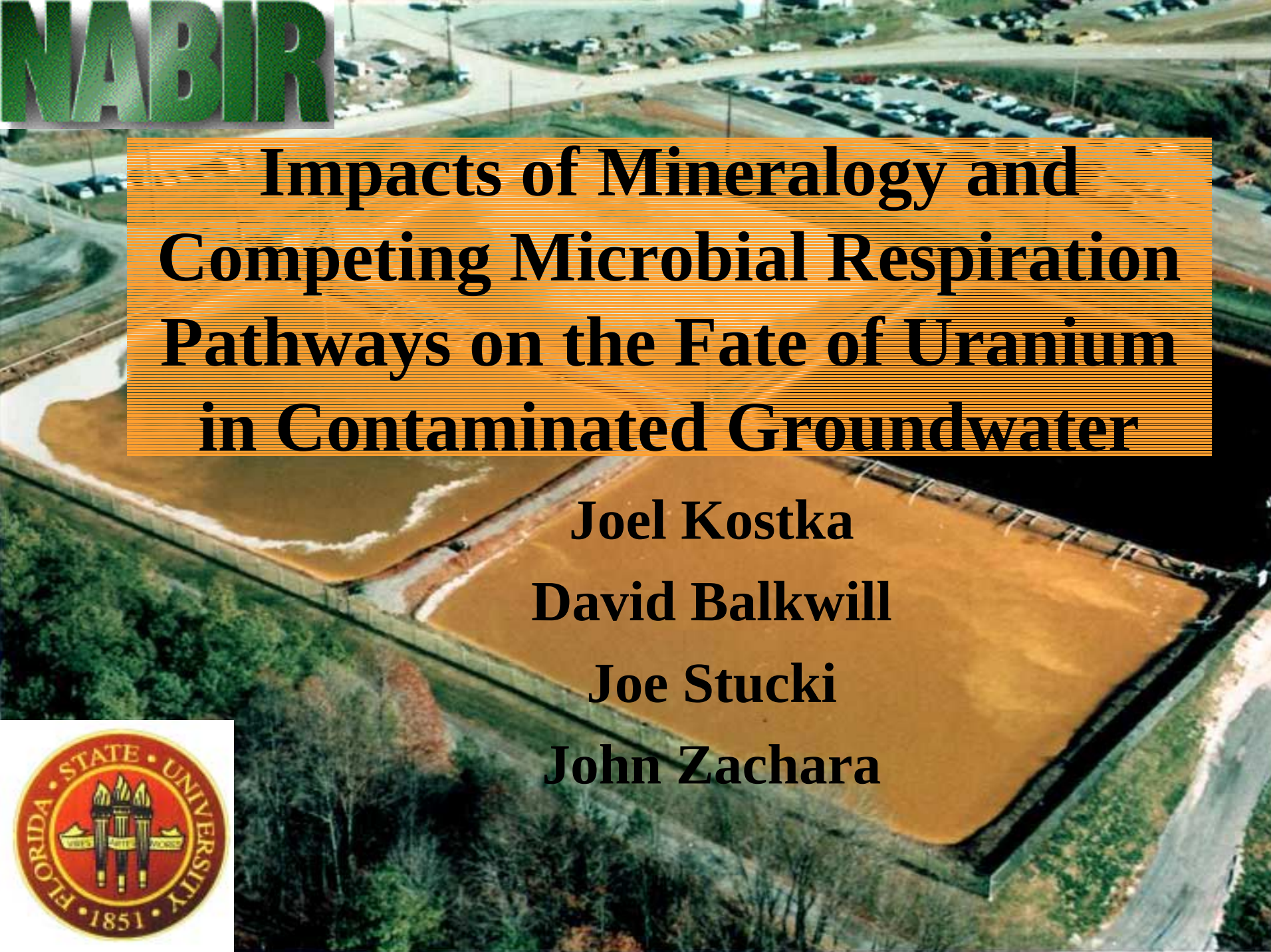


Impacts of Mineralogy and Competing Microbial Respiration Pathways on the Fate of Uranium in Contaminated Groundwater

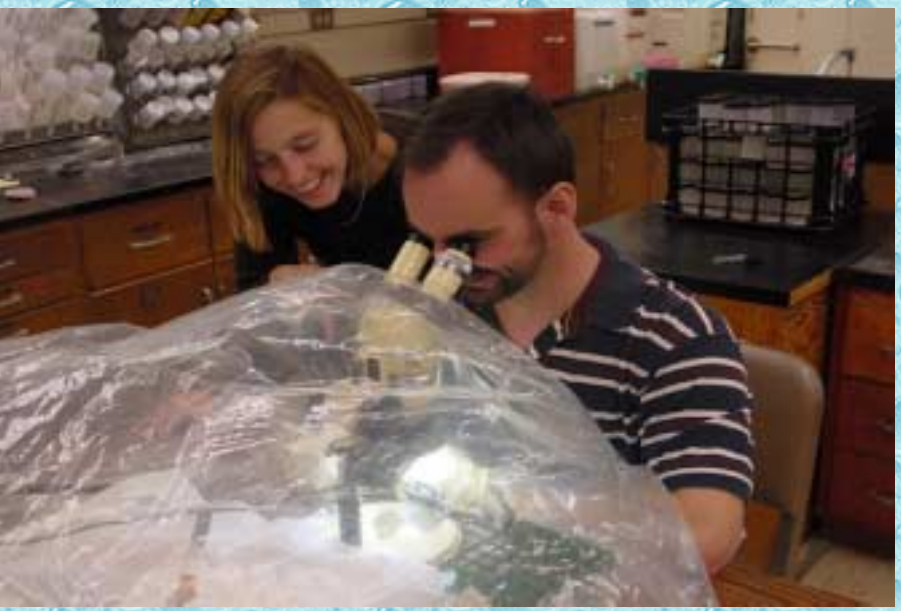
Joel Kostka

David Balkwill

Joe Stucki

John Zachara

NABIR



Acknowledgements

- Lainie (Ellen) Petrie
- Sherry Dollhopf
- Nadia North
- Dava Dalton
- Hayley Skelton
- Harold Adams
- David Watson and FRC staff
- Susan Pfiffner
- Jack Istok
- Lee Krumholz

NABIR

Overall Goal

Field-oriented project to understand microbially-mediated mechanisms controlling the biostimulation of U(VI) reduction/ immobilization in subsurface at FRC

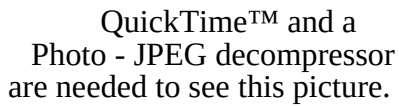
Objectives

- **Analyze Fe minerals, other redox-active constituents which may accelerate or inhibit U(VI) immobilization (Zachara, Stucki, Kostka)**
- **Characterize anaerobic microbial consortia likely to catalyze U(VI) reduction: enrichment cultures of FeRB and SRB (Kostka, Balkwill)**
- **Measure rates of carbon oxidation and electron acceptor utilization in anoxic sediment incubations (Kostka)**

Outline of Results

- Geochemistry, Fe mineral analysis
- Comparison of cultivatable FeRB
  cloning/ sequencing of 16S rRNA genes, T-RFLP of MPN enrichments
- Nitrate reduction in sediment microcosms
- Comparison of bkgd to contaminated

(Area 1), low pH to high pH sediments


QuickTime™ and a
Photo - JPEG decompressor
are needed to see this picture.

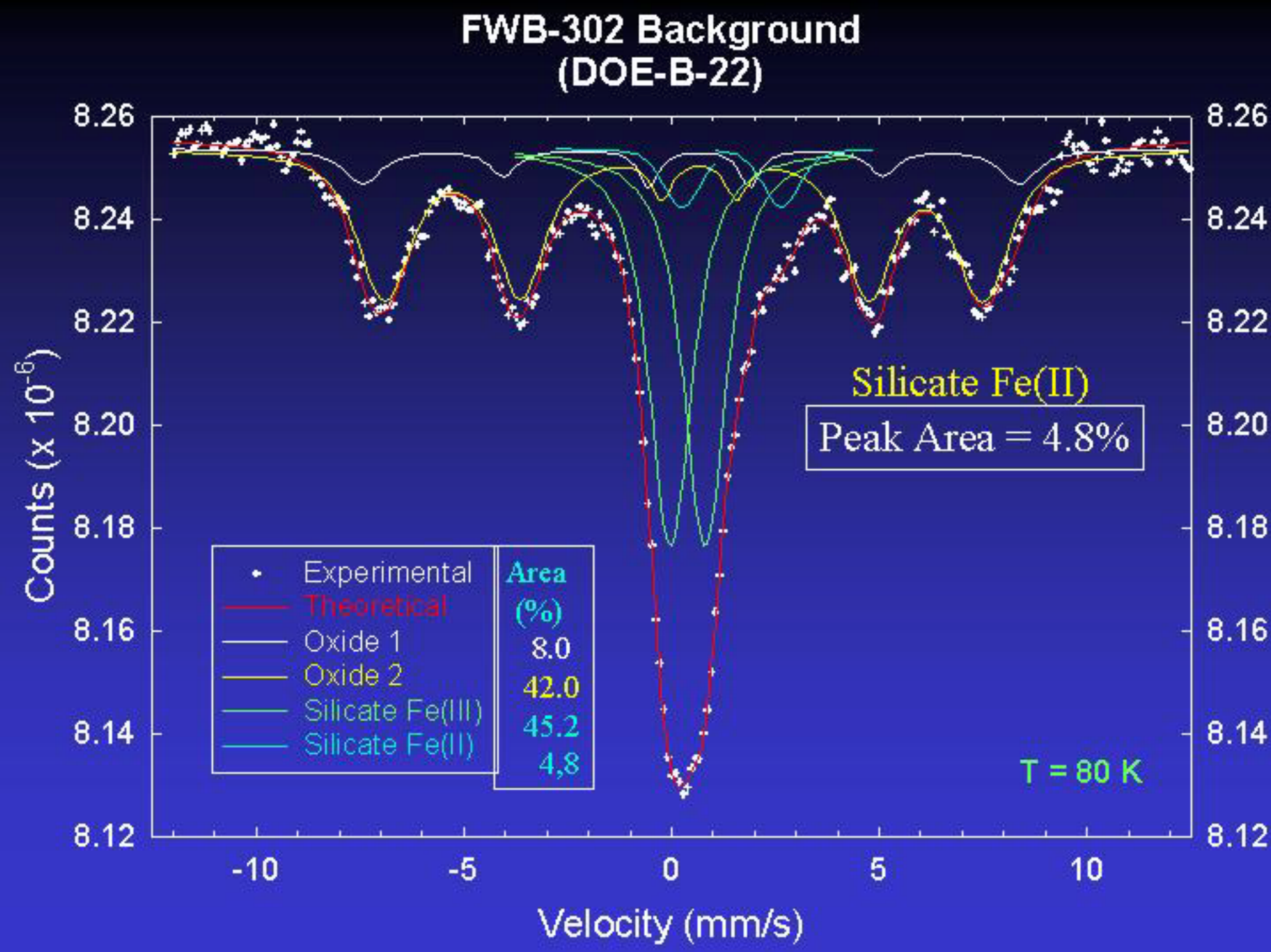
NABIR

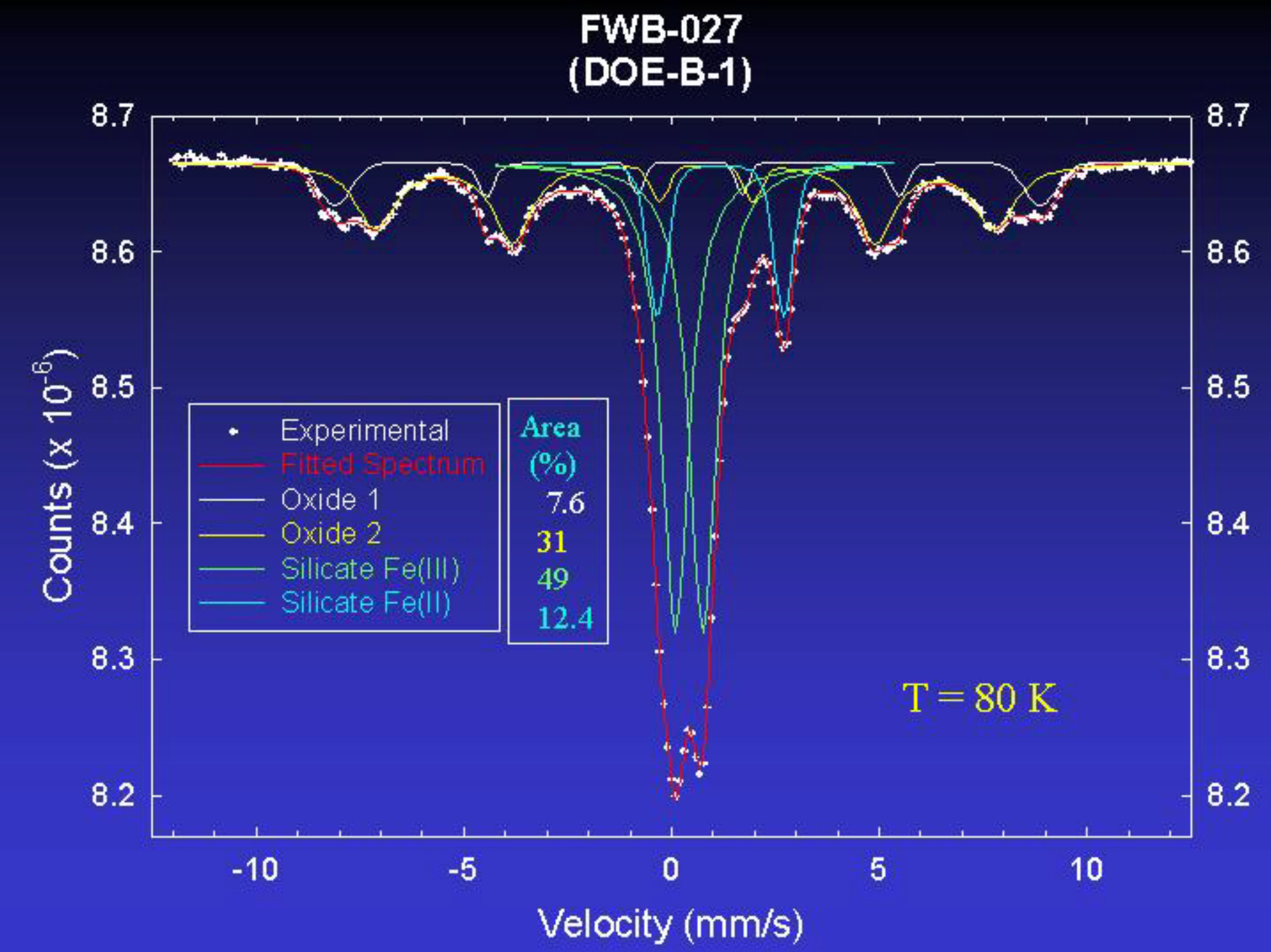
Groundwater chemistry

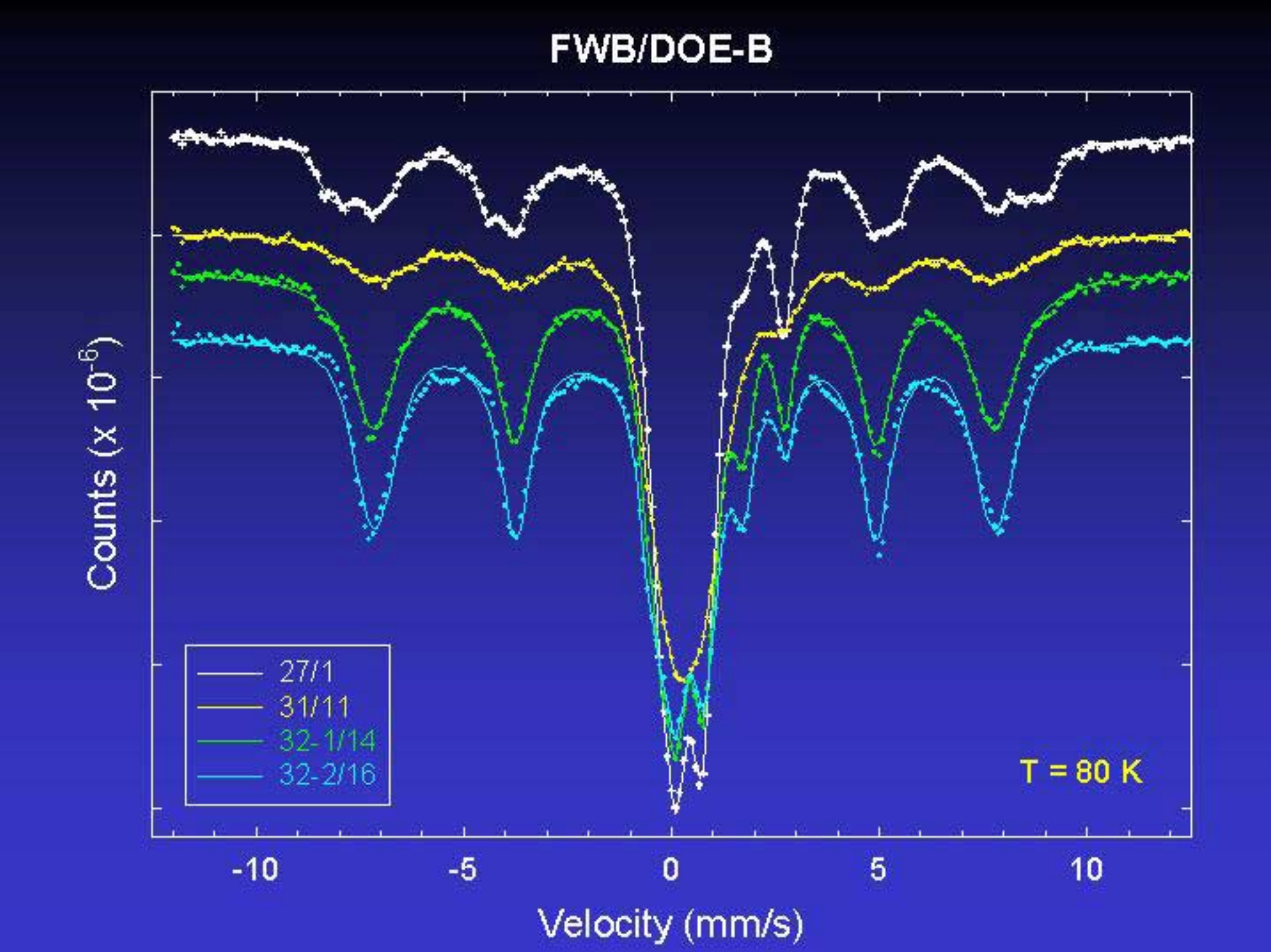
	Sample	pH	U(VI)	NO_3^-	NH_4^+
			(µg l^{-1})	(mM)	(µM)
Bkgd	300	6.8-7.6	0	0.002-0.003	----
Contam.	19	7.0	144-149	0.149-0.155	0-3
	20	5.2	122	----	----
	30	3.7	0	88.4-108	22-26
	32	5.4	975-1030	11.6-11.9	2-4

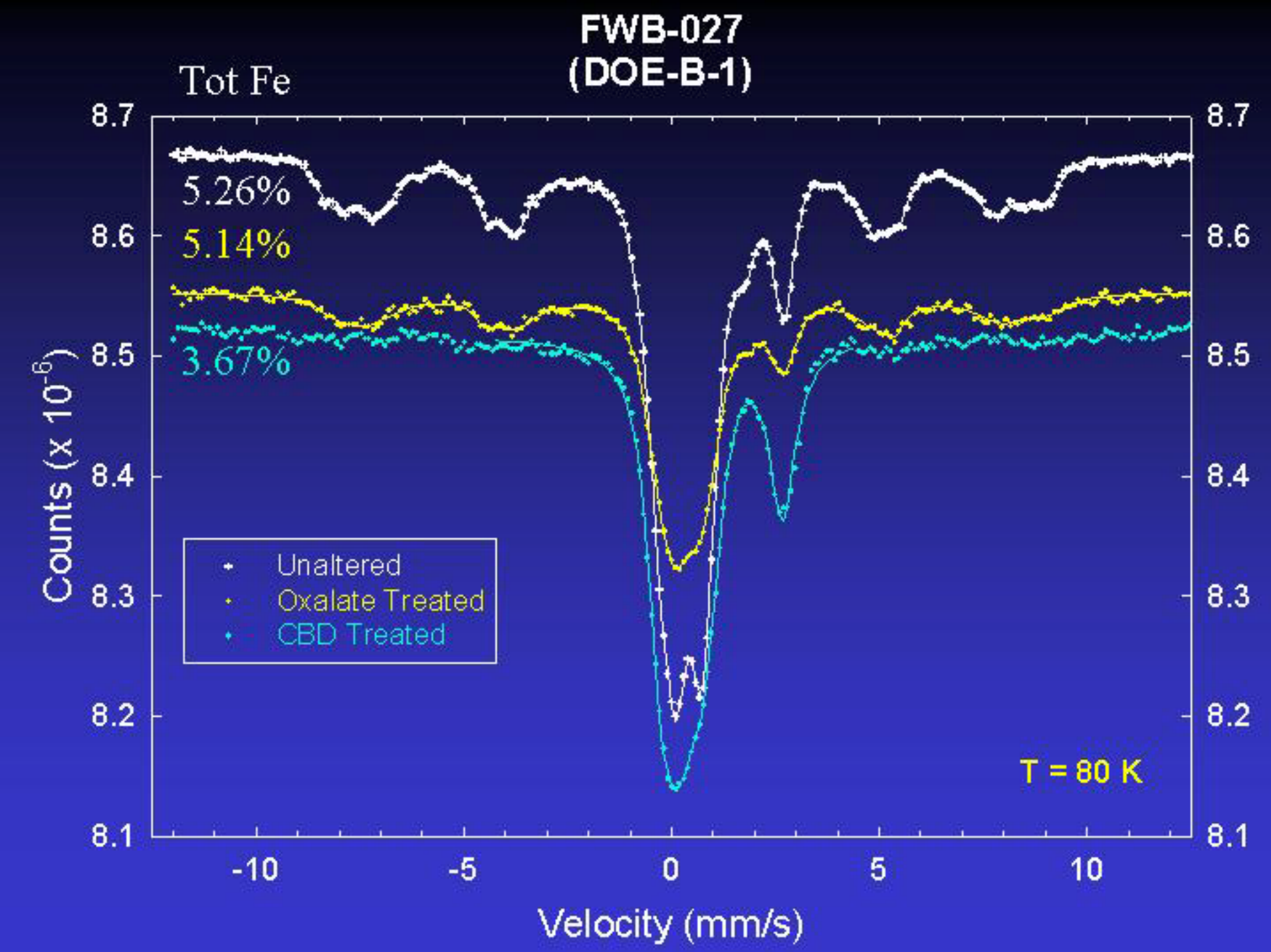
Sediment geochemistry

Sample	pH	NO_3 (umol/cm^3)	Fe -HCl (umol/cm^3)	Fe-Dithio. (umol/cm^3)
302-02	5.4	$1.02*10^{-2}$-$1.03*10^{-2}$	4.36	503.5
302-05	5.7	$1.84*10^{-5}$-$2.40*10^{-3}$	26.1	302.3
27	3.6	9.80-54.9	13.5	260.7
28	3.4	1.37-$1.80*10^{2}$	11.3	329.6
30	4.2	22.7-31.5	15.2	414.4
31	3.8	0.120-91.7	10.8	376.4
32	4.4	$1.67*10^{-2}$-17.2	18.9	407.9
33	3.6	13.6-295	14.5	467.7
34	3.8	3.28-70.5	16.3	325.0


FWB-302 Background
(DOE-B-22)
Counts (x 10^-6)
Velocity (mm/s)
8.26
8.24
8.22
8.20
8.18
8.16
8.14
8.12
-10
-5
0
5
10
Silicate Fe(II)
Peak Area = 4.8%
Experimental
Theoretical
Oxide 1
Oxide 2
Silicate Fe(III)
Silicate Fe(II)
Area (%)
8.0
42.0
45.2
4,8
T = 80 K


FWB-027
(DOE-B-1)
Counts (x 10^-6)
8.7
8.6
8.5
8.4
8.3
8.2
-10
-5
0
5
10
Velocity (mm/s)
Experimental
Fitted Spectrum
Oxide 1
Oxide 2
Silicate Fe(III)
Silicate Fe(II)
Area (%)
7.6
31
49
12.4
T = 80 K


FWB/DOE-B
Counts (x 10-6)
27/1
31/11
32-1/14
32-2/16
T = 80 K
-10
-5
0
5
10
Velocity (mm/s)


FWB-027
(DOE-B-1)
Tot Fe
5.26%
5.14%
3.67%
Unaltered
Oxalate Treated
CBD Treated
T = 80 K
Counts (x 10^-6)
Velocity (mm/s)
8.7
8.6
8.5
8.4
8.3
8.2
8.1
-10
-5
0
5
10

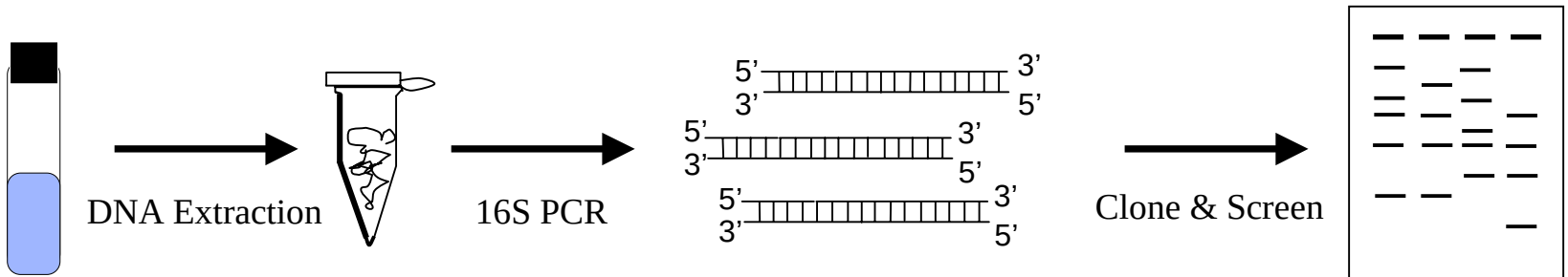
Iron Content After Treatment

Total Fe Content (wt. %) by Chemical Analysis			
Treatment	Unaltered	Oxalate	CBD
FWB-027	5.26	5.14	3.67
FWB-031	3.68	3.72	2.72
FWB-032-1	5.72	5.52	3.09
FWB-032-2	5.25	5.36	2.17

Comparison of Mössbauer and Chemical Analysis

Iron Oxide Component	Chemical Analysis (wt. %)	Peak Area (%)
FWB-027	30.2	38.6
FWB-031	26.1	29.2
FWB-032-1	46.0	58.8
FWB-032-2	58.7	63.1

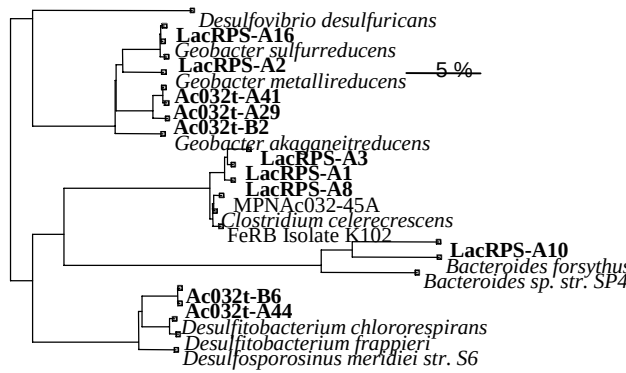
Cloning & Sequencing -FeRB

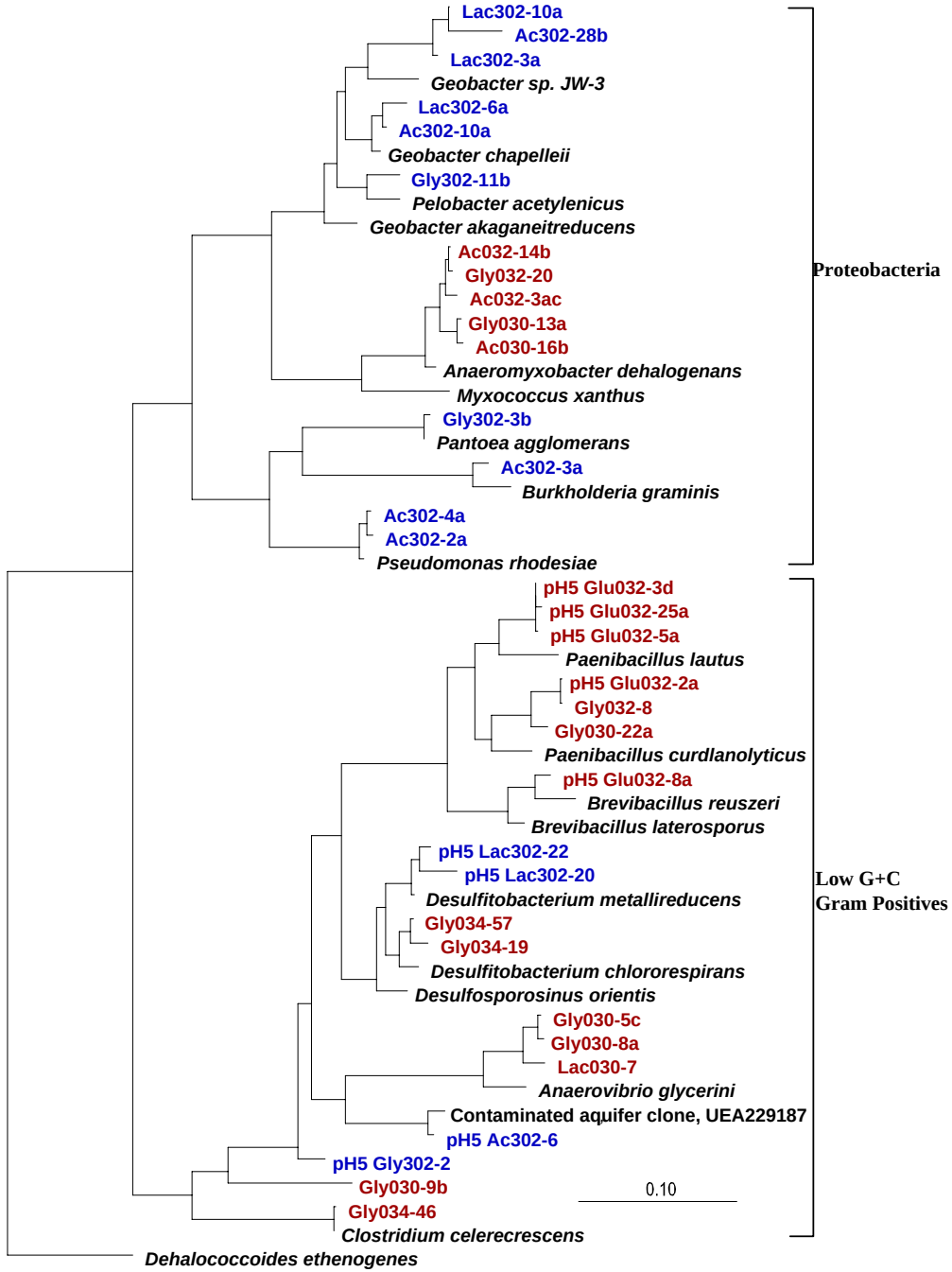


MPN Tube

- 20 MPN tubes were analyzed.
- Biomass and DNA concentrations were extremely low.
- All positive clones were screened (total of 708).
- Extraction, amplification, and cloning of sterile water was used as a negative control.

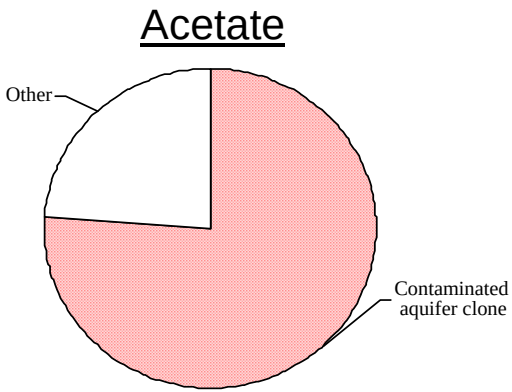
Sequence (1000 bp) & Analyze



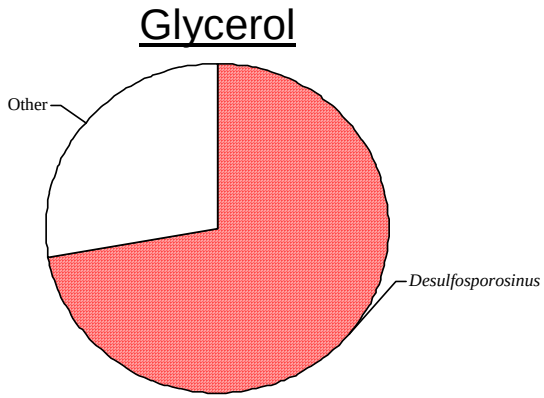

Lac302-10a
Ac302-28b
Lac302-3a
Geobacter sp. JW-3
Lac302-6a
Ac302-10a
Geobacter chapelleii
Gly302-11b
Pelobacter acetylenicus
Geobacter akaganeitreducens
Ac032-14b
Gly032-20
Ac032-3ac
Gly030-13a
Ac030-16b
Anaeromyxobacter dehalogenans
Myxococcus xanthus
Gly302-3b
Pantoea agglomerans
Ac302-3a
Burkholderia graminis
Ac302-4a
Ac302-2a
Pseudomonas rhodesiae
Proteobacteria
pH5 Glu032-3d
pH5 Glu032-25a
pH5 Glu032-5a
Paenibacillus lautus
pH5 Glu032-2a
Gly032-8
Gly030-22a
Paenibacillus curdlanolyticus
pH5 Glu032-8a
Brevibacillus reuszeri
Brevibacillus laterosporus
pH5 Lac302-22
pH5 Lac302-20
Desulfitobacterium metallireducens
Gly034-57
Gly034-19
Desulfitobacterium chlororespirans
Desulfosporosinus orientis
Gly030-5c
Gly030-8a
Lac030-7
Anaerovibrio glycerini
Contaminated aquifer clone, UEA229187
pH5 Ac302-6
pH5 Gly302-2
Gly030-9b
Gly034-46
Clostridium celerecrescens
Low G+C
Gram Positives
0.10
Dehalococcoides ethenogenes

Cultured at pH 4 to 5

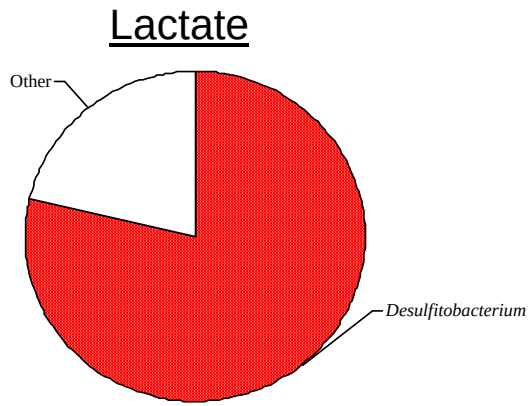
Background:



(21 clones)

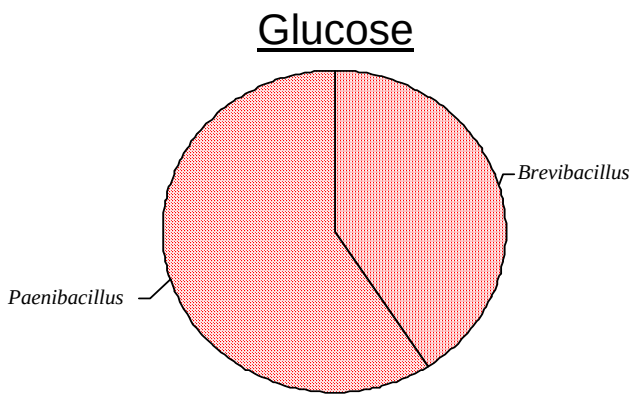


(18 clones)



(14 clones)

Contaminated:

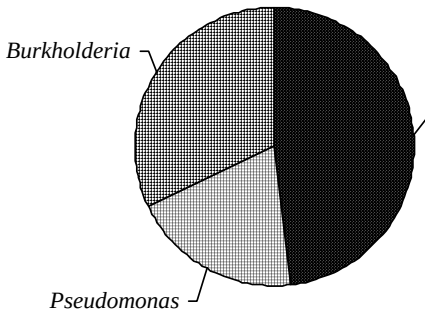


(86 clones)

Background:

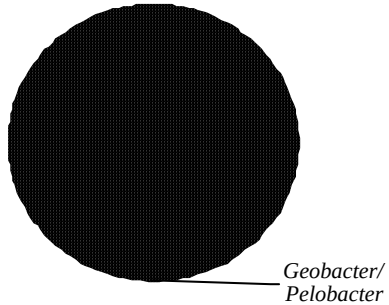
Cultured at pH 7

Acetate



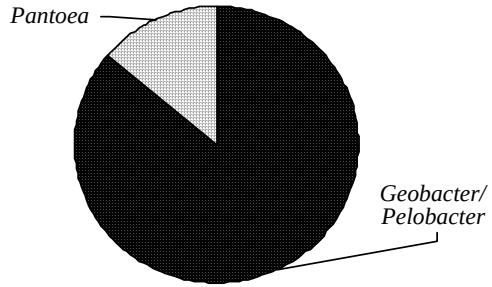
(50 clones)

Lactate



(39 clones)

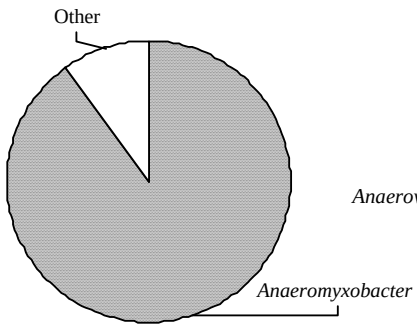
Glycerol



(49 clones)

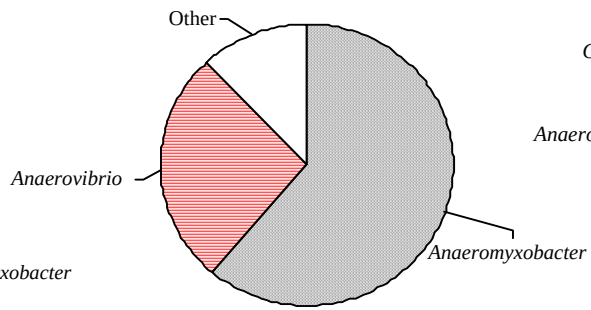
Contaminated:

Acetate



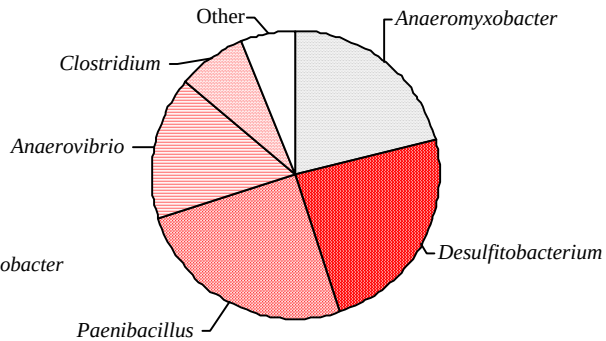
(152 clones)

Lactate



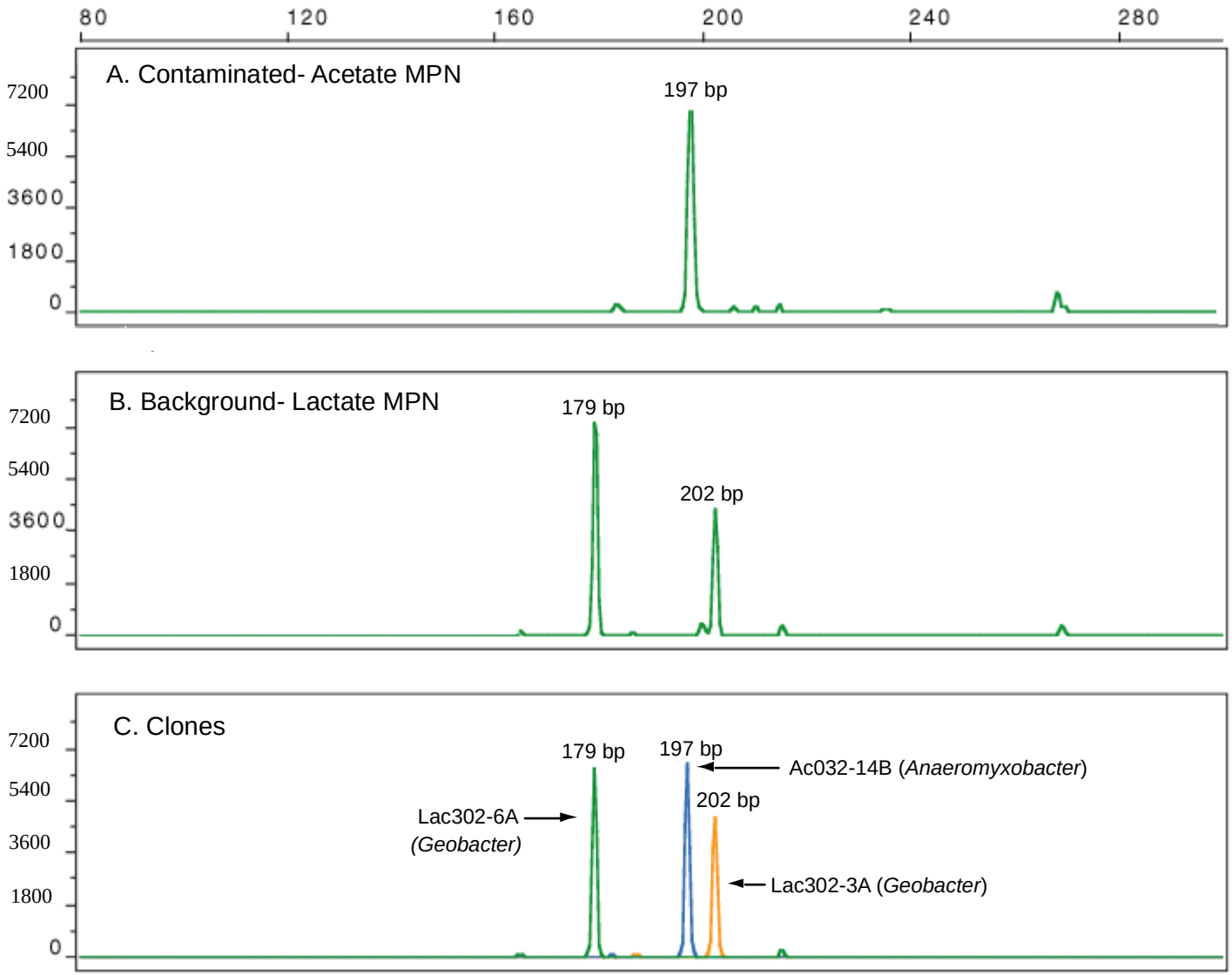
(41 clones)

Glycerol

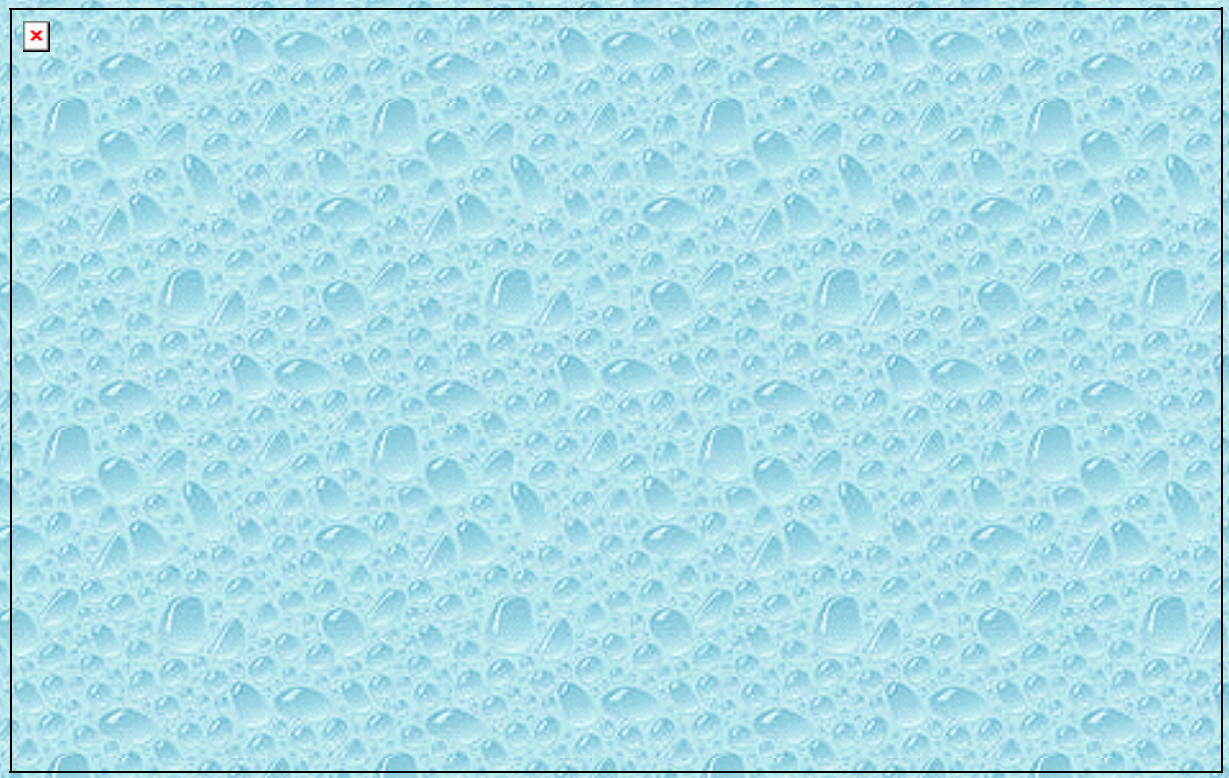


(238 clones)

Terminal Restriction Fragment Analysis



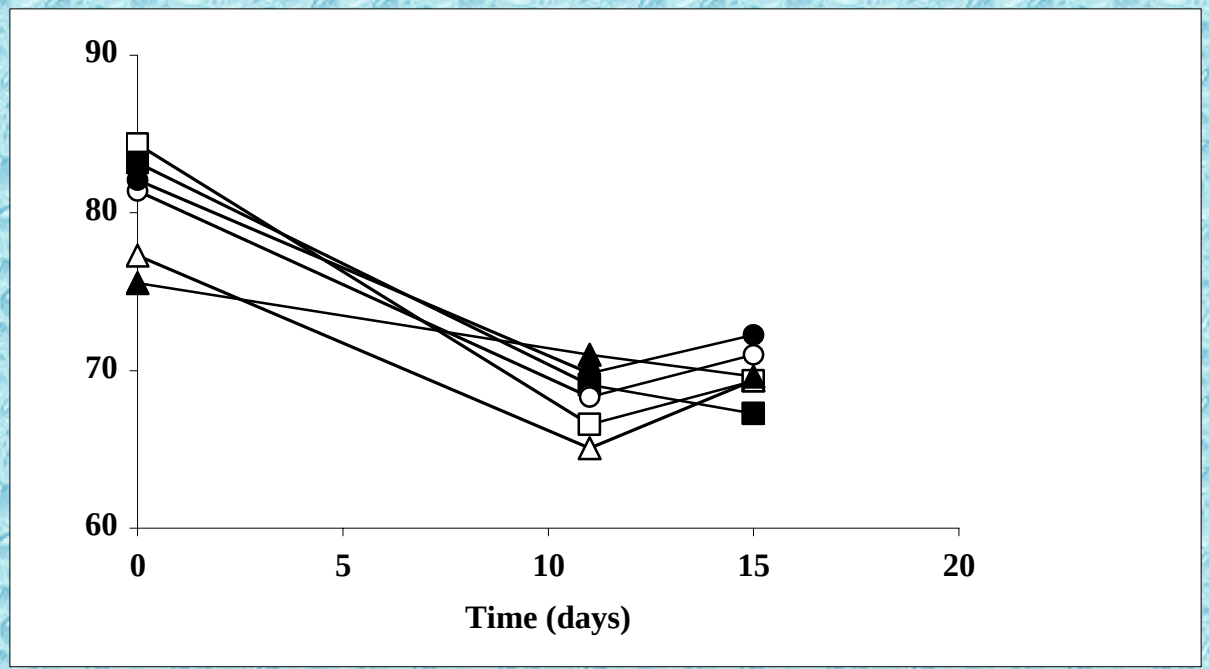
Denitrification Potential (acetylene block technique) FWB 18, pH = 6.4 to 6.6



Denitrification Potential Area 1

- FWB 18, at pH of 6 to 7, rates relatively high, ~ 100 nmol N g^{-1} d^{-1}
- FWB 27 to 32, at pH of 3 to 3.5, no denitrification detected

Nitrate depletion
FWB 30, pH = 3 to 3.5



Conclusions

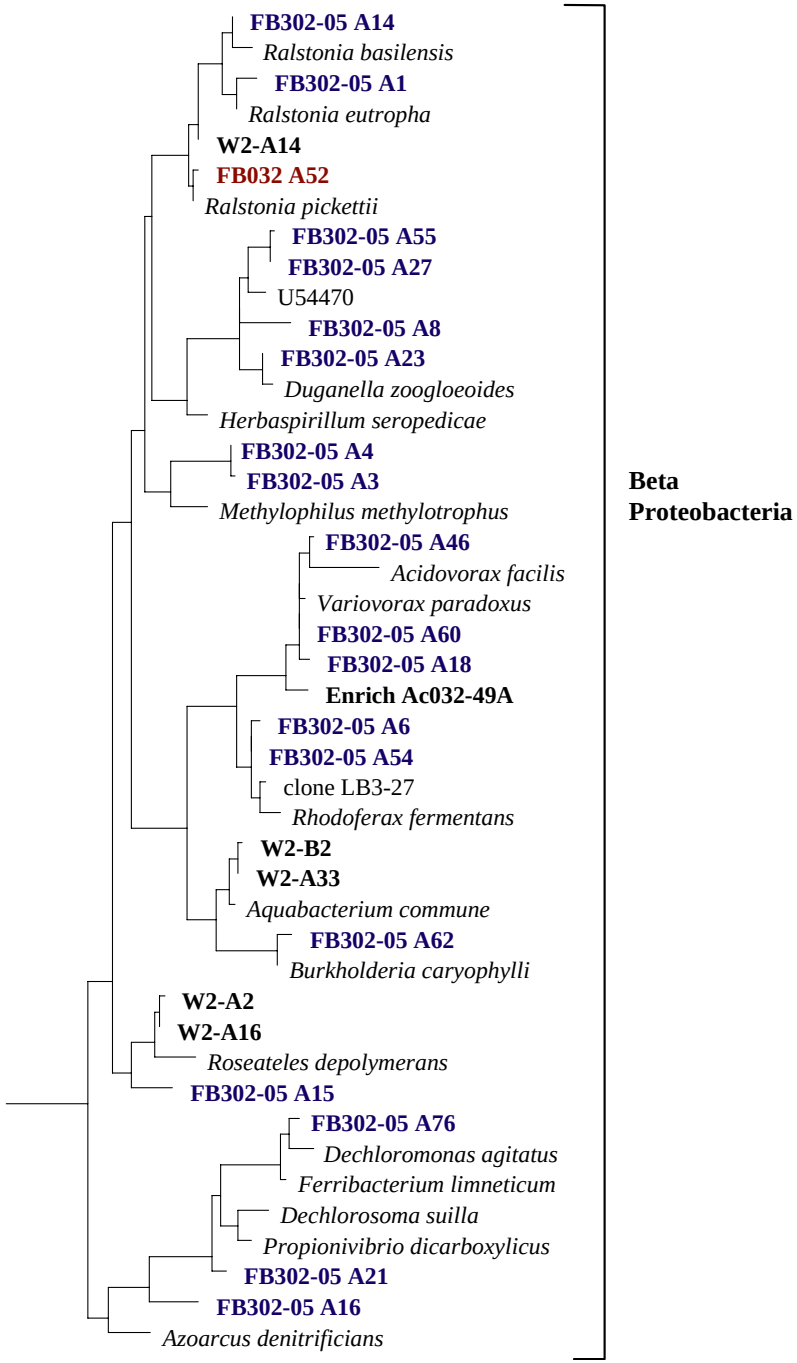
- Fe minerals in FRC subsurface mostly aluminosilicates and Al-substituted goethite, mineralogy does not change across pH gradient
- Diversity of culturable FeRB largely dependent upon pH
- Culturable FeRB at low pH predominated by Gram positives and organisms most closely related to *Anaeromyxobacter*
- Members of previously cultured FeRB groups only observed at high pH in bkgd sediments
- Denitrification potential dependent upon sediment pH with much higher rates measured at neutral pH sites within Area 1

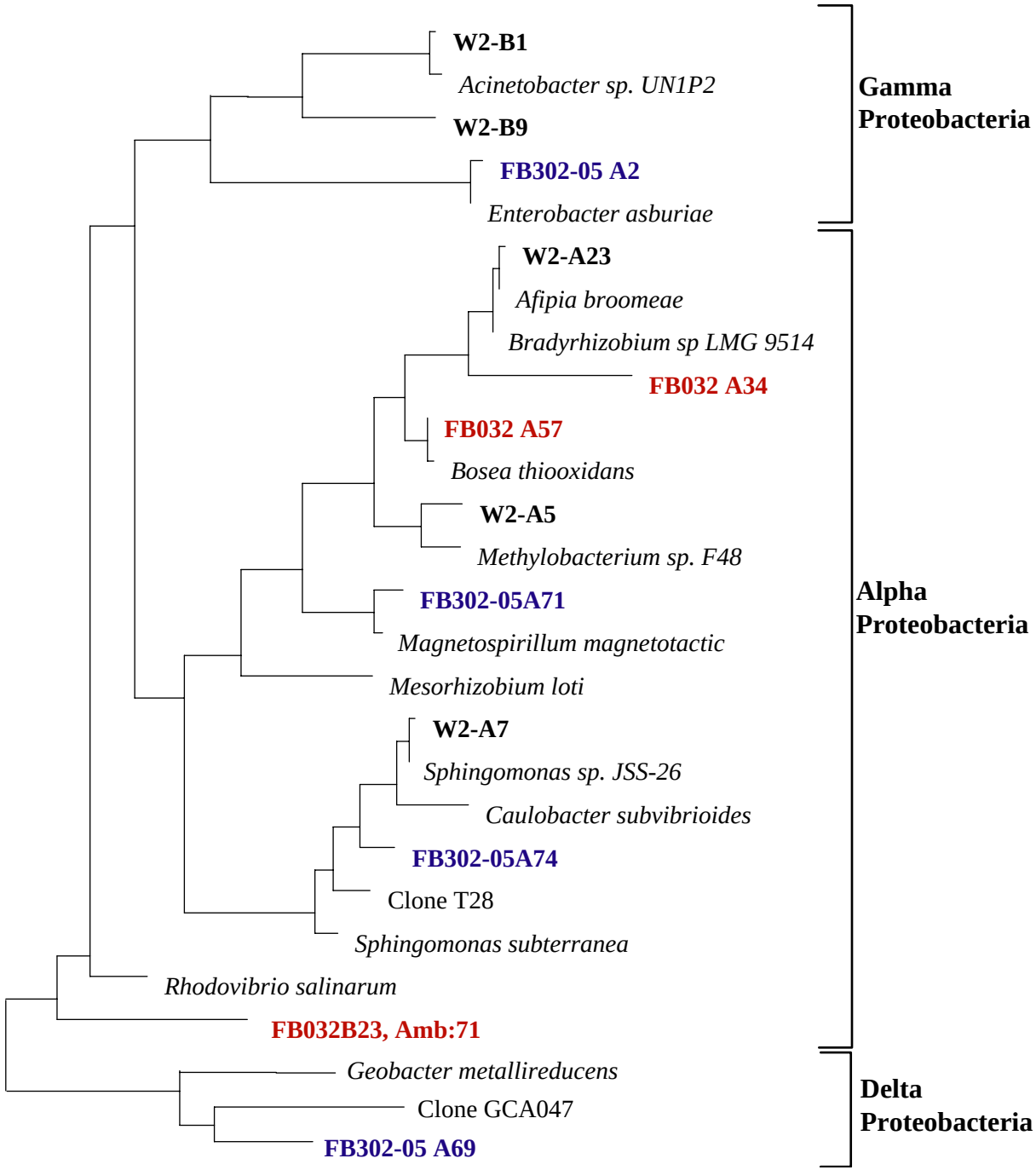
Implications

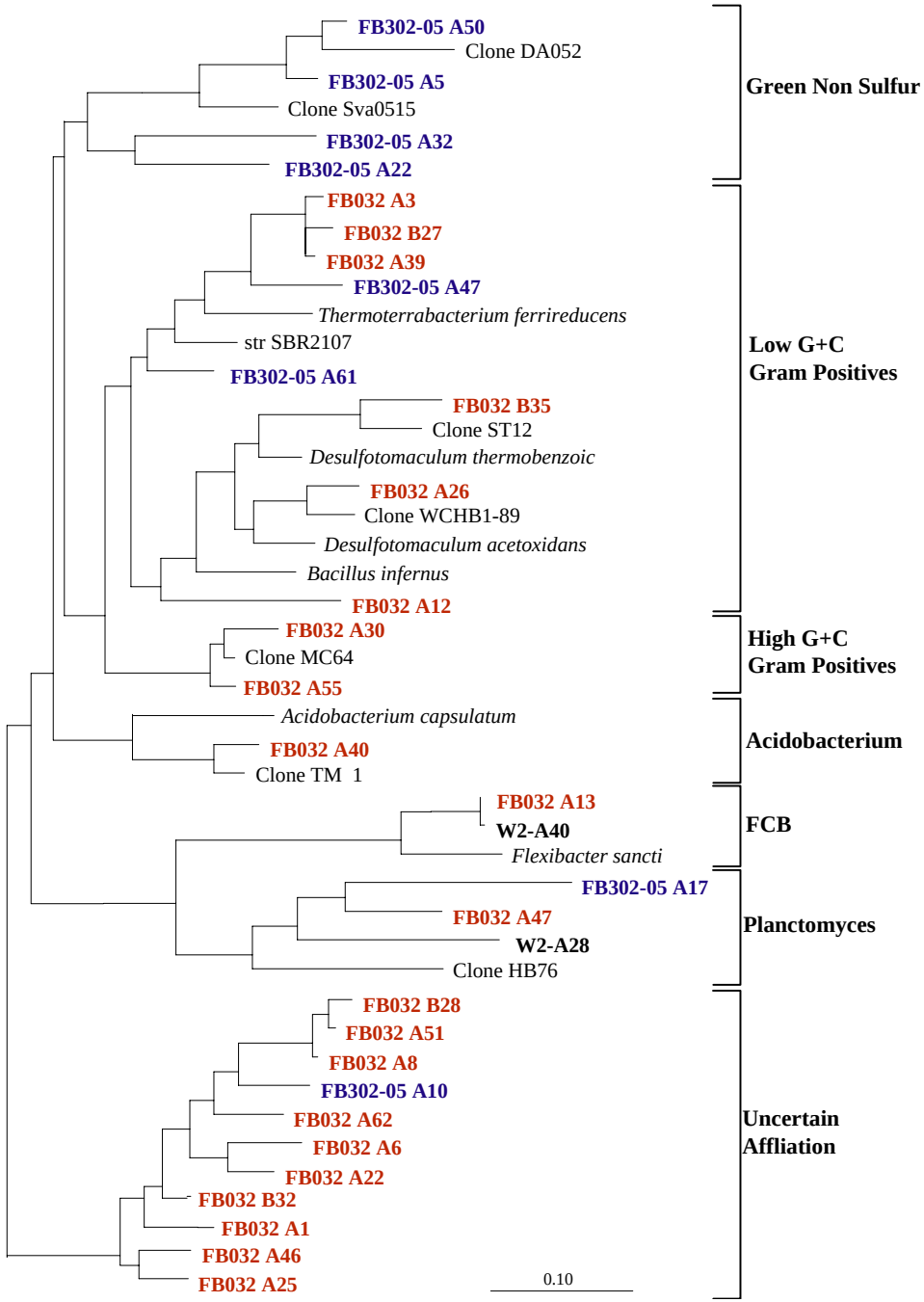
- Contaminated FRC subsurface is a heterogenous "extreme environment" where the metabolism of Fe-reducing bacteria is likely to be controlled by low pH and high NO_3^-
- New model Fe(III)-reducing organisms are needed for FRC where diversity different from past subsurface studies
- Results point to NO_3^- removal and neutralization of pH for establishment of conditions conducive to U(VI) reduction/ immobilization by FeRB
- Neutralization necessary prior to nitrate removal if denitrification is targeted pathway

Future Work

- Purify FeRB from Area 1 for use as model organisms
- Determine pathways/ controls of nitrate reduction at low pH
- Use more definitive molecular methods (real-time PCR) to study FeRB communities
- Combine approaches (mineral characterization, rate measurements, microbial community analysis) to determine interactions between Fe mineral transformation and U(VI) solubility


FB302-05 A14
Ralstonia basilensis
FB302-05 A1
Ralstonia eutropha
W2-A14
FB032 A52
Ralstonia pickettii
FB302-05 A55
FB302-05 A27
U54470
FB302-05 A8
FB302-05 A23
Duganella zoogloeoides
Herbaspirillum seropedicae
FB302-05 A4
FB302-05 A3
Methylophilus methylotrophus
FB302-05 A46
Acidovorax facilis
Variovorax paradoxus
FB302-05 A60
FB302-05 A18
Enrich Ac032-49A
FB302-05 A6
FB302-05 A54
clone LB3-27
Rhodoferax fermentans
W2-B2
W2-A33
Aquabacterium commune
FB302-05 A62
Burkholderia caryophylli
W2-A2
W2-A16
Roseateles depolymerans
FB302-05 A15
FB302-05 A76
Dechloromonas agitatus
Ferribacterium limneticum
Dechlorosoma suilla
Propionivibrio dicarboxylicus
FB302-05 A21
FB302-05 A16
Azoarcus denitrificians
Beta
Proteobacteria


W2-B1
Acinetobacter sp. UN1P2
W2-B9
FB302-05 A2
Enterobacter asburiae
W2-A23
Afipia broomeae
Bradyrhizobium sp LMG 9514
FB032 A34
FB032 A57
Bosea thiooxidans
W2-A5
Methylobacterium sp. F48
FB302-05A71
Magnetospirillum magnetotactic
Mesorhizobium loti
W2-A7
Sphingomonas sp. JSS-26
Caulobacter subvibrioides
FB302-05A74
Clone T28
Sphingomonas subterranea
Rhodovibrio salinarum
FB032B23, Amb:71
Geobacter metallireducens
Clone GCA047
FB302-05 A69
Gamma Proteobacteria
Alpha Proteobacteria
Delta Proteobacteria


FB302-05 A50
Clone DA052
FB302-05 A5
Clone Sva0515
FB302-05 A32
FB302-05 A22
FB032 A3
FB032 B27
FB032 A39
FB302-05 A47
Thermoterrabacterium ferrireducens
str SBR2107
FB302-05 A61
FB032 B35
Clone ST12
Desulfotomaculum thermobenzoic
FB032 A26
Clone WCHB1-89
Desulfotomaculum acetoxidans
Bacillus infernus
FB032 A12
FB032 A30
Clone MC64
FB032 A55
Acidobacterium capsulatum
FB032 A40
Clone TM 1
FB032 A13
W2-A40
Flexibacter sancti
FB302-05 A17
FB032 A47
W2-A28
Clone HB76
FB032 B28
FB032 A51
FB032 A8
FB302-05 A10
FB032 A62
FB032 A6
FB032 A22
FB032 B32
FB032 A1
FB032 A46
FB032 A25
0.10
Green Non Sulfur
Low G+C
Gram Positives
High G+C
Gram Positives
Acidobacterium
FCB
Planctomyces
Uncertain
Affliation

Summary - FeRB MPNs

- ~1800 tubes tested under a range of culture conditions (pH, [NO_3^-], C substrates, reductant)
- Growth detected in majority of bkgd vs. minority of contam. sediment samples
- Growth also limited in low pH MPNs
- Counts similar in background vs. contam. sediment
- Little growth detected in unwashed contaminated sediments, whereas washing had no effect on counts in background sediments

FeRB MPNs- Variety of Sediments

- Surface, rooted sediments in aquatic environments: 10^4 to 10^7 cells g^{-1}
- Aerated agricultural soils: 10^3 to 10^6 cells g^{-1}
- Subsurface sediments: 10^2 to 10^4 cells g^{-1}