



Leading Science · Lasting Solutions

How Treatability and Molecular Testing Saves Time, Money and Heartburn



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Remediation Seminars
Short Courses
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Research Triangle Park, NC

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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



THE WHAT, WHY AND HOW OF TREATABILITY STUDIES

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**
- **Site-specific remediation recommendations reported**



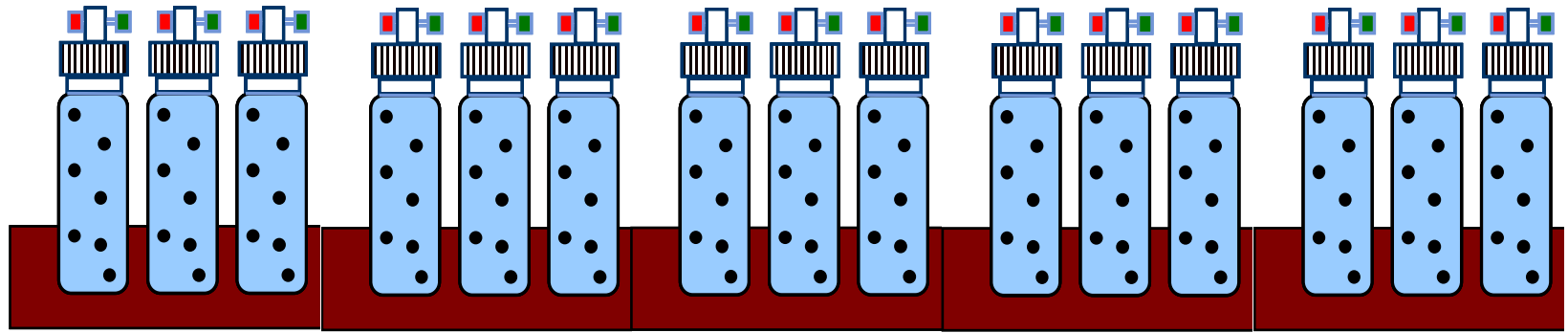
Treatability studies are typically microcosm or column tests for technologies including:

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





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
H₂/O₂ 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



TREATABILITY CASE STUDIES



Case Study: Denmark Site

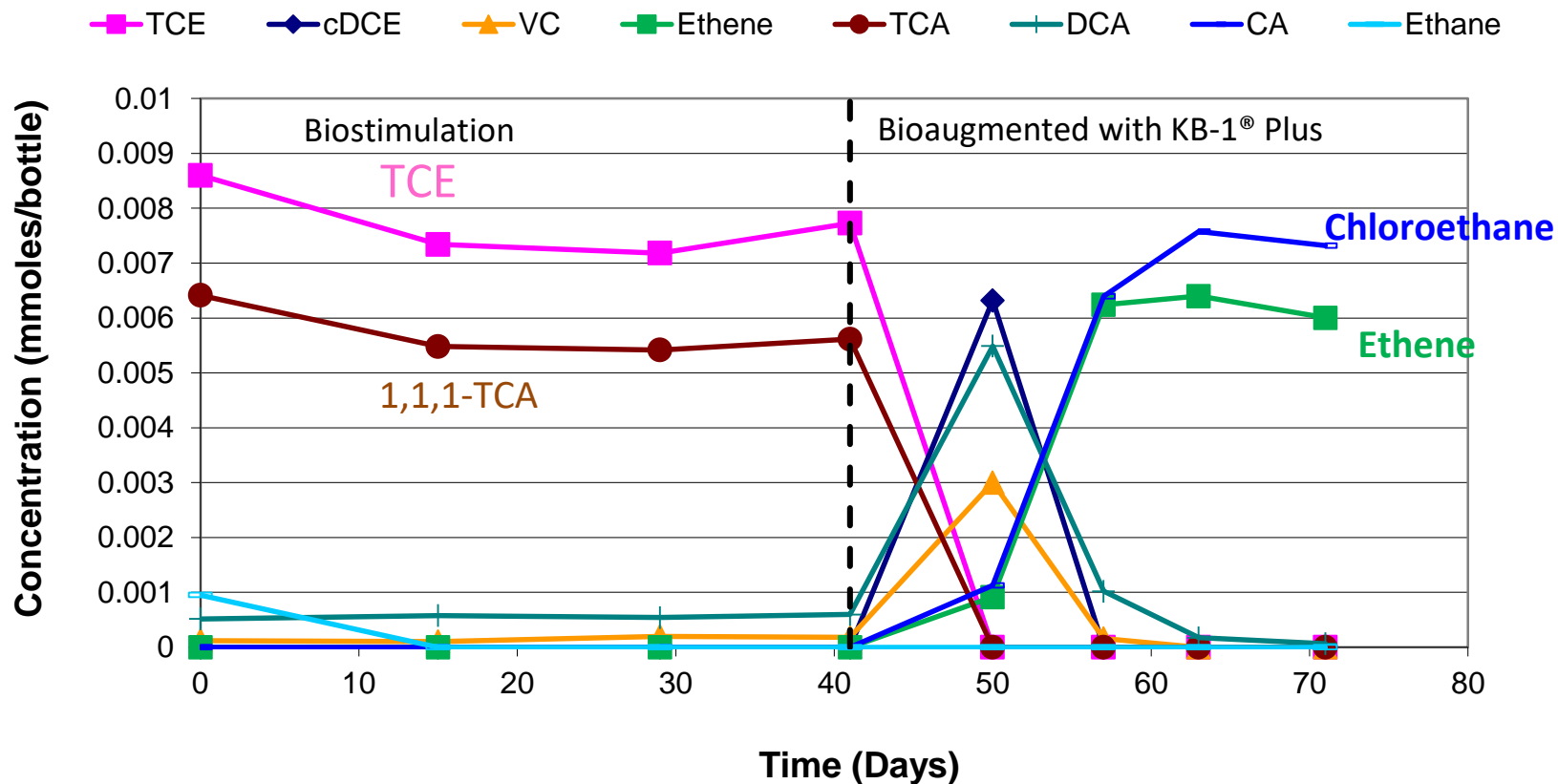
- Mixed chlorinated ethenes and ethanes
- 1,1,1-TCA (5 mg/L) and TCE (5mg/L)
- Can potential inhibition by 1,1,1-TCA be overcome?
- Is ISCO with persulfate viable remedial option?

Study Design:

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



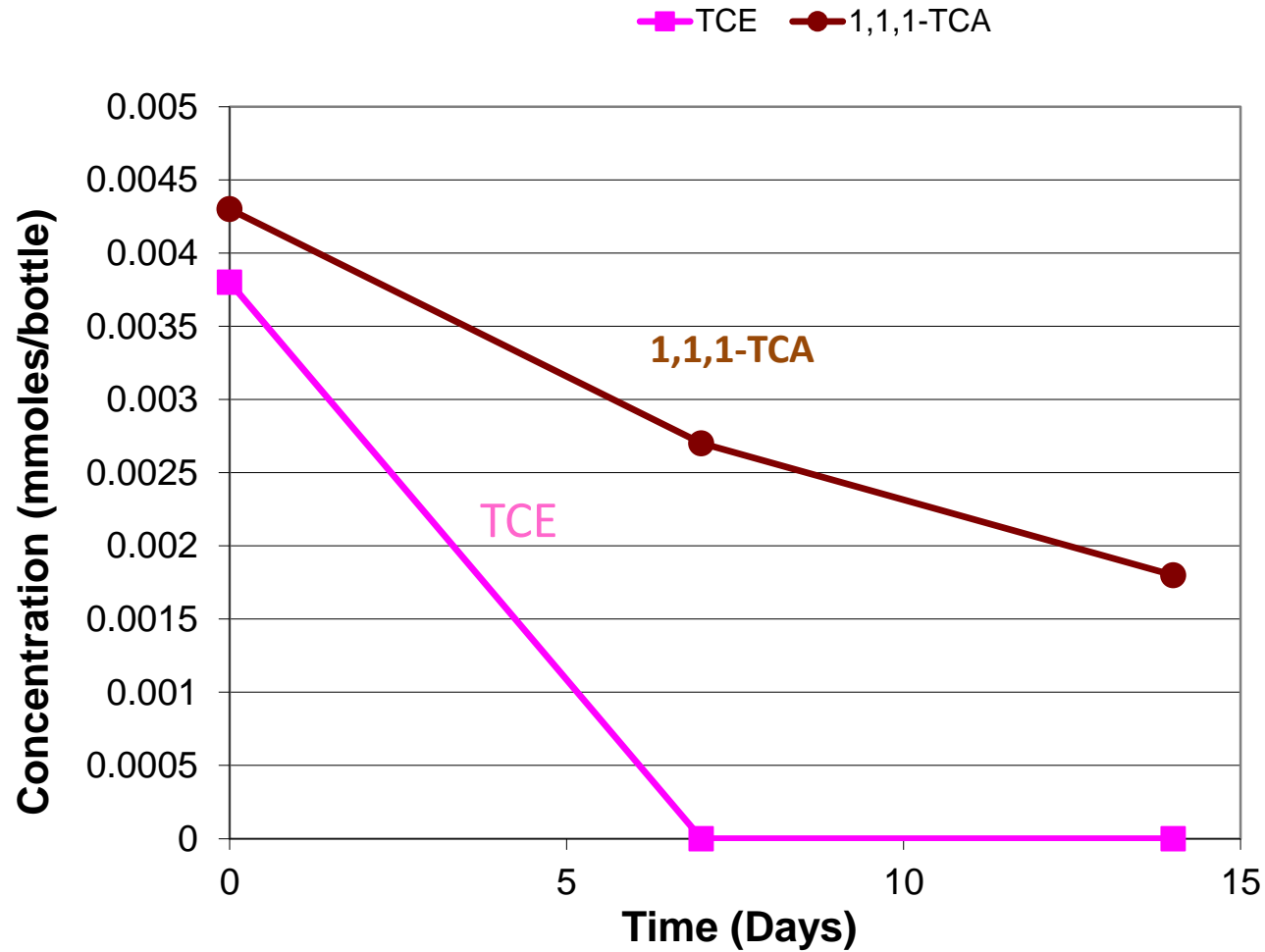
Case Study: Denmark Site





Case Study: Denmark Site

Activated Persulfate



Conclusions-Denmark Study

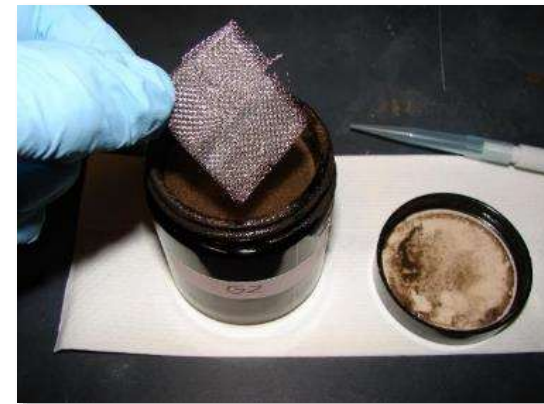
- Biostimulation alone=no dechlorination TCE/1,1,1-TCA
- KB-1[®] Plus bioaugmentation + biostimulation= rapid dechlorination-but with chloroethane accumulation
- Activated persulfate complete and rapid degradation of TCE slower and incomplete for 1,1,1-TCA

Based on study results enhanced bioremediation was selected as site remedy



Treatability Study for Active Cap Optimization

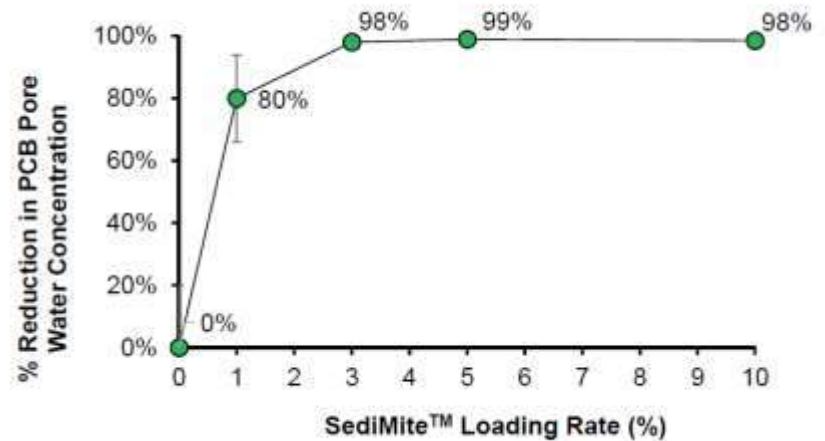
- Bench-scale treatability test to evaluate how much activated carbon (SediMite™) to add a PCB-impacted sediment
- PCB availability was measured because addition of the carbon changes availability not total PCB concentration
- Availability measured via SiREM passive samplers (SP3™) in site sediment amended with different SediMite™ loading rates





Case Study: PCB Active Cap Optimization

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



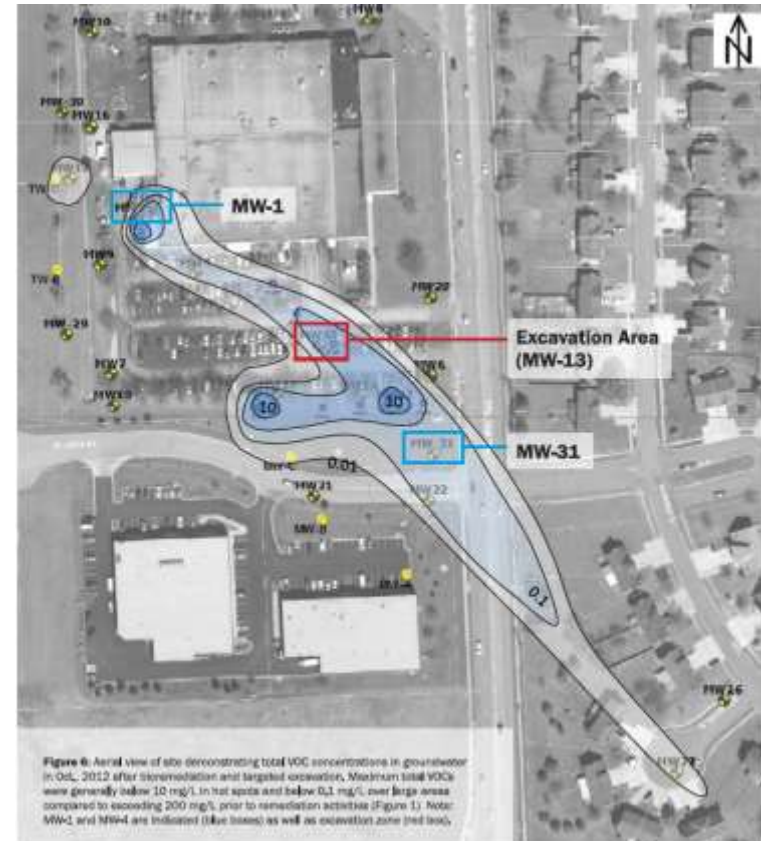
Study cost~\$10K findings saved more than \$300K in excess SediMite costs

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





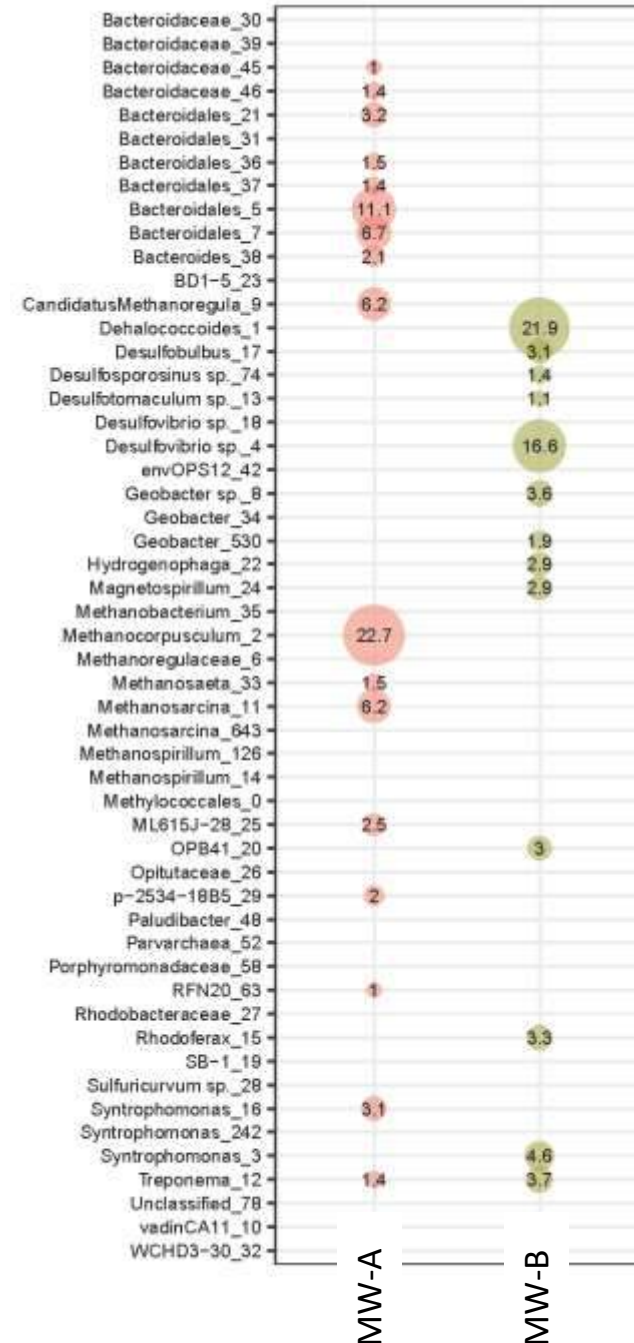
MOLECULAR GENETIC TESTING



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



Overview of Gene-Trac[®] qPCR Testing

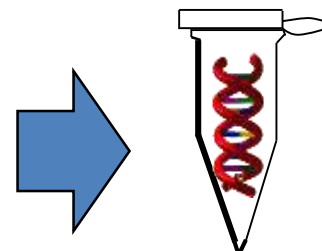


1) Groundwater Sampling

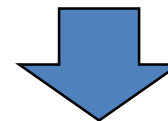
2) Transport 1L GW or field filter to Lab



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



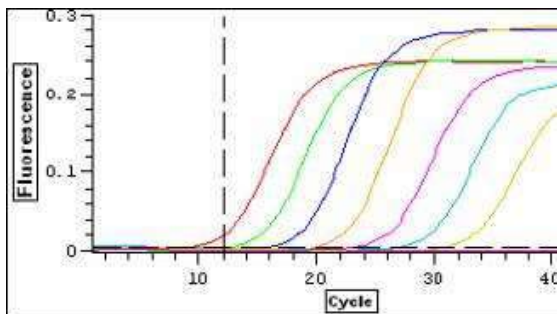
4) Extract DNA from filter



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



Tests available for a wide range of contaminant classes ...

Contaminant Class	Redox	Gene-Trac® Test Name	Target	Relevance	
Chlorinated Ethenes	Anaerobic	Dhc	<i>Dehalococcoides</i>	Dechlorinates PCE, TCE, all DCE isomers, VC	
		Dhb	<i>Dehalobacter</i>	Dechlorination of PCE & TCE to cDCE	
		Dem	<i>Desulfuromonas</i>	Dechlorination of PCE & TCE to cDCE	
		Dab	<i>Desulfitobacterium</i>	Partial dechlorination of PCE and TCE to cDCE	
		Geo	<i>Geobacter</i>	Dechlorinates PCE to cDCE/biogeochemical degradation	
		Dhg	<i>Dehalogenimonas</i>	Dechlorination of tDCE to VC and VC to ethene	
	Chloroethene FGA		Vinyl Chloride Reductase (<i>vcrA</i>)		Dechlorination of cDCE & VC to ethene
			BAV1 Reductase (<i>bvcA</i>)		Dechlorination of cDCE and VC to ethene
			Trichloroethene Reductase (<i>tceA</i>)		Dechlorination of PCE and TCE to cDCE and VC
	Aerobic	Polaromonas	<i>Polaromonas</i>		Aerobic dechlorination of cDCE
etn		<i>etnE</i>		Aerobic degradation of VC	
Chlorinated Ethanes	Anaerobic	Dhb	<i>Dehalobacter</i>	Dechlorinates 1,1,1-TCA/1,2-DCA /1,1,2-TCA/ 1,1,2,2-TeCA	
		Dhg	<i>Dehalogenimonas</i>	Dechlorinates 1,2- DCA, 1,1,2,2-TeCA , 1,1,2-TCA	
		Dhc	<i>Dehalococcoides</i>	Dechlorinates 1,2-DCA to ethene	
		Dab	<i>Desulfitobacterium</i>	Dechlorinates 1,1,2-TCA & 1,2-DCA	
		cfrA/dcrA	Dichloroethane Dehalogenase (<i>dcrA</i>)	Dechlorinates 1,1,1-TCA & 1,1-DCA	
	sMMO	Soluble Methane Monooxygenase	Co-metabolism of 1,1,1-TCA & 1,1-DCA by methanotrophs		
	Aerobic	PMO	Propane Monooxygenase	Co-metabolism of chlorinated ethanes by propanotrophs	
dhIA		Haloalkane Dehalogenase (<i>dhIA</i>)	Aerobic dechlorination of 1,2-DCA		
Chlorinated Methanes	Anaerobic	Dhb	<i>Dehalobacter</i>	Dechlorination of chloroform to DCM; DCM to acetate	
		cfrA/dcrA	Chloroform Reductase (<i>cfrA</i>)	Converts chloroform to dichloromethane	
	Aerobic	sMMO	Soluble Methane Monooxygenase	Co-metabolism of chloroform & dichloromethane	
Chlorinated Propanes	Anaerobic	Dhg	<i>Dehalogenimonas</i>	Converts TCP to allyl chloride; DCP to propene	
		Dhc	<i>Dehalococcoides</i>	Converts DCP to propene	
		Dhb	<i>Dehalobacter</i>	Converts DCP to propene	
		Dab	<i>Desulfitobacterium</i>	Dechlorination of TCP & DCP	
Chlorinated Benzenes	Anaerobic	Dhc	<i>Dehalococcoides</i>	Partial dechlorination of HCB/PCB	
		Dhb	<i>Dehalobacter</i>	Reductive dechlorination of DCB, MCB	
Chlorinated Phenols	Anaerobic	Dhc	<i>Dehalococcoides</i>	Dechlorination of 2,3-dichlorophenol, TCP and PCP	
PCBs	Anaerobic	Dhc	<i>Dehalococcoides</i>	Dechlorinates select Arochlor 1260 congeners	
		Dhb	<i>Dehalobacter</i>	Dechlorinates 2,3,4-trichlorobiphenyl; 2,3,4,5-tetrachlorobiphenyl	
		Dhg	<i>Dehalogenimonas</i>	Dechlorinates select Arochlor 1260 congeners	
BTEX	Anaerobic	SRB	Sulfate reducing bacteria (<i>dsrA</i>)	Partners to ORM-2 in anaerobic benzene degradation	
		ORM-2	<i>Deltaproteobacterium ORM-2</i>	Anaerobic benzene degrader (SO ₄ /CH ₄ reducing conditions)	
		Pepto-ben	Benzene degrading <i>Peptococcaceae</i>	Anaerobic benzene degrader under NO ₃ reducing conditions	
		abcA	Benzene Carboxylase (<i>abcA</i>)	Involved in benzene ring cleavage	
Fuel Oxygenates	Aerobic	MTBE/TBA	<i>Methylibium petroleiphilum</i> PM1	MTBE/TBE degrading microorganism	
			tert-butyl alcohol hydroxylase (<i>mdpJ</i>)	Active on TBA in aerobic MTBE degradation pathway	
			HIBA mutase (<i>hcmA</i>)	Active on 2-HIBA in aerobic MTBE degradation pathway	
1,4-Dioxane	Aerobic metabolism	1,4-dioxane	Dioxane monooxygenase (<i>dxmb</i>)	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 <i>Bacteria</i> -measure of total biomass	
		Arch	Archaea	Quantifies <i>Archaea</i> biomass	
		SRB	Sulfate reducing bacteria (<i>dsrA</i>)	Anaerobic hydrocarbon oxidation/biogeochemical reduction/MIC	
		NGS	<i>Bacteria/Archaea</i>	Comprehensive characterization of microbial communities	



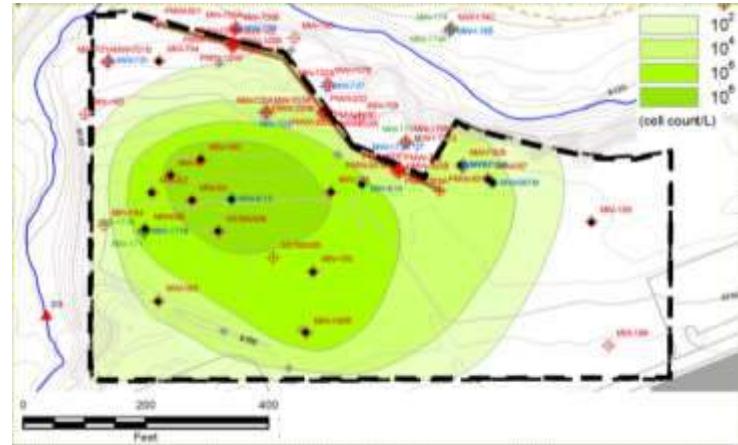
Uses of Molecular Genetic Testing in Bioremediation

Initial Assessment:

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

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?

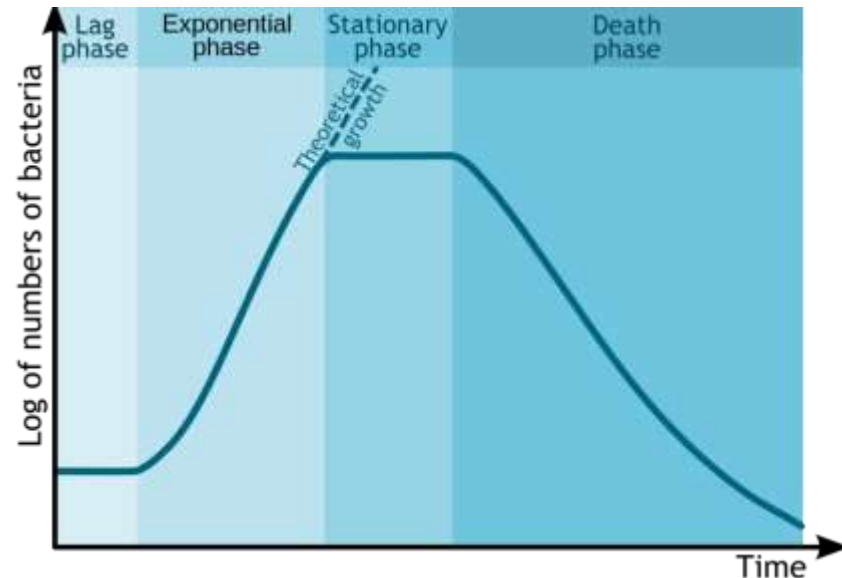


*Dhc concentrations at 8 Acre FEW AFB
KB-1 bioaugmented site as determined by
Gene-Trac® testing*

Dhc Growth Dynamics

- *Dhc* at a 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—e.g., DNAPL sites

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





COMPOUND SPECIFIC ISOTOPE ANALYSIS (CSIA)



Isotope Enrichment

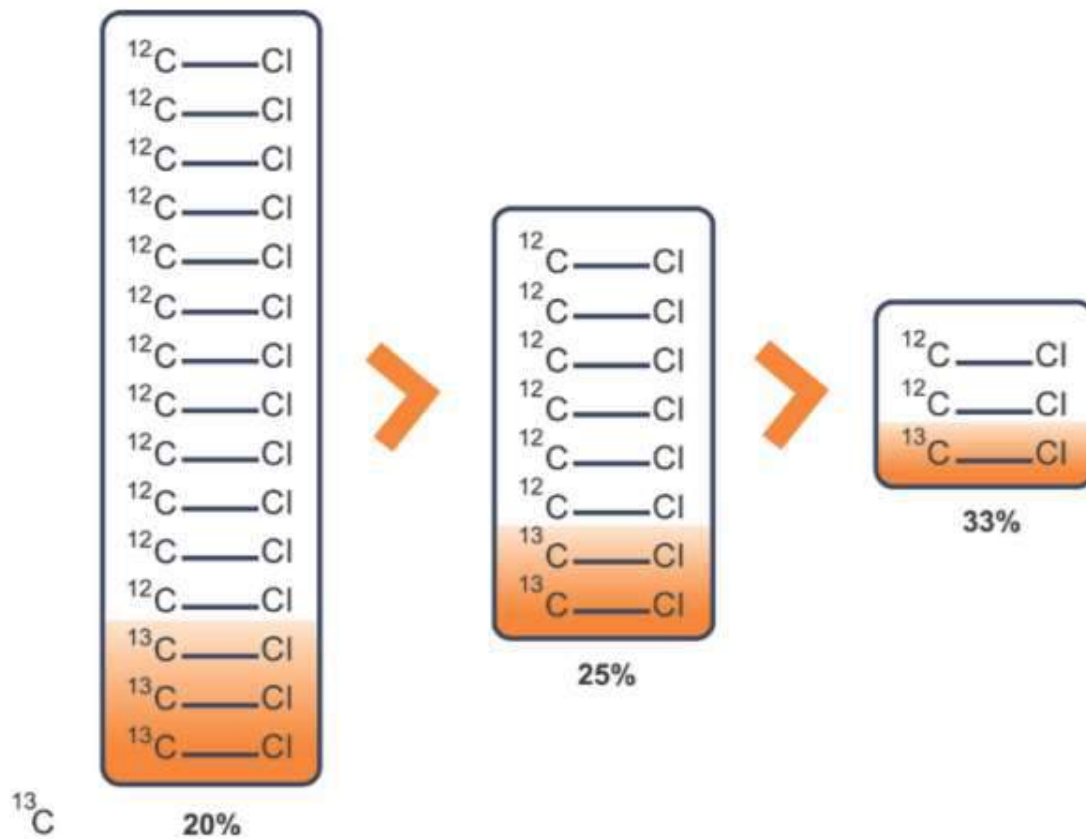
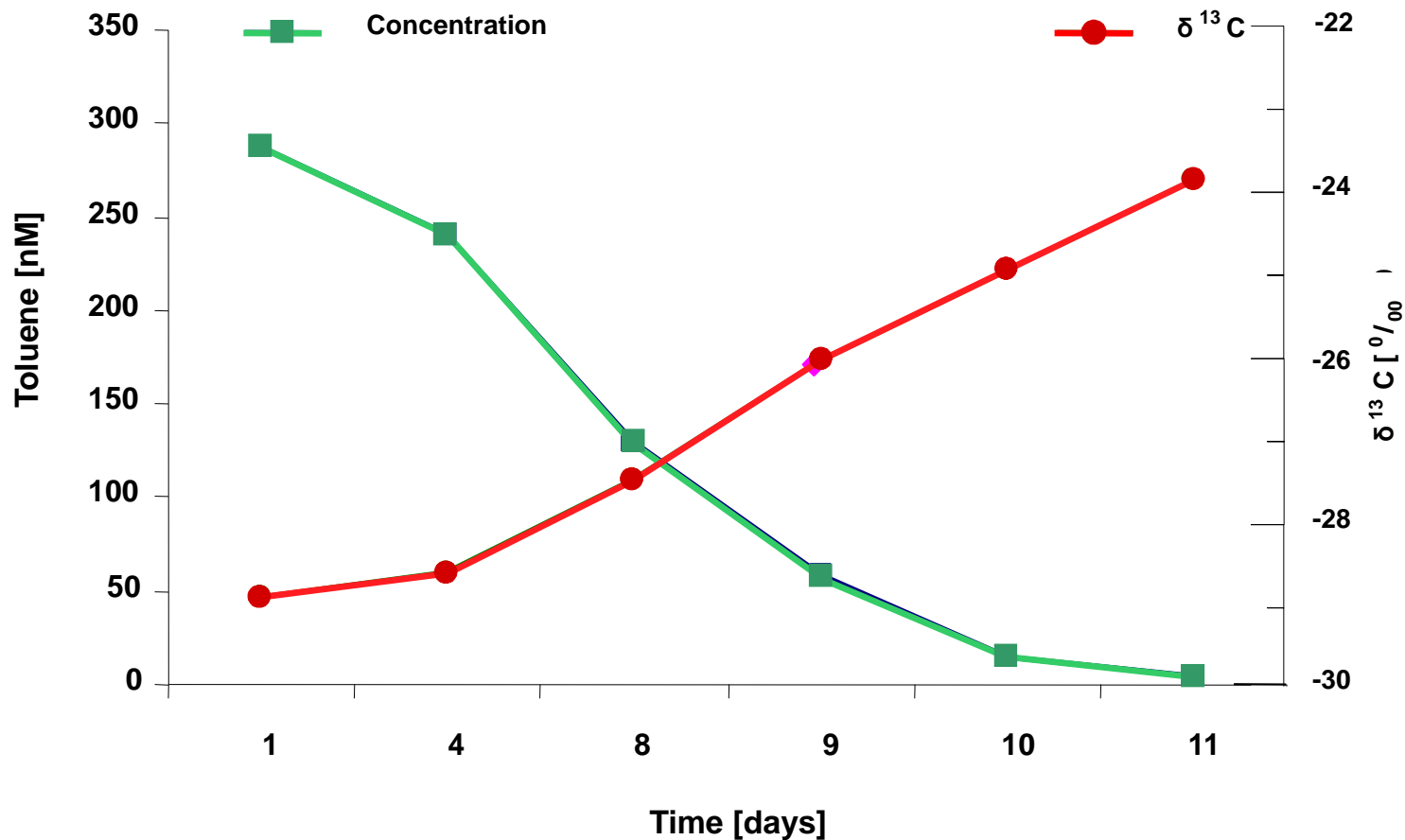


Figure 2. Illustration of ^{13}C enrichment during degradation of a contaminant with a C-Cl bond.

Source: Microseeps, Inc., 2010, used with permission.

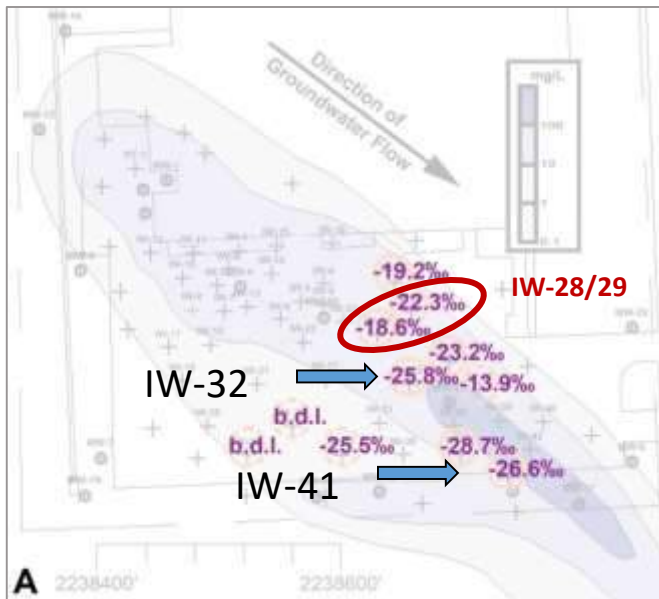


Degradation of toluene under sulfate reducing conditions

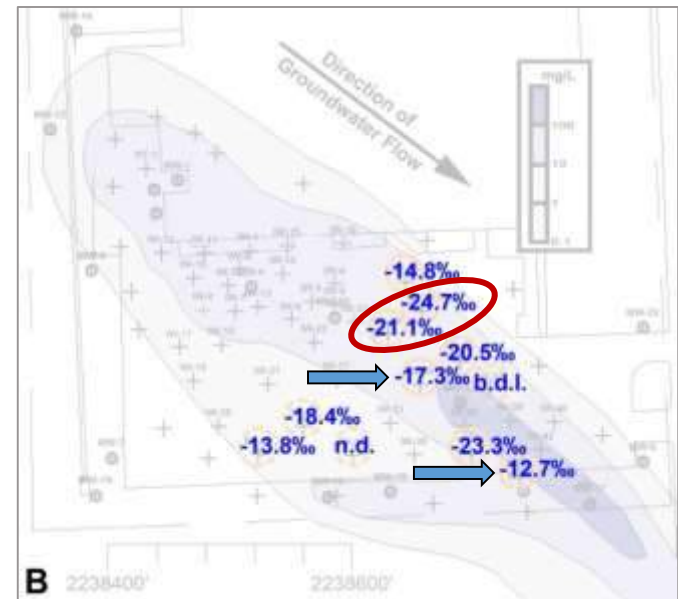


Use of Compound Specific Isotope Analysis (CSIA) to Confirm 1,1,1-TCA Degradation (Douglas, 2015)

Before Bioaugmentation



105 Days After Bioaugmentation



Well	$\Delta[1,1,1\text{-TCA}]$	$\Delta\delta^{13}\text{C}$	Estimate of Extent of Biodegradation
IW-24	-370 $\mu\text{g/L}$	+4.4‰	69% to 71%
IW-28	+100 $\mu\text{g/L}$	-2.5‰	Not dominant process
IW-29	+11400 $\mu\text{g/L}$	-2.4‰	Not dominant process
IW-32	+160 $\mu\text{g/L}$	+8.5‰	90% to 91% (even with increasing concentration)
IW-33	-150 $\mu\text{g/L}$	+2.7‰	52% to 54%
IW-38	-5930 $\mu\text{g/L}$	+5.4‰	77% to 79%
IW-41	-1130 $\mu\text{g/L}$	+13.9‰	98%



Summary and Conclusions

- Treatability and molecular testing aid planning and assessment
- Provide evidence that is not always available 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

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