Bio-immobilization of Radionuclides:

Results of Field Experiments and Biogeochemical Modeling

Dr. Jonathan ("Jack") Istok
Oregon State University
541-737-8547
Jack.Istok@orst.edu

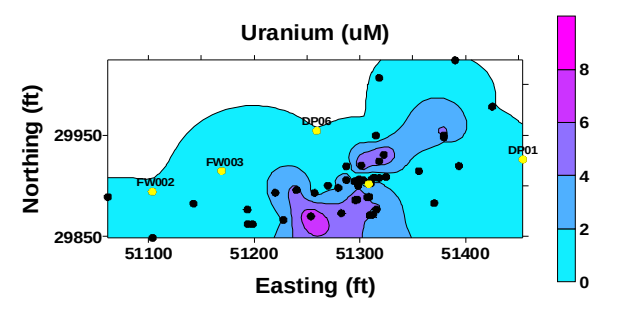
Oregon State University OSU
Pacific Northwest National Laboratory
Operated by Battelle for the U.S. Department of Energy
ornl Oak Ridge National Laboratory
William R. Wiley EMSL Environmental Molecular Sciences Laboratory
Argonne National Laboratory

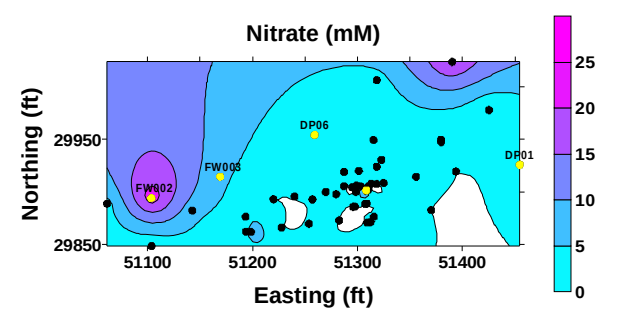
Part 1:
Field Experiments

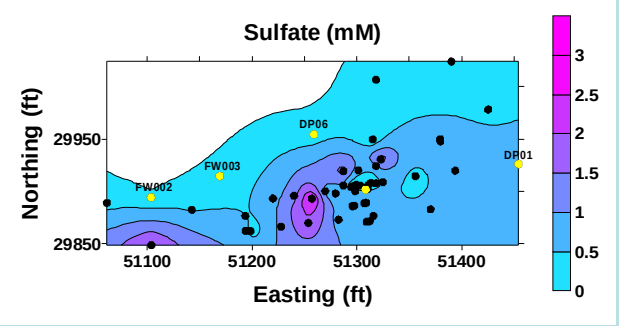
- Summary of results from research project *"Factors Controlling In Situ Uranium and Technetium Bioreduction at the NABIR Field Research Center"*
- *In situ* testing demonstrates potential for bio-immobilization of uranium and technetium under wide range of initial geochemical conditions
- Reoxidation of immobilized uranium and technetium identified as important technical issue
- Single-well, push-pull tests demonstrated as a simple, rapid, low-cost site characterization technology

Geochemical conditions at the FRC are highly spatially variable

FRC Area 2







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

Initial Conditions

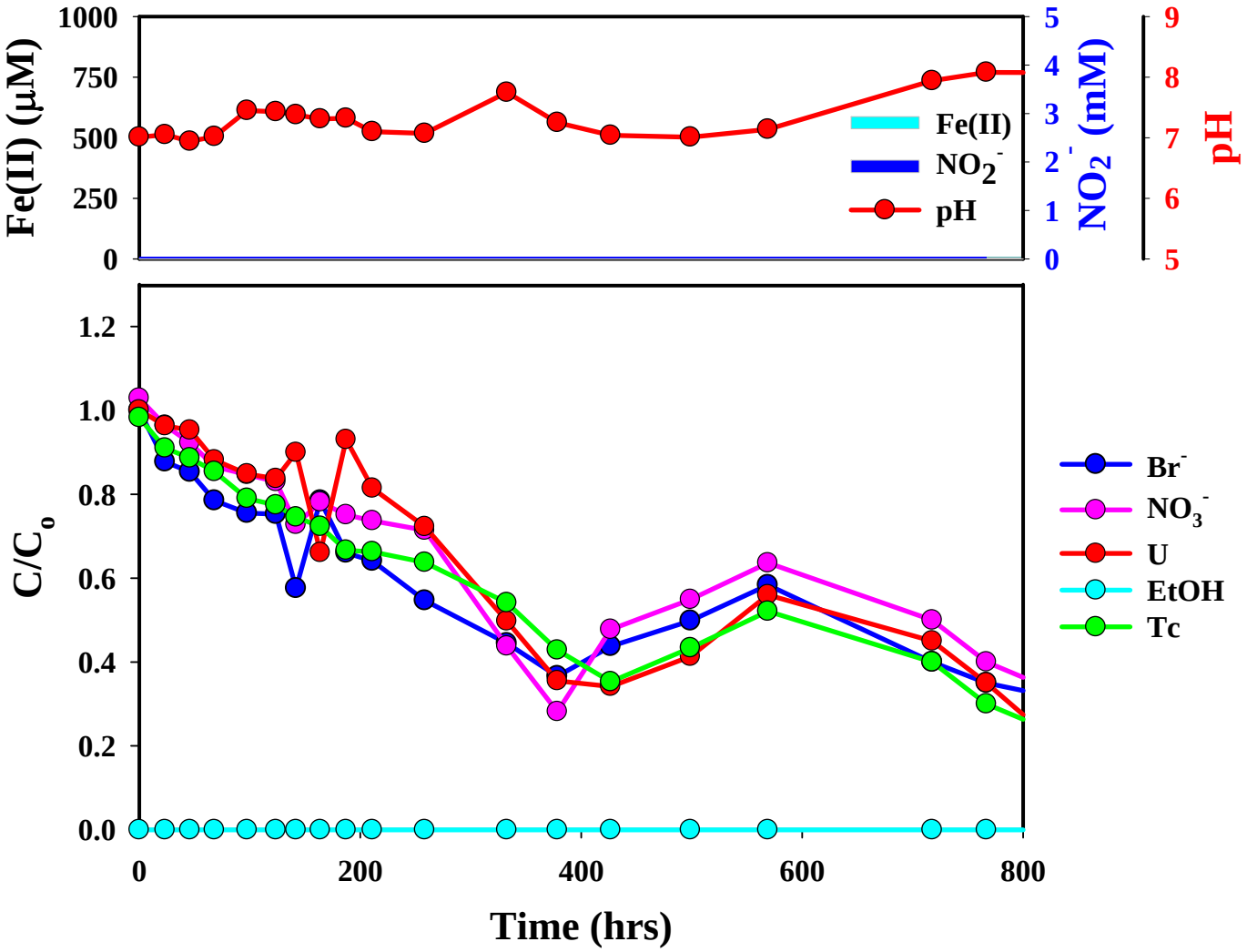
pH	NO_3^- (mM)	SO_4^{2-} (mM)	U(VI) (μM)	Tc(VII) (pM)
3.3-3.9	100-140	0-1	5-12	10000-15000
5.2-5.6	90-100	0-1	5-12	10000-15000
5.6-7.2	0-6	1-2	1-7	200-1000

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

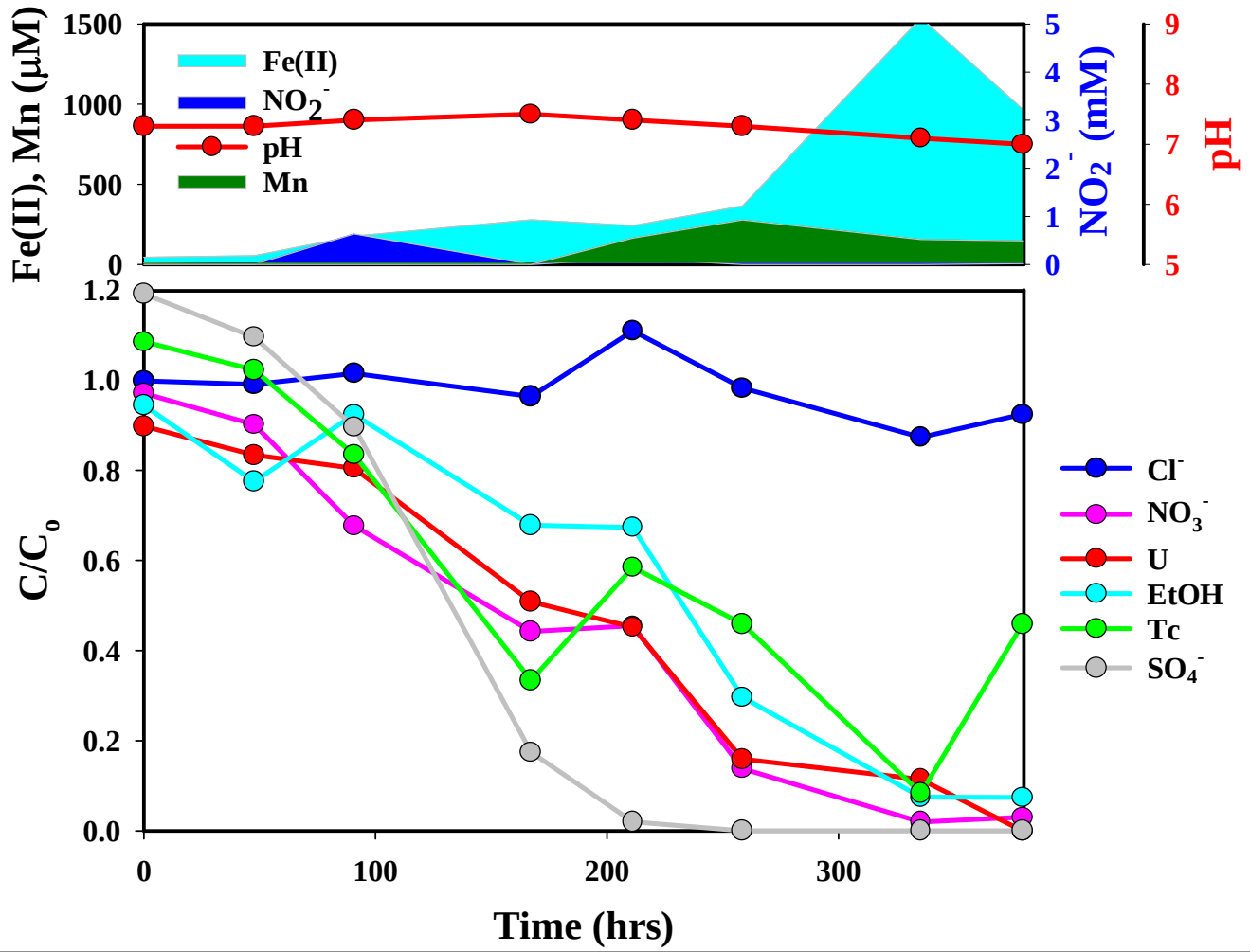
Typical test design

- **Collect 50-200 L site groundwater**
- **Amend with bromide tracer, +/- electron donor, +/-other amendments**
- **Inject by siphon**
- **Sample for 4-6 weeks after injection**
- **Plot concentration profiles**
- **Adjust for dilution**
- **Compute reaction rates**
- **104 Area 1 tests 105 Area 2 tests**
- **Total = 209**

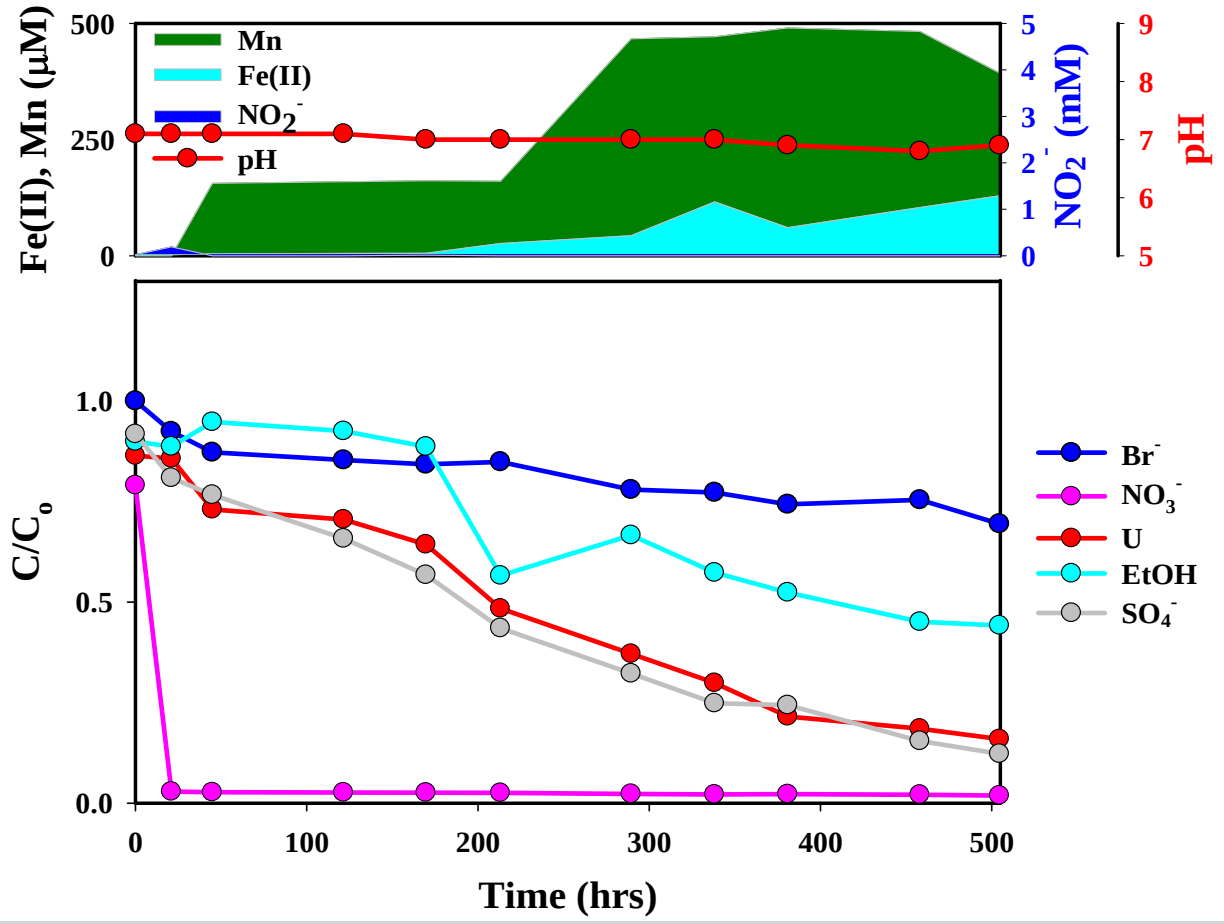
Microbial activity is electron donor limited; tests with no donor show only dilution losses



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



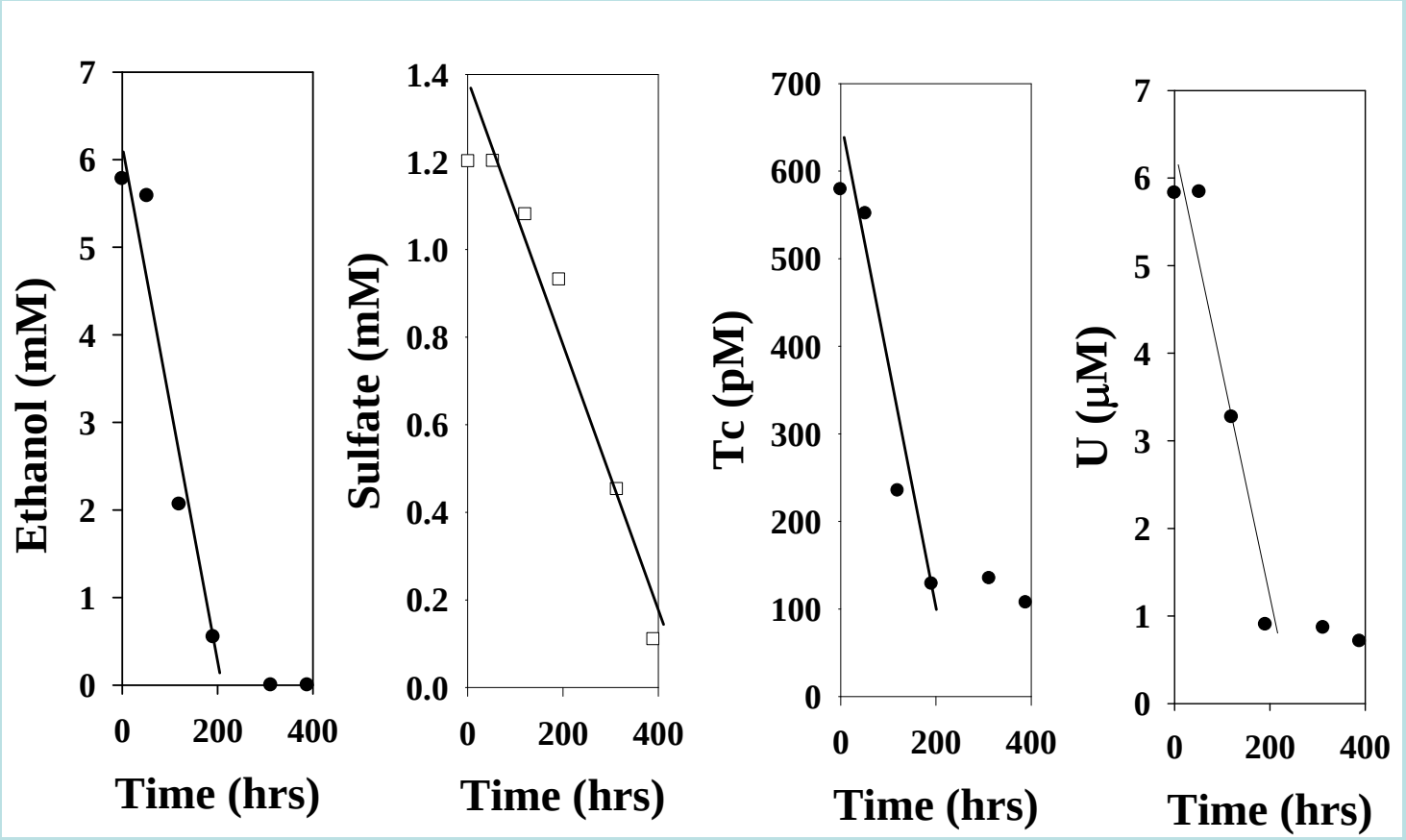
After biostimulation, microbial activity was similar in all environments tested including those with low initial pH



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, *in* situ 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

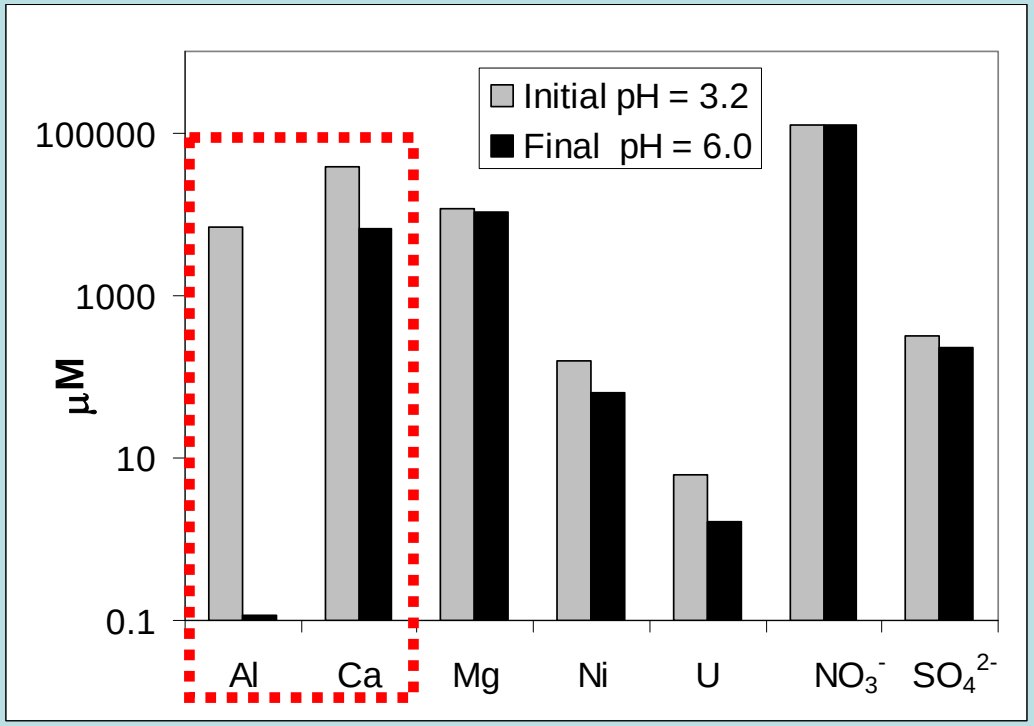
But *in situ* rates were very different from laboratory rates

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"

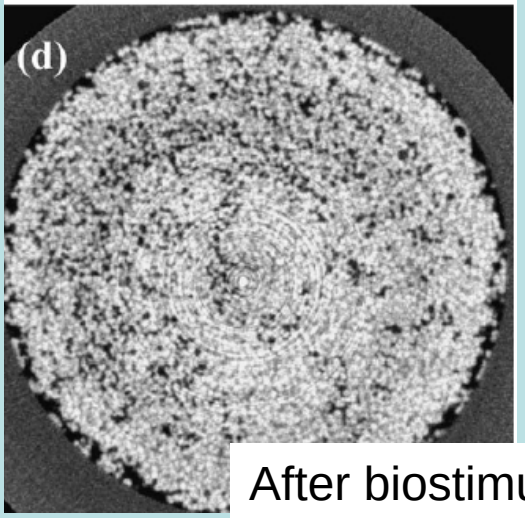
Enhanced microbial activity results in production of mineral precipitates, biomass, and gas

Titration of low pH groundwater

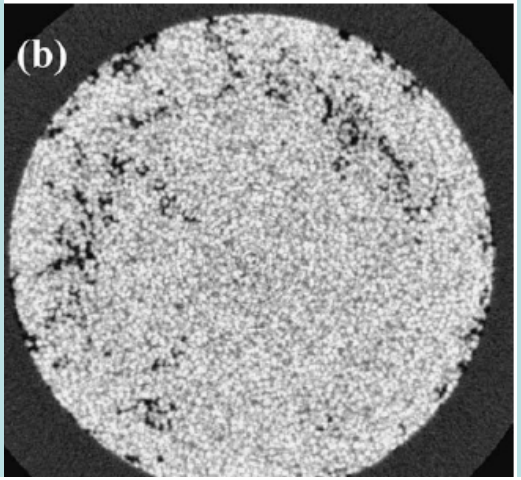


~ 2 g precipitates/L water

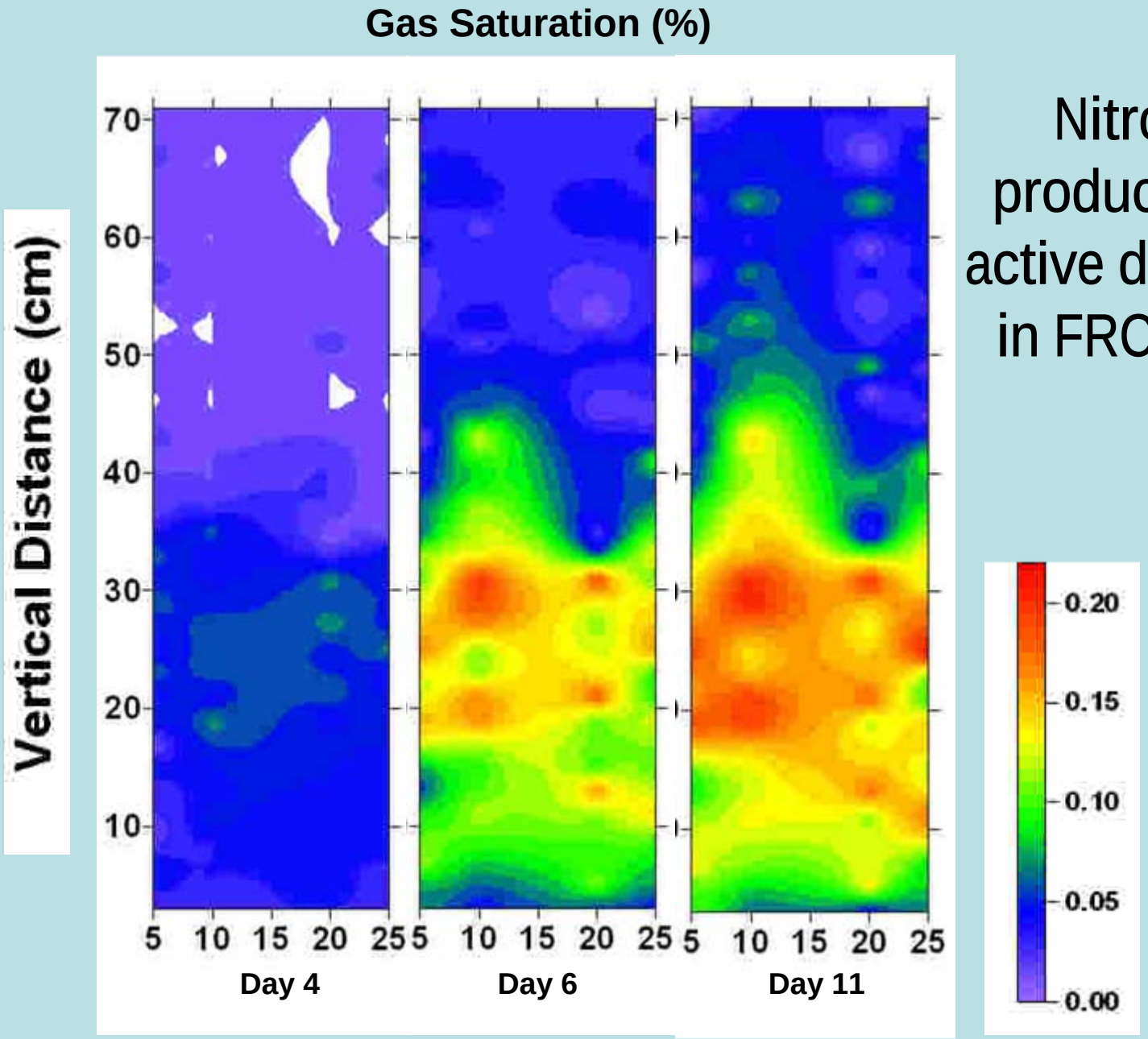
Before biostimulation



After biostimulation

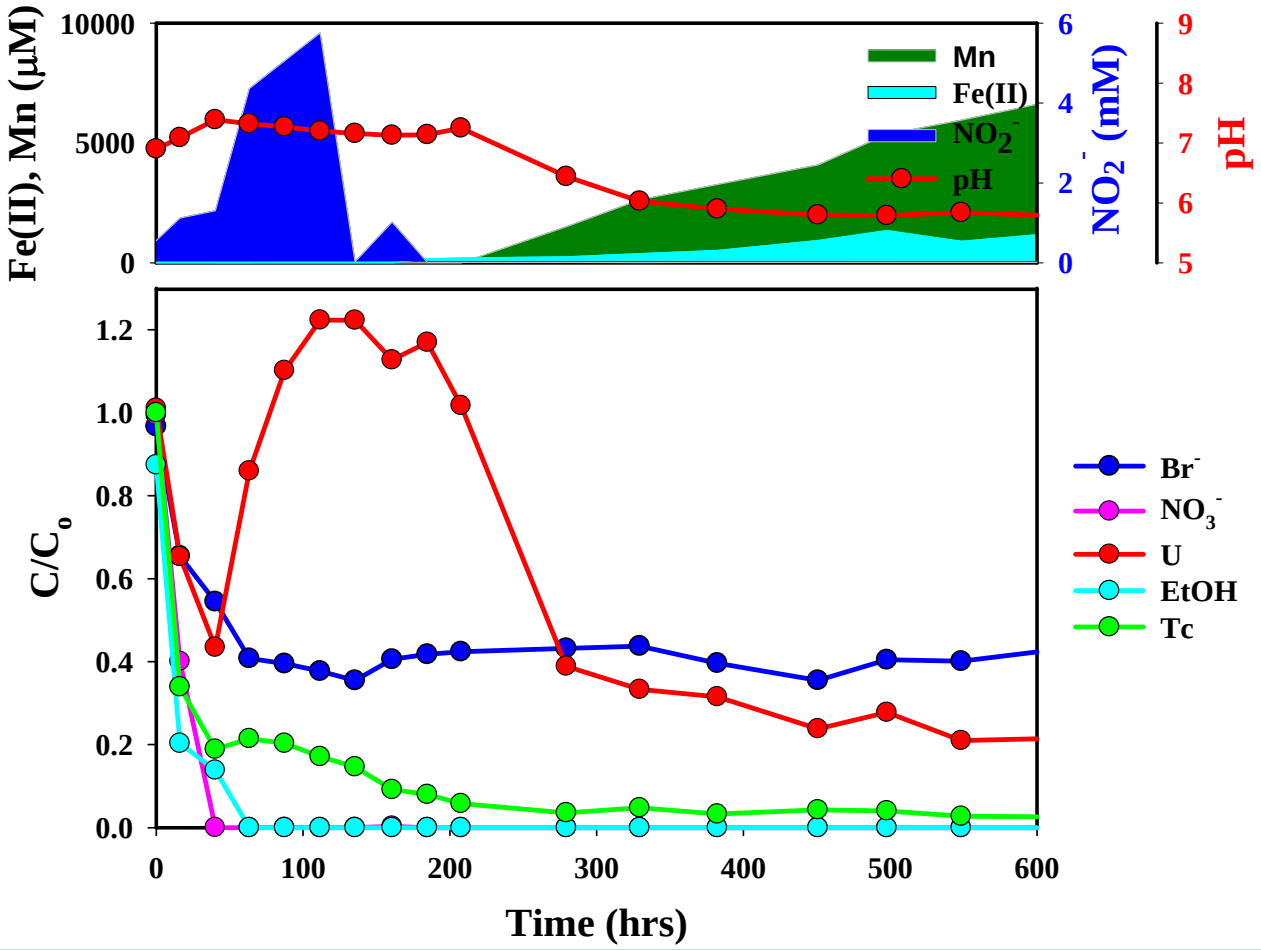


Gibbsite, calcite precipitates



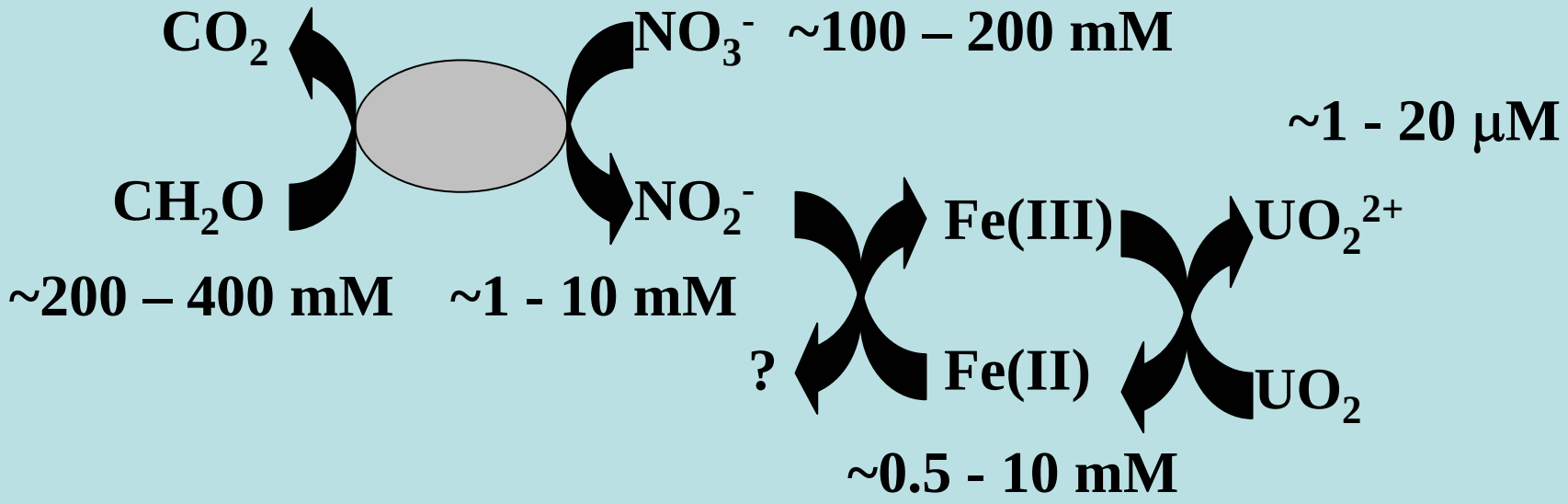
Nitrogen gas production during active denitrification in FRC sediments

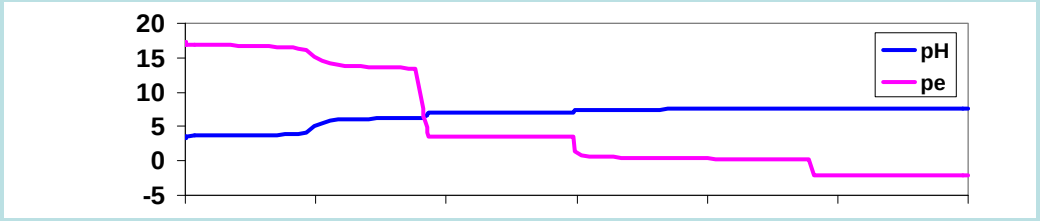
Addition of nitrate (and other oxidants) to previously reduced sediments reoxidizes and remobilizes U (but not Tc ?)

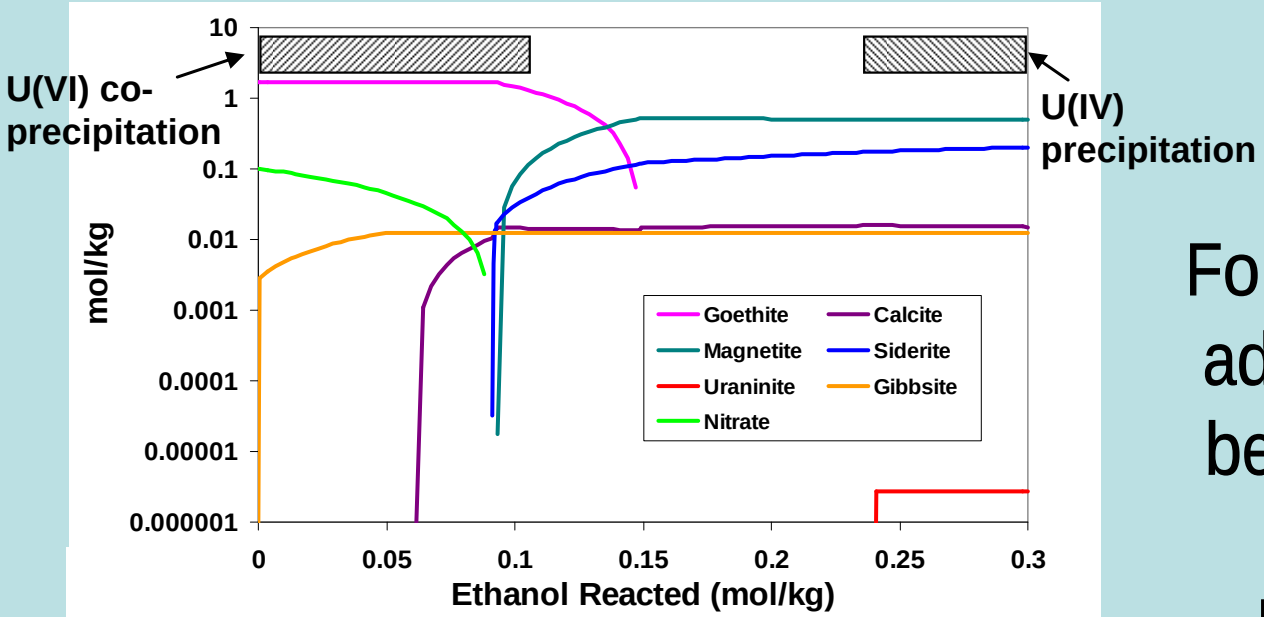


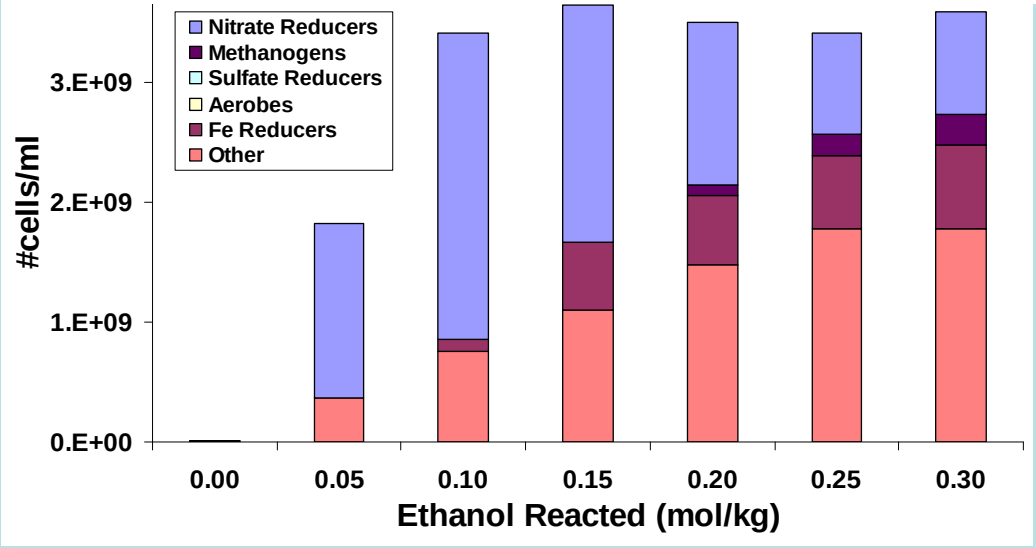
Addition of 100 mM NO_3^- to biostimulated sediments

Mechanisms of nitrate-dependent microbial U(IV) oxidation were identified using microbial isolates and a range of mineral systems









Form and amount of added substrates can be controlled to favor alternate bioimmobilization strategies

Part 2:
Biogeochemical modeling

- Results from research project *"Stability of U(VI) and Tc(VII) Reducing Microbial Communities to Environmental Perturbation: Development and Testing of a Thermodynamic Network Model"*
- Simple but powerful modeling approach was developed for predicting system response to donor additions or other perturbations
- Should prove useful for evaluating boimmobilization and related biotechnologies

Thermodynamic approach for predicting microbial growth

- **Assume organisms with all required metabolic capabilities are present**
- **Define a *synthetic microbial community* as a collection of microbial groups, each with a defined metabolism and growth equation**
- **Whichever groups can obtain the most energy in a particular '*thermodynamic niche*' grow**
  - **System specific combination of electron donors, electron acceptors, metabolic products, other geochemical variables**
  - **Growth predictions directly coupled to geochemical environment**

Growth equations

1 C-mol biomass = *a*(electron donor) + *b*(electron acceptor) + *c*(N source) + *d*(H_2O) + *e*(H^+) + …

Solve for stoichiometric coefficients using charge, elemental, and free energy balances

1 mol cells (Example denitrifer group, YDx = 0.41) =

6.1 CH_3COO^- +1 NH_4+ 5.8 NO_3^- + 3.7 H^+

-5.9 H_2O -7.2 HCO_3^- -2.9 N_2(aq)

MODELING APPROACH REQUIRES ONLY FOUR ADJUSTABLE PARAMETERS

(Gibbs energy dissipated per C-mol produced biomass for each growth substrate: ethanol, lactate, acetate, hydrogen)

Microbial groups in current thermodynamic database

Group	Acceptor Half-Reaction	Donor Half-Reaction	YDx
1	O_2/CO_2	Ethanol/CO_2	0.56
2		Acetate/CO_2	0.41
3		Lactate/CO_2	0.56
4		Ethanol/Acetate	0.14
5		Lactate/Acetate	0.14
6		H_2/H^+	0.13
7		CH_4/CO_2	0.55
8	NO_3^-/N_2	Ethanol/CO_2	0.27
9		Acetate/CO_2	0.41
10		Lactate/CO_2	0.27
11		Ethanol/Acetate	0.29
12		Lactate/Acetate	0.06
13		H_2/H^+	0.17
14	Fe^{3+}/Fe^{2+}	Acetate/CO_2	0.12
15		Ethanol/Acetate	0.13
16		Lactate/Acetate	0.13
17		H_2/H^+	0.07
18	SO_4^{2-}/HS^-	Acetate/CO_2	0.10
19		Ethanol/Acetate	0.04
20		Lactate/Acetate	0.04
21		H_2/H^+	0.07

Group	Acceptor Half-Reaction	Donor Half-Reaction	YDx
22	MnO_4^{2-}/Mn^{2+}	Acetate/CO_2	0.12
23		Ethanol/Acetate	0.29
24		Lactate/Acetate	0.06
25		H_2/H^+	0.15
26	CO_2/CH_4	Acetate/CO_2	0.02
27		H_2/H^+	0.02
28	H^+/H_2	Acetate/CO_2	0.11
29		Ethanol/Acetate	0.01
30		Lactate/Acetate	0.06
31	UO_2^{++}/U^{4+}	Acetate/CO_2	0.22
32		Ethanol/Acetate	0.19
33		H_2/H^+	0.12
34	CrO_4^{2-}/Cr^{+++}	Acetate/CO_2	0.32
35		Lactate/Acetate	0.06
36		H_2/H^+	0.12
37	TCO_4^-/TcO^{2+}	Acetate/CO_2	0.07
38		Ethanol/Acetate	0.06
39		H_2/H^+	0.04

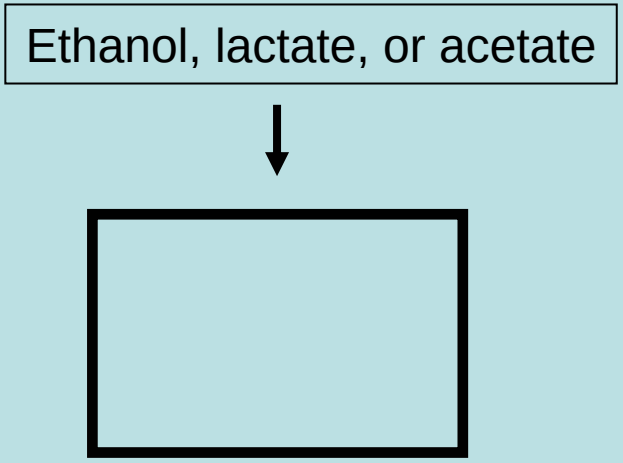
Model tested in four environments

	FRC Area 2 (mM)	FRC Area 1 (mM)	Old Rifle (mM)	Hanford 100 H (mM)
pH	6.4	3.3	7.3	7.8
O_2	0.1	0.1	0.0	0.1
NO_3^-	1.2	100.0	0.1	0.7
SO_4^{2-}	0.8	0.4	6.4	0.7
Iron oxides (mmol/kg)	306	361	124	233
Mn oxides (mmol/kg)	48	22	10	3
Ca	3.5	18.0	5.3	1.5
Mg	1.1	8.3	5.4	3.0
Al	-	12.0	-	-
HCO_3^-	0.1	0.0	0.1	0.1
U	4.9×10^{-3}	1.4×10^{-3}	5.25×10^{-4}	-
V	-	-	1.54×10^{-2}	-
Tc	4.1×10^{-7}	1.8×10^{-5}	-	-
Cr	1×10^{-3}	-	-	2.93×10^{-2}
Growth substrate	Ethanol	Ethanol	Acetate	Lactate (HRC)

Reaction path simulations

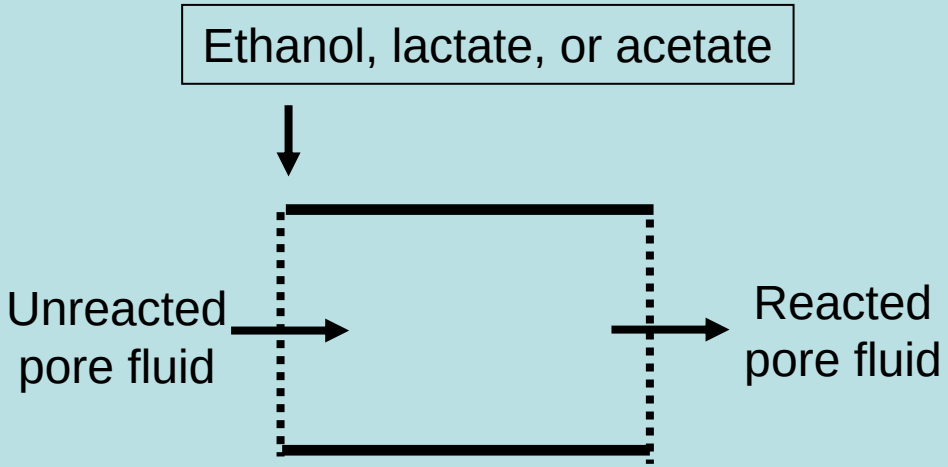
Growth substrate is added in small amounts and allowed to react until entire biogeochemical system is at minimum free energy = thermodynamic equilibrium

"Batch" (closed) Simulations



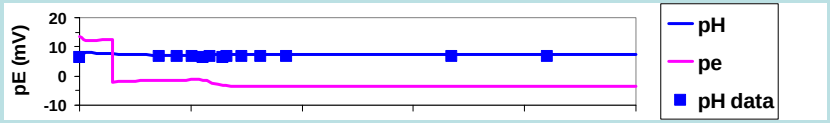
Laboratory microcosms/
Field push-pull tests

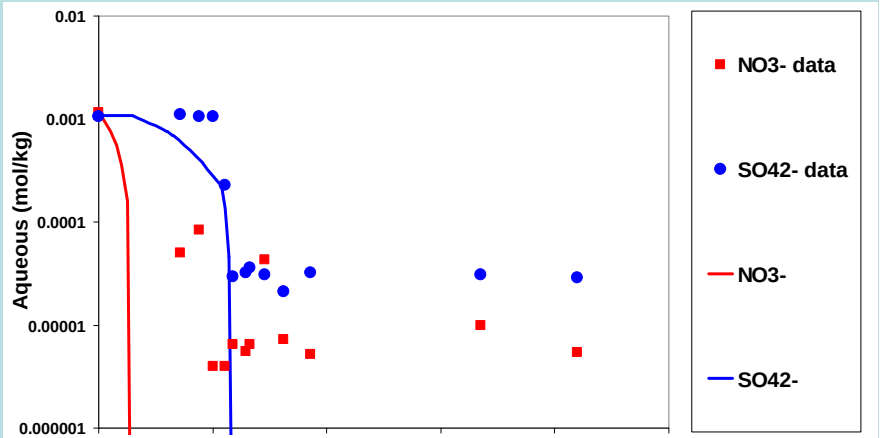
"Flush" (open) Simulations

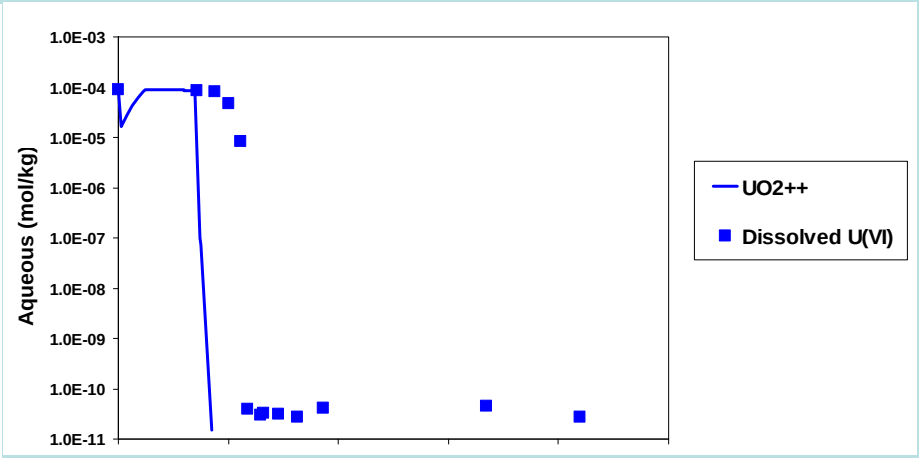


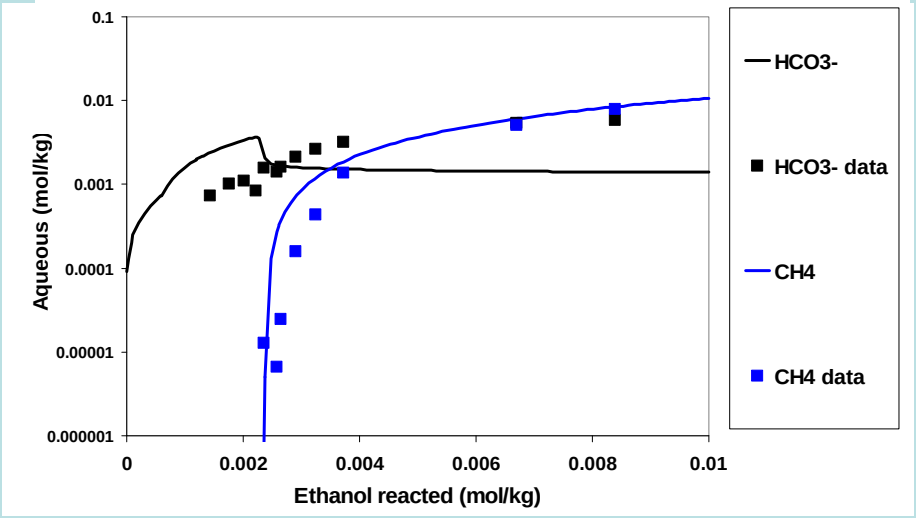
Laboratory columns/
Intermediate-scale physical models
Field natural gradient tests

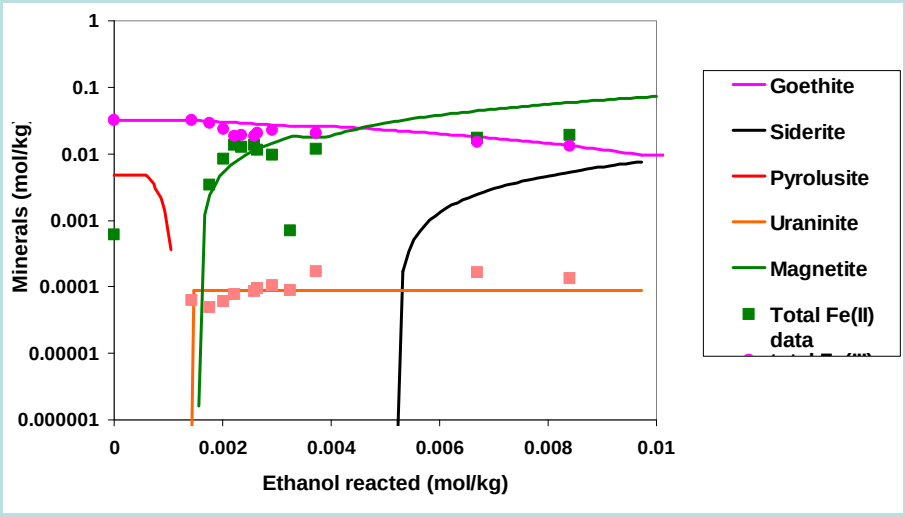
Example batch simulation for FRC Area 2 (data from Mohanty et al.)



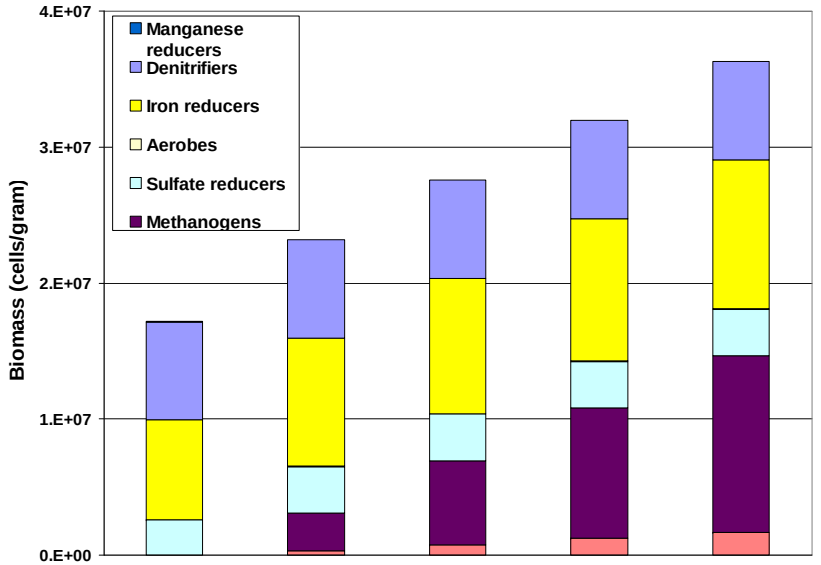


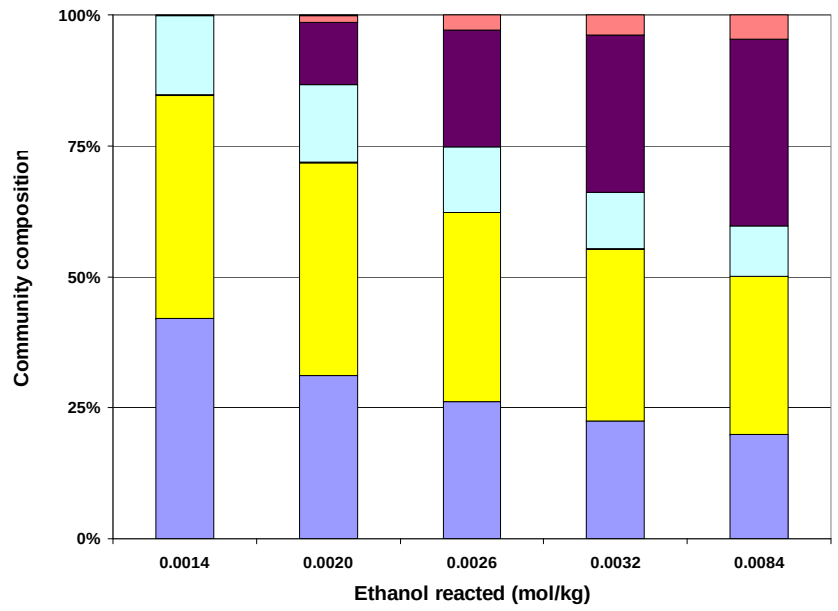




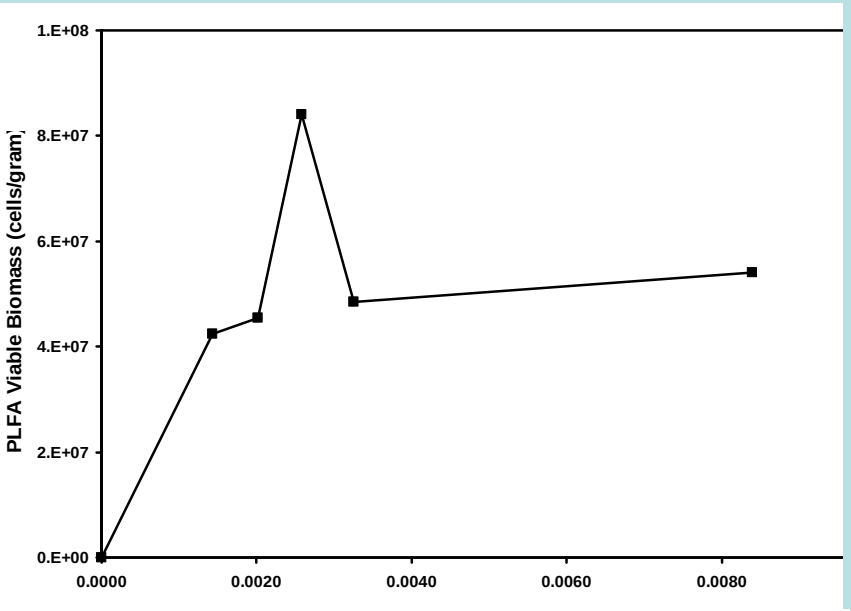


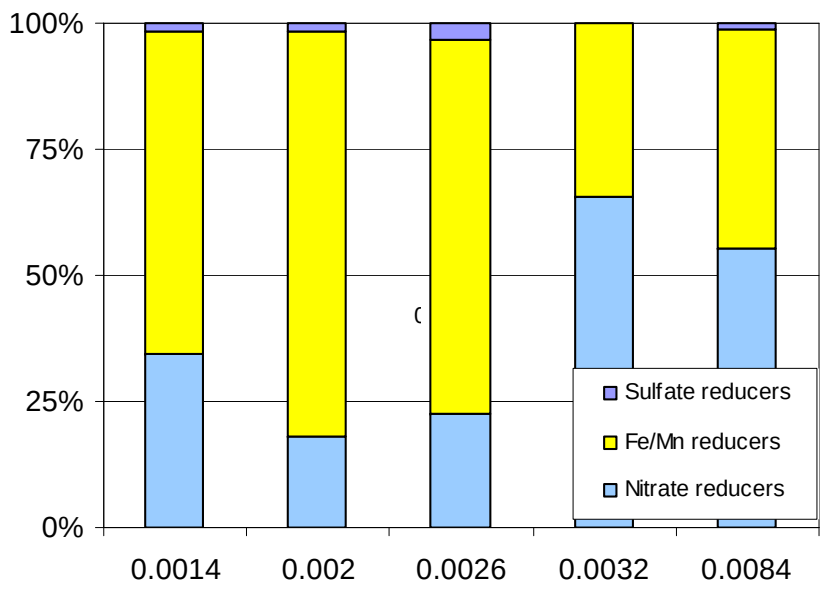
Predicted

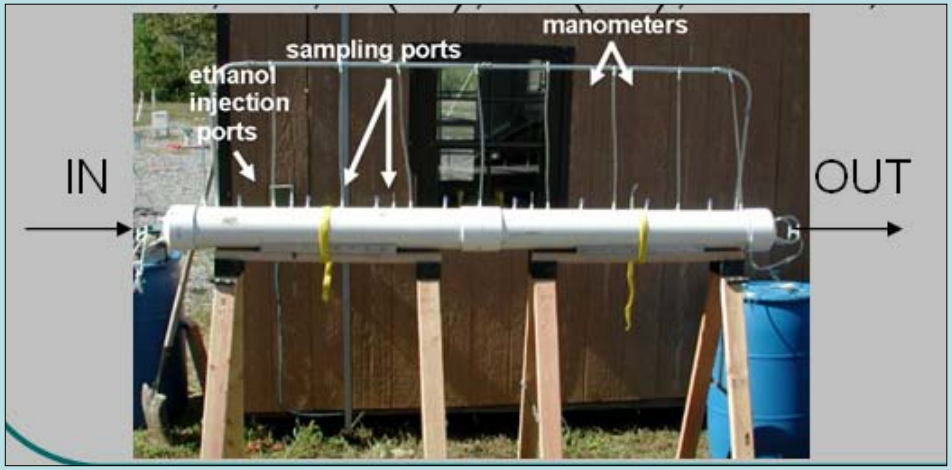




Observed

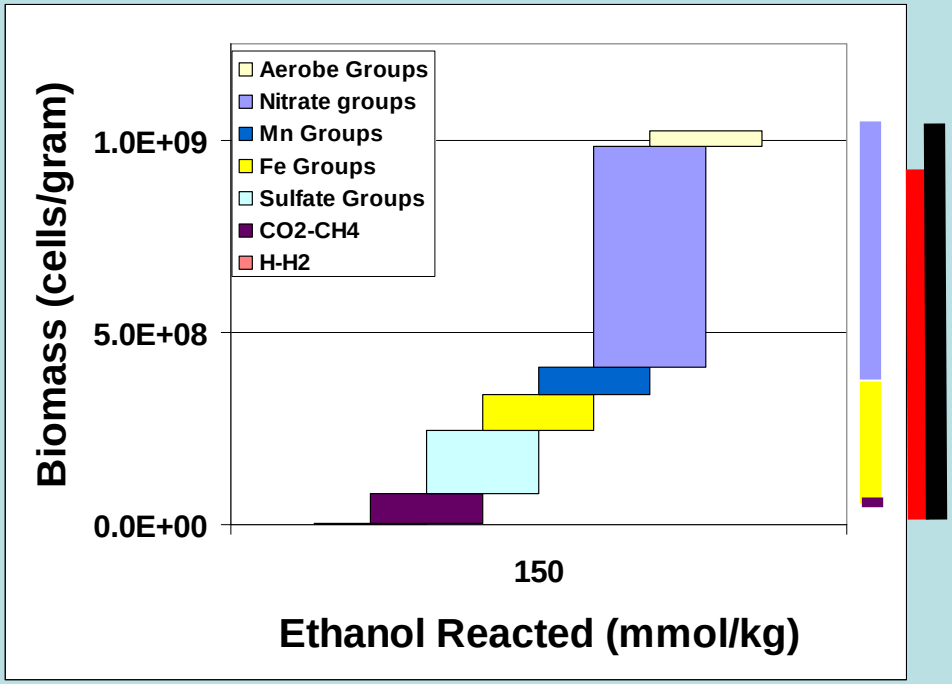






Example flush simulation fo 24 month mesocolumn experiment
Growth stimulated with ethanol
Complete mass balance

Comparison of Model Prediction with Sediment Microbial Analyses



Sediment analyses (n = 8)

PLFA Viable Biomass
= 8 x 10^8 cells/gram

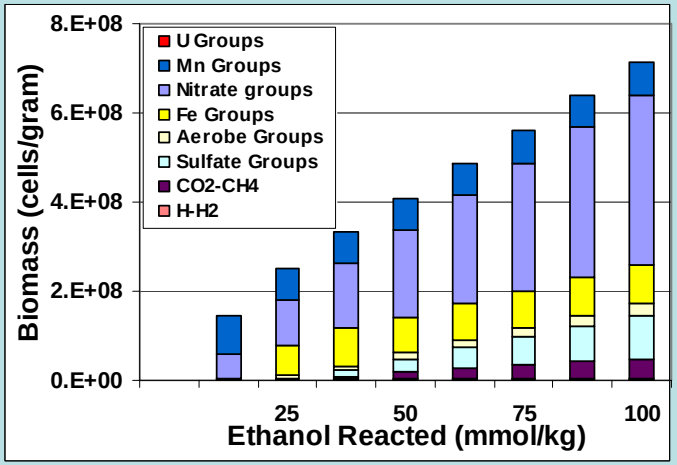
Eubacterial 16S n RNA
= 1. x 10^9 copies/gram

nirS + nirK
= 7 x 10^8 copies/gram

Iron/sulfate reducing bacteria
= 2 x 10^8 copies/gram

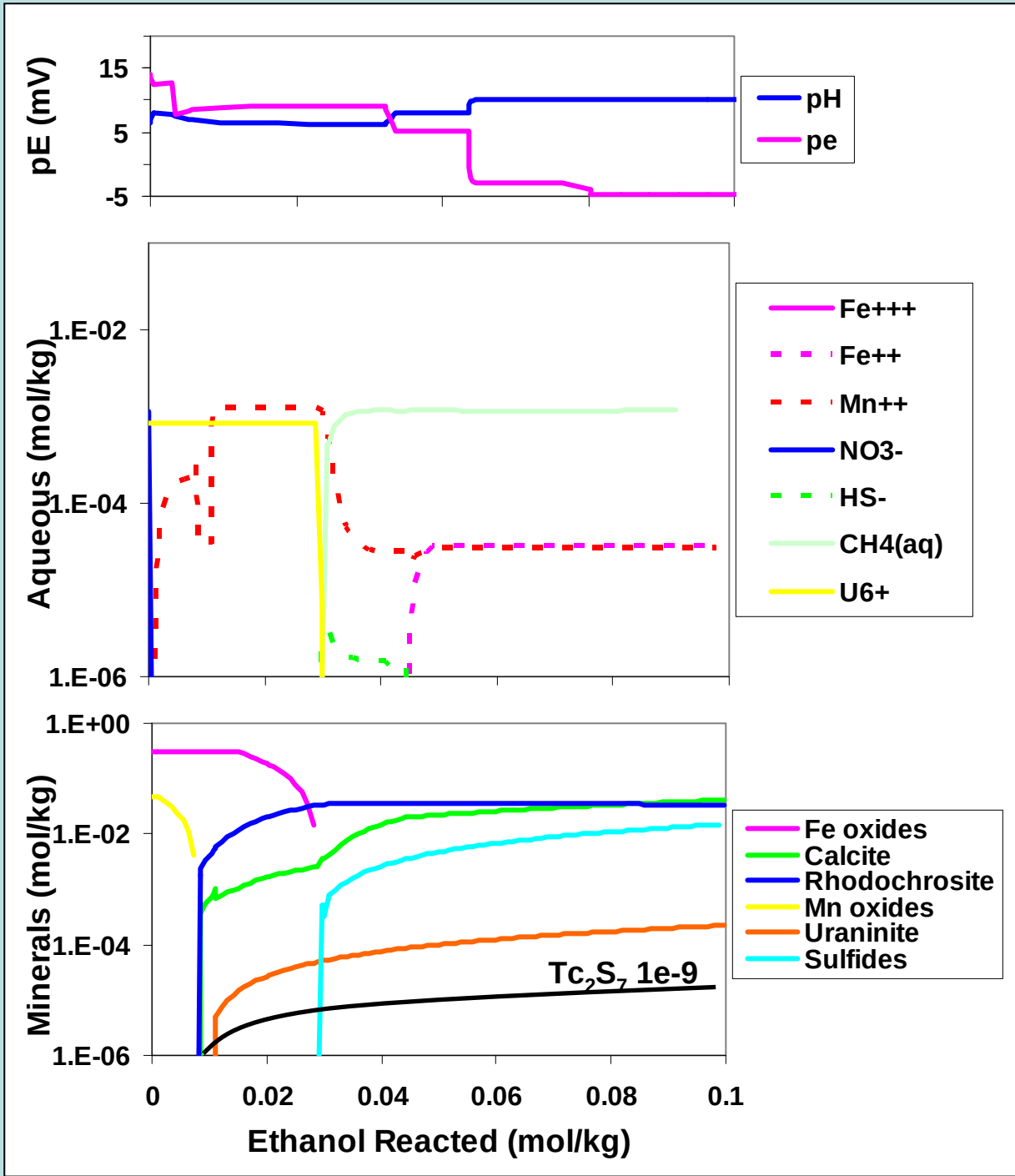
Geobacter
= 1 x 10^4 copies/gram

Methanogens
= 1 x 10^6 copies/gram



Model predicts major biogeochemical processes observed during experiment

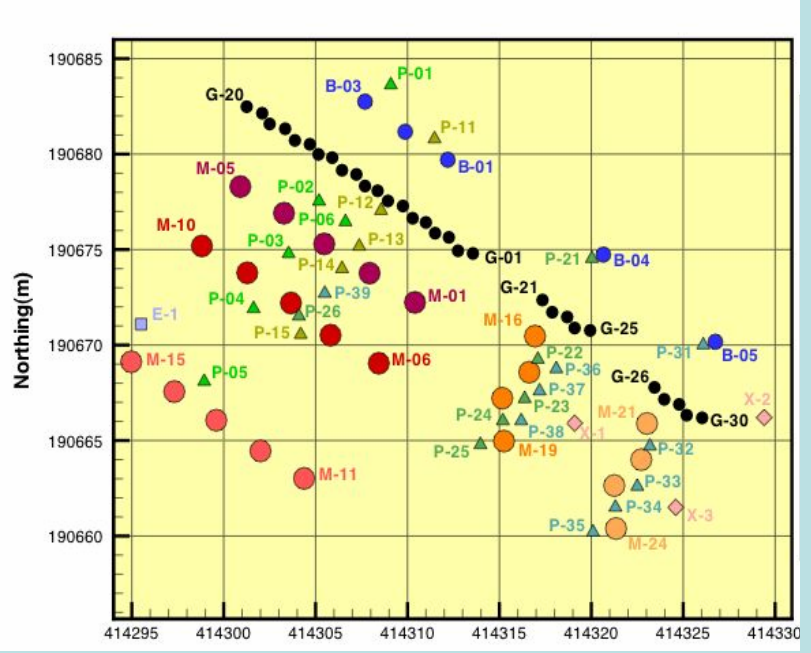
Reduction of soluble electron acceptors
Accumulation of Fe^{2+}, Mn^{2+}, HS^-, CH_4
Reduction of U(VI) and Tc(VII)

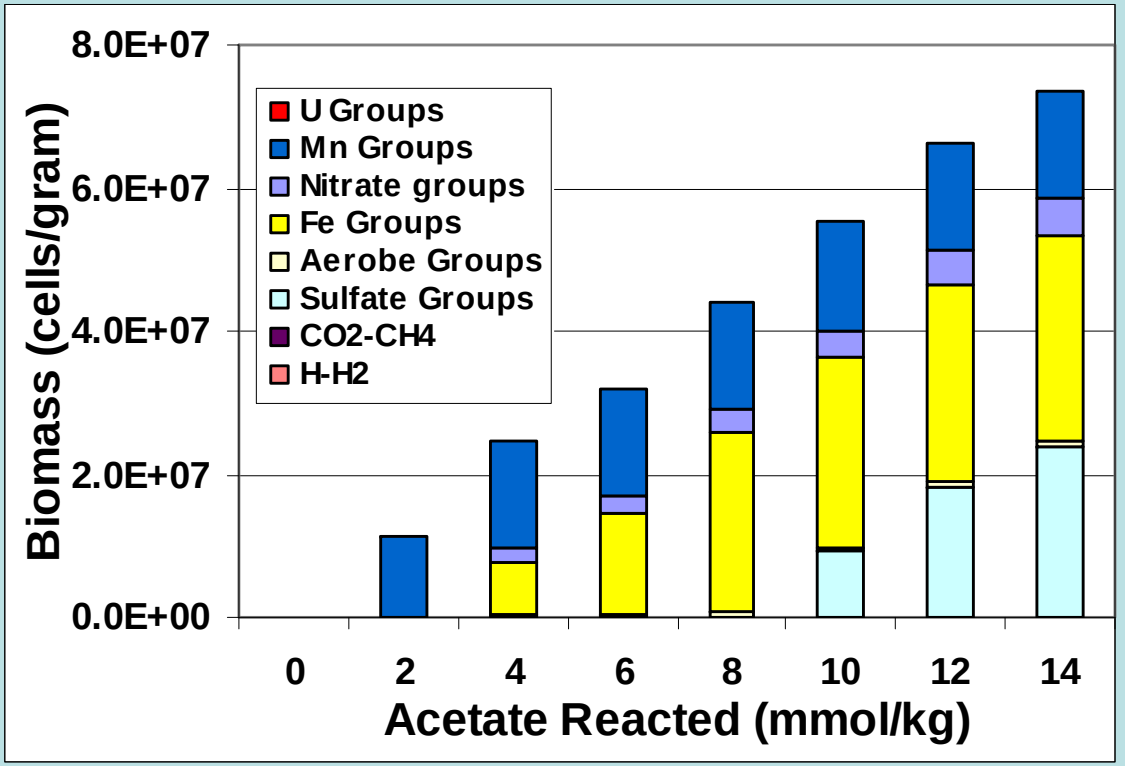


Example flush simulation for Old Rifle

- Microbial groups combined into major classes for plotting
- Many groups grow on microbially generated acetate and H_2

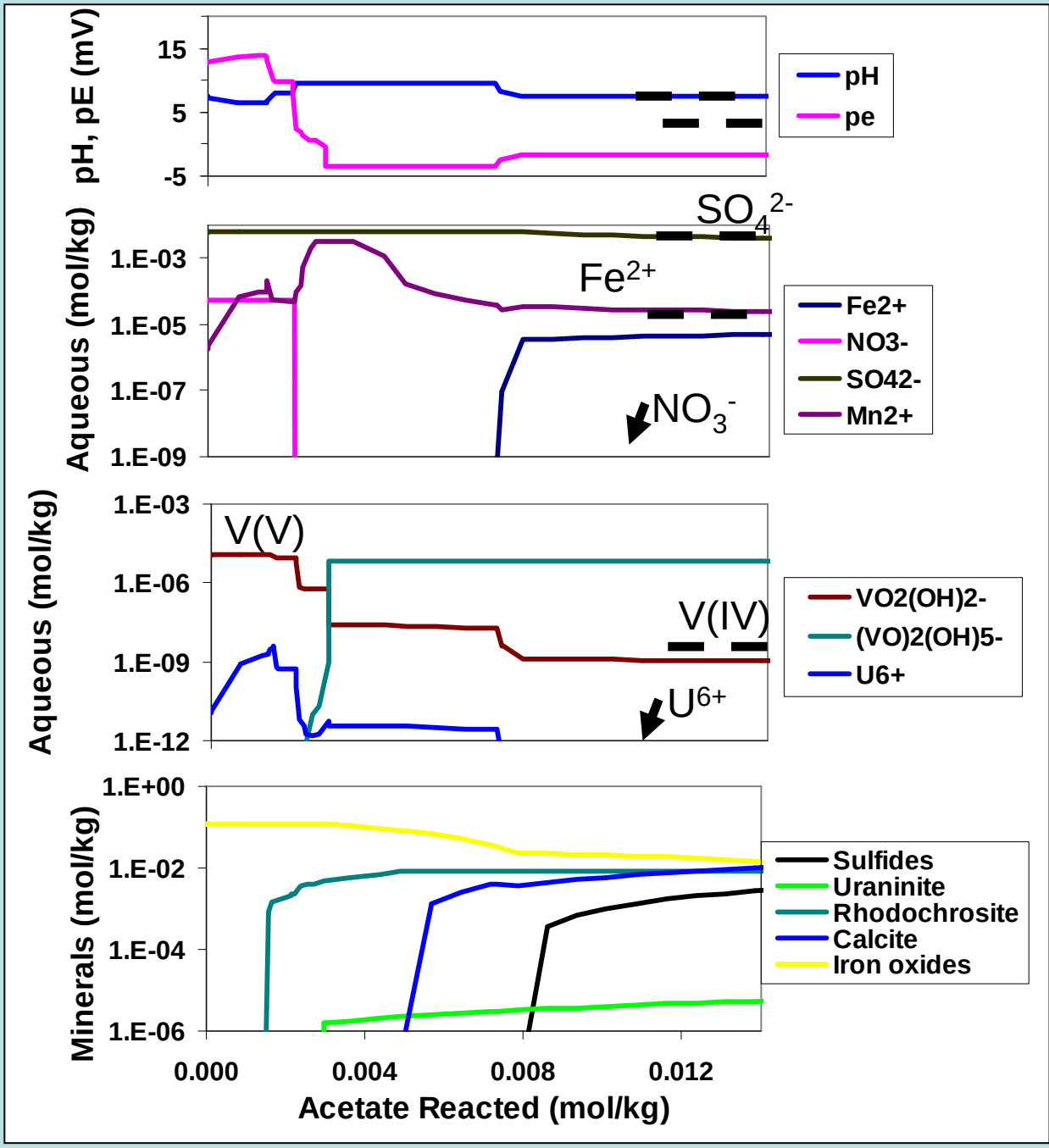
	(mM)
pH	7.3
O_2	0.0
NO_3^-	0.1
SO_4^{2-}	6.4
Iron oxides (mmol/kg)	124
Mn oxides (mmol/kg)	10
Ca	5.3
Mg	5.4
Al	-
HCO_3^-	0.1
U	5.25×10^{-4}
V	1.54×10^{-2}



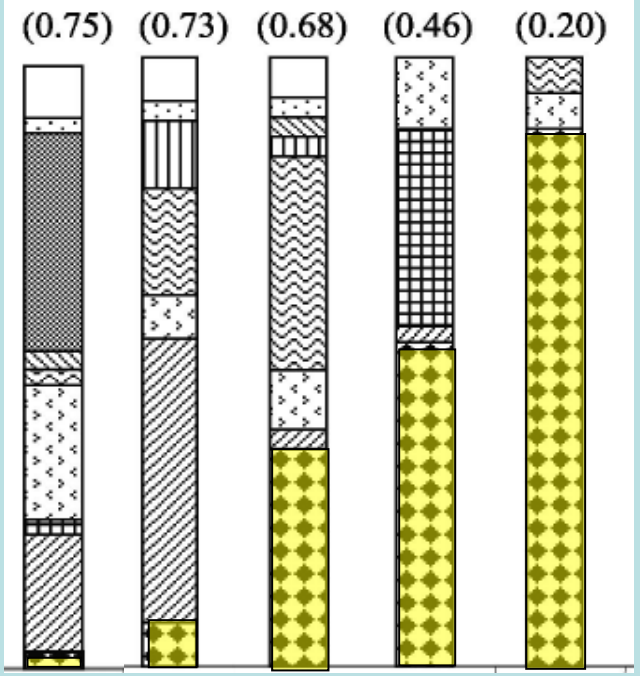


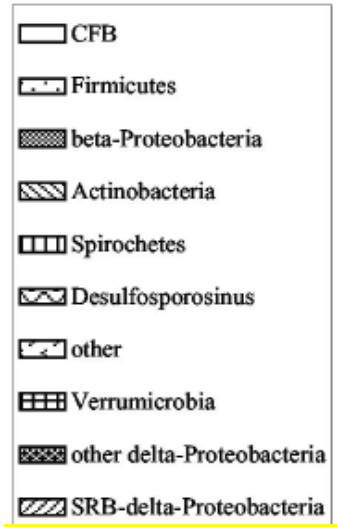
Model predicts major biogeochemical processes observed during experiment

Reduction of soluble electron acceptors
Accumulation of Fe^{2+}
Reduction of U(VI) and V(V)



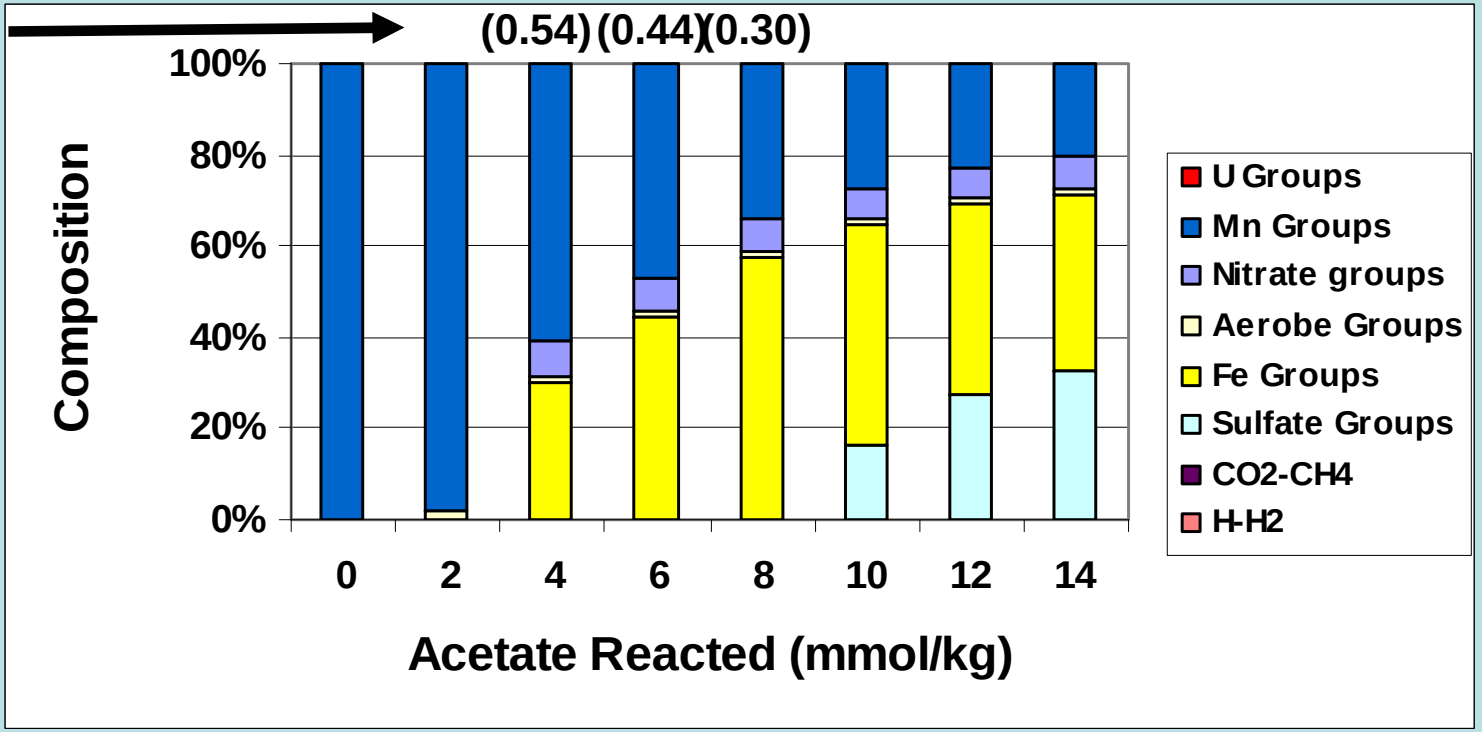
Anderson et al. (2003) Clone libraries from groundwater samples



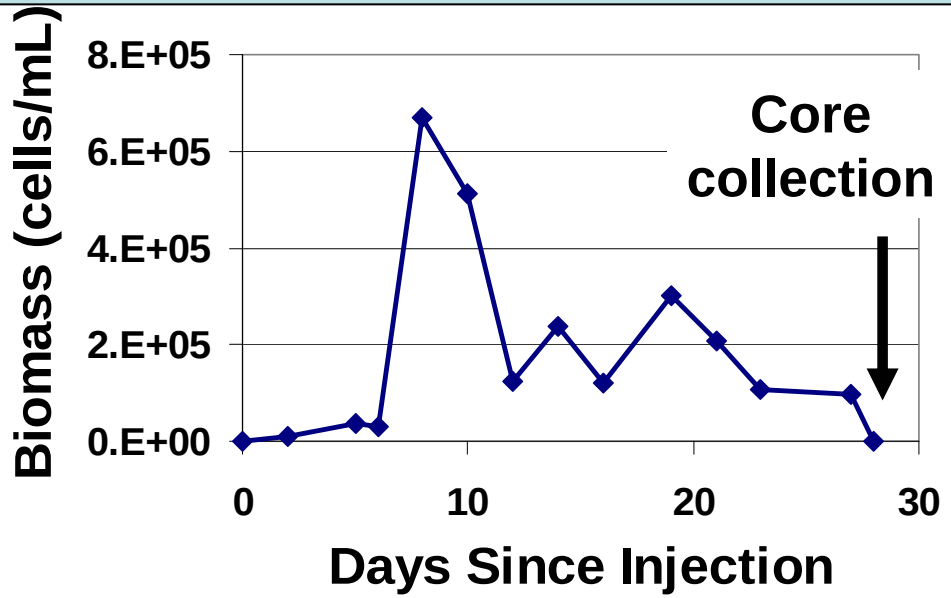


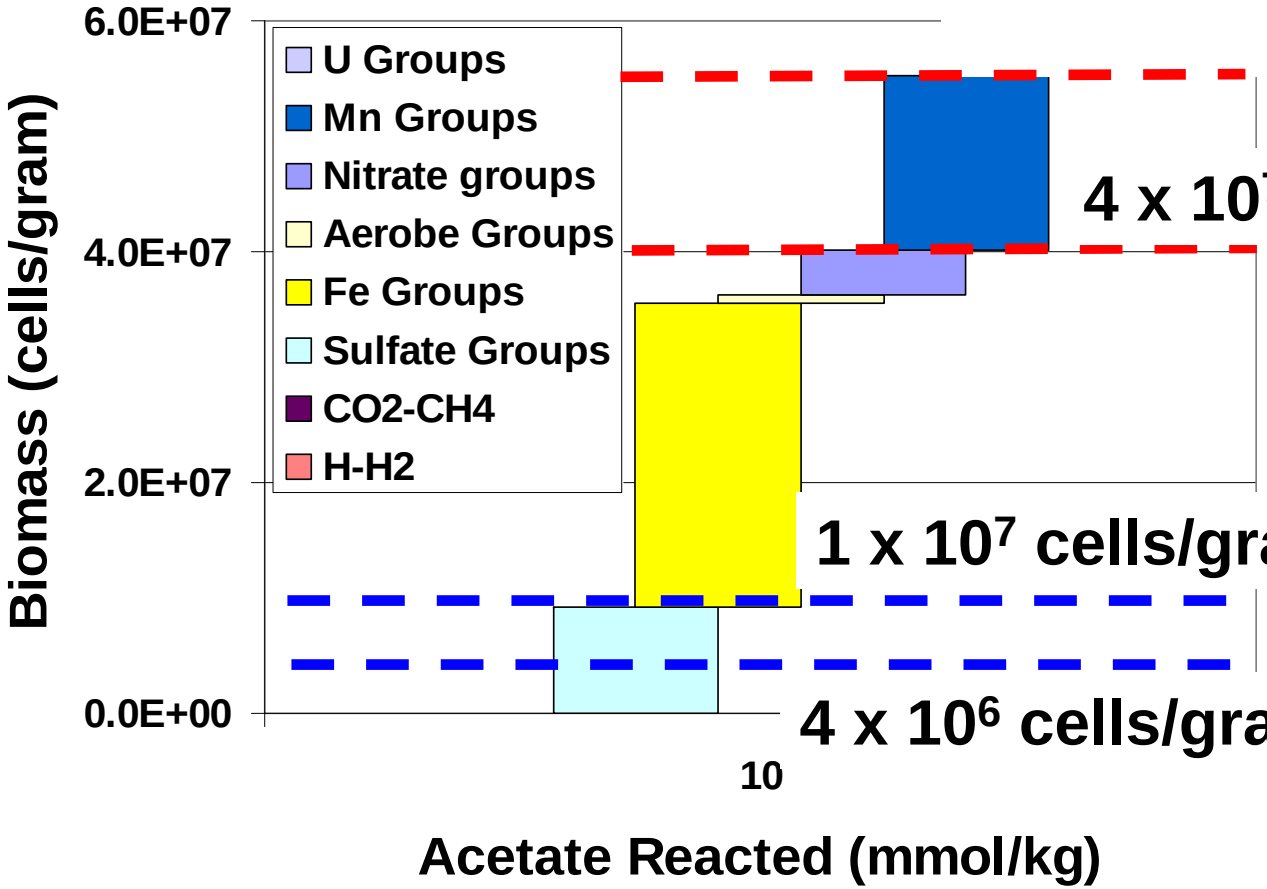
Geobacteraceae

(Community diversity index)



^{13}C Acetate Experiment





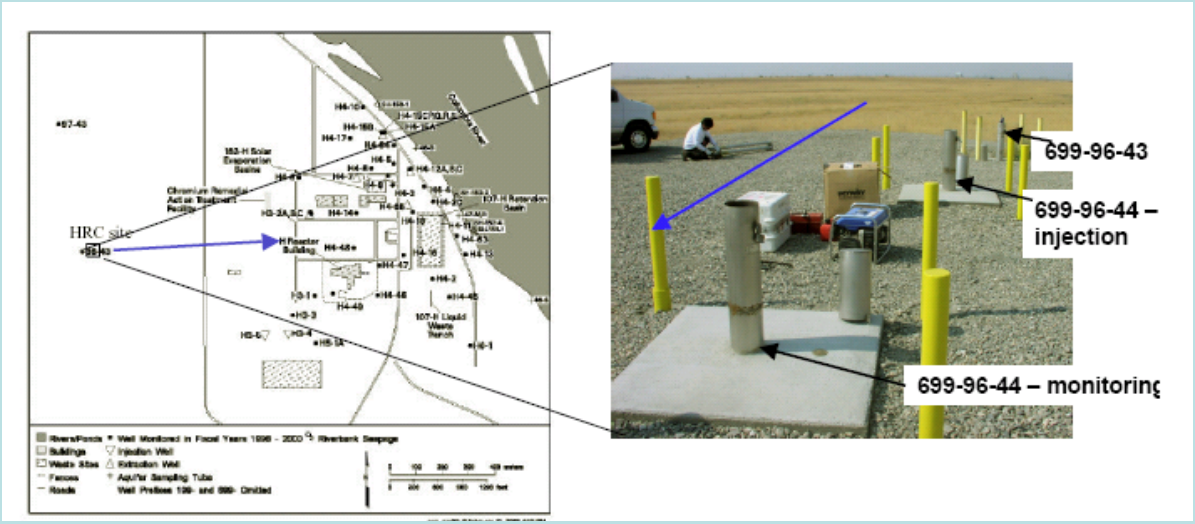
4×10^7 cells/gram

1×10^7 cells/gram

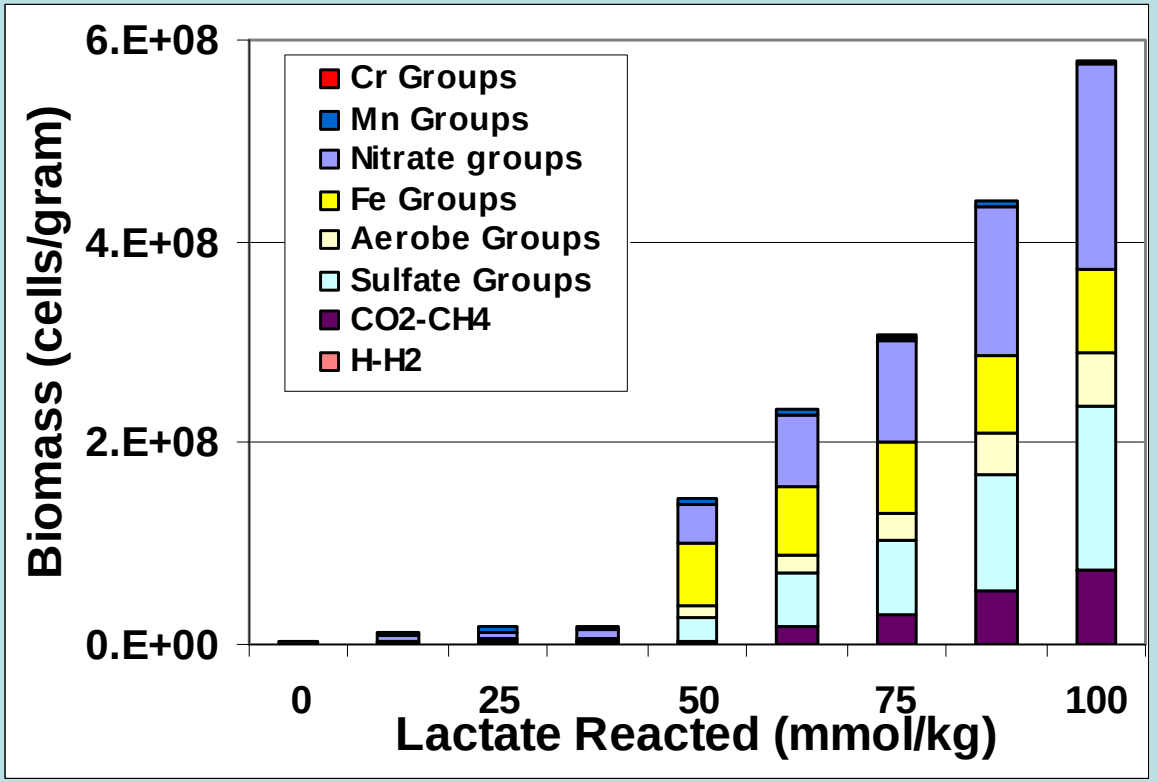
4×10^6 cells/gram

^{13}C PLFA Biomass from sediment cores

Example flush simulation for Hanford 100H

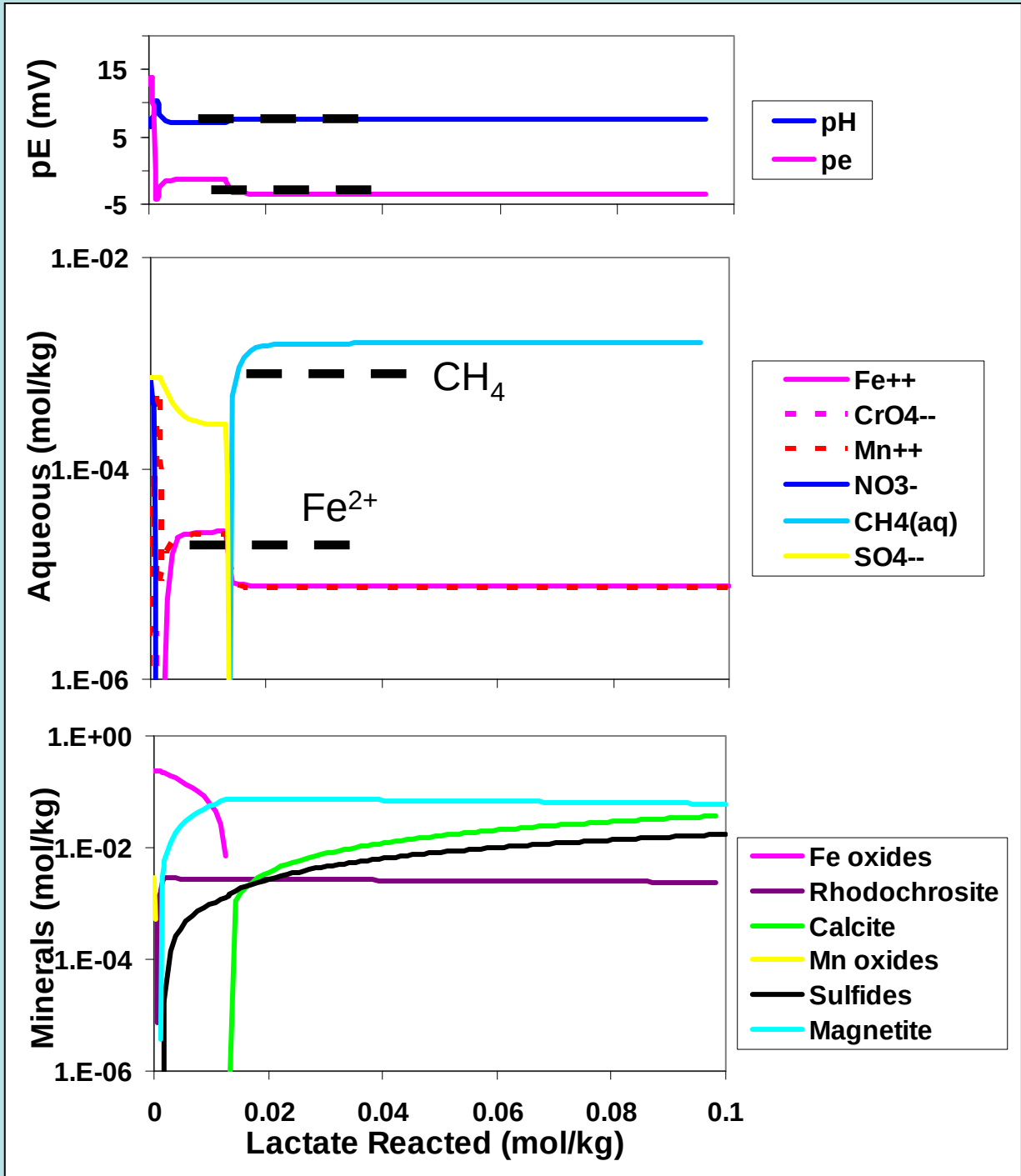


	(mM)
pH	7.8
O_2	0.1
NO_3^-	0.7
SO_4^{2-}	0.7
Iron oxides (mmol/kg)	233
Mn oxides (mmol/kg)	3
Ca	1.5
Mg	3.0
Al	-
HCO_3^-	0.1
Cr	2.93×10^{-2}

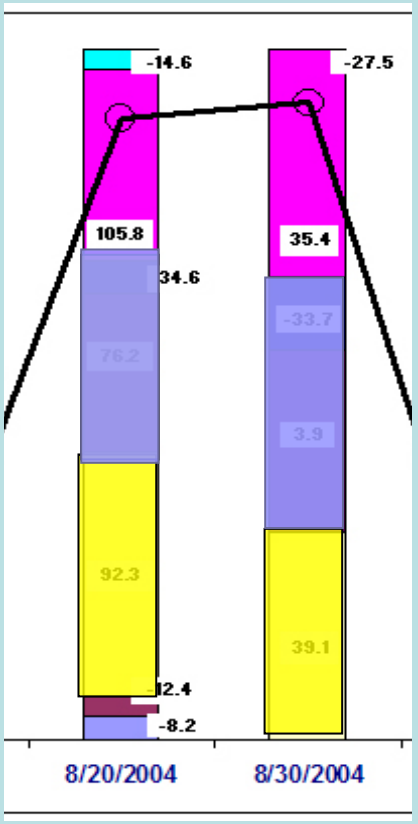


Model predicts major biogeochemical processes observed during experiment

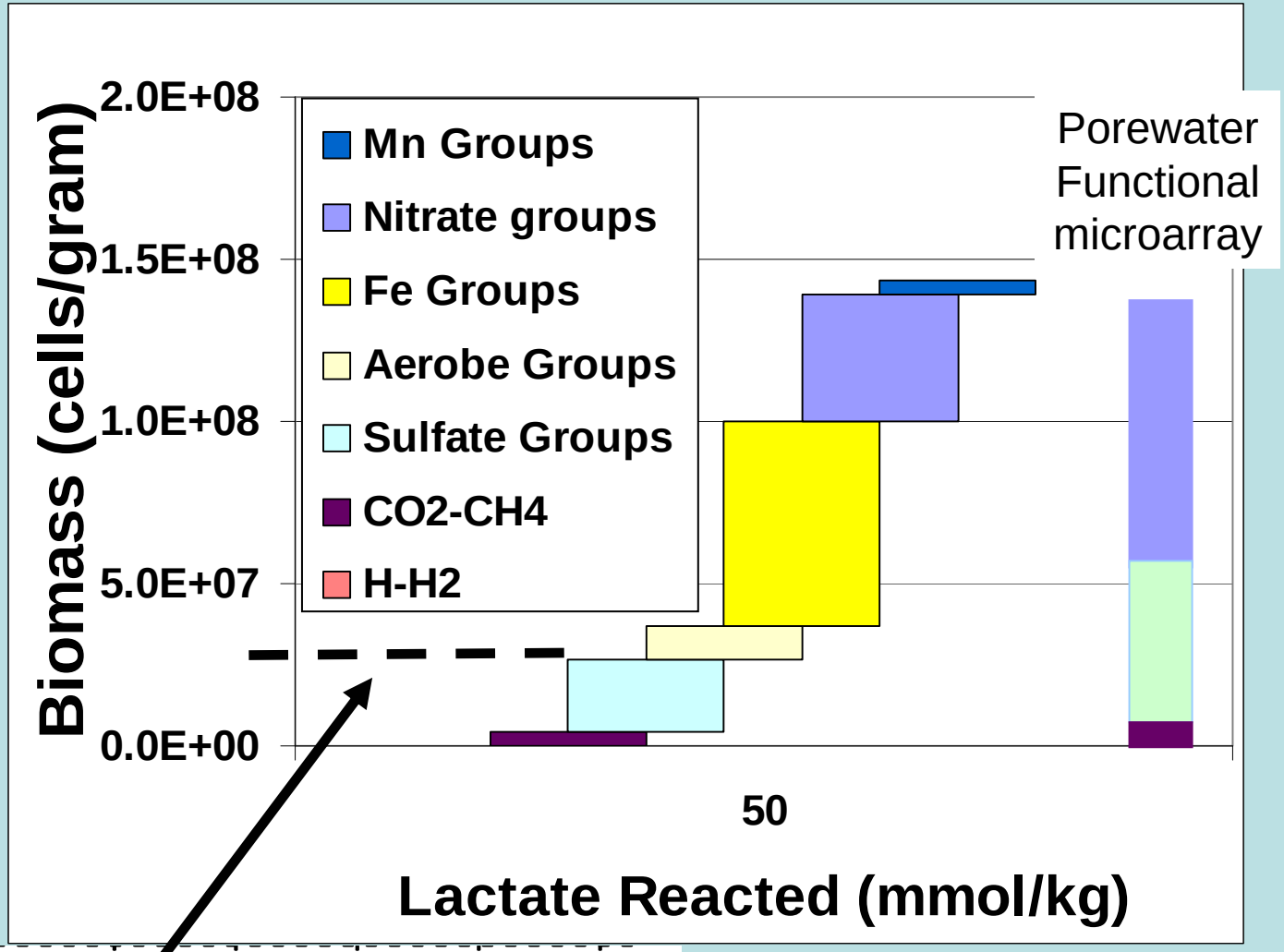
Reduction of soluble electron acceptors
Accumulation of Fe^{2+}, CH_4
Reduction of Cr(VI)

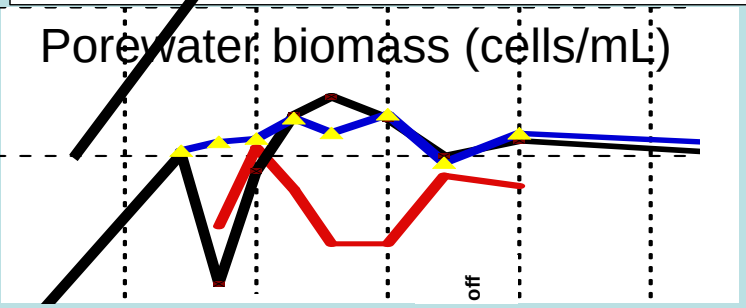


Porewater PLFA

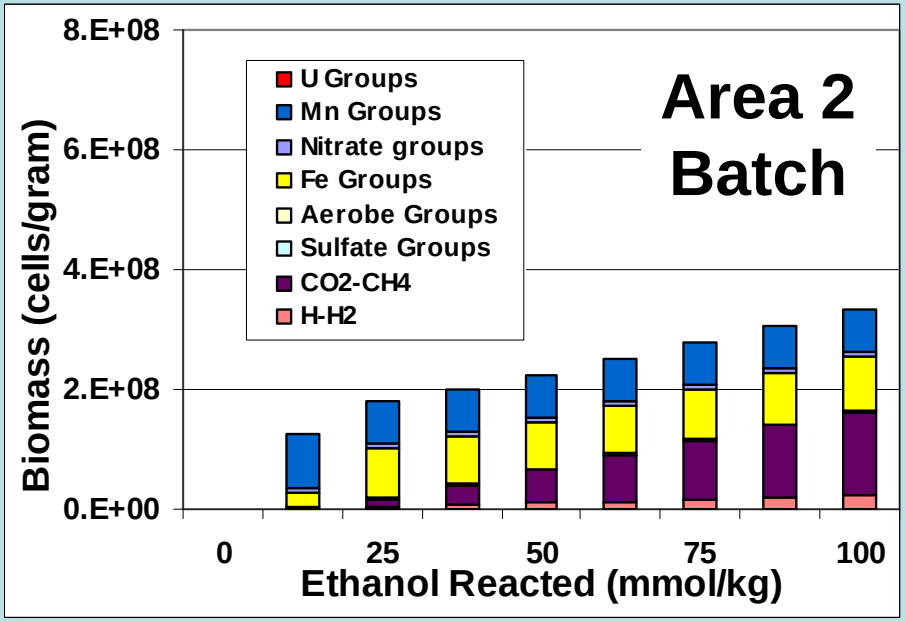


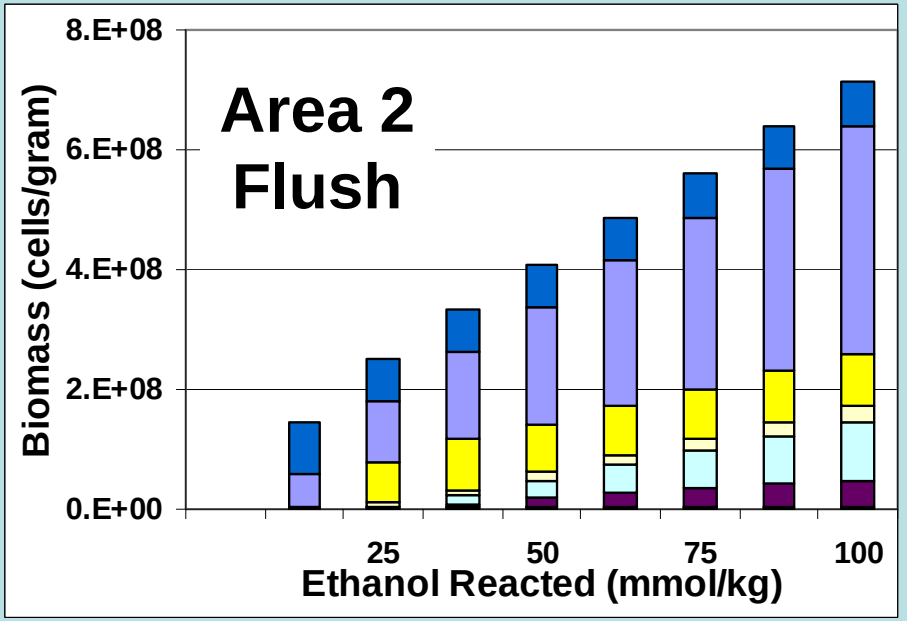
Porewater Functional microarray

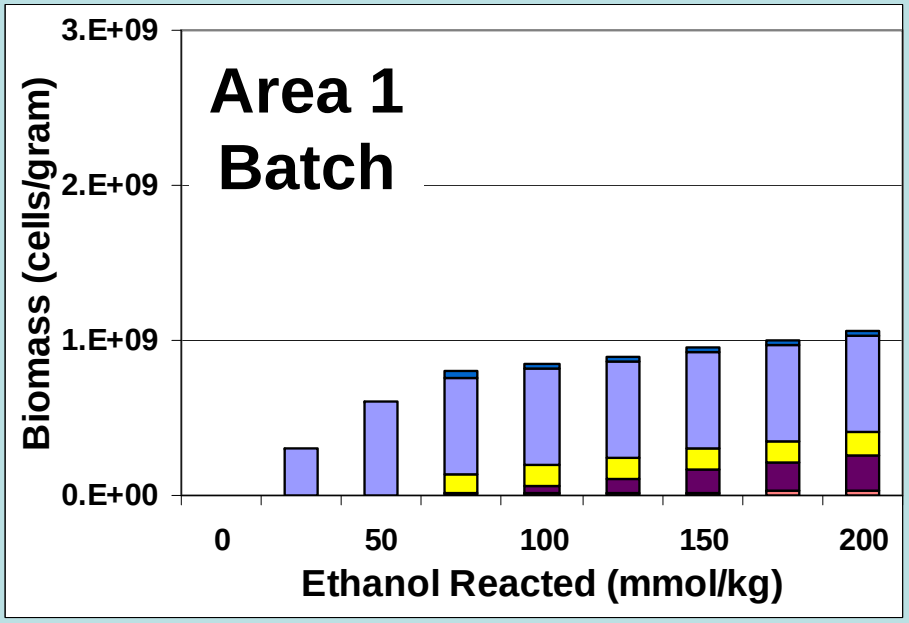


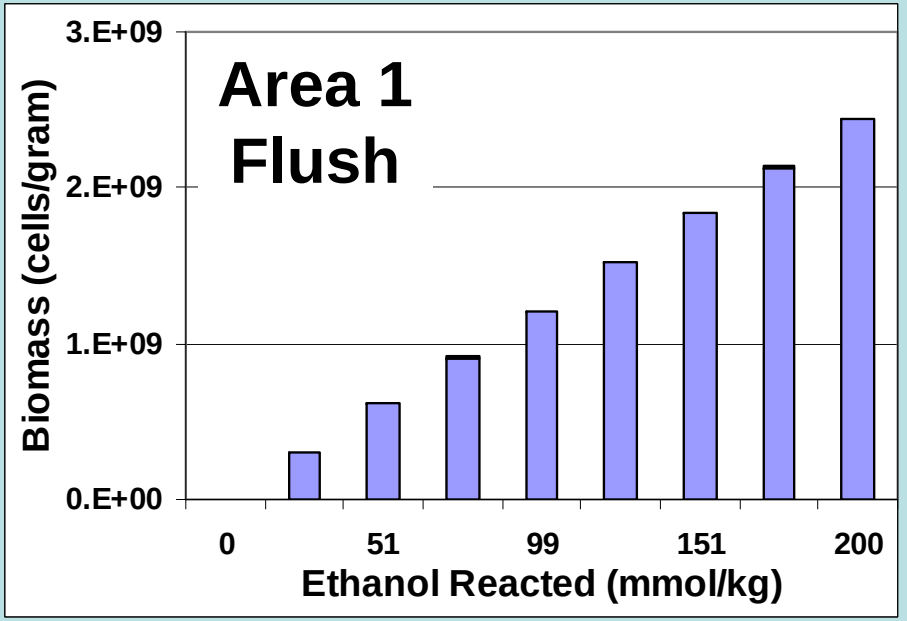


An interesting observation…









Conclusions

- ***In situ*** **experiments have demonstrated potential for bio-reduction in diverse geochemical environments**
- **Important Technical issues have been identified**
  - **Formation of mineral precipitates, biomass, and gas**
  - **Low stability of reduced sediments and contaminants following introduction of oxidants**
- **Single-well, push-pull tests demonstrated to be inexpensive, rapid, and effective method for detecting and quantifying effects of chemical amendments on the subsurface**

Conclusions

- ***In situ*** **experiments have demonstrated potential for bio-reduction in diverse geochemical environments**
- **Technical issues remain**
  - **Effects of mineral precipitates, biomass, and gas on sediment hydraulic properties**
  - **Stability of reduced sediments and contaminants following introduction of oxidants**
- **Single-well, push-pull tests demonstrated to be inexpensive, rapid, and effective method for detecting and quantifying effects of chemical amendments on subsurface**

Conclusions (cont.)

- **A new thermodynamic modeling approach that couples microbial growth with geochemical reactions can make useful predictions for the effects of chemical additions on complex, highly contaminated environments**
  - **Approach builds on well-known geochemical modeling techniques**
  - **Only required parameters are the free-energy dissipation for microbial growth on each substrate (e.g., ethanol, lactate, acetate, and hydrogen)**
  - **Fewer parameters makes it possible to model intact microbial communities in highly complex geochemical environments**
- **Initial porewater and sediment geochemistry data are only required inputs**

Conclusions (cont.)

- **Model predictions are in *qualitative* agreement with geochemical observations from laboratory batch experiments, field push-pull tests, intermediate-scale column experiments, and field natural gradient tests at three ERSP research sites**
  - **Consumption of electron acceptors (porewater and sediment)**
  - **Production of reduced metals and metabolic products (e.g. Fe^{2+}, Mn^{2+}, H_2, CH_4, Acetate)**
  - **Precipitation of sulfides, carbonates, and other minerals**
  - **Reductive precipitation of U, Tc, V, and Cr**
  - **Biomass increase (PLFA) and community composition (clone libraries)**
  - **Major microbial groups (PLFA, qPCR, functional microarray)**