Enhanced *In Situ* Bioremediation – State of the Practice

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Presentation Overview

▶ Introduction

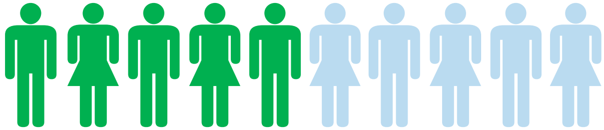
- Review of Enhanced Bioremediation
- Best Practices for Successful Application, Distribution, and Mixing of Amendments
- Technological Advances to Address Complex Sites
- Innovation in Amendment Formulations
- Best Practices and Advances in Monitoring
- BioPIC – A Tool to Select, Evaluate, and Convince
- Wrap-Up

Presentation Objectives

- Highlight recent technological advances in EISB to address complex sites
- Provide best practices to maximize and measure treatment effectiveness
- Identify long-term impacts and challenges of EISB
- Introduce a recently developed tool that can aid practitioners to select an appropriate bioremediation strategy

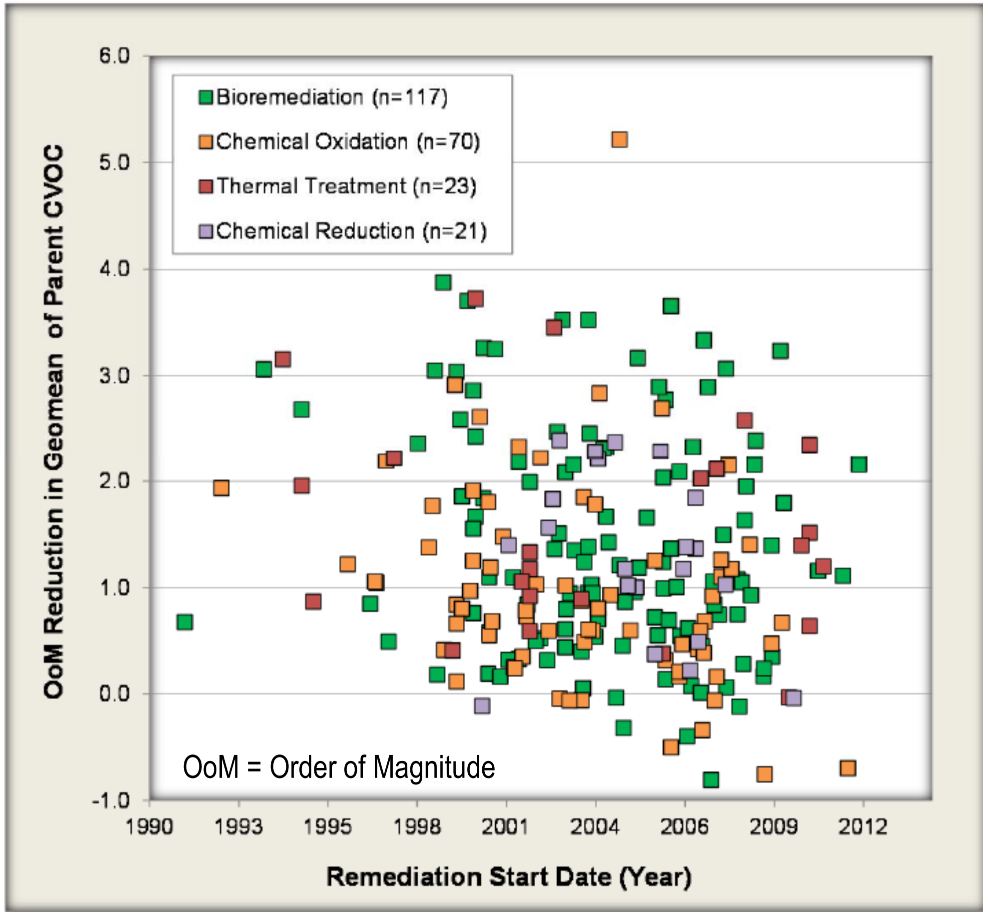
What's in it for me?

- Chlorinated solvents in groundwater remain a key issue at impacted sites and that enhanced *in situ* bioremediation (EISB) is a frequently selected treatment remedy
- EISB has been proven to be an effective technology, but there is much room for improvement



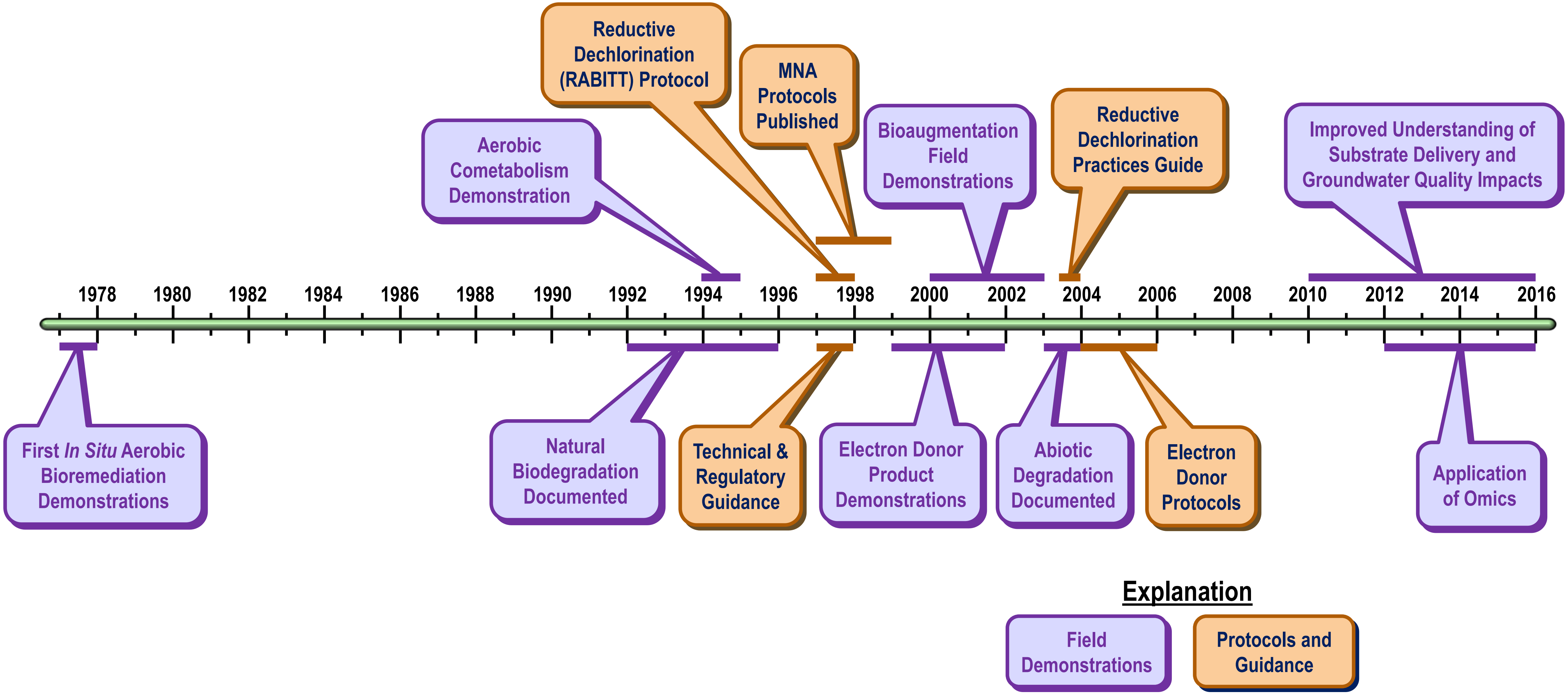
50% **Faced injection issues for *in situ* remedy**

30% **Using bioremediation technologies**



Source: *ESTCP ER-201120 Final Report*

History of Bioremediation



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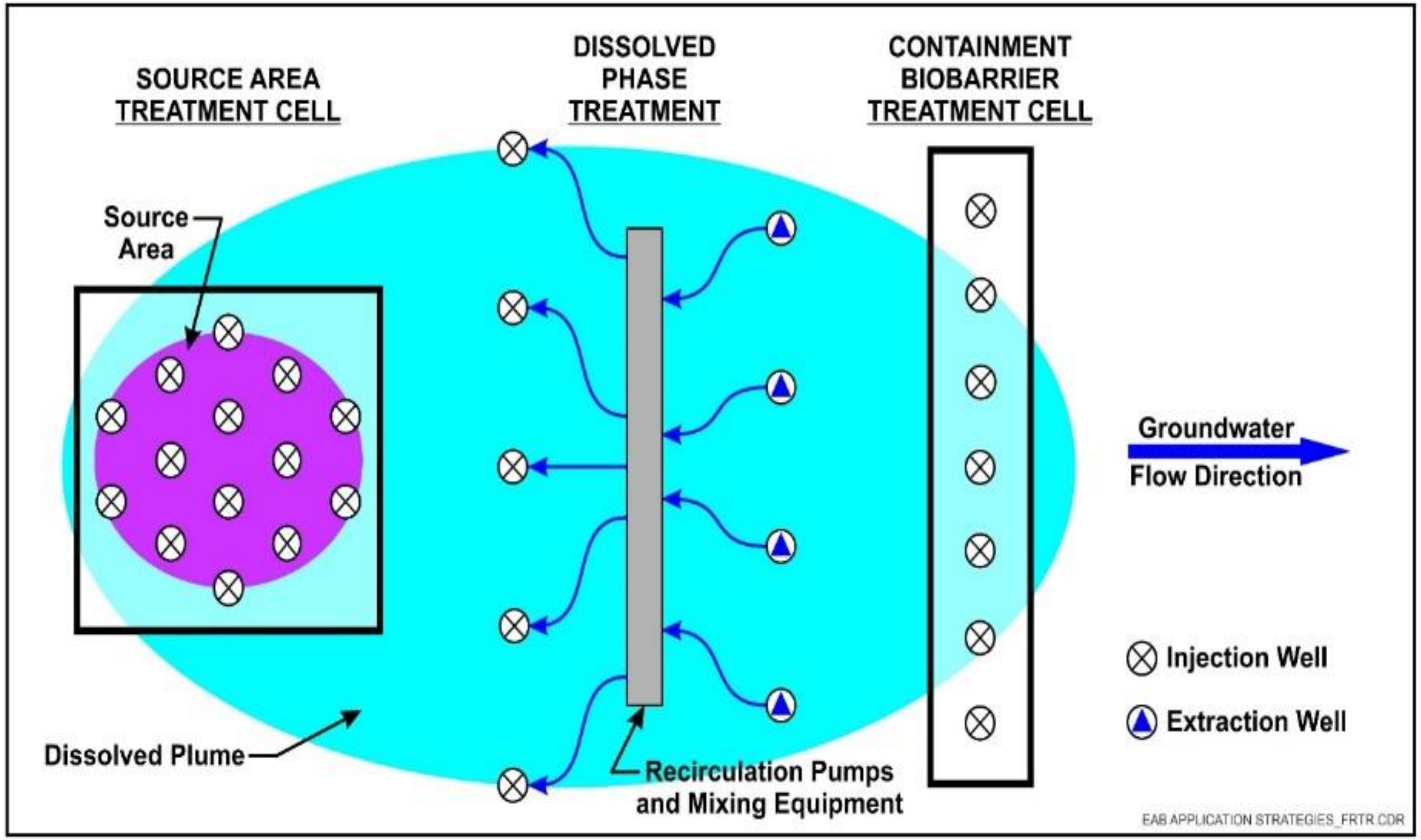
Enhanced *In Situ* Bioremediation (EISB)

- Relies on naturally occurring microbial processes via indigenous or amended (bioaugmented) microorganisms
- Generally relies on introduction of amendments
- Initially used for dissolved-phase plume treatment and control
- Increasingly used for source area treatment and control
- Complementary with other remedial technologies



Aerobic Biodegradation Process

Electron Donor

Electron Acceptor

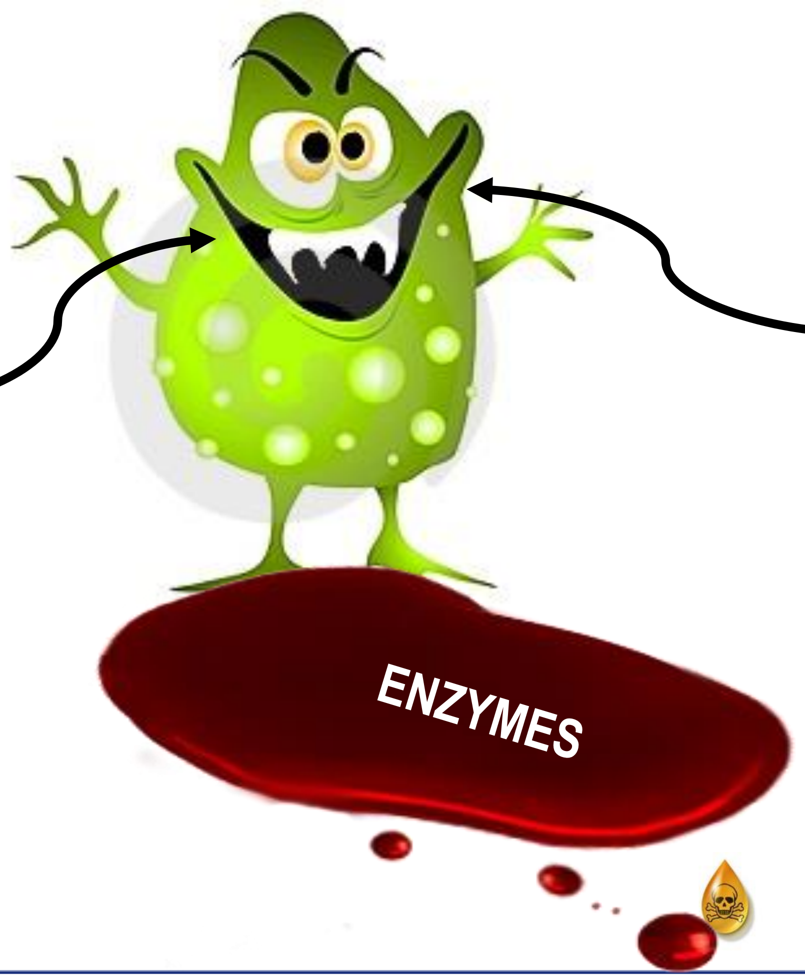
Oxidized Donor

Reduced Acceptor

Electron Donor + Electron Acceptor → Respiration Products + Energy

Other Common Biodegradation Processes

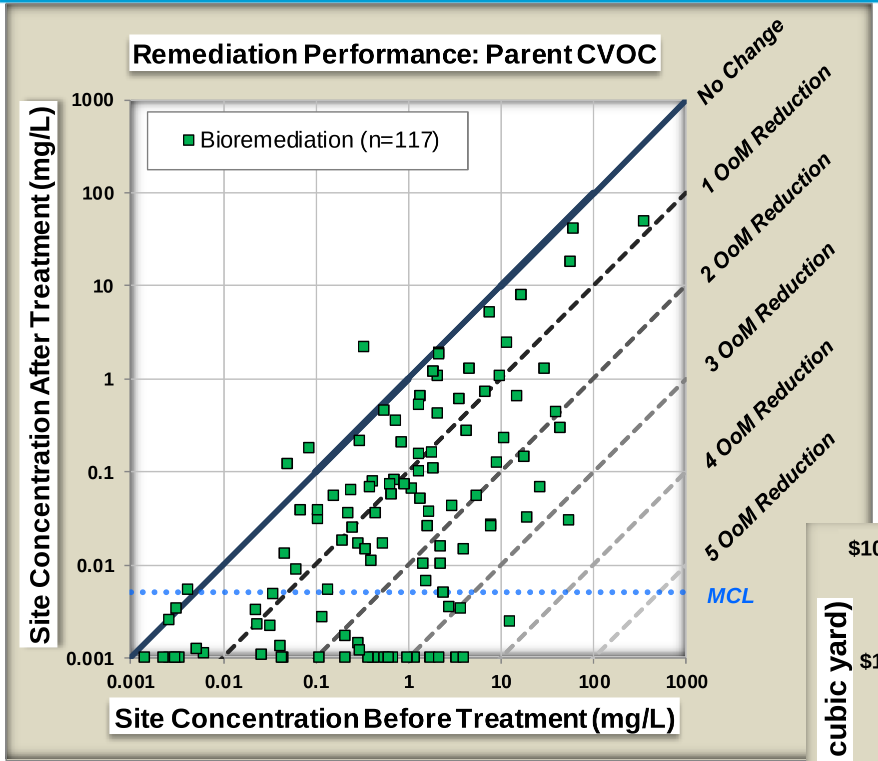
Process	***Electron Acceptors***	***Electron Donors***
Anaerobic Reductive Dechlorination	COCs (e.g., chlorinated ethenes)	Organic Compound (e.g., oil)
Anaerobic Oxidative Degradation	Nitrate & Sulfate	COCs (e.g., BTEX)
Cometabolic Degradation	Oxygen	Organic Compound

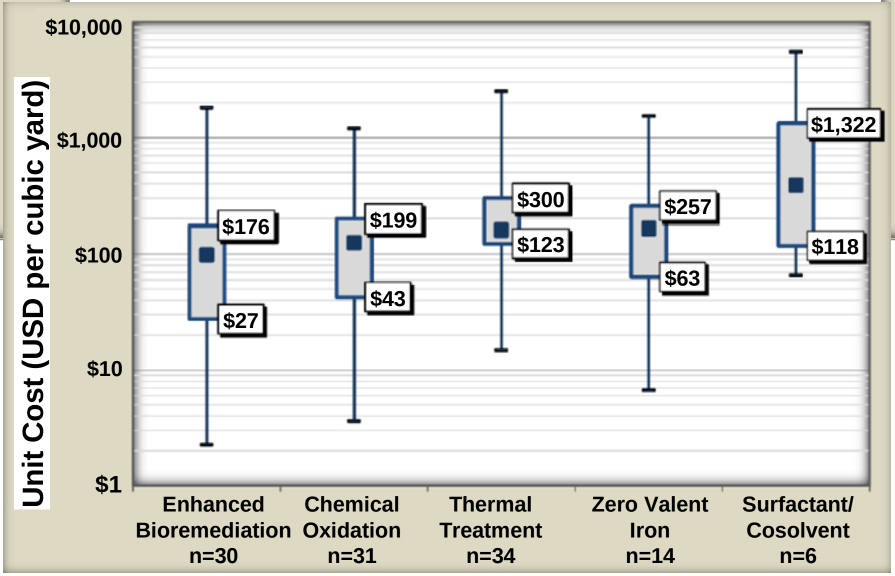


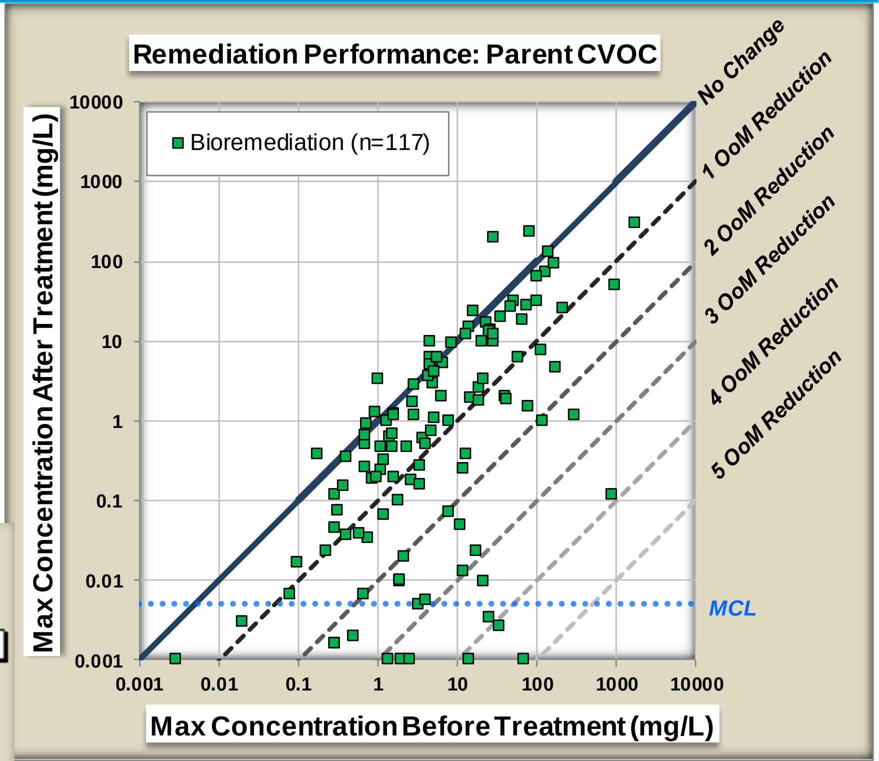
Contaminants of Concern Degraded by Each Process

Class	Common Contaminant	Aerobic Oxidation	Anaerobic		Cometabolic Degradation
			Oxidation	Reduction	
Petroleum Hydrocarbon Related	Non-halogenated alkenes/alkanes	X			
	BTEX	X	X		X
	Simple PAHs (e.g., naphthalene)	X	X		X
	Cyclic PAHs		X		X
	MTBE	X			X
Chlorinated Ethenes	PCE & TCE			X	X
	DCE & VC	X		X	X
Chlorinated Ethanes	1,1,1-TCA, 1,2-DCA, 1,1-DCA			X	X
Chlorinated Methanes	Carbon tetrachloride				X
	Chloroform & methylene chloride			X	X
Pesticides	Some Pesticides	X		X	X
Ethers	1,4-dioxane				X
Energetics	TNT	X			X

How effective is bioremediation? (ESTCP ER-201120)







Source: ESTCP Project 201120 (GSI, 2016)

Previous Related RITS

- Distribution of *In Situ* Amendments (Spring 2014)
- Environmental Molecular Diagnostics: Current Capabilities and Future Trends (Spring 2013)
- Application of Molecular Biological Tools for Site Remediation (Spring 2009)
- Bio – State of the Practice (Fall 2007)
- Anaerobic Bioremediation using Biobarriers (Fall 2006)

https://www.navfac.navy.mil/navfac_worldwide/specialty_centers/exwc/products_and_services/ev/erb/rits/pastrits.html

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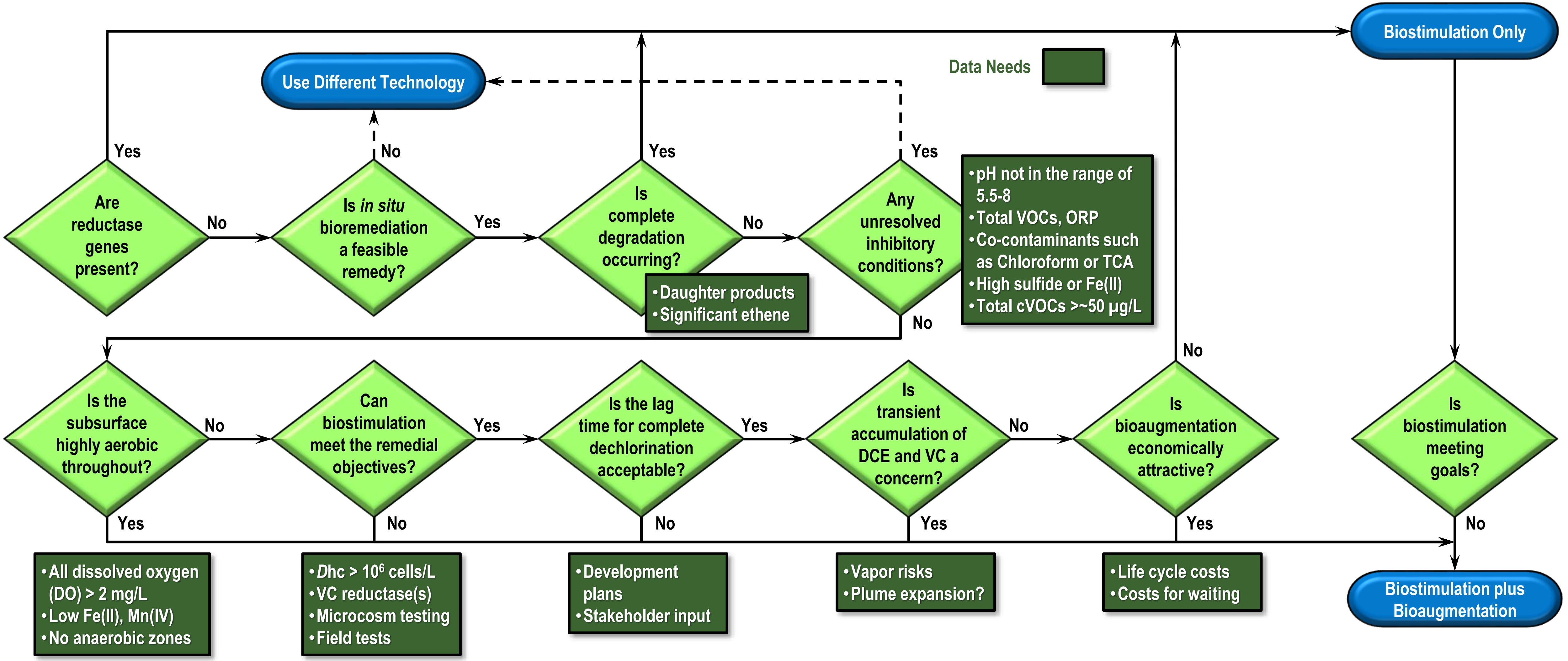
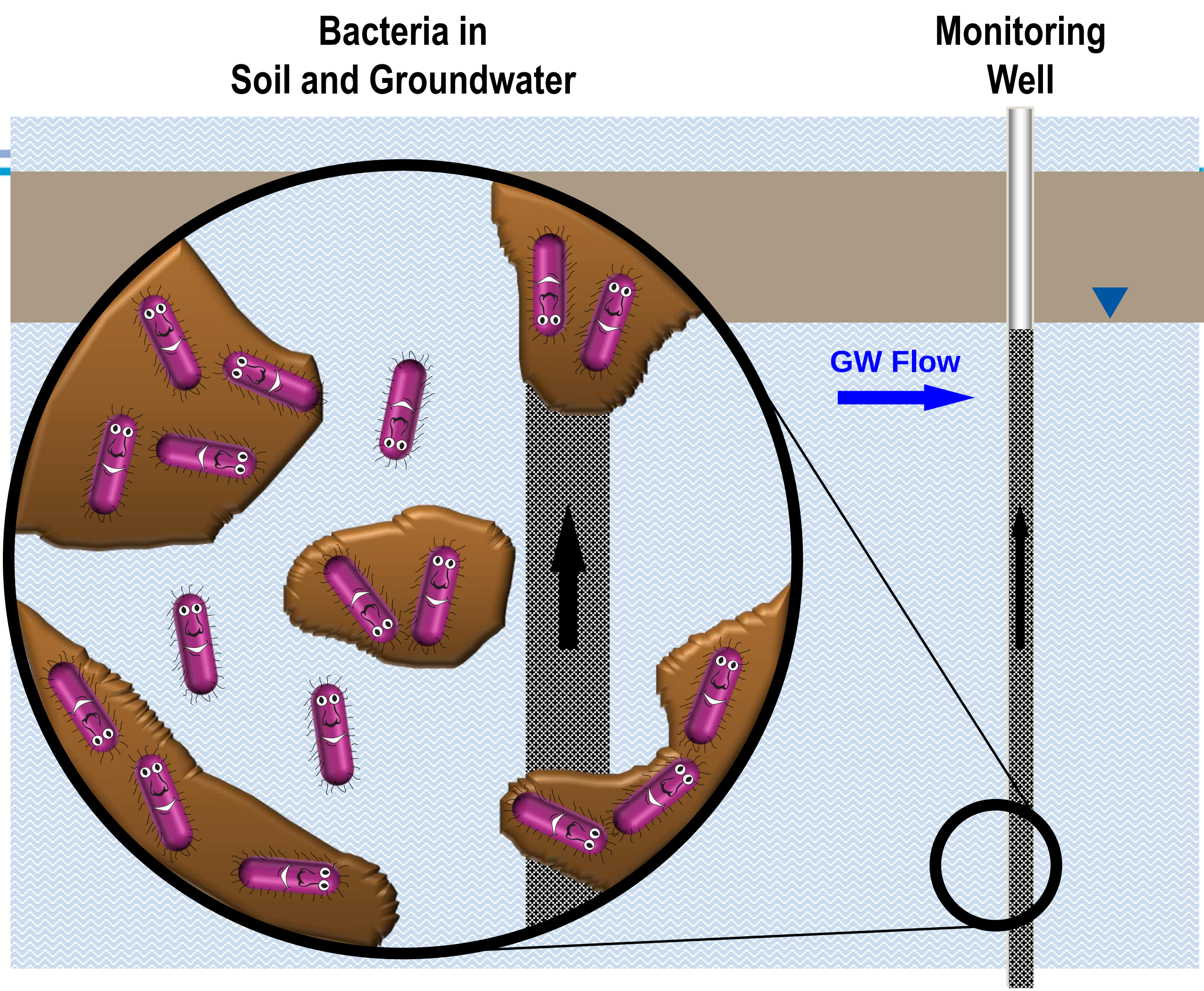
Are Conditions Favorable for Biostimulation and Bioaugmentation?



Source: Adapted from *BioPIC Final Report* (Lebron et. al, 2016)

Solid-Aqueous Phase Distribution Considerations

- Distribution of microbes between the solid and aqueous phase is not uniform
- qPCR analysis of groundwater can underestimate the total abundance of *DHC* cells by orders-of-magnitude (SERDP ER-1231)
  - Majority of cells during "non-growth" conditions may be in aqueous phase
  - Majority of cells may be attached to solids during/subsequent to biostimulation
- Sampling is biggest source of variability

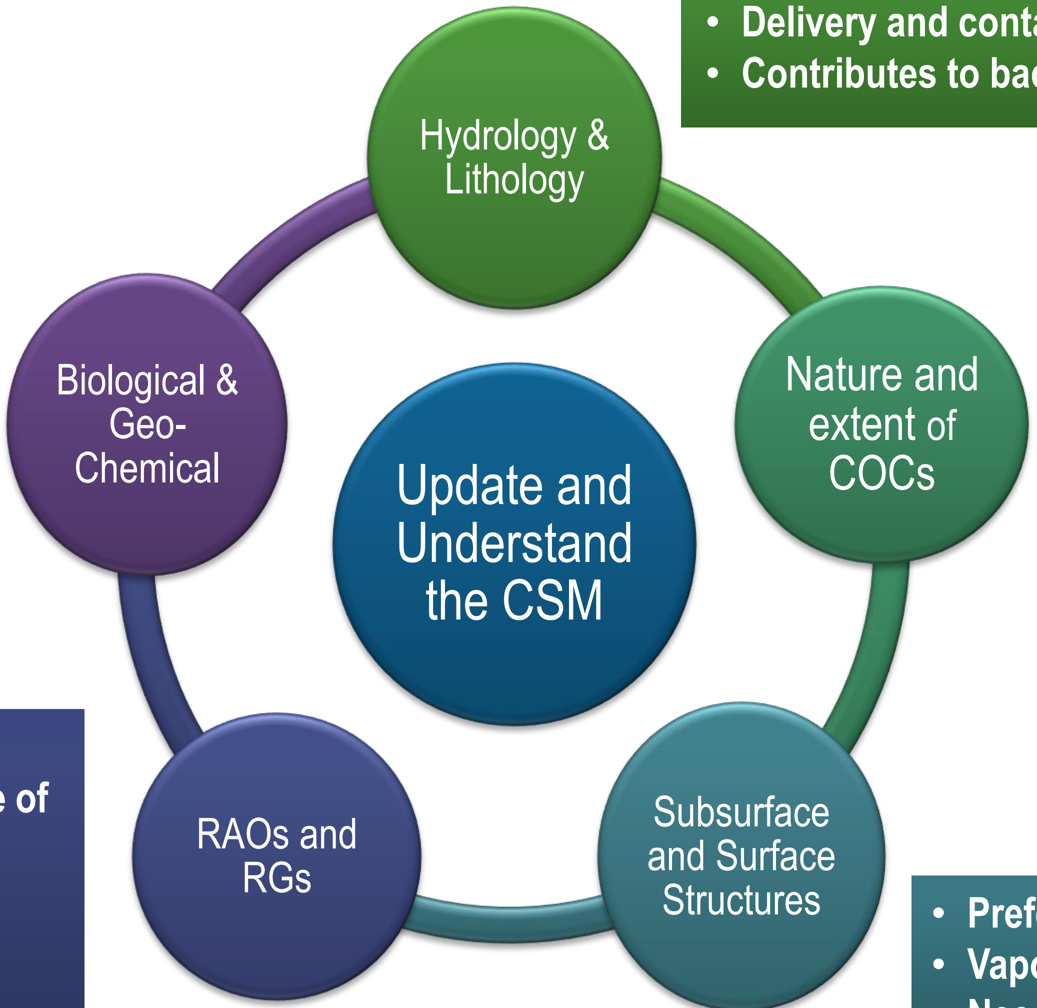


Key Point qPCR may underestimate cell density, but does not overestimate. Failure to detect sufficient levels of *DHC* in groundwater is not sufficient to prove complete dechlorination will not occur.

Key Conceptual Site Model Elements and Design Impacts

- Competing biological & chemical reactions
- Inhibitory conditions
  - pH
  - Alkalinity
  - Co-contaminants
  - DO
  - Low cVOC concentration

- Delivery and contact of amendments with COCs
- Contributes to back-diffusion and rebound



- Treatment design (e.g., treatment vs. containment) & method of amendment introduction
- Affects the demand for and type of ERD amendments
- Contributes to back-diffusion and rebound.
- DNAPL impacts type and extent of treatment
- Performance, duration, and number of amendment applications
- Remedy expectations
- Cost

- Treatment design
- Types, concentration, & volume of amendments
- Need for bioaugmentation
- Duration & number of injection events

- Preferential pathways
- Vapor intrusion mitigation
- Need for horizontal wells

Tools to Improve the CSM for EISB Applications

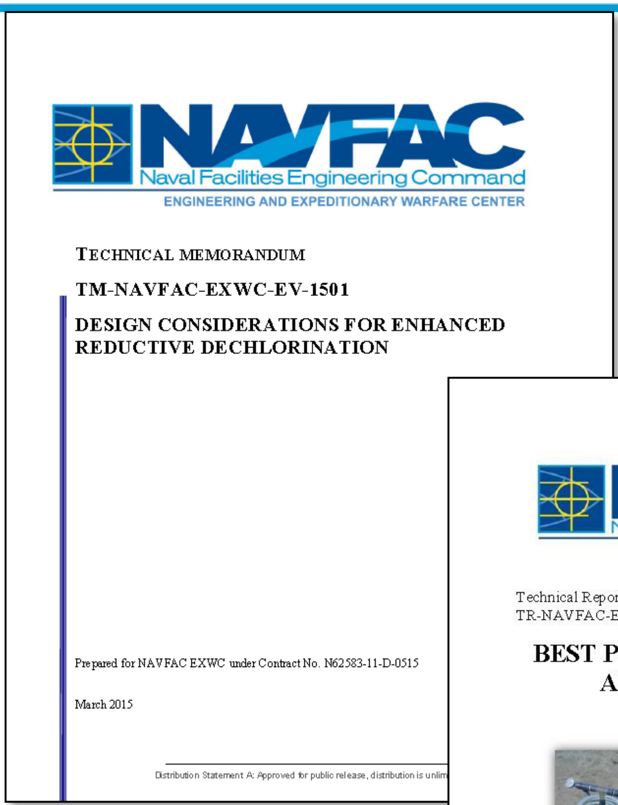
- High Resolution Site Characterization (RITS Spring 2015)
  - Groundwater profiling and soil coring
  - Geophysical tools
- Mass Flux Tools (RITS Spring 2011)
- Rock Matrix Characterization
- Molecular Diagnostic Tools (RITS Spring 2013)
  - CSIA (Compound specific isotope analysis)
  - qPCR (quantitative polymerase chain reaction)

Best Practice — **Apply high resolution site characterization and innovative diagnostic tools to improve the CSM and optimize the remedial design and performance**

Amendment Delivery & Contact – the Biggest Challenge

- No one approach, very site-specific
- Design should always be based on an **UP-TO-DATE** CSM
- Always develop an amendment delivery plan

Amendment Delivery Plan Minimum Requirements
• Amendment dosing and longevity • Number of injection events • Injection/extraction well layout • Equipment specifications • Procedures to introduce amendments • Process and performance monitoring requirements • Treatment milestones and endpoints • Contingencies for potential deviations


NAVFAC
Naval Facilities Engineering Command
ENGINEERING AND EXPEDITIONARY WARFARE CENTER

TECHNICAL MEMORANDUM

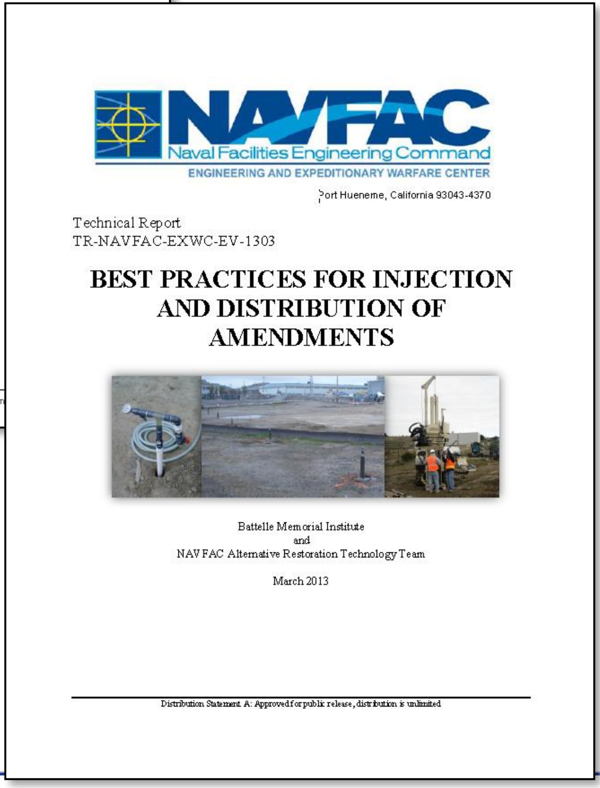
TM-NAVFAC-EXWC-EV-1501

DESIGN CONSIDERATIONS FOR ENHANCED REDUCTIVE DECHLORINATION

Prepared for NAVFAC EXWC under Contract No. N62583-11-D-0515

March 2015

Distribution Statement A: Approved for public release, distribution is unlim


NAVFAC
Naval Facilities Engineering Command
ENGINEERING AND EXPEDITIONARY WARFARE CENTER
Port Hueneme, California 93043-4370

Technical Report
TR-NAVFAC-EXWC-EV-1303

BEST PRACTICES FOR INJECTION AND DISTRIBUTION OF AMENDMENTS

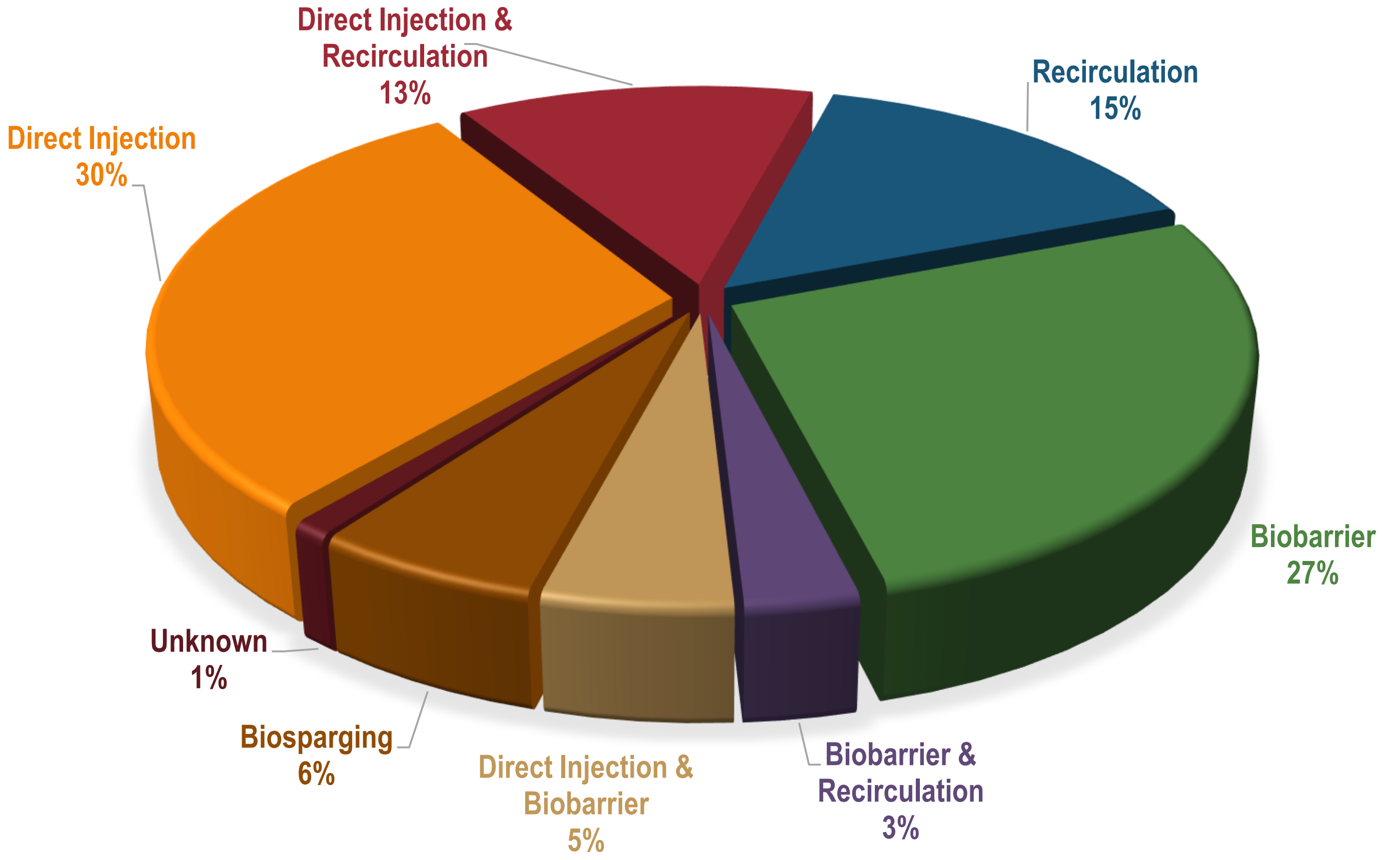
Battelle Memorial Institute
and
NAVFAC Alternative Restoration Technology Team

March 2013

Distribution Statement A: Approved for public release, distribution is unlimited

Substrate Delivery Design Approach

- Multiple approaches used
  - Direct injection
  - Recirculation
  - Hydraulic or pneumatic fracturing
- Consider site-specific factors
- Understand advantages & limitations
  - Radius of influence
  - Cost
  - Time
  - Amendment type & dosage

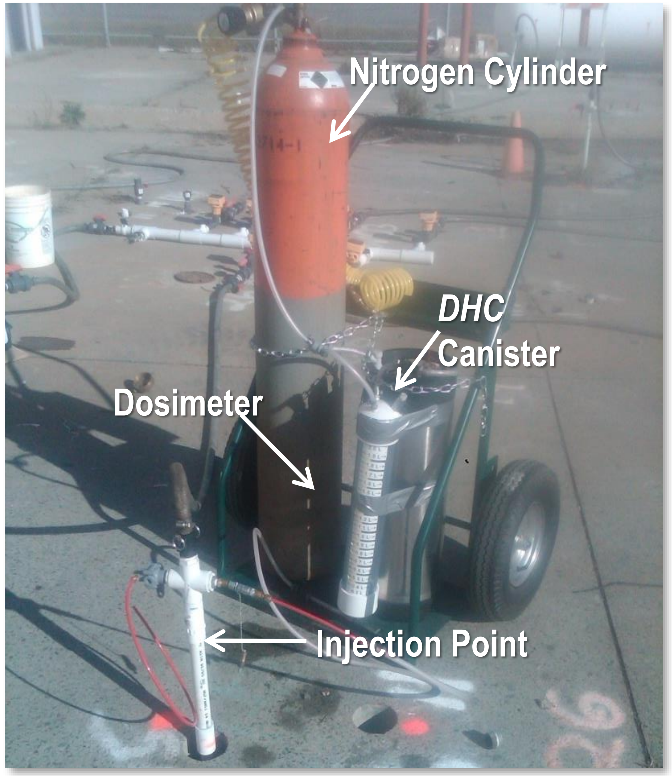


Bioremediation Remedies Selected 1998 – 2008 at NPL Sites

Source: Introduction to *In Situ* Bioremediation of Groundwater (EPA, 2015)

Best Practices for Delivery of Bioaugmentation Cultures

- Ensure appropriate aquifer conditions prior to introduction (pH, ORP, DO, TOC)
- Design and inject sufficient culture (10^7 cells/L as a rule of thumb)
- Introduce microbes anaerobically
  - Nitrogen or argon blanket
  - Sandwich between donor and/or anoxic water
- Wait and monitor



Bioaugmentation Equipment

Key Points

1. **Increasing culture dosage does not necessarily result in less time for dechlorination**
2. **Field applications demonstrate that within a few months *DHC* can proliferate and distribute downgradient over considerable distance**

Design Considerations for Buffering Agents

- pH generally declines during application due to formation of hydrochloric and carbonic acids
- Buffering capacity tests should be performed using site soil/groundwater
- Frequency of application and distribution may be different than electron donors
- Design tools that are available to estimate buffering requirements

Three Common Buffering Agents[1]

Sodium Bicarbonate	AquaBupH	Neutral Zone
• Highly soluble • Can increase sodium content of aquifer • Inexpensive • BUCHLORAC dosing tool available	• Alkaline buffering suspension & emulsified oil • More recalcitrant than bicarbonate • Design tool available from EOS Remediation	• Colloidal suspension of calcium carbonate • Highly recalcitrant

1) Other types of buffering agents are available. The Navy does not endorse any particular type. Selection should be based on site-specific conditions and project objectives.

Problems, Pitfalls, and Site-Specific Considerations

- Preferential pathways, daylighting of fluids, and media contact
- pH control
- Fouling
- Adverse byproducts and secondary water quality impacts
  - Methane and hydrogen sulfide
  - Long-term reduction of ORP
  - Mobilization of metals

Best Practices to Reduce Likelihood of Preferential Pathways

- Inject at low flowrates and pressures
- Offset injection locations between injection events if direct injection application
- Alternate injection/extraction well combinations if recirculation application
- Space points/wells more closely in low permeability formations

Key Point

Beware of overdosing! Work closely with substrate suppliers and use substrate design tools when possible.

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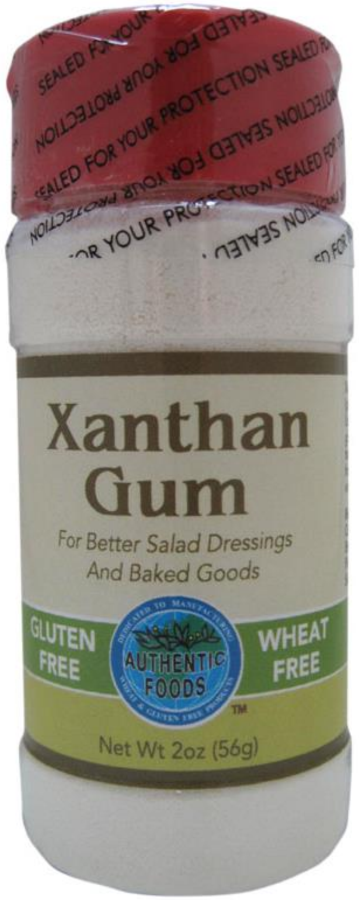
Technological Advances to Address Complex Sites

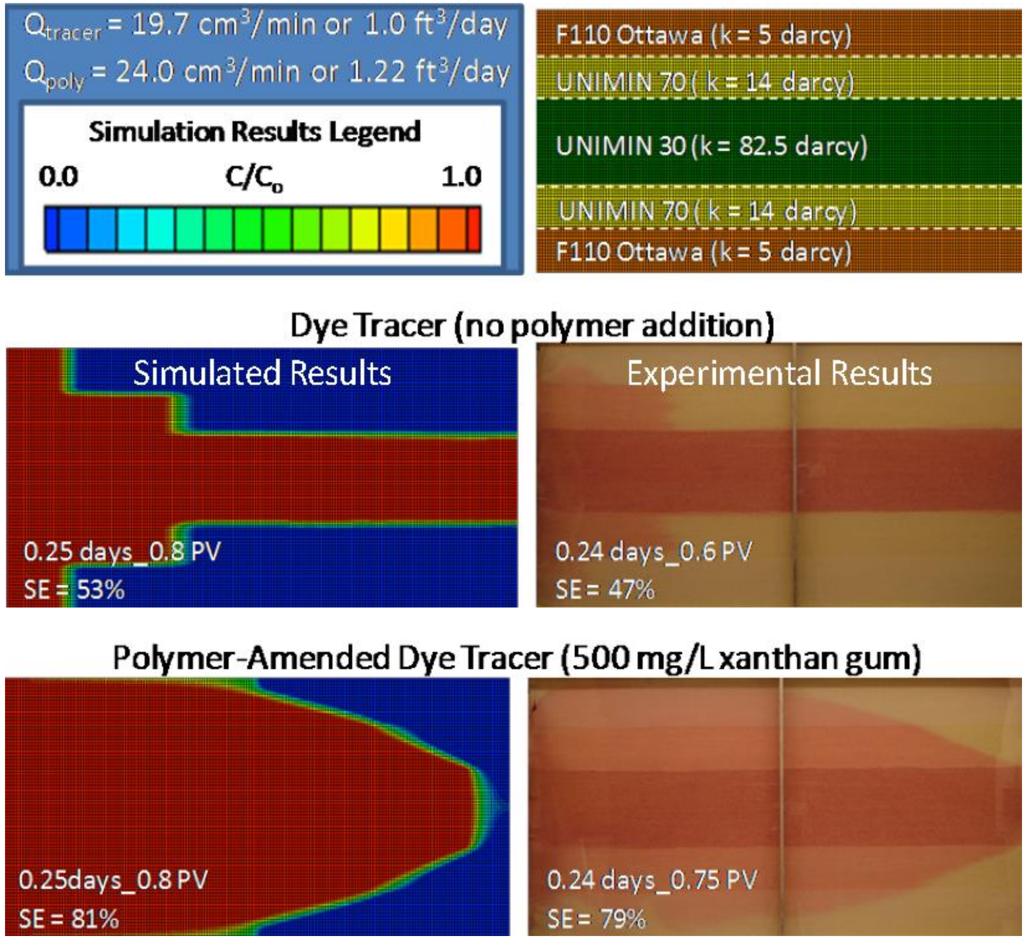
► **Application of Shear Thinning Fluids**

- Electrokinetic Bioremediation
- Heat-Enhanced Bioremediation
- Bioremediation in Fractured Bedrock
- Bioremediation of 1,4-Dioxane

Shear-Thinning Fluids

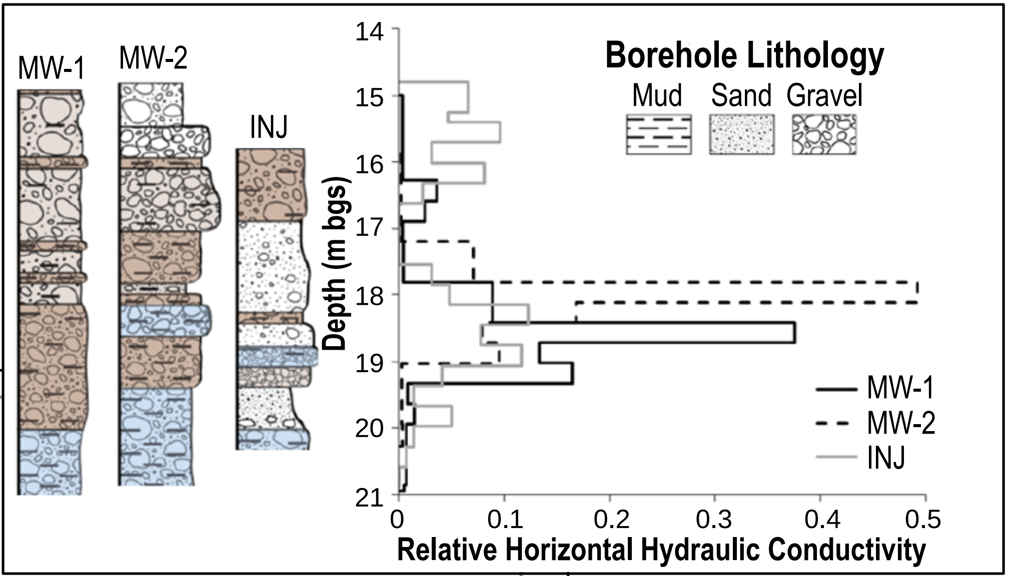


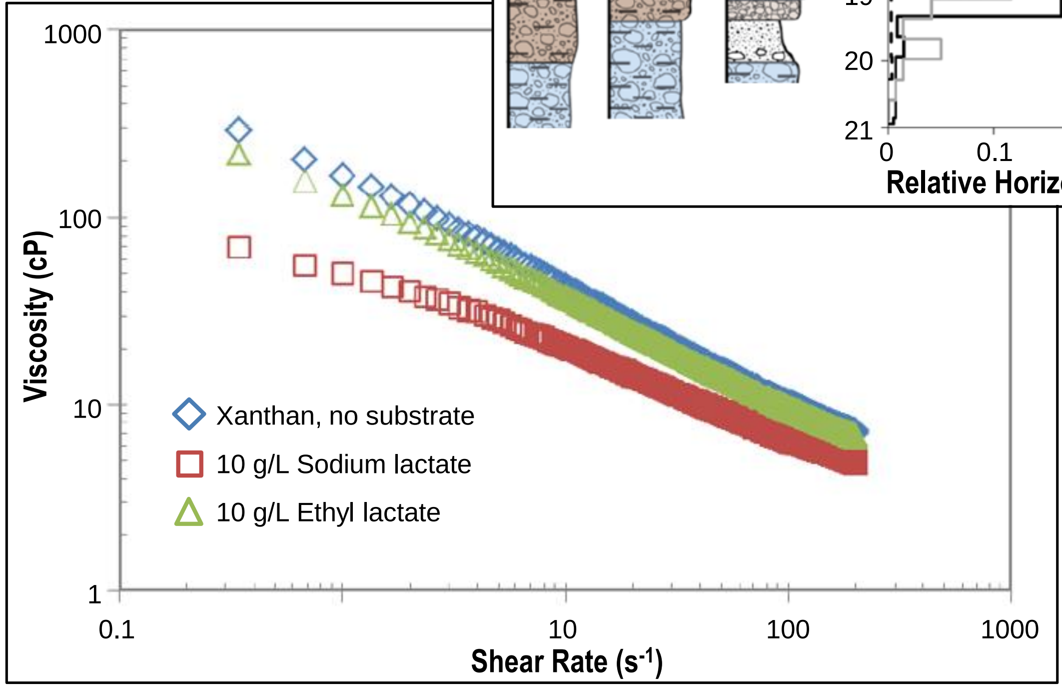
- Non-Newtonian
- Greater viscosity reduction in low-permeability zones compared to high-permeability zones
- Creates transverse gradient from high- to low-permeability zones
- Food-grade water soluble polymers
- Mix and apply with substrate



Using Shear Thinning Enhancements to Improve Distribution (ESTCP ER-200913)

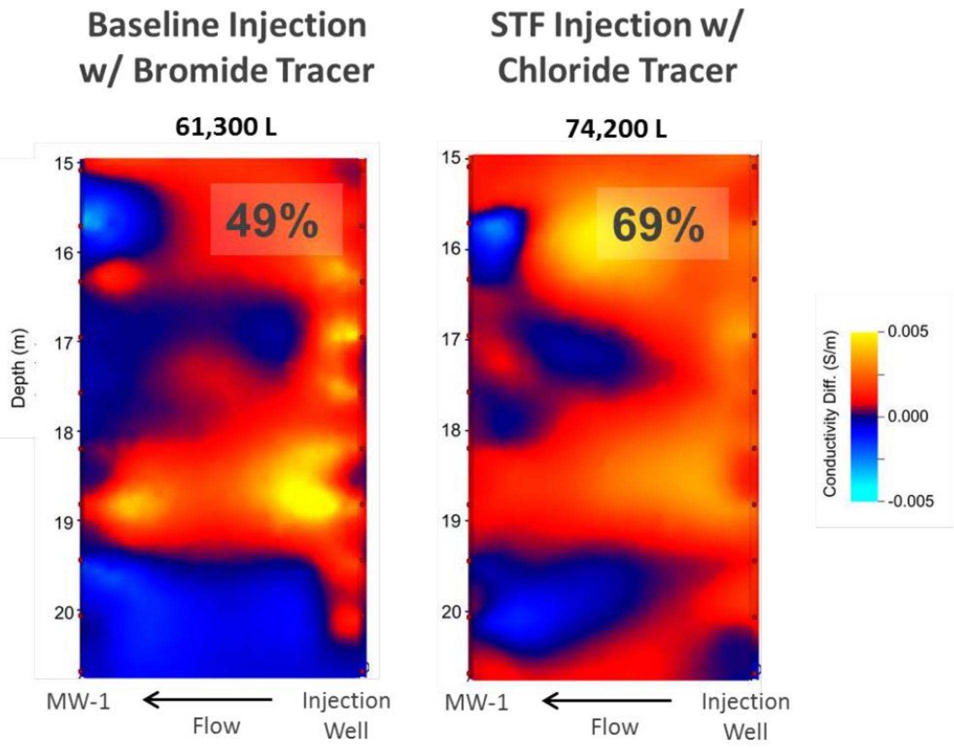
- Area D TCE plume at Joint Base Lewis McChord, WA
  - Glacial outwash & till with varying permeability
  - Low levels TCE present in till and higher silt zones
- Treatability tests to determine amendment type and dose
  - Ethyl lactate (1 g/L)
  - Chloride tracer (230 mg/L)
  - Xanthan gum (800 mg/L) injected





Using Shear Thinning Enhancements to Improve Distribution (ESTCP ER-200913) (cont.)

- Demonstrated improvement to uniformity of amendment distribution
- Evidence of enhanced persistence of TOC in low-permeability layers
- TCE effectively removed below action levels without rebound
- Lessons learned
  - Cannot directly inject into low k layers
  - Target aquifers with permeability contrasts <2 orders of magnitude and/or low-k layers thinner than ~0.5 m to facilitate distribution throughout low-k layer
  - Design injection pressure can be estimated by multiplying the water only injection pressure by the viscosity of the shear thinning fluid under the injection conditions

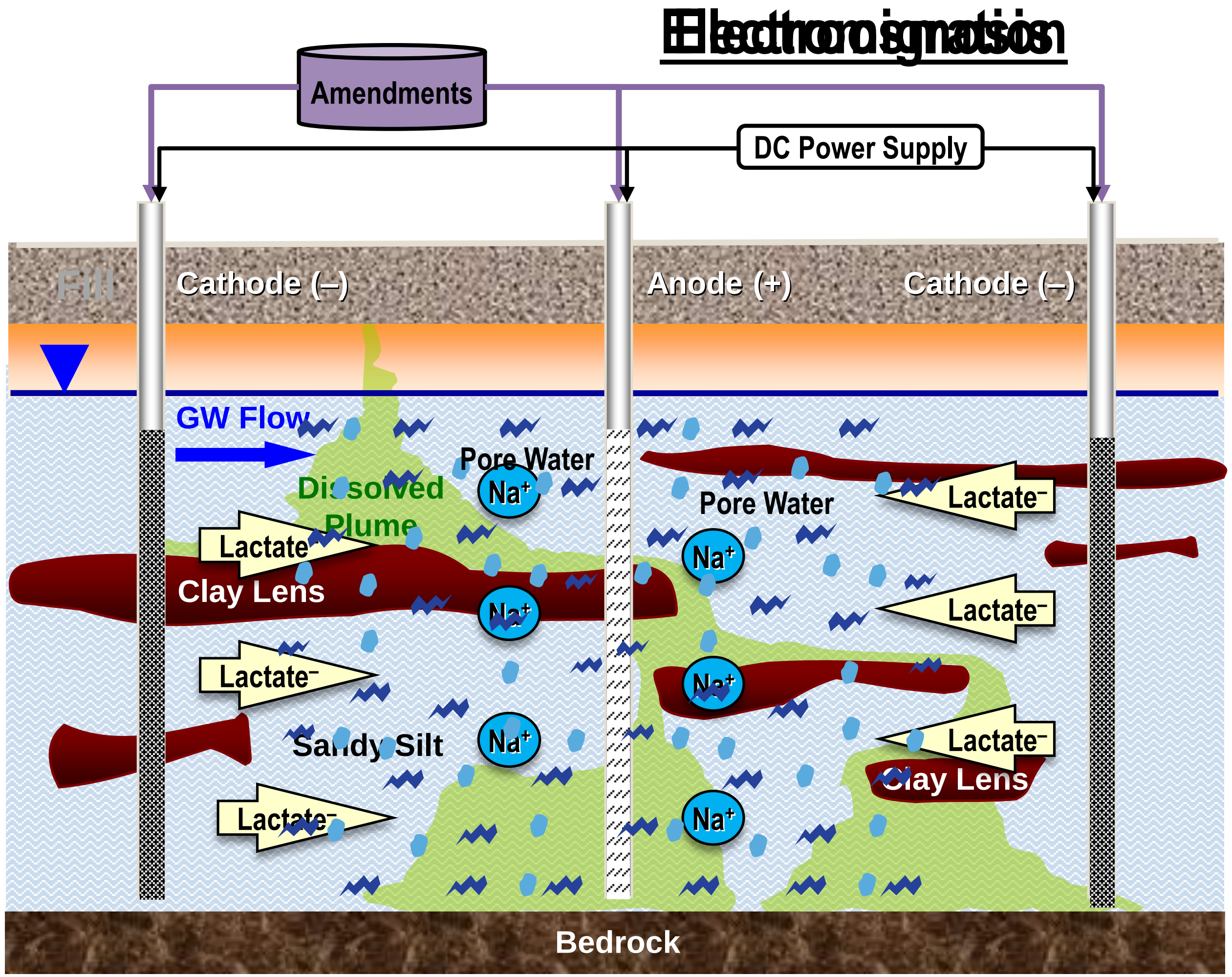


Technological Advances to Address Complex Sites

- Application of Shear Thinning Fluids
- ► **Electrokinetic Bioremediation**
- Heat-Enhanced Bioremediation
- Bioremediation in Fractured Bedrock
- Bioremediation of 1,4-Dioxane

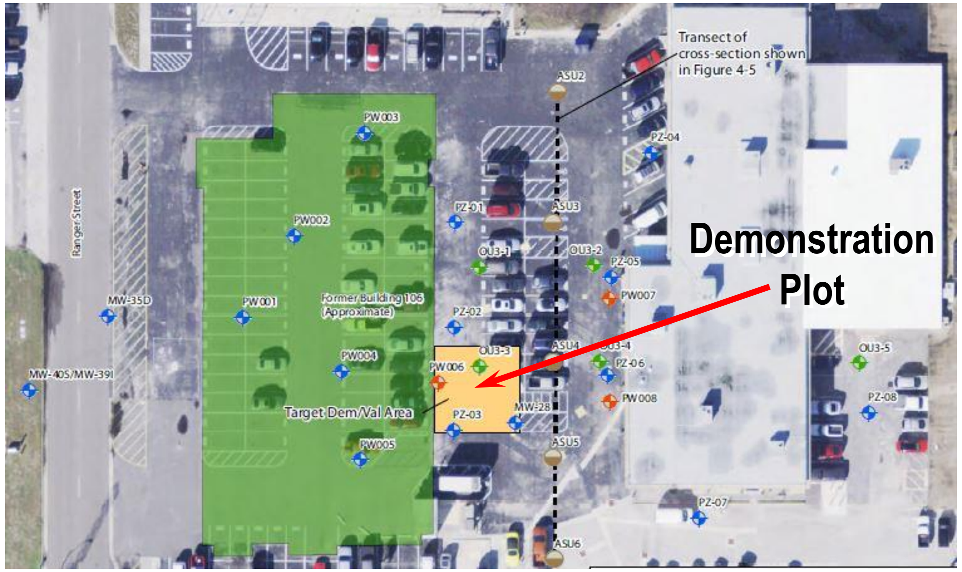
Electrokinetic (Ek) Bioremediation

- Low permeability silts and clays (K <10^{-7} m/s) present challenge for amendment distribution
- This technology leverages the electrical properties of the amendments to promote distribution
- Technology applies an electric current to facilitates electromigration and electro-osmosis
- Successfully demonstrated in Denmark to treat PCE
  - Generated lactate flow of 3 to 5 cm/day through clay

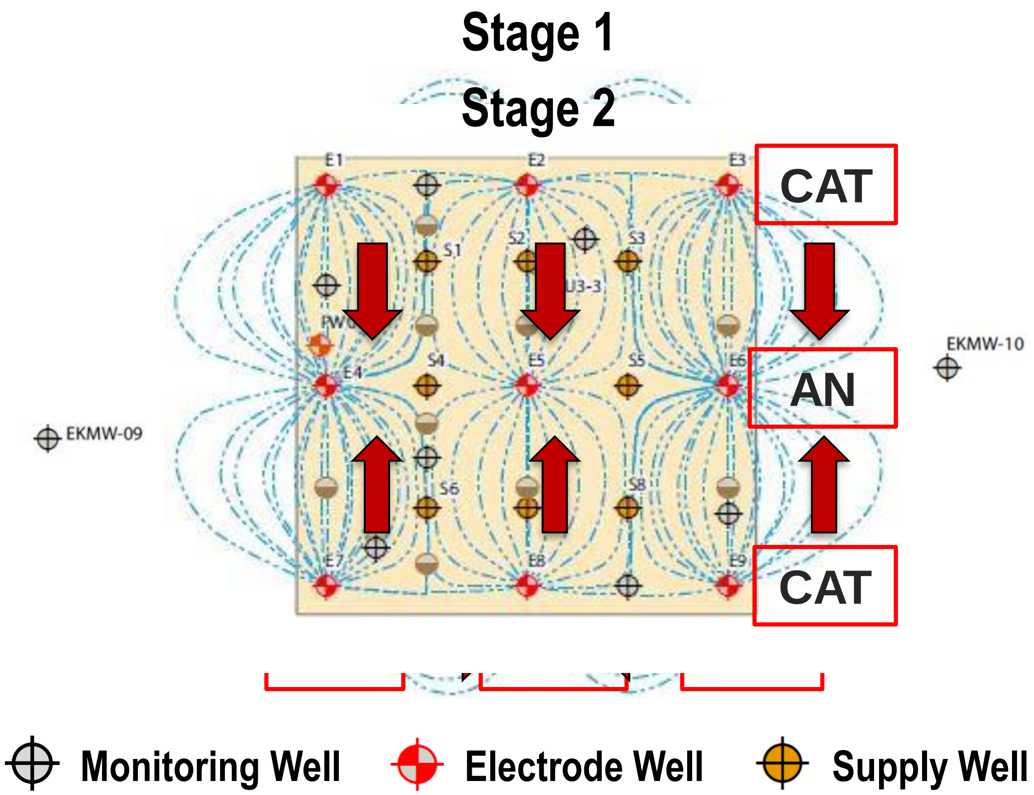


Ek-Bio Demonstration at NAS Jacksonville, FL (ESTCP ER-201325)

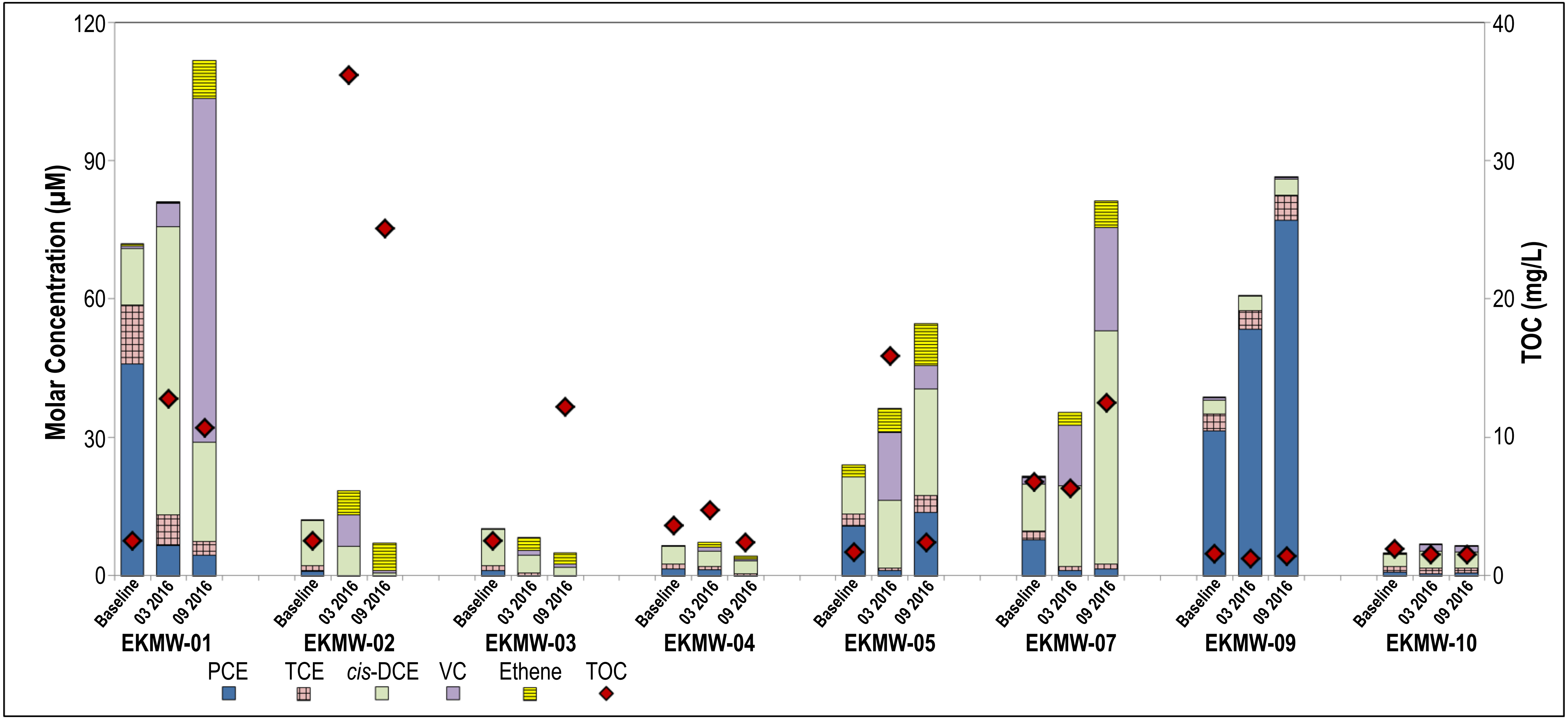
- Potassium lactate and KB-1 (2 Stages)
- Stage 1
  - 100 gallons, 60% lactate
  - 4 L KB-1 into each of 8 supply wells & 2 L KB-1 into each of 9 electrode wells
  - Introduced over 6 months



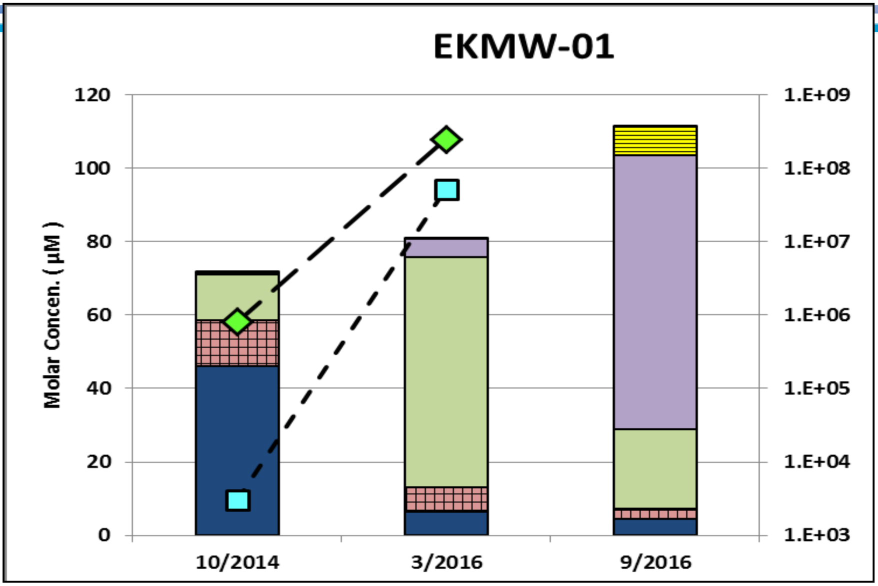
Source: Courtesy of Geosyntec

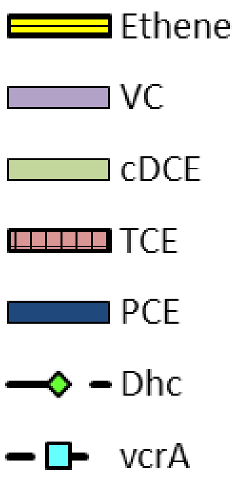


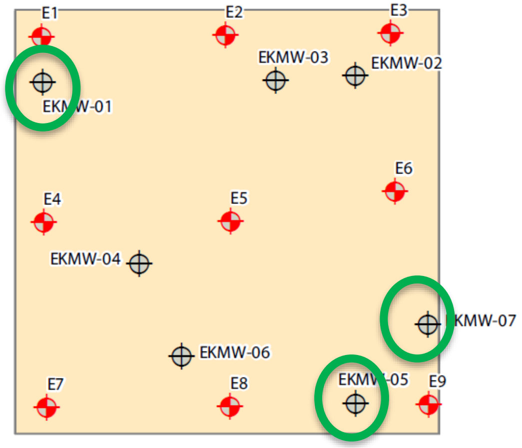
Ek-Bio Demonstration at NAS Jacksonville, FL (ESTCP ER-201325)
Preliminary Results

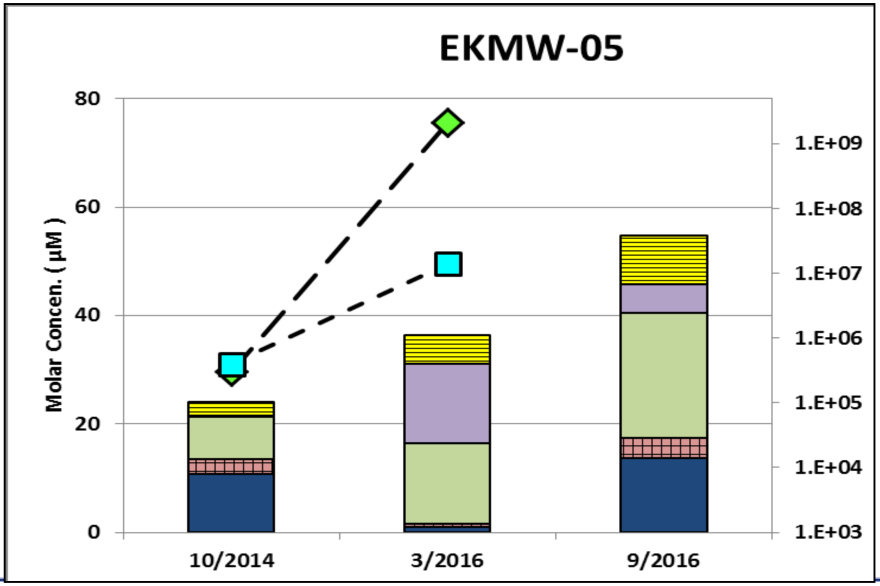


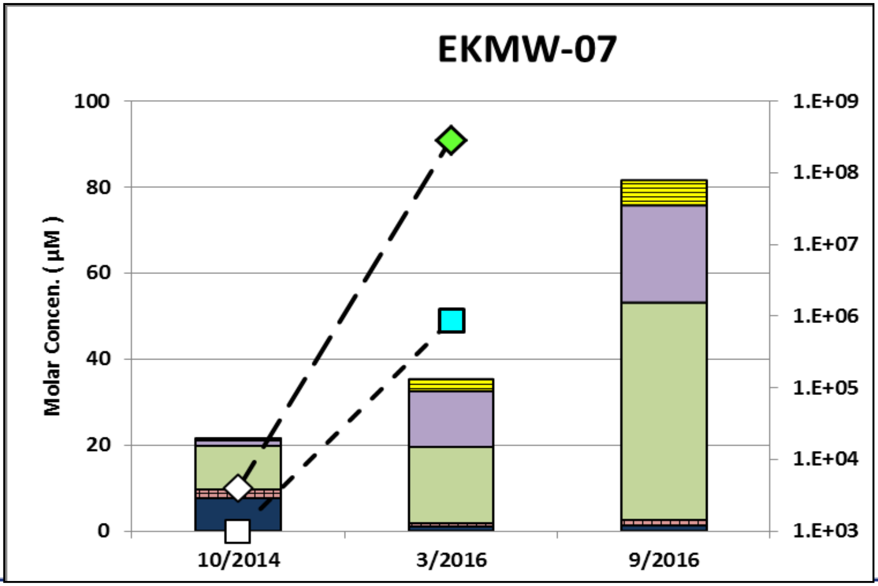
Ek-Bio Demonstration at NAS Jacksonville, FL (ESTCP ER-201325)
Preliminary Results (cont.)











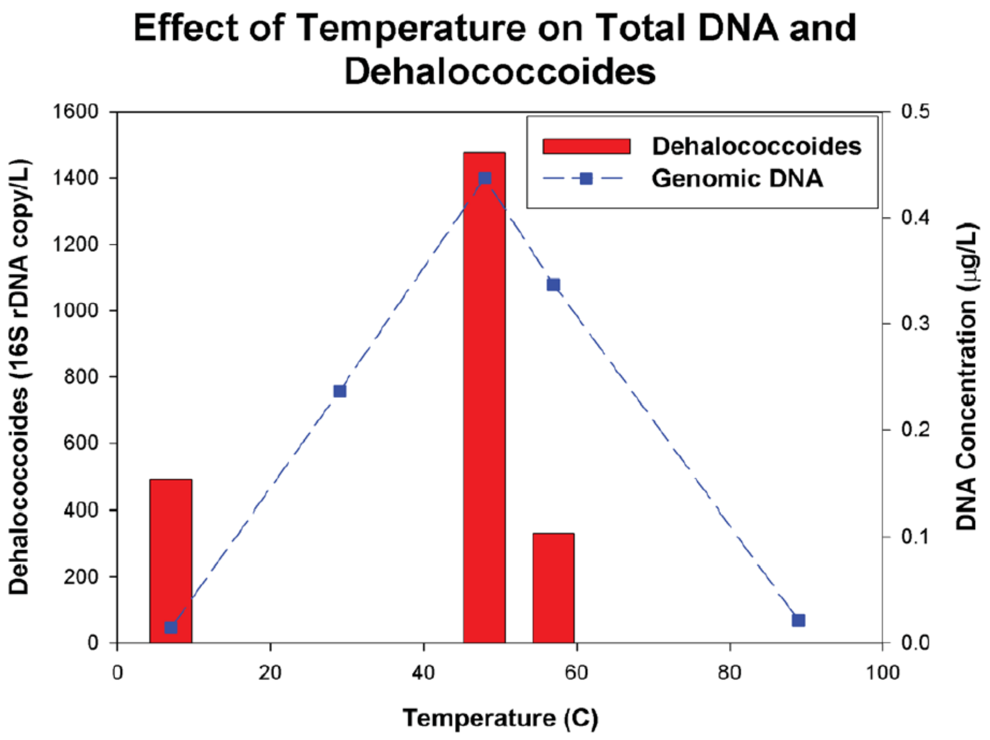
Technological Advances to Address Complex Sites

- Application of Shear Thinning Fluids
- Electrokinetic Bioremediation
- ► **Heat-Enhanced Bioremediation**
- Bioremediation in Fractured Bedrock
- Bioremediation of 1,4-Dioxane

Heat-Enhanced Bioremediation

Why heat?

- Biologically-mediated reaction kinetics increase
- Increased mass transfer rates
  - DNAPL dissolution
  - Desorption
  - Volatilization
- Aboveground treatment generally not required



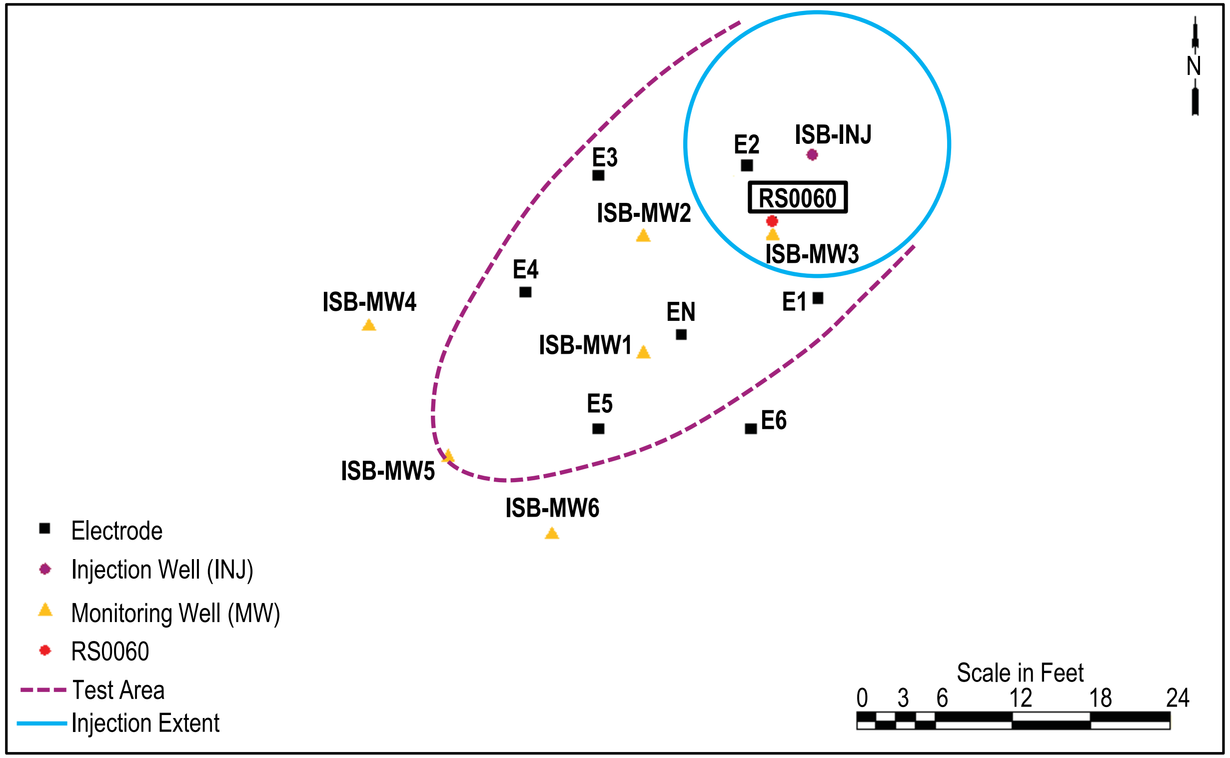
Source: ESTCP ER-200719 Final Report

Key Point

Increases in mass transfer rates for dissolution and volatilization must be balanced by the rate of contaminant degradation to prevent mobilization of COCs outside of the treatment area

Evaluation of Thermally Enhanced Bioremediation (ESTCP ER-200719)

- Comparison of EISB with and without heating
- Joint Base Lewis-McChord Landfill 2 (TCE, NAPL/residual saturation)

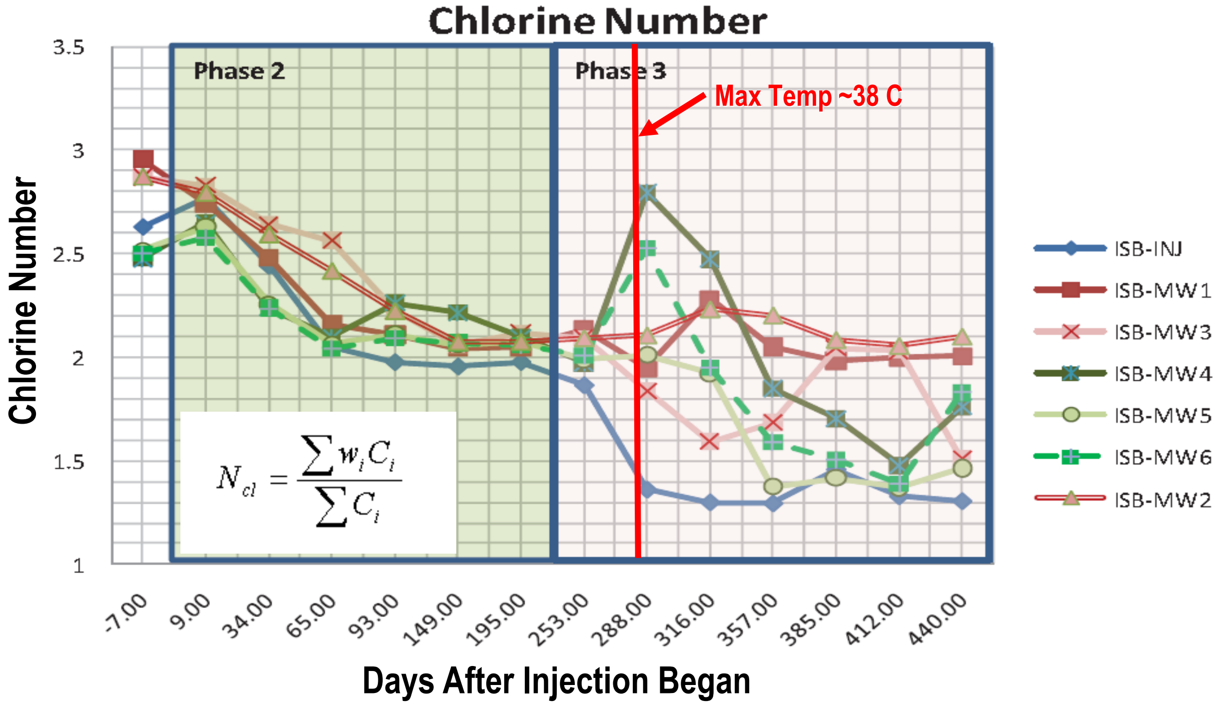


Source: ESTCP ER-200719 Final Report

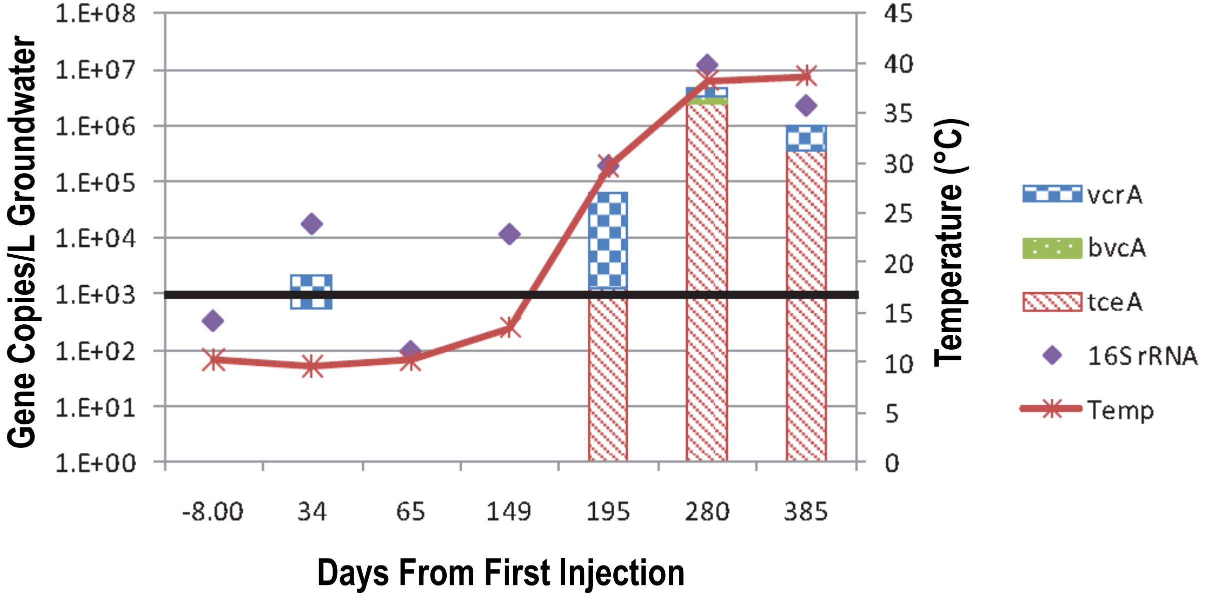
EISB Design

- Target treatment depth of 9 to 20 ft bgs
- Emulsified oil (EOS®) & AquaBupH (2 injections)
- Whey powder & sodium bicarbonate (9 injections)
- 1 injection well, 6 monitoring wells
- Seven 12-ft-long heating electrodes, spaced 11.5 ft apart, and installed to a depth of 20 ft bgs
- Low temperature heating (30º to 40º C) evaluated

Evaluation of Thermally Enhanced Bioremediation (ESTCP ER-200719) Results



$$N_{cl} = \frac{\sum w_i C_i}{\sum C_i}$$



Key Point

TCE treatment rate increased by a factor of 4.6 at 40ºC compared to rate at 10ºC based on empirical correlation

Technological Advances to Address Complex Sites

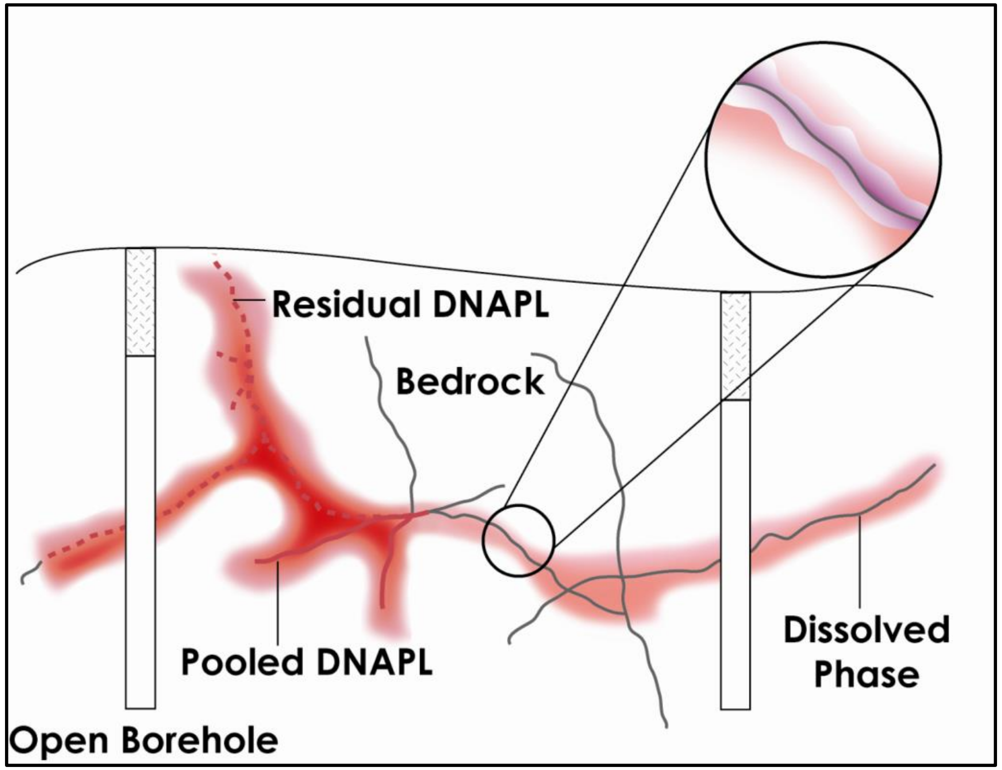
- Application of Shear Thinning Fluids
- Electrokinetic Bioremediation
- Heat-Enhanced Bioremediation

► **Bioremediation in Fractured Bedrock**

- Bioremediation of 1,4-Dioxane

Challenges of Bioremediation in Fractured Bedrock

- COC distribution is likely to be less well understood
- Remedial behavior will be less predictable
- Longer treatment duration required to address matrix-diffusion
- Low TOC and other naturally occurring electron acceptors
- Difficult to achieve good hydraulic connectivity to deliver amendments



Source: Courtesy of Geosyntec

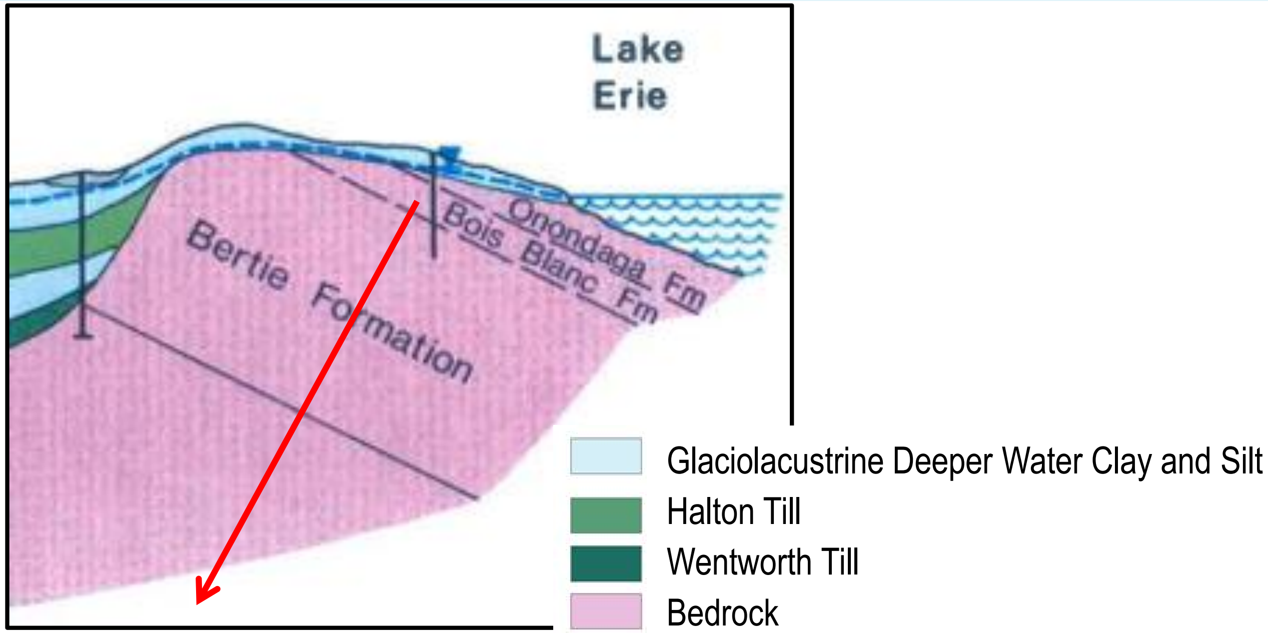
Best Practices for Applying Bioremediation in Fractured Bedrock

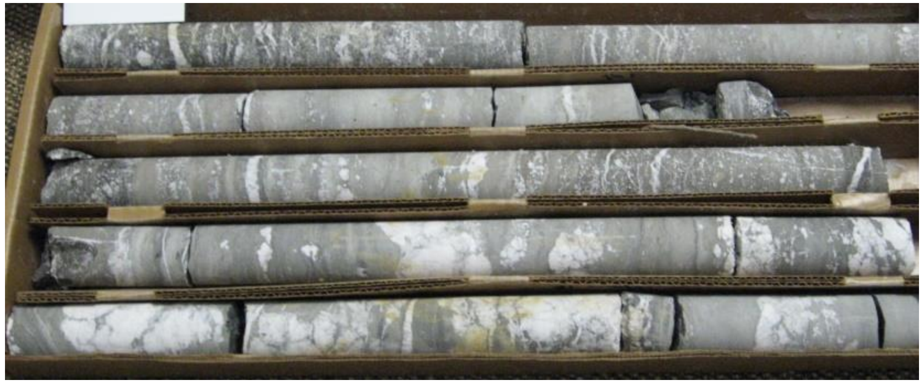
- Consider applying high resolution site characterization techniques
  - Identify horizontal and vertical extent of COCs in groundwater and bedrock
    - e.g., discrete sampling, rock matrix characterization
  - Groundwater flow pathways and velocities
    - e.g., borehole geophysics, heat pulse flow meter
- Inject into isolated zones, establish dense injection grids
- Use long-lasting amendments
- Inject amendments over extended intervals and/or plan for multiple (possibly frequent) injection events
- Consider alternative delivery techniques to facilitate contact & mixing
  - e.g., hydraulic fracturing, pull-push, horizontal barriers

Case Study Fractured Bedrock – Site Characterization

- Identify data gaps/uncertainty
  - Source (overburden, bedrock or both?)
  - How deep are impacts to bedrock?
  - Connectivity?
  - Do vertical fractures play a role?
- Characterization tools
  - Transducer deployment, geophysics, packer testing
  - Tracer testing

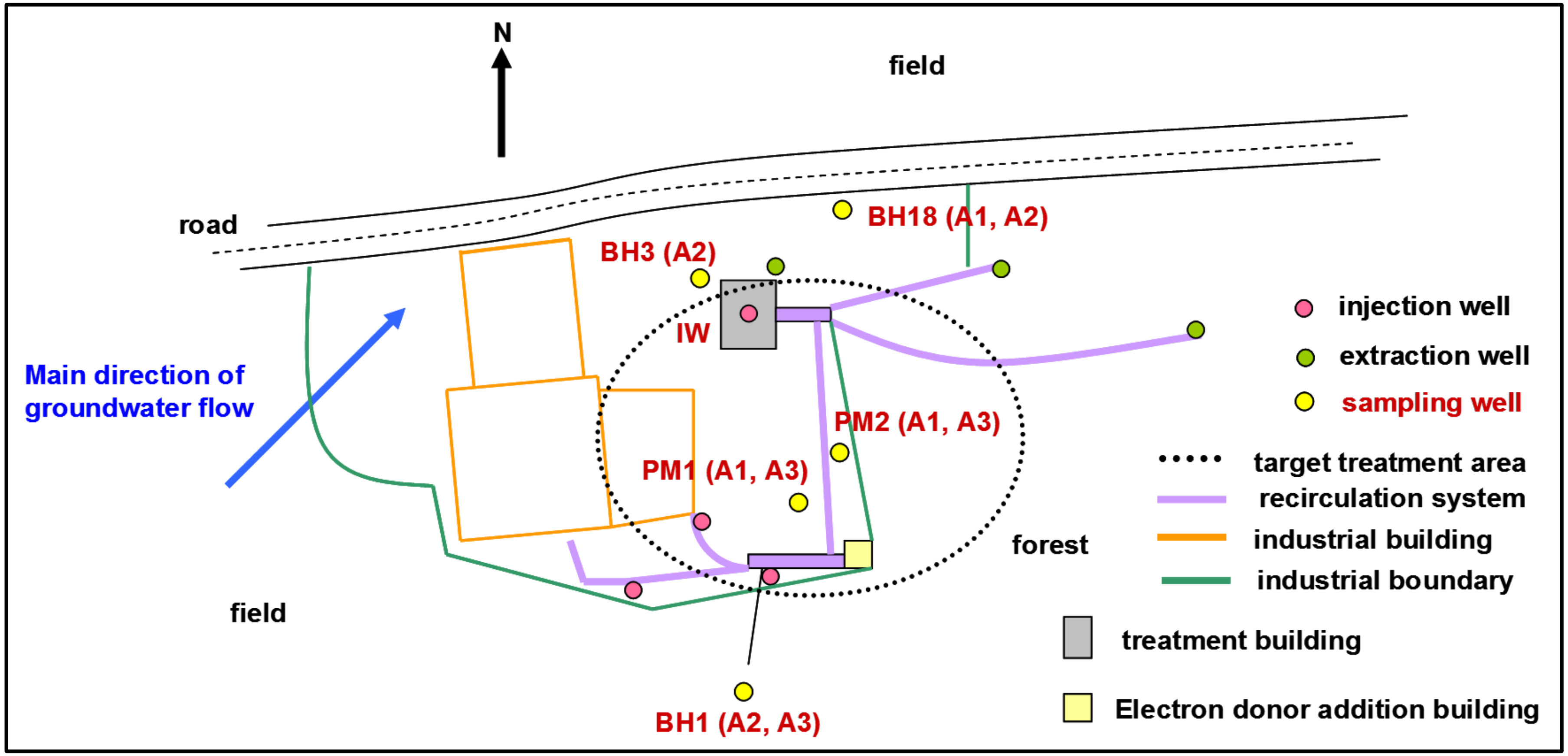
Case Study Courtesy of Geosyntec





Source: Courtesy of Geosyntec

Case Study Fractured Bedrock
Amendment Application

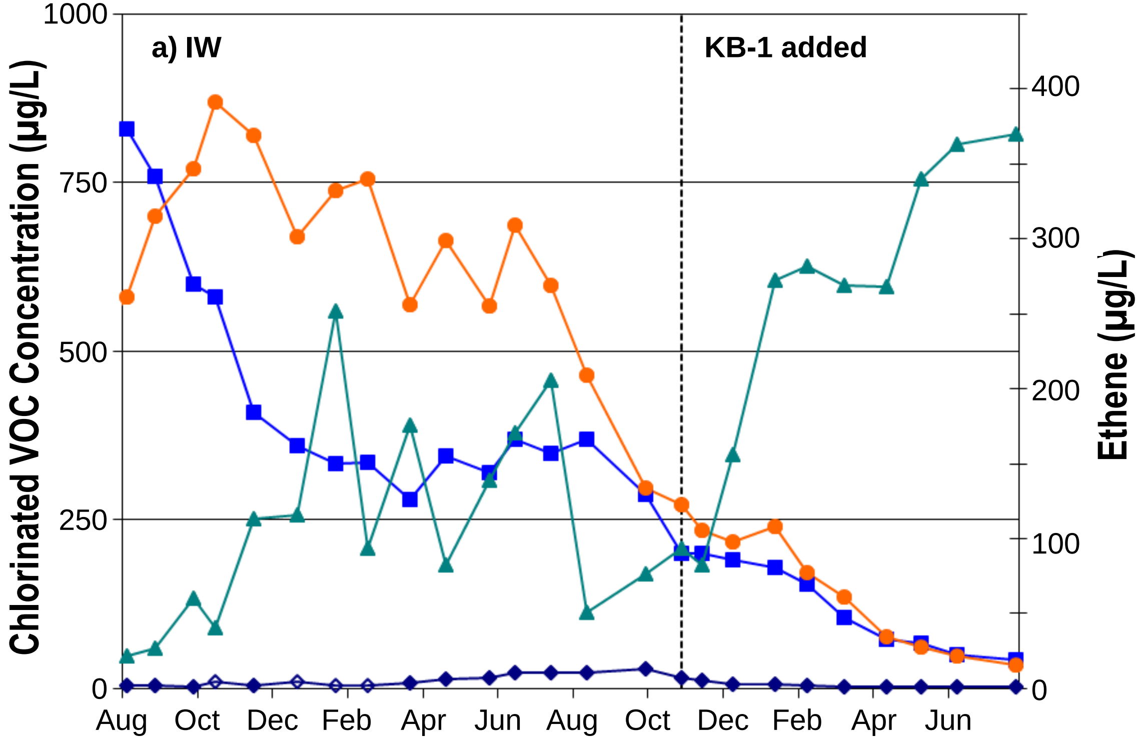


- Amendments
  - Ethanol (95%)
  - Chlorine Dioxide to control fouling
  - KB-1®
- Operation
  - Recirculation system controls groundwater flow and enhances flushing of source to reduce clean up times
  - Recirculation helps to keep nutrients and microbes in contaminated zones

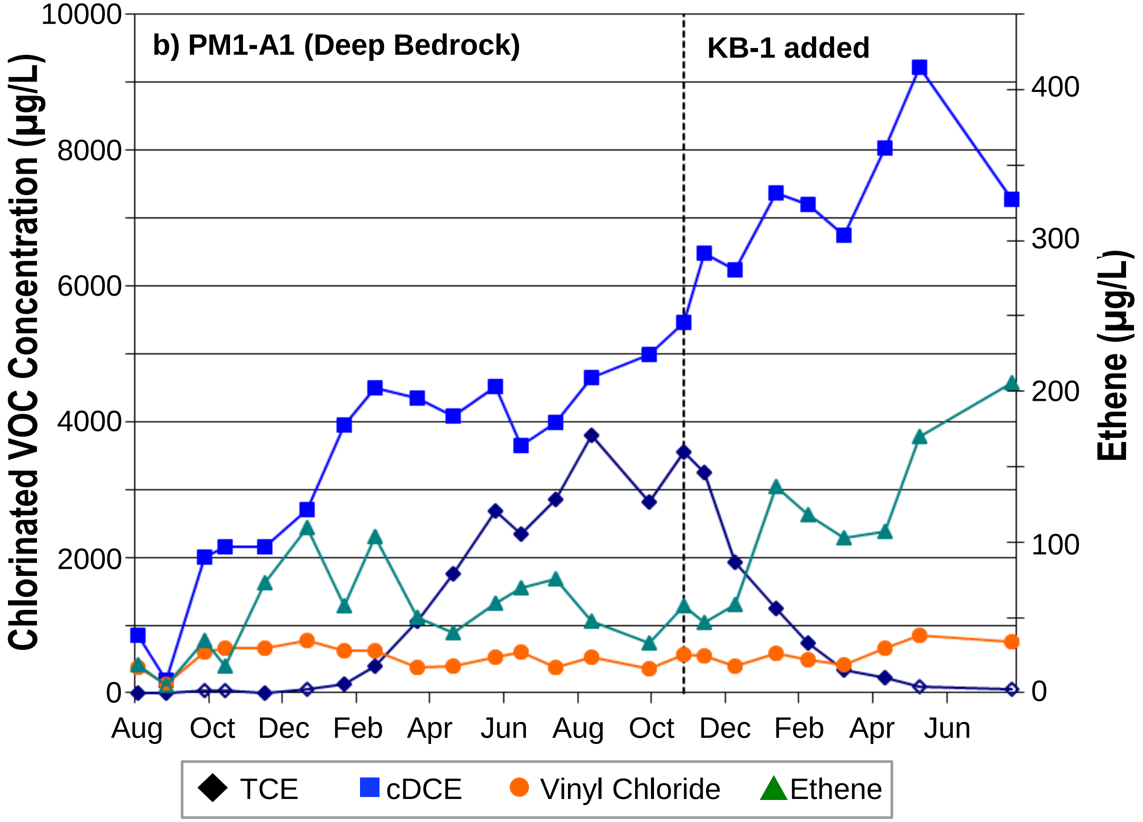
Source: Courtesy of Geosyntec

Case Study Fractured Bedrock
Results

Reinjected Groundwater



PM1-A1 Deep Bedrock



- Anaerobic conditions maintained
- Growth of bacteria observed concurrent with COC treatment
- Reduction of DCE/VC and **>4-fold increase in ethene concentration** after KB-1 bioaugmentation

Source: Courtesy of Geosyntec

Summary

- EISB well-suited to address challenging geologies
- Good characterization and creativity key to achieve goals
- May need to evaluate multiple approaches, expect iterative approach in challenging conditions
- Hydraulic control is important to deliver amendment to target zone in both bedrock and low-permeability media

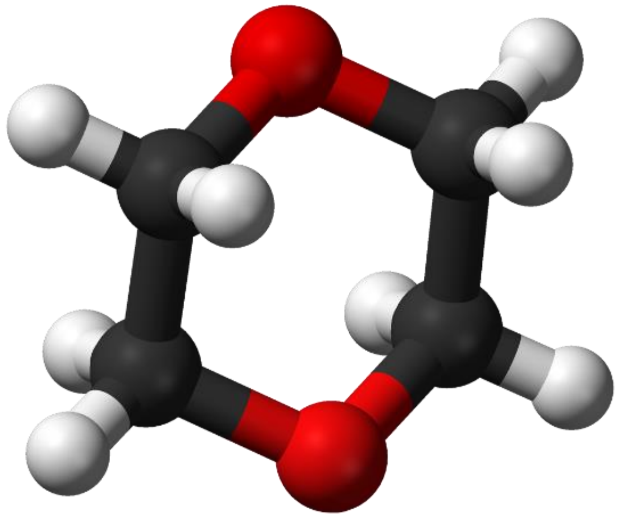
Technological Advances to Address Complex Sites

- Application of Shear Thinning Fluids
- Electrokinetic Bioremediation
- Heat-Enhanced Bioremediation
- Bioremediation in Fractured Bedrock

► **Bioremediation of 1,4-Dioxane**

Bioremediation of 1,4-Dioxane

- Direct metabolic oxidation
  - Dioxane serves as substrate
  - May be better suited for higher concentrations
  - *Pseudonocardia dioxanivorans sp*. Strain CB1190, *Rhodococcus sp*., *Amycolata sp*., *Mycobacterium vaccae*)
  - Bioaugmentation cultures now available
- Co-metabolic oxidation
  - Primary substrates include hydrocarbons such as ethane, propane, & toluene
  - May be more effective for lower concentrations
  - *Pseudonocardiacae* strain CB1190, *Pseudonocardia sp.* strain ENV487, *Mycobacterium sp*. ENV421, *Nocardia sp*. ENV425)



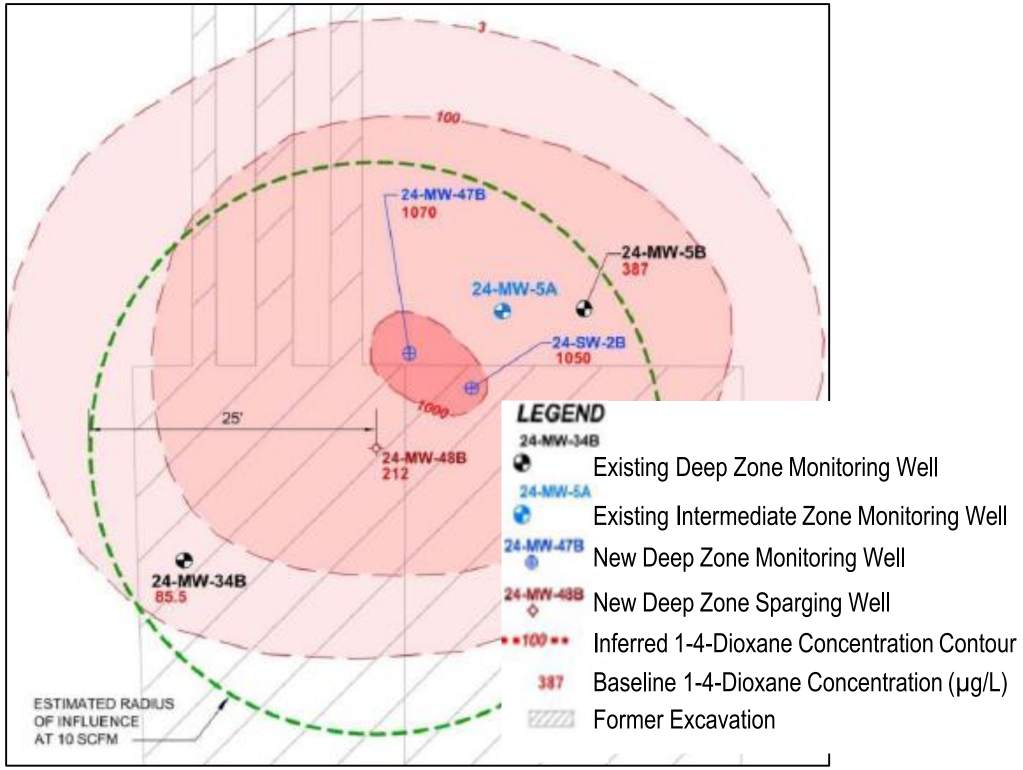
1,4-Dioxane ($C_4H_8O_2$)

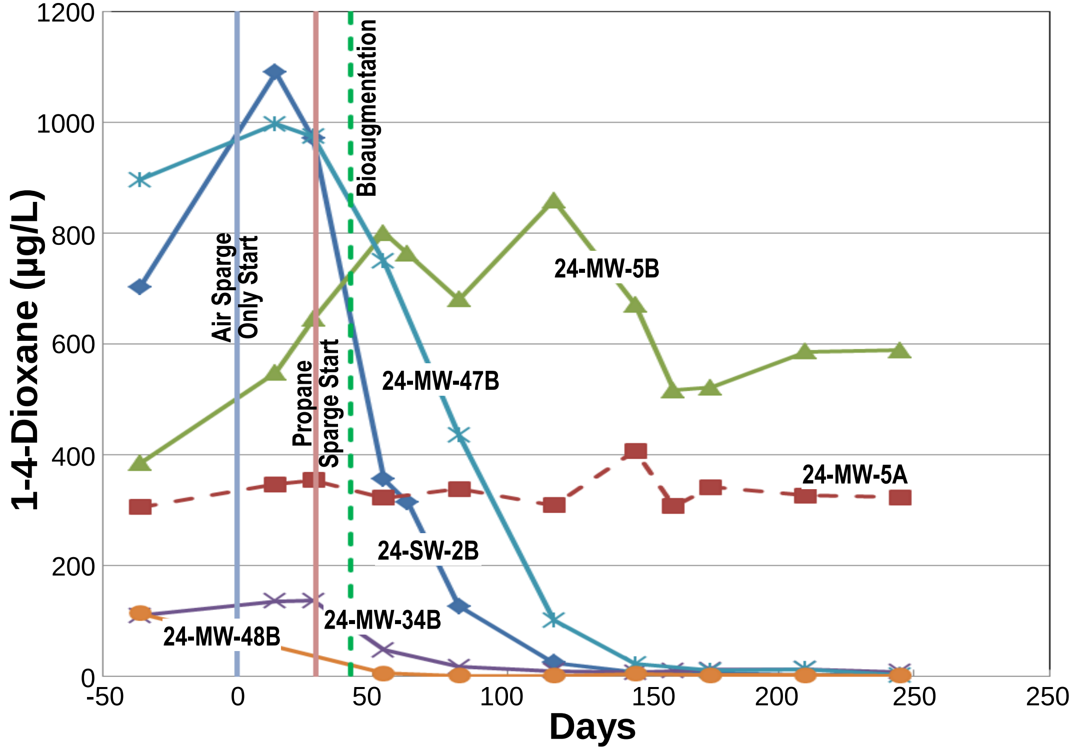
New Developments in 1,4-Dioxane Site Management (SERDP ER-2307)

- Most 1,4-dioxane plumes are dilute, but not as long as originally thought
- Storage in low permeability zones may contribute to 1,4-dioxane persistence
- There is evidence of attenuation at field sites
  - Positive correlation between increasing oxygen concentrations in groundwater and 1,4-dioxane attenuation
  - Negative correlation with high levels of metals and cVOCs
- Treatment train approach may be necessary
  - ISCO followed by EISB
  - Direct and co-metabolic aerobic treatment may be effective when 1,4-dioxane concentrations are high
- MNA may be a valuable tool for remediation at low concentration sites (e.g., <100 ppb)

Cometabolic 1,4-Dioxane Bioremediation – Vandenberg AFB, CA

Soil Sample Location		24-PMW-44	24-PMW-44	24-PMW-44	24-PMW-44	24-PMW-45	24-PMW-45	24-PMW-45	24-PMW-45	24-PMW-45	24-PMW-45
Sample Depth (ft bgs)		10-12	10-12	15-17.5	15-17.5	10-15	10-15	10-15	20-21	20-21	20-21
Amendment		propane	methane	propane	methane	propane	methane	ethane	propane	methane	ethane
1-4-Dioxane Concentration (µg/L)	Time = 0	376	334	405	383	341	366	362	682	350	373
	7 days	<12.5	356	<12.5	332	<12.5	356	<12.5	<12.5	305	101





Source: Lippincott, D.R., R. Steffan, S. Streger, J. Hinkle, J. Stromo, K. Gerber, and A. Nelson. 2014. "Field-scale Demonstration of *In Situ* 1,4-Dioxane Bioremediation," In *Proceedings of the Battelle Ninth International Conference on Remediation of Chlorinated and Recalcitrant Compounds,* Monterey, CA, May 19-22.

NAVFAC EXWC 1,4-Dioxane Remediation Research and Development

- **NESDI Project 545** – North Island, CA (Spring 2017)
  - – Biotreatment of 1,4-dioxane ‘source’ area (ppm concentrations)
  - – Conducting laboratory biotreatability tests
  - – Scale-up of best lab tech to be evaluated
  - – Pilot test
- **ESTCP Project ER-201733** – North Island, CA (Spring 2017)
  - – Comingled plume 1,4-dioxane (ppm levels) and chlorinated solvents
  - – Aerobic cometabolism with multiple primary substrates
  - – Potential limited effectiveness of single primary substrate (competitive inhibition)
- **Headquarters-Funded Project** – Bedford, MA; Brunswick, ME; North Island, CA; Whidbey Island, WA (Current)
  - – Testing for 1,4-dioxane biomarkers and performing laboratory MNA tests
  - – Two sites have already been sampled with the other two later in the spring

Other 1,4-Dioxane Research and Development

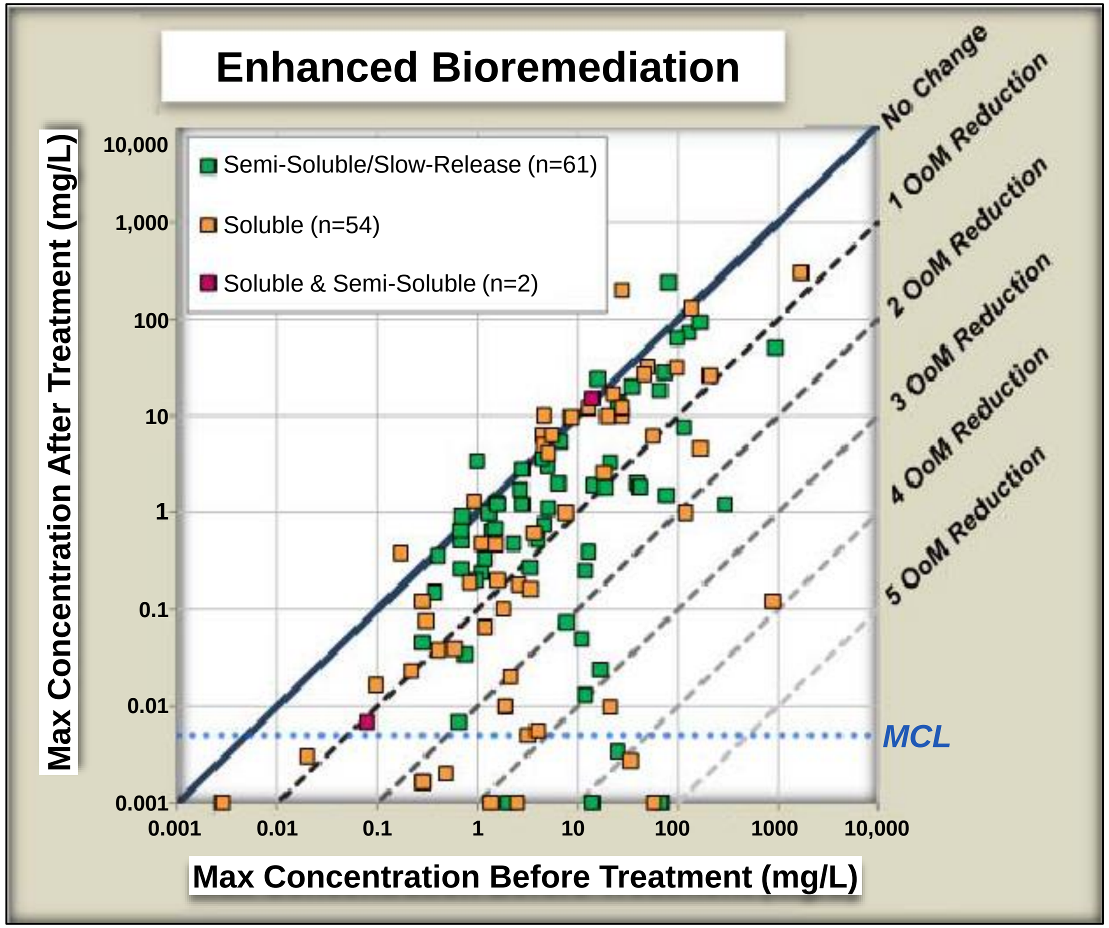
- Pilot test at Former Air Force Plant 3
  - Two biobarriers in sequence, upgradient anaerobic barrier (to produce methane), downgradient barrier to generate oxygen for methanotrophs to generate methane monooxygenase
- SERDP ER-2300 *In Situ* Biodegradation of 1,4-Dioxane: Effects of Metals and Chlorinated Solvent Co-Contaminants
- SERDP ER-2303 Evaluation of Branched Hydrocarbons as Stimulants for *In Situ* Cometabolic Biodegradation of 1,4-Dioxane and Its Associated Co-Contaminants
- SERDP ER-2306 – Cometabolic Aerobic Biodegradation of 1,4-Dioxane by Methanotrophs in a Co-Mingled Chlorinated Solvent Plume

Presentation Overview

- Introduction
- Review of Enhanced Bioremediation
- Best Practices for Successful Application, Distribution, and Mixing of Amendments
- Technological Advances to Address Complex Sites
- ► **Innovation in Amendment Formulations**
- Best Practices and Advances in Monitoring
- BioPIC – A Tool to Select, Evaluate, and Convince
- Wrap-Up

Electron Donors – Which Should I Use?

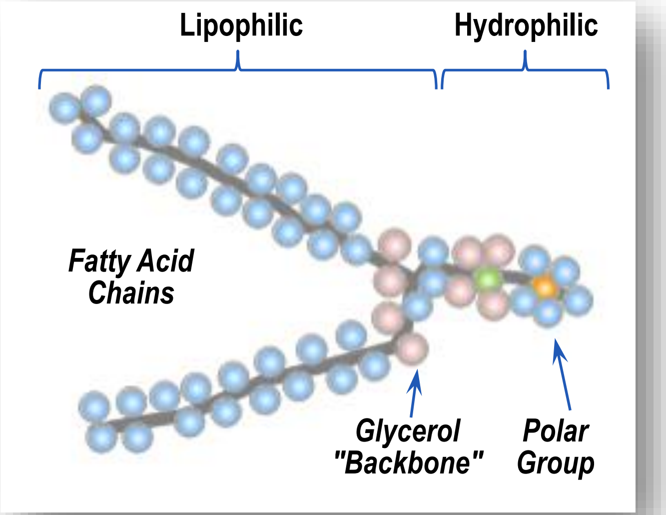
- Wide variety of electron donor amendments available
  - – Soluble substrates
  - – Slow release substrates
  - – Solid substrates
- Soluble and slow release demonstrated to perform similarly
- Type of amendment should be based on site-specific factors and objectives
- Work closely with vendors, use available dosing design tools

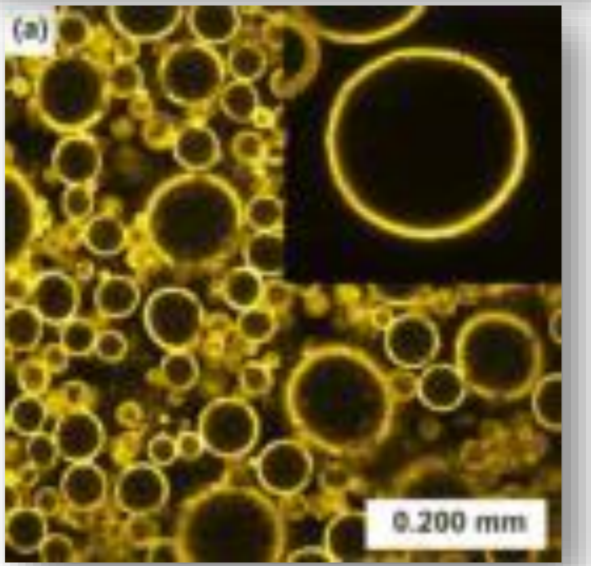


Source: *ESTCP ER-201120, Final Report*, GSI International, March 2016

Example – Emulsified Lecithin Substrate

- ELS™ – microemulsion lecithin-based food grade carbon
- Amphiphilic (has hydrophobic and hydrophilic ends)
- Composed of fast release and slow release electron donor
- Provides nitrogen and phosphorous
- Small size for easy distribution (60% <1 µm, 85% <2 µm)
- High yield of hydrogen





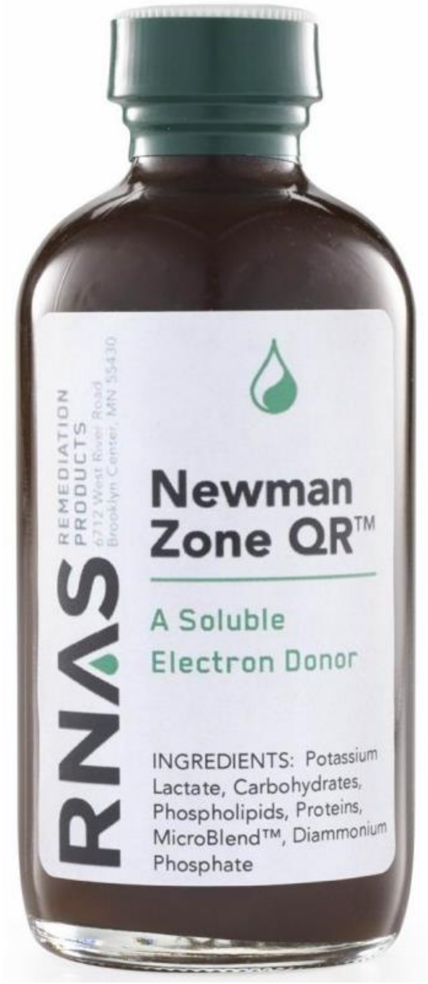
Source: Courtesy of Peroxychem

Quick Release Electron Donors

- Complex blend of food grade carbon source, nutrients, cofactors, and vitamins
- Average longevity (typically months)
- Stimulates rapid microbial growth
- RNAS (Newman Zone QR), EOS (EOS QR), Tersus (EDS QR) have developed proprietary formulas[1]

<u>Example – Newman Zone QR</u>

- Contains lactate, complex carbohydrates, phospholipids, soluble protein, Microblend™, and phosphate
- Viscosity similar to water
- Highly soluble in groundwater

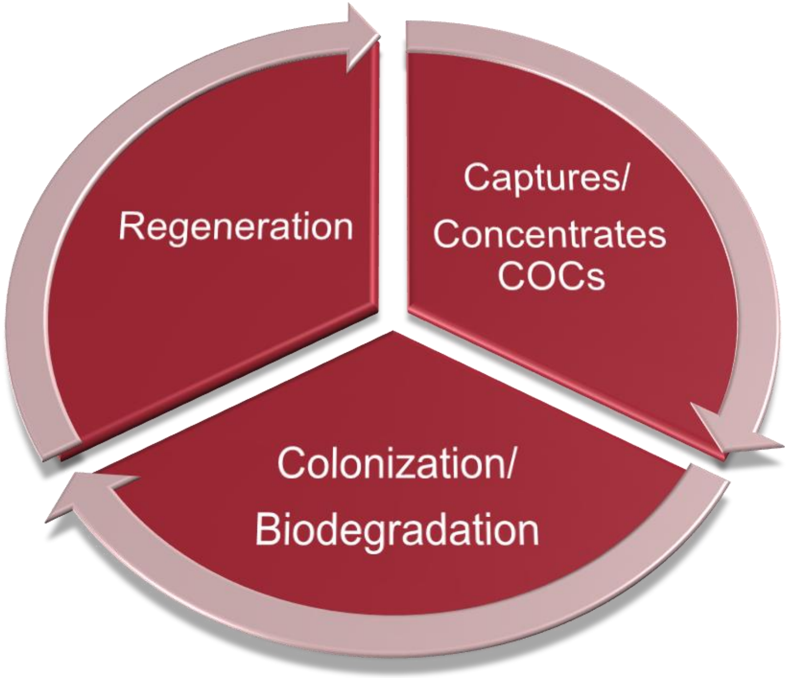


Source: Courtesy of RNAS

1) A wide range of electron donors are available. The Navy does not endorse any particular type. Selection should be based on site-specific conditions and project objectives.

PlumeStop® Colloidal Liquid Activated Carbon

- Colloidal biomatrix 1- to 2-µm particles of activated carbon
- Distributes well under low injection pressures (thin film of large carbon loading)
- Long lasting therefore addresses matrix diffusion
- Resists clumping
- No fracturing necessary
- No plugging of formation
- Can be applied in conjunction with an electron donor



Bioaugmentation Cultures

- Many bioaugmentation cultures to choose from
- Mixtures of a variety of bacteria
  - Can help address issues with co-contaminants
- Low-pH tolerant cultures more commonly available
  - KB-1 Plus (pH 5.8)
- Cultures continue to be developed and optimized
  - Aerobic degradation of 1,4-dioxane
  - Anaerobic benzene degradation

Pocket Reference for Commercially Available Bioaugmentation Cultures for Reductive Dechlorination

Vendor[1]	Culture	Target Contaminants	Other	Vendor Web Address
Sirem	KB-1	Chlorinated ethenes, ethanes, methanes, propanes, RDX, chloroflourocarbons	Well-suited for low pH (5.8 to 6.3) aquifers	http://siremlab.com/kb-1-kb-1-plus/
	KB-1 Plus			http://siremlab.com/kb-1-kb-1-plus/
Regenesis	BDI Plus	Chlorinated ethenes/ethanes		https://regenesis.com/remediation-products/
EOS Remediation	BAC-9	PCE, TCE, *cis*- & *trans*-DCE, VC, Freon 113, mixed plumes containing 1,1,1-TCA & 1,1,2-TCA, dichloroethane isomers, CT, chloroform, and bromine compounds		http://www.eosremediation.com/bac-9-product-information/
BCI Inc.	BCI-e	Chlorinated ethenes	Variations of cultures are available, which are not inhibited by chloroform, high PCE conc., TCA, and brackish water	http://www.bcilabs.com/s.bioaug.html
	BCI-a	TCA, 1,1-DCA, and chlorinated ethenes		
	BCI-t	Trichlorobenzene and Dichlorobenzenes		
Terra Systems	TSI DC	Chlorinated ethenes, ethanes, 1,1,1-trichloroethane, 1,1-dichloroethane, chloroethane, carbon tetrachloride and chloroform		http://www.terrasystems.net/bioremediation2.html
	TSI DC-TCA	Chlorinated ethenes, 1,1,1-trichloroethane (1,1,1-TCA), 1,1,2-trichloroethane (1,1,2-TCA), 1,2-dichloroethane (1,2-DCA), and 1,1-dichloroethane (1,1-DCA)	Equal concentrations Dhc and Dehalobactor	http://www.terrasystems.net/bioremediation2.html
Redox Tech	RTB-1	Chlorinated ethenes		http://www.redox-tech.com/Products/bioaugmentaion-with-rtb-1.html

1) Other cultures may be available. The Navy does not endorse any particular type. Selection should be based on site-specific conditions and project objectives.

Methane Inhibiting Amendments?

- Methane
  - Production indicates that the electron donor was consumed by methanogens instead of target microbes
  - Potential to create explosive concentrations of methane?
  - Vapor intrusion
  - Greenhouse gas
- Amendments formulated using red yeast rice extract
  - Contains statins, mono-unsaturated fatty acids, vitamins and nutrients
- Provect-CH_4
  - Water soluble powder
  - Persists 1 to 3 months in aquifer

Well Location	Time after Addition	CH_4 PID (ppm)	CH4 FID (ppm)
MW-4 (ABC Only)	0 min	297	>50,000
	5 min	439	>50,000
MW-207s (Provect CH_4)	0 min	82	Out of range
	5 min	41	1,599

Source: Courtesy of Provectus

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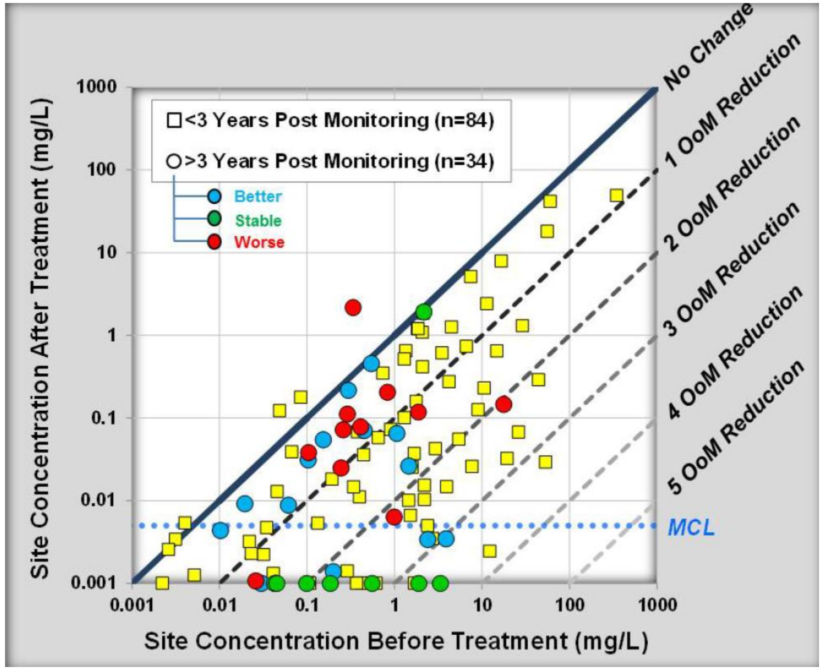
Process and Performance Monitoring Parameters

Measurement	Process	Performance	Common Evaluation Purposes
Pressures, Volumes, & Flowrates	X		Amendment dosage, formation of fractures, fouling
COC Concentrations		X	Treatment progress, rebound
Soil Gas Vapors	X	X	Biodegradation and vapor intrusion
Groundwater Levels	X	X	Distribution of amendments, preferential pathways, fouling, radius of influence
Groundwater Quality and Alkalinity		X	Amendment distribution, suitability of aquifer for survival and proliferation of microorganisms (e.g., suitable pH and buffering)
Total Organic Carbon	X	X	Electron donor distribution and supply
Visual Observations	X		Amendment distribution (e.g., presence in wells, gas bubbles)
Dissolved Hydrocarbon Gases		X	Degradation progress, high methane can be hazardous to bacteria and present health and safety issues.
Dissolved Metals		X	Evaluate redox conditions, metals mobilization
Bacteria & Gene Counts		X	Assess quantities of microorganisms and/or specific degradative genes

What period of long-term monitoring is adequate?

- No statistically significant trend that median concentration reduction is greater at sites with <3 years of monitoring compared to 3 to 12 years

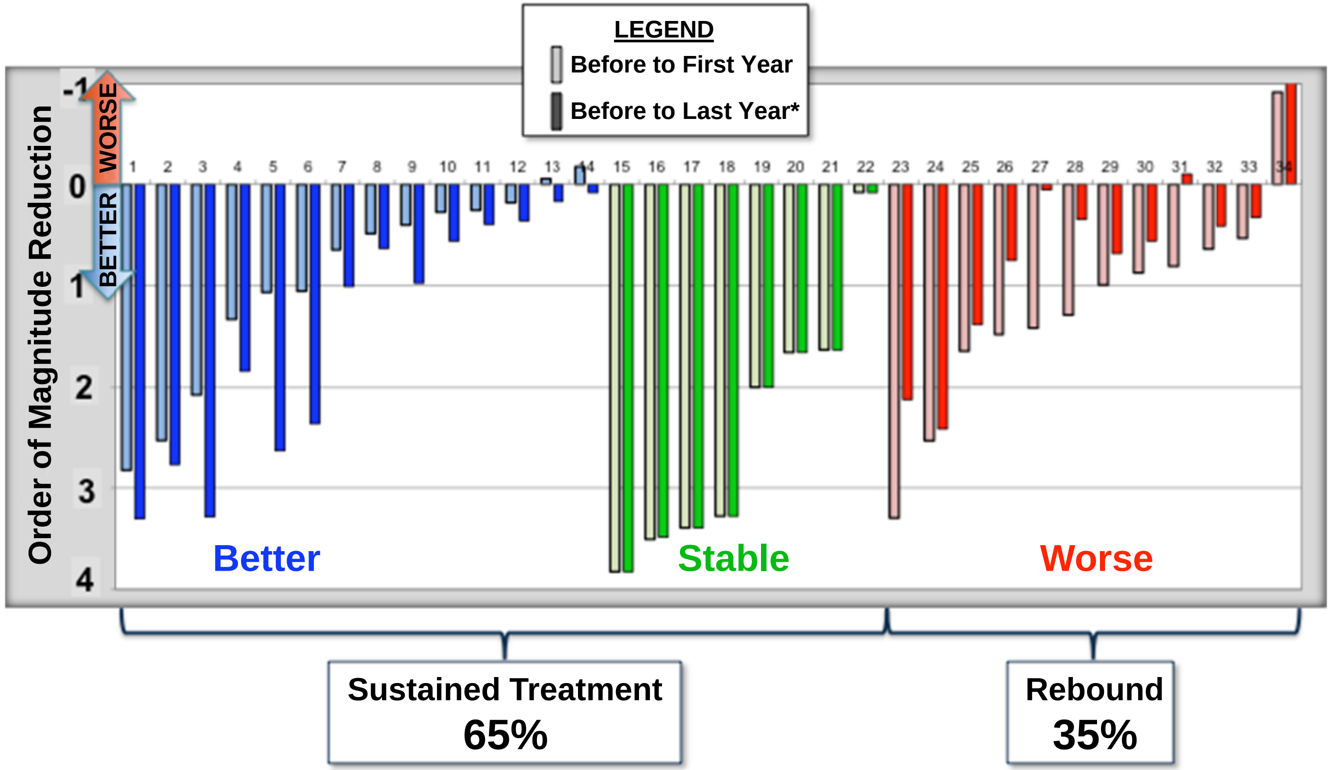


Source: ESTCP ER-201120

Key Point 3 years of long-term monitoring likely is sufficient to demonstrate efficacy of the remedy

What about monitoring for rebound?

- In general, sustained treatment observed at sites that achieved at least 2 OoM reduction within one year of treatment
- Only a small percentage of sites able to achieve an additional OoM after first year
- Extent of rebound, when occurred, was generally less than 1 OoM
- At all but 2 sites, concentration after final year was less than pretreatment value
- At sites where rebound occurred, median concentration reduction decreased from 90% to 67%



* Average time between first year and last year = 6 years

Source: *ESTCP ER-201120*

Monitoring of Secondary Water Quality Impacts (SWQI) (SERDP ER-2341)

- Application of enhanced bioremediation can have long-term impacts to groundwater
  - Potential adverse impacts on future groundwater use
  - Potential to exceed secondary maximum contaminant levels for iron, manganese, and total dissolved solids
  - Potential to mobilize hazardous compounds such as arsenic
  - Health and safety (e.g., formation of methane and hydrogen sulfide)
- Evaluated long-term performance data at 47 sites
  - Distinguished between upgradient, injection zone, and downgradient (<10, 10 to 25, 25, to 50, and >50 m) locations

SWQIs of Interest
Oxygen (O_2)
Nitrate (NO_3^-)
Sulfate (SO_4^{2-})
Manganese (MN^{2+})
Dissolved Iron (Fe^{2+})
Arsenic (As)
Methane (CH_4)
Sulfide (S^{2-})
Total Organic Carbon (TOC)
pH

SWQI (SERDP ER-2341)
Key Results

- Most SWQI attenuate rapidly outside of the injection area
- SWQIs unlikely to adversely impact potable water supplies
  - Significant SWQIs were located in or less than 10 m of the injection area
  - Large percentage (92%) of SWQIs were within cVOC plume
- Elevated levels of As were highly correlated with dissolved iron
- The reduced iron and sulfur and organic carbon will cause the downgradient aquifer to remain anoxic for years

SWQI	O_2	NO_3^-	SO_4^{2-}	S^{2-}	Fe^{2+}	MN^{2+}	As	CH_4	TOC	pH
Injection Area	NS	NS	-	+	+++	+++	+	++	+++	-
Downgradient (0 to 10 M)	NS	NS	NS	+	+	+++	+	+	++	NS
Downgradient >10 M	NS	NS	NS	NS	+	+	+	+	+	NS

Advanced Monitoring Tools to Assess EISB Performance

Tool	Overview	Example Questions Answered
CSIA	Analyze relative abundance of isotopes (^{13}C & ^{12}C)	Is biodegradation occurring?
qPCR	Quantification of target genes	Are necessary organisms present in sufficient quantity? What impact does amendment addition have on the community?
FISH	A flouresecent dye is appended to a particular gene of interest. Flourescent light emitted is used to determine abundance	What other microorganisms are present in the environment, and what impact do they have on the microorganisms or processes of interest? Does the microbial community change in response to an amendment?
Microarrays	Evaluate community composition based on the presence of 16S rRNA genes	How diverse is a community and what functional genes are present? What competing organisms are present?
SIP	Placement of isotopically modified (^{13}C & ^{15}N) contaminants in aquifer, followed by subsequent analysis of byproducts	Is biodegradation occurring? Can biodegradation occur under modified conditions? Are organisms present capable of degrading the contaminant?
Enzyme Activity Probes	Uses surrogate compounds that are transformed by target enzymes into distinct and readily detectable products.	Which known organisms are present and active? What is the rate of contaminant degradation?
Proteomics	Analysis of the proteins (enzymes) produced by a microbial community	Is a specific organism actively degrading the contaminant of concern?

http://www.itrcweb.org/emd-2/

Monitoring EISB with Omics

- Next generation sequencing
  - Differentiates community composition (qualitative)
  - Does not detect every genus
- qPCR (presence)
  - Quantitative measurement of specific organisms or functional genes
  - Need to know what you are looking for
- Proteomics (activity)
  - Provides a direct measurement of microbial activity
  - Are bacteria actively degrading COCs?

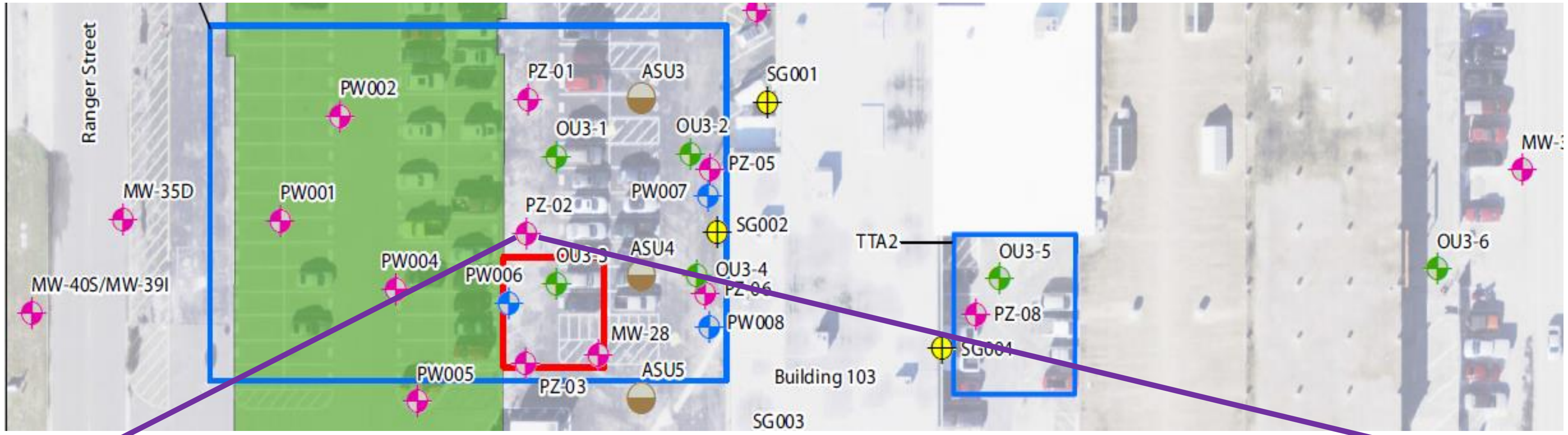
Omics Provides Answers

- **What is the composition of the microbial community and which organisms are thriving?**
- **How does the community change with time and/or environment?**
- **Should bioaugmentation be performed?**
- **Is active degradation occurring?**
- **Why is VC stall occurring?**
- **If/when should amendments be reapplied?**

Key Point — **Omics provides important lines of evidence to design and optimize EISB applications**

Case Study Naval Air Station, Jacksonville, FL

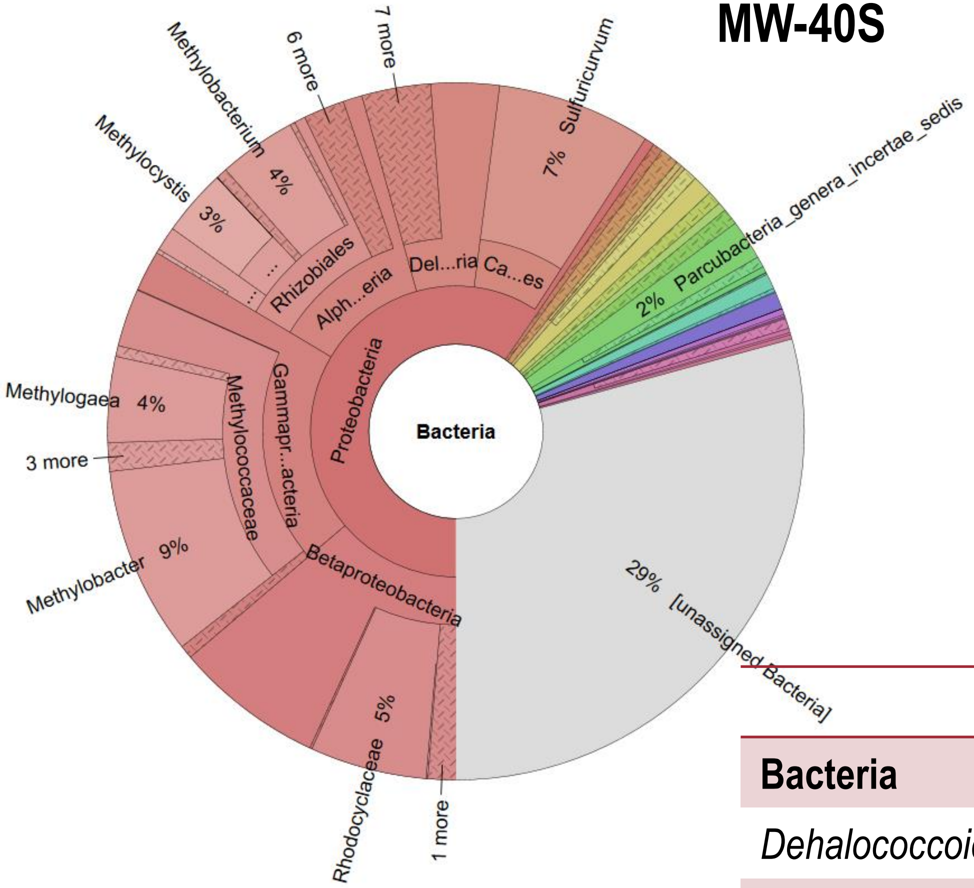
- Injections conducted in December 2013
- 50 injection points in two treatment areas (TTAs)
- Injection at 2 depth intervals
- ~146,610 gallons of 0.7% emulsified vegetable oil solution injected (~ 99% of design volume)
- ~100 liters of KB-1® injected



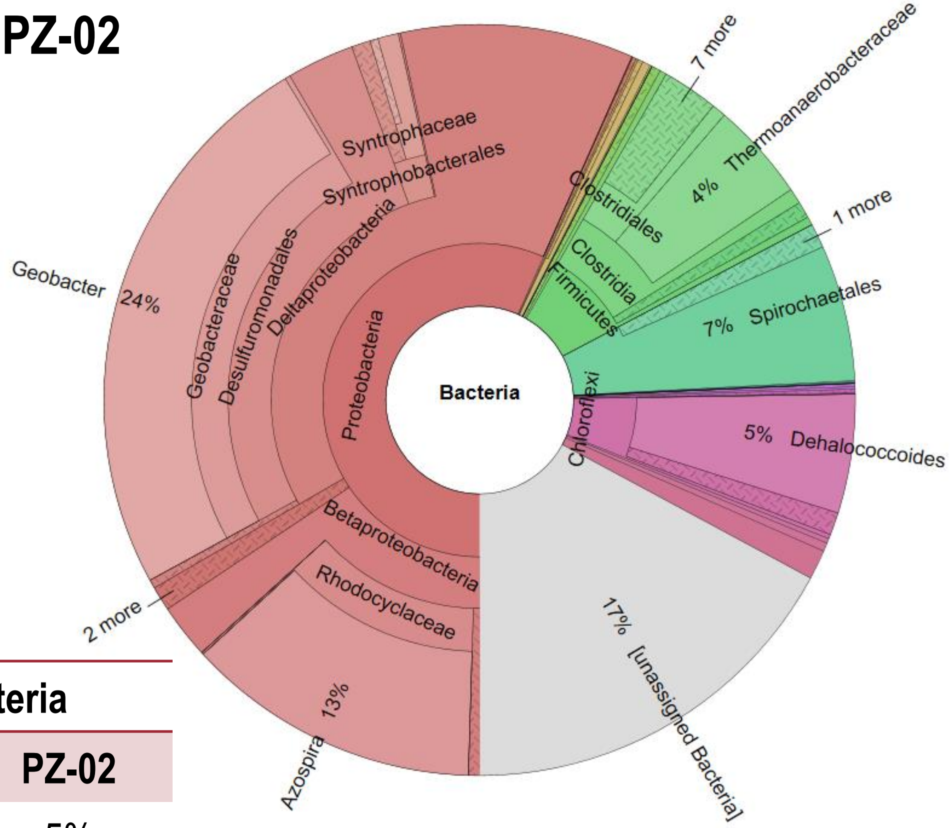
	PCE	TCE	*c*DCE	VC	Ethene	Methane	Sulfate	TOC	pH
Date	(µg/L)	(µg/L)	(µg/L)	(µg/L)	(µg/L)	(µg/L)	(mg/L)	(mg/L)	
13-Nov (Baseline)	14,000	10,100	16,100	256 J	NA	1,000	13	15	5.91
14-Mar	14,000	250	29,000	570	10 U	1,100	14	14	6.04
14-Jun	9,200	9,000	19,000	250	10U	940	17	8.8	5.92
14-Dec	50 UV	3,300	810	3,300	2,600	17,000	0.5	26	6.48
15-Jun	50 UJ	1,000	660	6,800	2,400	17,000	18	26	5.64

NAS Jacksonville Omic Results

MW-40S



PZ-02



	% of Bacteria	
Bacteria	**MW-40S**	**PZ-02**
Dehalococcoides	0.01%	5%
Geobacter	0.09%	24%
Methylobacter	9%	9%
Desulfuromonadales	0.09%	27%

NAS Jacksonville Omic Results (cont.)

- qPCR results indicated 3 x 10^7 C/L vcrA genes in treatment area
  - Increased exponentially December 2014
- vcrA and bvcA peptides detected at high levels in treatment area indicating active degradation was occurring

	PCE	TCE	*c*DCE	VC	Ethene	Methane	Sulfate	TOC	pH	vcrA Gene	vcrA Peptide	bvcA Peptide
Date	(µg/L)	(µg/L)	(µg/L)	(µg/L)	(µg/L)	(µg/L)	(mg/L)	(mg/L)				
13-Nov (Baseline)	14,000	10,100	16,100	256 J	NA	1,000	13	15	5.91	3,000		
14-Mar	14,000	250	29,000	570	10 U	1,100	14	14	6.04	6,000		
14-Jun	9,200	9,000	19,000	250	10U	940	17	8.8	5.92	4,000		
14-Dec	50 UV	3,300	810	3,300	2,600	17,000	0.5	26	6.48	3 x 10^7	Detected	Detected
15-Jun	50 UJ	1,000	660	6,800	2,400	17,000	18	26	5.64	7 x 10^7		

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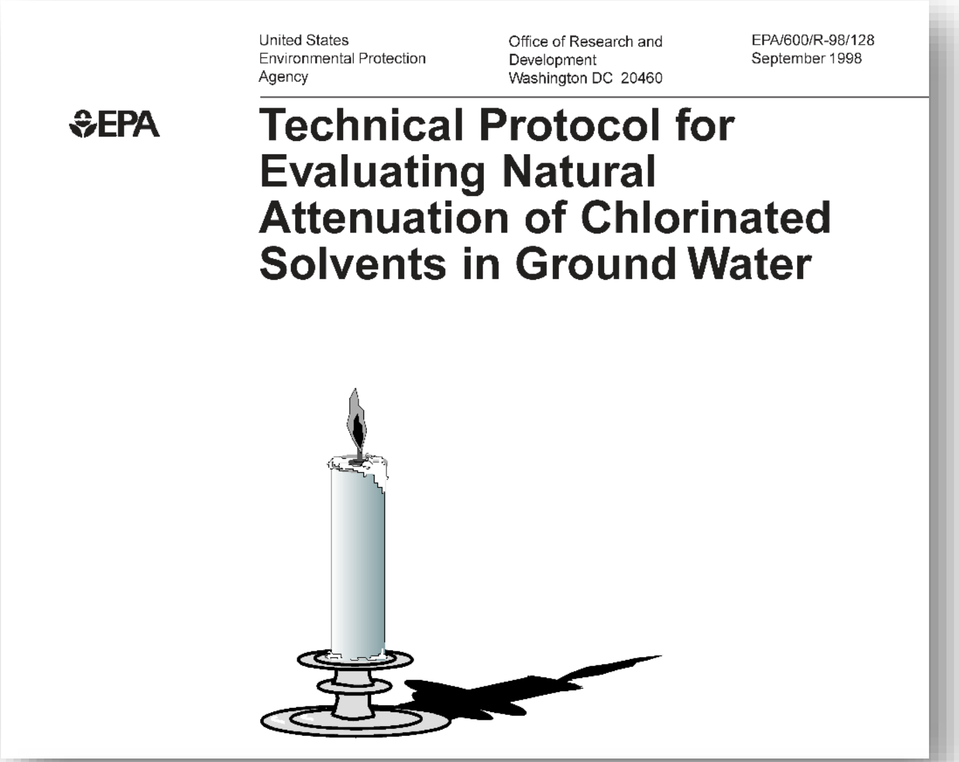
What is BioPIC (ESTCP ER-201129)?

- **Bio**remediation **P**athway **I**dentification **C**riteria
- Updated protocol for evaluating natural attenuation
- Guides users in the selection of MNA, biostimulation and/or bioaugmentation or other remedial technology
- Spreadsheet driven (Excel™)
- Currently limited to chlorinated ethenes



Based on EPA/600/R-98/128 (Sep 1998)

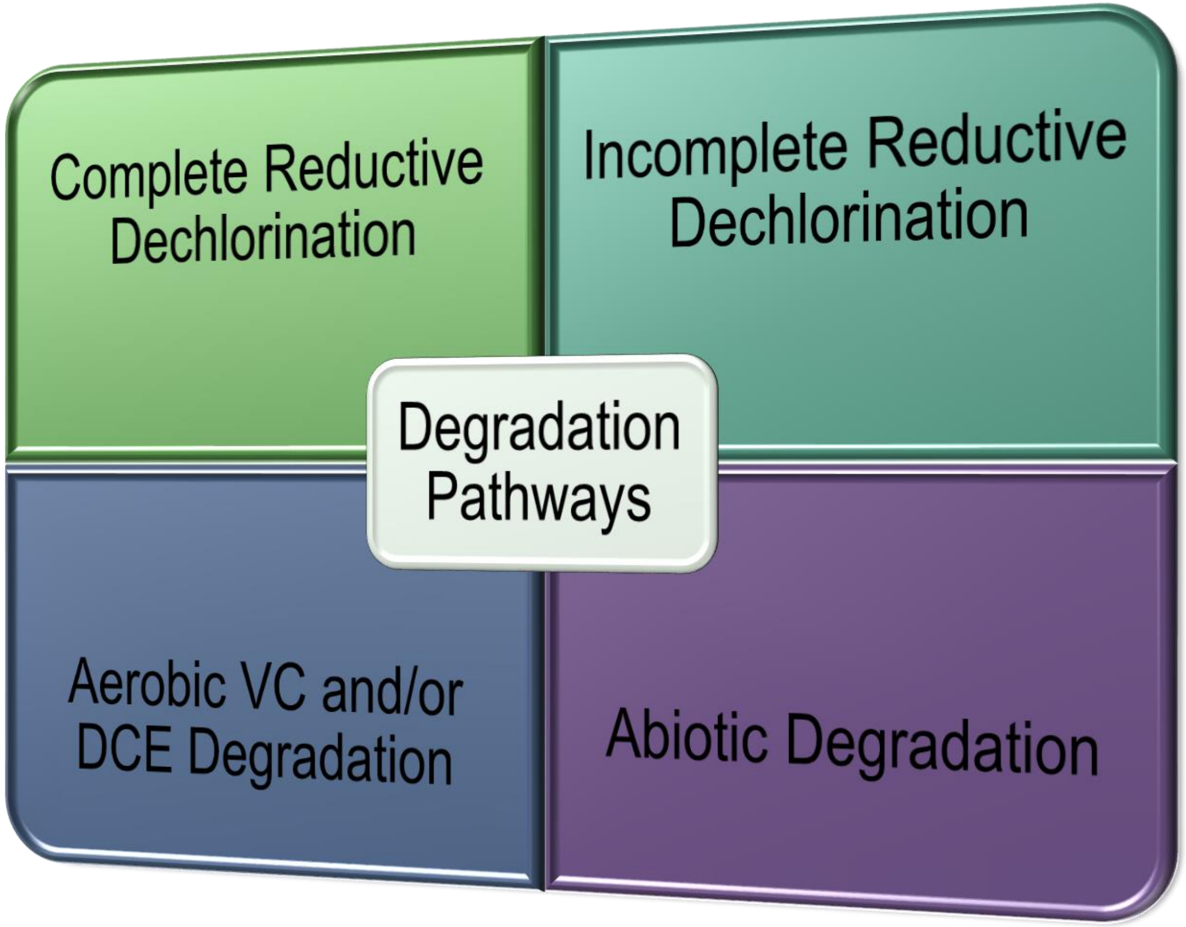
To Obtain BioPIC

https://serdp-estcp.org/content/search?cqp=Standard&SearchText=ER201129&x=0&y=0

Or search under ER-201129 Report

Reaction Pathways Covered in BioPIC

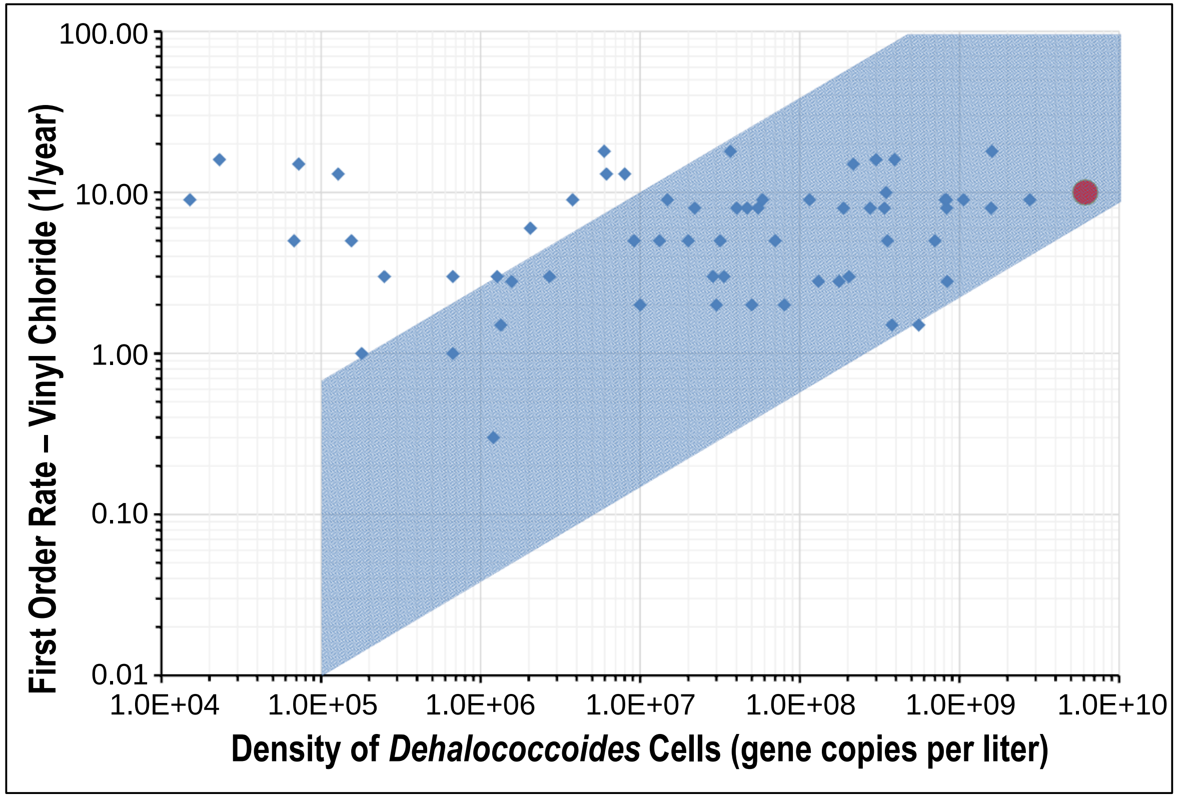
- Leverages relationships between biogeochemical parameters and degradation rates to deduce major degradation pathways
- Considers biotic AND abiotic processes
- Provides decision criteria for evaluating the need for biostimulation and bioaugmentation



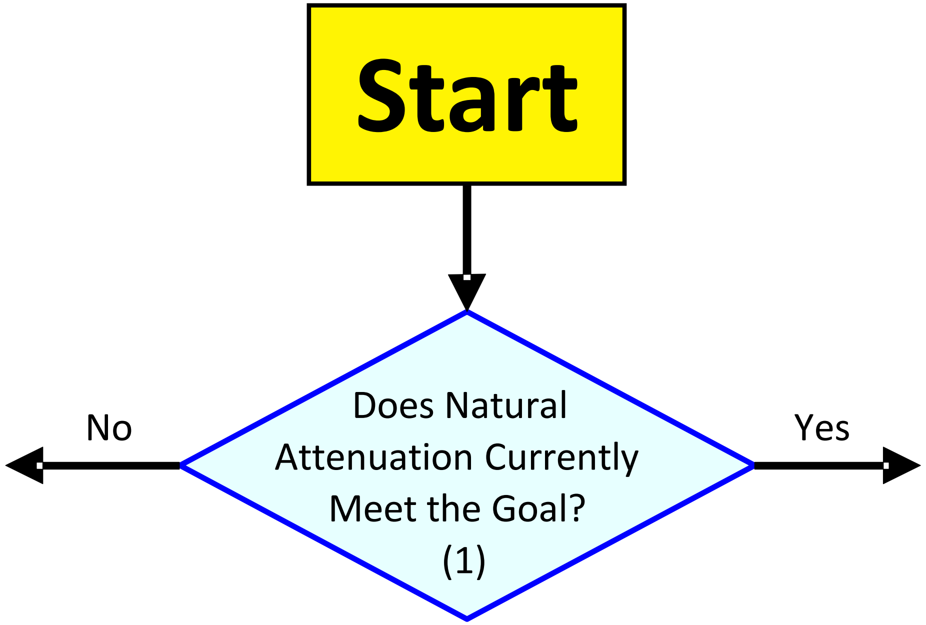
Parameters of Interest

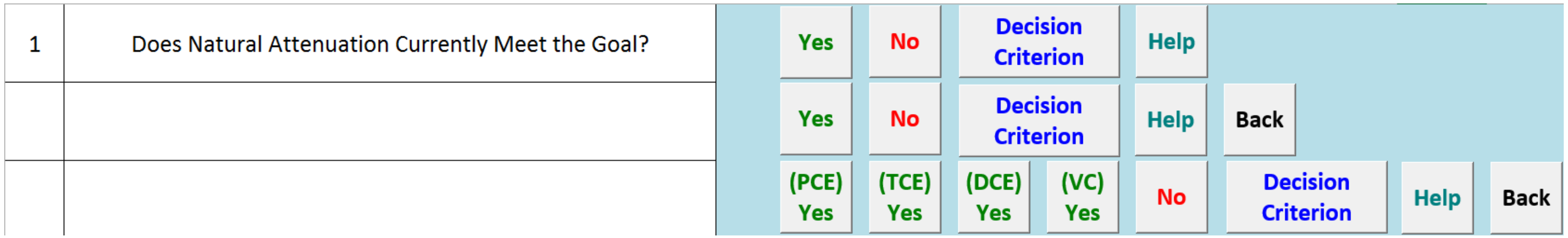
- Team compared attenuation rates of PCE, TCE, *cis*-DCE, and VC to a comprehensive list of bioremediation parameters
- DHC density (TCE, *c*DCE, and VC only), magnetic susceptibility, FeS, CH_4, and Fe (II) were found to have a direct correlation to attenuation rates
- BioChlor was used to calculate rate constants

Parameters of Interest Evaluated	
• *DHC* cell density	• *Fe (II)*
• *DHC*/total bacteria	• *Mn(II)*
• Rdases	• *CH_4*
• Rdases/*DHC*	• *Ethene*
• DO	• *TOC (in water)*
• ORP	• *VC conc.*
• Magnetic susceptibility	• *Rdases vs. VC conc.*
	• *FeS*

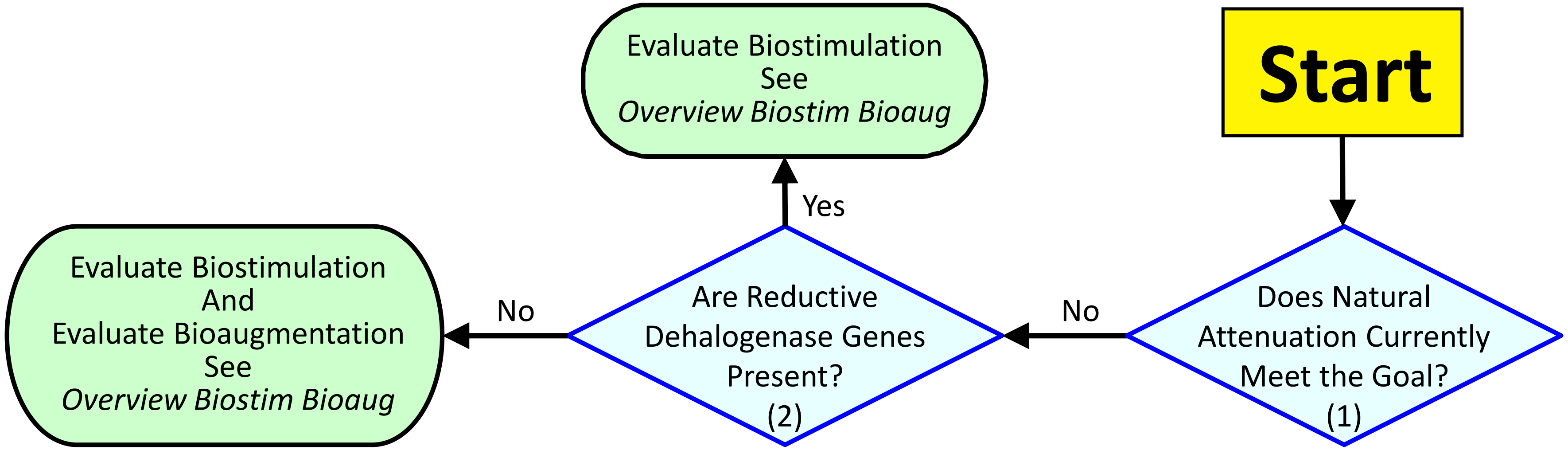


BioPIC Tour: 1st Question

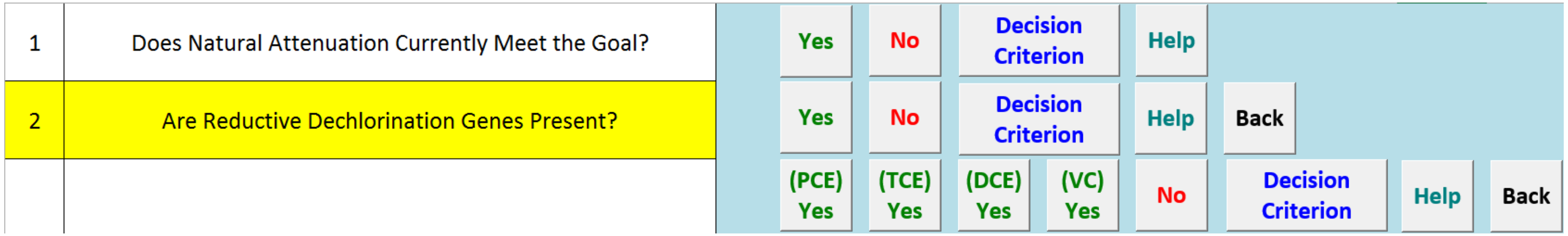




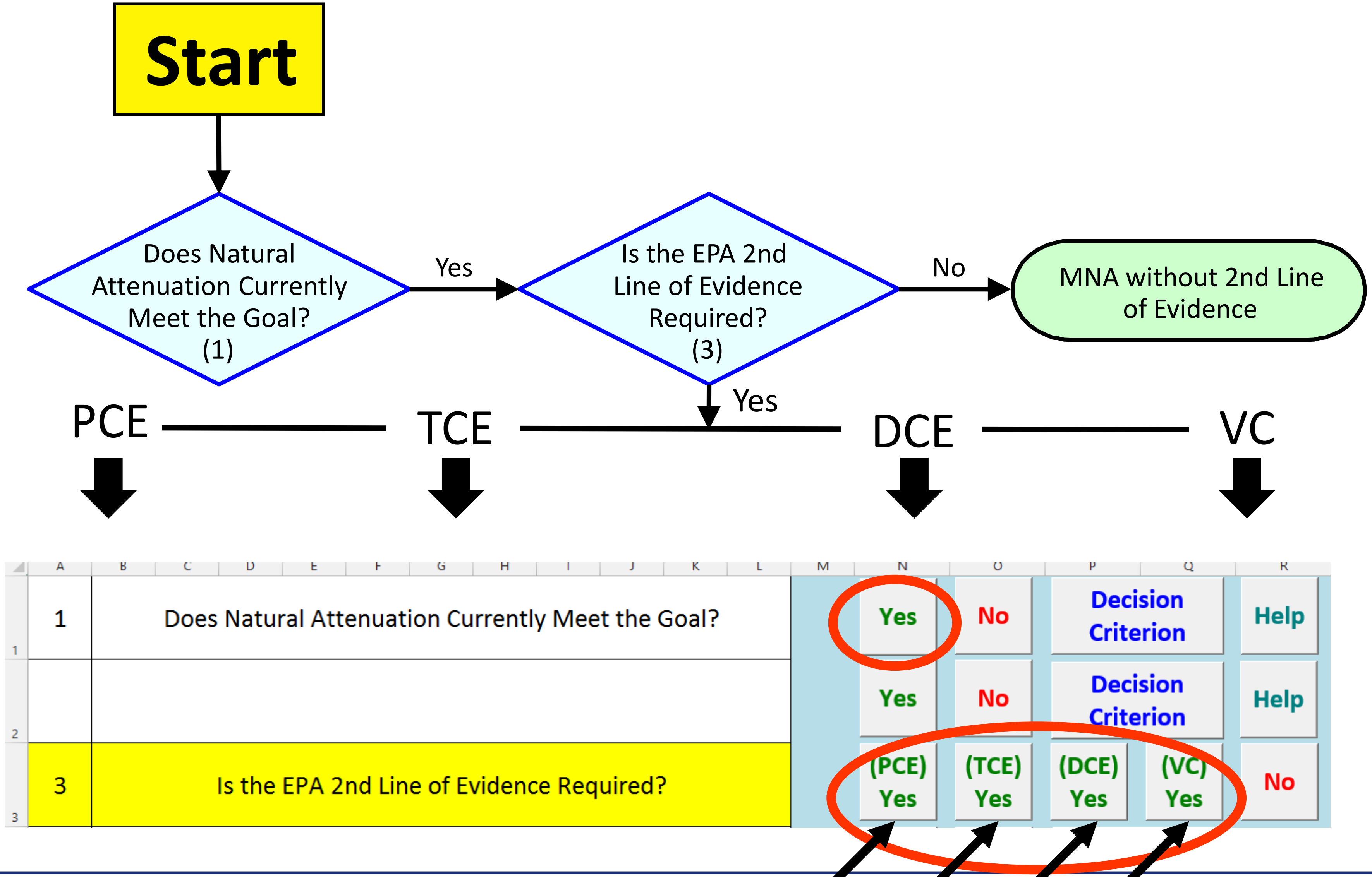
BioPIC Tour: 2nd Question



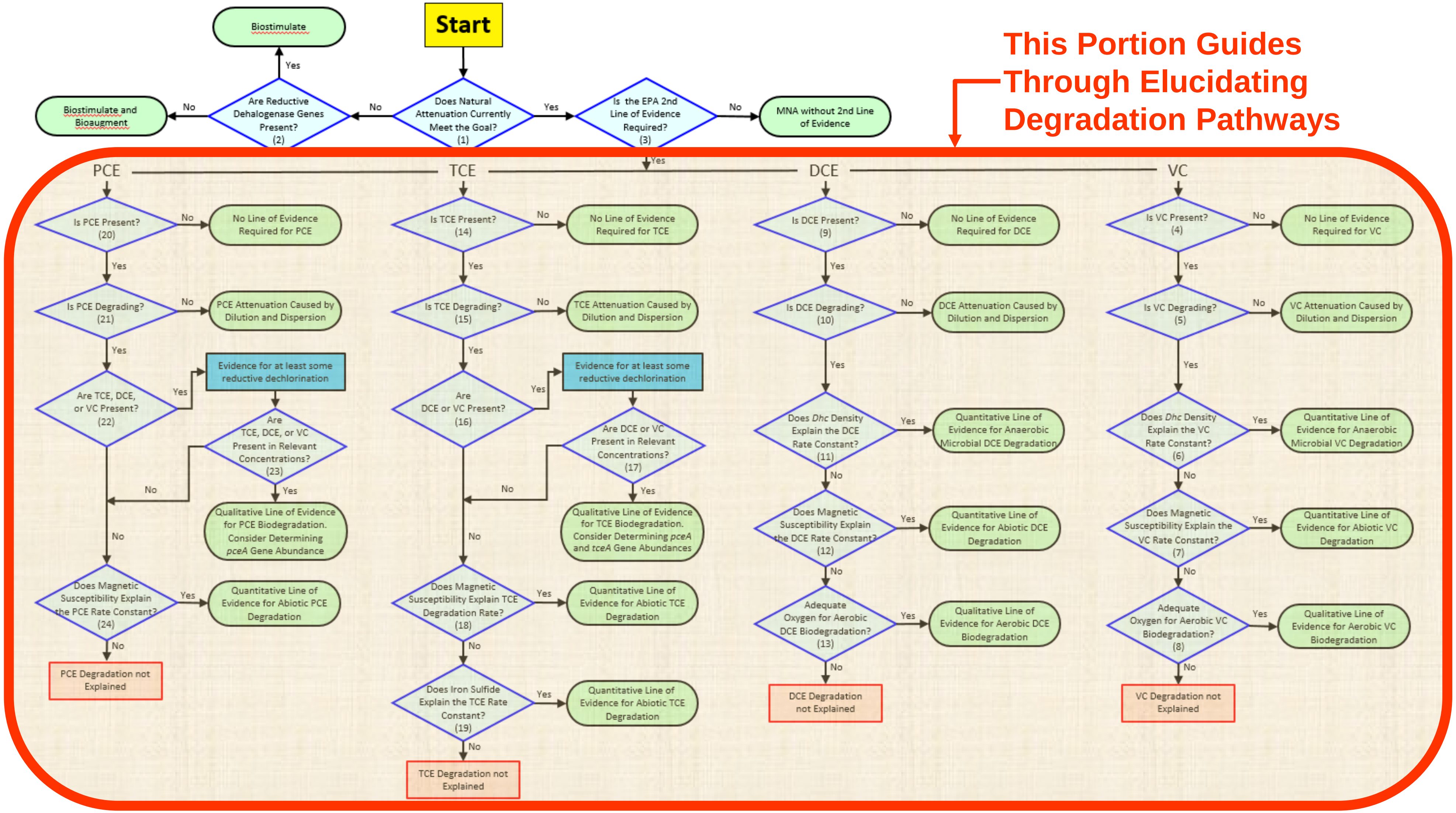
- **Decision Criteria: qPCR data is used to identify *DHC* presence**
- **If the density of the pceA, tceA, bvcA, or vcrA genes are greater than 1,000 gene copies per liter of groundwater, that gene is considered to be present**



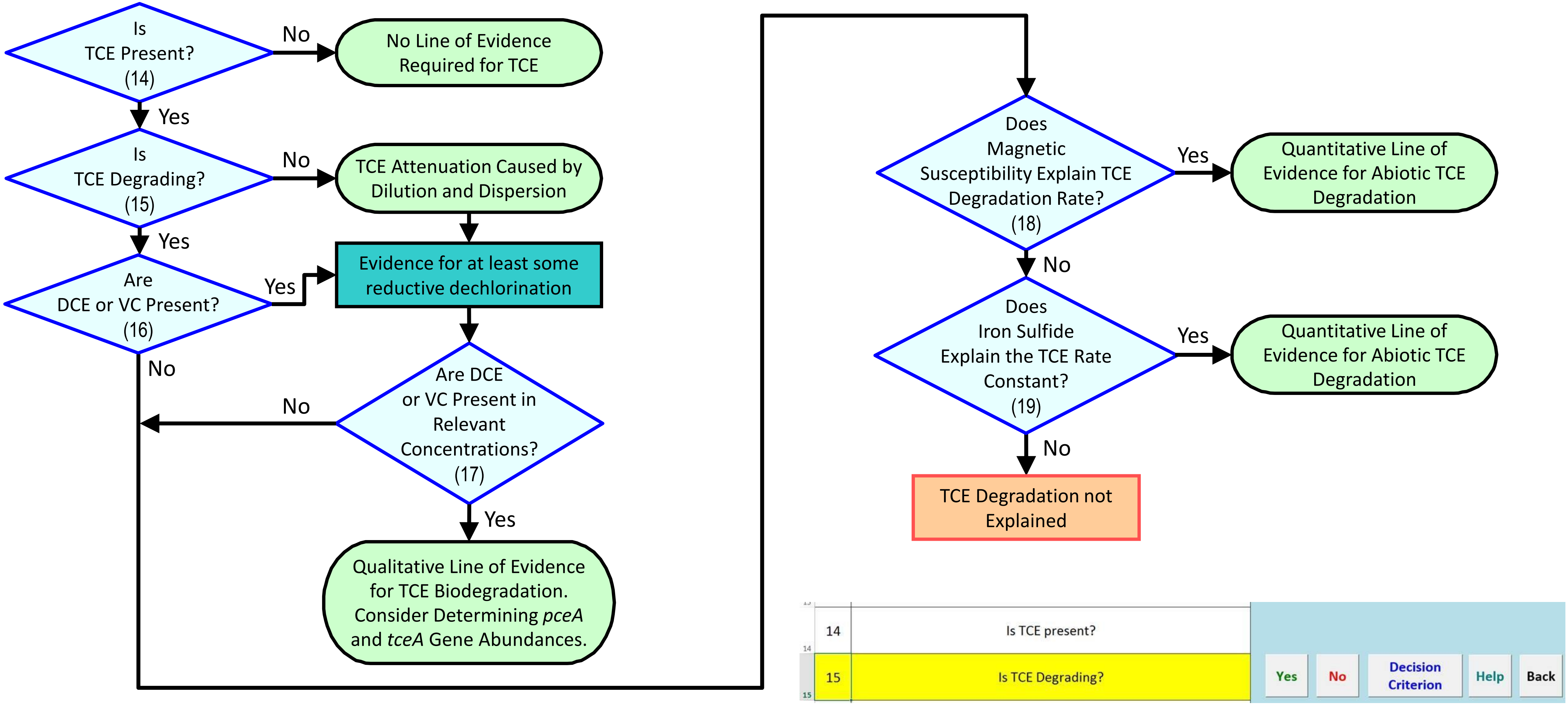
BioPIC Tour: 2nd Line of Evidence



BioPIC Tour: Identifying Pathways



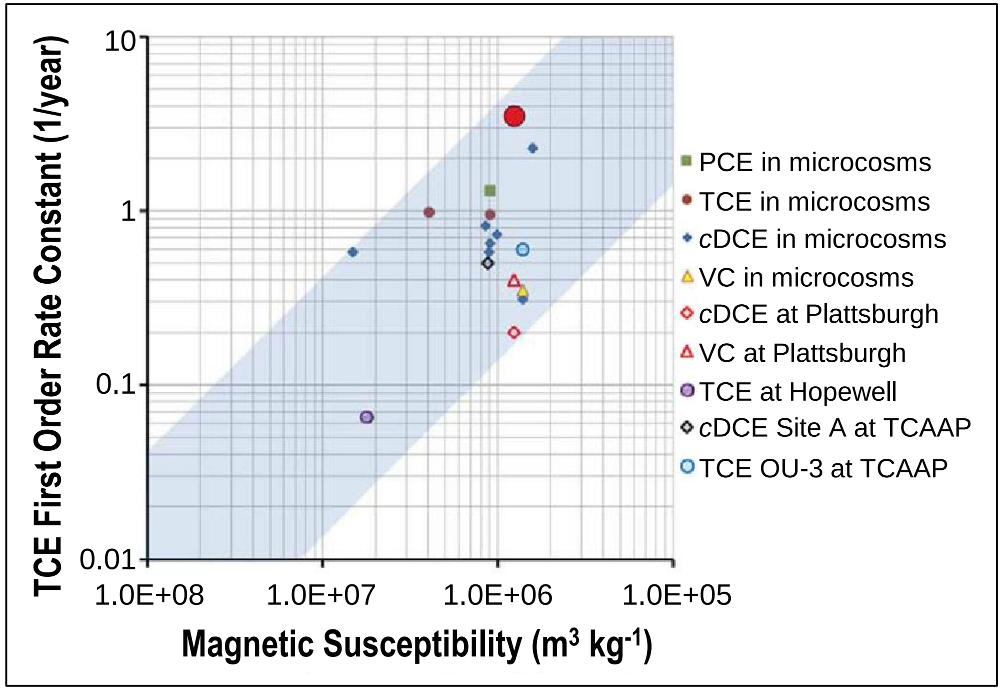
TCE Pathway


Is TCE Present? (14)
No
No Line of Evidence Required for TCE
Yes
Is TCE Degrading? (15)
No
TCE Attenuation Caused by Dilution and Dispersion
Yes
Are DCE or VC Present? (16)
Yes
Evidence for at least some reductive dechlorination
No
Are DCE or VC Present in Relevant Concentrations? (17)
No
Yes
Qualitative Line of Evidence for TCE Biodegradation. Consider Determining pceA and tceA Gene Abundances.
Does Magnetic Susceptibility Explain TCE Degradation Rate? (18)
Yes
Quantitative Line of Evidence for Abiotic TCE Degradation
No
Does Iron Sulfide Explain the TCE Rate Constant? (19)
Yes
Quantitative Line of Evidence for Abiotic TCE Degradation
No
TCE Degradation not Explained
14
Is TCE present?
15
Is TCE Degrading?
Yes
No
Decision Criterion
Help
Back

Required Data to Determine Pathways

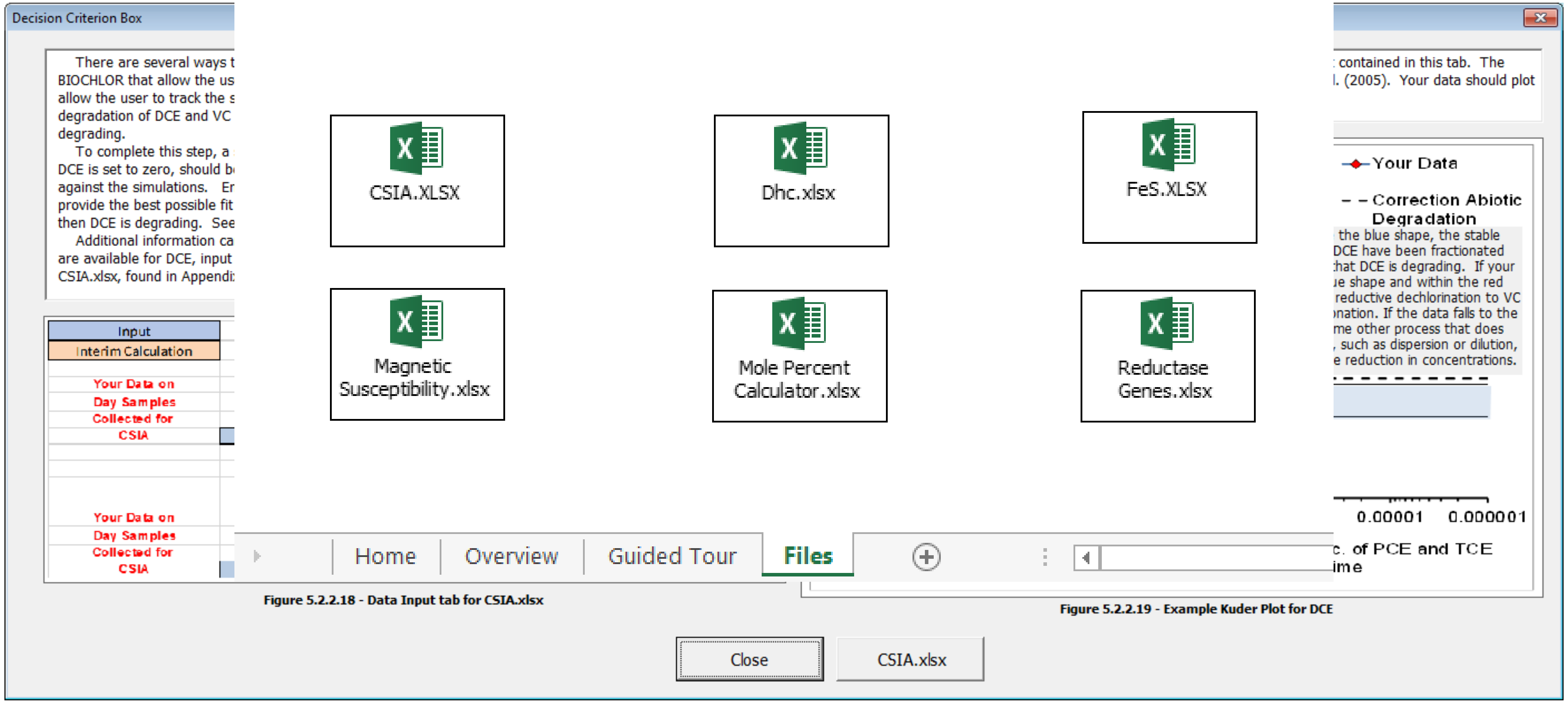
- Data needed to evaluate rate constants
  - Determine rate constant using BioChlor
- *DHC* density (for TCE, *c*DCE, and VC only) using qPCR
- Magnetic susceptibility either from a soil sample or water sample using a sonde (if abiotic pathways are suspected)
- FeS
- CH_4
- Fe(II)

Does Magnetic Susceptibility Explain TCE Degradation Rate?



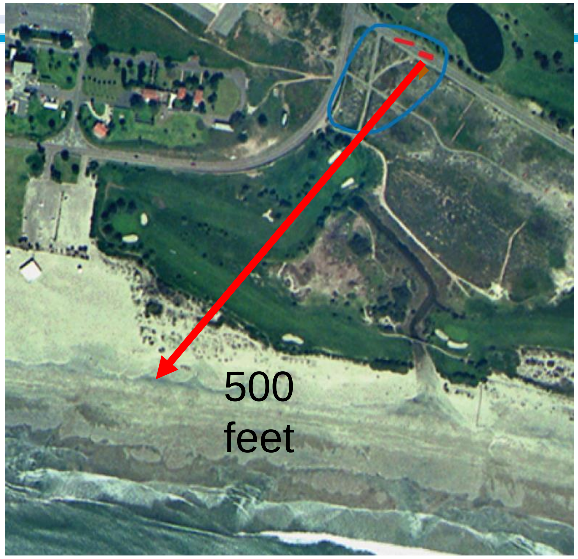
BioPIC Tour: Decision Criteria Files

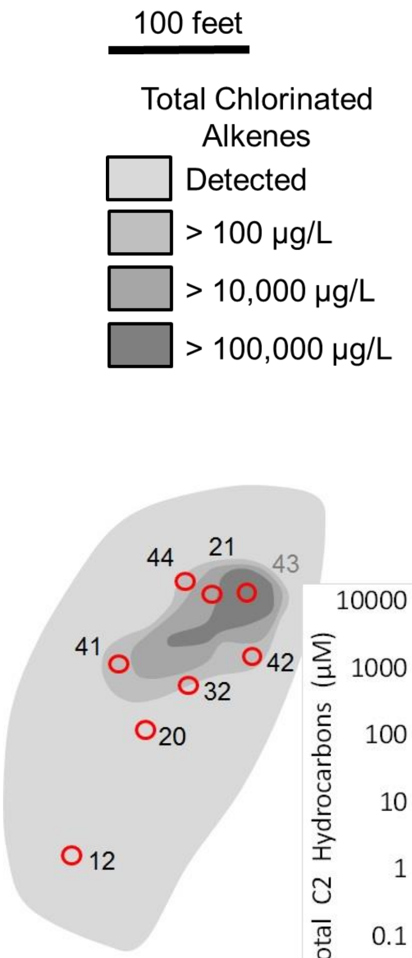
Decision Criterion Box Files

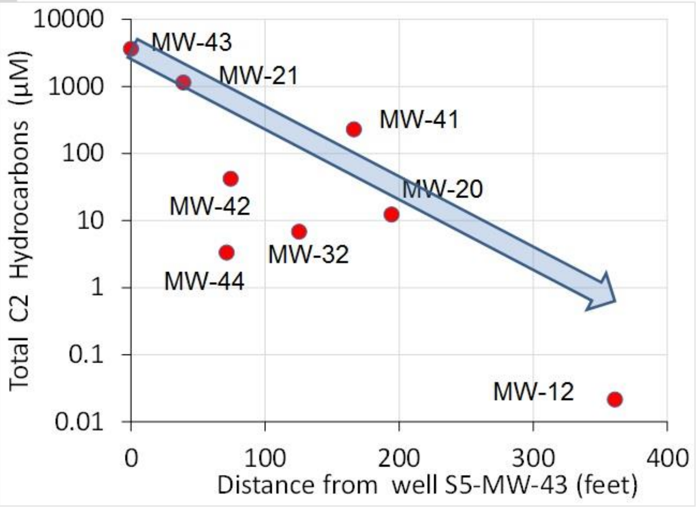


BioPIC Case Study Site 5, Unit 2 NASNI, CA

- Background
  - Served as a solid-waste disposal facility from 1940s until 1968
  - 1,000 to 2,000 tons of unseparated debris
  - Developed into a golf course in 1984
- Chlorinated ethene contamination
- Regulatory driver
  - Receptor is the high tide line (waters of State of California)
  - Will TCE, DCE and Vinyl Chloride (VC) reach the receptor at concentrations in excess of the surface water standards?

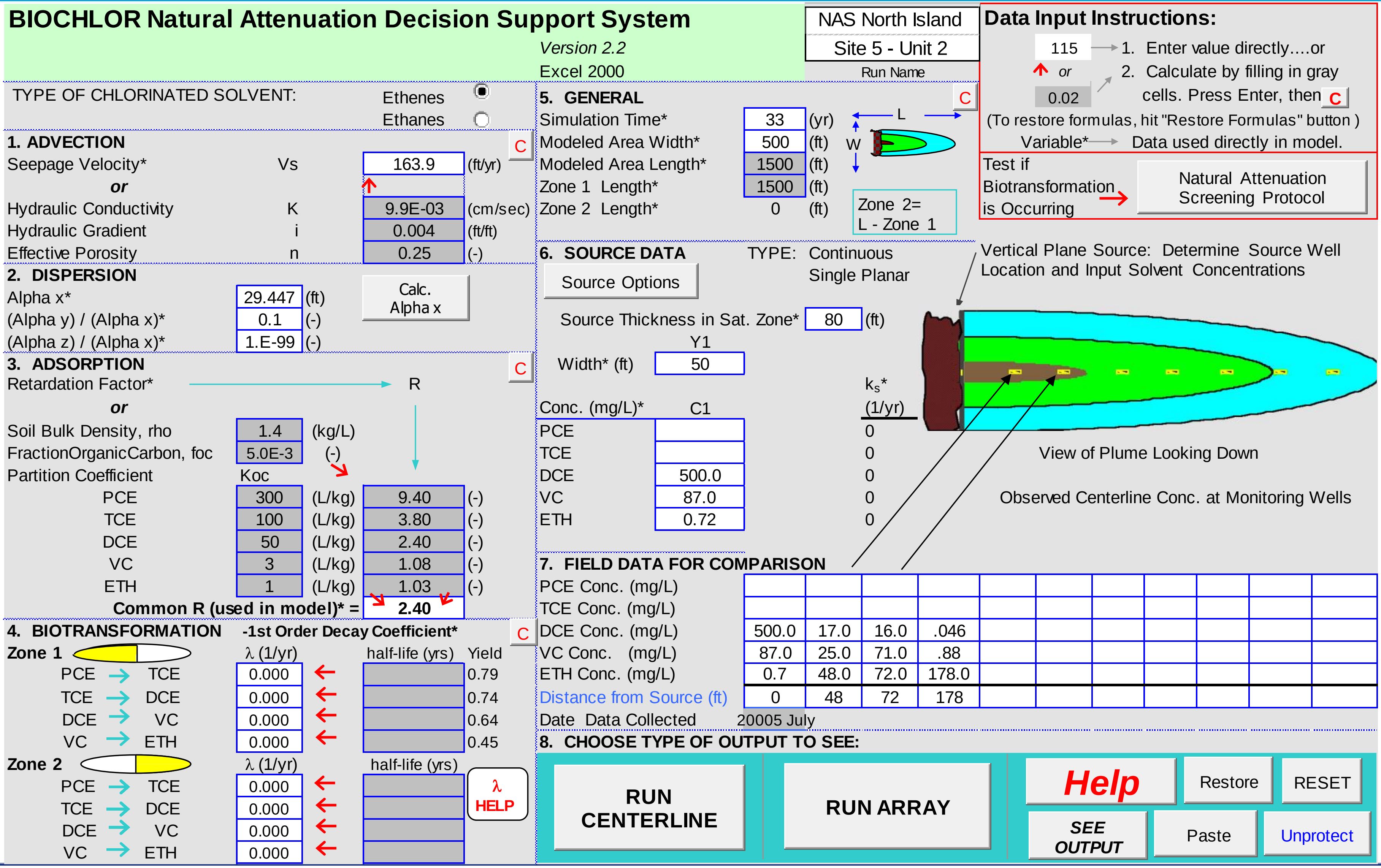






Case Study Site 5 NASNI

Does Natural Attenuation Address Site Goals?



BIOCHLOR Natural Attenuation Decision Support System

Version 2.2

Excel 2000

NAS North Island

Site 5 - Unit 2

Run Name

Data Input Instructions:

115 → 1. Enter value directly....or

or 2. Calculate by filling in gray

0.02 cells. Press Enter, then C

(To restore formulas, hit "Restore Formulas" button)

Variable* → Data used directly in model.

Test if Biotransformation is Occurring → Natural Attenuation Screening Protocol

TYPE OF CHLORINATED SOLVENT: Ethenes / Ethanes

1. ADVECTION

Seepage Velocity*	Vs	163.9	(ft/yr)
or			
Hydraulic Conductivity	K	9.9E-03	(cm/sec)
Hydraulic Gradient	i	0.004	(ft/ft)
Effective Porosity	n	0.25	(-)

2. DISPERSION

Alpha x*	29.447	(ft)
(Alpha y) / (Alpha x)*	0.1	(-)
(Alpha z) / (Alpha x)*	1.E-99	(-)

Calc. Alpha x

3. ADSORPTION

Retardation Factor* → R

or

Soil Bulk Density, rho	1.4	(kg/L)
FractionOrganicCarbon, foc	5.0E-3	(-)

Partition Coefficient

	Koc		R	
PCE	300	(L/kg)	9.40	(-)
TCE	100	(L/kg)	3.80	(-)
DCE	50	(L/kg)	2.40	(-)
VC	3	(L/kg)	1.08	(-)
ETH	1	(L/kg)	1.03	(-)

Common R (used in model)* = 2.40

4. BIOTRANSFORMATION -1st Order Decay Coefficient*

Zone 1	λ (1/yr)	half-life (yrs)	Yield
PCE → TCE	0.000		0.79
TCE → DCE	0.000		0.74
DCE → VC	0.000		0.64
VC → ETH	0.000		0.45

Zone 2	λ (1/yr)	half-life (yrs)
PCE → TCE	0.000	
TCE → DCE	0.000	
DCE → VC	0.000	
VC → ETH	0.000	

λ HELP

5. GENERAL

Simulation Time*	33	(yr)
Modeled Area Width*	500	(ft)
Modeled Area Length*	1500	(ft)
Zone 1 Length*	1500	(ft)
Zone 2 Length*	0	(ft)

Zone 2= L - Zone 1

6. SOURCE DATA TYPE: Continuous Single Planar

Source Options

Vertical Plane Source: Determine Source Well Location and Input Solvent Concentrations

Source Thickness in Sat. Zone* 80 (ft)

Width* (ft) Y1: 50

Conc. (mg/L)*	C1	k_s* (1/yr)
PCE		0
TCE		0
DCE	500.0	0
VC	87.0	0
ETH	0.72	0

View of Plume Looking Down

Observed Centerline Conc. at Monitoring Wells

7. FIELD DATA FOR COMPARISON

PCE Conc. (mg/L)				
TCE Conc. (mg/L)				
DCE Conc. (mg/L)	500.0	17.0	16.0	.046
VC Conc. (mg/L)	87.0	25.0	71.0	.88
ETH Conc. (mg/L)	0.7	48.0	72.0	178.0
Distance from Source (ft)	0	48	72	178
Date Data Collected	20005 July			

8. CHOOSE TYPE OF OUTPUT TO SEE:

RUN CENTERLINE | RUN ARRAY | Help | Restore | RESET | SEE OUTPUT | Paste | Unprotect

Case Study Site 5 NASNI

Optimum Rate Constants for Degradation of DCE

4. BIOTRANSFORMATION **-1st Order Decay Coefficient*** C

			λ (1/yr)	half-life (yrs)	Yield
Zone 1					
PCE	→	TCE	0.000		0.79
TCE	→	DCE	0.000		0.74
DCE	→	VC	17.000		
VC	→	ETH	10.000		
Zone 2			λ (1/yr)	half-life	
PCE	→	TCE	0.000		
TCE	→	DCE	0.000		
DCE	→	VC	0.000		
VC	→	ETH	0.000		

Model output for DCE to VC degradation rate of 17/year

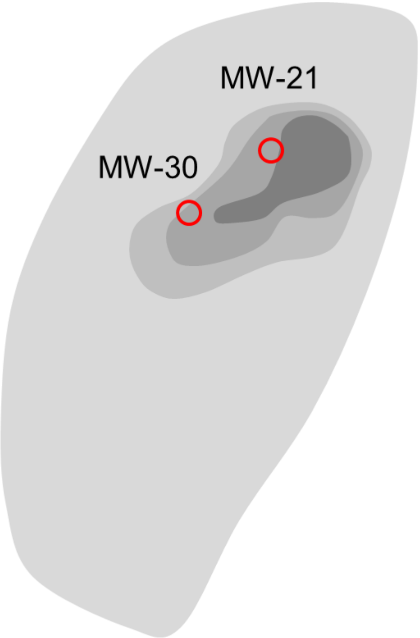
Key Point

Natural attenuation can be expected to keep concentrations of DCE and VC below the regulatory standard at the receptor

Case Study Site 5 NASNI

Are *DHC* bacteria responsible for the rate of reductive dechlorination?

qPCR data is used to identify *DHC* presence and density



10-6-2005

Density of Dhc 16s ribosomal DNA
Gene Copies per Liter

MW-21 has 6.15E + 09

MW-30 has 3.47E + 08

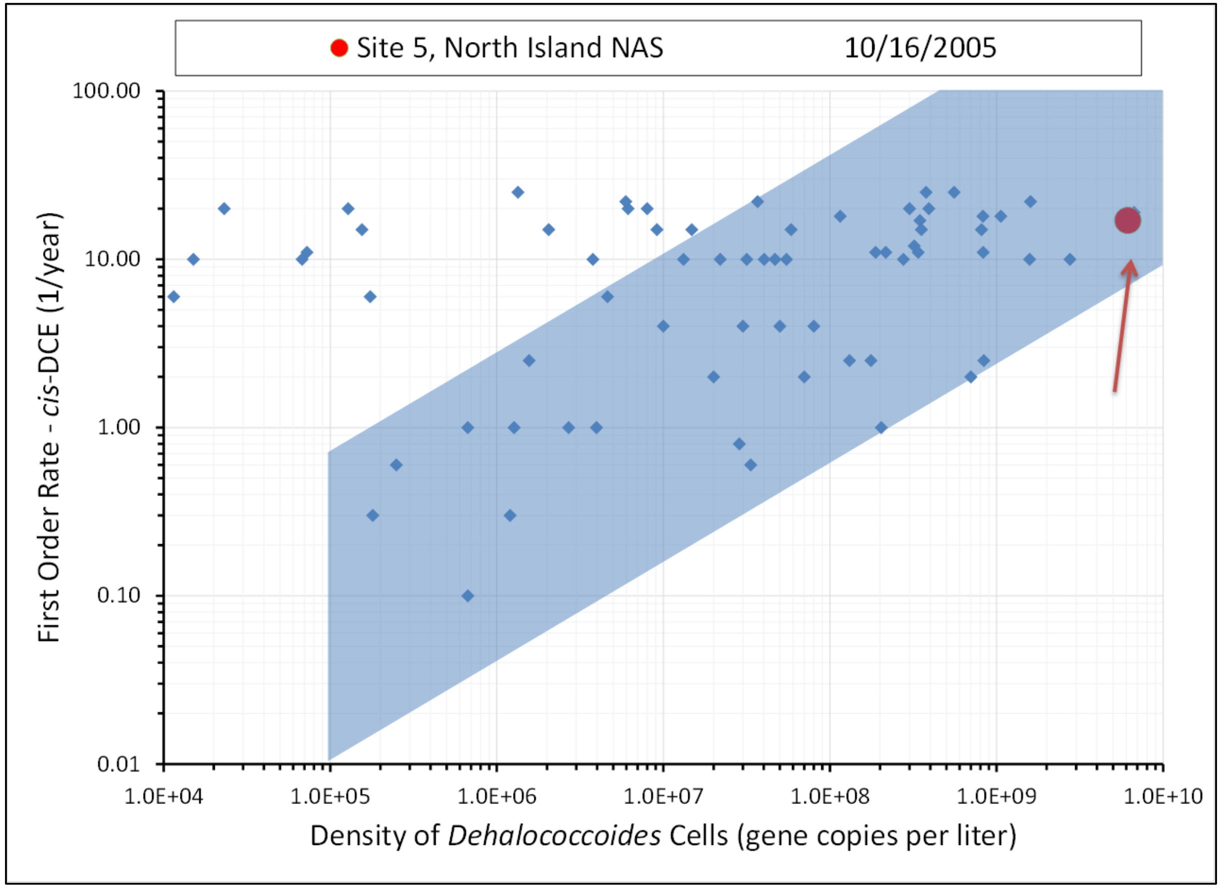
	Overwrite input cells with data specific to your site	The BASELINE rate constant is the slowest rate constant that is plausibly associated with *Dehalococcoides* DNA (*Dhc*)
	Input	
	First order rate constant for degradation per year	Fraction of rate constants in the benchmark data set that exceed the BASELINE to a extent than this rate constant
cis-DCE	17	>80%
Vinyl Chloride	10	>80%
	qPCR assay Gene copies per liter	
Dehalococcoides 16s rRNA	6.15E+09	
Location and Site	Site 5, North Island NAS	
Date	10/16/2005	

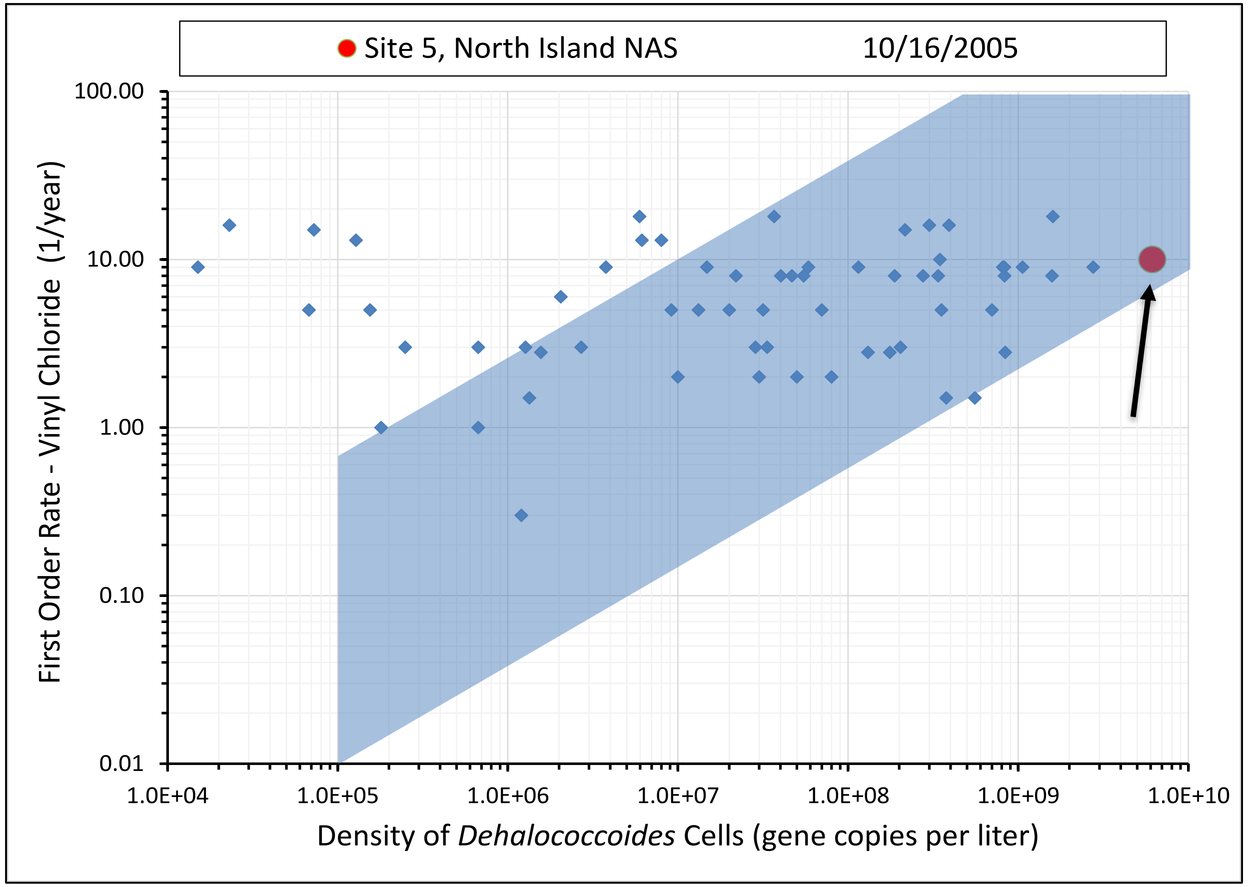
9	9	Is DCE present?
10	10	Is DCE Degrading?
11	11	Does Dhc Density Explain the DCE Rate Constant?

Yes | No | Decision Criterion | Help | Back

Case Study Site 5 NASNI

Determine if *DHC* are Responsible for the Dechlorination Rate





Presentation Overview

- Introduction
- Review of Enhanced Bioremediation
- Best Practices for Successful Application, Distribution, and Mixing of Amendments
- Technological Advances to Address Complex Sites
- Innovation in Amendment Formulations
- Best Practices and Advances in Monitoring
- BioPIC – A Tool to Select, Evaluate, and Convince

► Wrap-Up

Key Points and Take-Home Messages – EISB Design & Implementation

- Design of EISB applications must be developed based on site-specific conditions and RAOs/RGs
  - Wide range of amendments with varying properties
  - Bioaugmentation. Is it necessary?
  - Consider active, passive, and hybrid application techniques
- BioPIC is a useful tool to aid in design and implementation of an EISB remedy
- Achieving adequate contact between amendments and COCs remains one of the most challenging aspects of EISB
- Different amendments will achieve different radii of influence, will be consumed at different rates, and may need to be reapplied at different intervals

Summary of Key Site-Specific Factors that Impact Bioremediation Design and Amendment Distribution

Site-Specific Factor	Impact
Hydrogeology	• Amendments distribution and mixing • Amendment type (aqueous vs. slow release)
Nature and Extent of Contamination	• Overall remedial design and approach – Treatment vs. containment – Amendment types and dosage – Number, spacing, and design of wells/injection points – Frequency of re-application
Biological and Geochemical Conditions	• Need for bioaugmentation • Buffer requirements • Low temperatures (<10°C) can inhibit biological reactions • Metals can be solubilized
Location and Infrastructure	• Well/injection point design • Vapor monitoring and recovery
Remedial Action Objectives	• Type of system – Active vs. passive – Treatment vs. containment • Types and number of application of amendments • Frequency of re-application

Key Points and Take-Home Messages – Monitoring

- Always develop a monitoring plan
  - Monitor potential changes outside of the treatment area
  - Consider using high-resolution site characterization and innovative diagnostic tools
  - Monitor health and safety impacts
- Up to 3 years of post-application monitoring may be sufficient to assess performance and rebound
- Secondary groundwater quality impacts are not likely to occur outside of the treatment area; however, the treatment area may remain anoxic/reducing for years
- Metagenomics and proteomics are powerful monitoring tools that can be used to develop the CSM and monitor remedy performance

Key Point **Monitoring between and after injections is much more than monitoring changes in COC concentrations!**

Key Points and Take-Home Messages – Recent Developments

- New technologies such as Ek-Bio, heat-enhanced bioremediation, and application of shear thinning fluids are being developed, which can be used to address complex sites
- Formulations of amendments (electron donors, bioaugmentation cultures, and buffers) continue to be developed and optimized.
- Advanced MBTs, especially “omics” continue to be developed at a rapid rate and provide powerful tools to assess bioremediation (e.g. proteomics)
- 1,4-dioxane is degradable by direct and cometabolic mechanisms
  - Attenuation is positive correlation between increasing oxygen concentrations in groundwater, but negatively correlated with high levels of metals and cVOCs
  - Additional data/demonstrations needed and are underway

Future Directions

- Develop innovative cost-effective methods to ensure contact between the microbial community, electron donors, and contaminants
  - Fine-grained lower-permeability zones
- Improved understanding and modeling of bioaugmented cultures in aquifer
- Better understanding of long-term impacts of bioremediation
- Improved understanding and modeling of contributions of biotic versus abiotic processes

Additional Resources

- NAVFAC (2016) – Design Considerations for Enhanced Reductive Dechlorination
- SERDP (2016) – Numerical Modeling of Post Remediation Impacts of Anaerobic Bioremediation on Groundwater Quality (Borden)
- SERDP (2014) – Standardized Procedures for use of Nucleic Acid Based Tools (Lebron et al.)
- SERDP & ESTCP (2013) – Bioaugmentation for Groundwater Remediation (Stroo et al.)
- U.S. EPA (2013) – Introduction to *In Situ* Bioremediation of Groundwater
- SERDP & ESTCP (2010) – *In Situ* Remediation of Chlorinated Solvents (Stroo and Ward)
- ESTCP (2010) – Application of Nucleic Acid-Based Tools for Monitoring Monitored Natural Attenuation (MNA), Biostimulation and Bioaugmentation at Chlorinated Solvent Sites
- Interstate Technology and Regulatory Council (ITRC) (2008a) – Guidance on Enhanced Attenuation of Chlorinated Organics
- AFCEC (2007) – Protocol for *In Situ* Bioremediation of Chlorinated Solvents Using Edible Oil
- U.S. EPA (2006) – Engineering Issue on *In Situ* and Ex Situ Biodegradation Technologies for Remediation of Contaminated Sites

Additional Resources (cont.)

- ESTCP (2005) – Technology White Paper on Chlorinated Solvent Bioaugmentation
- AFCEC et al. (2004) – Principles and Practices of Enhanced Anaerobic Bioremediation of Chlorinated Solvents
- Mahendra S, Alvarez-Cohen L. 2005. Pseudonocardia dioxanivorans sp. nov., a novel actinomycete that grows on 1,4-dioxane. Int. J. Syst. Evol. Microbiol.
- Zenker, M.J., R.C. Borden, and M.A. Barlaz. 2003. "Occurrence and Treatment of 1,4-Dioxane in Aqueous Environments," Env. Eng. Sci., 20(5), pp. 423-432.
- Sales CM, Mahendra S, Grostern A, Parales RE, Goodwin LA, Woyke T, Nolan M, Lapidus A, Chertkov O, Ovchinnikova G, Sczyrba A, Alvarez-Cohen L. 2011. Genome sequence of the 1,4-dioxane-degrading Pseudonocardia dioxanivorans strain CB1190.
- Yuncu, B., J.L. Keener, R.C. Borden, S.D. Richardson, K. Glover, and A. Bodour. 2014. "Bioremediation of Commingled 1,4-Dioxane and Chlorinated Solvent Plumes," In
- Proceedings of the Battelle Ninth International Conference on Remediation of Chlorinated and Recalcitrant Compounds, Monterey, CA, May 19-22.

NAVFAC Points of Contact

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Questions and Answers