X-ray and Electron Micro(spectro)scopy Investigations of Spatial Distributions of Elemental Concentrations and Valence States in Bacteria

Ken Kemner[1] (PI), Shelly Kelly[1], Ed O'Loughlin[1], Max Boyanov[1], Ken Nealson[2], Susan Glasauer[3], Terry Beveridge[3], Barry Lai[1], Joerg Maser[1], Zhonghou Cai[1]

[1]Argonne National Laboratory, Argonne, Illinois; [2]University of Southern California, Los Angeles, CA; [3]University of Guelph, Guelph, Ontario, Canada

Bacteria and the extracellular material associated with them are thought to play a key role in determining a contaminant's speciation and thus its mobility in the environment. In addition, the metabolism and surface properties of bacteria can be quite different when the bacteria exhibit a planktonic (free-floating) versus a biofilm (surface-adhered) habit. The microenvironment at and adjacent to actively metabolizing cells also can be significantly different from the bulk environment. Thus, to understand the microscopic physical, geological, chemical, and biological interfaces that determine a contaminant's macroscopic fate, we must characterize, at micron and submicron length scales, the spatial distribution and chemical speciation of contaminants and elements that are key to biological processes. Hard x-ray microimaging is a powerful technique for highly sensitive element-specific investigations of complex environmental samples at the needed micron and submicron resolutions. Moreover, the greater spatial resolution of electron microscopy enables investigations of geomicrobial systems beyond those afforded by x-ray microprobes. Combining electron and x-ray microscopies provides even more advantages for investigating geomicrobial systems than using either technique alone. The objectives of the studies presented here are to (1) determine the spatial distribution, concentration, and chemical speciation of metals at, in, and near bacteria and bacteria-geosurface interfaces, (2) use this information to identify the metabolic and/or chemical processes occurring within the microbes, and (3) identify the interactions occurring near the interfaces among the metals, mineral, and bacterial surfaces, and bacterially produced extracellular materials under a variety of conditions. We have used x-ray fluorescence microscopy to investigate the spatial distribution of 3*d* transition metal elements in *Pseudomonas fluorescens* in free-floating (i.e., planktonic) and surface-adhered states. We have also investigated the valence state of Cr associated with these cells after exposure to toxic levels of Cr(VI) (1,000 ppm for ~6 hr). We have used electron and x-ray microscopies to investigate single *Shewanella oneidensis* MR-1 cells that have been shown to produce internal precipitates rich in biomineralized Fe [1]. The x-ray zone plate used in these microscopy experiments produced a focused beam with a cross section (and hence spatial resolution) of 0.15–0.30 μm.

Results from x-ray fluorescence imaging experiments with *P. fluorescens* indicate that the distribution of P, S, Cl, Ca, Fe, Ni, Cu, and Zn can define the location of the microbe. Quantitative analysis of the elemental concentrations within cells can indicate the viability and metabolic state of the cells. X-ray absorption near edge spectroscopy (XANES) investigations of the Cr valence state indicated two different modes of abiotic chemical reduction of the element (depending on the microbe's physiological state). Fe K-edge XANES measurements of internal precipitates in the *S. oneidensis* MR-1 cells identified the valence state of the precipitates. Additional Fe XANES measurements of precipitates external to the *S. oneidensis* MR-1 cells determined an average Fe valence state that is different from the internal average Fe valence state. These results will be presented, with a discussion of the use of combined x-ray and electron micro(spectro)scopies for identifying metabolic states of individual microbes within communities.

1. S. Glasauer, S. Langley, T.J. Beveridge, "Intracellular iron minerals in a dissimilatory iron-reducing bacterium," Science 295 117-119, 2002.