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Advances in Anaerobic Bioremediation of Benzene

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SiREM Core Service Areas

Remediation Testing

treatability studies

SiREMNA™

Characterization/Monitoring

- *Molecular Testing*

genetrac®

Bioaugmentation Cultures

KB-1®

- *Passive Samplers for Vapour and Pore Water*

WATERLOO MEMBRANE SAMPLER

SP3

Acknowledgements

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CO-OP

Ontario Genomics

NSERC CRSNG

GenomeCanada

Ontario

Mitacs

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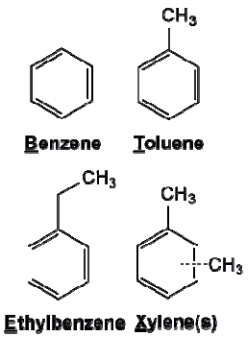
BTEX/Benzene Challenges

- 12,000 gas stations in Canada 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

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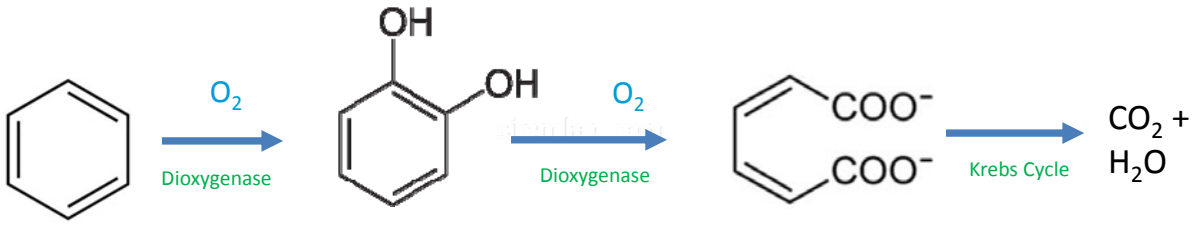
Aerobic Benzene Degradation



Aerobic Benzene Degradation – Energy Yield High

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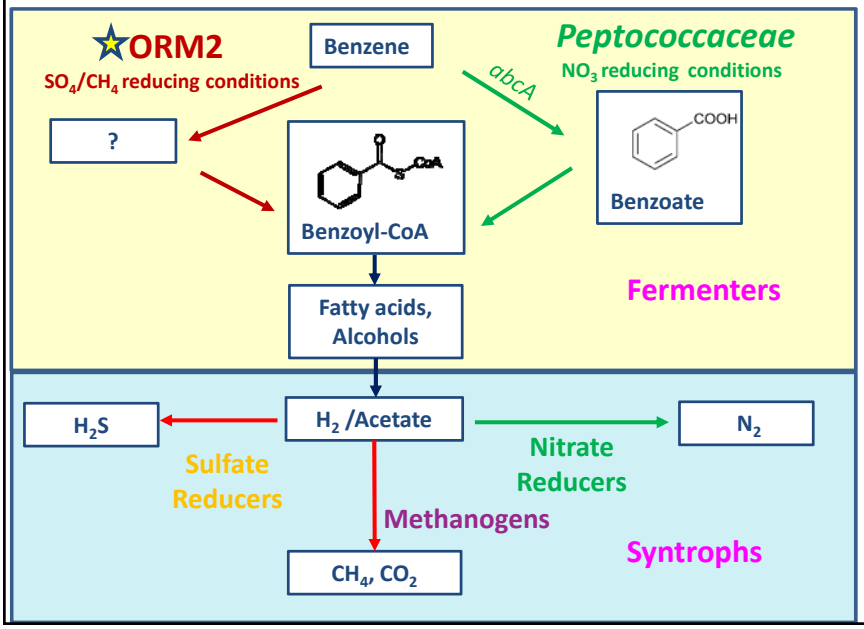
Anaerobic BTEX Degradation - a Team Effort



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

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))
- Anaerobic processes less likely to cause biofouling
- May be viable *in situ* remediation option for deep contamination

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Genomic Applications Partnership Program Project

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**

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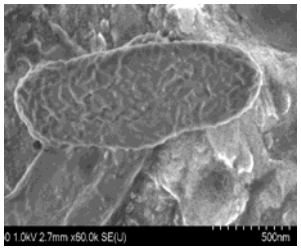
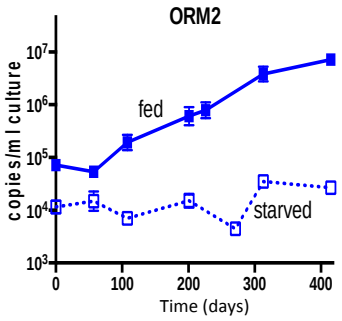
ORM2 Anaerobic Benzene Degrader

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





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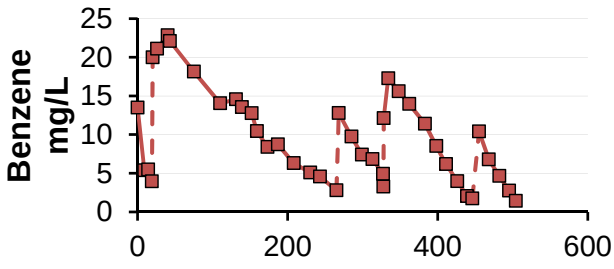
DGG-B Culture – ORM2's Home

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

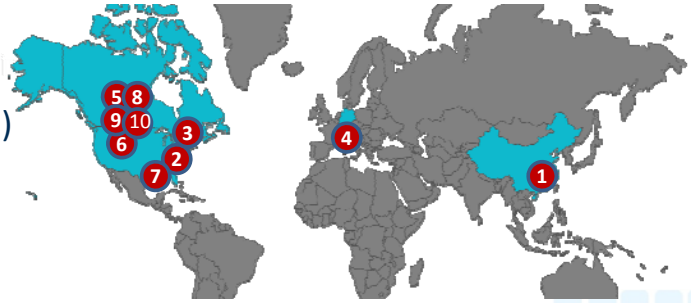


Treatability Testing Scope

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

Tested:

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



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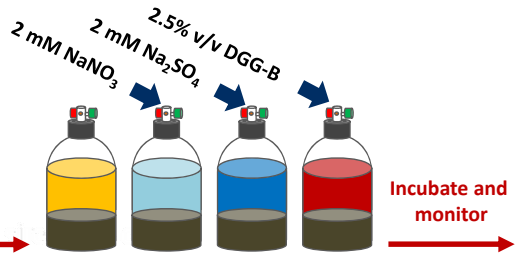
Treatability Testing

Crushed core sample (60 g)

Groundwater sample

Mix



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

Incubate and monitor

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

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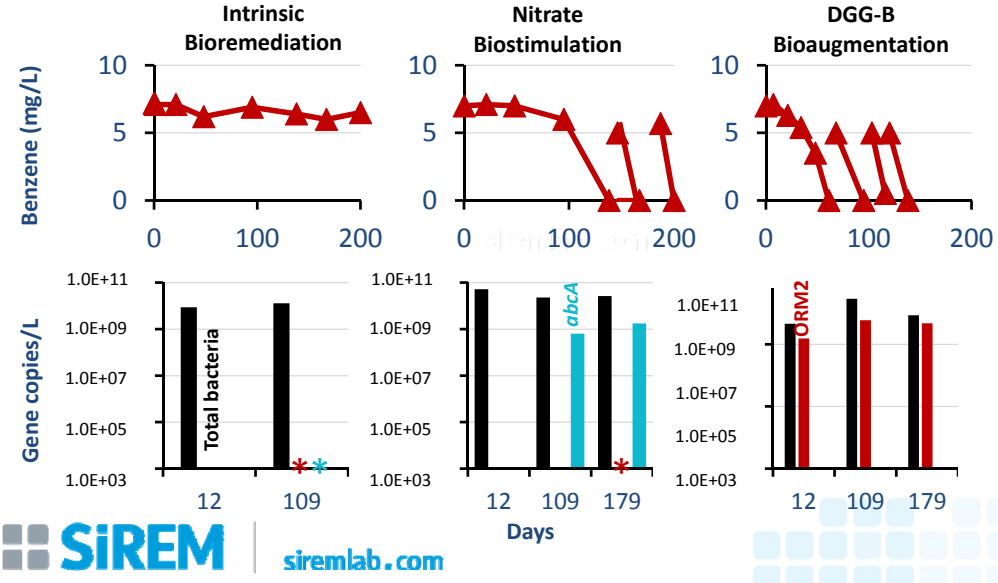
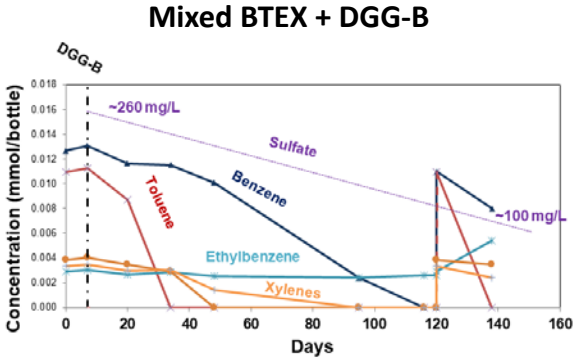
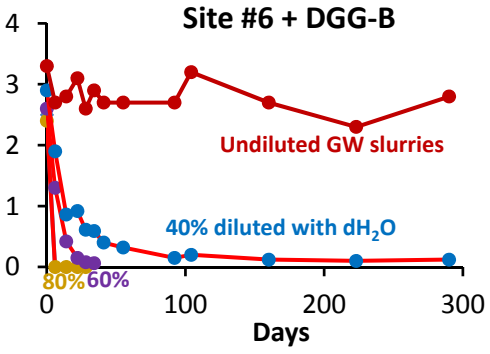
Treatability Test Results (Site #3, ON)



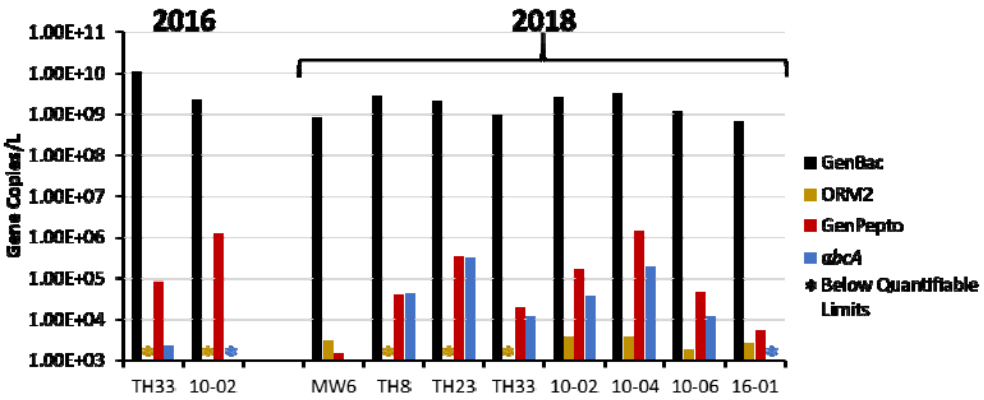
Lessons Learned

- 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



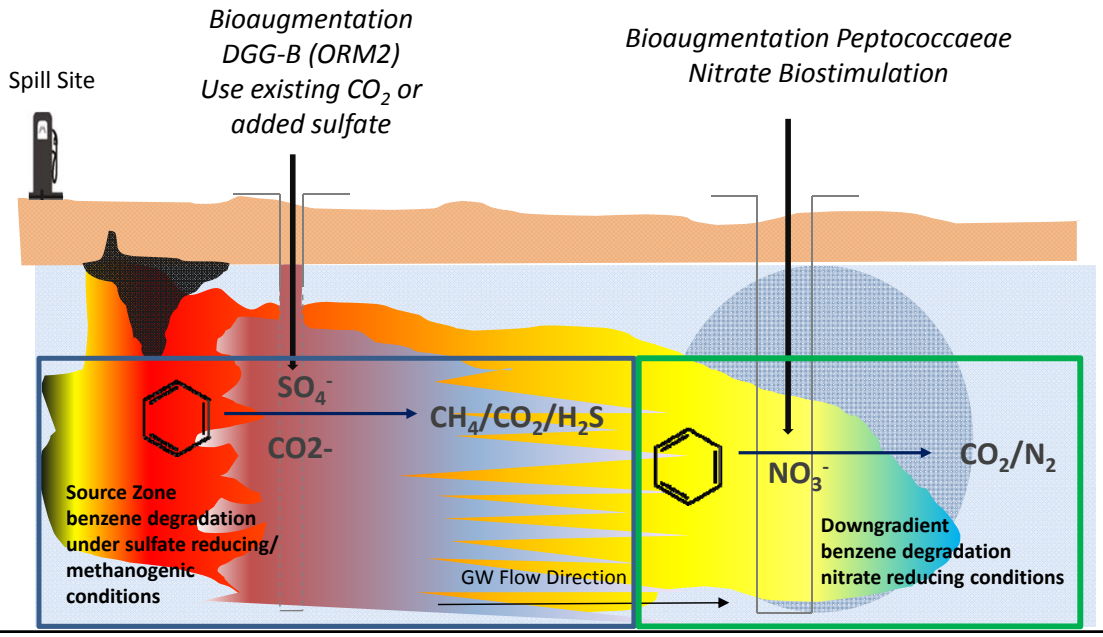


Indigenous Benzene Degraders Gas Station Site, SK



- Anaerobic benzene degraders (**ORM2, GenPepto**) and functional genes (***abcA***) detected in all wells
- Intrinsic benzene degraders comprise < 0.01% of total bacterial populations (**GenBac**).

Potential Anaerobic Field Approach for Benzene



Some Lessons Learned Anaerobic BTEX

- 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

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Upcoming Work…

- Identification of enzymatic pathways for benzene fermentation in ORM2 => improved molecular tools for monitoring anaerobic benzene
- Environment Canada Regulatory Approval
- Field applications of ORM2 benzene culture (2019) NJ, NC, SK
- Scale-up of existing TEX cultures to commercial volumes + development of associated molecular tests

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Thank you for your Attention!

Further Information

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