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

Jon R. Lloyd (PI), Jo Renshaw, Iain May, and Francis Livens

University of Manchester, UK

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 work with collaborators at the Argonne National Laboratory (Dr. Marianne Schiffer) and the University of Massachusetts (Dr. Derek Lovley and colleagues) 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 year of this grant we have focused on identifying the Tc(VII) reductase of *G. sulfurreducens*. Biochemical and genetic evidence have shown that the enzyme responsible for Tc(VII) reduction in *G. sulfurreducens* is a NiFe hydrogenase localized in the periplasm. Hydrogen was the sole electron donor used for Tc(VII) reduction, with CO profiling experiments suggesting the involvement of a NiFe hydrogenase. Treatment of whole cells with Cu(II), which is toxic to hydrogenases and can access the periplasm but cannot penetrate the cytoplasmic membrane, completely inhibited Tc(VII) reductase activity. Protease treatment of whole cells resulted in complete inhibition of insoluble metal reduction [e.g., Fe(III) oxides] on the surface of the cell but had no impact on Tc(VII) reduction. These results confirm that Tc(VII) is reduced in the periplasm (which is also supported by transmission electron microscopy studies) via a mechanism that is distinct from that used to reduce Fe(III) oxides. Finally, a *G. sulfurreducens* knockout mutant that is unable to synthesize the principal periplasmic NiFe hydrogenase (obtained from Derek Lovley's group in Amherst) was also unable to couple the oxidation of hydrogen to Tc(VII) reduction, unequivocally identifying the Tc(VII) reductase of *G. sulfurreducens*.

Additional experiments have focused on the mechanism for the reduction of key actinides, including U(VI), Np(V), and Pu(IV). We have previously shown that U(VI) is reduced via a mechanism that involves a periplasmic cytochrome c_7. Ongoing work aims to obtain baseline information on the reduction of other key actinides and metals by *G. sulfurreducens*, prior to determining the role of cytochrome c_7 and other redox active proteins on such transformations. The focus of our current research is to develop analytical tools for monitoring the reduction of penta- and tetravalent actinides in microbial culture, and we have now conducted preliminary experiments using Np(V) as a potential electron acceptor. We have also conducted experiments using an *E. coli* strain engineered to overproduce cytochrome c_7. Initial experiments suggest that this cytochrome confers the ability to reduce metals, including U(VI), on *E. coli*, and may give a useful model for determining the role of cytochrome c_7 in the reductive precipitation of contaminant metals.