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Leveraging Synergies of Molecular Biological Tools and Isotopic Analysis for MNA and Enhanced Bioremediation Assessment

Phil Dennis, Sandra Dworatzek and Brent Pautler (SiREM)
Anko Fischer and Kevin Kuntze (Isodetect)2

Presented by Phil Dennis
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Overview DNA and Isotopic Methods

Who is there?

Quantitative PCR

Quantify specific pre-selected targets:

- e.g., *Dehalococcoides*
- Functional genes e.g., VC-Reductase

Next Generation Sequencing

Characterize the entire microbial community

Isotopes $^{13}C/^{12}C$

Contaminant Degradation?

Compound Specific Isotope Analysis (CSIA)

Confirm contaminant degradation by enrichment of ^{13}C biotic and abiotic processes

Stable Isotope Probing (SIP)

Confirm biodegradation through addition of labelled compound e.g.,^{13}C benzene

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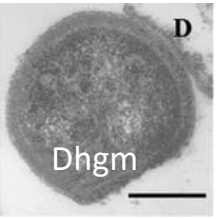
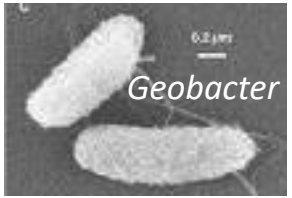
Chlorinated Solvent Degraders

Use chlorinated solvents in their respiratory metabolism to gain energy by reductive dechlorination or oxidation

Anaerobic

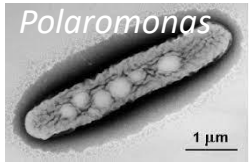
- *Dehalococcoides (Dhc):* all chloroethenes, 1,2-DCA
- *Dehalogenimonas (Dhgm):* tDCE, VC, chloropropanes,1,2-DCA
- *Geobacter*: PCE/TCE to cDCE

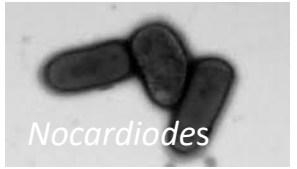




Aerobic

- *Polaromonas* – Some degrade cDCE aerobically
- *Nocardiodes* – Can degrade VC and ethene aerobically



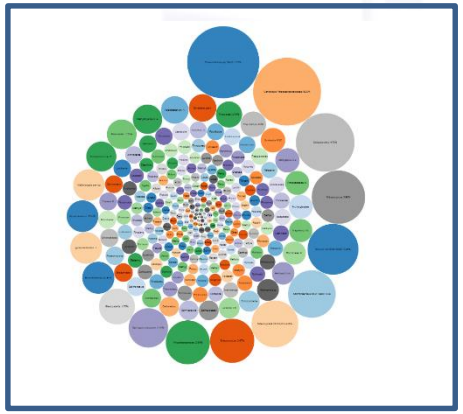


Molecular Biological Tools (MBTs)

Samples

DNA Extraction

Next Gen Sequencing



Microbial Community Profiles

qPCR

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Certificate of Analysis: Gene-Trac® Functional Gene Assay

Customer: John Smith, Some Company
Project: Some Project
Customer Reference: 013456789

SiREM Reference: S-XXXX
Report Date: 22-Jul-16
Data Files: iQ5-FGA-QPCR-XXXX
iQ5-DB-FGA-QPCR-XXXX

Table 1: Test Results

Sample ID	VC Reductase (*vcrA*)		BAV1 VC Reductase (*bvcA*)		TCE Reductase (*tceA*)	
	Percent *vcrA* [1]	Gene Copies/liter	Percent *bvcA* [1]	Gene Copies/liter	Percent *tceA* [1]	Gene Copies/liter
MW A	0.06 - 0.2 %	2×10^5	0.06 - 0.2 %	4×10^5	0.06 - 0.2 %	3×10^5
MW B	NA	6×10^3 U	NA	6×10^3 U	NA	6×10^3 U
MW C	0.007 - 0.02 %	2×10^4	NA	6×10^3 U	NA	6×10^3 U

See final page for notes.

Quantify gene copies of specific targets

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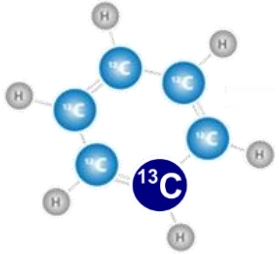
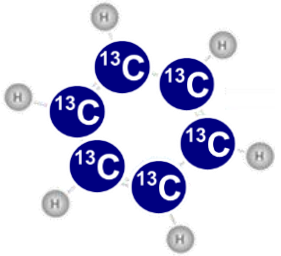
Stable Isotopes used in Two Ways

Natural abundance
^{13}C:^{12}C = 1:99

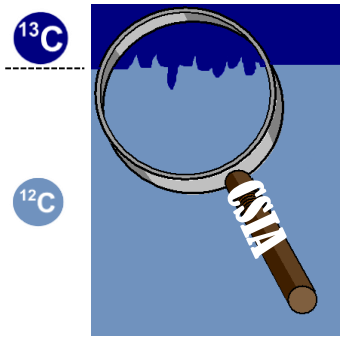
^{13}C-labeled Substrate
^{13}C:^{12}C = **99**:1

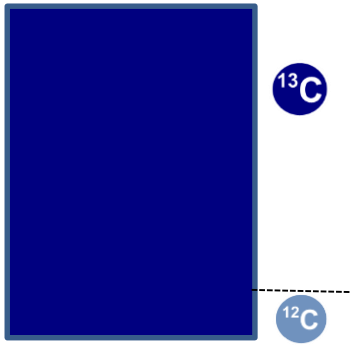
"CSIA"





"Stable Isotope Probing"





CO_2
biomass

^{13}C-CO_2
^{13}C-Biomass

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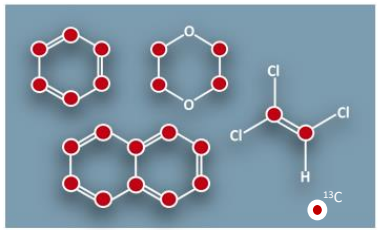
SIP with BACTRAP®

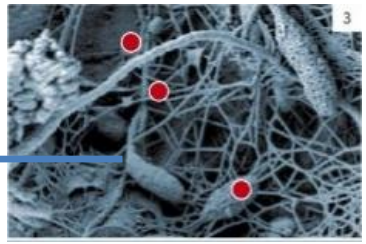
- ^{13}C-labeled contaminant adsorbed to *in situ* microcosms
- ^{13}C isotope signal finally detected in biomolecules from colonizing bacteria or in CO_2
- Tool to demonstrate mineralization of specific PAH, BTEX, Chlorinated ethene



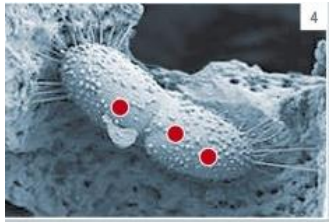
Label target compound with ^{13}C

Load carrier material with 13C-compound

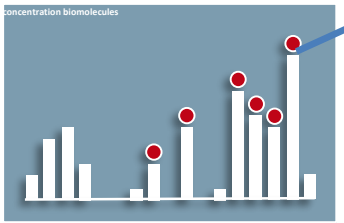
Incubate BACTRAPS in groundwater well



Microorganisms Colonize Carrier



^{13}C label will be assimilated during biodegradation



^{13}C in biomolecules (e.g., lipids) =biodegradation

Characterize microbial community with qPCR/NGS

Determination of isotope ratios by GC-IRMS

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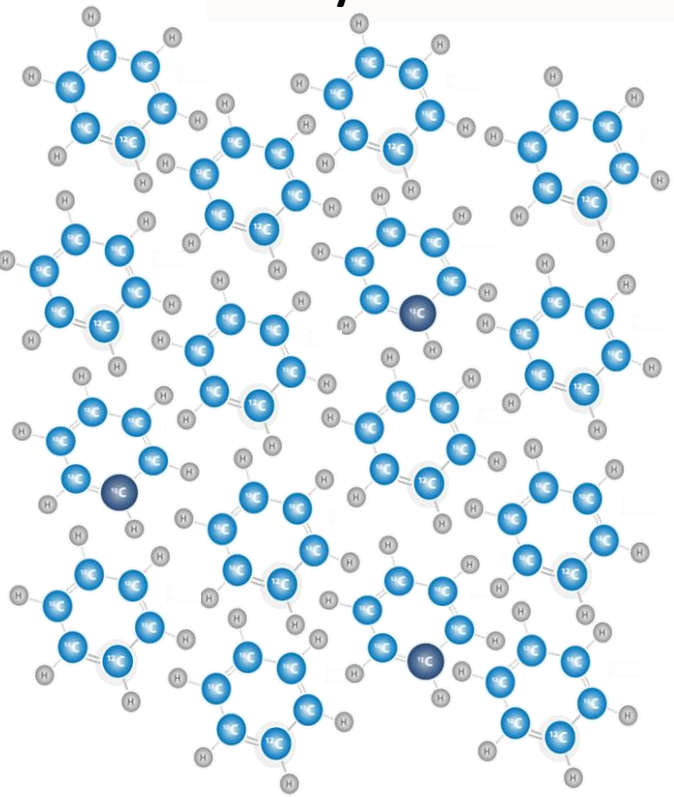
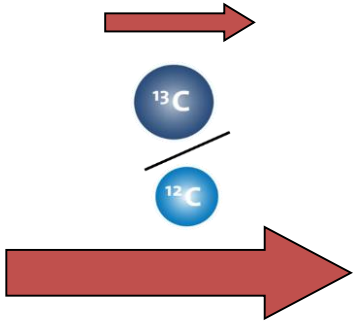
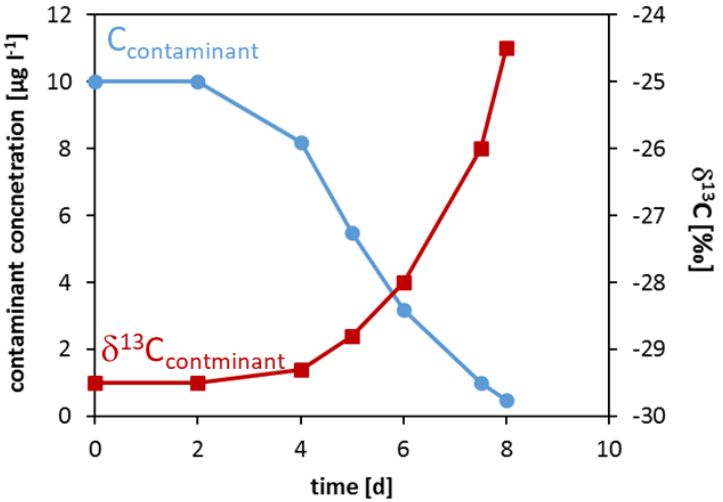
CSIA for evaluating *in situ* pollutant degradation

Pollutant molecules with **heavy isotopes** (^{13}C) are **degraded more slowly** and accumulate in the remaining pollutant pool.



DEGRADATION





In case of **degradation**, isotope values are getting more **positive**

What MBTs CSIA and SIP "Bring to the Table"

Molecular Biological Tools (MBTs)

- Very sensitive
- Quantify key organisms are increasing/decreasing/stable/?
- Can detect developing or potential biodegradation pathways

CSIA

- Integrates the extent of past degradation of target compounds
- Can be used to estimate long-term degradation rates
- Can differentiate reaction mechanisms (esp. Dual Isotope-CSIA)

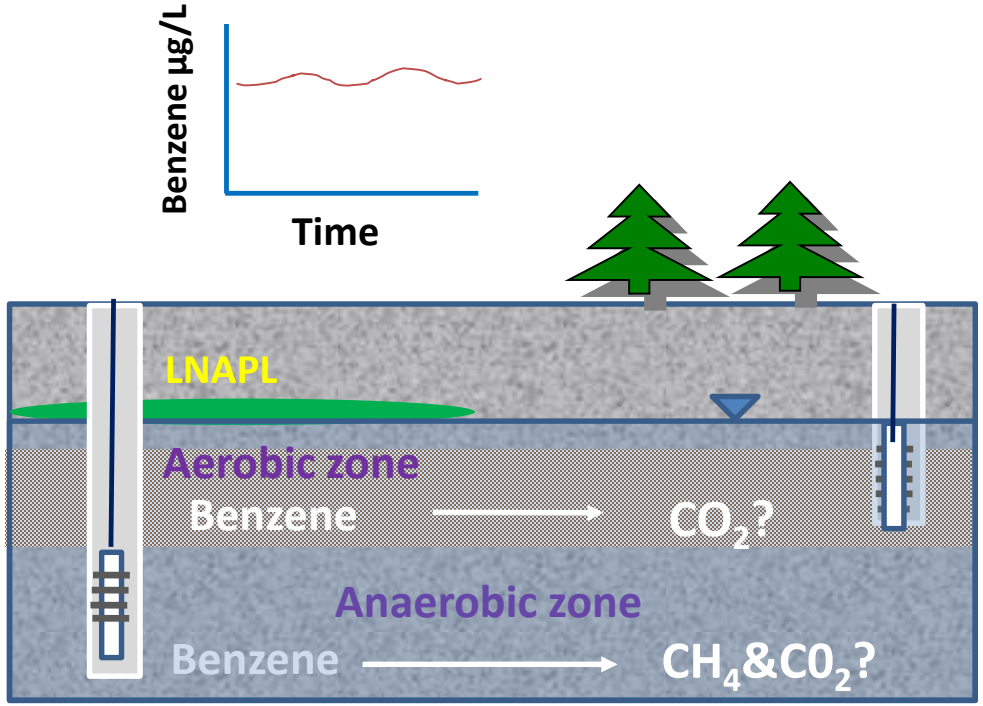
SIP

- Provides sensitive empirical evidence that degradation of a specific contaminant is occurring or could occur

Is Benzene Degrading/What Pathway?

- **Aerobic Zone**
  benzene biomarkers qPCR
  e.g., TDO ✓
- ^{13}C benzene enriched in aerobic zone? CSIA ✓
- ^{13}C CO_2 from Bactrap ✓

Aerobic Benzene Biodegradation ✓



- **Anaerobic Zone**
  benzene biomarkers qPCR
  e.g., *abcA* X
- CSIA ^{13}C benzene not enriched X
- ^{13}C benzene in Bactrap not detected in biomass/CO_2 X

Anaerobic Benzene Degradation Not Indicated X

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CASE STUDIES/EXAMPLES

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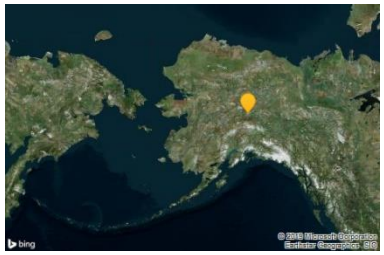
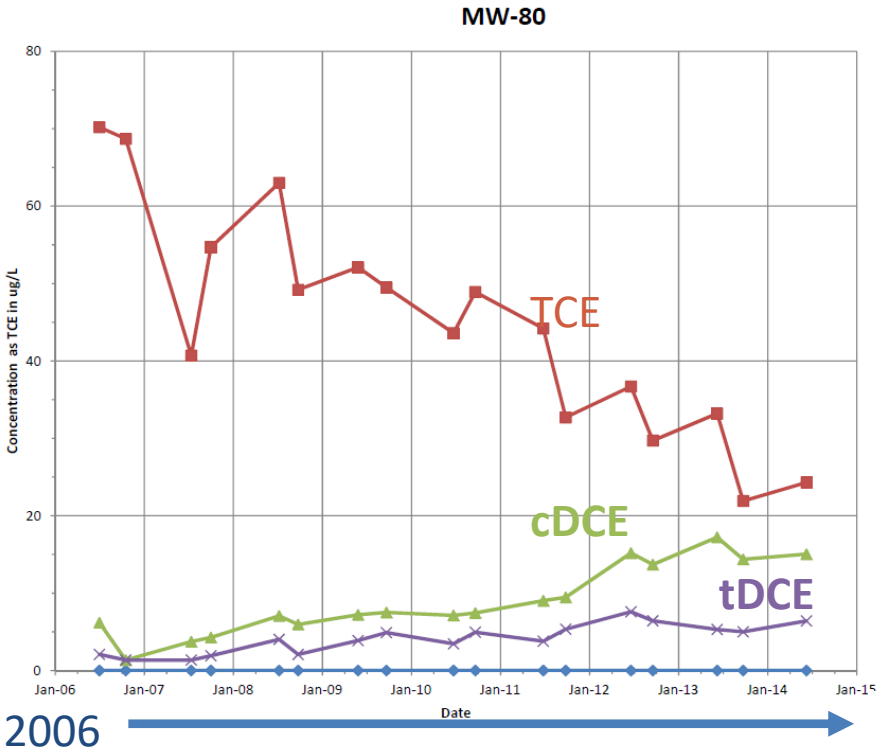
Alaska MNA Site



- Solvent and oil contamination related to pipeline support business maximum detected TCE ~150 µg/L in groundwater

- Compound specific isotope analysis (CSIA) and Gene-Trac NGS analysis study performed

Alaska MNA Site – CVOCs



2006 → 2015

- TCE concentrations declining
- cDCE accumulating
- tDCE accumulating
- No observed VC

- Is natural attenuation occurring? is degradation beyond cDCE occurring?

Alaska Site CSIA Results

Sample ID	Date Sampled	Trichloroethene		cis-Dichloroethene	
		Concentration (µg/L)	$\delta^{13}C$ (‰)	Concentration (µg/L)	$\delta^{13}C$ (‰)
MW-56	7/13/2017	5	-18.1 J	8	-25.6 J
MW-100	7/13/2017	3	-20.5 J	7	-30.9 J
MW-87	7/13/2017	12	-15.8	38	-24.5
MW-103	7/13/2017	90	-18.8	13	-25.0
MW-77	7/13/2017	28	-12.6	25	-24.1
MW-84	7/14/2017	12	-4.0	40	-22.6
MW-105	7/14/2017	41	-15.2	46	-20.8
MW-106	7/14/2017	13	-10.7	14	-24.5
MW-107	7/14/2017	11	-7.4	9	-26.0 J

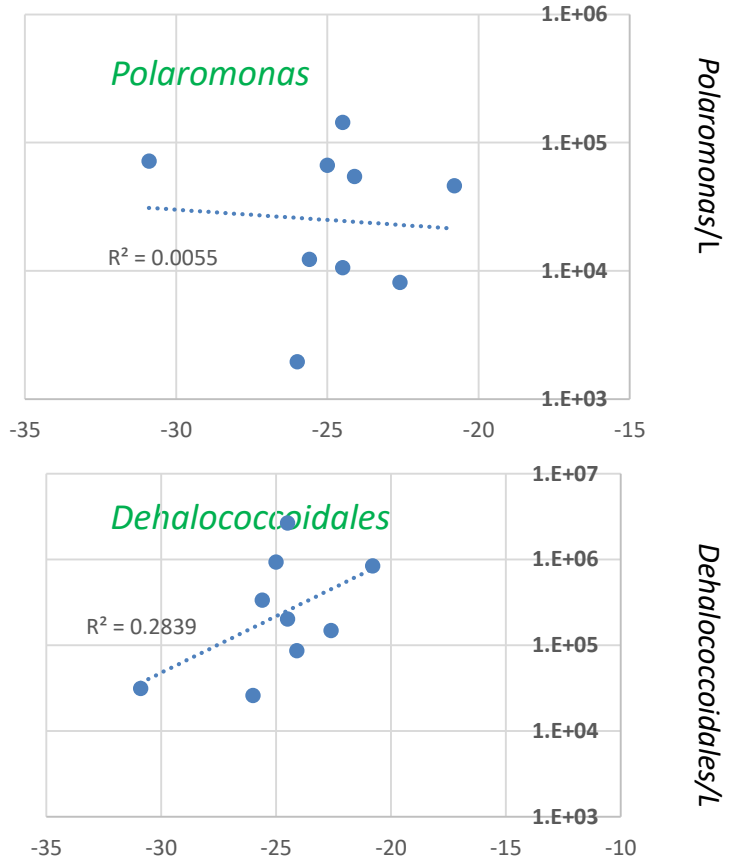
- Undegraded TCE -34‰ to -23‰ -Strong evidence for TCE degradation-source area wells show strongest ^{13}C enrichment
- Undegraded cDCE -29‰ to -22 ‰ -Evidence for cDCE degradation is inconclusive”-strongest at MW-105
- Is there further evidence for cDCE degradation based on microbial community?

Alaska Site NGS Results

- Low biomass in groundwater- consistent with a cold climate natural attenuation site
- High microbial diversity thousands of OTUs in site groundwater
- Potential dechlorinators:
  - *Dehalococcoidales* (anaerobic)
  - *Polaromonas* (aerobic cDCE)

cDCE Degradation at AK Site
Which Microbial Population?

- VC- Reductase (*vcrA/bvcA*) genes were not detected in high number.
- cDCE $\delta^{13}C$ not positively corelated with aerobic *Polaromonas* abundance
- cDCE $\delta^{13}C$ enrichment weakly corelated with *Dehalococcoidales* abundance
- Suggests that reductive pathway may be dominant biological cDCE pathway likely via reductive dechlorination

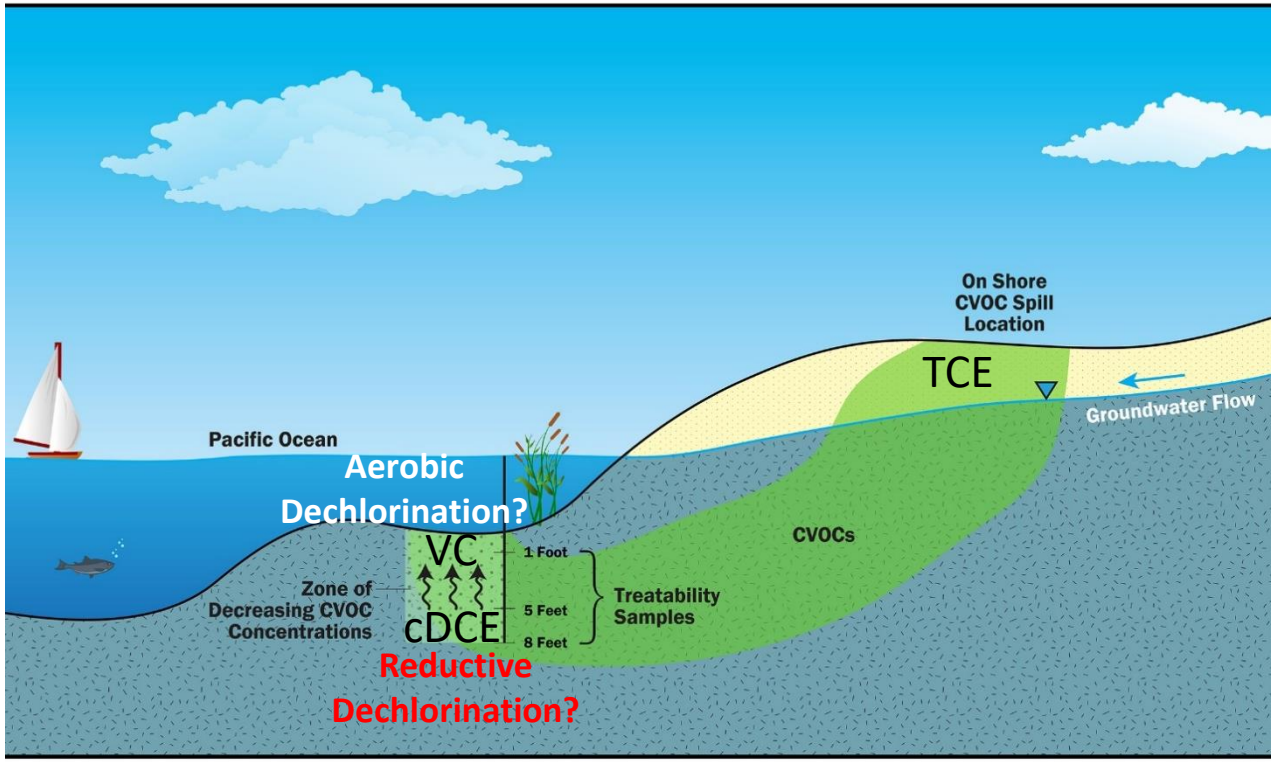


Alaska Site Conclusions

- Strong evidence for TCE degradation to cDCE based on CSIA
- tDCE from TCE *Dehalococcoidales* populations have been reported to produce tDCE and were present in substantial abundance in site groundwater
- Inconclusive evidence for cDCE degradation based on CSIA is more compelling due to correlation with obligate cDCE dechlorinating populations
- Based on correlations of CSIA and molecular data there is little evidence for substantial impacts of non-*Dehalococcoidales microbes* contributing to dechlorination processes for cDCE

California MNA Site SIP/qPCR Study[1]

Key question: is aerobic CVOC degradation occurring in shallow sediments?



Microbial community in sediments

- *Dehalococcoides* / *Dehalogenimonas* detected (reductive dechlorination) ethene ND
- *Polaromonas* not detected
- *etnE* detected (potential aerobic VC degradation?)

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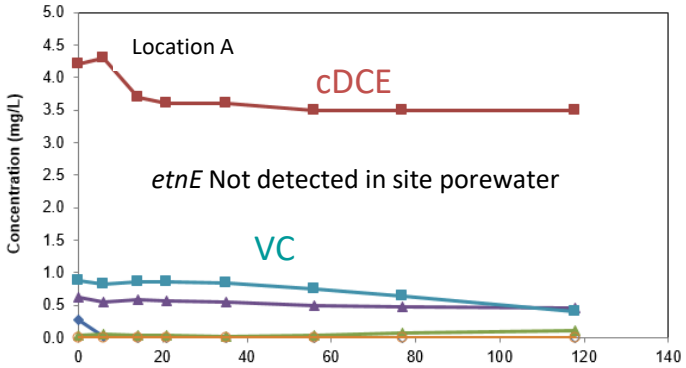
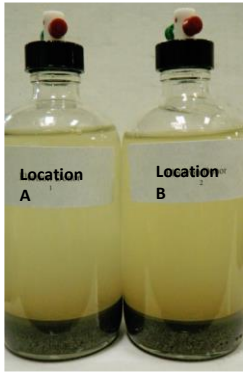
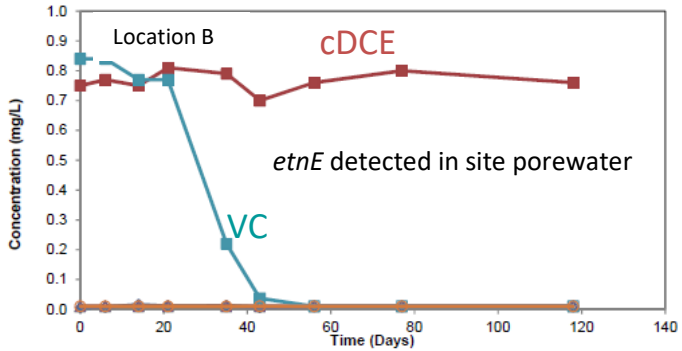
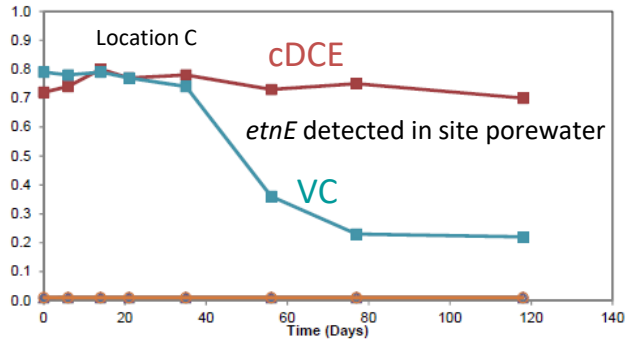
Microcosm Study





- Porewater & Sediments 1 ft depth
- Spiked with ^{13}C-VC & ^{13}C-cDCE
- O_2 added regularly
- Day 118 microcosms sacrificed for ^{13}C-CO_2 quantification





TCE cDCE tDCE
1,1-DCE VC Ethene

California Site ^{13}C SIP Data

Sample	$\delta^{13}C_{CO2}$ (‰)	Comment
Sterile Control-Location B		
1	-18.1	VC degradation to CO_2 not indicated
2	-14.9	
3	-18.6	
Oxygen Treatment-Location B		
1	**+29.5**	VC degradation to CO_2 indicated
2	**+27.8**	
3	**+31.5**	
Sterile Control-Location C		
1	-10.0	VC degradation to CO_2 not indicated
2	-6.9	
3	-7.3	
Oxygen Treatment-Location C		
1	**+32.3**	VC degradation to CO_2 indicated
2	**+41.6**	
3	-3.5	Replicate lagging

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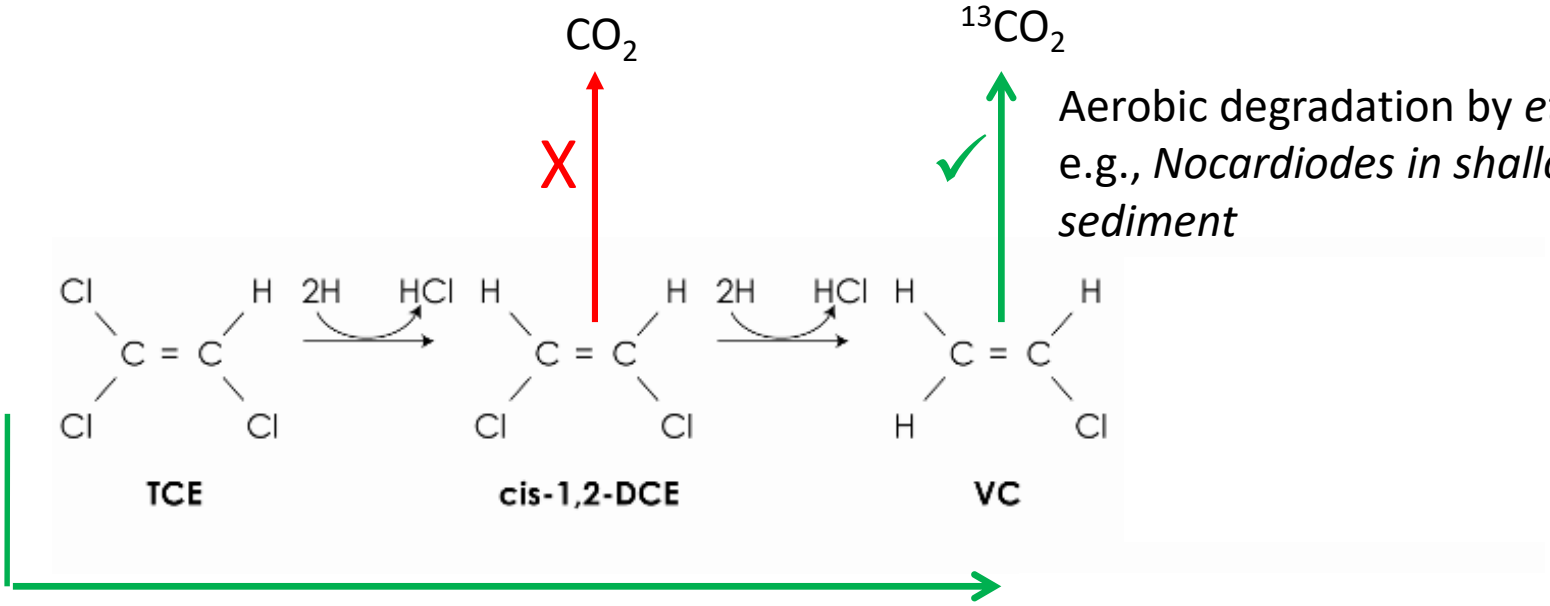
Confirmed Biodegradation Pathways CA Site



Combination of qPCR and SIP
Confirmed anaerobic/
aerobic pathways possible

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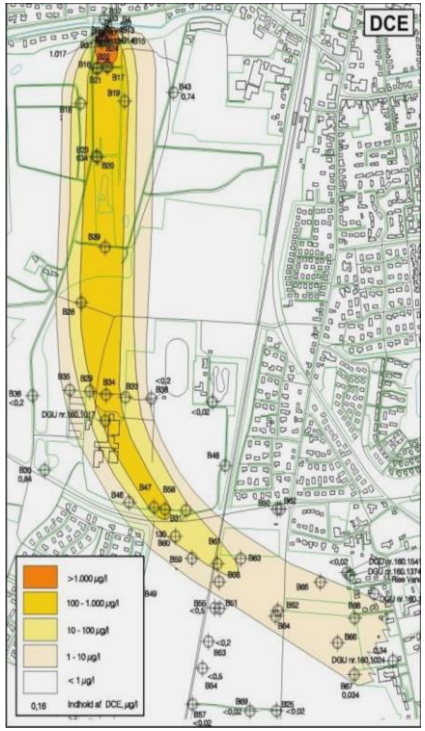
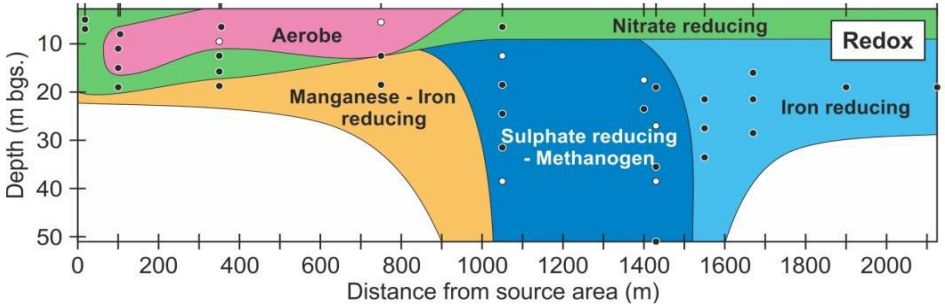
Denmark Site[2]

- PCE source from central dry-cleaning facility
- Source zone steam treatment –Downgradient MNA
- 2 km long plume mainly TCE and cDCE with range of geochemical conditions
- VC and ethene ND- Complete dechlorination?
- Study consisting of molecular tools, qPCR, NGS, dual Isotope CSIA





Conclusions Denmark Site

- *Dehalococcoides* and *Dehalogenimonas* dominated near source zone
- Of the >5,000 microorganisms identified by NGS an additional 13 potential; dechlorinators were flagged including *Geobacter*, *Polaromonas*, *Nocardiodes*
- Dual Isotope CSIA concluded that degradation processes for cDCE were primarily abiotic and mediated by pyrite FeS_2

"Analysis for microbial composition ... as well as dual stable isotopes has revealed high complexity in degradation processes and played an important role to substantiate the natural attenuation of the plume"$^{2-}$ Badin et al., 2016

Conclusions

- Molecular Tools and Isotopic Methods provide synergistic data that can clarify complex remediation scenarios

  - MNA
  - Abiotic degradation
  - Enhanced bioremediation

Thank you for Attending!

Questions?

Further Information
Phil Dennis,
(pdennis@siremlab.com) 519-515-0836

siremlab.com

Toll Free 1-866-251-1747

References

[1]Badin, Alice , Mette M. Broholm, Carsten S. Jacobsen , Jordi Palau , Philip Dennis , Daniel Hunkeler. 2016. ***Identification of abiotic and biotic reductive dechlorination in a chlorinated ethene plume after thermal source remediation by means of isotopic and molecular biology* tools.** *Journal of Contaminant Hydrology* 192 (2016) 1–19

[2] Smith, Simone, Neal Durant, and Amar Wadhawan Jeff Roberts and Jennifer Webb –Pete Stang, Greg Alyanakian, and John Willis –Trevet, LetaMaclean, Doug Roff, and Crispin Wanyoike, Bart Chadwick, Michael Pound. ***Development and Application of a $^{13}CO_2$ Method for Measuring Aerobic Mineralization of Vinyl Chloride in Marine Sediment Porewater Poster*** *Battelle Bioremediation Symposium 2017, Miami, FL*