Functional Metagenomics of Microbial Communities in Groundwater for a Bedrock Plume and Source Area

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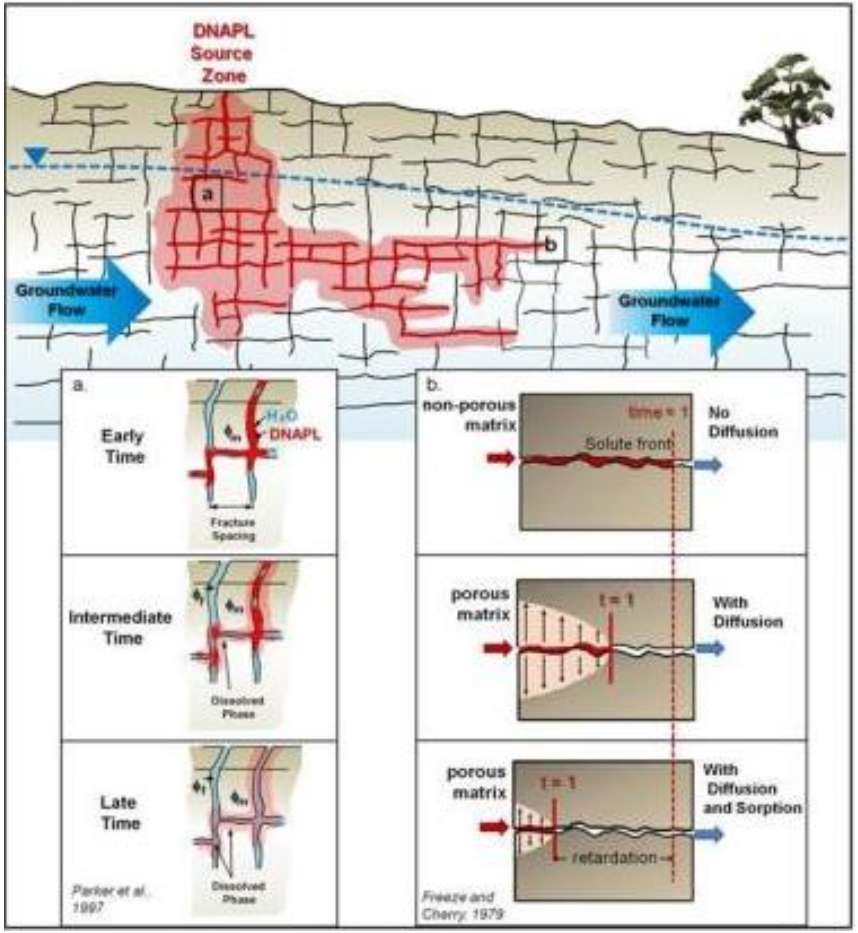
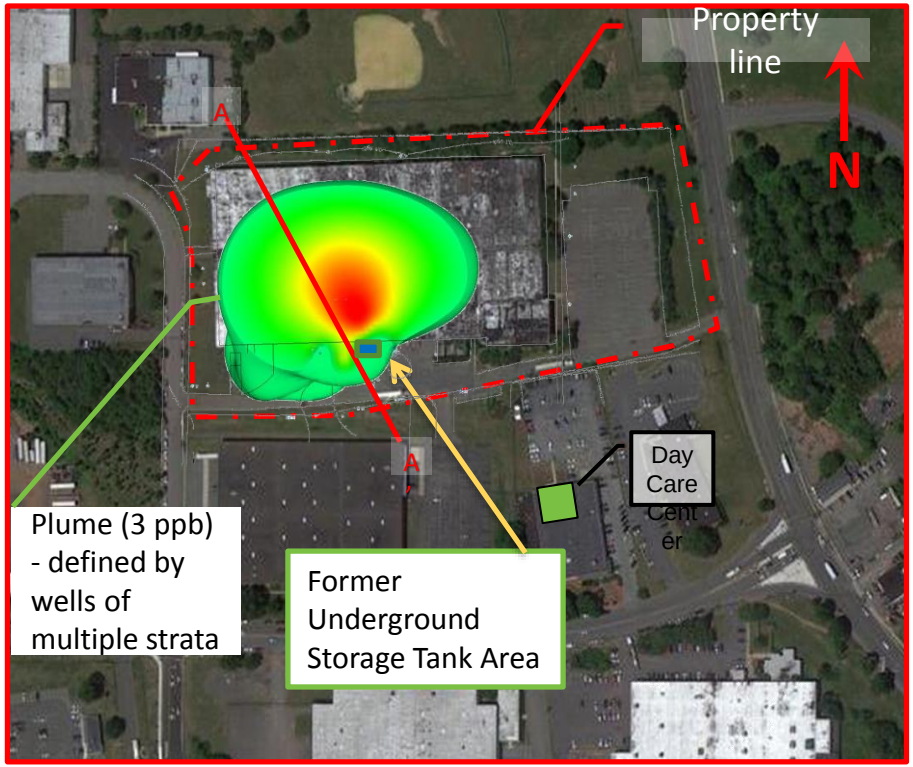
Problem?

- Site History
- Extent of Contamination
- Conceptual Site Model
- Site Remedial Strategy
- Advanced Characterization Tools & Data Evaluations
- Summary/Conclusions



Site Operational & Remedial Action History

- Pharmaceutical manufacturing -1976 to 2005
- Discharge of dichloromethane (DCM)
- DCM reached bedrock groundwater at 25 to 70 feet depth
- Shallow rock wells exhibit greatest contamination
- Presence of immobile residual contamination in rock matrix, but not free product
- GW P&T system operation from 1995 to 2009
- Evaluating attenuation since 2009



Surrounding area is industrial to the west and south. Commercial and residential areas to the east. Sensitive receptor to the south.

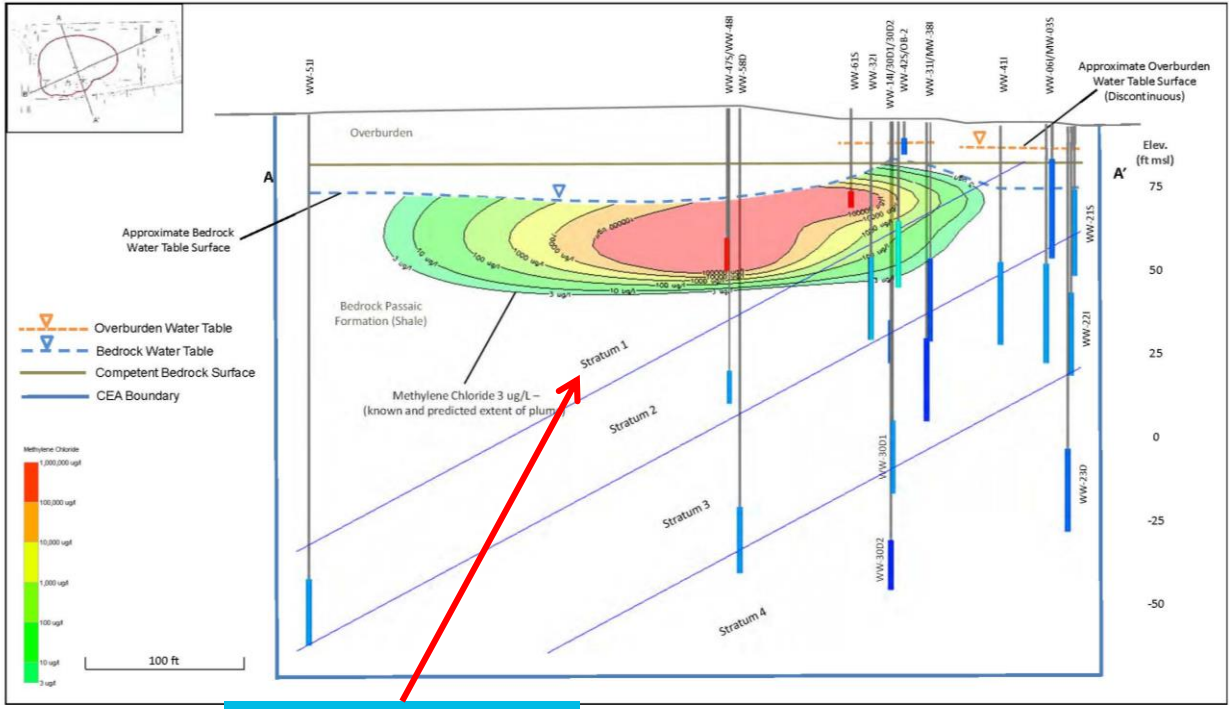
Site Geology

Shale Bedrock – primary permeability low to negligible

Layered sedimentary rock – layers (beds) dip gently northwest

Fractures along bedding and also higher angle fractures aligned northeast and northwest

Groundwater flows in fractures but low yielding



Site Remedial Strategy

1. Meeting groundwater standard of 3 ppb not practical from engineering perspective
2. Matrix contamination will diffuse into groundwater at concentrations >>3 ppb
3. Logistical constraints - most of contamination under warehouse
4. Seek Technical Impracticability (TI) Determination for source area (i.e. contaminated rock matrix/high dissolved phase plume)
5. Monitored Natural Attenuation (MNA) for dissolved phase plume surrounding source area
6. Regulators:

- ***MNA pursuit requires multiple lines of evidence***
- ***Require robust CSM to support passive groundwater remedy***

Advanced Characterization Tools to Develop CSM

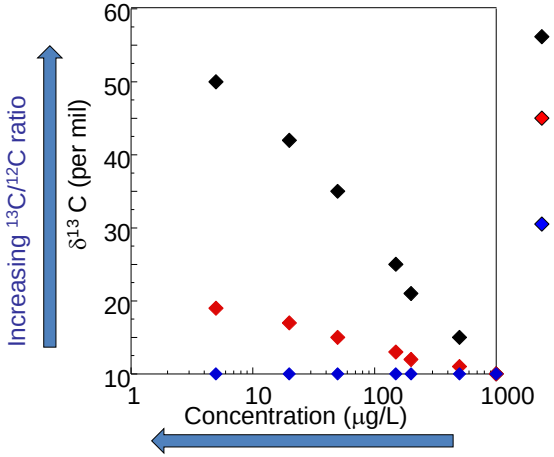
Informational Need	Characterization Tool
Evaluate Secondary Source- Contaminant Diffusion in Rock Matrix	Rock core analysis and diffusion modelling
Evaluate Contaminant Flux and Groundwater Flux in Transmissive Fracture Zones	Passive Flux Meter (PFM) and Hydraulic and Contaminant Fate and Transport Modelling
Evaluate Contaminant Biodegradation in the Source and Plume-	***Compound Specific Isotope Analysis (CSIA)***
Evaluate Contaminant Biodegradation in the Source and Plume-	***Microbial Metagenomics***

CSIA: Carbon Isotope Results

- Stable isotopes of carbon (C^{13}/C^{12}) analyzed from 8 wells
- Use Rayleigh model :

$$\delta^{13}C = \ln(C/C_0)*\varepsilon + \delta^{13}C_0$$

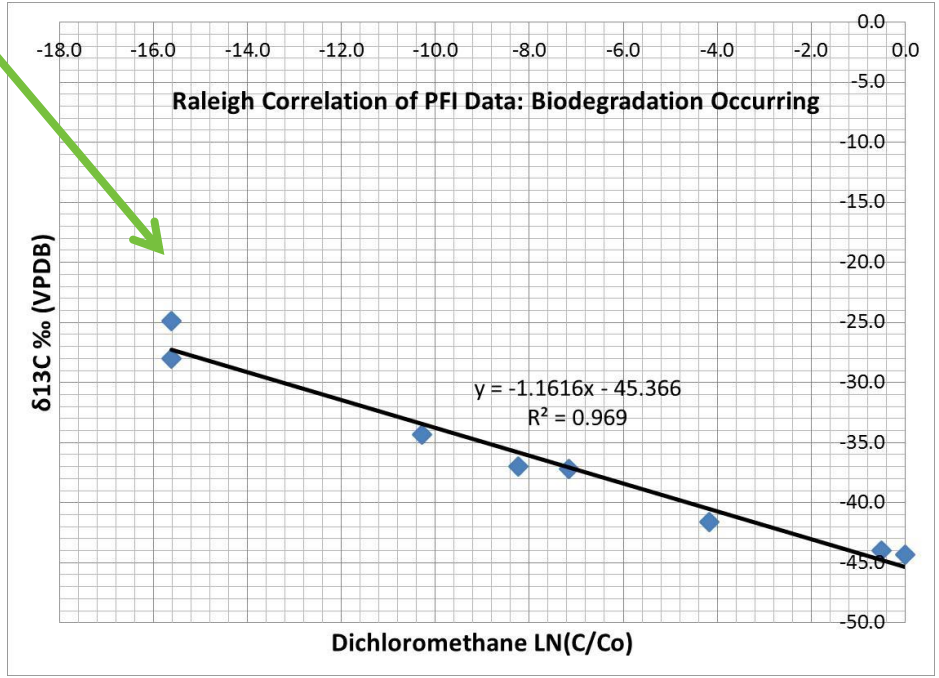
- ***Biodegradation occurring at the Site***



Scenario 1
degradation processes

Scenario 2
degradation processes

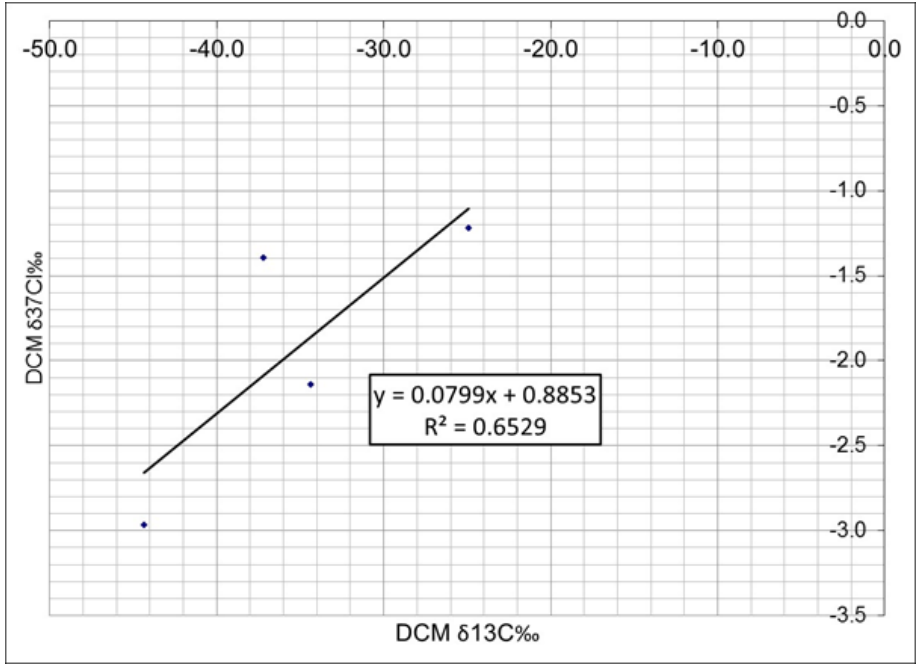
Scenario 3
No fractionation for dilution or adsorption



← Example of isotopic enrichment during contaminant degradation

CSIA: Carbon and Chlorine Isotope Results

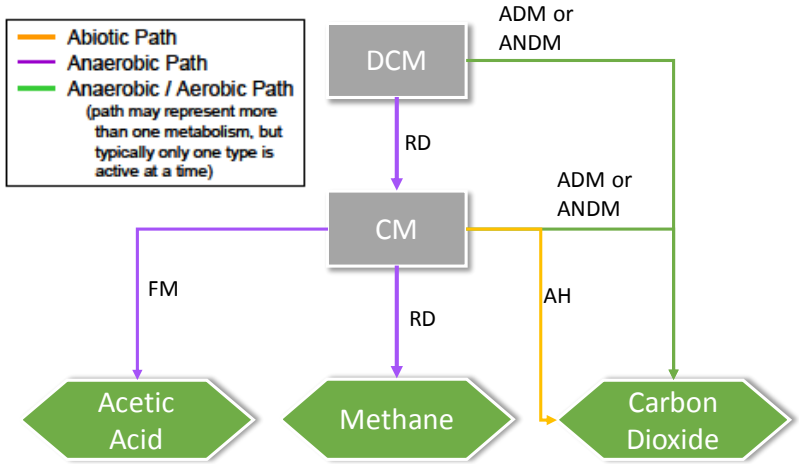
1. Stable isotopes of chlorine (Cl^{37}/Cl^{35}) analyzed from 4 wells
2. Linearity of results indicate a Rayleigh type fractionation
   - as DCM concentration drops, C13 and Cl37 ratios increased
   - consistent with both biotic and abiotic degradation
3. 2D-CSIA. C and Cl isotopes plotted in the X-Y format to determine a linear trend, with a slope that is often characteristic of a reaction mechanism:
   - S_N2 –type reaction
   - Cometabolic oxidation
   - Reductive dehalogenation



2D-CSIA trend indicates S_N2 - nucleophilic substitution, by -OH or by glutathione, mediated by various dehalogenase enzymes

Biodegradation Rate Estimates

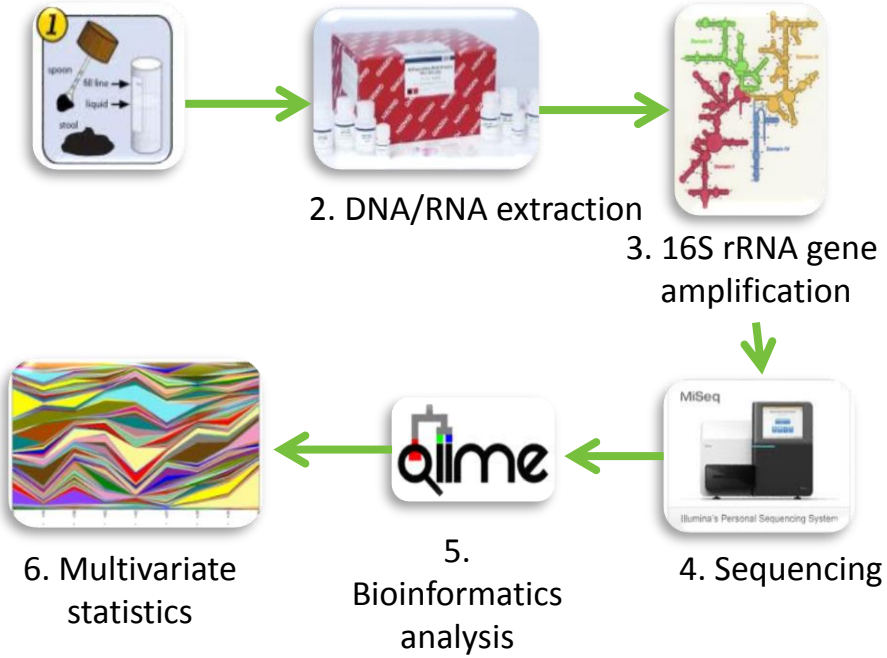
CSIA-derived Half Life Estimates			
Sample ID	ug/L	Aerobic- Oxic[1]	Anaerobic- Fermentation[2]
		DCM Half Life Mean (days)	
WW-61S	12000000	Source Well	
WW-47S	7200000	105	40
WW-37I	186000	13	5
WW-01I	3200	5	2
WW-33I	9300	5	2
WW-46I	414	4	2
WW-48I	2	2	1
WW-58D	2	3	1
1 Methods in EPA 2008			
2 Used Epsilon factors from Trueba-Santiso et. al 2017			



ADM- Aerobic Direct Metabolism
AH- Abiotic Hydrolysis
ANDM- Anaerobic Direct Metabolism
FM- Anaerobic Fermentation
RD- Reductive Dechlorination (Hydrogenolysis)

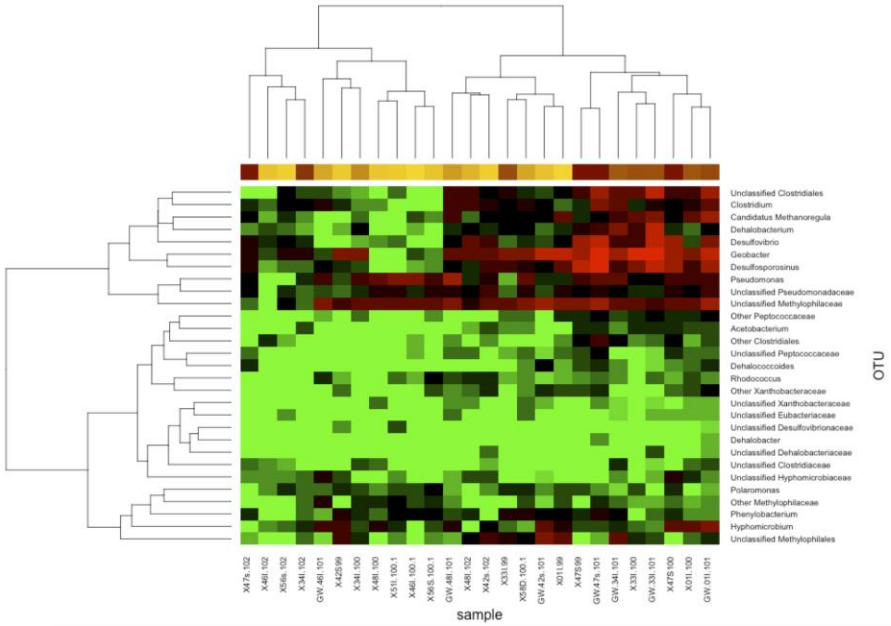
Microbiological DNA and RNA Analysis

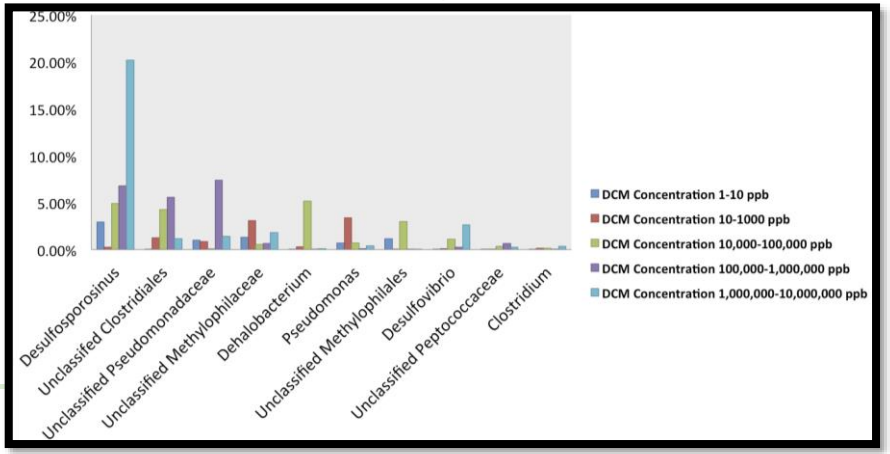
1. Ten wells - ranging from 0.89 - 9,800,000 ppb DCM concentrations
2. Groundwater samples (n=40) were collected quarterly between October 2013 and October 2014.
3. Samples were filtered and DNA and RNA was extracted.
4. This DNA was then subjected to Illumina-tag PCR and sequencing of the 16S rRNA gene.
5. 16S rRNA analysis and metatranscriptomics were conducted on 26 and 11 groundwater samples, respectively.



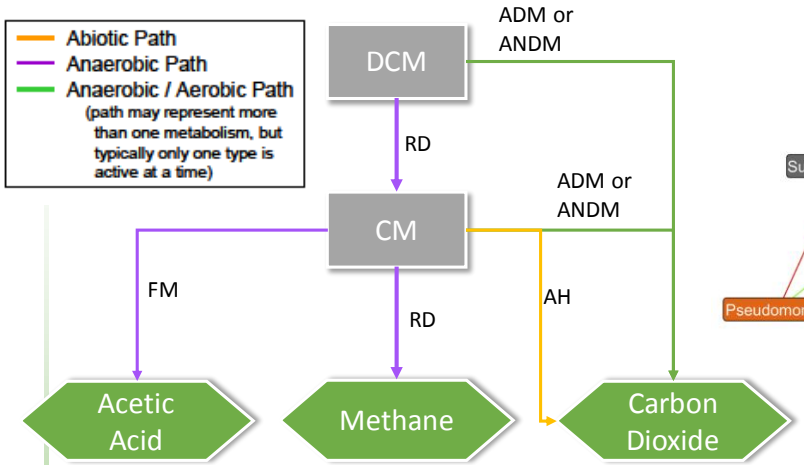
Metagenomics Results

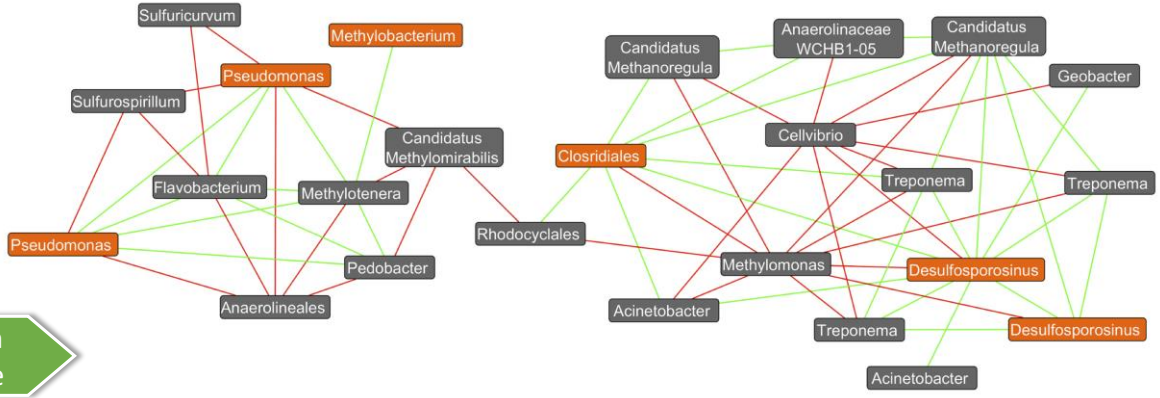
- Cell counts ranged from (1.44E+08 to 3.76E+06) were not statistically significant ($p > 0.05$) between samples with 200-800 OTUs identified.
- A two-way heatmap of DCM-degrading OTUs (40 identified) with DCM
- Near the source mechanisms are predominantly anaerobic, with higher abundances of *Desulfosporosinus, Desulfovibrio,* and unclassified Clostridiales taxa
- Increased abundance of aerobic degraders, such as the *Pseudomonas*, was noted in lower DCM concentration environments





DCM-Degrader Community Relationships

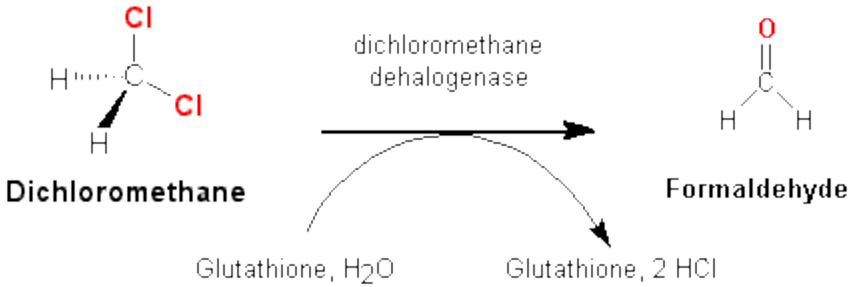


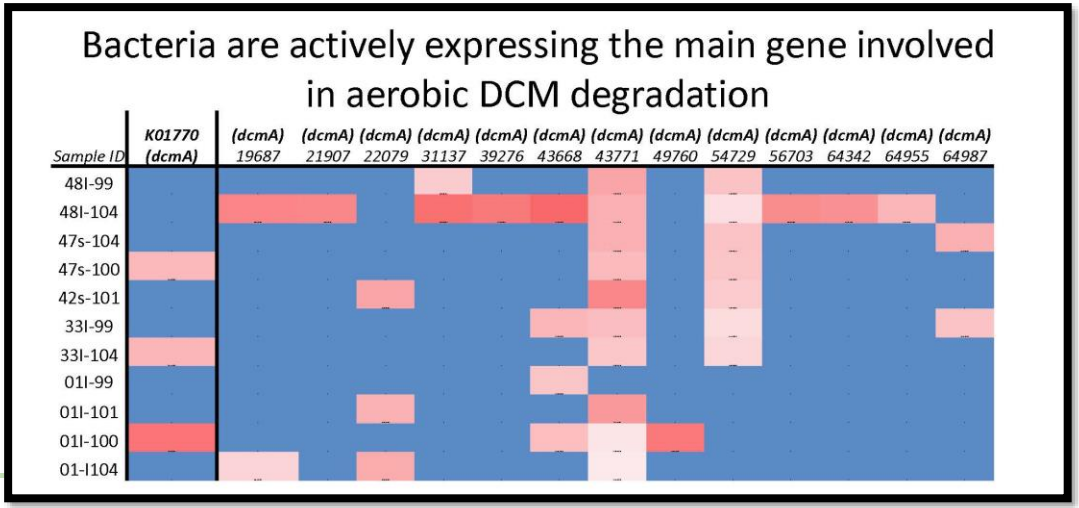


- Two distinct sub-networks were observed,
  - aerobic DCM degrading taxa (*Pseudomonas* and *Methylobacterium*)
  - anaerobic degraders (*Desulfosporosinus* and Clostridiales).

Microbiological Functional Gene Analysis

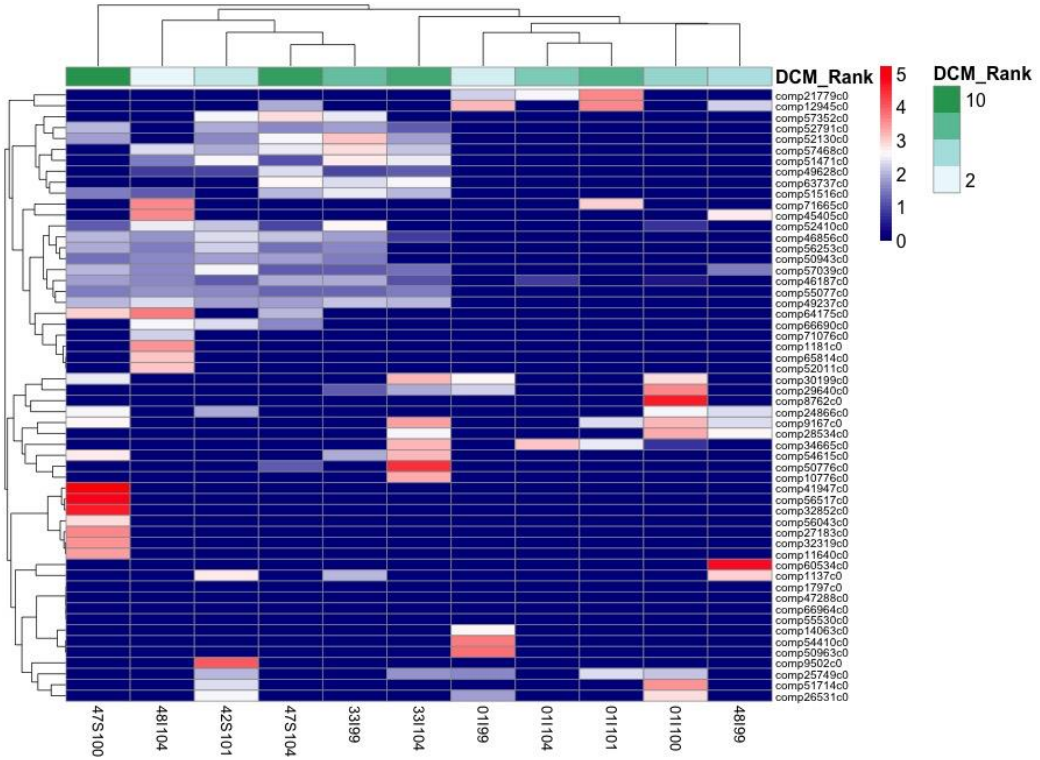
1. Dichloromethane dehalogenase (dcmA) is central in dehalogenation of dichloromethane in aerobic environments.
2. Known gene dcmA was not highly expressed ***but***
3. 14 novel dehalogenases were identified and have different expression patterns across differentially contaminated groundwater samples





Aerobic RNA Expression Heatmap

- A custom BLAST database - search for dehalogenase genes identified with metagenomics.
- rRNA transcripts revealed the unique expression profiles that clustered as a function of location and the severity of DCM contamination
- A random forest model used to evaluate expressed genes and/or physio-chemical parameters predictive of DCM concentration.
  - activation of chloroalkane/chloroalkene and methane metabolism pathways.



Biodegradation Rate and Gene Expression

- ***Aerobic degradation rates were correlated to both the known dcmA and novel dehalogenase transcripts:***
  - ***expression of the novel dehalogenases shared the highest correlation with degradation rates (Spearman rho: 0.48-0.72)***
  - ***previously identified/known dcmA genes (Spearman rho: -0.31)***.

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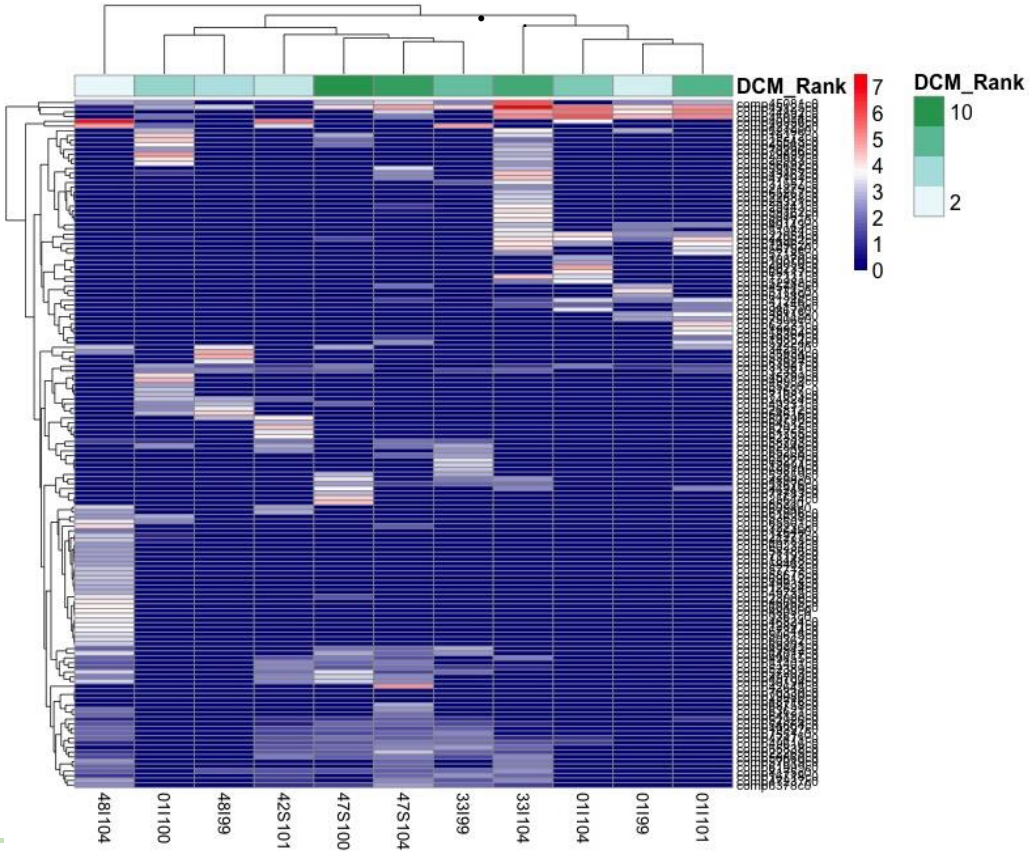
1 Methods in EPA 2008

2 Used Epsilon factors from Trueba-Santiso et. al 2017

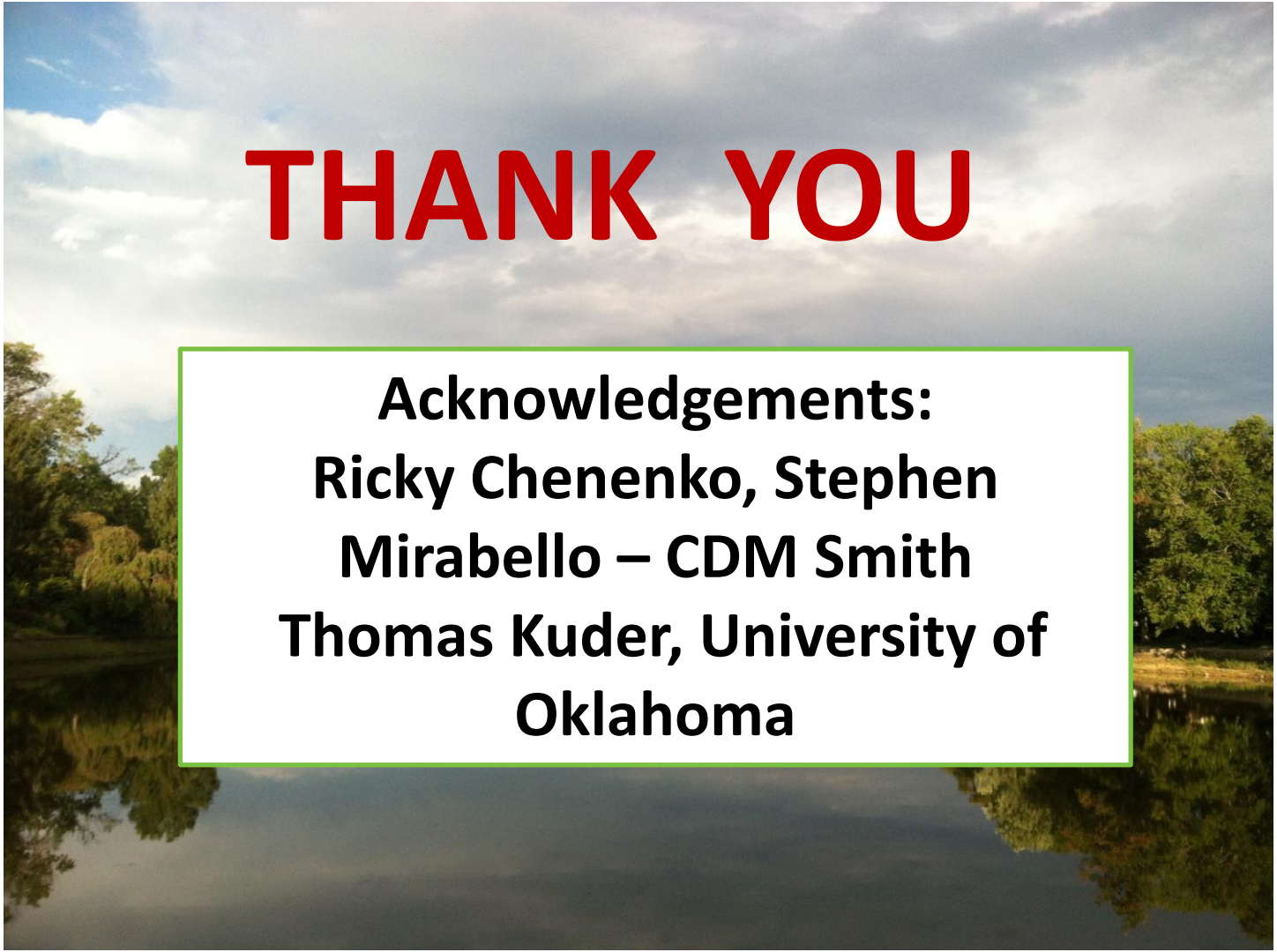
Anaerobic DCM Degradation

- A custom BLAST database was generated to search for genes related to the anaerobic degradation of DCM.
- Genes utilizing tetrahydrofolate cofactors were selected because tetrahydrofolate has been implicated in catalyzing the anaerobic degradation of DCM in prior studies (Leisinger and Braus-Stroymeyer, 1995).
- We can observe similar expression profiles within samples of similar DCM concentration/sampling well.
- 6 of the 10 most expressed tetahydrofolate cofactors fall within the *Desulfosporosinus* genome
- *Propionibacterium, Pseudomonas, Bacteroides* were observed as well



Summary

1. CSIA used to evaluate DCM biodegradation mechanism and rate
2. Metagenomics Results:
    1. Identified DCM degrading genes/organisms consistent with CSIA conclusions (Sn2 dehalogenase-mediated degradation)
    2. Also identified anaerobic DCM-degrading Desulfosporosinus and Propionibacterium
3. Metatranscriptomics Results:
    1. Dehalogenases were the most expressed genes in the profiles (consistent with CSIA and metagenomics)- found 14 novel dcmA genes
    2. Tetahydrofolate cofactors associated with Desulfosporosinus actively expressed
4. Metatranscriptomics hold much promise for identifying more relevant, site-specific biomarkers for predicting biodegradation potentials that potentially can be used to estimate biodegradation rates in situ.



THANK YOU

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