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Bioaugmentation-It's Not Just for TCE Anymore!

Phil Dennis

Remediation Seminars
Short Courses

November 13-14 2019
Greensboro, Research Triangle Park, NC

SiREM Major Service Areas

Remediation Testing

treatability studies

Characterization/Monitoring

- *Molecular Testing*

gene trac®

Bioaugmentation Cultures

KB-1®

- *Passive Samplers for Vapor and Pore Water*

WATERLOO MEMBRANE SAMPLER

SP3

Advantages of Enhanced Bioremediation for Chlorinated Solvents

- **Cost Effective:** As little as 1/3rd the cost of other options
- **Destroys Contaminants:** doesn't just move them
- **Prevents Rebound:** Once down concentrations stay down
- **Sustainable:** low carbon foot print/natural process/inobtrusive

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BIOAUGMENTATION

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The Basics of Enhanced Bioremediation

Injection of KB-1® each liter has ~100 billion Dhc cells

- **Biostimulation:** The addition of nutrients to stimulate microbial activity (e.g. electron donors)
- **Bioaugmentation:** the addition of beneficial microorganisms to improve the rate or extent of biodegradation
- **KB-1®and KB-1® Plus:** bioaugmentation cultures for remediation of chlorinated volatile organic compounds

Dehalococcoides (Dhc) as Featured in Wired Magazine

START

POLLUTION

Nature's Little Janitors

The more toxic crap human beings dump onto the planet, the trickier it gets to clean up. Bioremediation, the science of using living organisms to eat pollution, is an old concept, but biologists are expanding its reach with some new critters. UC Berkeley-based researchers recently reengineered a mustard plant to amp up its appetite for the heavy metal selenium, then put it to work cleaning up soil in California. And a British group used powdered water hyacinth root to clean arsenic out of Bangladeshi groundwater. Scientists are continually on the hunt for similarly helpful plants and microorganisms that thrive under conditions that would kill lesser beasts. Here are a half dozen currently on cleanup duty. – **Kate Ruder**

DHC
(*Dehalococcoides species*)
Found in: Upstate New York sewage
Eats: Chlorinated solvents
Used in: Naval weapons station and former electronics manufacturing plants in Pennsylvania

BRAKE FERN
(*Pteris vittata and Pteris cretica*)
Found in: An abandoned lumberyard for pressure-treated wood in Archer, Florida
Eats: Arsenic
Used in: Contaminated soil near a former chemical weapons facility in Washington, DC; drinking water in Albuquerque, New Mexico

GEOBACTER
(*Geobacter species*)
Found in: Potomac River sediment
Eats: Uranium and other heavy metals
Used in: Groundwater from a cold war-era uranium mine in Rifle, Colorado

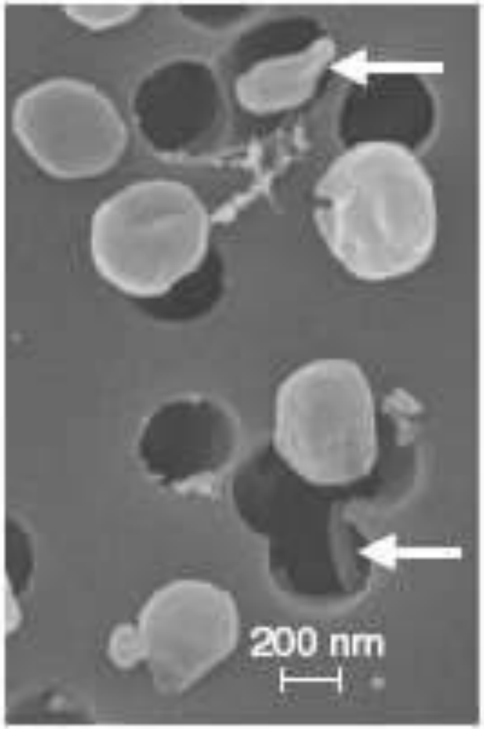
WATER HYACINTH
(*Eichhornia crassipes*)
Found in: Tropical and subtropical lakes and streams
Eats: Arsenic
Used in: Bangladeshi wells and groundwater

YELLOWSTONE EXTREMOZYME
(*Thermus brockianus*)
Found in: Hot springs at Yellowstone National Park
Eats: Hydrogen peroxide
Used in: Paper-pulp and textile wastewater tests in an Idaho lab

INDIAN MUSTARD PLANT
(*Brassica juncea*)
Found in: The lab
Eats: Selenium
Used in: Soil contaminated by agricultural runoff in the San Luis Drain in Mendota, California

Introduction to *Dehalococcoides (Dhc)*

- One of the smallest free living microbes ~0.5 µm, disk shaped
- Obligate anaerobes
- Degrader of a range of chlorinated compounds (chlorinated ethenes, propanes, dioxins, PCB's and more)
- Distributed throughout the world but not ubiquitous



***Dehalococcoides mccartyi* strain FL2**

KB-1® (101)

- Anaerobic bioaugmentation culture enriched from TCE site
- Non GMO/pathogen free
- Used to add *Dhc* to groundwater has ~100 billion *Dhc*/liter (L)
- Typically added at 1L : 35,000L culture : groundwater

Site Bioaugmentation Kit

Materials shipped to site- 20L vessel and injection tools

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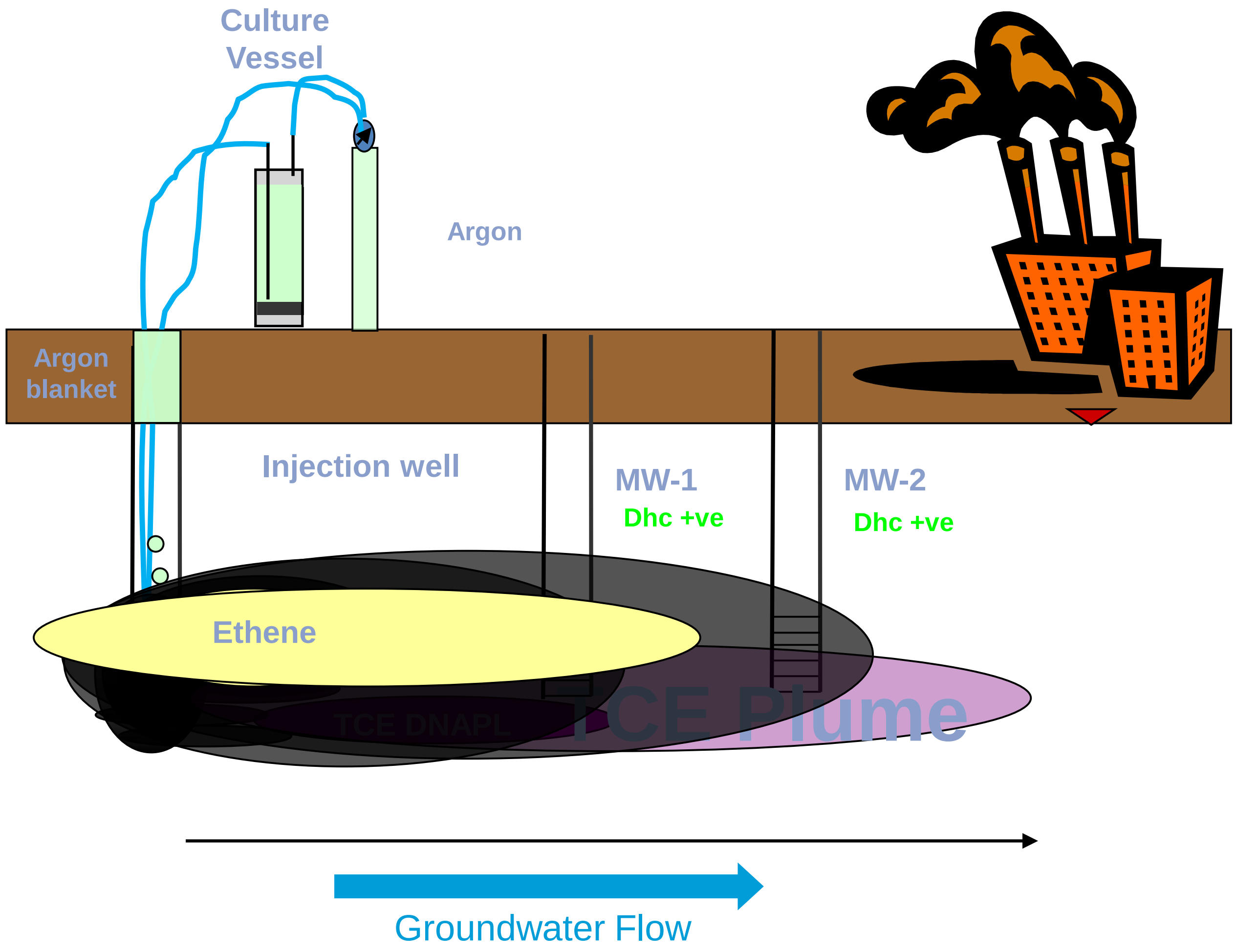
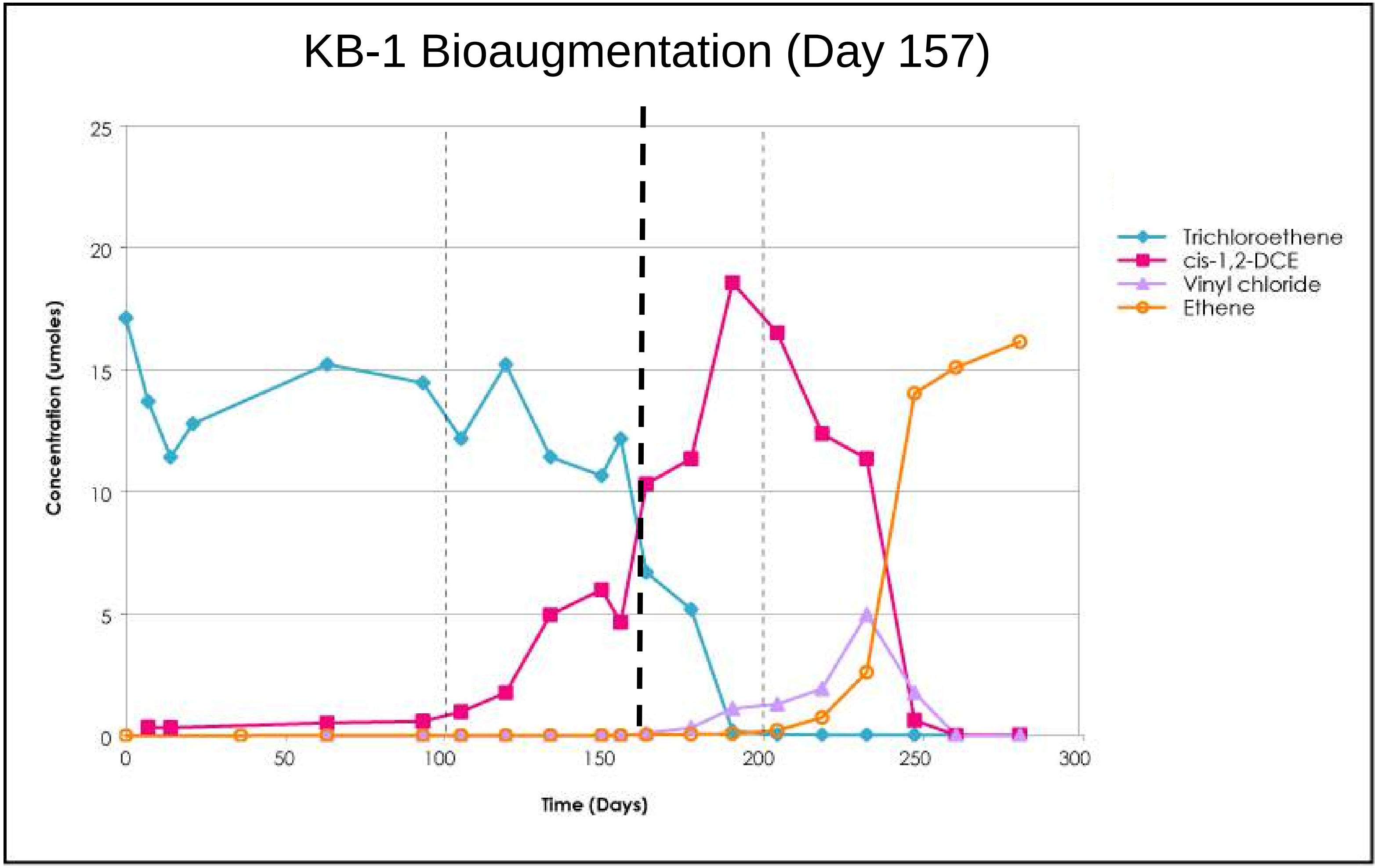
Bioaugmentation Culture Field Application



Impact of KB-1 Bioaugmentation California Site



Complete dechlorination of TCE to ethene only achieved after KB-1 added to groundwater

KB-1®/Plus Bioaugmentation Globally

More than 600 sites have been bioaugmented with KB-1® and KB-1® Plus cultures

Why Bioaugment?

- Complete degradation of chlorinated solvents will simply not occur in the absence of the right microorganisms
  -They must be introduced by bioaugmentation

- Where the right type of indigenous microorganisms are present but at low concentrations/poorly distributed-bioaugmentation can decrease the time-frame and costs required for site cleanup

- By increasing the speed and effectiveness of bioremediation can increase efficiency of electron donor use and decrease O&M costs including monitoring

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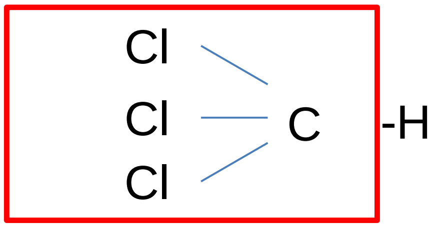
EXPANDING THE RANGE OF BIOAUGMENTATION

Conditions for Complete Reductive Dechlorination of Chlorinated Ethenes

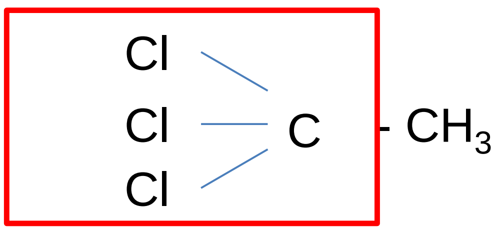
- Anaerobic: DO < 0.2 mg/L ORP <-75 mV
- Sufficient electron donor
- pH 6.0-8.5 (may be able to go a low as 5.5)
- Management of inhibitory co-contaminants (1,1,1-TCA/Chloroform/CFCs)
- The right bugs must be present

Examples of Compounds Observed to be Inhibitory to Reductive Dechlorination

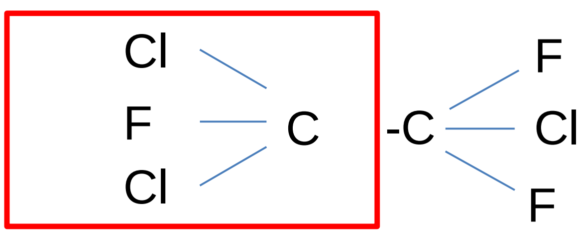
Tri-halogenated Compounds:



Chloroform
(also inhibits fermenters and methanogens)



1,1,1-trichloroethane



Chlorofluorocarbon (CFC 113)

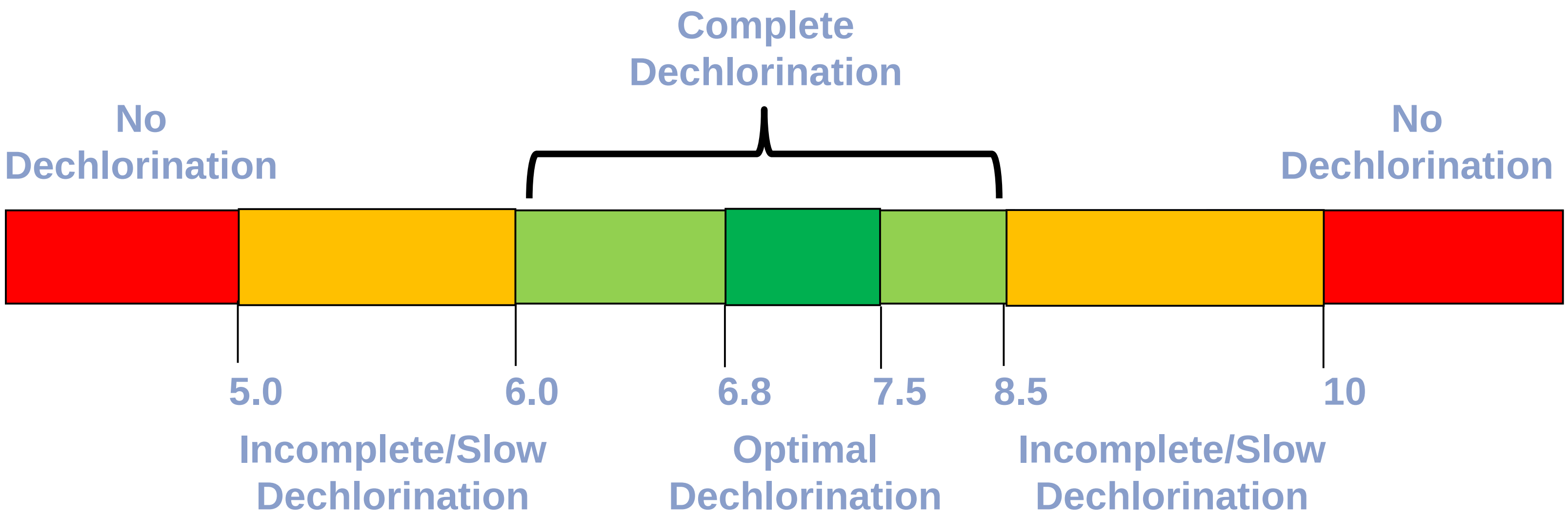
Inhibit *Dehalococcoides* by binding to reductive dehalogenases

KB-1 Plus cultures are used to overcome inhibition caused by these compounds

Bioaugmentation Cultures Available for Chlorinated and Other Compounds

Compound Class	Bioaugmentation Culture Available	Comment
Chlorinated ethenes	✓	PCE, TCE, DCE, VC
Low pH chlorinated ethenes	✓	Complete dechlorination to pH 5.7
Chlorinated ethanes	✓	1,2-DCA /1,1,1-TCA/TeCA
Chlorinated methanes	✓	Chloroform /Dichloromethane
CFCs	✓	Defluorination?
Chlorinated propanes	✓	TCP/DCP
Benzene	✓	Anaerobic Pathways-Initial field pilots in progress
TEX	For lab studies	Anaerobic pathways current research project
Chlorinated Benzenes	For lab studies	Aanerobic Pathways
1,4-Dioxane	For lab studies/ custom pilot	Aerobic

Impact of pH on Dechlorination



- **pH of 6.0-8.5 is <u>generally</u> required for dechlorination to ethene***
- **pH 6.8-7.5 is considered optimal range, 7.5 is best***
- **Sites with low pH more likely to accumulate cDCE/VC**
- **Low pH cultures may be of benefit where pH below 6.3**

*Rowlands, 2004

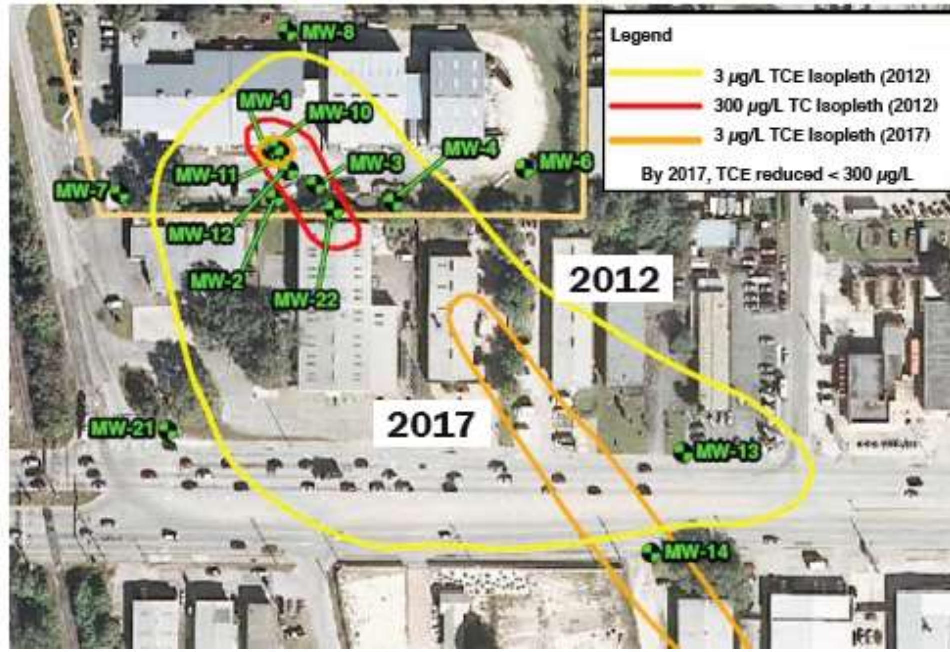
Low pH KB-1® Plus

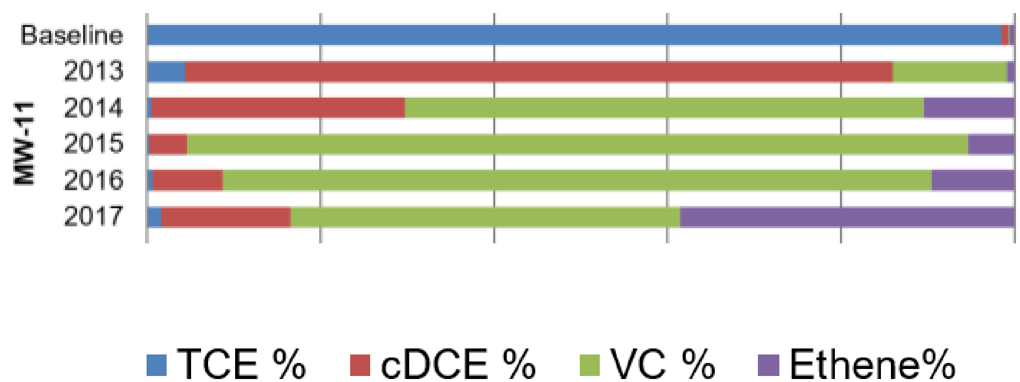
Image courtesy USGS

- Anaerobic bioaugmentation culture enriched from a wetland site with intrinsic pH~5.0
- Grown on TCE at progressively lower pH over a ~4 year period currently at pH 5.6-5.8

Low pH Site – South West Florida

- TCE source area up to 730 mg/L
- pH adjustment (calcium carbonate) proved challenging–long term pH was 5-6 in source area
- EISB: EVO/KB-1 Plus–low pH
- **2012-2017**
  - *Dhc* abundance in MW-11 (increased 2 orders of magnitude at pH 5.6-5.9)
  - 90% reduction in source zone TCE concentrations
  - Plume extent decreased from 5 acres -1.5 acres





Damasceno, S., 2017. A Case Study: Evaluation of Enhanced In Situ Bioremediation Performance in Low pH Aquifer. Battelle Bioremediation Symposium: Miami Florida.

Key Reductive Dechlorinators

Chlorinated ethenes/1,2-DCA ← ***Dehalococcoides***

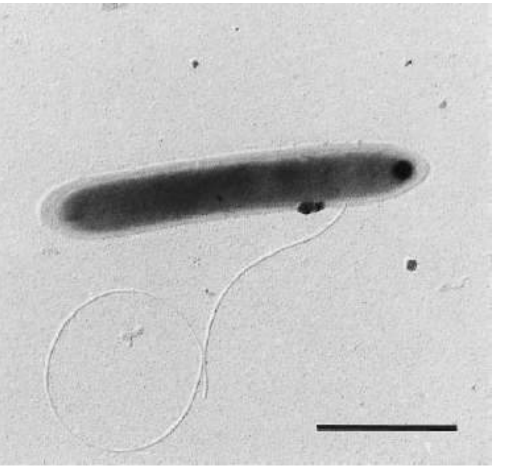
1,1,1-TCA /Chlorinated methanes ← ***Dehalobacter***

Chlorinated propanes /ethanes/ethenes ← ***Dehalogenimonas***

Dehalobacter (*Dhb*) Dechlorinates Tri-halogenated and other Compounds

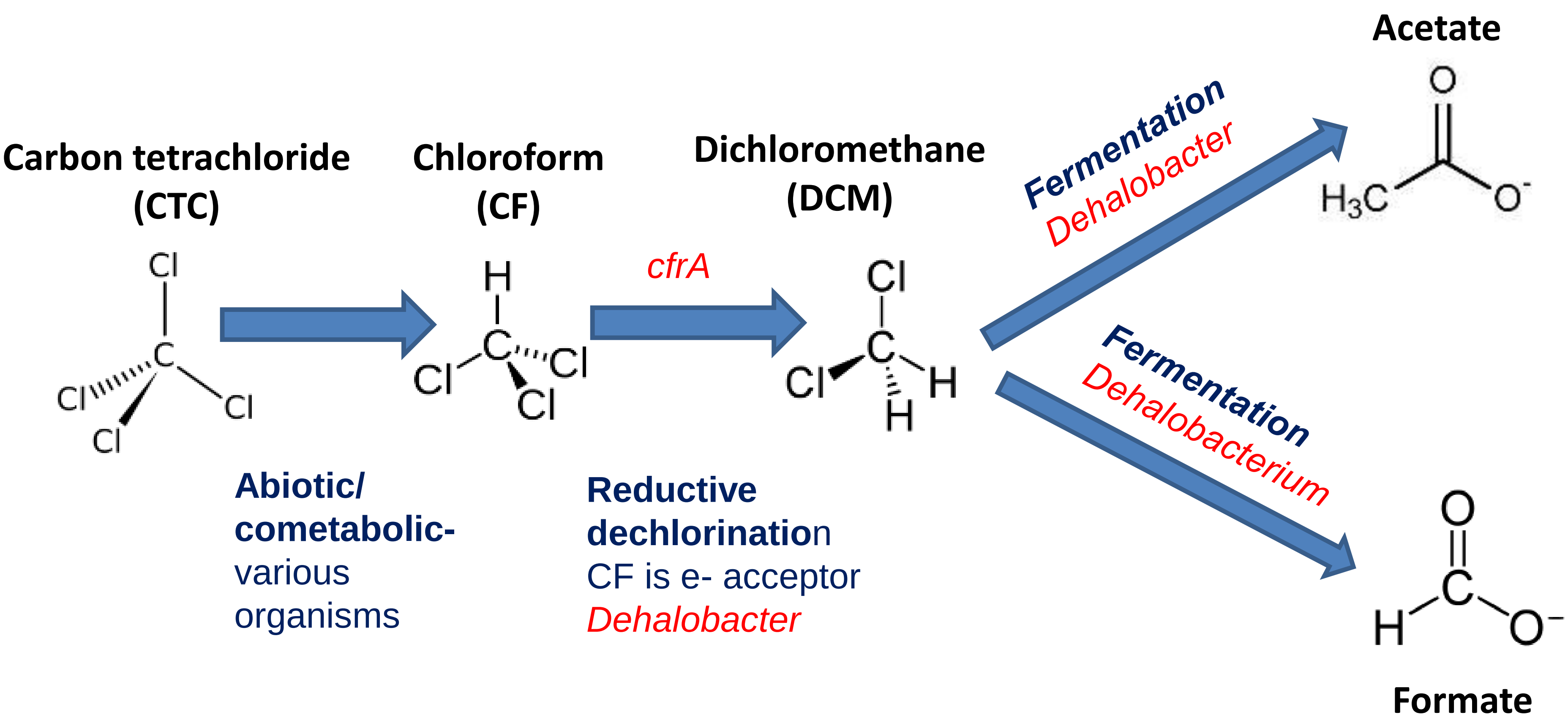
Dehalobacter activities include:

- 1,1,1-TCA degradation to CA
  (Grostern and Edwards, 2006)
- Chloroform to Dichloromethane *(cfrA)*
  (Grostern, Edwards, Duhamel and Dworatzek, 2010)
- DCM to acetate
  (Justicia-Leon et al., 2011)
- 1,1,2,2-TeCA to ethene
  (Manchester et al., 2012)

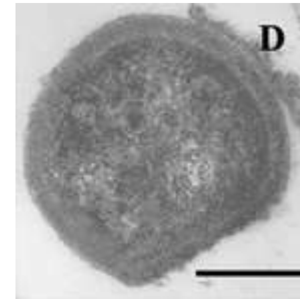


Dehalobacter restrictus

Complete Dechlorination of Carbon Tetrachloride Requires Several Mechanisms



Dehalogenimonas (Dhgm)



- Degrades Chlorinated propanes (1,2-DCP, 1,2,3-TCP) 1,2-DCA, *trans*-DCE- *Dhgm* is dihaloelimination specialist

1,2-DCA

Ethene

1,2-Dichloropropane

Propene

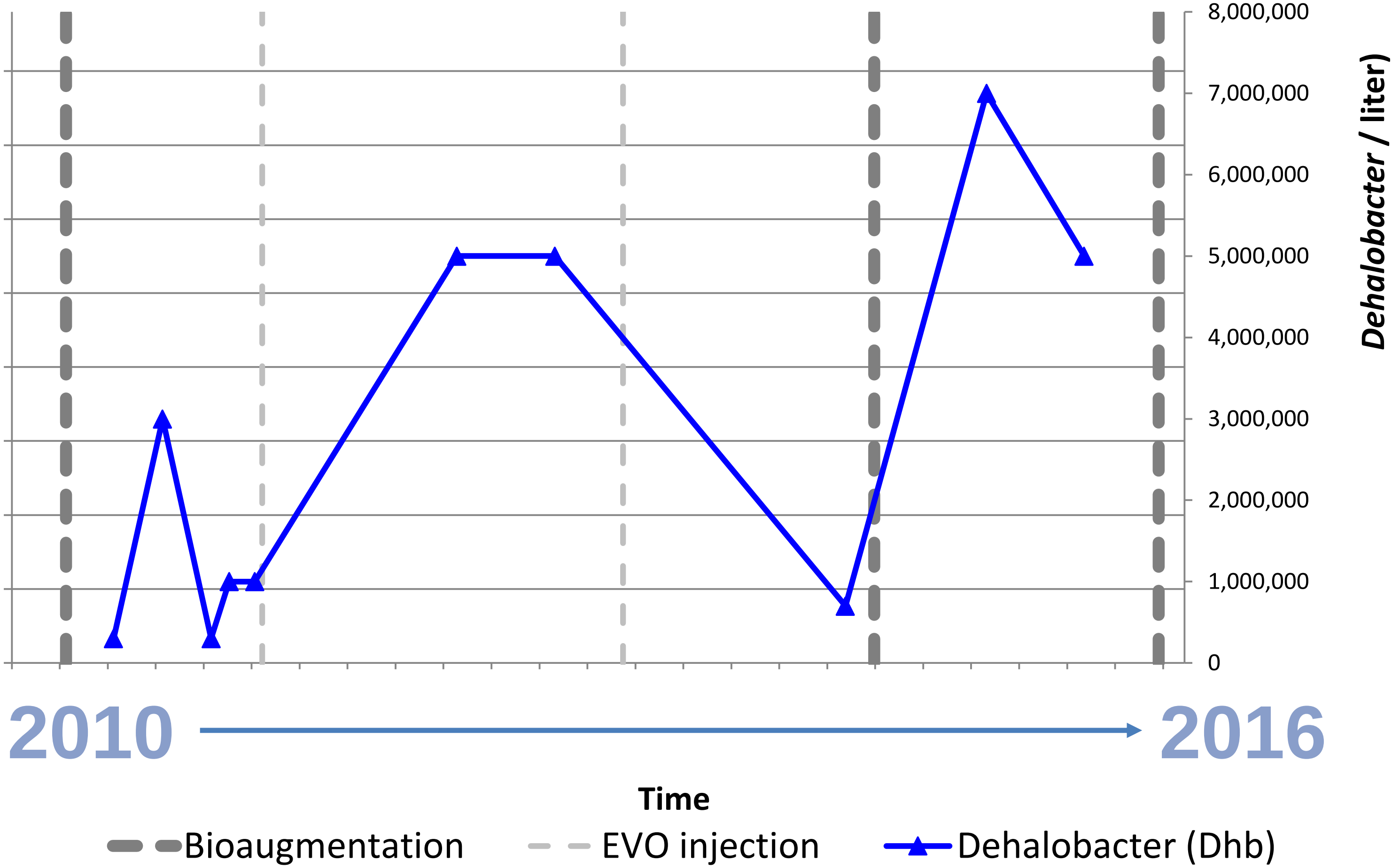
- *Dehalogenimonas sp.* recently reported to degrade VC to ethene (Yang et al., 2017)

Carbon Tetrachloride Remediation in Karst Aquifer–Eastern US Site

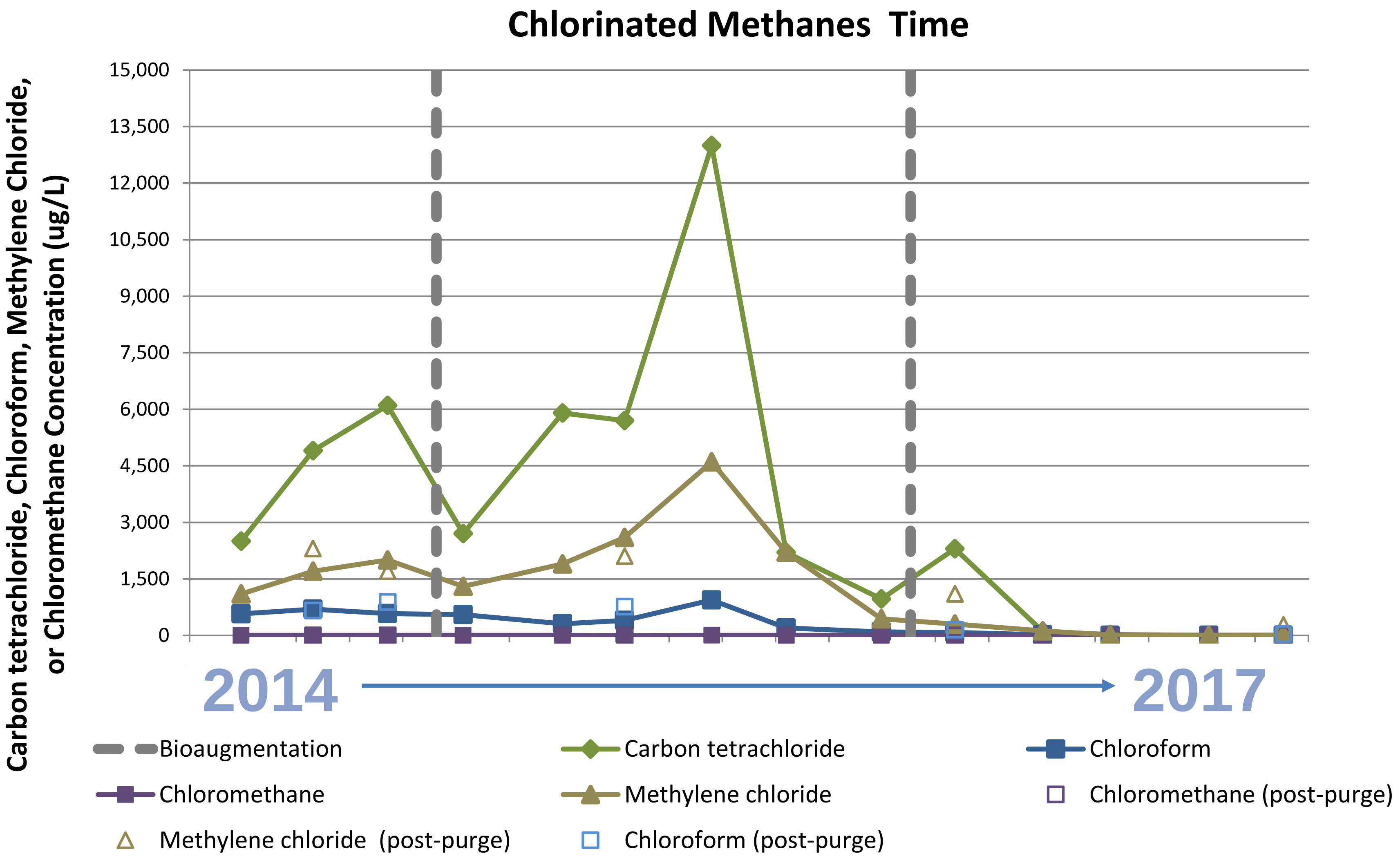
- Manufacturing site since 1940's
- Carbon tetrachloride as high as 50 mg/L in groundwater with recurring spikes
- EVO/Vitamin B12 /KB-1 Plus chlorinated methanes formulation
- Treatability test and push pull field test performed to verify remedy effectiveness
- Repeated bioaugmentation/biostimulation deemed good value by client for optimizing ongoing dechlorination

Tom Geiger et al. 2018 SUCCESSFUL BIOAUGMENTATION FOR DNAPL CARBON TETRACHLORIDE IN A KARST AQUIFER 11th Chlorinated and Recalcitrant Compounds Palm Springs CA

MW-47D-*Dehalobacter* Population



MW-HE-04 Results



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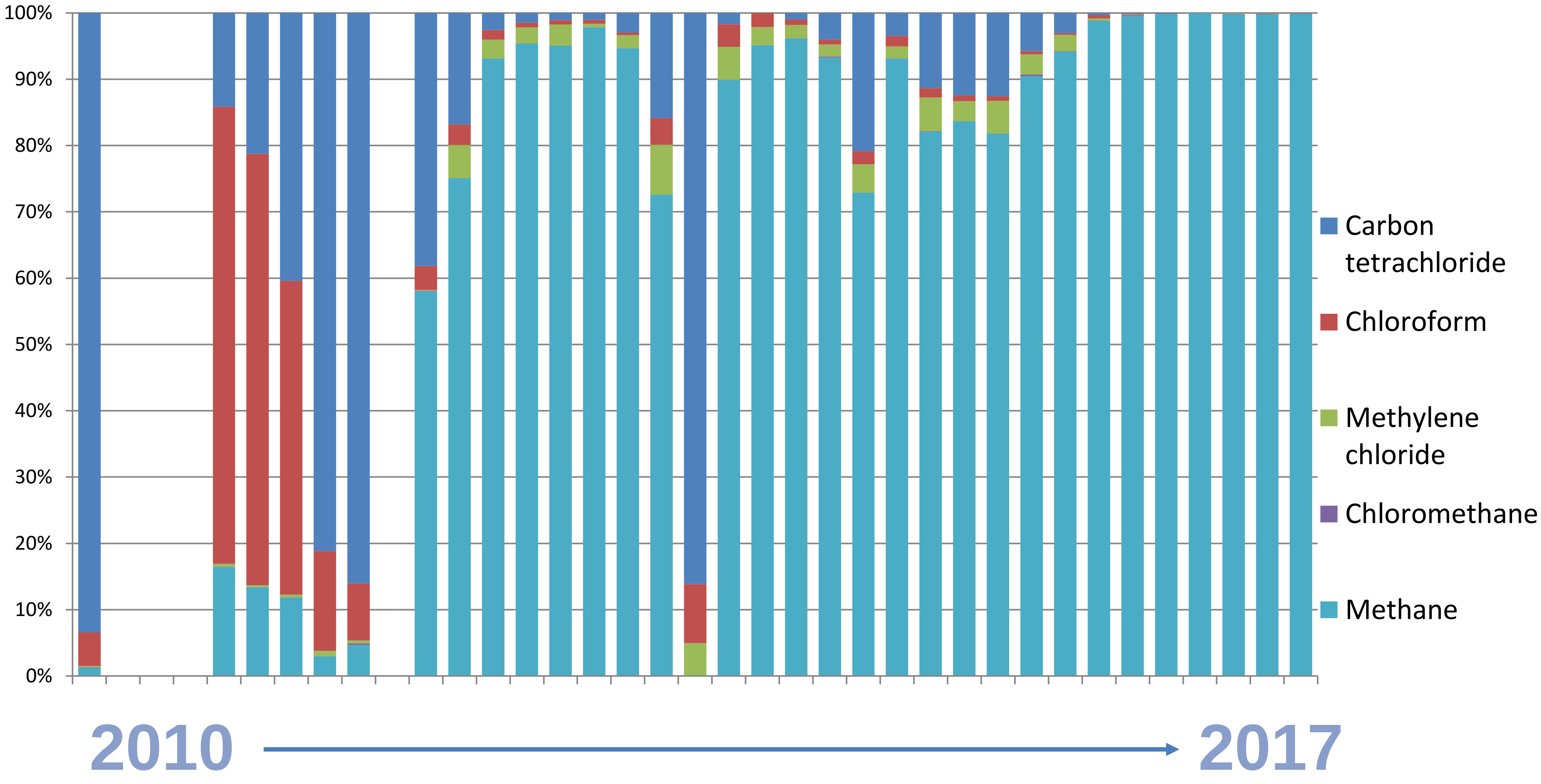
Carbon Tetrachloride Site
VOC Concentrations as % of Total (MW-47D)



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Summary

- Bioremediation offers significant benefits but can be challenging to optimize in some cases
- Bioaugmentation increases our ability to implement bioremediation dependably at a larger range of sites
- Bioaugmentation is routine for chlorinated ethenes/ethanes/methanes/low pH
- Novel cultures and approaches are expanding the range of conditions and compounds for which bioremediation is applicable

Further Information

siremlab.com

1-866-251-1747

519-515-0836

Phil Dennis: pdennis@siremlab.com

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