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Anaerobic Benzene Biodegradation - Insights from Treatability Studies and Molecular Tools

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Outline

- Introduction
- BTEX degradation and bioremediation
- Aerobic vs anaerobic
- Benzene degrading culture DGG-1
- Biomarkers
- Biotreatability studies
- Conclusions and Future work

SiREM Core Service Areas

Remediation Testing

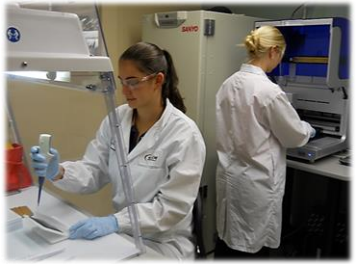
treatability studies

SiREMNA™

Characterization/Monitoring

- *Molecular Testing*

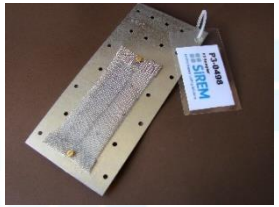
genetrac®



- *Passive Samplers for Vapor and Pore Water*

WATERLOO MEMBRANE SAMPLER

SP3



Bioaugmentation Cultures

KB-1®

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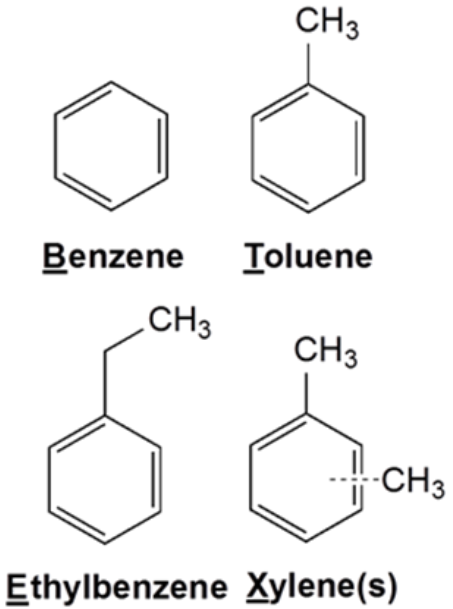
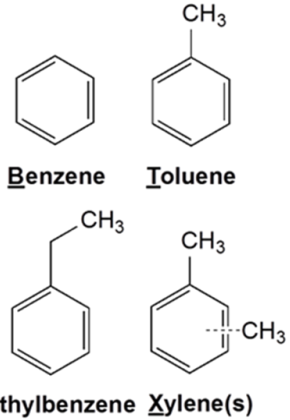
BTEX

- Petroleum hydrocarbons of primary concern in groundwater are benzene, toluene, ethylbenzene and xylenes (BTEX)

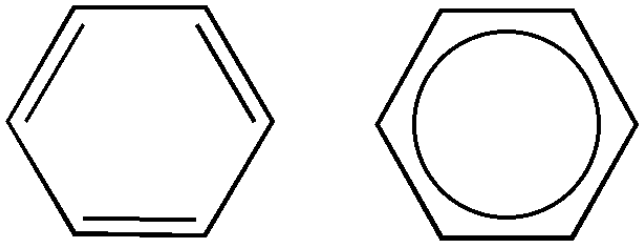
- BTEX comprises ~18% of gasoline



Benzene



- Potent carcinogen
- Particularly mobile in groundwater due to low sorption & high water solubility
- Most difficult BTEX compound to degrade anaerobically (unsubstituted ring structure)



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Bioremediation Approaches

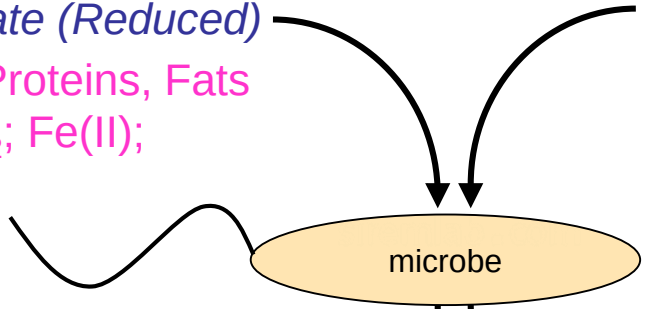
Category	Technology	Example Target Contaminants
Aerobic	Oxygen Addition Nutrient Addition	Petroleum Hydrocarbons, Pesticides
	Bioaugmentation	Petroleum Hydrocarbons, Pesticides
Anaerobic	Electron Donor Addition	Chlorinated Solvents, Perchlorate, Oxidized Metals, Explosives, Nitrate
	Bioaugmentation (KB-1® / KB-1® Plus/ DGG-1)	PCE, TCE, DCE, VC and 1,2-DCA Chlorinated ethanes and methanes: 1,1,1-TCA, carbon tetrachloride and chloroform; CFC-113 Benzene
	Electron Acceptor Addition	Petroleum Hydrocarbons
Cometabolic	Gas infusion, Bioaugmentation	1,4-Dioxane, NDMA, Chloroform, TCE, DCE, VC, MTBE, Creosote, >300 different compounds
Abiotic	Natural Attenuation Reduced Metals	Chlorinated solvents, Oxidized metals,

Anaerobic vs. Aerobic Respiration

Aerobic respiration
metabolic reactions and processes that take place in the cells of organisms that use oxygen as the terminal electron acceptor

Anaerobic respiration
metabolic reactions and processes that take place in the cells of organisms that use electron acceptors other than oxygen (e.g., sulfate, nitrate, iron, CO_2)

Overview of Microbial Metabolism



Electron Donor or Substrate (Reduced)

Sugars, Proteins, Fats
BTEX; H_2; Fe(II);

Electron Acceptor (Oxidized)

Oxygen,
Nitrate (NO_3), Sulfate (SO_4)
Fe(III), CO_2, chlorinated solvents

Electron Donor or Substrate (Oxidized)

CO_2, H_2O, Fe(III)

Electron Acceptor (Reduced)

Water
N_2, H_2S
Fe(II), CH_4,

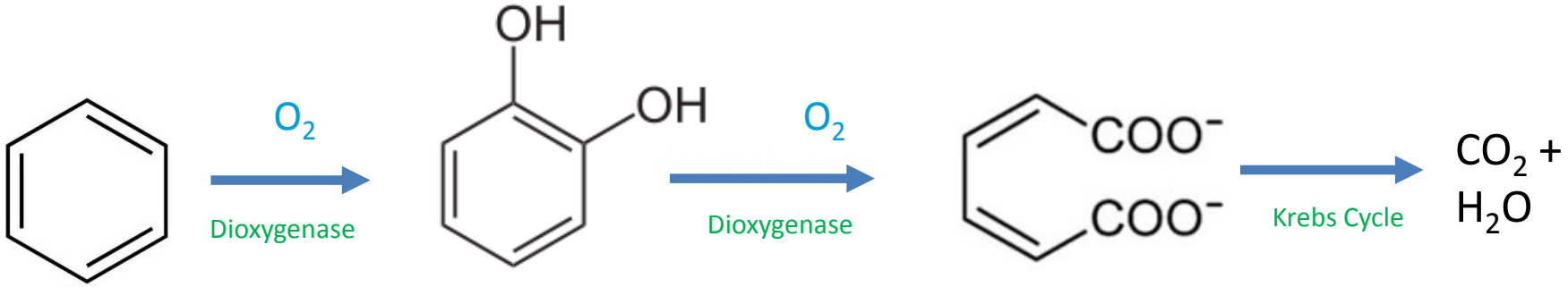
Aerobic BTEX Bioremediation

- Aerobic bioremediation approaches rely on availability or delivery of oxygen (e.g., peroxides, bioventing)

- When contamination is deep or under established reducing conditions, aerobic bioremediation can be difficult to establish and maintain

- Intrinsic microbial populations (e.g., *Pseudomonas*) are often capable of performing aerobic biodegradation
  - bioaugmentation not required

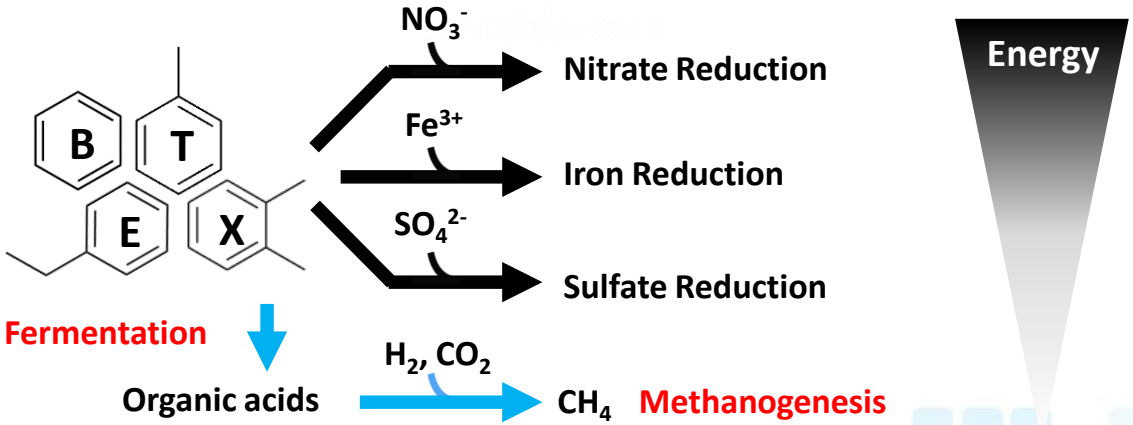
Aerobic Benzene Degradation



Aerobic Benzene Degradation - O_2 Required

Anaerobic BTEX Biodegradation

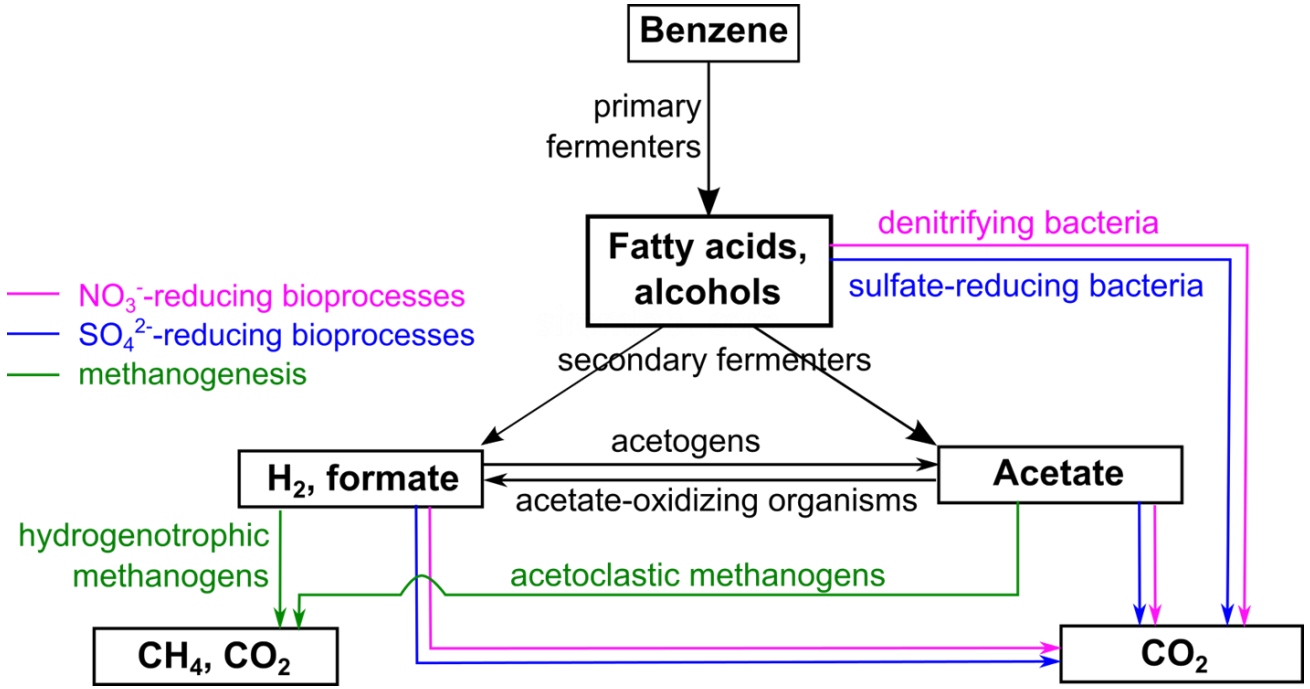
- In-situ BTEX biodegradation is often slow or even undetectable;
  - Slow growth rates and low energy yields
  - Geochemical factors (e.g., co-contaminants, limiting electron acceptors, etc.)
  - **Low abundance of intrinsic BTEX degraders (< 10^2/L or kg)**



Anaerobic BTEX Bioremediation (cont'd)

- Biodegradation of BTEX occurs under anaerobic conditions
    - Methanogenic
    - Nitrate reducing
    - Sulfate reducing
- Microbial populations – may be present at low concentration but growth is slow
- TEX degraders more ubiquitous than benzene degraders
- Benzene is biggest challenge due to its unsubstituted ring structure = need for bioaugmentation

Anaerobic Benzene Degradation



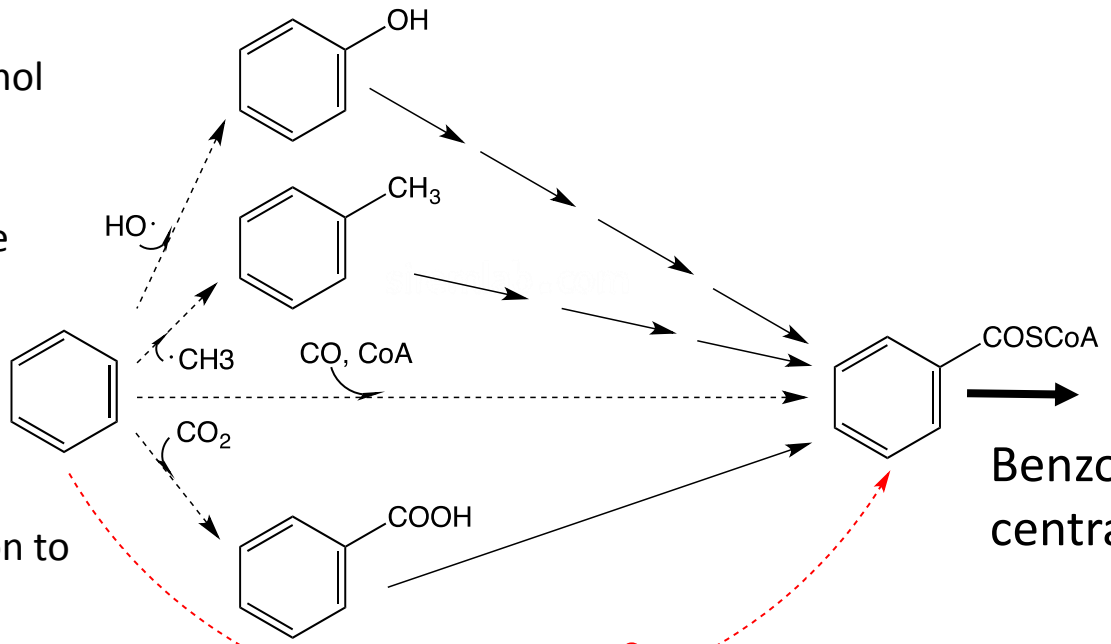
Anaerobic Benzene Activation Mechanisms

Hydroxylation to phenol

Methylation to toluene

Carbonylation to benzaldehyde

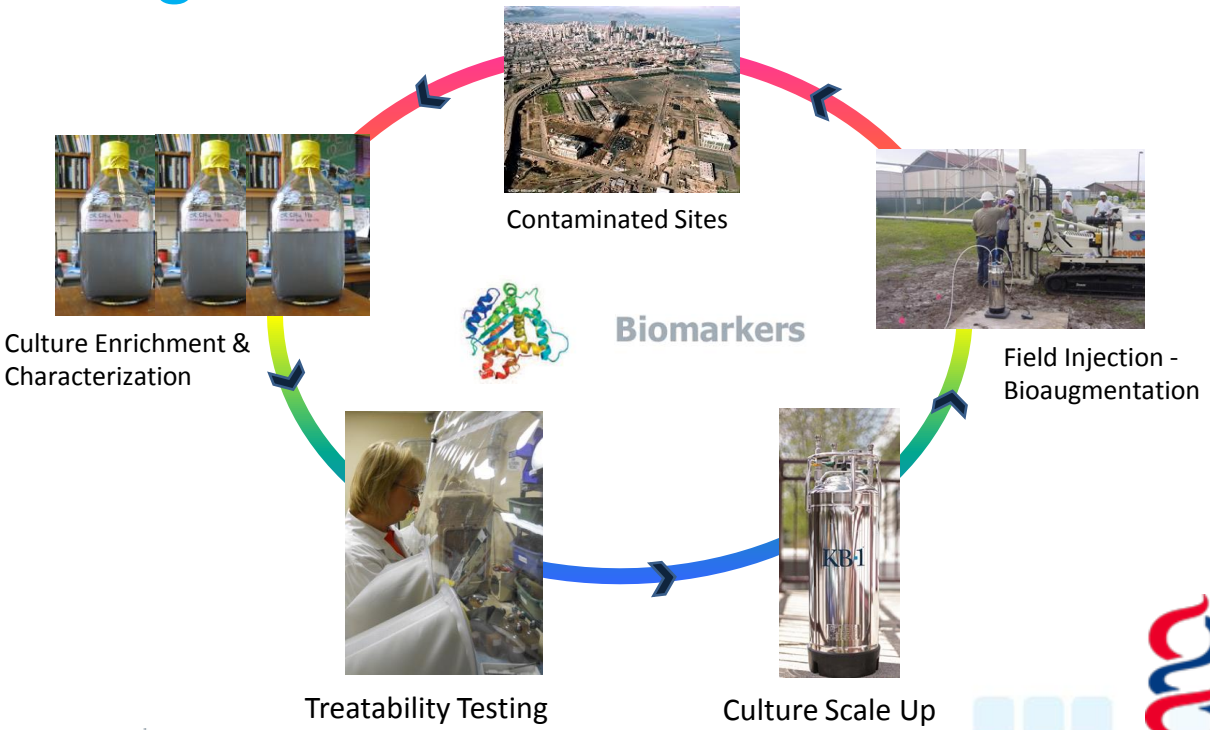
Carboxylation to benzoate



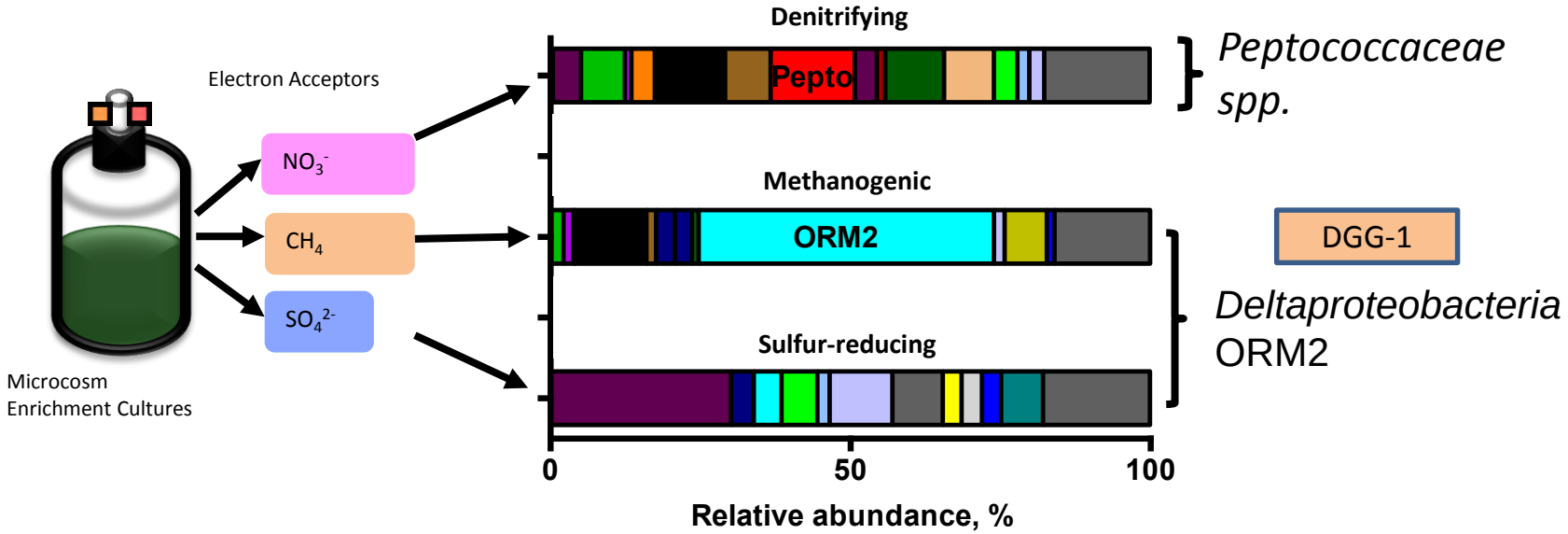
Benzoyl-CoA is a central metabolite

Other????

Culture Development & Use for Bioaugmentation



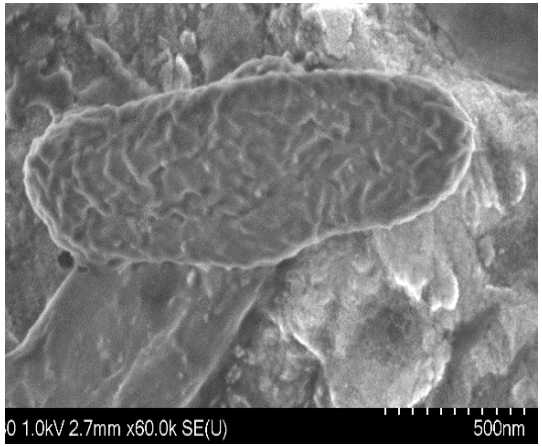
Anaerobic Benzene Degrading Cultures

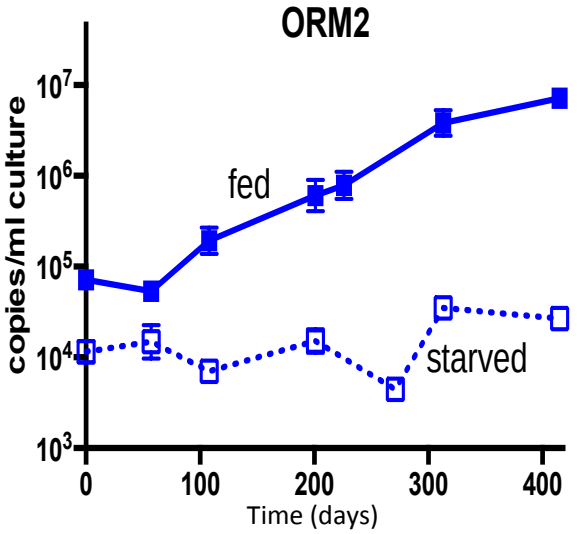


Credit to Dr. Fei Luo, University of Toronto for the slide and metagenomics work

ORM2

- Benzene specialist derived from an oil refinery site in 2003
- *Deltaproteobacterium*
- Slow growing ~ 30 day doubling time
- produces enzymes to ferment benzene





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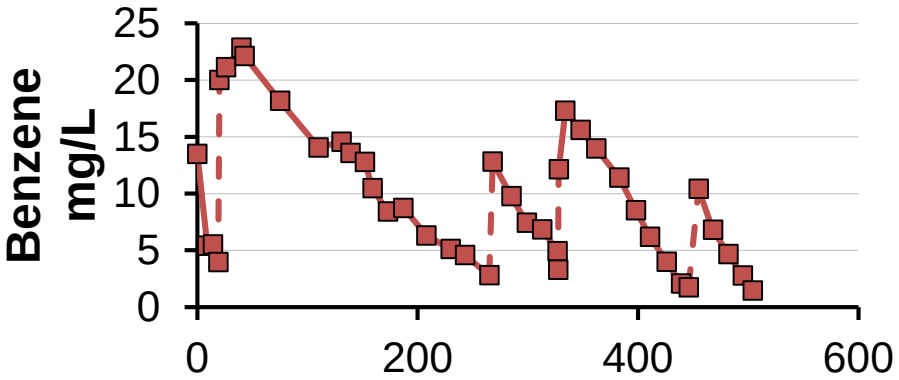
DGG-B Culture

- DGG-B successfully scaled up to commercial volumes
  - Benzene degradation rate = 4.0 µmol L^{-1} day^{-1}
  - 10^8 ORM2 copies/L required for active degradation (doubles every 30 days)



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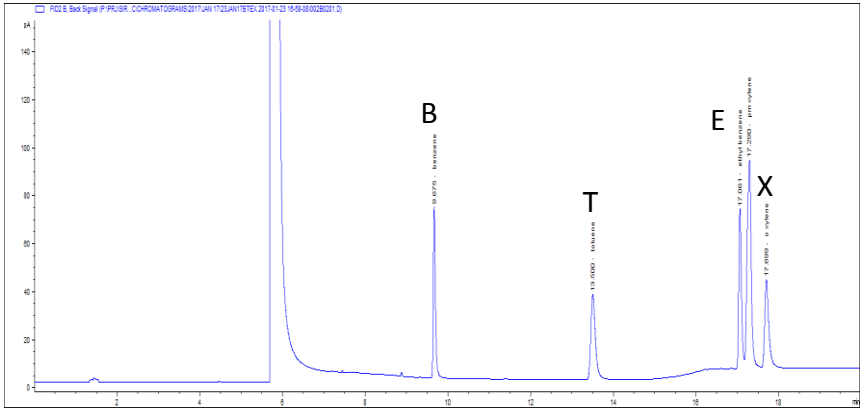
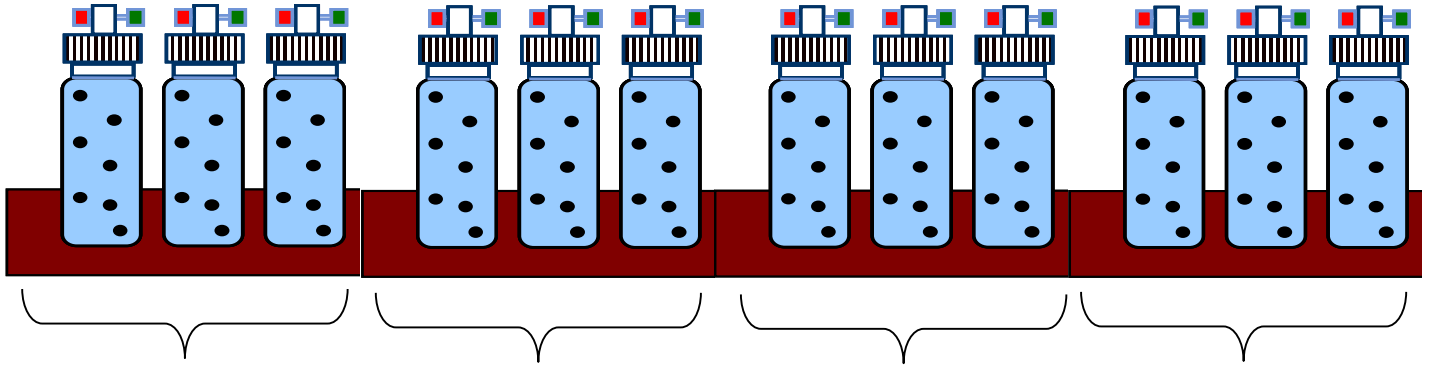
Anaerobic Biotreatability Testing

- Anaerobic conditions maintained during set up, incubation and sampling in glove bags filled with N_2, CO_2 and H_2 gas mixture



Batch Treatability Study Design Features



Sterile Control

Active Control

Biostimulation

Bioaugmentation + Biostimulation

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Treatability studies are custom designed for each site

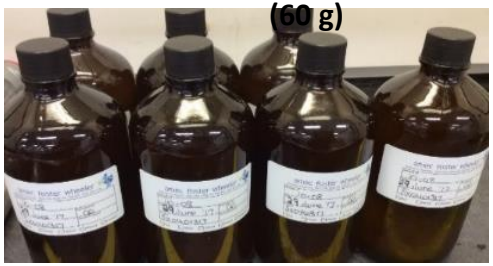
Treatability Testing

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

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

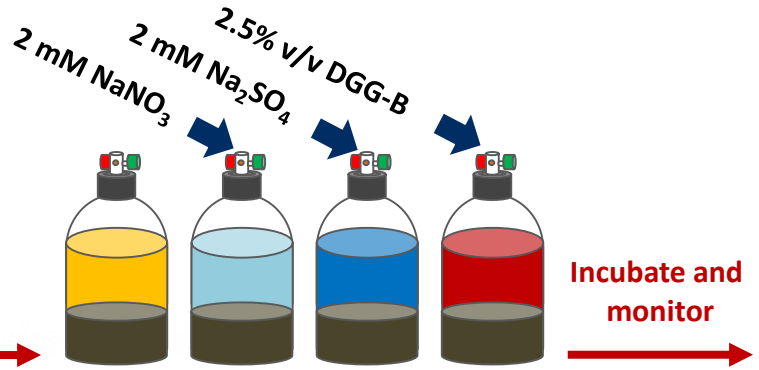
Treatability Testing

Crushed core sample (60 g)



Groundwater sample

Mix



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

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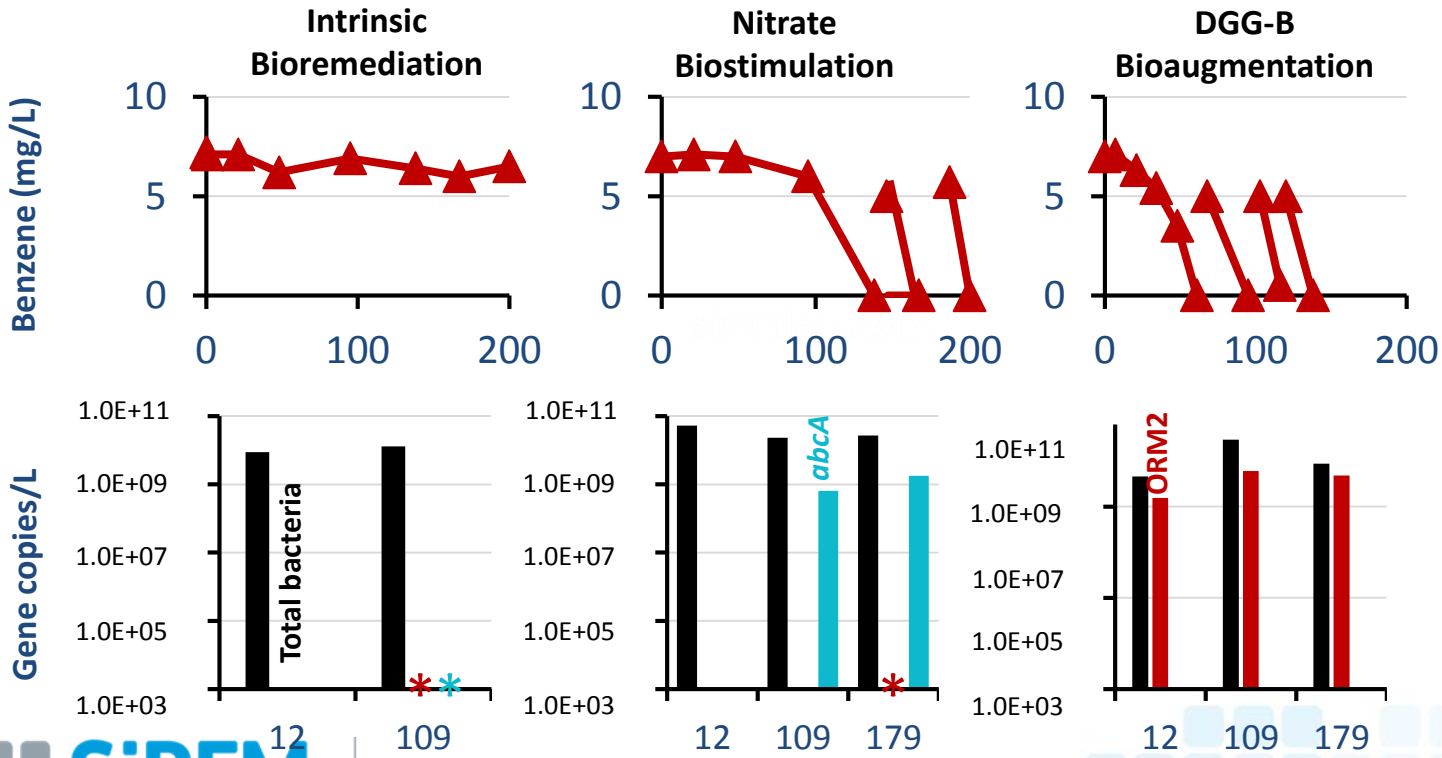
Treatability Test Results

Study #	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*			

* = Studies are ongoing

Treatability Test Results (Site #3, ON)



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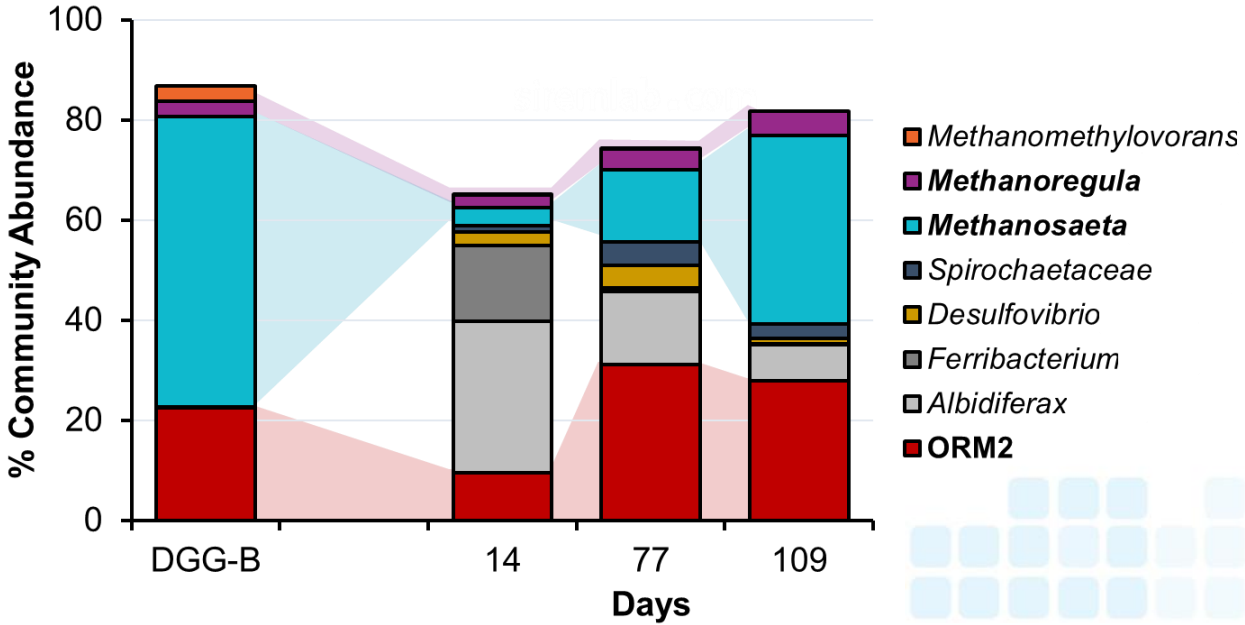
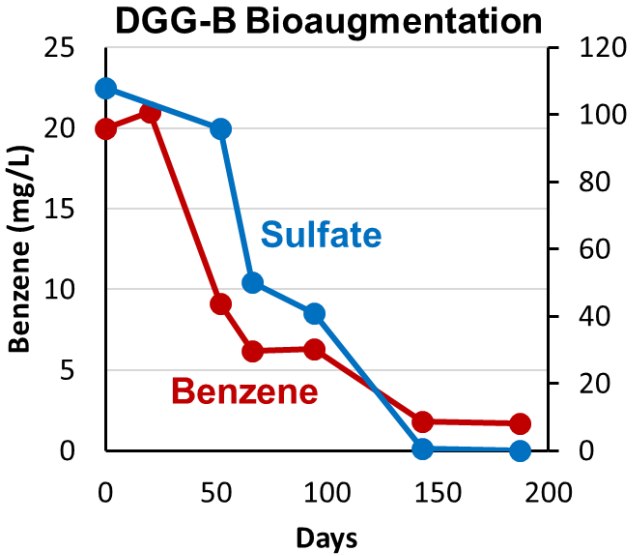
* = below quantifiable limits

Treatability Test Results (Site #3, ON)

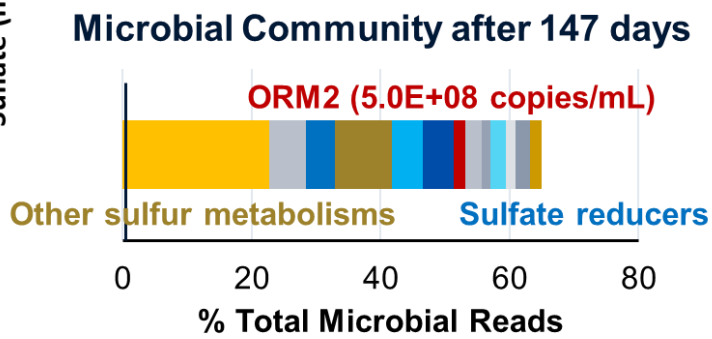
- 16S rRNA gene sequencing confirms enrichment of other key microbes from DGG-B post-bioaugmentation
  - **ORM2**, ***Methanosaeta***, and ***Methanoregula*** comprise **70%** of total microbial community reads vs **0.2%** (intrinsic bioremediation) by day 109



Treatability Test Results (Site #7, Louisiana)



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

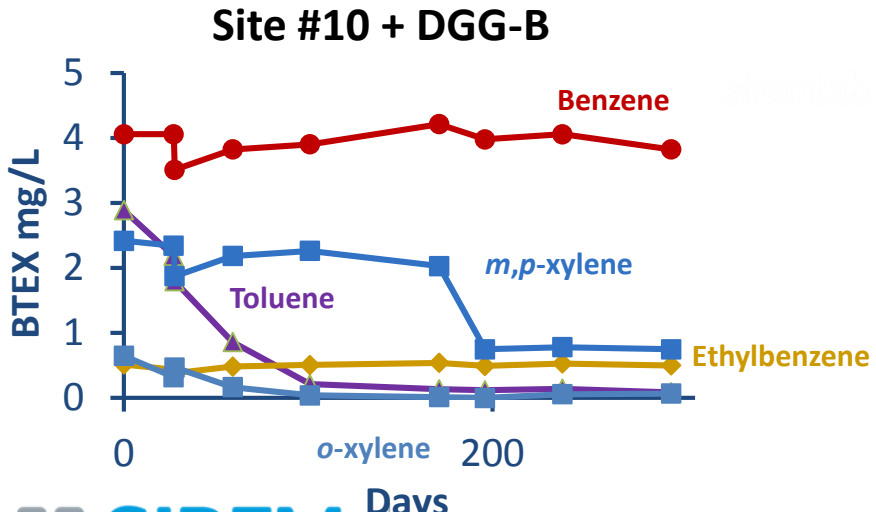


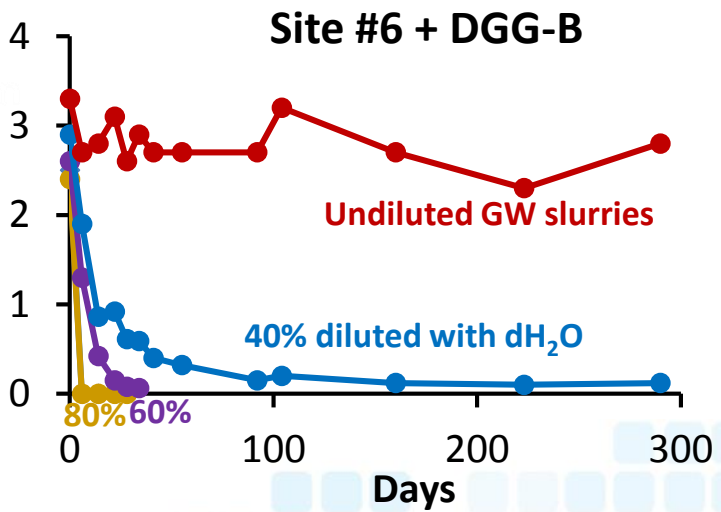
- Benzene degradation coupled to sulfate reduction

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

Lessons Learned

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

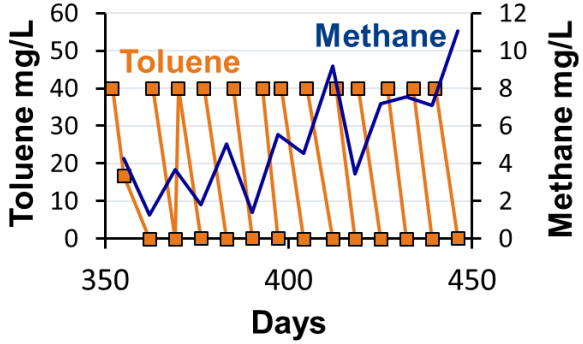




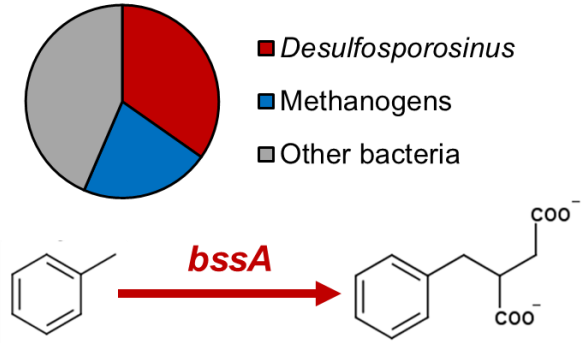
State of knowledge

Target pollutant	Culture developed	Key degraders & genes ID'ed?	Biomarkers developed?	Treatability tested?	Field tested?
Benzene	DGG-B	✓	✓	✓	In progress
	NRBC	✓	✓		
Toluene	DGG-T	✓	In progress		
***o*-Xylene**	DGG-X	✓	In progress		
Ethylbenzene	DGG-E	In progress			

DGG-T Activity



DGG-T Microbial Community



Future work

- Commercial pilot bioaugmentation testing
- On-going applied research
  - Complex sites – PHC, Harsh conditions
  - Complex contaminants
  - New partners University of Alberta, University of Waterloo, Imperial Oil

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- Jennifer Webb (SiREM)
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Thank you!

Further Information

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