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Advances in Anaerobic Benzene Bioremediation: Microbes, Mechanisms, and Biotechnologies

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RemTech, Virtual
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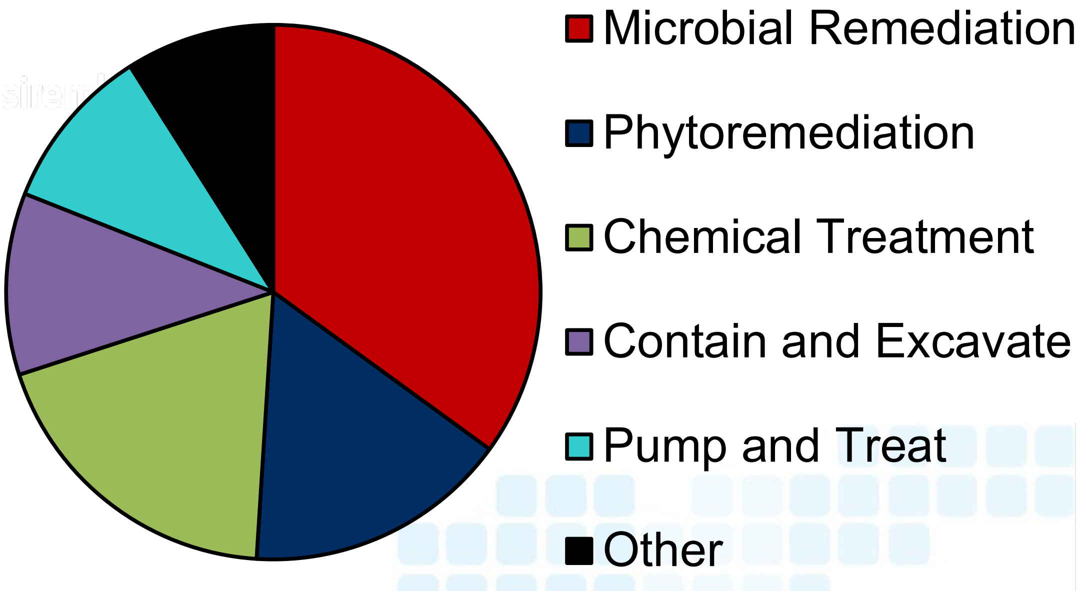
Introduction and Acknowledgements

- Sandra Dworatzek, Jennifer Webb (SiREM, Guelph, ON)
- Elizabeth Edwards, Nancy Bawa, Shen Guo and Courtney Toth (University of Toronto, Toronto, ON)
- Kris Bradshaw and Rachel Peters (Federated Co-operatives Ltd., Saskatoon, SK)
- Krista Stevenson (Imperial Oil, Sarnia, ON)

The Landscape of Hydrocarbon Bioremediation: A Lot Has Changed…

Microbial bioremediation is currently the most common technology used to remediate petroleum hydrocarbons



Adapted from Elekwachi et al., 2014 (J Bioremed Biodeg **5**)

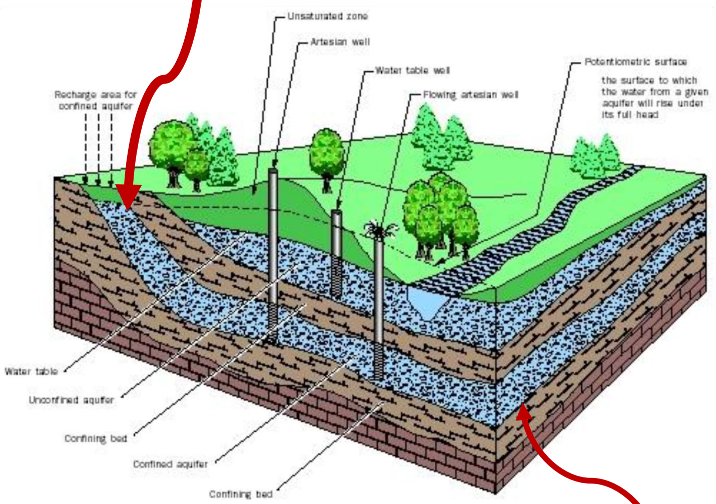
What Sites are Currently Being Targeted for Hydrocarbon Bioremediation?

Offshore Spills (mostly aerobic)

***Ex situ* Bioreactors (mostly aerobic)**

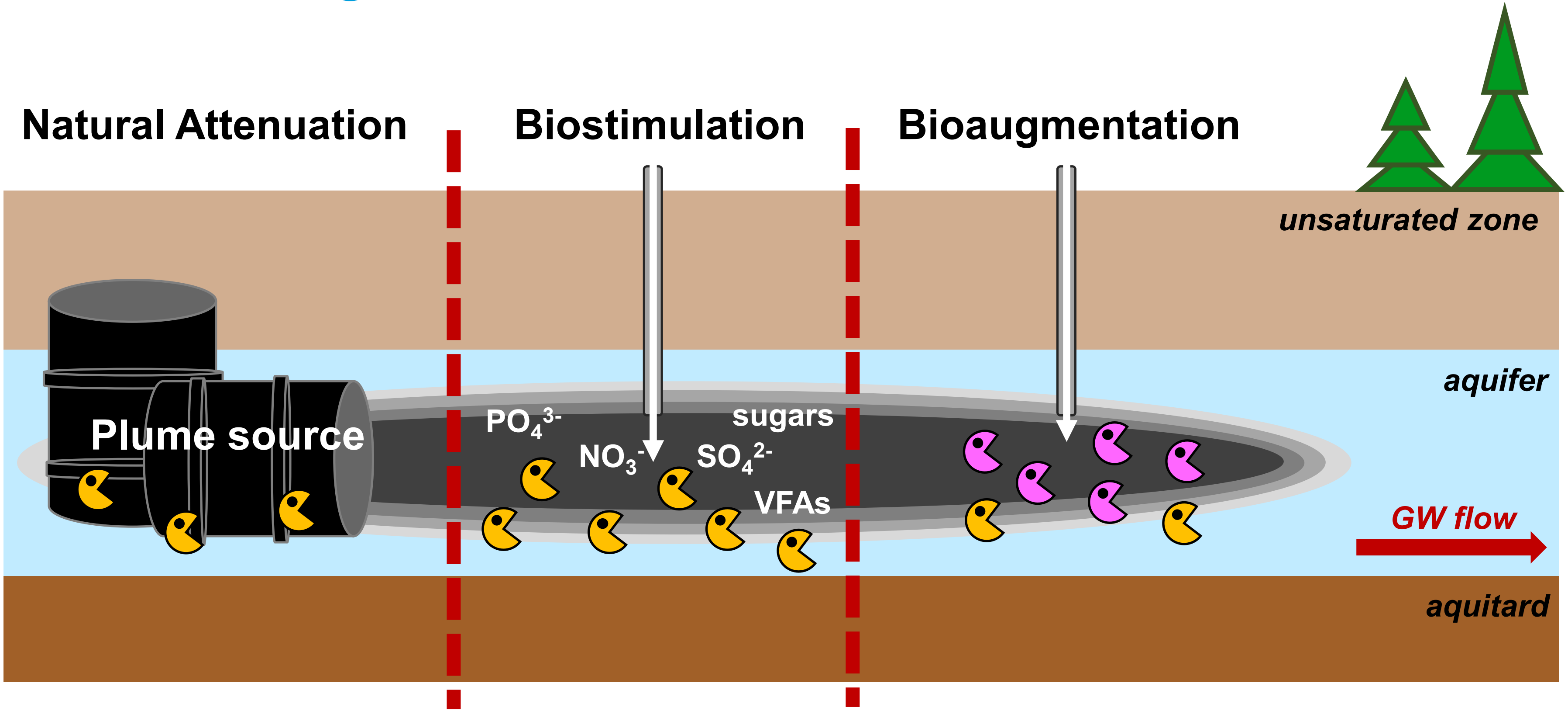
Tailings Ponds (aerobic and anaerobic)

4 Shallow Soils and Groundwater (aerobic)



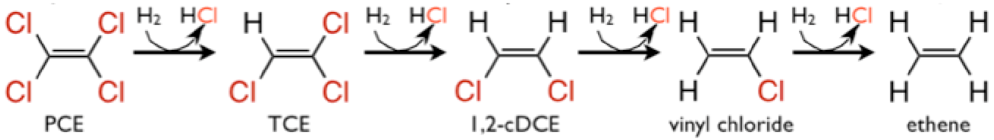
5 Deeper Groundwater (intrinsically anaerobic)

Groundwater Bioremediation Technologies Focusing on Anaerobic Microbial Processes



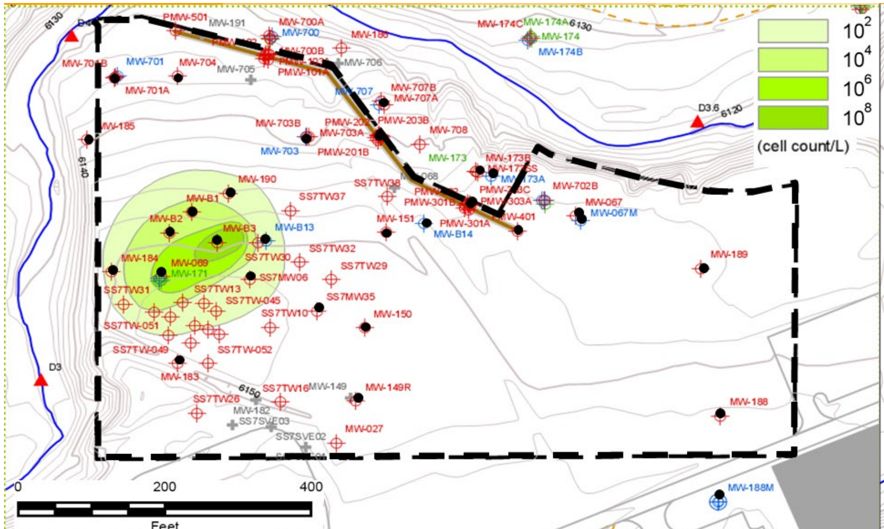
Bioaugmentation for anaerobic sites works!

***Dehalococcoides* (*Dhc*) bioaugmentation is widely accepted to improve reductive dehalogenation of chlorinated ethenes**



1 Month Post KB-1® Bioaugmentation

Dhc



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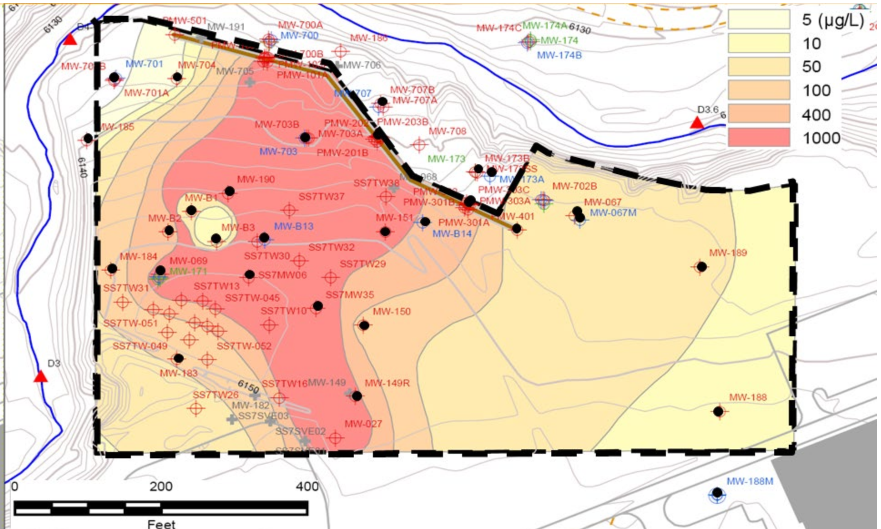

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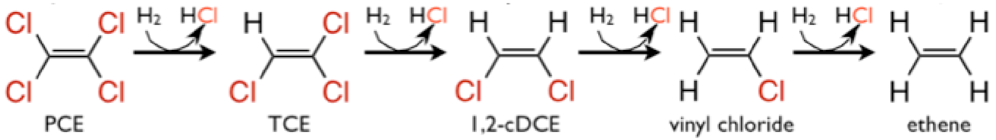
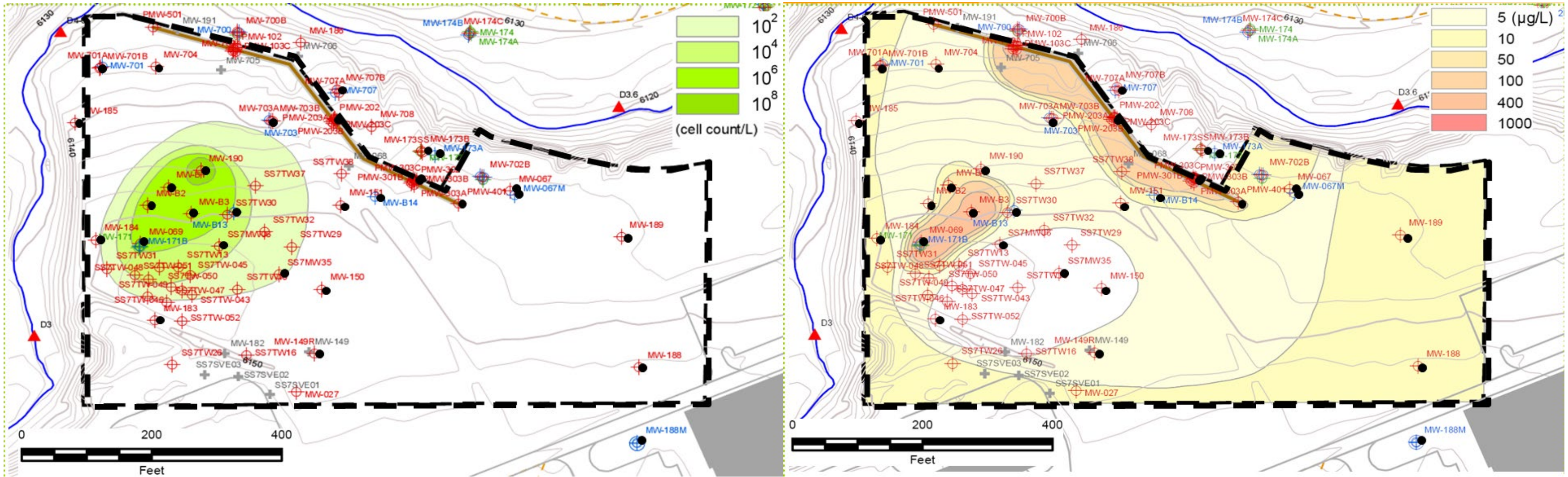


7 Months Post KB-1® Bioaugmentation

Dhc **TCE**



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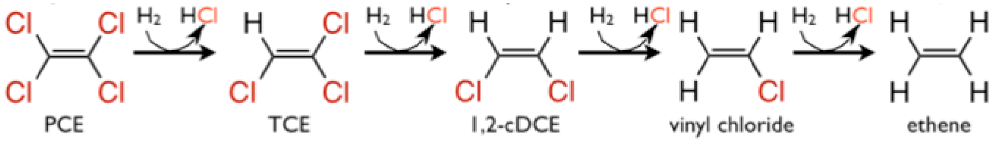
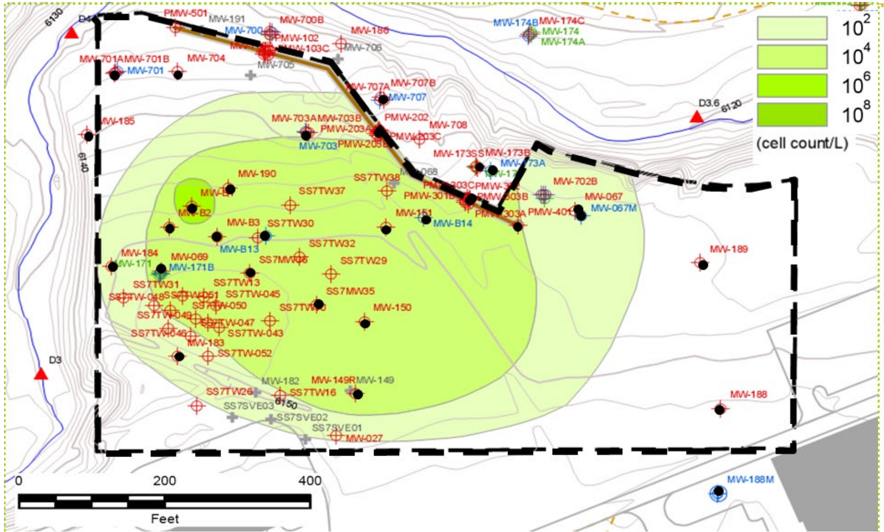
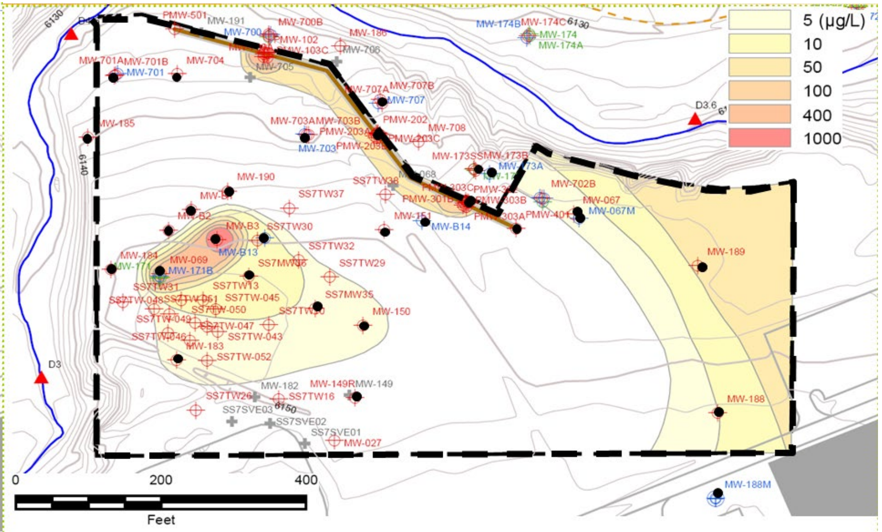


13 Months Post KB-1® Bioaugmentation

Dhc



TCE



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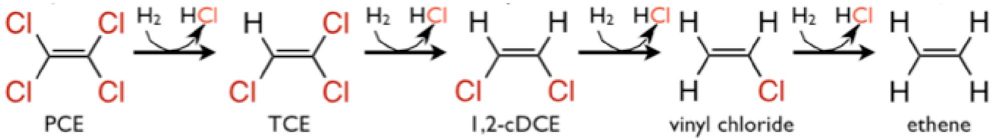
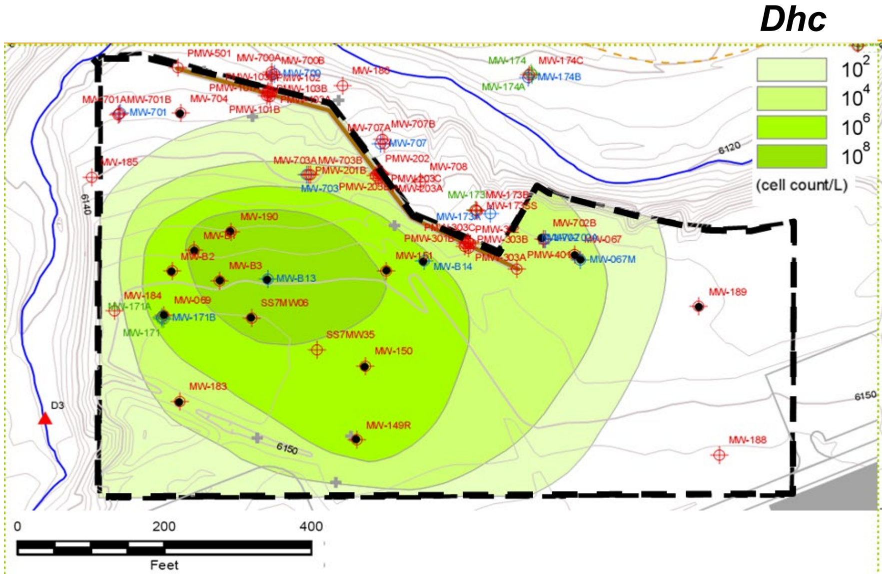
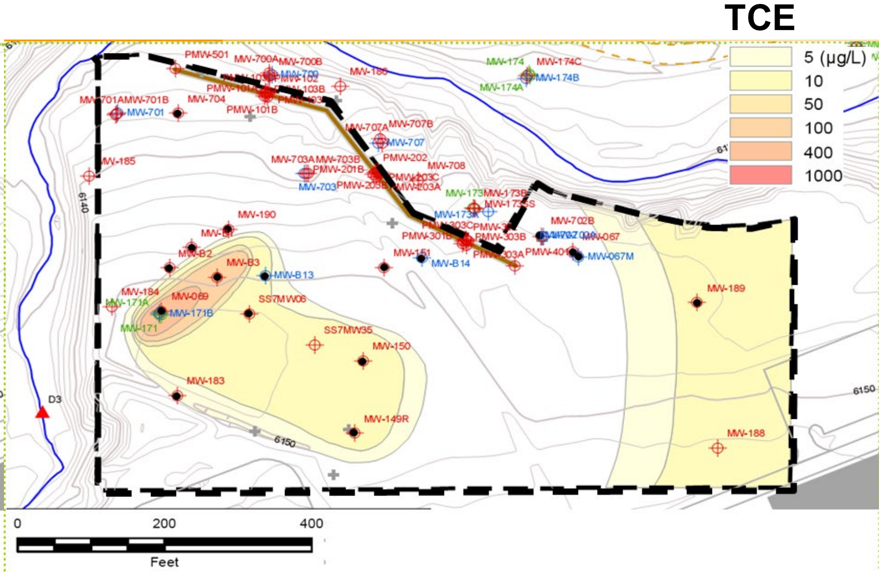


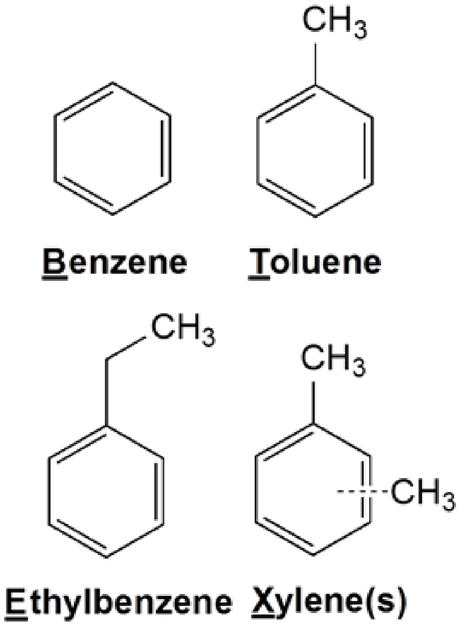
26 Months Post KB-1® Bioaugmentation





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:

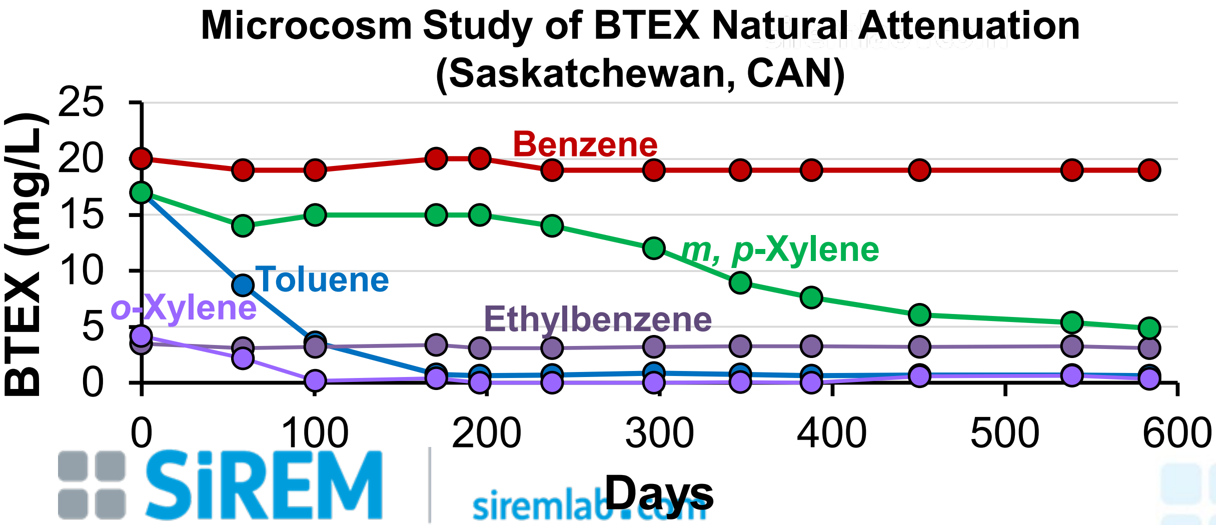
- Potent carcinogen
- Particularly mobile in groundwater due to low sorption & high water solubility
- Most difficult BTEX compound to degrade anaerobically (unsubstituted ring structure)
- Anaerobic conditions, bottleneck to site remediation

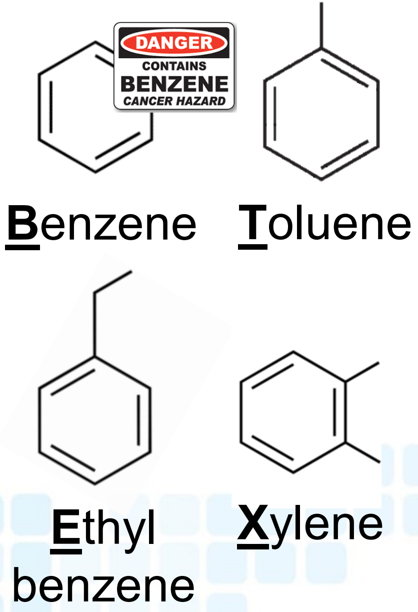
Why Go Anaerobic for BTEX?

- 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 are soluble, easier to apply/distribute compared to O_2 (e.g., epsom salts (sulfate))
- Viable *in situ* remediation option for deep contamination

Benzene as a Proof-of-Concept

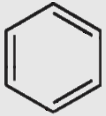
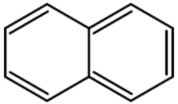
- Benzene is the **most difficult (and least understood)** BTEX compound to degrade anaerobically, and is often the **driver** for remediation efforts
- Intrinsic microbial processes can bioremediate benzene anaerobically, but are often **slow** or even **undetectable** *in situ*.





Hydrocarbons "Burn Through" O2

While aerobic processes are far more efficient thermodynamically, *anaerobic* processes can be used for *anoxic* sites

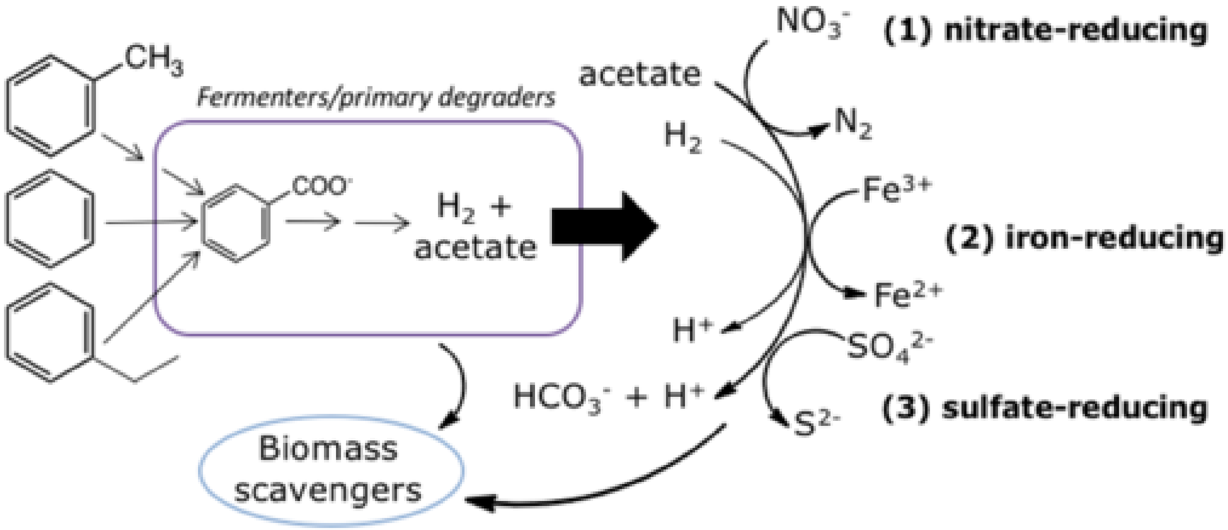
Hydrocarbon	Net Reaction	ΔG°' (kJ mol^{-1})
Benzene	$C_6H_6 + \mathbf{7.5}\ O_2 \rightarrow 6\ CO_2 + 3\ H_2O$	-3202
	$C_6H_6 + \mathbf{6}\ NO_3^- + 6\ H^+ \rightarrow 6\ CO_2 + 3\ N_2 + 6\ H_2O$	-3008
	$C_6H_6 + \mathbf{3.75}\ SO_4^{2-} + 7.5\ H^+ \rightarrow 6\ CO_2 + 3.75\ H_2S + 3\ H_2O$	-214
	$C_6H_6 + 4.5\ H_2O \rightarrow 3.75\ CH_4 + 2.25\ CO_2$	-135
Naphthalene	$C_{10}H_8 + \mathbf{12}\ O_2 \rightarrow 10\ CO_2 + 4\ H_2O$	-5093
	$C_{10}H_8 + \mathbf{9.6}\ NO_3^- + 9.6\ H^+ \rightarrow 10\ CO_2 + 4.8\ N_2 + 8.8\ H_2O$	-4782
	$C_{10}H_8 + \mathbf{6}\ SO_4^{2-} + 12\ H^+ \rightarrow 10\ CO_2 + 6\ H_2S + 4\ H_2O$	-313
	$C_{10}H_8 + 8\ H_2O \rightarrow 6\ CH_4 + 4\ CO_2$	-186
Hexadecane	$C_{16}H_{34} + \mathbf{24.5}\ O_2 \rightarrow 16\ CO_2 + 17\ H_2O$	-10392
	$C_{16}H_{34} + \mathbf{19.6}\ NO_3^- + 19.6\ H^+ \rightarrow 16\ CO_2 + 9.8\ N_2 + 26.8\ H_2O$	-9757
	$C_{16}H_{34} + \mathbf{12.25}\ SO_4^{2-} + 24.5\ H^+ \rightarrow 16\ CO_2 + 12.5\ H_2S + 17\ H_2O$	-632
	$C_{16}H_{34} + 7.5H_2O \rightarrow 12.25\ CH_4 + 3.75CO_2$	-372

Adapted from Widdel and Musat, 2010 (Handbook of Hydrocarbon and Lipid Microbiology, ed. K. N. Timmis)

Key Difference Between Bioremediation of Chlorinated Solvents vs Hydrocarbons

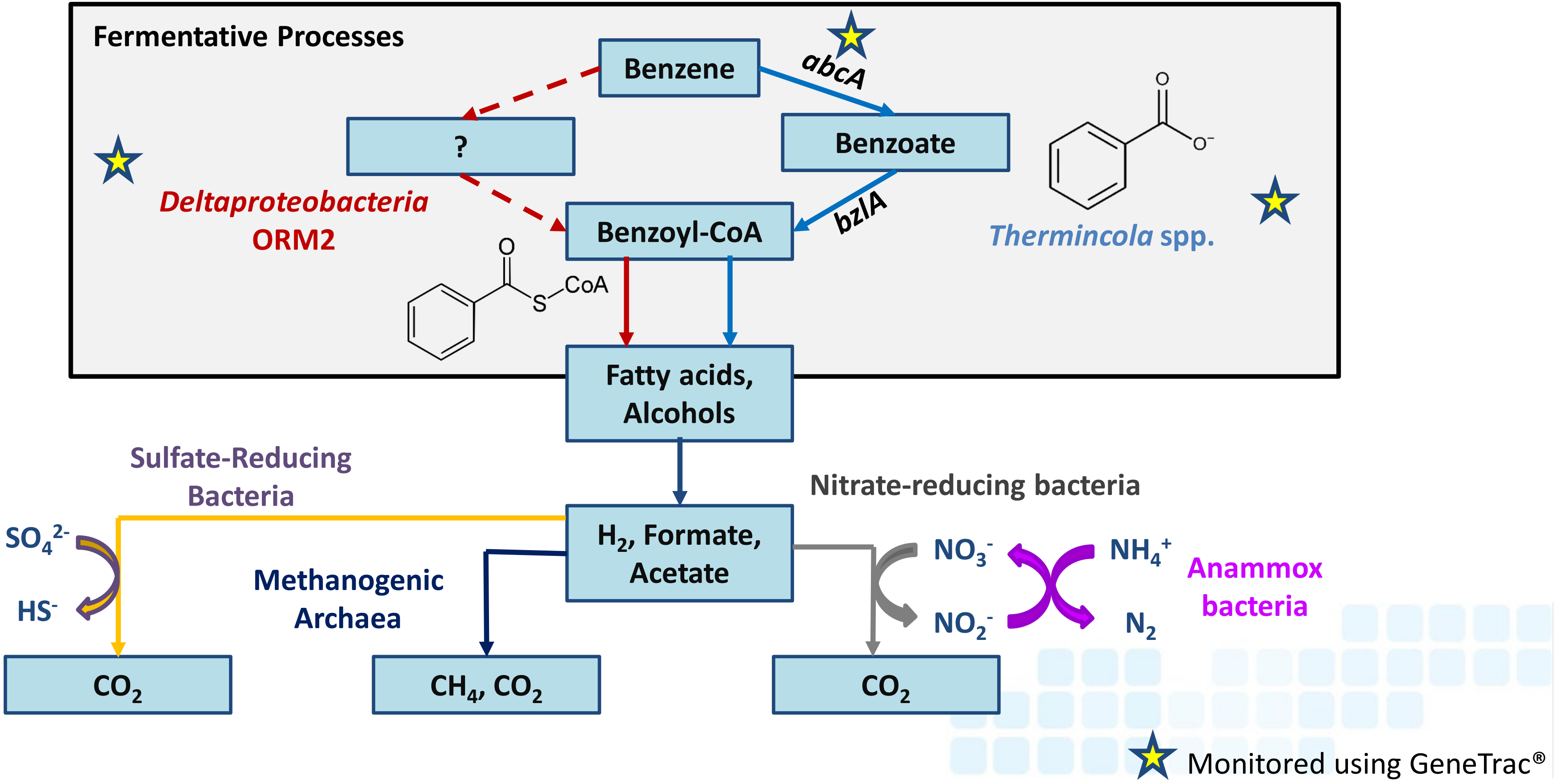
Hydrocarbons are *electron donors* rather than electron acceptors

- Adding carbon (sugars, VFAs, yeast extract) may not enhance bioremediation performance
- Adding electron acceptors does not always enhance bioremediation either



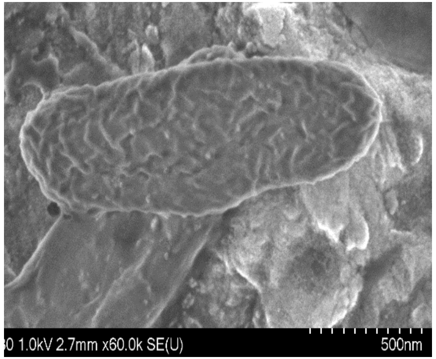
Gieg et al., 2014 (Curr Opin Biotechnol **27**)

Overview of Anaerobic Benzene Degradation



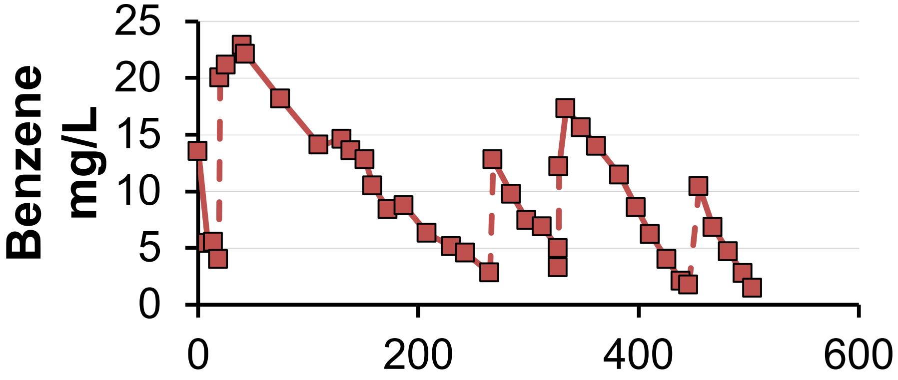
ORM2 Anaerobic Benzene Degrader

- 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

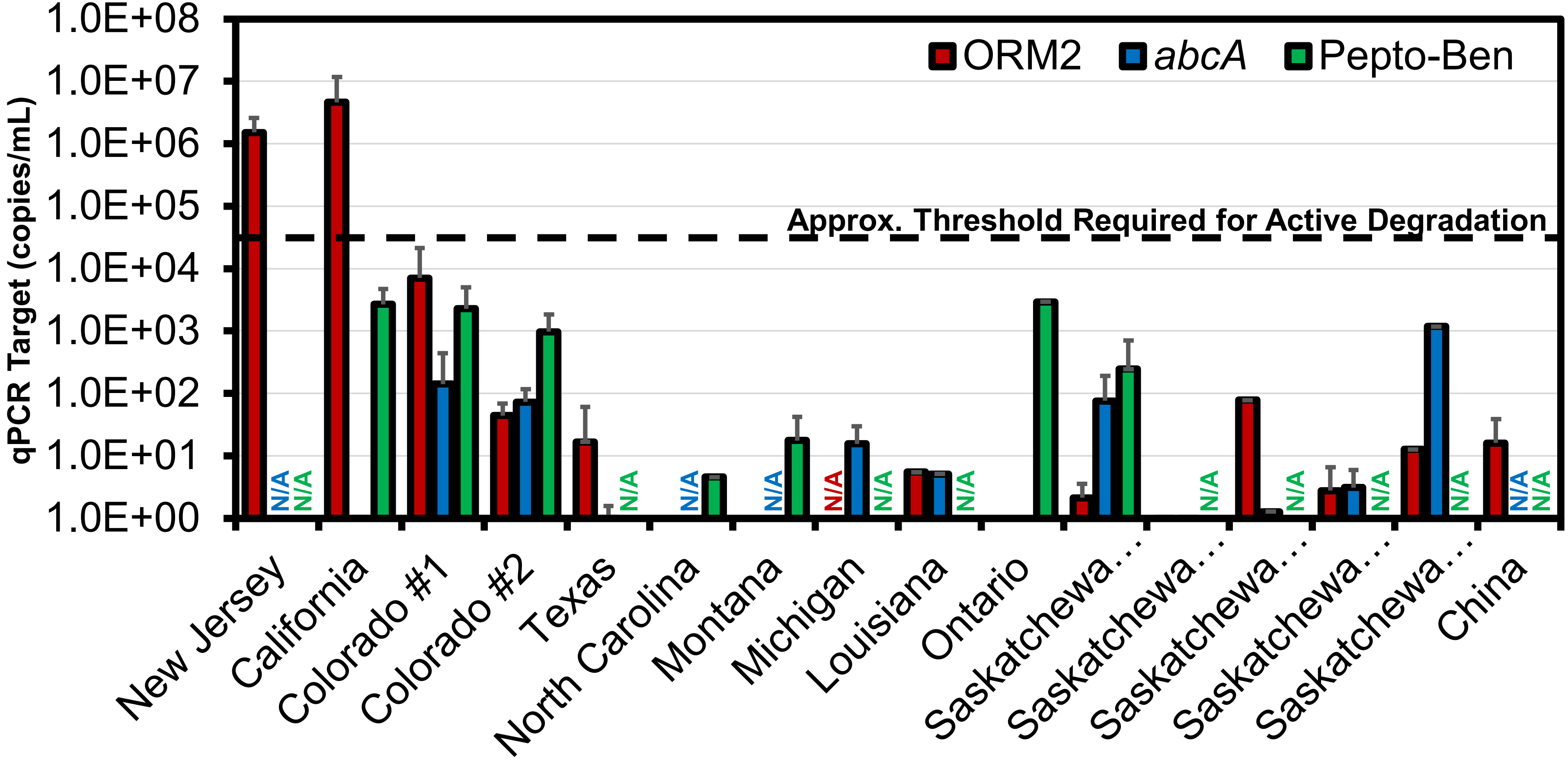


DGG-B Culture – ORM2's Home

- DGG-B successfully scaled up to commercial volumes
  - Benzene degradation rate = 0.6 mg /L/ day
  - 10^{11} ORM2/L



Anaerobic benzene degraders appear to be ubiquitous in anoxic benzene-contaminated environments, but in very low numbers

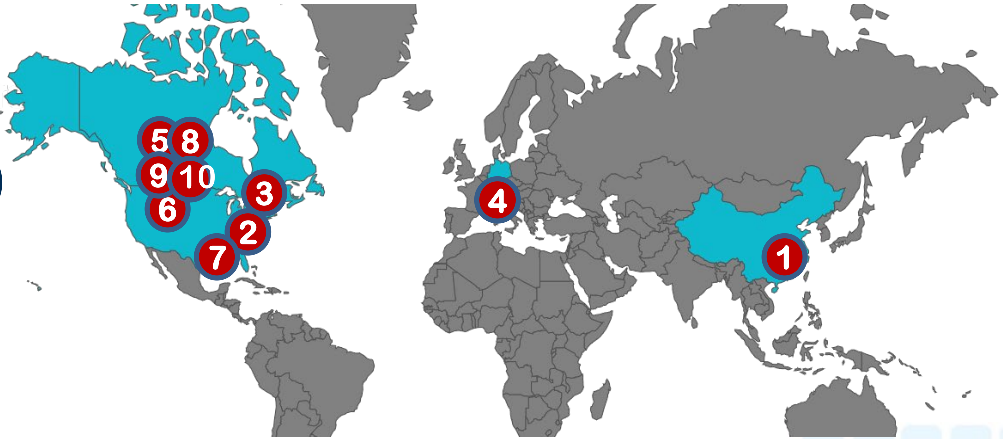


Treatability Testing Scope

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

Tested:

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

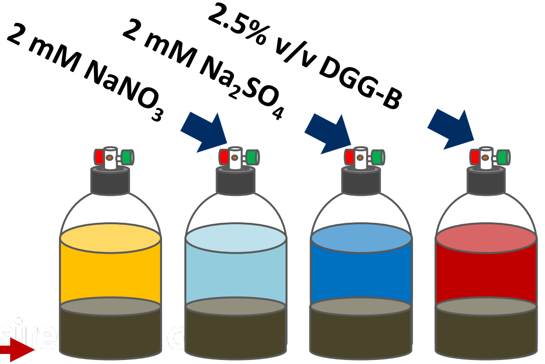


Treatability Testing

Homogenized core samples (60 g)

Groundwater sample

Mix



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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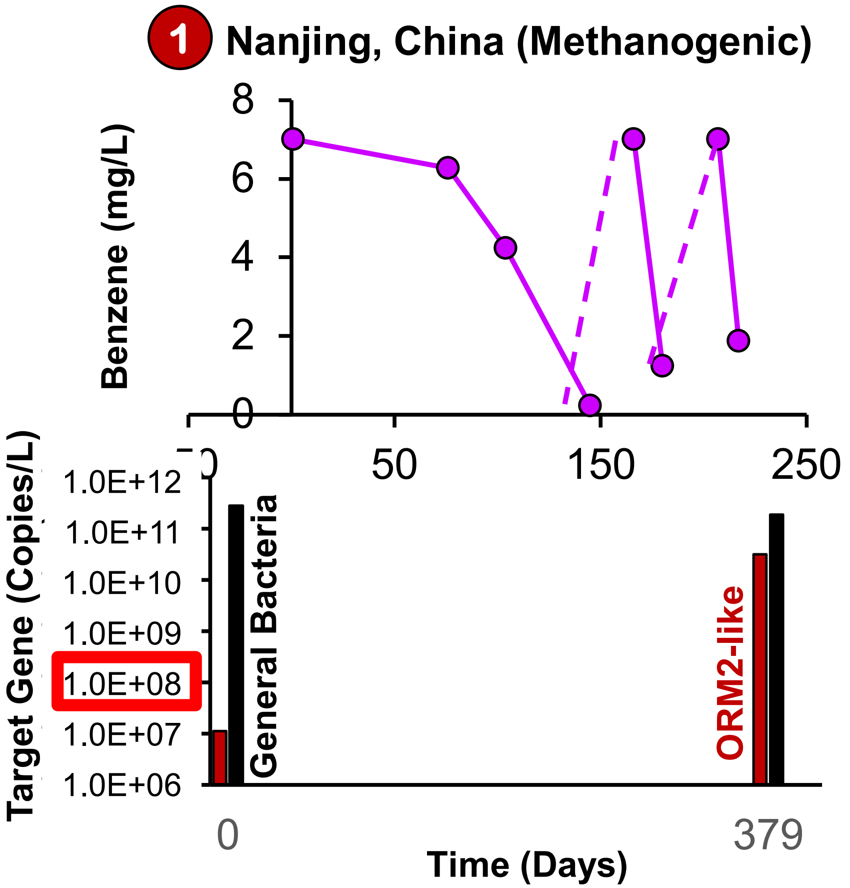
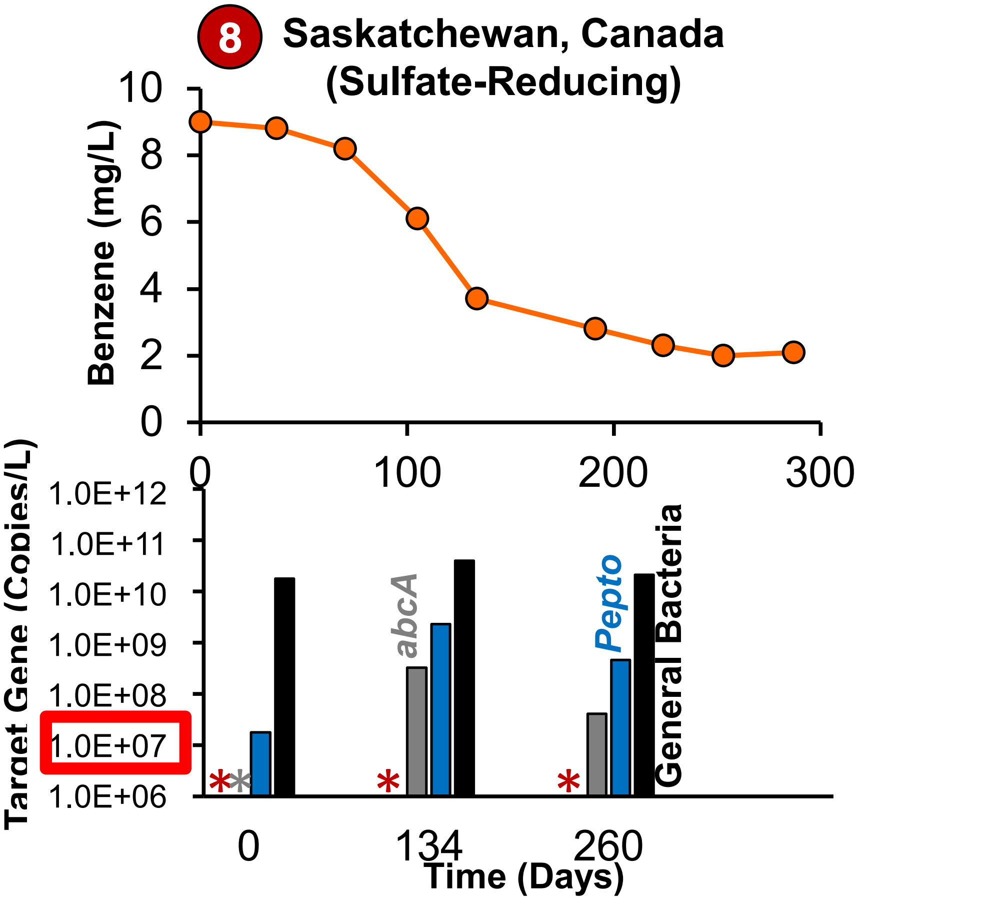
Current Treatability Studies Underway

Site Location	Target Substrate(s)	Culture(s) Tested	Treatability (Microcosm) Studies		Field Pilots
			Start Date	Successful Bioaug'?	Start Date
Saskatchewan	Benzene	DGG-B	Dec-2016	Yes	Nov-2019
Ontario	BTX	DGG-BTX	Jul-2019	Yes	Delayed
North Carolina	Benzene, MTBE	DGG-B	Jul-2019	Yes	Planning
Louisiana	Benzene	DGG-B	Nov-2019	Yes	Oct-2019
New Jersey	Benzene	DGG-B	Nov-2019	Yes (SO_4^{2+} + DGG-B)	Apr-2020
Saskatchewan	BTEX	DGG-B	Jan-2020		
New Jersey	Benzene	NRBC, DGG-B	May-2020		
New Jersey	Benzene, Chlorobenzene	NRBC, DGG-B	May-2020	NRBC (1/3 replicates)	
Alberta	Benzene	DGG-BX	Sep-2020		
Indiana	BTEX, Chlor. solvents	DGG-BTX, KB-1 Plus	--	--	Oct-2020

Intrinsic biodegradation correlates to known benzene degraders

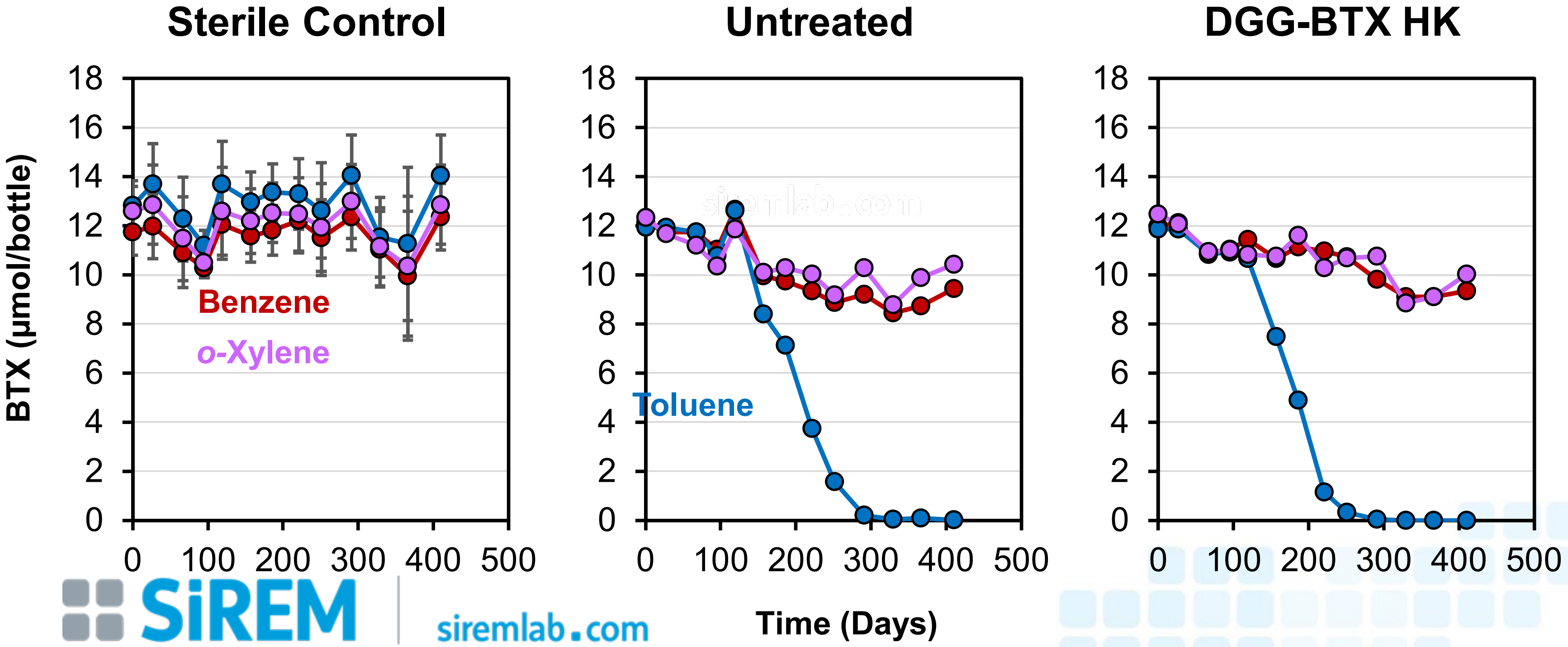




* = below quantifiable limits

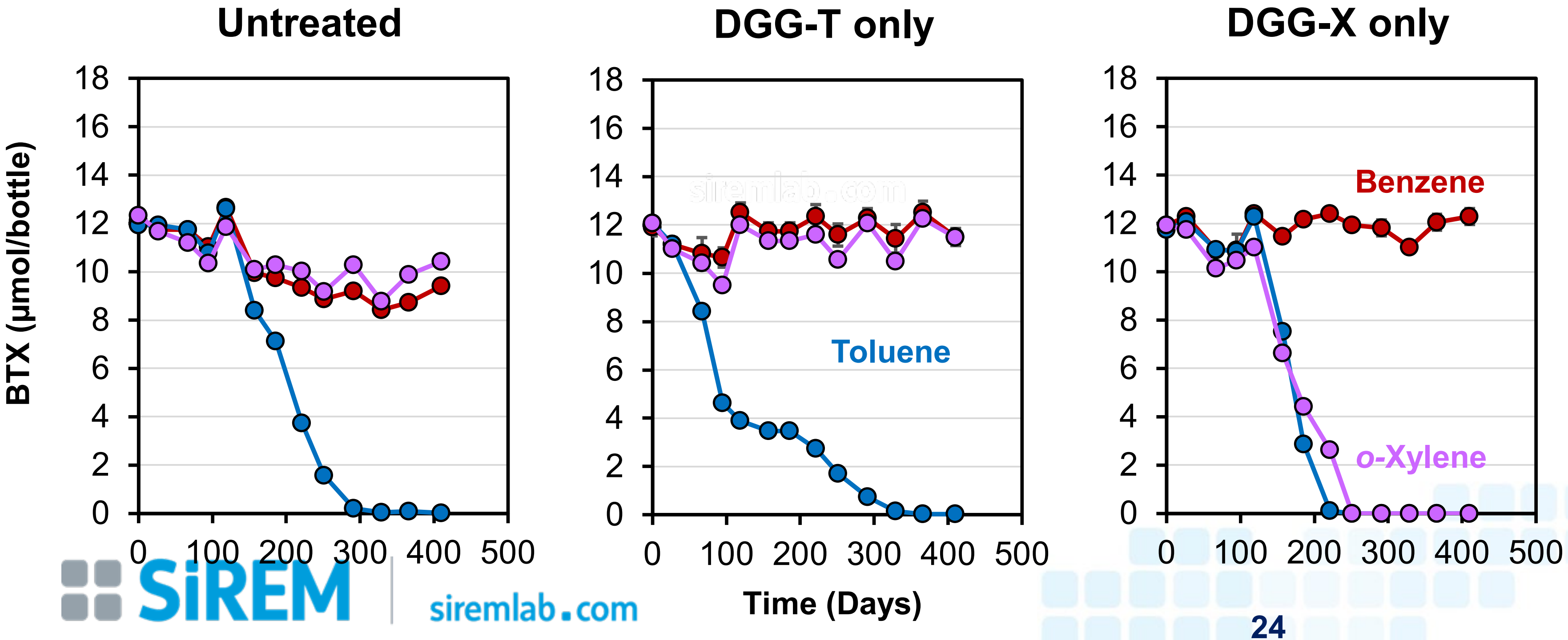
Borden II – Key Highlights

Methanogenic toluene degradation observed in untreated site materials. Injecting heat-killed (HK) culture did not enhance BTX bioremediation.



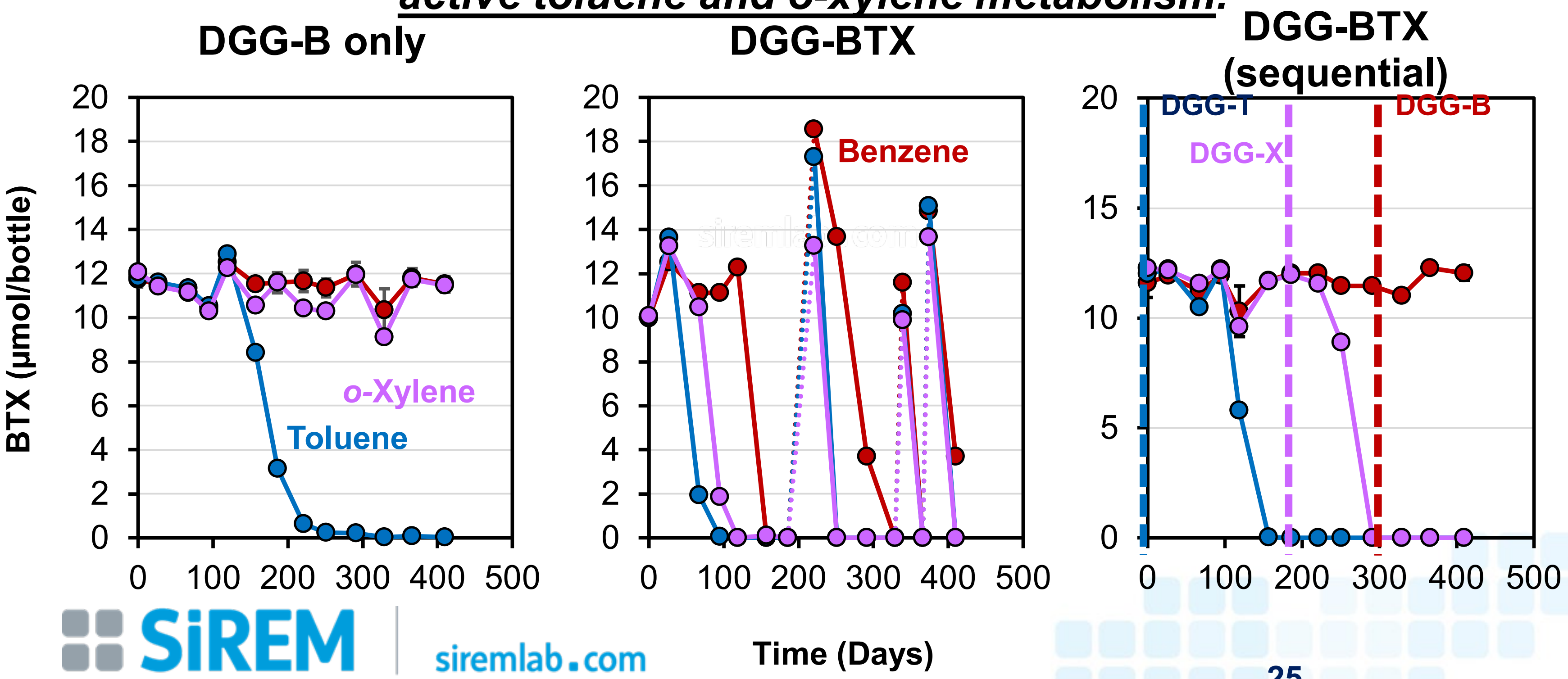
Borden II – Key Highlights

DGG-T enhanced rates of toluene degradation by up to ~ 63%.
DGG-X enhanced both toluene (~ 26%) and o-xylene degradation.

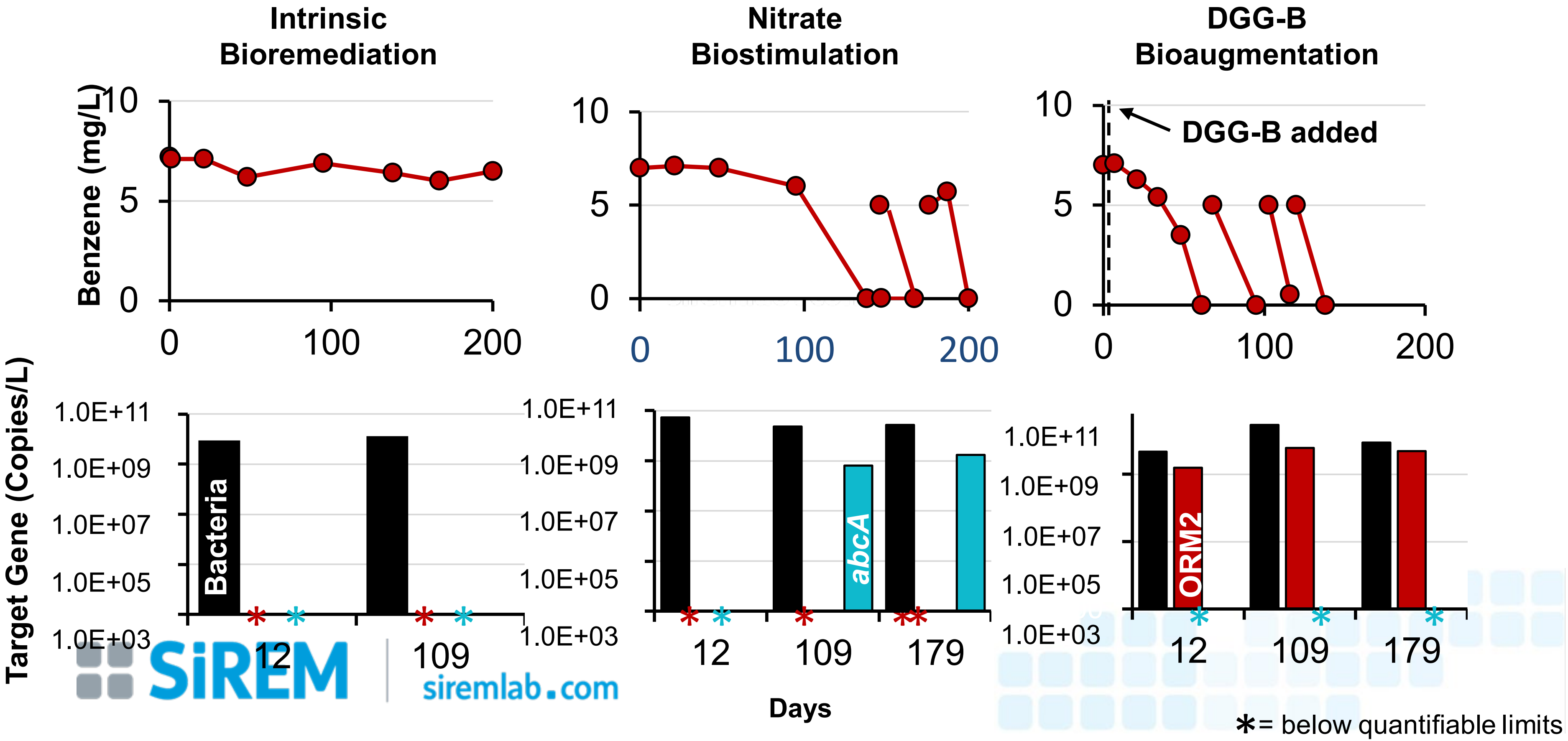


Borden II – Key Highlights

Benzene degradation was only observed in bioaugmented bottles with <u>active toluene and o-xylene metabolism</u>.



Target concentrations (≥10^8 copies/L)



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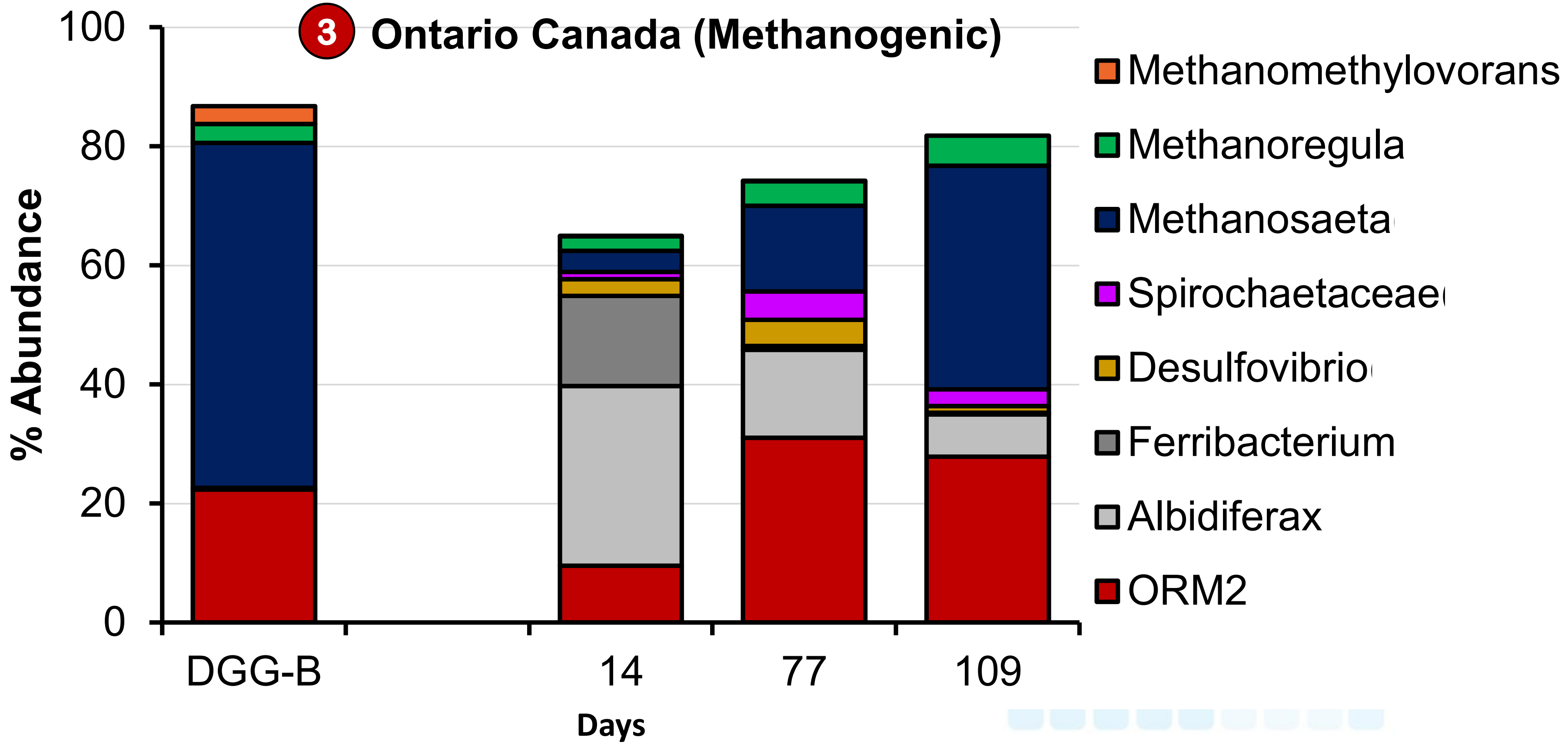
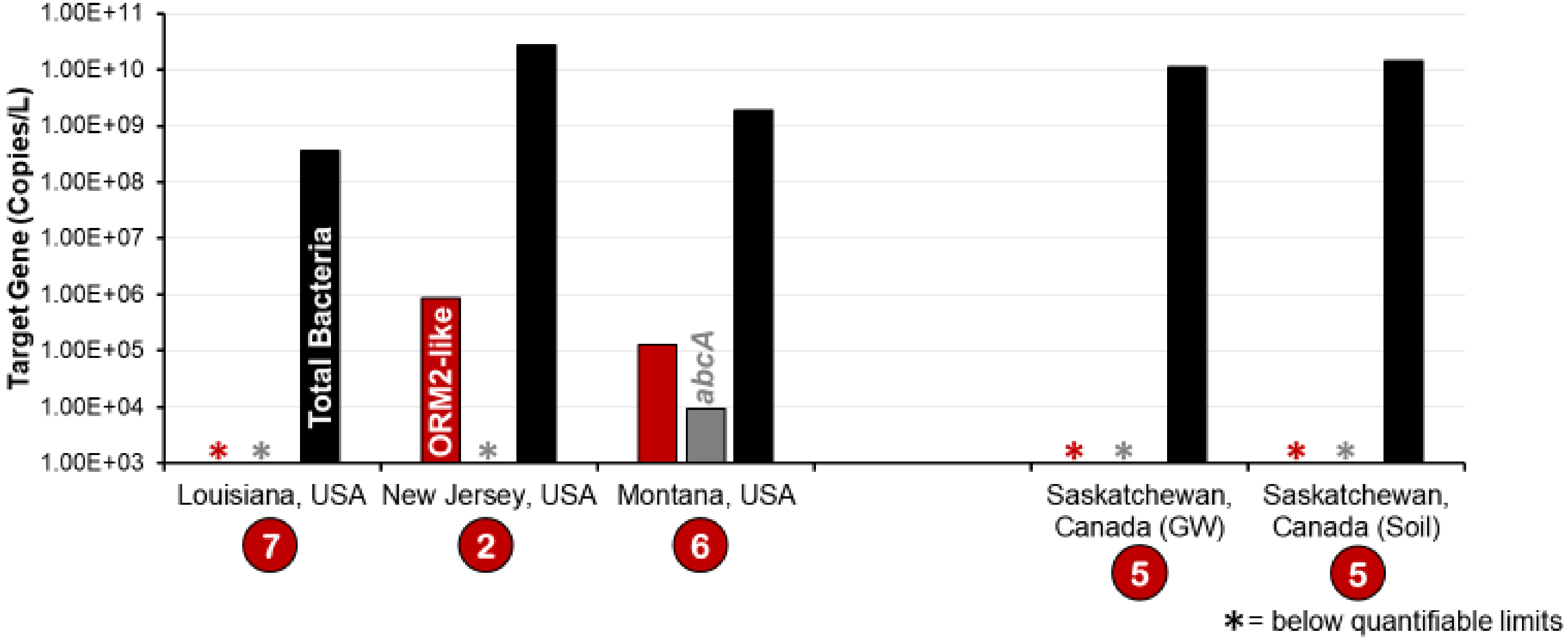
Bioaugmentation also increases the abundance of other key microbes



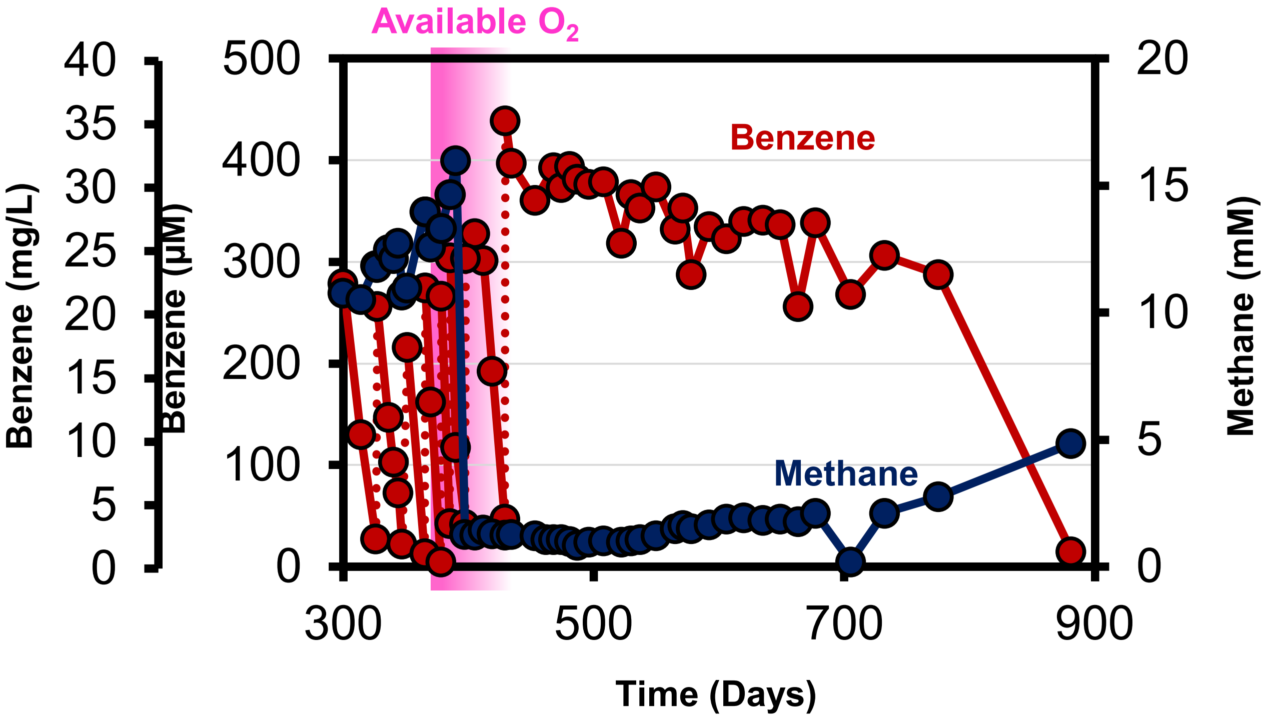
Benzene Persistence *In Situ* May Be Attributed to Low Abundance of Benzene Degraders

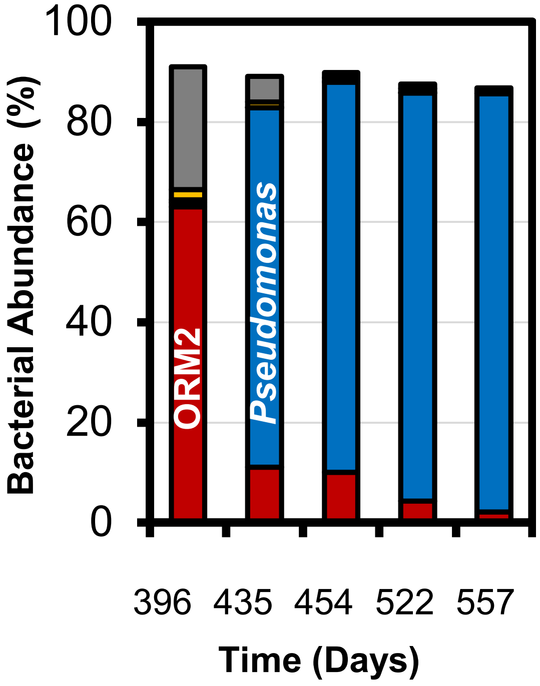
The overarching cause of this is now under investigation.



FAQs: Is Anaerobic Benzene Degradation Really a Strictly Anoxic Process?

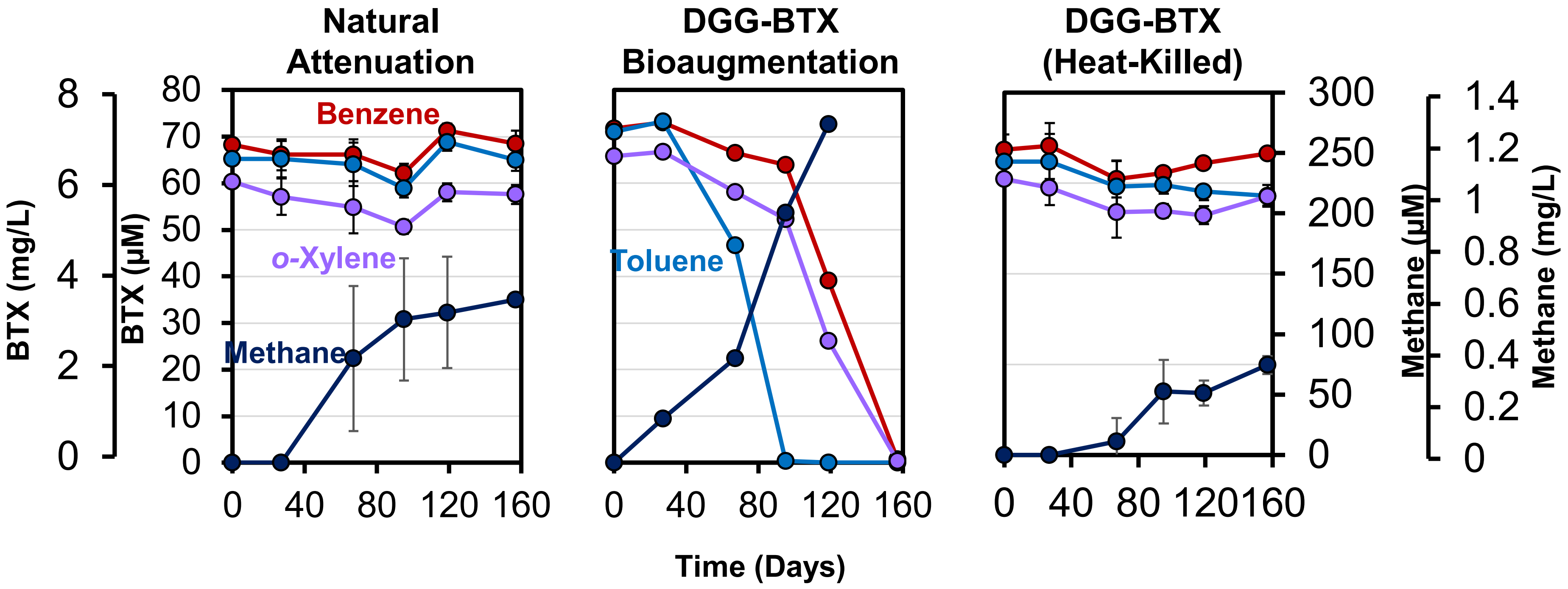
YES! Molecular oxygen stalls attenuation and poisons benzene fermenters





FAQs: Do Culture Nutrients/Dead Biomass Contribute to Faster BTEX Attenuation?

Laboratory data suggests NO – Active microbes/enzymes are required



Field Pilot Testing of DGG-BTM is Now Underway

- October 2019 (Louisiana, USA)
- November 2019 (Saskatchewan, CAN)
- March 2020 (New Jersey, USA)
- September 2020 (New Jersey, USA) DGG-Plus (B, T and X cultures)
- 2021– 2 more pilot tests – Alberta and Ontario plus private client sites

1st DGG-B™ Shipment: October 9th, 2019

Conclusions

- Treatability testing indicates $NO_3/SO_4/CO_2$ are suitable electron acceptors for BTEX degradation
- 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)
- Benzene degradation in the presence of TEX compounds slower than benzene alone-may need to treat TEX first

Take Home Message

1. Know your microbes;
   - *Deltaproteobacterium* ORM2 and *abcA*-harbouring *Thermincola* spp.

2. Know your numbers;
   - 10^4 to 10^5+ copies/mL are generally required for active anaerobic benzene degradation,

3. Bioaugmentation has the potential to be an effective benzene/BTEX bioremediation tool

Acknowledgements – Benzene/GAPP Team

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Questions?

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