Effect of Natural Organic Matter on Zinc Inhibition of Hematite Bioreduction by *Shewanella putrefaciens* CN32

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The effect of zinc on the biological reduction of hematite (α-Fe$_2$O$_3$) by the dissimilatory metal-reducing bacterium (*DMRB*) *Shewanella putrefaciens* CN32 was studied in the presence of four natural organic materials (NOMs). Experiments were performed under non-growth conditions with H$_2$ as the electron donor and zinc inhibition was quantified as the decrease in the 5 day extent of hematite bioreduction as compared to no-zinc controls. Every NOM was shown to significantly increase zinc inhibition during hematite bioreduction. NOMs were shown to alter the distribution of both biogenic Fe(II) and Zn(II) between partitioned (hematite and cell surfaces) and solution phases. To further evaluate the mechanism(s) of NOM-promoted zinc inhibition, similar bioreduction experiments were conducted with nitrate as a soluble electron acceptor, and hematite bioreduction experiments were conducted with manganese which was essentially non-inhibitory in the absence of NOM. The results suggest that Me(II)—NOM complexes may be specifically inhibitory during solid-phase bioreduction via interference of DMRB attachment to hematite through the formation of ternary Me(II)—NOM—hematite complexes.

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Introduction

Biological iron(III) reduction can be used to produce Fe(II)-rich reactive zones for the in situ treatment of contaminated groundwater. Iron(III) reduction can be stimulated by the addition of excess electron donor (e.g., lactate, acetate, ethanol) to promote the development of anaerobic conditions in a targeted subsurface location (1, 2). Additional amendments to promote iron(III) reduction could include macronutrients and, possibly, natural organic materials (NOM). NOM addition may be considered because these materials have been shown to stimulate the bioreduction of solid-phase iron (hydr)oxides (3, 4). Considering that NOM is known to complex metals (5–9), NOM addition could also conceivably decrease the toxicity of any metal co-contaminants.

The overarching premise of this study was to elucidate how NOM influences the inhibitory effects of heavy metal co-contaminants during biological iron(III) reduction. Zinc was selected for study because it is a common groundwater contaminant at U.S. Department of Energy facilities (10). A series of related studies have been conducted to examine the effect of zinc on the facultative dissimilatory metal-reducing bacterium (*DMRB*) *Shewanella putrefaciens* strain CN32. In one study, we showed that *S. putrefaciens* CN32 exhibited greater tolerance to zinc during solid-phase hematite reduction as compared to nitrate or oxygen reduction (11). We speculated that cells in biofilms on hematite surfaces could have developed greater resistance to zinc compared to freely dispersed cells. In the second study, we showed that amendments known to stimulate hematite reduction [anthraquinone-2,6-disulfonate (AQDS) and ferrozine] increased zinc toxicity (12). Mechanistic interpretations of these types of experiments are difficult because multiple factors (e.g., competition for electron acceptor, cell lethality) can exert either stimulatory or inhibition effects at the same time. However, we concluded that metal toxicity was more important than metal sorption in inhibiting hematite reduction.

Building on these studies, the purpose of this research was to investigate the combined effects of zinc and NOM on the biological reduction of hematite by *Shewanella putrefaciens* CN32. Due to the ability of NOM to react with both solid phases and soluble species, NOM could impact Me(II)—promoted inhibition of solid-phase bioreduction through a variety of mechanisms. For example, NOM complexation with Me(II) would reduce the “free” metal activity (i.e., Me$^{2+}$) in solution, leading to a reduction in overall microbial Me(II) toxicity. Reduction of free metal activity has been previously demonstrated using synthetic metal chelating compounds such as EDTA and NTA (13, 14). In addition, NOM may complex biogenic Fe(II), thereby enhancing solid-phase bioreduction through the prevention of surface passivation (4). Similar enhancement has been observed through the use of EDTA and NTA (15–17). To better differentiate these possible effects, similar experiments were conducted using nitrate to compare the systemic effect of zinc and NOM using a soluble electron acceptor in lieu of solid-phase hematite, and with a system containing hematite, NOM, and manganese to compare the inhibitory effects using a nontoxic Me(II), manganese.

Experimental Section

**Materials.** Hematite powder was obtained from J.T. Baker (Phillipsburg, NJ) and heated to 350 °C in air overnight before use to remove any residual organic carbon. The four NOMs tested from the International Humic Substances Society (IHSS) included the following: soil humic acid (SHA), Suwannee River humic acid (SRHA), Suwannee River fulvic acid (SRFA), and Suwannee River NOM (SRNOM). NOM suspensions dissolved in anaerobic 10 mM PIPES were added to each master reactor at the appropriate concentration. Aromaticity values for the NOMs were determined from $^{13}$C NMR spectra from a published report for the IHSS materials (18). Proton titration data for all materials were used to determine NOM acidity (19). Characteristics of the NOM materials are provided in Table 1.

**Bacterial Inoculum.** *Shewanella putrefaciens* CN32 was grown aerobiically on tryptic soy broth without dextrose (TSB-
TABLE 1. Characteristics of IHSS Natural Organic Materials Used in Current Study

<table>
<thead>
<tr>
<th>NOM source</th>
<th>organic radical content&lt;sup&gt;a&lt;/sup&gt; [spins g&lt;sup&gt;-1&lt;/sup&gt; × 10&lt;sup&gt;17&lt;/sup&gt;]</th>
<th>acidity at pH 6.8&lt;sup&gt;b&lt;/sup&gt; [meq g&lt;sup&gt;-1&lt;/sup&gt;]</th>
<th>Me(II) complexation capacity&lt;sup&gt;c&lt;/sup&gt; [mM Me(II) g&lt;sup&gt;-1&lt;/sup&gt;]</th>
<th>aromaticity [%]&lt;sup&gt;d&lt;/sup&gt;</th>
<th>C content [%]&lt;sup&gt;d&lt;/sup&gt;</th>
<th>O/C ratio&lt;sup&gt;d&lt;/sup&gt;</th>
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<tr>
<td>Soil HA</td>
<td>12.9</td>
<td>4.03</td>
<td>2.02</td>
<td>50</td>
<td>58.1</td>
<td>0.886</td>
</tr>
<tr>
<td>Suwannee River HA</td>
<td>1.5</td>
<td>3.97</td>
<td>1.99</td>
<td>37</td>
<td>52.6</td>
<td>0.809</td>
</tr>
<tr>
<td>Suwannee River FA</td>
<td>0.54</td>
<td>5.56</td>
<td>2.78</td>
<td>24</td>
<td>53.0</td>
<td>0.828</td>
</tr>
<tr>
<td>Suwannee River NOM</td>
<td>0.54</td>
<td>4.08</td>
<td>2.04</td>
<td>24</td>
<td>48.8</td>
<td>0.814</td>
</tr>
</tbody>
</table>

<sup>a</sup> From IHSS Standard and Reference Collection.  
<sup>b</sup> From proton titrations in 0.01 M NaCl provided by Ritchie and Perdue (19).  
<sup>c</sup> Me(II) complexation capacity calculated by assuming 1 mol of Me(II) consumes 2 equiv of NOM acidity (33). Suwannee River NOM–ZnII) complexation capacity was confirmed using 500 MWCO dialysis separation (Supporting Information Figure S1).  
<sup>d</sup> From Thorn et al. (18).

D) at 20 °C. Cells were harvested by centrifugation (4900g, 10 min, 20 °C) from a 16-hour-old culture (late log phase). The cells were washed three times in 10 mM 1,4-piperazineethanesulfonic acid (PIPES; pH 6.8) buffer with the final wash made with deoxygenated buffer to remove residual oxygen. Cell density was determined by absorbance at 420 nm.

Biorreduction Experiment Preparation. A “master reactor” approach was used to prepare all experiments to ensure consistent chemical and biological conditions (4, 11, 12). All preparations were performed within a Coy anaerobic chamber (Grass Lakes, MI). The reactor was prepared by combining the electron acceptor and the bacterial inoculum in a 250 mL serum bottle. All solutions (except the zinc and manganese stocks) were prepared in 10 mM PIPES (pH 6.8).

Before any NOM or metal addition to the master reactor, biotic no-NOM no-metal controls were prepared (in triplicate) by transferring 10 mL of the suspension into 20 mL amber serum bottles. The NOM was added before any metal to ensure that biotic with-NOM no-metal controls were prepared (in triplicate). Zinc or manganese was then incrementally added to the master reactor in an acidified, deoxygenated ZnCl<sub>2</sub> or MnCl<sub>2</sub> solution (1000 mg mL<sup>-1</sup> AAS certified standards) along with an equal volume of 0.1 N NaOH (for pH maintenance). At each concentration, 10 mL of the suspension was transferred into 20 mL amber serum bottles (in triplicate for zinc, duplicate for manganese). All serum bottles were sealed with Teflon-faced butyl rubber septa and aluminum crimp tops, and incubated outside the chamber in the dark at 20 °C on orbital shakers. Hydrogen from the anaerobic chamber atmosphere (97.5:2.5% N<sub>2</sub>/H<sub>2</sub>) was used as the electron donor in all experiments. The pH of the experiments never varied outside the range of 6.6–7.0.

Hematite Bioreduction. Experiments were performed with 2.0 g L<sup>-1</sup> hematite (0.0125 mol Fe<sub>2</sub>O<sub>3</sub> L<sup>-1</sup>), a final cell density of 10<sup>8</sup> cells mL<sup>-1</sup>, metal concentrations ranging from 0.02 to 0.23 mM total zinc or 0.02 to 1.82 mM total manganese, and the appropriate NOM (dissolved in 10 mM PIPES and added volumetrically). After 5 d incubation, reactors were sacrificed to measure dissolved and 0.5 N HCl extractable (total) Fe(II), dissolved and total metal, and pH.

Me(II) Distribution. Metal distribution results were determined using hematite bioreduction data described above. Partitioned Me(II) concentrations were operationally defined and calculated as the difference between the 0.5 N HCl extractable (total) and dissolved (both passing through 0.2 μm filter) metal concentrations, and are reported as mmoles of Me(II) per m<sup>2</sup> total sorbent surface area. Sorbent concentrations were 2.0 g L<sup>-1</sup> hematite and 0.064 g L<sup>-1</sup> cells, with cell mass estimated using an average cell weight of 6.4 × 10<sup>-10</sup> mg dry wt. cell<sup>-1</sup> (26) and surface area of 140 m<sup>2</sup> g<sup>-1</sup> (21, 22) for S. putrefaciens CN32. Hematite surface area was 9.04 m<sup>2</sup> g<sup>-1</sup> as measured by N<sub>2</sub>-BET (23).

Analytical Techniques. Fe(II) was reported as dissolved, total, and partitioned. For dissolved Fe(II), samples were filtered (0.2 μm cellulose acetate), combined with ferrozine (1.96 mM ferrozine in 50 mM HEPES, pH 8.0) inside the chamber, and Fe(II) was measured by ferrozine outside the chamber (24). Solution pH of the filtrate was determined in the anaerobic chamber using a combination pH electrode. For total Fe(II), an unfiltered sample was acidified with HCl to achieve a final solution normality of 0.5 N. The solution was mixed for ca. 24 h, filtered (0.2 μm), and Fe(II) in the filtrate was measured by ferrozine (24). Dissolved and total metal were measured from the corresponding dissolved and total Fe(II) filtrate samples by flame atomic absorption spectrometry (AAS) after preservation with conc. HNO<sub>3</sub>.

Data Analysis. Inhibition was defined as the percent change in bioreduction due to metal addition relative to its biotic no-metal control (11, 12, 25) and calculated as follows:

\[
\%\text{Inhibition} = \left(1 - \frac{\text{Bioreduction Extent with Me(II)}}{\text{Bioreduction Extent without Me(II)}}\right) \times 100
\]

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

\[
\text{IC}_{50} = 10^{\frac{\beta_0 - \beta_{i}}{\beta_i}}
\]

where \(\beta_0\) was the y-axis intercept of the metal versus percent inhibition regression line and \(\beta_i\) was the slope of the metal versus percent inhibition regression line, as adapted from the literature (11, 12, 25, 26). Metal toxicity as determined by direct measurements of cell viability, e.g., by BacLight staining in our previous studies (11, 12), was not possible in the presence of NOM due to interferences with BacLight stain.

Results

Effect of Zinc and NOM on Hematite Bioreduction. Total Fe(II) (0.5 N HCl extractable) produced after 5 d as a function of zinc addition for varied concentrations of IHSS SHA (15–250 mg L<sup>-1</sup>) and the with-SHA no-zinc controls are presented in Figure 1A. For each SHA concentration, biotic no-SHA no-zinc controls (results not shown) and biotic with-SHA no-zinc controls (data symbols on y-axis in Figure 1A) were also prepared. In the absence of zinc, all SHA concentrations ≥ 75 mg L<sup>-1</sup> stimulated hematite bioreduction compared to their corresponding no-SHA controls. For example, 250 mg L<sup>-1</sup> SHA stimulated Fe(II) production by 217% compared to its corresponding no-SHA control. Low concentrations of zinc stimulated Fe(II) production in the presence (<150 mg L<sup>-1</sup>) and absence of SHA (Figure 1A). Fe(II) production for the no-NOM control increased relative to its corresponding no-zinc control for
IC50 values based upon free Zn2+ values between 60 and 88% compared to the no-NOM control. Experiments performed using 2.0 g L−1 hematite, 106 cells mL−1 (S. putrefaciens CN32 under non-growth conditions) in 10 mM PIPES (pH 6.8). Values are mean of three replicates and error bars represent standard deviation (±standard deviation). Dashed lines (panel B) represent 95% confidence interval for total zinc ≥ 0.05 mM. Error bars smaller than symbol size not shown.

FIGURE 2. (A) 5 d 0.5 N HCl extractable Fe(II) production, and (B) inhibition of extent of hematite bioreduction as a function of total zinc for 150 mg L−1 SRFA, 250 mg L−1 SRHA, and no-NOM control. Experiments performed using 2.0 g L−1 hematite, 106 cells mL−1 (S. putrefaciens CN32 under non-growth conditions) in 10 mM PIPES (pH 6.8). Values are mean of three replicates and error bars represent standard deviation (±standard deviation). Dashed lines (panel B) represent 95% confidence interval. Error bars smaller than symbol size not shown.

Effect of Manganese and NOM on Hematite Bioreduction. Hematite bioreduction experiments using manganese instead of zinc were performed to determine whether Mn(II), having similar charge, molecular weight, and radius of hydration as Zn(II), inhibits hematite bioreduction similar to zinc. The manganese experiments were performed with 250 mg L−1 SRFA and 150 mg L−1 SRHA, both previously shown to increase zinc inhibition during hematite bioreduction (Figures 1 and 2). Manganese did not inhibit hematite bioreduction in the absence of NOM (open circles in Figure 3) except at the highest concentration studied (1.82 mM). The IC50 value was 3.06 mM total manganese for the no-NOM control (open circles in Figure 3) (J2). However, SRFA and SHA addition resulted in manganese inhibition, similar to the effects observed with zinc. The addition of 150 mg L−1 SRFA resulted in an IC50 of 0.16 mM total manganese, while 250 mg L−1 SHA resulted in IC50 of 0.85 mM total manganese (Table 3). Both IC50 concentrations for manganese were significantly higher (ca. > 2–10×) than those observed for zinc (i.e., manganese was less inhibitory than zinc).

Effect of NOM on Me(II) Distribution. 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 interpreting the results from these experiments. Me(II) inhibition of hematite reduction could be caused by...
either Me(II) toxicity leading to cell death or Me(II) and/or NOM partitioning leading to hematite surface blockage, or a combination of both. Divalent metal partitioning was calculated as the difference between the total and dissolved Me(II) measurements during the hematite bioreduction experiments. Freundlich isotherms were chosen to model the distribution of Fe(II) between solid (hematite and cell surfaces) and solution phases because they consistently provided greater correlation coefficients compared to Langmuir isotherms. For consistency, Freundlich isotherms were also used to model zinc and manganese distributions. Interpretation of these distribution results may be limited due to variable biogeochemical differences associated with the batch systems (e.g., competitive Me(II) partitioning, potential for biomineralization, variable Fe(II) production), however, Me(II) distributions provide critical information regarding bioreduction system behavior.

The effect of NOM on the distribution of Fe(II) during hematite bioreduction (in the absence of zinc) is presented in Figure 4A. The Fe(II) partitioning measurements (data points in Figure 4A) were calculated from 5 d hematite bioreduction experiments amended with the specified NOM concentration and variable zinc concentrations. Results are summarized in Table 2. The effect of NOM on the distribution of zinc during hematite bioreduction (in the presence of 0.012 to 0.51 mM biogenic Fe(II)) is presented in Figure 4B. A second “baseline” Fe(II) partitioning isotherm (solid line in Figure 4B) was developed from a series of no-NOM with-zinc controls (containing 0.020–0.23 mM total zinc). The Fe(II) partitioning measurements (data points in Figure 4B) were calculated from 5 d hematite bioreduction experiments amended with the specified NOM concentration and variable zinc concentrations. For each data set, the highest dissolved Fe(II) concentrations correspond to the lowest zinc concentrations tested for each NOM tested since increased zinc addition typically resulted in less Fe(II) production. The effect of NOM with-manganese on the distribution of Mn(II) during hematite bioreduction is presented in Figure 4D. The solid line represents a “baseline” Mn(II) partitioning isotherm developed from a series of no-NOM with-manganese controls (0.020–1.8 mM manganese). Mn(II) partitioning measurements (data points in Figure 4D) were calculated from 5 d hematite bioreduction

<table>
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<tr>
<th>NOM addition</th>
<th>total zinc IC₅₀ (mM)</th>
<th>effect on Fe(II) partitioning</th>
<th>effect on Zn(II) partitioning</th>
<th>NOM–Me(II) complexation capacity (mM)</th>
<th>total Me(II) (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 mg L⁻¹ SHA</td>
<td>0.078</td>
<td>+</td>
<td>+</td>
<td>0.030</td>
<td>0.14–0.27</td>
</tr>
<tr>
<td>75 mg L⁻¹ SHA</td>
<td>0.063</td>
<td>+</td>
<td>+</td>
<td>0.15</td>
<td>0.16–0.40</td>
</tr>
<tr>
<td>150 mg L⁻¹ SHA</td>
<td>0.050</td>
<td>+</td>
<td>var</td>
<td>0.30</td>
<td>0.23–0.25</td>
</tr>
<tr>
<td>250 mg L⁻¹ SHA</td>
<td>0.056</td>
<td>var (−)</td>
<td>0.50</td>
<td>0.22–0.47</td>
<td></td>
</tr>
<tr>
<td>150 mg L⁻¹ SRFA</td>
<td>0.069</td>
<td>0</td>
<td>var</td>
<td>0.42</td>
<td>0.17–0.25</td>
</tr>
<tr>
<td>250 mg L⁻¹ SRHA</td>
<td>0.085</td>
<td>0</td>
<td>0.50</td>
<td>0.27–0.56</td>
<td></td>
</tr>
</tbody>
</table>

* Defined as inhibitory concentration of Zn(II) that caused 50% decrease in 5 d extent of hematite bioreduction compared to with-NOM no-Zn(II) control (29). IC₅₀ value for no-NOM with-Zn(II) was 0.20 mM total zinc (17). Symbols: (+) always increased partitioning, (−) always decreased partitioning, (var) increased and decreased partitioning, (0) partitioning unchanged. Calculated from NOM acidity at pH 6.8 in 0.01 M NaCl solution (19) assuming 1 mol Me(II) consumes 2 equiv of acidity (32). Calculated as total biogenic Fe(II) produced + total Zn(II) added.
experiments with the specified NOM concentration and variable manganese concentrations. SHA addition increased Mn(II) partitioning at dissolved Mn(II) concentrations greater than 0.045 mM, while SRFA addition did not alter Mn(II) partitioning (Table 3).

**Discussion**

Results from this study demonstrate that all NOMs significantly increased zinc inhibition during hematite bioreduction, however, not all NOM addition resulted in increased zinc partitioning (Table 2). For example, SRFA did not effect Fe(II) partitioning except at very low dissolved Fe(II) concentrations (<0.03 mM; Figure 4A and B) and had little effect on Zn(II) (Figure 4C) or Mn(II) partitioning (Figure 4D), yet significantly increased both zinc and manganese inhibition of hematite bioreduction (Figures 2B and 3). As expected, Me(II)–NOM complexes did not inhibit nitrate bioreduction (using Zn(II); Supporting Information Figure S2), thus interactions between Me(II) and NOM in the absence of a solid-phase electron acceptor decreased inhibition. Surprisingly, NOM addition resulted in manganese inhibition when, in the absence of NOM, manganese did not inhibit bioreduction (Figure 3). Increased Me(II) partitioning cannot be the sole cause of NOM-promoted Me(II) inhibition because inhibition occurred with or without increased Me(II) partitioning. These results warrant further analysis regarding how Me(II)–NOM complexes specifically interfere with hematite bioreduction.

Our interpretation of these results suggests that the formation of hematite–Me(II)–NOM or cell–Me(II)–NOM complexes, or “ternary surface complexes” (28) either partitioned (to hematite and/or cell surfaces) or in solution, could be responsible for the inhibition effects observed. The formation of a ternary Me(II)–SHA–hematite surface complex has been described by Murphy et al. (28) where free metals are bound between hematite surface hydroxyls and ionized functional groups of NOM. While our operational definition of partitioned Me(II) concentration does not distinguish between the distribution of Me(II) to hematite or cells, or between the distribution of Me(II) or Me(II)–NOM complexes to these surfaces, we speculate that the preferential partitioning of Me(II)–NOM complexes (i.e., the formation

<table>
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<tr>
<th>NOM addition</th>
<th>total manganese IC₅₀ (mM)</th>
<th>effect on Mn(II) partitioning</th>
<th>NOM–Me(II) complexation capacity (mM)</th>
<th>total Me(II) (mM)</th>
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<tbody>
<tr>
<td>250 mg L⁻¹ SHA</td>
<td>0.85</td>
<td>+</td>
<td>0.50</td>
<td>0.64–1.1</td>
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<tr>
<td>150 mg L⁻¹ SRFA</td>
<td>0.16</td>
<td>var</td>
<td>0.42</td>
<td>0.24–0.97</td>
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</tbody>
</table>

* Defined as inhibitory concentration of Mn(II) that caused 50% decrease in 5 d extent of hematite bioreduction compared to with-NOM no-Mn(II) control (25). IC₅₀ value for no-NOM with-Mn(II) was 3.06 mM total manganese (12). IC₅₀ values were calculated from the least-squares lines obtained from the data presented in Figure 3. Symbols: (+) always increased partitioning, (−) always decreased partitioning, (var) increased and decreased partitioning, (0) partitioning unchanged. Calculated from NOM acidity at pH 6.8 in 0.01 M NaCl solution assuming 1 mol Me(II) can consume 2 equiv of acidity (33). Calculated as total biogenic Fe(II) produced + total Mn(II) added.

FIGURE 4. Distribution of (A) Fe(II) in the absence of zinc, and (B) Fe(II) in the presence of zinc (0.020–0.23 mM), (C) zinc in the presence of Fe(II) (0.012–0.51 mM), and (D) manganese in the presence of Fe(II) (0.060–0.71 mM) as a function of NOM during hematite bioreduction. Solid lines represent Freundlich isotherms for no-NOM, with Me(II) controls and dashed line represents 95% confidence intervals. Experiments performed using 2.0 g L⁻¹ hematite, 10⁹ cells mL⁻¹ (S. putrefaciens CN32 under non-growth conditions) in 10 mM PIPES (pH 6.8). Values are mean of three replicates and error bars represent standard deviation (±standard deviation). Error bars smaller than symbol size not shown.
of ternary Me(II)–NOM–hematite and/or Me(II)–NOM–
cell complexes) compared to Me(II) could magnify the
inhibitory effect of these complexes. It is also possible that
the partitioning of Me(II)–NOM complexes increased even
under conditions when operationally defined Me(II) parti-
tioning decreased. We speculate that the mechanism of NOM-
promoted Me(II) inhibition of hematite bioreduction could
be interference in attachment of DMRB to hematite due to
Me(II)–NOM complexes partitioned to either DMRB or
hematite, as shown in Figure 5.

Nitrate bioreduction experiments (Supporting Informa-
tion Figure S2) clearly showed that Me(II)–NOM complexes
were not inhibitory to S. putrefaciens CN32. Dialysis experi-
ments confirmed that Zn(II)–NOM complexes did form
under this solution chemistry (Supporting Information
Figure S1). Furthermore, because S. putrefaciens CN32 was
the sole sorbent in the nitrate bioreduction experiments,
Me(II)–NOM–DMRB ternary complexes (if formed) were
also not inhibitory to S. putrefaciens CN32. Instead, the nitrate
reduction results suggest that NOM acted as an effective
scavenger or complexing agent for minimizing bioavailable
free Zn²⁺. This was not the case during solid-phase hematite
reduction. While one would assume that NOM was again
effective at complexing free zinc, Zn(II)–NOM complexes
appeared to instead passivate the hematite surface, and
subsequently increased the overall inhibitory effect of zinc
during hematite bioreduction. Results from this study suggest
that these ternary Me(II)–NOM–hematite complexes formed
at the hematite surface passivate the surface and decrease
the bioavailability of surface Fe(III).

The enhancement of dissimilatory iron(III) reduction
through the addition of NOM and supplemental electron
donors and nutrients has been thought of as a viable
environmental remediation tool for in situ heavy metal and
radionuclide contaminant cleanup. The applicability of biologically mediating reducible metals [e.g., Cr(Vb) and
radionuclides (e.g., Tc(VII), U(VI)) has been proven under
both laboratory and field settings (29–32), however, results
from this study suggest that ancillary reactions between NOM
with other metal co-contaminants should be considered
when evaluating bioremediation applicability.

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Seed Grant program.

Supporting Information Available
Equilibrium dialysis and nitrate bioreduction results. This
material is available free of charge via the Internet at http://
pubs.acs.org.

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