# Breaking Benzene: Translating Decades of Anaerobic Bioremediation Research to Field Practice

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# Participating and Funding Organizations

BioZone
Centre for Applied Bioscience and Bioengineering

SiREM
Leading Science · Lasting Solutions

GenomeCanada

Ontario Genomics

UNIVERSITY OF TORONTO

Imperial

Ontario

UNIVERSITY OF ALBERTA

CO-OP

Mitacs

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InnoTech ALBERTA

ALBERTA INNOVATES

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Civil and Environmental Engineering, University of Waterloo

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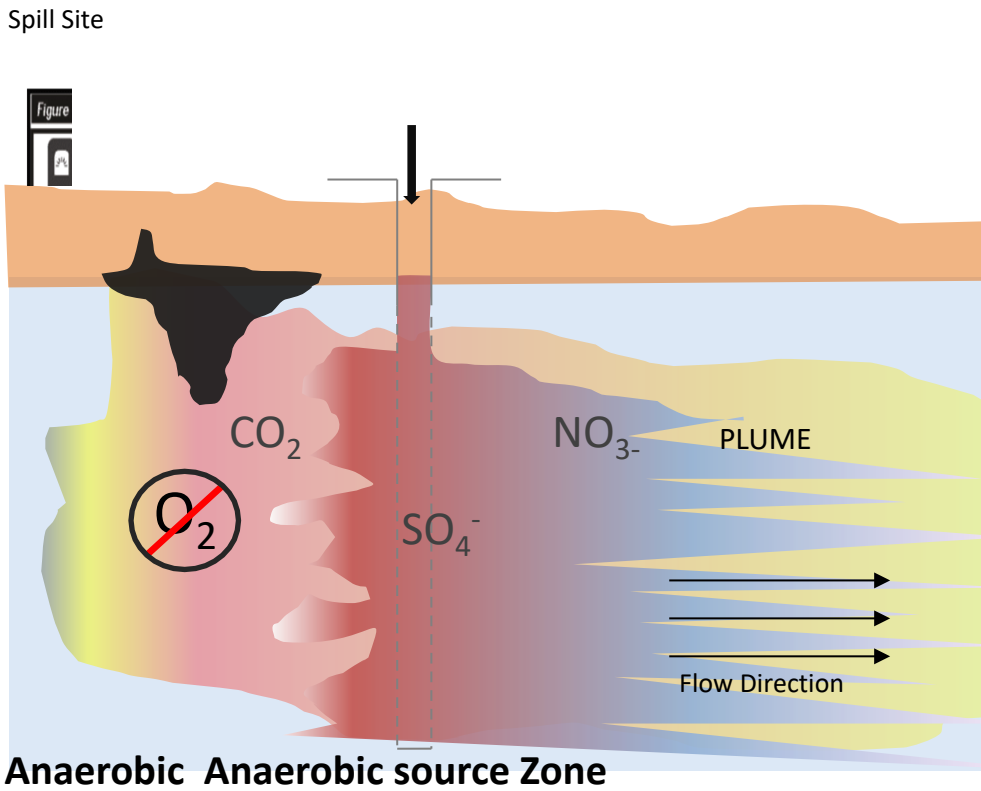
Innotech Alberta, Edmonton ON

***…and many others!***

# Why Go Anaerobic for BTEX?

- Hydrocarbon sites often anaerobic
  - High organic loading consumes $O_2$
  - Adding $O_2$ is challenging
- 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., epsom salts (sulfate)



# What Limits BTEX Biodegradation in Groundwater?

Hydrocarbon Properties? ✖

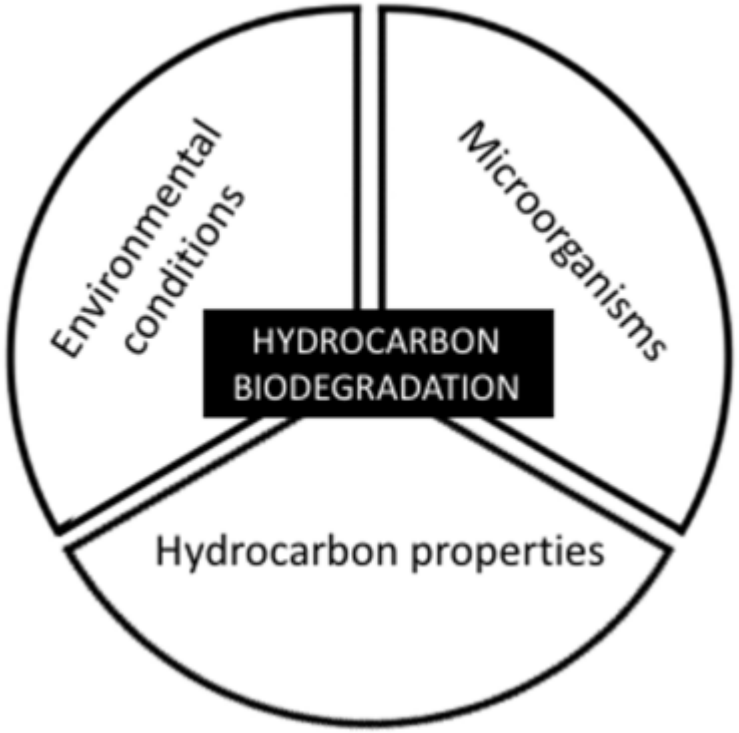
- BTEX is susceptible to biodegradation

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***



# Benzene-Degrading Enrichment Cultures

Elizabeth Edwards
Prof, UofT

Contaminated Site Material (1995)
(>20 Years of Enrichment in the Lab)

20+ years

**Site 1**

Swamp

$Sw\text{-}NO_3$

**Site 2**

Cartwright

$Cart\text{-}NO_3$

$Cart\text{-}SO_4$

$Cart\text{-}CH_4$

**Site 3**

Oil Refinery (OR)

$OR\text{-}CH_4$

Degrade: Benzene, Benzoate, Toluene, Phenol

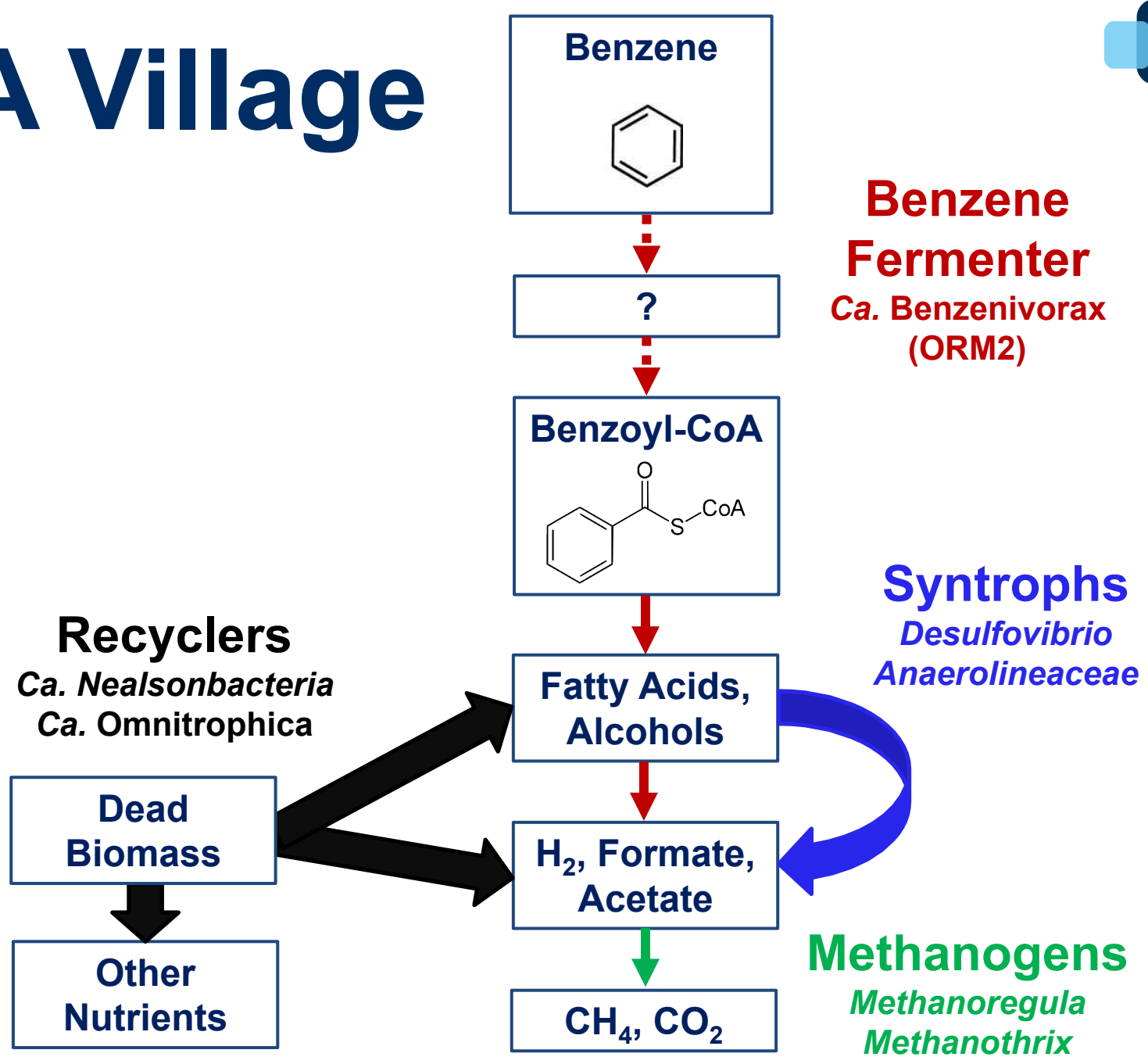
Degrade: Benzene

Nitrate-reducing

Sulfate-reducing or methanogenic

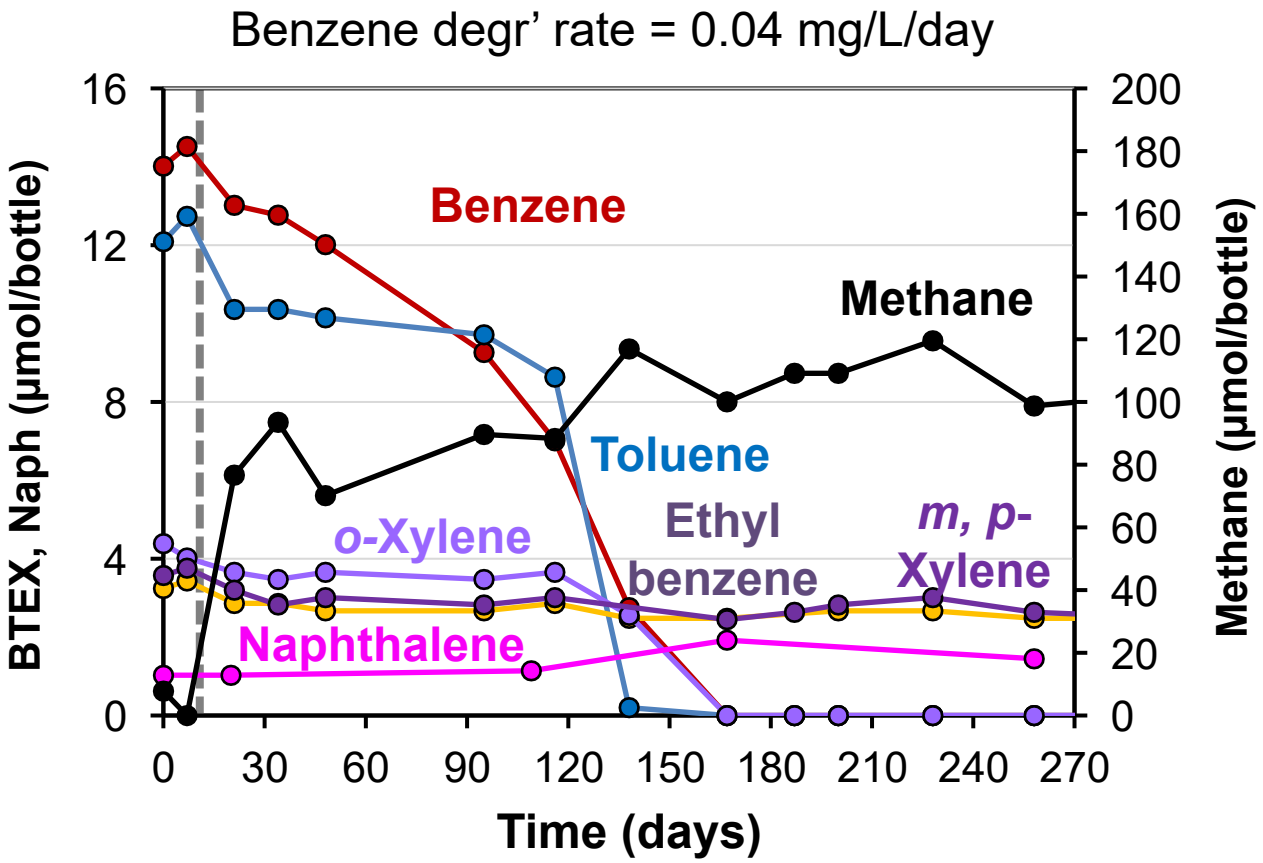
# It Takes A Village

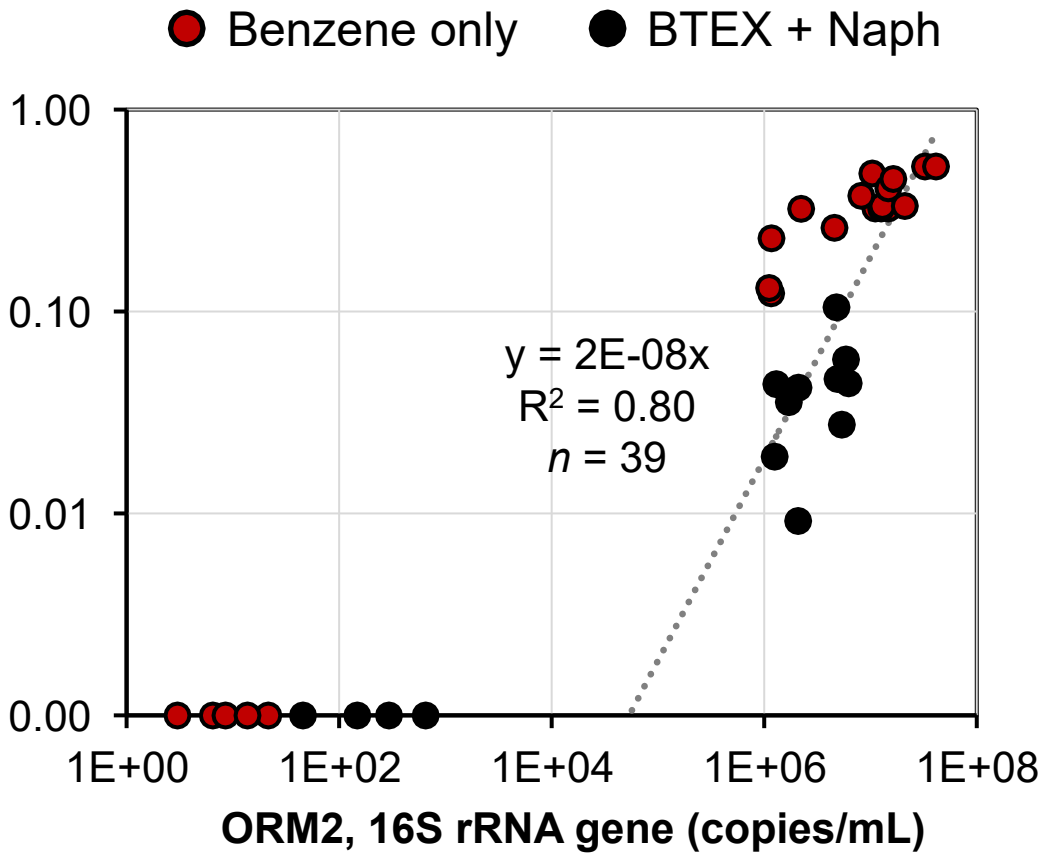
- "Syntrophs" help metabolize fermentation intermediates
- "Recyclers", convert dead biomass (proteins, carbohydrates, etc.) into fermentation intermediates and other nutrients



# Can We Make Things Better?

***Ca. Benzenivorax (ORM2) growth slows in the presence of hydrocarbon co-contaminants (especially toluene), resulting in slower rates of benzene degradation. <u>But why</u>?***



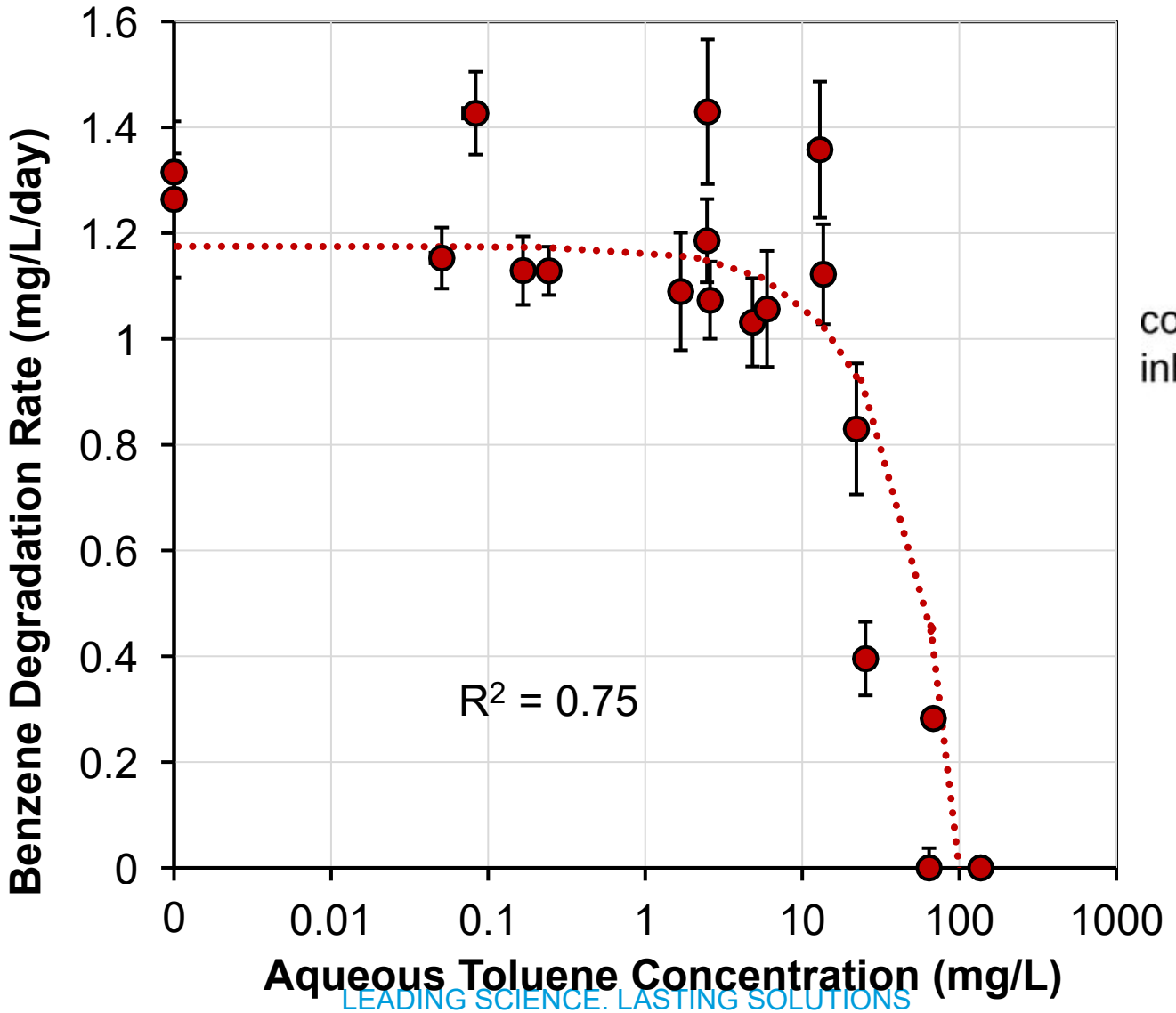


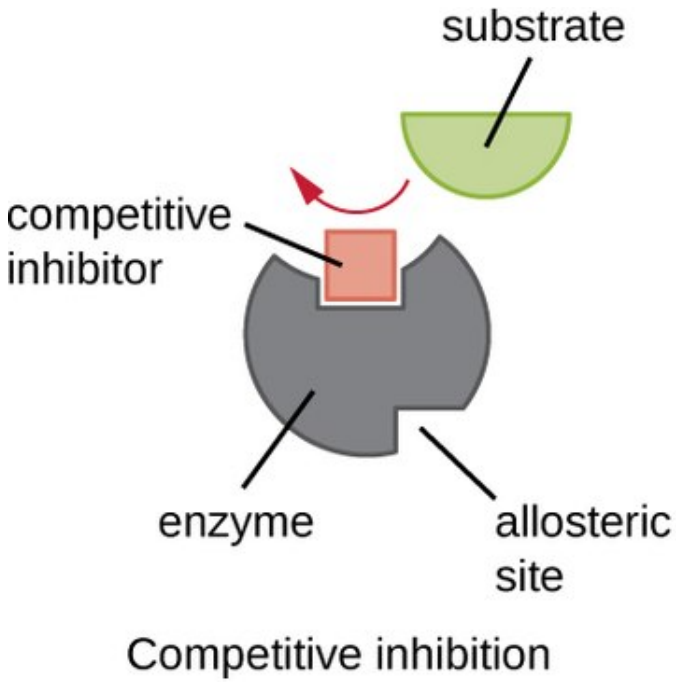

**Toth** et al. 2021 (Environ Sci Technol 55)

# Recent data suggests that toluene is a competitive inhibitor.

**Connie Zeng**
**BSc Student**

10 mg/L benzene
0 – 100 mg/L toluene



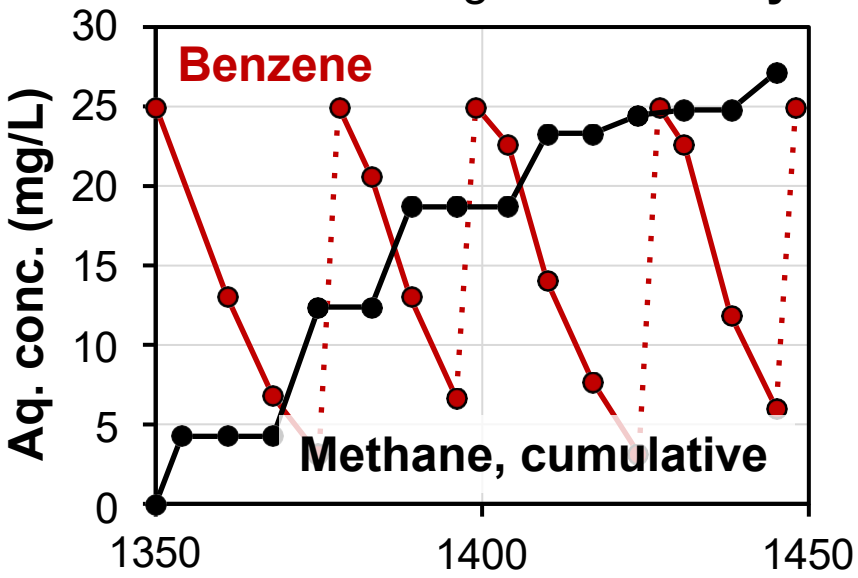




Competitive inhibition

# DGG PLUS™ Bioaugmentation Cultures

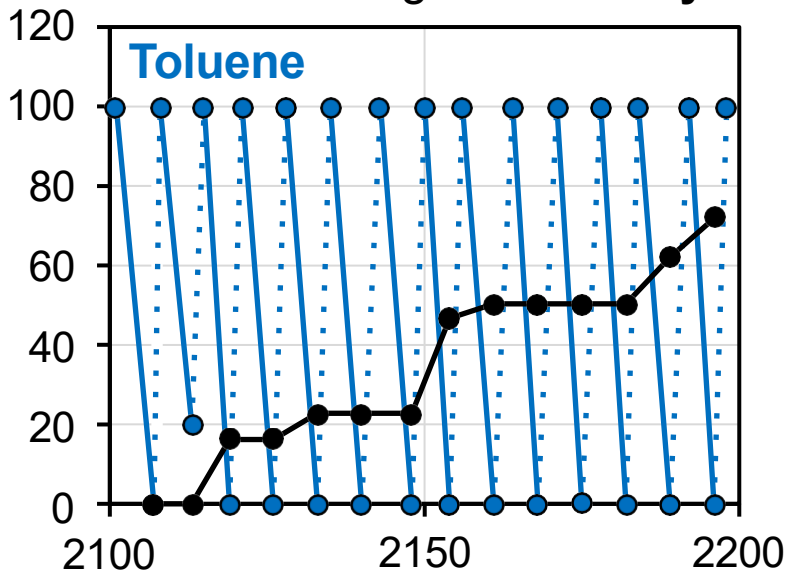
## DGG B™

Degr' rate = up to **3 mg/L/day**
ORM2 doubling time ~ **30 days**



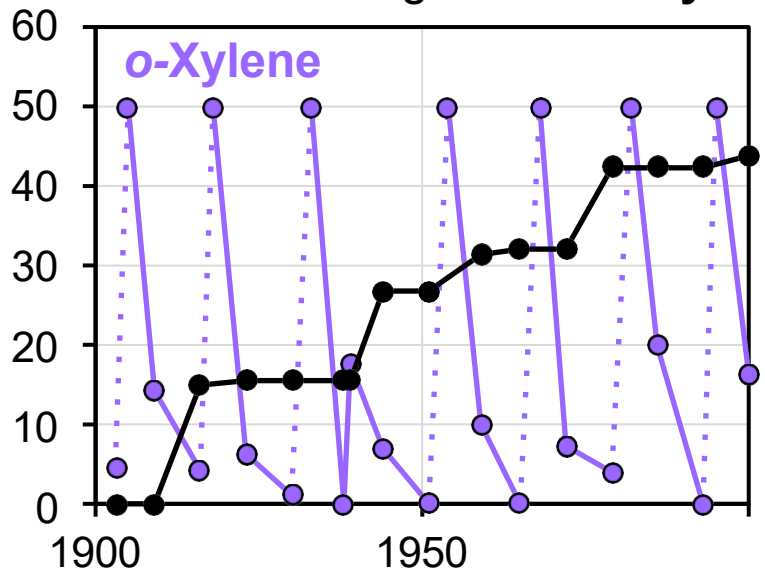

## DGG T™

Degr' rate = up to **48 mg/L/day**
DSP doubling time ~ **3 days**




## DGG X™

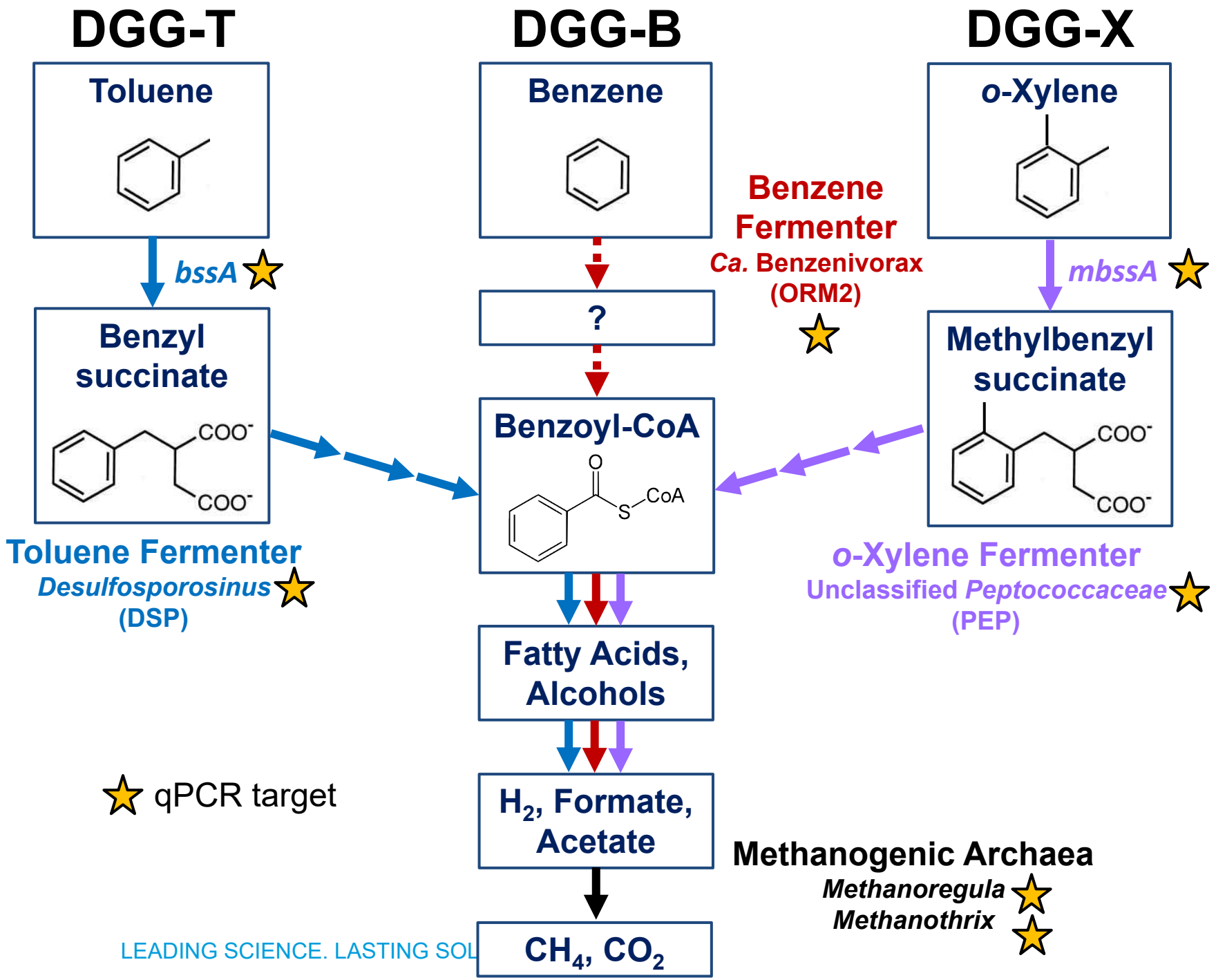
Degr' rate = up to **11 mg/L/day**
PEP doubling time ~ **7 days**



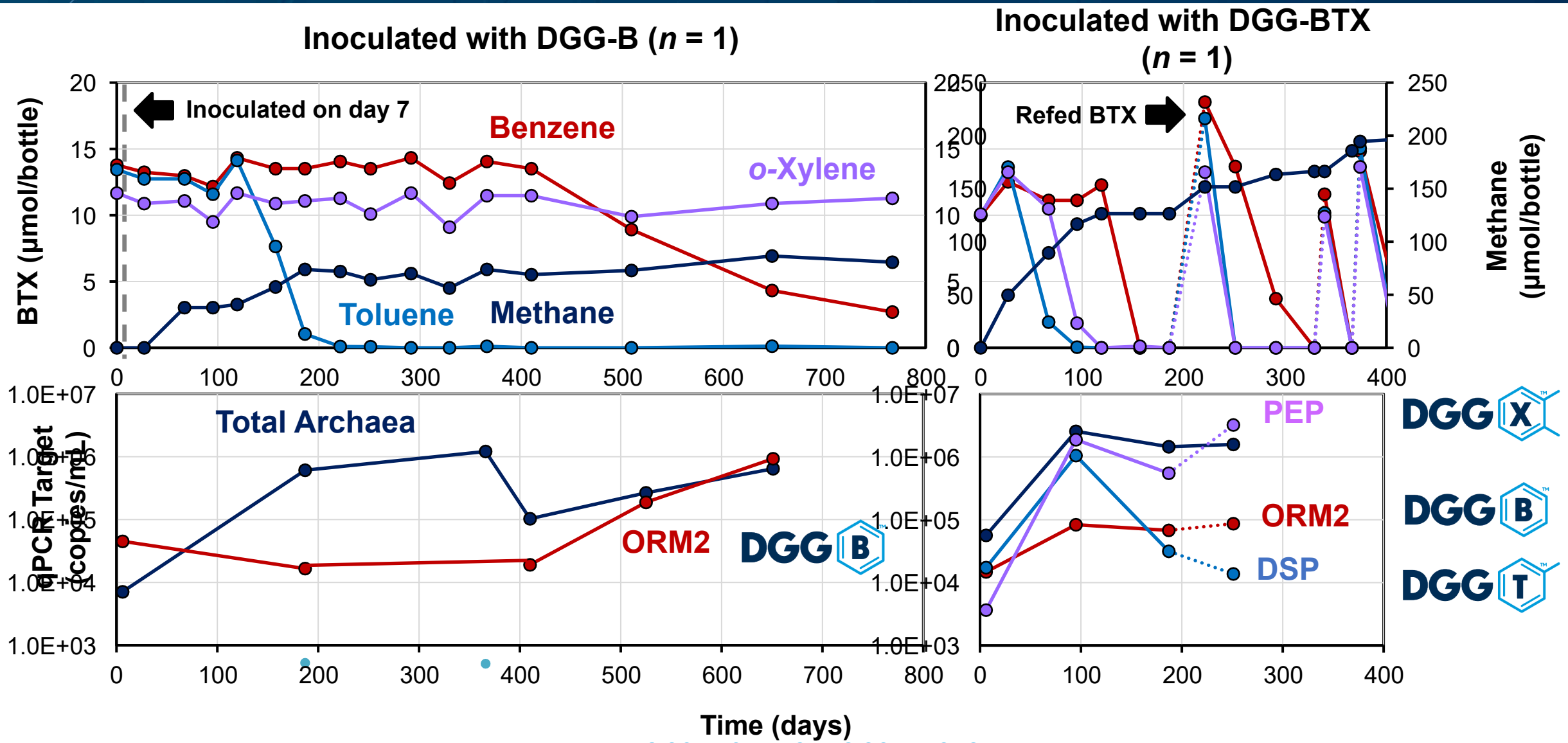

**Time (days)**

Edwards and Grbić-Galić 1994 (Appl Environ Microbiol 60),
**Toth** et al. (data in preparation)

- 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



# *Treatability studies support that co-contaminant treatment can improve the likelihood of benzene remediation success.*

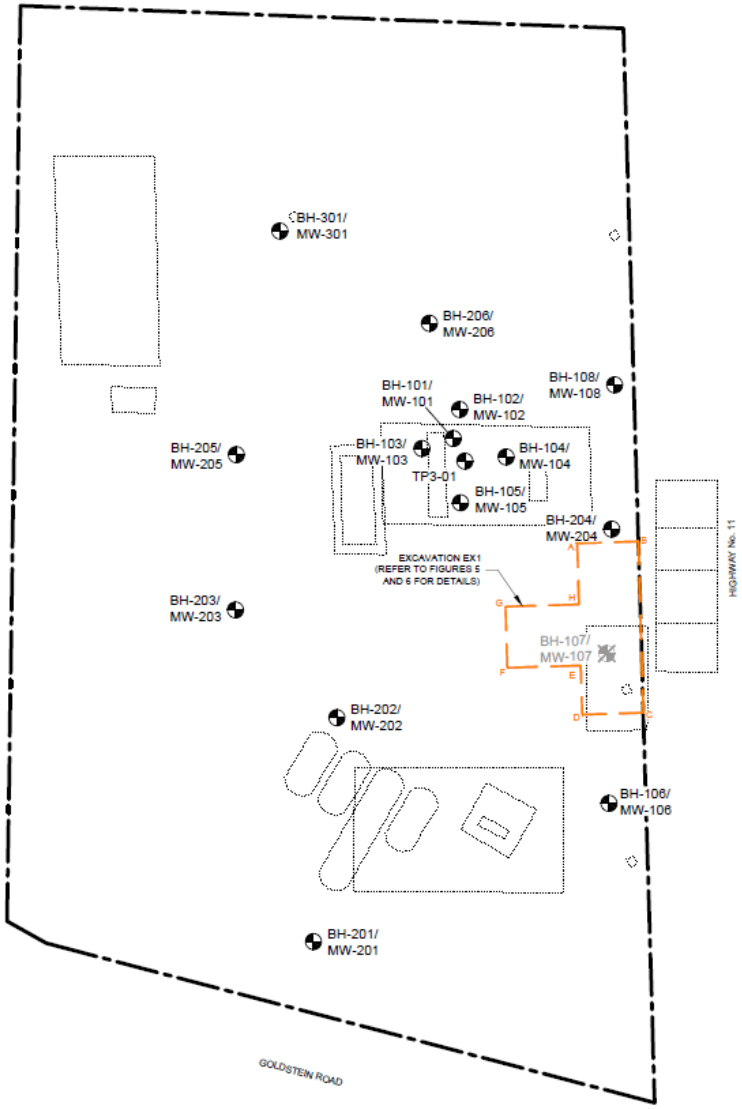



LEADING SCIENCE. LASTING SOLUTIONS

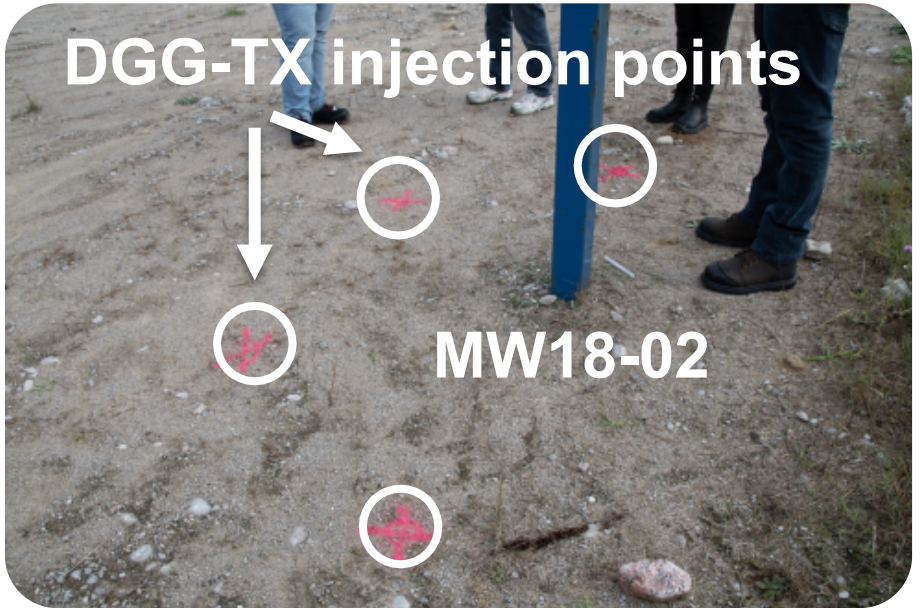
**Toth** et al. (data in preparation)

# Case Study: Ontario, Canada Site

- **1948–1995**: Operated as a highway gas station
- **May 1996**: Decommissioned & excavated ~15 feet bgs
  - Hydrocarbon vapours detected along east side of property
  - Soil & groundwater exceed TPH regulatory limits
- **Feb 2001**: Groundwater monitoring program begins
  - Intrinsic bioremediation cleaned up most of site
  - TPH contamination at BH-107 persisted
- **Dec 2018**: Soil around BH-107 excavated & backfilled
  - A new well was installed (MW18-02)
  - TPH contamination still detected post-excavation
- **Sep 2022**: DGG-TX was injected around MW18-02



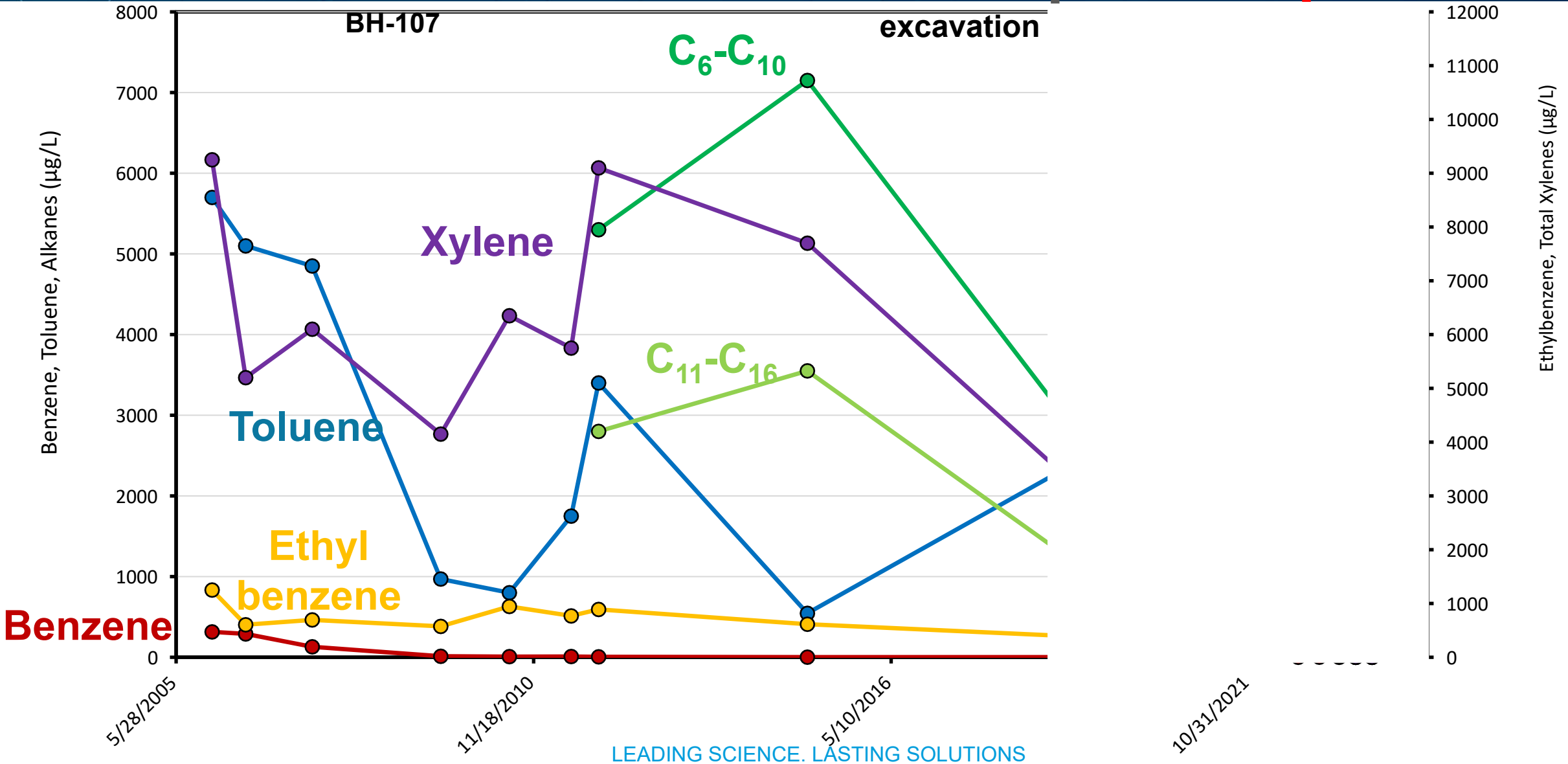
# ON Site: Culture Injection (Sep-2022)


DGG PLUS™

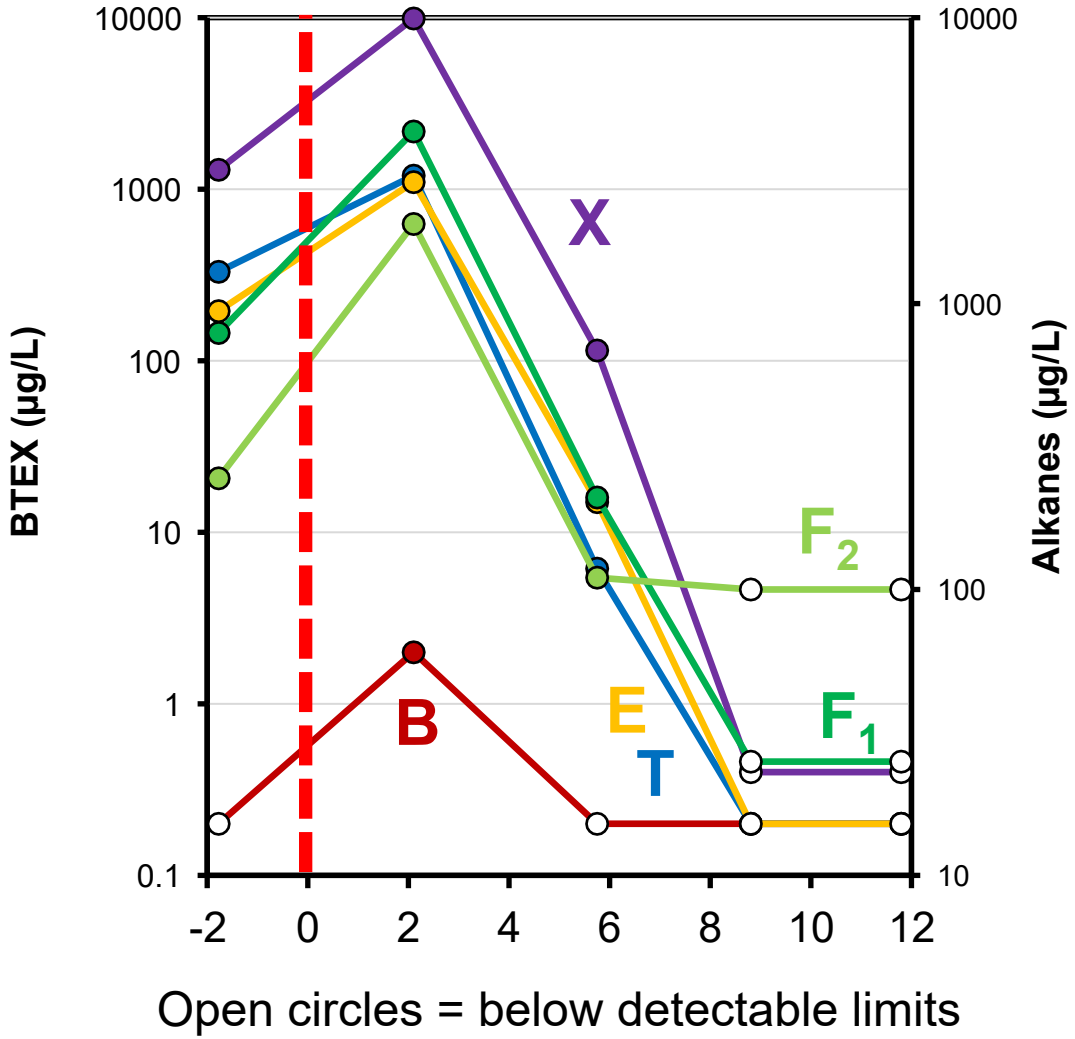
; SCIENCE. LASTING SOLUTIONS

# ON Site: Historical Groundwater Trends



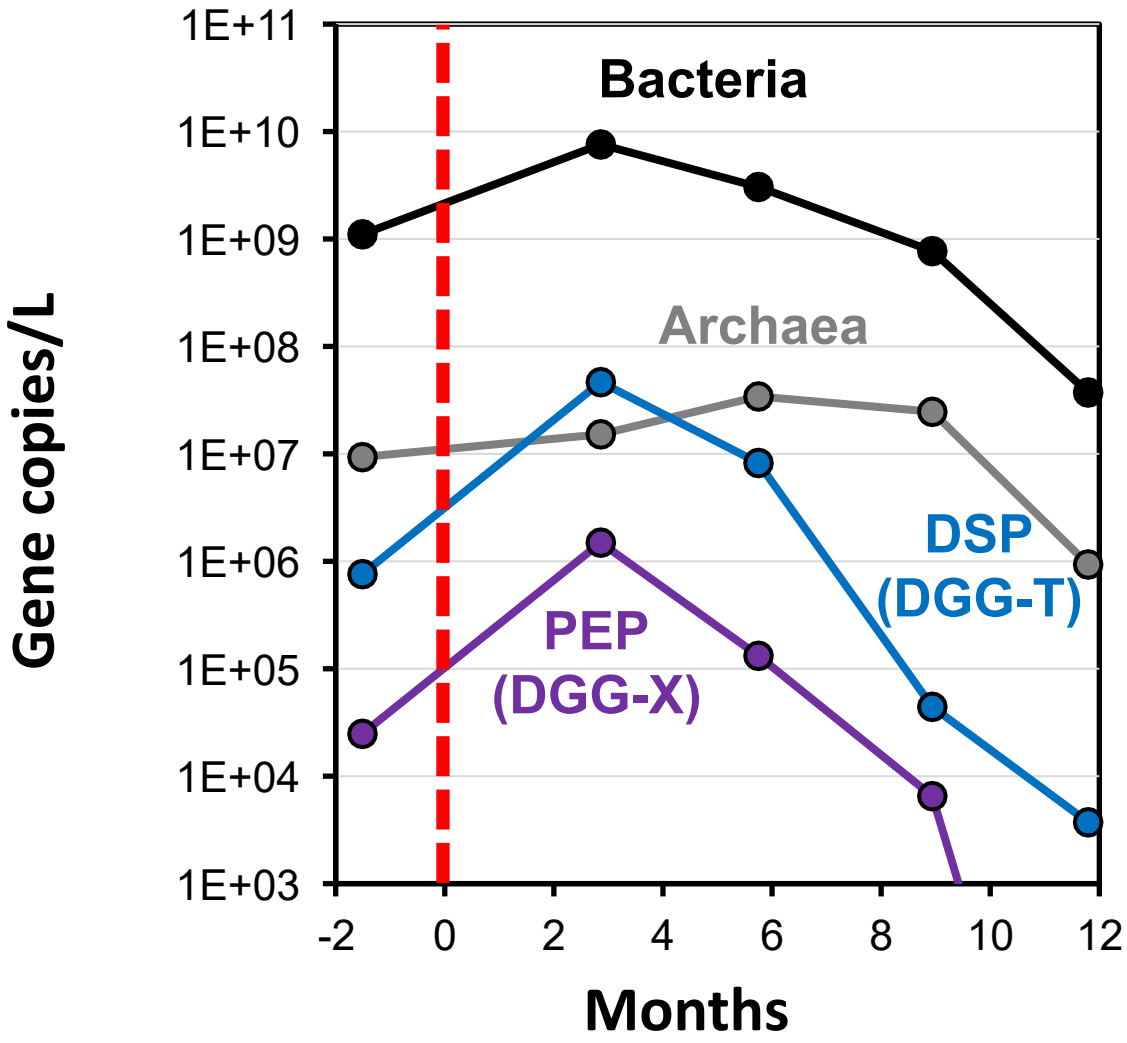
# Post-Bioaugmentation Results: MW18-02

- BTEX, alkanes and naphthalene undetectable in groundwater within 9 months of DGG-TX culture injection
- Only decreases in toluene and xylene were expected!

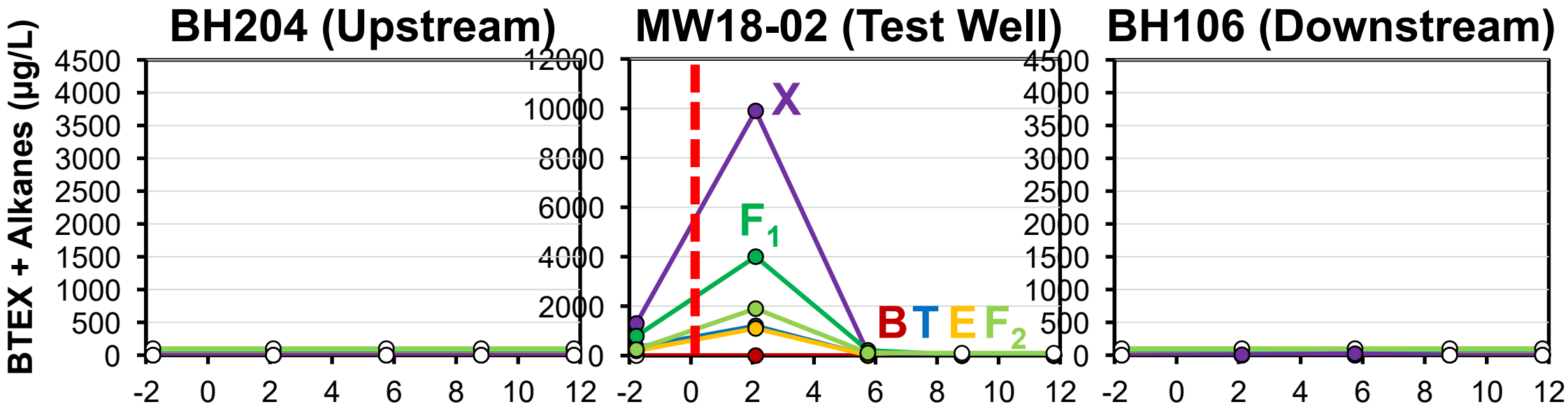


# Biomarker Assessment

- After bioaugmentation abundance of toluene (DSP) and xylene (PEP) degraders increased~60 fold
- Growth & decay of key biomarkers coincided with TPH trends.



# Did the Contamination Migrate?

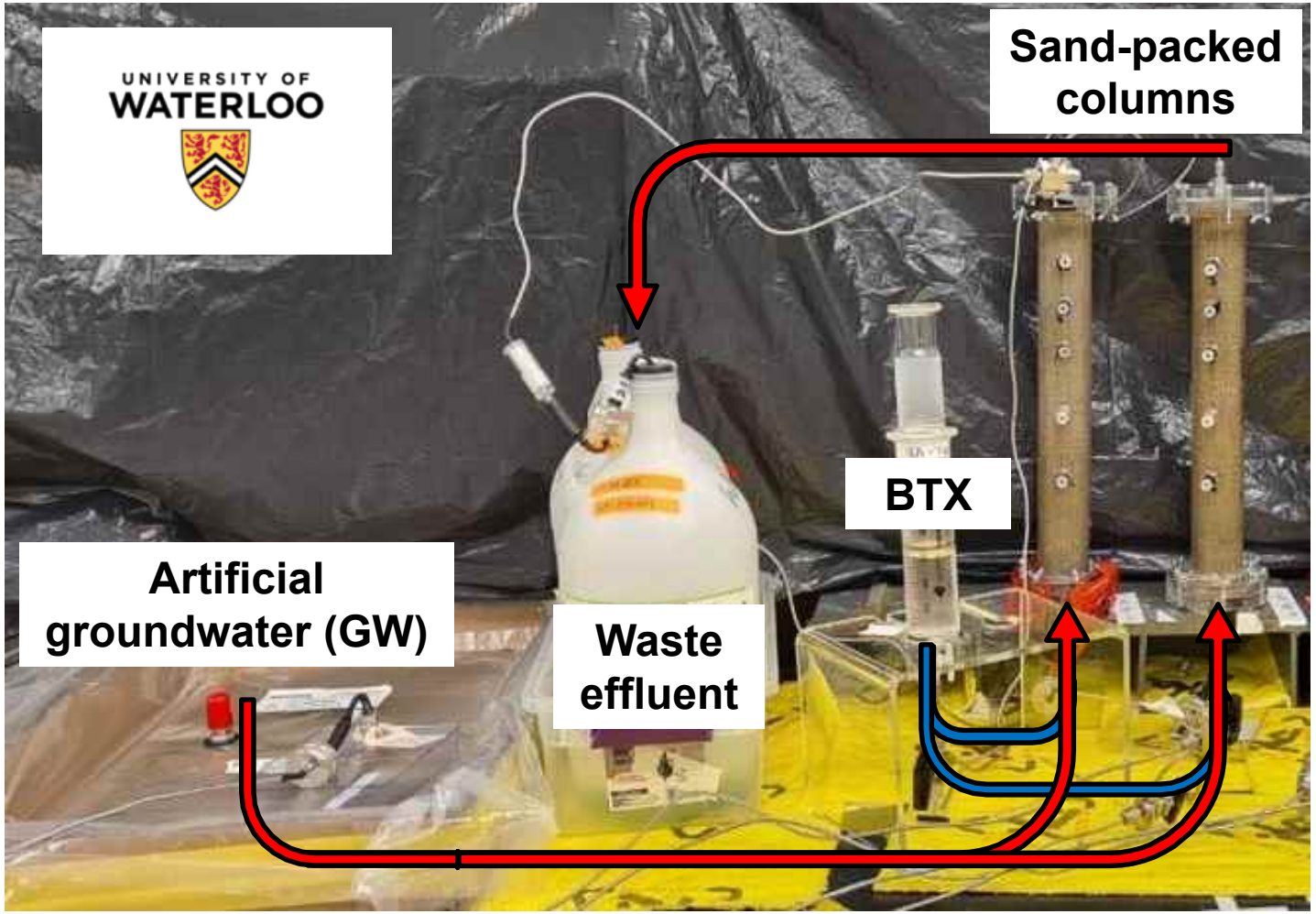



**Data from nearby monitoring wells suggest that the TPH plume did not migrate.**


# Success and a Question

- ✓ Achieving ND for TPH and BTEX was achieved 9 months after bioaugmentation with Toluene and Xylene culture
- ? Interestingly other TPH in addition to T an X were also degraded why?
  - Did removing T and X relieve toxicity/inhibition to the microbial community?
  - Did beneficial symbionts in DGG-Plus help indigenous TPH degraders?

# Validating Bioaugmentation Cultures Under Conditions of Flow



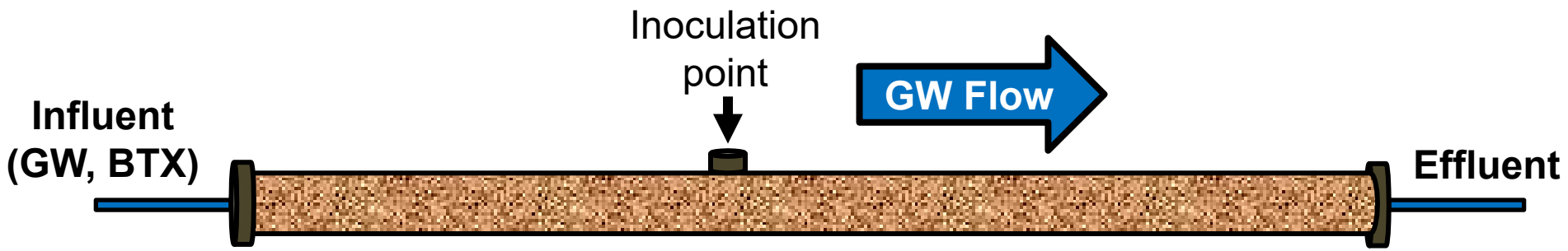


**Adam Schneider**
MEng Student

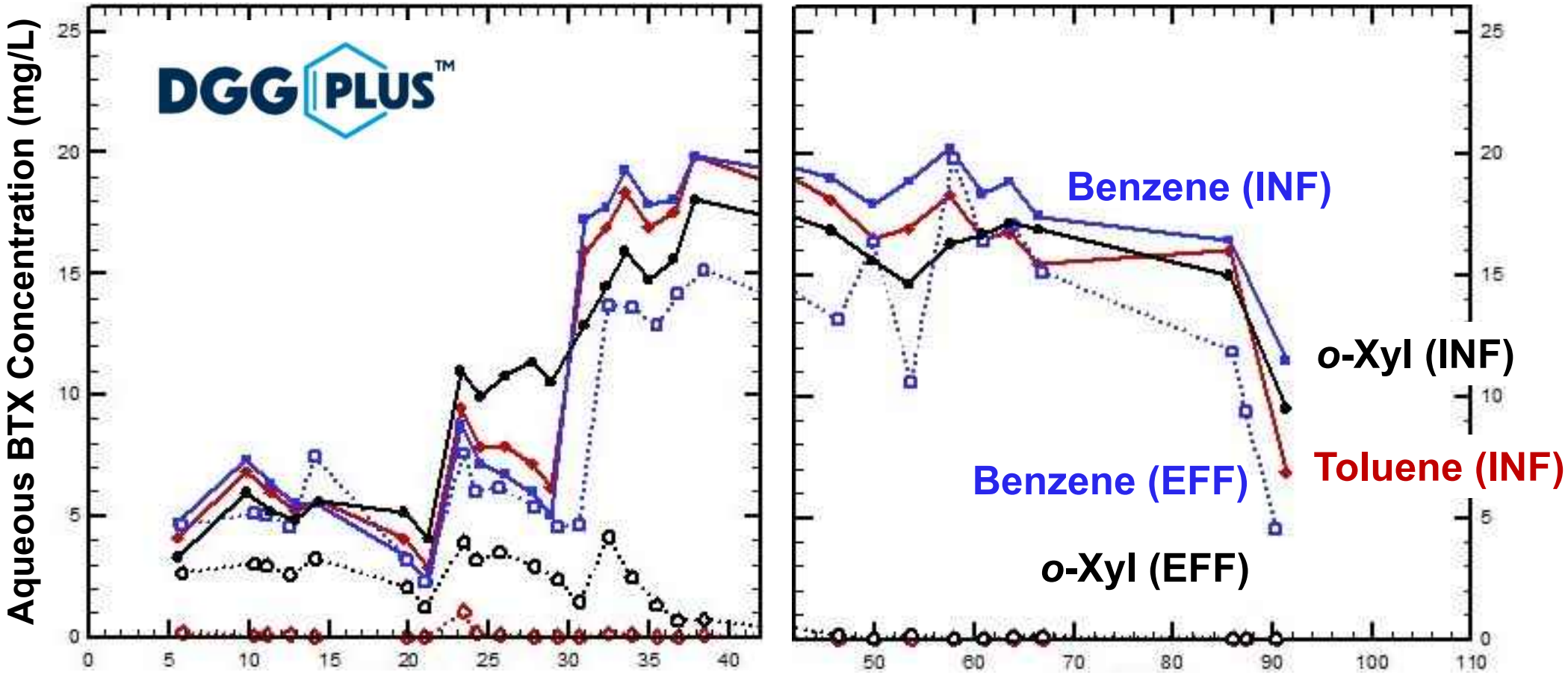
**Bill McLaren**
MEng Graduate

**Andrea Marrocco**
PhD Graduate

**Neil Thomson**
Prof., UWaterloo



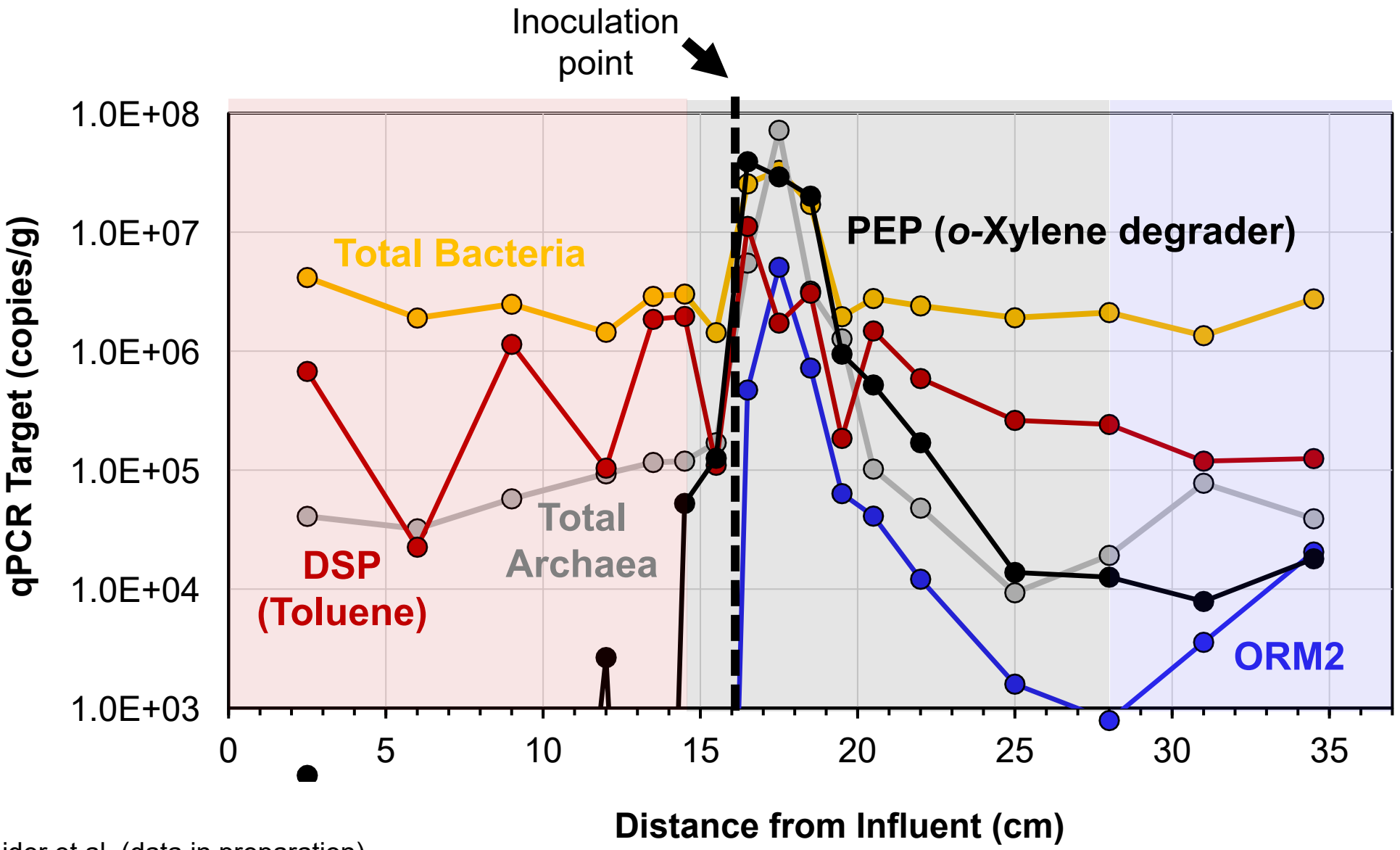

## Bioaugmented Column

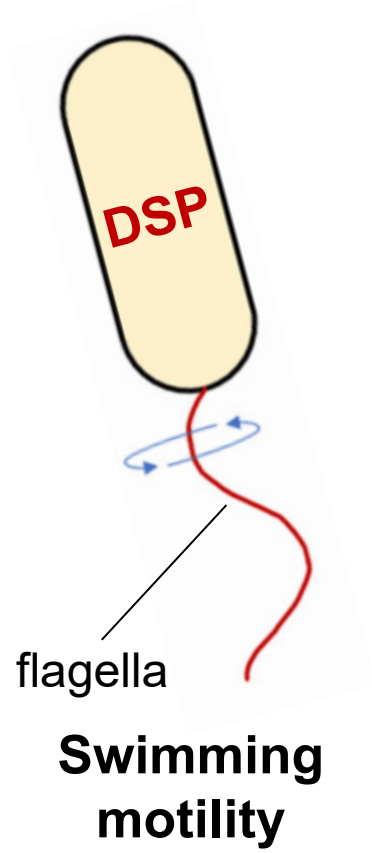





Schneider et al. (data in preparation)

# *End point qPCR shows evidence of DGG Plus culture dispersal into distinct sections of the bioaugmented column.*




Schneider et al. (data in preparation)

# Conclusions

- **Anaerobic BTX cultures can:**
  - Be scaled up to field volumes
  - Be successfully used for bioaugmentation
  - Impact and increase field rates
- **T and X degrade 1st first, B last**
- **T and X degraders can swim, B degrader is a “surfer”**
- **Adding B,T&X cultures seems may assist TPH degradation**

# Questions?

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