

# Role of Sulfhydryl Sites on Bacterial Cell Walls in the Biosorption, Mobility and Bioavailability of Mercury and Uranium

## Principle Investigator

Satish C. B. Myneni, Department of Geosciences, Princeton University, Princeton, NJ 08544; e-mail: smyneni@princeton.edu

## Co-Investigators

Jeremy Fein, Department of Civil Engineering & Geological Sciences, University of Notre Dame, Notre Dame, IA 46556; e-mail: fein@nd.edu

Bhoopesh Mishra, Argonne National Laboratory, Argonne, IL; e-mail: bhoopeshm@gmail.com

## Participated Researchers

Princeton University: R. Sanders, N. Crompton, M. Frith, E. Schoenfelt, C. M. Joe-wang  
University of Notre Dame: S.M.D Cheatham, J. Szymanowski, Q. Yu, R. Nell

## Publications

Nell, R.M., and Fein, J.B. (2016, submitted) Influence of sulfhydryl sites on metal binding by bacteria. *Geochim. Cosmochim. Acta*.

Mishra B., Shoenfelt E., You Q., Yee N., Fein J.B., Myneni S.C.B. (2016, submitted) Stoichiometry of mercury-thiol complexes on bacterial cell envelopes. *Chem. Geol.*

Dunham-Cheatham S., Farrell, B., Mishra B., Myneni S., and Fein J.B. The effect of chloride on the adsorption of Hg onto three bacterial species. *Chem. Geol.* 373, 106-114. (2014).

Dunham-Cheatham S., Mishra B., Myneni S., and Fein J.B. The effect of natural organic matter on the adsorption of mercury to bacterial cells. *Geochim. Cosmochim. Acta* 150, 1-10. (2014)

Myneni S.C.B., Hay M.B., Mishra B. Applications of scanning transmission X-ray microscopy in studying clays and their chemical interactions. In *Advanced Applications of Synchrotron Radiation in Clay Science*. Clay Mineral Society special volume, V 19 (2014).

Nell, R.M., Szymanowski J.E.S., and Fein, J.B. The effects of bacterial surface adsorption and exudates on HgO precipitation. *Geomicrobiol. Journal*. DOI: 10.1080/01490451.2015.1043411 (2015).

von der Heyden, B.P., Hauser E.J., Mishra B., Martinez G.A., Bowie A.R., Tyliczszak T., Mtshali T.N., Roychoudhury A.N., Myneni S.C.B. Ubiquitous presence of Fe(II) in aquatic colloids and its association with organic carbon. *Environ. Sci. & Technol. Letters* 1: 387-392 (2014).

Yu, Q., and Fein, J.B. Sulfhydryl binding sites within bacterial extracellular polymeric substances. *Environ. Sci. Technol.* 50, 5498-5505. (2016).

Yu, Q., and Fein, J.B. The effect of metal loading on Cd adsorption onto *Shewanella oneidensis* bacterial cell envelopes: The role of sulfhydryl sites. *Geochim. Cosmochim. Acta* 167, 1-10. (2015).

Yu, Q., Szymanowski, J., Myneni S.C.B., and Fein, J.B. Characterization of sulfhydryl

sites within bacterial cell envelopes using selective site-blocking and potentiometric titrations. *Chem. Geol.* 373, 50-58. ((2014)

Joe-Wong C., Shoenfelt E., Hauser EJ., Crompton N., Myneni SCB. Estimation of reactive thiol concentrations in dissolved organic matter and bacterial cell membranes in aquatic systems. *Environ. Sci. Technol.*, 46: 9854-9861 (2012).

### Presentations

The PIs and their group members gave over 20 research presentations on the obtained results in different international geochemistry conferences, and at different academic institutions.

### Research Objectives

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, has been largely overlooked. Previous macroscale metal sorption, and XAS studies have shown that carboxyl and phosphoryl functional groups to be the important metal binding groups on bacterial cell walls and the sulfhydryl groups were not considered. The goal of our investigation was to evaluate the density of the sulfhydryl sites on different bacterial cell membranes that are common to soil systems, the binding affinities of these reactive groups towards Hg, and how this binding modifies the speciation of Hg in the natural waters.

### Research Summary

The study used a variety of Gram-positive and Gram-negative bacterial species, and both macroscale and spectroscopic studies in exploring the cell membrane bound sulfhydryls (thiols) and their Hg interactions. The funded research lead to significant progress in elucidating the nature and importance of sulfhydryl sites within bacterial cell envelopes for binding metals at very low metal loadings. The research provided a foundation for understanding Hg binding onto bacterial cells, and the experiments lead to calculations of stability constants for the important Hg-bacterial surface complexes. We also quantified the role of natural organic matter (NOM), the key player known to play a predominant role in Hg speciation via sulfhydryl binding, in competing with bacterial cell envelope binding sites for the binding of Hg, and developed a model that can account for this competition over a wide range of Hg:NOM concentration ratios. Results from this study represent a crucial foundation for quantifying Hg binding onto bacteria and for relating Hg binding to processes such as Hg methylation, which may be controlled by Hg binding. In addition, these results are applicable to other metal-cell membrane interactions.

Specifically our findings from the funded project are as follows:

a) We developed fluorescence spectroscopy-, and potentiometric titration-based methods for the determination of sulfhydryls in the bacterial cell membranes. These methods are complementary, and can be applied to any types of cells. Our results suggest that the thiol density varies widely with the organism, and the growing conditions and the growth stages of the organism. Our studies suggest that the abundance of sulfhydryl sites on a range of environmentally-relevant bacterial species is relatively low- micromoles of

thiols/g of cells- when compared to other binding sites. However, as discussed later, this is significant for binding soft metals. In addition, we also found that sulfhydryl concentration is higher on bacterial species capable of Hg methylation.

b) Using the abundance of thiols in the cell membrane and Hg sorption studies, we arrived at the stability constants for Hg-sulfhydryl complexes that form within bacterial cell envelopes. These studies demonstrated that sulfhydryl sites dominate metal binding onto bacteria under low metal loading conditions, and the funded research was the first to quantify stability constants for metal-sulfhydryl complexes that form within bacterial cell envelopes. Our results indicate that these stability constants can be orders of magnitude greater than stability constants for non-sulfhydryl site complexes. Therefore, although the concentration of sulfhydryl sites within bacterial cell envelopes is typically a small fraction of the total binding site concentration, sulfhydryl site binding can dominate the budget of adsorbed metal under low metal loading conditions where all binding sites are available.

c) XAS studies of Hg binding to sulfhydryls on cell membranes showed a major variation with the bacterial species and Hg loadings. We found that Hg formed multidentate  $\text{Hg}(\text{thiol})_3$ - $\text{Hg}(\text{thiol})_2$  - Hg-thiol complexes progressively as a function of increasing Hg loadings in different cells. However, we did not observe  $\text{Hg}(\text{thiol})_3$  complexes in the case of methylating organism. These results are significant and have profound implications for Hg transport and methylation, and these trends should be tested for other bacterial species that are not considered in this study.

d) Using the methods we developed, we also attempted to quantify the concentration of sulfhydryl sites within bacterial extracellular polymeric substances (EPS). We found quite different results for different bacterial species studied: *Pseudomonas putida* possesses sulfhydryl sites exclusively within its EPS molecules, with the concentration of sulfhydryl sites on the cell wall below the detection limit of our approach. Conversely, *Shewanella oneidensis* has virtually no sulfhydryl sites within its EPS molecules, but has all of its sulfhydryl sites on its cell wall. Differences in the role of metals in each bacterial metabolism may account for the observed differences, but more data are needed to corroborate this interpretation.

In summary results obtained from this investigation provides quantitative methods for the estimation of the abundances of reactive sulfhydryls on cell membranes and their binding with Hg. These results have important implications on the uptake, speciation, transport and bioavailability of Hg in the environment.