The Biogeochemistry of Pu and U: Distribution of Radionuclides Affected by Microorganisms and their Siderophores, Reductants, and Exopolymers

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Radionuclide-contaminated environments, including the Rocky Flats Environmental Technology Site (RFETS) and the contaminated groundwater at the NABIR Field Research Center (FRC), are often oxic. Radionuclide distribution within such environments is affected by indigenous biogeochemical processes, including the metabolic activities of aerobic microorganisms, key members being the ubiquitous *Pseudomonas* and *Bacillus* genera. Because of the chemical similarities between the actinides, uranium (U) and plutonium (Pu), and iron (Fe), the metabolic processes of these microorganisms that affect the biogeochemistry of Fe could also significantly affect Pu and U distribution. We propose to determine the extent to which metabolic processes involved in Fe acquisition and in exopolymer production affect the distribution of Pu and U between the aqueous and solid phases. First, we will determine the equilibrium distribution of Pu and U between these phases, in the presence and absence of microorganisms, and in relation to Fe bioavailability. Second, using transposon mutagenesis, we will determine to what extent microbial processes (including siderophore production and metabolism, and exopolymer and reductant production) directly or indirectly influence aqueous/solid phase distribution of Pu and U.

We have continued our investigations of the redistribution of actinide species during aerobic dissolution of Fe-bearing minerals. One example is as follows: *Pseudomonas putida* is a common siderophore-producing bacteria in soil and subsurface environments. We began our experiments with *P. putida* by assessing the toxicity of adsorbed U(VI). Fe-deficient medium (FeDM) was prepared by adding the following analytical grade ingredients to 1.0 L of distilled water in acid-washed and rinsed flasks: 0.67 g of glycerol-2-phosphate; 1.0 g of NH_4Cl; 0.2 g of $MgSO_4 \bullet 7\ H_2O$; 0.05 g of $CaCl_2 \bullet 2\ H_2O$; 5.0 g of succinic acid disodium salt anhydrous, and 4.19 g MOPS. The medium was then chelexed with 10 g/L of chelating resin for one hour and filtered through a 0.22 µm membrane. After filtering, 0.125 mL of trace elements (0.005 g of $MnSO_4 \bullet H_2O$; 0.0065 g of $CoSO_4 \bullet 7\ H_2O$; 0.0023 g of $CuSO_4$; 0.0033 g of $ZnSO_4$; and 0.0024 g of MoO_3 per 100 mL of distilled, deionized water) was added to the medium. The pH of the medium was ~7.45. The medium was then transferred into acid-washed/rinsed 250 mL polycarbonate flasks to a final volume of 50 mL. Hematite (29 m^2/L) was added to the necessary flasks. The flasks were sterilized by autoclave, and filter-sterilized U(VI) (0.055 mM) was added, approximately 54 hours prior to inoculation. The initial cell density of *P. putida* was 3 x 10^{-6} cells/mL. The growth of *P. putida* was not greatly impacted by the addition of 0.055 mM U(VI) to media containing hematite. Growth may have been slightly inhibited in the cultures containing U(VI), but the maximum cell density was similar for both cultures. We are currently determining the redistribution of U in these experiments, and expect U to be mobilized by bacteria similar to previously reported studies.

The results of the research program will contribute to NABIR's stated needs to understand both "the principal biogeochemical reactions that govern the concentration, chemical speciation, and distribution of metals and radionuclides between the aqueous and solid phases" and "what alterations to the environment would increase the long term stability of radionuclides in the subsurface."