Organic Matter Mineralization with Reduction of Ferric Iron in Anaerobic Sediments

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The potential for ferric iron reduction with fermentable substrates, fermentation products, and complex organic matter as electron donors was investigated with sediments from freshwater and brackish water sites in the Potomac River Estuary. In enrichments with glucose and hematite, iron reduction was a minor pathway for electron flow, and fermentation products accumulated. The substitution of amorphous ferric oxyhydroxide for hematite in glucose enrichments increased iron reduction 50-fold because the fermentation products could also be metabolized with concomitant iron reduction. Acetate, hydrogen, propionate, butyrate, ethanol, methanol, and trimethylamine stimulated the reduction of amorphous ferric oxyhydroxide in enrichments inoculated with sediments but not in uninoculated or heat-killed controls. The addition of ferric iron inhibited methane production in sediments. The degree of inhibition of methane production by various forms of ferric iron was related to the effectiveness of these ferric compounds as electron acceptors for the metabolism of acetate. The addition of acetate or hydrogen relieved the inhibition of methane production by ferric iron. The decrease of electron equivalents proceeding to methane in sediments supplemented with amorphous ferric oxyhydroxides was compensated for by a corresponding increase of electron equivalents in ferrous iron. These results indicate that iron reduction can outcompete methanogenic food chains for sediment organic matter. Thus, when amorphous ferric oxyhydroxides are available in anaerobic sediments, the transfer of electrons from organic matter to ferric iron can be a major pathway for organic matter decomposition.

The microbial reduction of ferric iron [Fe(III)] to ferrous iron [Fe(II)] may have a key role in the iron cycle of aquatic environments and influence the exchange of nutrients and trace metals between sediments and the overlying water. The concentration of Fe(III) in sediments frequently exceeds that of other electron acceptors such as oxygen, nitrate, and sulfate, and thus there is the potential for significant nutrient release from organic matter mineralization with Fe(III) as the electron acceptor. Fe(III) reduction mediates phosphate and trace metal fluxes because these compounds are retained in the sediments by adsorption onto Fe(III) oxyhydroxides and are released when Fe(III) is reduced (2, 14).

Microbial Fe(III) reduction has not been studied as extensively as other anaerobic processes in sediments, such as sulfate reduction and methane production (5, 6, 13). Microbial food chains with sulfate reduction or methane production as the terminal process decompose complex organic matter to carbon dioxide or methane with the accumulation of only low steady-state concentrations of organic intermediates (for a review, see reference 8a). A similar metabolism of organic matter with Fe(III) as the electron acceptor is theoretically possible and is more thermodynamically favorable than the mineralization of organic matter with sulfate reduction or methane production as the terminal step (4). The accumulation of Fe(II) in sediments prior to sulfate reduction or methane production (4, 8) suggests that Fe(III)-reducing bacteria, or food chains of Fe(III)-reducing bacteria, can outcompete sulfate-reducing and methane-producing food chains for organic matter when Fe(III) is available.

However, there is no microbiological evidence for significant oxidation of organic matter with Fe(III) as the electron acceptor. Pure cultures of Fe(III)-reducing bacteria reduce small quantities of Fe(III) when the bacteria metabolize substrates such as glucose and malate to fermentation acids

and alcohols (6, 11). Most of the reducing equivalents and organic carbon are retained in the fermentation products. Other isolates weakly reduce Fe(III) with hydrogen as the electron donor (1, 5).

Studies with mixed microbial populations in freshwater sediments further indicate that only a small fraction of the reducing equivalents in organic matter can be transferred to Fe(III). In the profundal sediments of Belham Tarn, glucose, malate, and ethanol stimulated Fe(III) reduction, but common fermentation products such as acetate, propionate, and butyrate were inhibitory (6). The extent of Fe(III) reduction was minor in comparison with the reducing potential of the organic matter in the sediments (6).

Fe(III) reduction could be a significant process for organic matter mineralization if, in addition to the minor Fe(III) reduction which occurs during fermentation, the products of fermentation could also be metabolized with concomitant Fe(III) reduction. In anaerobic rice paddy soils, acetate is a major fermentation product (15), and the disappearance of added acetate and the production of $^{14}CO_2$ from [2-^{14}C]acetate are associated with the accumulation of Fe(II) (7). We report here that when Fe(III) was in an amorphous form, a wide range of fermentation products were metabolized with concomitant Fe(III) reduction. These results indicate that nonrecalcitrant organic matter can be completely mineralized with Fe(III) as the ultimate electron acceptor. This capability enables Fe(III) reduction to compete with methanogenic food chains for electron donors in sediments.

MATERIALS AND METHODS

Study site. Sediments from two sites in the Potomac River Estuary in Maryland were investigated. Tidal river sediments were collected at a water depth of 2 m (Fig. 1). This is a freshwater site with salinity of less than 0.5‰ and sulfate concentrations of less than 1 mM in the overlying water.
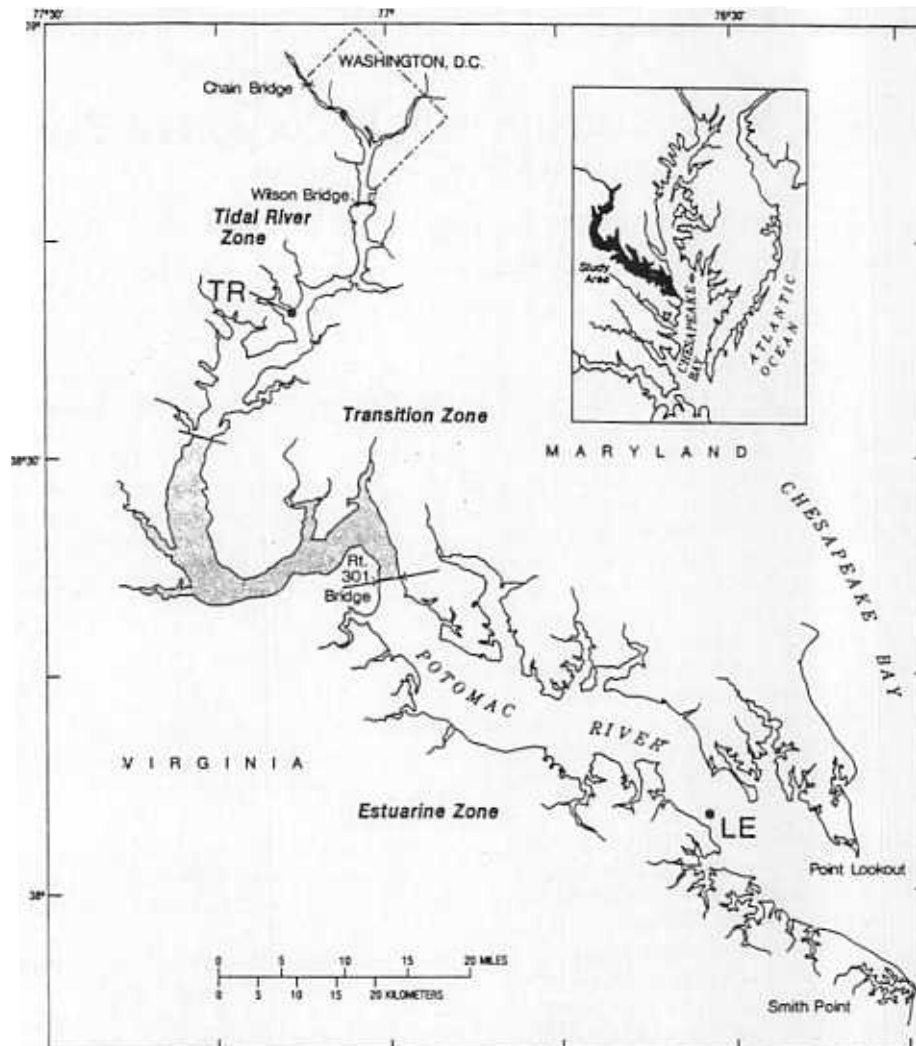
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FIG. 1. Location of tidal river (TR) and lower estuary (LE) sampling sites in the Potomac River Estuary.

Sediments were collected from the lower estuary at a depth of 10 m (Fig. 1). Salinity and sulfate concentrations in the overlying water were typically 11‰ and 10 to 15 mM, respectively. The brown, flocculent surface sediments were taken from Eckman dredge (tidal river) or petite Ponar dredge (lower estuary) samples and transported in sealed canning jars in the dark.

Enrichments. The freshwater enrichment medium contained the following constituents (in grams per liter of deionized water): $NaHCO_3$, 2.5; $CaCl_2 \cdot 2H_2O$, 0.1; KCl, 0.1; NH_4Cl, 1.5; NaH_2PO_4, 0.6; NaCl, 0.1; $MgCl_2 \cdot 6H_2O$, 0.1; $MgSO_4 \cdot 7H_2O$, 0.1; $MnCl_2 \cdot 4H_2O$, 0.005; $NaMoO_4 \cdot 2H_2O$, 0.001; and yeast extract (BBL Microbiology Systems), 0.05. The estuarine enrichment medium was similar to the freshwater medium, with the concentrations of the following constituents changed to the indicated levels (in grams per liter of deionized water): NaCl, 11.7; $MgCl_2 \cdot 6H_2O$, 5.3; and $CaCl_2 \cdot 2H_2O$, 1.0. The media were adjusted to pH 7 and then dispensed into 25-, 120-, or 160-ml Wheaton serum bottles. Hematite (Fe_2O_3 powder; J. T. Baker Chemical Co.) or amorphous ferric oxyhydroxide was added to provide ca. 250 mmol of Fe(III) per liter.

The media were bubbled with N_2-CO_2 (80:20) for several minutes. As with all gases used in the manipulations of enrichments and sediments, the gases were passed through a heated column of reduced copper filings to remove traces of oxygen. Each bottle of enrichment medium was sealed with a butyl rubber stopper (Bellco Glass, Inc.) and an aluminum crimp. The enrichments were initiated with a 10 to 20% inoculum of sediment and were incubated in the dark at 30°C.

Studies with whole sediment. Sediments were homogenized and transferred (5 or 10 ml) into 25-ml Wheaton serum bottles or anaerobic pressure tubes (Bellco Glass, Inc.) under N_2-CO_2 (93:7). Concentrated solutions of substrates and suspensions of various forms of Fe(III) were bubbled with the N_2-CO_2 mixture before they were added to the sediments. Each control received an equal volume of deionized water that had been bubbled with N_2-CO_2. Incubations were done at 20°C in the dark. The samples were incubated without shaking, except when the potential for methane production from hydrogen was determined. These samples were incubated horizontally with vigorous shaking on a wrist-action shaker.

Preparation of Fe(III) forms. Various forms of Fe(III) were synthesized by using modifications of previously described methods (9). Amorphous Fe(III) oxyhydroxide was formed by neutralizing a 0.4 M solution of $FeCl_3$ to a pH of 7 with NaOH. To form akaganeite (β-FeOOH), a 0.5 M $FeCl_3$ solution was incubated at 90°C for 16 h. Goethite (α-FeOOH)

was formed by adjusting a 0.4 M FeCl₃ solution to pH 12 with NaOH. After 1 week at room temperature, the solution was incubated at 90°C for 16 h. The Fe(III) forms were washed with deionized water until the chloride concentration in the associated water was less than 1 mM. The structure of the akaganeite and goethite and the amorphous nature of the Fe(III) oxyhydroxides were confirmed by X-ray diffraction analysis.

Measurement of Fe(II) production. After the enrichments had been transferred (10% inoculum) three to seven times, enrichment media with the $MgSO_4 \cdot 7H_2O$ omitted were inoculated. This procedure ensured that the sulfate concentration was less than 40 μM and eliminated significant chemical reduction of Fe(III) via sulfide production. In the initial studies with glucose, acetate, propionate, and hydrogen as substrates (Fig. 2), Fe(II) was measured by adding a 0.1-ml sample from the enrichment to 5, 10, or 20 ml of ferrozine (1 g/liter) in 50 mM HEPES (N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid) buffer (13). After being mixed for 15 s, the mixture was passed through a filter (Nuclepore Corp., pore diameter, 0.2 μm), and the A_{562} was determined. Fe(II) standards were prepared from ferrousethylenediammonium sulfate.

In all other experiments, enrichment (0.1 ml) or sediment (ca. 0.1 g) samples were added to preweighed vials containing 5 ml of 0.5 N HCl. The weight of the added sample was determined. After 15 min (enrichments) or 1 h (sediments), 0.1 ml was added to ferrozine in HEPES buffer, and Fe(II) was determined as above. During this procedure, Fe(II) was not oxidized and Fe(III) was not reduced, as determined by additions of $FeCl_2$ and amorphous Fe(III) oxyhydroxide to enrichments and sediments in control experiments.

Analytical techniques. Hydrogen was determined with a Gow Mac Series 550P gas chromatograph equipped with a thermal conductivity detector and dual 2-m stainless steel columns packed with Carbosieve S-II (Supelco, Inc.). Nitrogen (30 ml/min) was the carrier gas, and the oven temperature was 50°C. This system was occasionally used to determine methane concentrations, in which case the oven temperature was 140°C. Methane was routinely determined on a Series 2400 gas chromatograph (Fisher Scientific Co.) equipped with a flame ionization detector. Gases were separated on a stainless steel 1-m column of Porapak Q (Waters Associates, Inc.), with helium (30 ml/min) as the carrier. Volatile fatty acids were separated on a 2-m glass column of 10% SP-1200–1% H_3PO_4 on 80/100 Chromosorb W AW (Supelco, Inc.) with helium as the carrier. The chromatograph was a Shimadzu GC-mini 2 with a flame ionization detector. The temperatures of the injector and detector were 190°C, and the oven temperature was 120°C. The same column at 60°C was used to measure alcohols and acetone. Samples were acidified (pH < 3) with concentrated H_3PO_4 and filtered through a Nuclepore filter (pore diameter, 0.2 μm) before analysis.

Glucose concentrations were measured enzymatically with the glucose (HK) reagent kit (Sigma Chemical Co.) after the samples had been passed through a Nuclepore filter (pore diameter, 0.2 μm).

Sulfate in sediment pore water was determined with a Dionex Model 14 ion chromatograph equipped with an anion precolumn, separator column, and suppressor.

RESULTS

Fe(III) reduction in enrichments. Enrichments inoculated with surface sediments from the tidal river and lower estuary sites reduced more Fe(III) with amorphous Fe(III)

oxyhydroxide as the Fe(III) source than with hematite as the Fe(III) source (Fig. 2). Hematite is the Fe(III) form commonly used to study Fe(III)-reducing bacteria (10). With hematite, small quantities of Fe(II) accumulated with glucose, hydrogen, or propionate as the electron donor but not with acetate as the donor (Fig. 2A). Although the media were a bright red "oxidized" color and the potential substrates and yeast extract were the only added reducing agents, the substrates were primarily metabolized to methane in the hematite enrichments (Fig. 2C), even after 10 transfers of a 10% inoculum. In contrast, active Fe(III)-reducing enrichments were obtained with all four substrates and amorphous Fe(III) oxyhydroxide (Fig. 2B). Methane production was low (Fig. 2D) and was completely eliminated after 4 or 5 transfers.

Differences in metabolism in glucose enrichments with hematite and with amorphous Fe(III) oxyhydroxide were further examined (Fig. 3). Both enrichments fermented glucose to butyrate and acetate. No alcohols or acetone was detected. Hydrogen initially accumulated in the enrichments containing amorphous Fe(III) oxyhydroxide. In enrichments with hematite there was a slight reduction of Fe(III) during the metabolism of glucose. There was little further Fe(III) reduction after the glucose was consumed, butyrate was not further metabolized, and acetate was only consumed with concomitant methane production.

In enrichments with amorphous Fe(III) oxyhydroxide, Fe(III) reduction was 10-fold greater than in the hematite enrichments during the initial metabolism of glucose. Furthermore, acetate, butyrate, and hydrogen were metabolized with a concomitant increase in Fe(II) production in enrichments with amorphous Fe(III) oxyhydroxide. These results indicate that when highly crystalline Fe(III) is the potential electron acceptor, there is only a minor transfer of electrons from organic matter to Fe(III) during the fermentation, whereas fermentable substrates can be completely mineralized with amorphous Fe(III) as the electron acceptor because the products of fermentation also serve as electron donors for Fe(III) reduction.

The suitability of other Fe(III) forms as electron acceptors for the metabolism of fermentation products was investigated by adding a 10% (vol/vol) inoculum of tidal river sediments to freshwater enrichment medium containing sodium acetate (20 mM) and one of the following Fe(III) forms (initial Fe(III) concentration in millimoles per liter): amorphous ferric oxyhydroxide (250), akaganeite (300), or goethite (120). After 15 days the concentrations of Fe(II) from the reduction of the various Fe(III) forms were 100, 52, and 5 mM for amorphous Fe(III) oxyhydroxide, akagenite, and goethite, respectively. Therefore, amorphous Fe(III) oxyhydroxide was used in further enrichment studies.

As was expected from the results shown in Fig. 3, enrichments with butyrate as the electron donor actively reduced amorphous Fe(III) oxyhydroxide (Table 1). Neither acetate nor hydrogen was detectable as an intermediate during butyrate metabolism. Ethanol also stimulated Fe(III) reduction over that observed for the controls, in which yeast extract was the only potential electron donor. Methanol and trimethylamine stimulated Fe(III) reduction when the inoculum was tidal river sediments. Fe(III) was not reduced when the various enrichment media were not inoculated or when inoculated enrichments were autoclaved (121°C for 15 min) prior to incubation. Detailed stoichiometric studies of substrate metabolism and Fe(III) reduction await isolation of the bacteria, but with all substrates measured (analysis for trimethylamine was not done) the reduction of Fe(III) was
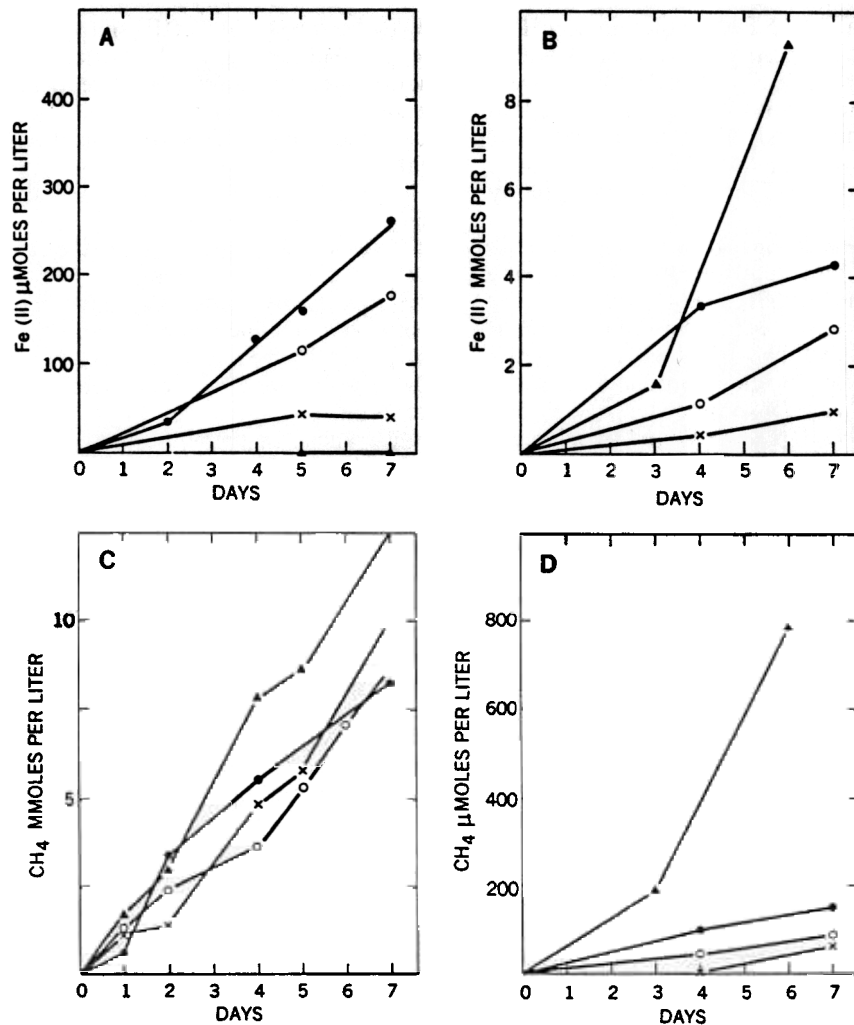
FIG. 2. Ferrozine-extractable Fe(II) and methane production in enrichments from the lower estuary with glucose (10 mM), acetate (20 mM), propionate (10 mM), or hydrogen (H_2-CO_2, 80:20) and hematite (A) and (C) or amorphous Fe(III) oxyhydroxide (B) and (D). Symbols: ●, glucose; ▲, acetate; ○, hydrogen; ×, propionate. Data are means of duplicate determinations on one representative culture from each treatment. Methane with hydrogen values were divided by 10 in panel (C). Note the differences in units on the y axes of the panels.
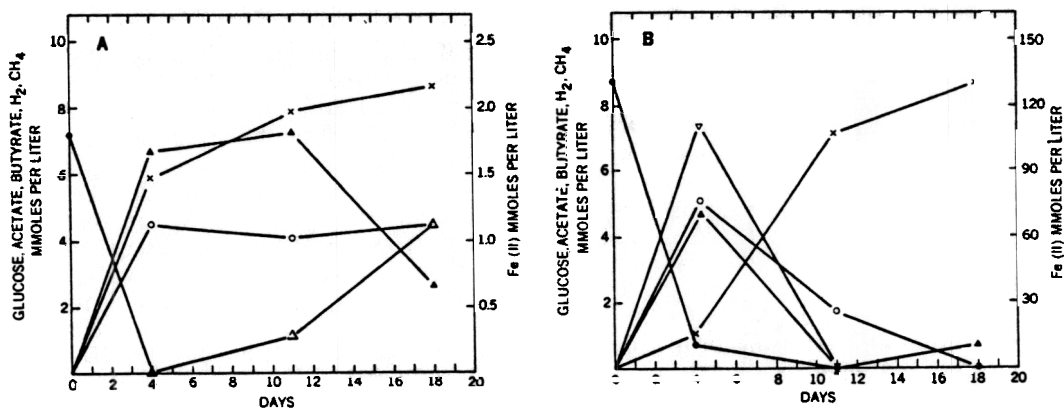

FIG. 3. Glucose metabolism in tidal river enrichments with hematite (A) or amorphous Fe(III) oxyhydroxide (B). Symbols: ●, glucose; ×, Fe(II); ▲, acetate; ○, butyrate; ▽ hydrogen; △, methane. Data are means of duplicate determinations on one culture from each treatment that was representative of four replicates. Note the difference in the scales for Fe(II). No hydrogen was produced in panel (A). No methane was produced in panel (B).

associated with a decrease in substrate concentration. Attempts to obtain enrichments with methane as the electron donor were unsuccessful. Fe(III) reduction was active in enrichments with such fermentable substrates as cellulose, trypticase, and benzoate (data not shown).

Fe(III) reduction in sediments. When freshly collected tidal river surface sediments were incubated under anaerobic conditions, Fe(II) accumulated (Table 2). The rate of Fe(II) accumulation was greatest during the initial 5 days of incubation and then declined with time. Sediments incubated for more than 30 days did not have measurable rates of Fe(II) accumulation. The addition of amorphous Fe(III) oxyhydroxide increased Fe(II) accumulation after the initial 14 days of incubation. Volatile fatty acids, hydrogen, and glucose stimulated Fe(III) reduction when amorphous Fe(III) oxyhydroxide was also added. Because the initial sulfate concentration in these sediments was 220 μM, only a minor fraction of the Fe(III) reduction could have been the result of metabolism of the substrates by sulfate reducers with the subsequent chemical reduction of Fe(III) by sulfide.

The potential for organic matter mineralization with concomitant Fe(III) reduction at in situ substrate concentrations was evaluated by assessing the ability of Fe(III) reduction to compete with methanogenic food chains for electron donors. Tidal river sediments were preincubated for 1 month or longer to deplete the Fe(III) available for reduction. The addition of amorphous Fe(III) oxyhydroxide to these sediments inhibited methane production from 50 to 90%, depending on the sediment sample. (Fig. 4 and 5; Table 3). However, when high concentrations of methanogenic substrates were provided, the rates of methane production in the sediments supplemented with Fe(III) were comparable to those in the controls (Fig. 4 and 5). Even after 15 days of incubation with added Fe(III), the potential for methane production from added acetate was equivalent to that in the controls, and added hydrogen was metabolized to methane

TABLE 2. Fe(II) production in tidal river sediments amended with electron donors and amorphous Fe(III) oxyhydroxide

Treatment[a]	Fe(II) produced (μmol/g of wet sediment)[b] after incubation for (days):		
	5	14	27
Control	9.4 ± 3.2	11.4 ± 0.3	14.0 ± 1.3
Control + Fe(III)	7.7 ± 3.2	13.6 ± 3.4	19.1 ± 0.3
Acetate	7.2 ± 1.2	13.2 ± 1.0	13.8 ± 1.1
Acetate + Fe(III)	7.2 ± 0.8	17.6 ± 0.7	28.5 ± 5.3
Propionate	8.2 ± 0.8	12.4 ± 0.3	15.1 ± 1.0
Propionate + Fe(III)	7.6 ± 1.4	16.0 ± 1.0	24.6 ± 1.2
Butyrate	7.8 ± 0.8	13.8 ± 1.7	14.9 ± 0.4
Butyrate + Fe(III)	6.3 ± 0.3	16.1 ± 1.2	32.7 ± 6.8
Hydrogen	9.8 ± 0.1	15.0 ± 0.4	16.5 ± 0.6
Hydrogen + Fe(III)	10.8 ± 0.6	20.1 ± 0.6	32.9 ± 0.6
Glucose	9.9 ± 0.1	13.2 ± 0.4	14.8 ± 0.4
Glucose + Fe(III)	10.0 ± 0.2	18.5 ± 0.4	28.4 ± 0.7

[a] Amendment amounts were 45 mmol of Fe(III), 20 mmol of acetate, 100 mmol of hydrogen, and 10 mmol of propionate, butyrate, and glucose per liter of wet sediment.

[b] Mean ± standard deviation (n = 3 for control, acetate, and propionate treatments; n = 2 for other treatments).

at a rate 80% of that in the controls after a brief lag (Fig. 4). A similar inhibition of methane production with the addition of amorphous Fe(III) oxyhydroxide and the relief of inhibition with added hydrogen or acetate was also observed in methane-producing sediments from the lower estuary which were collected from the interval in gravity cores immediately below the zone of sulfate reduction. The degree of inhibition of methane production with the various iron forms was amorphous Fe(III) oxyhydroxide > akaganeite > goethite > hematite (Fig. 5). None of the Fe(III) forms was toxic to methanogens because with the addition of hydrogen the rates of methane production in the Fe(III)-supplemented and control sediments were the same (Fig. 5). These results suggest that a lowered substrate availability inhibited methane production in the Fe(III)-supplemented sediments.

The diversion of electron flow from methane production to Fe(III) reduction was demonstrated by comparing the amounts of methane and Fe(II) produced in sediments with and without added Fe(III) (Table 3). The increase in the number of moles of Fe(II) produced in Fe(III)-supplemented sediments was ca. nine times the decrease in the number of moles of methane produced. Eight electron equivalents are required for the production of one mole of methane and one electon equivalent is required to reduce Fe(III) to Fe(II). Therefore, the addition of Fe(III) did not significantly alter the total quantity of organic matter mineralized, because the number of electron equivalents in the terminal products [Fe(II) and methane] was not significantly different in the two treatments. Reducing equivalents not used for methane production in sediments supplemented with Fe(III) were used to reduce Fe(III).

TABLE 1. Production of Fe(II) in enrichments with amorphous Fe(III) oxyhydroxide and various potential electron donors

Substrate[a]	Source of inoculum[b]	Fe(II) produced (mmol/liter) after incubation for (days):	
		7	14
None	LE		
	TR		
Butyrate	LE	6.3	27.4
	TR	0.0	24.0
Methanol	LE	0.3	2.5
	TR	3.5	28.1
Trimethylamine	LE	0.1	6.1
	TR	2.8	8.0
Ethanol	LE	28.1	46.3
	TR	22.2	80.8
Methane	LE	0.0	1.7
	TR	0.1	0.7

[a] Initial substrate concentrations: butyrate, 10 mM; methanol, 20 mM; ethanol, 20 mM; trimethylamine, 20 mM; methane, 101 kPa. Enrichments without added substrates did contain yeast extract as a potential electron donor. Results are the means of duplicate determinations on a single representative culture from each treatment.

[b] LE, Lower estuary; TR, tidal river.

DISCUSSION

The results demonstrate that Fe(III) reduction has the potential to be a major pathway for organic matter decomposition in anaerobic sediments. As much as 90% of the electron flow in methanogenic tidal river sediments was
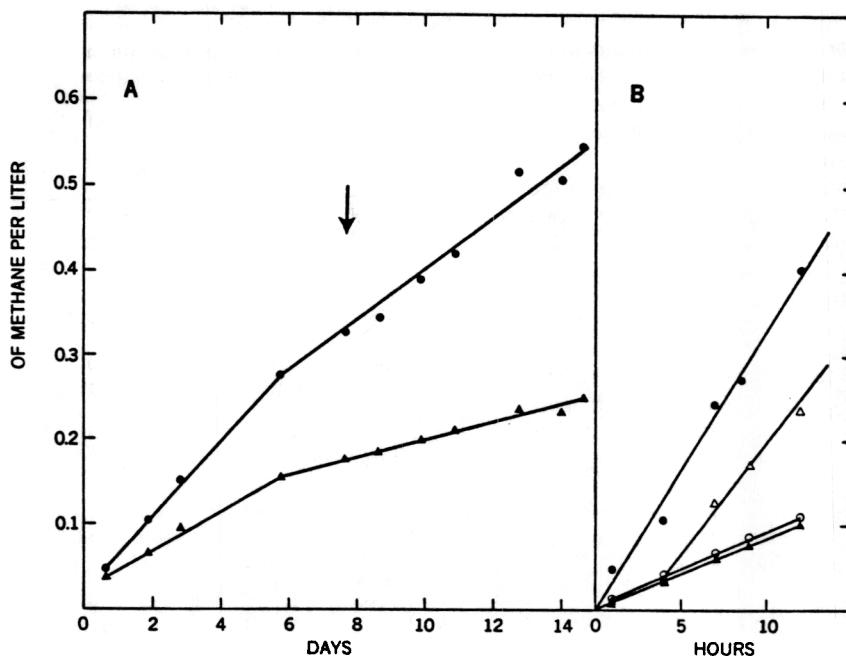
FIG. 4. Effect of addition of amorphous Fe(III) oxyhydroxide on methanogenesis in tidal river sediments. (A) Fe(III) (20 mmol/liter was added at time zero and where indicated by the arrow. Results are the means of six replicates for each treatment. Symbols: ●, controls with no added Fe(III); ▲, Fe(III) added. (B) After 15 days of incubation the headspace was flushed with N_2-CO_2 (93:7), and acetate (20 mM) or H_2-CO_2 (80:20, 101 kPa) was added. Results are representative examples of three replicates for each treatment. Symbols: ●, hydrogen added to controls; △, hydrogen added to Fe(III)-supplemented sediments; ○, acetate added to controls; ▲, acetate added to Fe(III)-supplemented sediments.

diverted from methane production to Fe(III) reduction when amorphous Fe(III) oxyhydroxide was added.

The diversion of electron flow to Fe(III) reduction with the addition of amorphous Fe(III) oxyhydroxide to methanogenic sediments demonstrates the capacity for Fe(III) reduction at in situ organic matter concentrations, but does not indicate the actual in situ electron donors for Fe(III) reduction. In contrast to studies on the substrates for sulfate reduction and methane production in sediments, studies on the in situ electron donors for Fe(III) reduction are limited by the lack of specific metabolic inhibitors (e.g., molybdate

for sulfate reduction and bromoethanesulfonic acid for methane production) and the lack of a unique carbon end product (i.e., methane for methane production). Because acetate and hydrogen are the major substrates for methane production in freshwater sediments (for a review, see reference 8a), the inhibition of methane production in Fe(III)-supplemented tidal river sediments suggests that acetate and hydrogen metabolism can be coupled with Fe(III) reduction at in situ concentrations of acetate and hydrogen. In addition, the metabolism of fermentable substrates and fermentation acids with concomitant Fe(III) reduction could result in lower
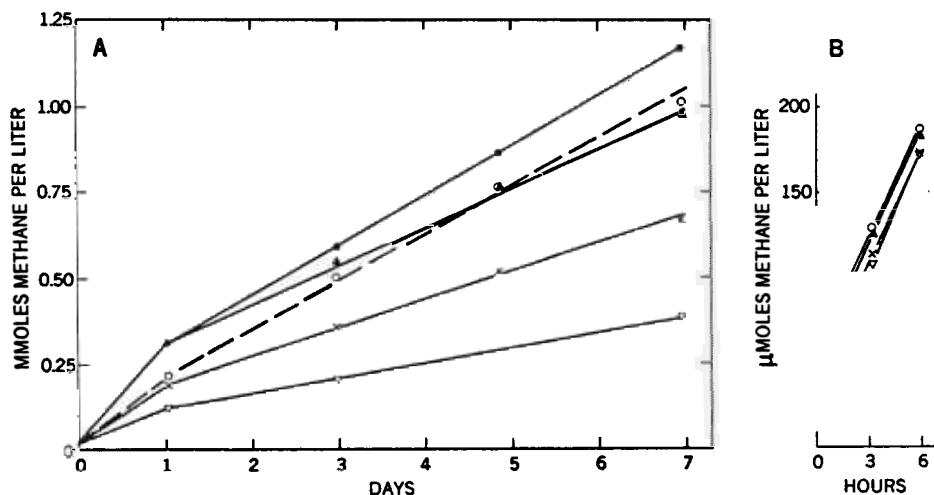

FIG. 5. Methane production in tidal river sediments supplemented with various Fe(III) forms. (A) Methane production during 7 days after Fe(III) supplementation. (B) After 7 days the headspace was flushed with N_2-CO_2, and H_2-CO_2 (80:20, 101 kPa) was added. Symbols [added Fe(III) concentrations in millimoles per liter]: ●, control (0); ○, hematite (100); ▲, goethite (100); ×, akaganeite (100), ▽, amorphous Fe(III) oxyhydroxide (50). In panel (B), control and hematite-supplemented sediments had the same methane values over time and are both indicated by ○ for clarity.

TABLE 3. Methane production and Fe(III) reduction in control and Fe(III)-supplemented tidal river sediments

Sediment treatment	Amt produced (μmol/g of wet sediment)[a]	
	Methane[b]	Fe(II)[c]
Control		
Fe(III)[d]		

[a] After 15 days of incubation; mean ± standard deviation ($n = 5$).

[b] Amount produced in control sediments minus amount produced in Fe(III)-supplemented sediments = 0.98 μmol/g.

[c] Amount produced in control sediments minus amount produced in Fe(III)-supplemented sediments = −9.28 μmol/g.

[d] Amorphous Fe(III) oxyhydroxide added to provide 50 μmol of Fe(III) per gram of wet sediment.

hydrogen and acetate production than when Fe(III) is not available. This would also diminish methane production.

The enrichment studies further demonstrated the potential for Fe(III) reduction with acetate and hydrogen, as well as with other fermentation products that are of lesser significance in sediments. These results indicate that when Fe(III) is in an amorphous form, Fe(III) reduction in sediments is not only a minor pathway for electron disposal by fermentative bacteria as was previously suggested (6). The products of fermentative Fe(III)-reducing bacteria and other fermenters can also be metabolized with concomitant Fe(III) reduction. The net result is a significant transfer of electrons to Fe(III) during the mineralization of organic matter.

The finding that Fe(III) reduction can outcompete methanogenic food chains for organic matter suggests a mechanism for the exclusion of methane production from the zone of Fe(III) reduction in sediments. The alternative explanation that Fe(III) is in some way toxic to methanogens was disproved. Methanogens were active in enrichment medium containing 250 mmol of Fe(III) per liter, and in sediments, the addition of methanogenic substrates relieved the inhibition of methane production by added Fe(III). The accumulation of Fe(II) before the reduction of sulfate in pore water depth profiles and incubated sediments suggests that Fe(III) can also outcompete sulfate reduction for electon donors (4, 8, 15). The mechanisms controlling competition between Fe(III) reduction and other anaerobic processes warrant further study.

Because fermentable substrates and fermentation products can be metabolized with concomitant Fe(III) reduction when Fe(III) is in a suitable form, the form, concentration, and availability of Fe(III) must be major factors controlling the proportions of carbon and electron flow that proceed through Fe(III) reduction in anaerobic sediments. The progressively greater inhibition of methane production in sediments by hematite, goethite, akaganeite, and amorphous Fe(III) oxyhydroxides corresponds to the suitability of these Fe(III) forms as electron acceptors for acetate metabolism. This indicates that the form of the Fe(III) controls the extent of organic matter mineralization with concomitant Fe(III) reduction at in situ organic matter concentrations, as well as

in enrichments. At least half of the nonsiliceous Fe(III) in freshwater and estuarine sediments is considered to be in an amorphous form (3, 12). The availability of the Fe(III) in sediments of the Potomac River Estuary as an electron acceptor for organic matter mineralization is currently under investigation.

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