



Deposited via The University of Leeds.

White Rose Research Online URL for this paper:

<https://eprints.whiterose.ac.uk/id/eprint/127218/>

Version: Accepted Version

---

**Article:**

Dunham-Cheatham, S, Mishra, B, Myneni, S et al. (2015) The effect of natural organic matter on the adsorption of mercury to bacterial cells. *Geochimica et Cosmochimica Acta*, 150. pp. 1-10. ISSN: 0016-7037

<https://doi.org/10.1016/j.gca.2014.11.018>

---

© 2015, Elsevier Ltd. Licensed under the Creative Commons Attribution NonCommercial-NoDerivatives 4.0 International <http://creativecommons.org/licenses/by-nc-nd/4.0/>

**Reuse**

This article is distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs (CC BY-NC-ND) licence. This licence only allows you to download this work and share it with others as long as you credit the authors, but you can't change the article in any way or use it commercially. More information and the full terms of the licence here: <https://creativecommons.org/licenses/>

**Takedown**

If you consider content in White Rose Research Online to be in breach of UK law, please notify us by emailing [eprints@whiterose.ac.uk](mailto:eprints@whiterose.ac.uk) including the URL of the record and the reason for the withdrawal request.

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

Sarrah Dunham-Cheatham<sup>1‡</sup>, Bhoopesh Mishra<sup>2</sup>, Satish Myneni<sup>3</sup>, and Jeremy B. Fein<sup>1</sup>

<sup>1</sup> *Department of Civil and Environmental Engineering and Earth Sciences, University of Notre Dame, Notre Dame, IN 46556, USA*

<sup>2</sup> *Department of Physics, Illinois Institute of Technology, Chicago, Illinois 60616, USA*

<sup>3</sup> *Department of Geosciences, Princeton University, Princeton, NJ 08544, USA*

## **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

‡ Corresponding author: dr.smdcheatham@gmail.com

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

33

34

### **Introduction**

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

44 Natural organic matter (NOM) is present in nearly every near-surface geologic system, and  
45 complexation reactions between metals and NOM can dramatically change the behavior of the  
46 metals in the environment (McDowell, 2003; Ravichandran, 2004). NOM molecules contain a  
47 range of functional group types, including carboxyl, phenol, amino, and sulfhydryl groups, that  
48 have the potential to create highly stable complexes with metal ions across the pH range (Ephraim,  
49 1992; Ravichandran et al., 1999; Drexel et al., 2002; Haitzer et al., 2002; Croué et al., 2003;  
50 Ravichandran, 2004). Hg binds strongly to the sulfhydryl groups present within the NOM structure  
51 (Dong et al., 2011; Muresan et al., 2011). The relative thermodynamic stabilities of Hg-NOM and  
52 Hg-bacteria complexes are not well known. Depending on these relative stabilities, the formation

53 of metal-NOM complexes may decrease adsorption of Hg to bacteria cell envelopes due to a  
54 competitive ligand effect, or under certain conditions may increase adsorption of Hg to bacteria  
55 due to ternary complexation with NOM. For example, investigating Pb, Cu, and Ni separately,  
56 Borrok et al. (2007) found that ternary metal-FA-bacteria complexes form, and that the importance  
57 of the complexes is strongly affected by pH. Conversely, Wightman and Fein (2001) found that  
58 the presence of NOM decreases the amount of Cd adsorbed to bacteria under mid- and high-pH  
59 conditions, and that the presence of Cd does not affect the adsorption of NOM to bacteria,  
60 suggesting that ternary complexes do not occur. No studies have been conducted to date to  
61 determine the effects of NOM on Hg binding to bacteria. However, because Hg forms strong  
62 complexes both with cell envelopes (Daughney et al., 2002; Mishra et al., 2011; Dunham-  
63 Cheatham et al.) and NOM (Loux et al., 1998; Ravichandran, 2004; Skyllberg et al., 2006), it is  
64 likely that significant changes to Hg adsorption behavior occur in the presence of NOM.

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

76

77

## Methods

### 78 **Experimental methods**

#### 79 *Bacterial growth and washing procedure*

80 *Bacillus subtilis* (a Gram-positive aerobic soil species) and *Shewanella oneidensis* MR-1  
81 (a Gram-negative facultative anaerobic species) cells were cultured and prepared following the  
82 procedures outlined in Borrok et al. (2007). Briefly, cells were maintained on agar plates consisting  
83 of trypticase soy agar with 0.5% yeast extract added. Cells for all experiments were grown by first  
84 inoculating a test-tube containing 3 mL of trypticase soy broth with 0.5% yeast extract, and  
85 incubating it for 24 h at 32 °C. The 3 ml bacterial suspension was then transferred to 1 L of  
86 trypticase soy broth with 0.5% yeast extract for another 24 h on an incubator shaker table at 32 °C.  
87 Cells were pelleted by centrifugation at 8100 g for 5 min, and rinsed 5 times with 0.1 M NaClO<sub>4</sub>.

88 *Geobacter sulfurreducens* (a Gram-negative species capable of Hg methylation) cells were  
89 cultured and prepared using a different procedure than detailed above. Cells were maintained in  
90 50 mL of anaerobic freshwater basal media (ATCC 51573) at 32 °C (Lovely and Phillips, 1988).  
91 Cells for all experiments were grown by first inoculating an anaerobic serum bottle containing 50  
92 mL of freshwater basal media, and incubating it for 5 days at 32 °C. Cells were pelleted by  
93 centrifugation at 8100 g for 5 minutes, and rinsed 5 times with 0.1 M NaClO<sub>4</sub> stripped of dissolved  
94 oxygen by bubbling a 85%/5%/10% N<sub>2</sub>/H<sub>2</sub>/CO<sub>2</sub> gas mixture through it for 30 minutes. After  
95 washing, each of the three types of bacteria was then pelleted by centrifugation at 8100 g for 60  
96 minutes to remove excess water in order to determine the wet mass so that suspensions of known  
97 bacterial concentration could be created. All bacterial concentrations in this study are given in

98 terms of gm wet biomass per liter. Bacterial cells were harvested during stationary phase, and all  
99 experiments were performed under non-metabolizing, electron donor-free conditions.

100

#### 101 *Adsorption experiments*

102 To prepare experiments, aqueous Hg, NOM, and suspended bacteria stock solutions were  
103 mixed in different proportions to achieve the desired final concentrations for each experiment. The  
104 experiments were conducted in sets with constant pH (at pH  $4.0 \pm 0.1$ ,  $6.0 \pm 0.1$ , or  $8.0 \pm 0.3$ ) and  
105 constant bacterial concentration ( $0.2 \text{ g bacteria L}^{-1}$  in all cases) at three different FA concentrations  
106 (0, 25, or  $50 \text{ mg L}^{-1}$ ), with Hg log molalities ranging from -6.30 to -5.00 ( $0.1$  to  $2.0 \text{ mg L}^{-1}$ ).

107 FA stock solutions were prepared in Teflon bottles by dissolving dried, powdered  
108 International Humic Substances Society Suwannee River FA Standard I in a  $0.1 \text{ M NaClO}_4$  buffer  
109 solution to achieve the desired final FA concentration for each experiment. A known mass of wet  
110 biomass was then suspended in the FA stock solution, and the pH of the FA-bacteria parent  
111 solution was immediately adjusted to the experimental pH using  $0.2 \text{ M HNO}_3$  and/or NaOH. To  
112 prepare experimental solutions, aliquots of the FA-bacteria parent solution were added  
113 gravimetrically to Teflon reaction vessels, followed by a small aliquot of commercially-supplied  
114  $1,000 \text{ mg L}^{-1}$  Hg aqueous standard to achieve the desired final Hg concentration. The pH of each  
115 suspension was again adjusted immediately to the experimental pH. The vessels were placed on  
116 an end-over-end rotator to agitate the suspensions for the duration of the experiment (2 h for *B.*  
117 *subtilis* and *G. sulfurreducens* and 3 h for *S. oneidensis* MR-1, as determined by initial kinetics  
118 experiments (results not shown)). The pH of the suspensions was monitored and adjusted every 15  
119 minutes throughout the duration of the experiment, except during the last 30 minutes, when the  
120 suspensions were undisturbed. At the completion of each experiment, the pH of the suspensions

121 was measured and the experimental suspensions were centrifuged at 8100 g for 5 minutes. The  
122 aqueous phase was collected for Hg analysis by inductively-coupled plasma optical emission  
123 spectroscopy (ICP-OES), and the solid phase of some of the runs was collected for XAS analyses.  
124 Duplicate experiments were performed for each experimental condition.

125

#### 126 *ICP-OES measurements*

127 ICP-OES standards were prepared gravimetrically by diluting a commercially-supplied  
128 1,000 mg L<sup>-1</sup> Hg aqueous standard with pH-adjusted 0, 25, or 50 mg L<sup>-1</sup> FA stock solution made  
129 in 0.1 M NaClO<sub>4</sub> so that the pH, ionic strength, and FA concentration of the standards closely  
130 matched that of the samples. We found significant interference when standards and samples were  
131 not closely matched in this way. The log molality of the Hg standards ranged from -6.60 to -5.00.  
132 The standards and samples were all stored in Teflon containers and analyzed with a Perkin Elmer  
133 2000DV ICP-OES at wavelength 253.652 nm within 1 day of collection. The set of standards was  
134 analyzed before and after all of the samples were analyzed, as well as after every 15 samples, to  
135 check for machine drift. Analytical uncertainty, as determined by repeat analyses of the standards,  
136 was ± 2.8% for the 0 mg L<sup>-1</sup> FA samples, ± 7.7% for the 25 mg L<sup>-1</sup> FA samples, and ± 9.5% for  
137 the 50 mg L<sup>-1</sup> FA samples. Neither standards nor samples were acidified prior to analysis. FA  
138 concentration strongly affected system performance and signal strength, likely due to spectral  
139 interferences caused by the FA molecule. For each pH and FA concentration condition studied, we  
140 conducted biomass-free control experiments to determine the extent of Hg loss due to adsorption  
141 onto the experimental apparatus as well as any interferences caused by the presence of FA during  
142 the ICP-OES analysis.

143

144 *XAS measurements*

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

152 Crystalline powder standards (cinnabar [red HgS] and mercuric acetate) were measured  
153 and used to calibrate the theoretical calculations against experimental data. Data from the standards  
154 were analyzed to obtain the S<sub>0</sub><sup>2</sup> parameter, where S<sub>0</sub><sup>2</sup> is the value of the passive electron reduction  
155 factor used to account for many-body effects in EXAFS. By fixing the values of S and O atoms to  
156 2 in cinnabar and mercuric acetate, we obtained S<sub>0</sub><sup>2</sup> values of 1.02 ± 0.05 and 0.98 ± 0.03,  
157 respectively. Hence, we chose to set the value of S<sub>0</sub><sup>2</sup> = 1.0 for all samples. Fitting of the powder  
158 standards to their known crystallographic structures (cinnabar and mercuric acetate) reproduced  
159 the spectral features in the entire fitting range (1.0–4.2 Å), and fitting parameters were in  
160 agreement with previously reported values. Only the paths necessary to model the solid standards  
161 were used for fitting the solution standards and the unknown Hg samples.

162 Two Hg species, Hg-cysteine and Hg-acetate, were utilized as solution-phase standards for  
163 Hg XAS analyses. First, an aqueous Hg<sup>2+</sup> standard was prepared from high-purity 5 mM Hg<sup>2+</sup> in  
164 5% HNO<sub>3</sub> and was then adjusted to pH 2.0 ± 0.1 for measurement by adding appropriate amounts  
165 of 5 M NaOH. A Hg-cysteine standard was prepared by adding cysteine to the aqueous Hg<sup>2+</sup>  
166 standard to achieve a Hg:ligand ratio of 1:100. The pH of the Hg-cysteine standard was adjusted

167 to  $5.0 \pm 0.1$  by adding appropriate amounts of 1 M or 5 M NaOH. A Hg-acetate standard was  
168 prepared by adding mercuric acetate salt to milliQ water and adjusting the pH to  $5.0 \pm 0.1$  by  
169 adding appropriate amounts of 1 M or 5 M NaOH.

170 Sulfur K-edge XANES spectra for biomass and FA samples were acquired at sector 9-BM  
171 of the Advanced Photon Source at Argonne National Laboratory using Lytle detector in  
172 fluorescence detection mode. At 9-BM, signal from higher order harmonics was removed by  
173 detuning the monochromator to 70% of maximum beam flux at 2472.0 eV. Energy calibration was  
174 performed by setting the first peak in the spectrum of sodium thiosulfate salt ( $\text{Na}_2\text{S}_2\text{O}_3$ ) to 2469.2  
175 eV. XANES spectra were measured between 2450 and 2500 eV. Step sizes in the near-edge region  
176 (2467-2482 eV) were 0.08 eV, and 0.2 eV in pre- and post- edge regions, respectively. Samples  
177 were smeared on carbon tape and the data were collected under a He atmosphere.

178 For this study, sulfur species are divided into three main categories and referred to as  
179 reduced S (below 2472 eV), sulfoxide S (near 2473.5 eV), and oxidized S (above 2476.5 eV).  
180 Accordingly, three commercially-supplied (Sigma Aldrich) S standards, cysteine, dimethyl  
181 sulfoxide (DMSO), and sodium dodecyl sulfate (NaDS), were used to fingerprint S speciation. S  
182 standards were mixed with a dry powder of polyacrylic acid (PAA) to achieve a mixture containing  
183 ~1% total S by mass. To perform S XANES measurements, a thin layer of a PAA-S standard  
184 mixture was smeared on a carbon tape. All standards were prepared within 12 hours of analysis.

185 To prepare Hg XAS samples, FA was reacted with Hg by diluting a commercially-supplied  
186  $1000 \text{ mg L}^{-1}$  Hg standard with a pH-adjusted  $50 \text{ mg L}^{-1}$  FA stock solution prepared in 0.1 M  
187  $\text{NaClO}_4$ . The log molalities of Hg investigated were -4.30 and -3.60 at both pH  $4.00 \pm 0.10$  and  
188  $8.00 \pm 0.10$  for each Hg concentration. *S. oneidensis* MR-1 biomass was also reacted with Hg in  
189 the presence and absence of FA to ascertain possible effects of FA on the Hg binding environment

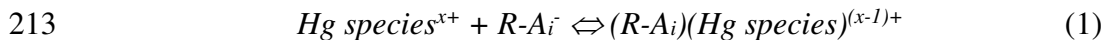
190 on the bacterial cell envelopes. Biomass was collected from the experiments with  $-5.30 \log M \text{ Hg}$ ,  
191 pH values  $4.00 \pm 0.10$  or  $8.00 \pm 0.10$ , and  $50 \text{ mg L}^{-1}$  FA. Samples were loaded into slotted Plexiglas  
192 holders that were subsequently covered with Kapton tape with a Kapton film sandwiched in  
193 between the tape and plexiglass to avoid direct contact of the sample with the tape adhesive.  
194 Samples were refrigerated until data collection. All measurements were conducted within 48 hours  
195 of sample preparation.

196 The data were analyzed by using the methods described in the UWXAFS package (Stern  
197 et al., 1995). Energy calibration between different scans was maintained by measuring Hg/Sn  
198 amalgam on the reference chamber concurrently with the fluorescence measurements of the  
199 biomass-bound Hg samples (Harris et al., 2003). The inflection point of the Hg  $L_{III}$ -edge (12.284  
200 KeV) was used for calibration. Data processing and fitting were done with the ATHENA and  
201 ARTEMIS programs (Ravel and Newville, 2005). The data range used for Fourier transformation  
202 of the  $k$ -space data was  $2.0\text{--}9.5 \text{ \AA}^{-1}$ . The Hanning window function was used with  $dk = 1.0 \text{ \AA}^{-1}$ .  
203 Fitting of each spectrum was performed in  $r$ -space, at  $1.2\text{--}3.2 \text{ \AA}$ , with multiple  $k$ -weighting ( $k^1$ ,  $k^2$ ,  
204  $k^3$ ) unless otherwise stated. Lower  $\chi^2$  (reduced chi square) was used as the criterion for inclusion  
205 of an additional shell in the shell-by-shell EXAFS fitting procedure.

206

### 207 **Thermodynamic modeling**

208 Surface-complexation models were constructed to model Hg binding with bacterial cell  
209 envelope functional groups and with those on the FA molecules, and to quantify the competition  
210 between the two. Observed adsorption reactions between aqueous Hg species and deprotonated  
211 bacterial cell envelope sites and/or FA binding sites were modeled according to the following  
212 generic reaction:



214 where ‘*Hg species*<sup>x+</sup>’ represents the specific aqueous Hg species considered, ‘*R-A<sub>i</sub>*<sup>-</sup>’ represents the  
 215 deprotonated cell or FA binding site, ‘*(R-A<sub>i</sub>)(Hg species)*<sup>(x-1)+</sup>’ represents the Hg-bacterial cell envelope  
 216 or Hg-FA complex, and the ‘*x*’ represents the charge of the aqueous Hg species. Stability constants  
 217 for each of the Hg-bacterial cell envelope and Hg-FA complexes are expressed as the  
 218 corresponding mass action equation for Reaction (1):

219 
$$K_{ads} = \frac{[(R - A_i)(\text{Hg species})^{(x-1)+}]}{a (\text{Hg species}^{x+}) [R - A_i^-]} \quad (2)$$

220 where *K<sub>ads</sub>* is the thermodynamic equilibrium constant for Reaction (1), the square brackets  
 221 represent concentrations in mol L<sup>-1</sup>, and *a* represents the activity of the species in parentheses.

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

236 sites, using the average values from Borrok and Fein (2004) as a model of the FA site speciation.  
237 The calculated acidity constant and site concentration for this generic site are listed in Table S1.

238

## 239 Results

### 240 *Adsorption experiments*

241 Consistent with previous studies of Hg adsorption onto bacteria (Daughney et al., 2002;  
242 Dunham-Cheatham et al.), we observed extensive adsorption of Hg onto the bacterial species  
243 studied in the absence of FA, with the extent of adsorption relatively independent of pH between  
244 pH 4 and 8 (Figure 1, top plots). For example, approximately 77% of the Hg in a 2 mg L<sup>-1</sup> Hg  
245 solution adsorbs at pH 4 onto 0.2 g L<sup>-1</sup> *S. oneidensis* MR-1, while approximately 75% adsorbs at  
246 pH 8. The presence of FA decreases the amount of Hg adsorbing to cell envelopes of each of the  
247 bacterial species and at each of the pH conditions studied (Figure 1, middle and bottom plots).  
248 With 50 mg L<sup>-1</sup> FA, the extent of adsorption at pH 4 decreases to 65%, and at pH 8 to 50%. Our  
249 experimental results also indicate that the three bacterial species studied here exhibit similar  
250 extents of Hg adsorption under each experimental condition, consistent with the observations from  
251 a number of previous studies (e.g. Cox et al., 1999; Yee and Fein, 2001; Borrok et al., 2005,  
252 Johnson et al., 2007). Our data suggest that as the concentration of FA increases, so does the  
253 amount of Hg remaining in solution. These results indicate that FA competes with the bacterial  
254 cells for the adsorption of Hg, and that the adsorption of Hg to FA results in a competitive ligand  
255 effect. As a result, less Hg is available for adsorption to proton-active functional groups on the  
256 bacterial cell envelope, and less Hg is removed from solution. These results are not surprising, as  
257 FA molecules contain sulfhydryl groups within their structure and sulfhydryl groups bind strongly  
258 with Hg (Xia et al., 1999; Hesterberg et al., 2001; Drexel et al., 2002; Haitzer et al., 2002; 2003),  
259 leading to effective competition with bacterial cell envelopes which also contain proton-active

260 sulfhydryl functional groups (Guiné et al., 2006; Mishra et al., 2007; 2009; 2010; 2011; Pokrovsky  
261 et al., 2012; Song et al., 2012; Colombo et al., 2013). In our experimental systems, FA binding  
262 sites outnumber those present on the bacteria. For example, 50 mg L<sup>-1</sup> FA corresponds to  
263 approximately 2.8 x 10<sup>-4</sup> moles of sites L<sup>-1</sup> (Borrok and Fein, 2004), while 0.2 g L<sup>-1</sup> *B. subtilis*  
264 biomass contains 4.7 x 10<sup>-5</sup> total moles of sites L<sup>-1</sup>. At pH 8, 50 mg L<sup>-1</sup> FA does diminish the extent  
265 of Hg adsorption, but only from approximately 75% (with no FA present) to 50%. It appears that  
266 given equal site concentrations, bacterial binding of Hg would dominate the competition with FA.

267

#### 268 *Hg XANES and EXAFS*

269 To probe the effect of FA on Hg binding mechanisms with bacterial biomass, we  
270 examined Hg-biomass binding at pH 4 and 8 in the presence and absence of a stoichiometric  
271 excess of FA (1 mg L<sup>-1</sup> Hg and 50 mg L<sup>-1</sup> FA) using Hg L<sub>III</sub> edge XANES and EXAFS. For the  
272 XAS studies, *S. oneidensis* MR-1 was chosen to represent the bacterial species used in this study.  
273 Figure 2 shows a comparison between Hg XANES for Hg bound : 1) to FA and to *S. oneidensis*  
274 MR-1 biomass at pH 4, 2) to *S. oneidensis* MR-1 biomass in the presence and absence of FA at  
275 pH 4, 3) to *S. oneidensis* MR-1 biomass in the presence and absence of FA at pH 8, and 4) to  
276 cysteine, and to acetate. Hg XANES data indicate that Hg is complexed with thiol groups in the  
277 Hg-biomass samples. Spectral features supporting this conclusion are the small pre-edge peak  
278 and the slight dip at 12300 eV in the Hg-biomass XANES data similar to that present in the Hg-  
279 cysteine data. This finding is consistent with a previous study which showed Hg binding with  
280 sulfhydryl groups on *B. subtilis* cell envelopes under similar experimental conditions (Mishra et  
281 al., 2011). The Hg-FA XANES data exhibits a small pre-edge peak similar to that present in the  
282 Hg-cysteine data, which confirms that Hg is bound predominantly with the high-affinity thiol

283 groups in FA under the experimental conditions. The absence of the dip in the Hg-FA XANES at  
284 12300 eV, however, indicates differences in the coordination environment of Hg between FA  
285 and bacterial biomass. To understand these subtle differences in XANES, a linear combination  
286 fitting of the first derivative of Hg-FA XANES data was performed which resulted in about 90%  
287 contribution from Hg-cysteine binding and about 10% contribution from Hg-carboxyl binding  
288 (figure S2a). The first derivative of Hg-cysteine standard reproduced the Hg-biomass data  
289 confirming that the entire budget of Hg complexation with biomass was accounted by  
290 complexation of Hg with thiols. XANES spectra of Hg reacted with *S. oneidensis* MR-1 biomass  
291 in the presence and absence of FA at pH 4 and 8 are reproducible, confirming that the binding  
292 mechanism of Hg with *S. oneidensis* MR-1 biomass does not change appreciably in the presence  
293 of FA.

294 Hg EXAFS results are consistent with the Hg XANES results described above. Differences  
295 between the coordination environment of Hg-FA and Hg-biomass is more pronounced in the  $k^2\chi(k)$   
296 EXAFS data (Figure S1). Low signal to noise ratio in the aqueous Hg-FA data does not allow for  
297 a meaningful Fourier Transform (FT) of the Hg-FA EXAFS data. EXAFS  $k^2\chi(k)$  and FT data  
298 between FA-bearing and FA-free Hg-biomass samples are similar (Figure S1), validating the Hg  
299 XANES results. Figure S2b shows a comparison between the FT Hg EXAFS data for Hg bound  
300 to *S. oneidensis* MR-1 biomass in the presence and absence of FA at both pH 4 and 8 and their  
301 corresponding EXAFS fits. EXAFS fitting parameters are shown in Table S2. It is worth pointing  
302 out that although Hg-cystein standard showed a bond distance of 2.32 Å, Hg-biomass samples at  
303 pH 4 and 8 had a bond distance of 2.35 Å. This should not be considered a discrepancy because  
304 Hg-S distances can vary from 2.32 to 2.36 Å for Hg(SR)<sub>2</sub> complexes (Manceau, and Nagy 2008).  
305 Similarly, Hg-S distances can range from 2.40 to 2.51 Å for Hg(SR)<sub>3</sub> complexes, and 2.50–2.61 Å for  
306 Hg(SR)<sub>4</sub> complexes.

307  
308

309           Taken together, Hg XANES and EXAFS results indicate that Hg binds predominantly to  
310 the high-affinity thiol groups on bacterial cell envelopes in the presence and absence of FA and  
311 Hg binding mechanisms with bacterial biomass do not change in the presence of FA, excluding  
312 the possibility of the formation of a ternary complex. Additionally, Hg XAS results show that pH  
313 does not affect the adsorption mechanism of Hg onto biomass in the presence of FA, which is  
314 consistent with the similar extent of Hg adsorption as a function of pH described above. However,  
315 it is important to note that Hg binding mechanisms with bacterial biomass may be affected by FA  
316 at high Hg loadings, where Hg is primarily bound to biomass via lower-affinity carboxyl functional  
317 groups. Hg XAS results suggest that S functional groups on *S. oneidnensis* MR-1 cell envelopes  
318 outcompete S functional groups in FA for Hg binding. In other words, on average Hg binding to  
319 FA appears weaker than Hg binding to bacterial biomass. S XANES was conducted to identify the  
320 differences in complexation behavior of Hg with S functional groups on FA and bacterial biomass.

321           S K-edge XANES is highly sensitive to changes in the electronic environment of the sulfur  
322 absorber (Xia et al., 1998). Although S K-edge XANES spectra were collected on a large number  
323 of standards, in this study we have divided S species into three main categories: reduced S (below  
324 2472 eV), sulfoxide S (near 2473.5 eV), and oxidized S (above 2476.5 eV). The S XANES spectra  
325 for cysteine, dimethyl sulfoxide (DMSO), and sodium dodecyl sulfate (NaDS) are shown in Figure  
326 3a. Species with very different S oxidation states, such as cysteine, sulfoxide, and ester sulfate, are  
327 easily resolved in the XANES spectrum. However, resolving one species from another within these  
328 three energy ranges is challenging. Reduced S species, including thiols, sulfides, polysulfides, and  
329 thiophenes, all give white-line features occurring between 2469 and 2472 eV. More extensive  
330 model libraries that include XANES spectra of organic and inorganic S compounds are available

331 in the literature (Myneni, 2002; Vairavamurthy, 1998).

332 Speciation of S in *S. oneidensis* MR-1 biomass was easily identified because the peak  
333 energy position of S XANES measurement on the biomass overlapped with the cysteine peak  
334 position (Figure 3b). Figure 3b shows the dramatic differences between S XANES on *S. oneidensis*  
335 MR-1 cells and Suwanee River FA. S XANES comparing FA with *S. oneidensis* MR-1 shows that  
336 nearly the entire S budget of the biomass is present as thiol groups, which are known to form strong  
337 bonds with Hg. However, FA has a range of reduced S (including reactive thiol) groups and a large  
338 fraction of oxidized S species, consistent with previous observations (Morra et al., 1997). Morra  
339 et al., 1997 suggest that a significant fraction of sulfur in SR Fulvic acid is found in oxidized (+5  
340 oxidation state) form, followed by smaller fractions in reduced forms (-0.3 ±1.0 and 1.7 oxidations  
341 states) respectively. Similarly, Einsiedl et al., 2007 used S XANES to estimate that soil FAs  
342 contain around 51% oxidized (S<sub>+4</sub>,S<sub>+5</sub>,S<sub>+6</sub>) and 49% reduced (S<sub>-1</sub>,S<sub>0</sub>,S<sub>+2</sub>) sulfur species. The  
343 reduced S species was dominated by thiols, thiophene and disulfide. Such a dramatic difference  
344 between the S budget of FA and bacterial biomass could result in diverse reactivities and stabilities  
345 of Hg-S complexes between the two. A detailed study of the reactivity and stability of Hg with FA  
346 and bacterial biomass is beyond the scope of this study.

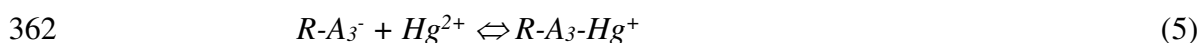
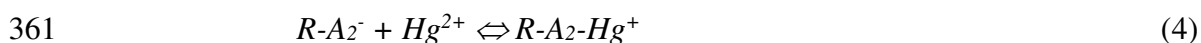
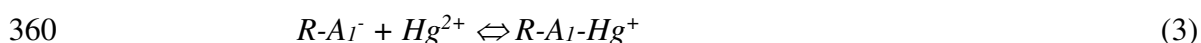
347

## 348 **Discussion**

349 The experimental results presented here suggest that bacterial cell envelope functional  
350 groups and FA functional groups exhibit different binding affinities for Hg under the experimental  
351 conditions. Hg binding onto the bacterial cell envelopes is extensive, and although Hg binds  
352 strongly with FA, especially with the sulfhydryl groups present within FA (Xia et al., 1999;  
353 Hesterberg et al., 2001; Drexel et al., 2002; Haitzer et al., 2002; 2003), the presence of even up to

354 50 ppm FA with only 0.2 g (wet mass) L<sup>-1</sup> of bacteria does not cause the speciation of Hg to be  
355 dominated by the FA. The results suggest there is a possibility for competition between the  
356 bacterial and FA binding sites for the available Hg.

357 In order to quantify the competitive binding, we use a semi-empirical surface complexation  
358 approach. First, we use the FA-free adsorption data at pH 4, 6, and 8 to solve for equilibrium  
359 constants for the following Hg<sup>2+</sup> adsorption reactions, respectively:



363 where  $R-A_1$ ,  $R-A_2$ , and  $R-A_3$  represent the bacterial functional groups with the three lowest pKa  
364 values, respectively. At pH 4, the  $R-A_1$  sites are the dominant deprotonated sites available for Hg<sup>2+</sup>  
365 binding for each bacterial species; at pH 6, both  $R-A_1$  and  $R-A_2$  sites are deprotonated; and at pH  
366 8,  $R-A_1$ ,  $R-A_2$ , and  $R-A_3$  sites likely contribute to the binding of Hg<sup>2+</sup>. Therefore, we used the pH 4  
367 data to constrain the stability constant value for Reaction (3) alone, then fixed that value and used  
368 the pH 6 data to solve for the stability constant value for Reaction (4) with a model that involved  
369 Reactions (3) and (4) simultaneously. We then used the values that we calculated for the stability  
370 constants for Reactions (3) and (4) and the pH 8 data to solve for the best-fitting value for Reaction  
371 (5) with a model that involved Reactions (3) - (5) simultaneously. This modeling approach assumes  
372 that Hg<sup>2+</sup> binding at a given pH occurs dominantly onto sites with pKa values lower than the pH  
373 of the experiments; that is, dominantly onto deprotonated sites. However, the resulting stability  
374 constant values, which are tabulated in Table 1, yield excellent fits to the FA-free Hg adsorption  
375 data as a function of pH and Hg loading (e.g., Figure 4). The calculated stability constants for each  
376 reaction for each bacterial species studied here are similar to each other. The log stability constant

377 values for Reaction (3) range from 7.3 for *B. subtilis* to 7.8 for *G. sulfurreducens*; those for  
378 Reaction (4) range from 11.2 for *S. oneidensis* MR-1 to 11.6 for both *B. subtilis* and *G.*  
379 *sulfurreducens*; and those for Reaction (5) range from 15.6 for *S. oneidensis* MR-1 to 16.5 for *G.*  
380 *sulfurreducens*. The fact that the stability constant values increase by four-to-five orders of  
381 magnitude from one site to the next likely is due to the simplified nature of the adsorption model.  
382 We assume that  $Hg^{2+}$  is the adsorbing aqueous Hg species under all pH conditions. However,  
383  $Hg(OH)_2$  is the dominant aqueous Hg species under the experimental conditions, and the  
384 concentration of  $Hg^{2+}$  is small and becomes smaller with increasing pH over the pH range of our  
385 experiments. Therefore, because the extent of adsorption is relatively pH independent, the stability  
386 constants that describe adsorption of  $Hg^{2+}$  onto bacterial binding sites must become larger with  
387 each site considered.

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

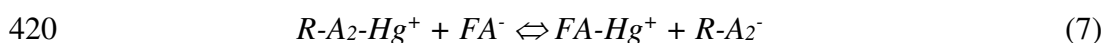


394 where  $FA^-$  represents the generic deprotonated site on the FA molecule. We modeled this site as a  
395 hybrid of the 4 sites used by Borrok and Fein (2004) to account for FA protonation behavior, with  
396 the pKa value of the hybrid FA site equal to the average of the pKa values used by Borrok and  
397 Fein (2004) and the site concentration equal to the average of the total of the 4 sites for all 9 FAs  
398 modeled by Borrok and Fein (2004). Clearly, modeling  $Hg^{2+}$  adsorption onto this hybrid generic  
399 FA binding site is a simplification of the complex binding environment of Hg on the FA molecule,

400 but it allows us to quantify the competition between the FA and the bacterial cell envelope, and to  
401 calculate quantitative estimates of the effects of each binding environment in more complex  
402 settings.

403 The calculated stability constants, tabulated in Table 1, yield an excellent fit to the observed  
404 effects of the presence of FA on Hg adsorption onto the bacteria studied here (e.g., Figure 4). The  
405 stability constants that we calculated for the three bacterial species are similar to each other and  
406 do not vary systematically between bacterial species. Additionally, the 25 mg L<sup>-1</sup> FA data yield  
407 calculated Hg-FA stability constant values that are not significantly different from those calculated  
408 using the 50 mg L<sup>-1</sup> FA data. The calculated stability constant values do change systematically  
409 with pH, with values increasing with increasing pH. This trend is likely a result of the  
410 oversimplification of our Hg-FA binding model; it is probable that the FA molecule contains  
411 multiple functional group types that deprotonate sequentially with increasing pH, not just the one  
412 site type that we assumed in our models. However, the calculated log stability constant values are  
413 not strongly dependent upon pH, with the largest spread being from 13.4 to 14.9 for the pH 4 to 8,  
414 25 mg L<sup>-1</sup> FA data for *B. subtilis*. Thus, the values in Table 1 can be used to yield reasonable  
415 estimates of the competition between bacteria and FA in the pH and FA:bacteria concentration  
416 ratio conditions studied here.

417 The calculated K values can be used to illustrate the direct competition between bacteria  
418 and FA for available aqueous Hg<sup>2+</sup>. For example, the competition reaction between bacterial site  
419 A<sub>2</sub> and the FA binding site can be expressed as:



421 where the log equilibrium constant for Reaction (7) can be calculated as the log K value for  
422 Reaction (6) minus the log K value for Reaction (4), or values of 2.4 for *B. subtilis*, 3.0 for *S.*

423 *oneidensis* MR-1, and 2.6 for *G. sulfurreducens* under pH 6 conditions with 50 mg L<sup>-1</sup> FA, 0.2 g  
424 L<sup>-1</sup> bacteria. These calculated equilibrium constant values for Reaction (7) can be used to quantify  
425 the distribution of Hg between bacterial and FA binding sites for conditions with different relative  
426 concentrations of each site type, and the large positive values suggest that on a mass normalized  
427 basis, bacterial binding of Hg is greater than that exhibited by FA. Although both bacteria and FA  
428 contain sulfhydryl binding sites that are especially effective at binding Hg, our results suggest that  
429 these sites may exhibit a higher density on bacteria than they do on FA. It is likely that the FA  
430 contains binding sites with a range of Hg binding constants. The binding constants determined by  
431 the simplified thermodynamic modeling described above represents an averaging of several site  
432 types, some of which have larger binding constants than do the binding sites on the cell envelopes,  
433 accompanied by a large number of site with binding constants that are smaller than those on the  
434 cell envelopes. The resulting averaged Hg binding constant that we calculate for the FA is similar  
435 to those that we calculate for the bacterial biomass. These general conclusions about the S binding  
436 mechanisms on the FA and on the bacteria are supported by our S XANES data, which demonstrate  
437 that the FA contains a wide range of S moieties while the bacterial biomass is dominated by a  
438 single thiol-type S moiety.

439

440

### **Conclusions**

441 The results from this study show that the presence of FA decreases the extent of Hg  
442 adsorption onto three different bacterial species through competitive binding of the Hg. The  
443 presence of the FA does not change the binding environment of Hg on the bacteria, indicating a  
444 lack of ternary complexation between the Hg, the FA, and the bacteria. The binding of Hg to both  
445 the bacteria and the FA under the experimental conditions is dominated by sulfhydryl binding to

446 both ligands, and the similarities between the binding environments likely results in the  
447 competitive balance between them. We use the experimental results to calibrate a quantitative  
448 semi-empirical model of the binding of Hg to bacteria and FA, and the stability constants that we  
449 calculate can be used to estimate the distribution and speciation of Hg in bacteria- and FA-bearing  
450 geologic systems. Because accessibility of Hg to bacteria for metabolic processes such as  
451 methylation may be controlled by adsorption, the stability constants calculated in this study may  
452 also be useful in estimating the bioavailability of Hg in soil and groundwater systems that contain  
453 significant concentrations of FA.

454

#### 455 **Acknowledgements**

456 Funding for this research was provided by a U.S. Department of Energy, Subsurface  
457 Biogeochemistry Research (SBR) grant. The experiments and analyses were performed at the  
458 Center for Environmental Science & Technology, University of Notre Dame. XAS measurements  
459 were obtained at the MRCAT-10-ID Beamline at the Advanced Photon Source (APS), Argonne  
460 National Laboratory. BM was supported by the Argonne Subsurface Scientific Focus Area project,  
461 which is part of the SBR Program of the Office of Biological and Environmental Research (BER),  
462 U.S. DOE under contract DE-AC02-06CH11357.

463

**Tables**

464 Table 1: Calculated log stability constant values for Reactions (3) - (6).

465

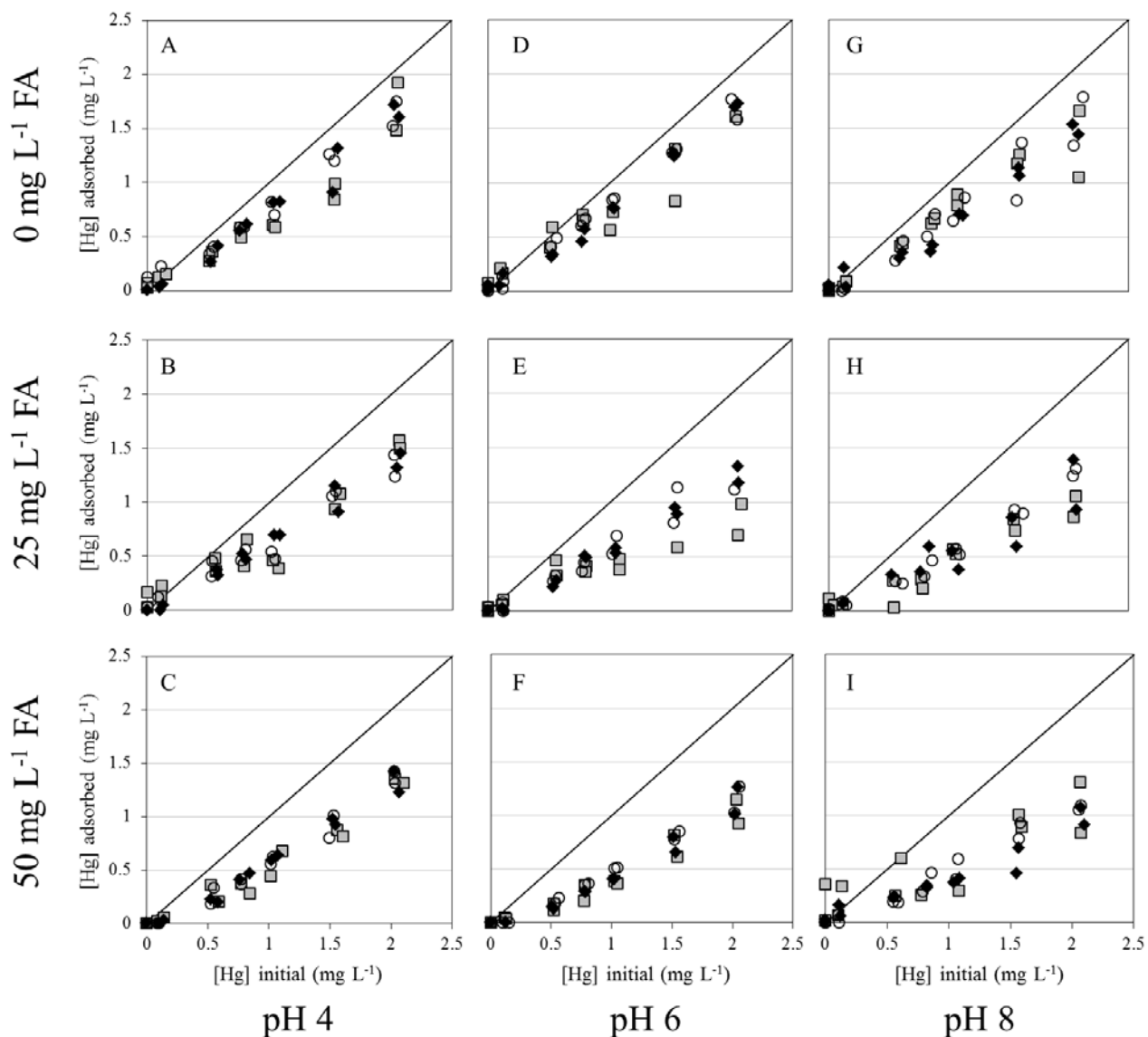
[FA] (mg L <sup>-1</sup> )	pH	Bacteria	Reaction (3) <sup>a</sup>	Reaction (4) <sup>b</sup>	Reaction (5) <sup>c</sup>	Reaction (6) <sup>d</sup>	
						25 mg L <sup>-1</sup> FA	50 mg L <sup>-1</sup> FA
0		<i>B. subtilis</i>	7.3 ± 0.1	11.6 ± 0.2	16.4 ± 0.1		
		<i>S. oneidensis</i> MR-1	7.6 ± 0.2	11.2 ± 0.1	15.6 ± 0.1		
		<i>G. sulfurreducens</i>	7.8 ± 0.2	11.6 ± 0.1	16.5 ± 0.1		
25, 50	4	<i>B. subtilis</i>				13.4 ± 0.2	13.4 ± 0.1
		<i>S. oneidensis</i> MR-1				13.8 ± 0.2	13.6 ± 0.3
		<i>G. sulfurreducens</i>				13.8 ± 0.1	13.6 ± 0.1
	6	<i>B. subtilis</i>				14.3 ± 0.1	14.0 ± 0.1
		<i>S. oneidensis</i> MR-1				14.4 ± 0.2	14.2 ± 0.3
		<i>G. sulfurreducens</i>				14.4 ± 0.2	14.2 ± 0.1
	8	<i>B. subtilis</i>				14.9 ± 0.2	14.4 ± 0.2
		<i>S. oneidensis</i> MR-1				14.9 ± 0.2	15.0 ± 0.4
		<i>G. sulfurreducens</i>				14.6 ± 0.3	14.6 ± 0.2
Average value:						14.3 ± 0.2	14.1 ± 0.2

466 <sup>a</sup>  $R-A_1^- + Hg^{2+} \Leftrightarrow R-A_1-Hg^+$ 467 <sup>b</sup>  $R-A_2^- + Hg^{2+} \Leftrightarrow R-A_2-Hg^+$ 468 <sup>c</sup>  $R-A_3^- + Hg^{2+} \Leftrightarrow R-A_3-Hg^+$ 

469 <sup>d</sup>  $FA^- + Hg^{2+} \Leftrightarrow FA-Hg^+$ . Both columns present the calculated log stability constant values for the adsorption of Hg to deprotonated FA, as expressed  
 470 in Reaction (6). The left column presents the values calculated from the 25 mg L<sup>-1</sup> FA data, and the right column presents the values calculated from  
 471 the 50 mg L<sup>-1</sup> FA data.

472  
473

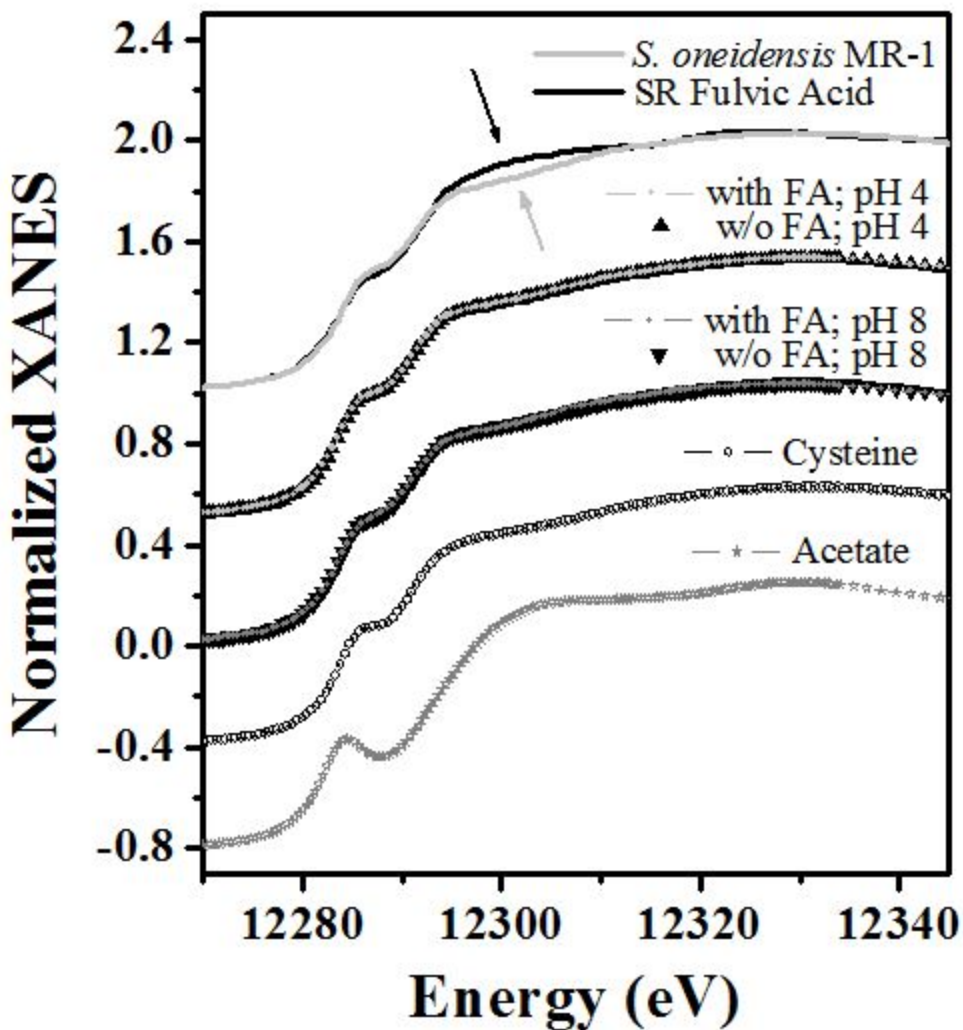
### Figures



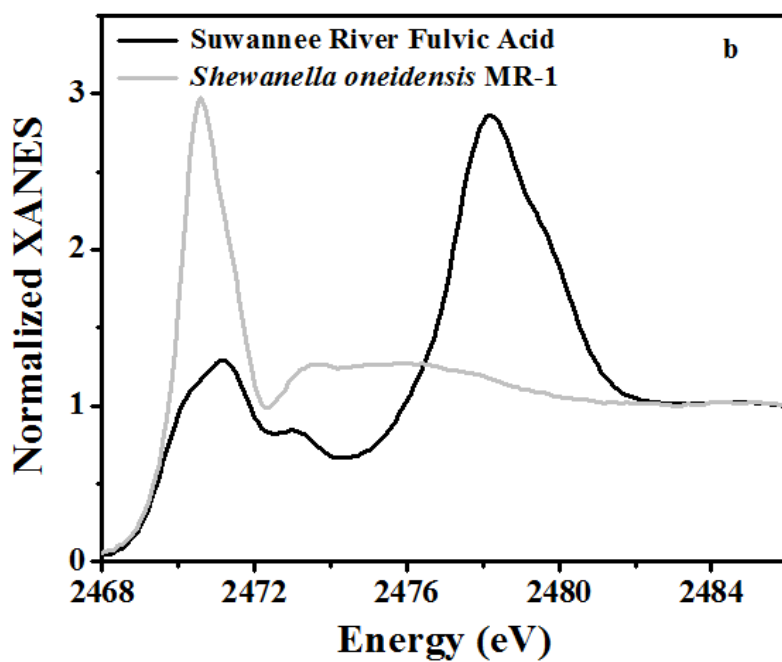
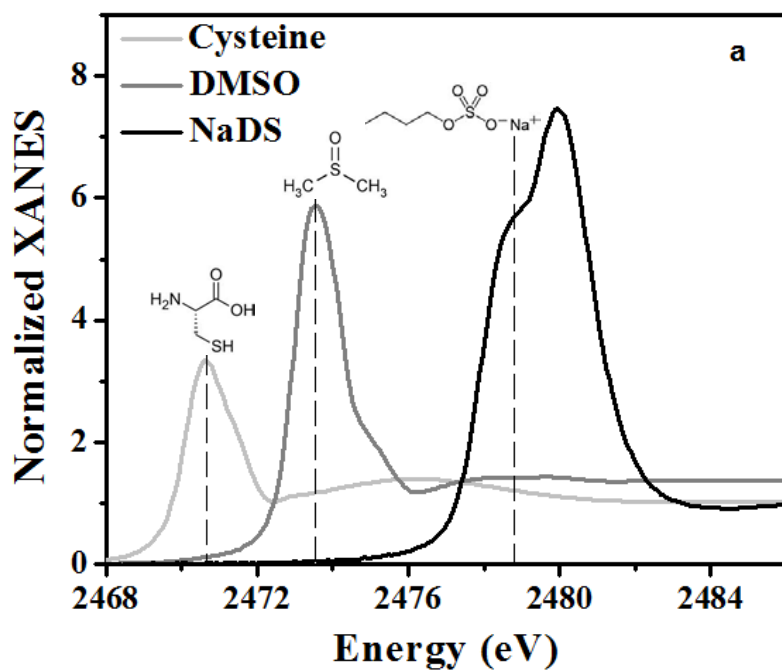
474

475 Figure 1: Aqueous chemistry results for Hg isotherms in the absence and presence of FA at pH 4  
476 (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-  
477 free controls, plots B, E, and H present the results for the 25 mg L<sup>-1</sup> FA experiments, and plots C,  
478 F, and I present the results of the 50 mg L<sup>-1</sup> FA experiments. *B. subtilis* is represented by the  
479 black-outlined, grey-filled squares, *S. oneidensis* MR-1 is represented by the solid black

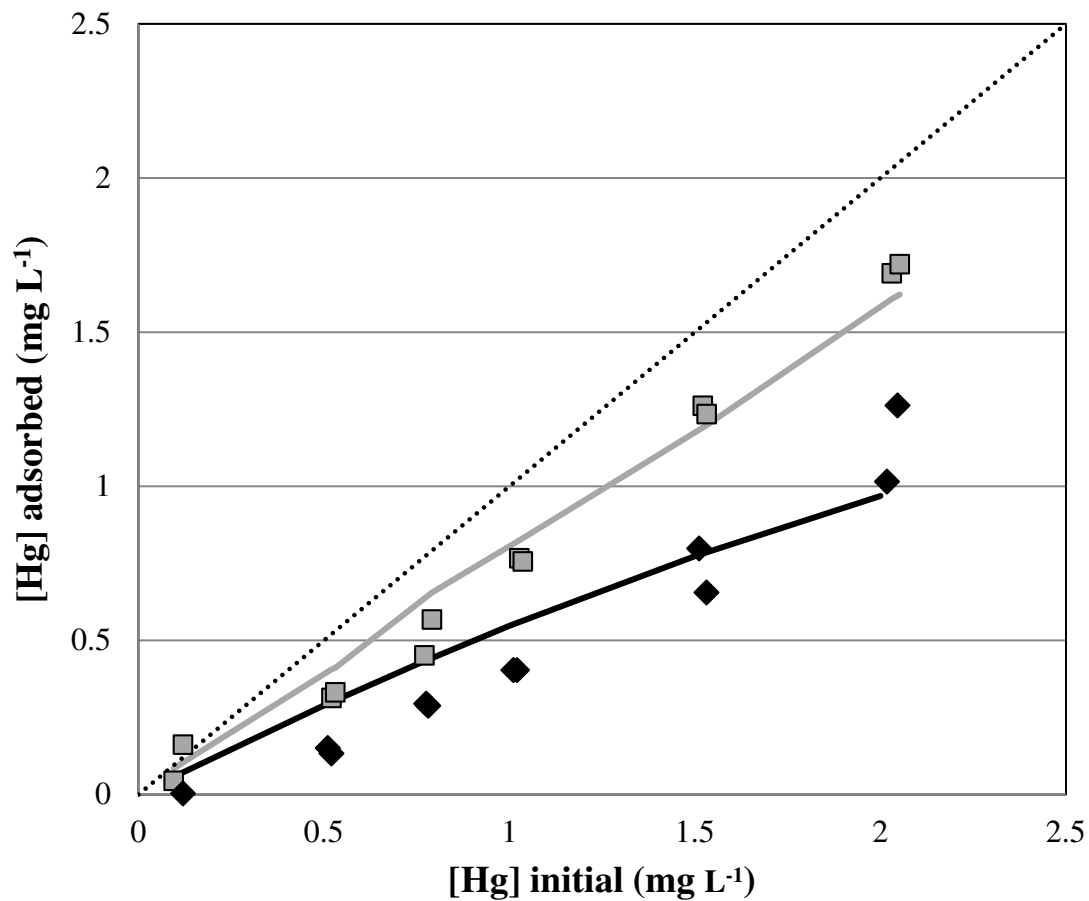
480 diamonds, and *G. sulfurreducens* is represented by the hollow circles. The black line on each plot  
481 represents 100% Hg adsorption under each experimental condition.



482  
 483 Figure 2: Hg L<sub>III</sub> edge XANES spectra of Hg bound to (from top to bottom) *S. oneidensis* MR-1  
 484 only at pH 4, FA only at pH 4, *S. oneidensis* MR-1 in the presence of 50 and 0 mg L<sup>-1</sup> FA at pH  
 485 4, *S. oneidensis* MR-1 in the presence of 50 and 0 mg L<sup>-1</sup> FA at pH 8, cysteine only, and acetate  
 486 only.  
 487



488  
 489 Figure 3: Sulfur K edge XANES spectra for a) cysteine, dimethyl sulfoxide (DMSO), and sodium  
 490 dodecyl sulfate (NaDS), and b) *S. oneidensis* MR-1 biomass and Suwannee River FA.



491

492 Figure 4: Representative model fits for *S. oneidensis* MR-1 at pH 6 under 0 mg L<sup>-1</sup> FA (grey  
 493 squares and grey curve) and 50 mg L<sup>-1</sup> FA (solid black diamonds and black curve) conditions.

494 The dotted line represents 100% Hg adsorption under each experimental condition.

495

497 Table S1: Hg reactions used in the speciation modeling.

Reaction	Log K
$\text{H}_2\text{O} - \text{H}^+ = \text{OH}^-$	-14.00 <sup>b</sup>
$\text{H}_2\text{CO}_3^0 - \text{H}^+ = \text{HCO}_3^-$	-6.355 <sup>a</sup>
$\text{H}_2\text{CO}_3^0 - 2\text{H}^+ = \text{CO}_3^{2-}$	-16.67 <sup>a</sup>
$\text{H}_2\text{CO}_3^0 - \text{H}_2\text{O} = \text{CO}_2^0$	2.770 <sup>b</sup>
$\text{Na}^+ + \text{H}_2\text{CO}_3^0 - 2\text{H}^+ = \text{NaCO}_3^-$	-15.41 <sup>b</sup>
$\text{Na}^+ + \text{H}_2\text{CO}_3^0 - \text{H}^+ = \text{NaHCO}_3^0$	-6.60 <sup>b</sup>
$\text{Na}^+ + \text{H}_2\text{O} - \text{H}^+ = \text{NaOH}^0$	-14.2 <sup>b</sup>
$\text{Hg}^{2+} + \text{H}_2\text{O} - \text{H}^+ = \text{HgOH}^+$	-3.40 <sup>a</sup>
$\text{Hg}^{2+} + 2\text{H}_2\text{O} - 2\text{H}^+ = \text{Hg}(\text{OH})_2^0$	-5.98 <sup>a</sup>
$\text{Hg}^{2+} + 3\text{H}_2\text{O} - 3\text{H}^+ = \text{Hg}(\text{OH})_3^-$	-21.1 <sup>a</sup>
$2\text{Hg}^{2+} + \text{H}_2\text{O} - \text{H}^+ = \text{Hg}_2(\text{OH})_3^+$	-3.30 <sup>b</sup>
$3\text{Hg}^{2+} + 3\text{H}_2\text{O} - 3\text{H}^+ = \text{Hg}_3(\text{OH})_3^{3+}$	-6.40 <sup>b</sup>
$\text{Hg}^{2+} + \text{H}_2\text{CO}_3^0 - 2\text{H}^+ = \text{HgCO}_3^0$	-3.91 <sup>a</sup>
$\text{Hg}^{2+} + \text{H}_2\text{CO}_3^0 - \text{H}^+ = \text{HgHCO}_3^+$	0.42 <sup>a</sup>
$\text{Hg}^{2+} + \text{H}_2\text{CO}_3^0 + \text{H}_2\text{O} - 3\text{H}^+ = \text{Hg}(\text{OH})\text{CO}_3^-$	-11.355 <sup>a</sup>
$\text{B}_1^- + \text{H}^+ = \text{B}_1\text{-H}^0$	
	<i>Bacillus subtilis</i> 3.30 <sup>c</sup>
	<i>Shewanella oneidensis</i> 3.30 <sup>d</sup>
	<i>Geobacter sulfurreducens</i> 3.36 <sup>e</sup>
$\text{B}_2^- + \text{H}^+ = \text{B}_2\text{-H}^0$	
	<i>Bacillus subtilis</i> 4.80 <sup>c</sup>
	<i>Shewanella oneidensis</i> 4.80 <sup>d</sup>
	<i>Geobacter sulfurreducens</i> 4.81 <sup>e</sup>
$\text{B}_3^- + \text{H}^+ = \text{B}_3\text{-H}^0$	
	<i>Bacillus subtilis</i> 6.80 <sup>c</sup>
	<i>Shewanella oneidensis</i> 6.70 <sup>d</sup>
	<i>Geobacter sulfurreducens</i> 6.49 <sup>e</sup>
$\text{FA}^- + \text{H}^+ = \text{FA-H}^0$	5.85 <sup>f</sup>

498 <sup>a</sup> Powell et al., 2005.499 <sup>b</sup> Martell and Smith, 2001.500 <sup>c</sup> Fein et al., 2005501 <sup>d</sup> Mishra et al., 2010502 <sup>e</sup> Dunham-Cheatham et al.503 <sup>f</sup> Calculated as the average of all reported pK<sub>a</sub> values in Table 2 from Borrok and Fein (2004).

504 Assumed total site concentration is the sum of the average site concentrations for the individual

505 FA sites: 5.50 x 10<sup>-3</sup> moles of sites per gram of humic substance.

506

507 Table S2: Best-fit values of Hg solution standards and Hg-biomass samples.

<b>Sample</b>	<b>path</b>	<b>N</b>	<b>R(Å)</b>	<b><math>\sigma^2</math> (<math>10^{-3}</math> Å<sup>2</sup>)</b>
HgAc	Hg-O	$1.78 \pm 0.32$	$2.06 \pm 0.01$	$10.9 \pm 0.9$
	Hg-C	$1.78^a$	$2.83 \pm 0.01$	$12.8 \pm 4.0$
Hg-cysteine	Hg-S	$1.88 \pm 0.21$	$2.32 \pm 0.01$	$10.5 \pm 1.2$
Hg-biomass (at pH 4)	Hg-S	$1.85 \pm 0.19$	$2.35 \pm 0.01$	$10.2 \pm 1.5$
Hg-biomass (at pH 8)	Hg-S	$1.70 \pm 0.15$	$2.35 \pm 0.01$	$11.0 \pm 1.3$

508 <sup>a</sup> Set to Coordination number of O for this sample.

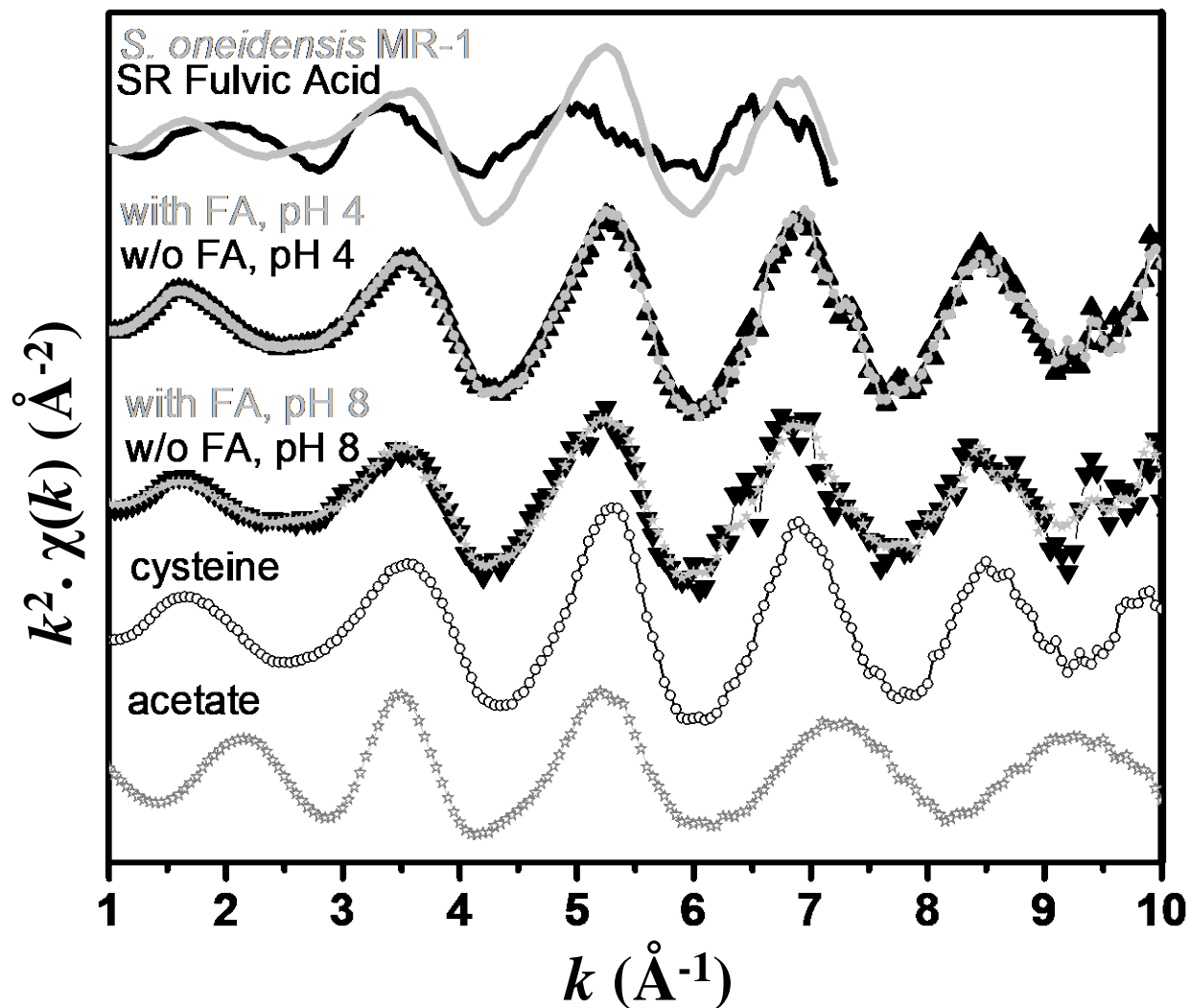
509

510

<b><u>Sample</u></b>	<b><u>path</u></b>	<b><u>CN</u></b>	<b><u>R(Å)</u></b>	<b><u><math>\sigma^2</math> (<math>10^{-3}</math> Å<sup>2</sup>)</u></b>	<b><u>E<sub>o</sub></u></b>	<b><u><math>\chi_v^2</math></u></b>	<b><u>R</u></b>
HgAc	Hg-O	$1.78 \pm 0.32$	$2.06 \pm 0.01$	$10.9 \pm 0.9$	$1.2 \pm 0.9$	48	0.63
	Hg-C	$1.78^a$	$2.83 \pm 0.01$	$12.8 \pm 4.0$			
Hg-cysteine	Hg-S	$1.88 \pm 0.21$	$2.32 \pm 0.01$	$10.5 \pm 1.2$	$2.8 \pm 0.6$	22	0.45
Hg-biomass (at pH 4)	Hg-S	$1.85 \pm 0.19$	$2.35 \pm 0.01$	$10.2 \pm 1.5$	$3.0 \pm 0.5$	30	0.55
Hg-biomass (at pH 8)	Hg-S	$1.70 \pm 0.15$	$2.35 \pm 0.01$	$11.0 \pm 1.3$			

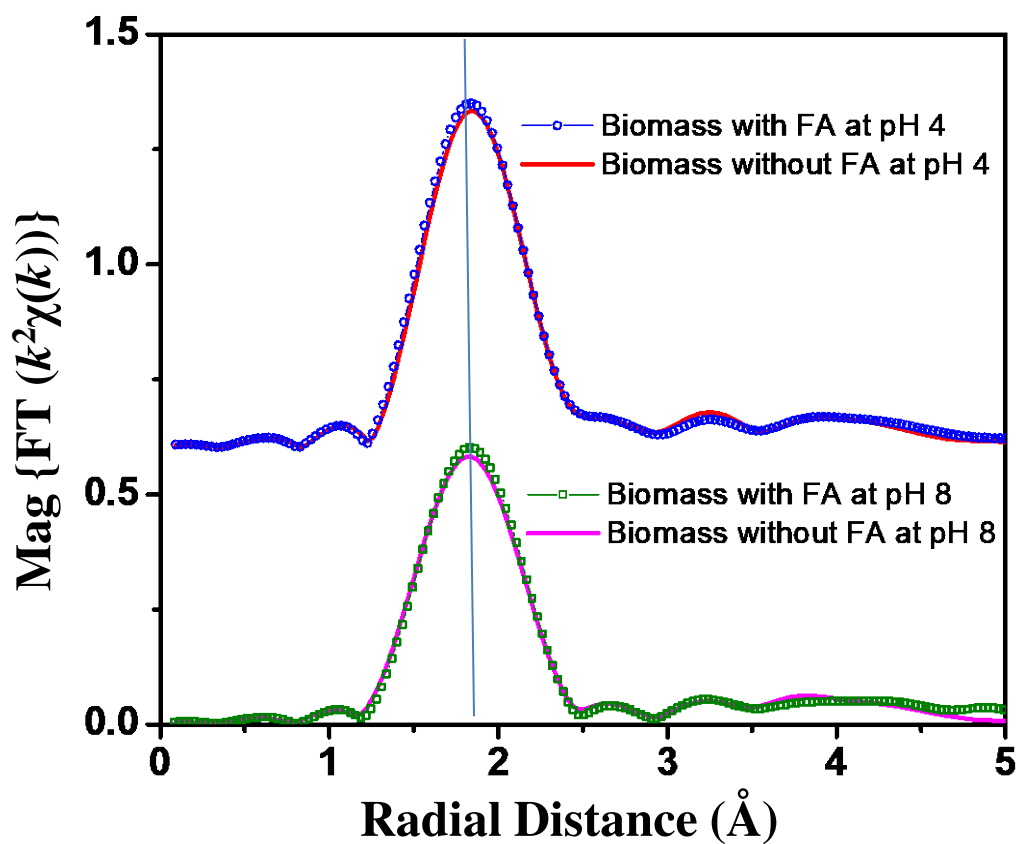
511 <sup>a</sup> Set to Coordination number of O for this sample.

512



513

514 Figure S1:  $k^2\chi(k)$  EXAFS data for Hg  $L_{III}$  edge EXAFS spectra of Hg bound to (top to bottom):  
 515 *S. oneidensis* MR-1 only at pH 4, FA only at pH 4, *S. oneidensis* MR-1 in the presence of 50 and  
 516 0  $\text{mg L}^{-1}$  FA at pH 4, *S. oneidensis* MR-1 in the presence of 50 and 0  $\text{mg L}^{-1}$  FA at pH 8, cysteine  
 517 only, and acetate only.



518

519 Figure S2: Magnitude of Hg L<sub>III</sub> edge EXAFS Fourier Transform (FT) data for Hg binding to *S.*

520 *oneidensis* MR-1 in the presence of 0 and 50 mg L<sup>-1</sup> FA at pH 4 (top), and 0 and 50 mg L<sup>-1</sup> FA at

521 pH 8 (bottom).

522

## References

- 524 Benoit J. M., Gilmour C. C., Mason R. P., and Heyes A. (1999) Sulfide Controls on Mercury  
525 Speciation and Bioavailability to Methylating Bacteria in Sediment Pore Waters. 33. 951-957.
- 526 Beveridge T. J., and Murray R. G. E. (1976) Uptake and retention of metals by cell walls of  
527 *Bacillus subtilis*. Journal of Bacteriology. 127. 1502-1518.
- 528 Borrok D and Fein J. B. (2004) Distribution of protons and Cd between bacterial surfaces and  
529 dissolved humic substances determined through chemical equilibrium modeling. *Geochimica  
530 et Cosmochimica Acta*. 68. 3043-3052.
- 531 Borrok D., Turner B. F., and Fein J. B. (2005) A universal surface complexation framework for  
532 modeling proton binding onto bacterial surfaces in geologic settings. *American Journal of  
533 Science*. 305. 826-853.
- 534 Borrok D., Aumend K. and Fein J. B. (2007) Significance of ternary bacteria-metal-natural organic  
535 matter complexes determined through experimentation and chemical equilibrium modeling.  
536 *Chemical Geology*. 238. 44-62.
- 537 Colombo M. J., Ha J., Reinfelder J. R., Barkay T., Yee N. (2013) Anaerobic oxidation of Hg(0)  
538 and methylmercury formation by *Desulfovibrio desulfuricans* ND132. *Geochimica et  
539 Cosmochimica Acta*. 112. 166-177.
- 540 Compeau G. C., and Bartha R. (1987) Effect of salinity on mercury-methylating activity of sulfate-  
541 reducing bacteria in estuarine sediments. *Applied and Environmental Microbiology*. 53. 261-  
542 265.
- 543 Cox J. S., Smith D. S., Warren L. A., and Ferris F. G. (1999) Characterizing heterogeneous  
544 bacterial surface functional groups using discrete affinity spectra for proton binding.  
545 *Environmental Science & Technology*. 33. 4514-4521.
- 546 Croué J.-P., Benedetti D., Violleau D., and Leenheer J. A. (2003) Characterization and Copper  
547 Binding of Humic and Nonhumic Organic Matter Isolated from the South Platte River:  
548 Evidence for the Presence of Nitrogenous Binding Sites. *Environmental Science &  
549 Technology*. 37. 328-336.
- 550 Daughney C. J., Siciliano S. D., Rencz A. N., Lean D., and Fortin D. (2002) Hg(II) adsorption by  
551 bacteria: A surface complexation model and its application to shallow acidic lakes and  
552 wetlands in Kejimikujik National Park, Nova Scotia, Canada. *Environmental Science &  
553 Technology*. 36. 1546-1553.
- 554 Dong W. M., Bian Y. R., Liang L. Y., and Gu B. H. (2011) Binding Constants of Mercury and  
555 Dissolved Organic Matter Determined by a Modified Ion Exchange Technique. *Environmental  
556 Science & Technology*. 45. 3576-3583.
- 557 Drexel R. T., Haitzer M., Ryan J. N., Aiken G. R., and Nagy K. L. (2002) Mercury(II) Sorption to  
558 Two Florida Everglades Peats: Evidence for Strong and Weak Binding and Competition by  
559 Dissolved Organic Matter Released from the Peat. *Environmental Science & Technology*. 36.  
560 4058-4064.
- 561 Dunham-Cheatham S., Farrell B., Mishra B., Myneni S., and Fein J. B. The effect of chloride on  
562 the adsorption of Hg onto three bacterial species. Manuscript submitted for publication.

- 563 Ephraim J. H. (1992) Heterogeneity as a concept in the interpretation of metal ion binding by  
564 humic substances. The binding of zinc by an aquatic fulvic acid. *Analytica Chimica Acta*. 267.  
565 39-45.
- 566 Fein J. B., Boily J.-F., Yee N., Gorman-Lewis D., and Turner B. F. (2005) Potentiometric titrations  
567 of *Bacillus subtilis* cells to low pH and a comparison of modeling approaches. *Geochimica et*  
568 *Cosmochimica Acta*. 69. 1123-1132.
- 569 Fein, J.B., 2006. Thermodynamic modeling of metal adsorption onto bacterial cell walls: current  
570 challenges. *Adv. Agron.* 90, 179–202.
- 571 Fortin D. and Beveridge T. J. (1997) Role of the bacterium *Thiobacillus* in the formation of  
572 silicates in acidic mine tailings. *Chemical Geology*. 141. 235-250.
- 573 Guiné V., Spadini L., Sarret G., Muris M., Delolme C., Gaudet J. P., and Martins J. M. F. (2006)  
574 Zinc sorption to three gram-negative bacteria: Combined titration, modeling, and EXAFS  
575 study. *Environmental Science & Technology*. 40. 1806-1813.
- 576 Haitzer M., Aiken G. R., and Ryan J. N. (2002) Binding of Mercury(II) to Dissolved Organic  
577 Matter: The Role of the Mercury-to-DOM Concentration Ratio. *Environmental Science &*  
578 *Technology*. 36. 3564-3570.
- 579 Haitzer M., Aiken G. R., and Ryan J. N. (2003) Binding of Mercury(II) to Aquatic Humic  
580 Substances: Influence of pH and Source of Humic Substances. *Environmental Science &*  
581 *Technology*. 37. 2436-2441.
- 582 Harris, H. H., Pickering, I. J., George, G. N. (2003) The Chemical Form of Mercury in  
583 Fish. *Science*. 301. 1203.
- 584 Hesterberg D., Chou J. W., Hutchison K. J., and Sayers D. E. (2001) Bonding of Hg(II) to Reduced  
585 Organic Sulfur in Humic Acid as Affected by S/Hg Ratio. *Environmental Science &*  
586 *Technology*. 35. 2741-2745.
- 587 Johnson K. J., Szymanowski J. E. S., Borrok D., Huynh T. Q., and Fein J. B. (2007) Proton and  
588 metal adsorption onto bacterial consortia: Stability constants for metal-bacterial surface  
589 complexes. *Chemical Geology*. 239. 13-26.
- 590 Kenney J. P. L. and Fein J. B. (2011) Cell wall reactivity of acidophilic and alkaliphilic bacteria  
591 determined by potentiometric titrations and Cd adsorption experiments. *Environmental*  
592 *Science & Technology*. 45. 4446-4452.
- 593 Loux, N. T. (1998) An assessment of mercury-species-dependent binding with natural organic  
594 carbon. *Chemical Speciation and Bioavailability*. 10. 127-136.
- 595 Lovely, D.R. and Phillips, E.J.P. (1988) Novel mode of microbial energy metabolism: organic  
596 carbon oxidation coupled to dissimilatory reduction of iron or manganese. *Applied and*  
597 *Environmental Microbiology*. 54. 1472-1480.
- 598 Martell A. E. and Smith R. M. (2001) NIST Critically selected stability constants of metal  
599 complexes, Version 6.0. NIST Standard Reference Database. 46. National Institute of  
600 Standards and Technology. Gaithersburg, MD.
- 601 McDowell W. H. (2003) Dissolved organic matter in soils – future directions and unanswered  
602 questions. *Geoderma*. 113. 179-186.

603 Mishra, B., Fein, J. B., Boyanov, M. I., Kelly, S. D., Kemner, K. M., Bunker, B. A. (2007)  
604 Comparison of Cd Binding Mechanisms by Gram-Positive, Gram-Negative and Consortia of  
605 Bacteria Using XAFS. AIP Conference proceeding. 882. 343-345.

606 Mishra B., Boyanov M. I., Bunker B. A., Kelly S. D., Kemner K. M., Nerenberg R., Read-Daily  
607 B.L., and Fein J. B. (2009) An X-ray absorption spectroscopy study of Cd binding onto  
608 bacterial consortia. *Geochimica et Cosmochimica Acta*. 73. 4311-4325.

609 Mishra B., Boyanov M., Bunker B. A., Kelly S. D., Kemner K. M., and Fein J. B. (2010) High-  
610 and low-affinity binding sites for Cd on the bacterial cell walls of *Bacillus subtilis* and  
611 *Shewanella oneidensis*. *Geochimica et Cosmochimica Acta*. 74. 4219-4233.

612 Mishra, B., O'Loughlin, E. J., Boyanov. M.B., Kemner, K. M. (2011) Binding of HgII to High-  
613 Affinity Sites on Bacteria Inhibits Reduction to Hg<sup>0</sup> by Mixed Fe<sup>II/III</sup> Phases. *Environmental*  
614 *Science & Technology*. 45. 9597-9603.

615 Morra, M.J., Fendorf, S. E., Brown, P. D. (1997) Speciation of sulfur in humic and fulvic acids  
616 using X-ray absorption near-edge structure (XANES) spectroscopy. *Geochimica et*  
617 *Cosmochimica Acta*. 61. 683-688.

618 Muresan B., Pernet-Coudrier B., Cossa D., and Varrault G. (2011) Measurement and modeling of  
619 mercury complexation by dissolved organic matter isolates from freshwater and effluents of a  
620 major wastewater treatment plant. *Applied Geochemistry*. 26. 2057-2063.

621 Myneni, S. C. B. (2002) Soft X-ray spectroscopy and spectromicroscopy studies of organic  
622 molecules in the environment. *Reviews in Mineralogy and Geochemistry*. 49. 485-579.

623 Pokrovsky O.S., Pokrovski G. S., Shirokova L.S., Gonzalez A. G., Emnova E. E., Feurtet-Mazel  
624 A. (2012) Chemical and structural status of copper associated with oxygenic and anoxygenic  
625 phototrophs and heterotrophs: possible evolutionary consequences. *Geobiology*. 10. 130-149.

626 Powell K. J., Brown P. L., Byrne R. H., Gajda T., Hefter G., Sjoberg S., and Wanner H. (2005)  
627 Chemical speciation of environmentally significant heavy metals with inorganic ligands. Part  
628 1: The Hg<sup>2+</sup>-Cl<sup>-</sup>, OH<sup>-</sup>, CO<sub>3</sub><sup>2-</sup>, SO<sub>4</sub><sup>2-</sup>, and PO<sub>4</sub><sup>3-</sup> aqueous systems. *Pure and Applied Chemistry*.  
629 77. 739-800.

630 Ravel, B., Newville, M. (2005) ATHENA, ARTEMIS, HEPHAESTUS: Data analysis for X-ray  
631 absorption spectroscopy using IFEFFIT. *Journal of Synchrotron Radiation*. 12. 537-541.

632 Ravichandran M., Aiken G. R., Ryan J. N., and Reddy M. M. (1999) Inhibition of precipitation  
633 and aggregation of metacinnabar (mercuric sulfide) by dissolved organic matter isolated from  
634 the Florida Everglades. *Environmental Science & Technology*. 33. 1418-1423.

635 Ravichandran M. (2004) Interactions between mercury and dissolved organic matter – a review.  
636 *Chemosphere*. 55. 319-331.

637 Skyllberg U., Bloom P. R., Qian J., Lin C.-M., and Bleam W. F. (2006) Complexation of  
638 Mercury(II) in Soil Organic Matter: EXAFS Evidence for Linear Two-Coordination with  
639 Reduced Sulfur Groups. *Environmental Science & Technology*. 40. 4174-4180.

640 Segre, C. U., Leyarovsky, N. E., Chapman, L. D., Lavender, W. M., Plag, P. W., King, A. S.,  
641 Kropf, A. J., Bunker, B. A., Kemner, K. M., Dutta, P., Duran, R. S., Kaduk, J. (2000) The  
642 MRCAT insertion device beamline at the Advanced Photon Source, CP521. Synchrotron

- 643 Radiation Instrumentation: Eleventh U.S. National Conference; Pianetta, P., Ed.; American  
644 Institute of Physics:New York, 419–422.
- 645 Song Z., Kenney J. P. L., Fein J. B., Bunker B. A. (2012) An X-Ray Absorption Fine Structure  
646 study of Au adsorbed onto the non-metabolizing cells of two soil bacterial species. *Geochimica  
647 et Cosmochimica Acta.* 86. 103-117.
- 648 Stern, E. A., Newville, M., Ravel, B., Yacoby, Y., Haskel, D. (1995) The UWXAFS analysis  
649 package philosophy and details. *Physica B.* 209. 117–120.
- 650 Vairavamurthy, A. (1998) Using X-ray absorption to probe sulfur oxidation states in complex  
651 molecules. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy.* 54. 2009-  
652 2017.
- 653 Westall J. C. (1982) FITEQL, A computer program for determination of chemical equilibrium  
654 constants from experimental data. Version 2.0. Report 82-02, Department of Chemistry,  
655 Oregon State University, Corvallis, OR, USA.
- 656 Wightman P. G. and Fein J. B. (2001) Ternary interactions in a humic acid-Cd-bacteria system.  
657 *Chemical Geology.* 180. 55-65.
- 658 Winfrey M. R. and Rudd J. W. M. (1990) Environmental factors affecting the formation of  
659 methylmercury in low pH lakes. *Environmental Toxicology and Chemistry.* 9. 853-869.
- 660 Xia K., Skyllberg U. L., Blears W. F., Bloom P. R., Nater E. A., and Helmke P. A. (1999) X-ray  
661 Absorption Spectroscopic Evidence for the Complexation of Hg(II) by Reduced Sulfur in Soil  
662 Humic Substances. *Environmental Science & Technology.* 33. 257-261.
- 663 Xia K., Weesner, F., Blears W.F., Helmke P.A., Bloom P.R., Skyllberg U.L. (1998) XANES  
664 Studies of Oxidation States of Sulfur in Aquatic and Soil Humic Substances. *Soil Science  
665 Society of America Journal.* 62. 124-1246.
- 666 Yee N. and Fein J. B. (2001) Cd adsorption onto bacterial surfaces: A universal adsorption edge?  
667 *Geochimica et Cosmochimica Acta.* 65. 2037.