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

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LBNL, UCB, PNNL, Regenesis

Earth Sciences Division, Ecology Department

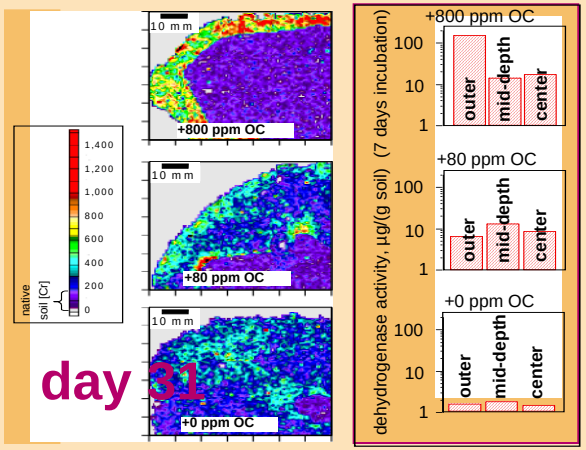
Mesoscale Studies on Cr(VI) Bioreduction that led to Field Studies

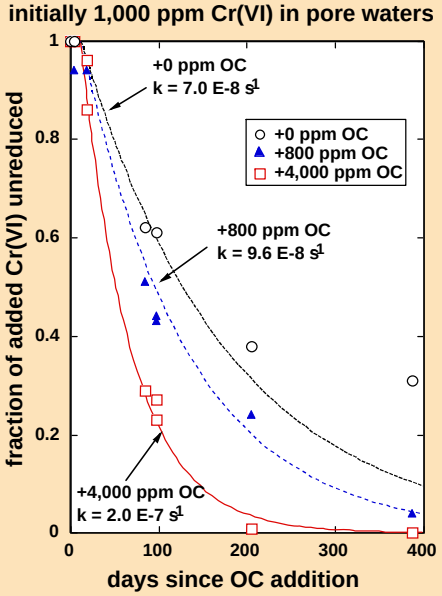
Jiamin Wan, Tetsu Tokunaga, Mary Firestone and Terry Hazen (NABIR supported 1998-2004)

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**

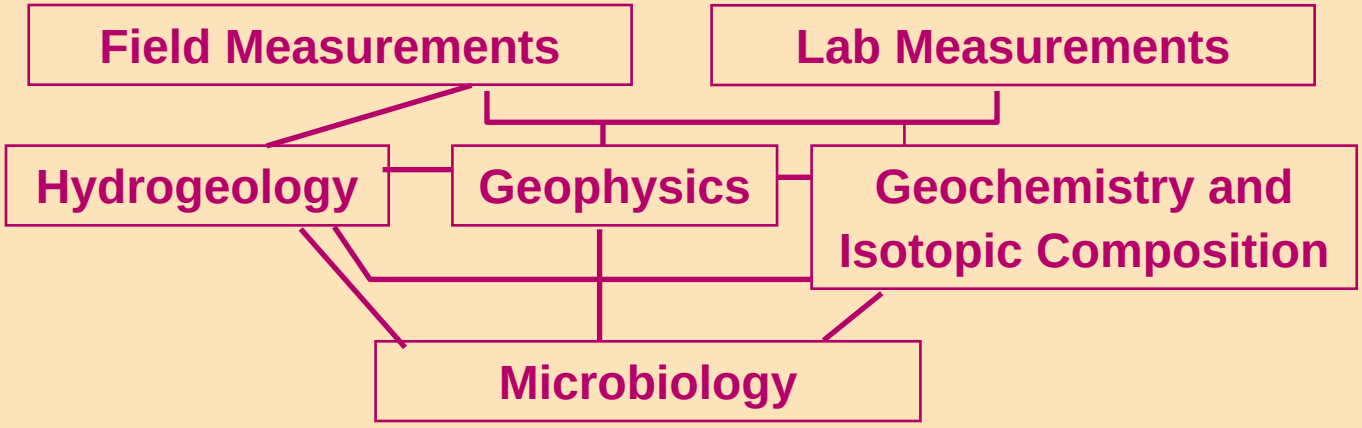
Multidisciplinary Team

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

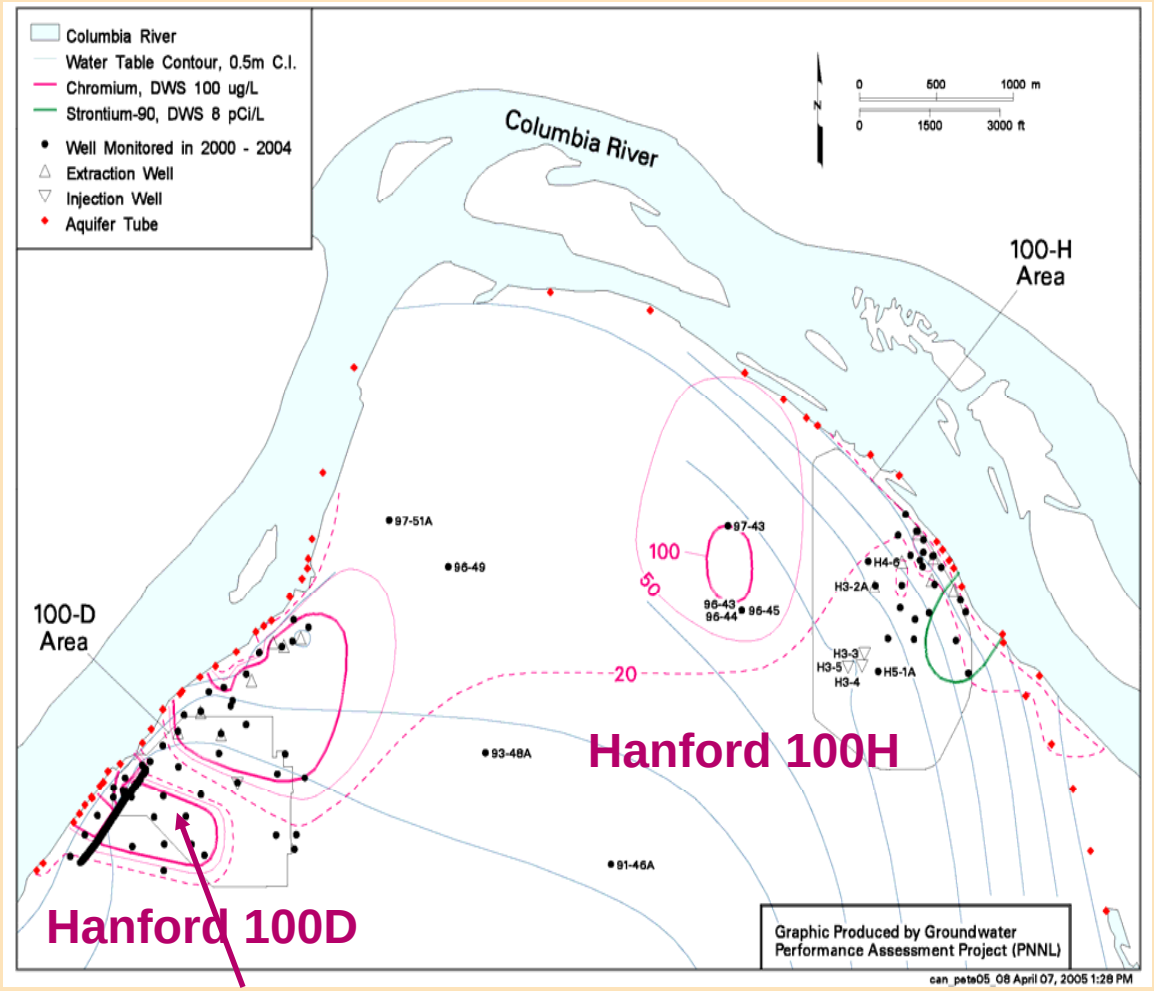
To carry out field investigations to assess the potential for immobilizing Cr(VI) in groundwater using lactate-stimulated bioreduction of Cr(VI) to Cr(III) at the Hanford 100H site, and to determine critical community structure changes and stressors that would enable control and predictions of fundamental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

Integrated Approach



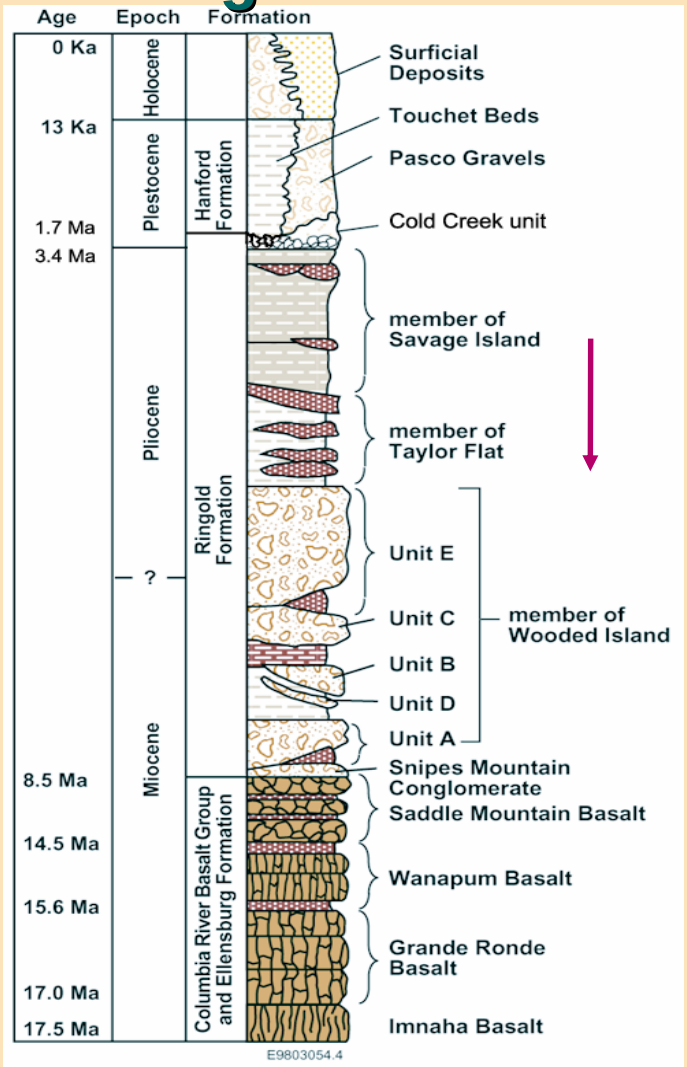
Hanford 100H Site Characterization

Cr Concentration Map



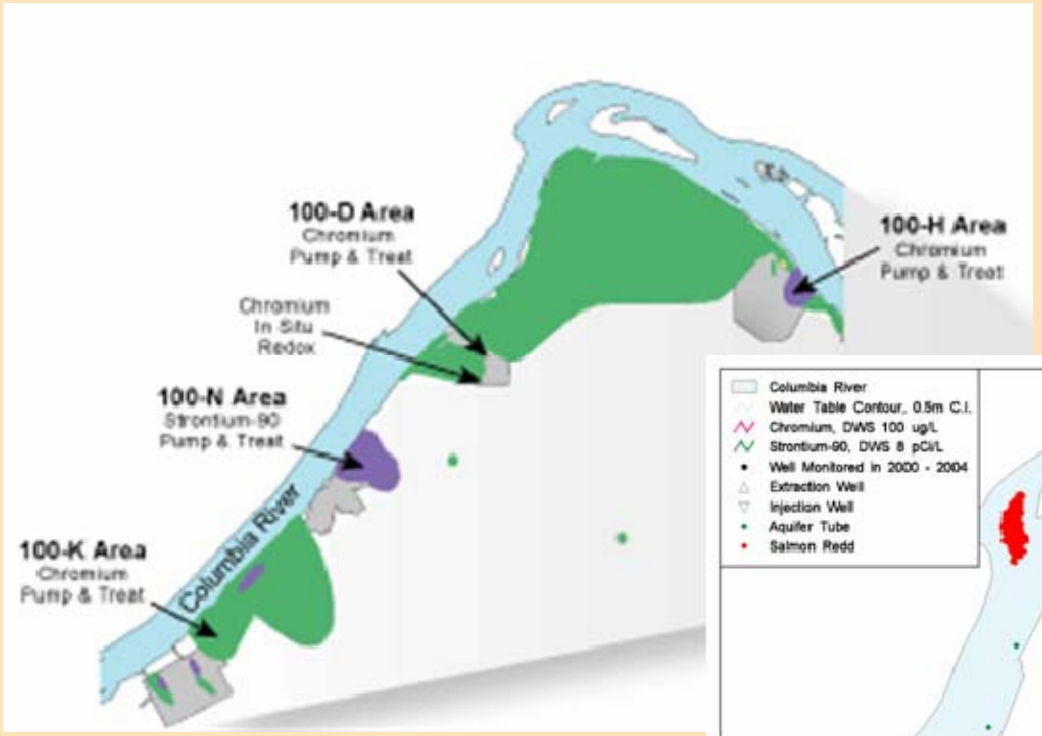
The Cr source is believed to be sodium dichromate ($Na_2Cr_2O_7.2H_2O$)

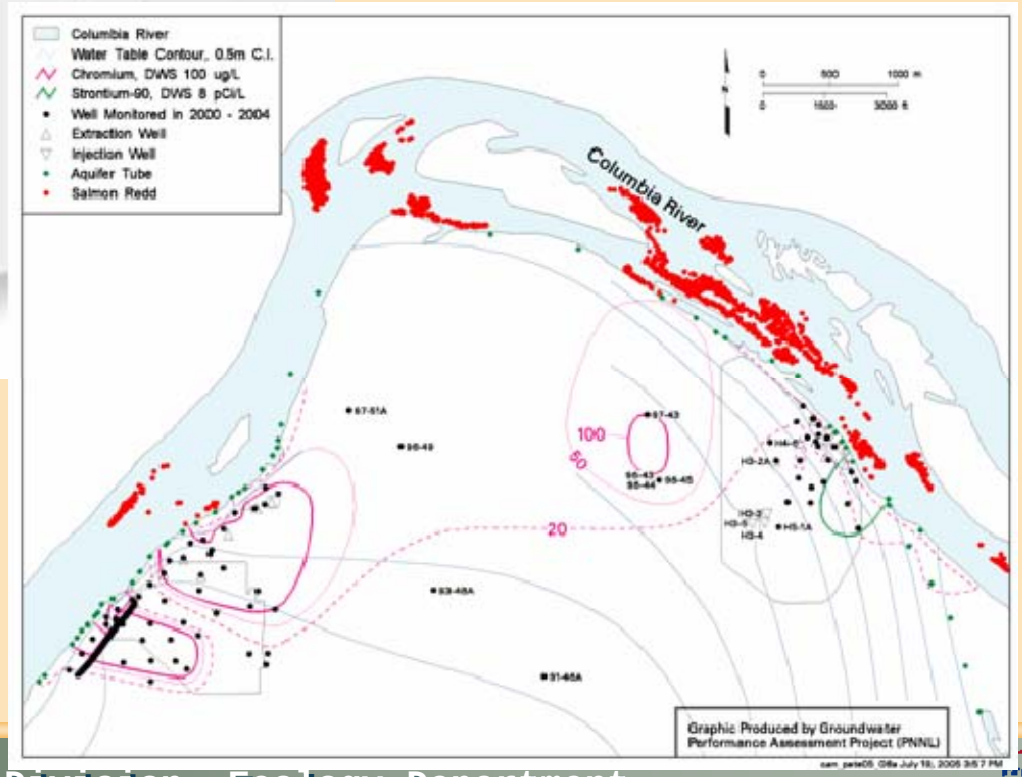
Lithological Column



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

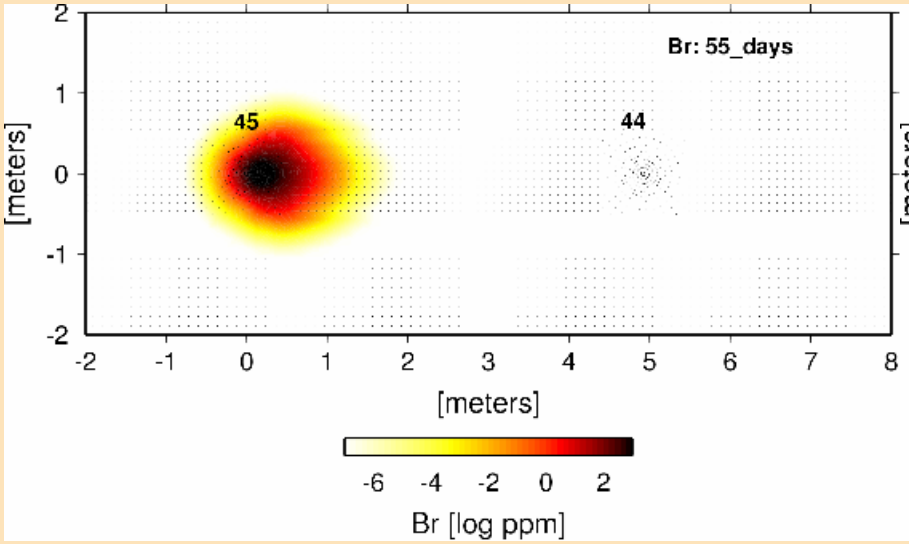
100 Area Hexavalent Chromium Plumes



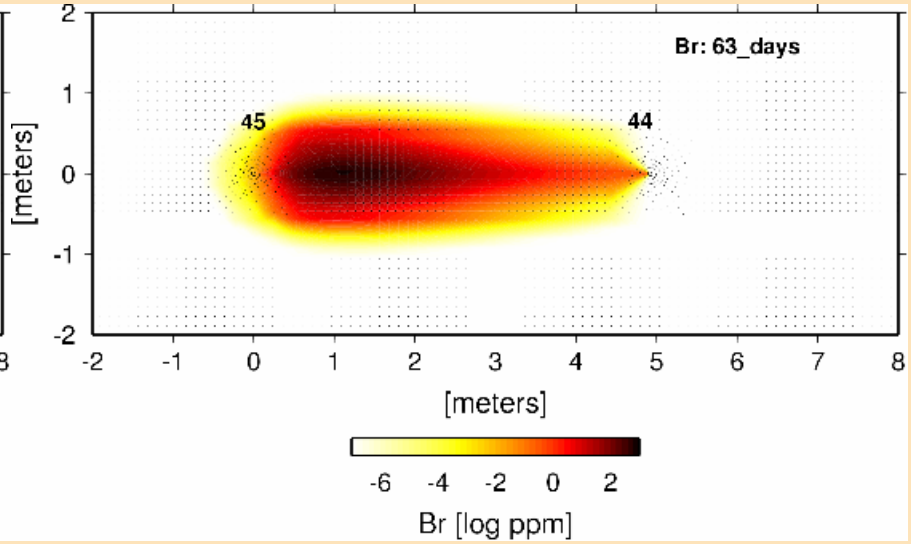


LiBr Injection (2/27/2004)

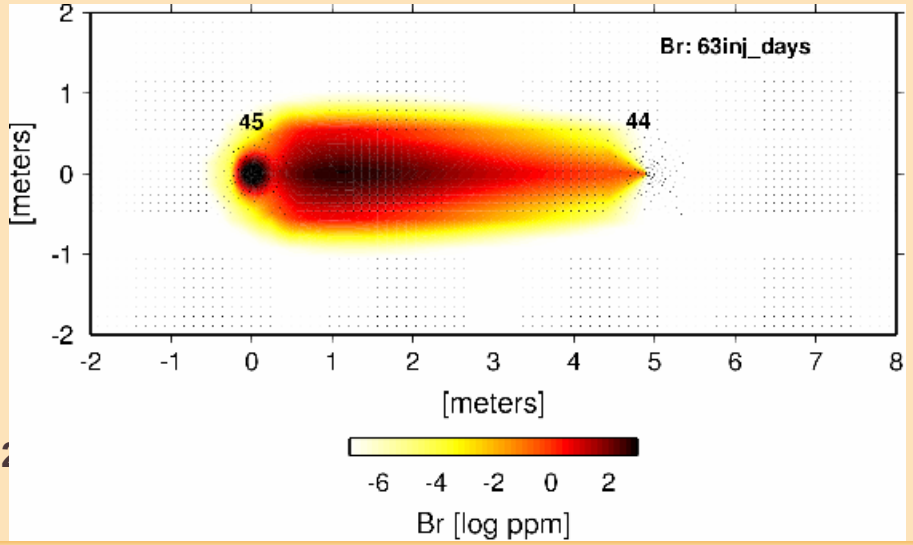
55 Days No pumping



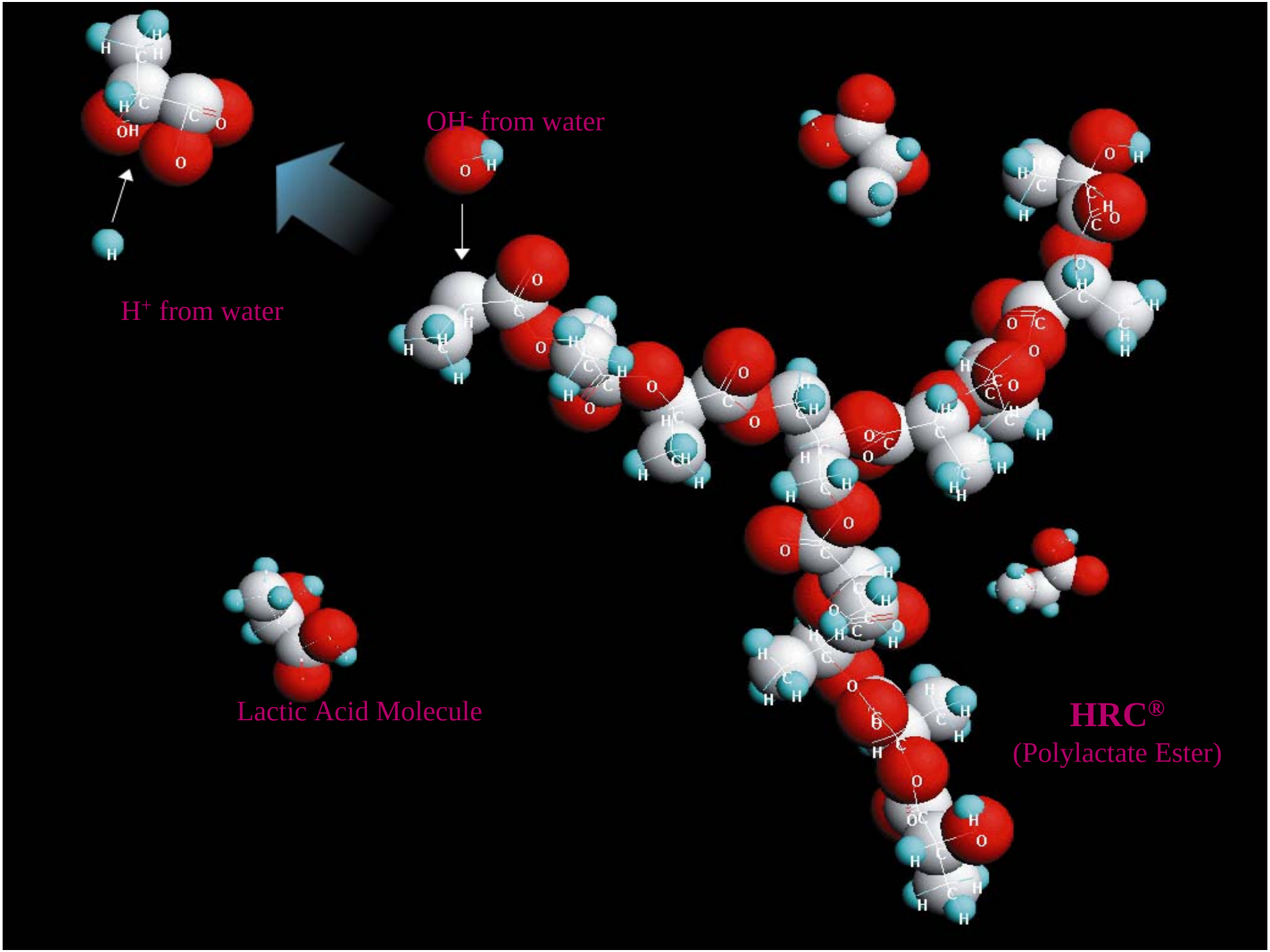
63 Days Pumping



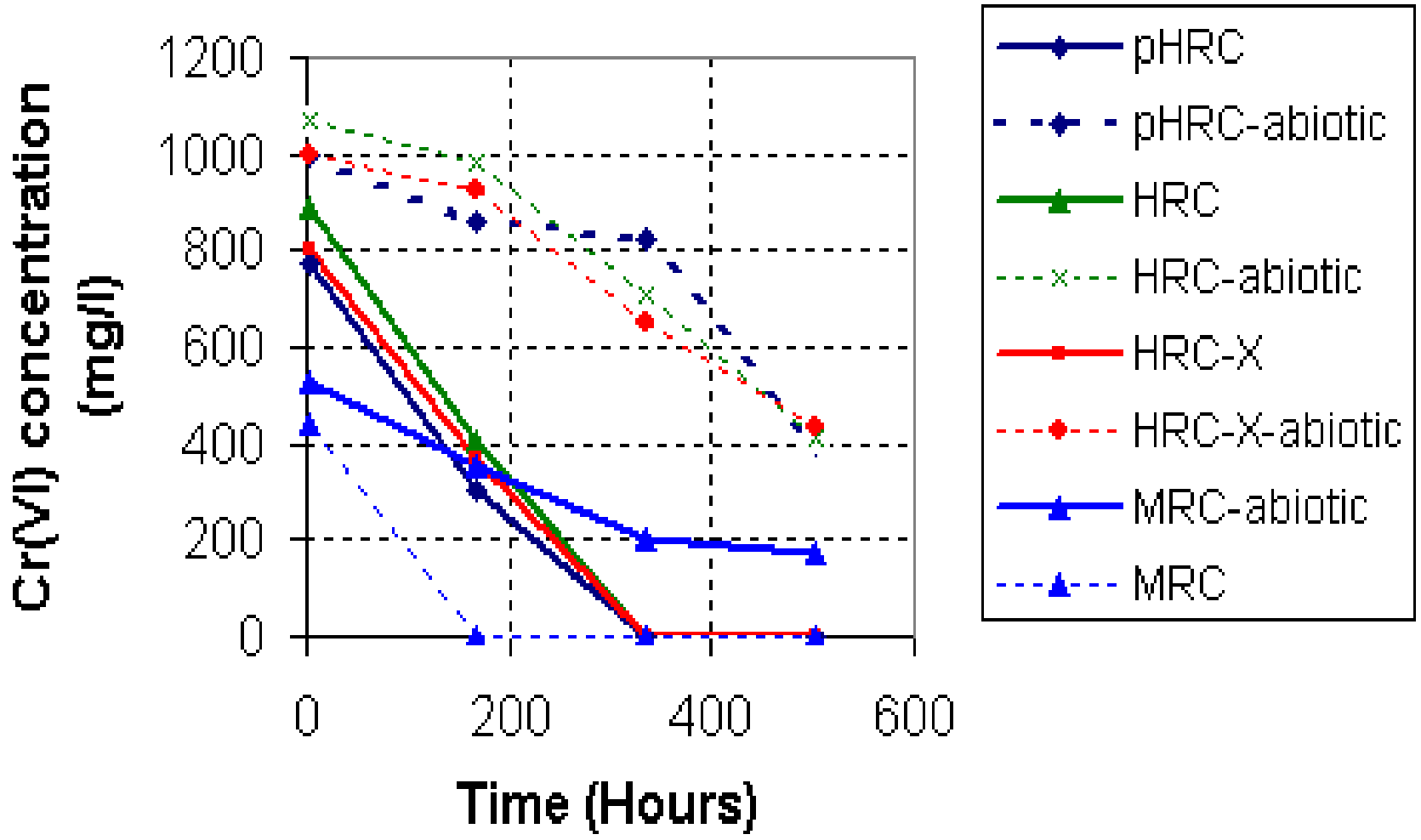
63 Days Pumping + 2nd Injection



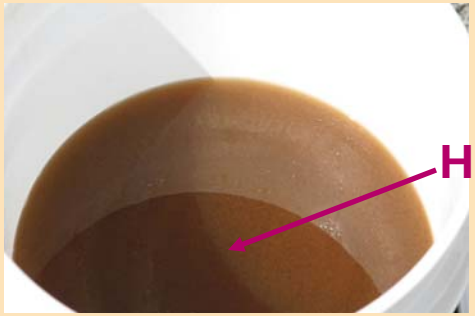
- LiBr Injection: 15.3 g/liter, 12 liters over 2 hours


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

Lactate-Induced Bioreduction of Cr(IV)

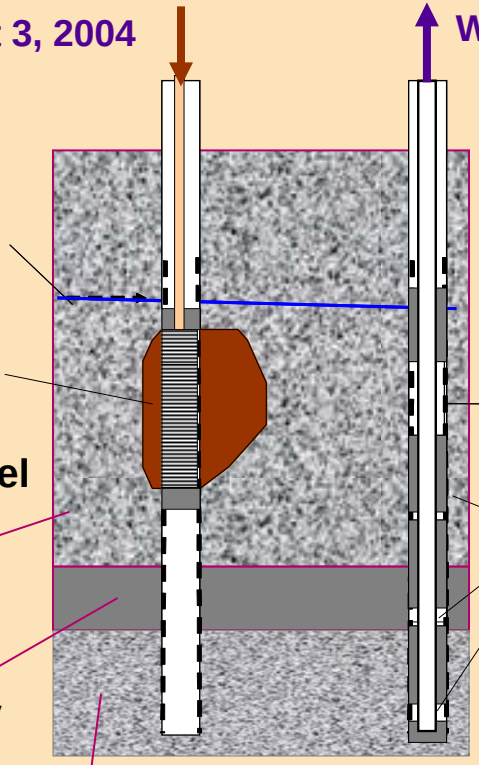


Field HRC Injection Test

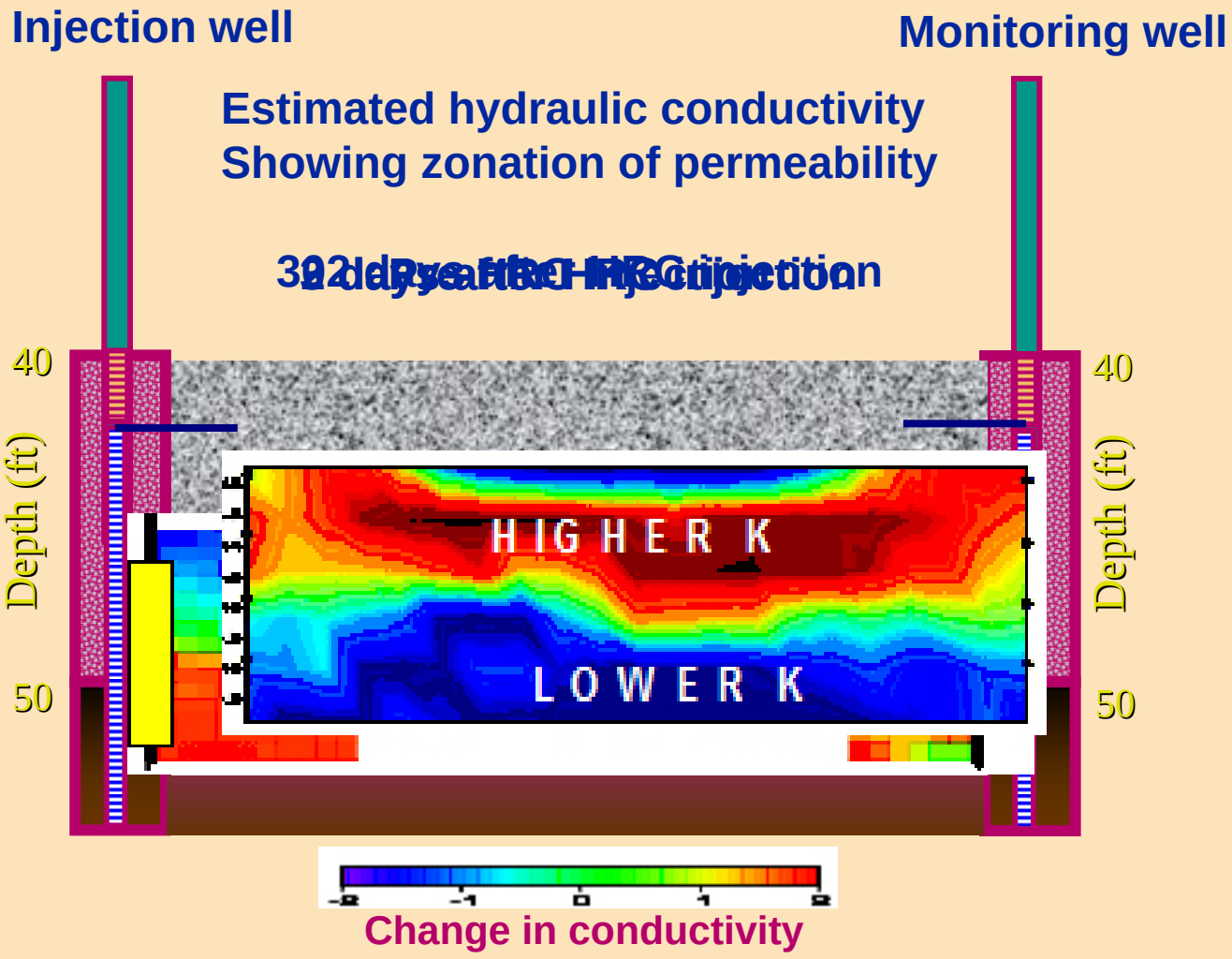


Injection of 40 lbs of ^{13}C-labeled HRC
Well 699-96-45, August 3, 2004

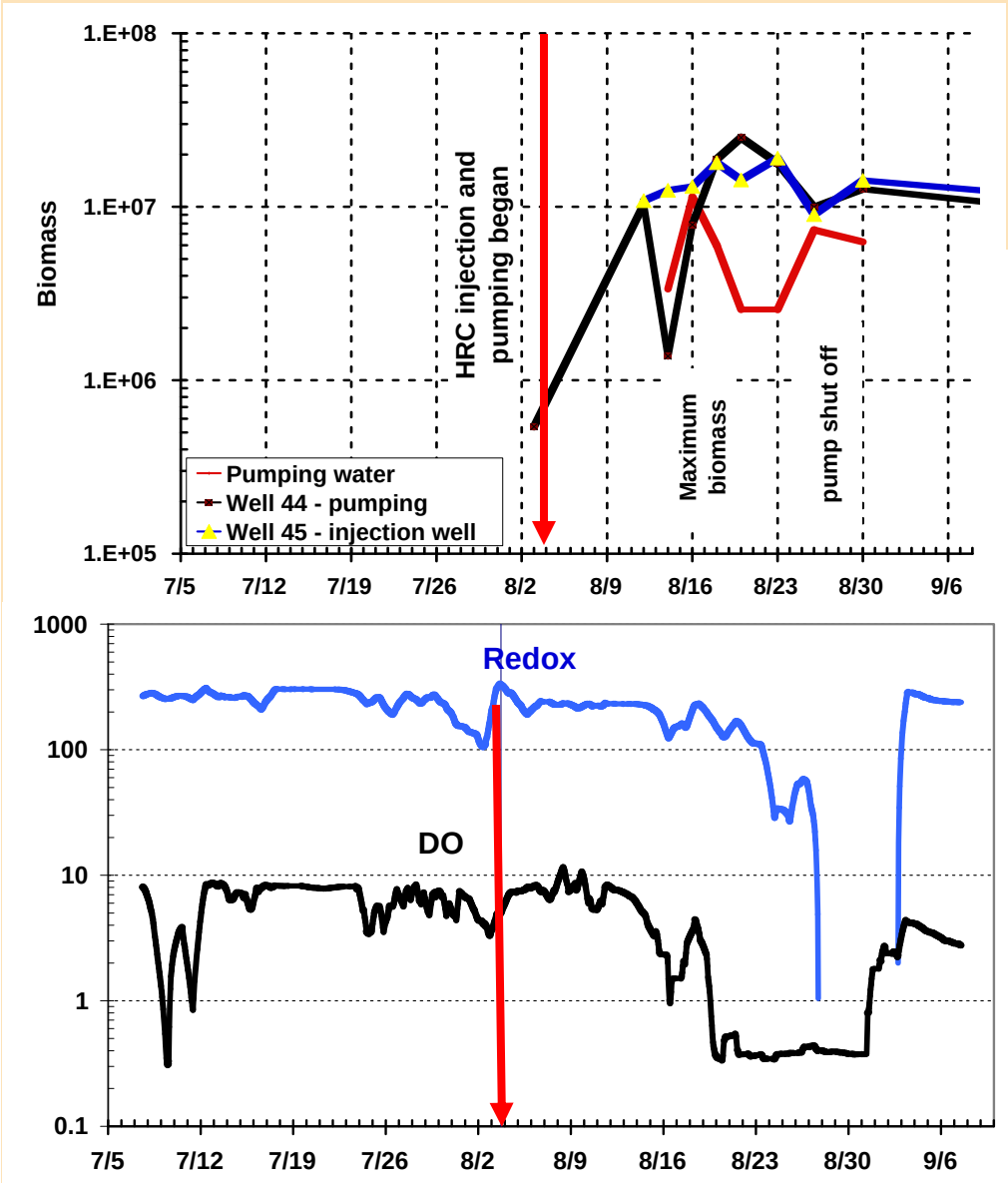
Pumping - 27 days
Well 699-96-44

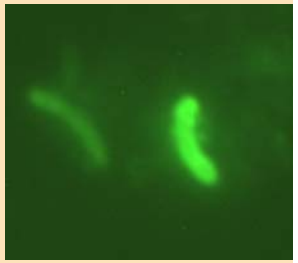


Non-invasive geophysical monitoring

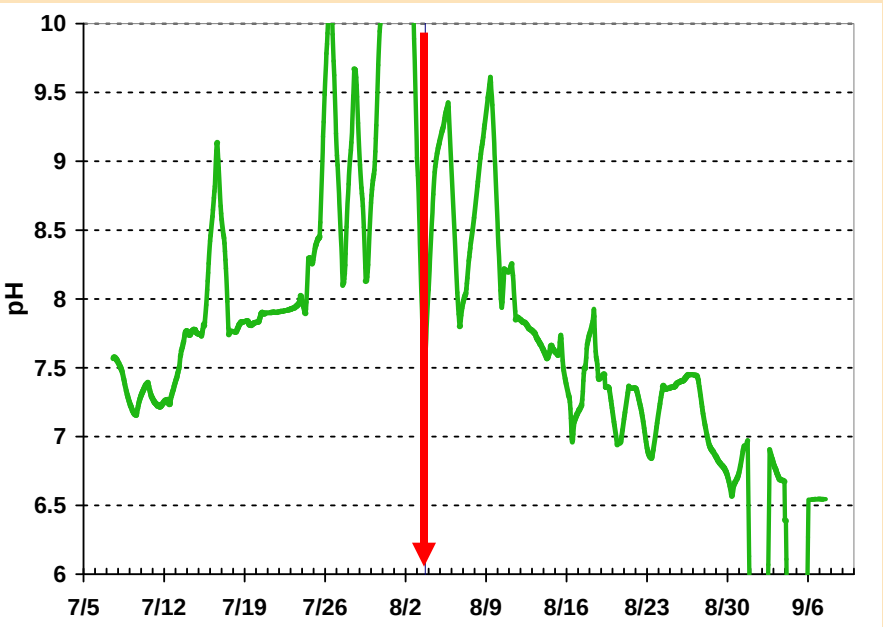


Results of HRC Biostimulation





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

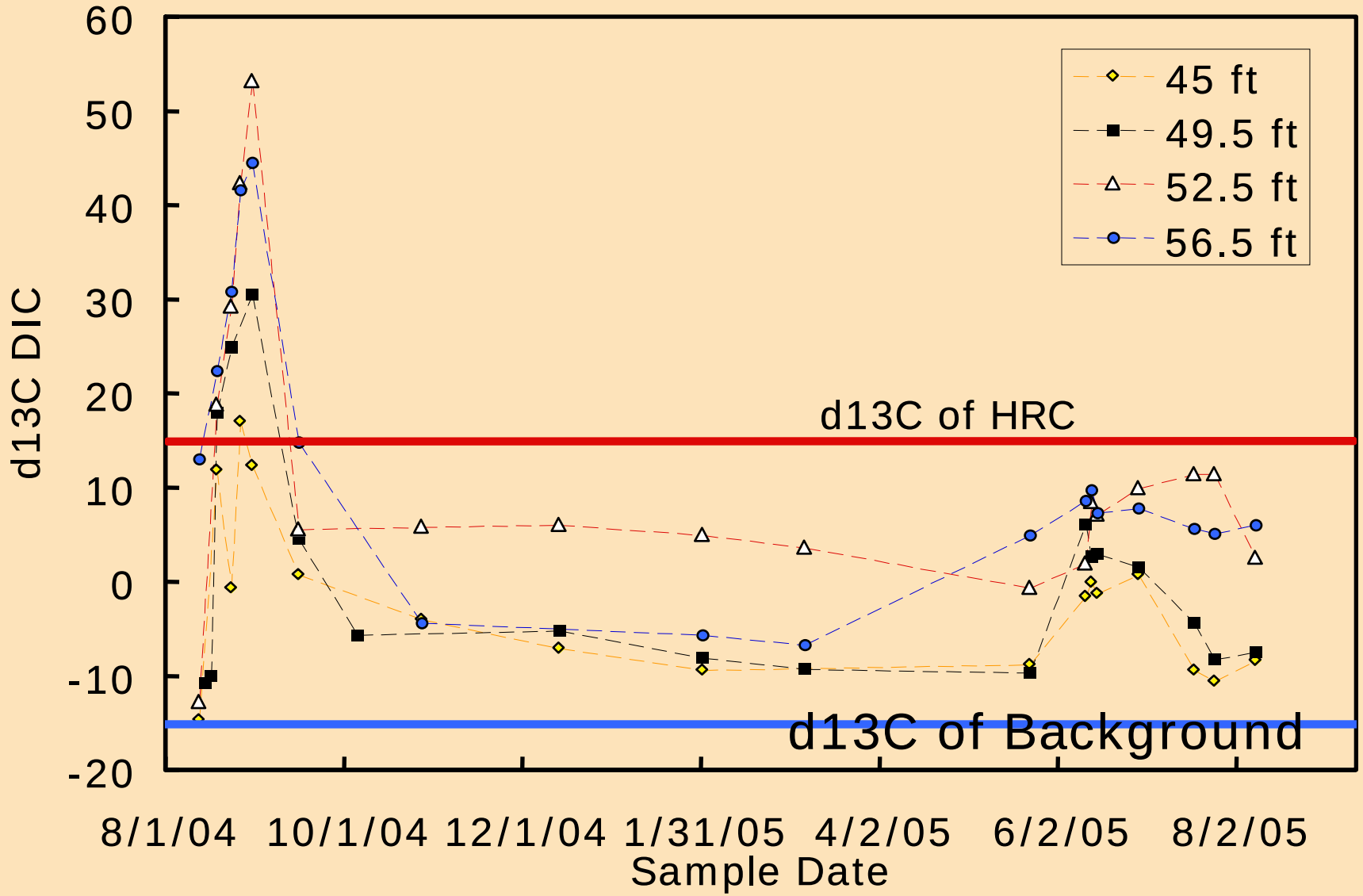


Redox dropped from 240 to -130 mV

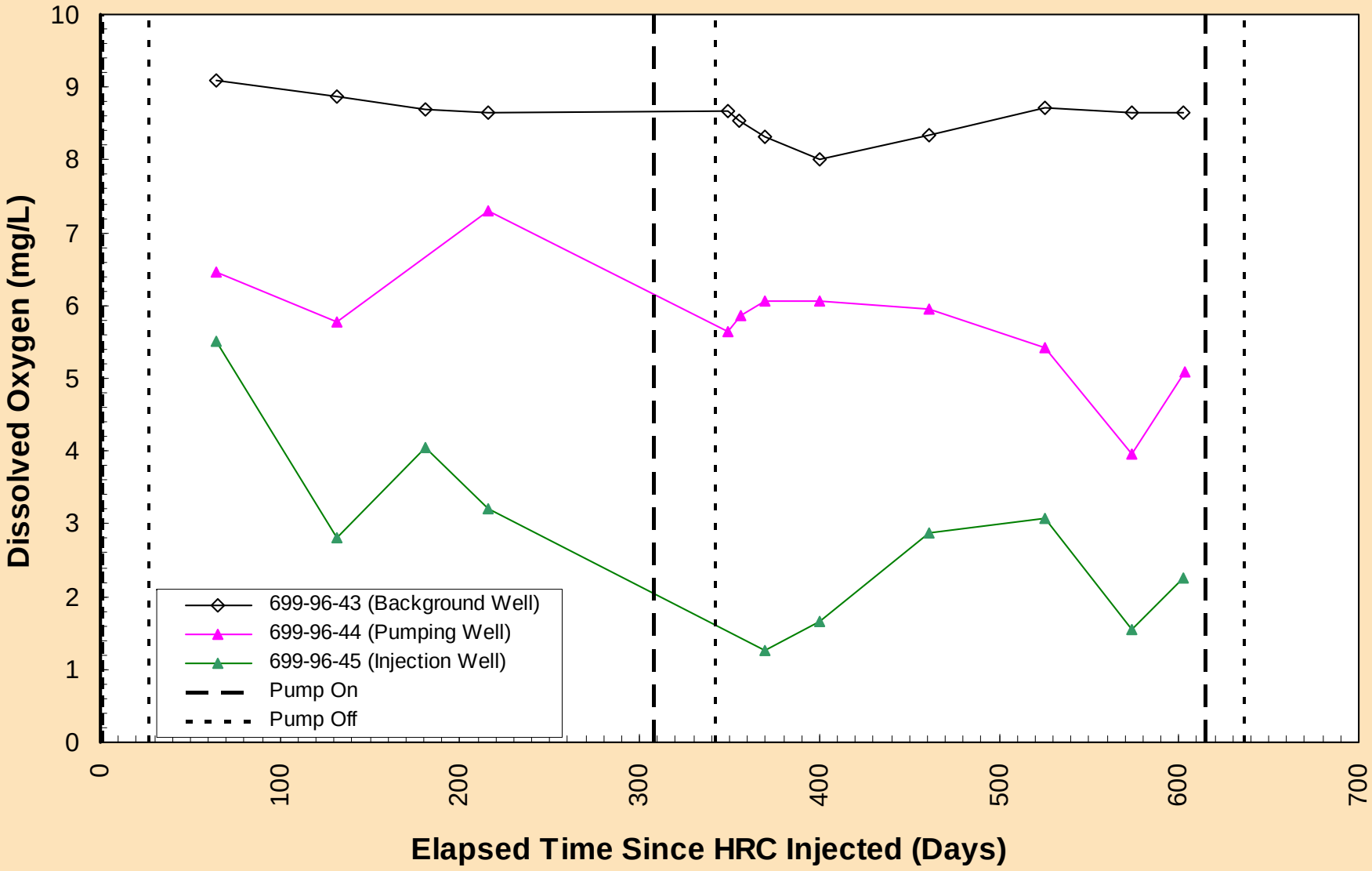
DO dropped from 9 mg/l (~100%) to 0.35 mg/l (4.5%)

Biogeochemical Evidence of Microbial Metabolism in Groundwater

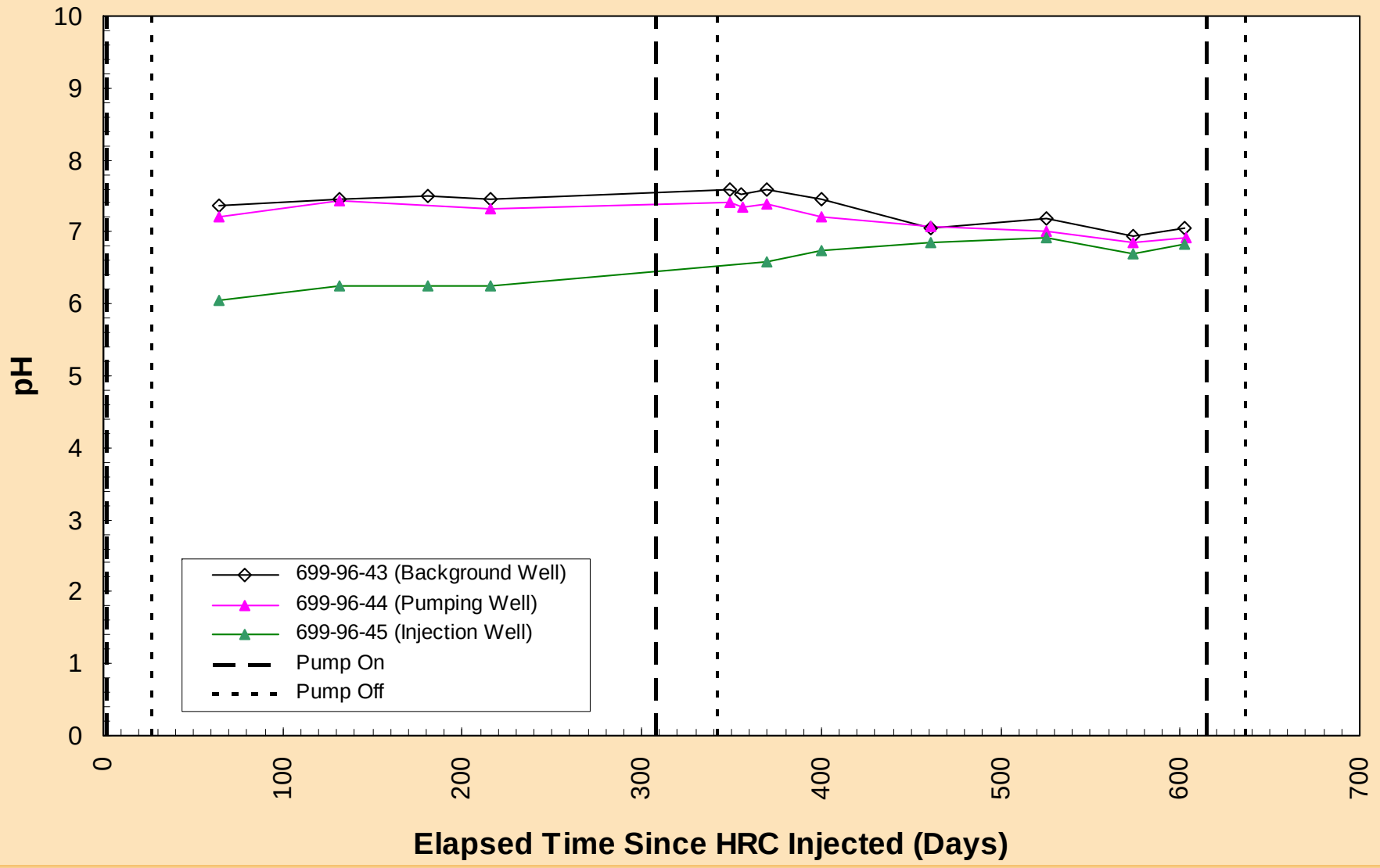
$d^{13}C$ of Dissolved Inorganic Carbon is Byproduct of HRC Metabolism



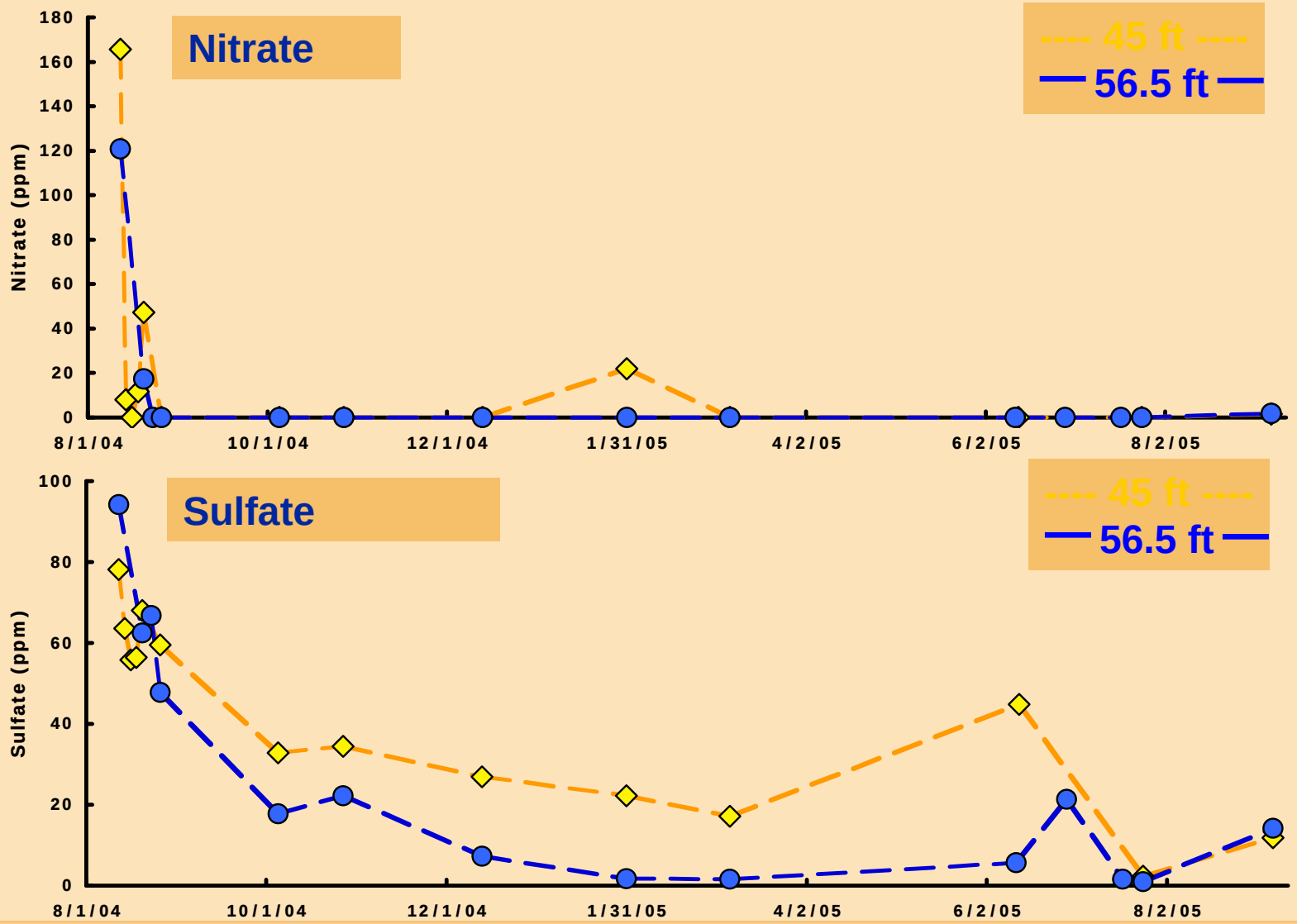
HRC Experiment, Monthly Sampling



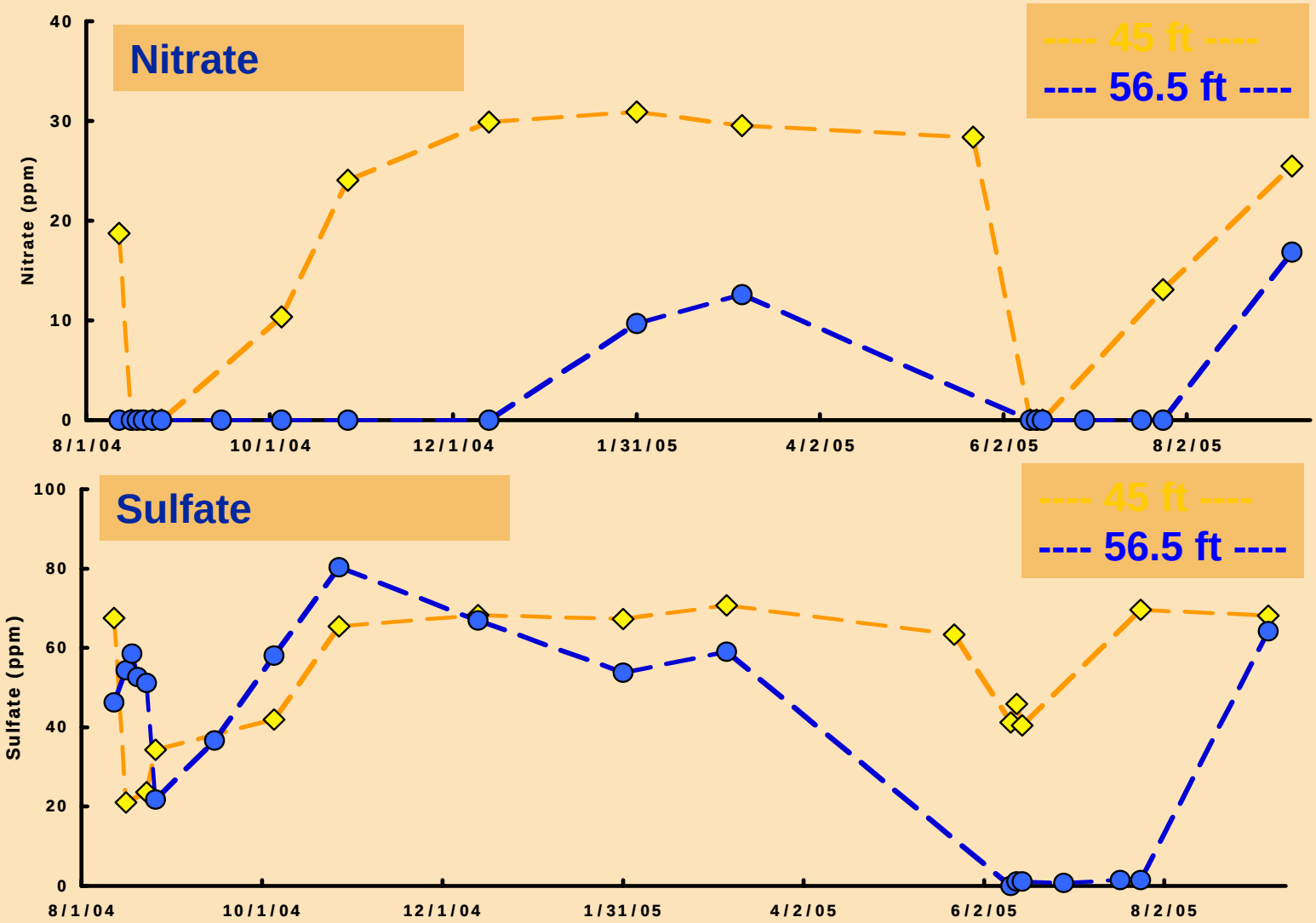
HRC Experiment, Monthly Sampling



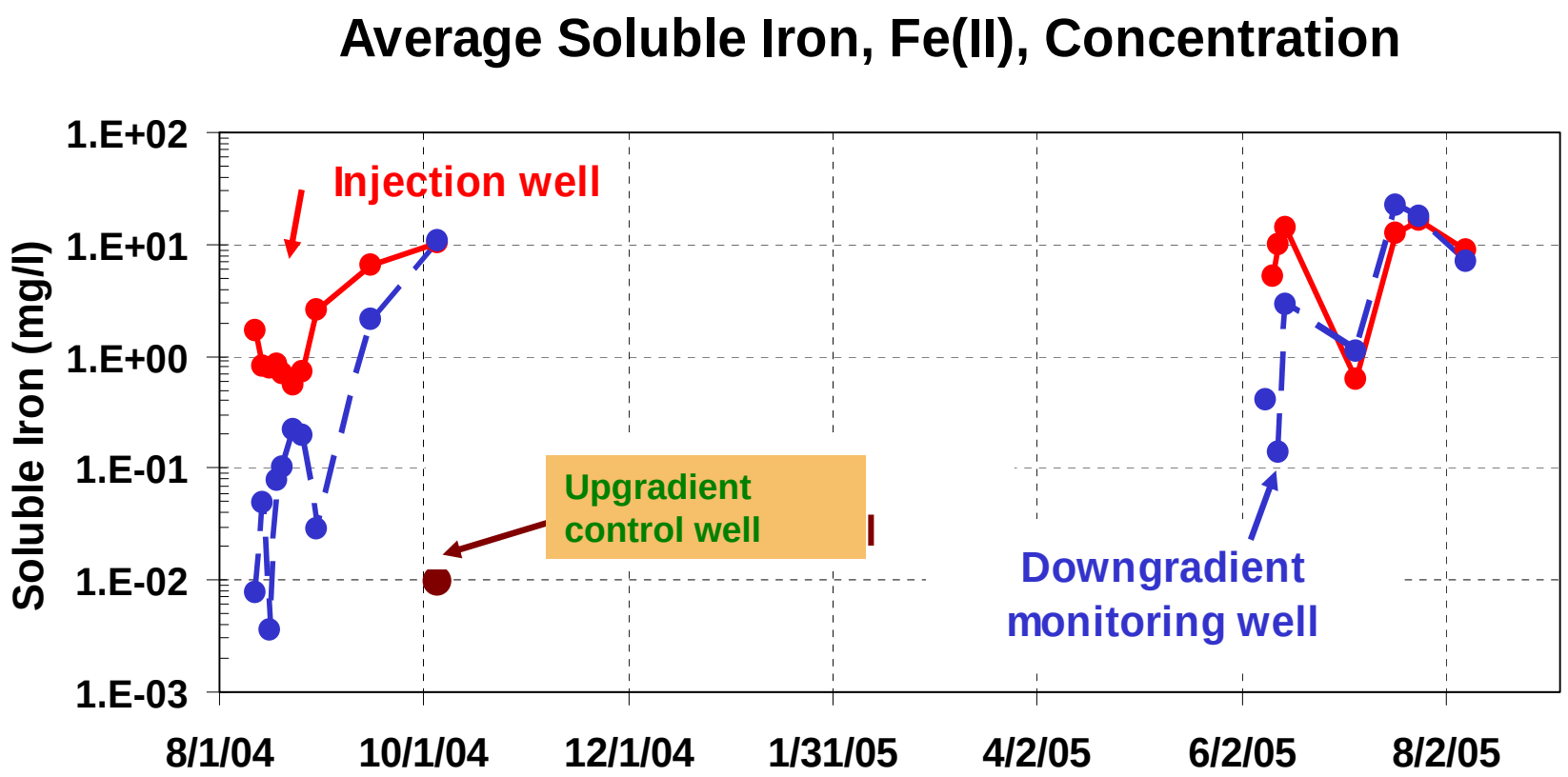
Geochemistry – Injection well



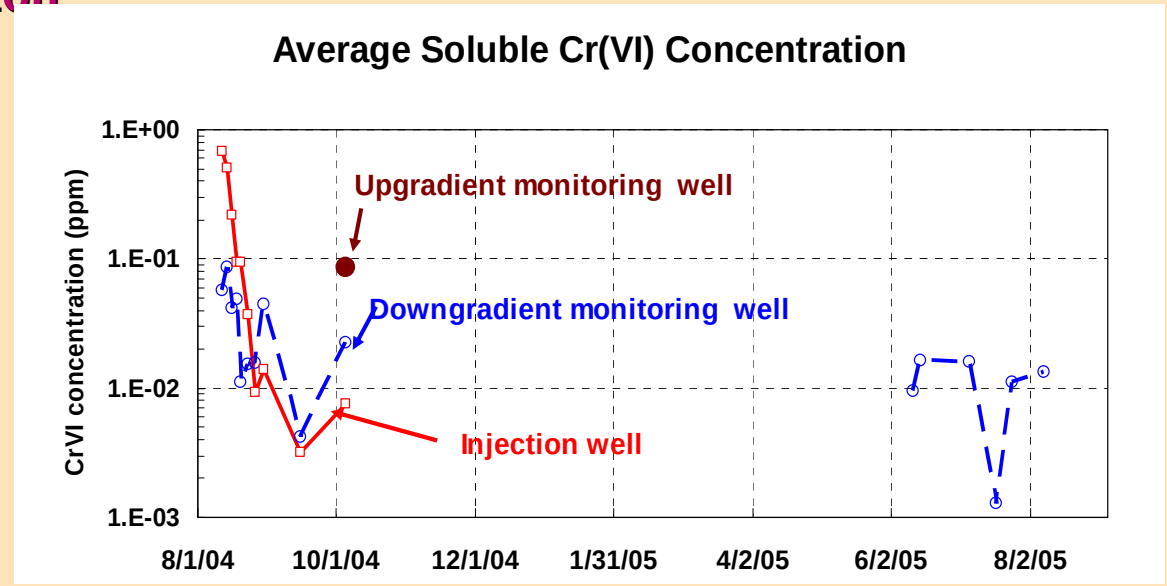
Geochemistry – Monitoring well

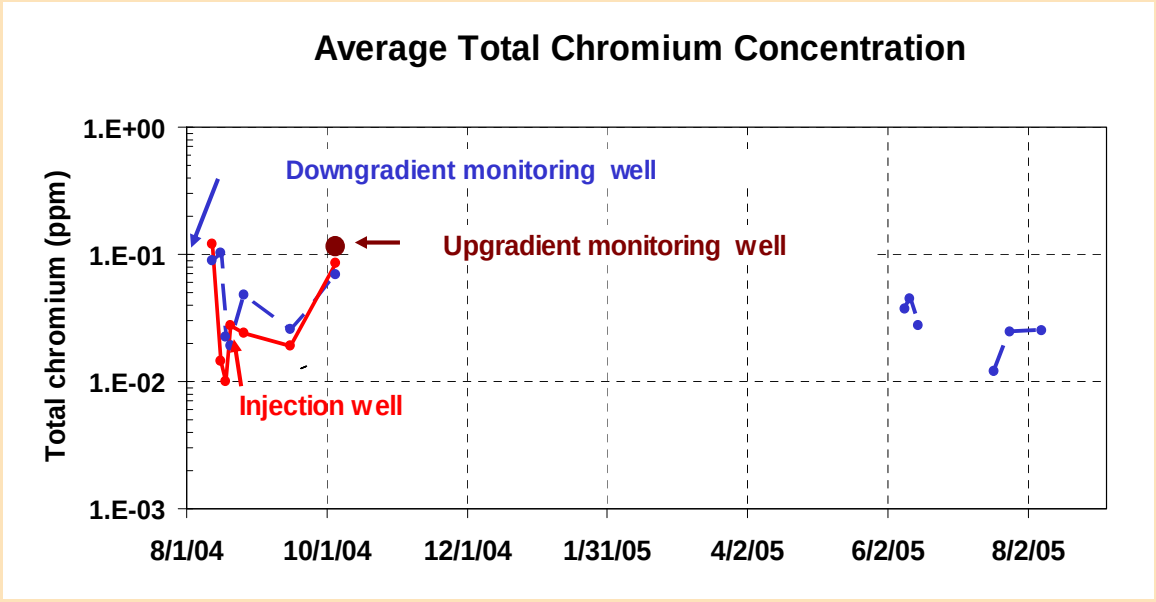


Geochemistry – Iron reduction

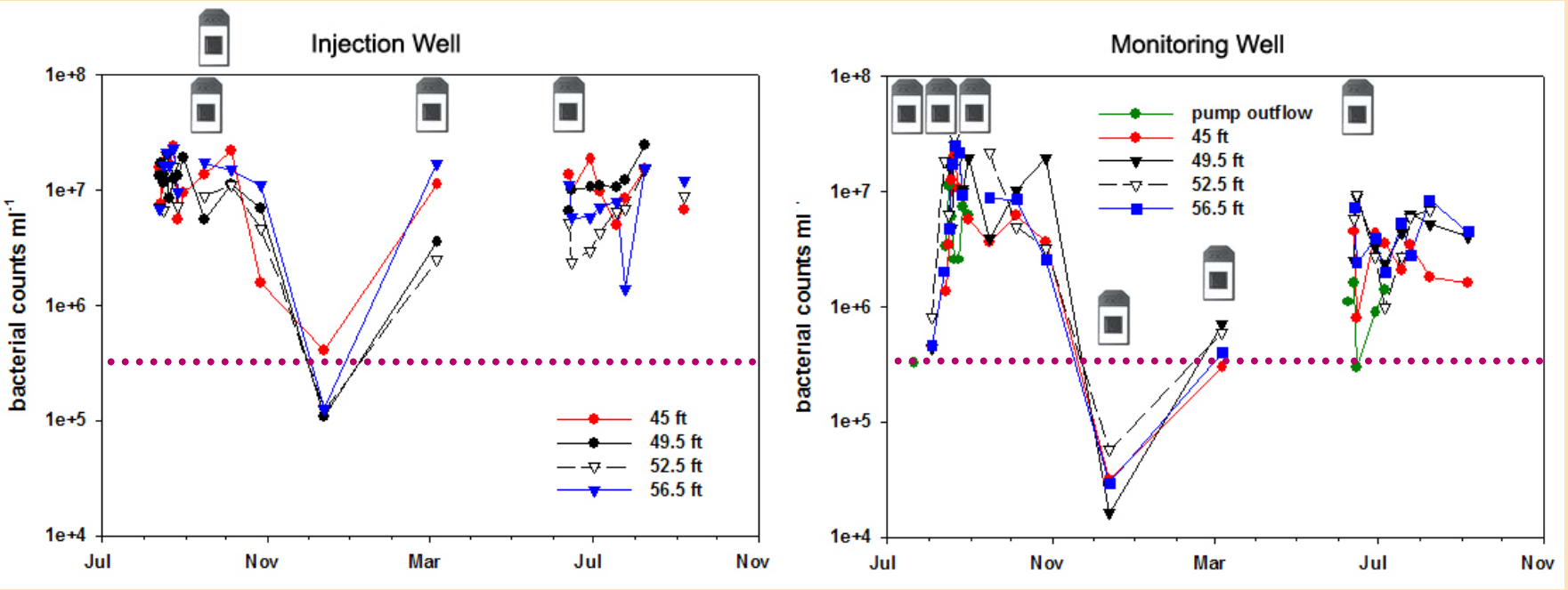


Changes of Cr(VI) Concentration in Groundwater after HRC Injection





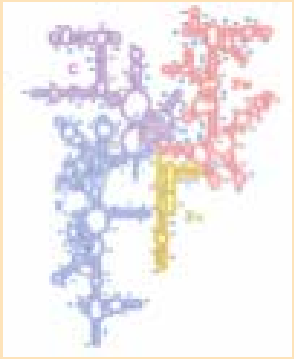
Bacterial biomass - Direct counts



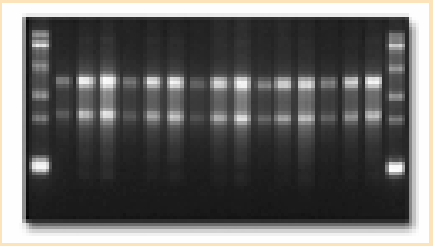
Bacterial biomass enriched rapidly by 2 orders of magnitude – remained elevated over one year later

PhyloChip

- **PhyloChip – 500,000 probes (300k target 16S)**



16S rRNA gene used as biomarker due to large database and availability of “universal” primers.

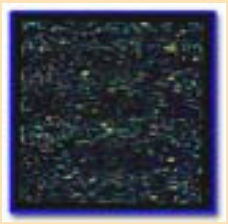


16S rRNA gene is amplified from genomic extract or 16S rRNA molecules are used directly

Amplicon pool fragmented, biotin labeled

Biotin labeled 16S hybridizes to its complement sequence on the array surface.

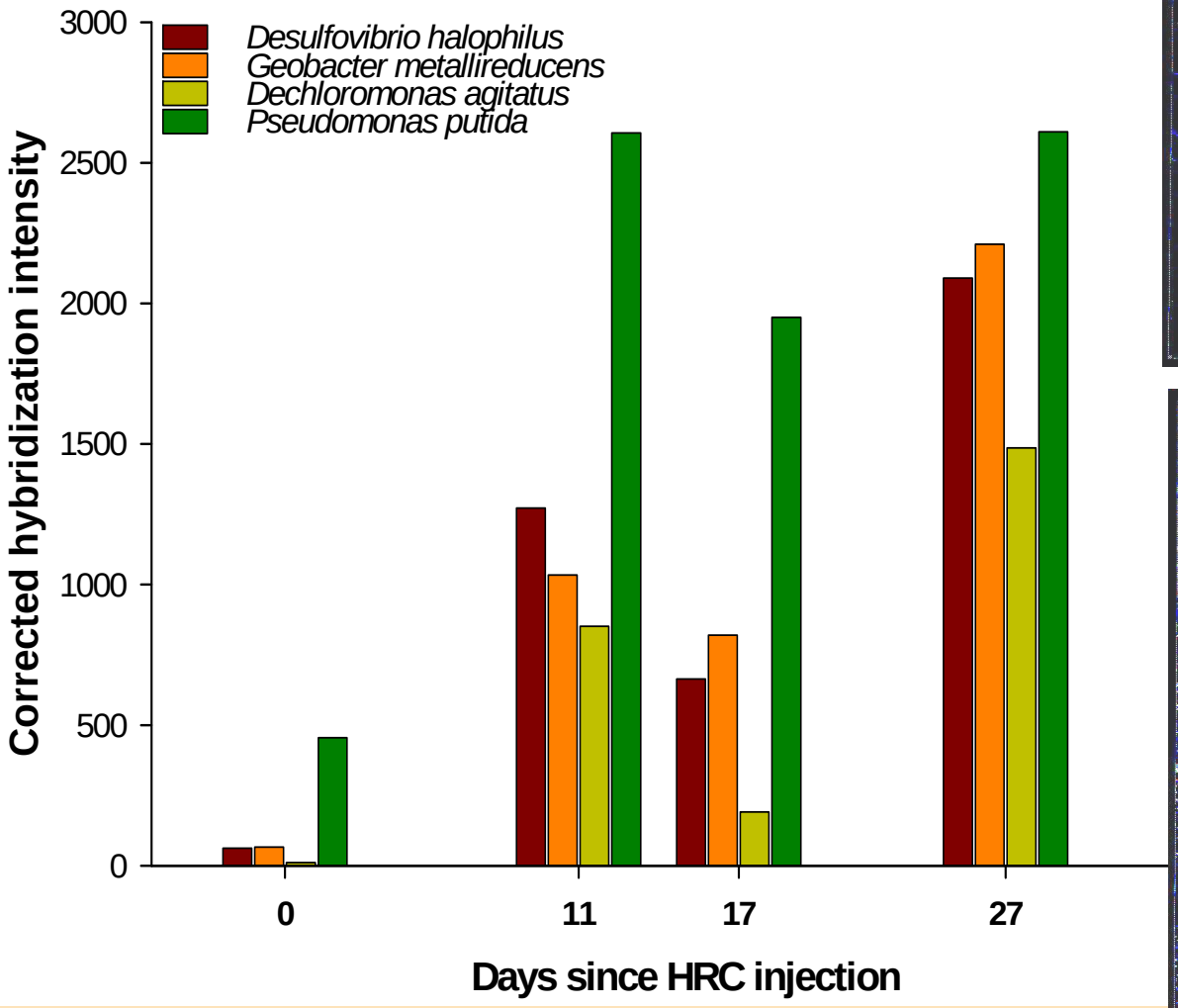
PhyloChip stained and washed using automatic fluidics station

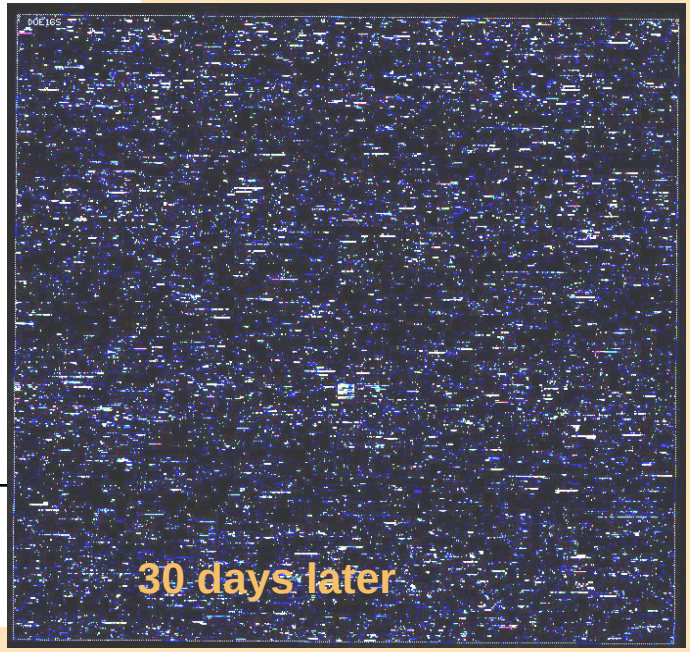


PhyloChip is scanned, fluorescence data analyzed and probe sets with >90% probes positive are considered present

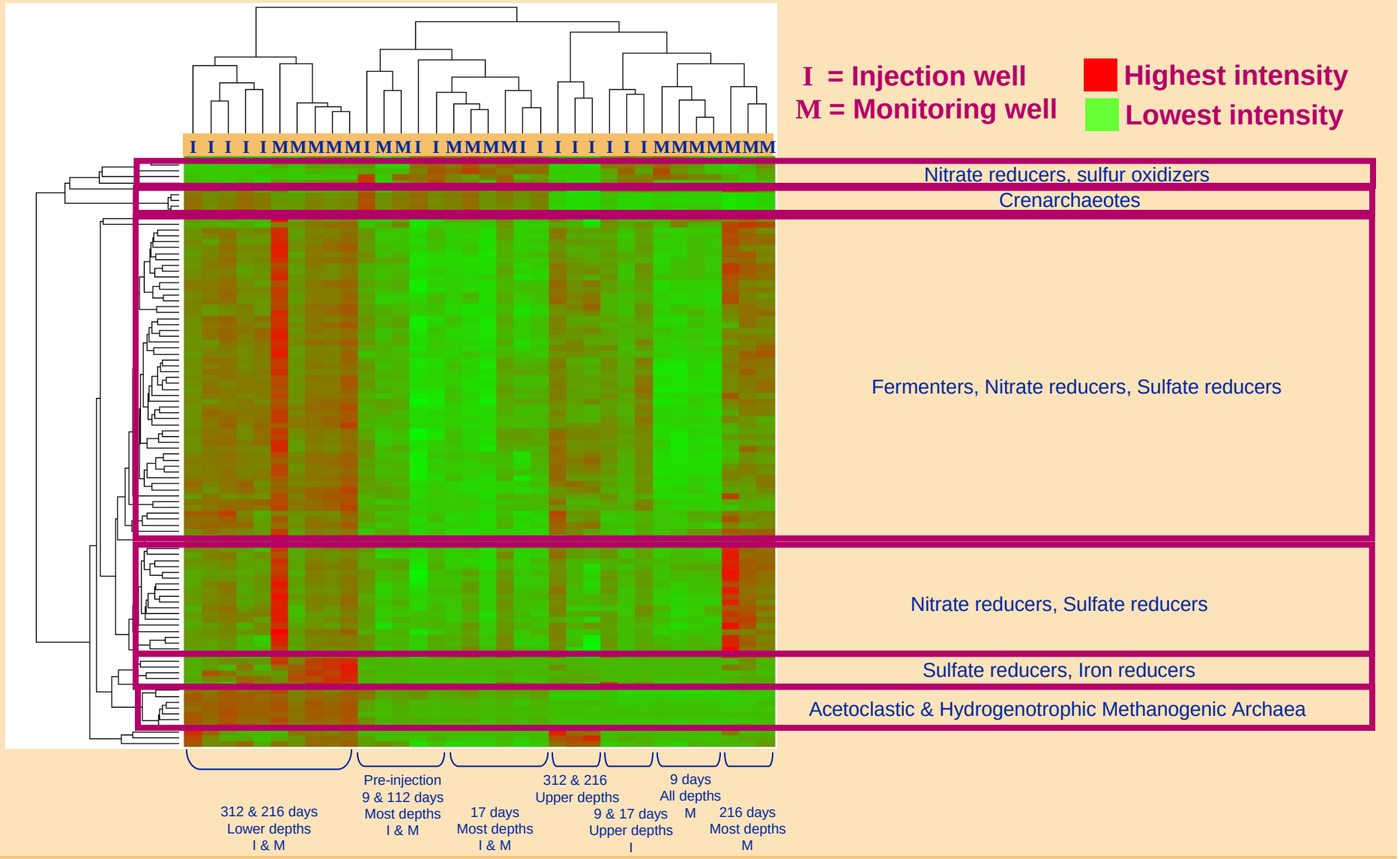
Microarray analysis of bacterial community changes during Cr(VI) remediation at Hanford 100H site:

Dynamics of some significant organisms.

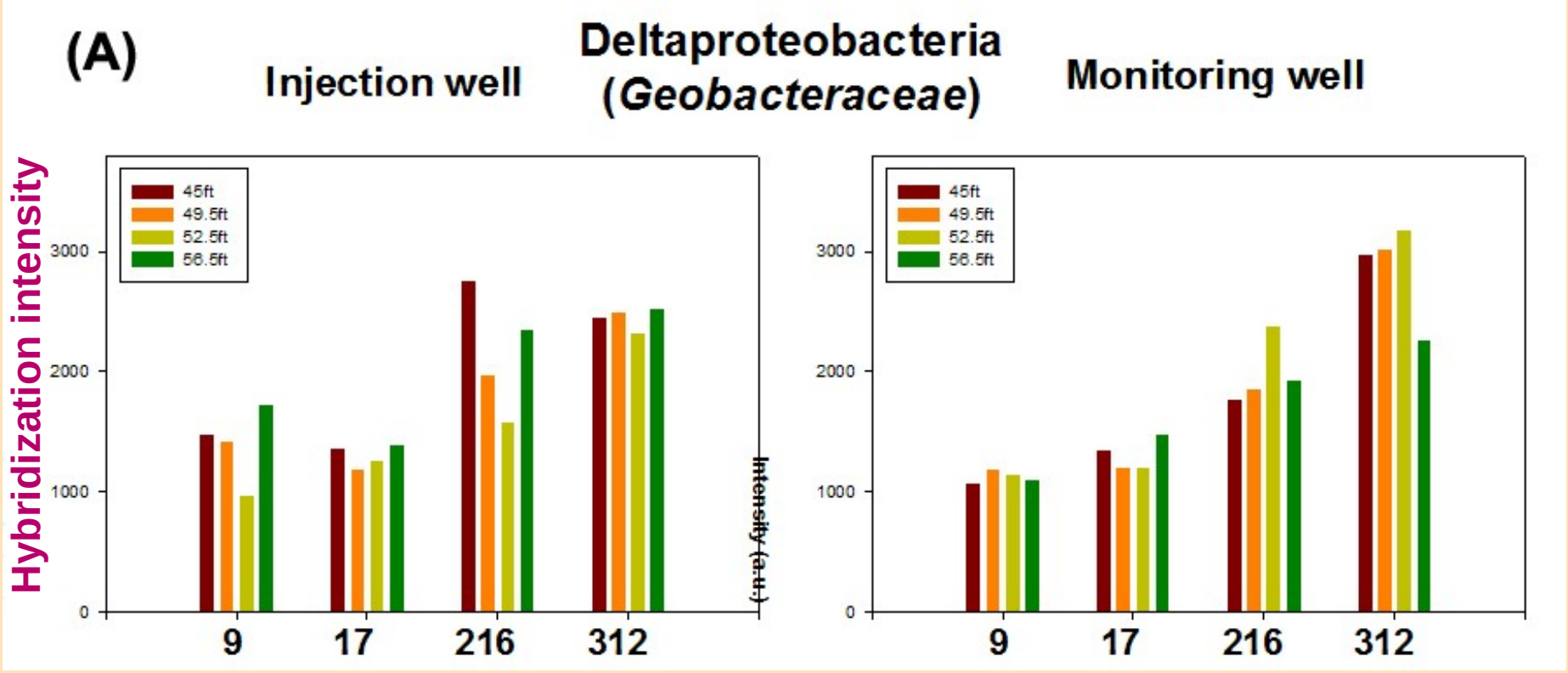




Data mining – Bidirectional clustering

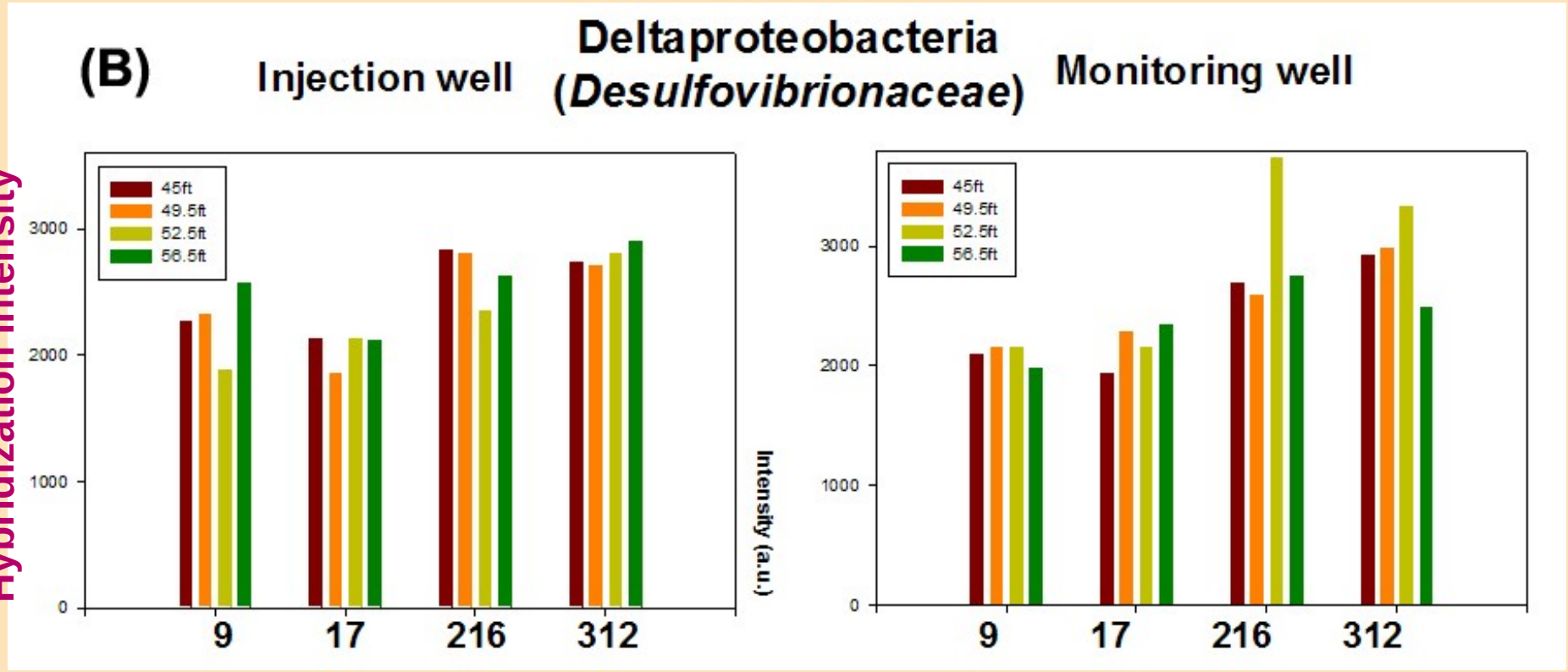


Functional groups – Iron reduction



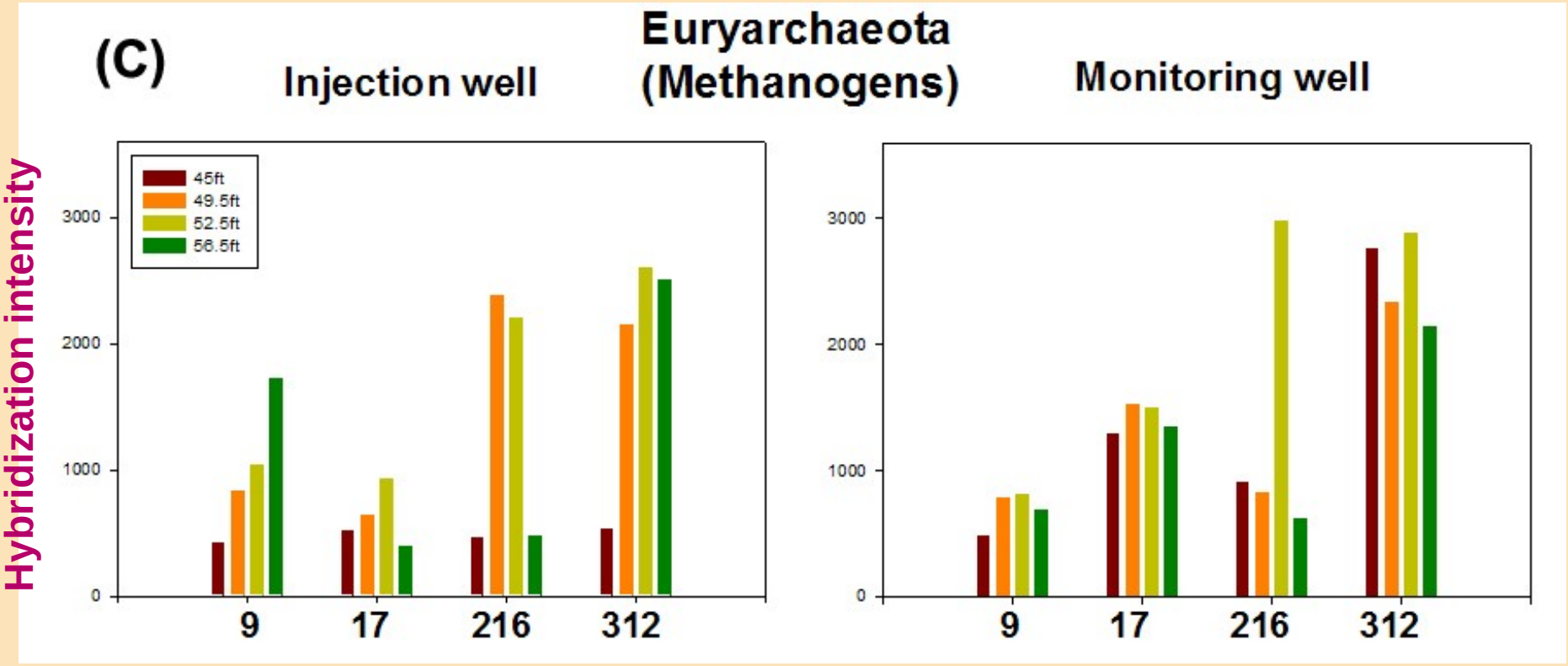
Fe(II) can abiotically reduce Cr(VI) to Cr(III)

Functional groups – Sulfate reduction



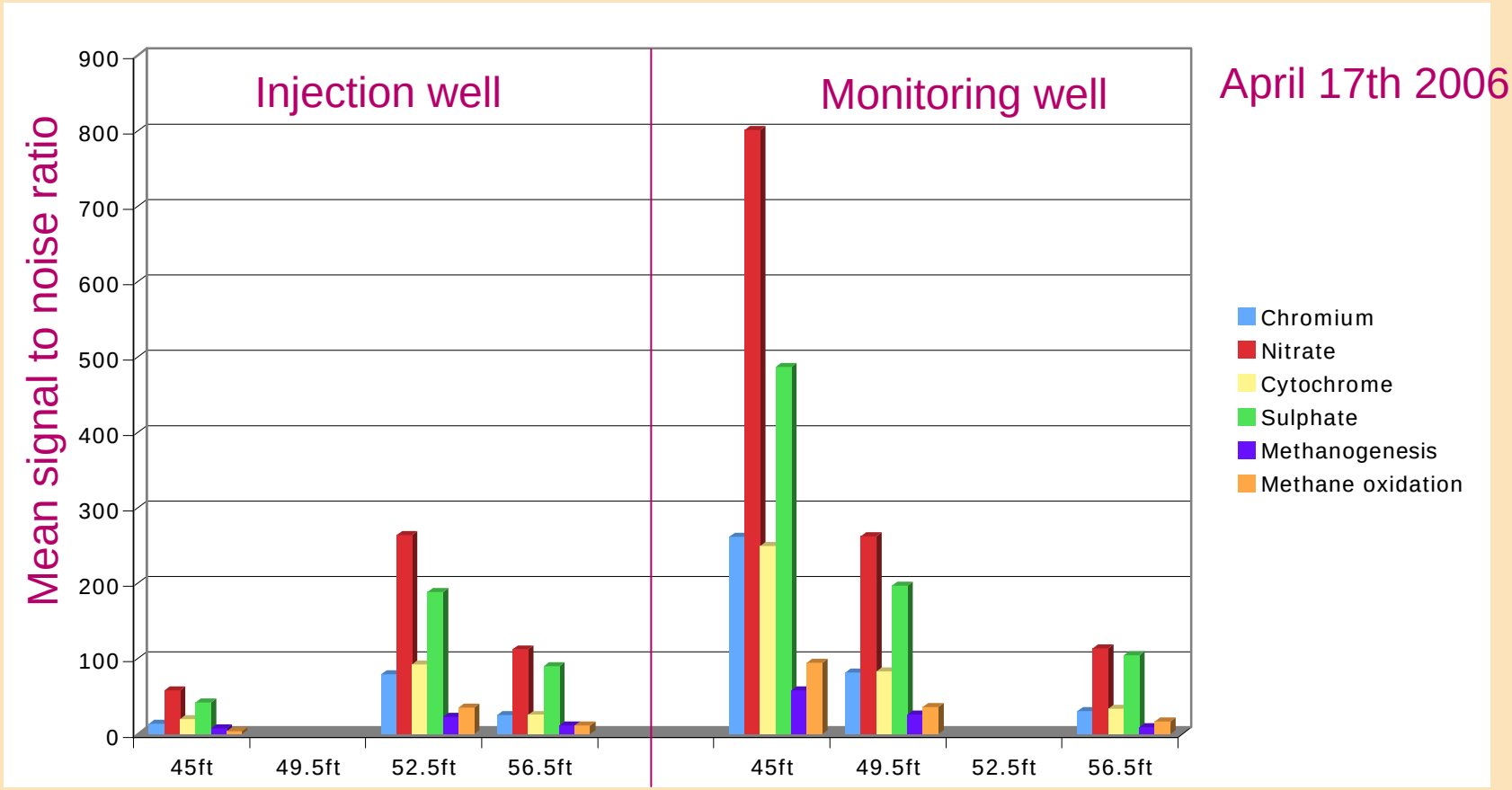
H_2S can abiotically reduce Cr(VI) to Cr(III)

Functional groups – Methanogenesis



Presence of methanogens indicates strongly reducing conditions

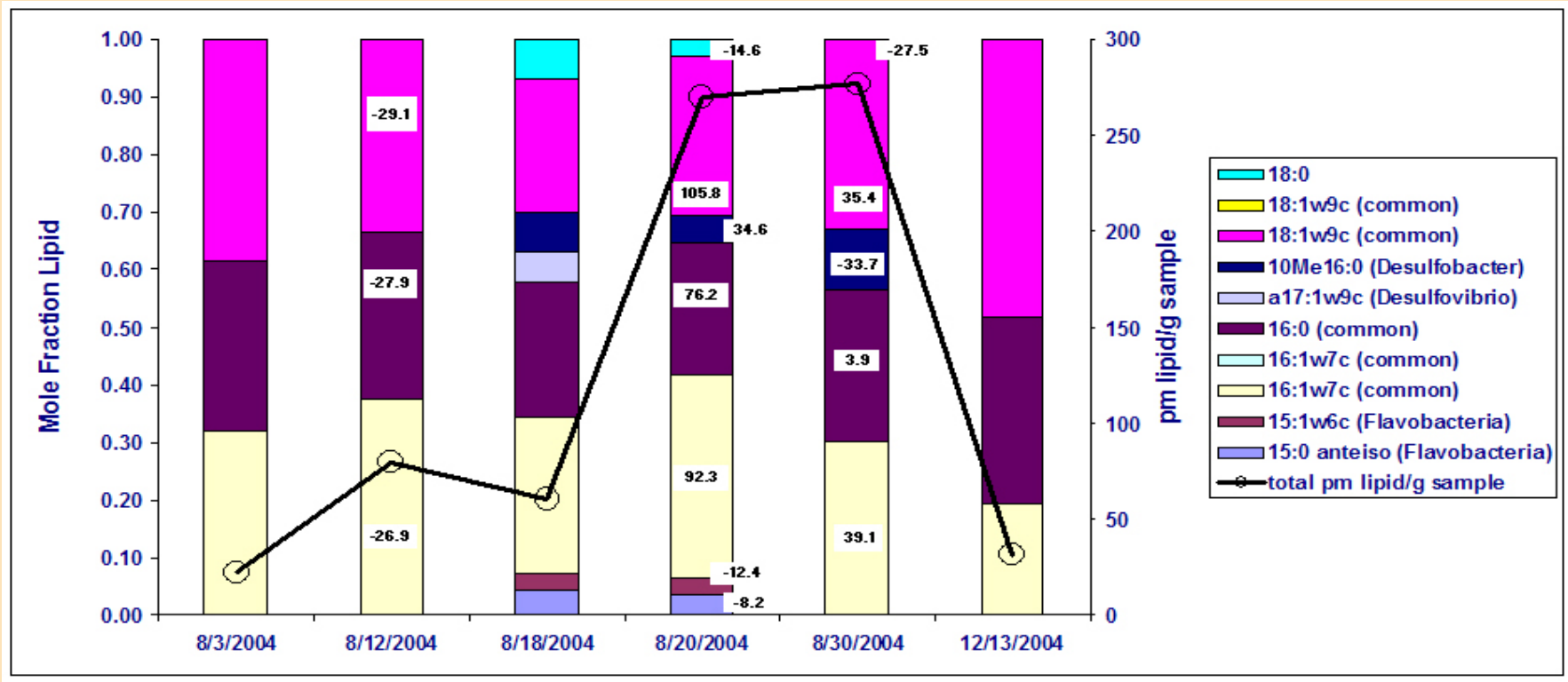
Functional microarray analysis



Nitrate, Sulfate, Iron reduction. Methanogenesis, Methane oxidation, Sulfur oxidation.
Many chromium tolerance/reduction genes.

Joe Zhou, Joy Van Nostrand - University of Oklahoma

^{13}C Phospholipid Analysis



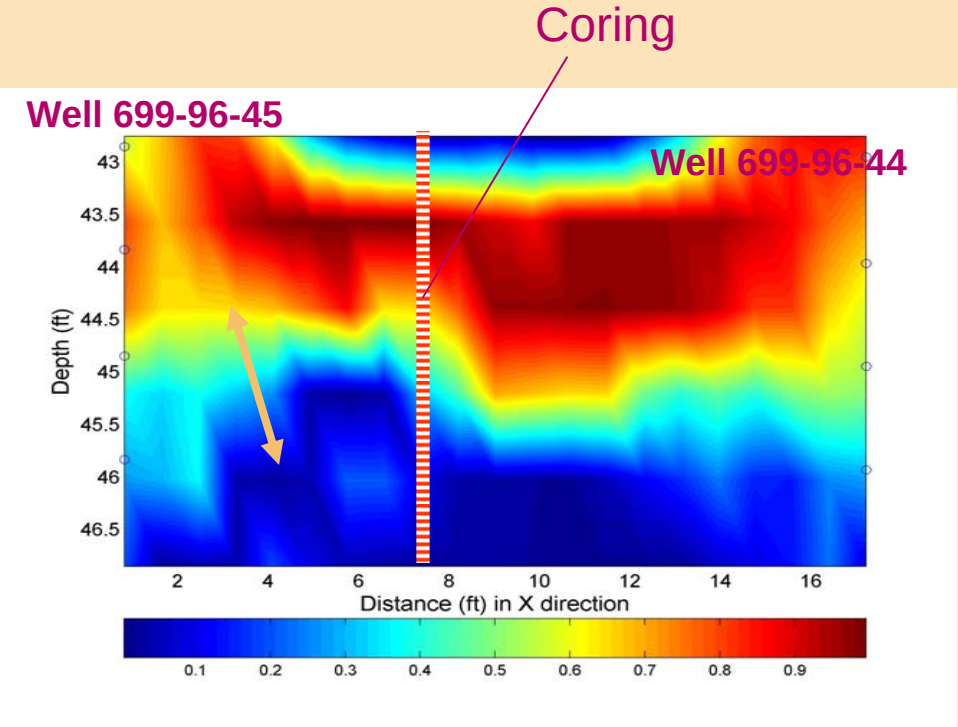
- General bacterial biomarkers indicate rapid enrichment in ^{13}C
- ^{13}C ratio is greater than expected (overall spiked HRC ratio was 15 per mil)
  - ^{13}C polylactate used as spike it is not esterified to glycerol backbone
  - it is released and consumed more rapidly
- Biomarkers for *Flavobacteriaceae*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

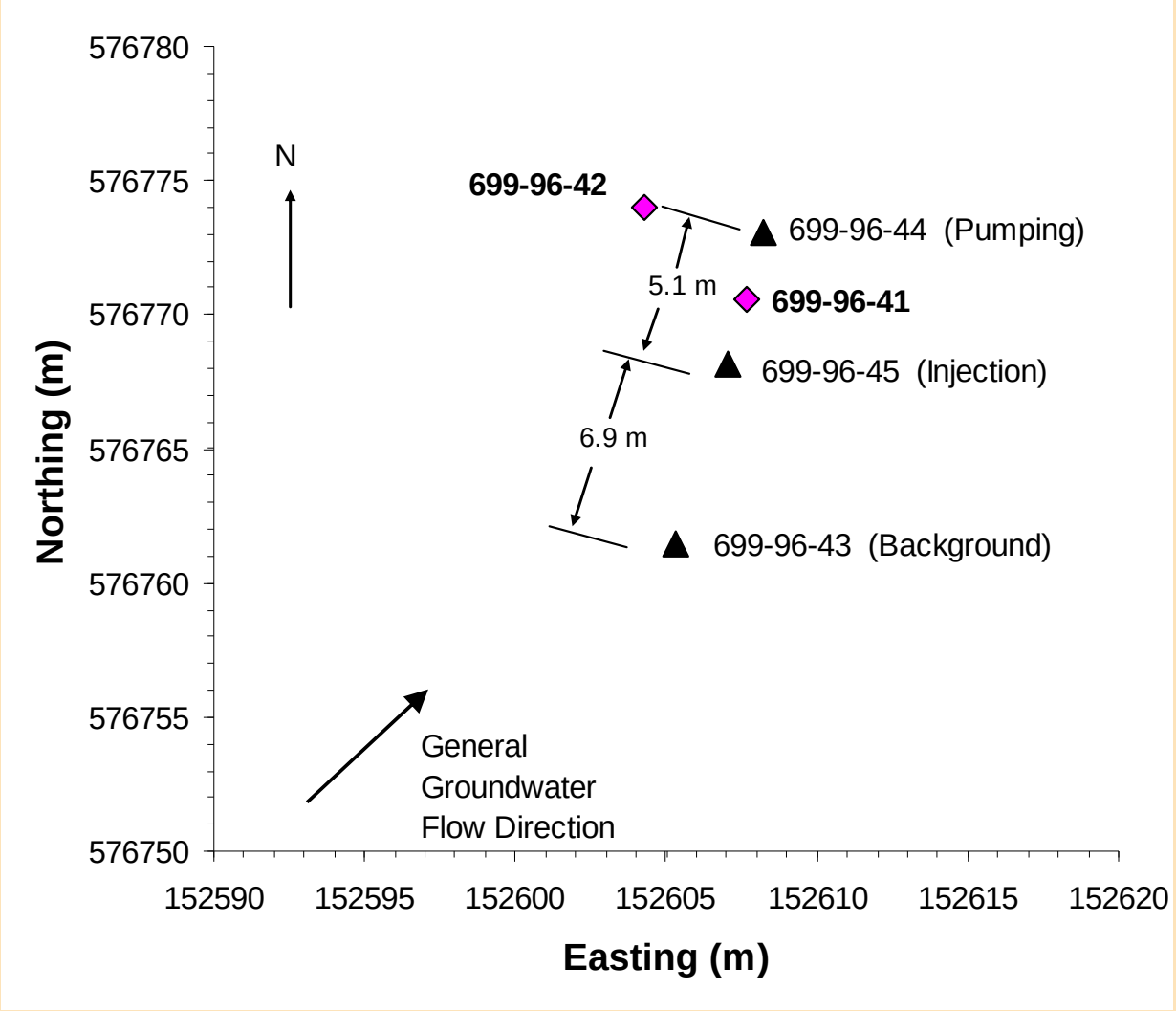
- Despite low initial microbial densities (<10^5 cells g^{-1}), HRC injection in the groundwater stimulated increase in the biomass up to 10^7 - 10^8 cells ml^{-1}
- Highly reducing conditions were achieved quickly with hierarchical depletion of electron acceptors O, NO_3, and Fe (III) (SO_4 was reduced 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.

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 installed over summer).



Layout of Wells at Cr Bioreduction Site



Contacts

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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