

**Final report for DOE Grant No. DE-SC0006609**  
**Persistence of Microbially Facilitated Calcite Precipitation as an *in situ***  
**Treatment for Strontium-90**

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This final report describes research that extends the multi-institutional 13 month Rifle, Colorado Field Campaign (DOE Grant No. DE-FG02-07ER64404, “Field Investigations of Microbially Facilitated Calcite Precipitation for Immobilization of Strontium-90 and Other Trace Metals in the Subsurface”) and is a supplement to the previously submitted final report (Smith *et al.* 2012a). For completeness, the earlier submitted final report is appended to this report.

## Persistence of Microbially Facilitated Calcite Precipitation as an *in situ* Treatment for Strontium-90

### Abstract

Subsurface radionuclide and metal contaminants throughout the U.S. Department of Energy (DOE) complex pose one of DOE's greatest challenges for long-term stewardship. One promising stabilization mechanism for divalent ions, such as the short-lived radionuclide  $^{90}\text{Sr}$ , is co-precipitation in calcite. We have previously found that nutrient addition can stimulate microbial ureolytic activity, that this activity accelerates calcite precipitation and co-precipitation of Sr, and that higher calcite precipitation rates can result in increased Sr partitioning. We have conducted integrated field, laboratory, and computational research to evaluate the relationships between ureolysis and calcite precipitation rates and trace metal partitioning under environmentally relevant conditions, and investigated the coupling between flow/flux manipulations and precipitate distribution.

A field experimental campaign conducted at the Integrated Field Research Challenge (IFRC) site located at Rifle, CO was based on a continuous recirculation design; water extracted from a down-gradient well was amended with urea and molasses (a carbon and electron donor) and re-injected into an up-gradient well. The goal of the recirculation design and simultaneous injection of urea and molasses was to uniformly accelerate the hydrolysis of urea and calcite precipitation over the entire inter-wellbore zone. The urea-molasses recirculation phase lasted, with brief interruptions for geophysical surveys, for 12 days and was followed by long-term monitoring which continued for 13 months. A post experiment core located within the inter-wellbore zone was collected on day 321 and characterized with respect to cation exchange capacity, mineral carbonate content, urease activity, *ureC* gene abundance, extractable ammonium (a urea hydrolysis product) content, and the  $^{13}\text{C}$  isotopic composition of solid carbonates. It was also subjected to selective extractions for strontium and uranium. Result of the core characterization suggest that urea hydrolysis occurred primarily within the upper portion of the inter-wellbore zone and that strontium was mobilized from cation exchange sites and subsequently co-precipitated with new  $\text{CaCO}_3$ .

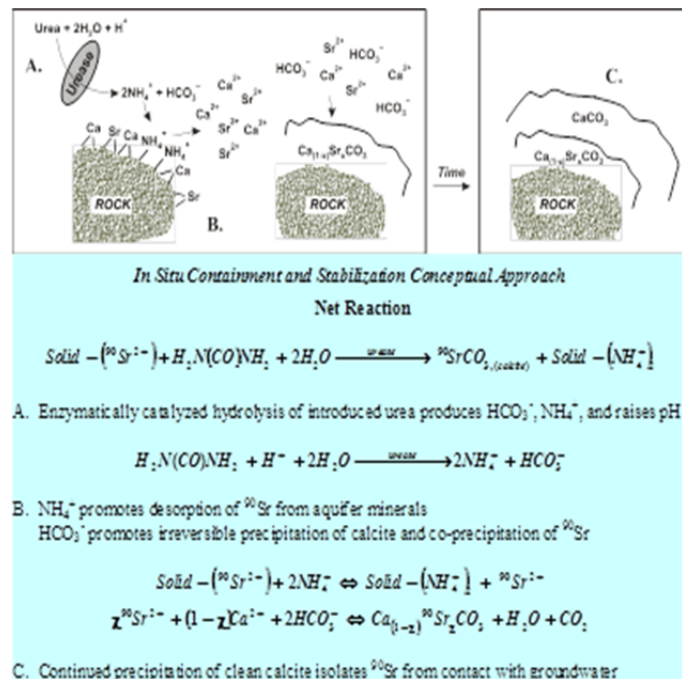
## Introduction

Subsurface radionuclide and trace metal contaminants throughout the U.S. Department of Energy (DOE) complex pose one of DOE's greatest challenges for long-term stewardship. One promising stabilization mechanism for divalent trace ions, such as the short-lived radionuclide  $^{90}\text{Sr}$ , is co-precipitation in calcite. Calcite, a common mineral in the arid western U.S., can form solid solutions with trace metals. We have conducted integrative, multi-disciplinary field-scale investigations of the potential for *in situ* stabilization and containment of radionuclides or contaminant metals (*e.g.*,  $^{90}\text{Sr}$ ) by their facilitated co-precipitation with calcium carbonate in groundwater and vadose zone systems. This research was conducted with the recognition that eventual successful implementation of any full scale *in situ* remediation, including monitored natural attenuation, requires 1) fundamental understanding of the biogeochemical processes that influence or control contaminant mobility, 2) the translation of that understanding into conceptual and numerical models that account for process coupling within the hydrogeologic setting, and 3) robust methods for measuring and monitoring relevant *in situ* processes to verify their occurrence and demonstrate long-term sustainability.

Our facilitated *in situ* stabilization approach, shown schematically in Figure 1, relies upon the hydrolysis of urea to cause the acceleration of calcium carbonate precipitation (and trace metal co-precipitation) by increasing groundwater pH and alkalinity. Subsurface urea hydrolysis is catalyzed by urease enzyme produced *in situ* by urea-hydrolyzing organisms, which are ubiquitous in the environment. Relying on *in situ* microbial activity rather than direct addition to introduce the carbonate mitigates the potential for premature mineral formation (*i.e.*, borehole plugging) and enables treatment of a wider area from a single injection point.

Because many arid western vadose zones and aquifers are saturated or even oversaturated with respect to calcite, the co-precipitation process has the potential to contain and stabilize the target contaminants for 100s to 1000s of years. Another advantage of the ureolysis approach is that the ammonium ions produced by the reaction can exchange with contaminant metals sorbed to subsurface mineral surfaces, thereby enhancing the susceptibility of the contaminants to re-capture in a more stable solid phase (co-precipitation rather than adsorption) and resulting in treatment of the solid as well as the aqueous phase. Treatment of the solid phase constitutes a major advantage compared to pump and treat type approaches for groundwater cleanup (Mackay and Cherry 1989).

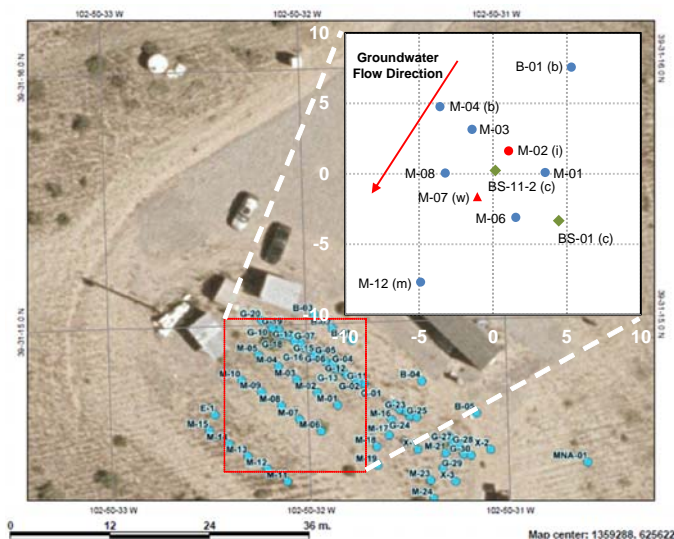
In our previous research we (i) demonstrated that ureolysis-driven calcite



**Figure 1.** Conceptual approach for *in situ* containment stabilization using ureolytic calcite precipitation and contaminant ( $^{90}\text{Sr}$  in this example) co-precipitation.

precipitation occurs under laboratory (Fujita *et al.* 2000; Ferris *et al.* 2004) and field conditions (Colwell *et al.* 2005; Fujita *et al.* 2008) and can incorporate substantially more strontium than abiotic precipitation (Fujita *et al.* 2004; Mitchell and Ferris 2005), (ii) developed and tested in the field the tools needed to determine *in situ* urease activity (expressed as ureolysis rates; Colwell *et al.* 2005) and (iii) developed biomolecular tools to track changes in subsurface microbial communities (Colwell *et al.* 2005; Petzke *et al.* 2006; Gresham *et al.* 2007). However, the coupling between ureolysis-driven calcite precipitation and advective groundwater fluxes remains poorly understood. Single well field experiments have been useful to elucidate microbial and biogeochemical processes under relevant *in situ* conditions while limiting the complexity introduced by advecting groundwater. Here we report on an expansion of our previous research, an investigation of ureolysis-driven calcite precipitation under conditions of forced advection groundwater flow. Specifically our field-based study was designed to assess the relationships between introduced reagents, changes in urease activity, calcite precipitation rates, mineral distribution, and hydrogeological-geochemical heterogeneity.

During 2010 and 2011 a field-scale advective experiment and associated long-term monitoring were conducted at a Uranium Mill Tailings Remedial Action (UMTRA) site in Rifle, CO<sup>1</sup>. The UMTRA site is the location for a Subsurface Biogeochemical Research (SBR) Integrated Field Research Challenge (IFRC) study. Although not a surrogate for a <sup>90</sup>Sr contaminated site, the Rifle site was selected for two reasons. First the biogeochemical and hydrologic characteristics of the site were compatible with the project research objectives. Second the project benefitted from the availability of existing research infrastructure (*e.g.*, wells, site-power, etc.) and synergies with ongoing IFRC and Lawrence Berkeley National Laboratory Scientific Focus Area research activities at the site. The results present here are limited to a study conducted on a post experimental core. Detailed results of the recirculation experiment campaign and associated long term monitoring have been previously submitted to DOE and are attached to this report.



**Figure 2.** Rifle, CO Integrated Field Research Challenge (IFRC) well field used for the experimental campaign. The inset shows the locations of background wells (b), the injection well (i), the extraction well (w), a monitoring well (m) and core collection (c). The coordinates are in meters and referenced to the midpoint of the inter-wellbore zone with positive coordinates being north and east of the midpoint.

<sup>1</sup> The Rifle site is a former uranium and vanadium mill tailings site located along the Colorado River, with a shallow unconfined alluvial aquifer generally at about 3.5 m below land surface (mbls). The aquifer is largely anoxic, and most of the research to date at the site has focused on bioreduction of uranium, using acetate injections to stimulate microbial activity. The website (<http://ifcrifle.pnl.gov/about/>) for the IFRC provides much more information about the site and previous research results.

## Methods

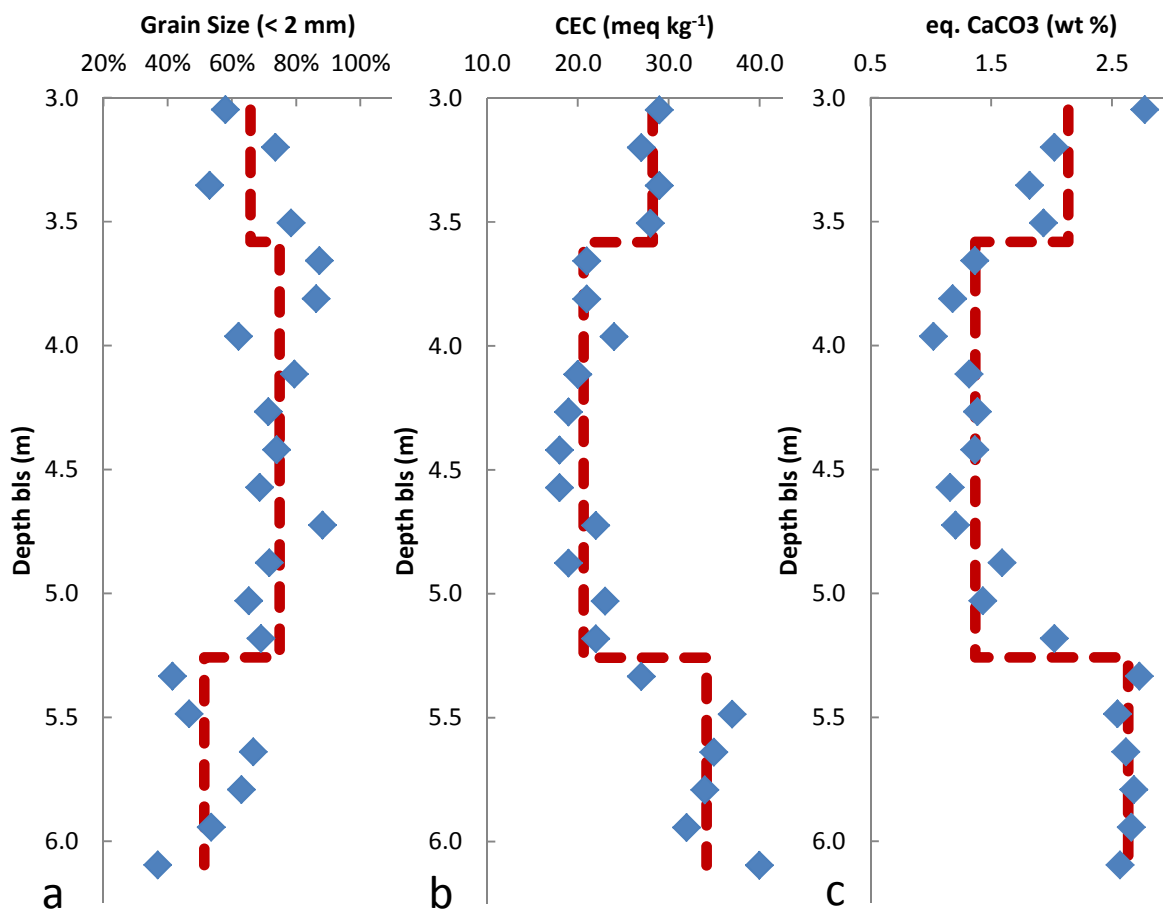
Our Rifle experimental campaign was conducted within an established IFRC experimental plot that was first used for uranium bioreduction experiments in 2002 (Anderson *et al.* 2003). Figure 2 shows the portion of the Rifle site where the 2002 plot is located. At this location the unconfined aquifer occurs at a depth of approximately 3 m below land surface (mbls) in alluvial sediments overlying the impermeable Wasatch formation at a depth of approximately 6.1 mbls. Although regional flow varies slightly with river stage, the nominal flow direction is to the southwest. Our experimental design was based on a continuous recirculation design; water extracted from down-gradient well M-07 was amended with urea and molasses (a carbon and electron donor) and re-injected into up-gradient well M-02. The goal of the recirculation design and simultaneous injection of urea and molasses was to uniformly accelerate the hydrolysis of urea and calcite precipitation over the entire inter-wellbore zone. This regimen is analogous to an *in situ* treatment focused on treating contaminated aquifer geomeedia rather than the groundwater.

The urea-molasses recirculation phase was initiated on 09/01/10 (time = 0) and continued, with brief interruptions for geophysical surveys, for 12 days. A total of 168,000 L of recirculated water amended with 42.5 kg of urea and 2.2 kg of molasses (metered into the recirculated water at a constant rate) was injected (nominal pumping rate 10.5 L min<sup>-1</sup>) over the 12 day recirculation period.

A single well (BS-11-2, Figure 2) located 1 meter down-gradient from the injection well (M-02) and that fully penetrated the fluvial aquifer was drilled by the Rifle IFRC 10 months after the urea-molasses recirculation phase (*i.e.*, day 321). At the time of drilling, the water table was at a depth of 2.9 m below land surface (bls). The well was cored over its entire 6 m depth. A visual inspection of the core was conducted in the field and the portion of the core below the water table was sampled at 15 cm intervals for chemical and microbial characterization as described below.

In addition, 4 sediment samples from a core (BS-01) collected in June 2010 (*i.e.*, day -67) outside of the inter-wellbore zone of the recirculation experiment were made available to us by the Rifle IFRC. These samples were composites of the 10 to 13 ft, 13 to 16 ft, 16 to 19 ft, and 19 to 20 ft intervals of the core and were also characterized as described below.

Cation exchange capacity was determined by the University of Idaho Analytical Sciences Laboratory using EPA Method 9081. The abundance of solid carbonates (measured as released CO<sub>2</sub> and reported as equivalent CaCO<sub>3</sub>) was determined by Actlabs Ltd. using an Eltra CW-800 Elemental Analyzer. Exchangeable ammonium was determined using a single step method adopted from Mackin and Aller (1984). Extractable trace metals were determined using a four step extraction (Tessier *et al.* 1979) that allows the assessment of trace metal partitioning among four operationally-defined sediment fractions: readily exchangeable, carbonates, iron and manganese oxyhydroxides, and oxidizable organics/sulfides. Values of  $\delta^{13}\text{C}$  for solid carbonates were determined by EA Atekwana (Oklahoma State University) by gas source isotope ratio mass spectroscopy (IRMS). Urease activity was measured using a <sup>14</sup>C labeled urea hydrolysis technique we had previously developed (Colwell *et al.* 2005). Bacterial *ureC* genes were quantified using a quantitative polymerase chain reaction (qPCR) assay with degenerate PCR primers targeting a 389-bp amplicon of the *ureC* gene (assay described in Fujita *et al.* 2010).



**Figure 3.** Variation with depth of (a) grain size, (b) cation exchange capacity, and total solid carbonate minerals reported as equivalent CaCO<sub>3</sub> for core BS-11-2. Dashed red vertical lines are average values over the indicated depth range. Dashed red horizontal lines show the 3 zones in the core as described in the text.

## Results and Discussion

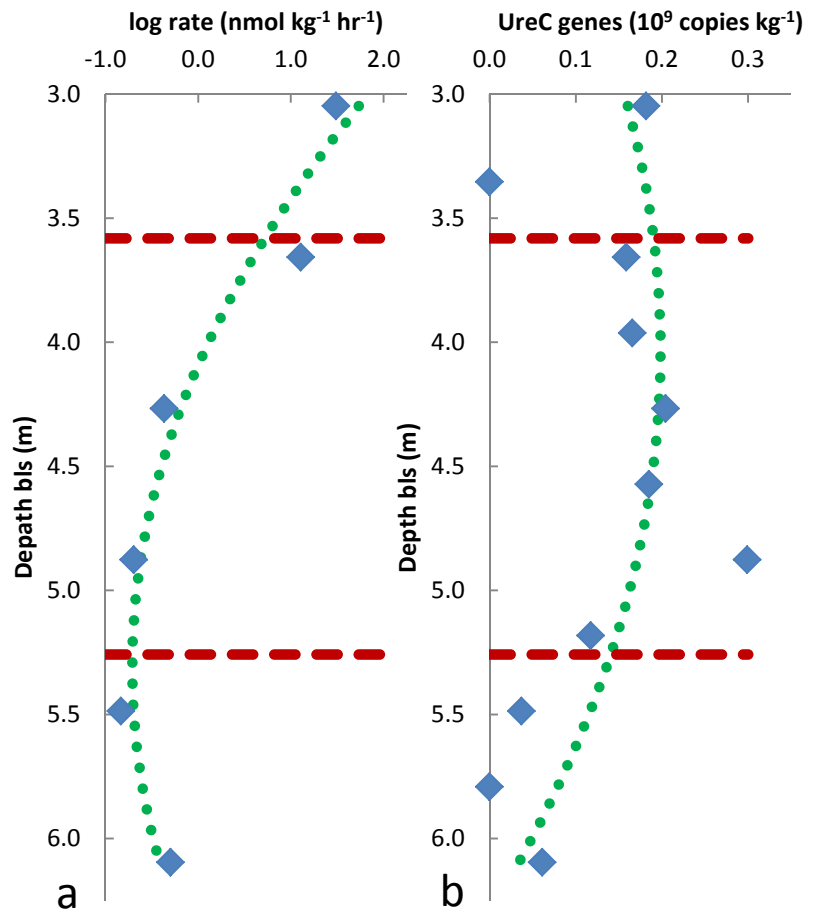
Figure 3 shows variations in key physical and chemical characteristic of the BS-11-2 core. Based on a combination of visual inspection of the core, and data on grain size (*i.e.*, < 2 mm), cation exchange capacity (a surrogate for clay content), CaCO<sub>3</sub> content, and organic carbon content (not shown), the core was divided vertically into three units. The uppermost unit was a sandy-cobble zone with some clay. This transitioned to a sandy zone with fewer cobbles at 3.6 m depth, and at greater depth (5.3 m) a dark, highly-reduced (as evidenced by organic carbon) cobble and sand zone occurred. The core bottomed in the underlying Wasatch formation.

The observed mean values for total carbonate minerals (reported as weight percent CaCO<sub>3</sub>) for the background BS-01 core (not shown) and for core BS-11-2 (Figure 3c) were  $1.4 \pm 0.8\%$  and  $1.9 \pm 0.6\%$ , respectively. The difference between the two cores was not statistically significant at the 90% or 50% confidence levels (t-test). We expected that urea hydrolysis would result in increased precipitation of calcite (Figure 1). However, the complete conversion to calcite of the urea that was injected during the recirculation period would add approximately 0.004% calcite to

the Rifle sediments (Smith *et al.* 2012a). Such a change in calcite content, assuming homogeneous distribution over the inter-wellbore zone, would be extremely difficult to observe directly given that the preexisting calcite content of the site ranges from ~0.6 to 2.8%.

Slug tests conducted in wells surrounding BS-11-2 by DR Newcomer (Pacific Northwest National Laboratory) prior to the recirculation period were combined with electromagnetic borehole flowmeter tests (Waldrop and Waldrop 2005) to estimate the hydraulic conductivity of the three zones identified in the core. The results, reported in Table 1, indicate that the hydraulic conductivity in the fluvial aquifer decreases with depth and that the hydraulic conductivity of the upper zone is 6 times that of the lowest zone.

Figure 4 shows the variation with depth of (a) estimated urease activity and (b) *ureC* gene abundance. The urease activity in the sediments from the upper meter of the aquifer was 30 times higher than the activity in sediments from the lower portion of the aquifer; the latter values are near background levels for the site (Smith *et al.* 2012a). The number of *ureC* gene copies also shows a decreasing trend (with scatter) with depth although with much less variation than for the urease activity (note the log scale for activity and the linear scale for gene copies). Although we observed a positive correlation between *ureC* gene counts and activity for initial measurements and during increases in biomass associated with the urea recirculation period Smith *et al.* 2012b), the long-term results obtained from the BS-11-2 core do not follow this pattern. DNA extraction from the long-term sediment samples was more difficult than from the samples collected prior to and during recirculation, and variation in extraction efficiency may have contributed some systematic biases to the core results. Another possible explanation for the apparent divergence from correlation between urease activity and gene numbers is that the long-term samples have higher extracellular urease protein loads, which would decouple the number of organisms with *ureC* genes from the urease activity.



**Figure 4.** Variation with depth of (a) estimated urease activity (expressed as hydrolysis rates) and (b) *ureC* gene copies for core BS-11-2. Green dotted lines are smoothed (LOESS regression) trends. Dashed red horizontal lines show the 3 zones in the core as described in the text.

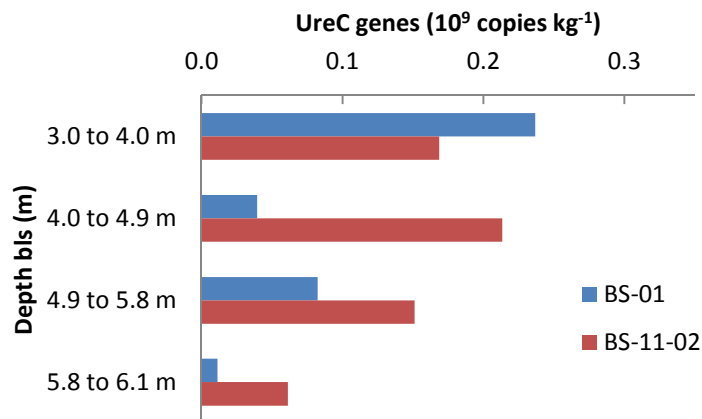


**Table 1.** Characteristic of fluvial sediments within the inter-wellbore zone.

Depth bls		<sup>1</sup> Cation Exchange Capacity (meq kg <sup>-1</sup> )	<sup>1</sup> CaCO <sub>3</sub> (wt. %)	<sup>2</sup> Hydraulic Conductivity (m d <sup>-1</sup> )	<sup>3</sup> Porosity (%)
top (m)	bottom (m)				
2.90	3.58	28.3	2.14	15.7	27
3.58	5.26	20.6	1.37	6.3	27
5.26	6.10	34.2	2.63	2.6	27
<sup>1</sup> This Study. <sup>2</sup> Estimated from slug tests (DR Newcomer, 12/16/210, person communication) and electromagnetic borehole flowmeter tests (Waldrop and Waldrop 2005) in surrounding wells. <sup>3</sup> Anderson <i>et al.</i> (2003).					
Rifle, CO UMTRA IFRC, Well/Core BS-11-2. Drilled 07/19/11. Water Table at 2.9 m bls. Wasatch Formation 6.1 m bls.					

The influence of the urea-molasses injection on *ureC* gene count was assessed by comparison of the BS-11-2 core and the background BS-01 core. The gene counts are similar in the upper portion of both cores (with BS-11-2 being 30% less than BS-02). However in the deeper portions of the core, the gene counts are 2 to 5 times higher in the core from the inter-wellbore zone when compared to the background core. These observations suggest that not only did the urea-molasses treatment increase the gene count by a factor of 2 to 5, but that the change persists for many months following the termination of the treatment.

Figure 6 shows the variation with depth of (a) extractable ammonium (a urea hydrolysis product) and (b)  $\delta^{13}\text{C}$  for solid carbonates (a possible indicator of urea hydrolysis induced CaCO<sub>3</sub> precipitation). The ammonium concentration is at or near zero in the upper portion of the core, and increases with depth. The ammonium results appear at odds with the observed urease activity results where the highest activities (and by inference the highest production of ammonium) occurred at the top of the core (Figure 4a). One explanation of this observation is that the ammonium distribution observed in the core 10 months after the recirculation phase does not reflect the distribution at the time of urea hydrolysis but reflects the redistribution of ammonium following the end of urea hydrolysis (urea was not observed in groundwater samples beyond one month after the recirculation phase). This is supported by the observed distribution of hydraulic conductivity (Table 1) in which the portion of the core with the highest ammonium concentration occurs where the hydraulic conductivity is



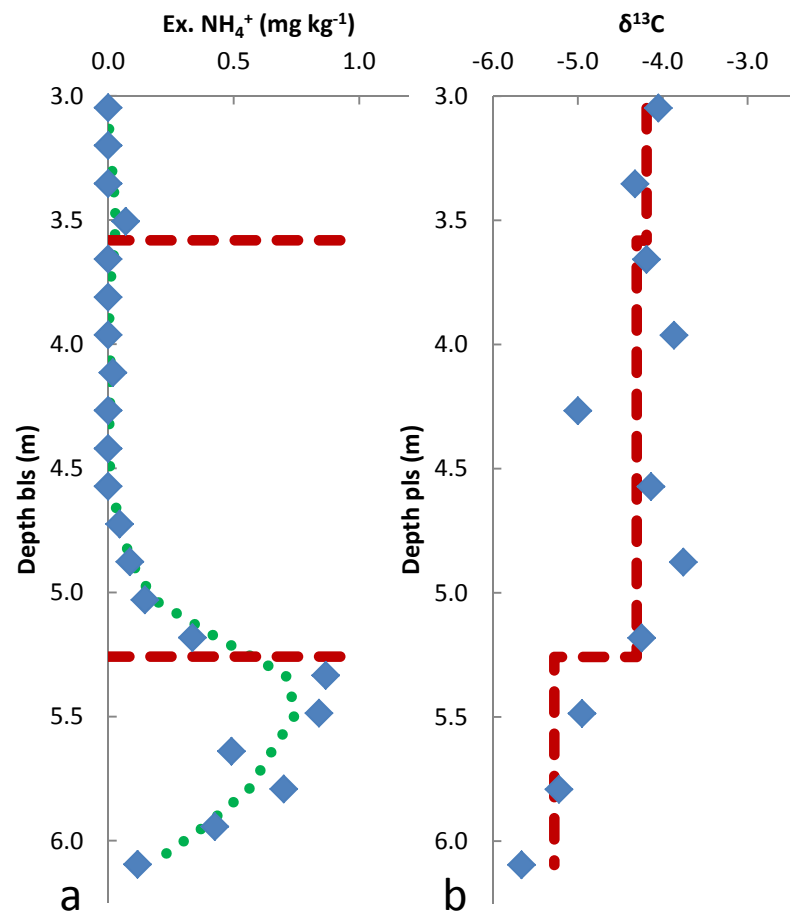
**Figure 5.** Comparison of *ureC* gene copy counts between the BS-11-2 core and the background BS-01 core. Values for BS-11-2 core are averages calculated over the reported depth intervals. Values for BS-01 are determined from composite sample over the reported depth interval.



low; one could presume that in the shallower sediments, with higher hydraulic conductivity, ammonium was more rapidly “flushed” from the zone by regional groundwater.

Figure 6b presents the variation in  $\delta^{13}\text{C}$  for solid carbonates with depth. The  $\delta^{13}\text{C}$  values are fairly uniform in the upper two zones within the core, but become increasingly negative toward the bottom of the core. Because the urea used for the experiment had a  $\delta^{13}\text{C}$  value ( $-40.7 \pm 0.4$  ‰; reflecting urea’s manufacture from natural gas) which was distinctly more negative than the ambient site groundwater value ( $-16.6 \pm 0.2$  ‰), one would expect that solid phase carbonate precipitated with carbon derived from urea hydrolysis would have a more negative  $\delta^{13}\text{C}$  value. Figure 7 shows a comparison of  $\delta^{13}\text{C}$  values for core BS-11-2 and background core BS-01. At all depth intervals the  $\delta^{13}\text{C}$  values for BS-11-2 are more negative than the background core, with the difference more pronounced at greater depth. Measurement of  $\delta^{13}\text{C}$  in the solid carbonates from the two cores showed mean values of  $-3.89$  ‰ (BS-01) and  $-4.49$  ‰ (BS-11-2). A t-test suggested that the observed difference was significant at the 90% confidence level but not at the 95% confidence level. The observation of lighter carbon in the post-recirculation core, while not conclusive, was consistent with the addition of a small amount of calcite containing carbonate derived from hydrolysis of the isotopically light urea.

Figure 8 shows the results of the 4 step selective extraction for (a) strontium and (b) uranium in BS-11-2 core. Strontium was observed in the readily exchangeable (EX), and carbonate (CARB) fractions but not in the iron and manganese oxyhydroxides (OXY), and oxidizable organics/sulfides (ORG) fractions. Uranium was observed in the readily exchangeable (EX), carbonate (CARB), and iron and manganese oxyhydroxides (OXY) fractions but not in the oxidizable organics/sulfides (ORG) fractions. The total extractable Sr (sum of 4 extraction steps) was uniform with depth. Figure 8a shows the fraction of Sr in the exchangeable



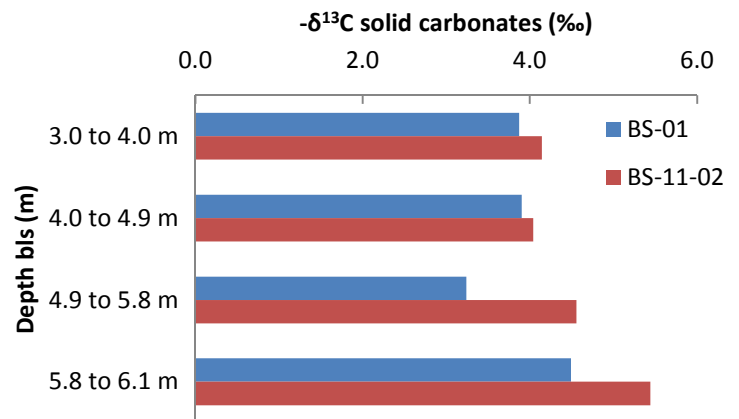
**Figure 6.** Variation with depth of (a) extractable ammonium (a urea hydrolysis product) and (b)  $\delta^{13}\text{C}$  for solid carbonates (a possible indicator of urea hydrolysis induced  $\text{CaCO}_3$  precipitation) in core BS-11-2. Green dotted lines are smoothed (LOESS regression) trends. Dashed red vertical lines are average values over the indicated depth range. Dashed red horizontal lines show the 3 zones in the core as described in the text.

phase. In the upper 60 cm of the core there is a slight depletion of the exchangeable Sr (EX) with a corresponding enrichment in carbonate Sr (CARB) relative to the remainder of the core. This result, while not conclusive is consistent with the release of strontium via ion exchange with ammonium for urea hydrolysis and the subsequent co-precipitation of strontium in calcite (see Figure 1).

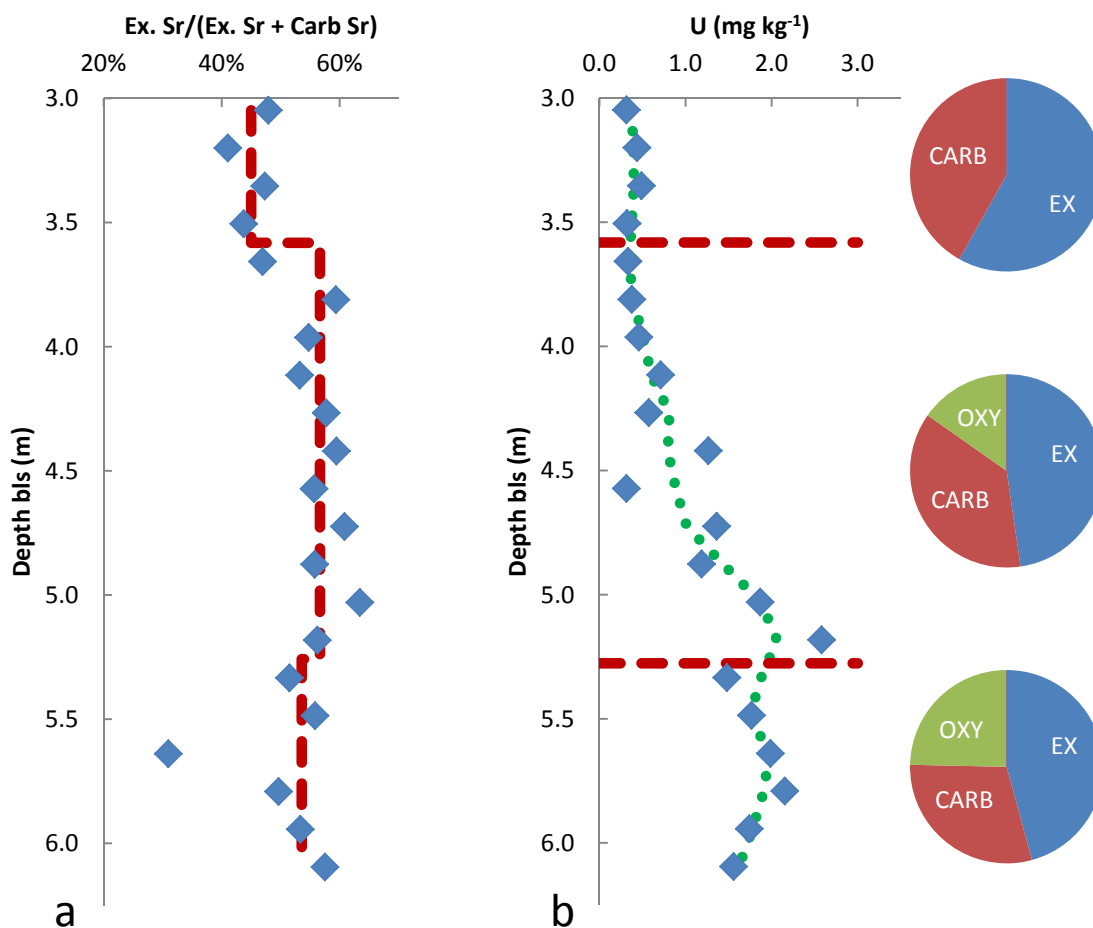
Figure 8b presents the variation with depth of the total extractable uranium as well as the average distribution of uranium between the 4 extractable phases. These results show an increase in uranium with depth and an increasing fraction of uranium associated with the iron and manganese oxyhydroxides (OXY). Although uranium studies are beyond the scope of this project, they are the focus of the Rifle IFRC. Given that the inter-wellbore zone had been used previously (2002) for uranium bioreduction experiments in which uranium associated with hydrous metal oxides was reduced, one can hypothesize that the results in Figure 8b reflect the reduction of hydrous metal oxides associated uranium to a recalcitrant phase (relative to the extraction procedure used here) in the upper hydraulically conductive portion of the aquifer. If confirmed, these results suggest that bioreduced uranium can persist under ambient aquifer conditions for at least a decade. Alternatively bioreduction may have resulted in the loss of hydrous metal oxide and the associated uranium has been transported down gradient. However, additional investigation of the core and the extraction procedures would be required to assess these hypotheses.

## Conclusions

Results of the core characterization suggest that urea hydrolysis occurred within the upper portion (and perhaps to a lesser degree in lower portion) of the inter-wellbore zone and that strontium was mobilized from cation exchange sites and subsequently co-precipitated with new  $\text{CaCO}_3$ . These results combined with those previously reported (Smith *et al.* 2012a, attached) suggest that calcite precipitation driven by urea hydrolysis can be effectively implemented and controlled at the field scale using a recirculation treatment design. Although the injected fluids and associated stimulation of urease activity may move beyond the inter-wellbore zone, significant urea hydrolysis was only observed in the inter-well bore zone. Furthermore, based on the observations of changes in carbon isotope ratios in the groundwater (Smith *et al.* 2012a) and the solids, the enhanced precipitation of calcite and/or other carbonates at very low level can be inferred to only have occurred in the inter-wellbore zone.



**Figure 7.** Comparison of  $\delta^{13}\text{C}$  values for solid carbonates between core BS-11-2 and the background core BS-01. Values for BS-11-2 core are averages calculated over the reported depth intervals. Values for BS-01 are determined from composite samples over the reported depth interval.



**Figure 8.** Variation with depth of extractable (a) strontium and (b) uranium. Pie diagrams show the distribution of extractable uranium between the operationally-defined sediment fractions; readily exchangeable (EX), carbonates (CARB), iron and manganese oxyhydroxides (OXY), and oxidizable organics/sulfides (ORG). Green dotted lines are smoothed (LOESS regression) trends. Dashed red vertical lines are average values over the indicated depth range. Dashed red horizontal lines show the 3 zones in the core as described in the text.

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Field Investigations of Microbially Facilitated Calcite Precipitation for Immobilization of Strontium-90 and Other Trace Metals in the Subsurface

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**Field Investigations of Microbially Facilitated Calcite Precipitation for Immobilization of Strontium-90 and Other Trace Metals in the Subsurface**  
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This final report is organized into 2 sections. The first section (page 2) describes the multi-institutional 13 month Rifle Colorado Field Campaign with an emphasis on the contributions to the campaign by the University of Idaho. The second section (page 16) of the report describes the overall accomplishments including a list of publications for the entire project.

## ASSESSING MICROBIALLY FACILITATED CALCITE PRECIPITATION AT THE FIELD SCALE: THE RIFLE, COLORADO FIELD CAMPAIGN

### Abstract

Subsurface radionuclide and metal contaminants throughout the U.S. Department of Energy (DOE) complex pose one of DOE's greatest challenges for long-term stewardship. One promising stabilization mechanism for divalent ions, such as the short-lived radionuclide  $^{90}\text{Sr}$ , is co-precipitation in calcite. We have previously found that that nutrient addition can stimulate microbial ureolytic activity that this activity accelerates calcite precipitation and co-precipitation of Sr, and that higher calcite precipitation rates can result in increased Sr partitioning. We have conducted integrated field, laboratory, and computational research to evaluate the relationships between ureolysis and calcite precipitation rates and trace metal partitioning under environmentally relevant conditions, and investigated the coupling between flow/flux manipulations and precipitate distribution.

A field experimental campaign conducted at the Integrated Field Research Challenge (IFRC) site located at Rifle, CO was based on a continuous recirculation design; water extracted from a down-gradient well was amended with urea and molasses (a carbon and electron donor) and re-injected into an up-gradient well. The goal of the recirculation design and simultaneous injection of urea and molasses was to uniformly accelerate the hydrolysis of urea and calcite precipitation over the entire inter-wellbore zone. The urea-molasses recirculation phase lasted, with brief interruptions for geophysical surveys, for 12 days followed by long-term monitoring which continued for 13 months. Following the recirculation phase we found persistent increases in urease activity (as determined from  $^{14}\text{C}$  labeled laboratory urea hydrolysis rates) in the upper portion of the inter-wellbore zone. We also observed an initial increase (approximately 2 weeks) in urea concentration associated with injection activities followed by decreasing urea concentration and associated increases in ammonium and dissolved inorganic carbon (DIC) following the termination of injection. Based on the loss of urea and the appearance of ammonium, a first order rate constant for urea hydrolysis of  $0.18 \text{ day}^{-1}$  with an associate  $R_f$  for ammonium of 11 were estimated. This rate constant is approximately 6 times higher than estimated for previous field experiments conducted in eastern Idaho. Additionally, DIC carbon isotope ratios were measured for the groundwater. Injected urea had a  $\delta^{13}\text{C}$  of  $-40.7 \pm 0.4 \text{ ‰}$  compared to background groundwater DIC of  $\delta^{13}\text{C}$  of  $-16.6 \pm 0.2 \text{ ‰}$ . Observed decreases in groundwater DIC  $\delta^{13}\text{C}$  of up to  $-19.8 \text{ ‰}$  followed temporal trends similar to those observed for ammonium and suggest that both the increase in ammonium and the shift in  $\delta^{13}\text{C}$  are the result of urea hydrolysis. Although direct observation of calcite precipitation was not possible because of the high pre-existing calcite content in the site sediments, an observed  $\delta^{13}\text{C}$  decrease for solid carbonates from sediment samples collect following urea injection (compared to pre-injection values) is likely the result of the incorporation of inorganic carbon derived from urea hydrolysis into newly formed solid carbonates.



## Introduction

Subsurface radionuclide and trace metal contaminants throughout the U.S. Department of Energy (DOE) complex pose one of DOE's greatest challenges for long-term stewardship. One promising stabilization mechanism for divalent trace ions, such as the short-lived radionuclide  $^{90}\text{Sr}$ , is co-precipitation in calcite. Calcite, a common mineral in the arid western U.S., can form solid solutions with trace metals. We have conducted integrative, multi-disciplinary field-scale investigations of the potential for *in situ* stabilization and containment of radionuclides or contaminant metals (e.g.,  $^{90}\text{Sr}$ ) by their facilitated co-precipitation with calcium carbonate in groundwater and vadose zone systems. This research recognized that eventual successful implementation of any full scale *in situ* remediation, including monitored natural attenuation, requires 1) fundamental understanding of the biogeochemical processes that influence or control contaminant mobility, 2) the translation of that understanding into conceptual and numerical models that account for process coupling within the hydrogeologic setting, and 3) robust methods for measuring and monitoring relevant *in situ* processes to verify their occurrence and demonstrate long-term sustainability.

Our facilitated *in situ* stabilization approach, shown schematically in Figure 1, relies upon the hydrolysis of urea to cause the acceleration of calcium carbonate precipitation (and trace metal co-precipitation) by increasing groundwater pH and alkalinity. Subsurface urea hydrolysis is catalyzed by urease enzyme produced *in situ* by urea-hydrolyzing organisms, which are ubiquitous in the environment. Relying on *in situ* microbial activity rather than direct addition to introduce the carbonate mitigates the potential for premature mineral formation (i.e., borehole plugging) and enables treatment of a wider area from a single injection point.

Because many arid western vadose zones and aquifers are saturated or even oversaturated with respect to calcite, the co-precipitation process has the potential to contain and stabilize the target contaminants for 100s to 1000s of years. Another advantage of the ureolysis approach is that the ammonium ions produced by the reaction can exchange with contaminant metals sorbed to subsurface minerals, thereby enhancing the susceptibility of the contaminants to re-capture in a more stable solid phase (co-precipitation rather than adsorption) and resulting in treatment of the solid as well as the aqueous phase. Treatment of the solid phase constitutes a major advantage compared to pump and treat type approaches for groundwater cleanup (Mackay and Cherry 1989).

In our previous research we (i) demonstrated that ureolysis-driven calcite

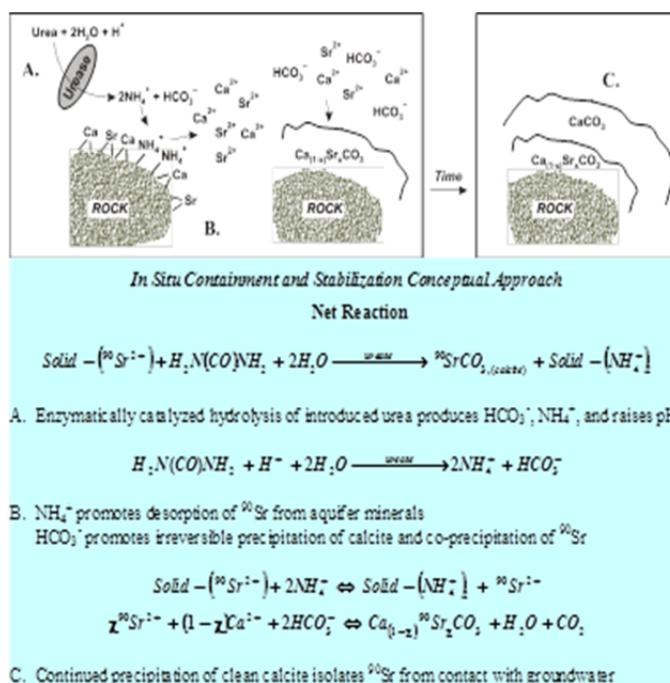


Figure 1. Conceptual approach for *in situ* containment stabilization using ureolytic calcite precipitation and contaminant ( $^{90}\text{Sr}$  in this example) co-precipitation.

precipitation occurs under laboratory (Fujita et al. 2000; Ferris et al. 2004) and field conditions (Colwell et al. 2005; Fujita et al. 2008) and can incorporate substantially more strontium than abiotic precipitation (Fujita et al. 2004; Mitchell and Ferris 2005), (ii) developed and tested in the field the tools needed to determine *in situ* urease activity (expressed as ureolysis rates; Colwell et al. 2005) and (iii) developed biomolecular tools to track changes in subsurface microbial communities (Colwell et al. 2005; Petzke et al. 2006; Gresham et al. 2007). However, the coupling between ureolysis-driven calcite precipitation and advective groundwater fluxes remains poorly understood. Single well field experiments have been useful to elucidate microbial and biogeochemical processes under relevant *in situ* conditions while limiting the complexity introduced by advecting groundwater. Here we report on an expansion of our previous research, an investigation of ureolysis-driven calcite precipitation under conditions of forced advection groundwater flow. Specifically our field-based study was designed to assess the relationships between introduced reagents, changes in urease activity, calcite precipitation rates, mineral distribution, and hydrogeological-geochemical heterogeneity. Our original research plan was centered upon conducting field experiments at the Vadose Zone Research Park (VZRP) at the Idaho National Laboratory (INL); the VZRP is an uncontaminated hydrogeological surrogate of the  $^{90}\text{Sr}$  contaminated Idaho Nuclear Technology and Engineering Center (INTEC) facility at INL. However, modifications to facility operations at INTEC (which provided water to the VZRP percolation ponds) altered the VZRP site hydrology to such an extent that the site was no longer suitable for our field experiments. As a result the field campaign was moved to a location in the city of Rifle, CO.

During 2010 and 2011 a field-scale advective experiment and associated long-term monitoring were conducted at a Uranium Mill Tailings Remedial Action (UMTRA) site in Rifle, CO<sup>1</sup>. The UMTRA site is the location for a Subsurface Biogeochemical Research (SBR) Integrated Field Research Challenge (IFRC) study. Although not a surrogate for a  $^{90}\text{Sr}$  contaminated site, the Rifle site was selected for two reasons. First the biogeochemical and hydrologic characteristics of the site were compatible with the project research objectives. Second the project benefitted from the

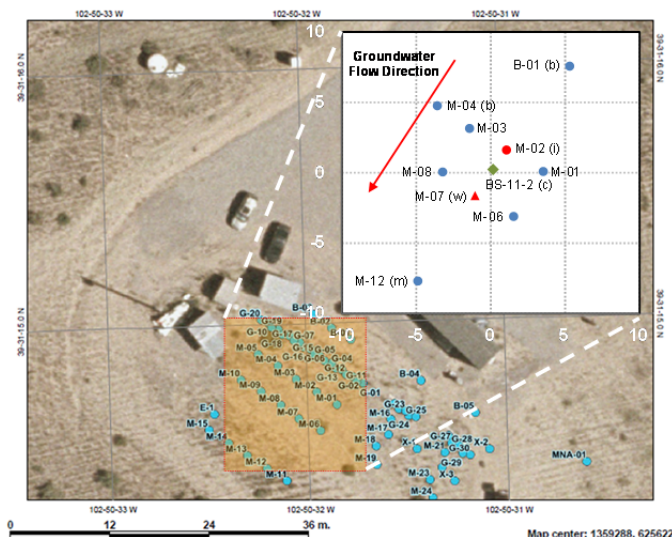


Figure 2. Rifle, CO Integrated Field Research Challenge (IFRC) well field used for the experimental campaign. The inset shows the locations of background wells (b), the injection well (i), the extraction well (w), a monitoring well (m) and core collection (c). The coordinates are in meters and referenced to the midpoint of the inter-wellbore zone with positive coordinates being north and east of the midpoint.

<sup>1</sup> The Rifle site is a former uranium and vanadium mill tailings site located along the Colorado River, with a shallow unconfined alluvial aquifer generally at about 3.5 m below land surface (mbls). The aquifer is largely anoxic, and most of the research to date at the site has focused on bioreduction of uranium, using acetate injections to stimulate microbial activity. The website (<http://ifcrifle.pnl.gov/about/>) for the IFRC provides much more information about the site and previous research results.

availability of existing research infrastructure (e.g., wells, site-power, etc.) and synergies with ongoing IFRC and Lawrence Berkeley National Laboratory Scientific Focus Area research activities at the site. In summary, the move from the VZRP to the Rifle site reflected a change in location for our research, not a change in our research objectives.

## Methods

Our Rifle experimental campaign was conducted within an established IFRC experimental plot that was first used for uranium bioreduction experiments in 2002 (Anderson et al. 2003). Figure 2 shows the portion of the Rifle site where the 2002 plot is located. At this location the unconfined aquifer occurs at a depth of approximately 3 m below land surface (mbls) in alluvial sediments overlying the impermeable Wasatch formation at a depth of approximately 6.1 mbls. Although regional flow varies slightly with river stage, the nominal flow direction is to the southwest. Our experimental design was based on a continuous recirculation design; water extracted from down-gradient well M-07 was amended with urea and molasses (a carbon and electron donor) and re-injected into up-gradient well M-02. The goal of the recirculation design and simultaneous injection of urea and molasses was to uniformly accelerate the hydrolysis of urea and calcite precipitation over the entire inter-wellbore zone. This regimen is analogous to an *in situ* treatment focused on treating contaminated aquifer geomeedia rather than the groundwater.

The urea-molasses recirculation phase was initiated on 09/01/10 (time = 0) and continued, with brief interruptions for geophysical surveys, for 12 days; long-term monitoring (collection of water samples at decreasing frequency, from twice weekly initially to monthly at the end) continued until day 418 (10/24/11). A total of 168,000 L of recirculated water amended with 42.5 kg of urea and 2.2 kg of molasses (metered into the recirculated water at a constant rate) was injected (nominal pumping rate  $10.5 \text{ L min}^{-1}$ ) over the 12 day recirculation period. Conservative tracer (KBr) tests were conducted at the start of the recirculation period and again at the end of the long-term monitoring.

Wells B-01 and M-04 served as locations for background sampling and well M-12 served as a down-gradient monitoring location. Other wells depicted in Figure 2 were used to support cross borehole geophysical tomography (seismic, electrical, and radar; not reported here). Because direct access to the geomeedia in order to examine the effects of treatment was limited, we incubated retrievable “packages” of Rifle sediment contained within permeable membranes in selected wells within the flow cell (wells B-01, M-02, and M-04). The packages suspended in the wells were termed “passive” sediments, to distinguish them from sediments contained within columns held in a cooler (to maintain groundwater temperature) at the surface through which groundwater was diverted just prior to injection; the sediments in the columns were termed “active” (Figure 3). The cooler also contained an additional set of “passive” sediments, where groundwater contact with the sediments was limited to diffusion from the exterior of the packages. A single core (BS-11-2, Figure 2), 1 meter down-gradient from the injection well M-02 was collected in July of 2011, 10 months after the active injection period for microbial and chemical characterization. In addition, sediment samples from a core (BS-01) that was collected outside of the flow cell prior to the initiation of our field campaign were made available to us.

Aqueous urea and ammonium were measured by ion chromatography, dissolved inorganic carbon (DIC) was measured with a total carbon analyzer, and bromide was measured by inductively coupled plasma - mass spectroscopy and ion chromatography. Urease activity was

measured using a  $^{14}\text{C}$  labeled urea hydrolysis technique we had previously developed (Colwell et al. 2005),  $\delta^{13}\text{C}$  values were determined by EA Atekwana (Oklahoma State University) by gas source isotope ratio mass spectroscopy (IRMS), and calcite content of sediments was estimated from the amount of evolved carbon dioxide associated with the IRMS measurements.

## Results and Discussion

Slug tests conducted by DR Newcomer (Pacific Northwest National Laboratory) after the completion of the recirculation period showed a 96% decrease in hydraulic conductivity in the injection well (M-02) compared to previous tests in 2006; the conductivity of the extraction well (M-07) was essentially unchanged (-3%). Based on a KBr tracer test (~46% recovery) conducted at the start of the recirculation period and shown in Figure 4a, we estimated an initial pore volume of approximately  $15.7\text{ m}^3$  for the inter-wellbore zone using the method described by Shook and Forsmann (2005). Results of a second KBr tracer test conducted approximately 13 months later, but with pumping and recirculating conditions similar to the recirculation period are also shown in Figure 4a and indicate reduction of the inter-wellbore zone pore volume to  $9.0\text{ m}^3$ . The apparent decrease in hydraulic conductivity is likely the result of increased biomass or mineral precipitation, or both, in the injection well. Because the maximum amount of calcite that could have potentially precipitated if the conversion of urea to calcite was 100% efficient ( $0.026\text{ m}^3$ ) was small compared to the observed reduction in the inter-wellbore zone pore volume ( $6.7\text{ m}^3$ ), the results must reflect the plugging of some of the slower flow paths and focusing of flow into more conductive flow paths rather than an absolute reduction in porosity. Figure 4b shows the relationship between normalized storage capacity (fractional inter-wellbore zone pore volume) and normalized flow capacity (fractional inter-wellbore zone flow). Examination of Figure 4b suggests not only that urea-molasses injection reduced the inter-wellbore zone pore volume, but that the hydraulic conductivity distribution of the remaining inter-wellbore zone changed. Prior to the urea-molasses injection (Sep-10), 40% of the inter-wellbore zone pore volume accounted for 80% of the flow. Thirteen months following injection (Oct-11), 40% of the inter-wellbore zone pore volume accounted for 65% of the flow. In a



Figure 3. Recirculation experiment showing the injection well (M-02) and the extraction well (M-07). On the left table in the upper photo are pump and flow controls. On the right table is the “cooler” that was modified to serve as a constant head reservoir for reinjection into M-02. Not shown is the metering pump that was used to deliver urea and molasses into the water pumped from M-07. The cooler also served as a constant temperature bath (lower photo) for sediment columns that were subjected to advecting groundwater and served as analogs for injection well aquifer sediments (“active” sediments).



perfectly homogeneous system 40% of the inter-wellbore zone pore volume would account for 40% of the flow.

Concentrations of urea and its hydrolysis products ammonium and dissolved inorganic carbon (DIC; 0.45 micron filter) in water samples collected from wells B-01 (background), M-02 (injection), M-07 (extraction), and M-12 (down-gradient monitoring) are presented in Figure 5. Because DIC was naturally present in the groundwater, the background concentration (composited from several wells) was subtracted to estimate the excess DIC resulting from urea hydrolysis. Figure 5 shows increasing urea concentrations in wells B-01, M-02, and M-07 during the recirculation period. Wells M-02 and M-07 showed steady increases over the recirculation period reflecting the constant addition of urea. The appearance of urea in the up-gradient background well B-01 was unexpected and suggests that the rate of injection was sufficient to push the injectate up-gradient because only a fraction of the aquifer's thickness was responsible for fluid transport. This interpretation is supported by the results of electromagnetic borehole flow meter testing in the injection well (M-02) that was conducted prior to our experimental campaign; the tests found that flow is not distributed evenly within the wellfield and that half of the flow occurs in the upper 0.5 m of the saturated thickness (Waldrop and Waldrop 2005). At the end of the recirculation period urea concentrations decreased over a period of days to weeks in wells B-01, M-02, and M-07. Urea made a delayed appearance in well M-12, located 7.2 meters down-gradient from the extraction well. The urea concentrations in M-12 continued to increase after the recirculation period with the maximum concentration occurring on day 34, reflecting advective transport of unhydrolyzed urea.

An important observation is the lack of ammonium detection in any of the wells during the recirculation period, suggesting that urea hydrolysis during this time was minimal. In addition, ammonium was never observed in the background well B-01 and M-04. Ammonium and excess DIC were observed for several months after the recirculation period within the inter-wellbore

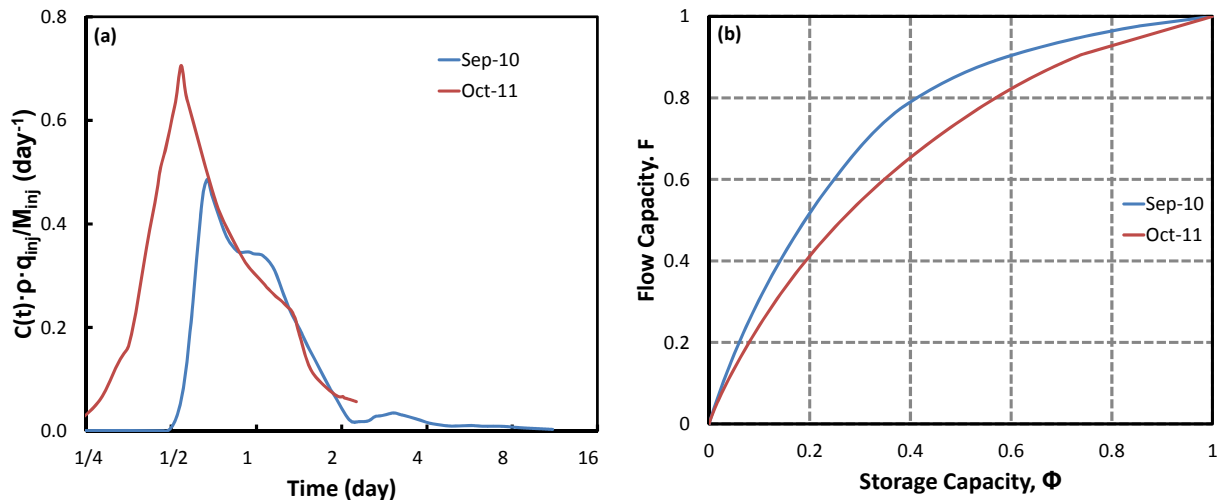


Figure 4. Deconvolved (i.e., effects of recirculation removed) normalized bromide ( $C(t) =$  concentration [m/m] at time  $t$ ,  $\rho =$  fluid density [m/v],  $q_{inj} =$  pumping rate [v/t],  $M_{inj} =$  total tracer mass [m]) tracer test results for a test conducted at the start of recirculation (Sep-10) and 13 months later at the end of long term monitoring. (a) Tracer breakthrough curves (time 0 is the start of tracer injection). (b) Fractional pore volume (Storage Capacity) and fractional flow (Flow Capacity). Data were analyzed using spreadsheet-based methods described in Shook and Forsmann (2005).

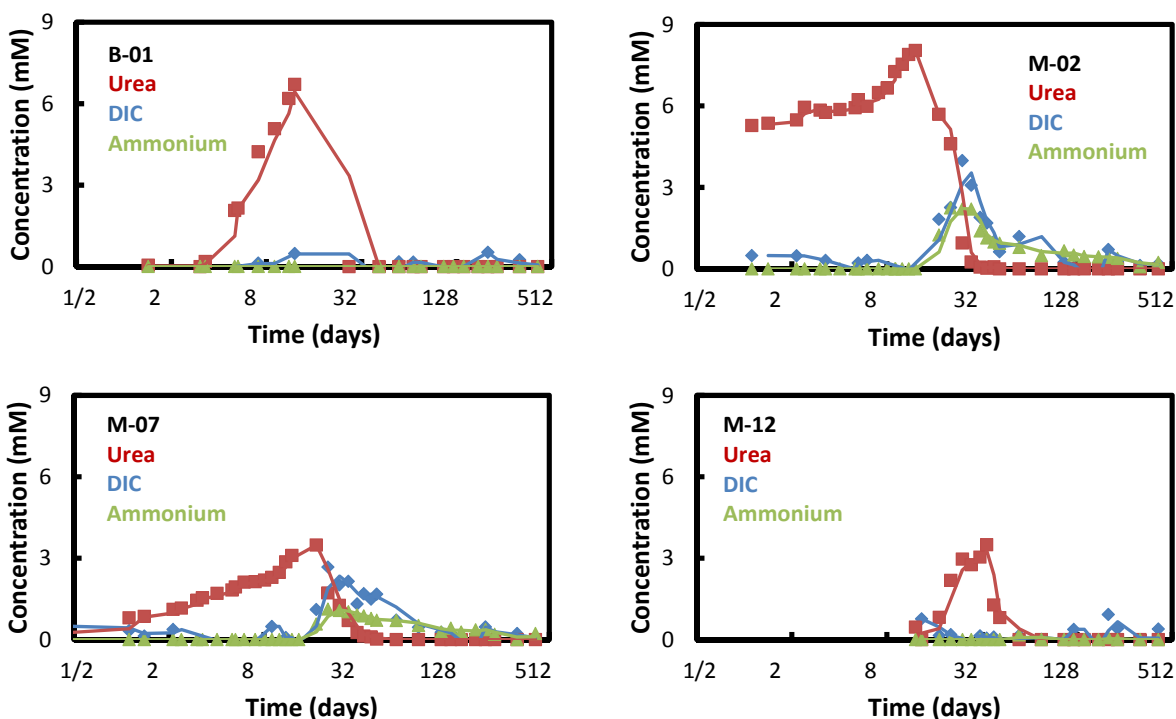


Figure 5. Concentrations of urea, excess dissolved inorganic carbon (DIC), and ammonium as a function of time in wells B-01, M-02, M-07 and M-12. Excess DIC (the amount resulting from urea hydrolysis) was estimated by subtraction of the average site background DIC from the measured concentrations. The lines are moving averages through the data.

zone (M-02 and M-07) with the molar concentration of excess DIC exceeding that for ammonium. Given that urea hydrolysis produces 2 moles of ammonium for every mole of DIC (Figure 1), the higher abundance of DIC relative to ammonium likely reflects the exchangeable adsorption of ammonium on aquifer sediments. This interpretation is supported by the continued presence of ammonium in the injection and extraction wells a year after the urea concentration dropped below detection. Ammonium was observed on two occasions in the down-gradient monitoring well M-12 (the low concentrations are difficult to discern on the scale used in Figure 5); both occurrences were after the observed urea concentration in the well had returned to below detection, suggesting that advective transport of ammonium from the recirculation cell (M-02 and M-07) rather than local urea hydrolysis could have been the source. In general the ammonium and DIC results are consistent with an interpretation in which significant urea hydrolysis was limited to the inter-wellbore zone.

Figure 6 presents the variation in  $\delta^{13}\text{C}$  over time for DIC. The urea used for the

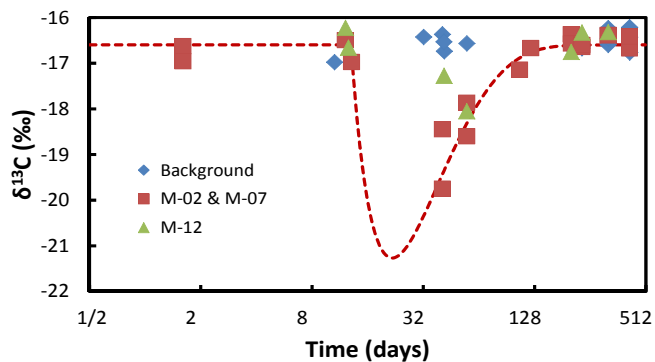


Figure 6. Changes in  $\delta^{13}\text{C}$  values for dissolved inorganic carbon as a function of time.

experiment had a  $\delta^{13}\text{C}$  value of  $-40.7 \pm 0.4 \text{ ‰}$ , distinctly more negative than the ambient site groundwater value of  $-16.6 \pm 0.2 \text{ ‰}$ , reflecting urea's manufacture from natural gas. A pronounced and prolonged decrease in  $\delta^{13}\text{C}$  was observed in the inter-wellbore zone (M-02 and M-07) during the same time period that excess DIC and ammonium appeared in the same wells (Figure 4). The down-gradient monitoring well M-12 also showed depletion of the  $^{13}\text{C}$  content of the DIC, likely the result of advective transport of DIC from the inter-wellbore zone. Due to sampling difficulties,  $\delta^{13}\text{C}$  values are not available for the days 13 through 40, the time period in which urea was decreasing in the flow cell (Figure 5). The dashed line in Figure 6 is a sketch of a possible breakthrough curve.

The urea and molasses treatment resulted in an enhanced level of urease activity as expressed by increases in measured  $^{14}\text{C}$  urea hydrolysis rates. As we have found at other locations (*e.g.*, Fujita 2010), the majority of urease activity at the Rifle site was associated with the sediments (*i.e.*, attached microorganisms) and much less activity was associated with the groundwater (*i.e.*, planktonic microorganisms). For example, at the end of the recirculation period (day 13) the urease activity for water collected from the injection well (M-02) was  $0.05 \pm 0.02 \text{ nmole L}^{-1} \text{ hr}^{-1}$  (pretreatment background of  $0.017 \pm 0.002 \text{ nmole L}^{-1} \text{ hr}^{-1}$ ) compared to a weighted average activity of  $63 \pm 11 \text{ nmole kg}^{-1} \text{ hr}^{-1}$  for the sediments (active). Figure 7 shows the urease activity for sediment samples incubated and collected as part of the Rifle campaign. Figure 7a shows urease activity for sediments deployed during the experiment. They include passive sediment samples (*i.e.*, transport was by diffusion) that were incubated and recovered on day 13, prior to the appearance of the ammonium and the decrease in  $\delta^{13}\text{C}$  associated with urea hydrolysis. The values for the passive samples were averages from the "Background" wells B-01 and M-04, as well as from the cooler maintained at the surface within the recirculation loop just before the injection ("Injection"). Figure 7a also presents the measured urease activity for the "long-term" passive sediments collected on day 322 from the background wells B-01 and M-04 as well as from the "Injection" well M-02. Also shown is average urease activity for the "active" sediment columns subjected to advective flow of urea and molasses (as shown in Figure 3) collected on day 13. Comparisons of the day 13 passive (Background (P) and Injection (P)) and active (Injection (A)) samples show the importance of advective nutrient flux in stimulating urease activity; the active sediments

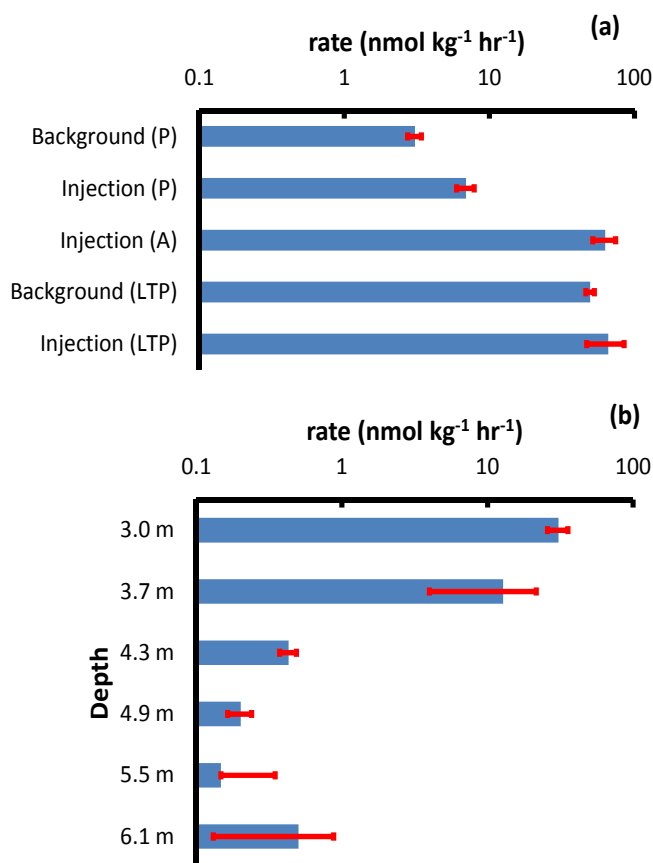


Figure 7. Urease activity reported as urea hydrolysis rates for active (A) and passive (P) sediment experiments, including "long-term" passive sediments incubated 322 days (LTP) (a) and for sediment samples from drill core BS-11-02 (b).



exhibited urease activity 18 times higher than the passive samples. All of the day 322 passive samples showed higher urease activity than the day 13 background samples, suggesting a long-term persistence of increased urease activity within the aquifer zone impacted by the urea and molasses injection. Figure 7b shows urease activity for samples from the post experiment core (BS-11-2). The activity in the sediments from the upper meter of the aquifer was 30 times higher than the activity in sediments from the lower portion of the aquifer. This result is consistent with our observations that the majority of the flow during the recirculation period was confined to the upper portion of the aquifer. The fact that the urease activities measured in the upper portion of the aquifer were similar to those observed for the long-term passive samples (Figure 7a) suggests that the use of long-term passive samples was a reasonable approach to access aquifer conditions.

Based on the time dependent changes of measured urea and ammonium concentrations the field-scale rate of urea hydrolysis can be estimated. However, to estimate the rate one must account for dispersion during transport and dilution (associated with well pumping) during sampling. This can be done by the inclusion of a conservative tracer; however because of the recirculation design of our experiment this was not practical. Instead we used a technique (described below) that used the generated ammonium as a non-conservative tracer with explicit consideration of adsorption.

Under conditions of first order kinetics, the rate of urea hydrolysis and ammonium production are given by

$$R_{urea} = \frac{d[urea]}{dt} = -k[urea] \quad (1)$$

$$R_{NH_4^+} = \frac{d[NH_4^+]^{total}}{dt} = 2k[urea] \quad (2)$$

Where  $R_i$  is the rate for the subscripted species,  $t$  is time and  $k$  is the first order rate constant. The concentrations  $[urea]$  and  $[NH_4^+]^{total}$  are expressed on a per liter of water basis. The concentration of urea at any given time,  $t$ , is given by integrating equation (1) from  $t = 0$  to  $t$

$$[urea] = [urea]_0 e^{-kt} \quad (3)$$

where  $[urea]_0$  is the concentration of urea at  $t = 0$ . The concentration of ammonium at time  $t$  is given by substitution of equation (3) into equation (2) and integrating from time  $t = 0$  to  $t$

$$[NH_4^+]^{total} = 2[urea]_0(1 - e^{-kt}) + [NH_4^+]_0^{total} \quad (4)$$

where  $[NH_4^+]_0^{total}$  is the total concentration of ammonium at  $t = 0$ . In the subsurface, ammonium can exchange with cations on the aquifer geomeedia and only a fraction of the total ammonium produced remains in solution.

$$f_{aq} = \frac{[NH_4^+]}{[NH_4^+]^{total}} = \frac{[NH_4^+]}{[NH_4^+] + [NH_4^+]^{rock}} \quad (5)$$

$$[NH_4^+]^{total} = \frac{[NH_4^+]}{f_{aq}}$$

where  $f_{aq}$  is the fraction of ammonium that remains in solution. In aquifer geomechanics subject to reversible linear sorption, the fraction of a sorbing constituent that remains in solution is related to the retardation factor  $R_f$  by (Palmer and Fish 1997)

$$R_f = \frac{1}{f_{aq}} \quad (6)$$

Substituting equations (5) and (6) into equation (4) under the condition of no initial ammonium yields

$$[NH_4^+] = \frac{2[urea]_0}{R_f} (1 - e^{-kt}) \quad (7)$$

Dividing equation (3) by (7) yields:

$$\frac{[urea]}{[NH_4^+]} = \frac{R_f}{2(e^{kt} - 1)} \quad (8)$$

The urea hydrolysis rate constant ( $k$ ) and the retardation factor for ammonium ( $R_f$ ) were estimated by a non-linear least square fit of equation (8) to  $[urea]/[NH_4^+]$  ratios as a function of time for water samples collected from the extraction well M-07. The extraction well was chosen for this analysis because it was at the down-gradient “end” of the urea-molasses treated inter-wellbore zone and the up-gradient water had been subject to urea-molasses treatment. For the injection well (M-02), once recirculation was ended, the up-gradient water entering the well would have been untreated. Figure 8 is a plot of  $\ln [urea]/[NH_4^+]$  as a function of time; the solid line is a least squares fit of equation (8) to the data with values for the first order rate constant ( $k$ ) of  $0.18 \text{ d}^{-1}$  and for the ammonium

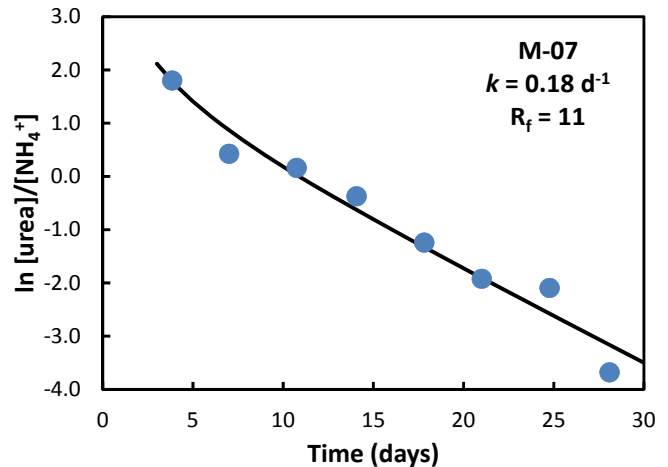


Figure 8. Changes in the ratio of urea to ammonium as a function of time for water samples collected from the extraction well, M-07.

Table 1. Urea hydrolysis rate constants and ammonium retardation factors derived from field experiments and laboratory samples (Owsley, ID, Colwell et al. 2005; UP-1, ID Fujita et al. 2008; VZRP, ID and Rifle, CO this study; INTEC, INL, ID, Fujita et al. 2009; 100-N, Hanford, WA, Fujita et al. 2010).

Field Experiment	1 <sup>st</sup> order $k$ (day <sup>-1</sup> )	$R_f$	Geomedia	Redox condition
Owsley, ID	0.006	11	Fractured basalt	Reducing
UP-1, ID	0.033	6.6	Fractured basalt	Oxidizing
VZRP, ID	0.034	-	Unconsolidated sediments	Oxidizing
Rifle, CO	0.18	11	Unconsolidated sediments	Reducing
<sup>14</sup> C Laboratory Measurements	1 <sup>st</sup> order $k$ (day <sup>-1</sup> )		Sample Type	Redox condition
INTEC, INL, ID	0.030	-	Water	Oxidizing
100-N, Hanford, WA	0.015	-	Water, Sediments	Oxidizing

retardation factor ( $R_f$ ) of 11.

Table 1 shows a comparison of the  $k$  and  $R_f$  values measured for the Rifle recirculation field experiment with values from other field experiments we have conducted as well as rate constants estimated based only on laboratory assays on samples collected from <sup>90</sup>Sr-contaminated DOE sites. As may be seen from the table the measured rate constant for the Rifle recirculation field experiment was 6 times higher than what we observed for a much shorter duration (~2 hours) recirculation experiment conducted in a perched water body at the INL Vadose Zone Research Park (VZRP), and a single well push-pull experiment conducted in the Snake River Plain Aquifer at University Place, Idaho Falls, Idaho (Fujita et al. 2008).

We expected that urea hydrolysis would result in precipitation of calcite (Figure 1). However as indicated earlier, the complete conversion to calcite of the urea that was injected during the recirculation period would produce 0.026 m<sup>3</sup> of calcite in an estimated pore volume of 15.7 m<sup>3</sup>. Assuming a porosity of 27% (Anderson et al. 2003) and a uniform distribution of precipitated calcite, the hydrolysis of urea would add approximately 0.004% (v/v) calcite to the Rifle sediments. Such a change in calcite content would be extremely difficult to

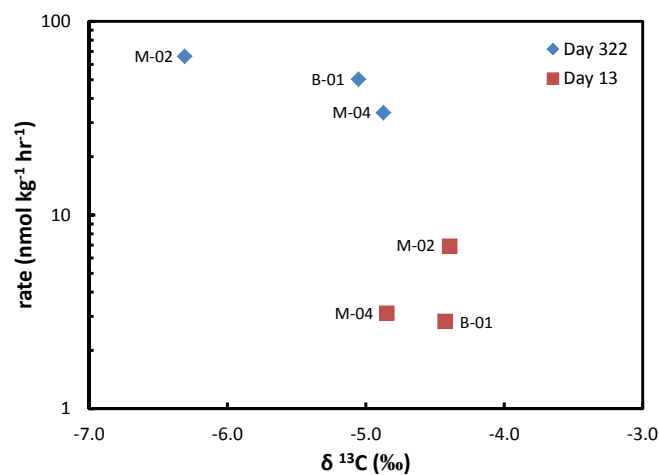


Figure 9. Urease activity (expressed as urea hydrolysis rate) and  $\delta^{13}\text{C}$  values for solid carbonates from passive sediment experiments.

observe directly given that the preexisting calcite content of the site ranges from ~0.6 to 2.0% (based on the amount of CO<sub>2</sub> liberated from sediment samples during carbon isotope determinations). However, because the source (at least in part) of the carbonate in any new calcite was the hydrolyzed urea,  $\delta^{13}\text{C}$  values of newly precipitated calcite should reflect the isotopically light carbon in the urea ( $\delta^{13}\text{C} = -40.7 \pm 0.4 \text{ ‰}$ ).

Examination of 4 samples from a core (BS-01) collected outside the flow cell and drilled prior to the initiation of recirculation and 11 samples from a core (BS-11-02) drilled 1 meter down-gradient of the injection well 10 months after the completion of recirculation showed mean values of 1.38% and 1.51% for total carbonate minerals reported as calcite, respectively. Although the core collected from the post recirculation period inter-wellbore zone had a slightly higher calcite content as expected, a t-test for the data showed that the difference between the two cores was not statistically significant at the 90% confidence level (or at the 50% level). Measurement of  $\delta^{13}\text{C}$  for the two cores showed mean values of -3.89 ‰ (BS-01) and -4.49 ‰ (BS-11-02) for the solid carbonates, with the mean  $\delta^{13}\text{C}$  value for the post-recirculation core being -0.60 ‰ lighter as expected if carbonate from hydrolyzed urea contributed to new calcite precipitation. A t-test suggests that the observed difference was significant at the 90% confidence level but not at the 95% confidence level. The observation of lighter carbon in the post recirculation core, while not conclusive, was consistent with the addition of a small amount of calcite that contains carbonate derived from the hydrolysis of isotopically light urea.

The passive sediment samples were also examined for evidence of calcite precipitation. Figure 9 shows  $\delta^{13}\text{C}$  values for carbonates and associated measured urease activities for passive sediment samples incubated in the injection (M-02) and background (B-01, M-04) wells. Day 13 samples showed similar urease activities and  $\delta^{13}\text{C}$  values for all samples. However, the day 322 sample from the injection well showed a significant decrease in  $\delta^{13}\text{C}$ , while the background well sediment samples showed smaller changes. The  $\delta^{13}\text{C}$  decrease is likely the result of the incorporation of inorganic carbon derived from hydrolyzed urea into solid carbonates in the M-02 sample. This interpretation is consistent with the fact that ammonium (a product of urea hydrolysis) was only observed in well M-02, although the measured urease activities for the three day 322 samples were similar to each other and elevated compared to day 13.

## Conclusions

Based on results presented here, several conclusions can be made regarding field-scale urea hydrolysis and associated calcite precipitation.

Under the condition of simultaneous injection of urea and molasses there is a significant lag time (at least 2 weeks) before the onset of urea hydrolysis, allowing a significant dispersal of injected urea before hydrolysis and associated calcite precipitation occurs. Stimulated urease activity and relatively high rates of urea hydrolysis can be realized by a recirculation treatment strategy and persist for long periods of time. Further, we confirmed that stimulated urease activity is predominantly associated with the aquifer geomechanics. The areal extent of increased urease activity was apparently larger than the area in which hydrolysis was actually observed which was limited by the distribution of urea at the time that significant hydrolysis started. We found that evidence of urea hydrolysis was readily available from chemical and isotopic signatures in the groundwater that persisted for months after urea in groundwater was undetectable.

Although indirect evidence based on carbon isotopes suggests that calcite precipitation occurred within the inter-wellbore zone, tracer and slug test results indicate that the impact on the inter-wellbore zone flow-field is substantially more pronounced than would be expected from just pore space filling by precipitated calcite. The results are consistent with preferential blocking of flow access to lower permeability zones and associated focusing of flow in the most conductive portion of the aquifer

In summary, our results suggest that calcite precipitation driven by urea hydrolysis can be effectively implemented and controlled at the field scale using a recirculation treatment design. Although the injected fluids and associated stimulation of urease activity may move beyond the inter-wellbore zone, significant urea hydrolysis was only observed in the inter-well bore zone. Furthermore, based on the observations of changes in carbon isotope ratios, the precipitation of calcite and/or other carbonates at very low level can be inferred to only have occurred in the inter-wellbore zone.

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**SUMMARY ACCOMPLISHMENTS AND PUBLICATIONS  
FOR  
FIELD INVESTIGATIONS OF MICROBIALLY FACILITATED CALCITE  
PRECIPITATION FOR IMMOBILIZATION OF STRONTIUM-90 AND OTHER  
TRACE METALS IN THE SUBSURFACE**

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Susan S. Hubbard, Lawrence Berkeley National Laboratory  
Timothy R. Ginn, University of California – Davis

Subsurface radionuclide and trace metal contaminants throughout the U.S. Department of Energy (DOE) complex pose one of DOE's greatest challenges for long-term stewardship. One promising stabilization mechanism for divalent trace ions, such as the short-lived radionuclide  $^{90}\text{Sr}$ , is co-precipitation in calcite. Calcite, a common mineral in the arid western U.S., can form solid solutions with trace metals. The rate of trace metal incorporation is a function of precipitation kinetics, metal adsorption on the calcite, the substrata upon which the calcite precipitates, and solid solution properties. The first factor is susceptible to manipulation using either abiotic or biotic means. We previously demonstrated that increasing the calcite precipitation rate by stimulating the activity of urea hydrolyzing microorganisms is a promising approach that can result in significantly enhanced Sr uptake. In limited field experiments we showed that with nutrient amendments we could stimulate ureolytic activity and calcite precipitation in groundwater. A particularly attractive characteristic of this approach is that it accelerates naturally occurring processes, rather than drastically altering *in situ* environmental conditions. This enhances the sustainability of the desired remediation results. The specific objectives of our research were to:

- Assess the field-scale relationships between introduced reagents, changes in urease activity, calcite precipitation rates, mineral distribution, and hydrogeological-geochemical heterogeneity, under conditions representative of DOE sites where  $^{90}\text{Sr}$  contamination occurs;
- Assess the sustainability of the treatment;
- Evaluate the potential for geophysical methods to detect and monitor the spatiotemporal distribution of calcite precipitation in the subsurface at the field-scale; and
- Develop modeling tools to enable optimal design of *in situ* remediation strategies based on engineered calcite precipitation, with particular attention to the effects of precipitation on flow re-routing.

We conduct integrated field, laboratory, and computational research to evaluate a) the relationships between urea hydrolysis rate, calcite precipitation rate under environmentally relevant conditions; and b) the coupling between flow/flux manipulations and calcite precipitate distribution and metal uptake. The primary emphasis was on field-scale processes, with the laboratory and modeling activities designed specifically to support the field studies. Initial field studies were conducted at the Vadose Zone Research Park (VZRP), an uncontaminated field research site at the Idaho National Laboratory (INL) that mimics conditions at the nearby Idaho Nuclear Technology and Engineering Center (INTEC), where significant  $^{90}\text{Sr}$  contamination has been identified in the perched water (vadose zone) and aquifer below the facility. However,



modifications to facility operations at INTEC (which provided water to the VZRP percolation ponds) altered the VZRP site hydrology to such an extent that the site was no longer suitable for our field experiments. As a result the field campaign was moved to a location in the city of Rifle, CO. Our research was organized into 5 interrelated tasks:

- Task 1. Site specific ion exchange model for VZRP
- Task 2. Development of RT-qPCR for *ureC* and *amoA* mRNA, and correlations with activity
- Task 3. Geophysical Laboratory Studies
- Task 4. Field Experiments
- Task 5. Coupled biogeochemical and flow modeling (field-scale)

Below is a description of the overall accomplishments (including a list of publications) of the full research team for the project.

### **Task 1. Site specific ion exchange model for VZRP (UI Lead)**

Status: Exchange experiments with  $\text{Sr}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Li}^+$ ,  $\text{Na}^+$  and  $\text{NH}_4^+$  and  $\text{Ca}^{2+}$ -saturated sediments collected from the VZRP were completed, and a model was developed and implemented in The Geochemist Workbench® and TOUGHREACT computer codes. In addition, Exchange experiments with  $\text{Sr}^{2+}$  and  $\text{NH}_4^+$  and  $\text{Ca}^{2+}$ -saturated sediments collected from the Rifle IFRC Site were also completed. These results are being combined with data collected by IFRC investigators to develop a site specific cation exchange model for the Rifle Site.

#### *Publication:*

- (1) Baker, L. L., Strawn, D. G. and R. W. Smith. Cation Exchange on Vadose Zone Research Park Subsurface Sediments, Idaho National Laboratory. *Vadose Zone Journal*. 9:476–485.

### **Task 2. Development of RT-qPCR for *ureC* and *amoA* mRNA, and correlations with activity (INL Lead)**

Status: Due to changes in staffing, we did not have the resources to support the considerable effort needed to develop methods to extract mRNA of sufficient quantity and quality from our field samples to apply reverse transcriptase techniques with reliable quantitation. However, we did enhance our qPCR techniques, developing plasmid standards for bacterial *ureC* and *amoA* gene quantitation, and also improved statistical protocols for analysis. These improved methods were applied to microbiological samples (groundwater and incubated solids) from the VZRP and Rifle field sites. We also collaborated with other researchers to expand our ammonia oxidizer analyses to archaeal organisms, and examined their numbers and diversity in field samples from a previous urea injection experiment.

#### *Publications:*

- (2) Reed, D.W., Smith, J.M., Francis, C.A. and Y. Fujita, Responses of Ammonia-Oxidizing Bacterial and Archaeal Populations to Organic Nitrogen Amendments in Low-Nutrient Groundwater. *Appl. Environ. Microbiol.*, 2010. 76(8): p. 2517-2523.

- (3) Rope, A. M., Reed, D. W., and Y. Fujita. Development of an Improved Standard for Quantification of the amoA Gene in Environmental Samples. *DOE Office of Science Journal of Undergraduate Research*. Accepted.

### **Task 3. Geophysical Laboratory Studies (LBNL Lead)**

Status: Geochemical and geophysical (complex electrical, seismic, and radar) measurements were simultaneously collected during laboratory flow-through column experiments with urea hydrolyzing organisms. The experiments suggested that electrical conductivity was most sensitive to the pore fluid changes (calcite precipitation induced in these experiments was purposefully minimal). To focus on the geophysical signature of calcite precipitation, experiments were performed whereby calcite precipitation was abiotically induced on glass beads. This experiment revealed a significant complex electrical phase response that indicated early precipitation of disseminated precipitates followed by a later stage of aggregation, which documented for the first time the potential of this method for remotely monitoring the evolution of calcite precipitation. In addition, dynamic synchrotron microtomography experiments were performed to assess the changes in pore structure associated with microbially mediated-calcite precipitation using a novel, flow-through mini bioreactor. These experiments have illustrated how calcite precipitation induced through urea hydrolysis impacts pore geometry and thus the overall porosity of the system.

#### *Publications:*

- (4) Wu, Y., S.S. Hubbard, K.H. Williams, and J. Ajo-Franklin. On the complex conductivity signatures of calcite precipitation. *Journal of Geophysical Research – Biosciences*. 2010. 115(G00G04): 10 pp., DOI: 10.1029/2009JG001129.
- (5) Wu Y, JB Ajo-Franklin, N Spycher, SS Hubbard, G Zhang, KH Williams, J Taylor, Y Fujita, and RW Smith. Geophysical Monitoring and Reactive Transport Modeling of Ureolytically-Driven Calcium Carbonate Precipitation. *Geochemical Transactions*. 2011. 12:7, DOI: 10.1186/1467-4866-12-7.

### **Task 4. Field Experiments (UI and INL Co-Leads)**

Status: Our initial field work was conducted at the Idaho National Laboratory Vadose Zone Research Park. We characterized background geochemistry and microbial ureolytic activity, and also conducted field injection experiments with molasses alone and molasses and urea to assess ureolytic activity with the addition of those amendments. The intention during these experiments was to assess the stimulation of ureolytic activity specifically, and not to induce extensive calcite precipitation. In addition, cross borehole tomographic radar, seismic, and complex electrical data were collected before, during, and after urea injection to characterize baseline *in situ* heterogeneity and to monitor the spatiotemporal distribution of biogeochemical transformations associated with the urea hydrolysis. Finally, a streamtube model was also developed and parameterized to describe the VZRP flow cell and to incorporate urea hydrolysis and calcite precipitation kinetics.

Following the changes in VZRP conditions that prevented us from continuing our field campaign to study calcite precipitation, the major focus of our field activities shifted to the Rifle, CO IFRC site. In addition to the activities described previously in this report, cross borehole seismic, electrical resistivity and radar tomography, as well as surface azimuthal electrical resistivity and

radar tomography surveys, were conducted before, during, and after urea injection to characterize baseline *in situ* heterogeneity and to monitor the spatiotemporal distribution of biogeochemical transformations associated with the urea hydrolysis. We found that fluid flow and urea hydrolysis were largely confined to the upper portion (0.5 m) of the inter-wellbore zone.

#### **Task 5. Coupled biogeochemical and flow modeling (field-scale) (UC-Davis Lead)**

Status: We developed a generalized ureolysis kinetic model based on prior published forms for urease enzyme as the ureolytic agent, and have applied it with inverse modeling techniques (UCODE parameter fitting software) to data from column experiments where microbial cells were the ureolytic agent. How kinetics derived for actual enzyme activities translate to analogous kinetics where the reaction is mediated by *in situ* cells containing the enzyme is not clear. Also, the distribution of *in situ* cells (either native or augmented) in the aquifer materials is unknown. Even in controlled column experiments an injected biomass distribution is only expected to be of exponential profile, if bacterial attachment is governed by filtration theory, and the parameters of the exponential profile are unknown. Consequently, we applied inverse modeling to determine effective parameters of an exponential biomass distribution along a column after injection of the bacterial cells. In this case we used a stop-flow approach, where the bacterial injection period was followed by a zero-flow interval in order to provide time for bacterial attachment to surfaces inside the column. We validated the fitted distribution by comparison with results from multiple column experiments and with post-experiment bioassay data. The nonuniform distribution that resulted was then used to successfully predict calcite precipitation distribution in space (along the column) and extent (over time). This work also engendered collaboration with the separately-funded J. DeJong lab at UC Davis. Through this collaboration an important element of engineering design for *in situ* calcite precipitation was established. We also applied stop-flow strategies to reagent injection. In this way we first distribute reagent uniformly through the column by injecting solutes at flow rates that are high with respect to reaction rate, and then follow this injection with another wait period to allow the reaction to proceed, again we hypothesized, more uniformly, in the absence of transport. We found that the combined stop-flow injection strategies used for both biomass distribution and reaction control resulted in a relatively uniform calcite precipitation along a 1D column. For comparison we also evaluated continuous injection of reagents and found a much more nonuniform distribution of precipitates. This is due to the approximately log-linear decrease in attached microbial concentration along the injection path that in turn arises from colloid filtration processes, the underlying theory of which says that the attachment rate is first-order kinetic (generating a log-linear distribution). By injecting reagents in *reverse* direction we make use of the then log-linear increasing (with respect to the direction of reagent injection) microbial cell concentration that increases the effective enzyme concentration involved in the ureolysis, and compensates for the reduction in reagent concentration along the flow path.

We also constructed a streamtube-ensemble model of the well-to-well injection-withdrawal scheme at VZRP and calibrated and tested it against bromide breakthrough and urea breakthrough data respectively. This model is being applied to our Rifle site field campaign data to evaluate the continuous travel time distribution associated with *reinjection* of withdrawal-well effluent into the injection well.

*Publications:*

- (6) Barkouki T.H., B.C. Martinez, B.M. Mortensen, T.S. Weathers, J.D. DeJong, N.F. Spycher, T.R. Ginn, Y. Fujita, and R.W. Smith. Forward and inverse biogeochemical modeling of microbially induced precipitation in 0.5m columnar experiments. *TOUGH Symposium 2009* (Berkeley, CA; September 14-16, 2009) (G. Moridis, C. Doughty, E. Sonnenthal, and C. Valladao, Organizers) Lawrence Berkeley National Laboratory, Berkeley, CA.
- (7) Barkouki, T.H., B.C. Martinez, B.M. Mortensen, T.S. Weathers, J.D. DeJong, T.R. Ginn, N.F. Spycher, R.W. Smith, and Y. Fujita. Forward and Inverse Bio-Geochemical Modeling of Microbially Induced Calcite Precipitation in 0.5m Column Experiments. *Transport in Porous Media*. 2011, 90:23-39, DOI: 10.1007/s11242-011-9804-z.
- (8) Spycher N.F., G Zhang, S Sengor, M Issarangkun, T.H. Barkouki, T.R. Ginn, Y Wu, R.W. Smith, S.S. Hubbard, Y. Fujita, R. Sani, B. Peyton. Application of TOUGHREACT V2.0 to Environmental Systems. *TOUGH Symposium 2009* (Berkeley, CA; September 14-16, 2009) (G. Moridis, C. Doughty, E. Sonnenthal, and C. Valladao, Organizers) Lawrence Berkeley National Laboratory, Berkeley, CA.
- (9) Martinez, B.C., Barkouki, T.H., Dejong, J.D., Ginn, T.R., Upscaling of microbial induced calcite precipitation in 0.5m columns: Experimental and modeling results, Geotechnical Special Publication 211, Proceedings Geo-Frontiers 2011, Advances in Geotechnical Engineering, pp. 4049-4059, 2011.

#### **DOE Site Specific Publications:**

Specific to the Idaho Nuclear Technology and Engineering Center (INTEC) at INL:

- (10) Fujita, Y., R.W. Smith, and J.L. Taylor. *In Situ* Calcite Precipitation for Contaminant Immobilization. in *In Situ and On-Site Bioremediation—2009. Tenth International In Situ and On-Site Bioremediation Symposium* (Baltimore, MD; May 5–8, 2009) (GB Wickramanayake and HV Rectanus, Chairs). Battelle Memorial Institute, Columbus, OH. [www.battelle.org/biosymp](http://www.battelle.org/biosymp) (ISBN 978-0-9819730-1-2) A-05.

Specific to the Hanford 100-N area:

- (11) Fujita, Y., J.L. Taylor, L.M. Wendt, D.W. Reed, and R.W. Smith. Evaluating the Potential of Native Ureolytic Microbes to Remediate a <sup>90</sup>Sr Contaminated Environment. *Environmental Science & Technology*. 2010. **44**:7652-7658.