Natural Organic Matter Inhibits Biological Uranium Reduction by *Shewanella putrefaciens* CN32

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The broad objective of our NABIR research project is to conduct experiments to generate/expand a kinetic/thermodynamic database for use in modeling the concomitant biological reduction of iron(III) and uranium(VI) in field sediments amended with natural organic matter (NOM). Reaction-based reactive transport models will be validated with laboratory experiments, and the validated models will be used for eventual field-scale predictions. The specific objectives of our poster-presented research were to examine the effect of several different NOMs on U(VI) bioreduction, resolve the mechanism by which the NOM affects this process, and collect kinetic data for rate formulation/parameterization purposes. Experiments were performed with the dissimilatory metal-reducing bacterium (DMRB) *Shewanella putrefaciens* CN32 in a $NaHCO_3$-buffered solution under a N_2-CO_2 atmosphere with 5 mM lactate as the sole electron donor and 100 μM uranyl acetate as the sole electron acceptor. A preliminary experiment conducted with cell densities of 10^7 and 10^8 cells mL^{-1} revealed that U(VI) was bioreduced to a lesser extent with the higher cell density, possibly due to greater biosorption of U(VI). U(VI) was measured as dissolved (0.2 μm filter) and carbonate-extractable by kinetic phosphorescence analysis (KPA). Precipitated solids retained on the 0.2 μm filter were analyzed by x-ray photoelectron spectroscopy (XPS) to confirm the presence of U(IV). Preliminary experiments were also conducted at "high" and "low" HCO_3^- concentrations (30 and 1 mM, respectively) such that two different inorganic U(VI) complexes were the dominant species, and revealed no difference in the rate and extent of bioreduction.

Five different NOMs were tested: (1) Field Research Center (FRC) forest soil humic acid, (2) FRC groundwater NOM, (3) IHSS Suwannee River humic acid, (4) IHSS Suwannee River NOM, and (5) IHSS soil humic acid. The electron shuttling compound anthraquinone-2,6-disulfonate (AQDS) was also tested. For all experiments, a biotic no-amendment control was always prepared. Results from the biotic no-amendment controls were highly reproducible and yielded a zero-order reduction rate of 14.4 ± 0.4 (n = 6) μmol U(VI) d^{-1} (with 10^7 cells mL^{-1} and $[U(VI)]_{initial}$ = 100 μM). The FRC groundwater NOM had no effect on the rate and extent of U(VI) bioreduction. However, with the other four NOMs, no measurable U(VI) bioreduction occurred after five days. AQDS increased the rate of U(VI) bioreduction. Two mechanisms are proposed to explain the inhibition of U(VI) bioreduction by NOM. First, NOM may complex U(VI), making it biologically unavailable or less available. Second, NOM may act as a competitive electron acceptor and not as an electron shuttle. In the second case, it is proposed that the reduction of U(VI) by NOM is either thermodynamically unfavorable or extremely slow. Additional work to improve our interpretation of these results include: U(VI)–NOM dialysis experiments to confirm that complexation can occur under these experimental conditions; U(VI) bioreduction with low concentrations of NOM to examine the competitive electron acceptor hypothesis; U(VI) bioreduction experiments with excess citrate to compare NOM results to conditions of known complexation/speciation.