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

Overall 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 100H site

Integrated Approach

Field Measurements
- Hydrogeology
- Geophysics
- Microbiology

Lab Measurements
- Geochemistry and Isotopic Composition
Critical Biogeochemistry

Chemical Species

Electron Acceptors

Dominant Terminal Electron Accepting Process

Aerobic Respiration | Denitrification | Iron (III) Reduction | Sulfate Reduction | Methanogenesis

Time

Distance from Source

Chemical Species:
- Organics
- SO₄
- NO₃
- Fe (II)
- H₂
- H₂S
- CH₄
- SO₄
- CO₂
- Mn (IV)
- Cr (VI)
- U (VI)
- Fe (III)
- PCE/TCE

Critical Biogeochemistry

Equivalents

pE

0

-10

+10

0
The Cr source is believed to be sodium dichromate (Na$_2$Cr$_2$O$_7$·2H$_2$O)
On-Site Data Collection

Flow cell with Br sensor

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

Sampling port
Br-Injection and Pumping Tests

$K_s = 3.7 - 7.4 \text{ m/day}$

Effective porosity = 0.2-0.26
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

Field HRC Injection Test

Injection at depths of 44 ft to 50 ft

Hanford sandy gravel and gravelly sand

Ringold clay

Ringold silt

Groundwater level

Pumping - 27 days
Well 699-96-44

Water samplers
Post-HRC Injection Changes in Electrical Conductivity

HRC Injection Zone

High $K_{sat}$

2 Days after HRC injection

3 DAYS

Hypothesis: Lactic acid

Hypothesis: Reaction halo due to formation of precipitates

30 DAYS

Groundwater Flow

Pump

CHANGE IN CONDUCTIVITY (mS/m)

2
Results of HRC Biostimulation

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

D. vulgaris (direct fluorescent antibody)
Isotopic Composition of Nitrate Indicates Denitrification

After Kendall and McDonnell, 1998

Biogeochemical Evidence of Microbial Metabolism in Groundwater

Isotopic Composition of Nitrate Indicates Denitrification

Kendall and McDonnell, 1998
Geochemical Evidence of Microbial Metabolism in Groundwater

HRC injection

Pumping stopped

Injection well

Downgradient Monitoring well

Upgradient Monitoring well

Soluble Iron Fe(II) (ppm)

Fe(III) + Microbial reduction → Fe(II)

Increase in Ferrous Concentration after the HRC Injection

Fe(II) + Cr(VI) → Cr(III) Precipitation
Acridine orange direct counts:
Injection well - 45

Sampling date
Aug  Sep  Oct  Nov  Dec  Jan

45.0 ft "yellow"
49.5 ft "black"
52.5 ft "white"
56.5 ft "blue"
Acridine orange direct counts:
Monitoring well - 44
PLFA biomass estimates

PLFA biomass estimates, injection well 45

PLFA biomass estimates, extraction well 44
PLFA: Community structure

44, yellow, community structure

time (days)

mole fraction lipid
PLFA: Selected Biomarkers

well 44, yellow depth

Fraction

day

Pseudomonas
Desulfovibrio sp.
Geobacter

well 44, white depth

Fraction

day

Pseudomonas
Desulfovibrio sp.
Geobacter

well 44, black depth

Fraction

day

Pseudomonas
Desulfovibrio sp.
Geobacter

well 44, blue depth

Fraction

day

Pseudomonas
Desulfovibrio sp.
Geobacter
DOE 16s rDNA microarray

- Rapidly detect the composition and diversity of microbes in an environmental sample
- Massive parallelism - 550,000 probes in a 1.28 cm² array
- All 9,900 species in 16S rDNA database
- Single nucleotide mismatch resolution

MATCH

MisMatch

cctagcatg Cattctgcata
cctagcatg Gattctgcata
Microarray analysis of bacterial community changes during Cr(VI) remediation at Hanford 100H site:

Dynamics of some significant organisms.
16S Microarray is Quantitative:
when comparing same OTU over time/treatment

~10^7 16S gene copies

~10^{11} 16S gene copies
16S rDNA GeneChip

- **Archaea**
  - Only Crenarchaeotes (non-thermophilic) detected
    - Dominated by one type – no cultured relative.

- **Bacteria**
  - Initial enrichment of denitrifiers
    - *Fulvimonas, Pseudomonas, Hyphomicrobium, Acidovorax, Aquaspirillum, Thauera, Azoarcus, Comamonas, Dechloromonas, Clostridium*.
  - Followed by enrichment of sulfate reducer(s)
13C of Dissolved Inorganic Carbon is Byproduct of HRC Metabolism

Possible CO$_2$ production

Biogeochemical Evidence of Microbial Metabolism in Groundwater

$\delta^{13}$C of HRC

$\delta^{13}$C of background water

$\delta^{13}$C of Dissolved Inorganic Carbon is Byproduct of HRC Metabolism
Changes in Cr(VI) Concentrations after the HRC Injection

- HRC injection
- Pumping stopped
- Upgradient Monitoring well
- Downgradient Monitoring well
- Injection well

Cr(VI) concentration over time:
- Injection well
- Upgradient Monitoring well
- Downgradient Monitoring well

Time: 8/2 to 10/11
Cr(VI) ppm: 1.E-03 to 1.E+00
Conclusions

1. Bacteria in Hanford groundwater is low but includes *Arthrobacter, Oxalobacter, Sporomusa* and *Pseudomonas* species. Under background conditions, the total microbial population is $<10^5$ cells g$^{-1}$.

2. Lactate increases biomass to $>10^8$ cells g$^{-1}$, generates highly reducing conditions, and enhances Cr(VI) removal from the pore solution.

3. Bacteria densities and diversity continued to increase for the first 6 weeks, followed by a decrease in the microbial diversity and density after 3 months.

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, followed by reduction of nitrate to non detect, and finally sulfate reduction. DO and nitrate began to return to background concentrations two months after HRC injection, despite groundwater 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.
Future Research

- 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
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