Mechanisms for the Reduction of Actinides and Tc(VII) in *Geobacter sulfurreducens*

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Uranium and technetium are the primary radioactive metals contaminating subsurface environments at U.S. Department of Energy (DOE) sites. Dissimilatory Fe(III)-reducing microorganisms can control the mobility of these contaminants through the enzymatic reduction of highly soluble U(VI) and Tc(VII) to insoluble tetravalent forms, which will precipitate from groundwater and be immobilized in the subsurface. The aims of this project are to use the tools of biochemistry and molecular biology to confirm the identities of the genes encoding the relevant U(VI) and Tc(VII) reductases in *G. sulfurreducens* and to elucidate the detailed mechanisms of U(VI) and Tc(VII) reduction by the corresponding enzymes. Furthermore, we aim to explore the range of other metals and radionuclides reduced by *Geobacter sulfurreducens*—including Np(V), Pu(IV), and Hg(II)—and identify the roles of the U(VI) and Tc(VII) reductases in the reduction of these other priority pollutants.

During the first two years of this grant, we have focused on identifying the Tc(VII) reductase of *G. sulfurreducens*, developing the analytical tools for monitoring the reduction of penta and tetravalent actinides in microbial culture, and characterizing the mechanisms of U(VI) and Np(V) reduction in detail. Extensive biochemical and genetic evidence, including studies with a defined mutant from Derek Lovley's group at the Univ. of Massachusetts, have shown that the enzyme responsible for Tc(VII) reduction in *G. sulfurreducens* is a NiFe hydrogenase localized in the periplasm. In collaboration with Mireille Bruschi's group in Marseille, France, we have also partially purified and characterized a suite of hydrogenases in *G. sulfurreducens* as a first step towards studying hydrogenase-mediated radionuclide reduction in vitro.

X-ray absorption spectroscopy (XAS) was also used to show that *G. sulfurreducens* reduces U(VI) by a one-electron reduction, forming an unstable U(V) species, which subsequently disproportionates to give insoluble U(IV). This was confirmed by challenging *G. sulfurreducens* with the Np(V) analogue, which is stable with respect to disproportionation and was not reduced by whole cells. Similar results were obtained in vitro using purified cytochrome c_7 from *G. sulfurreducens*, with hydrogenase as the electron donor for actinide reduction. This surprising degree of selectivity for hexavalent actinides illustrates the need for mechanistic understanding and care in devising in situ bioremediation strategies for complex wastes containing other redox-active actinides, including plutonium. In the final year of this program, we will explore the impact of *G. sulfurreducens* on plutonium in a range of oxidation states, confirm the role of *c*-type cytochromes in actinide reduction using in vivo and in vitro techniques (via collaboration with Marianne Schiffer at Argonne National Laboratory and Derek Lovley and colleagues at the Univ. of Massachusetts), and characterize the mechanism of reduction of other contaminant metals, including Cr(VI) and Hg(II). Studies on Cr(VI) reduction by *G. sulfurreducens* are already well advanced in our laboratory.