Thermodynamics and Growth:

Is it possible to predict changes in microbial community composition using equilibrium-based reaction paths ?

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Data used for ongoing model testing has been generously supplied by"

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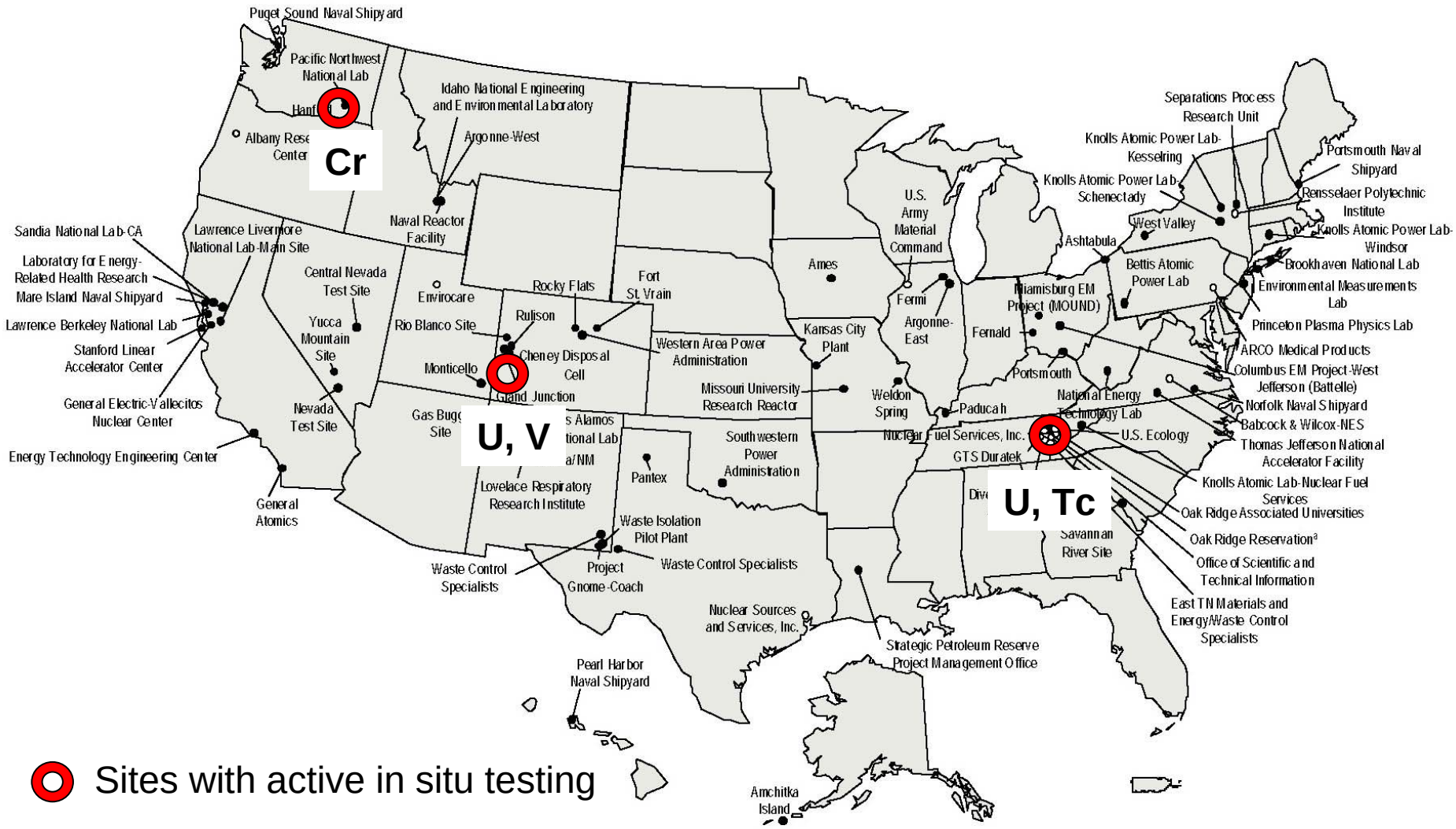
Why is the capability to predict microbial growth important ?

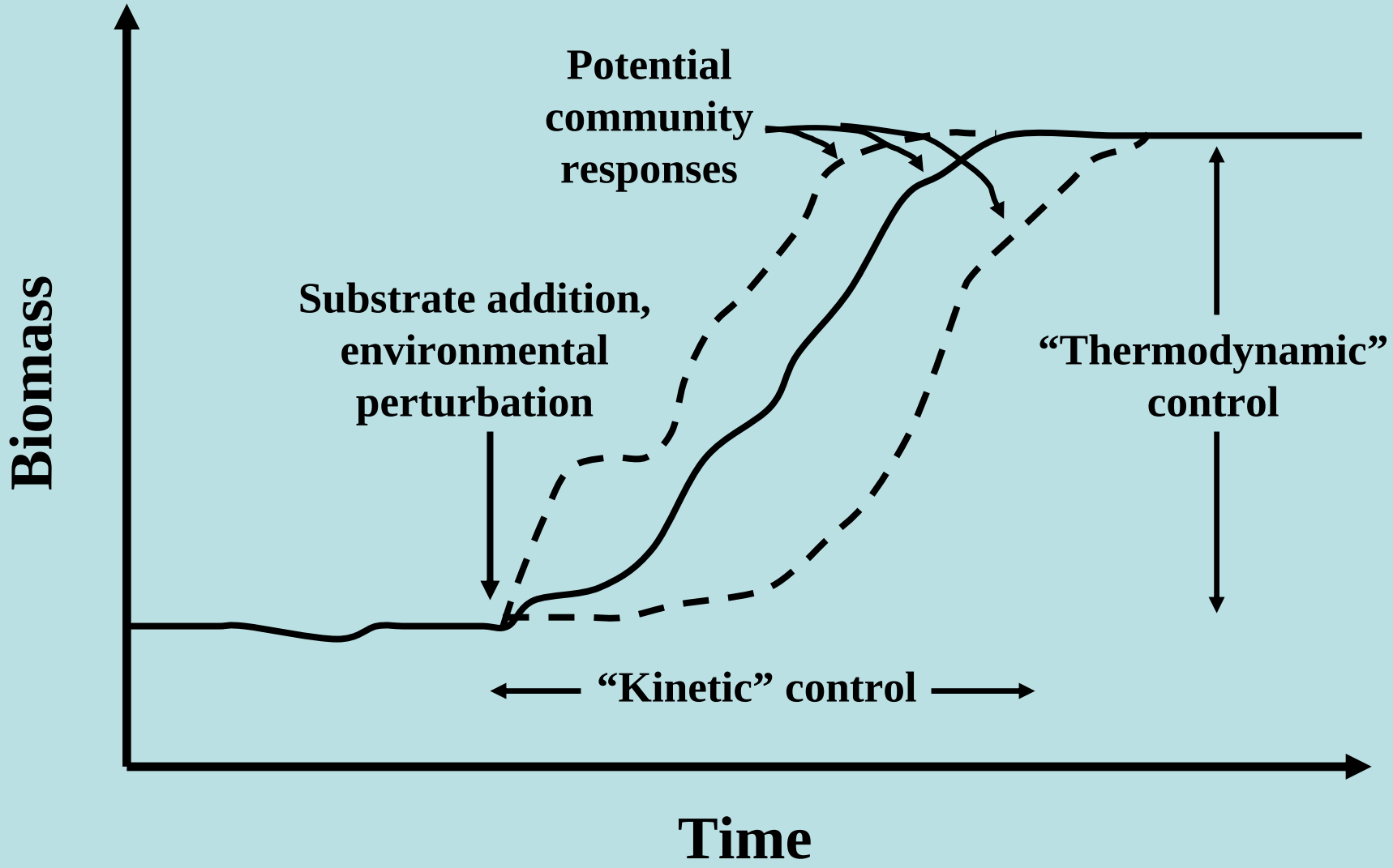
- **The number of contaminated sites is large and geochemical and contaminant conditions are highly variable from site-to-site**
- ***In situ* field research has been conducted at only a few locations**
- **Transferring results from the laboratory to the field or from one field site to the next has proven problematic**
- **Addressing these issues requires the continued development of process-based models:**
  - **Kinetic geochemical/transport models provide detailed descriptions but require many system-specific parameters**
  - **Thermodynamic models require fewer parameters…can they provide useful information ?**



Sites with active in situ testing

	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)

Equilibrium vs kinetic descriptions of microbial growth



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, growth yield, and overall growth equation**
- **Whichever groups can obtain the most energy from a growth substrate in a particular '*thermodynamic niche*' grow**
  - **System specific combination of electron donors, electron acceptors, metabolic products, other geochemical variables**
  - **Growth 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 balance, material balance, Gibbs energy balance, biomass yield

Biomass yield (Y_{DX}) estimated from Gibbs energy dissipated per C-mol produced biomass from compilations in:

Heijnen (1991) *Antonie van Leeuwenhock* **60**:235-256

Heijnen and van Dijken (1991) *Biotechnology and bioengineering* **39:**833-858

Growth equation examples

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

$6.1\ CH_3COO^- + 1\ NH_4 + 5.8\ NO_3^- + 3.7\ H^+$
$-5.9\ H_2O - 7.2\ HCO_3^- - 2.9\ N_2(aq)$

1 mol cells (Example iron reducer group, YDx = 0.07) =

$67.4\ H_2(aq) + 1\ NH_4^+ + 114.8\ Fe^{3+}$
$-114.8\ Fe^{2+} - 110.8\ H^+ - 13\ H_2O - 5\ HCO_3^-$

1 mol cells (Example sulfate reducer group, YDx = 0.10) =

$24.9\ CH_3COO^- + 22.5\ SO_4^{2-} + 1\ NH_4^+ + 1.5\ H^+$
$-3\ H_2O - 44.9\ HCO_3^- - 22.5\ HS^-$

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

Computational procedure

Define microbial groups

Write growth equation for each group (one Y_{DX} per group)

Compute ΔGs and logKs

MATLAB Program

↓

Add growth equations, ΔGs and logKs, and required substrates to thermodynamic data base

MATLAB Program

↓

Specify initial aqueous and sediment geochemistry

Select reaction path type

"Batch" vs "Flush"

React substrates and solve coupled microbial growth and geochemical reactions

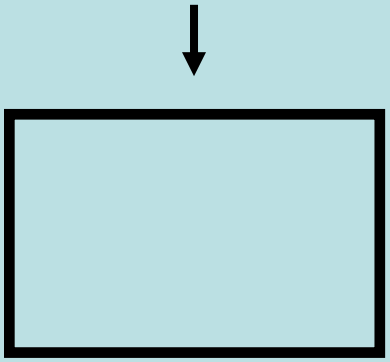
Geochemist's Work Bench

Equilibrium reaction paths

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

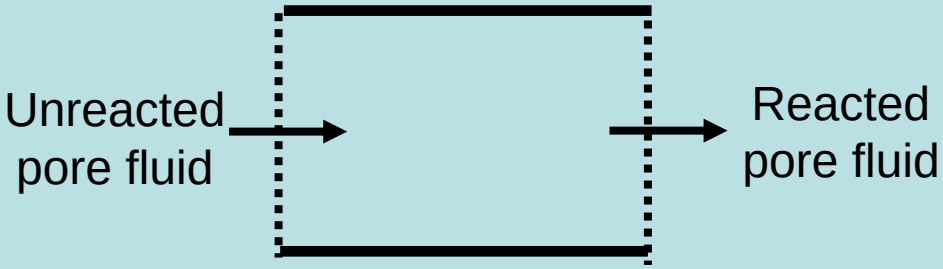
Ethanol, lactate, or acetate



Laboratory microcosms/
Field push-pull tests

"Flush" (open) Simulations

Ethanol, lactate, or acetate

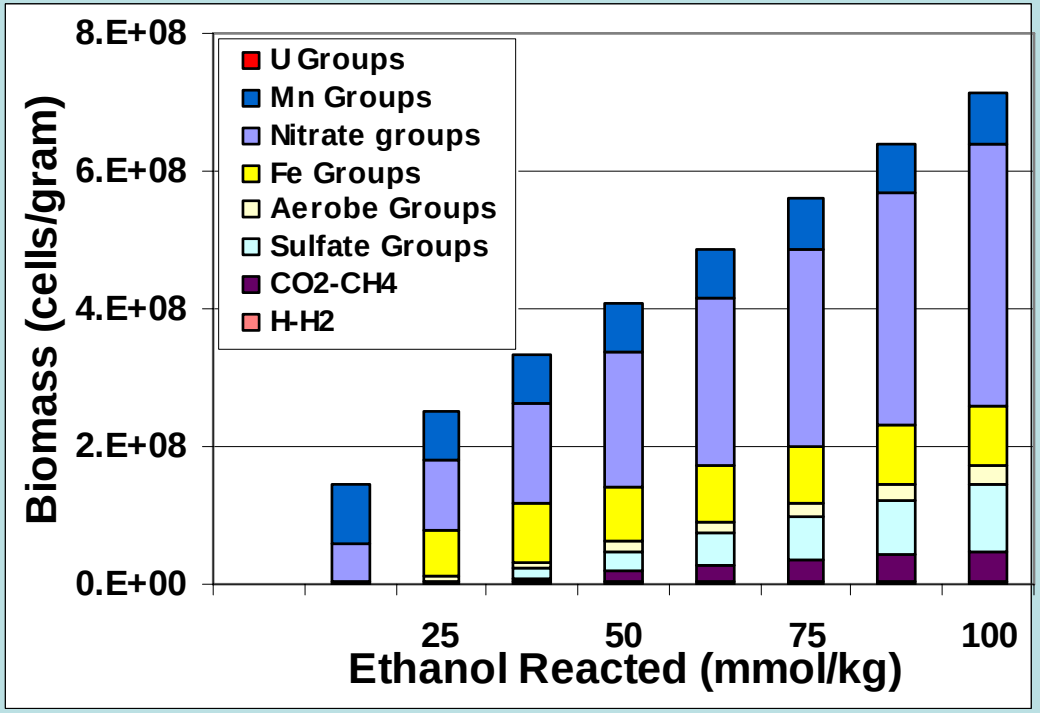


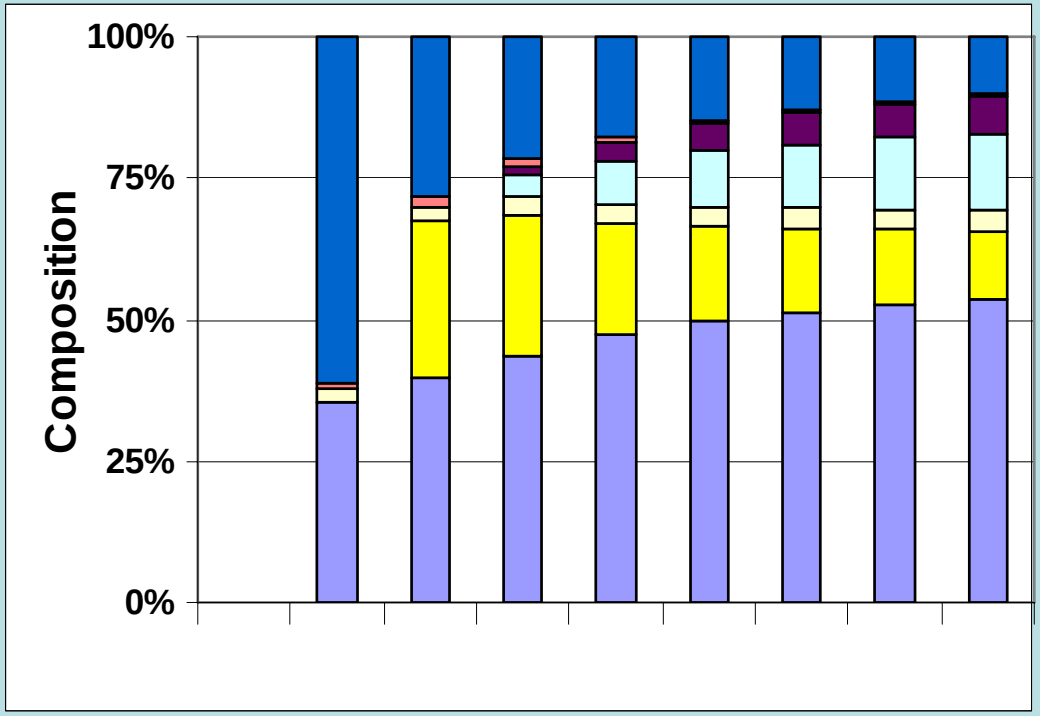
Laboratory columns/
Intermediate-scale physical models
Field natural gradient tests

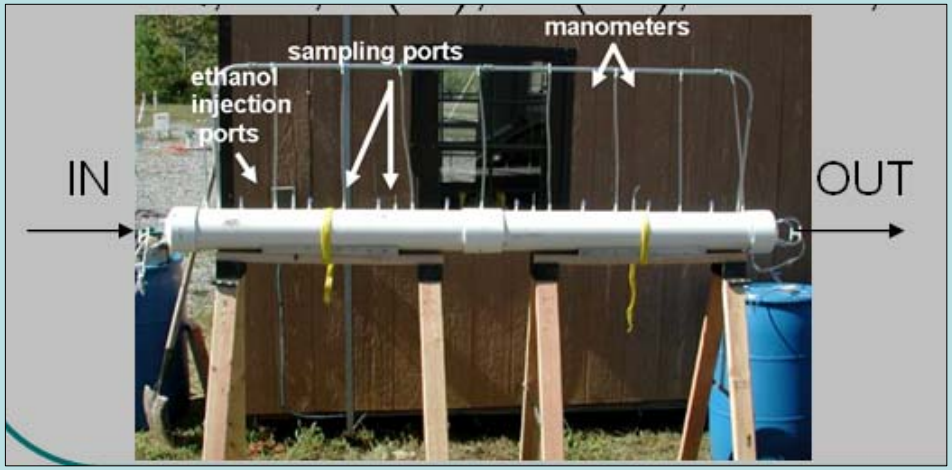
Example flush simulation for FRC Area 2

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

	(mM)
pH	6.4
O_2	0.1
NO_3^-	1.2
SO_4^{2-}	0.8
Iron oxides (mmol/kg)	306
Mn oxides (mmol/kg)	48
Ca	3.5
Mg	1.1
Al	-
HCO_3^-	0.1
U	4.9×10^{-3}
Tc	4.1×10^{-7}

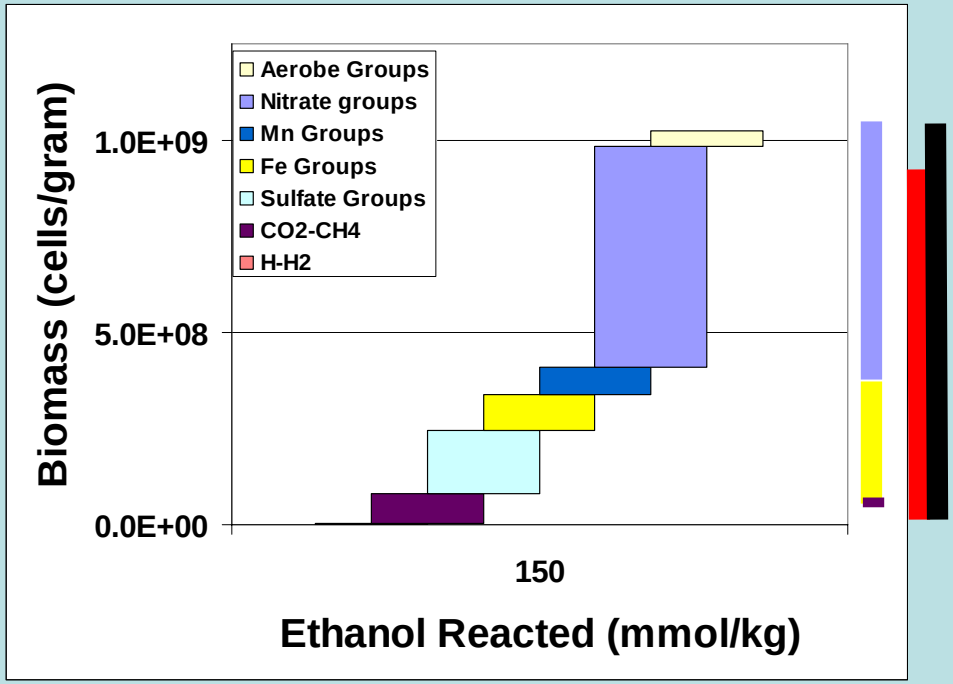






24 month experiment
809 L GW835 groundwater
Growth stimulated with ethanol
Complete mass balance

Comparison of Model Prediction with Sediment Microbial Analyses



Sediment analyses (n = 8)

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

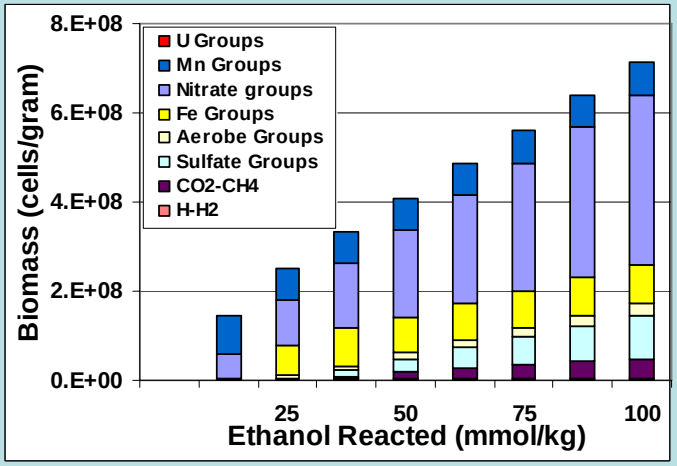
Eubacterial 16S n RNA
= 1. x 10^9 copies/gram

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

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

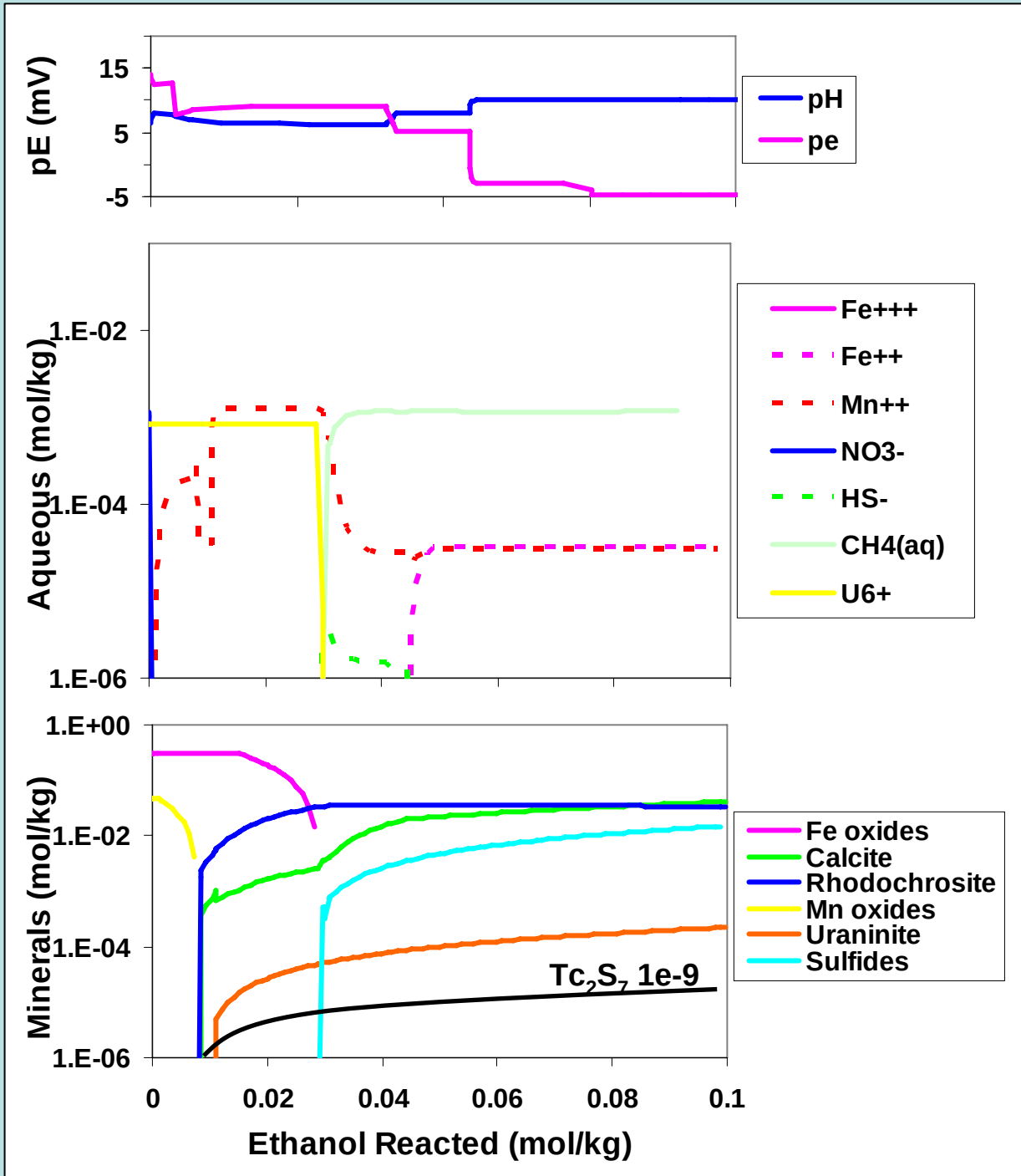
Geobacter
= 1 x 10^4 copies/gram

Methanogens
= 1 x 10^6 copies/gram



Model predicts major biogeochemical processes observed during experiment

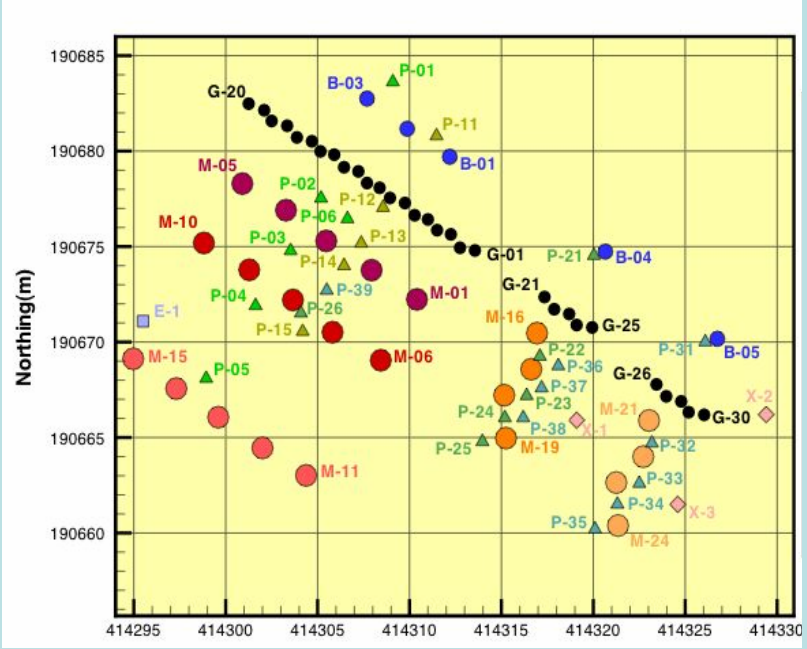
Reduction of soluble electron acceptors
Accumulation of Fe^{2+}, Mn^{2+}, HS^{-}, CH_4
Reduction of U(VI) and Tc(VII)

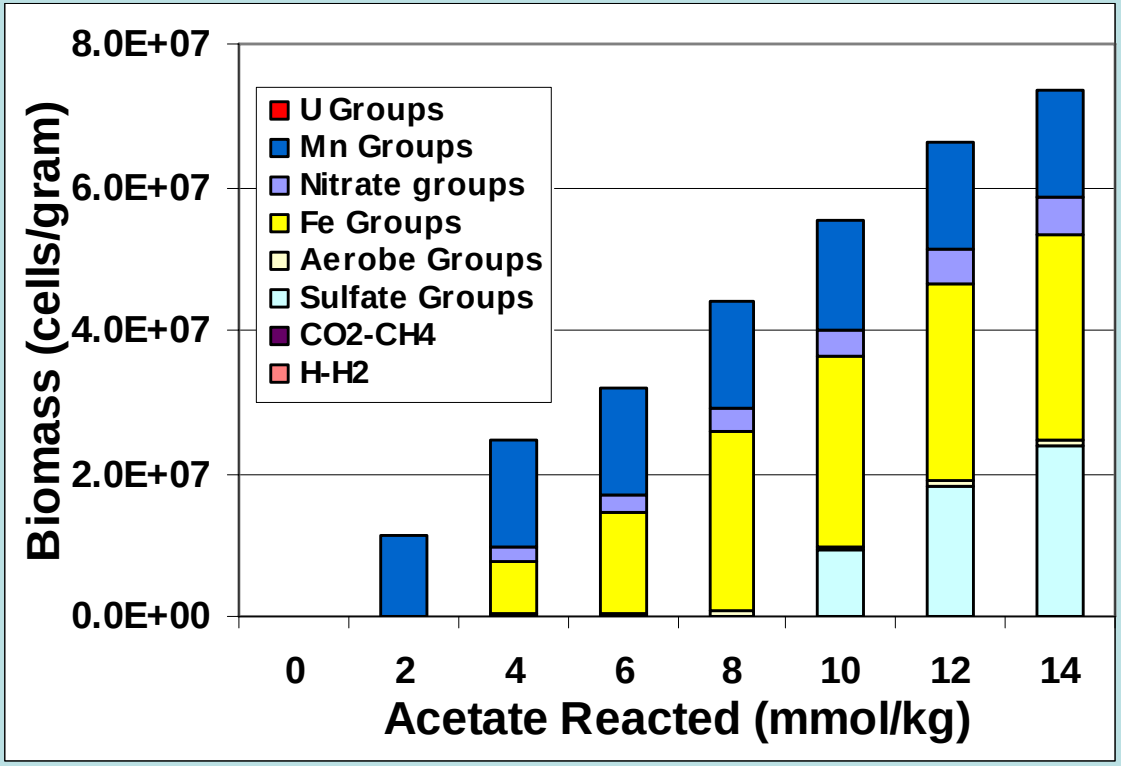


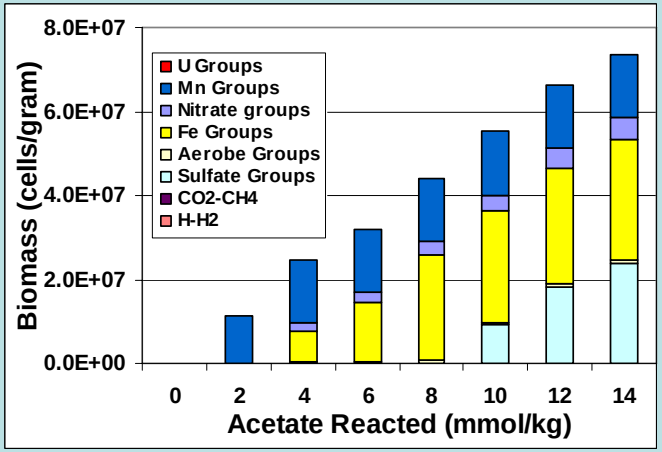
Example flush simulation for Old Rifle

- 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}







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Reduction of soluble electron acceptors
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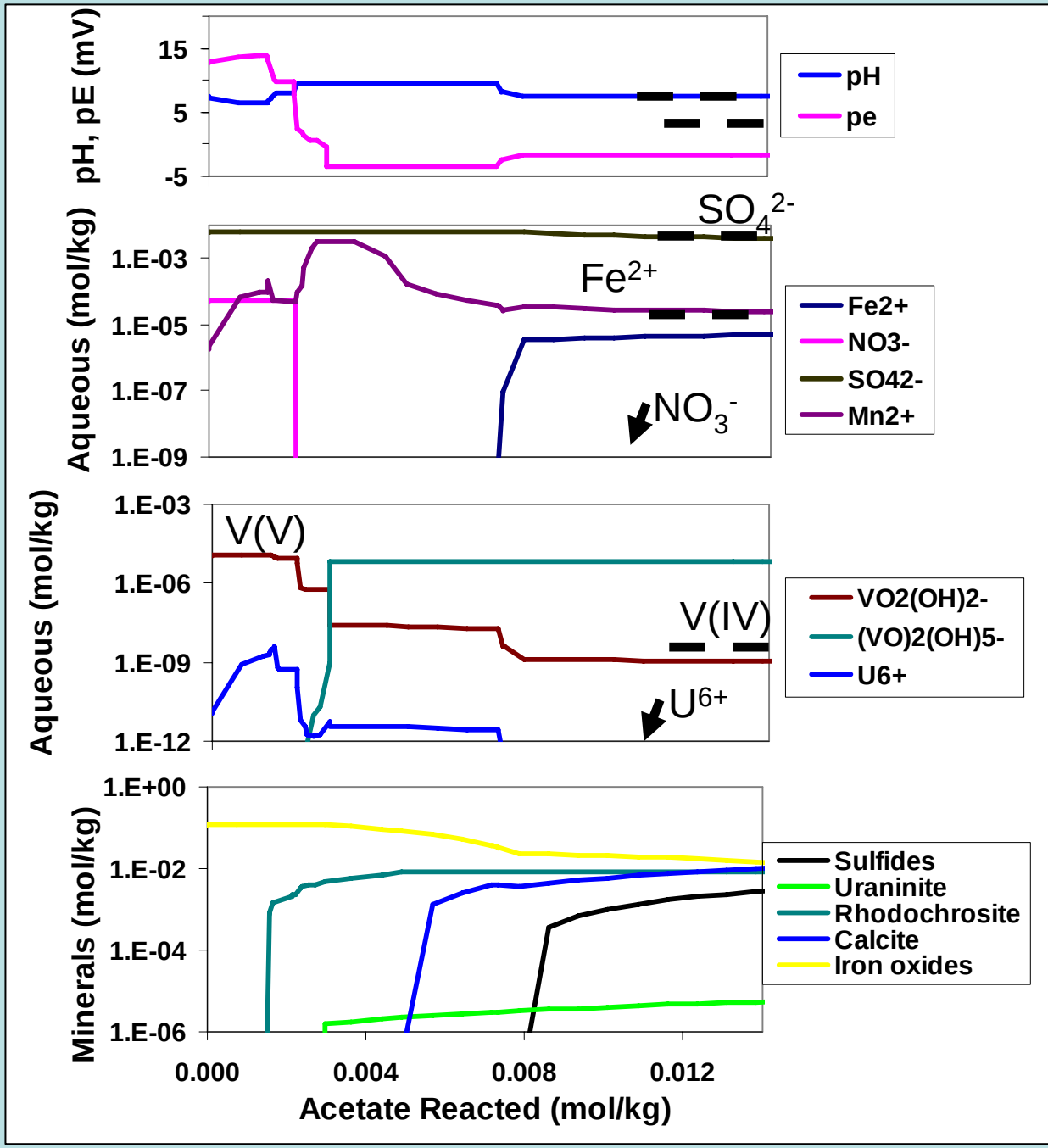
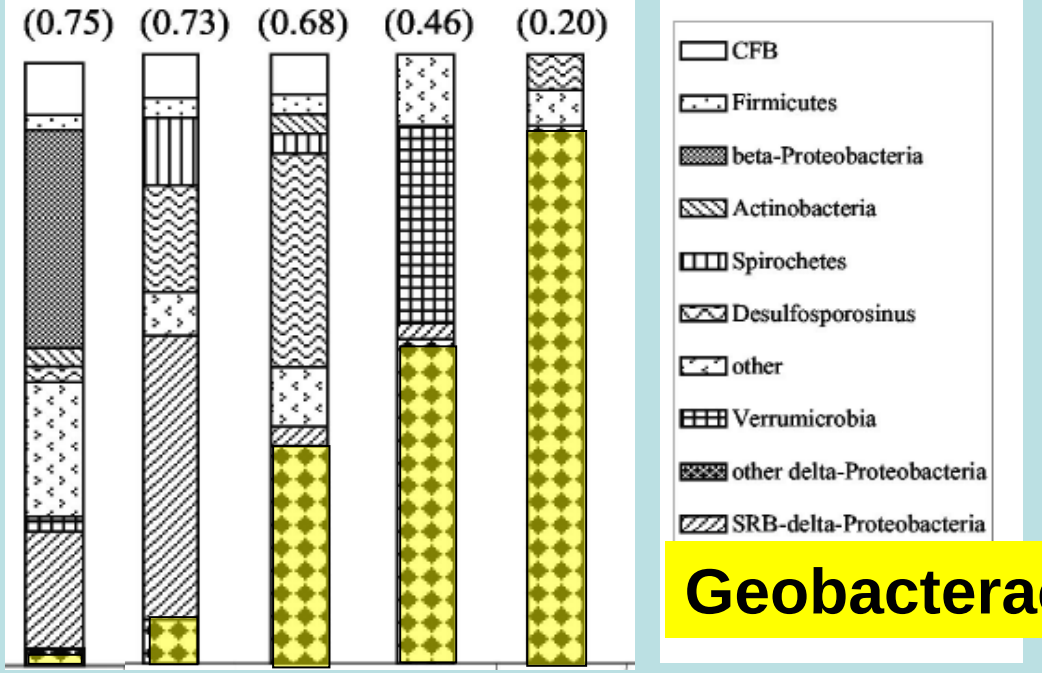
Reduction of U(VI) and V(V)

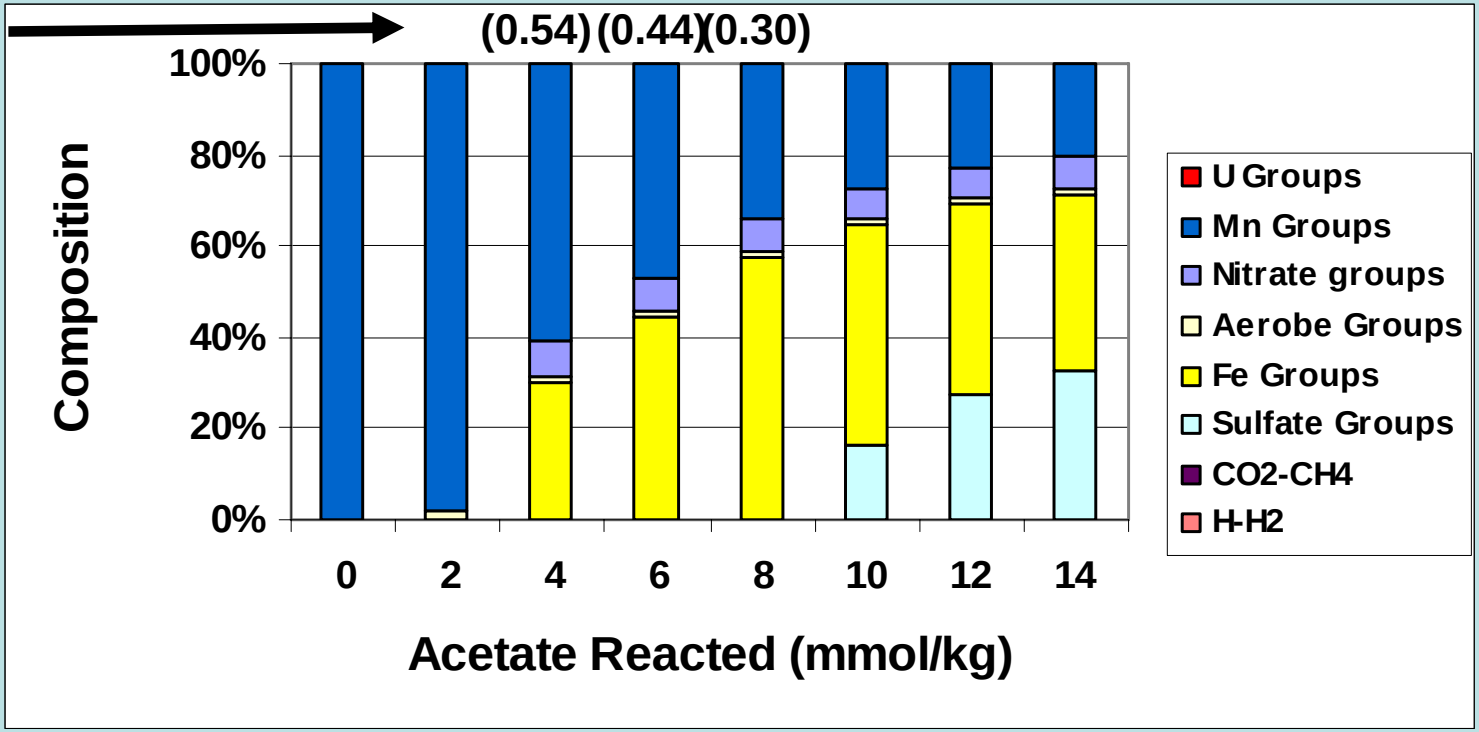


Anderson et al. (2003) Clone libraries from groundwater samples

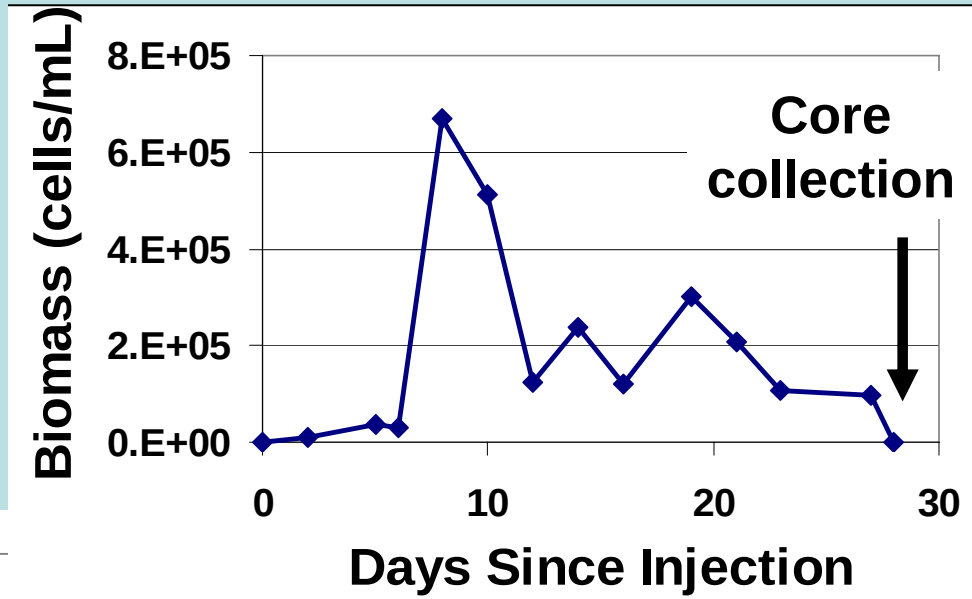


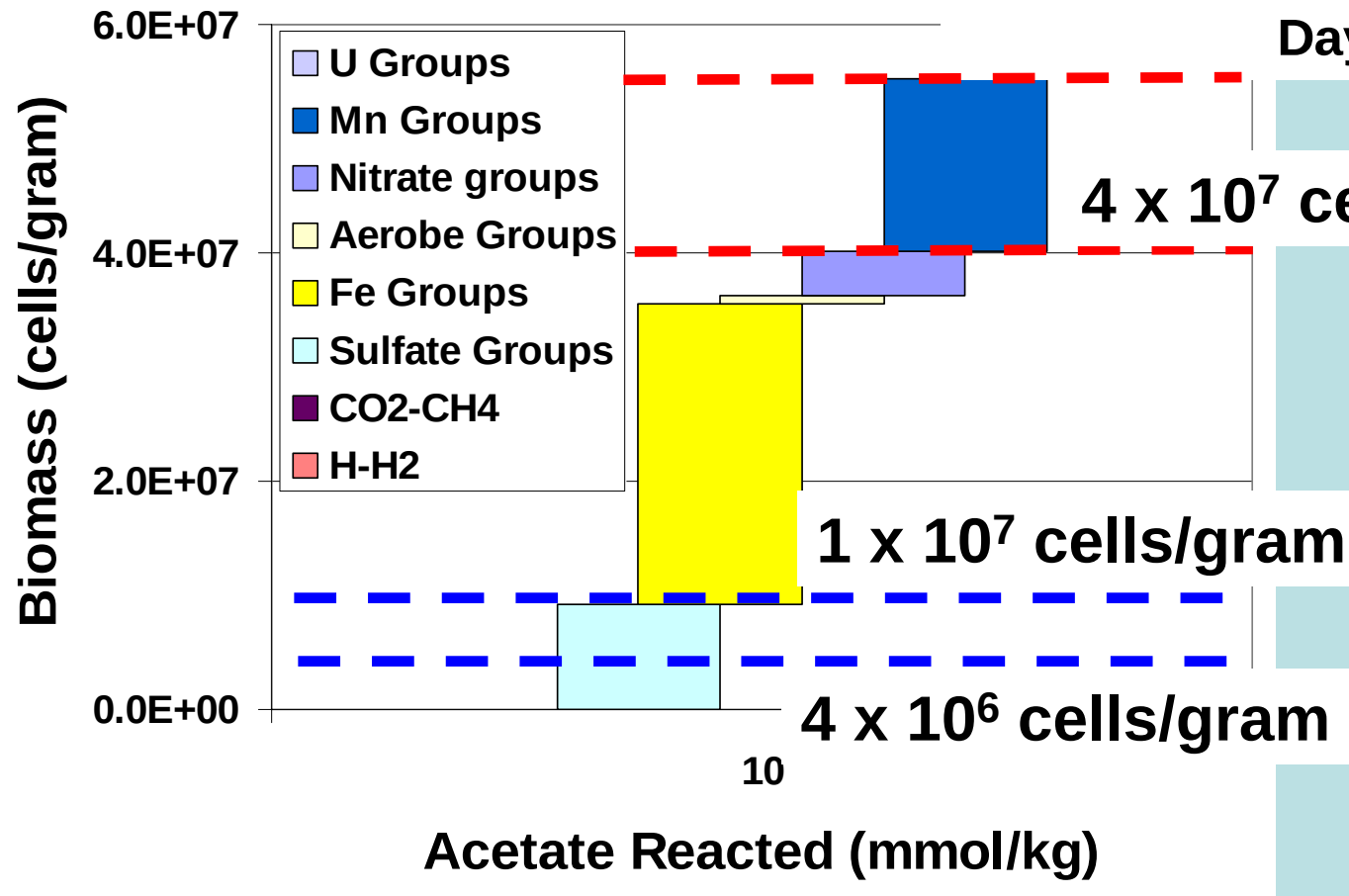
Geobacteraceae

(Community diversity index)



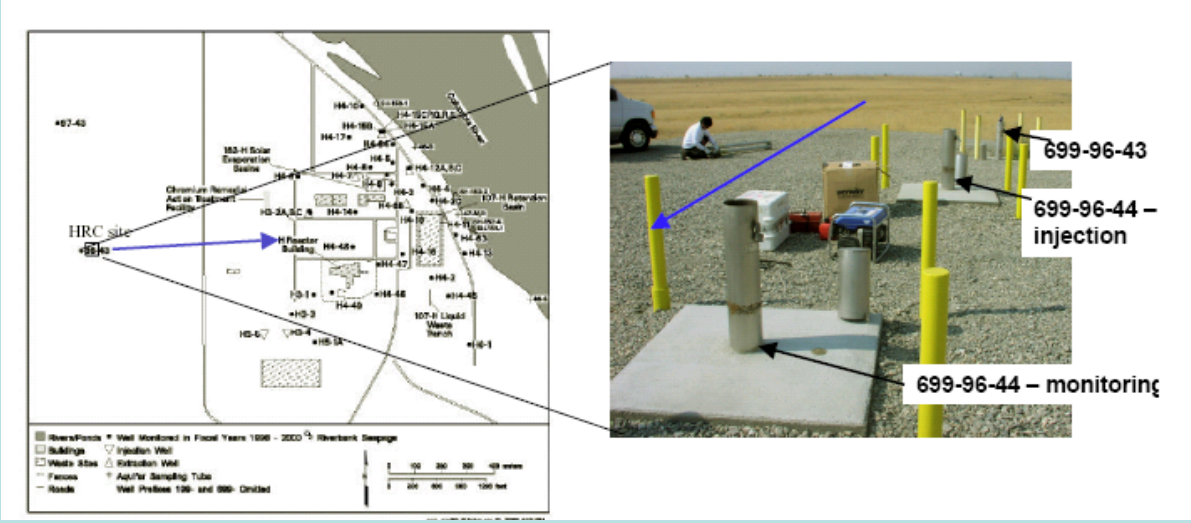
^{13}C Acetate Experiment



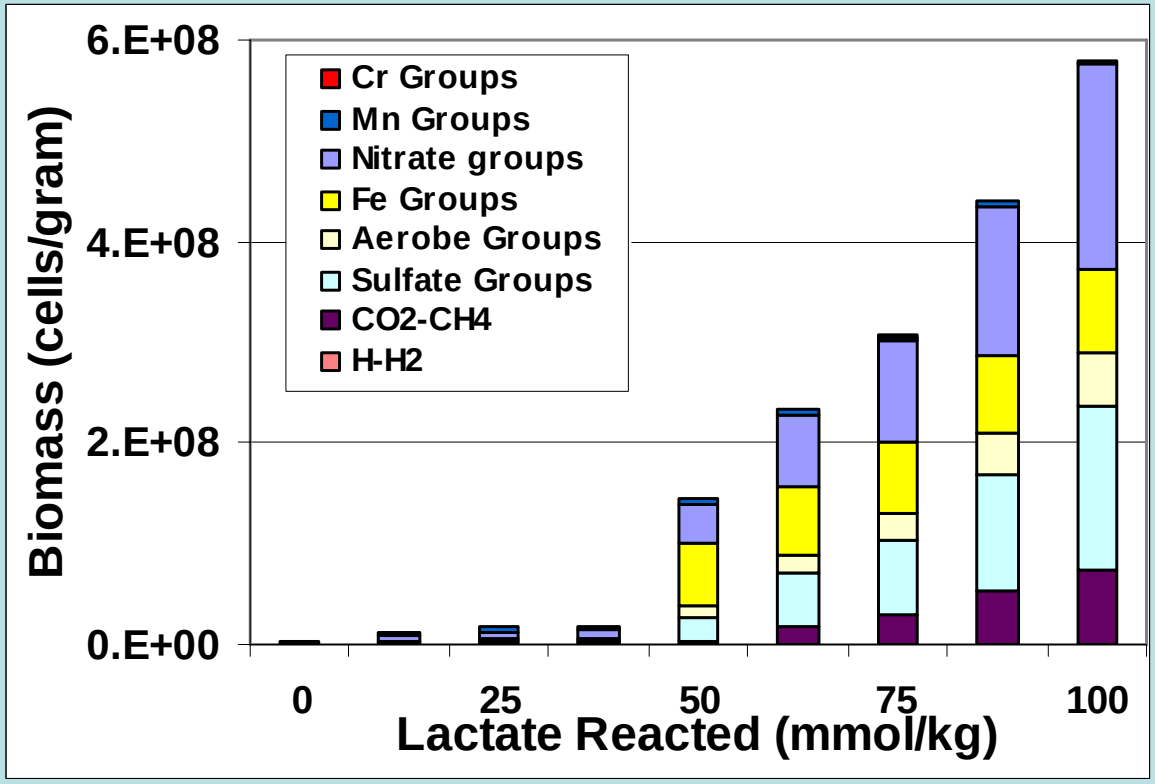


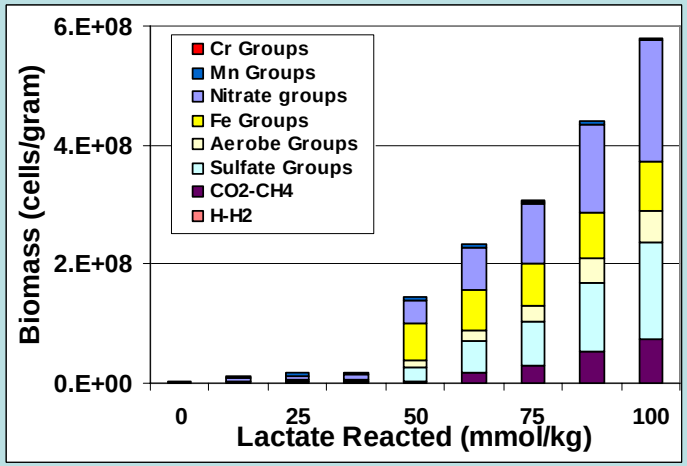
^{13}C PLFA Biomass from sediment cores

Example flush simulation for Hanford 100H



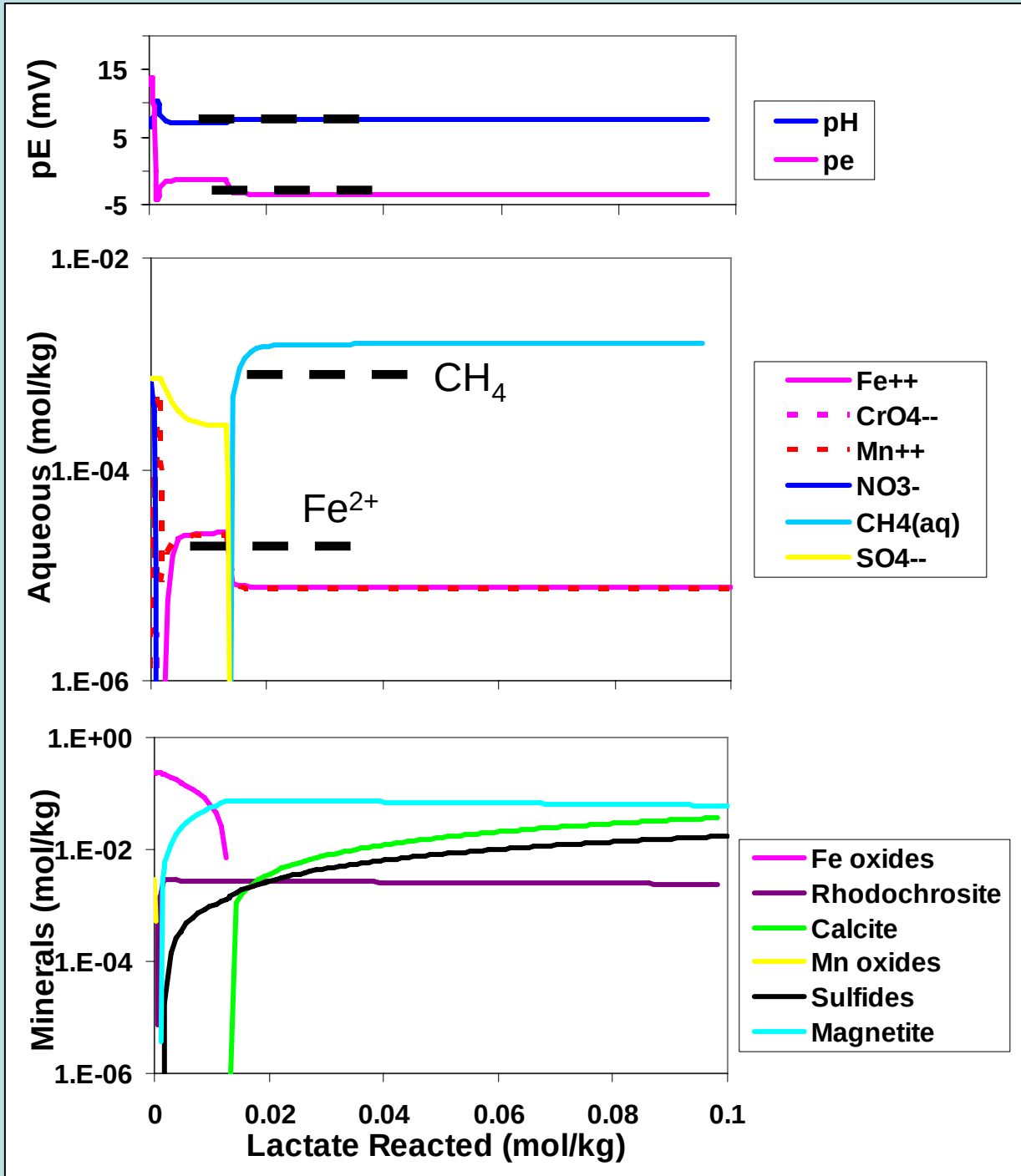
	(mM)
pH	7.8
O_2	0.1
NO_3^-	0.7
SO_4^{2-}	0.7
Iron oxides (mmol/kg)	233
Mn oxides (mmol/kg)	3
Ca	1.5
Mg	3.0
Al	-
HCO_3^-	0.1
Cr	2.93×10^{-2}

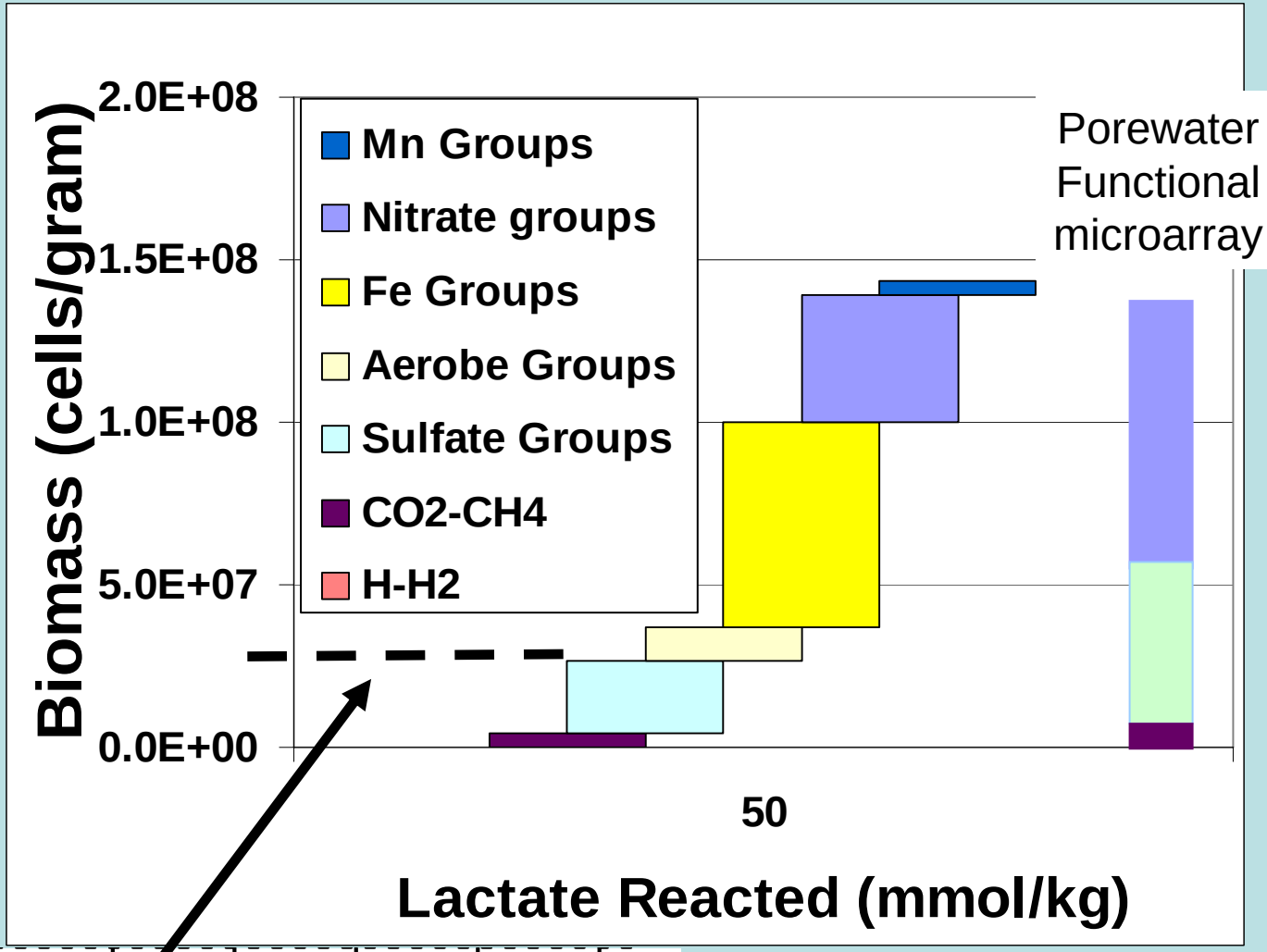




Model predicts major biogeochemical processes observed during experiment

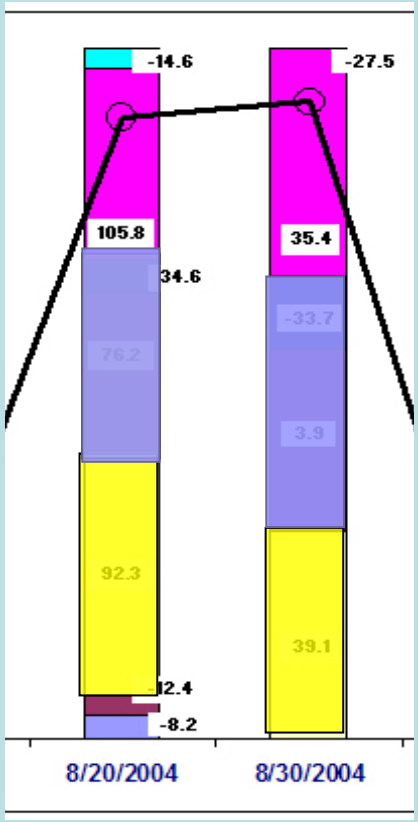
Reduction of soluble electron acceptors
Accumulation of Fe^{2+}, CH_4
Reduction of Cr(VI)



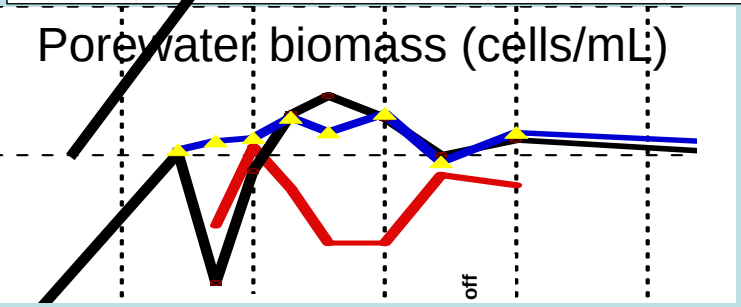


Porewater Functional microarray

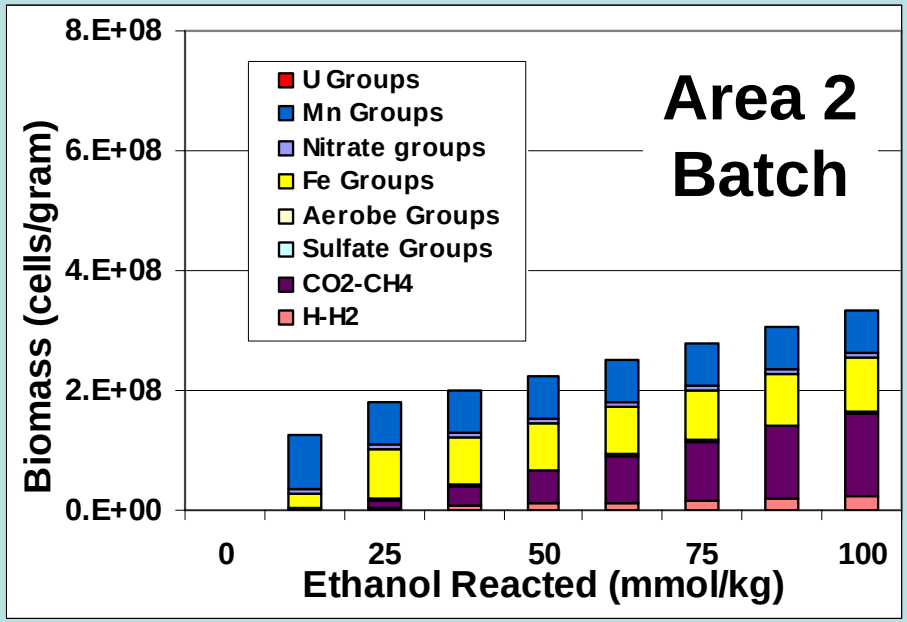
Porewater PLFA

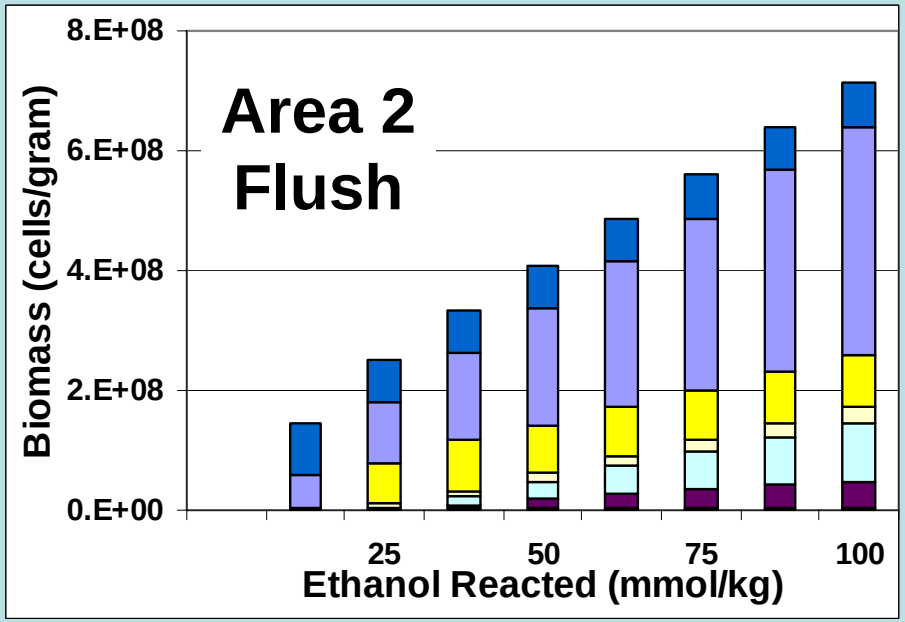


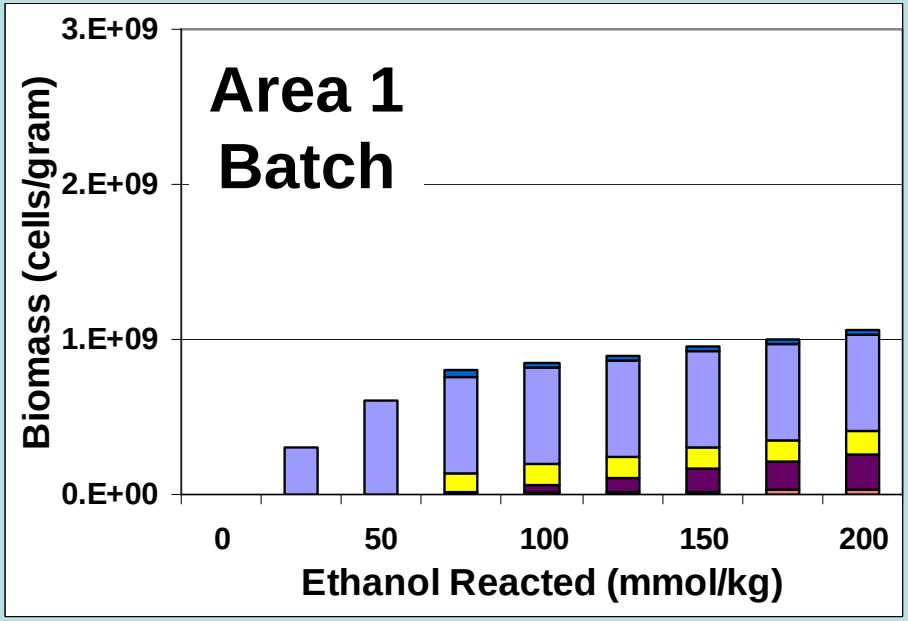
Porewater biomass (cells/mL)

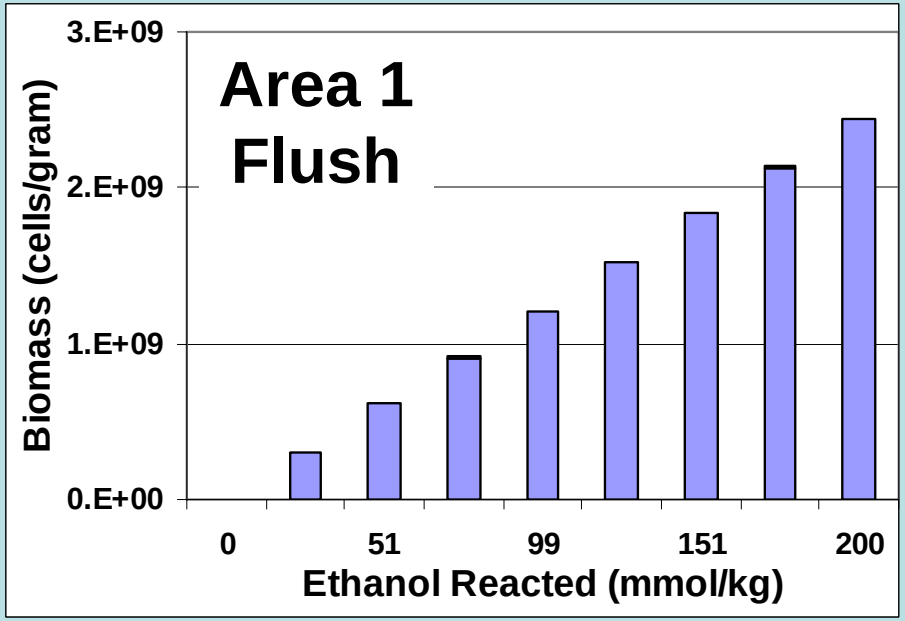


An interesting observation…









Conclusions

- **Thermodynamic approach provides quantitative, system-specific predictions for microbial growth coupled to porewater and sediment geochemistry**
- **Only required microbial parameter are the cell yields, YDxs, one for each microbial group**
- **Model predictions are in qualitative agreement with geochemical observations from many experimental systems (laboratory batch experiments, field push-pull tests, intermediate-scale column experiments, 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**

Conclusions (cont.)

- Model predictions closely matched biomass (PLFA) and community structure (PLFA, qPCR) data from intermediate-scale column experiments at FRC Areas 1 and 2
- Model accurately predicted shifts in microbial community structure at Old Rifle (porewater PLFA and clone libraries) but overestimated biomass (PLFA) in sediment cores
- Predictions for Hanford 100H match community structure (functional microarray and PLFA)
- Model provides insight into long-standing difficulty in transferring laboratory results to the field
  - Note on scaling: system composition scales exactly
- Model may be a useful tool for rapid preliminary testing of alternate biotechnologies across the DOE complex
  - Porewater and sediment geochemistry only required inputs