Hanford 100H Cr(VI) long-term Bioimmobilization

Terry C. Hazen

DOE BER Distinguished Scientist

Head, Ecology Department

Head, Center for Environmental Biotechnology

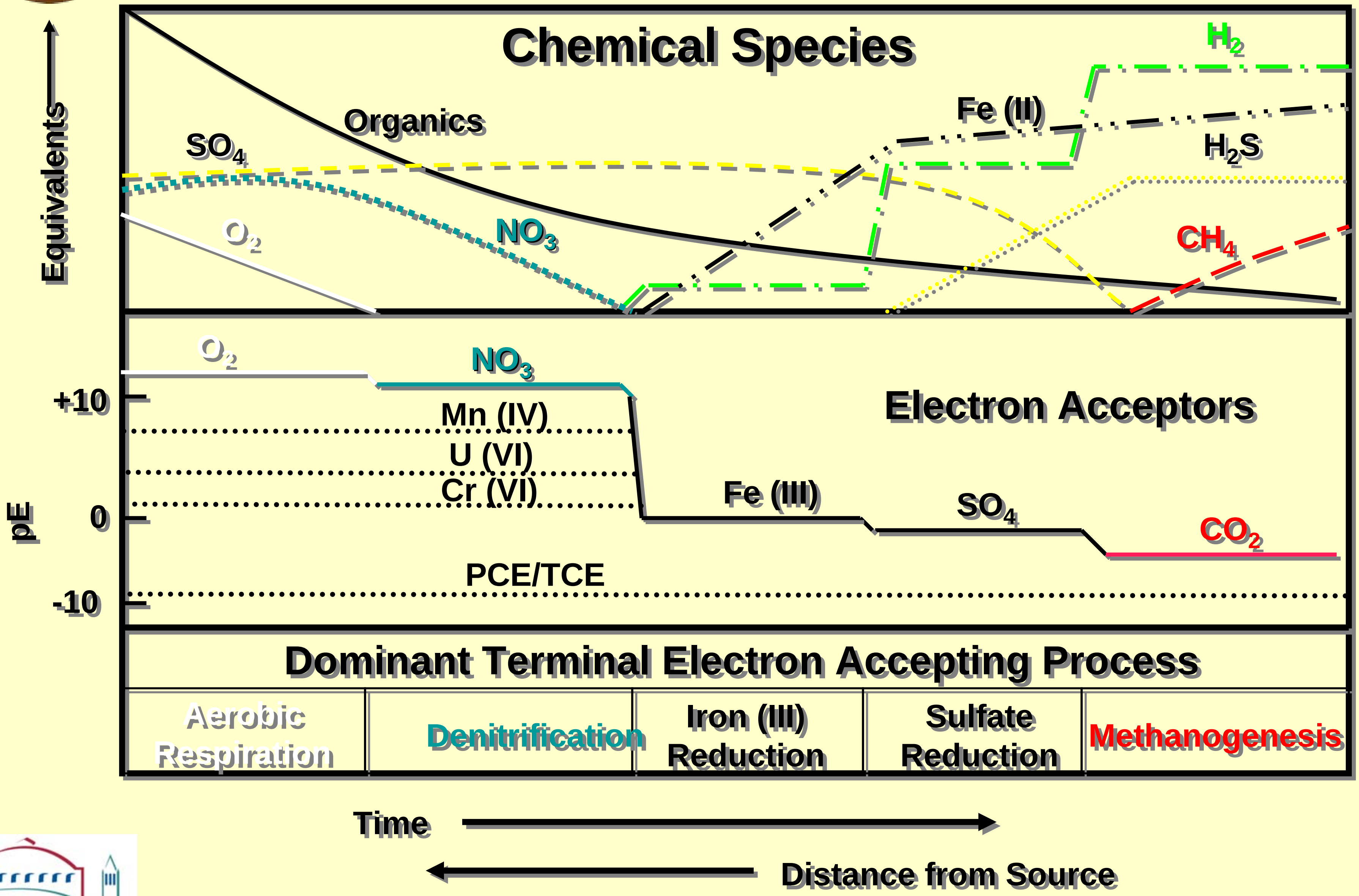
Co-Director, Virtual Institute for Microbial Stress and Survival

Lawrence Berkeley National Laboratory

Berkeley, California 94720

tchazen@lbl.gov   http://vimss.lbl.gov

Critical Biogeochemistry



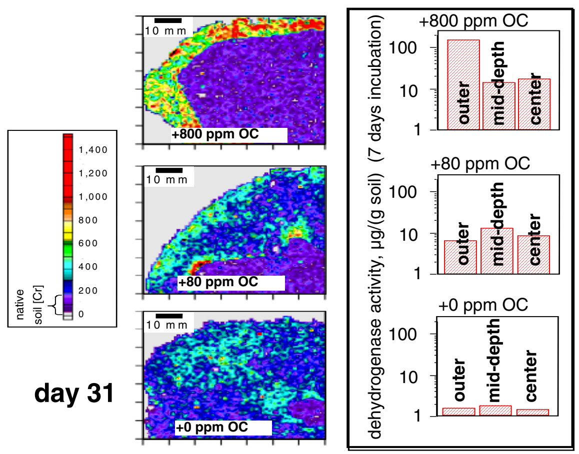
Cr(VI) Bioreduction Lab Studies

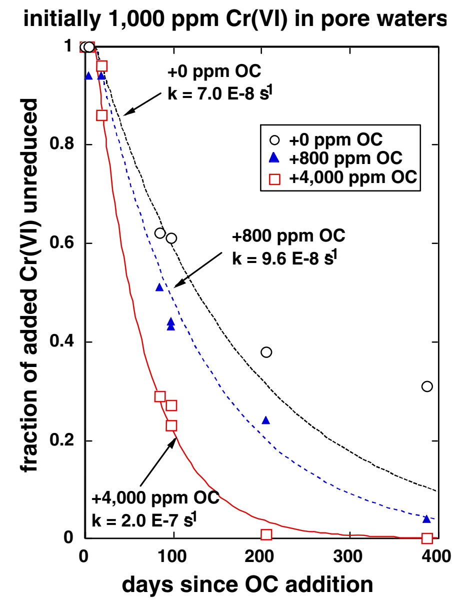
Jiamin Wan, Tetsu Tokunaga, Mary Firestone, Eoin Brodie and Terry Hazen (ERSP/NABIR supported 1998-2004)

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.



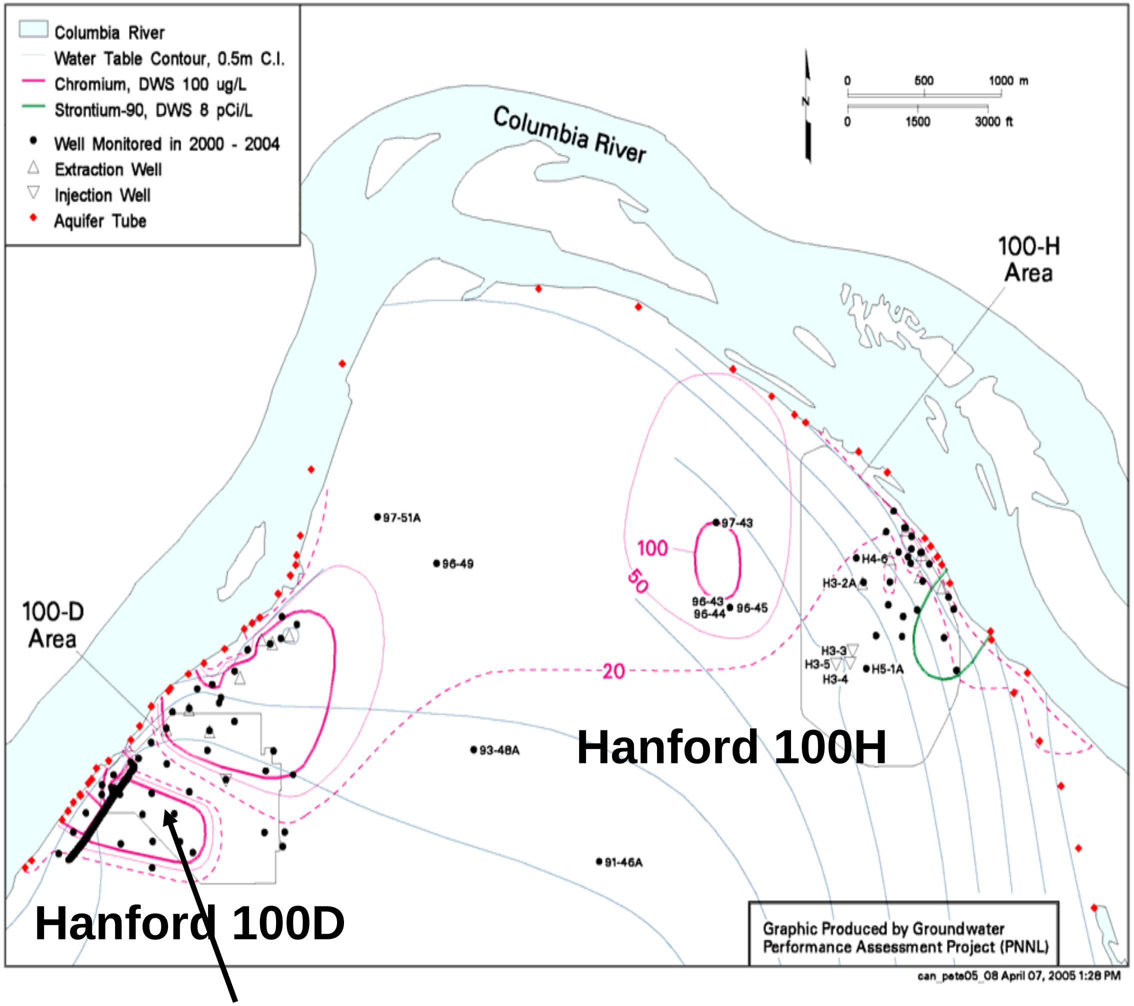


Fe(III) + Microbial reduction → **Fe(II)**

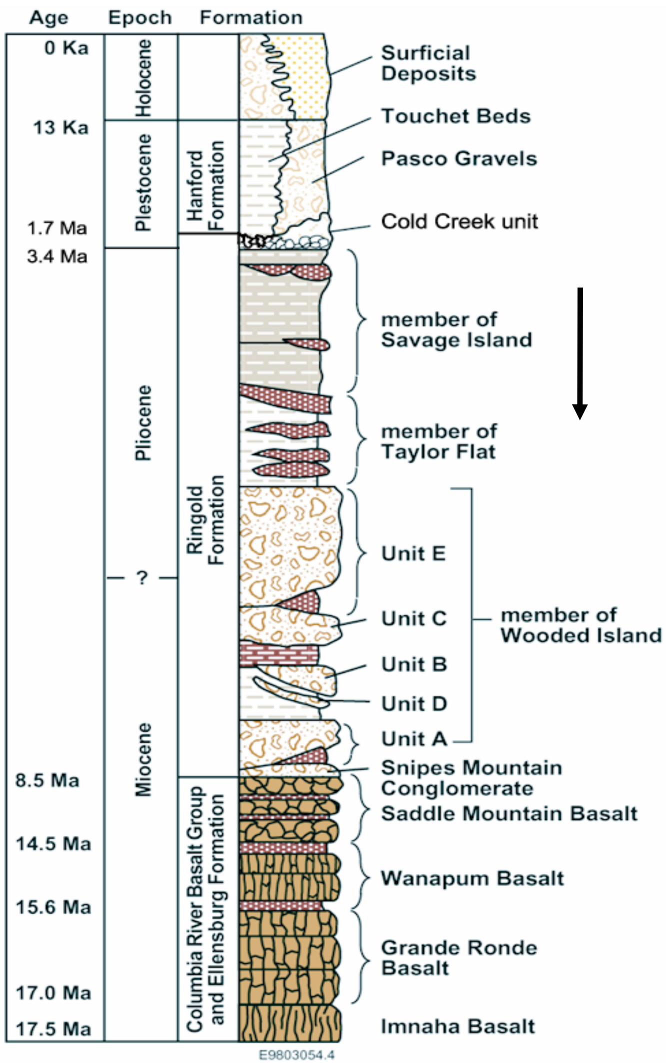
Fe(II) + **Cr(VI)** → **Cr(III) Precipitation**

Hanford 100H Site Characterization

Cr Concentration Map



Lithological Column

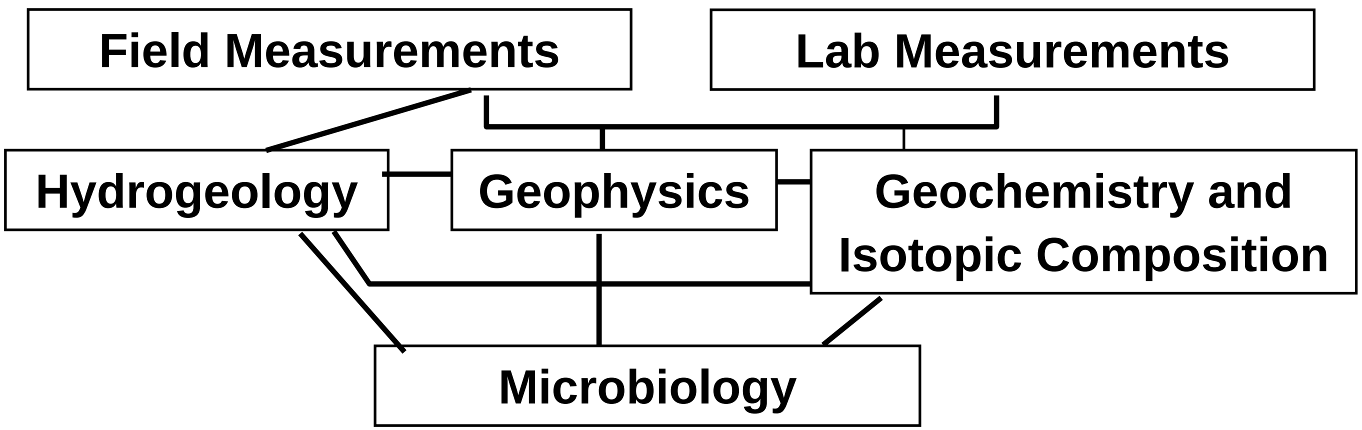


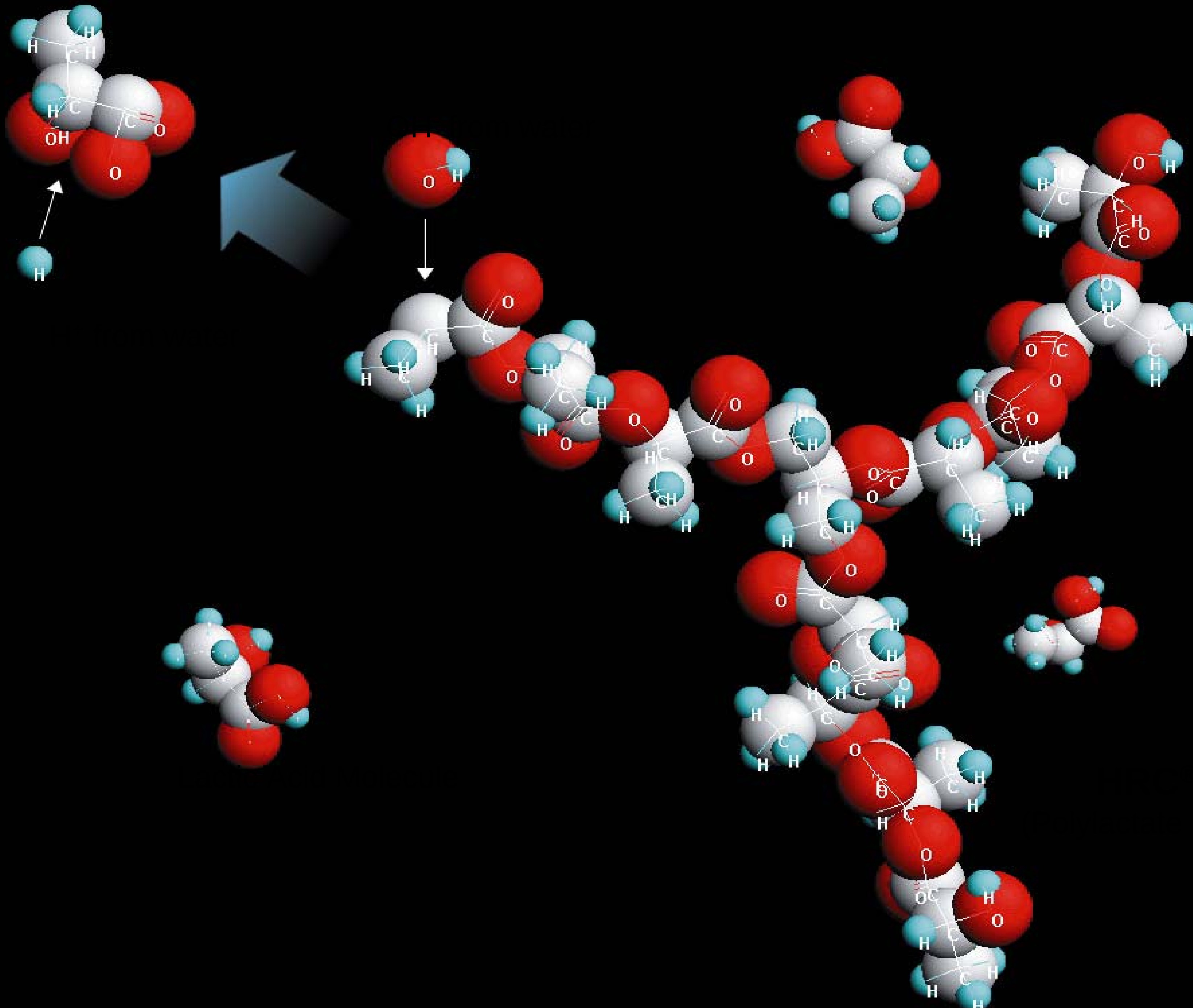
The Cr source is believed to be sodium dichromate ($Na_2Cr_2O_7.2H_2O$)

Overall Objective

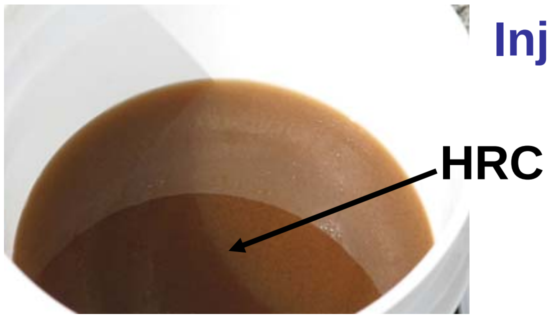
To carry out field investigations to assess the potential for immobilizing Cr(VI) in groundwater using lactate-stimulated bioreduction of Cr(VI) to Cr(III) at the Hanford 100H site, and to determine critical community structure changes and stressors that would enable control and predictions of fundamental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

Integrated Approach



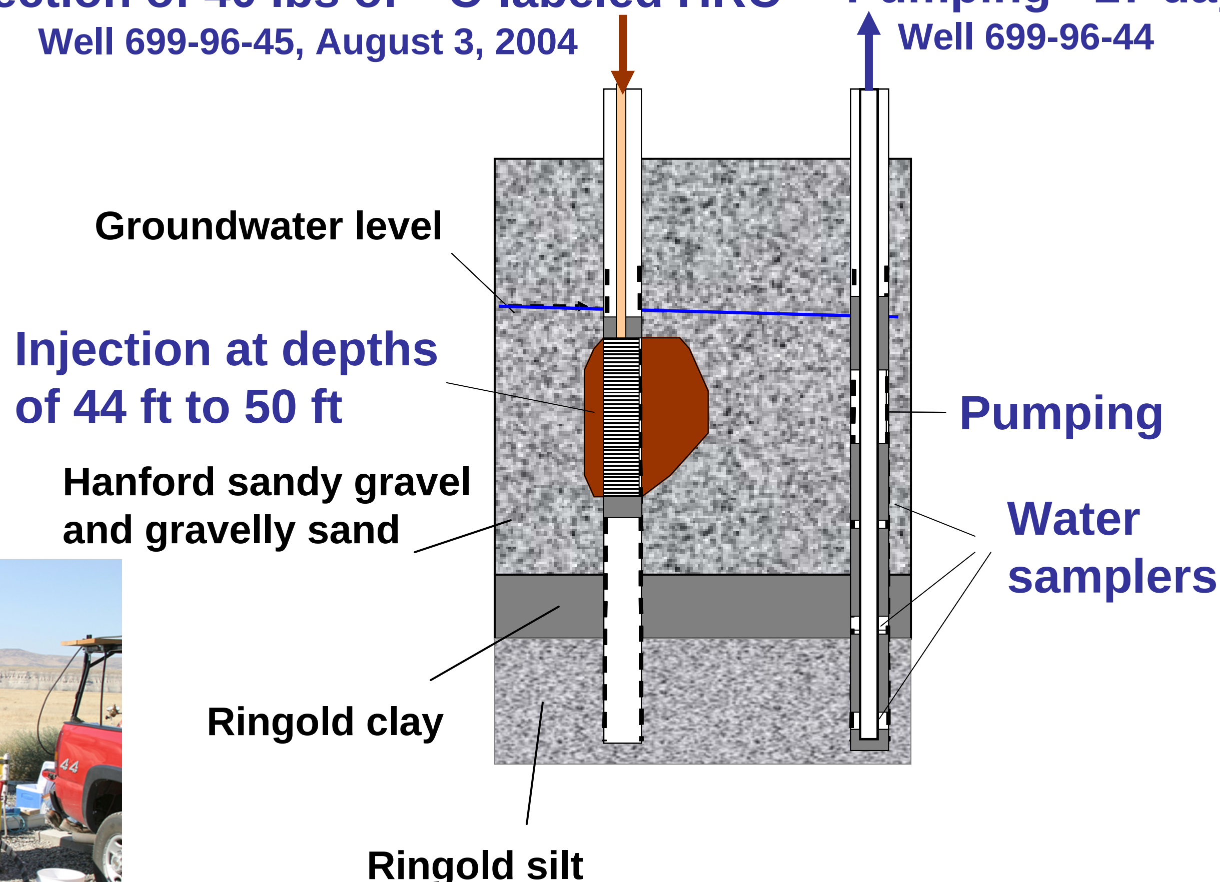


Field HRC Injection Test

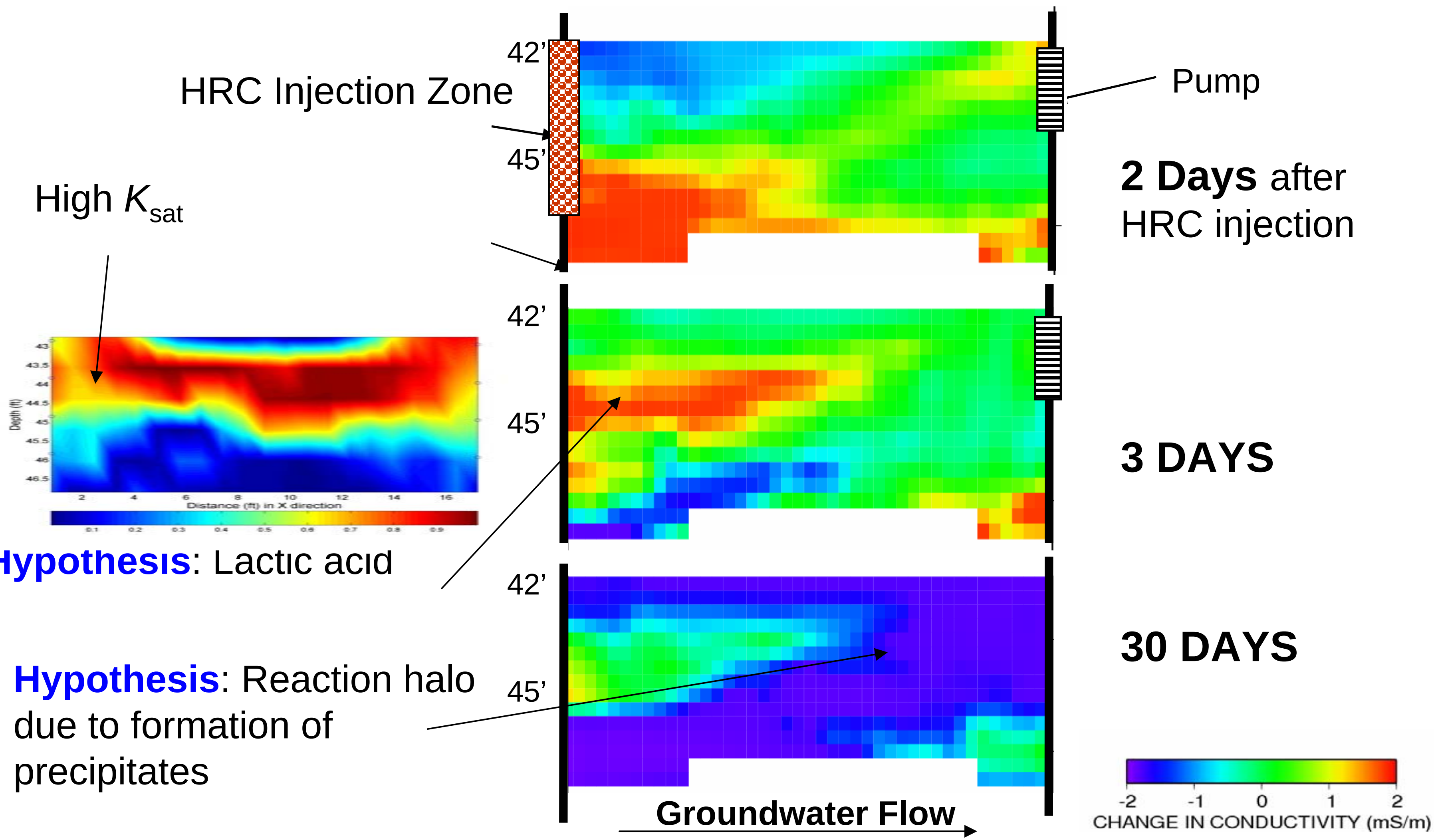


Injection of 40 lbs of ^{13}C-labeled HRC
Well 699-96-45, August 3, 2004

Pumping - 27 days
Well 699-96-44



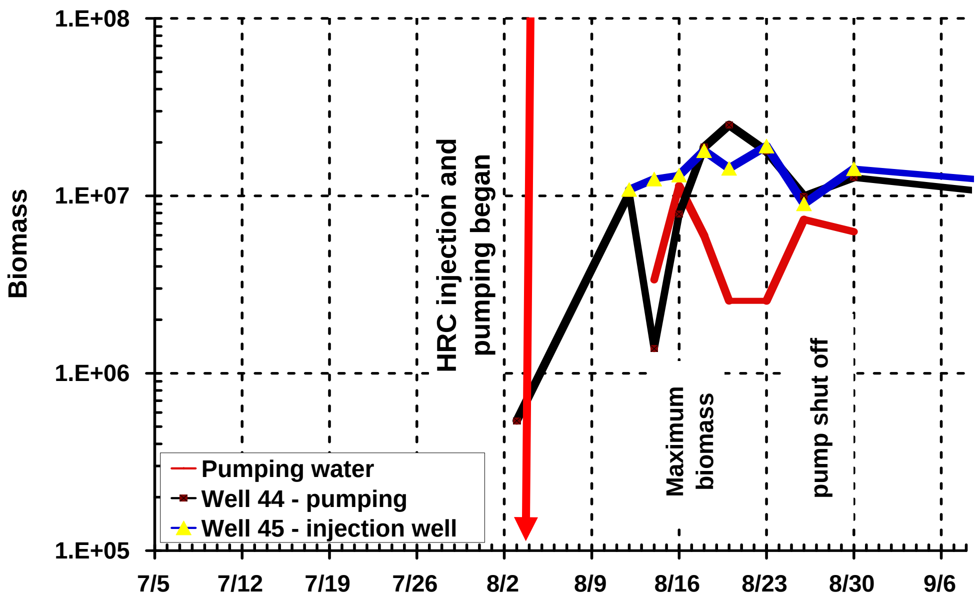
Post-HRC Injection Changes in Electrical Conductivity



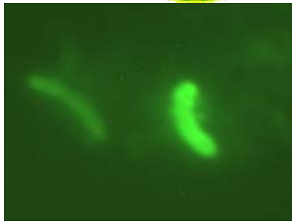
Hypothesis: Lactic acid

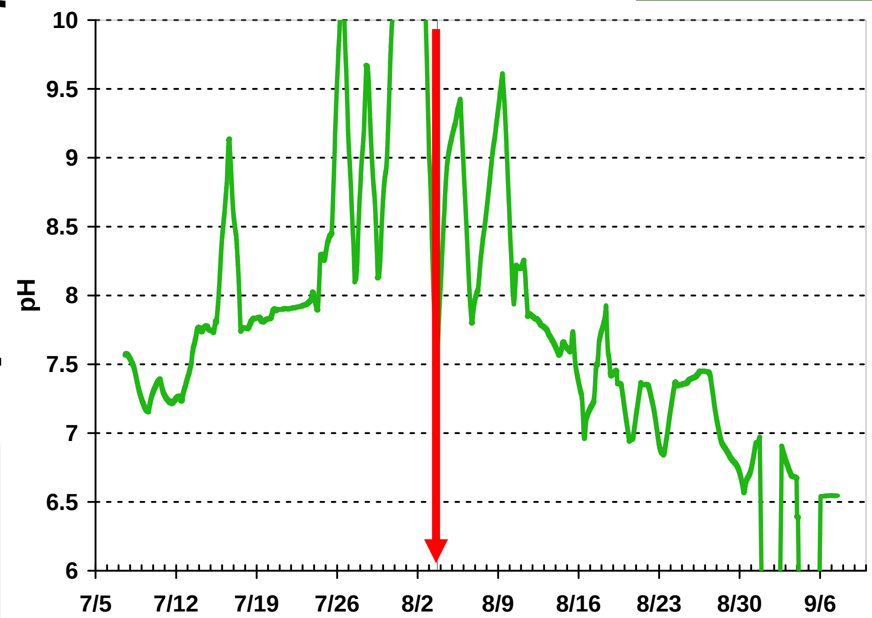
Hypothesis: Reaction halo due to formation of precipitates

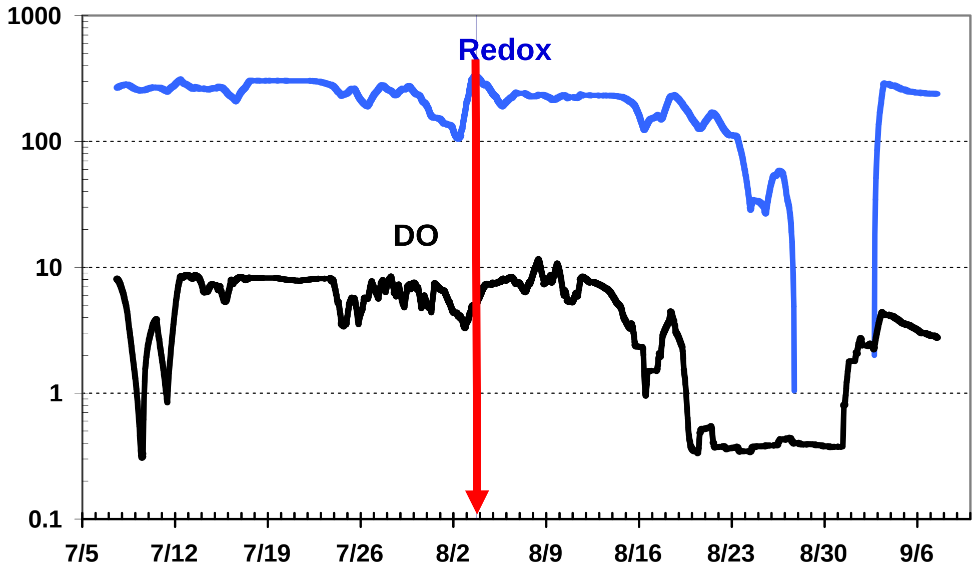
Results of HRC Biostimulation



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





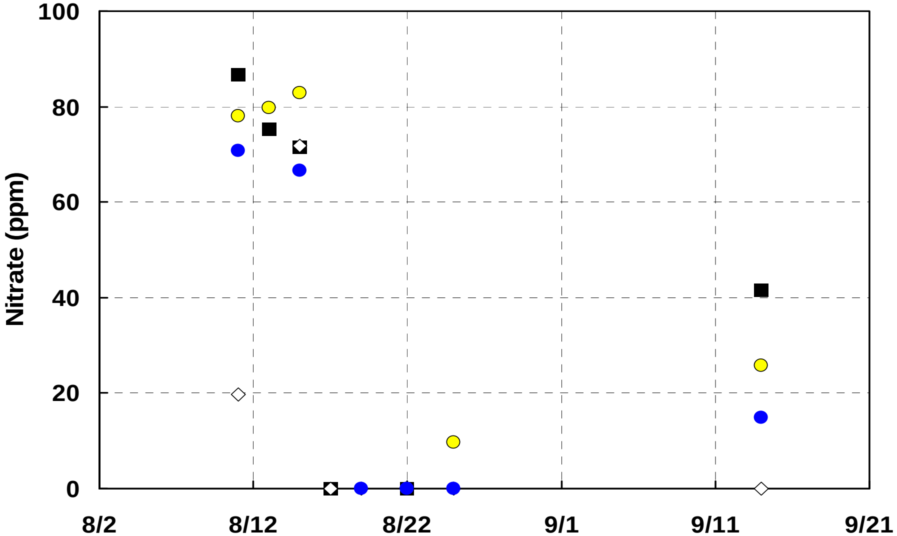


Redox dropped from 240 to -130 mV

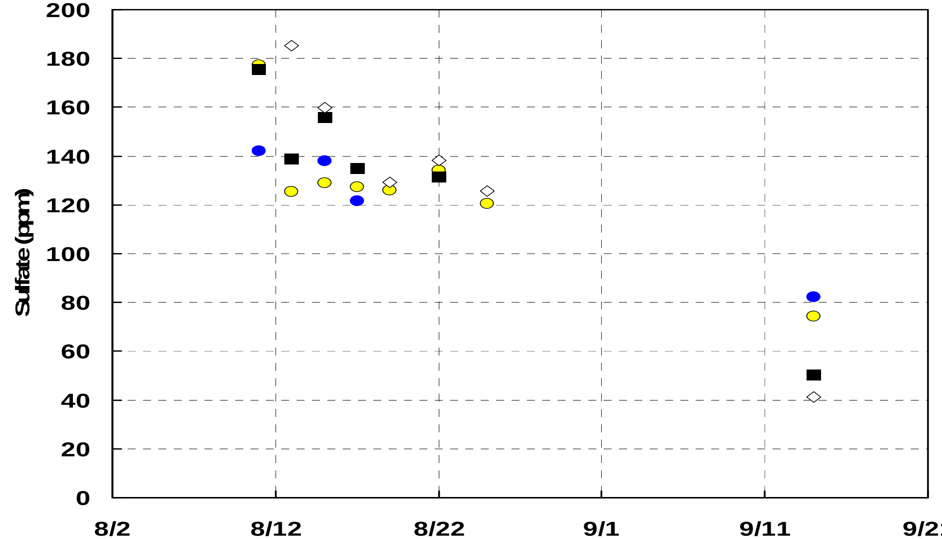
DO dropped from 9 mg/l (~100%) to 0.35 mg/l (4.5%)

Biogeochemical Evidence of Microbial Metabolism in Groundwater

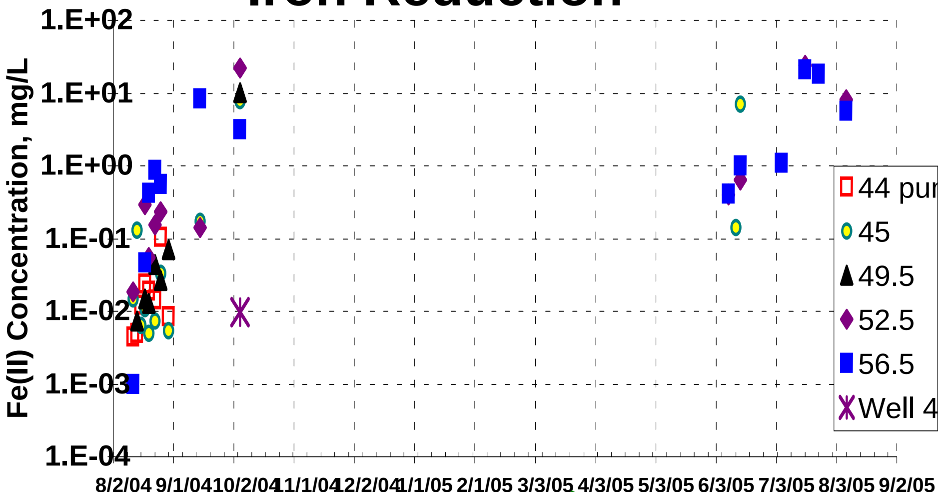
Denitrification



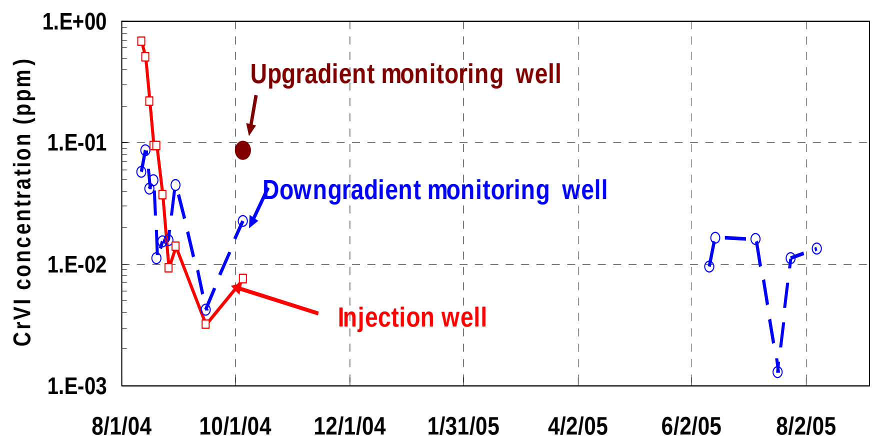
Sulfate reduction



Iron Reduction



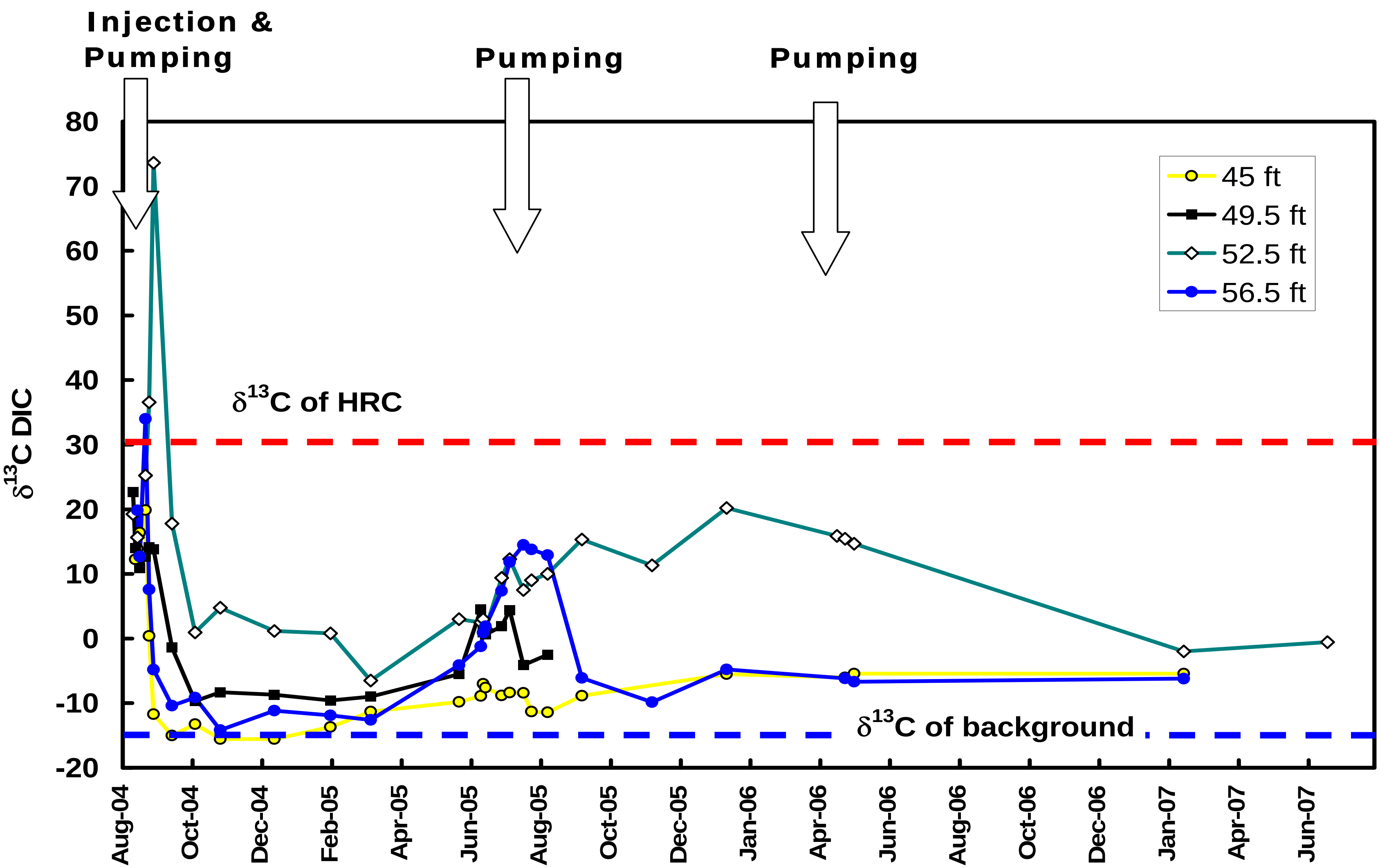
Average Soluble Cr(VI) Concentration



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

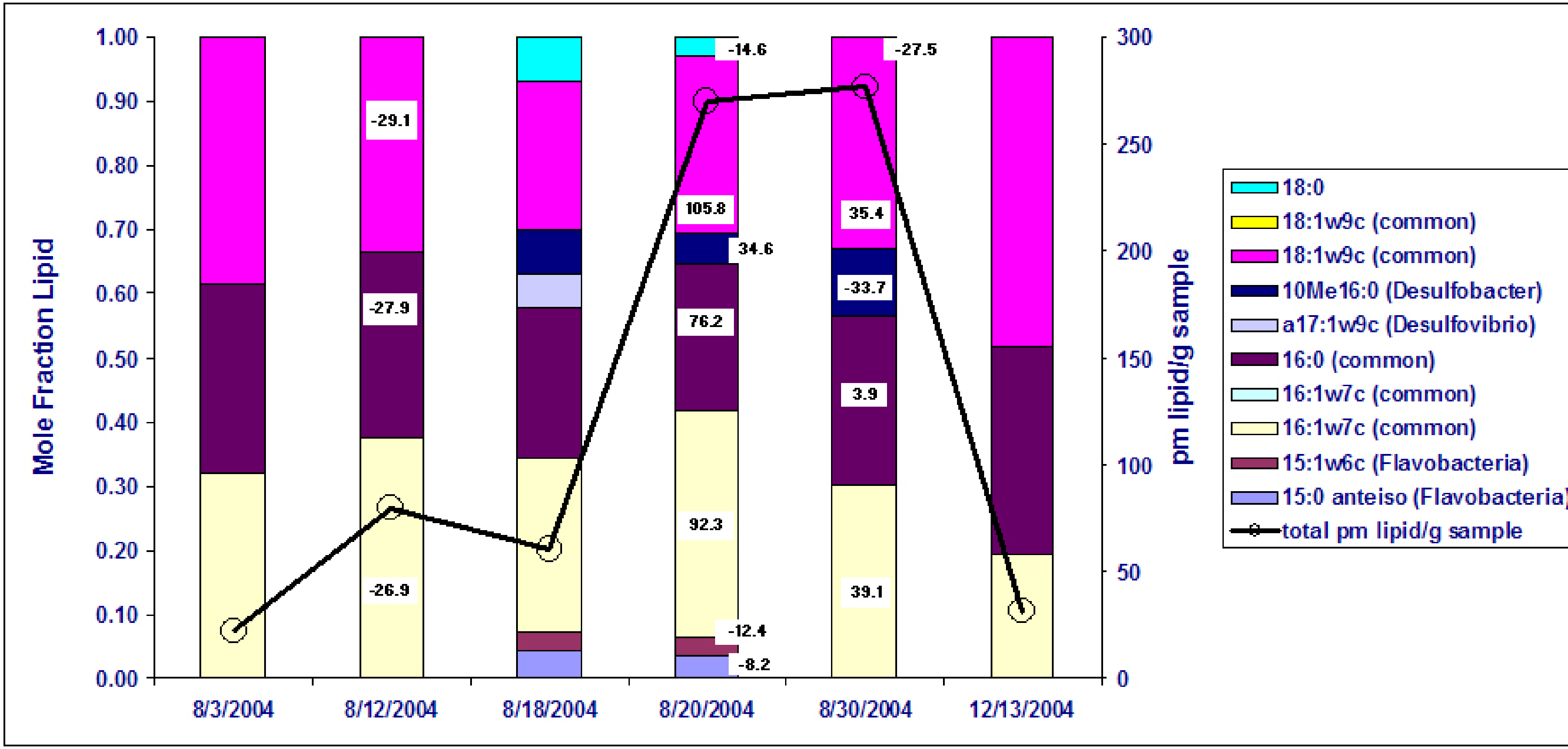
Fe(II) + Cr(VI) → Cr(III) Precipitation

Biogeochemical Evidence of Microbial Metabolism in Groundwater



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

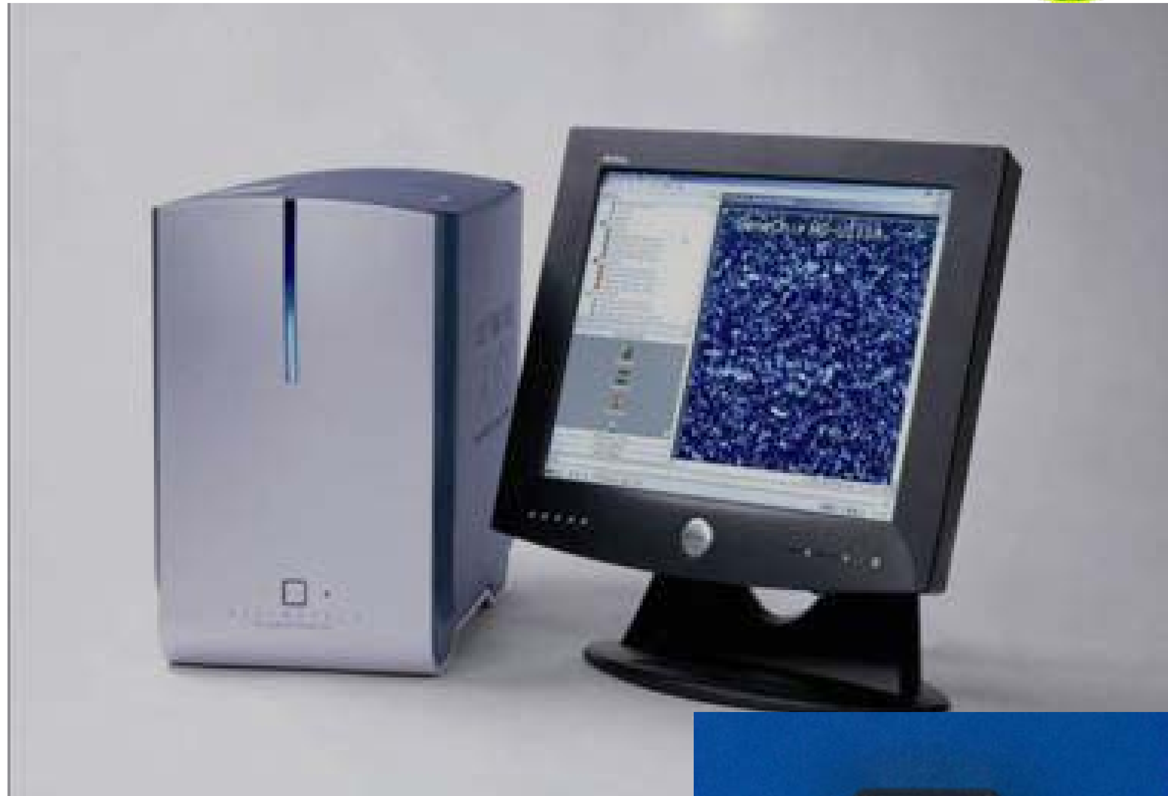
^{13}C Phospholipid Analysis



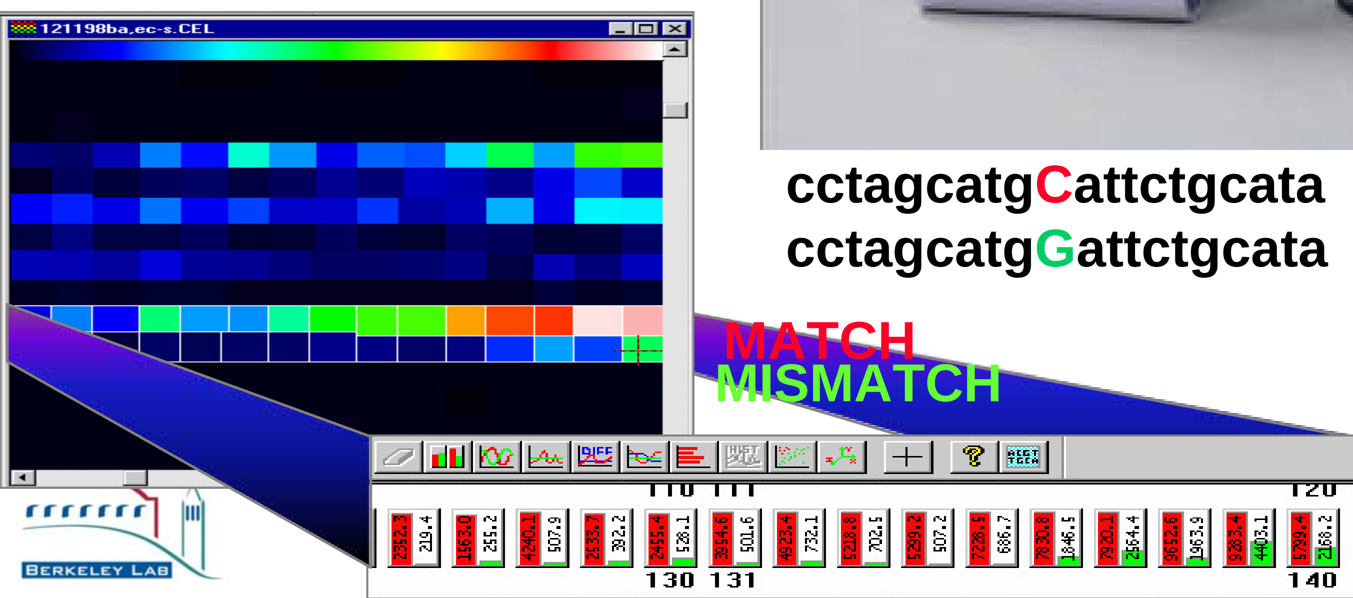
- **General bacterial biomarkers indicate rapid enrichment in ^{13}C**
- **^{13}C ratio is greater than expected (overall spiked HRC ratio was 15 per mil)**
  - **^{13}C polylactate used as spike it is not esterified to glycerol backbone**
  - **it is released and consumed more rapidly**
- **Biomarkers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

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



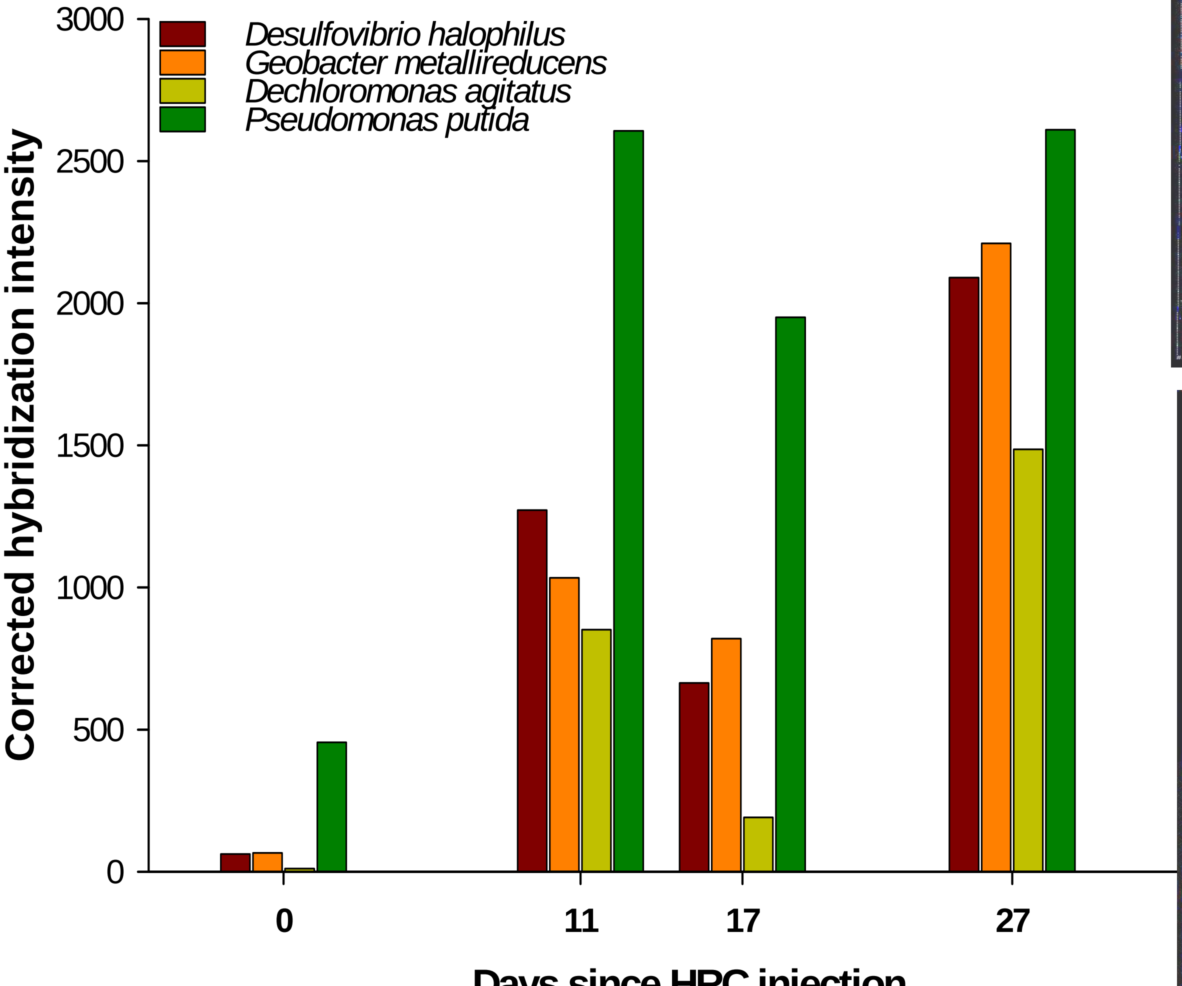
cctagcatgCattctgcata
cctagcatgGattctgcata

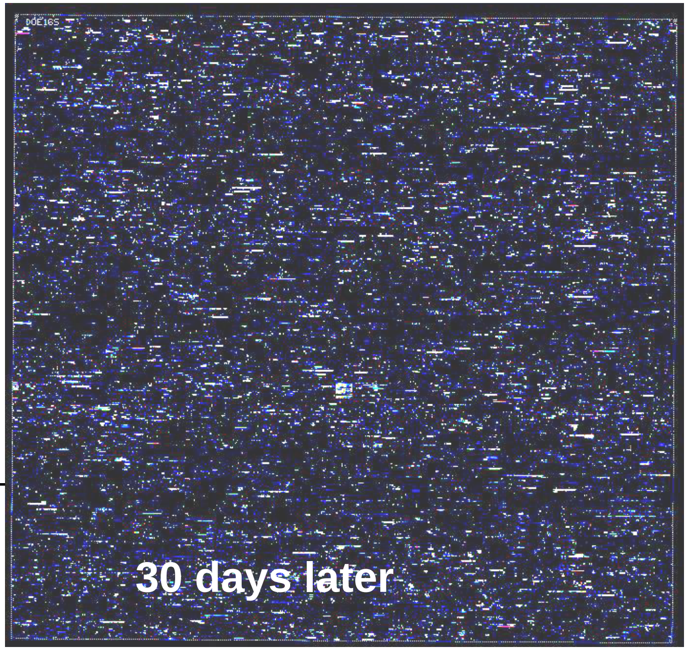


BERKELEY LAB

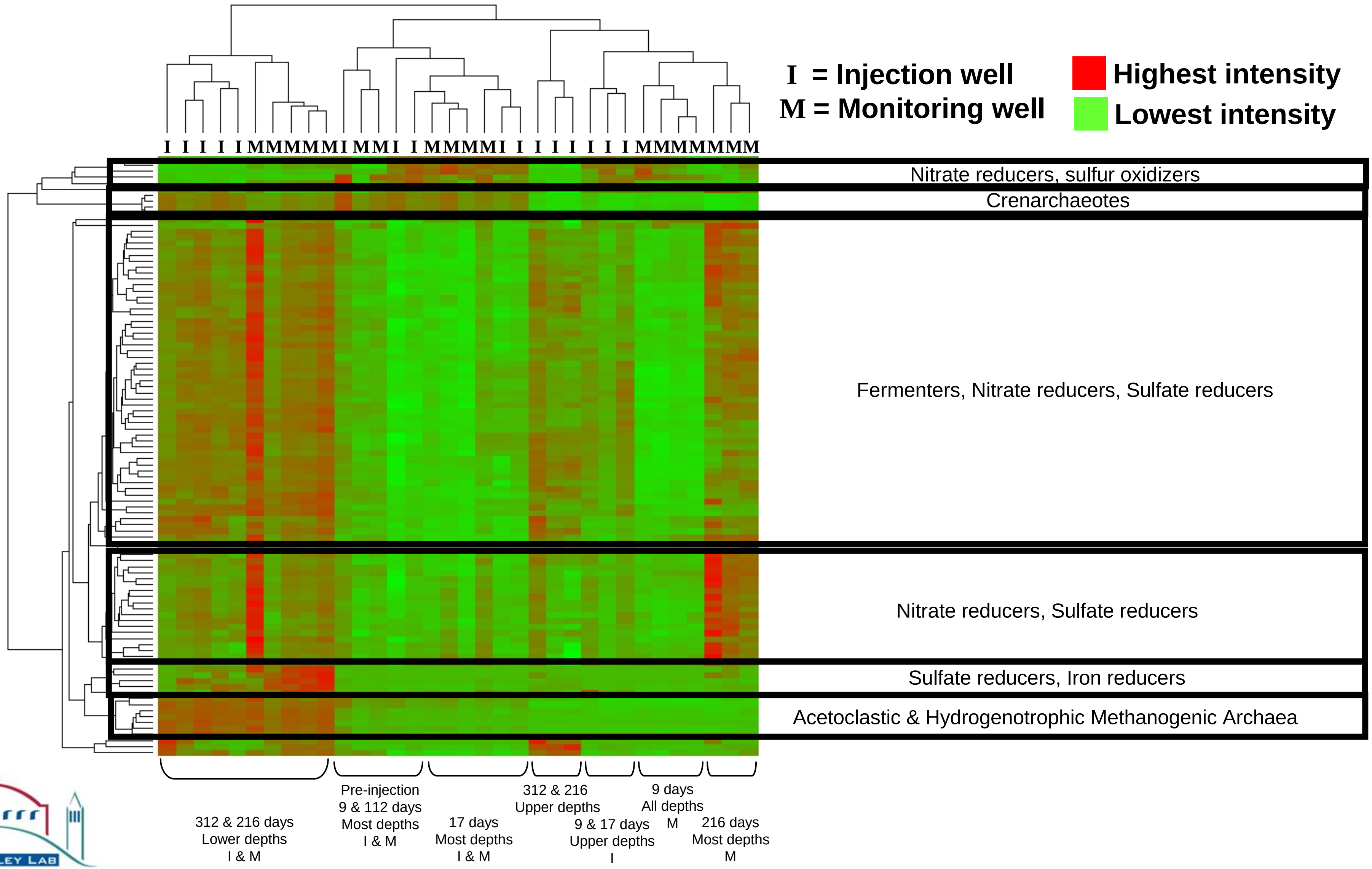
Microarray analysis of bacterial community changes during Cr(VI) remediation at Hanford 100H site:

Dynamics of some significant organisms.

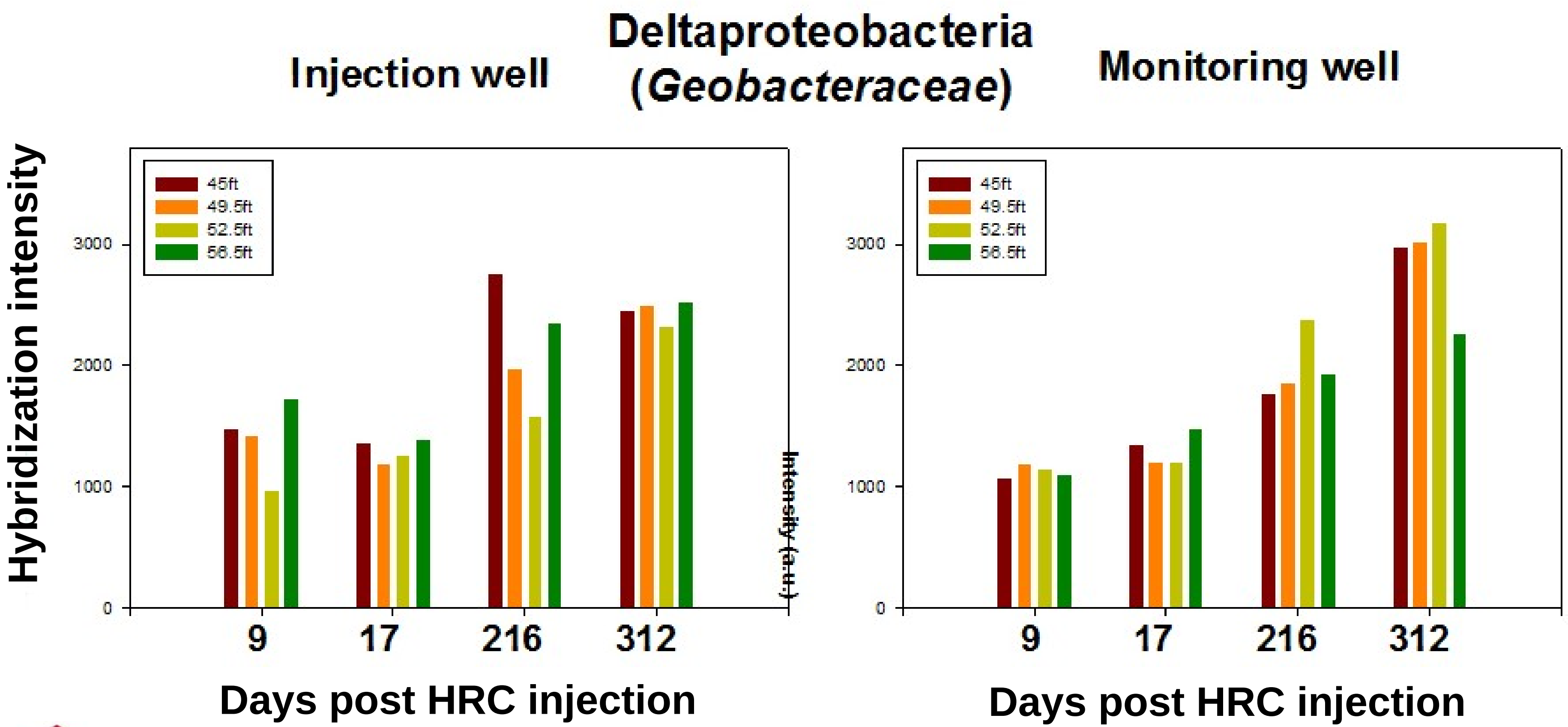




Data mining – Bidirectional clustering



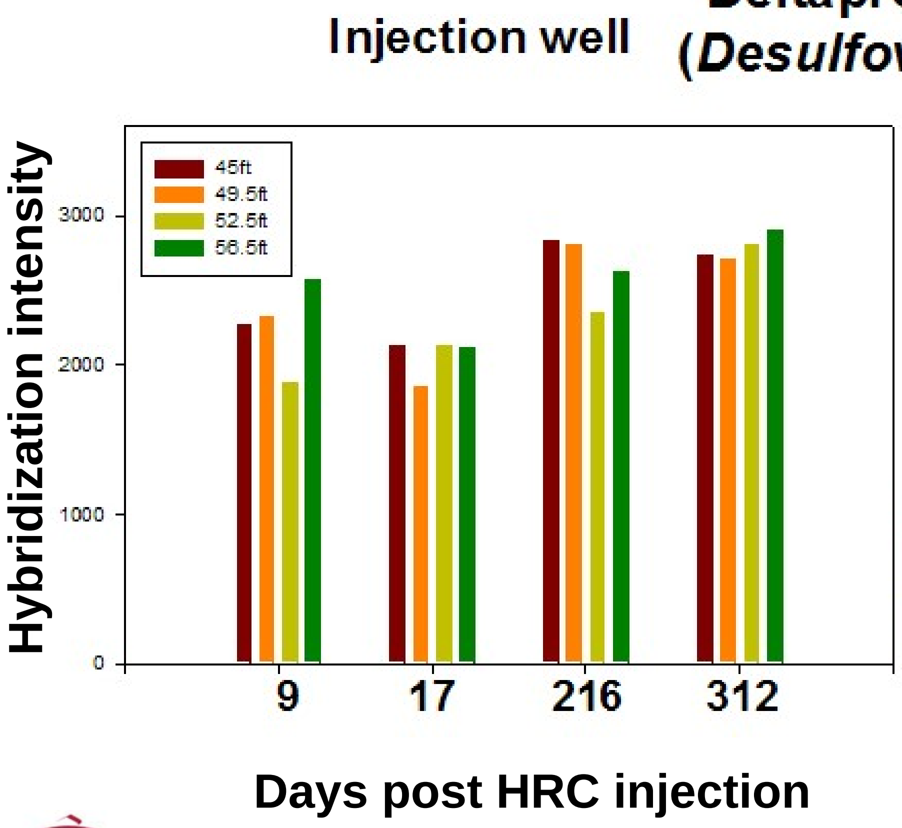
Functional groups – Iron reduction

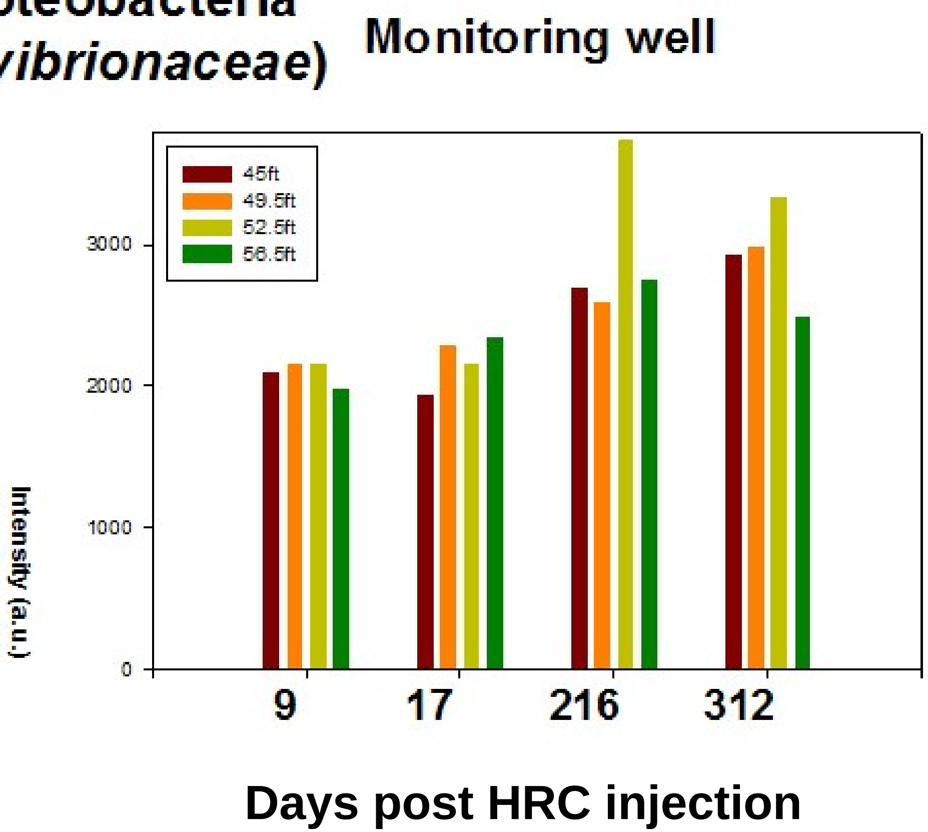


Fe(II) can abiotically reduce Cr(VI) to Cr(III)

Functional groups – Sulfate reduction

Deltaproteobacteria (*Desulfovibrionaceae*)



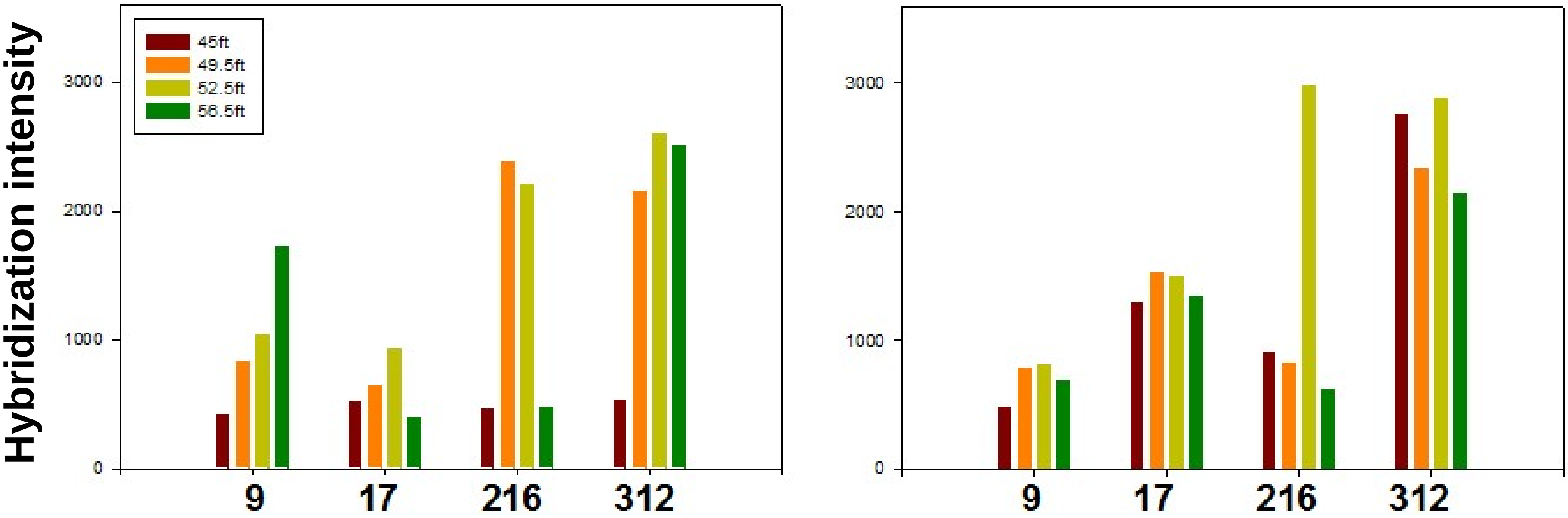


H_2S can abiotically reduce Cr(VI) to Cr(III)

Functional groups – Methanogenesis

Euryarchaeota (Methanogens)

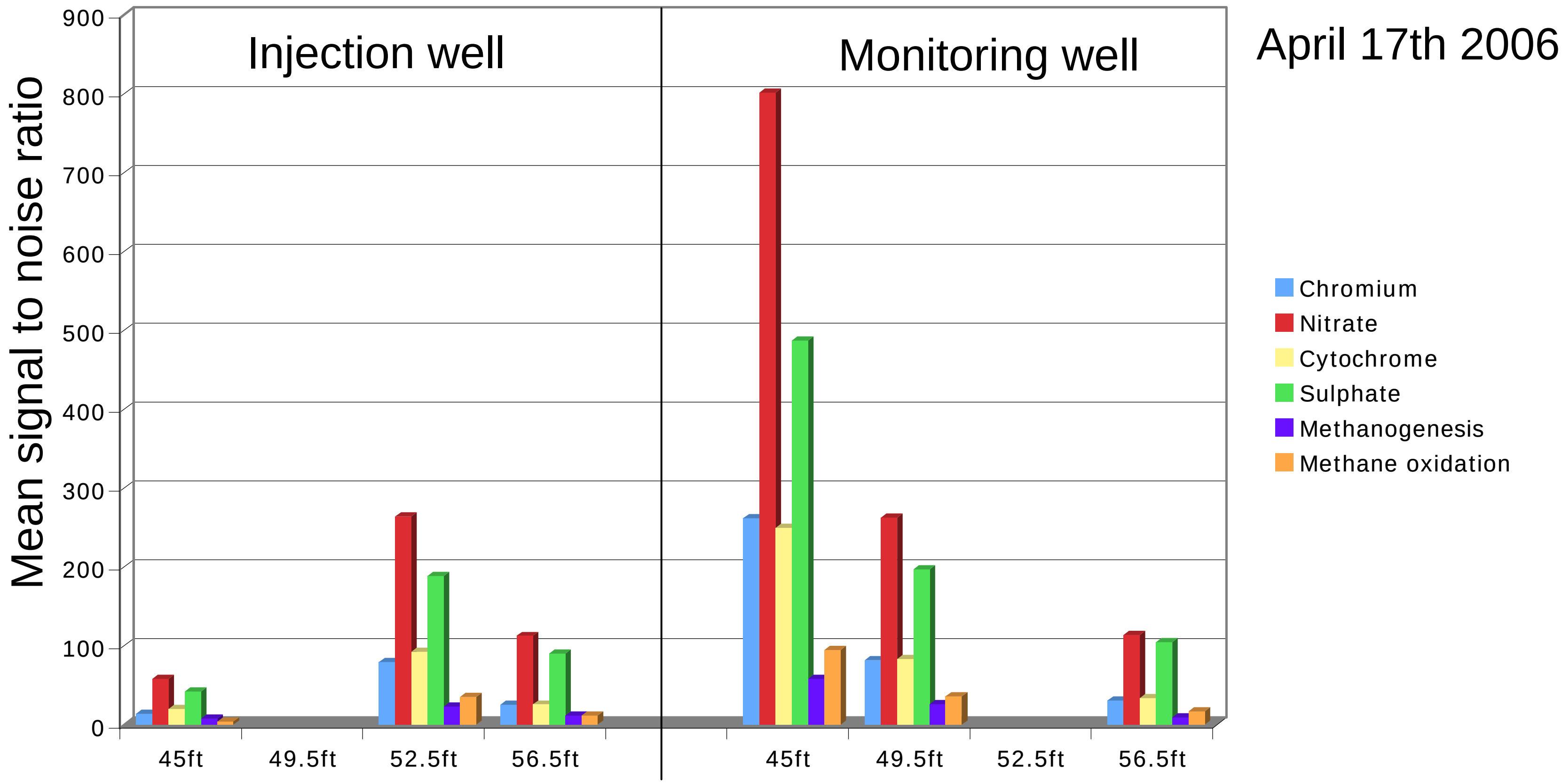
Injection well | **Monitoring well**



Days post HRC injection | **Days post HRC injection**

Presence of methanogens indicates strongly reducing conditions

Functional microarray analysis



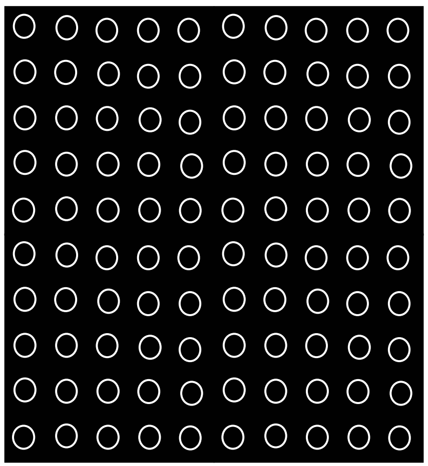
Nitrate, Sulfate, Iron reduction. Methanogenesis, Methane oxidation, Sulfur oxidation. Many chromium tolerance/reduction genes.

Joe Zhou, Joy Van Nostrand - Oklahoma

NanoSIMS + microarray indicates active organisms

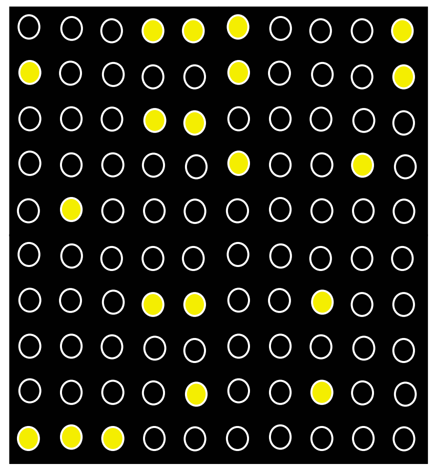
(Hoeprich, Pett-Ridge, Brodie, et al. New Genomics:GTL project)

Array spotted with universal 16S probe set



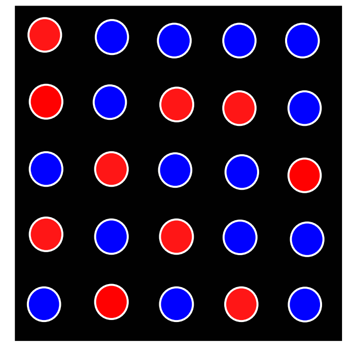
----100 μm----

Affymetrix PhyloChip Fluorescence Scan



rRNA profile shows species that are present in the community

System-specific NimbleGen chip + NanoSIMS ^{13}C:^{12}C analysis



Indicates subset of active community that consumed ^{13}C-label substrate

^{12}C rRNA

^{13}C rRNA

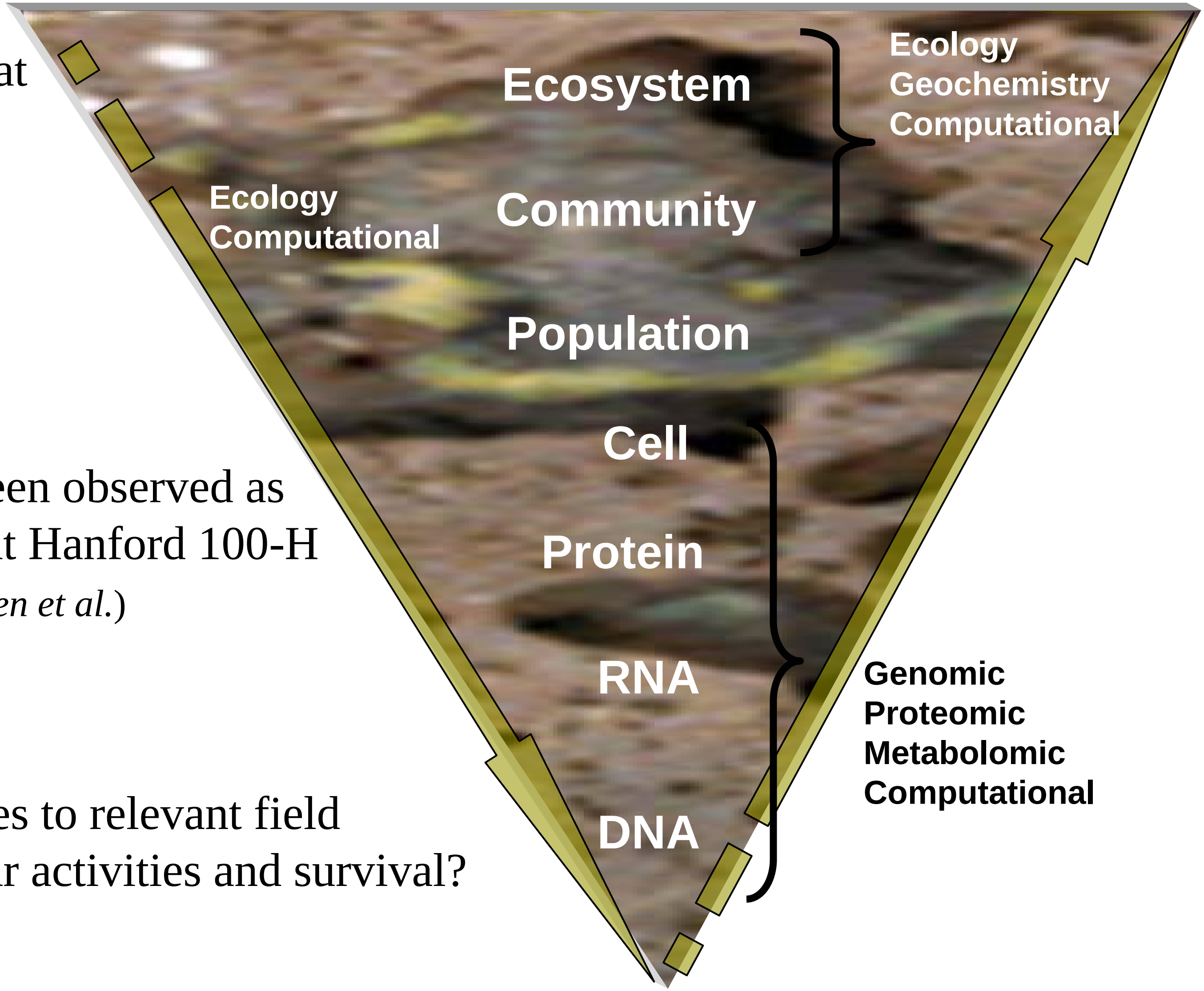
"Chip-SIP" yields identity and function from the same sample

Systems Biology to Elucidate Field Relevant Responses

Desulfovibrio are present at elevated numbers during biostimulation

Desulfovibrio spp. have been observed as predominant populations at Hanford 100-H during biostimulation (*Hazen et al.*)

How do cellular responses to relevant field conditions impact cellular activities and survival?



What is Known from Hanford 100H Lactate Biostimulation Experiment

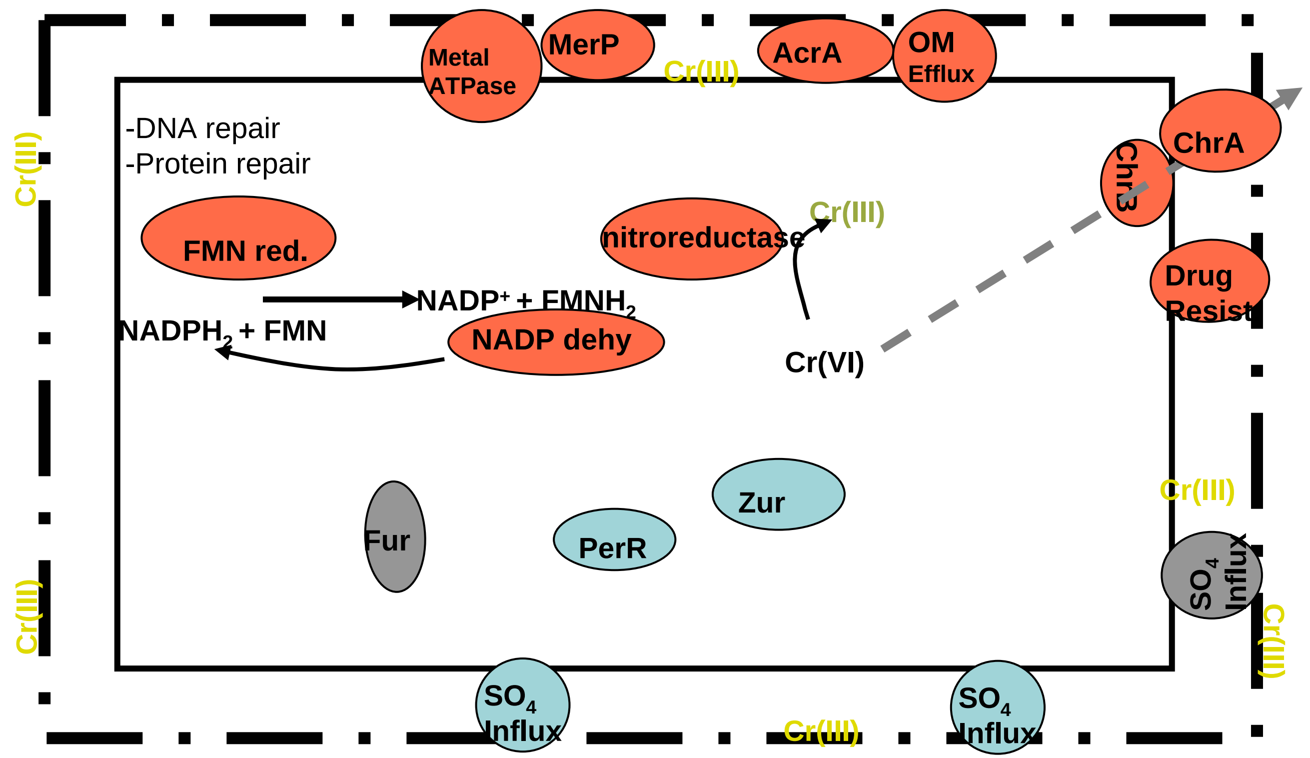
- *In situ* hydrogeological, geochemical (including radioactive and stable isotope analyses), geophysical measurements, and microbiological analyses of water samples and sediments provided detailed and robust interpretation of field-scale biogeochemical processes.
- The ^{13}C concentration, which was used to label the injected lactate, is essential to track and identify the presence of microbial metabolism and lactate degradation.
- A sequential depletion of competing terminal electron acceptors O^{2-}, NO_3^-, Fe^{3-},SO_4^{2-}, and transiently CO_2, creating a sustained dissolved, ferrous ion environment.
- A chemical reaction of ferrous ion with toxic and soluble Cr(VI) causes the formation of nontoxic and insoluble Cr(III)-Fe complexes.
- Cr(VI) concentration has remained below the drinking water standards for ~3 years after a single 40lb polylactate injection.
- The longevity of the Cr(VI) bioimmobilization indicates an efficacy of using lactate injection for controlling the Cr(VI) concentration in groundwater at many contaminated sites.

Environmental Remediation Sciences

BERKELEY LAB

What Cellular Systems are Involved in Cr(VI) Responses in *Desulfovibrio vulgaris* Hildenborough?

- Sulfate influx down-expressed
- Metal efflux up-expressed
- *chr*AB up-expressed
- FMN dependent nitroreductase, NADH dehydrogenase, and FMN reductase up-expressed



Klonowska, A., He*, Z., He, Q., Hazen*, T.C., Thieman, S.B., Alm*, E.J., Arkin*, A.P., Wall*,J.D., Zhou*, J. and Fields*, M.W. Global Transcriptomic Analysis of Chromium(VI) Exposure of *Desulfovibrio vulgaris* Hildenborough Under Sulfate-Reducing Conditions. (in press)

Acknowledgments

Adam Arkin, Eric Alm, Kat Huang, Dylan Chivian, Janet Jacobson, Jay Keasling, Aindrila Mukhopadhyay, Eoin Brodie, Sharon Borglin, Hoi-Ying Holman, Jil Geller, Elenor Woezi, Jenny Lin, Dominique Joyner, Rick Huang, Romy Chakraborty, Boris Faybishenko, Mark Conrad, Joern Larsen, Zouping Zheng, Gary Andersen, Todd DeSantis, Tetsu Tokunaga, Jiamin Wan, Susan Hubbard, Ken Williams, John Peterson, Natalie Katz, Jill Banfield, Tamas Torok, Seung Baek, Don Herman, Mary Firestone

Pacific Northwest National Laboratory: Phil Long, et al.

Regenesis: Steve Koenigsberg, Ana Willet

DOE Joint Genome Institute: Paul Richardson, Phil Hugenholtz

Missouri: Judy Wall, et. al.

Montana State University: Mathew Fields, et. al.

The University of Chicago: Stephen Sutton, Matthew Newville

Sandia National Laboratories: Anup Singh, et. al.

Jizhong Zhou, et. al.

Massachusetts Institute of Technology: Eric Alm

U Washington: David Stahl, et. al.

Oak Ridge National Laboratory: Martin Keller, et. al.