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X-ray Microscopy Analysis of Bacterial Cells

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The microenvironment within, at, and adjacent to actively metabolizing cell surfaces can be significantly different from the bulk environment. The behavior of nutrient elements and contaminants in such microenvironments can affect fundamental biochemical and biogeochemical processes that influence the macroscopic fates of contaminants. However, it is currently difficult to understand the biogeochemical interactions in such microenvironments because the chemistry there is difficult to define. Information about biochemical and biogeochemical interactions within microbes and at microbe-mineral interfaces is paramount for understanding fundamental biochemical processes, predicting the fates of contaminants, and designing effective bioremediation approaches.

Because biologically driven redox changes to metals and radionuclides result in changes in the oxidation state, chemical environment, or structural characteristics of the element of interest, x-ray absorption spectroscopy (XAS) techniques can provide significant insight into these transformations. Additionally, to adequately determine the oxidation state and chemical speciation of elements within microbes and at microbe-mineral interfaces, the dimensions of the x-ray probe must enable the majority of the x-rays to be positioned at these locations. Recent advances in synchrotron-based x-ray imaging and microspectroscopy have resulted in the development of a class of techniques that enable this. For example, elemental distributions in a microbial system can be mapped by using an x-ray fluorescence (XRF) microprobe, where the electron beam of an energy-dispersive x-ray microanalysis experiment is replaced by an apertured or focused x-ray beam from a synchrotron source (Figure 1) [1]. With the aid of synchrotron x-ray sources, x-ray microprobe techniques can be combined with x-ray spectroscopic techniques by using a monochromatic x-ray beam to record x-ray absorption spectra at the absorption edges of the element of interest at specific locations in the sample. Because such spectra are sensitive to the chemical and structural environment of the element, such "spectroscopic imaging" experiments can substantially improve understanding of the system or process of interest.

We have performed XRF imaging experiments of single hydrated *Pseudomonas fluorescens* bacteria at ambient temperature and pressure with 150 nanometer resolution [1]. Results from our studies have shown that the spatial distributions of P, S, Cl, Ca, and many of the 3d transition elements can identify the location of a single bacterium. We have also shown that comparison of the intensity of the XRF signal from the microbe to that for thin-film glass standards with known elemental area concentrations enables quantification of the concentration of each element in each specific microbe. Differences in elemental concentrations of many key biosignature elements, resulting from exposure to toxic levels of Cr(VI), indicate that this technique can distinguish between "healthy" and lysed

cells. X-ray microspectroscopy measurements of elevated Cr concentrations near single surface-adhered cells exposed to toxic levels of Cr(VI) demonstrated chemical reduction of the contaminant to Cr(III) and binding of Cr(III) to phosphoryl functional groups.

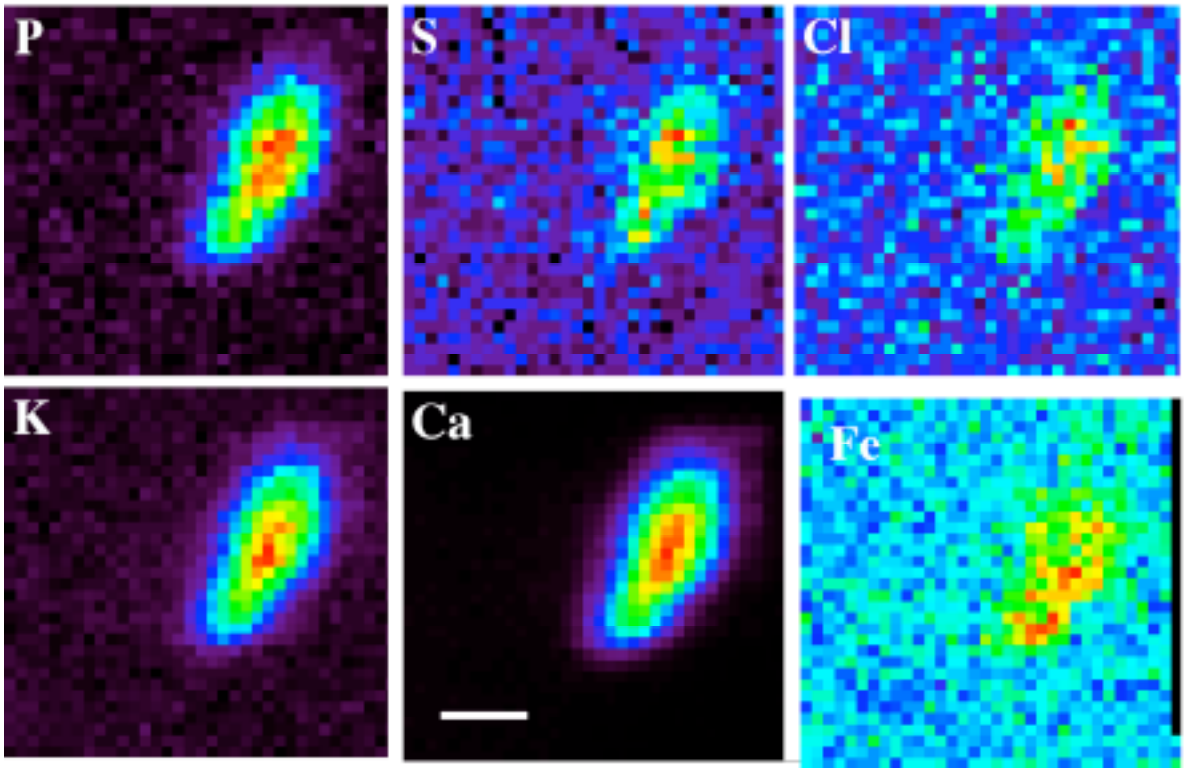

Fig. 1. XRF maps of elemental distributions within a *P. fluorescens* microbe. Scale bar = 1 micron.

We have imaged single hydrated *Shewanella oneidensis* MR-1 bacteria adhered to amorphous lepidocrocite thin films under anoxic conditions. The results indicate that the combination of the high brilliance of synchrotron and the use of high-resolution zone plates for focusing enables investigation of the spatial distribution and concentration of nutrient elements, as well as contaminant metals and radionuclides, at the mineral-microbe interface. We have also integrated electron and XRF microscopies to investigate identical locations (within 100 nm) in a number of mineral-metal-microbe systems. In these studies we have taken advantage of the strengths of each technique by using the superior spatial resolution of the electron microscope (relative to the x-ray microscope) and the superior elemental sensitivity of the x-ray microscope (relative to the electron microscope), along with the ability of the x-ray microscope to spatially probe chemical speciation. Results of these experiments will be presented.

References

[1] K. M. Kemner et al., Science 306 (2004) 686.

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