energy.gov
U.S. DEPARTMENT OF ENERGY

Field scale evaluation of biostimulation in the near source zone of the former S3 ponds at Oak Ridge

Stanford University

Craig Criddle
Scott Fendorf
Michael Fienen
Margy Gentile
Matt Ginder-Vogel
Peter Kitanidis
Jian Luo

Anna Michalak
Jennifer Nyman
Julie Stevens
Weimin Wu

Jack Carley

Oak Ridge National Laboratories

Craig Brandt
Scott Brooks
Sue Caroll
Matthew Fields
Baohua Gu
Philip Jardine
Tonia Melhourne
Terry Gentry

Ye-Kyoung Ku
Ken Lowe
Stacy Rast
Jeff Riggs
Jizhong Zhou
Yul Roh
Christopher Scaadt
Bobette Nourse

Universität Stuttgart

Olaf Cirpka
Frank-Andreas Weber

NABIR Field Research Center

David Watson

Retec

Robert Hickey
Raj Rajan
Dan Wagner

LBNL

Susan Hubbard
Ken Williams

Argonne

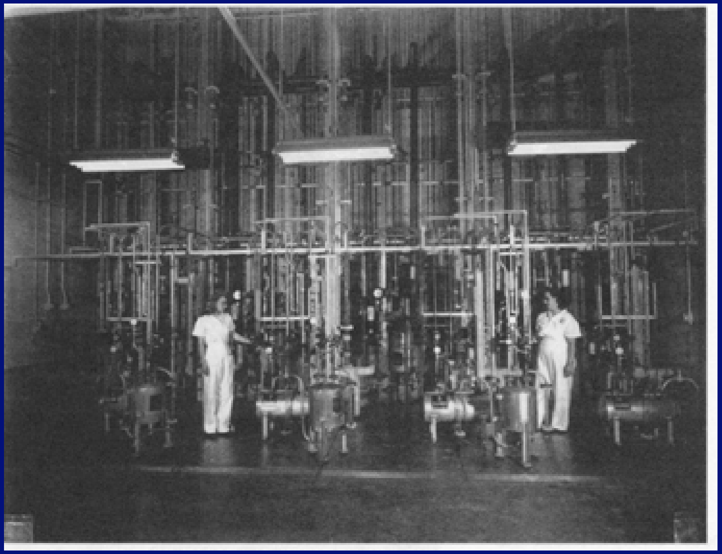
Ken Kemner

INEEL

Gill Geesey
David Cummings

Michigan State University

Terry Marsh



The Oak Ridge S3 ponds

- Depleted uranium
- Strong acids (HNO_3 and H_2SO_4)
- Halogenated solvents
- Heavy metals

1951-1984 : wastes stored in unlined ponds

Field research station

A large parking lot now covers the S3 ponds. This is the “source zone”.

Contaminants in groundwater near the S3 ponds

Inorganic Constituents	Concentrations	Organic Constituents	Concentrations
pH	3.4-3.6	BOD5	200 mg/L[*]
TIC	202-401 mg/L	COD	300 mg/L[*]
Chloride	249-298 mg/L	TOC	65-81 mg/L
Sulfate	843-1116 mg/L	2-Butanone	69-84 µg/L
Nitrate	7500-8963 mg/L	Acetone	340-700 µg/L
Nitrite	Low	Ethanol	200 µg/L
Uranium	42-51 mg/L	Chloroform	34-36 µg/L
Technetium-99	35-40 nCi/L (80-89 dpm/ml)	Tetrachloroethene	2100-3300 µg/L
Ni	11.5-14 mg/L	Trichloroethene	94-130 µg/L
Cd	0.45 mg/L	cis-1,2 Dichloroethene	700-740 µg/L
Al	541±47 mg/L	1,1,2-trichloro-1,2,2-trifluoroethane	1200-1500 µg/L
Ca	931±74 mg/L	Methylene chloride	39-42 µg/L
Mg	174±11 mg/L	Citric acid	~6 mg/L [#]
Mn	130±9 mg/L	Acetic acid	~120 mg/L [#]
Sb	<0.003 mg/L		
Cr	0.17 mg/L		
Pb	0.03 mg/L		
Se	0.02 mg/L		

[*] estimated value: a measurement is needed.

[#] values for MLS FW 100, 40’ depth.

Where we are

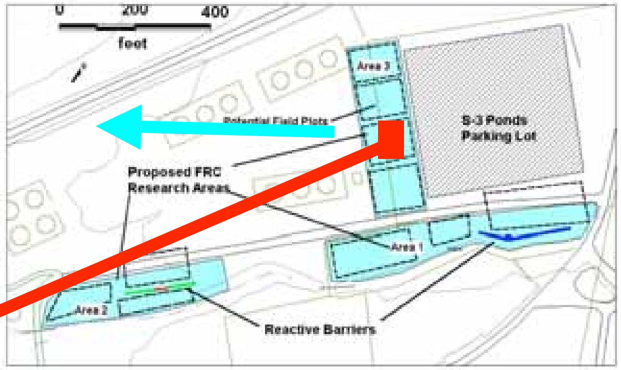
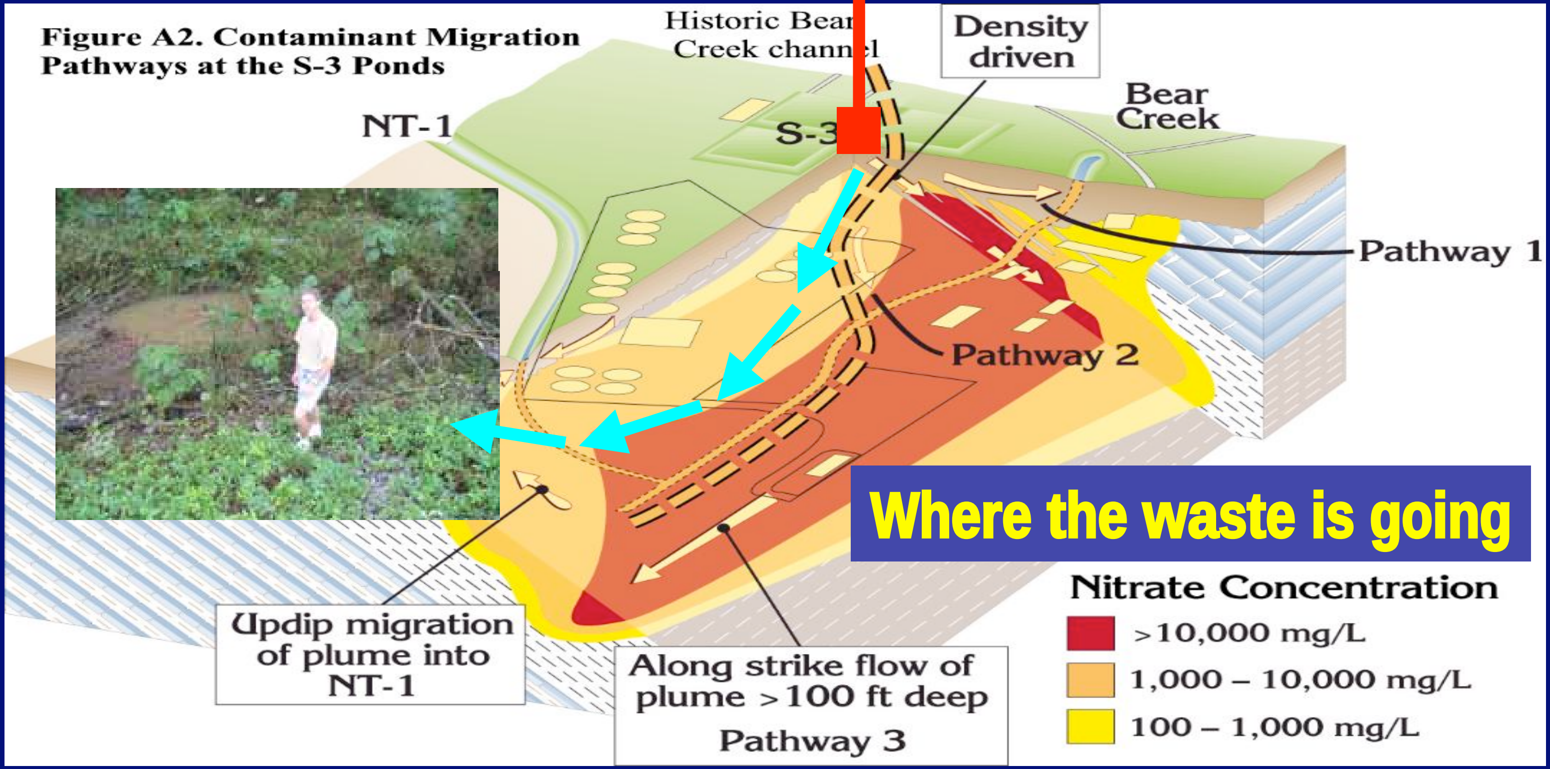


Figure A1. Location of potential field plots within Areas 1, 2, and 3

Figure A2. Contaminant Migration Pathways at the S-3 Ponds



Where the waste is going

Rationale for work near the source zone

The source zone is a reservoir of U(VI) for long-term groundwater and surface water contamination.

About 98% of the U(VI) in the near source zone is sorbed to solids or part of a solid phase.

The remaining 2% of U(VI) is dissolved in the groundwater at highly toxic levels (20-50 mg/L).

Conversion of solid-associated U(VI) into highly insoluble U(IV) will prevent dissolution and desorption, decreasing the time and cost of remediation.

PRIMARY OBJECTIVE

Evaluate the rates and mechanisms of U(VI) reduction by microbial populations

$$UO_2(CO_3) + H^+ + 2e^- = UO_2 + HCO_3^-$$

A few electrons goes a long ways:

- 119 mg U are reduced for every mmol of electrons transferred to U(VI).
- This is equivalent to 119 mg U reduced/mg H_2
- It is also equivalent to 119/8 = 14.9 mg U reduced/ mg COD

Hypotheses

- Biological reduction of U(VI) in the S-3 soils is a multistep process involving desorption/dissolution of U(VI), followed by uptake/reductive mineralization.

- Desorption/dissolution will typically limit the overall reduction rate, with highest rates observed under conditions that favor partitioning of U(VI) into the aqueous phase (i.e., elevated pH and TIC levels).

- Both metal- and sulfate-reducing bacteria will play a role in U reduction, with iron-reducing bacteria acting first followed by sulfate-reducing bacteria.

Chemistry considerations

Low pH (~3.5):

- buffered by Al^{3+} (~20 mM)

High U(VI):

~98% on the soil (~400 mg/kg)
~2% in groundwater(~ 40 mg/L)

High NO_3^-:

130-480 mM in groundwater - NO_3^- and denitrification intermediates inhibit U(VI) reduction (Senko et al., 2001)

High Ca^{2+}:

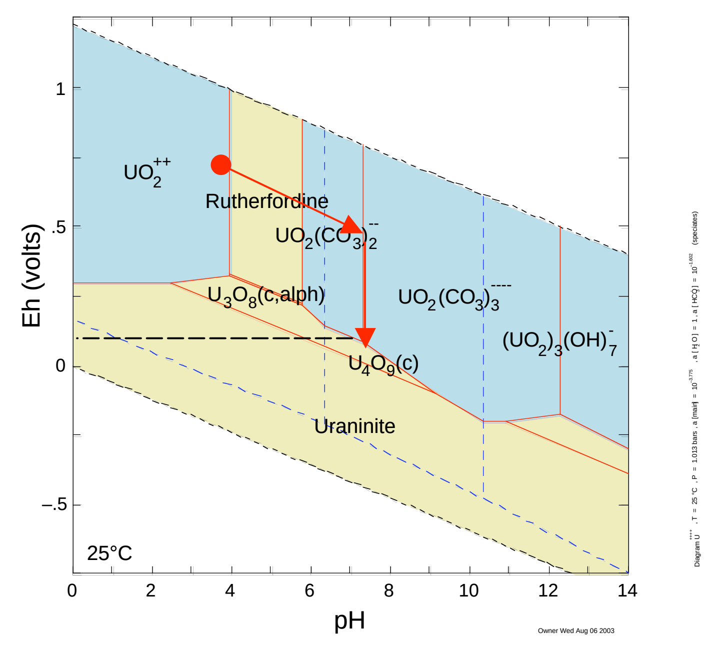
~20 mM in groundwater - Ca^{2+} inhibits U(VI) reduction at 5 mM (Brooks et al., 2003)

$UO_2(CO_3) + H^+ + 2e^- = UO_2 + HCO_3^-$ $E°' = +0.105$ V

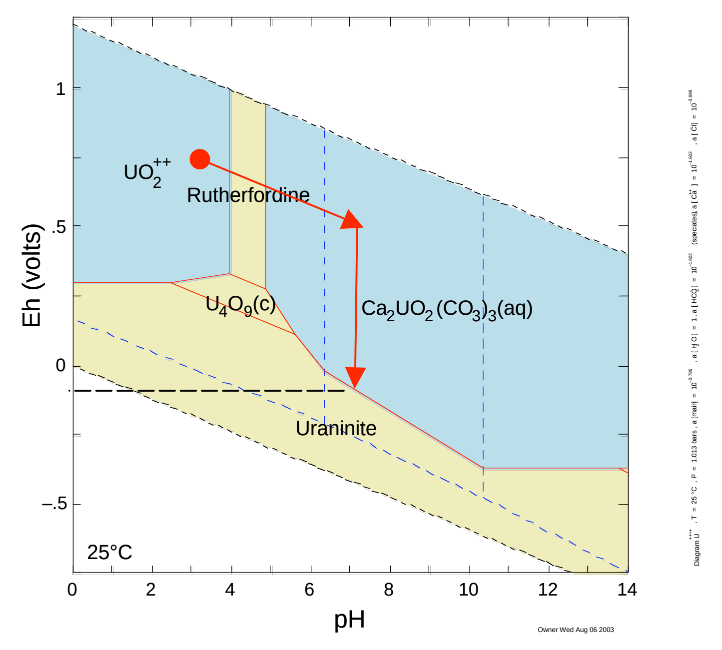
$Ca_2UO_2(CO_3)_3 + 2e^- = 2Ca^{2+} + UO_2 + 3CO_3^{2-}$ $E°' = -0.046$ V

Eh/pH Plot of Uranium Speciation (300 mg/L TIC, 40 mg/L U)

No Ca

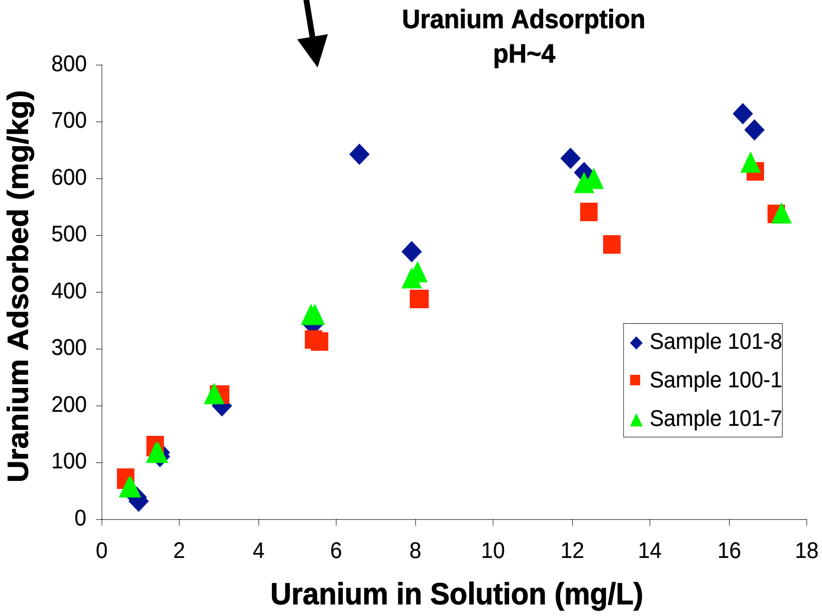


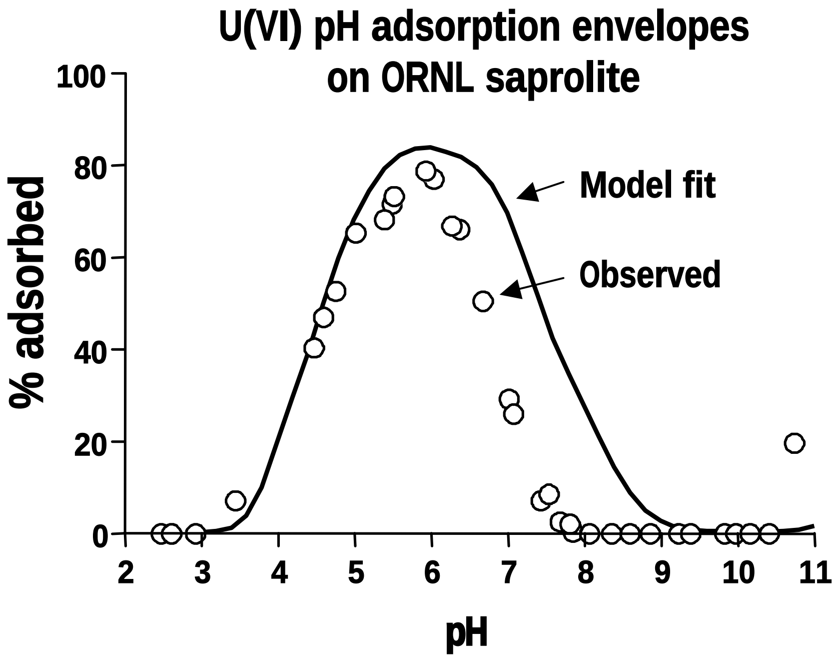
+ 20 mM Ca



Uranium adsorption

U sorption is concentration dependent.
It is also strongly pH dependent.





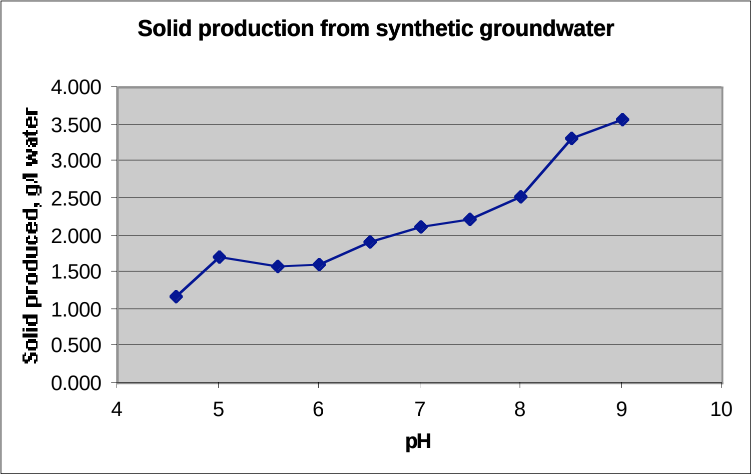
Potential clogging agents

- Aluminum hydroxide form at pH 5.
- Calcium and magnesium carbonates form at pH 7-9.
- N_2 gas forms during denitrification.
- High levels of biomass are produced during denitrification.

pH adjusted to 7 with 50% liquid from denitrifying batch cultures

pH adjusted to 7 with Na_2CO_3

pH adjusted to 7 with KOH

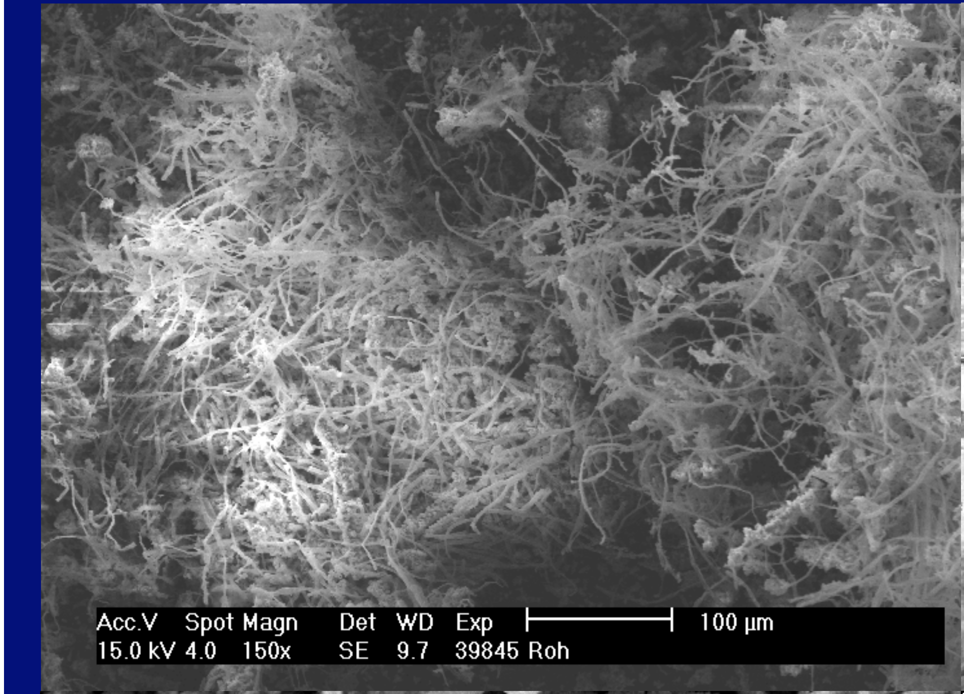


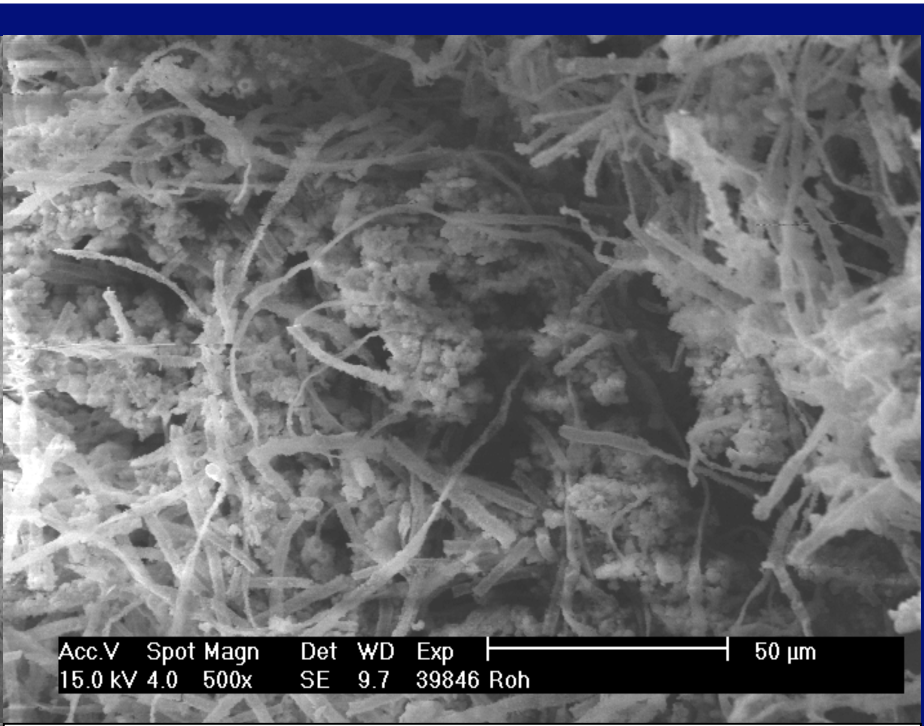
2 g/L solids produced

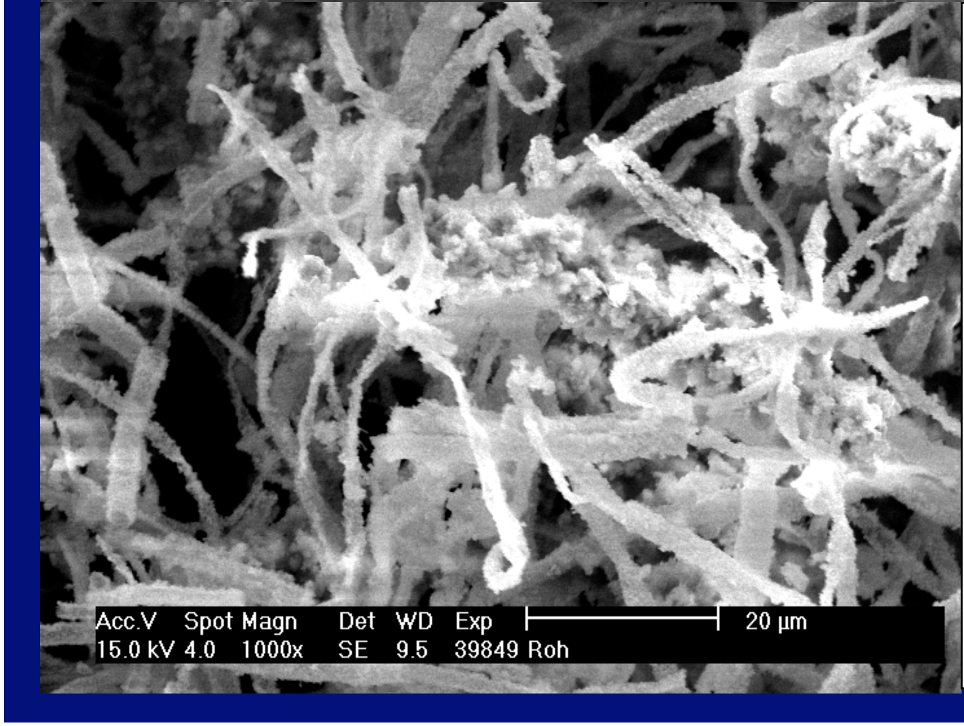
Clogging happens (and what to do about it)

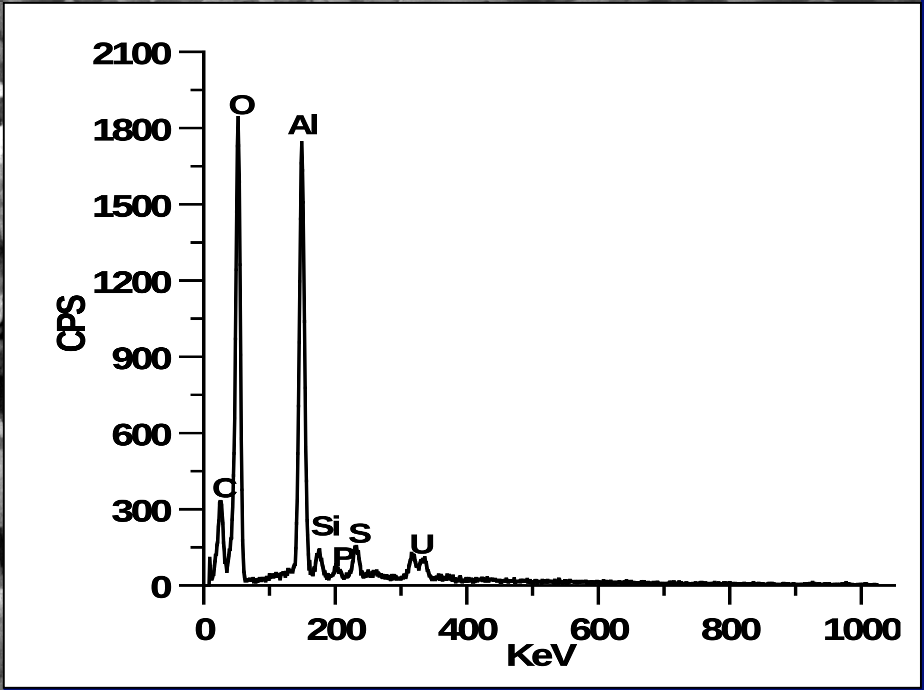
Clogged pump head screen. The white precipitate dissolved in a 2% HCl solution after 1.5 hour. Clogging observed even with < 4 mg/L Al.

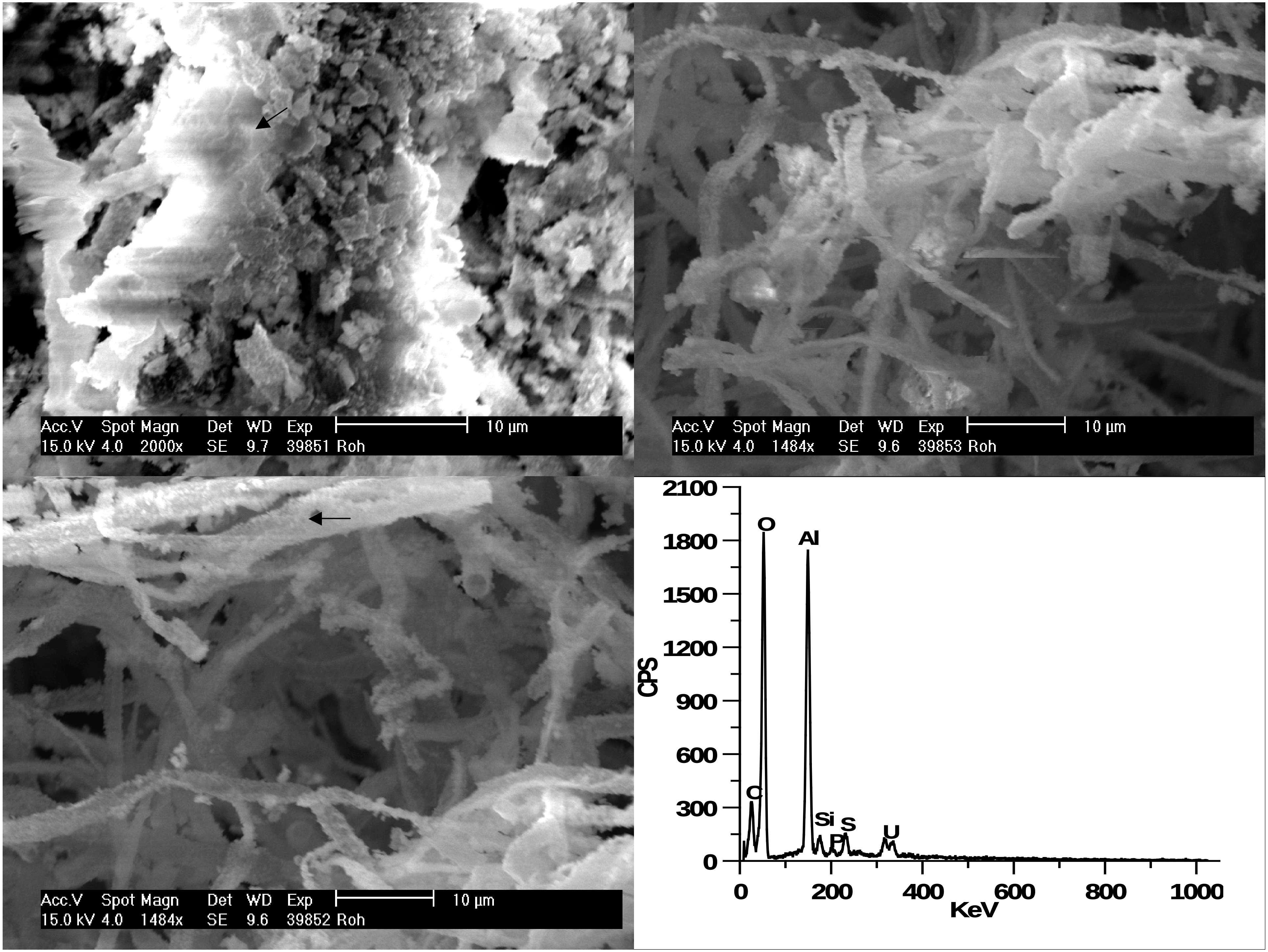
Surge block for cleaning wells


Acc.V Spot Magn Det WD Exp 100 µm
15.0 kV 4.0 150x SE 9.7 39845 Roh


Acc.V Spot Magn Det WD Exp 50 µm
15.0 kV 4.0 500x SE 9.7 39846 Roh


Acc.V Spot Magn Det WD Exp 20 µm
15.0 kV 4.0 1000x SE 9.5 39849 Roh


CPS
2100
1800
1500
1200
900
600
300
0
O
Al
C
Si
S
P
U
0
200
400
600
800
1000
KeV


Acc.V Spot Magn Det WD Exp 10 µm
15.0 kV 4.0 2000x SE 9.7 39851 Roh
Acc.V Spot Magn Det WD Exp 10 µm
15.0 kV 4.0 1484x SE 9.6 39853 Roh
Acc.V Spot Magn Det WD Exp 10 µm
15.0 kV 4.0 1484x SE 9.6 39852 Roh
CPS
2100
1800
1500
1200
900
600
300
0
O
Al
C
Si
P
S
U
0
200
400
600
800
1000
KeV

Geology

- Saprolite contains a highly interconnected fracture network with densities of 100-200 fractures/m. Fractures are < 5-10% of the total porosity, but carry >95% of the groundwater flow.

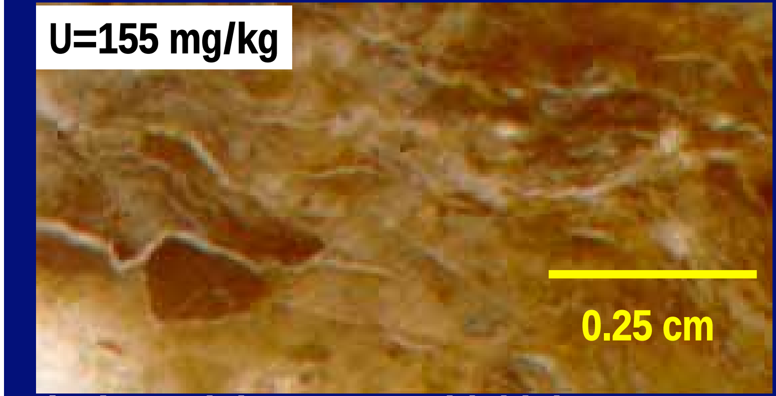
- The fractures surround a high porosity, low permeability matrix that is a source and sink for contaminants.

Overlying Saprolites

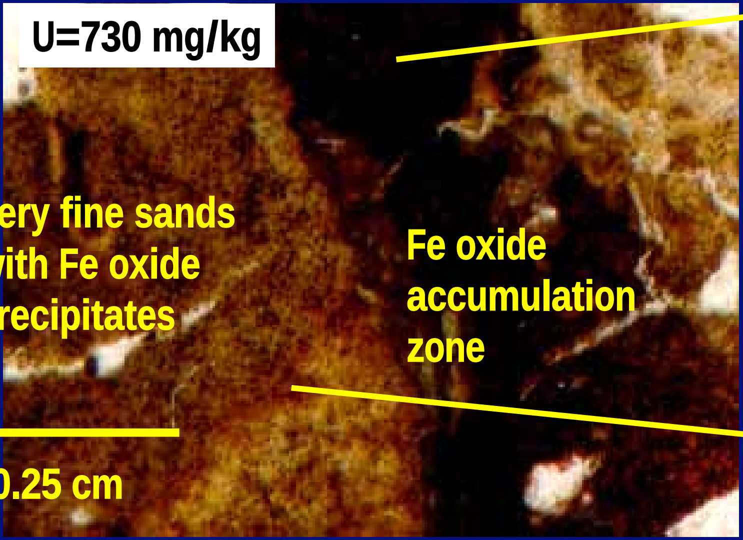
Underlying Bedrock

Core Mineralogical Evaluations

Overlying Gleyed leached flow zone with high U, low pH groundwater



Black precipitate Zone with higher pH and lower U in groundwater



Very fine sands with Fe oxide precipitates

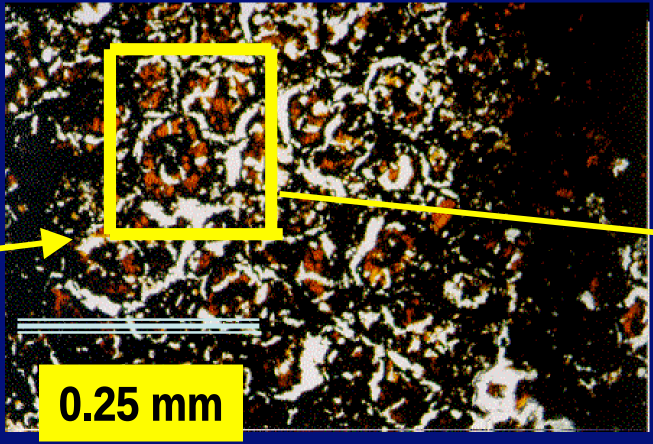
Fe oxide accumulation zone

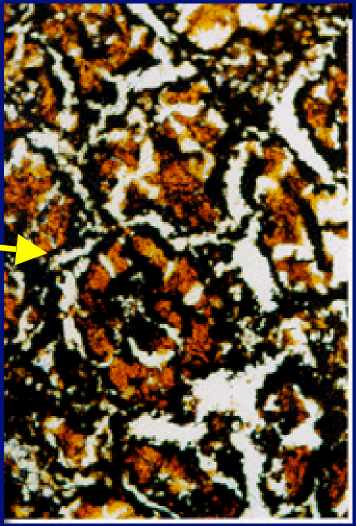
A high U zone was detected in the center of the test cell at a depth of 46'.

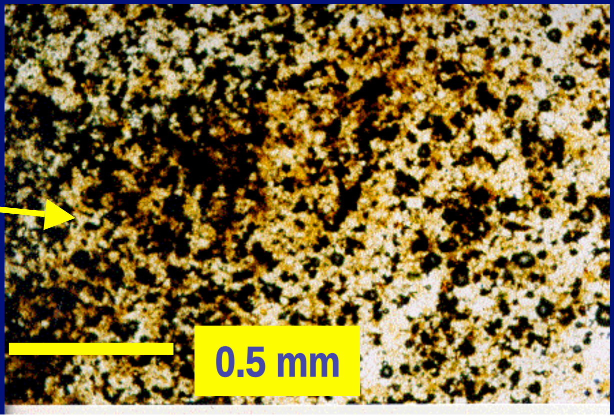
XRD results:

Gleyed Zone - Quartz, Vermiculite, Mica, HIV, Ca-feldspar

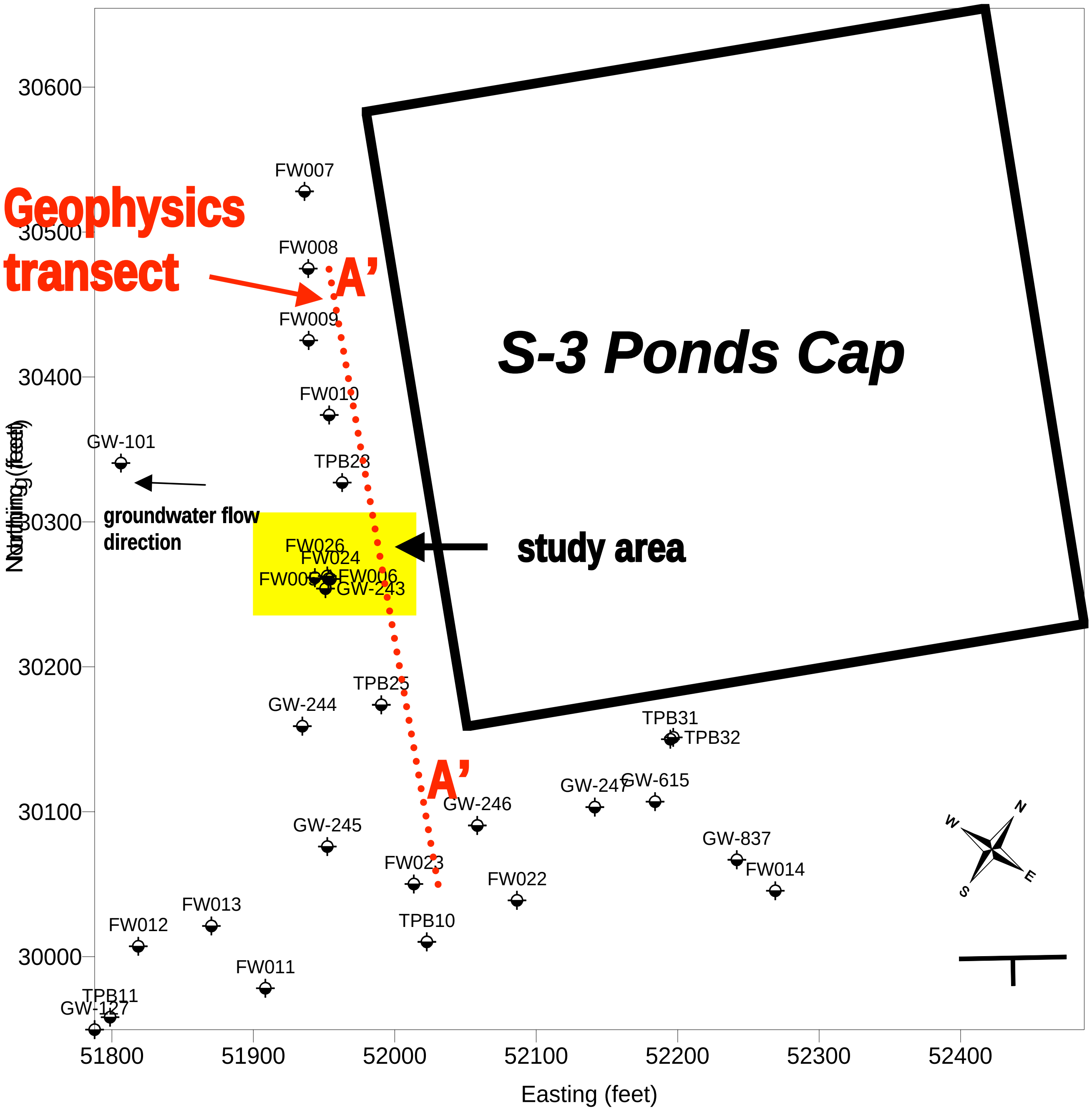
Black Zone - Quartz, Ca-feldspar, Vermiculite, Mica, Goethite







Phillips/Watson, 2003



Geophysics transect

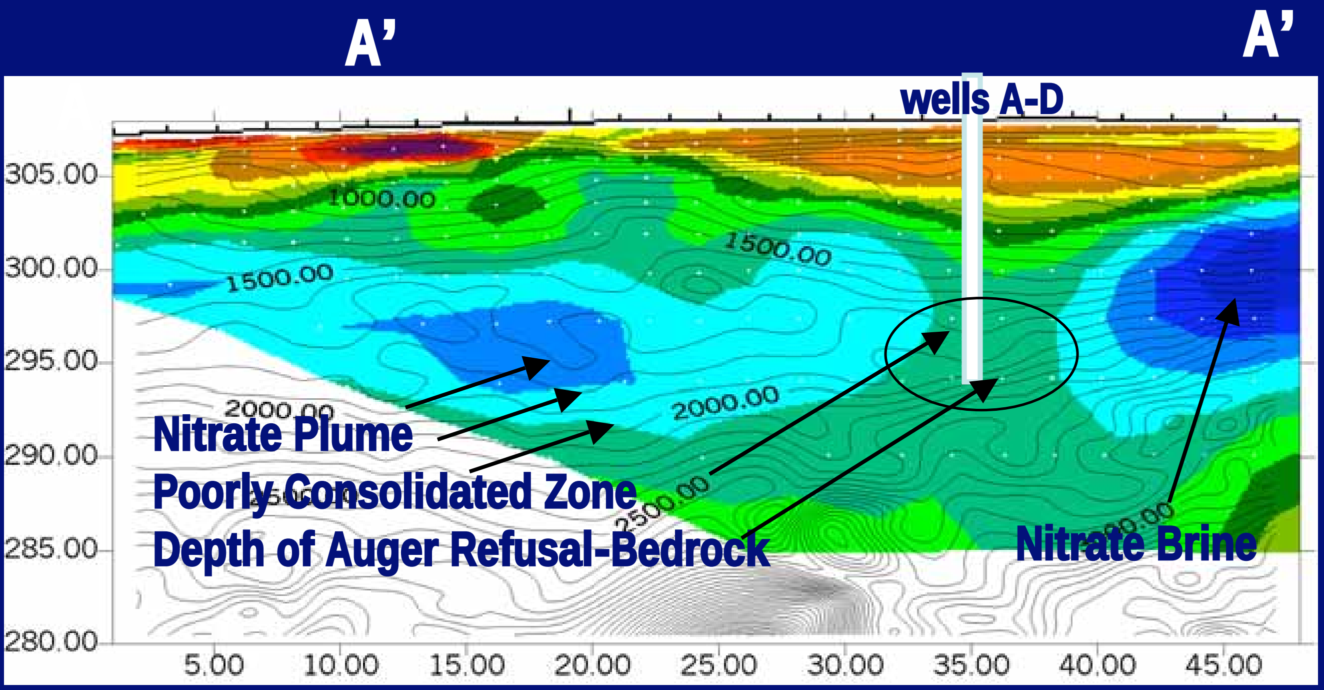
groundwater flow direction

study area

S-3 Ponds Cap

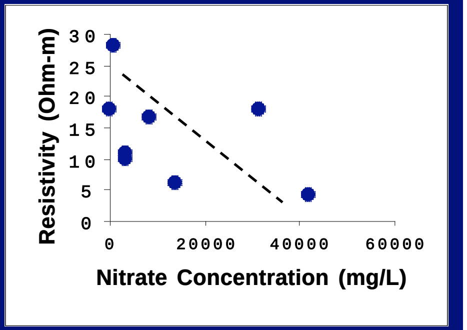
Geophysics was used to identify areas of contaminant transport

S-3 Ponds Cap
Surface Seismic/Electrical Resistivity
(Doll et al., *SAGEEP*, 2002).



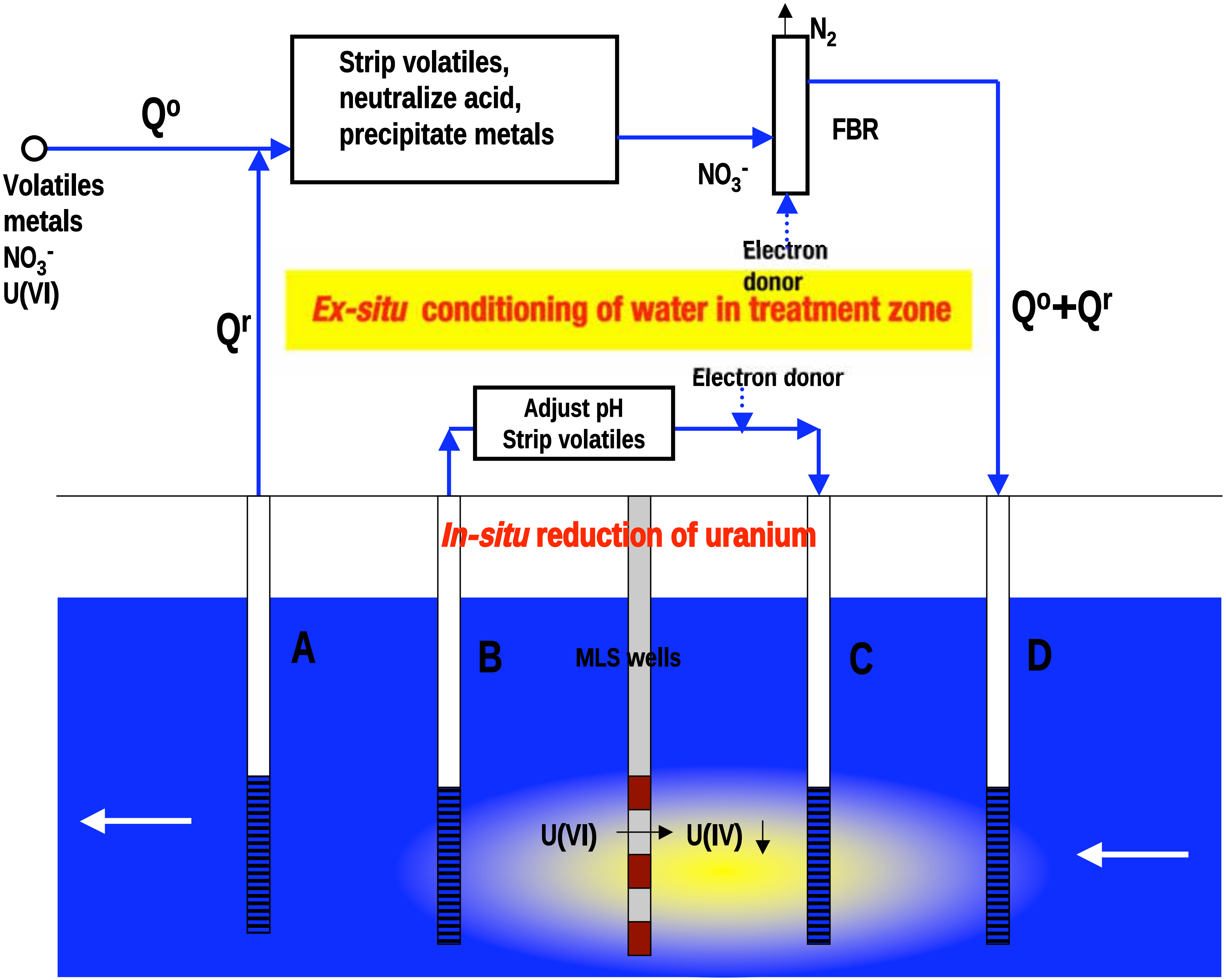
Nitrate Plume
Poorly Consolidated Zone
Depth of Auger Refusal-Bedrock
Nitrate Brine

Electrical Resistivity
🟦 Low (~4 Ohm-m)
🟥 High (~150 Ohm-m)



Low Resistivity ~ High Nitrate

Contour Lines: Seismic Velocity (m/s)



Q^o

Volatiles
metals
NO_3^-
U(VI)

Strip volatiles,
neutralize acid,
precipitate metals

N_2

FBR

NO_3^-

Electron donor

Q^r

Ex-situ conditioning of water in treatment zone

Q^o+Q^r

Electron donor

Adjust pH
Strip volatiles

In-situ reduction of uranium

A

B

MLS wells

C

D

U(VI) → U(IV) ↓

The "Big Top" where extracted groundwater is treated to enable metal reduction *in-situ*

Tanker for chemical sludge disposal

Bag filters for disposal of biomass

Inside the Big Top

Ex-situ conditioning of water in treatment zone

1. Precipitate Al and Ca
2. Remove NO_3^- by denitrification in FBR
3. Vacuum strip to remove VOCs and N_2

The aboveground treatment train

Vacuum stripper

Two-step
chemical precipitation

Fluidized bed reactor
(FBR)

FBR sampling and characterization

- Phylogenetic analyses
- Functional gene microarrays
- Functional monitoring

Well TPB16 enrichment

innoculum →

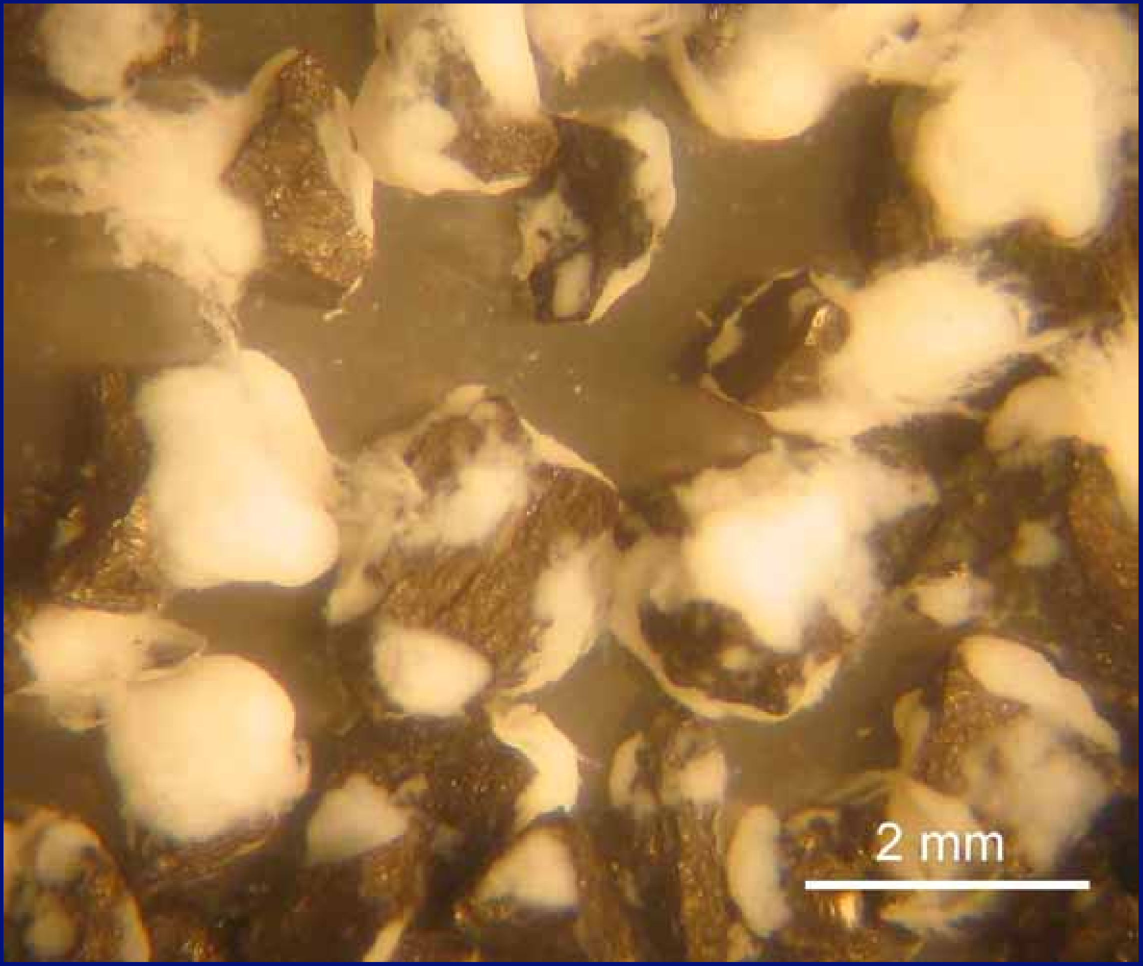
Two piilot scale FBRs

innoculum →

Full scale FBR

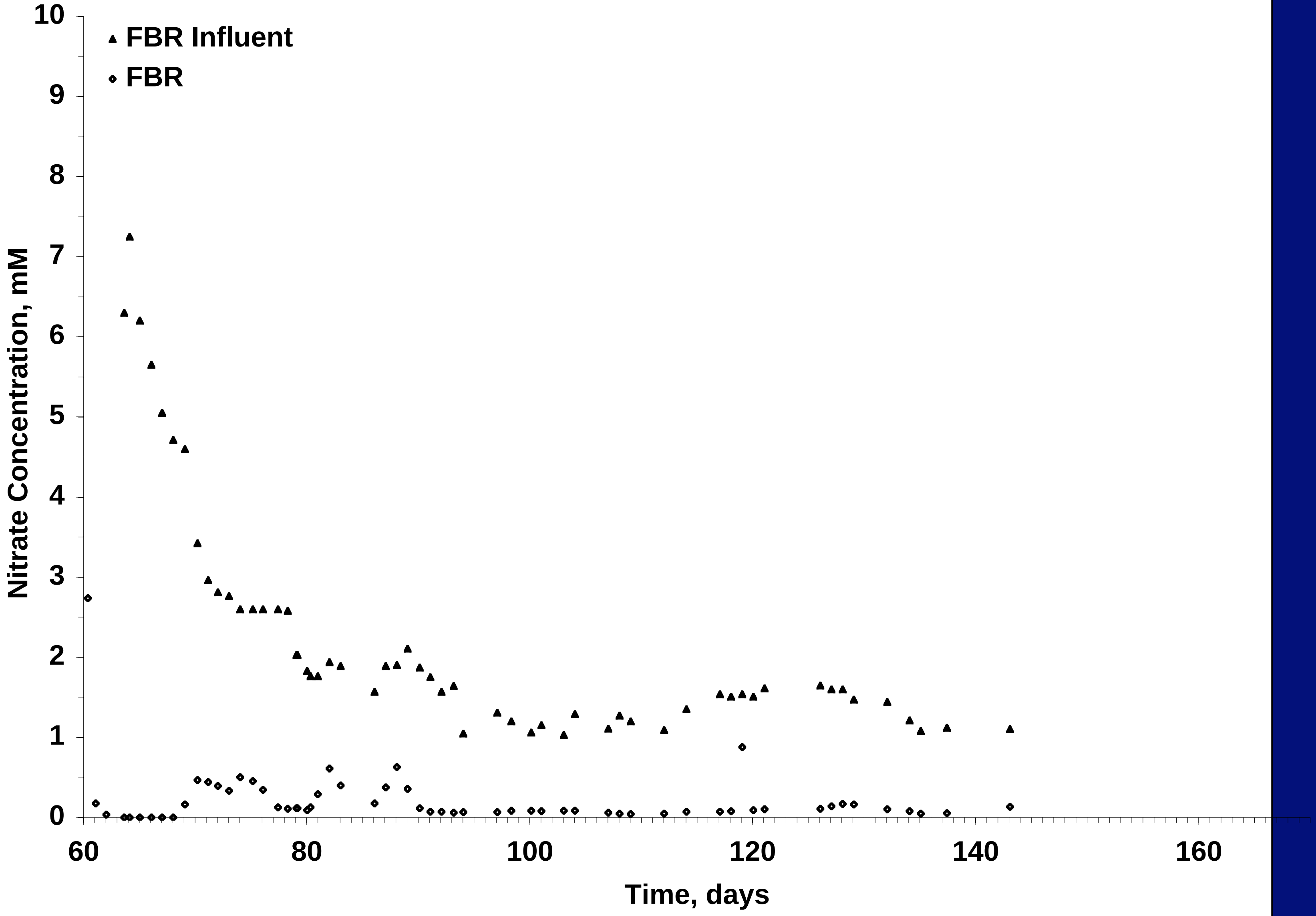
Fluidized Bed Reactor

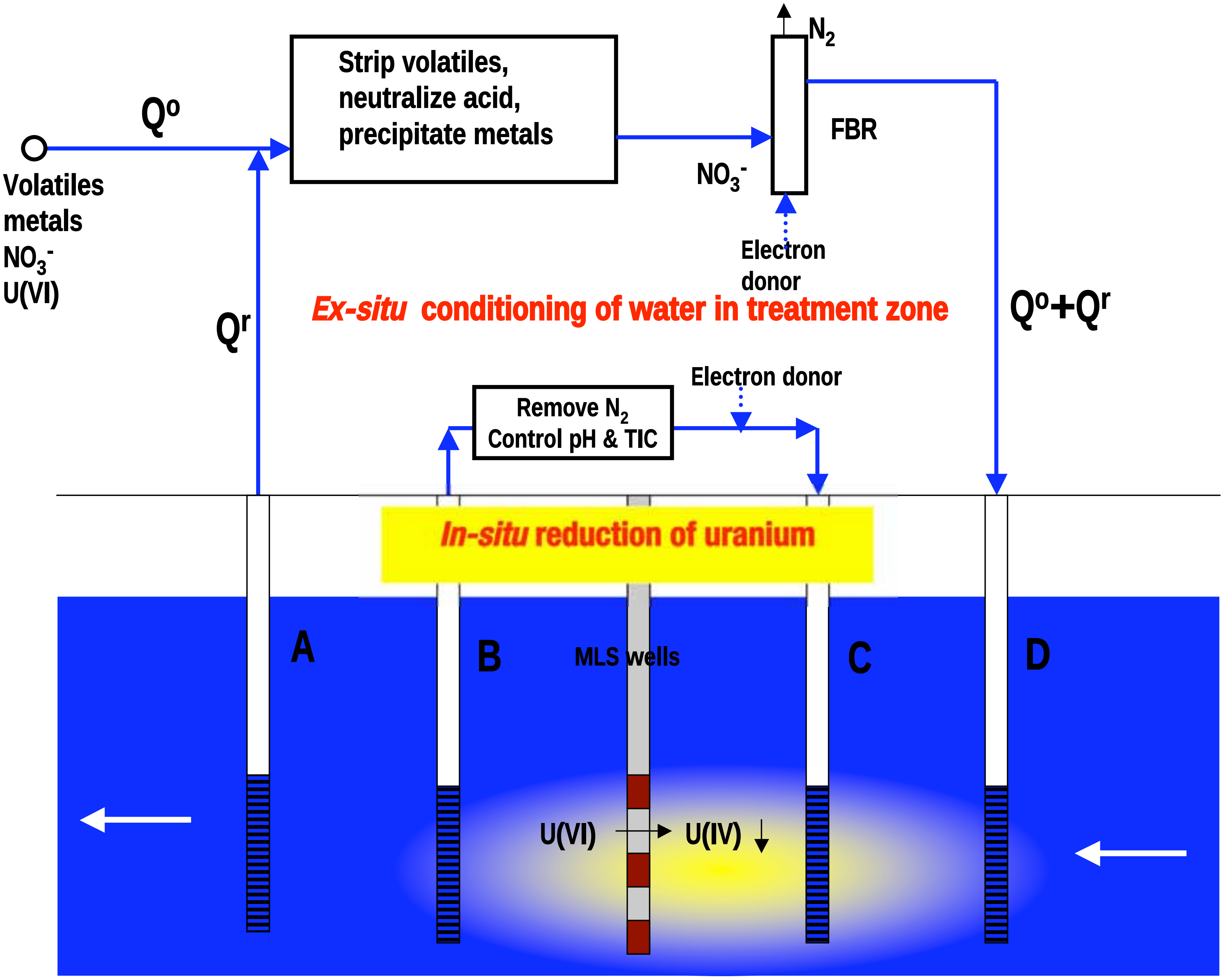
- Removes NO_3^- as N_2
- Efficient
- Cheap
- Raises pH
- Demonstrated in two continuous pilot-scale systems (pH 7.4 and 9.2)



Denitrifying biofilms growing on granular activated carbon in pilot scale FBR at Stanford. Some of the bacterial general found in this community include *Zoogloea*, *Xanthomonas*, *Dechloromonas, Dechlorosoma, and Sporumosa.*

FBR: nitrate removal





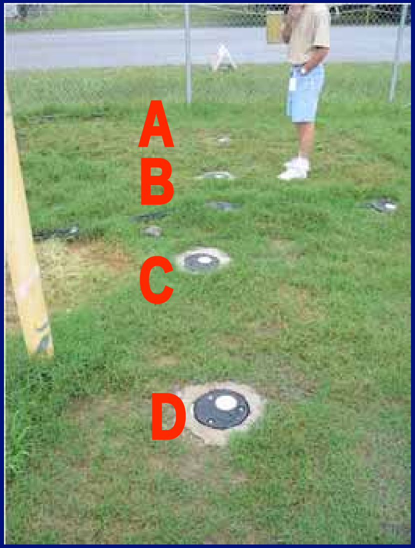
Strip volatiles, neutralize acid, precipitate metals

Remove N_2
Control pH & TIC

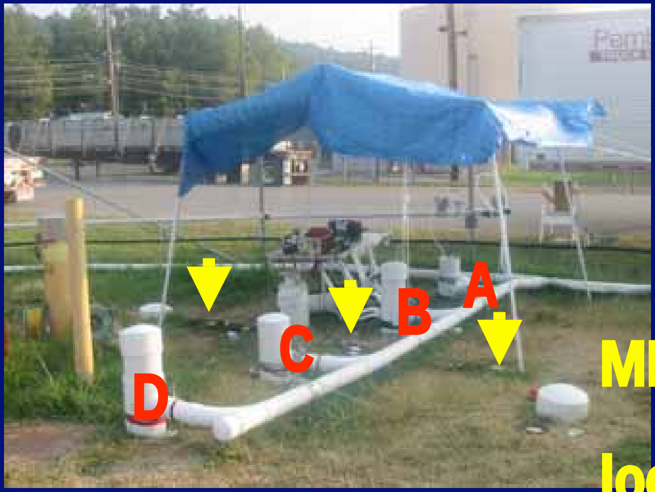
Ex-situ conditioning of water in treatment zone

In-situ reduction of uranium

Well layout



before plumbing

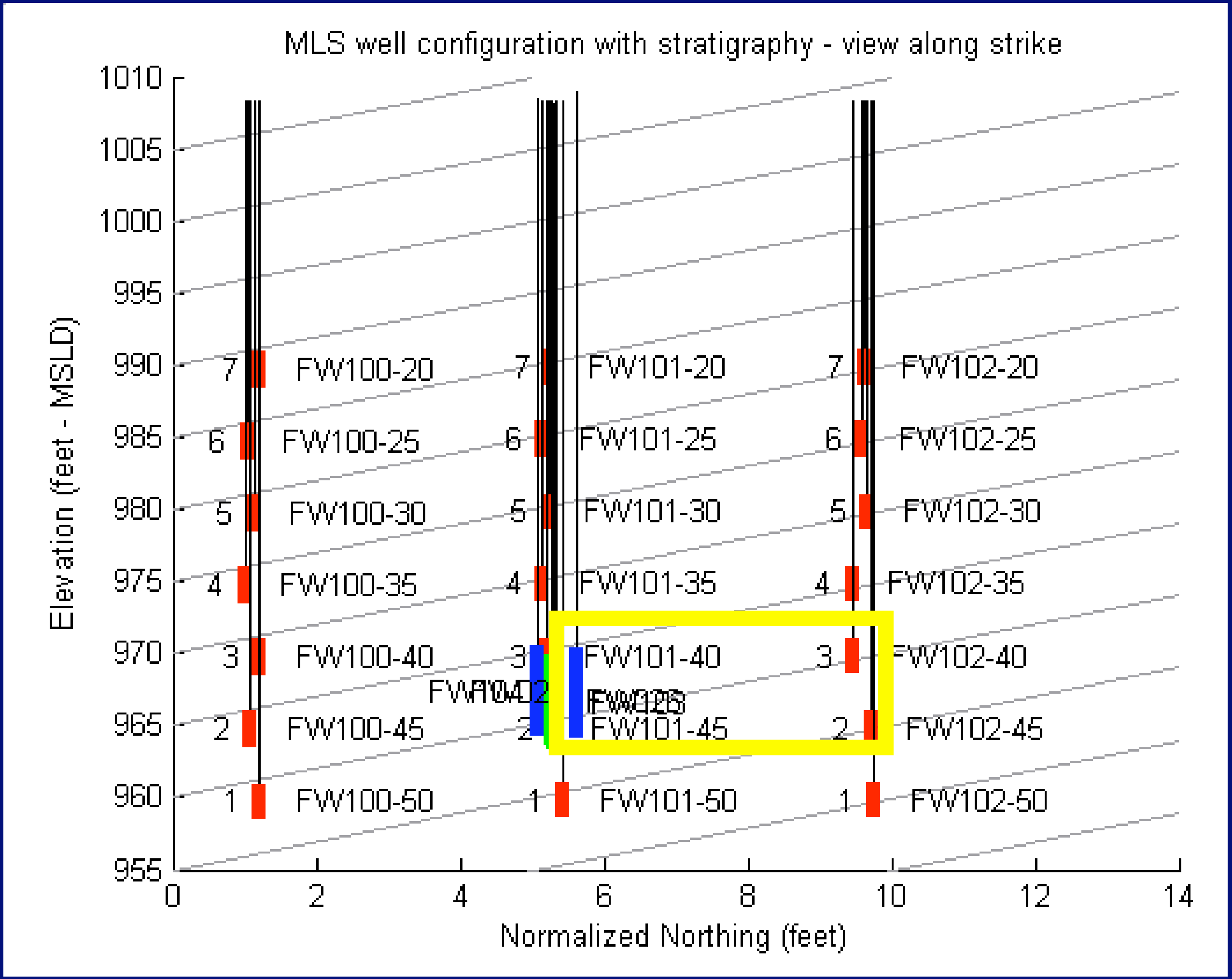


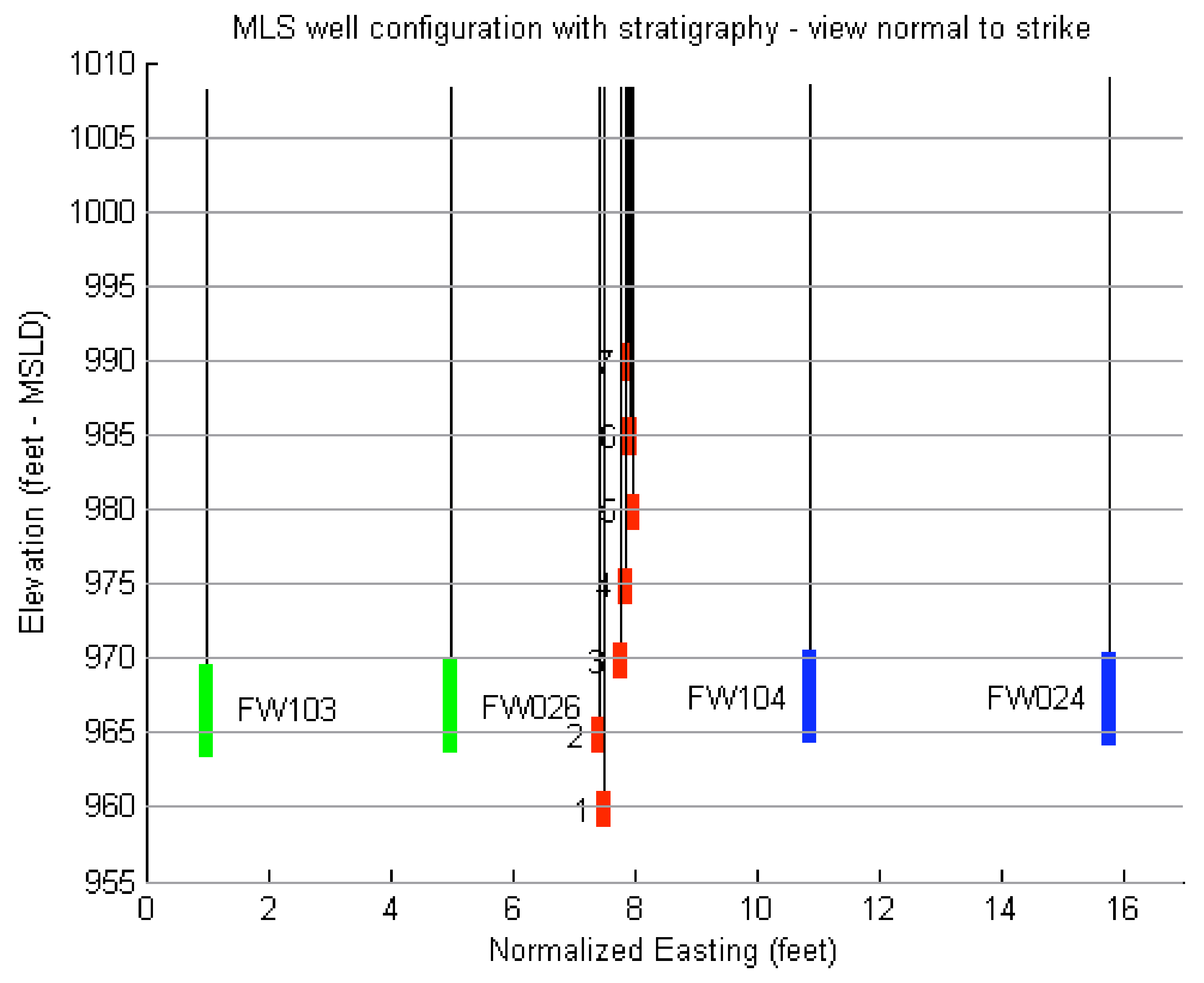
MLS well locations

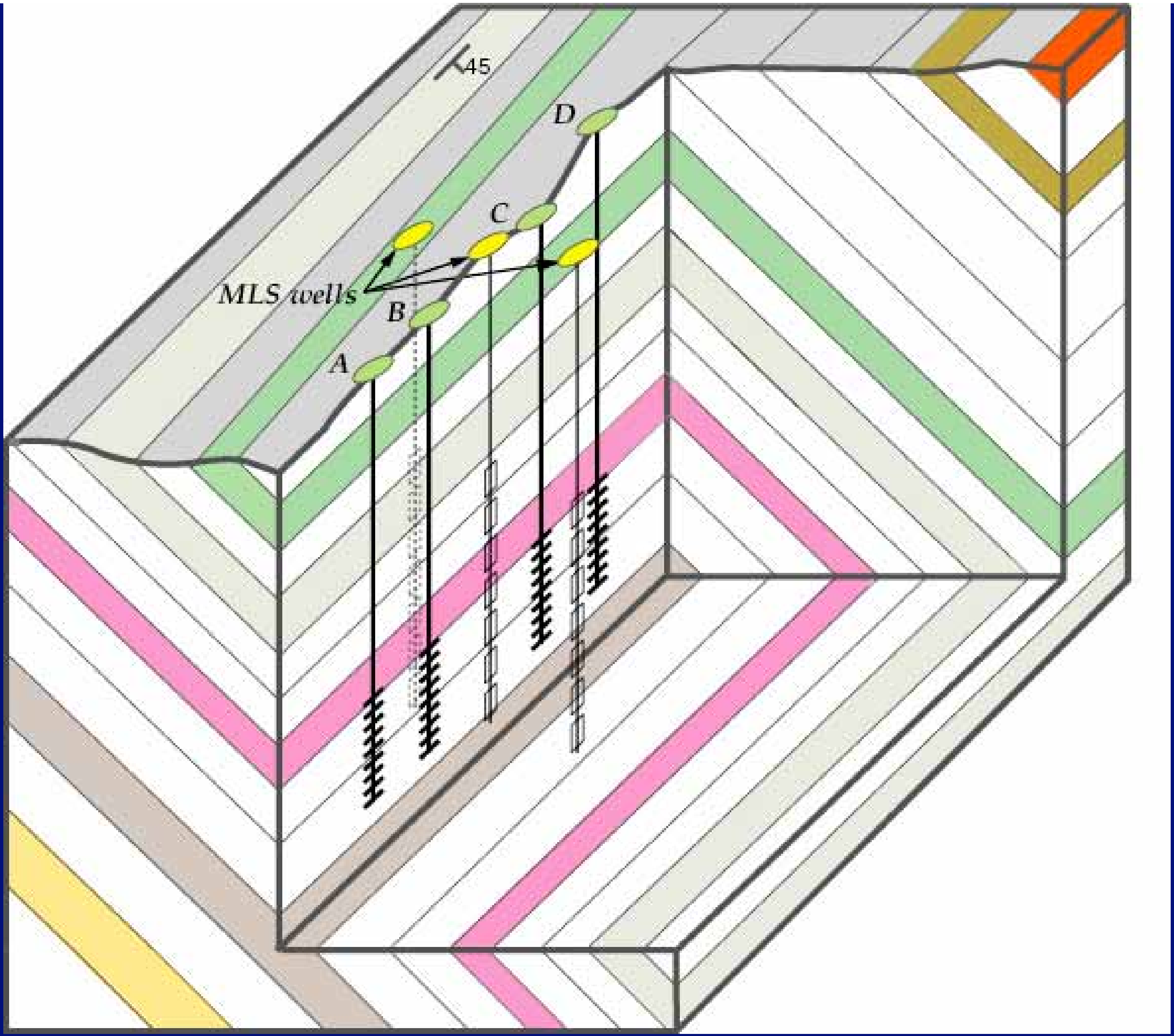
after plumbing

Skid with pumps and meters for wells inside Big Top

Multilevel sampling wells



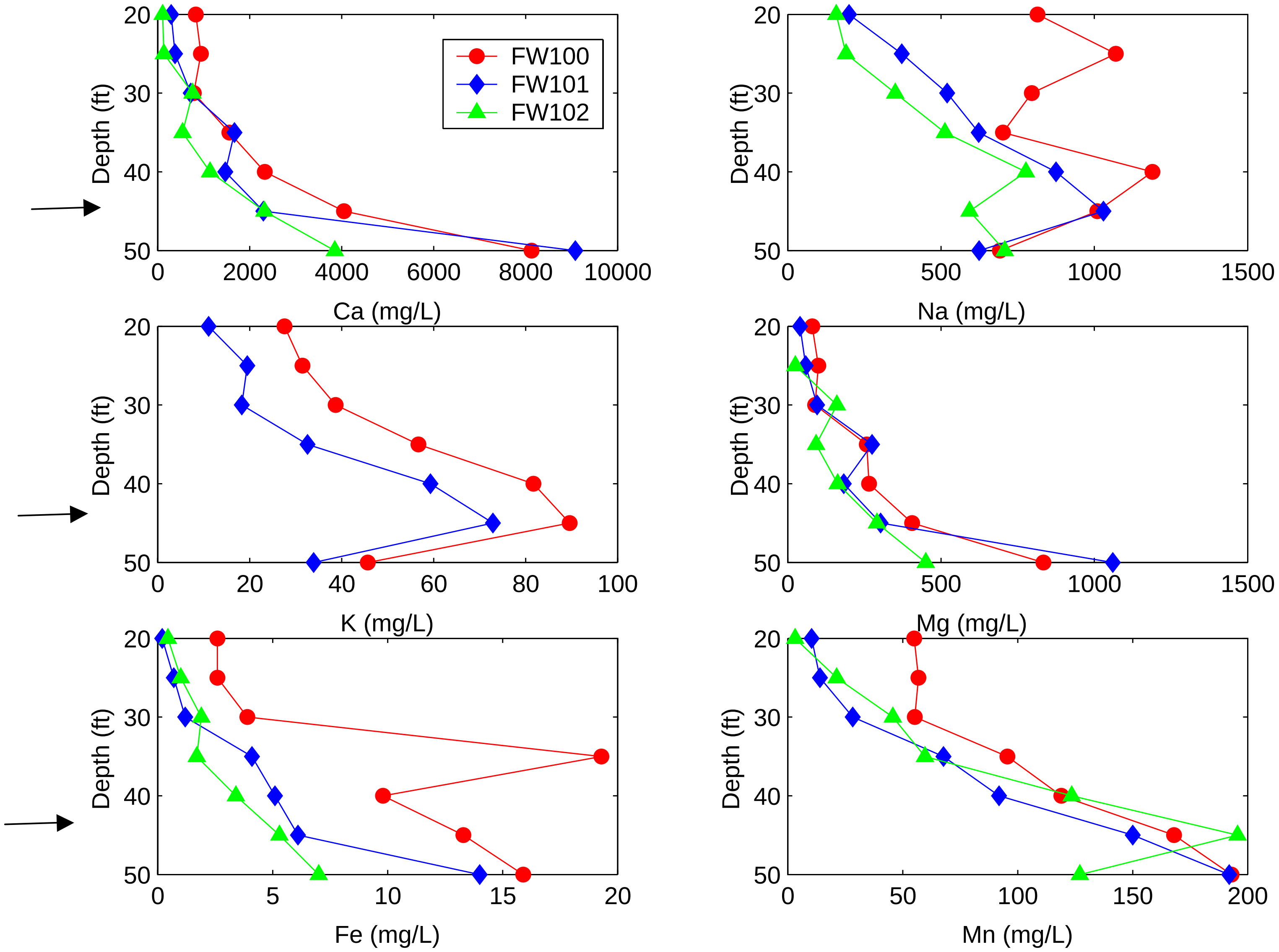

MLS well configuration with stratigraphy - view normal to strike
Elevation (feet - MSLD)
1010
1005
1000
995
990
985
980
975
970
965
960
955
7
6
5
4
3
2
1
FW103
FW026
FW104
FW024
0
2
4
6
8
10
12
14
16
Normalized Easting (feet)



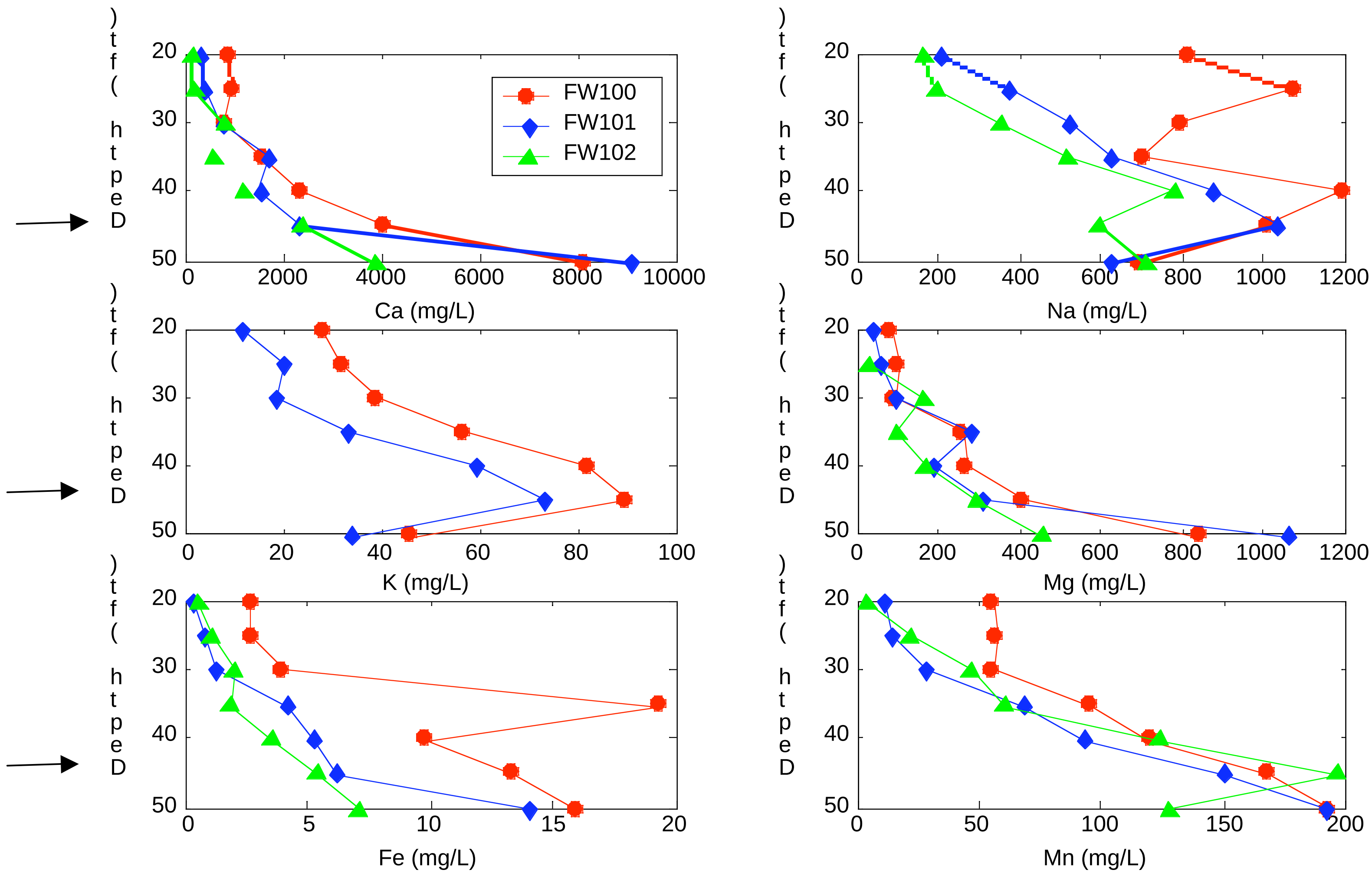
Screened Interval = 38-45’

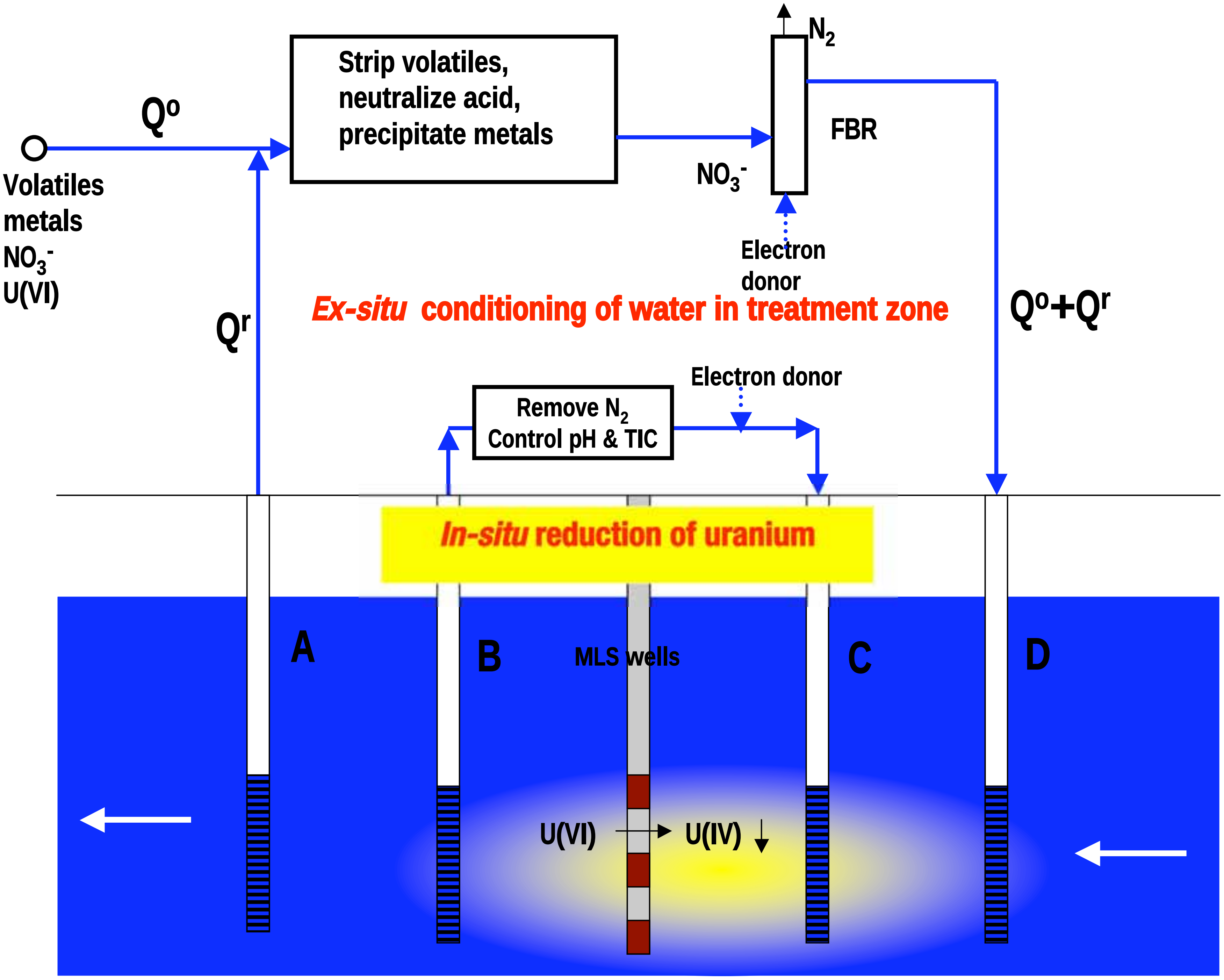
Cross-sectional view of the injection/extraction wells and the MLS wells.

Chemical profiles with depth at the MLS wells



Chemical profiles with depth at the MLS wells (cont)





Q^o

Volatiles
metals
NO_3^-
U(VI)

Strip volatiles,
neutralize acid,
precipitate metals

N_2

FBR

NO_3^-

Electron
donor

Q^r

Ex-situ conditioning of water in treatment zone

Q^o+Q^r

Electron donor

Remove N_2
Control pH & TIC

In-situ reduction of uranium

A

B

MLS wells

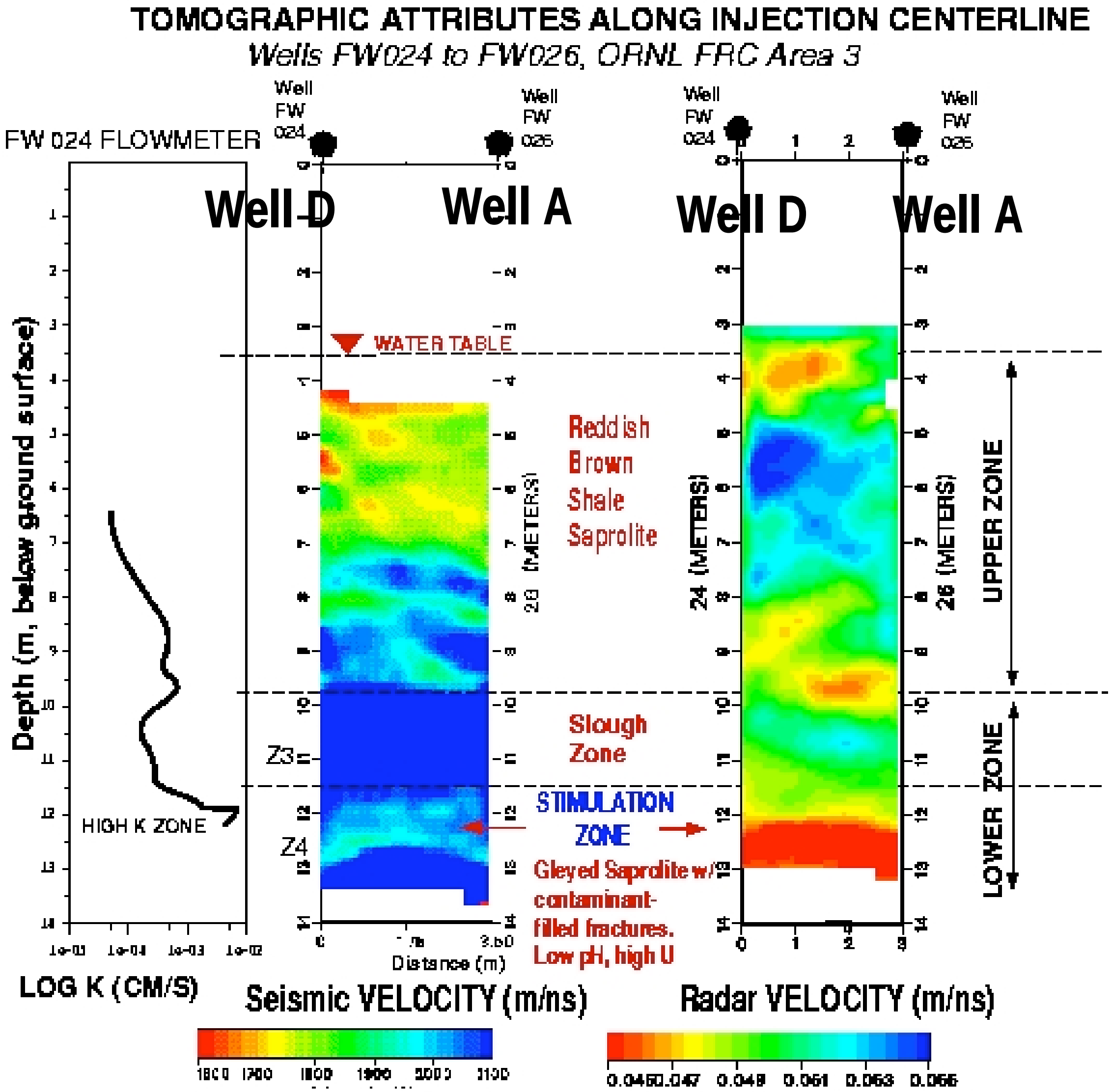
C

D

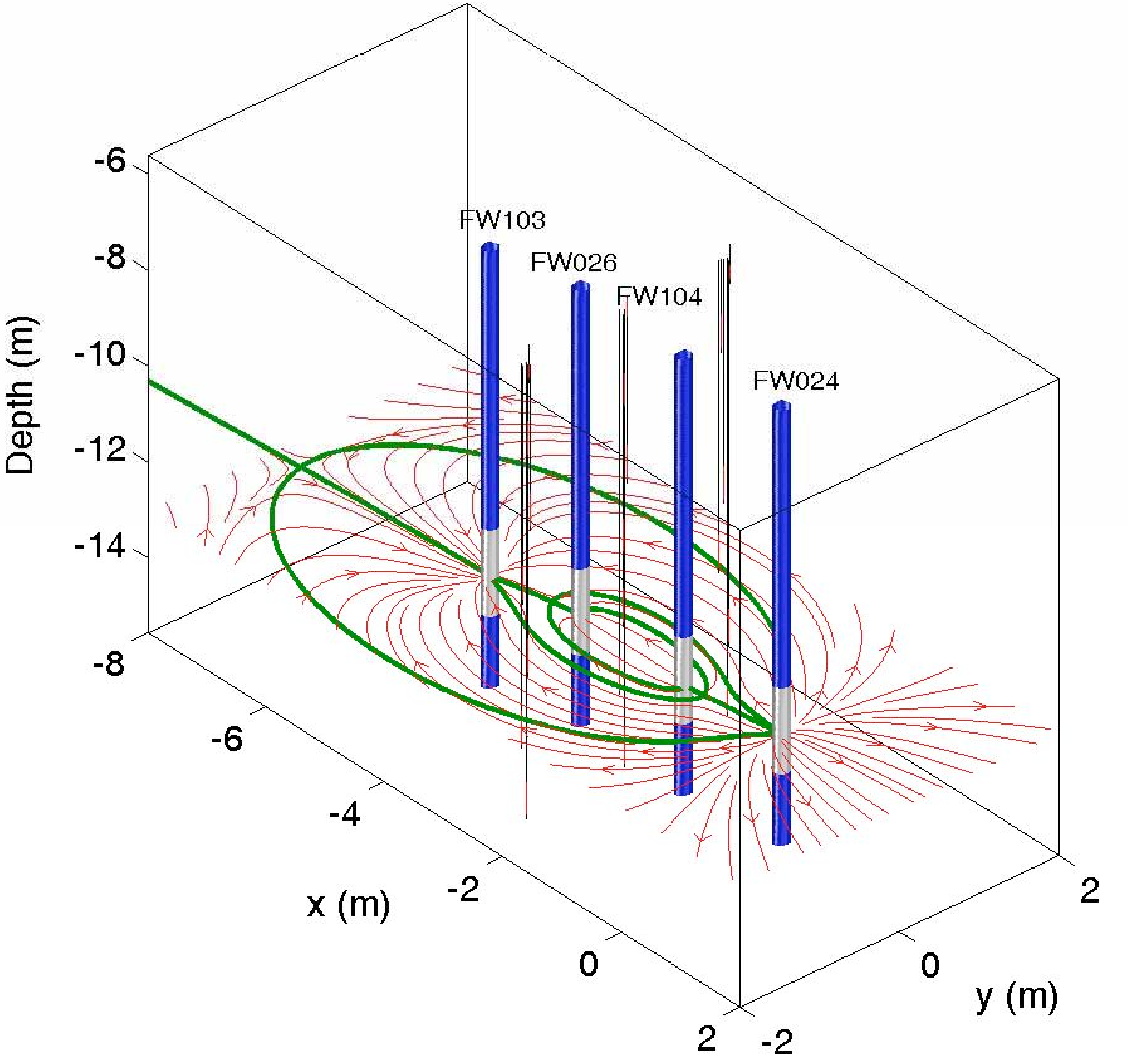
U(VI) → U(IV) ↓

Seismic and Radar Tomography

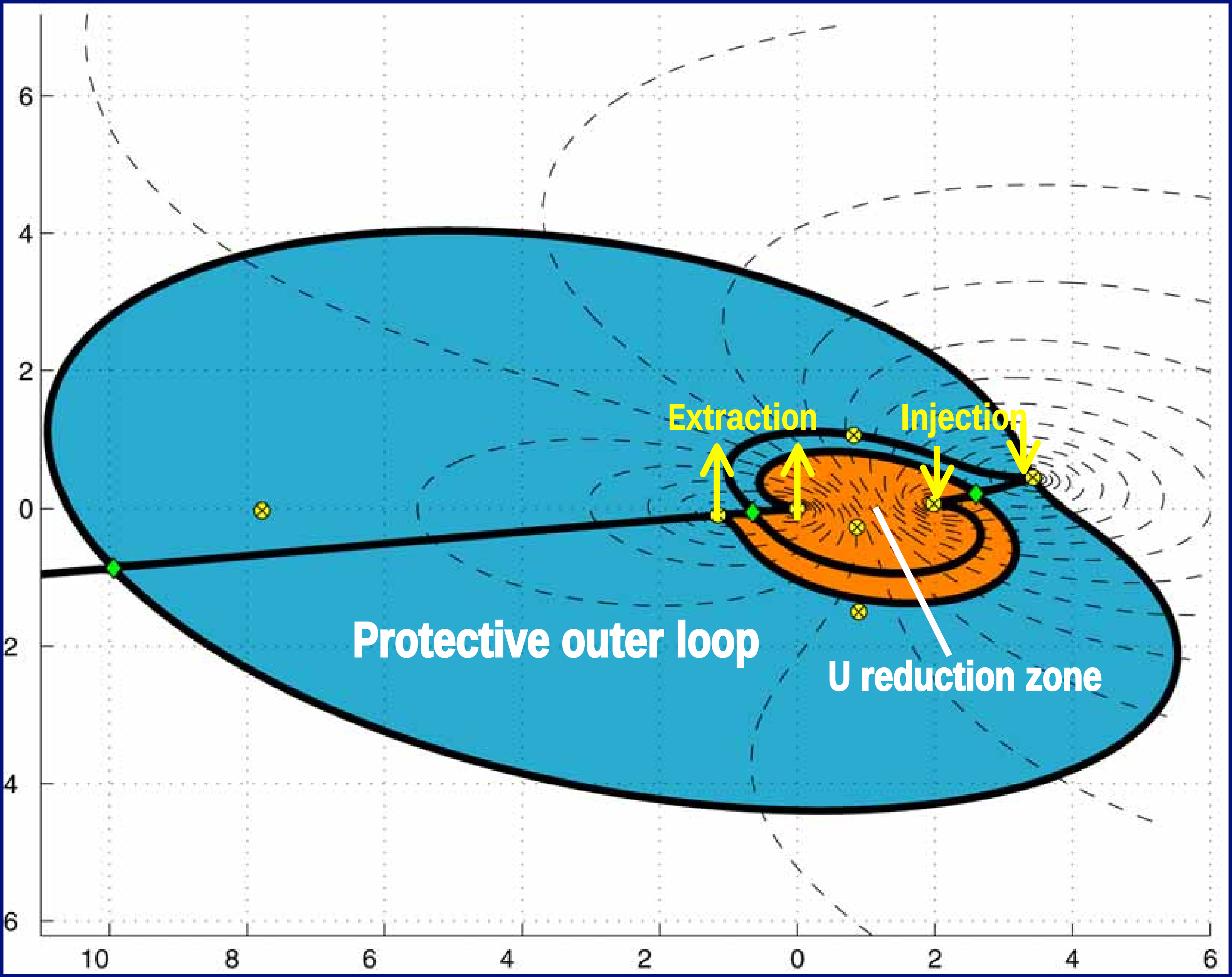
Mapping subsurface material heterogeneities using cross-borehole techniques.



Hubbard et al., 2003


FW103
FW026
FW104
FW024
Depth (m)
-6
-8
-10
-12
-14
-8
-6
-4
-2
0
2
x (m)
-2
0
2
y (m)

Regions of the subsurface



Overall Strategy

1. Perform a tracer study to determine connectivity of wells and residence time distribution. Obtain desorption rates from the rebound.

2. Flush outer and inner cell with clean water at pH 4 to remove Al, Ca, and most of the nitrate. Follow with flush at pH 5-6 to prepare for denitrification.

3. Stimulate denitrification *in-situ* and vacuum strip N_2 to remove residual nitrate.

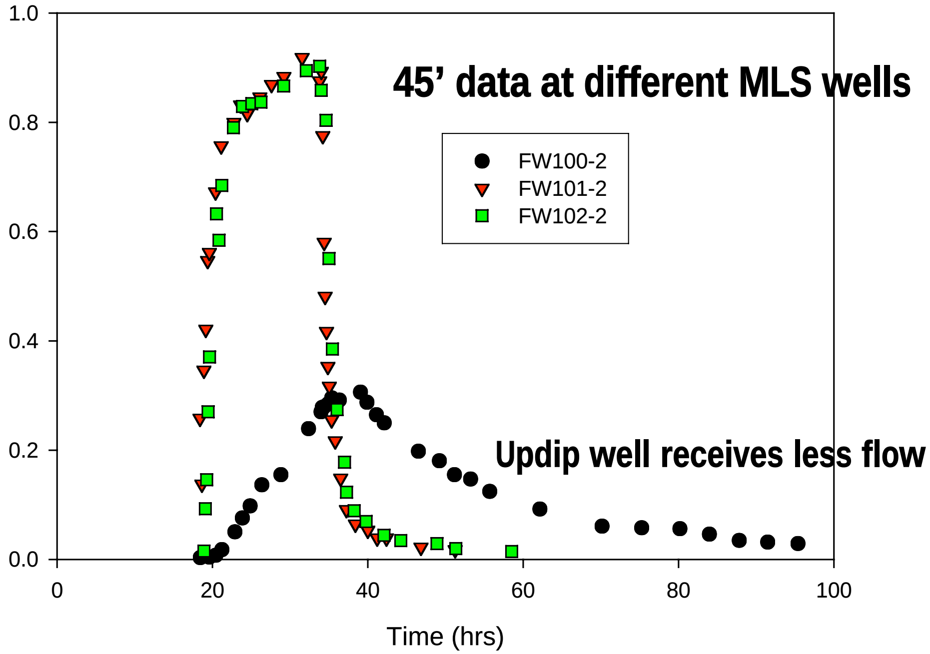
4. Increase pH of inner cell to mobilize U(VI) and add electron donor to inner cell to reduce U(VI).

Tracer study

A dual dipole tracer injection-withdraw test was conducted using $CaBr_2$ and $CaCl_2$ in an effort to create an inner and outer hydraulic cell.

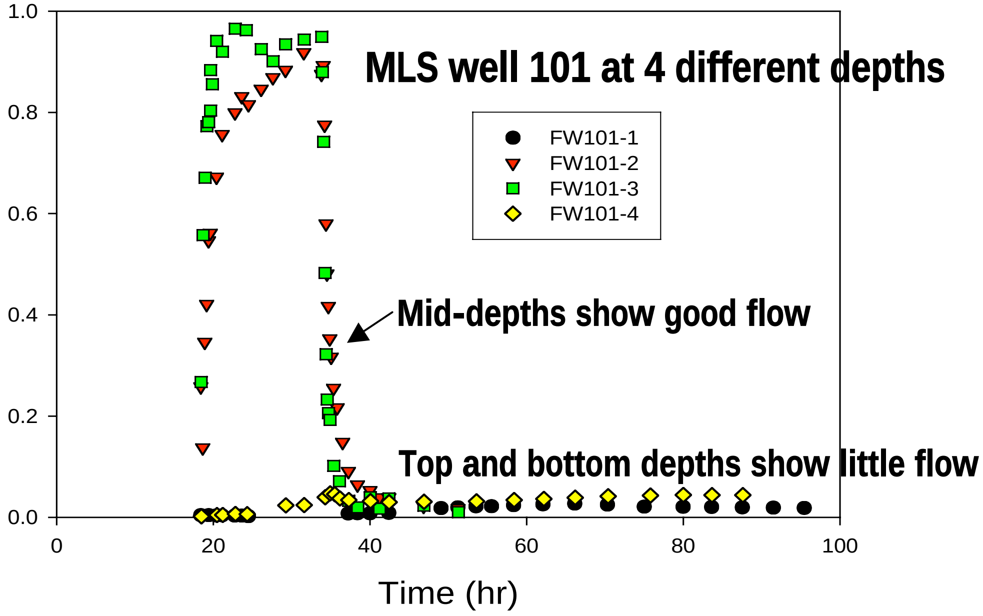
Results confirmed location and transport features of preferential flow regimes and slow flowing matrix regimes.

Experimental data was numerically simulated and the model used to design the *in situ* U bioreduction system.



45’ data at different MLS wells

Updip well receives less flow

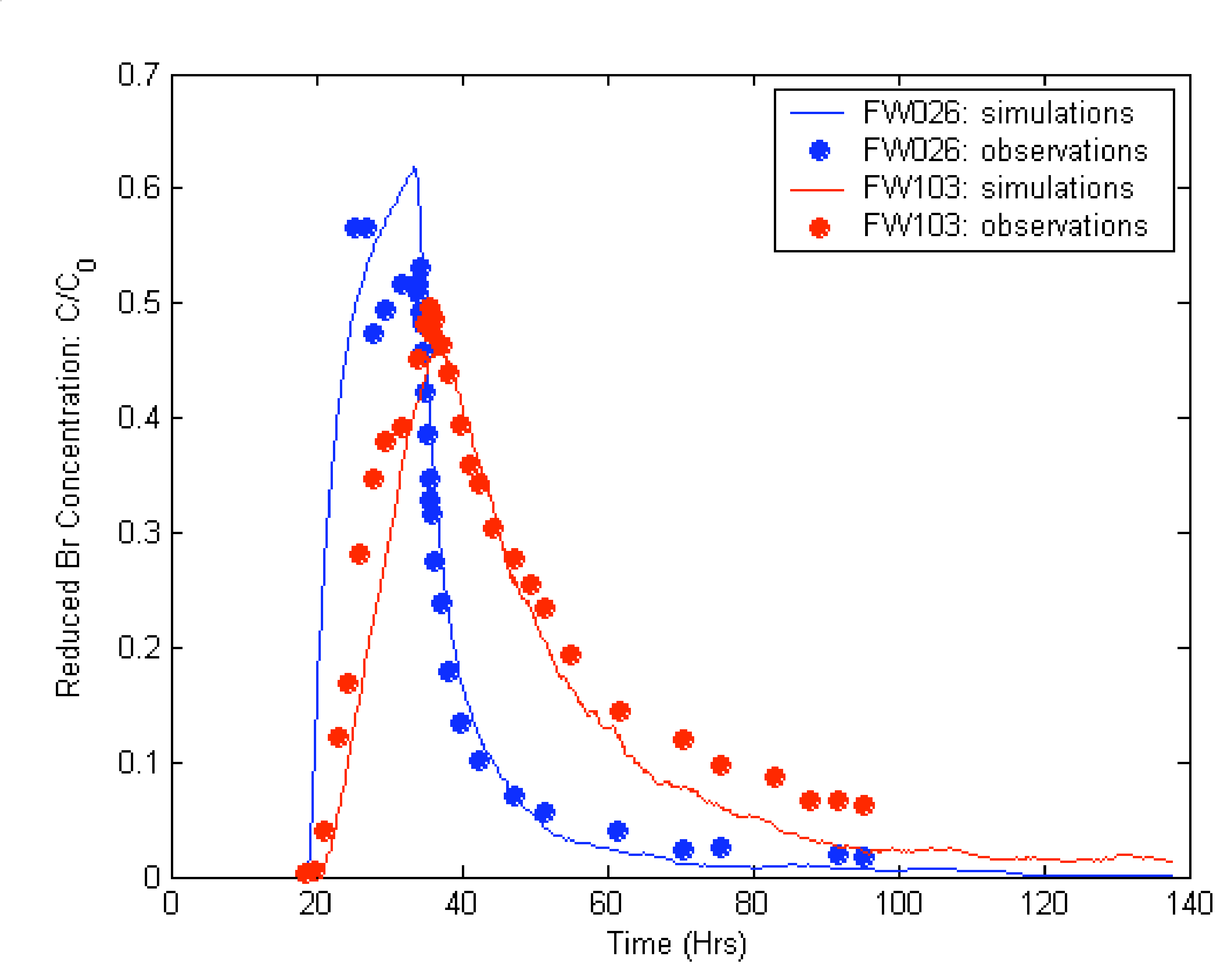


MLS well 101 at 4 different depths

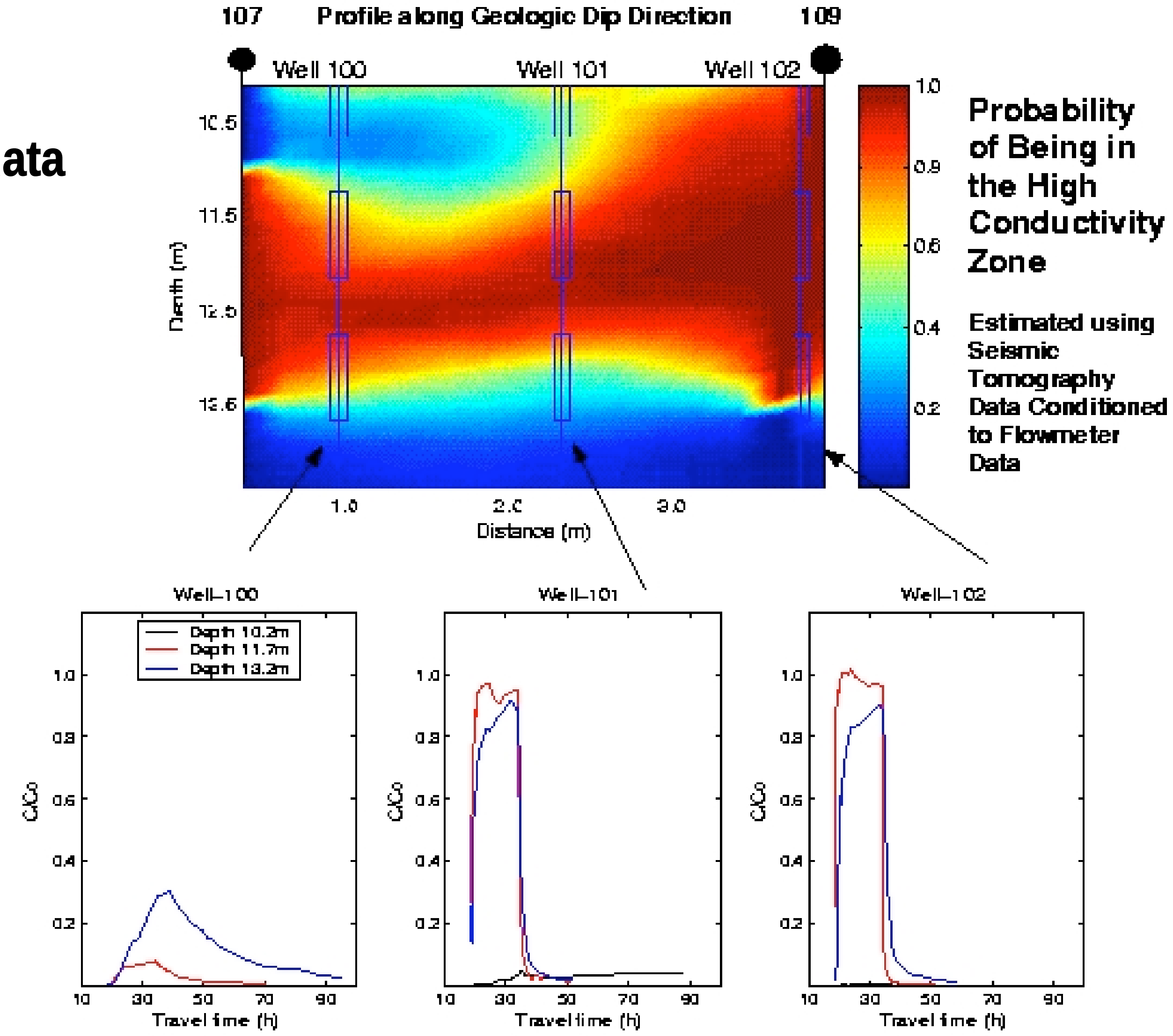
Mid-depths show good flow

Top and bottom depths show little flow

Tracer study simulations



Seismic tomography data complements tracer measurements.

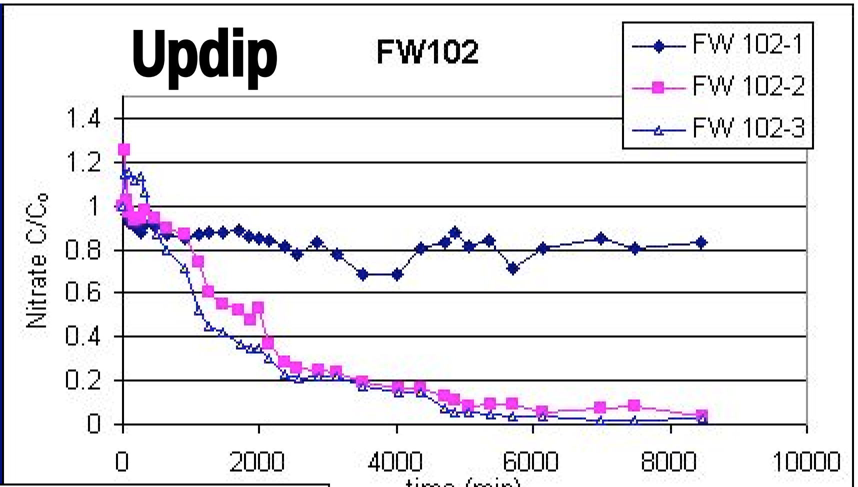


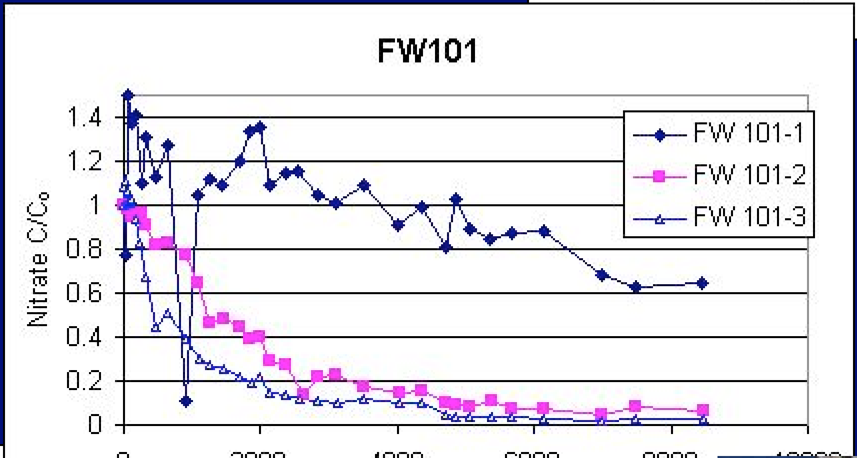
Tracer Breakthrough at 3 Multi-Level Samplers along Geologic Dip Direction

Hubbard et al., 2003

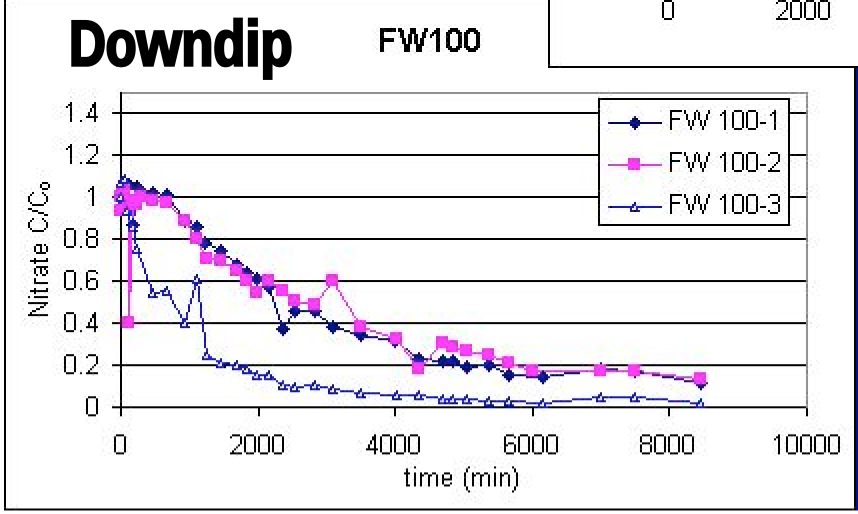
Mehlhorn et al., 2003

Effect of tracer clean water flush on nitrate in MLS wells





Mid-depths were flushed well
Bottom depth was poorly flushed

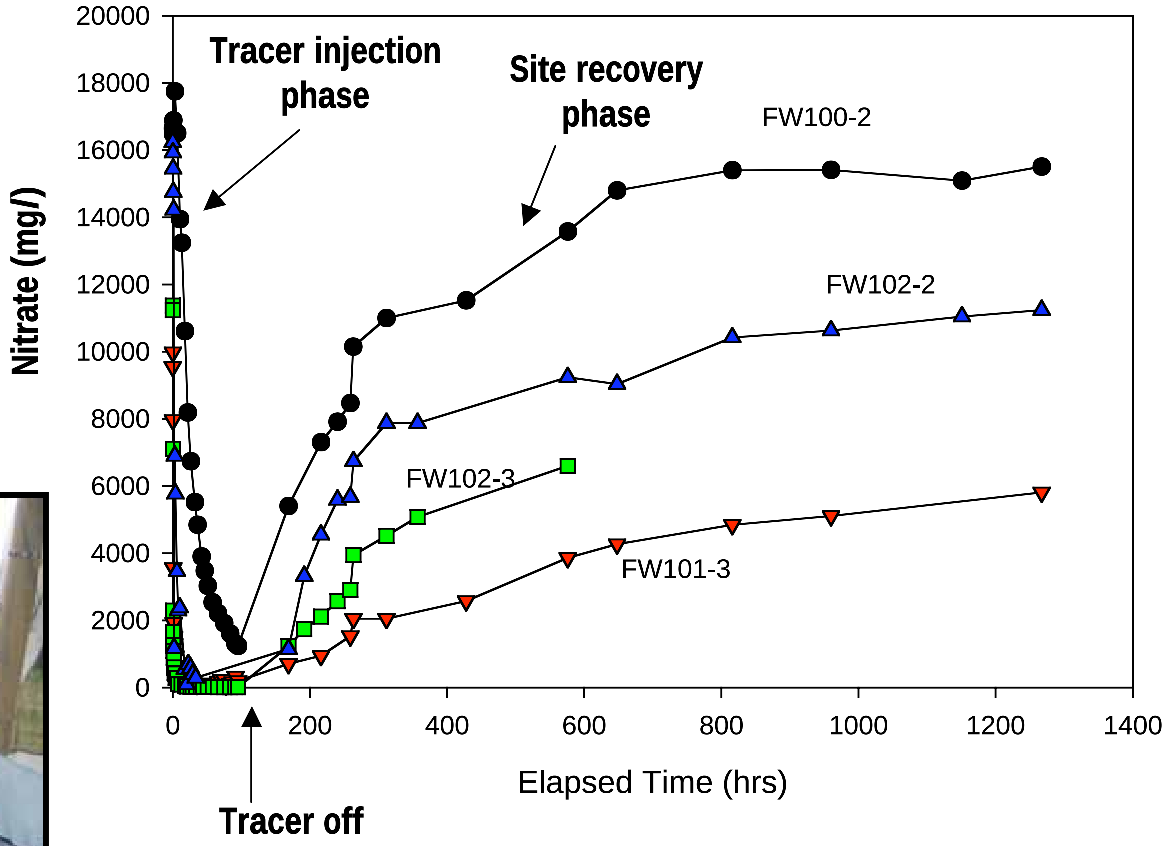


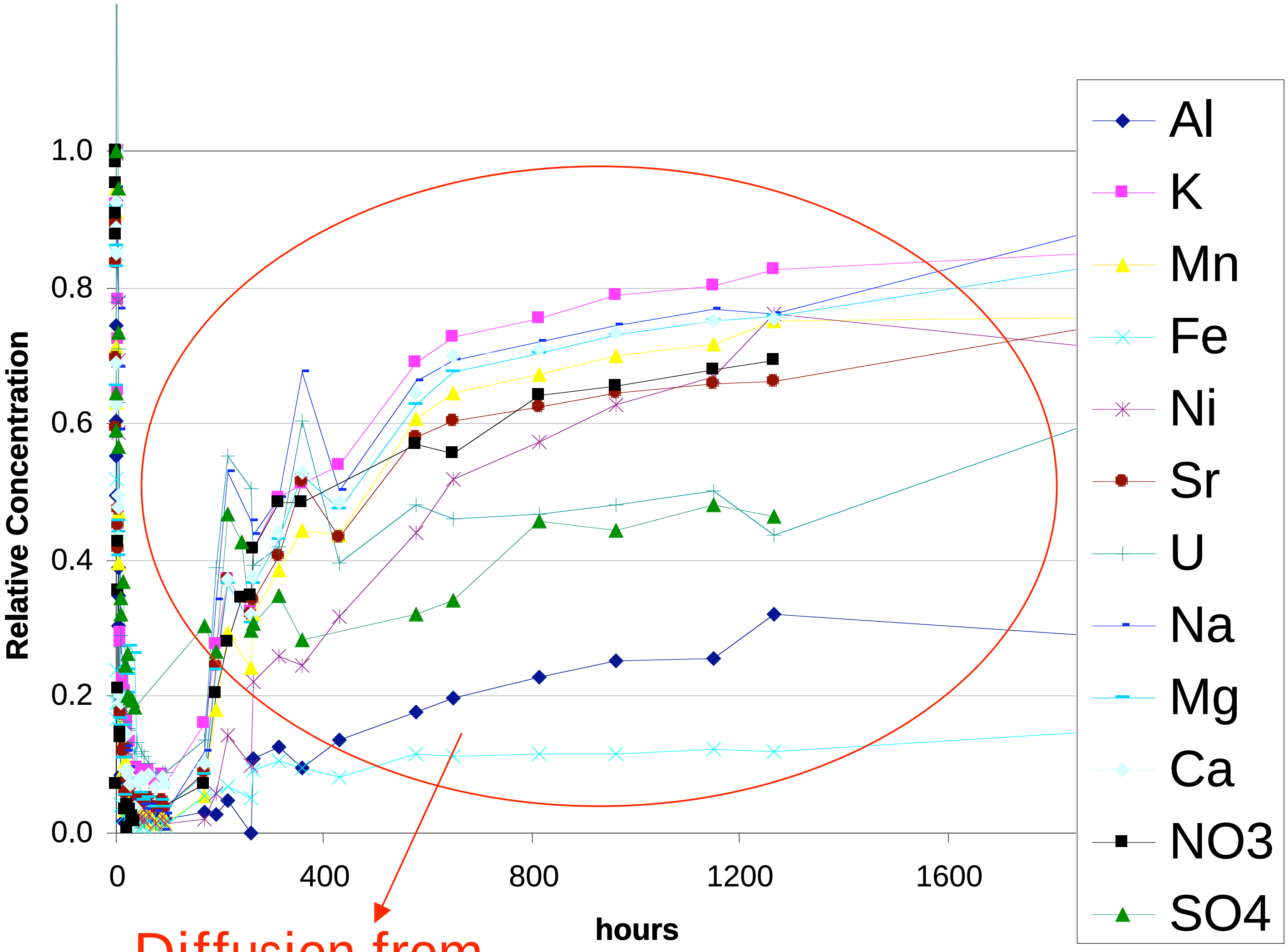
All depths were flushed

Natural gradient site recovery solute breakthrough

Natural gradient contaminant transport monitored during site recovery.

Quantification of solute residence times, direction of groundwater flow, and strike vs. dip interactions.





Diffusion from matrix

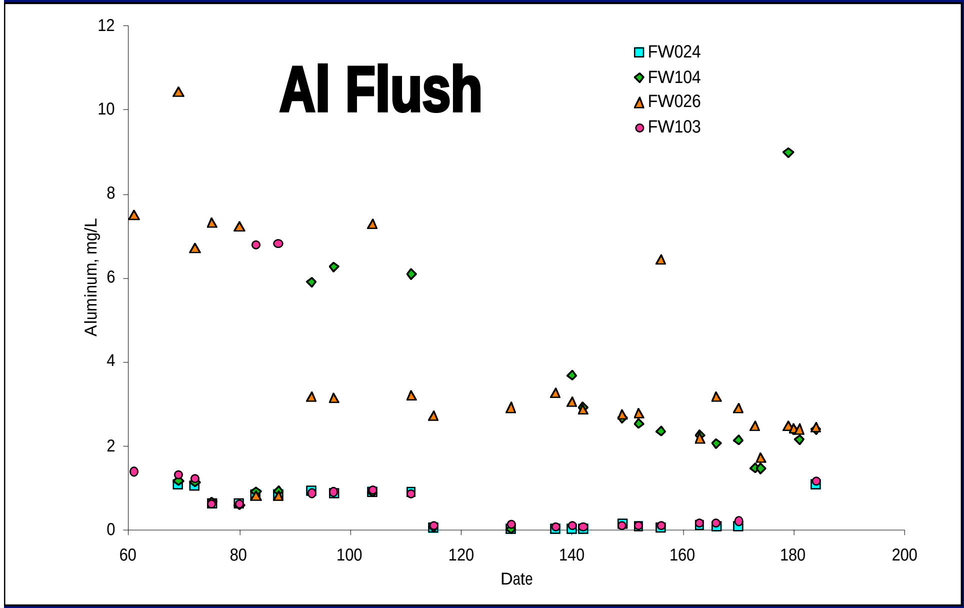
Overall Strategy

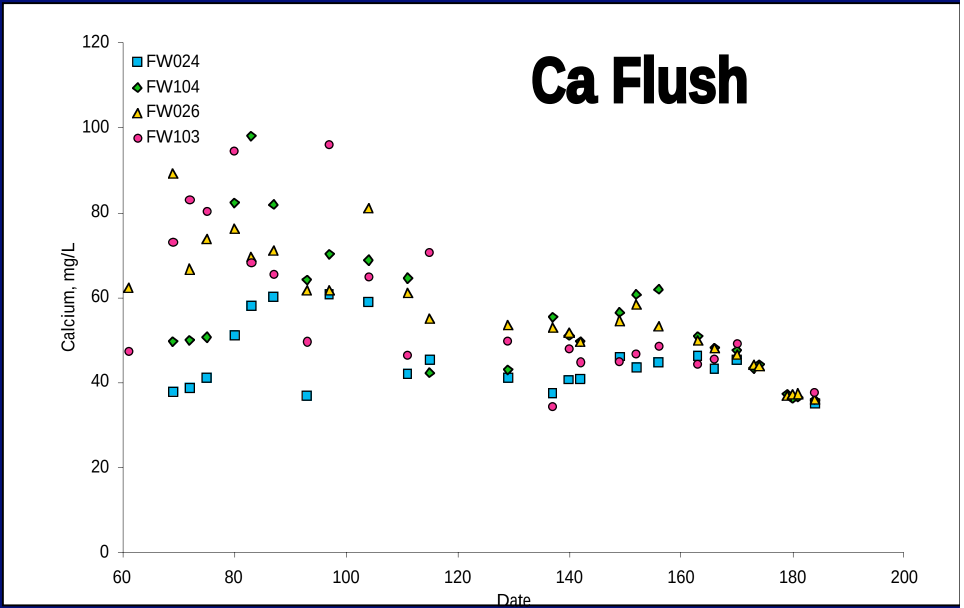
✓ 1. Perform a tracer study to determine connectivity of wells and residence time distribution. Obtain desorption rates from the rebound.

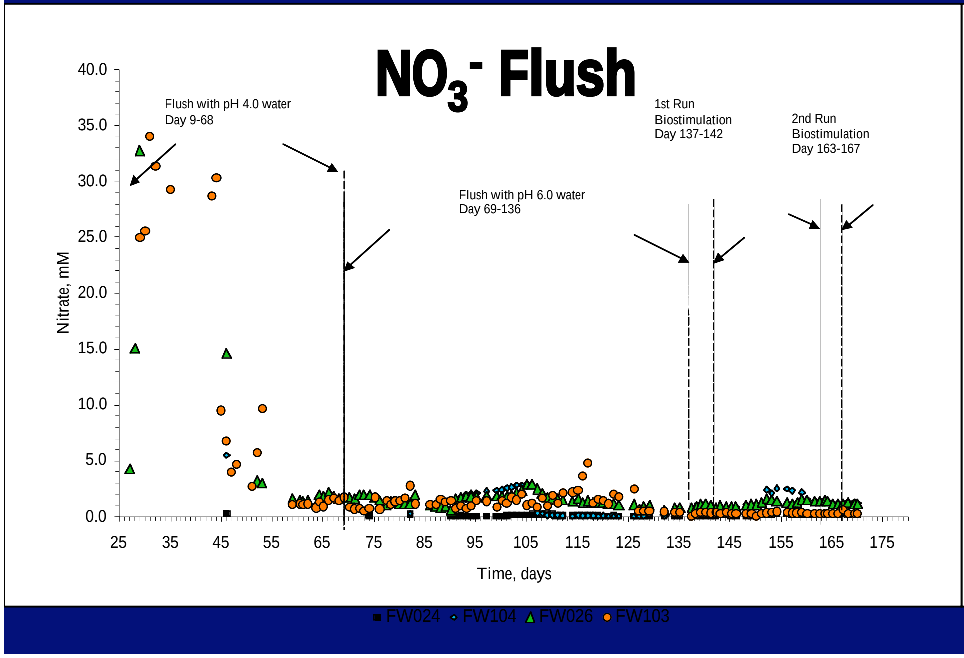
2. Flush outer and inner cell with clean water at pH 4 to remove Al, Ca, and most of the nitrate. Follow with flush at pH 5-6 to prepare for denitrification.

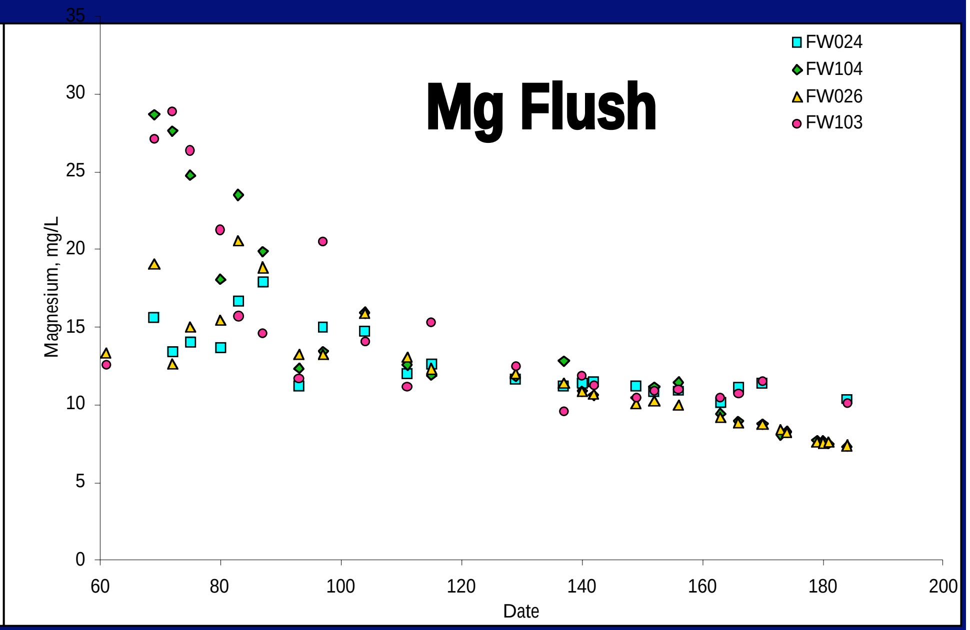
3. Stimulate denitrification *in-situ* and vacuum strip N_2 to remove residual nitrate.

4. Increase pH of inner cell to mobilize U(VI) and add electron donor to inner cell to reduce U(VI).

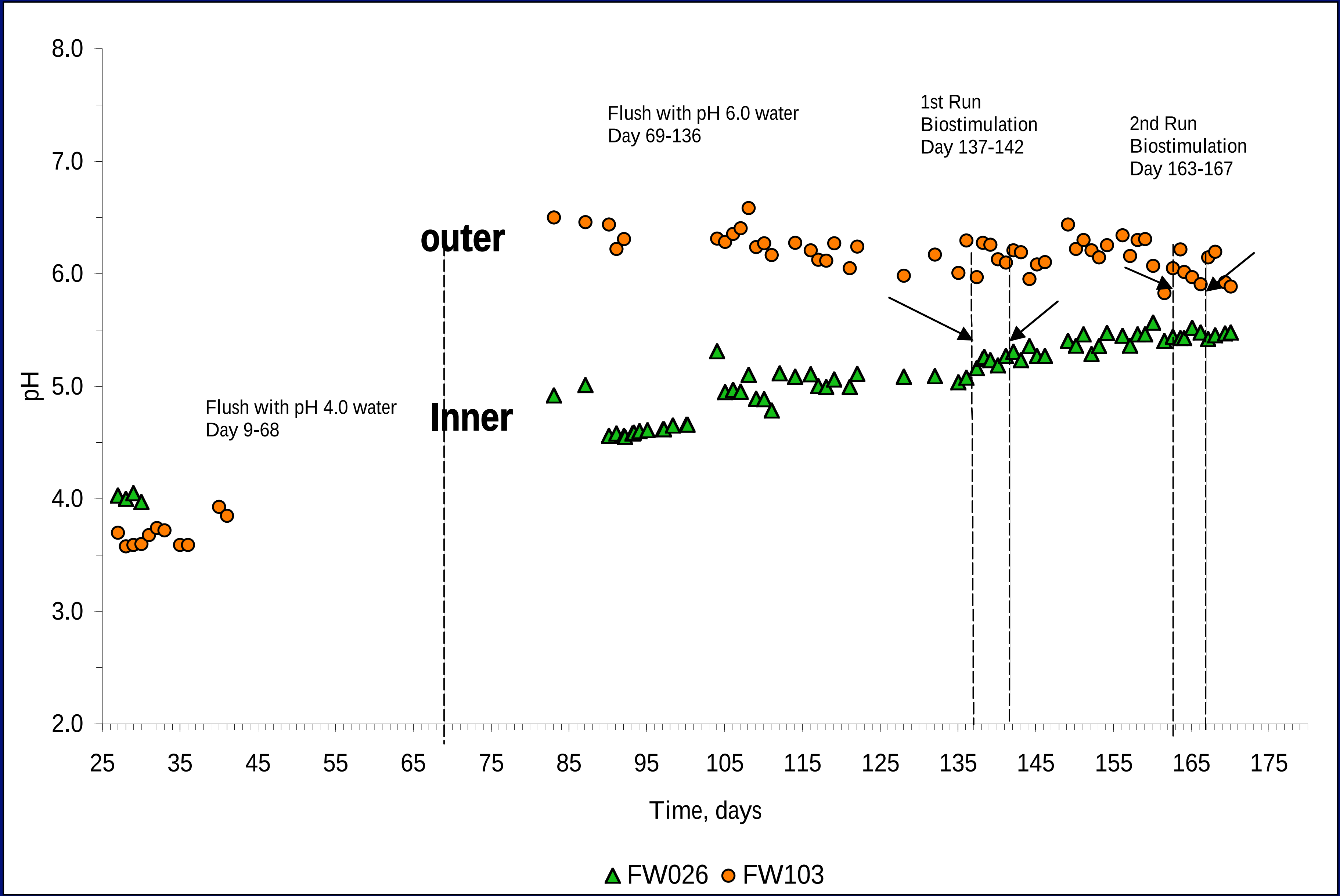

Al Flush
FW024
FW104
FW026
FW103
Aluminum, mg/L
0
2
4
6
8
10
12
60
80
100
120
140
160
180
200
Date


Ca Flush
FW024
FW104
FW026
FW103
Calcium, mg/L
0
20
40
60
80
100
120
60
80
100
120
140
160
180
200
Date


NO3- Flush
Flush with pH 4.0 water
Day 9-68
1st Run
Biostimulation
Day 137-142
2nd Run
Biostimulation
Day 163-167
Flush with pH 6.0 water
Day 69-136
Nitrate, mM
0.0
5.0
10.0
15.0
20.0
25.0
30.0
35.0
40.0
25
35
45
55
65
75
85
95
105
115
125
135
145
155
165
175
Time, days
FW024
FW104
FW026
FW103


Mg Flush
FW024
FW104
FW026
FW103
Magnesium, mg/L
0
5
10
15
20
25
30
35
60
80
100
120
140
160
180
200
Date

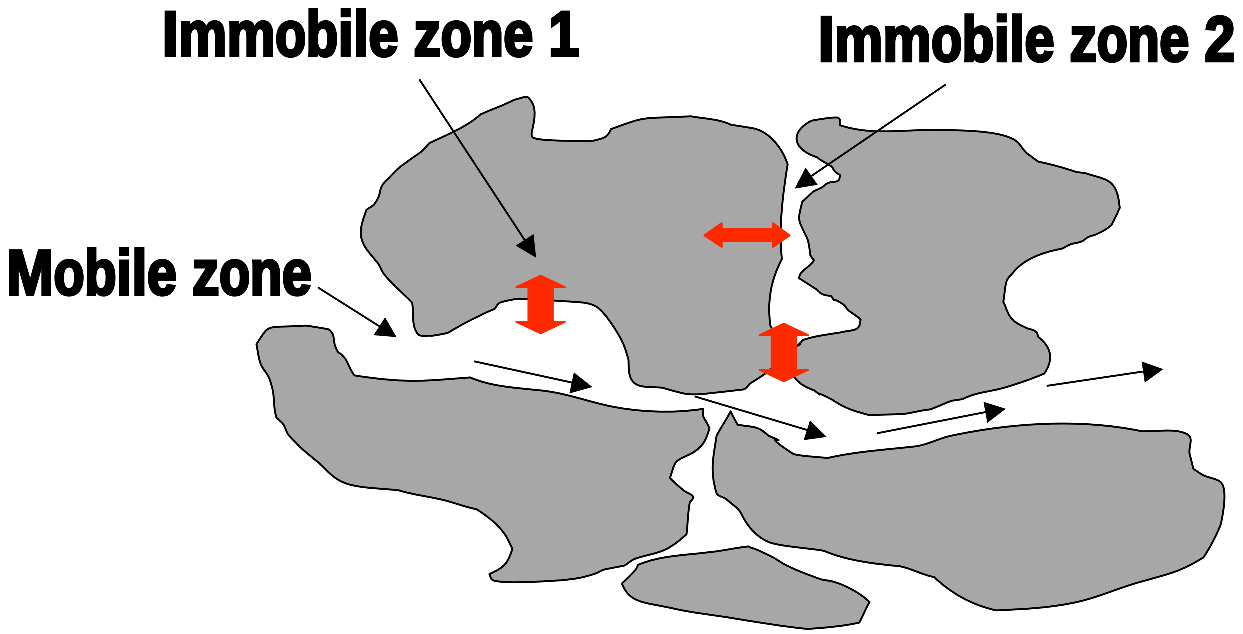
pH increase in inner and outer loop extraction well



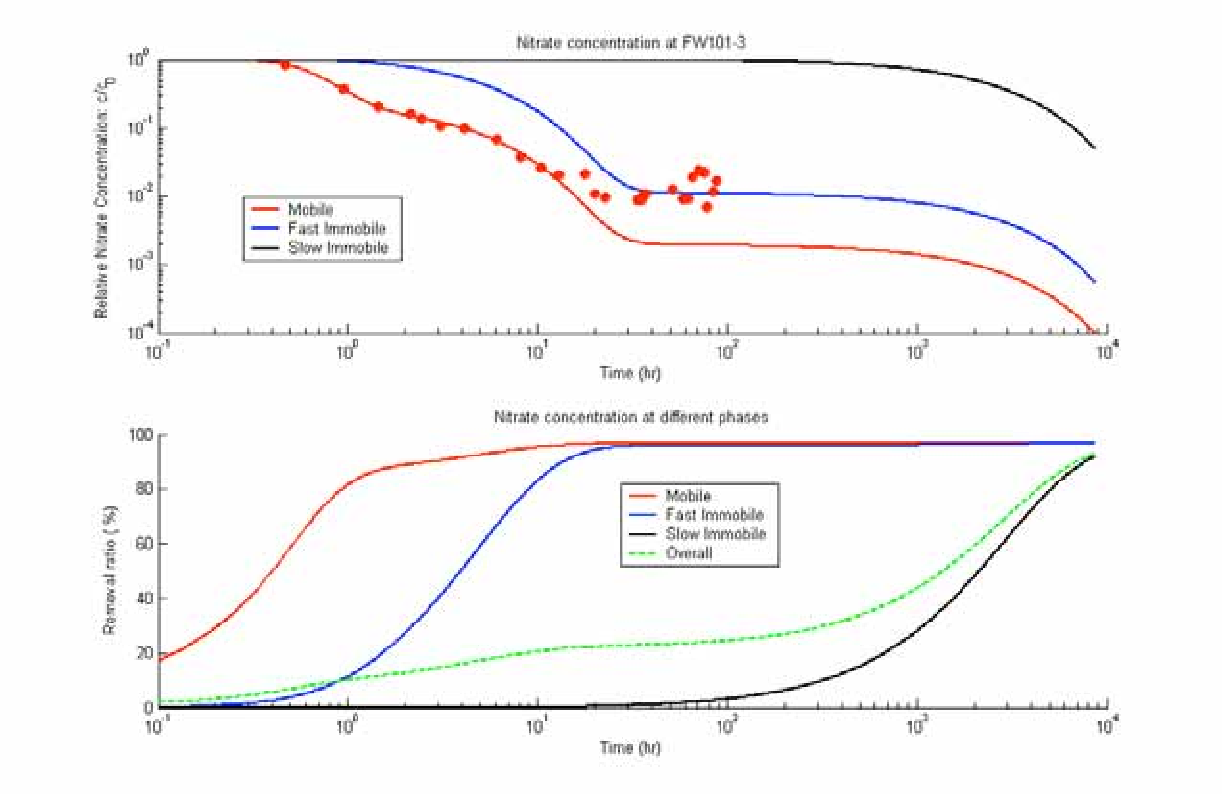
- **Kinetic Model**

  Assumptions

  - Kinetically controlled sorption/desorption
  - Kinetic mass transfer between two regions



Modeling of flushing



- The half-life of nitrate in the second immobile region is about 3 months. To deplete the second immobile zone would take about one year.

- The mobile region definitely responds to flushing and a low average Nitrate concentration can be maintained while removing the Nitrate as it enters the mobile zone.

Overall Strategy

✓ 1. Perform a tracer study to determine connectivity of wells and residence time distribution. Obtain desorption rates from the rebound.

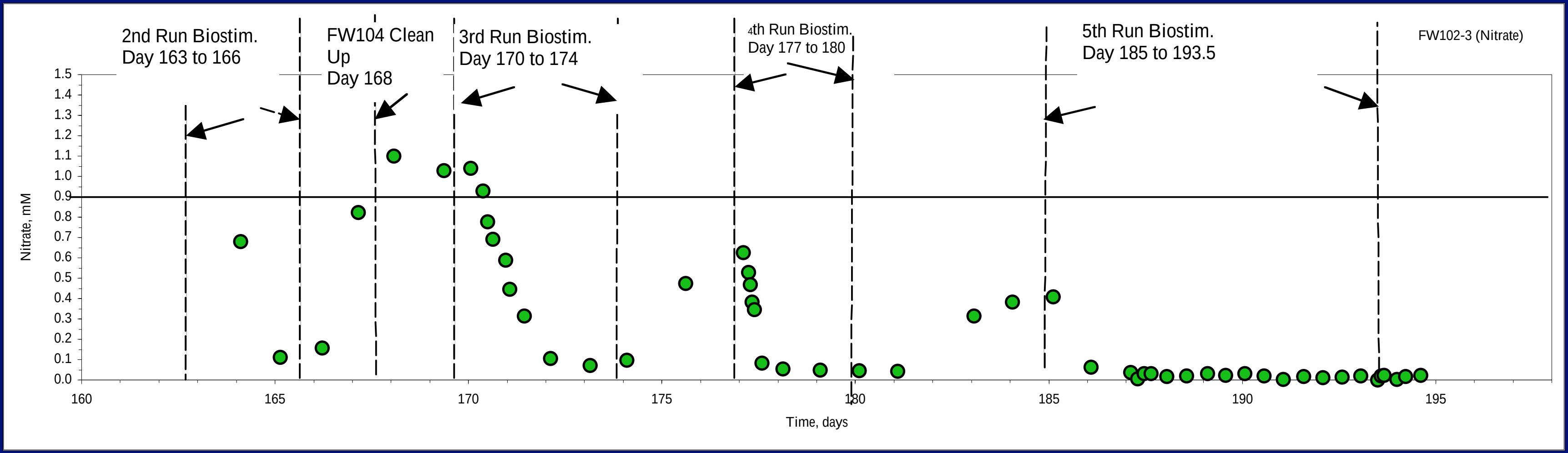
✓ 2. Flush outer and inner cell with clean water at pH 4 to remove Al, Ca, and most of the nitrate. Follow with flush at pH 5-6 to prepare for denitrification.

3. Stimulate denitrification *in-situ* and vacuum strip N_2 to remove residual nitrate.

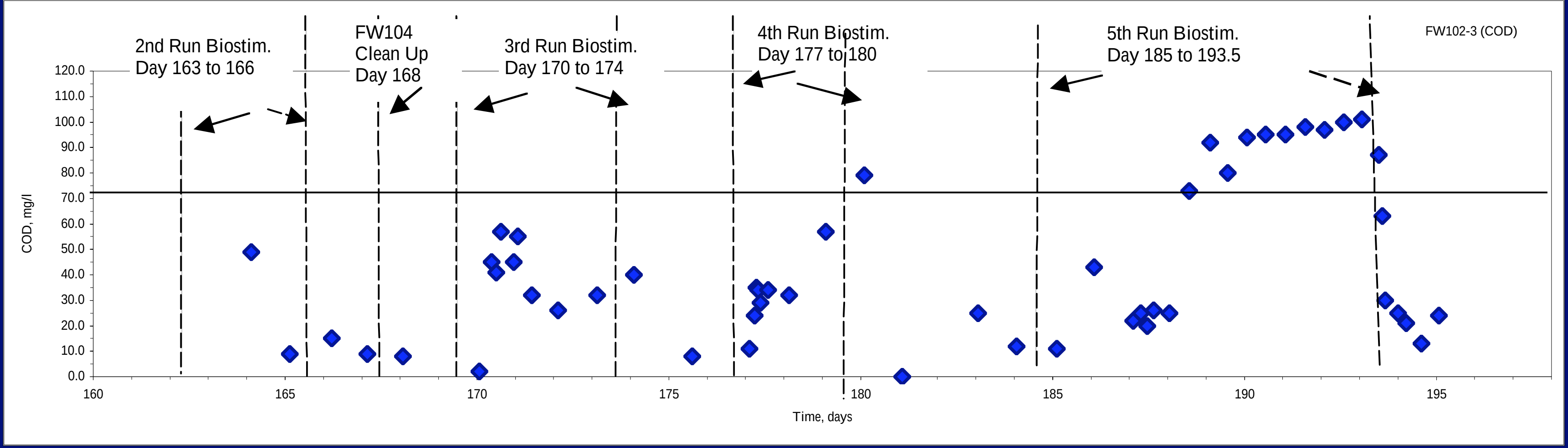
4. Increase pH of inner cell to mobilize U(VI) and add electron donor to inner cell to reduce U(VI).

MLS 102-3

NO_3^-

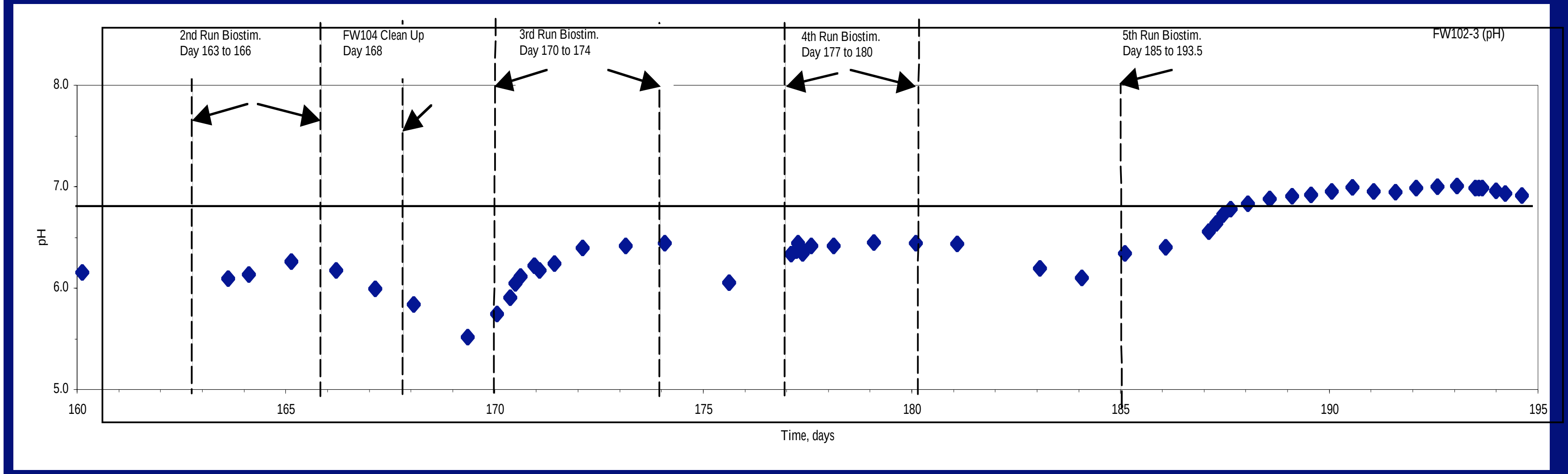


Soluble COD

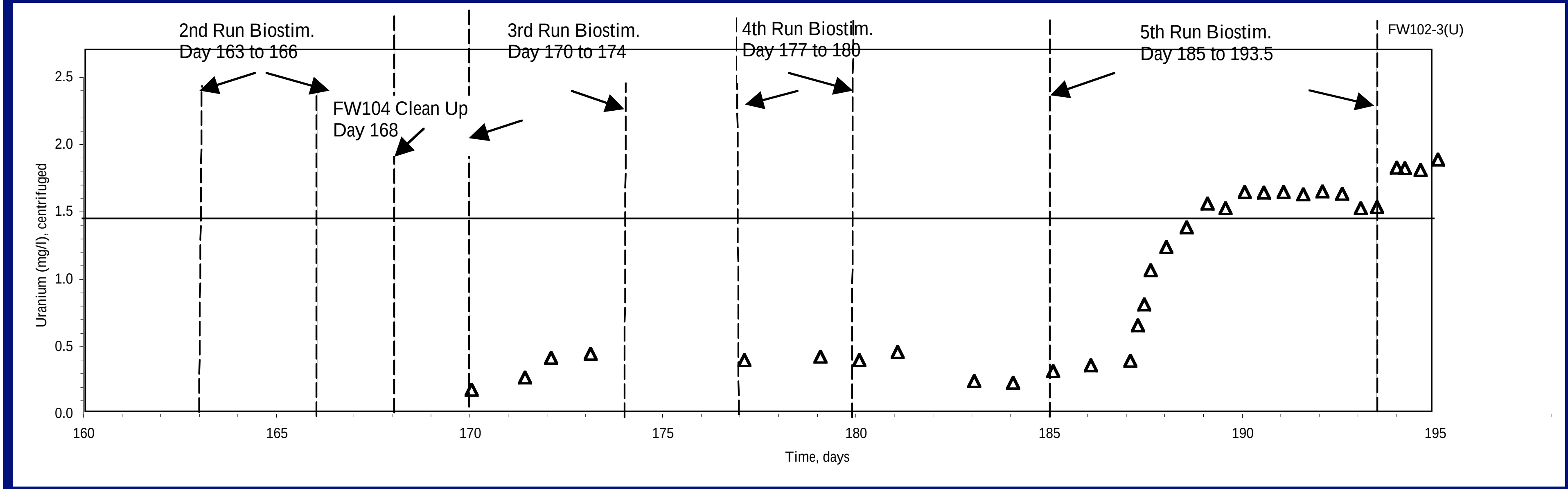


MLS 102-3

pH



Soluble U(VI)



Next up for the field

- Build up U-reducing biomass
- Increase pH but avoid clogging
- Drilling
- Oxidation experiments

In-line Uranium Immunosensor

This sensor has the ability to autonomously run a standard curve from stock reagents and prepare environmental samples for analysis. The sensor measures U(VI) at concentrations from 4-100 nm (0.6-24 ppb)

Optics, LED, and capillary bed containing particles with immobilized capture ligand.

Fluorescently labeled antibody and environmental contaminant are mixed in a disposable syringe.

Drive motor pushes the antibody-contaminant mixture over the capture ligand.

Handheld Immunosensor

Subsurface Microbiology

Monitoring of succession

Baseline analysis of low pH community

Slides on inner loop sidestream

Small packed columns

Filtered samples from inner loop - collected for microarray analysis and plate counts

U-reducing enrichments - phylogeny, kinetics

Phylogenetic analyses

Kinetic model development

Genes Included in New Functional Gene Array

Functional Gene	**# Genes Downloaded**	**# Genes with Unique Probes**	**# Genes with Group Probes**
Denitrification			
Nitrate reductase (narB)	34	10	22
Nitrate reductase (narG)	544	196	227
Nitrate reductase (napA)	145	56	81
Nitrate reductase (nasA)	120	57	55
Nitric oxide reductase (norB)	68	46	13
Nitrite reductase (nirS)	411	140	129
Nitrite reductase (nirK)	952	139	132
Nitrous oxide reductase (nosZ)	273	117	94
subtotal	2547	761	753
Other Processes			
Carbon fixation	in progress	in progress	in progress
Carbon polymer degradation	1243	828	263
Methane oxidation/reduction	1377	505	392
Metal resistance/reduction (Cd, Ni, etc.)	2263	1302	604
Nitrification	1258	51	361
Nitrogen fixation	2162	467	623
Organic remediation	5032	in progress	in progress
Sulfur reduction	989	478	345
Grand Total	>16871	>4392	>3341

Key points

- Site characteristics: high acidity, high nitrate, high sulfate, high metals. Organisms identified repeatedly at low pH: *Acidovorax, Burkholderia, Pseudomonas, Microbacterium, Clostridia*.

- Aluminum buffers the system at low pH and precipitates when the pH is increased. Our strategy is to remove it *ex-situ* by precipitation.

- Nitrate inhibits U(VI) reduction. Our strategy is to remove bulk nitrate *ex-situ* and residual nitrate *in-situ* (with ex-situ vacuum stripper).

- Calcium inhibits U(VI) reduction. Our strategy is to remove it *ex-situ* by precipitation.

More key points

- A nested recirculation scheme appears to protect the treatment zone from aluminum, nitrate, and acidity.
- Addition of ethanol to the inner loop effectively removes the nitrate
- We have preliminary evidence for microbial removal of U.

Insights and new tools

- Control of pH/TIC appears to be a useful strategy for increasing reduction, by increasing desorption/dissolution rates.

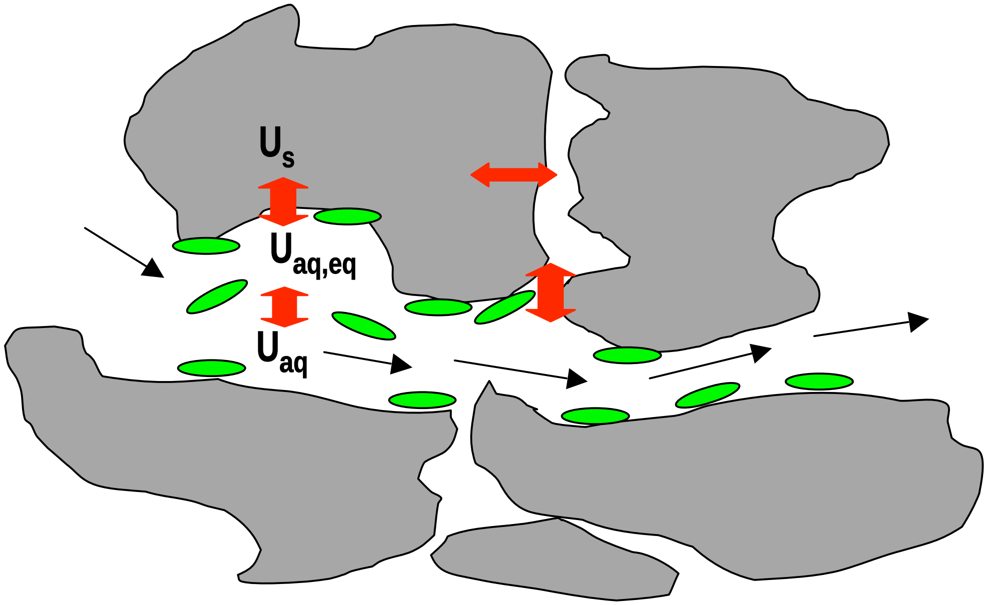
- The vacuum stripper with pH control will be a useful tool for management of U speciation and control of N_2.

- A two-stage process seems feasible for site remediation. This approach would have coupled ex-situ and in-situ elements.

- **Model - coupled mass transfer and reaction**

Assumptions

- Kinetically controlled sorption/desorption
- Kinetic mass transfer between two regions
- Microbial reduction of U(VI) in the mobile zone



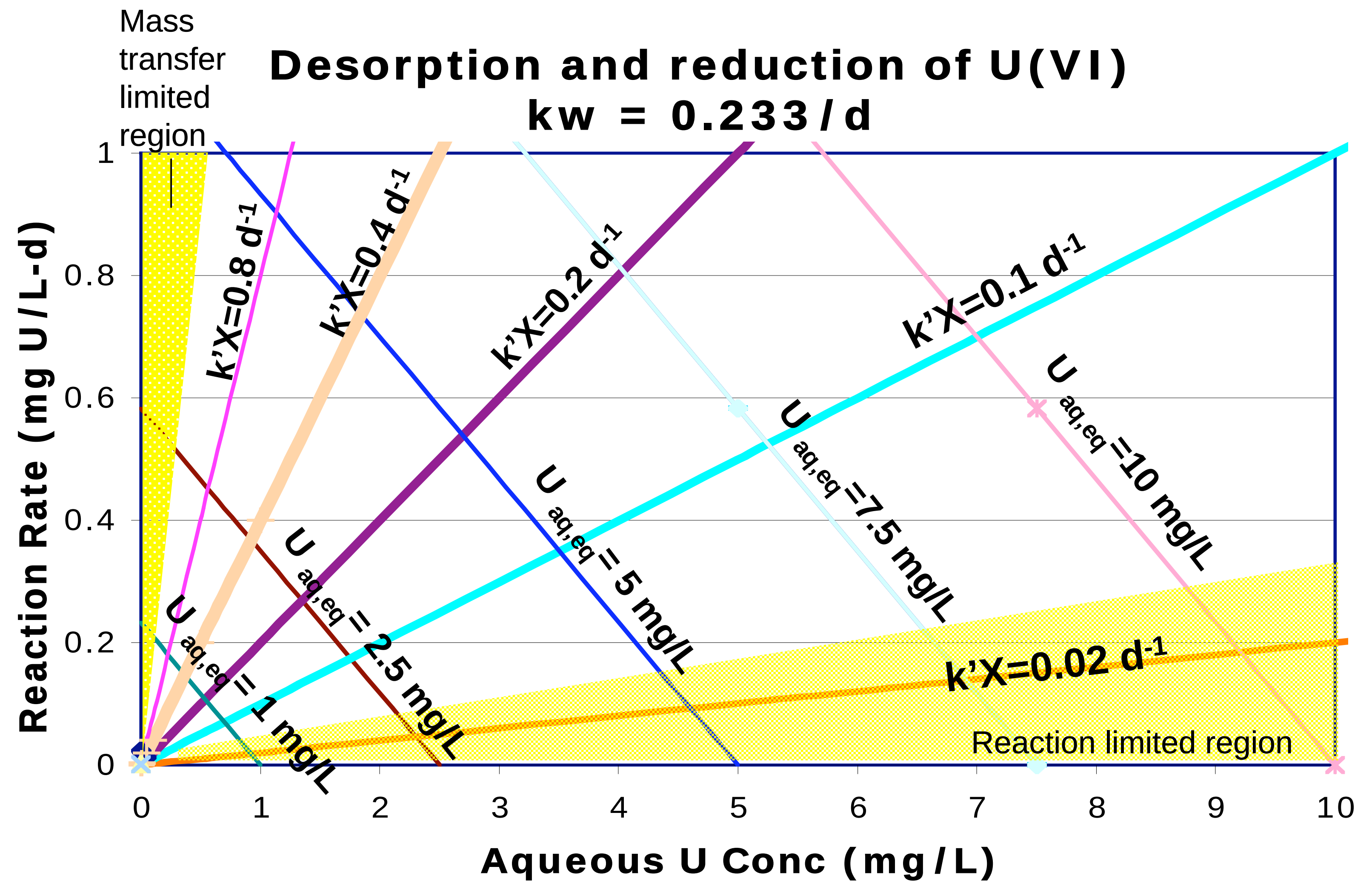
k_w is a lumped parameter accounting for mass transfer. It has units of time^{-1}. $U_{eq,aq}$ is the concentration of U in equilibrium with the solid phase concentration. It is a function of pH and TIC. X is biomass concentration, and k' is a pseudo second order rate coefficient .

Rate of mass transfer = $k_w(U_{aq,\ eq} - U_{aq})$

Rate of reduction = $k'X\ U_{aq}$

At steady state:

Rate of mass transfer = $k_w(U_{aq,eq}-U_{aq})$= Rate of reduction = $k'X\ U_{aq}$

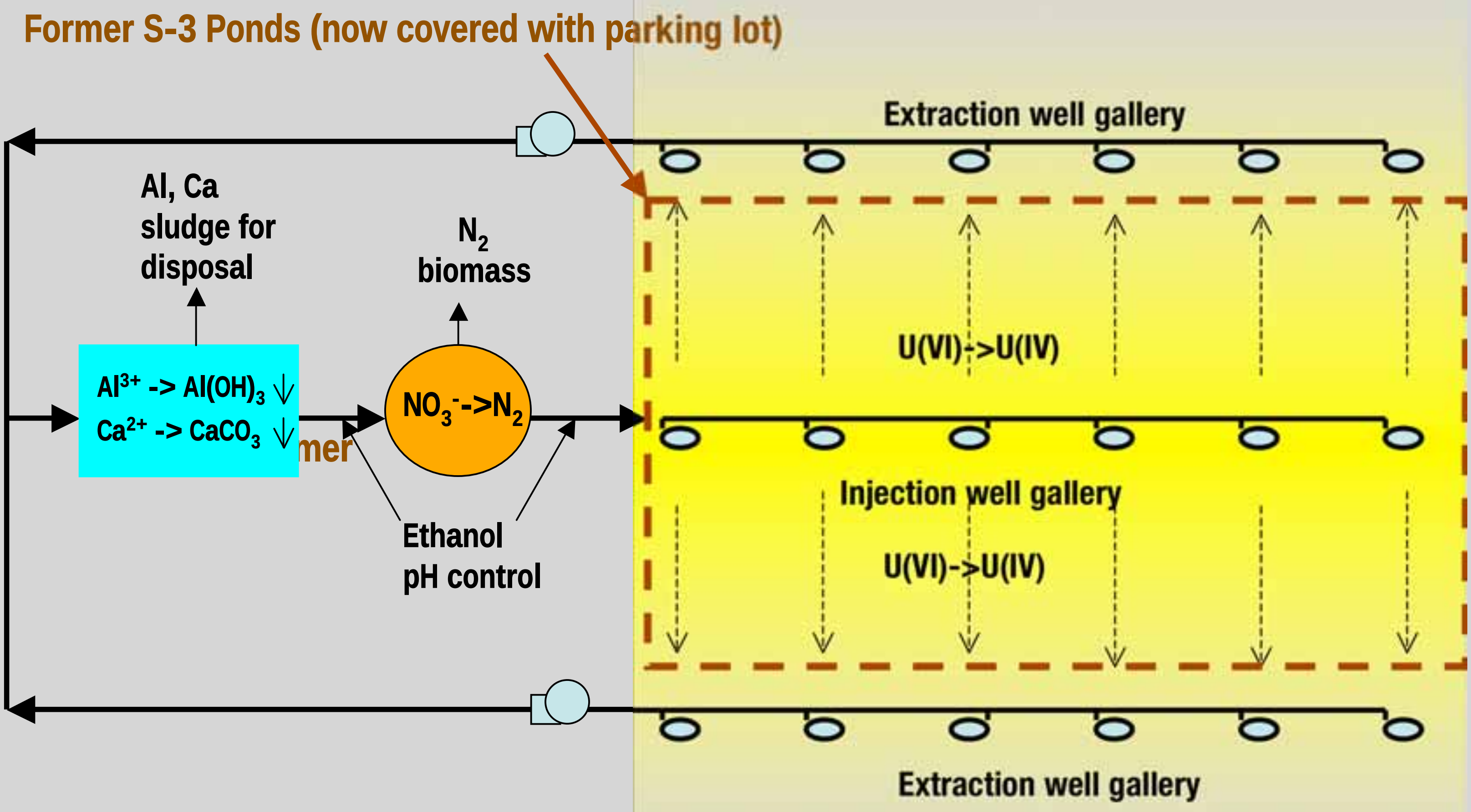


Preliminary calculations indicate that MT limitation is likely

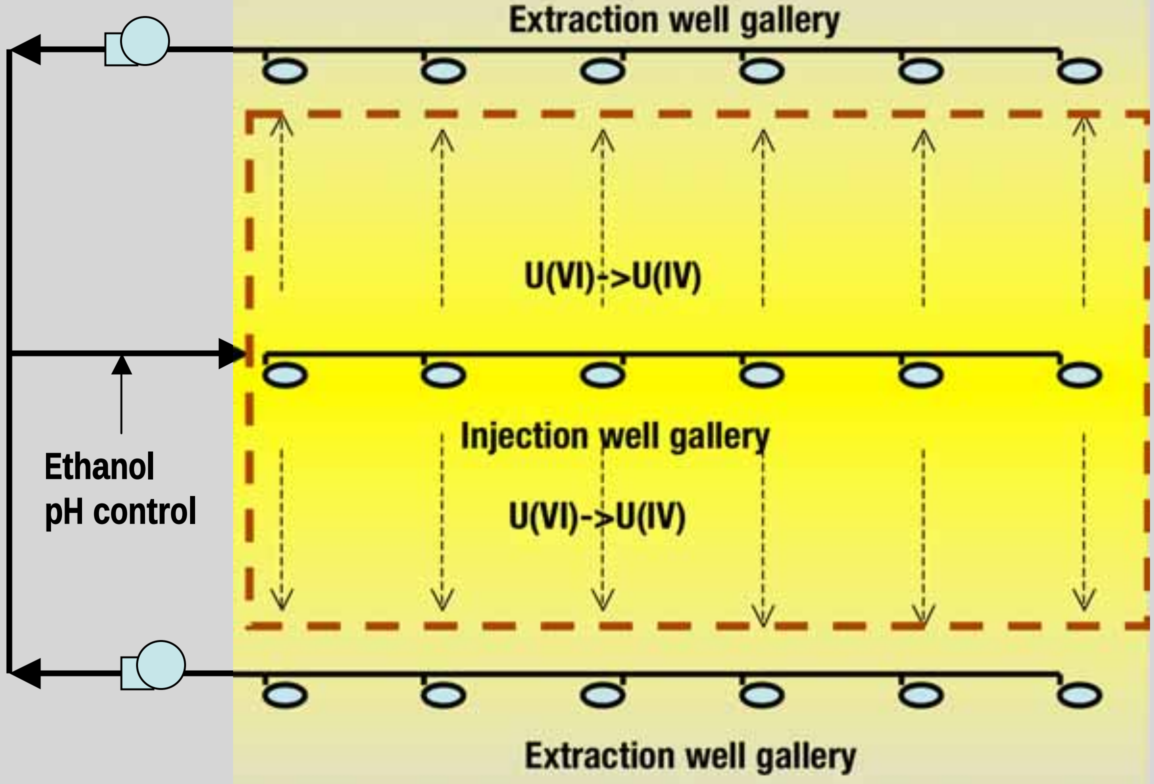
Newly added feature:

Vacuum stripper on the inner cell recirculation loop enables control of TIC and N_2

Stage 1 -removal of aluminum, calcium, nitrate

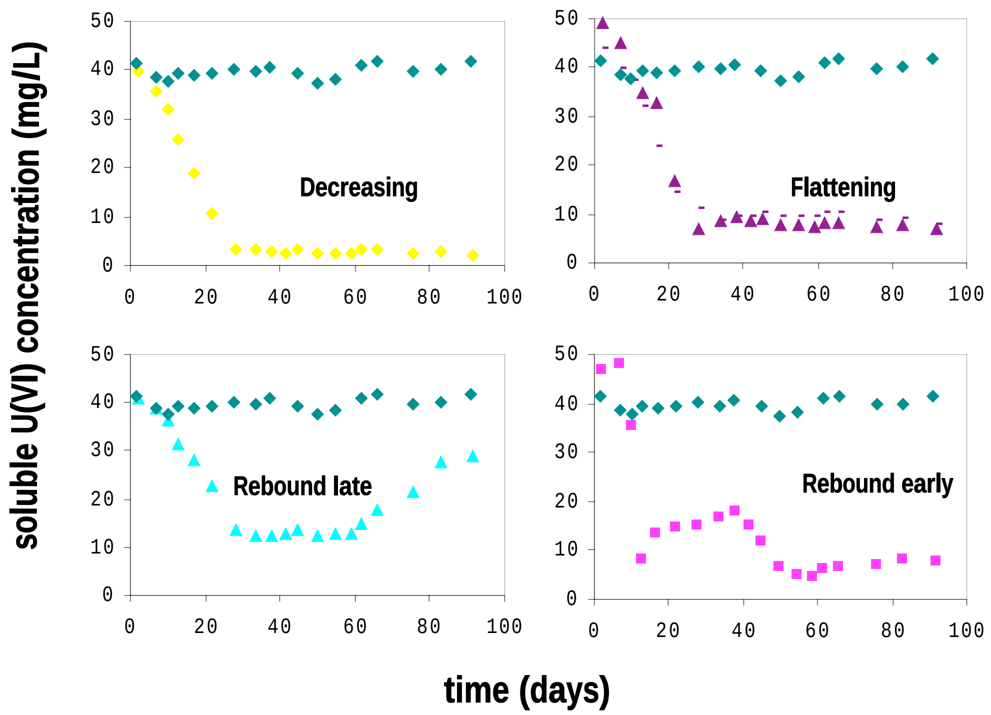


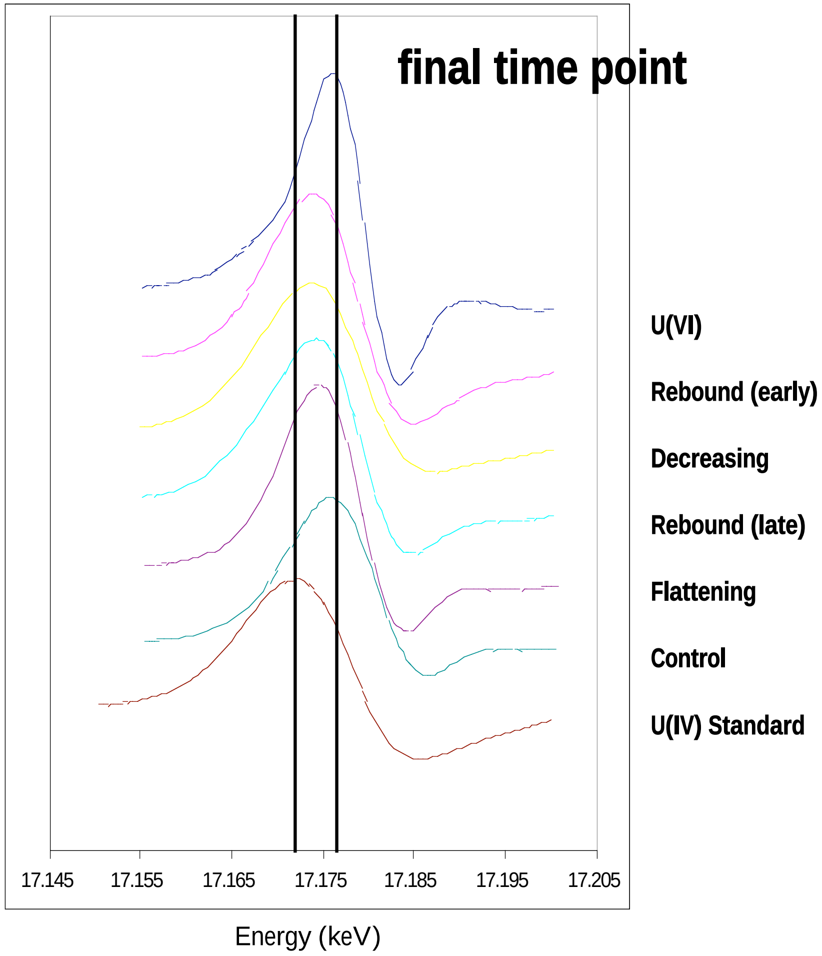
Stage 2 - conversion of U(VI) to U(IV)



Batch microcosm studies: ethanol biostimulation of U reduction in the presence of contaminated sediment

Variation in pattern of **soluble** uranium concentration over time:





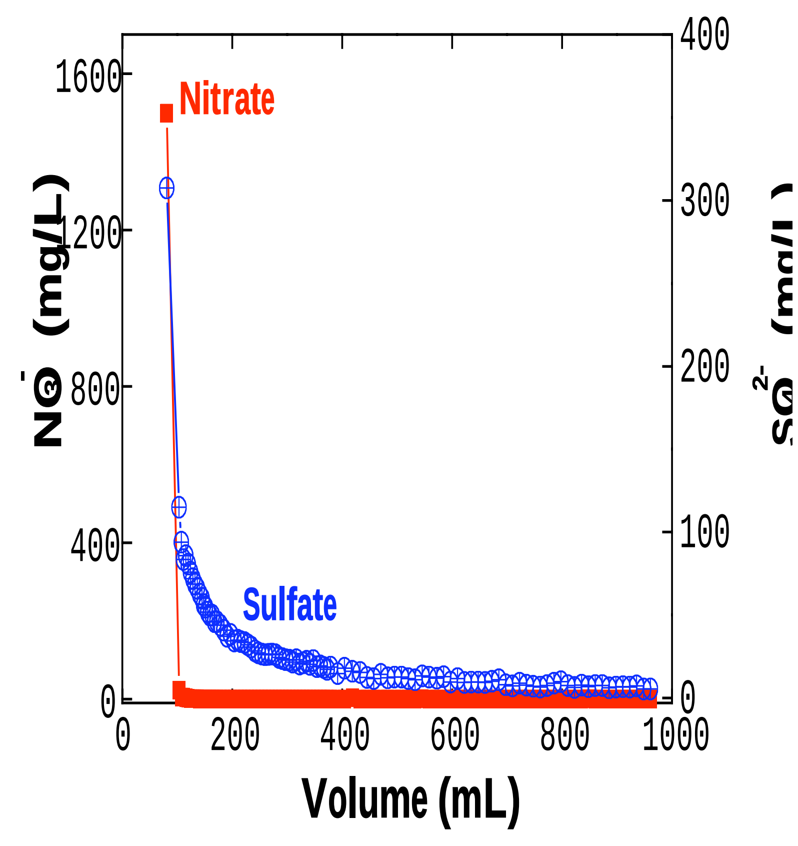
X-ray absorption spectroscopy shows the reduction of **solids-associated** uranium in viable, but not control, microcosms.

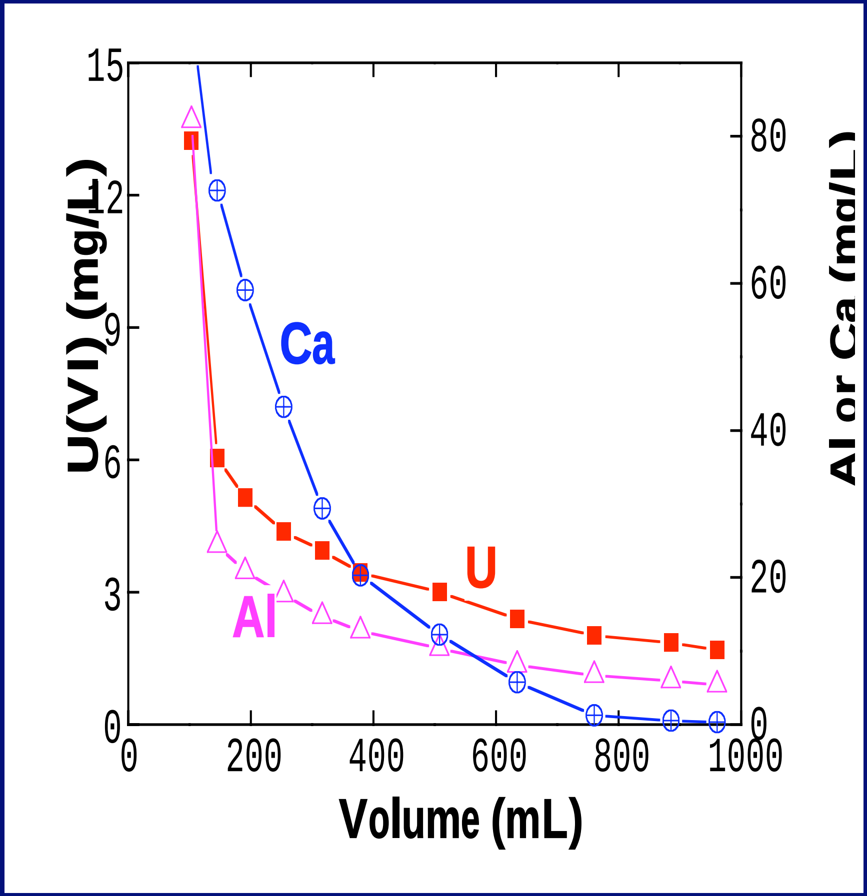
Column microcosm

- Source: a hot spot in our test plot, FWB 104, 38-43 ft.
- Total U(VI) ~500 mg/kg.
- Low pH (3.7), high Al, Ca, Ni, and others.
- High phosphate ~1000 mg/kg.

Step 1: Acid flush to remove Al and Ca

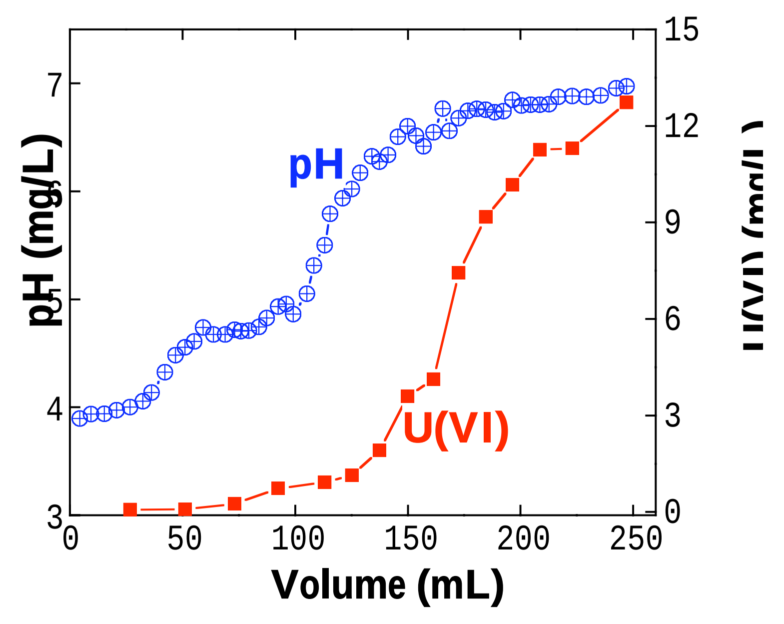
Flush with 10 mM KCl, pH ~4.

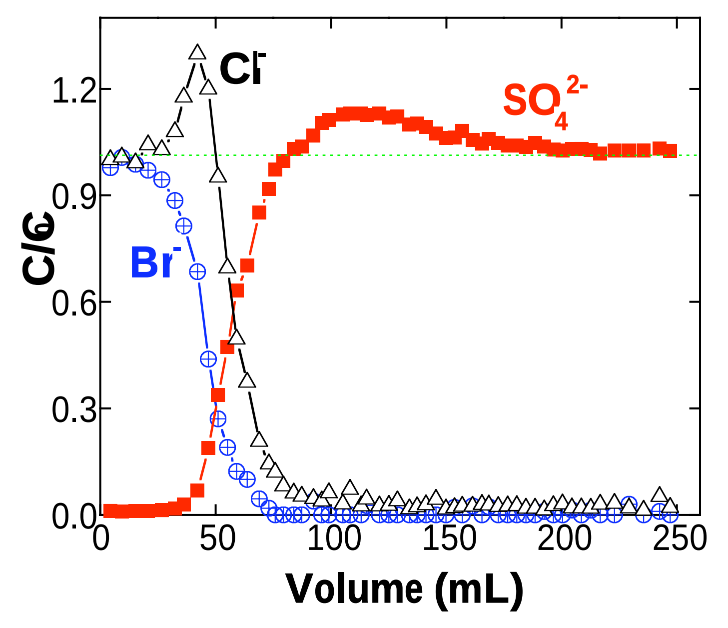




Step 2: pH adjustment + tracer test

Flush with 30 mM $NaHCO_3$, 15 mM K_2CO_3, 5 mM K_2SO_4, CO_2 purged, pH ~7.1.



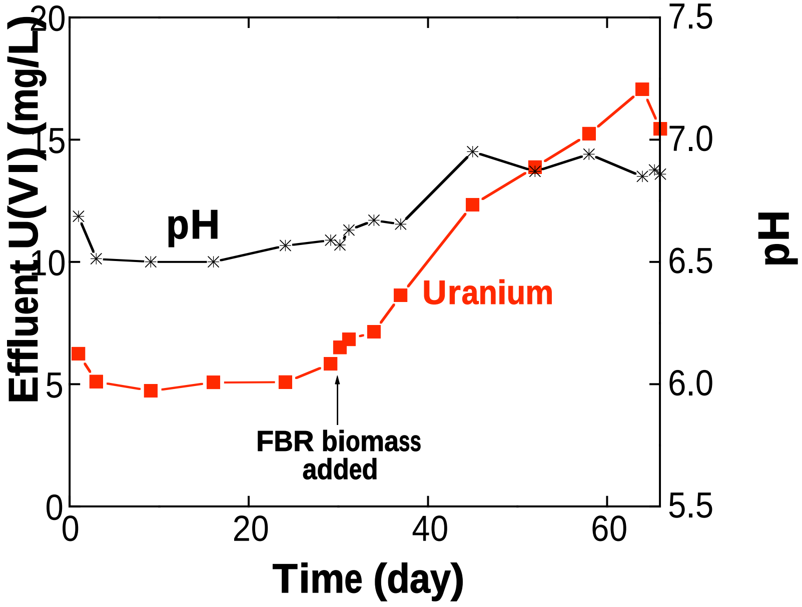


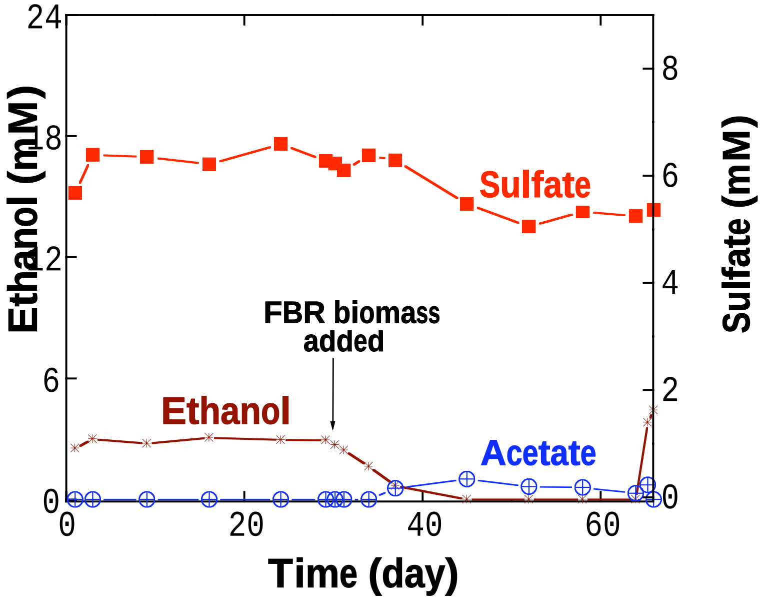
Step 3. Biostimulation

- Strictly anaerobic operation.
- Column was operated in a continuous recirculation mode to simulate field operations (0.2 mL/min).
- Biostimulation was achieved by periodic ethanol addition (~4 mM ethanol spikes, 30 mM $NaHCO_3$, 30 mM K_2CO_3, 5 mM K_2SO_4, CO_2 purged, pH ~7.1).

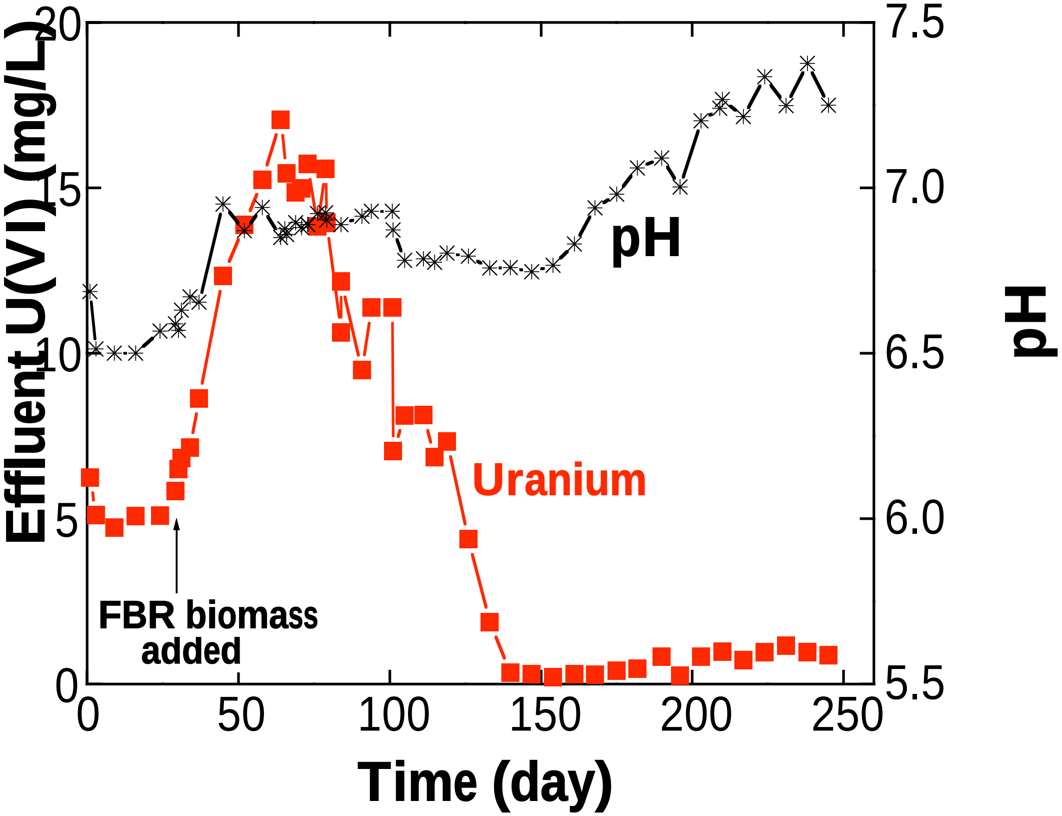
Sample collection reservoir within recirculation loop

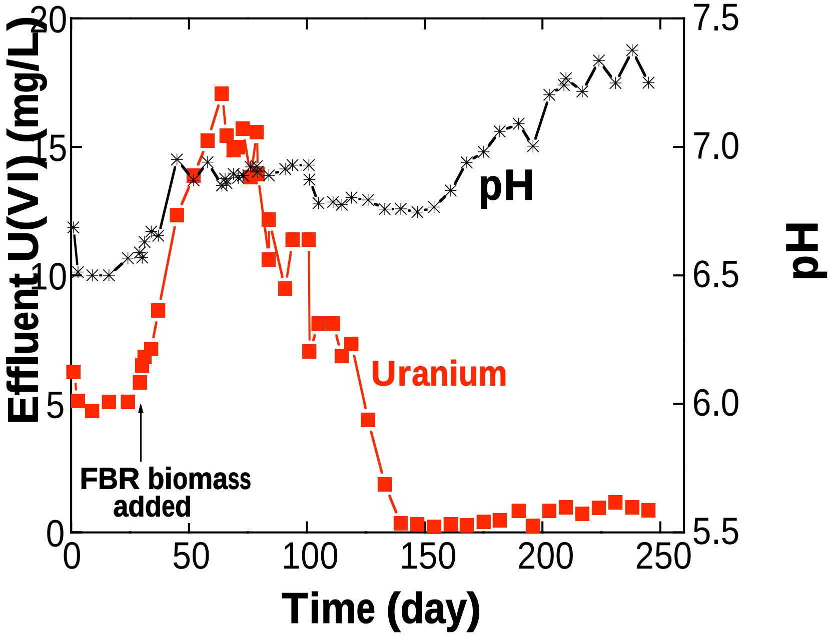
- No evidence of microbial activity for the first 30 days; pH at ~6.5. U(VI) at 5 mg/L.
- FBR biomass was added on day 30. U(VI) begins to increase, along with pH.
- Days 30-60: ethanol disappears first, acetate appears then slowly disapears as sulfate drops. Soluble U(VI) increases to 15 mg/L. U(VI) rises to 15 mg/L.



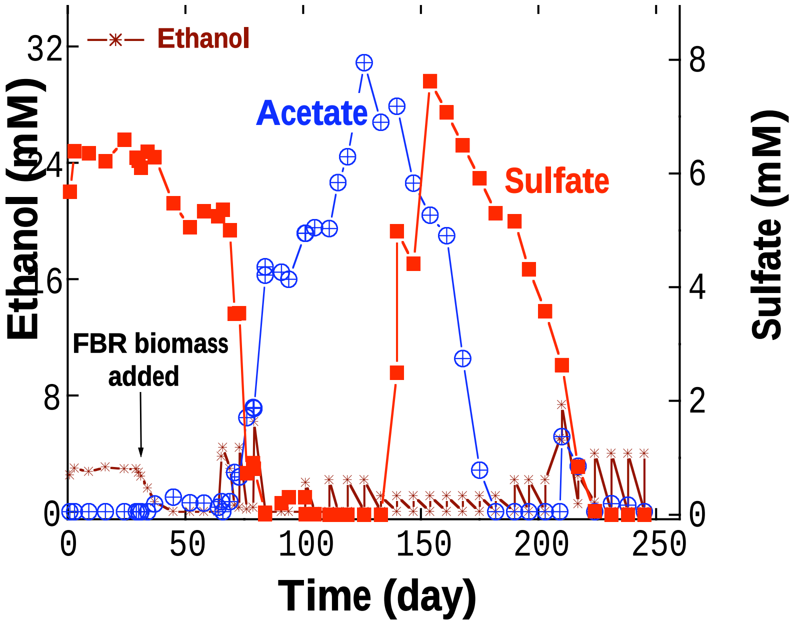


- **Days 60-130: U(VI) levels fall; pH remains high.**





Initially soluble U(VI) levels increased as pH increased



Days 60-120: acetate accumulates as U(VI) falls, no sulfate!

Day 130: sulfate added

Days 130-170: acetate falls, then sulfate

Days 170-220: ethanol added but no acetate formed, sulfate falls, pH increases dramatically, U(VI) starts to increase. Methane detected.

Before

After

Background levels of denitrifiers, metal-reducers, and sulfate-reducers on sediment (MPN/g)

	Nitrate reducers	Ferric citrate reducers	Sulfate reducers
FW-107 (13.2 m)	3500	46	240
FW-109 (15.4 m)	5400	1700	1100

Fields