Factors Controlling In Situ Uranium and Technetium Bioreduction at the NABIR Field Research Center

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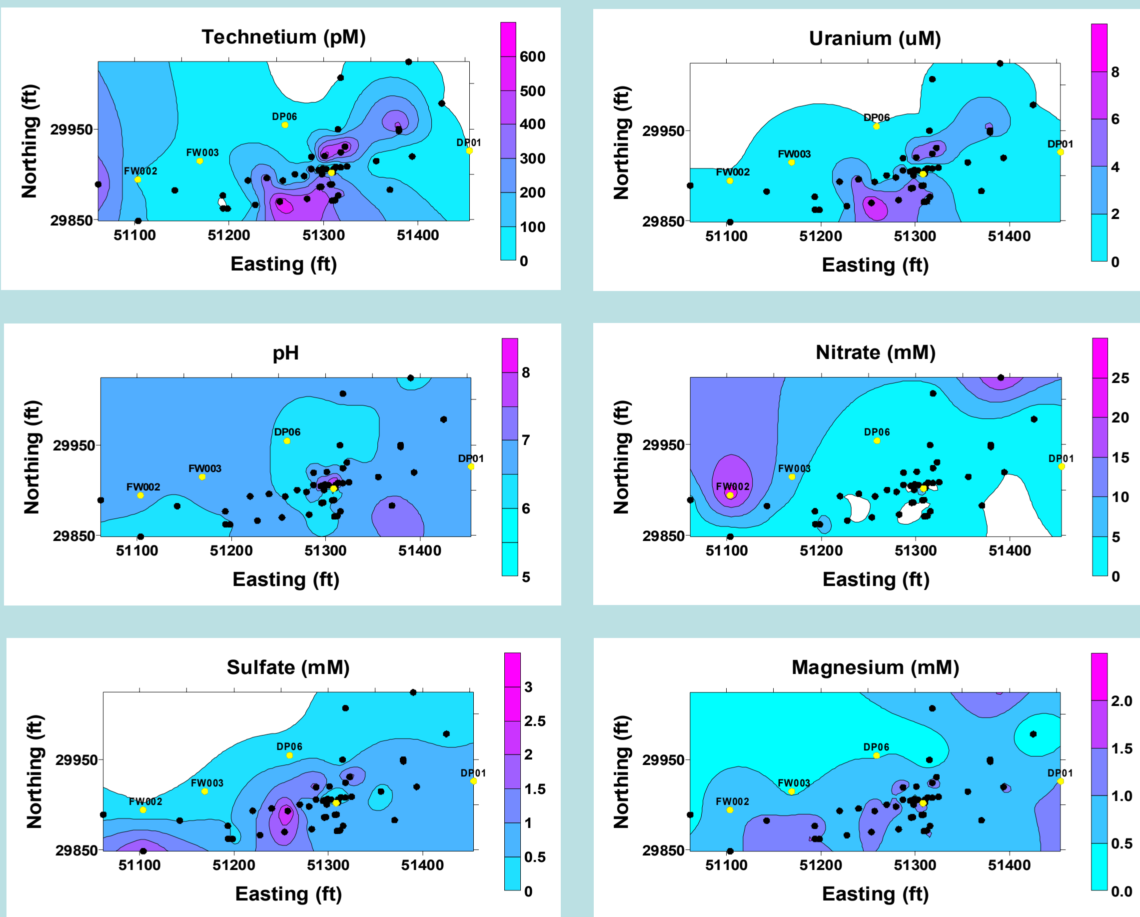
Overview

- Summary of In Situ Testing
- Preliminary results from coupled microbiological - geochemical modeling
- Recent experiments on fate of N_2 gas and precipitates
- Status of intermediate-scale physical models

Geochemical conditions at the site are highly spatially variable

Area 2



Area 1

Area 1 Groundwater

Well-ID	pH	Nitrate (mM)	Sulfate (mM)	Uranium (µM)	Technetium (pM)
FW015	3.4	149	2.2	10.3	10860
FW016	4.5	11	0.2	0.9	710
FW017	4.4		0.1	0.2	191
FW019	6.6	8	0.6	0.7	2288
FW020	4.6	75	1.1	1.4	165
FW021	3.3	142	0.4	5.8	18182
FW027	5.4	168	0.0	0.1	15466
FW028	4.4	167	0.1	9.6	7117
FW029	4.0	62	2.3	9.2	7390
FW030	3.5	145	0.0	4.2	12603
FW031	5.7	63	0.1	0.0	1205
FW032	5.2	23	0.0	0.0	942
FW033	5.9	14	0.7	0.3	1313
FW034	6.8	1	0.8	0.5	39

Field tests were conducted under a wide range of initial conditions in the shallow (< 8 m) subsurface

Initial Conditions

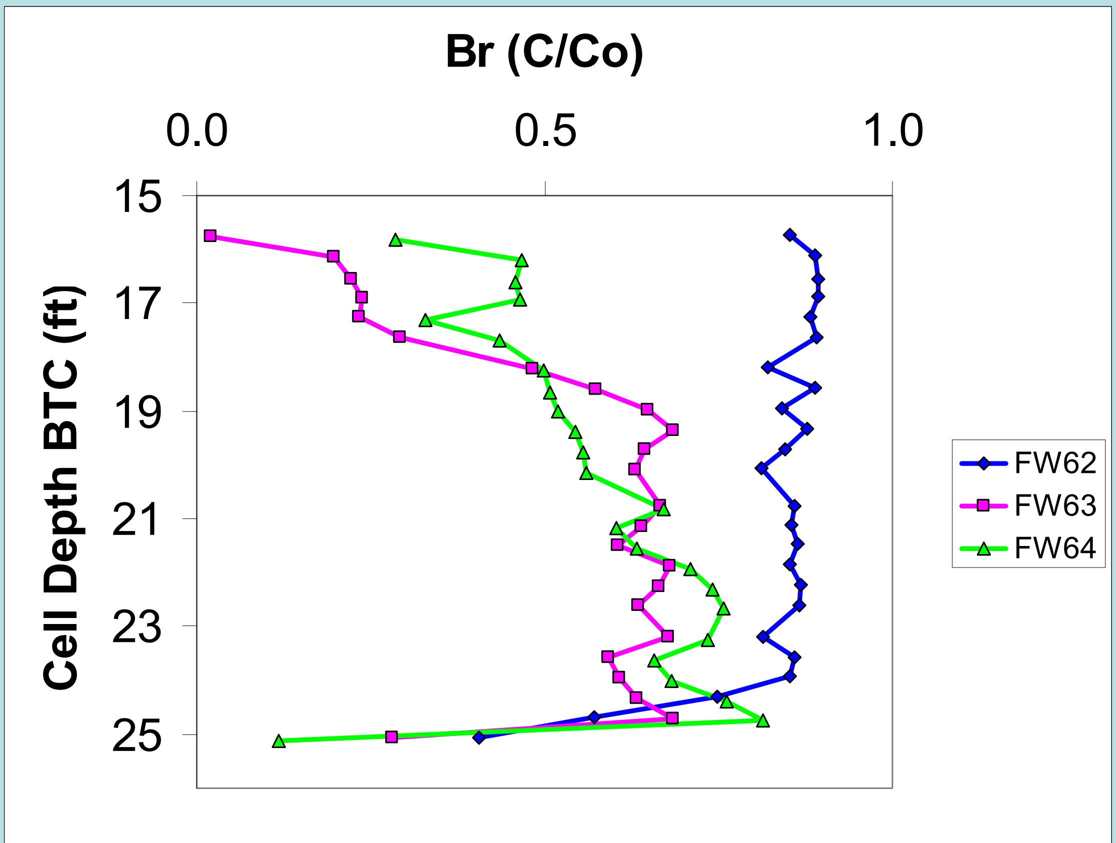
pH	NO_3^- (mM)	SO_4^{2-} (mM)	U(VI) (μM)	Tc(VII) (pM)
3.3-3.9	100-140	0-1	5-12	10000-15000
5.2-5.6	90-100	0-1	5-12	10000-15000
5.6-7.2	0-6	1-2	1-7	200-1000

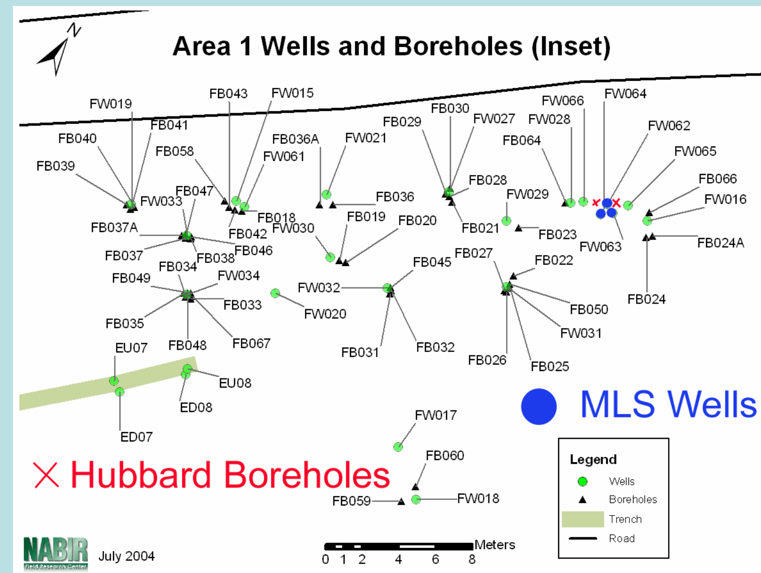
Microbial activity was detected and quantified using single-well, push-pull tests

Typical test design

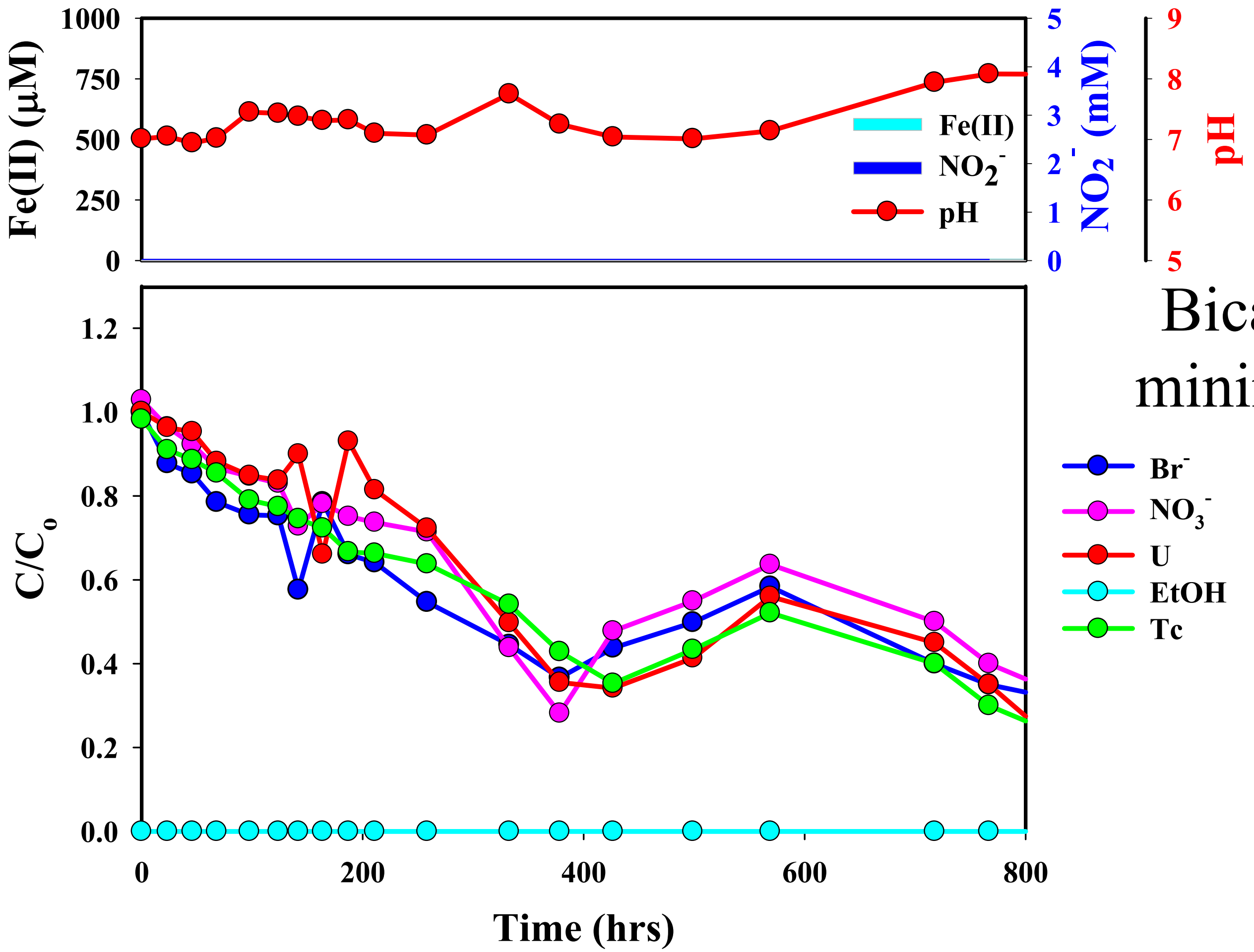
- Collect 50-200 L site groundwater
- Amend with bromide tracer, +/- electron donor, +/-other amendments
- Mix with 80:%:20% N2:CO2
- Inject by siphon
- Sample for 400-1200 hours after injection
- Plot concentration profiles
- Adjust for dilution
- Compute reaction rates
- 104 Area 1 tests 105 Area 2 tests
- Total = 209

Tracer test showing shape of interrogated volume



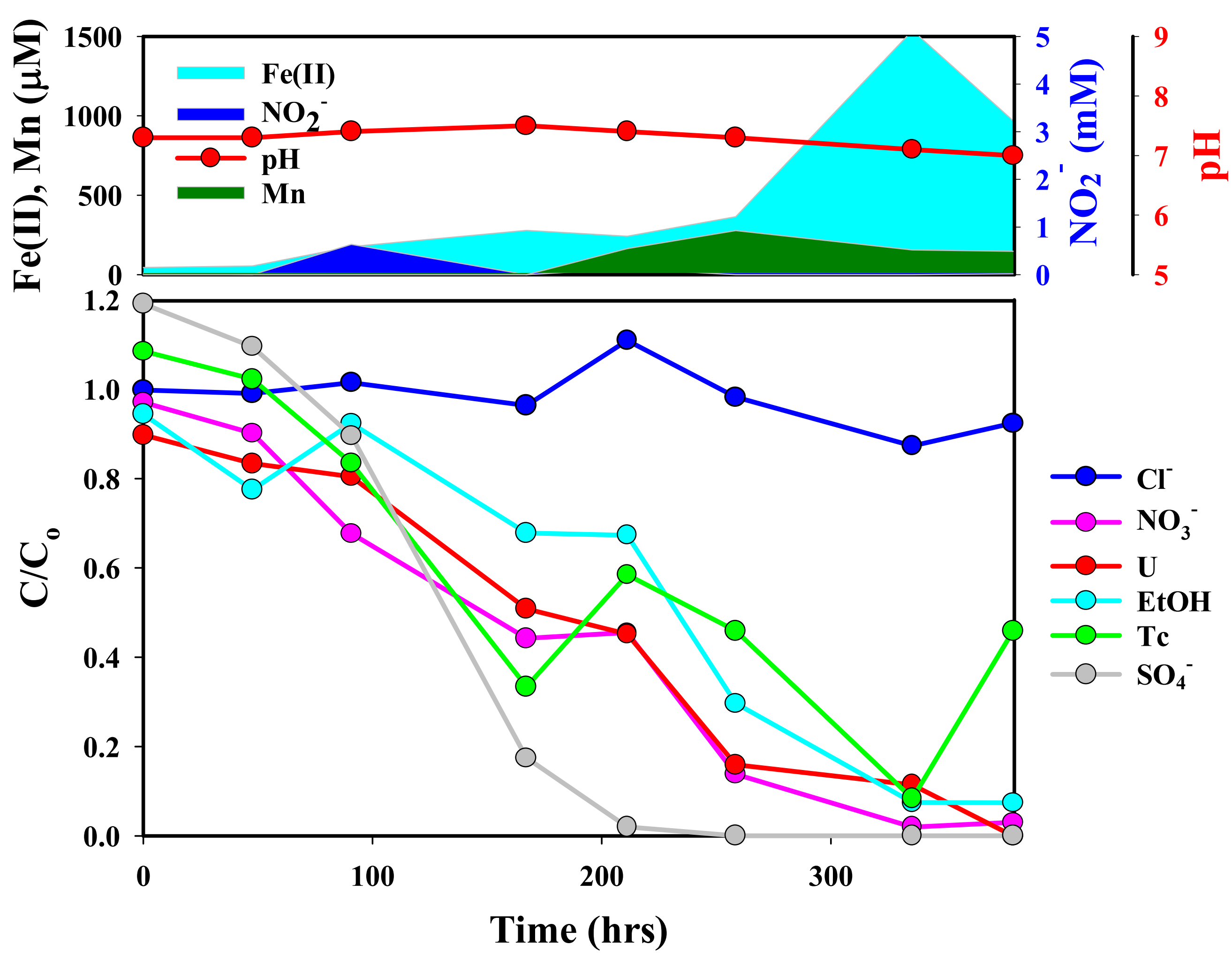


Microbial activity is electron donor limited; control tests with no added donor exhibit only dilution losses



Bicarbonate added to minimize U sorption to sediments

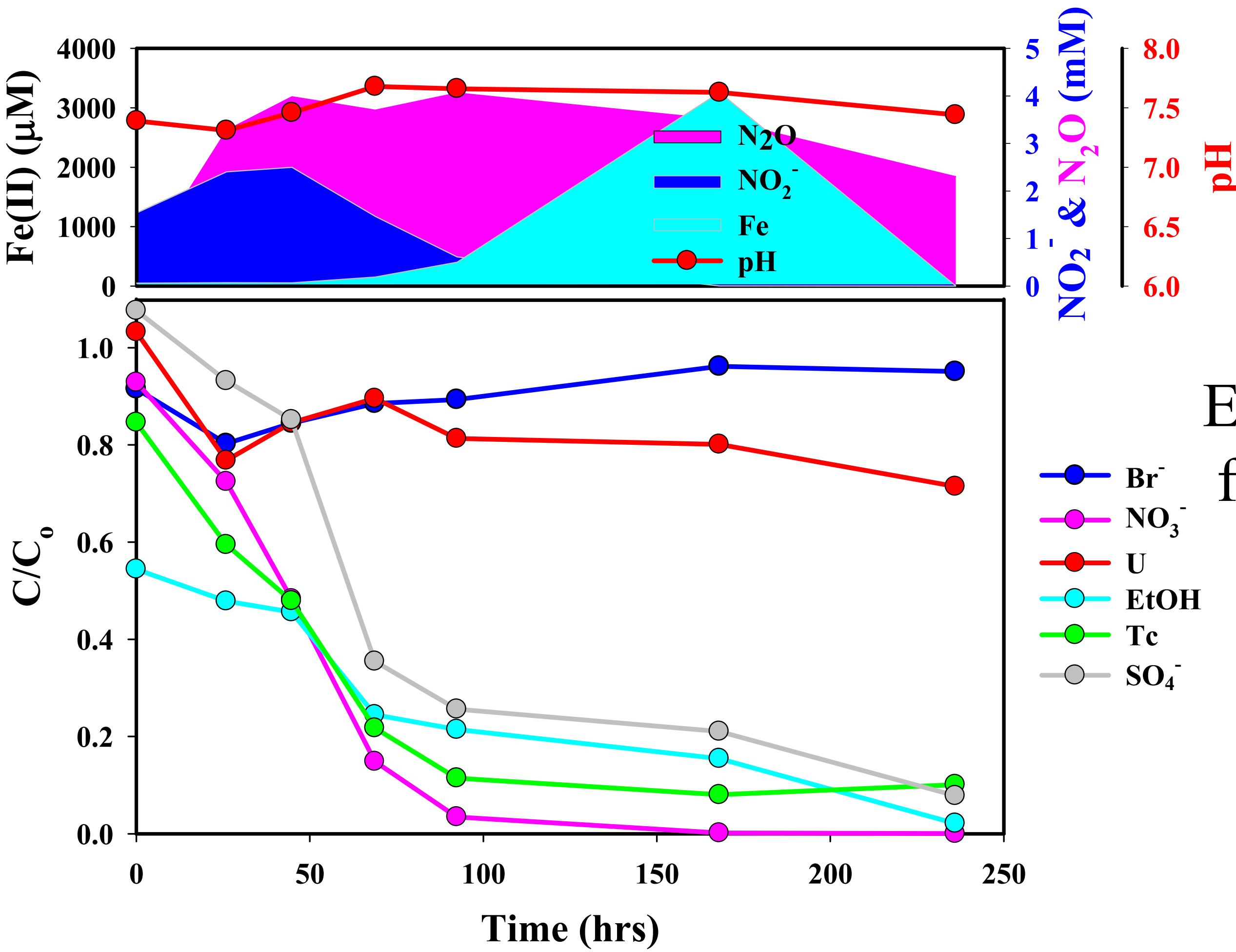
Microbial activity rapidly (~ weeks) stimulated in all environments tested with the addition of exogenous electron donor



Ethanol, glucose, acetate, SRS (emulsified vegetable oil) investigated; best results obtained with ethanol

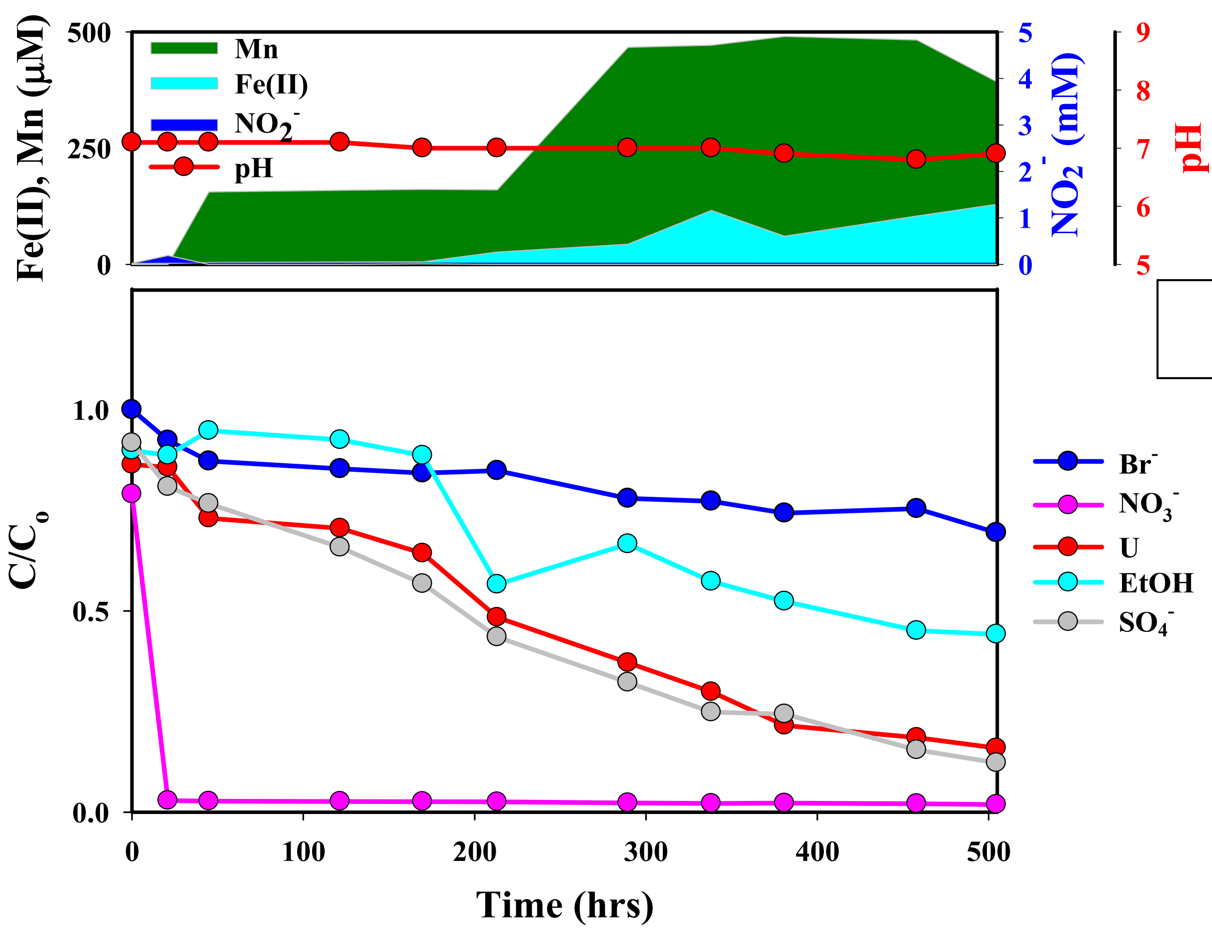
- Ethanol advantages…
- Inexpensive
- Stable
- Easy to deliver

Denitrification is main process responsible for observed nitrate loss



Example results from acetylene block tests

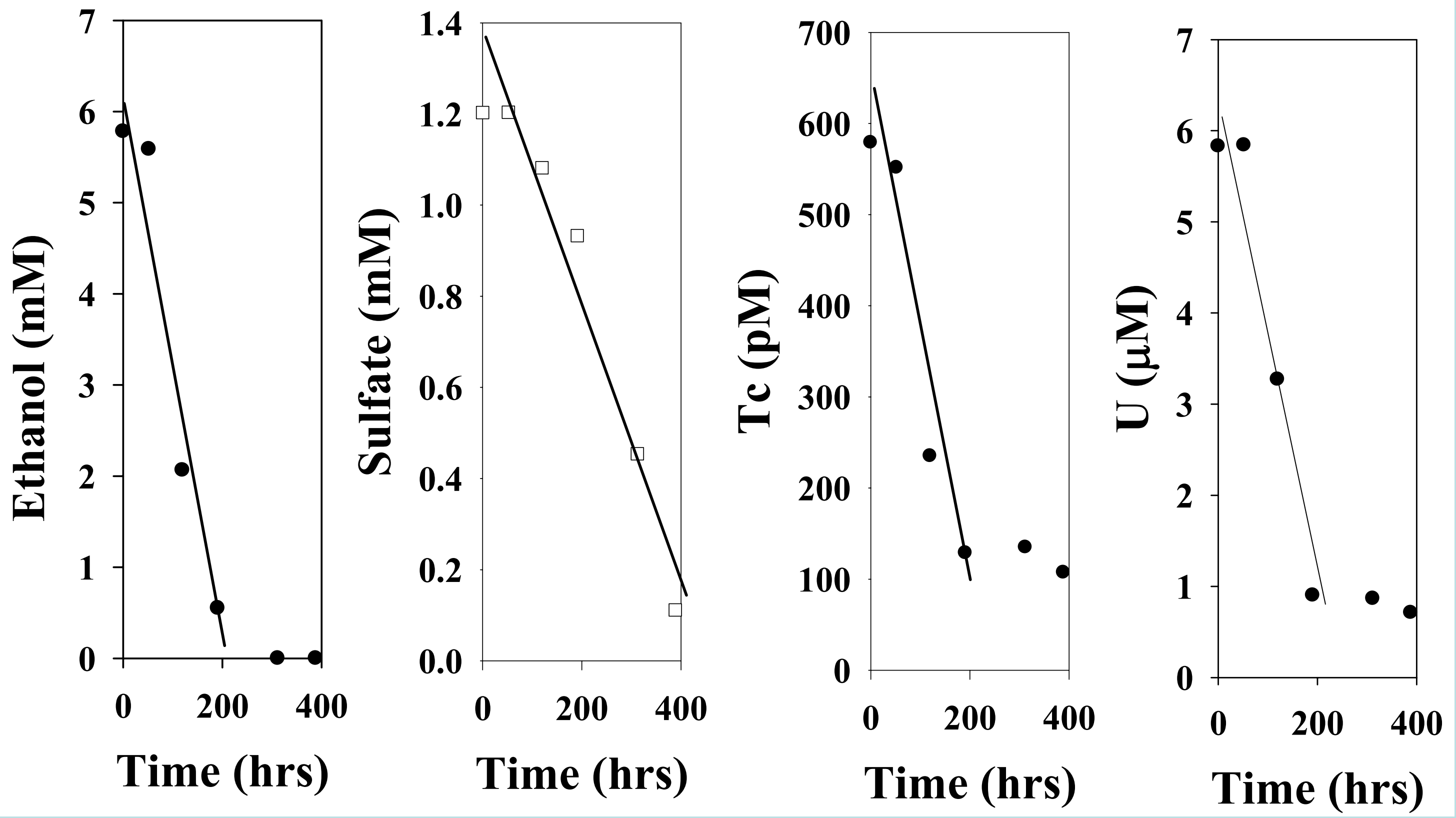
After biostimulation, microbial activity was similar in all environments tested including low initial pH



Initial pH ~ 3.8

In situ rates of microbial activity were determined for wide range of initial geochemical conditions

Example dilution adjusted concentration profiles



30 μM/hr 3 μM/hr 2.6 pM/hr 0.03 μM/hr

After biostimulation, rates of microbial activity were similar in all environments tested

In Situ Activity Measurements

Initial pH	EtOH (mM/hr)	NO_3^- (mM/hr)	SO_4^{2-} (mM/hr)	U(VI) (μM/hr)	U(IV) (μM/hr)	Tc(VII) (pM/hr)
3.3 – 3.9	0.3 – 1.0	0.1 – 0.4	0 – 0.01	$10^{-4} – 10^{-3}$	$10^{-3} – 10^{-2}$	4 – 30
5.2 – 5.6	0.3 – 4.0	0.3 – 4.0	0 – 0.01	$10^{-4} – 10^{-3}$	$10^{-3} – 10^{-2}$	10 – 150
5.6 – 7.2	0.1 – 2.0	0.1 – 2.0	0 – 0.03	$10^{-4} – 10^{-3}$	$10^{-3} – 10^{-2}$	4 - 10

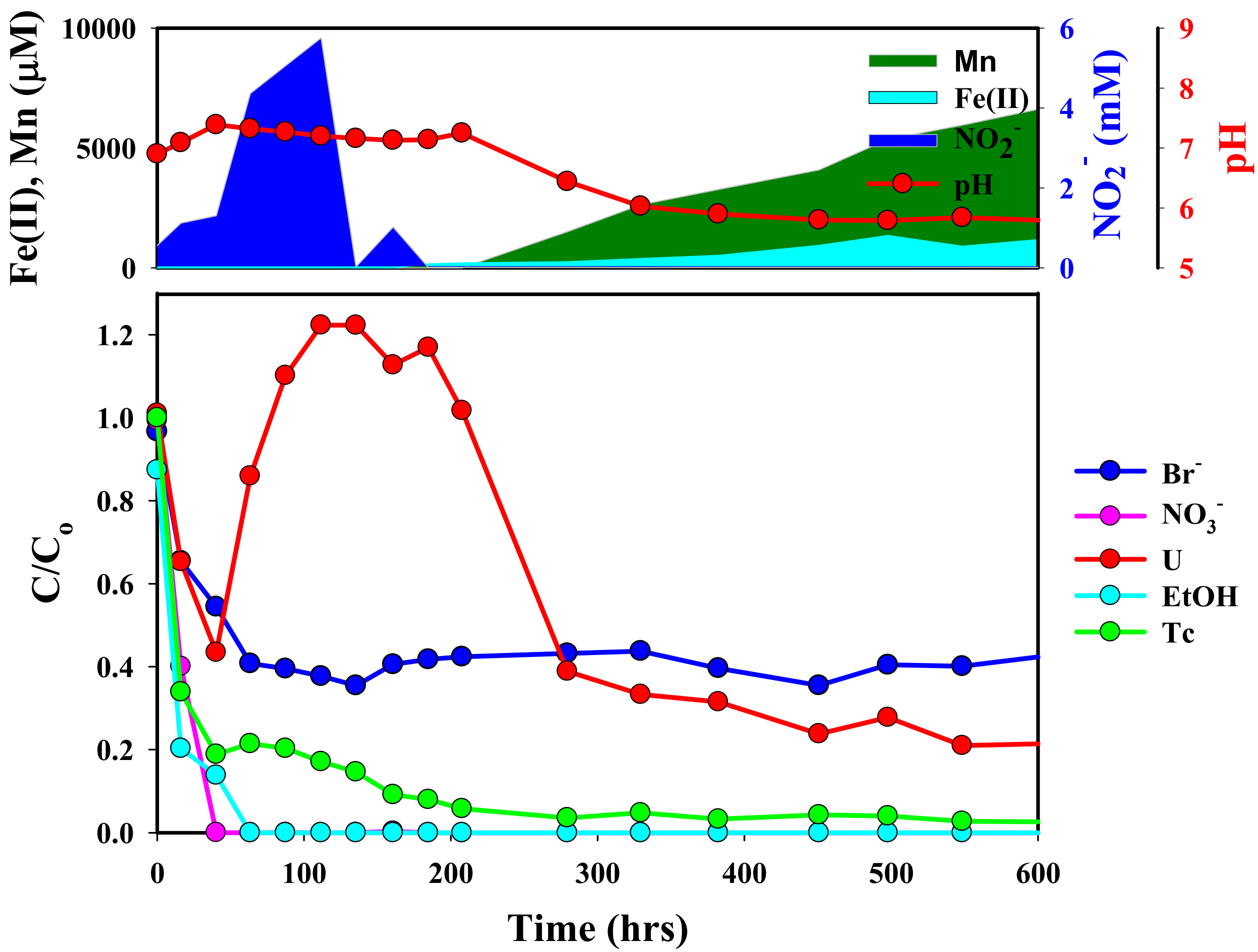
But *in situ* rates are very different from laboratory rates

U(VI) bioreduction — Tc(VII) bioreduction

Microcosm (uM/hr)	*In situ* (uM/hr)		Microcosm (pM/hr)	*In situ* (pM/hr)
135-690	0.001 - 0.04 (FRC) 0.001 - 0.002 (Rifle) 0.01 - 0.07 (Landfill) **10^5 – 10^6** **Smaller**		10,000 – 110,000	1 – 460 (FRC) **10^4 – 10^5** **Smaller**

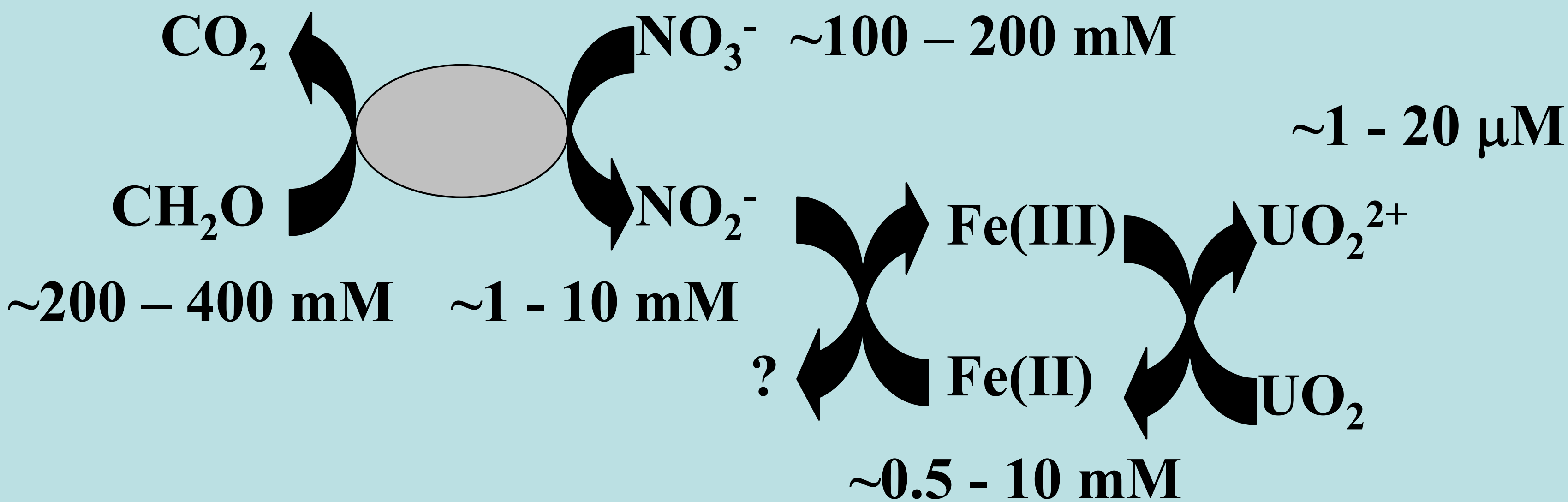
For more information see FRC Working Group Report "Rates and mechanisms of microbially mediated metal reduction"

Addition of nitrate to previously reduced sediments reoxidizes and remobilizes U but not Tc



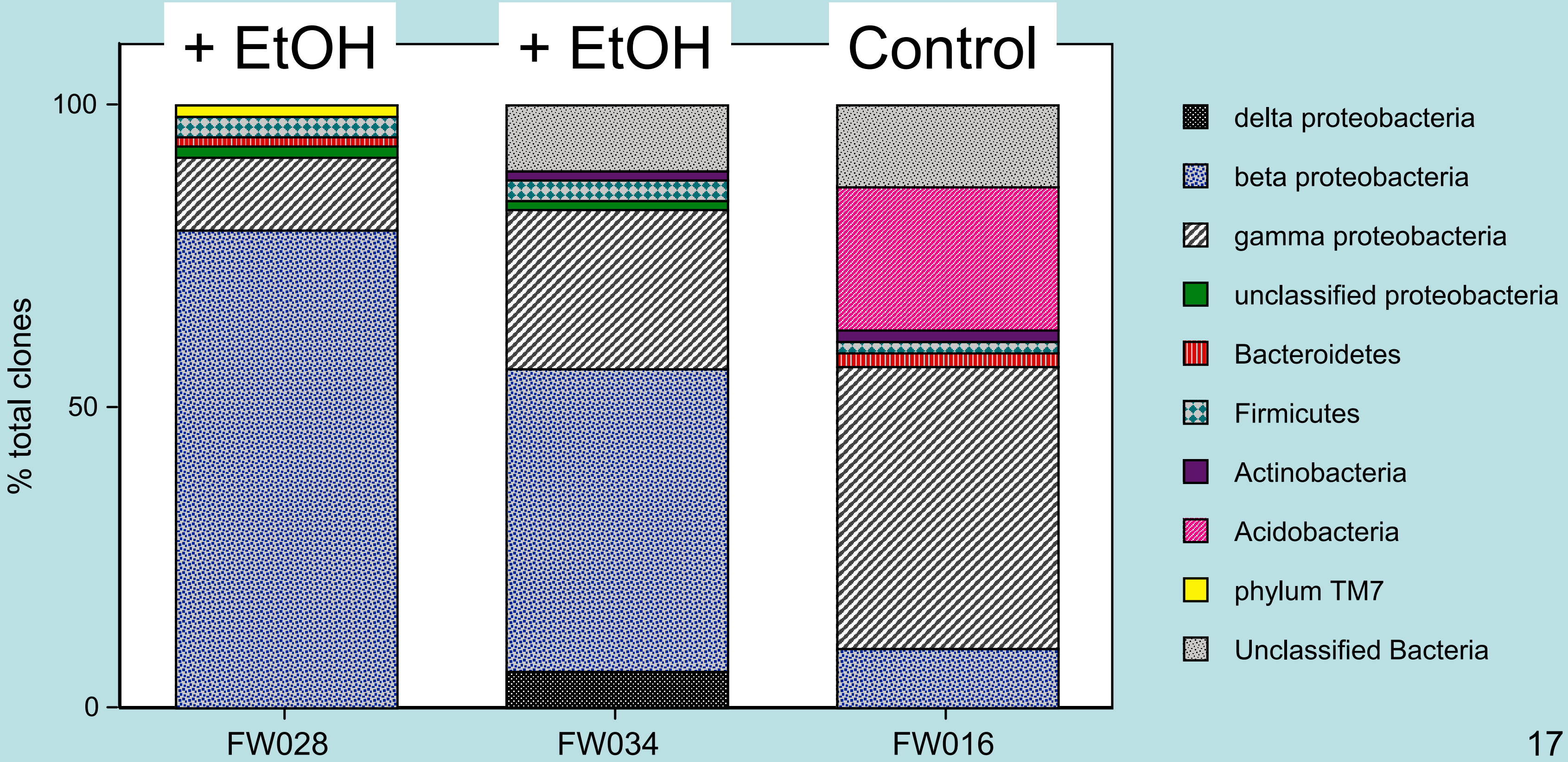
Addition of 100 mM NO_3^- to biostimulated sediments

Mechanisms of nitrate dependent microbial U(IV) oxidation investigated using microbial isolates and range of mineral systems



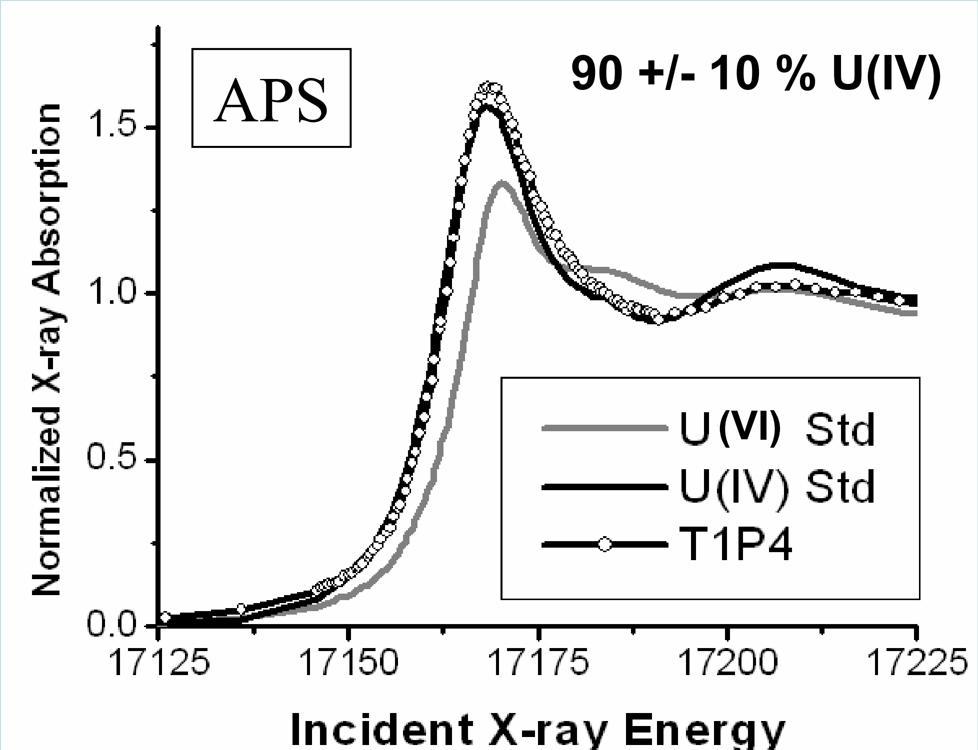
Ethanol additions stimulated the growth and activity of metal-reducing organisms

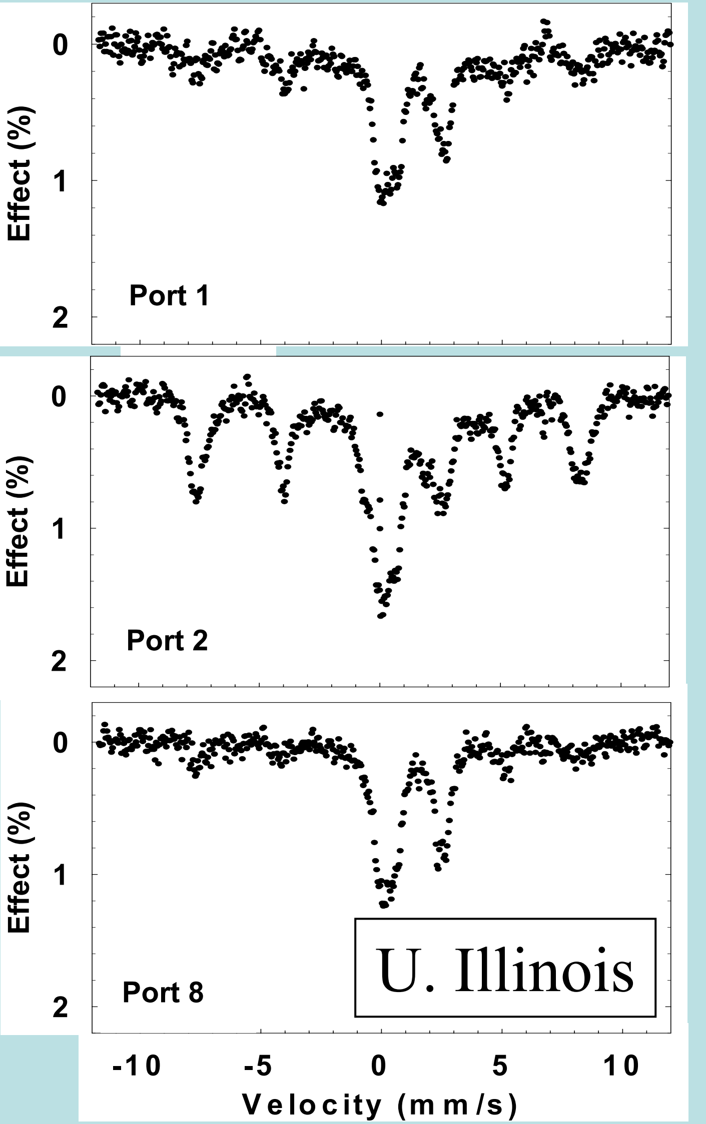
- **PLFA, DMA, DGGE, 16s rRNA; Q-PCR (groundwater, microbial samplers, sediments)**



Metal and radionuclide reduction supported by multiple lines of evidence

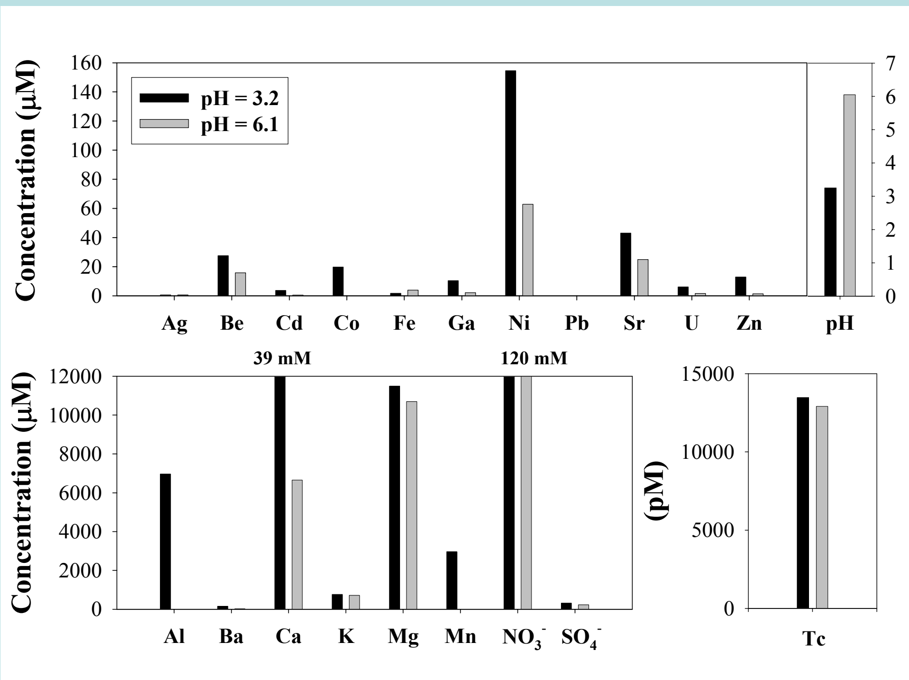
- Fe(II) and Mn(II) accumulation in groundwater
- Fe(II) and U(IV) accumulation in sediments





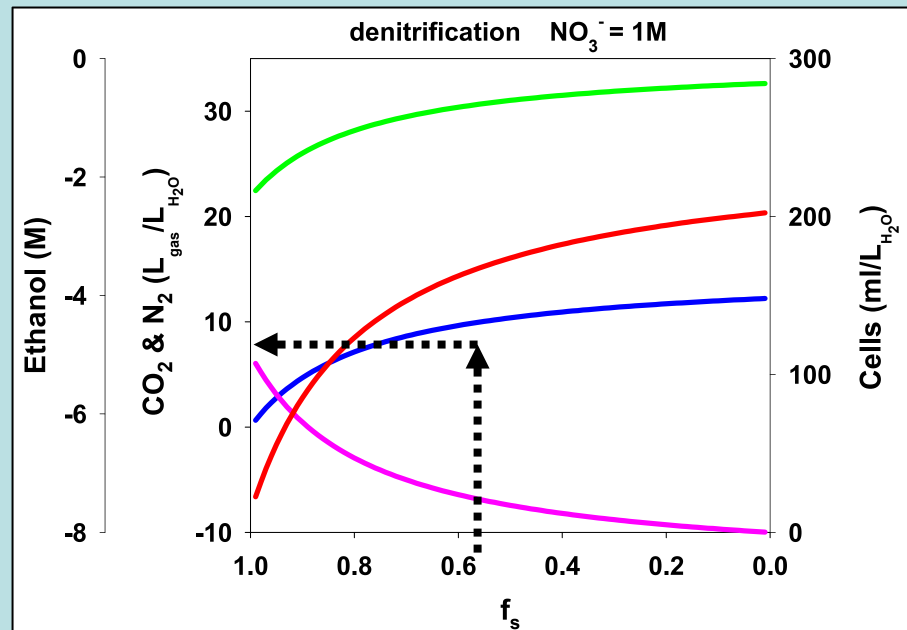
Enhanced microbial activity results in production of precipitates, biomass, and gas production

Titration of low pH groundwater



~ 2 g/L precipitates

Denitrification stoichiometry



200 mM NO_3^- > ~ 4.5 L N_2

Coupled microbiological and geochemical models describe many features of field experiments

- Results from NABIR project *"Stability of U(VI) and Tc(VII) Reducing Microbial Communities to Environmental Perturbation: Development and Testing of a Thermodynamic Network Model"*
- Growth equations and free energy values for defined microbial groups are computed and combined with existing chemical thermodynamic data bases
- Response of system to donor additions modeled with equilibrium reaction paths computed by minimizing overall system (microbiology and geochemistry) free energy (see poster for details)

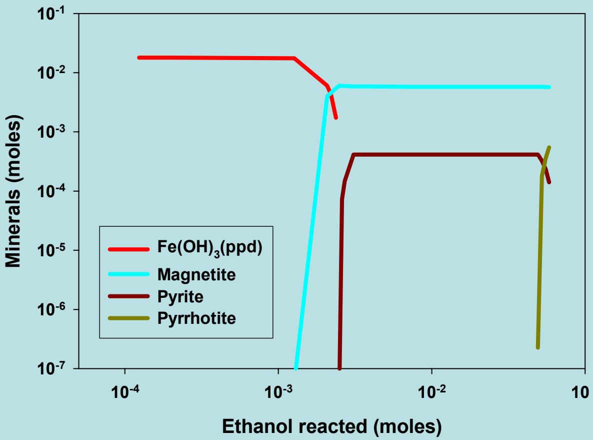
Area 2 Example - Bioreduction

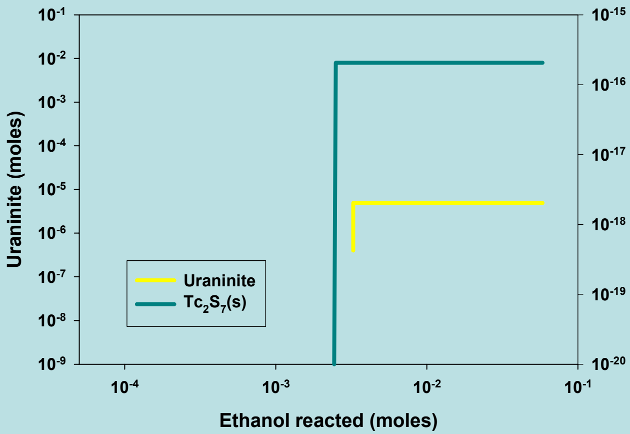
Geochemistry

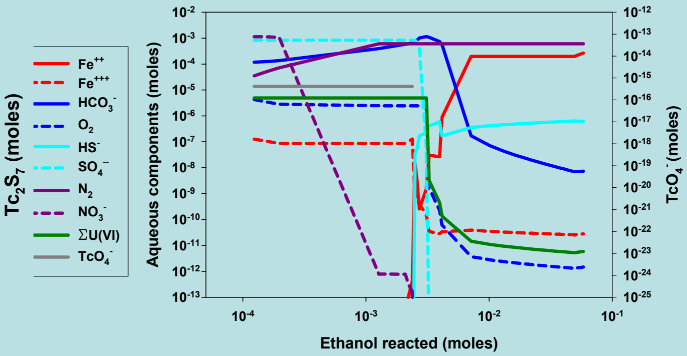
pH	6.4	
O_2	68	mM
NO_3^-	1.2	mM
SO_4^{2-}	0.83	mM
Fe(III)	17	mM
HCO_3^-	90	μM
Ca^{2+}	3.5	mM
Mg^{2+}	1.1	mM
K^+	0.12	mM
Al^{3+}	0.06	mM
Na^+	1.1	mM
Cl^-	0.65	mM
U	4.9	μM
Tc	411	pM

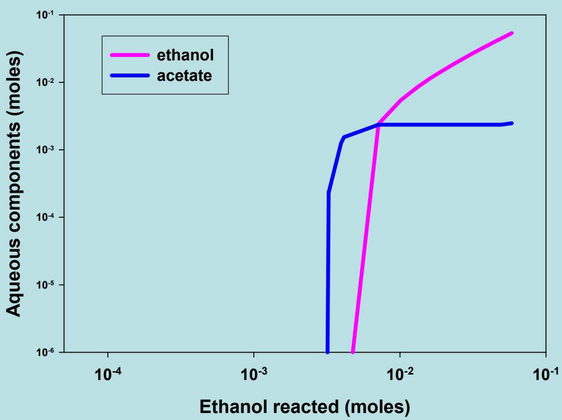
Microbiology

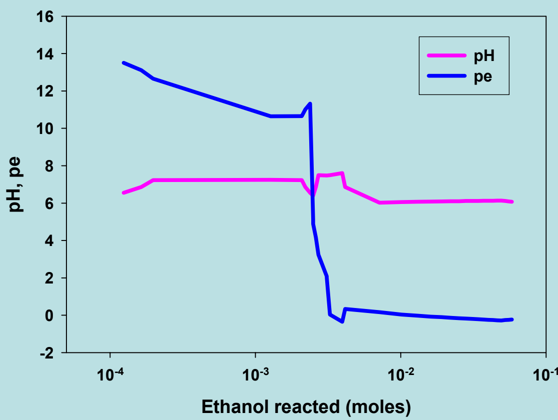
- NH_4^+ as nitrogen source
- Ethanol as electron donor
- Defined functional groups
  - "Aerobes"
  - "Denitrifers"
  - "Iron reducers"
  - "Sulfate reducers"
  - "Fermenters" (oxidize ethanol to acetate)
- Compute equilibrium reaction path with 60 mM ethanol

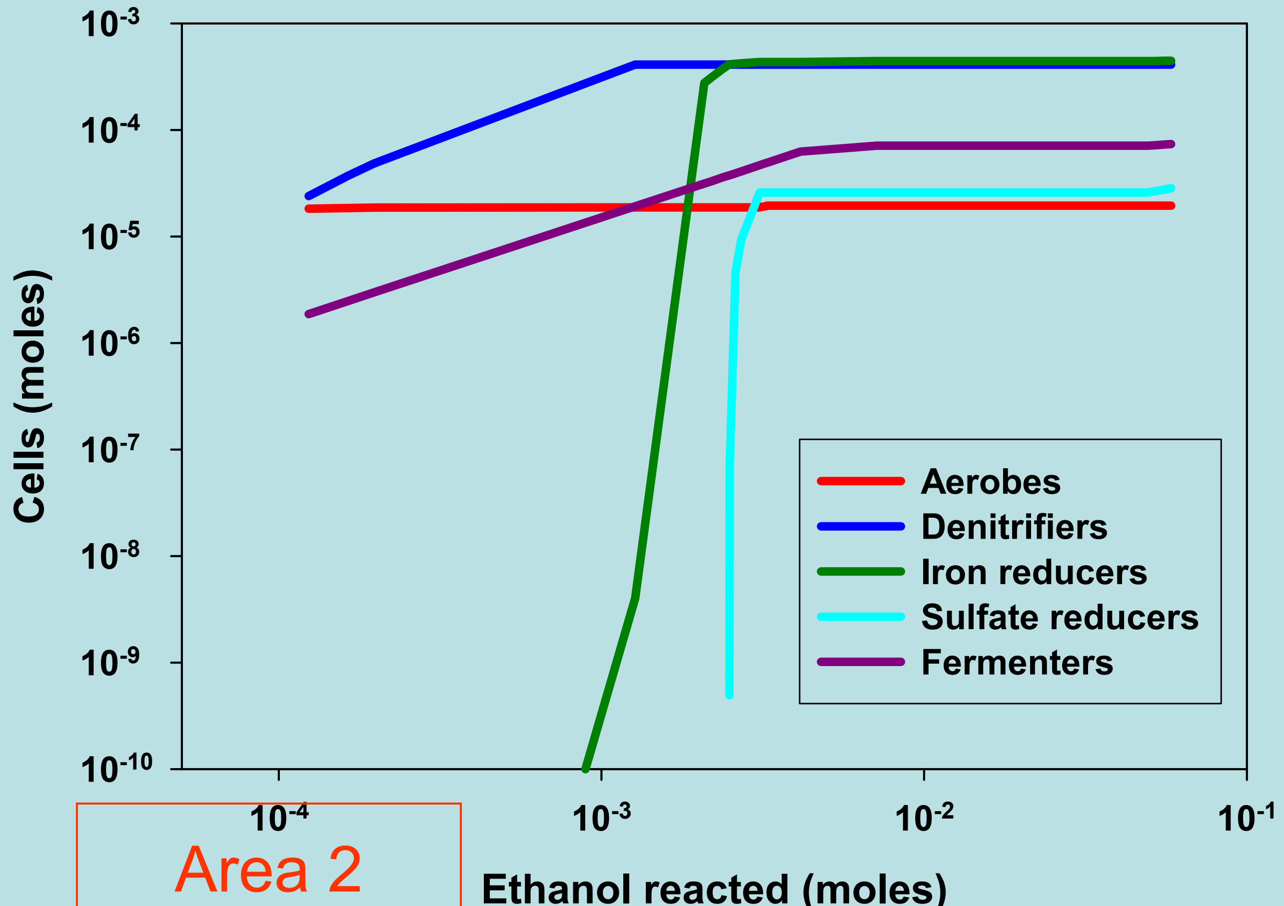

Minerals (moles)
Ethanol reacted (moles)
Fe(OH)3(ppd)
Magnetite
Pyrite
Pyrrhotite


Uraninite (moles)
Tc2S7 (moles)
Ethanol reacted (moles)
Uraninite
Tc2S7(s)


Aqueous components (moles)
TcO4- (moles)
Ethanol reacted (moles)
Fe++
Fe+++
HCO3-
O2
HS-
SO4--
N2
NO3-
ΣU(VI)
TcO4-


Aqueous components (moles)
Ethanol reacted (moles)
ethanol
acetate


pH, pe
Ethanol reacted (moles)
pH
pe


Cells (moles)
Ethanol reacted (moles)
Aerobes
Denitrifiers
Iron reducers
Sulfate reducers
Fermenters

Area 2

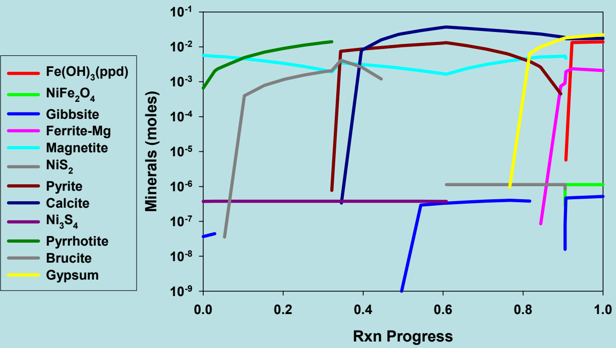
Predicted cell growth and minerals formed

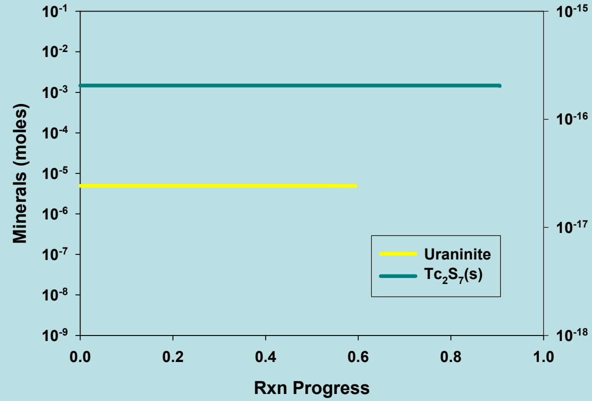
	(moles)	
Iron Reducers	4.5×10^{-4} *(46%)*	
Denitrifiers	4.1×10^{-4} *(42%)*	
Fermenters	7.4×10^{-5} *(7%)*	
Sulfate Reducers	2.7×10^{-5} *(3%)*	
Aerobes	2.0×10^{-5} *(2%)*	
Magnetite	5.9×10^{-3}	
Calcite	5.2×10^{-4}	
Pyrite	4.1×10^{-4}	
Uraninite	4.9×10^{-6}	
Tc_2S_7	9.7×10^{-8}	

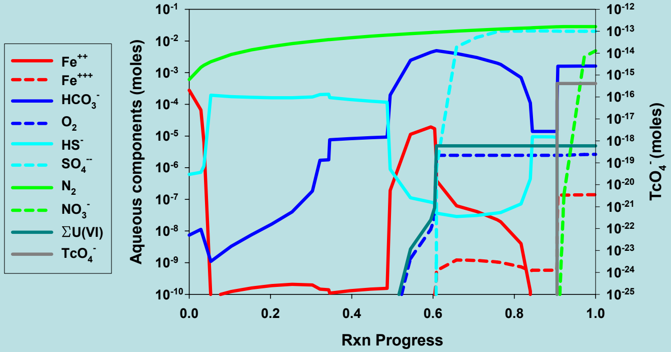
Area 2 Example - Reoxidation

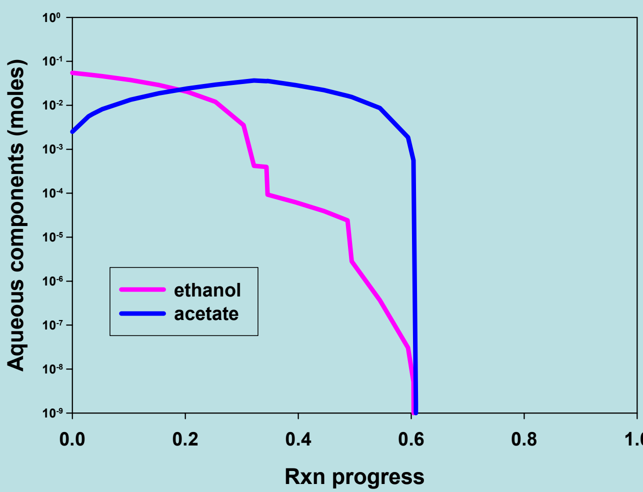
- **Reoxidation of previously reduced system by 50 volumes of original groundwater**

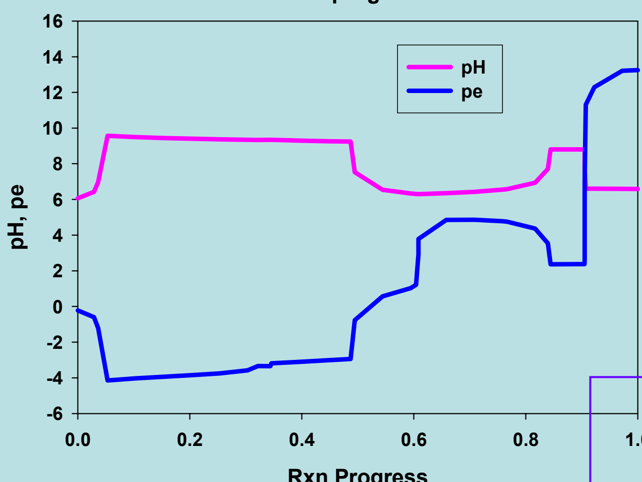
Reacting masses		
O_2	3.3	mg
NO_3^-	61	mM
SO_4^{2-}	42	mM
Ca^{2+}	176	mM
HCO_3^-	4.5	mM
Na^+	54	mM
Mg^{2+}	55	mM
K^+	6.2	mM
U	0.25	mM
Tc	21	nM

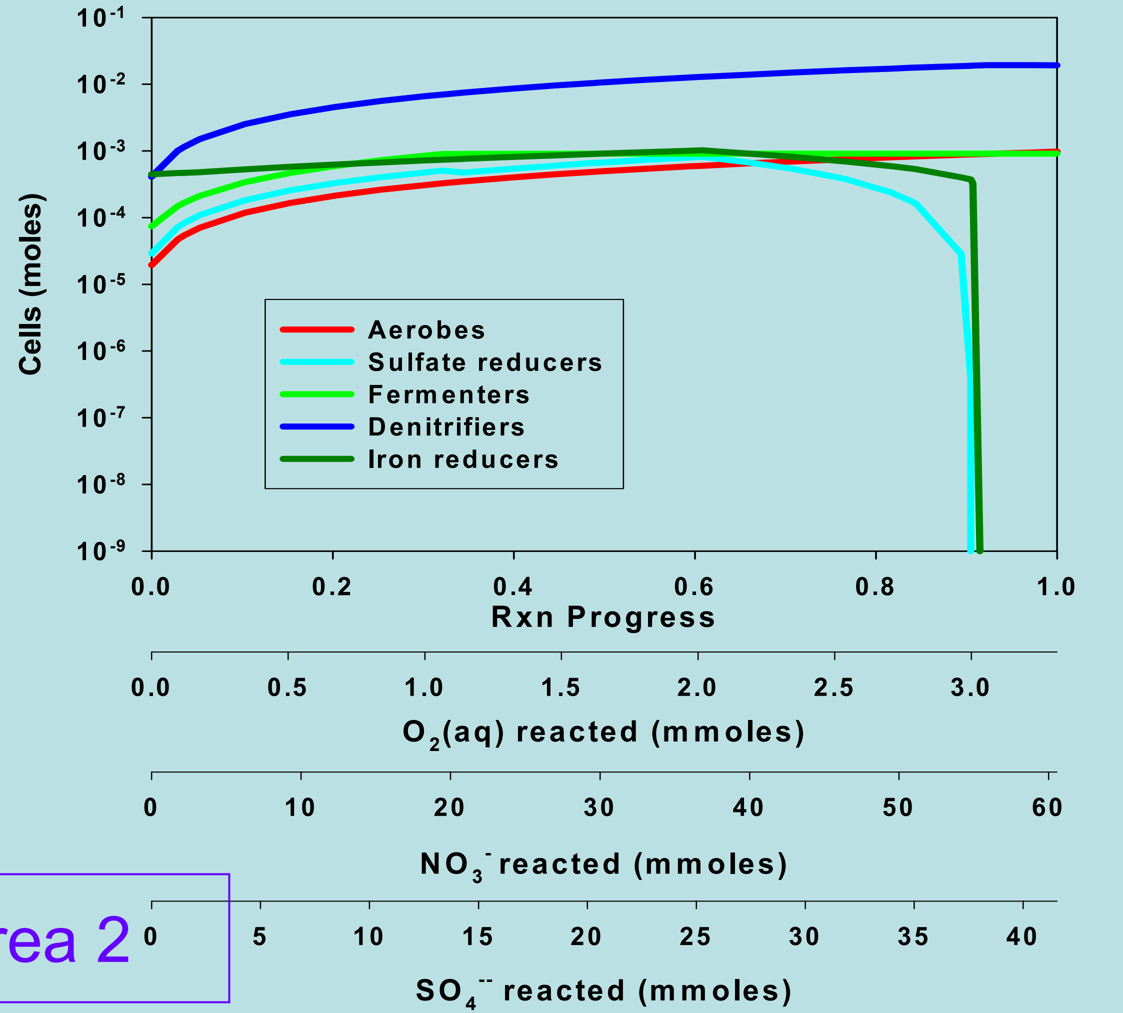

Fe(OH)3(ppd)
NiFe2O4
Gibbsite
Ferrite-Mg
Magnetite
NiS2
Pyrite
Calcite
Ni3S4
Pyrrhotite
Brucite
Gypsum
Minerals (moles)
Rxn Progress


Minerals (moles)
Tc2S7 formed (moles)
Uraninite
Tc2S7(s)
Rxn Progress


Fe++
Fe+++
HCO3-
O2
HS-
SO4--
N2
NO3-
ΣU(VI)
TcO4-
Aqueous components (moles)
TcO4- (moles)
Rxn Progress


Aqueous components (moles)
ethanol
acetate
Rxn progress


pH, pe
pH
pe
Rxn Progress


Cells (moles)
Aerobes
Sulfate reducers
Fermenters
Denitrifiers
Iron reducers
Rxn Progress
O2(aq) reacted (mmoles)
NO3- reacted (mmoles)
SO4-- reacted (mmoles)

Area 2

Predicted changes in microbial community composition after reoxidation (Area 2)

	Bioreduction (moles)	Reoxidation (moles)
Iron Reducers	4.5×10^{-4} *(45%)*	~ 0
Denitrifiers	4.1×10^{-4} *(42%)*	0.019 *(91%)*
Fermenters	7.4×10^{-5} *(7%)*	9.0×10^{-4} *(4%)*
Sulfate Reducers	2.7×10^{-5} *(3%)*	~ 0
Aerobes	2.0×10^{-5} *(2%)*	9.7×10^{-4} *(5%)*

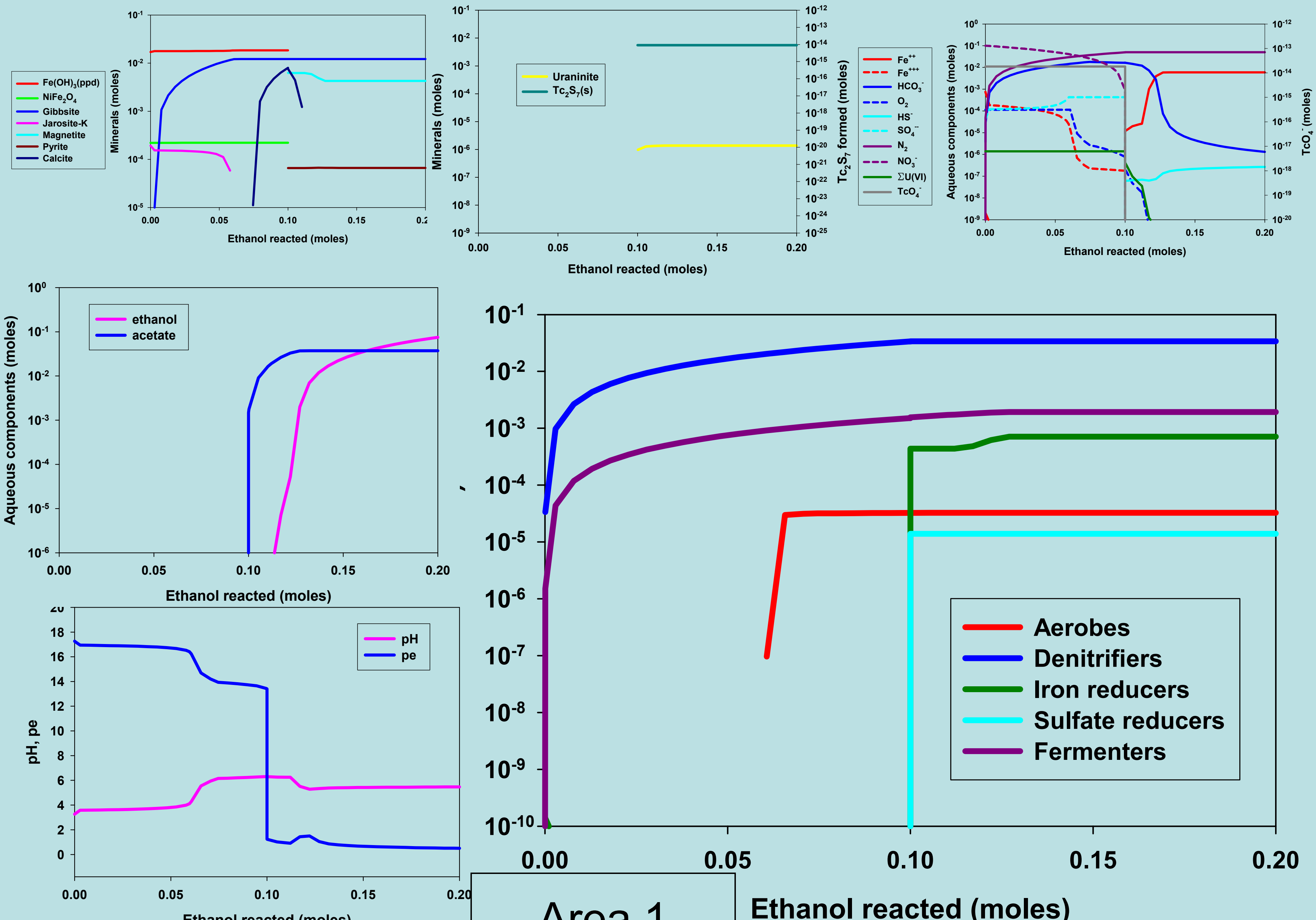
Area 1 Example - Bioreduction

Geochemistry

pH	3.3	
O_2	112	μM
NO_3^-	100	mM
SO_4^{2-}	0.43	mM
Fe(III)	18	mM
HCO_3^-	100	μM
Ca^{2+}	19	mM
Mg^{2+}	8.4	mM
K^+	1.0	mM
Al^{3+}	12	mM
Na^+	23	mM
Cl^-	7.9	mM
U	1.4	μM
Tc	22	nM

Microbiology

- NH_4^+ as nitrogen source
- Ethanol as electron donor
- Microbial groups
  - "Aerobes"
  - "Denitrifers"
  - "Iron reducers"
  - "Sulfate reducers"
  - "Fermenters" (oxidize ethanol to acetate)
- Compute equilibrium reaction path with 60 mM ethanol


Fe(OH)3(ppd)
NiFe2O4
Gibbsite
Jarosite-K
Magnetite
Pyrite
Calcite
Minerals (moles)
Ethanol reacted (moles)
Uraninite
Tc2S7(s)
Minerals (moles)
Tc2S7 formed (moles)
Ethanol reacted (moles)
Fe++
Fe+++
HCO3-
O2
HS-
SO4--
N2
NO3-
ΣU(VI)
TcO4-
Aqueous components (moles)
TcO4- (moles)
Ethanol reacted (moles)
ethanol
acetate
Aqueous components (moles)
Ethanol reacted (moles)
pH
pe
pH, pe
Ethanol reacted (moles)
Aerobes
Denitrifiers
Iron reducers
Sulfate reducers
Fermenters
Ethanol reacted (moles)

Area 1

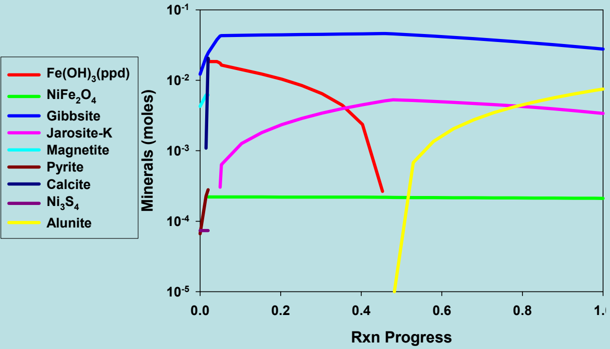
Predicted cell growth and minerals formed

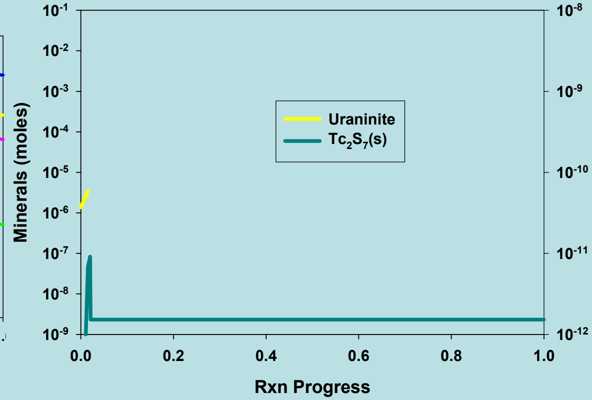
	(moles)
Iron Reducers	7.1×10^{-4} *(2%)*
Denitrifiers	3.4×10^{-2} *(93%)*
Fermenters	1.9×10^{-3} *(5%)*
Aerobes	3.4×10^{-5} *(.1%)*
Sulfate Reducers	1.4×10^{-5} *(.04%)*
Gibbsite	1.2×10^{-2}
Magnetite	4.3×10^{-3}
Pyrite	6.6×10^{-5}
Ni_3S_4	7.3×10^{-5}
Uraninite	1.4×10^{-6}
Tc_2S_7	9.1×10^{-8}

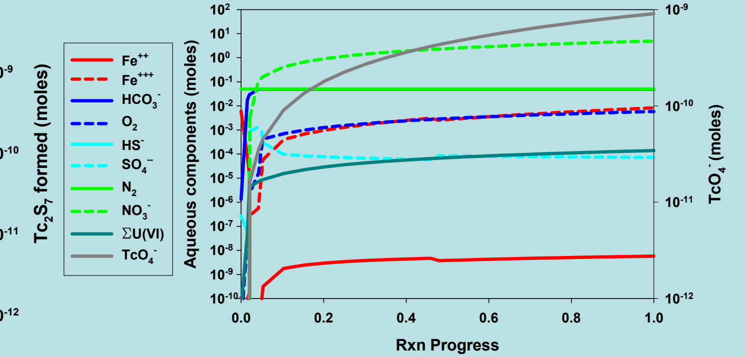
Area 1 Example - Reoxidation

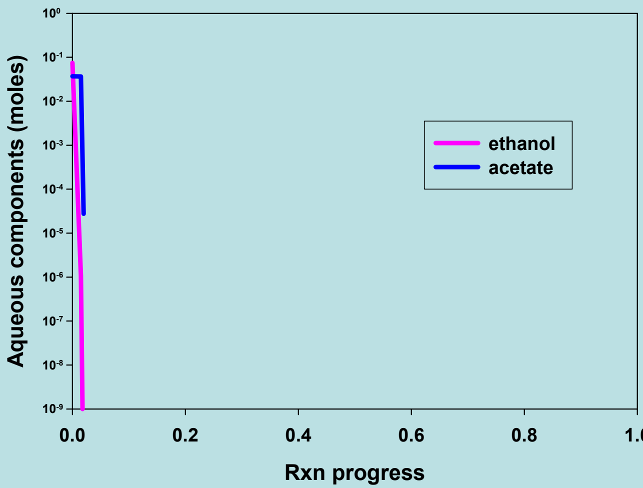
- **Reoxidation of previously reduced system by 50 volumes of original groundwater**

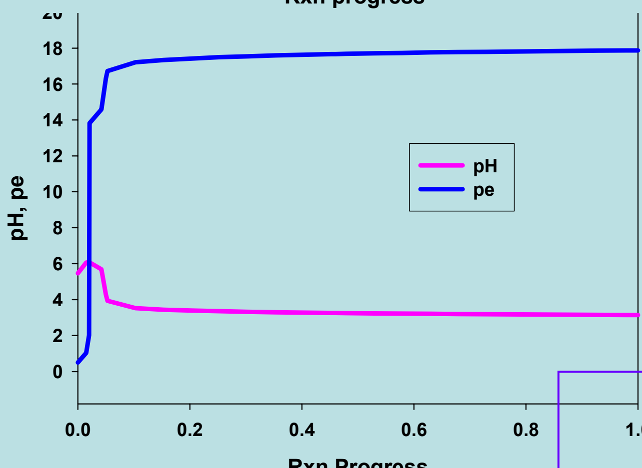
Reacting masses		
NO_3^-	5000	mM
SO_4^{2-}	21	mM
O_2	5.6	mM
Na^+	1136	mM
Ca^{2+}	926	mM
Al^{3+}	1107	mM
Mg^{++}	416	mM
K^+	49	mM
HCO_3^-	0.1	mM
U	0.07	mM
Tc	1.1	μM

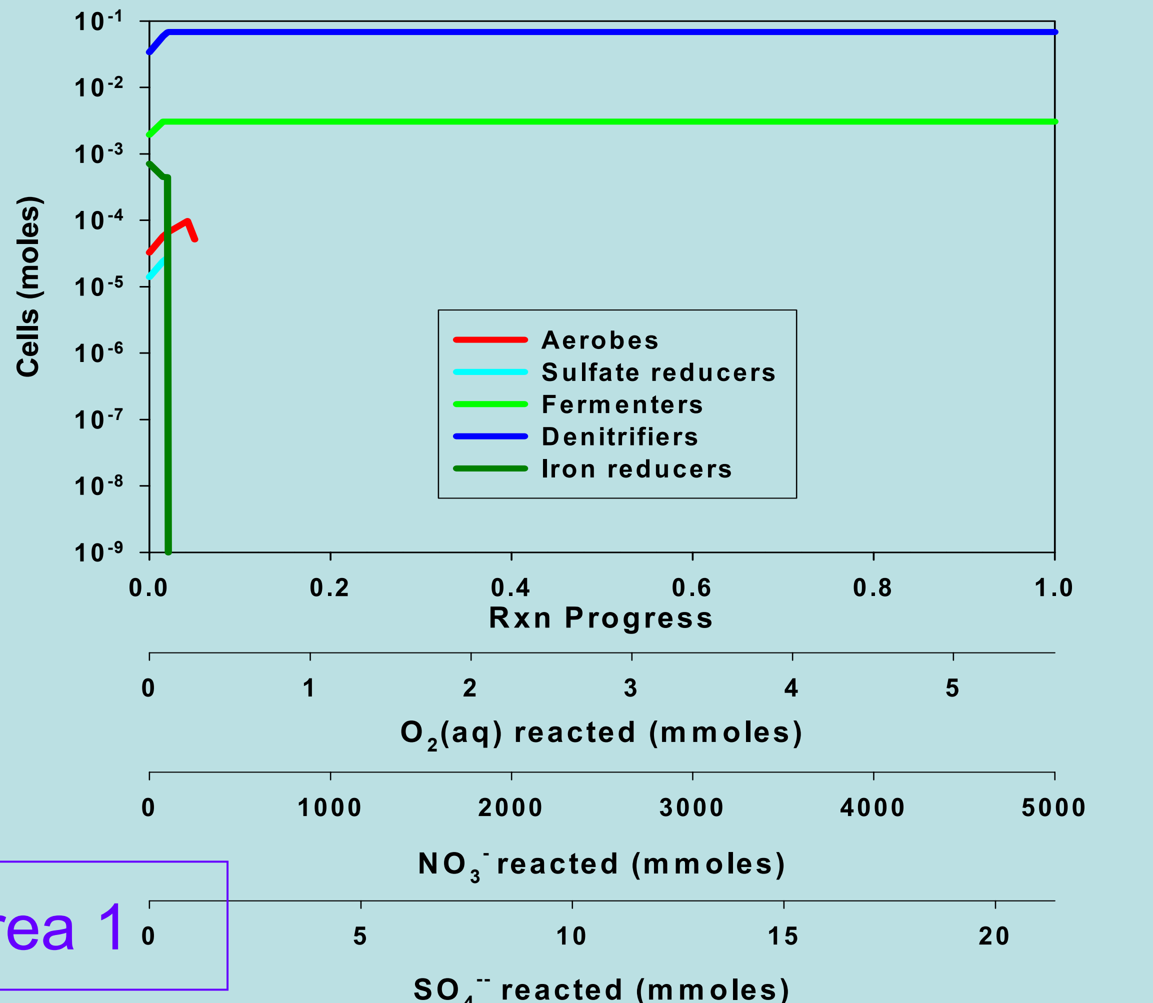

Fe(OH)3(ppd)
NiFe2O4
Gibbsite
Jarosite-K
Magnetite
Pyrite
Calcite
Ni3S4
Alunite
Minerals (moles)
Rxn Progress


Uraninite
Tc2S7(s)
Minerals (moles)
Tc2S7 formed (moles)
Rxn Progress


Fe++
Fe+++
HCO3-
O2
HS-
SO4--
N2
NO3-
ΣU(VI)
TcO4-
Aqueous components (moles)
TcO4- (moles)
Rxn Progress


ethanol
acetate
Aqueous components (moles)
Rxn progress

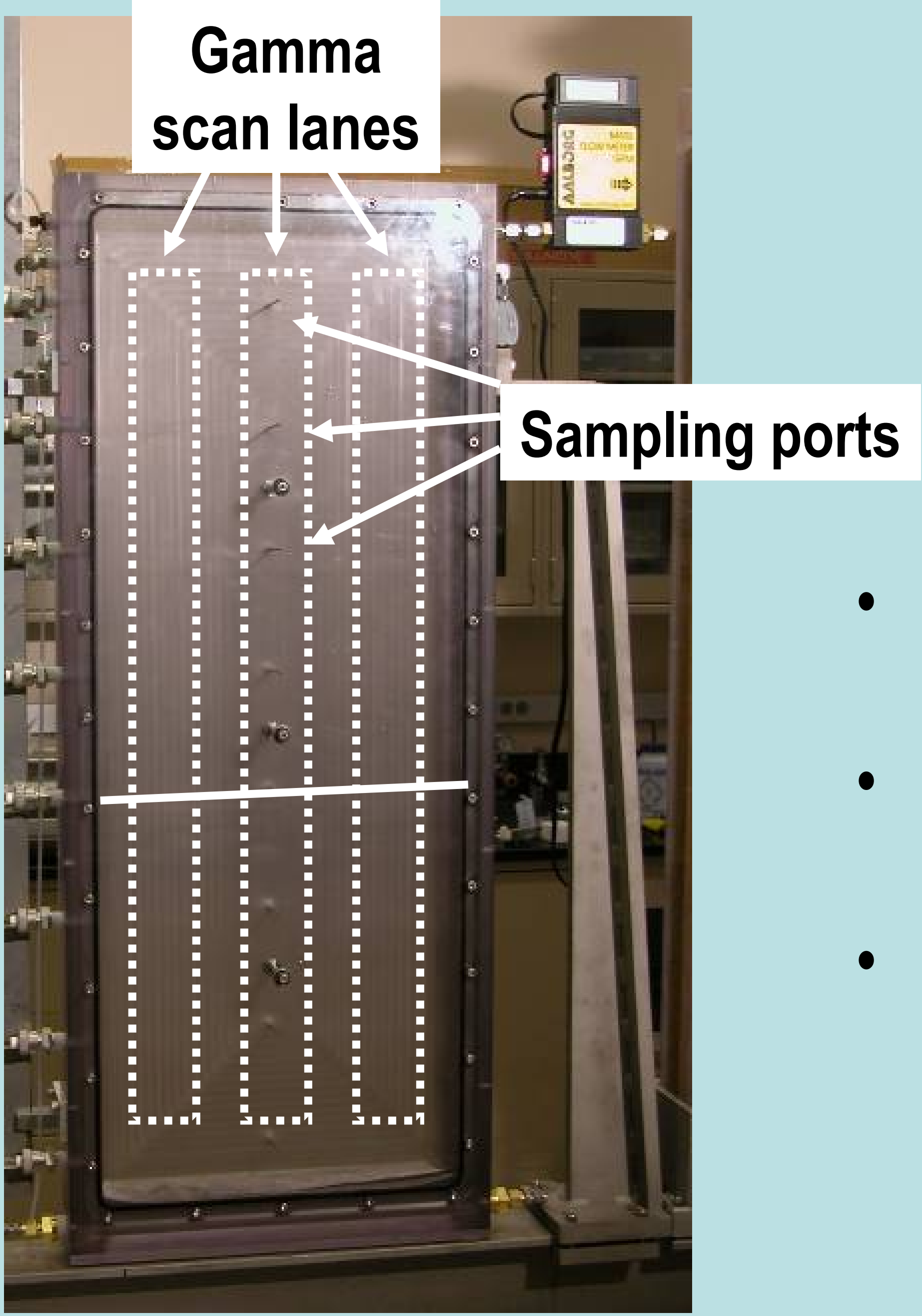

pH
pe
pH, pe
Rxn Progress


Aerobes
Sulfate reducers
Fermenters
Denitrifiers
Iron reducers
Cells (moles)
Rxn Progress
O2(aq) reacted (mmoles)
NO3- reacted (mmoles)
SO4-- reacted (mmoles)

Area 1

Predicted changes in microbial community composition after reoxidation (Area 1)

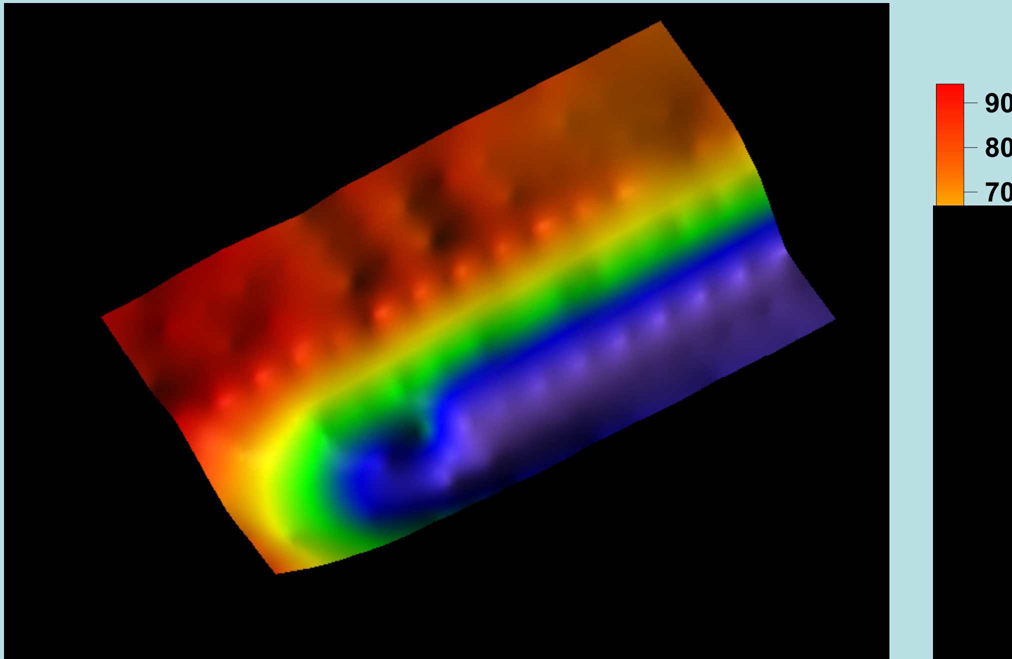
	Bioreduction (moles)	Reoxidation (moles)
Iron Reducers	7.1×10^{-4} *(2%)*	5.7×10^{-10} *(0%)*
Denitrifiers	3.4×10^{-2} *(93%)*	6.8×10^{-2} *(96%)*
Sulfate Reducers	1.4×10^{-5} *(.04%)*	-
Aerobes	3.4×10^{-5} *(.1%)*	-
Fermenters	1.9×10^{-3} *(5%)*	3.1×10^{-3} *(4%)*

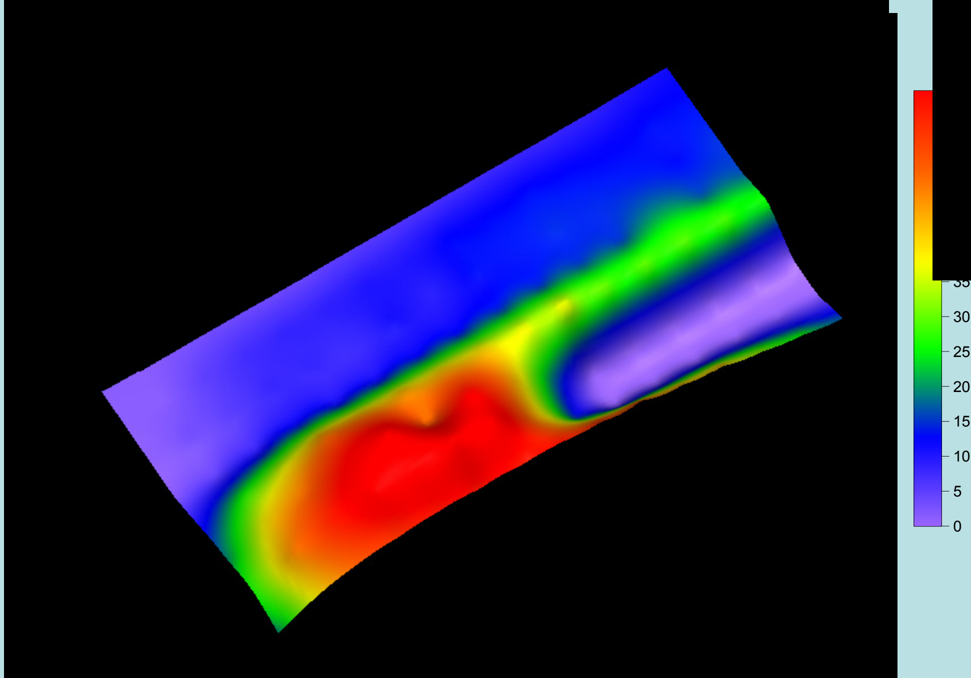


Fate of N_2 gas produced by denitrification

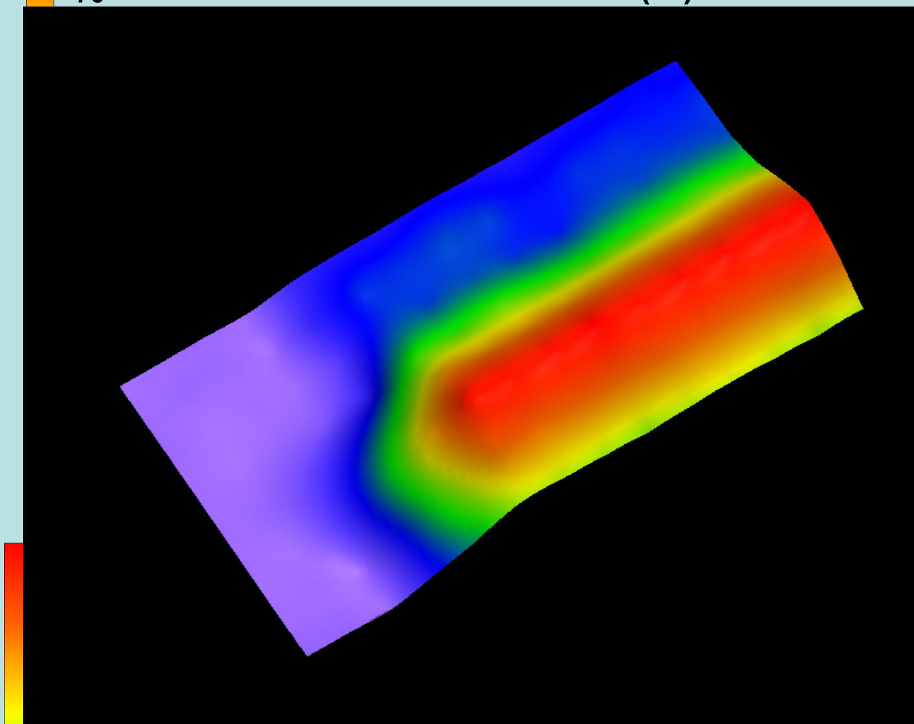
- FRC Background Sediment and Maynardsville Limestone
- Denitrifying activity stimulated with ethanol
- Gas and liquid saturations monitored to track fate of N_2 gas

Nitrate (mM)



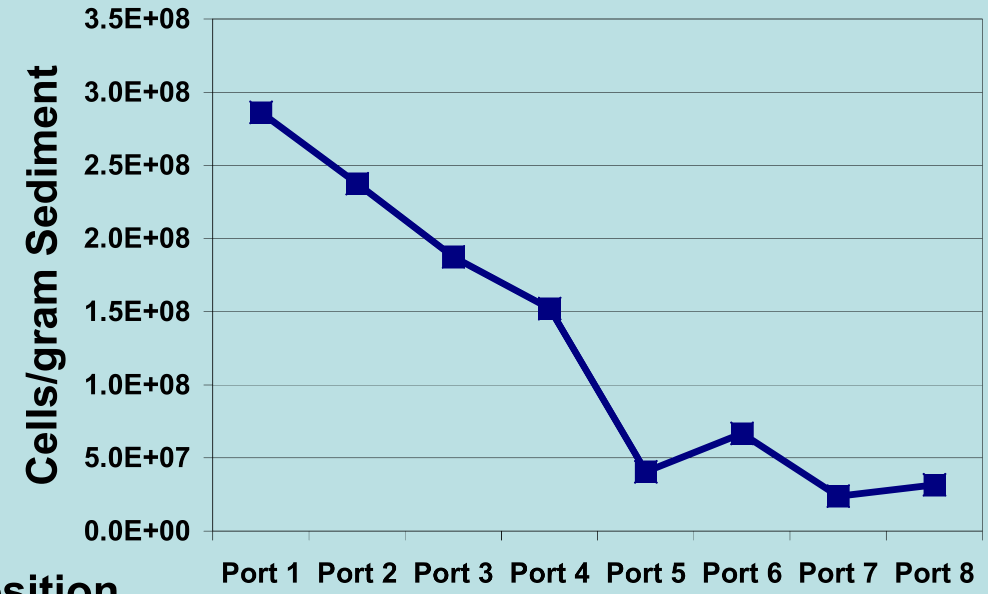


Gas Saturation (%)

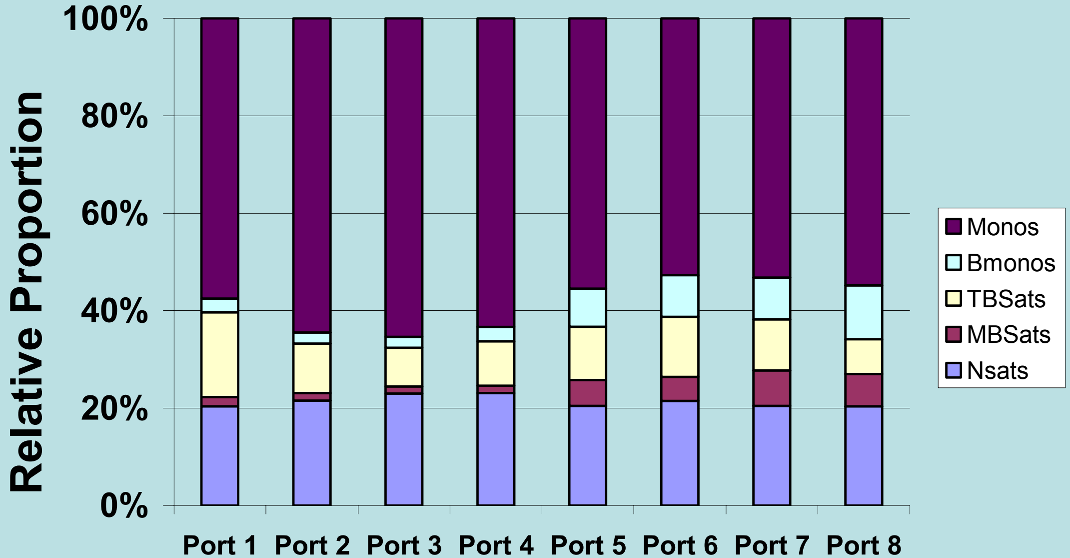


Measured Gas Saturations

Biomass Estimate PLFA

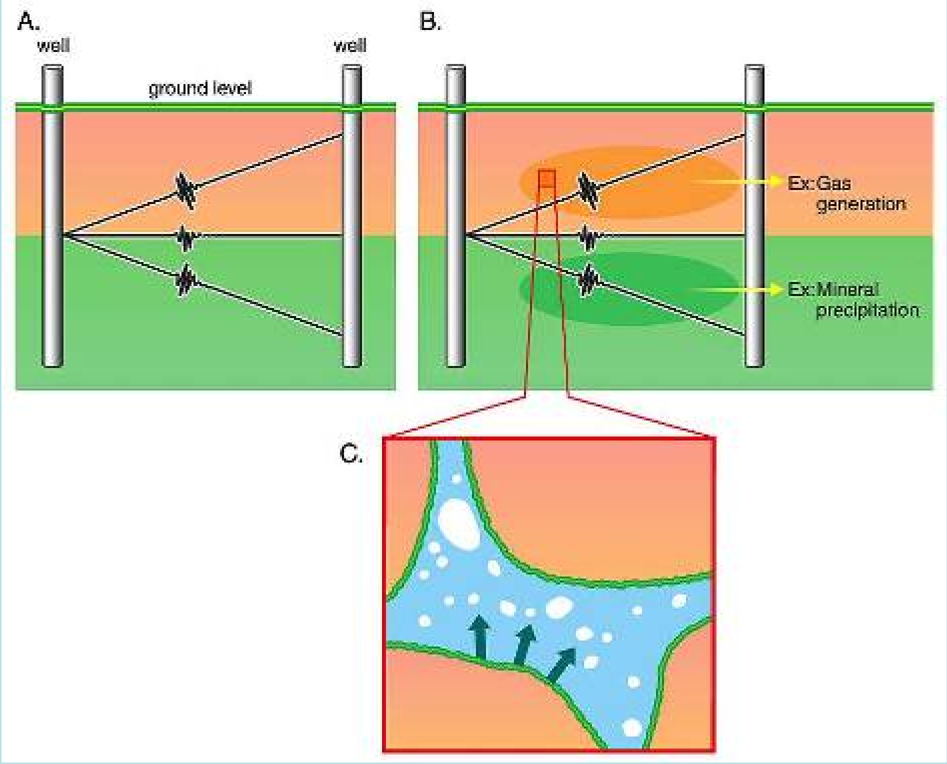


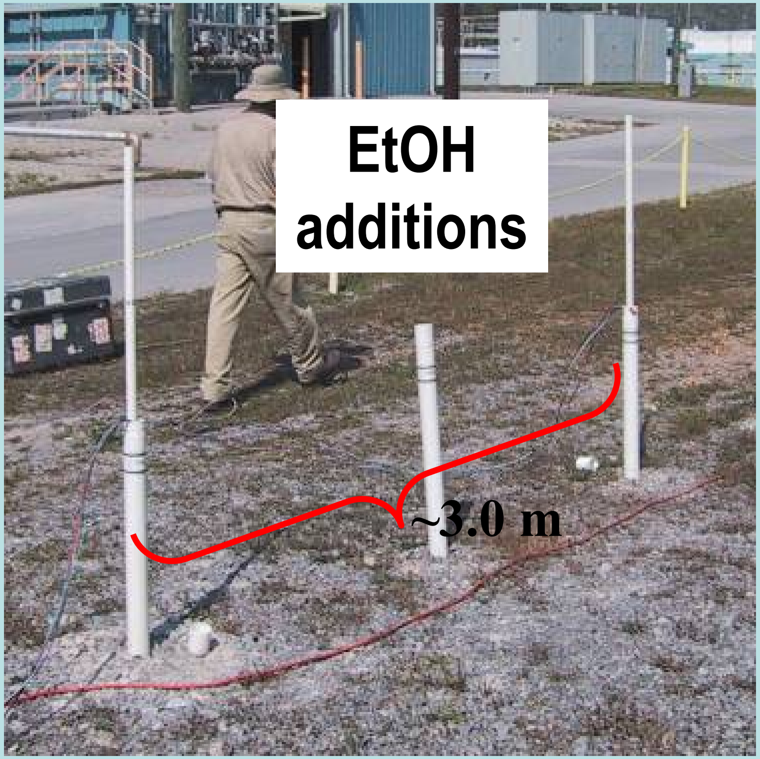
PLFA Community Composition



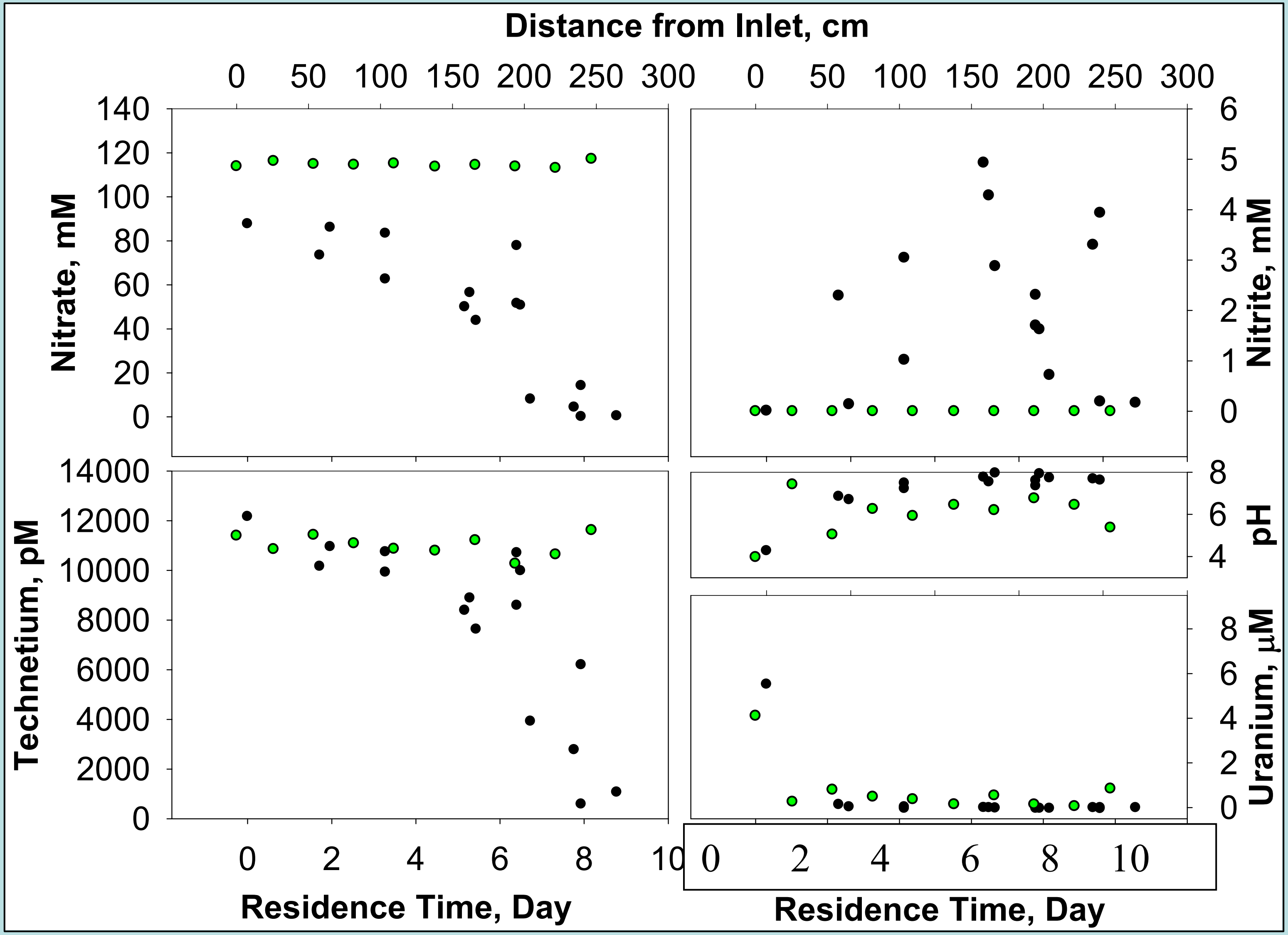
Range of methods being used in attempt to detect precipitate and gas formation during biostimulation

- See poster by Ken Williams and Susan Hubbard

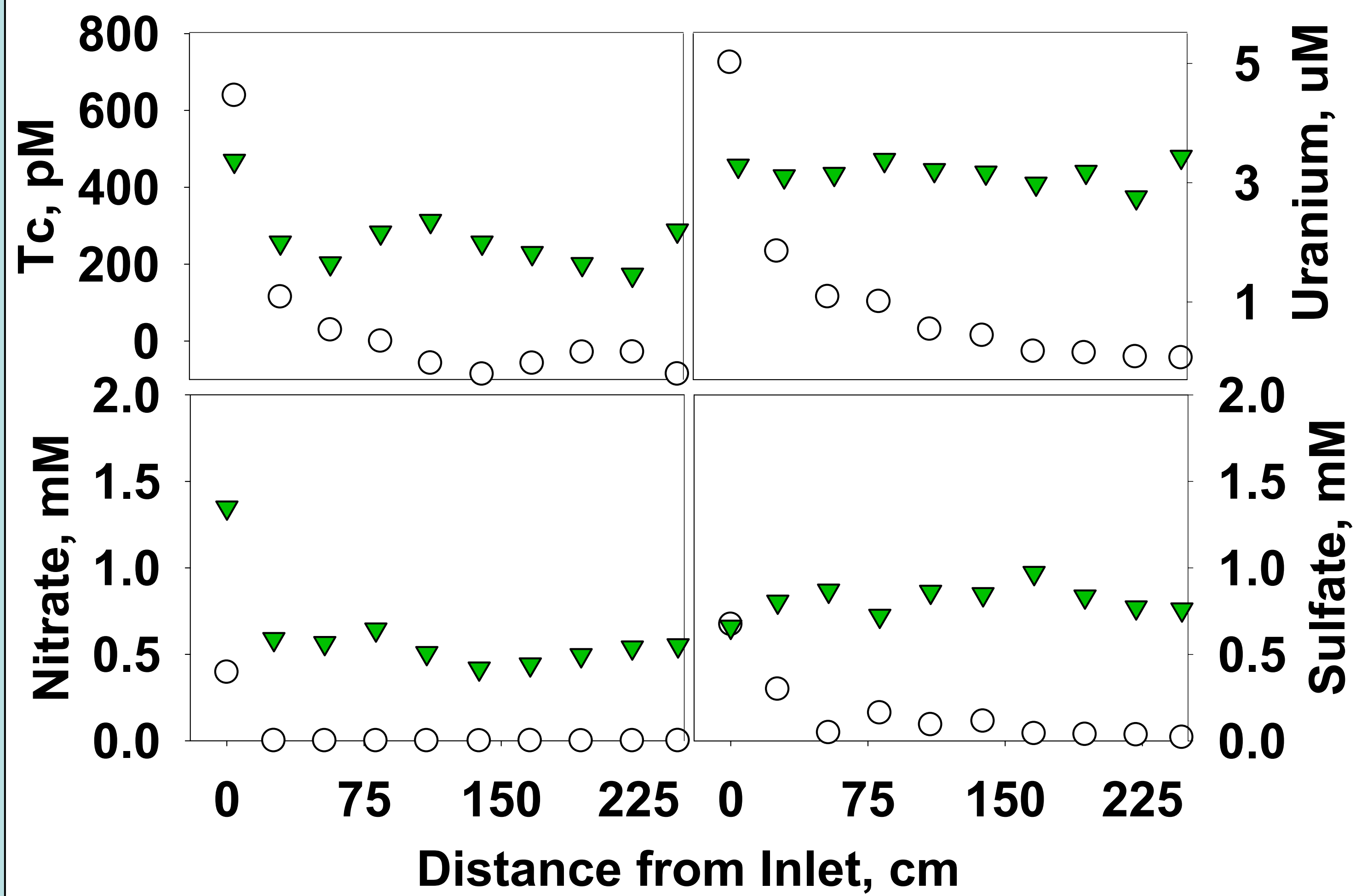




Status of intermediate-scale physical models: bioreduction in Area 1 (Mandy Michalsen)



Status of intermediate-scale physical models: bioreduction in Area 2



Status of intermediate-scale physical models: reoxidation in Area 2