Metabolomics, Lipidomics, and Metagenomics:

Multiple Lines of Evidence for Monitored Natural Attenuation

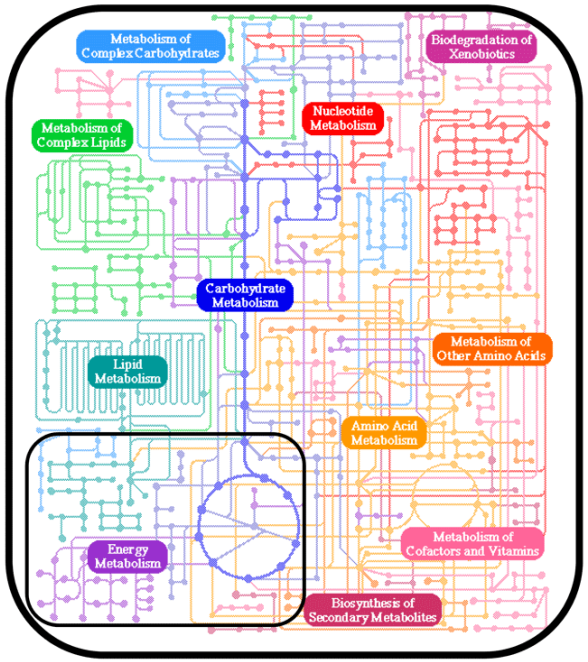
THE UNIVERSITY OF TENNESSEE

microbialinsights

Metabolomics

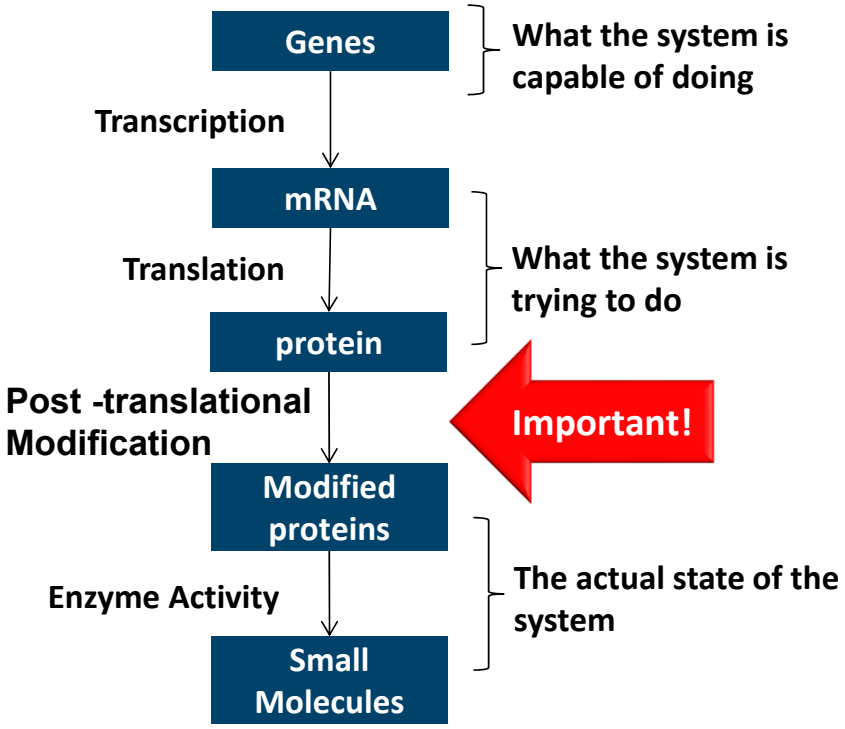
- Metabolomics: Systematic study of unique chemical fingerprints that result from specific cellular processes.
- Metabolome: The collection of all metabolites in a biological cell, tissue, organism, or environment.
- Metabolic Profiling: Instantaneous snapshot of cell physiology

Representation of the cellular metabolome



https://www.urmc.rochester.edu/labs/munger.aspx

Why Metabolomics?



Molecular Biology Tools (MBTs) like qPCR are well established and have become an integral component of remedy selection and performance monitoring

Metabolomics provides an opportunity to monitor directly the metabolism of contaminant degraders

Rabinowitz, J.D., (2007), *Expert Review or Proteomics*, 187-198

Evaluating MNA – Chlorinated Sites

VOCs & CSIA

- Daughter product formation
- Isotopic fractionation

qPCR

- Concentrations of sMMO, PHE, RMO
- Concentrations of DHC and RDases

DHC > 10^4 cells/mL

Generally useful rates (Lu et al., 2006)

Ethene production (MI Database)

Metabolomics

- Metabolite profiles that correlate with biodegradation activity
- Overall health of halorespiring community
- Specific metabolites?

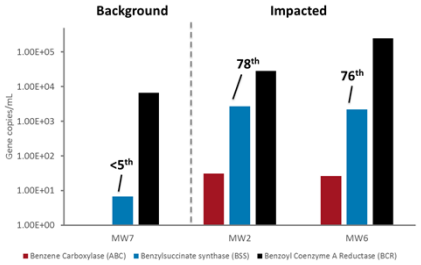
Evaluating MNA – Petroleum Sites

VOCs, Geochem & SIP

- Decreasing or stable trends
- Electron acceptor consumption
- ^{13}C incorporation into biomass and CO_2

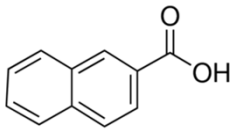
qPCR

- Concentrations of functional genes (e.g. TOD, BSS)
- Background vs. plume
- Low, medium, or high



Metabolomics

- Metabolite profiles that correlate with biodegradation activity
- Overall health
- Specific metabolites



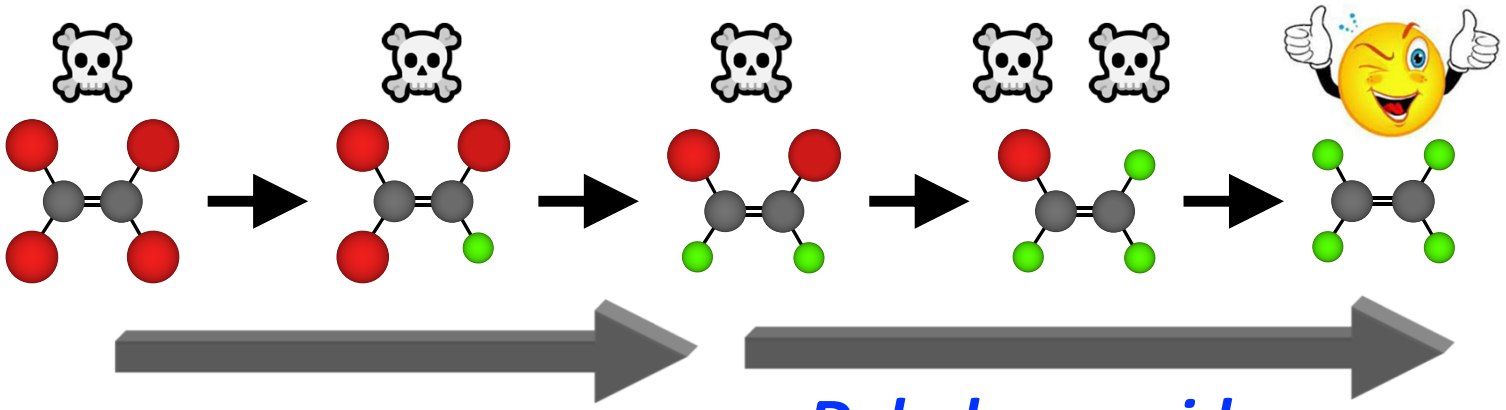
2-naphthoic acid
Phelps et al., 2002 "Metabolic biomarkers for monitoring anaerobic naphthalene biodegradation *in situ*".

Metabolomics at UTK

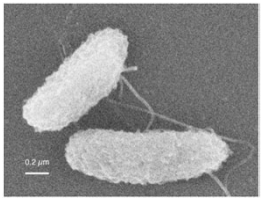
Older Platform	Current Platform	Current Platform	Current Platform	Newest Addition
HPLC-Triple Quadrupole	**UPLC-Orbitrap**	**UPLC-Orbitrap**	**GC or UPLC-Orbitrap**	**UPLC-Quadrupole-Orbitrap**
Capabilities: **~120 Known Water Soluble Compounds and Flux Analyses** **No Molecular Discovery** **2 h/sample**	**Capabilities:** **~150 Known Water Soluble Compounds and Flux Analyses** **~8,000 Spectral Features from Unknowns** **0.5 h/sample**	**Capabilities:** **~400 Known Lipid-Like Compounds and Flux Analyses** **~12,000 Spectral Features from Unknowns** **1.5 h/sample**	**Capabilities:** **Technology Development** **~10,000 Spectral Features from Unknowns** **Analysis of the Extracellular Matrix or for Discovery of Compound of Intermediate Molecular Weight**	**Capabilities:** **Molecular Characterization, Structural Elucidation, Proteomics, and Glycomics**

Halorespiring Bacteria

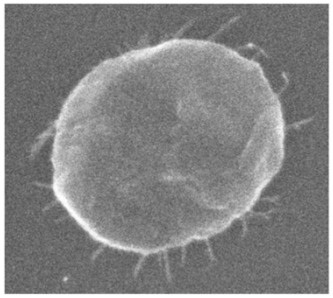
Stall? VC is more toxic!



Geobacter lovleyi, Dehalobacter, Sulfurospirillum, Desulfuromonas, Desulfitobacterium



Dehalococcoides

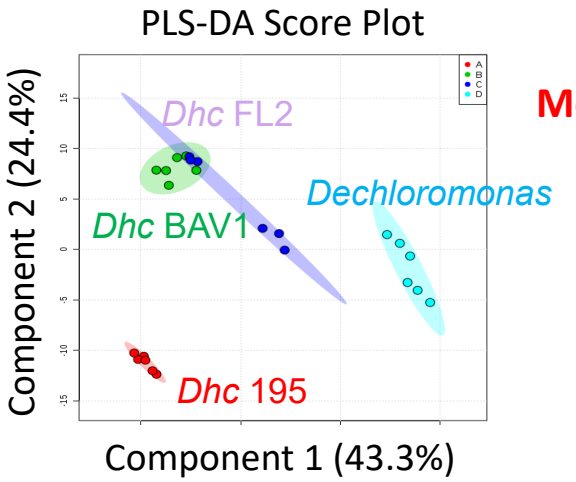


Löffler et al. 2013, IJSEM, 63:625

He et al. 2003, Nature, 424:62

First Steps: Metabolomics of Isolates

	***Dehalococcoides mccartyi* strain 195**	***Dehalococcoides mccartyi* strain BAV1**	***Dehalococcoides mccartyi* strain FL2**	***Dechloromonas aromatica* strain RCB**
Number Detected Metabolites*	3203	3255	3069	3014
Number Identified Metabolites	105	99	103	114
# Metabolites unique to strain	209	256	155	157



Metabolic profiles were different between *Dhc* and *Dechloromonas*

Metabolic profiles were even different between *Dhc* strains

Partial least squares – discriminant analysis (PLS-DA)

Next Steps: Metabolomics of SDC-9™

Two Experiments

- SDC-9 + Lactate + **cis-DCE**
- SDC-9 + Lactate + **VC**
- Both with Controls (No Addition, Media, Stock)

Sampling and Analysis

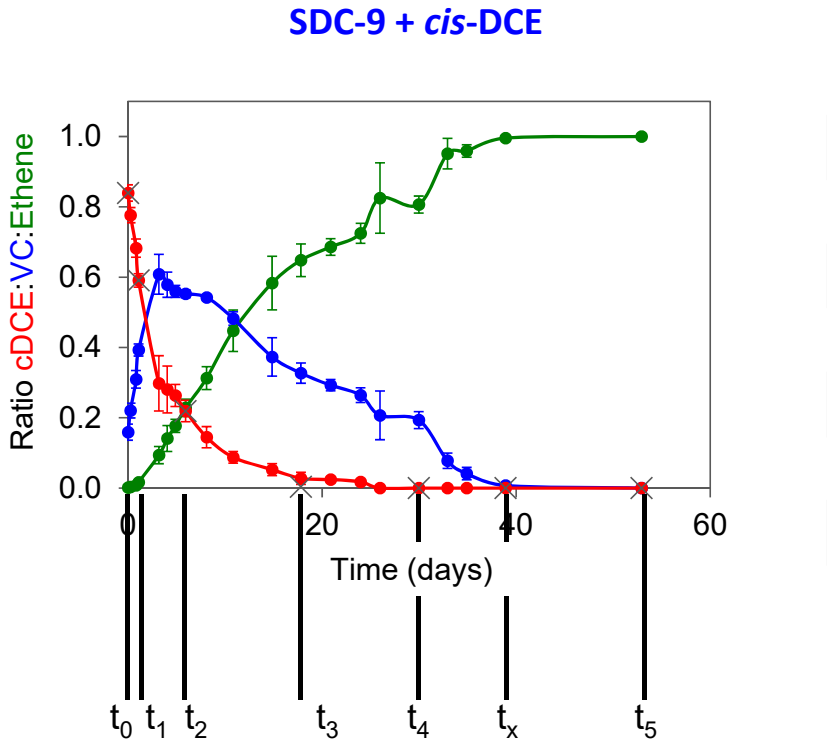
- Monitored over time based on extent of reductive dechlorination
- VOCs – Chlorinated ethenes
- qPCR – *Dhc* and Total Bacteria
- VFAs – Lactate
- Metabolomics

Next Steps: Metabolomics of SDC-9

		SDC-9 + *cis*-DCE		SDC-9 + VC	
Timepoint	**% Cl remaining**	**+ cDCE**	**No Addition**	**+ VC**	**No Addition**
$t_{initial}$	100%	n = 3		+ VC	No Addition
t_0	>90%	n = 5	n = 3	n = 5	
t_1	60% – 80%	n = 5		n = 5	n = 5
t_2	20% – 40%	n = 5		n = 5	n = 5
t_3	0%	n = 5	n = 3	n = 5	n = 5
t_4	5 days after t_3	n = 5		n = 5	n = 5
t_x	0% VC (+ cDCE only)	n = 3			
t_5	~25 days after t_3	n = 5	n = 3		
t_{stock}			n = 1	n = 5	n = 5
Media		n = 3			n = 1

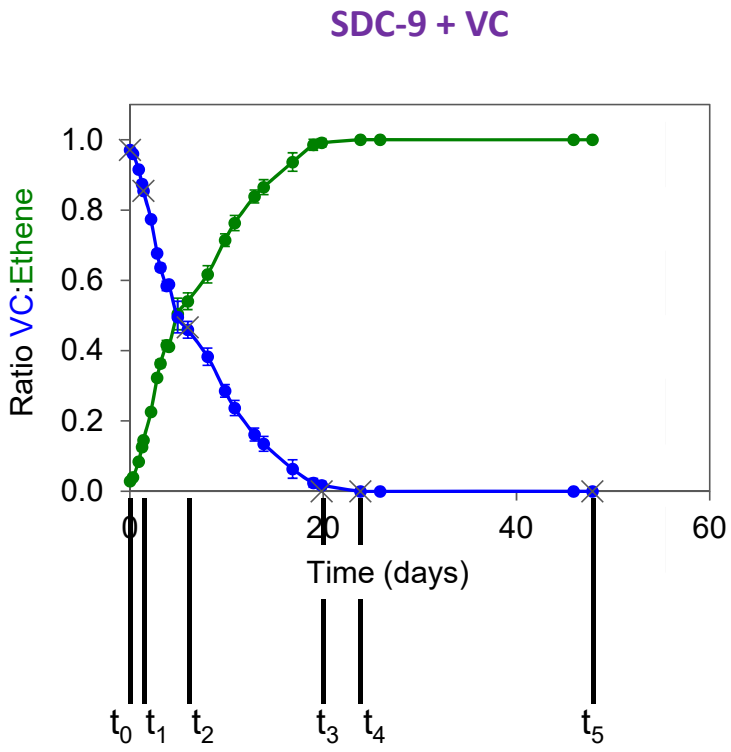
Kinetics of cis-DCE Dechlorination

Timepoint	% Cl remaining
$t_{initial}$	100%
t_0	>90%
t_1	60% – 80%
t_2	20% – 40%
t_3	0% cis-DCE
t_4	5 days after t_3
t_x	0% VC
t_5	~25 days after t_3

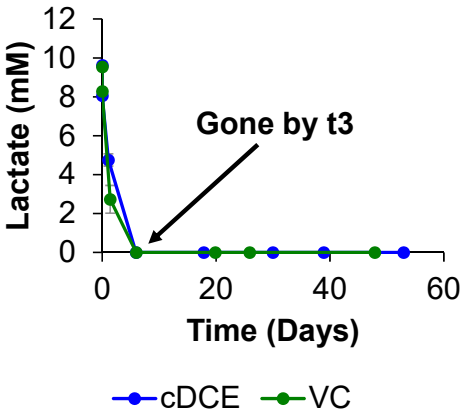


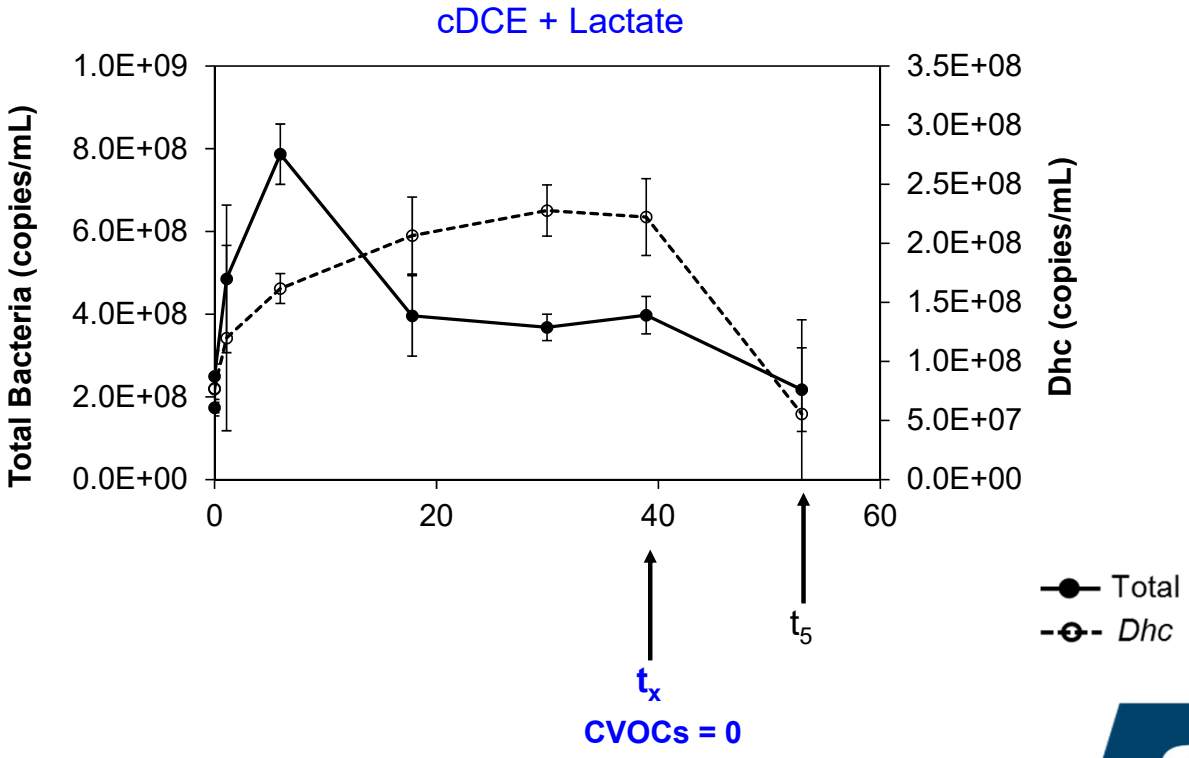
Kinetics of VC Dechlorination

Timepoint	% Cl remaining
$t_{initial}$	100%
t_0	>90%
t_1	60% – 80%
t_2	20% – 40%
t_3	0%
t_4	5 days after t_3
t_x	Not Applicable
t_5	~25 days after t_3

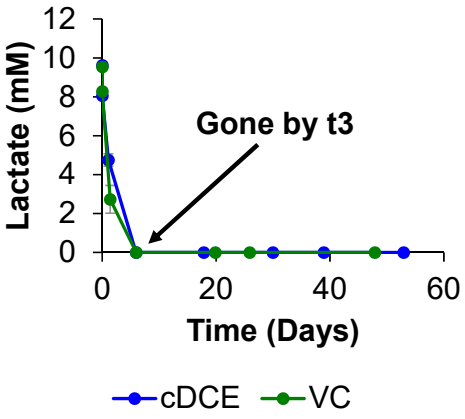


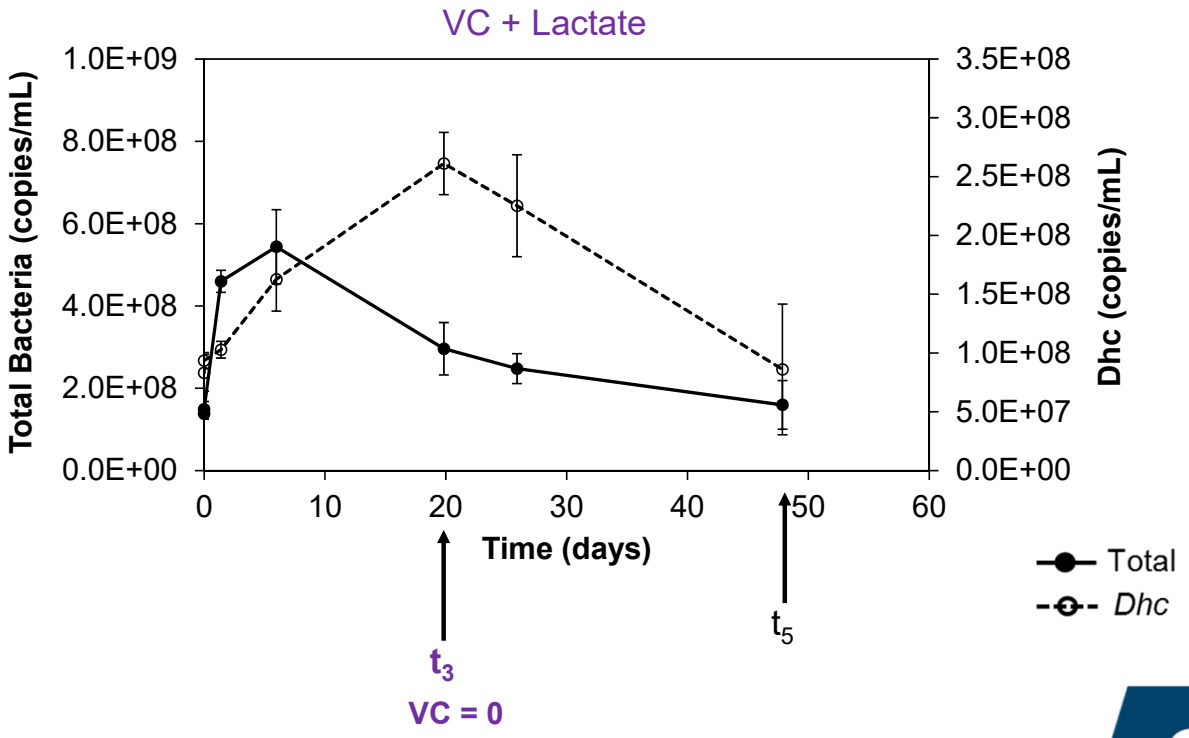
Kinetics & Growth: SDC-9 + cis-DCE





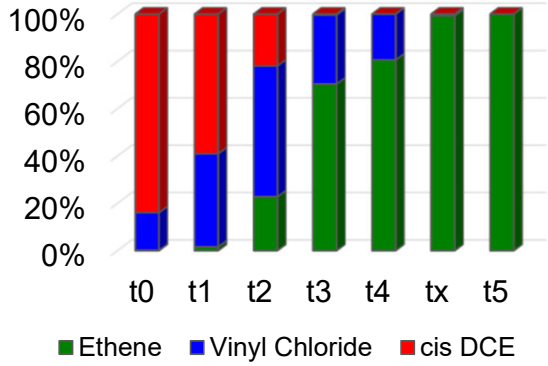
Kinetics & Growth: SDC-9 + VC





Metabolomics for cis-DCE Addition

	All Features	De-isotoped De-adducted	% Remaining	Knowns	% Known
$t_{initial}$	9,333	7,313	78.4%	225	3.08%
t_0	11,100	8,507	76.6%	224	2.63%
t_1	12,036	8,940	74.3%	232	2.60%
t_2	12,488	9,266	74.2%	232	2.50%
t_3	11,981	9,057	75.6%	224	2.47%
t_4	10,576	8,183	77.4%	222	2.71%
t_x	9,384	7,346	78.3%	217	2.95%
t_5	9,812	7,660	78.1%	204	2.66%



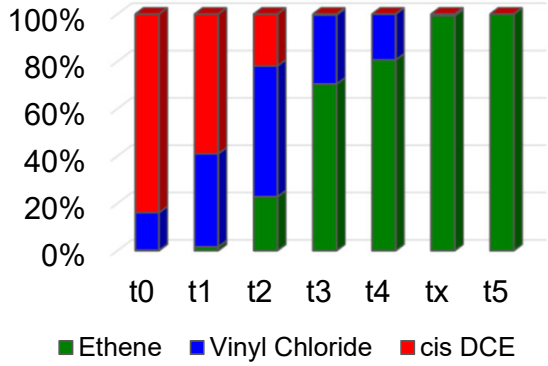
Metabolomics for cis-DCE Addition

	All Features	De-isotoped De-adducted	Knowns	
$t_{initial}$	9,333	7,313	225	$t_{initial}$
t_0	11,100	8,507	224	t_0
t_1	12,036	8,940	232	t_1
t_2	12,488	9,266	232	t_2
t_3	11,981	9,057	224	t_3
t_4	10,576	8,183	222	t_4
t_x	9,384	7,346	217	t_x
t_5	9,812	7,660	204	t_5

← Inactive

Active

← Inactive



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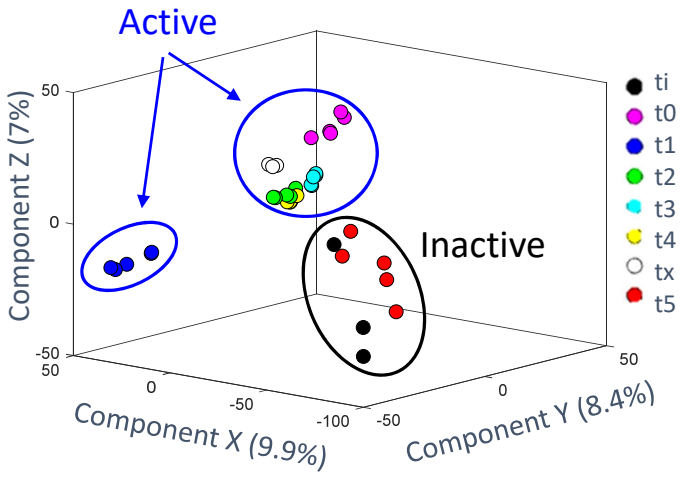
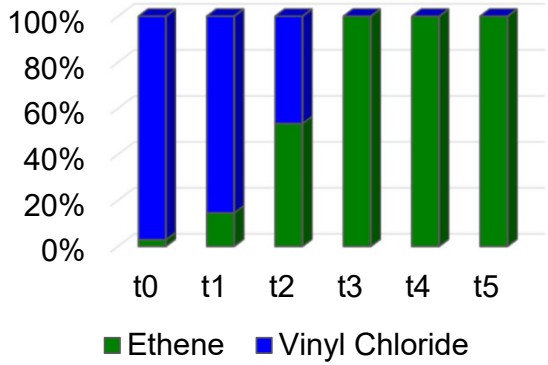


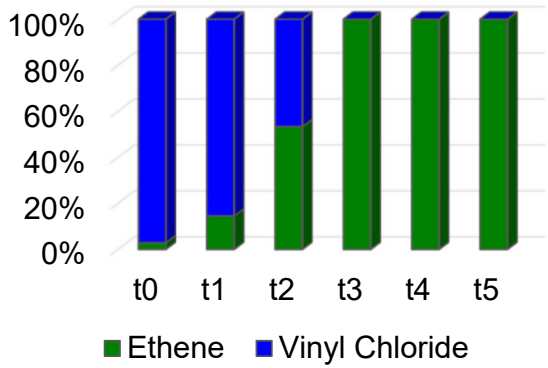
Metabolomics for VC Addition

	All Features	De-isotoped De-adducted	% Remaining	Knowns	% Known
$t_{initial}$	6,319	4,633	73.3%	226	4.88%
t_0	10,825	7,389	68.3%	227	3.07%
t_1	11,836	8,018	67.7%	230	2.87%
t_2	12,432	8,526	68.6%	233	2.73%
t_3	11,527	8,723	75.7%	224	2.57%
t_4	9,979	7,739	77.6%	219	2.83%
t_5	5,831	4,833	82.9%	216	4.47%



Metabolomics for VC Addition

	All Features	De-isotoped De-adducted	Knowns	
$t_{initial}$	6,319	4,633	226	← Inactive
t_0	10,825	7,389	227	
t_1	11,836	8,018	230	Active
t_2	12,432	8,526	233	Active
t_3	11,527	8,723	224	Active
t_4	9,979	7,739	219	
t_5	5,831	4,833	216	← Inactive



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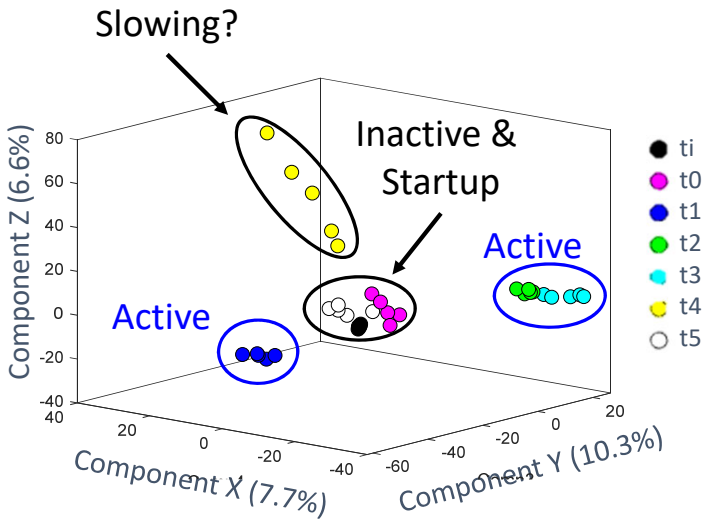


Ground Truthing the Method

qPCR Data:

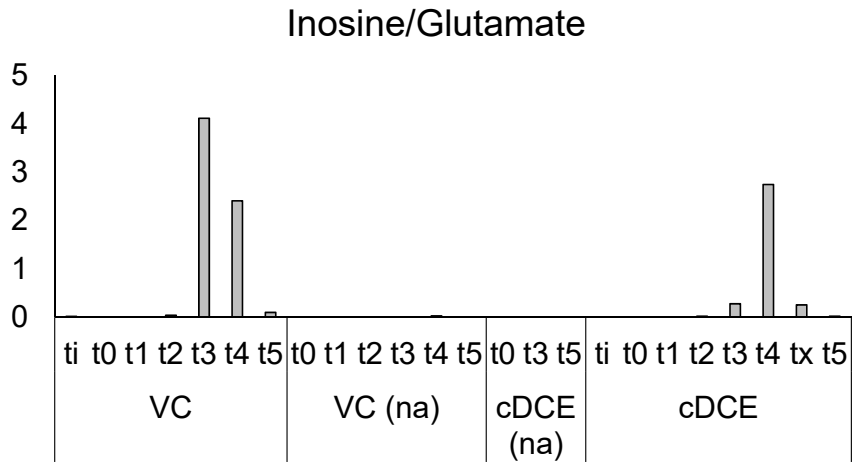
	TotalBac_16S	16S_DHC	tceA	vcrA	bvcA	fdhA
Well 1	2.04E+04	Detectable not quantifiable				
Well 2	1.37E+05	1.32E+02	1.10E+01	3.03E+01	2.88E+01	4.67E+01
Well 3	4.87E+05	3.44E+04	8.64E+02	5.16E+04	1.74E+04	4.57E+04
Well 4	2.83E+06	3.68E+05	1.88E+05	4.11E+05	8.04E+04	4.79E+05

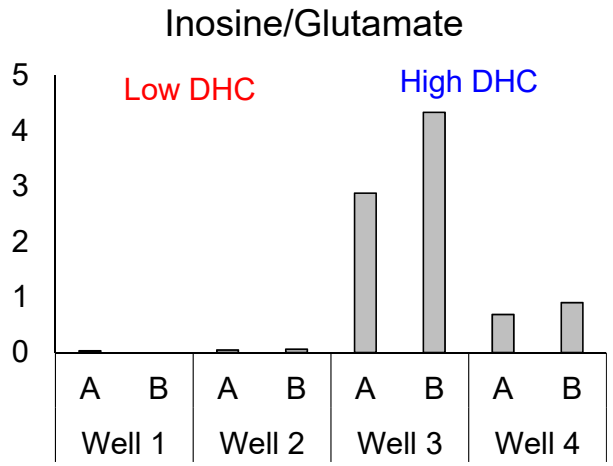
Metabolomics Data:

	All Features	De-isotoped De-adducted	% Remaining	Knowns	% Known
Well 1	11,139	7,815	70.2%	121	1.54%
Well 2	9,570	6,824	71.3%	110	1.61%
Well 3	8,875	6,421	72.3%	145	2.26%
Well 4	5,530	4,318	78.1%	140	3.24%

Finding Biomarkers: Preliminary Efforts

Dr. Campagna's lab has begun the analysis to determine whether metabolite signatures can be used to predict the state of remediation efforts, but much is left to be done.





Wells 3 and 4 are most likely undergoing active dechlorination based on qPCR data, and the glutamate normalized inosine ratios might be an easily measured biomarker for activity as well. Many more signatures are also likely.

Finding Biomarkers: Preliminary Efforts

SDC-9 PLSda Top 50 VIP scores			Detected in Groundwater Features		
Treatment	m/z	rt (min)	m/z	rt (min)	Retention time match
cDCE	160.0331	12.09	160.0331	12.12	Excellent
VC	271.0436	11.81	271.0436	11.66	Excellent
cDCE	272.0679	12.09	272.0679	12.08	Excellent
cDCE	249.0979	4.54	249.0979	3.61	Good
VC	353.0139	13.94	353.0139	16.84	Bad
VC	190.0768	1.27	190.0768	1.26	Excellent
VC	186.9568	6.36	186.9568	6.30	Excellent
VC	244.0654	2.91	244.0655	2.91	Excellent
VC	175.0026	0.78	175.0027	0.80	Excellent
cDCE	112.0484	14.31	112.0485	13.55	Good
cDCE	223.0743	13.34	223.0742	11.93	Fair
VC	151.0430	8.71	151.043	7.22	Fair
VC	190.9492	12.61	190.9491	12.63	Excellent
cDCE	138.0559	6.63	138.056	5.86	Good
VC	318.0111	0.77	318.0114	0.79	Excellent

Each of the compounds in the table were detected in cultures that had been exposed to chlorinated compounds.

Conclusions - Metabolomics

- Rapidly emerging technology
- Great potential to reveal activity
- Metabolic profiles can distinguish…
  - Active vs inactive
  - Degrading vs stalled
  - Healthy vs inhibited
- Ongoing identification of metabolic biomarkers
- Targeted metabolomics

Final Thoughts

Routine Lines of Evidence

- VOC trends
- Daughter products
- Geochemistry
- CSIA or SIP
- qPCR or QuantArray

Performing Additional Omics

- Complex sites
- High risk sites
- Emerging contaminants
- Routine evidence is inconclusive

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Thank you
for your time

Are there any final questions?

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