Inhibition of Biological Reductive Dissolution of Hematite by Ferrous Iron

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Bacterial dissimilatory iron reduction is self-inhibited by the production of ferrous [Fe(II)] iron resulting in diminished iron reduction as Fe(II) accumulates. Experiments were conducted to investigate the mechanisms of Fe(II) inhibition employing the dissimilatory metal-reducing bacterium *Shewanella putrefaciens* strain CN32 under nongrowth conditions in a system designed to minimize precipitation of ferrous iron minerals. After an initial period (ca. 1 day) of relatively rapid iron reduction, hematite reduction rates were controlled by mass transfer of Fe(II). Experiments in which hematite was equilibrated with Mn(II) prior to inoculation indicated that the observed inhibition was not due to Fe(II) sorption. At longer times, soluble Fe(II) accumulated such that the reaction was slowed due to a decreased thermodynamic driving force. The thermodynamic evaluation also supported the prior conclusion that hydrated hematite surface sites may yield substantially more energy during bioreduction than "bulk" hematite. For well-mixed conditions, the rates of hematite reduction were directly proportional to the biologically available reaction potential.

Introduction

The dissolution of ferric iron minerals by dissimilatory metal-reducing bacteria (DMRB) has been the focus of much recent study. One area of practical interest has been evaluating means of increasing the rate of iron reduction in natural systems for groundwater remediation purposes (*1*−*10*). Several potential mechanisms of stimulating iron reduction are available including the following: addition of redox intermediates (i.e. electron shuttling compounds), removal of biogenic "free" ferrous iron from the systems (via transport or reaction), and solubilization of ferric iron to make it more bioavailable, and addition of an electron donor. A fundamental problem in the evaluation of these various strategies has been a lack of understanding of what processes control the rate of iron reduction (*2*, *3*, *5*, *6*, *8*, *10*, *11*). Recent research has begun to clarify what might control the rate of iron reduction under various conditions. Comparisons of rate data from the literature are difficult due to differences in time scales, iron sources, organisms, and the chemical environments (*12*). The goal of this paper is to evaluate one such system and provide a conceptual model of reductive dissolution of ferric minerals.

The observed limitations on the extent of iron reduction in batch studies have been most signifiant with crystalline ferric iron minerals (*2*, *6*, *9*−*11*, *13*−*16*). Amorphous, less thermodynamically stable, ferric iron phases are generally observed to be more readily bioreducible (*3*, *11*, *17*−*20*). However, crystalline oxide/hydroxide minerals represent a substantial fraction of iron minerals in the subsurface (*11*, *21*). Their importance as ferric iron sources for bioreduction processes stems from the need to sustain iron reduction over long periods of time possibly past the point at which the more readily reducible ferric iron minerals are depleted (*11*). In the context of long-term remediation strategies (or in environments where crystalline materials are dominant) the reduction of crystalline ferric iron minerals may be very important (*22*).

In systems that have been undergoing iron reduction for some time it is likely that Fe(II) will be present at elevated aqueous concentrations, as an adsorbed material, and possibly as biologically produced (directly or indirectly) minerals. The influence of Fe(II) on crystalline oxide reduction is thus an issue that bears investigation in the context of sustained bioremediation efforts that rely on microbial iron reduction. In addition, amorphous ferric iron minerals can undergo Fe(II)-catalyzed phase transformations into more crystalline phases such as goethite and magnetite (*23*).

Several mechanisms have been proposed by which Fe(II) might interfere with the reduction of crystalline, and amorphous, ferric minerals, including interference by adsorbed Fe(II) with some step in the electron transfer or dissolution (*16*, *24*), formation of Fe(II) precipitates that act as an insulator and prevent electron transfer (*11*) or prevent dissolution of Fe(II) from the crystal lattice (*25*), thermodynamic constraints due to accumulation of Fe(II) (*10*, *16*), or a mass transfer limitation for either dissolution or reduction (*10*).

Bioreduction of minerals is subject to mass transfer and abiotic kinetic controls in addition to limitations due to microbial/enzyme kinetics. The abiotic reductive dissolution rate may be controlled by adsorption of the reductant, electron transfer, or the dissolution of the reduced species from the crystal lattice (*26*). Soluble biomolecules, biological surfaces, and other complexants (e.g. natural organic materials) which have an affinity for Fe(II) may decrease the activity of Fe^{2+}, thus increasing the thermodynamic driving force and the mass transfer of Fe(II).

Previous experiments demonstrated a relationship between increased mixing intensity and increased hematite reduction (*10*). The transport of Fe(II) was hypothesized to be rate limiting for hematite reduction after ca. 1 day of incubation. The accumulation of Fe(II) near the oxide-cell interface may inhibit hematite reduction due to a loss of thermodynamic driving force. Other proposed mechanisms of inhibition include formation of surface precipitates and interference of Fe(II) with bioreduction. The objectives of the present study are to determine which species are mass transfer limited and determine the mechanism of inhibition (i.e. thermodynamic versus interference due to sorption).

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Experimental Section

Microorganism and Culture Conditions. *Shewanella putrefaciens* strain CN32 was cultivated and prepared as previously described (*9*). Briefly, cells were grown at 20 °C aerobically on an orbital shaker (100 rpm) on tryptic soy broth (100 mL culture) without dextrose (Difco). Cells were harvested by centrifugation (3510 × *g*, 10 min, 15 °C) and triple washed with 50 mM PIPES (by centrifugation). The last wash was done anaerobically in 50 mM PIPES. The cell density was determined by absorbance at 420 nm.

Hematite. An iron oxide powder was obtained from J. T. Baker (Philipsburg, NJ) and identified by X-ray diffraction and Mössbauer spectroscopy to be hematite (α-Fe_2O_3) of greater than 99% purity. The hematite powder had an average particle diameter of 300 nm and a specific surface area of 9.04 $m^2\ g^{-1}$ (measured by 5-point N_2−BET). Hematite was heated to 550 °C in air overnight before use to remove any organic carbon. Hematite was added to 50 mM PIPES buffer (+30 μM phosphate in some experiments) at least 48 h prior to any experiment to allow for hydration.

Bioreduction Experiment Preparation. Bioreduction experiments were prepared as previously described unless noted (*9*). Briefly, nongrowth cultures (ca. 10^8 cells mL^{-1}) were incubated (20 °C on an orbital shaker unless otherwise noted) in crimp sealed serum bottles with a 97.5:2.5% N_2:H_2 headspace, 2.0 g L^{-1} hematite (25 mM as Fe), and 50 mM PIPES (+30 μM phosphate in some cases). The initial pH was 6.8, and the pH remained within the range of 6.6−7.2 during all experiments. The medium was designed to limit secondary mineral formation. Experiments with and without 30 μM of phosphate established that phosphate had no effect on the amount of biogenic Fe(II) produced or on the fraction in which Fe(II) was recovered (*9*). All preparations were performed in an anaerobic chamber. Unamended biotic controls containing only the inoculated basic test medium were run for all experiments. Unless otherwise noted all treatments and controls were run at least in triplicate and mixed at ca. 100 rpm on a shaker table. Uninoculated controls for each amendment were also tested, none of which produced Fe(II).

Functional Analogue Amendments. Ferrozine (J. T. Baker), a specific Fe(II) chelator, was added to experimental systems as a dry powder to achieve a final concentration of 1.47 mM (*27*). AQDS (a quinone known to be capable of acting as an intermediate in iron reduction by this organism) ($E^0_w = -184$ mV, pH 7) (Aldrich, Milwaukee, WI) was added volumetrically from a filtered (0.1 μm), aerated, concentrated stock solution (*28*).

Variable Mixing Speed Experiments. Nongrowth bioreduction cultures were incubated under different mixing speeds to study the effect of mass transfer on hematite bioreduction (*29*). The experimental systems used conditions identical to the standard (unamended) bioreduction experiments except that the cultures were incubated in 250 mL media bottles rather than crimp sealed serum vials. Two cultures were mixed on a magnetic mixer at two speeds (400 and 1200 rpm) and one was incubated statically. Experiments using these materials were performed in 50 mM PIPES buffer with 30 μM phosphate.

Ferric Citrate Reduction. Ferric citrate reduction was tested in a solution containing 1.4 mM ferric citrate prepared in 50 mM PIPES buffer (pH = 6.8). Anaerobic ferric citrate PIPES medium was inoculated in the anaerobic chamber to achieve a final cell density of 10^8 cells mL^{-1}. One set of triplicate cultures was mixed at a moderate intensity (ca. 400 rpm), and the other set was incubated statically. Samples were taken over time and extracted in 0.5 N HCl for 24 h. Ferrous iron analysis was done using a modified 1,10-phenanthroline method as described below.

Manganese and Chloramphenicol Addition. Manganese was added (from a concentrated $MnCl_2$ stock) to hematite suspensions 5 days prior to inoculation to allow for adsorption equilibrium. The 5-day equilibration period was based on studies of Fe(II) sorption to hematite (*30*). Manganese was measured spectrophotometrically using a modification of HACH method 8149 (*31*). The sample (20 μL) was added to a solution of 880 μL of distilled-deinoized water with 50 μL of alkaline cyanide reagent (HACH) and 50 μL of a 0.1% 1-(2-pyridylazo)-2-naphthol (PAN) reagent (HACH). Soluble and acid extractable (0.5 N HCl) Mn(II) were defined identically to the comparable Fe(II) fractions. Chloramphenicol was added to cultures from a concentrated stock solution (prepared in 33% methanol to dissolve the chloramphenicol) to achieve a final concentration of 100 μM. A 33% methanol blank solution was used to spike the parallel cultures not amended with chloramphenicol. The chloramphenicol concentration (100 μM) was found to completely inhibit aerobic growth in tryptic soy broth without dextrose (no visible growth after 5 days).

Variable Hydrogen Pressure Experiments. Cultures were prepared as described above (Bioreduction Experiment Preparation) except that the headspace of the serum bottles (120 mL) contained either 2% or 5% hydrogen, and the cultures were mixed at 400 rpm on a magnetic mixer.

Leonardite Humic Acid. Leonardite humic acid is a terrestrial humic acid available from the International Humic Substance Society (IHSS) which was previously found to stimulate biological hematite reduction while not reducing hematite under abiotic conditions (*10*). Cultures were amended with Leonardite humic acid at concentrations of 125, 250, 500, and 1000 mg L^{-1}. These cultures were sampled for Fe(II) after 8 h and 1, 2, and 5 days. Leonardite humic acid was previously demonstrated to not reduce hematite in the absence of bacteria (*10*).

Fe(II) Analysis. Fe(II) was reported as soluble (filtered through 0.2 μm), acid extractable (in 0.5 N HCl), and adsorbed which was determined using methods previously described, where adsorbed was defined as the acid extractable minus the soluble Fe(II) (*9*). Solution pH was determined by combination electrode on the remaining filtrate in the anaerobic chamber. Iron(II) analysis for the Fe(III)-citrate reduction experiments used a method based on 1,10-phenanthroline (*32*). The method utilized ammonium fluoride to eliminate interferences due to soluble Fe(III).

Results and Discussion

Bioreduction of hematite slowed as Fe(II) accumulated in the system (e.g., Figure 1). These observations are consistent with previously reported results (*10*). The addition of ferrozine (a ferrous iron complexant) resulted in almost no sorbed Fe(II) during the first day, a longer initial period of fast bioreduction, and an increased overall production of soluble and acid extractable Fe(II), as shown in Figure 1a. The addition of AQDS (an electron shuttling compound) resulted in a much faster initial rate of bioreduction and accumulation of about five times as much Fe(II) after 5 days, as shown in Figure 1b. Incremental bioreduction between 5 and 30 days was previously demonstrated to be insensitive to the presence of AQDS (*29*). The decreases in hematite reduction with time were not due to cell death, since more than 90% of cells were viable after 5 days as determined by direct microscopic counts using the *Bac*light LIVE/DEAD viability stain (Molecular Probes, Eugene, OR) (*9*). Note that *Bac*light only measures membrane integrity; however, this method has been used previously to diagnose viability of *Shewanella putrefaciens* (*33*−*35*).

Increasing the intensity of mixing was found to increase the rate of iron reduction (Figure 2). This was previously ascribed to a mass transport controlled process (*10*). Although

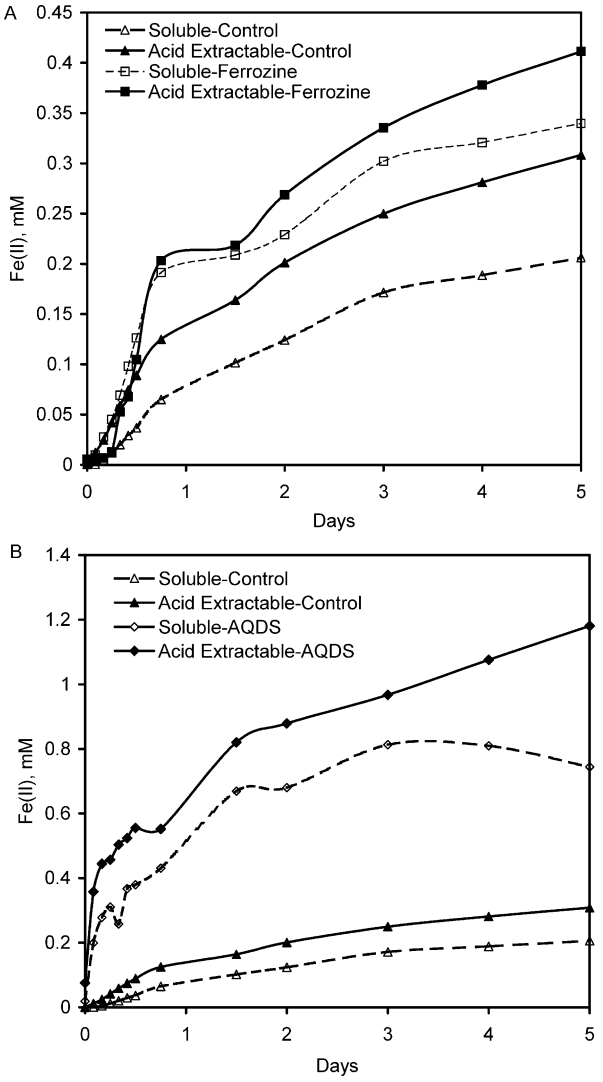

FIGURE 1. Acid extractable and soluble Fe(II) as a function of time in unamended biotic controls and cultures amended with (A) ferrozine (1.47 mM) and (B) AQDS (50 μM).

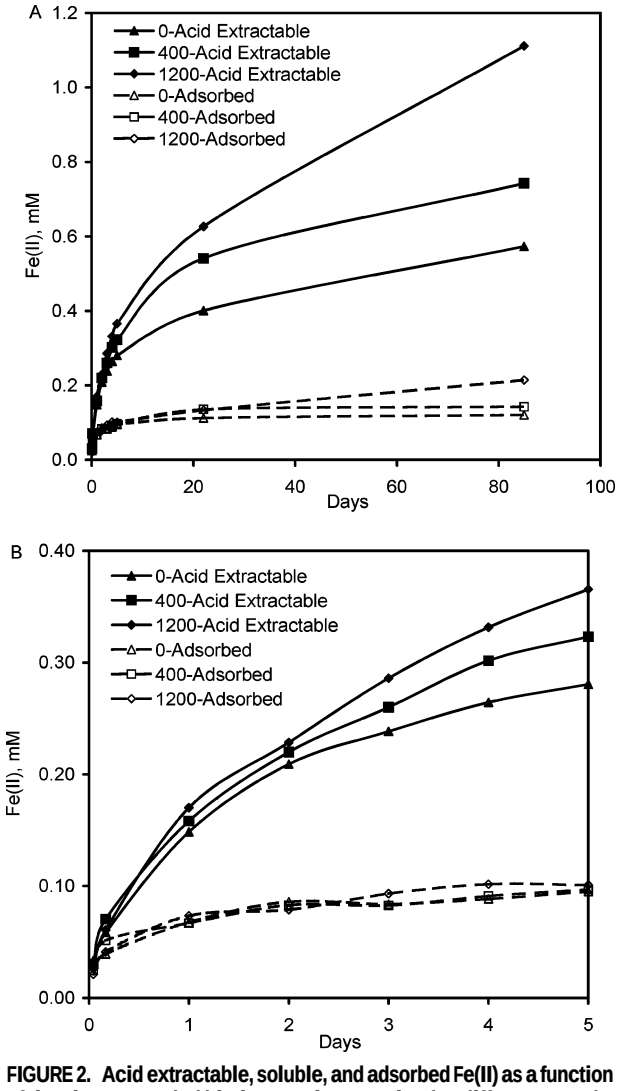

FIGURE 2. Acid extractable, soluble, and adsorbed Fe(II) as a function of time in unamended biotic experiments mixed at different speeds: (A) entire experiment and (B) first 5 days only. The numbers in the legend indicated the speed in RPM of the magnetic mixer. Zero indicates a static bottle. Reproduced with permission from *Environmental Science & Technology* 2002, *36*, 2897−2904. Copyright 2002 American Chemical Society.

total Fe(II) was greater for more intense mixing, nearly identical concentrations of adsorbed Fe(II) were observed for the three mixing conditions (Figure 2b), which is consistent with saturation of sorption sites for Fe(II). The maximum sorption in these experiments was consistent with previously published maximum sorption density for Fe(II) onto hematite (*30*) and *Shewanella putrefaciens* CN 32 (*12*, *24*). The disparity between Fe(II) produced at different mixing speeds generally increased over time, although Fe(II) produced in the static and 400 rpm cultures between days 22 and 85 was nearly identical.

These experiments (Figure 2) clearly demonstrated that bioreduction of hematite increased with improved mass transport conditions. However, the experiments did not identify the reactant or product whose transport was limiting nor the reaction basis for the rate limitation. The answer to the second question is critically dependent on the first, so these questions will be addressed in order.

Hematite reduction was tested with 0.02 and 0.05 atm partial pressure of H_2 (Figure 3) to test the hypothesis that reaction rate was limited by mass transfer of H_2. These cultures were mixed at 400 rpm with magnetic stir bars identical to those used in the variable mixing experiments (Figure 2). Initially (up to 1 day) there was a small but statistically distinguishable difference in bioreduction ($p =$ 0.047, two tailed t-test), with slightly more Fe(II) produced at 0.05 atm H_2 than at 0.02 atm H_2. No statistical differences existed between subsequent samples ($\alpha = 0.05$). Additionally, the zero-order reduction rates from 1 to 5 days were very similar (no statistically significant difference) for both sets of cultures (0.0367 μM Fe(II) min^{-1} for 0.05 atm H_2 and 0.0357 μM Fe(II) min^{-1} for 0.02 atm H_2). Thus, the rate of the reaction after the first day appeared to be independent of the supply of H_2 to the organisms.

Analogously, mixing significantly increased the rate of ferric citrate reduction, indicating a mass transport influence (Figure 4). Both static and mixed systems sustained bioreduction rates for ferric citrate that were far greater than for reduction of hematite, even for hematite in the presence of AQDS (Figure 1a). For ferric citrate, the consumption of H_2, based on the consumption of half of a mole of hydrogen per mole of Fe(III) reduced, was 3.9 μM min^{-1} ($t = 0-150$ min) and 1.5 μM min^{-1} ($t = 0-300$ min) for the mixed and static systems, respectively. These results confirmed that the supply of H_2 was greater than the maximum demand for H_2 in the hematite experiments (e.g., 1.2 μM H_2 min^{-1}, between $t =$ 0−2 h for the AQDS-amended hematite experiment, Figure

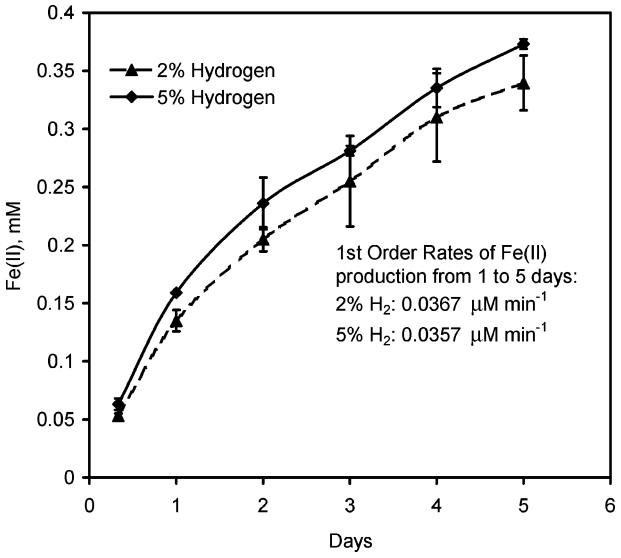

FIGURE 3. Acid extractable Fe(II) in hematite reducing cultures with 2 and 5% H_2 in the headspace. Values are means of three replicates (± standard deviation).

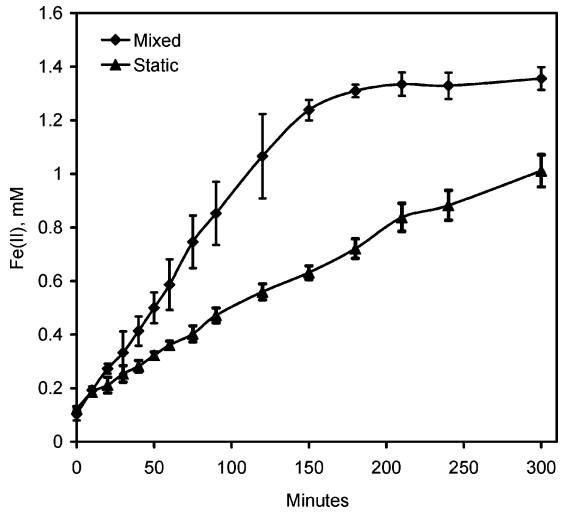

FIGURE 4. Acid extractable Fe(II) in Fe(III)-citrate reducing cultures. Values are means of three replicates (± standard deviation).

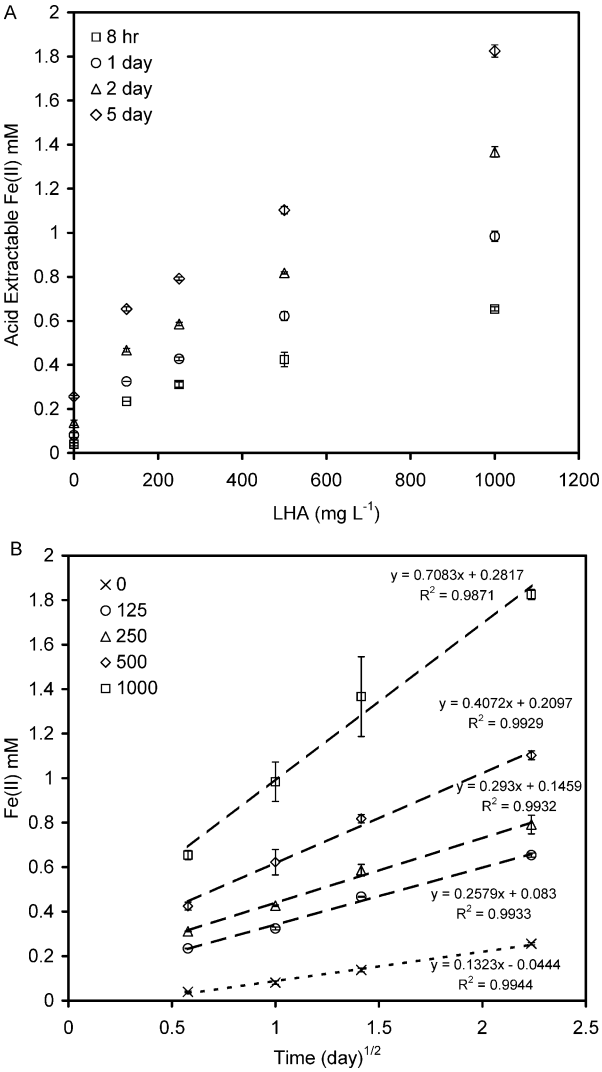

FIGURE 5. Acid extractable Fe(II) in hematite reducing cultures amended with Leonardite humic acid versus (A) humic acid concentration and (B) $time^{1/2}$. Values are means of three replicates (± standard deviation). Equations, lines, and r^2 values determined by least squares linear regressions. Numbers in legend indicate concentration of humic acid in mg L^{-1}.

1b). Based on these results, transport of H_2 was eliminated as a rate-determining step.

Transport of Fe(II) or H^+ might also limit the rate of bioreduction. Diffusive transport of H^+ is fast, and the experimental system was well buffered, so that activity of H^+ within the interfacial regions would have remained relatively constant. Therefore it is likely that mass transport of Fe(II) was the rate-limiting process for bioreduction of hematite under conditions that are described in Figure 2 (i.e., no amendments). There are several mechanisms by which bioreduction could be slowed due to high Fe(II) concentration at the reaction site. Mechanisms that required precipitation (e.g., coating the hematite or cell surface) were not considered because the system was undersaturated with respect to Fe-(II) precipitates and because all nonsoluble Fe(II) was accounted for by the previously measured extent of biosorption (*12*) and sorption to hematite (*30*).

Hematite reduction was also studied in cultures that were amended with variable amounts of Leonardite humic acid (LHA). LHA increased the rate and extent of Fe(II) production relative to unamended controls (Figure 5a), which was consistent with earlier experiments (*10*). Humic materials can enhance hematite bioreduction by electron shuttling or by Fe(II) complexation (*10*). It was previously demonstrated that LHA enhanced the rate and the extent of bioreduction substantially due to complexation of Fe(II) (*29*). Data from Figure 5a were also plotted in Figure 5b, where Fe(II) production is shown as a function of $t^{1/2}$. Straight lines were obtained, with the slope increasing with the concentration of LHA. These results are consistent with a diffusion controlled process as described by eq 1 (*36*)

$$C = C_0 + 2k_p t^{1/2} \quad (1)$$

where C = solution concentration at time t, C_0 = initial solution concentration, k_p = reaction rate constant [M $s^{-1/2}$] where M = mol L^{-3}, and t = time in seconds.

The LHA was increasingly effective in promoting hematite reduction over time, possibly due to the rate of reduction being increasingly controlled by mass transfer (Figure 5a). Complexation of Fe(II) in the bulk solution would increase the flux of Fe(II) from the interface by decreasing the bulk Fe^{2+} activity via complexation. LHA near the interface would tend to be "saturated" with Fe(II) faster than more LHA could diffuse into the interface making complexation more significant in the bulk (relative to near the interface) solution

as Fe(II) accumulated. Additionally the Fe(II)-LHA would also diffuse from the surface providing another means of removing Fe(II) from the interface. LHA would also decrease the activity of Fe^{2+} at the interface, with impacts that are discussed below.

Collectively the results were consistent with mass transport of Fe(II) as the most likely rate controlling process during the hematite bioreduction experiments presented, i.e., more intense mixing increased the rate of bioreduction of hematite (Figure 2), improved mass transfer of H_2 did not increase the rate of hematite bioreduction after 1 day (Figure 3), and there were good linear correlations between production of Fe(II) from hematite and $t^{1/2}$, and an increased slope (k_p) with increasing concentration of LHA (Figure 5).

If mass transfer were the rate-determining step, then inhibition of bioreduction could be due to any of the following mechanisms: (i) adsorption of Fe(II) and blocking of active dissolution sites such as kinks (*25*) on the hematite surface, (ii) interference with electron transfer from the cell to the oxide bound Fe(III), or (iii) decreased thermodynamic driving force due to accumulation of products at the interface.

The hypothesis that Fe(II) inhibited bioreduction by occupation of surface sites on either hematite or cells was tested by using Mn(II) as a surrogate for Fe(II). Mn(II) is not a product or reactant of hematite bioreduction, and it should not affect the thermodynamic driving force. Wilson (*37*) and Coughlin and Matsui (*38*) demonstrated significant sorption of Mn(II) by ferric oxides. Jeon et al. (*39*) showed that addition of Zn(II), Ni(II), or Cd(II) resulted in a 20−43% decrease in sorption of Fe(II) onto hematite, when sorbate to sorption site ratios were slightly lower than for the current work. As a result, it was hypothesized that Mn(II) might inhibit hematite reduction due to the sorption of the Mn(II) to cells or oxide surfaces. Mn(II) was added at three concentrations (0.125 mM, 0.25 mM, and 0.5 mM) and allowed to equilibrate with the hematite suspension prior to inoculation of cells, because sorption of Fe(II) to hematite is slow relative to sorption of Fe(II) to biomass (*12*, *30*). The two lower concentrations had very slight inhibitory effects, but 0.5 mM was stimulatory. The cause of this slight inhibition at low concentrations of Mn(II) is unknown. The stimulatory effect of 0.5 mM Mn(II) increased with time (Figure 6a). Adsorption of Fe(II) was decreased due to addition of Mn(II).

To evaluate whether the observed Mn(II) stimulation of iron reduction was linked to protein synthesis, cultures were amended with either 0.5 mM Mn(II), 0.1 mM of the protein synthesis inhibitor chloramphenicol (0.1 mM was found to inhibit growth of this strain), or both. Although Fe(II) production was partially inhibited by chloramphenicol, it did not prevent Mn(II) from stimulating hematite reduction (Figure 6b). The stimulation of hematite reduction by Mn-(II), therefore, appears to be a chemical effect rather than a biological/genetic effect (e.g. induction of a protein related to iron reduction). The Mn(II) experiments indicated that Fe(II) likely did not inhibit bioreduction of hematite by a sorption mechanism.

Data from the unamended experiments were evaluated to determine the possibility that the rate of bioreduction was controlled by thermodynamic constraints. In this regard, several investigators have reported that a minimum excess potential of 0.207 mV was required for microbial energy conservation (*16*, *40*, *41*). Liu et al. (*16*) reported that 22.7 kJ mol^{-1} of excess energy was required for bioreduction of goethite *by S. putrefaciens* CN32, based upon kinetic data.

The reaction potentials for the reduction of hematite, goethite, HFO, and an unknown species, denoted "hydrated hematite", are shown in Figure 7a for the range of Fe(II) concentrations that were observed (data from Figures 1 and 2). It was assumed, for these experiments without added LHA or ferrozine, that all dissolved Fe(II) was present as Fe^{2+}.

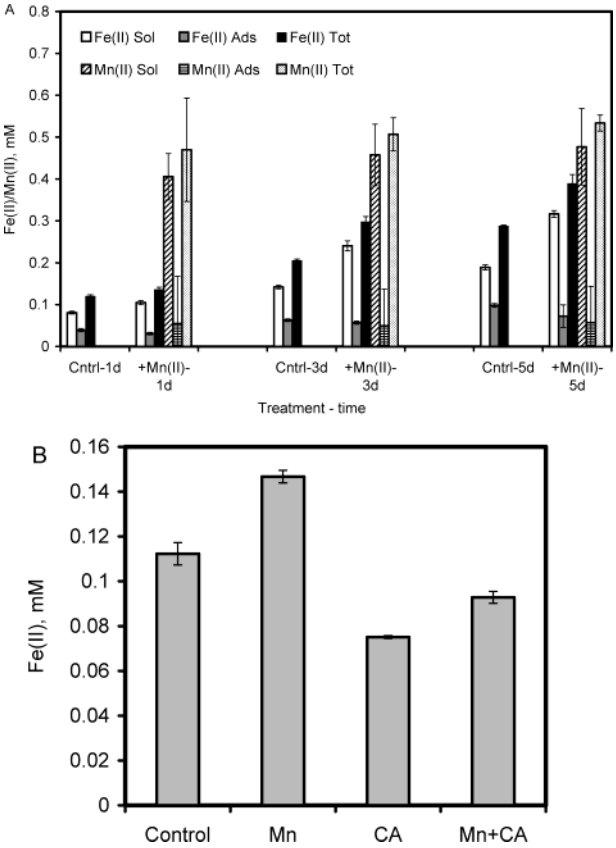

FIGURE 6. Acid extractable, soluble, and adsorbed Fe(II) and Mn(II) in hematite reducing cultures amended with (A) Mn(II) (0.5 mM) and (B) Mn(II) (0.5 mM) with and without chloramphenicol (0.1 mM). Samples were taken at 1, 3, and 5 days (A) or 1 day only (B). CA = chloramphenicol, Mn = preequilibrated for 5 days with Mn(II). Values represent means of six (A) or five (B) replicates (± standard deviation).

Reaction potentials in Figure 7a were also adjusted for temperature, ionic strength, and pH. In addition to curves for the well-described phases of ferric oxides, an additional curve was drawn such that the net driving force for reaction (reaction potential minus 0.207 V) became zero for the highest soluble Fe(II) concentrations that were observed. This phase was designated "hydrated hematite" and based on Figure 7a was considered to have a $E° = 0.844$ V, which is 0.114 V more reactive than hematite ($E° = 0.730$ V). Figure 7a shows that if it is assumed that $\{Fe^{3+}\}$ is controlled by equilibrium dissolution of hematite, then the reaction potential became less than 0.207 V (equivalent to a one-electron transfer of 20 kJ mol^{-1}).

Previous modeling of this experimental system (*S. putrefaciens* CN32, hydrogen, and hematite) required a standard potential of 0.10 V greater than hematite (0.730 mV, Visual Minteq v. 2.01) to accurately model the observed hematite reduction (without explicit consideration of the 20 kJ mol^{-1} excess energy) (*12*). HFO is even more soluble, with a standard potential of 0.960 V (Visual Minteq v. 2.01). The reaction potentials in Figure 7a indicate that the reaction potential within the cell−oxide−water interfacial region was likely between that of HFO and goethite, i.e., ferric oxide at the interface might exhibit reaction potential that is different than reaction potential for the three-dimensional bulk hematite (*42*). The phase hypothesized by Burgos et al. (*12*) to have a standard potential 0.10 V greater than hematite only became biologically unfavorable for reduction at the highest observed Fe(II) concentration (not shown). This phase could represent a hydrated surface species. Analogous behavior has been reported, e.g. interfacial aluminum oxide

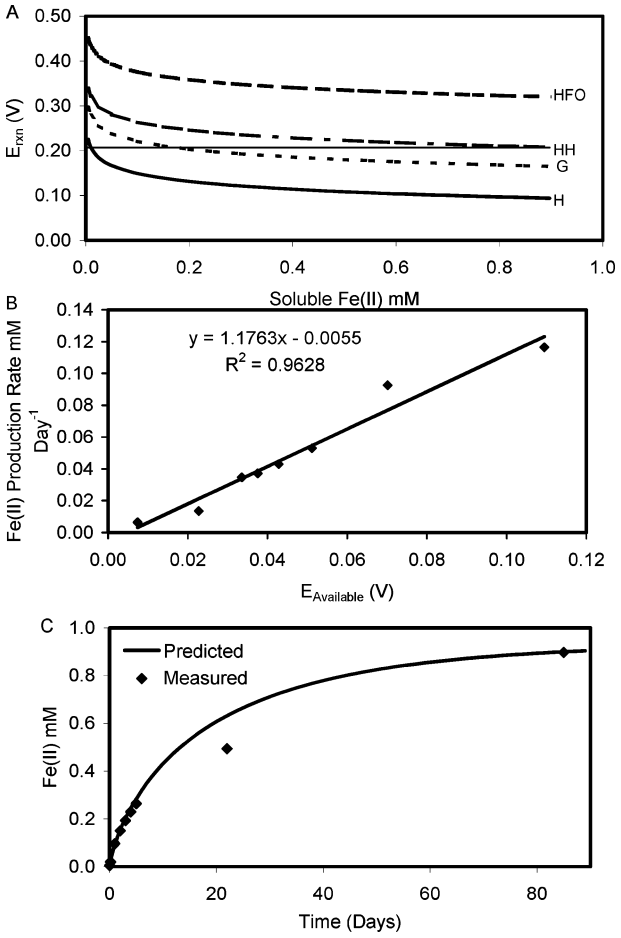

FIGURE 7. (A) Reaction potentials (E) for hematite "H", hydrated hematite "HH" (*12*), goethite "G", and hydrous ferric oxide "HFO" reduction as a function of soluble Fe(II) over the range of soluble Fe(II) measured in bioreduction experiments without amendments (Figures 1 and 2). Horizontal line is 0.207 mV biological potential threshold. (B) Rate soluble Fe(II) production as a function of $E_{Available}$. (C) Predicted and measured soluble Fe(II) using rate data from (B) and "hydrated hematite" from part (A). $T = 20$ °C, pH = 6.8, $P_{H2} = 0.025$ atm, $I = 0.05$ M, $\gamma_{Fe(II)} = 0.48$. Thermodynamic constants from Visual Minteq v. 2.01.

(from hydrated corundum α-Al_2O_3) exhibited sorption characteristics that were similar to amorphous AlOOH (*43*). There is sufficient energy due to hydration to force this transformation to a phase with lower interfacial tension with water but higher activity of Fe^{3+}. Hematite is readily hydrated and dehydrated and has a heat of immersion of 3.7×10^{-5} J cm^{-2} (after drying at 70 °C) (*44*). For 10 hydroxyl sites per nm^2 the heat of immersion would be 22.5 kJ mol^{-1}, or $\Delta E = 0.24$ V if all the energy were transferred to a more soluble and energetic surface (*42*). Based upon the present and prior studies (*12*, *42*), the analysis of Liu et al. (*16*), and theoretical considerations (*40*, *43*, *44*) the hydrated hematite surface may have a higher solubility of Fe^{3+} and a higher reaction potential that would occur for bulk hematite.

Biological hematite reduction results in accumulation of Fe(II) in the interface between water and either hematite or cells. Transport of Fe(II) away from the surface by mixing would achieve a higher reaction potential at the cell oxide interface. Roden et al. demonstrated the dramatic effect of advective flow on the biological reduction of goethite coated sand (*8*). The previously demonstrated influence of solid and soluble Fe(II) complexing agents (*6*) is also consistent with Fe(II) transport limitations.

It was previously hypothesized that Fe(II) accumulation and the concomitant decrease in thermodynamic driving force was controlling microbial hematite reduction kinetics (*10*). Average soluble Fe(II) production per time was calculated based upon the 1200 rpm mixed culture (Figure 2). The high mixing provided the best mass transfer and should have resulted in the smallest difference between the bulk and interfacial activities of Fe^{2+}. The biologically available potential was defined as the calculated reaction potential minus 0.207 V and was denoted $E_{Available}$. Fe(II) production was linearly related to $E_{Available}$ (Figure 7b).

The reaction rates were predicted to be first order with respect to the reaction potential, where reaction potential was defined as the biologically available potential associated with the measured pH and Fe(II) (at 1200 rpm) and hydrated hematite (0.114 V more reactive than hematite). The agreement between predicted and observed values was good (Figure 7c), indicating that for well-mixed conditions the rate was directly proportional to the available energy of reaction $E_{Available}$. The only parameter manipulated was the reaction potential for the "hydrated hematite" phase.

The bioreduction of hematite in the present study appeared to transition from kinetic to thermodynamic control. This was previously hypothesized (*10*), and the present study strengthens this conceptual model. Fe(II) transport appeared to be limiting the rate of hematite reduction. Based upon the influence of Mn(II) on hematite reduction, the mechanism of Fe(II) inhibition was likely thermodynamic rather than a physical "blocking" effect due to sorption. Thermodynamic analysis of the biological feasibility of hematite reduction demonstrated that hydrated hematite surfaces were more energetic than the "bulk" material. The reduction of hydrated hematite surface may have a free energy yield that is intermediate between "bulk" hematite and amorphous ferric oxide/hydroxides. Bioreduction kinetics appeared to be first order with respect to biologically available reaction potential.

Environmental Implications. Stimulation of biological iron reduction is a proposed strategy for bioremediation. Successful application of biostimulation must consider all of the factors that could control the kinetics of this process. Mass transfer is often neglected as a possible rate-limiting step in bench studies. This work has demonstrated that mass transfer may have implications in natural systems where electron donors are added in order to stimulate bioreduction.

Hematite was used in this study. Hematite is the most stable of the ferric oxides and therefore the most resistant to bioreduction. Crystalline oxides may represent an important source of iron for long term bioremediation, persisting after the amorphous phases have been depleted. Bioreduction of crystalline ferric iron minerals may be slowed or stopped due to thermodynamic limitations such as those described in this paper, i.e., due to local product [i.e. Fe(II)] accumulation or due to decreased biologically available reaction potential. Hydrated hematite surfaces may be more favorable for reduction than previously recognized.

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