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The Impact of Bacterial Strain on the Products of Dissimilatory Iron Reduction

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Abstract

Three bacterial strains from the genus *Shewanella* were used to examine the influence of specific bacteria on the products of dissimilatory iron reduction. Strains CN32, MR-4 and W3-18-1 were incubated with HFO (hydrous ferric oxide) as the terminal electron acceptor and lactate as the organic carbon and energy source. Mineral products of iron reduction were analyzed using X-ray powder diffraction, electron microscopy, coulometry and susceptometry. Under identical nutrient loadings, iron reduction rates for strains CN32 and W3-18-1 were similar, and about twice as fast as MR-4. Qualitative and quantitative assessment of mineralized end products (secondary minerals) indicated that different products were formed during experiments with similar reduction rates but different strains (CN32 and W3-18-1), and similar products were formed during experiments with different iron reduction rates and different strains (CN32 and MR-4). The major product of iron reduction by strains CN32 and MR-4 was magnetite, while for W3-18-1 it was a mixture of magnetite and iron carbonate hydroxide hydrate (green rust), a precursor to fougérite. Another notable difference was that strains CN32 and MR-4 converted all of the starting ferric iron material into magnetite, while W3-18-1 did not convert most of the Fe³⁺ into a recognizable crystalline material. Biofilm formation is more robust in W3-18-1 than in the other two strains used in this study. The differences in mineralization may be an indicator that EPS (or another cellular product from W3-18-1) may interfere with the crystallization of magnetite or facilitate formation of green rust. These results suggest that the relative abundance of mineral end products and the relative distribution of these products are strongly dependent on the bacterial species or strain catalyzing iron reduction.

2. INTRODUCTION

Iron is one of the most abundant elements on earth, making up about 5 weight percent of the earth's crust. Under present-day surface conditions, iron can occur as one of several oxyhydroxides, carbonates, phosphates or silicates and form a major constituent of some rocks and sediments (Fagel et al., 2005; Lepp, 1975; Postma, 1977). The structure of these minerals can influence their susceptibility to reduction and oxidation reactions, thus influencing the biogeochemical cycling of iron in soils and sediments (Huber and Garrels, 1953; Postma, 1977).

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It is well known that bacteria can play an important role in iron biogeochemical cycling (Lovley, 1991; Lovley and Phillips, 1986a; Lovley and Phillips, 1986b; Nealson, 1997; Nealson et al., 2002; Nealson et al., 1983; Nealson and Saffarini, 1994). The process by which bacteria can couple the oxidation of organic carbon to the external reduction of iron oxide is known as dissimilatory iron reduction (DIR). The secondary mineral products of DIR can have an impact on the mobilization or sequestration of organic and inorganic pollutants, as well as the subsequent geochemical cycling of iron in soil and sedimentary environments (Cooper et al., 2006; Croal et al., 2004; Zachara et al., 2001; Zachara et al., 2002).

There are several factors thought to control the products of DIR. Researchers have postulated that the amount of Fe^{2+} in a system during the early stages of reduction can have an impact on the subsequent mineralization (Fredrickson et al., 2003; Hansel et al., 2003; Hansel et al., 2004). Low initial concentrations of Fe^{2+} can promote goethite accumulation and inhibit magnetite precipitation, even if Fe^{2+} concentrations increase at a later time (Hansel et al., 2003). Under laboratory conditions, it is thought that the type of buffer used is largely responsible for the identity of the reduced iron oxide biominerals (Fredrickson et al., 1998; Zachara et al., 2002). A conceptual model has also been proposed where the rate of supply and total concentration of Fe^{2+} is the primary determinant of secondary mineralization products (Zachara et al., 2002). These authors have suggested that bacteria have no influence on the formation of secondary iron oxide minerals beyond producing a supply of Fe^{2+} .

While dissimilatory iron reducers are ubiquitous in nature, most laboratory-based work has been done on strains from *Geobacter* and *Shewanella* (Croal et al., 2004; Crosby et al., 2007; Lovley et al., 2004; Lovley et al., 1998; Lovley and Phillips, 1988; Myers and Nealson, 1990; Nealson and Saffarini, 1994; Roden, 2004; Roden, 2006). The *Shewanella* genus encompasses several strains that have been shown capable of DIR (Fredrickson et al., 1998; Lloyd, 2003; Myers and Nealson, 1990; Venkateswaran et al., 1998; Weber et al., 2006). *Shewanella* are gram-negative, facultative anaerobes belonging to the γ -proteobacteria. They have a rapid generation time under aerobic conditions, and are highly adaptable to dynamic environmental conditions, making them ideal for physiologic studies. The majority of work done investigating the factors that affect the formation of secondary iron minerals within *Shewanella* has been done on *S. putrefaciens* CN32 (Crosby et al., 2007; Dong et al., 2000; Fredrickson et al., 1998; Hansel et al., 2003; Liu et al., 2001a; Liu et al., 2001b; Zachara et al., 2001; Zachara et al., 2002). In this contribution, we compare the secondary iron mineral products of three different *Shewanella* strains, using lactate as the electron donor, in order to explore the influence of bacterial strain on mineral formation.

3. Materials and Methods

3.1 Bacterial Cultures

The *Shewanella* strains used in this study are listed in Table 1. The bacteria were inoculated directly from frozen stocks into Luria-Bertani (LB) broth and grown overnight in a 15°C incubator shaking at 125rpm. The cultures were washed with defined medium to remove traces of LB. Medium composition is listed in Table 2 and has been shown to support the growth of several strains of *Shewanella*.

Prior to inoculation, the medium was filter-sterilized with a 0.2 μm PES vacuum filtration system (Nalgene). Medium (18mL) was then added to serum bottles along with 2mL of 0.2 M hydrous ferric oxide (20mM final concentration). The bottles were purged with pure N_2 , plugged with butyl-rubber stoppers and crimp sealed. Washed cells were injected into the sealed serum bottles with a syringe using a 21-gauge needle to achieve a final cell concentration of approximately 1×10^8 cells/mL. Triplicate cultures were incubated at 15°C and sampled at defined intervals for up to 800 hours.

3.2 Preparation of Hydrous Ferric Oxide

A stock solution of 0.2 M hydrous ferric oxide (HFO) was prepared according to the method of Cornell and Schwertmann (1996). Ferric chloride hexahydrate (54g of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$) was dissolved into 18M Ω water (2L). NaOH pellets were added to bring the pH up to approximately 7, causing precipitation of the dissolved ferric iron. The precipitated iron slurry was washed repeatedly with 18M Ω water to remove any trace salts and brought to a final volume of 1L. The resulting material was analyzed via X-ray diffraction to confirm the production of HFO.

3.3 Fe^{2+} Analysis

At selected time points, bottles were removed from the incubator and placed in an anaerobic glove box (Coy Labs, N_2/H_2 headspace). Aliquots (250 μL) were extracted with a 21-gauge syringe needle and placed into micro-centrifuge tubes. These samples were centrifuged at 10600 $\times$ g rcf for 2 minutes. The supernatant was placed into a separate tube containing 250 μL of 1N HCl. This sample was used to determine the aqueous Fe^{2+} concentration. The pellet was re-suspended with 250 μL of anaerobic 18M Ω water and 250 μL of 1N HCl. This solution was used for determining the HCl-extractable solid phase and sorbed Fe^{2+} . Both portions of the reduced iron were quantified using the ferrozine spectrophotometric assay as described by Stookey (1970). Additionally, a single drop of the collected 250 μL aliquots was added to pH paper for determination of the pH at each time point.

3.4 Mineralogical Analysis

Samples were prepared in a similar fashion for all analyses done to determine mineral identity, concentration and morphology. All samples were collected in the glove box. Prior to sampling, the bottles were vigorously shaken and then quickly sampled to obtain a uniform distribution of particles. Approximately 2 ml of material was collected from the anaerobic serum bottles using 21-gauge needles. In an attempt to remove organics and salts from the collected material, samples were washed 2–3 $\times$ with anaerobic 18M Ω water. Replicates of three samples had a precision of about 20%.

3.4.1 Environmental Scanning Electron Microscopy—Approximately 10 μL of the washed samples were placed onto a polycarbonate filter and allowed to air dry under anaerobic conditions. Samples were kept under anaerobic conditions until the time of analysis. These samples were used to determine biomineral morphology by use of an environmental scanning electron microscope (ESEM, Hitachi TM-1000 Table Top Microscope).

3.4.2 Susceptometry—Magnetic susceptibility was utilized in order to determine the relative amount of magnetite in the collected samples. This technique has been previously used to quantify sedimentary magnetite as well as magnetite produced by magnetotactic bacteria and by dissimilatory iron reducers (Hesse, 1994; Leslie et al., 1990; Vali et al., 2004). Comparisons of the remanence for synthetic magnetite (Sigma-Aldrich), hematite, siderite, HFO and biogenic green rust produced susceptibility values for magnetite that were orders of magnitude larger than those of the other compounds (Salas, 2008), indicating that this technique could be used to measure the amount of magnetite produced in these experiments. The remaining material from the washed samples was collected for analysis. Samples were transferred into micro-centrifuge tubes and allowed to dry under anaerobic conditions. The dried samples were then weighed, placed into small plastic containers and analyzed on a susceptometer (Kappabridge KLY-4S). The resulting values for the tested biominerals were compared to standards constructed from a synthetic magnetite (Sigma-Aldrich) in order to derive the mass of magnetite found in the biological samples. After analysis, these samples were placed in serum bottles, sealed and air was evacuated prior to coulometric analysis.

3.4.3 Coulometry—Coulometry was used to quantify the amount of solid-phase inorganic carbon found in the samples. The samples used for susceptometry measurements were reweighed, placed into serum bottles, stoppered and sparged with 100% N₂. Sulfuric acid (400 µl of 20%) was added to each sample to dissolve the solid material and release any solid inorganic carbon as carbon dioxide. The acidified samples were placed on a shaker rotating at 105 rpm and left overnight to ensure complete dissolution of the sample. Once the samples had dissolved, the serum bottles were connected in-line to a coulometer (UIC, Inc. Coulometrics model 5012) via two 21-gauge needles serving as an inlet and outlet, using nitrogen as a carrier gas

3.4.4 XRD—At the end of the experiment, samples were collected in order to characterize the major biomineral products produced by the tested bacterial strains. An aliquot of 3 mL was collected, washed repeatedly with anaerobic 18MΩ water to remove any organics and salts, and air-dried under anaerobic conditions. Samples remained under anaerobic conditions until the time of analysis. A small amount of dried material was analyzed by powder X-ray diffraction on a Rigaku R-Axis Spider curved imaging plate microdiffractometer utilizing monochromatic Mo Kα radiation.

4. RESULTS

4.1 Reduced Iron Oxide Biominerals

ESEM images comparing the morphology of the biomineral products produced by the three bacterial strains tested in these experiments are shown in Figure 1. Both CN32 and MR-4 produced nanocrystalline grains. In the case of CN32, these grains were morphologically similar to those previously reported (Zachara et al., 2002). Strain W3-18-1 produced both nanocrystalline material, and hexagonal plates (Fig 1B). Powder XRD analysis revealed that all three strains produced magnetite (Fig. 2). In the case of W3-18-1, XRD results indicated that a second mineral was formed. Peak patterns for this second product were consistent with iron carbonate hydroxide hydrate (green rust), a precursor to fougérite (Génin et al., 2005).

4.2 Comparison of HFO Reduction Rates

Reduction of HFO through 800 hours is shown in Figure 3. HCl (0.5N) is considered to be an effective solvent for most biogenic solid phase Fe²⁺, including magnetite (Fredrickson et al., 2003). As such, measurements of HCl-extractable Fe²⁺ are used here as an indicator of total reduced iron production. The rates of Fe³⁺ reduction for all three strains through 100 hours were comparable. After 100 hours, there was a divergence in rates. The rate of reduction by *S. putrefaciens* CN32 appeared to be constant through 400 hours, followed by a slightly slower rate for hours 400–800. Rates of reduction by strain W3-18-1 were similar to CN32, although W3-18-1 showed a somewhat higher rate between 100 and 200 hours. There was a lag in the reduction rate for strain MR-4 between 100 and 300 hours, after which the rate of reduction increased.

The amount of dissolved Fe²⁺ that accumulated during HFO reduction was different for the three strains (Fig. 4). Strains CN32 and W3-18-1 showed a similar pattern of increase through 400 hours. At 800 hours, however, the amount of aqueous phase Fe²⁺ was lower in the samples with strain CN32. The concentration of aqueous phase reduced iron continued to increase steadily for W3-18-1 through the duration of the experiments. Strain MR-4 also showed a steady increase in aqueous phase Fe²⁺, although it was approximately 5 times lower than the other two strains. In all cases the concentration of Fe²⁺ found in solution constituted less than 5% of the total reduced iron in the samples.

4.3 Relative Magnetite Concentrations

The pattern of magnetic susceptibility for strain CN32 showed a rapid increase followed by a plateau after 400 hours (Fig. 5). Magnetic susceptibility of the products of iron reduction by strain MR-4 showed an exponential increase with time. In contrast, strain W3-18-1 produced materials that showed no significant change in response to the applied magnetic field beyond 100 hours. However, even in this case, the susceptibility values were larger than that of the controls, indicating that a transformation of some of the starting ferric iron oxide material has taken place. There are various factors that can influence the susceptibility of a material, such as mineral concentration, mineral composition, crystal size and crystal shape (Dearing, 1999). While grain size can play a role in the degree to which a sample will respond to an applied magnetic field, microscopy does not suggest large differences in the morphology of the magnetite products.

4.4 Inorganic Carbon Concentrations

Solid phase inorganic carbon present in the samples at the end of the experiment are shown in Figure 6. Values for strains CN32 and MR-4 were within the range of the background values measured in the negative control samples. Almost five times as much particulate inorganic carbon was measured in the samples inoculated with strain W3-18-1. Given the conditions in these cultures, the only expected solid-phase carbonate would be an iron carbonate of some kind. Based on the information gathered from XRD analysis, the iron carbonate is in the form of iron carbonate hydroxide hydrate. By using the formula weight of iron carbonate hydroxide hydrate (636 g/mol, $\text{Fe}_6(\text{OH})_{12}\text{CO}_3 \cdot 2\text{H}_2\text{O}$) and an iron to carbon ratio of 6, we conclude that approximately 15% of the starting ferric iron material has been incorporated into green rust.

4.5 Degree of Saturation

The degree of saturation for green rust, magnetite, and siderite was calculated to assess whether differences in the mineral products could be attributed to differences in saturation state. Calculations were done for the conditions at the end of the experiment, using the following reactions:

Because detailed thermodynamic data for green rust is limited, calculations were done for 25°C, rather than 15°C (Table 4). Activity coefficients for solutes were calculated from the ionic strength and the Davies equation, but ignored the role of any ion complexing. Calculations assume that some HFO remained throughout the experiment, which may not be certain. However, Fe^{2+} concentrations typically rose throughout the experiment, indicating the presence of some bioavailable Fe^{3+} in the cultures. XRD did not show the presence of more crystalline ferric (hydr)oxides (Fig. 2), and susceptometry data did not detect a loss of magnetite (Fig. 5). Additionally, the concentrations of all solutes during the second half of the incubations did not change by more than a factor of 3 for any of the relevant ions. Given the very high results for saturation (Table 3), these assumptions should not be significant.

Results show that the solution is supersaturated with respect to all three phases. Magnetite is the most super-saturated phase and should be the most stable (Table 3, Fig. 7). Either magnetite or green rust could easily precipitate if nucleation barriers could be overcome, unless kinetics were too slow or crystal growth were inhibited. Although magnetite is the most highly supersaturated mineral in all treatments, the relative supersaturation of green rust is greatest in experiments with W3-18-1. In this case, the relative supersaturation is approximately an order of magnitude greater than with the other two strains. Siderite is modestly supersaturated in all three treatments.

5. DISCUSSION AND SUMMARY

For CN32 and MR-4, the products of DIR consist almost entirely of magnetite. This is in agreement with previous results in which *Shewanella* strains were incubated under similar conditions (Fredrickson et al., 2003; Fredrickson et al., 1998; Zachara et al., 2001) (Table 3). Based on susceptometry data, and the amount of reduced iron produced (Fig. 3), we estimate that CN32 converted all of the starting material into crystalline magnetite, while MR-4 converted approximately one third of the hydrous ferric oxide into magnetite. Strain W3-18-1 also produced magnetite, however, the amount of magnetite produced was much smaller, with the major product of iron reduction being green rust. Additionally, unlike strains CN32 and MR-4, a majority of the reduced Fe^{2+} was not remineralized into any form detectable by the methods used in these experiments. Thus, under similar conditions, only strain W3-18-1 produced a detectable iron carbonate mineral product. The production of iron carbonate material by iron reducing bacteria under laboratory conditions has been reported by other researchers (Fredrickson et al., 1998; Mortimer and Coleman, 1997; Roh et al., 2006; Zachara et al., 2002). However, the conditions under which these minerals were produced involved large amounts of CO_2 in the headspace, and probably far higher saturation states for green rust and siderite.

It has been shown that increased concentrations of Fe^{2+} can have an inhibitory effect on the bioreduction of ferric iron (Liu et al., 2001b; Roden and Urrutia, 2002). This inhibition would influence the remineralization and subsequent identity of mineral products by reducing the amount of reduced iron being introduced into solution (Roden and Urrutia, 2002). However, the starting amounts of Fe^{2+} used in those experiments were larger than the concentration of aqueous Fe^{2+} reported here. The relative rates of Fe reduction do not decrease with increasing Fe^{2+} in any consistent pattern, either for a single strain or across strains, so it seems unlikely that this factor was critical in the experiments done in our study.

Organics can interact strongly with both ferric and ferrous forms of iron (Banwart, 1999; Gonye and Carpenter, 1974; Gu et al., 1995; Hernandez and Newman, 2001; Kraemer et al., 2005; Lee et al., 2007). If various *Shewanella* strains form different secondary metabolites during lactate metabolism, and these chelate either form of iron, solute activities might differ in experiments with each strain of bacteria. However, this seems unlikely, because genetic information suggests that processing of lactate by each strain occurs via identical metabolic pathways. Furthermore, work done with HPLC (Salas, 2008) does not suggest that there are major differences in the production of secondary metabolites.

Phosphates are known to interact strongly with iron hydroxides and can impact both Fe^{3+} reduction and subsequent biomineralization (Fredrickson et al., 1998; Kukkadapu et al., 2004). Borch et al (2007) have reported that increasing phosphate concentrations correlate with decreasing rates of Fe^{2+} production, and that the major reduced iron biomineral in these systems is magnetite. However, the presence of millimolar levels of phosphate can stabilize green rust and inhibit magnetite formation (Bocher et al., 2004; Glasauer et al., 2003; Zachara et al., 2002). The concentration of phosphate used in these experiments ($\sim 800 \mu\text{M}$) did not appear to

inhibit magnetite formation (Table 3). Nevertheless, more work is needed to determine if these strains respond differently to phosphate concentration.

Incubation temperature may have played a role in the nature of biominerals produced in these experiments, as it has been shown that incubation temperature can affect the rate at which bacteria reduce iron and the iron biominerals they produce (Roh et al.; Zhang et al., 1998). Work done in our lab comparing the reduction of iron by CN32 at 15° C and 30° C showed that through 100 hours, reduction at 15° C is roughly half the rate of reduction at 30° C (data not shown). However, CN32 produced magnetite under both temperatures. This is consistent with previous work reporting that between 18–37° C, the mineral products of iron reduction by *S. sp.* PV-4 are unchanged, even though the temperature extremes are suboptimal for this particular strain (Roh et al., 2006). While the variation in biomineral products reported here might be attributable to differences in optimal temperature for each strain, a more thorough examination of the effects of temperature variability on mineral formation by these strains is needed.

While green rust is highly super-saturated under these conditions (Table 3), a stability diagram for magnetite, siderite and green rust indicates that the major product of iron reduction in these experiments should be magnetite (Fig. 7). The results observed with strains CN32 and MR-4 are in agreement with this prediction. However, in the case of W3-18-1 there is a significant amount of green rust although magnetite has a greater stability (Fig. 8, Table 3). The factor(s) responsible for these differences remain to be definitively elucidated, but our results suggest they do not involve differences in rates of Fe^{3+} reduction, types of excreted metabolites, medium pH, medium Fe^{2+} concentration, or CO_2 concentration in the medium headspace. Other factors might include differences in extracellular polysaccharides or biofilm formation.

Biofilms are known to be intimately involved with secondary mineral formation (Brown et al., 1999; Sawicki et al., 1995). Results from our laboratory indicate that there are differences in the degree of biofilm production by the three *Shewanella* strains tested. Biofilm formation by W3-18-1 is robust, while it is much weaker for strains CN32 and MR-4 (Bretschger, 2008). In the case of W3-18-1, the biofilm may have retarded diffusion of Fe^{2+} into the bulk solution, creating a microenvironment in which conditions were far from the chemical composition of the bulk system. It is also possible that specific compounds excreted by strain W3-18-1 have served to stabilize green rust or facilitate its nucleation. Polymeric substances have been shown to alter the conditions for crystal growth, leading to the stabilization of various minerals, some of which are thermodynamically unstable under Earth -surface conditions (Braissant et al., 2003; Douglas and Yang, 2002; Ueshima and Tazaki, 2001). Although some work has been done characterizing the EPS of strains MR-4 and CN32 (Korenevsky et al., 2002), there is no information on the compounds extruded by W3-18-1. Further work is needed to understand how biofilms and specific EPS compounds influence iron biomineral identity. It may also be that differences in EPS and biofilm formation can influence the pathways by which dissimilatory iron reduction leads to magnetite production. Although the microbially-mediated transformation of hydrous ferric oxide to magnetite has been described as a solid-state conversion (Hansel et al., 2003), other researchers have suggested that at low temperatures, the chemical conversion of HFO to magnetite can proceed via other intermediates such as green rust or ferrous hydroxide in a dissolution-precipitation reaction (Kahani and Jafari, 2008; Sumoondur et al., 2008). This would suggest that the formation of magnetite by dissimilatory iron reducers might also proceed through such a dissolution-precipitation reaction. In this case, the green rust detected in the W3-18-1 cultures may be a transitory phase, but its transformation to magnetite may be inhibited by EPS (or another metabolite) produced by W3-18-1. This is noteworthy, as the presence of green rust might influence subsequent iron biogeochemical cycling. While green rust is considered to be metastable with respect to magnetite and siderite (Génin et al., 1998), it is also a precursor to lepidocrocite (Schwertmann and Fechter, 1994).

In addition, its presence in some soils indicates that it does possess some temporal stability (Génin et al., 2005; Trolard et al., 1997). Thus, while the primary products of DIR may undergo further transformation as environmental conditions change, the availability of iron and the identity of succeeding iron minerals may be influenced by these early stage precipitates.

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Figure 1.

Environmental scanning electron micrographs of reduced iron oxide biominerals produced by the strains used in this experiment. A. Magnetite produced by strain CN32 (white arrow, and surrounding field of view); B. Magnetite/Iron carbonate hydroxide hydrate mixture produced by strain W3-18-1. Iron carbonate indicated by white arrows.

Figure 2. XRD patterns for bominerals and standards. X-axes are values in two-theta. Note for peaks for CN32 are somewhat sharper than for the other two strains.

Figure 3.

Total HCl-extractable reduced iron-oxide produced by the tested *Shewanella* strains using HFO as the electron acceptor and lactate as the electron donor. Error bars indicate the standard error of the mean for 4–6 replicates at each time point. The scale on the right represents the HCl-extractable Fe^{2+} , in micromoles, for each 250 μl sample collected for analysis.

Figure 4.

Concentrations of reduced aqueous phase iron. Data for each sample is based on 4 to 9 independent replicates. Error bars indicate the standard error for each time point. The scale on the right represents the dissolved Fe^{2+} , in micromoles, for each 250 μl sample collected for analysis.

Figure 5. Semi-log plot of susceptibility values (χ) for the tested *Shewanella* strains. Strains are represented by the following symbols: (▲) *S. putrefaciens* CN32; (■) *S. sp.* MR-4; (●) *S. putrefaciens* W3-18-1; (◆) negative control. Error bars indicate the standard deviation of the mean for each time point.

Figure 6. Box plot of solid phase inorganic carbon in the collected 2mL samples produced by the bacterial strains used in these experiments. The error bars represent the range of data for each strain.

Figure 7. Thermodynamic stability field of hydrous ferric oxide (assumed stoichiometry: $\text{Fe}(\text{OH})_3$), magnetite (Fe_3O_4), green rust ($\text{Fe}_6(\text{OH})_{12}\text{CO}_3 \cdot 2\text{H}_2\text{O}$) and siderite (FeCO_3) as a function of Fe^{2+} concentration and pH. A $[\text{HCO}_3^-]$ of 0.03M was assumed, based on buffer concentration.

Figure 8.

Thermodynamic stability field of hydrous ferric oxide (assumed stoichiometry: Fe(OH)_3), magnetite (Fe_3O_4), and siderite (FeCO_3) as a function of Fe^{2+} concentration. A pH of 8.4 was assumed, based on the average from data gathered in these experiments (table 2.3). The (●) symbol represents a HCO_3^- concentration of 0.03M and a Fe^{2+} concentration of 8.7mM for W3-18-1, 7.1mM for CN32 and 4.4mM for MR-4

Table 1

Bacterial strains used in dissimilatory iron reduction experiments^I

Strain	Strain Origin	Biofilm Formation	Lipopolysaccharide Layer	Putative Metal Reducing genes*
<i>Shewanella putrefaciens</i> CN32	Uranium Mine, New Mexico	Weak	Short, rough	11 heme <i>omcB</i>
<i>Shewanella</i> sp. MR-4	Black Sea Water Column	Weak	Smooth	<i>omcA1</i> , <i>mttD</i> , <i>mttE</i> , <i>mttF</i>
<i>Shewanella putrefaciens</i> W3-18-1	Pacific Ocean Marine Sediments	Strong	ND	11 heme <i>omcB</i>

^I Strains provided by the Pacific Northwest National Lab;

* In addition to *tomrA*, *mttB* and *mttC*; ND = No Data

Table 2Medium Composition^{1,2}

Chemical Description	Concentration (moles/L)
NaHCO ₃	3.1×10 ⁻²
Ammonium chloride	2.84×10 ⁻²
Potassium chloride	1.34×10 ⁻³
Sodium phosphate monobasic	8.32×10 ⁻⁴
Sodium chloride	3.0×10 ⁻²
Sodium Lactate	2.0×10 ⁻²
Biotin (d-biotin)	8.19×10 ⁻⁸
Folic acid	4.53×10 ⁻⁸
Pyroxine HCl	4.86×10 ⁻⁷
Riboflavin	1.33×10 ⁻⁷
Thiamine HCl 1.0 H ₂ O	1.41×10 ⁻⁷
Nicotinic acid	4.06×10 ⁻⁷
d-pantothenic acid, hemicalcium salt	2.10×10 ⁻⁷
B12	7.38×10 ⁻¹⁰
p-aminobenzoic acid	3.64×10 ⁷
Thioctic acid	2.42×10 ⁻⁷
L-glutamic acid	1.36×10 ⁻⁴
L-arginine	1.15×10 ⁻⁴
DL-serine	1.90×10 ⁻⁴
Nitritotriacetic acid	7.85×10 ⁻⁵
Magnesium sulfate heptahydrate	1.21×10 ⁻⁴
Manganese sulfate monohydrate	2.96×10 ⁻⁸
Sodium chloride	1.71×10 ⁻⁴
Ferrous sulfate heptahydrate	3.60×10 ⁻⁶
Calcium chloride dihydrate	6.80×10 ⁻⁶
Cobalt chloride hexahydrate	4.20×10 ⁻⁶
Zinc chloride	9.54×10 ⁻⁶
Cupric sulfate pentahydrate	4.01×10 ⁻⁷
Aluminum potassium disulfate dodecahydrate	2.10×10 ⁻⁷
Boric acid	1.62×10 ⁻⁶
Sodium molybdate dihydrate	1.03×10 ⁻⁶
Nickel chloride hexahydrate	1.01×10 ⁻⁶
Sodium tungstate	7.58×10 ⁻⁷

¹ Adapted from Fredrickson, et al (1998);² pH is not adjusted, and typically stabilizes at approximately 8.6

Table 3

Summary of iron reduction rates and DIR product distribution

	<i>S. putrefaciens</i> CN32	<i>S. species</i> MR-4	<i>S. putrefaciens</i> W3-18-1
Initial Amount of Hydrous Ferric Oxide (μmoles Fe)	400	400	400
Major Reduced Iron Products	Magnetite (Fe ₃ O ₄)	Magnetite (Fe ₃ O ₄)	Magnetite (Fe ₃ O ₄) Green Rust (Fe ₆ (OH) ₁₂ CO ₃ *2H ₂ O)
Major Iron Products (μmoles Fe)	Fe ₃ O ₄ 400 +/- 60	Fe ₃ O ₄ 140 +/- 16	Fe ₃ O ₄ 43 +/- 10 Fe ₆ (OH) ₁₂ CO ₃ *2H ₂ O 66 +/- 2
Reduction Rate 0–100 hours (μmol/L Fe*day) ^a	225 +/- 24	203 +/- 30	325 +/- 52
Reduction Rate 100–400 hours (μmol/L Fe*day) ^a	329 +/- 7	106 +/- 6	424 +/- 1
Reduction Rate 400–800 hours (μmol/L Fe*day) ^a	135 +/- 31	112 +/- 27	151 +/- 9
Green Rust Degree of Saturation (Ω) ^b	3.49X10 ¹⁶	2.36X10 ¹⁴	6.93X10 ¹³
Magnetite Degree of Saturation (Ω) ^b	5.03X10 ¹⁴	4.06X10 ¹⁴	7.31X10 ¹⁴
Siderite Degree of Saturation (Ω) ^b	5.05X10 ²	4.08X10 ²	1.16X10 ³
Final pH	8.4 +/- 0.1	8.4 +/- 0.3	8.2 +/- 0.2

^a Rates were calculated using $(\text{Fe}^{2+}_{i+1} - \text{Fe}^{2+}_i) / (t_{i+1} - t_i)$, where Fe^{2+} corresponds to the amount of reduced iron measured at each time point i , t corresponds to the time at point i , and i corresponds to the collection times of 0, 100, 400 and 800 hours.

^b Ω was calculated using IAP/K_{sp} , where $\text{IAP} = \{[\text{Fe}^{2+}]^4[\text{HCO}_3^-]\} / \{[\text{H}^+]\}^7$ for green rust, $\{[\text{Fe}^{2+}] / [\text{H}^+]\}^2$ for magnetite and $\{[\text{Fe}^{2+}]^4[\text{HCO}_3^-]\} / \{[\text{H}^+]\}$ for Siderite. K_{sp} for each species was calculated from $\ln K_{\text{eq}} = -\Delta G^0 / RT$.

Table 4

Thermodynamic Constants Used in Calculations

Species	ΔG°_f (KJ/mol)
Solids	
Fe(OH) ₃	-699 ¹
Fe ₃ O ₄	-1012.6 ¹
Fe ₆ (OH) ₁₂ CO ₃ *2H ₂ O	-4059.9 ²
FeCO ₃	-666.7 ¹
Liquid	
H ₂ O	-238 ¹
Aqueous	
Fe ²⁺	-78.87 ¹
HCO ₃ ⁻	-586.8 ¹

¹From Stumm and Morgan, 1996;²From Drissi, et al, 1995. Activity coefficients: Fe²⁺ = 0.394, HCO₃⁻ = 0.769