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

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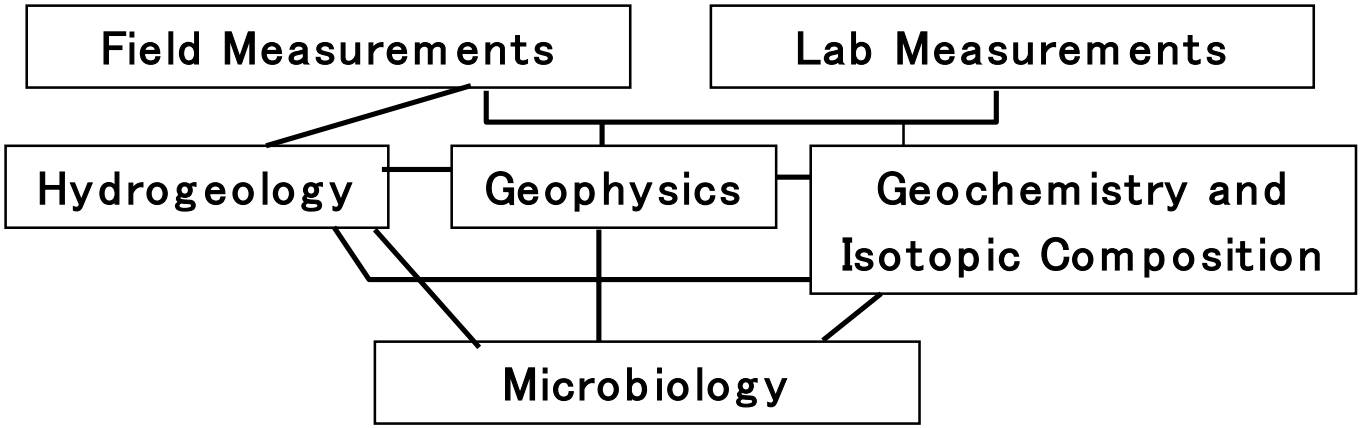
Overall Objective

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



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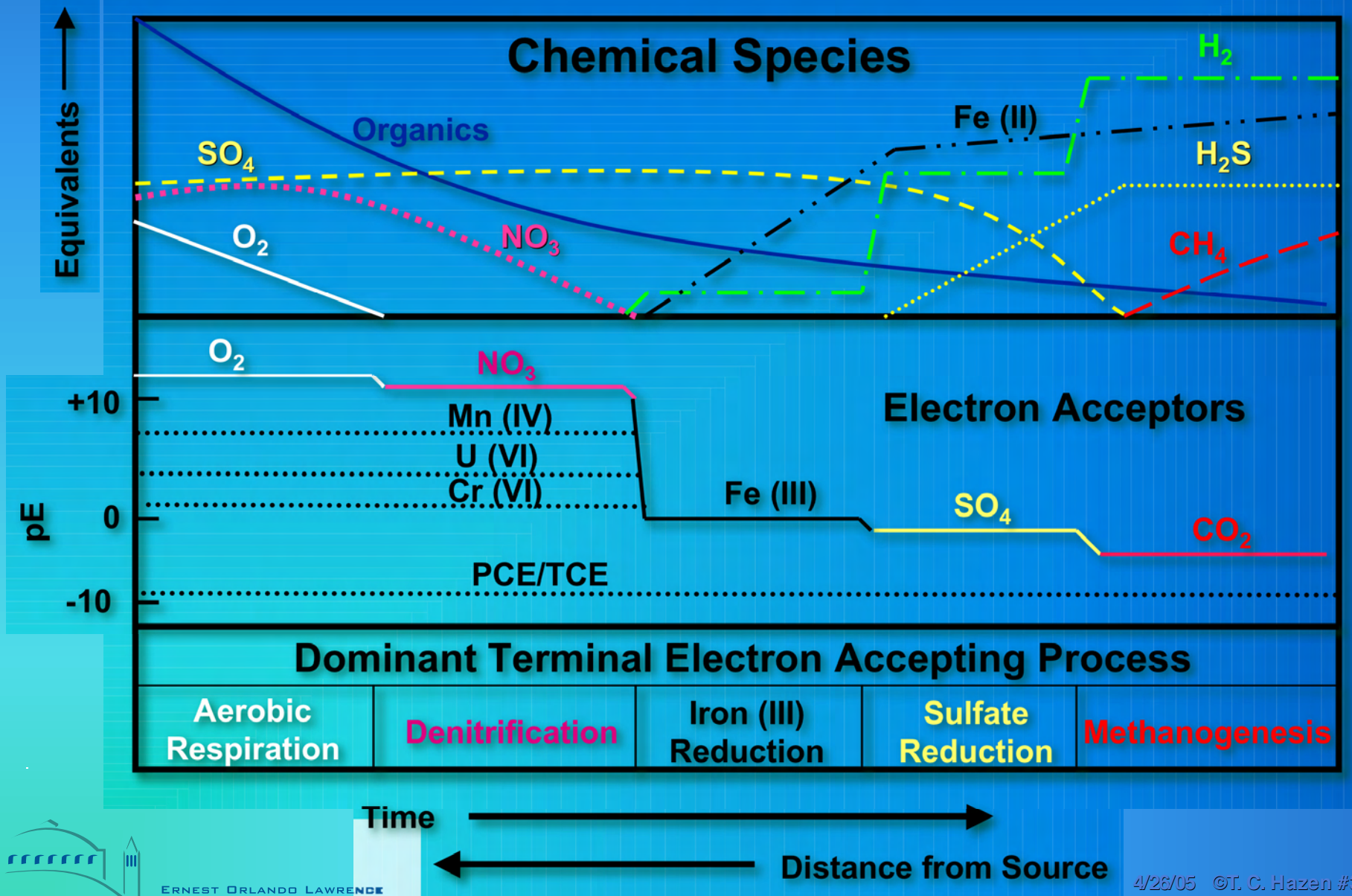
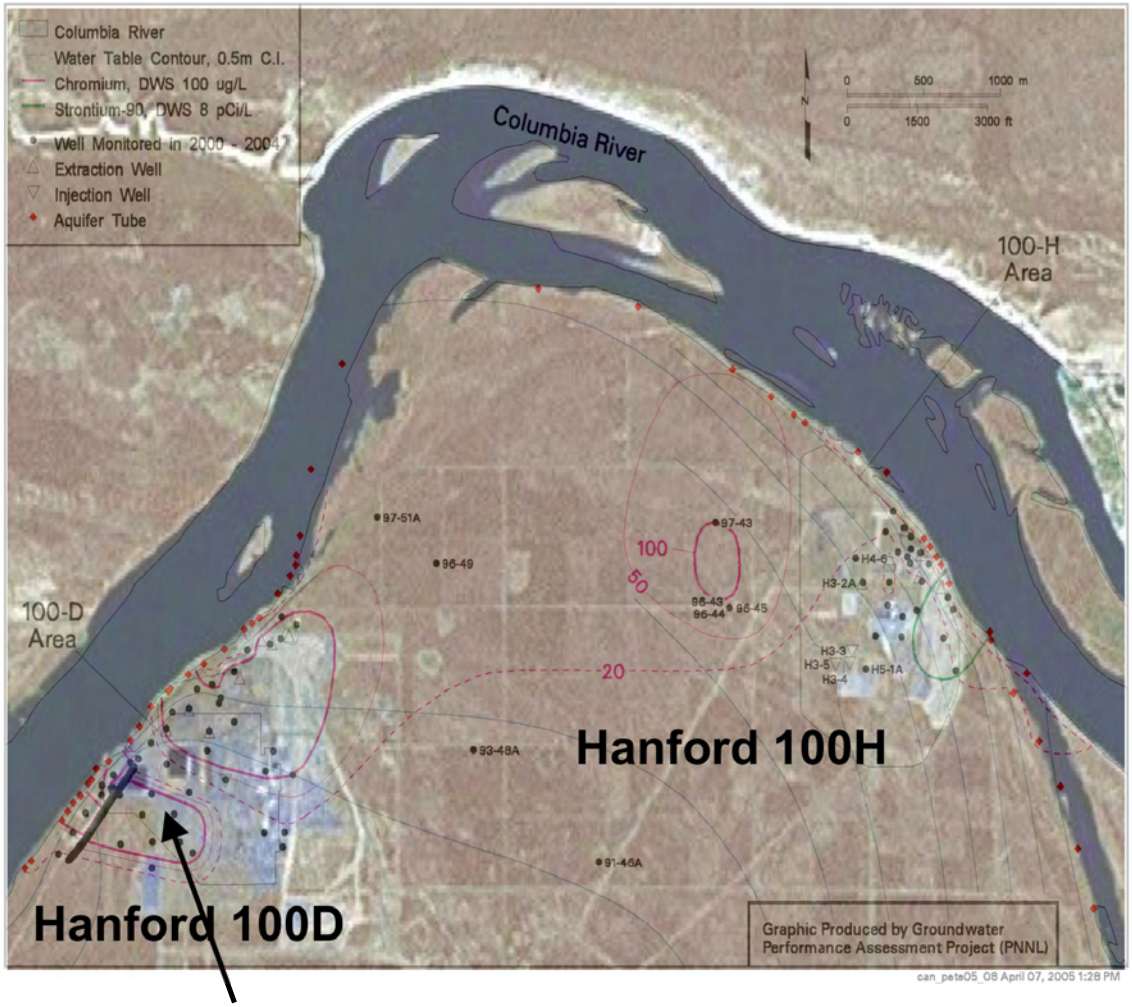
Critical Biogeochemistry

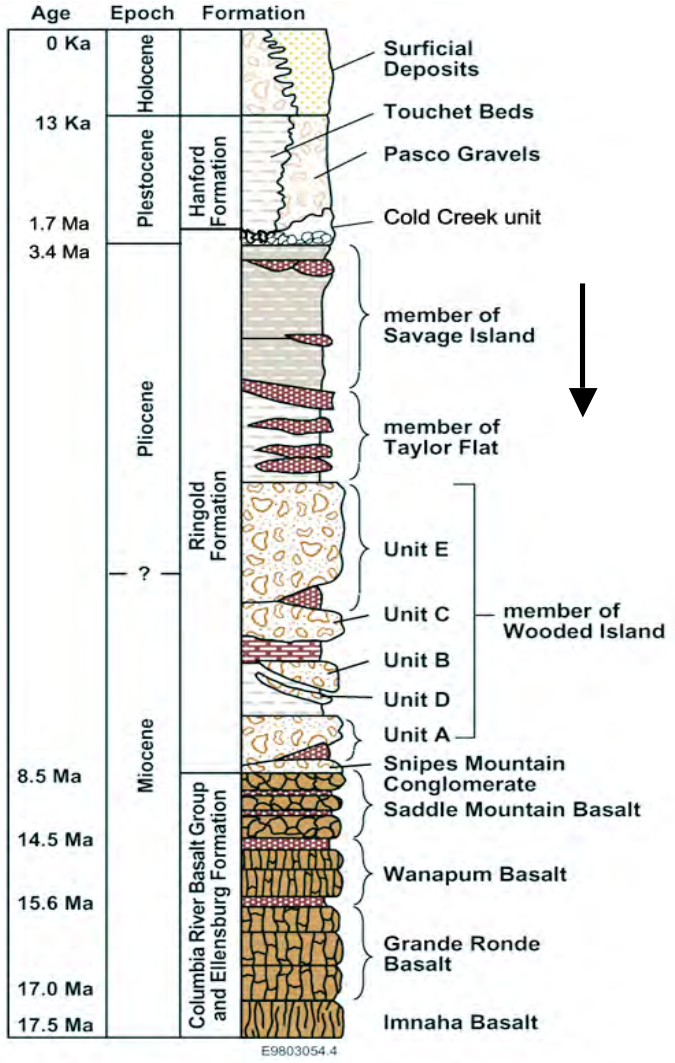


Hanford 100H Site Characterization

Cr Concentration Map

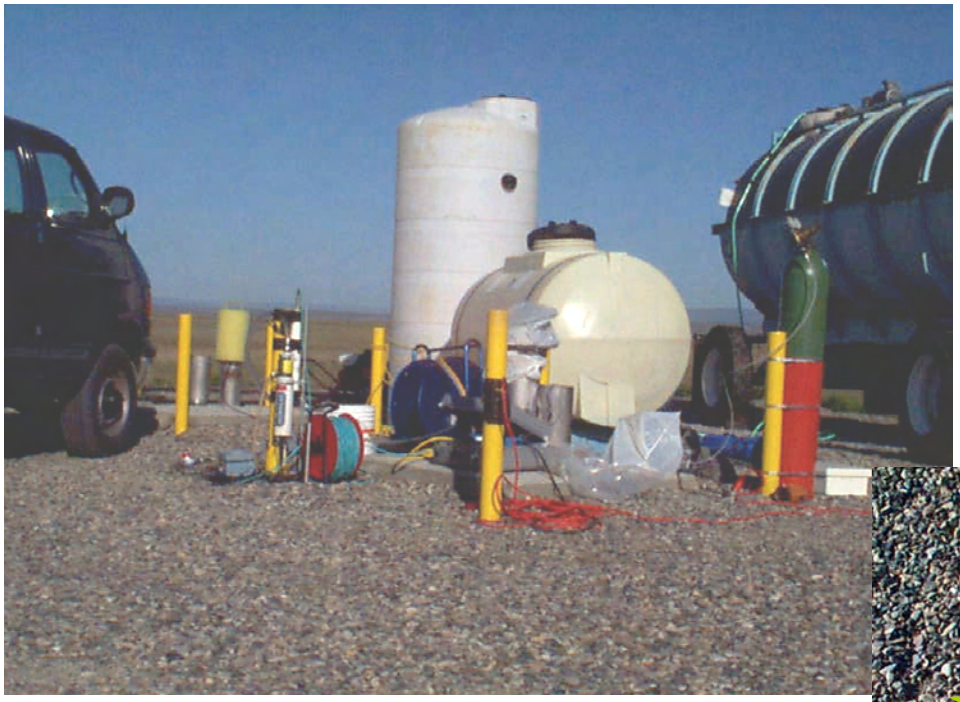


Lithological Column

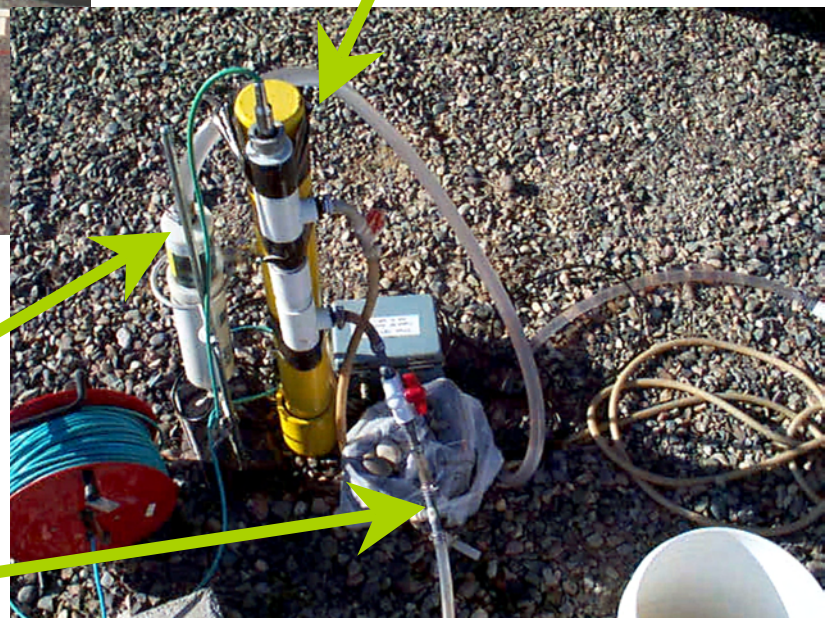


The Cr source is believed to be sodium dichromate ($Na_2Cr_20_7.2H_2O$)

On-Site Data Collection



Flow cell with Br sensor



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

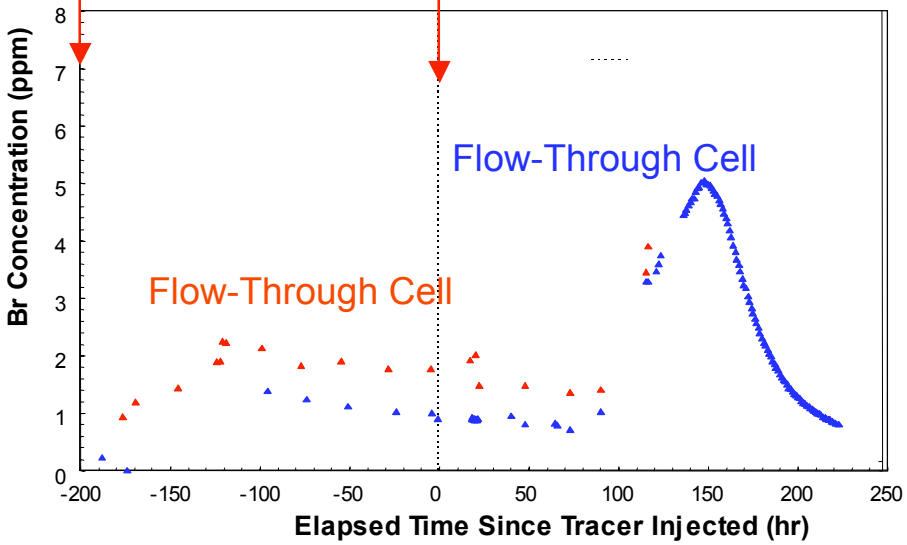
Sampling port

Br-Injection and Pumping Tests

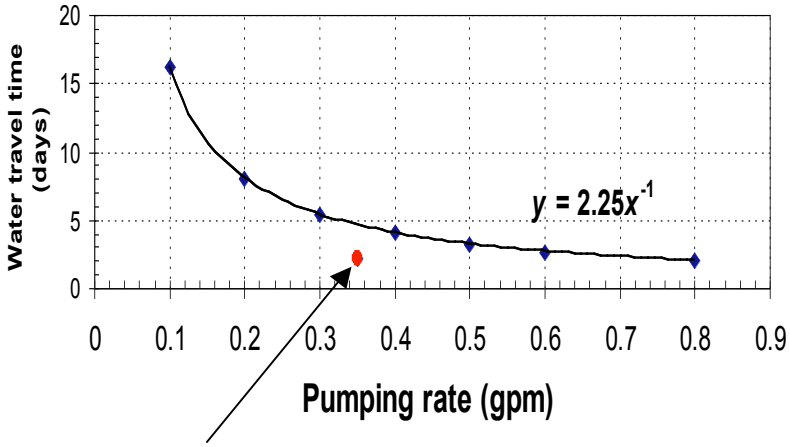
Pumping started

Tracer injection

Br Ion-Selective Electrode Measurements



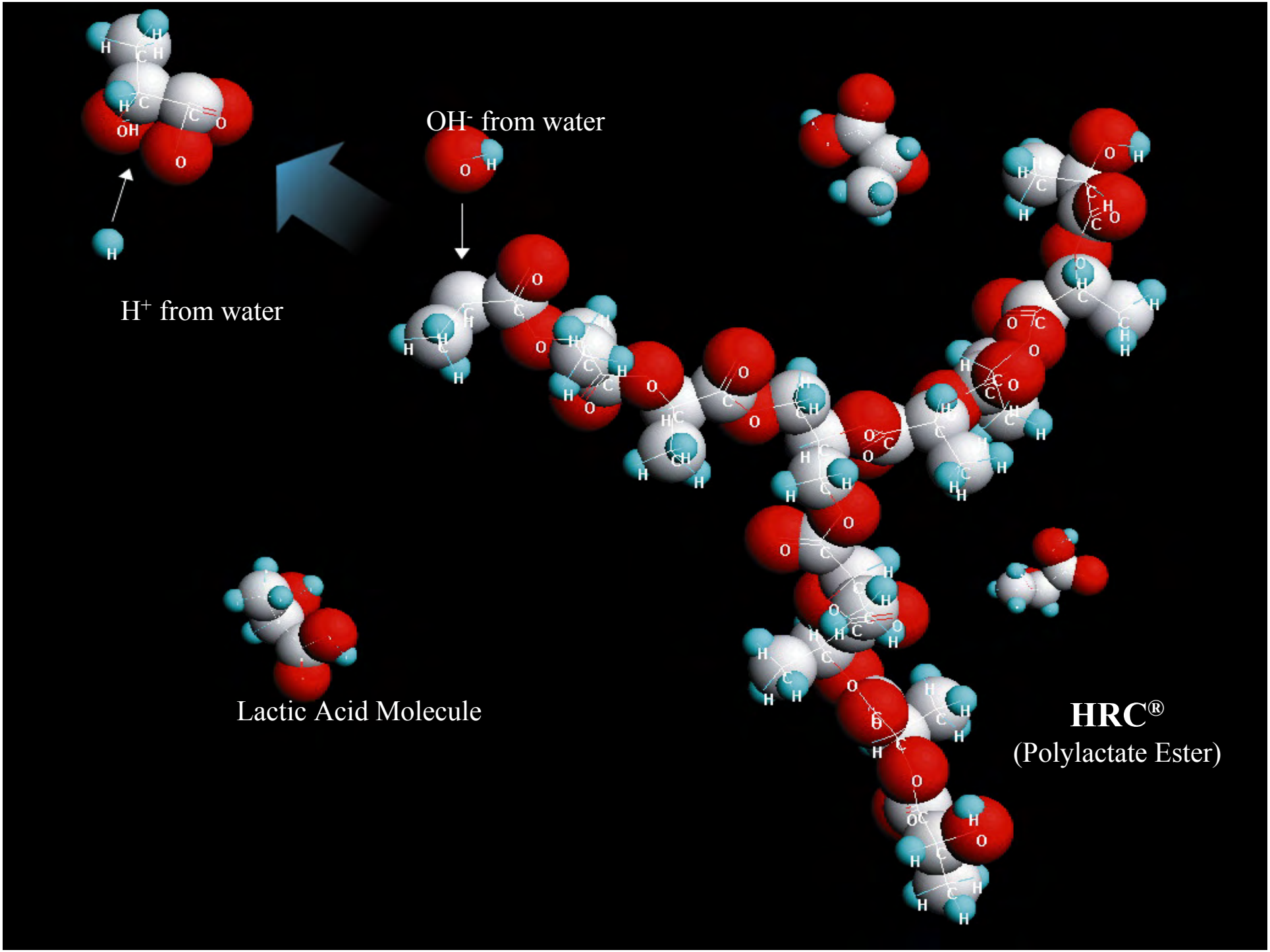
Water travel time between the injection and pumping wells vs. the pumping rate



For a distance of 4 m from the pumping well, q=0.35 gpm, water travel time is 2.75 days

K_s=3.7-7.4 m/day

Effective porosity = 0.2-0.26


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

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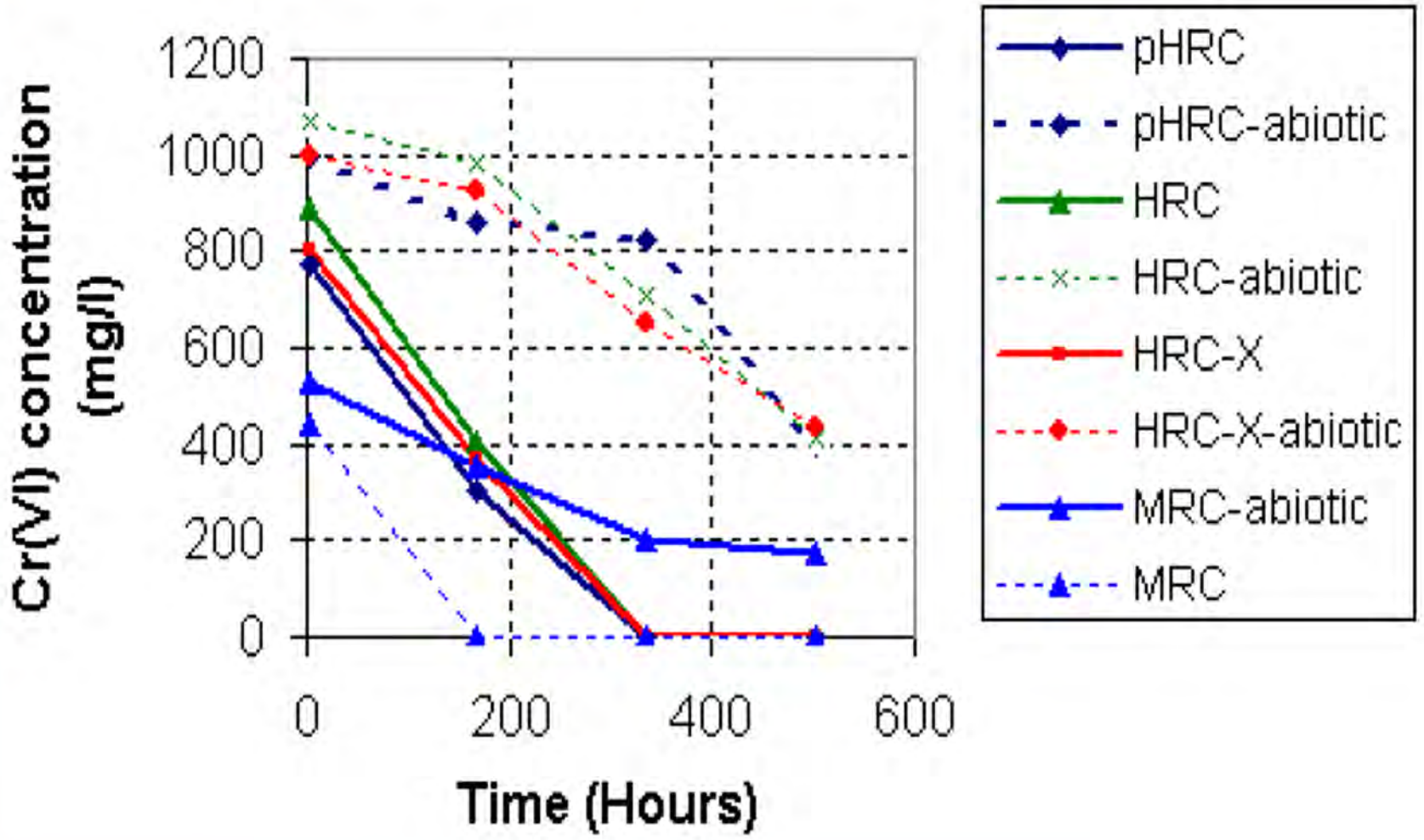
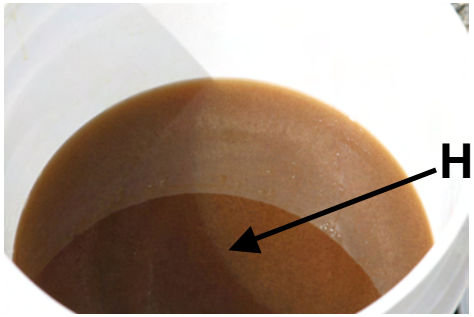
Lactate-Induced Bioreduction of Cr(IV)

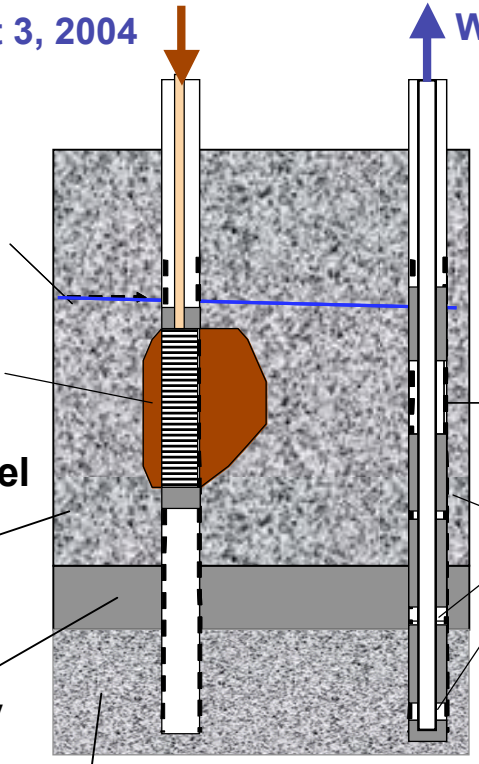


Field HRC Injection Test



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

Pumping - 27 days
Well 699-96-44



Groundwater level

Injection at depths of 44 ft to 50 ft

Pumping

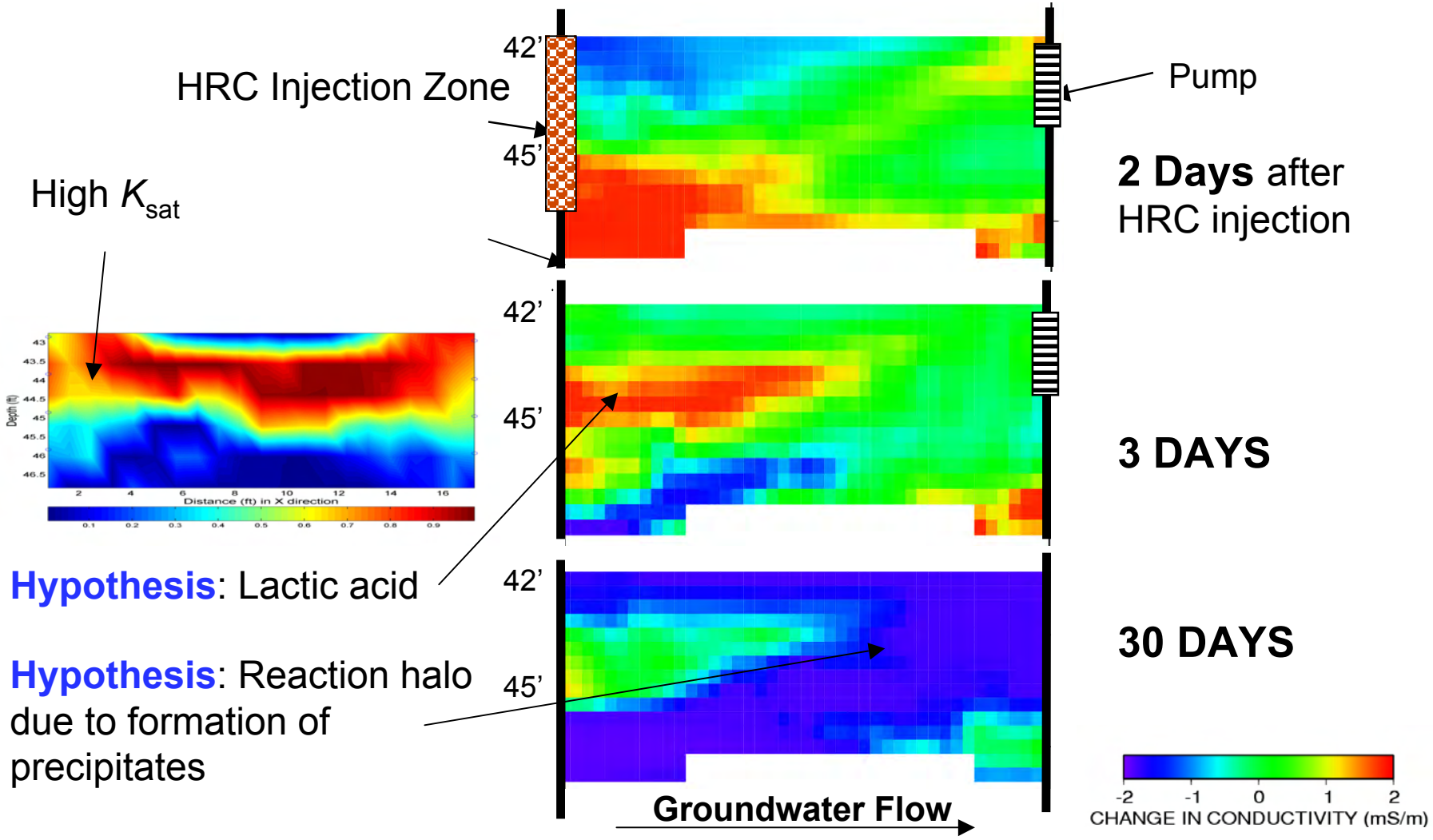
Hanford sandy gravel and gravelly sand

Water samplers

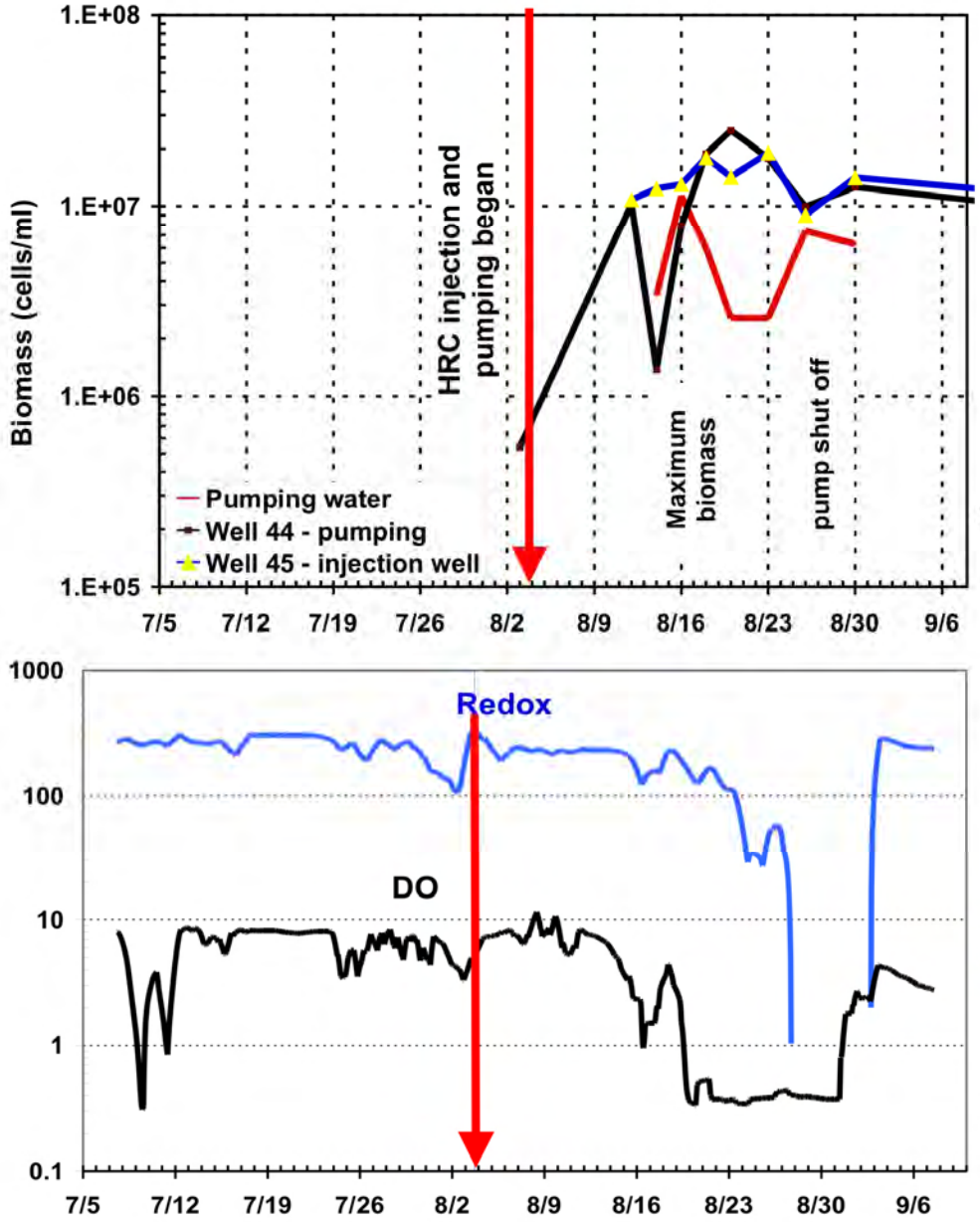
Ringold clay

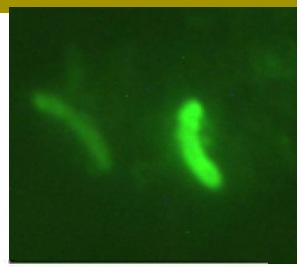
Ringold silt

Post-HRC Injection Changes in Electrical Conductivity

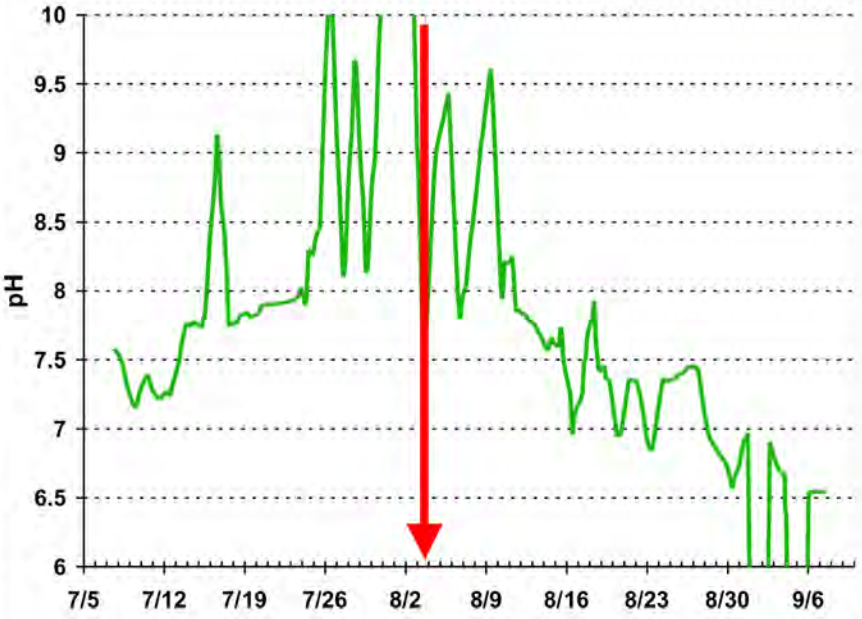


Results of HRC Biostimulation





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

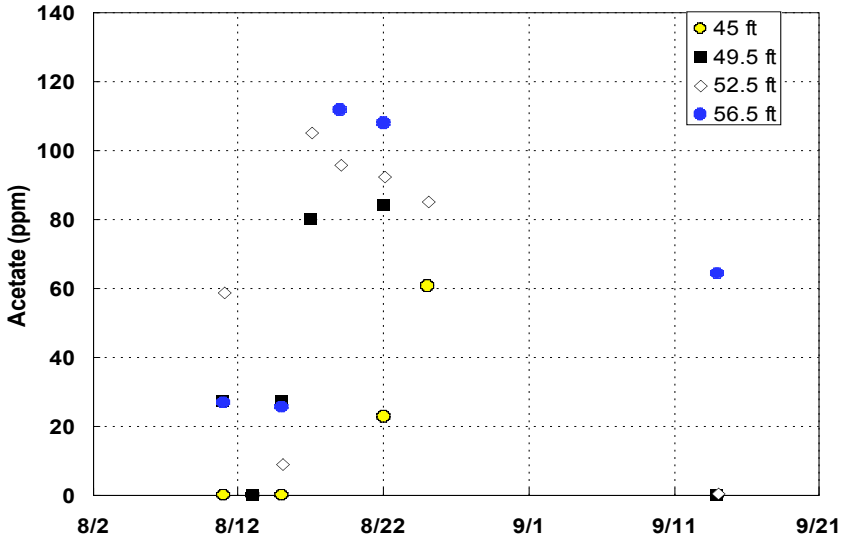


Redox dropped from 240 to -130 mV

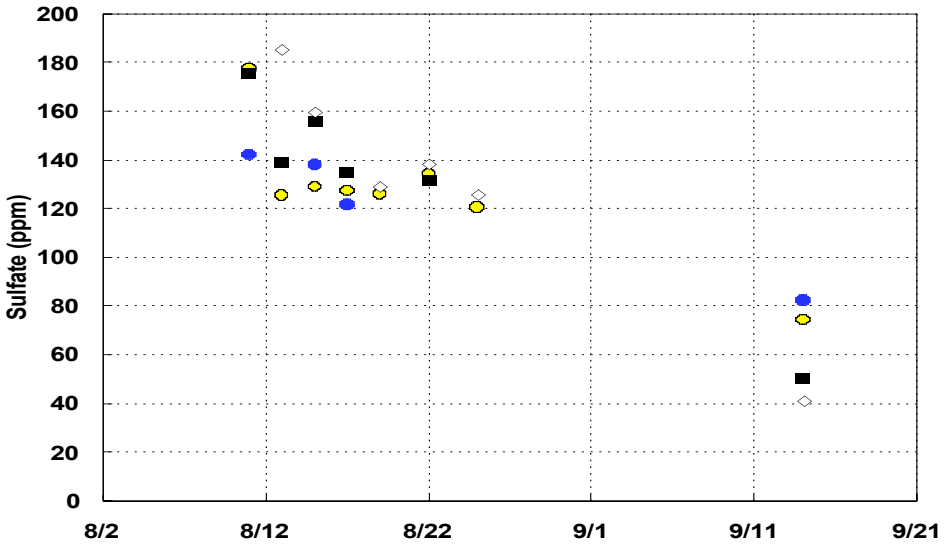
DO dropped from 9 mg/l (~100%) to 0.35 mg/l (4.5%)

Biogeochemical Evidence of Microbial Metabolism in Groundwater

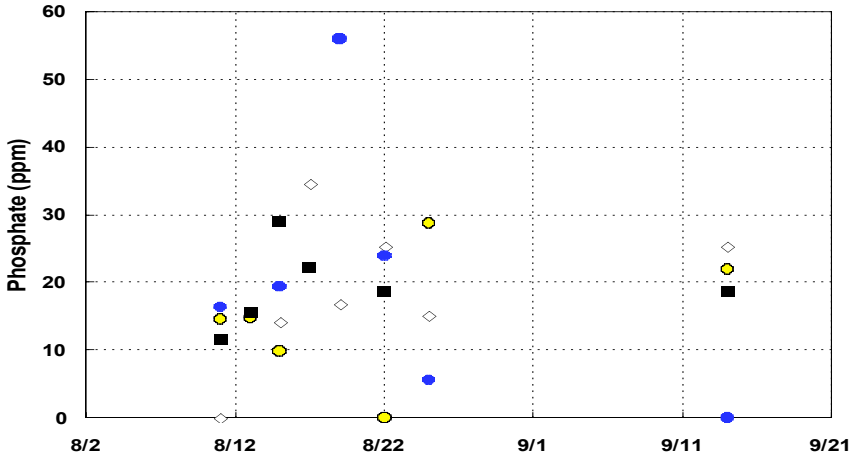
Acetate increase



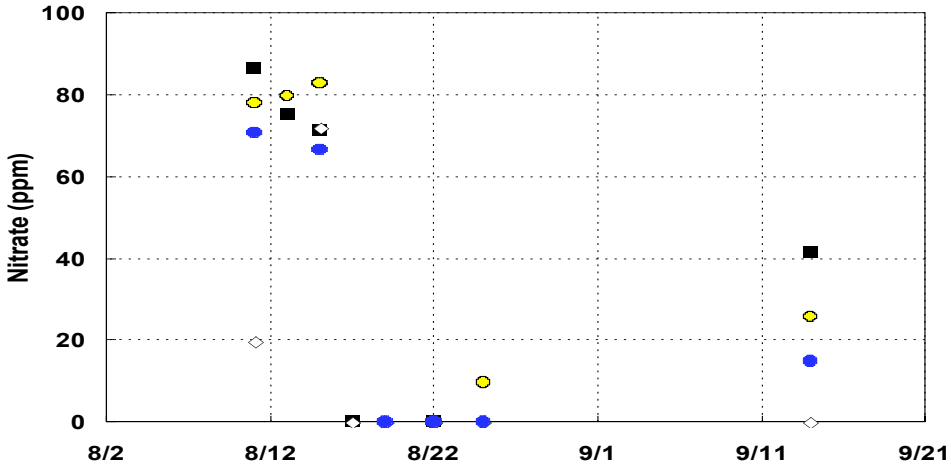
Sulfur reduction



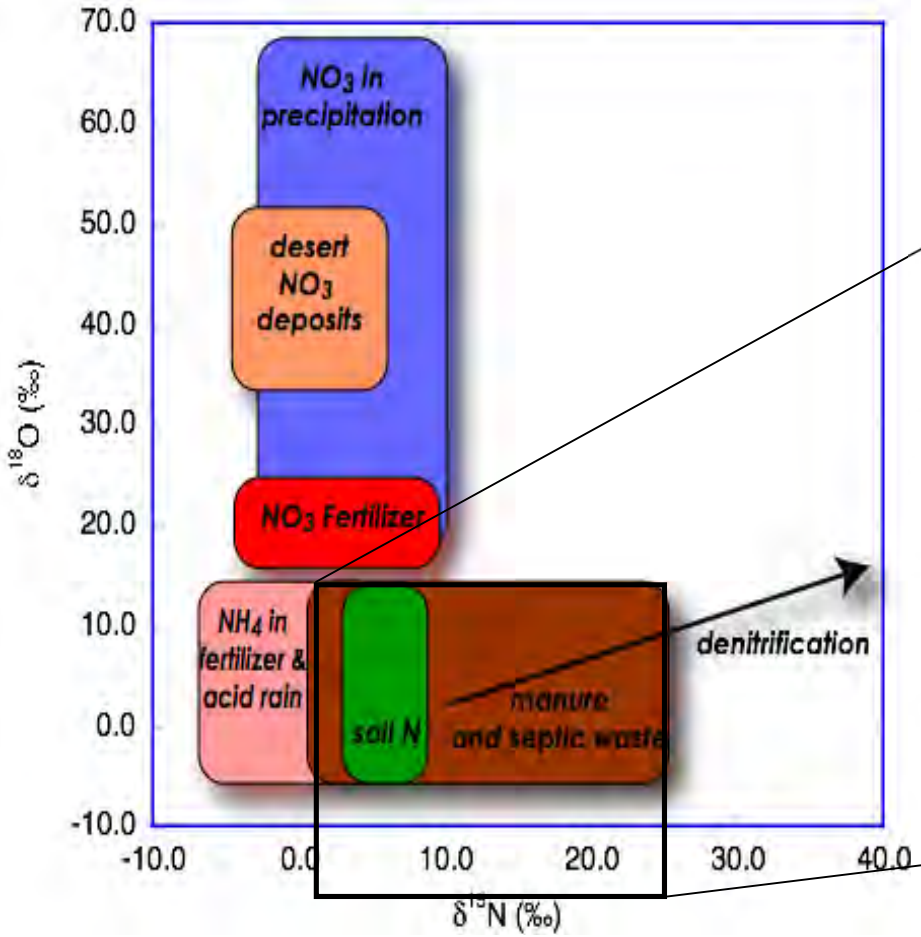
Phosphate increase

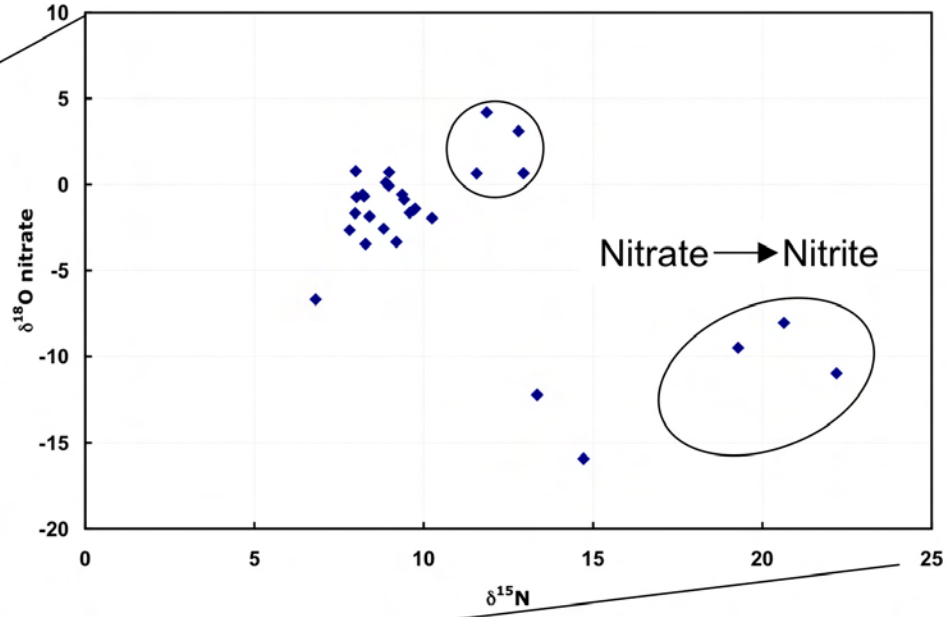


Nitrate decrease



Biogeochemical Evidence of Microbial Metabolism in Groundwater

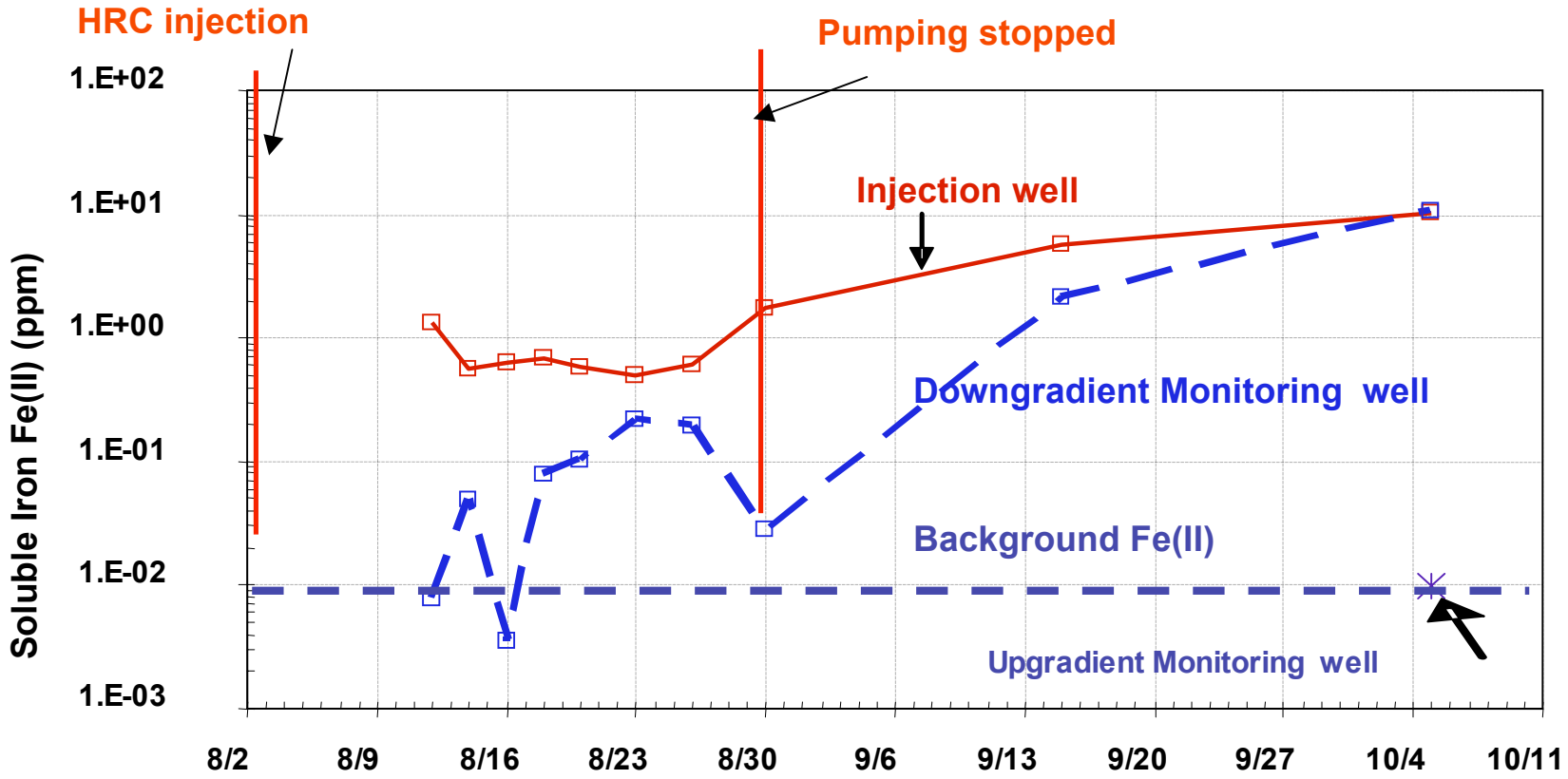




Kendall and McDonnell, 1998

Isotopic Composition of Nitrate Indicates Denitrification

Geochemical Evidence of Microbial Metabolism in Groundwater



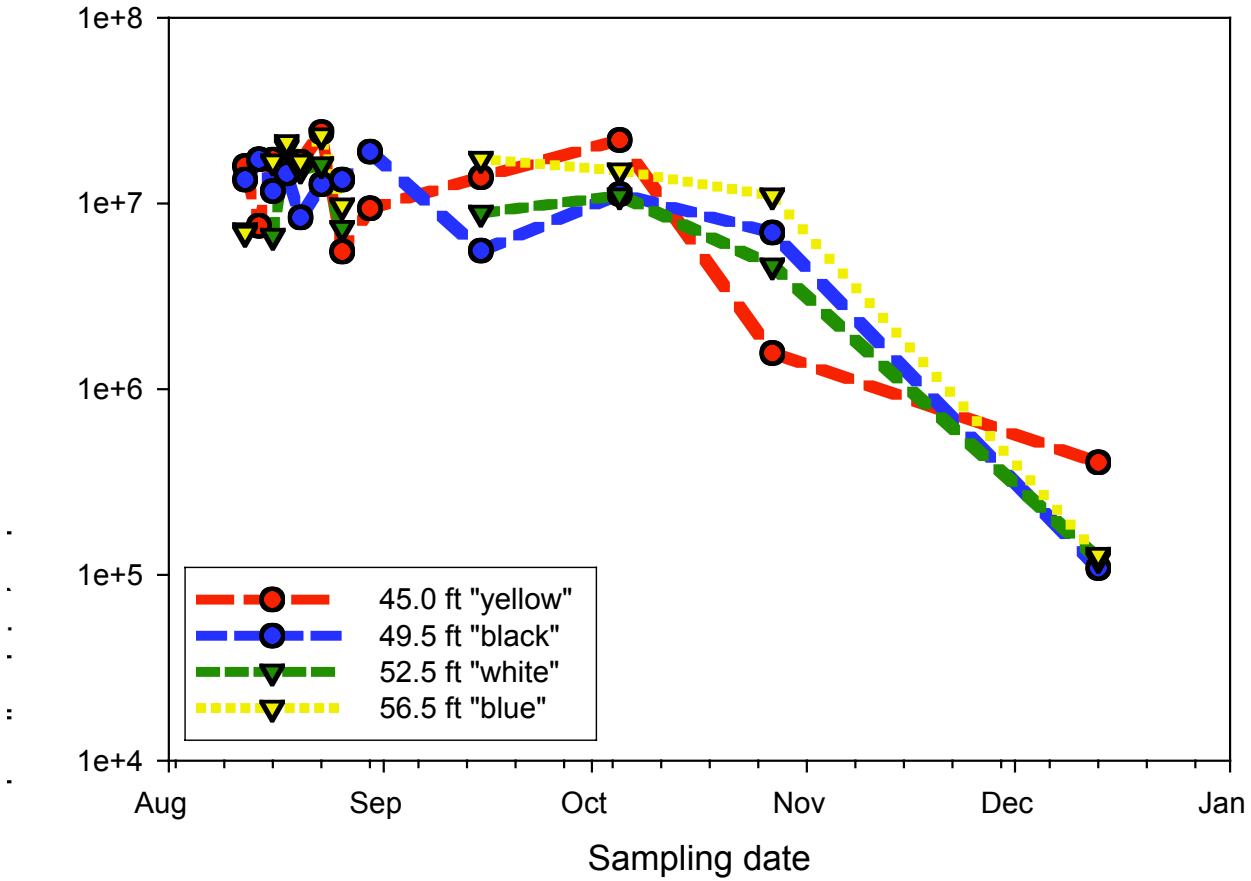
Fe(III) + Microbial reduction → Fe(II)

Increase in Ferrous Concentration after the HRC Injection

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

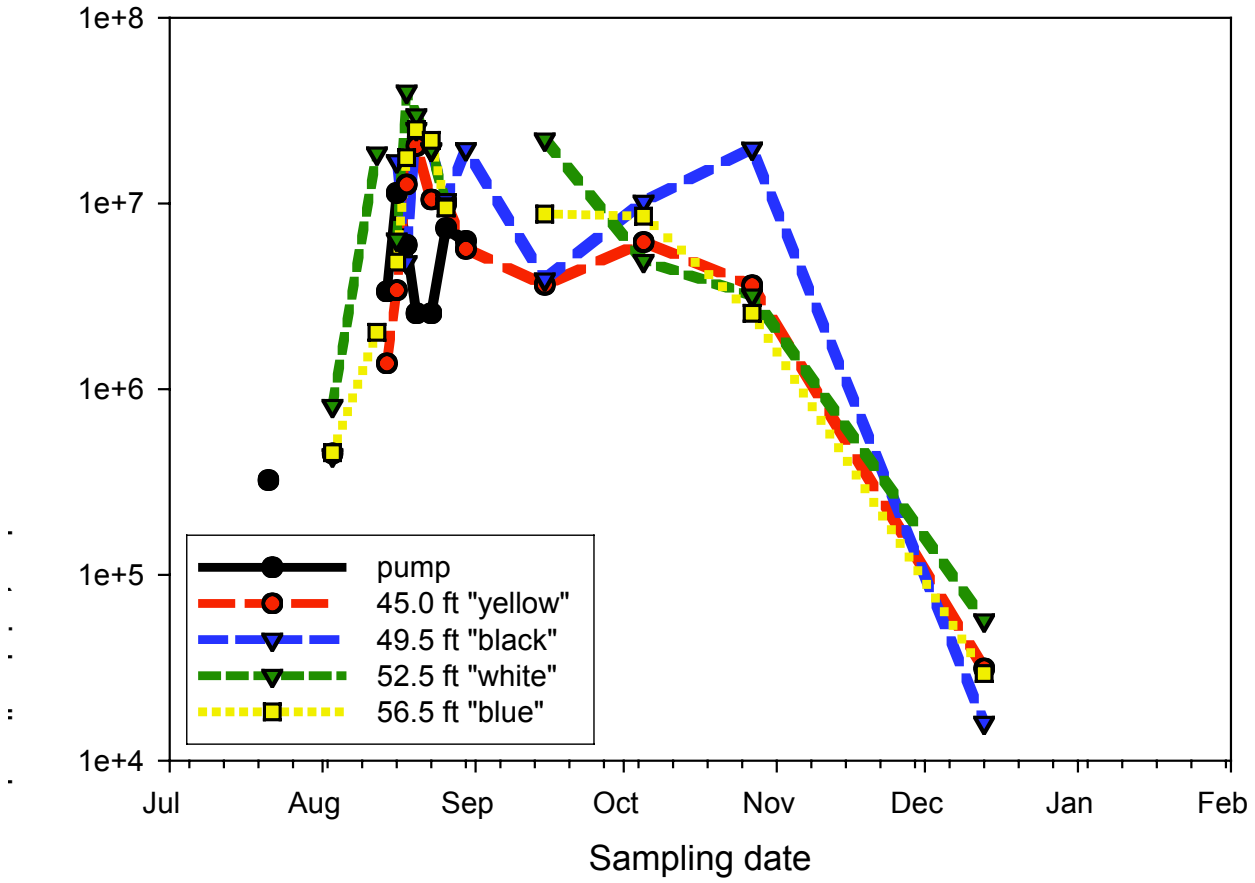
Acridine orange direct counts:

Injection well - 45

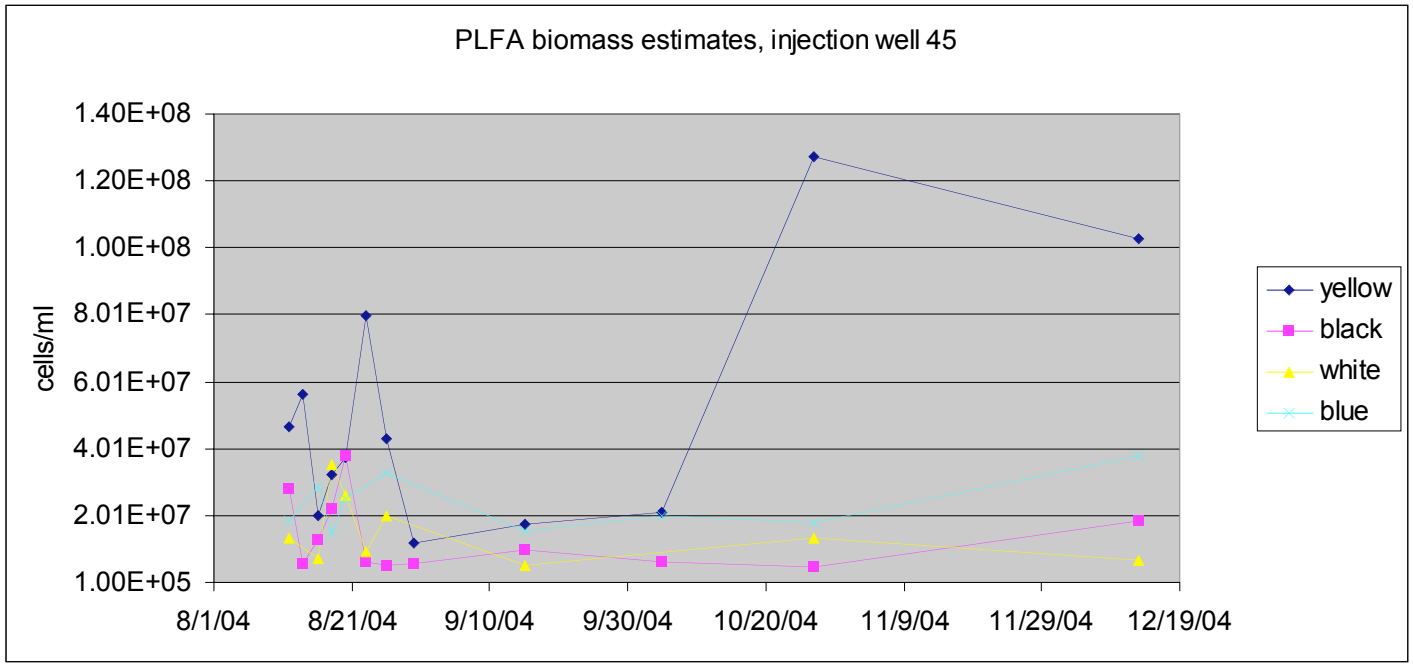


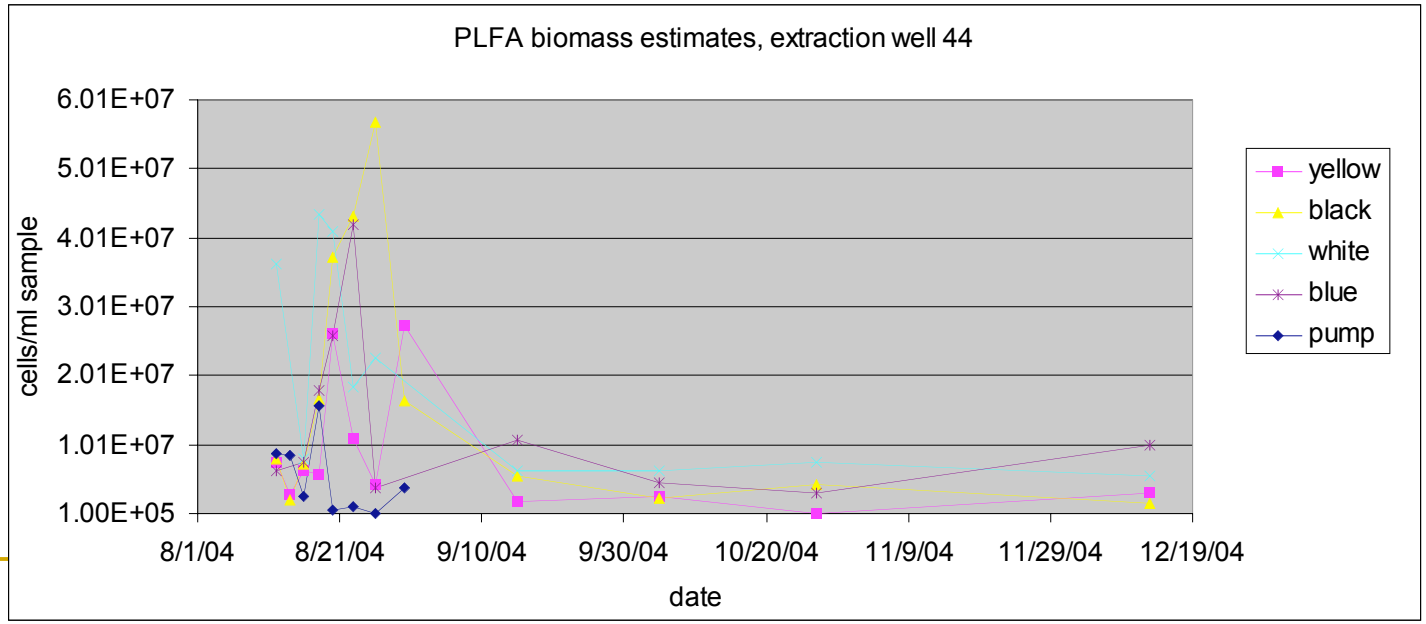
Acridine orange direct counts:

Monitoring well - 44

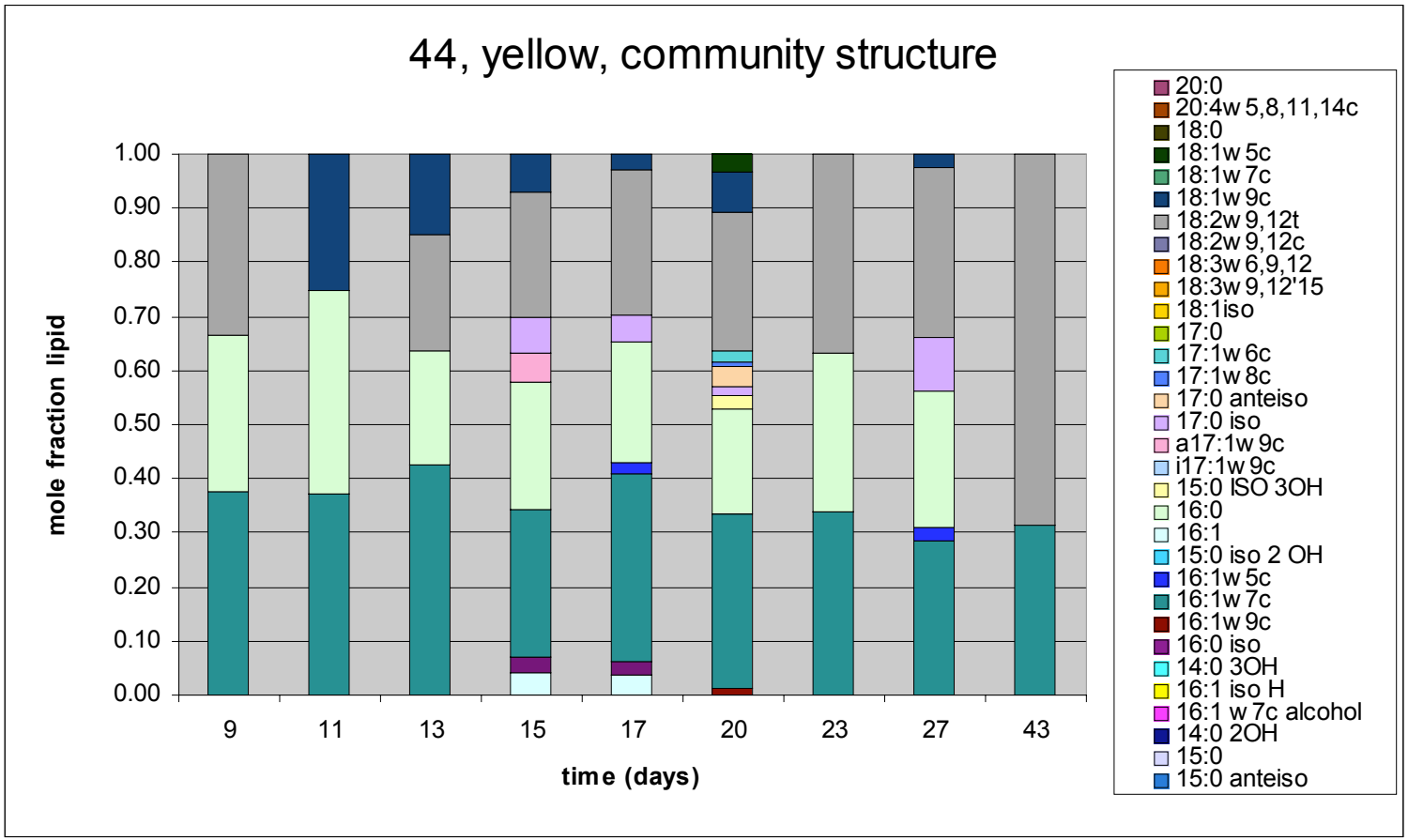


PLFA biomass estimates

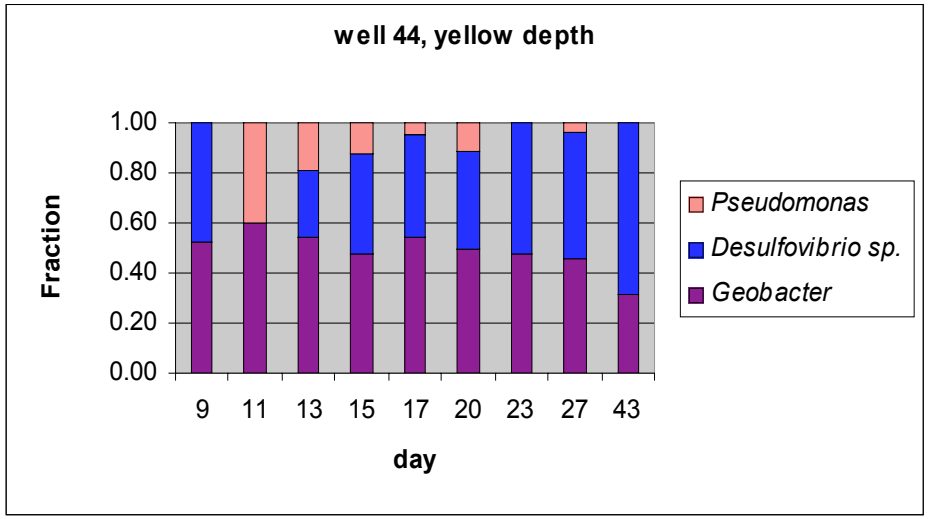


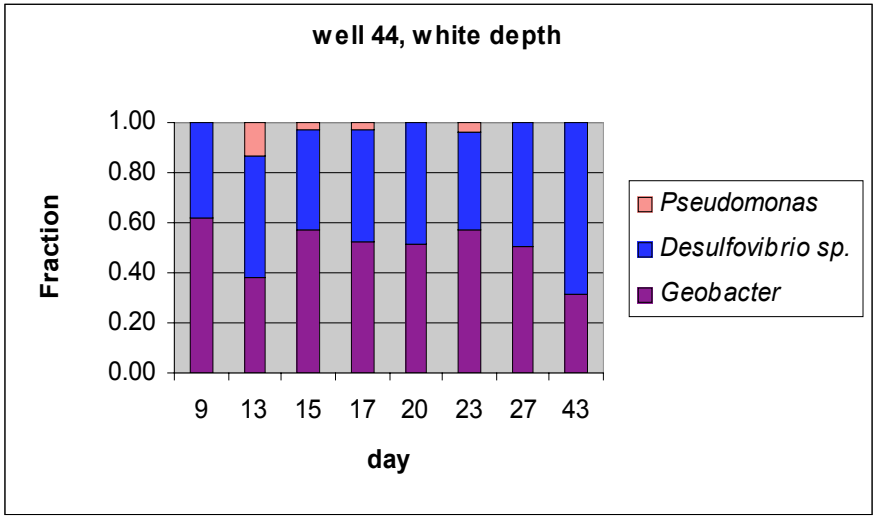


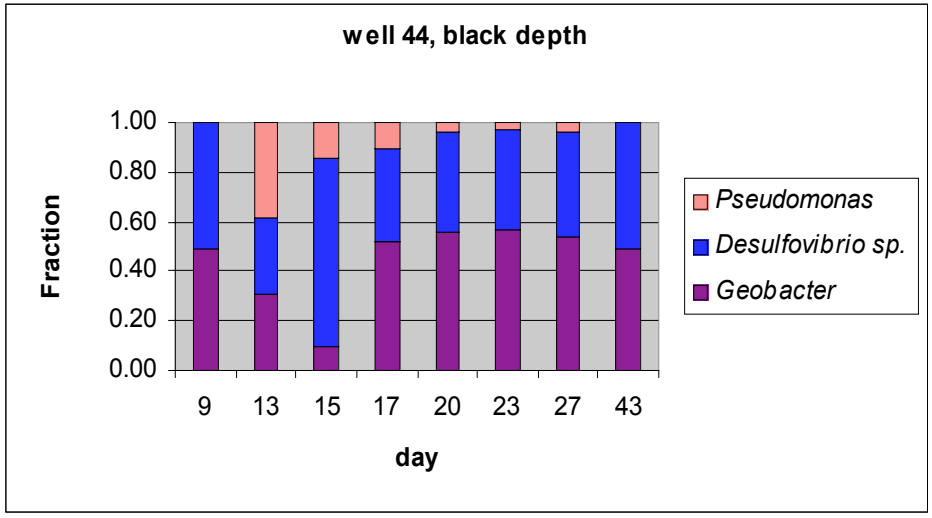
PLFA: Community structure

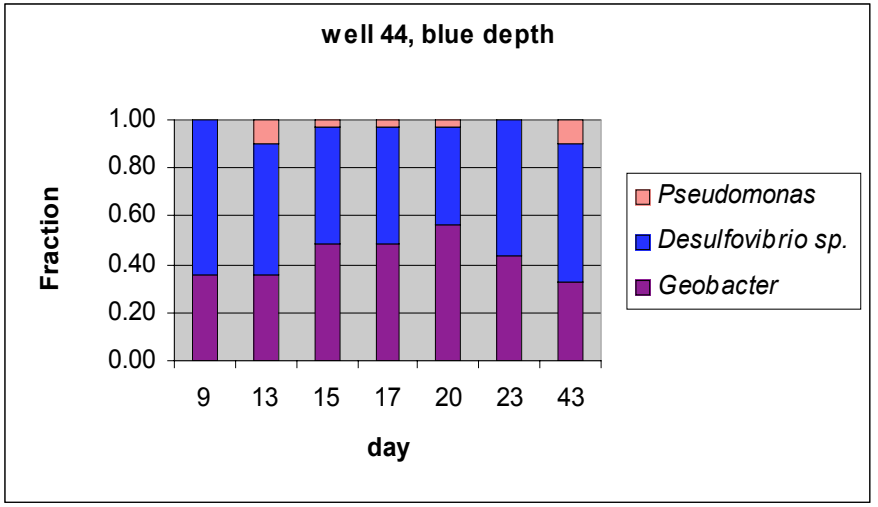


PLFA: Selected Biomarkers









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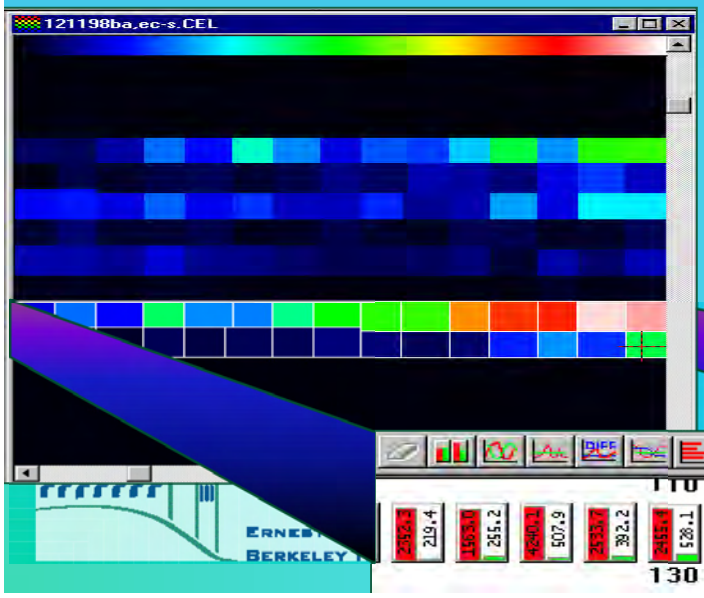
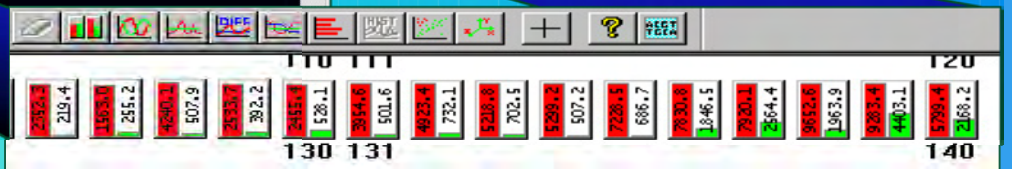
DOE 16s rDNA microarray

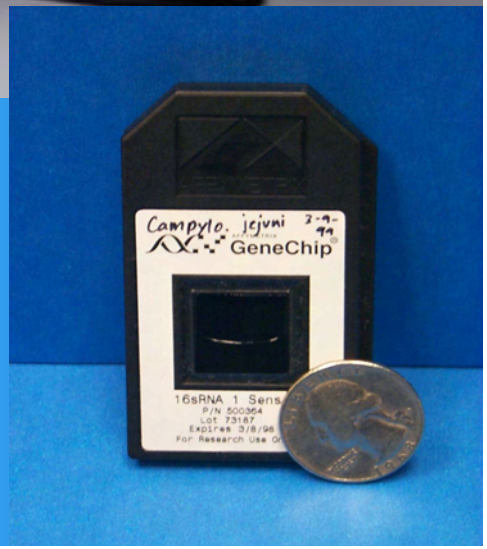
- Rapidly detect the composition and diversity of microbes in an environmental sample
- Massive parallelism - 550,000 probes in a 1.28 cm^2 array
- all 9,900 species in 16S rDNA database
- Single nucleotide mismatch resolution



cctagcatgCattctgcata
cctagcatgGattctgcata

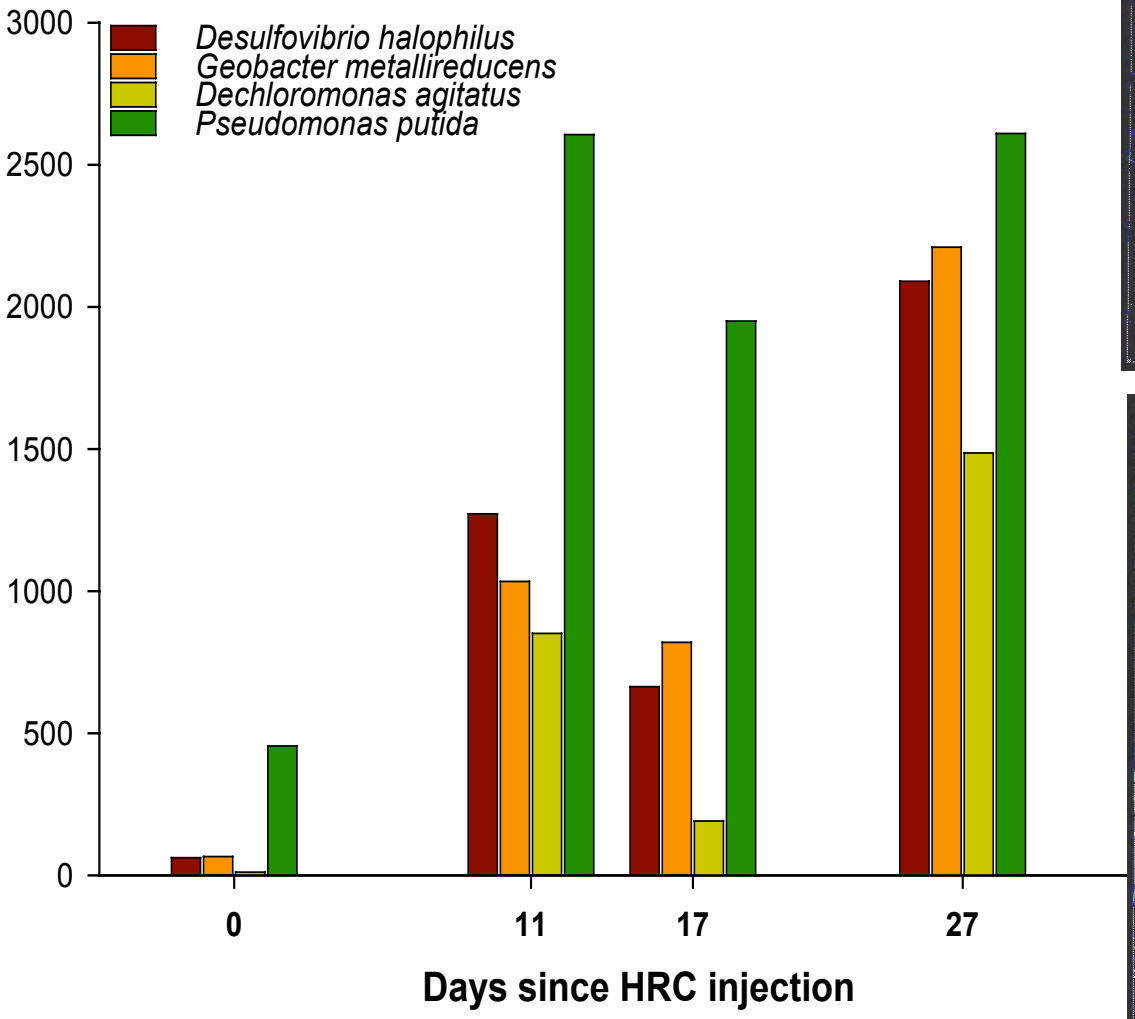
MATCH
MISMATCH

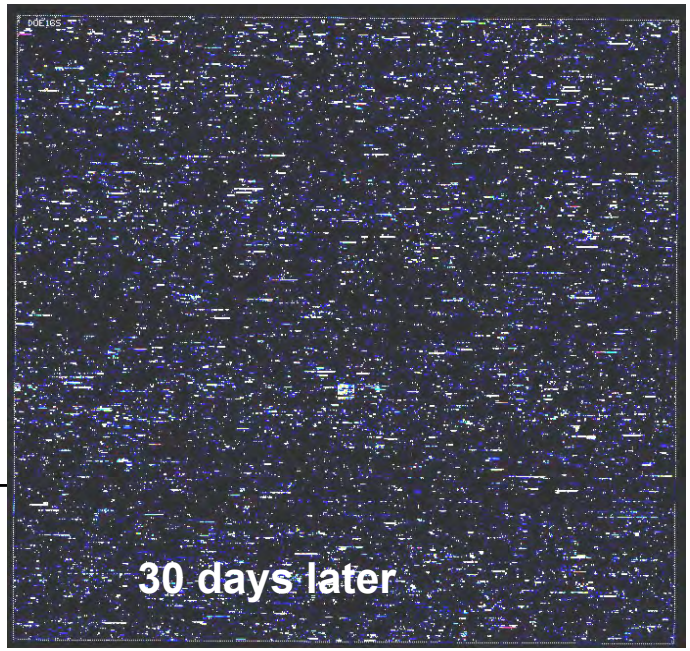




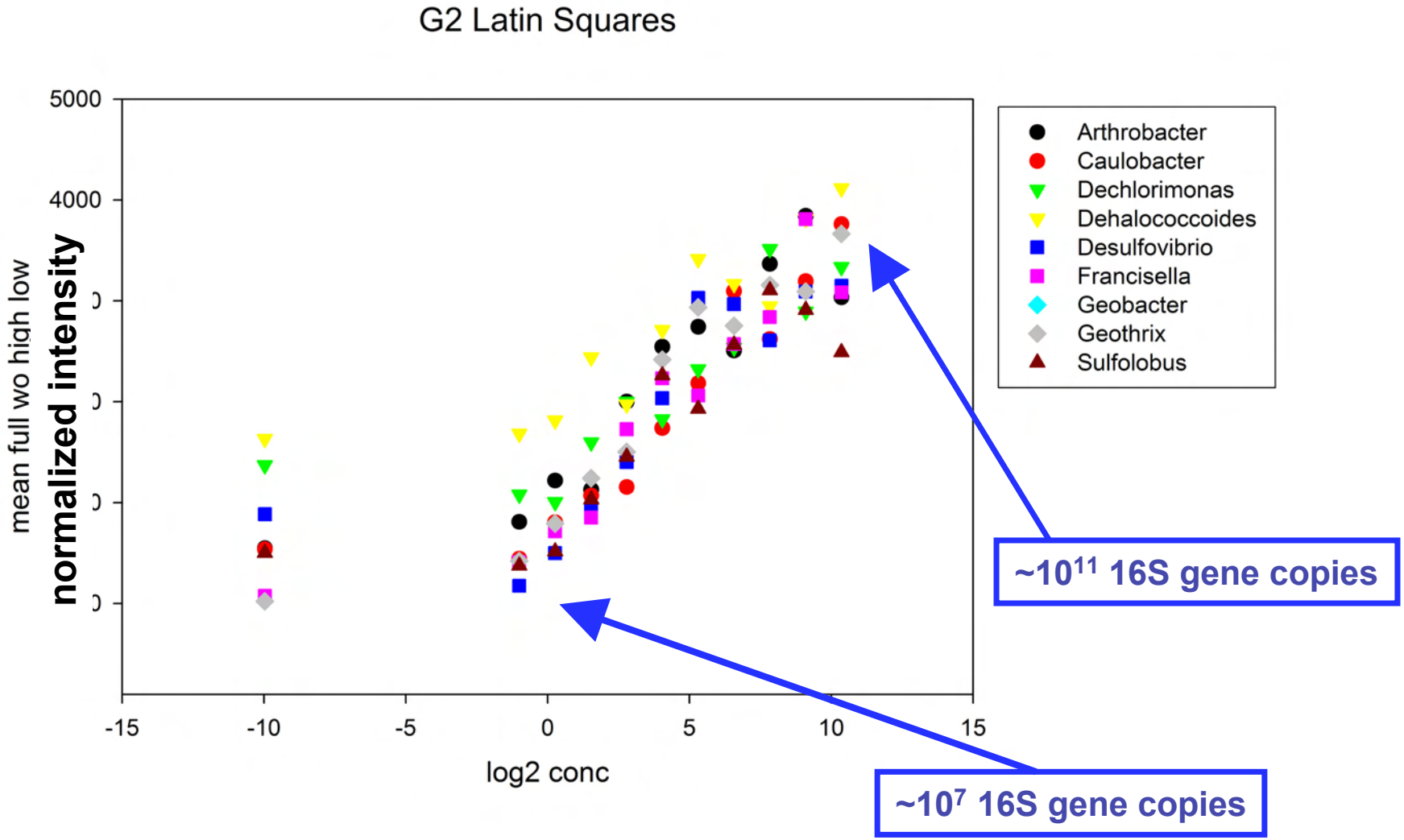
Microarray analysis of bacterial community changes during Cr(VI) remediation at Hanford 100H site:

Dynamics of some significant organisms.





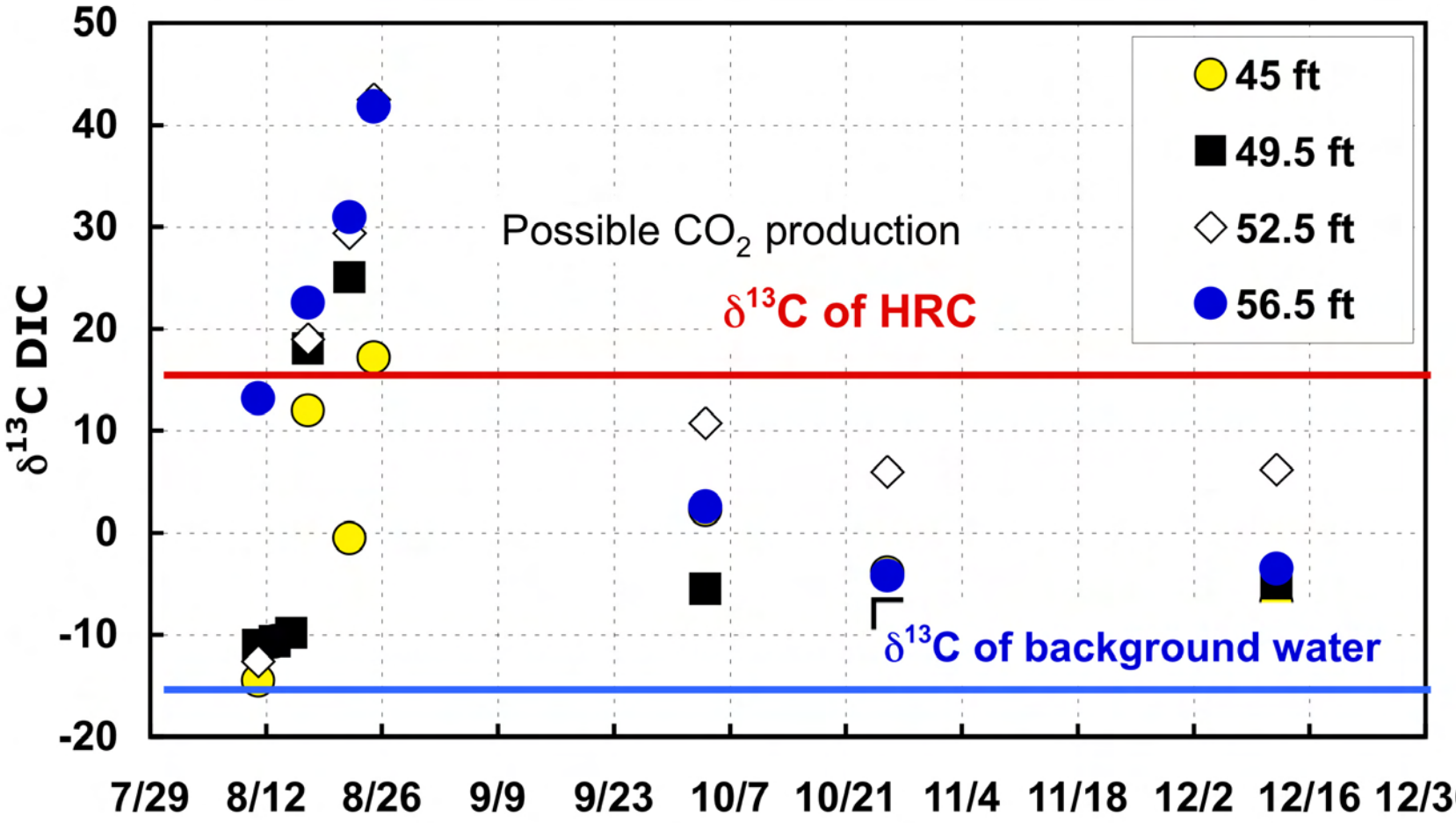
16S Microarray is Quantitative:
when comparing same OTU over time/treatment



16S rDNA GeneChip

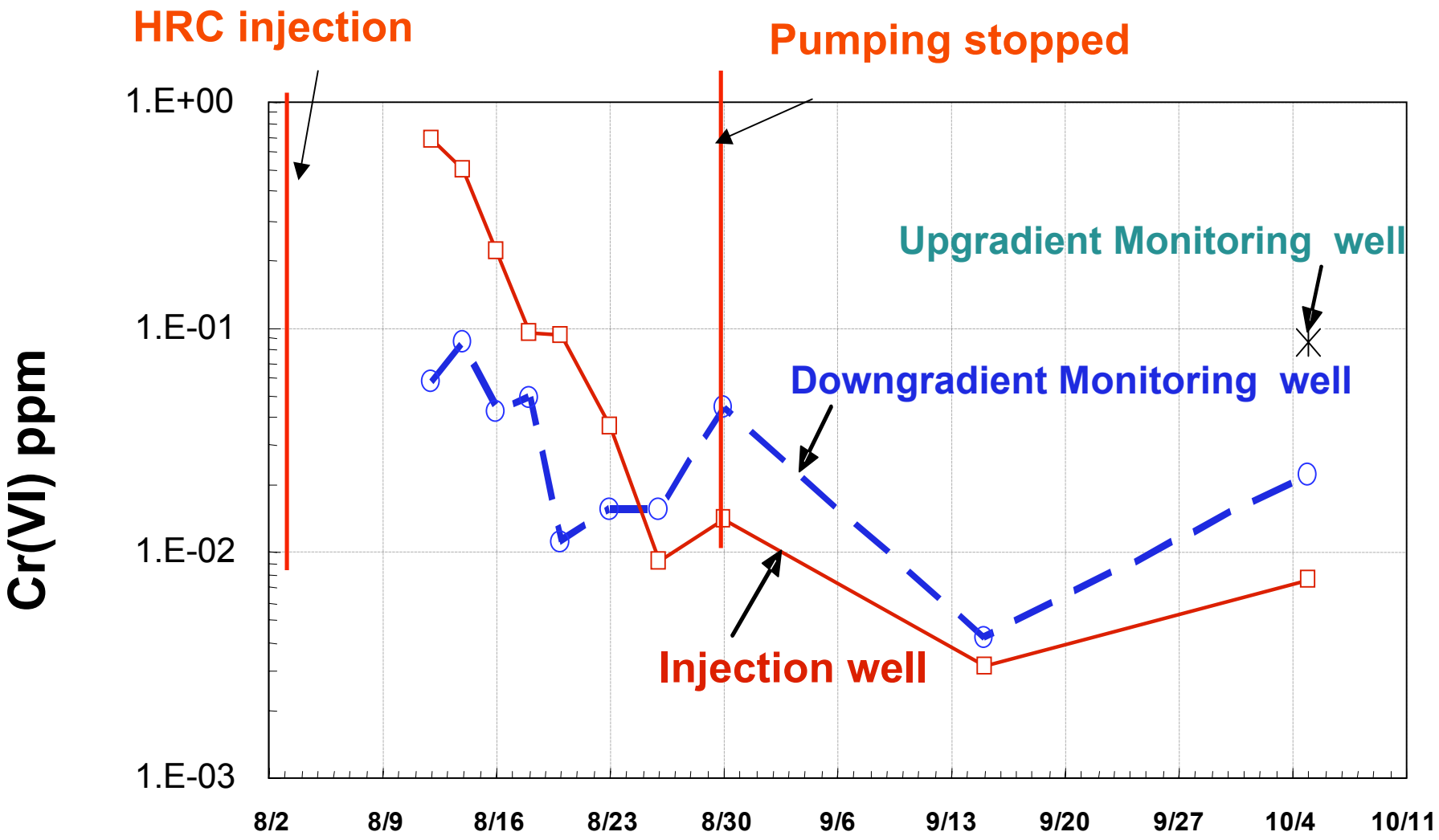
- Archaea
  - Only Crenarchaeotes (non-thermophilic) detected
    - Dominated by one type – no cultured relative.
- Bacteria
  - Initial enrichment of denitrifiers
    - *Fulvimonas, Pseudomonas, Hyphomicrobium, Acidovorax, Aquaspirillum,Thauera, Azoarcus, Comamonas, Dechloromonas, Clostridium*.
  -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

Biogeochemical Evidence of Microbial Metabolism in Groundwater



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

Changes in Cr(VI) Concentrations after the HRC Injection



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 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.

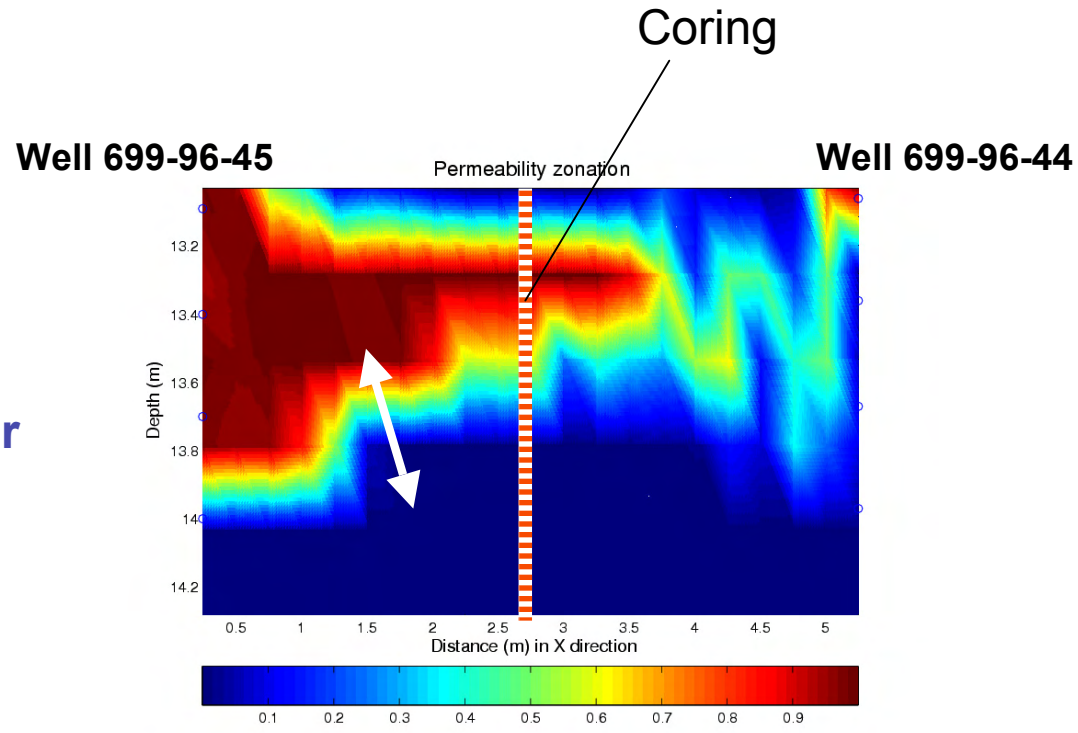
6. $\delta^{13}C$ ratios in dissolved inorganic carbon confirmed microbial metabolism of HRC. $\delta^{13}C$ ratios remain above background values after 6 months. Increases in carbon isotope ratios of DIC in Well 44 are coincident with increases in bromide, chloride and acetate and decreases in nitrate. 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.

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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Hanford 100H Chromium Project

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

Center for Environmental Biotechnology

http://www-esd.lbl.gov/CEB

Environmental Remediation Technology Program

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

Ecology Department

http://www-esd.lbl.gov/ECO

DOE Natural and Accelerated Bioremediation Research Program

http://www.lbl.gov/NABIR