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

Terry C. Hazen, B. Faybishenko, E. Brodie, D. Joyner, S. E. Borglin, R. Chakraborty, M. Conrad, T. Tokunaga, J. Wan, S. Hubbard, K. Williams, J. Peterson, M. Firestone, G. Andersen, T. DeSantis, P. E. Long, D. R. Newcomer, A. Willett, and S. Koenigsberg

LBNL, UCB, PNNL, Regenesis

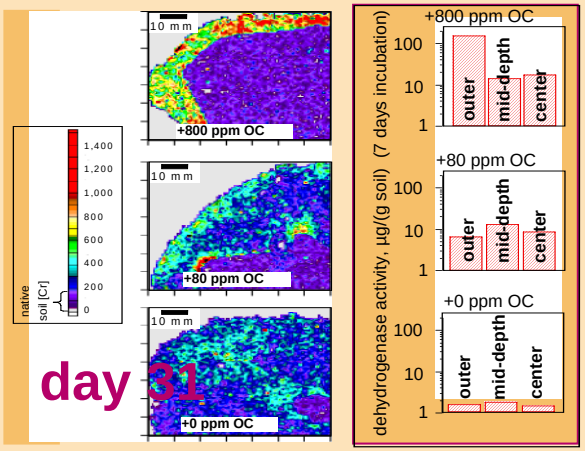
Mesoscale Studies on Cr(VI) Bioreduction that led to Field Studies

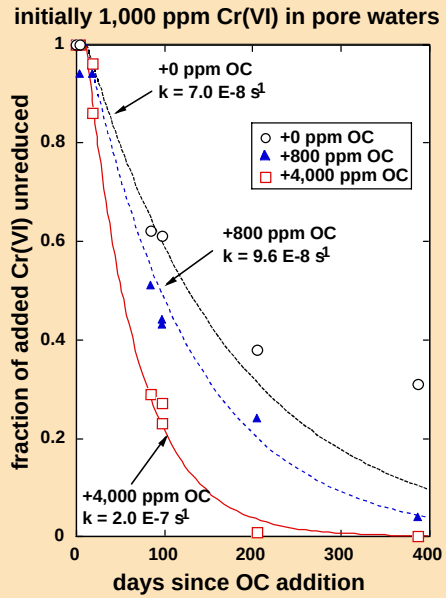
Jiamin Wan, Tetsu Tokunaga, Mary Firestone and Terry Hazen (NABIR supported 1998-2004)

Tokunaga, T. K. J. Wan, M. K. Firestone, T. C. Hazen, K. R. Olson, D. J. Herman, S. R. Sutton, and A. Lanzirotti. 2003. *In-situ* reduction of Cr(VI) in heavily contaminated soils through organic carbon amendment. J. Environ. Qual. 32:1641-1649.

Tokunaga, T. K., J. Wan, T. C. Hazen, E. Schwartz, M. K. Firestone, S. R. Sutton, M. Newville, K. R. Olson, A. Lanzirotti, and W. Rao. 2003. Distribution of chromium contamination and microbial activity in soil aggregates. J. Environ. Qual. 32:541-549.

Tokunaga, T. K., J. Wan, M. K. Firestone, T. C. Hazen, E. Schwartz, S. R. Sutton, and M. Newville. 2001. Chromium diffusion and reduction in soil aggregates. Environmental Science & Technology 35:3169-3174.





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

Fe(II) + **Cr(VI)** → **Cr(III) Precipitation**

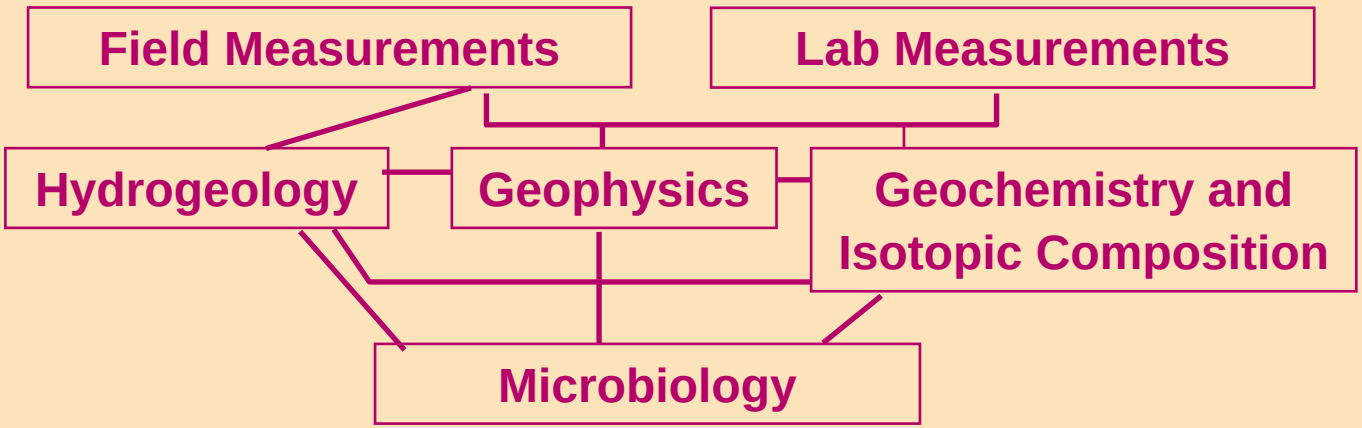
Multidisciplinary Team

Scientific Field	LBNL	PNNL	Regenesis
Microbiology	Terry Hazen, Eoin Brodie, Sharon Borglin, Dominique Joyner, Mary Firestone		
Hydrogeology	Boris Faybishenko, Jiamin Wan, Tetsu Tokunaga	Philip E. Long, Bruce Bjornstad	
Geophysics	Susan Hubbard, Ken Williams, John Peterson,		
Geochemistry	Mark Conrad	Tom Resch, Kirk Cantrell	
Field and technical support	Victor Gruol, Phil Rizzo	Darrell Newcomer	Steve Koenigsberg, Anna Willet, Kevin Lapus

Overall Objective

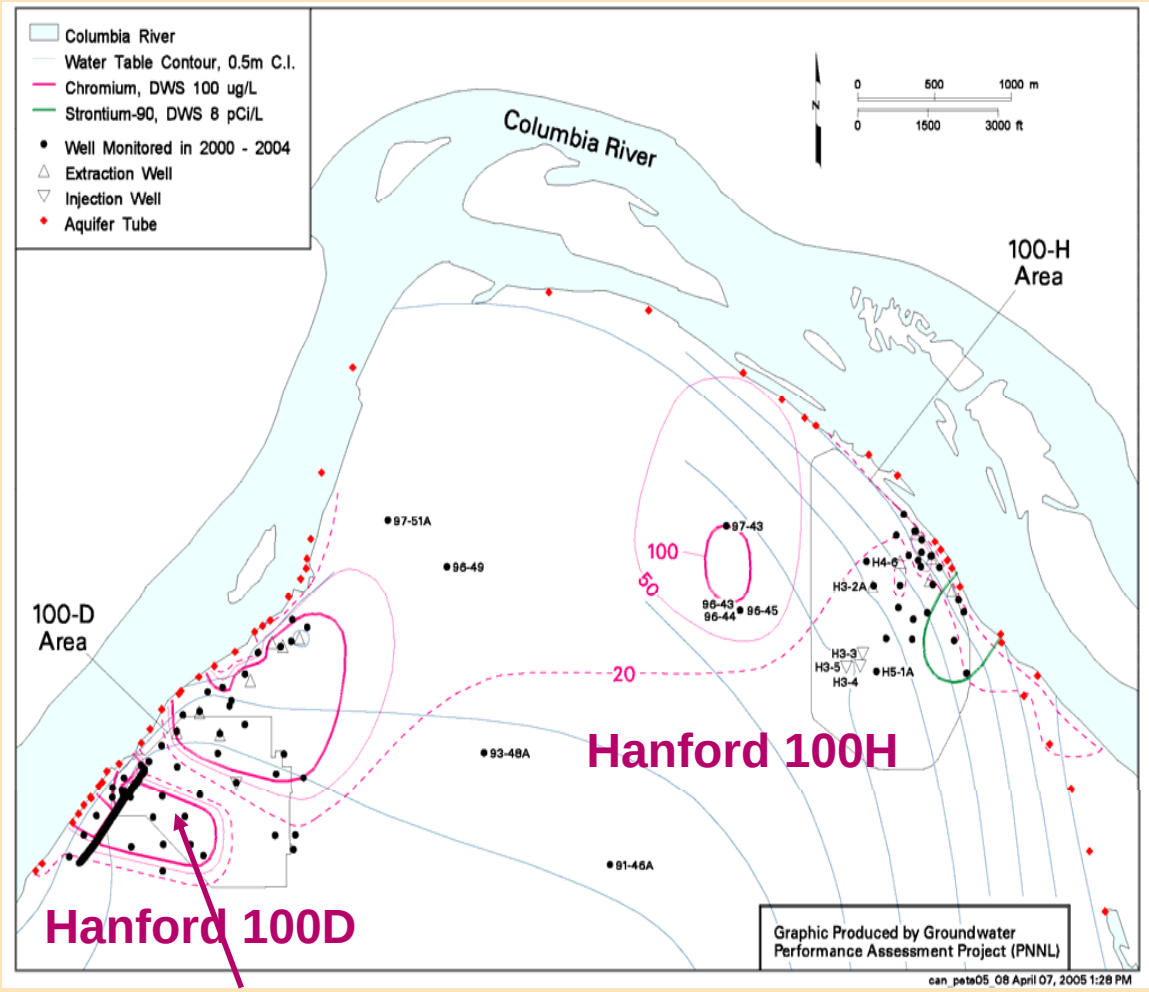
To carry out field investigations to assess the potential for immobilizing Cr(VI) in groundwater using lactate-stimulated bioreduction of Cr(VI) to Cr(III) at the Hanford 100H site, and to determine critical community structure changes and stressors that would enable control and predictions of fundamental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

Integrated Approach



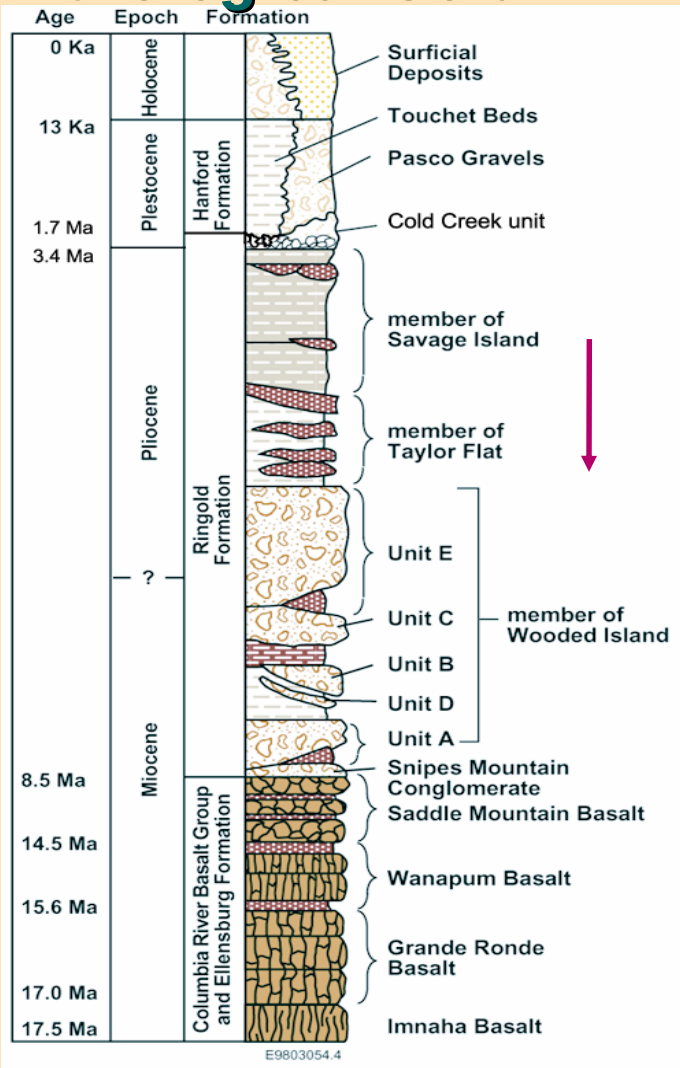
Hanford 100H Site Characterization

Cr Concentration Map



The Cr source is believed to be sodium dichromate ($Na_2Cr_20_7.2H_2O$)

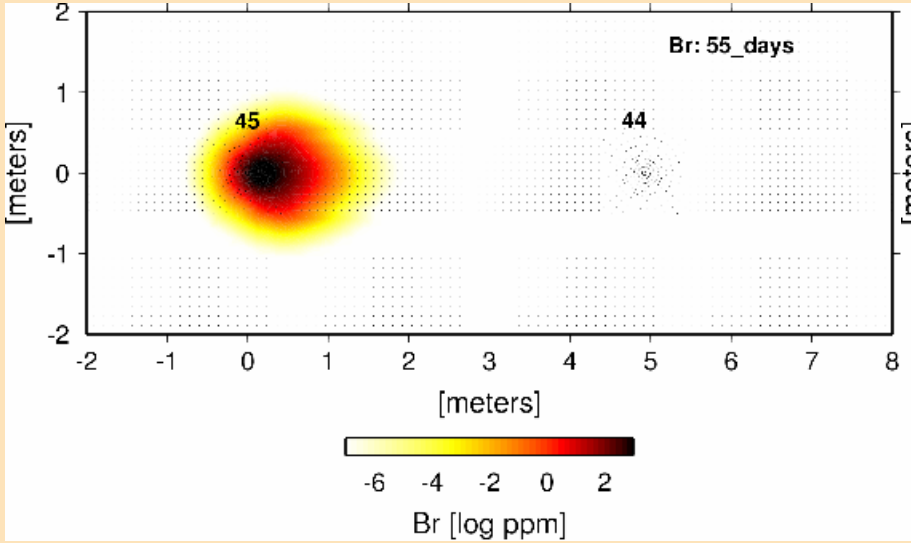
Lithological Column



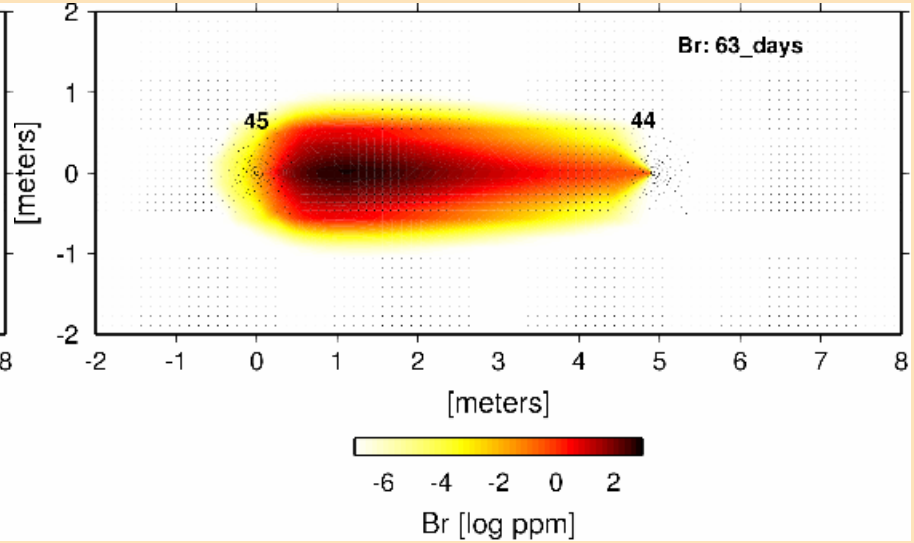
http://esd.lbl.gov/ERT/hanford100h/

LiBr Injection (2/27/2004)

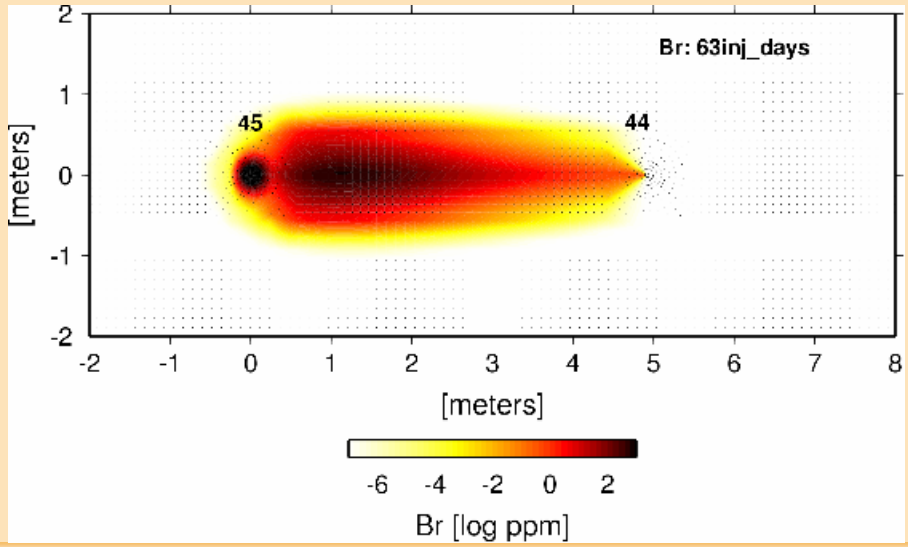
55 Days No pumping



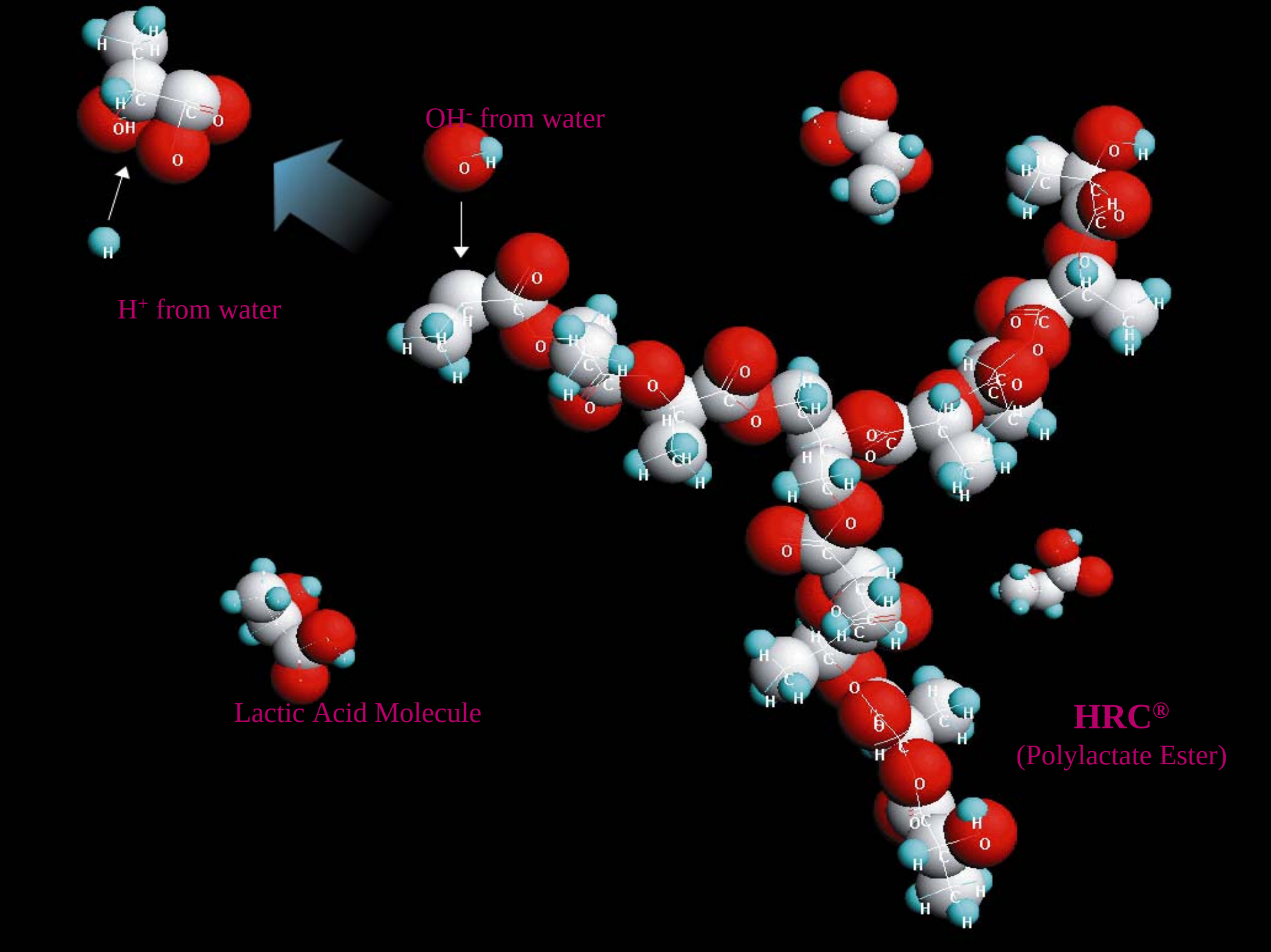
63 Days Pumping



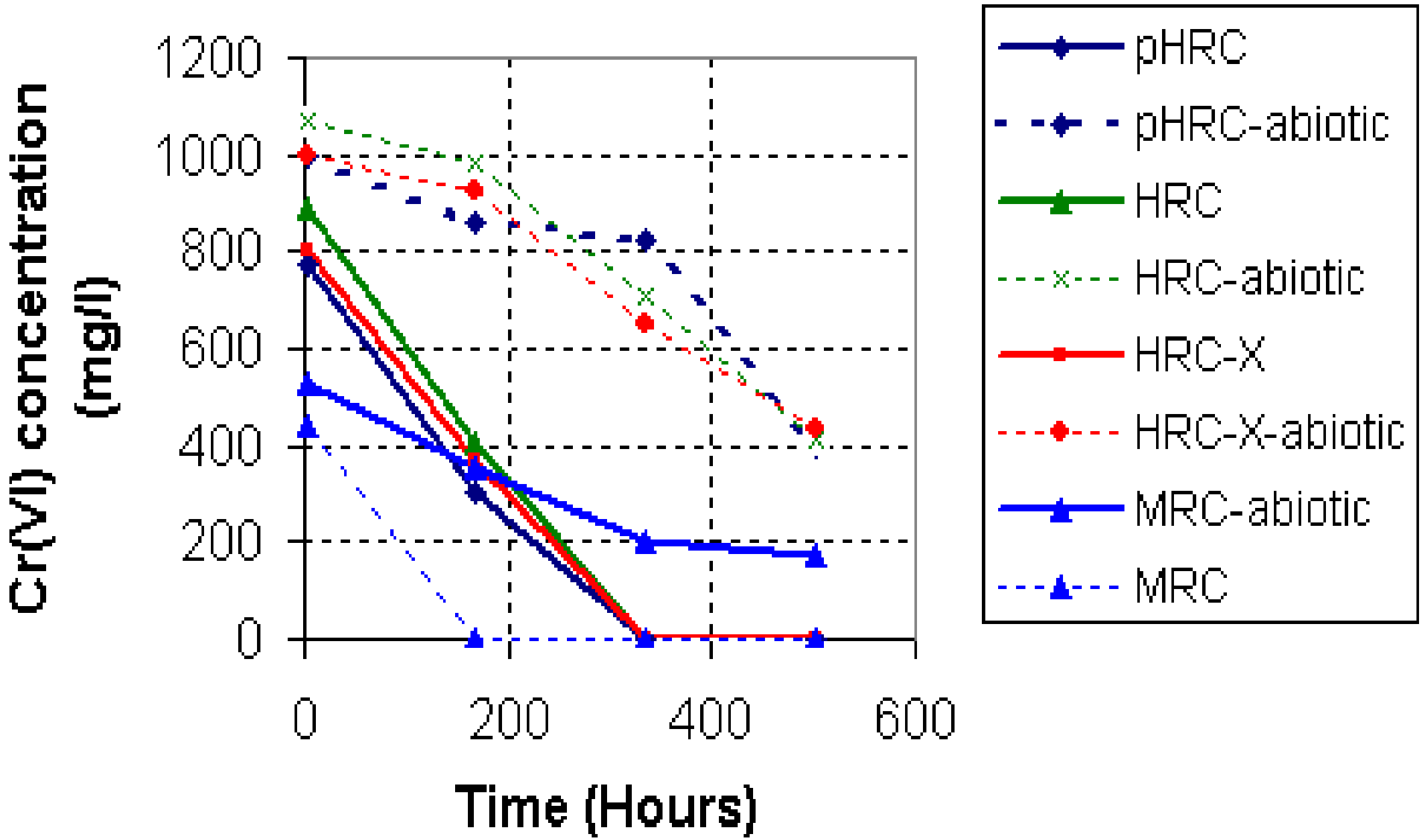
63 Days Pumping + 2nd Injection



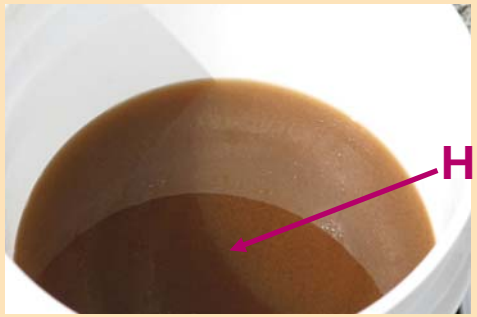
- **LiBr Injection: 15.3 g/liter, 12 liters over 2 hours**


OH^- from water
H^+ from water
Lactic Acid Molecule
HRC®
(Polylactate Ester)

Lactate-Induced Bioreduction of Cr(IV)

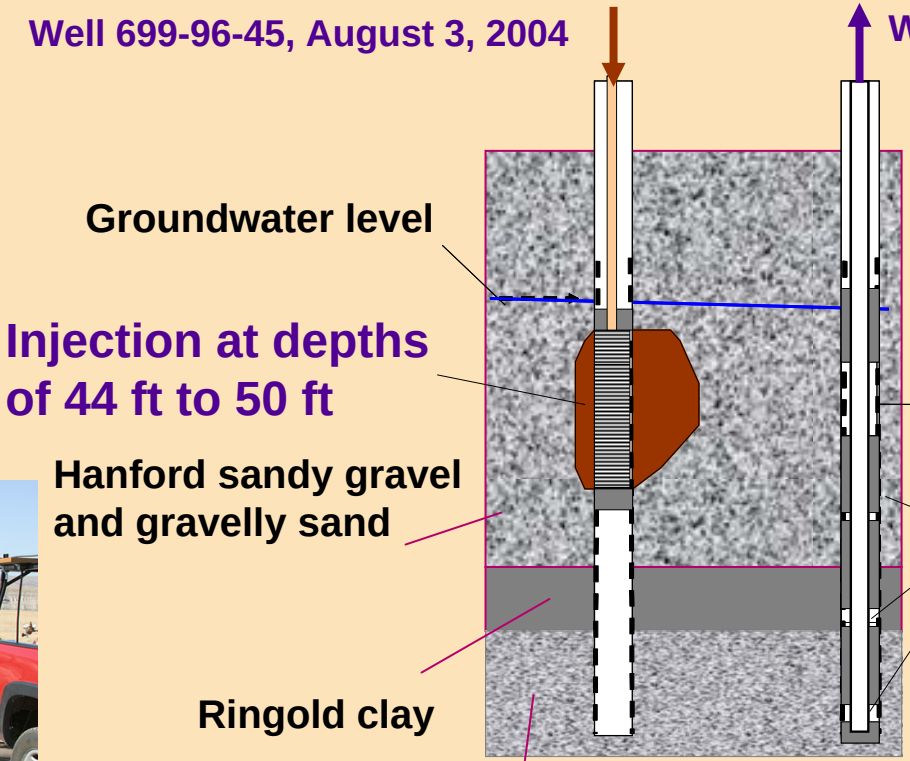


Field HRC Injection Test



Injection of 40 lbs of ^{13}C-labeled HRC
Well 699-96-45, August 3, 2004

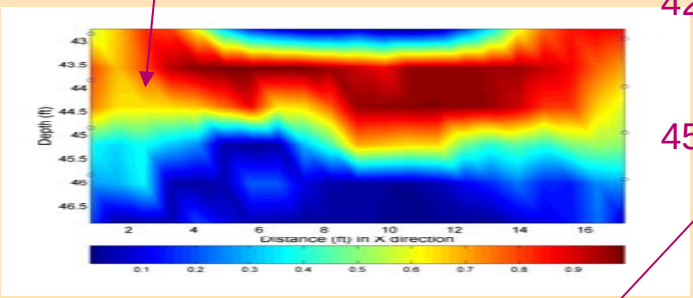
Pumping - 27 days
Well 699-96-44



Post-HRC Injection Changes in Electrical Conductivity

HRC Injection Zone

High K_{sat}



Hypothesis: Lactic acid

Hypothesis: Reaction halo due to formation of precipitates

42’
45’

Pump

2 Days after HRC injection

42’
45’

3 DAYS

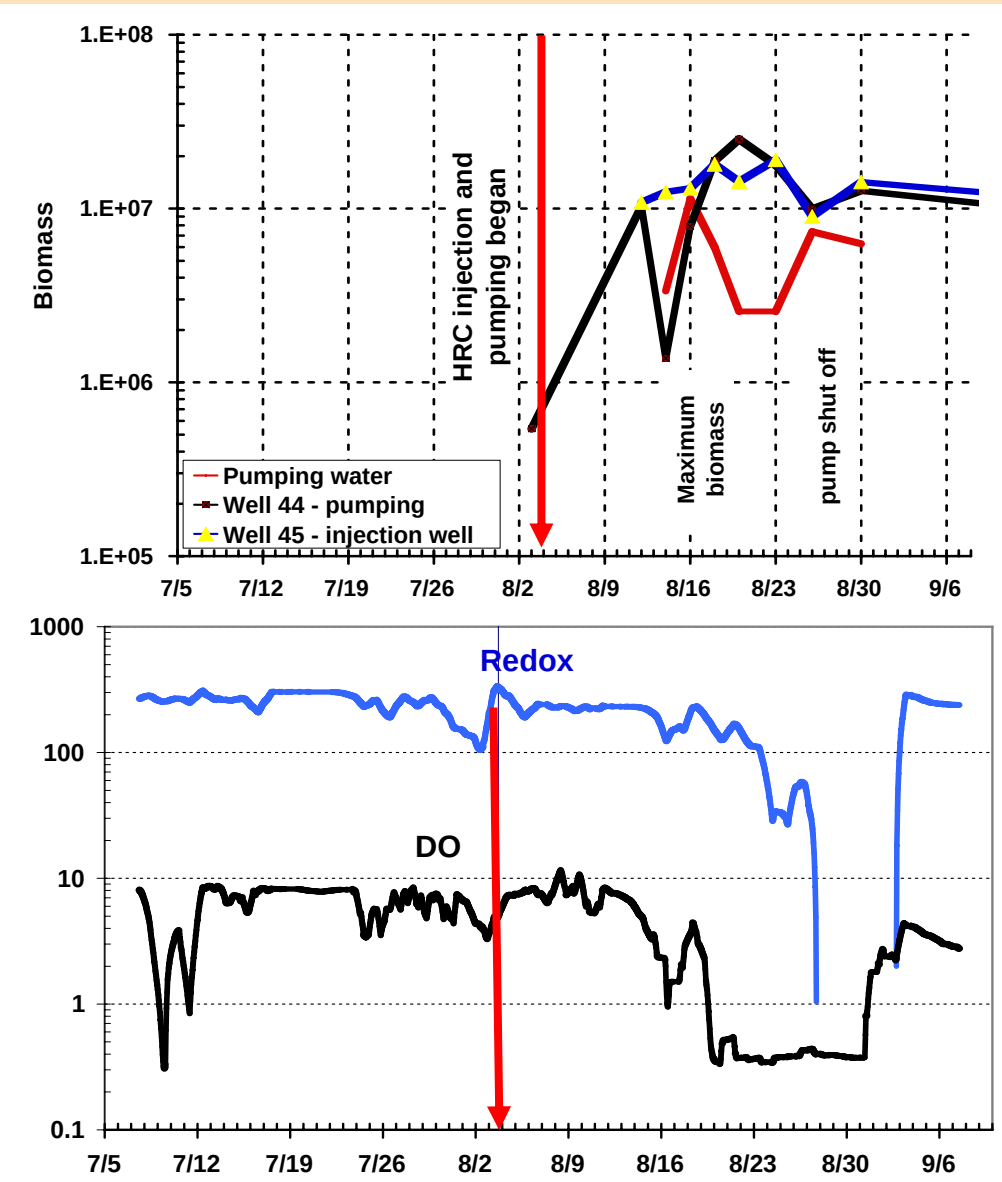
42’
45’

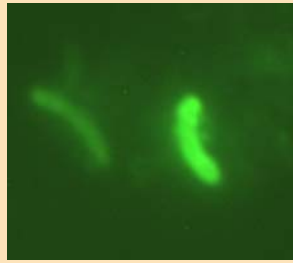
30 DAYS

Groundwater Flow

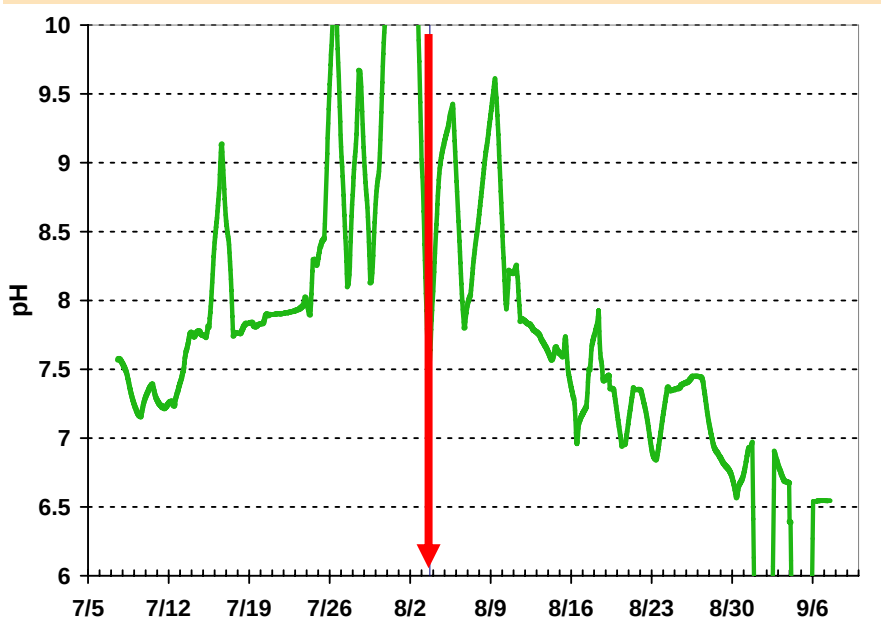
-2 -1 0 1 2
CHANGE IN CONDUCTIVITY (mS/m)

Results of HRC Biostimulation



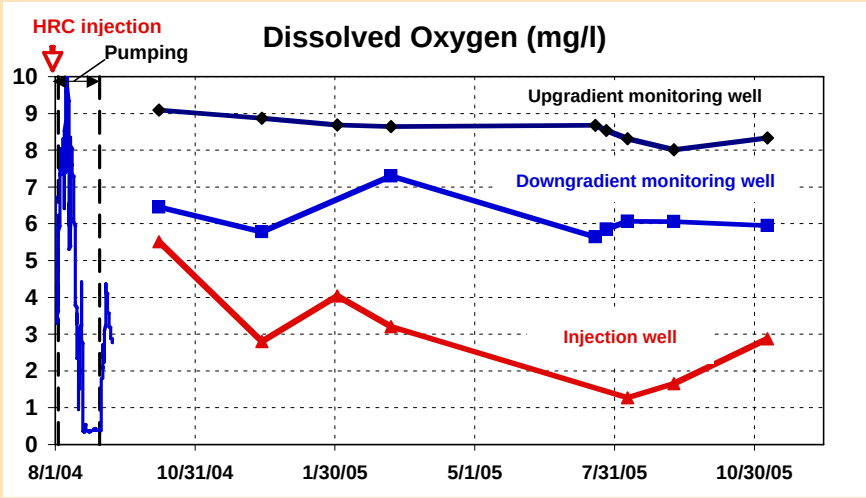


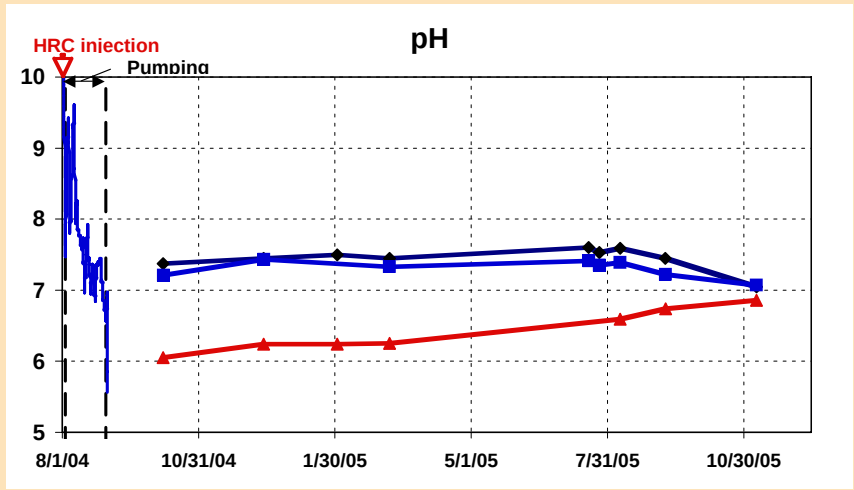
D. vulgaris **(direct fluorescent antibody)**

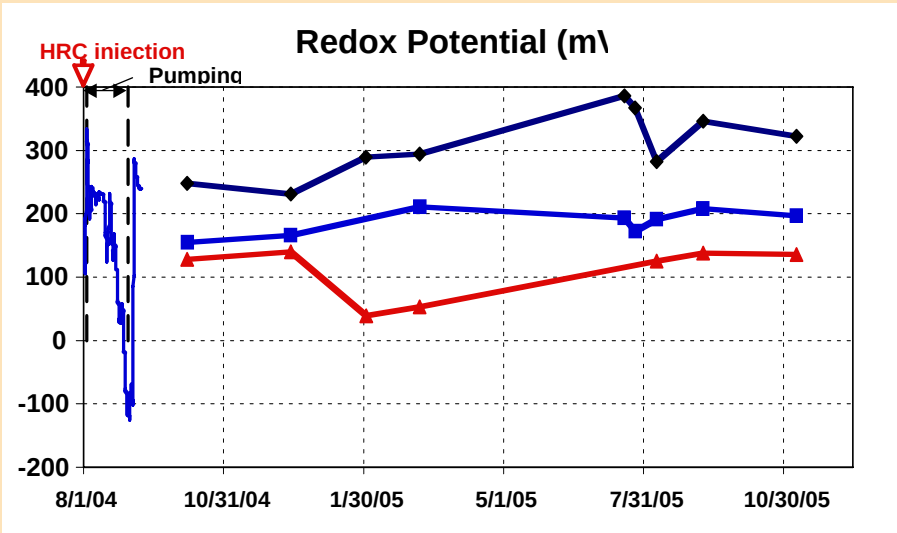


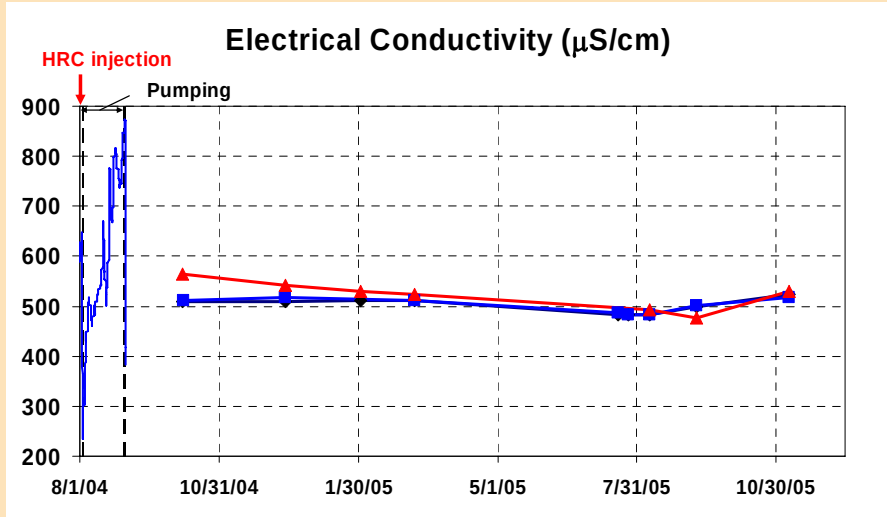
Redox dropped from 240 to -130 mV

DO dropped from 9 mg/l (~100%) to 0.35 mg/l (4.5%)


HRC injection
Pumping
Dissolved Oxygen (mg/l)
Upgradient monitoring well
Downgradient monitoring well
Injection well
10
9
8
7
6
5
4
3
2
1
0
8/1/04
10/31/04
1/30/05
5/1/05
7/31/05
10/30/05

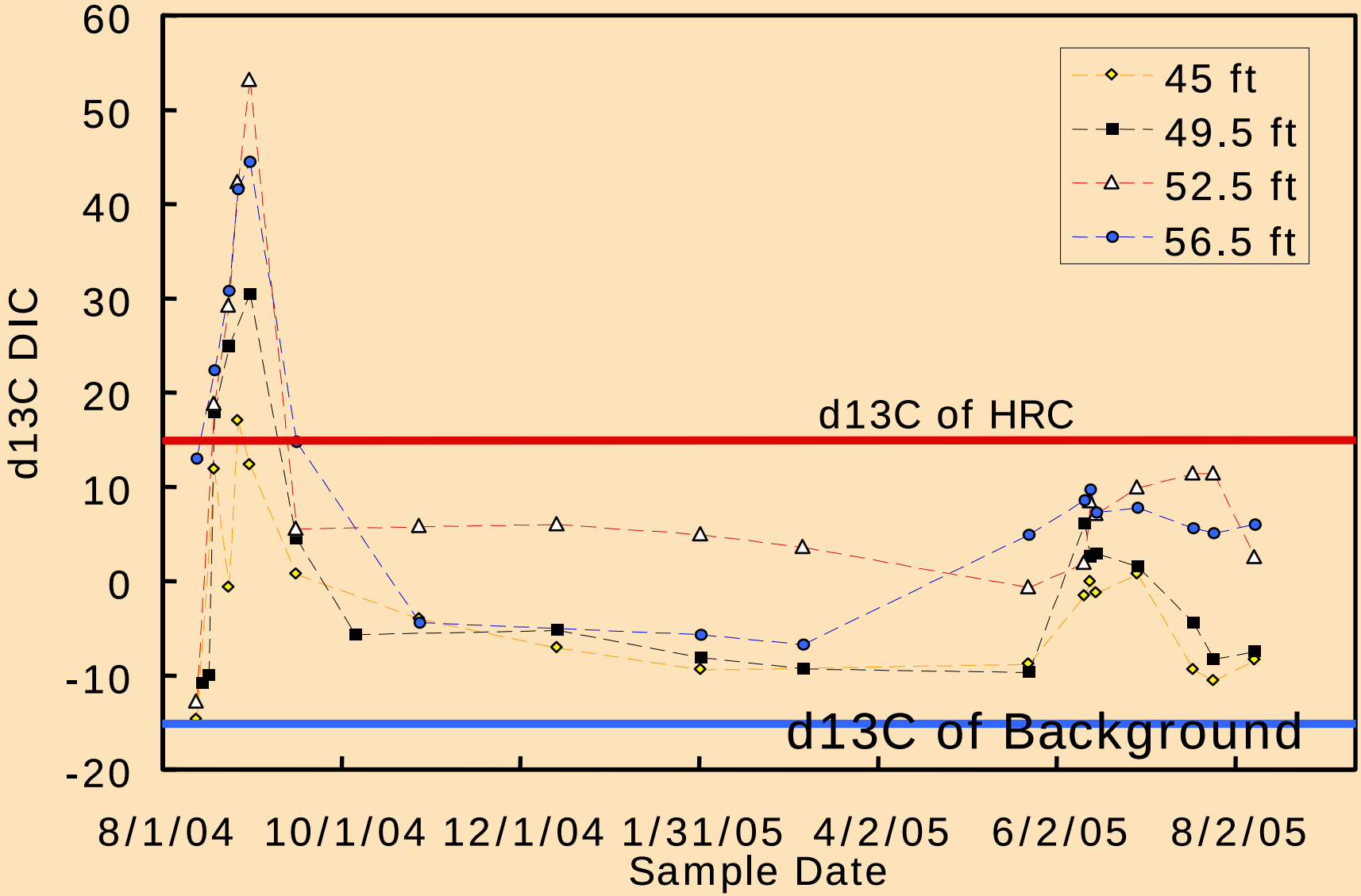

HRC injection
Pumping
pH
10
9
8
7
6
5
8/1/04
10/31/04
1/30/05
5/1/05
7/31/05
10/30/05


HRC injection
Pumping
Redox Potential (m\
400
300
200
100
0
-100
-200
8/1/04
10/31/04
1/30/05
5/1/05
7/31/05
10/30/05


HRC injection
Pumping
Electrical Conductivity (µS/cm)
900
800
700
600
500
400
300
200
8/1/04
10/31/04
1/30/05
5/1/05
7/31/05
10/30/05

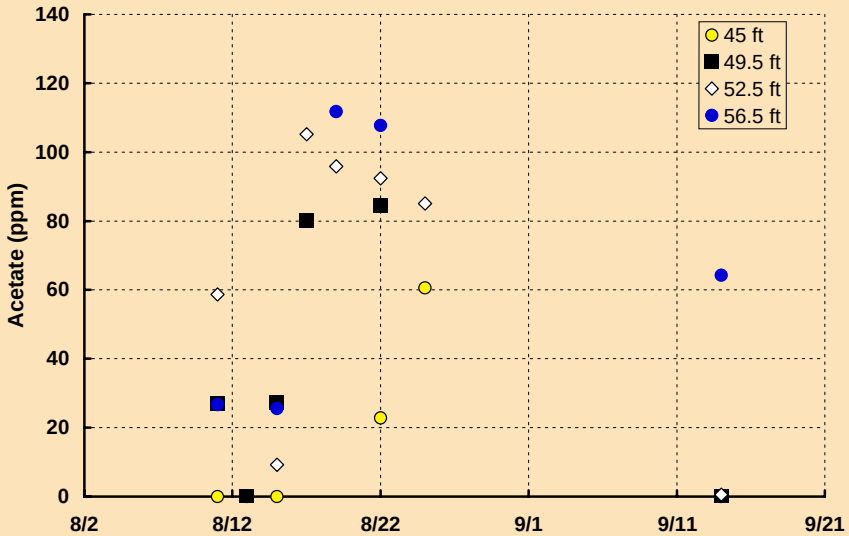
Biogeochemical Evidence of Microbial Metabolism in Groundwater

$d^{13}C$ of Dissolved Inorganic Carbon is Byproduct of HRC Metabolism

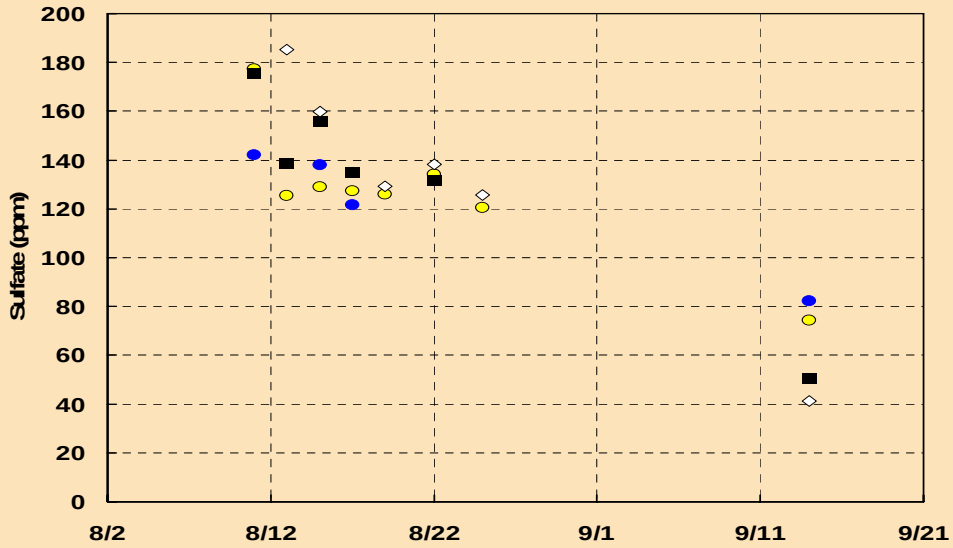


Biogeochemical Evidence of Microbial Metabolism in Groundwater

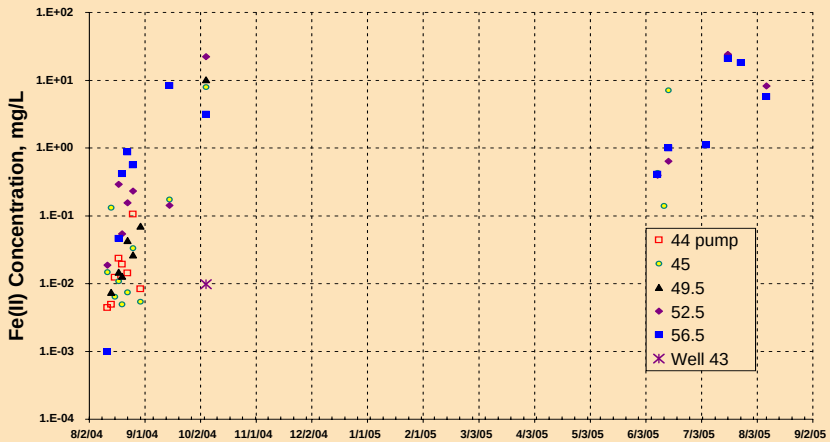
Acetate Increase



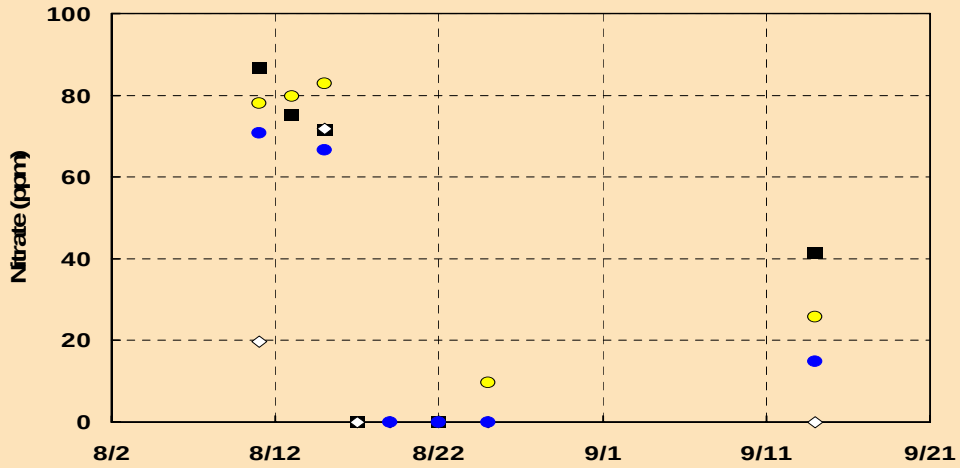
Sulfate reduction



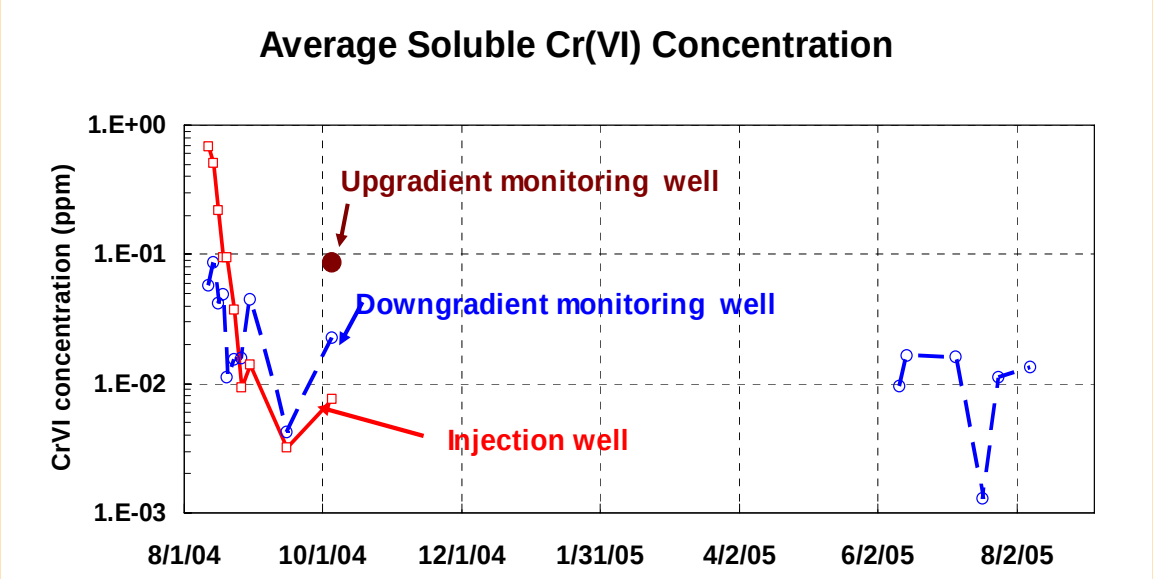
Fe(II) Increase

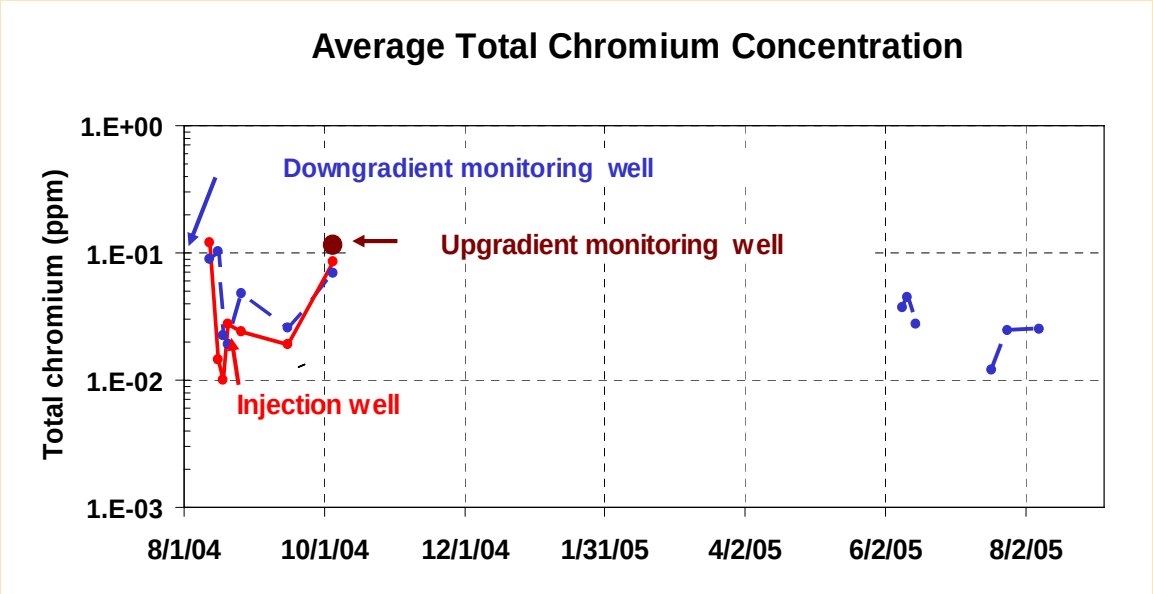


Nitrate decrease



Changes of Cr(VI) Concentration in Groundwater after HRC Injection





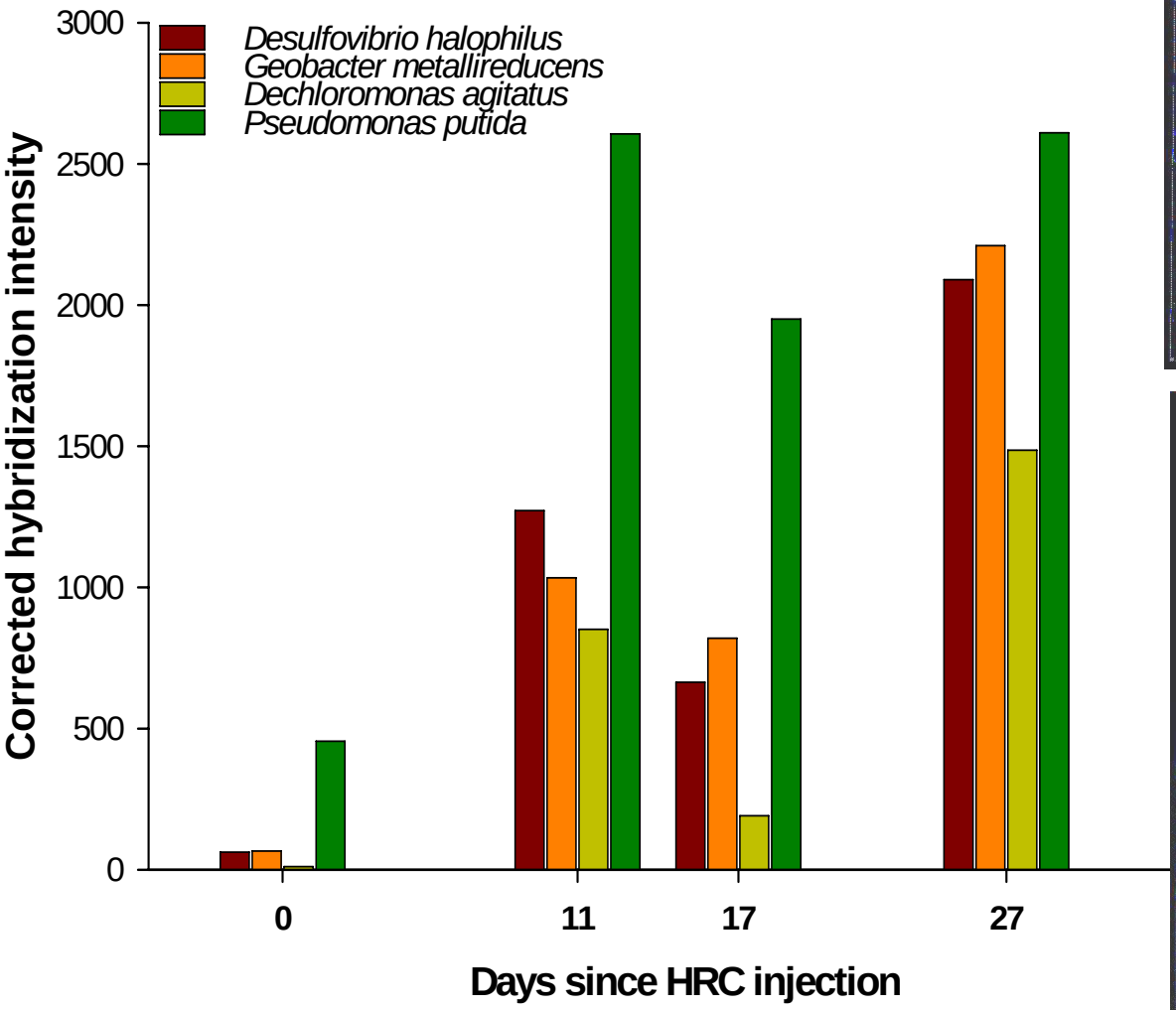
Combined High Density Microarray Analysis and ^{13}C Phospholipid Analysis

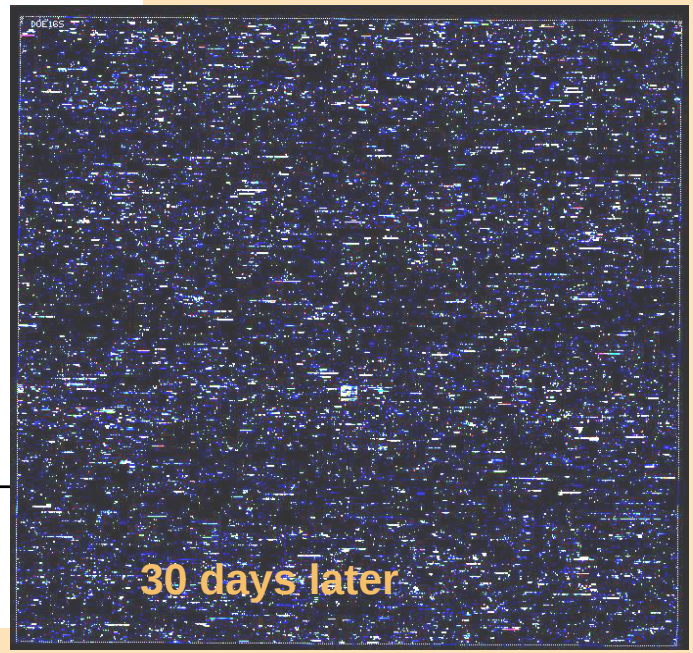
Approach

- 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

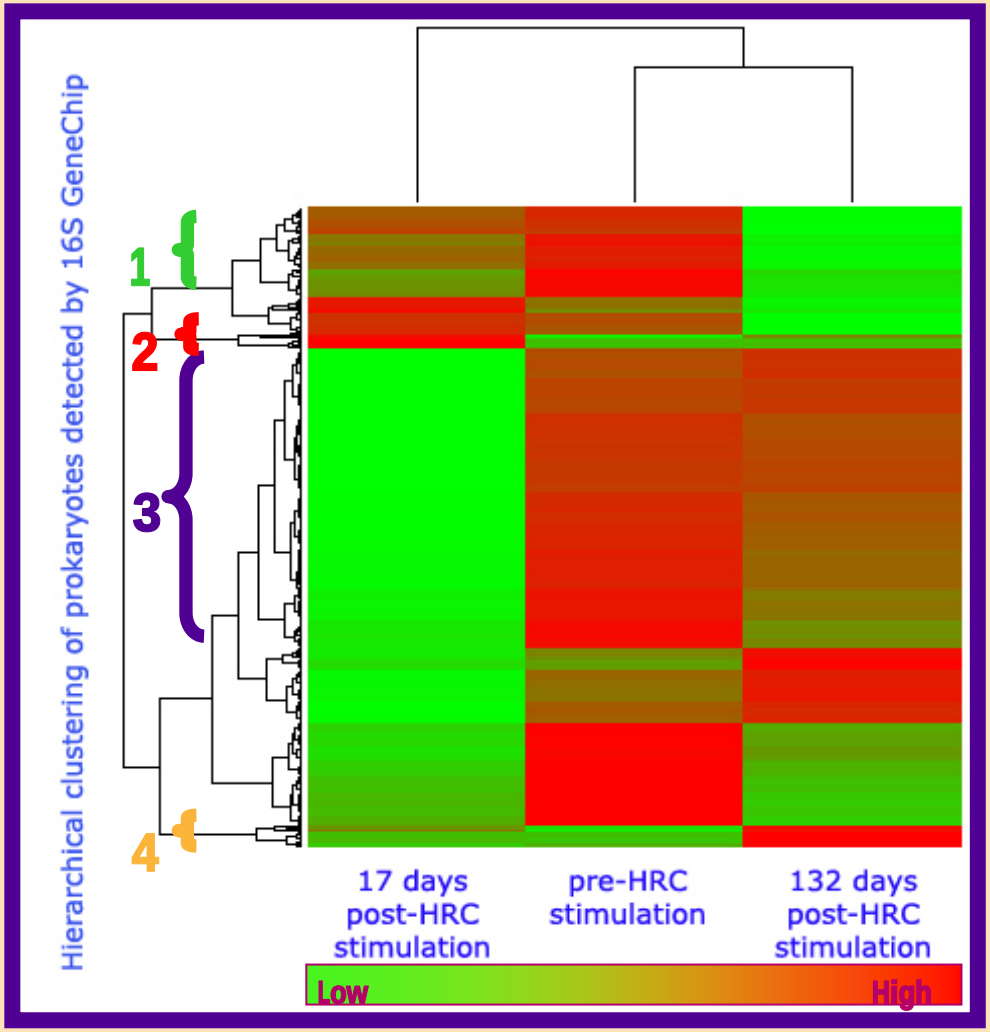
Microarray analysis of bacterial community changes during Cr(VI) remediation at Hanford 100H site:

Dynamics of some significant organisms.





High Density Microarray Analysis



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

Group1 organisms decline
Pseudomonas, Burkholderia (Denitrifiers)
Acidithiobacillus, Thiothrix (Sulfur oxidizers)
Leptothrix (Iron oxidizer)

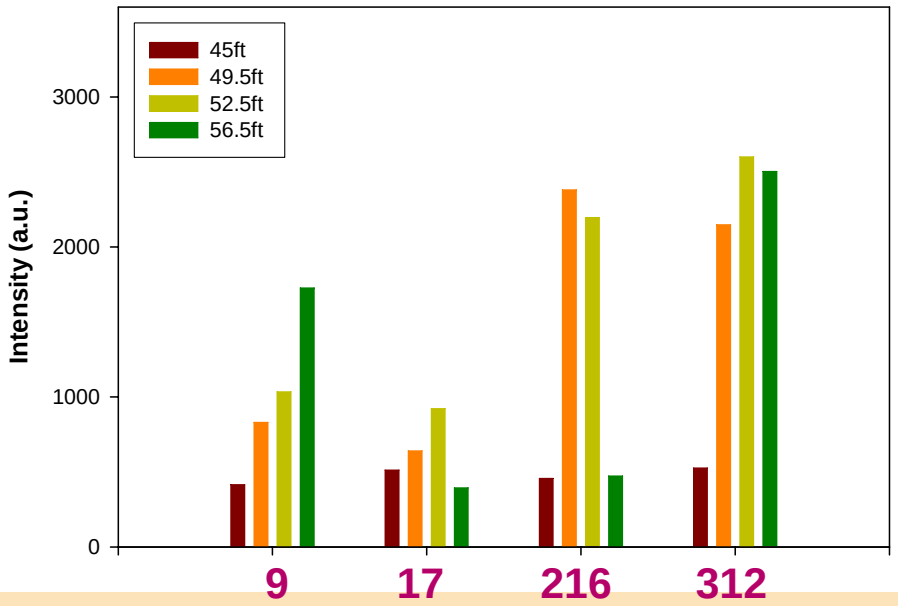
Group2 organisms increase then decline
Acidovorax, Thauera (denitrifiers)
Flavobacteria (aerobes, use glycerol)

Group3 organisms decline then return
Mainly oligotrophic bacteria

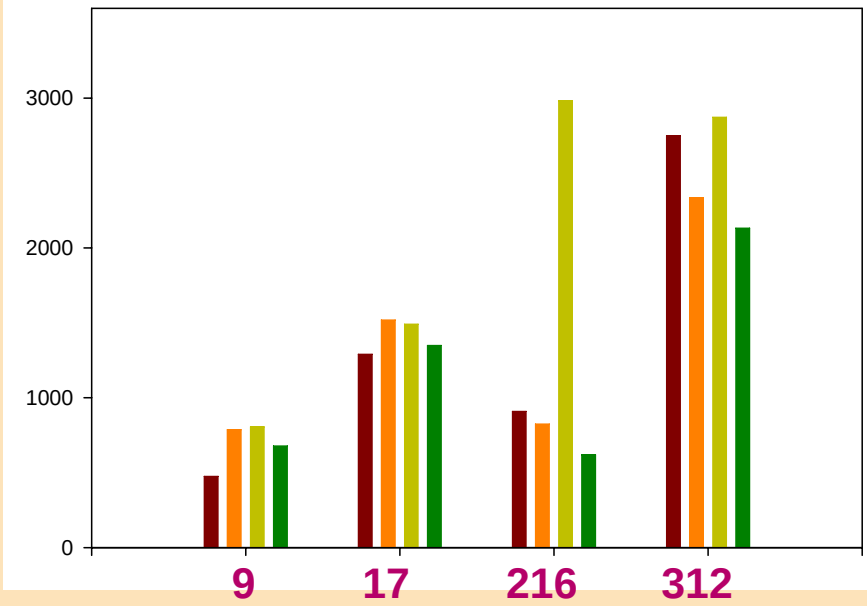
Group 4 organisms increase in late stages
Legionella, Chlamydophila, Flectobacillus.

Euryarchaeota (Methanogens)

Injection well



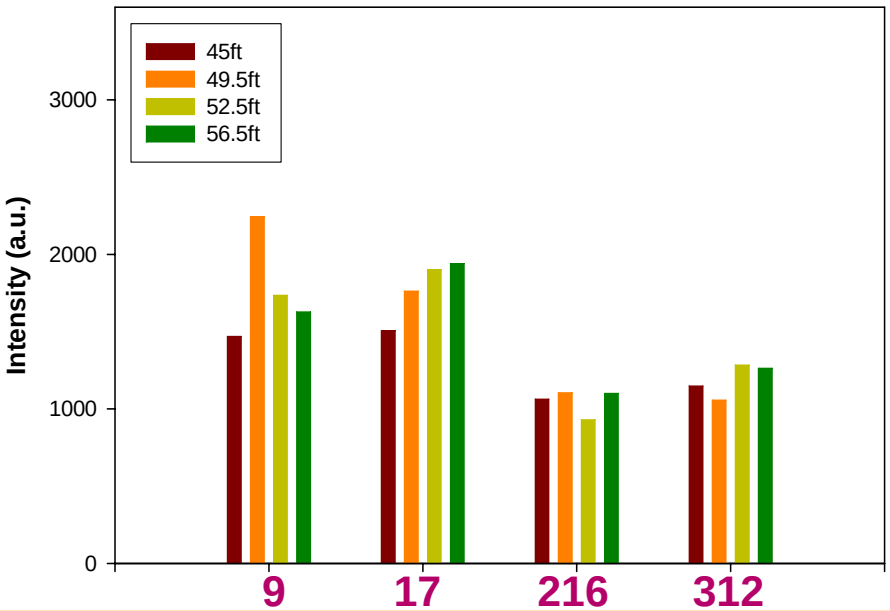
Monitoring well



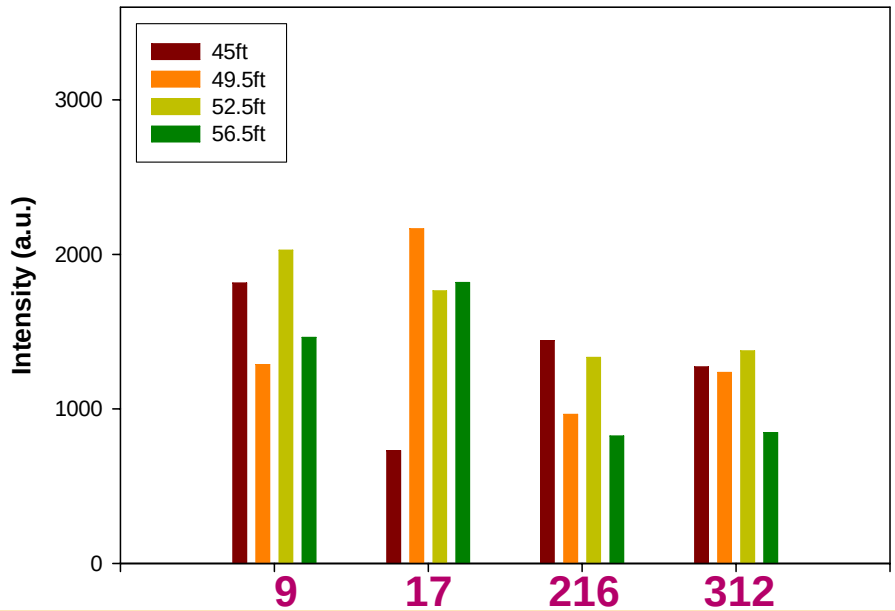
Comment – methanogenic conditions 312 days after single HRC injection

Bacteroidetes (*Flavobacteriaceae*)

Injection well



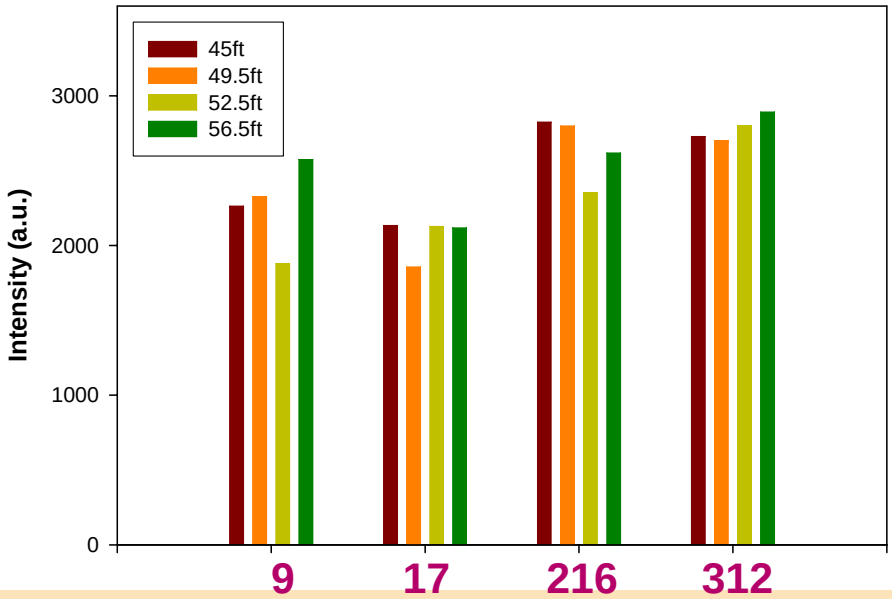
Monitoring well



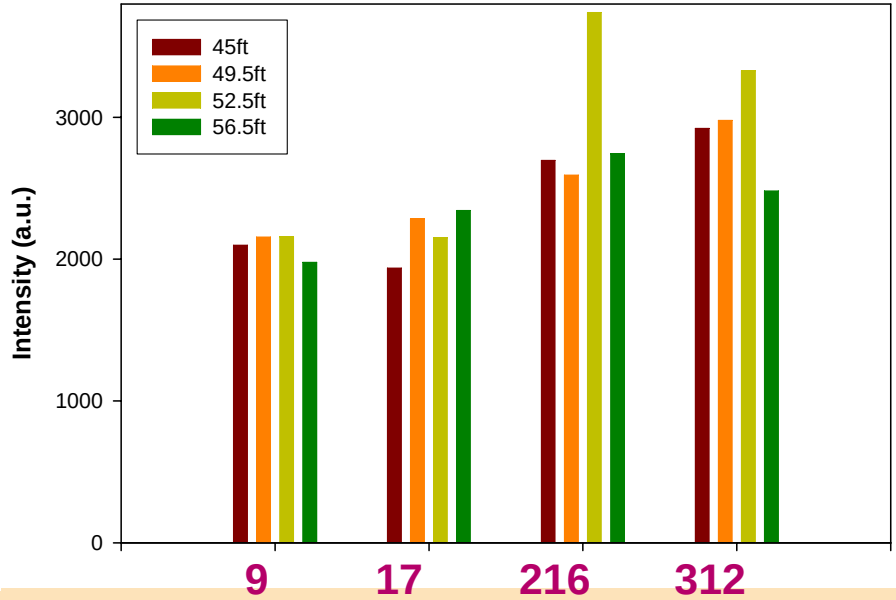
Comment - Initial enrichment of Flavobacteria but declining over time
Do not use lactate – but use glycerol – hence no ^{13}C detected in their PLFAs

Deltaproteobacteria (*Desulfovibrionaceae*)

Injection well

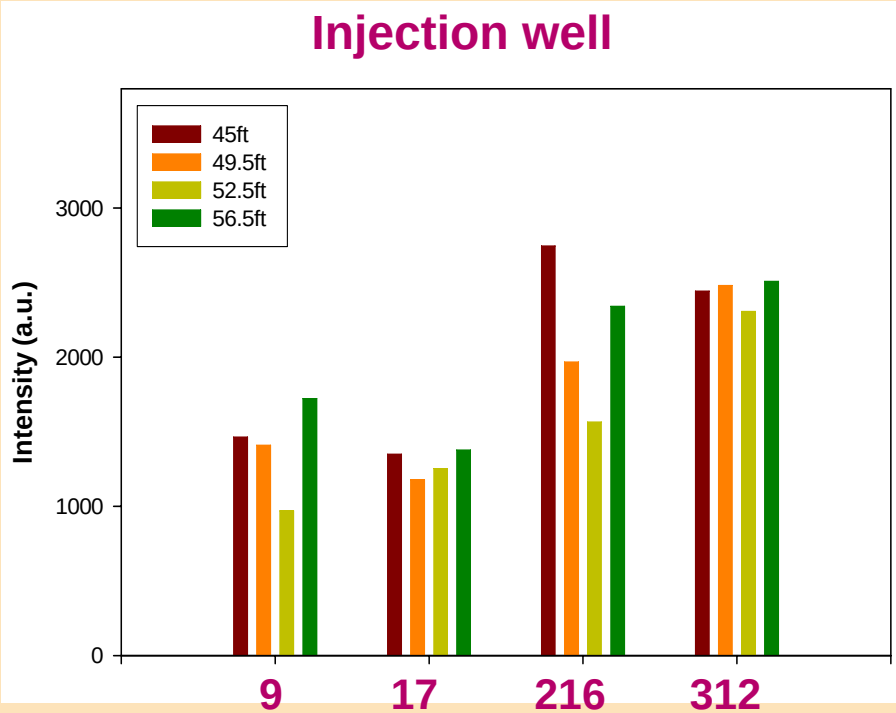


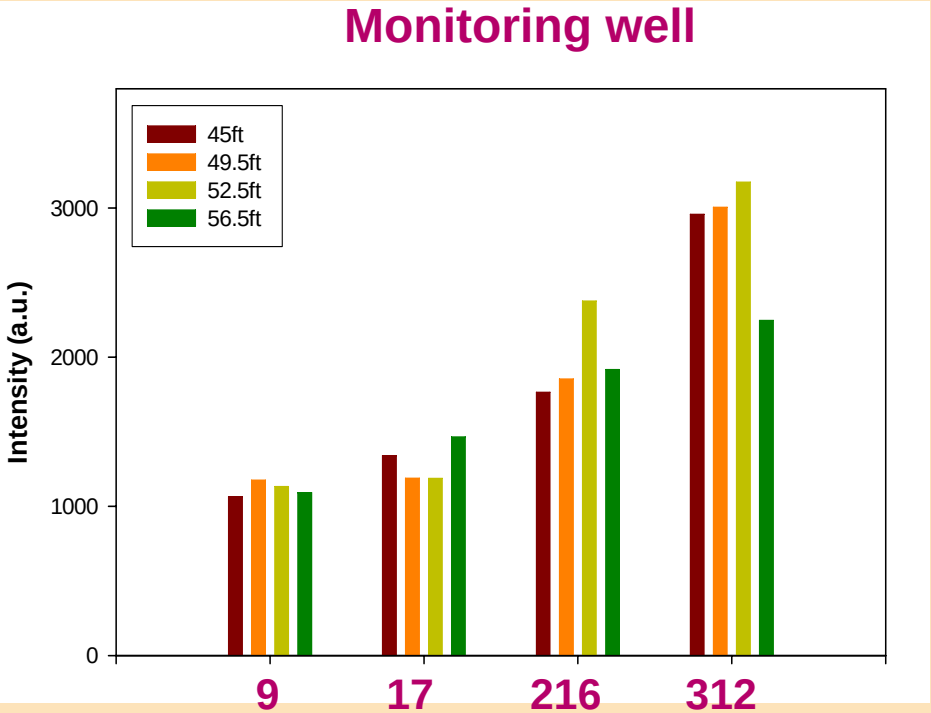
Monitoring well



Comment – continuous presence of Desulfovibrio –
may help maintain chromium reducing conditions by producing H_2S - observed

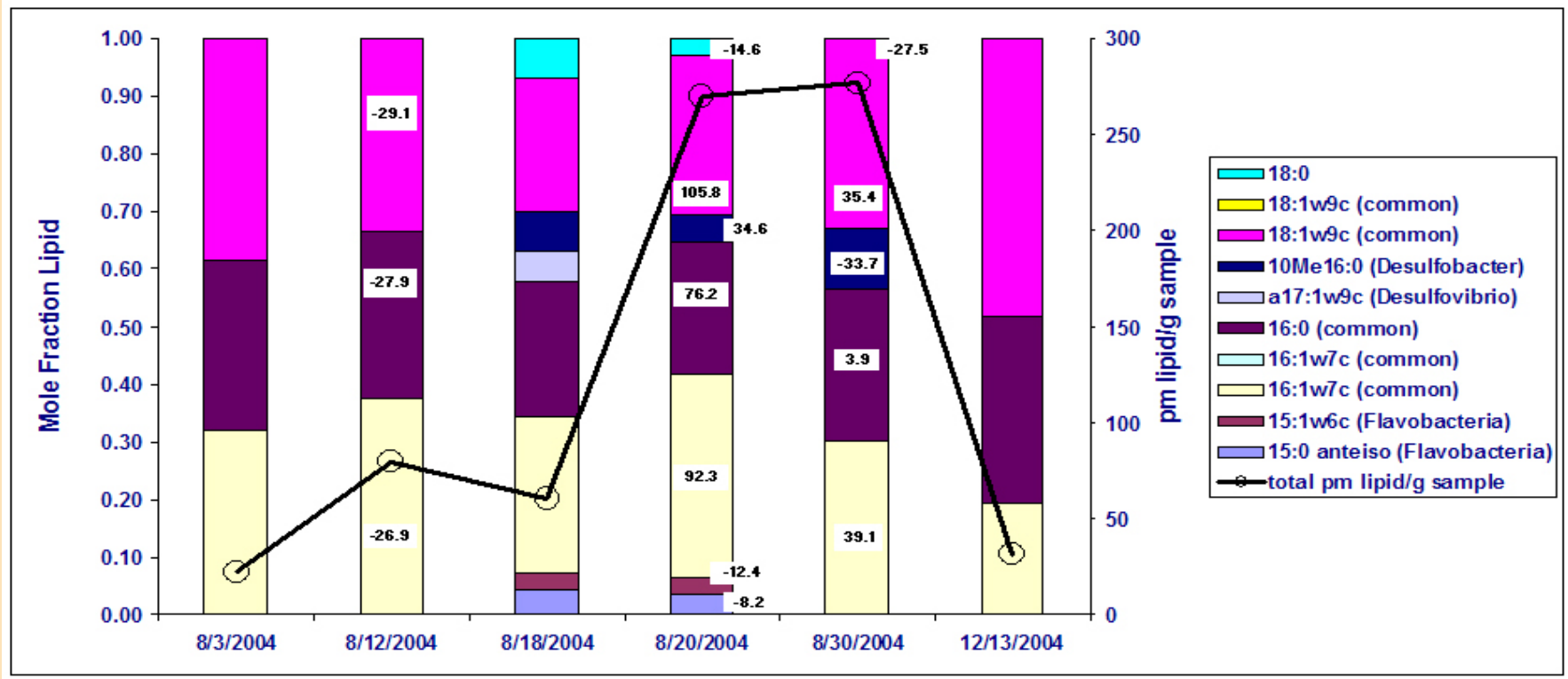
Deltaproteobacteria (*Geobacteraceae*)





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

^{13}C Phospholipid Analysis



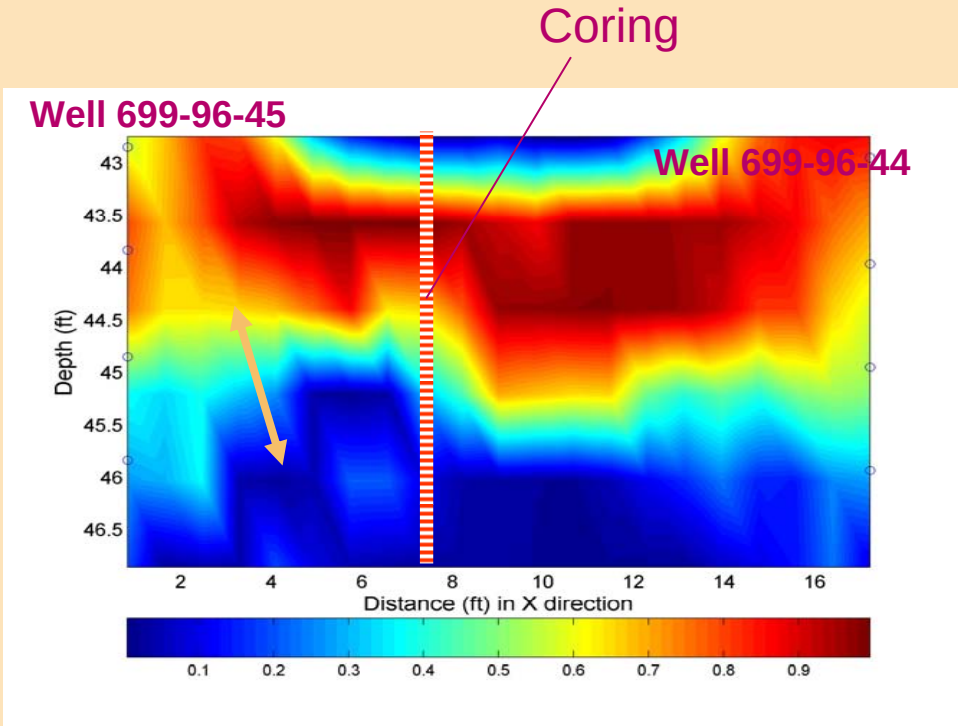
- General bacterial biomarkers indicate rapid enrichment in ^{13}C
- ^{13}C ratio is greater than expected (overall spiked HRC ratio was 15 per mil)
  - ^{13}C polylactate used as spike it is not esterified to glycerol backbone
  - it is released and consumed more rapidly
- Biomarkers for *Flavobacteriaceae* 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).



Contacts and Publications

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