Monitoring Microbial Uranium Reduction at the Oxic-Anoxic Interface

University-Led Research

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This collaborative, multi-investigator research project aims to develop innovative molecular and biogeochemical tools to enhance understanding of the microbiology controlling the redox state of metals and radionuclides in subsurface environments and near oxic-anoxic interfaces. Diverse groups of microorganisms affect the oxidation state of metals and, therefore, affect many biogeochemical processes including carbon turnover and the mobility of toxic radionuclides in subsurface environments. Relevant to uranium (U) transport are bacteria that can reduce the relatively water-soluble and mobile hexavalent U(VI) to the less-soluble U(IV) redox state.

We have applied physical, chemical and biological tools to analyze the fate of U under various geochemical conditions. Using EXAFS analysis we found that phosphate concentrations and the specific metal-reducing bacterial population can affect the form of reduced U(IV), indicating that a diversity of mononuclear U(IV) forms may be commonly formed during bioreduction. Using high precision mass spectrometry, we have also shown that significant U isotopic fractionation occurs during U(VI) reduction. During microbial reduction, the U(VI) remaining in solution becomes isotopically lighter; however, different U(VI)-reducing species resulted in different fractionation extents, and a new *Shewanella* isolate reduced U(VI) efficiently but without fractionation. These results indicate that different U reduction pathways/mechanisms operate that yield different products and can lead to varying amounts of isotopic fractionation. *c*-type cytochromes have been implicated in electron transfer to oxidized metal species, and using metaproteomic analysis we identified specific proteins expressed in cultures of *Anaeromyxobacter dehalogenans* strain 2CP-C, *Geobacter daltonii* strain FRC-32, and *Shewanella oneidensis* strain MR-1. Cytochromes expressed in response to the presence of specific electron acceptors provide information about the physiological state of the microbe, thus making predictions of metal-reducing activity possible.

To better investigate the spatial relationship associated with flow in a groundwater system we have completed the design of a rectangular Plexiglass flow-through column that will allow the simultaneous measurements of nucleic acid and protein biomarkers, U isotopes, and *in situ* EXAFS and XANES in a zone of controlled redox transition changes (i.e., the oxic-anoxic interface). The combined application of these tools will monitor the responses of metal-reducing bacteria and advance our understanding of the relevant microbiology and processes that determine the fate of electron donors (e.g., carbon) and electron acceptors (e.g., oxidized metals and radionuclides, oxygen) near oxic-anoxic transition zones.

Application of the new tools to DOE IFRC or relevant field sites will demonstrate their value to assess, monitor, and predict *in situ* reductive processes. The comprehensive understanding of the mechanisms and pathways affecting metal speciation, in particular U precipitation and mobilization, will contribute to the development of models that predict the long-term fate of metals in redox transition zones.