Promoting Uranium Immobilization by the Activities of Microbial Phosphatases

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Abstract

The overall objective of this project is to examine the activity of non-polar phosphatases present in naturally occurring subsurface environments for the purpose of promoting uranium immobilization of subsurface uranium (\(\text{U(VI)}\)) precipitates. Specifically, we hypothesize that the precipitation of \(\text{U(VI)}\) phosphates may be promoted through the microbial release and in-situ precipitation of \(\text{PO}_4^{3-}\) or as a result of direct immobilization and reactive transport. An experimental approach has been designed to examine the effect of phosphatase activity on uranium precipitation. A subset of subsurface isolates were selected based on their ability to immobilize \(\text{U(VI)}\). Two FRC (Fresno River Contaminated) isolates exhibited enhanced phosphatase activity relative to the \(\text{U(VI)}\) activity indicated the majority (75 of 100) of the initial uranium in biogenic incubations.

Uranium Biomineralization at pH 5.5

- Electron microscopy of FRC Rahnella sp revealed membrane blebbing during incubation in simulated groundwater.
- Extracellular \(\text{U(VI)}\) precipitation
- Cell-associated \(\text{U(VI)}\) precipitation

Background soil without G3P (b), Renatured whole cell G3P (c), Renatured whole cell G3P reveals a significant increase in phosphatase activity relative to the control.

Phosphate Liberation

- Visible Cell Counts

\(\text{Y9-2} + \text{U(VI)} \rightarrow \text{Y9-2} + \text{cell associated U(VI)} + \text{cell associated phosphate}\)

- Microbial phosphatase screening and functional assays

- Time 0 h
- 1 h post U(VI) addition
- 2 h post U(VI) addition
- 3 h post U(VI) addition
- 4 h post U(VI) addition
- 5 h post U(VI) addition
- 6 h post U(VI) addition
- 7 h post U(VI) addition
- 8 h post U(VI) addition
- 9 h post U(VI) addition
- 10 h post U(VI) addition
- 11 h post U(VI) addition
- 12 h post U(VI) addition
- 13 h post U(VI) addition
- 14 h post U(VI) addition
- 15 h post U(VI) addition
- 16 h post U(VI) addition
- 17 h post U(VI) addition
- 18 h post U(VI) addition
- 19 h post U(VI) addition
- 20 h post U(VI) addition
- 21 h post U(VI) addition
- 22 h post U(VI) addition
- 23 h post U(VI) addition
- 24 h post U(VI) addition
- 25 h post U(VI) addition
- 26 h post U(VI) addition
- 27 h post U(VI) addition
- 28 h post U(VI) addition
- 29 h post U(VI) addition
- 30 h post U(VI) addition
- 31 h post U(VI) addition
- 32 h post U(VI) addition
- 33 h post U(VI) addition
- 34 h post U(VI) addition
- 35 h post U(VI) addition
- 36 h post U(VI) addition

Uranium precipitation in biogenic incubations.

\(\text{Y9-2} + \text{U(VI)} \rightarrow \text{Y9-2} + \text{cell associated phosphate}\)

Neighboring spotting analysis of acid phosphatase gene. Sequences were obtained by the use of TASMER-Li and the dynamic programming by the Cross-Approach (DPA) algorithm using Gap A and Class B control phytase, respectively (a) and (b). Alignments generated for each class of acid phosphatase gene were analyzed for similarity and specificity to the query phytase. Type I, Type II, Type III, and Type IV acid phosphatase gene sequences are shown for control and query phytase sequences. Simulations for each class of acid phosphatase gene are shown for the substrate and 0.05 mg/ml methyl green (D), (E).