Role of Sulfhydryl Sites on Bacterial Cell Walls in the Biosorption, Mobility and Bioavailability of Mercury and Uranium
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Introduction & Research Goals

Bacteria are ubiquitous in a wide-range of low temperature aqueous systems, and can strongly affect the distribution and transport of metals and radionuclides in the environment. However, the role of metal adsorption onto bacteria, via the reactive cell wall functional groups, is perhaps more common and has been largely overlooked. Macroscale studies have been conducted on metal adsorption onto selected bacteria species, and only recently molecular scale X-ray Absorption Spectroscopy (XAS) studies have also been performed to understand the binding mechanisms of metals. All these previous studies have shown carboxyl and phosphoryl functional groups to be the important metal binding groups on bacterial cell walls. However, our preliminary X-ray absorption spectroscopy studies indicated the presence of sulfhydryl groups and identified their chemical state in bacteria and cell surface isolates. Our studies also indicate that chalcophilic soft metal ions, such as Cd^{2+}, can bind to these groups in preference to the more abundant carboxyl and phosphoryl groups on cell walls when metal concentration is low. In addition these thiols can alter the soluble U(VI) to insoluble U(IV).

The goal of this exploratory study is to provide a quantitative and mechanistic understanding of the impact of bacterial sulfhydryl groups on the bacterial uptake, speciation, methylation and bioavailability of Hg and redox changes of uranium. The relative concentration and reactivity of different functional groups present on bacterial surfaces will be determined, enabling quantitative predictions of the role of biosorption of Hg under the physicochemical conditions found at contaminated DOE sites.The hypotheses we propose to test in this investigation are as follows-

1) Sulfhydryl groups on bacterial cell surfaces modify Hg speciation and solubility, and play an important role, specifically in the sub-micromolar concentration ranges of metals in the natural and contaminated systems.

2) Sulfhydryl binding of Hg on bacterial surfaces significantly influences Hg transport into the cell and the methylation rates by the bacteria.

3) Sulfhydryls on cell membranes can interact with hexavalent uranium and convert to insoluble tetravalent species.

4) Bacterial sulfhydryl surface groups are inducible by the presence of metals during cell growth.

Our studies focused on the first hypothesis, and we examined the nature of sulfhydryl sites on three representative bacterial species: *Bacillus subtilis*, a common gram-positive aerobic soil species; *Shewanella oneidensis*, a facultative gram-negative surface water species; and *Geobacter sulfurreducens*, an anaerobic iron-reducing gram-negative species that is capable of Hg methylation; and at a range of Hg concentration (and Hg:bacterial concentration ratio) in which these sites become important. A summary of our findings is as follows-

- Hg adsorbs more extensively to bacteria than other metals. Hg adsorption also varies strongly with pH and chloride concentration, with maximum adsorption occurring under circumneutral pH conditions for both Cl-bearing and Cl-free systems. Under these conditions, all bacterial species tested exhibit almost complete removal of Hg from the experimental solutions at relatively low bacterial concentrations (Fig. 1).
- Synchrotron based X-ray spectroscopic studies of these samples indicate that the structure and the coordination environment of Hg surface complexes on bacterial cell walls change dramatically- with sulfhydryls as the dominant Hg-binding groups in the

micromolar and submicromolar range, and carboxyls and phosphoryls dominating at high micromolar concentrations (Fig. 2).

- Hg interactions change from a trigonal or T-shaped HgS_3 complex to HgS or HgS_2 type complexes as the Hg concentration increases in the submicromolar range. Although all bacterial species studied exhibited the same types of coordination environments for Hg, the relative concentrations of the complexes change as a function of Hg concentration (Fig. 2).

Hg Reactions with Bacterial Surfaces: Details of Macroscopic Sorption Measurements

During the past year, we conducted a range of bulk adsorption experiments involving aqueous Hg^{2+} and several representative species of non-metabolizing bacteria in order to determine the extent of passive adsorption, and the reversibility of the adsorption reactions. We conducted experiments as a function of pH and Cl^- concentration in order to determine the effects of bacterial surface site protonation and aqueous Hg-chloride and Hg-hydroxide complexation on the extent of Hg adsorption onto bacterial cell walls. We also conducted experiments as a function of the Hg:bacteria concentration ratio in order to probe the adsorption behavior in the low-loading conditions under which sulfhydryl binding of Hg dominates. We conducted all of these experiments using three representative bacterial species: *Bacillus subtilis*, *Shewanella oneidensis*, and *Geobacter sulfurreducens*. We conducted potentiometric titration experiments using the *Geobacter* species in order to determine the concentrations and acidity constants for the important binding sites on the *Geobacter* cell wall. These properties have been determined by our group in the past for both *B. subtilis* and *S. oneidensis*. The adsorption experiments in this study probe whether cell wall structural differences manifest themselves as differences in Hg adsorption behavior and binding mechanisms, and the experiments also probe whether Hg methylating bacteria exhibit significantly different Hg adsorption behavior than non-methylating species, and whether the newly-characterized sulfhydryl site plays a controlling role in bacterial Hg methylation.

Our experiments demonstrate extensive and strong Hg adsorption onto each of the species of bacteria tested (Figure 1). For example, under similar experimental conditions to those shown in Figure 1, *B. subtilis* and *S. oneidensis* would adsorb more than an order of magnitude less Cd and Pb than they do Hg under most pH conditions studied (Borrok and Fein, 2005). The extent of Hg adsorption varies strongly with pH and chloride concentration, with maximum adsorption occurring under circumneutral pH conditions for Cl-bearing systems, and at approximately pH 4-5 in Cl-free systems. Figure 1 depicts the effects of pH and chloride on the extent of Hg adsorption by *B. subtilis* and *S. oneidensis*.

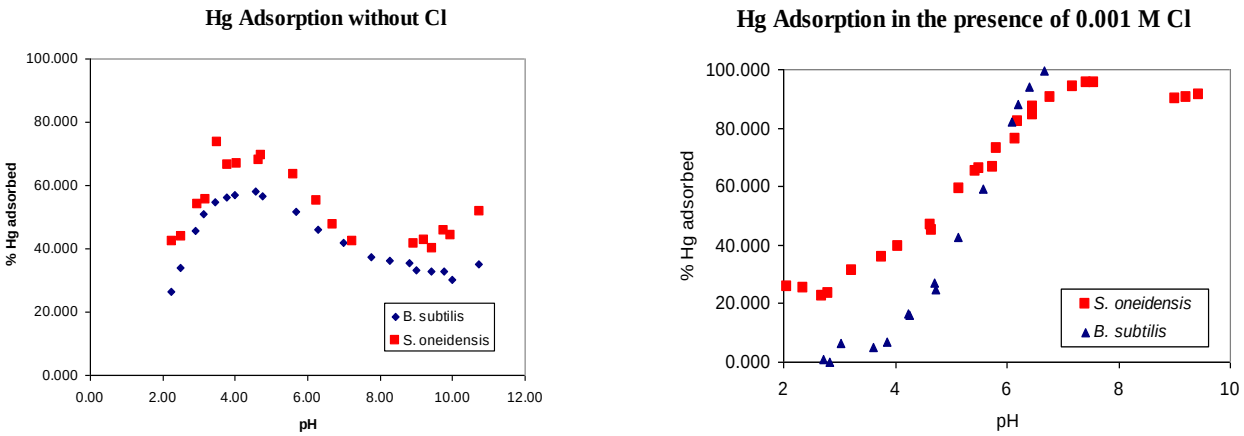

Figure 1. Adsorption of Hg onto gram-positive *B. subtilis* (blue symbols) and gram-negative *S. oneidensis* (red squares) cells, in the absence (left) and presence of 0.001 M Cl (right). Experimental conditions: 0.2 g/L cells; 75 μM Hg; 0.1 M $NaClO_4$ electrolyte to buffer ionic strength.

In general, the bacterial species tested exhibit similar bulk adsorption behaviors, although *S. oneidensis* does remove up to 10 – 20% more Hg from solution than *B. subtilis*. In the absence of Cl^-, the pH dependence is more complex than the behavior of other metal cations (e.g., Fein et al., 1997; Fein et al., 2001), with the extent of adsorption increasing from pH 2 to 4, and in general decreasing from 4 to 9. The pH behavior likely reflects both changes to the speciation of the cell wall functional groups and changes in the aqueous Hg speciation as a function of pH. Interestingly, with the addition of Cl^- to the system, the bulk adsorption behavior changes dramatically to that typical of metal cation adsorption behavior, even though the dominant aqueous Hg species are neutral or negatively charged. The addition of Cl^- significantly decreases Hg adsorption onto the bacteria under low pH conditions, and dramatically increases the extent of Hg adsorption above pH 6. We use the experimental measurements, in conjunction with the EXAFS results from this study, to construct site-specific surface complexation models of Hg adsorption onto bacteria that constrain the thermodynamic stability of the important Hg-bacterial surface complexes. We can use the results not only to compare the thermodynamic stabilities of the surface complexes of one bacterial species to another, but also to compare the Hg bacterial complex stabilities to those of other metals studied previously. Preliminary results from the surface complexation modeling suggest that both aqueous and bacterial surface speciation affect Hg adsorption, and the modeling yields stability constants for the Hg-bacterial surface complexes that are similar in magnitude to those for U-bacterial complexes, suggesting that Hg can out-compete a wide range of divalent metal cations for available binding sites on bacterial cell walls under most conditions of geologic interest.

Hg Reactions with Bacterial Surfaces: Structure and Coordination Environment of Hg on Bacteria Surfaces.

To evaluate the structure of Hg on bacterial surfaces, we examined Hg reactions with *Bacillus subtilis*, and *Shewanella oneidensis*. We exposed these bacterial suspensions (10^{10} $cellsL^{-1}$) to different concentrations of Hg^{2+} (120 nM to 350 μM) around pH 6. The ratio of Hg concentration to bacterial cell densities used in these experiments are similar to those

encountered in contaminated soils and ground water (Schaefer et al., 2004). The structure and coordination environments of Hg on bacterial surfaces was analyzed using synchrotron based X-ray Absorption Near Edge Structure (XANES), and Extended X-ray Absorption Fine Structure spectroscopy (EXAFS) spectroscopy at the Hg L_3 edge. The chemical forms of thiol groups of bacteria and their cell wall isolates were identified using XANES Spectroscopy at the S K-edge. The S-XANES spectroscopy studies were also conducted on the intact cells and their cellwall isolates of *Shewanella oneidensis* and *Geobacter sulfurreducens* grown under nitrate, fumerate, and Fe reducing conditions.

Our studies indicate that Hg adsorbs strongly on all examined bacterial surfaces, and their uptake of Hg from aqueous solutions was almost complete. The Hg-XANES spectra indicate that the electronic state and coordination environment of Hg complexes on bacterial surfaces change significantly as a function of Hg concentration above 0.5 μM, and with minimal changes below this concentration. The Hg-EXAFS spectra indicate that Hg complexes entirely with cysteine groups at the nanomolar and low micromolar concentrations, and carboxyls and/or phosphoryls at high micromolar concentrations (Fig. 2). In addition, the structure of cysteine complex changes from primarily HgS_3 to HgS_2 and HgS (where S = cysteine) complex as the bacteria were exposed to Hg at sub-micromolar concentration (Fig. 2). This result takes special significance in light of the fact that other studies have shown that the tolerance level of Hg toxicity for *Shewanella oneidensis* is around 0.5 μM.

Based on the spectral features of the $\chi(k)$ (phase shift and shape of oscillations) and real part of the Fourier transformed data (increase in nearest neighbor bond length), coordination environment of Hg can be broadly divided into three concentration ranges, a) 350-50 μM, b) 25-5.0 μM, and c) 2.5-0.5 μM (Fig. 2b and 2c). The Hg-EXAFS analysis indicates that Hg complexes bind predominantly with cysteine groups below 25 μM and with carboxyls and/or phosphoryls groups above 25 μM Hg concentrations. However, the average coordination environment of Hg changes dramatically from Hg-S for 25 μM to Hg-S_2 for 15-5.0 μM and Hg-S_3 for 0.5 μM Hg concentrations. These changes are reflected in average bond length of the nearest neighbor atom which increases from 2.02 Å for 350 μM sample to 2.51 Å for 0.5 μM sample. The combination of coordination number, bond length, and Debye-Waller parameter (σ^2) makes a clear case for the formation of T-shaped distorted trigonal Hg-S_3 type complex at Hg concentration less than 0.5 μM Hg.

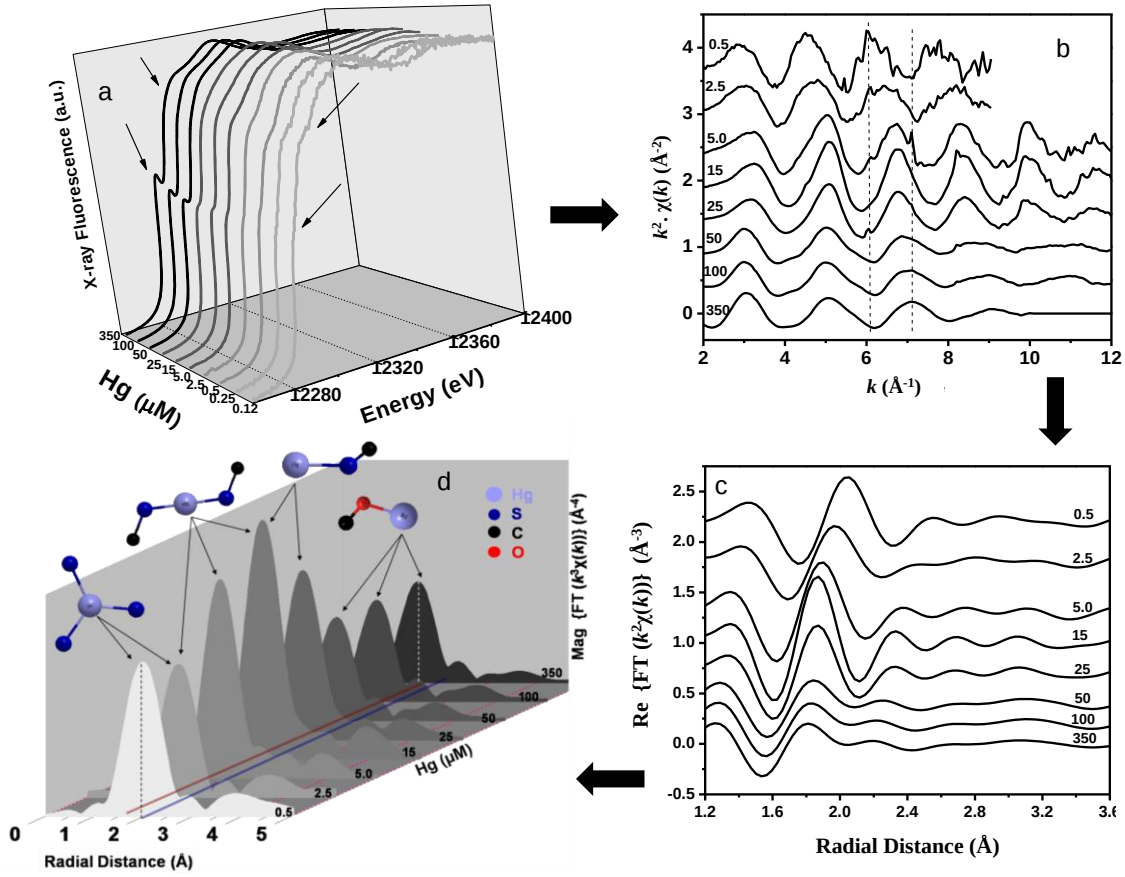

Figure 2. a) Hg L_3 edge XANES, b) k^2 weighted $\chi(k)$ data, c) real part of the Fourier transform, and d) Fourier transform magnitude of EXAFS data for Hg adsorption to *Shewanella oneidensis* as a function of adsorbed Hg concentration at pH 5.5 (± 0.2). The red and blue lines in the Fourier transform magnitude of EXAFS data correspond to 2.02 and 2.51 Å (phase corrected), respectively. A systematic change in the binding of Hg from Hg-S_3, Hg-S to Hg-carboxyl complex was observed with increasing Hg concentration in both XANES (pointed with thin arrows in "a") and EXAFS spectra, a trend that was observed for all bacterial species examined. Cell density in this study was 10^{10} cell/L.

The Hg-reacted bacterial cells and their cell wall isolates also indicate that their S-XANES spectra change significantly as a function of increasing Hg concentration. These spectral changes are similar to those associated with the deprotonation and Hg-complexation of cysteine (Fig. 3). Together, the Hg-EXAFS and XANES, and S-XANES spectroscopy studies indicate that Hg forms stable HgS_3 complexes with the thiol group in cysteine on bacterial cell surfaces.

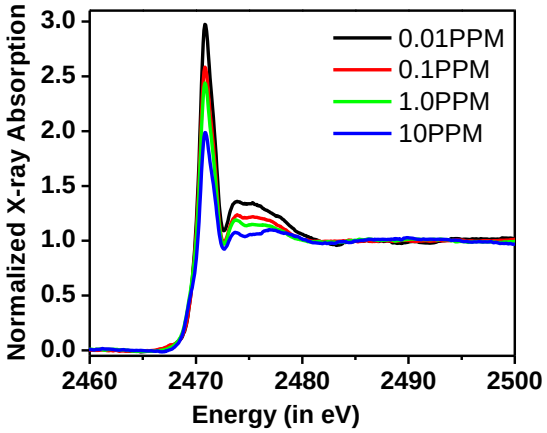

Figure 3: Normalized S-XANES spectra for *Shewanella oneidensis* (10^{10} cell/L) exposed to varying amounts of Hg. Experiments were conducted at pH 5.5 (± 0.2). As the Hg concentration increases, spectral changes in S indicate that the electronic state of reduced S changes. The intensity drop at ~ 2471 eV, and the appearance of small shoulder at 2470 eV indicate cysteine deprotonation, and Hg complexation (Myneni, 2002).

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