Biogeochemical Processes Controlling Microbial Reductive Precipitation of Radionuclides

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For microbial reductive precipitation of radionuclides to be useful for groundwater remediation, bacteria must effectively reduce target contaminants, and reduced products must be stable to reoxidation by naturally occurring oxidants present in the system. This project focuses on identifying and quantifying the coupled biogeochemical reactions that control the reductive precipitation of U(VI) and Tc(VII).

Bacterial U(VI) reduction. U precipitates were observed by transmission electron microscopy (TEM) in association with cell periplasm and outer membrane. Subsequent time course analyses indicated periplasm-associated $UO_{2(s)}$ is eventually localized to cell exterior as aggregates of a fine-grained precipitate and in association with fiberlike features that radiate from the cell surface. This led to a hypothesis that metal-reducing *Shewanella* have an active mechanism for exporting nm-sized $UO_{2(s)}$ from the cell envelope. The type II secretion pathway, the terminal branch of the general secretion pathway (GSP), is responsible for the extracellular secretion of proteins including toxins and hydrolytic enzymes. An *S. oneidensis* MR-1insertional mutation in the *gspD* gene (provided by A. Belieav) was characterized with regards to U(VI) reduction. The MR-1 *gspD* mutant was found to reduce U(VI), but at a reduced rate relative to the wild type. When thin sections of the *gspD* mutant were examined by TEM, nm-sized $UO_{2(s)}$ was localized to cell periplasm and the outside of the outer membrane; extracellular U solids were nearly absent. In contrast, MR-1 cells treated in an identical manner initially contained both extracellular and periplasmic $UO_{2(s)}$, but upon extended incubation, U was localized to the cell exterior. These results suggest U(VI) enters the periplasm through the outer membrane as a uranyl carbonate complex and is subsequently reduced. The poorly crystalline nm-sized $UO_{2(s)}$ particles that form are subsequently exported by the cell via an unknown mechanism, and the $UO_{2(s)}$ is removed from the cell surface via a secreted protein. Although additional work is required to establish the precise mechanisms, the results have significant implications with regards to the fate and transport of microbially reduced radionuclides.

Manganese oxides. Mn(III,IV) oxides are among the most powerful, naturally occurring oxidants and may promote the oxidative dissolution of uraninite ($UO_{2,cr}$) and TcO_2. Thermodynamic calculations predict, and batch laboratory experiments confirm, that manganese oxides oxidize UO_2 and TcO_2. Nevertheless, the rate at which this solid–solid redox reaction occurs is poorly known. The oxidation of biogenic uraninite (precipitated and harvested from cultures of *Shewanella putrefaciens* CN32) by well-characterized manganese oxides has been measured. Experiments were conducted at pH 6.9 in 30 mM $NaHCO_3$, using an initial UO_2 concentration of 250 μM and initial Mn-oxide concentration of 100 m^2/L. The measured ratio of Mn(II):U(VI) produced agreed well with the expected ratio based on direct measurement of the oxidation state of Mn and U in the respective starting solids. Production of U(VI) in the presence of the Mn(IV) oxides $MnO_{2,am}$, nsutite (γ-MnO_2), and the mixed oxide hausmannite (Mn_3O_4) was described well by a single pseudo-first-order product curve, whereas U(VI) production in the presence of the Mn(III) oxides manganite (γ-MnOOH) and bixbyite (Mn_2O_3) were described by two simultaneous pseudo-first-order product curves ($P < 0.001$). Addition of Mn(II) to vials containing Mn_2O_3 or pre-reduction of Mn_2O_3 with AH_2DS had no effect on the rate of U(VI) production. By contrast, treating γ-MnO_2 with AH_2DS prior to introducing UO_2 resulted in a significantly faster rate of U(VI) production; the enhanced rate of U(VI) production was evident with repeated additions of UO_2 to the system. We continue to examine the kinetics of UO_2 oxidation as a function of solution composition and are conducting similar experiments using continuous flow through methods.