NABIR

Biostimulation of Microbial Metabolism in Acidic FRC Subsurface Sediments

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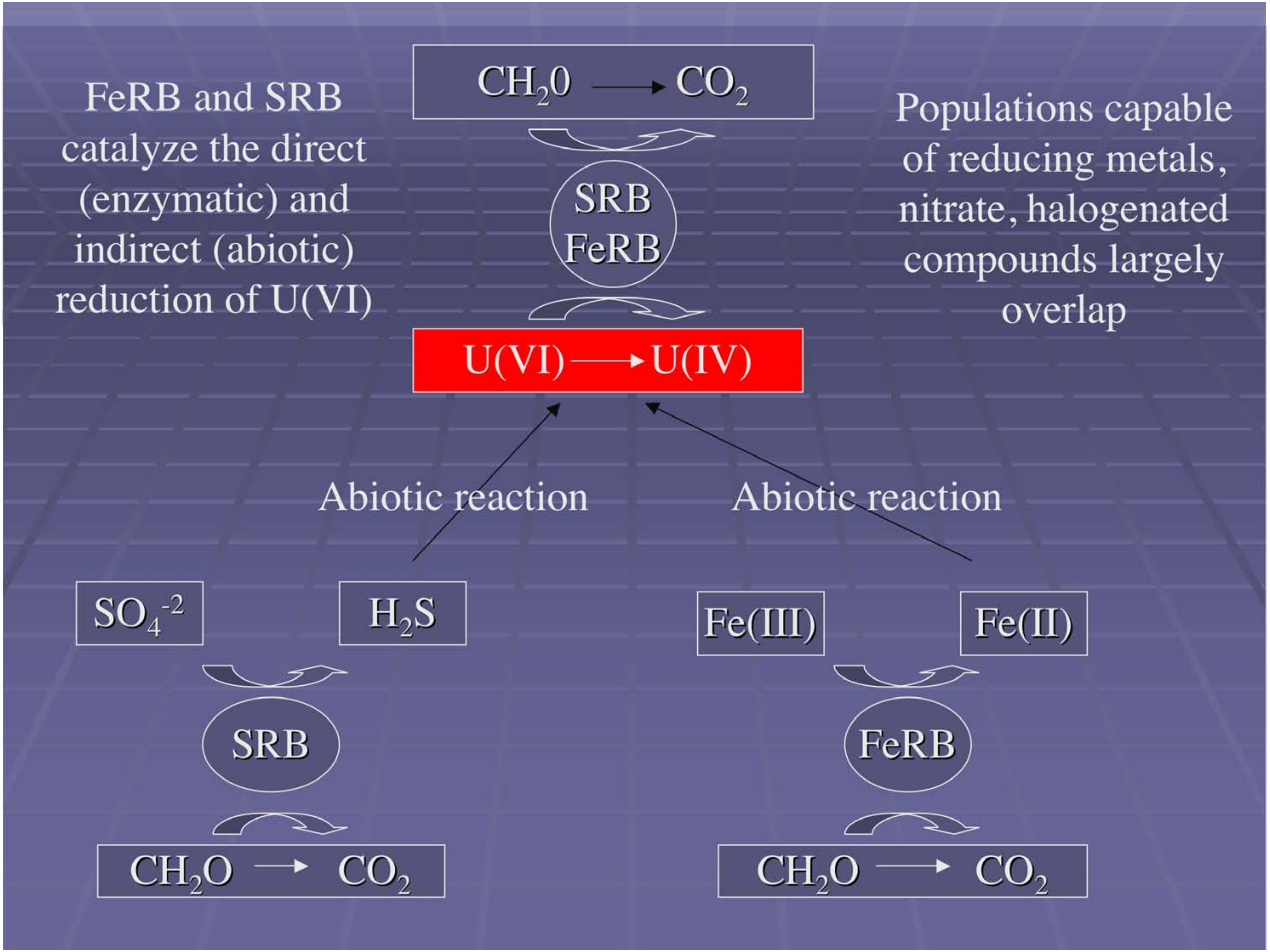
Outline

- Introduction
- Microcosm Experiments
  - Electron acceptor utilization
  - Electron donor metabolism
  - Pathways
- Conclusions
- Future work

Intro to FRC

- FRC = harsh environment for microorganisms; Ex. pHs often 3-4 in contaminated areas
- Upon addition of electron donor and pH neutralization, extensive nitrate and metal reduction have been observed
- Thus, communities believed to be limited by: low C, pH and high nitrate, toxic metals
- What we don't know… a great deal.


FeRB and SRB catalyze the direct (enzymatic) and indirect (abiotic) reduction of U(VI)
$CH_20 \rightarrow CO_2$
SRB
FeRB
$U(VI) \rightarrow U(IV)$
Populations capable of reducing metals, nitrate, halogenated compounds largely overlap
Abiotic reaction
Abiotic reaction
SO_4^{-2}
H_2S
Fe(III)
Fe(II)
SRB
FeRB
$CH_2O \rightarrow CO_2$
$CH_2O \rightarrow CO_2$

Objectives

- Experimentally determine the controls or mechanisms of microbial metabolism under close to *in situ* conditions in microcosms
- Determine rates and pathways of dominant terminal-electron-accepting processes
- Compare electron donors for their biostimulation potential
- Provide inputs for reactive transport modeling to improve U bioremediation strategies

Biostimulation Experiments - Hypotheses

- For bioremediation (immobilization) of U(VI), environmental conditions of subsurface must be altered to stimulate U(VI) reduction
- U(VI) reduction rate is most likely limited by pH and the abundance of nitrate, electron donor
- Upon pH neutralization and electron donor addition, indigenous microbial communities will be stimulated to reduce NO_3^-/ Fe(III)/ Mn(IV)/ U(VI)

Objectives of *In situ* Biostimulation Experiment

- To determine structure/ function relationships of metal-reducing bacteria and competing heterotrophs during *in situ* bioremediation in acidic subsurface environments
- Quantify microbial activity using geochemical analysis of groundwater/ sediments (push-pull activity tests)
- In parallel, quantify the change in the abundance/ diversity of sedimentary microbial communities using cultivation-independent methods
  - Quantitative MPN (most probable number)-PCR
  - Cloning and sequencing of 16S rRNA genes

In Situ Biostimulation using Push-Pull Activity Tests Jack Istok - OSU

- Biostimulation: addition of electron donors to increase microbial activity
- Push-pull activity tests: wells were injected with site groundwater, bicarbonate, an inert tracer, and an electron donor (glucose or ethanol)
- Groundwater chemistry was monitored over time to determine the kinetics of electron donor and acceptor utilization
- Sediment cores collected in the zone of influence surrounding wells, before and after electron donor addition

Treatments - Sediment Microcosms

- Area 1 sediments - pH 4, pH 5-7
- Neutralization with bicarbonate
- Washing to remove toxic metals
- Area 1 groundwater or distilled water
- Variety of electron donors

Initial Sediment Geochemistry

Sample	pH	Nitrate ($\mu mol/cm^3$)	Fe-HCl ($\mu mol/cm^3$)	Fe-Dithionite ($\mu mol/cm^3$)
30	4.2	22.7-31.5	15.2	414.4
31	4.8	0.1-91.7	10.8	376.4
32	4.4	0.02-17.2	18.9	407.9
33	3.6	13.6-295	14.5	467.7
34	3.8	3.3-70.5	16.3	325.0

Incubations at Bitök

Slurry	pH	Nitrate (mM)	EDs
1	5.5-6	0-0.1	Ctrl, Lac, Glu, EtOH, H_2
2	5.5-6	20-25	Ctrl, Lac, Glu, EtOH, H_2
3	4.3	20-25	Ctrl, Glu, EtOH
4	7.0	20-25	Ctrl, Glu, EtOH
5	6.8	1-2.5	Ctrl, Glu, EtOH
6	7.0	1-2.5	Ctrl, Glu, EtOH

Analytical Measurements

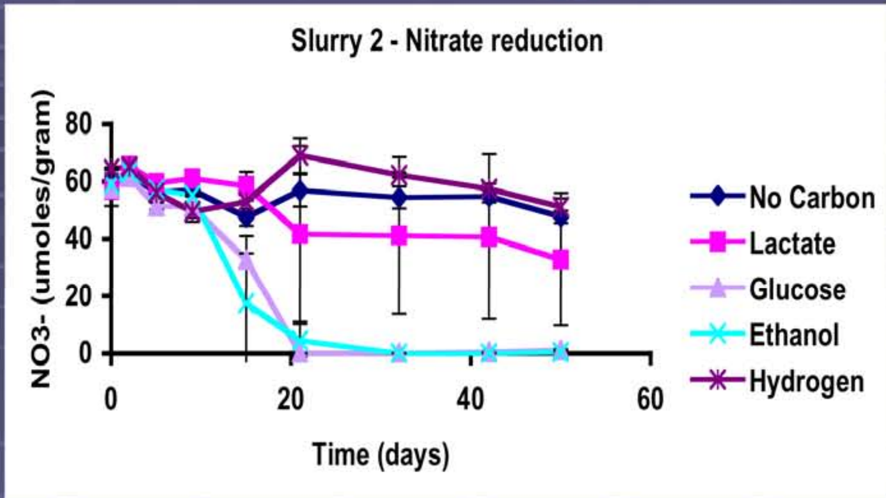
- Gases - CO_2, H_2, CH_4, N_2
- Carbon substrates (HPLC)
- Nitrate
- Nitrite
- Fe(II)
- Sulfate
- pH

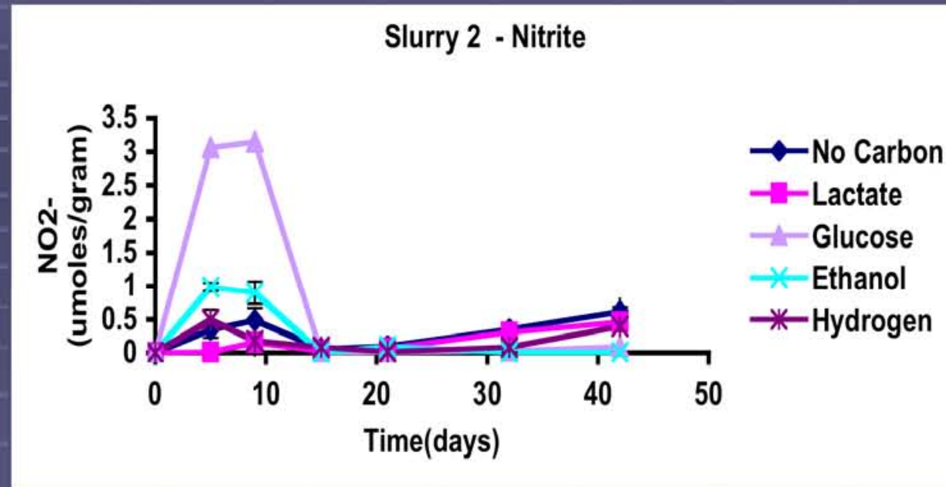
Area 1 Slurries

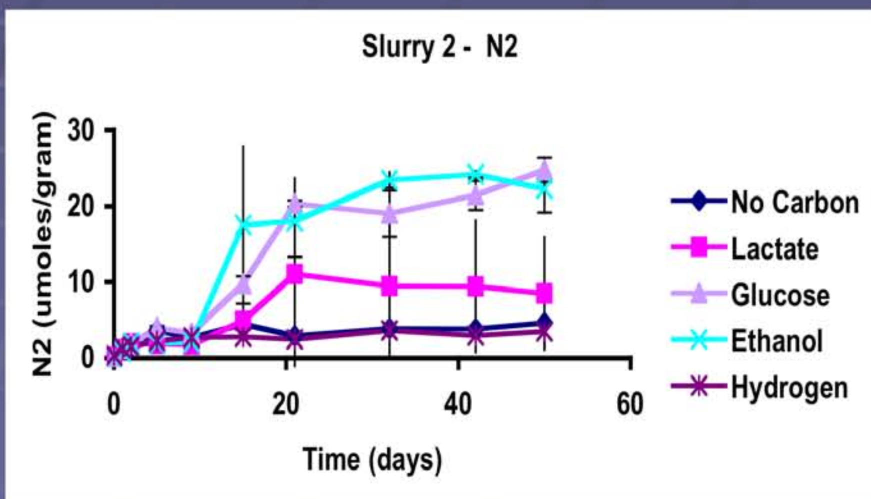
- Biostimulation clearly demonstrated
- Activity and geochemistry varies even within core
- Microbial groups display similar heterogeniety?

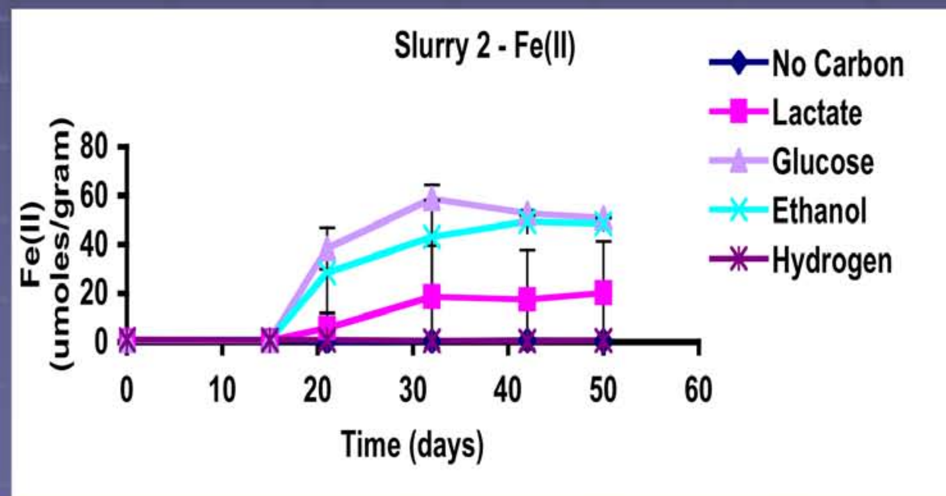

No C-2
Lac-2
Glu-2
EtOH-2
H_2-2
Slurry 2
(neutral sediment + nitrate)
Day 35

Sed. pH 5-6 - TEAPs



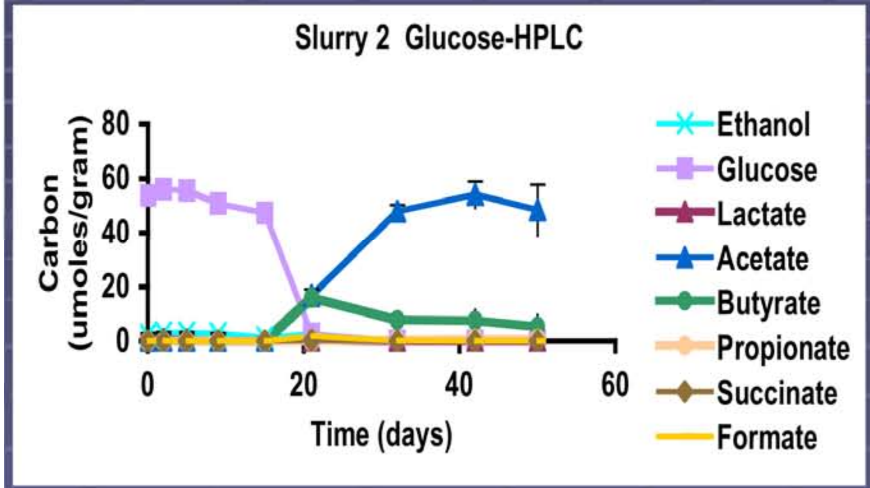


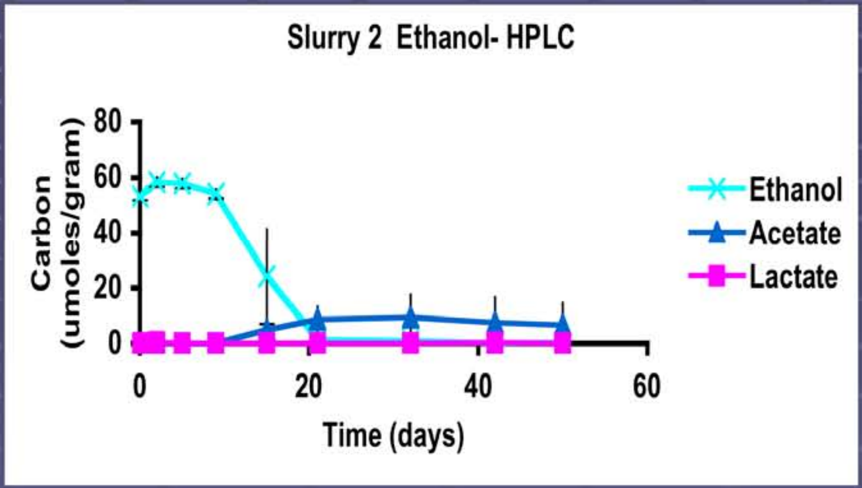


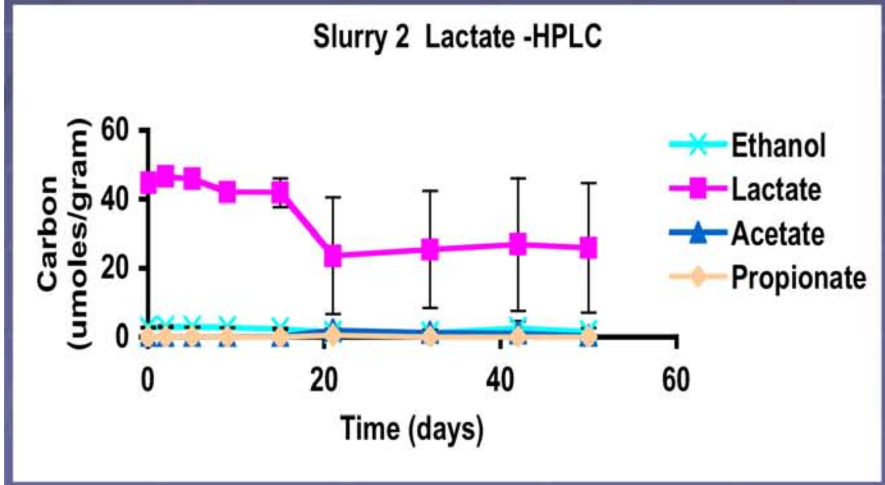


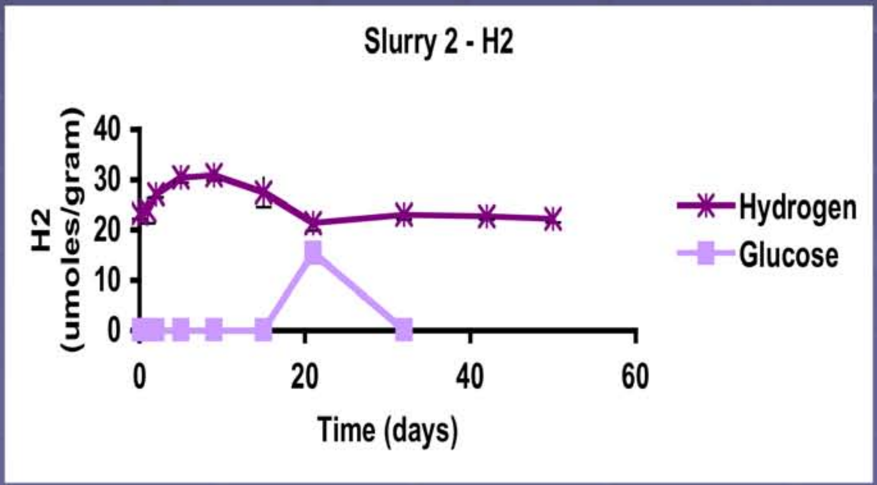
✓ Complete denitrification followed by substantial Fe(III) reduction

Sed. pH 5-6 - Donors





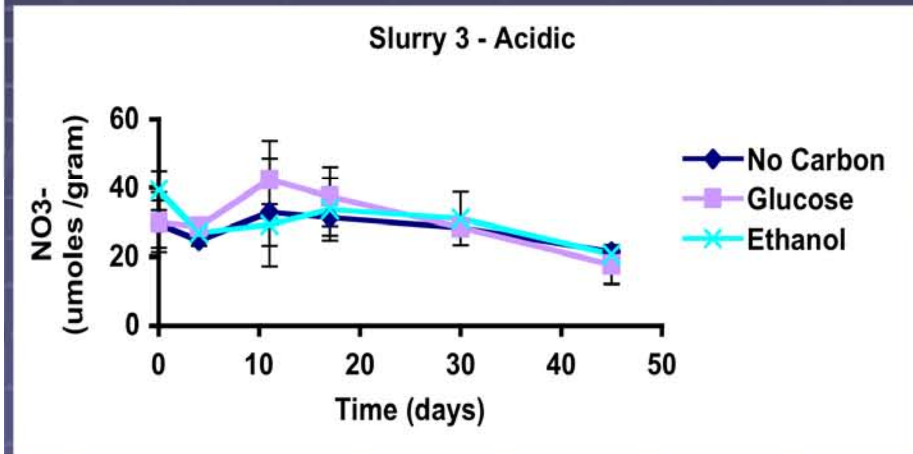


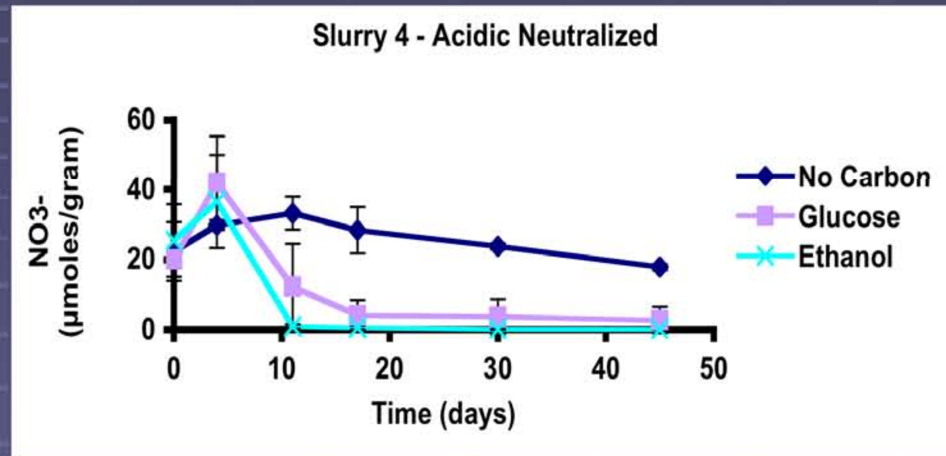


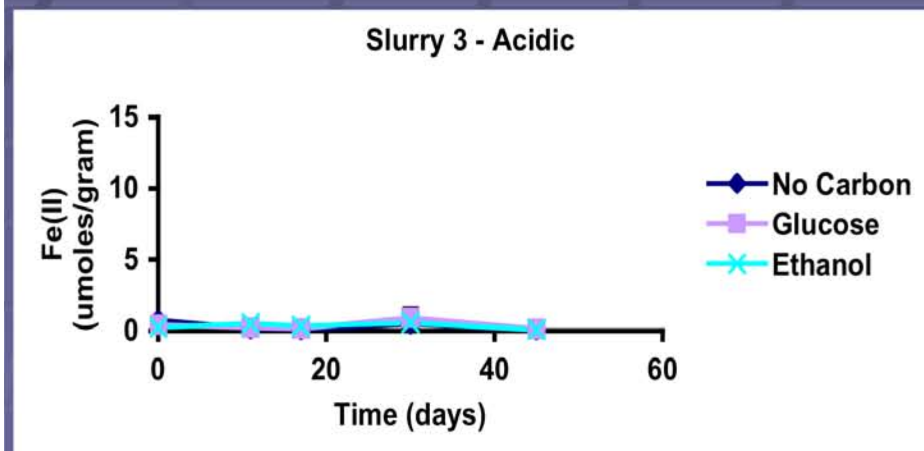
✓ Glucose oxidized to acetate; ethanol completely oxidized; lactate and hydrogen not as effective electron donors

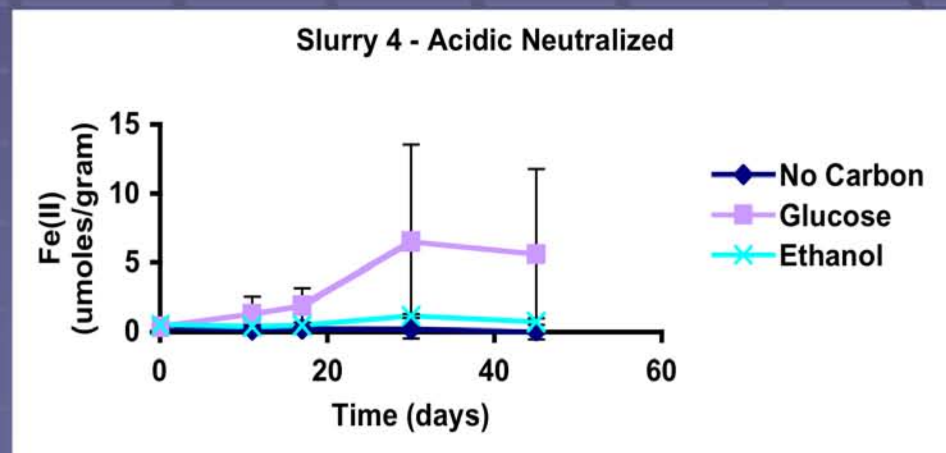

Slurry 3
Acidic, Acidic neut
washed, washed neut
Glucose
Acidic
Acidic Neut
washed
washed neut
Acidic Glu-1
A-4 12-5
W Glu-2
12/5
12/5

Sed. pH 4 - TEAPs



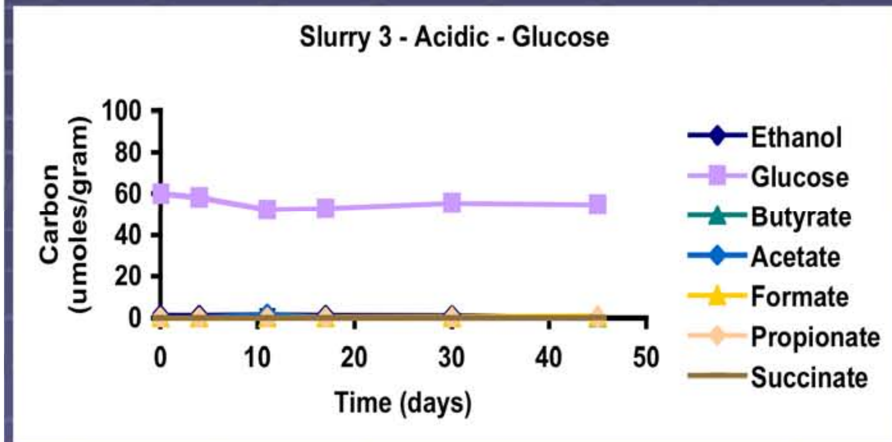


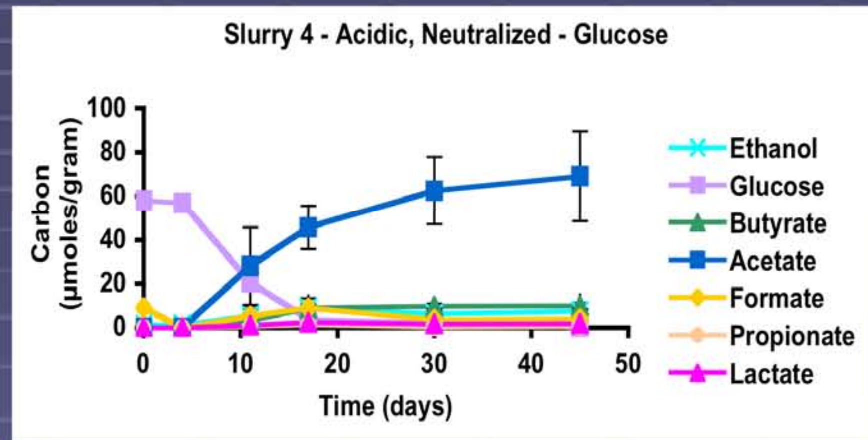


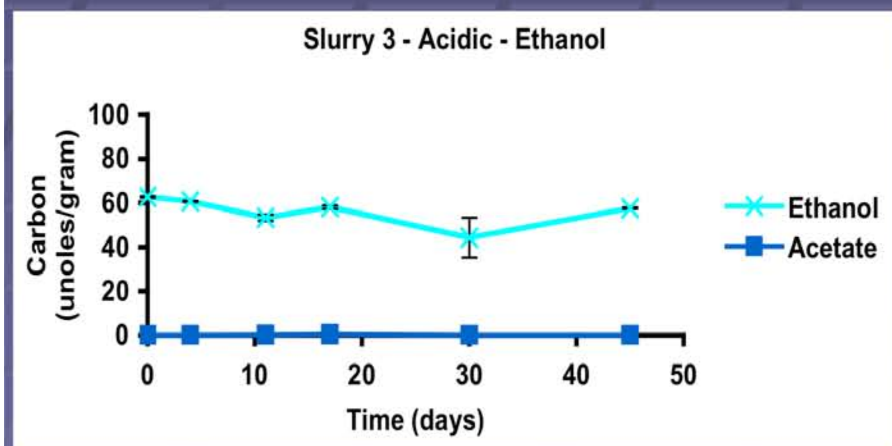


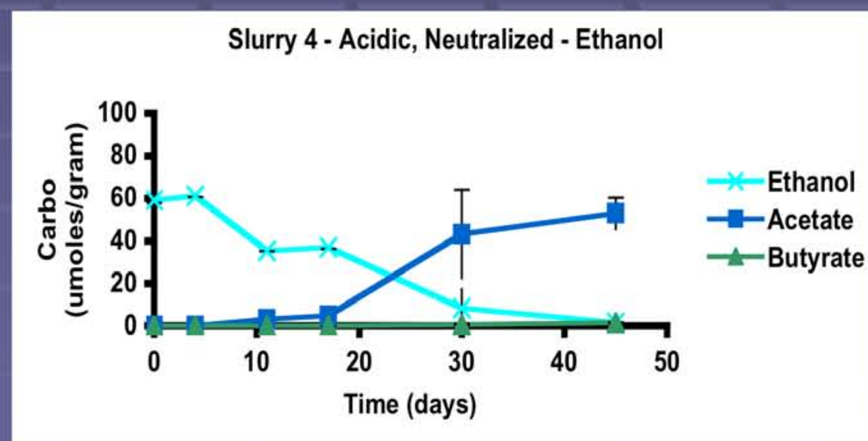
✓ Activity at pH 7 but not at pH 4; nitrate reduction dominates; no Fe(III) reduction coupled to ethanol oxidation

Sed. pH 4 - Donors



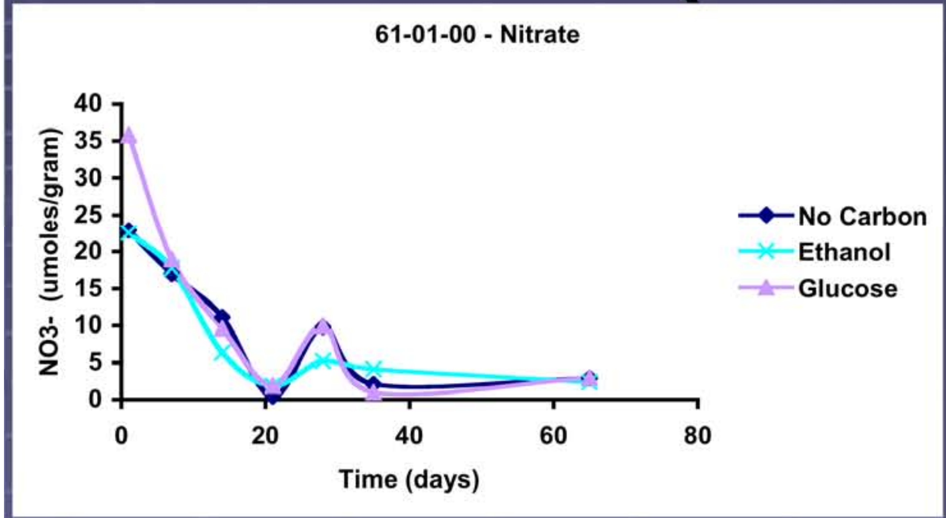


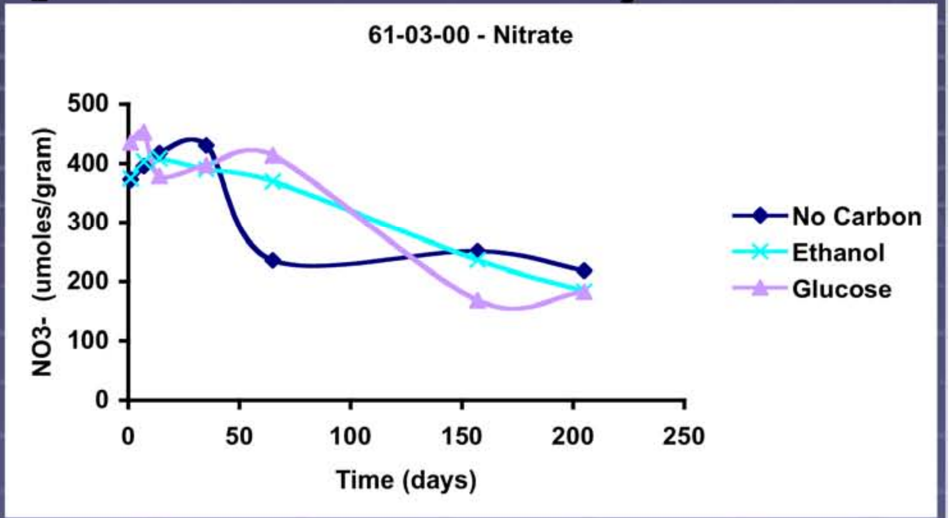


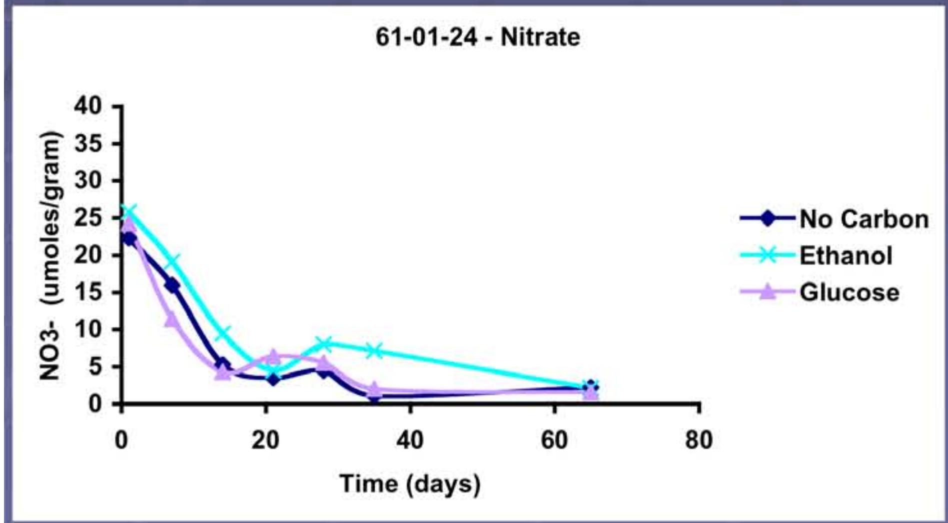


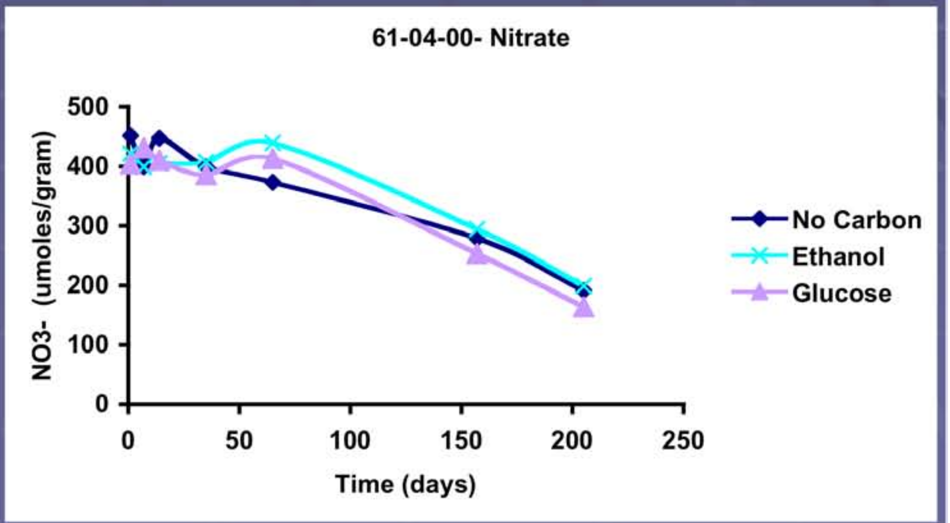
- ✓ Activity at pH 7 but not at pH 4; acetate is main product

Nitrate Reduction at High Nitrate Levels (Sed. pH 4 or 5.5)





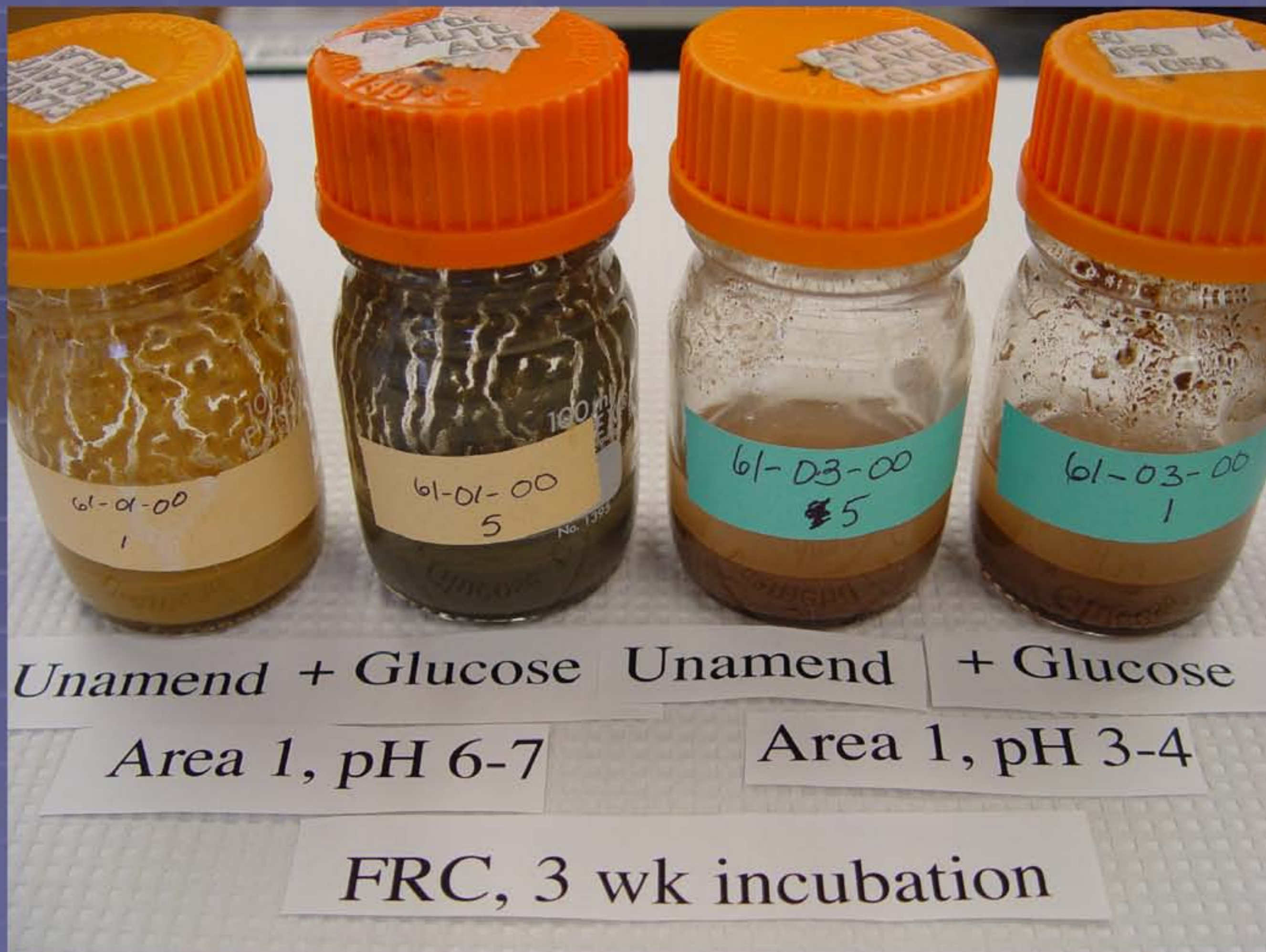




- ✓ Rates similar at 25 mM and 400 mM initial nitrate concentrations (pH 7)


Unamend
+ Ethanol
+ Glucose
FRC, 3 wk incubation
Area 1, pH 6-7, 8/03


61-01-00
1
61-01-00
5
61-01-24
1
61-01-24
5
Unamend
+ Glucose
Unamend
+ Glucose
FRC, 3 wk incubation
Area 1, pH 6-7, 8/03


61-01-00
1
61-01-00
5
61-03-00
5
61-03-00
1
Unamend
+ Glucose
Unamend
+ Glucose
Area 1, pH 6-7
Area 1, pH 3-4
FRC, 3 wk incubation

Conclusions

- Metabolism inhibited under acidic conditions (pH 4)
- Electron donor/ acceptor appear to be secondary controls to pH
- FRC subsurface contains some as yet unidentified indigenous electron donor/ carbon substrate
- > sediment pH 5 or upon neutralization, classic microbial food web operates
- Nitrate and Fe(III) reduction are dominant TEAPs

Conclusions

- Electron donors vary in their biostimulation potential:

Glucose > Ethanol > Lactate > Hydrogen

- Pathways (along with activity, rates) also vary according to initial sediment pH and electron donor
- Acidic conditions shift nitrate-reducers toward incomplete reduction pathways, away from denitrification
- High nitrate concentrations do not inhibit metabolism in neutralized acidic sediments
- Ethanol did not stimulate substantial Fe(III) reduction in neutralized acidic sediments

Suggestions for Future Work

- Quantify U(VI) reduction in microcosm samples
- Quantify change in Fe mineralogy
- Identify “active” members of microbial communities
- Further characterization/ quantification of environmental parameters limiting metabolism (Ni, Al)

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