



The Use of DNA Technologies in Determining the Biotreatability of Chlorinated Aliphatic Hydrocarbons

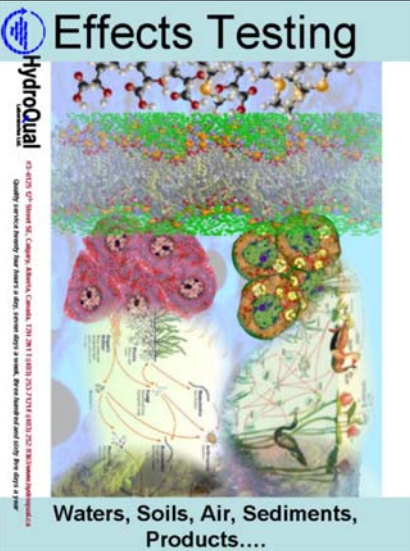
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R&D Coordinator

HydroQual Laboratories Ltd.



HydroQual Laboratories Ltd



- Wholly-owned subsidiary of Golder Associates Ltd.
- HydroQual uses biological testing for measuring the effect of toxicants and their impact on various links in the food chain
- Aims at evaluating and/or improving environmental health
- Expertise in various fields of the natural sciences (microbiology, biochemistry, toxicology, ecology, botany and zoology).
- ISO 17025:2005 (CAEAL), EPA, AIHA

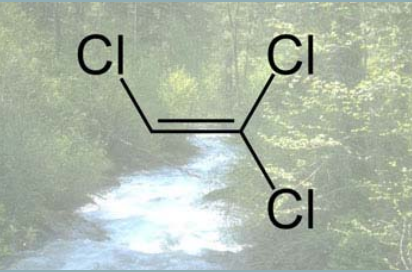


Biotreatability Studies

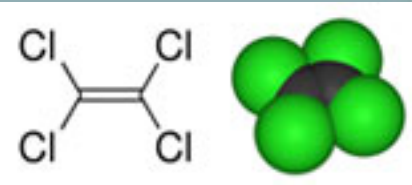


- Bench scale testing
 - provide key information with small amount of material (lower cost, etc)
- Various types of contaminants
 - Hydrocarbons, VOCs, and other organics
 - Metals
- Test multiple treatments, determine dosage, additional amendments
- Strategy may vary based on
 - Bench test results
 - Legislation

TCE and PCE



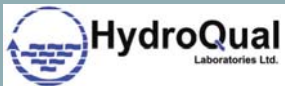
- Chlorinated Aliphatic Hydrocarbons
- Were commonly used in industrial processes including dry cleaning
- Heavier than water - DNAPL
- Recalcitrant for remediation



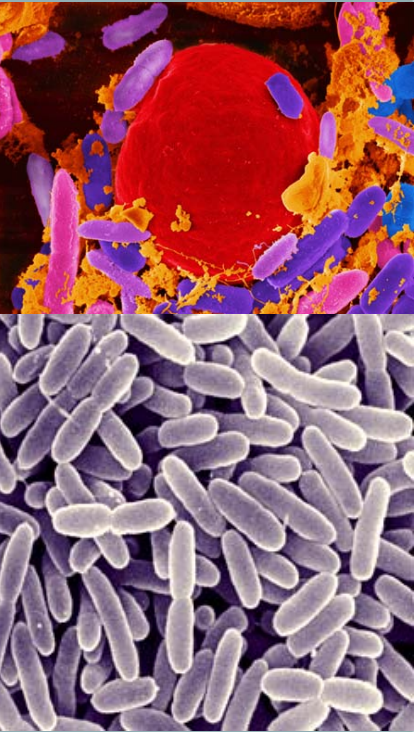
TCE/PCE Remediation Strategies



- Chemical oxidation - permanganate
- Aerobic bioremediation - adding oxygen-producing chemicals
- Abiotic remediation - nZVI, ferrous iron
- Anaerobic biostimulation and augmentation - reducing ORP and increasing hydrogen, methane
 - all methods benefit from greater quantities of contaminant in the aqueous phase.
 - use of surfactants, dispersants, chelators, emulsifiers.



Biostimulation

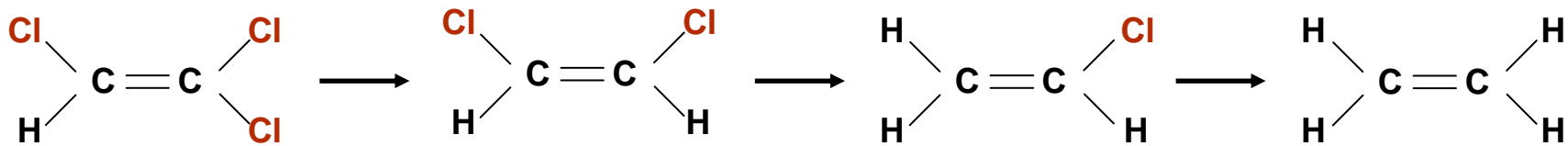


- Addition of nutrients that will reduce redox potential and oxygen.
- Bacteria involved require hydrogen, methane, acetate, or formate as electron donors
 - can be provided directly or indirectly
 - carbon compounds that are food for methanogens that produce the above as waste products.
- Bioaugmentation = adding bacteria
 - Illegal in Canada

Anaerobic dehalorespiration

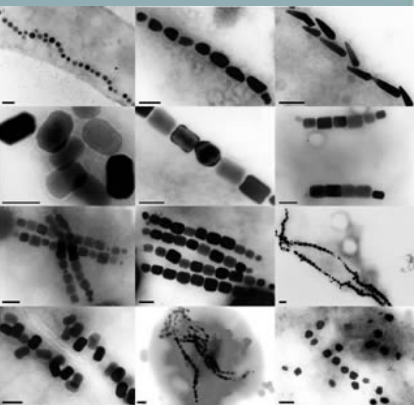
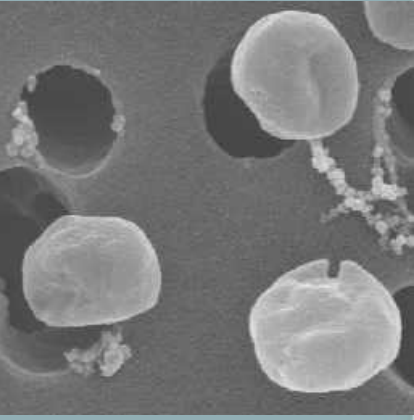
- Pathway for degradation of TCE to ethylene.

TCE → cis-Dichloroethylene → Vinyl Chloride → Ethylene



- Often remediation stalls at cis-DCE or VC.

Bacteria

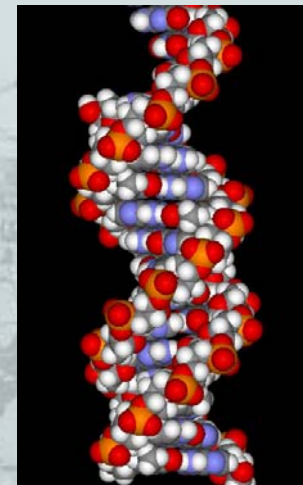
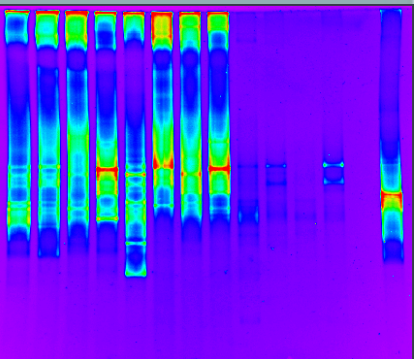
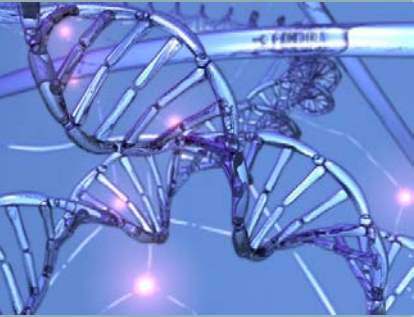


- *Dehalobacter restrictus*
- *Desulfitobacterium metallireducens*
- *Dehalospirillum* spp.
- *Desulfuromonas chloroethenica*
- *Desulfobacterium* spp.
- *Clostridium bifermentans*
- *Dehalococcoides ethanogenes* (Dhc)

- Most stop at DCE, only Dhc can degrade TCE to ethylene.

Detection Methods

- Culture in the lab
 - Slow – 3 weeks or more
 - Many are unculturable
- Analytical detection of daughter products
 - Indirect
- DNA analysis
 - Fast
 - Complete
 - More sensitive
 - Direct

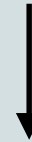


All living things have DNA

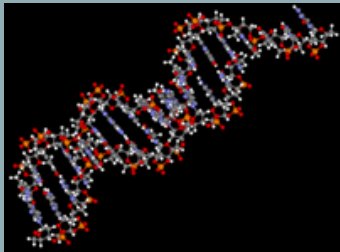
- DNA encodes for all cellular processes
 - Proteins and enzymes
 - Cellular machinery
 - Cellular structure



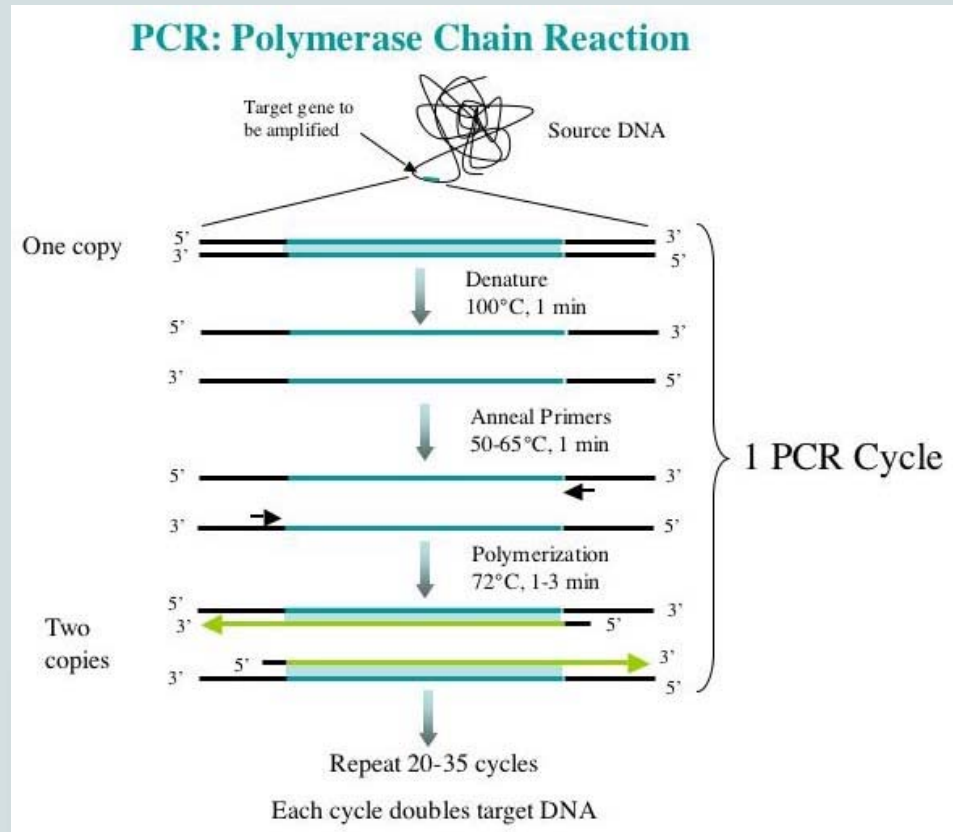
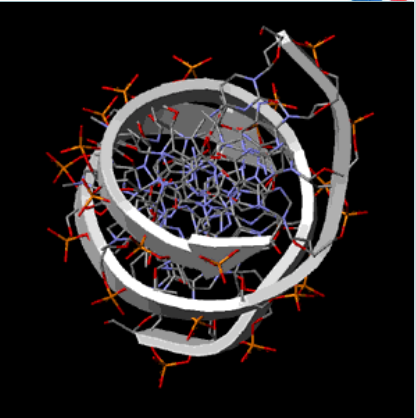
DNA isolation



- Break open the bacteria
- Specifically isolate DNA from everything else

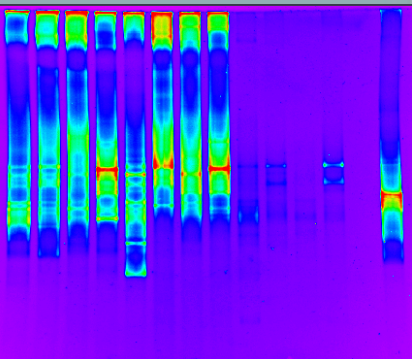
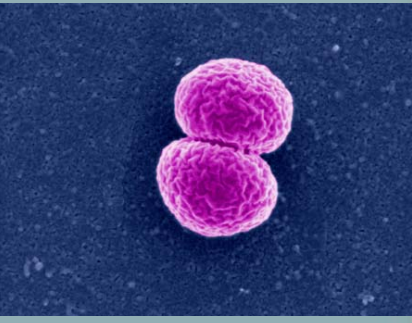


- What's needed
 - Thermophilic enzyme = Taq
 - DNA bases – AGCT
 - Primers – DNA targets



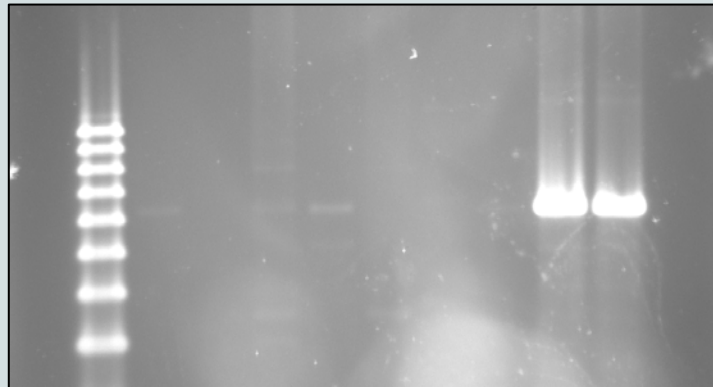
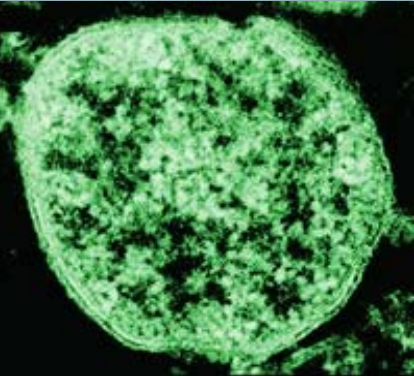
Detection Methods

- PCR - standard or quantitative
 - Can detect Dhc specifically
 - Detect other known bacteria
- DNA profiling and sequencing
 - Bacterial diversity and monitoring
 - Sequence members of the population for ID



Biotreatability Case Studies

- Strictly anaerobic
- Sites had been pretreated with nZVI
- Groundwater and soil provided
- Dhc was present in initial samples



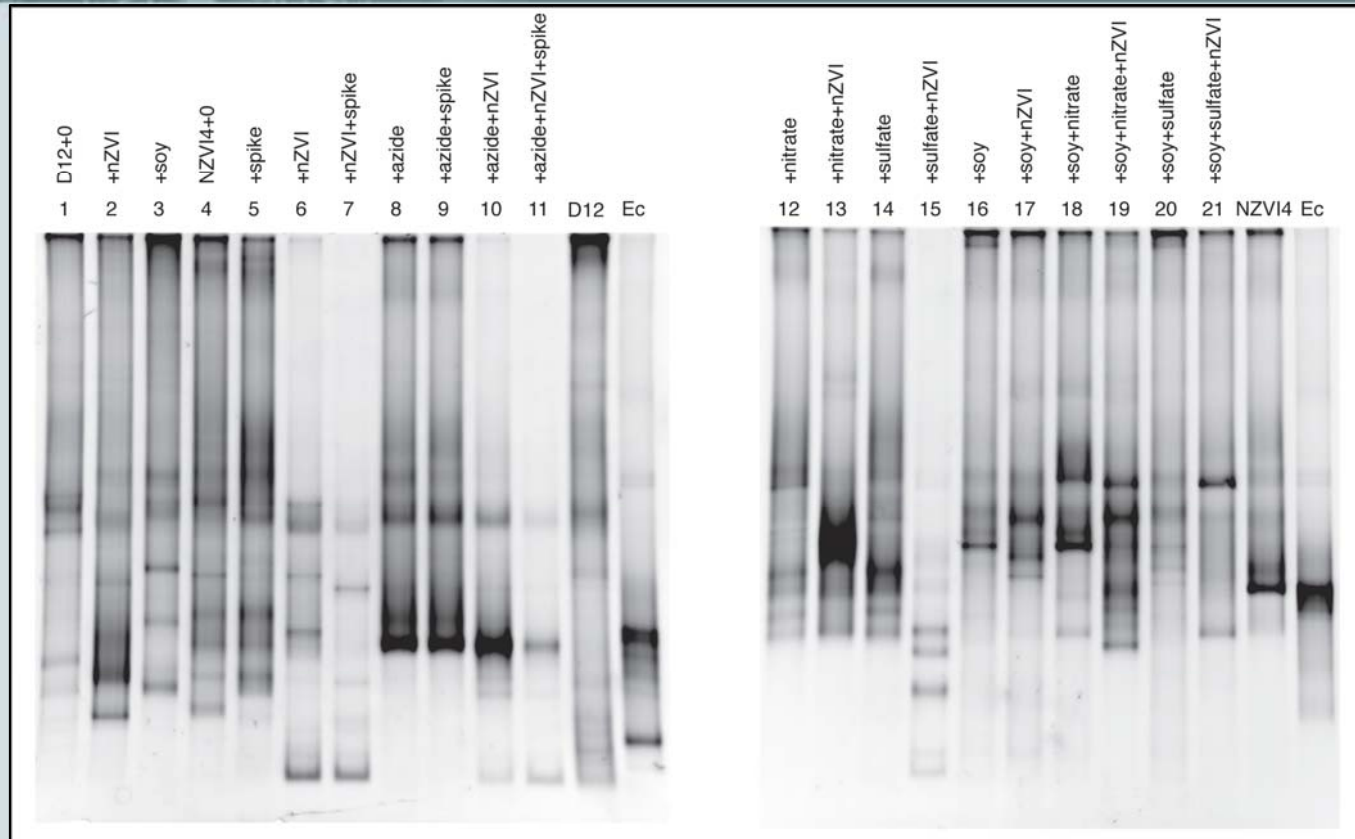
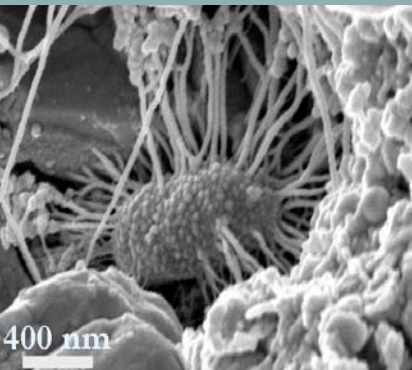
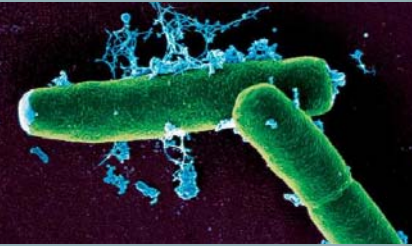
Case 1

Test Design Matrix for Anaerobic Benzene Bio-degradation								
Reactor		Chlorinated Hydrocarbon	Nano-scale Zero-Valent Iron	Sodium Azide	Nitrate	Sulfate	Soy Protein	Treatment/Target Populations
1	D12	-	-	-	-	-	-	D12 control
2	D12	-	+	-	-	-	-	control + nZVI
3	D12	-	-	-	-	-	+	control + soy powder
4	NZVI4	-	-	-	-	-	-	NZVI4 control
5	NZVI4	+	-	-	-	-	-	control + spike
6	NZVI4	-	+	-	-	-	-	nZVI
7	NZVI4	+	+	-	-	-	-	nZVI + spike
8	NZVI4	-	-	+	-	-	-	abiotic control
9	NZVI4	+	-	+	-	-	-	abiotic control + spike
10	NZVI4	-	+	+	-	-	-	abiotic nZVI control
11	NZVI4	+	+	+	-	-	-	abiotic nZVI control + spike
12	NZVI4	-	-	-	+	-	-	<i>Geobacter</i> + <i>Dechloromonas</i>
13	NZVI4	-	+	-	+	-	-	<i>Geobacter</i> + <i>Dechloromonas</i> + nZVI
14	NZVI4	-	-	-	-	+	-	<i>Geobacter</i> + <i>Desulfobacterium</i>
15	NZVI4	-	+	-	-	+	-	<i>Geobacter</i> + <i>Desulfobacterium</i> + nZVI
16	NZVI4	-	-	-	-	-	+	soy protein
17	NZVI4	-	+	-	-	-	+	soy protein + nZVI
18	NZVI4	-	-	-	+	-	+	soy protein + nitrate
19	NZVI4	-	+	-	+	-	+	soy protein + nitrate + nZVI
20	NZVI4	-	-	-	-	+	+	soy protein + sulfate
21	NZVI4	-	+	-	-	+	+	soy protein + sulfate + nZVI



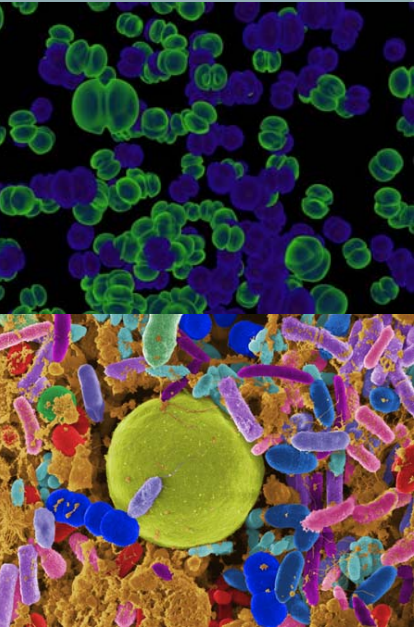
- Anaerobic set up
- 350 to 400 mL of GW
- Analysis at 2 weeks, 1 and 2 months
- Chemical, population, and bacterial ID by PCR

Case 1



- After 2 months, we detected differences in the bacterial populations with the addition of nZVI, soy and nitrate.
- Comparing analytical, population, and ID data, we recommended nitrate rather than sulfate as an amendment.

Case 2

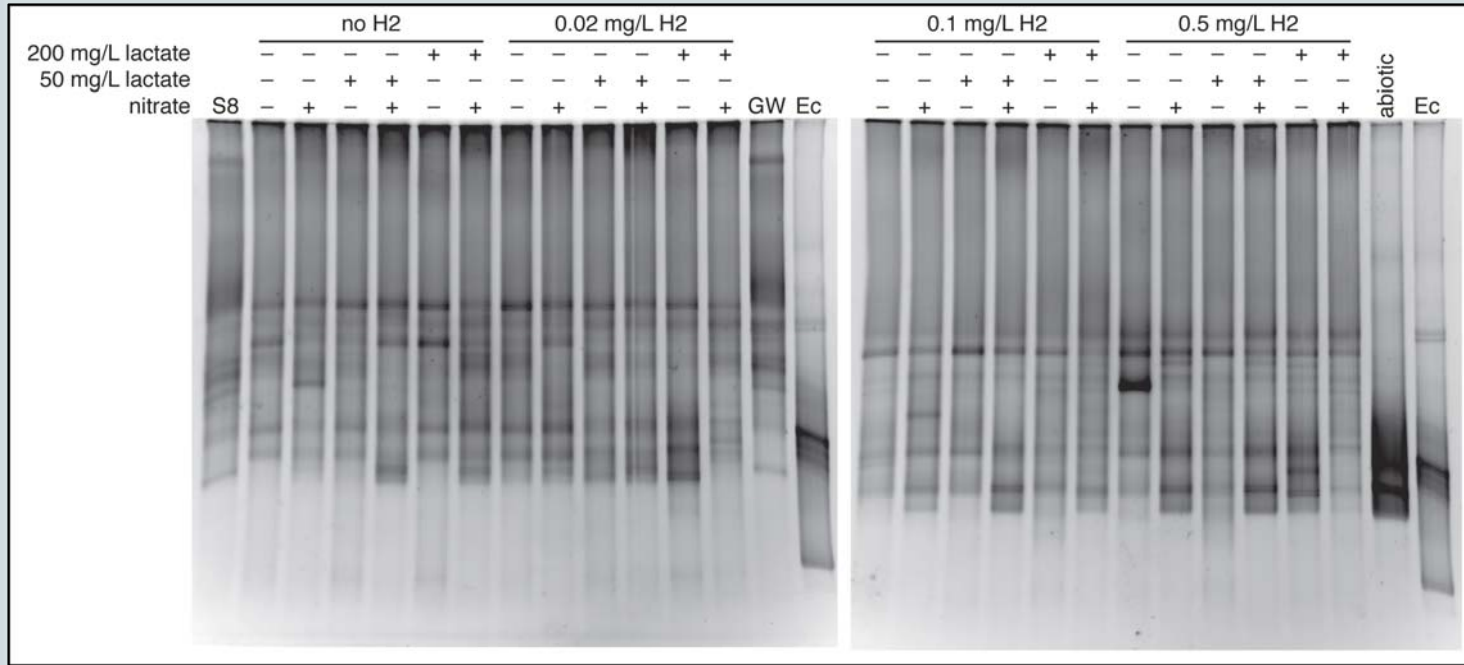
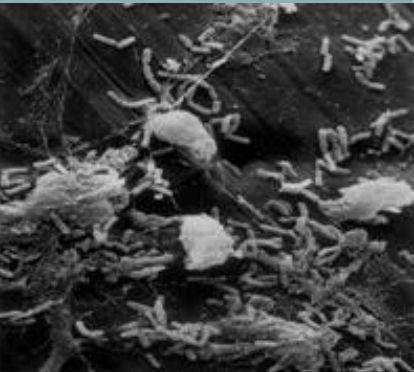


Test Matrix							
test	H2			lactate		nitrate	Comments
	0.02 mg/L	0.1 mg/L	0.5 mg/L	50 mg/L	200 mg/L	200 mg/L	
1	-	-	-	-	-	-	control
2	-	-	-	-	-	+	
3	-	-	-	+	-	-	
4	-	-	-	+	-	+	
5	-	-	-	-	+	-	
6	-	-	-	-	+	+	
7	+	-	-	-	-	-	
8	+	-	-	-	-	+	
9	+	-	-	+	-	-	
10	+	-	-	+	-	+	
11	+	-	-	-	+	-	
12	+	-	-	-	+	+	
13	-	+	-	-	-	-	
14	-	+	-	-	-	+	
15	-	+	-	+	-	-	
16	-	+	-	+	-	+	
17	-	+	-	-	+	-	
18	-	+	-	-	+	+	
19	-	-	+	-	-	-	
20	-	-	+	-	-	+	
21	-	-	+	+	-	-	
22	-	-	+	+	-	+	
23	-	-	+	-	+	-	
24	-	-	+	-	+	+	
25	-	-	-	-	-	-	abiotic control

- Anaerobic set up
- 10 grams of soil plus 100 mL of GW
- Hydrogen was added as a gas and dissolved into GW
- Incubated for 1, 2 weeks, 1 and 2 months

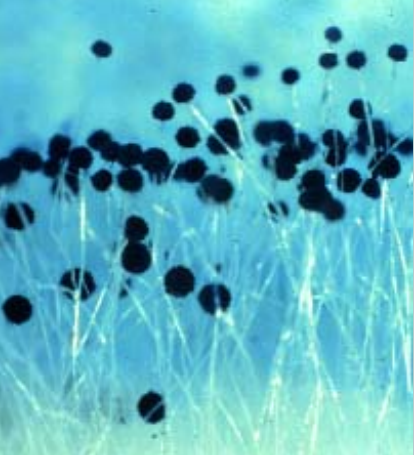
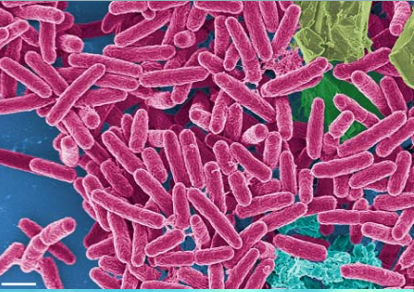


Case 2

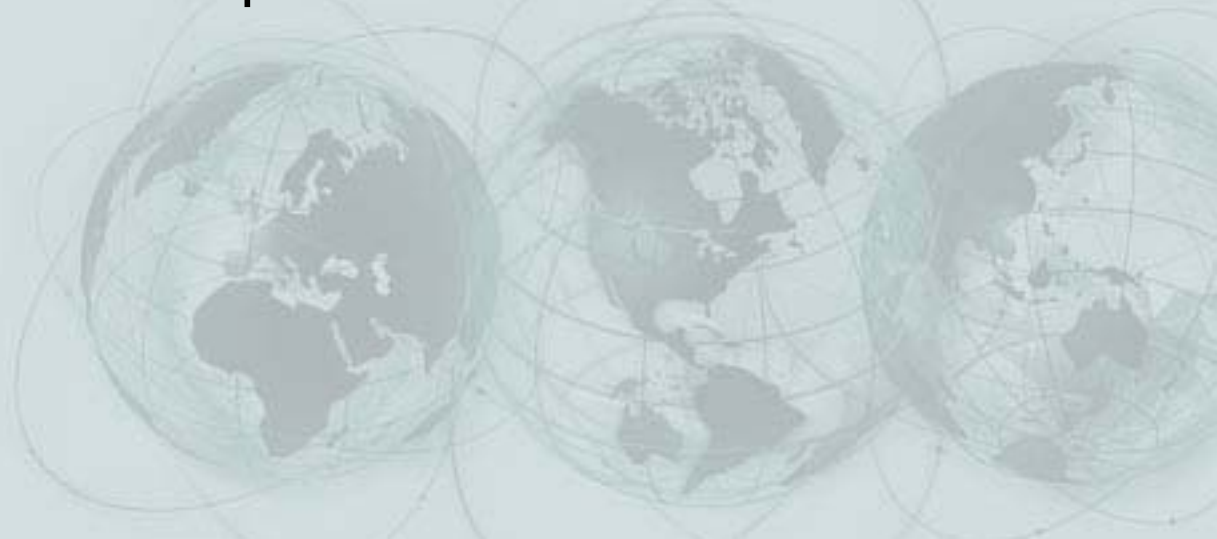


- 1 month analysis
- Chemical, population and Dhc PCR were analyzed.
- Most of the degradation was found to be abiotic from the residual nZVI.

Future Directions

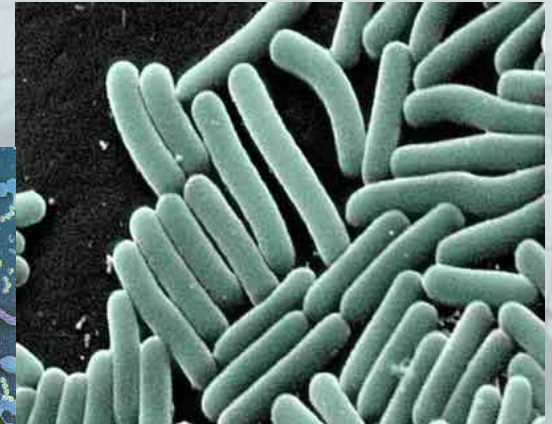
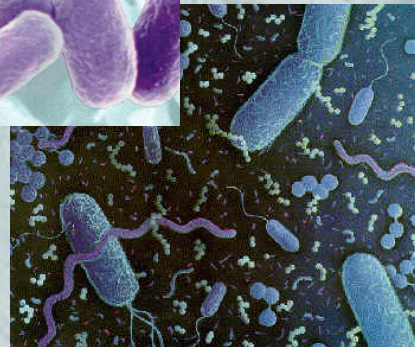
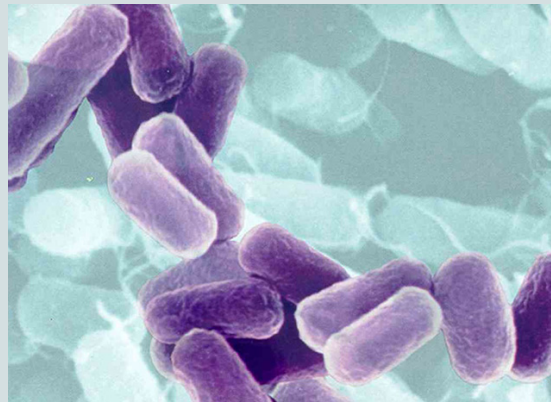


- Total microbial profiling of environments
 - Classes of bacteria
 - *Archaea*
 - Fungi and Moulds
 - Algae
- Provides a complete picture of microbial populations, which might need to act as a consortium to produce the desired effect



Specific Detection by PCR

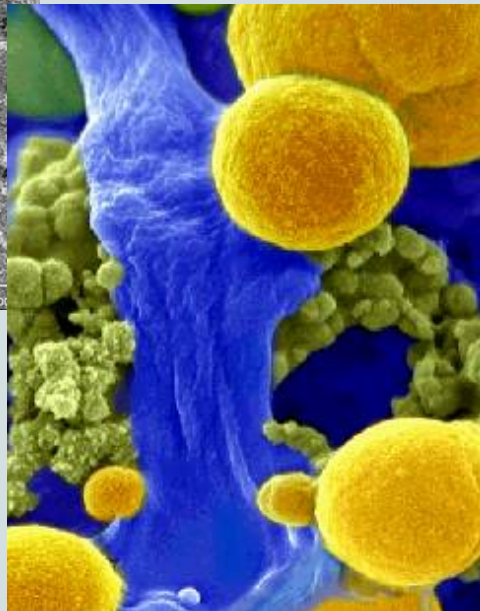
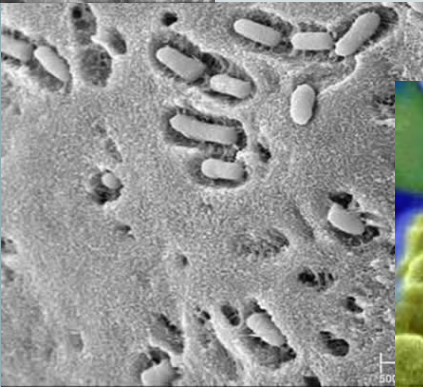
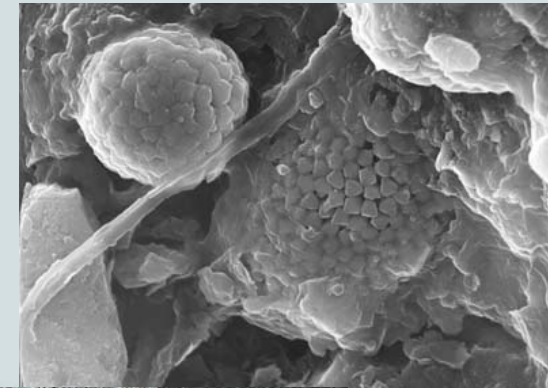
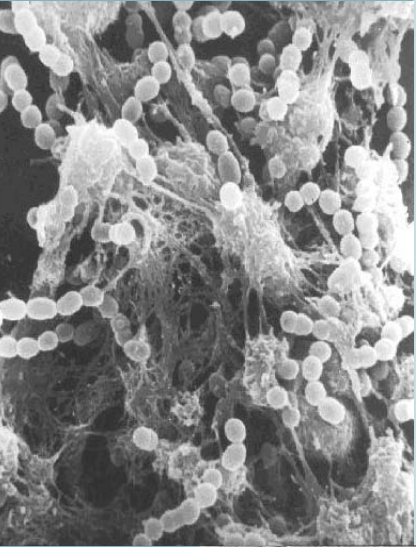
- Hydrocarbon-degrading bacteria
- BTEX-degrading bacteria
- PAH-degrading bacteria



Other Uses of PCR

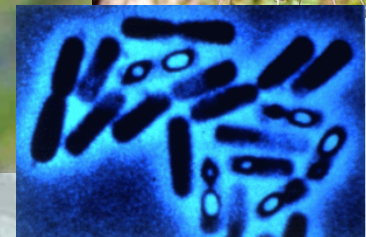
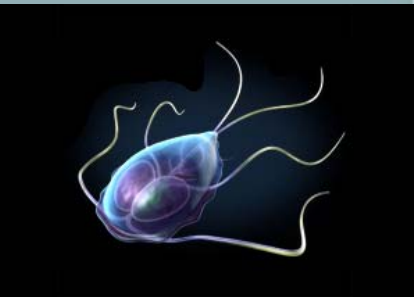
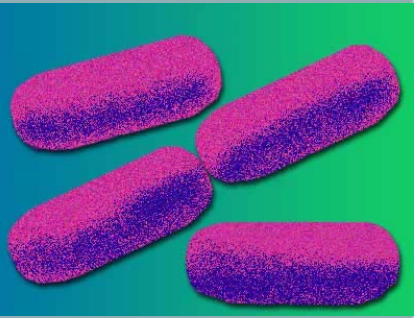
➤ Biocorrosion Analysis

- Acid-producing bacteria
- Sulfate-reducing bacteria



Summary

- Come talk to us!
- Come talk to us!
- Come talk to us!



Questions?



➤ Contact information

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