Using Molecular Biological Tools to Predict Rates of Anaerobic BTEX Biodegradation



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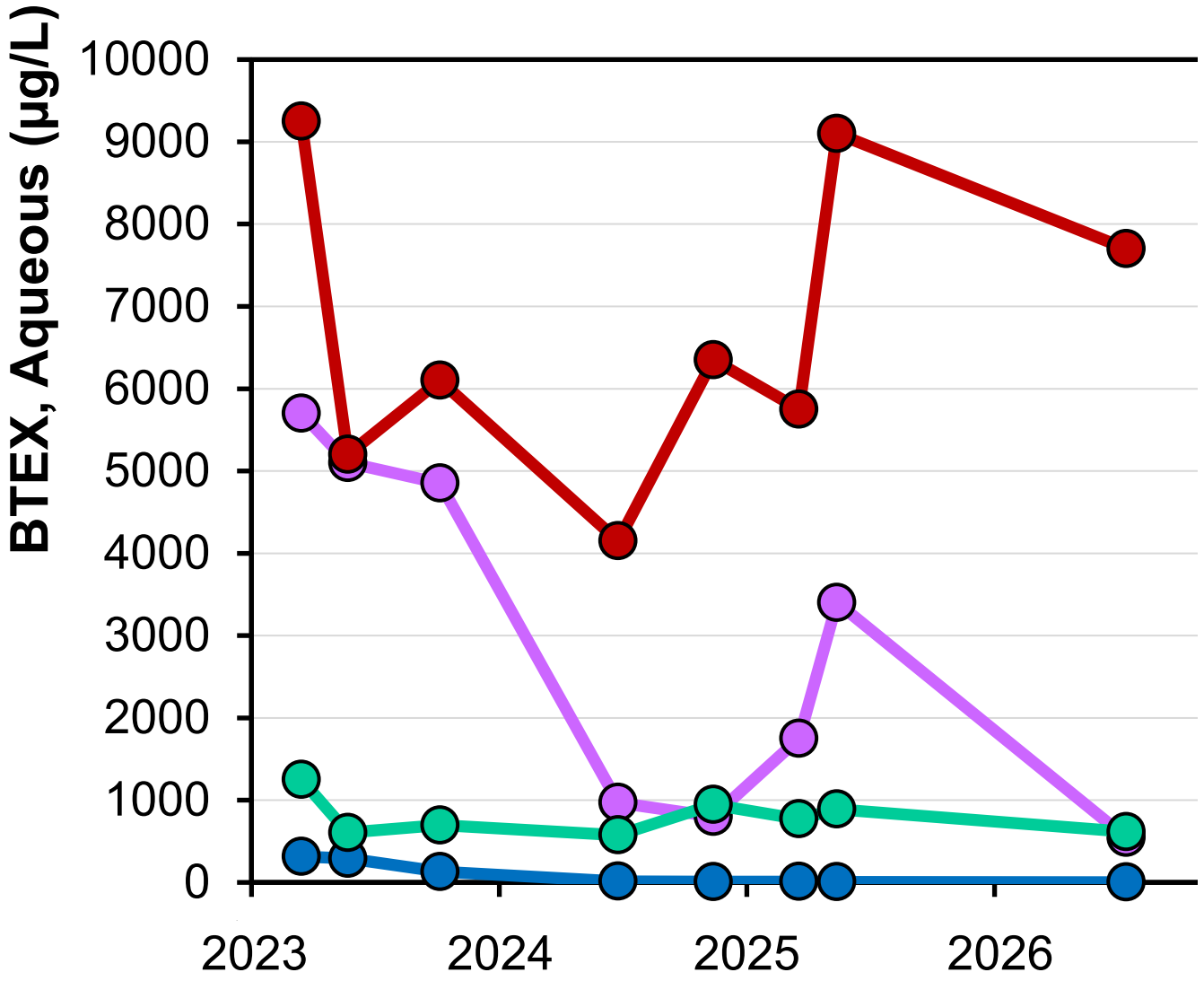
[3]Department of Civil and Environmental Engineering, **University of Alberta**

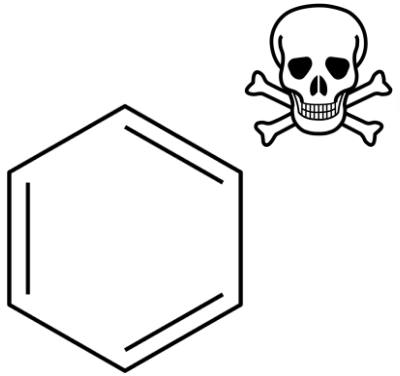
[4]**SiREM**, 130 Stone Road West, Guelph, Ontario

October 15th, 2026

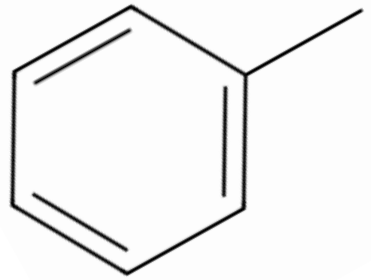
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BTEX Contamination in Groundwater

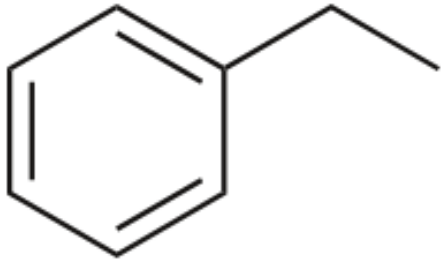




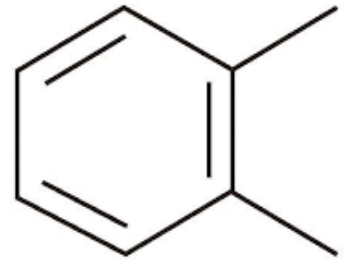
Benzene



Toluene

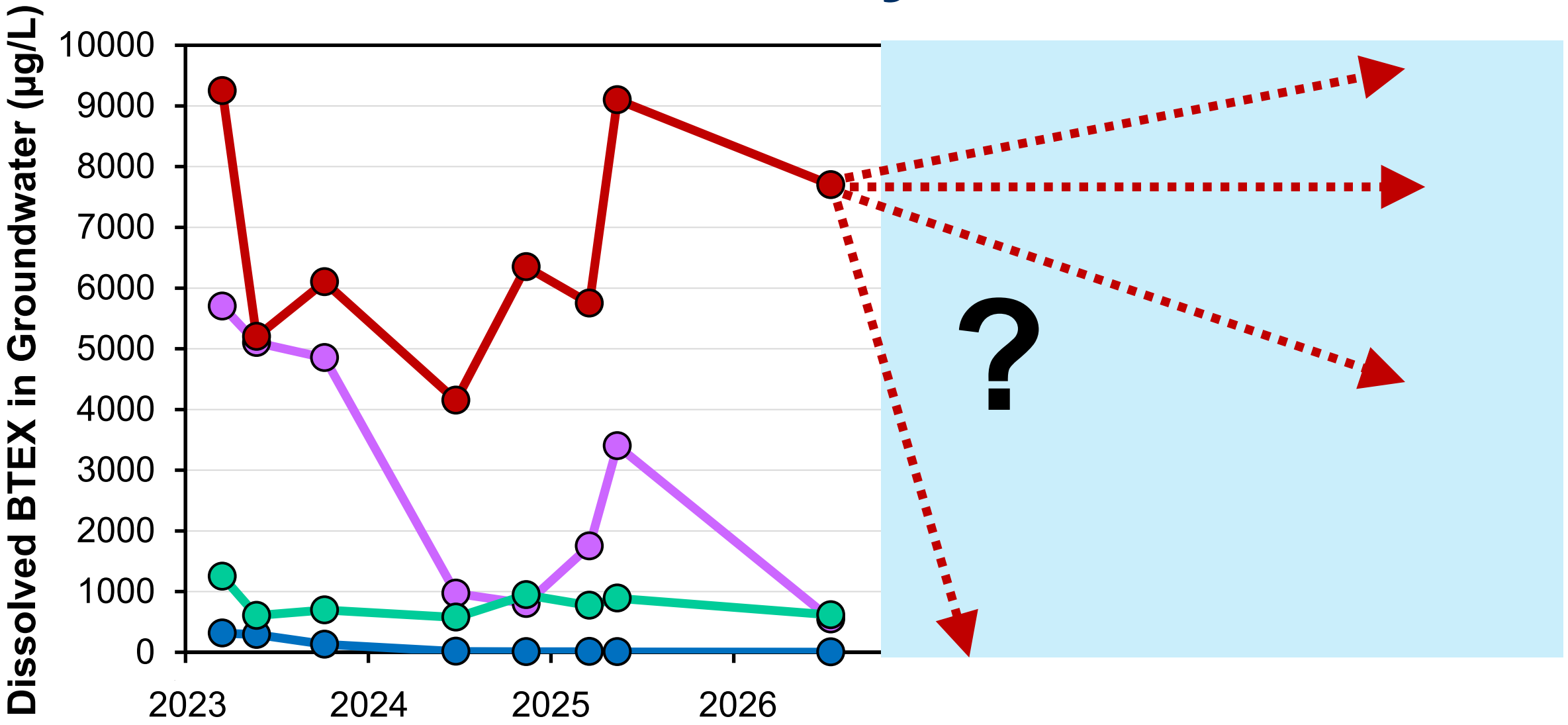


Ethyl benzene



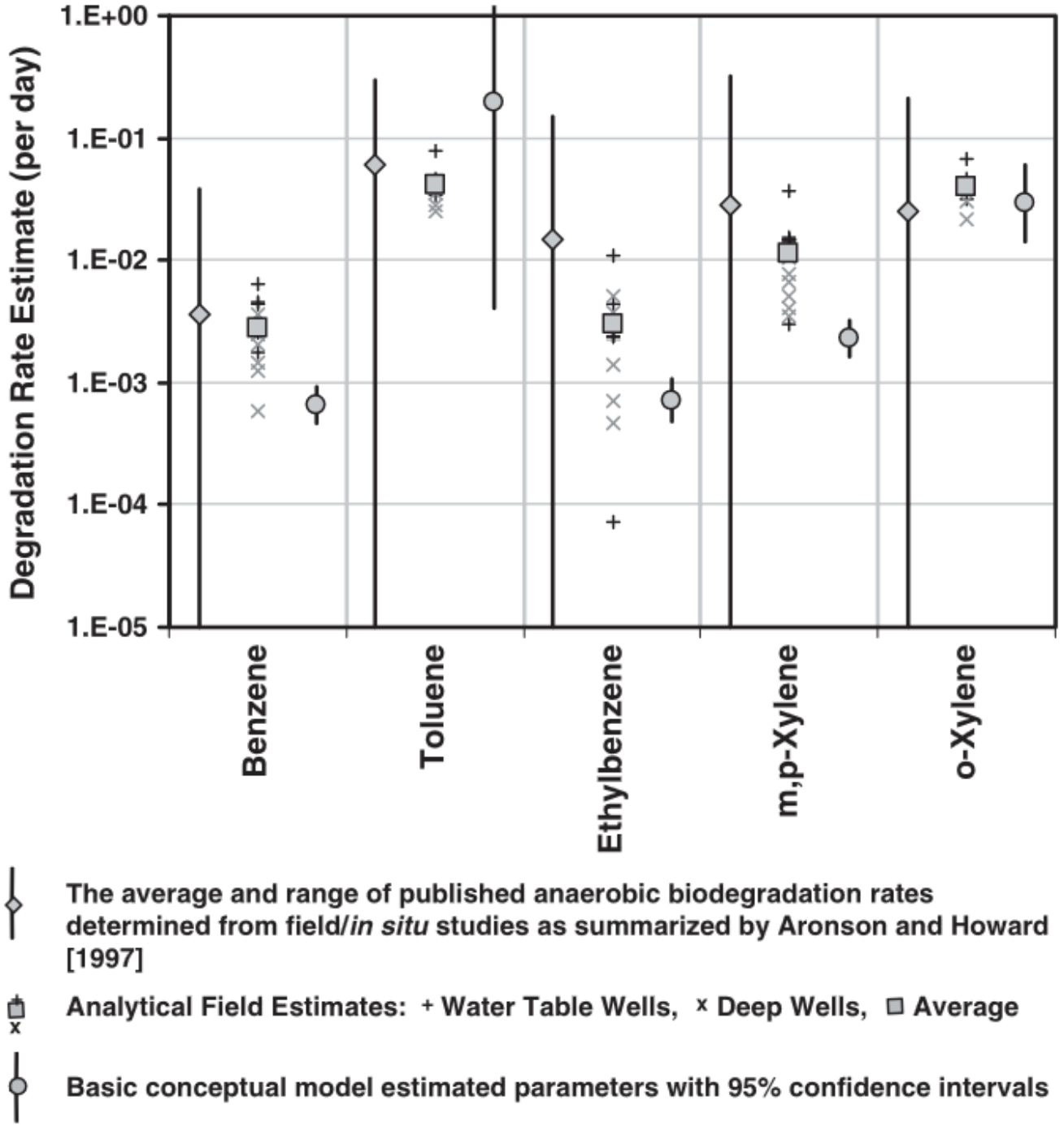
Xylene

Forecasting Bioremediation Trends is No Easy Task



- Different methods of estimating biodegradation rates can produce substantially different results, even when applied to the same contaminated site.

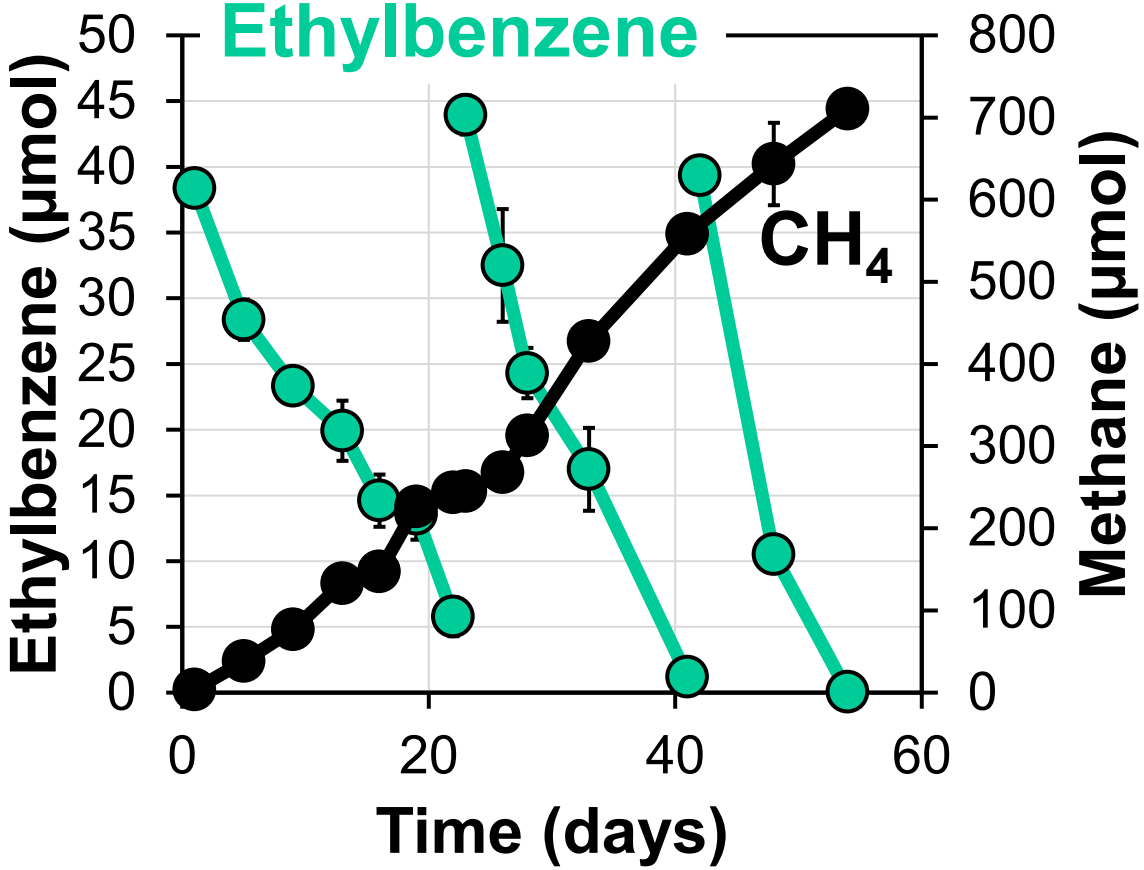
- Ex) First order rate constant estimates at a crude oil spill site in Bemidji, MN

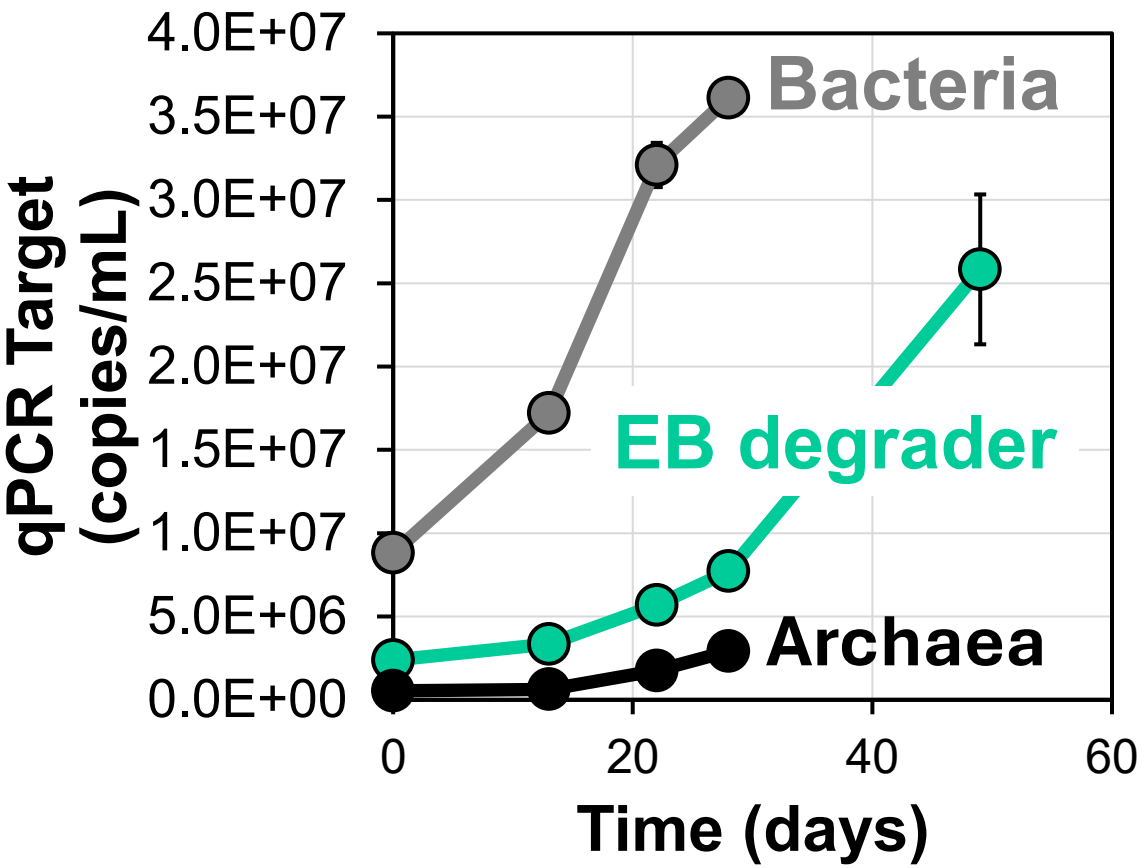


Essaid et al. 2003 (J Contam Hydrol 63)

Can Microbial Measurements Improve Biodegradation Models?

Goal: Link microbial <u>abundance</u> and <u>growth</u> to biodegradation rates

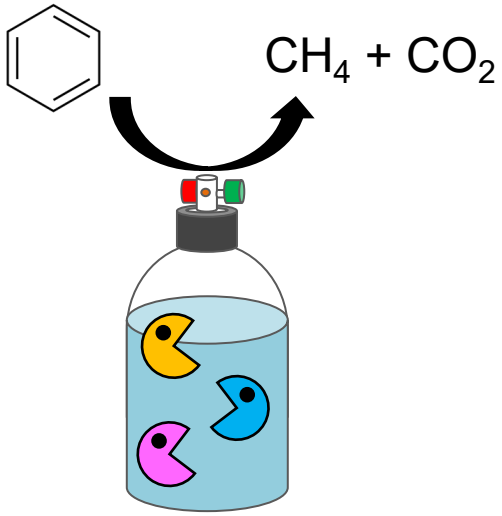




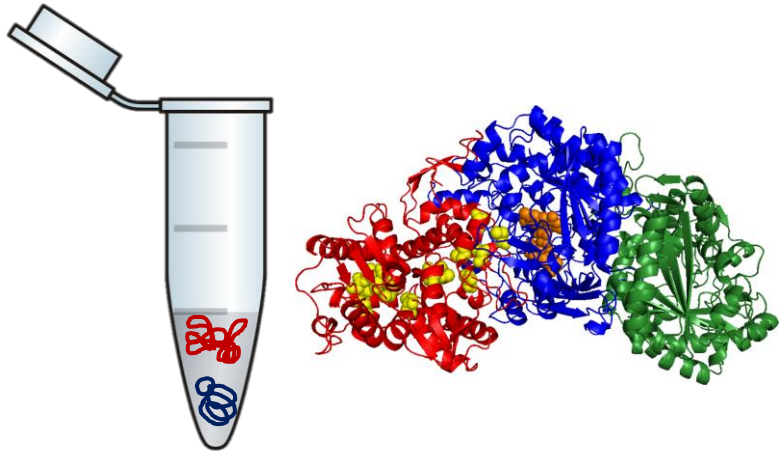
Today's Presentation

A story of how studying microbial cultures led to the development of <u>quantitative</u> molecular tools for monitoring anaerobic BTEX bioremediation

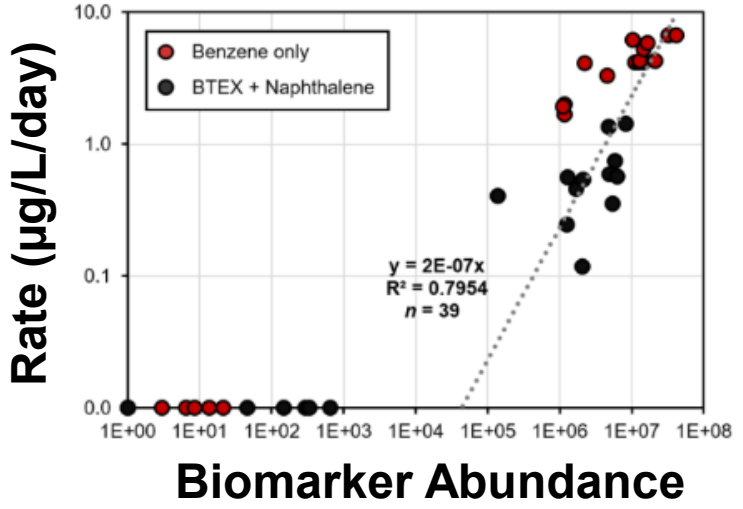
Part 1: Culture Development



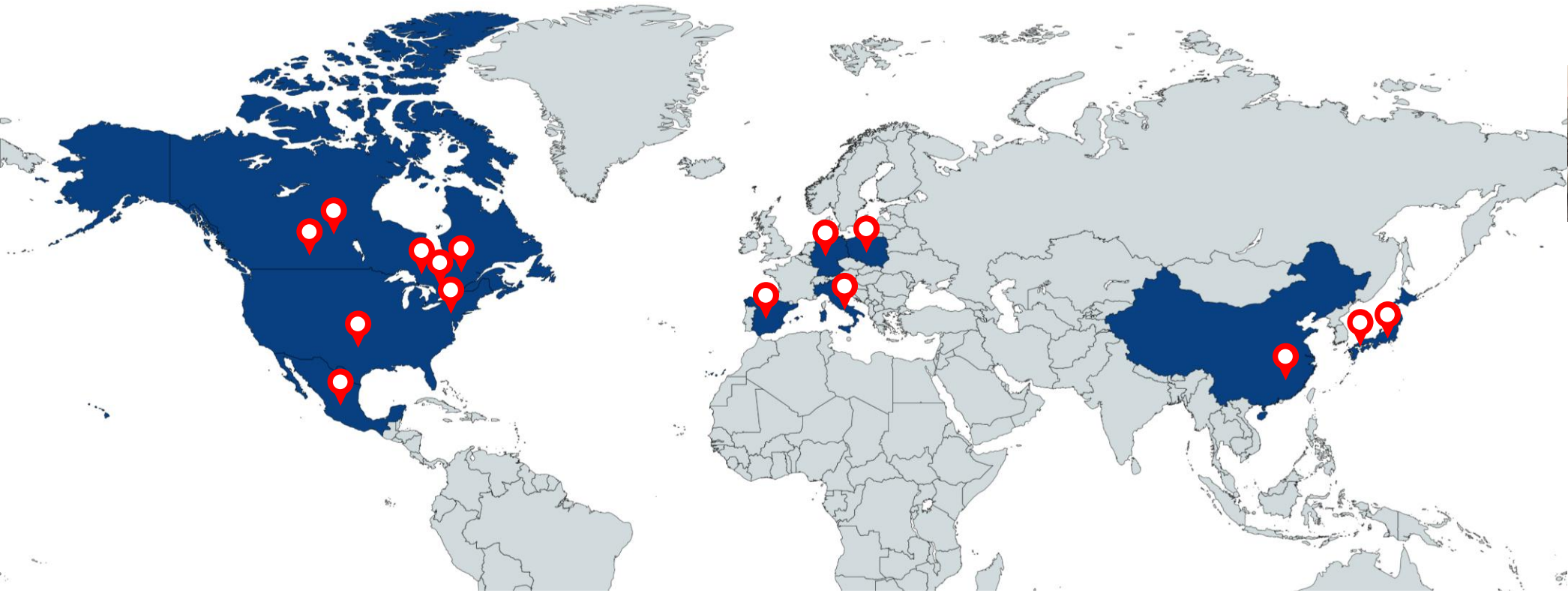
Part 2: Biomarker Discovery



Part 3: Biomarker-Rate Relationships



Enriching for Anaerobic BTEX-Degrading Microbes





Contaminated sediments

+



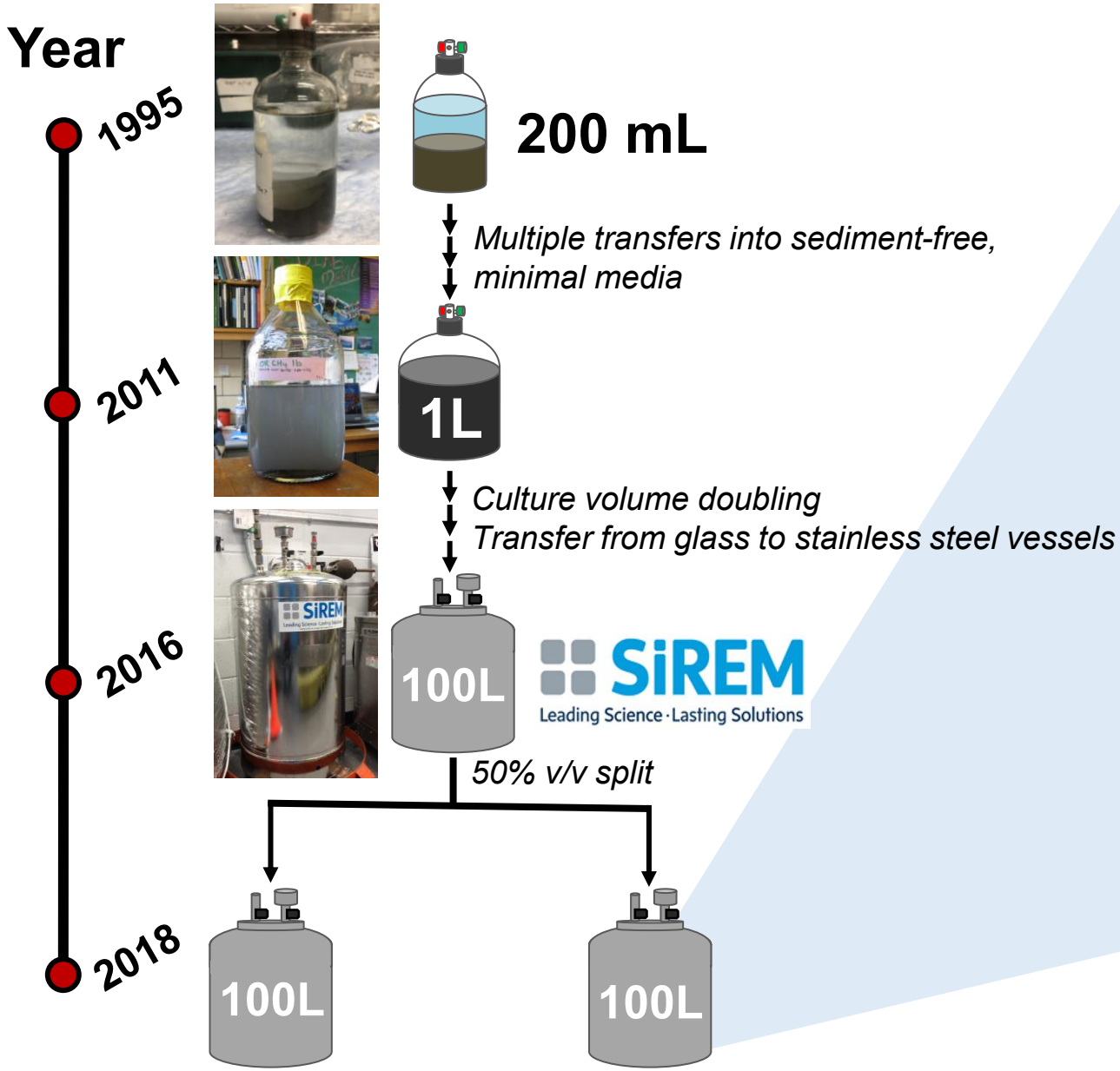
Contaminated groundwater

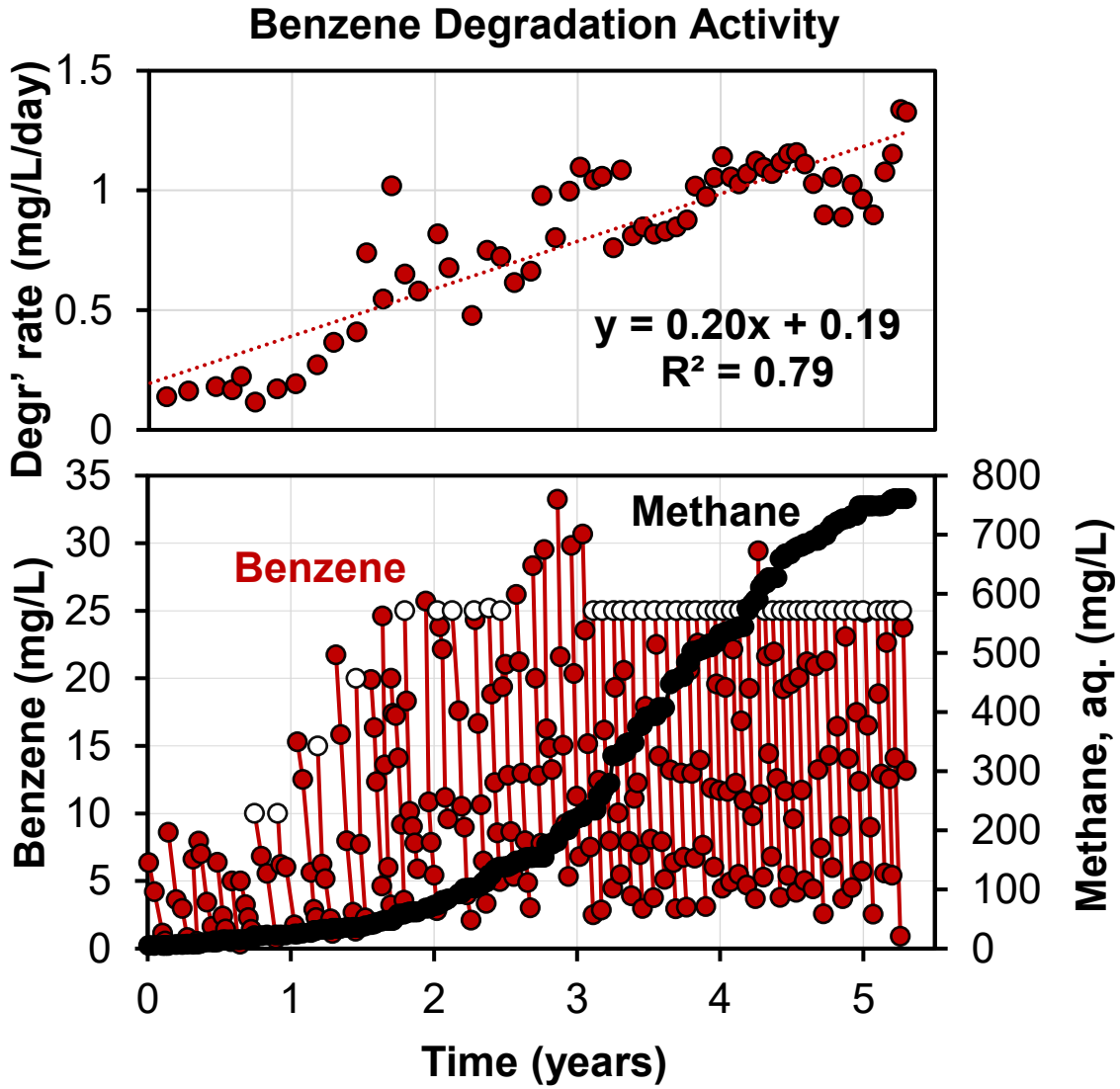


Enrichment culture

Nales et al. 1998 (Bioremediat J 2); Herrmann et al. 2008 (FEMS Microbiol Ecol 63), Sakai et al. 2009 (J BioSci Bioeng 108), Toth et al. 2021 (Environ Sci Technol 55)

DGG-B: A Methanogenic Culture that Degrades Benzene





Open circles = Target aqueous benzene concentration
Closed circles = Measured timepoint

Toth et al. 2021 (Environ Sci Technol 55)

Other Methanogenic Cultures Established

DGG T™

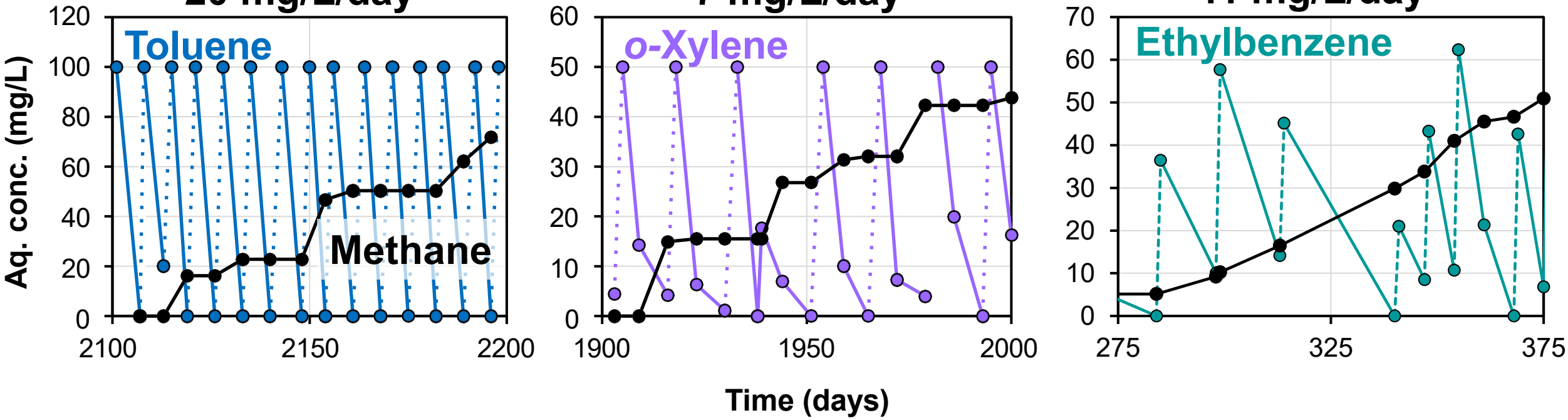
Degr' rate = up to
20 mg/L/day

DGG X™

Degr' rate = up to
7 mg/L/day

DGG E™

Degr' rate = up to
11 mg/L/day



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

Example Molecular Biological Tools

DNA





(Meta)genomic Sequencing

Who's there, and what can they do?

Proteins





Proteomics

What are they doing?

DNA





Amplicon Sequencing

Who's there, and in what proportions?

DNA





Quantitative / Digital PCR

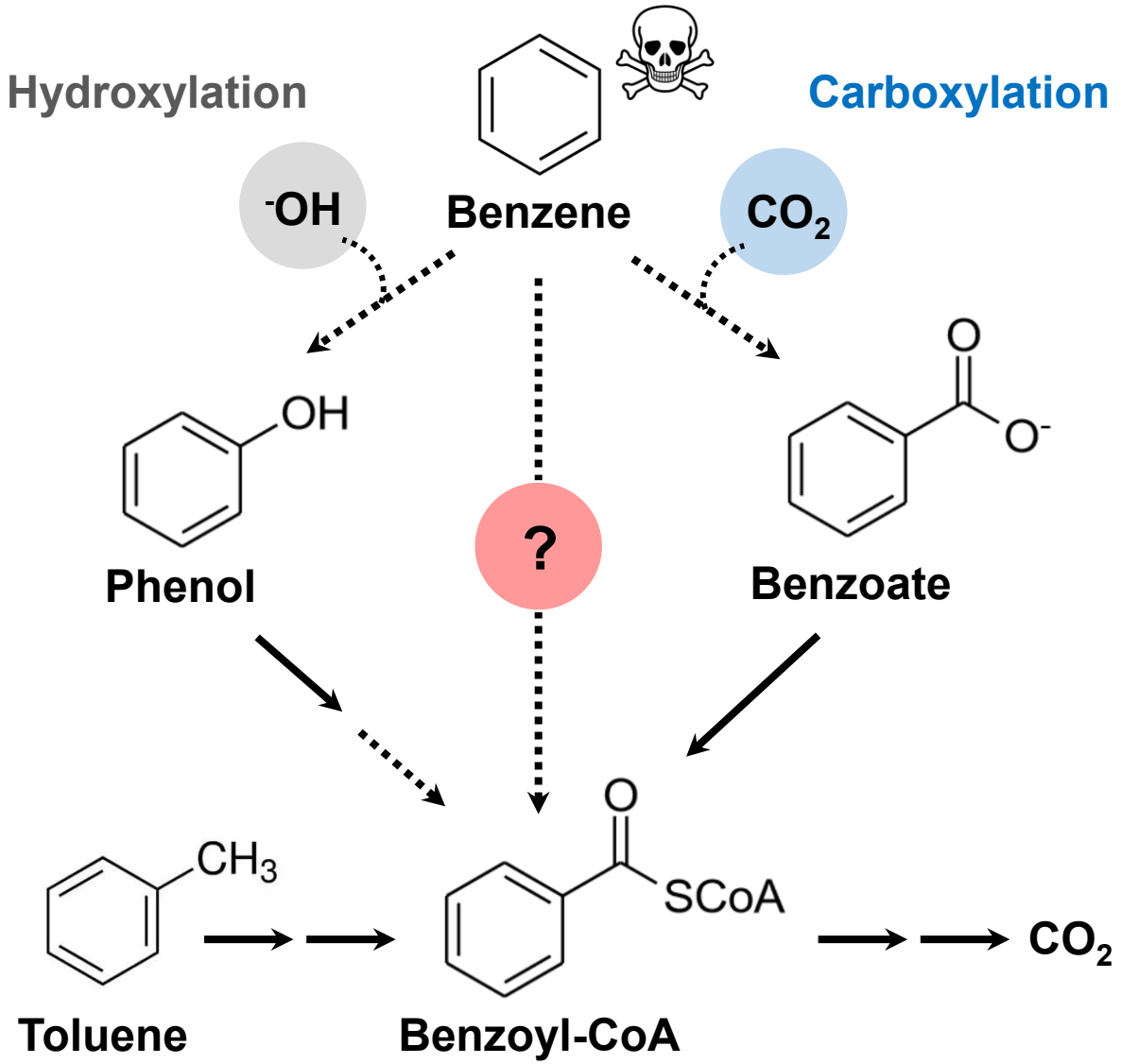
How many copies of specific genes are there?

Non-targeted → **Targeted**

Molecular Biological Tools in Action

- Benzene is notoriously slow to degrade in anoxic environments

- Until recently, we had no idea;
  1. Which ***microbes*** were involved,
  2. Which ***enzymes*** were involved,
  3. What ***biochemical reactions*** were involved



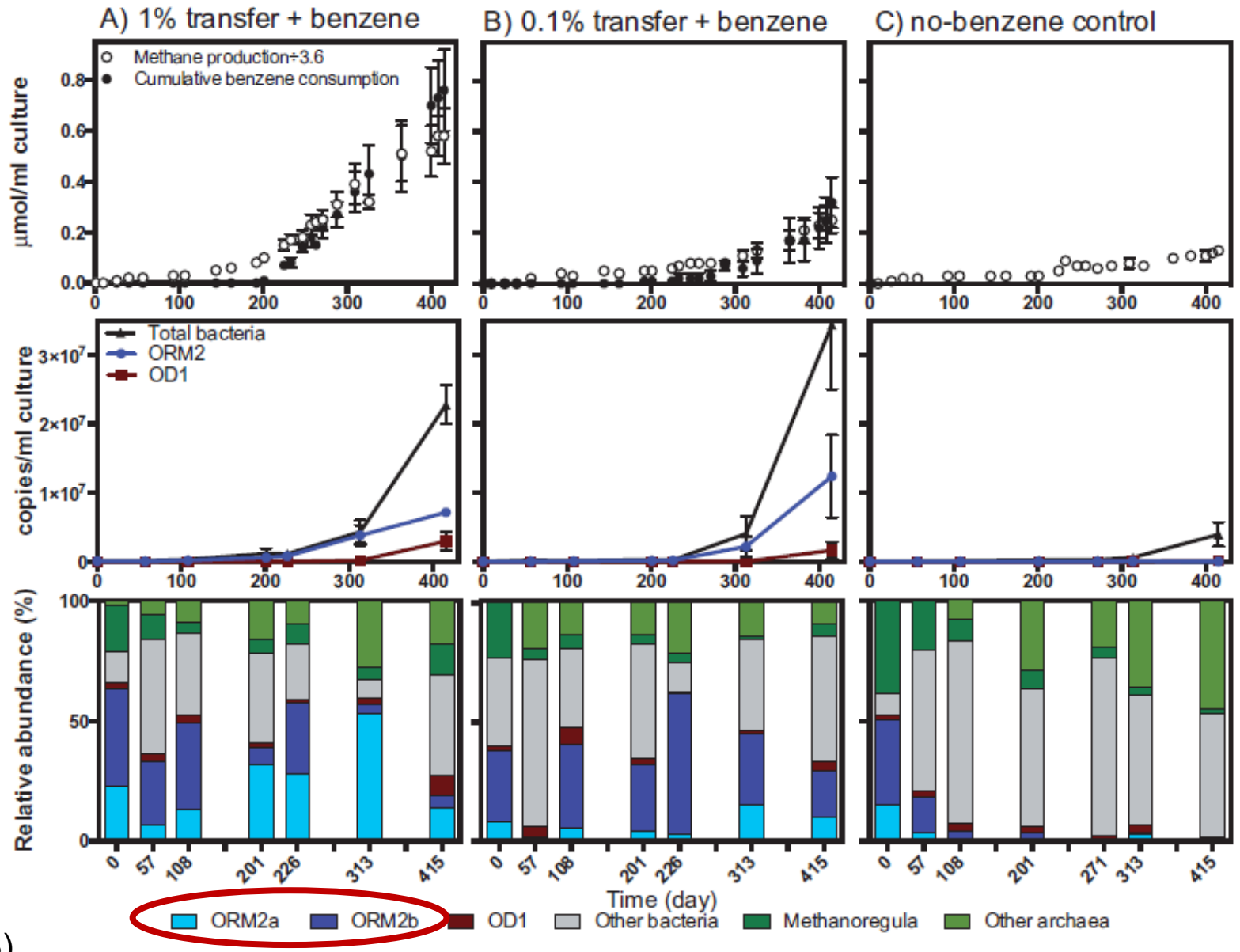
⋯➤ = Unknown or unverified biochemical reaction

Growth experiments & targeted molecular analyses enabled us to identify putative benzene-degrading bacteria in DGG-B

Benzene degradation activity

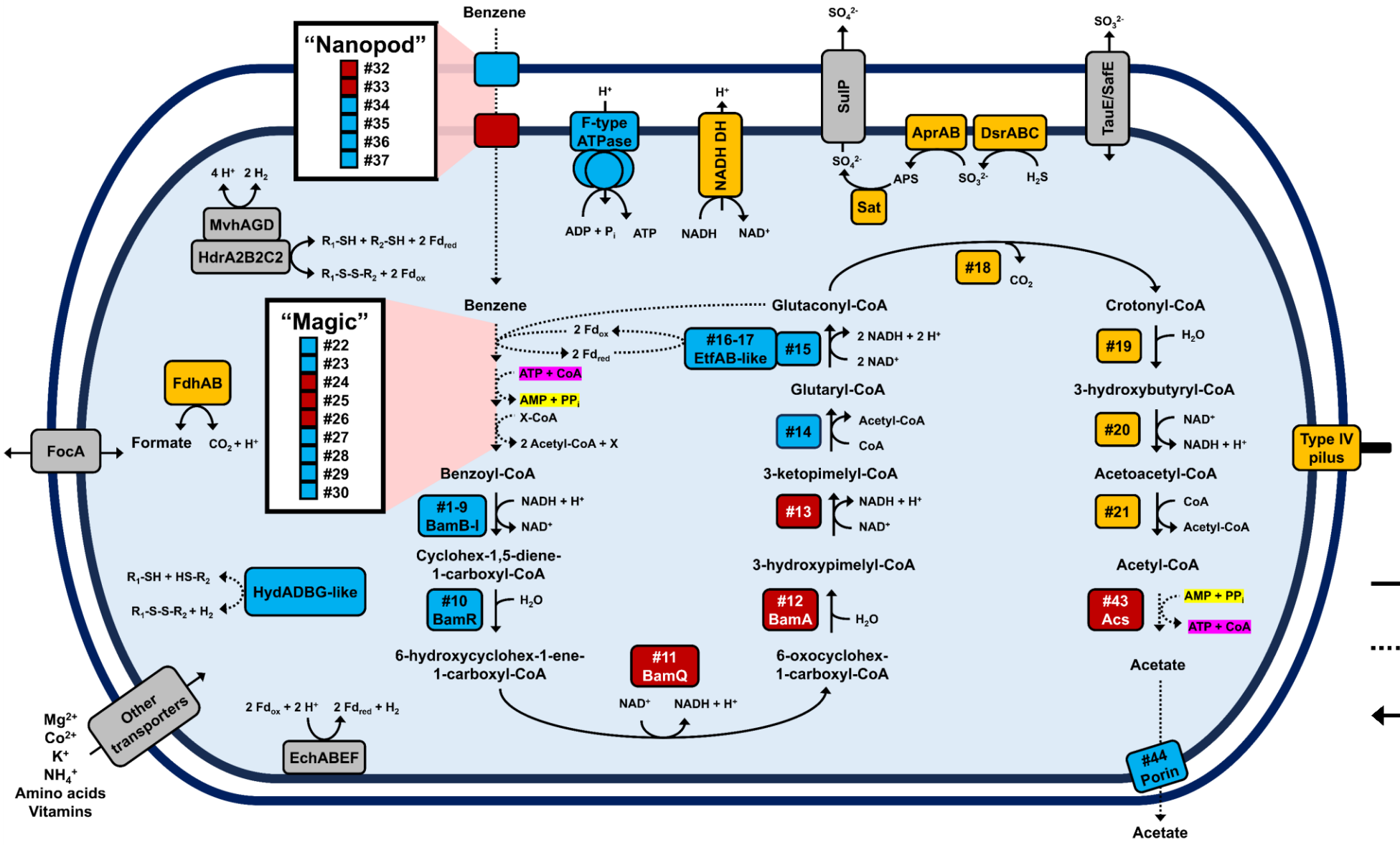
Quantitative PCR

Amplicon sequencing



Luo et al. 2016 (Environ Microbiol 18)

Using metagenomics and proteomics, novel enzymes and metabolic processes involved in anaerobic benzene degradation were identified in ORM2



Protein Abundance

- High (top 3%)
- Medium
- Low
- Not detected

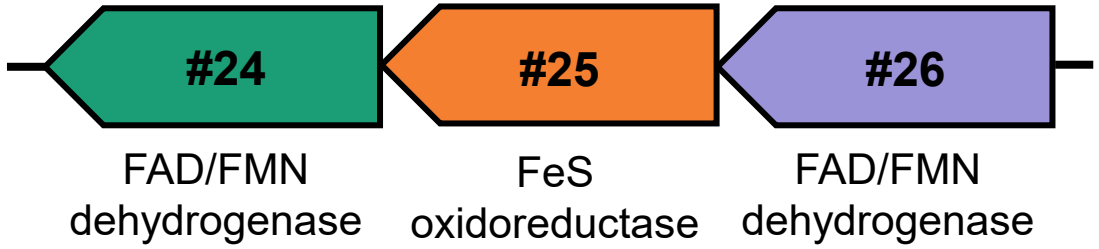
Arrow Legend

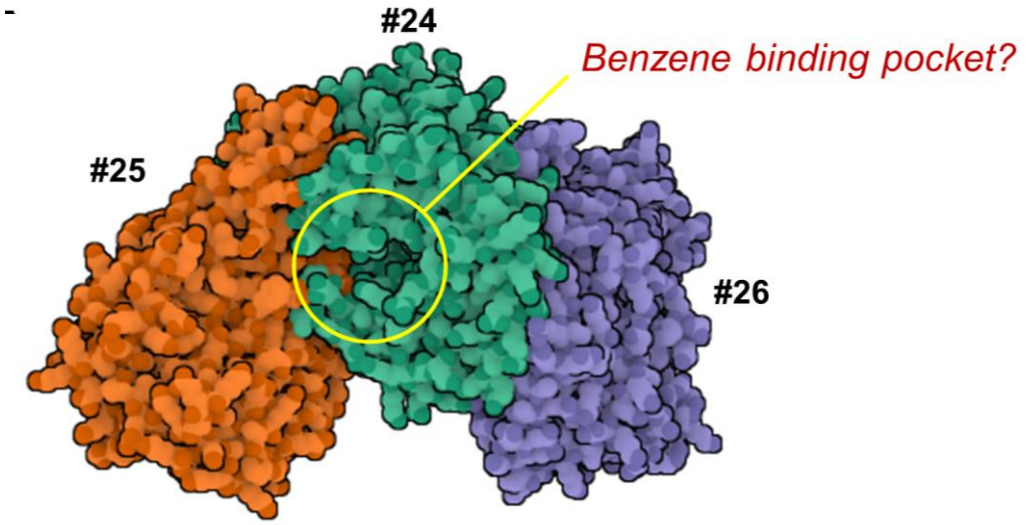
- Known reaction
- Putative reaction
- Unknown reaction direction

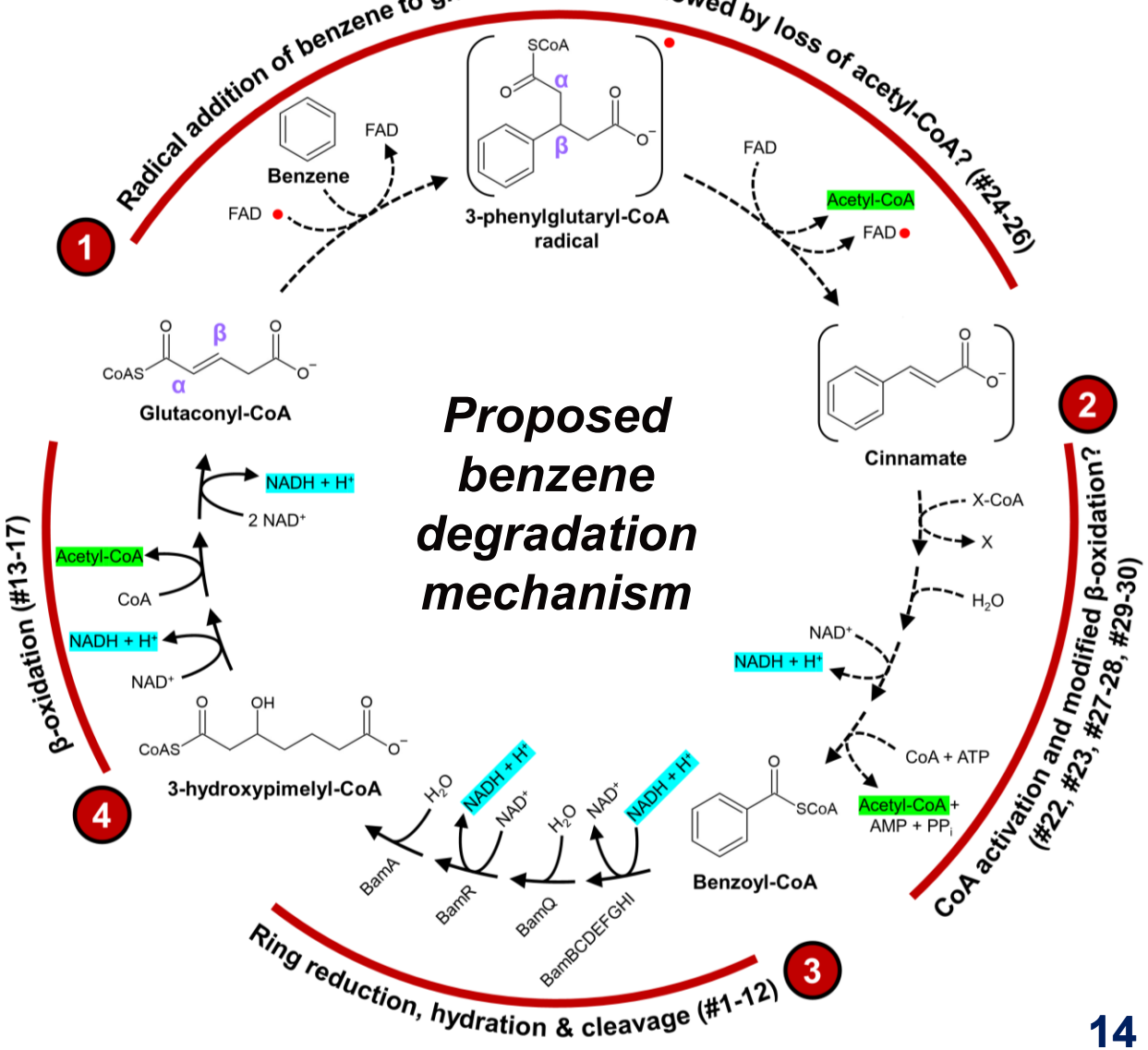
Toth et al. 2023 (Microbiol Resour Announc 12); Toth et al. 2026 (Appl Envjron Microbiol, 92)

Biomarker Discovery

Genes predicted to encode for the "Magic" benzene activation enzyme complex in ORM2

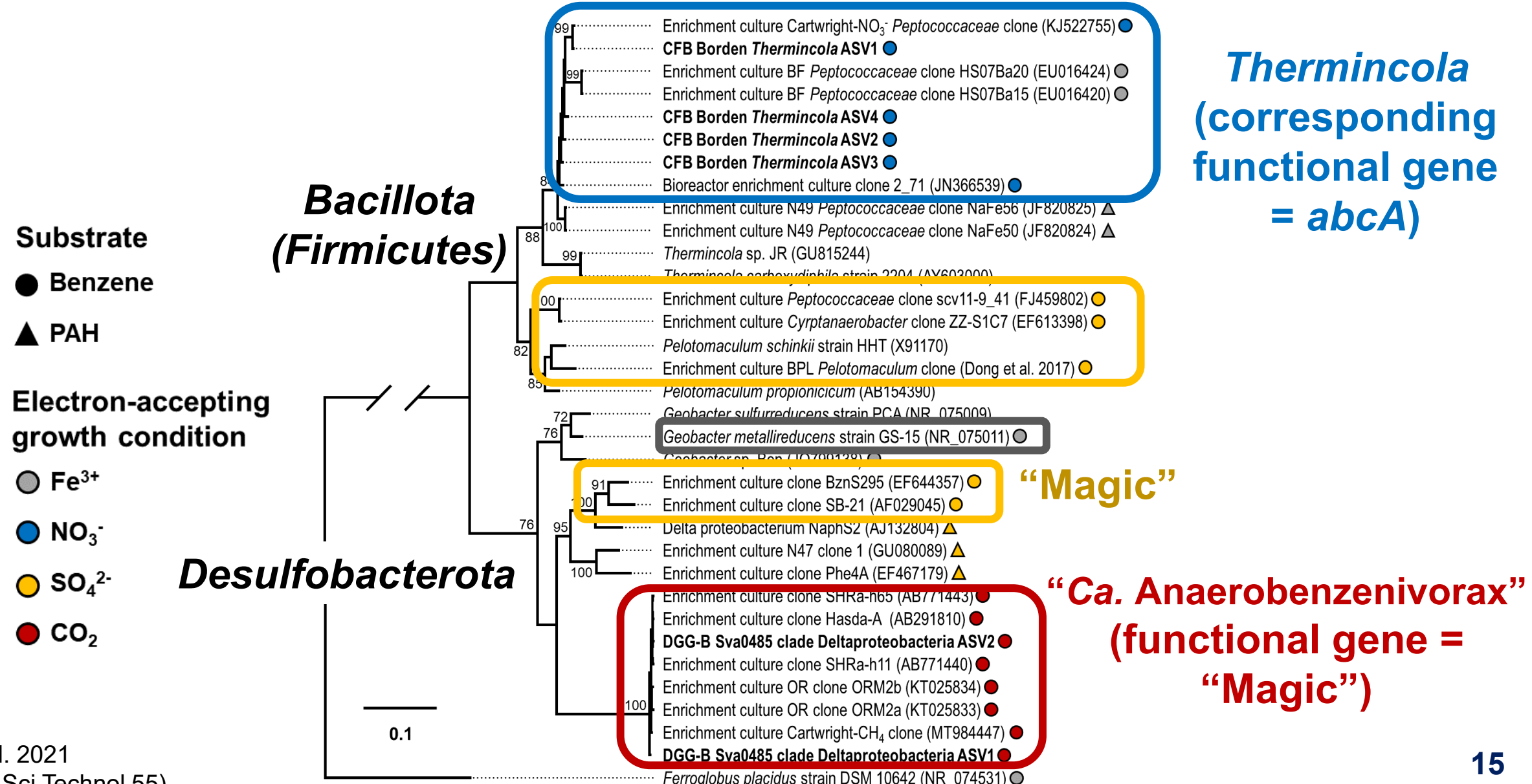






Toth et al. 2026 (Appl Envjron Microbiol, *ahead of press*)

Anaerobic benzene degraders and functional genes are highly specialized. This is also true for other anaerobic BTEX degraders & genes.



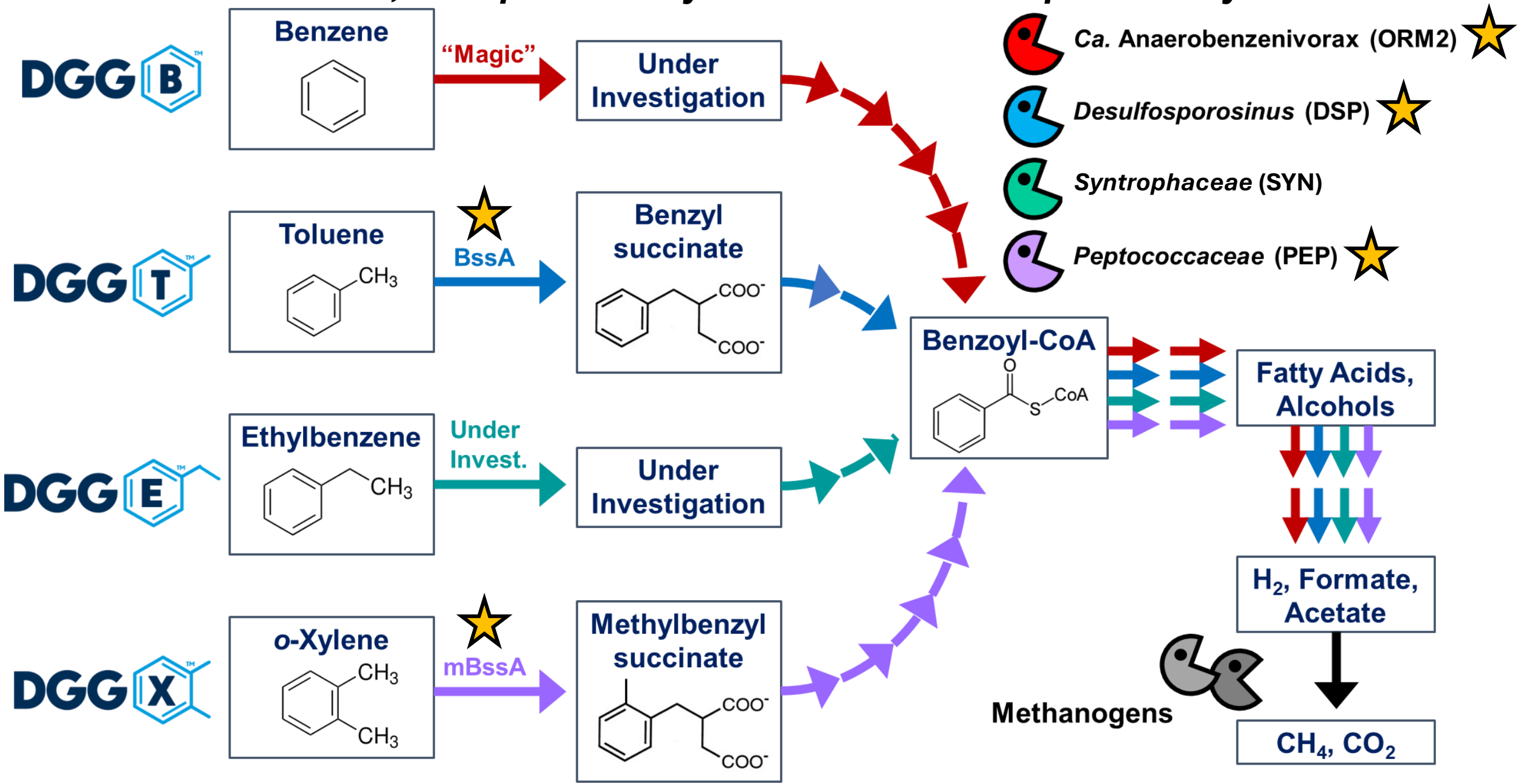
Thermincola (corresponding functional gene = *abcA*)

"Magic"

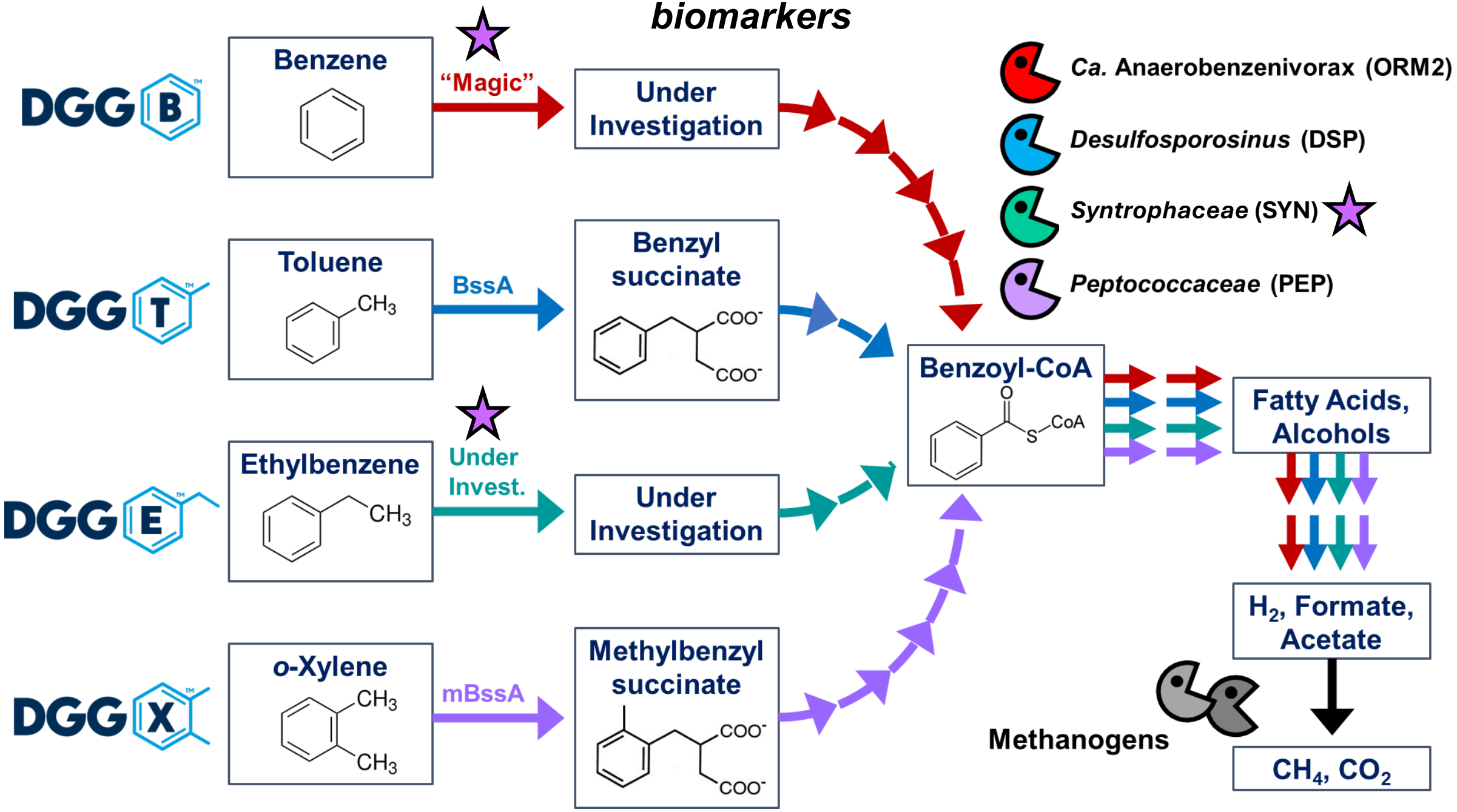
"*Ca.* Anaerobenzenivorax" (functional gene = "Magic")

Toth et al. 2021
(Environ Sci Technol 55)

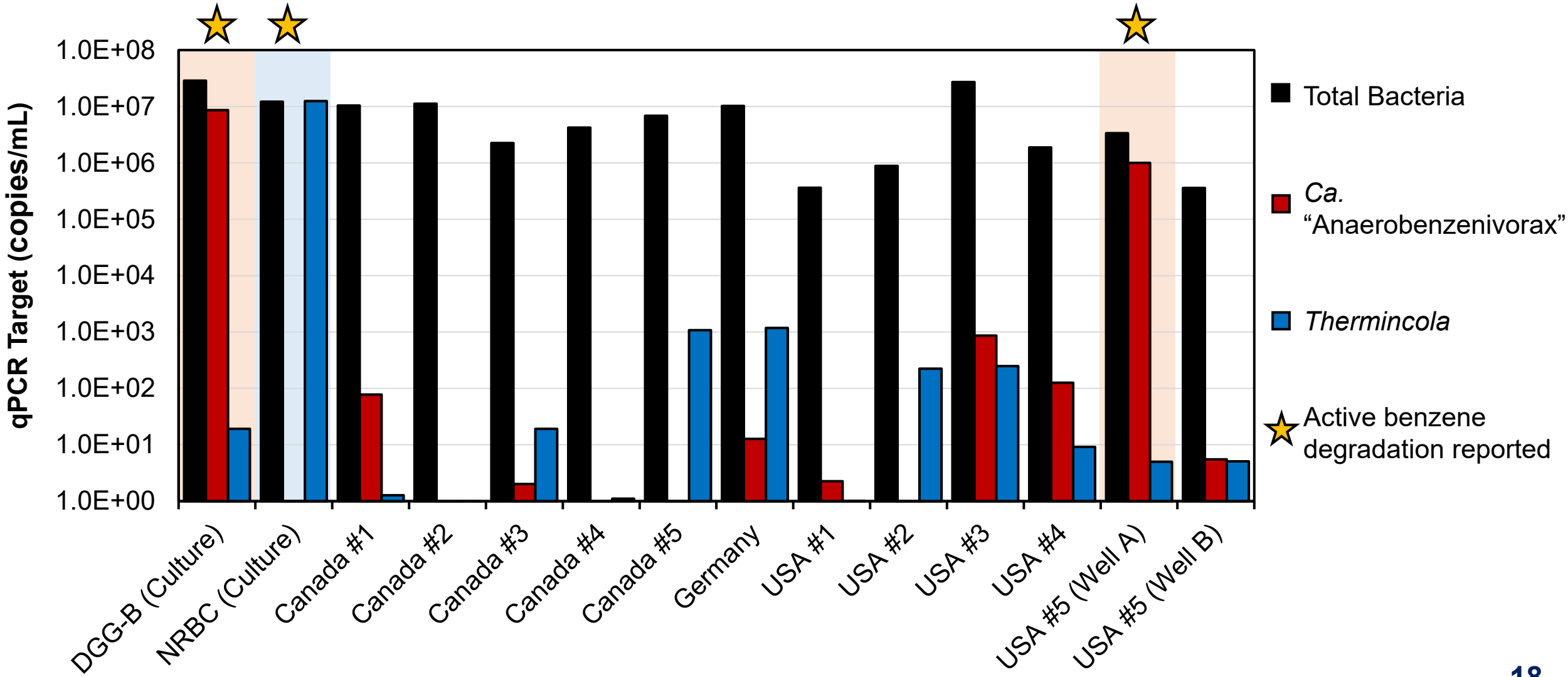
Key microbes, functional genes, and metabolic pathways have been characterized in most DGG cultures, and qPCR assays have been developed for key biomarkers



Characterization and qPCR assay development is underway for the remaining biomarkers



The same microbes in our cultures are naturally present at contaminated field sites, but usually in low abundances. This may (partially) explain slow biodegradation rates at contaminated field sites



Linking Microbial Abundance, Growth, and Biodegradation Rates

Benzene contaminated



Crushed core sample (60 g)



Groundwater sample (200 mL)

Aqueous BTEX concentrations depend on site materials

Mix in glove box, let stand 1 – 4 weeks

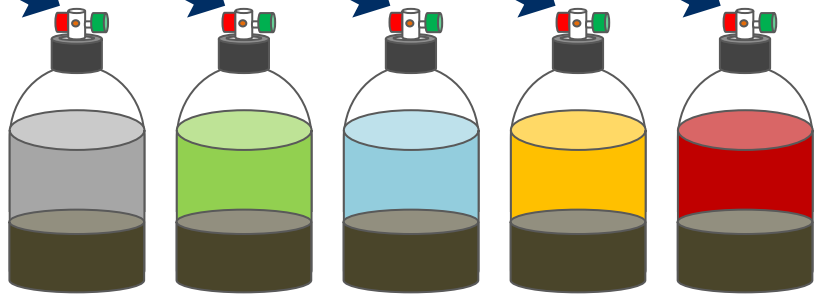
$HgCl_2$+NaN_3, Autoclave

Untreated

$NaNO_3$

Na_2SO_4

1% v/v DGG-B

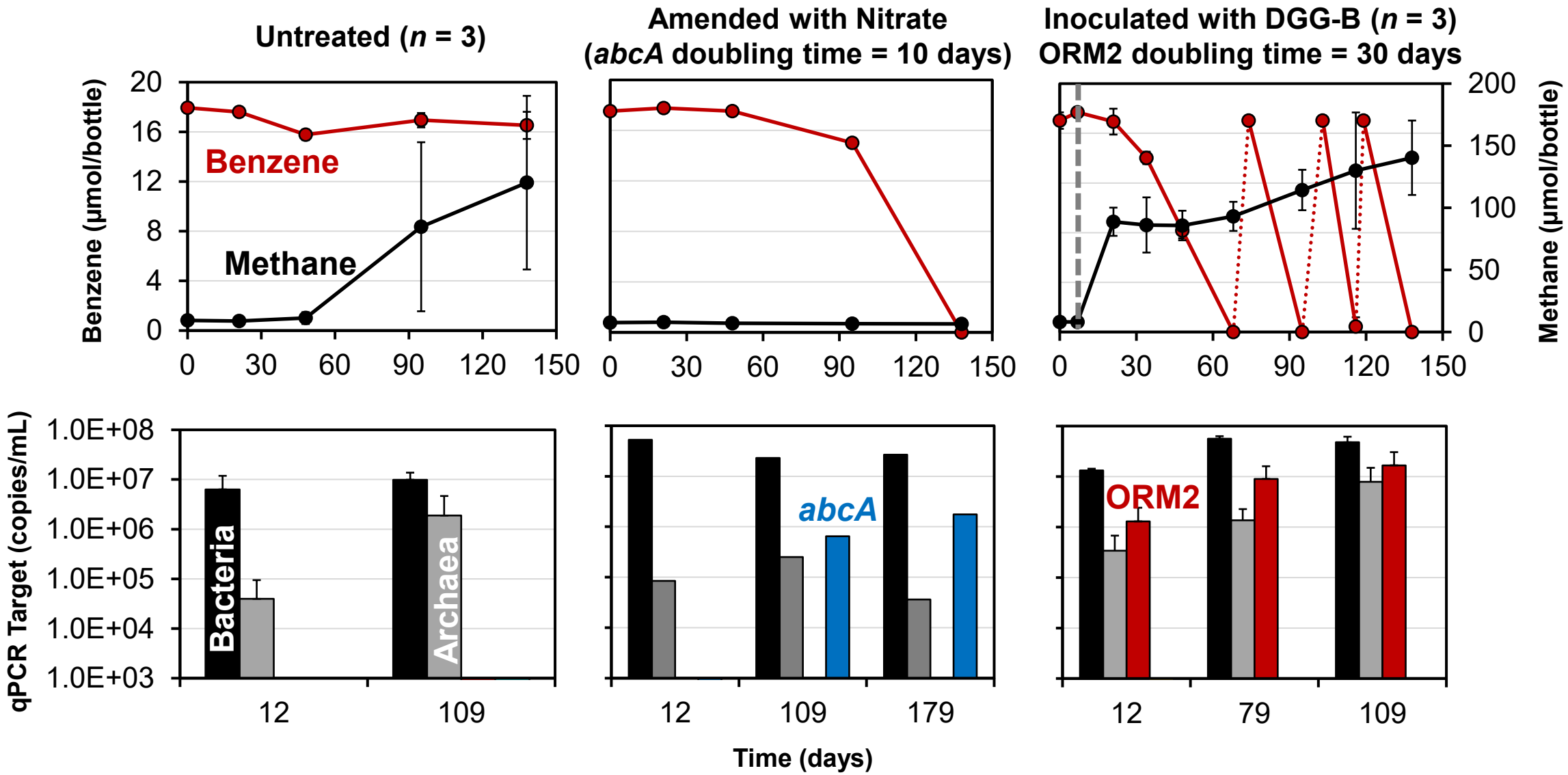


230 mL groundwater slurries
20 mL headspace (10% CO_2 / 90% N_2)



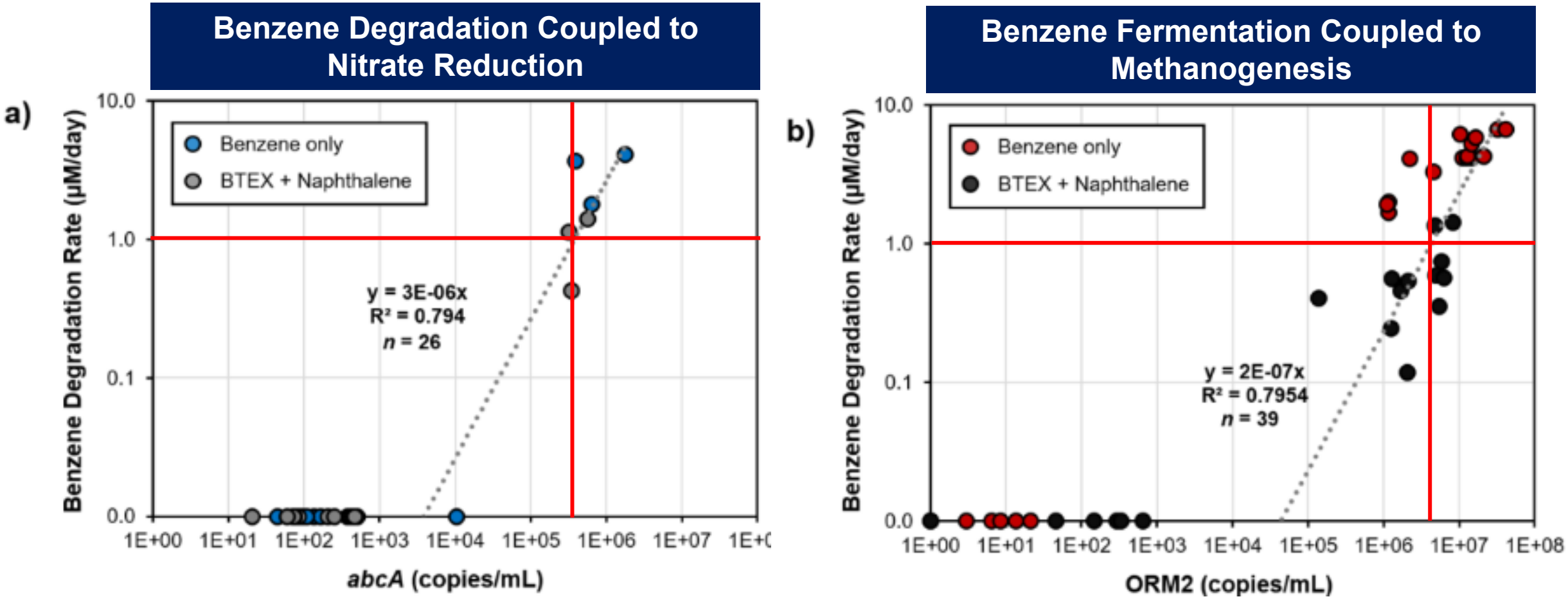
Incubate and monitor over 1-2 years

20+ microcosm studies have shown that the onset of anaerobic benzene and BTEX biodegradation coincides with growth of key microbes and functional genes



Toth et al. 2021 (Environ Sci Technol 55)

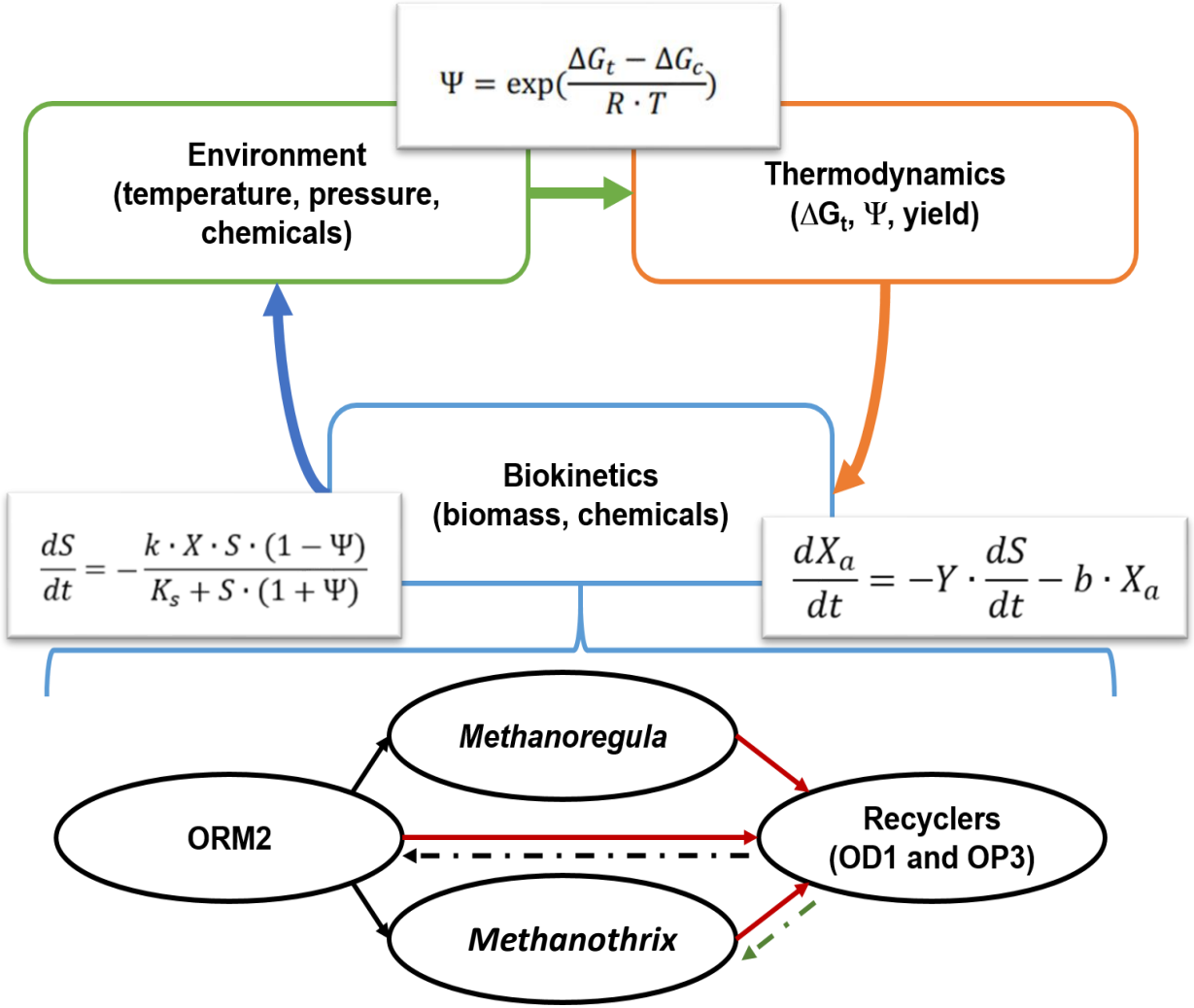
Biodegradation rates were shown to be empirically tied to abundances of key microbes and functional genes. This relationship also extends to other BTEX compounds

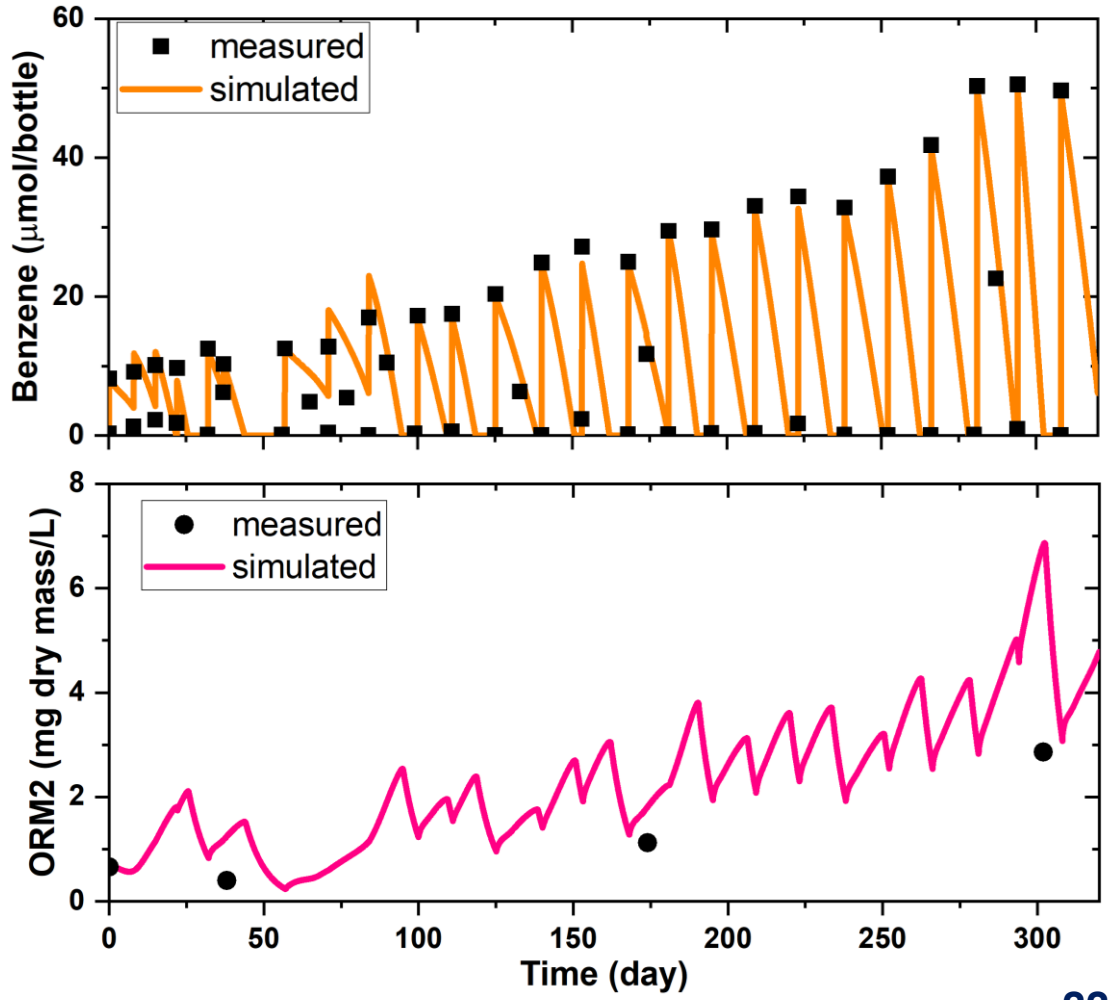


Toth et al. 2021 (Environ Sci Technol 55)

Multi-Phase Biokinetics Modeling

Preliminary modelling studies incorporating molecular data have closely reproduced observed anaerobic benzene biodegradation and microbial growth

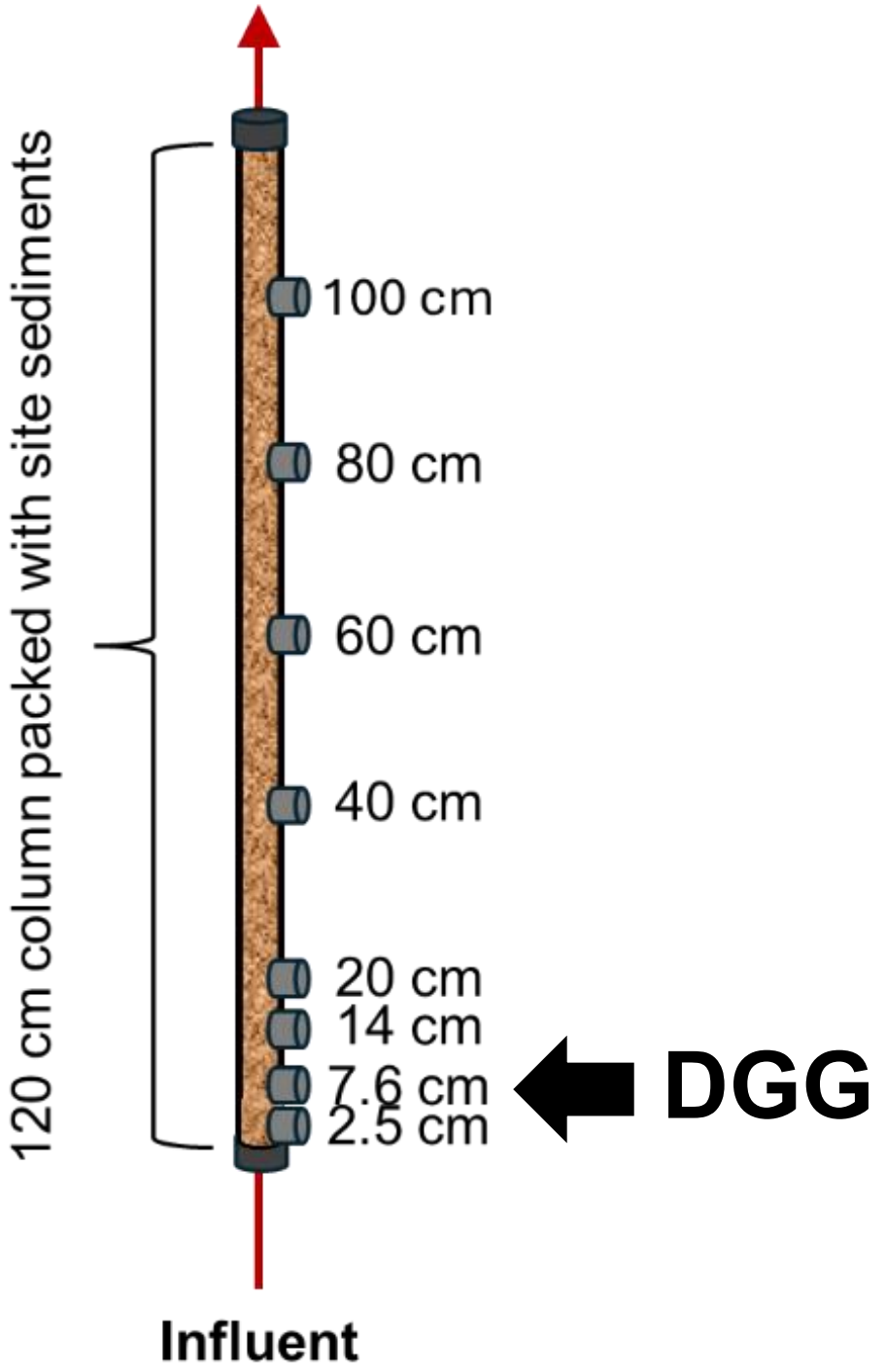




Liang 2026 (PhD Thesis)

What's Next?

- New column studies are being constructed to determine first order BTEX biodegradation rate constants for each DGG culture
- Molecular-predicted biodegradation rates will be compared to measured rates along the column flow path
- Two associated field studies will further evaluate whether biomarker–rate relationships can reliably estimate in situ biodegradation rates



Take Home Messages

Anaerobic BTEX biodegradation is driven by specialized microorganisms that can be enriched, characterized, and monitored using molecular biological tools.

Microbial biomarker abundance can be linked to biodegradation rates. Experimentally established relationships provide a new approach for estimating anaerobic benzene biodegradation rates

Integrating molecular biomarkers with geochemical data has the potential to improve bioremediation forecasts. Ongoing column and field studies will evaluate how well these relationships translate to in situ conditions.

Acknowledgements (Best Team Ever!)

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Civil and Environmental Engineering, University of Waterloo



And many others

And thank you! Any Questions?



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