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Microbial Iron Redox Cycling in a Circumneutral-pH Groundwater Seep▽†

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The potential for microbially mediated redox cycling of iron (Fe) in a circumneutral-pH groundwater seep in north central Alabama was studied. Incubation of freshly collected seep material under anoxic conditions with acetate-lactate or H_2 as an electron donor revealed the potential for rapid Fe(III) oxide reduction (ca. 700 to 2,000 μmol liter^{-1} day^{-1}). Fe(III) reduction at lower but significant rates took place in unamended controls (ca. 300 μmol liter^{-1} day^{-1}). Culture-based enumerations (most probable numbers [MPNs]) revealed significant numbers (10^2 to 10^6 cells ml^{-1}) of organic carbon- and H_2-oxidizing dissimilatory Fe(III)-reducing microorganisms. Three isolates with the ability to reduce Fe(III) oxides by dissimilatory or fermentative metabolism were obtained (*Geobacter* sp. strain IST-3, *Shewanella* sp. strain IST-21, and *Bacillus* sp. strain IST-38). MPN analysis also revealed the presence of microaerophilic Fe(II)-oxidizing microorganisms (10^3 to 10^5 cells ml^{-1}). A 16S rRNA gene library from the iron seep was dominated by representatives of the *Betaproteobacteria* including *Gallionella*, *Leptothrix*, and *Comamonas* species. Aerobic Fe(II)-oxidizing *Comamonas* sp. strain IST-3 was isolated. The 16S rRNA gene sequence of this organism is 100% similar to the type strain of the betaproteobacterium *Comamonas testosteroni* (M11224). Testing of the type strain showed no Fe(II) oxidation. Collectively our results suggest that active microbial Fe redox cycling occurred within this habitat and support previous conceptual models for how microbial Fe oxidation and reduction can be coupled in surface and subsurface sedimentary environments.

Changes in iron (Fe) redox state are linked to carbon and energy flow as well as the behavior of various inorganic compounds in modern soils and sediments. Microorganisms play a pivotal role in the Fe redox cycle in such environments (29, 35, 39). A growing body of literature indicates that aerobic lithotrophic Fe(II)-oxidizing bacteria (FeOB) can contribute significantly to circumneutral-pH Fe(II) oxidation (4, 9, 15, 23, 25, 34) and that microbial catalysis can dominate Fe(II) oxidation in diffusion-limited reaction systems (32, 34). Microbial catalysis is strictly required for anaerobic nitrate-dependent Fe(II) oxidation (36), since an abiotic reaction between Fe(II) and nitrate does not take place under typical near-surface conditions (40).

Circumneutral-pH Fe(II) oxidation produces Fe(III) oxide mineral phases which can function as electron acceptors for anaerobic respiration by dissimilatory Fe(III)-reducing bacteria (FeRB) (8, 37). This metabolism is widespread among prokaryotic taxa (19) and plays a key role in oxidation of natural organic compounds and in the bioremediation of organic and metal contaminants in the subsurface (18). The coupling of Fe(III) oxide reduction to oxidation of organic carbon or H_2 leads to release of Fe(II) into the aqueous phase. When the oxidative and reductive parts of the Fe redox cycle come together with ongoing input of energy, a self-sustaining microbial community based on Fe redox cycling may develop. Sustained microbial Fe redox cycling has been proposed in various redox interfacial environments like groundwater Fe seeps (8), plant roots (10), the sediment-water interface in circumneutral-pH (29, 33) and acidic (24) aquatic ecosystems, and hot springs and hydrothermal vents (16a, 24a).

Here we present data that support the existence of a sustained microbial Fe redox cycle in a circumneutral-pH groundwater Fe seep in north central Alabama. Potential microbial involvement in Fe redox cycling was assessed by most probable number (MPN) enumerations, in vitro Fe(III) reduction experiments, and isolation of representative Fe(III)-reducing and Fe(II)-oxidizing microorganisms. A simple kinetic model was used to explore the impact that decay of dead chemolithotrophic biomass coupled to Fe(III) reduction could have on rates of Fe turnover.

MATERIALS AND METHODS

Field site description and sampling. Groundwater containing dissolved Fe(II) is seeping out from a sandstone outcrop near the University of Alabama in Tuscaloosa (see Fig. S1 in the supplemental material). A layer of precipitated Fe(III) oxide accumulates where the water comes into contact with the atmosphere. The source of the Fe(II) in the sandstone aquifer is unknown but is likely either pyrite oxidation or dissimilatory Fe(III)-reducing activity in the subsurface.

The pH and aqueous Fe content of the seep waters were measured on 11 occasions between October 2004 and April 2005. pH was measured with an Accumet model 20 (Fisher Scientific) pH meter equipped with an Orion combination electrode. Aqueous Fe(II) and Fe(III) concentrations were determined by adding 0.1 ml of 6 M HCl to 1 ml of filtered sample and speciating the Fe using Ferrozine as previously described (28). The Fe(II) and Fe(III) contents of the seep material itself were similarly determined by extracting 1-ml samples of homogenized material in 5 ml of 1 M HCl.

In vitro Fe(III) reduction experiments. Fresh seep material was collected in sterile 250-ml centrifuge bottles, and 10 g (wet weight) was transferred to sterile 50-ml serum bottles, which were closed with black rubber stoppers and degassed with N_2-CO_2 (80:20) for 15 min. Production of 0.5 M HCl-extractable Fe(II) was measured over time in the absence and presence of exogenous electron donors (5 mM lactate–5 mM acetate or 10% H_2 in the headspace). Samples were taken with sterile, N_2-flushed syringes. All incubations were done in triplicate.

MPN enumerations. A three-tube MPN technique was used to enumerate organisms capable of different types of energy metabolism. MPN values were

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calculated from standard MPN tables (42). Aerobic and anaerobic heterotrophs were grown in tryptic soy broth (0.25%) medium (Difco Laboratories, Detroit, MI); tubes were scored positive on the basis of visual turbidity. A carbonate-buffered (pH 6.8) freshwater (FW) medium (41) was used for cultivation of FeRB and aerobic FeOB. For enumeration of FeRB, either natural Fe(III) minerals (freeze-dried and autoclaved) from the Fe seep or synthetic amorphous Fe(III) oxide (21) was utilized at a final concentration of ca. 20 mmol liter^{-1}. Either H_2 (10% in headspace) or a mixture of 5 mM acetate and 5 mM lactate was used as the electron donor. Tubes for Fe(III) reduction were scored positive by measurement of Fe(II) formation. FeOB were enumerated in Fe(II)-O_2 opposing-gradient cultures as described elsewhere (34). Tubes were scored positive on the basis of the formation of a compact growth band of cells plus Fe(III) oxide, compared to the more diffuse band of Fe(III) oxide precipitation that formed in uninoculated controls (6). All MPN tubes were incubated for between 1 and 4 weeks at room temperature.

Enrichment and isolation studies. FeRB were enriched starting with the highest-dilution MPN tubes that were positive for growth (10^{-1} or 10^{-2} for H_2 and 10^{-3} to 10^{-5} for acetate-lactate) by continuous transfer on 5 mM acetate-lactate or H_2 (10% in the headspace). Isolates from the acetate-lactate enrichments were obtained by the roll tube method (14) adapted for use with solid-phase Fe(III) oxide (31). The isolation medium contained 40 mmol liter^{-1} of natural Fe(III) oxide and a mixture of 5 mM acetate and lactate. Tubes were incubated for 5 weeks at 30°C; small black and white colonies appeared after 3 to 4 weeks. Some (but not all) of the white colonies were surrounded by a clearing zone. Between 10 and 20 colonies of each type were picked from the 10^{-5} to 10^{-7} dilution and transferred into fresh medium containing 75 mmol liter^{-1} natural Fe(III) oxide, 0.1% yeast extract, and either 5 mM acetate or 5 mM lactate as the electron donor. FeOB were purified from the highest-dilution MPN tubes that were positive for growth (10^{-2} and 10^{-3}) by extinction to dilution in Fe(II)-O_2 opposing-gradient medium as previously described (34).

Growth experiments. Growth of representative FeRB isolates was tested in FW medium with natural or synthetic Fe(III) oxide. Acetate, lactate, or glucose as the electron donor was added from sterile stock solutions. The cultures were grown for at least 10 generations prior to conducting the growth experiments. Samples were collected for 0.5 M HCl-extractable-Fe determination and for direct bacterial counts with acridine orange. Solid-phase Fe(III) oxides were dissolved by oxalate extraction prior to staining as described previously (20). FeOB growth experiments were initiated by inoculating fresh Fe(II)-O_2 opposing-gradient tubes with 50 μl of the growth band from a previous culture. Triplicate tubes were sacrificed at each sampling point. Prior to sampling, the headspace of each tube was flushed with N_2 for 10 min to remove residual O_2. The tubes were then restoppered and homogenized by shaking for 20 min. Samples were collected for determination of 0.5 M HCl-extractable Fe and for direct bacterial counts with acridine orange following oxide dissolution in oxalate. The potential for aerobic Fe(II) oxidation by the type strain of *Comamonas testosteroni* (M11224) was similarly evaluated.

DNA extraction and clone library. DNA was extracted from seep material and cultures with the SoilKit from MoBio according to the manufacturer's instructions. DNA yield was quantified by 1.5% gel electrophoresis. All gels were stained for 20 min with ethidium bromide and examined with a Geldoc system and Quantity One software (Bio-Rad). The universal bacterial primer set consisting of GM3 (5′-AGAGTTTGATCMTGGC-3′; positions 8 to 24) and GM4R (5′-TACCTTGTTACGACTT-3′; positions 1492 to 1507) (22) was used to amplify almost the entire 16S rRNA gene from chromosomal DNA. PCR was performed with an iCycler Q (Bio-Rad) as follows. Twenty picomoles of each primer, 200 μM nucleotide mixture, 5× TaqEnhancer (Eppendorf, Hamburg, Germany), 1× PCR buffer, and 1 U of *Taq* DNA polymerase (Eppendorf) were adjusted to a final volume of 50 μl. Template DNA (4 μl) was added after being preheated to 70°C. Thermocycling consisted of 35 cycles of 95°C for 60 s, 44°C for 60 s, and 72°C for 90 s (7 min on the final cycle). PCR products were cloned using the pGEM-T vector and *Escherichia coli* JM109 competent cells according to the manufacturer's instructions (Promega). Recombinant transformants were selected by blue and white screening. 16S rRNA gene sequences were obtained from the University of Wisconsin-Madison Biotechnology Center. The sequences were checked for potential chimeras with the Ribosomal Database Project CHECK_CHIMERA program; sequences identified as chimeric were discarded. Each sequence was compared to GenBank using BLAST (1) as well as the Ribosomal Database Project II (3) in order to identify the closest relative. A value of 95% similarity was used as a conservative cutoff for assignment of genus level phylogenetic affiliation (12).

Nucleotide sequence accession numbers. The final clone library and pure culture 16S rRNA gene sequences have been submitted to GenBank under accession numbers FM877969 to FM878000.

TABLE 1. MPN values for different types of microorganisms in Fe seep material determined on two different dates

Physiological group	MPN[a](cells ml^{-1}) range(s)[b] determined on:	
	December 2004	April 2005
Aerobic heterotrophs	9.1×10^6–2.0×10^8	4.2×10^5–3.6×10^7
Anaerobic heterotrophs	4.9×10^7–1.1×10^9	5.3×10^4–1.6×10^7
Aerobic Fe(II) oxidizers	3.3×10^3–2.4×10^4	7.1×10^3–5.3×10^5
Fe(III) reducers with acetate-lactate	3.3×10^5–2.4×10^6, 7.4×10^3–5.3×10^4	2.3×10^3–1.3×10^6, 3.4×10^2–4.2×10^4
Fe(III) reducers with H_2	4.3×10^2–1.1×10^4, 3.2×10^2–6.2×10^3	3.5×10^2–5.8×10^4, 9.6×10^1–2.5×10^2

[a] For Fe(III) reducers with acetate-lactate and H_2, ranges are results of tests in FW with natural/synthetic Fe(III) oxide.

[b] Ranges represent 95% confidence intervals for three-tube MPN determinations.

RESULTS AND DISCUSSION

In situ parameters. Aqueous Fe(II) and Fe(III) concentrations ranged from 1 to 3 μM and 2 to 6 μM, respectively, over a 170-day monitoring period; the pH remained between 5.9 and 6.7 (see Fig. S2A in the supplemental material). Approximately 70% (7.07 mmol/g) of the dry mass of the mat material was soluble in 0.5 M HCl. The bulk 0.5 M HCl-extractable-Fe content of the mat material ranged from 85 to 115 mmol liter^{-1}; Fe(II) comprised 2 to 10% of total 0.5 M HCl-extractable Fe (see Fig. S2B in the supplemental material).

MPN determinations revealed high numbers (10^4 to 10^9 cells ml^{-1}) of aerobic and anaerobic heterotrophs in the seep material; densities of culturable FeRB and FeOB ranged from 10^2 to 10^6 and 10^3 to 10^5 cells ml^{-1}, respectively (Table 1). The April 2005 MPNs for the various physiological groups were lower by 1 or 2 orders of magnitude than those from October 2004. A 16S rRNA gene clone library revealed the presence of a variety of organotrophic proteobacteria as well as recognizable betaproteobacterial lithotrophs (*Gallionella* and *Leptothrix* spp.) in the seep material (Table 2). Phylotypes of commonly recognized FeRB such as *Geobacter* or *Shewanella* spp. were not obtained.

Fe(III) reduction. In vitro Fe(III) reduction assays (Fig. 1) together with the MPN results revealed the presence of FeRB capable of metabolizing both natural and exogenous electron donors. Natural electron donors may include organic matter produced by lithotrophic FeOB, as well as dissolved and/or particulate materials from the surrounding terrestrial ecosystem. Although previous studies have demonstrated the potential for Fe(III) reduction during anaerobic incubation of groundwater seep materials amended with exogenous electron donors (8), this is the first study to document in vitro Fe(III) reduction through metabolism of endogenous electron donors. The amorphous Fe(III) oxides present in the seep materials were favorable electron acceptors for FeRB growth, as indicated by the significantly higher FeRB MPNs obtained using natural versus synthetic amorphous Fe(III) oxide (Table 1). Similar results were obtained in studies employing Fe(III) oxides produced by a nitrate-reducing Fe(II)-oxidizing enrichment culture (37). The seep material was converted to a black nonmagnetic phase during microbial reduction; X-ray diffraction analysis revealed the formation of a hydrocarbonate green rust (see Fig. S3 in the supplemental material).

TABLE 2. Composition of the 16S rRNA gene clone library from the Fe seep[a]

Taxon	No. of clones	Similarity (%)
Alphaproteobacteria		
Sphingomonas	8	97–99
Hyphomicrobium	4	99
Pedomicrobium	2	99
Sinorhizobium	14	99
Methylocella	2	92
Phyllobacterium	2	90
Uncultured *Alphaproteobacteria*	10	92–96
Betaproteobacteria		
Leptothrix	2	98
Gallionella	33	96–99
Rhodocyclus	3	99
Comamonas	6	98–99
Variovorax	10	93–96
Uncultured *Betaproteobacteria*	20	95–99
Gammaproteobacteria		
Aeromonas	10	98–99
Methylomonas	2	92
Uncultured *Gammaproteobacteria*	2	99

[a] A total of 130 clones were sequenced (*Alphaproteobacteria*, 42 clones; *Betaproteobacteria*, 74 clones; *Gammaproteobacteria*, 14 clones).

Although FeRB such as *Geobacter* and *Shewanella* spp. were not detected in the 16S rRNA gene clone library (Table 2), *Geobacter*-related sequences were amplified from second or third transfers of Fe(III)-reducing enrichment cultures derived from tubes having highly positive MPNs with acetate-lactate and H_2 as the electron donors (see Table S1 in the supplemental material). Stable Fe(III) oxide-reducing enrichments were obtained from tubes having highly positive MPNs with 5 mM acetate-lactate as the electron donor. Enrichment cultures derived from tubes having highly positive MPNs with H_2 as the sole electron donor (no added organic carbon) grew for only a few generations.

Acetate-lactate enrichment cultures were used to inoculate roll tubes containing natural Fe(III) oxide from the seep. Black colonies transferred out of the roll tubes grew by Fe(III) reduction with acetate (see Fig. S4A in the supplemental material) but not lactate (data not shown) as the electron donor. The 16S rRNA gene sequences (>1,400 bp) from five of these isolates were the same and showed 99% similarity with that of *Geobacter* sp. strain CLFeRB (11). Transfer of white colonies with visible clearing zones from the Fe(III)-reducing roll tubes revealed Fe(III) reduction with lactate but not acetate as the electron donor (data not shown). The 16S rRNA gene sequences of these organisms were 100% similar to each other and revealed the highest similarity (98%) to "*Shewanella saccharophila*" (2), the first described glucose-oxidizing FeRB. To our knowledge this is the first demonstration of the simultaneous isolation of *Geobacter* and *Shewanella* species from the same starting enrichment culture.

The *Shewanella* isolate (denoted strain IST-21) grew with glucose as an electron donor for Fe(III) oxide reduction (see Fig. S4B in the supplemental material); no growth was observed on glucose alone or on glucose with 20 mM nitrate (data not shown). Acetate accumulated during oxidation of glucose with Fe(III) (see Fig. S4B in the supplemental material), whereas no acetate was produced under aerobic conditions (data not shown). The ratios of Fe(II) and acetate produced to glucose consumed were 7.5 ± 0.3 and 1.9 ± 0.1 mmol, respectively (see Fig. S4C in the supplemental material). These results agree with those of Coates et al. (2) for "*S. saccharophila*" and are consistent with the following reaction: $C_6H_{12}O_6 + 8Fe(OH)_3 + 12H^+ \rightarrow 2CH_3COO^- + 2HCO_3^- + 8Fe^{2+} + 20H_2O$. As discussed by Coates et al. (2), this reaction provides a mechanism whereby "*S. saccharophila*" and acetate-oxidizing FeRB (e.g., *Geobacter* spp.) could function cooperatively in Fe(III) reduction. This finding, together with (i) the demonstrated presence of abundant anaerobic (fermentative) heterotrophs (Table 1) and (ii) the isolation of a *Bacillus* strain (IST-38) capable of reducing Fe(III) with tryptic soy broth and yeast extract during fermentative metabolism (see Fig. S4D in the supplemental material), provides explicit confirmation of the existence of a microbial community capable of degrading complex organic carbon with Fe(III) oxide as the terminal electron acceptor. The seep material also hosted significant numbers of H_2-oxidizing FeRB (Table 1), which are expected to play a role in organic carbon metabolism (17).

Fe(II) oxidation. Densities of culturable FeOB were comparable to the those of culturable FeRB (Table 1). Microscopic investigation of fresh Fe seep material (data not shown) revealed two obvious morphotypes: sheaths, as known from *Leptothrix ochracea* (38), and stalks, produced by *Gallionella* species (13). Both structures were often associated with Fe oxides. Although the 16S rRNA gene clone library revealed the presence of these well-known neutrophilic FeOB in the seep material (Table 2), no *Gallionella* or *Leptothrix* spp. were isolated by the Fe(II)-O_2 opposing-gradient cultivation method. Such results are commonly observed in cultivation-based studies of neutrophilic aerobic Fe(II) oxidation (5).

A significant number of *Comamonas*-related 16S rRNA gene sequences were obtained in the clone library (Table 2). A *Comamonas* strain (IST-3) was isolated from the 10^{-3} dilution in the FeOB MPN series from December 2004. This strain grew in opposing-gradient cultures only in the presence of a Fe(II) source (Fig. 2A and B). Rates of Fe(III) deposition for inoculated and chemical controls were comparable, in agreement with previous observations (6, 32). The lack of cell growth in controls lacking Fe(II) ruled out the possibility of

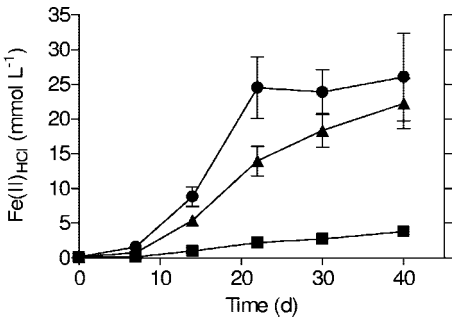

FIG. 1. In vitro Fe(III) reduction during anaerobic incubation of Fe seep material. Data points show the means ± standard deviations of triplicate bottles supplemented with nothing (■), 5 mM acetate-lactate (●), or 10% H_2 in the bottle headspace (▲). Rates of Fe(III) reduction (after a ca. 1-week lag phase) were approximately 300, 2,000, and 700 μmol liter^{-1} day^{-1} for the three treatments, respectively.

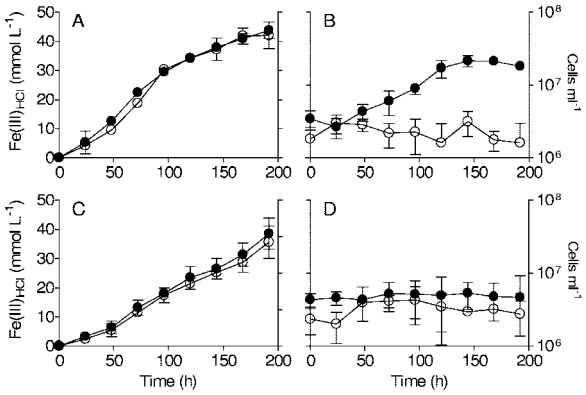

FIG. 2. Fe(III) oxide accumulation and cell growth of *Comamonas* sp. strain IST-3 (A and B) and *Comamonas testosteroni* M11224^T (C and D) in Fe(II)-O_2 opposing-gradient cultures. Filled and open circles show results for inoculated and uninoculated controls, respectively. Data points show the means ± standard deviations of triplicate cultures.

growth through energy-generating metabolism of agar impurities or H_2 from the incubation setup procedure in the anaerobic chamber. Sequencing of the 16S rRNA gene of this culture revealed 100% similarity to the type strain of *C. testosteroni* (M11224). However, no growth of the type strain was observed with or without Fe(II) (Fig. 2C and D).

Although strict autotrophic conditions were imposed during isolation and growth experiments with *Comamonas* sp. strain IST-3, the possibility of CO_2 fixation was not directly tested. A whole-genome sequence for *C. testosteroni* strain KF-1 has been determined by the DOE Joint Genome Institute and is available in the GenBank genome database. Analysis of the annotated *C. testosteroni* KF-1 genome revealed no evidence for enzymes involved in CO_2 fixation such as ribulose-1,5-bisphosphate carboxylase, fumarate reductase, α-ketoglutarate synthase, or ATP-dependent citrate lyase. It therefore seems likely that the isolated *Comamonas* strain is able to grow by oxidation of Fe(II) with O_2 only under mixotrophic conditions, i.e., when a source of organic carbon is available to facilitate growth. This organic carbon may be delivered from trace impurities in the agar or by thermal breakdown of the agar during autoclaving. The observed growth yield of strain IST-3 [ca. 5×10^5 cells per μmol Fe(II) oxidized] was ca. 10-fold lower than those observed for autotrophic FeOB (32), which is consistent with the idea that growth of the isolate was limited by the availability of a fixed carbon source. Our findings expand the range of *Betaproteobacteria* capable of circumneutral-pH Fe(II) oxidation (7).

Microbial Fe redox cycling. Culture-based enumeration and pure culture studies demonstrated the existence of Fe redox cycling microbiota in the groundwater seep materials. Although in situ rates of Fe(III) reduction and Fe(II) oxidation are unknown, simple calculations suggest that Fe reduction and oxidation could proceed at comparable rates. Fe(III) was reduced at ca. 300 μmol liter^{-1} day^{-1} during incubation of unamended seep material under anoxic conditions (Fig. 1); this probably represents a maximum value, as aerobic respiration was excluded. Rates of Fe(II) oxidation can be approximated based on observed FeOB cell densities and cell-specific rates of Fe(II) oxidation. The latter were obtained from the growth experiment with *C. testosteroni* IST-3 shown in Fig. 2A and B, which indicated that ca. 10 μmol Fe(II) per tube was oxidized between 125 and 175 h when the cell density was approximately constant at ca. 2×10^7 cells per tube. At the calculated cell-specific rate of Fe(II) oxidation of ca. 1×10^{-8} μmol Fe(II) cell^{-1} h^{-1}, ca. 10^6 cells ml^{-1} would be required to achieve a Fe(II) oxidation rate equal to the rate of Fe(III) reduction observed in unamended seep materials. A 10-fold-smaller FeOB cell density (ca. 10^5 cells ml^{-1}) would be required if the cell-specific oxidation rate observed for strain TW2 (32) is used. FeOB cell densities as high as 10^5 cells ml^{-1} were documented in the seep materials (Table 1), which suggests that rates of biological Fe(II) oxidation could have approached the observed in vitro Fe(III) reduction rates.

Our data are consistent with the ongoing operation of a microbial Fe redox cycle in the seep materials, in which rates of Fe oxidation and reduction are roughly in balance. These findings agree with results from FeRB/FeOB cocultures that demonstrated the potential for coupled Fe oxidation and reduction activity at the aerobic-anaerobic interface (29). The seep system provides a model for how coupled Fe oxidation and reduction could take place in a wide variety of surface and subsurface environments on Earth, as well as subsurface environments on Mars, where reduced fluids may contact O_2- or NO_3^--bearing water or water vapor (16).

A simple kinetic model was constructed to explore quantitatively the potential coupling of Fe(II) oxidation and Fe(III) reduction in the groundwater seep materials. Although the model does not explicitly simulate the seep system, it provides insight into the role that Fe redox cycling could play in total rates of Fe turnover. The model consists of seven compartments (see Fig. S5 and Table S2 in the supplemental material) connected by a system of kinetic reactions (see Tables S3 and S4 in the supplemental material) for Fe(II) oxidation, Fe(III) oxide reduction, and the growth and decay of FeOB and FeRB. Fe(II) was assumed to enter the system at a rate of 500 μM day^{-1}, which led to accumulations of Fe(III) oxide (several 10s of mmol liter^{-1}) comparable to those observed during in situ development of the Fe mat (see Fig. S2B in the supplemental material). The incoming Fe(II) was oxidized according to a pseudo-first-order rate constant (100 day^{-1}) in the midrange of those recently documented for similar groundwater seep systems (25). All Fe(II) oxidation was presumed to be coupled to autotrophic FeOB growth, consistent with studies of FeOB growth and Fe(II) oxidation in diffusion-limited gradients (34). Recent studies of biotic (untreated) versus abiotic (azide-treated) Fe(II) oxidation in Fe(II)-spiked groundwater seep materials similar to those examined here indicated that on average more than one-half of Fe(II) oxidation could be attributed to microbial catalysis (25). FeOB biomass was assumed to undergo slow first-order decay at a rate (0.05 day^{-1}) typical of low-energy-yield metabolic pathways (26). Dissolved organic carbon (DOC) was released from the decaying FeOB biomass according to a first-order rate constant of 0.1 day^{-1} based on rates of organic carbon (baker's yeast) turnover in FW wetland sediments (30). The released DOC was in turn oxidized via Fe(III) reduction according to a simple kinetic framework developed for simulation of amorphous Fe(III) oxide reduction and other redox pathways in the same FW

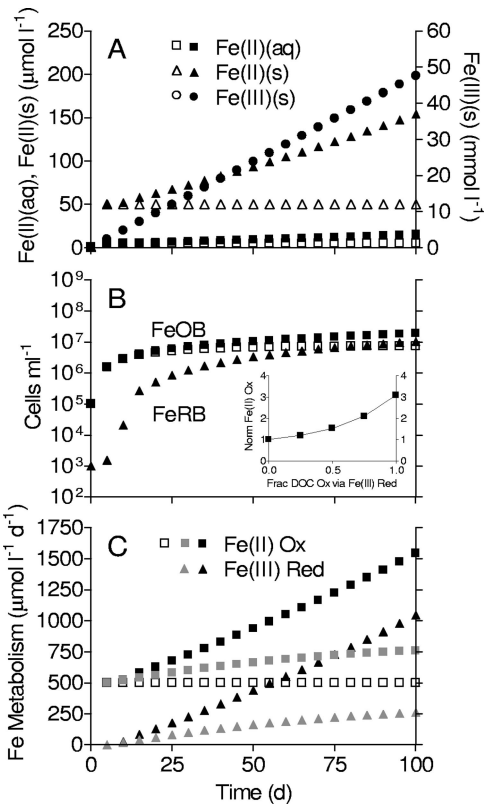

FIG. 3. Results of the microbial Fe redox cycling model (see Fig. S5 and Tables S2 to S5 in the supplemental material) simulations with (filled symbols) and without (open symbols) Fe(III) reduction activity. Gray symbols in panel C show results of simulation in which only one-half of the decay of dead autotrophic biomass is mediated by Fe(III) reduction. The inset in panel B shows rates of Fe(II) oxidation, normalized to the baseline rate of aqueous Fe(II) input (500 μmol liter^{-1} day^{-1}), as a function of the fraction of dead autotrophic biomass decay mediated by Fe(III) reduction.

wetland sediments (27). The Fe(II) produced was assumed to partition between the aqueous and solid phases according to a simple linear isotherm with a K_d (sorption coefficient) of 10, which produced aqueous-/solid-phase Fe(II) ratios consistent with in situ observations. A complete list of parameter values and their origin is given in Table S5 in the supplemental material.

A series of 100-day simulations was conducted to assess how Fe(III) reduction coupled to decay of autotrophic FeOB biomass could affect total Fe turnover within the reaction system. For the sake of simplicity, solid-phase materials [Fe(III) oxide, solid-phase Fe(II), and cell biomass] were assumed to be retained throughout the simulation. The calculations showed that under these conditions rates of Fe(II) oxidation increase linearly with time in conjunction with the onset of Fe(II) regeneration coupled to Fe(III) oxide reduction (Fig. 3C). Rates of Fe(II) oxidation were accelerated by a factor of up to 3 when DOC produced by FeOB decay was directly coupled to Fe(III) reduction (Fig. 3B, inset). Lower but significant stimulation of Fe(II) oxidation took place when the fraction of FeOB decay coupled to Fe(III) reduction decreased, as would be the case if aerobic heterotrophic microorganisms were active.

The key outcome of this exercise is the quantitative verification that recycling of Fe through coupled oxidative and reductive pathways can increase the overall rate of Fe turnover. Such recycling is likely to take place in virtually all redox interfacial environments, and hence detection and analysis of Fe redox-based microbial life in such environments should always consider both Fe(II)-oxidizing and Fe(III)-reducing physiologies.

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