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How Treatability and Molecular Testing Saves Time, Money and Heartburn

Phil Dennis

Remediation Seminars
Short Courses

May 8-10 2018
Hartford, CT/Boston, MA

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Two Key Questions

- What is the best remediation approach?

- Once implemented is the remediation strategy working?

Treatability and molecular testing help to answer both questions

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THE WHAT, WHY AND HOW OF TREATABILITY STUDIES

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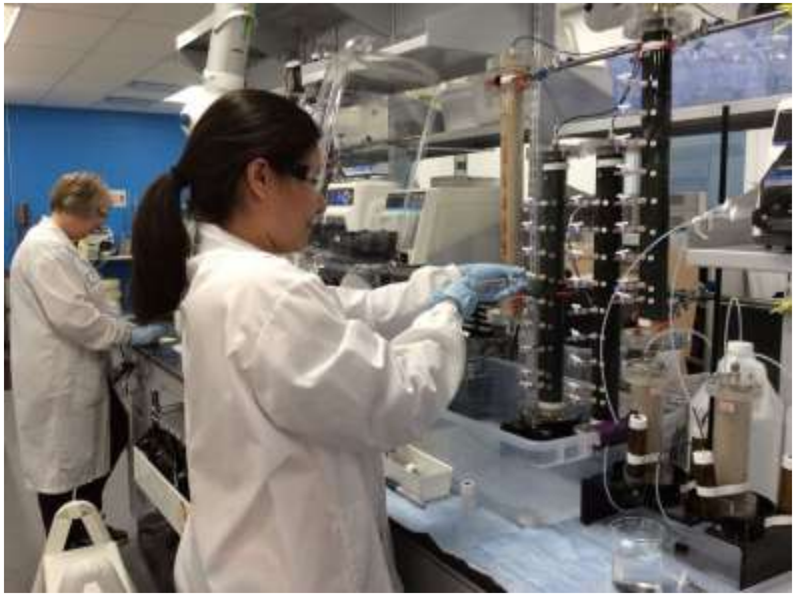
What is a Treatability Test?

- Laboratory “bench-scale” testing
- Use site soil, sediment or rock and groundwater
- Microcosms or columns constructed using site materials
- Customize treatment variables to meet site specific needs
- Monitor contaminant degradation under various conditions

treatability studies

Treatability studies are typically microcosm or column tests for technologies including:

- Anaerobic and aerobic bioremediation
- In situ chemical reduction (e.g., ZVI)
- In situ chemical oxidation
- Sediment remediation



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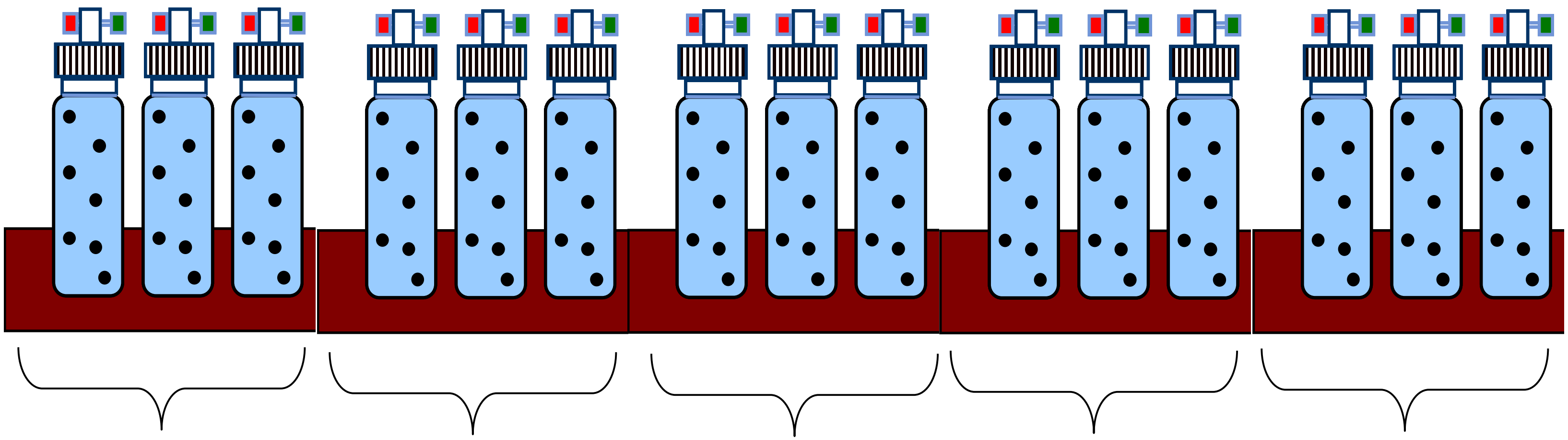
Microcosm Study Typical Design



Sterile Control
autoclaved and poisoned to inhibit microbes measure possible abiotic losses

Active Control
unamended

Biostimulation
addition of organic electron donors

Bioaugmentation+ Biostimulation
addition of known degrading populations e.g., KB-1

Gas Addition
H_2/O_2 addition etc. To measure impact of gas infusion /cometabolic processes e.g. propane addition

Treatability studies are custom designed for each site

What Treatability Studies Can Tell You?

- Electron donor/acceptor/cometabolite consumption
- Degradation intermediates/pathways
- Effect of controlling variables (e.g., pH, redox, amendment addition, inhibitory effects, oxidant demand, persulfate activators)
- Residence time/longevity for PRBs
- Contaminant degradation rates/lag times
- Insight into pilot–test design

Why Use a Treatability Test?

- Allows evaluation of multiple remedial options prior to field implementation
- Optimization of a selected remedy
- Studies are flexible allowing changes “on the fly” in the lab
- Regulatory approval for injections is not required
- Manageable, incremental risk from lab to pilot to full-scale
- Reassures stakeholders that the selected remediation approach is feasible prior to field implementation

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TREATABILITY CASE STUDIES

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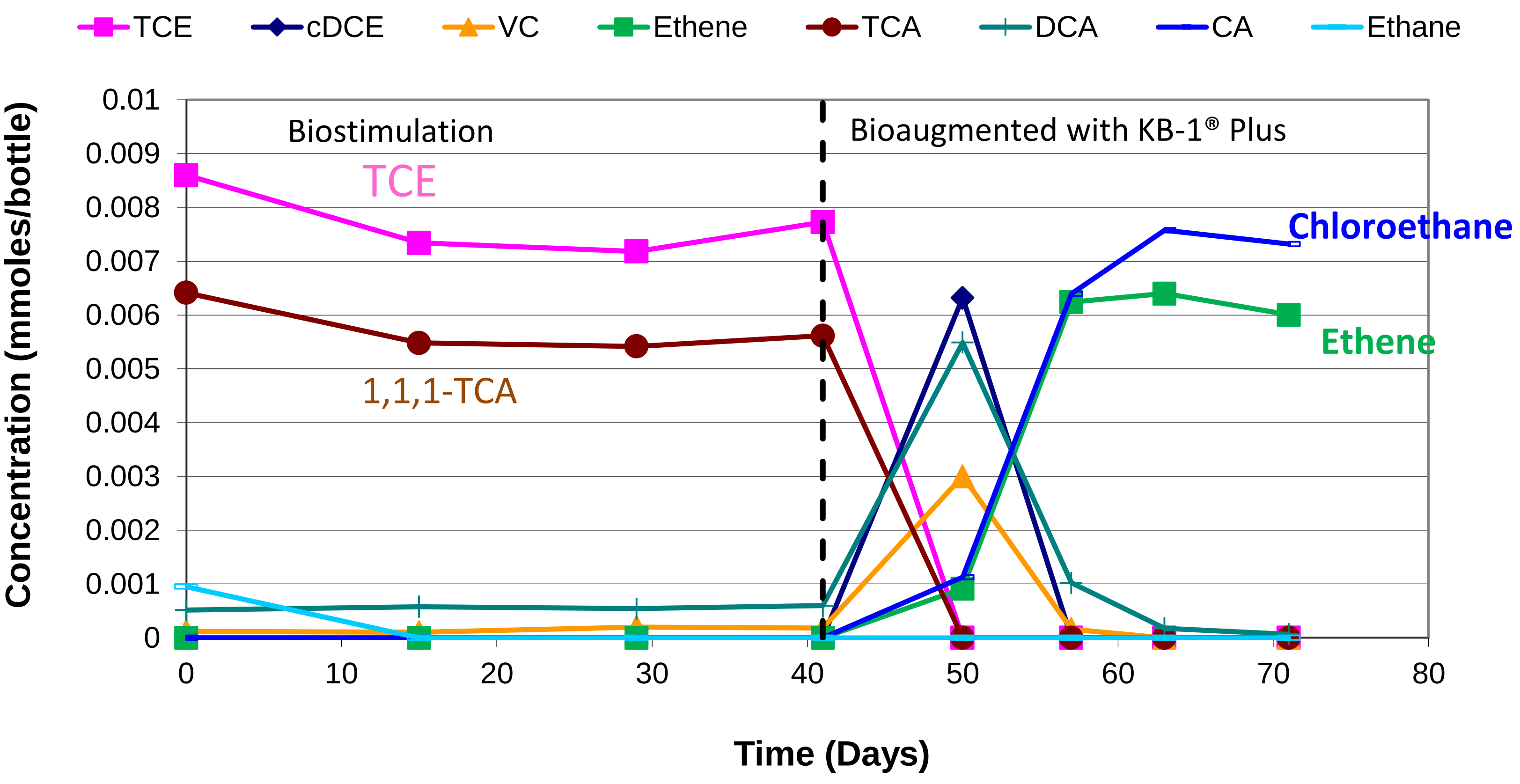
Case Study: Denmark Site

- Mixed chlorinated ethenes and ethanes
- 1,1,1-TCA (5 mg/L) and TCE (5mg/L)
- Can potential inhibition by 1,1,1-TCA be overcome?
- Is ISCO with persulfate viable remedial option?

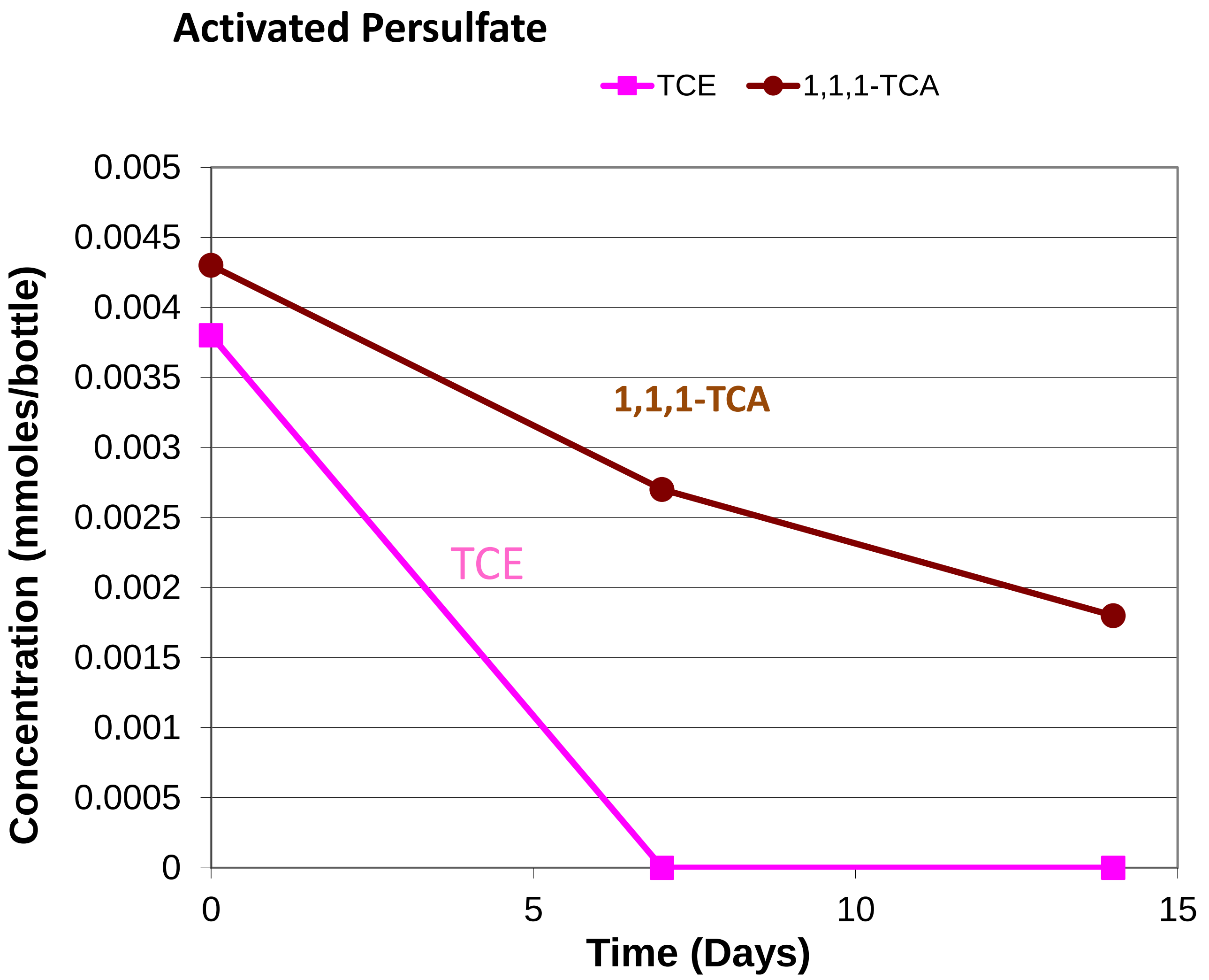
Study Design:

- Anaerobic Sterile Control
- Anaerobic Active Control
- EVO Amended/KB-1® Plus Bioaugmented
- Base Activated Persulfate

Case Study: Denmark Site



Case Study: Denmark Site



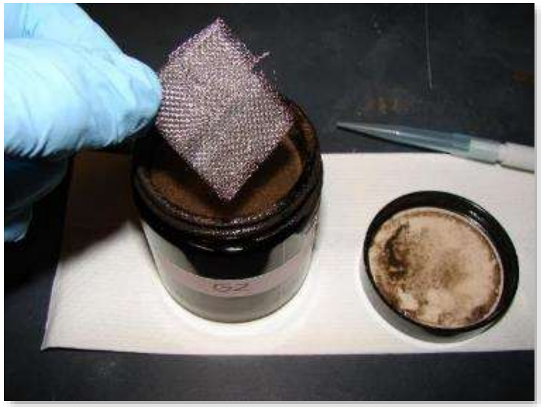
Conclusions-Denmark Study

- Biostimulation alone=no dechlorination TCE/1,1,1-TCA
- KB-1® Plus bioaugmentation + biostimulation= rapid dechlorination-but with chloroethane accumulation
- Activated persulfate complete and rapid degradation of TCE slower and incomplete for 1,1,1-TCA

Based on study results enhanced bioremediation was selected as site remedy

Treatability Study for Active Cap Optimization

- Bench-scale treatability test to evaluate how much activated carbon (SediMite™) to add a PCB-impacted sediment



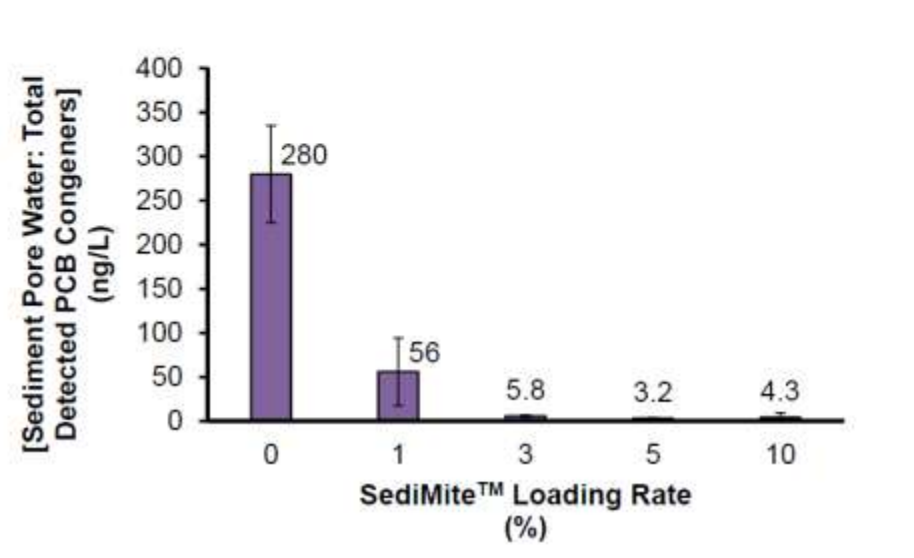
- PCB availability was measured because addition of the carbon changes availability not total PCB concentration

- Availability measured via SiREM passive samplers (SP3™) in site sediment amended with different SediMite™ loading rates

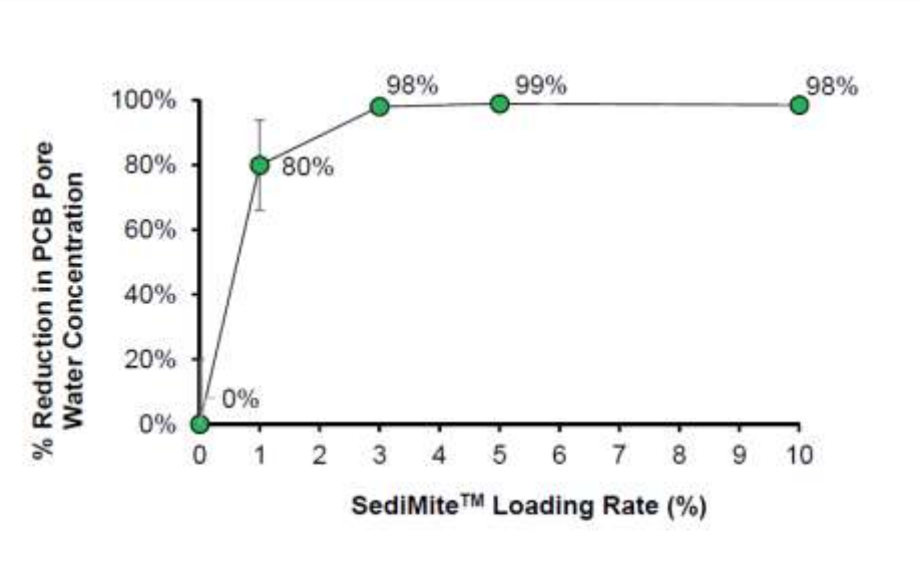
Image courtesy of SediMit

Case Study: PCB Active Cap Optimization

- Study results revealed significant reduction in PCB availability even at low SediMite loadings (1-3%)



Study cost~$10K findings saved more than $100K in excess SediMite costs



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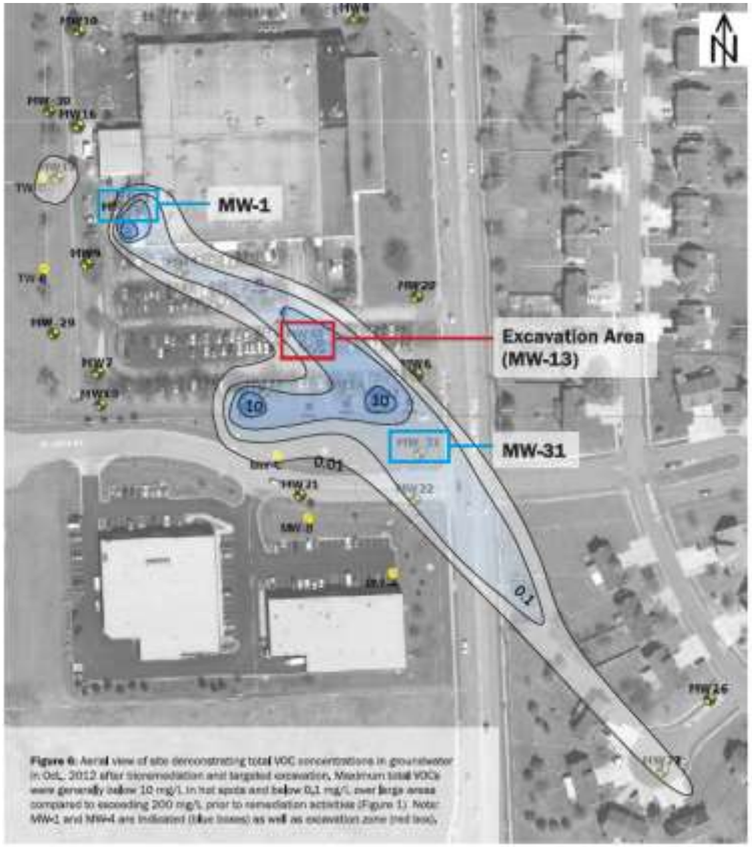
Treatability Testing Aided Decision Making

Kansas site with high concentration mixed VOCs including dichloromethane

- MW-1:10 mg/L DCM attenuated successfully
  MW-13: 200 mg/L DCM-degradation not observed

- Treatability testing indicated that >160 mg/L DCM was not biodegradable with available bioaugmentation cultures

- 500 tons of soil in MW-13 area removed in 2009 to remove DCM source area



Study justified moving quickly to excavation saved time and money on likely futile bioremediation attempt

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MOLECULAR GENETIC TESTING

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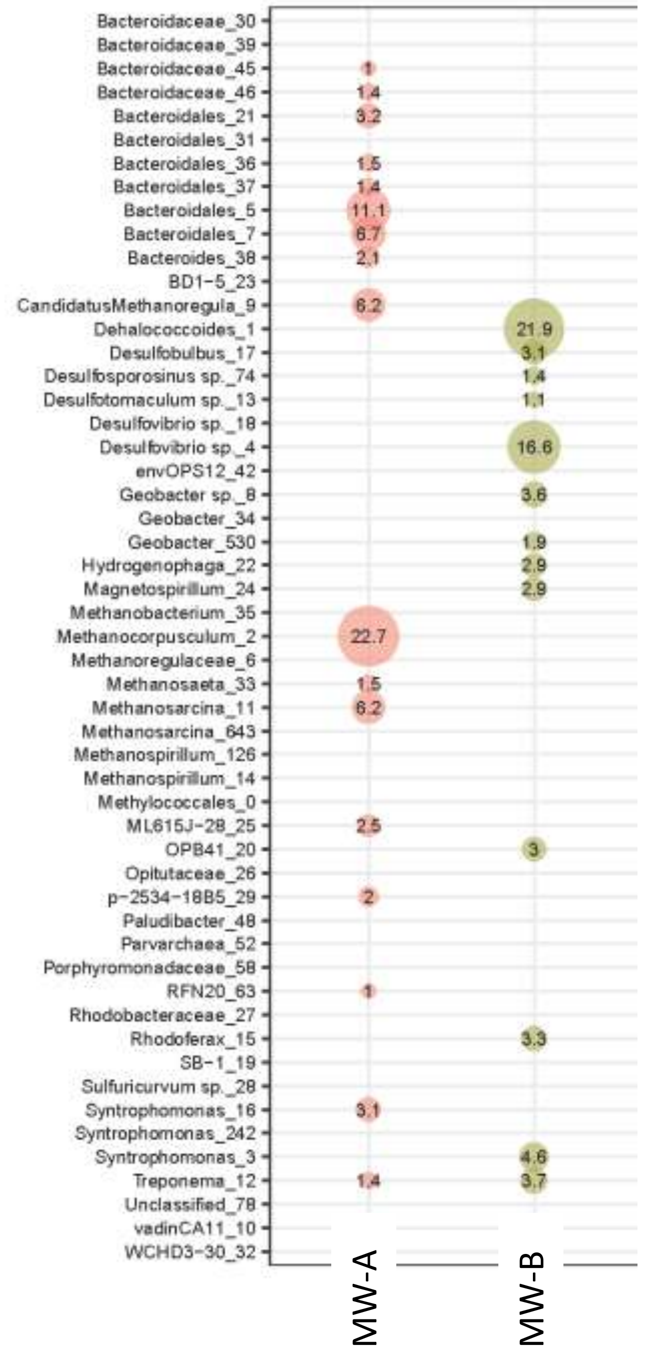
Molecular Genetic Testing

- For site remediation typically DNA based tests on groundwater/soil
- Quantitative polymerase chain reaction (qPCR) tests used to quantify specific microorganisms and functional genes critical to bioremediation processes
- Next generation sequencing (NGS) to characterize entire microbial population

Bubble plot output from NGS report (right) indicates the relative proportion of the major microbes in a sample



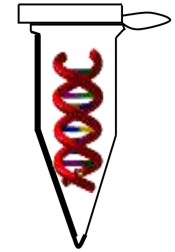
SiREM genetrac

Overview of Gene-Trac® qPCR Testing

1) Groundwater Sampling

2) Transport 1L GW or field filter to Lab

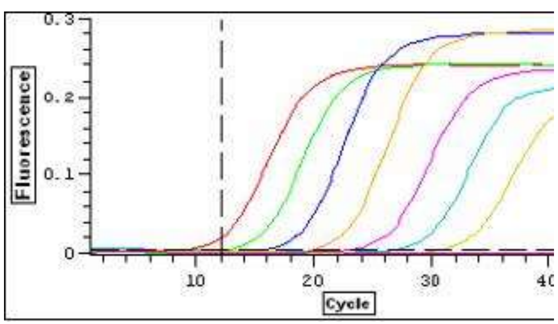
3) Filter groundwater water samples (NA for field filter)



4) Extract DNA from filter

5) Assemble PCR Reactions

6) PCR amplify specific genes (e.g., 16S rRNA/*vcrA*) with targeted primers in qPCR Machine



7) qPCR output used to calculate gene copies /L groundwater

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Tests available for a wide range of contaminant classes …

Contaminant Class	Redox	Gene-Trac® Test Name	Target	Relevance
Chlorinated Ethenes	Anaerobic	Dhc	*Dehalococcoides*	Dechlorinates PCE, TCE, all DCE isomers, VC
		Dhb	*Dehalobacter*	Dechlorination of PCE &TCE to cDCE
		Dsm	*Desulfuromonas*	Dechlorination of PCE & TCE to cDCE
		Dsb	*Desulfitobacterium*	Partial dechlorination of PCE and TCE to cDCE
		Geo	*Geobacter*	Dechlorinates PCE to cDCE/biogeochemical degradation
		Dhg	*Dehalogenimonas*	Dechlorination of tDCE to VC and VC to ethene
		Chloroethene FGA	Vinyl Chloride Reductase (*vcrA*)	Dechlorination of cDCE & VC to ethene
			BAV1 Reductase (*bvcA*)	Dechlorination of cDCE and VC to ethene
			Trichloroethene Reductase (*tceA*)	Dechlorination of PCE and TCE to cDCE and VC
	Aerobic	Polaromonas	*Polaromonas*	Aerobic dechlorination of cDCE
		etn	*etnE*	Aerobic degradation of VC
Chlorinated Ethanes	Anaerobic	Dhb	*Dehalobacter*	Dechlorinates 1,1,1-TCA/1,2-DCA /1,1,2-TCA/ 1,1,2,2-TeCA
		Dhg	*Dehalogenimonas*	Dechlorinates 1,2- DCA, 1,1,2,2-TeCA , 1,1,2-TCA
		Dhc	*Dehalococcoides*	Dechlorinates 1,2-DCA to ethene
		Dsb	*Desulfitobacterium*	Dechlorinates 1,1,2-TCA &1,2-DCA
		cfrA/dcrA	Dichloroethane Dehalogenase (*dcrA*)	Dechlorinates 1,1,1-TCA & 1,1-DCA
	Aerobic	sMMO	Soluble Methane Monooxygenase	Co-metabolism of 1,1,1-TCA & 1,1-DCA by methanotrophs
		PMO	Propane Monooxygenase	Co-metabolism of chlorinated ethanes by propanotrophs
		dhlA	Haloalkane Dehalogenase (*dhlA*)	Aerobic dechlorination of 1,2-DCA
Chlorinated Methanes	Anaerobic	Dhb	*Dehalobacter*	Dechlorination of chloroform to DCM; DCM to acetate
		cfrA/dcrA	Chloroform Reductase (*cfrA*)	Converts chloroform to dichloromethane
	Aerobic	sMMO	Soluble Methane Monooxygenase	Co-metabolism of chloroform & dichloromethane
Chlorinated Propanes	Anaerobic	Dhg	*Dehalogenimonas*	Converts TCP to allyl chloride; DCP to propene
		Dhc	*Dehalococcoides*	Converts DCP to propene
		Dhb	*Dehalobacter*	Converts DCP to propene
		Dsb	*Desulfitobacterium*	Dechlorination of TCP & DCP
Chlorinated Benzenes	Anaerobic	Dhc	*Dehalococcoides*	Partial dechlorination of HCB/PCB
		Dhb	*Dehalobacter*	Reductive dechlorination of DCB, MCB
Chlorinated Phenols	Anaerobic	Dhc	*Dehalococcoides*	Dechlorination of 2,3-dichlorophenol, TCP and PCP
PCBs	Anaerobic	Dhc	*Dehalococcoides*	Dechlorinates select Arochlor 1260 congeners
		Dhb	*Dehalobacter*	Dechlorinates 2,3,4-trichorobiphenyl; 2,3,4,5-tetrachlorobiphenyl
		Dhg	*Dehalogenimonas*	Dechlorinates select Arochlor 1260 congeners
BTEX	Anaerobic	SRB	Sulfate reducing bacteria (*dsrA*)	Partners to ORM-2 in anaerobic benzene degradation
		ORM-2	*Deltaproteobacterium ORM-2*	Anaerobic benzene degrader (SO_4/CH_4 reducing conditions)
		Pepto-ben	Benzene degrading *Peptococcaceae*	Anaerobic benzene degrader under NO_3 reducing conditions
		abcA	Benzene Carboxylase (*abcA*)	Involved in benzene ring cleavage
Fuel Oxygenates	Aerobic	MTBE/TBA	*Methylibium petroleiphilum* PM1	MTBE/TBE degrading microorganism
			tert-butyl alcohol hydroxylase (*mdpJ*)	Active on TBA in aerobic MTBE degradation pathway
			HIBA mutase (*hcmA*)	Active on 2-HIBA in aerobic MTBE degradation pathway
1,4-Dioxane	Aerobic metabolism	1,4-dioxane	Dioxane monooxygenase (*dxmb*)	Energy yielding 1,4-dioxane degradation
		1,4-dioxane	Aldehyde Dehydrogenase	Energy yielding 1,4-dioxane degradation
	Aerobic Cometabolism	pMMO	Particulate Methane Monooxygenase	Co-oxidation of 1,4-dioxane in presence of methane
		sMMO	Soluble Methane Monooxygenase	Co-oxidation of 1,4-dioxane
		PMO	Propane Monooxygenase	Co-oxidation of 1,4-dioxane in presence of propane
Nitrogen	Anaerobic	Anammox	Major anammox genera	Anaerobic co-removal of ammonium and nitrite
Prokaryotic Groups	Variable	Universal	Bacteria	Quantifies *Bacteria*-measure of total biomass
		Arch	Archaea	Quantifies *Archaea* biomass
		SRB	Sulfate reducing bacteria (*dsrA*)	Anaerobic hydrocarbon oxidation/biogeochemical reduction/MIC
		NGS	*Bacteria/Archaea*	Comprehensive characterization of microbial communities

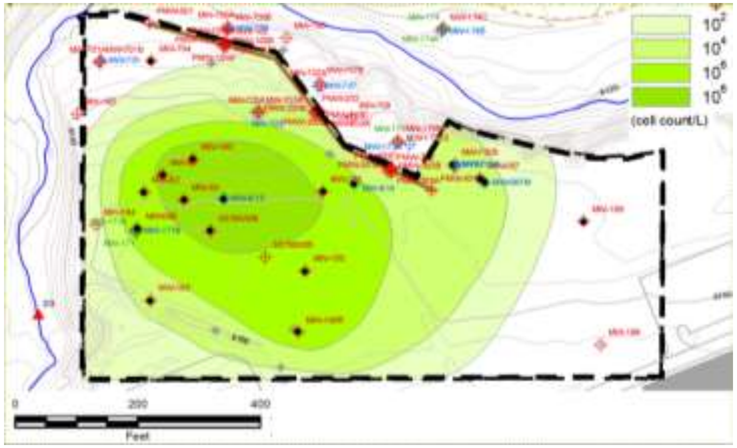
Specific Advantages of Molecular Genetic Testing

- **Establishes Causation**: Provides information on **why** dechlorination and other processes are occuring
- **Sensitive:** can detect changes in microbiology before geochemical changes observed (e.g., *Dhc* increases months before ethene detected)
- **Spatially Discrete:** microbes typically more localized than their metabolic products (e.g., methanogens vs. methane)
- **Time Averaged:** microbial community changes their DNA degrading is relatively slow-can be used to identify events that occurred previously (e.g., influx of aerobic water indicated by increased aerobes)

Uses of Molecular Genetic Testing in Bioremediation

Initial Assessment:

- Are the required microorganisms indigenous to the site?
- Is MNA feasible?
- Is bioaugmentation required?



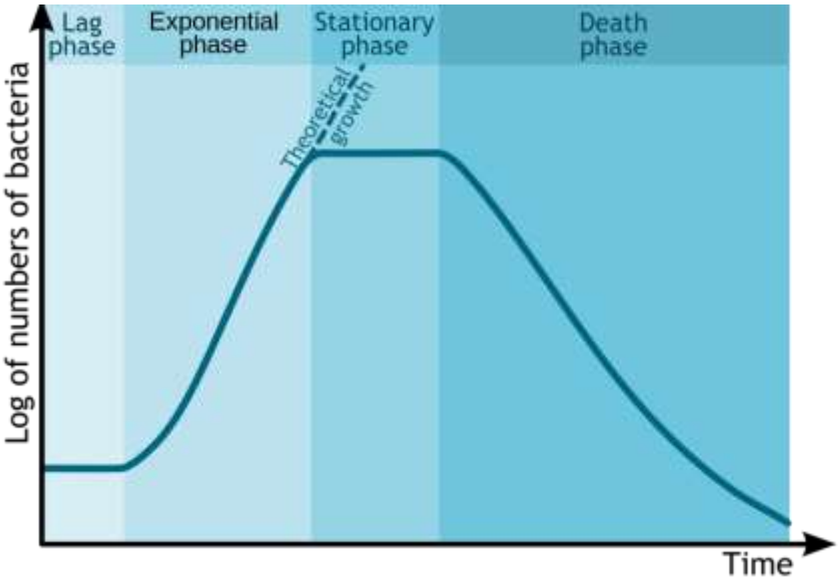
Dhc concentrations at 8 Acre FEW AFB KB-1 bioaugmented site as determined by Gene-Trac® testing

Ongoing Monitoring:

- Impact of site amendments?
- Increases growth rate and spread of biodegradative microbes
- Assess impacts of negative events (e.g., redox changes, pH declines)
- Is remediation progressing effectively at all locations?

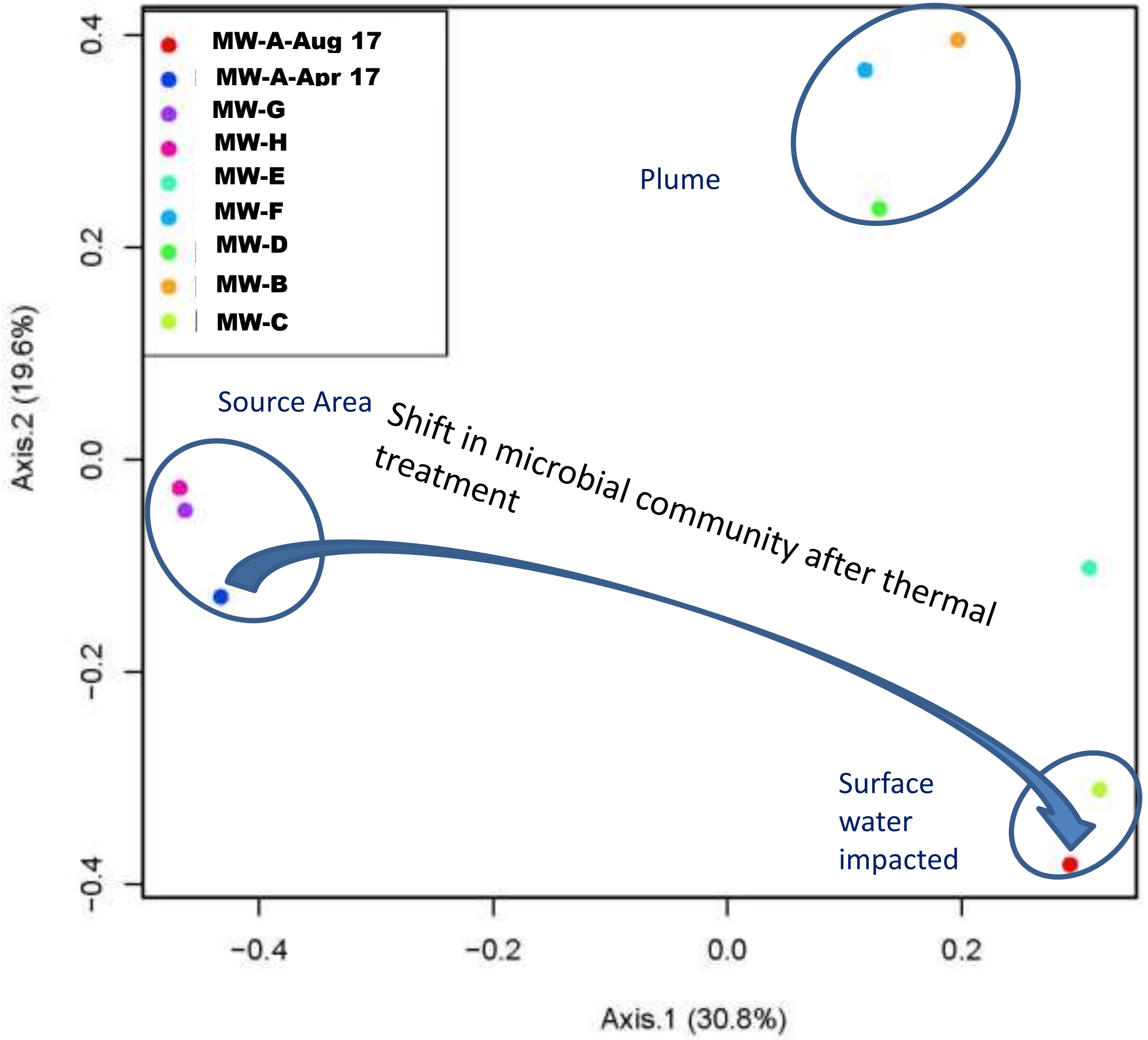
Dhc Growth Dynamics

- *Dhc* at site move through microbial growth curve
- *Dhc* in groundwater commonly range from ND to billions (e.g., 10^9) per liter
- Ethene is dependably observed at $>10^7$ *Dhc* per liter
- Wide range of in situ *Dhc* doubling times observed–indicator of health of population and the suitability of conditions
- Changes in *Dhc* population may occur even where VOC or ethene numbers are not changing



Dhc testing gives advance notice and ongoing assessment of suitability of site conditions for reductive dechlorination

NGS Cluster Analysis Demonstrates Impact of Thermal Treatment at MGP Site



Summary and Conclusions

- Treatability and molecular testing aid planning and assessment
- The tests provide evidence that is not always available/clear from other types of testing
- The costs of this type of tests are often offset by O&M savings due to improved planning & implementation
- Decreased uncertainty as treatability data provides preview of success prior to field implementation
- Molecular data provides performance preview and assessment during remedy implementation

= Less Stress!

Further Information

siremlab.com

1-866-251-1747
519-515-0836

Phil Dennis: pdennis@siremlab.com