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# Field Applications of Anaerobic BTX Bioaugmentation Cultures

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# 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

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# Participating and Funding Organizations

BioZone
Centre for Applied Bioscience and Bioengineering

Imperial

MITACS

UNIVERSITY OF TORONTO

UNIVERSITY OF ALBERTA

InnoTech ALBERTA

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UNIVERSITY OF WATERLOO

CO-OP

Ontario

Ontario Genomics

GenomeCanada

ALBERTA INNOVATES

# Acknowledgements – Best Team Ever!


**Dr. Elizabeth Edwards, Dr. Courtney Toth, Dr. Fei Luo, Shen Guo, Nancy Bawa, Xu (Charlie) Chen, Johnny Xiao, Kan Wu, Jingzhi (Amanda) Liang, Dr. Camilla Nesbø, Dr. Olivia Molenda, Line Lomheim, and Vinthiya Paramananthasivam**
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SiREM, Guelph ON

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Civil and Environmental Engineering, University of Waterloo

**Dr. Karen Budwill**
Innotech Alberta, Edmonton ON


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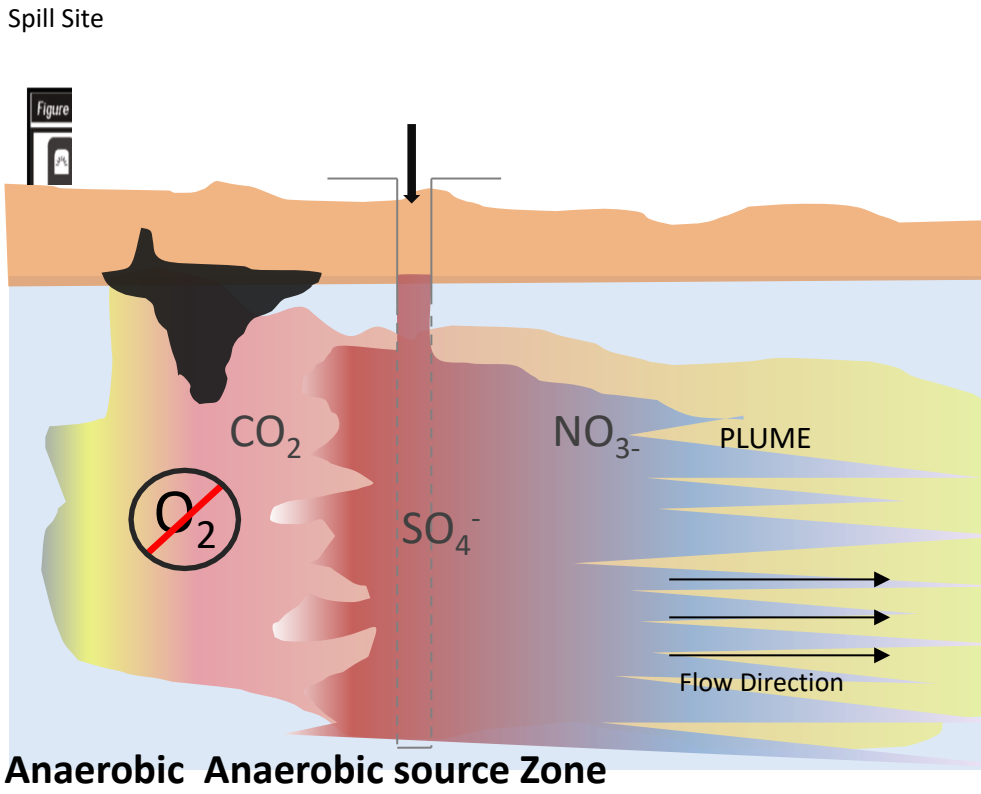
***…and many others!***

# Why Go Anaerobic for BTEX?

- Hydrocarbon sites often anaerobic
  - High organic loading consumes $O_2$
  - Adding $O_2$ is hard!

- Anaerobic $e^-$ acceptors ($NO_3/SO_4/CO_2$)
  - 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



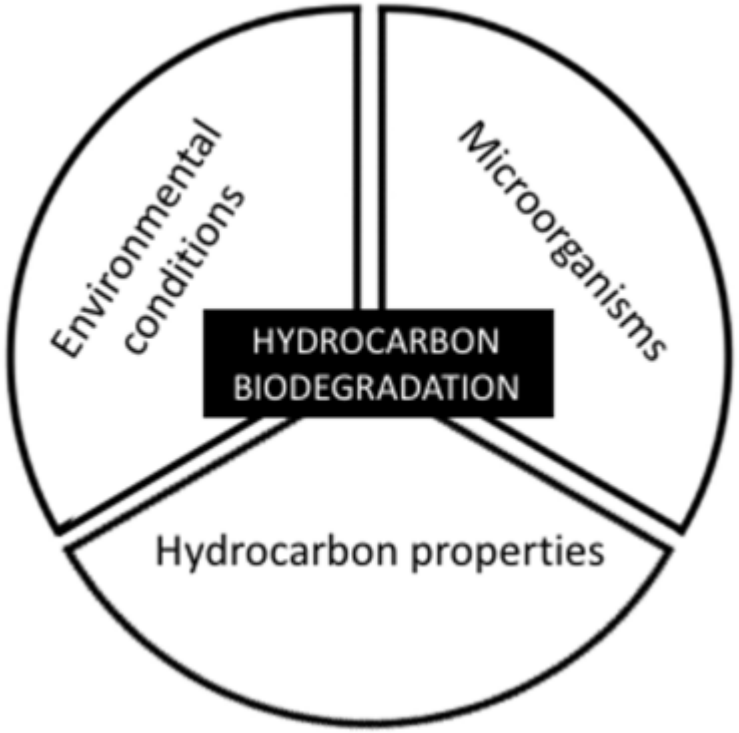
# What Limits BTEX Biodegradation?

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***

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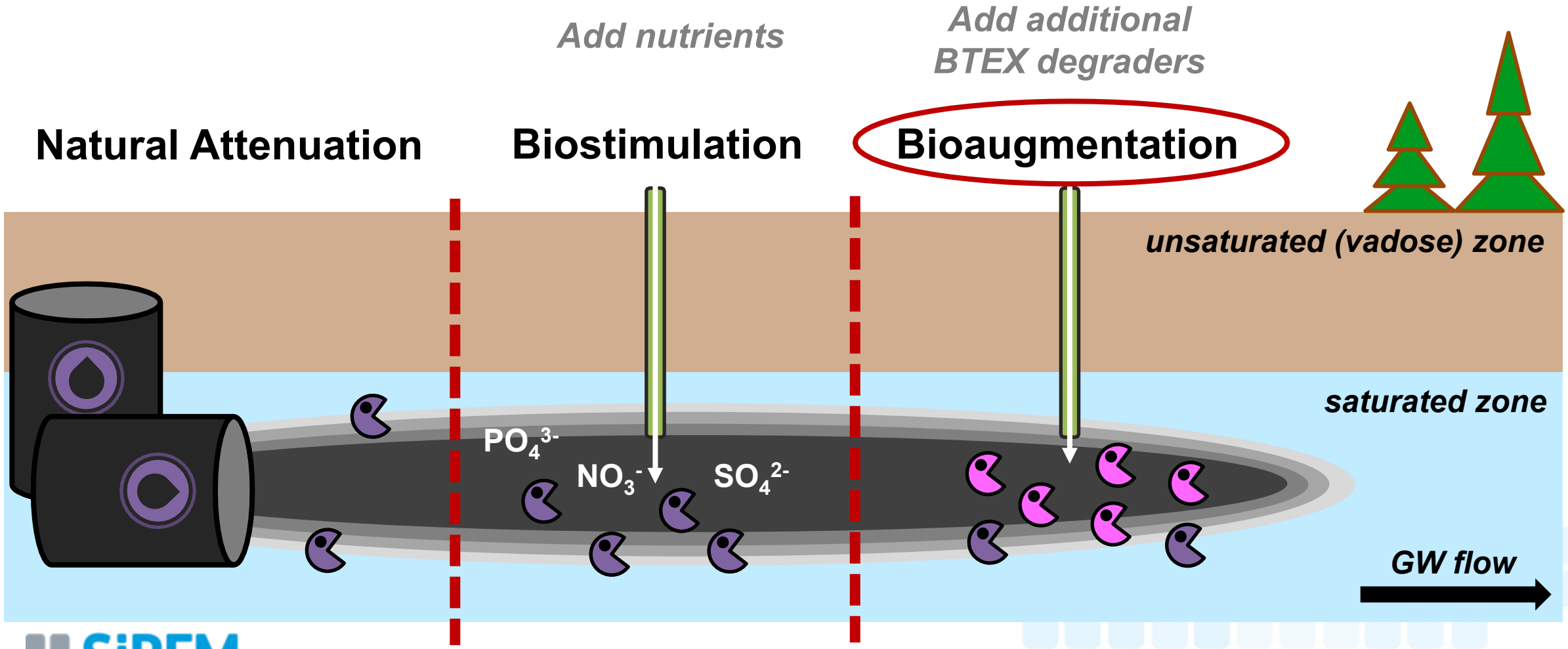
# How Can We Reliably Increase Concentrations of BTEX Degraders?

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# 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.

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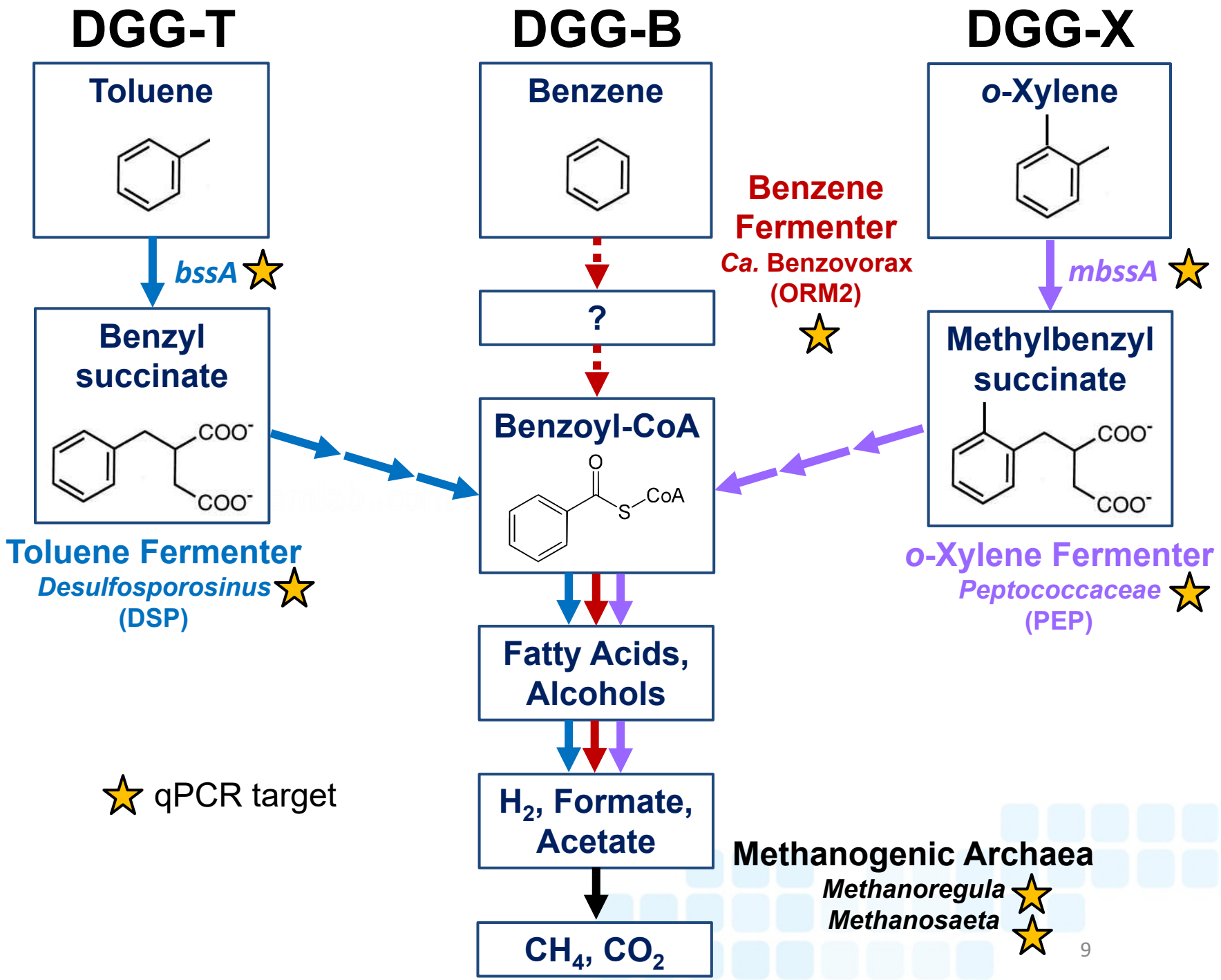
- The key microbes in each culture include one **hydrocarbon fermenter** and **2 methanogens**
- Key microbes & functional genes are monitored by **qPCR** and **NGS**
- Metagenomes have been sequenced and **reconstructed genomes** are being analyzed

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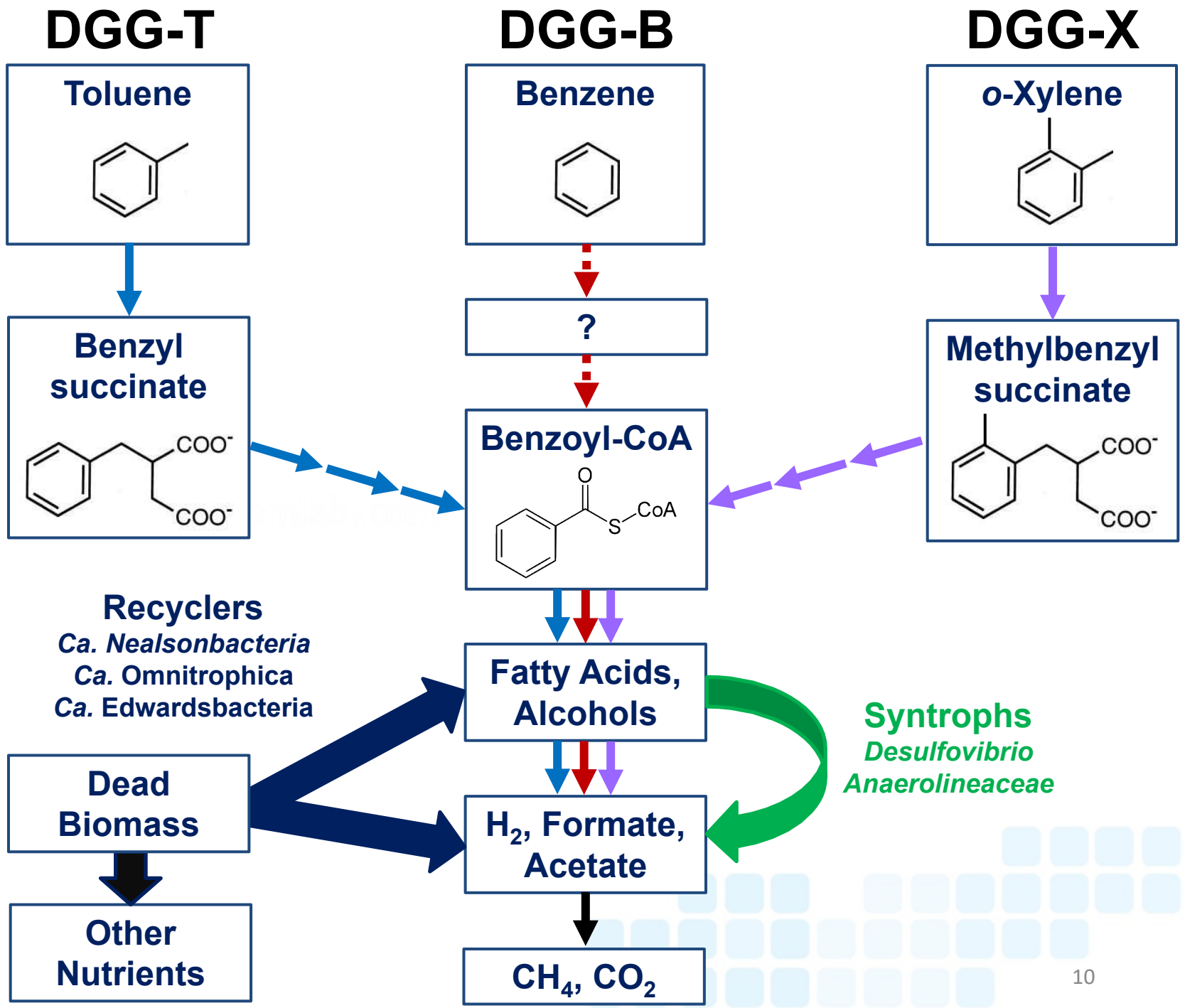
- "**Syntrophs**" help metabolize fermentation intermediates

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



# DGG-Plus Bioaugmentation Cultures

DGG B™

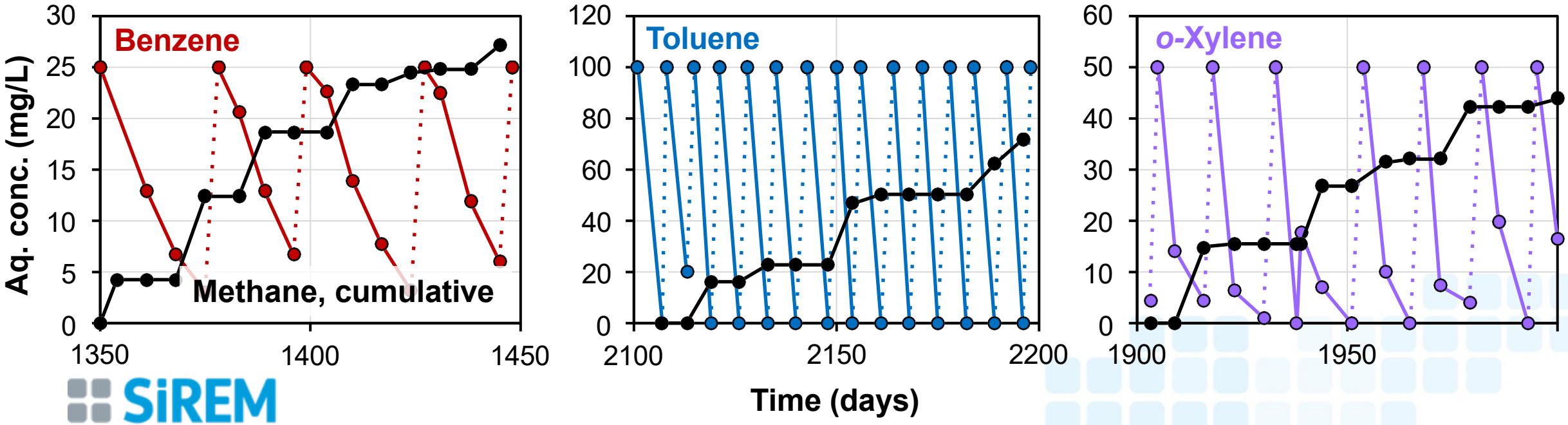
Degr' rate = ~ **1.3 mg/L/day**

DGG T™

Degr' rate = ~ **25 mg/L/day**

DGG X™

Degr' rate = ~ **9 mg/L/day**

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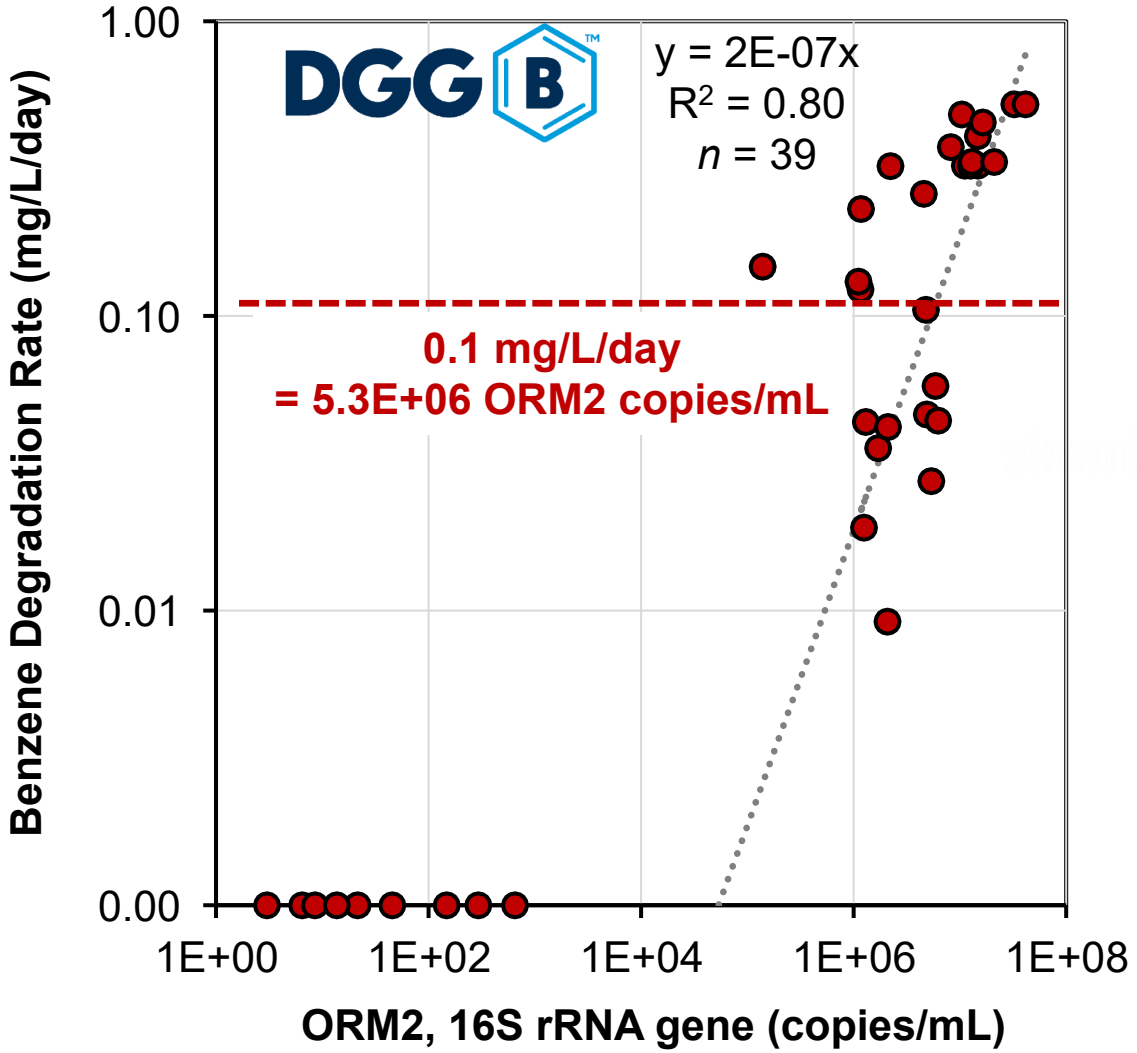
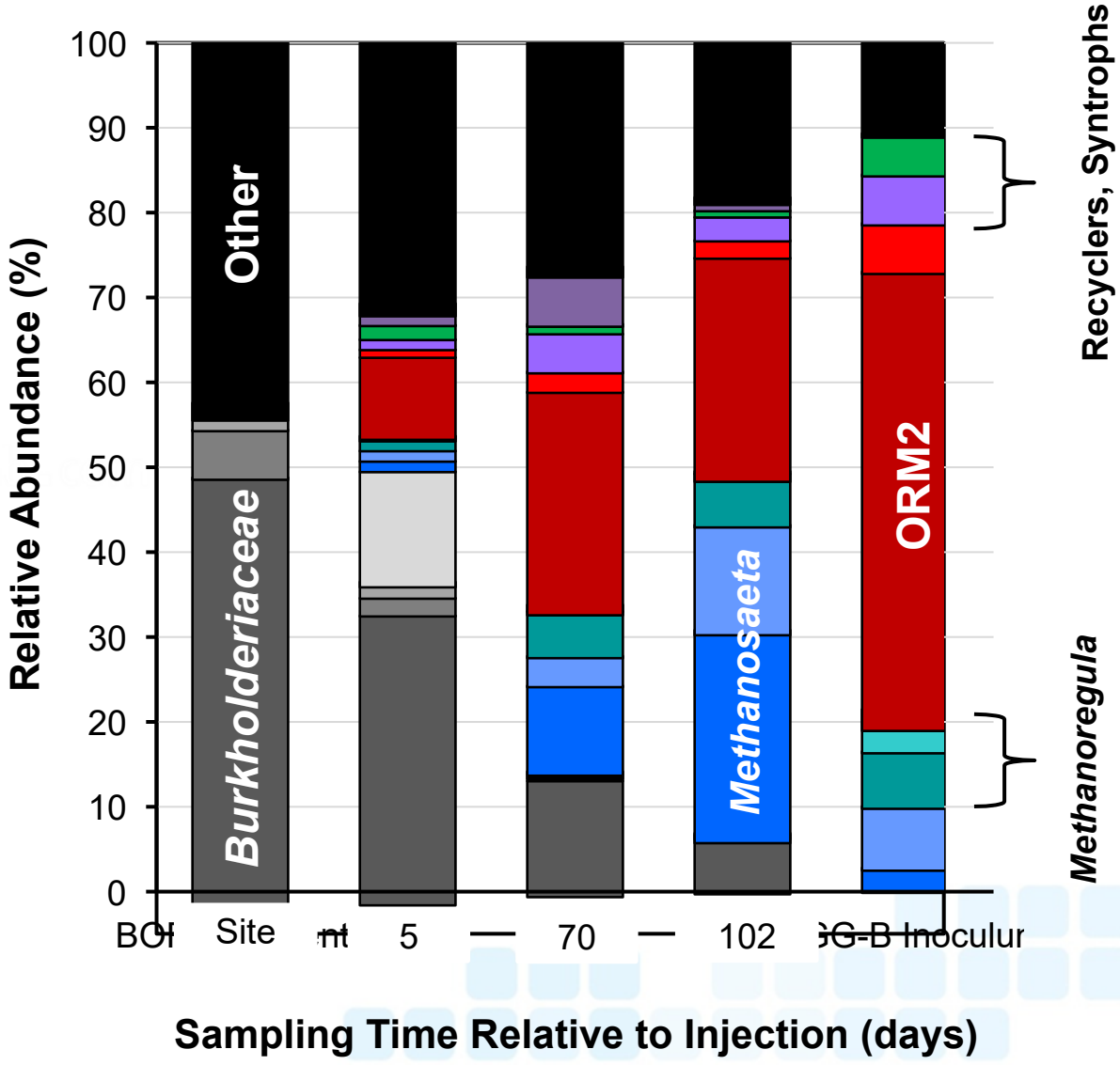
# *In DGG-B, rates of benzene degradation correlate with conc. of ORM2.*

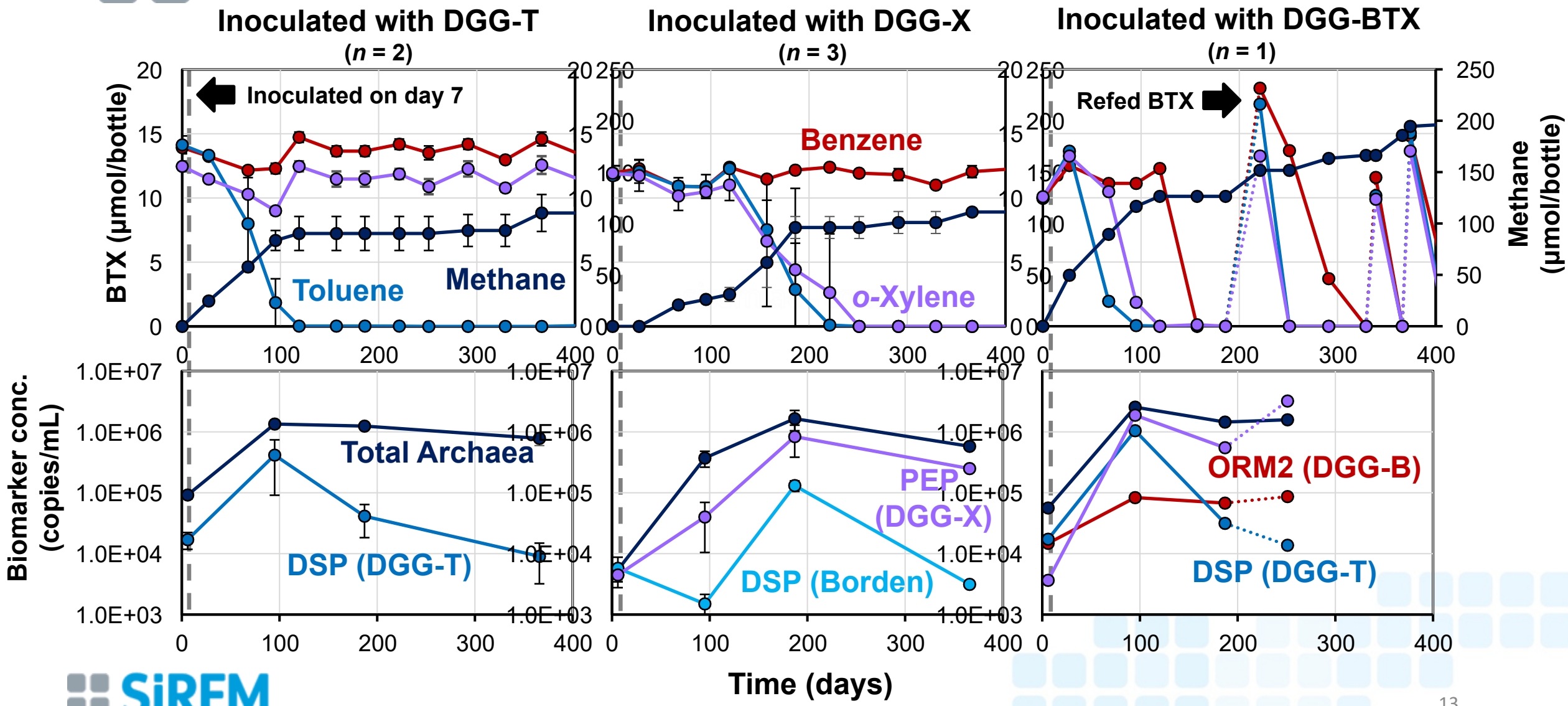
*Growth of methanogens, syntrophs & recyclers is also needed to sustain degradation.*




Toth et al. 2021 (Environ Sci Technol 55)

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

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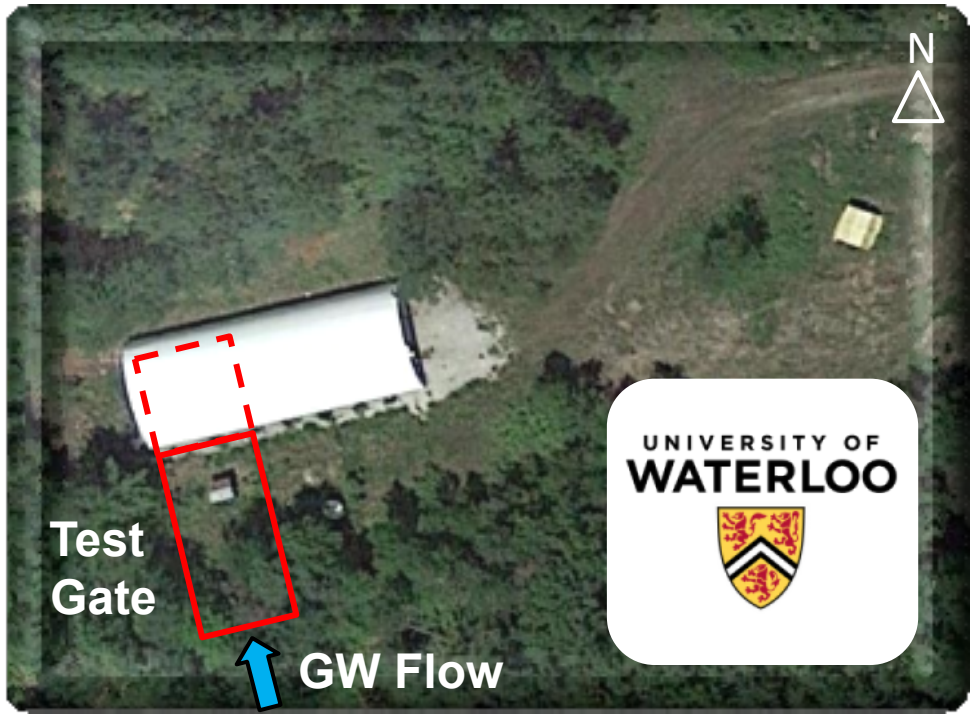
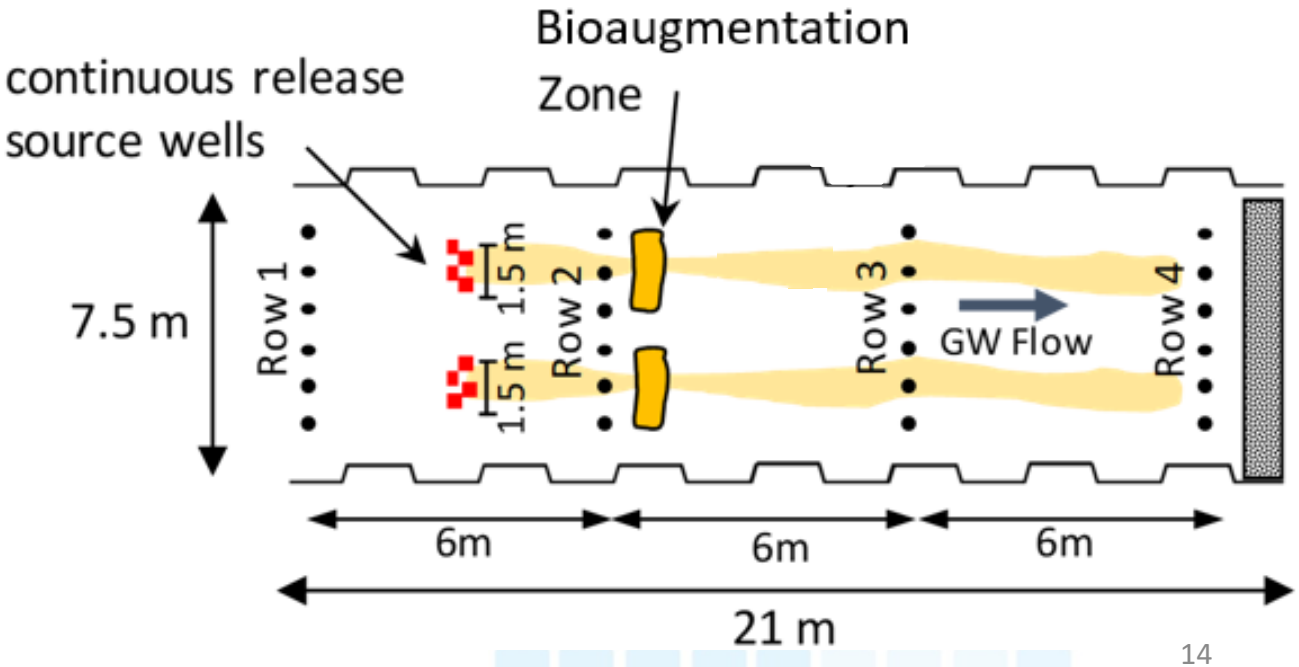
# Upcoming Field Pilot at CFB Borden

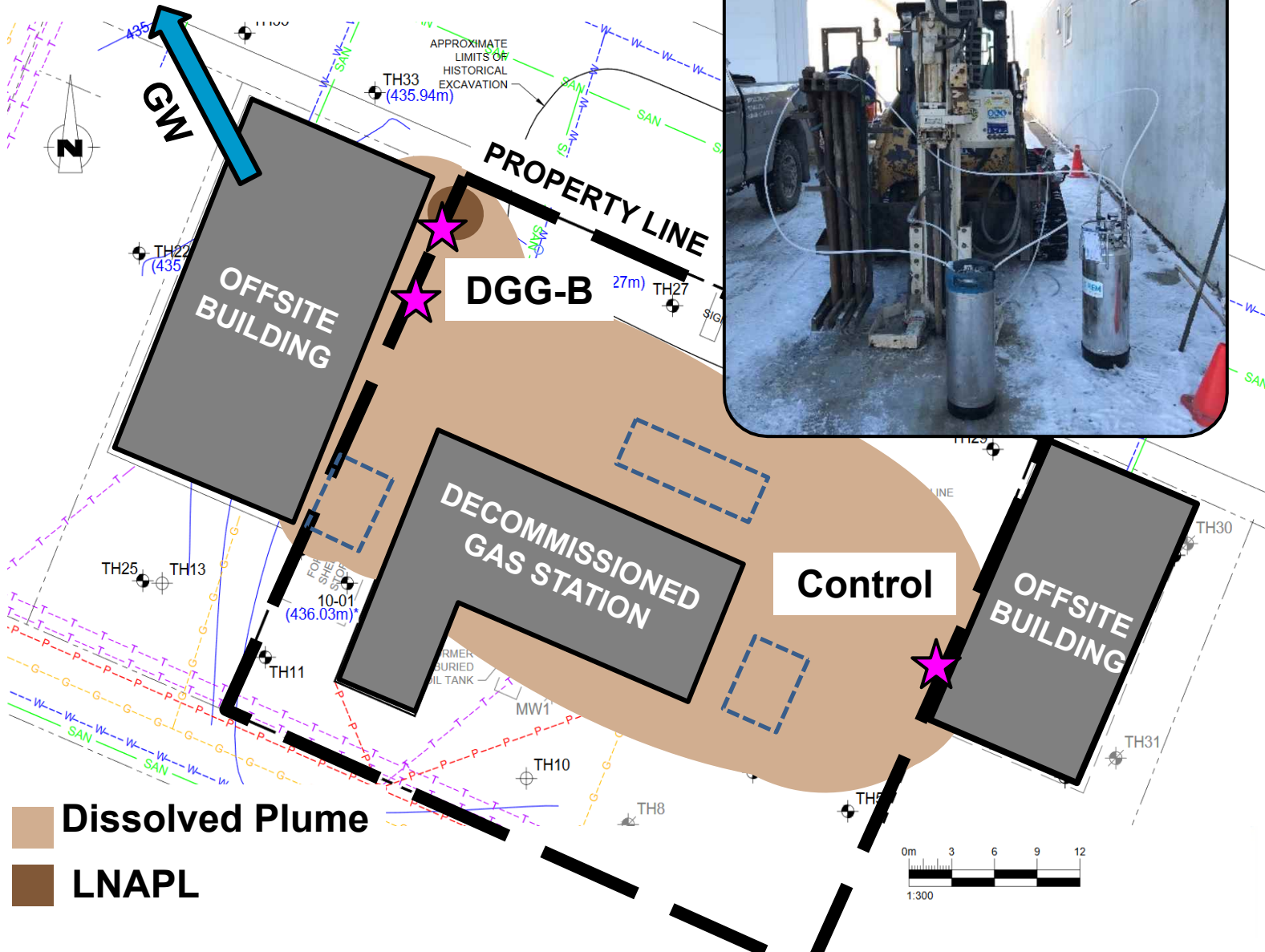
- Pilot consists of a gated system supplied with BTEX and includes 2 test lanes
  - **Lane 1** will be injected with **DGG-BTX** (enhanced BTX degradation expected)
  - **Lane 2** will only receive **DGG-T** (only toluene degradation expected)
  - Ethylbenzene = negative control (no enhanced degradation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



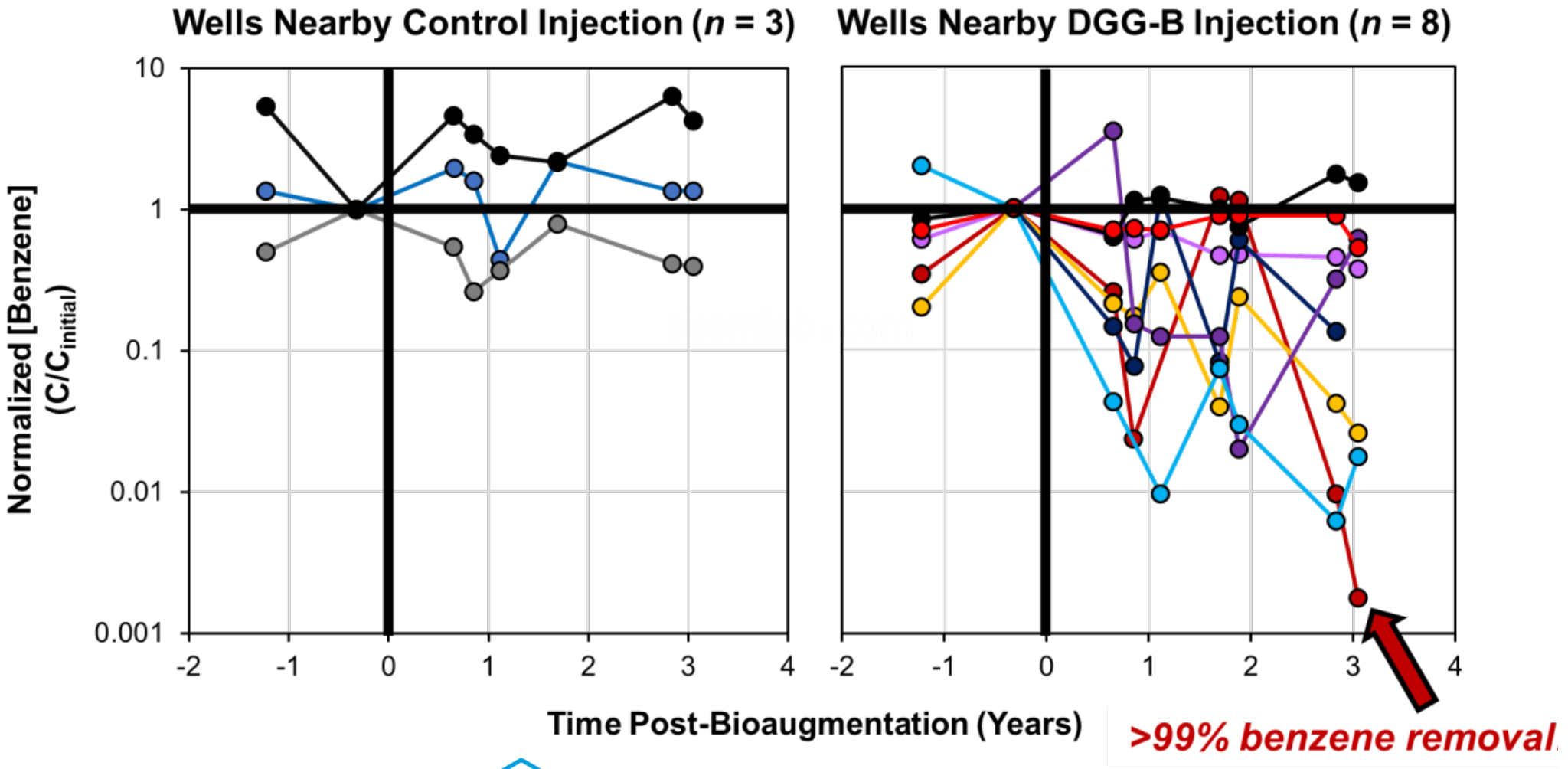



# SK Site Bioaugmentation

- 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

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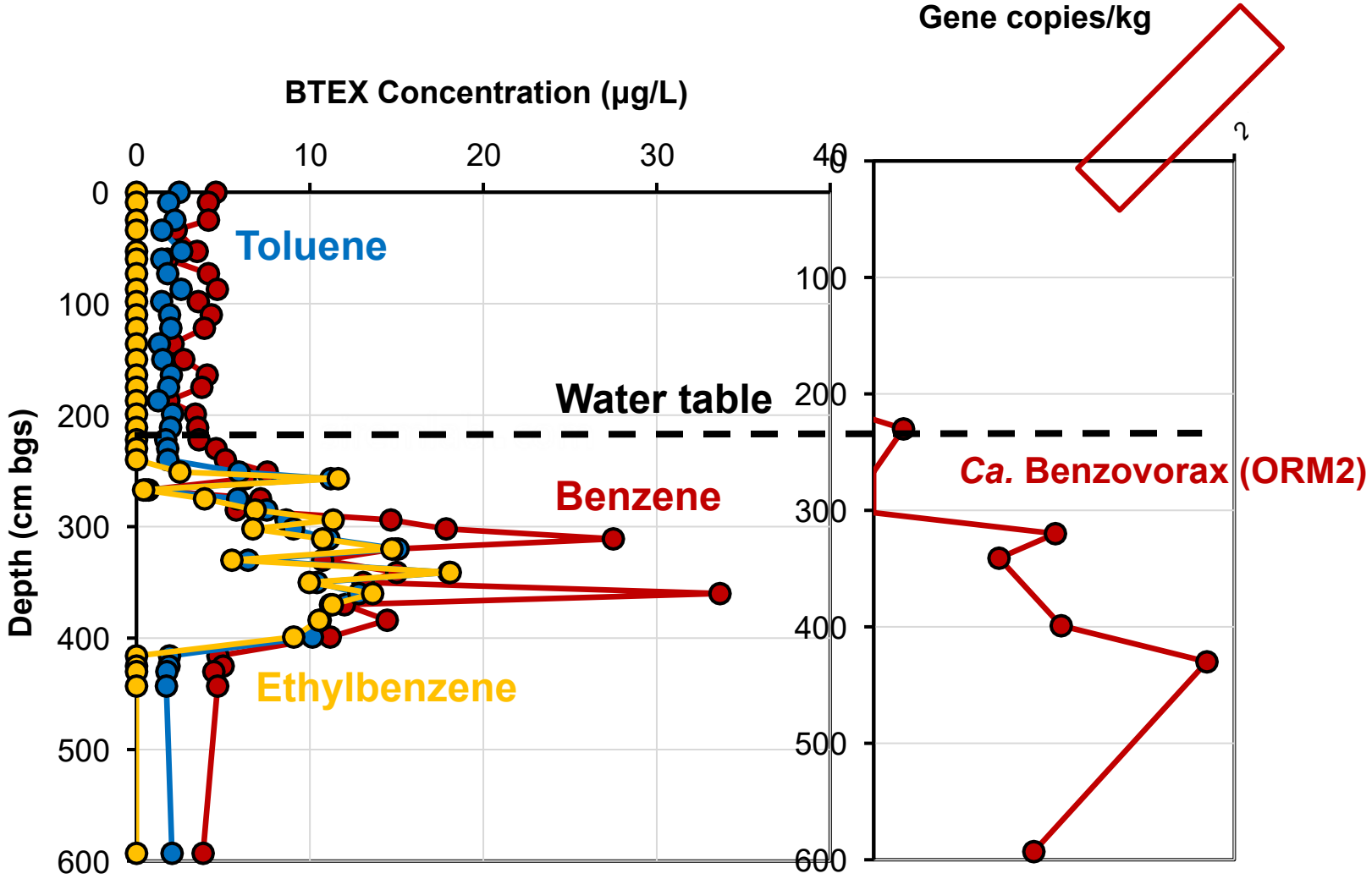
DGG PLUS™

# *Ca.* Benzovorax vs Benzene in Cores from SK site

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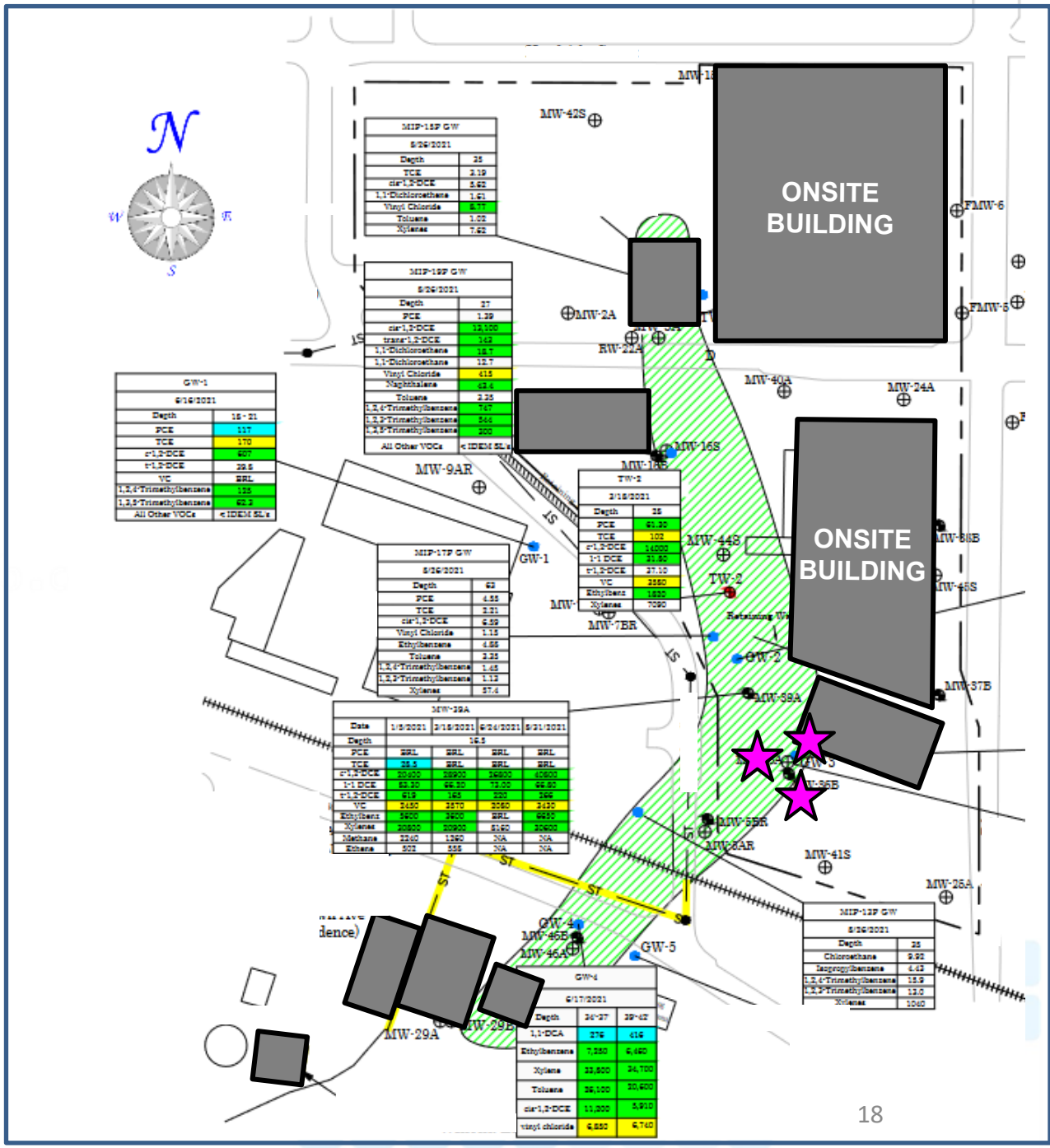
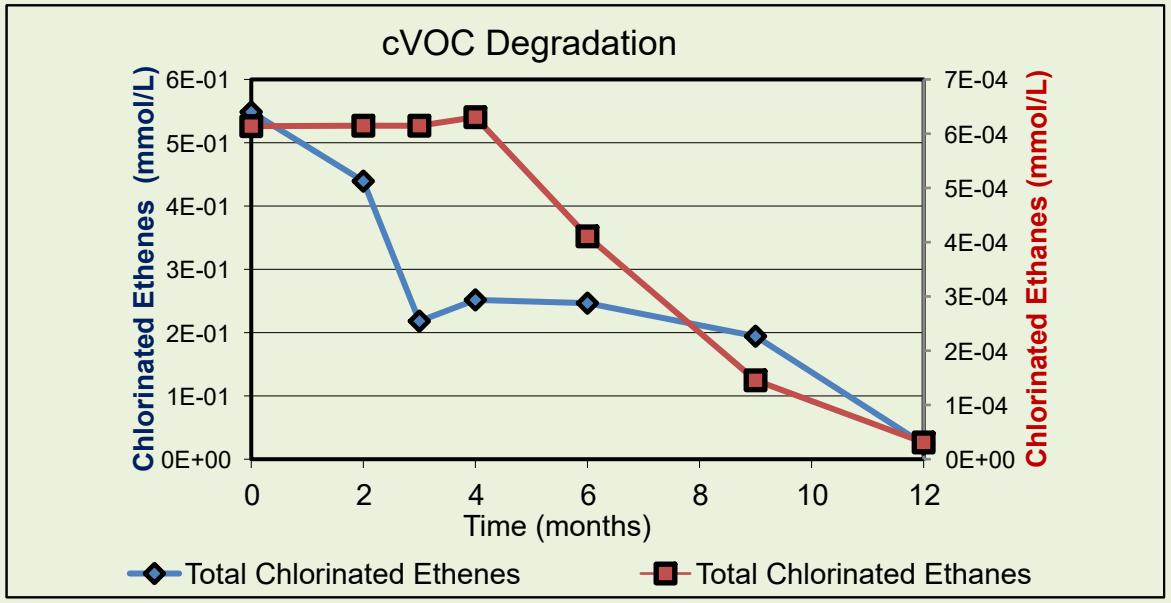
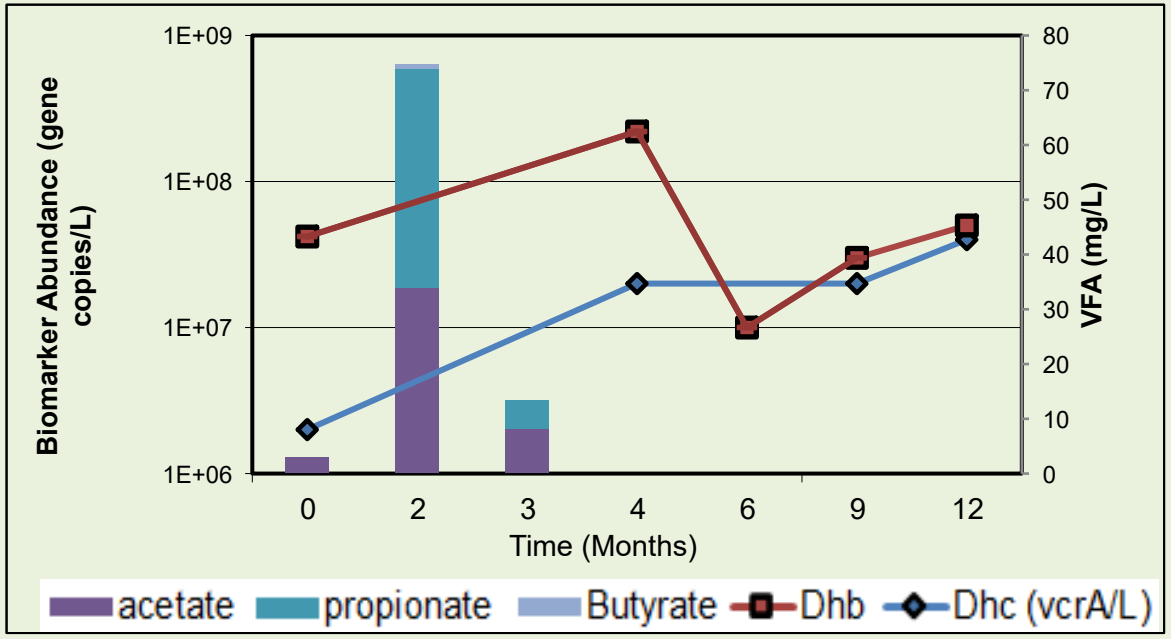
# US Field Pilot

- 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

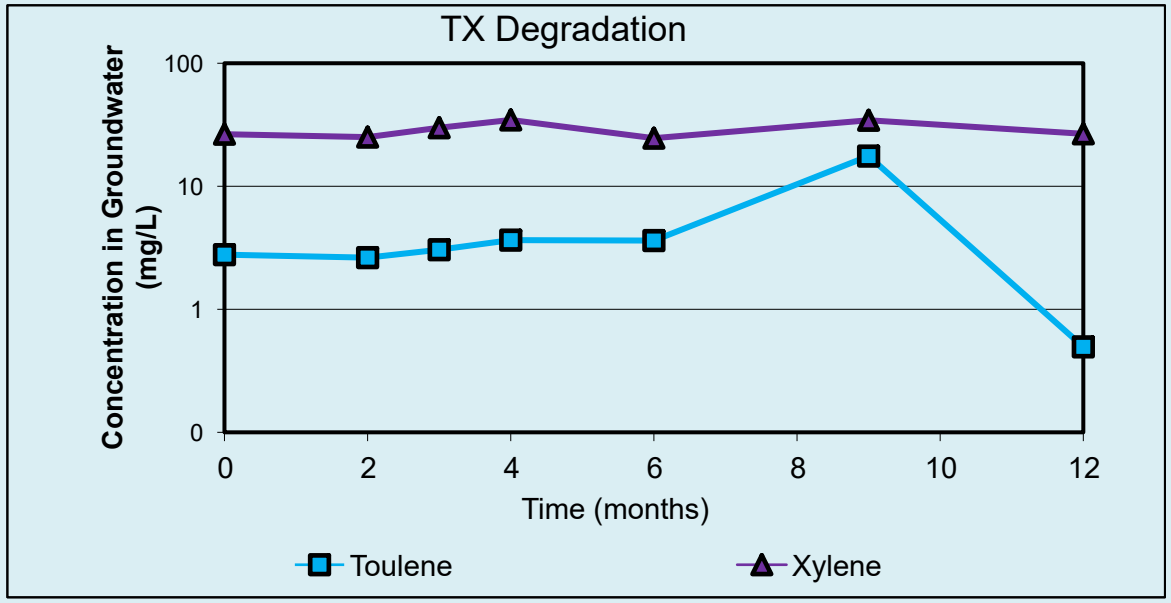


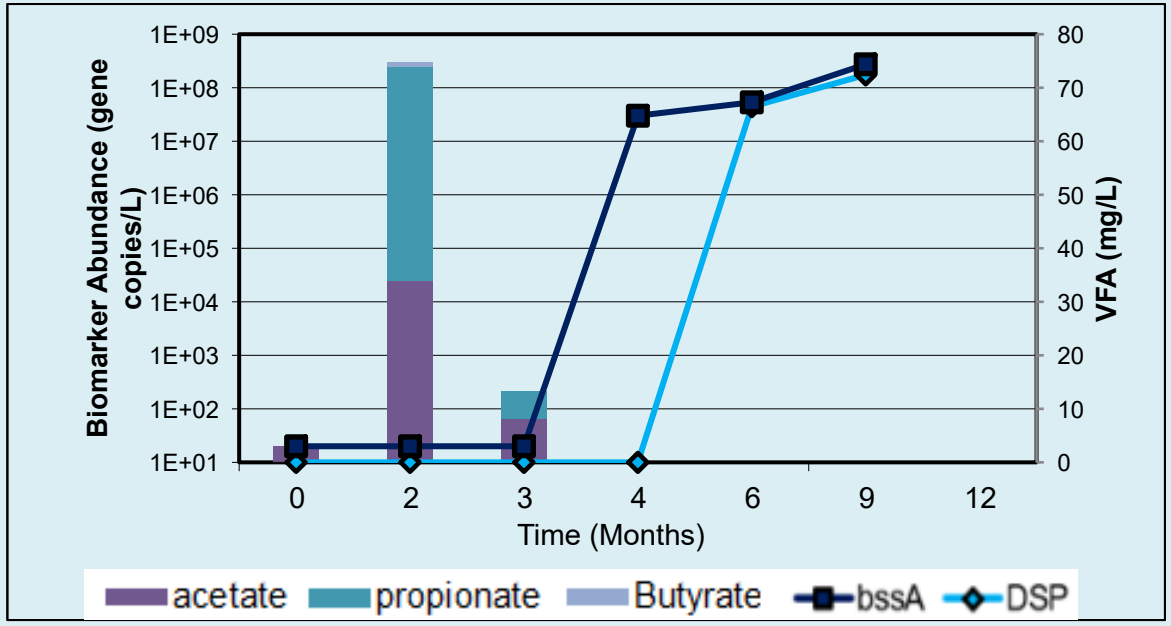
# CHLORINATED ETHENES & ETHANES




# 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 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

Ontario

- Culture applications performed in Ontario, Saskatchewan and British Columbia and 3 US States

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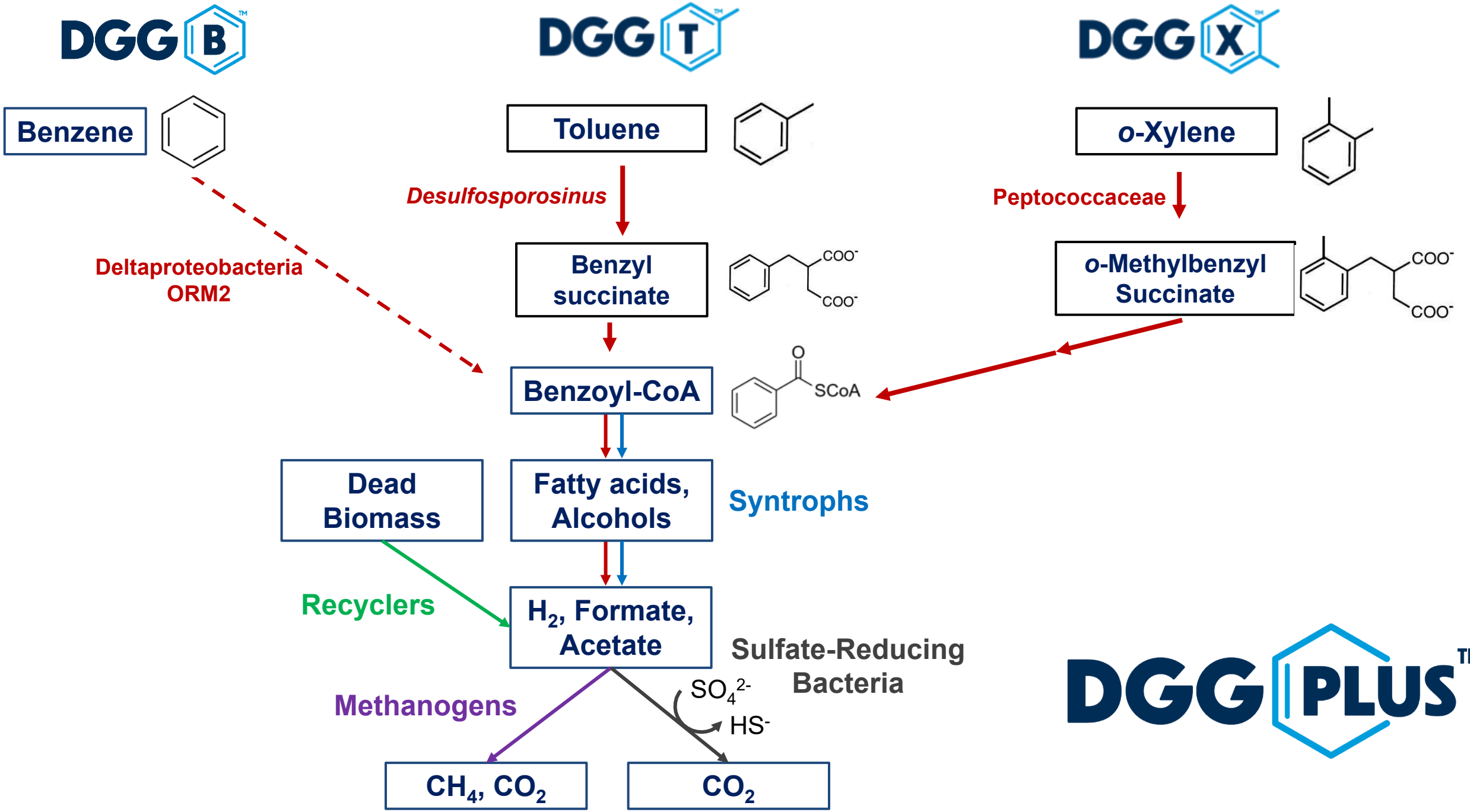
DGGB
DGGT
DGGX
Benzene
Toluene
o-Xylene
Desulfosporosinus
Peptococcaceae
Deltaproteobacteria
ORM2
Benzyl
succinate
o-Methylbenzyl
Succinate
COO-
COO-
Benzoyl-CoA
O
SCoA
Dead
Biomass
Fatty acids,
Alcohols
Syntrophs
Recyclers
H2, Formate,
Acetate
Sulfate-Reducing
Bacteria
Methanogens
SO4 2-
HS-
CH4, CO2
CO2
DGGPLUS™

# Take Home Message

- Microbes responsible for anaerobic benzene 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.

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# Questions?
## siremlab.com

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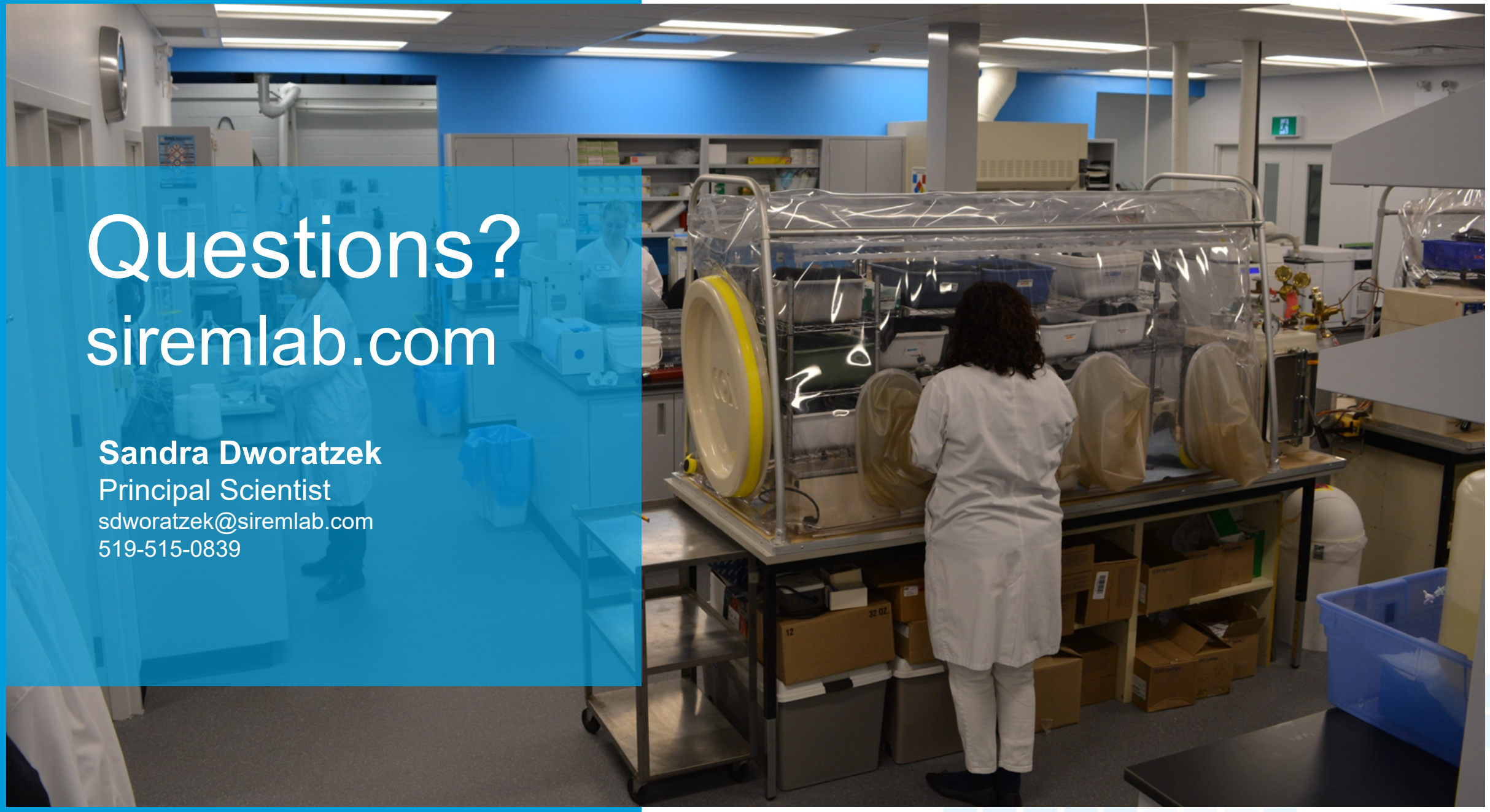

# US Field Pilot KB-1 / DGG-Plus