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Availability of Ferric Iron for Microbial Reduction in Bottom Sediments of the Freshwater Tidal Potomac River

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The distribution of Fe(III), its availability for microbial reduction, and factors controlling Fe(III) availability were investigated in sediments from a freshwater site in the Potomac River Estuary. Fe(III) reduction in sediments incubated under anaerobic conditions and depth profiles of oxalate-extractable Fe(III) indicated that Fe(III) reduction was limited to depths of 4 cm or less, with the most intense Fe(III) reduction in the top 1 cm. In incubations of the upper 4 cm of the sediments, Fe(III) reduction was as important as methane production as a pathway for anaerobic electron flow because of the high rates of Fe(III) reduction in the 0- to 0.5-cm interval. Most of the oxalate-extractable Fe(III) in the sediments was not reduced and persisted to a depth of at least 20 cm. The incomplete reduction was not the result of a lack of suitable electron donors. The oxalate-extractable Fe(III) that was preserved in the sediments was considered to be in a form other than amorphous Fe(III) oxyhydroxide, since synthetic amorphous Fe(III) oxyhydroxide, amorphous Fe(III) oxyhydroxide adsorbed onto clay, and amorphous Fe(III) oxyhydroxide saturated with adsorbed phosphate or fulvic acids were all readily reduced. Fe_3O_4 and the mixed Fe(III)-Fe(II) compound(s) that were produced during the reduction of amorphous Fe(III) oxyhydroxide in an enrichment culture were oxalate extractable but were not reduced, suggesting that mixed Fe(III)-Fe(II) compounds might account for the persistence of oxalate-extractable Fe(III) in the sediments. The availability of microbially reducible Fe(III) in surficial sediments demonstrates that microbial Fe(III) reduction can be important to organic matter decomposition and iron geochemistry. However, the overall extent of microbial Fe(III) reduction is governed by the inability of microorganisms to reduce most of the Fe(III) in the sediment.

Microbial reduction of ferric iron [Fe(III)] is a potentially important process in iron geochemistry and organic matter mineralization of aquatic sediments (7, 9, 13, 14, 16, 27, 32). However, the rates of Fe(III) reduction and factors controlling these rates have not been adequately assessed. The rate of Fe(III) reduction in marine sediments was measured with diluted sediments that were exposed to air prior to anaerobic incubation (27). This treatment oxidized ferrous iron [Fe(II)] and may have caused the formation of Fe(III) compounds that were not available in undisturbed sediments. Previous studies which estimated Fe(III) reduction from the accumulation of Fe(II) in the water overlying the sediment (13, 24, 31) may have seriously underestimated the rate of Fe(III) reduction, since much of the Fe(II) produced during the reduction of Fe(III) remains in solid forms (2, 23). Sedimentary iron profiles at some sites suggest significant Fe(III) reduction, but the persistence of solid iron with depth in other studies has suggested that much of the Fe(III) in sediments is resistant to microbial reduction (3, 6, 9, 15, 24, 31, 32).

The extent of Fe(III) reduction in the freshwater reach of the tidal Potomac River is of interest, since it can influence the pathways of organic-matter decomposition (16) and the exchange of phosphate and trace metals between the sediment and the overlying water (1, 10, 29). Since Fe(III) reduction can outcompete methanogenic (16) and sulfate-reducing food chains (manuscript in preparation) for organic matter in sediments in which Fe(III) is available for reduction, the availability of microbially reducible Fe(III) is expected to be the major factor controlling Fe(III) reduction in anaerobic, nitrate-depleted sediments. Surface-sediment samples from a freshwater site in the tidal Potomac River that were collected under aerobic conditions contained microbially reducible Fe(III) (16), and other studies (3, 11) have suggested that deeper sediments may contain amorphous Fe(III) oxyhydroxides, a form of Fe(III) that Fe(III)-reducing bacteria in these sediments readily reduce (16).

The purpose of the study reported in this paper was to determine the distribution of Fe(III) and its availability for microbial reduction in freshwater sediments of the Potomac River Estuary. The results indicate that, although there are sufficient quantities of microbially reducible Fe(III) for Fe(III) reduction to be an important pathway for anaerobic electron flow in sediments near the sediment-water interface, most of the sediment Fe(III) is resistant to microbial reduction and persists with depth in the sediment. Potential reasons for the inability of microorganisms to reduce this Fe(III) were investigated.

MATERIALS AND METHODS

Sediment incubations. Sediment samples were collected from the freshwater reach of the tidal Potomac River at the previously described site in Gunston Cove (16). For depth profiles, sediments were sampled with a Benthos gravity corer that was fitted with an aluminum pole for coring by hand and plastic core liners (inner diameter, 6.7 cm). Within 30 min of collection, the sediments were placed in a portable extrusion apparatus (W. Andrle and E. Callender, manuscript in preparation). Under N_2, the sediment was extruded from the top of the core, and appropriate depth intervals were transferred to 25-ml serum bottles which were then capped with butyl rubber stoppers (Bellco Glass, Inc.). Upon return to the laboratory (after ca. 2 h), the headspaces

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of the bottles were flushed with N_2-CO_2 (93:7). All sediment incubations were at 20°C in the dark.

Surface sediment samples were also collected with an Eckman dredge. Canning jars were completely filled with brown flocculent surface sediments and sealed with a lid. The sediments were transferred to 1-liter or 500-ml Erlenmeyer flasks under a stream of N_2-CO_2 (93:7), homogenized by shaking, dispensed into 120- or 25-ml serum bottles, and incubated for at least 1 month to deplete the microbially reducible Fe(III).

A 100-ml subsample of this sediment was exposed to air with continuous mixing with a magnetic stir bar for 1 week at room temperature. Deionized water was added periodically to prevent drying of the sediment. The sediments that had been exposed to air were mixed in 25-ml serum bottles under N_2-CO_2 (93:7) with an equal volume (10 ml) of sediments that had been maintained under anaerobic conditions. The anaerobic sediments were added to ensure that Fe(III)-reducing organisms were present.

The capacity of sediments that were depleted of microbially reducible Fe(III) to reduce amorphous Fe(III) oxyhydroxide and amorphous Fe(III) oxyhydroxide with adsorbed phosphate was determined by adding 0.6 g of a thick slurry of the Fe(III) forms to 25-ml serum bottles. The bottles were flushed with N_2-CO_2 (93:7) for 5 min, and 10 ml of sediment was added.

Enrichment culture. The previously described enrichment culture which metabolizes acetate with the concomitant reduction of amorphous Fe(III) oxyhydroxide (16) was subcultured for more than 15 transfers (10% inoculum) and then used to inoculate media with various Fe(III) forms. The previously described (16) freshwater mineral medium with yeast extract (0.05 g/liter) and a N_2-CO_2 (80:20) gas phase was supplemented with a final concentration of 50 mM sodium acetate. Incubations were at 30°C in the dark.

Fe(III) forms. Amorphous Fe(III) oxyhydroxide, akaganeite, and goethite were synthesized as described previously (16) and added to enrichment medium at final concentrations of 200 to 300, 240, and 140 mmol of Fe(III) per liter, respectively. Hematite (Fe_2O_3 powder; J. T. Baker Chemical Co.), Fe_3O_4 powder (Alfa Products), and $FePO_4 \cdot 2H_2O$ (Alfa Products) were added at final concentrations of 350, 585, and 210 mmol of Fe(III) per liter, respectively.

Ferrihydrite was formed from microbial metabolism of ferric citrate (26). The medium contained (in grams per liter of deionized water) the following: ferric citrate, 5.0; NH_4Cl, 0.4; NaH_2PO_4, 0.15; $NaHCO_3$, 0.05; $CaCl_2$, 0.05; KCl, 0.05; NaCl, 0.05; $MgCl \cdot 6H_2O$, 0.05; $MgSO_4$, 0.05; and yeast extract, 0.025. The medium was adjusted to pH 7, inoculated with 0.2 g of sediment per liter, and incubated at room temperature with stirring on a magnetic stirrer for 3 days. The solid Fe(III) was collected by centrifugation and added to acetate enrichment medium at 200 mmol of Fe(III) per liter.

Amorphous Fe(III) oxyhydroxide was coated onto clay as previously described (18). Colloidal kaolinite (mean particle diameter, 1 μm; Fe_2O_3 content, 0.3%) (Fisher Scientific Co.) was suspended in 0.2 M $FeCl_3$. The pH was adjusted to 3.5 with NaOH. After 30 min, the pH was adjusted to 7. The clay was collected and washed, with centrifugation, until the chloride concentration in the supernatant was less than 1 mM. The final concentration of Fe(III) on the clay was 1.8% (wt/wt), which was comparable to the oxalate-extractable Fe(III) content of the sediments. Fe(III)-coated clay was added to acetate enrichment medium at 65 mmol of Fe(III) per liter.

Fulvic acids were extracted from the sediment with 0.5 N NaOH and passed over a cation exchange column as previously described (25). Amorphous Fe(III) oxyhydroxide was saturated with adsorbed fulvic acids by mixing a solution of the fulvic acids and a slurry of amorphous Fe(III) oxyhydroxide at pH 7. Adsorption of the fulvic acids was noted by the removal of the yellow color from the filtrate (Nuclepore filter; pore diameter, 0.2 μm) of this mixture. More fulvic acid solution was added to the mixture until a strong yellow color could be detected in the filtrate. The Fe(III) was collected by centrifugation and added to enrichment medium at 225 mmol of Fe(III) per liter.

Phosphate was adsorbed onto amorphous Fe(III) oxyhydroxide by suspending amorphous Fe(III) oxyhydroxide in excess 250 mM NaH_2PO_4 (pH 7). The Fe(III) was collected by centrifugation and washed with deionized water six times. The material had a molar ratio of Fe(III) to phosphate of 1.9.

Analytical techniques. HCl-extractable Fe(II) was determined as described previously (16). Approximately 0.1 ml from enrichments or 0.1 g of sediment was added to a preweighed vial containing 5 ml of 0.5 N HCl. The weight of the added sample was determined. After 15 min (enrichments) or 1 h (sediments) at room temperature, 0.1 ml of the mixture was added to 5 or 10 ml of ferrozine (1 g/liter) in 50 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) buffer (pH 7). After being mixed for 15 s, the mixture was filtered through a Nuclepore filter (pore diameter, 0.2 μm), and the A_{562} was determined (28). Fe(II) standards were prepared from ferrous ethylenediammonium sulfate. Fe(II) was not oxidized and Fe(III) was not reduced during this procedure, as determined by additions of $FeCl_2$ and amorphous Fe(III) oxyhydroxide to enrichments and sediments in control experiments. Preliminary experiments indicated that the HCl extraction method was superior to the previously described ferrozine extraction method (27) in extracting solid Fe(II) forms that were produced from Fe(III) reduction.

The oxalate extraction method (17) was modified to measure oxalate-extractable Fe(III) (in preparation). Briefly, sediment (ca. 0.3 g) was added under a stream of N_2-CO_2 (93:7) to a preweighed and N_2-flushed 25-ml serum bottle which contained a Teflon-coated magnetic stir-star. The weight of the added sediment was determined. The bottle was wrapped in aluminum foil to exclude light. A N_2-flushed solution (10 ml) of 28 g of ammonium oxalate and 15 g of oxalic acid per liter of deionized water (final pH, 3.2) was added through the butyl rubber stopper. The bottles were placed on a magnetic stirrer overnight (ca. 16 h) at room temperature. A 0.1-ml portion was removed and added to ferrozine in HEPES buffer for determination of Fe(II), as outlined above. The remaining extract was filtered through a Nuclepore filter (pore diameter, 0.2 μm), diluted in 5% HCl, and analyzed for total iron by atomic absorption. Oxalate-extractable Fe(III) was calculated as the difference between total oxalate-extractable iron and oxalate-extractable Fe(II). Added Fe(II) ($FeCl_2$) was not oxidized and added Fe(III) [amorphous Fe(III) oxyhydroxide] was not reduced by this procedure.

Soluble reactive phosphate in filtered oxalate extracts was determined by the previously described method (22), in which excess ammonium molybdate is added to eliminate the interference of oxalate with the ascorbic acid-molybdenum blue assay (20).

Methane was analyzed by gas chromatography on a Shimadzu GC-mini 2 with a flame ionization detector. Gases

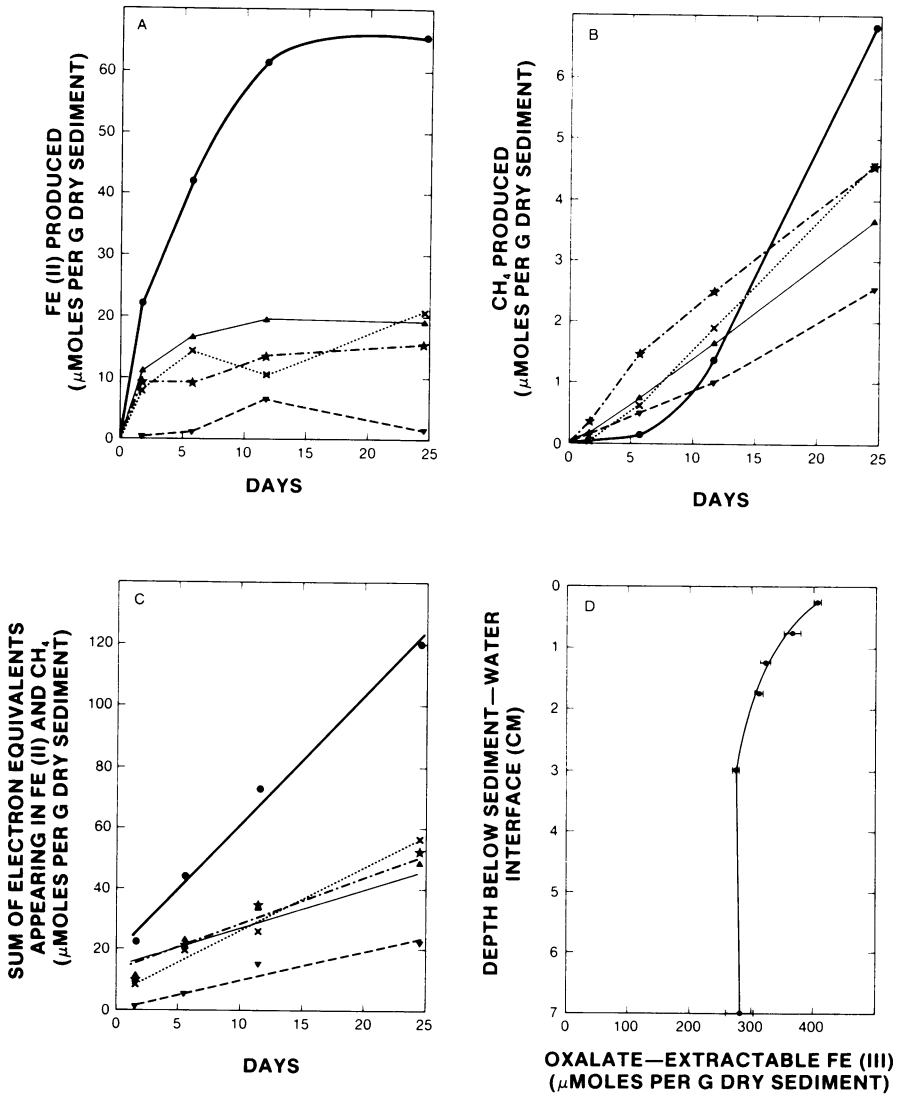

FIG. 1. (A) Fe(III) reduction, (B) methane production, (C) total terminal electron flow to Fe(II) and methane, and (D) depth profile of oxalate-extractable Fe(III) in Gunston Cove sediments sampled in October 1985. Initial HCl-extractable Fe(II) concentrations (in micromoles per gram) were 188, 235, 239, 247, and 215 for the 0- to 0.5-, 0.5- to 1.0-, 1.0- to 1.5-, 1.5- to 2-, and 2- to 4-cm intervals, respectively. The sum of electron equivalents was calculated from the stoichiometry of one electron per Fe(III) reduced and eight electrons per methane produced. Symbols: ●, 0 to 0.5 cm; ×, 0.5 to 1 cm; ▲, 1 to 1.5 cm; *, 1.5 to 2 cm; ▼, 2 to 4 cm.

were separated on a 1-m column of Porapak Q (Waters Associates, Inc.) at 120°C with N_2 (30 ml/min) as the carrier.

RESULTS

Fe(III) distribution and Fe(III) reduction in sediments. In incubated sediments, Fe(III) reduction was most active in the 0- to 0.5-cm depth interval (Fig. 1A). Although there was a slight increase in Fe(II) concentrations in the first 1.5 days of incubation in sediments from depths of between 0.5 and 2 cm, the increases after 1.5 days were 5% or less of the Fe(II) concentrations at 1.5 days. This is considered to be insignificant, since it is within the 6% error in Fe(II) determinations on sediments on sequential days. The change in the level of Fe(II) over the entire incubation period in the 2- to 4-cm interval (Fig. 1A) and lower depths (data not shown) was also not greater than the potential error in the Fe(II) measurements.

Methane was produced without a lag in the 1.0- to 1.5-, 1.5- to 2-, and 2- to 4-cm depth intervals (Fig. 1B). The active methane production in these sediments and those at deeper intervals (not shown) suggested that the lack of Fe(III) reduction at these depths after 1.5 days of incubation was not due to a lack of suitable electron donors for Fe(III) reduction, since Fe(III) reduction can utilize the same electron donors as methanogenic food chains (16).

There was an initial lag in methane production in the sediments from the 0- to 0.5- and 0.5- to 1.0-cm depth intervals (Fig. 1B). Although the rate of methane production was approximately linear in the 0.5- to 1.0-cm interval after 1.5 days, methane production lagged in the 0- to 0.5-cm interval until between 5 and 10 days of incubation, when the rate of Fe(III) reduction declined. The transition from

Fe(III) reduction to methane production in the 0- to 0.5-cm interval did not alter the rates of organic matter decomposition as the rate of electron flow from organic matter to terminal electron-accepting products [Fe(II), methane] was linear during the transition period (Fig. 1C). These results suggest that in the 0- to 0.5-cm depth interval, Fe(III) reduction effectively competed with methane production for electron donors until the Fe(III) that was available for microbial reduction was depleted.

The rate of electron flow from organic matter to the terminal electron-accepting products in the 0- to 0.5-cm interval was more than twice the rate in the rest of the upper 2 cm and more than four times higher than the rate in sediments deeper than 2 cm (Fig. 1C). Over the first 11.5 days of incubation (before the depletion of the microbially reducible Fe(III) in the 0- to 0.5-cm interval), the electron flow through Fe(III) reduction in the 0- to 0.5-cm depth interval alone was 92% of the electron flow to methane production in the entire upper 4 cm.

Oxalate-extractable Fe(III) declined over the top 4 cm and then remained constant (Fig. 1D). Corresponding with the decline in oxalate-extractable Fe(III) in the surface sediments was an increase in Fe(II) extractable in 0.5 N HCl (Fig. 1), which suggested that the loss of oxalate-extractable Fe(III) with depth was the result of the reduction of Fe(III) to Fe(II). Measurements made in June 1985 indicated that oxalate-extractable Fe(III) persisted to at least 20 cm (data not shown). If it is assumed that the rate of Fe(III) input to the surface sediments was constant over the period during which these sediments were deposited, then the profile of oxalate-extractable Fe(III) indicates that Fe(III) is reduced in situ in the upper 4 cm but not at lower depths. In the sediment incubations, less than 80% of the oxalate-extractable Fe(III) in the 0- to 0.5-cm depth interval (Fig. 1D) was reduced before the rate of Fe(III) reduction declined and active methane production started (Fig. 1A and B) and none of the nearly 300 μmol of oxalate-extractable Fe(III) per g of dry sediment in the 2- 4-cm depth interval were reduced. Thus, both the sediment incubations and the depth profiles of oxalate-extractable Fe(III) indicated that most of the oxalate-extractable Fe(III) in the sediments was not available for microbial reduction.

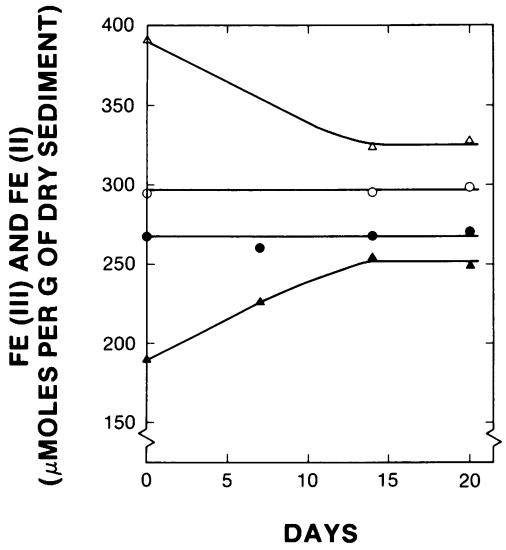

FIG. 2. HCl-extractable Fe(II) (●) and oxalate-extractable Fe(III) (○) in reduced sediments that had been preincubated to deplete microbially reducible Fe(III) prior to the incubation period shown here. Fe(II) (▲) and Fe(III) (△) were measured during anaerobic incubation of a mixture of equal volumes of oxidized and reduced sediment.

TABLE 1. Reduction of synthetic amorphous Fe(III) oxyhydroxide and synthetic amorphous Fe(III) oxyhydroxide with adsorbed phosphate in sediments preincubated to deplete microbially reducible Fe(III)

Addition	Amt of Fe(II)[a] after incubation for:	
	0 days[b]	14 days
None	286 ± 4	289 ± 3
Fe(III)[c]	297 ± 5	346 ± 6
Fe(III)-PO_4[d]	300 ± 1	374 ± 4

[a] Amount expressed in micromoles of Fe(II) per gram of dry sediment; mean ± standard deviation (n = 4).
[b] Fe(II) measured ca. 2 h after the addition of Fe(III).
[c] Amorphous Fe(III) oxyhydroxide added to give an initial concentration of 180 μmol of added Fe(III) per g of dry sediment.
[d] Amorphous Fe(III) oxyhydroxide saturated with adsorbed phosphate added to give an initial concentration of 200 μmol of added Fe(III) per g of dry sediment.

Similar distributions of Fe(III) and Fe(III) reduction were observed in sediments collected in February 1986 and June 1985.

Studies on Fe(III) availability. Surface sediments which had been incubated to deplete the microbially reducible Fe(III) still had substantial Fe(III) concentrations which were not reduced by extended incubation (Fig. 2). When these sediments were exposed to air, Fe(II) was oxidized to Fe(III). When the oxidized sediments were mixed with an equal volume of sediment that had been maintained under anaerobic conditions, more than half of the newly formed Fe(III) was reduced under anaerobic conditions within 15 days (Fig. 2). There was a 1:1 correspondence between the decrease in oxalate-extractable Fe(III) and the increase in HCl-extractable Fe(II) over time, which indicated that the oxalate extracts included all of the Fe(III) available for reduction and that the HCl extracts included all of the Fe(II) produced during Fe(III) reduction. Fe(III) was not reduced in mixtures of oxidized and reduced sediments if the sediment mixtures were autoclaved (121°C, 15 min) prior to incubation (data not shown).

The persistence of Fe(III) in the sediments shown in Fig. 2 was not the result of a lack of suitable electron donors for Fe(III) reduction, since added synthetic amorphous Fe(III) oxyhydroxide was actively reduced (Table 1). Adsorption of phosphate onto amorphous Fe(III) oxyhydroxide did not seem to be the factor limiting Fe(III) reduction in the sediment, since synthetic amorphous Fe(III) oxyhydroxide saturated with adsorbed phosphate was reduced as readily as unmodified amorphous Fe(III) oxyhydroxide (Table 1).

Potential reasons for oxalate extraction of Fe(III) that was unavailable for microbial reduction from sediments were investigated further under more defined conditions with the previously described enrichment culture which metabolizes acetate with the concomitant reduction of amorphous Fe(III) oxyhydroxide (16). There was a strong correlation between oxalate extractability and the ability of the culture to reduce pure Fe(III) oxides and oxyhydroxides (Fig. 3). Amorphous Fe(III) oxyhydroxide, which was oxalate extractable, was actively reduced until ca. half of the added Fe(III) was reduced (Fig. 3 and 4). The crystalline Fe(III) oxides and hydroxides were sparingly extracted in oxalate and poorly reduced (Fig. 3). Microbially produced ferrihydrite and

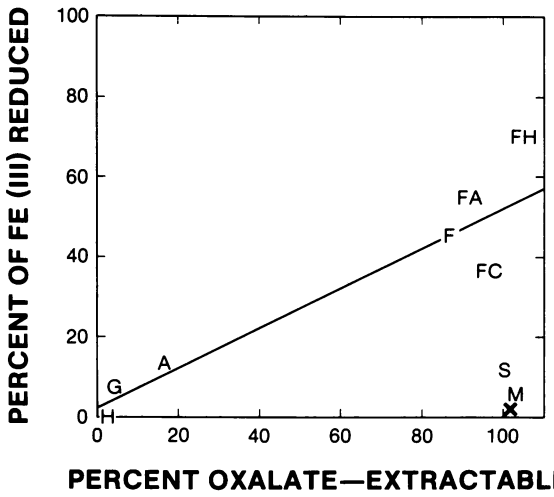

FIG. 3. Oxalate extractability of various Fe(III) forms and the percentage of the Fe(III) reduced to Fe(II) by the acetate enrichment culture after 55 or more days of incubation. Regression line (r^2 = 0.99) is for data from hematite, goethite, akaganeite, and amorphous Fe(III) oxyhydroxide. Symbols: H, hematite; G, goethite; A, akaganeite; F, amorphous Fe(III) oxyhydroxide; FC, amorphous Fe(III) oxyhydroxide adsorbed onto clay; FA, amorphous Fe(III) oxyhydroxide with adsorbed fulvic acids; FH, ferrihydrite; S, $FePO_4 \cdot 2H_2O$; M, Fe_3O_4; X, mixed Fe(III)-Fe(II) material produced in the enrichment culture from the reduction of amorphous Fe(III) oxyhydroxide.

amorphous Fe(III) oxyhydroxide with adsorbed fulvic acids were oxalate extractable and were actively reduced by the culture (Fig. 3 and 4). Less Fe(III) could be included in the culture medium when the Fe(III) source was amorphous Fe(III) oxyhydroxide adsorbed onto clay, because of the added volume of the clay. Despite this, the amorphous Fe(III) oxyhydroxide adsorbed onto clay was actively reduced without a lag (Fig. 4). The amorphous Fe(III) oxyhydroxide adsorbed onto clay was oxalate extractable, and the percentage of added Fe(III) that was ultimately reduced was comparable to that of unmodified amorphous Fe(III) oxyhydroxide (Fig. 3).

Reagent $FePO_4 \cdot 2H_2O$ did not fit the positive correlation between oxalate extractability and the availability of Fe(III) for microbial reduction, since the compound was highly oxalate extractable but was poorly reduced (Fig. 3). However, $FePO_4 \cdot 2H_2O$ can account for little of the oxalate-extractable Fe(III) that was unavailable for reduction in sediments, since the molar Fe(III)/phosphate ratio in oxalate extracts of the sediments shown in Fig. 2 was 9.4 ± 1.1 (n = 4), much higher than the ratio for $FePO_4 \cdot 2H_2O$ or other ferric phosphate minerals that might be found in freshwater sediments (11, 21).

The reduction of only ca. half of the added Fe(III) in cultures with amorphous Fe(III) oxyhydroxide (Fig. 3) was unexpected, since the initial acetate concentration in the medium should have provided enough reducing power for all of the Fe(III) to be reduced. The Fe(III) that persisted with extended incubation was in a magnetic, black solid. X-ray diffraction analysis indicated that the material contained magnetite (Fe_3O_4) and siderite ($FeCO_3$) (E. Landa, personal communication). The material was extracted in oxalate (Fig. 3). The Fe(III)/Fe(II) ratio of the extract was 1.6. The material was not reduced when incubated with fresh acetate enrichment medium and a new inoculum from an active enrichment (Fig. 4). Reagent Fe_3O_4 was also extracted in oxalate but was not reduced by the culture (Fig. 3).

DISCUSSION

The results demonstrate that, although the surficial sediments of Gunston Cove contain significant quantities of microbially reducible Fe(III), most of the oxalate-extractable Fe(III) in the sediments is not available for microbial reduction. Fe(III) reduction was restricted to the surficial sediments even though electron donors for Fe(III) reduction were available at greater depths and oxalate-extractable Fe(III) persisted to depths of at least 20 cm. Despite this restriction, Fe(III) reduction was as important a pathway for anaerobic electron flow as methane production when sediments from the 0- to 4-cm depth interval were incubated under anaerobic conditions, because the zone of Fe(III) reduction was within the most active zone of organic matter decomposition.

The microbially reducible Fe(III) in the surficial sediments is considered to be amorphous Fe(III) oxyhydroxide or the poorly ordered Fe(III) oxyhydroxide, ferrihydrite (5). None of the other Fe(III) forms that were added to enrichment cultures or sediments in this and a previous study (16) were readily reducible. The production of microbially reducible Fe(III) when reduced sediments were exposed to air further supports the above conclusion, since the oxidation of the Fe(II) in anaerobic pore water results in the production of amorphous Fe(III) oxyhydroxide (8).

Oxalate was initially chosen as the Fe(III) extractant because oxalate extracts the amorphous iron forms available for microbial reduction in soils (19) and because of the strong correlation between oxalate extractability and the suscepti-

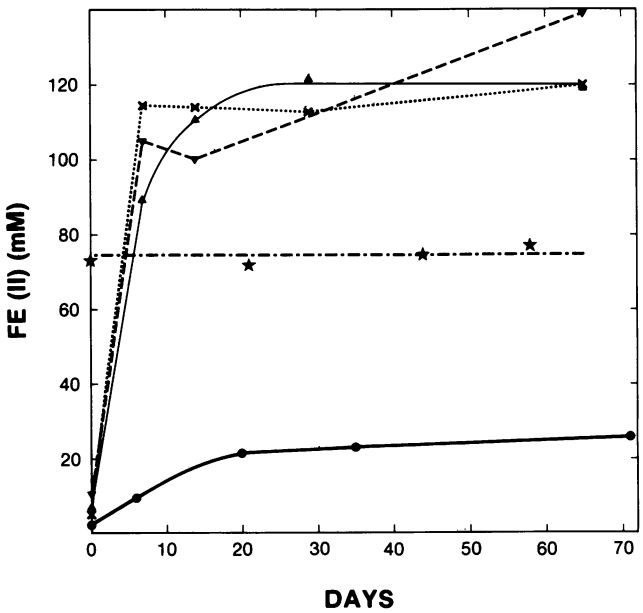

FIG. 4. HCl-extractable Fe(II) produced from the reduction of various Fe(III) forms in acetate enrichment cultures. Symbols: ×, amorphous Fe(III) oxyhydroxide; ●, amorphous Fe(III) oxyhydroxide coated on clay; ▲, amorphous Fe(III) oxyhydroxide with adsorbed fulvic acids; ▼, ferrihydrite; *, mixed Fe(III)-Fe(II) compounds produced in enrichment culture from the reduction of amorphous Fe(III) oxyhydroxide. Note that the initial Fe(III) concentration in the enrichment with Fe(III) coated on clay was only 65 mmol/liter compared with 200 mmol/liter or more for the other Fe(III) forms.

bility of pure Fe(III) oxides and oxyhydroxides to reduction in the enrichment culture. Although the microbially reducible Fe(III) in sediments is contained in oxalate extracts, oxalate extractability is not a good indicator of the availability of sediment Fe(III) for microbial reduction, since poorly reducible Fe(III) forms were also extracted.

A potential explanation of the resistance of oxalate-extractable sediment Fe(III) to reduction was that constituents of the sediment combine with amorphous Fe(III) oxyhydroxides and block Fe(III) reduction. Amorphous Fe(III) oxyhydroxide may be adsorbed onto clays and adsorb phosphate and organic matter in sediments (1, 4, 11, 12, 30). However, modification of synthetic amorphous Fe(III) oxyhydroxide to simulate these conditions did not alter the susceptibility of the amorphous Fe(III) oxyhydroxide to reduction. The resistance of the oxalate-extractable Fe(III) to microbial reduction suggests that, contrary to previous reports (3, 11), most of the oxalate-extractable Fe(III) in the sediments of the freshwater tidal Potomac River is not in any of the generally conceived forms of amorphous Fe(III) oxyhydroxide. If it were, Fe(III) reducers would reduce it, since the preservation of Fe(III) in the sediments is not the result of a lack of suitable electron donors.

The oxalate-extractable Fe(III) that is resistant to microbial reduction might be a mixed Fe(III)-Fe(II) compound. Thermodynamic calculations based on measurements of Fe(II), pH, and E_h suggest that mixed Fe(III)-Fe(II) compounds such as $Fe_4(OH)_{10}$ and $Fe_3(OH)_8$ are the predominant Fe(III) forms in reduced sediments and soils (2, 22). Like the Fe_3O_4 powder and the mixed Fe(III)-Fe(II) compound(s) produced from the reduction of amorphous Fe(III) oxyhydroxide in the enrichment culture, the mixed Fe(III)-Fe(II) compound(s) in reduced sediments might be oxalate extractable but unavailable for microbial reduction. If so, the enrichment culture with amorphous Fe(III) oxyhydroxide may provide a model for the preservation of oxalate-extractable Fe(III) in sediments. In this model, the oxalate-extractable Fe(III) at the sediment-water interface is primarily microbially reducible amorphous Fe(III) oxyhydroxide. As in the enrichment culture, the Fe(II) produced from Fe(III) reduction interacts with the remaining amorphous Fe(III) oxyhydroxide to form mixed Fe(III)-Fe(II) compounds that are oxalate extractable but that resist further reduction. Consistent with this model is the observation that oxalate extracts of sediments without readily reducible Fe(III) have a Fe(III)/Fe(II) ratio of ca. 1. Verification of this model, or any other, will require improved analytical techniques to define the structure of Fe(III) in anaerobic sediments. Preliminary X-ray diffraction and Mössbauer spectroscopy analyses of Gunston Cove sediments have failed to detect any Fe(III) minerals.

Definitive identification of the microbially reducible Fe(III) form in sediments is also necessary so that more accurate estimates of in situ rates of Fe(III) reduction can be made in short-term, whole-core incubations with the appropriate form of a radioactive Fe(III) tracer. Although the bottle incubations were an important adjunct to the depth profiles of oxalate-extractable Fe(III) for the identification of the zones where Fe(III) was available for reduction, this method suffers from the typical shortcomings of bottle incubations. Processes which require rapid replenishment of external electron acceptors (oxygen, nitrate, sulfate) were eliminated in the incubations. All of these processes could influence the in situ distribution and rates of Fe(III) reduction. Thus, there is as yet no verification that the rates of Fe(III) reduction in bottle incubations were the same as the rates in situ.

The limitation of the most active site of Fe(III) reduction to surficial sediments, despite the persistence of Fe(III) to greater depths, as found in Gunston Cove and another site in the freshwater tidal Potomac River (unpublished data), may be typical of freshwater environments. Flux measurements of Fe(II) indicate that the most active zone of Fe(III) reduction in Lake 227 sediments is at the sediment-water interface (7) and Fe(III), resistant to microbial reduction, persists in the upper 20 cm of these sediments (6).

In summary, most of the oxalate-extractable Fe(III) in the freshwater sediments of the tidal Potomac River is not available for microbial reduction. Active Fe(III) reduction is restricted to the surficial sediments even though oxalate-extractable Fe(III) persists to depths of at least 20 cm. The preservation of Fe(III) within the sediments provides a mechanism for the high capacity of anaerobic freshwater sediments in the Potomac River Estuary to retain phosphate (3). Although thin, the zone of active Fe(III) reduction is within the most active site of organic-matter decomposition and in the zone for exchange of phosphate and trace metals with the overlying water. Thus, microbial Fe(III) reduction can have an important role in the nutrient and metal dynamics of the Potomac River.

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