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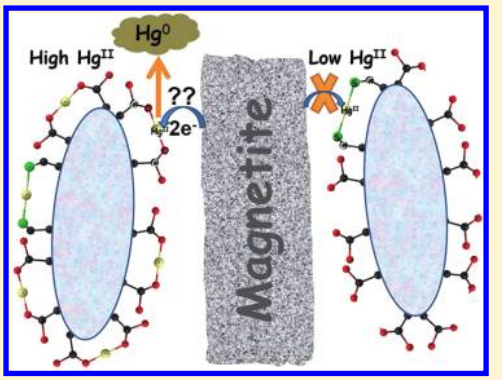
Binding of Hg^{II} to High-Affinity Sites on Bacteria Inhibits Reduction to Hg^0 by Mixed $Fe^{II/III}$ Phases

Bhoopesh Mishra,* Edward J. O'Loughlin, Maxim I. Boyanov, and Kenneth M. Kemner

Biosciences Division, Argonne National Laboratory, Argonne, Illinois 60439, United States

Supporting Information

ABSTRACT: Magnetite and green rust have been shown to reduce aqueous Hg^{II} to Hg^0. In this study, we tested the ability of magnetite and green rust to reduce Hg^{II} sorbed to 2 $g \cdot L^{-1}$ of biomass (*Bacillus subtilis*), at high (50 μM) and low (5 μM) Hg loadings and at pH 6.5 and 5.0. At high Hg:biomass loading, where Hg^{II} binding to biomass is predominantly through carboxyl functional groups, Hg L_{III}-edge X-ray absorption spectroscopy showed reduction of Hg^{II} to Hg^0 by magnetite. Reduction occurred within 2 h and 2 d at pH 6.5 and 5.0, respectively. At low Hg:biomass loading, where Hg^{II} binds to biomass via sulfhydryl functional groups, Hg^{II} was not reduced by magnetite at pH 6.5 or 5.0 after 2 months of reaction. Green rust, which is generally a stronger reductant than magnetite, reduced about 20% of the total Hg^{II} bound to biomass via sulfhydryl groups to Hg^0 in 2 d. These results suggest that Hg^{II} binding to carboxyl groups does not significantly inhibit the reduction of Hg^{II} by magnetite. However, the binding of Hg^{II} to biomass via sulfhydryl groups severely inhibits the ability of mixed $Fe^{II/III}$ phases like magnetite and green rust to reduce Hg^{II} to Hg^0. The mobility of heavy metal contaminants in aquatic and terrestrial environments is greatly influenced by their speciation, especially their oxidation state. In the case of Hg, reduction of Hg^{II} to Hg^0 can increase Hg mobility because of the volatility of Hg^0. Since Hg is typically present in aquatic and terrestrial systems at low concentrations, binding of Hg^{II} to high-affinity sites on bacteria could have important implications for the potential reduction of Hg^{II} to Hg^0 and the overall mobility of Hg in biostimulated subsurface environments.



INTRODUCTION

Mercury (Hg) is a contaminant of global concern, as bioaccumulation of methylmercury poses significant risk to aquatic ecosystems and human health.[1] Although elemental mercury (Hg^0) is far less reactive and toxic than the water-soluble ionic Hg^{II} species, the high mobility of Hg^0 (due to low vapor pressure) and the relative ease of oxidization of Hg^0 to Hg^{II} render Hg^0 an environmental hazard. Historical records from lake sediments provide compelling evidence that long-range atmospheric transport of Hg^0 results in significant inputs of Hg to remote areas.[2] The reduction of Hg^{II} to Hg^0 results from both abiotic and microbially mediated processes and is a key component of global Hg biogeochemical cycling.[3] In soils and sediments, the reduction of Hg^{II} to Hg^0 is generally attributed to direct microbial processes.[4] However, abiotic reduction pathways,[5−9] including photoreduction,[10,11] can also contribute significantly to Hg^{II} reduction to Hg^0.

The mobility of heavy metal contaminants in aquatic and terrestrial environments is greatly influenced by their speciation, especially their oxidation state. For example, Cr^{VI}, U^{VI}, and Tc^{VII} species tend to be more soluble and hence mobile than Cr^{III}, U^{IV}, and Tc^{IV} species. Thus, stimulating the in situ activity of native metal-reducing bacteria by the addition of organic substrates (e.g., acetate, ethanol) could potentially immobilize many heavy metals and radionuclides in contaminated environments. However, the activity of metal-reducing bacteria (Fe^{III}-reducing bacteria in particular) can lead to the reduction of Fe^{III} oxides to Fe^{II}-bearing phases such as magnetite, vivianite, siderite, and green rust,[12,13] some of which can be effective reductants for Hg^{II} to Hg^0 reduction.[10,11] Thus, promotion of metal-reducing conditions for immobilization of heavy metals and radionuclides can lead to increased mobility of Hg.

Understanding the geochemical processes that mediate Hg transformations in aquatic and terrestrial environments is necessary to predict its fate and transport. O'Loughlin et al.[10] showed the reduction of Hg^{II} to Hg^0 by green rust and suggested that other Fe^{II} phases may also reduce Hg^{II}. Indeed, reduction of Hg^{II} by magnetite has recently been reported.[11] Aqueous Hg^{II} reduction by magnetite occurs within minutes, and reaction rates increase with increasing magnetite surface area and solution pH.[11] The same study showed that chloride, an environmentally important inorganic ligand with strong binding affinity for Hg^{II}, inhibits the rate and extent of Hg^{II} reduction by magnetite. Although the reduction of aqueous Hg^{II} to Hg^0 by green rust and magnetite establishes the potential for abiotic Hg^{II} reduction

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under Fe^{III}-reducing conditions, the redox properties of Hg can be profoundly altered by the presence of organic ligands.[14,15] Field observations indicate the effect of organic ligands by showing the coexistence of Hg^{II} with high levels of Fe^{II} in groundwater containing very low levels of chloride ions.[16] It is possible that strong complexation of Hg^{II} with organic ligands significantly affects its availability for reduction by magnetite and other reactive $Fe^{II/III}$ minerals resulting from the activity of Fe^{III}-reducing bacteria. Hence, the effect of organic ligands on Hg^{II} reduction by magnetite and other Fe^{II}-bearing minerals must be evaluated to improve understanding of the geochemical processes that influence Hg transformations in the subsurface.

Studies on the speciation of Hg^{II} indicate that complex organic ligands such as natural organic matter (NOM) form stable Hg complexes through their sulfhydryl, carboxyl, and amine groups.[17−23] X-ray absorption spectroscopy (XAS) has shown that Hg^{II} interacts strongly with bacterial cell envelope through sulfhydryl and carboxyl functional groups.[24] A systematic study of Cd^{II} binding to both gram-positive and gram-negative bacteria suggests that Cd^{II} (and Hg^{II}, which has similar coordination properties) binds to the high-affinity sulfhydryl groups (about 2% of total functional groups), followed by much higher extents of adsorption to the more abundant carboxyl and phosphoryl groups at higher metal:biomass ratios.[25] Because typical Hg concentrations in contaminated environments are low and cell density in biostimulated environments may be high, preferential binding of Hg^{II} to sulfhydryl groups on bacterial cells could significantly impact the availability of Hg^{II} for reduction. This bacterial binding can affect the overall redox behavior of Hg^{II}, in both natural environments and bioremediation settings where Hg can be a cocontaminant with other metals and radionuclides that are being immobilized. Understanding the interplay between factors influencing the reduction of Hg^{II} by reactive Fe^{II} phases in the presence and absence of biomass will improve understanding of abiotic Hg^{II} reduction in contaminated environments.

We have investigated the effects of Hg binding to bacteria on the reduction of Hg^{II} to Hg^0 by magnetite and green rust. We hypothesized that sorption of Hg^{II} to biomass would inhibit abiotic reduction of Hg^{II} by mixed $Fe^{II/III}$ phases. To test this hypothesis, after Hg^{II} adsorption to *Bacillus subtilis* (a common soil bacterium that is neither a methylator [i.e., cannot produce methylmercury] nor a dissimilatory metal reducer) at different metal:biomass ratios, we introduced a stoichiometric excess of magnetite or green rust, then used synchrotron XAS to determine the speciation and coordination environment of solid-phase-associated Hg. Experiments were done as a function of pH (5.0 and 6.5), total Hg concentration (5 and 50 μM), and reaction time (2 h to 2 months).

METHODS AND MATERIALS

Bacterial Growth Conditions. The procedures for growth and washing of *B. subtilis* 168 for use in this study were similar to those described earlier.[25,26] Briefly, *B. subtilis* was cultured in tryptic soy broth with 0.5% yeast extract and incubated for 24 h at 32 °C on a shaker. The cells were collected by centrifugation (5800 × *g* for 60 min) and rinsed five times with 0.1 M $NaClO_4$ (the background electrolyte used in the Hg^{II} sorption experiments). The resulting cell density, reported on a wet mass basis, corresponds to approximately 8 times the dry mass of the cells.

Hg^{II} Adsorption to Biomass. Washed bacteria were suspended in Teflon centrifuge tubes in 0.1 M $NaClO_4$ electrolyte at 32 °C to form a suspension of 2 g·L^{-1} of bacteria (wet mass). Hg^{II} was added from a stock solution created from a commercially available (GFS Chemicals) reagent grade 5 mM Hg^{II} standard solution in 5% HNO_3, which was titrated to pH 3.0 with 1 M NaOH. The pH of each system (pH 5 or 6.5) was adjusted with 1 M HNO_3 or NaOH, and the systems were allowed to react for 3 h on a shaker. The pH (±0.3 pH units) was monitored every 15 min and adjusted as required with aliquots of 1 M HNO_3 or NaOH.

After 3 h of reaction, the suspensions were centrifuged, and the bacterial pellet was retained for analysis by X-ray absorption fine structure (XAFS) spectroscopy. The supernatant was filtered (0.45 μm) using nylon membrane (Millipore filter), acidified, and analyzed for dissolved Hg^{II} by inductively coupled plasma-optical emission spectroscopy (ICP-OES; Perkin-Elmer) with matrix-matched standards. The amount of Hg adsorbed to bacteria was calculated by subtracting the concentration of Hg remaining in solution from the total Hg concentration in the experimental system.

Reaction of Biomass-Bound Hg^{II} with Magnetite/Green Rust. Magnetite and hydroxysulfate green rust (GR_{SO4}), a green rust containing SO_4^{2-} as the interlayer anion, were synthesized as described by Cornell and Schwertmann.[27] After 3 h of reaction time between Hg^{II} and the biomass, magnetite or green rust was added at a molar ratio of Hg^{II}:Fe^{II} = 1:50. The system was rotated end-over-end at 20 rpm. All reactions were carried out in an anoxic glovebox (Coy) containing an atmosphere of 5% H_2 and 95% N_2. After reaction for 2 h, 2 d, or 2 months, subsamples of the suspension were centrifuged under anoxic conditions. Pellets containing biomass and Fe oxides were retained for Hg XAFS analysis within 2 h.

Hg XAS Measurements and Data Analysis. Hg L_{III}-edge X-ray absorption near edge structure (XANES) and extended X-ray absorption fine-structure (EXAFS) spectroscopy measurements were performed at the MRCAT sector 10-ID beamline,[28] Advanced Photon Source, Argonne National Laboratory. Details of the XAS experiments, standards, and data analysis are in the Supporting Information.

RESULTS AND DISCUSSION

Hg^{II} Complexation with Biomass. Hg^{II} concentrations in the supernatants of samples without reductant were below the ICP-OES detection limit (0.05 μM), indicating complete removal of Hg from solution by sorption to biomass. Figure 1a,b compares the XANES and k^2-weighted $\chi(k)$ EXAFS data for Hg standards with Hg^{II} complexed to biomass at 5 μM (Hg_L-bio) and 50 μM (Hg_H-bio) Hg^{II} loadings, at pH 5.0. The spectra indicate that Hg is complexed via sulfhydryl groups in the Hg_L-bio sample. Spectral features supporting this conclusion are the small pre-edge peak and the slight dip at 12 300 eV in the XANES, as well as the large amplitude and the phase of oscillations in the k^2-weighted $\chi(k)$ data (which are similar for Hg_L-bio and Hg-cysteine data). Similarly, a strong pre-edge peak at 12285 eV and a peak at 12300 eV in the XANES spectra, combined with smaller amplitudes of oscillation in the k^2-weighted $\chi(k)$ data, suggest that Hg in the Hg_H-bio sample is predominantly complexed via carboxyl groups. The differences between the amplitudes and bond distances of Hg_L-bio and Hg_H-bio and their similarities with Hg-cysteine and Hg-acetate solution standards, respectively, are further illustrated in the magnitude and real part of the Fourier transforms shown in Figure S1a,b (Supporting Information).

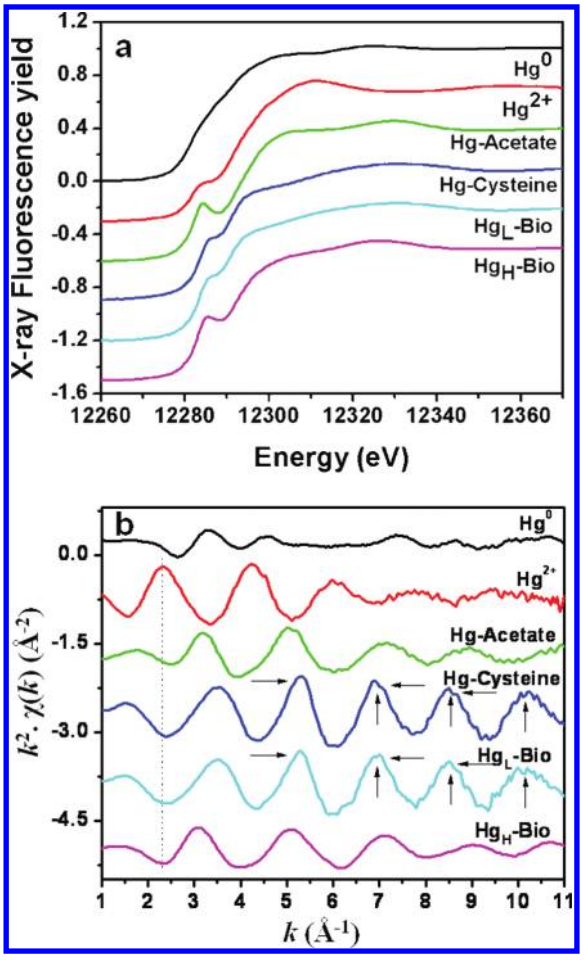

Figure 1. (a) Hg L_{III}-edge XANES spectra of Hg^{II} sorbed to biomass samples at high (Hg_H-bio) and low (Hg_L-bio) Hg^{II} loadings, with XANES spectra of Hg standards for comparison. (b) k^2-weighted $\chi(k)$ spectra of Hg L_{III}-edge EXAFS for high and low loadings of Hg^{II} sorbed to biomass samples, with k^2-weighted $\chi(k)$ spectra of Hg standards for comparison.

The first derivative of XANES data comapring Hg_H-bio and Hg_L-bio samples shown in Figure S1c of the SI also suggest that Hg in the Hg_H-bio and Hg_L-bio samples is predominantly complexed via carboxyl and sulfhydryl groups respectively.[29] No differences in spectra are observed between pH 6.5 and 5.0 for the same metal to biomass ratio (Figure S2 in Supporting Information shows Hg_L-bio at pH 5 and 6.5), consistent with previous finding that the Hg^{II} binding mechanism to biomass does not change over this pH range.[24]

The EXAFS data from samples Hg_L-bio and Hg_H-bio and the Hg standards were modeled quantitatively as described in the SI, by using simultaneous multiple *k*-weight fits and multiple sample fits. Best-fit values are in Table 1, and the fits are shown in Figure S3 (SI). The best fit for Hg_L-bio was with 1.85 (±0.18) S atoms at 2.32 (±0.01) Å in the first shell. Inclusion of an O/N atom in the first shell or a C atom in the second shell did not significantly improve the fit (see SI).

The best fit for Hg_H−bio was with 1.65 (±0.24) O atoms at 2.06 (±0.01) Å in the first shell. Inclusion of a C atom (1.58 ± 0.36) in the second shell significantly improved the fit. However, the Hg−C distance for the Hg_H−bio sample was 3.05 (±0.02) Å—much longer than the Hg−C distance determined for Hg−acetate solution standard (2.83 ± 0.01 Å)—suggesting the formation of a carboxyl with α-hydroxy carboxylic acid or a malate-type coordination geometry, consistent with previous findings.[22,24] In summary, the EXAFS results suggest an inner-sphere binding mechanism of Hg^{II} to biomass. At low metal:biomass, Hg^{II} binds to sulfhydryl groups, followed by carboxyl groups on bacterial biomass at higher metal:biomass ratios, consistent with previous findings.[24] Preferential binding of Hg^{II} to sulfhydryl groups at low metal:biomass, followed by carboxyl groups at higher metal:biomass, has also been observed for Hg^{II} complexation with NOM.[22,30−32]

Reaction of Biomass-Bound Hg^{II} with Magnetite. Hg sorbed to biomass under the conditions described above was reacted with magnetite ($Hg_{H/L}$-bio-magnetite). At pH 6.5, over 90% of the added Hg^{II} in the Hg_H-biomagnetite sample was reduced to Hg^0 by magnetite after only 2 h (see Figures 2a and S4 of the SI), indicating a rapid, possibly minute-scale reaction rate. This result is consistent with a previous study of Hg^{II} reduction by magnetite in the absence of biomass.[11] In contrast to the nearly complete reduction of Hg^{II} at pH 6.5 within 2 h, only 60% of the added Hg^{II} was reduced to Hg^0 at pH 5.0 over the same reaction period (data not shown), indicating slower reaction kinetics at pH 5 than at pH 6.5. However, almost complete reduction was observed at pH 5.0 after 2 d, and the sample remained reduced after 2 months (see Figures 2a, 2b, and S4 of the SI). Slower kinetics of reduction of Hg^{II} by magnetite at pH 5.0 than at pH 6.5 have also been observed with Hg^{II}in aqueous solution.[11] The similarity of reduction of aqueous Hg^{II} and Hg^{II} sorbed to biomass under Hg_H−bio-magnetite conditions suggests that complexation to biomass via carboxyl groups does not significantly affect the susceptibility of Hg^{II} to reduction by magnetite.

Hg^{II} in the Hg_L-bio-magnetite sample was not reduced to Hg^0 by magnetite after 2 d or 2 months of reaction time at pH 6.5 and 5.0 (Figures 2c and 2d). XANES spectra of the Hg_L-bio sample with magnetite after 2 d and 2 months at pH 5.0 and 6.5 match well with the Hg_L-bio spectrum, suggesting that Hg^{II} bound to the sulfhydryl groups on biomass was not reduced by magnetite after 2 months (Figure 2c and 2d). This is also confirmed by the EXAFS data (Figures S2 and S4, Supporting Information).

Effect of Magnetite Concentration. The stoichiometry of Hg^{II}:Fe^{II} was fixed at 1:50 for all experiments described above, while the concentration of biomass remained constant at 2 g·L^{-1} (wet mass). This resulted in a stoichiometric ratio of biomass: magnetite in the Hg_L−bio-magnetite system ten times that of the Hg_H−bio-magnetite system. To test the possibility that coating of the magnetite surface by biomass reduced reactivity in the Hg_L-bio-magnetite system, we increased the Hg^{II}:Fe^{II} stoichiometric ratio to 1:500 in the Hg_L-bio-magnetite system at pH 6.5, where magnetite effectively reduced Hg^{II} to Hg^0 within 2 h. XANES spectra collected after 2 d reproduced the spectral features of the Hg_L−bio or the Hg_L−bio-magnetite (1:50) spectra, confirming that Hg^{II} reduction to Hg^0 by magnetite is inhibited by binding of Hg^{II} to sulfhydryl groups on biomass, rather than by possible interaction between the bacteria and magnetite (Figure S5 of the SI).

Reaction of Biomass-Bound Hg^{II} with Green Rust. In light of the inability of magnetite to reduce Hg^{II} bound to sulfhydryl groups on biomass, we repeated the experiment with green rust instead of magnetite as the reductant. Green rusts contain up to 75% Fe^{II}, are generally stronger reductants than magnetite,[33−35] and readily reduce many heavy metals and radionuclides,

Table 1. Best-Fit Values for Solution Standards and Hg-bio Samples

sample	path	N	R(Å)	$\sigma^2(10^{-3}$ Å$^2)$	ΔE_0(eV)
Hg^{2+}	Hg−O	6.12 ± 0.65	2.30 ± 0.01	15.1 ± 3.5	−2.0 ± 1.2
HgAc	Hg−O	1.78 ± 0.32	2.06 ± 0.01	10.9 ± 0.9	3.2 ± 1.8
	Hg−C	1.78 [a]	2.83 ± 0.01	12.8 ± 4.0	
Hg_H−bio	Hg−O	1.65 ± 0.24	2.06 [b]	10.9 [b]	3.2 [b]
	Hg−C	1.58 ± 0.24	3.05 ± 0.02	12.8 [b]	
Hg−cysteine	Hg−S	1.88 ± 0.21	2.32 ± 0.01	10.5 ± 1.2	−1.7 ± 0.9
Hg_L−bio	Hg−S	1.85 ± 0.18	2.32 [c]	10.5 [c]	−1.7 [c]

[a] Fixed this value to be the same as O based on crystallographic data. [b] This variable was set to be equal to the HgAc standard during the simultaneous fit. [c] This variable was set to be equal to the Hg-cysteine standard during the simultaneous fit.

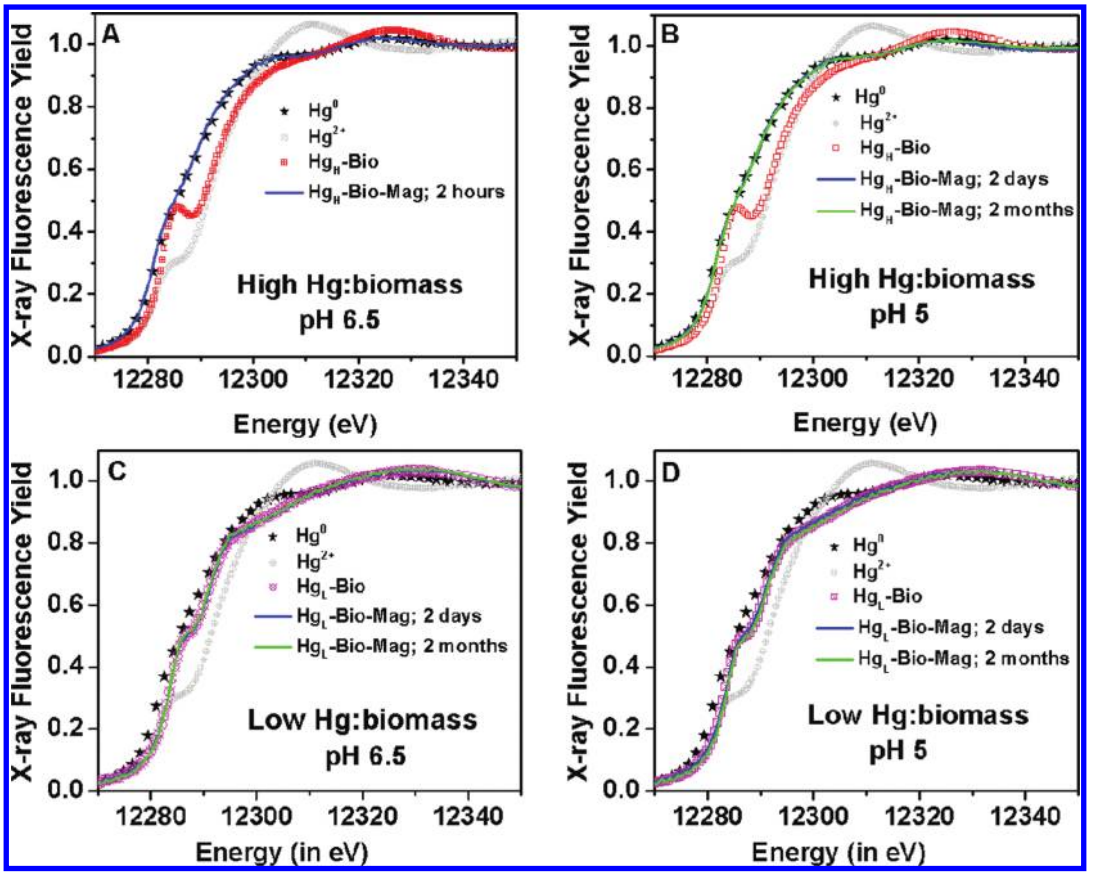

Figure 2. Top: Hg L_{III}-edge XANES spectra at high Hg:biomass ratio (Hg_H−bio) reacted with magnetite at pH 6.5 for (a) 2 h or (b) 2 d and 2 months at pH 5.0, with data for Hg^0, Hg^{2+}, and Hg_H−bio samples. Bottom: Hg L_{III}-edge XANES spectra at low Hg:biomass ratio (Hg_L−bio) reacted with magnetite for 2 d and 2 months at (c) pH 6.5 and (d) pH 5.0, with data for Hg^0, Hg^{2+}, and Hg_H−bio samples. The 2-d spectrum is not clearly visible, because 2-d and 2-month spectra overlap.

including Hg^{II}.[10,36−38] At pH 6.5, partial reduction of Hg^{II} to Hg^0 by green rust was observed after 2 d (Figure 3), under the experimental conditions where no reduction was observed with magnetite (5 μM Hg^{II}, 2 g·L^{-1} biomass, and 250 μM Fe^{II} as green rust). A linear combination fit of the XANES spectrum revealed that while 80% of the Hg^{II} added to the system remained bound to biomass as a Hg−cysteine complex, about 20% of the Hg^{II} was reduced to Hg^0. First derivative of the Hg XANES spectrum, which is usually more senstive to changes in oxidation state of Hg than Hg XANES, also confirm this observation (Figure S6 of the SI). We did not conduct this experiment at pH 5.0, because green rust becomes unstable at pH 5.[39]

Summary of Reduction Results. Results of the reactions of magnetite and green rust with Hg^{II} complexed to biomass under different conditions are compiled in Table 2. The uncertainty in the XANES analyses is about 10%; therefore, although XANES data indicate 100% reduction in the Hg_H−bio-magnetite system, up to 10% of the Hg in the solid phase might remain oxidized. The results for the Hg_L−biomagnetite system (5 μM added Hg^{II}) indicate that the same amount of sulfhydryl-bound Hg probably remains oxidized in the Hg_H−bio-magnetite system (50 μM added Hg^{II}). The specific mechanism by which binding to sulfhydryl groups inhibits Hg^{II} reduction is not clear. Specifically, this study did not distinguish whether the inihibition is

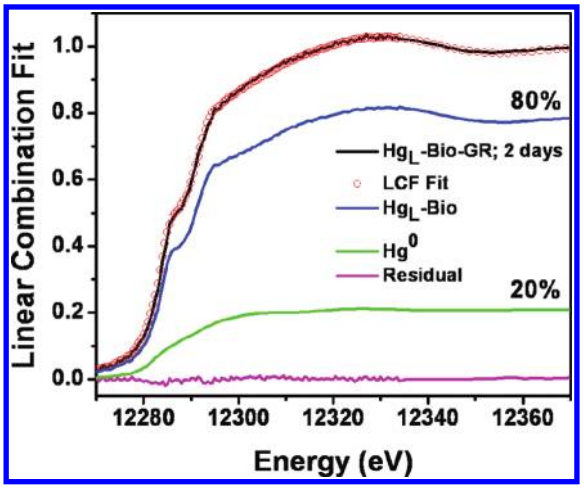

Figure 3. Linear combination fit of the XANES for the low Hg:biomass sample (Hg_L—bio) reacted with green rust at pH 6.5 (±0.2) for 2 d. Of the total Hg^{II} sorbed to biomass, 20% was reduced to Hg^0, while 80% of the sorbed Hg^{II} remained as Hg-cysteine complex after 2 d.

Table 2. Hg XANES and EXAFS Analysis Results for High And Low Loadings of Biomass-Sorbed Hg Sample Reacted with Magnetite and Green Rust at pH 6.5 and 5.0 (±0.2) for Different Reaction Times[a]

sample pH	50 (μM Hg adsorbed to 2 g/L *Bacillus subtilis,* and reacted with magnetite (2.5 mM Fe^{II})	5 μM Hg adsorbed to 2 g/L *Bacillus subtilis* and reacted with magnetite/GR(250 μM Fe^{II})
5.0	2 h-60% reduced 2 days, fully reduced 2 months, fully reduced	2 days, not reduced 2 months, not reduced
6.5	2 h, fully reduced	2 days, not reduced 2 months, not reduced 2 days (2.5 mM Fe^{II}), not reduced 2 days (with Green Rust), 20% reduced

[a] The uncertainty in XANES analysis is about 10%.

because sulfhydryl-bound Hg^{II} cannot be reduced by magnetite or because the high binding constant of Hg—cysteine complexes severely constrains the concentration of dissolved Hg^{II}. Additional studies are required to identify the exact mechanism of electron transfer for the reduction of Hg^{II} to Hg^0.

Implications for Subsurface Hg Biogeochemistry. The results of our study are relevant to the fate of Hg^{II} in the presence of Fe^{II} species in suboxic and anoxic environments. Reducing conditions are commonly encountered in natural aquatic environments. In addition, organic substrates have been injected into the subsurface and groundwater for the biostimulation of native metal-reducing bacteria, to promote in situ bioremediation by the reduction and potential immobilization of metals and radionuclides (e.g., Cr^{VI}, Tc^{VII}, U^{VI}); however, when Hg is present as a cocontaminant, creation of reducing conditions may have undesired consequences for the speciation and mobility of Hg. Nonetheless, our results show that when conditions are favorable for Hg sorption to sulfhydryl groups on biomass, Hg^{II} is unlikely to be reduced to Hg^0 by Fe^{II} species.

Our studies involved relatively high concentrations of Hg and biomass to enable spectroscopic analyses; however, concentrations of Hg in natural and contaminated geologic settings seldom exceed the nanomolar range. Biomass cell density in natural aquatic environments can also be orders of magnitude lower than those used in this study. However, since the ratio of Hg:biomass determines the nature of Hg^{II} complexation to biomass, the results of this study would be applicable under similar Hg:biomass ratios in the environment. For example, a natural environment with 5 nM Hg^{II} and 2 mg·L^{-1} biomass would likely exhibit same behavior as the 5 μM Hg and 2 g·L^{-1} biomass conditions in our study. At lower Hg concentrations or alternatively at higher biomass density, Hg^{II} forms more stable $Hg(cysteine)_2$ and $Hg(cysteine)_3$ complexes,[24] which would likely further limit the availability of Hg^{II} complexed with biomass for reduction by mixed $Fe^{II/III}$ phases. Hence, the biostimulation of a subsurface environment would likely inhibit the reduction of Hg^{II} to Hg^0 by mixed $Fe^{II/III}$ phases.

The use of a model gram-positive aerobic bacterium in this study should not limit the applicability of our results to more complex systems. Sulfhydryl functional groups are ubiquitous in natural environments and have very high affinity for Hg. The relative abundances of functional groups corresponding to the deprotonation constant of cysteine (8.5 ± 1.0), obtained from potentiometric titration data of *B. subtilis*, *Shewanella oneidensis* MR-1, and *Geobacter sulfurreducens*, are 1.0:1.5:2.0, respectively.[25,26,40] Moreover, complexation of Hg^{II} with sulfhydryl groups is not unique to bacterial biomass. Previous studies have shown Hg^{II} binding with natural and dissolved organic matter to be dominated by sulfhydryl groups.[17,22,29,41] Recent work has shown that complexation of Hg^{II} with sulfhydryl groups is also prevalent in sulfide-rich environments.[42]

Reduction of Hg^{II} to Hg^0 is a complex biogeochemical phenomenon, with competing microbial and abiotic redox pathways playing a role in surface and subsurface environments. Our results provide new insight into aspects of Hg biogeochemistry necessary for an effective assesment of Hg^{II} reduction and remobilization in surface and near-subsurface environments. Previous studies have shown a decline in the availability of Hg^{II} to mercuric reductase and in the rate of bacterial Hg^{II} reduction to Hg^0 with increased cell density.[43,44] Although both microbial and abiotic reductions of Hg^{II} are less likely under biostimulated conditions because of sorption of Hg^{II} to the high-affinity binding sites of biomass—given variations in cell structure, affinity, and biochemical processes—it might not be unreasonable to observe an increase in Hg^0 budgets via enzymatic reduction under such conditions. Clearly, additional studies are required to assess the long-term stability of Hg^{II} bound to biomass in natural systems. Moreover, iron in the form of Fe^{II} sorbed to clays and other minerals is far more common in the environment than Fe^{II} mineral phases like magnetite and green rust; hence, it is also important to evaluate the potential for sorbed Fe^{II} to reduce Hg^{II} complexed with sulfhydryl ligands.

■ ASSOCIATED CONTENT

Supporting Information. Details regarding the experimental procedures, EXAFS data collection and analysis; additional figures for XANES, derivative of XANES, and EXAFS data and their fits. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author
*E-mail: bmishra@anl.gov.

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