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The effect of natural organic matter on the adsorption of mercury to bacterial cells

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Abstract

We investigated the ability of non-metabolizing Bacillus subtilis, Shewanella oneidensis MR-1, and Geobacter sulfurreducens bacterial species to adsorb mercury in the absence and presence of Suwanee River fulvic acid (FA). Bulk adsorption and X-ray absorption spectroscopy (XAS) experiments were conducted at three pH conditions, and the results indicate that the presence of FA decreases the extent of Hg adsorption to biomass under all of the pH conditions studied. Hg XAS results show that the presence of FA does not alter the binding environment of Hg adsorbed onto the biomass, regardless of pH or FA concentration, indicating that ternary bacteria-Hg-FA complexes do not form to an appreciable extent under the experimental conditions, and that Hg binding on the bacteria is dominated by sulfhydryl binding. We use the experimental results to calculate apparent binding constants for Hg onto both the bacteria and the FA. The calculations yield similar binding constants for Hg onto each of the bacterial species studied. The calculations also indicate similar binding constants for Hg-bacteria and Hg-FA complexes, and the values of these binding constants suggest a high degree of covalent bonding in each type of complex, likely due to the presence of significant concentrations of sulfhydryl functional groups on each. S XAS confirms the presence of sulfhydryl sites on both the FA and bacterial cells, and demonstrates the presence of a wide range of S moieties on the FA in contrast to the bacterial biomass, whose S sites are dominated by thiols. Our results suggest that although FA can compete

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with bacterial binding sites for aqueous Hg, because of the relatively similar binding constants for the types of sorbents, the competition is not dominated by either bacteria or FA unless the concentration of one type of site greatly exceeds that of the other.

**Introduction**

Heavy metals, such as Hg, adsorb to proton-active functional groups on bacterial cell envelopes (e.g., Beveridge and Murray, 1976; Fortin and Beveridge, 1997; Daughney et al., 2002; Fein, 2006; Kenney and Fein, 2011), affecting the speciation and distribution of these metals in geologic systems. Recent studies (e.g., Guiné et al., 2006; Mishra et al. 2007; 2009; 2010; 2011; Pokrovsky et al., 2012; Song et al., 2012; Colombo et al., 2013) have shown that at least some bacterial cell envelopes contain proton-active sulfhydryl functional groups. Because Hg binds readily and strongly to sulfur compounds (Compeau and Bartha, 1987; Winfrey and Rudd, 1990; Benoit et al., 1999), bacterial adsorption of Hg may dramatically affect the distribution, transport and fate of Hg in geologic systems.

Natural organic matter (NOM) is present in nearly every near-surface geologic system, and complexation reactions between metals and NOM can dramatically change the behavior of the metals in the environment (McDowell, 2003; Ravichandran, 2004). NOM molecules contain a range of functional group types, including carboxyl, phenol, amino, and sulfhydryl groups, that have the potential to create highly stable complexes with metal ions across the pH range (Ephraim, 1992; Ravichandran et al., 1999; Drexel et al., 2002; Haitzer et al., 2002; Croué et al., 2003; Ravichandran, 2004). Hg binds strongly to the sulfhydryl groups present within the NOM structure (Dong et al., 2011; Muresan et al., 2011). The relative thermodynamic stabilities of Hg-NOM and Hg-bacteria complexes are not well known. Depending on these relative stabilities, the formation
of metal-NOM complexes may decrease adsorption of Hg to bacteria cell envelopes due to a competitive ligand effect, or under certain conditions may increase adsorption of Hg to bacteria due to ternary complexation with NOM. For example, investigating Pb, Cu, and Ni separately, Borrok et al. (2007) found that ternary metal-FA-bacteria complexes form, and that the importance of the complexes is strongly affected by pH. Conversely, Wightman and Fein (2001) found that the presence of NOM decreases the amount of Cd adsorbed to bacteria under mid- and high-pH conditions, and that the presence of Cd does not affect the adsorption of NOM to bacteria, suggesting that ternary complexes do not occur. No studies have been conducted to date to determine the effects of NOM on Hg binding to bacteria. However, because Hg forms strong complexes both with cell envelopes (Daughney et al., 2002; Mishra et al., 2011; Dunham-Cheatham et al.) and NOM (Loux et al., 1998; Ravichandran, 2004; Skyllberg et al., 2006), it is likely that significant changes to Hg adsorption behavior occur in the presence of NOM.

In this study, we use bulk adsorption and Hg X-ray absorption spectroscopy (XAS) experiments, conducted as a function of pH and FA concentration, using intact non-metabolizing bacterial cells to study Hg binding onto three different bacterial species and to compare the ability of bacteria to adsorb mercury in the presence and absence of a fulvic acid (FA). We use the experimental results to calculate apparent stability constants for Hg-bacteria and Hg-FA complexes, allowing for quantitative modeling of the competitive binding that can occur between bacteria and FA in more complex settings. This study examined both Gram-positive and Gram-negative bacterial species in order to determine if cell envelope structure affects the binding reactions, and one species was a Hg methylator, which we examined in order to determine if the extent or nature of Hg binding onto that species differed from that exhibited by the non-methylators.
Methods

Experimental methods

Bacterial growth and washing procedure

*Bacillus subtilis* (a Gram-positive aerobic soil species) and *Shewanella oneidensis* MR-1 (a Gram-negative facultative anaerobic species) cells were cultured and prepared following the procedures outlined in Borrok et al. (2007). Briefly, cells were maintained on agar plates consisting of trypticase soy agar with 0.5% yeast extract added. Cells for all experiments were grown by first inoculating a test-tube containing 3 mL of trypticase soy broth with 0.5% yeast extract, and incubating it for 24 h at 32 °C. The 3 ml bacterial suspension was then transferred to 1 L of trypticase soy broth with 0.5% yeast extract for another 24 h on an incubator shaker table at 32 °C. Cells were pelleted by centrifugation at 8100 g for 5 min, and rinsed 5 times with 0.1 M NaClO₄.

*Geobacter sulfurreducens* (a Gram-negative species capable of Hg methylation) cells were cultured and prepared using a different procedure than detailed above. Cells were maintained in 50 mL of anaerobic freshwater basal media (ATCC 51573) at 32 °C (Lovely and Phillips, 1988). Cells for all experiments were grown by first inoculating an anaerobic serum bottle containing 50 mL of freshwater basal media, and incubating it for 5 days at 32 °C. Cells were pelleted by centrifugation at 8100 g for 5 minutes, and rinsed 5 times with 0.1 M NaClO₄ stripped of dissolved oxygen by bubbling a 85%/5%/10% N₂/H₂/CO₂ gas mixture through it for 30 minutes. After washing, each of the three types of bacteria was then pelleted by centrifugation at 8100 g for 60 minutes to remove excess water in order to determine the wet mass so that suspensions of known bacterial concentration could be created. All bacterial concentrations in this study are given in
terms of gm wet biomass per liter. Bacterial cells were harvested during stationary phase, and all experiments were performed under non-metabolizing, electron donor-free conditions.

Adsorption experiments

To prepare experiments, aqueous Hg, NOM, and suspended bacteria stock solutions were mixed in different proportions to achieve the desired final concentrations for each experiment. The experiments were conducted in sets with constant pH (at pH 4.0 ± 0.1, 6.0 ± 0.1, or 8.0 ± 0.3) and constant bacterial concentration (0.2 g bacteria L⁻¹ in all cases) at three different FA concentrations (0, 25, or 50 mg L⁻¹), with Hg log molalities ranging from -6.30 to -5.00 (0.1 to 2.0 mg L⁻¹).

FA stock solutions were prepared in Teflon bottles by dissolving dried, powdered International Humic Substances Society Suwannee River FA Standard I in a 0.1 M NaClO₄ buffer solution to achieve the desired final FA concentration for each experiment. A known mass of wet biomass was then suspended in the FA stock solution, and the pH of the FA-bacteria parent solution was immediately adjusted to the experimental pH using 0.2 M HNO₃ and/or NaOH. To prepare experimental solutions, aliquots of the FA-bacteria parent solution were added gravimetrically to Teflon reaction vessels, followed by a small aliquot of commercially-supplied 1,000 mg L⁻¹ Hg aqueous standard to achieve the desired final Hg concentration. The pH of each suspension was again adjusted immediately to the experimental pH. The vessels were placed on an end-over-end rotator to agitate the suspensions for the duration of the experiment (2 h for *B. subtilis* and *G. sulfurreducens* and 3 h for *S. oneidensis* MR-1, as determined by initial kinetics experiments (results not shown)). The pH of the suspensions was monitored and adjusted every 15 minutes throughout the duration of the experiment, except during the last 30 minutes, when the suspensions were undisturbed. At the completion of each experiment, the pH of the suspensions
was measured and the experimental suspensions were centrifuged at 8100 g for 5 minutes. The aqueous phase was collected for Hg analysis by inductively-coupled plasma optical emission spectroscopy (ICP-OES), and the solid phase of some of the runs was collected for XAS analyses. Duplicate experiments were performed for each experimental condition.

**ICP-OES measurements**

ICP-OES standards were prepared gravimetrically by diluting a commercially-supplied 1,000 mg L\(^{-1}\) Hg aqueous standard with pH-adjusted 0, 25, or 50 mg L\(^{-1}\) FA stock solution made in 0.1 M NaClO\(_4\) so that the pH, ionic strength, and FA concentration of the standards closely matched that of the samples. We found significant interference when standards and samples were not closely matched in this way. The log molality of the Hg standards ranged from -6.60 to -5.00. The standards and samples were all stored in Teflon containers and analyzed with a Perkin Elmer 2000DV ICP-OES at wavelength 253.652 nm within 1 day of collection. The set of standards was analyzed before and after all of the samples were analyzed, as well as after every 15 samples, to check for machine drift. Analytical uncertainty, as determined by repeat analyses of the standards, was ± 2.8% for the 0 mg L\(^{-1}\) FA samples, ± 7.7% for the 25 mg L\(^{-1}\) FA samples, and ± 9.5% for the 50 mg L\(^{-1}\) FA samples. Neither standards nor samples were acidified prior to analysis. FA concentration strongly affected system performance and signal strength, likely due to spectral interferences caused by the FA molecule. For each pH and FA concentration condition studied, we conducted biomass-free control experiments to determine the extent of Hg loss due to adsorption onto the experimental apparatus as well as any interferences caused by the presence of FA during the ICP-OES analysis.
XAS measurements

Hg L\textsubscript{III}-edge X-ray absorption near edge structure (XANES) and extended X-ray absorption fine-structure spectroscopy (EXAFS) measurements were performed at the MRCAT sector 10-ID beamline (Segre et al., 2000), Advanced Photon Source, at Argonne National Laboratory. The continuous-scanning mode of the undulator was used with a step size of 0.5 eV and an integration time of 0.1 sec per point to decrease the radiation exposure during a single scan. Additionally, measurements were made at different spots on the samples to further decrease the exposure time. Hg XAS measurements were collected as described in Mishra et al. (2011).

Crystalline powder standards (cinnabar [red HgS] and mercuric acetate) were measured and used to calibrate the theoretical calculations against experimental data. Data from the standards were analyzed to obtain the S\text{02} parameter, where S\text{02} is the value of the passive electron reduction factor used to account for many-body effects in EXAFS. By fixing the values of S and O atoms to 2 in cinnabar and mercuric acetate, we obtained S\text{02} values of 1.02 ± 0.05 and 0.98 ± 0.03, respectively. Hence, we chose to set the value of S\text{02} = 1.0 for all samples. Fitting of the powder standards to their known crystallographic structures (cinnabar and mercuric acetate) reproduced the spectral features in the entire fitting range (1.0–4.2 Å), and fitting parameters were in agreement with previously reported values. Only the paths necessary to model the solid standards were used for fitting the solution standards and the unknown Hg samples.

Two Hg species, Hg-cysteine and Hg-acetate, were utilized as solution-phase standards for Hg XAS analyses. First, an aqueous Hg\textsuperscript{2+} standard was prepared from high-purity 5 mM Hg\textsuperscript{2+} in 5\% HNO\textsubscript{3} and was then adjusted to pH 2.0 ± 0.1 for measurement by adding appropriate amounts of 5 M NaOH. A Hg-cysteine standard was prepared by adding cysteine to the aqueous Hg\textsuperscript{2+} standard to achieve a Hg:ligand ratio of 1:100. The pH of the Hg-cysteine standard was adjusted
to 5.0 ± 0.1 by adding appropriate amounts of 1 M or 5 M NaOH. A Hg-acetate standard was
prepared by adding mercuric acetate salt to milliQ water and adjusting the pH to 5.0 ± 0.1 by
adding appropriate amounts of 1 M or 5 M NaOH.

Sulfur K-edge XANES spectra for biomass and FA samples were acquired at sector 9-BM
of the Advanced Photon Source at Argonne National Laboratory using Lytle detector in
fluorescence detection mode. At 9-BM, signal from higher order harmonics was removed by
detuning the monochromator to 70% of maximum beam flux at 2472.0 eV. Energy calibration was
performed by setting the first peak in the spectrum of sodium thiosulfate salt (Na$_2$S$_2$O$_3$) to 2469.2
eV. XANES spectra were measured between 2450 and 2500 eV. Step sizes in the near-edge region
(2467-2482 eV) were 0.08 eV, and 0.2 eV in pre- and post- edge regions, respectively. Samples
were smeared on carbon tape and the data were collected under a He atmosphere.

For this study, sulfur species are divided into three main categories and referred to as
reduced S (below 2472 eV), sulfoxide S (near 2473.5 eV), and oxidized S (above 2476.5 eV).
Accordingly, three commercially-supplied (Sigma Aldrich) S standards, cysteine, dimethyl
sulfoxide (DMSO), and sodium dodecyl sulfate (NaDS), were used to fingerprint S speciation. S
standards were mixed with a dry powder of polyacrylic acid (PAA) to achieve a mixture containing
~1% total S by mass. To perform S XANES measurements, a thin layer of a PAA-S standard
mixture was smeared on a carbon tape. All standards were prepared within 12 hours of analysis.

To prepare Hg XAS samples, FA was reacted with Hg by diluting a commercially-supplied
1000 mg L$^{-1}$ Hg standard with a pH-adjusted 50 mg L$^{-1}$ FA stock solution prepared in 0.1 M
NaClO$_4$. The log molalities of Hg investigated were -4.30 and -3.60 at both pH 4.00 ± 0.10 and
8.00 ± 0.10 for each Hg concentration. *S. oneidensis* MR-1 biomass was also reacted with Hg in
the presence and absence of FA to ascertain possible effects of FA on the Hg binding environment
on the bacterial cell envelopes. Biomass was collected from the experiments with -5.30 log M Hg, pH values 4.00 ± 0.10 or 8.00 ± 0.10, and 50 mg L⁻¹ FA. Samples were loaded into slotted Plexiglas holders that were subsequently covered with Kapton tape with a Kapton film sandwiched in between the tape and plexiglass to avoid direct contact of the sample with the tape adhesive. Samples were refrigerated until data collection. All measurements were conducted within 48 hours of sample preparation.

The data were analyzed by using the methods described in the UWXAFS package (Stern et al., 1995). Energy calibration between different scans was maintained by measuring Hg/Sn amalgam on the reference chamber concurrently with the fluorescence measurements of the biomass-bound Hg samples (Harris et al., 2003). The inflection point of the Hg L₃-edge (12.284 KeV) was used for calibration. Data processing and fitting were done with the ATHENA and ARTEMIS programs (Ravel and Newville, 2005). The data range used for Fourier transformation of the k-space data was 2.0–9.5 Å⁻¹. The Hanning window function was used with dk = 1.0 Å⁻¹. Fitting of each spectrum was performed in r-space, at 1.2-3.2 Å, with multiple k-weighting (k¹, k², k³) unless otherwise stated. Lower $\chi_r^2$ (reduced chi square) was used as the criterion for inclusion of an additional shell in the shell-by-shell EXAFS fitting procedure.

**Thermodynamic modeling**

Surface-complexation models were constructed to model Hg binding with bacterial cell envelope functional groups and with those on the FA molecules, and to quantify the competition between the two. Observed adsorption reactions between aqueous Hg species and deprotonated bacterial cell envelope sites and/or FA binding sites were modeled according to the following generic reaction:
$Hg_{\text{species}}^{x+} + R-A_i^− \rightleftharpoons (R-A_i)(Hg_{\text{species}})^{(x-1)+}$ \hspace{1cm} (1)

where ‘$Hg_{\text{species}}^{x+}$’ represents the specific aqueous Hg species considered, ‘$R-A_i$’ represents the deprotonated cell or FA binding site, ‘$(R-A_i)(Hg_{\text{species}})^{(x-1)+}$’ represents the Hg-bacterial cell envelope or Hg-FA complex, and the ‘$x$’ represents the charge of the aqueous Hg species. Stability constants for each of the Hg-bacterial cell envelope and Hg-FA complexes are expressed as the corresponding mass action equation for Reaction (1):

$$K_{\text{ads}} = \frac{[(R-A_i)(Hg_{\text{species}})^{(x-1)+}]}{a(Hg_{\text{species}}^{x+})[R-A_i^−]} \hspace{1cm} (2)$$

where $K_{\text{ads}}$ is the thermodynamic equilibrium constant for Reaction (1), the square brackets represent concentrations in mol L$^{-1}$, and $a$ represents the activity of the species in parentheses.

We used FITEQL 2.0 (Westall, 1982) for the equilibrium thermodynamic modeling of the adsorption data, using the aqueous speciation equilibria and equilibrium constants given in Table S1, and using the Davies equation within FITEQL to calculate activity coefficients. Because all of our experiments were conducted at the same ionic strength, we applied a non-electrostatic model to account for the Hg adsorption data. Bacterial site concentrations and acidity constants used in the calculations for $B. subtilis$, for $S. oneidensis$ MR-1, and for $G. sulfurreducens$ are from Fein et al. (2005), Mishra et al. (2010), and Dunham-Cheatham et al., respectively. The objective of the modeling exercise was not to construct precise site-specific mechanistic binding models, but rather to provide a quantitative means of estimating the competitive binding of bacteria and FA under a range of relative concentration conditions. Toward this end, because specific binding constants for Hg with each site type on the FA molecule are not known, we modeled Hg binding with the FA as a single complexation reaction between Hg$^{2+}$ and the deprotonated form of a generic FA site. We assumed that this generic binding site exhibits an acidity constant equal to the average of the acidity constants of all of the FA sites, with a site concentration equal to the total concentration of all FA sites.
sites, using the average values from Borrok and Fein (2004) as a model of the FA site speciation. The calculated acidity constant and site concentration for this generic site are listed in Table S1.

**Results**

**Adsorption experiments**

Consistent with previous studies of Hg adsorption onto bacteria (Daughney et al., 2002; Dunham-Cheatham et al.), we observed extensive adsorption of Hg onto the bacterial species studied in the absence of FA, with the extent of adsorption relatively independent of pH between pH 4 and 8 (Figure 1, top plots). For example, approximately 77% of the Hg in a 2 mg L\(^{-1}\) Hg solution adsorbs at pH 4 onto 0.2 g L\(^{-1}\) *S. oneidensis* MR-1, while approximately 75% adsorbs at pH 8. The presence of FA decreases the amount of Hg adsorbing to cell envelopes of each of the bacterial species and at each of the pH conditions studied (Figure 1, middle and bottom plots). With 50 mg L\(^{-1}\) FA, the extent of adsorption at pH 4 decreases to 65%, and at pH 8 to 50%. Our experimental results also indicate that the three bacterial species studied here exhibit similar extents of Hg adsorption under each experimental condition, consistent with the observations from a number of previous studies (e.g. Cox et al., 1999; Yee and Fein, 2001; Borrok et al., 2005, Johnson et al., 2007). Our data suggest that as the concentration of FA increases, so does the amount of Hg remaining in solution. These results indicate that FA competes with the bacterial cells for the adsorption of Hg, and that the adsorption of Hg to FA results in a competitive ligand effect. As a result, less Hg is available for adsorption to proton-active functional groups on the bacterial cell envelope, and less Hg is removed from solution. These results are not surprising, as FA molecules contain sulfhydryl groups within their structure and sulfhydryl groups bind strongly with Hg (Xia et al., 1999; Hesterberg et al., 2001; Drexel et al., 2002; Haitzer et al., 2002; 2003), leading to effective competition with bacterial cell envelopes which also contain proton-active
sulphhydryl functional groups (Guiné et al., 2006; Mishra et al., 2007; 2009; 2010; 2011; Pokrovsky et al., 2012; Song et al., 2012; Colombo et al., 2013). In our experimental systems, FA binding sites outnumber those present on the bacteria. For example, 50 mg L$^{-1}$ FA corresponds to approximately 2.8 x 10$^{-4}$ moles of sites L$^{-1}$ (Borrok and Fein, 2004), while 0.2 g L$^{-1}$ *B. subtilis* biomass contains 4.7 x 10$^{-5}$ total moles of sites L$^{-1}$. At pH 8, 50 mg L$^{-1}$ FA does diminish the extent of Hg adsorption, but only from approximately 75% (with no FA present) to 50%. It appears that given equal site concentrations, bacterial binding of Hg would dominate the competition with FA.

**Hg XANES and EXAFS**

To probe the effect of FA on Hg binding mechanisms with bacterial biomass, we examined Hg-biomass binding at pH 4 and 8 in the presence and absence of a stoichiometric excess of FA (1 mg L$^{-1}$ Hg and 50 mg L$^{-1}$ FA) using Hg L$_{III}$ edge XANES and EXAFS. For the XAS studies, *S. oneidensis* MR-1 was chosen to represent the bacterial species used in this study. Figure 2 shows a comparison between Hg XANES for Hg bound: 1) to FA and to *S. oneidensis* MR-1 biomass at pH 4, 2) to *S. oneidensis* MR-1 biomass in the presence and absence of FA at pH 4, 3) to *S. oneidensis* MR-1 biomass in the presence and absence of FA at pH 8, and 4) to cysteine, and to acetate. Hg XANES data indicate that Hg is complexed with thiol groups in the Hg-biomass samples. Spectral features supporting this conclusion are the small pre-edge peak and the slight dip at 12300 eV in the Hg-biomass XANES data similar to that present in the Hg-cysteine data. This finding is consistent with a previous study which showed Hg binding with sulphhydryl groups on *B. subtilis* cell envelopes under similar experimental conditions (Mishra et al., 2011). The Hg-FA XANES data exhibits a small pre-edge peak similar to that present in the Hg-cysteine data, which confirms that Hg is bound predominantly with the high-affinity thiol...
groups in FA under the experimental conditions. The absence of the dip in the Hg-FA XANES at 12300 eV, however, indicates differences in the coordination environment of Hg between FA and bacterial biomass. To understand these subtle differences in XANES, a linear combination fitting of the first derivative of Hg-FA XANES data was performed which resulted in about 90% contribution from Hg-cysteine binding and about 10% contribution from Hg-carboxyl binding (figure S2a). The first derivative of Hg-cysteine standard reproduced the Hg-biomass data confirming that the entire budget of Hg complexation with biomass was accounted by complexation of Hg with thiols. XANES spectra of Hg reacted with S. oneidensis MR-1 biomass in the presence and absence of FA at pH 4 and 8 are reproducible, confirming that the binding mechanism of Hg with S. oneidensis MR-1 biomass does not change appreciably in the presence of FA.

Hg EXAFS results are consistent with the Hg XANES results described above. Differences between the coordination environment of Hg-FA and Hg-biomass is more pronounced in the $k^2\chi(k)$ EXAFS data (Figure S1). Low signal to noise ratio in the aqueous Hg-FA data does not allow for a meaningful Fourier Transform (FT) of the Hg-FA EXAFS data. EXAFS $k^2\chi(k)$ and FT data between FA-bearing and FA-free Hg-biomass samples are similar (Figure S1), validating the Hg XANES results. Figure S2b shows a comparison between the FT Hg EXAFS data for Hg bound to S. oneidensis MR-1 biomass in the presence and absence of FA at both pH 4 and 8 and their corresponding EXAFS fits. EXAFS fitting parameters are shown in Table S2. It is worth pointing out that although Hg-cystein standard showed a bond distance of 2.32 Å, Hg-biomass samples at pH 4 and 8 had a bond distance of 2.35 Å. This should not be considered a discrepancy because Hg-S distances can vary from 2.32 to 2.36 Å for Hg(SR)$_2$ complexes (Manceau, and Nagy 2008). Similarly, Hg-S distances can range from 2.40 to 2.51 Å for Hg(SR)$_3$ complexes, and 2.50–2.61 Å for Hg(SR)$_4$ complexes.
Taken together, Hg XANES and EXAFS results indicate that Hg binds predominantly to the high-affinity thiol groups on bacterial cell envelopes in the presence and absence of FA and Hg binding mechanisms with bacterial biomass do not change in the presence of FA, excluding the possibility of the formation of a ternary complex. Additionally, Hg XAS results show that pH does not affect the adsorption mechanism of Hg onto biomass in the presence of FA, which is consistent with the similar extent of Hg adsorption as a function of pH described above. However, it is important to note that Hg binding mechanisms with bacterial biomass may be affected by FA at high Hg loadings, where Hg is primarily bound to biomass via lower-affinity carboxyl functional groups. Hg XAS results suggest that S functional groups on *S. oneidnensis* MR-1 cell envelopes outcompete S functional groups in FA for Hg binding. In other words, on average Hg binding to FA appears weaker than Hg binding to bacterial biomass. S XANES was conducted to identify the differences in complexation behavior of Hg with S functional groups on FA and bacterial biomass.

S K-edge XANES is highly sensitive to changes in the electronic environment of the sulfur absorber (Xia et al., 1998). Although S K-edge XANES spectra were collected on a large number of standards, in this study we have divided S species into three main categories: reduced S (below 2472 eV), sulfoxide S (near 2473.5 eV), and oxidized S (above 2476.5 eV). The S XANES spectra for cysteine, dimethyl sulfoxide (DMSO), and sodium dodecyl sulfate (NaDS) are shown in Figure 3a. Species with very different S oxidation states, such as cysteine, sulfoxide, and ester sulfate, are easily resolved in the XANES spectrum. However, resolving one species from another within these three energy ranges is challenging. Reduced S species, including thiols, sulfides, polysulfides, and thiophenes, all give white-line features occurring between 2469 and 2472 eV. More extensive model libraries that include XANES spectra of organic and inorganic S compounds are available.
Speciation of S in *S. oneidensis* MR-1 biomass was easily identified because the peak energy position of S XANES measurement on the biomass overlapped with the cysteine peak position (Figure 3b). Figure 3b shows the dramatic differences between S XANES on *S. oneidensis* MR-1 cells and Suwanee River FA. S XANES comparing FA with *S. oneidensis* MR-1 shows that nearly the entire S budget of the biomass is present as thiol groups, which are known to form strong bonds with Hg. However, FA has a range of reduced S (including reactive thiol) groups and a large fraction of oxidized S species, consistent with previous observations (Morra et al., 1997). Morra et al., 1997 suggest that a significant fraction of sulfur in SR Fulvic acid is found in oxidized (+5 oxidation state) form, followed by smaller fractions in reduced forms (-0.3 ±1.0 and 1.7 oxidations states) respectively. Similarly, Einsiedl et al., 2007 used S XANES to estimate that soil FAs contain around 51% oxidized (S^+4, S^+5, S^+6) and 49% reduced (S^-1, S_0, S^+2) sulfur species. The reduced S species was dominated by thiols, thiophene and disulfide. Such a dramatic difference between the S budget of FA and bacterial biomass could result in diverse reactivities and stabilities of Hg-S complexes between the two. A detailed study of the reactivity and stability of Hg with FA and bacterial biomass is beyond the scope of this study.

**Discussion**

The experimental results presented here suggest that bacterial cell envelope functional groups and FA functional groups exhibit different binding affinities for Hg under the experimental conditions. Hg binding onto the bacterial cell envelopes is extensive, and although Hg binds strongly with FA, especially with the sulfhydryl groups present within FA (Xia et al., 1999; Hesterberg et al., 2001; Drexel et al., 2002; Haitzer et al., 2002; 2003), the presence of even up to
50 ppm FA with only 0.2 g (wet mass) L\(^{-1}\) of bacteria does not cause the speciation of Hg to be dominated by the FA. The results suggest there is a possibility for competition between the bacterial and FA binding sites for the available Hg.

In order to quantify the competitive binding, we use a semi-empirical surface complexation approach. First, we use the FA-free adsorption data at pH 4, 6, and 8 to solve for equilibrium constants for the following Hg\(^{2+}\) adsorption reactions, respectively:

\[
\begin{align*}
R-A_1^- + \text{Hg}^{2+} & \Leftrightarrow R-A_1\text{-Hg}^+ \\
R-A_2^- + \text{Hg}^{2+} & \Leftrightarrow R-A_2\text{-Hg}^+ \\
R-A_3^- + \text{Hg}^{2+} & \Leftrightarrow R-A_3\text{-Hg}^+
\end{align*}
\]

where \(R-A_1, R-A_2, \text{ and } R-A_3\) represent the bacterial functional groups with the three lowest pKa values, respectively. At pH 4, the \(R-A_1\) sites are the dominant deprotonated sites available for Hg\(^{2+}\) binding for each bacterial species; at pH 6, both \(R-A_1\) and \(R-A_2\) sites are deprotonated; and at pH 8, \(R-A_1, R-A_2, \text{ and } R-A_3\) sites likely contribute to the binding of Hg\(^{2+}\). Therefore, we used the pH 4 data to constrain the stability constant value for Reaction (3) alone, then fixed that value and used the pH 6 data to solve for the stability constant value for Reaction (4) with a model that involved Reactions (3) and (4) simultaneously. We then used the values that we calculated for the stability constants for Reactions (3) and (4) and the pH 8 data to solve for the best-fitting value for Reaction (5) with a model that involved Reactions (3) - (5) simultaneously. This modeling approach assumes that Hg\(^{2+}\) binding at a given pH occurs dominantly onto sites with pKa values lower than the pH of the experiments; that is, dominantly onto deprotonated sites. However, the resulting stability constant values, which are tabulated in Table 1, yield excellent fits to the FA-free Hg adsorption data as a function of pH and Hg loading (e.g., Figure 4). The calculated stability constants for each reaction for each bacterial species studied here are similar to each other. The log stability constant
values for Reaction (3) range from 7.3 for \textit{B. subtilis} to 7.8 for \textit{G. sulfurreducens}; those for Reaction (4) range from 11.2 for \textit{S. oneidensis} MR-1 to 11.6 for both \textit{B. subtilis} and \textit{G. sulfurreducens}; and those for Reaction (5) range from 15.6 for \textit{S. oneidensis} MR-1 to 16.5 for \textit{G. sulfurreducens}. The fact that the stability constant values increase by four-to-five orders of magnitude from one site to the next likely is due to the simplified nature of the adsorption model. We assume that Hg$^{2+}$ is the adsorbing aqueous Hg species under all pH conditions. However, Hg(OH)$_2$ is the dominant aqueous Hg species under the experimental conditions, and the concentration of Hg$^{2+}$ is small and becomes smaller with increasing pH over the pH range of our experiments. Therefore, because the extent of adsorption is relatively pH independent, the stability constants that describe adsorption of Hg$^{2+}$ onto bacterial binding sites must become larger with each site considered.

Site-specific Hg binding constants have not been determined for Suwanee River FA, so we could not compare the measured effects of the presence of FA with those we would predict from speciation calculations. However, we used the measured extents of Hg adsorption in the presence of FA to calculate empirical generic site Hg binding constants for the FA. That is, we modeled the Hg binding onto the FA with the following single site reaction:

\begin{equation}
\text{FA}^- + \text{Hg}^{2+} \rightleftharpoons \text{FA-Hg}^+
\end{equation}

where FA$^-$ represents the generic deprotonated site on the FA molecule. We modeled this site as a hybrid of the 4 sites used by Borrok and Fein (2004) to account for FA protonation behavior, with the pKa value of the hybrid FA site equal to the average of the pKa values used by Borrok and Fein (2004) and the site concentration equal to the average of the total of the 4 sites for all 9 FAs modeled by Borrok and Fein (2004). Clearly, modeling Hg$^{2+}$ adsorption onto this hybrid generic FA binding site is a simplification of the complex binding environment of Hg on the FA molecule,
but it allows us to quantify the competition between the FA and the bacterial cell envelope, and to
calculate quantitative estimates of the effects of each binding environment in more complex
settings.

The calculated stability constants, tabulated in Table 1, yield an excellent fit to the observed
effects of the presence of FA on Hg adsorption onto the bacteria studied here (e.g., Figure 4). The
stability constants that we calculated for the three bacterial species are similar to each other and
do not vary systematically between bacterial species. Additionally, the 25 mg L\(^{-1}\) FA data yield
calculated Hg-FA stability constant values that are not significantly different from those calculated
using the 50 mg L\(^{-1}\) FA data. The calculated stability constant values do change systematically
with pH, with values increasing with increasing pH. This trend is likely a result of the
oversimplification of our Hg-FA binding model; it is probable that the FA molecule contains
multiple functional group types that deprotonate sequentially with increasing pH, not just the one
site type that we assumed in our models. However, the calculated log stability constant values are
not strongly dependent upon pH, with the largest spread being from 13.4 to 14.9 for the pH 4 to 8,
25 mg L\(^{-1}\) FA data for *B. subtilis*. Thus, the values in Table 1 can be used to yield reasonable
estimates of the competition between bacteria and FA in the pH and FA:bacteria concentration
ratio conditions studied here.

The calculated K values can be used to illustrate the direct competition between bacteria
and FA for available aqueous Hg\(^{2+}\). For example, the competition reaction between bacterial site
A\(_2\) and the FA binding site can be expressed as:

\[
R-A_2-Hg^+ + FA^- \leftrightarrow FA-Hg^+ + R-A_2^- \tag{7}
\]

where the log equilibrium constant for Reaction (7) can be calculated as the log K value for
Reaction (6) minus the log K value for Reaction (4), or values of 2.4 for *B. subtilis*, 3.0 for *S.*
oneidensis MR-1, and 2.6 for G. sulfurreducens under pH 6 conditions with 50 mg L⁻¹ FA, 0.2 g L⁻¹ bacteria. These calculated equilibrium constant values for Reaction (7) can be used to quantify the distribution of Hg between bacterial and FA binding sites for conditions with different relative concentrations of each site type, and the large positive values suggest that on a mass normalized basis, bacterial binding of Hg is greater than that exhibited by FA. Although both bacteria and FA contain sulphydryl binding sites that are especially effective at binding Hg, our results suggest that these sites may exhibit a higher density on bacteria than they do on FA. It is likely that the FA contains binding sites with a range of Hg binding constants. The binding constants determined by the simplified thermodynamic modeling described above represents an averaging of several site types, some of which have larger binding constants than do the binding sites on the cell envelopes, accompanied by a large number of site with binding constants that are smaller than those on the cell envelopes. The resulting averaged Hg binding constant that we calculate for the FA is similar to those that we calculate for the bacterial biomass. These general conclusions about the S binding mechanisms on the FA and on the bacteria are supported by our S XANES data, which demonstrate that the FA contains a wide range of S moieties while the bacterial biomass is dominated by a single thiol-type S moiety.

Conclusions

The results from this study show that the presence of FA decreases the extent of Hg adsorption onto three different bacterial species through competitive binding of the Hg. The presence of the FA does not change the binding environment of Hg on the bacteria, indicating a lack of ternary complexation between the Hg, the FA, and the bacteria. The binding of Hg to both the bacteria and the FA under the experimental conditions is dominated by sulphydryl binding to
both ligands, and the similarities between the binding environments likely results in the 
competitive balance between them. We use the experimental results to calibrate a quantitative 
semi-empirical model of the binding of Hg to bacteria and FA, and the stability constants that we 
calculate can be used to estimate the distribution and speciation of Hg in bacteria- and FA-bearing 
geologic systems. Because accessibility of Hg to bacteria for metabolic processes such as 
methylation may be controlled by adsorption, the stability constants calculated in this study may 
also be useful in estimating the bioavailability of Hg in soil and groundwater systems that contain 
significant concentrations of FA.

Acknowledgements

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National Laboratory. BM was supported by the Argonne Subsurface Scientific Focus Area project, 
which is part of the SBR Program of the Office of Biological and Environmental Research (BER), 
U.S. DOE under contract DE-AC02-06CH11357.
Table 1: Calculated log stability constant values for Reactions (3) - (6).

<table>
<thead>
<tr>
<th>[FA] (mg L(^{-1}))</th>
<th>pH</th>
<th>Bacteria</th>
<th>Reaction (3)(^a)</th>
<th>Reaction (4)(^b)</th>
<th>Reaction (5)(^c)</th>
<th>Reaction (6)(^d)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td><strong>B. subtilis</strong></td>
<td>7.3 ± 0.1</td>
<td>11.6 ± 0.2</td>
<td>16.4 ± 0.1</td>
<td>13.4 ± 0.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>S. oneidensis MR-1</strong></td>
<td>7.6 ± 0.2</td>
<td>11.2 ± 0.1</td>
<td>15.6 ± 0.1</td>
<td>13.8 ± 0.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>G. sulfurreducens</strong></td>
<td>7.8 ± 0.2</td>
<td>11.6 ± 0.1</td>
<td>16.5 ± 0.1</td>
<td>13.8 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td><strong>B. subtilis</strong></td>
<td></td>
<td></td>
<td></td>
<td>14.3 ± 0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>S. oneidensis MR-1</strong></td>
<td></td>
<td></td>
<td></td>
<td>14.4 ± 0.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>G. sulfurreducens</strong></td>
<td></td>
<td></td>
<td></td>
<td>14.4 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td><strong>B. subtilis</strong></td>
<td></td>
<td></td>
<td></td>
<td>14.9 ± 0.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>S. oneidensis MR-1</strong></td>
<td></td>
<td></td>
<td></td>
<td>14.9 ± 0.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>G. sulfurreducens</strong></td>
<td></td>
<td></td>
<td></td>
<td>14.6 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td><strong>B. subtilis</strong></td>
<td></td>
<td></td>
<td></td>
<td>14.6 ± 0.3</td>
</tr>
</tbody>
</table>

Average value: 14.3 ± 0.2  14.1 ± 0.2

\(^a\) \(R \cdot A_1^- + Hg^{2+} \Leftrightarrow R \cdot A_1^- \cdot Hg^+\)
\(^b\) \(R \cdot A_2^- + Hg^{2+} \Leftrightarrow R \cdot A_2^- \cdot Hg^+\)
\(^c\) \(R \cdot A_3^- + Hg^{2+} \Leftrightarrow R \cdot A_3^- \cdot Hg^+\)
\(^d\) \(FA^- + Hg^{2+} \Leftrightarrow FA \cdot Hg^+\). Both columns present the calculated log stability constant values for the adsorption of Hg to deprotonated FA, as expressed in Reaction (6). The left column presents the values calculated from the 25 mg L\(^{-1}\) FA data, and the right column presents the values calculated from the 50 mg L\(^{-1}\) FA data.
Figure 1: Aqueous chemistry results for Hg isotherms in the absence and presence of FA at pH 4 (A, B, C), pH 6 (D, E, F), and pH 8 (G, H, I). Plots A, D, and G present the results for the FA-free controls, plots B, E, and H present the results for the 25 mg L\(^{-1}\) FA experiments, and plots C, F, and I present the results of the 50 mg L\(^{-1}\) FA experiments. *B. subtilis* is represented by the black-outlined, grey-filled squares, *S. oneidensis* MR-1 is represented by the solid black
diamonds, and *G. sulfurreducens* is represented by the hollow circles. The black line on each plot represents 100% Hg adsorption under each experimental condition.
Figure 2: Hg L\textsubscript{III} edge XANES spectra of Hg bound to (from top to bottom) \textit{S. oneidensis} MR-1 only at pH 4, FA only at pH 4, \textit{S. oneidensis} MR-1 in the presence of 50 and 0 mg L\textsuperscript{-1} FA at pH 4, \textit{S. oneidensis} MR-1 in the presence of 50 and 0 mg L\textsuperscript{-1} FA at pH 8, cysteine only, and acetate only.
Figure 3: Sulfur K edge XANES spectra for a) cysteine, dimethyl sulfoxide (DMSO), and sodium dodecyl sulfate (NaDS), and b) *S. oneidensis* MR-1 biomass and Suwannee River FA.
Figure 4: Representative model fits for *S. oneidensis* MR-1 at pH 6 under 0 mg L\(^{-1}\) FA (grey squares and grey curve) and 50 mg L\(^{-1}\) FA (solid black diamonds and black curve) conditions. The dotted line represents 100% Hg adsorption under each experimental condition.
**Supplemental Information**

Table S1: Hg reactions used in the speciation modeling.

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Log K</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{H}_2\text{O} - \text{H}^+ = \text{OH}^- )</td>
<td>-14.00 (^b)</td>
</tr>
<tr>
<td>( \text{H}_2\text{CO}_3^{0} - \text{H}^+ = \text{HCO}_3^- )</td>
<td>-6.355 (^a)</td>
</tr>
<tr>
<td>( \text{H}_2\text{CO}_3^{0} - 2\text{H}^+ = \text{CO}_3^{2-} )</td>
<td>-16.67 (^a)</td>
</tr>
<tr>
<td>( \text{H}_2\text{CO}_3^{0} - \text{H}_2\text{O} = \text{CO}_2^{0} )</td>
<td>2.770 (^b)</td>
</tr>
<tr>
<td>( \text{Na}^+ + \text{H}_2\text{CO}_3^{0} - 2\text{H}^+ = \text{NaCO}_3^- )</td>
<td>-15.41 (^b)</td>
</tr>
<tr>
<td>( \text{Na}^+ + \text{H}_2\text{CO}_3^{0} - \text{H}^+ = \text{NaHCO}_3^{0} )</td>
<td>-6.60 (^b)</td>
</tr>
<tr>
<td>( \text{Na}^+ + \text{H}_2\text{O} - \text{H}^+ = \text{NaOH}^0 )</td>
<td>-14.2 (^b)</td>
</tr>
<tr>
<td>( \text{Hg}^{2+} + \text{H}_2\text{O} - \text{H}^+ = \text{HgOH}^+ )</td>
<td>-3.40 (^a)</td>
</tr>
<tr>
<td>( \text{Hg}^{2+} + 2\text{H}_2\text{O} - 2\text{H}^+ = \text{Hg(OH)}_2^{0} )</td>
<td>-5.98 (^a)</td>
</tr>
<tr>
<td>( \text{Hg}^{2+} + 3\text{H}_2\text{O} - 3\text{H}^+ = \text{Hg(OH)}_3^- )</td>
<td>-21.1 (^a)</td>
</tr>
<tr>
<td>( 2\text{Hg}^{2+} + \text{H}_2\text{O} - \text{H}^+ = \text{Hg}_2\text{(OH)}_3^{3+} )</td>
<td>-3.30 (^b)</td>
</tr>
<tr>
<td>( 3\text{Hg}^{2+} + 3\text{H}_2\text{O} - 3\text{H}^+ = \text{Hg}_3\text{(OH)}_3^{3+} )</td>
<td>-6.40 (^b)</td>
</tr>
<tr>
<td>( \text{Hg}^{2+} + \text{H}_2\text{CO}_3^{0} - 2\text{H}^+ = \text{HgCO}_3^{0} )</td>
<td>-3.91 (^a)</td>
</tr>
<tr>
<td>( \text{Hg}^{2+} + \text{H}_2\text{CO}_3^{0} - \text{H}^+ = \text{HgHCO}_3^{+} )</td>
<td>0.42 (^a)</td>
</tr>
<tr>
<td>( \text{Hg}^{2+} + \text{H}_2\text{CO}_3^{0} + \text{H}_2\text{O} - 3\text{H}^+ = \text{Hg(OH)CO}_3^- )</td>
<td>-11.355 (^a)</td>
</tr>
<tr>
<td>( \text{Bi}^- + \text{H}^+ = \text{Bi-H}^0 )</td>
<td>3.30 (^c)</td>
</tr>
<tr>
<td>( \text{Bacillus subtilis} )</td>
<td></td>
</tr>
<tr>
<td>( \text{Shewanella oneidensis} )</td>
<td></td>
</tr>
<tr>
<td>( \text{Geobacter sulfurreducens} )</td>
<td>3.36 (^e)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Log K</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{B}_2^- + \text{H}^+ = \text{B}_2\text{-H}^0 )</td>
<td>4.80 (^c)</td>
</tr>
<tr>
<td>( \text{Bacillus subtilis} )</td>
<td></td>
</tr>
<tr>
<td>( \text{Shewanella oneidensis} )</td>
<td>4.80 (^d)</td>
</tr>
<tr>
<td>( \text{Geobacter sulfurreducens} )</td>
<td>4.81 (^e)</td>
</tr>
</tbody>
</table>

<table>
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<tr>
<th>Reaction</th>
<th>Log K</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{B}_3^- + \text{H}^+ = \text{B}_3\text{-H}^0 )</td>
<td>6.80 (^c)</td>
</tr>
<tr>
<td>( \text{Bacillus subtilis} )</td>
<td></td>
</tr>
<tr>
<td>( \text{Shewanella oneidensis} )</td>
<td>6.70 (^d)</td>
</tr>
<tr>
<td>( \text{Geobacter sulfurreducens} )</td>
<td>6.49 (^e)</td>
</tr>
<tr>
<td>( \text{FA}^- + \text{H}^+ = \text{FA-H}^0 )</td>
<td>5.85 (^f)</td>
</tr>
</tbody>
</table>

\(^a\) Powell et al., 2005.  
\(^b\) Martell and Smith, 2001.  
\(^c\) Fein et al., 2005  
\(^d\) Mishra et al., 2010  
\(^e\) Dunham-Cheatham et al.  
\(^f\) Calculated as the average of all reported pK\(_a\) values in Table 2 from Borrok and Fein (2004).  

Assumed total site concentration is the sum of the average site concentrations for the individual FA sites: 5.50 x 10\(^{-3}\) moles of sites per gram of humic substance.
Table S2: Best-fit values of Hg solution standards and Hg-biomass samples.

<table>
<thead>
<tr>
<th>Sample</th>
<th>path</th>
<th>N</th>
<th>R(Å)</th>
<th>$\sigma^2$ (10^{-3} Å²)</th>
<th>$E_0$</th>
<th>$\chi^2$</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>HgAc</td>
<td>Hg-O</td>
<td>1.78 ± 0.32</td>
<td>2.06 ± 0.01</td>
<td>10.9 ± 0.9</td>
<td>1.2 ± 0.9</td>
<td>48</td>
<td>0.63</td>
</tr>
<tr>
<td></td>
<td>Hg-C</td>
<td>1.78\textsuperscript{a}</td>
<td>2.83 ± 0.01</td>
<td>12.8 ± 4.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hg-cysteine</td>
<td>Hg-S</td>
<td>1.88 ± 0.21</td>
<td>2.32 ± 0.01</td>
<td>10.5 ± 1.2</td>
<td>2.8 ± 0.6</td>
<td>22</td>
<td>0.45</td>
</tr>
<tr>
<td>Hg-biomass (at pH 4)</td>
<td>Hg-S</td>
<td>1.85 ± 0.19</td>
<td>2.35 ± 0.01</td>
<td>10.2 ± 1.5</td>
<td>3.0 ± 0.5</td>
<td>30</td>
<td>0.55</td>
</tr>
<tr>
<td>Hg-biomass (at pH 8)</td>
<td>Hg-S</td>
<td>1.70 ± 0.15</td>
<td>2.35 ± 0.01</td>
<td>11.0 ± 1.3</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{a} Set to Coordination number of O for this sample.
Figure S1: $k^2 \chi(k)$ EXAFS data for Hg L$_{\text{III}}$ edge EXAFS spectra of Hg bound to (top to bottom): 

- *S. oneidensis* MR-1 only at pH 4, FA only at pH 4, *S. oneidensis* MR-1 in the presence of 50 and 0 mg L$^{-1}$ FA at pH 4, 
- *S. oneidensis* MR-1 in the presence of 50 and 0 mg L$^{-1}$ FA at pH 8, 
- cysteine only, and 
- acetate only.
Figure S2: Magnitude of Hg L\textsubscript{III} edge EXAFS Fourier Transform (FT) data for Hg binding to \textit{S. oneidensis} MR-1 in the presence of 0 and 50 mg L\textsuperscript{-1} FA at pH 4 (top), and 0 and 50 mg L\textsuperscript{-1} FA at pH 8 (bottom).
References


Westall J. C. (1982) FITEQL, A computer program for determination of chemical equilibrium constants from experimental data. Version 2.0. Report 82-02, Department of Chemistry, Oregon State University, Corvallis, OR, USA.


