Mining and human use of uranium has created hundreds of sites globally where improperly disposed radionuclides and other toxic metals contaminate groundwater supplies and threaten human health. Non-invasive remediation strategies are desired to remove or immobilize the contaminants as invasive methods are costly, cause more environmental harm and introduce more chemicals to an already polluted site [1]. Bio-remediation strategies using environmental bacteria isolated from the contaminated site are non-invasive and effective, but have limitations in persistence. Understanding the mechanisms and mineral end-points of the bacterial-based bioremediation strategies will help alleviate limitations.

Bacterial-based bioremediation strategies rely on either adsorption to cell-associated polymers in the extracellular matrix or the chemical reduction of uranium to an insoluble mineral. Uranium in the six oxidation state (UO$_2^{2+}$) is soluble in water, while U(IV) is insoluble [2]. To immobilize uranium, U(VI) can be sequestered by cell-associated polymers or be reduced to minerals. Some species of bacteria produce sugar-based Extracellular Polymeric Substance (EPS) or long pili that adsorb U(VI) [3]. In the absence of long polymers, U(VI) can adsorb to the cell surface. Many organisms also have the capability of reducing uranium and this can be done in conjunction with adsorption [2]. The stability and effectiveness of the bioremediation strategies depend on the location and end-point uranium mineral formed [1].

Electron microscopy (EM) in combination with elemental analysis is uniquely suited to address open questions around the cellular mechanisms of uranium bioremediation approaches. In this work we used EM and microanalysis techniques to visualize and characterize how two different bacteria sequester uranium. The first organism is *Streptomyces* sp.MOE6 and was isolated from the soil in Columbia, MO. MOE6 produces large quantities of Extracellular Polymeric Substance (EPS) as it grows. The MOE6 EPS has been isolated and shown to adsorb uranium and other metals strongly (Elnahas & Majumder, unpublished data). We used Transmission Electron Microscopy (TEM) to visualize uranium chelation by the EPS. EPS was isolated from the organism, purified and lypholized. When ready for use, EPS was rehydrated and then mixed with uranium. EPS with or without uranium was on the carbon coated TEM grid and negative stained. The EPS was visualized and measured. The EPS polymers were highly branched, several microns long and nanometers thin (Fig. 1). Metal was observed to be bound by the EPS. Next we plan to use High Resolution Scanning Transmission Electron Microscopy (HRSTEM) with Electron Energy Loss Spectroscopy (EELS) to determine the nature of the interaction between metal and EPS. Imaging of whole-cell samples will also be pursued.

Secondly, we tested *Desulfovibrio vulgaris* Hildenborough (DvH), which is a model Sulfate-Reducing Bacterium (SRB) that has been shown to reduce and precipitate uranium (Majumder, unpublished data). Mechanisms of uranium reduction have been proposed in the intra and extra-cellularly, but it has not been verified in uranium is found inside the cell [2, 4]. DvH grown with and without uranium was visualized by ESEM coupled with X-ray Dispersive Spectroscopy (EDS), specifically the FEI Quanta
600 FEG Environmental Scanning Electron Microscope (ESEM) by aliquoting diluted culture onto a silicon wafer under low vacuum. Uranium mineral precipitate was observed next to the bacteria and in some instances had discrete ball-like structure (Fig. 2). EDS spectra and mapping confirmed the presence of uranium in the precipitate, but did not detect any uranium inside of the bacteria (Fig. 2). HRSTEM will be employed next for resolution on the Angstrom scale. EM and microanalysis initial characterization of these two bacterial systems has provided important mechanistic clues for remediation applications.

References:


Figure 1. Isolated EPS visualized by TEM. Individual polymers are micrometers long.

Figure 2. ESEM and EDS of DvH interacting with U. Image, spectra and elemental map.