Tackling Benzene Contamination with Anaerobic Microbes

Molecular Genomic Approaches for Tracking *In Situ* Anaerobic Benzene Degradation

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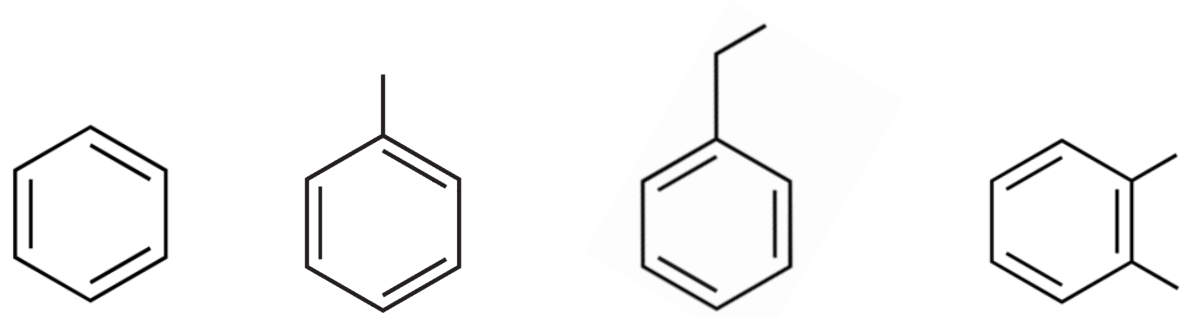
Bioremediation Symposium 2019

Introduction

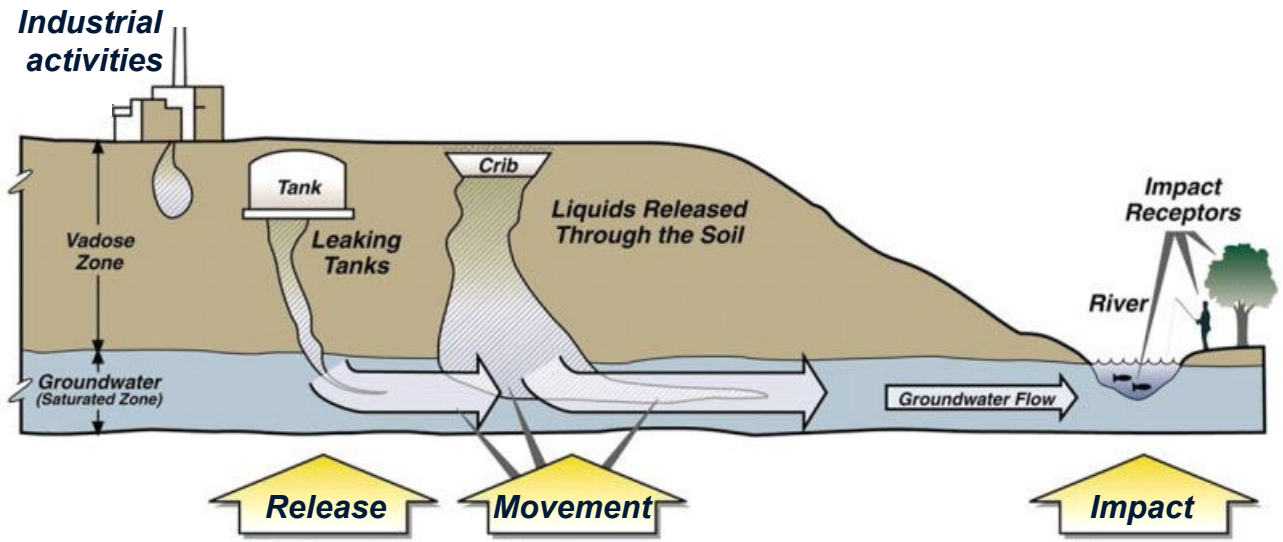
BTEX are petroleum-derived aromatic hydrocarbons and widespread groundwater pollutants.



Benzene **T**oluene **E**thylbenzene **X**ylene

Groundwater contamination with **benzene** is of primary concern;

- Potent **carcinogen**,
- **Mobile** pollutant due to low sorption to sediments & high water solubility,
- Drinking water limit of ≤ 5 µg/L (5 PPB)



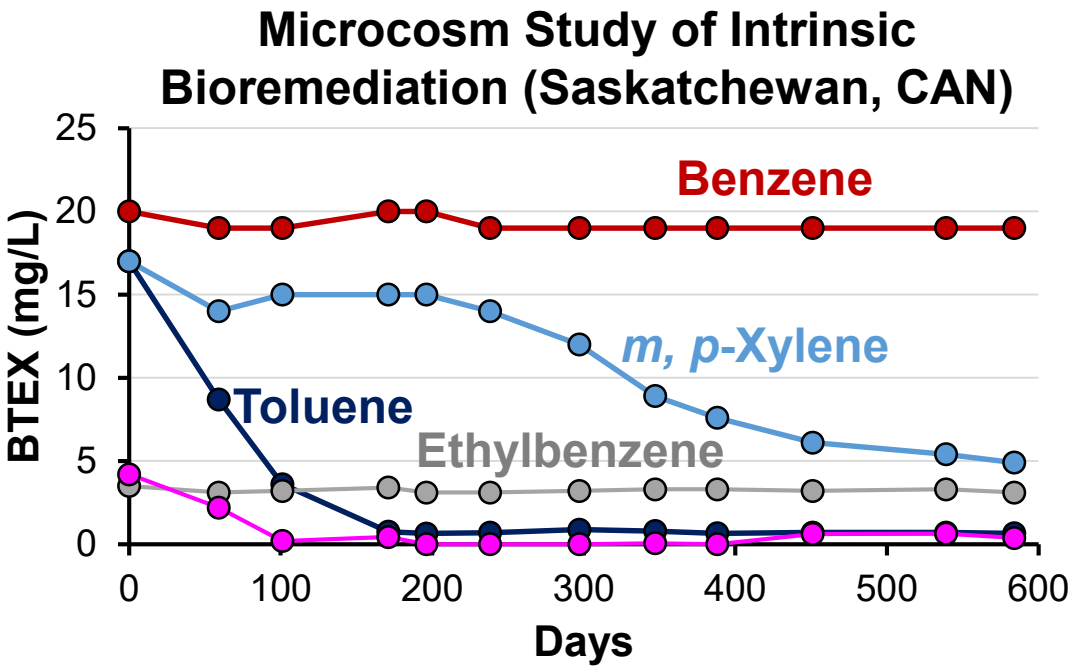
Adapted from Gee et al., 2007 (Vadose Zone J., 6)

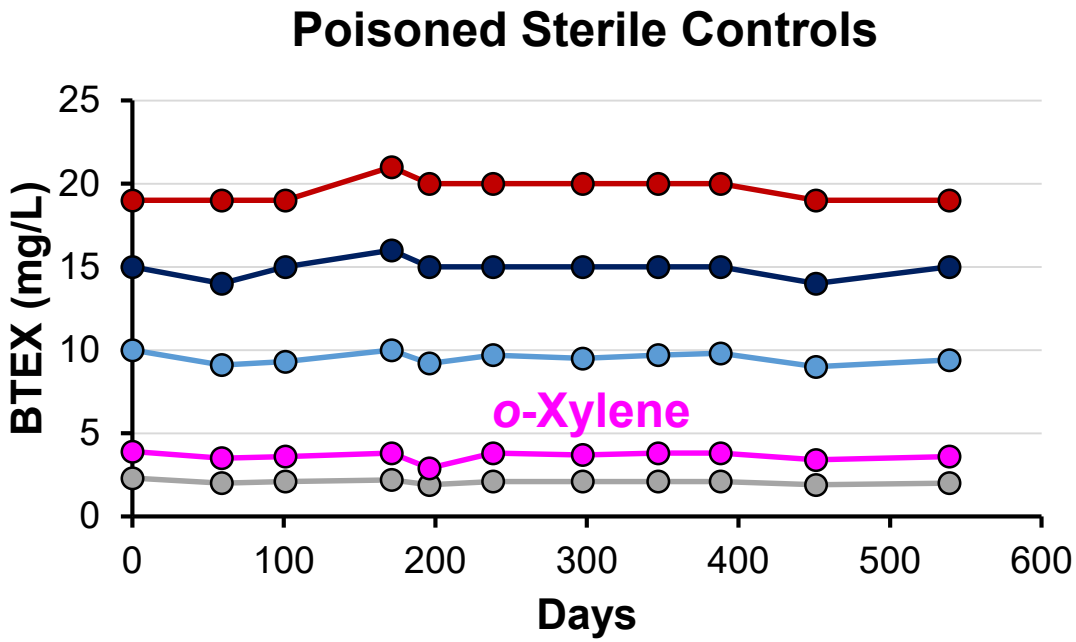
The Fate of Benzene in Groundwater

Contaminant plumes in aquifers usually become **anoxic** due to the low solubility and rapid microbial consumption of oxygen.

Benzene is the **most difficult** BTEX compound to biodegrade anaerobically.

Toluene > *o*-Xylene > *m*, *p*-Xylene, > Ethylbenzene > Benzene

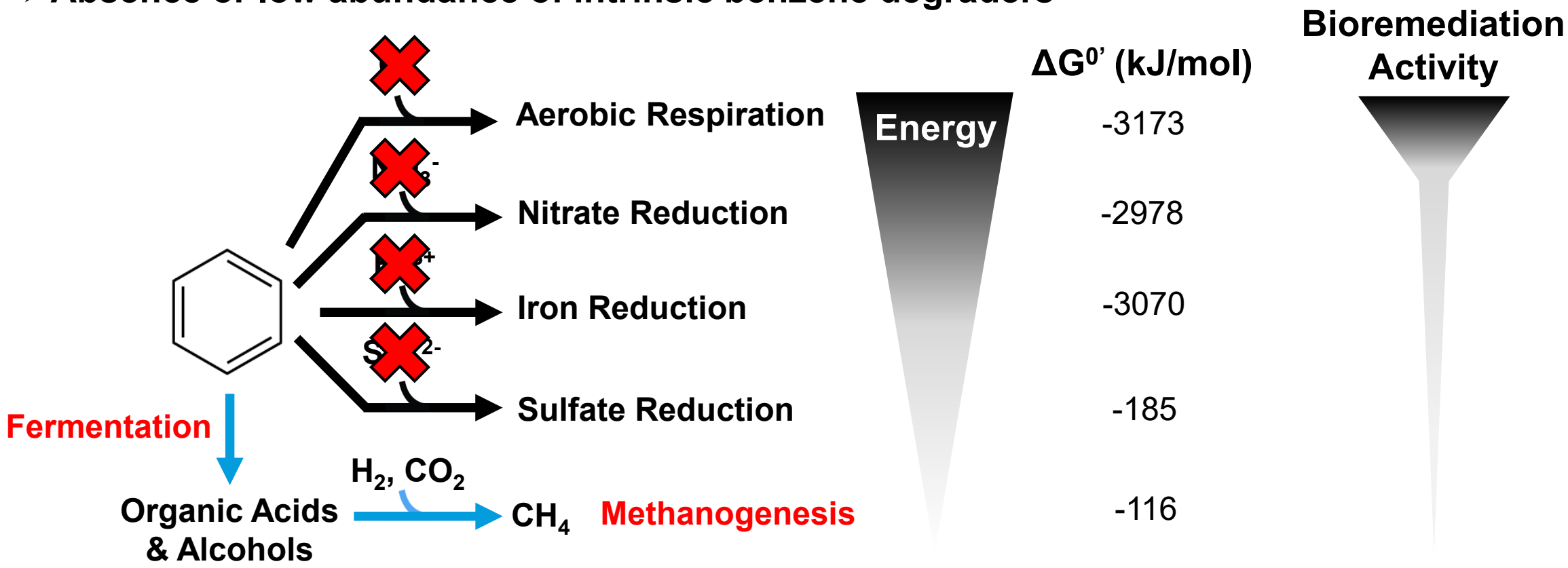




Challenge

Naturally-occurring (**intrinsic**) microbial processes can remediate benzene anaerobically and *in situ*, but are often slow or even undetectable.

- Slow growth rates and low energy yields
- Limiting oxygen or alternative electron acceptors
- **Absence or low abundance of intrinsic benzene degraders**



How Can We Reliably Track *In Situ* Anaerobic Benzene Degradation?

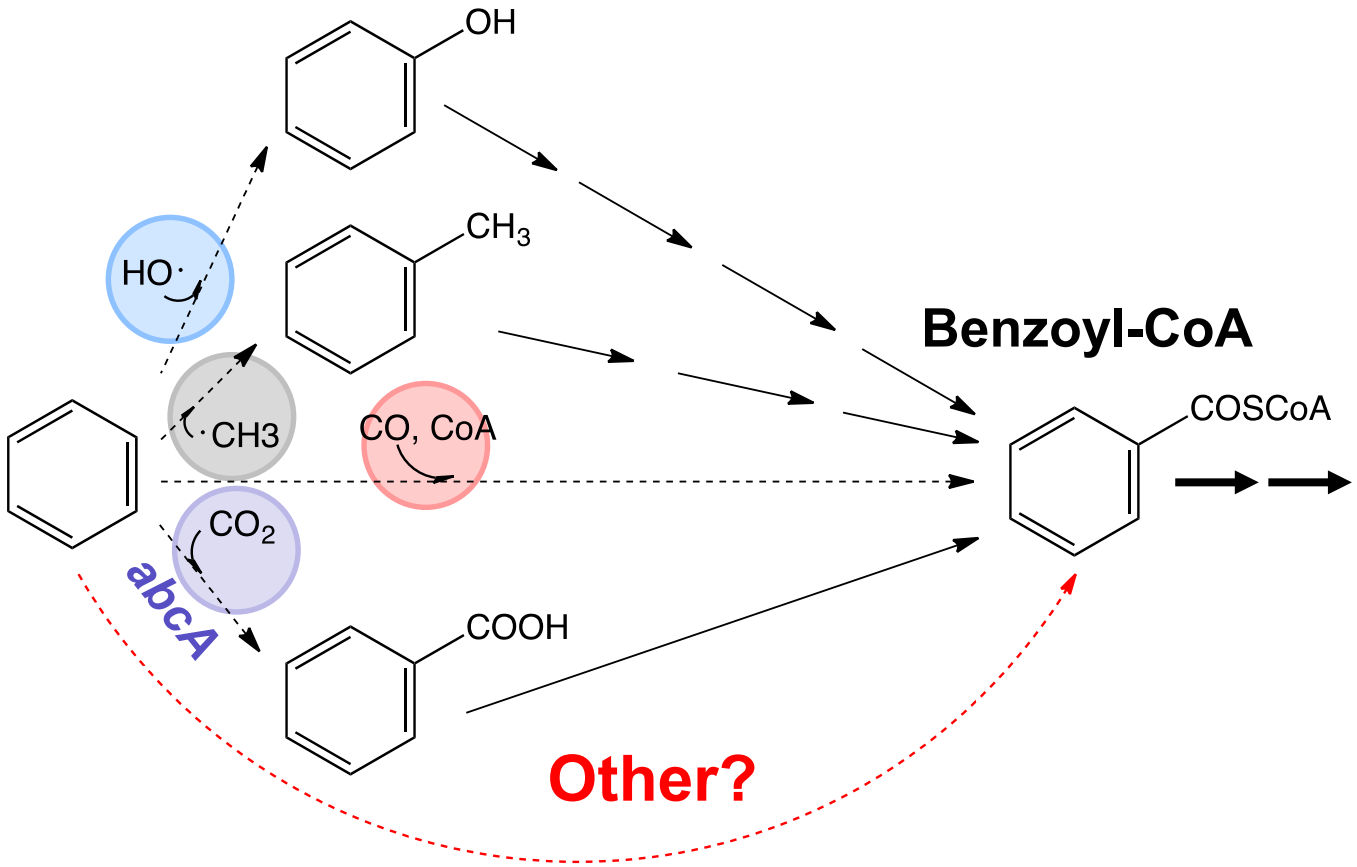
Hypothesis: Track the growth of benzene-degrading microorganisms instead!

Hydroxylation

Methylation

Carbonylation?

Carboxylation



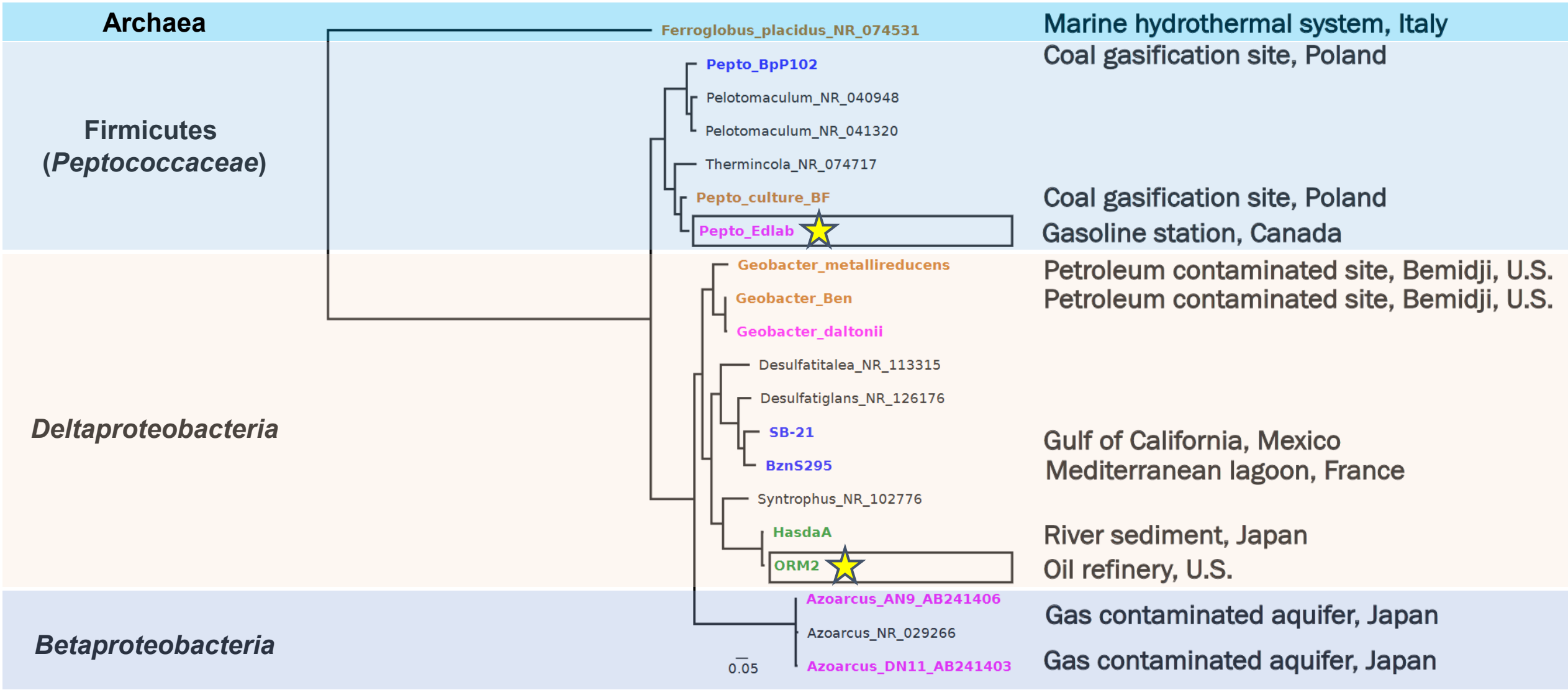
abcA = Anaerobic Benzene Carboxylase, catalytic subunit

Research Objective and Success Criteria

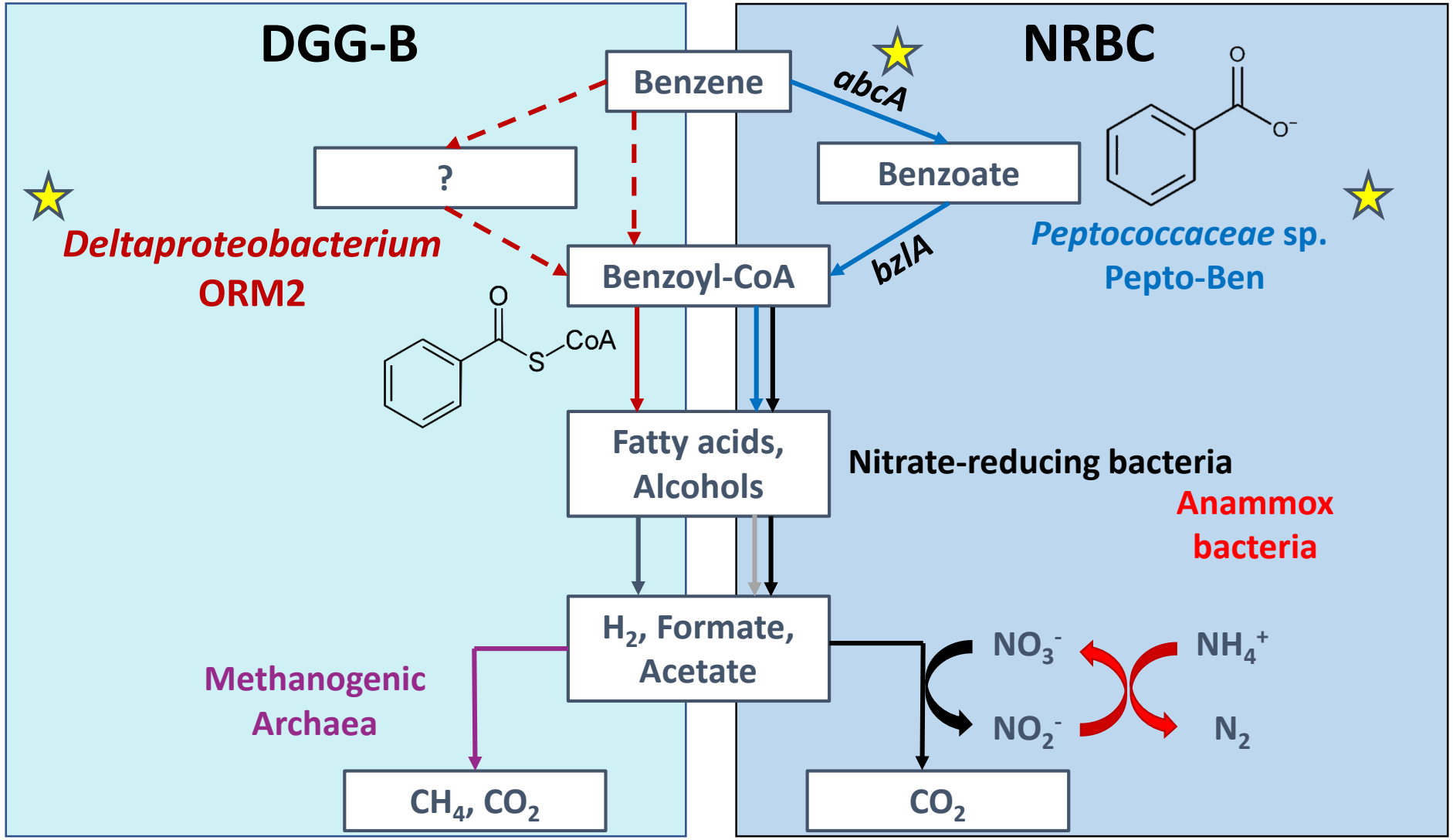
Design a series of molecular genomic tests that can be used to detect and quantify key anaerobic benzene-degrading microorganisms;

i. In established benzene-degrading cultures;
ii. BTEX-contaminated site materials (enriched in lab microcosms); and,
iii. BTEX-contaminated site materials (*in situ* field samples).

Known Anaerobic Benzene Degraders



Developing Molecular Genomic Tools From Established Anaerobic Benzene-Degrading Cultures

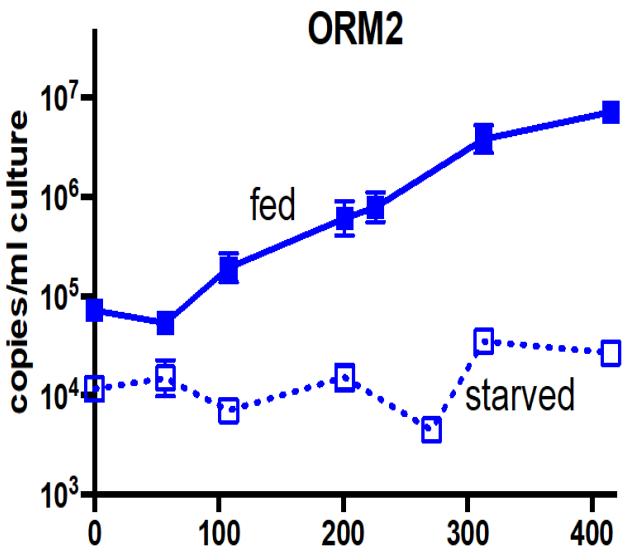


bzlA = Benzoyl-CoA Ligase

Developing Molecular Genomic Tools

ORM2 and Pepto-Ben are the only microbes in DGG-B and NRBC, respectively whose growth are **directly correlated to anaerobic benzene degradation**.

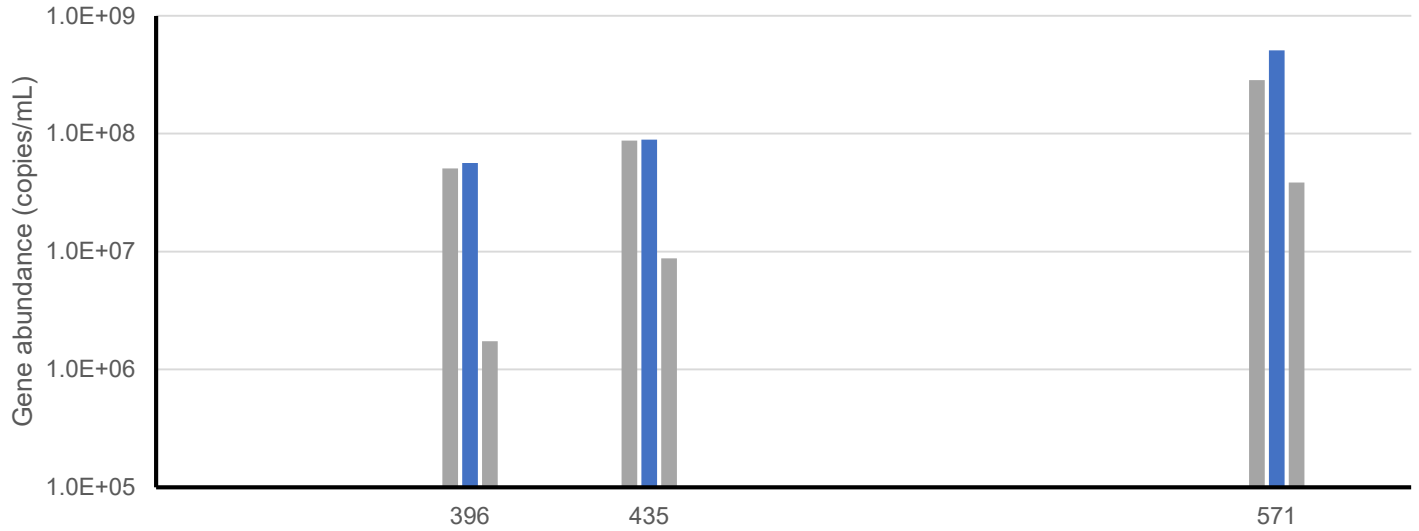
**Pepto.* primers capture a broader range of organisms

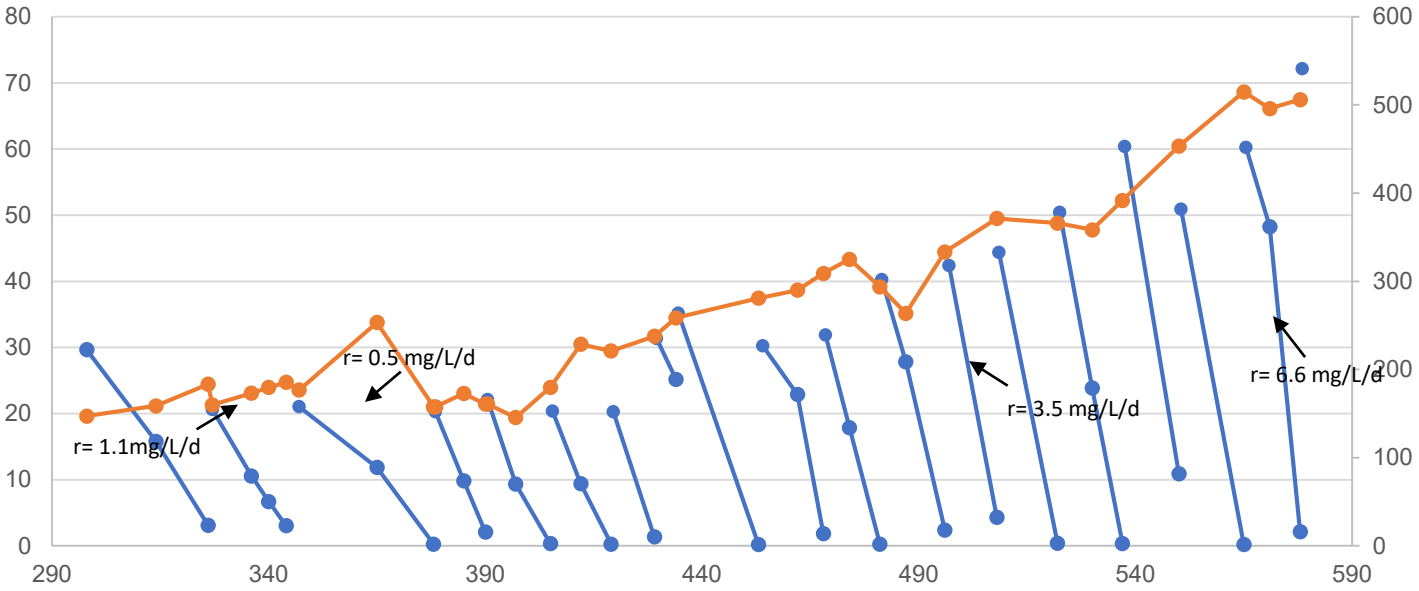


Fei Luo, PhD

		ORM2	*abcA*	*Peptococcaceae**	General Bacteria
Target		16S rRNA	Functional gene	16S rRNA	16S rRNA
PCR efficiency (E)		95.4 ± 4.7%	98.6 ± 4.3%	93.6 ± 3.2%	95.6 ± 3.0%
R^2 on standard curve		> 0.99	> 0.99	> 0.99	> 0.99
Primer specificity	Perfect matches	6	0	240	> 5.5E+05
	1 bp mismatch	9	0	275	>1.0E+06
	2 bp mismatches	12	0	312	>1.1E+07

Luo et al. (2014 and 2016)


1.0E+09
1.0E+08
1.0E+07
1.0E+06
1.0E+05
Gene abundance (copies/mL)
396
435
571

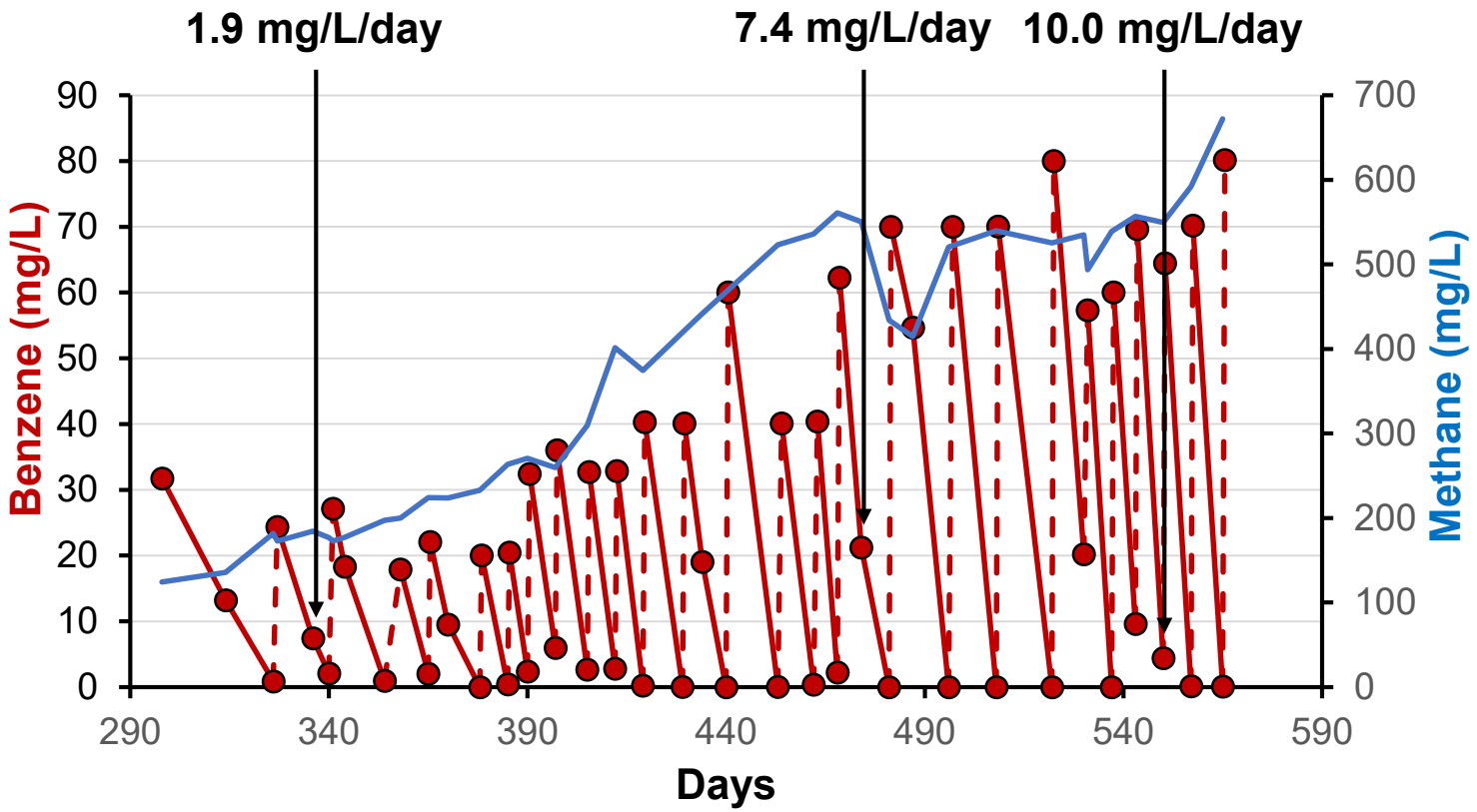

80
70
60
50
40
30
20
10
0
600
500
400
300
200
100
0
290
340
390
440
490
540
590
r= 1.1mg/L/d
r= 0.5 mg/L/d
r= 3.5 mg/L/d
r= 6.6 mg/L/d

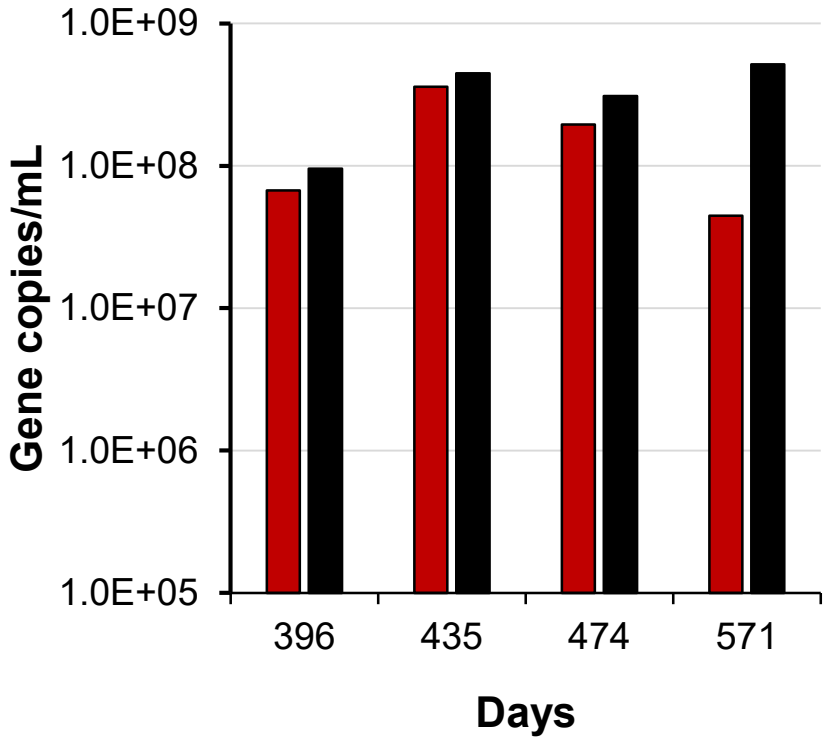
What is Contributing To The Persistence of Benzene in Anaerobic Environments?

DGG-B

✖ Slow growth rates due to low energy yields

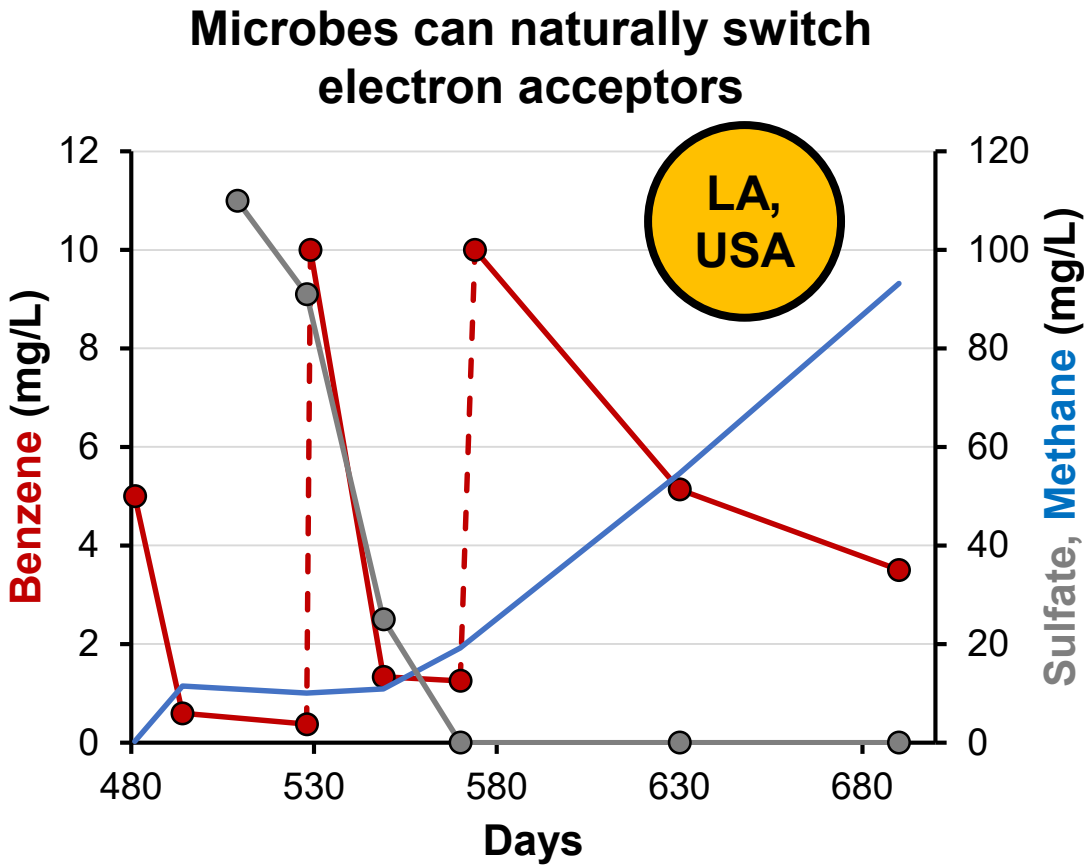
Anaerobic benzene degradation rates accelerate with increasing counts of benzene degraders

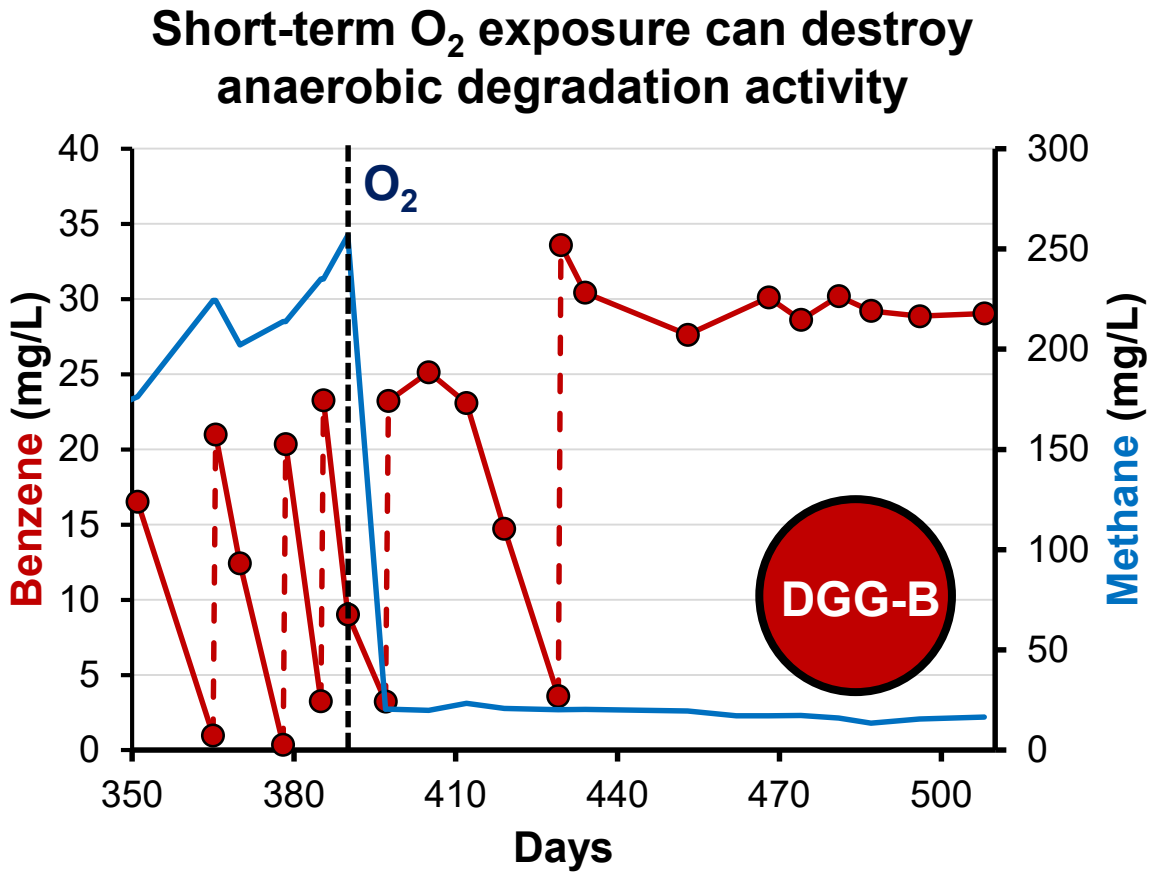




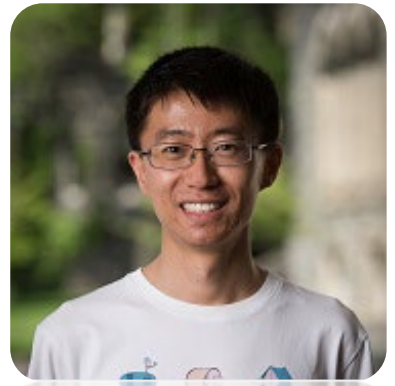
What is Contributing To The Persistence of Benzene in Anaerobic Environments?

✖ Limiting oxygen or alternative electron acceptors



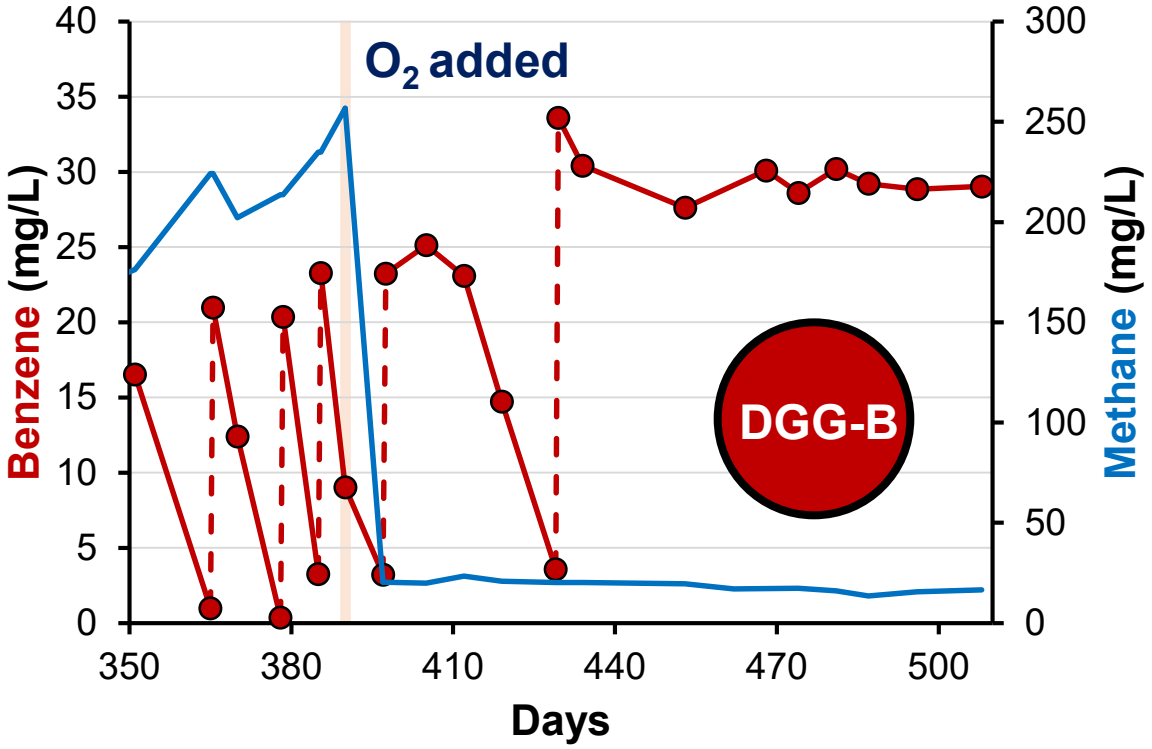


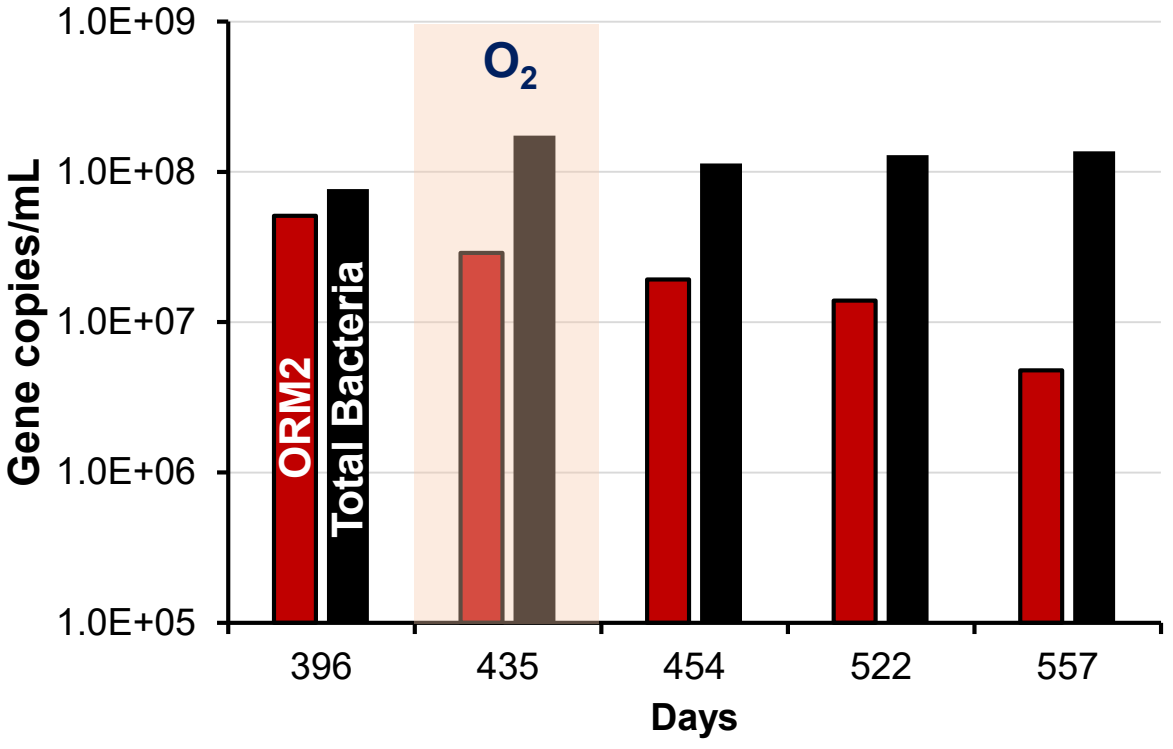
What is Contributing To The Persistence of Benzene in Anaerobic Environments?



✖ Limiting oxygen or alternative electron acceptors

Short-term O_2 exposure kills anaerobic benzene degraders





Guo et al. (in preparation)

Acknowledgements

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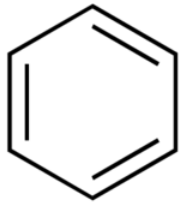
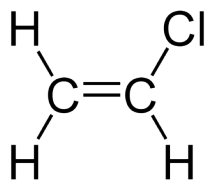


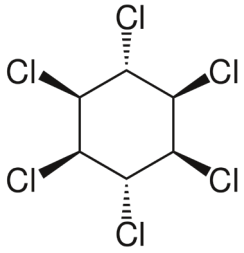
Lab Validation of DGG-B™
Thursday, Session E9
10:30 AM

Sandra Dworatzek, MSc
SiREM



Genomic Studies of Dechlorination Enzymes
Today, Poster #87

Nadia Morson, PhD Student
UofT



Lab Evidence of Microbial HCH Dechlorination
Today, Poster #88

Suly Rambinaising, PhD Student
UofA (Guadaloupe)

Thank You For Your Attention

Tackling Benzene Contamination with Anaerobic Microbes

Contact Us!

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