Investigations of HRC®-Stimulated Bioreduction of Cr(VI) at Hanford 100H

T.C. Hazen, B. Faybishenko, D. Joyner, S. Borglin, E.Brodie, S. Hubbard, K. Williams, J. Peterson, J. Wan, T. Tokunaga, M.Firestone (LBNL, University of California-Berkeley), P.E. Long and D. Newcomer (Pacific Northwest National Lab), S. Koenigsberg and A. Willet (Regenesis, Ltd.)

Pacific Northwest National Laboratory
...delivering breakthrough science and technology

REGENESIS

NABIR

http://www-esd.lbl.gov/ERT/hanford100h/

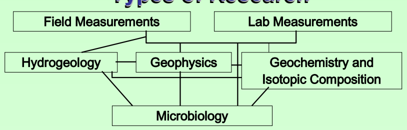
Hypothesis

Lactate (Hydrogen Release Compound—HRC™) injection into chromium contaminated groundwater through an injection well will cause indirect or direct bioreduction of chromate [Cr(VI)] and precipitation of insoluble species of [Cr(III)] on soil particles, probably catalyzed at oxide surfaces, at the field scale.

Objective

To carry out field investigations to assess the potential for immobilizing and detoxifying chromium-contaminated groundwater using lactate-stimulated bioreduction of Cr(VI) to Cr(III) at the Hanford Site's 100-H Area field site.

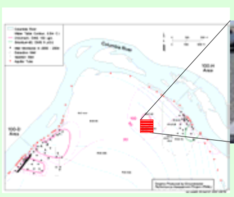
Types of Research



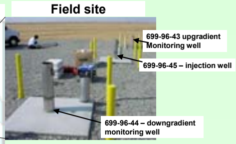
- Drilling, coring, and completion of two new 60 ft deep boreholes
- Development of a conceptual model of background conditions
- Microbial and lactate-induced laboratory and field studies
- Geophysical characterization
- Water sampling
- Hydraulic measurements

I. Background Geological, Hydrological, Geophysical, and Microbial Conditions at Hanford 100H

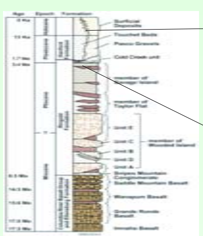
Chromium in groundwater at Hanford 100D area is source at Hanford 100H area



Field site



Lithological Column



Cores
(4 in. diameter)

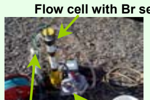
38.5 ft - Hanford gravelly sand

54 ft
Ringold silt

Access tube for geophysical survey

Groundwater sampling and packer inflation using argon gas

Flow cell with Br sensor

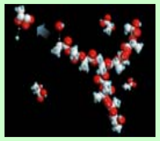


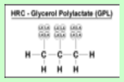
Sampling port

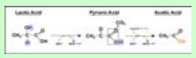
Sampling tube for methane

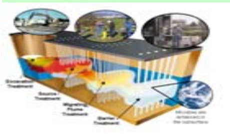
Flow cell with a multiparameter probe (pH, DO, conductivity, temperature)

II. Lactate/Polylactate (HRC™) Properties







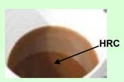


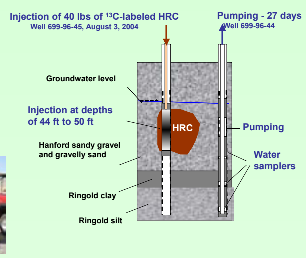
- HRC properties
  - a polymer of lactic acid (glycerol polylactate)
  - fermented by indigenous microorganisms to produce H_2, an electron donor
  - in the subsurface, slowly releases lactic acid to stimulate anaerobic bacteria and ferment the lactic acid to gain carbon and energy

HRC, when injected into chromium contaminated groundwater:

- Generates electron donors (lactate and hydrogen) for microbial production of reducing conditions to stimulate precipitation of Cr(III) solids
- Stimulates microbial reduction and production of species that can chemically reduce Cr(VI) to Cr(III) like Fe(II) and hydrogen sulfide.
- Causes the microbial population to remove the oxygen, nitrate, sulfate and other competing electron acceptors, thus, depressing the redox potential in the aquifer, affecting the transformation of Cr(VI) species to Cr(III) species, which are precipitated to precipitate on soil particle surfaces or colloids.

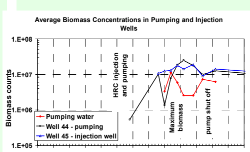
III. Pilot Field Experiment of Groundwater Biostimulation Using HRC Injection

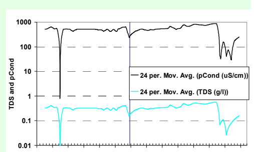


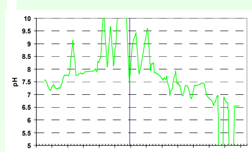


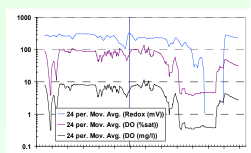
- All in all 11 gal of water were used as a primer to fill the injection hose before the injection, dilute HRC, and as a chaser after the HRC injection.
- Br breakthrough occurred 7 days after the injection, and the maximum was reached 11 days after the injection.
- Microbial cell counts reached the maximum 13-17 days after the injection.

III. Field Experiment Results



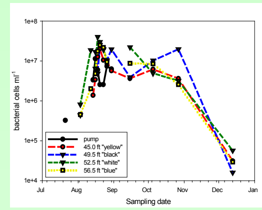




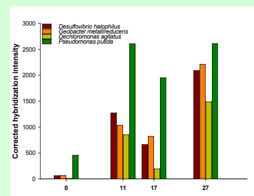


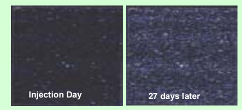
III.1. Microbial Analyses

- Phospholipid fatty acid analyses (PLFA)
- Terminal restriction fragment length polymorphism (T-RFLP) with primers for Fe and sulfate reducers, and nitrate dissimilatory reactions;
- Live/dead direct counts;
- TEA, ED, DOC, DIC, CO2, O2 Limiting nutrients, e.g., N, P, S, Fe;
- Nitrogen and oxygen isotope ratio; $^{53}Cr/^{52}Cr$ ratios
- Clone libraries
- 16S rDNA GeneChip
- Direct rRNA analysis by microarray
- Novel PCR independent analysis of microbial communities (Bacteria and Archaea)

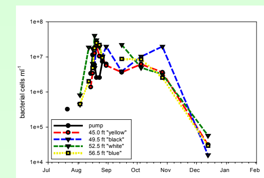


- Archaea
  - Only Crenarchaeotes (non-thermophilic) detected
    - Dominated by one type with no cultured relative.
- Bacteria Initial enrichment of denitrifiers
  - *Fulvimonas, Pseudomonas, Hyphomicrobium, Acidovorax, Aquaspirillum,Thauera, Azoarcus, Comamonas, Dechloromonas, Clostridium.*

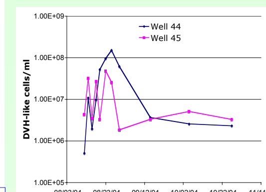




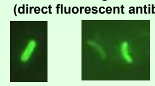
16S rDNA Clone library & qPCR



Sequencing at JGI
⇩
1000+ clones
⬇
Phylogenetics
⬇
qPCR
⬇
Metagenomics?

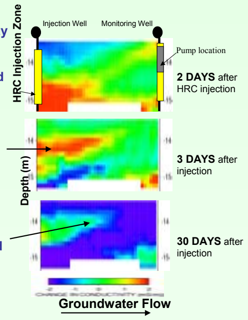


D. vulgaris
(direct fluorescent antibody)



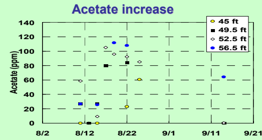
III.2. Post-HRC Injection Changes in Electrical Conductivity

- Changes in Electrical Conductivity (relative to data collected prior to HRC injection) were estimated using crosshole radar velocity and attenuation information.
- After the HRC injection, the data suggest that HRC products, such as lactic acids, quickly occupy a high hydraulic conductivity zone.
- After approximately 10 days, a 'reaction front' occurs downgradient from the HRC zone emplacement, which is interpreted to be associated with the development of precipitates or biofilms.

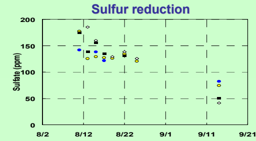


III.3. Geochemical and Isotopic Evidence of Biostimulation

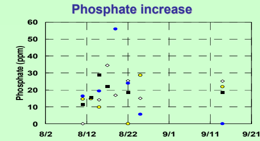
Acetate increase



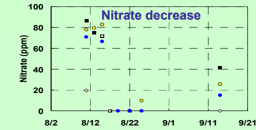
Sulfur reduction

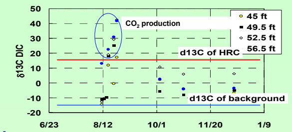


Phosphate increase

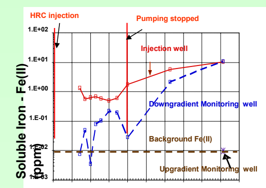


Nitrate decrease

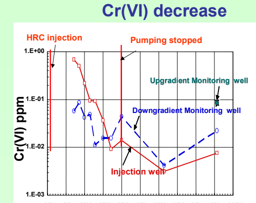




Ferrous increase



Cr(VI) decrease



IV. Key Findings

1. Several types of bacteria are present in the Hanford sediments, including *Arthrobacter, Oxalobacter, Sporomusa* and *Pseudomonas* species. Under background conditions, the total microbial population is <10^5 cells g^{-1}.
2. Different types of HRC™ and metal remediation compound (MRC™) could generate biostimulation and increase biomass to >10^8 cells g^{-1}, generate highly reducing conditions, and enhance Cr(VI) removal from the pore solution.
3. Pilot field-scale biostimulation of the groundwater using shows microbial cell counts reached the maximum of 2×10^7 cells g^{-1} 13 to 17 days after the injection and continued to increase for the first 6 weeks, followed by the decrease in the microbial diversity.
4. DO dropped from 8.2 to 0.35 mg/l, redox potential from 240 to -130 mV, and pH from 8.9 to 6.5. DO and nitrate began to return to background concentrations two months after HRC injection, despite ground water bacterial densities remaining high (>10^7 cells/ml).
5. Geophysical investigations show that HRC products (such as lactic acids) injected into groundwater can be detected using radar and seismic survey, and that even small variations in hydrogeological heterogeneity may influence the distribution of the amendment and its products.
6. $\delta^{13}C$ ratios in dissolved inorganic carbon confirmed microbial metabolism of HRC. $\delta^{13}C$ ratios remain above background values after 6 months. Increases in carbon isotope ratios of DIC in Well 44 are coincident with increases in bromide, chloride and acetate and decreases in nitrate. The source of chloride is likely from the HRC.
7. Hydrogen sulfide production was first observed after about 20 days post-injection, which corresponds with the enrichment of a *Desulfovibrio* species (sulfate reducer) identified using 16S rDNA microarray and monitored by direct fluorescent antibodies.
8. Cr(VI) concentrations in the monitoring and pumping wells decreased significantly and remained below up-gradient concentrations even after 6 months, when redox conditions and microbial densities had returned to background levels.
9. Directions of ongoing research are aimed at the evaluation of sustainability of chromium biostimulation, including the assessment of Cr(III) reoxidation, the evaluation of the HRC amount and distribution in the subsurface, and the need for pumping.

Selected Publications:

Hazen, T.C.. D. Joyner, S.Borglin, B.Faybishenko, J.Wan, T.Tokunaga, M.Conrad, C.Rios-Velazquez, J.Malave-Orengo, R.Martinez-Santiago, M.Firestone, E.Brodie, PE. Long, A.Willet, and S.Koenigsberg, Functional Microbial Changes During Lactate-Stimulated Bioreduction of Cr(VI) to Cr(III) in Hanford 100H Sediments, Abstract submitted to the Fourth International Conference "Remediation of Chlorinated and Recalcitrant Compounds, May 24-27, 2004.

Linde, N., S. Finsterle, and S. Hubbard, Inversion of hydrological tracer test data using tomographic constraints, EOS Spring Supplement, Montreal, Canada, May 2004.

Tokunaga TK. Wan JM. Firestone MK. Hazen TC. Olson KR. Herman DJ. Sutton SR. Lanzirotti A. In situ reduction of chromium(VI) in heavily contaminated soils through organic carbon amendment, Journal of Environmental Quality. 32(5):1641-1649, 2003a.

Tokunaga TK. Wan JM. Hazen TC. Schwartz E. Firestone MK. Sutton SR. Newville M. Olson KR. Lanzirotti A. Rao W., Distribution of chromium contamination and microbial activity in soil aggregates, Journal of Environmental Quality. 32(2):541-549, 2003b.

Acknowledgement: Funded by Joint Natural and Accelerated Bioremediation (NABIR) Program, and the Office of Science and Technology, Office of Environmental Management of DOE. Support of the field work by V.Gruol and P.Rizzo (LBNL), B. Bjornstad, T.Resch, and K.Cantrell (PNNL) is very much appreciated.