High-Resolution X-ray Fluorescence Imaging of Bacteria for Environmental Science

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Introduction

X-ray microscopy and imaging in the hard x-ray regime has emerged as one of the most important applications of high-brilliance third-generation synchrotron sources, such as the Advanced Photon Source. With the advent of high-resolution microfocusing optics, such as Kirkpatrick-Baez mirrors,[1,2] and Fresnel zone plates,[3] it is now possible to focus 8-keV x-rays to a spot size of only 150 nanometers with a gain of > 30,000 in the flux density.[4] This means 10^9-10^{10} photon/sec can be delivered into a tight submicron spot. With the improved performance in both x-ray sources and optics, it is then possible to build practical x-ray microprobes, which provide many new applications that have never been considered previously. One such application is the environmental study of the interaction between bacteria and heavy-metal contaminants.

Understanding the fate of heavy-metal contaminants in the environment[5] 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. Here we present 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 2-ID-D x-ray microscopy beamline.

Methods

We have used hard x-ray phase zone plates to investigate the spatial distribution of 3*d* elements in single hydrated *Pseudomonas fluorescens* bacteria adhered to a Kapton film. Another layer of Kapton film was used to enclose the bacteria and maintain their hydrated condition. The samples were mounted on a computer-controlled XY mechanical 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. Spatial maps of several elements were obtained by scanning the sample in 0.15-mm steps through the focused monochromatic x-ray beam and integrating the selected Kα fluorescence for 5 sec/pt.

Results and Discussion

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 to 1000 ppm Cr(6+) in solution for 6 hours. Observation

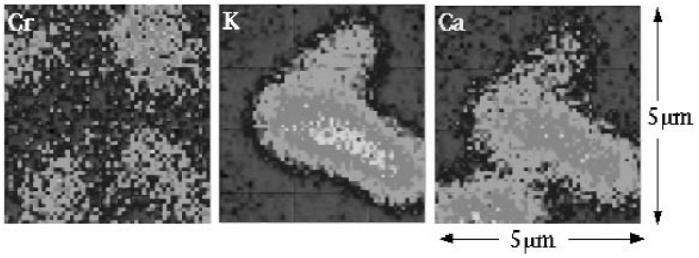

FIG. 1. Elemental maps of hydrated Pseudomonas fluorescens *bacterium treated with Cr(VI) solution.*

of these images indicates that monitoring the spatial distribution of the K and Ca Kα fluorescent radiation coming from the sample enables identification of the rod-shaped *Pseudomonas fluorescens* as well as the extracellular exudes associated with it. Additionally, comparison of the distribution of Cr to K or Ca indicates that the majority of the Cr in this sample is associated extracellularly. These results indicate that the majority of the Cr(VI) that was introduced to the sample was probably not actively metabolized. Finally, although these results demonstrate the utility of imaging hydrated bacteria at ambient temperature, in the future, a cryostat may be required to quick-freeze the samples in order to reduce the effects of radiation damage when performing spectromicroscopy studies.

Acknowledgments

Use of the Advanced Photon Source and SRICAT was supported by the U.S. Department of Energy, Office of Science, Office of Basic Energy Sciences, under Contract No. W-31-109-ENG-38. Additional support was provided by the U.S. Department of Energy's Office of Biological and Environmental Research, Natural and Accelerated Bioremediation Program.

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