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Competitive Mechanisms for Inhibition of Sulfate Reduction and Methane Production in the Zone of Ferric Iron Reduction in Sediments

DEREK R. LOVLEY* AND ELIZABETH J. P. PHILLIPS
Water Resources Division, U.S. Geological Survey, Reston, Virginia 22092

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Mechanisms for inhibition of sulfate reduction and methane production in the zone of Fe(III) reduction in sediments were investigated. Addition of amorphic iron(III) oxyhydroxide to sediments in which sulfate reduction was the predominant terminal electron-accepting process inhibited sulfate reduction 86 to 100%. The decrease in electron flow to sulfate reduction was accompanied by a corresponding increase in electron flow to Fe(III) reduction. In a similar manner, Fe(III) additions also inhibited methane production in sulfate-depleted sediments. The inhibition of sulfate reduction and methane production was the result of substrate limitation, because the sediments retained the potential for sulfate reduction and methane production in the presence of excess hydrogen and acetate. Sediments in which Fe(III) reduction was the predominant terminal electron-accepting process had much lower concentrations of hydrogen and acetate than sediments in which sulfate reduction or methane production was the predominant terminal process. The low concentrations of hydrogen and acetate in the Fe(III)-reducing sediments were the result of metabolism by Fe(III)-reducing organisms of hydrogen and acetate at concentrations lower than sulfate reducers or methanogens could metabolize them. The results indicate that when Fe(III) is in a form that Fe(III)-reducing organisms can readily reduce, Fe(III)-reducing organisms can inhibit sulfate reduction and methane production by outcompeting sulfate reducers and methanogens for electron donors.

Insight into the mechanisms that control the distribution of microbial redox processes is central to understanding the geochemistry of anaerobic aquatic environments. Three of the most important redox reactions in natural anaerobic environments are the oxidation of organic matter with the reduction of Fe(III), the oxidation of organic matter with the reduction of sulfate, and the conversion of organic matter to carbon dioxide and methane (21, 22, 28). These three processes are generally considered mutually exclusive, with no sulfate reduction or methane production until Fe(III) reduction is complete and no methane production until sulfate is depleted (3, 21, 22, 28).

There are three major steps in the metabolism of organic matter in sediments in which sulfate reduction or methane production is the terminal electron-accepting step (13, 26): (i) metabolism of fermentable substrates to the major fermentation products, acetate and hydrogen, and to minor products, such as propionate and butyrate; (ii) metabolism of fatty acids larger than acetate either by sulfate reducers or acetogenic proton-reducing bacteria; and (iii) metabolism of hydrogen and acetate by sulfate reducers or methanogens. When sulfate does not limit the metabolism of sulfate-reducing bacteria, they divert most of the carbon and electron flow away from the methanogenic food chain. A small part of the inhibition of methane production may result from sulfate reducers metabolizing fermentable subtrates (2, 25) as well as aromatic compounds and fatty acids larger than acetate (19, 26). However, significant quantities of acetate and hydrogen continue to be produced in the presence of sulfate (26). Thus, the inhibition of methane production by sulfate reduction ultimately depends on the ability of sulfate reducers to outcompete methanogens for hydrogen and acetate. Sulfate reducers prevent methane production from hydrogen and acetate by maintaining the concentrations of hydrogen and acetate at levels too low for methanogenic bacteria to metabolize them (9, 10, 13, 27). The minor methane production that is sometimes observed in the zone of sulfate reduction is primarily the result of methanogens metabolizing methylamines (7, 18).

A previous study indicated that Fe(III)-reducing bacteria can divert carbon and electron flow away from methanogenic food chains when Fe(III) is in the appropriate form (14). Addition of synthetic amorphic iron(III) oxyhydroxide to sediments in which methane production was the terminal electron-accepting process inhibited methane production 50 to 90%. The decrease in electron flow to methane production was completely compensated for by an increase in electron flow to Fe(III) reduction. Fe(III) was not toxic to methanogenic bacteria, as Fe(III) additions did not affect the potential for methane production from hydrogen and acetate when these substrates were added in excess. Field studies demonstrated that methane production was inhibited in freshwater sediments of the Potomac River until the microbially reducible Fe(III) in the sediments was reduced (15).

The purpose of this study was to determine the mechanisms by which sulfate reduction is inhibited in the zone of Fe(III) reduction. The results demonstrate that Fe(III)-reducing bacteria can outcompete sulfate-reducing as well as methanogenic food chains for organic matter in sediment. When Fe(III) is present as a coating of amorphic iron(III) oxyhydroxide on clay surfaces, Fe(III)-reducing bacteria maintain concentrations of hydrogen and acetate far below levels found in sediments in which sulfate reduction or methane production is the terminal electron-accepting process, and sulfate reduction and methane production are inhibited by over 95%. Since Fe(III) does not affect the potential of sulfate reducers and methanogens to metabolize when electron donors are available in excess, we concluded

* Corresponding author.

that the ability of Fe(III)-reducing bacteria to metabolize electron donors at concentrations below those that can be metabolized by sulfate reducers and methanogens is the major factor which inhibits sulfate reduction and methane production in the zone of Fe(III) reduction of sediments.

MATERIALS AND METHODS

Sediment source. Sediments were collected with an Eckman dredge at the previously described freshwater site in the Potomac River near the mouth of Gunston Cove (14). As previously described (15), the brown, flocculent surface sediments were collected and incubated under N_2-CO_2 (93:7, vol/vol) in 1-liter bottles, in the dark at 20°C, for a month or more to deplete sulfate and microbially reducible Fe(III). After this preincubation, methane production was the terminal electron-accepting process in these sediments.

Effect of Fe(III) on sulfate reduction. Sediment (ca. 150 ml) was transferred under N_2-CO_2 (93:7, vol/vol) into a 160-ml serum bottle which was then capped with a butyl rubber stopper (Bellco Glass, Inc., Vineland, N.J.). To establish a sediment in which sulfate reduction was the predominant terminal electron-accepting process, sodium sulfate was added to a final sulfate concentration of 5 mM. The addition of sulfate inhibited methane production by more than 95%. The sediments were incubated for 24 days, and 10-ml samples were then dispensed into 25-ml serum bottles under N_2-CO_2. The sulfate concentration at this time was 3.1 mM. Amorphic iron(III) oxyhydroxide [final concentration, 50 μmol of Fe(III) per g (wet weight) of sediment] was added to the sediment by adding 1 ml of a slurry of amorphic iron(III) oxyhydroxide particles under N_2-CO_2. The amorphic iron(III) oxyhydroxide was synthesized as previously described (14). Control sediments received 1 ml of water. Before being added to the sediments, both the slurry of amorphic iron(III) oxyhydroxide and the water were bubbled with N_2-CO_2 to remove O_2. Samples were withdrawn under N_2-CO_2 over time and were analyzed for Fe(II) and sulfate as described below.

An artificial sediment of clay coated with amorphic iron(III) oxyhydroxide was synthesized as previously described (14). The final concentration of Fe(III) on the clay was 1.8% (wt/wt). A slurry of the Fe(III)-coated clay (64% water by weight) was bubbled with N_2-CO_2 to remove O_2. Equal quantities (10 ml) of the slurry of Fe(III)-coated clay and the preincubated natural sediment were mixed in 25-ml serum bottles under N_2-CO_2. This provided a concentration of amorphic iron(III) oxyhydroxide that corresponded to the concentration of microbially reducible Fe(III) in the surface sediments of Gunston Cove (16). As a control without microbially reducible Fe(III), a clay suspension with a water content of 64% but without the Fe(III) coating was mixed with natural sediment. The bottles were sealed with butyl rubber stoppers. Methane concentrations were measured over time as outlined below. Preliminary studies had demonstrated that the addition of the suspension of clay without the Fe(III) coating had no effect on the rate at which natural sediments produced methane or on the potential for methane production with added excess hydrogen.

To examine the effect of Fe(III)-coated clay on sulfate reduction, sediment mixtures were prepared as described above. Sodium sulfate (2mM, final concentration) was then added from a 200 mM solution that had been bubbled with N_2-CO_2. Sediment samples were withdrawn over time for sulfate analysis as described below.

After 3 weeks of incubation in the presence of Fe(III)-coated clay, the potential for methane production and sulfate reduction was determined by transferring 3-ml samples of the sediment mixtures to 25-ml serum bottles under N_2-CO_2, sealing the bottle with a butyl rubber stopper and an aluminum crimp, adding 20 ml of hydrogen, and incubating the sediments horizontally on a wrist action shaker.

To measure hydrogen uptake by the sediments, 6-ml samples of sediment were transferred under N_2-CO_2 to anaerobic pressure tubes (Bellco Glass). The tubes were sealed with a butyl rubber stopper and an aluminum crimp. Hydrogen was added to a pressure of about 100 Pa in the headspace. The tubes were incubated horizontally on a wrist action shaker. Hydrogen was measured as outlined below.

All incubations were at 20°C in the dark.

Analytical techniques. The amount of Fe(II) was determined by the previously described method (14), in which Fe(II) is extracted with 0.5 N HCl and the HCl-soluble Fe(II) is measured with ferrozine.

The amount of methane was determined by gas chromatography with a flame ionization detector as previously described (15).

For hydrogen measurements, the gases were separated on a 1-m-long stainless steel column (⅛-in. [ca. 3.1-mm] outside diameter) packed with Carbosieve SII (100/120 mesh; Supelco, Inc.). The oven temperature was 80°C. The carrier gas was nitrogen at 20 ml/min. Hydrogen was measured with an RGD2 reduction gas detector (Trace Analytical). A hydrogen partial pressure of 1 Pa in the headspace is equivalent to approximately 9.9×10^{-6} atm of hydrogen or a dissolved hydrogen concentration of 8 nM.

For sulfate determinations, 1- to 1.5-ml samples of sediment were centrifuged and the supernatant was passed through a Versapor filter (0.45-μm pore size; Gelman Sciences, Inc., Ann Arbor, Mich.). Sulfate was determined by injecting a 25-μl sample of the filtrate onto a Supelcosil LC-IC column (4.6-mm inside diameter, 10 cm long; Supelco). The eluant was 3 mM phthallic acid (pH 5.0) at a flow rate of 1.0 ml/min. Sulfate was quantified by indirect photometric chromatography (24) by monitoring the A_{298} of the column effluent.

Acetate concentrations were determined as previously described (11). Briefly, interstitial water was collected by centrifugation and made basic (pH > 10) with NaOH. The water was evaporated. The acetate was dissolved in 200 μl of 10% phosphoric acid and quantified by gas chromatography with a flame ionization detector.

RESULTS

Addition of synthetic amorphic iron(III) oxyhydroxide to sediments in which sulfate reduction was the terminal electron-accepting process inhibited the rate of sulfate reduction about 90% (Fig. 1). Inhibition of sulfate reduction was accompanied by stimulation of Fe(III) reduction. Whereas the measured accumulation of Fe(II) in sediments without added Fe(III) was not greater than the analytical error in the Fe(II) measurements, there was a significant accumulation of Fe(II) over time in sediments with added Fe(III). Total electron equivalents proceeding to sulfate reduction and Fe(III) reduction in the sediments with added Fe(III) were 108% of the electron equivalents going to the terminal processes in control sediments after 9 days of incubation and were 92% after 22 days. Thus, the addition of Fe(III) did not alter overall rates of organic matter decomposition. Inhibition of sulfate reduction resulted from a diversion of electron flow away from sulfate reduction and to Fe(III) reduction. A similar experiment demonstrated that when amorphic

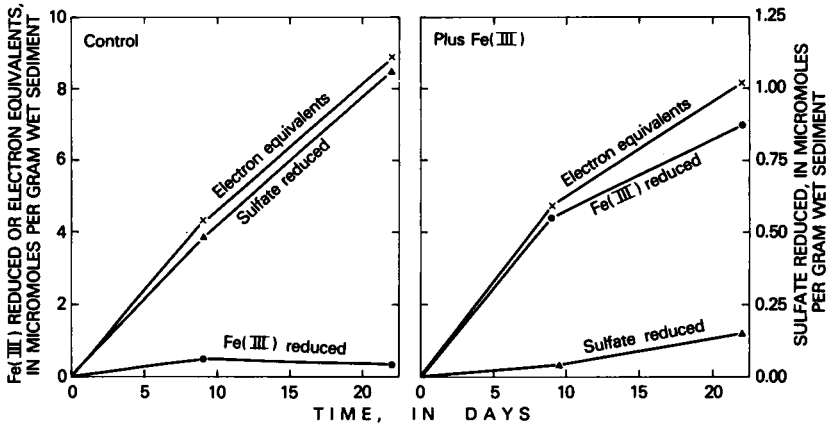

FIG. 1. Effect of added synthetic amorphic iron(III) oxyhydroxide on sulfate reduction. Electron equivalents were calculated as micromoles of Fe(III) reduced + (8 × micromoles of sulfate reduced). Datum points are means of five replicates of each sediment treatment.

iron(III) oxyhydroxide was added to methanogenic sediments, the decline in electron flow to methane production was compensated for by a corresponding increase in electron flow to Fe(III) reduction (14).

A potential mechanism for the decrease in the rate of sulfate depletion in sediments with added Fe(III) was the continued reduction of sulfate in the presence of Fe(III), but with the sulfide produced reoxidized to sulfate with the reduction of Fe(III). Such a mechanism, rather than a direct diversion of electron flow from sulfate reduction to Fe(III) reduction, could theoretically yield the results shown in Fig. 1. To test for Fe(III) oxidation of sulfide to sulfate, Fe(III) and sulfide were added to reduced sediments similar to those used for the experiment reported in Fig. 1, but which had not received any sulfate additions. The initial sulfate concentration was 30 μM. Sulfate remained at 30 μM following the addition of Fe(III) (50 μmol/g [wet weight] of sediment). The addition of sulfide (1 mM, final concentration) did not change the sulfate concentration either immediately or after 3 days of incubation. These results indicated that, in the study reported in Fig. 1, Fe(III) did not reoxidize sulfide to sulfate.

To more closely simulate the amorphic iron(III) oxyhydroxide found in sediments, a synthetic sediment consisting of amorphic iron(III) oxyhydroxide coated on clay was generated. When a slurry of the Fe(III)-coated clay was mixed with natural sediments, both sulfate reduction and methane production were inhibited (Fig. 2). However, sediments receiving additions of clay without the Fe(III) coating actively reduced sulfate (Fig. 2A) or produced methane if sulfate was not added (Fig. 2B). In sediments without added sulfate, the methane produced in sediments with added Fe(III) was less than 4% of the methane produced in sediments without added Fe(III). Sediments which received sulfate additions, with or without added Fe(III), produced methane at the same rate as the sediments with added Fe(III) (data not shown), which suggested that methanogens were metabolizing small quantities of noncompetitive substrates (7, 18) in the presence of Fe(III) or sulfate.

When the sediments contained added clay, we were unable to measure Fe(II) with sufficient precision to quantitatively estimate the rate of Fe(III) reduction. However, Fe(III) was reduced in the sediments with added amorphic iron(III) oxyhydroxide coated on clay, as shown by a progressive loss of the tan color characteristic of amorphic iron(III) oxyhydroxide.

Inhibition of sulfate reduction and methane production in the presence of Fe(III) was the result of substrate limitation rather than a direct toxic effect of amorphic iron(III) oxyhydroxide on sulfate-reducing or methanogenic bacteria. Even after 3 weeks of incubation in the presence of Fe(III), sulfate was reduced and methane was produced when hydrogen was added to subsamples of the sediments (Fig. 2).

Sediments with amorphic iron(III) oxyhydroxide coated onto clay as the electron acceptor had much lower steady-state concentrations of hydrogen and acetate than did sediments in which sulfate reduction was the terminal electron-accepting process (Table 1). Methanogenic sediments had higher hydrogen and acetate concentrations than did sulfate-reducing sediments. The hydrogen partial pressures in sulfate-reducing and methanogenic sediments without added clay were 0.08 ± 0.007 Pa and 0.80 ± 0.04 Pa (n = 3), respectively. This demonstrated that the added clay without Fe(III) had no effect on the hydrogen concentrations maintained by sulfate reducers and methanogens.

The lower acetate and hydrogen levels in sediments with Fe(III) could be attributed to the ability of the Fe(III)-reducing organisms to metabolize these substrates to lower concentrations. When hydrogen (Fig. 3) or 100 μM acetate (data not shown) were added to the Fe(III)-reducing sediment, the hydrogen and acetate were rapidly consumed and the amounts of hydrogen and acetate returned to concentrations characteristic of Fe(III)-reducing sediments.

DISCUSSION

The results demonstrate that competition for electron donors among the organisms that catalyze Fe(III) reduction, sulfate reduction, and methane production is a major factor controlling the distribution of these three important anaerobic redox reactions. When Fe(III) was present in the appropriate form, Fe(III)-reducing organisms diverted electron flow away from sulfate reduction and methane production even though there were populations of potentially active sulfate reducers and methanogens in the sediments used in this study. It is expected that in the Fe(III) reduction zone of most natural sediments, Fe(III)-reducing organisms will face even less competitive pressure from sulfate reducers and methanogens than in the studies reported here, because, before the onset of Fe(III) reduction, the oxygen, nitrate, and Mn(IV) present will inhibit the growth of sulfate reducers and methanogens (28).

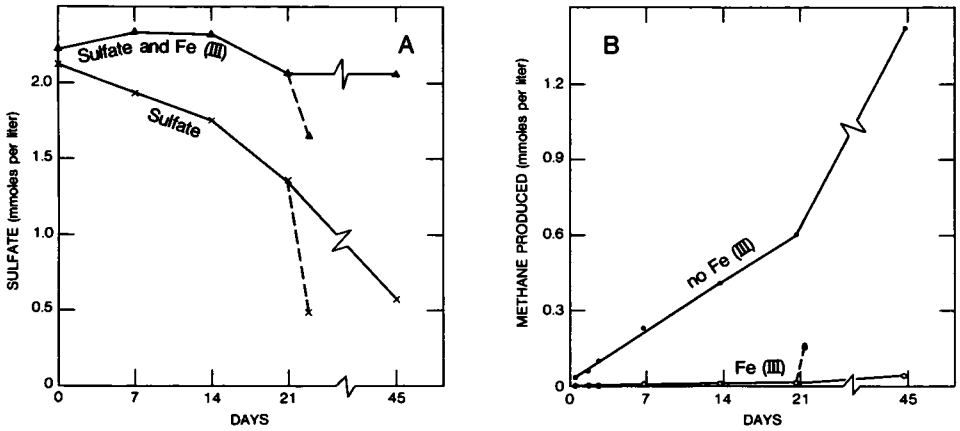

FIG. 2. Effect of amorphic iron(III) oxyhydroxide-coated clay on sulfate reduction and methane production. (A) Sulfate depletion in sediments that received additions of sulfate and clay suspension with or without Fe(III) coating. (B) Methane production in sediments that received additions of a clay suspension with or without Fe(III) coating. The dashed lines indicate sulfate depletion and methane production in subsamples of sediment that, at ca. 21 days of incubation, were incubated under excess hydrogen. Because the reaction rates were not completely synchronous in replicate treatments, the data presented are from one bottle of each sediment treatment and are representative of triplicates of each treatment.

Effect of Fe(III) form on competition. Fe(III) reduction, sulfate reduction, and methane production are not necessarily mutually exclusive. The proportion of carbon and electron flow that proceeds to Fe(III) reduction is as dependent on the form of the Fe(III) present as it is on the Fe(III) concentration. Crystalline Fe(III) forms, which Fe(III) reducers can only slowly reduce, do not permit Fe(III) reducers to effectively compete for electron donors in sediments (14). With discrete amorphic iron(III) oxyhydroxide particles as the Fe(III) form, Fe(III) reduction was either the dominant or an important electron-accepting process, but sulfate reduction and methane production coexisted with Fe(III) reduction under these conditions (14; this study). Fe(III) reducers competed most effectively when Fe(III) was present as a coating of amorphic iron(III) oxyhydroxide on clay.

Most of the amorphic iron(III) oxyhydroxide in natural sediments probably exists as a coating on clay (4). The clay may make Fe(III) more biologically available by stabilizing it in an amorphic form (8). Also, more surface area of the amorphic iron(III) oxyhydroxide may be exposed in clay coatings than when the amorphic iron(III) oxyhydroxide is in discrete particles.

The effect of the Fe(III) form on the ability of Fe(III)-reducing organisms to compete with methanogens and sulfate reducers is evident in sediments in the Potomac River estuary. Selective extraction techniques (16) have suggested that amorphic iron(III) oxyhydroxide, presumably present as a coating on clay, is the form of Fe(III) which permits Fe(III)-reducing bacteria to outcompete methanogenic bacteria in the top centimeter of freshwater sediments of the Potomac River (15). However, below the surficial sediments, Fe(III) reducers cannot outcompete methane production (freshwater site) or sulfate reduction (brackish-water site), even though the sediments contain high concentrations of Fe(III), because the Fe(III) is in forms unavailable for microbial reduction (15, 16, 20).

Competition for electron donors. Fe(III)-reducing organisms may divert electron flow from sulfate reduction and methane production at several levels of metabolism of organic matter. Fe(III)-reducing organisms that metabolize fermentable substrates with Fe(III) reduction (6, 17, 23) may divert to Fe(III) reduction some electron flow that otherwise

TABLE 1. Hydrogen and acetate concentrations in sediments in which methane production, sulfate reduction, or Fe(III) reduction was the predominant terminal process

Sediment type	Concn of:	
	Hydrogen (Pa)	Acetate (μM)
Methane producing[a]	0.82 ± 0.07[b]	5.2 ± 0.8
Sulfate reducing[c]	0.17 ± 0.09	2.2 ± 0.2
Fe(III) reducing[d]	0.03 ± 0.02	0.5 ± 0.1

[a] Sediments amended with clay only.
[b] Mean ± standard deviation (n = 3).
[c] Sediments amended with clay and 2 mM sulfate.
[d] Sediments amended with clay coated with amorphic iron(III) oxyhydroxide.

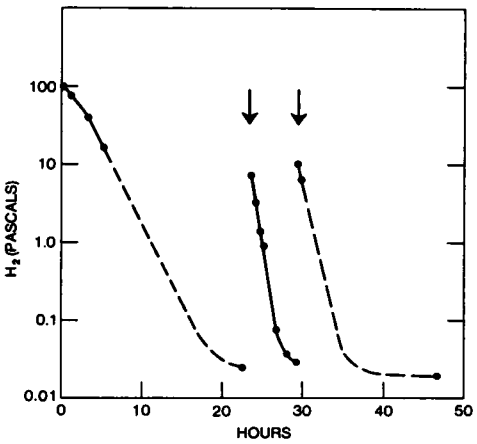

FIG. 3. Hydrogen uptake in sediments containing amorphic iron(III) oxyhydroxide-coated clay. Hydrogen was added at the start of incubation and at the times indicated with arrows. Because reaction rates were not completely synchronous in replicate treatments, the data presented are from one tube of sediment and are representative of results observed in five individual incubations.

would go to sulfate reduction or to fermentation products. However, the evidence indicates that no single Fe(III)-reducing organism is capable of completely oxidizing a fermentable substrate, such as glucose, to carbon dioxide. The known Fe(III)-reducing organisms which metabolize fermentable substrates transfer only a minor portion (1 to 3%) of the electron equivalents in fermentable substrates to Fe(III) (9a). Most of the electron equivalents from the fermentable substrates are found in fermentation products. These findings suggest that for Fe(III)-reducing organisms to significantly inhibit sulfate reduction and methane production, they must outcompete sulfate reducers and methanogens for fermentation products.

Fe(III)-reducing organisms capable of growing on hydrogen (1, 5) and acetate (D. R. Lovley, J. F. Stolz, G. L. Nord, and E. J. P. Phillips, manuscript submitted) have been isolated, and minor fermentation products such as butyrate and ethanol support Fe(III) reduction in enrichment cultures (14). Although Fe(III) reducers may divert some electron flow from sulfate reduction and methane production via the metabolism of minor fermentation products, competition for the major fermentation products, hydrogen and acetate, is expected to be most significant.

The results presented here are consistent with the hypothesis that Fe(III)-reducing organisms can prevent hydrogen and acetate uptake by sulfate reducers and methanogens by maintaining the concentration of hydrogen and acetate at levels too low for those organisms to metabolize. A lack of electron donors caused inhibition of sulfate reduction and methane production in the presence of added Fe(III), since the processes were restored with the addition of hydrogen or acetate (14) (Fig. 2 and data not shown). The steady-state concentrations of hydrogen and acetate in sediments in which Fe(III) reduction was the terminal electron-accepting process were much lower than those in sulfate-reducing or methanogenic sediments (Table 1). This was the result of hydrogen and acetate metabolism by Fe(III) reducers because, as previously discussed (10), the steady-state concentrations of intermediates such as hydrogen and acetate are solely dependent on the physiological characteristics of the organisms consuming the intermediates. Studies of uptake of added hydrogen and acetate confirmed the ability of Fe(III)-reducing organisms to metabolize hydrogen and acetate to concentrations well below those observed in sulfate-reducing and methanogenic sediments.

Geochemical implications. In addition to providing a conceptual model to aid in the understanding of the distribution of Fe(III) reduction, sulfate reduction, and methane production in anaerobic aquatic environments, the results presented here give further evidence for the concept (10, 13) that each hydrogen- and acetate-consuming process in anaerobic environments has characteristic hydrogen and acetate concentrations associated with it under steady-state conditions. Sulfate-reducing and methanogenic sediments from a eutrophic lake had hydrogen concentrations similar to those reported here (10). Kinetic analysis of acetate uptake (12) as well as theoretical considerations (13) previously indicated that the concentration of acetate should be lower in sulfate-reducing sediments than in methanogenic sediments. From these results, we predict that the profiles of hydrogen and acetate in sediments should follow a progression with depth from lowest concentrations in the Fe(III)-reducing zone, to higher concentrations as terminal metabolism switches from Fe(III) reduction to sulfate reduction, to highest concentrations in the methanogenic zone. Depth profiles which are qualitatively consistent with this pattern have recently been reported (K. M. Kuivila, Ph.D. dissertation, University of Washington, Seattle, 1986), but further investigation is warranted.

In summary, electron donor competition among Fe(III)-reducing, sulfate-reducing, and methanogenic bacteria controls the distribution of Fe(III) reduction, sulfate reduction, and methane production in anaerobic environments. When electron donors and acceptors are not limiting to these three processes, they can take place simultaneously. However, the rate at which fermentative bacteria can metabolize the complex particulate organic matter in sediments appears to limit the rate of supply of electron donors to the terminal electron-accepting processes, as shown by the finding that the rate of electron flow from organic matter to terminal processes is the same whether Fe(III), sulfate, or carbon dioxide is the electron acceptor (12, 14, 15; this study). When sufficient microbially reducible Fe(III) is available, Fe(III) reducers outcompete sulfate-reducing and methanogenic food chains for the electron donors. When sulfate is not limiting, sulfate reduction outcompetes methane production.

Whether similar competitive mechanisms influence the interactions of Mn(IV) and nitrate reduction with Fe(III) reduction, sulfate reduction, and methane production needs to be determined.

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