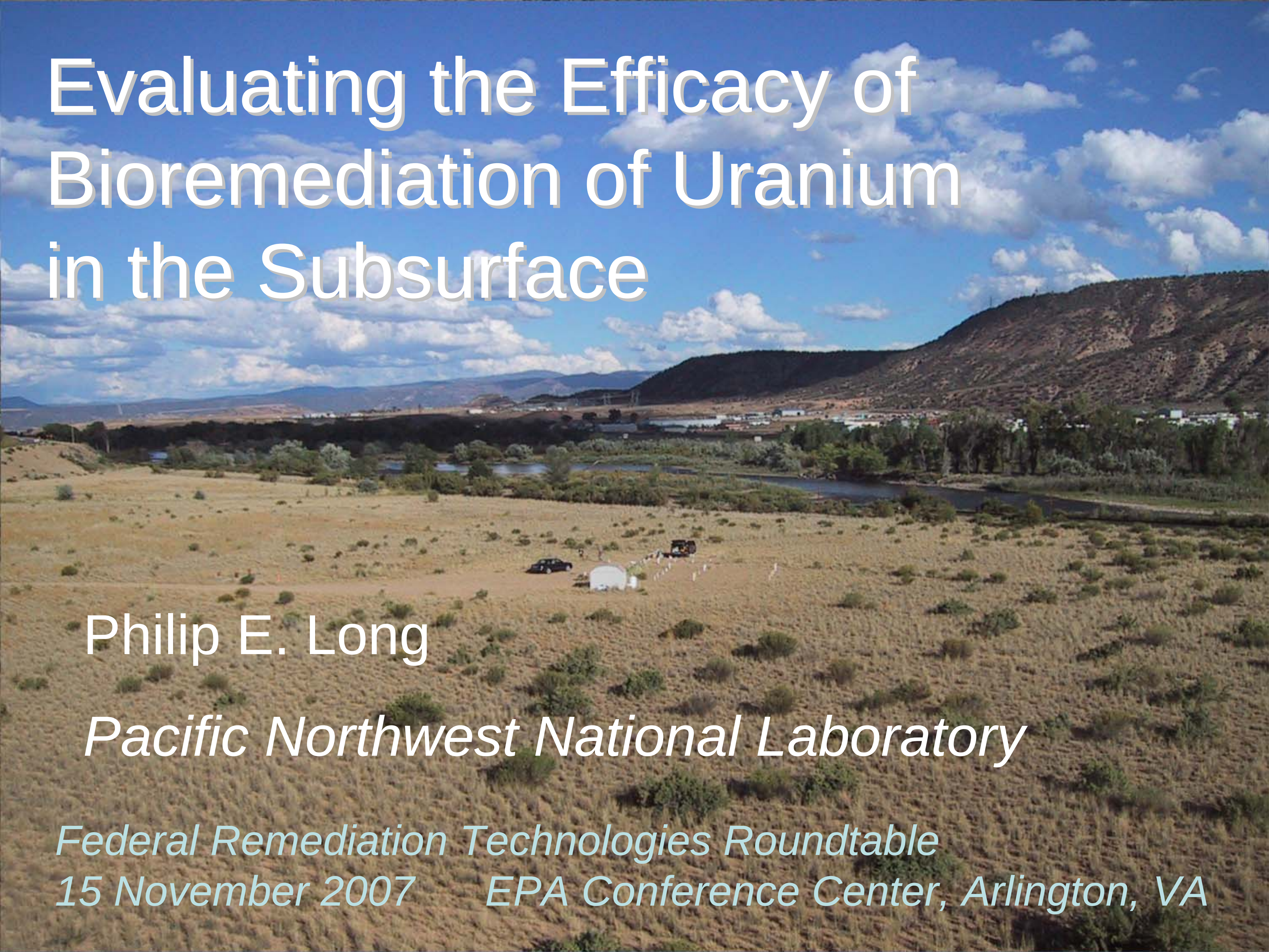


Evaluating the Efficacy of Bioremediation of Uranium in the Subsurface

Philip E. Long

Pacific Northwest National Laboratory

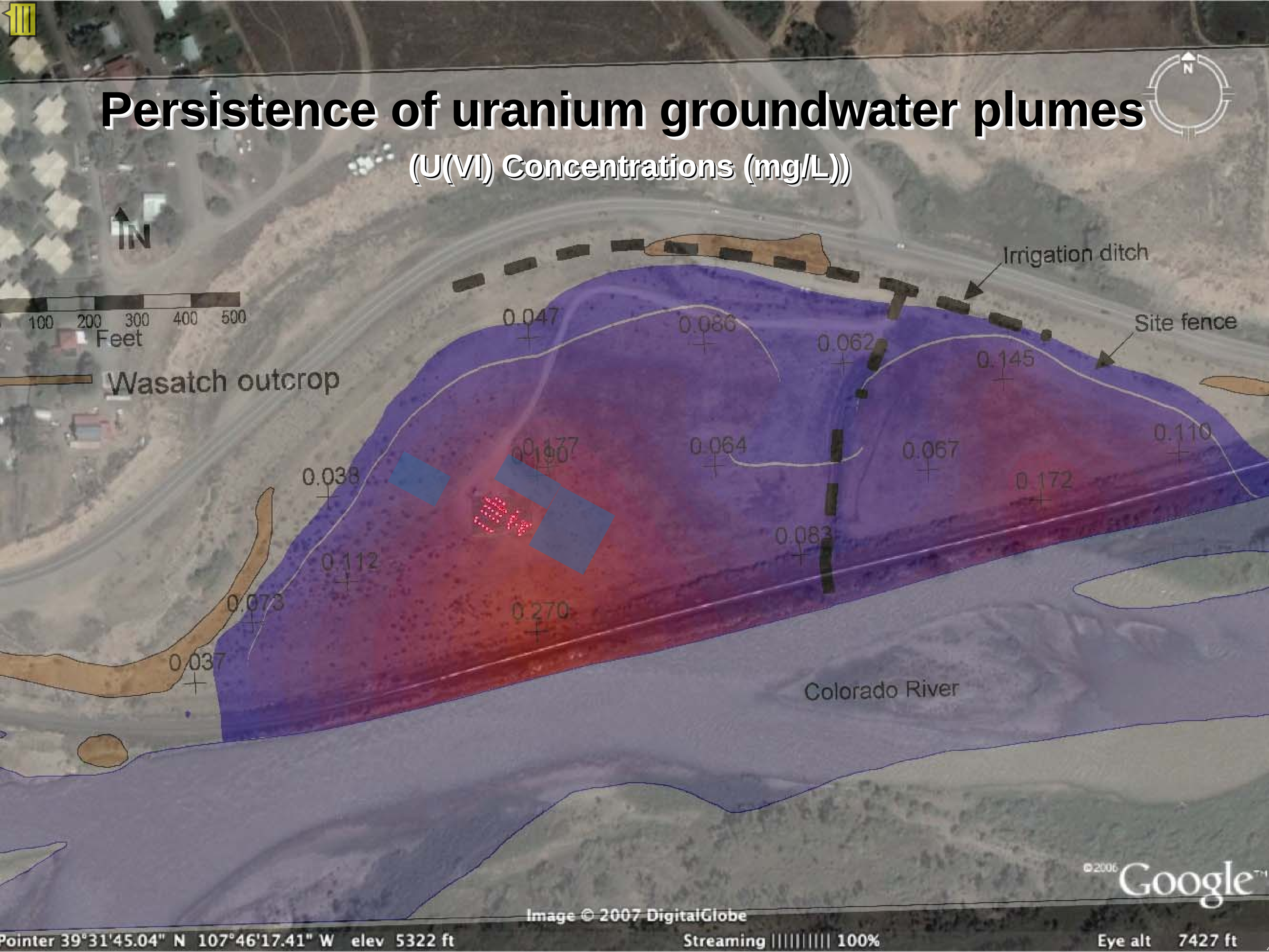
Federal Remediation Technologies Roundtable
15 November 2007 EPA Conference Center, Arlington, VA

Outline of presentation

- Background: persistence of uranium groundwater plumes
- Concept for uranium bioremediation
- Approach to development of a mechanistic understanding of subsurface mobility of uranium at the field scale
- Evaluation of uranium bioremediation
- Future directions and developments

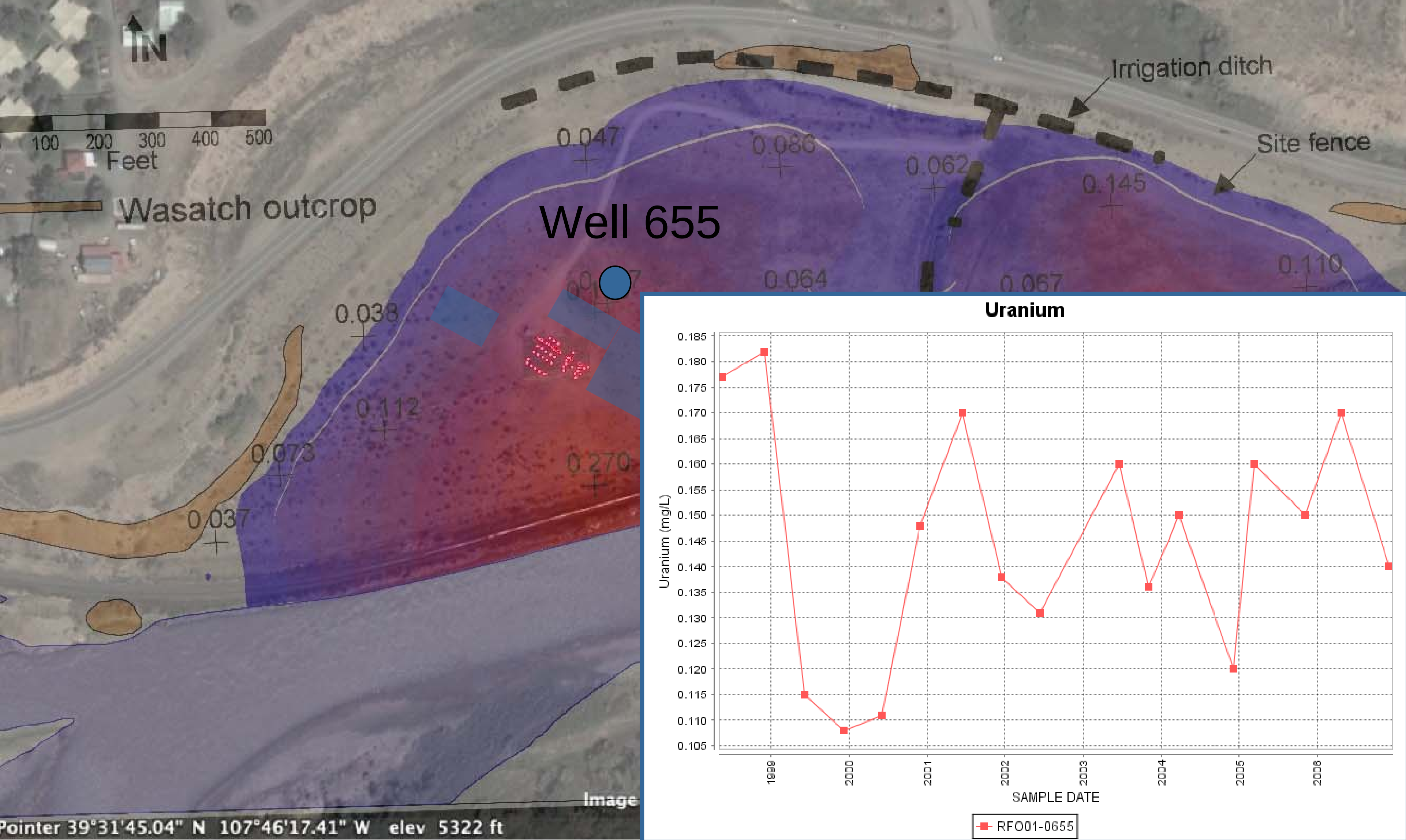
Persistence of uranium groundwater plumes

(U(VI) Concentrations (mg/L))



Persistence of uranium groundwater plumes

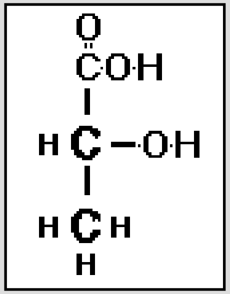
(U(VI) Concentrations (mg/L))



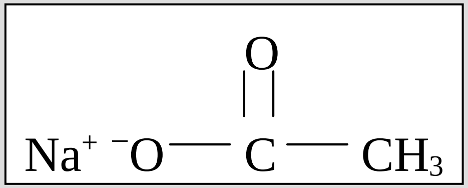
Concept for U(VI) bioreduction

- U(VI) is the mobile valence state of uranium
- Reduced uranium, U(IV), is insoluble as uraninite
- Reduction of U(VI) to U(IV) within aquifers could precipitate and immobilize uranium
- Lab studies suggest simple strategy to promote U(VI) reduction in contaminated aquifers:
  - add acetate as an electron donor to stimulate dissimilatory metal-reducing microorganisms
  - U(VI) is reduced concurrently with Fe(III)

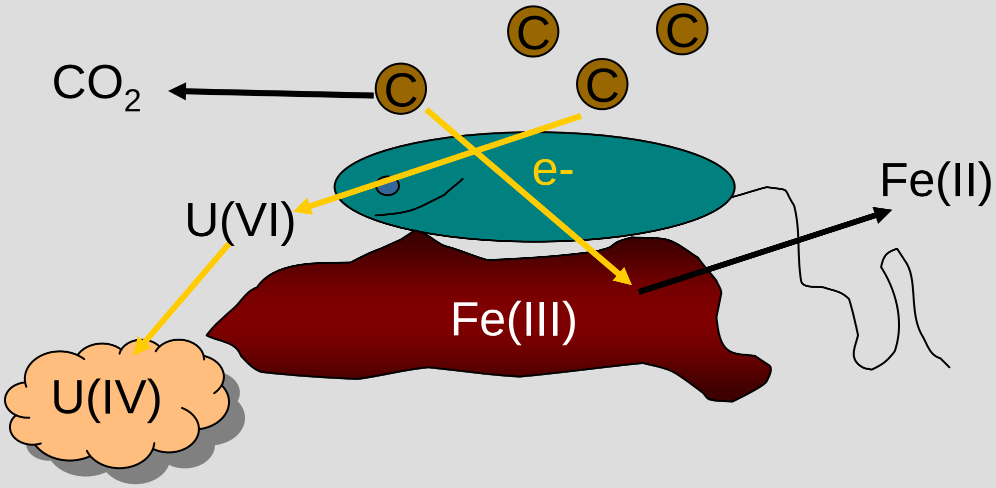
Lactate structure

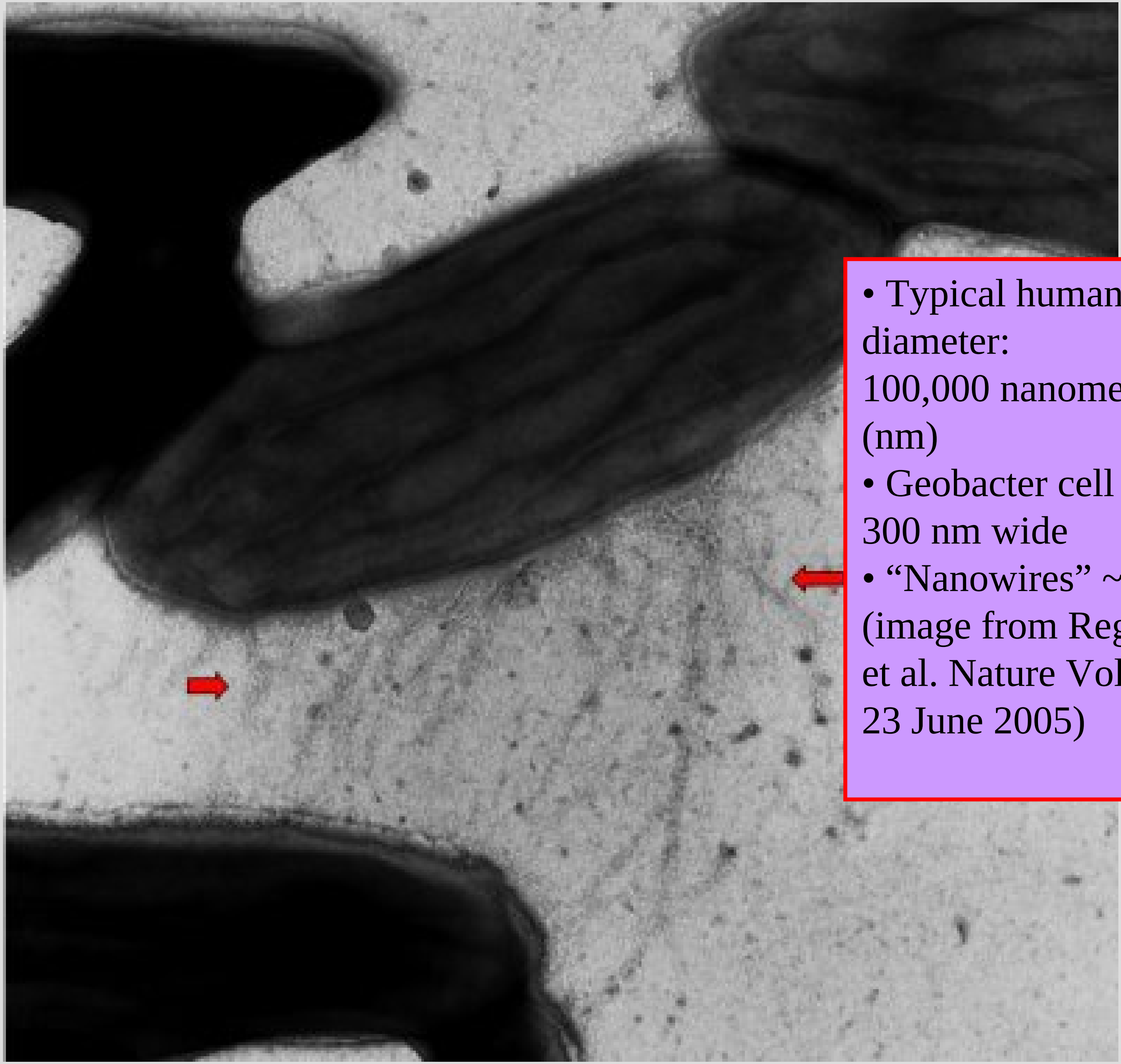


Acetate structure



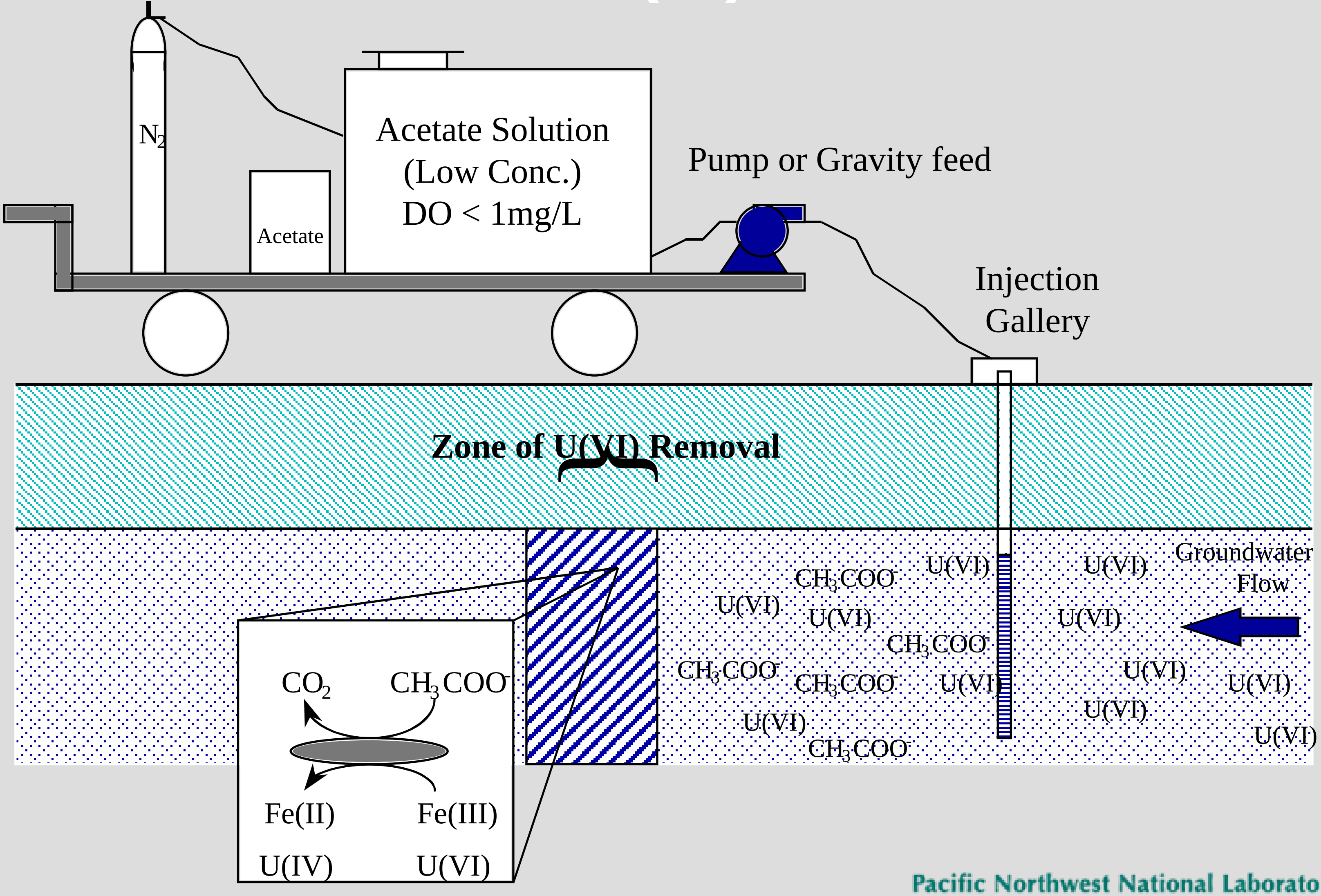
$$Na^{+}\ {}^{-}O-\overset{\overset{O}{\|}}{C}-CH_3$$





- Typical human hair diameter: 100,000 nanometers (nm)
- Geobacter cell at left 300 nm wide
- “Nanowires” ~10 nm (image from Reguera et al. Nature Vol 435, 23 June 2005)

Implementation of *in situ* bioremediation of U(VI)



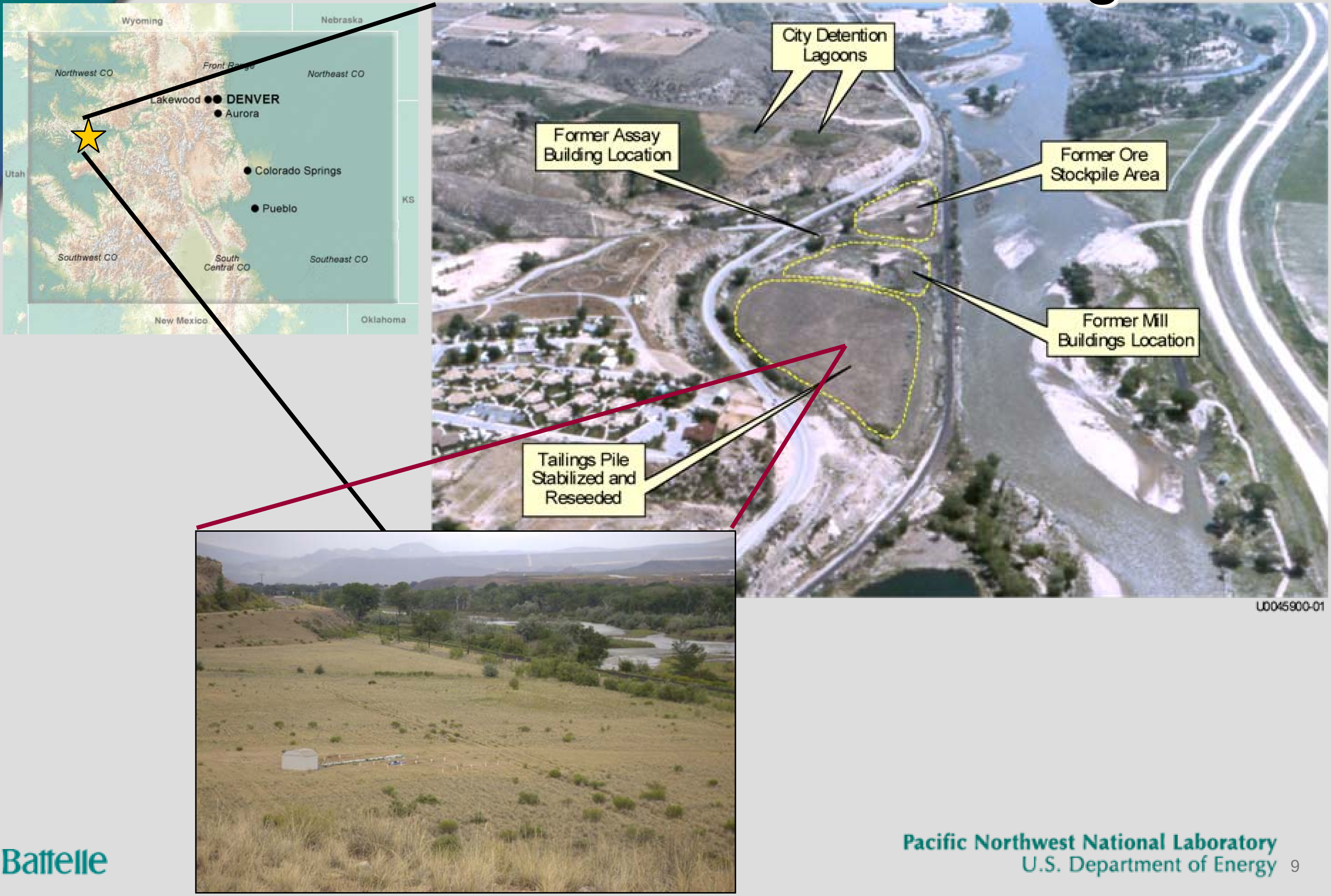
Context for metal and radionuclide bioremediation

~Decreasing cost →

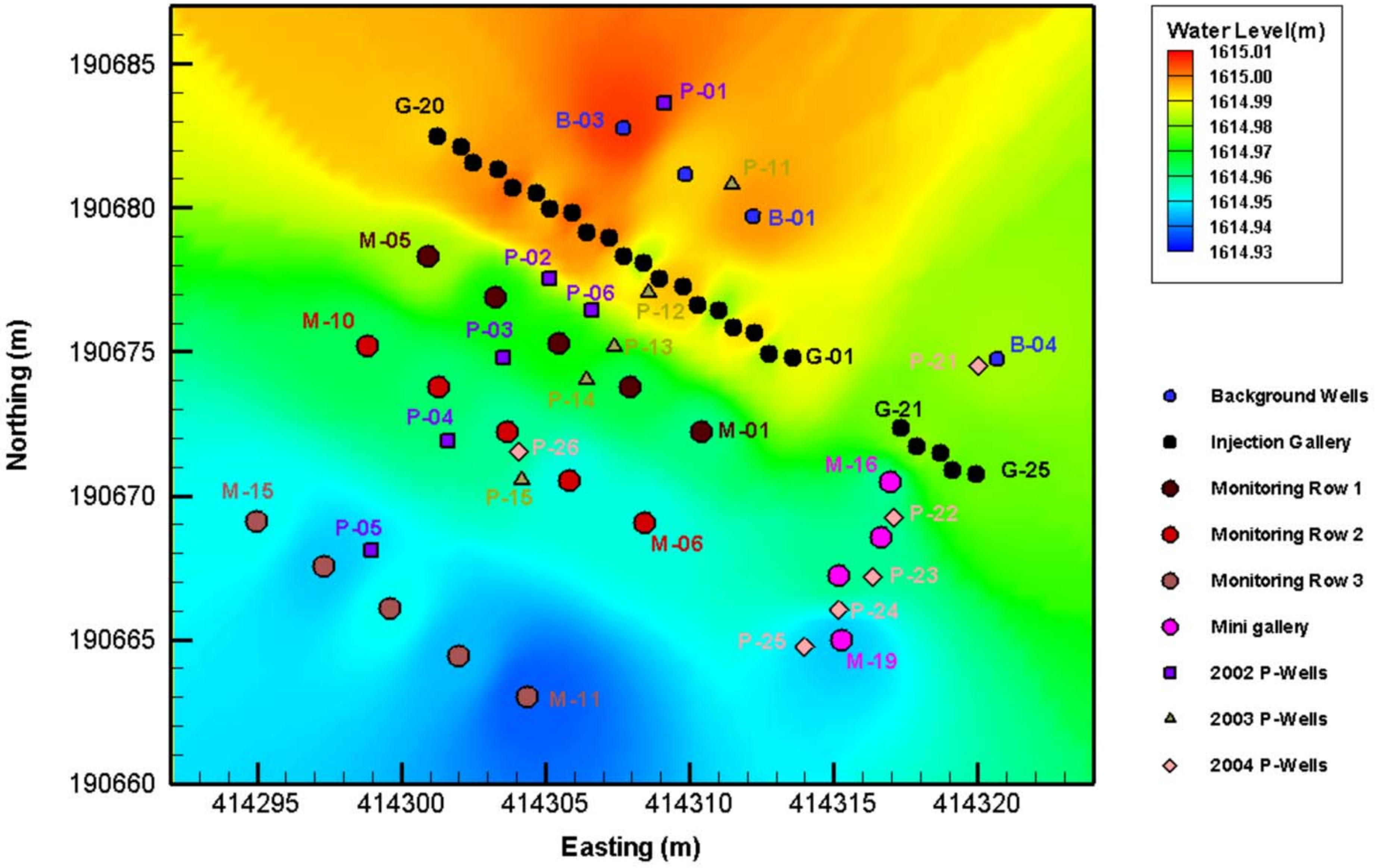
Active ↔ Passive

		Bioremediation			
Remedial approach	Pump and treat	Forced gradient amendment	Natural gradient displacive amendment	Natural gradient non-displacive amendment	Monitored natural attenuation
Comments	Usually the most costly, may be required for hydraulic control	Cost for maintaining gradient. Control on flow direction	Difficult to separate injectate dilution from microbial effects	Minimal dilution effect. Limited donor concentration	Contaminants may not respond in desired time frame

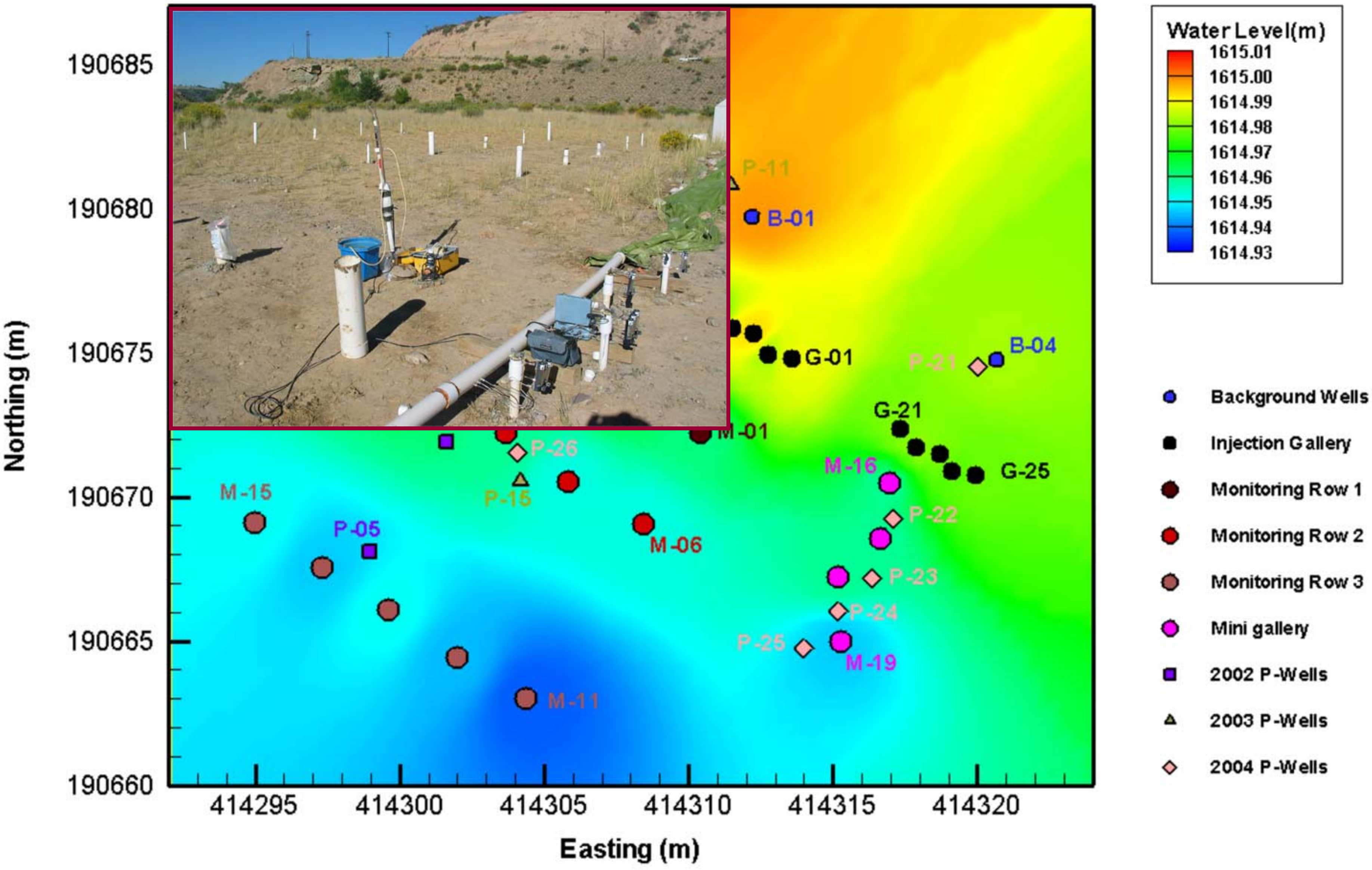
Context for field bioremediation research at the Old Rifle Uranium Mill Tailings Site



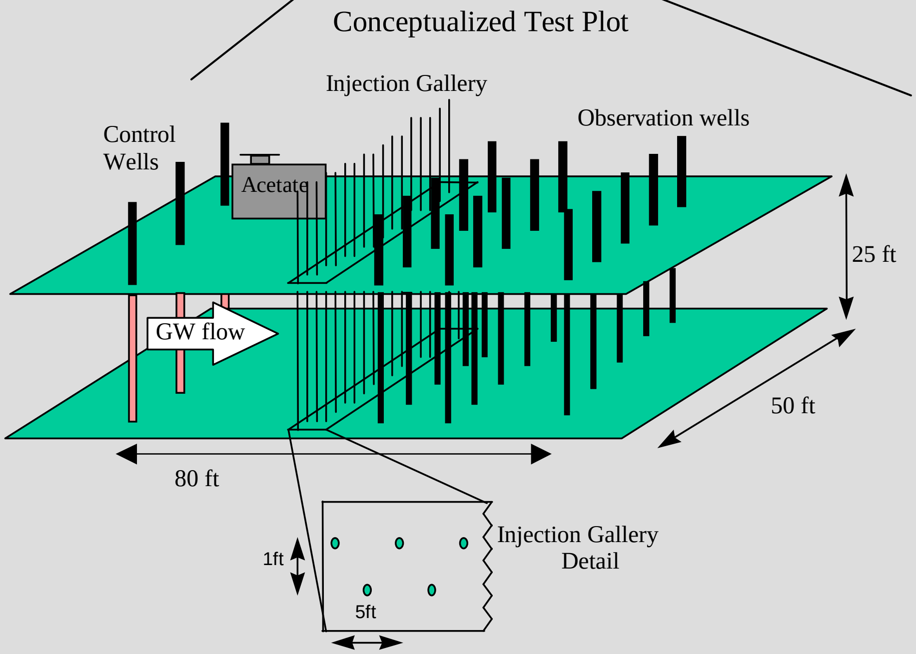
Map of NABIR Biostimulation Well Field and Water Table Contours (04/07/05) Old Rifle UMTRA Site, Rifle, CO

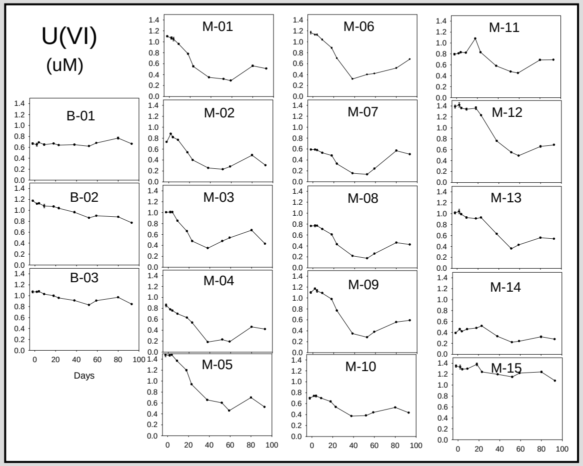


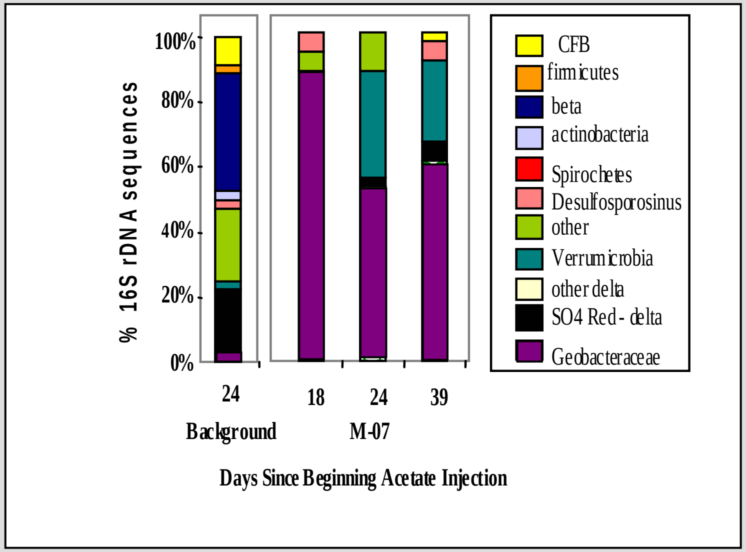
Map of NABIR Biostimulation Well Field and Water Table Contours (04/07/05) Old Rifle UMTRA Site, Rifle, CO



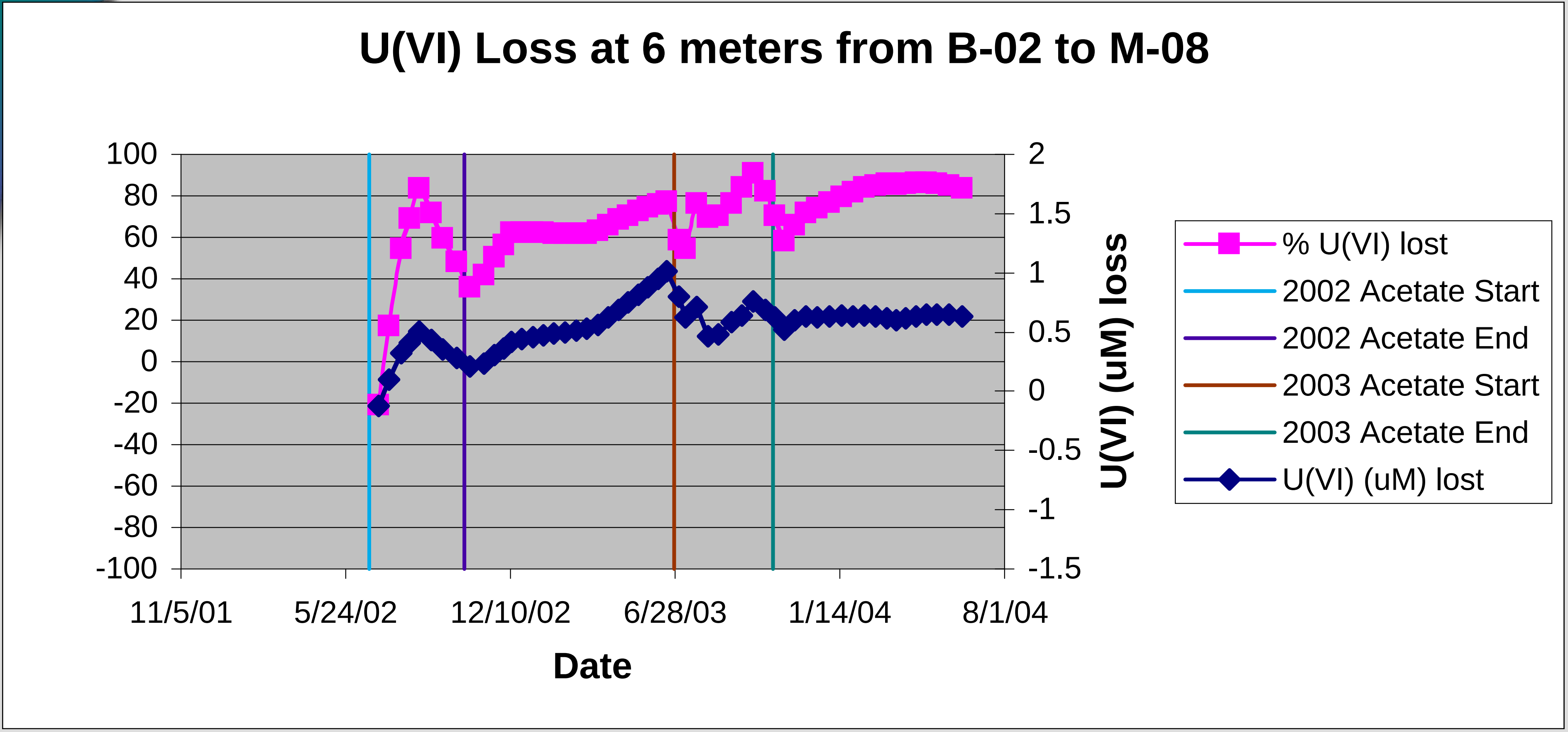
Previous work at the Rifle Site



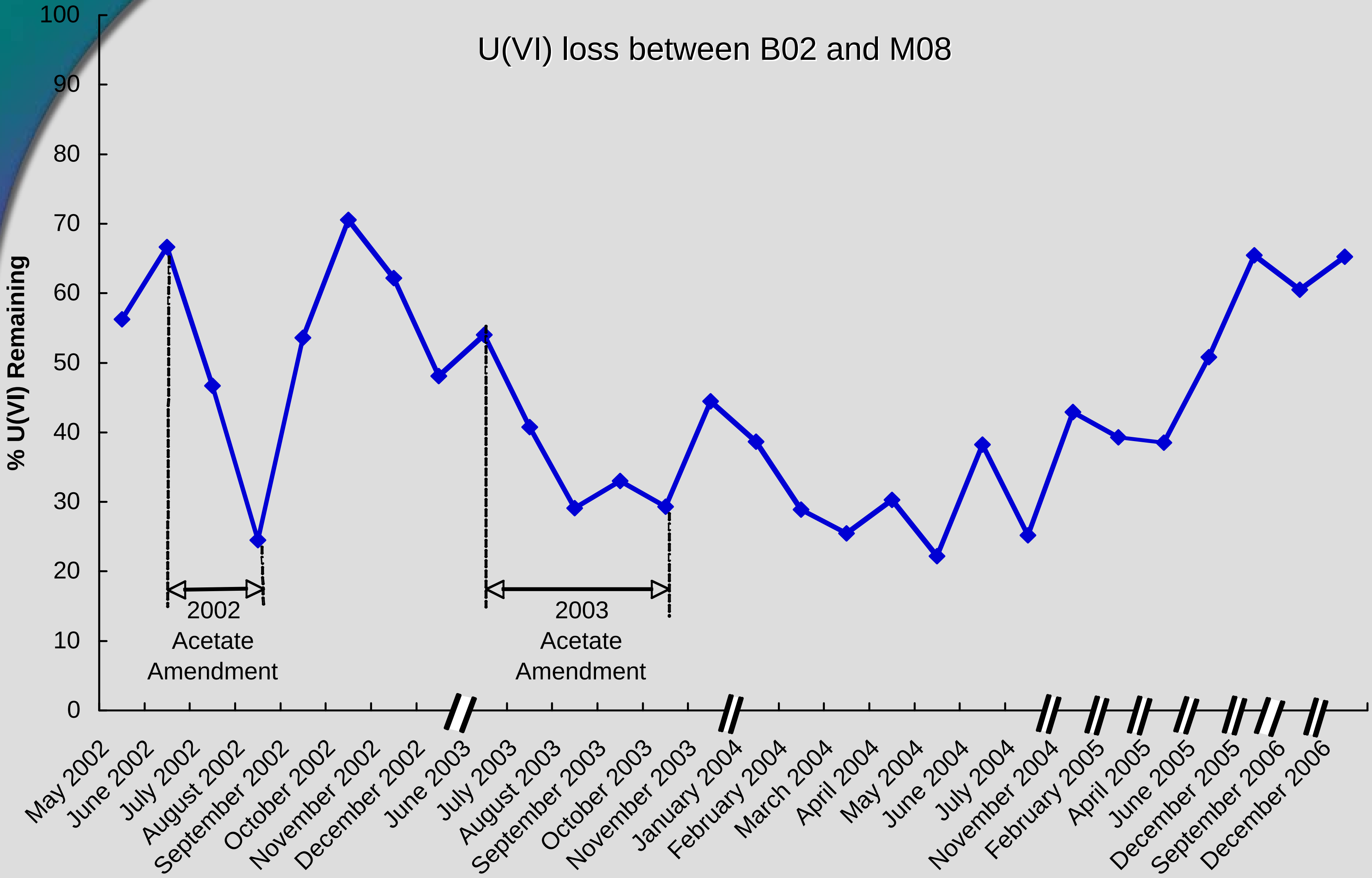




U(VI) loss for both 2002 and 2003 experiments



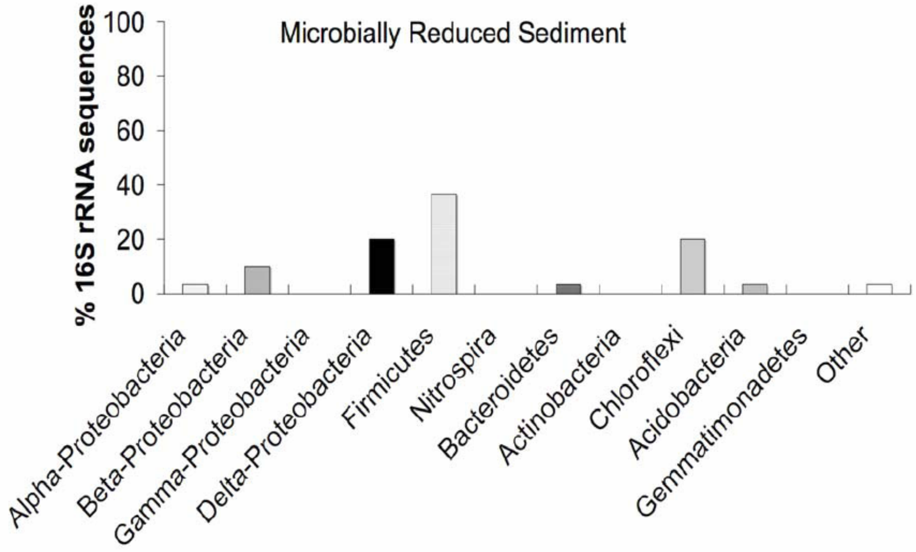
The Unexpected Continued Removal of U(VI)

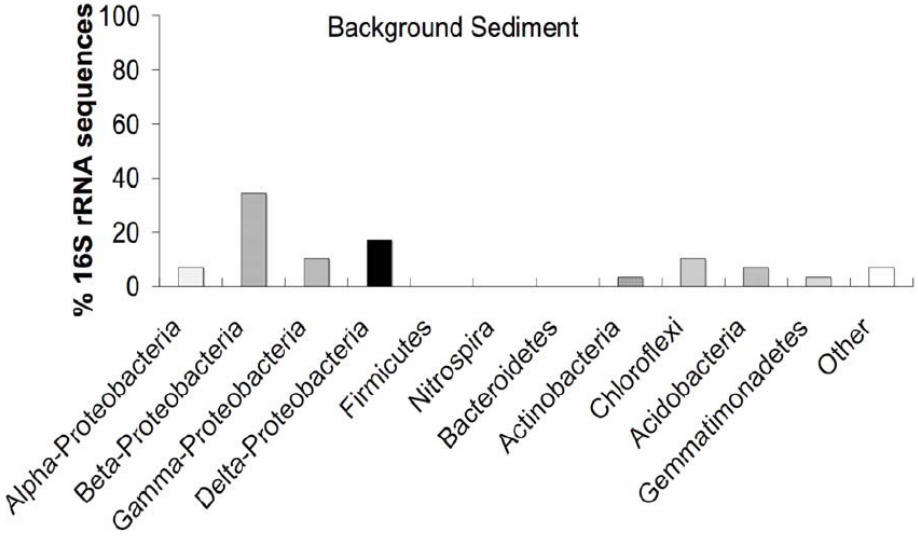


Source: N'Guessan *et al.* 2007 *ES&T in review*

Firmicutes predominate in post-amendment reduced sediment

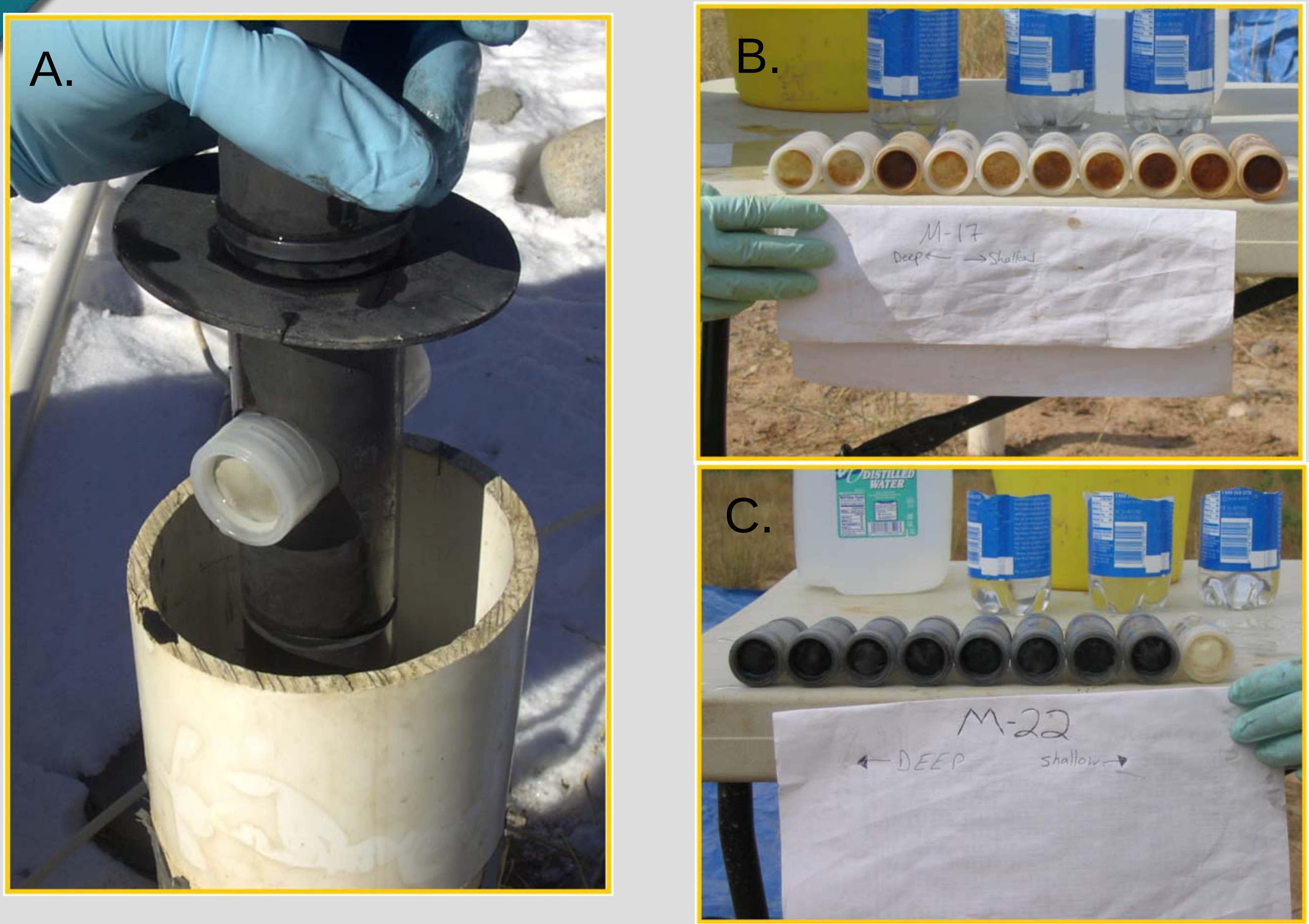
Distribution of 16S rRNA gene sequences





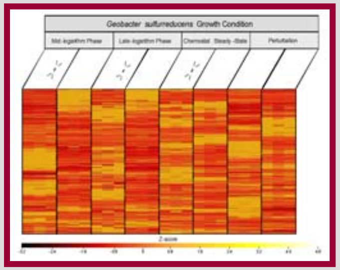
Source: N’Guessan *et al.* 2007 *ES&T in review*

Passive multilevel samplers. A. Cell on support rod being lowered into monitoring well. B. MLS cells from a background well. C. MLS cells from a treatment zone well undergoing sulfate reduction.



Rifle Integrated Field Challenge Site:

- Objective: development of a mechanistic understanding of subsurface mobility of uranium at the field scale
- Testing hypotheses relating to:
  - Extension of Fe-reducing conditions
  - U(VI) Sorption under reducing conditions
  - Mechanisms for post-biostimulation U removal
  - Rates of natural bioreduction of U
- Key approaches:
  - Mechanisms of U bioreduction illuminated by protein expression
  - Relative contribution of biotic processes and abiotic uranium immobilization processes evaluated (e.g. U bioreduction and U sorption)
  - Correlation of subsurface geochemical processes with geophysical monitoring of subsurface redox status associated with bioreduction
  - Comprehensive reactive transport modeling of uranium mobility in the subsurface



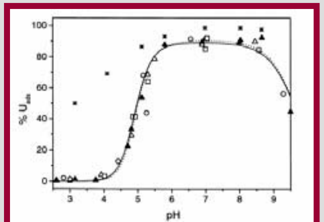
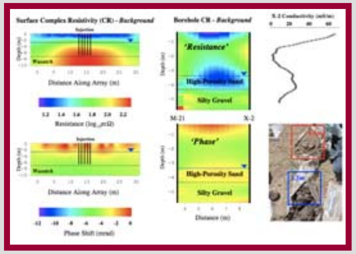
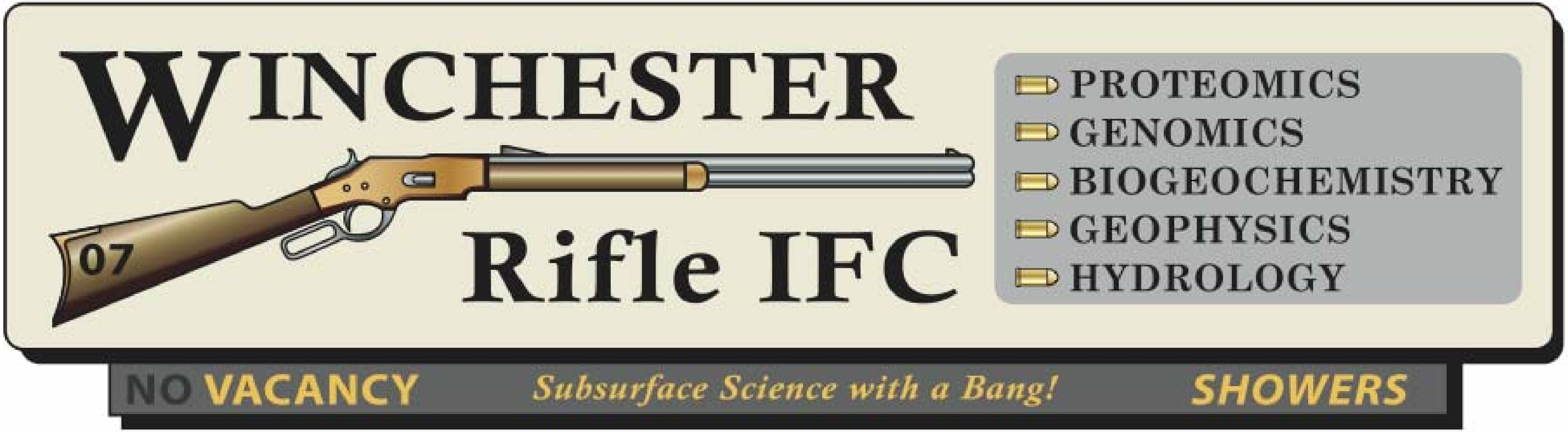


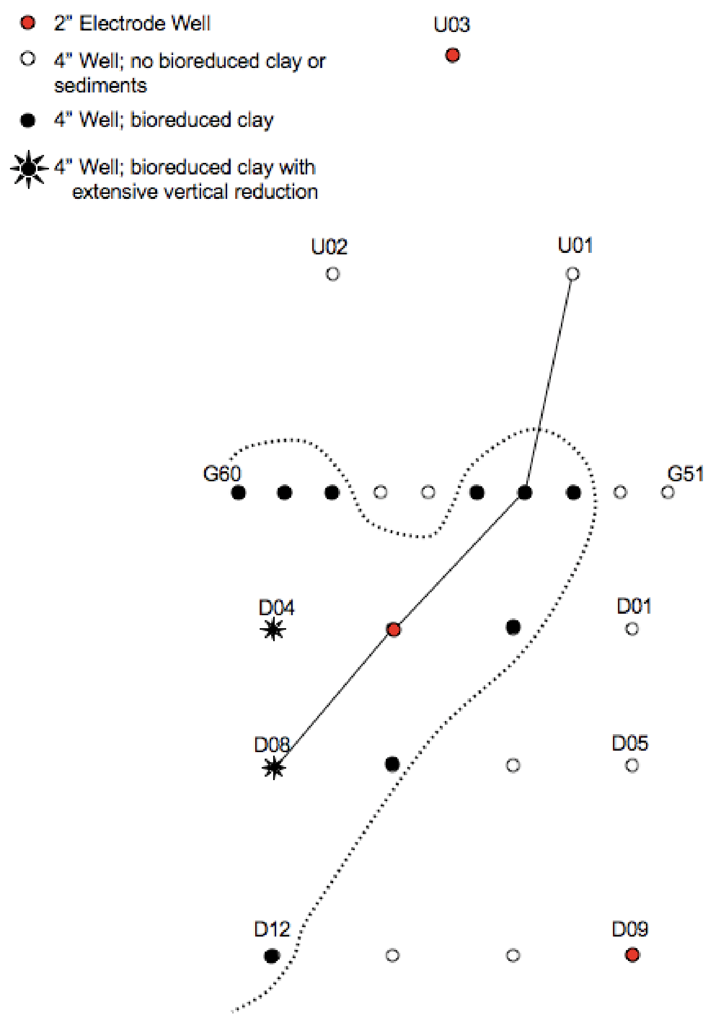
Fig. 5. Sorption edge at different ionic strengths: (▲) I = 0.1 mol/L, 1 d; (△) I = 0.1 mol/L, 4 h; (*) I = 0.1 mol/L, 3 months. (□) I = 0.2 mol/L, 4 h; (○) $I = 1 \cdot 10^{-3}$ mol/L, 4 h. The lines correspond to the modelling of the experimental data at I = 0.1 mol/L: (- - -) Model 1 and (—) Model 2. Solid to liquid ratio 2 g/L and $[U] = 4.4 \cdot 10^{-7}$ mol/L.

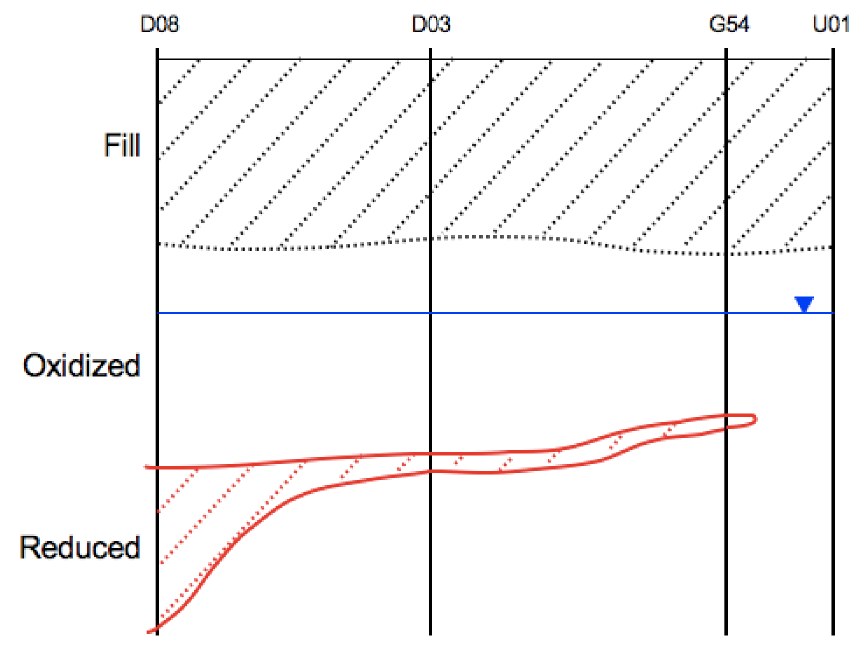




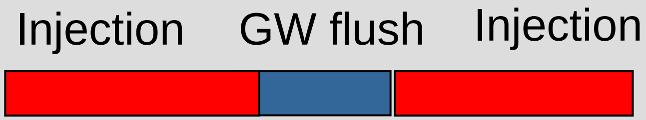
- 2007 field experiment
  - Replicated earlier field experiments showing U(VI) bioreduction by Geobacter
  - Intentionally limited acetate during part of the experiment
  - Successfully generated samples for proteomic and metagenomic analysis
  - Data sets include: U(VI) removal rates, hydraulic conductivity, hydrogeophysical monitoring, gene expression data, mineralogical changes, in situ incubators/sensors
  - Direct access to naturally bioreduced sediment (and uranium?)

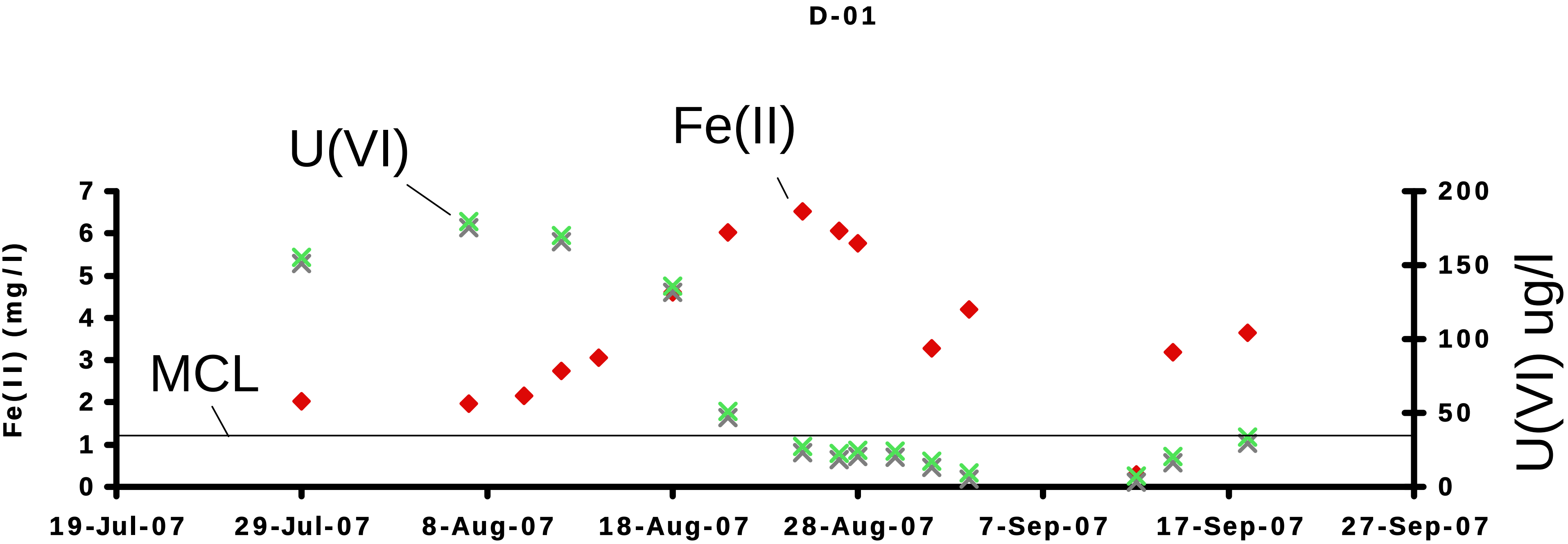
Winchester Well Layout and Distribution of Reduced Sediments



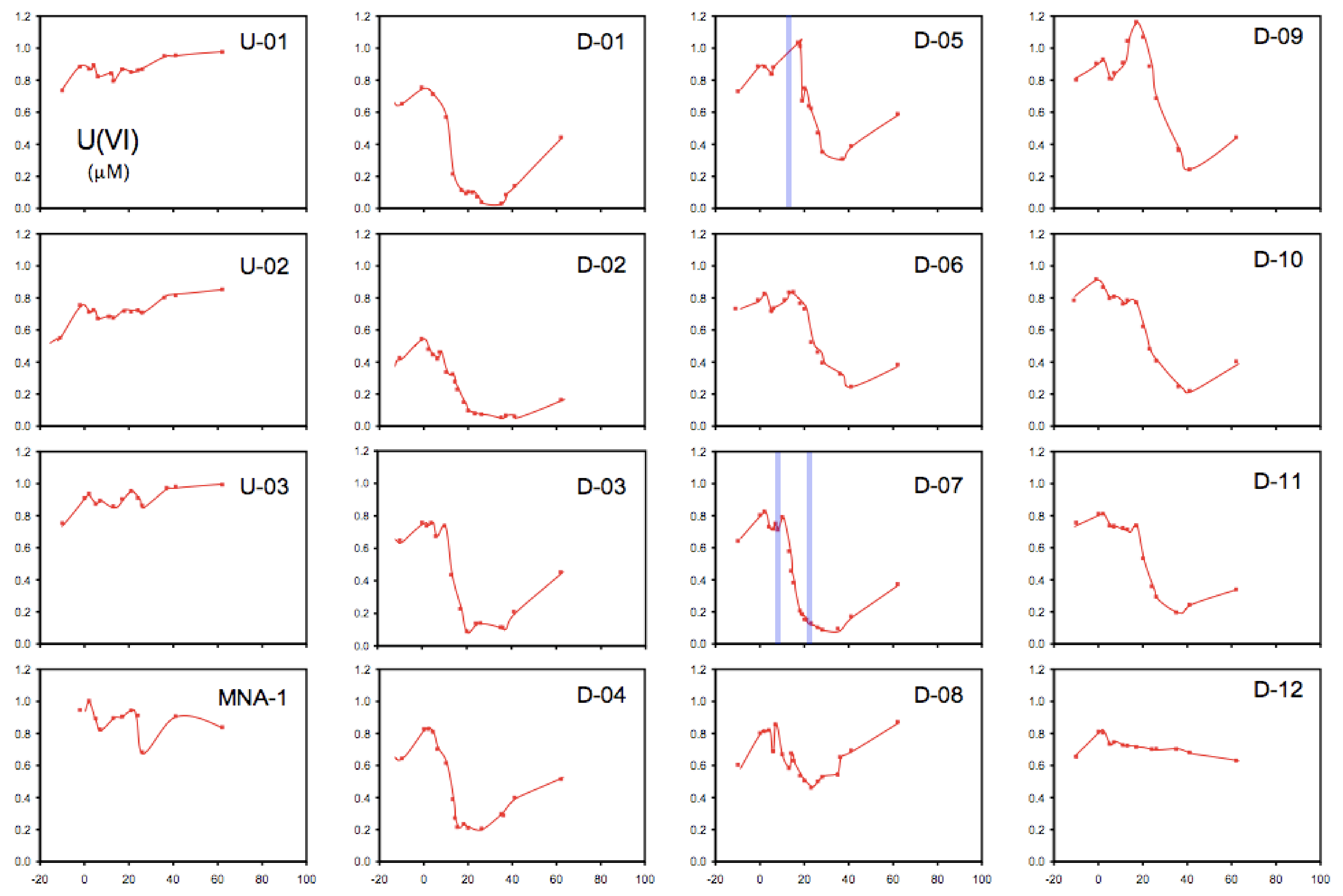


Fe(II) and U(VI) trends





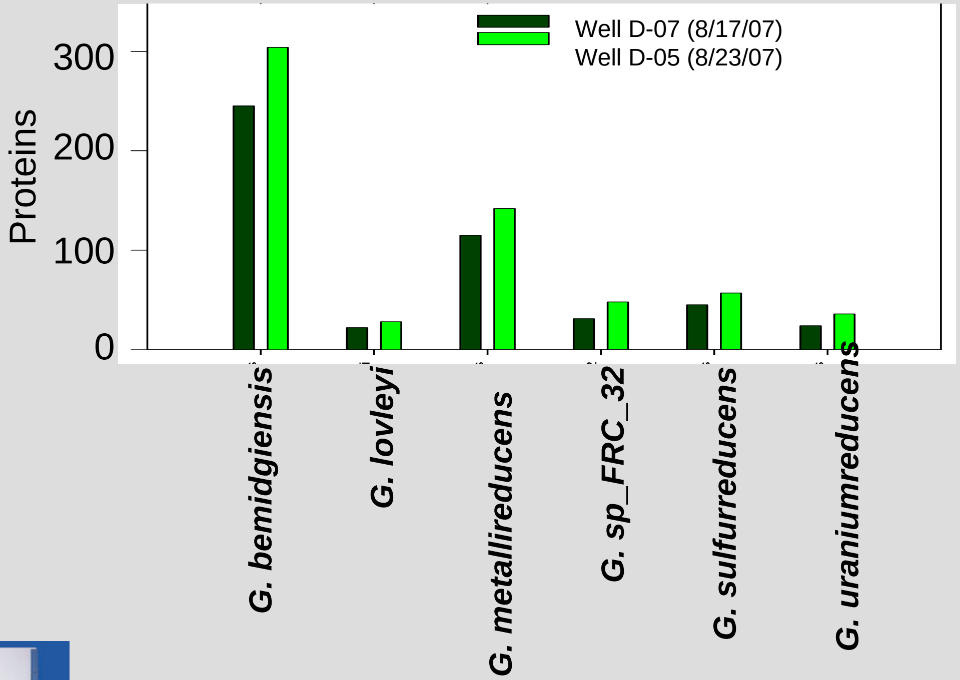
Uranium Concentration (μM) as a function of time (days)



Battelle

Pacific Northwest National Laboratory
U.S. Department of Energy

Proteomic Sampling and Characterization of the Microbial Community Structure and Dynamics During Electron Donor Amendment at the Rifle IFC



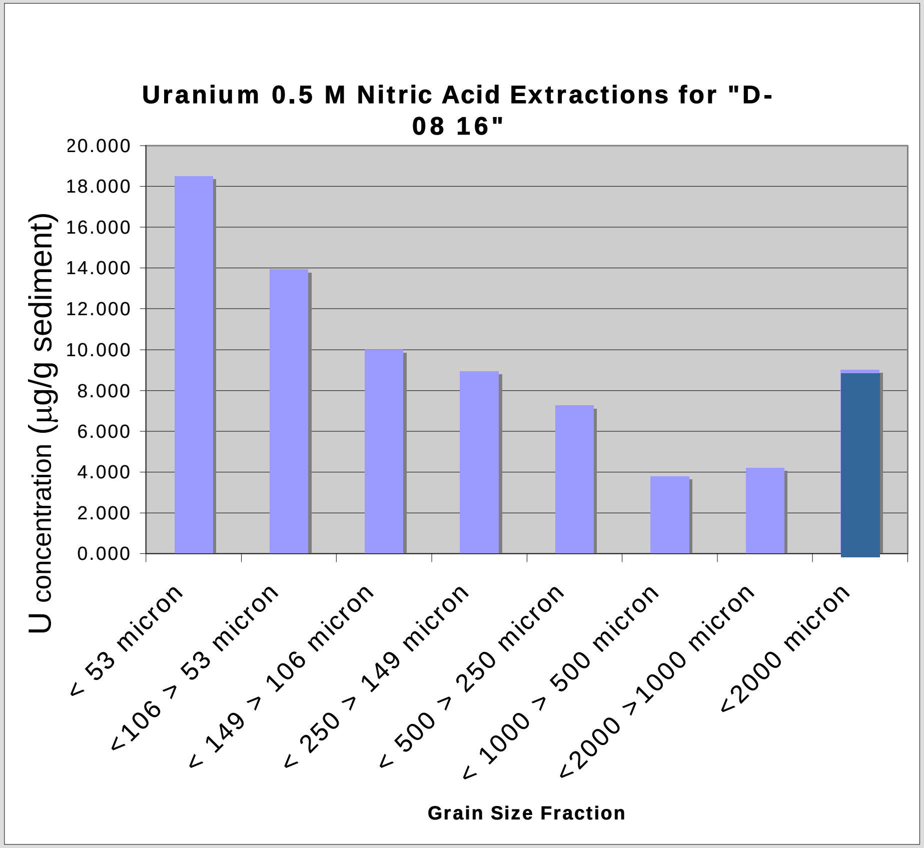
- In groundwater from downgradient wells, proteins from six different Geobacter species were detected where G. bemidjiensis was the most abundant organism
- Protoemic data are also being used to characterize the microbial community from stimulated sediments
- Proteomic data are being obtained both at EMSL/PNNL and ORNL, and analyzed jointly with UC Berkeley

Evaluation of uranium bioremediation

- Characterization
- Conceptual model development
- Monitoring
- Numerical modeling

Characterization

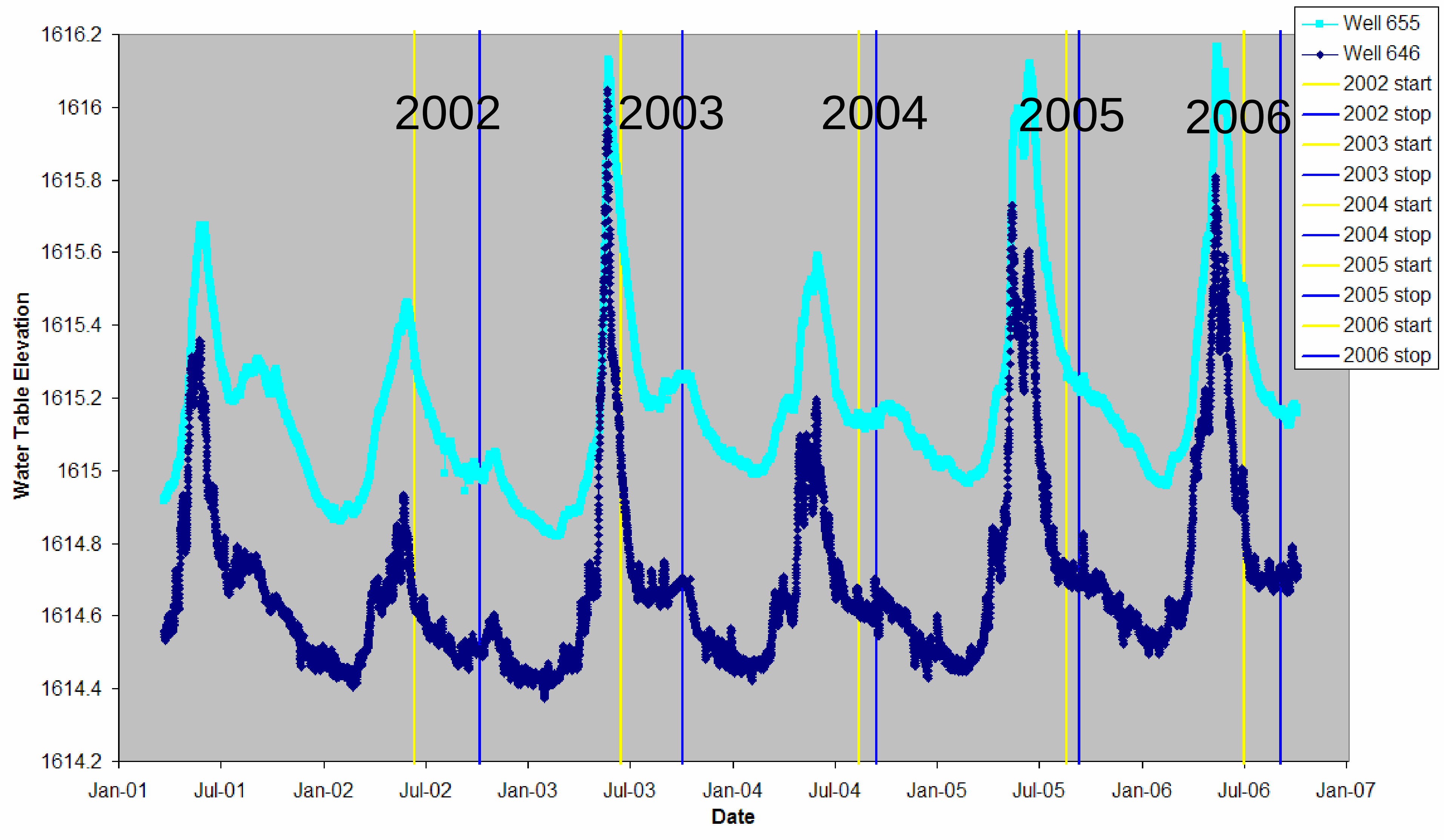
Importance of Sediment Grain Size to U sorption



Monitoring

- Seasonal changes
- Appropriate spatial coverage
- Real time monitoring
- Event-based sampling
- Passive in situ geochemical and biological sampling

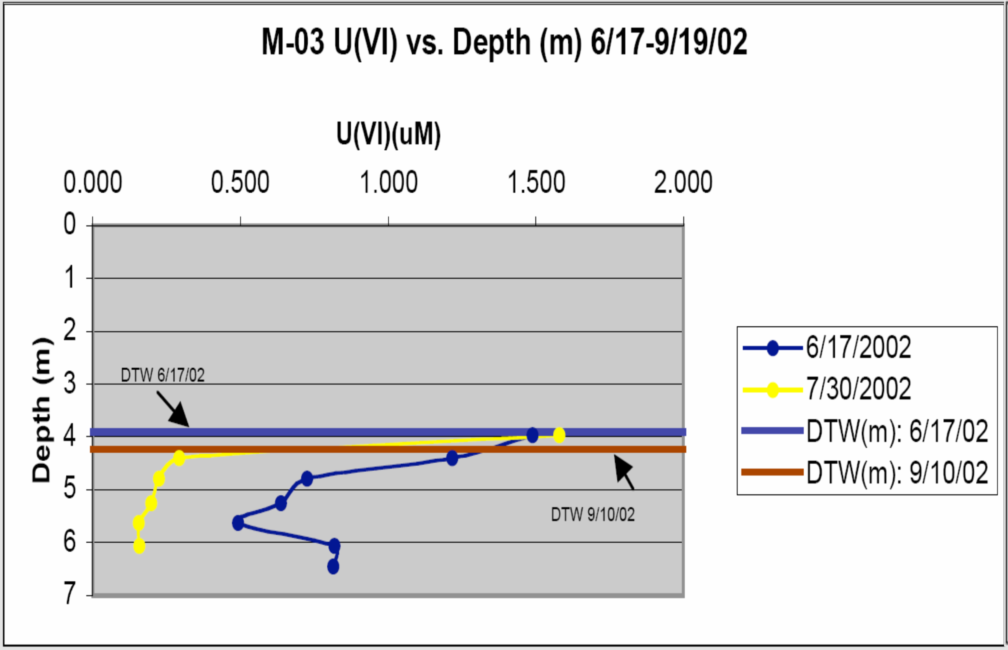
Water Table Elevation, Rifle Site 2001 to 2006

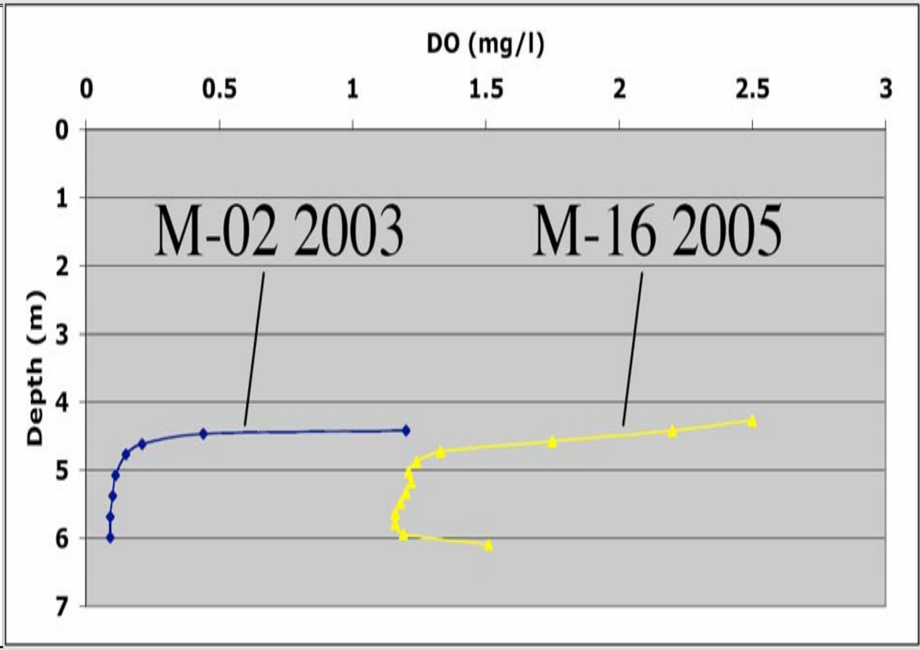


Stratified Water Chemistry

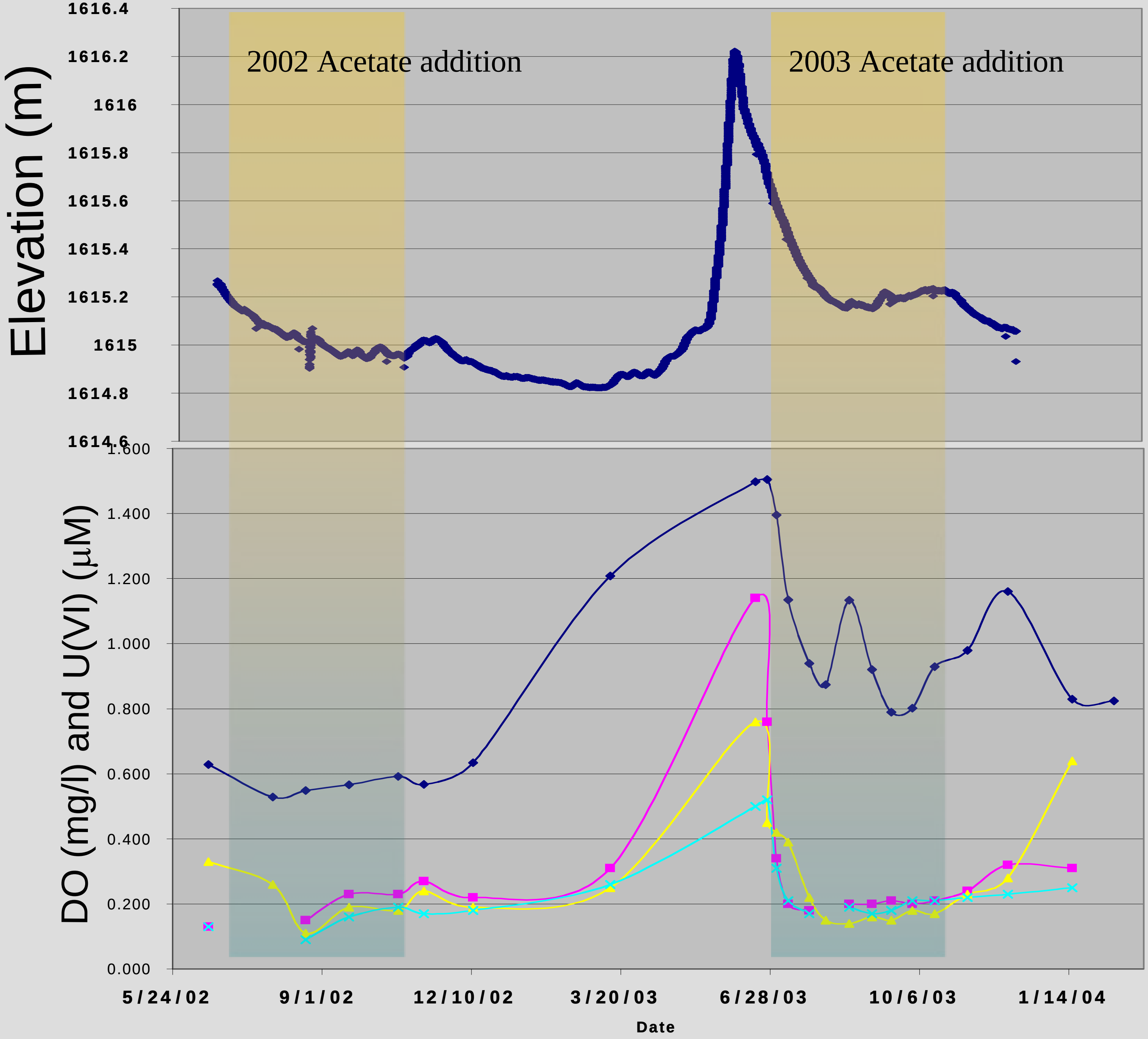
Depth-dependent U(VI) and DO

- Highest DO and U(VI) near the water table
- Issues
    - Oxygen diffusion through water table
    - Background utilization of DO
    - Screened interval of wells





B-01 Water table elevation



Biogeochemical Reaction Network

- Multisite, multicomponent uranium surface complexation model
  - $SSOH + UO_2^{2+} = SSOUO_2^{+} + H^{+}$ Log K = 6.798
  - $SOH + UO_2^{2+} = SOUO_2^{+} + H^{+}$ Log K = 5.817
  - $WOH + UO_2^{2+} = WOUO_2^{+} + H^{+}$ Log K = 2.57
  - $SSOH + UO_2^{2+} + H_2O = SSOUOOH + 2H^{+}$ Log K = -0.671
  - $SOH + UO_2^{2+} + H_2O = SOUOOH + 2H^{+}$ Log K = -2.082
  - $WOH + UO_2^{2+} + H_2O = WOUOOH + 2H^{+}$ Log K = -5.318
- 23 uranium aqueous complexation reactions
- 91 abiotic species, minerals, surface sites
- 3 energetics-based acetate-oxidizing TEAP reactions with biomass yield
- Dual Monod Rate Law with thermodynamic constraints

Iron TEAP

$0.125CH_3COO^{-} + 0.6FeOOH(s) + 1.155H^{+} + 0.02NH_4^{+} = 0.02BM\_iron + 0.6Fe^{++} + 0.96H_2O + 0.15HCO_3^{-}$

$$R = k[\text{free surface sites}]\frac{[\text{Ac}]}{\text{K}_{\text{Ac}} + [\text{Ac}]}\left(1 - \exp\left(\Delta G_r^0 + 2.3RT\log\left(\frac{\{Fe^{2+}\}^8\{HCO_3^{-}\}^2}{\{H^{+}\}^{15}\{CH_3COO^{-}\}}\right) - \Delta G_{\min}\right)/RT\right)$$

Sulfate TEAP

$0.125CH_3COO^{-} + 0.0057H^{+} + 0.0038NH_4^{+} + 0.1155SO_4^{--} = 0.0038BM\_sulfate + 0.0114H_2O + 0.231HCO_3^{-} + 0.1155HS^{-}$

$$R = k\frac{[SO_4^{2-}]}{K_{so4} + [SO_4^{2-}]}\frac{[\text{Ac}]}{\text{K}_{\text{Ac}} + [\text{Ac}]}\left(1 - \exp\left(\Delta G_r^0 + 2.3RT\log\left(\frac{\{HS^{-}\}\{HCO_3^{-}\}^2}{\{SO_4^{2-}\}\{CH_3COO^{-}\}}\right) - \Delta G_{\min}\right)/RT\right)$$

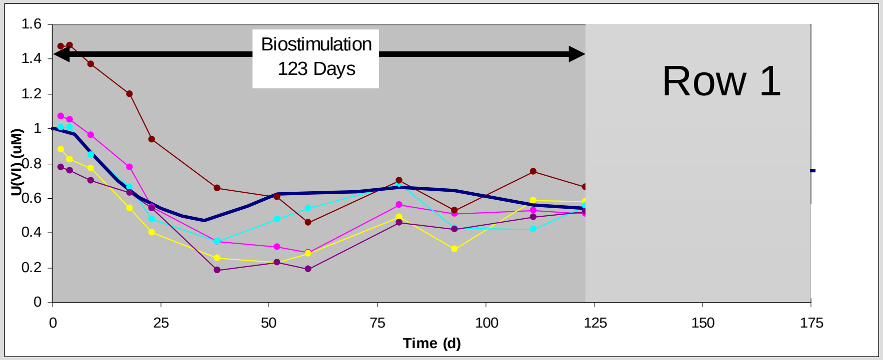
Uranium TEAP

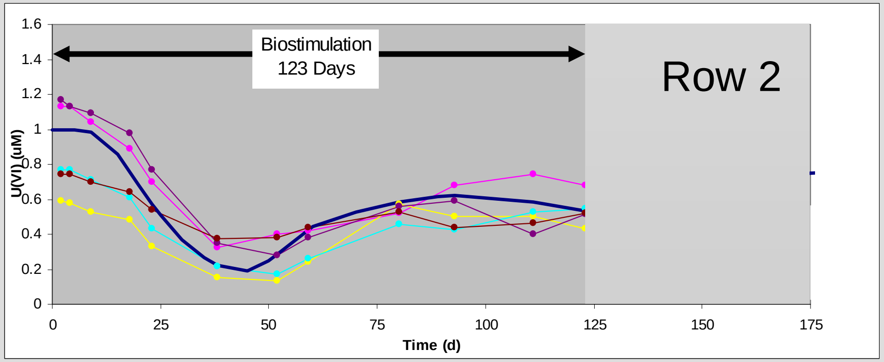
$0.1250CH_3COO^{-} + 0.3538H_2O + 0.0113NH_4^{+} + 0.3875UO_2^{++} = 0.0113BM\_iron + 0.855H^{+} + 0.1938HCO_3^{-} + 0.3875UO_2(s)$

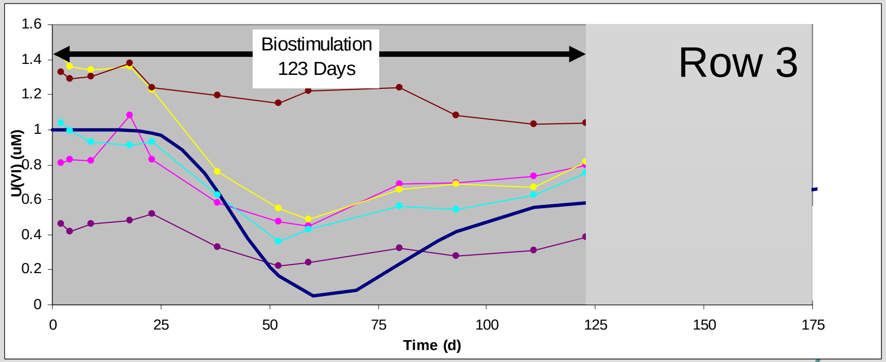
$$R = k\frac{[U(VI)aq]}{K_U + [U(VI)aq]}\frac{[\text{Ac}]}{\text{K}_{\text{Ac}} + [\text{Ac}]}\left(1 - \exp\left(\Delta G_r^0 + 2.3RT\log\left(\frac{\{H^{+}\}^9\{HCO_3^{-}\}^2}{\{UO_2^{2+}\}^4\{CH_3COO^{-}\}}\right) - \Delta G_{\min}\right)/RT\right)$$

Reactive Transport Modeling of Uranium Bioreduction

- Initial aqueous U(VI) spatially variable
- Initial timing of aqueous U(VI) removal reproduced by model
- Uranium rebound is slower than model prediction
- Considerations
  - Sulfate reducers removing some uranium
  - Uranium adsorption retarding front

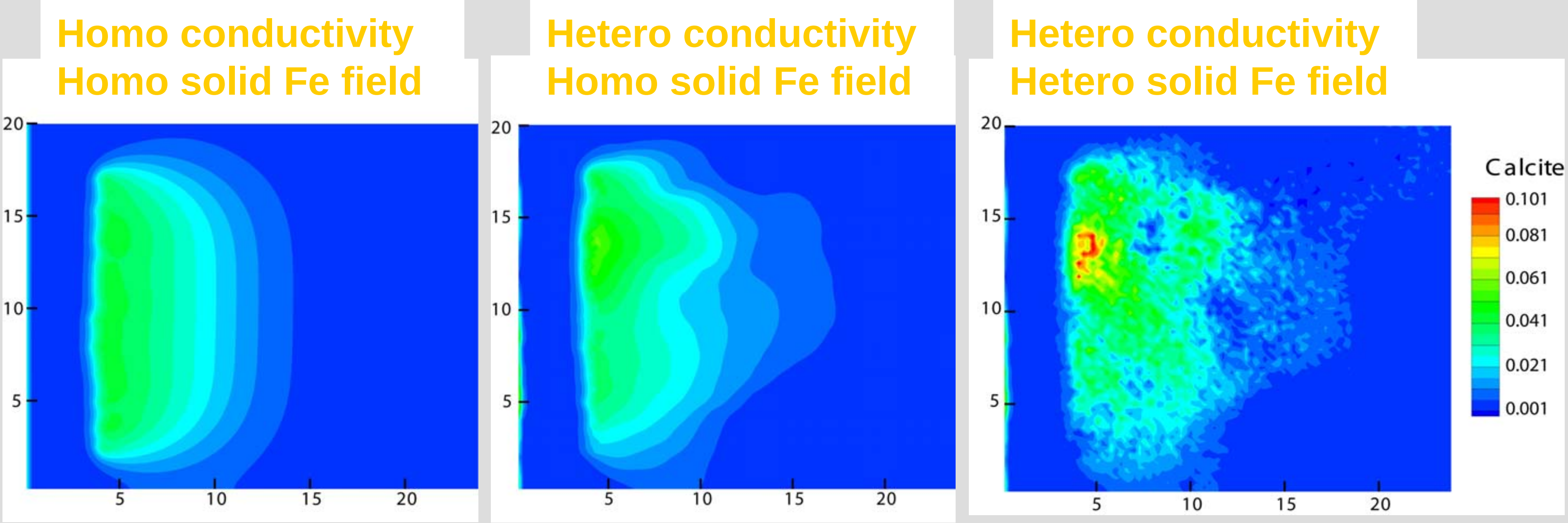






Exploring effects of physical and chemical heterogeneities on spatial patterns of calcite precipitation

All three cases: same average flow velocity, and same total solid iron content
Heterogeneous conductivity field obtained from inverse modeling of tracer data
Heterogeneous Fe content: negative correlation with conductivity



Homo conductivity
Homo solid Fe field

Hetero conductivity
Homo solid Fe field

Hetero conductivity
Hetero solid Fe field

Physical and chemical heterogeneities lead to locally larger amounts of calcite precipitation, therefore increases the possibility of clogging.

Carl Steefel, Li Li (LBNL)

Future directions and developments

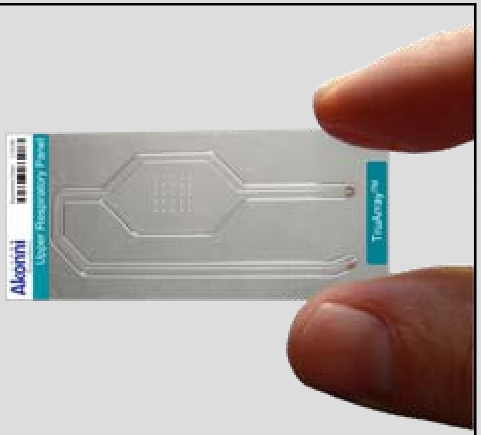
- In-field monitoring of selected genes and proteins
- Coupling of reactive transport models with in silico microbial models

Field Portable Microarray Analysis of Groundwater and Sediment

ERSP Science

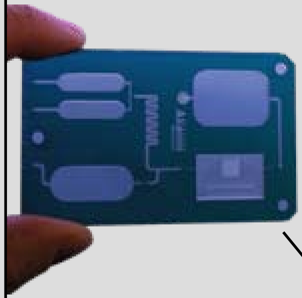
- *Breadboard lab instrument*
- *Automated methods for direct detection of RNA*
- *Environmentally relevant samples, biomass and sample sizes*
- *Environmentally relevant and vetted microarray probes*
- *Statistical techniques for converting noisy data into actionable information*

Rifle IFC Science/ Generation 1 Products



- *End-to-end kits*
- *Large-volume sample preparation device and chemistry*
- *Amplification cocktails and reagents*
- *Field portable analysis instrument*
- *Semi-automated algorithms and software*

Rifle IFC/DOE SBIR Generation 2 Products



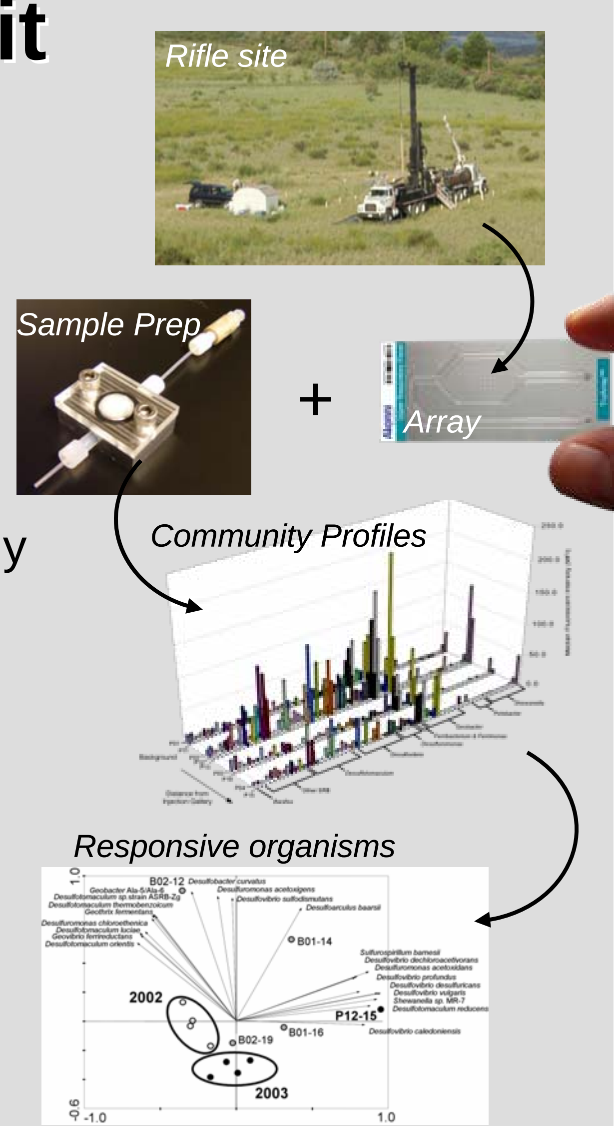
Sample-to-answer integrated cartridges

Portable controller instrument = fluidics, microarray analysis, automated reporting. Insert cartridge and press “go”

Darrell P. Chandler, Chief Science Officer
Akonni Biosystems

Field-Portable Kit

Process flow

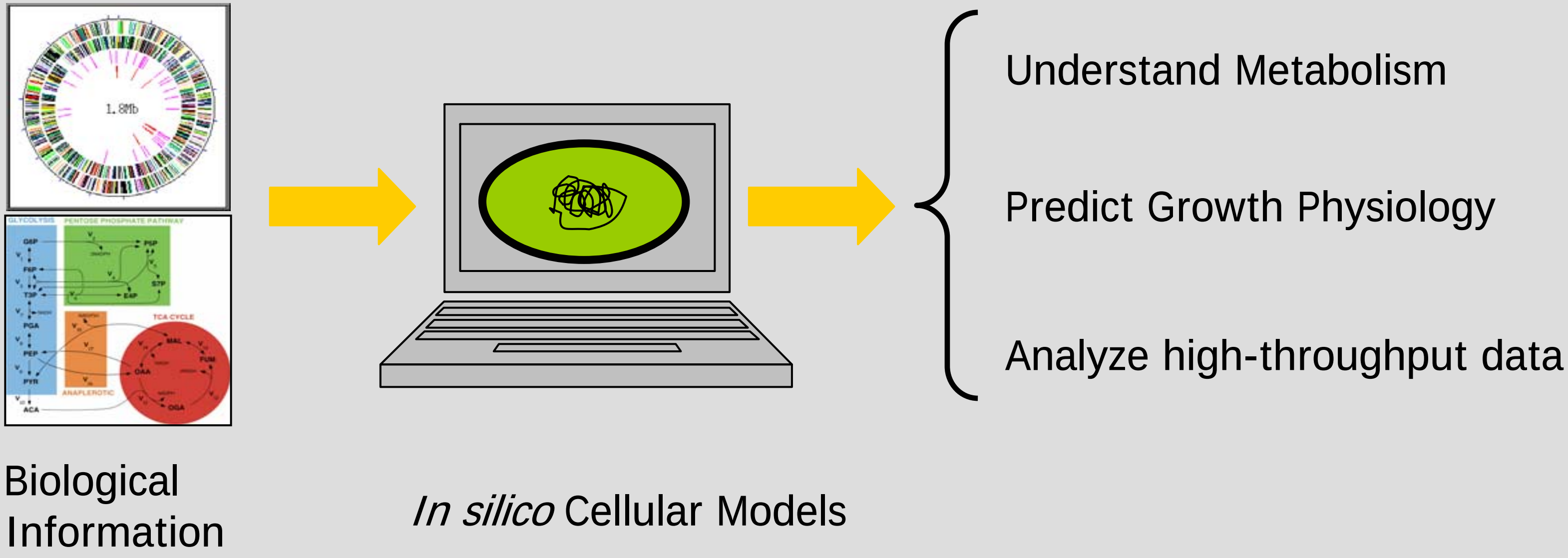


- Collect and concentrate sample
  - Groundwater or sediment
- Bead-beater lysis (5 min)
- Universal, flow-through nucleic acid purification and concentration preparation (15 min)
  - Will isolate both DNA and RNA simultaneously
- Simultaneous asymmetric amplification + microarray hybridization in same flow cell (120 min)
  - Single-pot DNA or RNA amplification and labeling
- Wash (5 min)
- Imaging, data extraction, analysis and reporting (5 min)
- = 2.5 hours, sample-to-answer microbial community profiling

Constraint-Based *In Silico* Modeling

- Genetic characterization of reaction pathways
- Laboratory characterization of flux constraints
- Optimization under specific conditions

Slide material from R. Mahadevan (Univ. of Toronto)



Understand Metabolism

Predict Growth Physiology

Analyze high-throughput data

Biological Information

In silico Cellular Models

Conclusions on U(VI) bioremediation (natural gradient, non-displacive)

- Effectively removes U(VI) from groundwater
- Additional field-scale research needed understand mechanisms and durability
- Precise monitoring of microbial activity on the horizon
- Electron donor pulsing and “engineering” of specific precipitates *in situ* may enhance long-term stability
- Compatible with monitored natural attenuation
- Amenable to regulatory evaluation

Acknowledgements

- Funding for documenting the prerequisite characterization, conceptual model requirements, and monitoring requirements is provided by the U.S. Nuclear Regulatory Commission
- Funding for all three IFC’s (Rifle, CO, Hanford 300 Area, and ORNL) is provided by the U.S. Department of Energy, Office of Science, Biological and Environmental Research, Environmental Remediation Sciences Division
- Pacific Northwest National Laboratory is operated by Battelle Memorial Institute for the United States Department Of Energy under Contract DE-AC06-76RL01830

U.S.NRC

Office of Science
U.S. DEPARTMENT OF ENERGY

Rifle IFC Project Participants

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