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Applications of Anaerobic Petroleum Hydrocarbon Bioremediation




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Acknowledgements – Benzene/GAPP Team

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Krista Stevenson
Imperial Oil Limited, Sarnia ON

Kris Bradshaw, and Rachel Peters
Federated Co-Operatives Limited, Saskatoon SK

Introduction to SiREM

Founded in 2002 in Guelph, ON

Provide products and testing services to support and improve site remediation

Further information: siremlab.com

SiREM Service Areas

Treatability Testing

treatability studies

SiREMNA™

Characterization/Monitoring

- *Molecular Testing*

gene trac®

Bioaugmentation

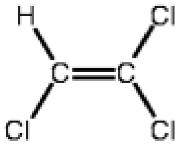
KB-1®

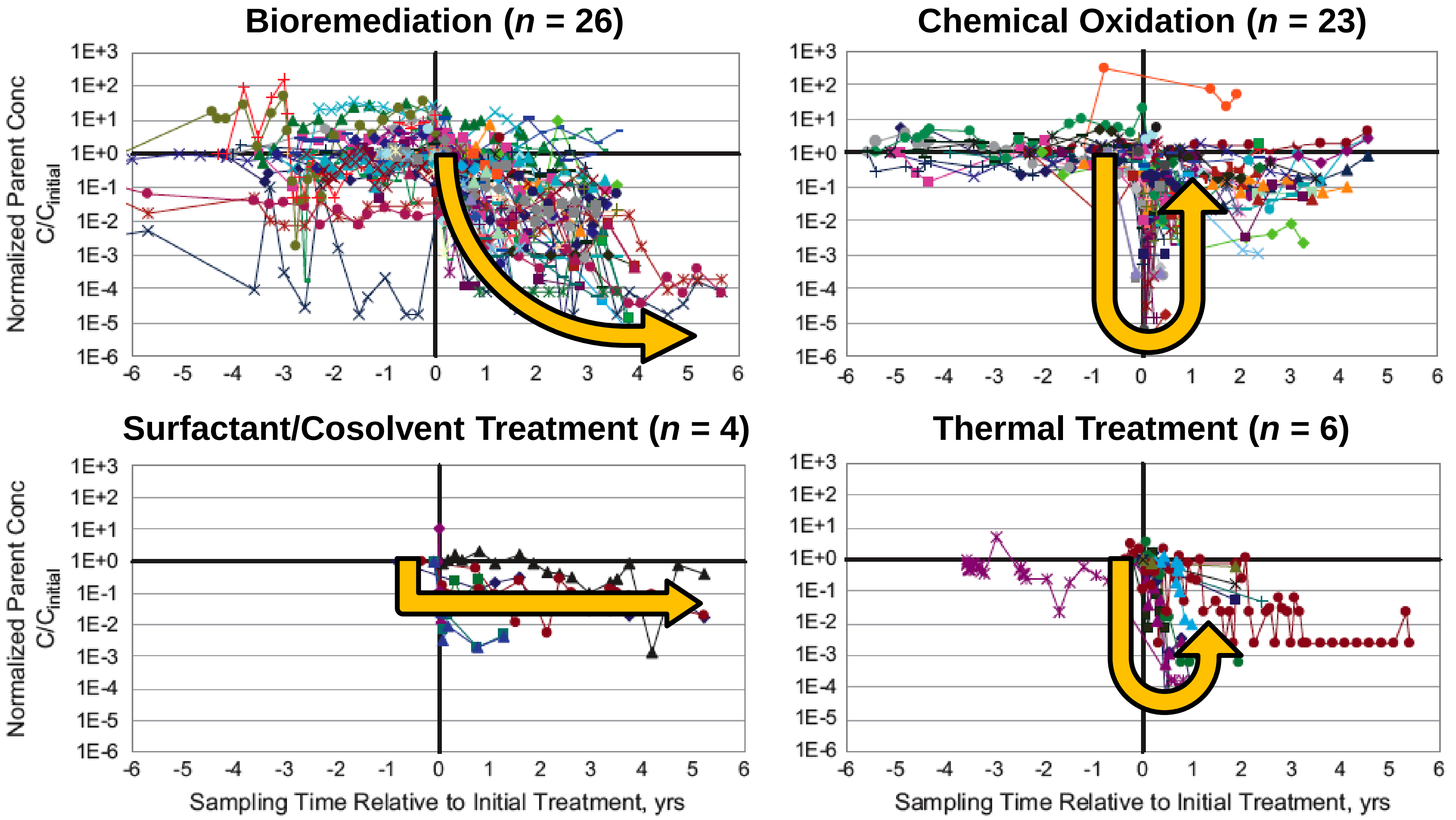
- *Passive Samplers for Vapor and Pore Water*

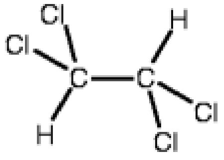
WATERLOO MEMBRANE SAMPLER

SP3

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







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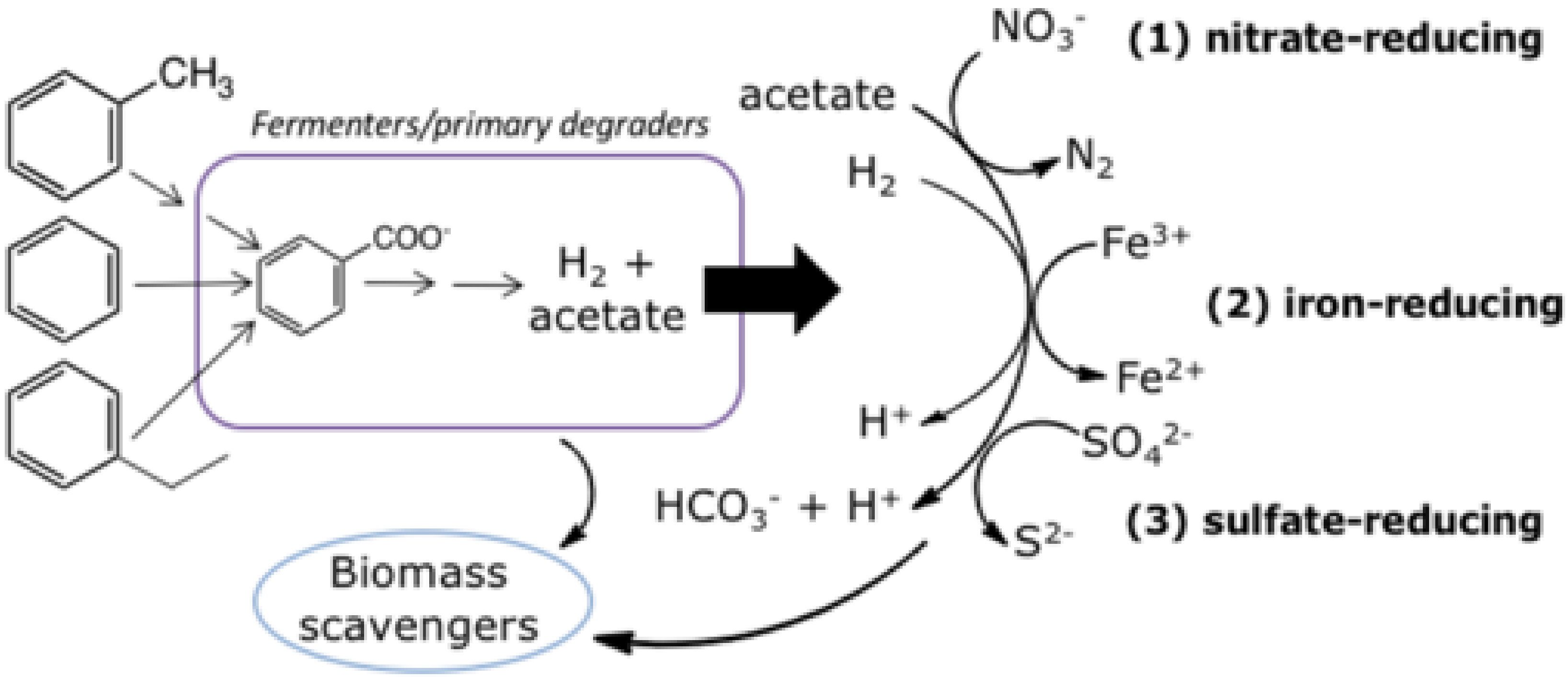
Why Go Anaerobic for BTEX?

- 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

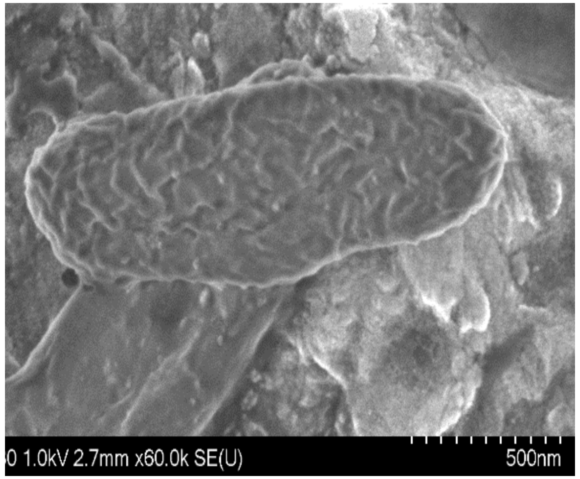
Hydrocarbons are *electron donors* rather than electron acceptors

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



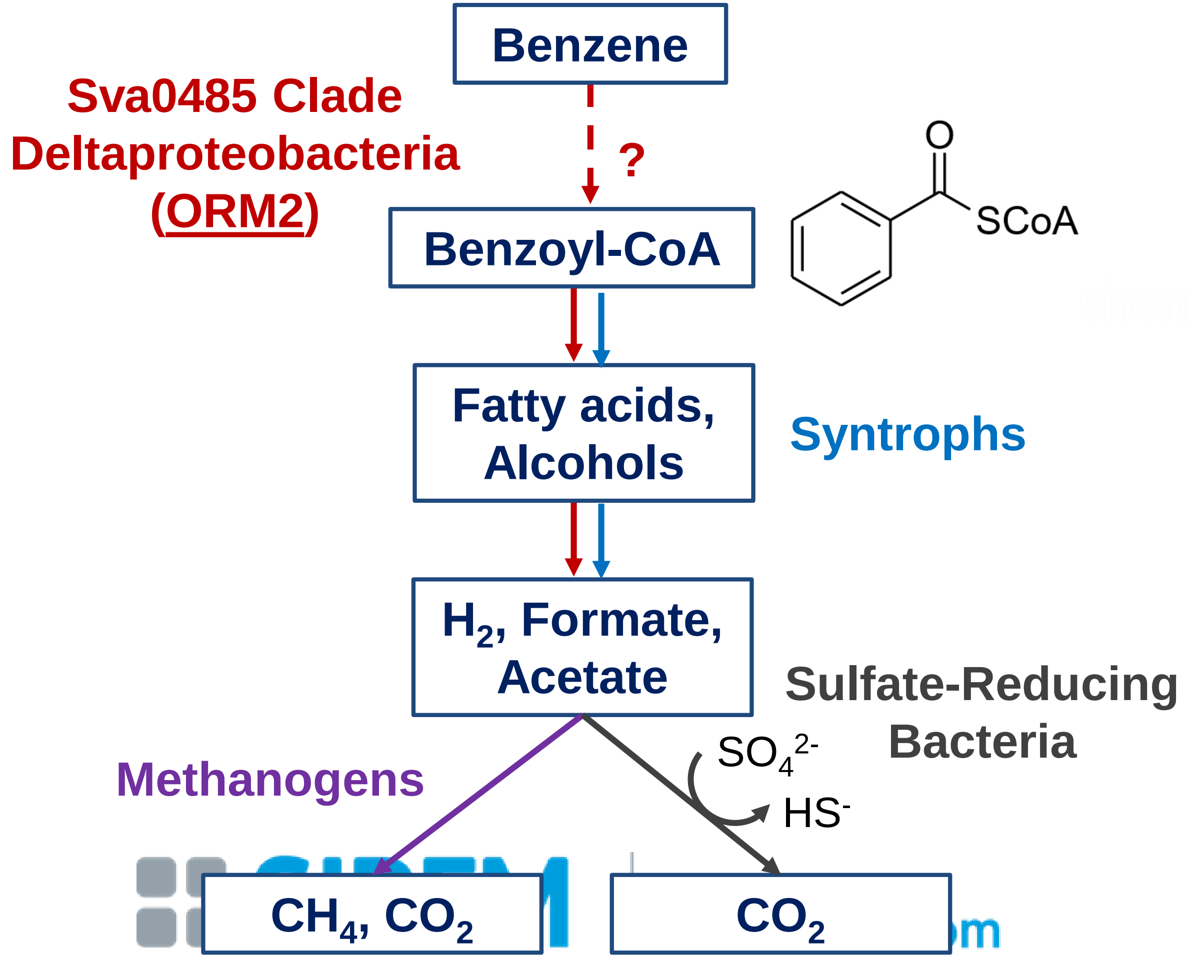
Gieg et al., 2014 (Curr Opin Biotechnol **27**)

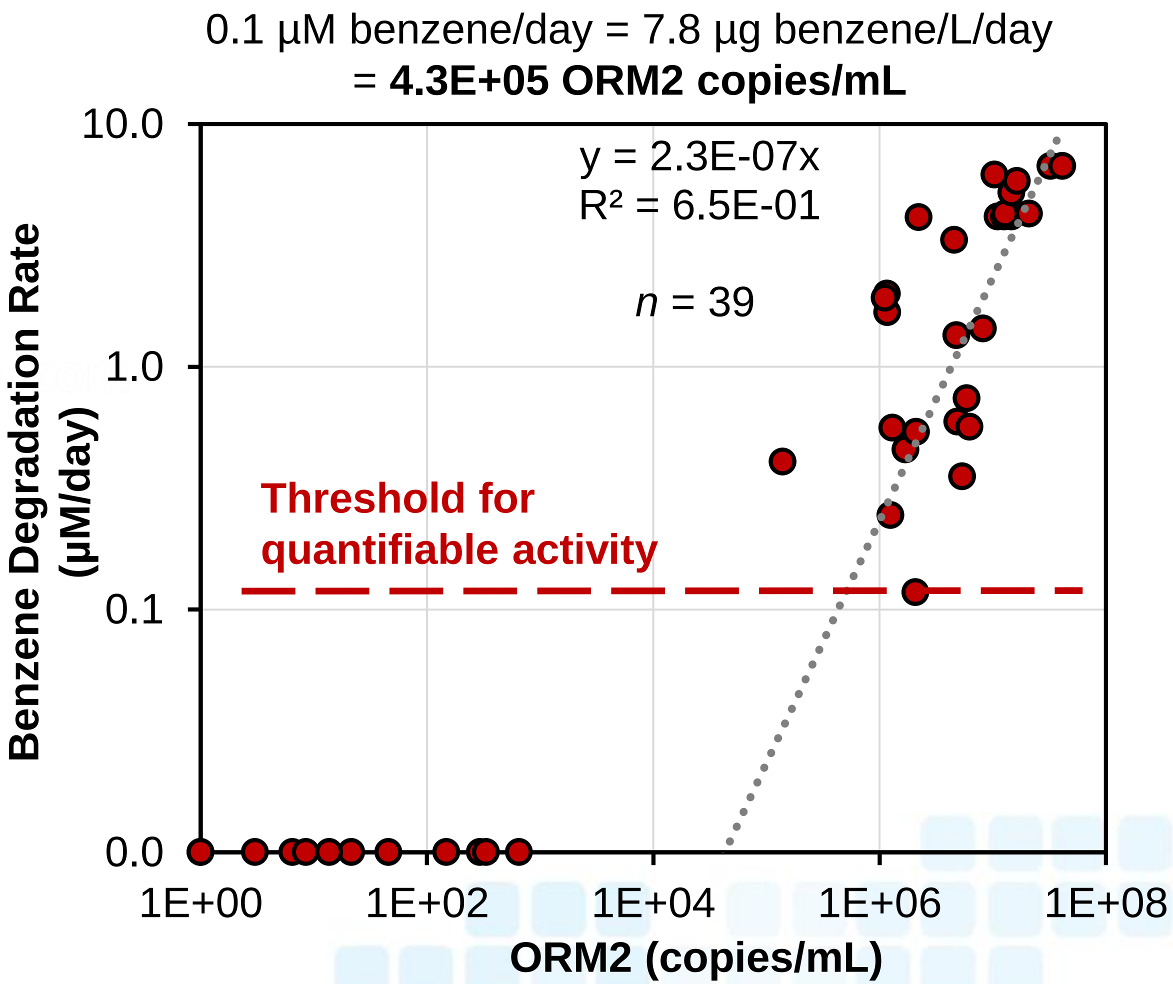
ORM2 Anaerobic Benzene Degrader



- Benzene specialist derived from an oil refinery site in 2003
- ORM2 is a *Deltaproteobacterium*
- Produces enzymes that ferment benzene
- Slower growing ~ 30 day doubling time

Rates of Anaerobic Benzene Degradation is Linked to Concentrations of Key Microbes





Adapted from Toth et al. 2021 (Environ Sci Technol, *in press*)

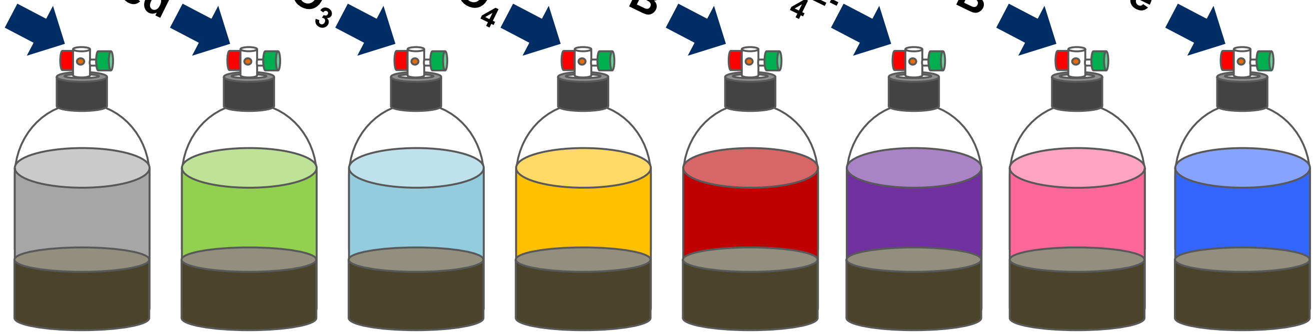
Treatability Study Experimental Setup

BTEX contaminated

Crushed core sample (60 g)

Groundwater sample (200 mL)

Mix in glove box, let stand 1 – 4 weeks

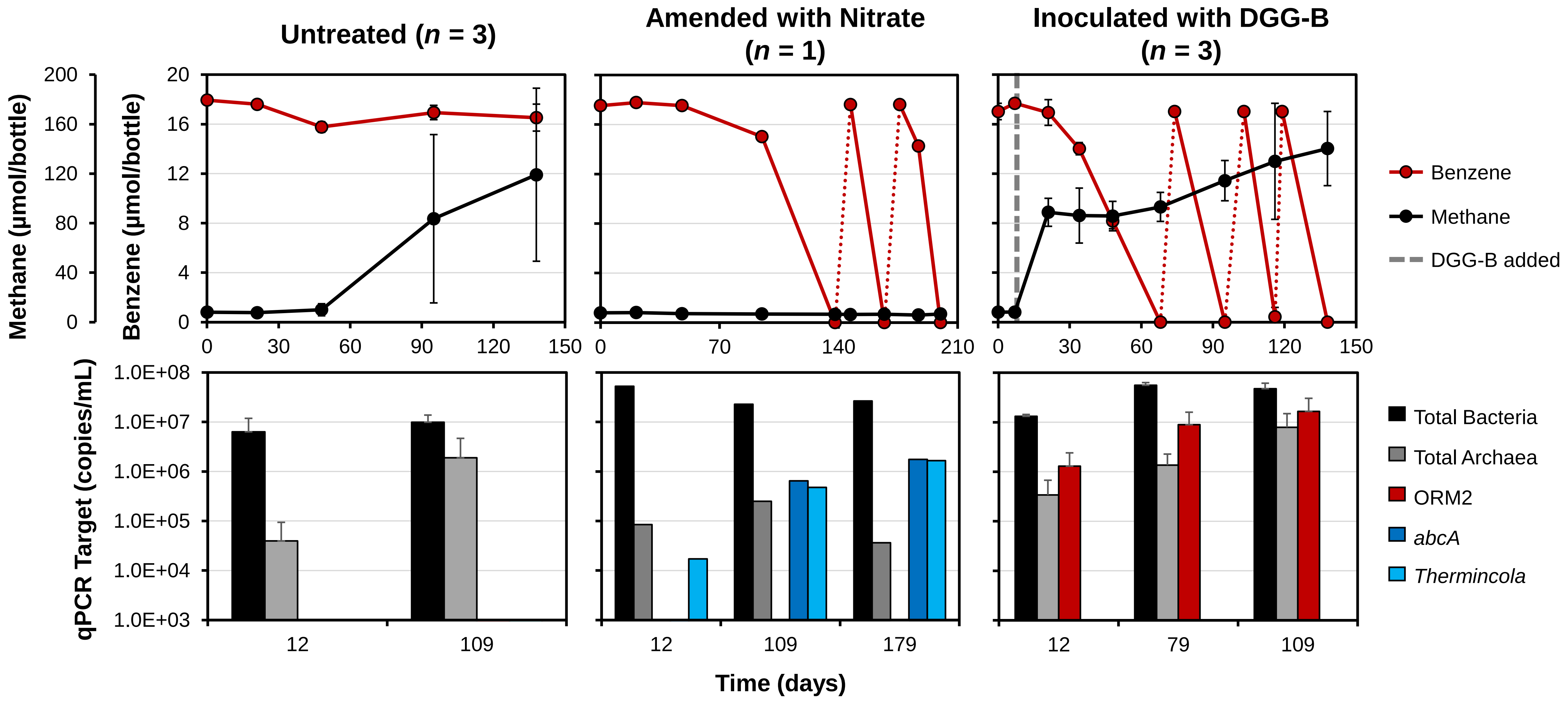


Incubate and monitor over 1-2 years

230 mL groundwater slurries
20 mL headspace (10% CO_2 / 90% N_2)

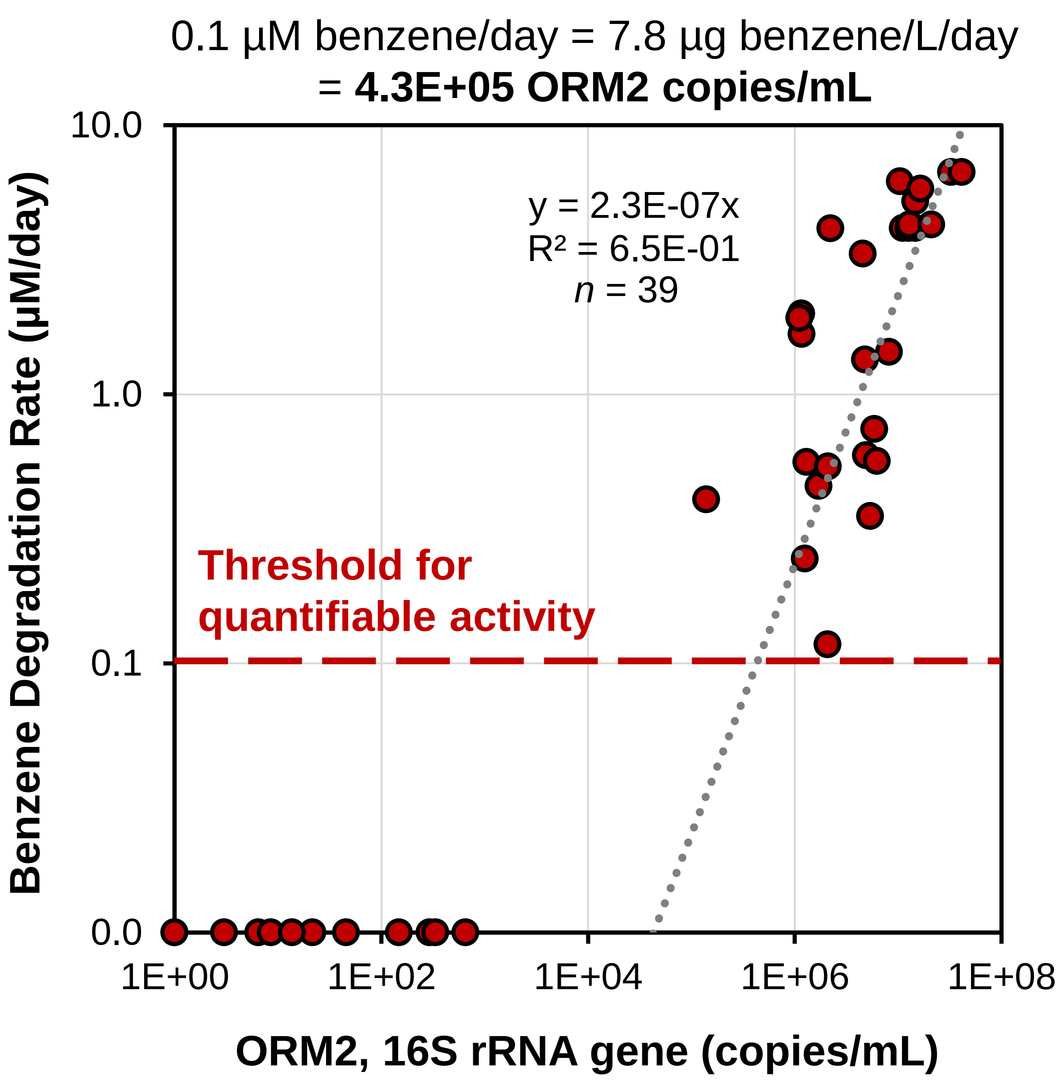
Aqueous BTEX concentrations depend on site materials

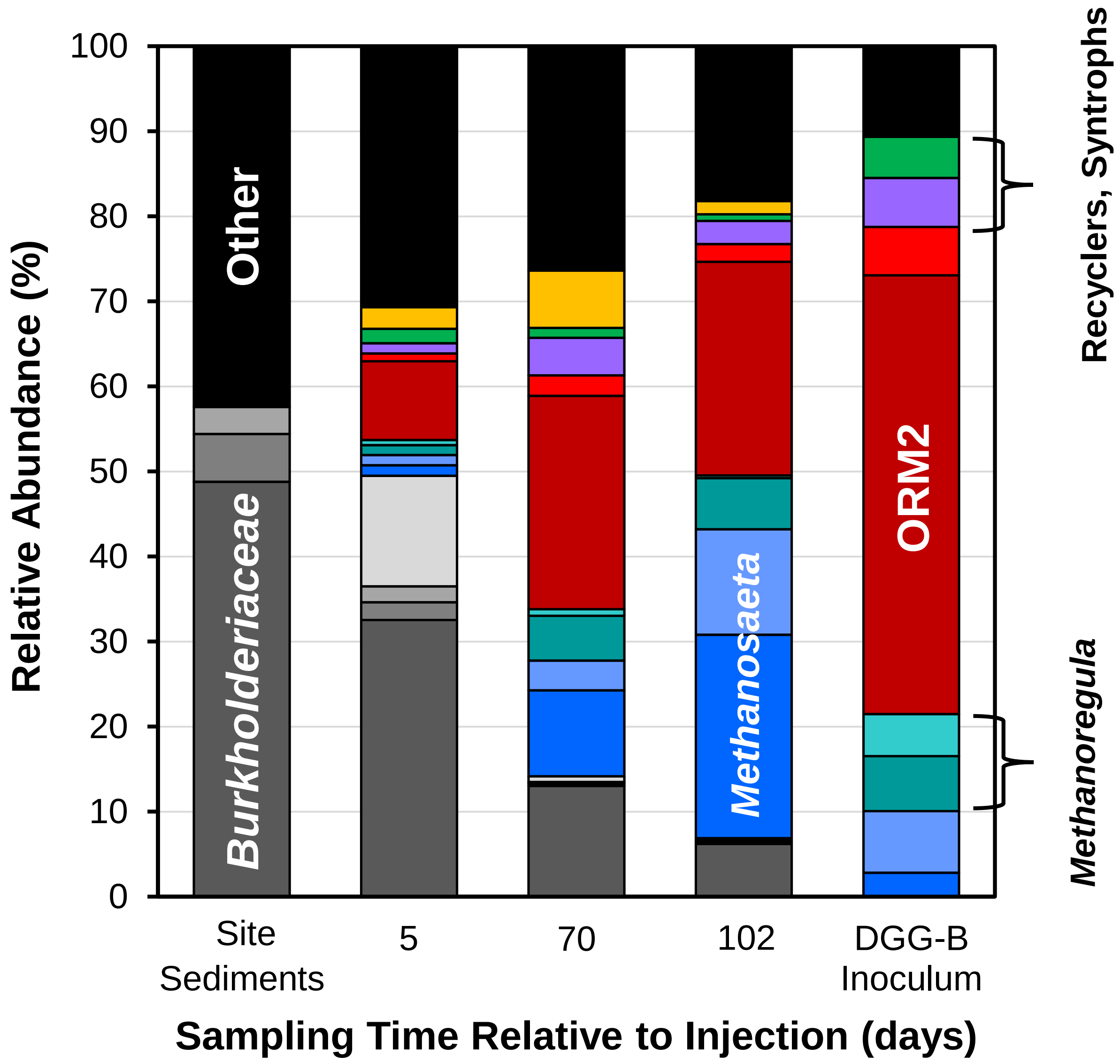
Treatability Test Results



Toth et al. 2021 (Environ Sci Technol 55)

DGG-B Genomic Monitoring Tools



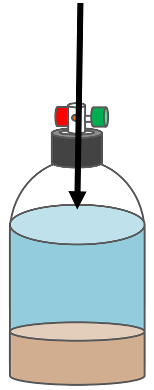


Toth et al. 2021 (Environ Sci Technol 55)

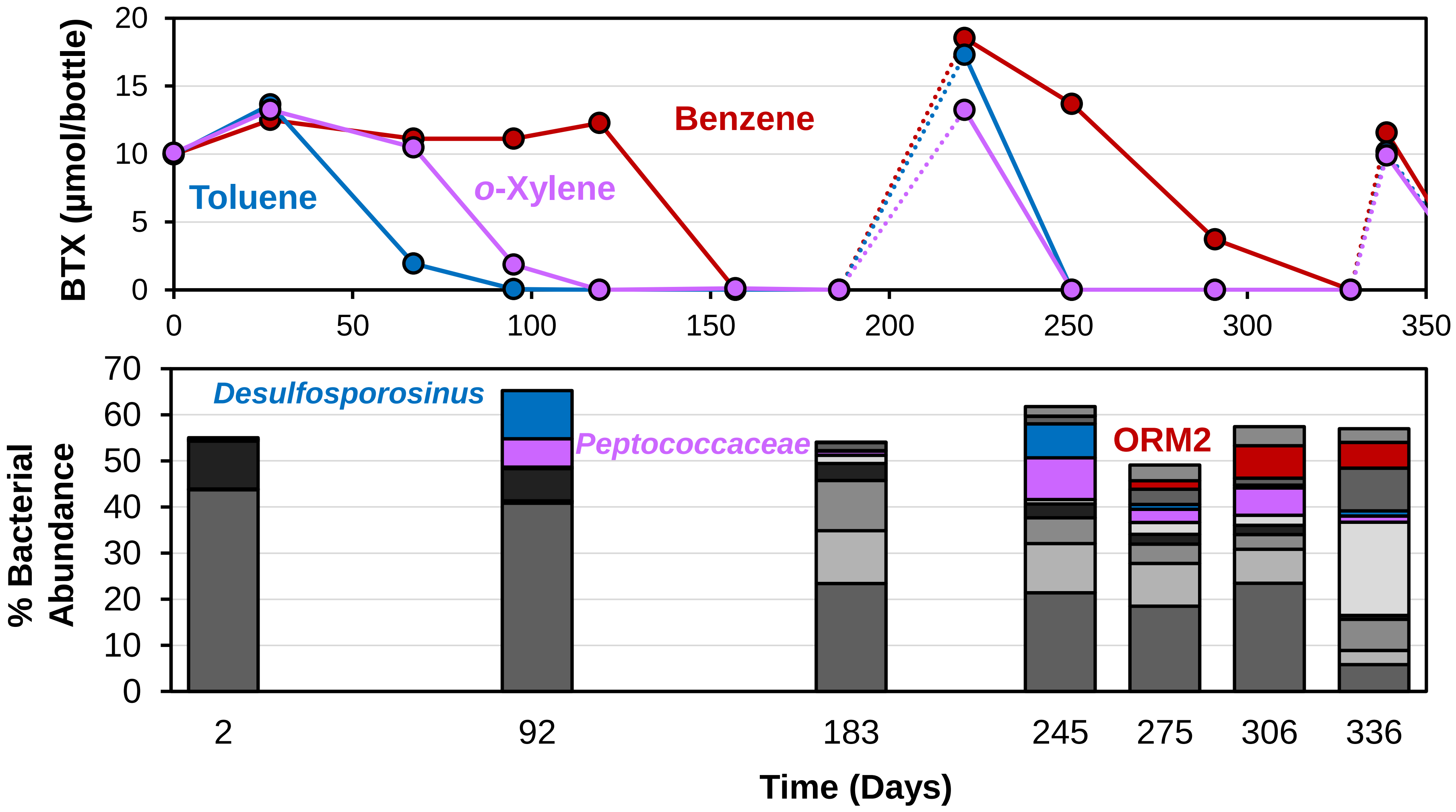
Concentrations of Key Microbes May Also Control Rates of Anaerobic TEX Biodegradation

Additional testing and qPCR analyses are underway

DGG B™
DGG T™
DGG X™



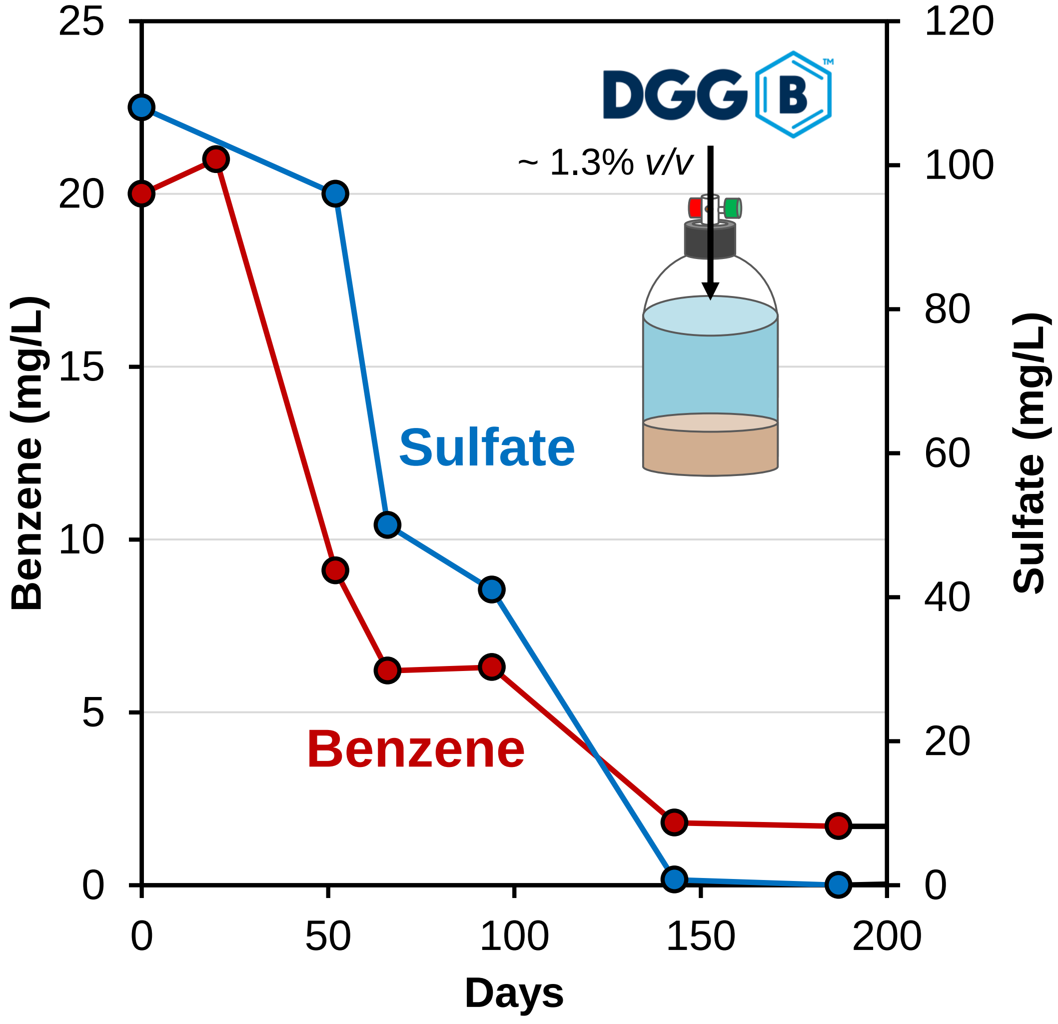
BTX-contaminated sediments and GW (~ 6 mg/L each)



Treatability Testing with DGG-B

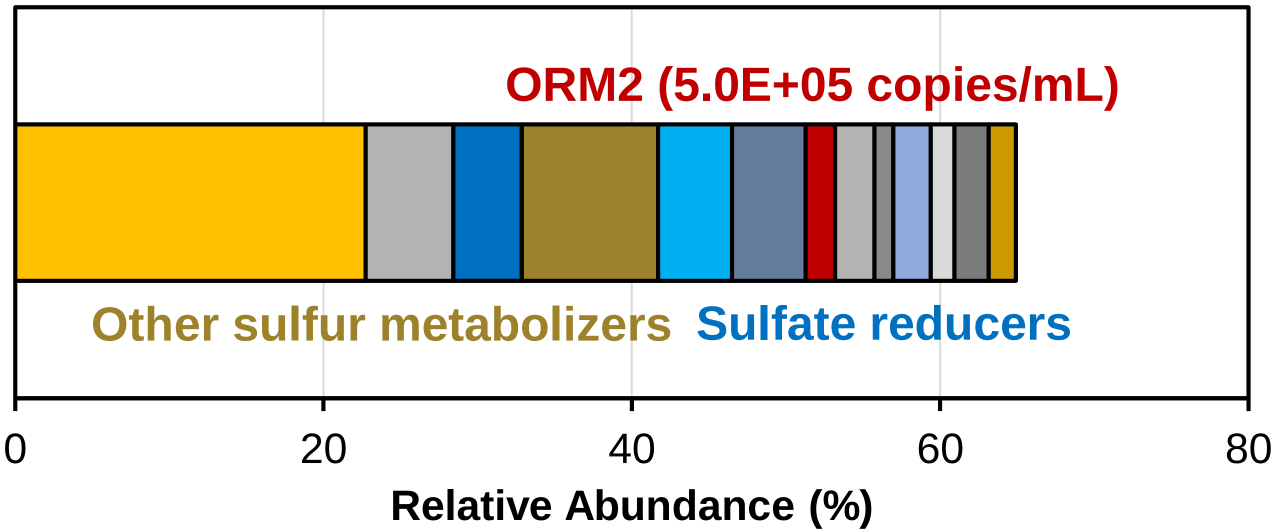
Benzene metabolism coupled to sulfate reduction was observed in DGG-B inoculated bottles

$$C_6H_6 + 3.75\ SO_4^{2-} + 3\ H_2O \rightarrow 6HCO_3^- + 3.75\ HS^- + 2.25\ H^+$$



Total Benzene loss (µM)	Sulfate loss (µM)	Sulfate/ Benzene Ratio
234	812	3.5

Microbial Community Composition after 147 days



What Limits BTEX Biodegradation in Groundwater?

<u>Hydrocarbon Properties?</u> ✖

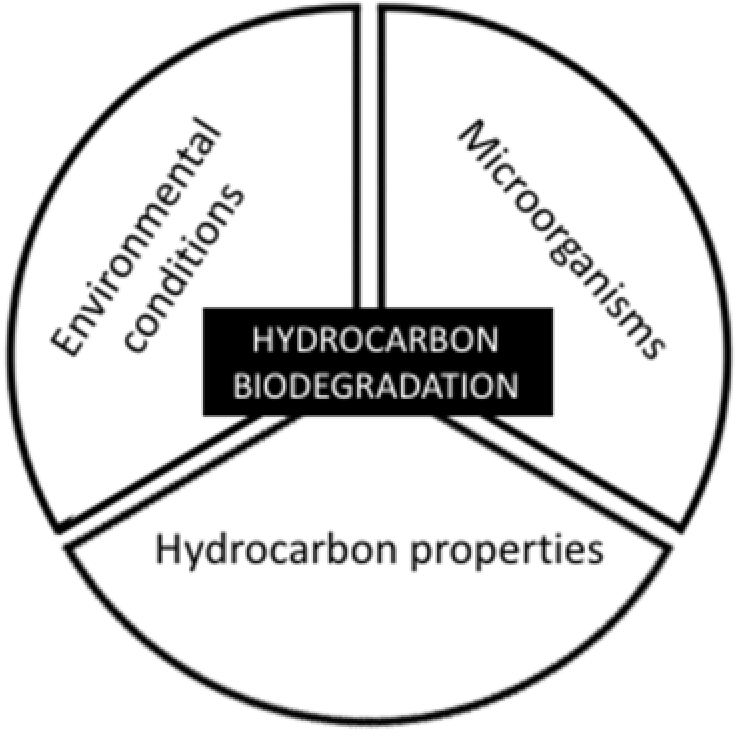
- BTEX is susceptible to biodegradation

<u>Environmental Conditions?</u> ***unlikely***

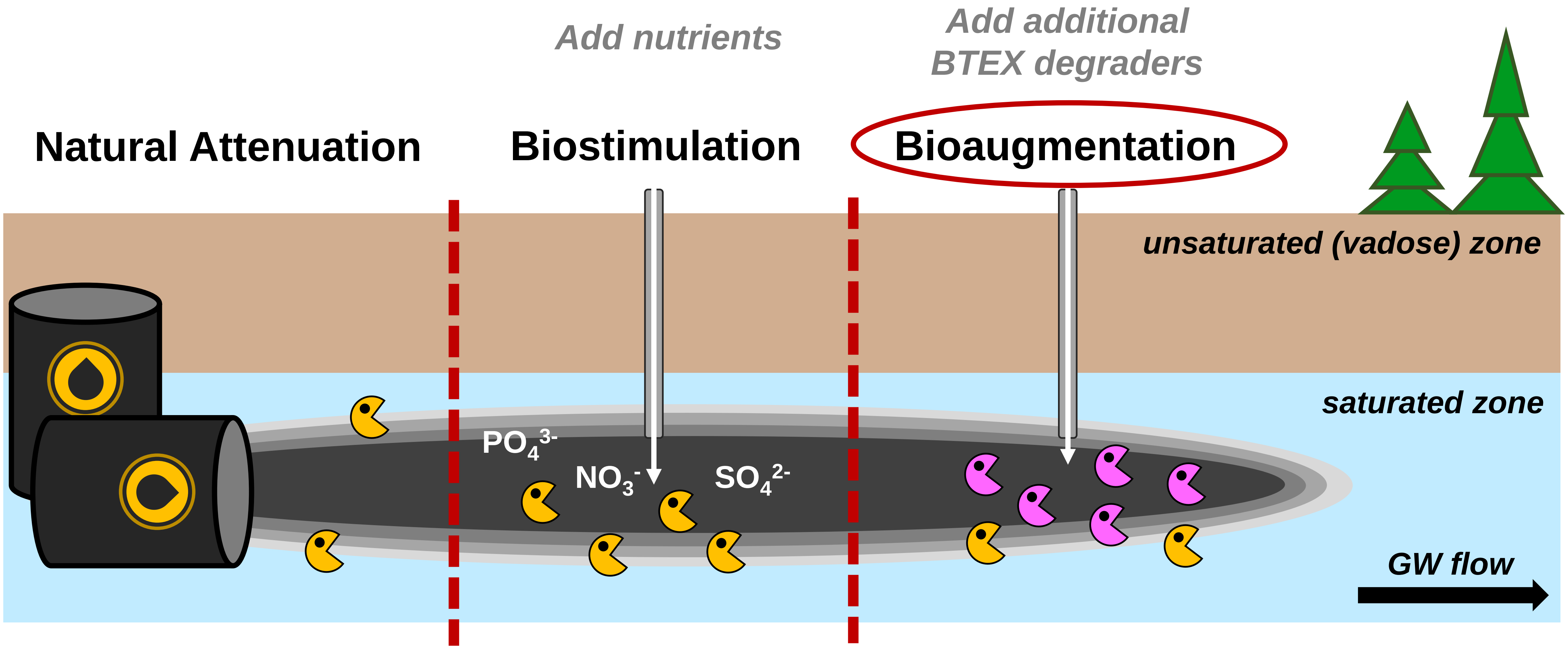
- Biodegradation occurs under all major electron-accepting conditions (O_2, Fe^{3+}, NO_3^-, SO_4^{2-}, CO_2)
- Nutrients are recycled over time
- pH, °C, co-contaminants may ↓ degradation rates

<u>Microorganisms?</u> **?**

- BTEX degraders are ubiquitous in nature…
- ***…but they aren't always in sufficient quantities***



How Can We Reliably Increase Concentrations of BTEX Degraders?



Project Goal & Success Criteria

In field trials, demonstrate the efficacy of anaerobic bioaugmentation cultures to treat BTEX-contaminated groundwater

1. Groundwater BTEX concentrations must decrease post-bioaugmentation, relative to untreated (control) wells;
2. BTEX loss/depletion should be sustained over the posttreatment monitoring period (***years!***); and,
3. Enrichment of bioaugmented organisms (ORM2, etc.) should be evident over time.

Field Pilot Site Overview

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

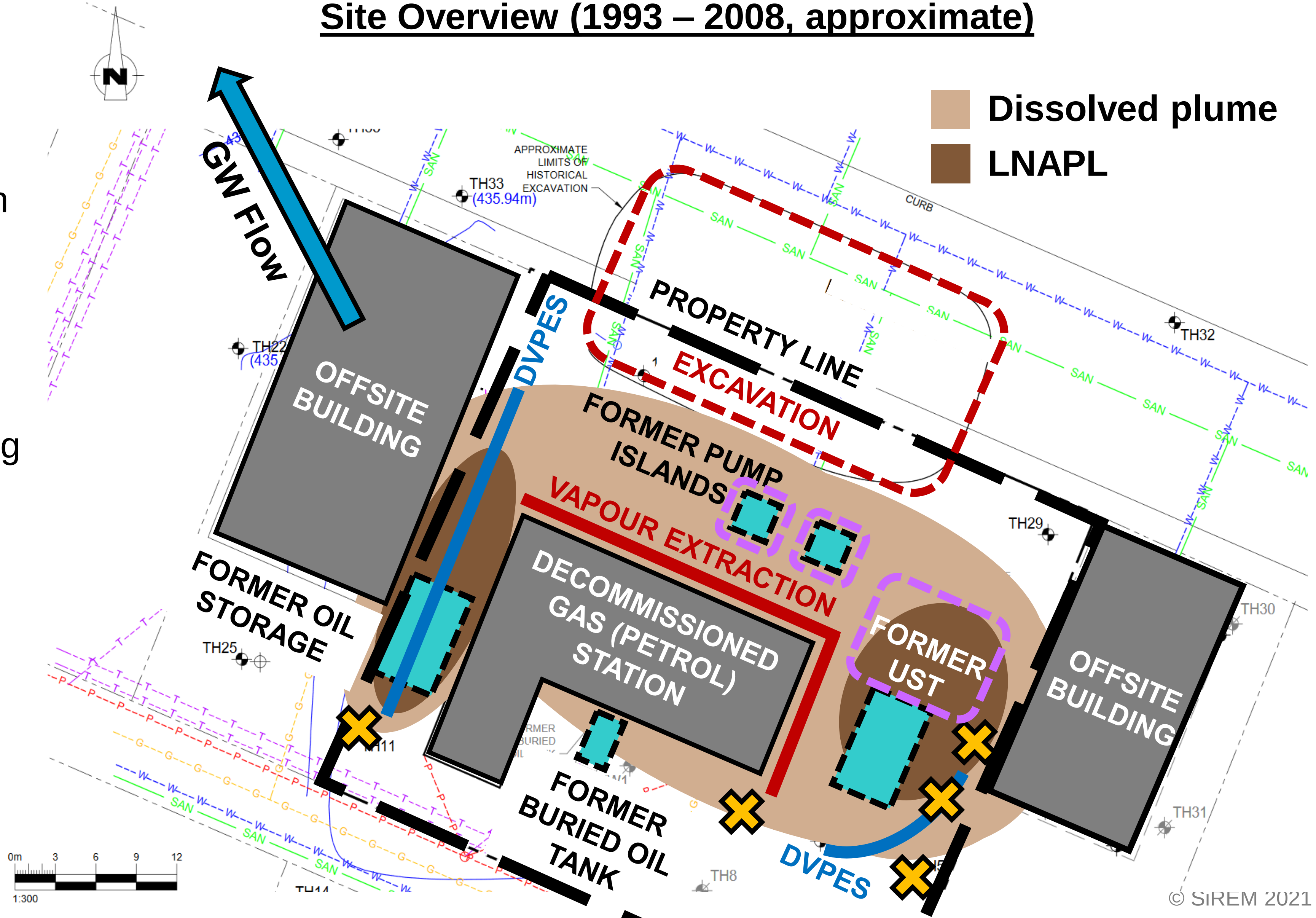
2005-'06: Dual phase vacuum extraction system (DVPES) use

2007-'08: More excavations, purging

2008: Site remediated?

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

Site Overview (1993 – 2008, approximate)



Field Pilot Site Overview

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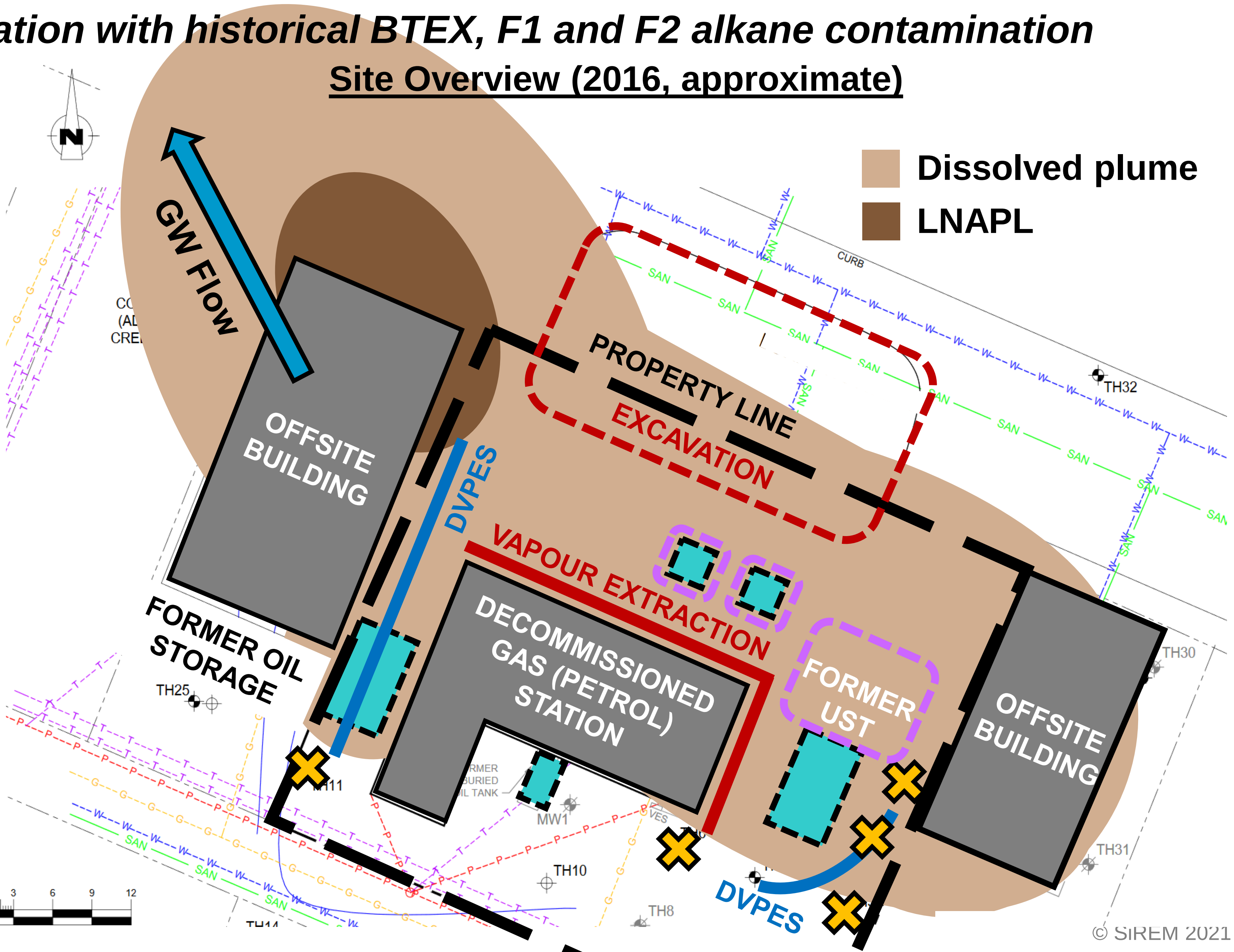
~~**2008**: Site remediated?~~

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

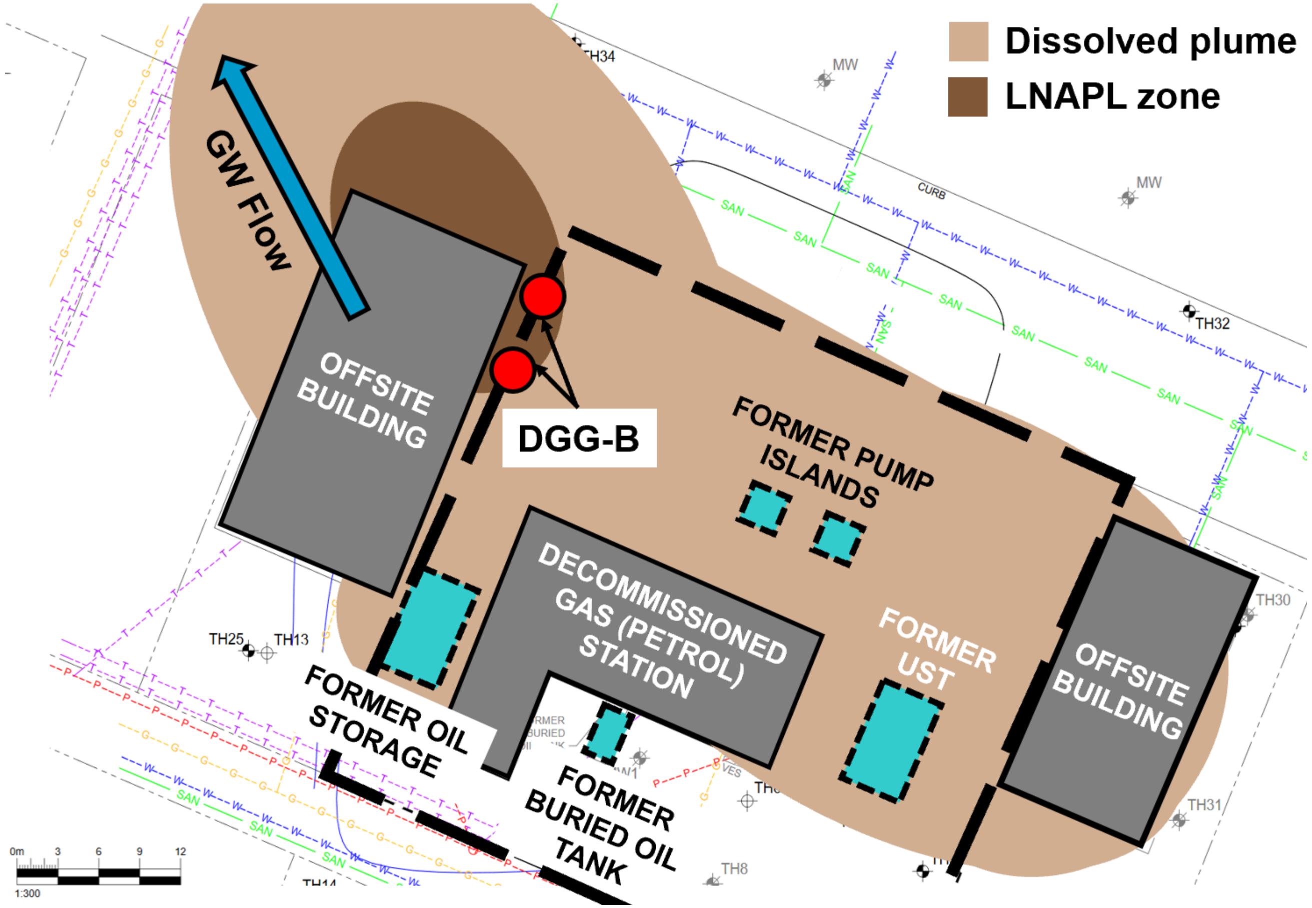
Site Overview (2016, approximate)

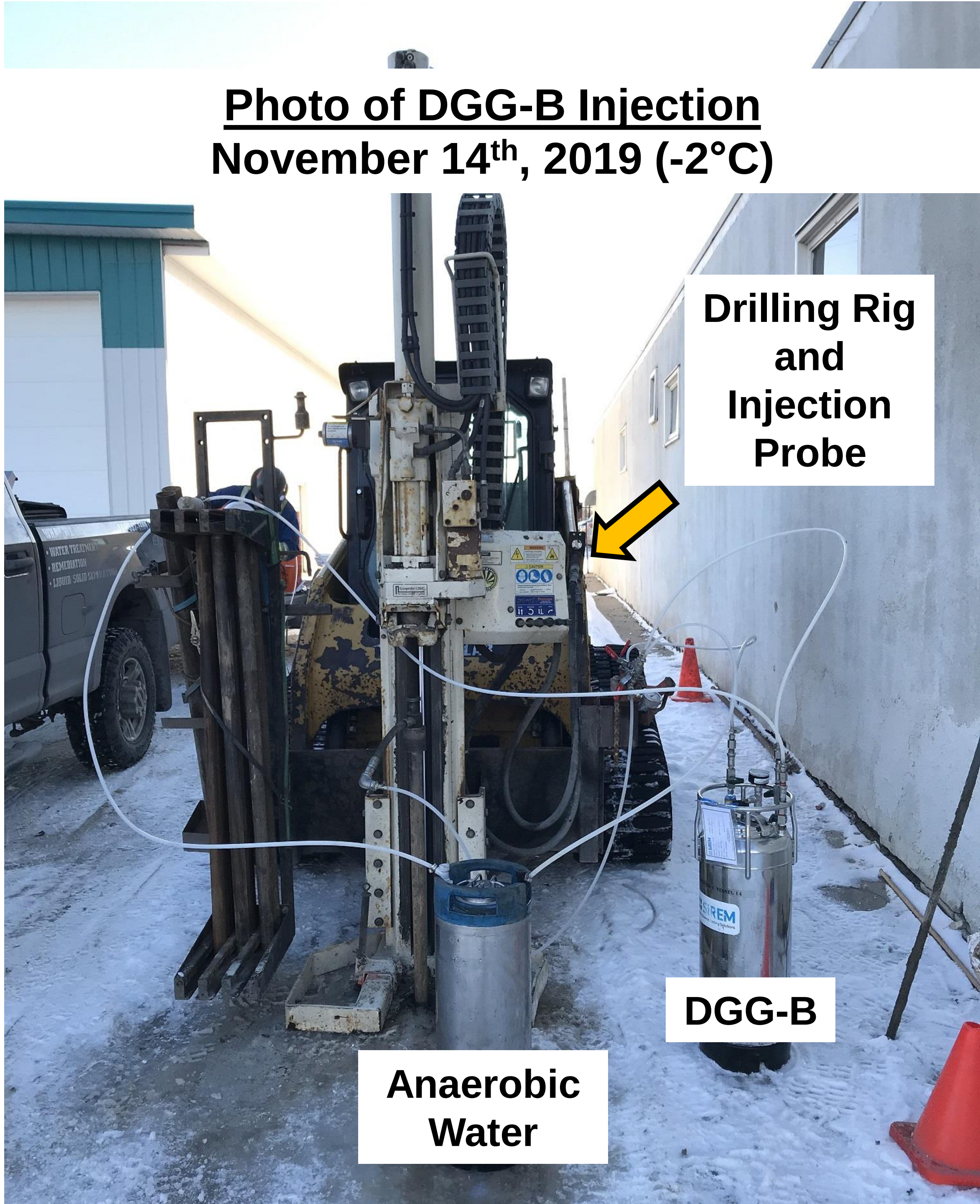


Field Injection Overview

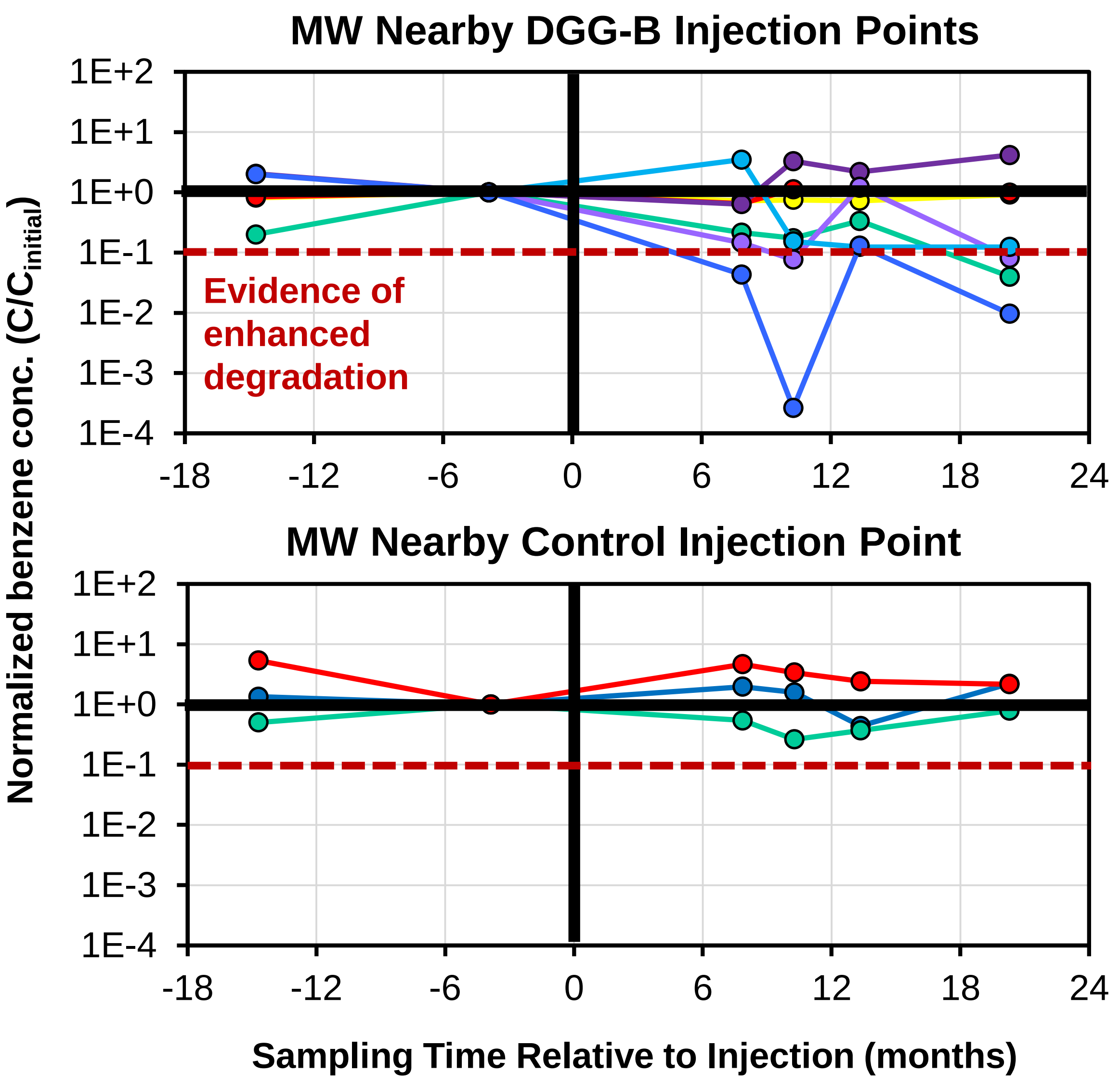
DGG-B was injected at two direct push points (10 L each) in the LNAPL zone 5 m apart

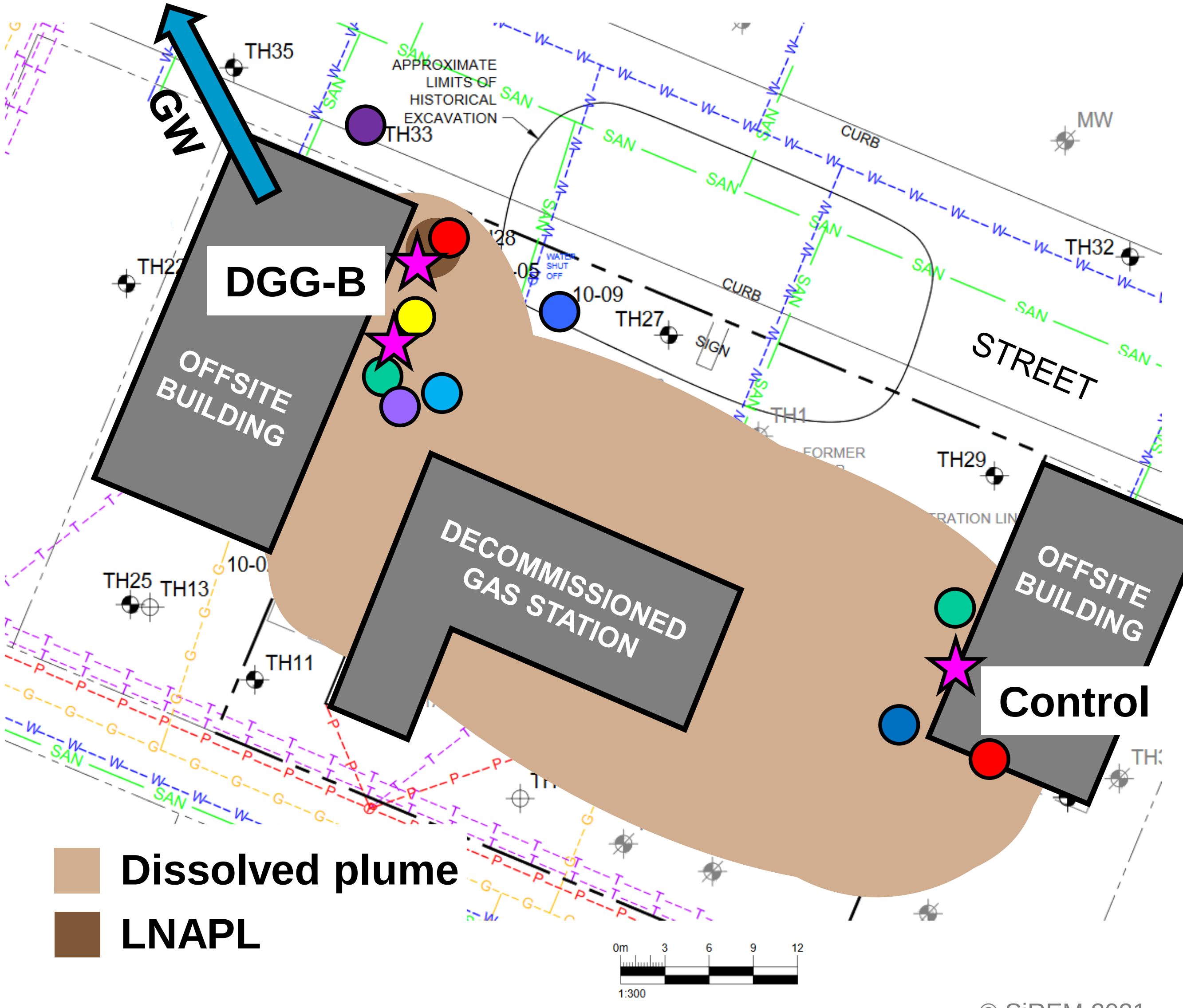
The study was designed to treat 20,000 L of groundwater (~ 1200 ft^3; 34 m^3)





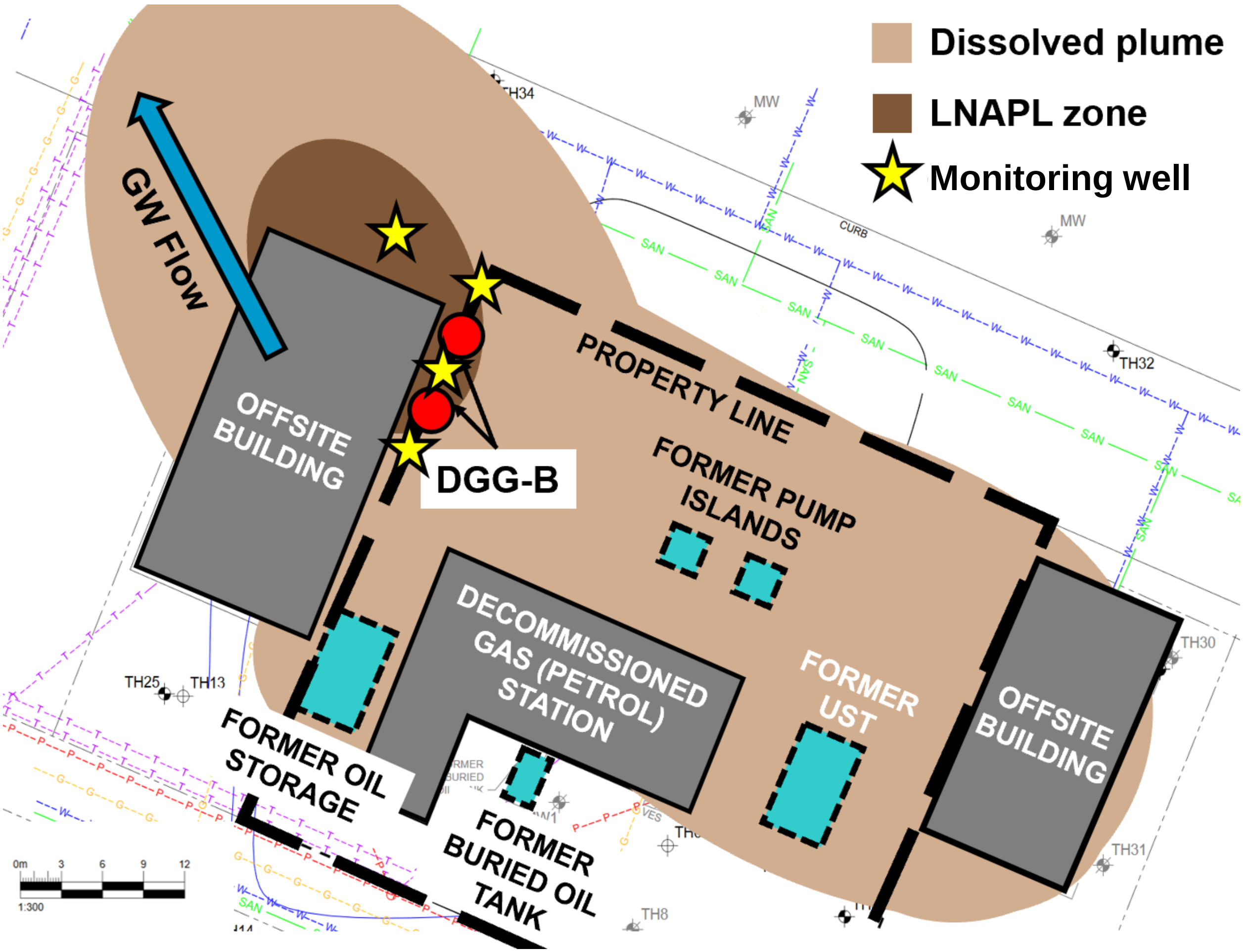
Reduction of Benzene Observed in Monitoring Wells

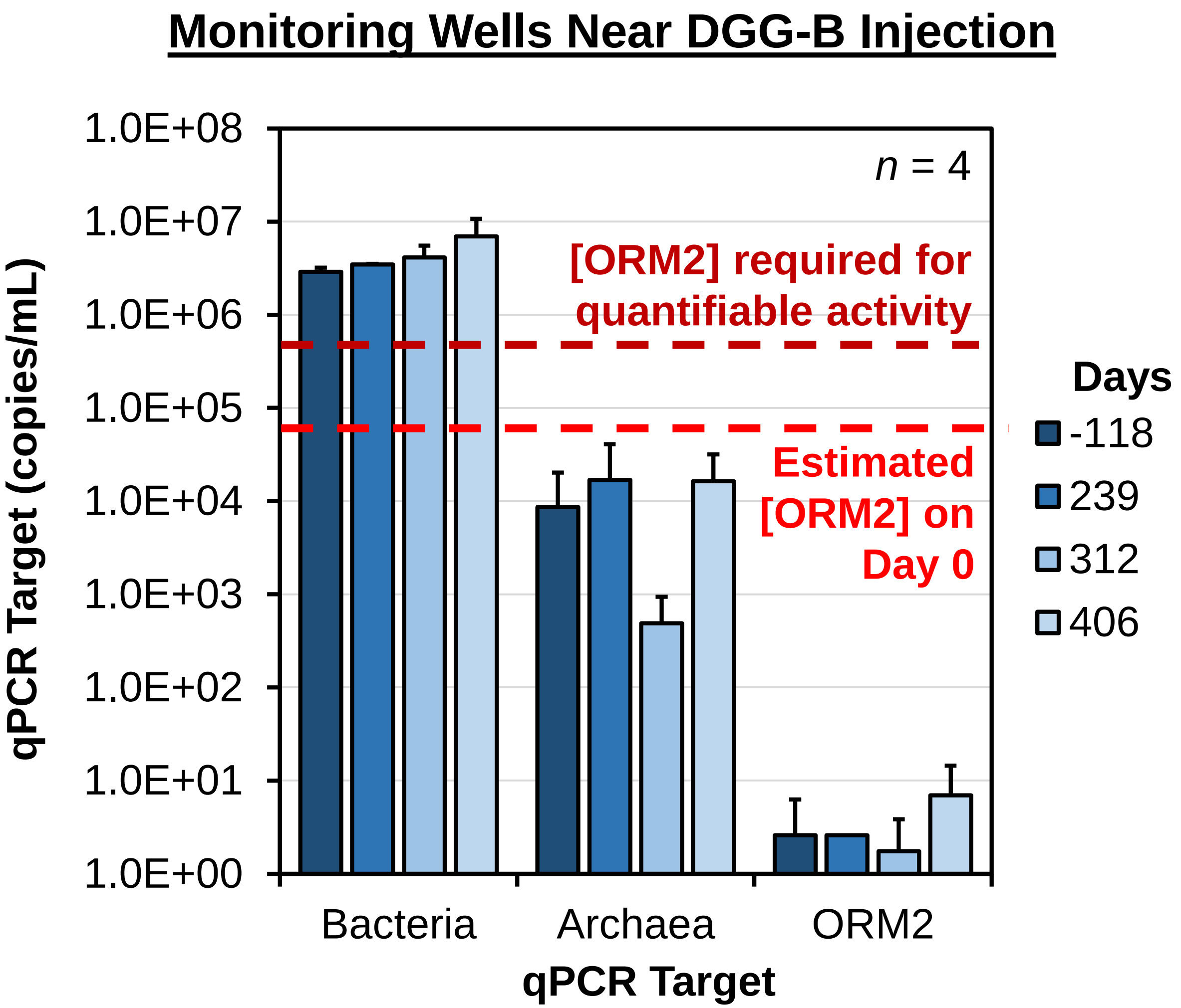




What about ORM2?

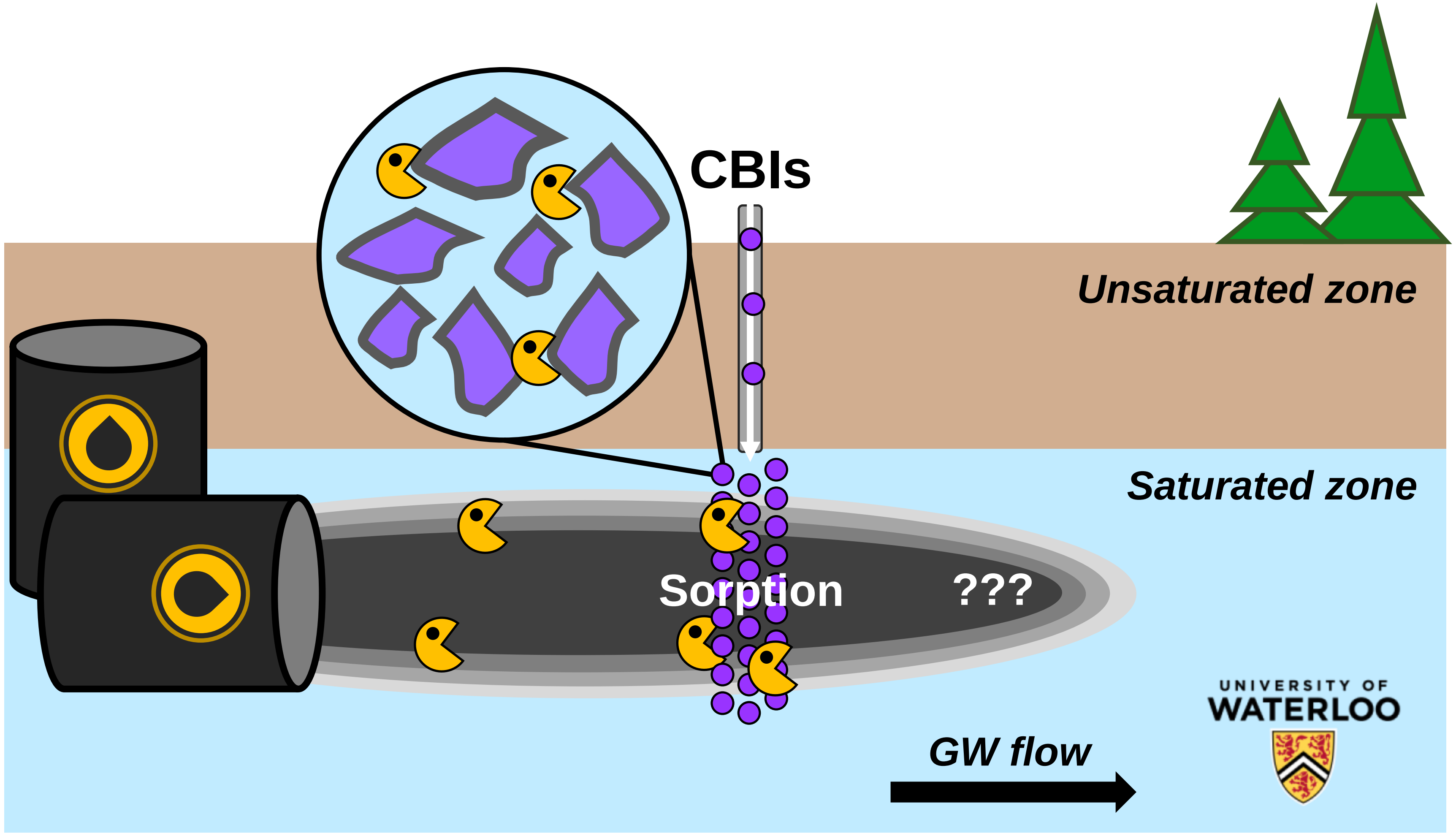
No enrichment of ORM2 has yet to be observed. Perhaps DGG-B did not survive post-injection and/or was poorly dispersed? If cells survived, are they attached to sediments?





Can We Improve Bioaugmentation Success?

Strategy #4) Immobilize BTEX on Carbon-Based Injectates (CBIs), encourage localized growth of anaerobic degraders. This technology could be combined with bioaugmentation



Andrea Marrocco

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Adam Schneider

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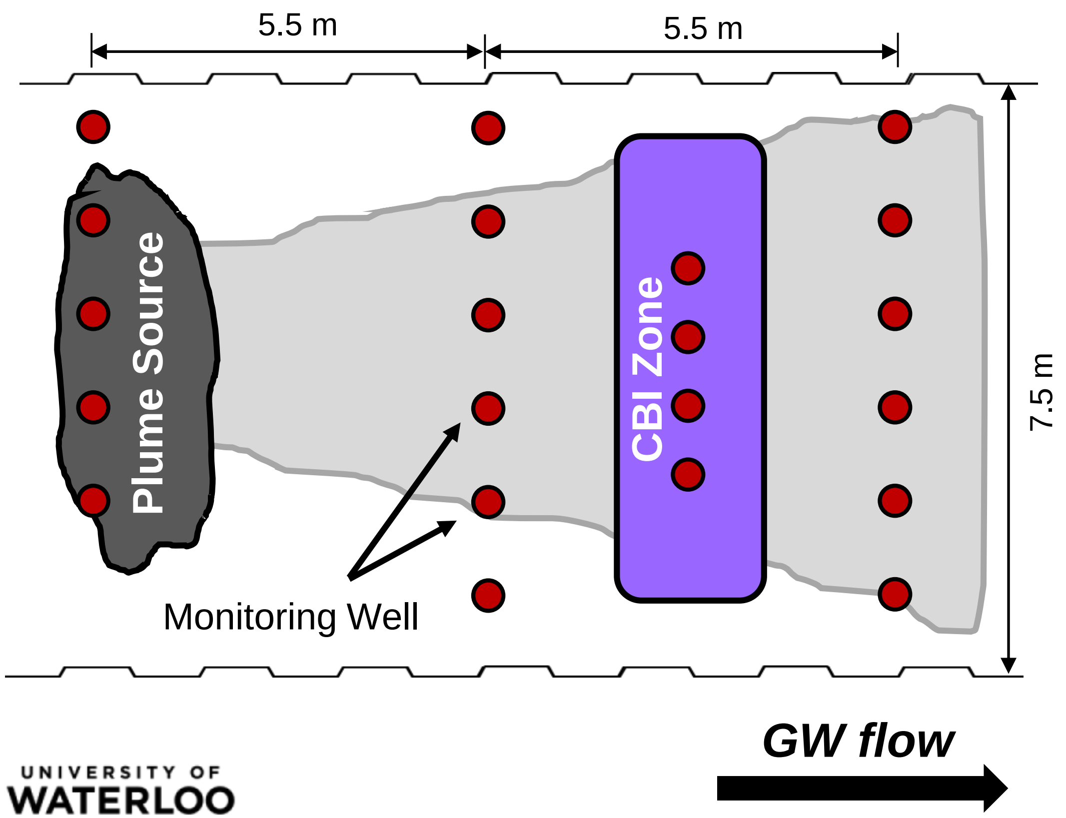
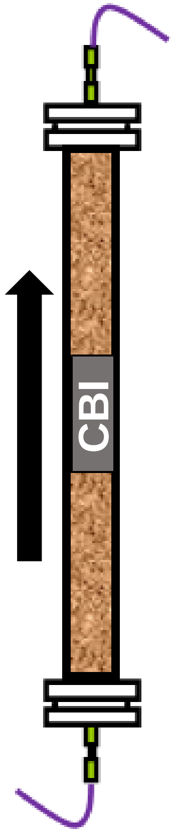
Can We Improve Bioaugmentation Success?

Strategy #4) Immobilize BTEX on Carbon-Based Injectates (CBIs), encourage localized growth of anaerobic degraders. This technology could be combined with bioaugmentation

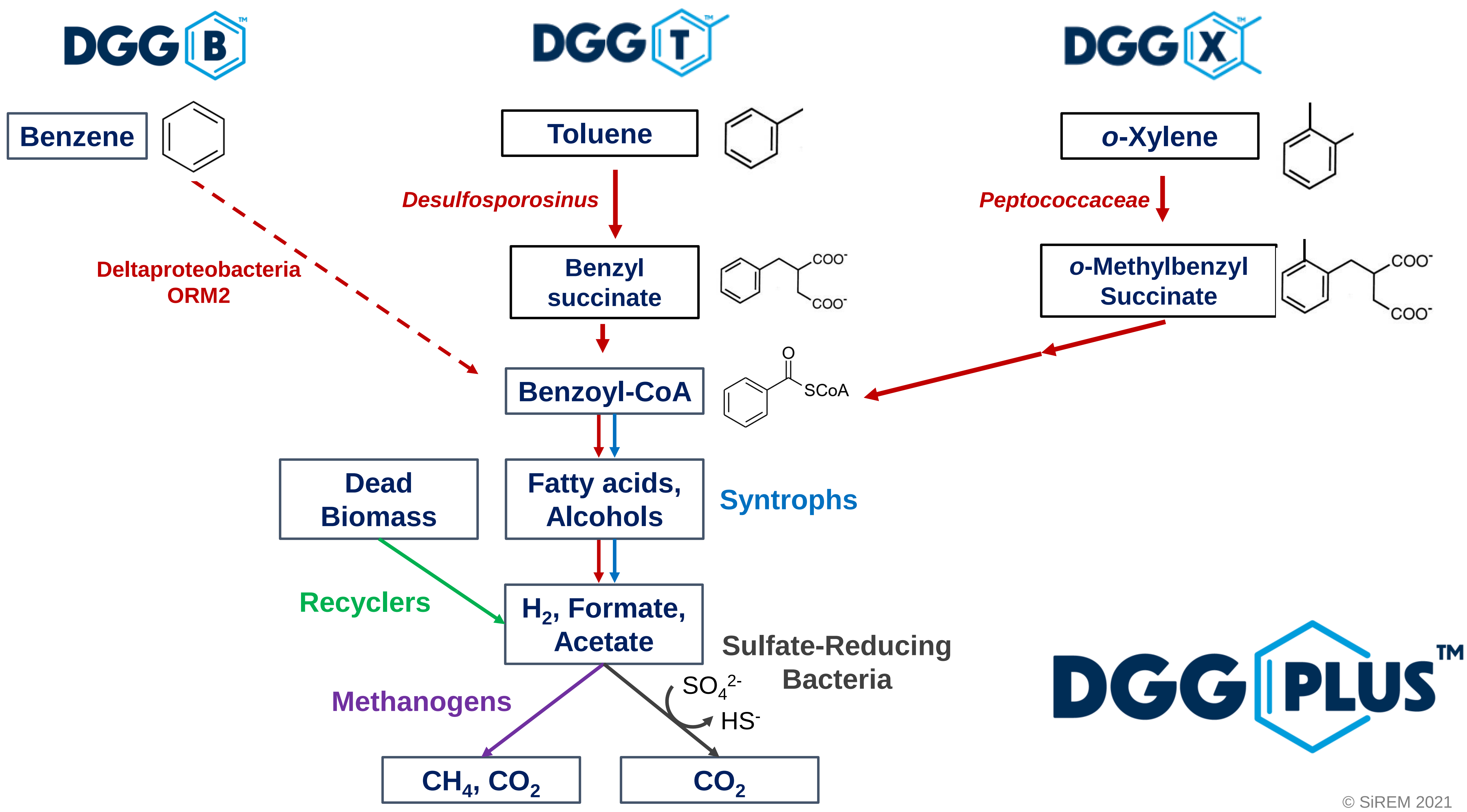
CBI Field Pilot (Underway)



CBI + DGG-B Microcosm & Column Studies (Underway)



UNIVERSITY OF WATERLOO


DGG B
DGG T
DGG X
Benzene
Toluene
o-Xylene
Desulfosporosinus
Peptococcaceae
Benzyl succinate
COO-
COO-
o-Methylbenzyl Succinate
COO-
COO-
Deltaproteobacteria ORM2
Benzoyl-CoA
O
SCoA
Dead Biomass
Fatty acids, Alcohols
Syntrophs
Recyclers
H2, Formate, Acetate
Sulfate-Reducing Bacteria
SO4 2-
HS-
Methanogens
CH4, CO2
CO2
DGG PLUS
TM
© SiREM 2021

Take Home Messages

1. Anaerobic BTEX biodegradation requires high concentrations of active, specialized microbes;
   - 4.3 x 10^5 ORM2 copies/mL required for benzene

2. These microbes have been scaled up to commercial volumes for field use

3. Our data supports that bioaugmentation can be used to treat BTEX in anaerobic systems;

4. Patience is a virtue

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DGG B

DGG-B Anaerobic Benzene Bioaugmentation Culture

Benzene

Benzene is found naturally in petroleum, and is used extensively in the synthesis of a wide range of materials and chemicals. It is also a frequent groundwater contaminant that is highly toxic and carcinogenic and thus a top priority for remediation efforts.

- Benzene is a specific problem – as it is more toxic and less biodegradable than other TEX (toluene, ethylbenzene and xylenes) compounds (particularly under anaerobic conditions)
- Benzene is also produced from the dechlorination of pesticides (lindane) and chlorobenzenes
- Under anaerobic conditions, **benzene is a substantial bottleneck** to achieving site cleanup goals.

Aerobic benzene biodegradation occurs readily; however, injection of sufficient oxygen into the subsurface is often prohibitively expensive. **Anaerobic microbial transformation processes offer an attractive remediation alternative** and have been successfully applied to chlorinated solvents. Why not benzene and other monoaromatic hydrocarbon compounds?

Closely related compounds, like toluene, ethyl benzene and xylenes, are also readily degraded anaerobically and the microbes that biodegrade these compounds are widely distributed.

The DGG-B benzene-degrading culture is currently available for testing in laboratory treatability studies and pilot testing.

Introducing DGG-B

At the University of Toronto, Elizabeth Edwards' lab has been studying anaerobic degradation of aromatic compounds for decades. A benzene-utilizing methanogenic culture was derived from an oil refinery site and couples benzene oxidation with methanogenesis. The culture consistently converts benzene to methane and carbon dioxide (CO_2), at a rate between 0.4 to 1.0 milligrams per liter per day (mg/L/day) and has a doubling time of 30 days. The DGG-B culture can promote benzene degradation under methogenic or sulfate reducing conditions. Methanogenic benzene degradation occurs syntrophically by benzene degraders, fermentative bacteria and methanogens. A Deltaproteobacterium, designated ORM2, has been identified as the key benzene degrading organism.

Quantitative PCR (qPCR) tools are available to track the abundance of the primary benzene-degrading strain ORM2

qPCR tools have been developed for potential biomarker genes for anaerobic benzene biodegradation. A variety of biomarkers including Gene-Trac® ORM2 and SRB, can be used to assess the potential benzene degrading populations that may be present in the environment, either naturally occurring or as a result of biostimulation or bioaugmentation. Contact SiREM for the most current list of applicable biomarkers to test.

Additionally if benzene is attenuating under nitrate electron acceptor conditions, SiREM has a Gene-Trac® test to quantify the presence of Peptococcaceae, the microbes that have been associated with this degradation pathway.

Contact SiREM for more information on our anaerobic benzene cultures and treatability testing options.

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Questions

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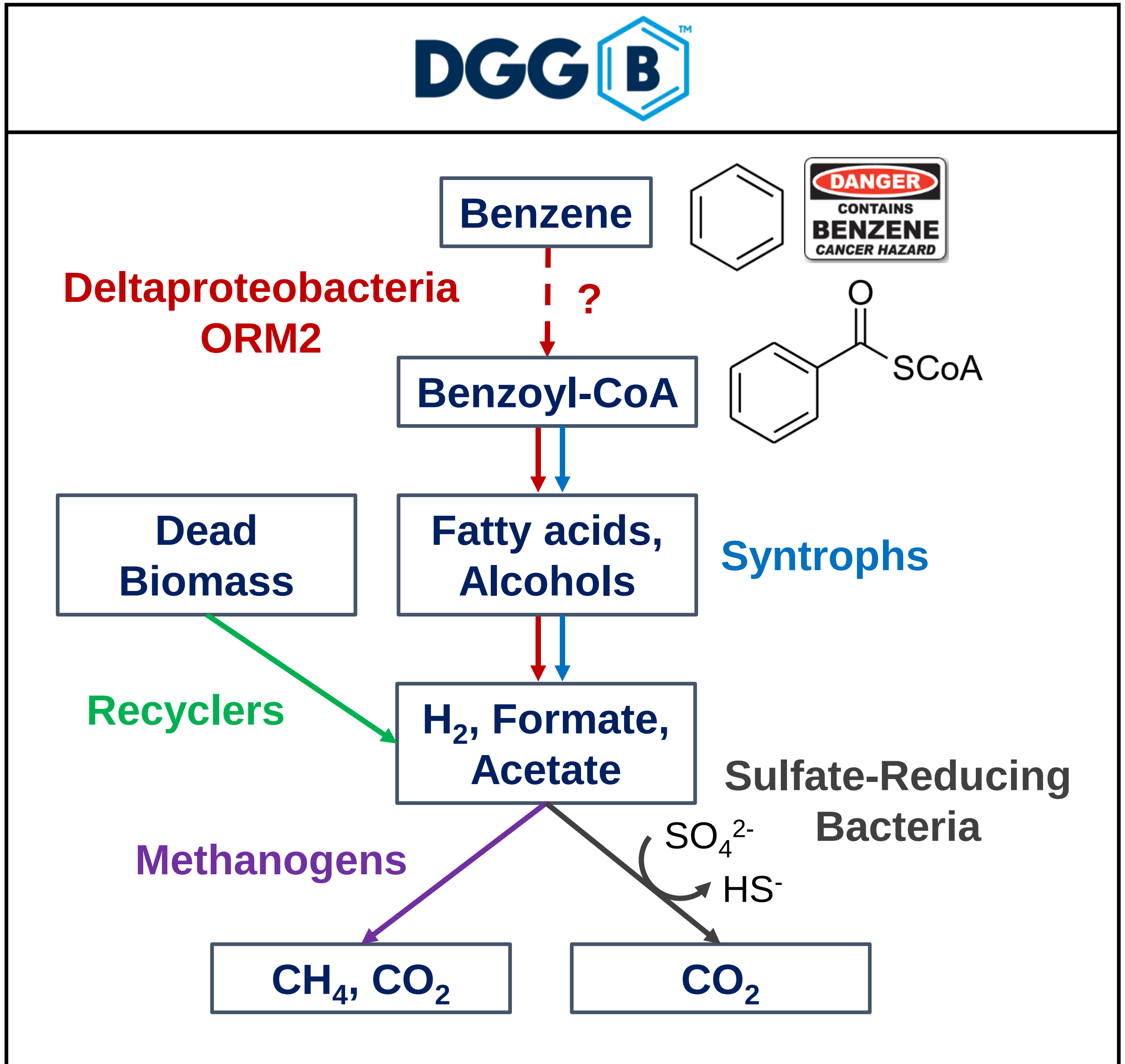
https://www.siremlab.com/advanced-bioaugmentation-cultures/

Objective: evaluate performance of bioaugmentation for anaerobic benzene treatment



Culture Scale-up & Growth

Lab Treatability Testing

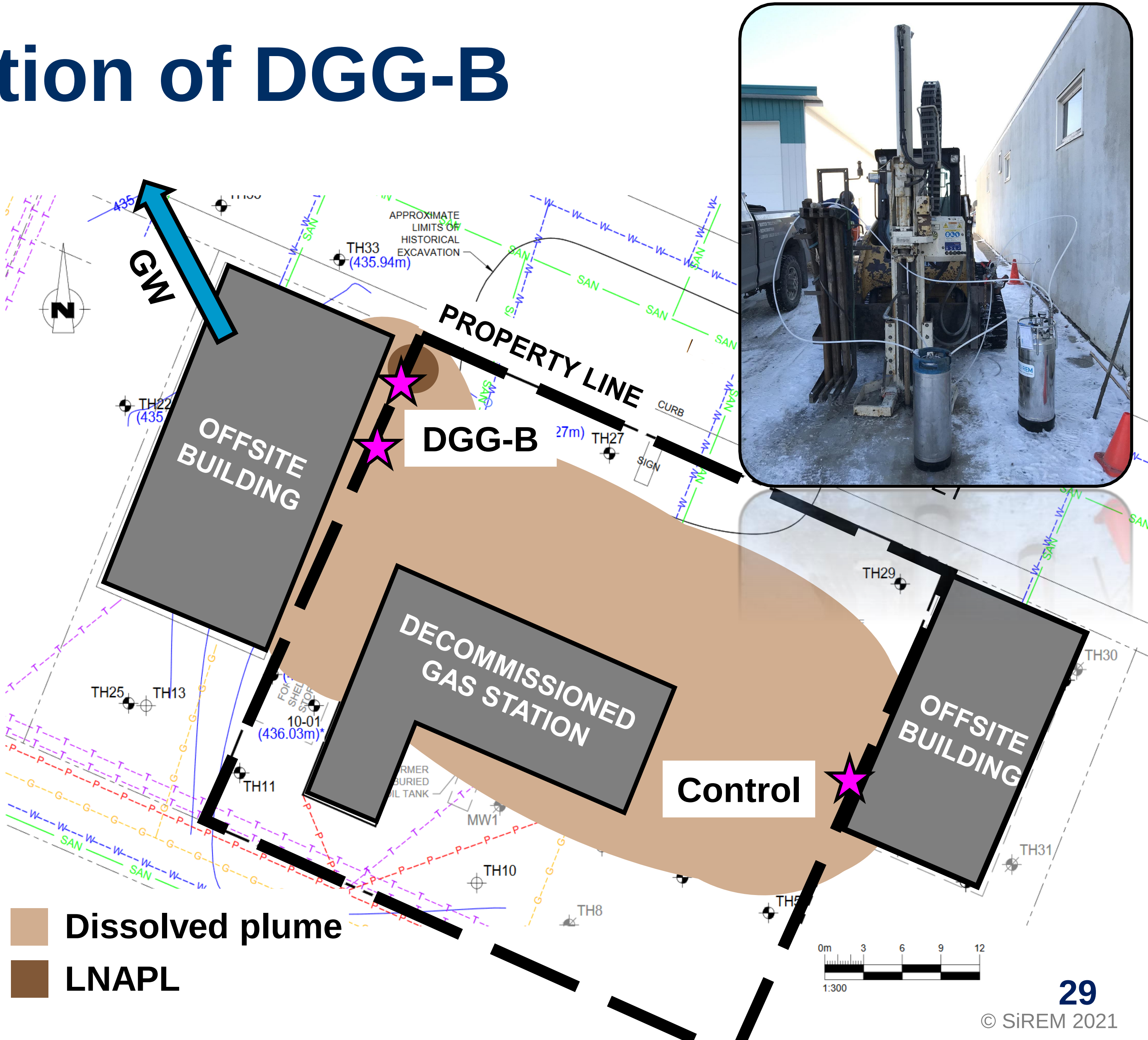
Develop Genomic Monitoring Tools

Regulatory Approval

Field Application of Culture

Field Application of DGG-B

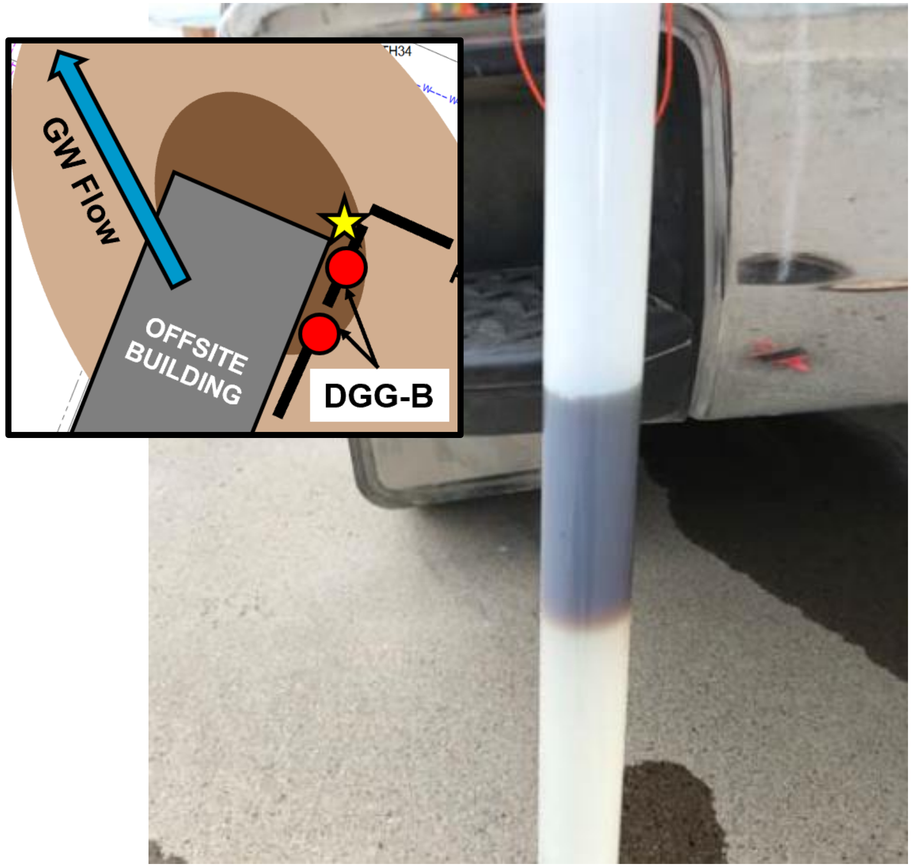
- ➢Decommissioned gasoline (petrol) station with historical BTEX, F1 and F2 contamination
  - Benzene conc. vary (< 0.01 – 20 mg/L)
- ➢DGG-B was injected at 2 points (10 L each) near NW corner of property
- ➢A **control well** injected with heat-killed DGG-B (10 L) was established on E edge of property



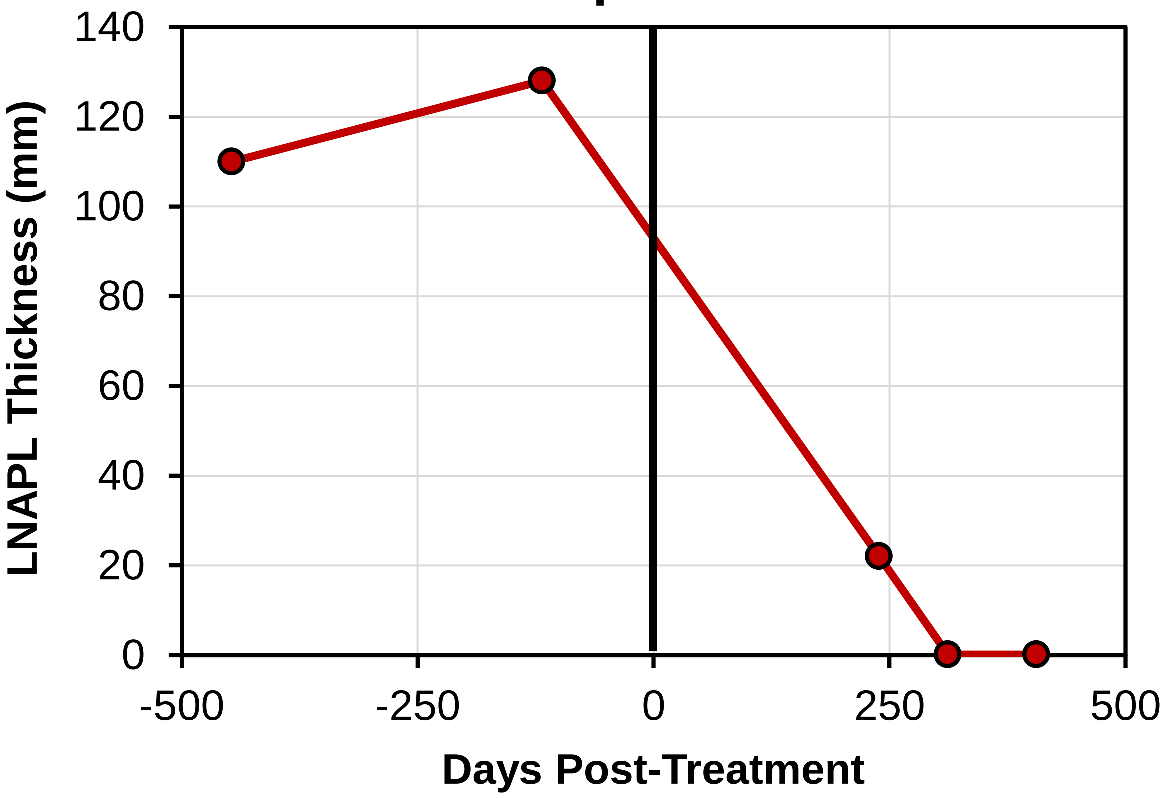
Decrease in LNAPL Thickness?

BTEX biodegradation activity might be masked by LNAPL re-dissolving into the surrounding groundwater. To be verified this year.

Photo of LNAPL layer in Monitoring Well TH28



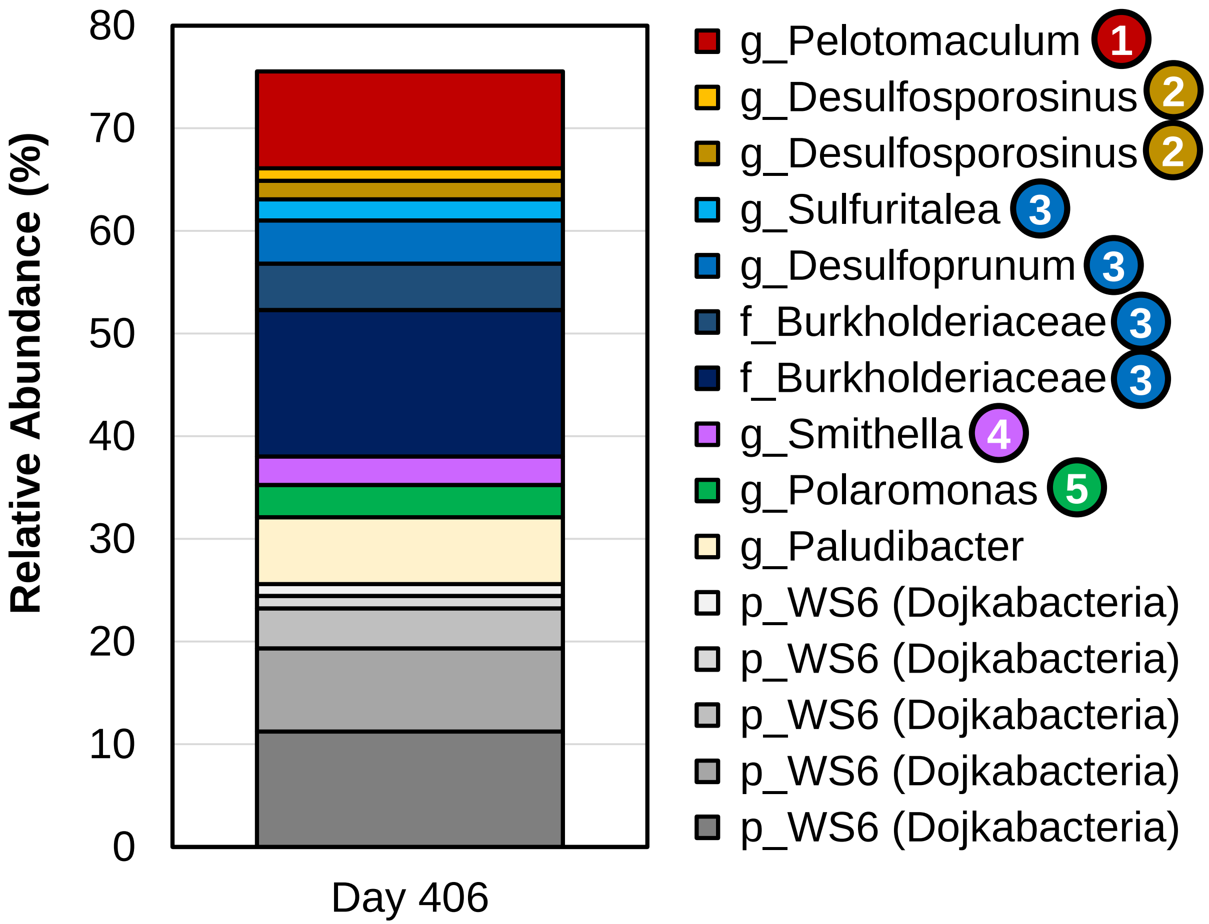
LNAPL Depletion in TH28



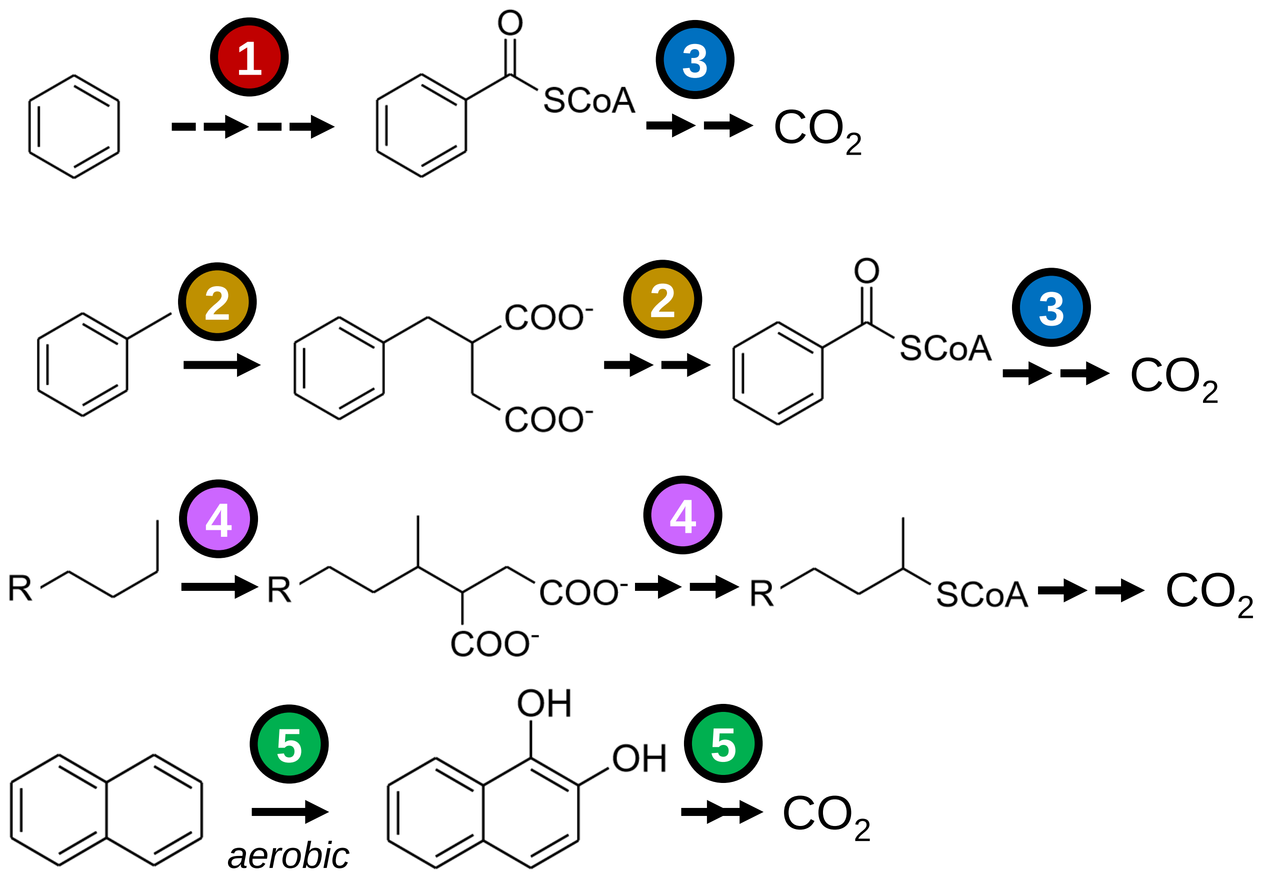
16S rRNA Gene Amplicon Sequencing of TH28

Several putative hydrocarbon degraders were identified, but most appear to be indigenous in origin (i.e., NOT from DGG-B)

Top 15 ASVs



Possible Metabolisms (Based on Taxonomy)



Field Injection Parameters

	Field Pilot (per injection point)	Microcosm Study
Input Parameters		
Concentration of ORM2 in DGG-B Inoculum (**copies/mL**)	5.2E+07	2.7E+07
Volume of DGG-B added per well (**L**)	10	2.5E-03
Minimum Dispersed Concentration of ORM2 (DGG-B) at Time of Injection		
Groundwater pore volume (PV, **L**)	10195	0.2
Number of ORM2 cells inoculated per well ($N_{KB-1} = C_{KB-1}*V_{KB-1}$, **copies**)	5.2E+11	5.2E+07
Minimum dispersed [ORM2] in groundwater ($C_{PV} = N_{ORM2}/PV$, **copies/mL**)	5.1E+04	3.4E+05
Expected Lag Time Prior to Onset of Active Biodegradation		
Minimum [ORM2] to achieve quantifiable activity (C_{viable}, **copies/mL**)	4.3E+05[a]	
# ORM2 doublings to reach viable concentration [$N_D = \ln(C_{viable}/C_{PV})/\ln(2)$]	6.4	0.7
Estimated time required to achieve one ORM2 population doubling (T_D, **days**)	50[a]	
Estimated lag time prior to onset of active degradation ($T_{max\ lag} = T_D*N_D$, **days**)	154	18
	Lag time (weeks) 22.1	**Lag time (weeks) 2.6**

[a]From Toth et al. 2021 (Environ Sci Technol, *in press*)

Lessons Learned & Take Home Messages

1. Anaerobic BTEX bioremediation requires high concentrations of active, specialized microbes;
   - 10^7 to 10^8+ copies/L required for benzene

2. Even with bioaugmentation, it is challenging to get/keep their numbers up;
   - Re-bioaugmentation occurred in Summer 2021
   - Additional measures will be included to understand what is happening to DGG-B post-bioaugmentation
   - What else can we do to improve bioaugmentation success?

3. Patience is a virtue – *this study will take years*

Thank You for Your Attention

Tackling Benzene Contamination with Anaerobic Microbes

Contact Us!

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