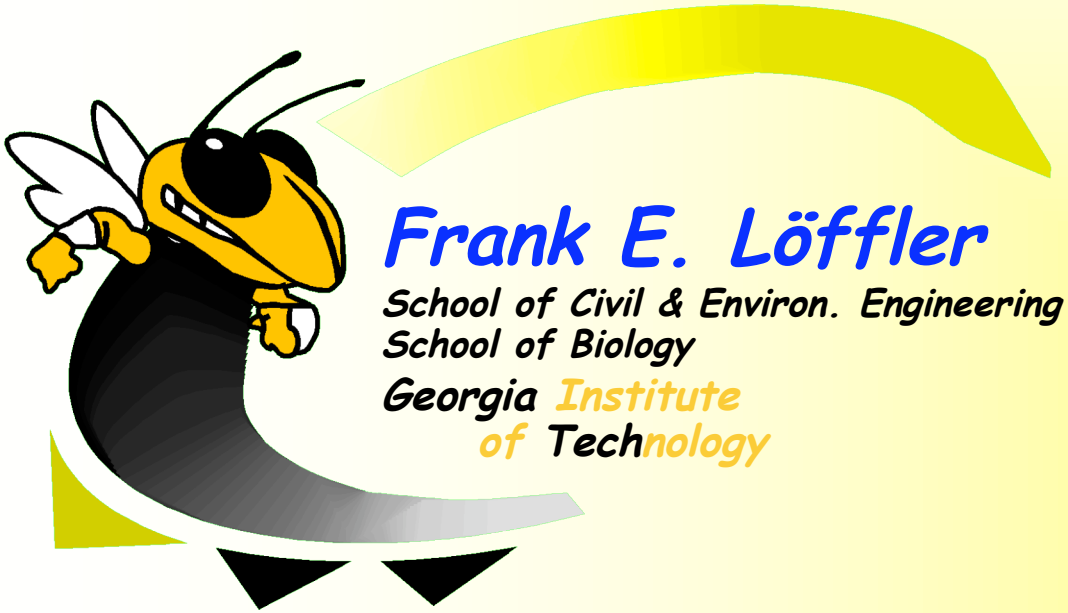
Dissimilatory Metal Reduction by *Anaeromyxobacter*



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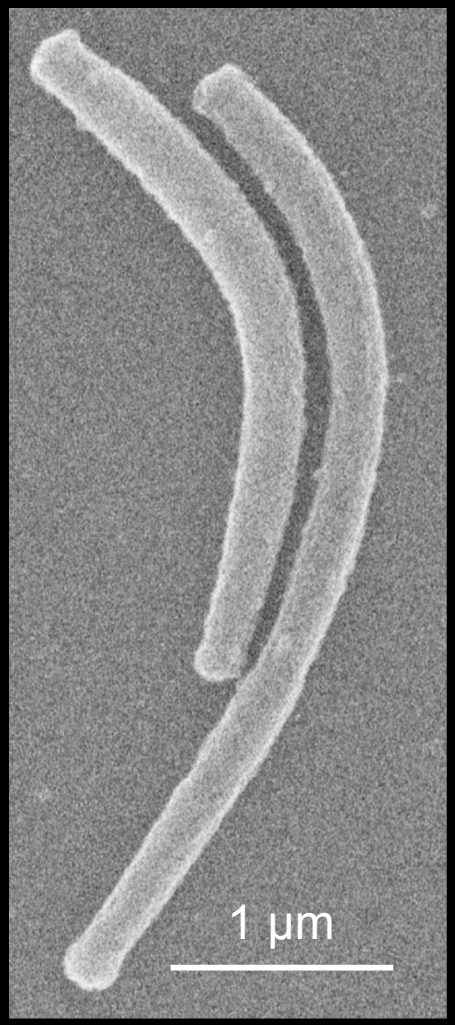
Department of Geology
University of Illinois at Urbana/Champaign

Anaeromyxobacter dehalogenans Strain 2CP-C - A True Versaphile



Electron Acceptors

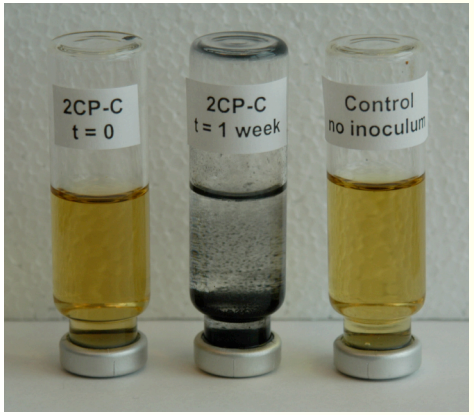
- Halophenols
- Oxygen
- Nitrate
- Nitrite
- N_2O
- Fumarate
- Humics
- Soluble and insoluble oxidized metal species
- U(VI)
- Tc(VII)

Electron Donors

- Acetate
- Hydrogen
- Succinate
- Pyruvate
- Formate
- Lactate
- YE
- Fe(II)

Anaeromyxobacter - Metal Reduction

Yellow-Brownish

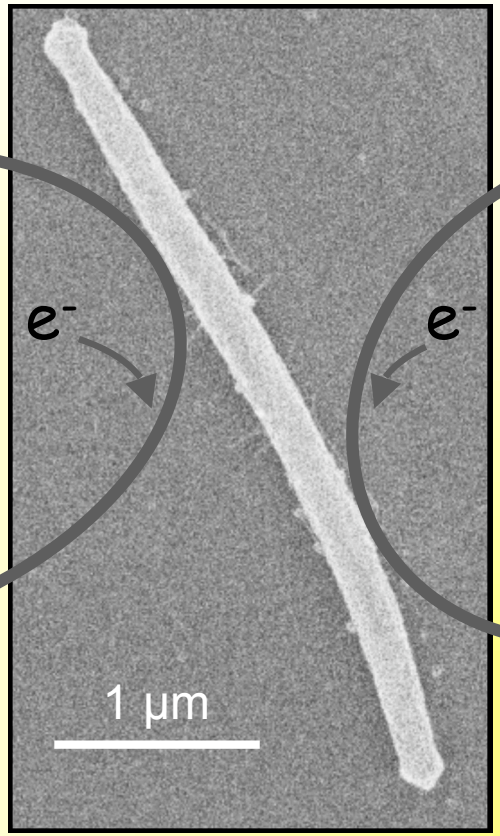


Clear

Fe^{3+} → Fe^{2+}

U^{6+} → U^{4+}

e^- e^-



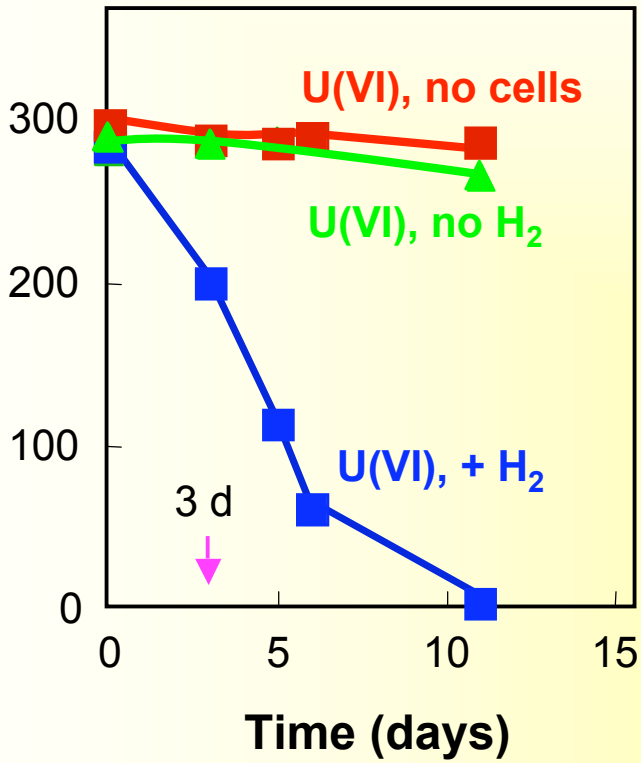
oxidized
soluble
mobile

reduced
insoluble
immobile

***Anaeromyxobacter dehalogenans* strain 2CP-C**

He & Sanford, 2003. Appl. Environ. Microbiol., 69:2712-2718

U(VI) Reduction Requires H_2

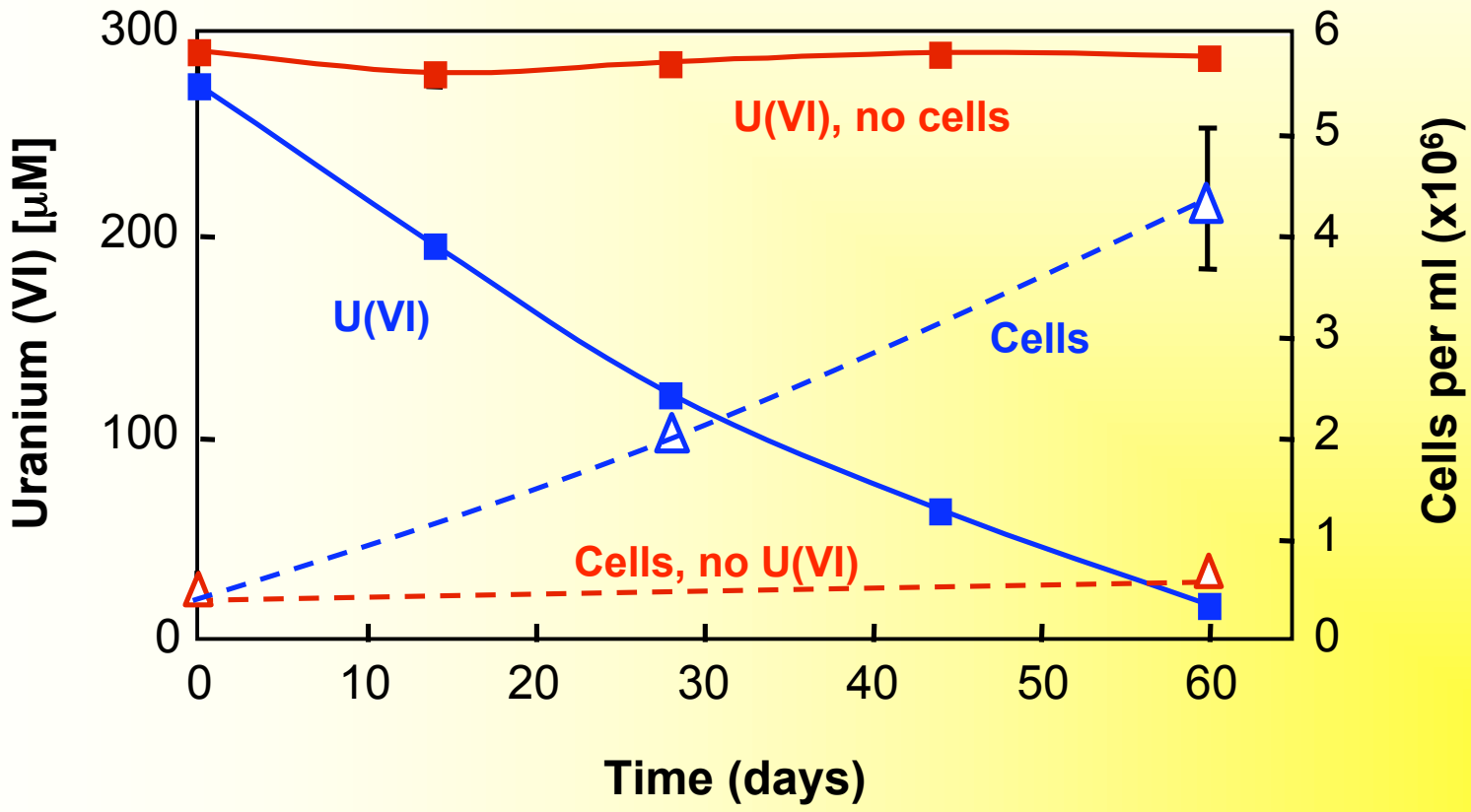


Acetate/NO_3^- (0.5 mM),
2% inoculum
U(VI), NO_3^- (0.5 mM), **± H_2**

Wu et al., 2006. Appl. Environ. Microbiol. 72:3608-3614.

A. dehalogenans Couples Growth to Uranium (VI) Reduction

Mineral salts medium, acetate, H_2
2% (v/v) inoculum



Energetics of U(VI) Reduction and Other Growth-Supporting Redox Couples

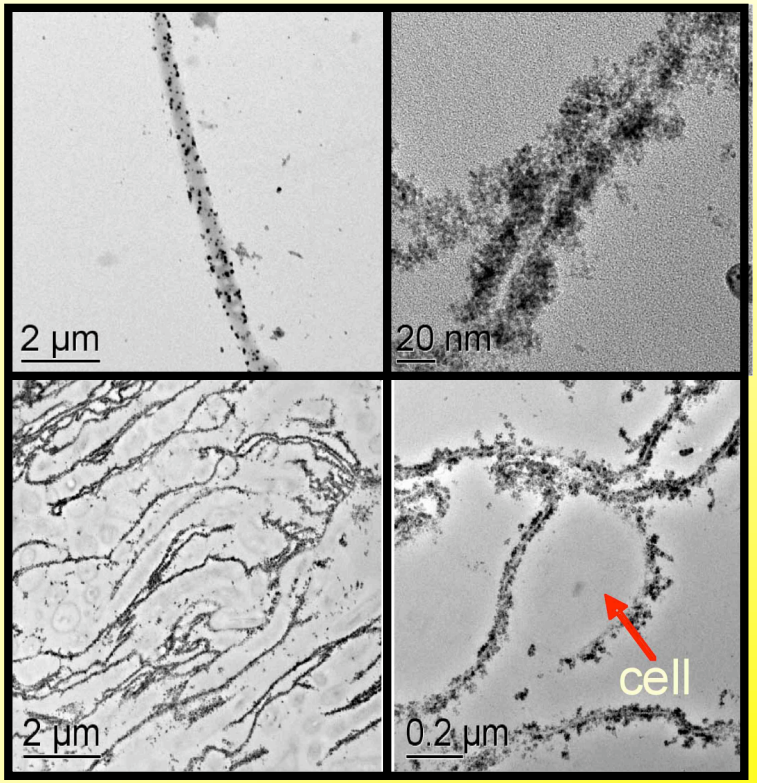
Reaction	$\Delta G°'$ (kJ/mole e^-)	ΔG (kJ/mole e^-)		
		pH 6	pH 7	pH 8
U(VI) → U(IV)				
$0.5\ UO_2(CO_3)_2^{-2} + 0.5\ H_2 \rightarrow 0.5\ UO_2 + HCO_3^-$	-51.2	-48.3	-48.3	-48.4
$0.5\ UO_2(CO_3)_3^{-4} + 0.5\ H^+ + 0.5\ H_2 \rightarrow 0.5\ UO_2 + 1.5\ HCO_3^-$	-45.8	-51.5	-48.6	-45.9
$0.5\ UO_2(CO_3)_2^{-2} + 0.125\ CH_3COO^- + 0.5\ H_2O \rightarrow 0.5\ UO_2 + 0.125\ H^+ + 1.25\ HCO_3^-$	-39.55	-40.3	-41	-41.7
$0.5\ UO_2(CO_3)_3^{-4} + 1.375\ H^+ + 0.125\ CH_3COO^- + 0.5\ H_2O \rightarrow 0.5\ UO_2 + 1.75\ HCO_3^-$	-34.15	-43.5	-41.3	-39.2
Other electron-accepting processes				
$0.5\ Fe_2O_{3\ (HFO)} + 2\ H^+ + 0.5\ H_2 \rightarrow Fe^{+2} + 1.5\ H_2O$	-38.79	-73	-61.6	-50.2
$\alpha\text{-}FeO(OH)_{goethite} + 2\ H^+ + 0.5\ H_2 \rightarrow Fe^{+2} + 2H_2O$	17.05	-17.2	-5.8	5.6
$0.5\ Fumarate + 0.5\ H_2 \rightarrow 0.5\ Succinate$	-43.0	-51.8	-51.8	-51.8
$0.125\ NO_3^- + 0.5\ H_2 + 2\ H^+ \rightarrow 0.125\ NH_4^+ + 0.375\ H_2O$	-75.0	-67.8	-69.3	-70.7

Growth Yield Comparisons

EA	2CP-C cells produced per μmole e^- transferred to EA (x 10^7)
Fumarate	14.9 ± 1.2
Fe(III)	6.26 ± 0.09
2-CP	5.16 ± 0.21
Nitrate	17.5 ± 0.17
U(VI)	0.77 ± 0.10 0.82 ± 0.32

Sanford et al., 2006. Growth-Linked Reduction of Hexavalent Uranium. Submitted.

- Strain 2CP-C growth yield with U(VI) lower than free energy considerations predict
- Similar observations made with *Geobacter lovleyi* and *Geobacter sulfurreducens*



Pacific Northwest National Laboratory
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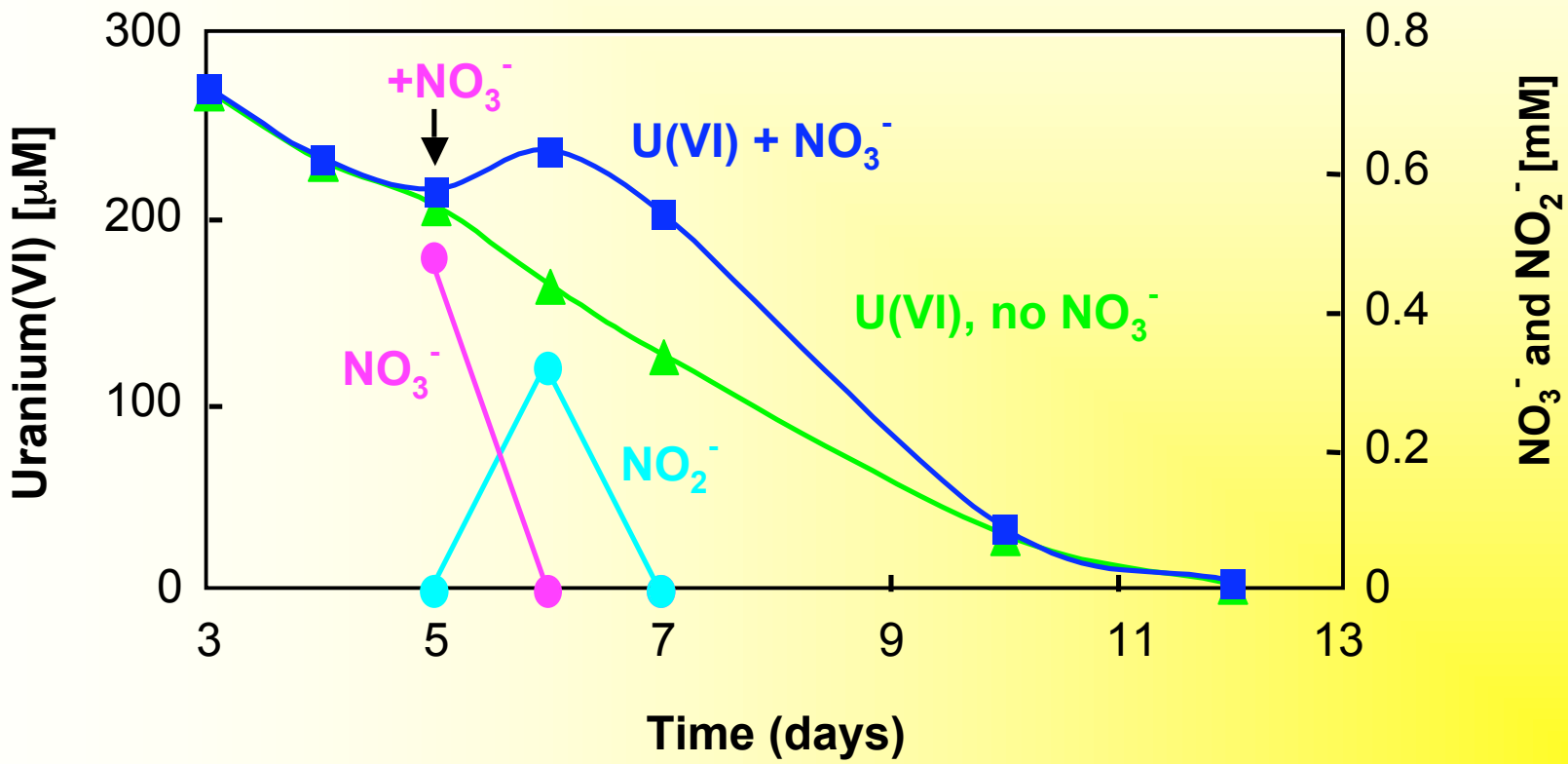
Alice Dohnalkova

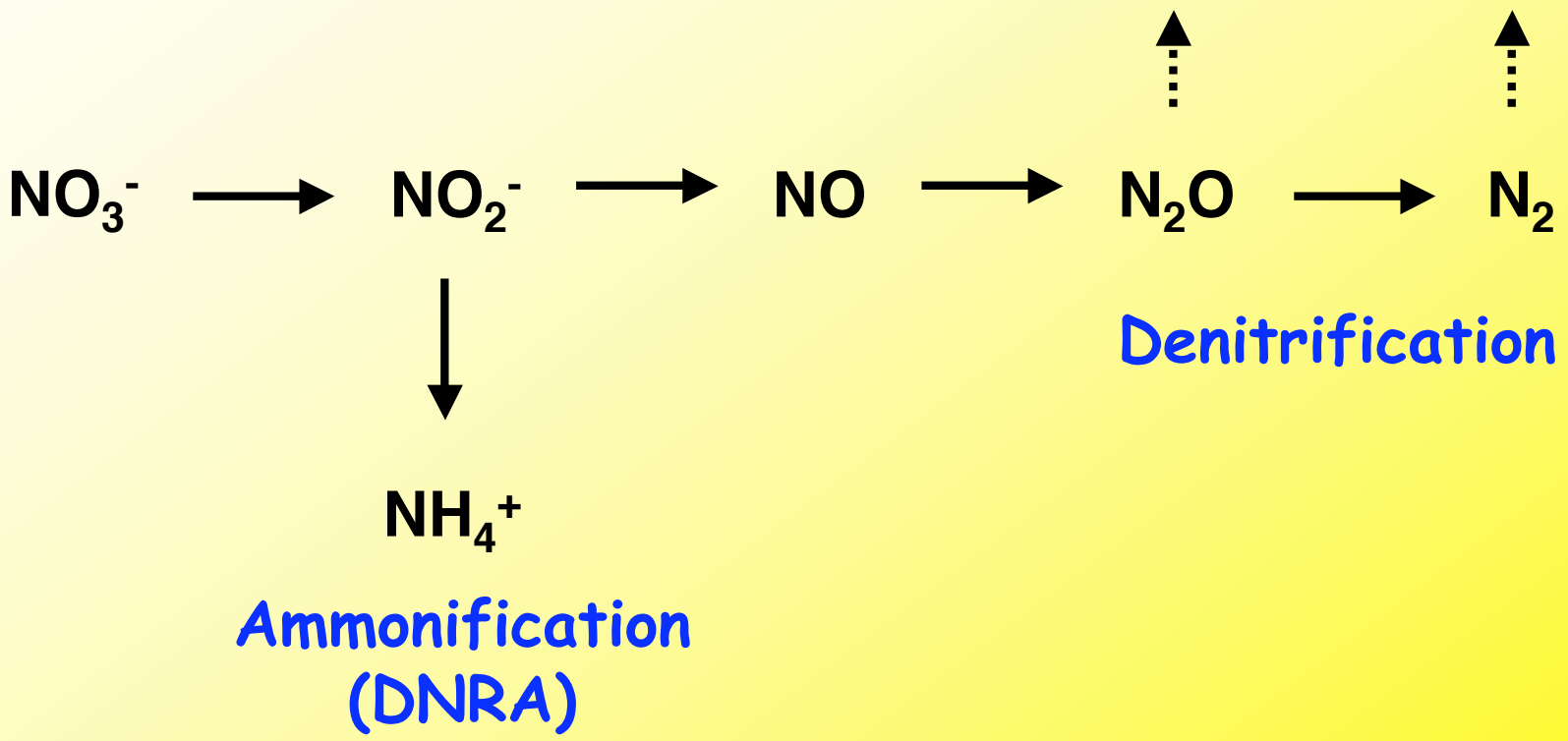
Effect of Nitrate on Uranium (VI) Reduction by *A. dehalogenans*

2CP-C grown with acetate/NO_3^- (0.5 mM)

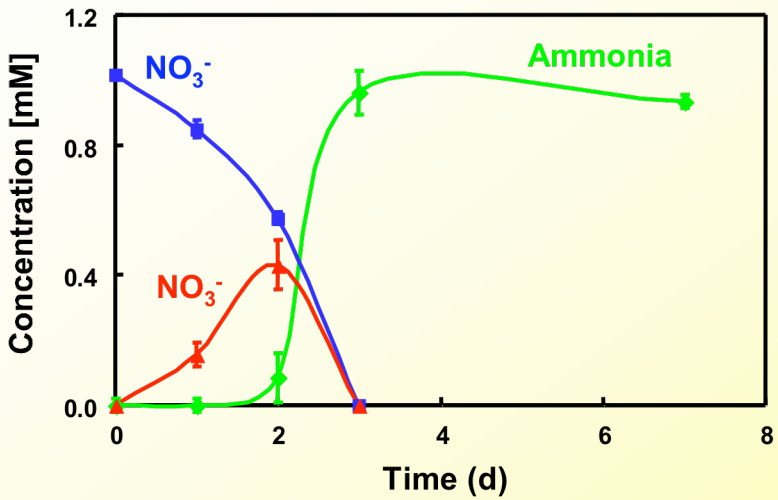
U(VI) and H_2 added after NO_3^- had been consumed (day 3)



Anaerobic Nitrogen Metabolism: Denitrification & DNRA



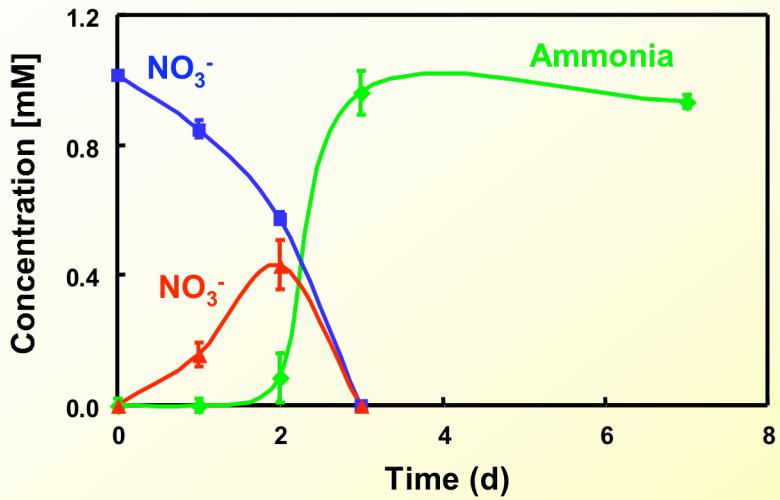
Anaeromyxobacter: DNRA and N_2O Reduction



NO_3^- → NO_2^- → NO → N_2O → N_2

NO_2^- → NH_4^+

Anaeromyxobacter: DNRA and N_2O Reduction

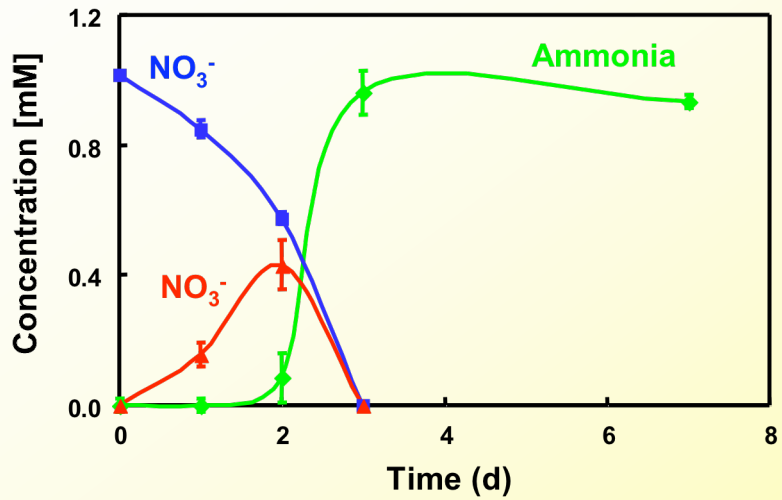


Greenhouse gas flux?

NO_3^- → NO_2^- ⇸ NO → N_2O —*nosZ*→ N_2

NO_2^- → NH_4^+

Anaeromyxobacter: DNRA and N_2O Reduction

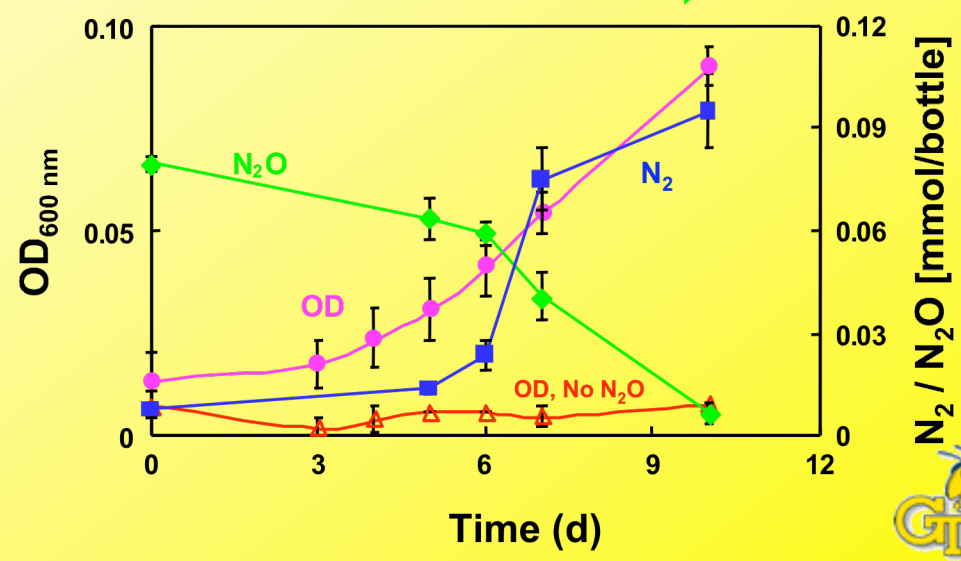


Greenhouse gas flux?

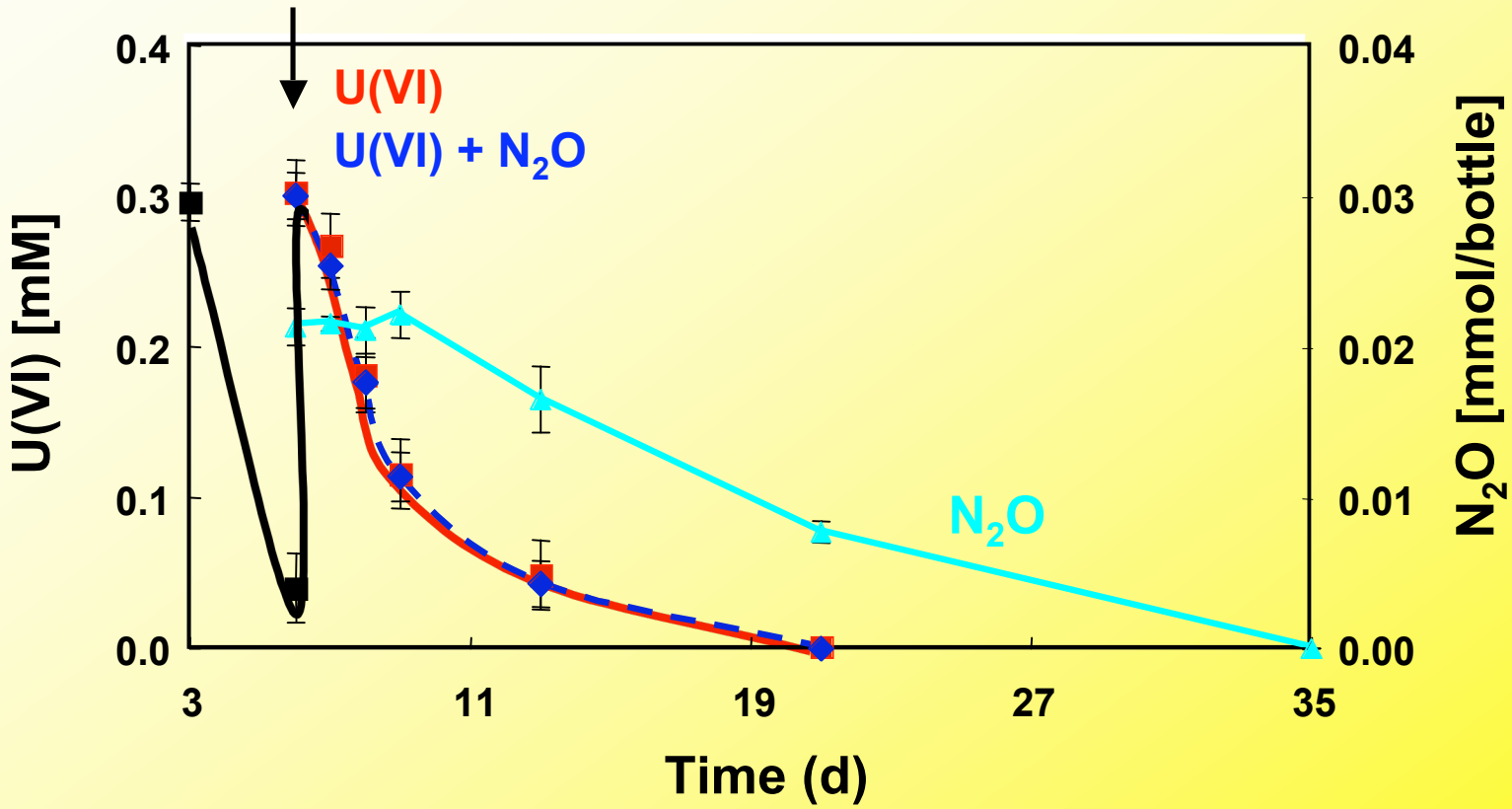
nosZ

$NO_3^- \rightarrow NO_2^- \not\rightarrow NO \rightarrow N_2O \rightarrow N_2$

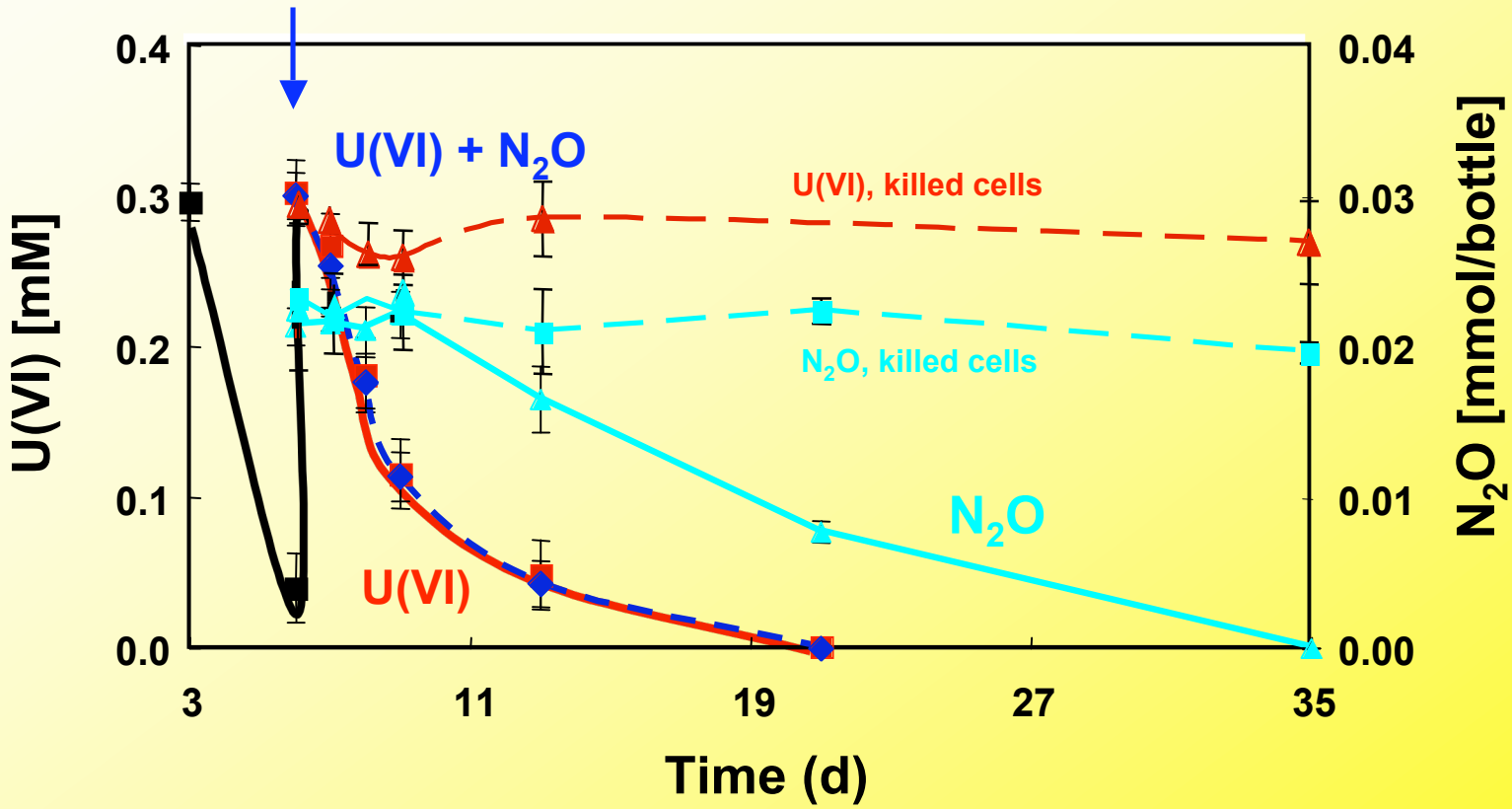
$NO_2^- \rightarrow NH_4^+$



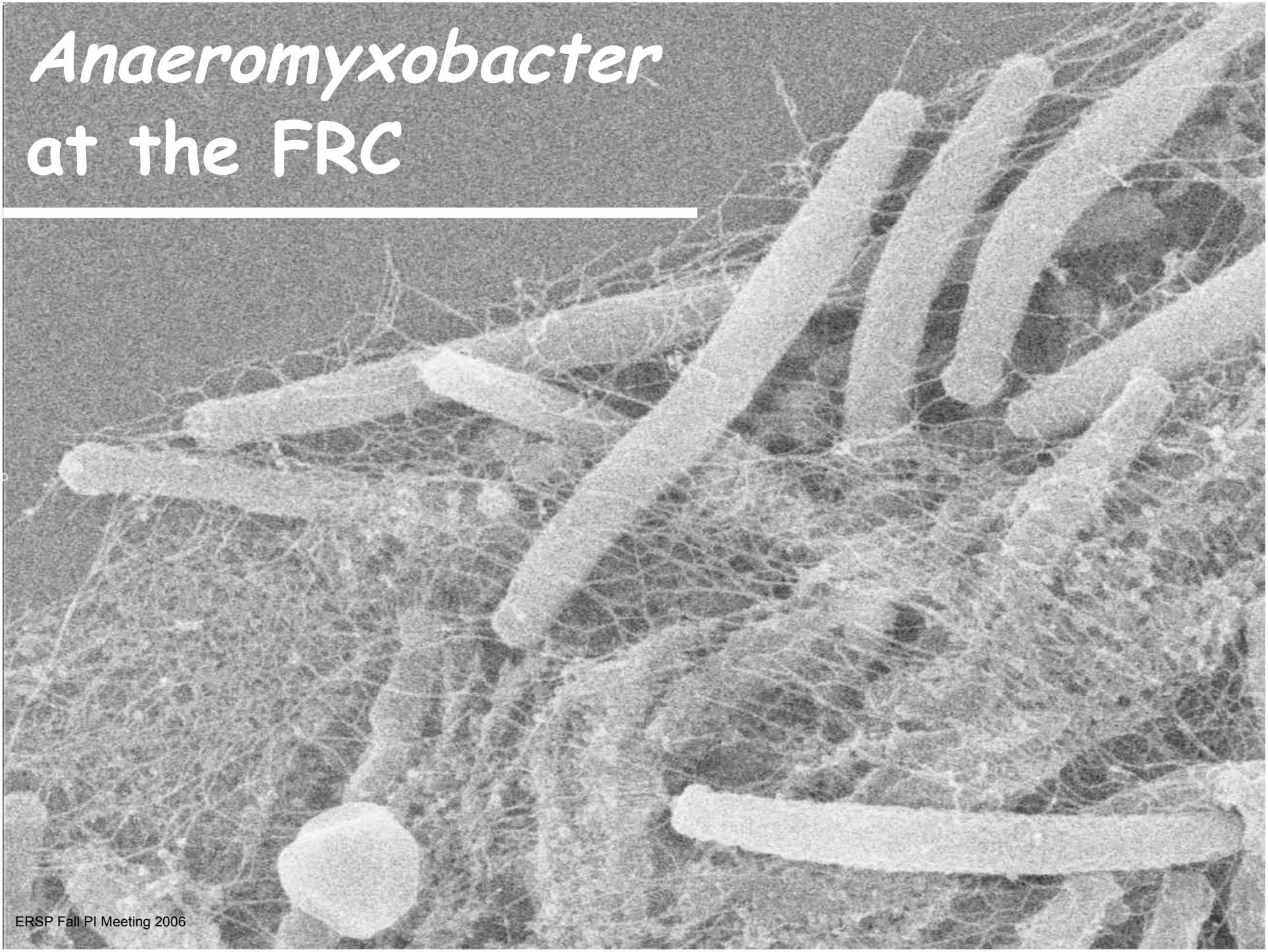
Effects of N_2O on U(VI) Reduction



Effects of N_2O on U(VI) Reduction

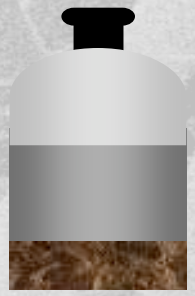


Anaeromyxobacter at the FRC



Anaeromyxobacter at the FRC

Well	Adeh
FW100-1	√
FW101-1	√
FW101-3	√
FW102-1	√
FW102-2	√
FW102-4	√
FW103	√
FW104	√
FW105	√
FW106	√
FW024	√
FW029	√
TPB16	√

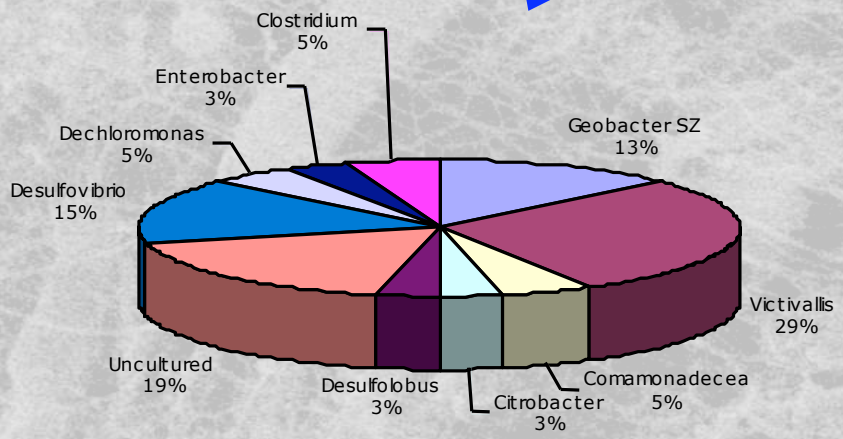


Microcosms (FW034)

Fumarate, NO_3^-, Acetate, H_2

Bacterial 16S rRNA gene-targeted approach

Anaeromyxobacter 16S rRNA gene-targeted approach

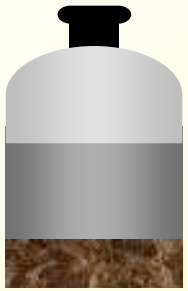


All positive clones contained *Anaeromyxobacter* 16S rRNA gene fragments

Anaeromyxobacter sequences grouped with the 2CP-C and GT-FRC clusters

Anaeromyxobacter at the FRC

FW034
FW032

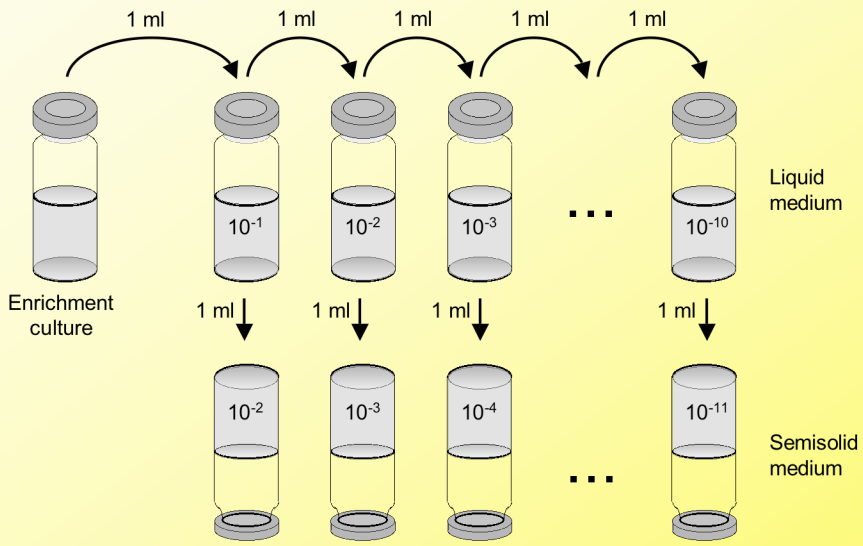


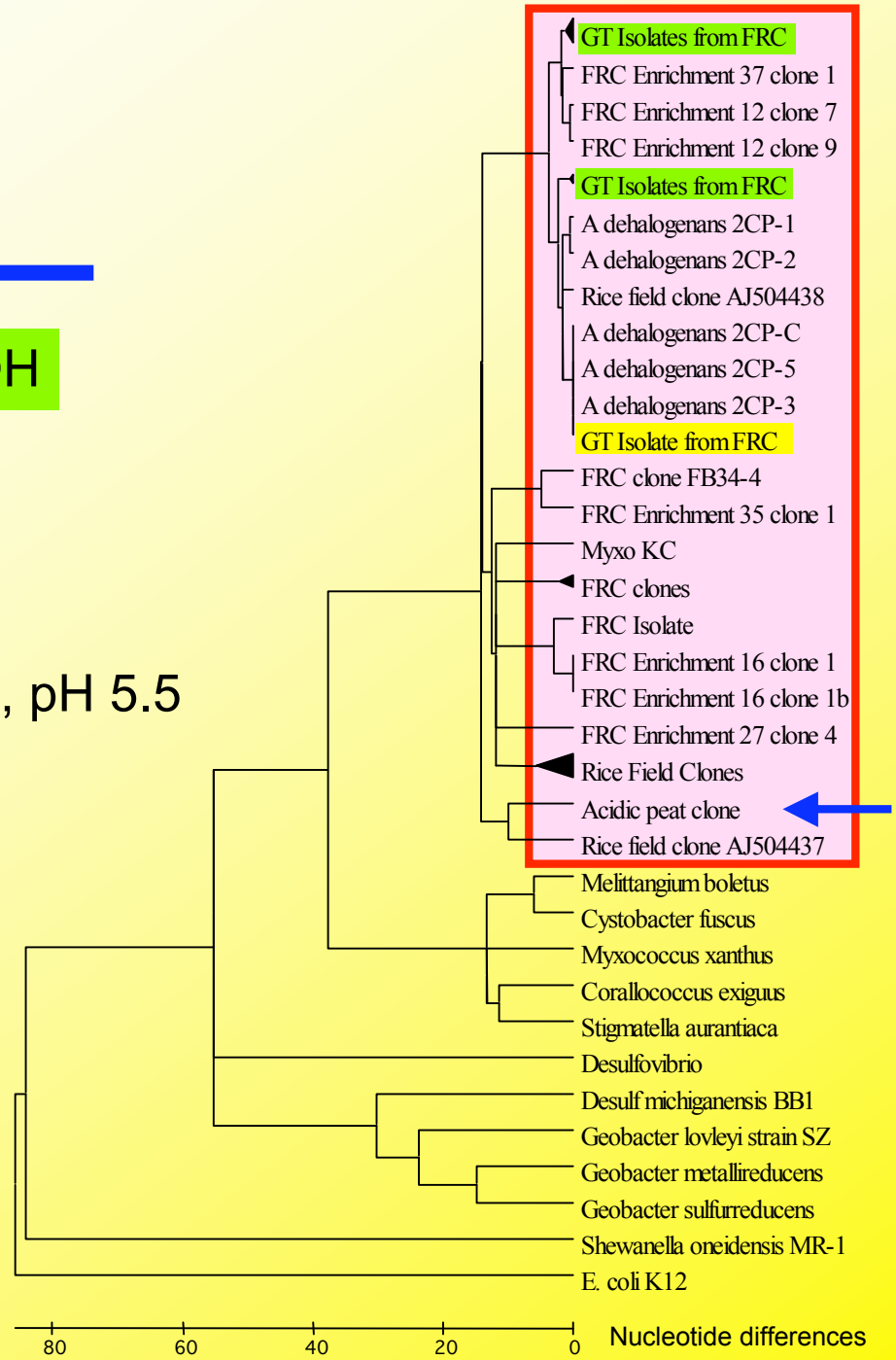
Microcosms

→ FeOOH

→ 2-CP

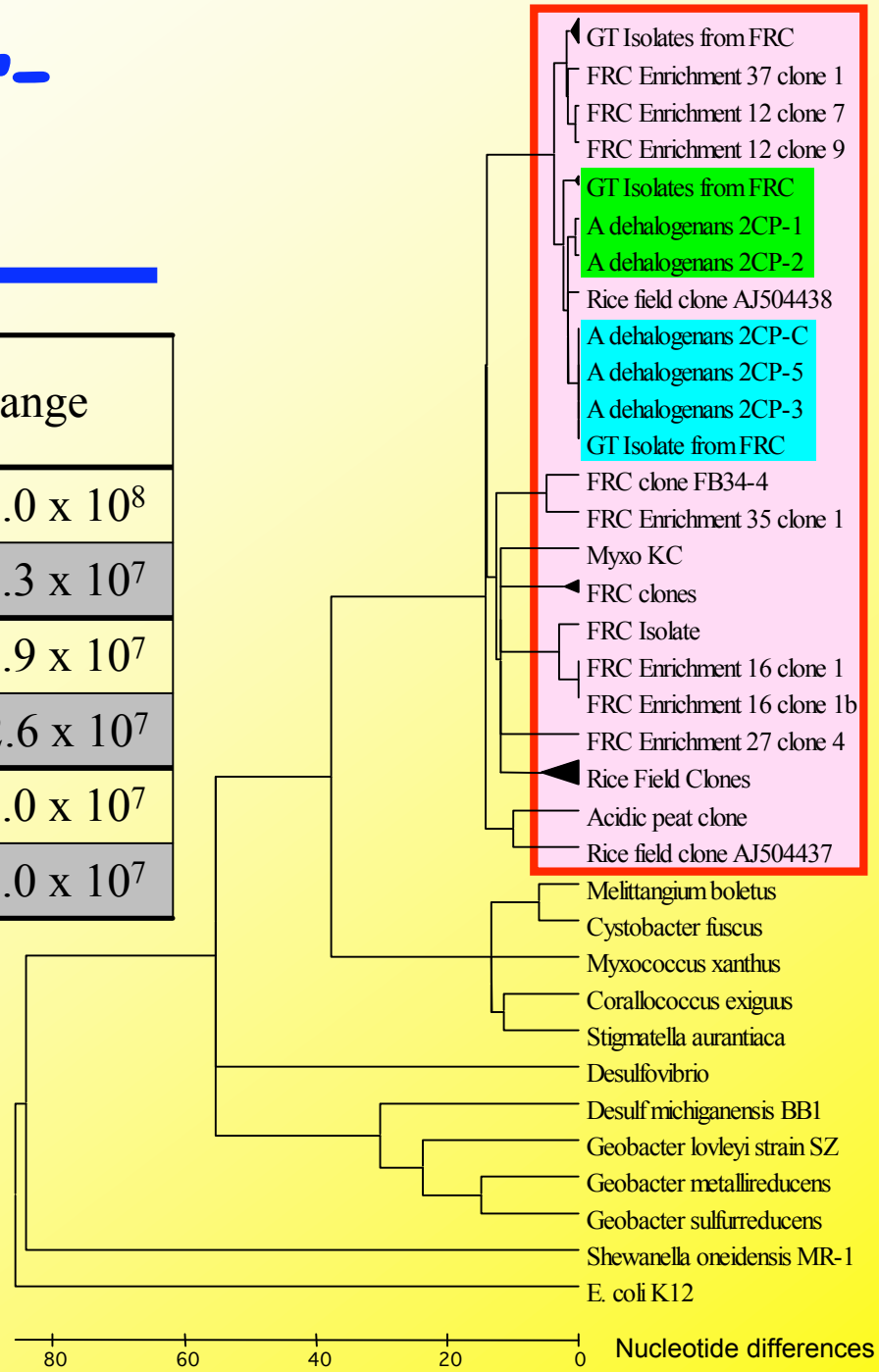
→ Fe(III), pH 5.5



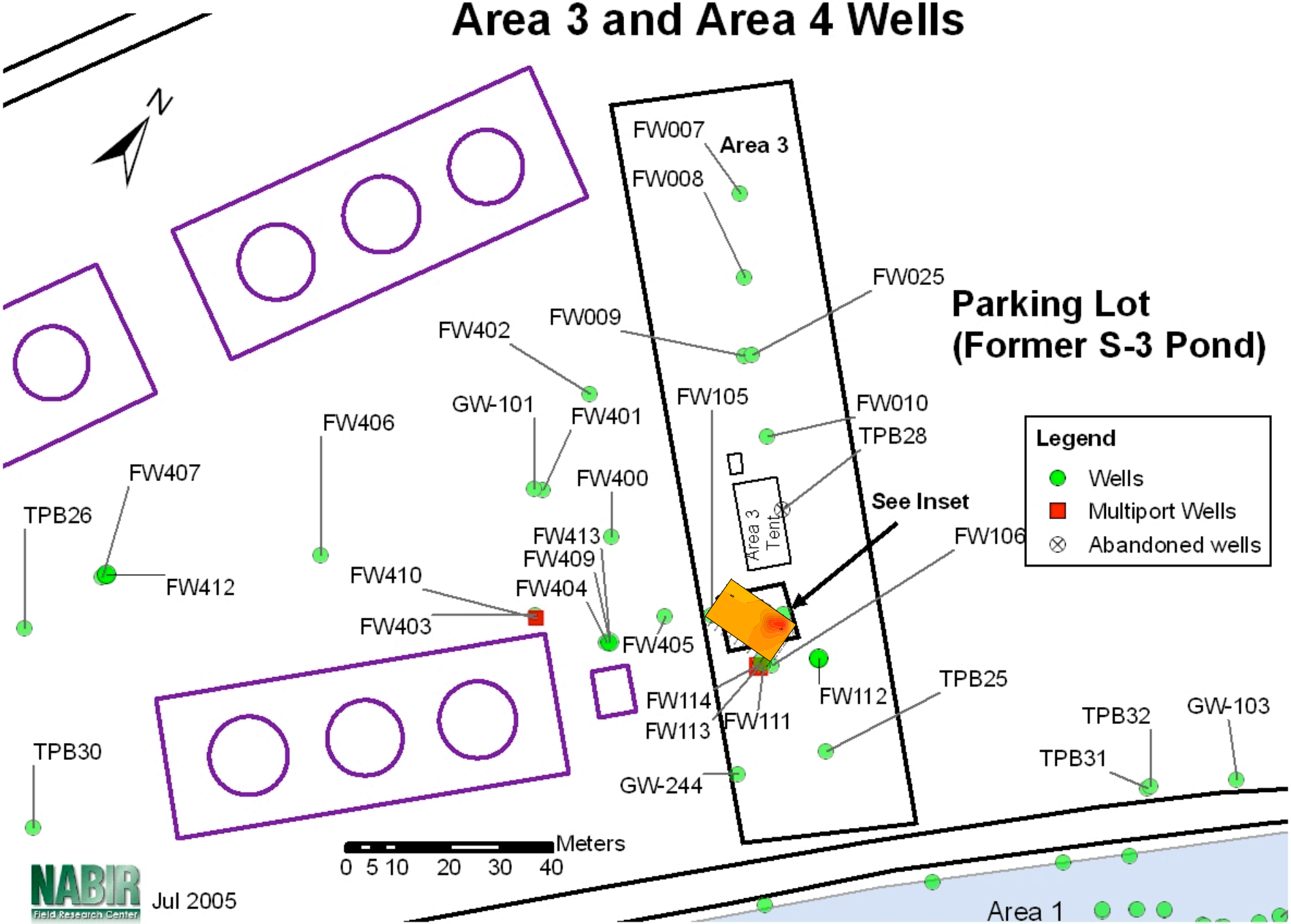


Anaeromyxobacter-Targeted qPCR

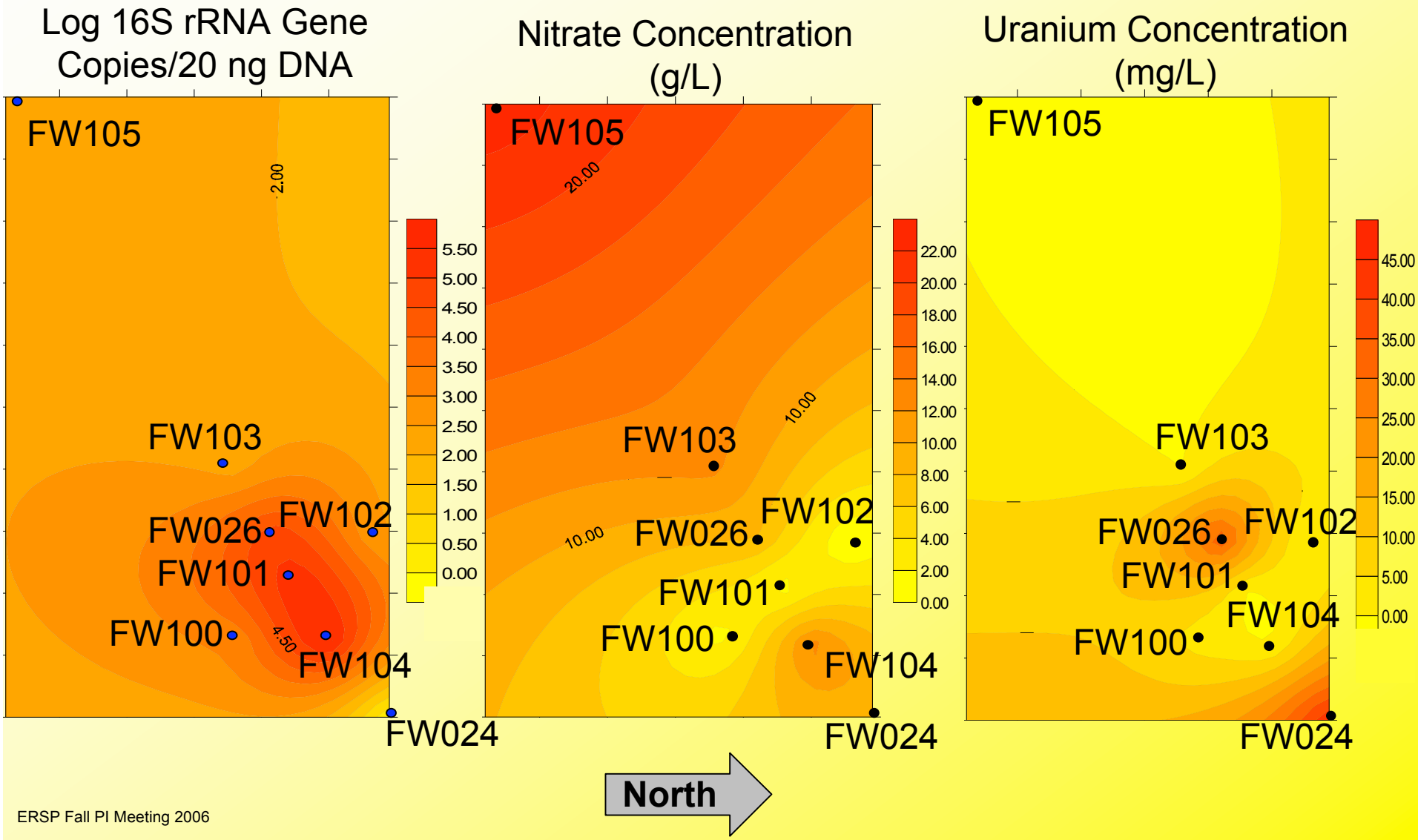
16S rRNA Gene Specificity	PCR	Linear range
Adehal	Singleplex	1.0×10^3-1.0×10^8
	Multiplex	5.8×10^2-8.3×10^7
2CP-1	Singleplex	7.9×10^1-7.9×10^7
	Multiplex	3.9×10^2 -2.6×10^7
2CP-C	Singleplex	3.0×10^2-3.0×10^7
	Multiplex	3.7×10^2-2.0×10^7



Area 3 and Area 4 Wells



Anaeromyxobacter Abundance in FRC Soil



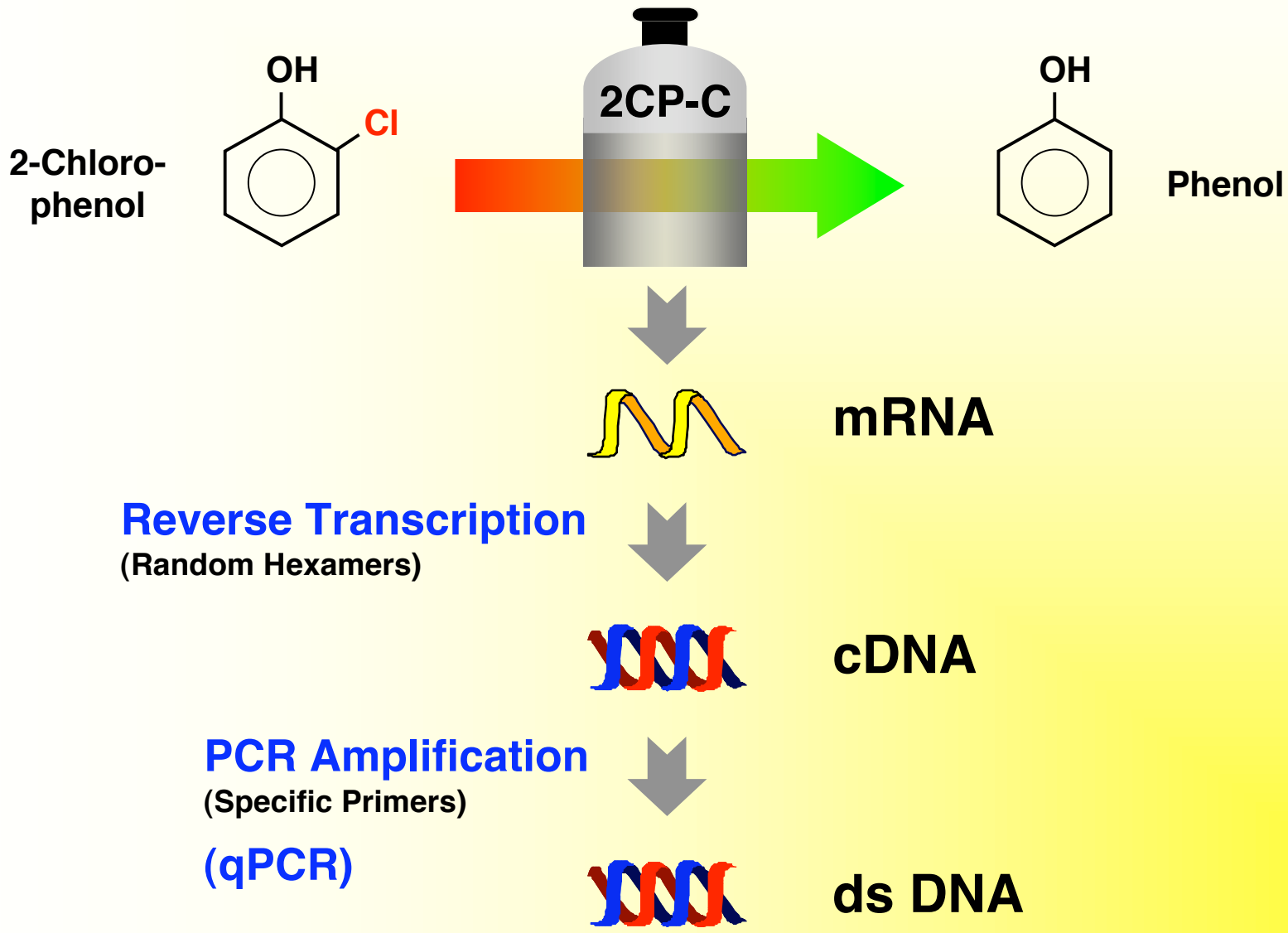
Genome Analysis

Anaeromyxobacter dehalogenans Strain 2CP-C

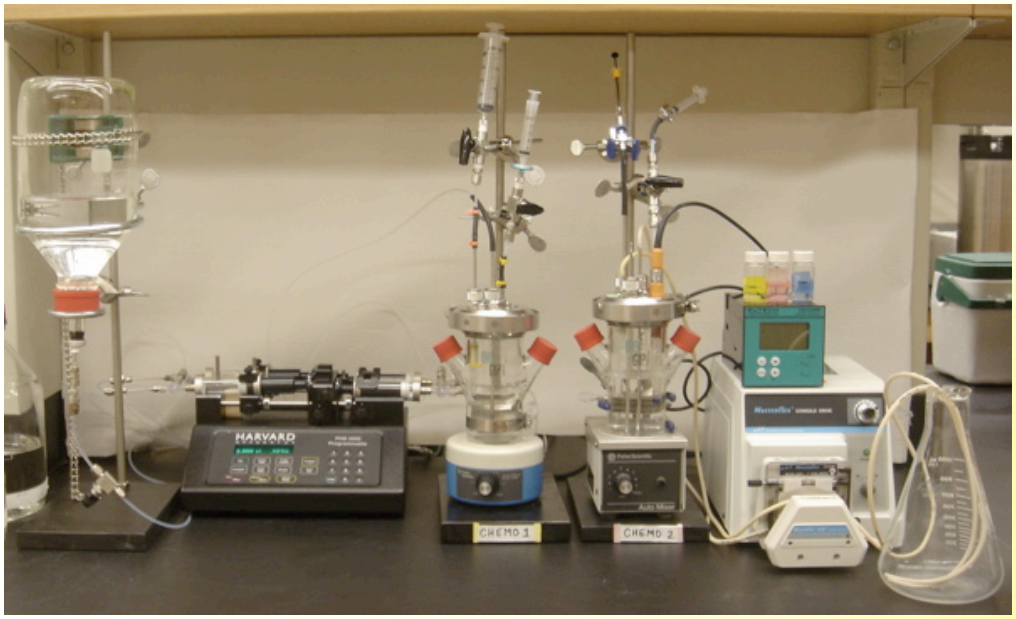
- 5,013,479 nt (circular)
- 74.9% G+C
- At least 5 prophages
- 2 paralogous rRNA gene clusters
- Gene clusters for type-IV pilus-based motility
- Gene clusters for flagellar motility
- 7 chemotaxis gene clusters
- 18 genes encoding MCPs

- SOD (Fe/Mn-type)
  4 rubrerythrin paralogs
  1 diheme-cyt-c peroxidase
  Catalase absent
- 69 genes with conserved CXX(XX)CH motifs
  - 17 genes > 10 CXX(XX)CH motifs
  - 1 with 40 CXX(XX)CH motifs
  - 1 with 26 CXX(XX)CH motifs
- 42 genes with CXXCXXXCP motifs (mostly Fe_4S_4 ferredoxins)
  (incl. 2 RDase genes)
  Myxococcus: 17 such genes

Biomarker Transcripts and RT-qPCR



Identification of U(VI) Reduction Biomarker Genes



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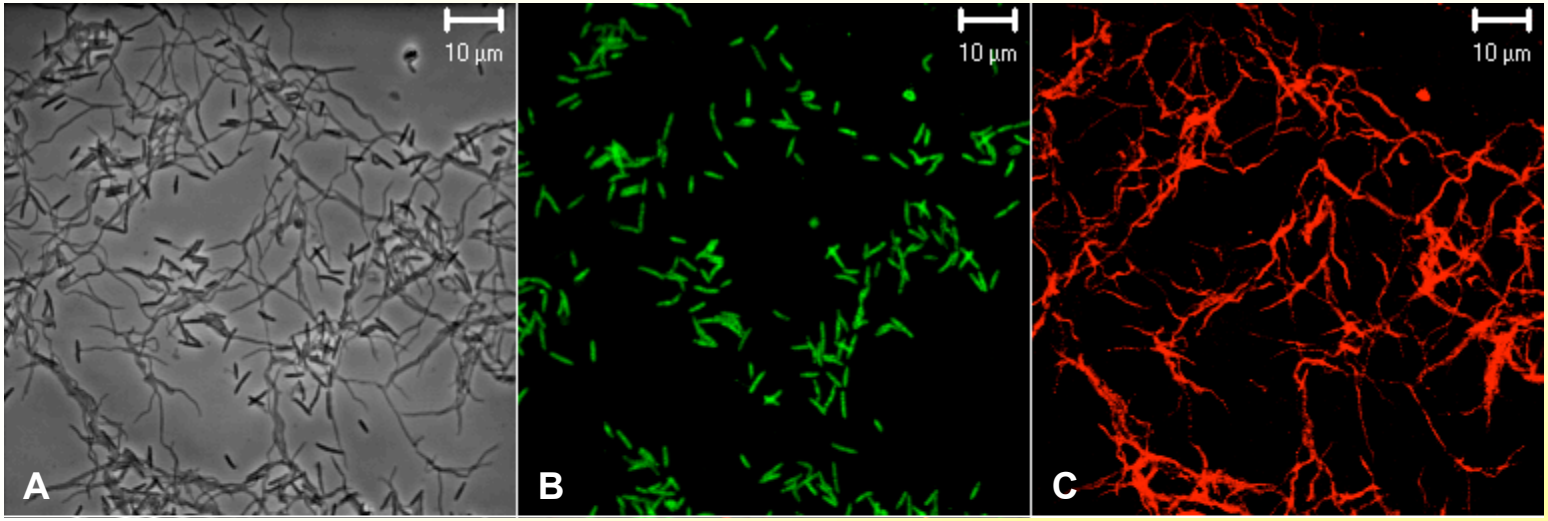
Alex Beliaev
Jim Fredrickson

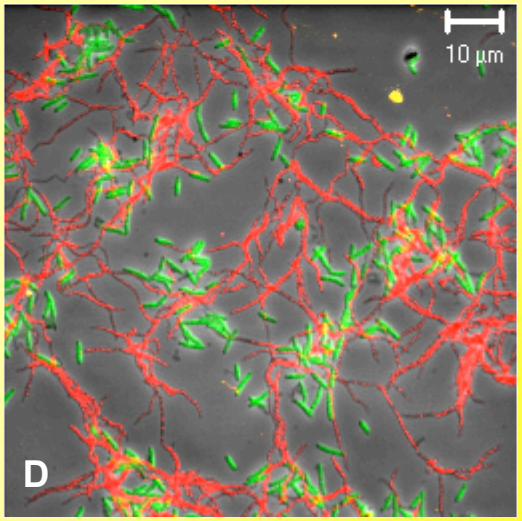
Approach:

- Chemostats cultures (EAs: Fe(III), U(VI), fumarate, 2-CP)
- "Steady-state" mRNA pool
- *Anaeromyxobacter dehalogenans* strain 2CP-C whole genome microarrays

Design of FISH Approaches for *Anaeromyxobacter* Visualization

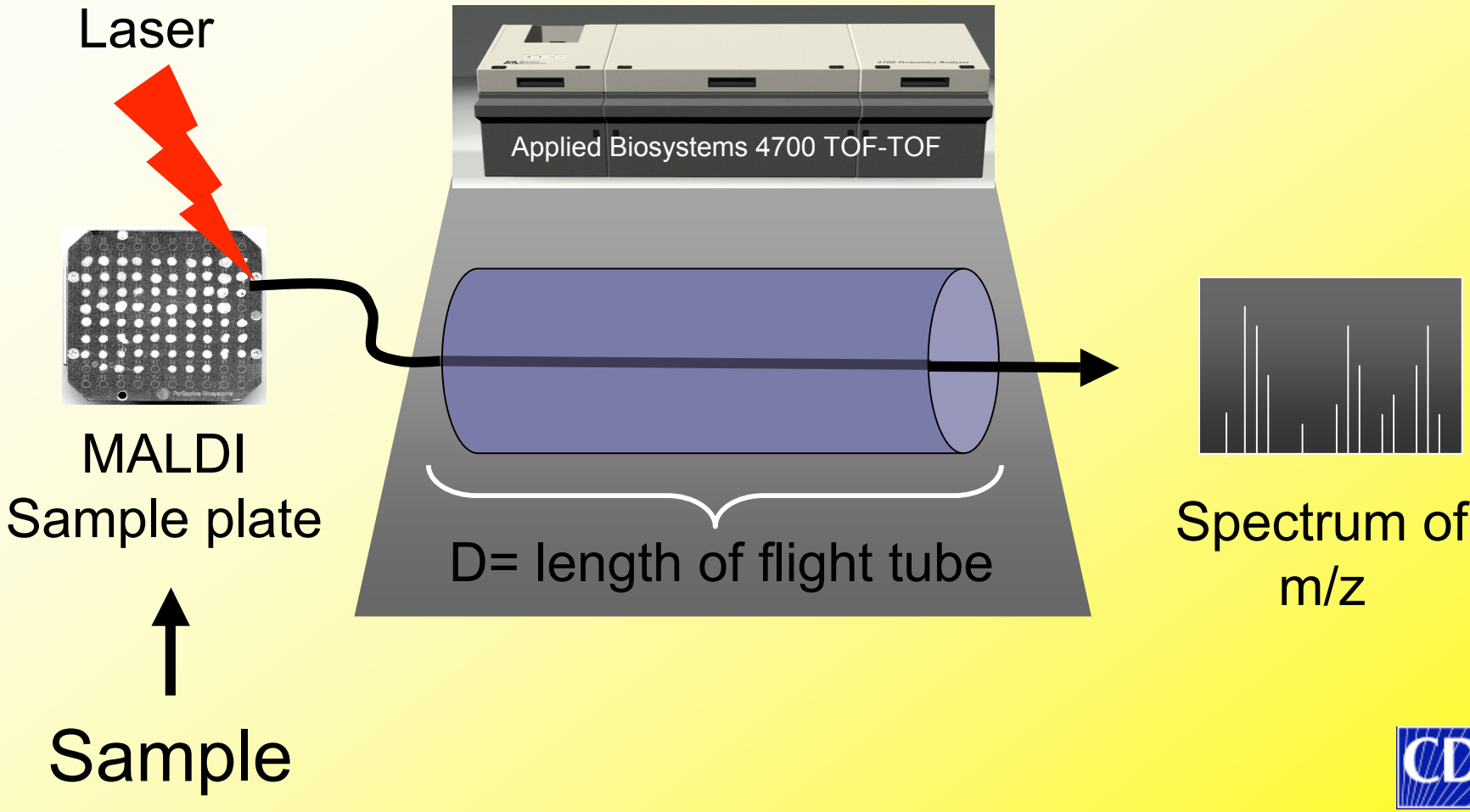


A: Phase contrast
B*: Myxococcus* probe (green)
C: *Anaeromyxobacter* probe (red)
D: A+B+C

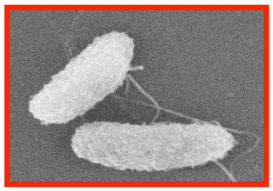


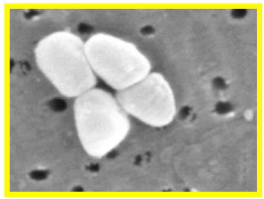
TECHNISCHE UNIVERSITÄT MÜNCHEN

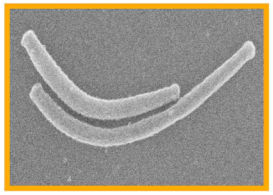
MALDI-TOF Mass Spectrometry

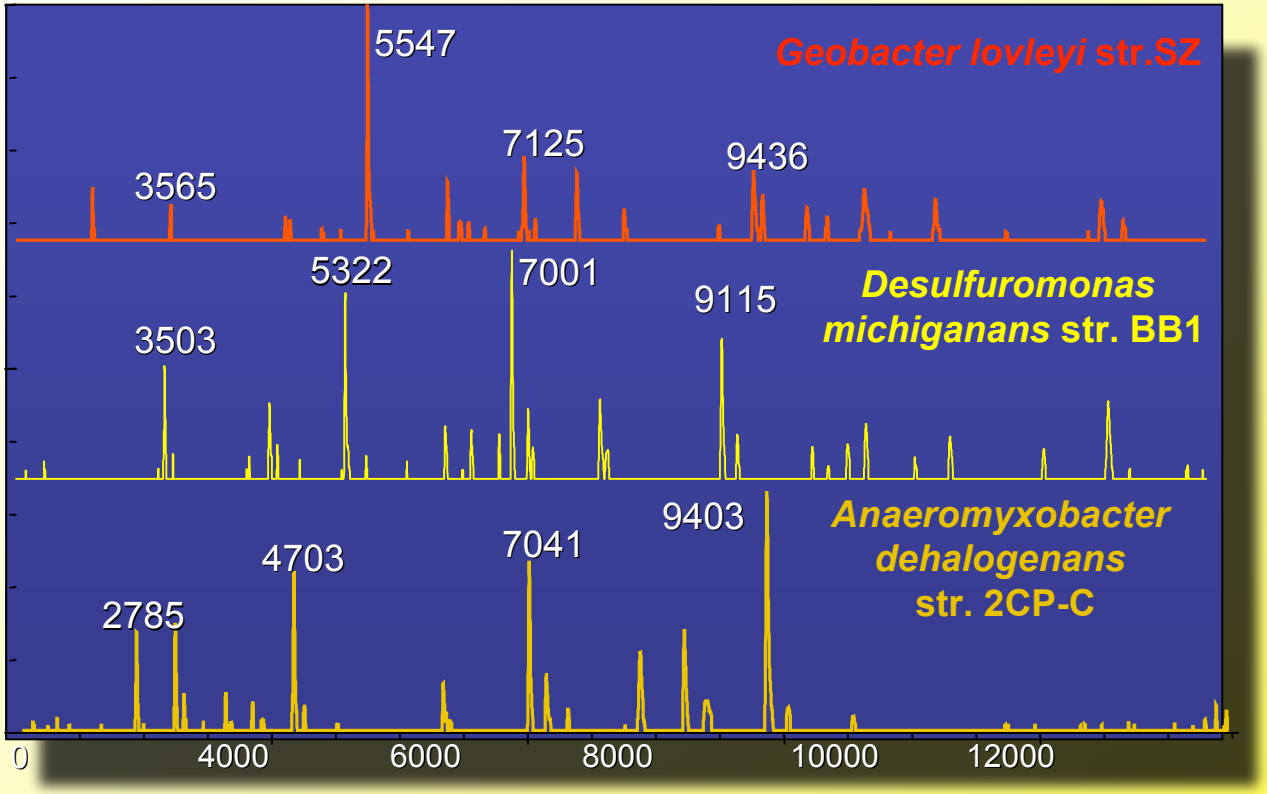


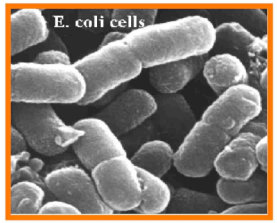
MALDI-TOF MS: Genus Specificity

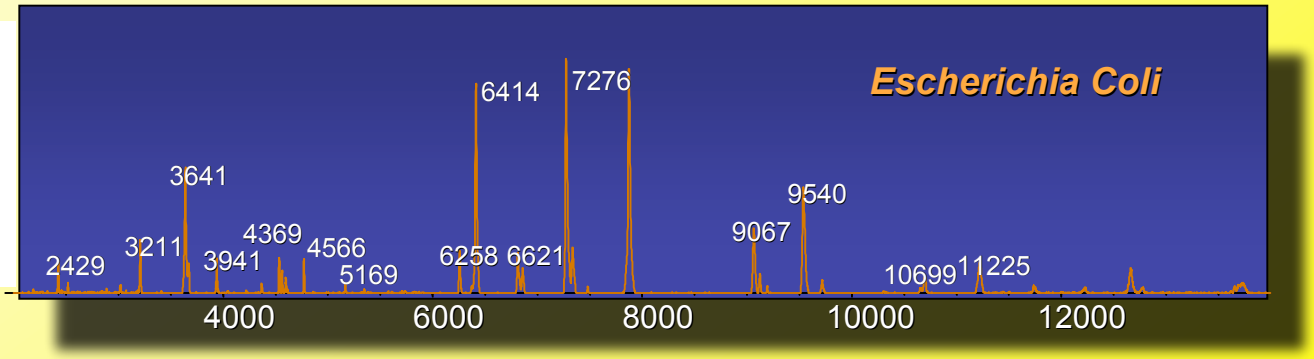






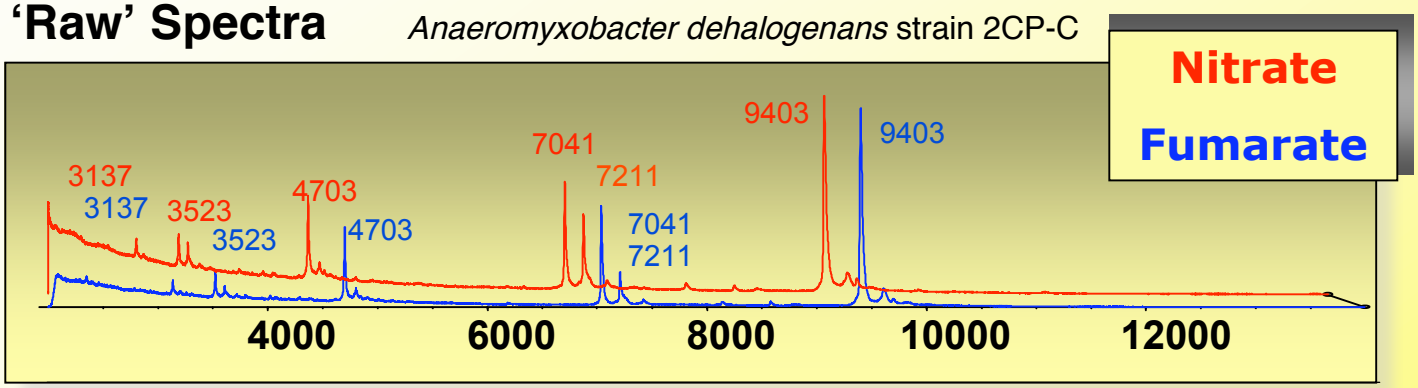




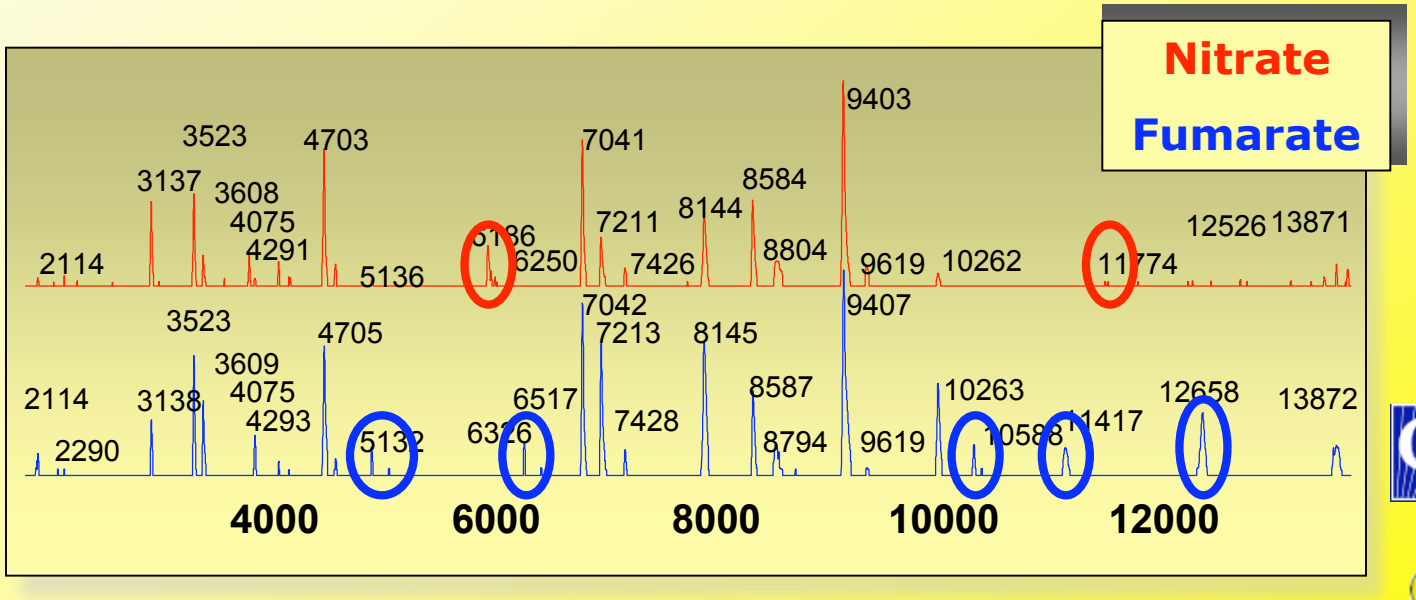


Whole Cell MALDI-TOF MS Gives Clues to Metabolic Activity

'Raw' Spectra *Anaeromyxobacter dehalogenans* strain 2CP-C



Smoothed and Denoised Spectra

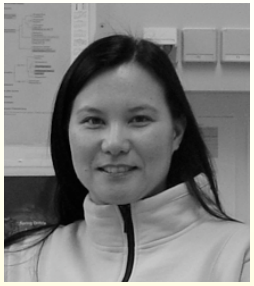


Take Home Messages

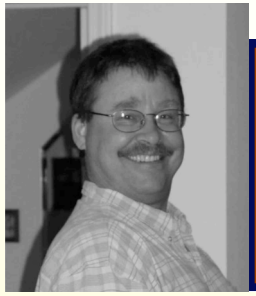
- *Anaeromyxobacter* spp.
  - native to the FRC
  - metabolically versatile facultative anaerobes
  - grow on the expense of U(VI) reduction
  - contribute to NO_3^- and N_2O redution
  - multiple isolate available
- Specific qualitative and quantitative 16S rRNA gene-targeted tools
  - multiplex PCR
  - FISH
- Microarrays identify process-specific biomarkers
  - RT qPCR (multiplex)
  - advanced FISH

The Team

Natuschka Lee



Robert Sanford



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Alex Beliaev
Jim Fredrickson
Matt Marshall
David Culley