Use Of The High-Energy X-ray Microprobe At The Advanced Photon Source To Investigate The Interactions Between Metals And Bacteria

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Abstract. Understanding the fate of heavy-metal contaminants in the environment is of fundamental importance in the development and evaluation of effective remediation and sequestration strategies. Among the factors influencing the transport of these contaminants are their chemical speciation and the chemical and physical attributes of the surrounding medium. 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 microenvironment at and adjacent to actively metabolizing cell surfaces can be significantly different from the bulk environment. Thus, the spatial distribution and chemical speciation of contaminants and elements that are key to biological processes must be characterized at micron and submicron resolution in order to understand the microscopic physical, geological, chemical, and biological interfaces that determine a contaminant's macroscopic fate. Hard X-ray microimaging is a powerful technique for the element-specific investigation of complex environmental samples at the needed micron and submicron resolution. An important advantage of this technique results from the large penetration depth of hard X-rays in water. This advantage minimizes the requirements for sample preparation and allows the detailed study of hydrated samples. This paper presents results of studies of the spatial distribution of naturally occurring metals and a heavy-metal contaminant (Cr) in and near hydrated bacteria (*Pseudomonas fluorescens*) in the early stages of biofilm development, performed at the Advanced Photon Source Sector 2 X-ray microscopy beamline.

ENVIRONMENTAL RESEARCH

Chemical contamination of soil and groundwater is a universal problem of immense complexity and great global concern. Sources of contamination include past and present agricultural and industrial activities, operations at national defense sites, and mining and manufacturing processes. Assessment of thousands of hazardous waste sites in the United States alone (including over 1,200 on the National Priority List) has identified the presence of an array of toxic substances. These include heavy metals (such as Pb, Cr, As, Zn, Cu, Cd, Ba, Ni, and Hg), radionuclides (including U, Pu, Sr, Cs, Co, and Tc), and potentially hazardous anions such as arsenate, chromate, and selenate.[1] The

restoration of soils and groundwaters that have been contaminated by combinations of these stable and radioactive substances (e.g., mixed wastes) presents significant scientific and engineering challenges, because the interactions among the contaminants are unknown.

THE NEED FOR SUBMICRON HIGH-ENERGY X-RAY MICROPROBES IN ENVIRONMENTAL AND MICROBIOLOGY RESEARCH

A current focal point of molecular environmental science involves the pathways, products, and kinetics of chemical reactions of contaminant species with inorganic and organic compounds, plants, and organisms in the environment.[1] These reactions often occur at aqueous solution-solid interfaces and can have many different results. The contaminant can be precipitated from the solution to the solid interface, transformed into a different species, incorporated into a solid phase, or released from the solid surface into the solution. Such interfacial reactions play a very important role in the transport and dispersal of toxic species in soils and natural waters. Therefore, discovering what is occurring at these interfacial surfaces is key to understanding the bioavailability of many contaminants. Despite this importance, these surfaces and their associated chemical reactions are not well understood. Consequently, little is known about the mechanisms by which biota, in particular microorganisms, determine the speciation, forms, reaction rates, and distribution of contaminants in soils and groundwater.

The heterogeneity of most environmental samples makes their study very difficult. Because environmental samples are almost always hydrated, high-energy X-rays having the ability to penetrate water are very useful. In addition, it can be valuable to probe both sides of the interfaces in these heterogenous samples to elucidate transformations that result in the movement of the contaminant across the interface. Thus, the smallest possible probe is needed to analyze the homogeneous region on either side of the interface selectively. These requirements make the use of micron and submicron X-ray beams advantageous. One difficulty encountered in investigating the contaminant-microbe interface is sample heterogeneity. For instance, in such a system, the metal contaminant may be bound in a variety of ways: (1) in solution, (2) to extracellular material, (3) within cell membrane regions, or (4) within the bacterium. To study the spatial distribution and chemical speciation of a contaminant metal at the microbe-metal interface and thus to elucidate the interactions occurring at this interface, the dimension of the X-ray probe must allow the vast majority of the X-rays to be positioned at the contaminant-microbe-mineral interface. The size of most bacteria is approximately 1 μm. Therefore, to investigate the speciation and spatial distribution of elements associated with bacteria, the dimensions of the X-ray probe must be smaller than 1 μm. Finally, although soft X-ray microprobes have considerable utility for studying biological samples,[2] hard X-ray microprobes provide improved fluorescence yields, better penetration of hydrated samples, and access to the K edges of third-row and heavier elements. Many of these elements are important nutrients, micronutrients, and environmental contaminants.

Third-generation X-ray sources such as the Advanced Photon Source (APS), where our experiments were performed, provide an increase in brilliance of approximately three orders of magnitude compared to second-generation synchrotron X-ray sources. In addition, advances in microfabrication technologies have resulted in X-ray phase zone plates[3] with spatial resolution better than 0.20 μm and focusing efficiency better than 33%. The combination of the increased brilliance of X-ray beams provided by the APS and improved zone plate fabrication technology provides unique capabilities in X-ray microscopy and spectromicroscopy.

THE USE OF X-RAY MICROPROBES FOR INVESTIGATING THE ROLE OF BACTERIA AND THEIR ORGANIC EXUDATES IN CONTAMINANT TRANSPORT

The objectives of our studies are (1) to determine the spatial distribution and chemical speciation of metals near bacteria-geosurface interfaces and (2) to use this information to identify the interactions occurring near these interfaces among the metals, mineral surfaces, and bacterially produced extracellular materials under a variety of conditions. We have used hard X-ray phase zone plates to investigate the spatial distribution of 3*d* elements in a single hydrated *Pseudomonas fluorescens* bacterium adhered to a Kapton film. The zone plate used in these microscopy experiments produced a focused beam of cross section 0.15 μm^2 and had an effective focal length of 12.5 cm at 10.0 keV. The samples were mounted on a computer-controlled XYZ piezo stage at 10 degrees to the incident beam, thus negligibly affecting the X-ray footprint on the sample in the horizontal dimension. The intensity of the fluorescence radiation from the sample was monitored by a single-element solid-state detector that enables efficient detection of fluorescent X-rays with energies greater than 1.5 keV. The elements were mapped by scanning this sample in 0.15-μm steps through the focused monochromatic X-ray beam and integrating the selected Kα fluorescence for 5 sec/pt. The total data collection time was approximately 6 hours.

Figure 1 shows results of the X-ray microprobe measurements, qualitatively indicating the spatial distributions of Cr, K, and Ca in and near a hydrated *Pseudomonas fluorescens* bacterium, adhered to a Kapton film at ambient temperature, that was exposed for 6 hours to Cr 1000 ppm in solution. Observation of these images indicates that monitoring the spatial distribution of the K and Ca Kα fluorescent radiation coming from the sample enabled identification of the rod-shaped *Pseudomonas fluorescens*, as well as the extracellular exudes associated with it. In addition, comparison of the distribution of Cr with that of K or Ca indicates that the majority of the Cr in this sample was associated extracellularly. Because most of the Cr remained outside the cell, the Cr(VI) was probably not actively metabolized. Finally, although these results demonstrate the utility of imaging hydrated bacteria at ambient temperature, a cryostat might be required to quick-freeze the samples in future spectromicroscopy studies in order to reduce the effects of radiation damage.

SUMMARY

We have demonstrated the utility of X-ray microbeams, particularly those produced by hard X-ray phase zone plates, for investigating biological and environmental systems. Specifically, we have illustrated the use of submicron hard X-ray beams (0.15 μm) for determining the spatial distribution of metals in a hydrated bacterium that was exposed to Cr 1000 ppm in solution for six hours. The further development of these techniques for such applications promises to provide unique opportunities in the field of microbiology and environmental research.

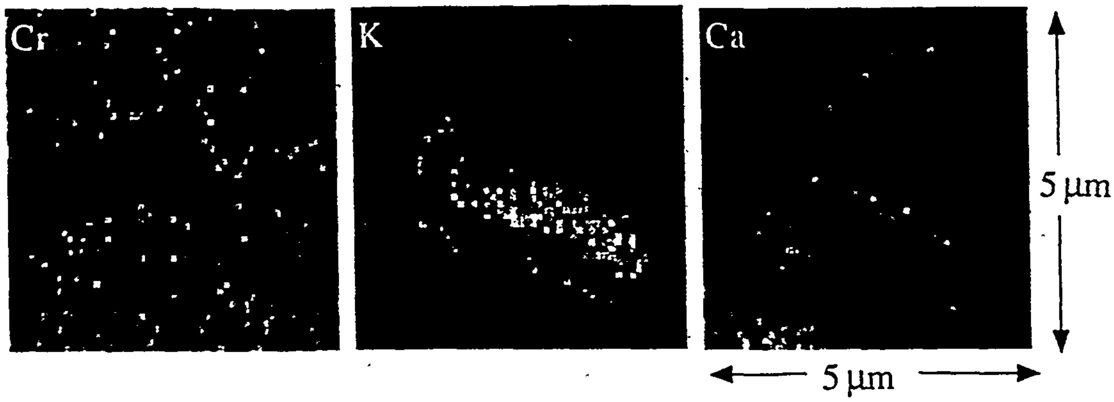

FIGURE 1. Elemental maps of a hydrated *Pseudomonas fluorescens* bacterium treated with Cr(VI) solution. See text for further details.

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