Leading Science · Lasting Solutions

Bioaugmentation – A Canadian Perspective

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Introduction to SiREM

Founded in 2002 in Guelph, ON
Expanded to Knoxville, TN in 2020

Provide products and testing services to support and improve site remediation

Further information: siremlab.com

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Enhanced Bioremediation Tools

Injection of KB-1 at a site in BC

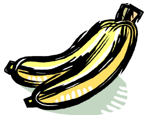
- **Biostimulation**: addition of electron donors, acceptors, pH adjustment, trace nutrients etc.

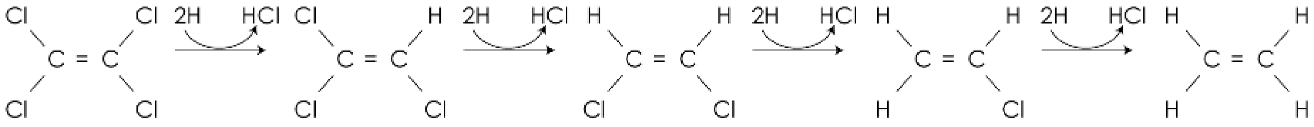
- **Bioaugmentation:** addition of beneficial microorganisms to improve biodegradation. KB-1®: DGG-B bioaugmentation cultures for chlorinated ethenes/benzene
- **Treatability Studies** determine in lab if biodegradation will occur (e.g., at cold temperatures) or can be optimized
- **Molecular Tests** to track growth and spread of biodegraders

- **Enhanced Distribution**
  - Tight DPT injection grids/multiple lifts
  - Hydraulic fracturing
  - Electrokinetics
  - Groundwater recirculation systems

Biodegradation of Chlorinated Ethenes By Reductive Dechlorination

Can accumulate if *DHC or DHG* are absent or wrong strain





PCE TCE cis-1,2-DCE VC Ethene

Dehalobacter Dehalospirillum
Desulfitobacterium Desulfuromonas

+*Dhc, Dhg*

Dehalococcoides (Dhc)
Dehalogenimonas (Dhg)

Ethene (aka. ethylene) is non-toxic, produced by many fruit to stimulate ripening

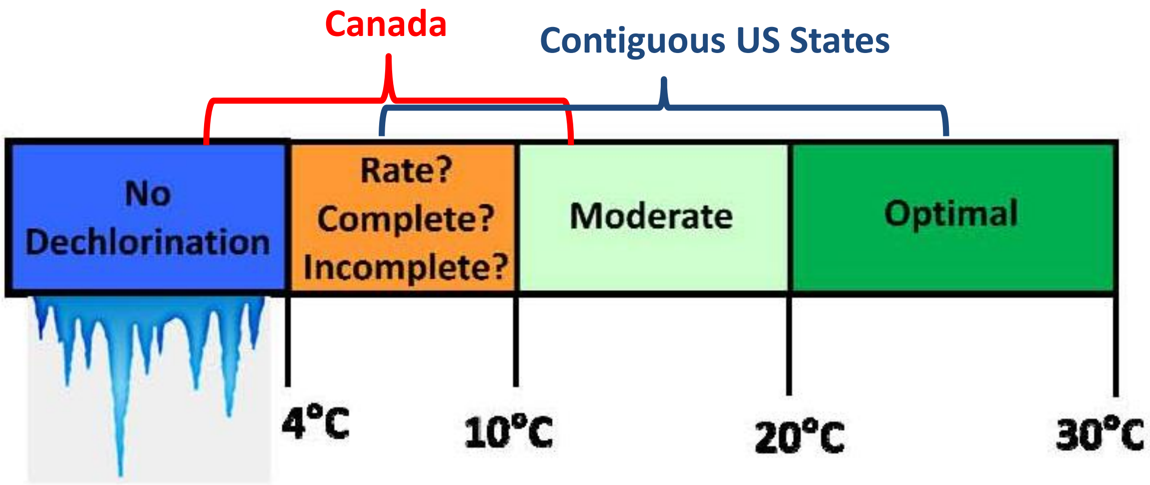
Advantages of Enhanced In Situ Bioremediation (EISB)

- Cost Effective: As little as 1/3rd the cost of other in situ remediation options
- Destroys Contaminants: doesn't just move them
- High Concentrations Treatable: Including DNAPL/LNAPL sites
- Resistant to Rebound: Once down concentrations tend to stay down
- Sustainable: low carbon foot-print/natural process/inobtrusive
- Compatible with remote sites-no utility or maintenance requirements

Reasons Why EISB Less Used in Canada Compared to USA?

- **Use of Groundwater for Drinking water?** 30% Canada/55% USA
- **Regulatory/Legal Landscape?** Federal Approvals/Government Funding/Cleanup Regulations
- **Number of sites?** >300,000 routinely estimated in the US, in Canada 22,000 Federal Government sites alone -total number unclear
- **Climate?-** is cold climate/groundwater limiting use of bioremediation in Canada
- **Geology?**- Is Canada's geology more challenging for *in situ* remediation

Average Groundwater Temperature and Dechlorination Rates



Is complete PCE/TCE dechlorination to ethene practical below 10°C ? Yes!

Can petroleum hydrocarbons degrade below 10°C? Yes!

References Bradley *et al*., 2005; 2007 Friis *et al*., 2006; Fletcher *et al.,* 2010

Performance of Bioaugmentation for Chlorinated Solvents at Varying Groundwater Temperatures

Site	Average Groundwater Temperature °C	Time post-bioaugmentation to 1st ethene detection (or 10X pre-bioaug. ethene)	Maximum Dhc Detected/L
Florida	25	3 months	6.0E+09
California	20	2 months	2.0E+09
Denmark	10	4 months	3.0E+07
Northern Ontario	8.3	13 months	4.0E+08
Alaska	6.9	11 months	9.0E+08

- High Dhc concentrations achieved (>10^7 /L) associated with ethene production
- Ethene detected ~ 1 year after bioaugmentation at cold water sites
- VC Half lives ~ 120-44 days at 8-10 °C vs. 60-35 days at 15-20 °C
- Bioremediation below 10 °C slower but feasible

*Scheutz *et al.*, 2008 note site had indigenous Dhc

SiREM Bioaugmentation Capabilities

Commercially available:

- Chlorinated ethenes
- Chlorinated ethanes
- Chlorinated methanes
- Chlorinated propanes
- CFCs
- RDX
- Benzene , Toluene , Xylene (anaerobic)

KB-1®

KB-1 plus®

DGG PLUS™

MADE IN CANADA · FABRIQUÉ AU CANADA

In development available for treatability testing/custom scale up:

- 1,4-dioxane (aerobic)
- Sulfolane (aerobic)
- And others

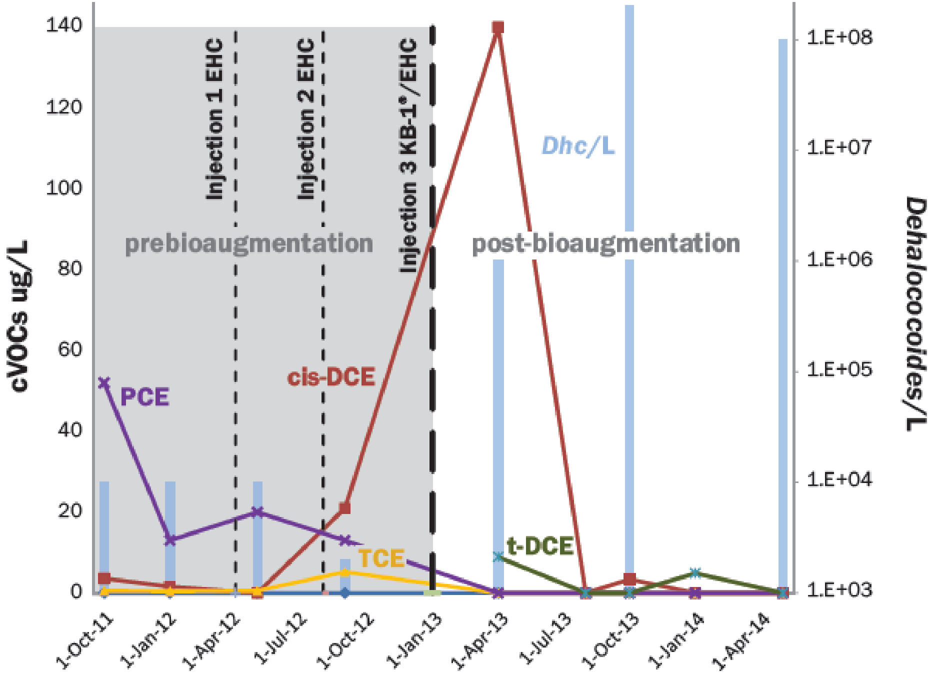
ask us for latest developments

Case Study 1: Low Permeability GTA Site

- Former drycleaner
- 1,350 m^3 of saturated material
- PCE up to 150 µg/L some incomplete dechlorination
- Silty sand geology
- Low permeability (K= 10^{-7} m/s)
- 2,600 kg EHC at 29 locations, ~ 3m centres using high pressure grout pump/direct push
- 20 L KB-1, 9 injection locations

Injection of KB-1 at GTA Site
-Photo Courtesy of Terraprobe Inc.

Case Study 1: Low Permeability GTA Site

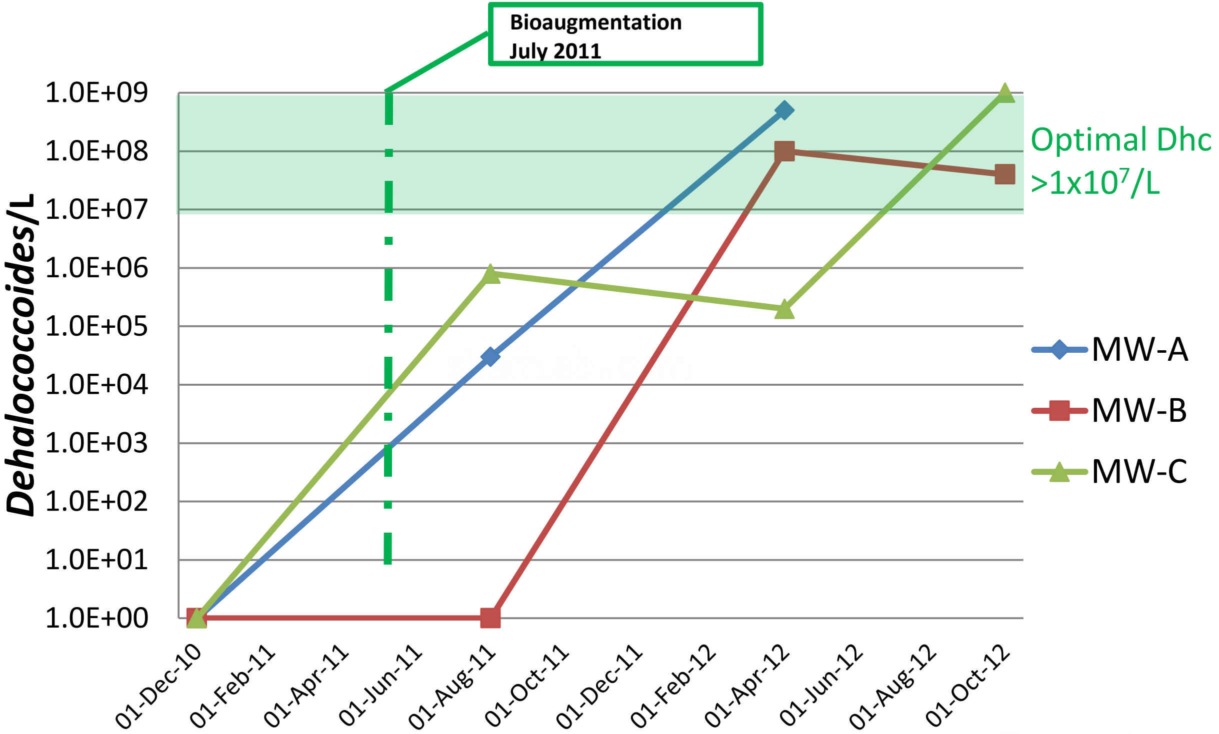


- Dhc was trace to ND prior to bioaugmentation
- Dhc increased 10,000- fold post bioaugmentation
- Within 1.5 years of bioaugmentation 95% cVOC removal observed
- Site remediation activities completed based on meeting Table 3 standards in monitoring wells

Case Study 2: Enhanced Bioremediation at BC Site

- PCE site with up to 25,000 µg/L
- Classified as high risk by BC MOE high concentration PCE
- E^- Donors: ethanol, cheese whey, emulsified soy bean oil
- Bioaugmented 39 locations (13 points 3 lifts) 2011 with 21 liters KB-1
- Some locations with pH challenges (i.e.,< 6.0) pH neutralization required

Case Study 2: Enhanced Bioremediation at BC Site



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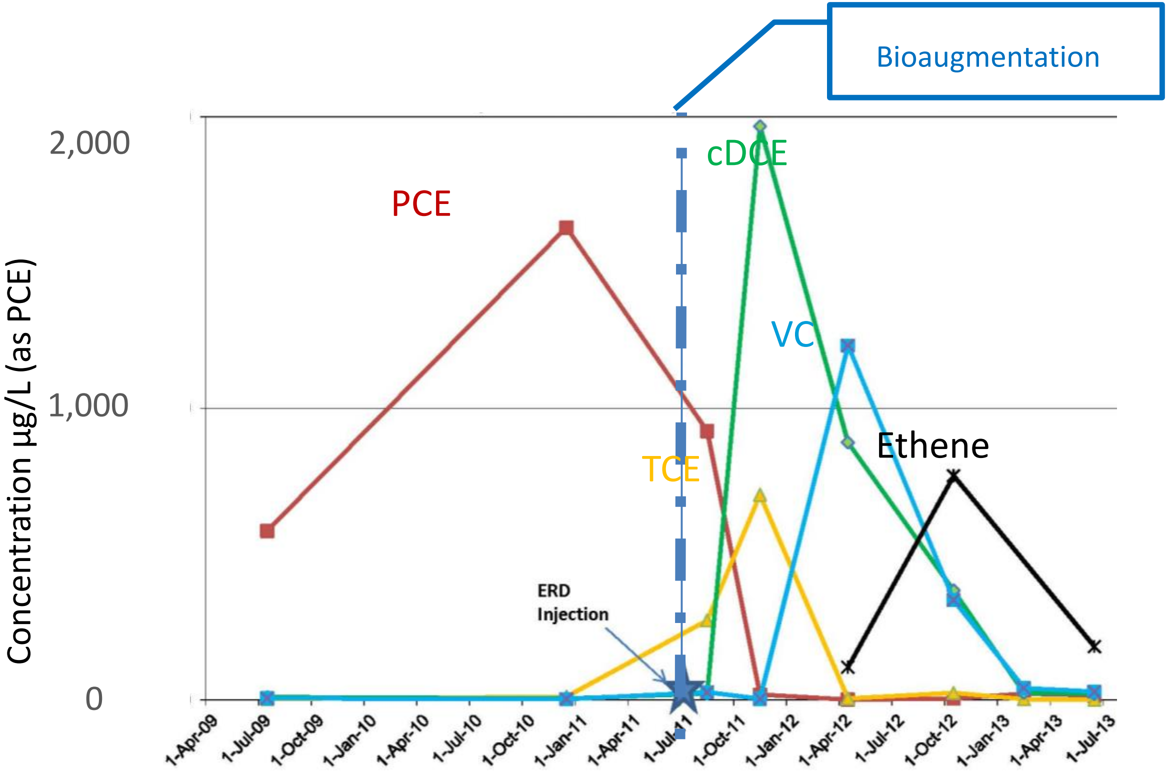

Greater than 90% reduction in PCE and TCE within 2 years of EISB with complete dechlorination to ethene site was reclassified from high risk to low risk by BC MOE

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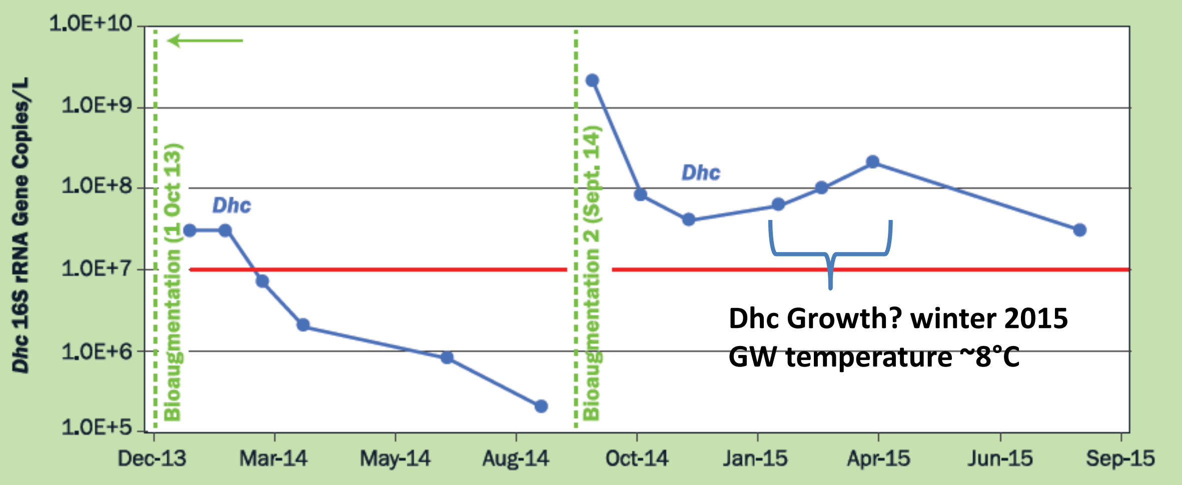
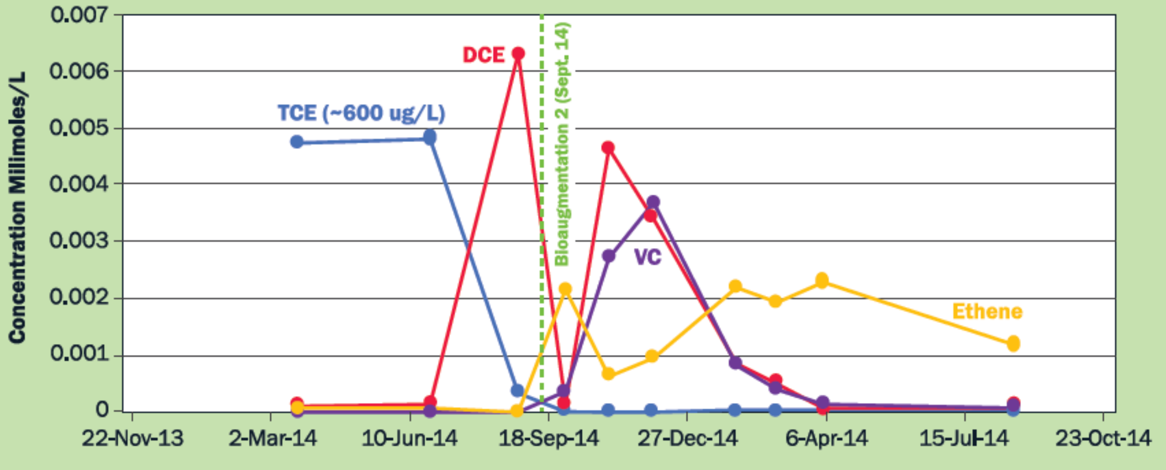
Case Study 3: Ottawa, Federal Site

- Pilot Test at Former Gloucester Landfill – Average GW temp. 10°C
- Injection well - total chlorinated VOCs 1,240 µg/L to ND/low ppb within 8 months of bioaugmentation vinyl chloride $t_{1/2}$ = 44 days
- Dhc spread downgradient was 9m in ~450 days =2 cm/day (6 cm/day is more typical at warmer sites)





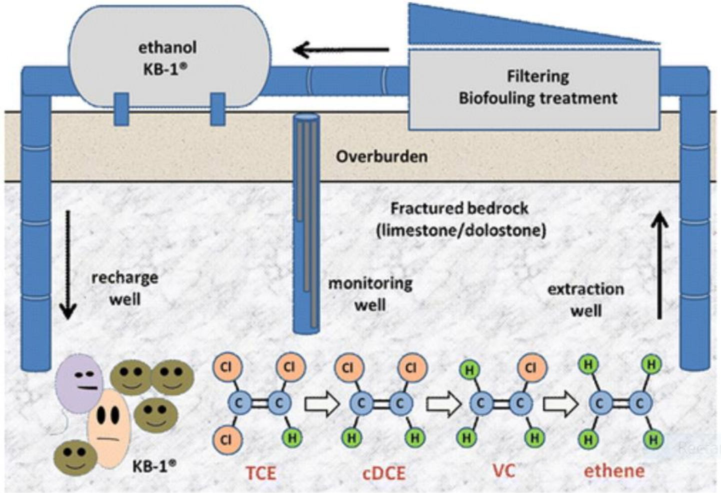
Some Challenging Canadian Geology

- **Fractured Rock** (Canadian Shield/Niagara Escarpment)
- **Glacial deposits/clay** (Great Lakes/St. Lawrence Lowlands)
- **Alluvial Silts and Clays**
- **Peat Lands** –anaerobic and low pH, low permeability- mainly in northern regions

Distribution challenges

Approaches are available to implement EISB in challenging geologies

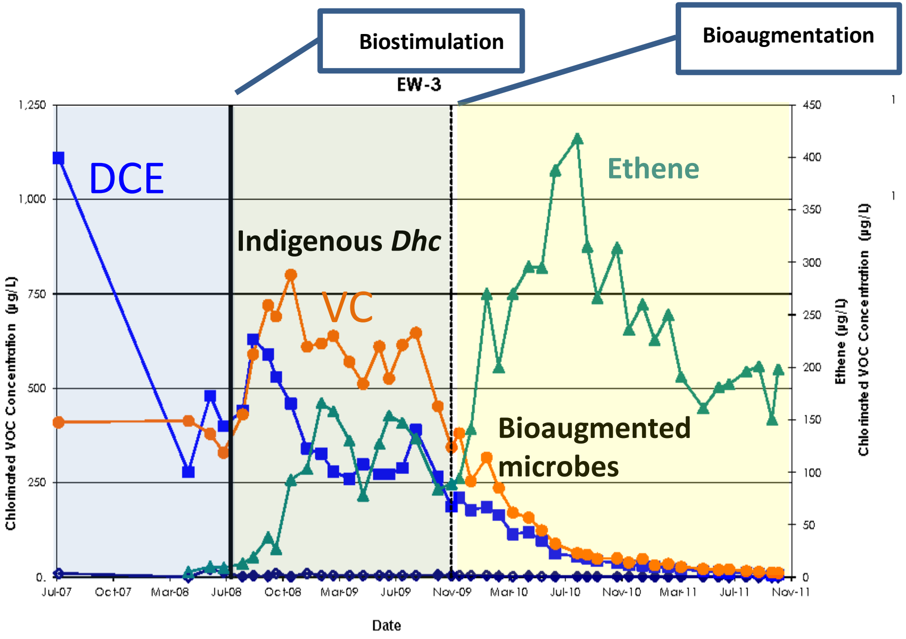
EISB at Fractured Rock Site, Fort Erie ON



- Recirculation system controls groundwater flow and enhances flushing of source to reduce clean up times
- Ethanol and KB-1 were injected via recirculation system
- Recirculation contains nutrients and microbes in contaminated zones

SUSTAINABLE DEVELOPMENT TECHNOLOGY CANADA™

Extraction Well Results, Fractured Rock Site, Fort Erie ON



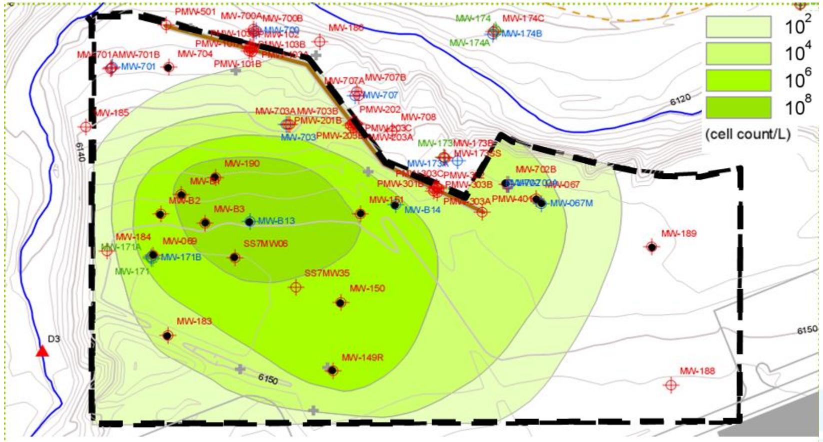
- Accelerated decrease in DCE/VC and >4-fold increase in ethene observed after bioaugmentation
- Bioaugmentation effective at Fractured rock site even with indigenous *Dhc* at site

Amendment Distribution Challenges

- E.g., low permeability glacial deposits in highly industrialized regions such as Southern Ontario, Fort McMurray, Alberta

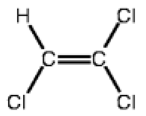
- Spread of remediation amendments can be challenging under low permeability conditions

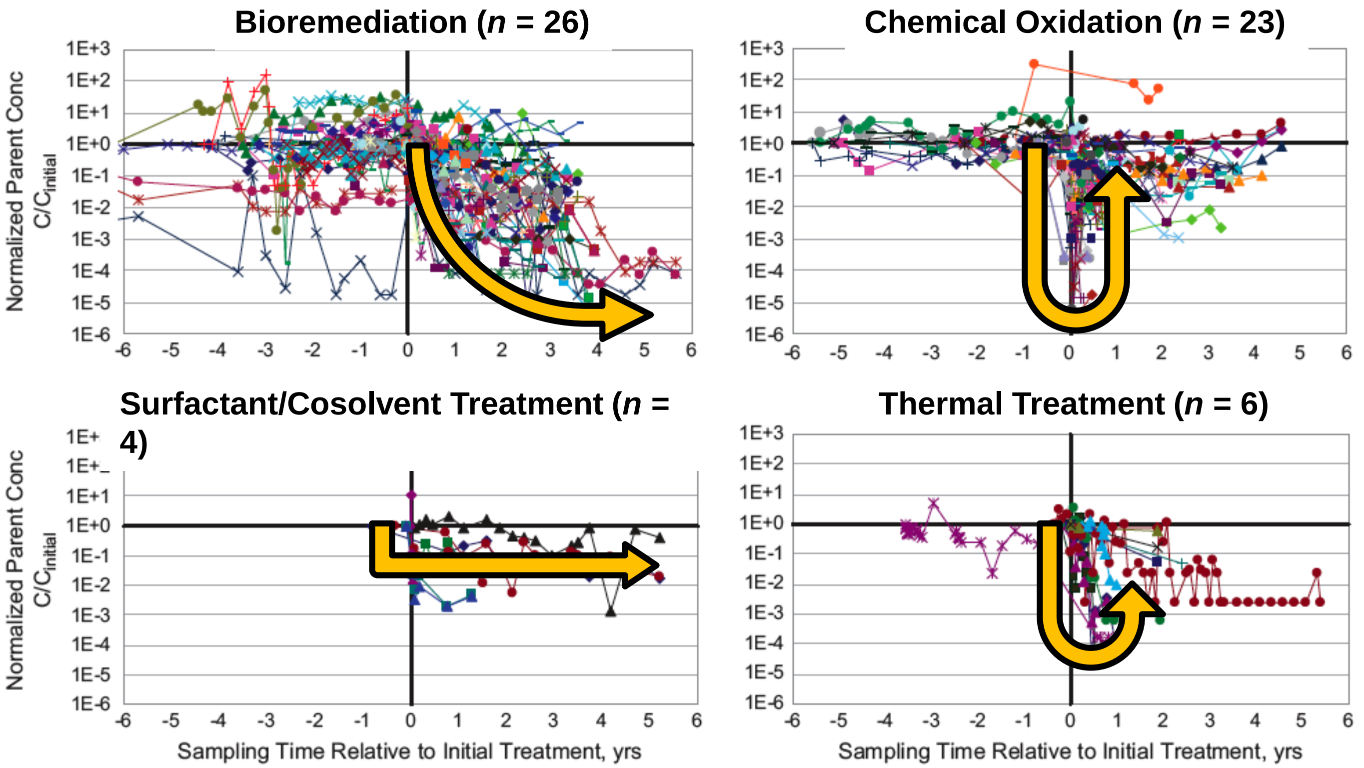
- Technical solutions include:
  - **High density direct push injection grids**
  - **Bio-barriers**–<u>contaminants move </u>through bioactive zone
  - **Hydraulic fracturing-** create amendment expressways
  - **Electrokinetics**- move amendments with electricity

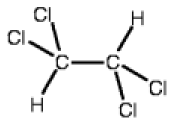


Dehalococcoides at clay site where hydraulic fracturing was used to assist in distribution of these dechlorinators across this 4-hectare site

Bioaugmentation has been an effective treatment for chlorinated solvents. Can it be used to treat other contaminants







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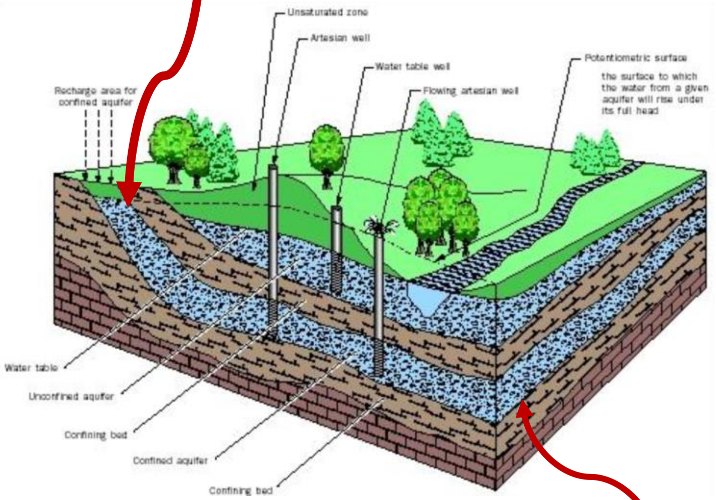
What Sites are Currently Being Targeted for Hydrocarbon Bioremediation?

Offshore Spills
(mostly aerobic)

***Ex situ* Bioreactors**
(mostly aerobic)

Tailings Ponds
(aerobic and anaerobic)

4 Shallow Soils and Groundwater
(aerobic)



5 Deeper Groundwater
(intrinsically anaerobic)

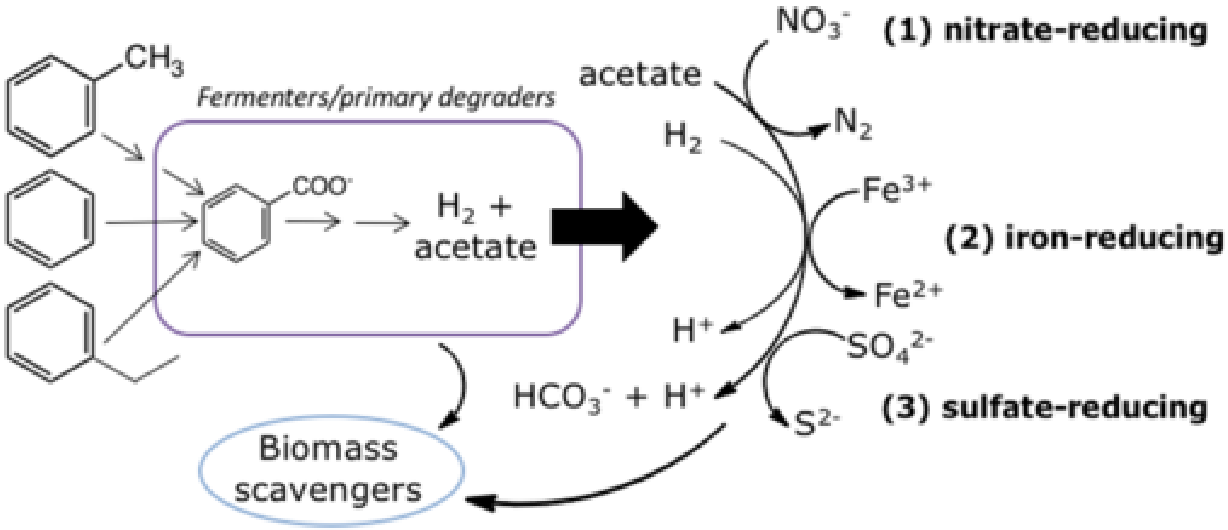
Why Go Anaerobic for BTEX?

- Hydrocarbon sites can go anaerobic - high organic loading consumes O_2
- Electron acceptors (NO_3/SO_4/CO_2) often already present in subsurface
- Anaerobic electron acceptors are soluble, easier to apply/distribute compared to O_2 (e.g., epsom salts (sulfate))
- Viable *in situ* remediation option for deep contamination

Key Difference Between Bioremediation of Chlorinated Solvents vs Hydrocarbons

Hydrocarbons are *electron donors* rather than electron acceptors

- Adding carbon (sugars, VFAs, yeast extract) may not enhance bioremediation performance
- Adding electron acceptors does not always enhance bioremediation either



Gieg et al., 2014 (Curr Opin Biotechnol **27**)

DGG™ Bioaugmentation Cultures

Anaerobic & methanogenic cultures that degrade benzene (DGG-B), toluene (DGG-X) and *o-xylene (DGG-X)*

DGG B™

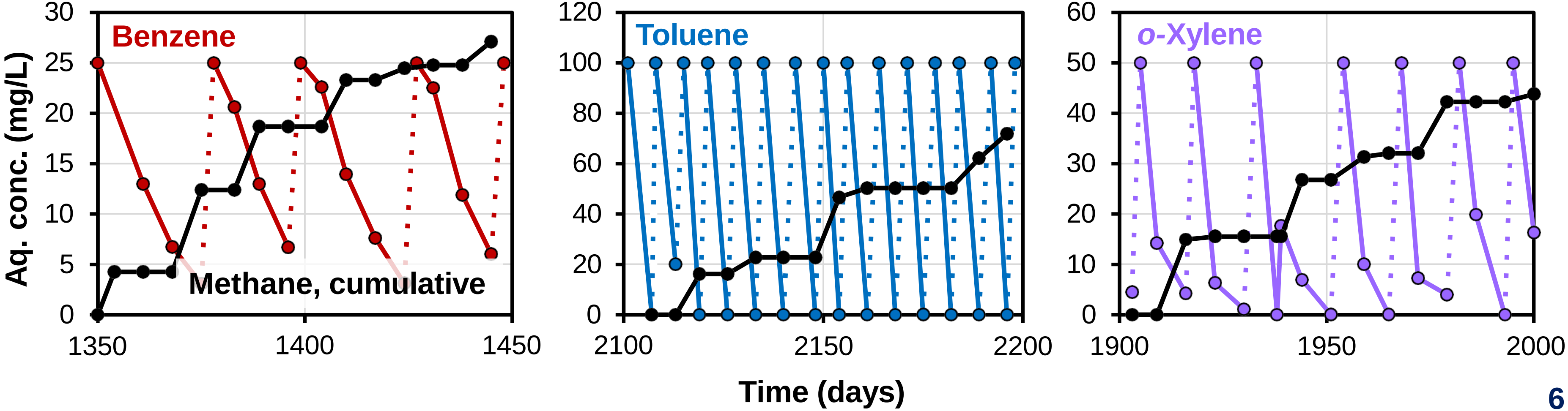
Degr' rate = ~ **1.3 mg/L/day**
= **0.5 mL/year per L culture**

DGG T™

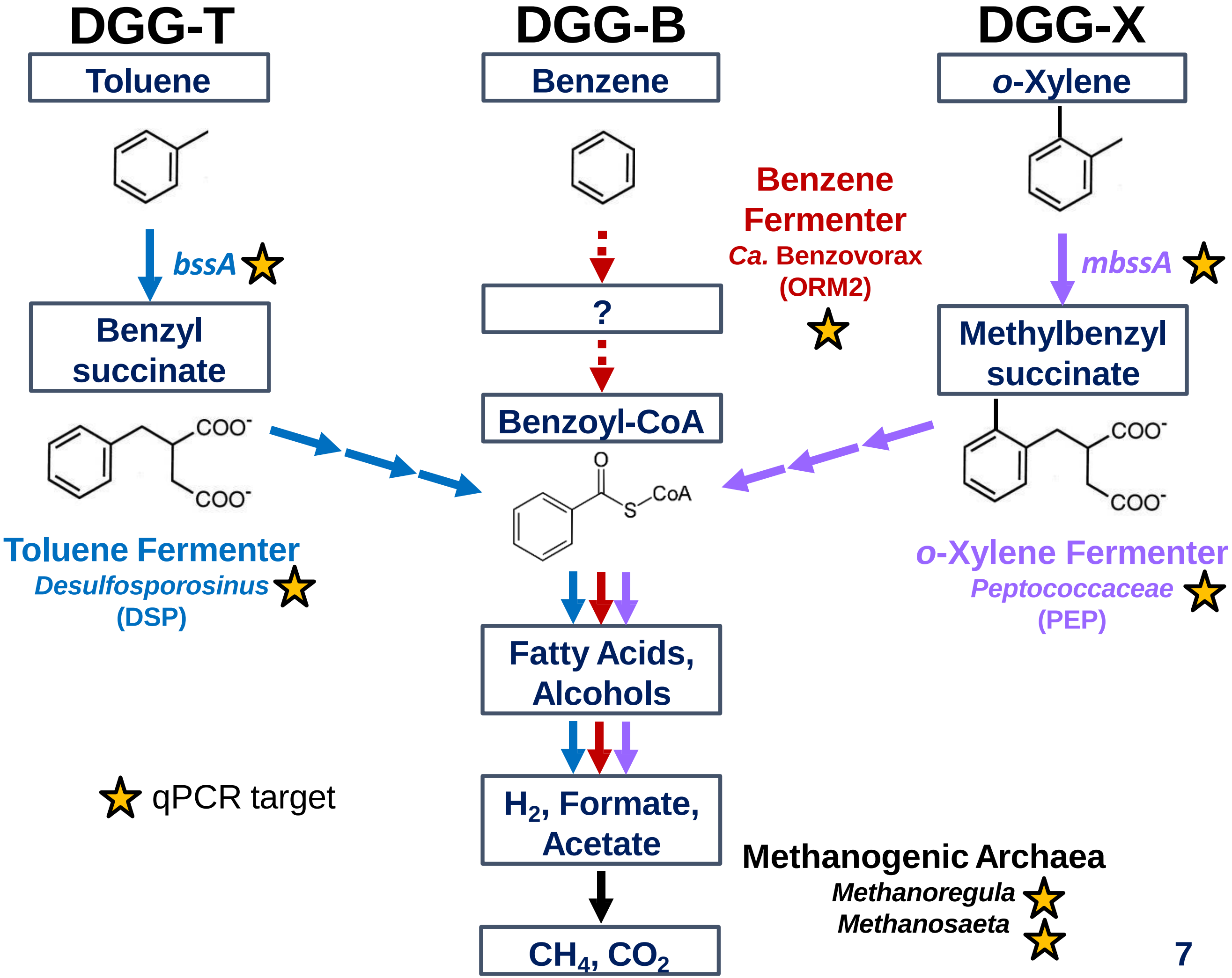
Degr' rate = ~ **25 mg/L/day**
= **11 mL/year per L culture**

DGG X™

Degr' rate = ~ **9 mg/L/day**
= **3.8 mL/year per L culture**

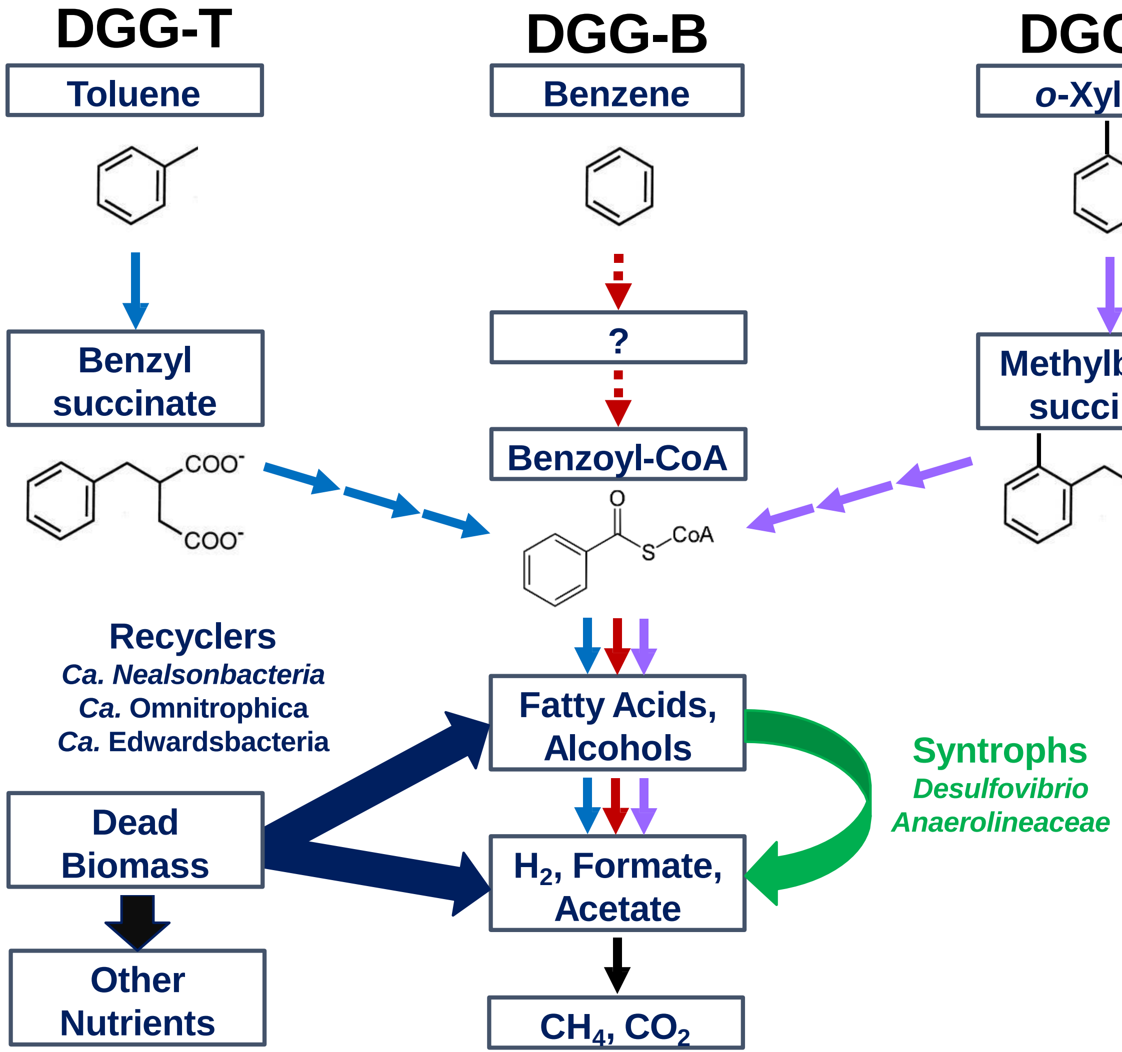


- The key microbes in each culture include one **hydrocarbon fermenter** and **2 methanogens**
- Key microbes & functional genes are monitored by **qPCR** and **NGS**
- Metagenomes have been sequenced and **reconstructed genomes** are being analyzed



- "**Syntrophs**" help metabolize fermentation intermediates

- "**Recyclers**" transform dead biomass (proteins, carbohydrates, etc.) back into useful culture nutrients



Case Study 4: Saskatchewan Field Pilot Site

Decommissioned gas station with historical BTEX, F1 and F2 alkane contamination

Site Timeline

1993: Leaks detected from UST, oil storage, pump islands

1993: Excavation, vapour extraction line installation

2005: Fertilizer injection

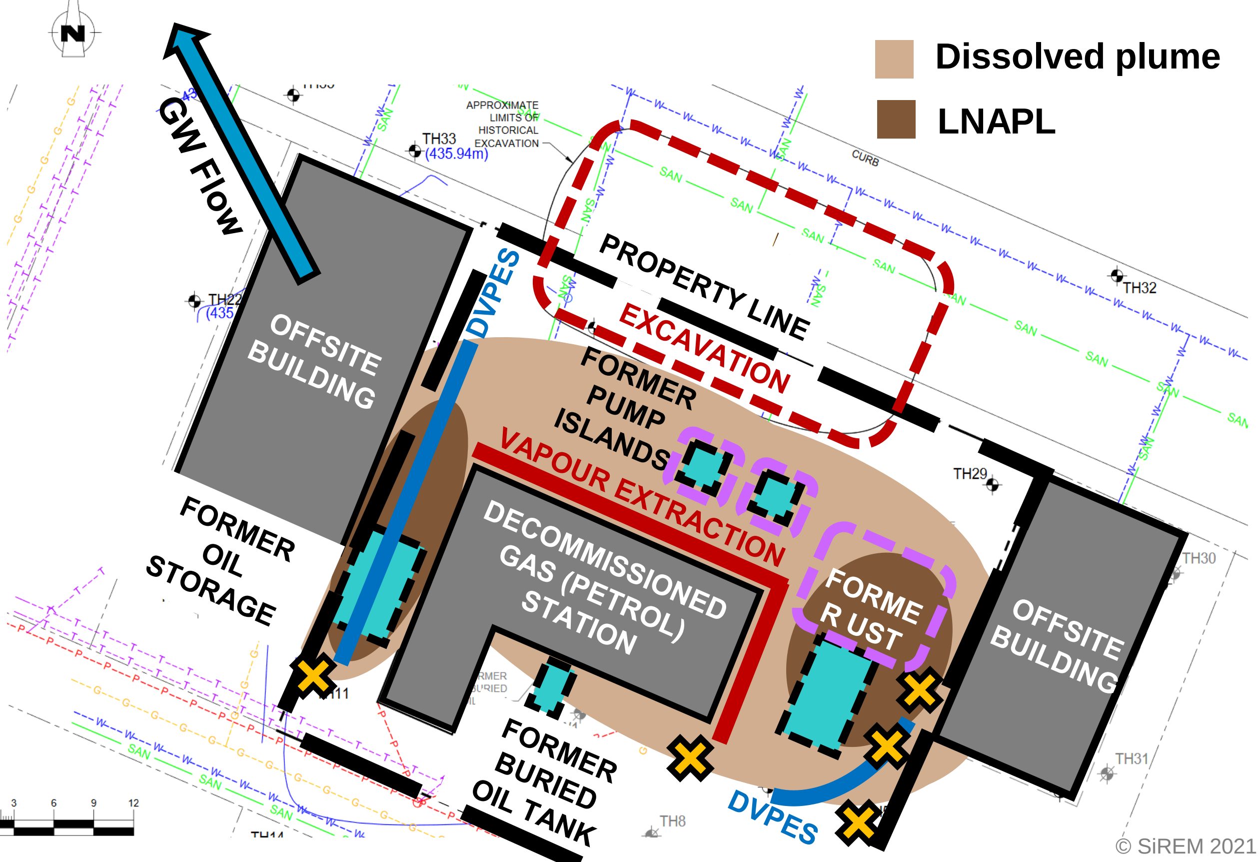
2005-'06: Dual phase vacuum extraction system (DVPES) use

2007-'08: More excavations, purging

2008: Site remediated?

LNAPL = light non-aqueous phase liquid
UST = underground storage tank

Site Overview (1993 – 2008, approximate)



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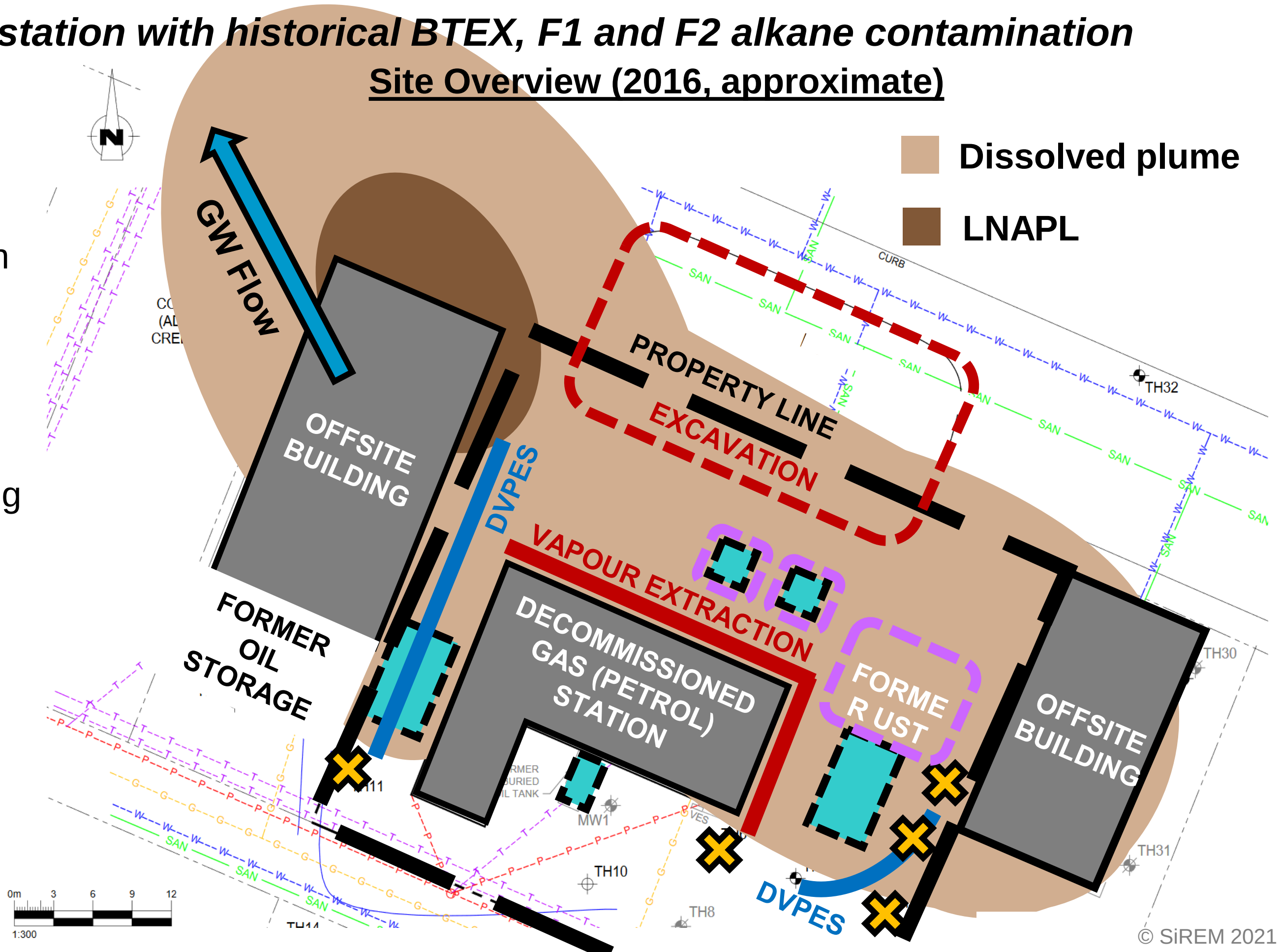
Site Overview (2016, approximate)



Field Injection Overview

DGG-B was injected at two direct push points (10 L each) in the LNAPL zone 5 m apart

The study was designed to treat 20,000 L of groundwater (~ 1200 ft^3; 34 m^3)

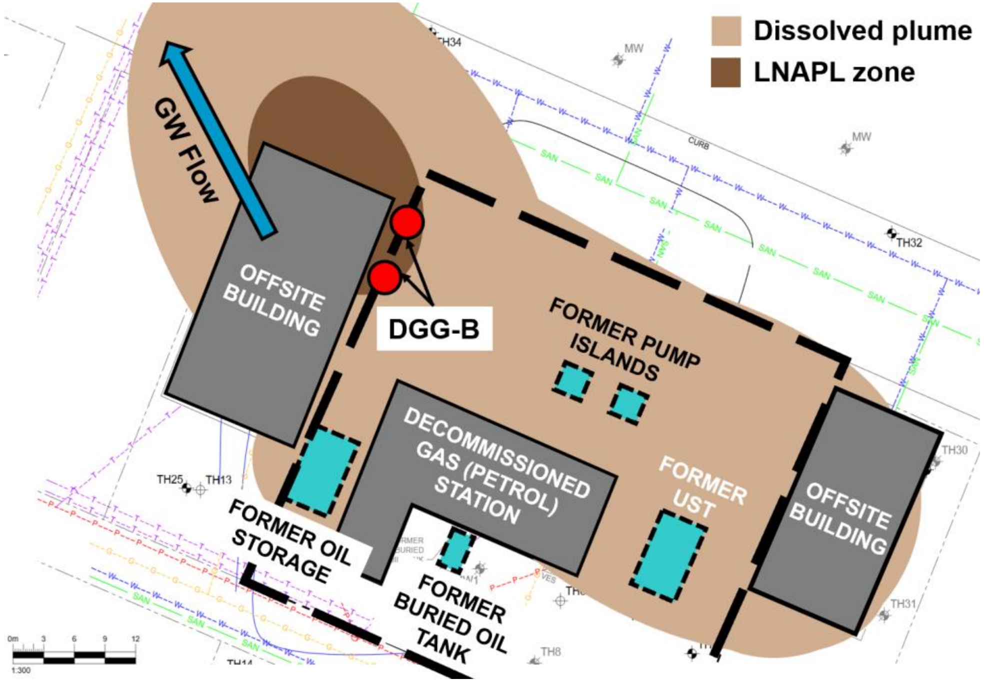
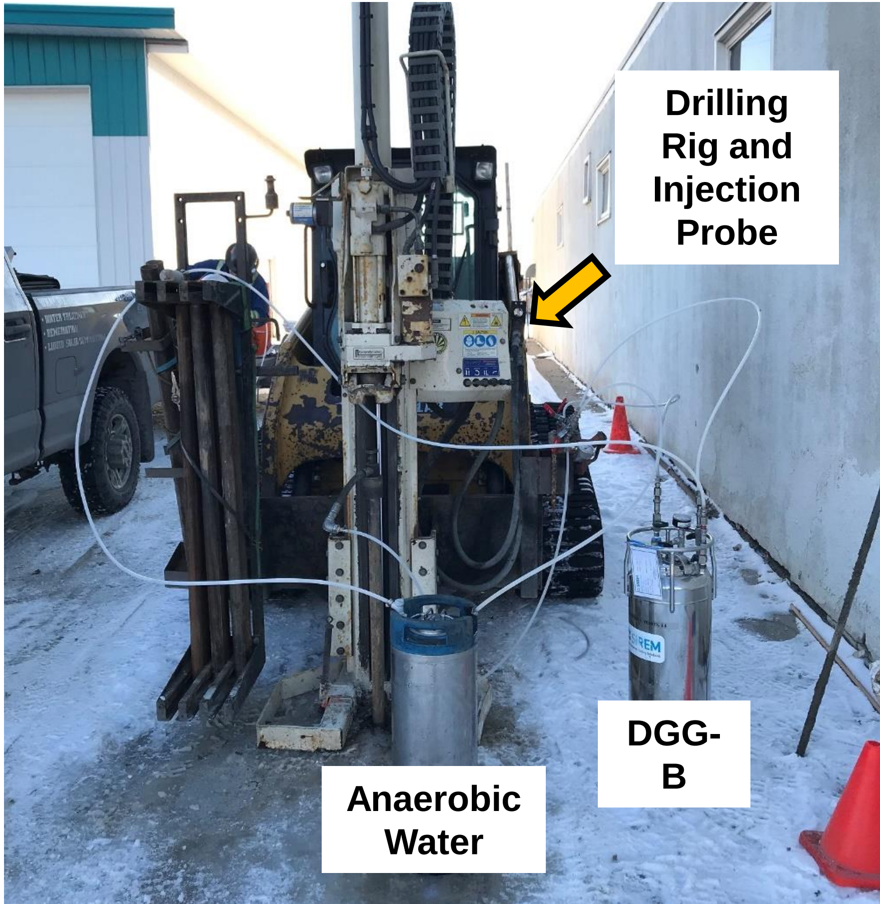
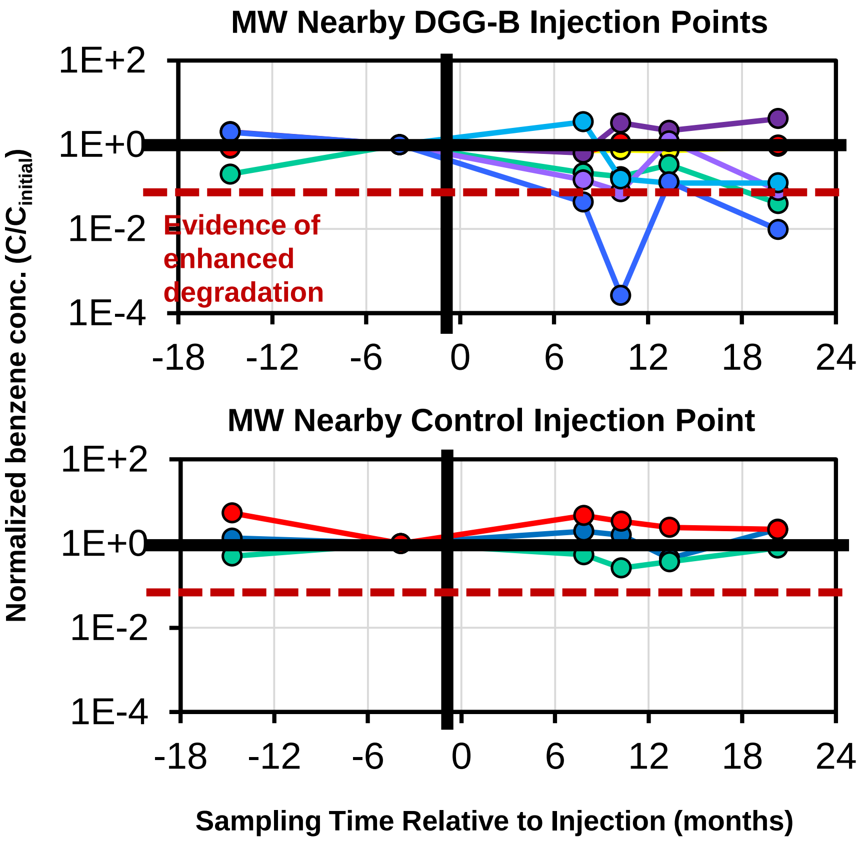


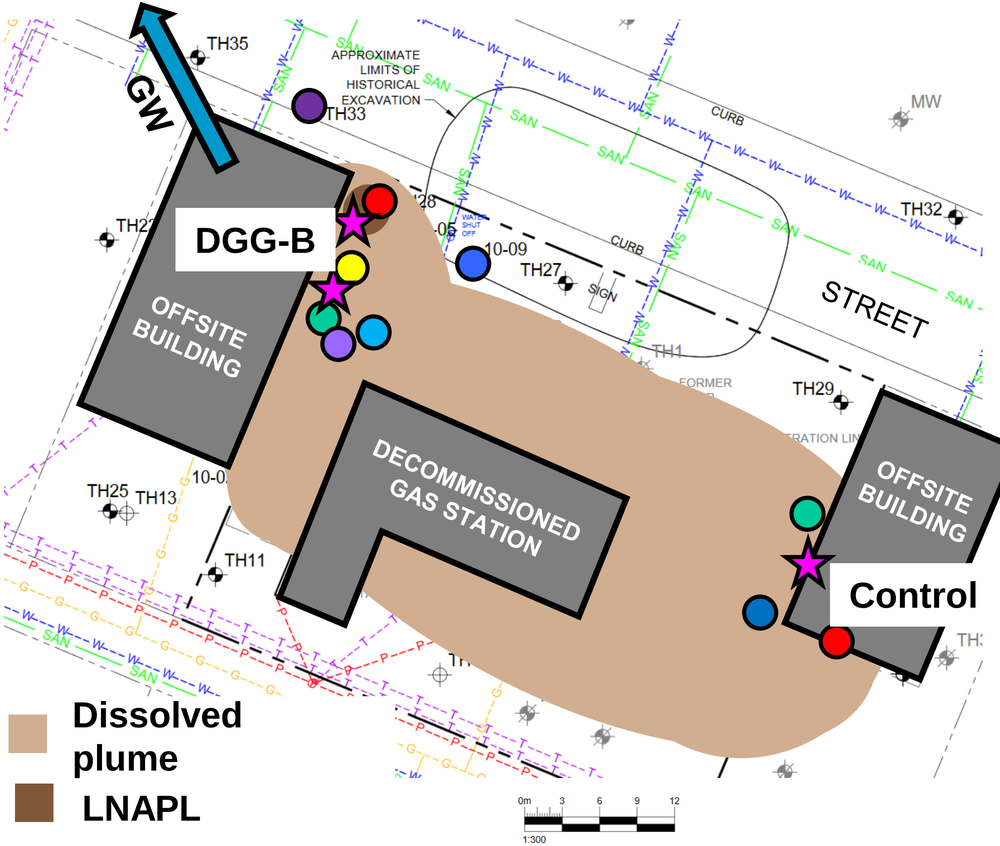
Photo of DGG-B Injection

November 14th, 2019 (-2°C)



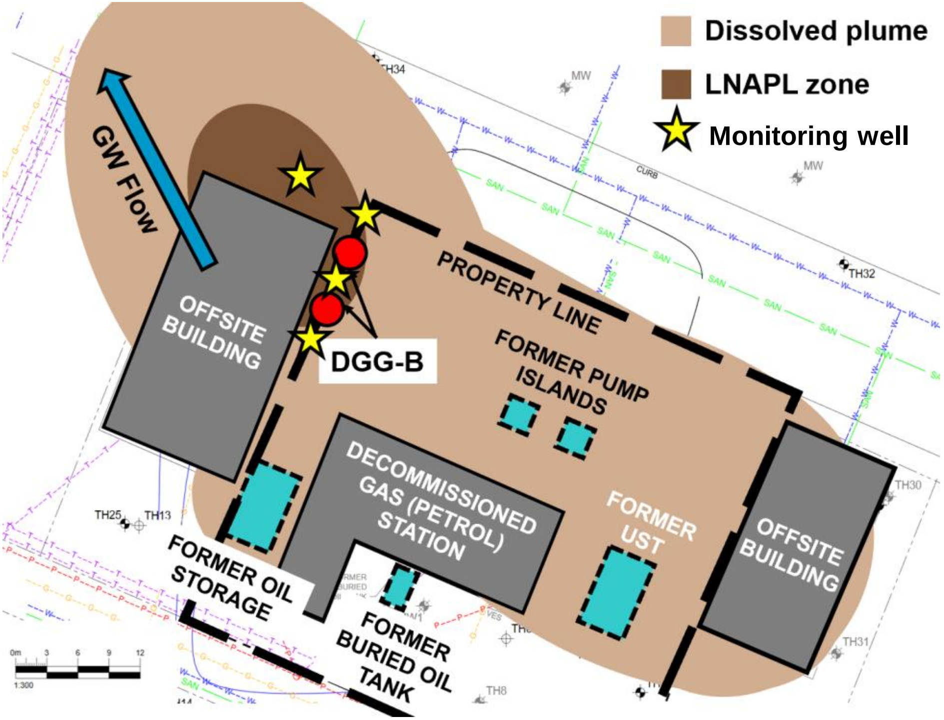
Reduction of Benzene Observed in Monitoring Wells

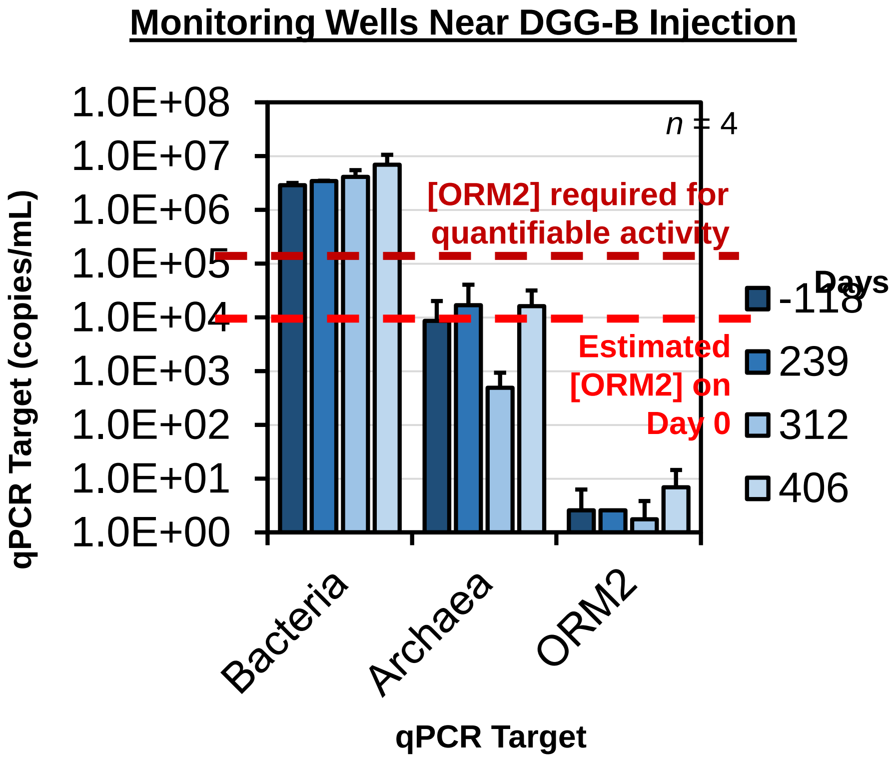




What about ORM2?

No enrichment of ORM2 has yet to be observed. Perhaps DGG-B did not survive post-injection and/or was poorly dispersed? If cells survived, are they attached to sediments?





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Questions?

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