Bioremediation Approaches for Sustained Uranium Immobilization
Independent of Nitrate Reduction

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Abstract

The daunting prospect of complete nitrate removal at DOE sites such as the FRC provides strong incentive to explore bioremediation strategies that will allow for uranium bioreduction and stabilization in the presence of nitrate. Typical in-situ strategies involving the stimulation of metal reducing bacteria are hindered by the low pH environment and require that the persistent nitrate be continuously transformed. This project investigates the possibility of stimulating nitrate-indifferent pH-tolerant organisms to achieve non-specific bioreduction of U(VI) despite nitrate.

Enrichments from FRC Area 2 sediments were prepared using a variety of electron donors (ethanol, glyceral, hydrogen, and glycerol) and MOPS/TRIS buffers at pHs ranging from 4.9 to 7. Successful enrichments containing 10-20 mM methanol have demonstrated the nearly complete reduction of uranium (90% reduction at ~10 ppm) with very little loss of nitrate (less than 10% loss at ~500 ppm) from pH 4.9-6.5. Many higher pH enrichments also demonstrated similar U reduction capacity with 5-30% nitrate loss. Bacterial 16S RNA genes from successful enrichments at pH 5.7-6.7 were amplified and sequenced for phylogenetic analysis. A majority of clone sequences retrieved from enrichment cultures were comprised of Clostridia, Clostidioides-like organisms and Bacteroidetes. Further experiments tested the stability of ~2 ppm U(VI) in nitrate or nitrite solutions. When added to water with oxygen removal, U(VI) was stable and oxidized only when exposed to air. The presence of nitrite (100 ppm) or nitrate (1000 ppm) did not induce measurable oxidation over the several timescales of measurements.

U(VI) stability experiments

U(VI) stability experiments were conducted by adding U(VI), Fe(II), or reasurin to pressure tubes containing sterile MINWO water. The water was either used directly, degassed, or deoxygenated, and various tubes containing nitrate (1000 ppm) or nitrite (100 ppm). In contrast with Fe(II) (Fig 3), no U(VI) oxidation has been observed in either nitrate or nitrite solutions after 100 days.

FRC enrichments

Enrichments from ERSP Field Research Center sediments were prepared with a modified phosphate-buffered basal medium, an electron donor (H2, methanol, ethanol, or glycerol), MOPS/TRIS buffers at pHs between 4.9 and 6.2, NaN3O (800-1000 ppm), and NaHCO3. These enrichments exhibiting growth were transferred to fresh modified PBMB medium with added nitrate and uranium (VI) in pressure tubes, again at various pHs with a series of electron donors. Bicarbonate was added in an amount consistent with FRC conditions (0.5-10 ppm, depending on pH).

Summary and Implications

• This research is investigating innovative biotransformation strategies to provide long-term stability of immobilized metals and radionuclides without requiring complete nitrate removal in low pH environments.

• The presence of nitrite or nitrate did not induce measurable U(VI) oxidation over the timescale of several months.

• Enrichments from FRC sediments have demonstrated the nearly complete reduction of uranium (~90% reduction at ~10 ppm) with very little loss of nitrate from pH 4.9-6.2.

• A majority of clone sequences retrieved from enrichment cultures were comprised of Clostridia, Clostidioides-like organisms and Bacteroidetes.

• As long as anaerobic conditions are maintained, this research demonstrates the potential for uranium reduction without complete and consistent nitrate removal through the activity of organisms such as the Clostridia, even if only a small fraction of the total reducing equivalents are directed toward uranium reduction.

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