

Imaging and analysis of biominerals and nanostructures associated with bacterial membranes

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Electron microscopy-based imaging and analytical methods have been used in characterization of the process of metal reduction by the dissimilatory metal-reducing bacteria *Shewanella oneidensis* MR-1. This deep subsurface organism is capable of reducing various metals and radionuclides, such as uranium (VI) and technetium (VII) into their less mobile forms as a mode of energy-yielding respiration. This capacity makes *Shewanella* an attractive candidate for potential *in-situ* remediation of uranium contaminated soils and groundwater.

Ultrastructure, composition, and mechanisms of cellular and extracellular components involved in metal reduction and precipitation are not completely understood. In order to evaluate the role of the Type II secretion (T2S) general secretion pathway in enzymatic reduction of U, experiments comparing a wild type cultures with mutants lacking key components of the T2S were conducted. Electron microscopy showed evidence of different sites of deposition of reduced nanocrystalline U(IV) material in the wild type and mutants. Newly formed biominerals were not only coupled with the cell membranes and periplasm, but also linked to extracellular filamentous structures. Evaluation of these structures, as well as analysis of minerals and proteins associated with solid phase uranium will have important implications for the fate and transport of bioreduced radionuclides in the subsurface.

Metal adsorption onto bacterial cell walls: Testing universal adsorption behavior over a wide range of bacterial diversity

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Bacteria are present in virtually all near-surface environments, and adsorption onto their cell walls can significantly affect metal speciation and mobility in those systems. However, the number of species present in geologic systems is enormous and undetermined. Modeling the effects of bacterial surface adsorption in natural systems would be an impossible task if each bacterial species exhibited unique adsorption behavior. Yee and Fein (2001) observed similar adsorption behavior for a range of gram-positive and gram-negative species of bacteria. However, the bacteria that they investigated came from only two phyla. Similarly, Borrok et al. (2004) observed similar behavior for a range of natural consortia. However, their study did not determine the genetic diversity present in each consortia. More studies, using a wider diversity of bacteria, are necessary to determine whether all bacteria adsorb metals to a similar extent.

We performed potentiometric titrations and metal adsorption experiments using bacteria from 5 phyla. The bacteria we used included acidophiles, psychrophiles, and thermophiles. All experiments were conducted using a 0.1 M NaClO₄ electrolyte to buffer ionic strength. The titrations were performed using an automated burette under a N₂ atmosphere. Small aliquots of NaOH were added to a bacterial solution and the pH was measured after each addition. Metal (Cd, Pb, and Sr) adsorption experiments were conducted as functions of pH and solute:sorbent ratio. We use the titration results to constrain the acidity constants and site concentrations of each species, and the metal adsorption results constrain the values of the thermodynamic stability constants for the important metal-bacterial surface complexes.

Our data shows that genetically diverse bacteria show similar behavior to a remarkable extent despite known differences in their cell wall structure. This implies we can use a single model to predict metal sorption onto cell walls.

References

- Borrok D.M., Fein J.B., and Kulpa C.F. (2004) *Geochim. Cosmochim. Acta* **68**, 3231-3238.
Yee N., and Fein J.B. (2001) *Geochim. Cosmochim. Acta* **65**, 2037-2042.