Molecular mechanisms and kinetics of microbial anaerobic nitrate-dependent U(IV) and Fe(II) oxidation

University-Led Research

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In this project, we are combining molecular genetic, spectroscopic, and microscopic techniques with kinetic and reactive transport studies to describe and quantify biotic and abiotic mechanisms underlying anaerobic, nitrate-dependent U(IV) and Fe(II) oxidation, which influences the long-term efficacy of *in situ* reductive immobilization of uranium at DOE sites. In these studies, *Thiobacillus denitrificans*, an autotrophic bacterium that catalyzes anaerobic U(IV) and Fe(II) oxidation, is used to examine coupled oxidation-reduction processes under either biotic (enzymatic) or abiotic conditions in batch and column experiments with biogenically produced $U^{IV}O_2$(s). Research is focused on identifying the primary redox proteins that catalyze metal oxidation, environmental factors that influence protein expression, and molecular-scale geochemical factors that control the rates of biotic and abiotic oxidation.

Biogenic UO_2(s) was synthesized under anaerobic conditions (using *S. oneidensis* strain MR-1) following previously published methods. Mixtures of biogenic UO_2(s) and quartz were packed into 1 mL or 5 mL polypropylene columns. For experiments in the presence of *T. denitrificans,* cells were harvested anaerobically, washed and resuspended, mixed with quartz and biogenic UO_2(s), and packed into the columns. Oxidation experiments were carried out in the dark at 25±2ºC in an anaerobic glove box (10%H_2, 90%N_2) in buffered solution (MOPS, pH ~ 7.2). In abiotic experiments, UO_2(s) oxidation by nitrate (up to 20 mM) was relatively slow; higher dissolved U concentrations were observed in abiotic experiments with nitrite (up 20 mM). Reactive transport modeling (using Crunchflow) of abiotic column experiments, which included sorption of U(VI) in the column, simulated steady-state U release. In the presence of *T. dentrificans* and 10 mM nitrate, higher rates of dissolved U release were observed compared with abiotic controls, suggesting that *T. dentrificans* catalyzed the oxidative dissolution of UO_2(s). Characterization by synchrotron XAS showed higher fractions of U(VI) compared to U(IV) in post-experiment solids reacted with higher dissolved nitrite concentrations. Structural analysis of biogenic UO_2(s) by EXAFS suggested variable particle size and/or a variable degree of local structure disorder. Spatially resolved characterization of samples reacted with nitrite and Fe(II) solutions by SXTM showed association of U with C and Fe around edges of UO_2(s) particles. Experiments examining Fe(II) and Fe(III) in coupled redox reactions are in progress.

Current work with *T. denitrificans* focuses on determining the enzymes responsible for anaerobic, nitrate-dependent Fe(II) oxidation. We previously reported that two *c*-type cytochromes, Tbd_0187 and Tbd_0146, were involved in anaerobic nitrate-dependent U(IV) oxidation in *T. denitrificans*. However, we have found that these same mutants were *not* defective in nitrate-dependent Fe(II) oxidation. Here we report on efforts to identify genes involved in anaerobic Fe(II) oxidation in *T. denitrificans* based on screening of its Tn*5* transposon mutant library; over 10,000 mutants have been screened to date, with defects in Fe(II) oxidation as great as 40%. One mutant that is 27% defective in Fe(II) oxidation relative to the wild-type has the Tn*5* transposon inserted in a putative *c*-type cytochrome. The insertion is downstream of the CXXCH heme-binding motif. A deletion knockout mutant for the disrupted gene is under construction, and more mutants from the library are being screened.