

Field Applications of Anaerobic BTX Bioaugmentation Cultures



Sandra Dworatzek, M.Sc.

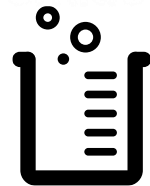
Jennifer Webb and Corey
Scales, Elizabeth Edwards, and
Courtney Toth

1 June 2023

Introduction to SiREM



BSL-2 Laboratory
Locations in
Knoxville, TN and
Guelph, ON



35 staff
4 PhD, 10 MSc.
Chemists, microbiologists,
molecular biologists,
environmental scientists,
engineers

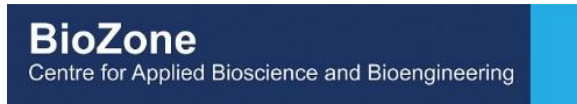


Canada, US and
International Project
Experience





Participating and Funding Organizations



UNIVERSITY OF
TORONTO



UNIVERSITY OF
ALBERTA



InnoTech
ALBERTA



SiREM

Leading Science · Lasting Solutions

UNIVERSITY OF
WATERLOO



Ontario



Ontario Genomics



GenomeCanada

ALBERTA
INNOVATES





Acknowledgements – Best Team Ever!

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Chemical Engineering and Applied Chemistry, University of Toronto

Sandra Dworatzek, and Jennifer Webb

SiREM, Guelph ON

Dr. Ania Ulrich, Korris Lee, Amy-Lynne Balaberda, Erika Strytveen, and Dr. Mian Nabeel Anwar

Civil and Environmental Engineering, University of Alberta

Dr. Neil Thomson, Andrea Marrocco, Griselda Diaz de Leon, Bill McLaren, and Adam Schneider

Civil and Environmental Engineering, University of Waterloo

Dr. Karen Budwill

Innotech Alberta, Edmonton ON

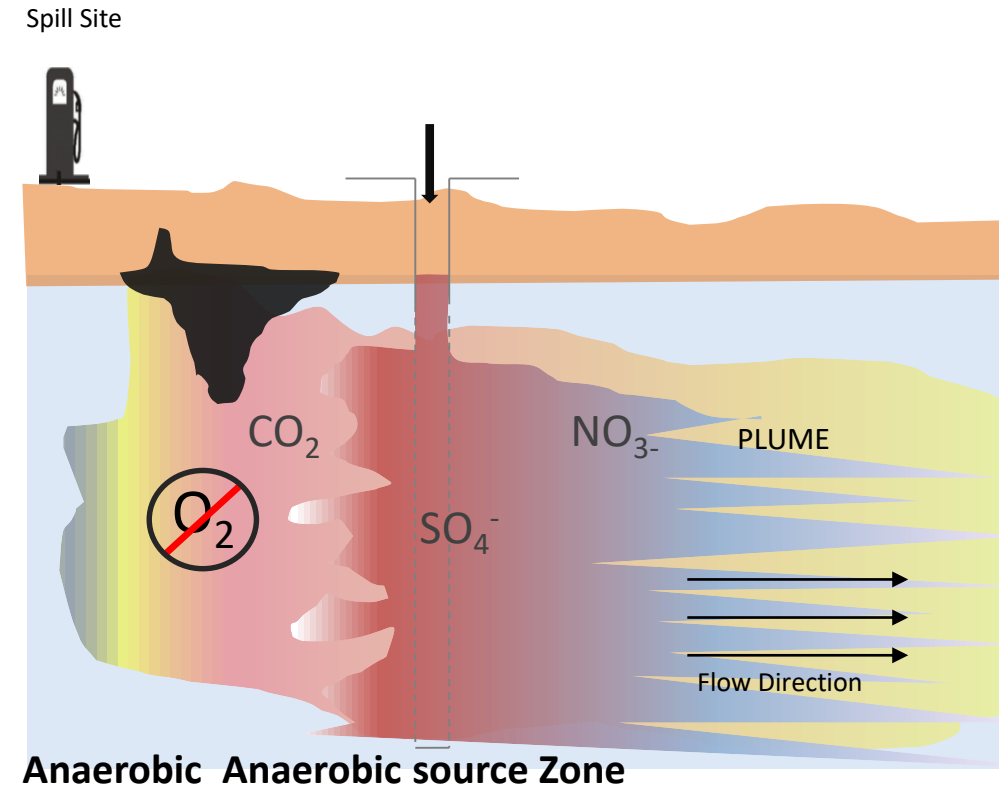


...and many others!



Why Go Anaerobic for BTEX?

- Hydrocarbon sites often anaerobic
 - High organic loading consumes O_2
 - Adding O_2 is hard!
- Anaerobic e^- acceptors ($NO_3^-/SO_4^{2-}/CO_2$)
 - often already present in subsurface
 - more soluble, easier to apply compared to O_2 (e.g., sulfate)
- Viable *in situ* remediation option for deep contamination





What Limits BTEX Biodegradation?

Hydrocarbon Properties? ❌

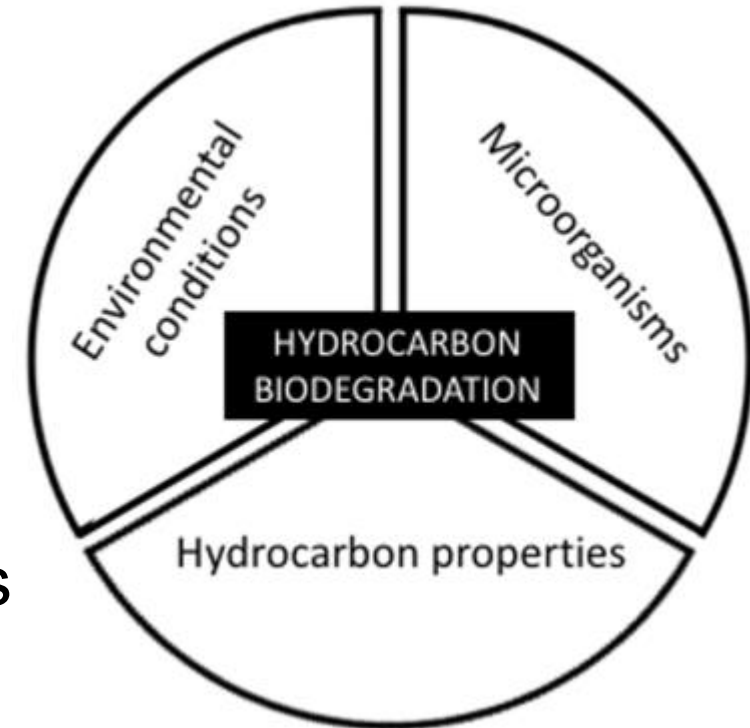
- BTEX is susceptible to biodegradation

Environmental Conditions? *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

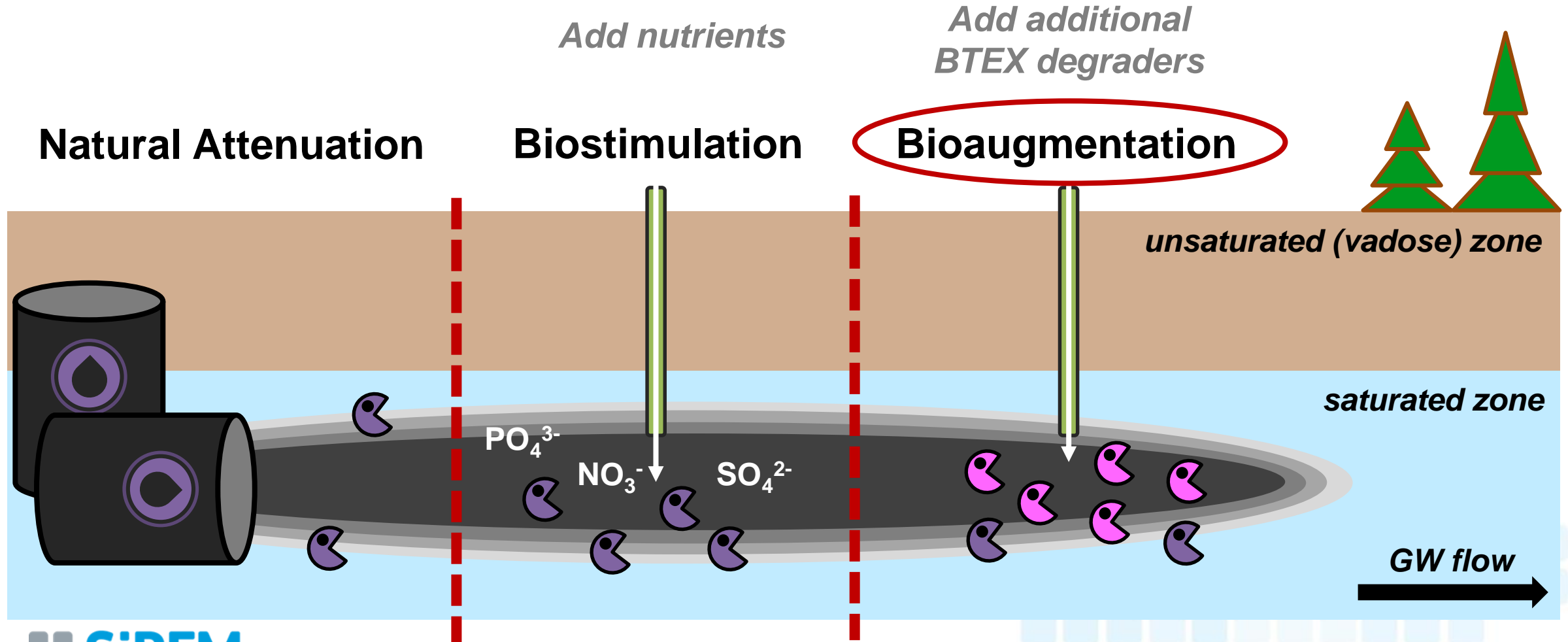
Microorganisms? ?

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





How Can We Reliably Increase Concentrations of BTEX Degraders?

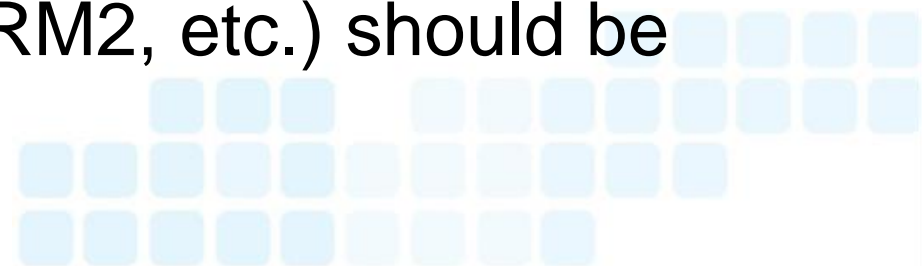




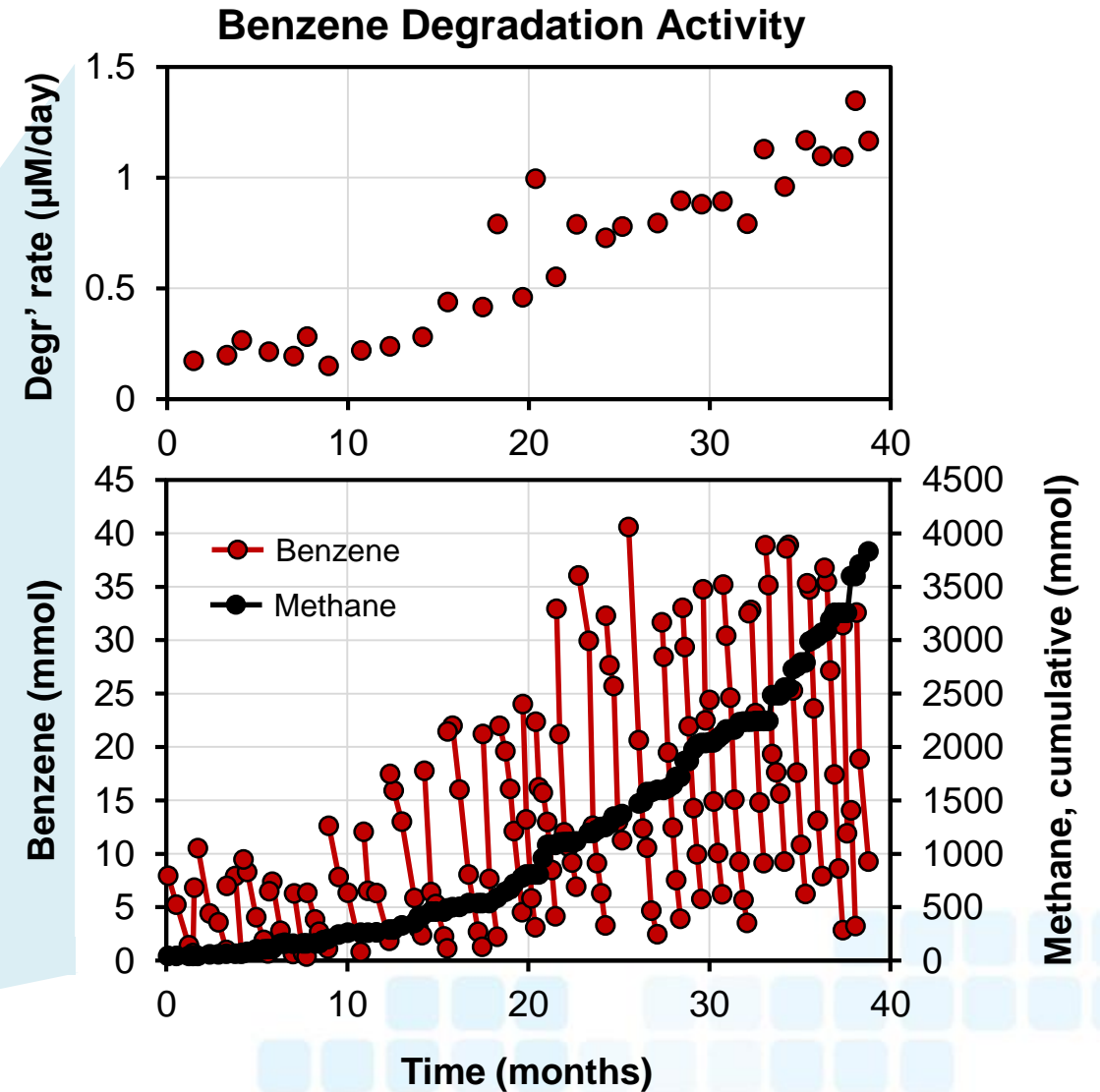
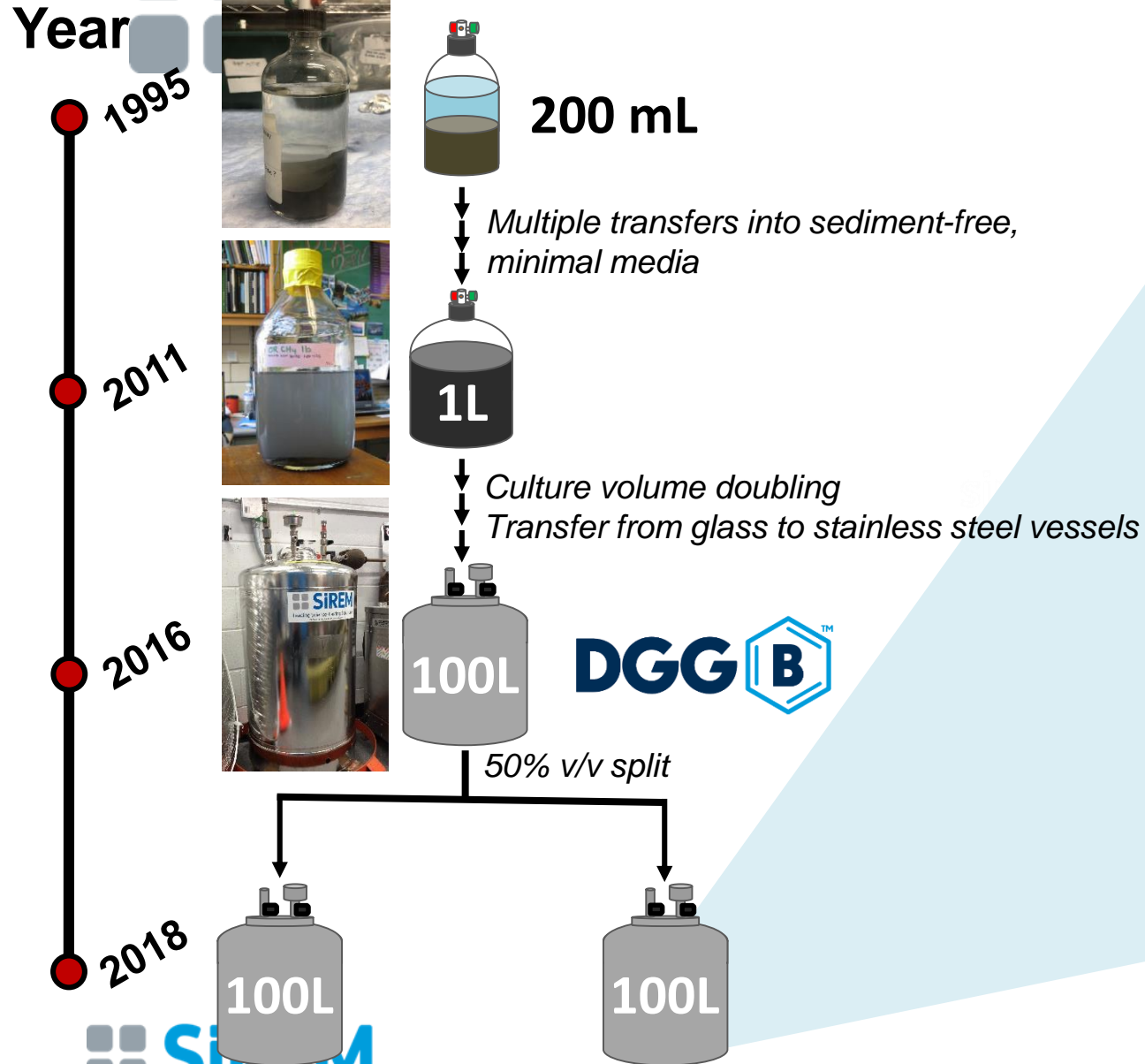
Project Goal & Success Criteria

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.

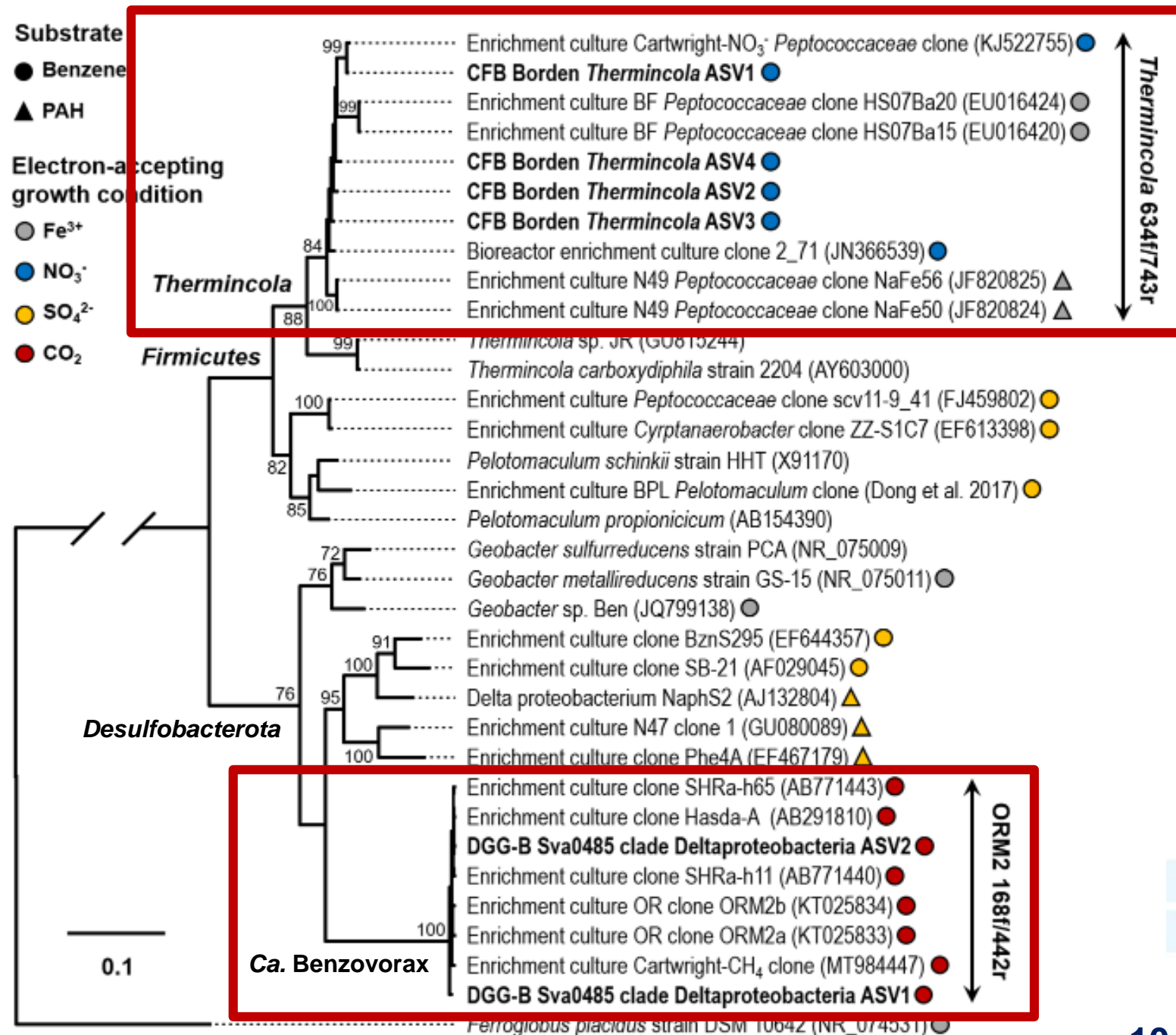


Methanogenic Benzene-Degrading Culture Scale-Up



First “Aha!” Moment: Anaerobic Benzene Degraders Belong to Distinct Clades

- qPCR primers were later designed to capture two clades of interest – ORM2 and *Thermincola*



Ulrich and Edwards 2003 (Environ Microbiol 5)

Luo et al. 2016 (Environ Microbiol 18)

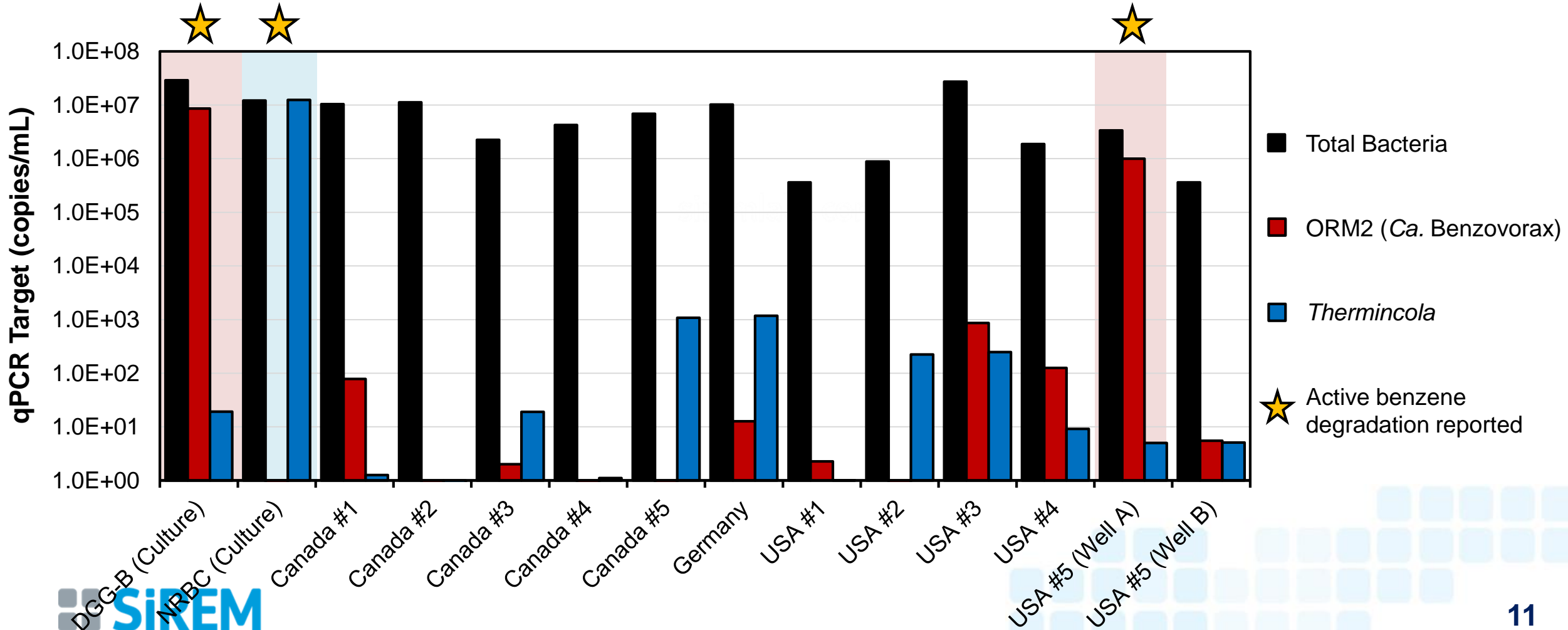
Toth et al. 2021 (Environ Sci Technol 55)

Toth et al. (data in preparation)

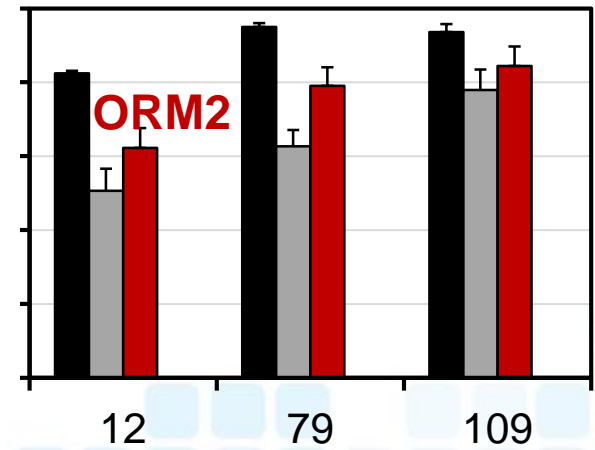
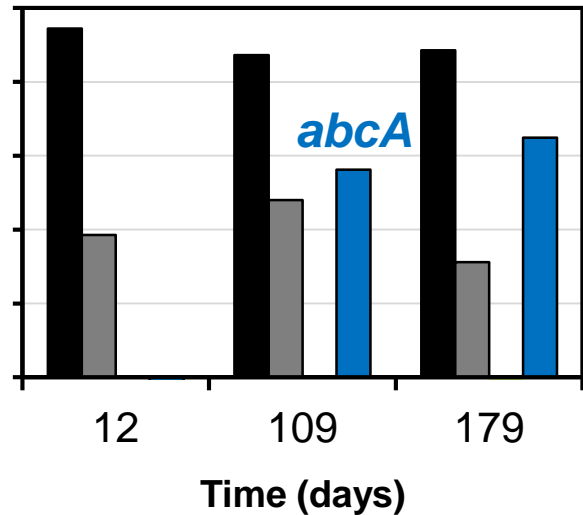
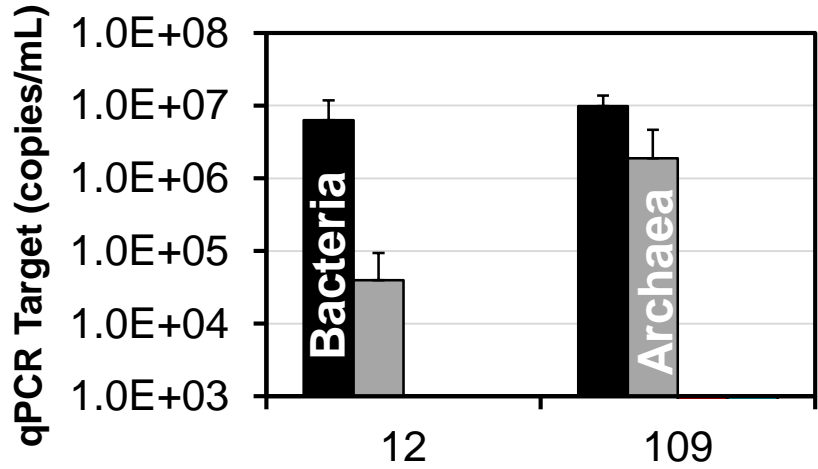
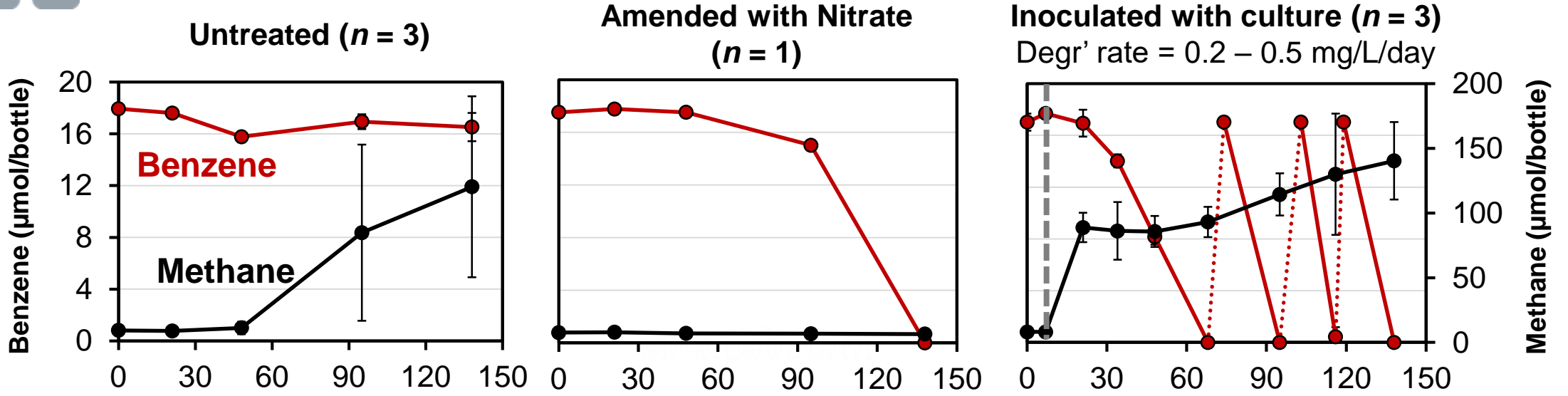


Second “Aha!” Moment: Anaerobic Benzene Degradation is a Number’s Game

Key microbes are often present at contaminated sites but often in low abundances

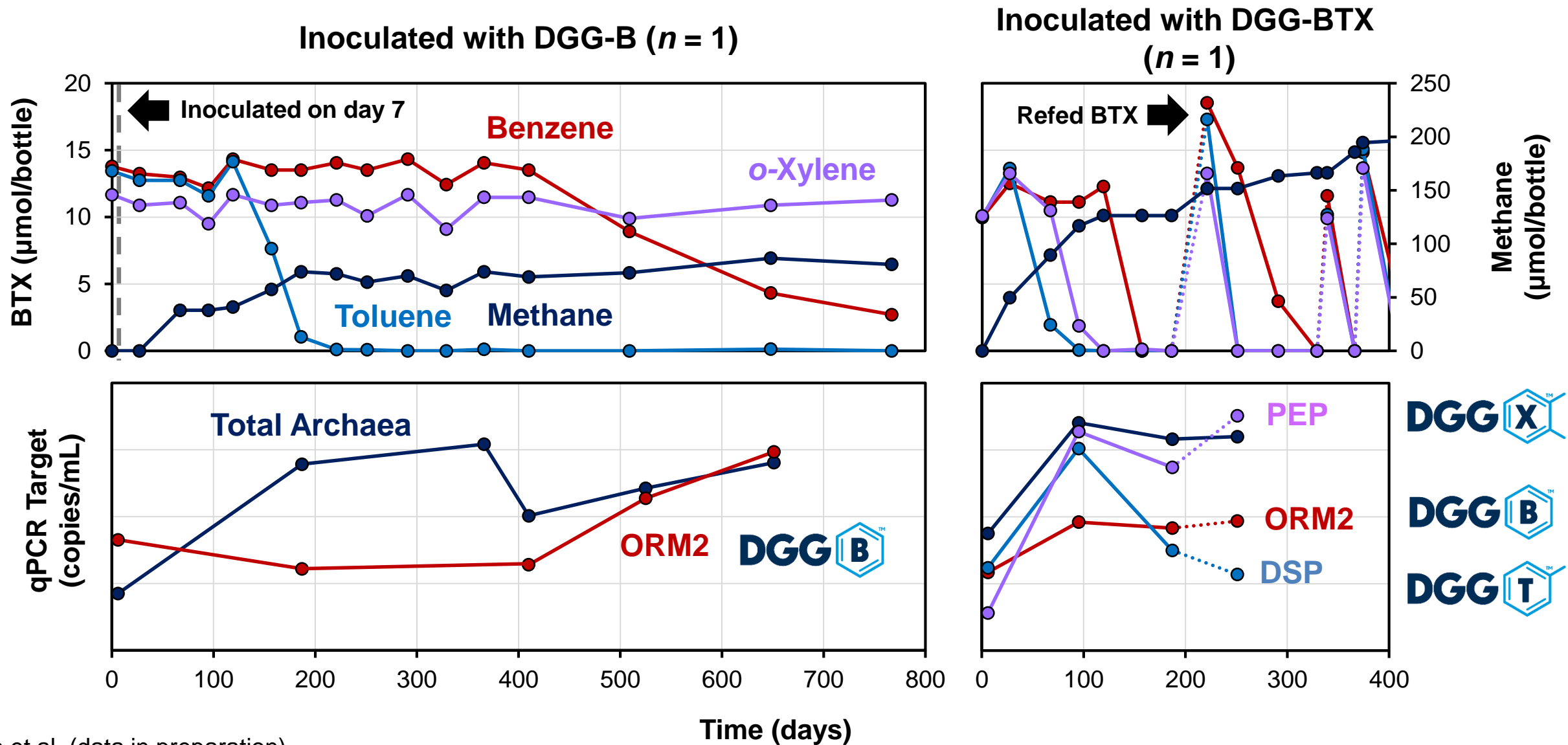


20+ experiments (microcosms & column studies) have supported that bioaugmentation can be effective for anaerobic treatment of benzene.

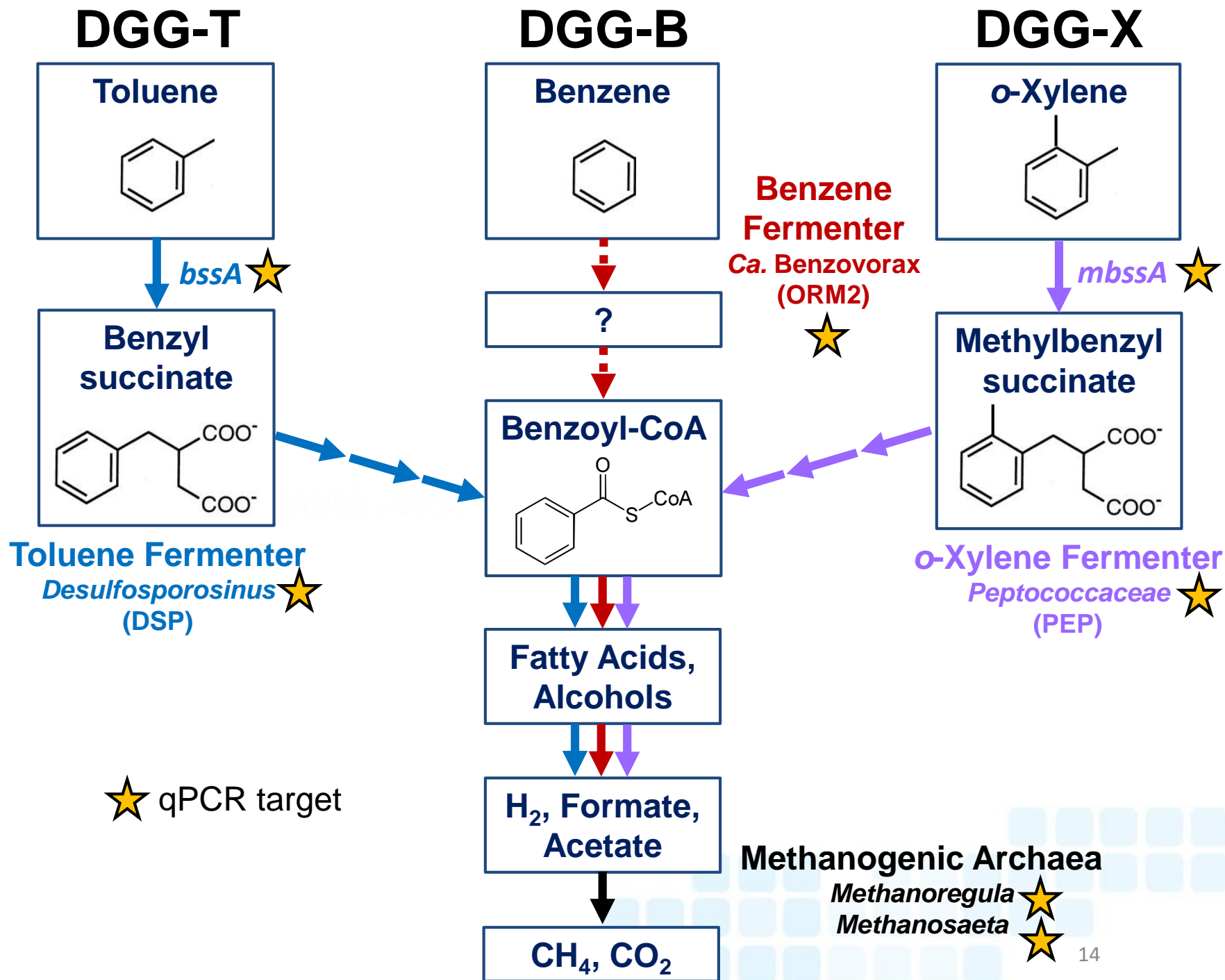


3rd “Aha!” Moment: Teamwork Makes the Dream Work

Co-contaminant treatment can improve the likelihood of benzene remediation success

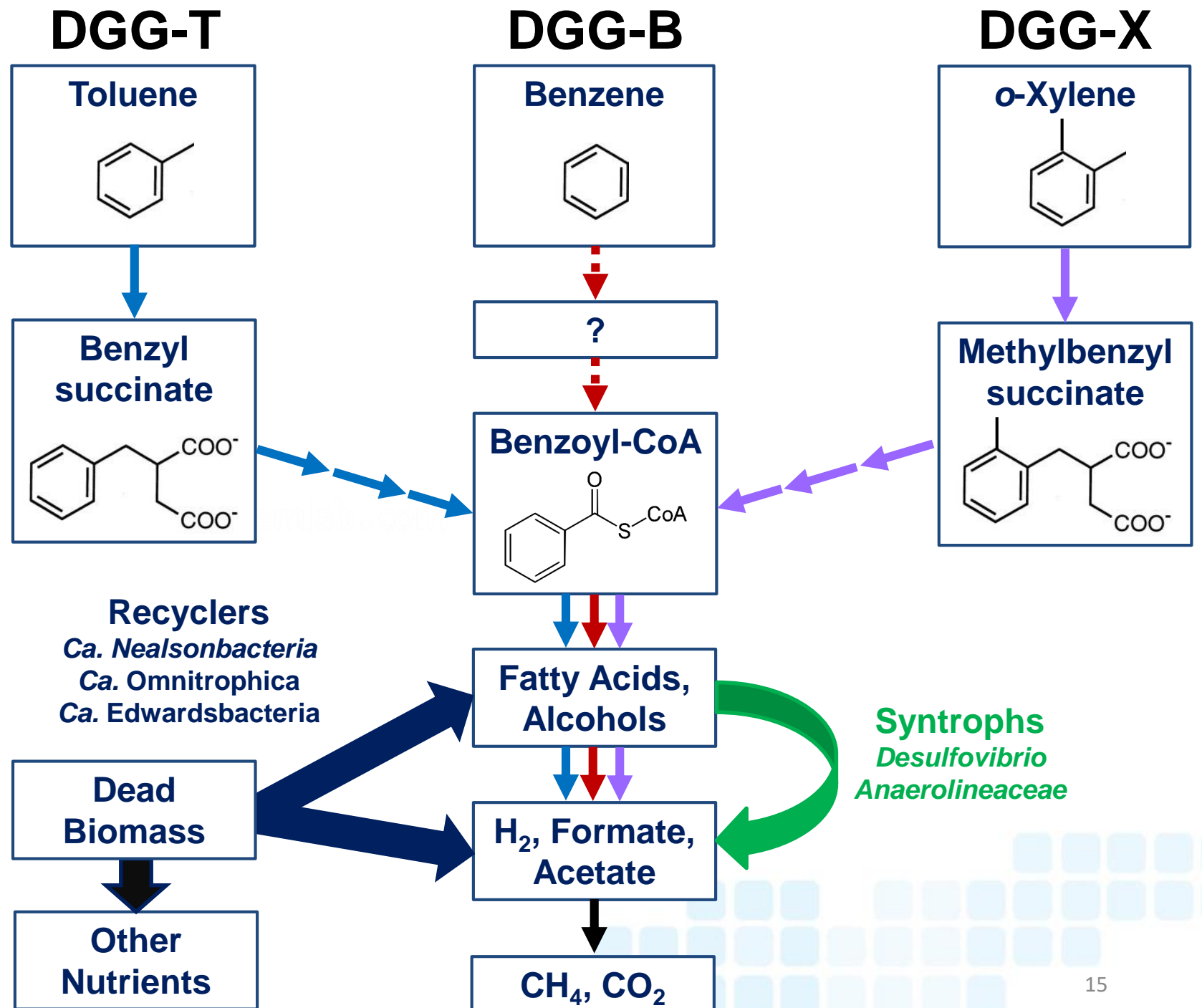


- The key microbes in each culture include one **hydrocarbon fermenter** and **2 methanogens**
- Key microbes & functional genes are monitored by **qPCR** and **NGS**
- Metagenomes have been sequenced and **reconstructed genomes** are being analyzed





- “**Syntrophs**” help metabolize fermentation intermediates
- “**Recyclers**” transform dead biomass (proteins, carbohydrates, etc.) back into useful culture nutrients





DGG-Plus Bioaugmentation Cultures



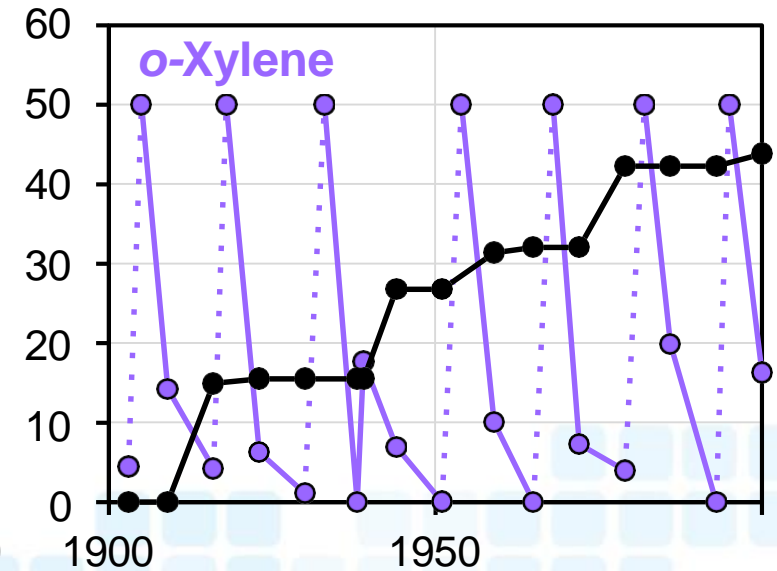
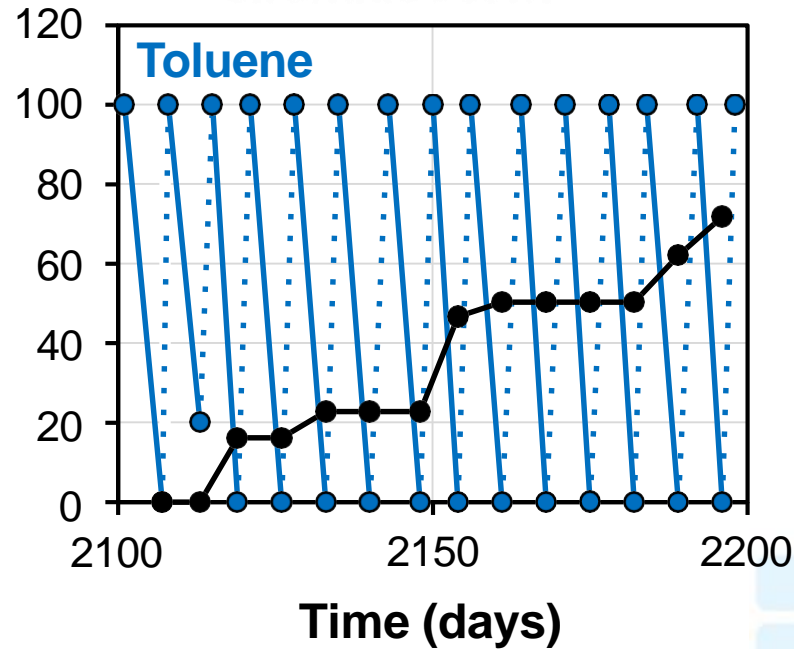
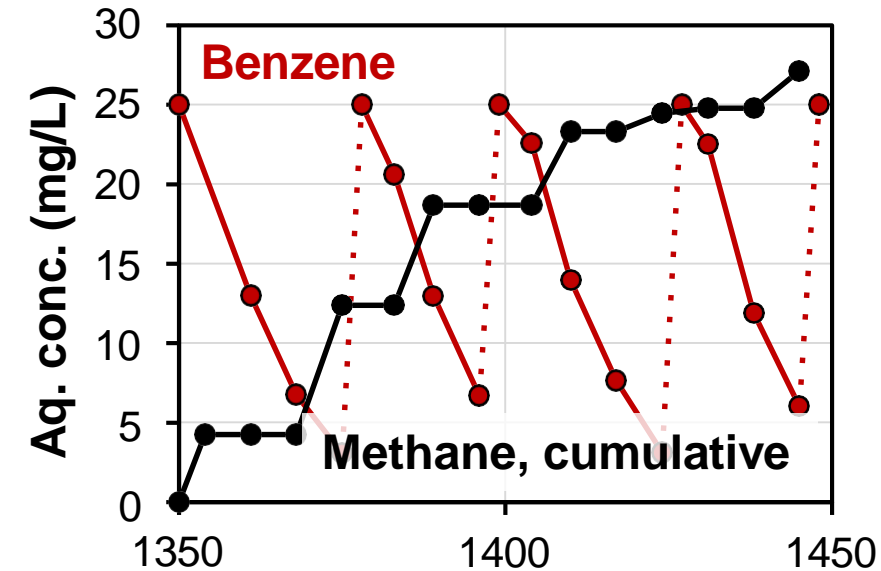
Degr' rate = ~ 1.3 mg/L/day



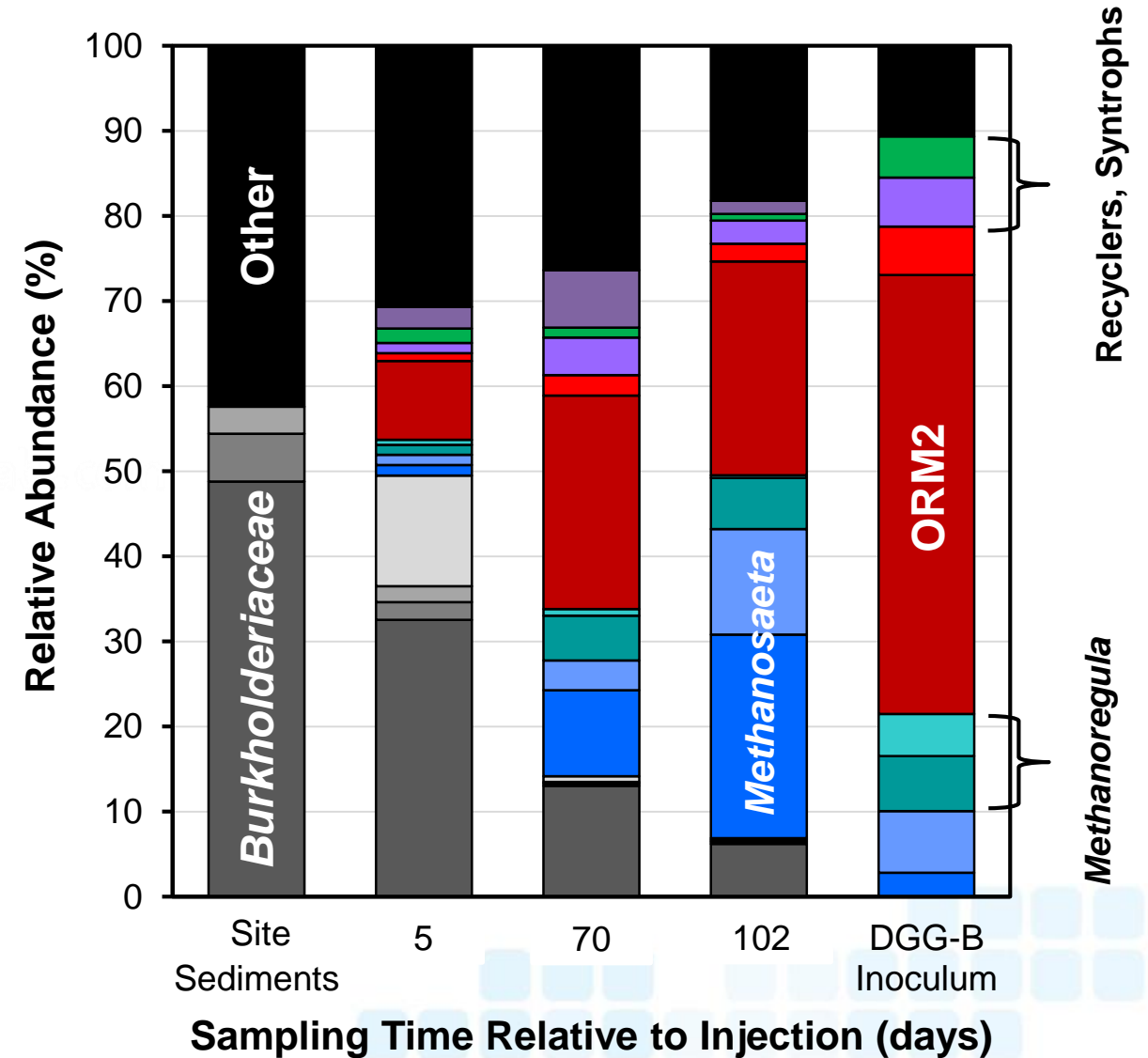
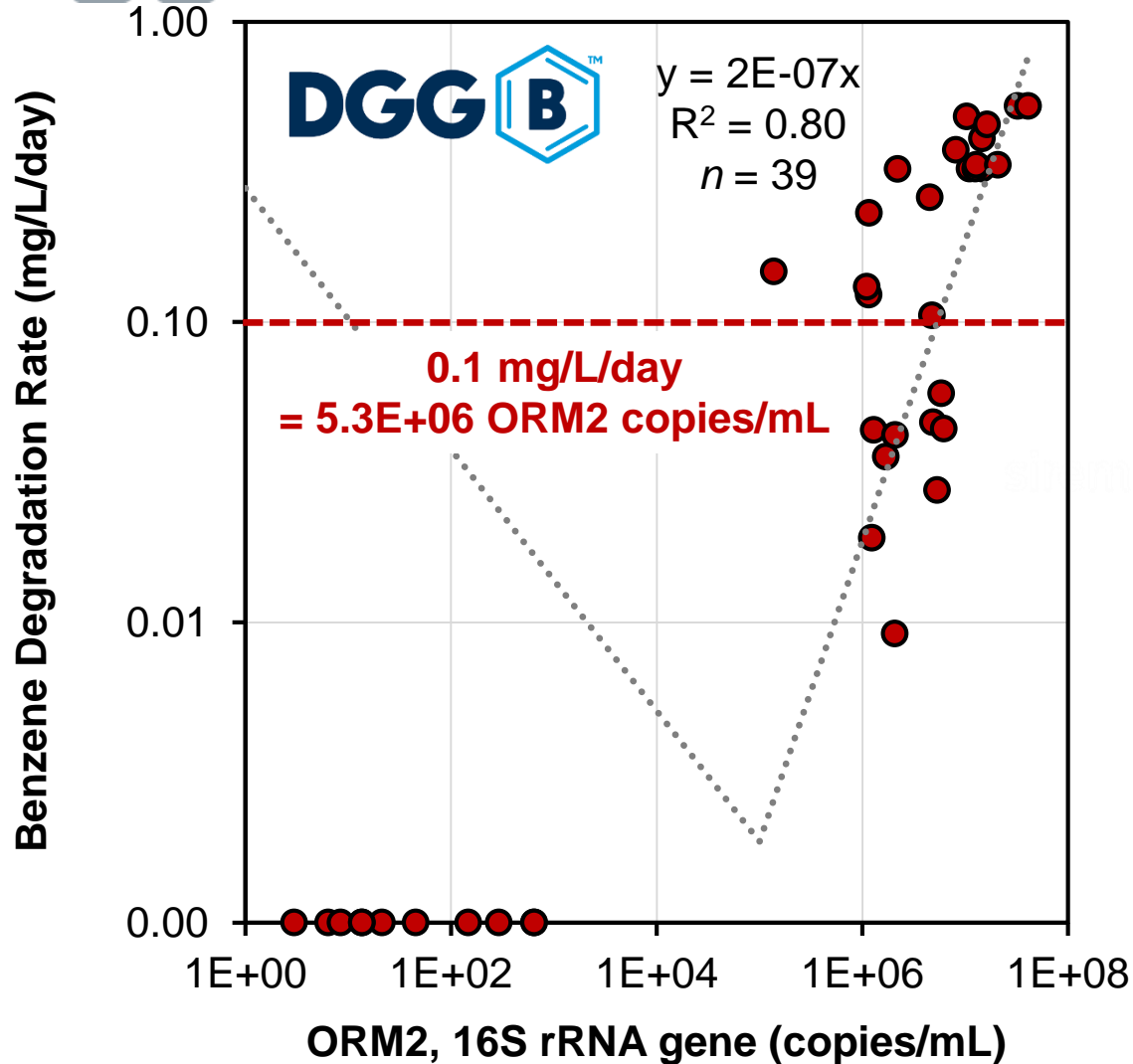
Degr' rate = ~ 25 mg/L/day



Degr' rate = ~ 9 mg/L/day



In DGG-B, rates of benzene degradation correlate with conc. of ORM2.
Growth of methanogens, syntrophs & recyclers is also needed to sustain degradation.



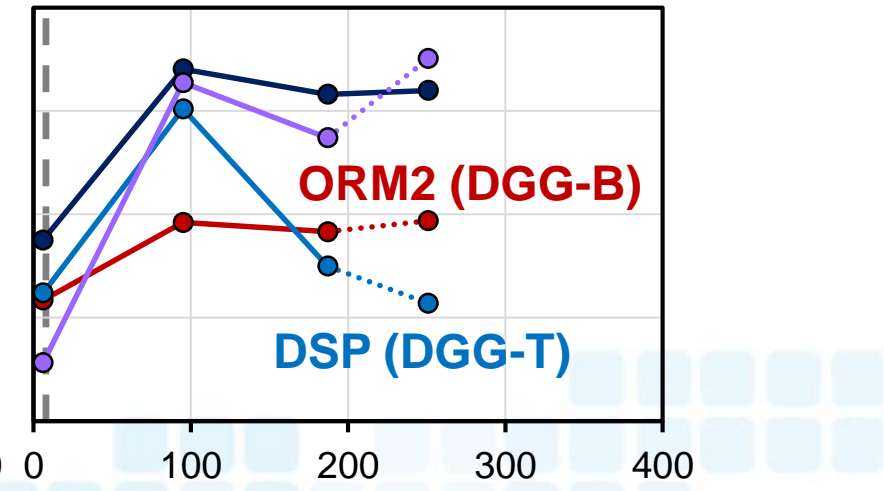
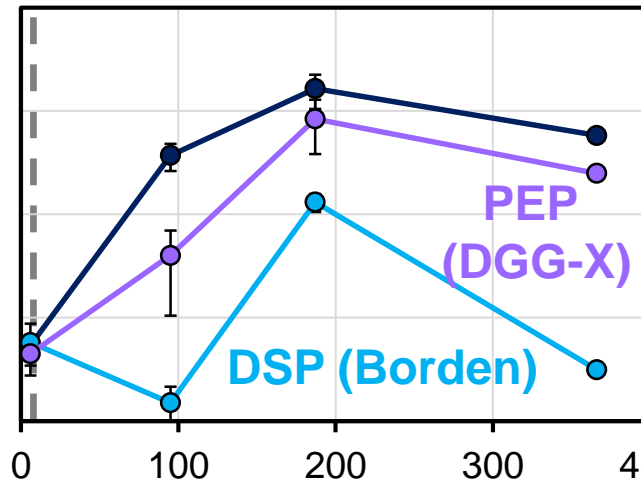
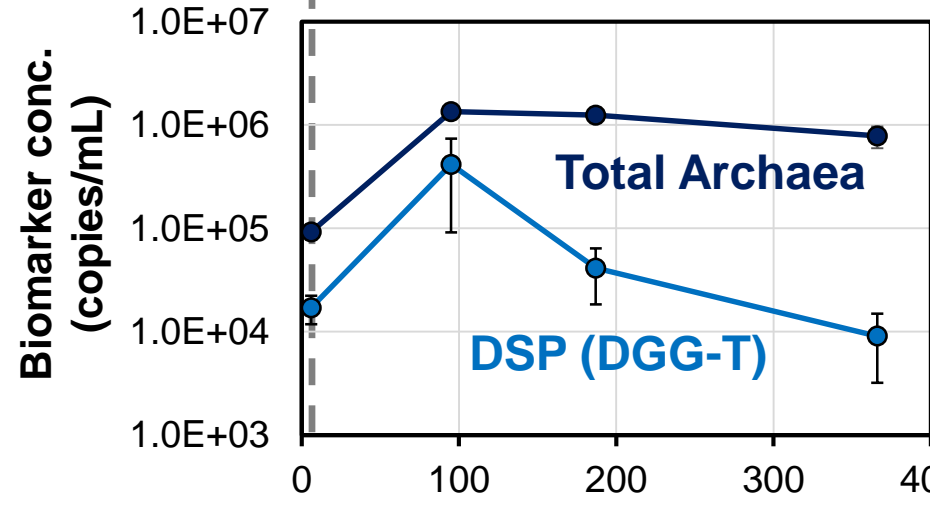
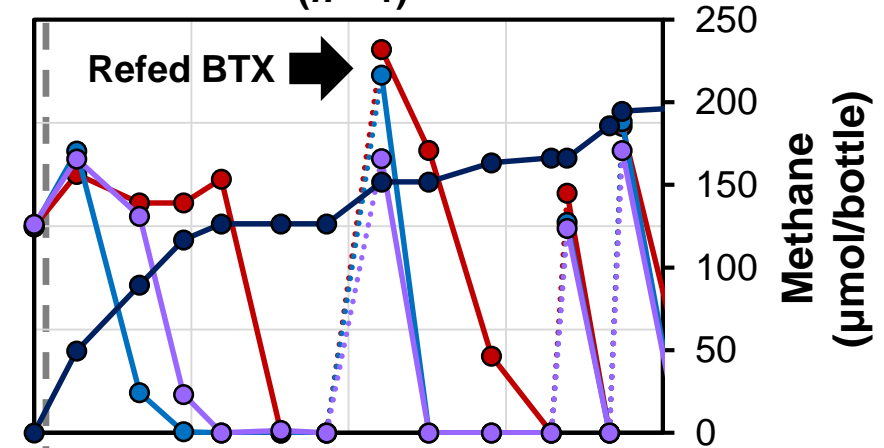
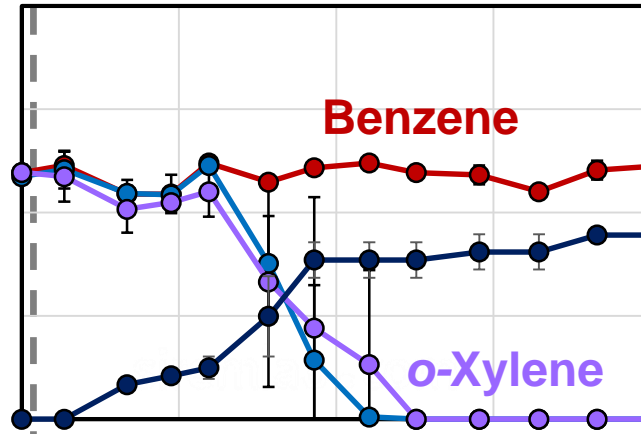
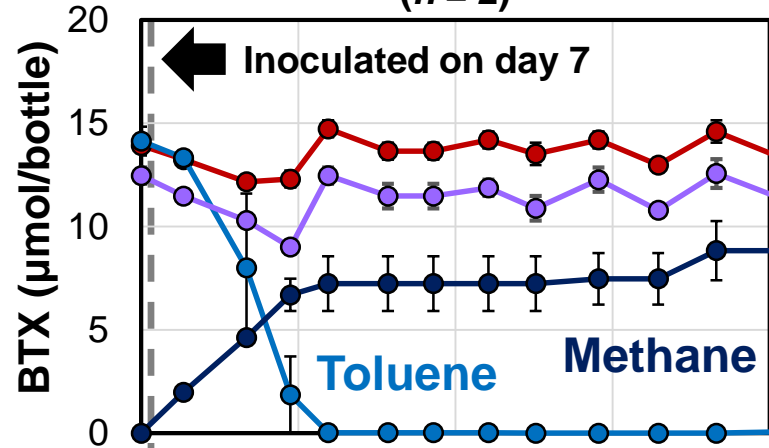


DGG Plus: fast, complete & reproducible BTX depletion.
Degradation order: toluene > o-xylene > benzene.

Inoculated with DGG-T
(n = 2)

Inoculated with DGG-X
(n = 3)

Inoculated with DGG-BTX
(n = 1)

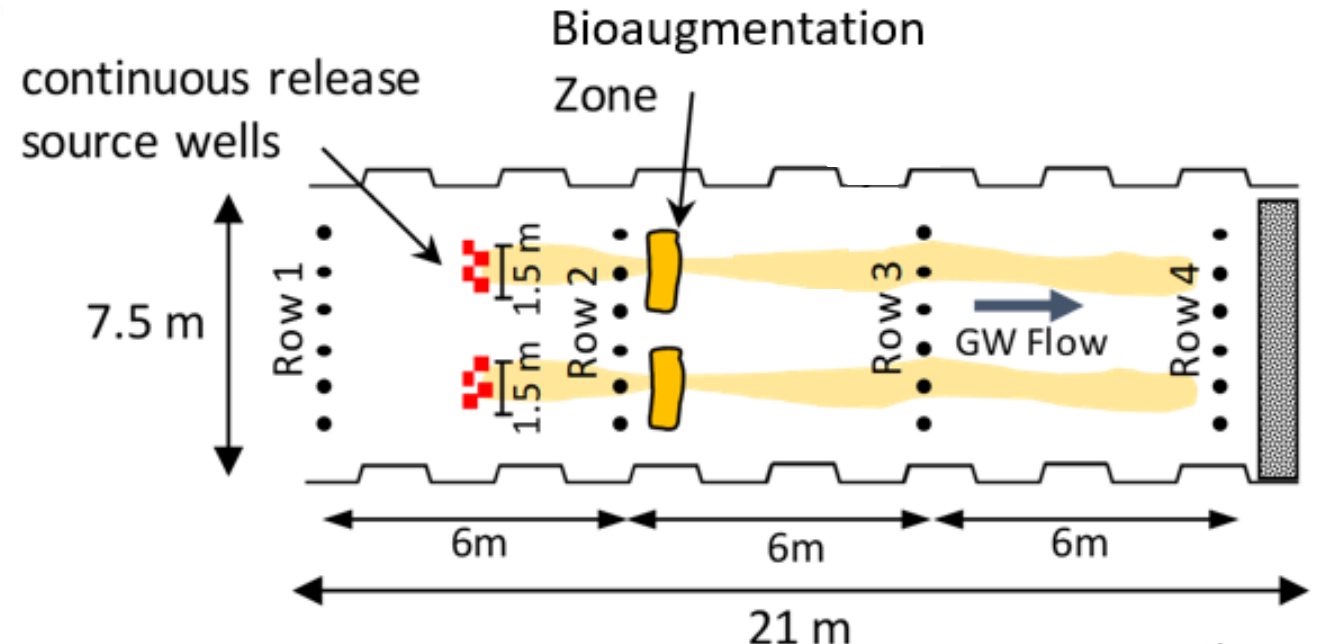
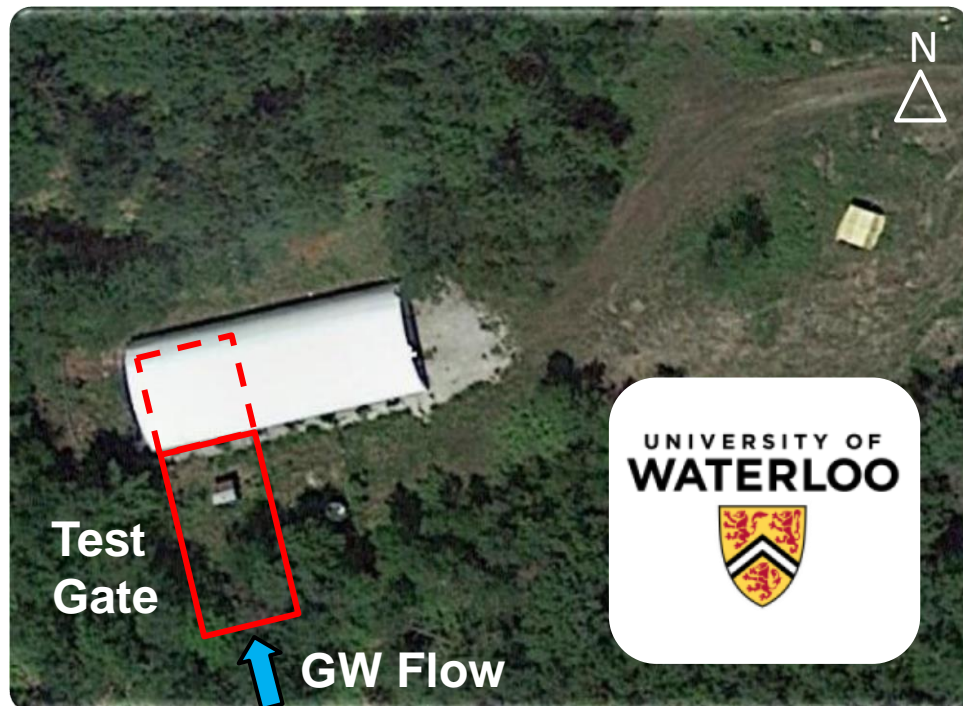


Time (days)



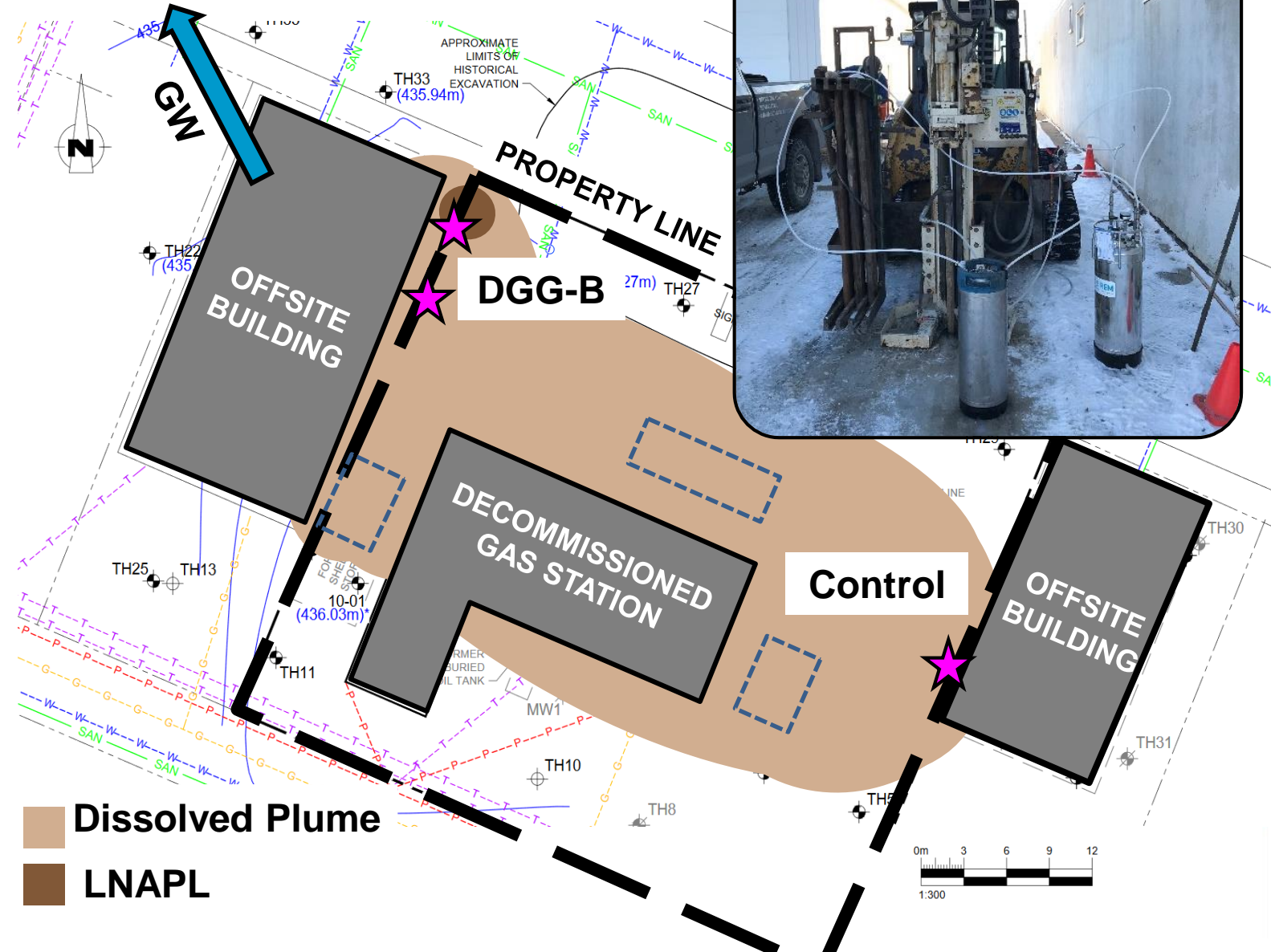
Upcoming Field Pilot at CFB Borden

- Pilot consists of a gated system supplied with BTEX and includes 2 test lanes
 - **Lane 1** will be injected with **DGG-BTX** (enhanced BTX degradation expected)
 - **Lane 2** will only receive **DGG-T** (only toluene degradation expected)
 - Ethylbenzene = negative control (no enhanced degradation expected)



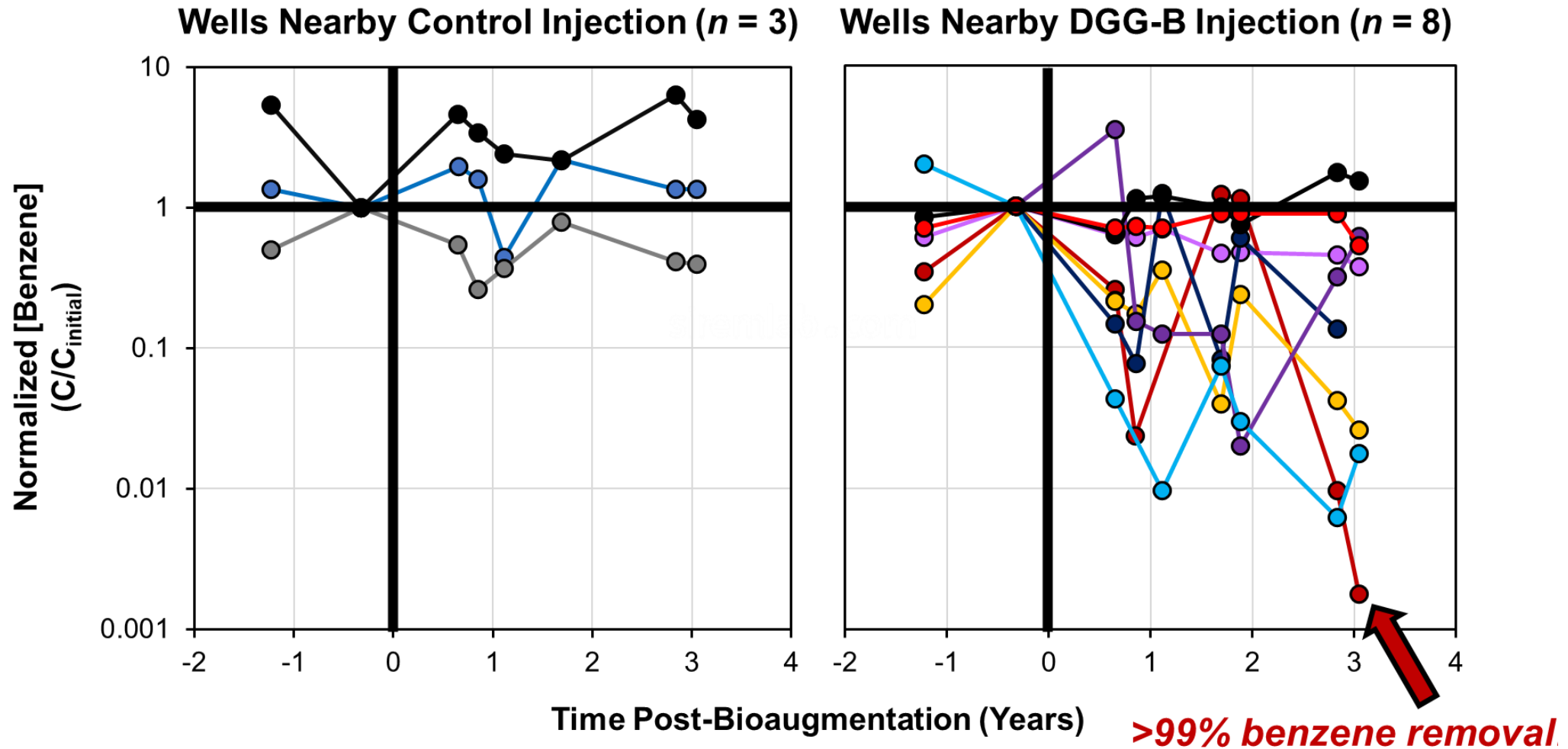
SK Site Bioaugmentation

- Former gas station with BTEX, F1 and F2 contamination
- Various remediation approaches 1993-2008
- 2019 benzene still (< 0.01 – 20 mg/L)
- DGG-B™ injected at 2 points (10 L each, near NW corner of property)
- A control well injected with heat-killed DGG-B™



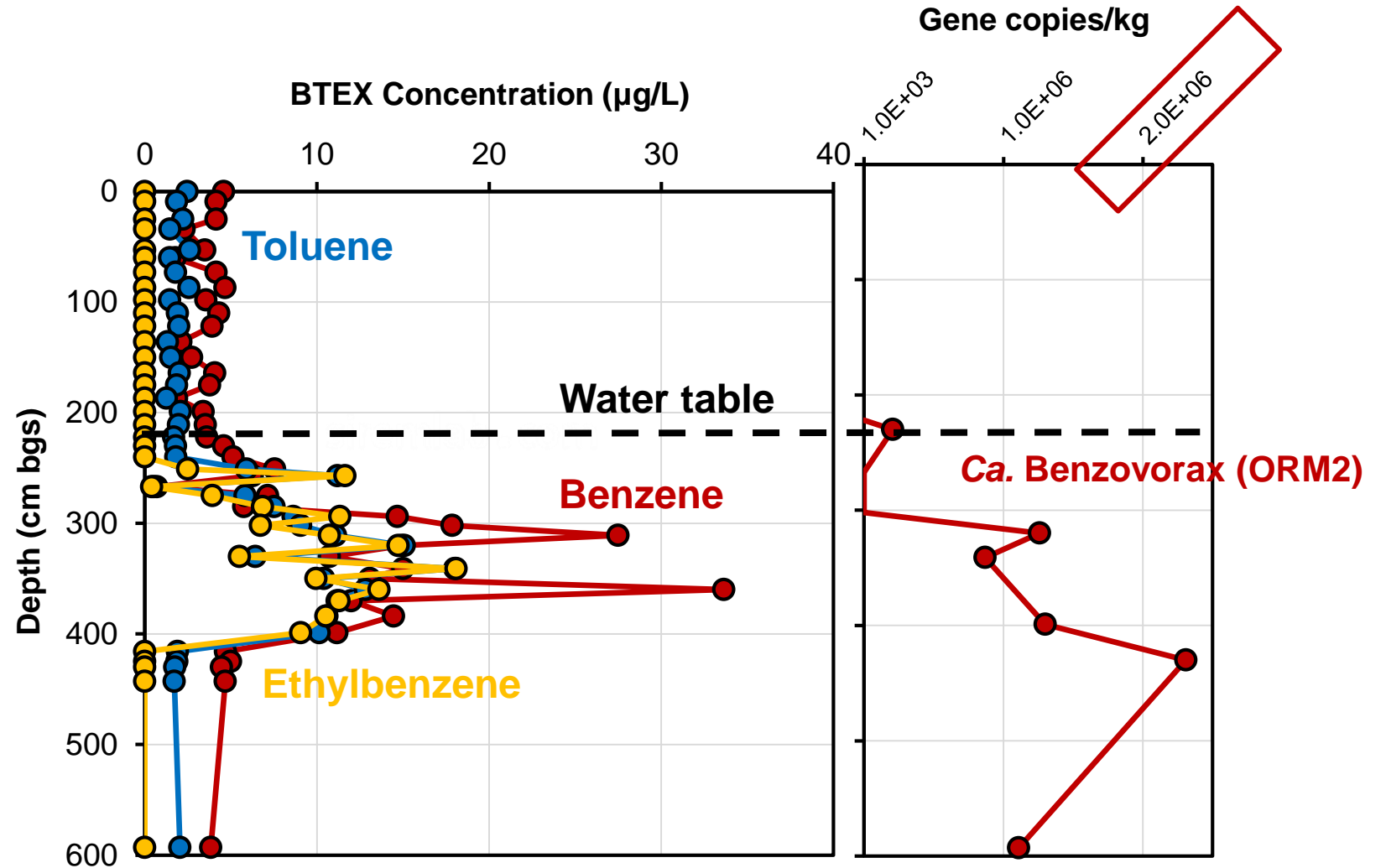


Benzene Reductions at SK Site





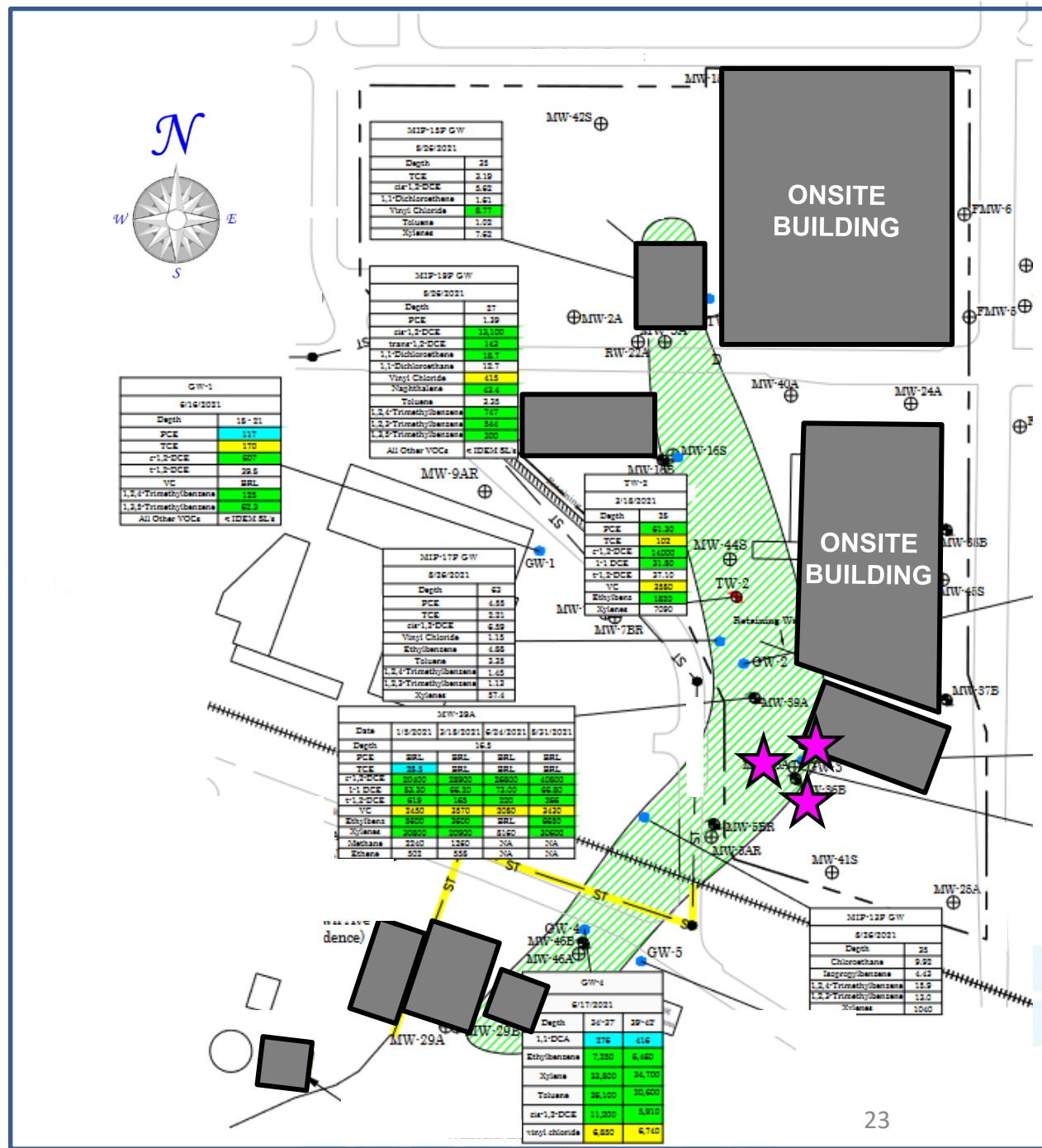
Ca. Benzovorax vs Benzene in Cores from SK site





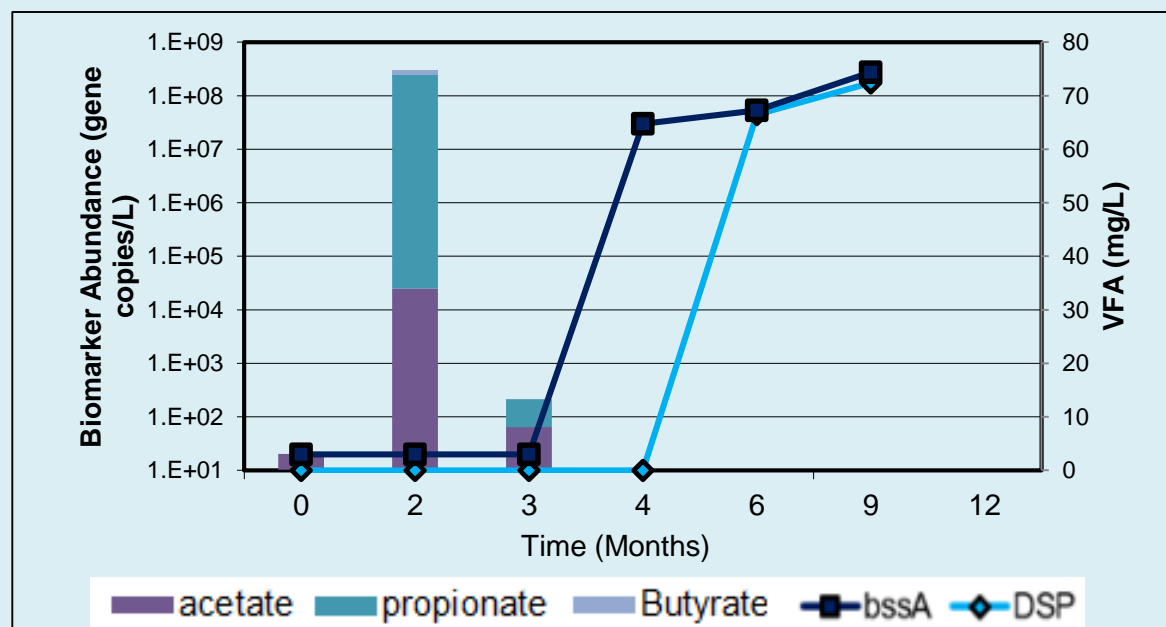
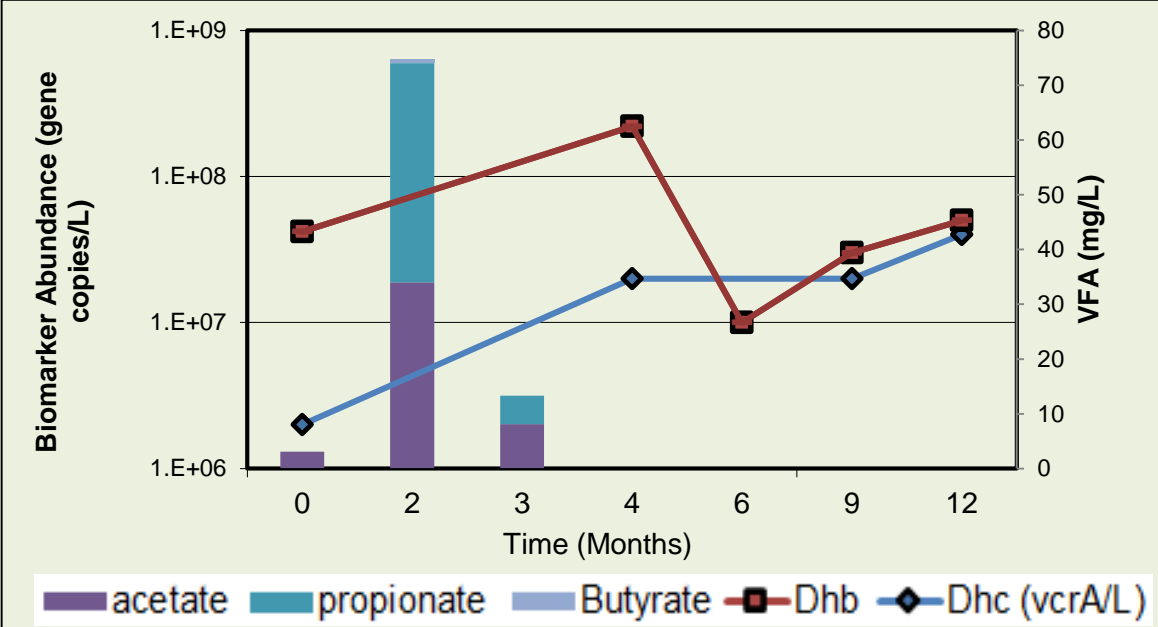
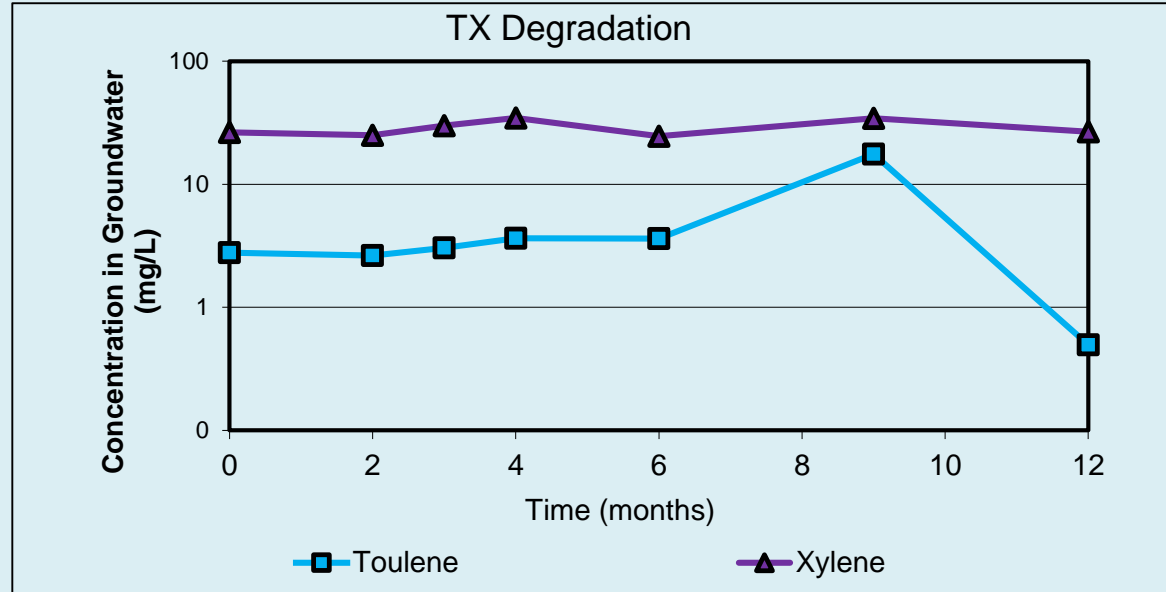
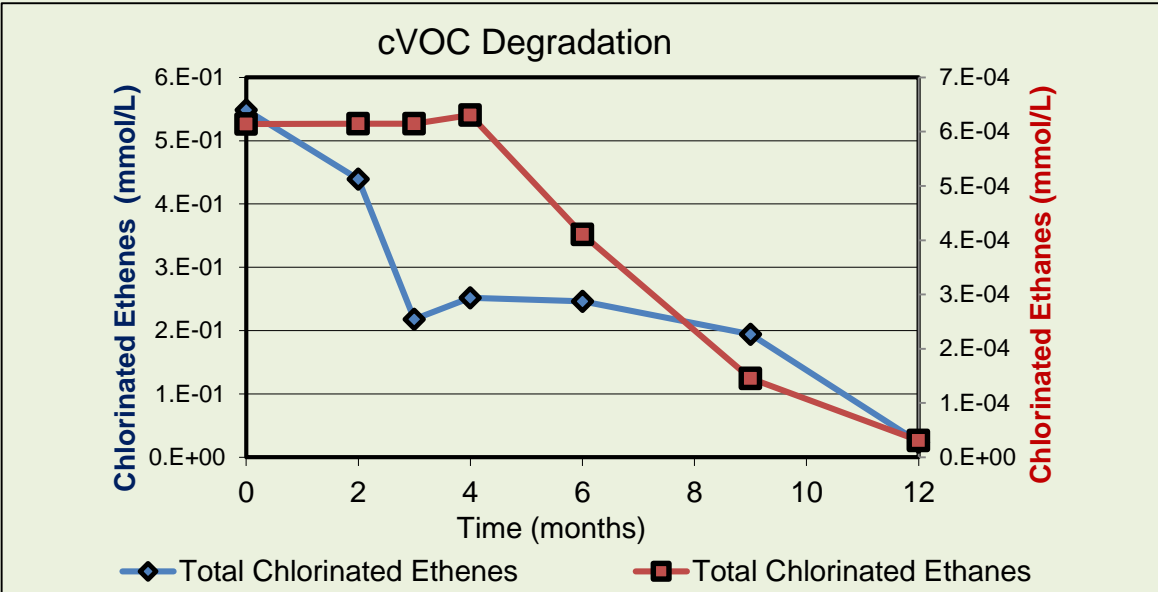
US Field Pilot

- Chemical manufacturing site, groundwater contaminated with chlorinated ethanes, ethenes and TEX
 - **Green** = exceeds drinking water limits
 - **Blue** = exceeds residential vapor limits
 - **Yellow** = exceeds industrial vapor limits
- In Sept 2020, a blend of KB-1® Plus and DGG Plus was injected at 3 points (★) near the center of the plume core
- Fall 2022 – full-scale bioaugmentation proceeded



CHLORINATED ETHENES & ETHANES

TOLUENE & XYLENE



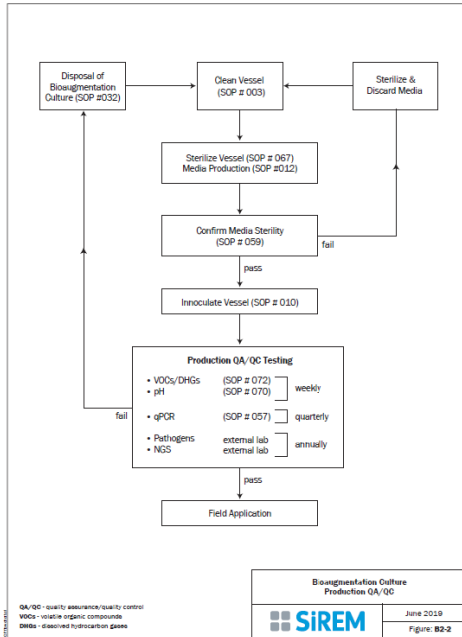


DGG Plus Culture Field Testing Overview

Site Reference	Target Substrate(s)	Culture(s) Tested	Treatability Testing	Field Pilots		
			Successful Bioaug'?	Start Date	Culture volume	Successful Bioaug'?
Louisiana	Benzene	DGG-B	Yes	Oct-19	60 L	Site not recently sampled
FCL-4 (SK)	Benzene	DGG-B	Yes	Nov-19	10 L	Positive results
New Jersey	Benzene	DGG-B	Yes, only when combined with SO ₄ ²⁻	Apr-20	145 L (+SO ₄ ²⁻)	Client reported positive results
Mid-east US	TEX, chlorinated compounds	DGG-BTX, KB-1®	--	Sept-20 (pilot)	3 - 6 L each	Yes, for KB-1 & DGG-T, DGG-X under evaluation
				Aug-22 (full scale)	~100 L each	Results pending
Ontario	TEX, F1-F2 alkanes	DGG-TX	Yes	Sept-22	10 L each	Results pending
British Columbia ^a	Xylene	DGG-X	--	Jan-23	60 L	Results pending
Borden (ON)	BTX	DGG-BTX	Yes	Spring-23	TBD	
Illinois	BTX	DGG-BTX	--	Spring-23	20L	

Regulatory Considerations Before Field Testing

New Substance Notification (NSN) approved in 2022



SAFETY DATA SHEET

1. CHEMICAL IDENTIFICATION AND COMPANY INFORMATION

Product Name: DGG-B
 Company Info: SIREM
 130 Stone Rd. W., Guelph, Ontario, Canada, N1G 3Z2
 Phone: 519-822-2265
 Toll Free, North America: 1-866-251-1747
 Fax: 519-835-3470
www.siremlab.com

Emergency Phone Number: 519-822-2265 (for 24/7 assistance, contact poison center hotline in your jurisdiction).

Description: Microbial inoculum (non-pathogenic, non-hazardous) in growth media consisting of a dilute aqueous solution of mineral salts and nutrients.

Recommended Use: Bioremediation of contaminated groundwater.

Restrictions on Use: DGG-B product intended for laboratory research and field applications for cleanup of contaminated groundwater. Products are not intended to be used as human or animal therapeutics, cosmetics, agricultural or pesticide products, food additives, or as household chemicals.

2. HAZARDS IDENTIFICATION

GHS Classification: Not classified as "hazardous" per OSHA 29 CFR 1910.1200, "Hazard Communication".

GHS Label elements, including hazard and precautionary statements: Not Applicable.

HMS	Health	Flammability	Physical Hazard	Personal Protection
Rating:	1	0	0	5*
HFPA	Health	Flammability	Reactivity	Special Hazard
Rating:	1	0	0	N/A

* B = Safety Glasses, Gloves.

A review of available data indicates minimal potential for health effects related to normal use of this product. Microbial components are non-pathogenic. The product is not expected to be a health hazard as a result of inhalation of mists, ingestion or skin contact. Eye contact may result in mild irritation/redness. Normal hygiene precautions should be observed, including eye protection, skin protection, and hand washing. The potential exists for individuals with hypersensitivity to biological materials to exhibit allergic sensitivity to biological components of this product (see Section 4, "First Aid Measures").

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 Date: 25 April 2019
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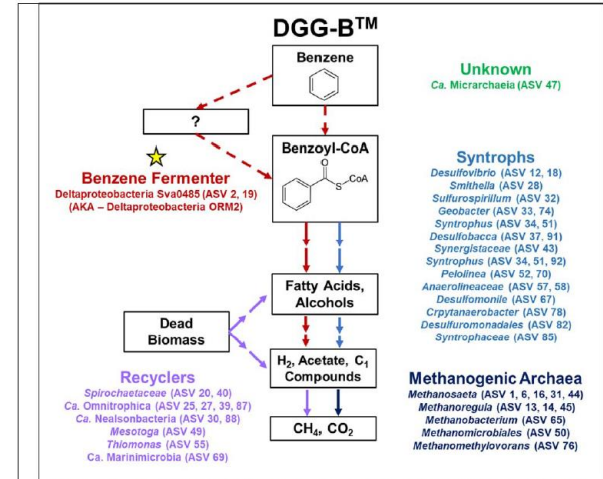


Figure B.1-3: Proposed working model for methanogenic benzene metabolism by DGG-B™

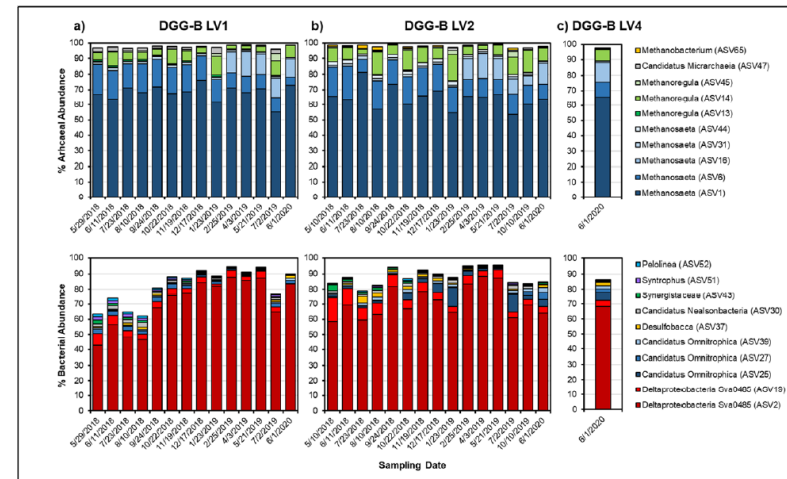
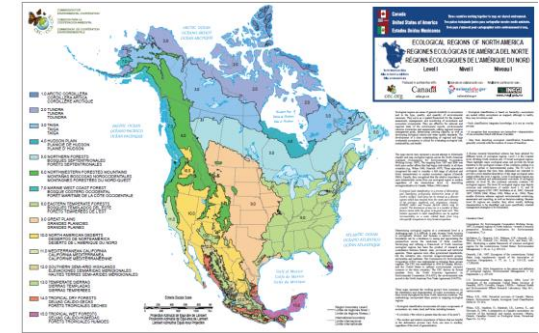


Figure B.1-4: Time course 16S rRNA gene amplicon sequencing demonstrating the stability of major archaeal and bacterial ASVs in DGG-B™ (2018 – 2020)

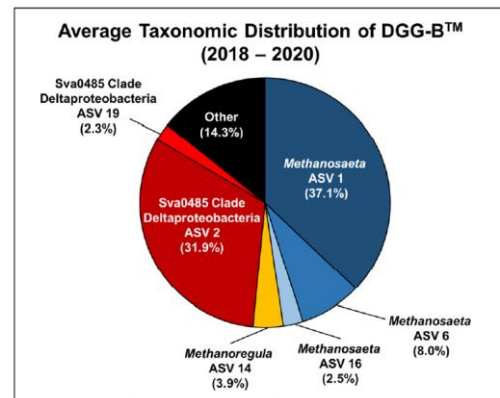


Figure B.1-1: Taxonomic distribution of dominant ASVs (> 2% average relative abundance) in DGG-B™.



Contents of bioremediation field injection kit, includes vessel in over pack, hose, regulator, scale fittings and miscellaneous tools

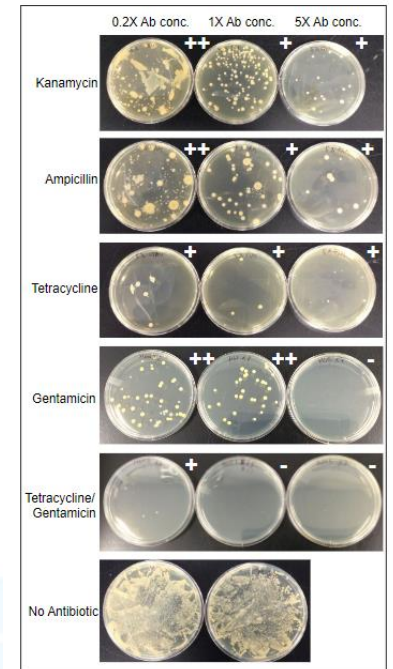


Figure B.5-2: Elimination of visible DGG-B™ colonies on aerobic Mueller-Hinton Agar (7 days at 22 °C) by specific antibiotics and concentrations.



Regulatory Approvals for DGG-Plus Culture

- Successful New Substances Notification
 - Fall 2022 DGG-Plus on Domestic Substances List
 - Federal Approval in Canada



- Ontario Mobile ECA now list DGG-Plus Culture
- Culture applications performed in Ontario, Saskatchewan and British Columbia and 3 US States



DGG B™



Deltaproteobacteria
ORM2

DGG T™



Desulfosporosinus



Dead Biomass

Fatty acids, Alcohols

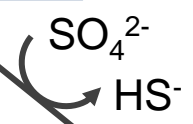
Syntrophs

Recyclers

H₂, Formate, Acetate

Sulfate-Reducing Bacteria

Methanogens



DGG X™



Peptococcaceae



DGG PLUS™



Take Home Message

- Microbes responsible for anaerobic PHC degradation are specialists;
 - Ca. *Benzovorax* are uniquely adapted to benzene.
 - *Desulfosporosinus* are adapted to toluene
 - *Peptococcaceae* are adapted to o-xylene
- Increasing abundances of these specialists is important to increase rates
- Pre- or co-treatment of non-targeted compounds may be necessary.





Questions? siremlab.com

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