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Advances in Anaerobic Benzene Bioremediation

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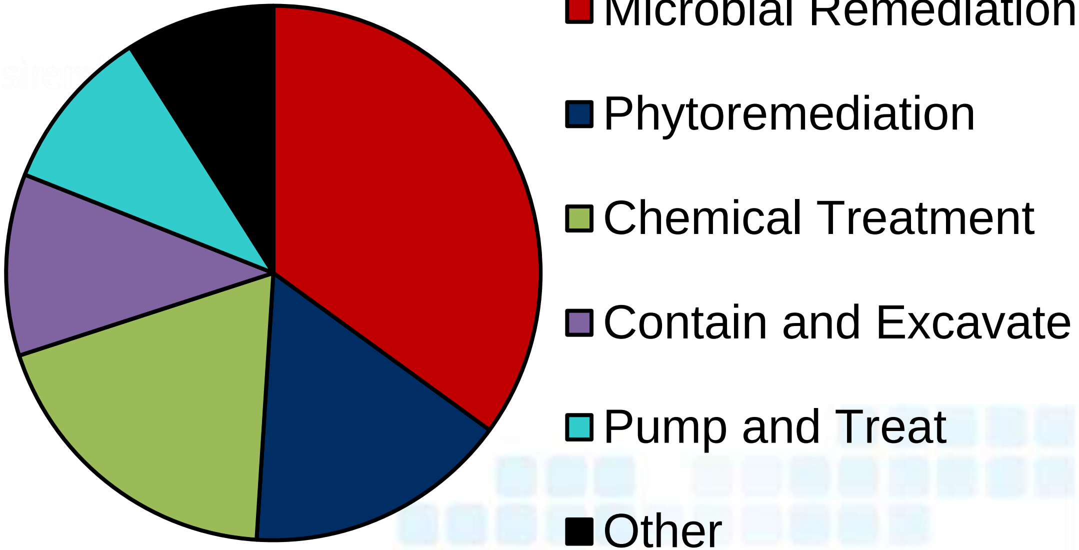
The Landscape of Hydrocarbon Bioremediation: A Lot Has Changed…

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



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

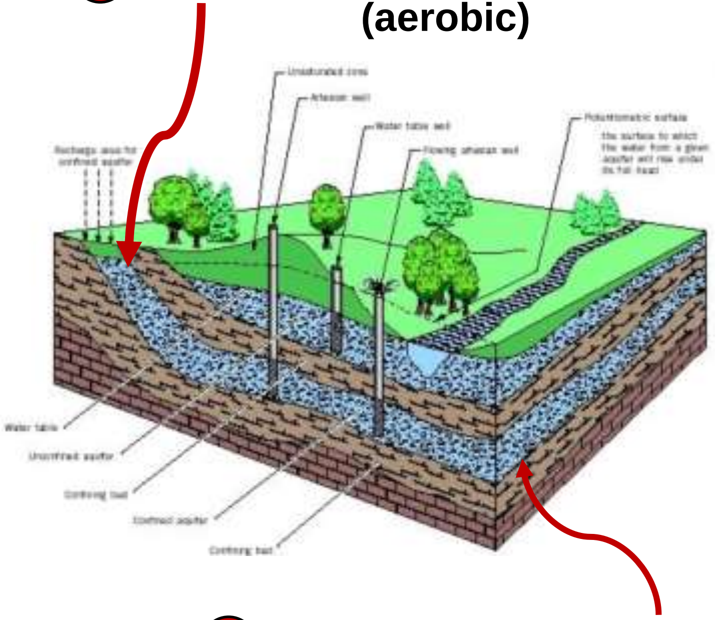
What Sites are Currently Being Targeted for Hydrocarbon Bioremediation?

Offshore Spills
(mostly aerobic)

Ex situ Bioreactors
(mostly aerobic)

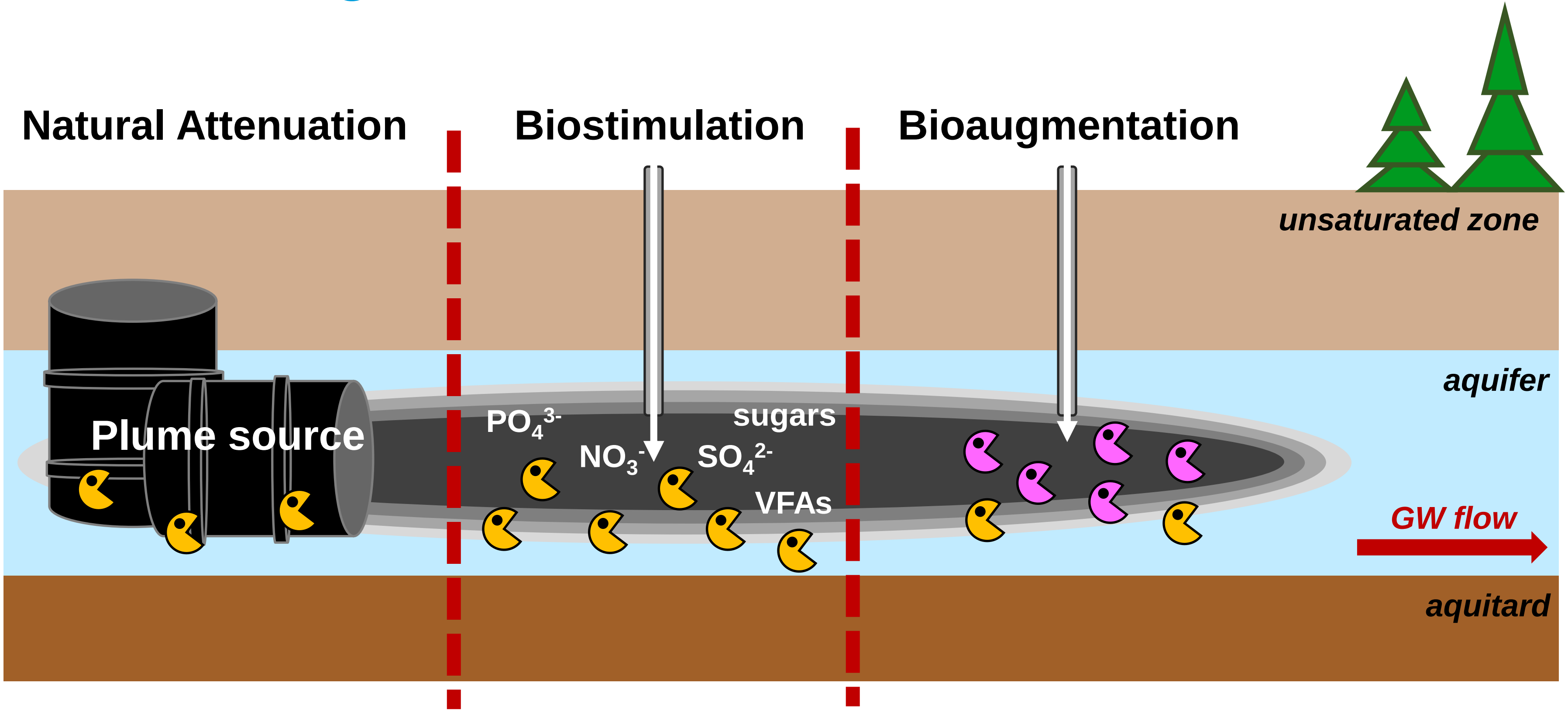
Tailings Ponds
(aerobic and anaerobic)

4 Shallow Soils and Groundwater
(aerobic)



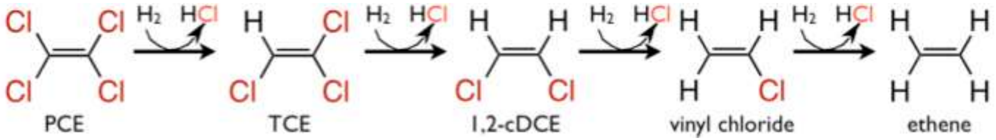
5 Deeper Groundwater
(intrinsically anaerobic)

Groundwater Bioremediation Technologies Focusing on Anaerobic Microbial Processes



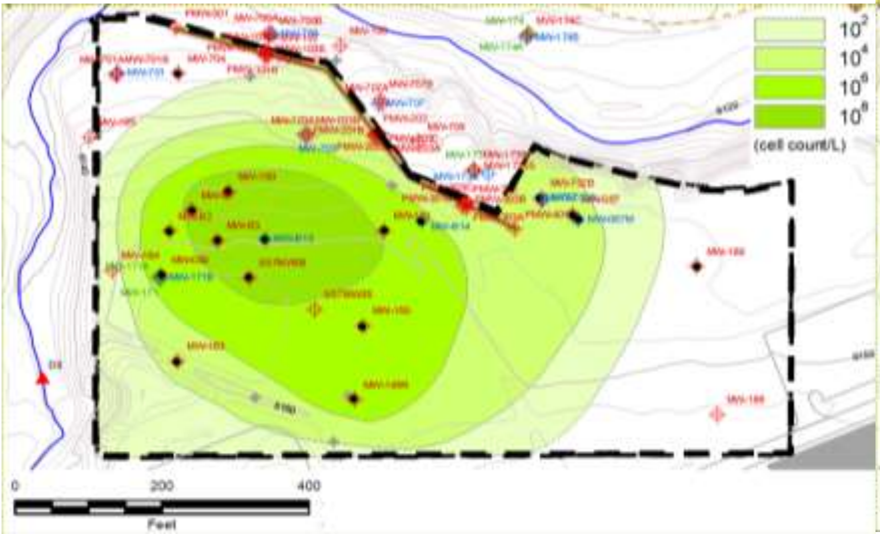
Bioaugmentation for anaerobic sites works!

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

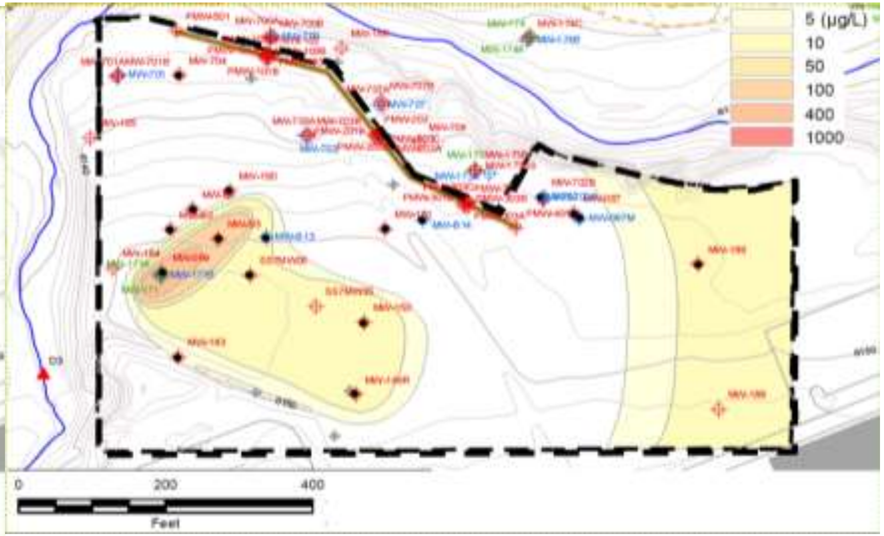


26 Months Post KB-1® Bioaugmentation

Dhc

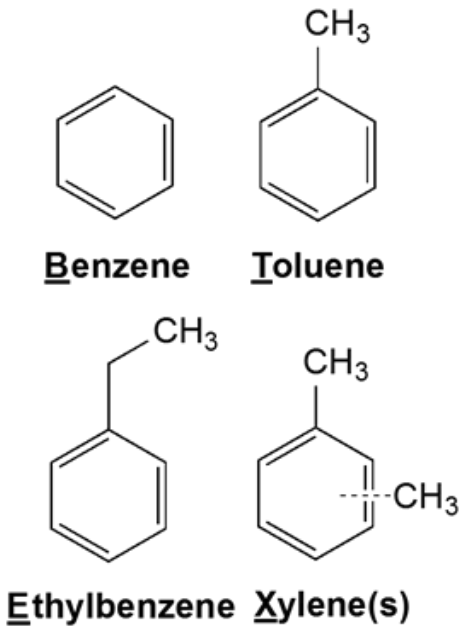


TCE



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:

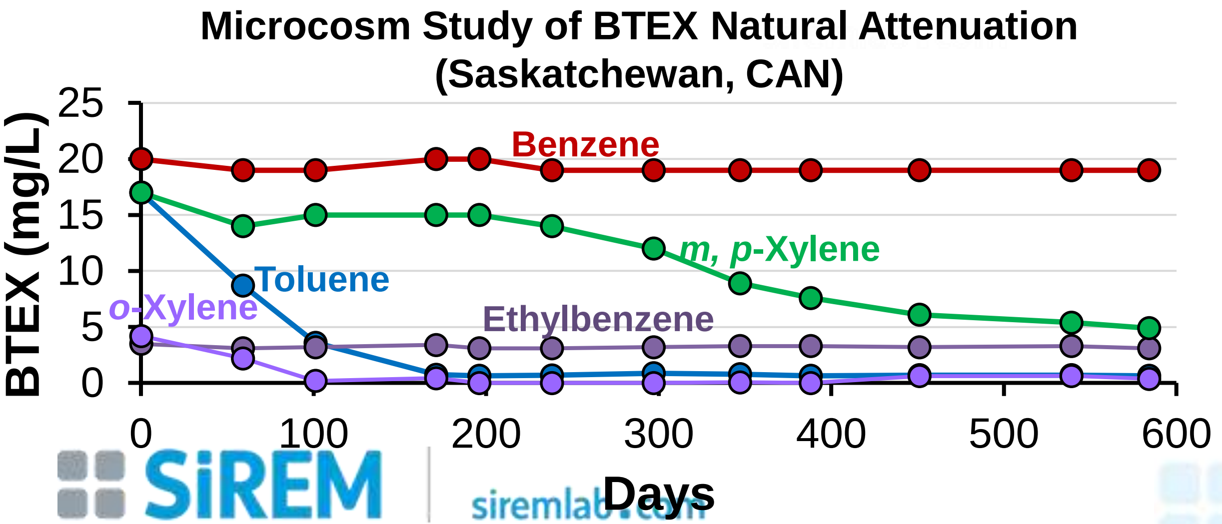
- Potent carcinogen
- Particularly mobile in groundwater due to low sorption & high water solubility
- Most difficult BTEX compound to degrade anaerobically (unsubstituted ring structure)
- Anaerobic conditions, bottleneck to site remediation

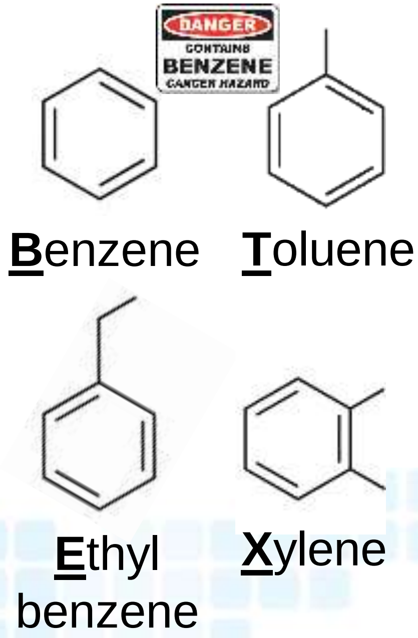
Why Go Anaerobic for BTEX?

- 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

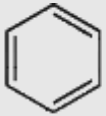
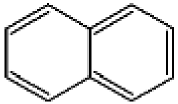
- Benzene is the **most difficult (and least understood)** BTEX compound to degrade anaerobically, and is often the **driver** for remediation efforts
- Intrinsic microbial processes can bioremediate benzene anaerobically, but are often **slow** or even **undetectable** *in situ*.





Hydrocarbons "Burn Through" O2

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

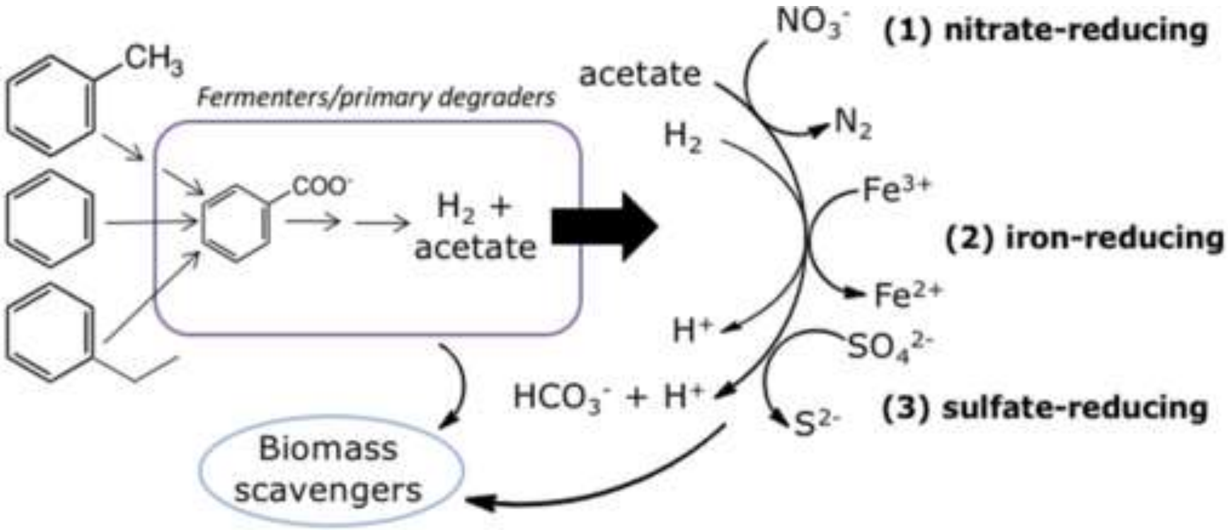
Hydrocarbon	Net Reaction	ΔG°' (kJ mol^{-1})
Benzene	C_6H_6 + **7.5** O_2 → 6 CO_2 + 3 H_2O	-3202
	C_6H_6 + **6** NO_3^- + 6 H^+ → 6 CO_2 + 3 N_2 + 6 H_2O	-3008
	C_6H_6 + **3.75** SO_4^{2-} + 7.5 H^+ → 6 CO_2 + 3.75 H_2S + 3 H_2O	-214
	C_6H_6 + 4.5 H_2O → 3.75 CH_4 + 2.25 CO_2	-135
Naphthalene	$C_{10}H_8$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



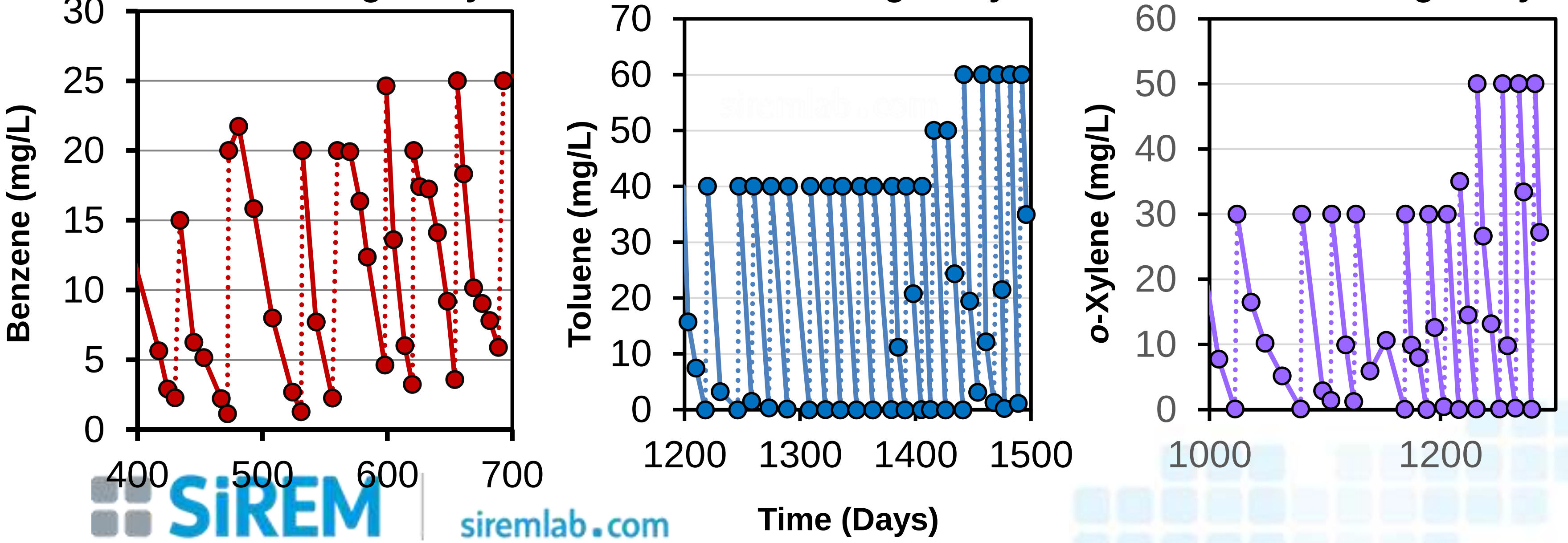
Gieg et al., 2014 (Curr Opin Biotechnol)

Culture Overview

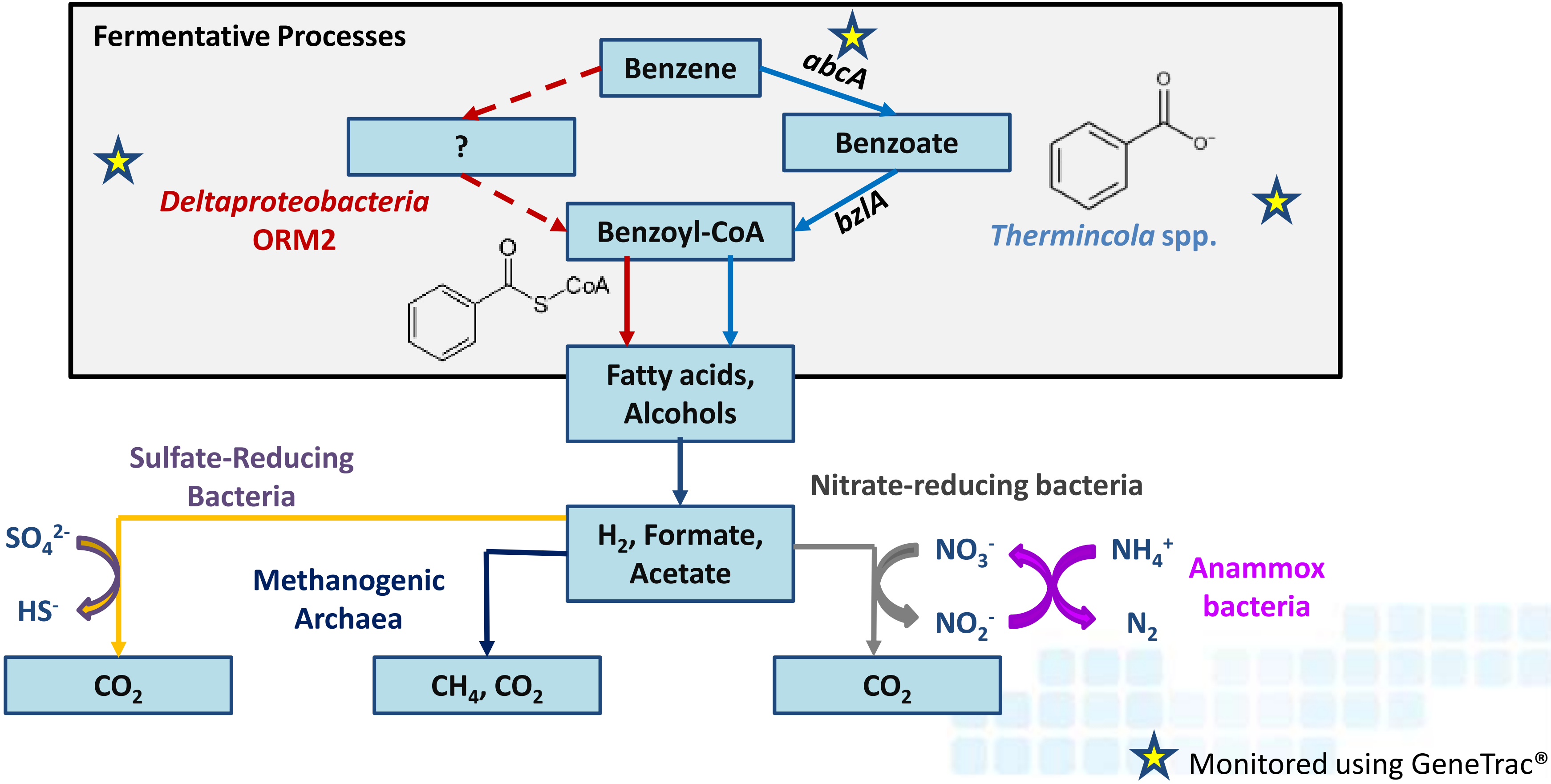
DGG B ~ 0.6 – 2.0 mg/L/day

DGG T ~ 3.2 – 5.7 mg/L/day

DGG X ~ 1.1 – 4.0 mg/L/day



Overview of Anaerobic Benzene Degradation

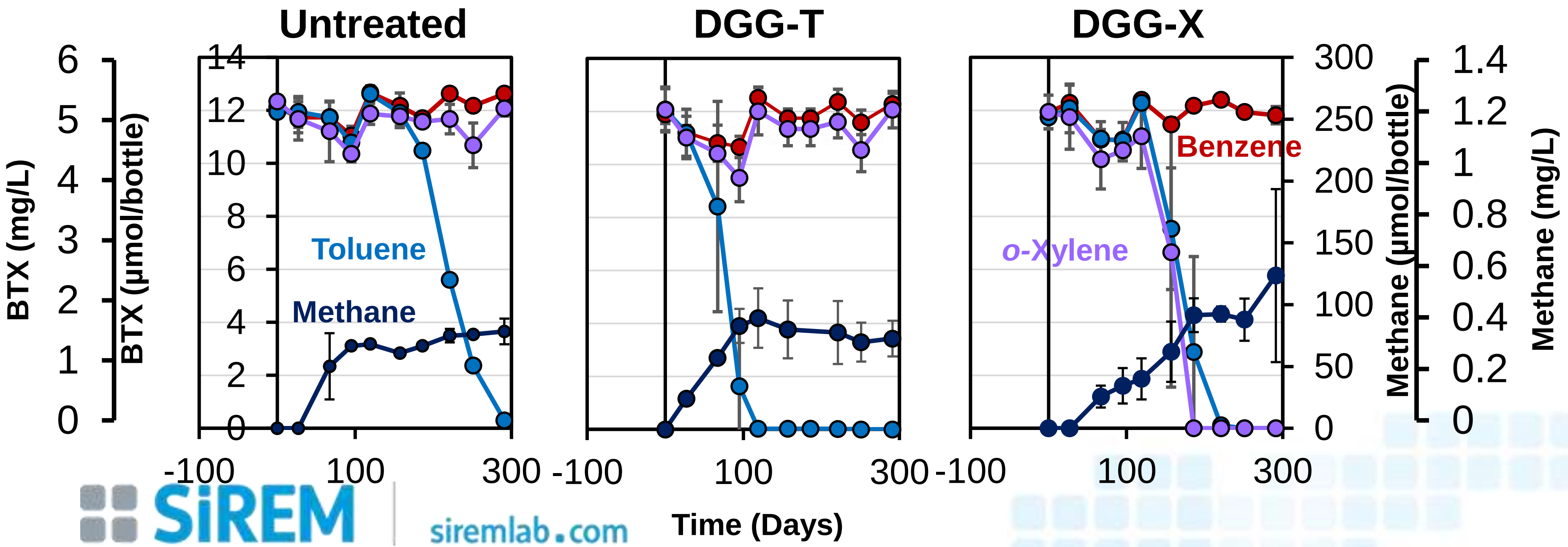


ORM2 Anaerobic Benzene Degrader

- 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

Screening DGG-T and DGG-X

Both cultures enhanced anaerobic bioremediation of their target hydrocarbon in preliminary microcosm treatability tests



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Treatability Testing Scope

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

Tested:

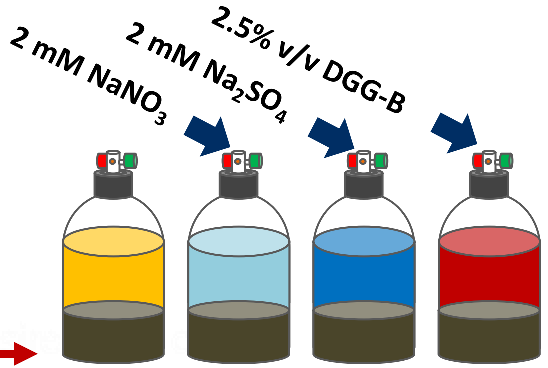
- Intrinsic bioremediation
- Biostimulation (nitrate or sulfate)
- DGG-B bioaugmentation

Treatability Testing

Homogenized core samples (60 g)

Groundwater sample

Mix



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

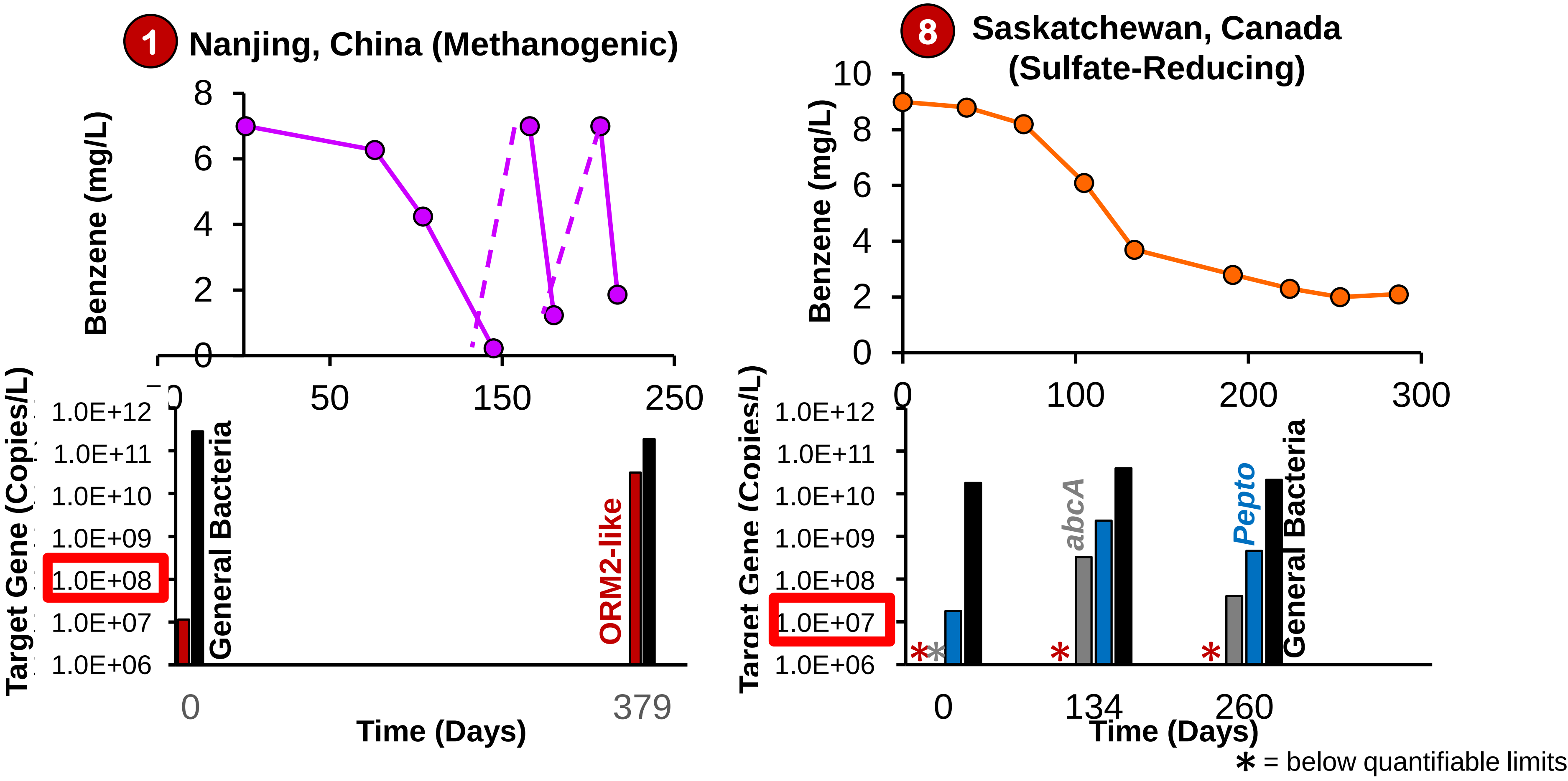
Incubate and monitor

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

Current Treatability Studies Underway

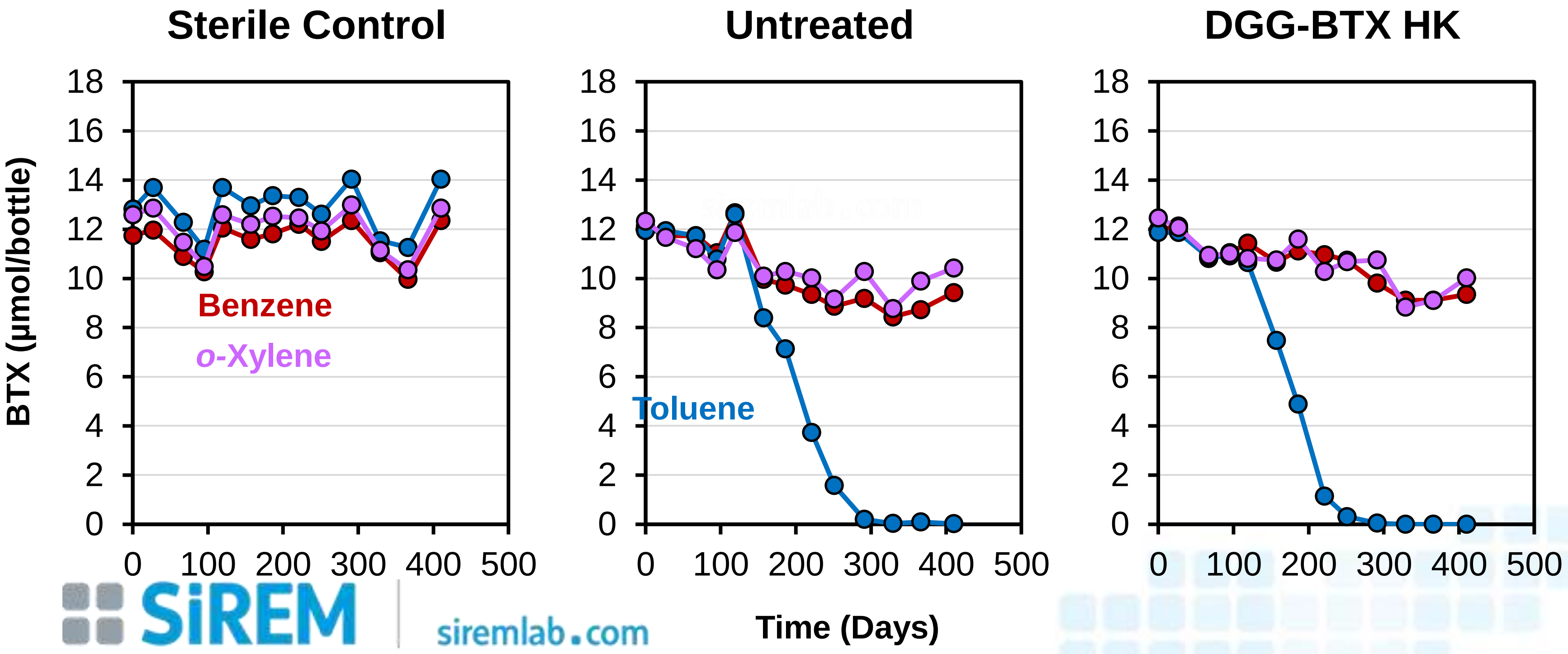
Site Location	Target Substrate(s)	Culture(s) Tested	Treatability (Microcosm) Studies		Field Pilots
			Start Date	Successful Bioaug'?	Start Date
Saskatchewan	Benzene	DGG-B	Dec-2016	Yes	Nov-2019
Ontario	BTX	DGG-BTX	Jul-2019	Yes	2021
North Carolina	Benzene, MTBE	DGG-B	Jul-2019	Yes	Planning
Louisiana	Benzene	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



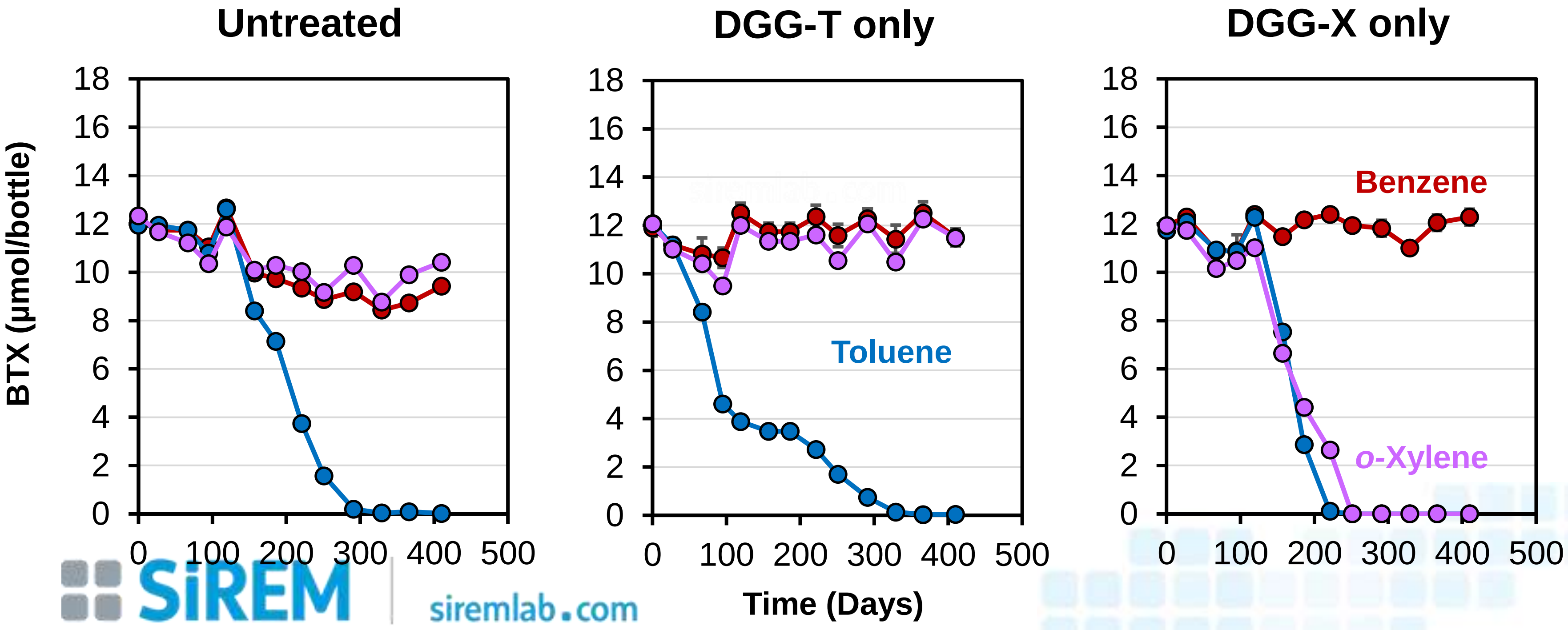
Borden II Study – Key Highlights

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



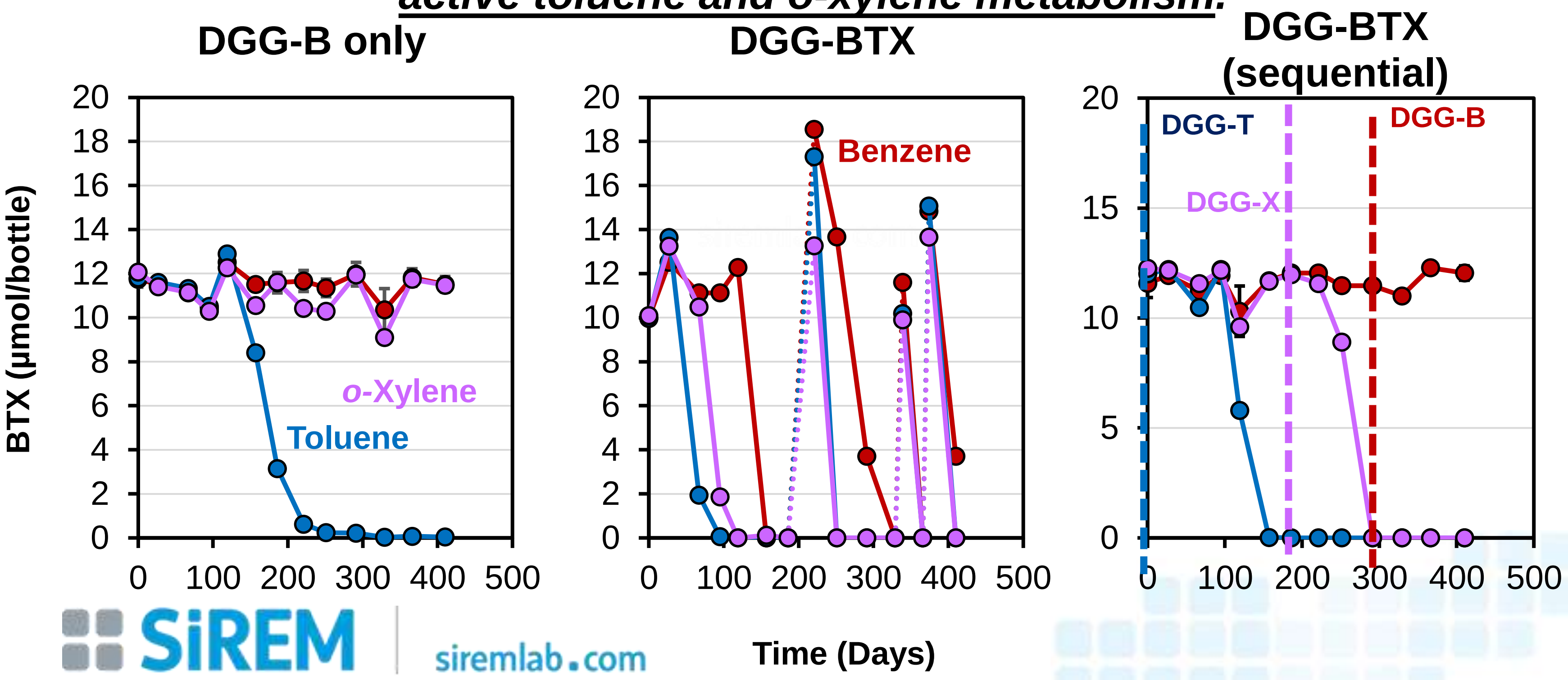
Borden II – Key Highlights

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

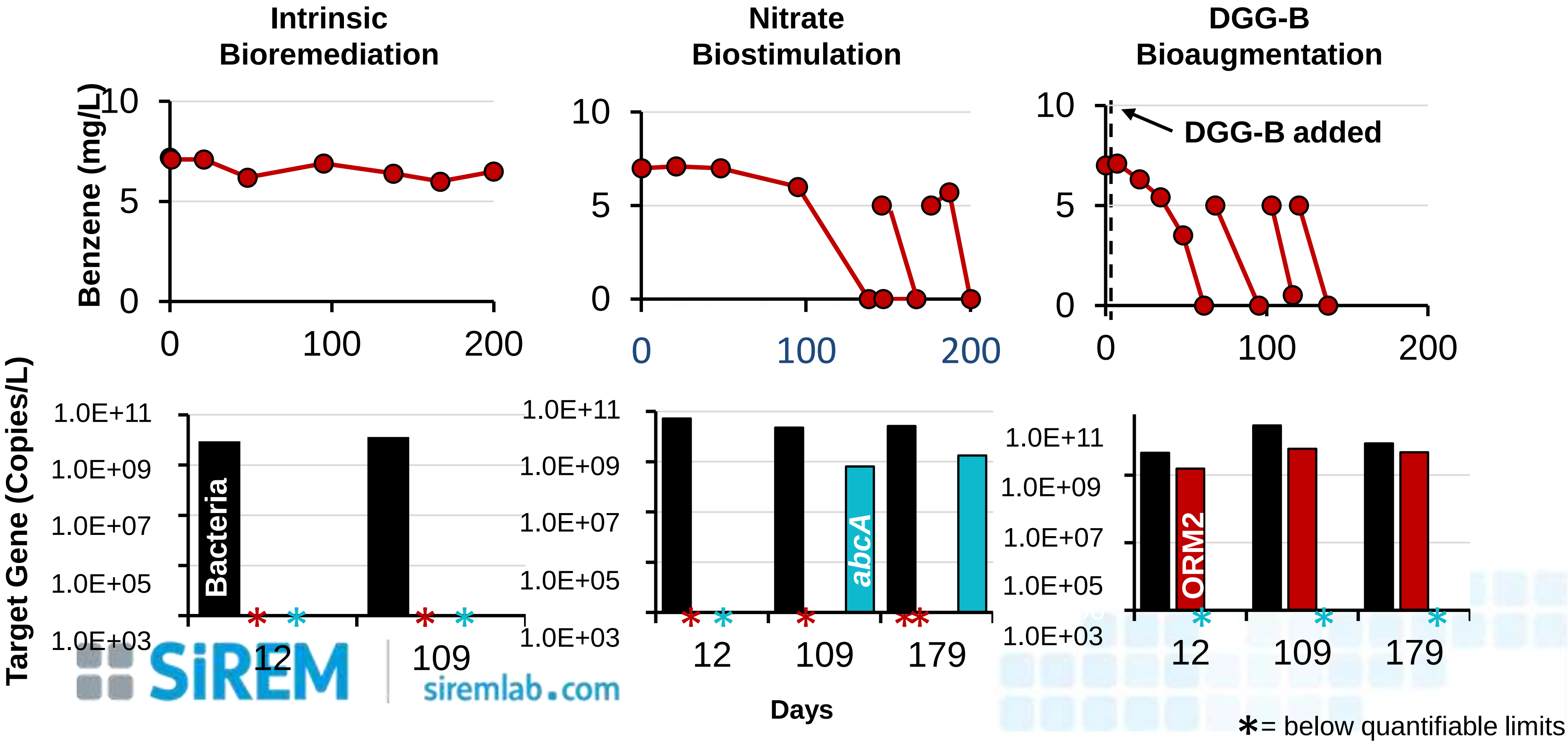


Borden II – Key Highlights

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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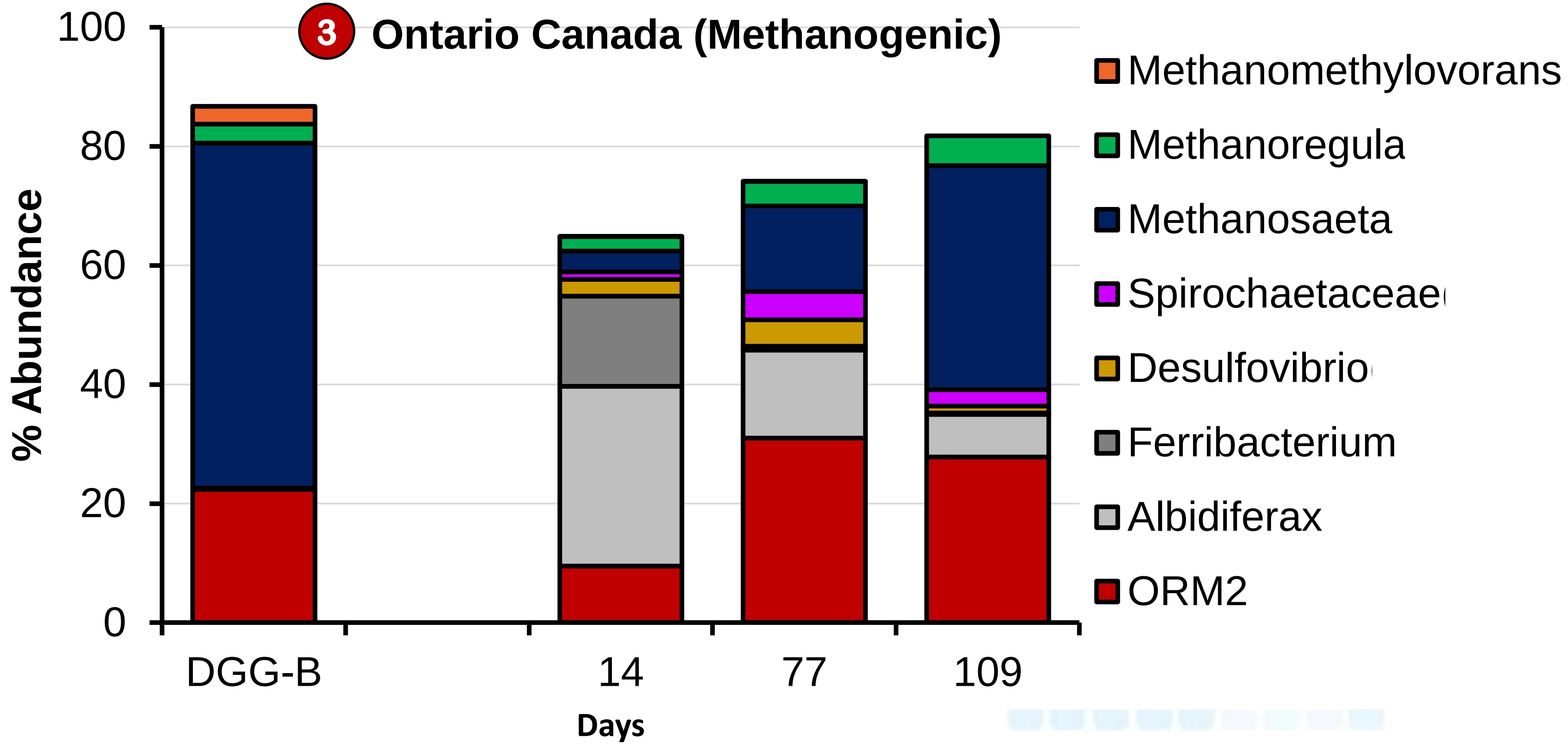
Bioaugmentation also increases the abundance of other key microbes



Field Pilot Testing of DGG-B & DGG-Plus

- 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

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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

Acknowledgements – Benzene/GAPP Team

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Federated Co-Operatives Limited, Saskatoon SK

Questions?

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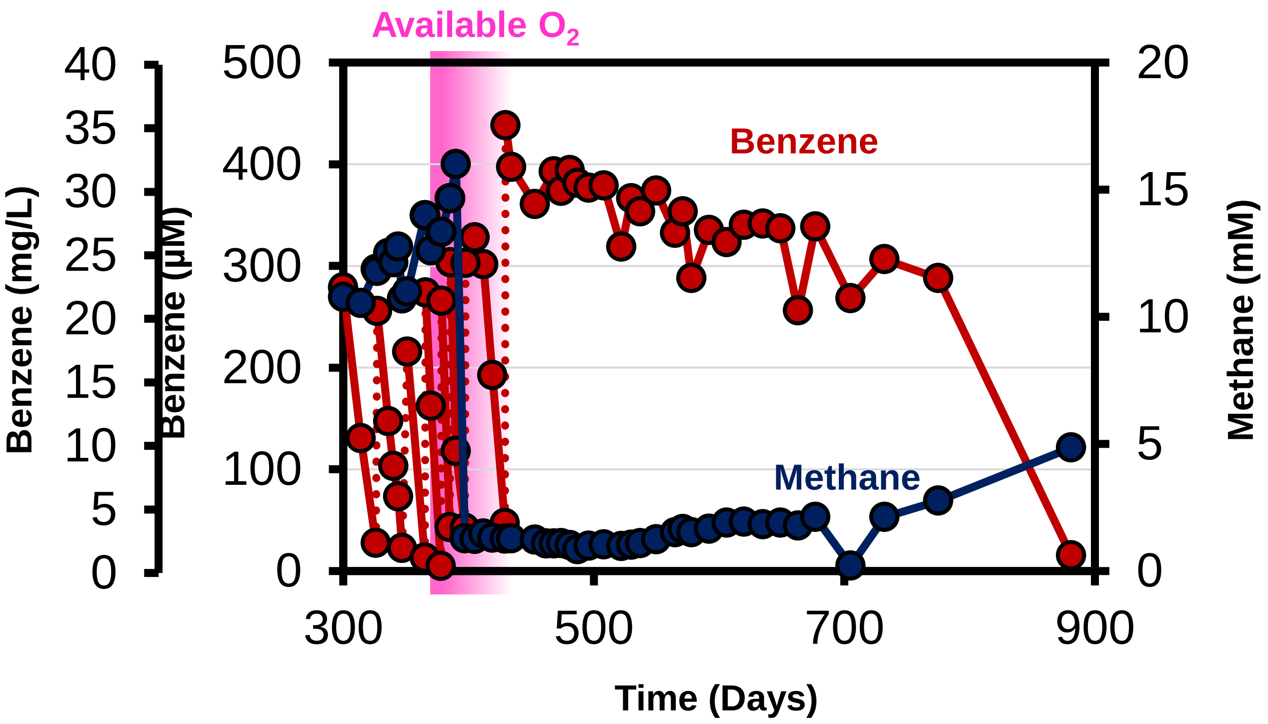
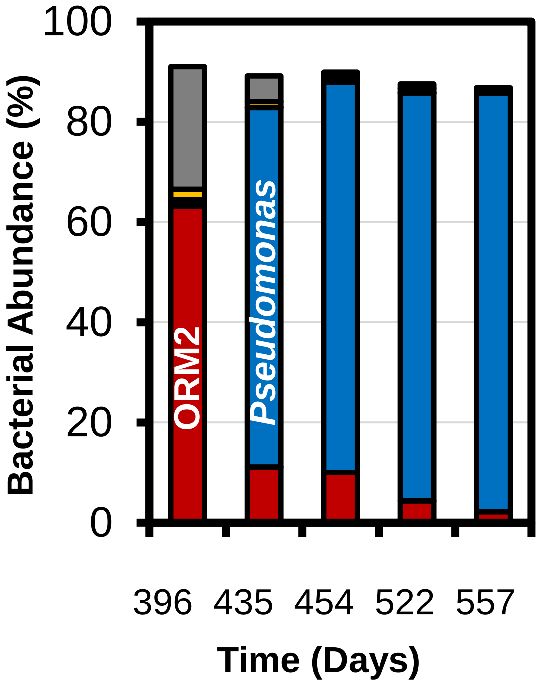
Back up slides

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