Assessing the Potential for In Situ Bioimmobilization of ^{99}Tc at the Hanford Site

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Introduction

Microorganisms, either directly or indirectly, can alter the oxidation states of uranium and technetium resulting in their precipitation as sparingly soluble solid-phases. This process, in concept, can render these contaminants immobile for long time periods. ^{99}Tc is a radionuclide that contributes significantly to estimates of future human health risk at the Hanford Site because of its longevity and mobility in the subsurface environment. It exists at high concentrations (up to 30,000 pCi/L) in the central areas of the site where the groundwater table is deep, and is predicted to move to the Columbia River within the next decade. It also has been observed at lower concentrations (600 pCi/L) in shallow groundwater near the river in the 100 H area.

The purpose of this project is to assess the feasibility of stimulating the *in situ* subsurface microbiota at the Hanford Site to reduce and immobilize ^{99}Tc. The concept and approach proposed has evolved from NABIR-funded research that is maturing to the point that it is now appropriate to pursue site-specific research to establish field-scale proof-of-concept. Although this project focuses on assessment of biostimulation approaches for reducing and immobilizing ^{99}Tc in the shallow groundwater system of the 100 H area at the Hanford Site, it is anticipated that the information will be applicable to other contaminants and site conditions at Hanford, and possibly elsewhere, within the DOE weapons production complex.

The initial objective of the project is to determine if indigenous microorganisms in aquifer sediments at Hanford can be stimulated to either directly or indirectly, via Fe(II), immobilize ^{99}Tc. If this is shown to be the case, two additional objectives will be addressed:

- Devise an electron donor addition strategy for stimulating indigenous microorganisms to immobilize ^{99}Tc *in situ*, and
- Evaluate the feasibility and develop a research plan including design parameters, if warranted, for an *in situ* biostimulation experiment at the Hanf

Methods

The potential for stimulating the reduction of $Tc(VII)O_4^-$ and/or sediment-associated Fe(III) by indigenous subsurface microorganisms was initially investigated in batch microcosm experiments. Following sediment retrieval, sediment-artificial groundwater slurries with UP-1 or 300 Area sediments were constructed and amended with various electron donors or acceptors (acetate, lactate, glucose, or fumarate; 5 mM each) and nutrients (NH_4Cl, KH_2PO_4, & K_2HPO_4; 50 µM each) to determine whether microbial growth could be readily stimulated. A subset of microcosms for each sediment and amendment combination received 20 _M $Tc(VII)O_4^-$ as NH_4TcO_4 or 5 mM Fe(III) as hydrous ferric oxide (HFO) to evaluate the potential for indigenous subsurface organisms to reduce Tc(VII) and/or Fe(III).

Microcosms consisted of 2 g sediment per 10 ml artificial groundwater incubated at ambient temperature and 100 rpm in stoppered pressure tubes flushed with oxygen-free N_2/CO_2 (80:20). Samples were obtained at select time points and analyzed for soluble Tc and Fe(II) and 0.5 N HCl-extractable Fe(II). ^{99}Tc in solution (<0.2 µm) was measured using liquid scintillation counting (0.292 Mev, beta). For Fe(II) measurements, sediment or culture medium was extracted with 0.5 N HCl for 1 h or 22 h and analyzed by the ferrozine method. Cell growth was measured by direct counting.

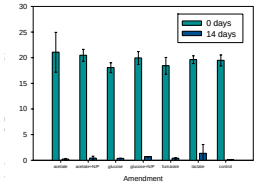
Hanford UP-1 Sediment Sample Collection and Cultivation Results

To investigate biostimulation in laboratory experiments, fresh Hanford sediments were collected in Nov. 2002. Well 299-W19-46 was drilled in the 200 Area of the Hanford Site for the recovery of core UP-1. Groundwater was encountered at 255.85 feet below ground surface and a split spoon sampler was driven from 260 to 262.5 ft (40% recovery) to collect subsurface sediments from the unconfined aquifer. Aseptic core sampling techniques were used. Sediment core samples were transferred to an anaerobic chamber are were used in microcosm and enrichment cultivations.

Location of the Hanford site and the 200 Area

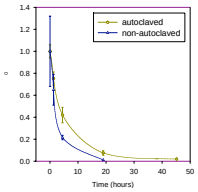
Location of the well 299-W19-46

Changes in Soluble ^{99}Tc Concentrations in UP-1 Sediment Microcosms



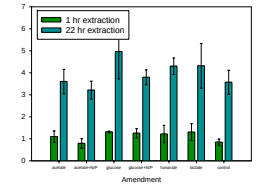
Soluble ^{99}Tc levels decreased substantially within 14 days in all UP-1 sediment microcosms including the unamended control.

Rates of Soluble ^{99}Tc Decrease in Live and Autoclaved UP-1 Sediment



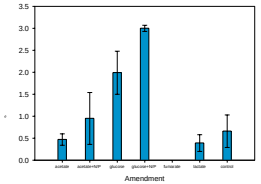
Rates of ^{99}Tc loss from solution were rapid in both live and autoclaved sediments indicating the presence of an abiotic reductant, possibly Fe(II).

Fe(II) Concentrations in UP-1 Sediment microcosm experiments



Comparisons of 1 hr vs. 22 hr HCl extractions demonstrates a time-dependence on the extent of Fe(II) extraction. These results suggest the presence of a crystalline Fe(II) phase that is weak acid soluble.

Hydrogen Concentrations in UP-1 Sediment Microcosms

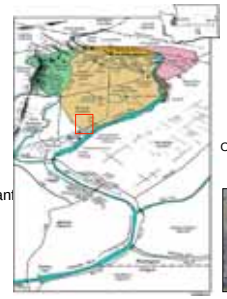


All sediment microcosms exhibited significant accumulations of H_2, with the notable exception of the fumarate treatment in which the H_2 concentration was below detection. The glucose treatments produced 3 – 4.5 times more H_2 than the unamended control.

Hanford 300 Area Sample Collection and Cultivation Results

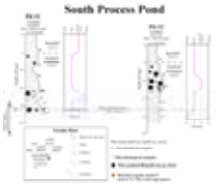
Sediment samples were collected from directly above the water table and adjacent to the Columbia River. Open pit excavations were employed to minimize sample disturbance and fracturing of basalt clasts. Hanford Formation sediments were collected aseptically and were used in anaerobic microcosm and enrichment experiments.

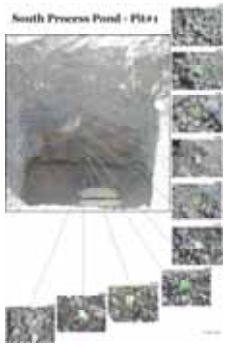
Location of the Hanford 300 Area and pit excavations.



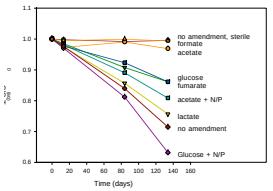
Open pits were excavated with the aid of a backhoe beneath the 300 Area Process Ponds in April, 2003.

The Hanford Formation in this area generally consisted of loose, clast-supported, muddy sandy gravel; lenses of matrix-supported gravelly sand occurred sporadically. Material filling the matrices between gravel clasts was variable and consisted of a poorly to moderately sorted mixture of clay, silt and/or fine to coarse sand. The gravel and sand particles were predominantly basalt. Rip-up clasts and beds of fine-grained Ringold Formation silt/clay were also present in zones of the Hanford Formation.





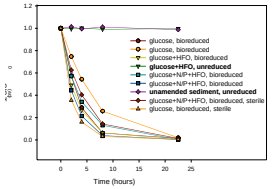
^{99}Tc reduction in South Pond Microcosms



Sediment microcosm experiments required >3 months incubation prior to Tc reduction. Sediments amended with glucose and N/P displayed the most extensive Tc reduction.

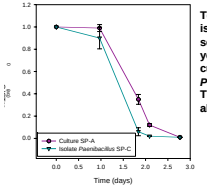
Examples of bioreduced and unreduced sediment microcosms.

^{99}Tc reduction by South Pond Enrichments



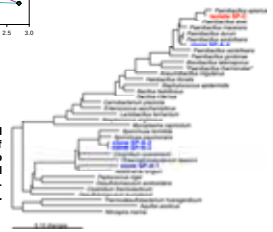
Following 4+ months incubation, many sediment enrichment cultures amended with glucose and hyrous ferric oxide (HFO) displayed growth stimulation and were visibly reduced. Reduced and non-reduced cultures were subsequently assayed for Tc reduction. These incubations exhibited rapid Tc reduction relative to unamended controls and sediments which were not previously reduced. Autoclaved, bioreduced sediment-cell suspensions reduced Tc at a rate similar to live cultures suggesting that Tc reduction by these organisms is an indirect reaction.

^{99}Tc reduction by a South Pond Isolate and Mixed Culture



Tc-reducing microorganisms were further isolated by dilution and streaking on solid medium containing glucose and yeast extract. A mixed enrichment culture of *Sporomusa, Acetonema,* and *Paenibacillus* species quickly reduced Tc. The new isolate *Paenibacillus* SP-C was also found to reduce Tc and HFO.

Cloning, sequencing, and phylogenetic analyses of 16S rDNAs was used to assess the microbial composition of Tc-reducing cultures.



Summary and Conclusions

- High concentrations of HCl-extractable Fe(II) within the UP-1 core material at the initial time of sampling suggests that disturbances during coring may have exposed fresh Fe(II)-bearing silicate mineral (e.g. basalt fragments) surfaces. This disturbance may have also contributed to H_2 generation by iron-silicate minerals as indicated by elevated H_2 measurements in the microcosm experiments including the unamended control.
- Rates of Tc reduction in UP-1 sediment microcosms under abiotic and non-sterilized conditions were rapid and comparable, suggesting that the reduction of Tc was largely abiotic.
- The observed rapid Tc reduction may be due to solid-phase associated Fe(II) resulting from exposure of fresh Fe(II) silicate surfaces during coring.
- Relatively high H_2 concentrations were observed in unamended UP-1 sediments, possibly due to abiotic processes resulting from freshly exposed basalt surfaces as previously reported (Bjornstad et al. 1994*). H_2 was below detection in fumarate amended microcosms, probably due to H_2 consumption by fumarate respiring microorganisms. H_2 production by glucose-fermenting microorganisms was also detected.
- Sediment sample collections at the 300 Area Process Pond used less-disruptive methods to avoid fracturing basalt clasts. The results of sediment microcosm experiments imply that sampling artifacts due to clast fracturing and H_2 production were minimized.
- Microcosm experiment results suggest that >3 months is required to biostimulate microbial activity using subtle methods in the 300 Area environment. Once the sediments are bioreduced, Tc reduction is rapid and extensive.
- Our conclusion from these results is that the highly aerobic nature of Hanford sediments near the water table has resulted in very low numbers of anaerobic microbes, hence 3 months is required for biostimulation. Samples from deeper in the aquifer or closer to the river may biostimulate more rapidly and contain higher levels of endogenous activity.
- Future experiments will focus on the Tc plume in the 100 H Area, using single-well push-pull tests to assess biostimulation to reduce Tc under in situ conditions. Long incubation times relative to groundwater flow rates will be addressed by repeated electron donor amendment. Push-pull tests will be conducted in collaboration with Jack Istok (Oregon State University).

*Bjornstad, B.N., McKinley, J.P., Stevens, T.O., Rawson, S.A., Fredrickson, J.K., and Long, P.E. (1994) Generation of hydrogen gas as a result of drilling within the saturated zone. *Ground Water Monitor. Remed.* Fall: 140-147.

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