Idaho National Engineering and Environmental Laboratory

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

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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:

$$H_2NCONH_2 + 2H_2O \rightarrow 2NH_4^+ + CO_3^{2-}$$

$$Ca^{2+} + CO_3^{-} \rightarrow CaCO_3(s)$$

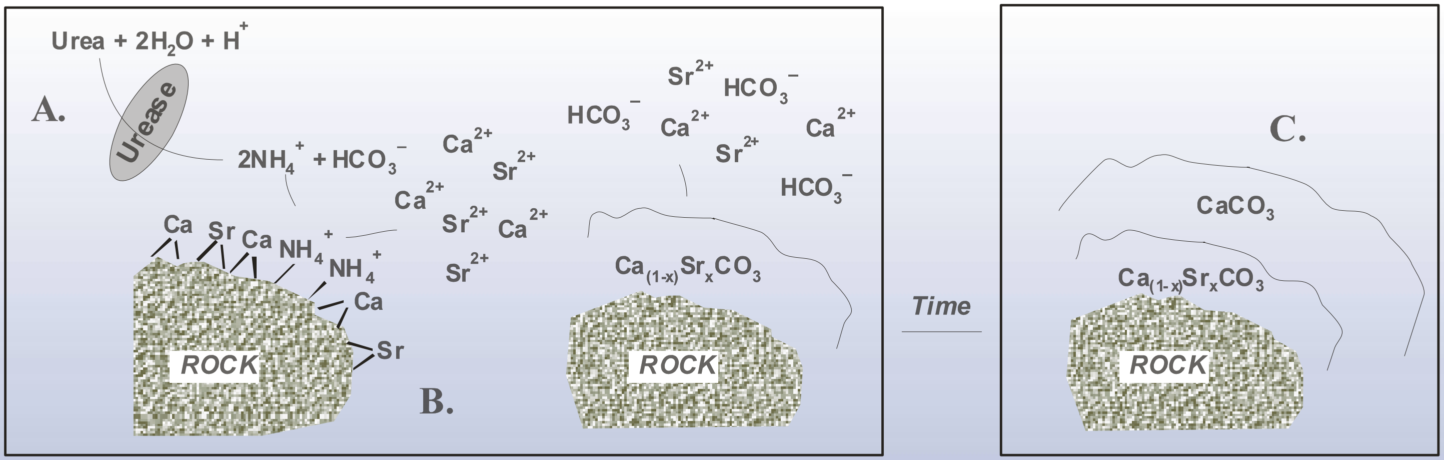
- Focus of our research thus far has been immobilization of Strontium-90:

$$(1-\chi)Ca^{2+} + \chi Sr^{2+} + CO_3^{2-} \Leftrightarrow Ca_{(1-\chi)}Sr_{\chi}CO_3 (s)$$

Why use urea hydrolyzing bacteria?

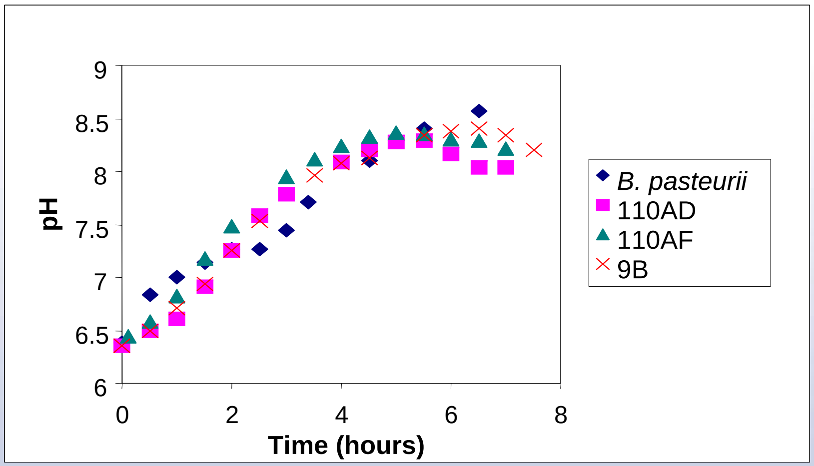
- Urea is a common nitrogen source, and hydrolyzing capability is widespread in the microbial world.
- All water samples from the Snake River Plain Aquifer (> 10 different locations) that have been tested for urease activity have been positive, and urea hydrolyzing bacteria are readily isolated.
- We 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.

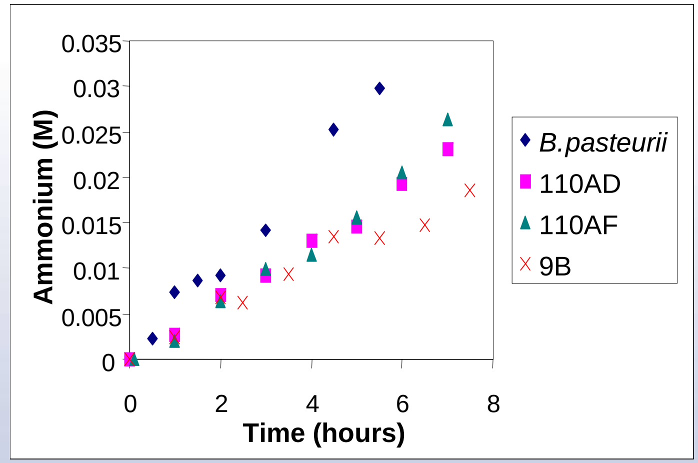
Conceptual approach for remediation



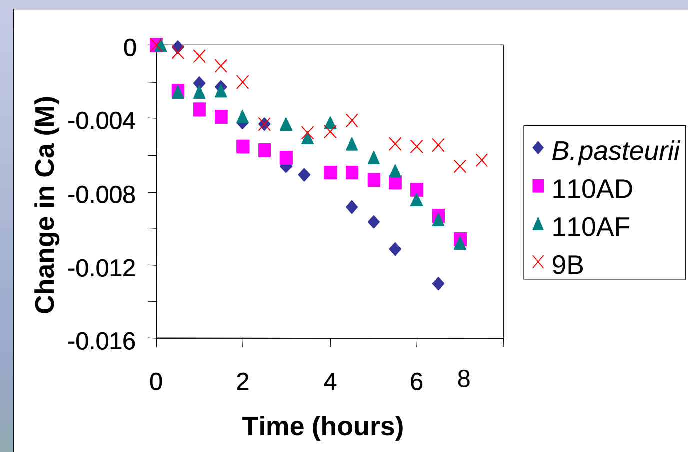
A. Hydrolysis of urea produces NH_4^+, HCO_3^- and raises pH.
B. NH_4^+ promotes desorption of Sr, Ca from mineral surfaces. HCO_3^- promotes precipitation of calcite, co-precipitation of Sr.
C. Continued precipitation of calcite isolates Sr from contact with groundwater.

Initial experiments: proof of principle

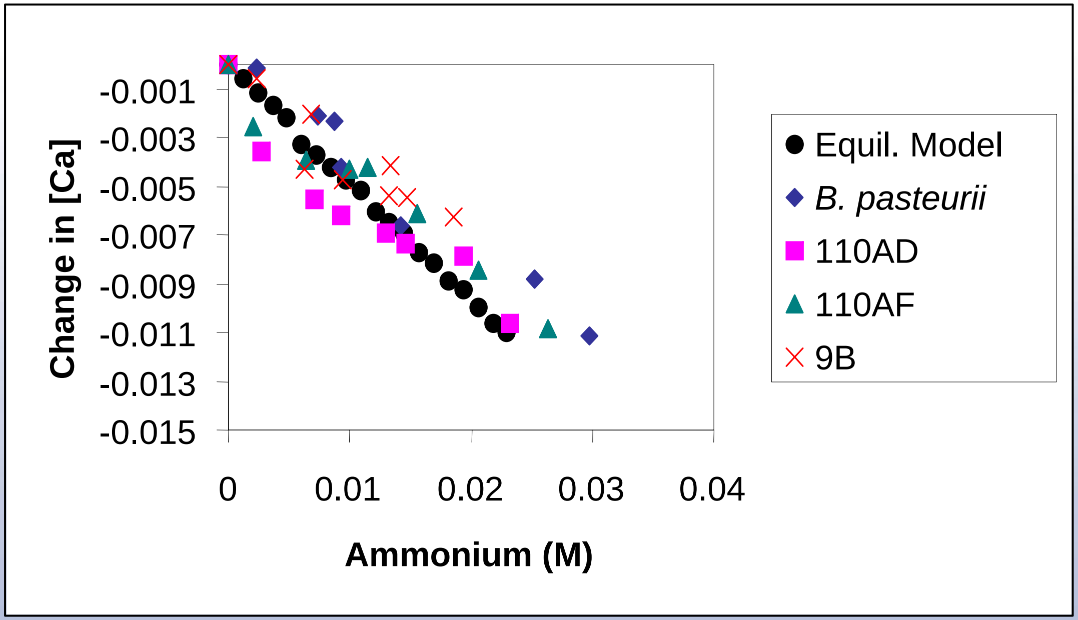




- *Batch experiments in artificial medium supplied with calcium and urea; initial log of saturation index -1.7.*
- *Used* Bacillus pasteurii *and SRPA bacterial isolates*
- *pH, calcium, ammonium monitored for 8 hours.*



System follows equilibrium model



$H_2NCONH_2 + 2H_2O \rightarrow 2NH_4^+ + CO_3^{2-}$ (1)

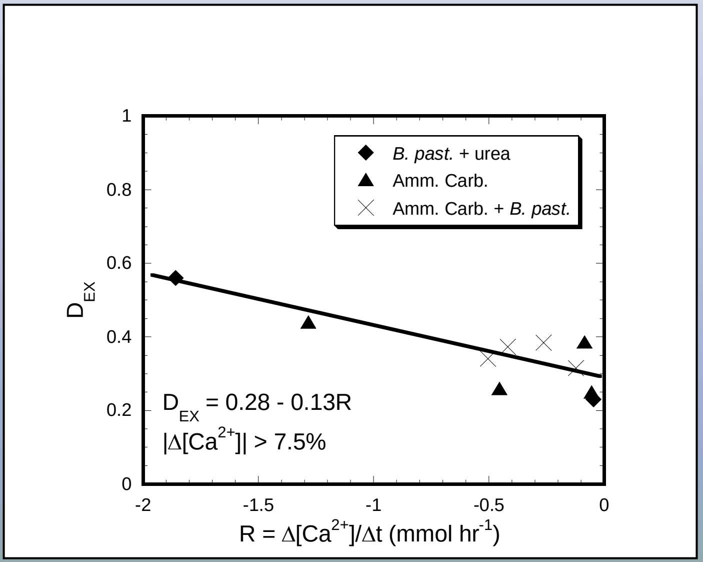
$Ca^{2+} + CO_3^{2-} \rightarrow CaCO_3$ (2)

$H_2NCONH_2 + 2H_2O + Ca^{2+} \rightarrow 2NH_4^+ + CaCO_3$ (3)

Strontium uptake is enhanced

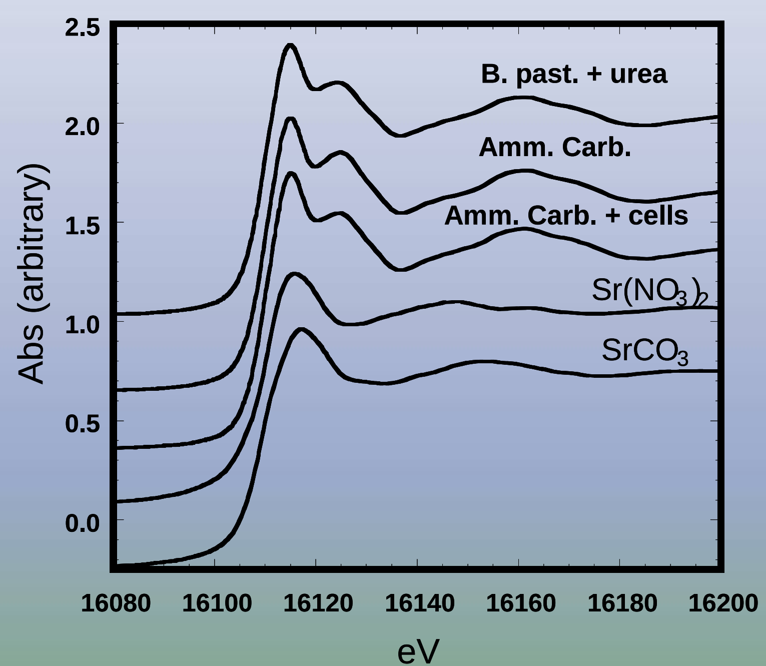
- When Sr is included, it is removed concurrently with Ca, and biogenically generated calcite takes up more Sr than abiotically generated calcite.
  - Higher D_{EX} correlated with higher precipitation rates.

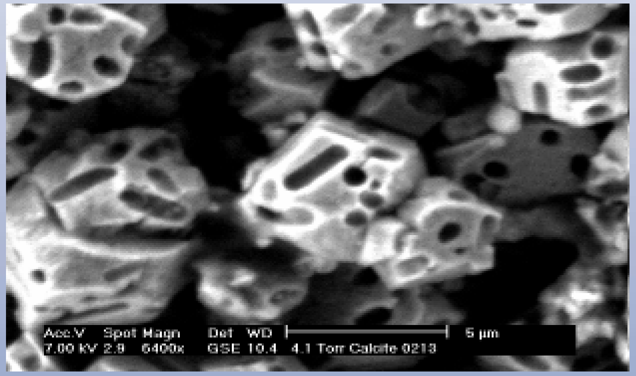
Sample generated by	Sr:Ca
B. Pasteurii + urea	0.044
Amm. Carb.	0.032
Amm. Carb. + *B. pasteurii*	0.027



Strontium is held tightly within calcite

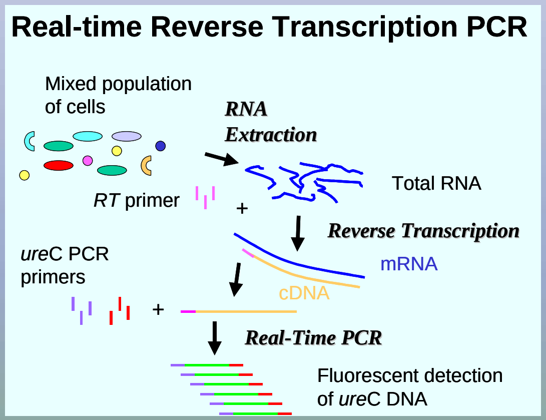
- X-ray Absorption Near Edge Structure (XANES) spectroscopy shows Sr substitutes for Ca in calcite lattice.
  - Solid solution formation is beneficial for long-term immobilization.





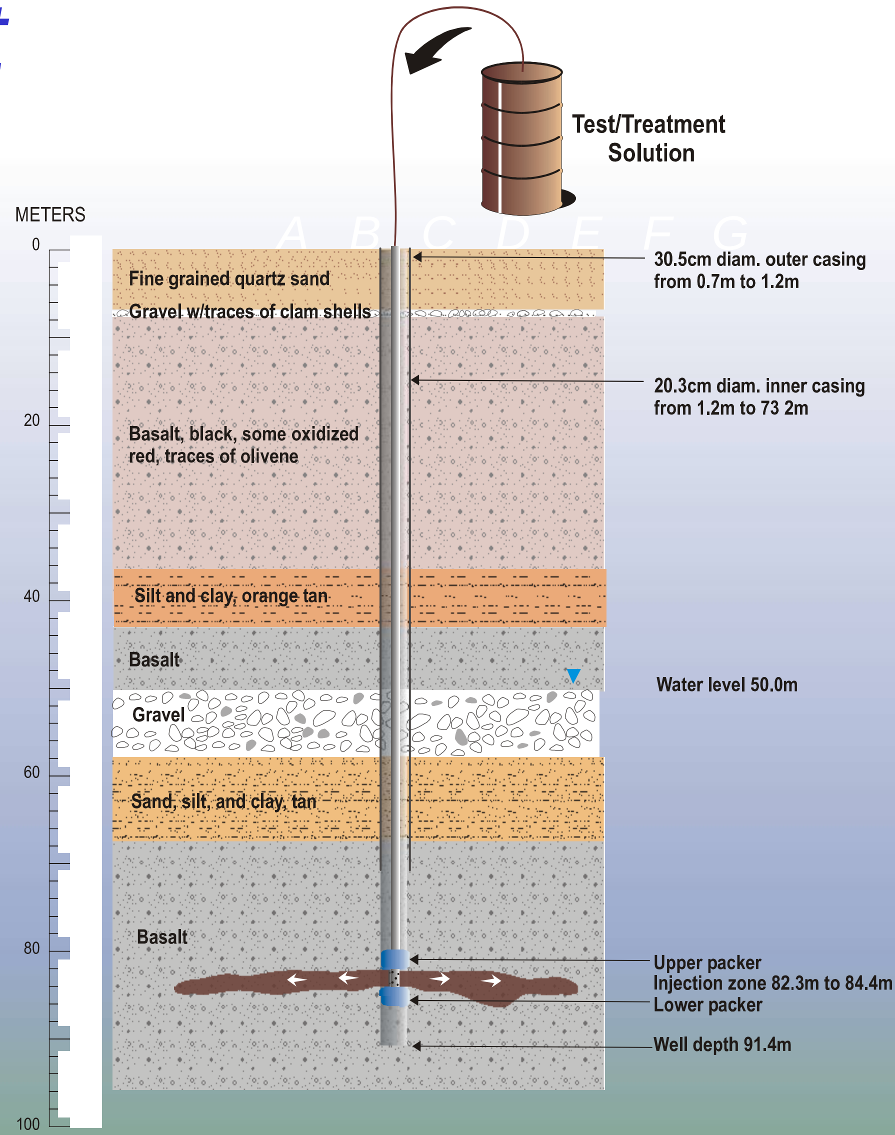
In the field, to verify the process we rely on urease activity

- 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 *ure*C mRNA transcripts, using real-time PCR.



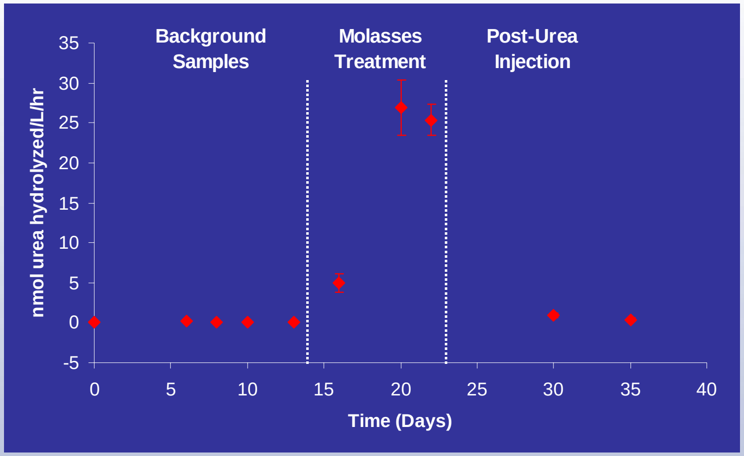
Idaho Falls field test

- 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.

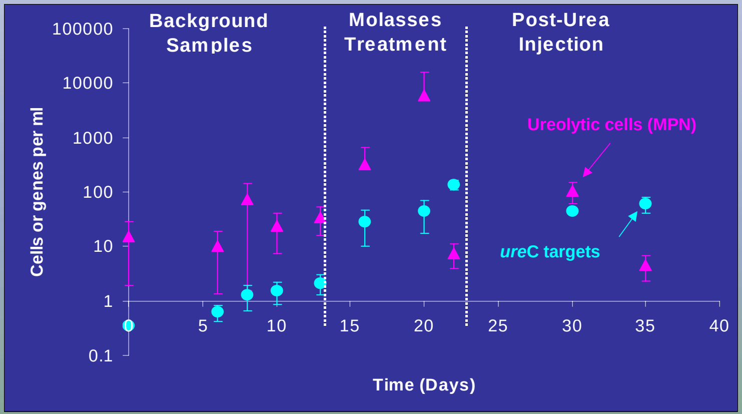


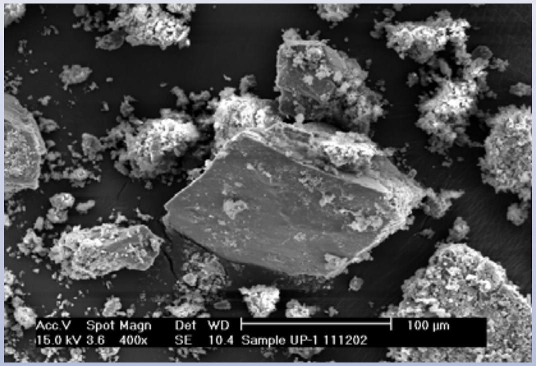
Field test results – urease activity was stimulated and calcite precipitated.

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.

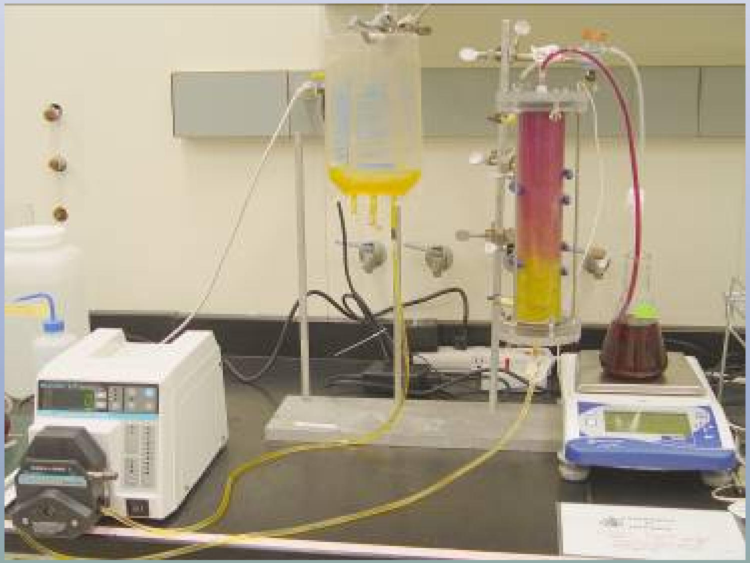
Other Activities

- 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 *ure*C 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 *in situ* generation of reactants.
- Extracellular urease immobilized on Eupergit® 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