

Iron isotope fractionation during microbial reduction of iron: The importance of adsorption

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ABSTRACT

In experiments investigating the causes of Fe isotope fractionation, the $\delta^{56/54}\text{Fe}$ value of Fe(II) remaining in solution ($\text{Fe(II)}_{(\text{aq})}$) after reduction of Fe(III) (goethite) by *Shewanella putrefaciens* is $\sim -1.2\%$ relative to the goethite, in agreement with previous research. The addition of an electron shuttle did not affect fractionation, suggesting that Fe isotope fractionation may not be related to the kinetics of the electron transfer. Furthermore, in abiotic, anaerobic $\text{FeCl}_{2(\text{aq})}$ experiments in which approximately one-third of $\text{Fe(II)}_{(\text{aq})}$ is lost from solution due to adsorption of Fe(II) onto goethite, the $\delta^{56/54}\text{Fe}$ value of $\text{Fe(II)}_{(\text{aq})}$ remaining in solution is shifted by -0.8% relative to FeCl_2 . This finding demonstrates that anaerobic nonbiological interaction between Fe(II) and goethite can generate significant Fe isotope fractionation. Acid extraction of sorbed Fe(II) from goethite in experiments reveals that heavy Fe preferentially sorbs to goethite. Simple mass-balance modeling indicates that the isotopic composition of the sorbed Fe(II) pool is $\sim +1.5\%$ to $+2.5\%$ heavier than Fe in the goethite [$\sim 2.7\%$ – 3.7% heavier than aqueous Fe(II)]. Mass balance is also consistent with a pool of heavy Fe that is not released to solution during acid extraction.

Keywords: isotope geochemistry, iron, iron oxides, goethite, reduction, adsorption.

INTRODUCTION

It has been hypothesized that Fe isotopes could document biological activity in ancient and extraterrestrial environments (Beard et al., 1999). The genesis of this hypothesis was the observation that the $\delta^{56/54}\text{Fe}$ value of $\text{Fe(II)}_{(\text{aq})}$ released by Fe-reducing bacteria was -1.3% relative to the Fe(III) in ferrihydrite or hematite (Beard et al., 1999, 2003), as well as the observation that the $\delta^{56/54}\text{Fe}$ value of sedimentary rocks varies over a range of $\sim 4\%$, while igneous and metamorphic rocks are relatively invariable (Beard et al., 2003). However, a number of studies demonstrated that nonbiological fractionation of Fe isotopes is also possible at low temperatures (Anbar et al., 2000; Brantley et al., 2001; Bullen et al., 2001; Johnson et al., 2002), and more study of the chemistry of fractionation is warranted.

The fate of biogenic Fe(II) has received much attention (e.g., Fredrickson et al., 2001; Roden and Urrutia, 2002; Zachara et al., 2001, 2002). In experiments, as much as 50% of aqueous biogenic Fe(II) precipitates as vivianite or siderite if HPO_4^{2-} or HCO_3^- is present (Fredrickson et al., 1998; Zachara et al., 2001)

and/or adsorbs to surfaces of Fe(III) solids or bacteria (Liu et al., 2001; Roden and Urrutia, 2002). It is currently unknown whether these reactions cause fractionation of Fe isotopes. Adsorbed Fe(II) is easily sampled with a 0.5 M HCl extraction, which preserves the concentration and redox state of Fe(II) without reduction of the Fe(III) oxide (Fredrickson et al., 1998; Lovley and Phillips, 1986). The combined soluble and adsorbed ferrous iron extracted [$\text{Fe(II)}_{(\text{acid ext.})}$] represents total biogenic Fe(II) (Roden and Zachara, 1996; Royer et al., 2002).

We conducted experiments to test the importance of adsorption in Fe isotope fractionation during the bioreduction of Fe. These include anaerobic Fe(II) adsorption experiments in the absence of microbially mediated Fe(III) reduction. We also carry out bioreduction experiments with *Shewanella putrefaciens* CN32. Reduction of Fe(III) is thought to occur upon adhesion of the cell to the mineral surface or by electron shuttling by small redox-active organic molecules without bacteriamineral contact (Nevin and Lovley, 2000; Newman and Kolter, 2000). Through study of *S. putrefaciens*, we examine the importance of adsorption to cells and of electron shuttles in Fe isotope fractionation. We also conduct “in vitro” experiments in which Fe(III) is reduced

by using outer-membrane fractions isolated from *S. oneidensis*.

METHODS

Microbial experiments were conducted in triplicate in an anaerobic chamber at $\sim 27^\circ\text{C}$ using synthetic goethite (4.5 mg/mL, Schwertmann and Cornell, 2000) and 30 mM lactate as the electron acceptor and donor, respectively. Less than 5% of the goethite was reduced. Stock cultures of *S. putrefaciens* CN32, grown aerobically in LB broth, were washed three times and resuspended in deaerated media. The experiments (all nongrowth) consisted of 60 mL of 10 mM 1,4-piperazinediethanesulfonic acid (PIPES)-buffered media (Table 1) at pH 7.5 with 10^8 cells/mL. The media contained no phosphate and bicarbonate to minimize Fe(II) mineral precipitation (Zachara et al., 2001). Anthraquinone-2,6-disulfonate (AQDS; 0.1 mM) was added to one set of cultures as an alternative electron shuttle. Controls containing goethite + media \pm AQDS and media + *S. putrefaciens* \pm AQDS were monitored concurrently.

After 7 days, an aliquot of the homogenized culture slurry was collected, filtered (0.2 μm), and acidified (pH < 2) for analysis of aqueous Fe [$\text{Fe}_{(\text{aq})}$]. An aliquot of the homogenized culture slurry was also collected, acidified with HCl to 0.5 M, agitated for 16 h, and then filtered (0.2 μm) to extract adsorbed Fe(II). After all samples were collected, the goethite was deposited on a 1 μm filter and dried oxygen free.

Adsorption of Fe(II) onto *S. putrefaciens* or goethite was examined in 24 h experiments with initial concentrations of 2.15 mM Fe(II) (approximately the maximum [$\text{Fe(II)}_{(\text{acid ext.})}$] in bioreduction experiments). Fe(II) stock, prepared anaerobically by adding 30 mL deaerated media to 1.066 g of $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$, was added to deaerated 10 mM PIPES at pH 7.5 with either 4.5 mg/mL goethite, 10^8 cells/mL, or no additives (control). Biosorption of Fe(II) onto *S. putrefaciens* CN32 equilibrates in < 30 min (Liu et al., 2001).

Outer membrane fractions of *S. oneidensis* MR-1 (Myers and Myers, 1993) were used for in vitro reductions. Incubations contained a

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TABLE 1. Fe ISOTOPE (RELATIVE TO IRMM-14) AND CONCENTRATION MEASUREMENTS

Experimental condition	$\delta^{56}\text{Fe}_{(\text{aq})}$ (No. of measmts.)	$\Delta^{56}\text{Fe}_{(\text{aq})}^*$	Fe(II) _(aq) or Fe(II) _(acid ext.) (mM)	Fe _{total} (mM)	% Fe(II) ads. [†]
Error (2 σ)	$\pm 0.1\text{‰}$	$\pm 0.14\text{‰}$	$\pm 5\%$	$\pm 5\%$	
Goethite 1	-0.23‰ (10)	0			
Goethite 2	-0.35‰ (2)	0			
Microbial Fe(III) reduction					
Soluble iron	-1.41‰ (3)	-1.18‰	0.609	0.643	
Acid extraction	-0.61‰ (4)	-0.38‰	0.860	0.801	29
Microbial Fe(III) reduction with AQDS					
Soluble iron	-1.48‰ (3)	-1.25‰	1.72	1.61	
Acid extraction	-0.50‰ (2)	-0.27‰	2.32	2.14	26
In vitro Fe(III) reduction					
Soluble iron	-0.20‰ (2)	0.15‰	0.875	0.879	
Acid extraction	-0.30‰ (2)	0.06‰	1.22	1.42	28
Fe(II) adsorption control					
Soluble iron	-0.40‰ (2)	0‰	2.12	2.16	
Acid extraction			2.28	2.24	
Fe(II) adsorption on goethite					
Soluble iron	-1.20‰ (4)	-0.81‰	1.28	1.25	
Acid extraction			2.10	1.97	39
Fe(II) adsorption on <i>Shewanella putrefaciens</i>					
Soluble iron	-0.30‰ (3)	0.10‰	1.92	1.99	
Acid extraction			2.10	2.07	9

Note: Microbial Fe(III) reduction—Goethite 1; solution: 28.0 mM NH₄Cl, 1.34 mM KCl, 0.68 mM CaCl₂, 50.0 mM NaClO₄, 30.0 mM lactate, 10 mM PIPES (pH 7.5), 10⁸ cells/mL *Shewanella putrefaciens*, 4.5 mg/mL goethite. Microbial Fe(III) reduction with AQDS—Goethite 1; solution: same as first, except 0.1 mM AQDS was added. In vitro Fe(III) reduction—Goethite 2; solution: same as first, except 0.1 mg/mL outer membrane fragments were added instead of live bacteria. Fe(II) adsorption control—solution: 10 mM PIPES (pH 7.5). Fe(II) adsorption on goethite—Goethite 2; solution: 10 mM PIPES (pH 7.5), and 4.5 mg/mL goethite. Fe(II) adsorption on *S. putrefaciens*—solution: 10 mM PIPES (pH 7.5), and 10⁸ cells/mL *S. putrefaciens*. AQDS = anthraquinone-2,6-disulfonate; PIPES = 1,4-piperazinediethanesulfonic acid.

* $\Delta^{56}\text{Fe}_{(\text{aq})} = \delta^{56}\text{Fe}_{\text{aqueous}} - \delta^{56}\text{Fe}_{\text{Fe(III) source}}$; where $\delta^{56}\text{Fe}_{\text{Fe(III) source}}$ is the isotopic composition of either goethite or the adsorption control solution (see text).

[†]% Fe(II) ads. = $[\text{Fe(II)}_{(\text{acid ext.})} - \text{Fe(II)}_{(\text{aq})}]/\text{Fe(II)}_{(\text{acid ext.})}$.

suspension of 0.1 mg outer membrane and 4.5 mg goethite per milliliter. Reduction, initiated by addition of sodium formate (10 mM) in 10 mL total solution, continued for 5 h. The 10 mL was split for analysis of dissolved Fe and dissolved + acid-extracted Fe.

Fe(II)_(aq) (analyzed with ferrozine; Lovley and Phillips, 1986) and total dissolved Fe (analyzed with inductively coupled plasma-atomic emission spectrophotometry) were measured in all solutions. Minerals were identified by X-ray diffraction (XRD, Rigaku Geigerflex) and by using a magnetic property measurement system (MPMS, Quantum Design, Institute for Rock Magnetism).

Fe isotopes were measured by using a multicollector-inductively coupled plasma-mass spectrometer (ThermoFinnigan Neptune, Washington State University). Cu was added to samples and standards as an element spike, and standard-sample-standard bracketing was used to control for variations in instrument mass fractionation (Arnold et al., 2004; Maréchal et al., 1999). The Neptune was operated at resolution $M/\Delta M \approx 2500$ to resolve isobaric interferences (Arnold et al., 2004). An in-house gravimetric standard of known isotopic composition was analyzed once for every 3–4 unknowns, and data were only collected when this standard was $\pm 0.1\text{‰}$ of

expected. The $\delta^{56/54}\text{Fe}$ values were calculated relative to the international Fe standard IRMM-014 (Taylor et al., 1992).

$$\delta^{56/54}\text{Fe} = \left[\frac{(^{56}\text{Fe}/^{54}\text{Fe})_{\text{sample}}}{(^{56}\text{Fe}/^{54}\text{Fe})_{\text{IRMM}}} - 1 \right] \times 1000. \quad (1)$$

The isotopic fractionation relative to initial Fe was also calculated:

$$\Delta^{56/54}\text{Fe} = \delta^{56/54}\text{Fe}_{\text{solution}} - \delta^{56/54}\text{Fe}_{\text{initial}}, \quad (2)$$

where $\delta^{56/54}\text{Fe}_{\text{initial}}$ is either $\delta^{56/54}\text{Fe}_{\text{goethite}}$ or $\delta^{56/54}\text{Fe}_{\text{FeCl}_2(\text{aq})}$. The external precision of measurements (2 σ) was better than $\pm 0.1\text{‰}$.

RESULTS

Two goethite samples, with specific surface areas (30.6 m²/g) and isotopic compositions (Table 1) identical within error, were used.

The $\Delta^{56/54}\text{Fe}$ for Fe_(aq) remaining in solution during bioreduction of goethite was identical within error for cultures with and without AQDS (Table 1). This isotopic signature (-1.2‰) is similar to that reported for Fe_(aq) during bioreduction by *Shewanella alga* (Beard et al., 1999, 2003). In contrast, the

$\delta^{56/54}\text{Fe}$ value of Fe_(aq) after in vitro Fe reduction was unchanged from that of the goethite (Table 1).

The Fe(II) concentration in solution increased by 26%–29% following addition of 0.5 M HCl. This acid extraction solubilizes biogenic Fe(II) present as precipitate or adsorbed to cells and minerals (Roden and Zachara, 1996; Royer et al., 2002). Consistent with Zachara et al. (2001), solids in our experiments contained no Fe(II) phases, as demonstrated by XRD (only goethite detected) and MPMS (goethite with $\sim 0.25\%$ hematite both before and after reduction). Therefore, the increase in Fe(II) concentration is attributed to Fe(II) adsorbed on bacteria and/or goethite (Table 1).

Values for [Fe(II)_(aq)] and total aqueous Fe concentrations ([Fe]_{total}) (Table 1) were generally identical ($\pm 2\sigma$), as expected because the HCl extraction preserves the redox state of Fe (Fredrickson et al., 1998; Lovley and Phillips, 1986). The average [Fe]_{total} after acid extraction of the experimental controls containing goethite was 0.04 mM, indicating that $\leq 2\%$ – 5% of the Fe extracted was derived from goethite dissolution. Values of [Fe]_{total} measured after acid extraction of the controls containing cells without goethite were below detection, documenting that Fe from lysed cells was insignificant.

The Fe isotope compositions of the 0.5 M HCl acid extractions of cultures with and without AQDS were similar, but $\sim 1.0\text{‰}$ heavier than Fe_(aq) prior to extraction (Table 1). In contrast, the isotopic compositions of the soluble and acid-extracted in vitro Fe did not significantly differ from each other or from goethite.

The isotopic composition of the FeCl_{2(aq)} used in the adsorption experiments (-0.4‰ relative to IRMM-14) was measured on a FeCl₂ control solution incubated 24 h in an experimental vessel without cells or goethite. After 24 h, 39% \pm 5% of the Fe(II)_(aq) adsorbed to goethite and 9% \pm 5% to *S. putrefaciens* cells (Table 1). Isotopically, Fe(II)_(aq) in equilibrium with *S. putrefaciens* cells did not differ significantly from the FeCl₂ control. In contrast, Fe(II)_(aq) remaining in solution after contact with goethite in the adsorption experiment was isotopically lighter (-0.81‰) than the FeCl₂ control (Table 1). After 0.5 M HCl extraction, the [Fe(II)_(acid ext.)] was within error of the 2.15 mM starting concentration, documenting that oxidation of Fe was insignificant and that all adsorbed Fe(II) was recovered by extraction in abiotic experiments.

DISCUSSION AND CONCLUSIONS

The isotopic fractionation observed here between Fe_(aq) and goethite during bioreduction,

~ -1.2‰, compares well with values for ferrihydrite and hematite reduction by *S. alga* (Beard et al., 1999, 2003). Surprisingly, fractionation was similar between cultures with and without AQDS, even with large [Fe(II)_(aq)] differences (Table 1). *S. putrefaciens* reduces Fe substrates by direct contact, by use of a shuttle such as AQDS, or by production of its own shuttle (Newman and Koller, 2000; Rosso et al., 2003). Given that AQDS increases the rate of reduction of solid Fe(III) by shuttling electrons without cell-mineral contact (Royer et al., 2002), reduction in the AQDS-amended cultures is likely dominated by electron shuttling. Therefore, the similarity of fractionation with and without AQDS indicates that the mechanisms of fractionation were similar in both cultures. This finding suggests that if there is a kinetic isotope effect during reduction, the electron transfer must not be the rate-limiting step.

Observed Fe isotope fractionation during abiotic adsorption of Fe(II) demonstrates unambiguously that $\Delta^{56/54}\text{Fe}_{\text{aq-Fe(III) source}}$ of -0.5‰ to -1‰ can be generated without cells. This isotope effect results from interaction between Fe(II)_(aq) and the goethite surface. To explore this, we assume $f_{\text{ads}} + f_{\text{aq}} = 1$, where f_{ads} is the fraction of Fe(II) adsorbed by the end of the experiment and f_{aq} is the fraction that remains dissolved. This treatment is most rigorous for Fe(II) in the abiotic experiment, where strict mass balance between dissolved and adsorbed Fe(II) was demonstrated (Table 1). The following discussion assumes that HCl extraction quantitatively releases all adsorbed Fe(II) (Table 1).

If we assume no isotopic exchange between Fe(II)_(aq) or Fe(II) adsorbed [Fe(II)_(ads)] with Fe(III) in goethite, then for the abiotic adsorption experiment,

$$\delta^{56/54}\text{Fe}_{\text{total}} = f_{\text{aq}}\delta^{56/54}\text{Fe}_{\text{aq}} + f_{\text{ads}}\delta^{56/54}\text{Fe}_{\text{ads}} \quad (3)$$

Taking $\delta^{56/54}\text{Fe}_{\text{total}} = \delta^{56/54}\text{Fe}_{\text{FeCl}_2} = -0.40\text{‰}$, $\delta^{56/54}\text{Fe}_{\text{aq}} = -1.20$, and $f_{\text{ads}} = 0.39$ (Table 1), we obtain $\delta^{56/54}\text{Fe}_{\text{ads}} = +0.85\text{‰}$.

If adsorbed and Fe(II)_(aq) equilibrate continuously, then $\Delta_{\text{ads-aq}} = 10^3 \ln(\alpha) = 2.05$, and the equilibrium fractionation factor, α , is ~1.0021. Alternatively, it is possible that a Rayleigh-type process (e.g., surface aging) irreversibly isolates Fe from the dissolved pool after adsorption. In such a case, we calculate $\alpha \approx 1.0016$.

Similar results are obtained from the *S. putrefaciens* experiments without and with AQDS. We calculate that $\delta^{56/54}\text{Fe}_{\text{ads}} = +2.66\text{‰}$ and $+3.33\text{‰}$, respectively (assuming $\delta^{56/54}\text{Fe}_{\text{total}} = \delta^{56/54}\text{Fe}_{\text{goethite}}$). For continuous equilibrium, $\Delta_{\text{ads-aq}} = 4.07$ and 4.81 , and

hence $\alpha \approx 1.0041$ and 1.0048 , respectively. The Rayleigh case predicts similar α values: 1.0035 and 1.0042 , respectively.

The *S. putrefaciens* results are semiquantitatively consistent with the abiotic adsorption experiment. While there are some systematic differences that exceed analytical uncertainties, the data from both types of experiments can be explained by partitioning of heavy Fe to the adsorbed pool, with fractionation factors of 1.0020 – 1.0050 . The gross agreement in these experiments suggests a significant abiotic adsorption contribution to isotope fractionation in the *S. putrefaciens* experiments.

Assuming the adsorption behavior is similar during the bioreduction and adsorption experiments, the relative proportions of dissolved and previously adsorbed Fe in our extraction solutions are representative of the proportions of dissolved and adsorbed Fe in the experiment. In this case $\delta^{56/54}\text{Fe}_{\text{total}} = \delta^{56/54}\text{Fe}_{\text{(acid ext.)}}$ in equation 3, which yields $\delta^{56/54}\text{Fe}_{\text{ads}} = +1.35\text{‰}$ and $+2.29\text{‰}$ in the experiments without and with AQDS, respectively. These values are substantially smaller than those calculated above from the overall experimental mass balance ($+2.66\text{‰}$ and $+3.33\text{‰}$, respectively). Therefore, a substantial fraction of the heavy Fe was lost from solution during the bioreduction experiment into a pool other than HCl-extractable Fe(II)_(ads); the exact isotopic composition of this pool is unknown.

The driving force for this isotope effect could be an equilibrium Fe isotope fractionation between Fe(II)_(aq) and Fe(II)_(ads). Equilibrium Fe isotope fractionations have been predicted (Polyakov and Mineev, 2000; Schauble et al., 2001) and probably observed in other systems (Anbar et al., 2000; Bullen et al., 2001; Johnson et al., 2002). Fe exchange between two ligands in chemical equilibrium will result in the isotopically heavy Fe preferentially binding with the stronger ligand (Schauble et al., 2001). Because the dominant Fe(II) species in our solutions has six weakly bonded water molecules, goethite surface complexation of Fe(II) may represent a stronger bonding environment that preferentially attracts heavy Fe(II) from solution. This hypothesis suggests that minerals with stronger Fe(II) surface complexes will yield a larger fractionation when in equilibrium with Fe(II)_(aq).

We have not yet included the possibility that fractionation may result from isotope exchange between dissolved or adsorbed Fe(II) and Fe(III) in goethite. Such exchange would require modification of equation 3 to include a third pool of isotopically exchangeable Fe. Exchange between Fe(II) and Fe(III) is also possible. For example, if isotopically heavy, adsorbed Fe(II) (atom A) reduces adjacent

Fe(III) (atom B), the extracted atom B would be isotopically lighter than the originally adsorbed atom A. In this way, a heavy isotopic pool (represented by atom A) could remain on the goethite. Such exchange could favor preferential accumulation of heavy Fe in the unextracted Fe(III) pool because equilibrium isotope exchange between Fe(II) and Fe(III) favors heavy Fe in the oxidized complex (Johnson et al., 2002; Schauble et al., 2001).

Loss of isotopically heavy Fe may also be related to structural alteration of goethite. Increased duration of Fe(II) adsorption onto Fe(III) solids reduces the yield of the $0.5 M$ HCl extraction to solubilize Fe(II)_(ads) (Coughlin and Stone, 1995; Jeon et al., 2003), perhaps because of formation of magnetite, alteration of the FeOOH surface to form a distinct Fe(II)-FeOOH structure, or Fe(II) diffusion into the solid (Tronc et al., 1992; Coughlin and Stone, 1995; Cooper et al., 2000; Jeon et al., 2003). Although no secondary minerals were observed here, other structural effects may have resulted in incomplete extraction of Fe(II) in the bioreduction experiments. Because the calculated fractionations (equation 3) were derived from 24 h adsorption experiments (where complete extraction was demonstrated) and then compared to the 7 d biological experiments (where complete extraction may not have been attained), incomplete extraction may contribute to the discrepancy between calculated and observed fractionations.

If biological reduction is the primary cause of Fe isotope fractionation, then isotope effects should be apparent in the in vitro (outer membrane) experiments. The extent of Fe reduction and adsorption in the in vitro experiment was similar to that observed in vivo (experiments with live cells). However, the isotopic composition in vitro did not differ significantly from that of the goethite.

The lack of fractionation in Fe_(aq) released in vitro was in contrast to all other experiments involving goethite despite the similarity in the fraction of Fe adsorbed in vitro to that adsorbed in vivo. The outer membrane fragments are not removed by filtering and the Fe(II) adsorbed to the outer membrane in solution should remain in solution. However, it is uncertain whether the adsorption of the outer membrane to the surface of goethite may have altered the adsorption behavior of Fe(II) in this system. The lack of fractionation may also be explained by the short duration of the in vitro experiments: 5 h (in vitro) versus 24 h (adsorption experiments) and 7 d (bioreduction experiments). The in vitro solutions may not have equilibrated long enough with respect to a time-dependent fractionation process occurring at the goethite surface. If re-

action time was a factor, Fe_(aq) released in vitro should show a lesser degree of fractionation than the adsorption or bioreduction experiments; however, this is not apparent. These hypotheses need to be addressed in greater detail by future experiments.

The observation of a significant isotopic fractionation during abiotic reaction of dissolved and mineral-bound Fe is consistent with the observation by Bullen et al. (2001) of an ~-1‰ fractionation during the oxidation of Fe(II) and precipitation of Fe(III). They and others (Johnson et al., 2002) interpreted these data as reflecting a combination of equilibrium and kinetic effects unrelated to adsorption. However, in light of our data, interactions between Fe(II)_(aq) and Fe(II)_(ads) seem likely to contribute. In systems where ferric oxide solids are present, the oxidation rate of Fe(II) is controlled by the oxidation of Fe(II)_(ads) rather than Fe(II)_(aq) (Dempsey et al., 2001). Hence, it seems likely that Fe(II) was adsorbing to the Fe(III) solids in the systems described by Bullen et al. (2001), in a manner analogous to the abiotic adsorption experiment reported here.

In conclusion, goethite Fe(III) reduction experiments with *S. putrefaciens* CN32 show that the δ^{56/54}Fe value of Fe(II)_(aq) is similar to *S. alga* reduction of ferrihydrite and hematite. However, an isotope effect associated with adsorption may account for much of the fractionation in the Fe(II)-goethite system, regardless of microbial activity. The in vitro reduction of Fe by using membrane fragments of *S. oneidensis* also did not result in Fe isotope fractionation, which further argues against the existence of an Fe isotope biosignature. Isotopically heavy Fe sorbed to goethite surfaces must be accounted for in bioreduction experiments aimed at the identification of Fe isotope fractionation; similarly, sorption must be included in discussions of natural Fe isotope fractionations.

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