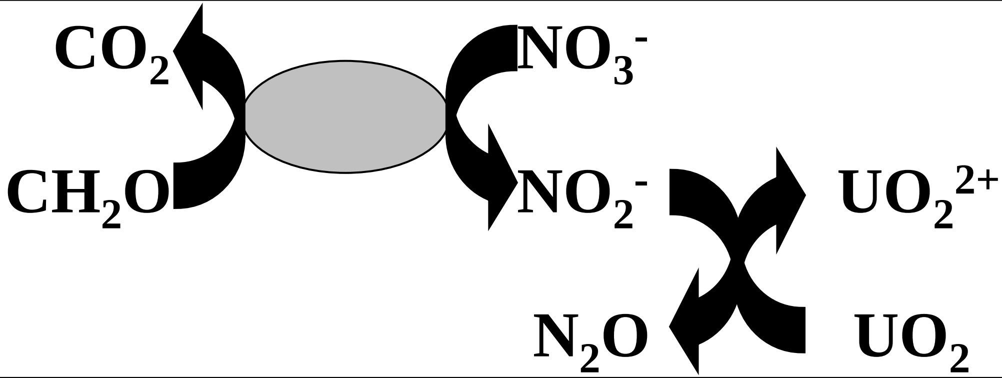
Microbiology/Microbial Ecology

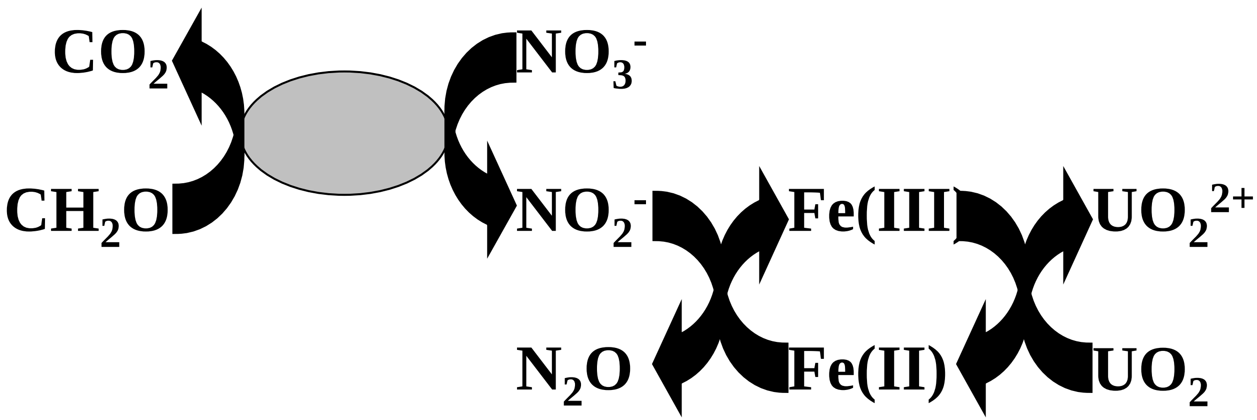
- Mechanism of U(IV) oxidation
- Mechanism of Denitrification.
- Ecology of Denitrifying community.

Lee Krumholz
University of Oklahoma

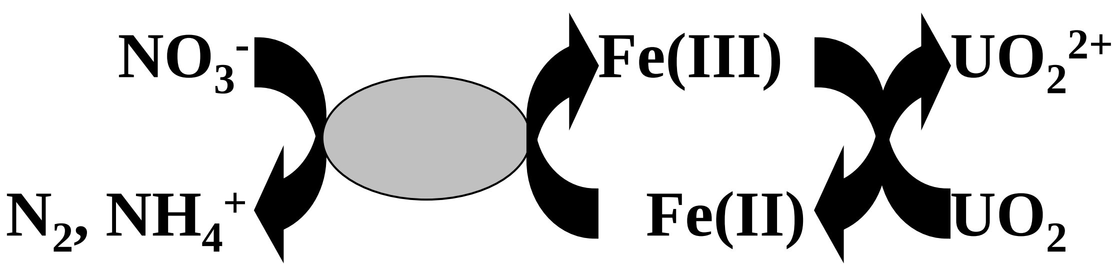
Mechanisms of NO_3^--dependent U(IV) oxidation



U(IV) oxidation by reactive denitrification intermediates



U(IV) oxidation by reactive denit. intermediates and Fe



U(IV) oxidation by Fe(III) from nitrate-dependent Fe(II)-oxidizing bacteria

“Abiotic” U(IV) oxidation

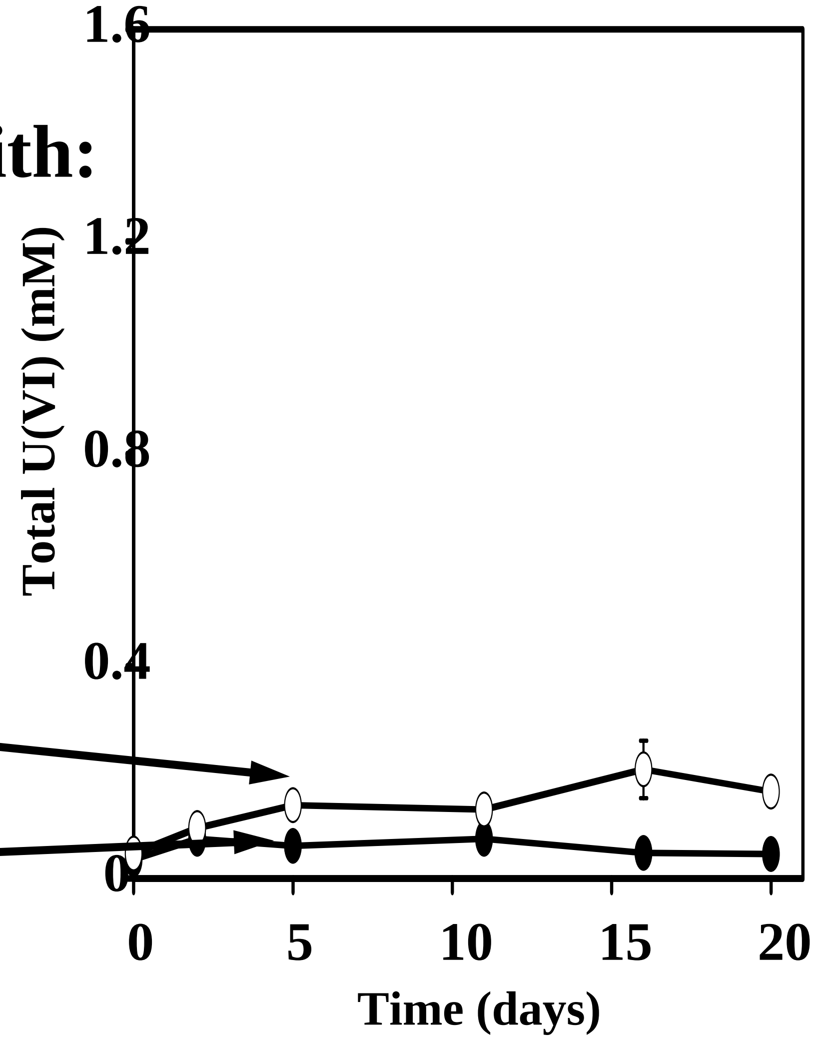
Incubate biogenic U(IV) with:

-Nitrite with 0.3 mM Fe(II)

-Hydrous ferric oxide (HFO)

-Nitrite only

-No oxidant



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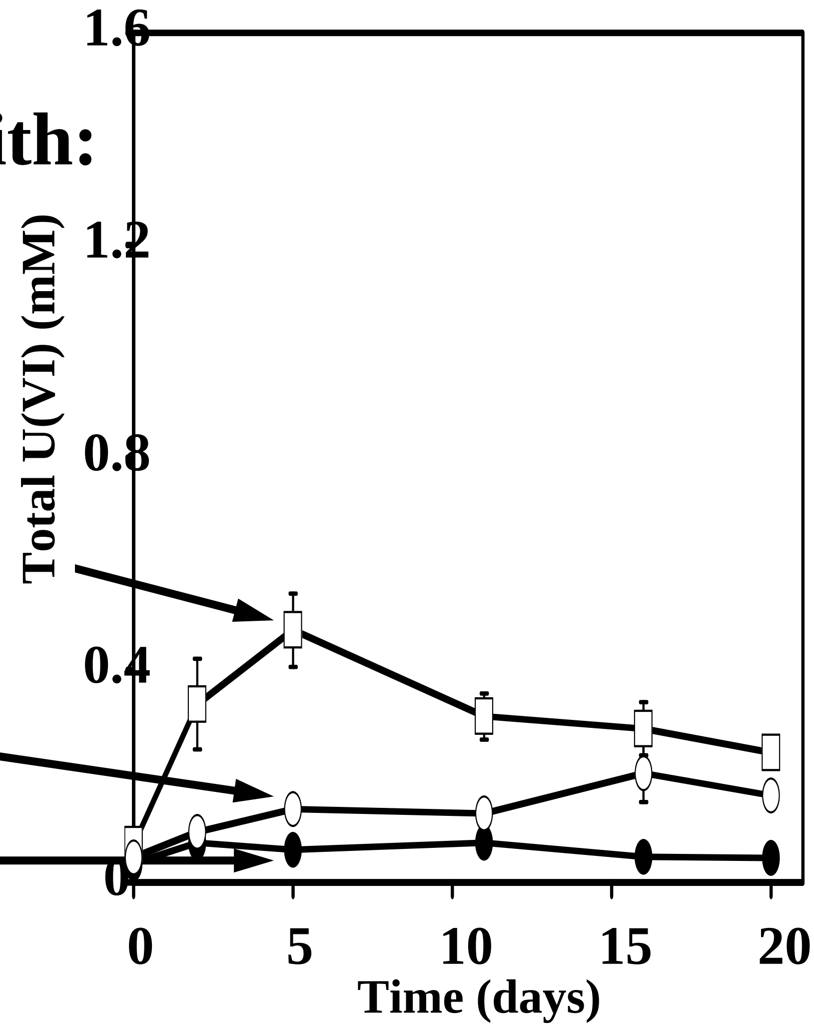

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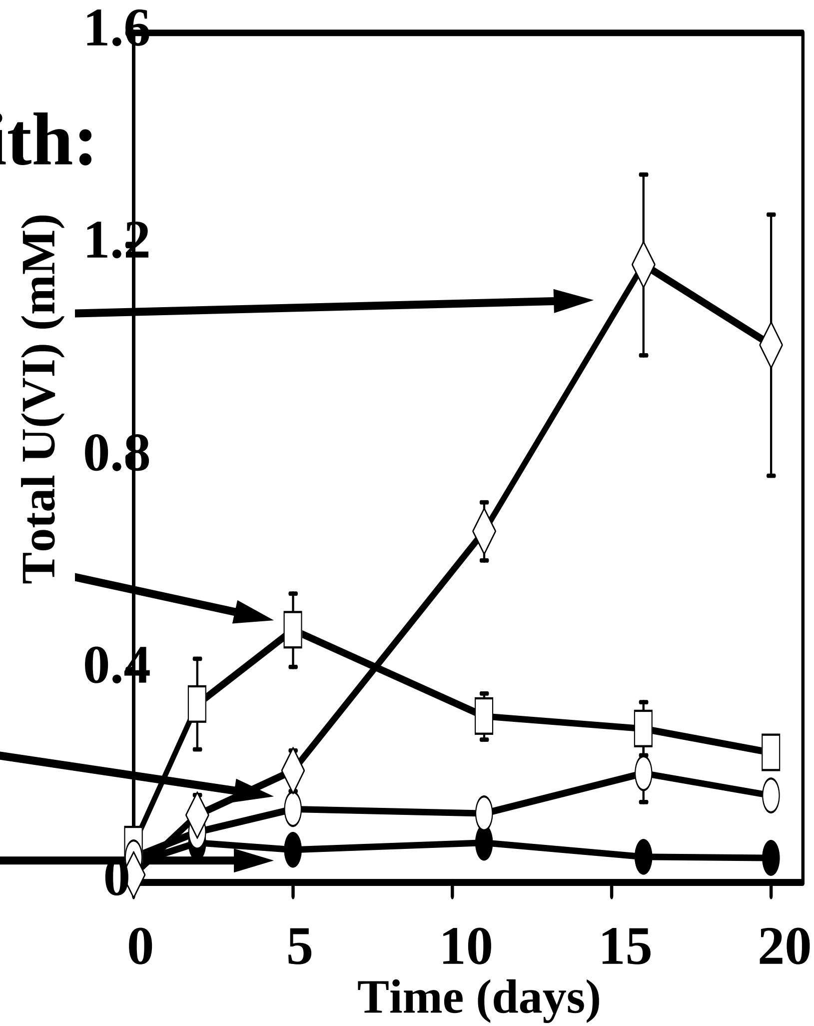
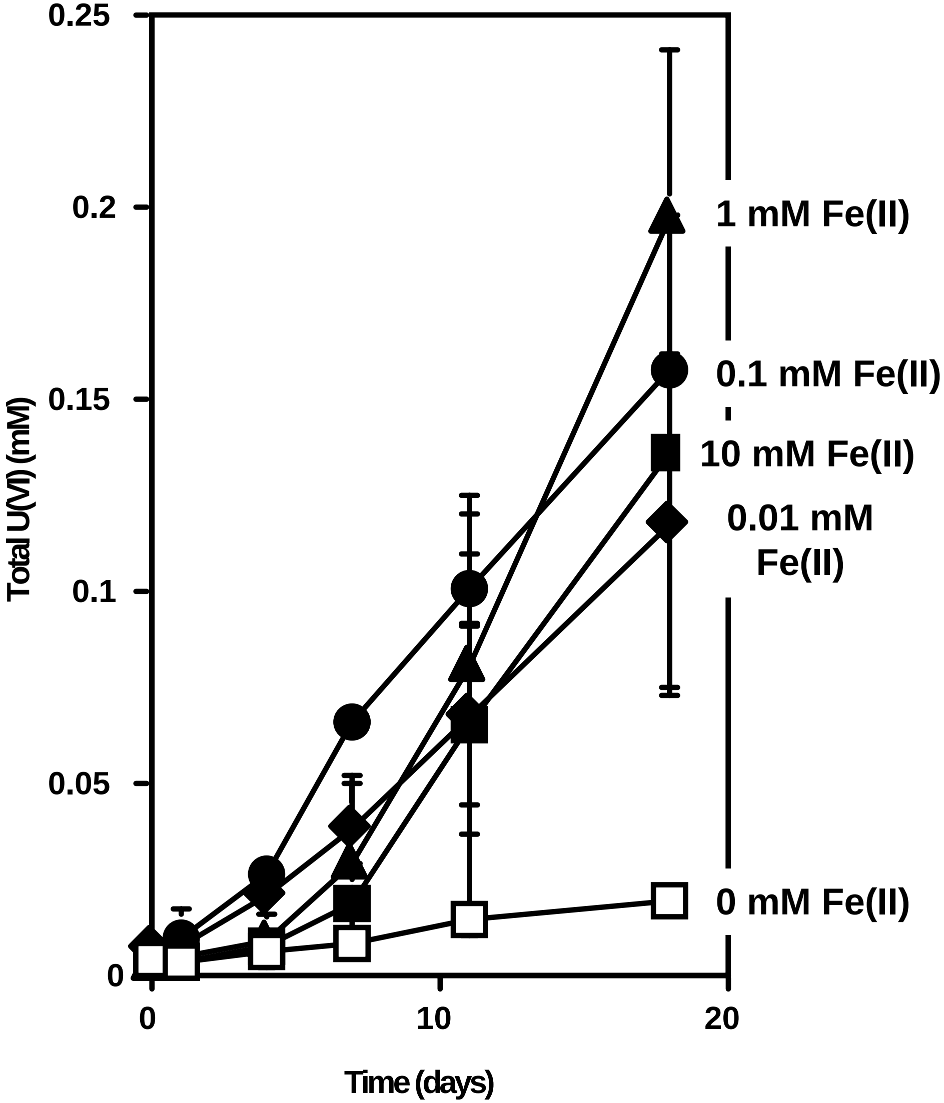
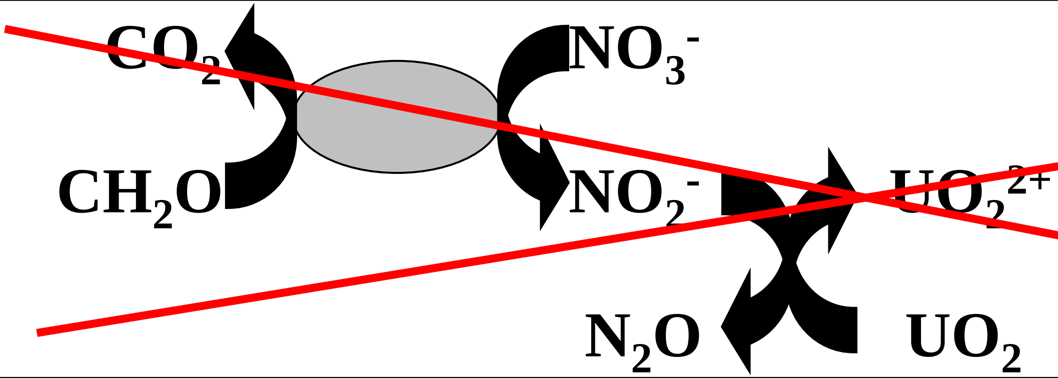


U(IV) oxidation by nitrite with various concentrations of Fe(II)

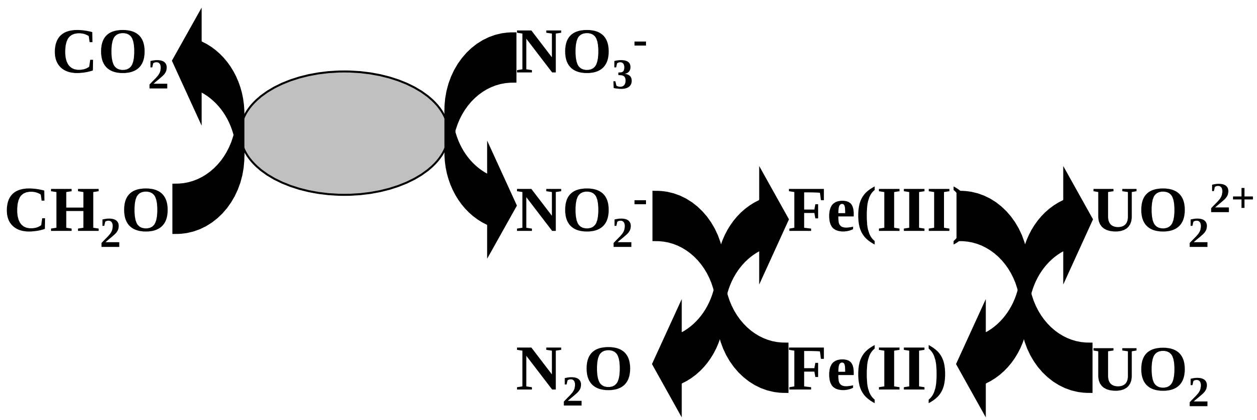


- **U(IV) oxidation by nitrite is independent of [Fe(II)] (unless Fe(II) in excess of NO_2^-)**
- **Fe shuttles electrons from U(IV) to nitrite**

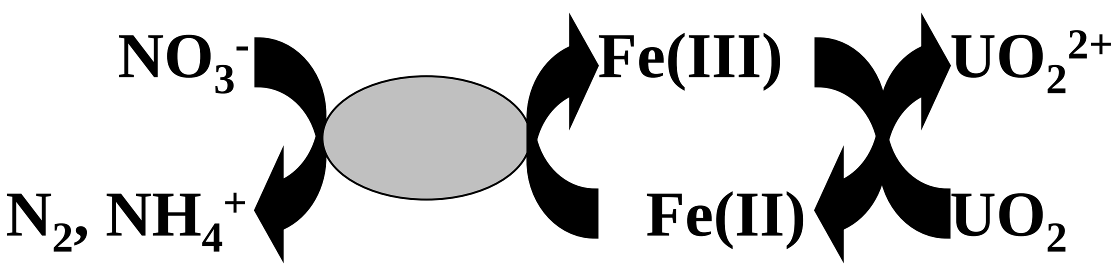
Mechanisms of NO_3^--dependent U(IV) oxidation



U(IV) oxidation by reactive denitrification intermediates

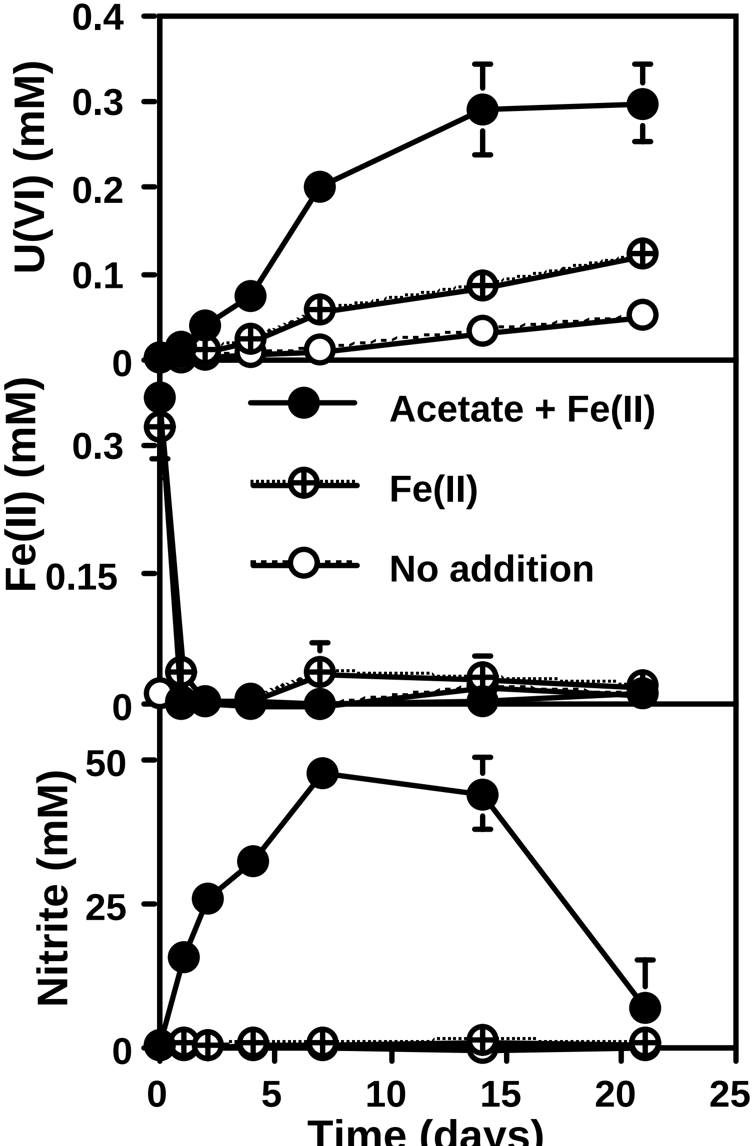


U(IV) oxidation by reactive denit. intermediates and Fe



U(IV) oxidation by Fe(III) from nitrate-dependent Fe(II)-oxidizing bacteria

U(IV) oxidation in FRC groundwater by resting cells of FRC isolate



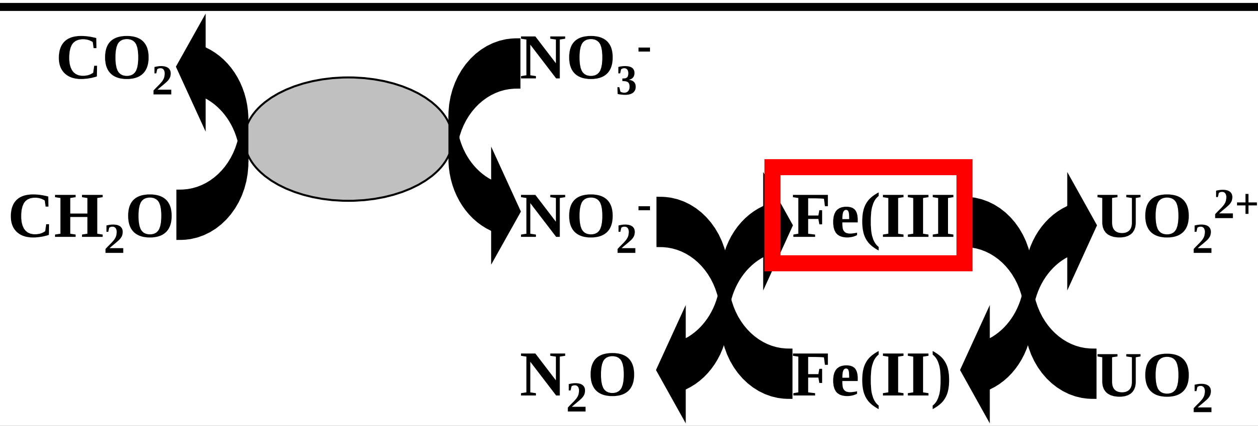
-U(IV) completely oxidized with acetate addition

-NO_2^- accumulated to high levels

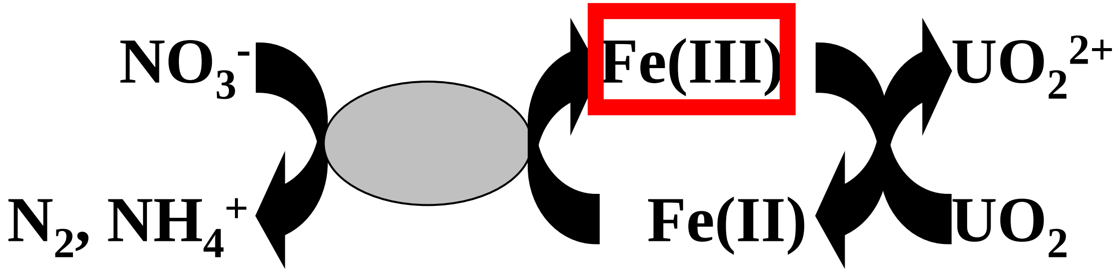
-Fe(II) rapidly oxidized in BOTH cases

-Less U(IV) oxidized under Fe(II)-oxidizing conditions

All Fe(III)s are not created equal

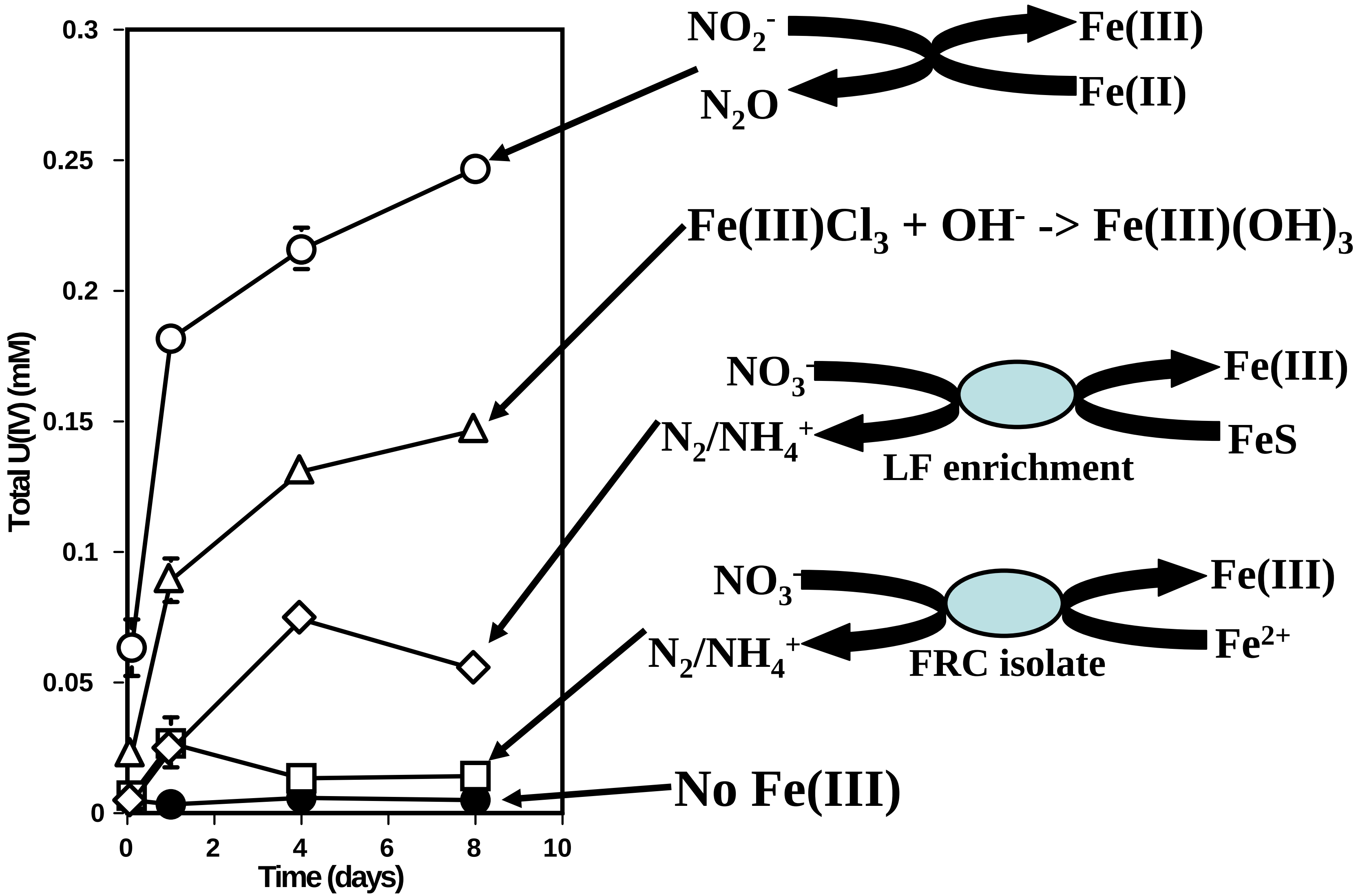


U(IV) oxidation by reactive denitrification intermediates

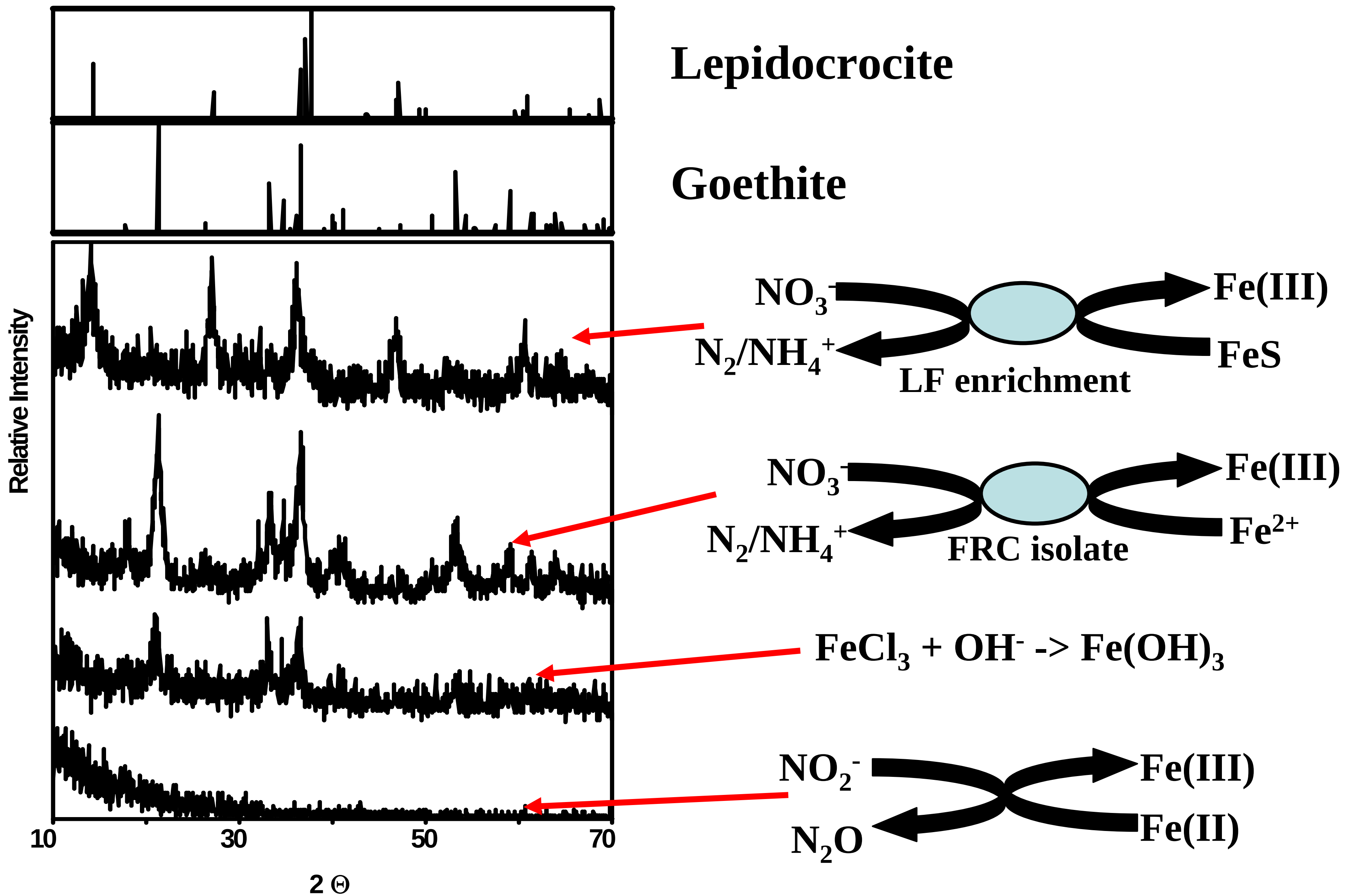


U(IV) oxidation by Fe(III) from nitrate-dependent Fe(II)-oxidizing bacteria

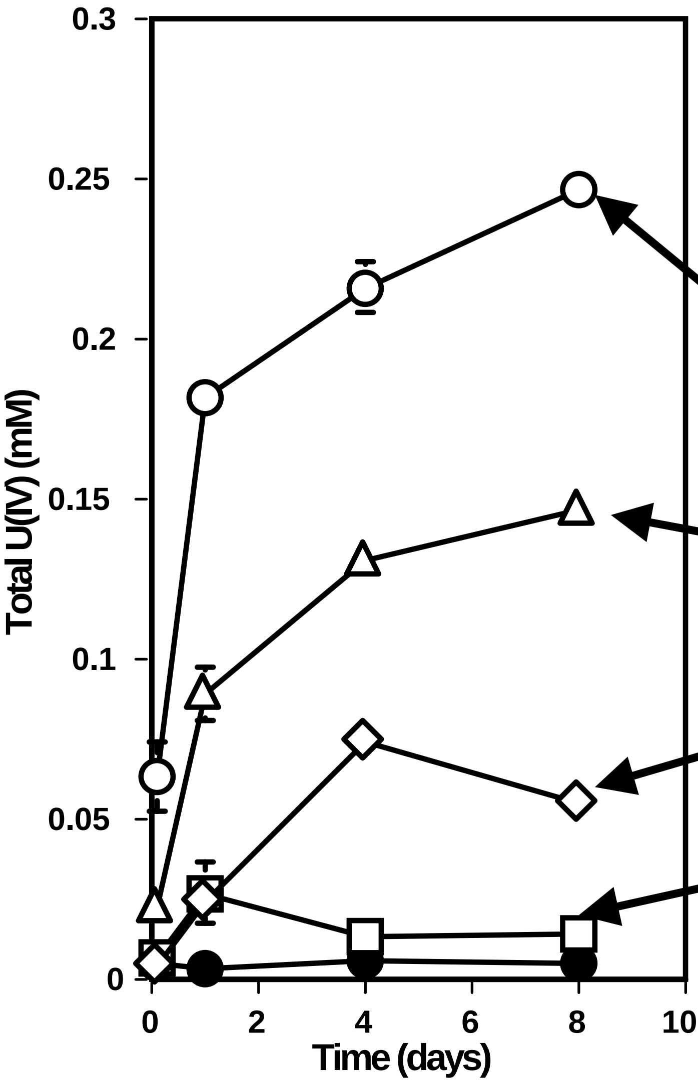
U(IV) oxidation by Fe(III) minerals



Characterization of biogenic and abiogenic Fe(III) minerals

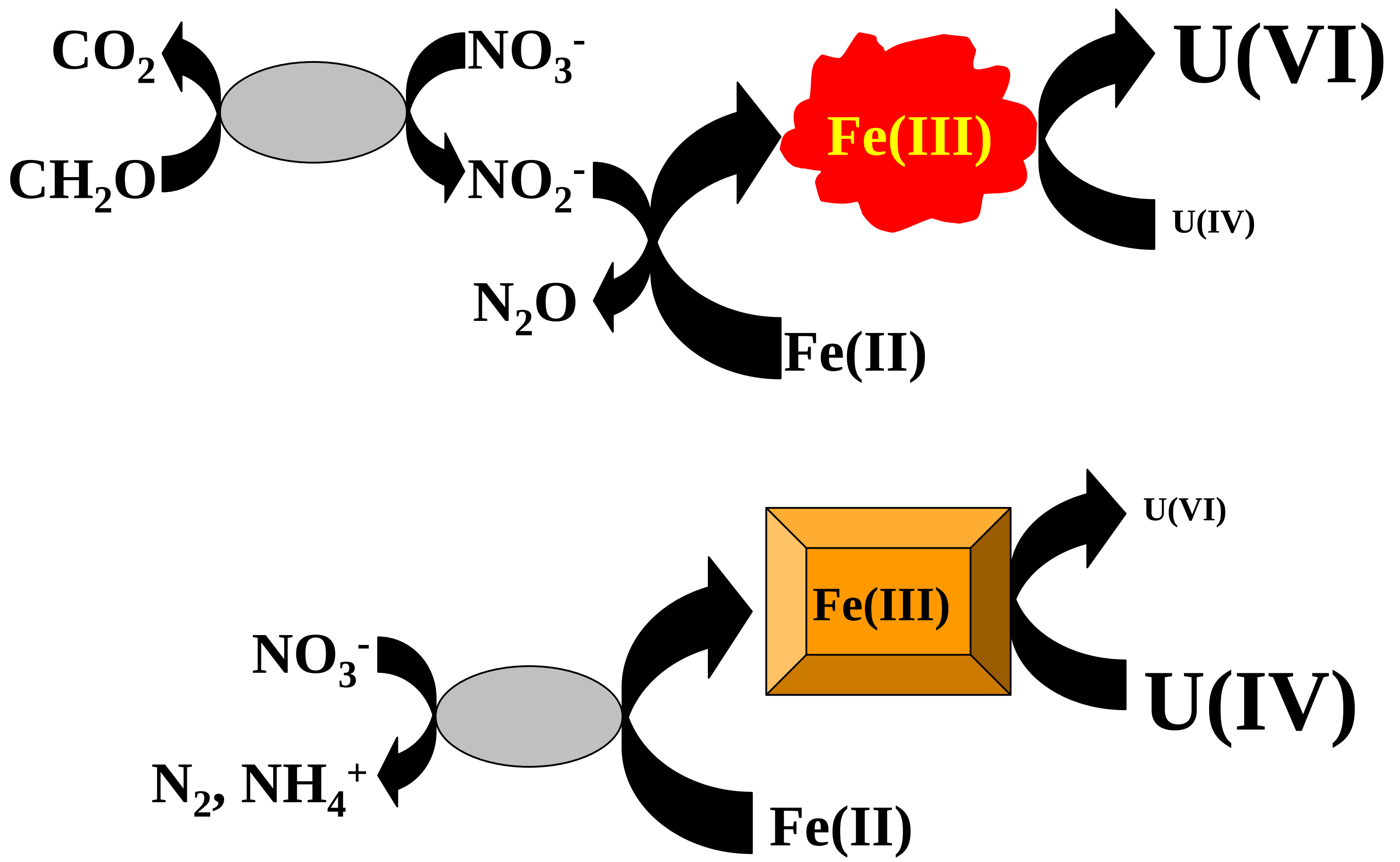


U(IV) oxidation by Fe(III) minerals



	E_H (V) (from Roden, 2003)	Surface Area (m^2/g)
HFO (Fe^{2+}/NO_2^-)	+0.34	113.8
HFO (hydrolysis of $FeCl_3$)	+0.34	87.9
(Bio)-lepidocrocite	-0.04	109.3
(Bio)-Goethite	-0.14	26.1

U(IV) oxidation by amorphous Fe(III) vs. crystalline Fe(III)



Question

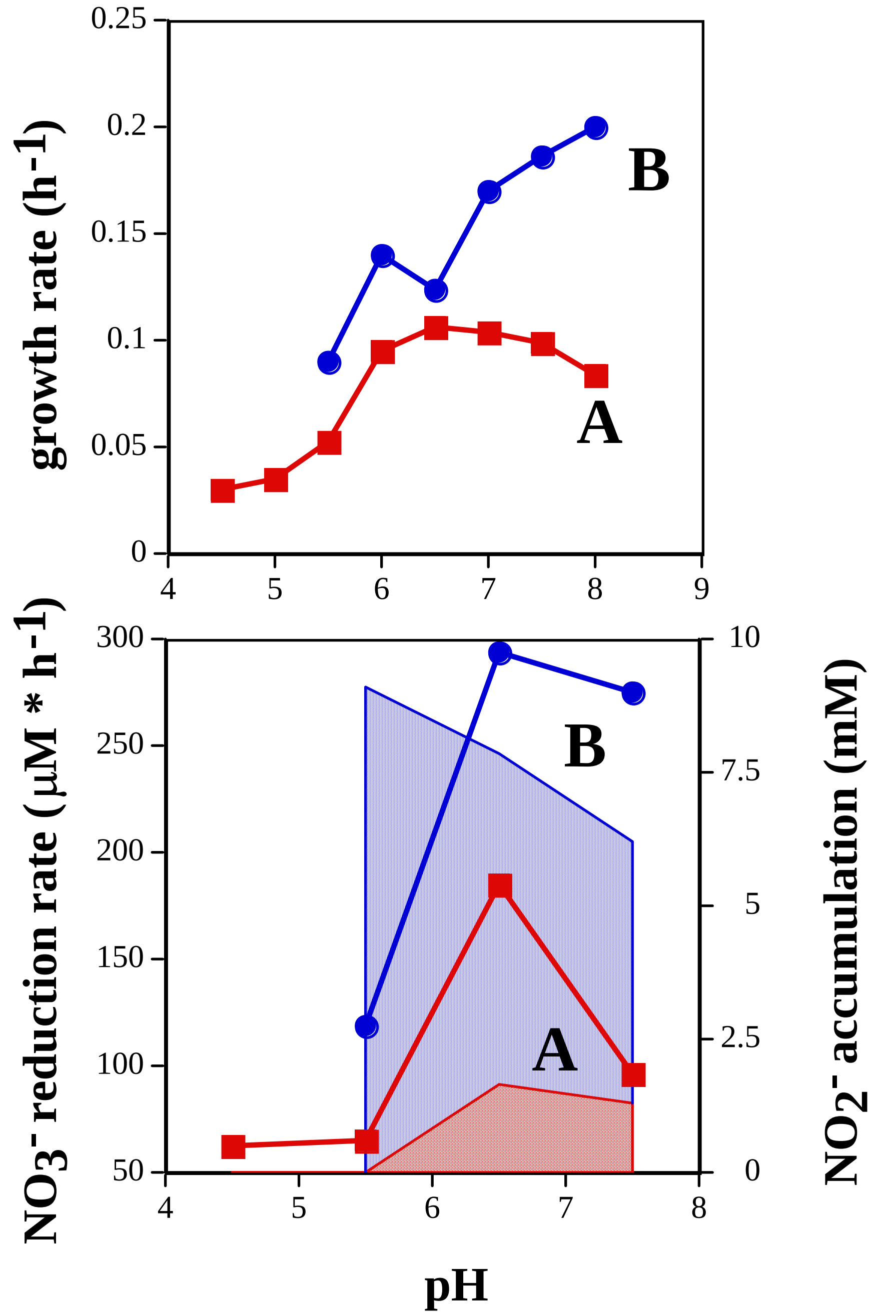
- What are the dominant nitrate reducing bacteria (under acidic conditions)?
- What is the mechanism for nitrate reduction during ethanol biostimulation?

Isolates from FRC

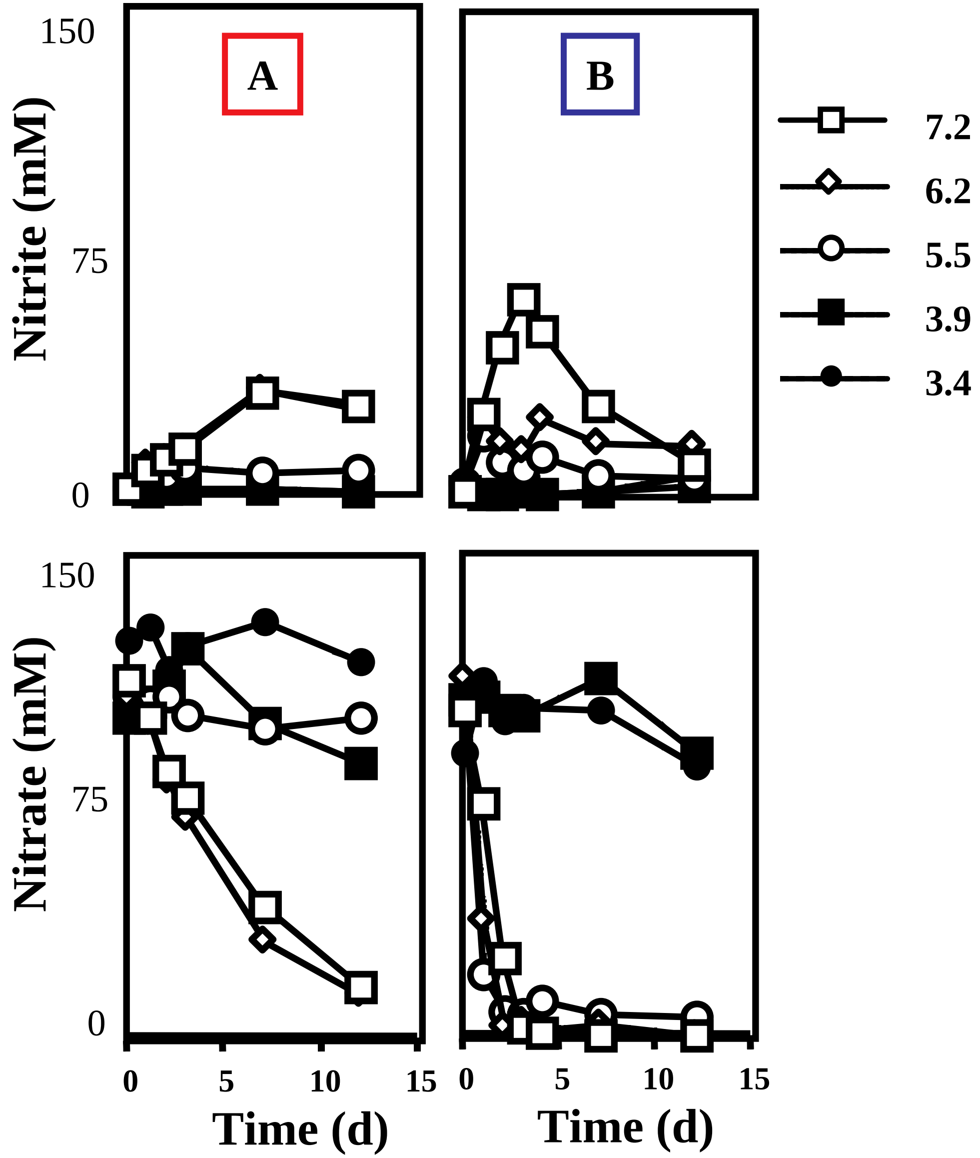
- Several strains were isolated by direct plating from biostimulated FRC groundwater on glucose or acetate.
- Identified by rRNA sequencing as
  - *Agrobacterium*
  - *Pseudomonas*
  - *Klebsiella*

pH tolerance of FRC isolates under laboratory conditions

- 10 mM NO_3^-
- 5mM glucose
- pHs 4.5-8.0



pH tolerance of FRC isolates in FW021 groundwater



Nitrate and Nitrite Reductase Assays

NO_3^-
↓ Nar/Nap
NO_2^-
↓ Nir (K/S)
NO
↓ Nor
N_2O
↓ Nos
N_2

	Isolate	BV^+/MV^+ Assay Results	Nitrite Reductase PCR results
A	**GN32#1**	**1.12 (Nap)**	**nirK**
A	**GN32#2**	**0.95 (Nap)**	**nirK**
B	**GN33#1**	**2.56 (Nap and Nar)**	**nirK**
B	**AN33#1**	**2.83 (Nap and Nar)**	**-**

II. Microbial Community of FRC

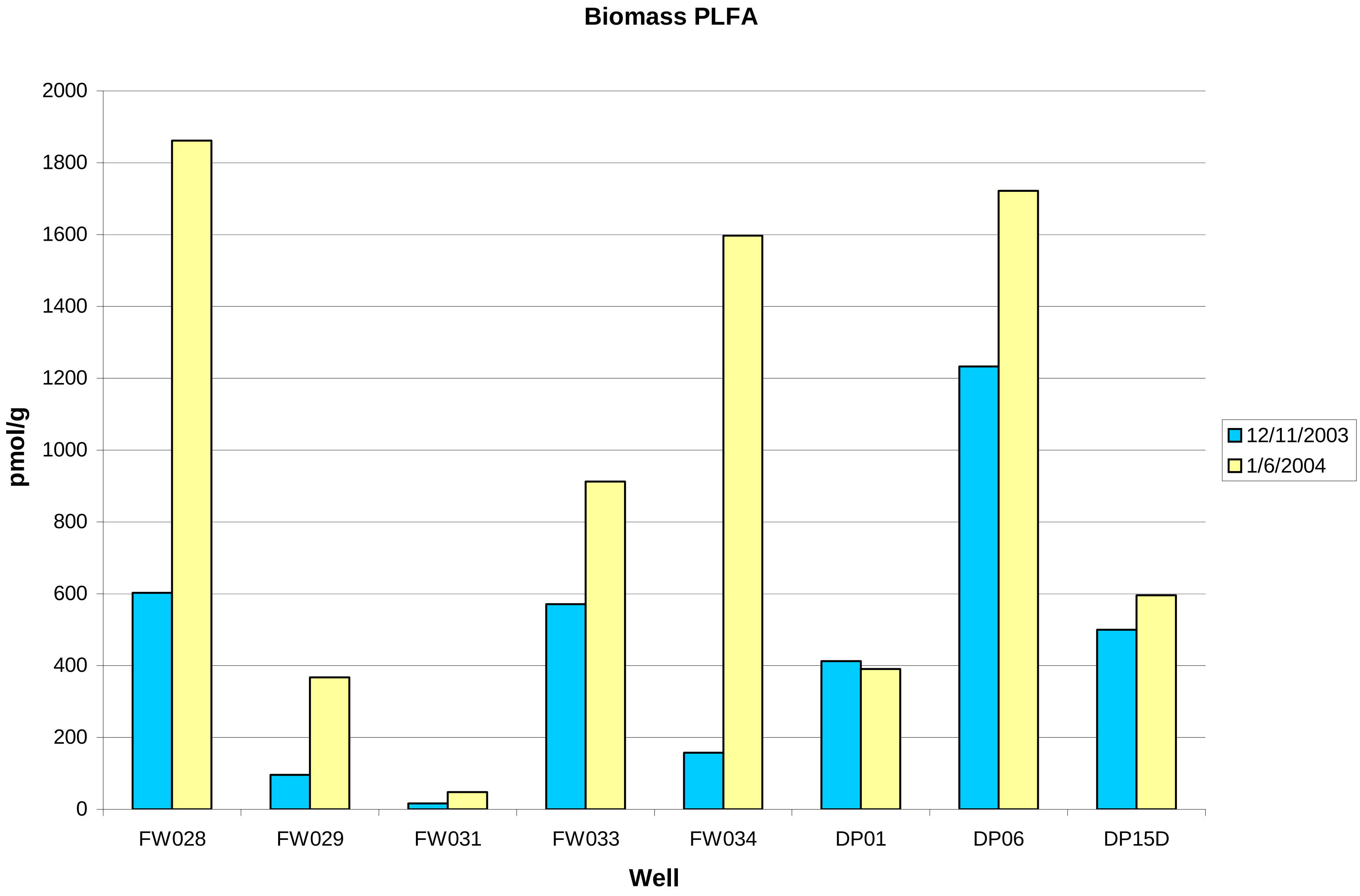
Clone libraries

Characterization of nitrate reducing microbial communities.

Goal: To identify acid tolerant NO_3^- reducing bacteria.

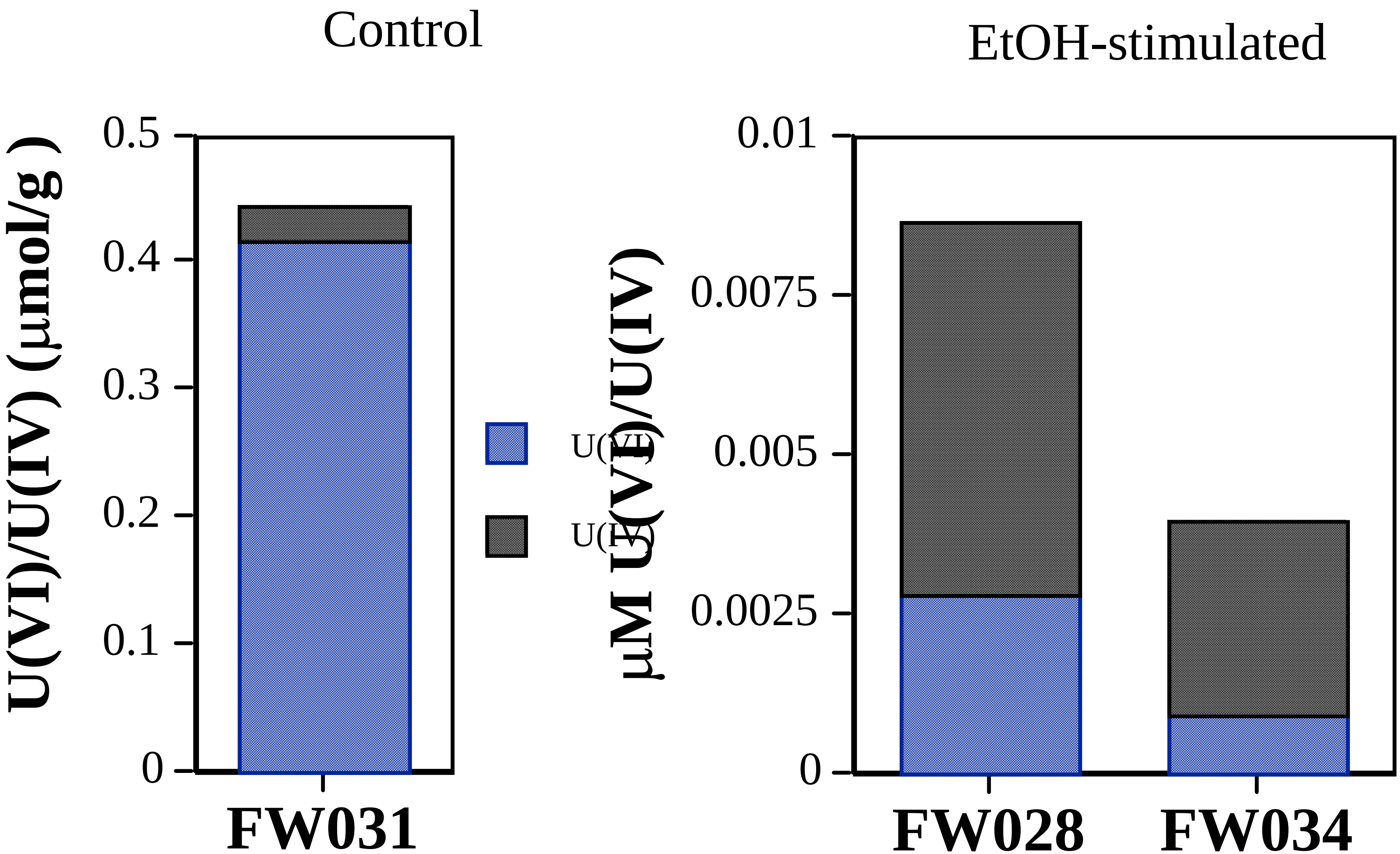
Experiment: Push pull tests: November, 2003
DNA extracted from core material nearby wells

Treatment Groups	Well location	Initial mM Nitrate	Depth
Area2, stimulated	DP15D	0.79	18'
Area2, stimulated	DP06	0.25	18'
Area2, unstimulated	FW003	0.45	19'
Area1, stimulated	FW028	131.33	22'
Area1, stimulated	FW034	132.44	15'
Area1, unstimulated	FW031	137.38	12'

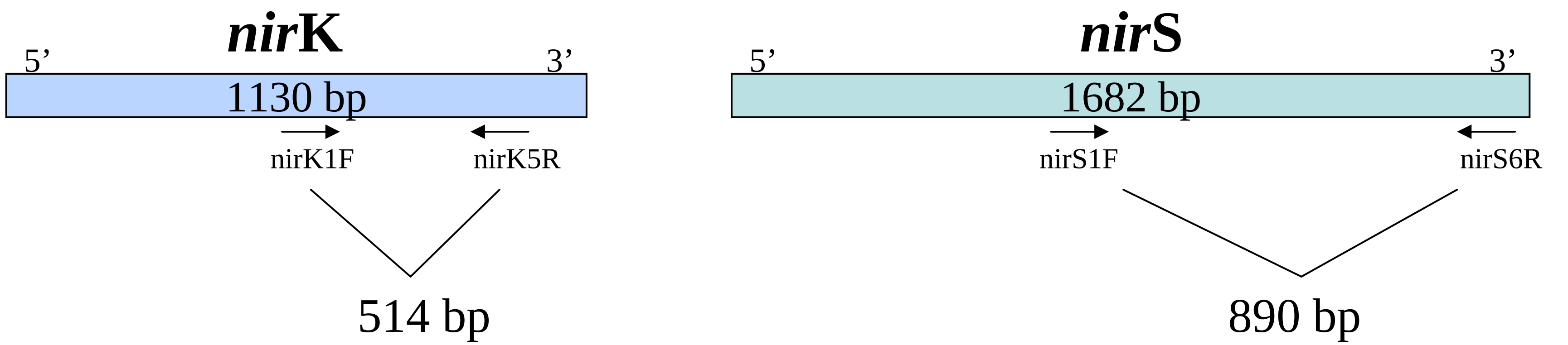


Biomass PLFA
pmol/g
2000
1800
1600
1400
1200
1000
800
600
400
200
0
FW028
FW029
FW031
FW033
FW034
DP01
DP06
DP15D
Well
12/11/2003
1/6/2004

U(IV) -- Area 1 cores



PCR amplification of *nir*K and *nir*S genes



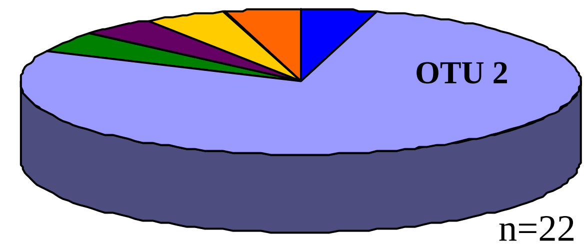
PCR results

Treatment Groups	Well location	16S	nirK	nirS
Area2, stimulated	DP15D	+	+	-
Area2, stimulated	DP06	+	-	+
Area2, unstimulated	FW003	+	-	-
Area1, stimulated	FW028	+	+	+
Area1, stimulated	FW034	+	+	+
Area1, unstimulated	FW031	+	+	-

Primers designed by Braker et al. AEM (1998) Vol. 64, p. 3769-3775

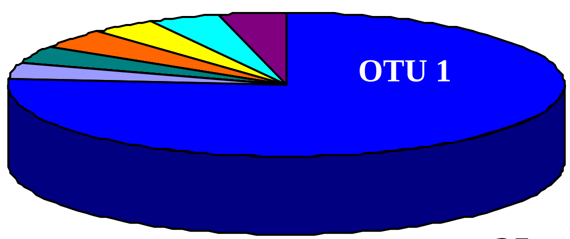
nirK clone libraries
13 OTU s

DP-15D

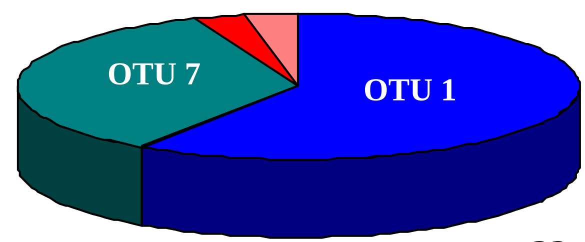


Area 2
EtOH
stimulated

FW028

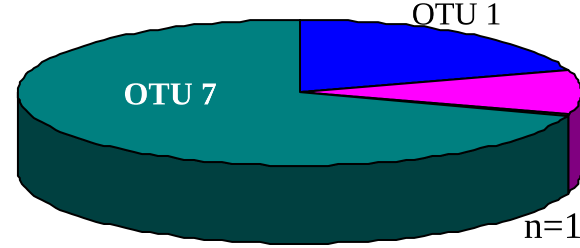


FW034



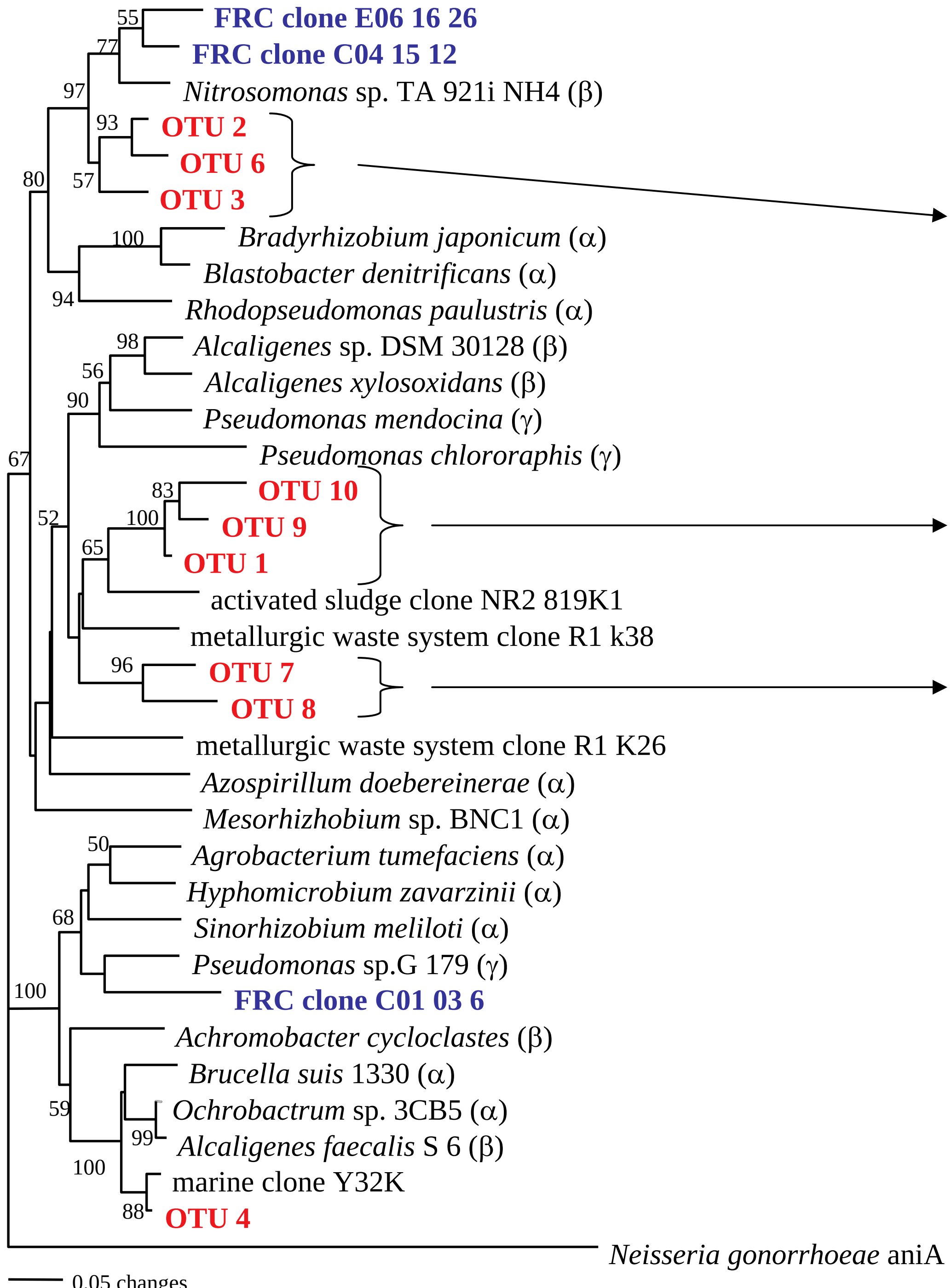
Area 1
EtOH
stimulated

FW031



Area 1
(donor
control)

nirK tree



n = 20
86% of DP15D clones
(stimulated, **Area2**)

n = 44
70% of FW028 and FW034 clones
(stimulated, **Area 1**)

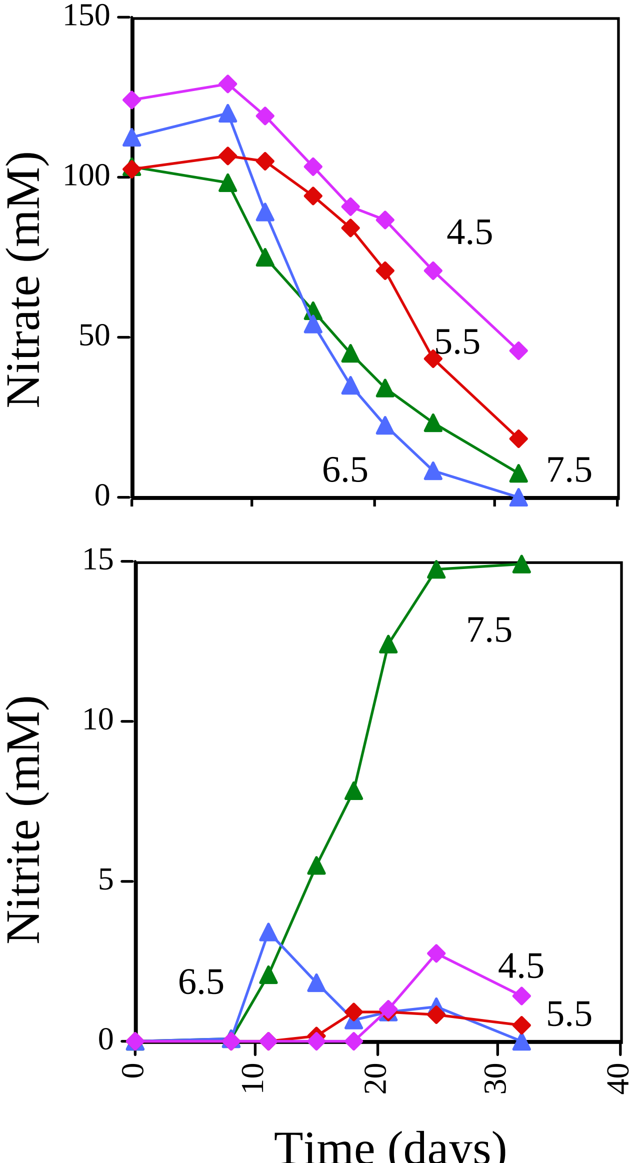
n = 20
70% of FW003 clones
(unstimulated, **Area 1**)

II. Microbial Community of FRC

B. Nitrate Reducing Enrichments

Purpose: to enrich and isolate organisms able grow under FRC biostimulation conditions at a range of pHs

Experiment:
- 100 mM EtOH
- 100 mM NO_3^-
- pHs 4.5-7.5
- Stimulated sediment from Area 1 (FB064, pH 4.28, 104.7 mM NO_3^-)



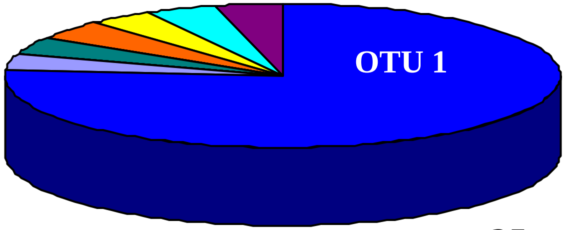
Isolate Results

•All new isolates are closely related to each other (based on both nirK and 16S sequences)

• All new isolates have nirK gene (not nirS)

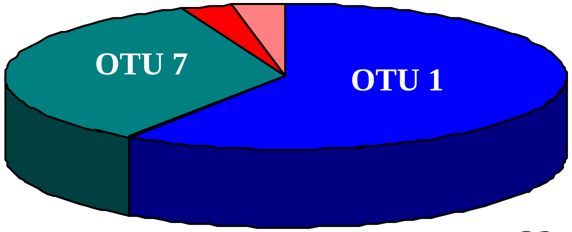
•nirK sequences of isolates are 99% similar to OTU 1 from the nirK clone libraries **-- This is the dominant clone sequence for both ethanol-stimulated Area 1 sites**.

FW028



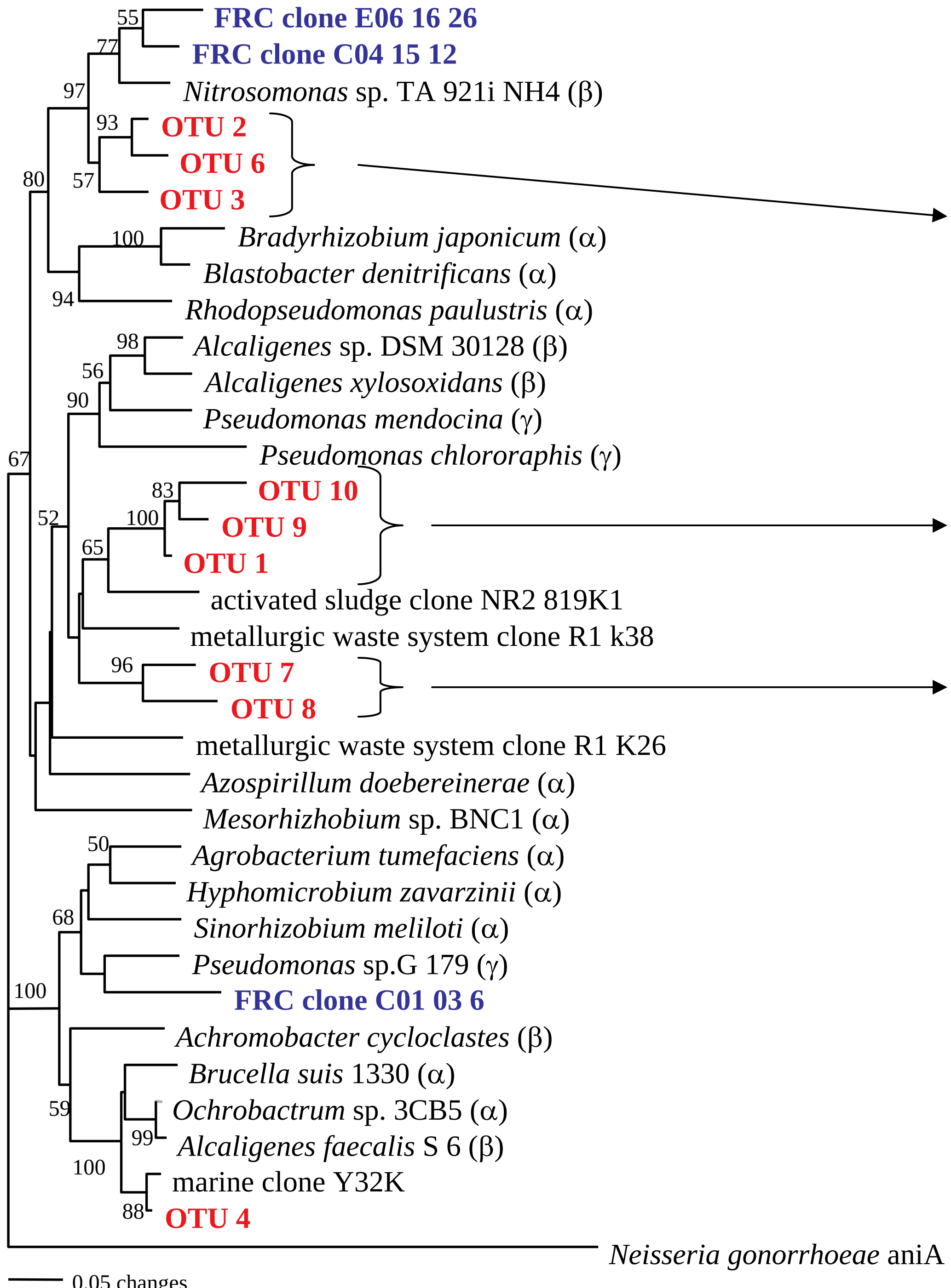
n=25

FW034



n=32

nirK tree



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86% of DP15D clones
(stimulated, **Area2**)

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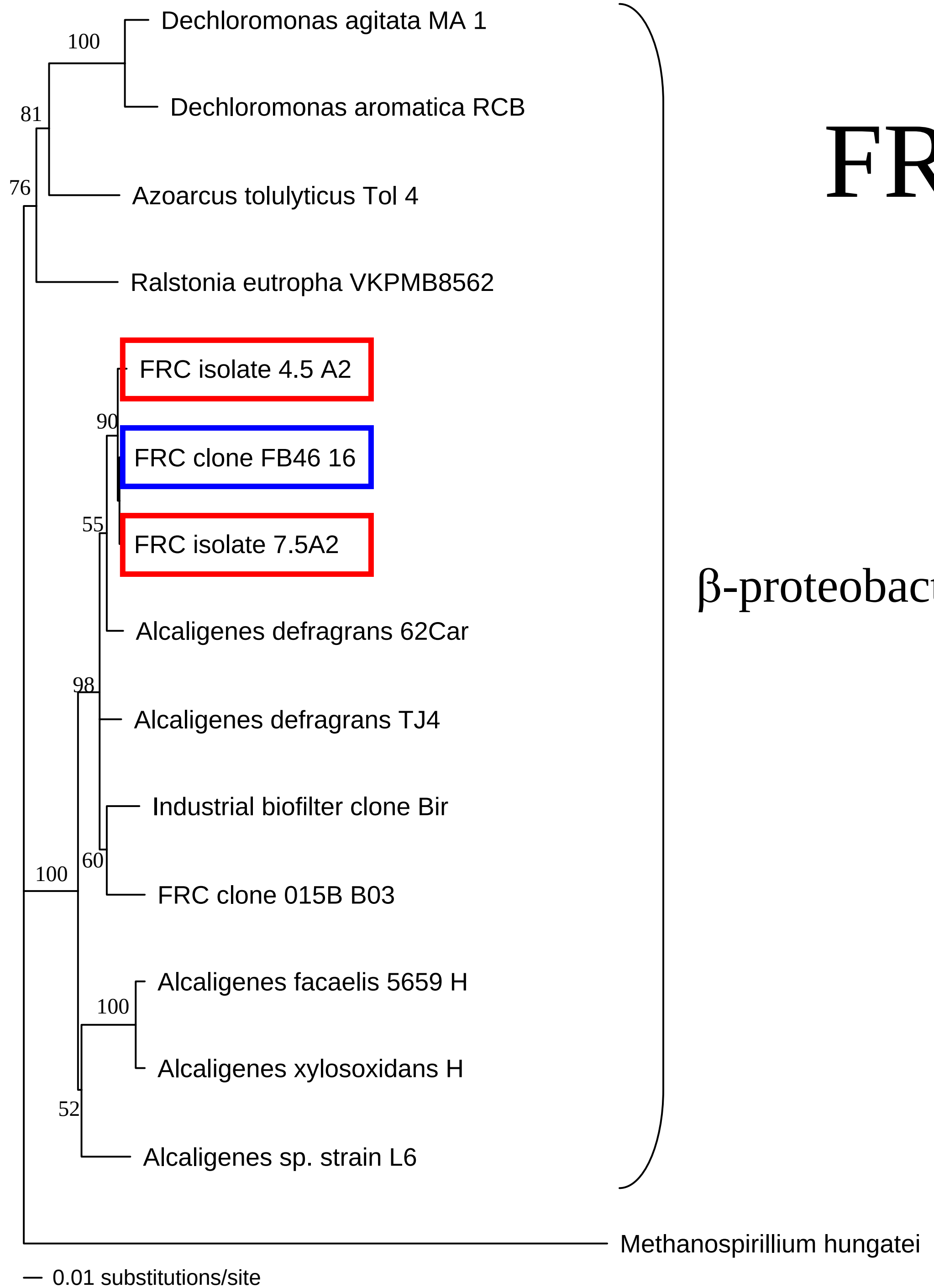
FRC Isolates



Alcaligenes defragrans

- Previously isolated from activated sludge
- All strains degrade terpenes.

Future Directions

- Aluminum tolerance
- pH tolerance.
- U(IV) oxidation

Acknowledgements

NABIR Program

Ph.D. Students

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Anne Spain

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