Microbially Facilitated Calcite Precipitation for Remediation of Sr-90 (EMSP Project 87016)

Yoshiko Fujita
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# People, Funding

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Project Description

- Investigation of potential for urea hydrolyzing bacteria to facilitate calcite precipitation and co-precipitation (immobilization) of trace metals and radionuclides.

- Urea hydrolysis yields ammonium, bicarbonate, and increases pH, promoting calcite precipitation:

\[
H_2NCONH_2 + 2H_2O \rightarrow 2NH_4^+ + CO_3^{2-}
\]
\[
Ca^{2+} + CO_3^{-} \rightarrow CaCO_3(s)
\]

- Focus of our research thus far has been immobilization of Strontium-90:

\[
(1-\chi)Ca^{2+} + \chi Sr^{2+} + CO_3^{2-} \Leftrightarrow Ca_{(1-\chi)}Sr_{\chi}CO_3(s)
\]
Why use urea hydrolyzing bacteria?

- Urea is a common nitrogen source, and hydrolyzing capability is widespread in the microbial world.
- All water samples from the Snake River Plain Aquifer (> 10 different locations) that have been tested for urease activity have been positive, and urea hydrolyzing bacteria are readily isolated.
- We can take advantage of these ubiquitous “reactors” to generate the reactants we need (carbonate, ammonium) in situ:
  - Prevents instantaneous precipitation in the well;
  - Allows distribution of reactants farther away from the point of injection.
Conceptual approach for remediation

A. Hydrolysis of urea produces $\text{NH}_4^+$, $\text{HCO}_3^-$ and raises pH.
B. $\text{NH}_4^+$ promotes desorption of Sr, Ca from mineral surfaces. $\text{HCO}_3^-$ promotes precipitation of calcite, co-precipitation of Sr.
C. Continued precipitation of calcite isolates Sr from contact with groundwater.
**Initial experiments: proof of principle**

- **Batch experiments in artificial medium supplied with calcium and urea; initial log of saturation index -1.7.**
- **Used Bacillus pasteurii and SRPA bacterial isolates**
- **pH, calcium, ammonium monitored for 8 hours.**
System follows equilibrium model

\[ \text{H}_2\text{NCONH}_2 + 2\text{H}_2\text{O} \rightarrow 2\text{NH}_4^+ + \text{CO}_3^{2-} \quad (1) \]
\[ \text{Ca}^{2+} + \text{CO}_3^{2-} \rightarrow \text{CaCO}_3 \quad (2) \]
\[ \text{H}_2\text{NCONH}_2 + 2\text{H}_2\text{O} + \text{Ca}^{2+} \rightarrow 2\text{NH}_4^+ + \text{CaCO}_3 \quad (3) \]
Strontium uptake is enhanced

- When Sr is included, it is removed concurrently with Ca, and biogenically generated calcite takes up more Sr than abiotically generated calcite.
- Higher $D_{EX}$ correlated with higher precipitation rates.

<table>
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<th>Sample generated by</th>
<th>Sr:Ca</th>
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<td>B. Pasteurii + urea</td>
<td>0.044</td>
</tr>
<tr>
<td>Amm. Carb.</td>
<td>0.032</td>
</tr>
<tr>
<td>Amm. Carb. + B. pasteurii</td>
<td>0.027</td>
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$$D_{EX} = 0.28 - 0.13R$$
$$|\Delta[Ca^{2+}]| > 7.5\%$$
Strontium is held tightly within calcite

- X-ray Absorption Near Edge Structure (XANES) spectroscopy shows Sr substitutes for Ca in calcite lattice.
- Solid solution formation is beneficial for long-term immobilization.
In the field, to verify the process we rely on urease activity

- Because we can’t see the calcite precipitating in the subsurface, we instead focus on urease activity.
  - Can recover groundwater, and check if urease activity is stimulated.
- We estimate in situ rates of ureolysis using a $^{14}$C tracer technique in the laboratory.
- We have also developed methods to detect and quantify urease genes (ureC subunit) and ureC mRNA transcripts, using real-time PCR.
Idaho Falls field test

- Research well at University Place (UP-1).
- Packers isolated 2m zone of consolidated basalt.
- Three volumes of dilute molasses (0.00075%) solution injected over 2 weeks, then one injection of 50 mM urea.
Field test results – urease activity was stimulated and calcite precipitated.

Ureolysis rates increased ~ 250X.

Ureolytic cell numbers increased ~ 100-200X.

Scanning electron micrograph of precipitate collected from well following urea injection. XRD confirmed presence of calcite.
Other Activities

- Evaluation of ureolytic activity and potential for remediation at Hanford 100-N Area.
- Static incubation field experiment to recover solid precipitates, biomass; planning push pull tests at the INEEL Vadose Zone Research Park.
- Continued development of real time RT-PCR for ureC mRNA transcripts.
- Development of methods for assaying nitrifying activity in groundwater.
- Investigation of the effects of precipitation on flow in porous media.
  - Could lead to strategies for manipulating distribution of precipitates in subsurface.
Coupling between precipitation and fluid transport

Currently using ureolytically driven calcite precipitation as a model system for \textit{in situ} generation of reactants.

- Extracellular urease immobilized on Eupergit\textsuperscript{®} C beads, mixed in quartz sand.

Initially flow faster than urea hydrolysis kinetics (low Damkohler number); then let column sit overnight with no flow.

Urea hydrolysis rate $>$ flow rate
**Coupling Project – cont.**

- Also using experiments to test geophysical characterization tools (induced polarization) and X-ray tomography for imaging precipitate distribution.

- Project aims ultimately to develop numerical models to describe coupling between precipitation and flow (work with L. Lake, U. Texas).

- For more information on coupling project, contact George Redden, reddgd@inel.gov