Field Investigations of Lactate-Stimulated Bioreduction of Cr(VI) to Cr(III) at Hanford 100H


LBNL, UCB, PNNL, Regenesis
Mesoscale Studies on Cr(VI) Bioreduction that led to Field Studies

Jiamin Wan, Tetsu Tokunaga, Mary Firestone and Terry Hazen (NABIR supported 1998-2004)


# Multidisciplinary Team

<table>
<thead>
<tr>
<th>Scientific Field</th>
<th>LBNL</th>
<th>PNNL</th>
<th>Regenesis</th>
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<tbody>
<tr>
<td><strong>Microbiology</strong></td>
<td>Terry Hazen, Eoin Brodie, Sharon Borglin, Dominique Joyner, Mary Firestone</td>
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<td><strong>Hydrogeology</strong></td>
<td>Boris Faybishenko, Jiamin Wan, Tetsu Tokunaga</td>
<td>Philip E. Long, Bruce Bjornstad</td>
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<td><strong>Geophysics</strong></td>
<td>Susan Hubbard, Ken Williams, John Peterson,</td>
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<td><strong>Geochemistry</strong></td>
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<td>Darrell Newcomer</td>
<td>Steve Koenigsberg, Anna Willet, Kevin Lapus</td>
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Overall Objective

To carry out field investigations to assess the potential for immobilizing Cr(VI) in groundwater using lactate-stimulated bioreduction of Cr(VI) to Cr(III) at the Hanford 100H site, and to determine critical community structure changes and stressors that would enable control and predictions of fundamental biogeochemistry that enables this bioremediation strategy for Cr(VI)

Integrated Approach

Field Measurements
- Hydrogeology

Lab Measurements
- Geophysics
- Geochemistry and Isotopic Composition
- Microbiology
The Cr source is believed to be sodium dichromate ($\text{Na}_2\text{Cr}_2\text{O}_7\cdot2\text{H}_2\text{O}$).

http://esd.lbl.gov/ERT/hanford100h/
LiBr Injection (2/27/2004)

55 Days No pumping

63 Days Pumping

63 Days Pumping + 2nd Injection

- LiBr Injection: 15.3 g/liter, 12 liters over 2 hours
Lactic Acid Molecule

H+ from water

OH- from water

HRC®
(Polylactate Ester)
Lactate-Induced Bioreduction of Cr(IV)
Injection of 40 lbs of $^{13}$C-labeled HRC
Well 699-96-45, August 3, 2004

Injection at depths of 44 ft to 50 ft

Groundwater level

Pumping - 27 days
Well 699-96-44

Hanford sandy gravel and gravelly sand

Ringold clay

Ringold silt

Water samplers
Post-HRC Injection Changes in Electrical Conductivity

Hypothesis: Lactic acid

Hypothesis: Reaction halo due to formation of precipitates
Results of HRC Biostimulation

**D. vulgaris** (direct fluorescent antibody)

Redox dropped from 240 to -130 mV
DO dropped from 9 mg/l (~100%) to 0.35 mg/l (4.5%)
Dissolved Oxygen (mg/l)

- Injection well
- Upgradient monitoring well
- Downgradient monitoring well

pH

- HRC injection
- Pumping

Redox Potential (mV)

- HRC injection
- Pumping

Electrical Conductivity (μS/cm)

- HRC injection
- Pumping
Biogeochemical Evidence of Microbial Metabolism in Groundwater

d\textsuperscript{13}C of Dissolved Inorganic Carbon is Byproduct of HRC Metabolism

- Biogeochemical Evidence of Microbial Metabolism in Groundwater
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Biogeochemical Evidence of Microbial Metabolism in Groundwater

**Acetate Increase**

- Fe(II) Increase
- Sulfate reduction
- Nitrate decrease

**Acetate (ppm)**

- 0
- 20
- 40
- 60
- 80
- 100
- 120
- 140

**Fe(II) Concentration, mg/L**

- 1.0E-04
- 1.0E-03
- 1.0E-02
- 1.0E-01
- 1.0E+00

**Sulfate (ppm)**

- 0
- 20
- 40
- 60
- 80
- 100
- 120
- 140
- 160
- 180
- 200

**Nitrate (ppm)**

- 0
- 20
- 40
- 60
- 80
- 100
- 120
- 140
- 160
- 180
- 200
Changes of Cr(VI) Concentration in Groundwater after HRC Injection

Average Soluble Cr(VI) Concentration

Average Total Chromium Concentration
Combined High Density Microarray Analysis and $^{13}$C Phospholipid Analysis

Approach

- Statistical analysis & data reduction used to mine vast quantities of data
- Organisms assigned to groups based on covariance (similar response to treatment)
- Combined with geochemical data and PLFA this yields insight into functional role/niche of specific organisms
Microarray analysis of bacterial community changes during Cr(VI) remediation at Hanford 100H site:

Dynamics of some significant organisms.

Days since HRC injection

Corrected hybridization intensity

<table>
<thead>
<tr>
<th>Days since HRC injection</th>
<th>Desulfovibrio halophilus</th>
<th>Geobacter metallireducens</th>
<th>Dechloromonas agitatus</th>
<th>Pseudomonas putida</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>3000</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>11</td>
<td>1500</td>
<td>1000</td>
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<td>17</td>
<td>2000</td>
<td>2000</td>
<td>2000</td>
<td>2500</td>
</tr>
<tr>
<td>27</td>
<td>2500</td>
<td>2500</td>
<td>2500</td>
<td>3000</td>
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Injection Day

30 days later
Hierarchical clustering and heatmap plot of 16S GeneChip analysis of microbial community sub-families detected during chromate bioremediation. PCA groups are indicated by brackets.

**Bacteria and Archaea Detected**

Grouped according to response to HRC during chromate remediation

**Group 1 organisms decline**
- Pseudomonas, Burkholderia (Denitrifiers)
- Acidithiobacillus, Thiothrix (Sulfur oxidizers)
- Leptothrix (Iron oxidizer)

**Group 2 organisms increase then decline**
- Acidovorax, Thauera (denitrifiers)
- Flavobacteria (aerobes, use glycerol)

**Group 3 organisms decline then return**
- Mainly oligotrophic bacteria

**Group 4 organisms increase in late stages**
- Legionella, Chlamydophila, Flectobacillus.

**High Density Microarray Analysis**

Hierarchical clustering of prokaryotes detected by 16S GeneChip.
Euryarchaeota
(Methanogens)

Injection well

Monitoring well

Days post HRC Injection

Days post HRC Injection

Comment – methanogenic conditions 312 days after single HRC injection
**Bacteroidetes**
*(Flavobacteriaceae)*

### Injection well

- **Intensity (a.u.)**
  - 0
  - 1000
  - 2000
  - 3000
- **Days post HRC Injection**
  - 9
  - 17
  - 216
  - 312
- **Comment** - Initial enrichment of Flavobacteria but declining over time

### Monitoring well

- **Intensity (a.u.)**
  - 0
  - 1000
  - 2000
  - 3000
- **Days post HRC Injection**
  - 9
  - 17
  - 216
  - 312
- **Comment** - Initial enrichment of Flavobacteria but declining over time

Do not use lactate – but use glycerol – hence no $^{13}$C detected in their PLFAs
Deltaproteobacteria
(Desulfovibrionaceae)

Comment – continuous presence of Desulfovibrio – may help maintain chromium reducing conditions by producing H$_2$S - observed
Deltaproteobacteria (Geobacteraceae)

**Injection well**

**Monitoring well**

Comment – continuous presence of Geobacter – may help maintain chromium reducing conditions by producing Fe(II) – observed also
**13C Phospholipid Analysis**

- General bacterial biomarkers indicate rapid enrichment in 13C.
- $^{13}$C ratio is greater than expected (overall spiked HRC ratio was 15 per mil).
  - $^{13}$C polylactate used as spike is not esterified to glycerol backbone.
  - It is released and consumed more rapidly.
- Biomarkers for *Flavobacteriaceae* increased following injection but showed minimal enrichment with $^{13}$C.
  - *Flavobacteria* do NOT typically utilize lactate, but may use glycerol (backbone, unlabeled).
Major Findings to Date

- Despite low initial microbial densities (<10^5 cells g^-1), HRC injection in the groundwater stimulated increase in the biomass up to 10^7 - 10^8 cells ml^-1.
- Highly reducing conditions were achieved quickly with hierarchical depletion of electron acceptors O, NO_3, and Fe (III) (SO_4 was reduced but never depleted except transiently months later), sulfate reduction has been sustained to for the last 20 months.
- SIP analysis confirmed microbial metabolism of HRC and PLFA indicated which group of organisms was utilizing the electron donor.
- Geophysical measurements were capable of characterizing hydrogeological conditions and monitoring the HRC distribution in groundwater.
- Biostimulation has not yet had an effect on subsurface flow.
- Cr(VI) was reduced to drinking water standards after increases in Fe(II), and has remained low for the last 20 months.
- Microbial community structure changes indicate dominance by sulfate reducers and iron reducers that are apparently maintaining Fe(II) and Cr(VI) reduction.
Future Research

- Metagenome Sequence by JGI
- Metagenome (large Insert and small insert clone libraries using MDA) by Diversa
- Isolation and sequencing of Desulfovibrio strains by JGI in the Lab Sequencing Program
- Mass transfer between high and low permeability zones
- Changes in hydraulic properties of sediments after HRC injection
- Evaluation of the potential for Cr(III) reoxidation
- Development of a numerical code TOUGH Bio-React
- Monitoring and new field tests (2 new wells in May).
Dr. Terry C. Hazen  tchazen@lbl.gov
Hanford Project  http://esd.lbl.gov/ERT/hanford100h/
ERSP  http://www.lbl.gov/ERSP
Hazen Lab  http://www-esd.lbl.gov/ECO/Hazenlab/index.htm
Ecology Department  http://www-esd.lbl.gov/ECO
Center for Environmental Biotechnology  http://www-esd.lbl.gov/CEB
Virtual Institute for Microbial Stress and Survival  http://vimss.lbl.gov