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Validation of Anaerobic Benzene Bioaugmentation Approaches Through Bench Scale Treatability Studies




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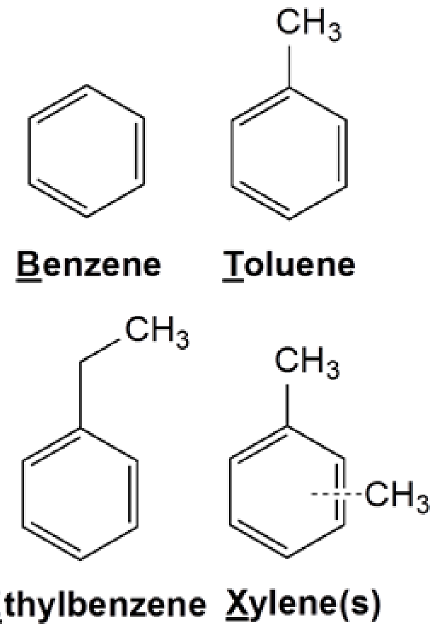
BTEX/Benzene Challenges

- Retail gas stations, refineries and fuel handling stations among potential sources

- BTEX comprises ~18% of gasoline
  - Benzene is typically around 1%



Benzene:

- Potent carcinogen
- Particularly mobile in groundwater due to low sorption & high water solubility
- Most difficult BTEX compound to degrade anaerobically (unsubstituted ring structure)
- Under anaerobic conditions, bottleneck to site remediation

Why Go Anaerobic for BTEX?

- Hydrocarbon sites can go anaerobic - high organic loading consumes O_2
- Electron acceptors (NO_3/SO_4/CO_2) often already present in subsurface
- Anaerobic electron acceptors soluble, easier to apply/distribute compared to O_2 (e.g., epsom salts (sulfate))
- May be viable *in situ* remediation option for deep contamination

Genomic Applications Partnership Program (GAPP) Project 2016 -2019

Overview of Project

1

BTEX Culture Scale Up

2

Treatability Testing

3

Genomics/ Development of Molecular Tools

4

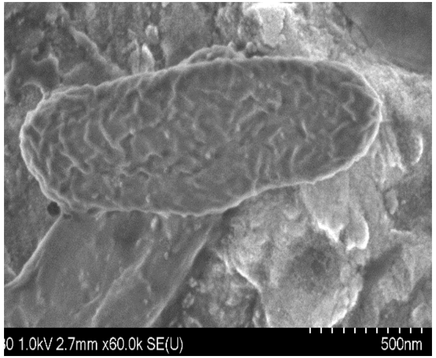
Federal NSN Approval
*underway

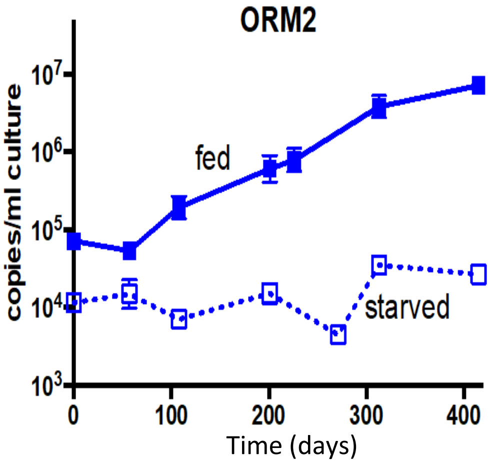
5

Field Pilot Application
*planning stages

ORM2 Anaerobic Benzene Degrader

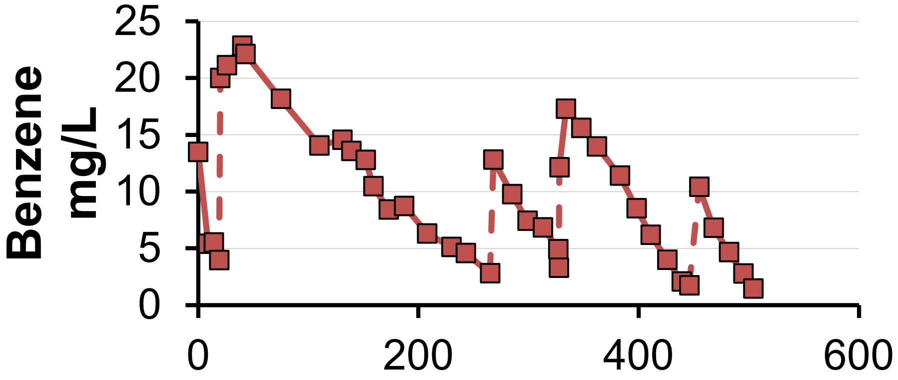
- Benzene specialist derived from an oil refinery site in 2003
- ORM2 is a *Deltaproteobacterium*
- Produces enzymes that ferment benzene
- Slower growing ~ 30 day doubling time



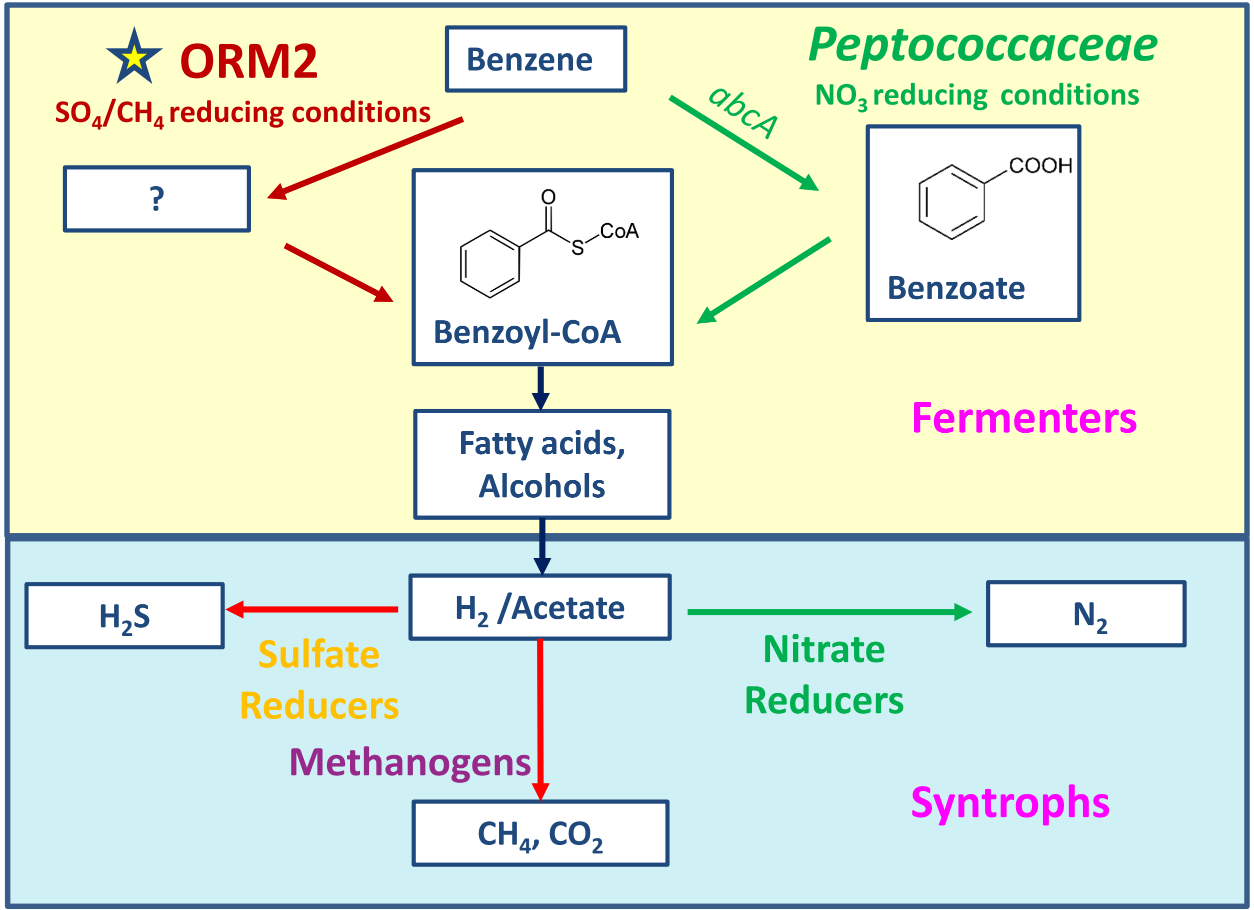


DGG-B Culture – ORM2's Home

- DGG-B successfully scaled up to commercial volumes
  - ➢ Benzene degradation rate = 0.3 mg /L/ day
  - ➢ 10^{10} ORM2/L



Anaerobic BTEX Degradation - a Team Effort



Benzene fermentation is energetically viable only when metabolites (e.g., H_2 and acetate) removed by:

- **Methanogens**
- Sulfate reducers
- Nitrate reducers

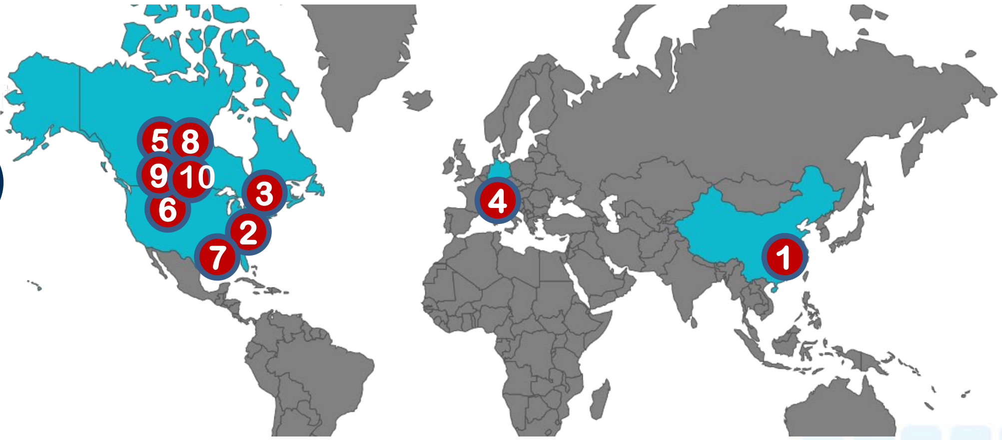
Energy yield lower than aerobic pathways

Treatability Testing Scope

BTEX-contaminated materials from 10 sites were assessed for their anaerobic benzene bioremediation potential

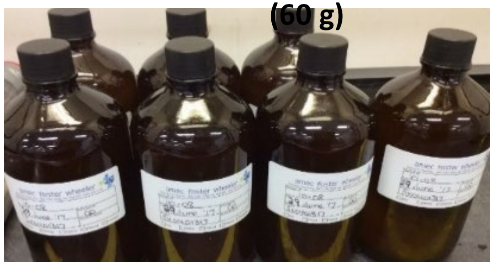
Tested:

- Intrinsic bioremediation
- Biostimulation (nitrate or sulfate)
- DGG-B bioaugmentation



Treatability Testing

Homogenized core samples (60 g)



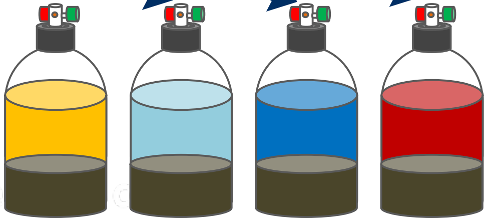
Groundwater sample

Mix

2 mM $NaNO_3$

2 mM Na_2SO_4

2.5% v/v DGG-B



Incubate and monitor

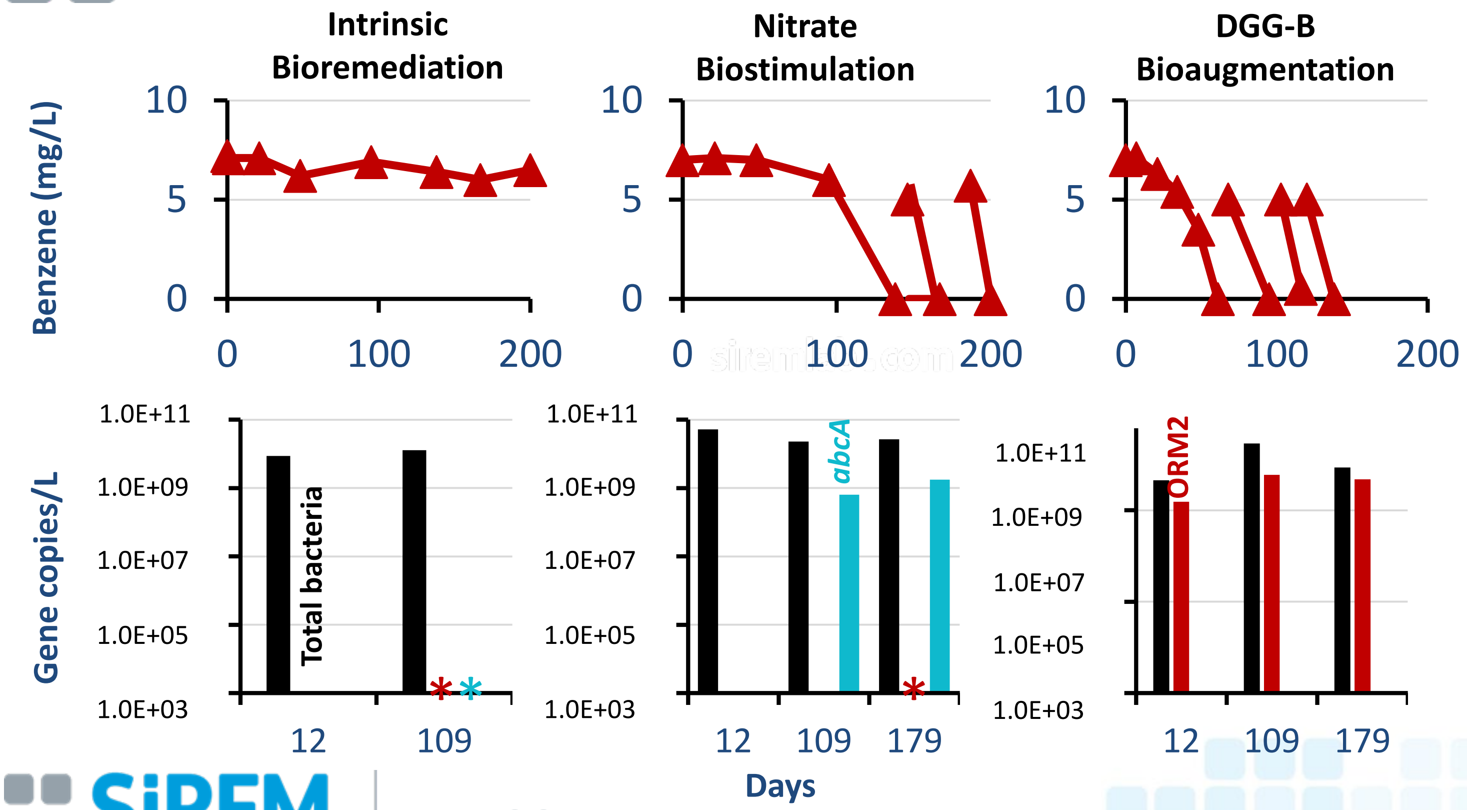
200 mL groundwater slurries
50 mL headspace (10% CO_2 / 90% N_2)

*Aqueous BTEX concentrations ranged between 0.1 – 20 mg/L, depending on site

Treatability Study Results

Site #	Location	Successful Bioremediation Strategy		
		Intrinsic Bioremediation	Biostimulation	Bioaugmentation
1	Nanjing, China	✓		✓
2	New Jersey, USA			
3	Ontario, Canada		✓	✓
4	Germany			✓
5	Saskatchewan, Canada	✓		✓
6	Montana, USA			
7	Louisiana, USA*	✓		✓
8	Saskatchewan, Canada*	✓		✓
9	Saskatchewan, Canada*			✓
10	Saskatchewan, Canada*			

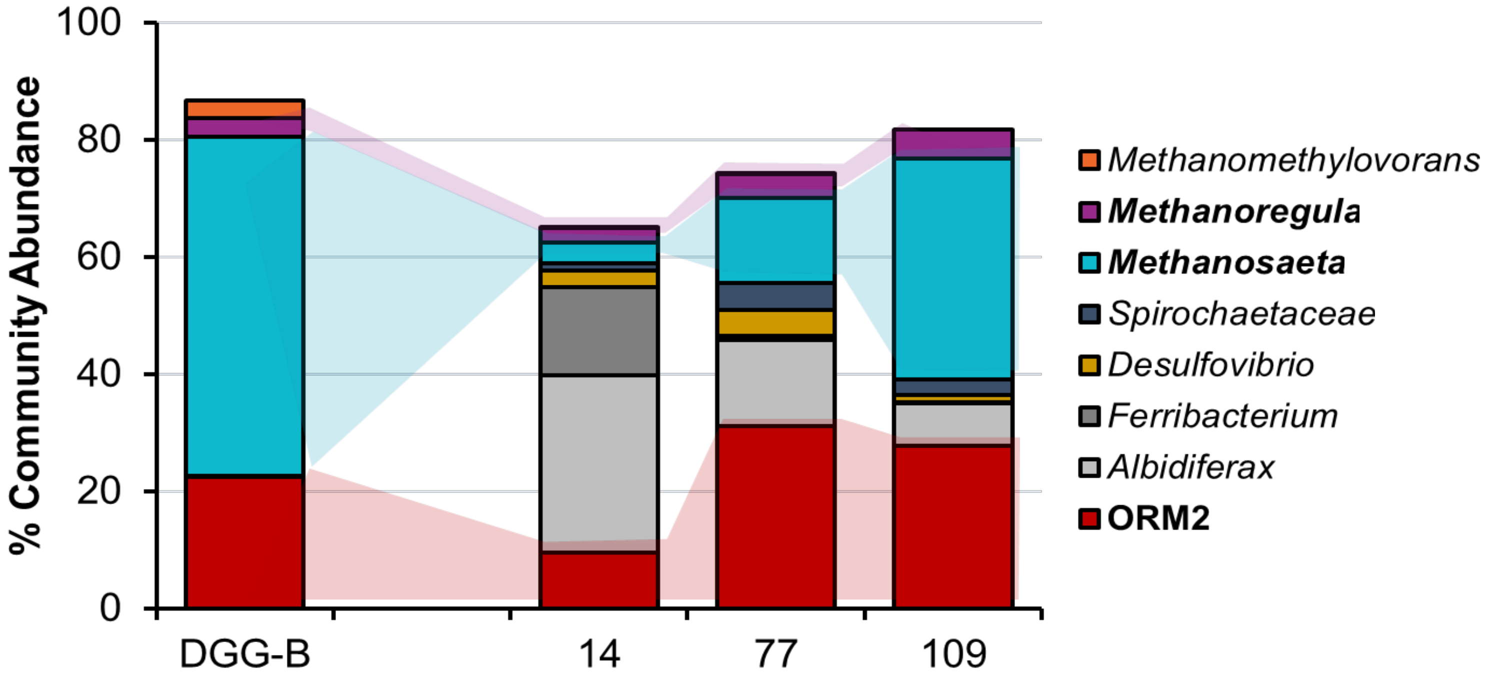
Treatability Test Results (Site #3, ON)



* = below quantifiable limits

Treatability Test Results (Site #3, ON)

- Microbial community sequencing confirms enrichment of key DGG-B microbes post-bioaugmentation
  - **ORM2**, ***Methanosaeta***, and ***Methanoregula*** comprised **70%** of 16S rRNA microbial reads

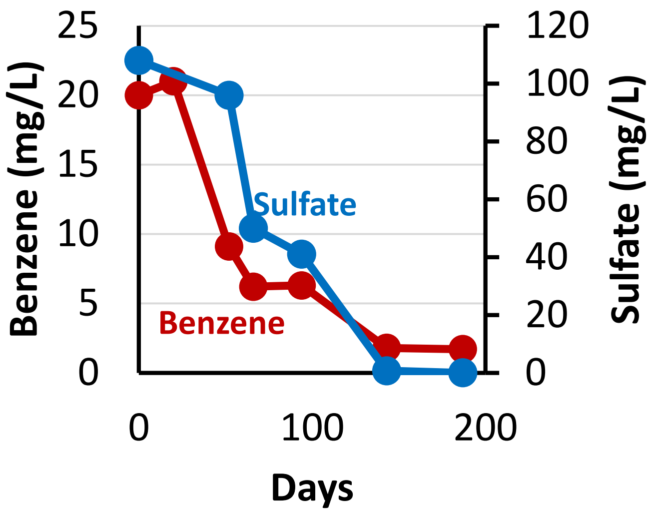


Treatability Test Results (Site #7, SK)

- ORM2 can couple benzene degradation to SO_4^{2-} reduction

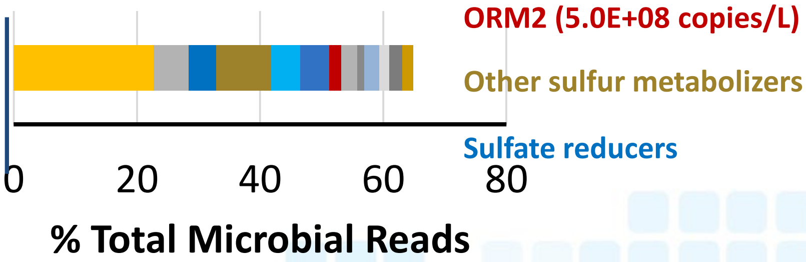
$$C_6H_6 + 3.75\ SO_4^{2-} + 3\ H_2O \rightarrow 6HCO_3^- + 3.75\ HS^- + 2.25\ H^+$$

DGG-B Bioaugmentation



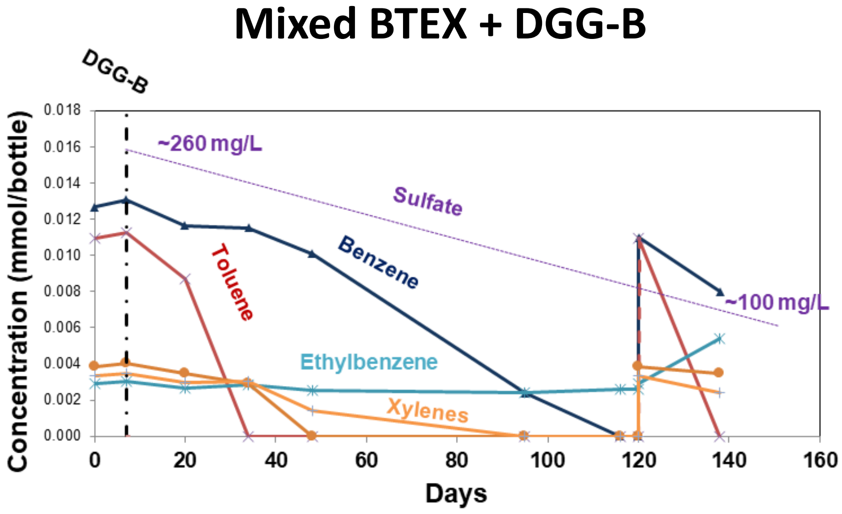
Total Benzene loss (µM)	Sulfate loss (µM)	Sulfate/ Benzene Ratio
234	812	3.5

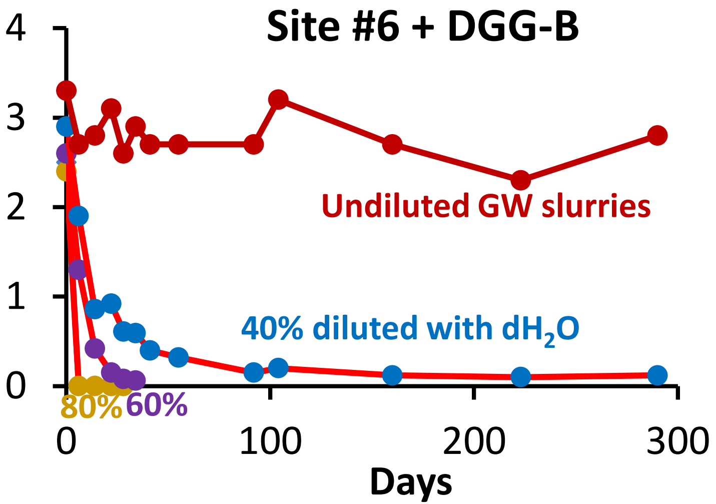
Microbial Community after 147 days



Lessons Learned

- Effective benzene degradation may require pre-treatment of TEX
- Other (unknown) factors can decrease degradation efficiency of DGG-B
  - e.g., Other petroleum hydrocarbons, salinity, metals





Conclusions

- Treatability testing indicates $NO_3/SO_4/CO_2$ are suitable electron acceptors
- Indigenous benzene degraders widely detected but at low proportions (<0.01%) and much lower than optimal abundance (10^7-10^8/L)
- Bioaugmentation possibly required even where indigenous benzene degraders present (slow growth rates) - Application volumes may be higher than other cultures
- Benzene degradation in the presence of TEX compounds slower than benzene alone-may need to treat TEX first

Upcoming Work…

- Identification of enzymatic pathways for benzene fermentation in ORM2 => improved molecular tools for monitoring anaerobic benzene
- Field applications of ORM2 benzene culture (2019)
- Scale-up of existing TEX cultures to commercial volumes, bench scale testing + development of associated molecular tests

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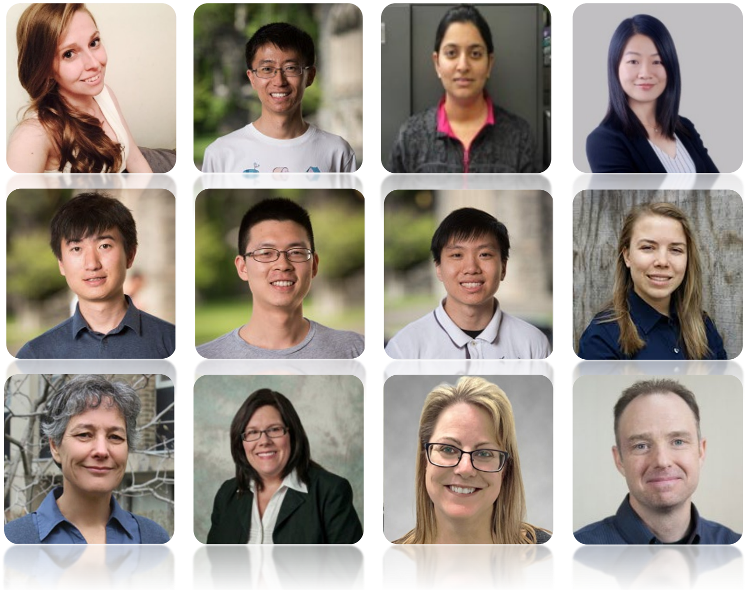

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Thank you for your Attention!

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