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Adsorption of methylmercury onto *Geobacter bemidjensis* Bem

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22 Abstract

23 The anaerobic bacterium *Geobacter bemidjensis* Bem has the unique ability to both produce and
24 degrade methylmercury (MeHg). While the adsorption of MeHg onto bacterial surfaces can
25 affect the release of MeHg into aquatic environments as well as the uptake of MeHg for
26 demethylation, the binding of MeHg to the bacterial envelope remains poorly understood. In this
27 study, we quantified the adsorption of MeHg onto *G. bemidjensis* and applied X-ray absorption
28 spectroscopy (XAS) to elucidate the mechanism of MeHg binding. The results showed MeHg
29 adsorption onto *G. bemidjensis* cell surfaces was rapid and occurred via complexation to thiol
30 functional groups. Titration experiments yielded cell surface thiol concentrations of 3.2 to 6.4
31 $\mu\text{mol/g}$ (wet cells). A one-site adsorption model with MeHg binding onto thiol sites provided
32 excellent fits to adsorption isotherms conducted at different cell densities. The log K binding
33 constant of MeHg onto the thiol sites was determined to be 10.2 ± 0.2 . These findings provide a
34 quantitative framework to describe MeHg binding onto bacterial cell surfaces and elucidate the
35 importance of bacterial cells as possible carriers of adsorbed MeHg in natural aquatic systems.

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43 **Introduction**

44 Anaerobic bacteria play a central role in the production and degradation of
45 methylmercury (MeHg) in the environment.¹⁻³ In anoxic systems, net MeHg accumulation has
46 been shown to be controlled by the balance of Hg methylation and MeHg demethylation rates,^{4, 5}
47 which are strongly influenced by microbe-mercury interactions. Adsorption of MeHg onto
48 bacterial cells is an important process that occurs during both mercury methylation and
49 demethylation.⁶⁻¹⁰ MeHg adsorption onto Hg-methylating bacteria can affect the release of
50 MeHg into aquatic environments,⁸ while MeHg adsorption onto methylmercury degrading
51 bacteria may limit the uptake of MeHg for enzymatic demethylation processes.⁹⁻¹³ Currently, the
52 adsorption affinity and binding mechanism of MeHg onto bacterial cells are poorly understood.

53 Previous studies have found that thiol functional groups on bacterial surfaces are reactive
54 moieties involved in heavy metal binding.¹⁴⁻²⁰ Despite the low abundance of thiol sites relative to
55 the total metal binding sites in bacterial membranes, metals such as Cd(II) preferentially bind to
56 thiol functional groups under low metal loading conditions.¹⁹ At environmentally relevant
57 metal:biomass ratios, thiol sites also dominate the adsorption of Zn(II) and Ni(II).¹⁷ Recently, Yu
58 and Fein (2017)²⁰ showed that the adsorption of Hg(II), Cd(II), and Au(III) onto the Gram
59 positive bacterium *Bacillus subtilis* is strongly enhanced by the elevated concentration of thiol
60 sites within the cell envelopes. To date, the role of thiol functional groups in the adsorption of
61 MeHg onto bacterial surface has not been characterized.

62 *Geobacter bemidjensis* Bem is a subsurface bacterium^{21, 22} that has been reported to both
63 produce and degrade methylmercury.^{10, 23} This gram-negative anaerobe carries the mercury
64 methylation genes *hgcAB*,^{10, 24} which enables *G. bemidjensis* to convert inorganic Hg(II) to
65 MeHg. Additionally, in contrast to other *Geobacter* species, strain Bem also carries a homolog of

66 the alkylmercury lyase gene *merB*,¹⁰ which confers the ability to demethylate MeHg. Both of
67 these mercury methylation and demethylation pathways are intracellular reactions that require
68 transport across the cell envelope to occur. In the case of mercury methylation, MeHg produced
69 by the bacterium can bind onto cell surface ligands thus influencing the release of MeHg into the
70 dissolved phase. For methylmercury degradation, adsorption of MeHg onto the cell envelope can
71 affect MeHg uptake and transport into the cell. A quantitative understanding of MeHg adsorption
72 on cell surface functional groups would thus improve our ability to model Hg transformations by
73 *G. bemidjensis* and the distribution of MeHg during methylation and demethylation.

74 In this study, we conducted bulk adsorption experiments and spectroscopic analyses to
75 investigate MeHg adsorption onto *G. bemidjensis* Bem. The objectives of this study were to (1)
76 quantify the extent of MeHg adsorption onto *G. bemidjensis* cells; (2) characterize the local
77 coordination environment of methylmercury adsorbed onto *G. bemidjensis* cells; and (3)
78 develop a surface complexation model to describe the MeHg adsorption reaction. The results of
79 this study provide new insights into the mechanism of MeHg adsorption onto an
80 environmentally-relevant Hg methylating and MeHg-degrading bacterium.

81

82 **Materials and Methods**

83 **Bacterial Culture and Assay Conditions:** Strain *Geobacter bemidjensis* Bem (gifted
84 from B. Gu, Oakridge National Lab) was cultured at 30 °C under a nitrogen (N₂) headspace in
85 ATCC Medium 1957 containing 40 mM fumarate and 30 mM acetate at pH 6.8. Cells were
86 harvested at late exponential phase in an anaerobic glovebox, centrifuged for 8 min at 7900 g,
87 and washed three times in 0.5 mM deoxygenated hypotonic MOPS buffer adjusted to 7.0 ± 0.2

88 with NaOH. Cells were then re-suspended in the MOPS buffer to a desired cell density for the
89 MeHg adsorption experiments.

90 **Methylmercury Adsorption Experiments:** A methylmercury chloride stock solution
91 (50 μM) was prepared by diluting a standard solution (1000 ppm; Alfa Aesar) with
92 deoxygenated Ultrapure Water ($18.2 \text{ M}\Omega \text{ cm}^{-1}$) and stored in an ultrahigh-purity N_2 atmosphere.
93 The MeHg stock solution was analyzed for both total mercury (THg) and MeHg, and the purity
94 was determined to be >99% MeHg before experimentation. Inside a glove box (Coy; 5:95 $\text{H}_2:\text{N}_2$
95 headspace), MeHg adsorption experiments were initiated by adding known amounts of MeHg to
96 each foil-wrapped serum bottles containing 100 mL *G. bemidjiensis* washed cells suspension,
97 capped with a Teflon-lined butyl rubber stopper and sealed with aluminum caps. The reactors
98 were then shaken at 60 rpm at 30 °C. To examine the adsorption kinetics, individual reactors
99 were sacrificed at specific time points for mercury analyses. Aliquots were taken for THg, total
100 methylmercury (TMeHg), cell associated Hg ($\text{MeHg}_{\text{cell}}$), aqueous Hg (MeHg_{aq}), and intracellular
101 Hg (MeHg_{in}) measurements. $\text{MeHg}_{\text{cell}}$ samples were collected by filtering the cell suspensions
102 through 0.2 μm IsoporeTM polycarbonate membrane filters. MeHg_{aq} was determined by
103 analyzing the filtrate of samples filtered through 0.2 μm PVDF syringe filters. MeHg_{in} was
104 determined by removing the adsorbed MeHg using a wash protocol modified from Schaefer et al.
105 (2009).²⁵ Briefly, the adsorbed MeHg was removed by rinsing filtered cell suspensions with 5
106 mL of an oxalic acid-EDTA-Cl buffer twice and then with 5 mL 100 mM cysteine for 5 min. The
107 concentration of adsorbed Hg (MeHg_{ads}) was calculated as the difference between $\text{MeHg}_{\text{cell}}$ and
108 MeHg_{in} . Finally, TMeHg measurements were performed and compared with THg values to
109 verify that methylmercury degradation did not occur during the experiment.

110 To determine MeHg binding constants, adsorption isotherm experiments were performed
111 by adding 10-500 nM MeHg to individual batch reactors containing cell densities ranging from 3
112 g/L to 0.03 g/L (wet wt). After 2 h of equilibration, samples were collected from each reactor and
113 digested for THg, MeHg_{cell}, and MeHg_{aq} analyses. MeHg adsorption experiments at 3 g/L cells
114 were repeated using *G. bemidjiensis* cells treated with 200 μM of Thiol Fluorescent Probe IV
115 (TFP-4) (EMD Millipore Corporation) to block cellular thiol sites.^{26, 27} Cells were treated with
116 TFP-4 in a glove box and shaken in the dark for 2 h then washed three times with MOPS buffer
117 before the adsorption experiment. Adsorption isotherms were modeled using FITEQL 2.0²⁸ to
118 determine the apparent equilibrium constant of MeHg binding onto *G. bemidjiensis* and TFP-4
119 treated cells. R² values were calculated to determine the goodness of fit.

120 **Mercury Analysis:** TMeHg samples were preserved in 0.1 M H₂SO₄ at 4 °C, distilled by
121 a Tekran 2750 gas manifold and heating system (EPA method 1630) and analyzed using Tekran
122 2700 gas chromatography coupled to cold vapor atomic fluorescence spectroscopy
123 (GC-CVAFS). All other samples were digested in bromine monochloride to a final
124 concentration of 0.02 M at 60 °C for at least 48 h and then analyzed by either cold vapor atomic
125 fluorescence spectrometry using a Brooks Rand MERX Total Mercury Analytical System (EPA
126 Method 1631) or cold vapor atomic absorption using a Leeman Laboratories Hydra AA Mercury
127 Analyzer (EPA Method 245.1).

128 **Determination of Thiol Functional Groups on Cell Surface:** The abundance of thiol
129 functional groups on the cell surface of *G. bemidjiensis* was quantified by the fluorescence-based
130 method of Joe-Wong et al. (2012) using qBBr (monobromo(trimethylammonio)bimane bromide)
131 (Sigma-Aldrich).²⁹ QBBr is a positively charged compound that forms highly fluorescent
132 thioether bonds with exposed thiol moieties on the cell surface whose fluorescence increases

133 prominently upon conjugation.³⁰ The positive charge of the molecule makes it difficult to pass
134 through the inner membrane, thus allowing qBBr to preferential react with cell envelope thiol
135 sites. In the titration experiment, qBBr was added at specific concentrations (0-35 μ M) to *G.*
136 *bemidjiensis* cell suspensions (3 g/L) and then allowed to react in the dark for 2 h in a 30°C
137 shaker. The fluorescence was then measured in 1-mL quartz cuvettes at an excitation wavelength
138 of 400 nm and peak emission intensity of 465 nm with a Molecular Devices Plate Reader.

139 In addition to the fluorescence approach, potentiometric titrations of *G. bemidjiensis* cell
140 suspensions were also performed to quantify the total surface site concentration.³¹ This acid-base
141 titration method measures the concentration of proton-active sites within bacterial cell envelopes.
142 The difference in the proton-active site concentration before and after the thiols are blocked
143 using a qBBr treatment was used to determine the concentration of thiol sites within the cell
144 envelope. Cells were treated with \sim 1 mM qBBr for 2 h in the dark and washed three times with
145 0.1 M NaCl to remove excess qBBr before the titration experiments. Titrations were conducted
146 using an autotitrator assembly with 10 g/L *G. bemidjiensis* or qBBr-treated cells suspended in 0.1
147 M NaCl. Aliquots of 1 M HCl were added to the cell suspensions to adjust the pH to 3.0,
148 followed by titration with 1 M NaOH to raise the pH to 9.7. These base titrations were the
149 portion of the dataset used in the titration modeling. Three titrations experiments were conducted
150 for *G. bemidjiensis*, and then triplicate experiments were repeated for qBBr-treated cells. All of
151 the titrations were performed under a N₂ gas headspace to exclude atmospheric CO₂. The
152 titration data were modeled using FITEQL 2.0 to determine the thiol concentration and acid
153 dissociation constant (pKa) of the proton-active surface functional groups. The number of
154 surface sites was systematically varied in the model to determine the number of discrete sites

155 required to fit the experimental data. The goodness of fit of each tested model was evaluated by
156 comparing the values of the residual function, $V(Y)$, from the FITEQL 2.0 output.

157 **X-ray Absorption Spectroscopy:** *G. bemedjiensis* cells with adsorbed MeHg were
158 analyzed using X-ray absorption spectroscopy. Cell suspensions (3 g/L) were reacted with 50 nM
159 or 500 nM MeHg for 2 h, pelleted by centrifugation, and freeze dried. After grinding the freeze
160 dried cells to a fine powder in an anaerobic chamber, the samples were placed in Teflon sample
161 holders, covered with Kapton thin film, and sealed with Kapton tape. The samples were then
162 placed in deoxygenated containers in slotted flexiglass sample holder sandwiched between
163 Kapton tape separated by thin Kapton film and shipped to the Advanced Photon Source at
164 Argonne National Laboratory for XAS analysis. Hg LIII-edge EXAFS spectra were collected at
165 beamline 13-ID-E, GeoSoilEnviroCARS, using a Si (111) monochromatic crystal with a 13-
166 element germanium detector. Samples were run under a N_2 atmosphere at ambient temperature
167 and pressure. Energy calibration was performed such that the first inflection points of Au foil and
168 HgSn amalgam were assigned as 11 919 and 12 284 eV, respectively. The beam was focused to
169 $200 \mu\text{m} \times 200 \mu\text{m}$. Quick scans (5 min/scan) were performed on MeHg samples collecting 3
170 scans each at over 20 separate spots for each sample to limit the beam-induced chemistry and get
171 highly reproducible scans. Data were processed using ATHENA and ARTEMIS.³² The k-space
172 data range used for these analyses are 2.5 to 8.0 \AA^{-1} , using a Hanning window function dk set to
173 1.0. S_0^2 of 0.9 was used with during simultaneous fitting of 68 $\mu\text{g/g}$ and 455 $\mu\text{g/g}$ MeHg-cell
174 samples at multiple (1, 2, and 3) k weighting. Statistically significant lower reduced chi-square
175 was used for inclusion of another atom in shell by shell fitting approach. R-factor of 1.5% was
176 obtained for a fit range of 1.1 to 3.0 \AA in Fourier transformed R space.

177

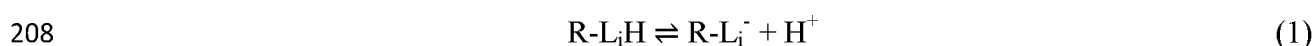
178 **Results and Discussion**

179 **MeHg adsorption onto *G. bemidijensis* Bem cell surfaces.** Kinetics experiments showed
180 that *G. bemidijensis* rapidly sorbed MeHg, with approximately 80% of MeHg sorbing in 15
181 minutes (Fig. 1A). MeHg sorption was concurrent to the removal of MeHg from solution. To
182 determine the intracellular versus surface-bound distribution of cell-associated MeHg, a 100 mM
183 cysteine solution was used to desorb MeHg on the cell surface. Approximately 95-100% of the
184 sorbed MeHg was extracted by reaction with cysteine (Fig. 1B), indicating that almost all the
185 MeHg was surface-bound and less than 5% of the MeHg was internalized. Adsorption isotherms
186 were then conducted at different cell densities to determine the affinity of MeHg adsorption onto
187 *G. bemidijensis* (Fig. 2). The data showed that the concentration of adsorbed MeHg increased
188 linearly with the aqueous MeHg concentration (Fig 2A-C). To test if thiol sites are involved in
189 MeHg adsorption, a thiol-specific probe TFP-4 was used to block the thiol sites associated with
190 the bacterial cells. The TFP-4 treated cells adsorbed markedly less MeHg (Fig. 2D) compared to
191 untreated cells (Fig. 2A). The decrease in MeHg adsorption was attributed to the loss of thiol
192 complexation in the TFP-4 treated cells.

193 **Quantification of thiol sites.** Two independent types of experiments were conducted to
194 determine the concentration of thiol sites on cell surface of *G. bemidijensis*. First, we conducted
195 a titration experiment using the fluorescent probe qBBr which binds specifically to sulfhydryl
196 sites within the cell envelope. When reacted with the qBBr fluorophore, the fluorescence
197 emission increased steeply and linearly with increasing qBBr concentration until the cell surface
198 thiol sites became saturated with qBBr (Fig. 3A). Further additions of qBBr showed weak
199 fluorescence emission with increasing qBBr concentration, as this excess qBBr remained
200 unbound to thiol sites and hence did not strongly fluoresce. The best-fit lines of the two linear

201 regions of the qBBr titration curve showed an inflection point at 3.2 $\mu\text{mol/g}$ wet cells,
202 corresponding to the concentration of reactive thiols on the cell surface of *G. bemidjensis*.

203 In the second experiment, we conducted acid-base potentiometric titrations with *G.*
204 *bemidjensis* washed cell suspensions to quantify the concentration of proton-active sites on the
205 cell surface (Fig 3B). Replicate titrations yielded an average total site concentration of $253.0 \pm$
206 $2.9 \mu\text{mol/g}$ (wet weight). The titration curves were modeled using FITEQL 2.0 to determine
207 individual site concentrations according the reaction:



209 where $[\text{R-L}_i\text{H}]$ and $[\text{R-L}_i^-]$ represent the concentrations of the *i*th neutral and deprotonated
210 organic acid functional group on the bacterial cell surface, respectively. The acidity constant of
211 each surface site type was quantified with the corresponding mass action equation:

$$212 \quad K_{a,i} = \frac{[\text{R-L}_i^-] a_{\text{H}^+}}{[\text{R-L}_i\text{H}]} \quad (2)$$

213 A four site model provided the best-fit to the titration data. The best-fit model yielded pKa
214 values of 3.9 ± 0.4 , 5.4 ± 0.3 , 7.2 ± 0.2 , and 9.6 ± 0.1 with corresponding site concentrations of
215 76.0 ± 7.6 , 70.3 ± 11.1 , 37.9 ± 4.0 and $68.8 \pm 5.1 \mu\text{mol/g}$, respectively (Table 1). We repeated
216 the titrations with cells that were treated with qBBr to block the thiol sites on the cell surface,
217 and the resulting titration curves were fit with a four site model. The model yielded very similar
218 pKa values to those from the titrations using the untreated cells (Table 1). A decrease of
219 approximately $6.4 \mu\text{mol/g}$ was observed in the site concentration for site 4 when cell surface
220 thiols were blocked by qBBr. A previous study also showed significant changes in concentration
221 of site 4 (pKa 9.2–9.4) of bacterial strains *Bacillus licheniformis*, *Bacillus subtilis*, *Bacillus*

222 *cereus*, *Shewanella oneidensis* and *Pseudomonas fluorescens* when thiols were blocked by
223 qBBr.³¹ Furthermore, the pKa value of site 4 is similar to that of thiol molecules such as cysteine,
224 glutathione and some proteins, with values ranging from 8.06 to 10.75.³³ Therefore, we suggest
225 that site 4 likely represents a mixture of site types, some of which are thiol functional groups,
226 and all of which exhibiting similar pKa values. Together with the qBBr fluorescence titration
227 experiment (Fig. 3A), we estimate the concentration of cell surface thiols to be between 3.2 and
228 6.4 $\mu\text{mol/g}$.

229 **XAS Analysis of Adsorbed MeHg.** X-ray absorption spectroscopy was performed to
230 examine the local binding environment of the adsorbed MeHg. The first derivative of normalized
231 X-ray absorption near edge structure (XANES) and the X-ray absorption fine structure (XAFS)
232 spectra of *G. bemedijensis* reacted with 50 nM and 500 nM MeHg are displayed in Figure 4A and
233 B, respectively. The amount of MeHg adsorbed to those two samples were 68 $\mu\text{g/g}$ and 455 $\mu\text{g/g}$,
234 respectively. Derivative of the XANES spectra of the two samples were nearly identical and
235 both spectra exhibited two main peaks (Fig 4A). The energy difference between the two peaks
236 was used to calculate the ΔE value. Because a larger ΔE corresponds to Hg-ligand complexes
237 with ionic character and a smaller ΔE to complexes with more covalent character,³⁴ we used this
238 value to infer the local bonding environment of MeHg. The derivative of the XANES spectra
239 showed a ΔE value of 7.0 eV, which is similar to ΔE values of Hg-cysteine₂ ($\Delta E = 7.2$ eV)^{35, 36}
240 and MeHg-cysteine ($\Delta E = 7.7$ eV).³⁷ K^2 weighted $\chi(k)$ spectra of the 68 $\mu\text{g/g}$ and 455 $\mu\text{g/g}$
241 samples were nearly identical and no phase shift in the k^2 weighted $\chi(k)$ XAFS oscillations
242 between the two samples was observed (Fig. 4B). This indicates that the stoichiometry of MeHg-
243 cell complexes was the same at the two different MeHg loadings. Both 68 $\mu\text{g/g}$ and 455 $\mu\text{g/g}$
244 samples were best fit with 0.9 +/- 0.1 sulfur atom at ~2.34 angstrom and 0.9 +/- 0.1 carbon atom

245 at ~ 2.03 angstrom, indicating that the Hg-C bond in MeHg remained intact when adsorbed on the
 246 bacterial surface and that MeHg was bonded exclusively to thiol sites of *G. bemidjensis* (Fig 4C
 247 and 4D, Table 4). No changes in the Hg-S bond distance was observed suggesting that the
 248 MeHg-S complexes were the same in both samples (Fig 4C and 4D). Notably, the binding
 249 distance and the ratios of Hg atoms to C and S atoms are similar to MeHg-DOM bonding.³⁸⁻⁴³

250 **MeHg Adsorption Constant.** Because the XAFS data showed MeHg binding to the thiol
 251 functional groups with a 1:1 MeHg:site stoichiometry (Fig 4B, Table 3), the adsorption
 252 isotherms were used to determine a stability constant for the MeHg-thiol complex formed on the
 253 cell surface using the following reaction:



255 where R-L₄-MeHg is the MeHg adsorbed onto the cell surface, and R-L₄⁻ is the thiol portion of
 256 site 4 with a pKa value of 9.5 and a thiol concentration of 6.4 $\mu\text{mol/g}$ as determined by the acid-
 257 base titration experiments. The stability constant was quantified with the corresponding mass
 258 action equation:

$$259 \quad K_{\text{MeHg-L}_4} = \frac{[\text{R-L}_4\text{-MeHg}]}{[\text{R-L}_4^-] a_{\text{MeHg}^+}} \quad (4)$$

260 The MeHg binding constant was remarkably consistent for three adsorption data sets. At cell
 261 concentration of 3 g/L, 0.6 g/L and 0.3 g/L, the adsorption data yielded $\log K_{\text{MeHg-L}_4}$ values of
 262 10.1 ± 0 , 10.3 ± 0.1 , and 10.2 ± 0.1 , respectively (Table 2). The model fits displayed in Figure 2
 263 show good agreement with the experimental data. The one-site model for MeHg binding onto R-
 264 L₄⁻ provided excellent fits to all three adsorption isotherms with r^2 values greater than 0.95
 265 (Table 2). No improvement in fit was obtained using a two-site model by including an additional

266 site for MeHg binding. Combining all data sets, the average log K value for the one site model
267 was 10.2 ± 0.2 . This binding constant is comparable to log K values of MeHg complexation to
268 aquatic humic and fulvic acids which range from 10.7 to 14.8 for binding reactions with the same
269 stoichiometry as is shown in Reaction 3.^{38, 44-46}

270 We then modeled the adsorption isotherm of the thiol-blocked cells (Fig. 2D). First, we
271 tested a one-site adsorption model with MeHg binding to site 1. The resulting log K value for
272 MeHg binding was 3.8 with a corresponding r^2 value of 0.96. Because our data were not
273 collected as a function of pH, the binding site for MeHg is not well-constrained. Therefore, we
274 also considered MeHg binding to alternate cell surface sites (e.g., sites 2, 3 or 4). The resulting
275 calculated binding constant was the highest for site 4 with a log K value of 6.4 (Table 2). None
276 of the models with the alternate sites improved the goodness of fit. Including an additional site
277 for adsorption using a two-site model also did not improve the goodness of fit. Because site 1 has
278 the lowest pKa value and the highest site density, the concentration of deprotonated sites at the
279 pH conditions of the adsorption experiments for site 1 is greater than the other sites. Thus, we
280 tentatively assign site 1 as the binding site for MeHg onto the thiol-blocked cells. We note that
281 the stability constant for this R-L₁-MeHg complex (log K = 3.8) is significantly lower than the
282 R-L₄-MeHg complex (log K = 10.2), thereby offering an explanation as to why MeHg binds
283 exclusively to thiol sites as observed in the EXAFS experiments. Although site 1 is much more
284 abundant than the thiols in site 4, the relative K values indicate that the thiol sites exhibit an
285 affinity to bind MeHg that is orders of magnitude greater than that exhibited by site 1. Since
286 MeHg binding to site 1 is much weaker than MeHg complexation to the thiol sites, we propose
287 that site 1 likely corresponds to a weak organic acid ligand such as a carboxyl functional group.

288

289 **Environmental Implications**

290 The rapid and extensive adsorption of MeHg onto *G. bemidjensis* indicates that cell
291 surfaces are reactive interfaces that can accumulate MeHg produced by Hg-methylating bacteria.
292 The adsorption model presented in this study provides a quantitative framework to understand
293 MeHg binding to the cell envelope of *Geobacter* species during MeHg production. Previous
294 studies have shown that approximately 80% of the MeHg produced by *G. bemidjensis* is
295 adsorbed onto cell surfaces¹⁰ and more than 90% of MeHg is bound to *G. sulfurreducens* PCA.⁸
296 The amount of adsorbed MeHg measured in these previous experiments is in agreement with the
297 distribution of MeHg predicted by our surface complexation model. Because MeHg binds
298 strongly to cell surfaces during methylation, the fate of this adsorbed MeHg is expected to be
299 tied to that of the bacterial cells. For example, if the Hg-methylating bacteria are mobile, then the
300 adsorbed MeHg would be carried along with the particulate phase for environmental transport.
301 MeHg is known to be partitioned onto particulate matter in aquatic environments,^{47, 48} where
302 bacterial cells typically represent a significant fraction of the particulate phase.^{49, 50} Conversely,
303 if the bacterial cells are attached to sediment surfaces or form biofilms, then the adsorbed MeHg
304 would have limited environmental mobility.

305 Our results suggest that the concentration of cellular thiols is an important factor that
306 controls the extent to which bacterial cells can act as carriers of adsorbed MeHg. The
307 concentration of thiols on bacterial surfaces appears to vary between bacterial species. Whereas
308 *G. bemidjensis* has at most 6.4 $\mu\text{mol/g}$ of cell surface thiol sites, the closely related Hg-
309 methylating bacterium *G. sulfurreducens* PCA has at least 10 times higher reactive thiols in its
310 cell envelope.¹⁶ In comparison, the non Hg-methylating bacteria *B. cereus*, *B. subtilis* and *S.*
311 *oneidensis* have intermediate cell surface thiol concentrations of 16.6, 22.5, and 33.1 $\mu\text{mol/g}$.³¹

312 Notably, these values are in the same order of magnitude as thiol concentrations found in solid
313 organic particles ($\sim 22 \mu\text{mol/g}$),⁵¹ and hence bacteria are likely to be able to compete effectively
314 with solid organic particles for available MeHg in aquatic systems.

315 The desorption of MeHg from the *G. bemidijensis* is facilitated by aqueous complexation
316 with strong ligands such as cysteine (Fig. 1B). Likewise, in natural systems the release of MeHg
317 from bacterial surfaces would be enhanced by the presence of low-molecular-weight thiol
318 compounds or other high affinity aqueous ligands. This can occur through interactions with
319 certain humic and fulvic acids which have been shown to complex MeHg with binding constants
320 of $\log K > 14$.^{38, 44, 46} Natural waters containing high concentrations of dissolved thiols and
321 aqueous sulfide⁵² would also favor the desorption process. Interestingly, MeHg desorption from
322 Hg-methylation bacteria may stimulate additional methylation to occur. Lin et al. (2015)⁸ found
323 that the amount of MeHg produced by *G. sulfurreducens* PCA increases when cysteine is added
324 to desorb cell-bound MeHg. Thus, adsorption-desorption reactions may play an important but
325 poorly understood role in affecting net methylmercury production by *Geobacter* species. The
326 stability constants determined in this study will improve quantitative models to describe the
327 competition of environmentally-relevant ligands in MeHg binding and for estimating the fate and
328 distribution of MeHg in the environment.

329 MeHg adsorbed to marine particles containing bacterial cells have been shown to
330 demethylate MeHg,⁴⁸ suggesting an interplay between adsorption and MeHg degradation. In
331 experiments with *G. bemidijensis* Bem, Lu et al. (2016)¹⁰ observed that the concentration of
332 adsorbed MeHg decreases during MeHg degradation. It is currently unknown if adsorbed MeHg
333 is bioavailable and directly internalized for demethylation. Recently, Ndu et al. (2016)¹²
334 demonstrated that non-specific binding of MeHg to cellular ligands decreases the rates of MeHg

335 degradation, thus suggesting that adsorbed MeHg is unavailable for direct uptake. In this case,
336 adsorbed MeHg must first desorb into the aqueous phase and then re-enter the cell via passive or
337 active transport for demethylation. Identification of the cell surface molecules that harbor the
338 high affinity MeHg binding sites and elucidation of the MeHg uptake pathway in *G. bemidjensis*
339 are needed to better understand the role of adsorption in the MeHg demethylation process.

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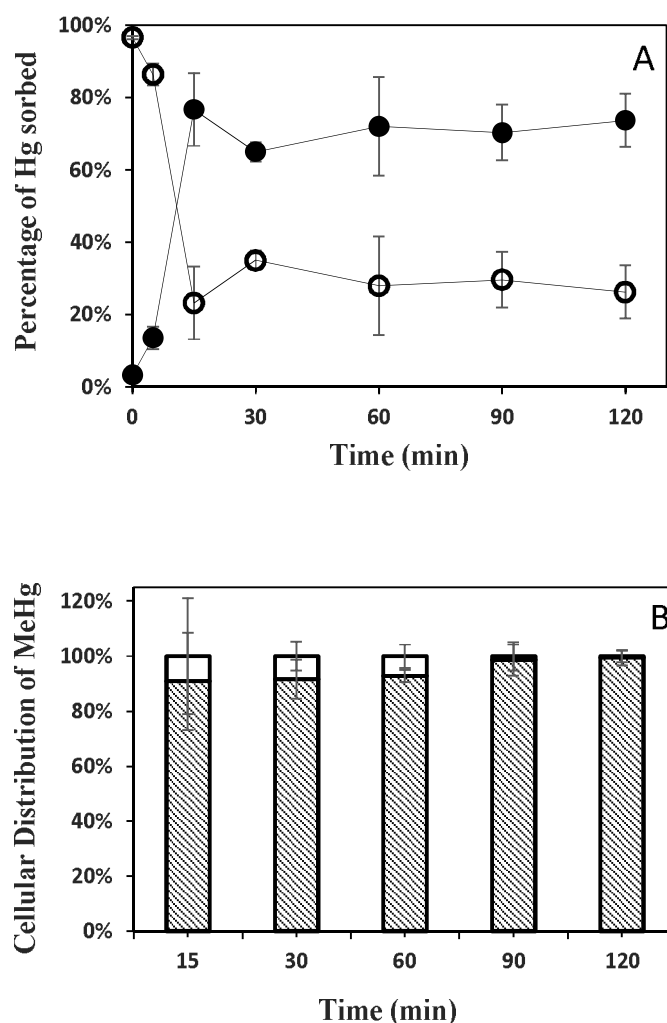


Figure 1. MeHg adsorption onto *Geobacter bemidjiensis* Bem. (A) MeHg adsorbed on *G. bemidjiensis* Bem (closed circles) and the aqueous concentration of MeHg remaining in solution (open circles); (B) Intracellular MeHg (solid white) and cell surface adsorbed MeHg (shaded pattern). Experiments were conducted in MOPS buffer with cell density of 3 g/L (wet wt) and a total MeHg concentration of 50 nM. Error bars represent 1σ values of triplicate experiments.

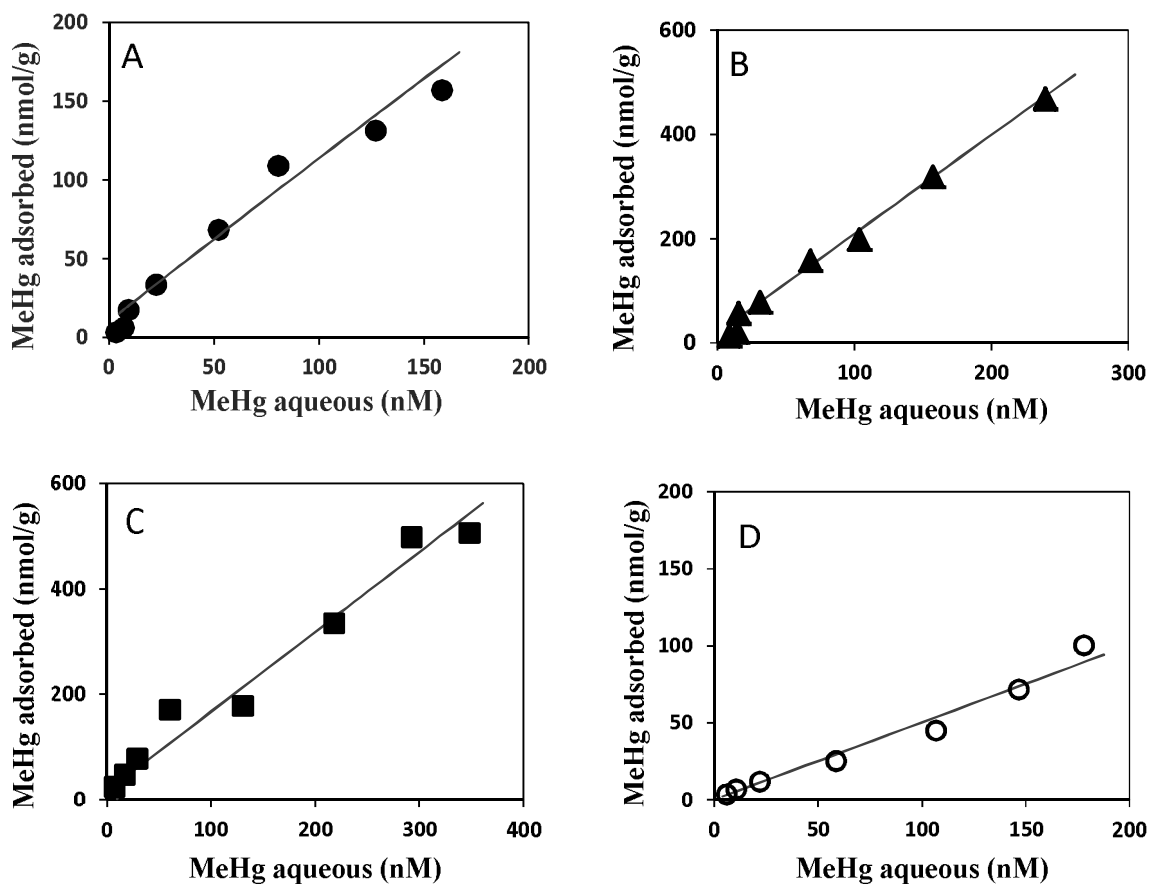


Figure 2. MeHg adsorption isotherms conducted at different cell densities: (A) 3 g/L, (B) 0.6 g/L, (C) 0.3 g/L. (D) MeHg adsorption isotherm conducted with TFP-4 treated cells (3 g/L). The solid black lines represent the best fitting one-site adsorption model.

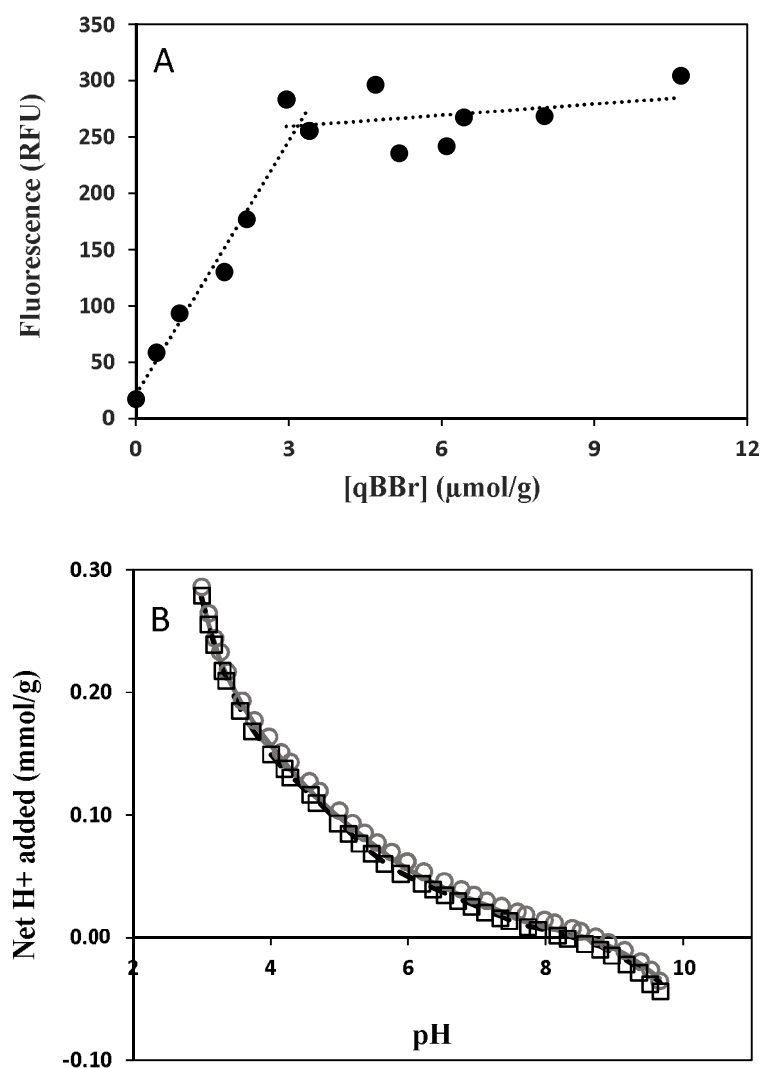


Figure 3. Quantification of reactive thiols on the cell surface of *G. bemitdjensis*. (A) Fluorescence intensities of *G. bemitdjensis* reacted with varying qBBr concentrations. Experiments were conducted at a cell density of 3 g/L and each data point represents an individual experiment. (B) Representative acid-base titration curves of *G. bemitdjensis* cells (open circles) and thiol-blocked cells (open squares). The best-fitting 4-site nonelectrostatic surface complexation model for *G. bemitdjensis* and thiol-blocked cells are depicted by the solid and dashed curves, respectively.

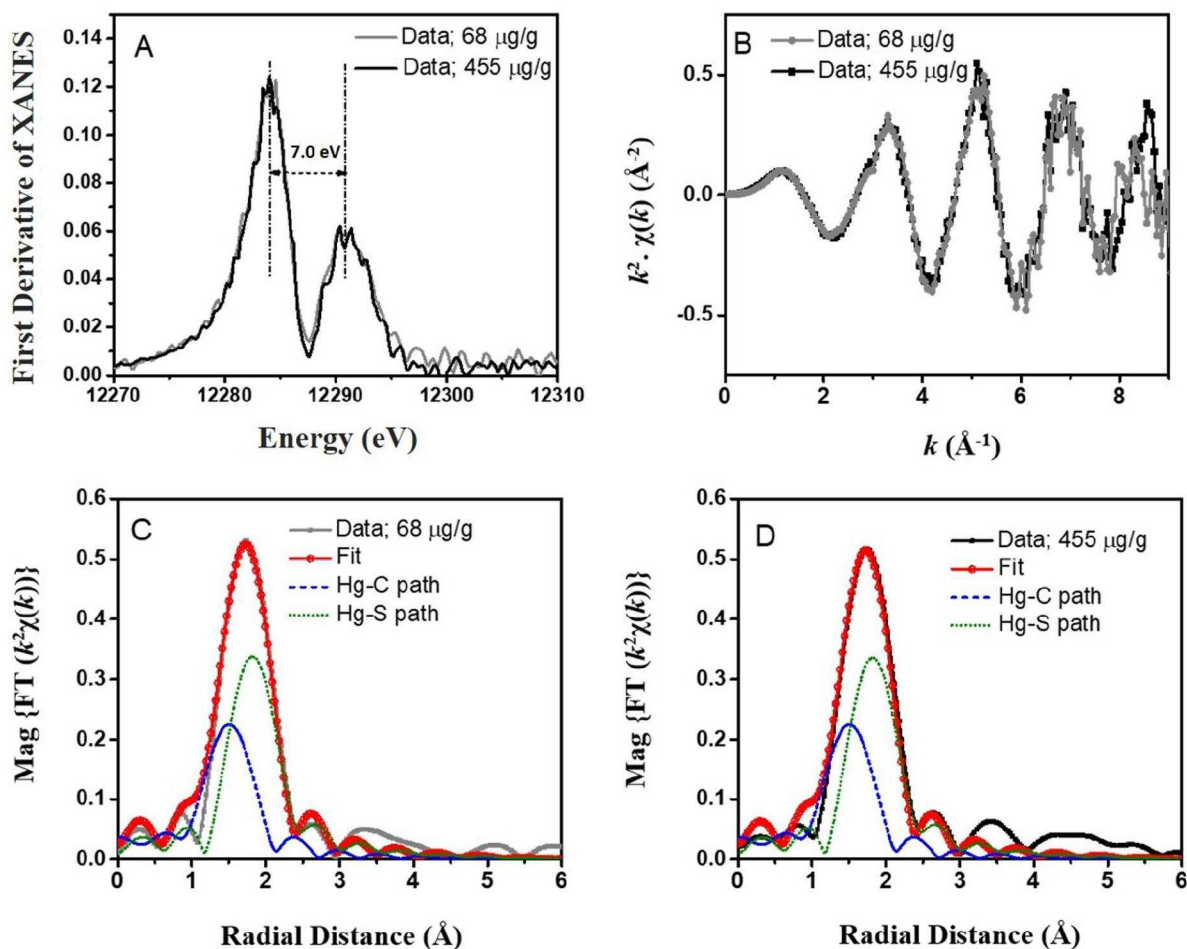


Figure 4. Structure characterization and fit of MeHg-reacted *G. bemidjensis* by X-ray absorption spectroscopy analysis. (A) Derivative of normalized XANES; (B) The k^2 -weighted EXAFS data of 68 $\mu\text{g/g}$ and 455 $\mu\text{g/g}$ MeHg adsorbed bacterial samples; (C) Magnitude of EXAFS Fourier-transformed data and along with Hg-C and Hg-S signal contributions for 68 $\mu\text{g/g}$ samples; and (D) Magnitude of EXAFS Fourier-transformed data and along with Hg-C and Hg-S signal contributions for 455 $\mu\text{g/g}$ sample.

Table 1. Surface site concentrations and acidity constants of *G. bemidjensis* with and without qBBr treatment

	pKa ₁	Sites 1 ($\mu\text{mol/g}$)	pKa ₂	Site 2 ($\mu\text{mol/g}$)	pKa ₃	Site 3 ($\mu\text{mol/g}$)	pKa ₄	Site 4 ($\mu\text{mol/g}$)
Untreated	3.9 \pm 0.4	76.0 \pm 7.6	5.4 \pm 0.3	70.3 \pm 11.1	7.2 \pm 0.2	37.9 \pm 4.0	9.6 \pm 0.1	68.8 \pm 5.1
qBBr-Treated	3.8 \pm 0.2	73.3 \pm 7.5	5.2 \pm 0.1	77.4 \pm 5.3	7.1 \pm 0.1	38.6 \pm 1.0	9.5 \pm 0.1	62.4 \pm 8.4

Table 2. Log K values for MeHg adsorption onto *G. bemidjensis* Bem and thiol-blocked cells.

	Cell density ^a	Surface site	Log K ^b	R ²
<i>Geobacter bemidjensis</i>	3 g/L	Site 4	10.1 \pm 0.2	0.95
	0.6 g/L	Site 4	10.3 \pm 0.1	0.98
	0.3 g/L	Site 4	10.2 \pm 0.1	0.96
Thiol-blocked <i>Geobacter bemidjensis</i>	3 g/L	Site 1	3.8 \pm 0.1	0.96
	3 g/L	Site 2	3.8 \pm 0.1	0.96
	3 g/L	Site 3	4.5 \pm 0.1	0.96
	3 g/L	Site 4	6.4 \pm 0.1	0.96

^awet weight.^bLog K adsorption constants with corresponding 1 σ uncertainty.

Table 3. XAFS best fit values for simultaneous modeling of MeHg adsorbed onto *G. bemidjiensis*

	Path	N	R (Å)	$\sigma^2 \times 10^{-3}$ (Å ²)	ΔE (eV)	S_0^2
68 $\mu\text{g/g}$ MeHg	Hg-C	0.9 ± 0.1	2.03 ± 0.01	3.3 ± 2.1	1.2 ± 1.4	0.9
	Hg-S	0.9 ± 0.1	2.34 ± 0.01	1.8 ± 1.1		
455 $\mu\text{g/g}$ MeHg	Hg-C	0.9 ± 0.1	2.03 ± 0.01	3.3	1.2 ± 1.4	0.9
	Hg-S	0.9 ± 0.1	2.34 ± 0.01	1.8		