Total Microbial Profiling… Because Sometimes Bacteria Just Aren't Enough.

P. Stenroos*, A. L. Douglas, J. S. Goudey, and L.L.R. Marques
HydroQual Laboratories Ltd
***Alberta Ingenuity r&D Associate**

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Outline

- Athabasca oil sands
- Bioremediation
- Methods
- AIF r&D associateship project
- Results
- Conclusions

The Athabasca oil sands

- The process used to extract oil from the oil sands requires large amounts of water and caustics

- The products of this process are bitumen and tailing slurry

- Creation of lakes and fens are viable reclamation options for mine wastes.

- One of the major concerns is the release of naphthenic acids and other potentially toxic substances from the tailings over time.

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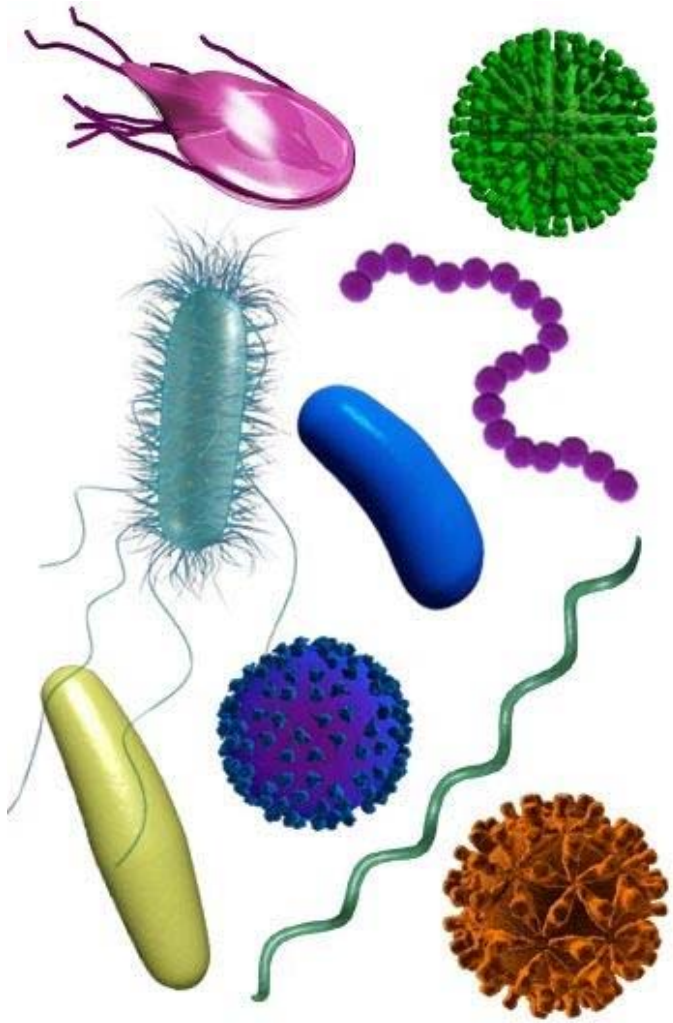
Microbial Bioremediation

- Natural indigenous microbes have the ability to remediate pollutants
- Cooperative effect between the total microbial community
- Bacteria, fungi, archaea might be equally important in the process



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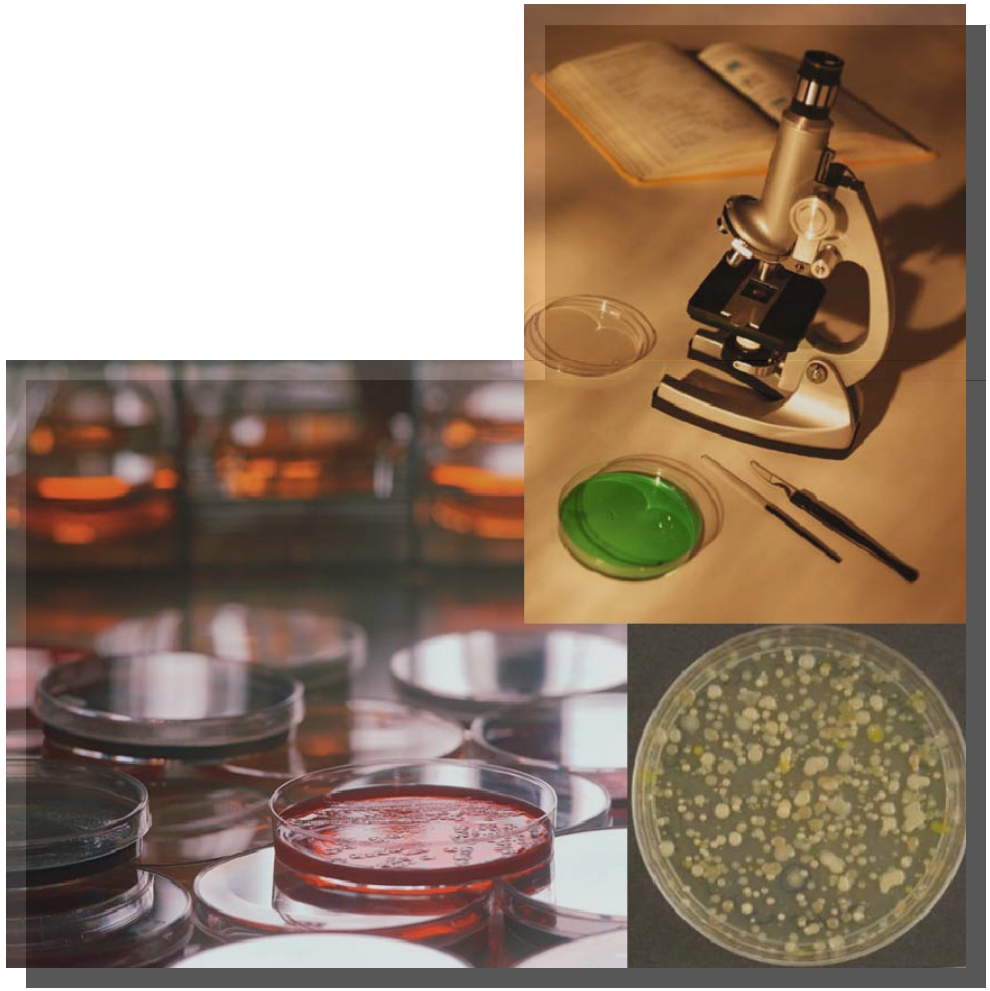
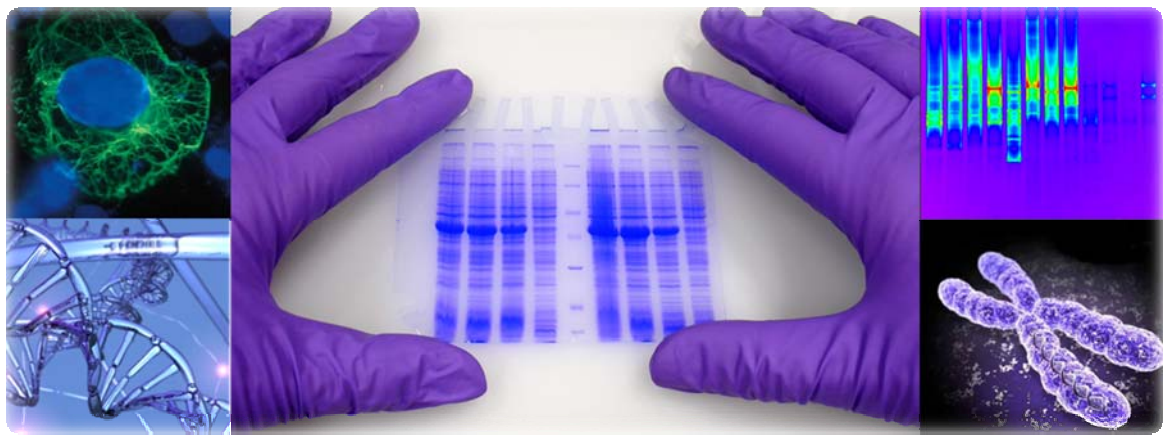
Conventional techniques

- Culturing techniques e.g plating, MPN
  - Inherent biases
  - Slow process
  - <1% can grow in lab conditions



Molecular techniques

- Rapid
- More complete
- No cultivation required
- Only small sample sizes required
- We can analyze bacteria, archaea, algae, fungi
- Complement to other analyses



DNA Fingerprinting

- Identify individuals within a population
  - CSI, genetic testing, genealogy
- Create a fingerprint of the population
  - Monitor population changes

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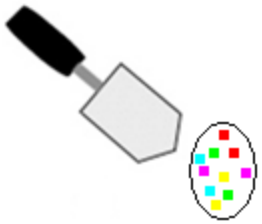
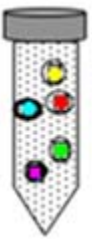
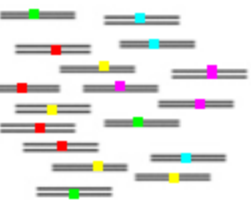
How Do You Fingerprint DNA?



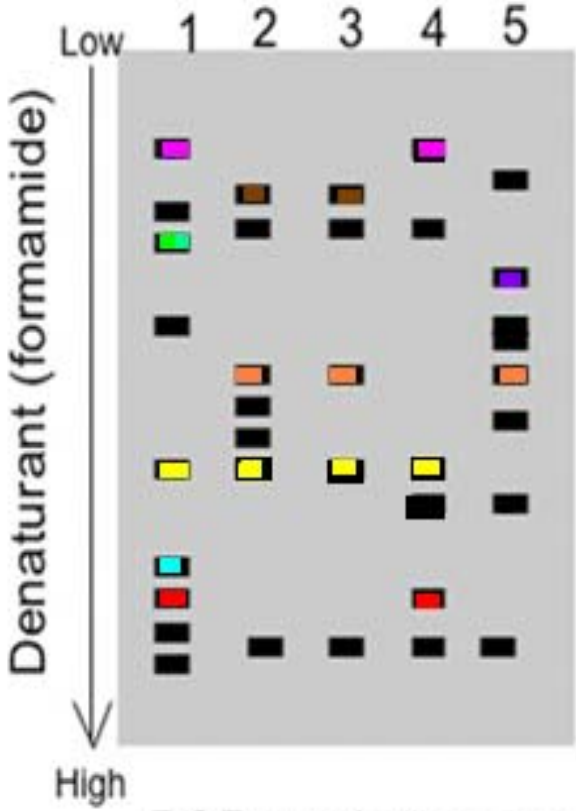
Collect sample



Extract nucleic acids from sample
(DNA, RNA or both)



Amplify target gene
(e.g., 16S rRNA gene)
using PCR



Separate PCR products using a denaturing gradient

PCR product separation by composition and not size

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Limitations of the molecular techniques

- Can’t detect metabolic activities
- It can’t exclude dead bacteria

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Total microbial profiling of tailing ponds

- 7 different ecosystems (tailing ponds)
  - Comparing the total microbial profile over time using DNA profiling techniques
- Total microbial community profile includes not only bacteria but also archaea, fungi, cyanobacteria and algae
- Because Sometimes Bacteria Just Aren't Enough.

Field work

Water and sediment sampling from an experimental tailings pond

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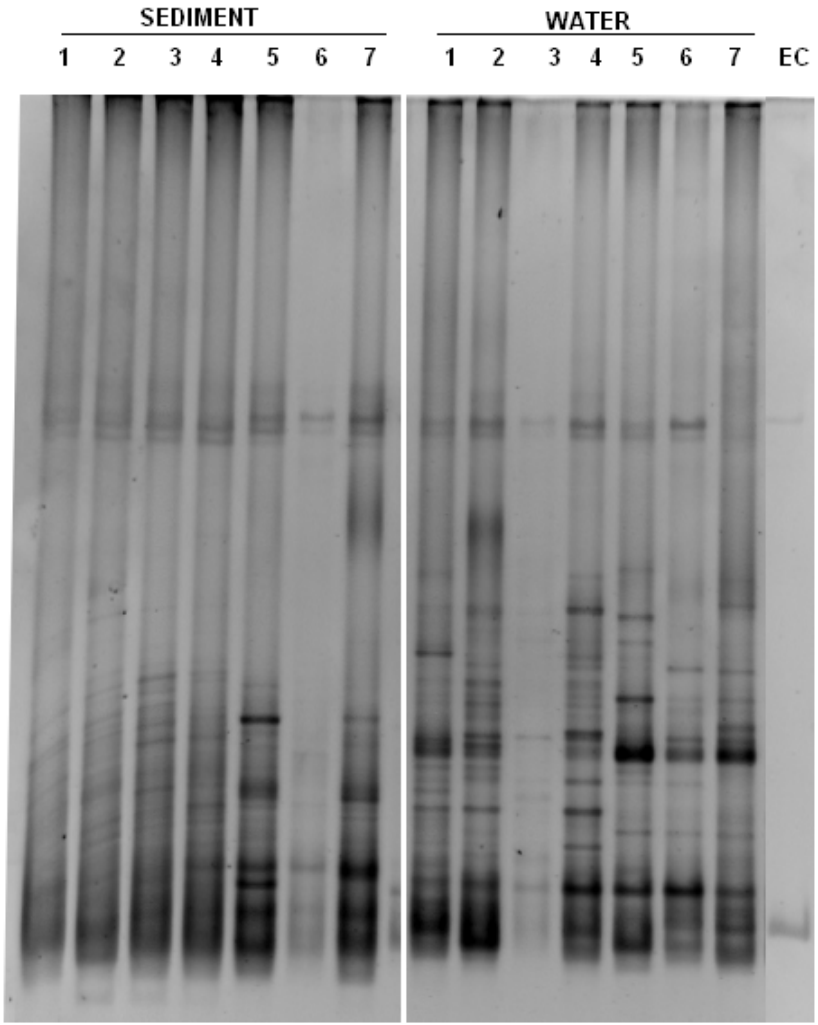
Bacterial profile



- The number, precise position and intensity of the bands gives an estimate of the number and relative abundance of dominant species in the samples

- EC: *E.Coli* positive control
- 1-7: Number of tailing pond

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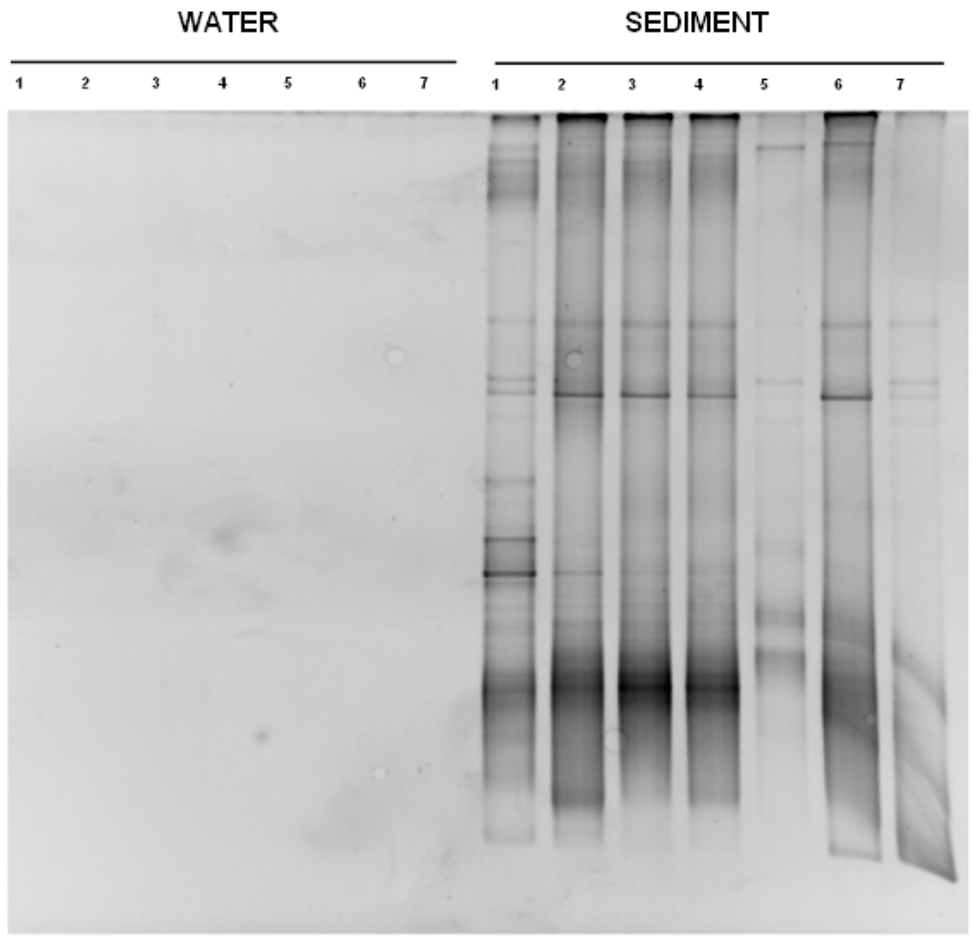
Archaeal profile

- Archaea is also know as extremophiles

- Live in some of the most extreme environments on the planet

- Involved in biodegradation of hydrocarbons



OCTOBER 2008
ARCHAEA

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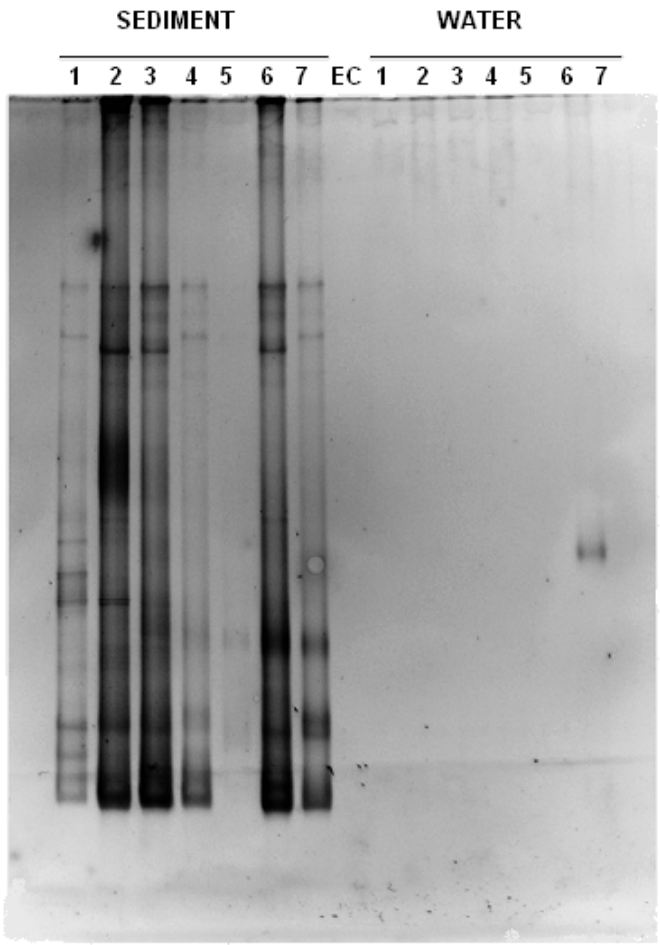
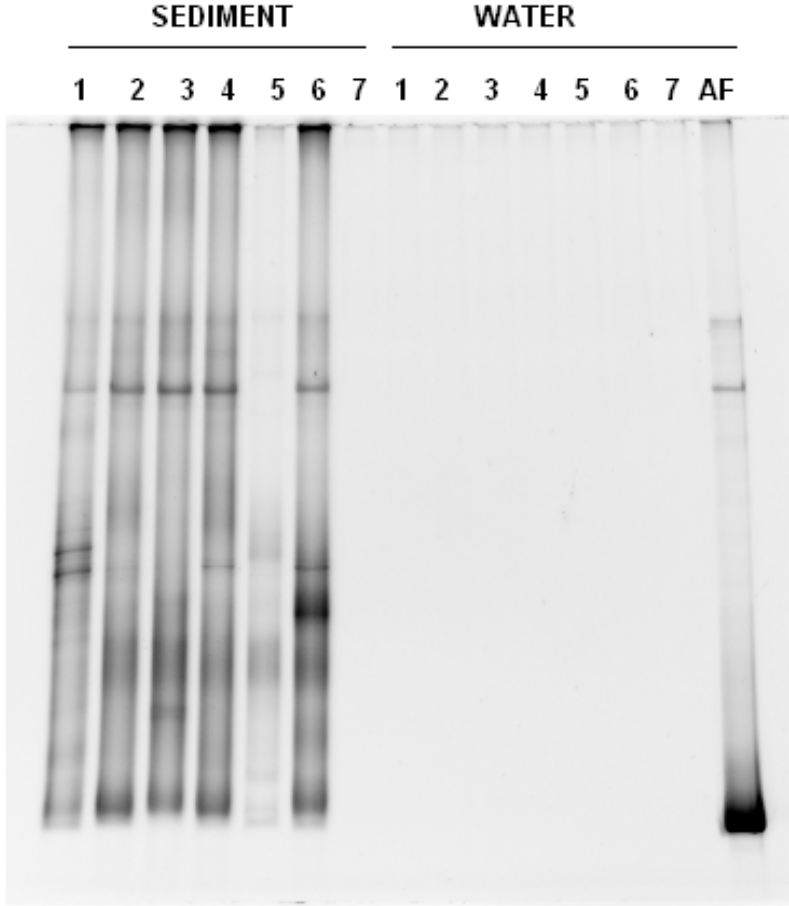
Archaeal profile



May 2009
Archaea



August 2009
Archaea

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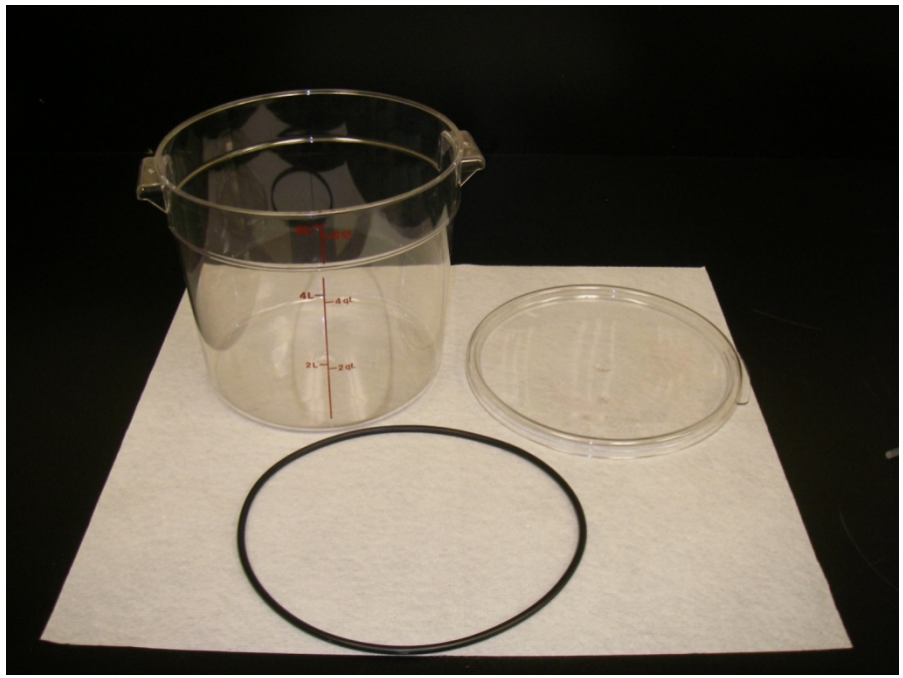
Results

- Seasonal variations in the bacterial and archaeal populations
- No detection of archaea in pond water
- We are in the process of analysing the fungal and cyanbacterial populations in all of our samples collected

Microcosms

Characterization of the community growing in the lab in microcosms

- Duplicates
  - One anaerobic and one aerobic
  - Same pond, same water & sediment
- Stored in dark at 18-22 ˚C



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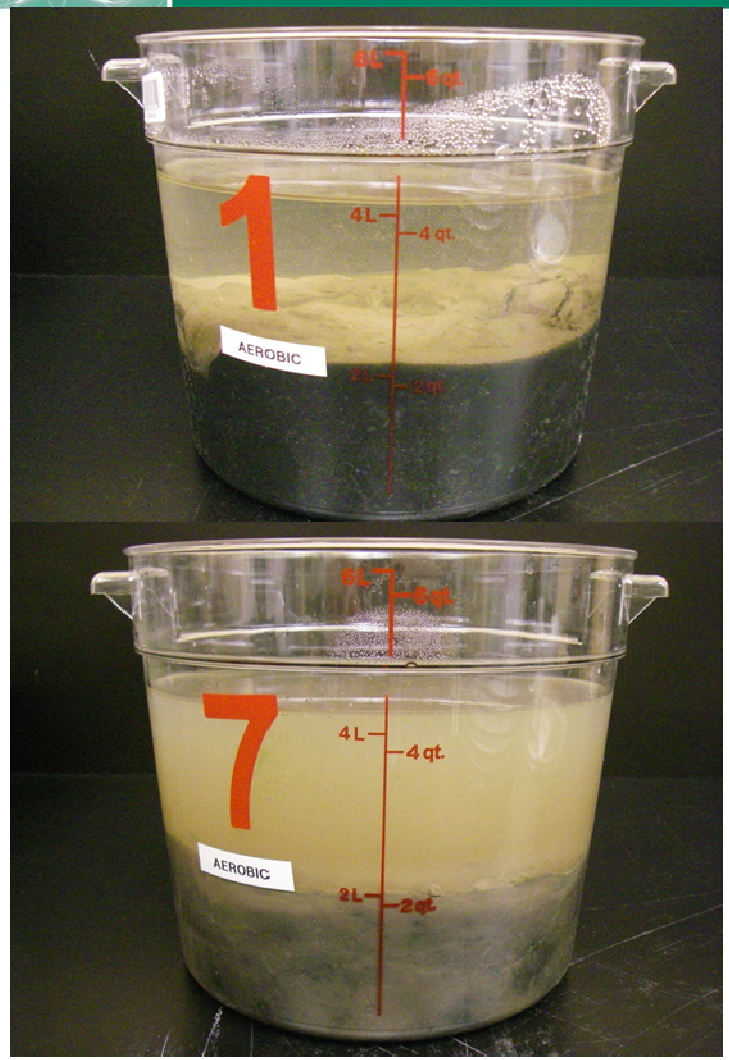
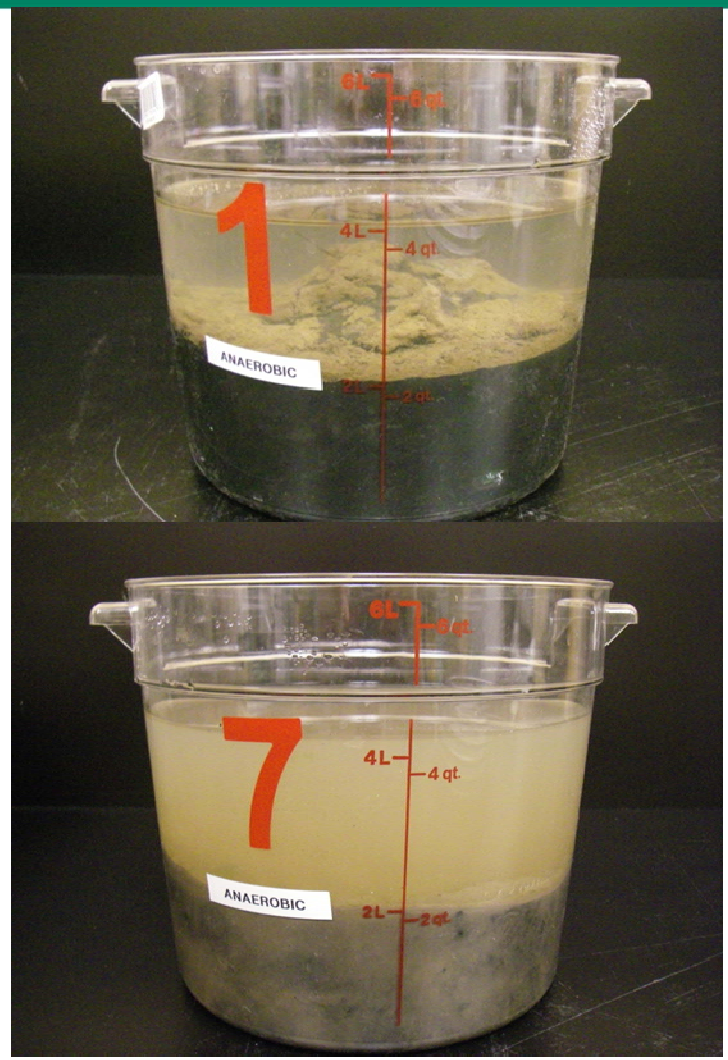
Microcosms Day 14





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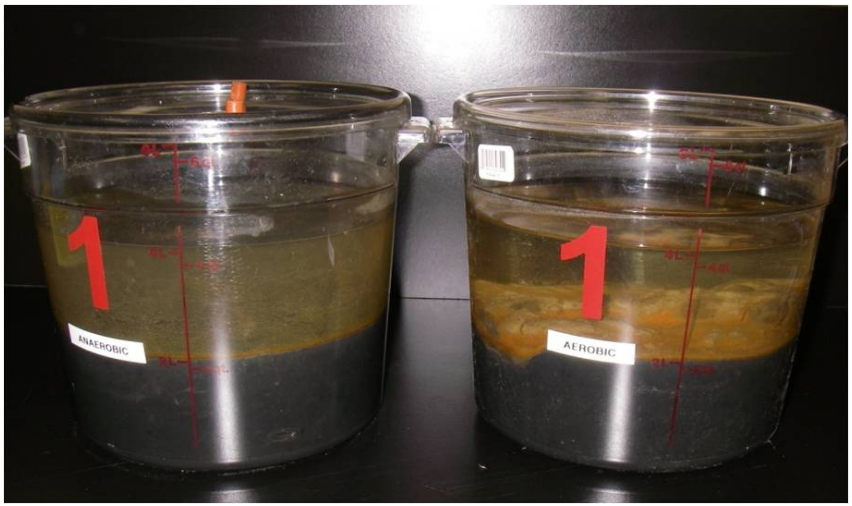
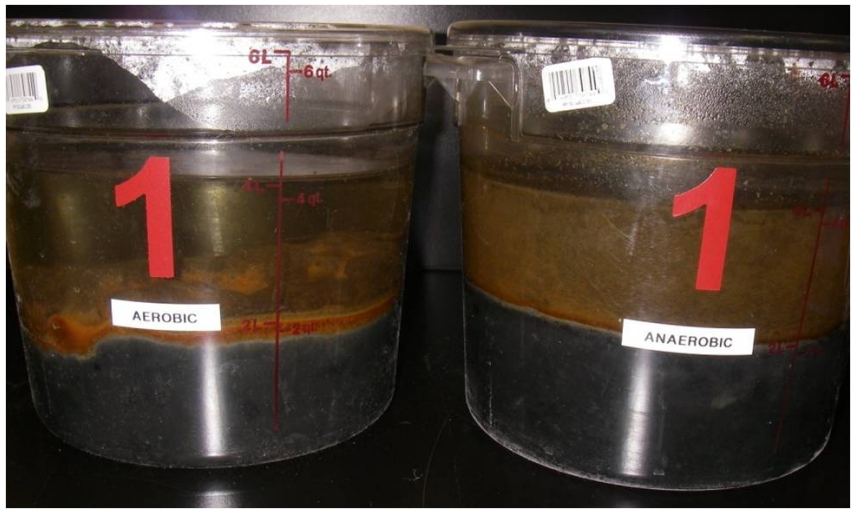
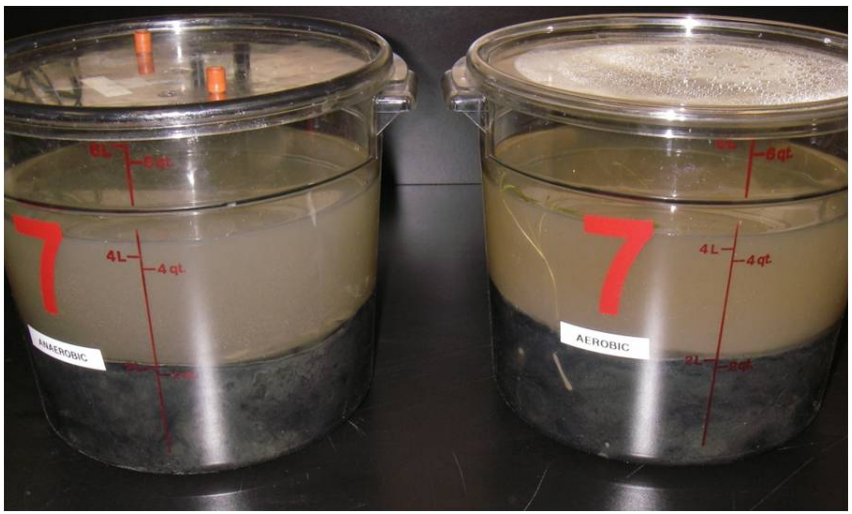
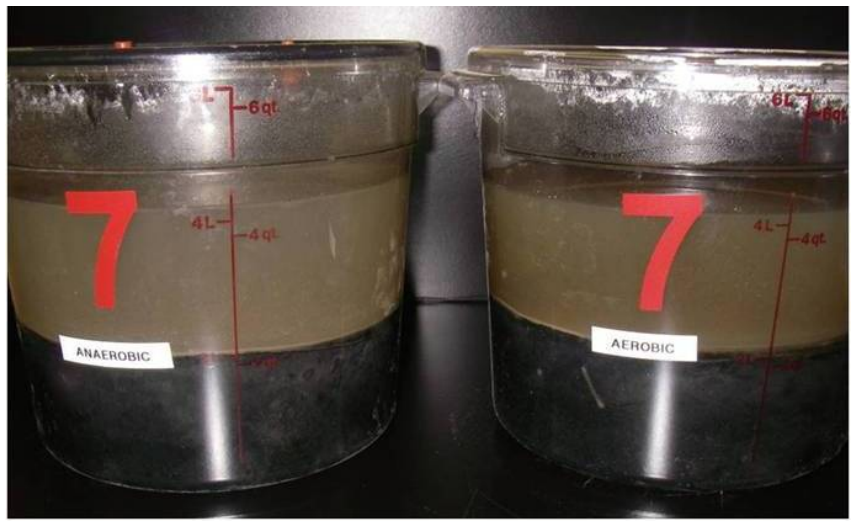
2 months later 7 months later









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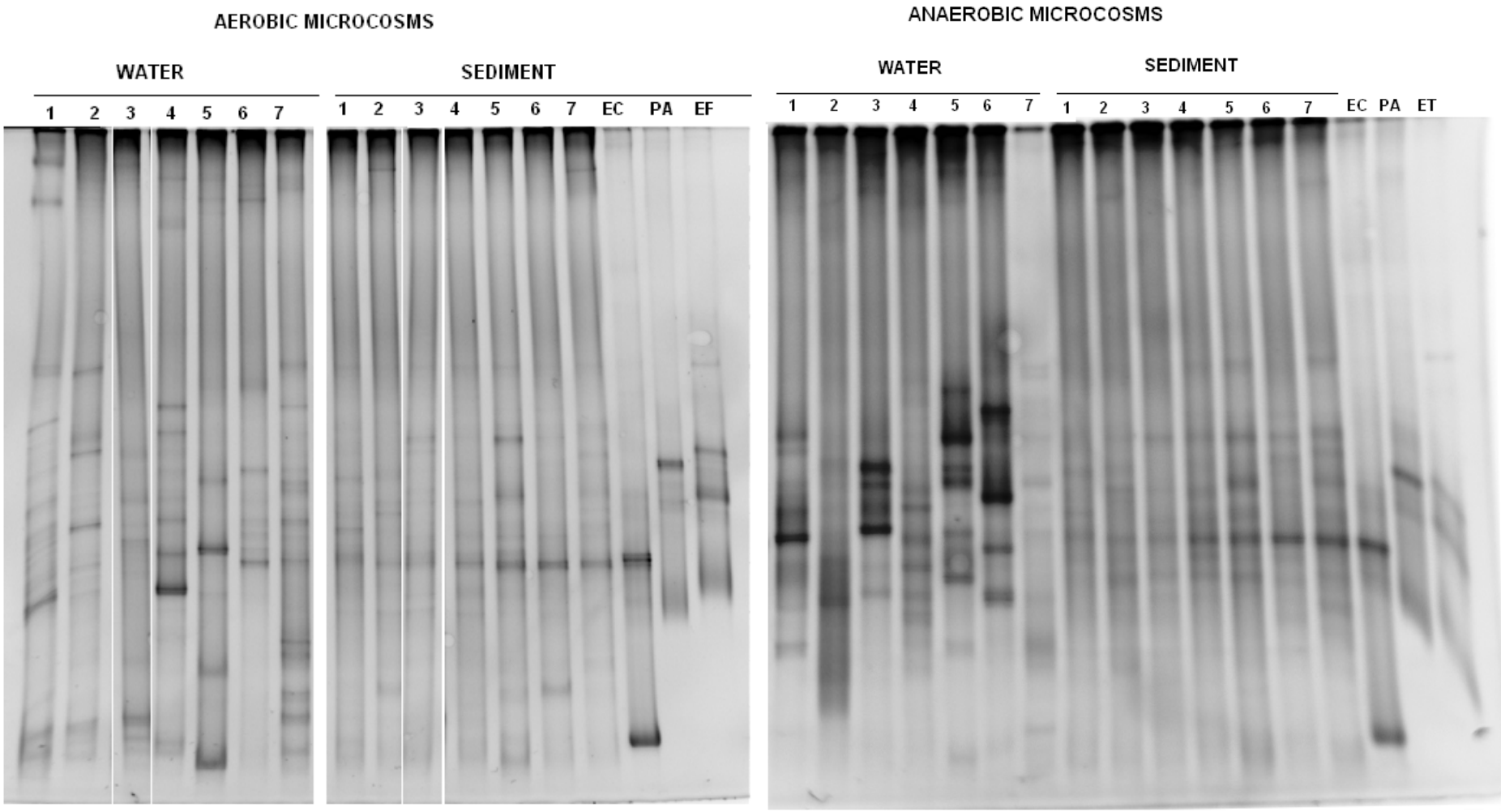
Bacterial profile of Aerobic vs Anaerobic microcosms



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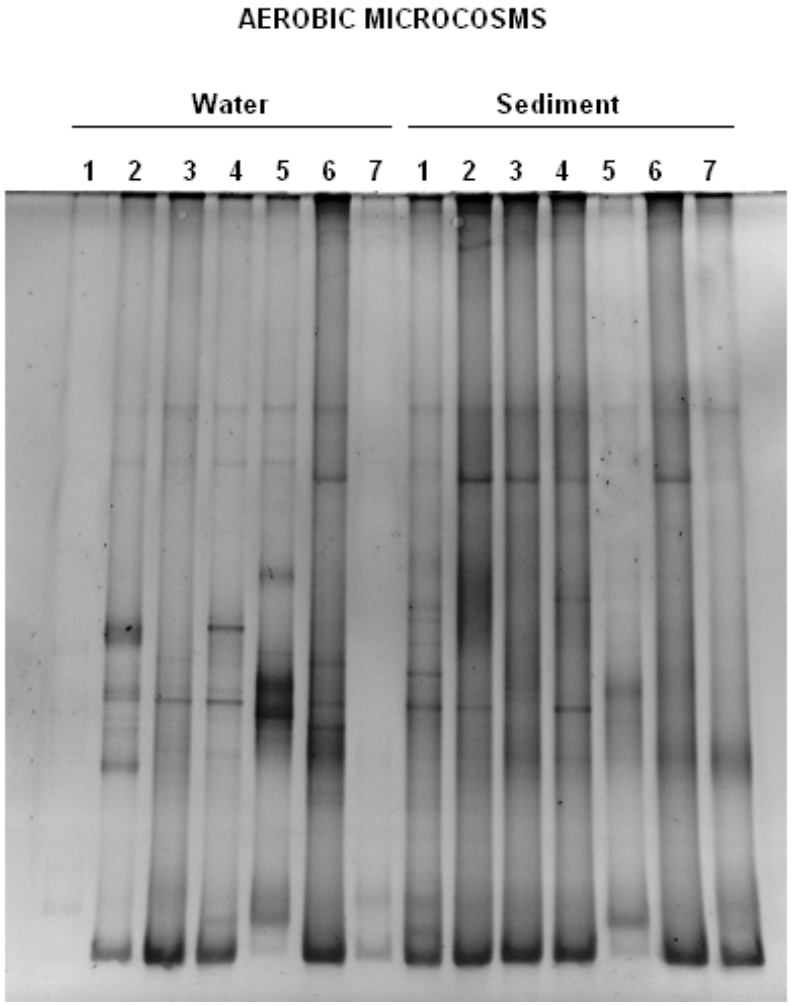
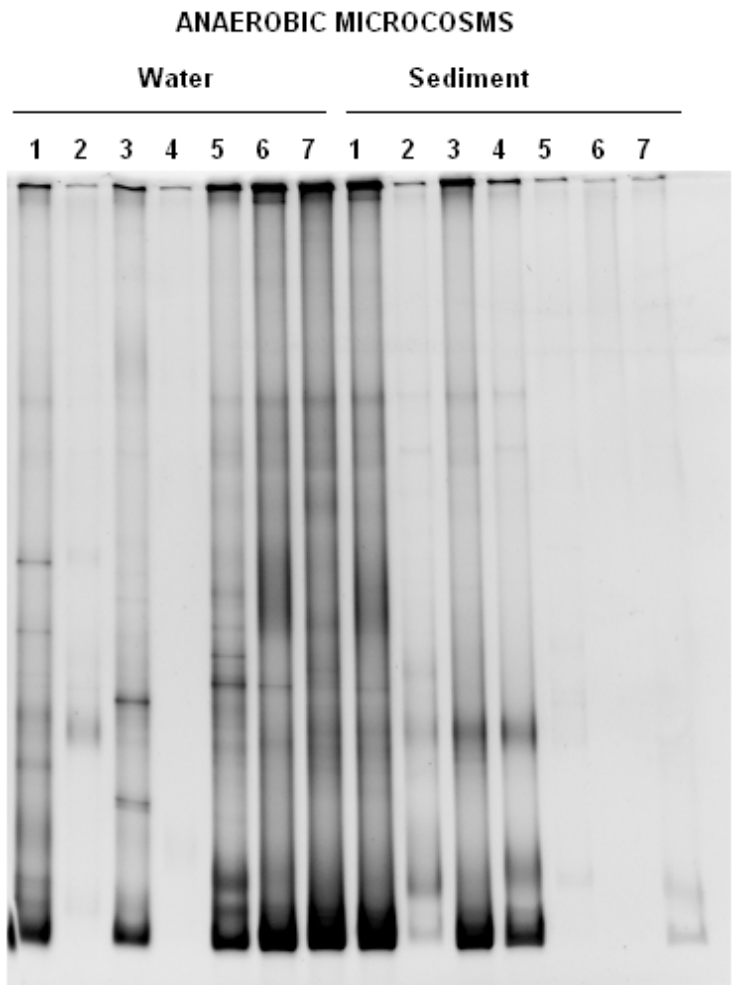
Archaeal profile for microcosms





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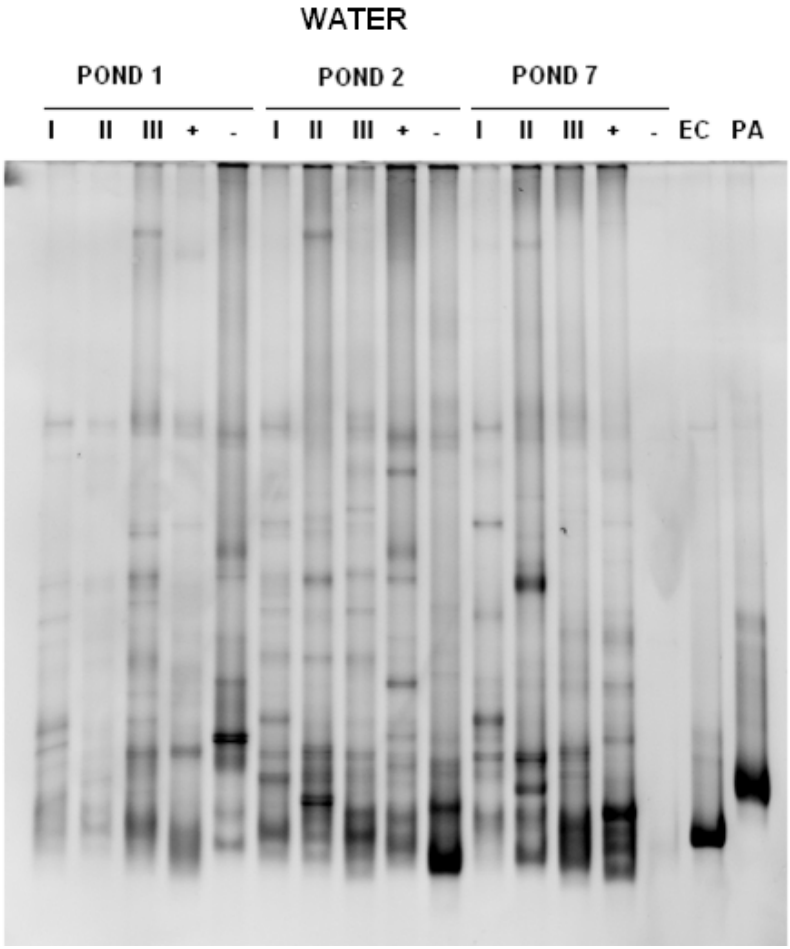
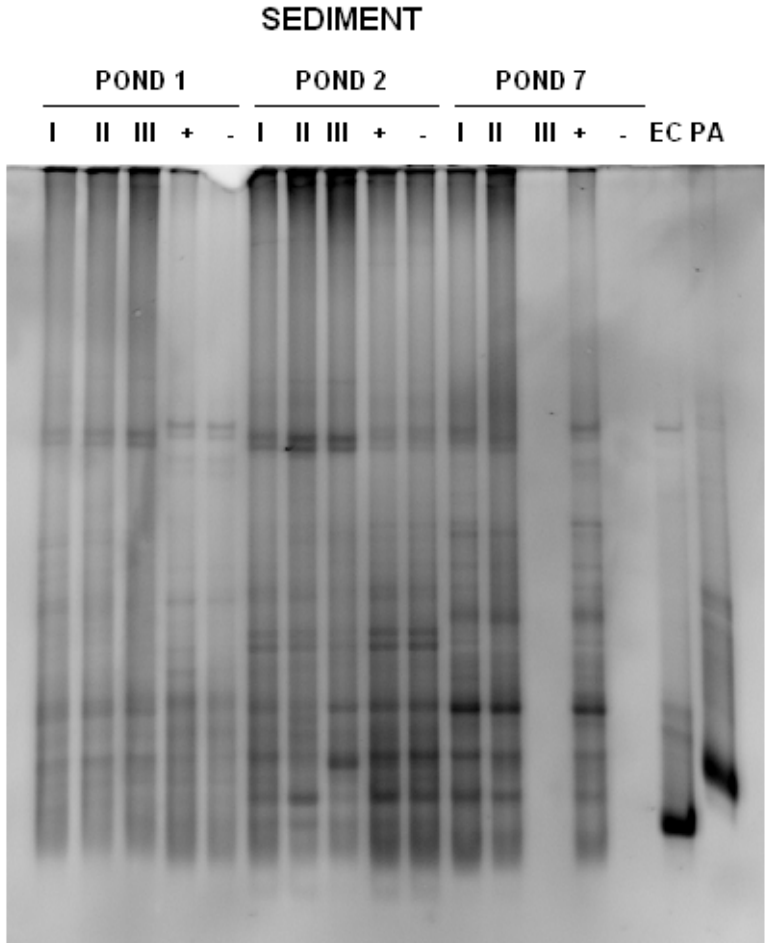
Bacterial profile comparison





I- October field sample, II-May , III-August , + Aerobic Microcosm, - Anaerobic Microcosm, EC & PA positive control

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Conclusions

- Bacterial communities found in the microcosms have not changed from the source material
  - Opportunities for remediation testing
- No archaea detected in test pond water, archaea detected in microcosm water
  - Is it the depth difference?
- Future directions include analysis of fungi and cyanobacteria

Acknowledgement

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Questions?

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Contact information
Pernilla Stenroos
(403) 253-7121
PStenroos@golder.com

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