Biogeochemical Conditions Favoring Magnetite Formation during Anaerobic Iron Reduction

P. E. BELL, A. L. MILLS,* AND J. S. HERMAN

Department of Environmental Sciences, University of Virginia, Charlottesville, Virginia 22903

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Several anaerobic bacteria isolated from the sediments of Contrary Creek, an iron-rich environment, produced magnetite when cultured in combinations but not when cultured alone in synthetic iron oxyhydroxide medium. When glucose was added as a carbon source, the pH of the medium decreased (to 5.5) and no magnetite was formed. When the same growth medium without glucose was used, the pH increased (to 8.5) and magnetite was formed. In both cases, Fe²⁺ was released into the growth medium. Geochemical equilibrium equations with E₀ and pH as master variables were solved for the concentrations of iron and inorganic carbon that were observed in the system. Magnetite was predicted to be the dominant iron oxide formed at high pHs, while free Fe²⁺ or siderite were the dominant forms of iron expected at low pHs. Thus, magnetite formation occurs because of microbial alteration of the local E₀ and pH conditions, along with concurrent reduction of ferric iron (direct biological reduction or abiological oxidation-reduction reactions).

The Contrary Creek arm of Lake Anna, an impoundment in central Virginia, receives acid mine drainage from several abandoned pyrite mines (10, 27, 28). Pyrite was extracted from massive sulfide deposits until about 1920, when the sulfur domes of the Gulf Coast region were opened. Oxidation of pyrite, an iron disulfide mineral, leads to the production of iron- and sulfate-rich acid mine drainage. Mixing of the acidic, iron-rich mine drainage with the less acidic lake water results in the deposition of an abundant iron oxyhydroxide floc layer (ca. 1-cm thick) called “yellow boy” on the surface of the sediments. Results of previous studies (10, 12; P. E. Bell, A. T. Huntley, and A. L. Mills, submitted for publication) on this system have documented the importance of anaerobic bacterial processes in the sediments of this portion of Lake Anna in neutralization of the incoming acidity.

Buffering capacity against sulfate acidity is generated in anoxic hypolimnion and sediments of acidified freshwater lakes (11, 12, 36), and the mechanism of this buffering is often attributed to bacterial SO₄²⁻ reduction (1, 12, 19, 36). Proton consumption can be stoichiometrically balanced by the removal of sulfate from the water column (1, 35), but for neutralization to be permanent, the sulfide generated from sulfate reduction must be removed from the system as a mineral phase or as H₂S (gas) to prevent reoxidation and proton regeneration. Sulfide is often sequestered from the system in iron minerals. Obviously, this process requires the presence of reduced iron.

Because of the high concentration of iron in the Contrary Creek arm of Lake Anna, oxidized iron is a potential terminal electron acceptor for anaerobic metabolism. Additionally, iron may be reduced abiotically by the reducing conditions caused by microbial metabolism. The reduced iron that results from direct or indirect microbial metabolism plays an important role in mineral formation in the sediments.

While the occurrence of biological iron reduction is well documented (14–18, 21, 22, 31, 38), little information is available on the role of iron-reducing bacteria in local geochemistry. Lovley and Phillips (22; D. R. Lovley and E. J. P. Phillips, Abstr. Meet. Am. Soc. Limnol. and Oceanogr. 1987, p. 48) first observed the formation of magnetite during anaerobic bacterial iron reduction. The presence of reduced iron in sediments and anoxic hypolimnion of lakes from disparate regions indicates that iron is generally available as an active redox species and is a potentially important participant in local mineral solubility equilibria in many locations (5, 30, 41, 43).

We have begun to examine anaerobic bacteria that reduce iron and to investigate their role in carbon and electron flow, neutralization of incoming acidity, and mineral solubility equilibria. Several anaerobic bacteria were isolated from the sediments of Contrary Creek and characterized. These organisms reduced iron when cultured in combinations in synthetic iron oxyhydroxide media, but not when cultured individually. In agreement with Lovley and Phillips (Abstr. 1987 meet. Am. Soc. Limnol. and Oceanogr., p. 48, 1987), the reduction of iron resulted in the generation of magnetite, with a concurrent increase in the pH of the growth medium, when the organisms were grown with yeast extract and acetate as carbon sources. In contrast, the pH decreased, no magnetite was formed, and more Fe²⁺ was released into the growth medium when glucose was added to the medium. The results suggest that the generation of magnetite is the biogeochemical outcome of bacterial alteration of the environment which influences local solubility equilibria, a process termed biologically induced mineral formation (23).

**MATERIALS AND METHODS**

Isolation and characterization of organisms. Anaerobic bacteria were isolated from the sediments of Contrary Creek by using enrichment cultures and a serum bottle modification of the Hungate technique using butyl rubber stoppers (6, 26). Sediment material was serially transferred in iron enrichment media.

The growth medium contained the following, in grams per liter, unless otherwise indicated: FeCl₃, 2.5; NH₄Cl, 1.25; Fe(NH₄)₆(SO₄)₂·6H₂O, 0.002; NaHCO₃, 7.5; yeast extract, 0.5; sodium acetate, 2.5; resazurin, 4 ml of a 0.025% aqueous solution; trace vitamin solution, 10 ml; trace mineral solution, 10 ml; salt solution, 25 ml. Salt solution consisted of the following, in grams per liter: KH₂PO₄, 6.0; (NH₄)₂SO₄, 6.0;
TABLE 1. Response of anaerobic isolates from the sediments of the Contrary Creek arm of Lake Anna to tests on API 20A strips

<table>
<thead>
<tr>
<th>Test</th>
<th>D</th>
<th>S</th>
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<tbody>
<tr>
<td>Indole</td>
<td>+</td>
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<td>Urea</td>
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<td>Lactose</td>
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<td>Salicin</td>
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<td>Motility</td>
<td>+</td>
<td>0</td>
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<td>O2</td>
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<td>SO4^- reduction</td>
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*a Symbols: +, positive reaction to the test; 0, no reaction to the test.

NaCl, 12.0; MgSO4 · 7H2O, 2.6; CaCl2 · 2H2O, 0.16. Trace vitamin solution consisted of the following, in milligrams per 100 ml: thiamine hydrochloride, 1.0; riboflavin, 0.5; nicotinic acid, 0.5; DL-calcium pantothenate, 0.5; vitamin B12, 0.01; para-aminobenzoic acid, 0.5; pyridoxine hydrochloride, 0.2; pyridoxine hydrochloride, 0.2; thiamine hydrochloride, 0.5; riboflavin, 0.5; nicotinic acid, 0.5. Trace mineral solution consisted of the following, in milligrams per 100 ml: nitricitric acid, 150; MgSO4 · 7H2O, 300; MnSO4, 2H2O, 30; NaCl, 100; FeSO4 · 7H2O, 10; CoSO4, 10; CaCl2 · 2H2O, 10; ZnSO4, 10; CuSO4 · 5H2O, 1; AlK(SO4)2, 1; H2BO3, 1; Na2MoO4 · 2H2O, 1; NiCl2 · 6H2O, 2; NaSe, 2.

The trace mineral solution was prepared by first dissolving the nitritric acid in deionized water (boiled first and cooled under a stream of N2) and adjusting the pH to 6 with KOH. The minerals were then added and the solution was sterilized by filtration through a filter (pore diameter, 0.2 μm). The vitamin solution was filter sterilized in a similar fashion. A 20% (wt/vol) glucose solution was prepared separately in degassed deionized water and filter sterilized. The final concentration of glucose used in these experiments was 2% (wt/vol). All incubations were at 35°C with an H2 atmosphere of 30 lb/in2. In all cases the same bottle was sampled sequentially with time.

Characterization of the isolates was performed by using API 20A strips (Analytab Products, Plainview, N.Y.). Nitrate reduction was tested by using the media described by MacPadden (24). The organisms were tested in medium B, described by Postgate (33), to determine if they could use sulfate as a terminal electron acceptor.

Analytical techniques. Ferrous iron was determined by the Ferrozine method (37), which was modified by first acidifying the samples with 10% H2PO4. Portions (10 to 100 μl) of the sample were added to 10 μl of acid and allowed to stand for at least 1 min before 5 ml of Ferrozine reagent was added. Samples were filtered through a membrane filter (pore size, 0.45 μm) before the A562 was read. Sulfide was measured by the colorimetric method described by Cline (4).

Organic acids were analyzed by gas chromatography with a gas chromatograph (model 3700; Varian Instruments, Inc.) equipped with a flame ionization detector. The acids were separated on a 2-m glass column packed with 10% SP-1200–1% H3PO4 on 80/100 mesh Chromosorb WAW (Supelco, Inc.), with N2 as the carrier. The temperature of the injector and detector was 190°C, and the column was held at 120°C for volatile fatty acids and at 150°C for nonvolatile fatty acids. Flow rates of the gasses were as follows: N2, 30 ml·min⁻¹; H2, 30 ml·min⁻¹; and air, 300 ml·min⁻¹.

Glucose was measured enzymatically with a glucose reagent kit (HK: Sigma Chemical Co., St. Louis, Mo.). Bacterial counts were obtained by using the acridine orange direct count method (13). Specimens were prepared for powder X-ray diffraction analysis by the camera method summarized by Kraus et al. (20).

E0-pH diagram and mineral solubility equilibria calculations. An E0-pH diagram (see Fig. 4) was derived by using the equations reported by Garrels and Christ (7) for the iron-water-carbonate system, the free energy of formation values reported by Robie et al. (34), concentrations of ferrous iron from culture filtrate (filter pore size, 0.45 μm), and the total inorganic carbon that was present in the experimental system. Ionic strength was calculated by using the Davies equation (39) to obtain activity coefficients for the major ions in solution. The low and high Fe2+ concentrations that were used to prepare the diagram were the values calculated from the addition of Fe(NH4)2(SO4)2 (5.1 μM) and the highest value obtained by Ferrozine analysis of the culture filtrate (181 μM), respectively. The siderite boundaries were derived by first using the initial bicarbonate concentration added to the cultures (45 mM) as total aqueous carbonate and solving for contours of CO3²⁻ activity, and then solving for contours of siderite in equilibrium with the appropriate activities of carbonate and ferrous iron. To test whether the added organic carbon made a contribution to the H2CO3⁻–HCO3⁻–CO3²⁻ system that would alter the E0-pH diagram, the diagram was redrawn by using carbonate activities determined as the added inorganic carbon plus the amount of carbon added if the organic matter was completely mineralized to CO2. Boundaries in both diagrams were nearly identical.

The saturation state of the medium with respect to a number of minerals was determined by using a geochemical equilibrium model (WATERQF) and the calculated and analytical concentrations of ions, pH, and E0 of the system (29, 32, 40). Sulfide was produced in low concentrations in the iron medium (1.69 × 10⁻⁶ M). This value was used as input for WATERQF.

RESULTS

Biochemical characterization. All of the organisms were gram-negative rods, and the group was physiologically diverse (Table 1). Only one isolate (strain E) grew in the presence of O2, and this organism was also the only one tested that reduced NO3⁻ to NO2⁻. Strain U was identified as Bacteroides ureolyticus by comparison of the responses to the various tests with the data in the API 20A (Analytab) taxonomy tables. Strain D was a fastidious anaerobe; it did not produce catalase and did not hydrolyze gelatin or any sugars. This organism produced sulfide from cysteine, pro-
duced indole from tryptophan, and formed subterminal spores. Strain S produced acid from glucose, formed subterminal spores, and produced sulfide from cysteine. The methanogen, strain CA, produced methane from CO₂ + H₂ but not from formate or acetate. Sulfate was not used as a terminal electron acceptor by any of the organisms alone or in combinations.

**Growth of organisms and release of ferrous iron.** When organisms D, S, and E were cultured alone and in combinations, ferrous iron was released into the medium only in the combined cultures (Fig. 1A). The medium supported the growth of strain S alone, but not that of strain D alone (Fig. 1B). Strain D is a fastidious anaerobe, and since there was no reducing agent added to the medium that was used, conditions may have been too oxidizing for the organism to initiate growth. Metabolic products of organism E probably lowered the Eh enough for organism D to thrive. The pH of all cultures started at 7.4, and by the end of the incubation period the pH of those combined cultures containing Fe²⁺ increased to 8.5. The pH of the monocultures, which produced no Fe²⁺, remained at 7.4. In the cultures that reduced iron, the orange iron oxyhydroxide precipitate first turned darker orange-brown and then black. Uninoculated controls exhibited no bacteria, no iron reduction, and no change in the color of the iron oxyhydroxide. The increase in Fe²⁺ in the cultures that were positive for iron reduction was linear with time and did not approximate the growth of the cells. Examination of the cultures by phase-contrast microscopy revealed that most of the bacteria were attached to the iron oxyhydroxide particles, and the amorphous particles tended to become more aggregated with time.

**Magnetite formation by anaerobic cultures.** Reduction of iron occurred when the isolates were incubated together in various combinations, but not when incubated in monoculture (Table 2). X-ray diffraction analysis of the black material yielded one major band indicating reflection close to 0.253 nm, which is the most intense reflection for magnetite (Fe²⁺Fe³⁺₆O₁₄). Magnetic material could also be collected by using a steel paper clip. Magnetite formation was observed only in the mixed cultures.

**Effect of glucose on iron reduction and magnetite formation in combined cultures.** Because some of the organisms could use glucose, it was added to the growth medium to determine if a change in the carbon source had an effect on iron reduction. The rate of increase of Fe²⁺ released into the medium when cells were cultured with glucose was greater than that in the same medium without the sugar (Fig. 2). The final pH (after 5 days of incubation) in the medium with glucose was always lower than the initial pH (initial pH, 7.4; final pH, ≤5.8), and no magnetite was formed. Two of the cultures (S-E, S-U) without glucose had a final pH of >8.2, and magnetite was present. When strains E and U were cultured together without glucose, the final pH was 7.4 and no magnetite was observed (i.e., there was no black precipitate). The linear increase in Fe²⁺ occurred at a rate similar to the linear decrease in glucose in combined cultures S-E and S-U (Fig. 3). Culture S-E used glucose at a faster rate than did S-U. Both S and E produced acid from glucose when cultured alone, so it was not unexpected that the organisms acted synergistically with respect to glucose depletions and acid production when cultured together. All cultures produced pyruvate, lactate, oxaloacetate, fumarate, propionate, isobutyrate, n-butyrate, and isovalerate. The quantities of acid produced were insufficient to account for the decrease in the pH of the medium. When strains S and U were cultured together without glucose, n-valerate was produced. There were no consistent patterns of organic acid formation among the cultures; that is, the cultures with glucose did not always produce more of these acids than did the cultures without the sugar added. All cultures exhibited an increase in organic acid production with time.

**Magnetite formation as a function of Eh and pH.** When mineral stability was examined as a function of both Eh and pH, it was apparent that at high pH and low Eh, magnetite was the dominant mineral in an iron-carbonate-water system, as predicted (Fig. 4). The less stable iron oxyhydroxides were not considered when the diagram shown in Fig. 4 was drawn because of the lack of consistent thermodynamic data for these metastable mineral phases.

At the beginning of the experiments, the log \( \Delta G'_f \) (i.e., log of ferrous ion activity) was −5.76, the pH was 7.4, and the Eh was in the range of 0 to −100 mV (resazurin in the medium was colorless). In freshly prepared medium, precipitated iron oxyhydroxide was dominant, and with time this

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Response of isolate*</th>
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<tbody>
<tr>
<td>D</td>
<td>+</td>
</tr>
<tr>
<td>S</td>
<td>+</td>
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<tr>
<td>E</td>
<td>+</td>
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<td>CA</td>
<td>ND</td>
</tr>
<tr>
<td>U</td>
<td>0</td>
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* Symbols: +, formation of black magnetic material; ±, formation of dark orange-brown material; 0, no change in the orange iron hydroxide; ND, not done.
Magnetite formation during anaerobic iron reduction

FIG. 2. Release of ferrous iron into anaerobic synthetic iron oxyhydroxide medium. The pH values given are those of the medium at the end of the experiment. All cultures started with a pH of 7.4.

Because of the presence of large amounts of phosphate and sulfate in the medium, as well as the complexities involved in drawing a multielement $E_h$-pH diagram, a geochemical equilibrium model was solved for the system. The results of the model indicate that the medium liquid phase should be supersaturated with respect to many minerals besides magnetite and siderite. Theoretically stable mineral phases that include, for example, the phosphate mineral vivianite [$\text{Fe}_3\text{(PO}_4)_2 \cdot 8\text{H}_2\text{O}$]; iron sulfide minerals pyrite ($\text{FeS}_2$), mackinawite ($\text{FeS}_1$), and greigite ($\text{Fe}_2\text{S}_3$); as well as iron oxide minerals goethite [$\text{FeO(OH)}$], hematite ($\text{Fe}_2\text{O}_3$), and maghemite ($\text{Fe}_2\text{O}_3$, which has a different crystal orientation than hematite) might be expected to form in this system. The only crystalline mineral phase observed, however, was magnetite, which had the highest saturation index of any of the mineral phases predicted by WATEQF. Sulfides were not considered because none of the bacteria were capable of dissimilatory sulfate reduction (although trace amounts of sulfide were formed from amino acid fermentation). Sulfate is not reduced abiotically at low temperature and pressure (8) and, thus, was not considered in this experimental system.

**DISCUSSION**

There are three main consequences of biologic iron reduction to consider: (i) the importance of iron reduction in carbon and electron flow, (ii) the importance of iron reduction in neutralizing acidity contained in acid mine drainage and acid rain, and (iii) the importance of iron reduction in the formation of magnetite.

**Importance of iron reduction in carbon and electron flow.** Lovley and Phillips (21, 22) have suggested that iron reduction is as important as methanogenesis in anaerobic carbon

![FIG. 3. Changes in ferrous iron and glucose concentrations with time in synthetic iron oxyhydroxide medium. The cultures are the same as those in Fig. 2. Note the difference in scale for iron and glucose.](image-url)

Material was converted to the more stable hematite (or goethite). Siderite and magnetite are not important phases under initial conditions.

By the end of the experiment, the log $a_{\text{Fe}^{2+}}$ was $-4.39$ and the $E_h$ was probably well below $-200$ mV (methanogenesis occurred in those cultures containing the methanogen strain CA). The geochemical effect of increasing the concentration of $\text{Fe}^{2+}$ was to shift the equilibrium boundaries to the left, that is, to extend the magnetite stability field to a more acidic pH and to move the siderite field (Fig. 4) to a more acidic pH.

When the pH was high (ca. 8.5), physical conditions at the end of the experiments were well within the magnetite stability field. Given the same $E_h$ and a pH of 7.4, conditions were still within the magnetite field, but were approaching the siderite field. Again, at the same $E_h$ but with a pH of 5.7, conditions were near the equilibrium boundary of siderite and ferrous iron. While the presence of siderite was pre-
and electron flow in the sediments of the Potomac River. The addition of ferric iron to mixed cultures from those sediments inhibited methanogenesis (the reduction of iron is more energetically favorable than is the reduction of CO2). Magnetite was formed as a product in the mixed cultures, and in pure culture (Lovley and Phillips, Abstr. Meet. Am. Soc. Limnol. and Oceanogr. 1987). The authors postulated that much of the Fe(III) found in situ exists as magnetite or other mixed ferric-ferrous minerals, and is therefore unavailable for use as a terminal electron acceptor by bacteria.

Results of the present study suggest that in the sediments of the Potomac River, magnetite is unlikely to be the major mineral product of iron reduction. Typical levels of reactants in the pore water of Potomac River sediments near the site sampled by Lovley and Phillips (22) are 1 to 2 mM Fe2+, 30 to 60 mM HCO3−, and a pH of 7.0 (S. D. Goodwin. M.S. thesis, University of Virginia, Charlottesville. 1980). These values are similar to those used to calculate the Eh-pH diagram (Fig. 4). Thus, the diagram should reasonably approximate the equilibrium conditions of the sediments in the Potomac River. At pH 7.0, the equilbrium is near the siderite-magnetite boundary, suggesting that both mineral phases would be present in the absence of sulfide. Results of the present study indicate that magnetite occurs because of local Eh-pH conditions and levels of reactants in the system, and not because of any direct microbiological precipitation.

The sulfate concentration in the overlying water near the site sampled by Lovley and Phillips (21) approached 1 mM. At that concentration, sulfate reduction in the sediment can produce abundant sulfide in the pore water. The presence of sulfide favors the formation of iron sulfide phases at low Eh and neutral pH, and the calculations of Eh-pH relationships given by Garrels and Christ (7) suggest that in the Potomac River sediments, the dominant ferrous ferrous phases that would be expected were siderite and iron sulfides, not magnetite. Therefore, carbon and electron flow would probably not be limited by sequestration of Fe(III) in magnetite.

The potential of iron-reducing bacteria as processors of carbon and electrons remains largely unexplored. In the present study, cultures produced high concentrations of organic acids, particularly pyruvate, which plays a pivotal role in carbon and electron flow. Complete understanding of how anaerobic systems function requires further quantification of the kinetics and products of iron reduction.

**Importance of iron reduction in neutralizing acidity contained in acid mine drainage and acid rain.** Neutralization of acidity in freshwater systems is often explained by alkalinization generated by sulfate reduction (1, 27, 35). However, the reduced sulfide must be sequestered within the system (or released as H2S [gas]) for proton removal and alkalinity generation to be permanent. The reaction for the combined process of iron and sulfate reduction is as follows:

\[
2\text{Fe(OH)}_3(\text{solid}) + \text{SO}_4^{2-} + \text{CH}_3\text{COO}^- + \text{H}_2 = \text{FeS (solid)} + \text{Fe}^{2+} + 3\text{OH}^- + 2\text{HCO}_3^- + 3\text{H}_2\text{O}
\]

A large part of the alkalinity generated in this reaction comes from the dissociation of Fe(OH)3, illustrated as follows:

\[
2\text{Fe(OH)}_3(\text{solid}) = 2\text{Fe}^{2+} + 6\text{OH}^-.
\]

From this point of view it can be stated that both processes, sulfate and iron reduction, are necessary to keep the end products of each reaction, ferrous iron and sulfide, from being reoxidized. The reoxidation would consume the generated alkalinity. Thus, it seems that both processes are important in the generation of alkalinity in strongly acidified lakes.

**Importance of iron reduction in the formation of magnetite.**

![Diagram](image-url)

**FIG. 4.** Eh-pH stability fields for iron, hematite, magnetite, and siderite in water at 25°C, 1 atm total pressure, and total dissolved carbonate activity of 10^{-1.35}. The solid line for iron represents ferrous iron activity for initial experimental conditions in the medium (log a_{Fe^{2+}} = -5.76). The dashed line represents ferrous iron activity in the low-pH medium (log a_{Fe^{2+}} = -4.39). There was essentially no difference in the Fe^{2+} lines generated for low- and high-pH Fe^{2+} iron concentrations. The hatched area represents the siderite stability field at the high Fe^{2+} concentration.

Many organisms contain magnetite, although the mechanism for the formation of the crystals is unknown. Magnetite has been found in the radula of chitons (23), skulls of pigeons (42), abdomens of bees (9), dolphin skulls (44), and "magnetosomes" of magnetotactic bacteria (2). As shown from the results of this study, it is possible that Fe^{2+} is secreted into alkaline inclusions or organelles under conditions that favor magnetite deposition.

Biological iron reduction may be an important mechanism in the formation of magnetite deposits in a variety of sediments and sedimentary rocks, as well as in biodegraded oil (3, 25). The formation of extracellular deposits of magnetite seems to be an indirect product of microbial metabolism and not a direct product of specific enzymatic action (i.e., there is no "magnetite-ase"). The organisms alter the local Eh and pH conditions which, in turn, shift local mineral solubility equilibria. This phenomenon has been called biologically induced mineralization (mineralization is used here as the geologic term which refers to formation of a mineral) and is considered to be a phylogenetic precursor to other more active forms of biominalization such as organic matrix-mediated mineralization that occurs in the formation of bivalve shells and bones (23). The precipitation is mediated by organisms altering local Eh conditions, pH conditions, or both (either intra- or extracellularly) and creating
conditions of supersaturation with respect to a mineral phase. Minerals produced from biologically induced mineralization have crystal habits similar to those produced when the minerals are precipitated from an inorganic solution. Organic matrix-mediated mineralization occurs when an organism lays out an organic matrix and actively pumps ions into the region of this matrix. The organic material contains "knobs" or foci for crystal nucleation and induces crystal formation and growth. All aspects of the mineral character (e.g., type, crystal orientation, substitution with other atoms) are under genetic control. The crystals formed by organic matrix-mediated mineralization have crystal habits different from those precipitated from inorganic solution and are clearly biological in origin (23).

We have shown that assemblages of anaerobic bacteria can induce the formation of extracellular deposits of magnetite at high pH and low Eh, but not at low pH and low Eh. These organisms reduce iron from iron oxyhydroxides either directly as a terminal electron acceptor or indirectly from reduced organic compounds. Magnete formation is the result of biologically mediated mineralization: that is, the organisms alter local Eh and pH conditions as well as generated reduced iron. When the pH is high the stable iron phase in a reducing iron-carbonate-water system is magnetite. At neutral pH, the stable ferrous phase is siderite. The Fe²⁺ concentration increases as the conditions become more acidic. If sulfur is added to the system, magnetite and siderite are largely replaced by iron sulfide minerals. Extracellular formation of magnetite is probably not an important process in nature, where there is an abundant supply of sulfide, but may be important in alkaline anaerobic systems.

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