Rates of Nitrate Reduction, Iron Reduction, and Substrate Utilization in Contaminated Subsurface Sediment Microcosms

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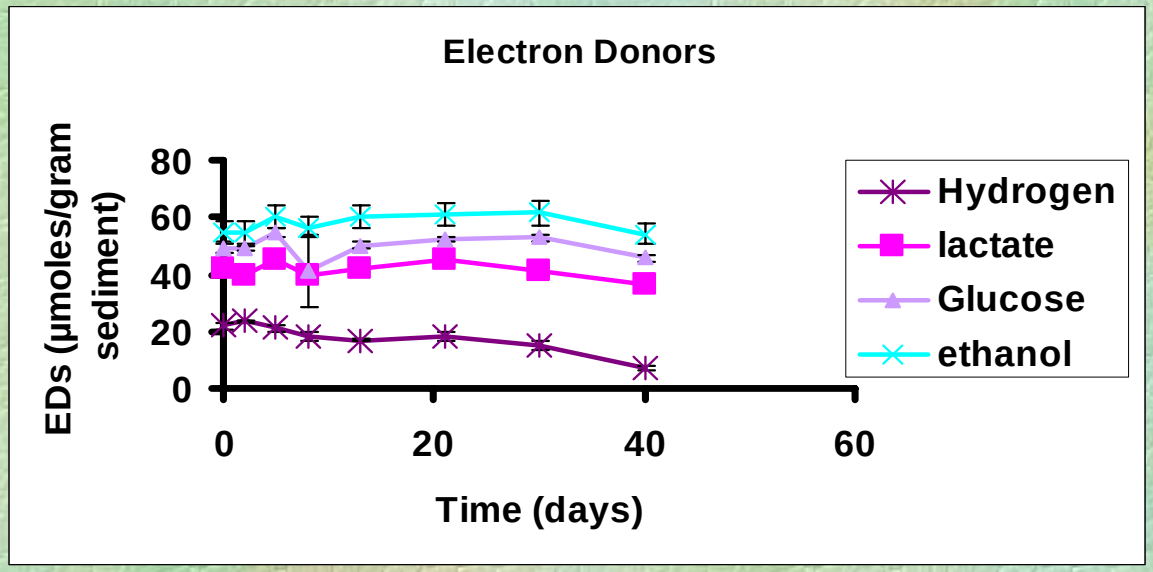
Objectives

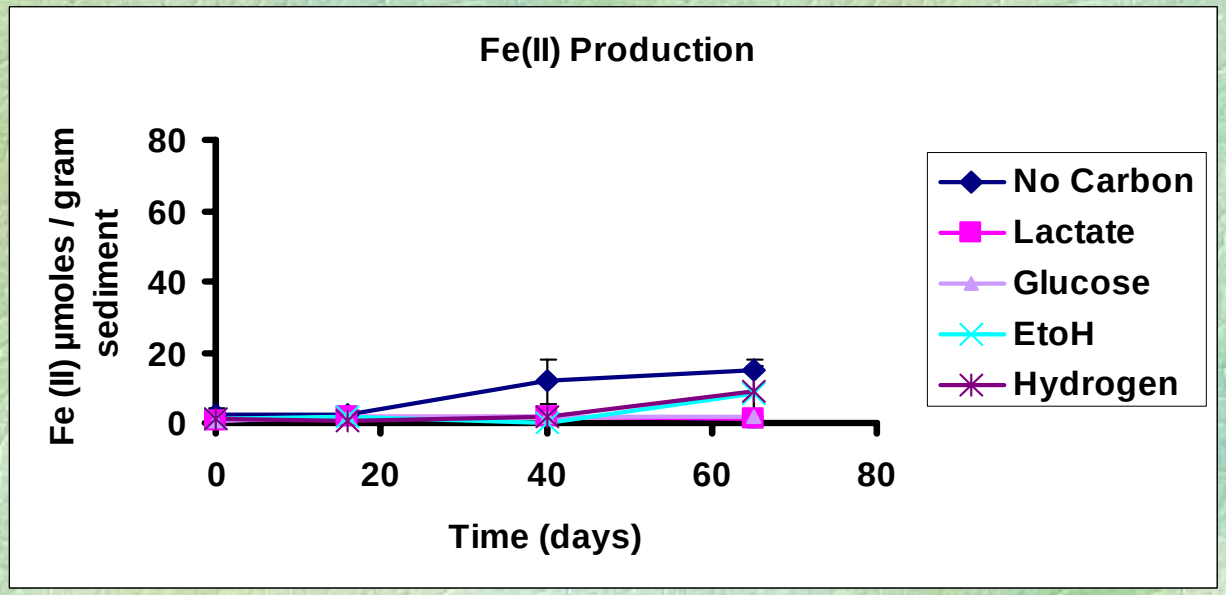
- Understand which environmental parameters are governing microbial activity (pH, nitrate, toxic metals)
- Study effects of addition of neutralizing agents
- Determine primary TEAP
- Study effects of varying pH and nitrate concentrations on TEAP
- Determine rates of nitrate reduction, iron reduction, and substrate utilization

Sediment Microcosms

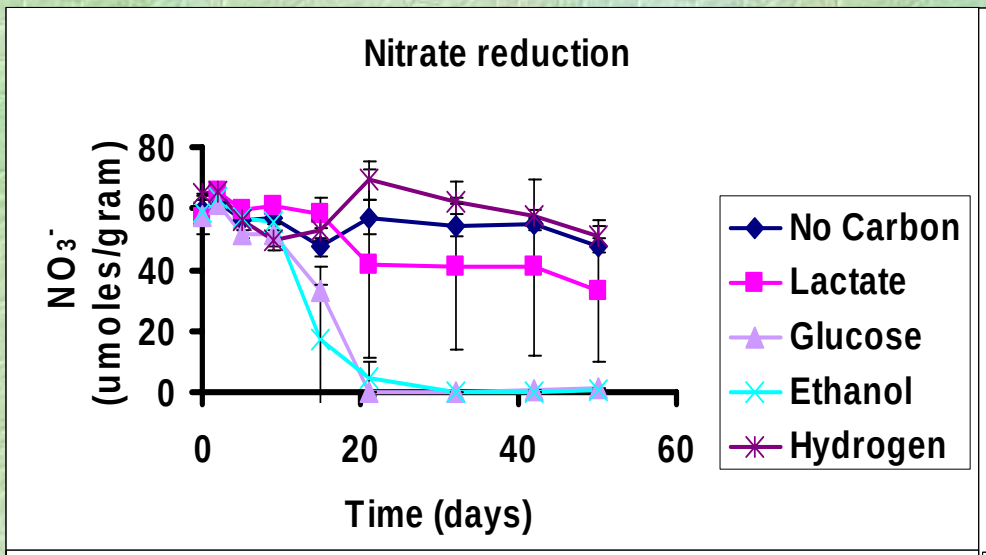
- -Absence vs presence of nitrate in neutral pH sediments (effect of nitrate)
- -Comparison of high/low nitrate concentrations - (effect of high nitrate)
- -Acidic sediments vs neutralized acidic sediments (effect of pH; neutralization)
- -Washed microcosms vs washed neutralized microcosms (absence of soluble toxins; effects of neutralization vs pH)

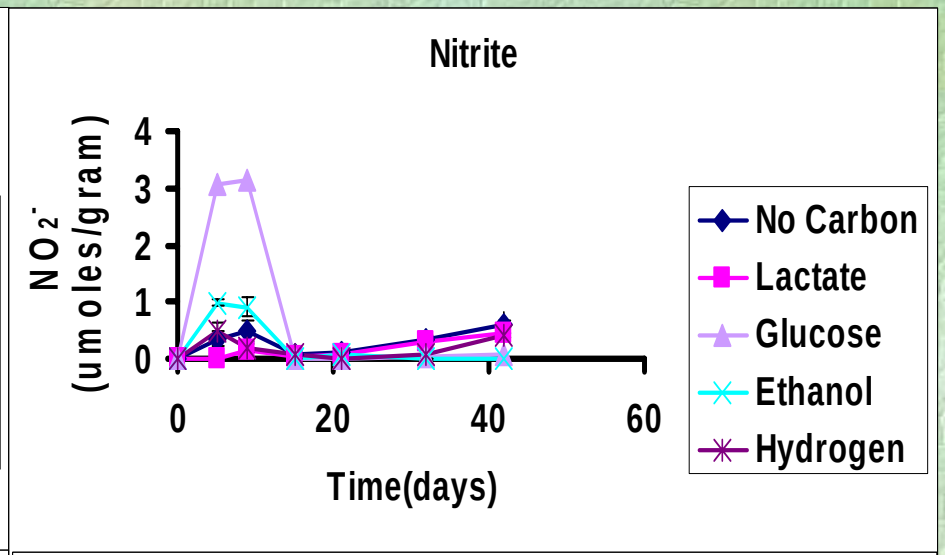
Results- No Nitrate (pH 5.5-6) Substrate Utilization / TEAP

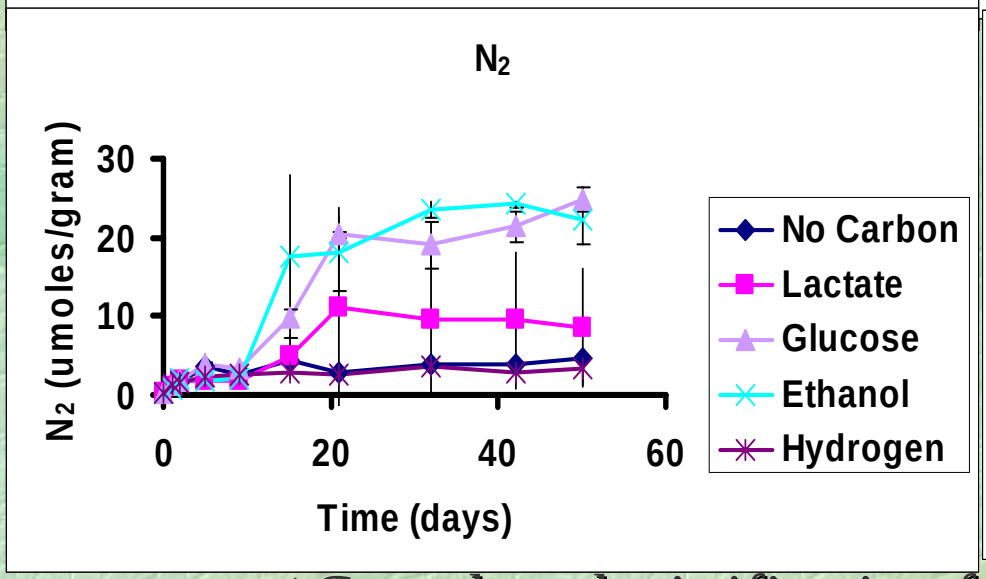


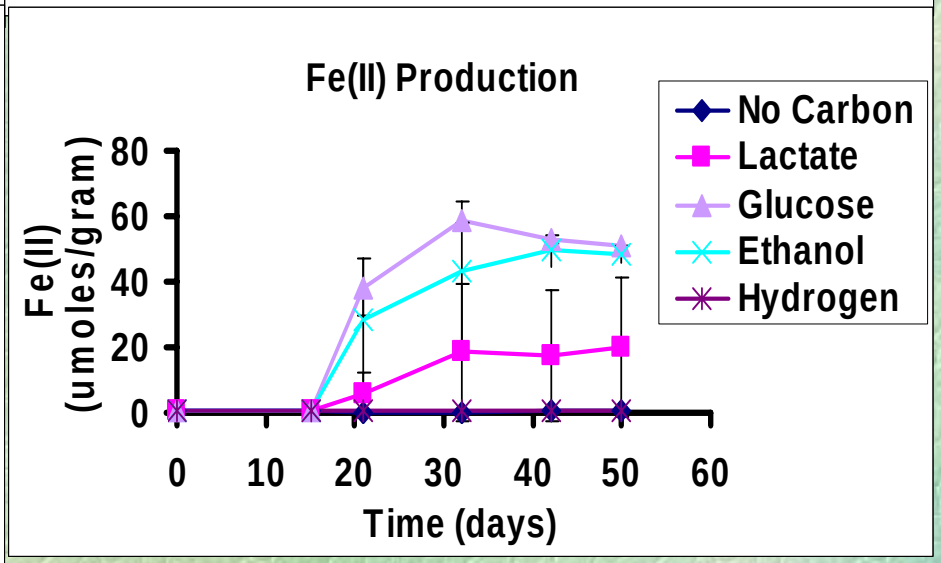


Results: Nitrate Present(pH 5-6)- TEAPs



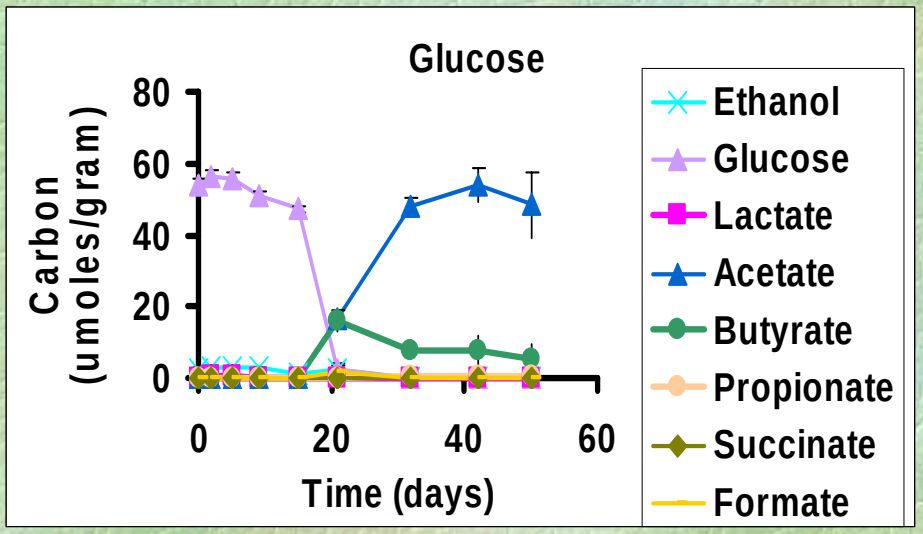


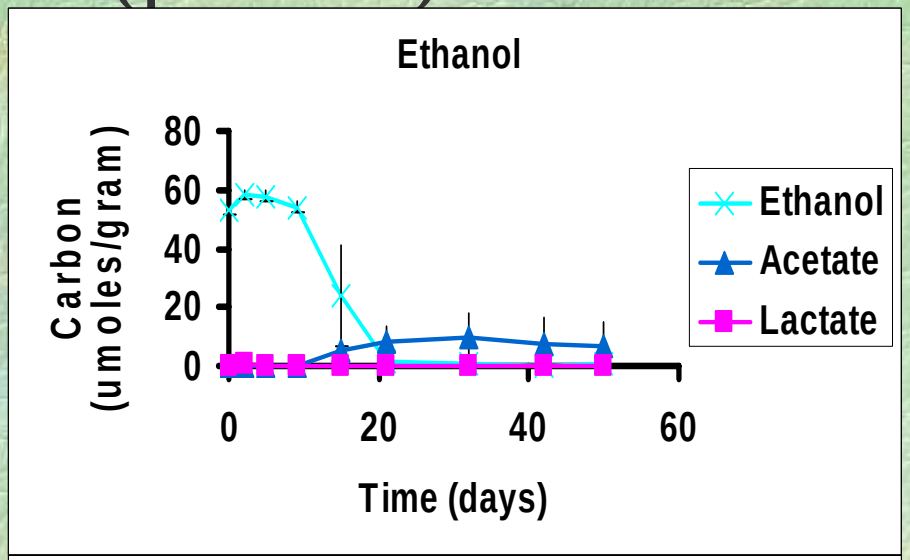


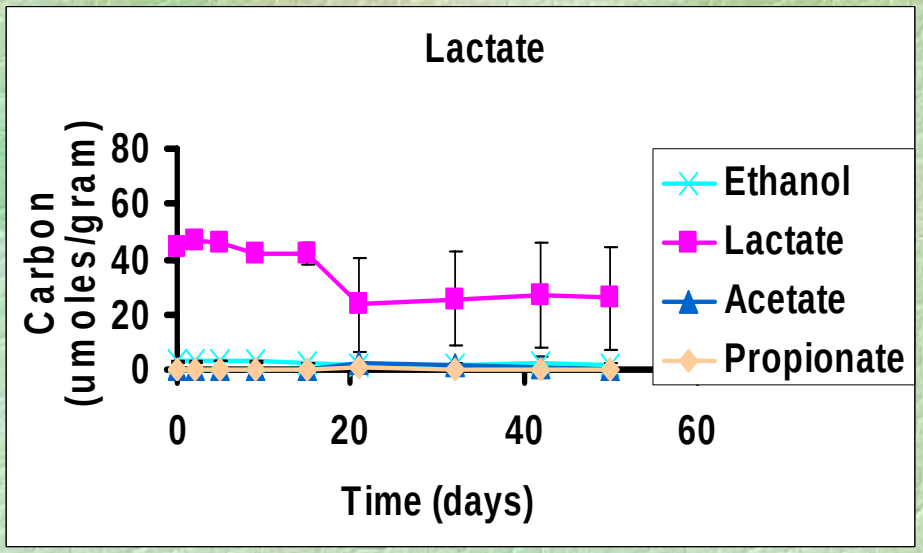


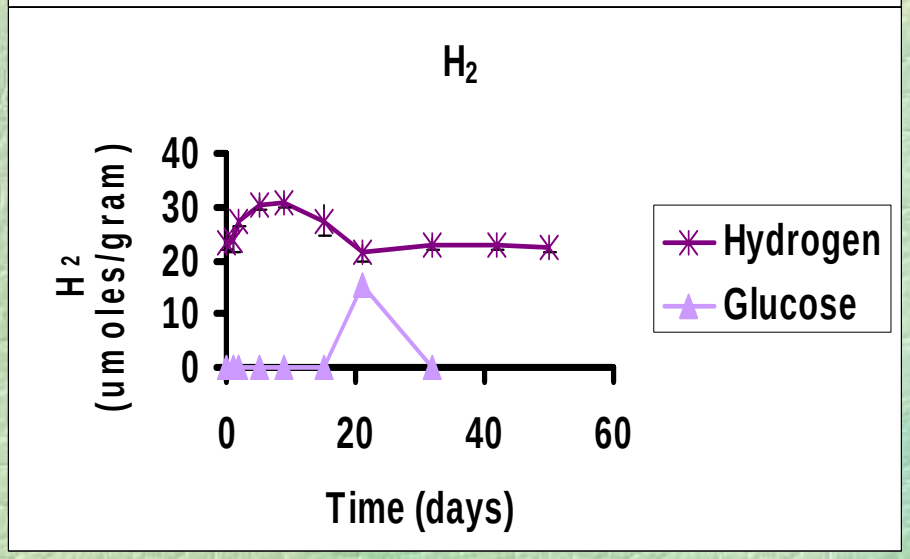
✓ Complete denitrification followed by substantial Fe(III) reduction

Results: Nitrate Presence(pH 5-6)- Donors









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

Results: Nitrate Presence(pH 5-6)- Carbon Balance

-150 µmoles/g carbon detected in CO_2

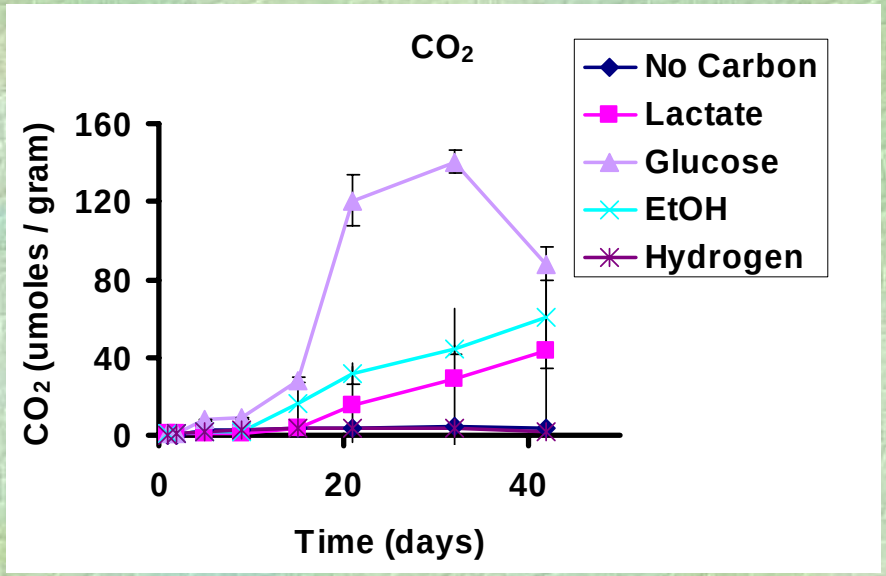
- 10 µmoles/ gram butyrate produced (40 carbons)

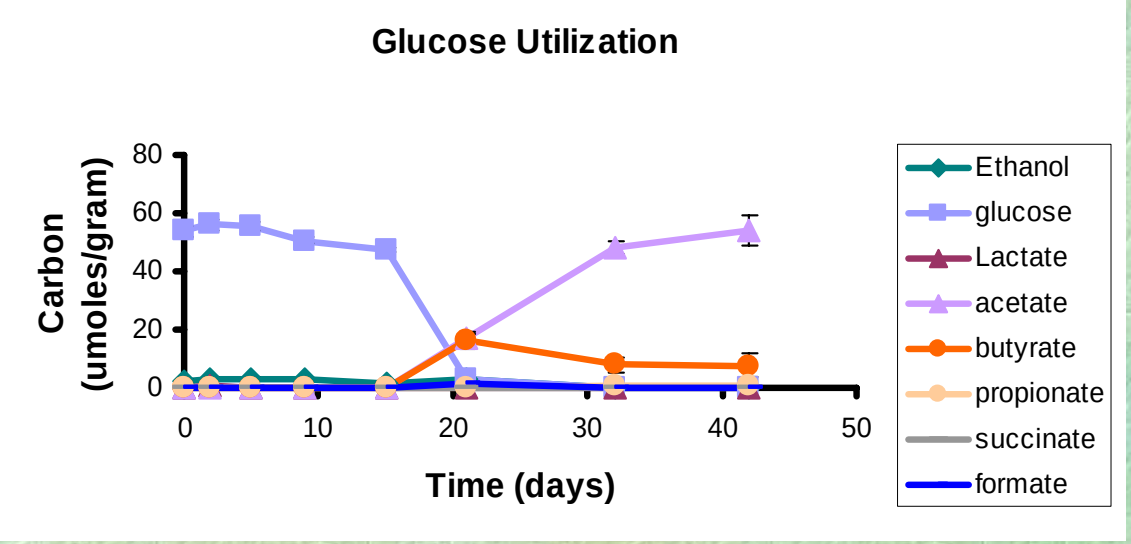
-50 µmoles/gram acetate produced (100 carbons)

150+40+100=290

- 50 µmoles/gram glucose consumed

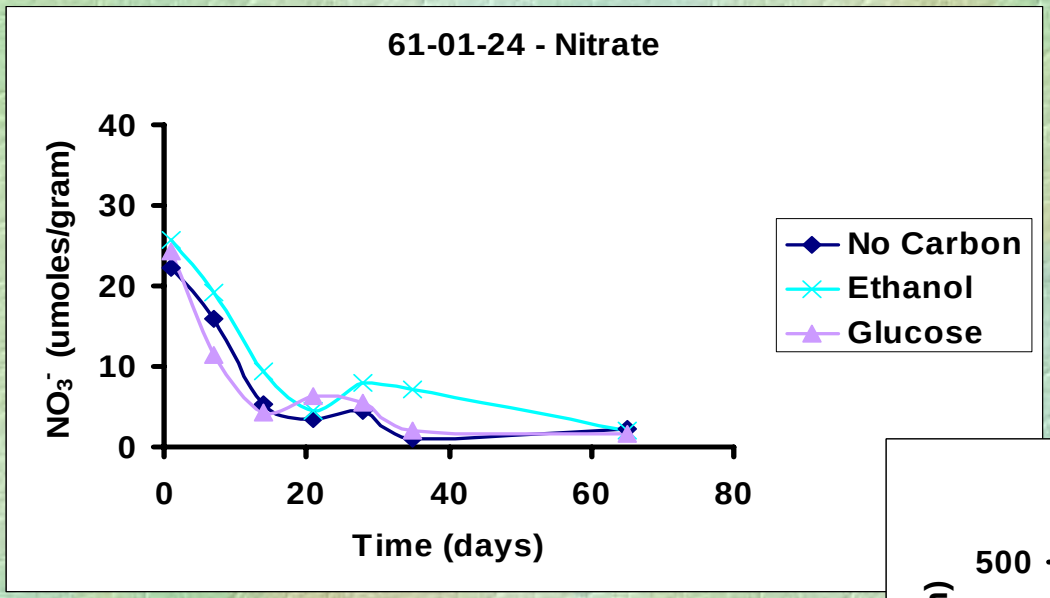
(= 97% carbon recovery)

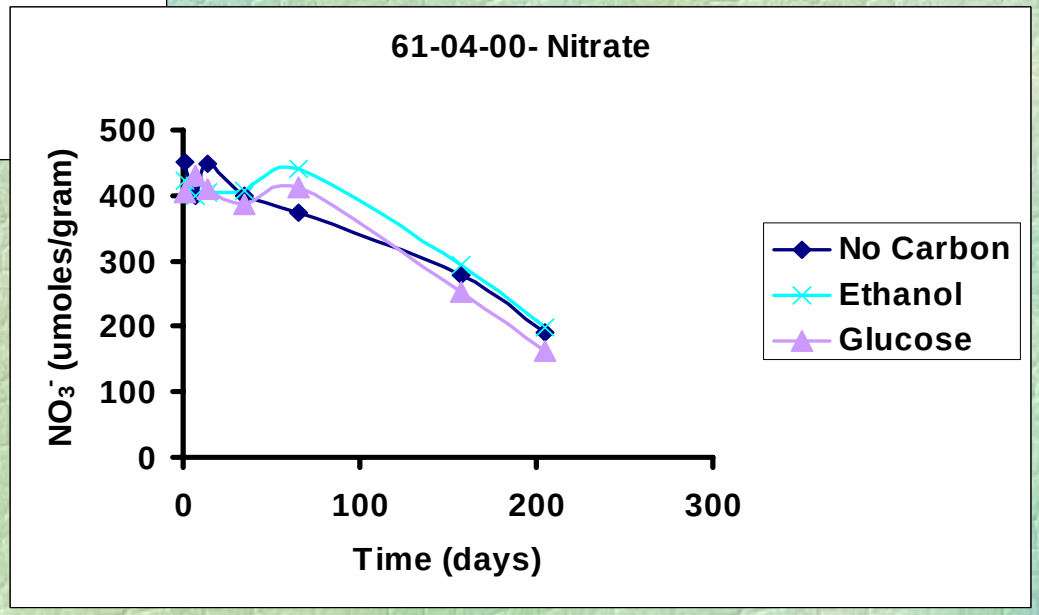




No C-2
12/2/03 17
5 Lac-2
01-01-00 12-2-03 20
8 Glu-2
01-01-00 12/2/03 23
11 EtOH-2
01-01-00 12/2/03 26
14 H2-2
01-01-00 12/2/03 29
Slurry 2
(neutral sediment + nitrate)
Day 35

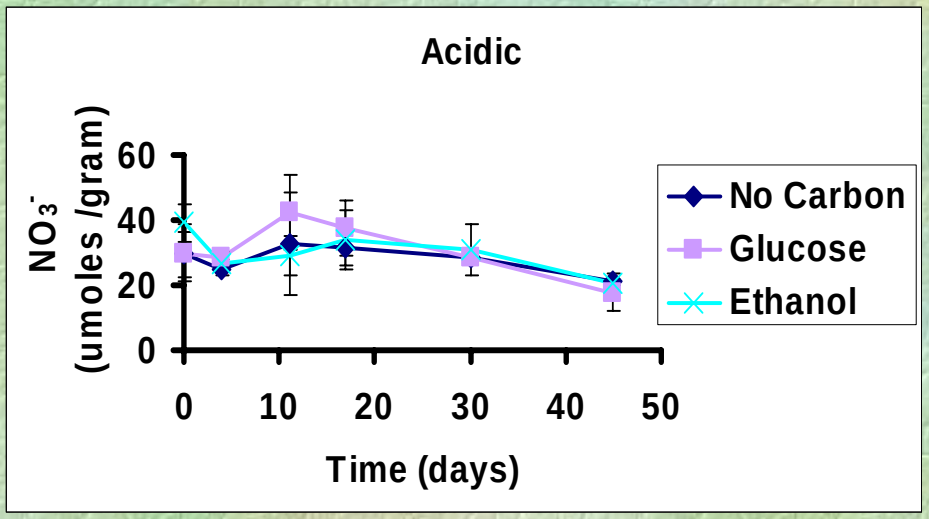
Nitrate Reduction -Variable Nitrate Concentrations (neutral or neutralized sediment)

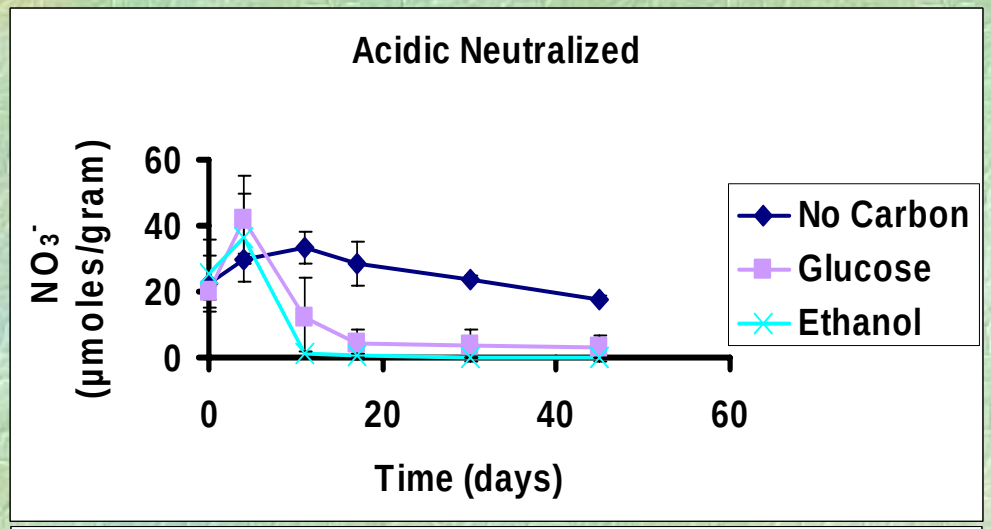


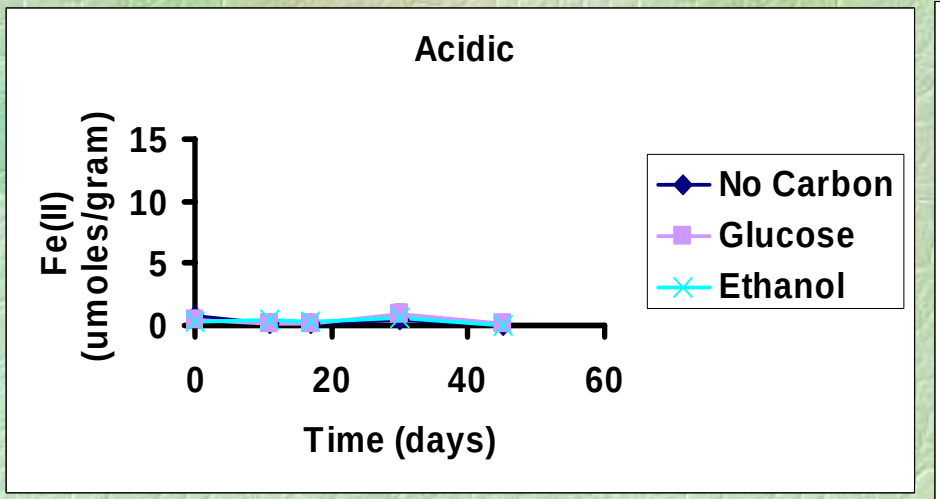


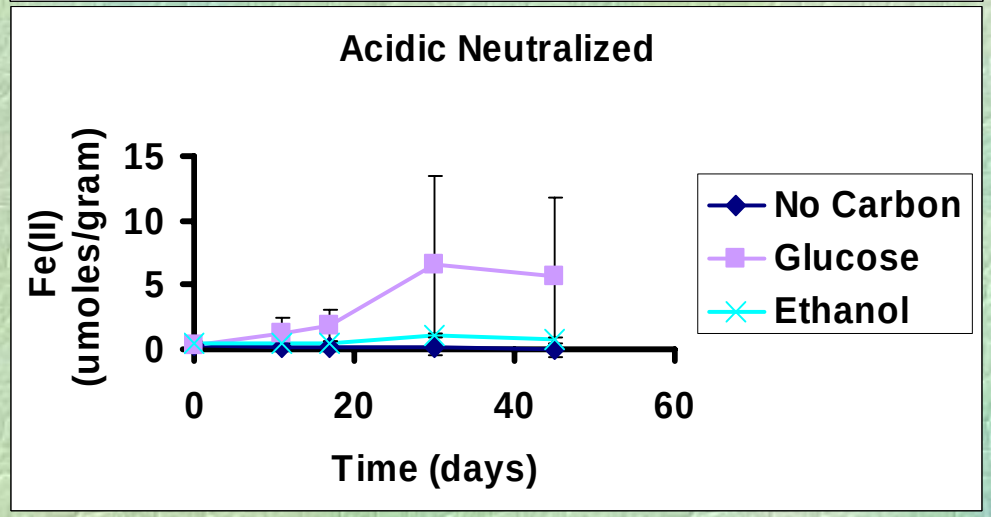
✓ Rates similar at 25 umoles/g and 400 umoles/g initial nitrate concentrations (pH 7)

Results: Acidic vs. Acidic Neutralized - TEAPs



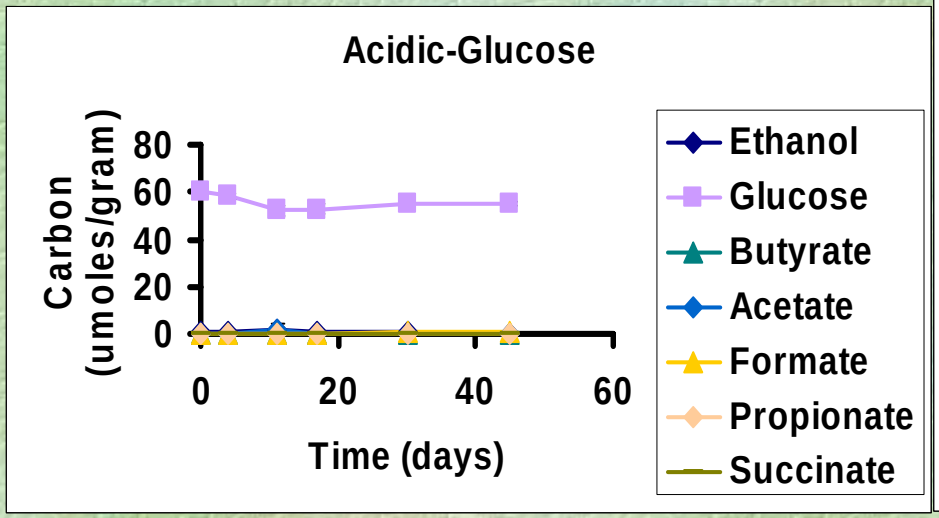


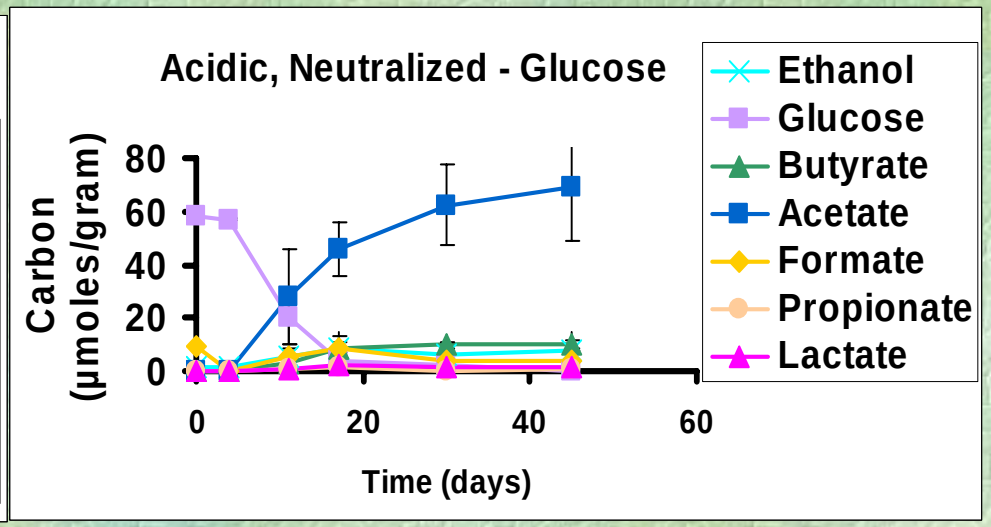


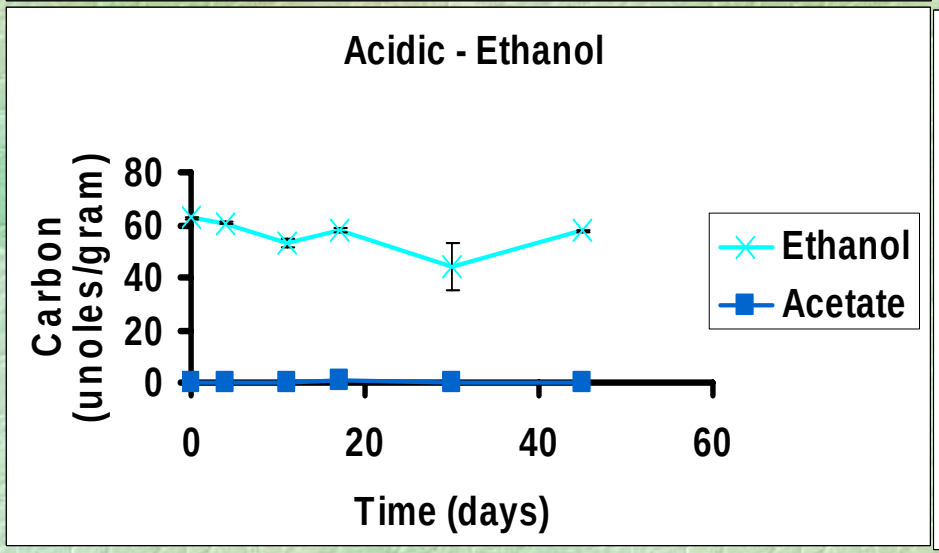


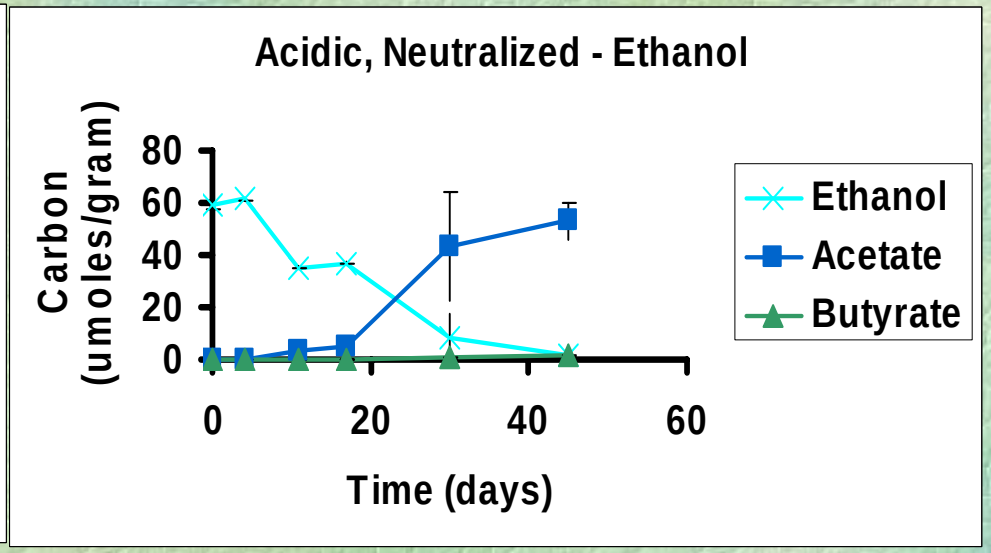
✓ Activity at pH 7 but not at pH 4; nitrate reduction dominates; no Fe(III) reduction coupled to ethanol oxidation

Results: Acidic vs. Acidic Neutralized - Donors





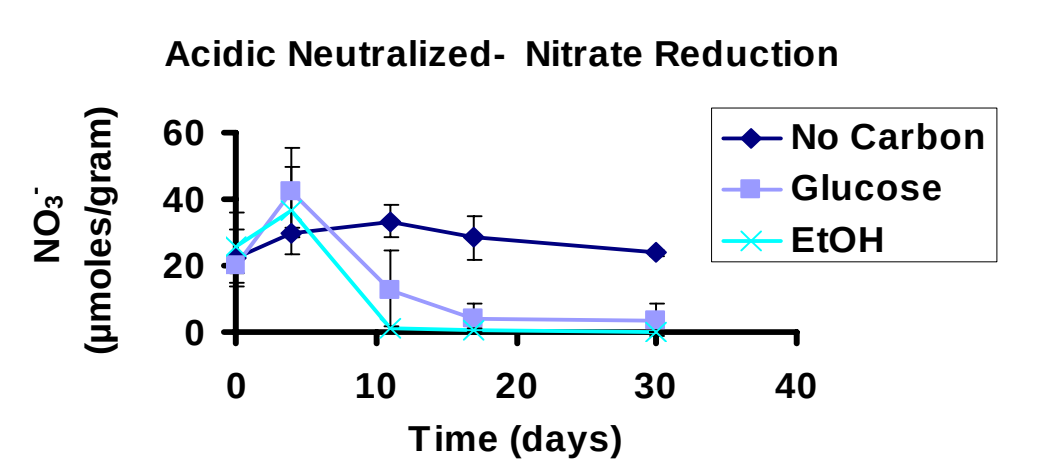


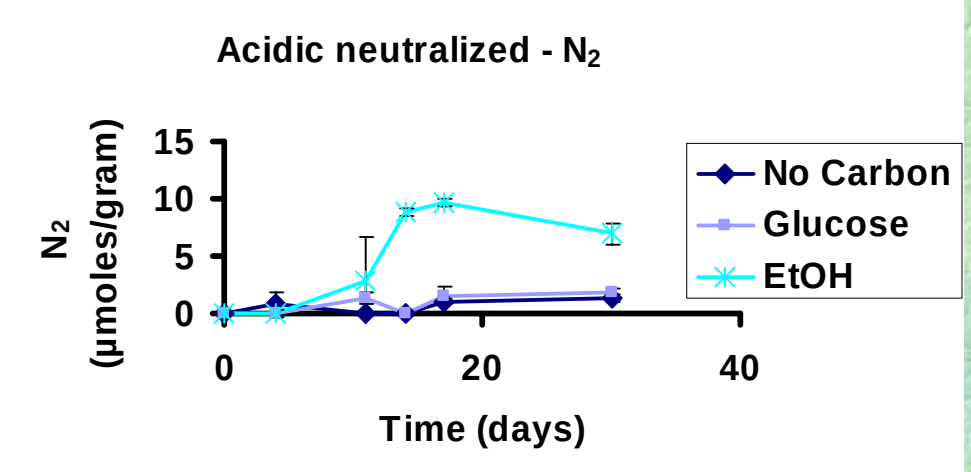


✓ Activity at pH 7 but not at pH 4; acetate is main product

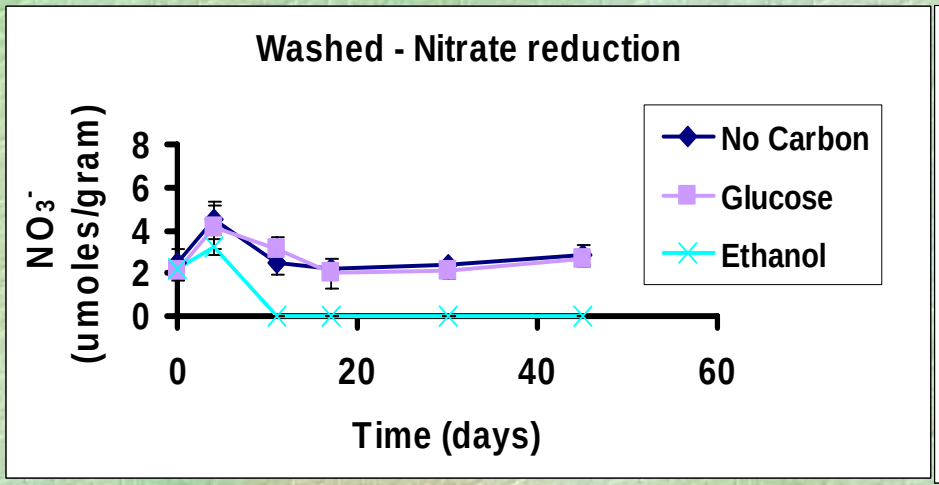
Results -Nitrate Reduction Pathway - Acidic Neutralized)

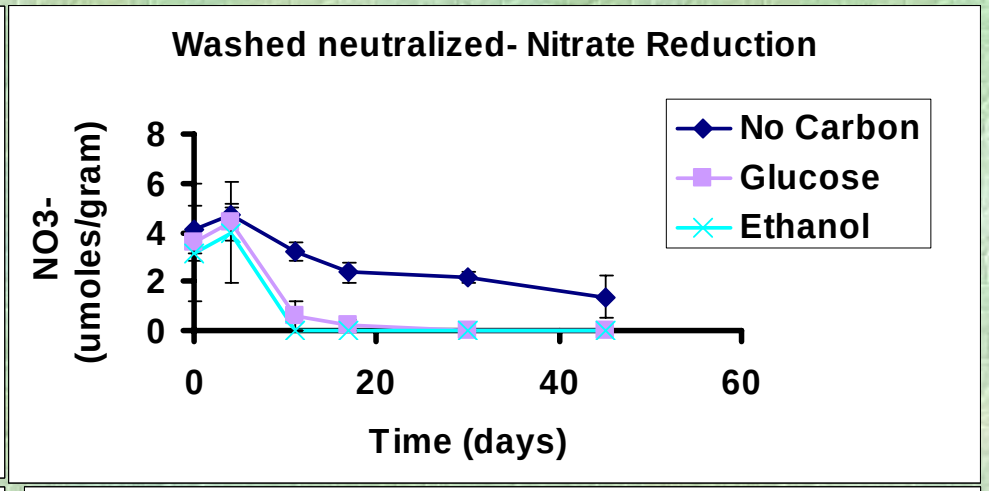
- Nitrate was reduced in glucose and ethanol supplemented microcosms.
- 4-5x more N_2 was produced in ethanol supplemented microcosms than in glucose microcosms.

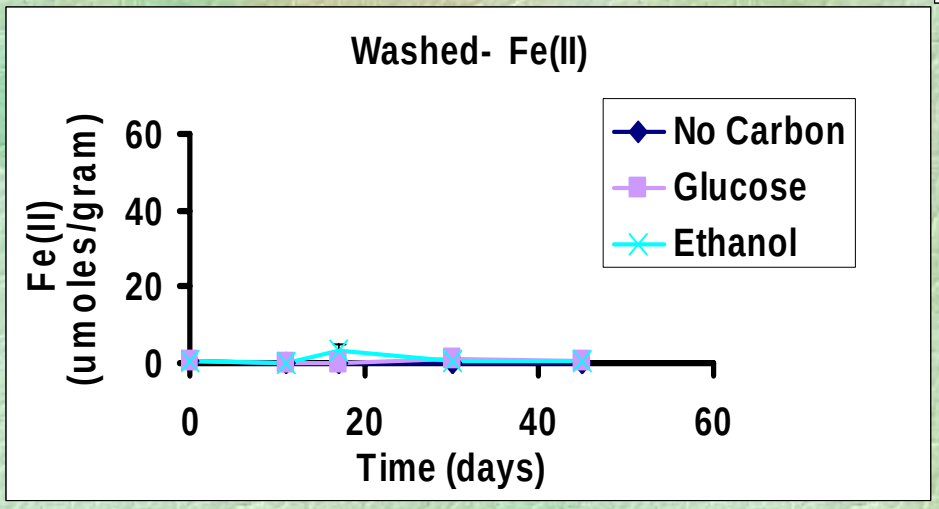


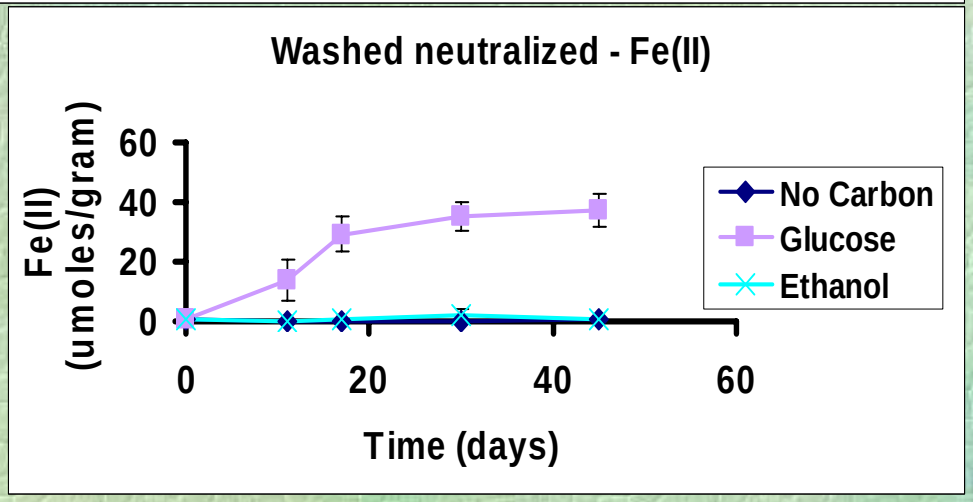


Results -Washed vs Washed Neutralized - TEAPs

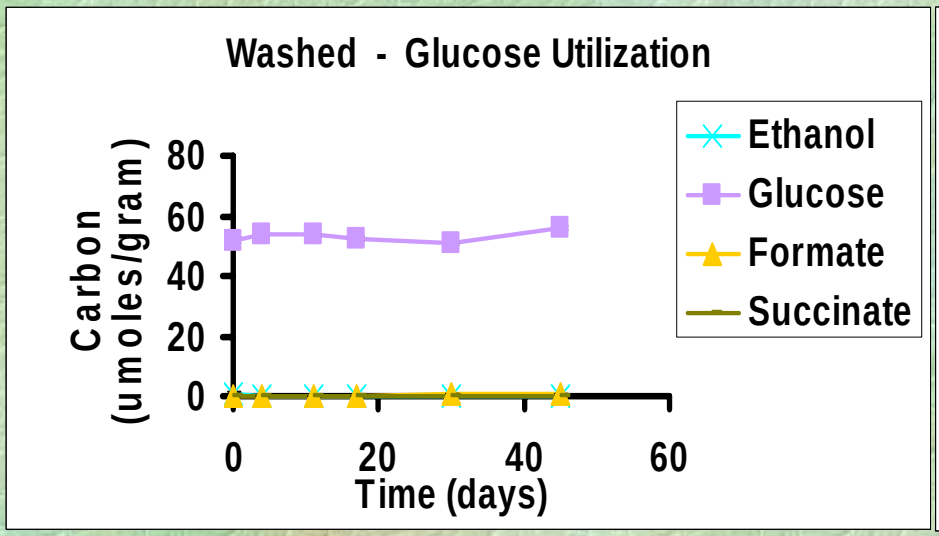


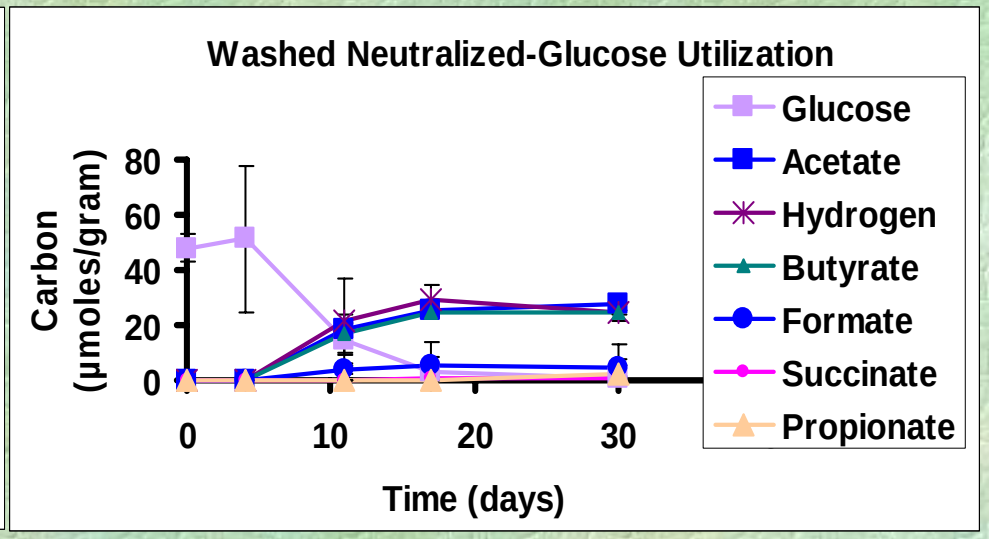


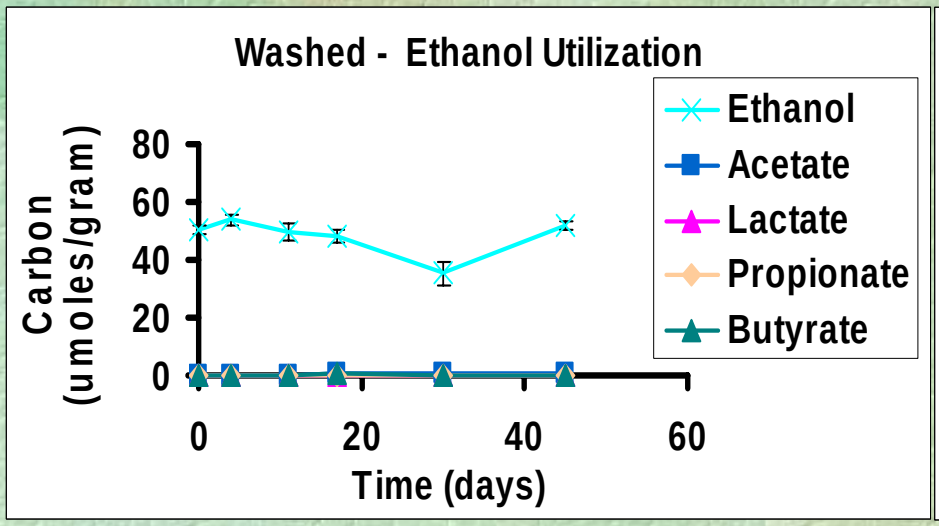


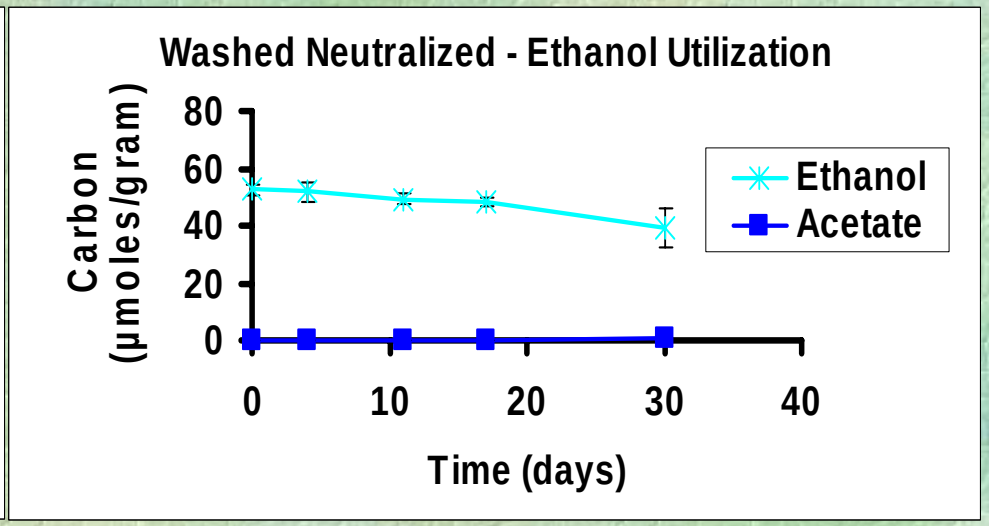


Results -Washed vs Washed Neutralized-Donors









Slurry 3
washed, neut
washed
12/22/03
WASHED, NEUtralized
WASHED

Comparison of Nitrate Reduction Rates

- Varying nitrate:
  - 15 mM nitrate - 0.7-1.3 μmoles/gram sediment/d
  - 250 mM nitrate* -0.7-1.3 μmoles/gram sediment/d
- Neutral sediment (20-25 mM nitrate)
  - 2.7-2.8 μmoles/gram sediment/d (glucose; ethanol)
- Acidic sediment *(10-15 mM nitrate)
  - 0.1-0.3 μmoles/gram sediment/d
- Acidic neutralized sediment (10-15 mM nitrate)
  - 1.7-2.8 μmoles/gram sediment/d (glucose; ethanol)
- Washed sediment (1.5-2.0 mM nitrate)
  - 0.3 μmoles/gram sediment/d (ethanol)
- Washed neutralized sediment (1.5-2mM nitrate)
  - 0.2-0.3 μmoles/gram sediment/d (glucose; ethanol)

*nitrate not completely reduced

Comparison of Fe(III) Reduction Rates

- Neutral sediment, no nitrate
  - 0.25 μmoles/gram sediment/d (no carbon)
- Neutral sediment containing nitrate
  - 1.4μmoles/gram sediment/d (glucose; ethanol)
- Neutralized acidic sediment
  - 0.3 μmoles/gram sediment/d (glucose)
- Washed Neutralized acidic sediment
  - 0.8 μmoles/gram sediment/d (glucose)

Comparison of Substrate Utilization Rates

- Neutral, no nitrate
  - <0.3 μmoles/gram sediment/d (glucose, ethanol, H_2, lactate)
- Neutral, with nitrate
  - 1.1 μmoles/gram sediment/d (glucose, ethanol, 1 lactate bottle)
- Acidic
  - <0.15 μmoles/gram sediment/d (glucose, ethanol)
- Acidic neutralized
  - 1.3 μmoles/gram sediment/d (glucose, ethanol)
- Washed
  - <0.1 μmoles/gram sediment/d (glucose, ethanol)
- Washed neutralized
  - 1.1 μmoles/gram sediment/d (glucose)

Microcosm Activity

Microcosm	Amendments	Nitrate reduction rates ($\mu moles g^{-1} d^{-1}$)	Fe(II) production rates ($\mu moles g^{-1} d^{-1}$)	Substrate Utilization ($\mu moles g^{-1} d^{-1}$)
1	No Carbon	NA	0.25	0.09
1	Lactate	NA	0.01	0.10
1	Ethanol	NA	0.15	0.25
1	Glucose	NA	0.02	0.07
1	Hydrogen	NA	0.15	0.13
2	No Carbon	0.26	0.00	0.19
2	Lactate	0.57	0.56	0.40
2	Ethanol	2.70	1.36	1.11
2	Glucose	2.84	1.44	1.10
2	Hydrogen	0.27	0.00	0.06
3	No Carbon	0.13	0.00	0.01
3	Ethanol	0.28	0.00	0.11
3	Glucose	0.26	0.00	0.12
4	No Carbon	0.18	0.00	0.10
4	Ethanol	2.82	0.04	1.30
4	Glucose	1.68	0.28	1.28
5	No Carbon	0.02	0.00	0.18
5	Ethanol	0.25	0.01	0.00
5	Glucose	0.01	0.05	0.00
6	No Carbon	0.07	0.00	0.14
6	Ethanol	0.32	0.00	0.01
6	Glucose	0.22	0.82	1.09

Conclusions

- Electron donors vary in their biostimulation potential:

  Glucose > Ethanol > Lactate > Hydrogen
- Nitrate and Fe(III) reduction are dominant TEAPs
- Pathways (along with activity, rates) vary according to initial sediment pH, electron donor, and nitrate concentrations
- Acidity of the sediment inhibits all tested microbial activities.
- High nitrate concentrations do not inhibit metabolism in neutralized acidic sediments
- Acidic conditions shift glucose utilizing nitrate-reducers toward incomplete reduction pathways, away from denitrification
- Ethanol does not stimulate substantial Fe(III) reduction in neutralized acidic sediments
- Neutralization with bicarbonate greatly increases activity, thus suggesting that carbonates have a greater influence on activity than pH alone.

Acknowledgements

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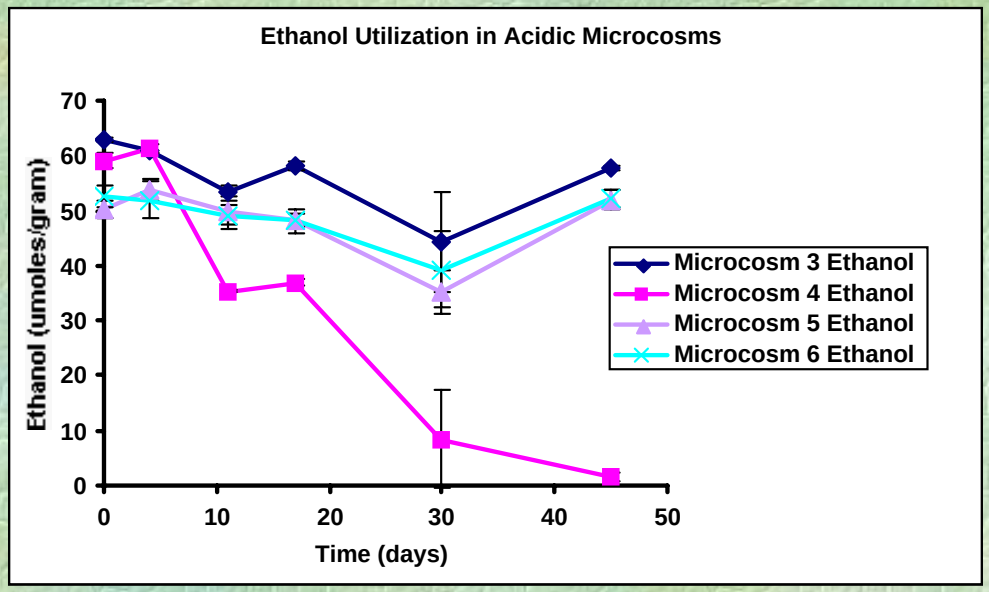
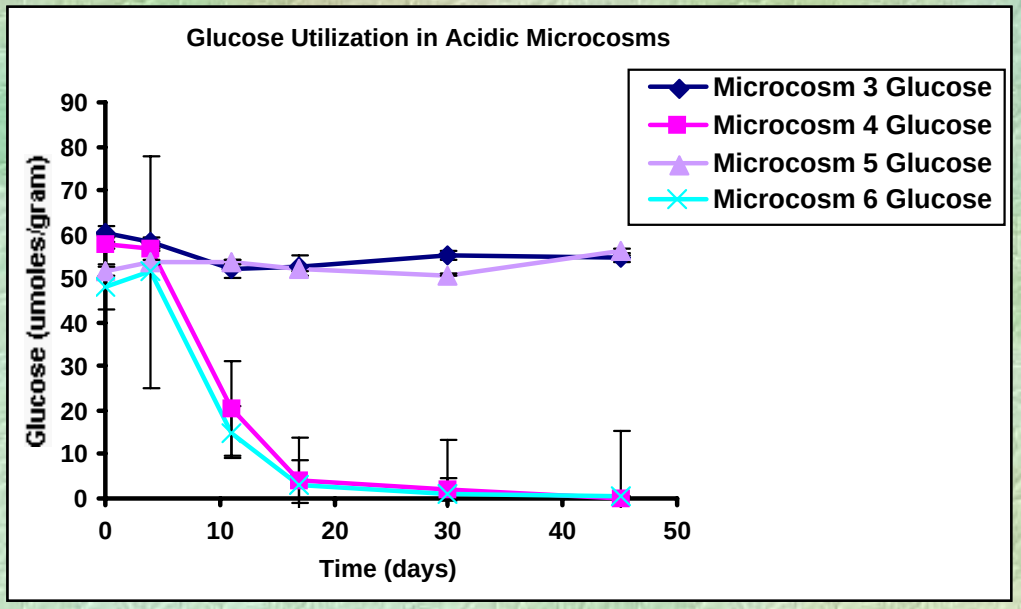
Slurry 3
Acidic, Acidic neut
washed, washed neut
Glucose
Acidic
Acidic Neut
washed
washed neut
Acidic Glu-1
A-4 12-5
Acidic Glu-3
neut AN-6
12-5
W Glu-2
1215
WN Glu1
1215

Comparison of Fe(II) production

Slurry	Fe(II) –µmoles/gram	ED
1	9-15	Ctrl, EtOH, H_2
2	20-60	Glu, EtOH,Lac
3	0	
4	7	Glu
5	1-3	EtOH, Glu
6	35-40	Glu, Ctrl

With acidic sediment, Fe(II) is not produced as long as EtOH is present in either washed, neutralized or non affected microcosms.

Substrate utilization