Final Executive Summary Report-SER Uranium research fund

Enhancing Bioremediation of in-situ Uranium Aquifers through Uranium and Carbon Isotopic Tracing of Biologic Activity

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Abstract

A 30-day microcosm, laboratory experiment identified tryptone as a viable biostimulant to promote bioremediation and valence reduction of uranium (VI to IV) for restoration of in-situ recovered (ISR) uranium aquifers at Cameco's Smith Ranch-Highland site. The experiment merely fed naturally-occurring bacteria within the collected drill-core material and did not introduce any new strains. The addition of tryptone as a bio-stimulant produced a 53% to 68% decrease in the concentration of soluble uranium. Phospholipid fatty acid (PLFA) data demonstrated a clear increase in microbial biomass amongst the tryptone-treated microcosms and established that the *Geobacter* species of bacteria was most likely responsible for reduction of U(VI) in this system.

The experiment also established that uranium isotopic measurements from monitoring well waters, specifically fractionations in $^{238}U/^{235}U$, are useful metrics in monitoring the progress of the bioremediation and establishing that valence reduction has occurred. Results from carbon isotopic measurements were also encouraging but are not yet definitive for monitoring bacterial activity in this application.

Details on the microcosm experiment are briefly described below; more complete details can be found in the associated Final Technical Report.

The positive results from the microcosm laboratory experiment have led to a follow-up, longer-term column experiment (funded separately by SER) to better mimic field applications, and a field trial currently underway at the Smith Ranch-Highland site. Preliminary results from the follow-up column study indicate up to 90% decrease in

soluble uranium through bio-stimulation. Full reports on these subsequent studies will follow their completions.

Introduction

Restoration efforts following in-situ recovery (ISR) of uranium are required by licensing agencies (Wyoming State DEQ and US EPA) and are critical for minimizing the overall environmental impacts of ISR uranium mining operations. Current restoration methods include aquifer sweeps to flush out remaining mining lixiviant and mining byproducts along with chemical treatments to reestablish a reducing environment and to drive reprecipitation of heavy metals brought into solution during ISR operations. These current methods are costly and often result in large amounts of consumptive water loss, so improved restoration strategies may improve the economics of uranium mining, streamline the mining to restoration process, and further minimize environmental impact.

Uranium exists commonly in two valence states, U(VI) and U(IV). The oxidized U(VI) form is relative soluble and typically the form of dissolved uranium, while the reduced U(IV) form is highly insoluble in most waters (Langmuir, 1978). Natural fluctuations and anthropogenic alterations to the redox state of surrounding fluids control the transportation of uranium, its deposition in roll-front deposits, in-situ recovery mining, and ultimately mine site restoration. Thereby, altering the redox state of the environment will alter the fate of uranium found within that environment.

Naturally occurring bacteria have been shown to be capable of reducing the valence state of uranium from U(VI) to U(IV), accelerating its precipitation (Cheng *et al.*, 2012; Gorby & Lovley, 1992; Phillips *et al.*, 1995; Uhrie *et al.*, 1996). There is a growing recognition that bacteria may have played an important role in the original uranium roll-front deposition (Boberg, 1981; Cheng *et al.*, 2012), which should allow them to play a similar role in ISR post-mining restoration.

Bioremediation is the use of living organisms to facilitate the clean-up of environmental contamination (USGS, 2011). This restoration strategy is an attractive alternative to physical and chemical restoration treatments as it is potentially quicker, less expensive and fixes the metals better. Biostimulation is one form of bioremediation that operates by stimulating the growth of an environment's naturally occurring organisms (often bacteria) by providing them with nutrients to accelerate the remediation (USGS, 2011). This remediation strategy has been investigated for 30 plus years and utilized on a number of contaminants including petroleum hydrocarbons and volatile organic compounds (Litchfield, 2005; Lorah *et al.*, 2008; Scow & Hicks, 2005; Song *et al.*, 2002). Potential applications to uranium and other metal contamination sites have been explored for at least 20 years with controlled laboratory experiments on soil samples (Gorby & Lovley, 1992; Hatzinger, 2004; Hatzinger, 2005; Lorah *et al.*, 2008; Lovley & Phillips, 1992; Merroun *et al.*, 2005; N'Guessan *et al.*, 2008; Phillips *et al.*, 1995) and a few small scale in-situ field experiments (Anderson *et al.*, 2003; Bopp *et al.*, 2010; Istok *et al.*, 2004; Ortiz-Bernad *et al.*, 2004; Wu *et al.*, 2007). In addition to uranium remediation, studies have demonstrated effective precipitation of other heavy metals, such as chromium, selenium, vanadium, or arsenic, from the reducing conditions promoted through biostimulation (Cheng *et al.*, 2012; Hatzinger, 2004; Hatzinger, 2005; Ortiz-Bernad *et al.*, 2004).

Although the biological fixation of uranium and other heavy metals has been demonstrated in the numerous laboratory studies noted above, the type of biostimulant and its subsequent efficacy appear to differ from site-to-site (e.g. Anderson *et al.*, 2003; Hatzinger, 2004). This is likely due to many factors including but not limited to initial

microbial community composition and transformations, intrinsic factors of the mine sediment, different levels of traditional remediation strategies applied, and differences in the aquifer water utilized (i.e. uranium level, total dissolved solids level, chemical treatment, etc.) (Luo *et al.*, 2007). Therefore, determining the proper biostimulant to produce this reducing situation and alter the redox state of the environment is vital to success in this restoration system.

Along with identifying an appropriate biostimulant for the environment specific to Cameco, Inc.'s Smith Ranch-Highland (SRH) site near Douglas, WY, the project tested isotopic fractionations as markers of biological reducing activity. Isotopic fractionation has been demonstrated to occur differently in biologically-induced systems as compared to abiotic systems (Bopp *et al.*, 2010; Botz *et al.*, 1996). This is largely due to enzymatic selection of reactants having a higher affinity for select isotopes over others, which is not a phenomenon observed in abiotic systems. Our plan was to monitor uranium- and carbon-isotopic stable isotope ratios with the goal of observing a measurable $^{238}U/^{235}U$ ratio shift or $^{13}C/^{12}C$ dissolved inorganic carbon (DIC) ratio shift that correlated with biological reduction of U(VI) to U(IV).

Microcosm design and implementation

A previous microcosm experiment using Smith Ranch-Highland cores and waters had tested 11 potential biostimulants (Hatzinger 2004). A significant result from this experiment was that neither acetate nor methanol was particularly effective at reducing soluble uranium on these SRH materials even though they had proven useful in Colorado and Tennessee sites. Of the remaining biostimulants tested, cheese whey and safflower

oil with ethanol showed the most extensive reduction of soluble uranium, although cheese whey temporarily clogged the wells during a subsequent field trial.

John Willford, UW Academic Professional Lecturer in the Department of Microbiology, was recruited to organize the microcosm experiment at UW. He selected two nutrient amendments as our biostimulant nutrients for the new microcosm study: 1) safflower oil with methanol and 2) tryptone. Tryptone is an enzymatic digest of casein, and was selected as an alternative to cheese whey as tryptone is completely water-soluble to at least 2% concentration. Tryptone's other advantages include: a) it has the same protein source as cheese whey (casein), b) it has a high nitrogen value amongst available peptones, and c) it provides the highest iron content for a milk-derived peptone (Merck, 2010). Iron (Fe) was included as a factor as it has been well demonstrated that Fe(III)-reducing microbes play a significant role in the reduction of U(VI) (Cheng *et al.*, 2012; Gorby & Lovley, 1992; Lloyd & Renshaw, 2005).

Cored sediment and waters from a post-mining SRH site were combined in 125 ml serum bottles under ultra-pure nitrogen gas conditions. Each bottle received 40g of aquifer core sample and 100mL of liquid amendment. Two different waters were used from SRH, a high total dissolved solids (TDS) water with approximately 5 mg/L uranium, and a low TDS water, with approximately 1 mg/L uranium. Bottles were set up with each water type and either no amendments, tryptone, or safflower, for a total of 6 sample types. Bottles were rolled continuously and sampled in triplicate every 5 days for 30 days total time. The water from the sampled microcosms was filtered prior to uranium and selenium concentration, uranium isotopic and carbon isotopic measurements. The

solids from the sampled microcosms were saved for phospholipid fatty acid (PLFA) and mineralogic determinations.

Results

Microcosms amended with tryptone showed decreases in uranium concentrations from 53% to 68% in low TDS and high TDS waters, respectively (Figure 1). Results from control (no amendments) and safflower microcosms show no decrease in uranium concentrations over the 30 day experiment, in fact concentrations in the high TDS no-add and safflower samples increased, probably due to liberation of labile uranium by the physical rolling of the samples.

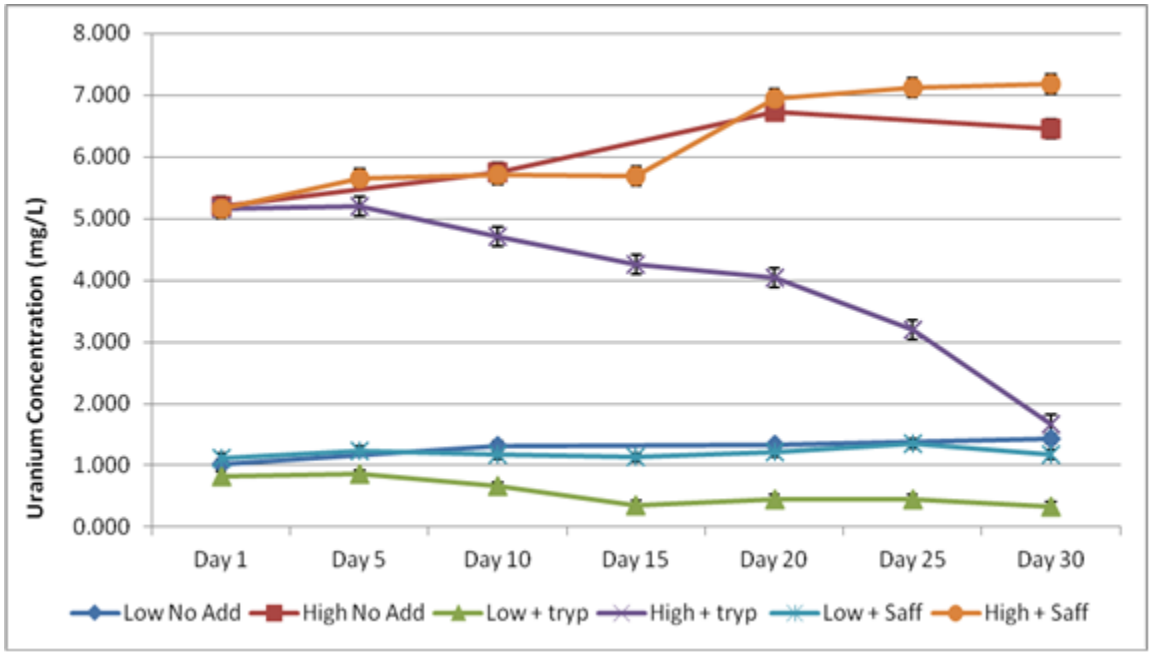

Figure 1. Measured soluble uranium concentrations in microcosm study. The error bars indicate standard error calculated using a general linear model. With spike recovery rates ranging from 86% to 110%, the data show little interference from the background matrix on measurability. Abbreviations: Low TDS water (Low), High TDS water (High), no nutrient (No Add), Tryptone (tryp), Safflower Oil with methanol (Saff).

The phospholipid fatty acid (PLFA) data demonstrate a clear increase in microbial biomass amongst the tryptone-treated microcosms (Figure 2). The increases in biomass correlate well with the periods of decrease in the concentration of soluble uranium. PLFA measurements coupled with carbonate concentration determinations (not shown) indicate that the safflower-amended microcosms did have some increased biological metabolic activity near the end of the study, so it is possible that there would have been some decrease in uranium with safflower in a longer-term study. PLFA analyses also provided a clearer picture of which specific organisms may be involved in reducing U(VI) to U(IV) within this specific environmental system. For the SRH samples in these conditions, it appears that *Geobacter* species were more active than those from either the *Desulfobacteraceae,* or *Shewanellaceae* families.

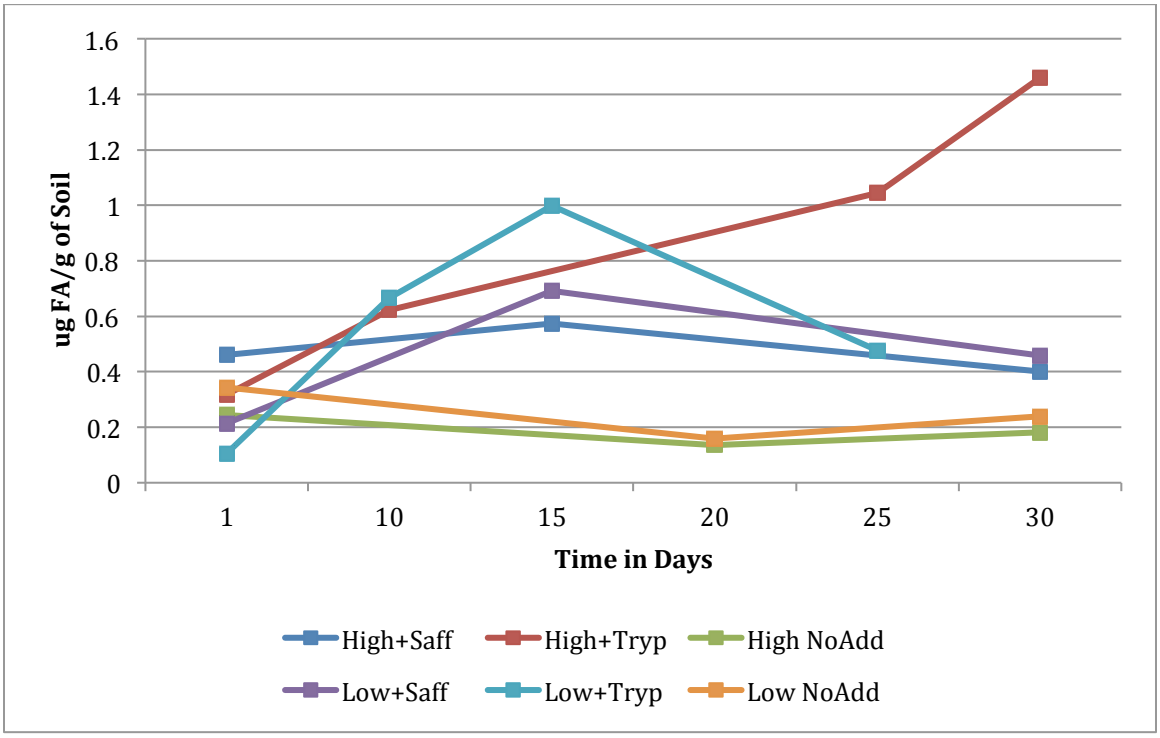

Figure 2. Microbial Biomass in µg of fatty acid/g of soil. Not all bottles were selected for PLFA analysis, which is why not all time points are represented. Abbreviations: Low TDS water (Low), High TDS water (High), Tryptone (tryp), Safflower Oil with methanol (Saff).

Uranium isotopic measurements showed a marked decrease in $^{238}U/^{235}U$ values for the high TDS waters (Figure 3), which correlates well with decrease in soluble uranium during shift in redox conditions. After purifying uranium on ion exchange columns in the clean lab at UW, isotopic ratios were measured on an inductively-coupled plasma multi-collector mass spectrometer (MC-ICP-MS) at UW. Analytical reproducibility was determined to be 0.07 permil, and the isotopic fractionation in the high TDS tryptone waters was 10 times this value, nearly 1 permil (Figure 3).

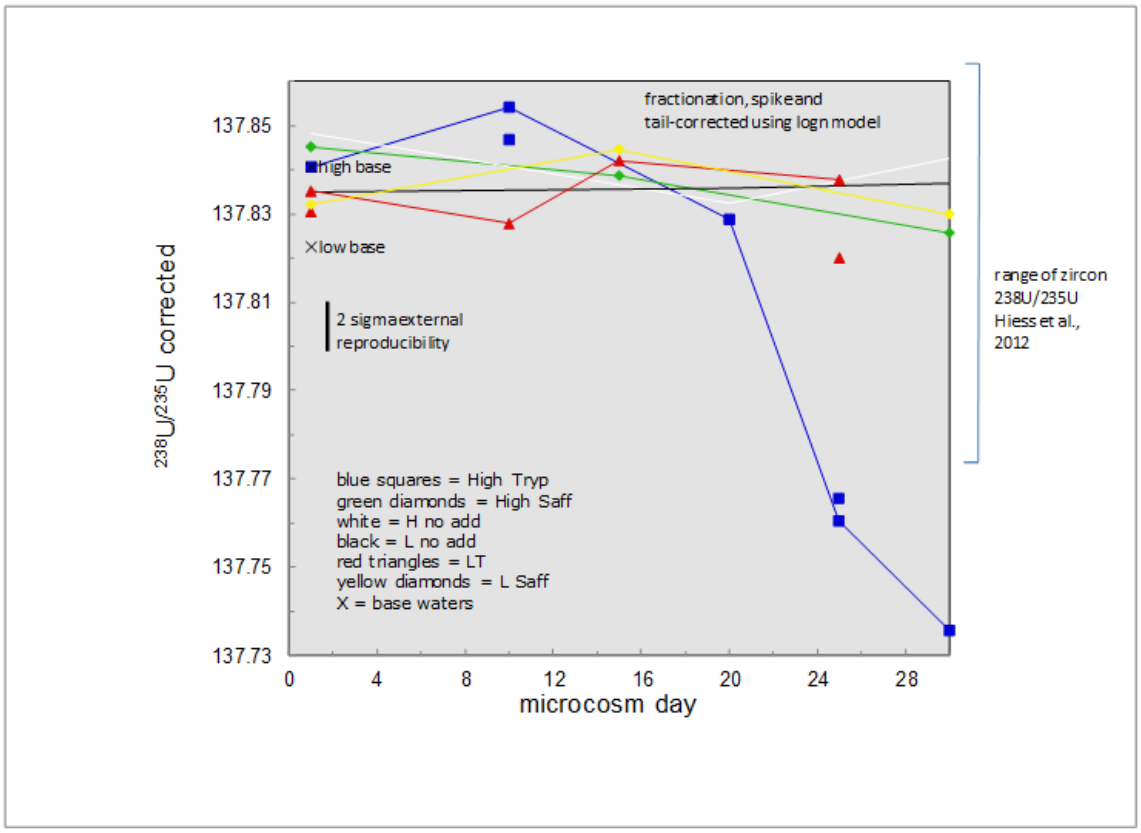

Figure 3. $^{238}U/^{235}U$ ratio values. A designation of a 2 sigma error bar size is included on the left side of the graph. The bracket on the left side of the graph designates the naturally-occurring $^{238}U/^{235}U$ ratio range determined utilizing zircon (Hiess *et al.*, 2012). Abbreviations: Low TDS water (L, LT), High TDS water (H, High), Tryptone (T, tryp), Safflower Oil with methanol (Saff).

This degree of fractionation exceeds that of most abiotic reservoirs on Earth, based on measured $^{238}U/^{235}U$ recorded in zircon, a relatively common uranium-rich silicate mineral (Hiess et al., 2012), although comparable fractionations have been recorded in uranium ore deposits and other biologically-active uranium sites (Rademacher et al., 2006; Bopp et al., 2009; 2010). Uranium isotopic results for the low TDS tryptone waters, safflower-amended waters and both high and low TDS controls show no fractionation outside of analytical uncertainty. Uranium isotopic fractionation appears to be a reliable measure of bacterially-induced valence reduction in cases with significant starting concentrations of uranium (the high TDS in this experiment), although it may not be sensitive enough to detect this effect for waters with lower starting concentrations.

In addition to these quantitative measurements, there were a number of qualitative observations that indicate a shift from oxidizing to reducing conditions in the tryptone-amended microcosms. These include strong sulfur odors, suggesting the reduction of sulfur to hydrogen sulfide (H_2S), beginning at day 10 and increasing in strength up to at least day 25, and the appearance of black precipitates first observed between days 18 and 25 suggesting the presence of iron sulfide (FeS) produced from biological reduction of sulfate. Accordingly, an overall darkened appearance was observed in most of the bottles suspected to have an altered redox state. The safflower-amended and control bottles did not show any of these changes and are likely to have remained in an oxidizing state.

Summary

The results from this microcosm experiment are very encouraging and indicate that a strategy of simply feeding the existing bacteria in a post-mining ISR field may

substantially decrease the soluble uranium and regenerate a chemically-reducing environment in the mined aquifer. It also appears that the active bacteria effectively reduce the uranium valence state from soluble U(VI) to insoluble U(IV), thereby fixing the uranium and other heavy metals such as selenium and iron. The valence reduction in this scenario can also be effectively tracked from the monitoring well waters using uranium isotopic measurements without requiring any post-remediation coring.

The positive results from this study led to a follow-up, 9-month column style experiment in 2014 and a field trial currently underway at Smith Ranch-Highland. Results of these two subsequent experiments will be available once the experiments are complete, but the preliminary results from the column experiment indicate even higher percentages of removal of soluble uranium (90% or more) than were seen in the microcosm experiment.

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