

Zinc and Manganese Inhibition of Biological Hematite Reduction

James J. Stone,^{1*} William D. Burgos,² Richard A. Royer,³ and Brian A. Dempsey²

¹*Department of Civil and Environmental Engineering
Rapid City, SD 57701*

²*Department of Civil and Environmental Engineering
The Pennsylvania State University
University Park, PA 16802*

³*Environmental Technology Laboratory
General Electric Company
Niskayuna, NY 12309*

ABSTRACT

The effects of zinc and manganese on the reductive dissolution of hematite by the dissimilatory metal-reducing bacterium (DMRB) *Shewanella putrefaciens* CN32 were studied in batch culture. Experiments were conducted with hematite (2.0 g L^{-1}) in 10 mM PIPES (pH 6.8), and H_2 as the electron donor under nongrowth conditions ($10^8 \text{ cell mL}^{-1}$), spiked with zinc (0.02–0.23 mM) or manganese (0.02–1.8 mM) and incubated for 5 days. Zinc inhibition was calculated based on the 5-day extent of hematite bioreduction in the absence and presence of zinc. Zinc inhibition of hematite bioreduction increased with anthraquinone-2,6-disulfonate (AQDS), a soluble electron shuttling agent, and ferrozine, a strong Fe(II) complexant. Both amendments would otherwise stimulate hematite bioreduction. These amendments did not significantly increase zinc sorption, but may have increased zinc toxicity by some unknown mechanism. At equal total Me(II) concentrations, zinc inhibited hematite reduction more than manganese and caused greater cell death. At equal sorbed Me(II) concentrations, manganese inhibited hematite reduction more than zinc and caused greater cell death. Results support the interpretation that Me(II) toxicity was more important than Me(II) sorption/surface blockage in inhibiting hematite reduction.

Key words: iron reduction; *Shewanella*; zinc; manganese; AQDS; inhibition; complexation

INTRODUCTION

THE DISSIMILATORY REDUCTION of Fe(III) has been recognized as a viable option for the *in situ* bioremediation of contaminated sediments and aquifers (Lovley *et al.*, 1996; Fredrickson *et al.*, 1998; Urrutia *et al.*, 1998,

1999). The abundance of ferric-containing minerals found within most sediments can greatly influence the fate and transport of organic and inorganic contaminants. While microbial iron reduction may be a feasible option for these remediation efforts, little is known about bioreduction processes in the presence of heavy metal cocon-

*Corresponding author: Department of Civil and Environmental Engineering, South Dakota School of Mines and Technology, 501 East Saint Joseph Street, Rapid City, SD 57701. Phone: 605-394-2443; Fax: 605-394-5171; E-mail: James.Stone@sdsmt.edu

taminants. Previous studies have shown that the rate or extent of microbial Fe(III) reduction is influenced by the presence of heavy metals such as Cu(II) (Markwiese and Colberg, 2000), Ni(II) (Fredrickson *et al.*, 2001; Parmar *et al.*, 2001), Co(III) (Zachara *et al.*, 2001), and Zn(II) (Cooper *et al.*, 2000; Stone *et al.*, 2006), primarily due to heavy metal toxicity/lethality to dissimilatory metal-reducing bacteria (DMRB). Metal–microbe interactions are well documented (Baath, 1989; Kushner, 1993; Gadd, 1996; Warren and Haack, 2001), and may exert a significant influence on the effectiveness of bioremediation at contaminated sites.

We recently reported on zinc inhibition of aerobic growth, nitrate bioreduction, ferric citrate bioreduction, and hematite bioreduction with *Shewanella putrefaciens* CN32 (Stone *et al.*, 2006). *S. putrefaciens* is a Gram-negative facultative anaerobe capable of reducing crystalline and soluble iron oxides (Cooper *et al.*, 2000; Zachara *et al.*, 2001; Royer *et al.*, 2002a, 2002b), nitrate, and a variety of toxic metals and radionuclides such as Cr(VI) (Fendorf *et al.*, 2000; Wielinga *et al.*, 2001), U(VI) (Brooks *et al.*, 2003), and Tc(VII) (Liu *et al.*, 2002a; Wildung *et al.*, 2000). Our experiments revealed that soluble electron acceptors were more sensitive to the presence of zinc than solid-phase hematite, and that low concentrations of zinc stimulated only solid-phase hematite bioreduction. We speculated that the formation of zinc-tolerant biofilms on hematite surfaces was a possible mechanism for the differences observed (Costerton *et al.*, 1995; Teitzel and Parsek, 2003; Stone *et al.*, 2006).

The enhancement of hematite bioreduction by two soluble amendments, anthraquinone-2,6-disulfonate (AQDS), and ferrozine, was reported by Royer *et al.* (2002b). AQDS can serve as an alternative soluble electron acceptor and shuttle electrons between DMRB and solid-phase ferric oxides (Lovley *et al.*, 1996; Fredrickson *et al.*, 1998; Zachara *et al.*, 1998; Hacherl *et al.*, 2001). Ferrozine can complex Fe(II) (Stookey, 1970), thus decreasing Fe(II) sorption onto oxide or cell surfaces and limiting the occurrence of Fe(II)-promoted “surface passivation” (Royer *et al.*, 2002a). The role of AQDS as an electron shuttling quinone has been well documented (Fredrickson *et al.*, 1998; Zachara *et al.*, 1998; Kieft *et al.*, 1999); however, its function and effectiveness in the presence of divalent metals other than biogenic Fe(II) is relatively unknown. Fredrickson *et al.* (2001) reported that AQDS did not enhance the bioreduction of Ni(II)- or Co(III)-substituted goethite by *S. putrefaciens* CN32. This lack of enhancement, compared to increased bioreduction in the absence of metals, was attributed to sorbed Ni(II) or Co(II/III) that occupied goethite surface sites and thus slowed reoxidation of reduced AQDS (AH₂DS) by goethite.

Divalent metals could inhibit the bioreduction of solid-phase metal oxides by at least two possible mechanisms. Me(II) toxicity leading to cell death could decrease metal oxide bioreduction. Me(II) sorption blocking bioreducible metal oxide surface sites could also decrease metal oxide bioreduction. The objectives of this study were to (1) determine the predominant mechanism of Me(II) inhibition of hematite bioreduction using zinc and manganese, and (2) determine the effectiveness of amendments known to stimulate hematite bioreduction in the presence of zinc.

EXPERIMENTAL PROTOCOLS

All experimental procedures have been previously described (Royer *et al.*, 2002a; Stone *et al.*, 2006); however, important descriptions are provided below.

Micro-organism and culture conditions

Shewanella putrefaciens CN32 was grown aerobically on tryptic soy broth without dextrose at 20°C. Cells were harvested by centrifugation (4,900 × *g*, 10 min, 20°C) from a 16-h-old culture (late log phase). The cells were washed three times in 10 mM 1,4-piperazinediethanesulfonic acid (PIPES; pH = 6.8), with the final wash deoxygenated to remove residual oxygen. Cell density was determined by absorbance at 420 nm.

Chemicals

Hematite powder was obtained from J.T. Baker (Phillipsburg, NJ) and heated to 550°C in air overnight before use to remove any residual organic carbon. AQDS (Sigma-Aldrich, St. Louis, MO) and ferrozine (J. T. Baker) were used as received, and filtered (0.2 μm) stock solutions were prepared in PIPES buffer.

Hematite bioreduction

A “master reactor” approach was used to ensure consistent chemical and biological conditions in each series of experiments (Royer *et al.*, 2002b; Stone *et al.*, 2006). A master reactor was prepared by first combining the hematite and inoculum in a 120-mL serum bottle. All solutions (except the zinc and manganese stocks) were prepared in 10 mM PIPES (pH 6.8). Before metal or amendment addition, three 10-mL aliquots were removed, which served as biotic no-amendment, no-metal controls. The amendment (final concentration of 1.46 mM ferrozine or 50 μM AQDS) was then added and three 10-mL aliquots were removed which served as biotic with-amendment, no-zinc controls. Zinc was incrementally added to the master reactor from an acidified, deoxy-

generated ZnCl₂ solution [1,000 mg L⁻¹ atomic absorption spectrometry (AAS) certified standard; VWR, West Chester, PA] along with an equal volume of 0.1 N NaOH (for pH maintenance). For each zinc concentration, 10 mL of the suspension from the master reactor was transferred to 20-mL amber serum bottles (in triplicate) within the anaerobic chamber (97.5:2.5% N₂H₂), sealed, and incubated in the dark at 20°C on orbital shakers (150 rpm) outside of the anaerobic chamber for 5 days. Total zinc concentrations ranged from 0.02 to 0.23 mM. Hydrogen from the anaerobic chamber atmosphere was used as the sole electron donor in all experiments. For manganese experiments, no amendments were tested and total Mn(II) concentrations ranged from 0.02 to 1.82 mM (prepared using 1,000 mg L⁻¹ MnCl₂ AAS certified standard; VWR). After a 5-day incubation period, reactors were sacrificed to measure dissolved and 0.5 N HCl extractable (adsorbed plus dissolved) (Jeon *et al.*, 2003) Fe(II), dissolved and 0.5 N HCl extractable metal, viable freely dispersed cells, and pH. Both dissolved and 0.5 N HCl extractable samples were filtered (0.2 μm) and Fe(II) was measured by ferrozine (Stookey, 1970). Dissolved and 0.5 N HCl extractable metal concentrations were measured from the corresponding dissolved and extractable Fe(II) filtrate samples by flame AAS after preservation with conc. HNO₃. Solution pH of the dissolved filtrate was determined in the anaerobic chamber using a combination pH electrode. The pH of the experiments never varied outside the range of 6.6–7.0.

Cell viability was measured using the *Ba*light bacterial viability kit (Molecular Probes, Eugene, OR). Viable cells were recognized by an apple green fluorescence indicating SYTO 9 stain absorption, while nonviable cells were recognized by red fluorescence caused by propidium iodide uptake, quenching the green SYTO 9 fluorescence (Sani *et al.*, 2001; Hu *et al.*, 2003; Teitzel and Parsek, 2003; Stone *et al.*, 2006). Viable cell counts were made at 64× magnification with a Zeiss epifluorescence microscope (Zeiss, Jena, Germany). Freely dispersed cell viability was calculated based upon the average from five field counts (each field being 2.64 × 10⁻⁴ cm²) for each sample (one per reactor). Cells visibly attached to hematite particles (i.e., localized biofilms) were not counted due to uncertainties related to stain uptake.

Zinc and manganese partitioning

Experiments were performed to determine the partitioning of zinc and manganese to 10⁸ viable cells mL⁻¹, 2.0 g L⁻¹ hematite during bioreduction experimental conditions described above. Dissolved Me(II) and 0.5 N HCl extractable Me(II) concentrations were determined from incubated serum bottles. Sorbed metal concentrations were operationally defined as the difference between the

0.5 N HCl extractable and dissolved (both passing through a 0.2-μm filter) metal concentrations measured after a 24-h reaction period. Incubation periods ranged from 1 to 5 days for zinc, and 5 days for manganese. For zinc experiments, variable biogenic Fe(II) concentrations ranged from 0.09–0.30 mM 0.5 N HCl extractable Fe(II). For manganese experiments, variable biogenic Fe(II) concentrations ranged from 0.10–0.20 mM 0.5 N HCl extractable Fe(II). Since our experiments contained two sorbents (hematite and cells), sorbed Me(II) concentrations were calculated as the difference between the 0.5 N HCl extractable and dissolved Me(II) concentrations and are reported as mmol of Me(II) per m² total sorbent surface area. Sorbent concentrations were 2.0 g L⁻¹ hematite and 0.064 g L⁻¹ cells, with cell mass estimated using an average cell weight of 6.4 × 10⁻¹⁰ mg dry wt. cell⁻¹ (Liu *et al.*, 2002b) and surface area of 140 m² g⁻¹ (Fein *et al.*, 1997; Sokolov *et al.*, 2001) for *S. putrefaciens* CN32. Hematite surface area was 9.04 m² g⁻¹ measured by N₂-BET (Jeon *et al.*, 2001).

Data analysis

Inhibition was defined as the percent change in the 5-day extent of hematite bioreduction due to metal addition relative to its biotic no-metal control (Sani *et al.*, 2001) and calculated as:

%Inhibition

$$= \left(1 - \frac{\text{Bioreduction Extent with Me(II)}}{\text{Bioreduction Extent without Me(II)}} \right) \times 100 \quad (1)$$

where the bioreduction extent was measured after 5 days for hematite reduction. It is important to note that for each series of experiments (with or without amendment), inhibition was calculated relative to its corresponding biotic no-metal control. The inhibitory concentration of metal required for 50% reduction in microbial metal reduction (IC₅₀) was calculated according to the following equation:

$$\text{IC}_{50} = 10^{\left(\frac{50 - \beta_0}{\beta_1} \right)} \quad (2)$$

where β₀ was the y-axis intercept of the metal vs. percent inhibition regression line and β₁ was the slope of the metal vs. percent inhibition regression line.

RESULTS AND DISCUSSION

Impact of ferrozine and AQDS on zinc inhibition

Figure 1A presents biogenic Fe(II) production (0.5 N HCl extractable) after 5 days as a function of zinc addi-

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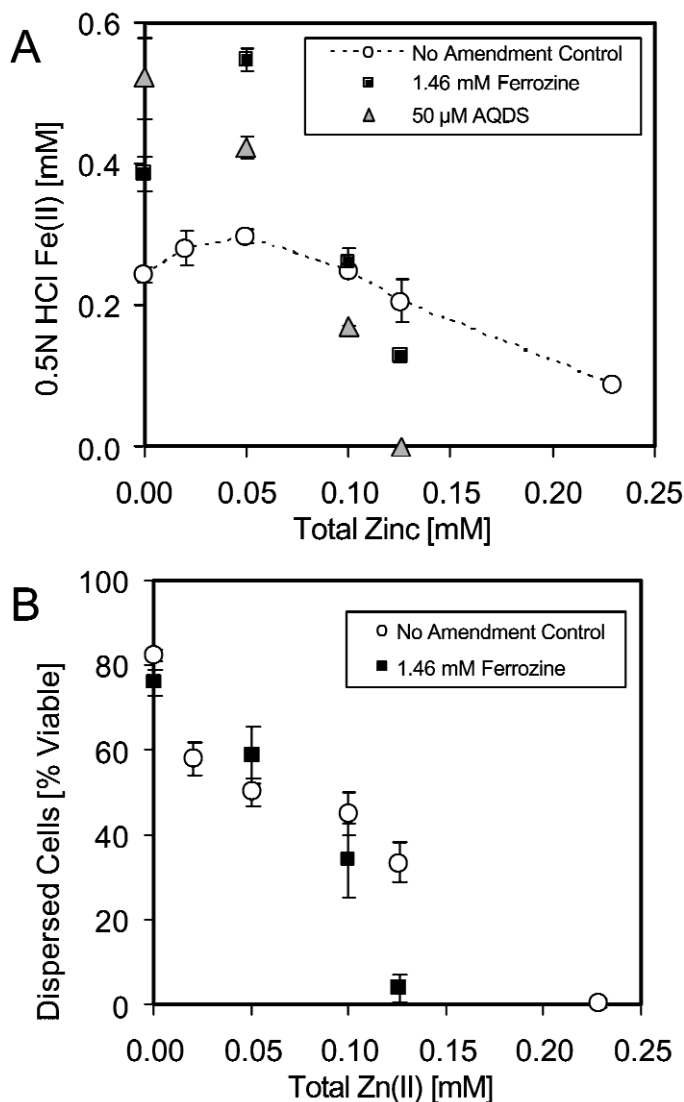


Figure 1. (A) 0.5 N HCl extractable Fe(II) production, and (B) freely dispersed cell viability for *Baclight* LIVE/DEAD cell staining as a function of total zinc addition for 1.46 mM ferrozine, 50 μ M AQDS, and no-amendment control. Experiments conducted with 2.0 g L⁻¹ hematite, 10⁸ cells mL⁻¹ under nongrowth conditions in 10 mM PIPES (pH 6.8). Values are means of (A) three replicates and (B) five replicates (\pm standard deviation). Error bars smaller than symbol size not shown.

tion. The biotic no-amendment control series (o symbols) is included for comparative purposes, and was prepared independently from the amendment experiments. Data points on the y-axis of Fig. 1A represent biotic no-zinc controls for each series. These values were used in Equation (1) (fraction denominator) to determine inhibition at each zinc concentration, and the corresponding inhibition values were used in Equation (2) to calculate the IC₅₀ values for each experimental condition (Fig. 2). The two amendment concentrations (1.46 mM ferrozine and 50 μ M AQDS) were selected because they enhanced hematite bioreduction (in the absence of zinc) (Royer *et*

al., 2002b). In the absence of zinc, both amendments stimulated hematite bioreduction (by 59 and 116% for ferrozine and AQDS, respectively) compared to the no-amendment control.

Low concentrations of zinc (≤ 0.05 mM) stimulated Fe(II) production for both ferrozine and the no-amendment controls (Fig. 1A). For the no-amendment controls, Fe(II) production increased by 22% with 0.05 mM total zinc compared to the no-amendment no-zinc control. Ferrozine addition increased Fe(II) production by 42% with 0.05 mM total zinc compared to the ferrozine no-zinc control. Fe(II) production for ferrozine and the no-

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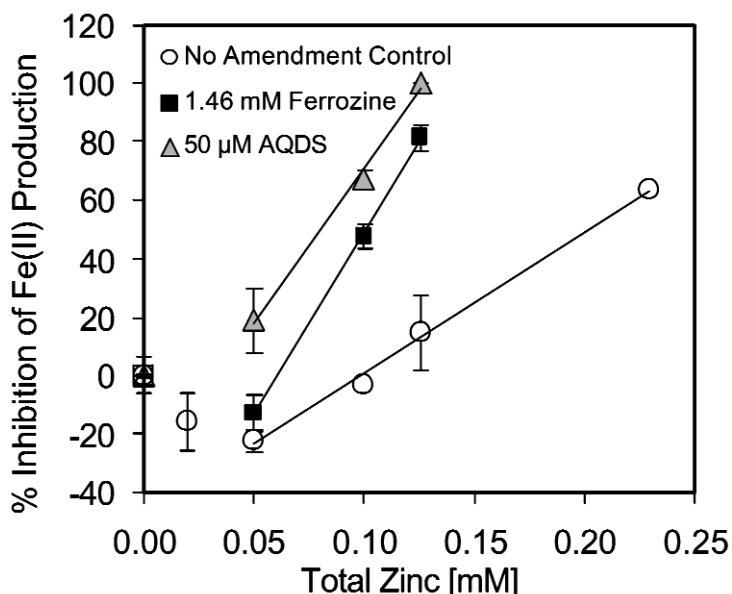


Figure 2. Percent inhibition of 0.5 N HCl extractable Fe(II) production as a function of total zinc for 1.46 mM ferrozine, 50 μM AQDS, and no-amendment control. Experiments conducted with 2.0 g L^{-1} hematite, $10^8 \text{ cells mL}^{-1}$ under nongrowth conditions in 10 mM PIPES (pH 6.8). Solid lines represent least-squares regression for total zinc $\geq 0.05 \text{ mM}$. Values are means of three replicates (\pm standard deviation). Error bars smaller than symbol size not shown.

amendment controls conformed to a Type II dose-response curve (stimulation followed by inhibition) as defined by Welp and Brummer (1997), where maximum Fe(II) production occurred at low zinc concentrations while higher zinc concentrations resulted in decreased Fe(II) production. In the presence of AQDS, Fe(II) production decreased by 23% with 0.05 mM total zinc compared to the AQDS no-zinc control. Zinc addition at concentrations greater than 0.05 mM inhibited hematite bioreduction in the absence and presence of both amendments.

Increasing zinc concentrations resulted in decreased freely dispersed cell viability (Fig. 1B). In no-amendment controls without zinc, 82% of the cells were viable after 5 days (o data point on the y-axis), whereas no freely dispersed viable cells remained in the presence of 0.23 mM total zinc. Similar results were observed for the ferrozine-containing control cultures; however less total zinc (0.13 mM) was required to achieve nearly no freely dispersed viable cells after 5 days. It is unknown why ferrozine increased zinc toxicity. Cell viability counts were not performed for the AQDS containing cultures due to interferences between the *Ba*light stain and AQDS.

The inhibition of hematite bioreduction vs. total zinc for each experimental series is presented in Fig. 2. The regression lines were calculated only for total zinc concentrations that resulted in increased inhibition ($\geq 0.05 \text{ mM}$ total zinc) compared to the corresponding no-zinc

control. The IC_{50} value for the no-amendment controls was 0.20 mM total zinc. Amendment addition lowered the IC_{50} (i.e., zinc became more toxic) to 0.10 and 0.081 mM total zinc for ferrozine and AQDS, respectively. IC_{50} values were also calculated based on dissolved zinc concentrations (results not shown). However, IC_{50} values could not be calculated based on free Zn^{2+} concentrations because zinc complexation reactions and constants with ferrozine and AQDS are presently unknown. A summary of the IC_{50} values and the corresponding regression equations are presented in Table 1.

Fe(II) and Zn(II) partitioning

The distribution of the divalent metals Fe(II), Zn(II), and Mn(II) between the solid (hematite and cell surfaces) and solution phases is important for interpretative reasons. Me(II) inhibition of hematite reduction could be caused by either Me(II) toxicity leading to cell death or Me(II) sorption leading to hematite surface blockage, or a combination of both. Sorbed Me(II) concentrations were calculated as the difference between the 0.5 N HCl extractable and dissolved Me(II) concentrations and are reported as mmol of Me(II) per m^2 total sorbent surface area (hematite and cells). A “baseline” Fe(II) sorption isotherm (solid line in Fig. 3A) was developed from a series of hematite bioreduction experiments performed in the absence of any amendment (i.e., ferrozine, AQDS) or

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Table 1. Summary of zinc IC_{50}^a values for hematite bioreduction by *S. putrefaciens* CN32.

Experimental condition	Inhibition equation ^b	Total zinc IC_{50}
No amendments	$y = 485.9x - 48.1; R^2 = 0.996$	0.20 mM total zinc
1.46 mM ferrozine	$y = 1239x - 75.2; R^2 = 0.999$	0.10 mM total zinc
50 μ M AQDS	$y = 1058x - 35.2; R^2 = 0.999$	0.081 mM total zinc

All experiments were performed with 2.0 g L⁻¹ hematite and 10⁸ cells mL⁻¹ in 10 mM PIPES (pH 6.8) at 20°C; ^adefined as inhibitory concentration of zinc where a 50% reduction of 5-day extent of Fe(II) production occurred relative to a corresponding no-zinc control; ^b IC_{50} values were calculated from the least-squares lines obtained from the data presented in Fig. 2.

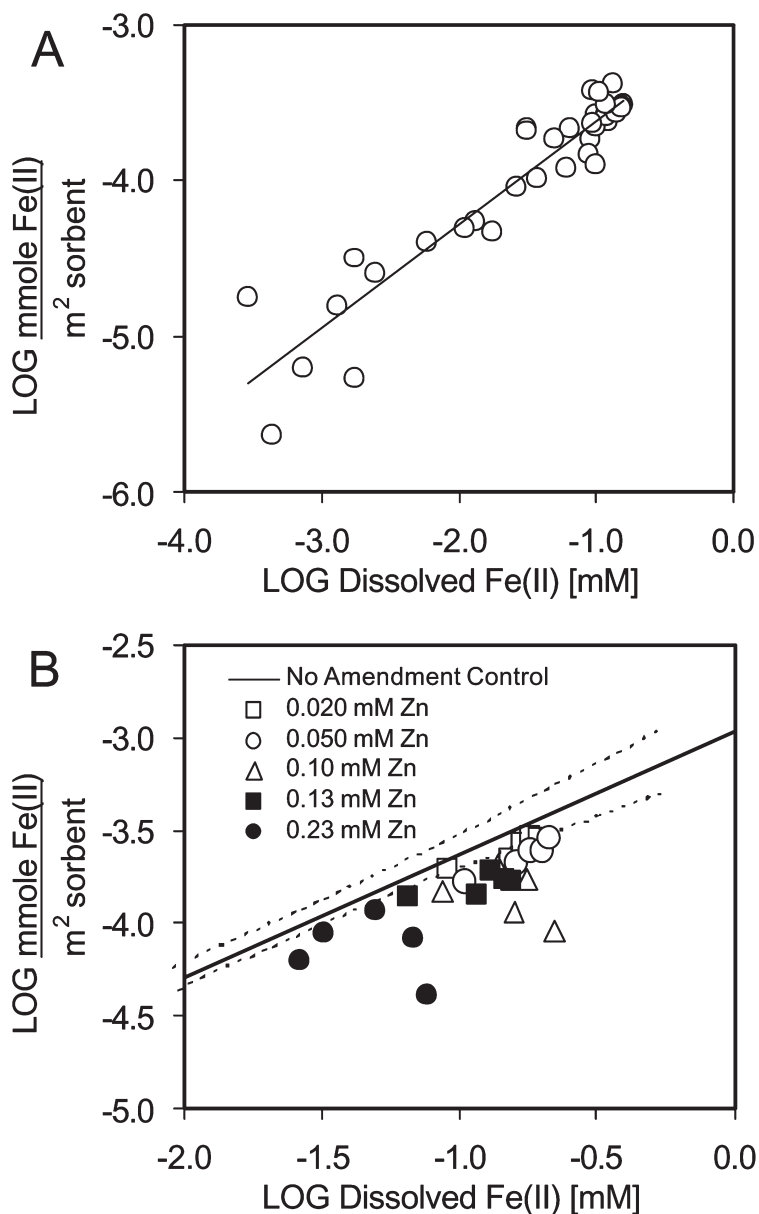


Figure 3. Fe(II) partitioning during hematite bioreduction for (A) no-amendment, no-metal controls, and (B) no-amendment, with-zinc samples. For both panels, solid line is Fe(II) Freundlich isotherm for the no-amendment, no-metal control, and dashed lines represents 95% confidence intervals. In A, symbols represent individual 5-day measurements. In B, symbols represent individual 1-5-day measurements.

metal (i.e., zinc, manganese). These experiments were performed with variable hematite concentrations (0.25–2.0 g L⁻¹) and a constant initial cell concentration of 10⁸ cells mL⁻¹. All data presented in Fig. 3A were determined from 1- to 5-day incubations. A Freundlich sorption isotherm ($R^2 = 0.891$) was selected over a Langmuir isotherm (not shown; $R^2 = 0.735$) due to a higher correlation coefficient. For consistency, Freundlich isotherms were used to describe metal partitioning for all results presented in this study (Table 2). The sorption isotherm for Fe(II) was similar to abiotic results reported by Jeon *et al.* (2003).

The effect of zinc on the partitioning of Fe(II) onto hematite and cells during hematite bioreduction is presented in Fig. 3B. The solid line represents the “baseline” no-zinc sorption isotherm (i.e., regression line from Fig. 3A), while the dashed lines represent the 95% confidence intervals associated with the isotherm equation. Dissolved Fe(II) measurements were taken daily for up to 5 days. The highest dissolved Fe(II) concentration for each zinc concentration corresponded to the 5-day incubation. The majority of data points fell below the Fe(II) baseline isotherm ($p < 0.05$) suggesting that zinc decreased the partitioning of Fe(II) onto hematite and cells. The higher total zinc concentrations (0.10, 0.13, and 0.23 mM) produced the greatest decrease in Fe(II) partitioning, especially for the 5-day results, which had the highest dissolved Fe(II) concentrations.

Impact of amendments on Fe(II) and Zn(II) partitioning

The effects of 1.46 mM ferrozine or 50 μM AQDS on Fe(II) partitioning are presented in Fig. 4A. The solid line represents the “baseline” Fe(II) sorption isotherm in the

absence of amendments (from Fig. 3A) with the corresponding 95% confidence intervals. These data show that ferrozine significantly reduced ($p < 0.05$) Fe(II) partitioning to hematite and cells for all measured dissolved Fe(II) concentrations. Ferrozine has a reported maximum Fe(II) complexation capacity of 0.33 mol Fe(II) mol⁻¹ ferrozine (Stookey, 1970) which, for the experimental conditions, was 0.48 mM Fe(II). This complexation capacity exceeded the total biogenic Fe(II) concentration produced in all but one condition (0.55 mM Fe(II) with 0.05 mM zinc; Fig. 1A). AQDS also decreased ($p < 0.05$) Fe(II) partitioning for two of the three conditions presented in Fig. 4A.

The effect of the amendments on zinc partitioning to hematite and cells are presented in Fig. 4B. The “baseline” no-amendment with-biogenic-Fe(II) zinc sorption isotherm was derived from results presented in Fig. 3B while dashed lines represent 95% confidence intervals. These data show that, with one exception, zinc sorption was not significantly affected by ferrozine or AQDS. The one exception was the lowest zinc concentration when Fe(II) production was highest. For both amendments, zinc sorption did not increase compared to the no-amendment controls; therefore, sorbed zinc blocking hematite surface sites was not likely an important mechanism of inhibition of bioreduction.

Impact of manganese

Fe(II) production was consistent for manganese concentrations up to 1.82 mM, where 0.5 N HCl extractable Fe(II) decreased from an average of 0.17 ± 0.014 mM ($n = 7$) to 0.10 mM (Fig. 5A). Fe(II) production in the biotic no-manganese control was 0.17 ± 0.017 mM ($n = 3$) 0.5 N HCl extractable Fe(II). Cell viability results in-

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Table 2. Summary of Freundlich isotherm parameters^a for 5-days Me(II) sorption in 10 mM PIPES, pH 6.8. Fe(II) sorption data shown in Fig. 3A; zinc and manganese sorption data shown in Fig. 6.

Metal	Sorbent	Equation	R ²
Fe(II)	Hematite + <i>S. putrefaciens</i> ^{b,c}	LOG $q = 0.663 \cdot \text{LOG } C - 2.96$	0.891
Zinc	Hematite + <i>S. putrefaciens</i> ^{c,dd}	LOG $q = 0.421 \cdot \text{LOG } C - 3.18$	0.957
Manganese	Hematite + <i>S. putrefaciens</i> ^{c,e}	LOG $q = 0.486 \cdot \text{LOG } C - 3.71$	0.884

^aValues derived using Freundlich isotherm equation: $\text{LOG } q = \log K_f + 1/n \text{ LOG } C$, where K_f = Freundlich adsorption capacity factor, and $1/n$ = Freundlich adsorption intensity factor; ^bResults from no-amendment no-zinc hematite bioreduction experiments (Stone *et al.*, 2006); ^cCells estimated from 10⁸ cells mL⁻¹ concentration of cells with 6.4×10^{-10} mg cell⁻¹ (Liu *et al.*, 2002b). Cell surface area estimated as 140 m² g⁻¹ (Fein *et al.*, 1997; Sokolov *et al.*, 2001); 2.0 g L⁻¹ hematite with estimated surface area of 9.04 m² g⁻¹ (Jeon *et al.*, 2001). ^dResults from no-amendment with-zinc hematite bioreduction experiments. Total biogenic Fe(II) ranged from 0.088–0.30 mM; ^eResults from no-amendment with-manganese hematite bioreduction experiments. Total biogenic Fe(II) ranged from 0.10–0.20 mM.

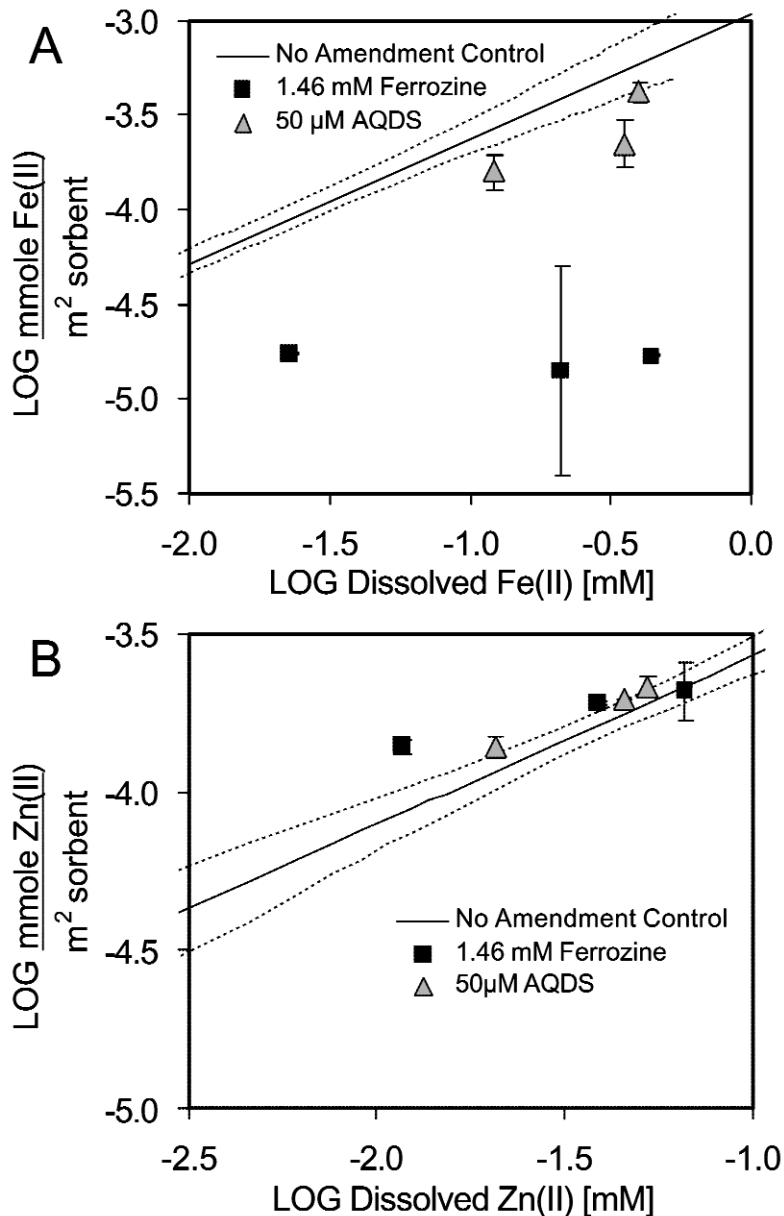


Figure 4. Distribution of (A) Fe(II), and (B) zinc in the presence of 1.46 mM ferrozine or 50 μ M ADQS during hematite bioreduction. In A, solid line is Fe(II) Freundlich isotherm for the no-amendment, no-zinc control, and dashed lines represents 95% confidence intervals. In B, the solid line is zinc Freundlich isotherm for the no-amendment, with-zinc samples and dashed lines represents 95% confidence intervals. Symbols represent 5-day mean values ($n = 4$ for A, $n = 3$ for B). Error bars represent standard deviations, not shown when smaller than symbol size.

indicated that manganese addition was not particularly lethal to cells (>80% viable) up to 0.91 mM manganese, beyond which viability decreased to 31% for 1.82 mM manganese.

The effect of manganese on the partitioning of Fe(II) onto hematite cells is presented in Fig. 5B. The solid line represents the “baseline” Fe(II) sorption isotherm (from Fig. 3A) in the absence of manganese and the

dashed lines represent 95% confidence intervals. Total manganese concentrations for these Fe(II) partitioning data ranged from 0.02 to 1.82 mM, while Fe(II) ranged from 0.10–0.20 mM 0.5 N HCl extractable Fe(II). The results show that manganese had no systematic effect on Fe(II) partitioning, in contrast to the decrease in Fe(II) partitioning observed in the presence of zinc (Fig. 3B).

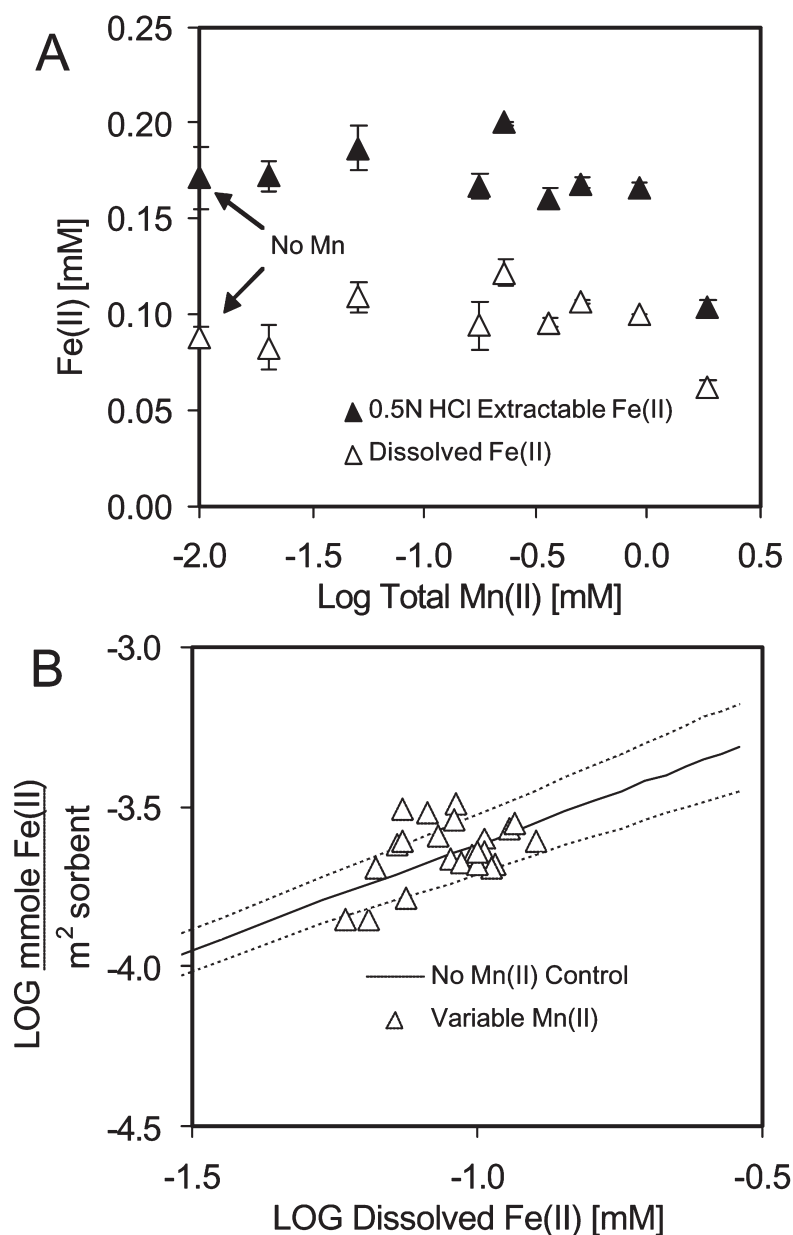


Figure 5 Effect of manganese on 5-day hematite bioreduction. (A) 0.5 N HCl extractable and dissolved Fe(II) production, and (B) distribution of Fe(II) as a function of total manganese. Experiments conducted with 2.0 g L^{-1} hematite, $10^8 \text{ cells mL}^{-1}$ in 10 mM PIPES (pH 6.8). In B, solid line represents Fe(II) Freundlich isotherm for no-amendment, no-manganese control, and dashed lines represent 95% confidence interval. Symbols represent mean values ($n = 2$) for A and individual measurements for B. Error bars represent standard deviations, not shown when smaller than symbol size.

Compared to zinc, manganese was far less lethal to *S. putrefaciens* CN32. At the highest manganese concentration tested (1.82 mM) 31% of cells were viable after a 5-day incubation period, while 0.23 mM zinc left 0% of the cells viable. Compared to zinc, manganese sorbed far less to hematite and cells (Fig. 6). At identical total Me(II) concentrations of 0.23 mM, zinc decreased the 5-

day extent of hematite bioreduction by 63.8%, while manganese increased hematite bioreduction by 17.0% (Fig. 7). Also, with 0.23 mM total Me(II), zinc left 0% of the cells viable while manganese left >80% of the cells viable. At near-equal sorbed Me(II) concentrations (0.155 and $0.169 \mu\text{mol Me(II)} \text{ m}^{-2}$ surface area for zinc and manganese, respectively), zinc increased the 5-

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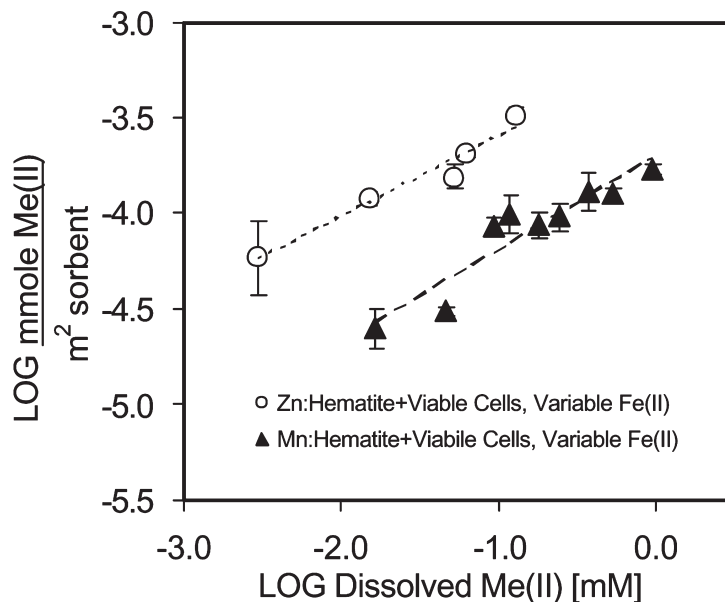


Figure 6. Distribution of zinc and manganese with no amendments for 2.0 g L^{-1} hematite, 10^8 viable cells mL^{-1} , and variable biogenic Fe(II) (0.088–0.30 mM for zinc, 0.10–0.20 mM for manganese). Symbols represent mean values ($n = 3$) and error bars represent standard deviations, not shown when smaller than symbol size.

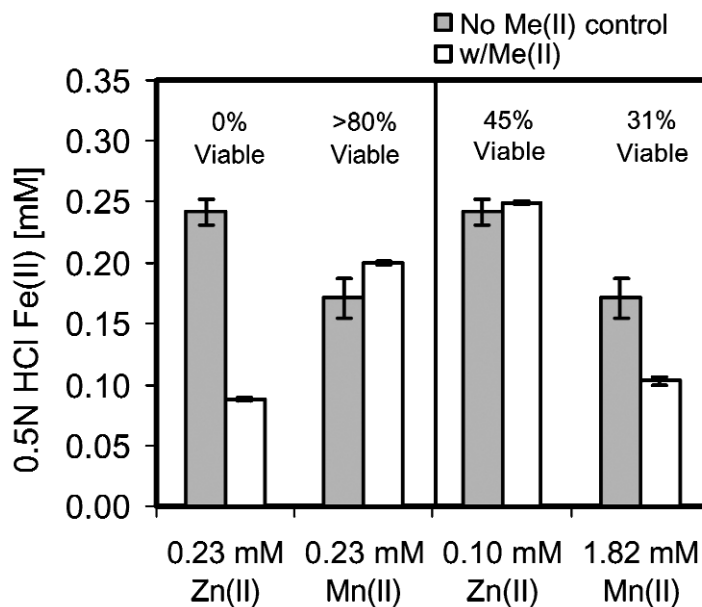


Figure 7. Comparison of zinc and manganese(II) on hematite bioreduction based on (left panel) equal total Me(II) concentrations, and (right panel) near-equal sorbed Me(II) concentrations. Open bars represent 5-day extent of hematite bioreduction in the presence of Me(II), and shaded bars represent 5-day extent of hematite bioreduction in corresponding no-Me(II) biotic controls. Biogenic Fe(II) measured as 0.5 N HCl extractable. In the right panel, the sorbed zinc concentration was $0.155 \mu\text{mol Zn(II) m}^{-2}$ and the sorbed manganese concentration was $0.169 \mu\text{mol Mn(II) m}^{-2}$. Error bars represent standard deviation. Percent viable estimates from viable 5-day freely dispersed cell counts.

extent of hematite bioreduction by 2.8% while manganese decreased hematite bioreduction by 39.8%. Also, at near-equal sorbed Me(II) concentrations, zinc left 45% of the cells viable while manganese left 31% of the cells viable. The comparisons made with these divalent cations consistently demonstrate that Me(II) toxicity/cell lethality was more important than Me(II) sorption/surface blockage in inhibiting hematite bioreduction.

SUMMARY

The relationships between sorbed and “free” Me(II) were assessed to predict Me(II) inhibition during the bioreduction of solid-phase iron(III) oxide. Previous studies suggest that “shielding” of zinc by solid-phase electron acceptors (i.e., hematite) may be responsible for the decreased sensitivity of free Zn^{2+} compared to aqueous electron acceptors. However, results from this study indicate increased surface sorbed Me(II) may not be responsible for the inhibition effects observed during hematite bioreduction. The addition of AQDS and ferrozine amendments did not increase Me(II) sorption, thus suggesting sorbed Me(II) likely was not responsible for the increased inhibition effects observed. Dissimilarities between zinc and manganese inhibition effects for both Me(II) addition and surface partitioning behavior further show Me(II) toxicity appears more important than Me(II) sorption for inhibiting hematite bioreduction. These results further advance the understanding of Me(II) inhibition mechanisms that occur during biological remediation efforts. Recognizing the interactions between Me(II) and bioreduction enhancement amendments is critical for the determination of effective bioremediation strategies

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