# Who's coming to dinner? Microbial phylogenetic analyses of various subsurface petroleum well environments for MEOR applications


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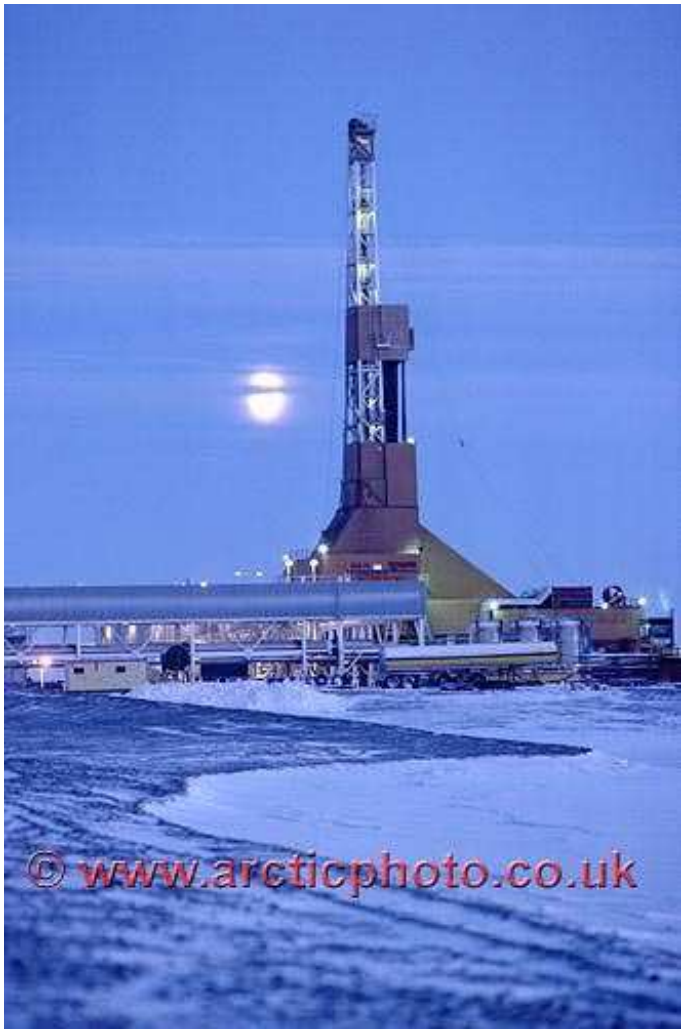
*"Putting science to work…."*

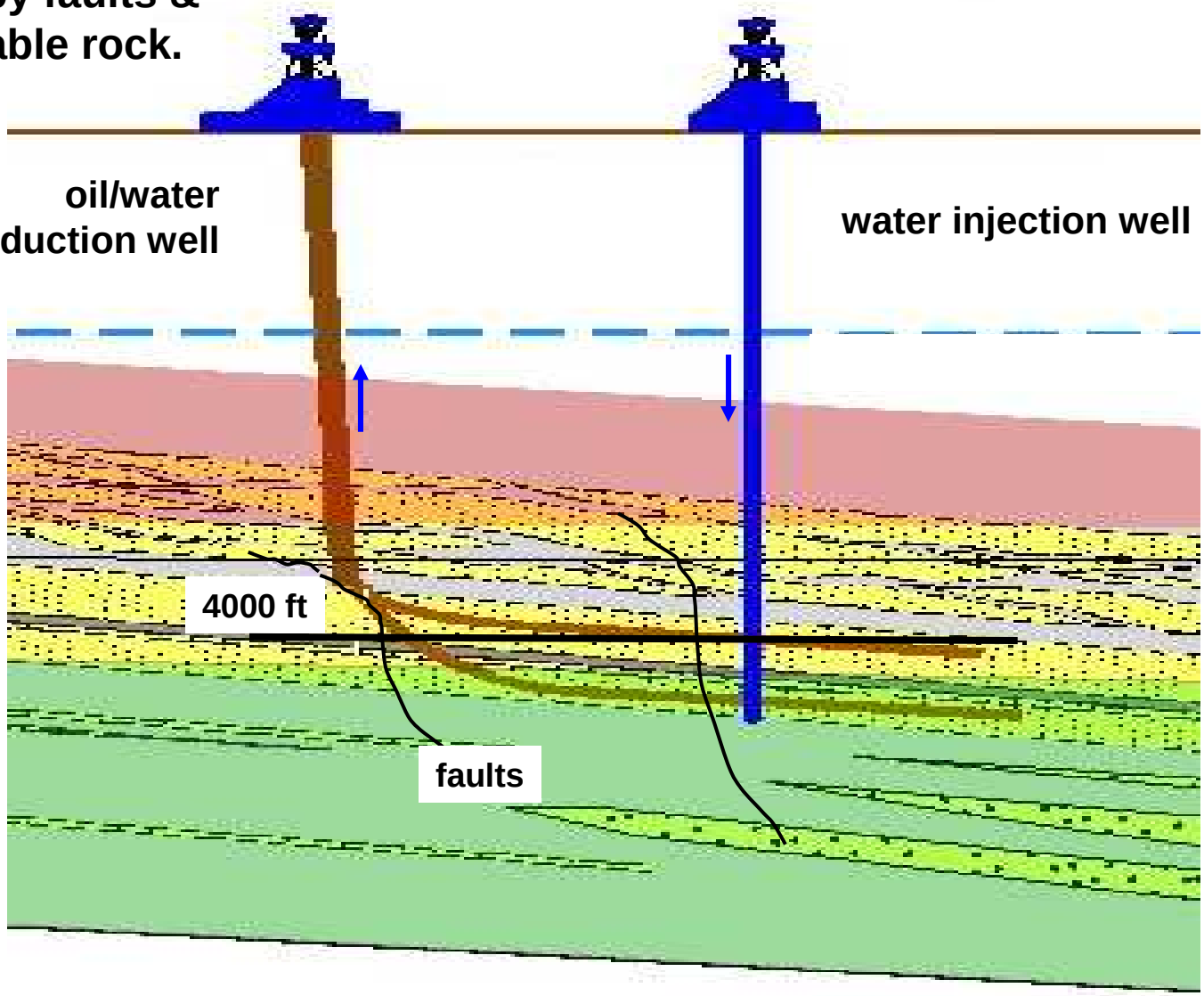



Milne Point, North Slope, Alaska, 2007

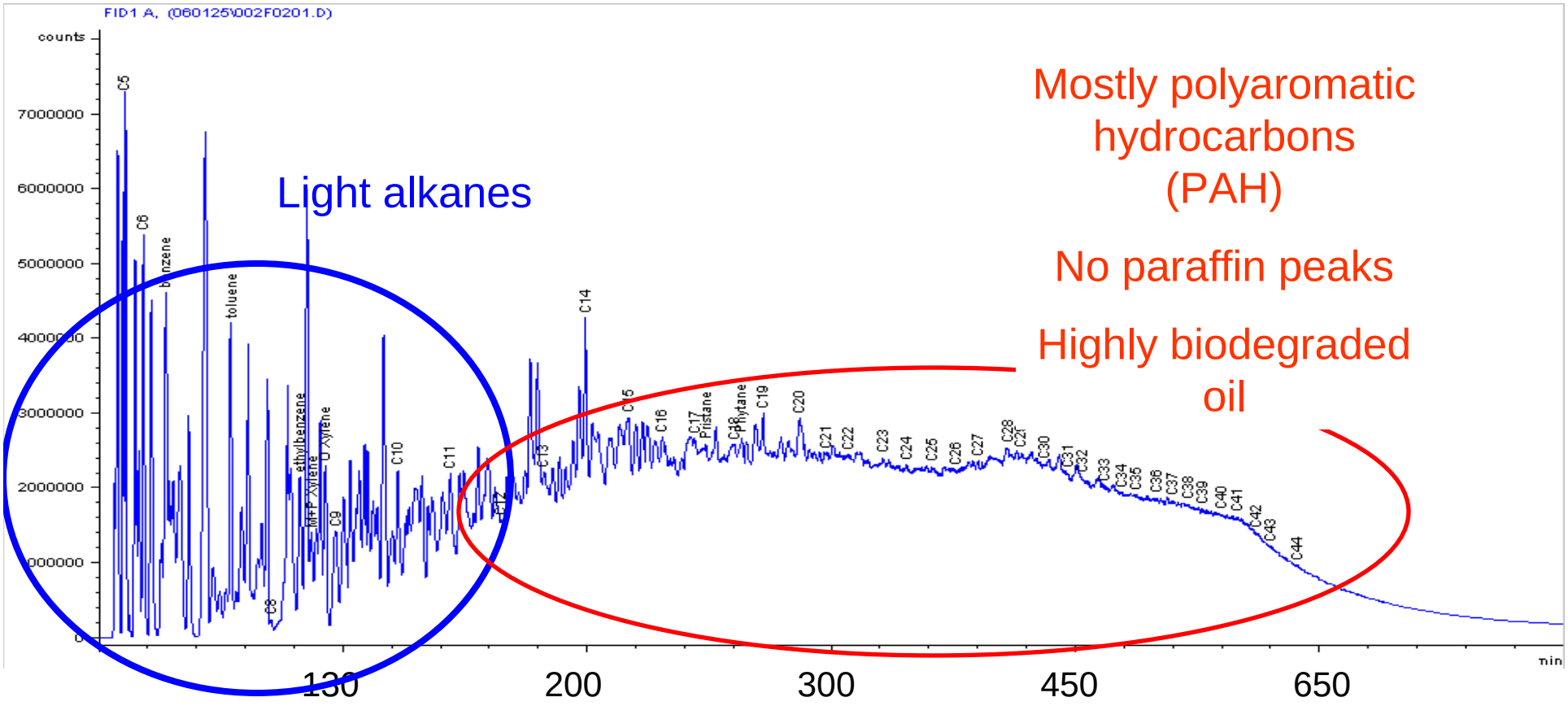
**Oil reservoirs are sand layers bounded by faults & impermeable rock.**

***Our focus*:**

***Add nutrients and microbes to injection water.***



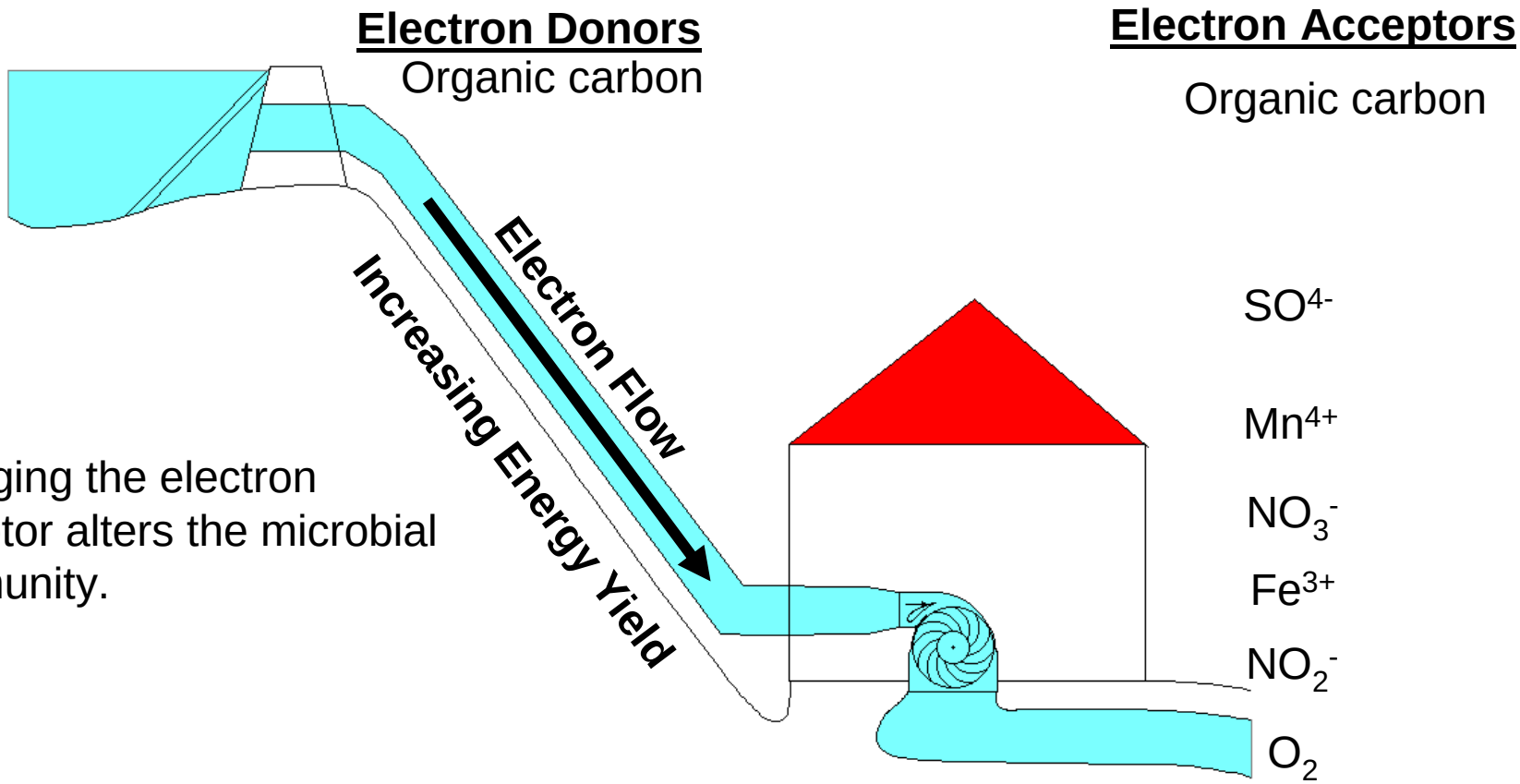
Up to 47,000 compounds in “petroleum”




~ mw

Milne Point oil

Many e- acceptors exist, the choice dictates the type of microbes that will predominate, biomass produced, and over all bioactivity.
Microbial Growth is Driven by the Redox differential

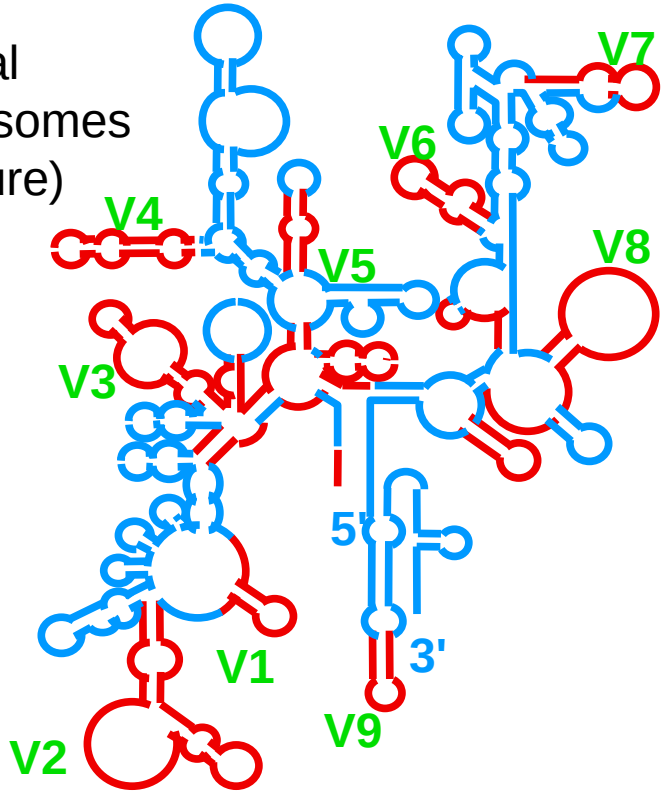



Changing the electron acceptor alters the microbial community.

# Secondary Structure of 16S rRNA

## Variable and Conserved Regions

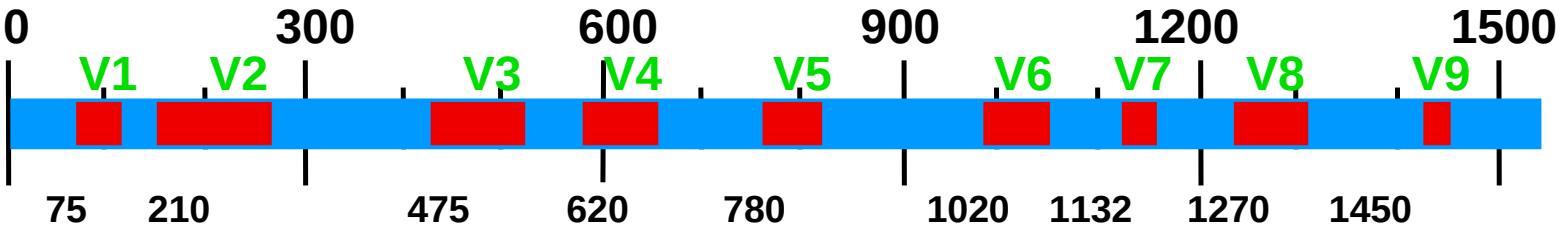
16SrRNA structural component of ribosomes (protein manufacture)




— = Conserved Regions

— = Variable Regions

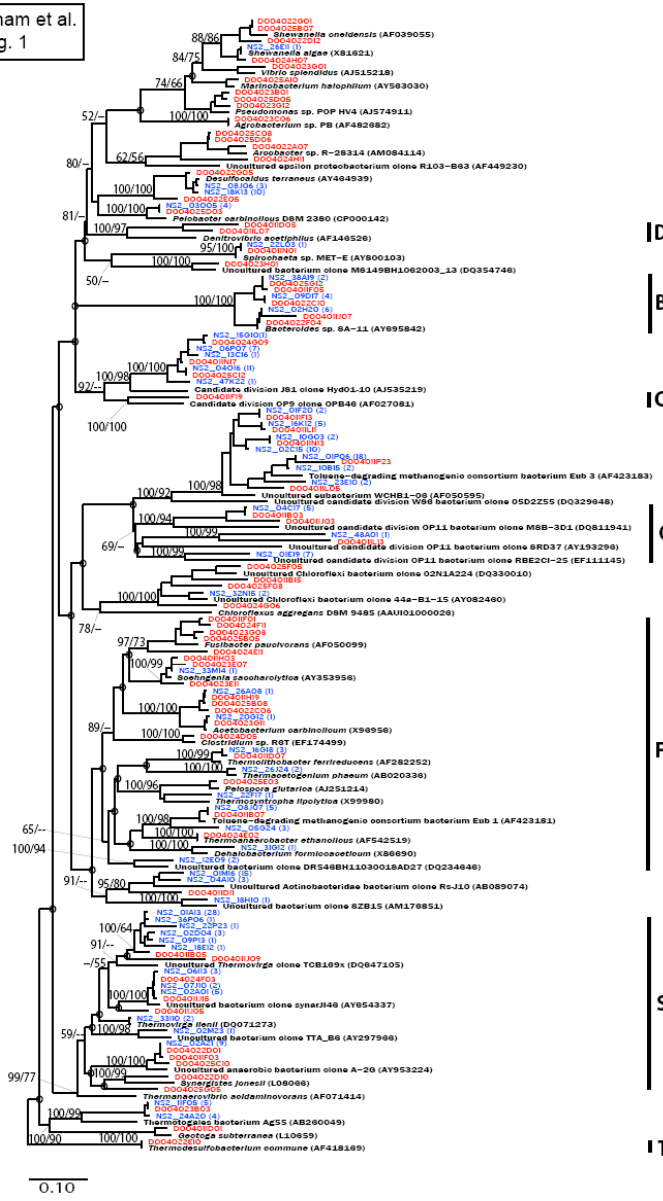
V# = Most Variable Regions




= Conserved Regions = Variable Regions V# = Most Variable Regions

***DMA alliance:*** Dupont's Microbially Enhanced Oil Recovery team in collaboration with Vinh Pham in the lab of Ed Delong at MIT.

Pham et al.
Fig. 1




Environmental Microbiology (2009) 11(1), 176–187 doi:10.1111/j.1462-2920.2008.01751.x

## Characterizing microbial diversity in production water from an Alaskan mesothermic petroleum reservoir with two independent molecular methods

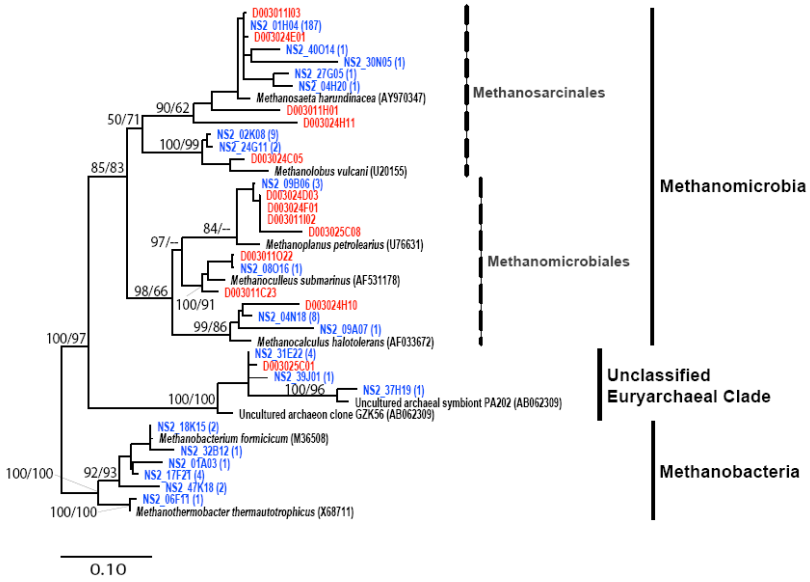
Vinh D. Pham,[1] Linda L. Hnatow,[2] Shiping Zhang,[2] Robert D. Fallon,[2] Scott C. Jackson,[2] Jean-Francois Tomb,[2] Edward F. DeLong[1]** and Sharon J. Keeler[2]*
[1]Department of Civil and Environmental Engineering, Massachusetts Institute of Technology, Cambridge, MA 02139, USA.
[2]Central Research and Development, E.I. DuPont de Nemours, Wilmington, DE 19880, USA.

Summary

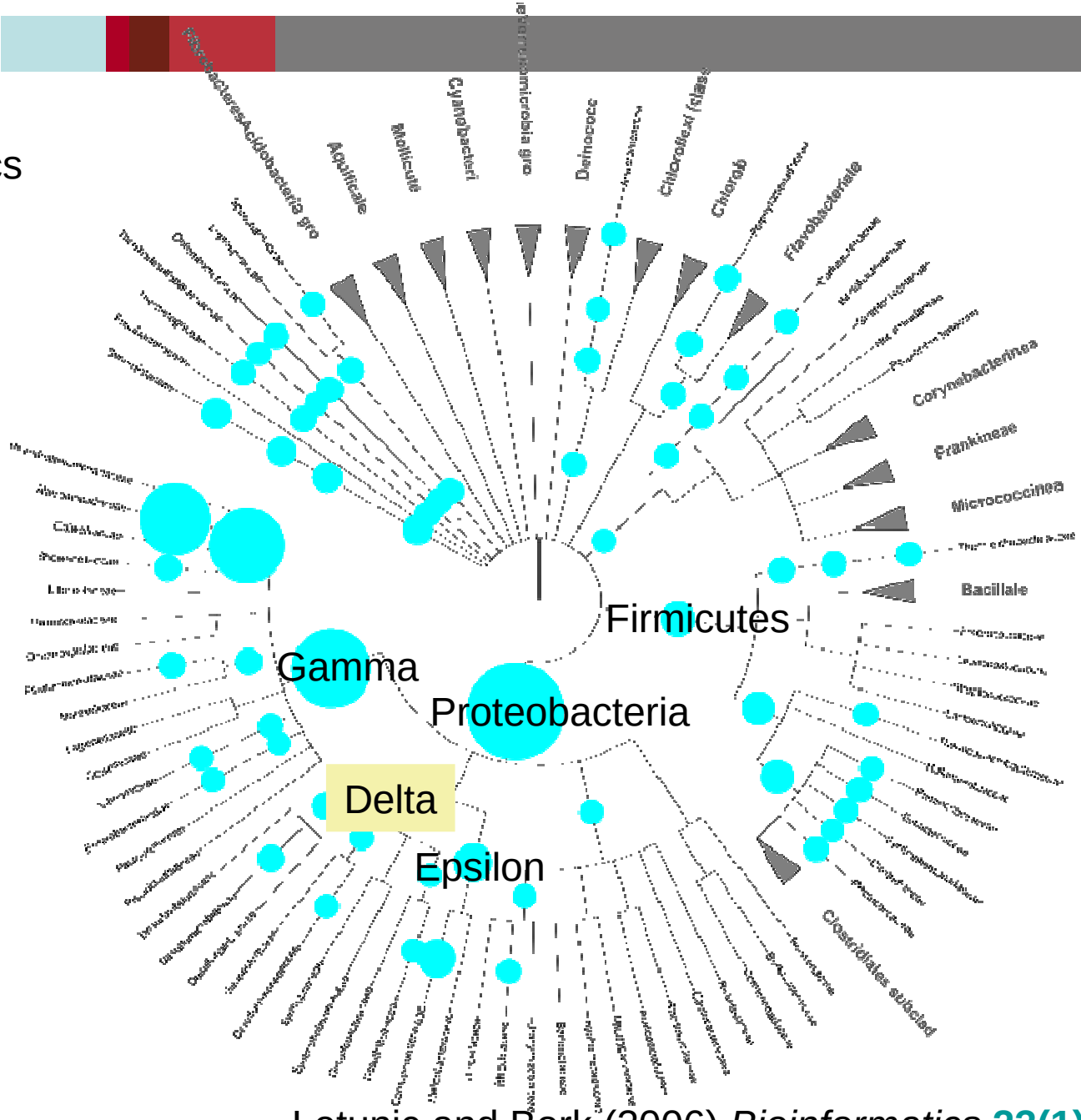
chain, which leads to methane production as the primary terminal electron sink.

Introduction

Recent interest in the study of petroleum reservoir microbiota has been fuelled by an acceleration in global energy demand, and by the recognition that microbial biodegradation of oil hydrocarbons has significant implications for oil quality and production (Head *et al.*, 2003). For example, most oil fields are degraded to varying extents,



ITOL graphics



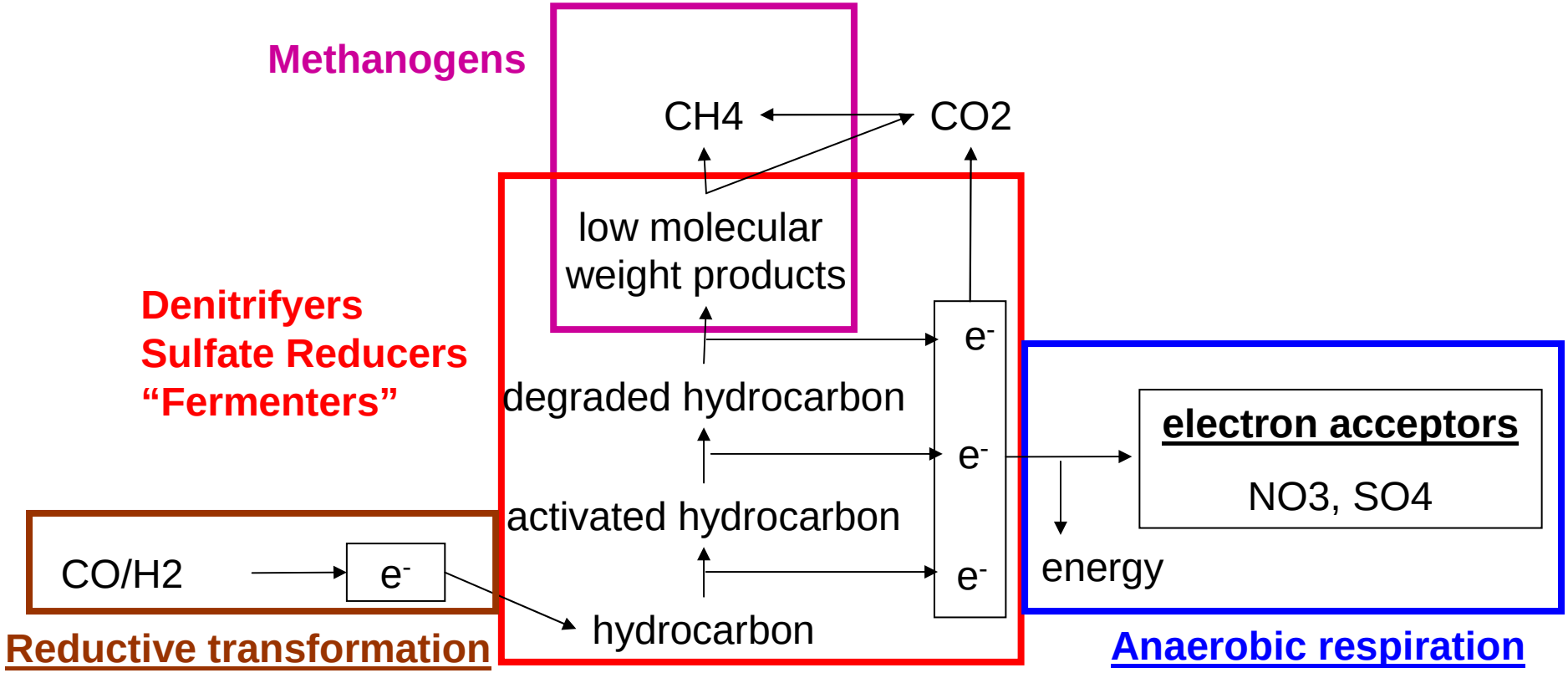

Letunic and Bork (2006) *Bioinformatics* **23(1)**:127-8

# A hydrocarbon "food chain"

A microbe must

- Acquire nutrients & carbon
- Generate energy
- Balance electrons

Exploitation of resources in an anaerobic environment often requires cooperative groups, consortia.



# Summary of North Slope reservoir system phylogenetics.

- Lack of sulfate reducing organisms by molecular methods
  - Low or transient level of sulfate in system
- High relative abundance of methanogens

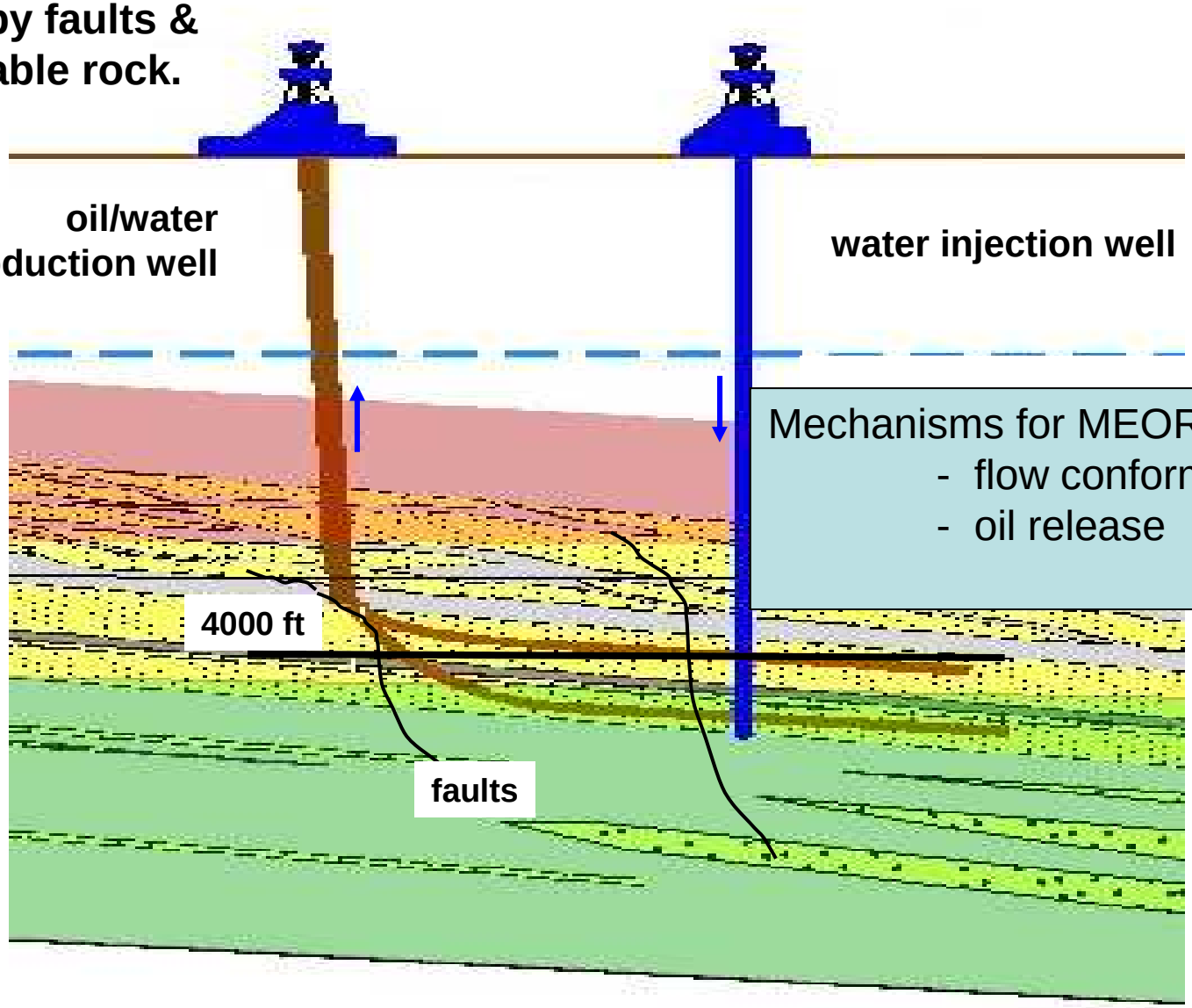
(might otherwise be outcompeted for acetate and H2 by SRBs)

- High abundance of acetogenic Firmicutes (fermenters)
- Transiently high level of acetate, together suggest a mutual metabolic codependency on acetate in this system.
- Many genera found in association with other petroleum environments suggest they are autocthonous.
- Did see single facultative species as ‘weeds’ in some samples, not true anaerobes and may have been introduced with injection water or may have PCR preference.
  - Although some of these were found in non-injected well systems
- Mostly non- halophilic species, ½ seawater salinity in this system.
- Sizable portion of uncultivated species found in extreme environments
- Isolated two species for potential use in MEOR.

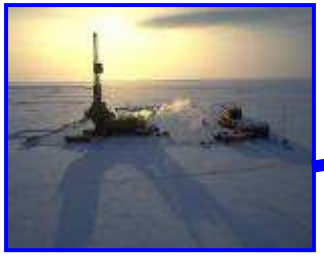
**Oil reservoirs are sand layers bounded by faults & impermeable rock.**

***Our focus*:**

***Add nutrients and microbes to injection water.***




Mechanisms for MEOR
- flow conformance
- oil release



North Slope, Alaska


Saskatchewan, Canada

Alberta Canada

East Texas

# Rationale for Subterranean Salt Water and Oil Reservoirs


Western Interior Seaway during the mid-Cretaceous, about 100 million years ago (USGS)

Widespread carbonate deposition suggests that the Seaway was warm and tropical, with abundant calcareous algae

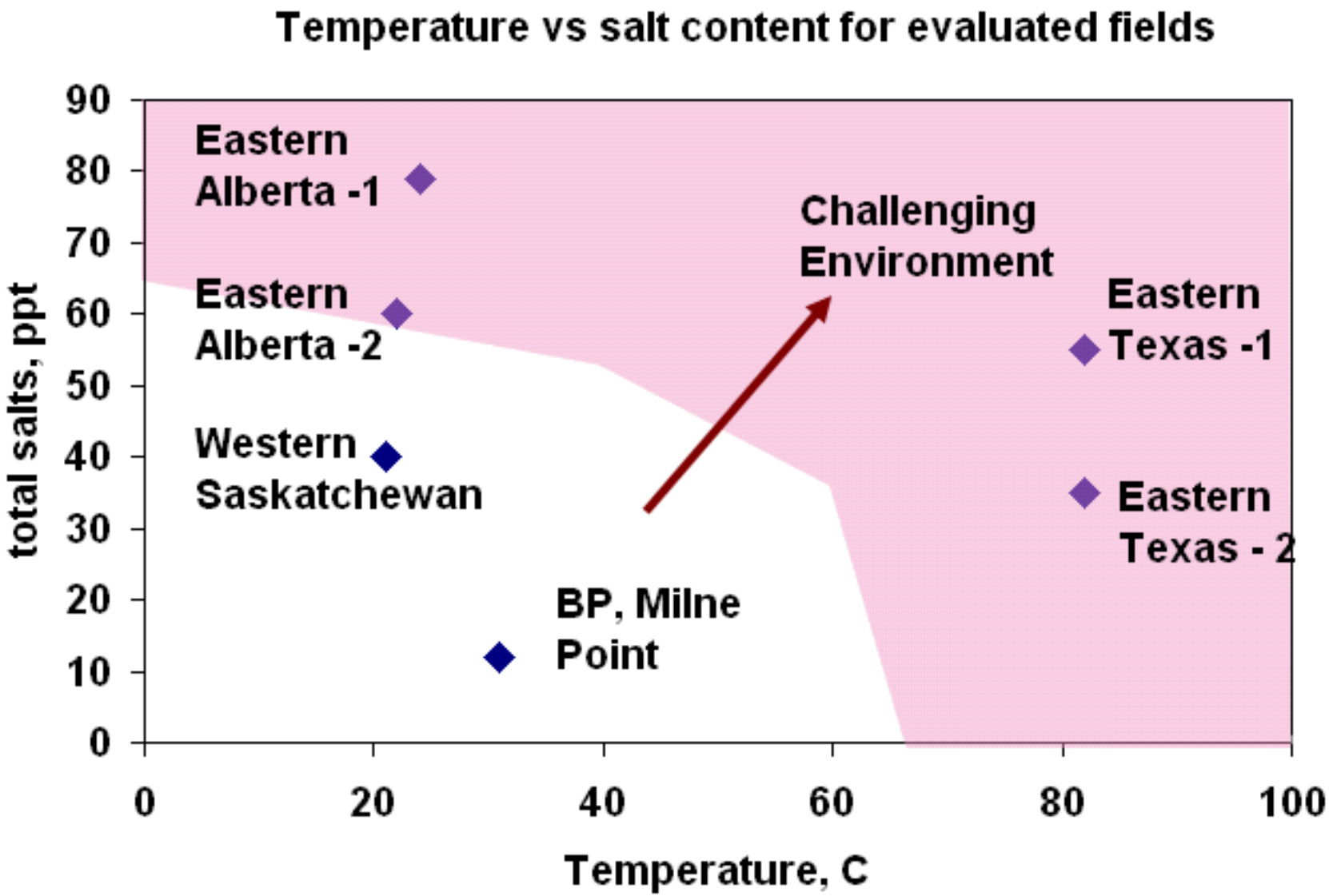
Formation of reefs that later form petroleum reservoirs

# Geology of well systems analyzed.

| site | location | formation | age | geological origin | max thickness |
|---|---|---|---|---|---|
| Milne Point | North Slope Alaska | Schrader Bluff sandstone | Upper Cretaceous | shoreface and marine sands | Var. |
| Texas | east Texas | Paluxy sandstone | Lower Cretaceous | river channel or delta "shoestring" sands | 40 feet |
| Saskatchewan | southwest Saskatchewan | Lloydminster sandstone | Lower Cretaceous | delta and shoreface sands | 100 feet |
| Alberta | east Alberta | Sparky sandstone | Lower Cretaceous | shoreface sands, locally cut by river or delta channels | 60 feet |

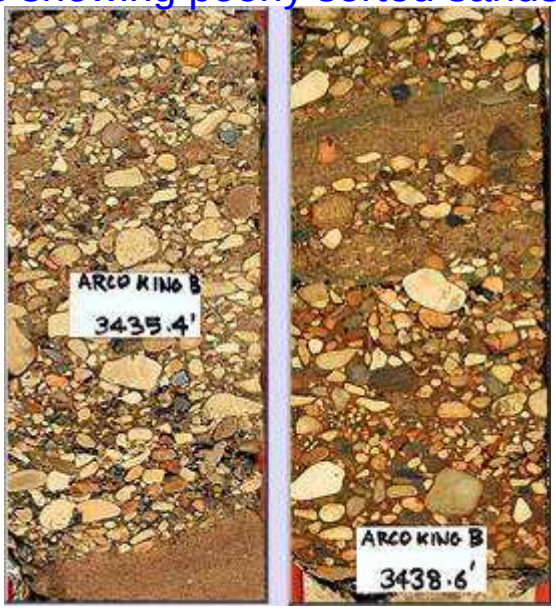
# Summary of well systems analyzed

| Oil well | Salinity (PPT) | Temp (℃) | Oil type | Sulfur |
|---|---|---|---|---|
| **Milne Point** | 15 | 25-30 | PAH dominant<br>15cP no S | No or low sulfate |
| **Texas** | 26,55 | 80 | Aliphatic, rich, 7 cP, | sour, 200-300 ppm |
| **Saskatchewan** | 36 | 25-30 | Heavy, Weathered,<br>1100 cP | 20-30 ppm<br>low sulfur |
| **Alberta** | 60-80 | 25-30 | Moderately heavy weathered oil 56 cP. | "sweet" oil, low organic sulfur, fluctuates. |

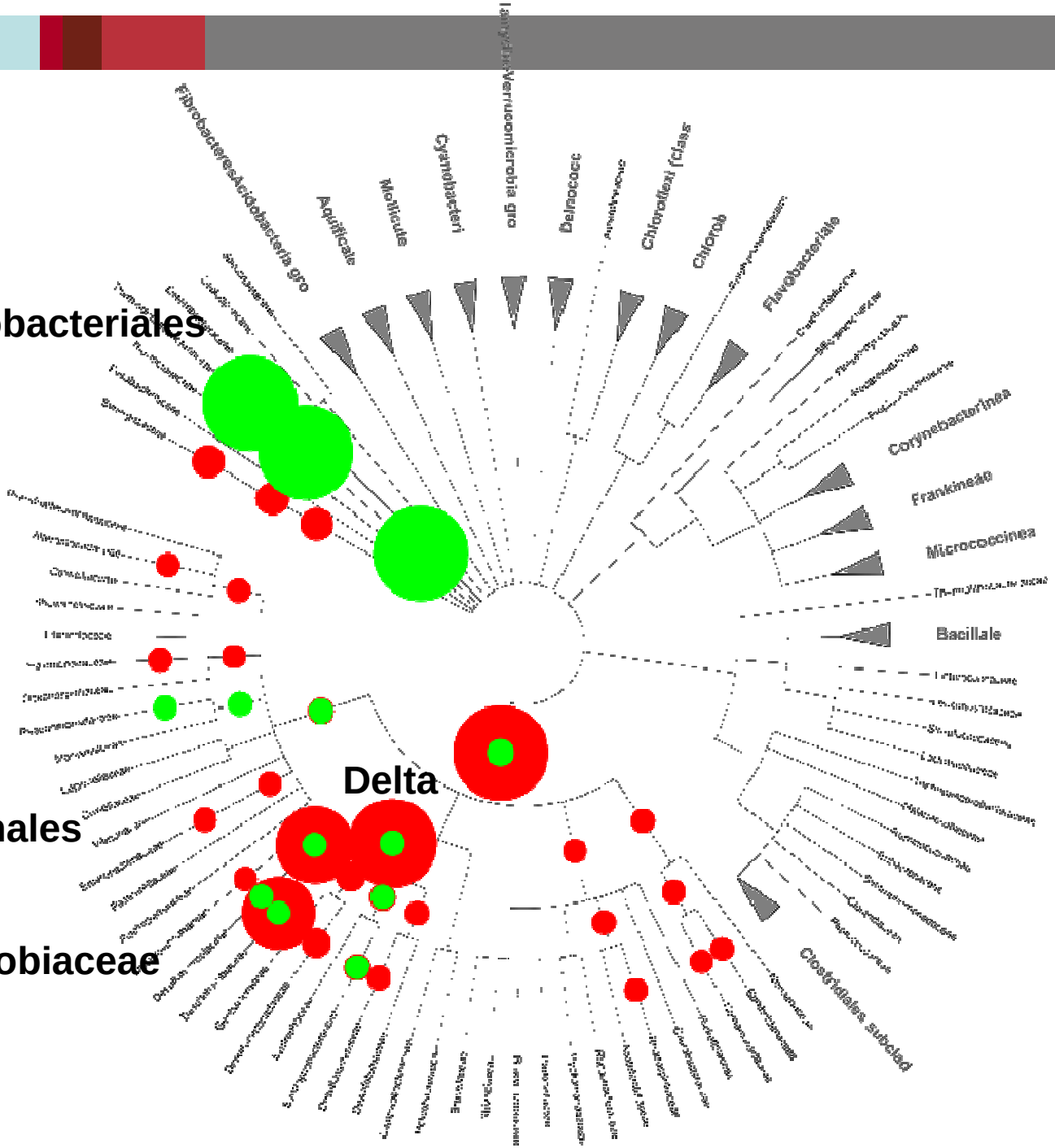

Temperature vs salt content for evaluated fields
total salts, ppt
90
80
70
60
50
40
30
20
10
0
0
20
40
60
80
100
Temperature, C
Eastern Alberta -1
Eastern Alberta -2
Western Saskatchewan
BP, Milne Point
Challenging Environment
Eastern Texas -1
Eastern Texas - 2

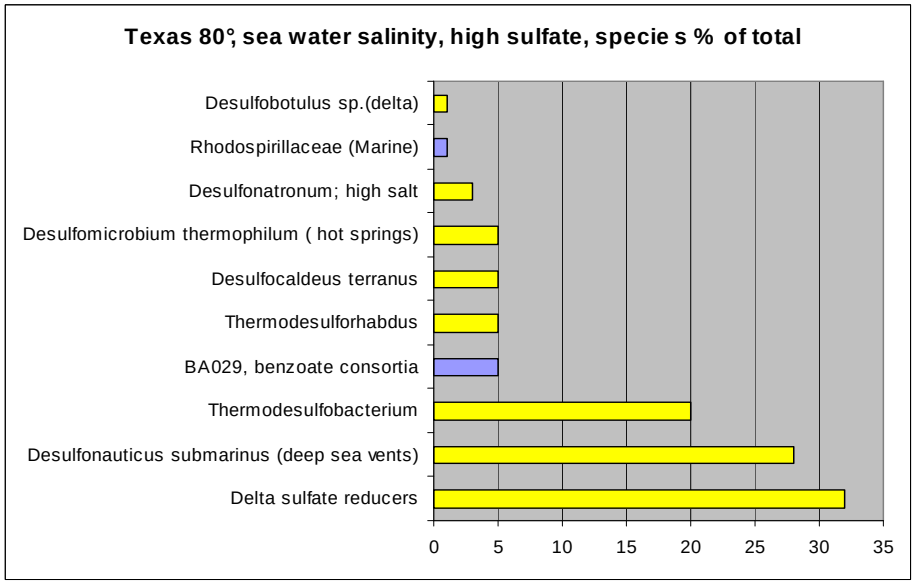
# East Texas

cores showing poorly sorted sands



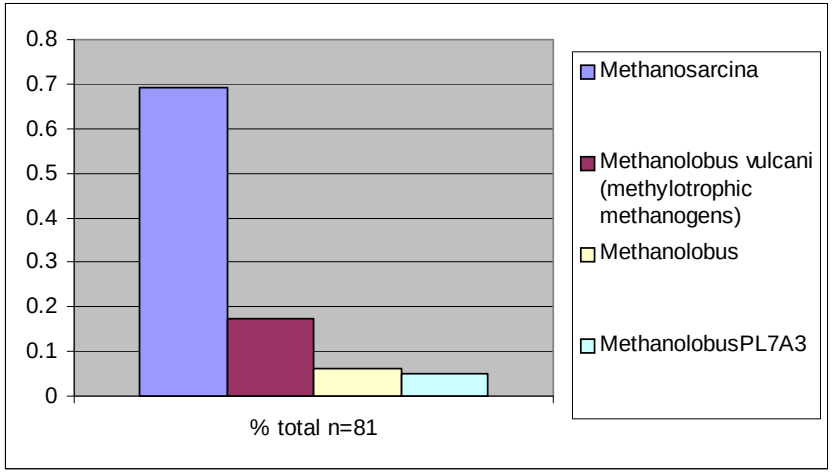
East
Texas


Thermodesulfobacteriales
Delta
Desulfovibrionales
Desulfohalobiaceae
Corynebacterinea
Frankineae
Micrococcinea
Bacillale
Clostridiales subclad
Aquificale
Mollicute
Cyanobacteri
Deinococc
Chlorob
Flavobacteriale

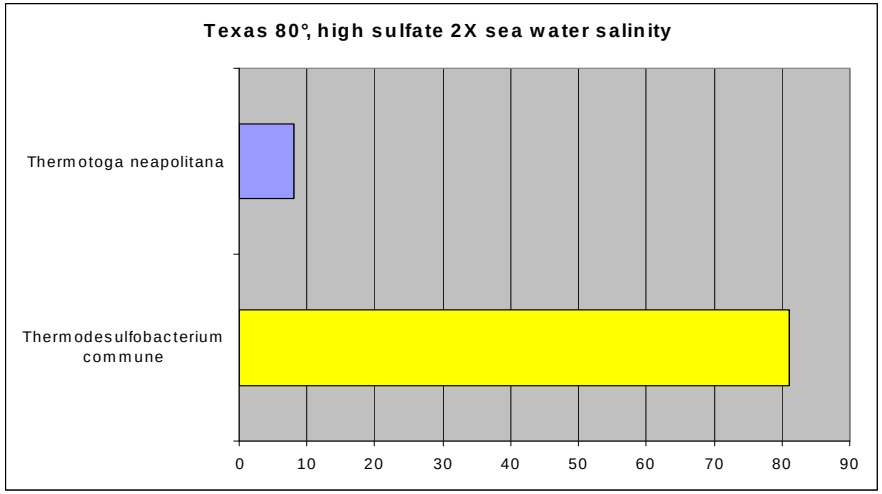



Halophilic, thermophilic SRBs predominate

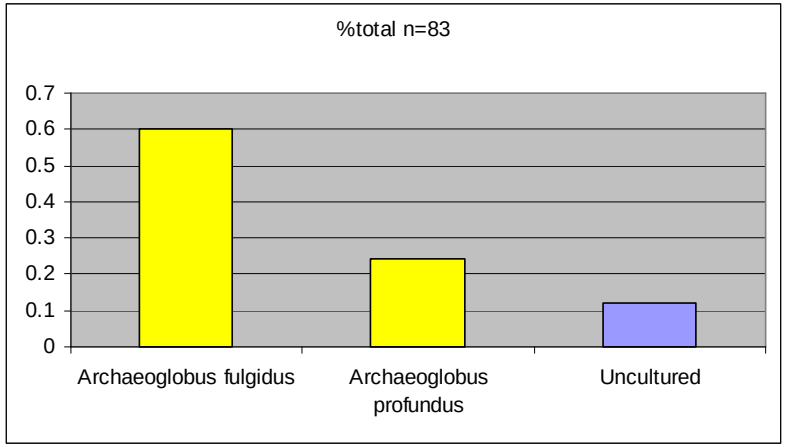
East Texas



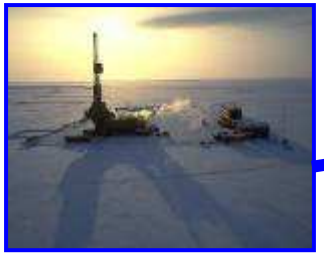

Acetogenic archael community




High salinity, high temperature, few species




Archael sulfate reducing community
One genera-Archaeglobus



North Slope, Alaska


Saskatchewan, Canada

Alberta Canada

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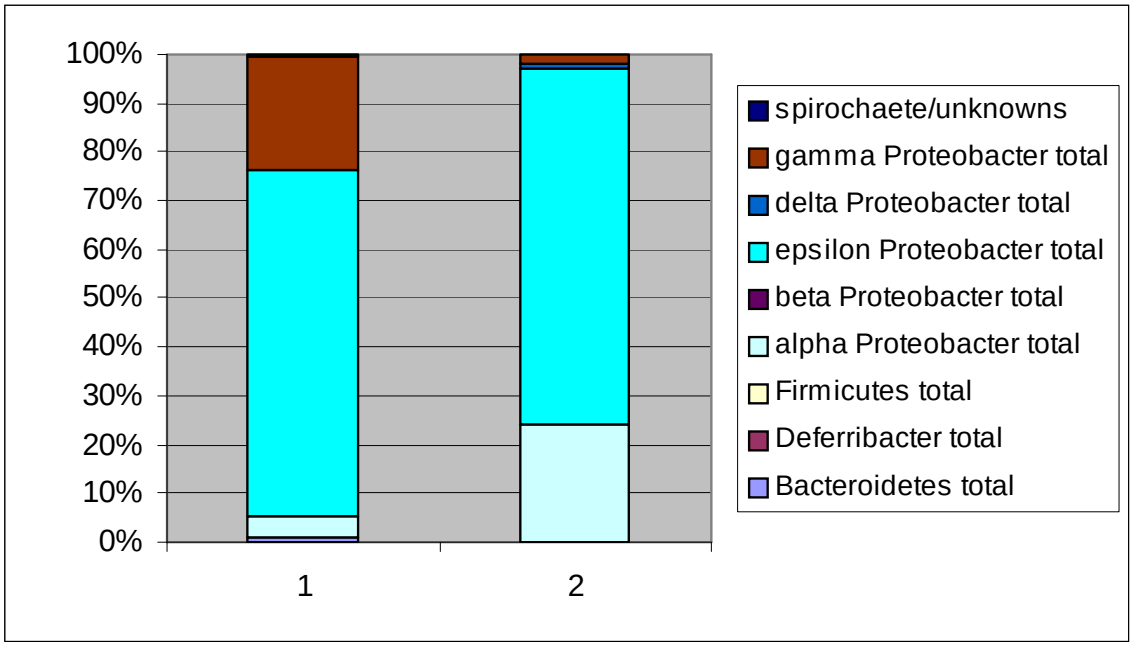
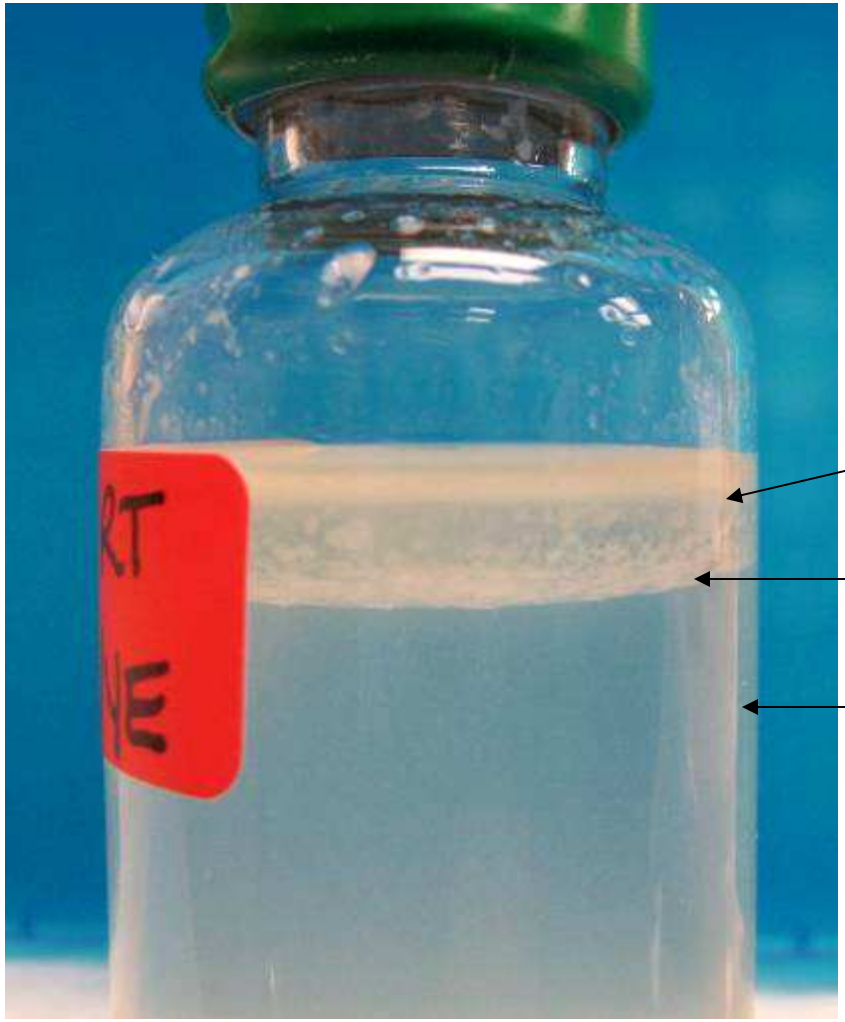



Epsilon genera dominate

| % ot total | NCBI hit | Phylogenetic Identification |
|---|---|---|
| 47.8 | Methanolobus taylorii | Note these organisms predominated in the North Slope system also. Thought to be methylotrophic methanogens |
| 15.6 | Methanosaeta | acetoclastic methanogens Note these organisms predominated in the North Slope system also |

Isolation of halotolerant biofilm producers by sequential
Isolation of oil/water interface




Corn oil layer
Carbon source

Growth at interface

Aqueous media
Saline with nitrate

# Isolation of halotolerant biofilm producers by sequential isolation of oil/water interface

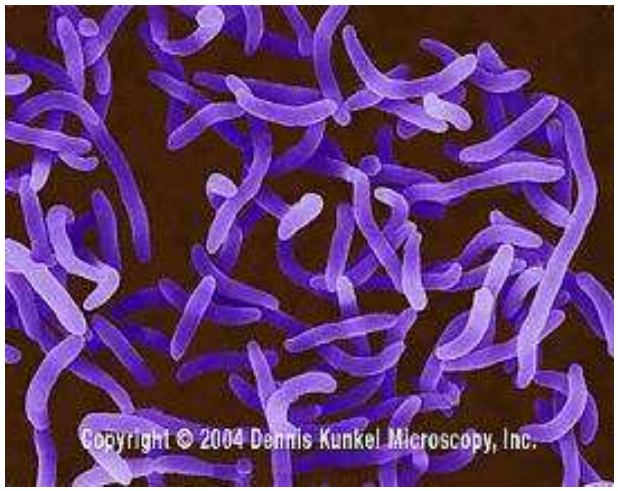
- Plated onto LB + nitrate for anaerobic colony isolations
- 16S rDNA identification of species recovered.

- *Mangroveibacter, Vibrio, Pseudomonas sp., Morganella, Enterobacter-* all facultative, some salt tolerant.

- Review literature on clinical occurrence of these genera.

- *Vibrios*-
  - known salt water organisms,\
  - investigated non-pathogenic strains

- *Pseudomonas sp*- halotolerant



# Analysis of Vibrio physiology to determine species.

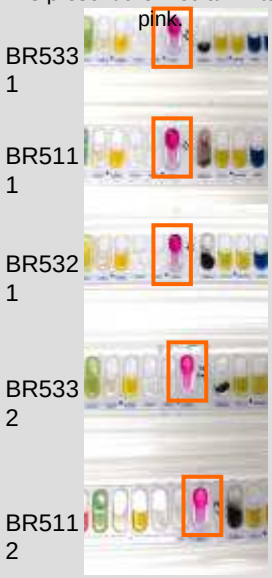
**API 20E test strips:** Strips consist of 20 microtubes containing dehydrated substrates. The tests are inoculated with a bacterial suspension that reconstitutes the media. During incubation (35℃ for 21 hours) metabolism produces co lor changes that are either spontaneous due to pH differences, or end products that are revealed by the addition of reagents.

| Organism | 4 | 5 | 6 | 7 | 15 | 22 |
|---|---|---|---|---|---|---|
| *Vibrio*—clinical species | | | | | | |
| *V. alginolyticus* | + | + | – | V | + | – |
| *V. carchariae* | – | + | – | + | + | – |
| *V. cholerae* | + | + | – | + | + | – |
| *V. cincinnatiensis* | – | (+) | – | – | + | + |
| *V. damsela* | + | V | + | – | (–) | – |
| *V. fluvialis* | – | – | + | – | + | + |
| *V. furnissii* | – | – | + | – | + | + |
| *V. hollisae* | – | – | – | – | – | + |
| *V. metschnikovii* | + | + | V | – | + | – |
| *V. mimicus* | – | + | – | + | – | – |
| *V. parahaemolyticus* | – | + | – | + | – | + |
| *V. vulnificus* | – | + | – | + | (–) | – |
| *Vibrio*—nonclinical | | | | | | |
| *V. aestuarianus* | – | V | + | – | + | – |
| *V. anguillarum* | + | – | (+) | – | + | (+) |
| *V. campbellii* | – | + | – | (–) | – | – |
| *V. cincinnatiensis* | (+) | – | V | – | V | – |
| *V. diazotrophicus* | – | – | – | – | + | + |
| *V. fischeri* | – | V | – | – | – | – |
| *V. gazogenes* | V | – | – | – | + | + |
| *V. harveyi* | – | + | – | (+) | + | – |
| *V. logei (15°C)* | – | + | – | V | (–) | – |

Farmer, J. and Hickman-Brenner, F. "The Genera Vibrio and Photobacterium." *Prokaryotes* (2006) 6:533. (www.springerlink.com/content/l0503382706745n8/fulltext.pdf)

**4: Voges-Proskauer**

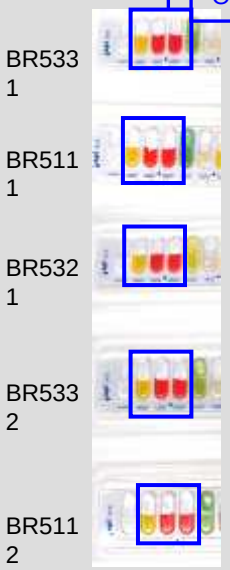
A test for the formation of acetylmethylcarbinol (acetoin) from fermentation of glucose. Solutions of α-naphthol, potassium hydroxide, and creatinine are added. If acetoin is present the media will turn pink.




•Voges-Proskauer was positive (pink) for all of our *Vibrio* strains. This is a *Vibrio alginolyticus* characteristic per the Prokaryotes table.

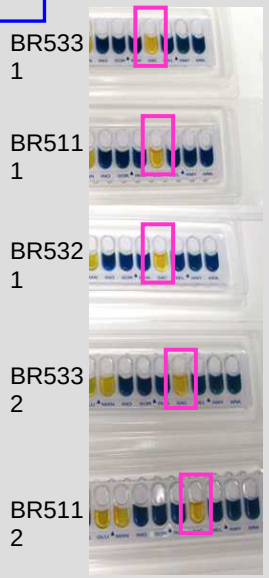
**5: Lysine decarboxylase (2-day)**
**6: Arginine dihydrolase (2-day)**
**7: Ornithine decarboxylase (2-day)**

Decarboxylation is shown by an alkaline reaction (red color of the pH indicator).




•ADH was negative (yellow), LDC was positive (red), and ODC was positive (red). All are *Vibrio alginolyticus* and *Vibrio harveyi* characteristics per the Prokaryotes table.

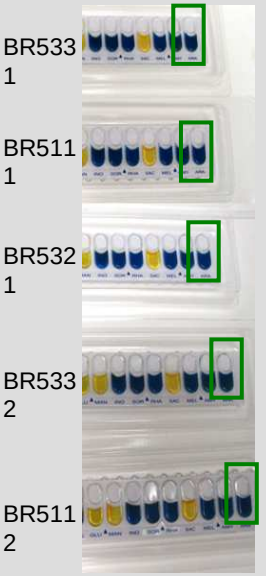
**15: Sucrose Fermentation**




•Sucrose fermentation was positive (yellow) for all of our *Vibrio* strains. This is a *Vibrio alginolyticus* and *Vibrio harveyi* characteristic per the Prokaryotes table.

**22: L-Arabinose fermentation**

Fermentation is shown by an acid reaction (yellow color of the pH indicator).




•Arabinose fermentation was negative (blue). This is a *Vibrio alginolyticus* and *Vibrio harveyi* characteristic per the Prokaryotes table.

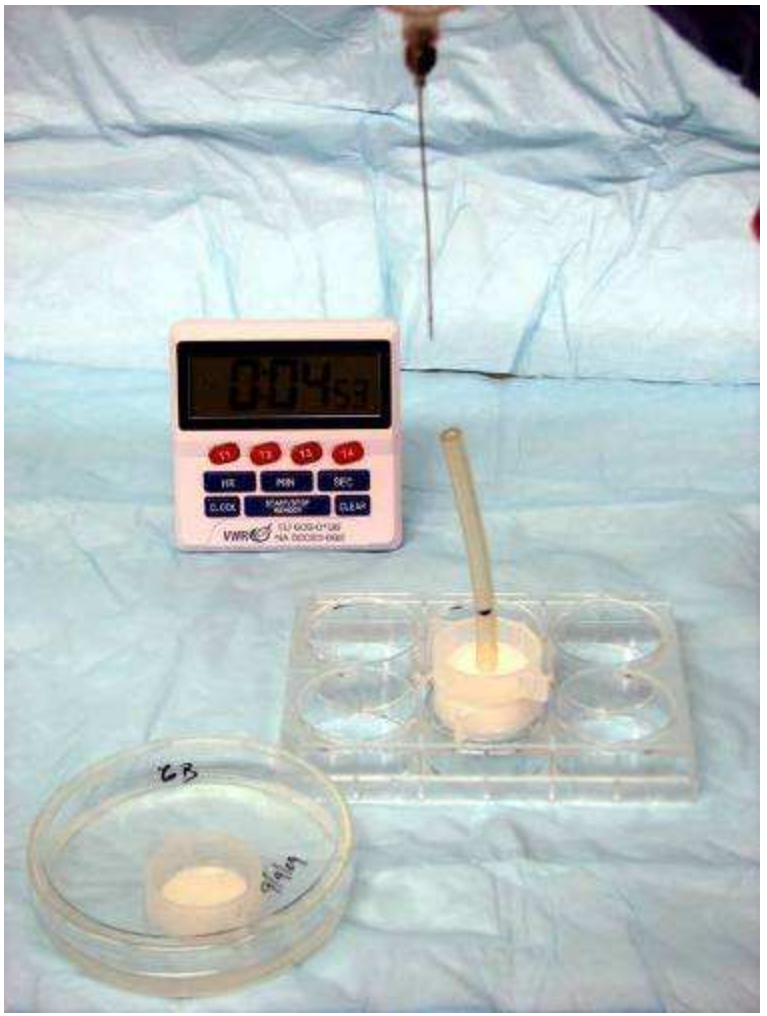
# Flow Conformance Assay

25 mm glass frits are mounted in a filter holder and treated with an inoculum of the isolated strain.

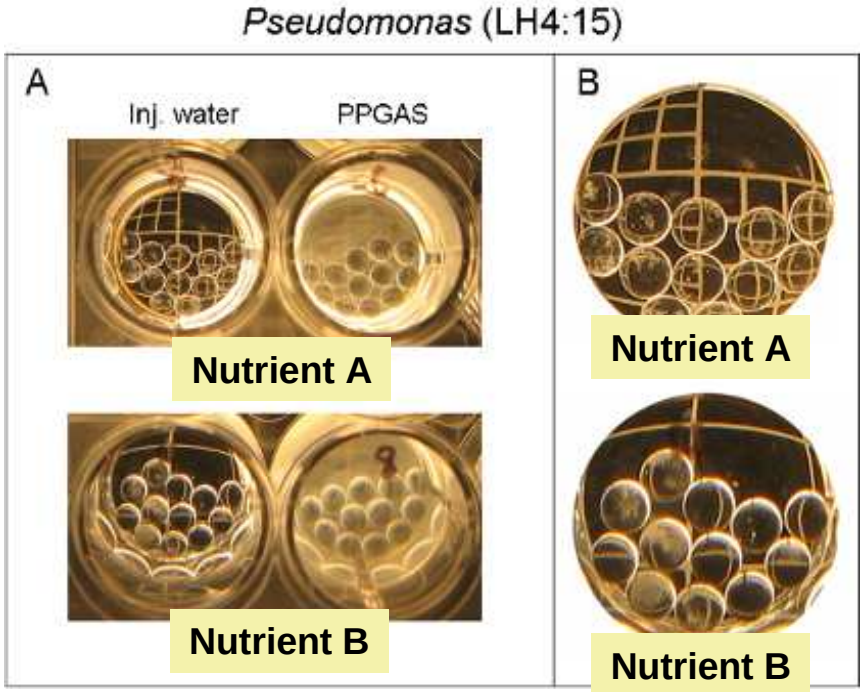
These glass frits are incubated in the medium/inoculum mix for a period of time under the desired conditions (e.g. anaerobically and at temp.)

After incubation the frits are screwed into a flow test assembly.

A fixed volume of water is allowed to pass through the filter test assembly and the flow rate is recorded.

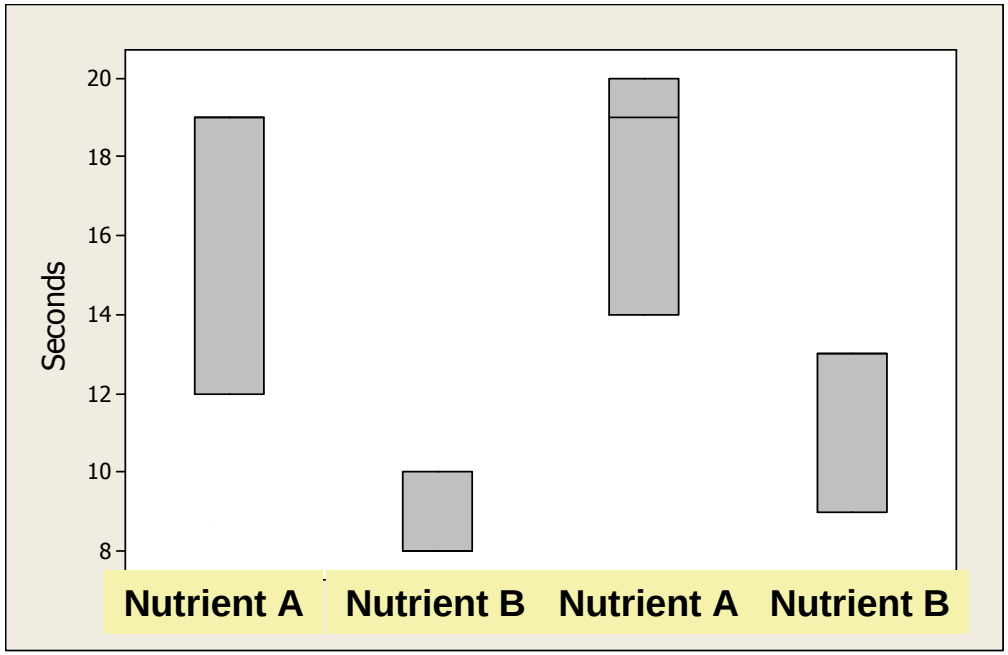


# *Pseudomonas sp.* biofilm formation

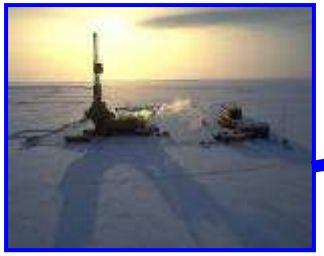



Preferential biofilm formation on nutrient A

Filter plugging assay- Pseudomonas sp.




1:2C/N 4:1C/ N



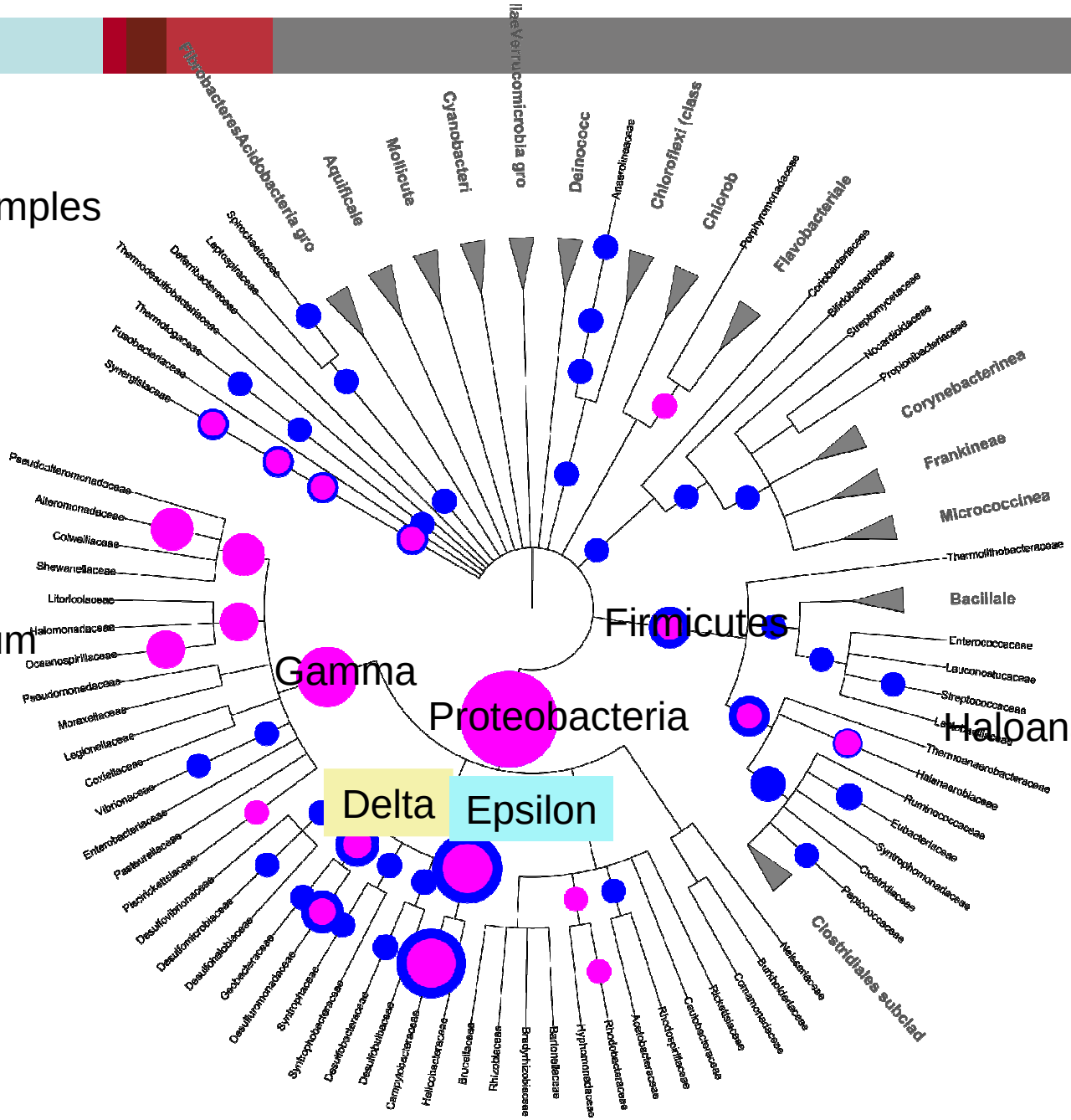
North Slope, Alaska

Saskatchewan, Canada

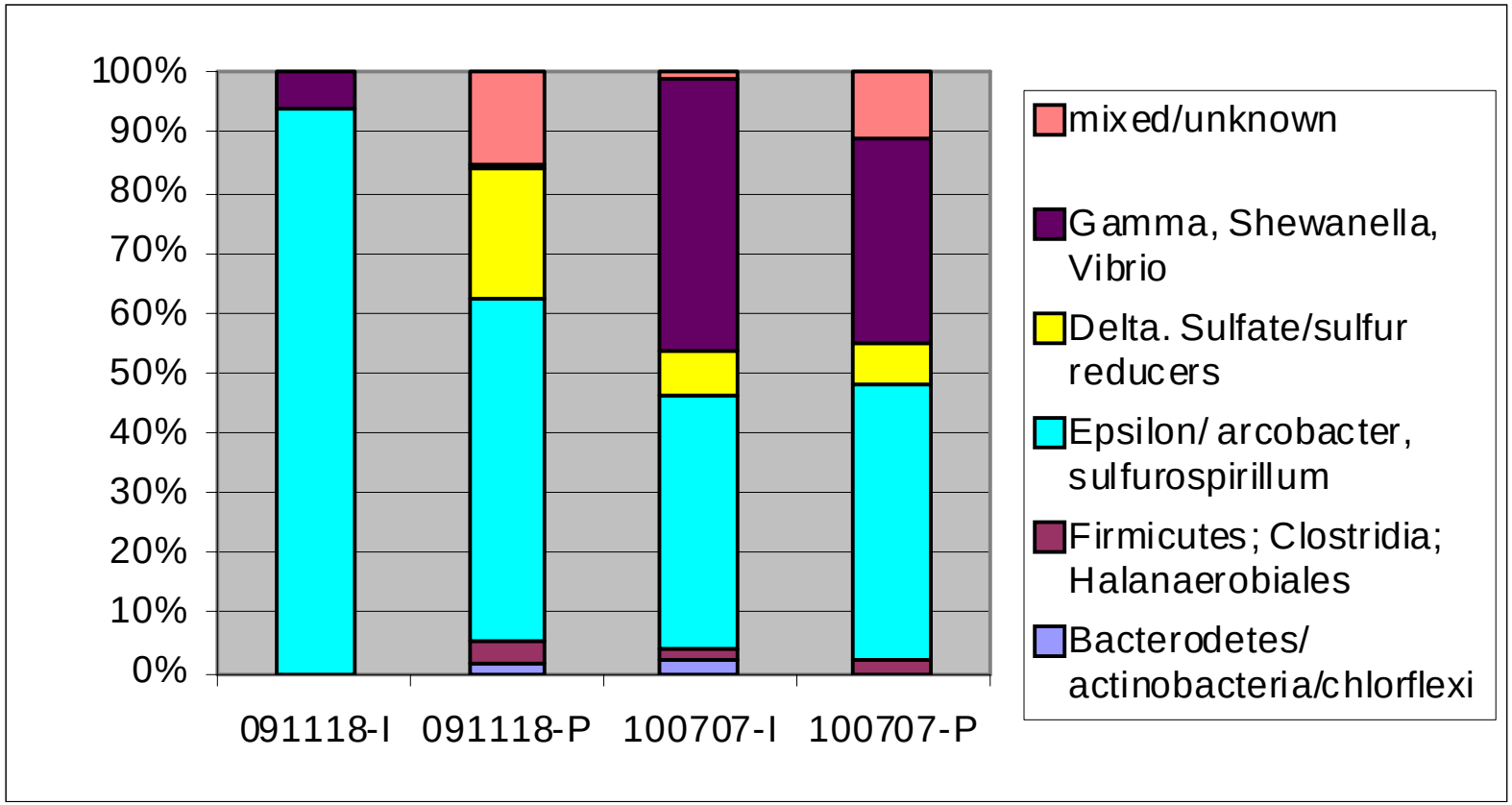
Alberta Canada

East Texas

Alberta
Two well samples



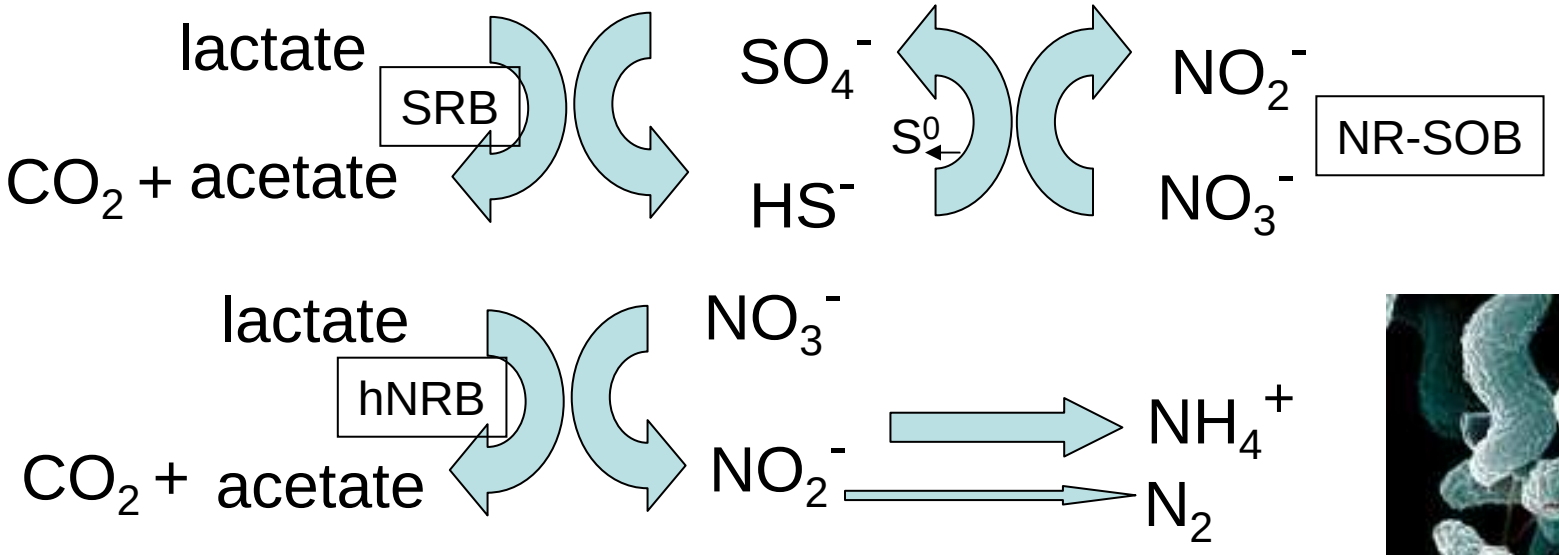
# Alberta water samples- epsilons dominate



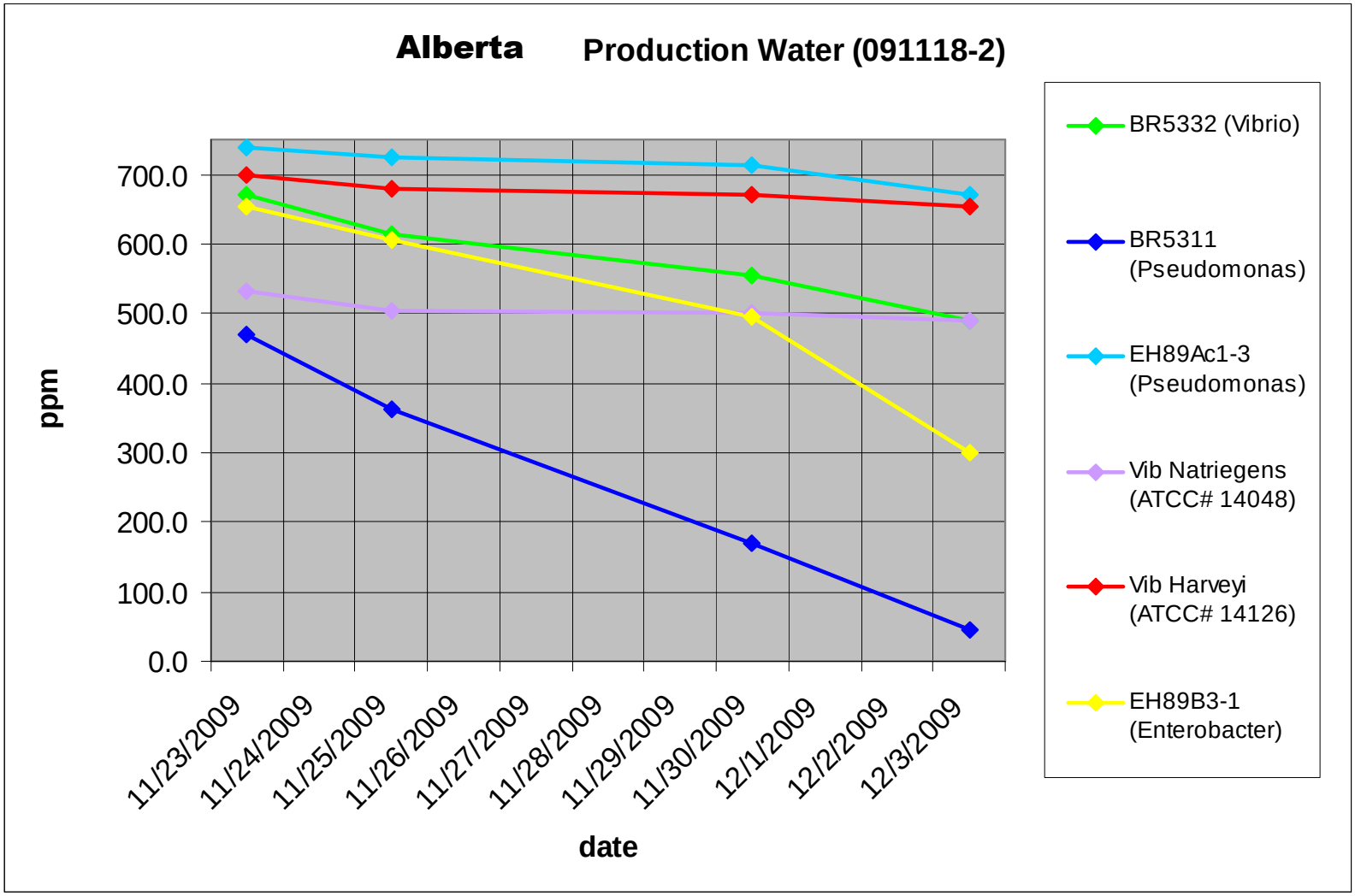

091118-1 = well #1 injection
091118-2 = well #1 production

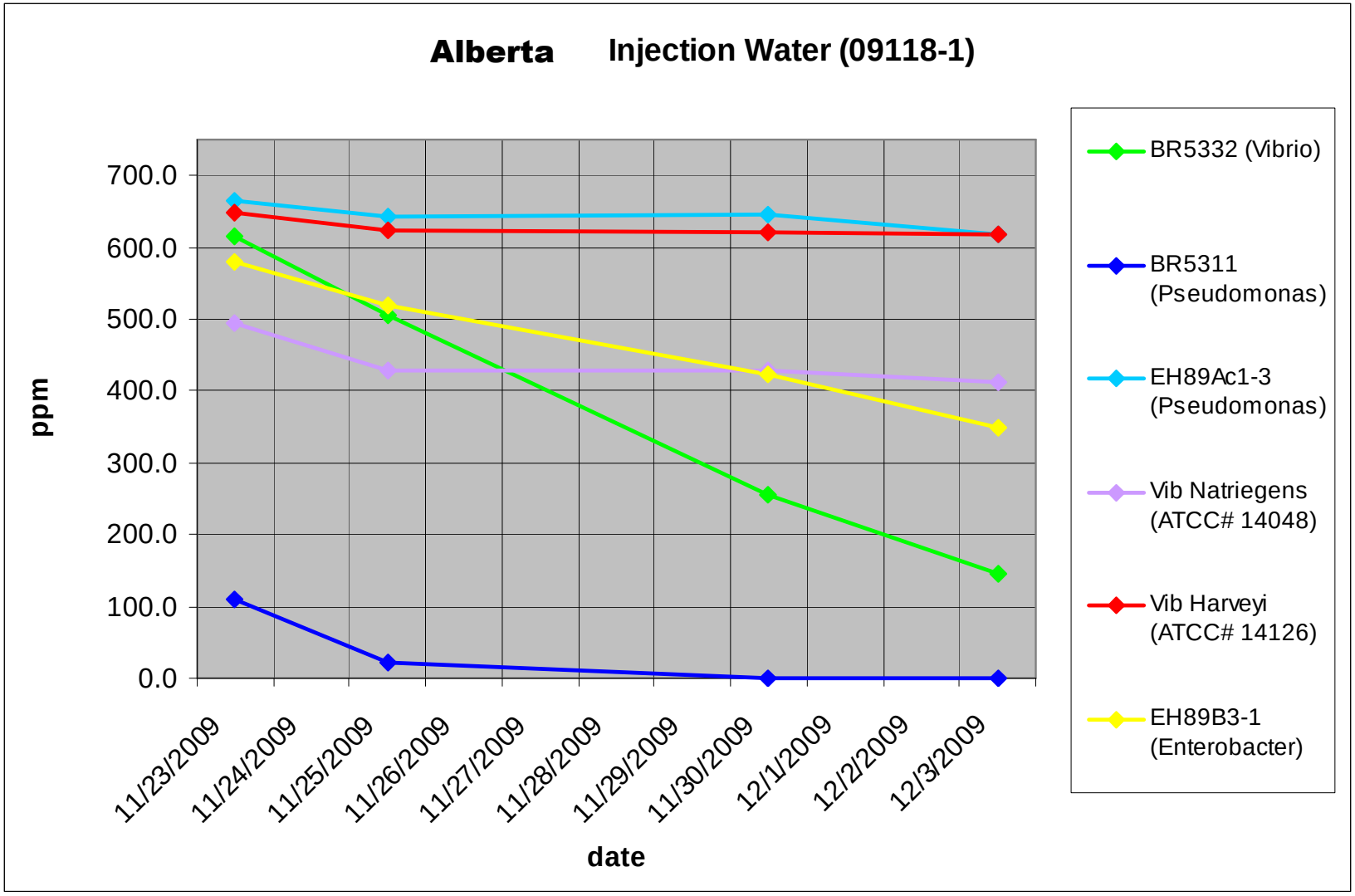
100707-1 = well #2 injection
100707-2 = well # 2 production

# Sulfur cycle by Sulfurospirillum (Epsilon Proteobacter)



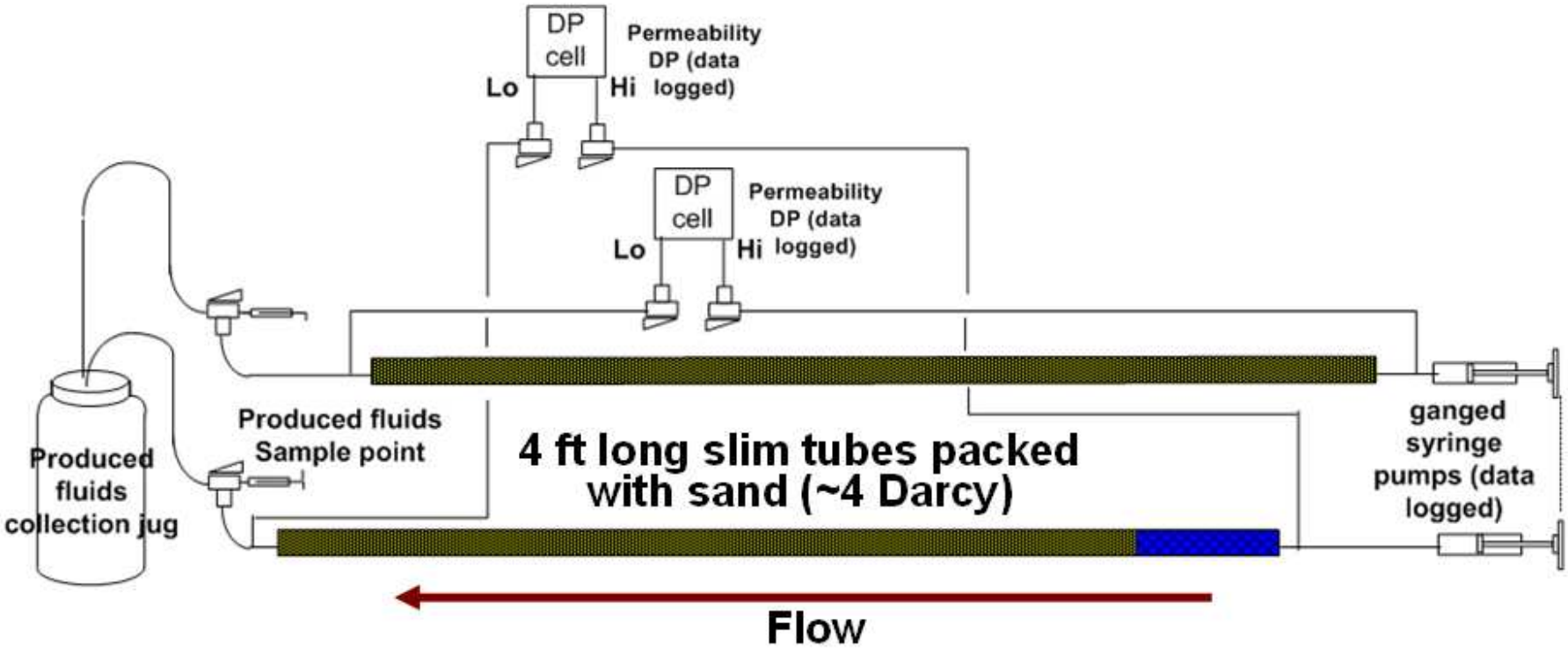

Hubert and Voordouw, 2007,
Applied and Environmental Microbiology


Alberta
Production Water (091118-2)
ppm
700.0
600.0
500.0
400.0
300.0
200.0
100.0
0.0
11/23/2009
11/24/2009
11/25/2009
11/26/2009
11/27/2009
11/28/2009
11/29/2009
11/30/2009
12/1/2009
12/2/2009
12/3/2009
date
BR5332 (Vibrio)
BR5311 (Pseudomonas)
EH89Ac1-3 (Pseudomonas)
Vib Natriegens (ATCC# 14048)
Vib Harveyi (ATCC# 14126)
EH89B3-1 (Enterobacter)

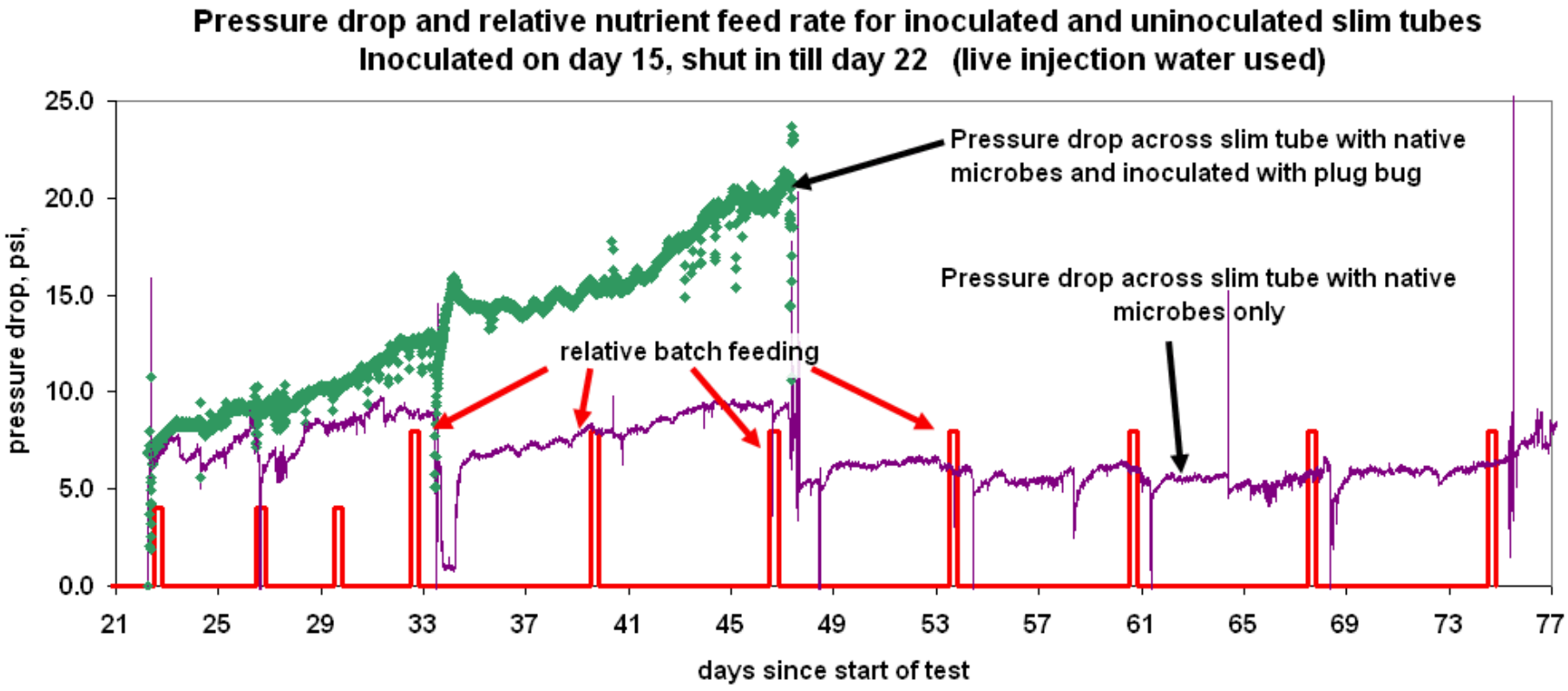

Alberta Injection Water (09118-1)
ppm
700.0
600.0
500.0
400.0
300.0
200.0
100.0
0.0
11/23/2009
11/24/2009
11/25/2009
11/26/2009
11/27/2009
11/28/2009
11/29/2009
11/30/2009
12/1/2009
12/2/2009
12/3/2009
date
BR5332 (Vibrio)
BR5311 (Pseudomonas)
EH89Ac1-3 (Pseudomonas)
Vib Natriegens (ATCC# 14048)
Vib Harveyi (ATCC# 14126)
EH89B3-1 (Enterobacter)

# Permeability modification – slim tube tests (hydraulically constrained)

- Once we have confirmation using the glass frits, we inoculate slim tubes and measure plugging in a continuously flowing system (live injection water)
- → Needed to refine nutrient dosing for well test



# Permeability modification – slim tube tests (hydraulically constrained)




*Pseudomonas sp.*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