fatty acid use. However, in my experiments net fatty acid accumulation in molybdate-inhibited sediments was not affected by addition of amorphous Fe (III). Although iron reducing bacteria can use these substrates, they were not important in these experiments.

Lovley et al. (1989) have demonstrated that *Altermonas putrefaciens* metabolizes lactate or pyruvate to acetate using Fe (III) or Mn (IV) as electron acceptors. In both Great Bay and Flax Pond sediments, we did not see an increase

in acetate accumulation associated with Fe (III) additions, which could be attributed to lactate or pyruvate metabolism.

Lovley et al. (1989) also have shown that di-hydrogen and formate can be important substrates and electron donors for Fe (III) reduction. Although di-hydrogen, formate, pyruvate, and lactate were not measured in our experiments, TMC data suggest that carbon substrates are not dominant decomposition intermediates in the environments tested. It has recently been reported that *Desulfovibrio desulfuricans* readily reduces iron (III) using acetate as an electron donor (Coleman et al. 1993). In addition other sulfate reducing species have been shown to use hydrogen in the reduction of iron. In the later case, with nonlimiting conditions (i.e. a balance of hydrogen, sulfate and reducible iron in the culture environment), sulfate and iron (III) were reduced simultaneously in culture. At low hydrogen concentrations it is suggested that Fe (III) may be the preferred electron acceptor. Although hydrogen was not measured in this study, it is usually produced during fermentation reactions, and should not have been limiting in experiments where molybdate was used as an inhibitor of sulfate reduction (Zehnder 1988). In experiments where iron (III) was added without inhibiting sulfate reduction, iron (II) production did not occur until sulfate was depleted (Figs. 1b, 1c). When sulfate is depleted, hydrogen may be used by sulfate reducers as an electron donor. Where sulfate is available in the presence of Fe (III), sulfate reduction appears to be the preferred mode of metabolism. In a transition zone where sulfate is found at low concentrations and hydrogen is available, Fe (III) reduction by sulfate reducers may occur. However, presumably, all iron (III) would be previously reduced in this zone. Estimates of hydrogen metabolism in Flax Pond (Novelli et al. 1987) suggest that it is not an important intermediary in the natural system though, hydrogen may have accumulated in sediments in the experiments done here. These data suggest that the iron reduction seen in flax Pond and Great Bay sediments is due primarily to chemical reactions involving sulfide (and possible bacterial reduction using hydrogen as an electron donor) rather than direct enzymatic reduction associated with carbon decomposition.

Competition for electron acceptors between bacteria and inorganic reactions

Although both amorphous and crystalline forms of iron are reactive with sulfides, iron accessible to bacterial for reduction is predominantly the amorphous oxyhydroxide in marine environments (Hines et al. 1984; Lovley & Phillips 1987a). The oxihydroxide may be reduced by bacteria or an abiotic reaction with sulfide. Consequently, a competition for Fe (III) may occur between bacterial enzymatic and inorganic chemical reactions. Since both direct bacterial reduction and chemical reduction of Fe (III) by hydrogen sulfide rely on a surface association with reactive iron (Tugel et al. 1986; Bell et al. 1987; Lovley 1991), iron reduction may be viewed as a competition between bacteria (uptake kinetics) and inorganic chemical reactions (chemical kinetics) for active sites available on iron oxyhydroxide solid

phases. These reductions may co-occur provided the abundance of oxyhydroxide is sufficient.

The high mobility of surface sediments in the Amazon river basin (Aller et al. 1986) would permit a rapid regeneration of amorphous iron oxyhydroxide affording a readily available supply of iron for iron reducing bacteria. This is one environment where one might expect iron reduction and carbon turnover to be coupled.

In addition, in lower salinity regions of estuaries and in freshwater sediment where SO_4^{2-} is low (Bricker & Troup 1975; Kuivila & Murray 1984) one finds extended zones of iron reduction. In areas where sulfate is limiting (such as in a fresh water environment) (Kelly & Schindler 1984), bacterial iron reduction and concurrent carbon turnover may be favored. In contrast, highly sulfitic, marine environments would favor chemical reduction.

In a linear depiction of zonal distribution of terminal electron-accepting processes in aquatic sediments, organic carbon degradation coupled to iron reduction and sulfate reduction occurs sequentially in associated zones (Froleich et al. 1979, Berner 1980, Lovley 1991). However, in nature, iron reduction and sulfate reduction processes are not necessarily separated spatially. Sulfide rapidly reduces amorphous iron oxyhydroxide, producing reduced iron, and bacteria which reduce iron must be directly associated with iron oxyhydroxide particles (Tugel et al. 1986). These particles may be initially introduced by bioturbation, or adjective mixing into microzones in an otherwise sulfitic environment. The concentration of sulfide and its proximity to oxidized iron particles in any environment, affects the availability of iron particles to bacterial reduction. In the experiments found in this paper, iron was added in excess and stirred into the sediment to facilitate availability of the oxyhydroxide to sedimentary bacteria. Iron reducing bacteria may outcompete sulfate reducing bacteria in a pure culture system under controlled conditions of electron acceptor and donor supply as well as in some sediment systems (Lovley & Phillips 1987b; Jones 1983; Jones et al. 1984). However, in environments where significant sulfide is generated, it is possible that the majority of dissolved iron found in pore waters is present as a result of chemical equilibria, chemical reductions and not direct bacterial reduction.

Only by the measurement of carbon sources or alternatively by the measurement of enzymatic activity, in conjunction with analysis of inorganic pools, can one be sure of an accurate portrayal of the mechanism of reduction in any environment.

Inorganic phases associated with reduced iron production

It appears that the major pathway for iron reduction in the environments studied does not occur via direct reduction involving carbon turnover. In addition to reduction mechanisms discussed previously, dissolved iron production in an environment may reflect chemical dissolution and precipitation phenomena. Solid phases may be initially present in the sediment or may be

produced as a result of iron reduction. In anoxic environments solid phase iron sulfides equilibrate with sulfide in the surrounding pore waters (e.g. eq. 3). This equilibration process may involve a second molecule of sulfide, S_8, polysulfide or another proton donor (i.e. HCO_3^-, CO_3^{-2}).

$$FeS + H^+ = Fe^{+2} + HS^-. \quad (3)$$

$$K_{sp} = (Fe^{+2})\ (HS^-)\ /\ (H^+)$$

The amount of dissolved iron in pore waters may reflect dissolution of such phases and may depend mainly on pH, total dissolved sulfide and the solubilities of the solid phase(s) present, and the availability of oxidants. In order to determine the potential contribution on mineral dissolution to Fe (II) production, ion activity products for solid-phase sulfides, including amorphous FeS, mackinawite, greigite, pyrite, and siderite ($FeCO_3$) were calculated using pore water solute concentrations and assuming a generalized equilibrium after Emerson et al. 1983) (data not shown). This method has been used previously (Giblin & Howarth 1984) to evaluate the possibility of mineral precipitation or dissolution in marine sediments.

In all of the experiments performed, ion activity products indicate pore water supersaturation with regard to pyrite. When Fe (III) is added in the incubation experiments, S_8, and polysulfide form in response to reactions of the Fe (III) with sulfide. This aides in FeS_2 formation (Rickard 1975; Luther 1991). Under anoxic conditions of the experiment FeS_2 will not redissolve and the Fe (II) in solution will be primarily in equilibrium with FeS and carbonate phases.

For the initial iron addition experiment done in this study (Flax Pond), variations found in log IAP primarily reflect variations in sulfide concentrations. Differences in the relative amounts of dissolved iron generated at high and low temperature in Flax Pond sediments may reflect equilibrium of Fe-sulfide phases with respect to surrounding pore waters.

In the two molybdate inhibition experiments done in Flax Pond, for subsamples where amorphous Fe (III) was added alone to the sediment, initial supersaturation with respect to pyrite and undersaturation with respect to other Fe-S phases was observed. Then, concurrent with continued production of sulfade in pore waters, there was a change in saturation state. In experiments where both molybdate and Fe (III) were added, supersaturation with respect to FeS (amorphous) was usually seen. Changes observed in FeS saturation between these two experiments may help explain differences in the appearance of dissolved iron.

IAP calculations done for the Great Bay sediments illustrate similar trends to those calculated for Flax Pond. Dissolved iron production in Flax Pond sediments can be explained by dissolution and precipitation of solid-phase sulfides. This is also true for Great Bay sediments. However in Great Bay, there is also possible utilization of carbon substrate concurrent with iron reduc-

tion. This indicates that a small proportion of iron in this environment may be directly reduced.

Conclusions

1. In environments tested iron reduction and sulfate reduction occurred concurrently.
2. In the sediments tested, sulfate reducing reactions were sufficient to account for all of the Fe (II) measured.
3. Although there is some evidence for carbon turnover associated with iron reduction, in the environments tested it is not the dominant mechanism.
4. Chemical equilibria, chemical reductions, and reductions associated with di-hydrogen may play a role in reduction of iron in the tested environments.
5. Iron reduction associated with carbon metabolism may be important in environments where: amorphous iron (III) is plentiful and continually generated, minimizing the competition between sulfide and bacteria for active oxyhydroxide surfaces; and in areas where sulfide production is low relative to iron-oxyhydroxide concentrations.

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