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Investigation of the Potential for 90Sr Immobilization in INTEC Perched Water Via Microbially Facilitated Calcite Precipitation

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TECHNICAL APPROACH

The goal of this work is to evaluate the applicability of a biogeochemical sequestration approach for remediation of ^{90}Sr contamination in perched water zones underlying the Idaho Nuclear Technology and Engineering Center (INTEC). The approach is based on the accelerated co-precipitation of the contaminant in calcite, where the acceleration is catalyzed by the microbial urea hydrolysis. We have previously demonstrated the potential for this remediation mechanism to immobilize strontium (1,2). Urea hydrolysis promotes calcite precipitation (and trace metal co-precipitation) by increasing groundwater pH and alkalinity. Ureolysis is catalyzed by the urease enzyme, which is produced by many environmental microorganisms. In the Snake River Plain Aquifer, which is saturated with respect to calcite (3), any co-precipitated ^{90}Sr should be effectively sequestered over the long-term, even after return to pre-manipulation conditions. Another advantage of the ureolysis approach is that the NH_4^+ ions produced by the reaction can exchange with cations sorbed to subsurface minerals, thereby enhancing the availability of the radionuclides for re-capture via a more stable mechanism (co-precipitation rather than adsorption).

In the first half-year of this 1.5 yr LDRD project, we assayed whether ureolytic organisms were present in perched water collected from INTEC and the INL Vadose Zone Research Park (VZRP), by estimating *in situ* ureolysis rates. The VZRP is a field research site that serves as an uncontaminated analog site for INTEC, with similar hydrogeologic features. The results indicated that significant ureolytic activity exists at both locations (Fujita, FY05 LDRD Final Report). In fact, higher rates of ureolysis were measured in the contaminated perched water than in the INTEC groundwater or in uncontaminated water from the VZRP. These results suggested that the proposed remediation strategy was potentially suitable for the contaminated INTEC perched water zones. In FY06, the goal was to conduct laboratory batch studies using ^{90}Sr-contaminated interbed sediments from INTEC to evaluate whether urea hydrolysis would lead to changes in ^{90}Sr partitioning. In a real field setting, we would rely on the native microbial community, but because available INTEC materials had been archived for 13 years and without regard for microbial preservation, for these experiments we elected to add urea hydrolyzing microorganisms enriched from VZRP sediments collected in 2005. In addition, we tested the effect of adding a calcite precipitation inhibitor, to see whether inhibiting calcite precipitation could promote aragonite formation and lead to greater Sr immobilization, because aragonite can incorporate more Sr than calcite.

This LDRD project was aimed at developing knowledge that is specific to the INTEC contamination problem, as well as generating more fundamental understanding of contaminant fate and transport processes in complex heterogeneous subsurface systems.

TECHNICAL OBJECTIVES

The technical objectives of the second year of this LDRD project were the following:

- Enrich a urea-hydrolyzing mixed microbial community from sediments collected from the VZRP.
- Use that mixed culture in laboratory experiments to test whether microbial ureolysis could cause a change in partitioning of ^{90}Sr in INTEC interbed sediments, and also test the effect of adding a calcite precipitation inhibitor, N,N,N',N'-ethylenediaminetetra(methylenephosphonic) acid (ENTMP).

RESULTS AND ACCOMPLISHMENTS

Microbial culture enrichment, preservation and characterization of ureolytic activity. We obtained material from an alluvium-basalt contact zone (~ 50-60 ft below land surface; where perched water would accumulate) that was cored at the VZRP in the spring of 2005; the core had not been collected or initially stored with special precautions to preclude external microbial contamination, but was the best available material for our use. Since receipt by the PI (Fujita), in early July 2005, the core had been stored at 4ºC. Approximately 8 g (wet weight) of material was incubated in 50 ml of sterile media containing 0.05% (w/v) yeast extract and 0.05% molasses (w/v), at 22ºC with shaking. Serial transfers (total of 5) were made every 2 to 4 days into fresh media, to remove sediment. The ability of the culture to hydrolyze urea was confirmed by inoculation into Urea R Broth (Difco); a change in color of the broth from orange to pink indicates urea hydrolysis. Aliquots of the culture, collected after 24 hours of growth (late log phase), were frozen (-80ºC) with glycerol (50%) as cryoprotectant. Recovery of the cells after freezing was confirmed by plating thawed cells on agar plates containing 0.05% yeast extract and 0.05% molasses. Three colony morphologies were observed, although >90% of the colonies observed were of a single morphology: yellow, opaque, 1-3 mm diameter, round, and drop-like with a smooth margin.

A urea hydrolysis rate was estimated by measuring the production of ammonium under nutrient-limiting conditions in overnight-grown (15 to 19 hour) cultures. Stock cultures of frozen cells were grown in the sterile medium with 10mM urea, the cells were harvested at late log phase by centrifugation, and then washed with sterile dilute media. The cells were re-suspended (in triplicate) in sterile dilute media at an approximate density of 8.5×10^9 cfu per ml and incubated aerobically for two hours at 22°C with shaking. Actual starting cell densities were confirmed using dilution/spread plate cell counts. Samples were aseptically recovered from the shaking flasks at 10 and 20 minute intervals and NH_4^+ was quantified by ion chromatography. A gross urea hydrolysis rate was estimated assuming: 1) zero-order kinetics, 2) complete hydrolysis of one mole of urea to two moles of ammonium, and 3) all cells hydrolyzed urea and at equivalent rates. The average rate was 2×10^{-11} µmol urea cfu^{-1} min^{-1}. This value was used to estimate the appropriate starting cell density for the experiments with INTEC sediments.

Experiments with INTEC sediments. The goal of these experiments was to determine whether microbial ureolysis would result in changes in the distribution of ^{90}Sr between three distinct geochemical compartments. The three compartments, operationally defined, were as follows:

1. Ion Exchangeable ^{90}Sr: extracted by 0.5M NH_4Cl for 16 h, at a solid:water ratio of 1:5.
2. Carbonate-bound ^{90}Sr: extracted by 1M Na acetate/acetic acid (pH 5) for 6 h, solid:water 1:5.
3. Residual ^{90}Sr (e.g, clays): extracted by 4M HNO_3, 16 h at 80°C, solid:water 1:5.

We hypothesized that the fraction of ^{90}Sr in the carbonate fraction would increase, at the expense of the ion exchangeable ^{90}Sr, as a result of induced calcite precipitation. We also hypothesized that the shift might be even more pronounced with ENTMP, because aragonite, another polymorph of $CaCO_3$, might be produced. Sr partitions approximately ten times more strongly into aragonite than into calcite (4).

Sediments were obtained from interbed core material retrieved from a well (perched monitoring well MW-2) drilled southeast of the INTEC Tank Farm in September 1993. The core, from a depth of approximately 110-111 ft, had been stored in a lexan core barrel at the INTEC core library since collection. The reported ^{90}Sr concentration in the core was 40 nCi/g (5). The core material was transported to a laboratory at the Reactor Technology Complex (RTC) and 18 subsamples were removed. For each of the 18 samples, approximately 5g soil was combined with simulated ground water (2) at a solid liquid ratio of 1:5. Samples 1-6 were designated as control samples. In addition to the simulated ground water, samples 7-12 were amended with cells from the mixed culture (final cell concentration of 1.28E + 09 cfu/ml),10 mM urea, 0.0005% yeast extract, and 0.00075% molasses. Samples 13-18 were prepared similarly to samples 7-12 but also had 5ppm ENTMP. The samples were incubated at room temperature in Erlenmeyer flasks with caps (to allow air exchange) for 10 days on a shaker table. After the incubation period, slurries were centrifuged to settle the solids and the supernatant was decanted, filtered and saved for ion chromatography analysis (for determination of NH_4^+, Ca, K, Na, Mg). One sediment sample from each treatment was washed in methanol (to prevent salt formation upon drying), dried at room temperature, and saved for x-ray diffraction (XRD) analysis. The remaining 15 samples were subjected to the sequential extraction procedure described above. Each extract was run through an Eichrom Sr- separation column using Eichrom procedure SRW01 R.1.4 in an effort to separate ^{90}Sr from other nuclides and interfering ions. Collected ^{90}Sr was then measured continuously on a scintillation counter for 10 days to measure the rate of ^{90}Y ingrowth.

Contrary to our expectations, we did not find any significant differences in the distribution of ^{90}Sr when comparing the three treatments (controls, added cells+nutrients, added cells+nutrients+ENTMP) and no calcite was detected by XRD. However, measurements of pH and NH_4^+ indicated that urea was hydrolyzed, and the average calcite saturation index was 1.3. This suggests that calcite precipitation kinetics were slow, and/or that calcite precipitation was inhibited. There was no significant difference between the flasks with ENTMP and those without. It is possible that the short incubation time (necessitated by delays in starting the experiment due to logistical complications, and then a required cutoff date due to the end of the fiscal year) was insufficient for precipitation to go to completion. The concentration of dissolved organics (cell metabolic products, molasses) may have inhibited precipitation; LeBron and Suarez (1996) have reported that dissolved organic carbon (DOC) can have a significant effect on precipitation rates (6). Unfortunately DOC measurements were not available for our experiments.

One of the interesting results obtained in this project was the observed distribution of ^{90}Sr between the three operationally defined fractions: ion exchange, carbonate and residual. (Measurements of ^{90}Sr remaining in sediment after the third extraction step indicated that < 1% of total ^{90}Sr was not captured by the extractions.) Figure 1 (Fujita-LDRDFY06final report fig 1.tif) shows a comparison of ^{90}Sr distribution between the fractions in the INTEC sediments and total Sr distribution in the VZRP sediments (extracted directly, without incubation in synthetic groundwater). The major portion of the ^{90}Sr is in ion exchange sites, which perhaps reflects the recent contamination history. In contrast, more of the total stable Sr is in the carbonate fraction.

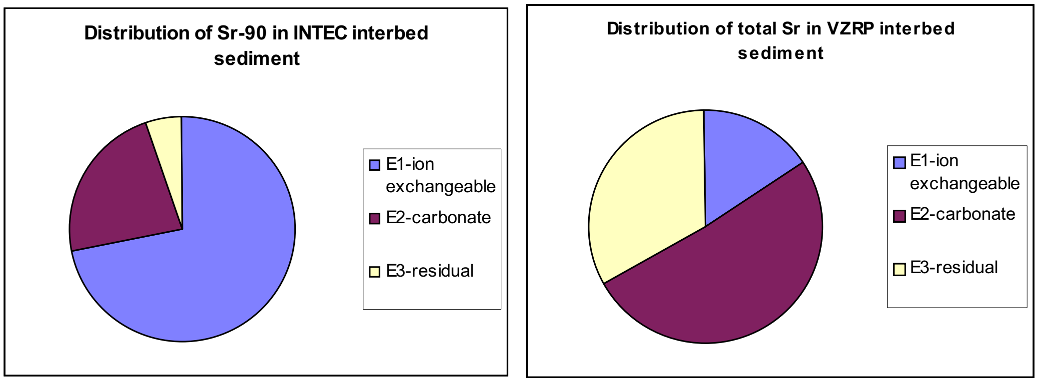

Figure 1. Comparison of distribution of Sr-90 in INTEC sediments and total Sr in VZRP sediments.

SUMMARY AND CONCLUSIONS

We were able to successfully enrich a urea hydrolyzing mixed culture from VZRP interbed sediments, and this remains available for future use. Unfortunately, our experiments with the INTEC interbed sediments indicated that although urea was hydrolyzed and the calcite saturation index was increased, calcite precipitation was not observed by the time the experiment was terminated, after 10 days. It is unknown whether the time was simply insufficient, or whether some inhibitor (besides ENTMP) was responsible. An interesting future experiment would be to add some calcite crystals to the supernatant solutions that appear to be supersaturated, to see if the presence of the calcite surfaces would be enough to overcome the inhibition; this would indicate that the inhibition was at the point of nucleation, rather than crystal growth. Alternatively, just re-measuring the supernatant solutions at some later time point would be instructive. From this project we also obtained an indication of the distribution of ^{90}Sr in INTEC interbed sediments between three different operationally defined geochemical compartments: ion exchange sites, carbonate phases, and residual. The majority of the ^{90}Sr appears to be in the ion exchange sites, in contrast to the situation observed with total Sr in uncontaminated VZRP sediments, where most of the Sr is in carbonates. This knowledge will be useful for future work at INTEC, for example perhaps for an Environmental Remediation Sciences Program (ERSP) field research center at INTEC; a proposal to investigate ^{90}Sr fate and transport at INTEC was submitted to an ERSP call in July 2006.

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