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BIOREMEDIATION APPROACHES AND TOOLS FOR BENZENE REMEDIATION UNDER ANAEROBIC CONDITIONS

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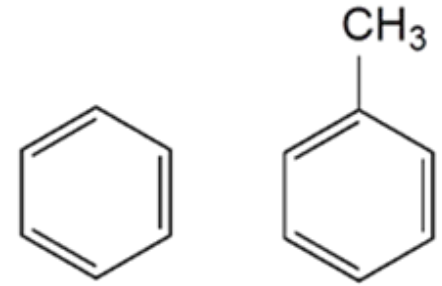
Outline

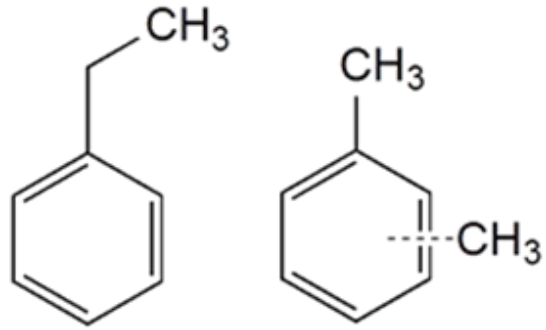
- Introduction –
- BTEX degradation and bioremediation
- Aerobic vs anaerobic
- Benzene degrading culture DGG-1
- Biomarkers
- Biotreatability studies
- Conclusions and Future work

BTEX Compounds

- Petroleum hydrocarbons of primary concern in groundwater are benzene, toluene, ethylbenzene and xylenes (BTEX)
- BTEX comprises ~18% of gasoline
- Benzene in particular is problematic potent carcinogen/ very mobile and most difficult to degrade under anaerobic conditions



Benzene Toluene



Ethylbenzene Xylene(s)

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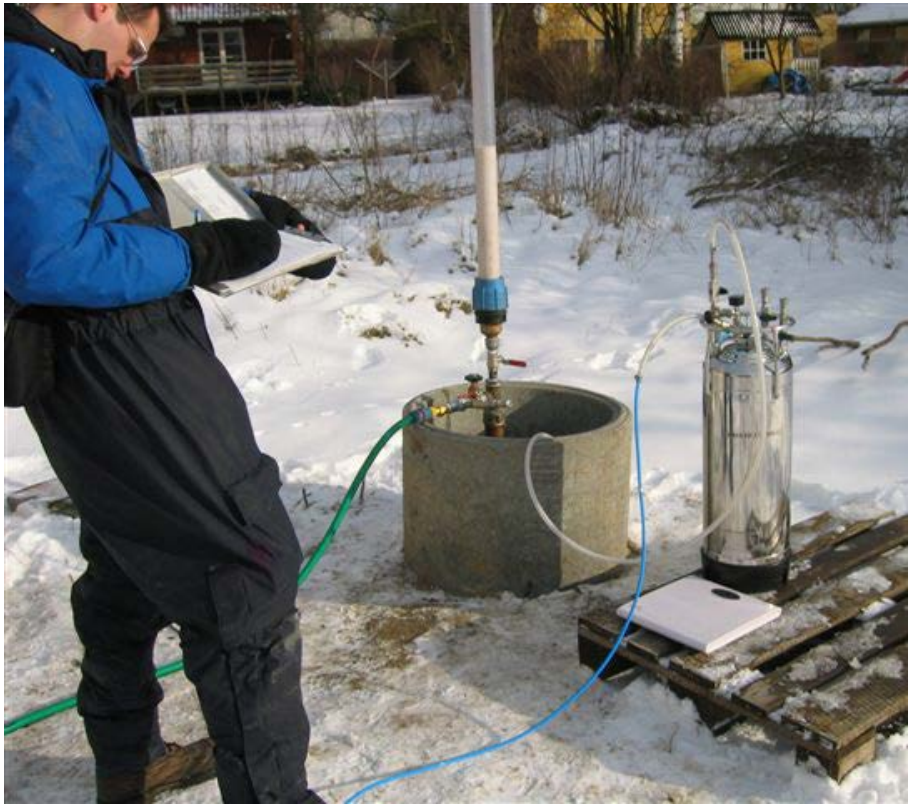
Remediation Approaches

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 such as 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,

Bioremediation



- **Biostimulation**: addition of amendments to increase biodegradation e.g., electron donors, electron acceptors, nutrients, etc.

- **Bioaugmentation:** addition of beneficial microorganisms to improve biodegradation

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, BTEX)

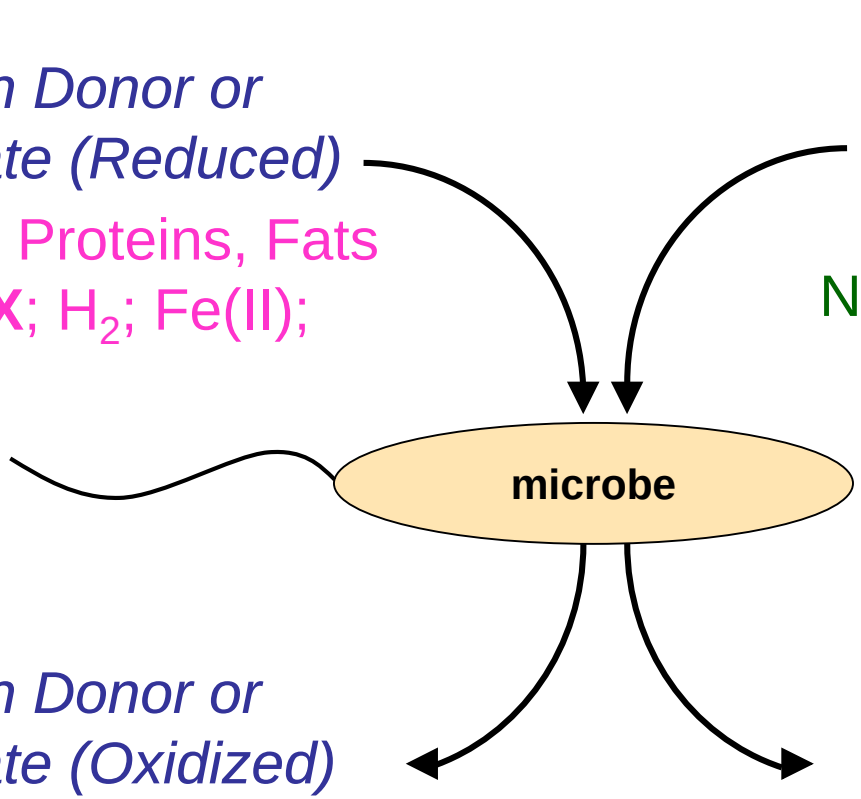
Overview of Microbial Metabolism

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)

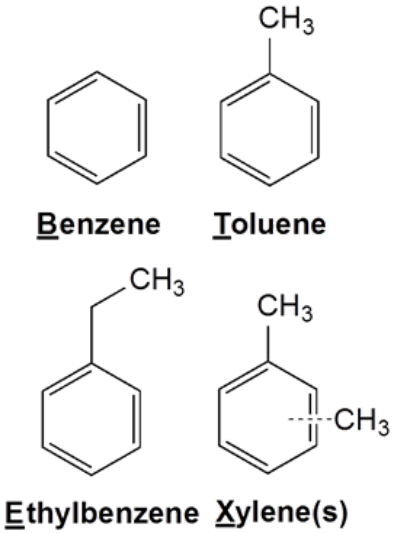
CO_2, H_2O, Fe(III)

Electron Acceptor (Reduced)

Water
N_2, H_2S
Fe(II), **CH_4**,

BTEX Bioremediation

- Aerobic bioremediation approaches rely on delivery of oxygen.
- Intrinsic microbial populations often capable of performing aerobic biodegradation.
- When contamination is deep or under naturally induced reducing conditions aerobic bioremediation can be difficult to establish and maintain.



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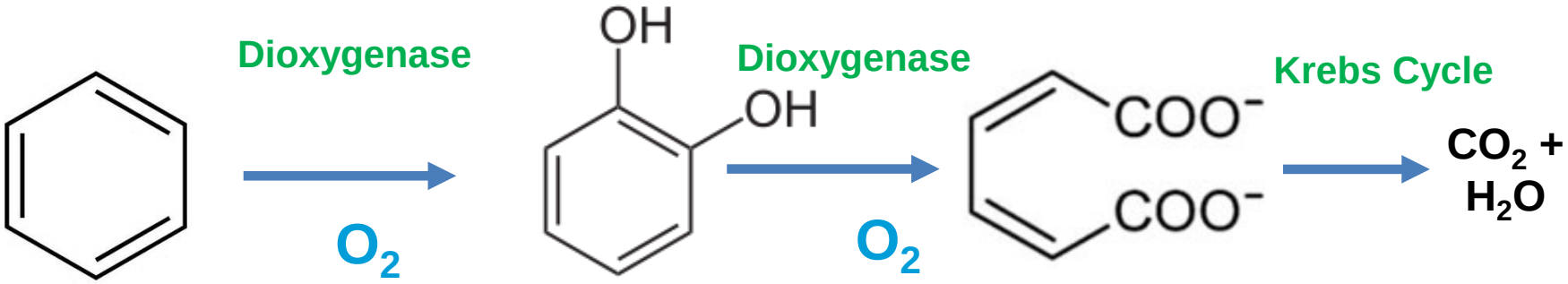
Aerobic Benzene Degradation



Aerobic processes effective but when contamination is deep or under established reducing conditions, aerobic bioremediation can be difficult to establish and maintain

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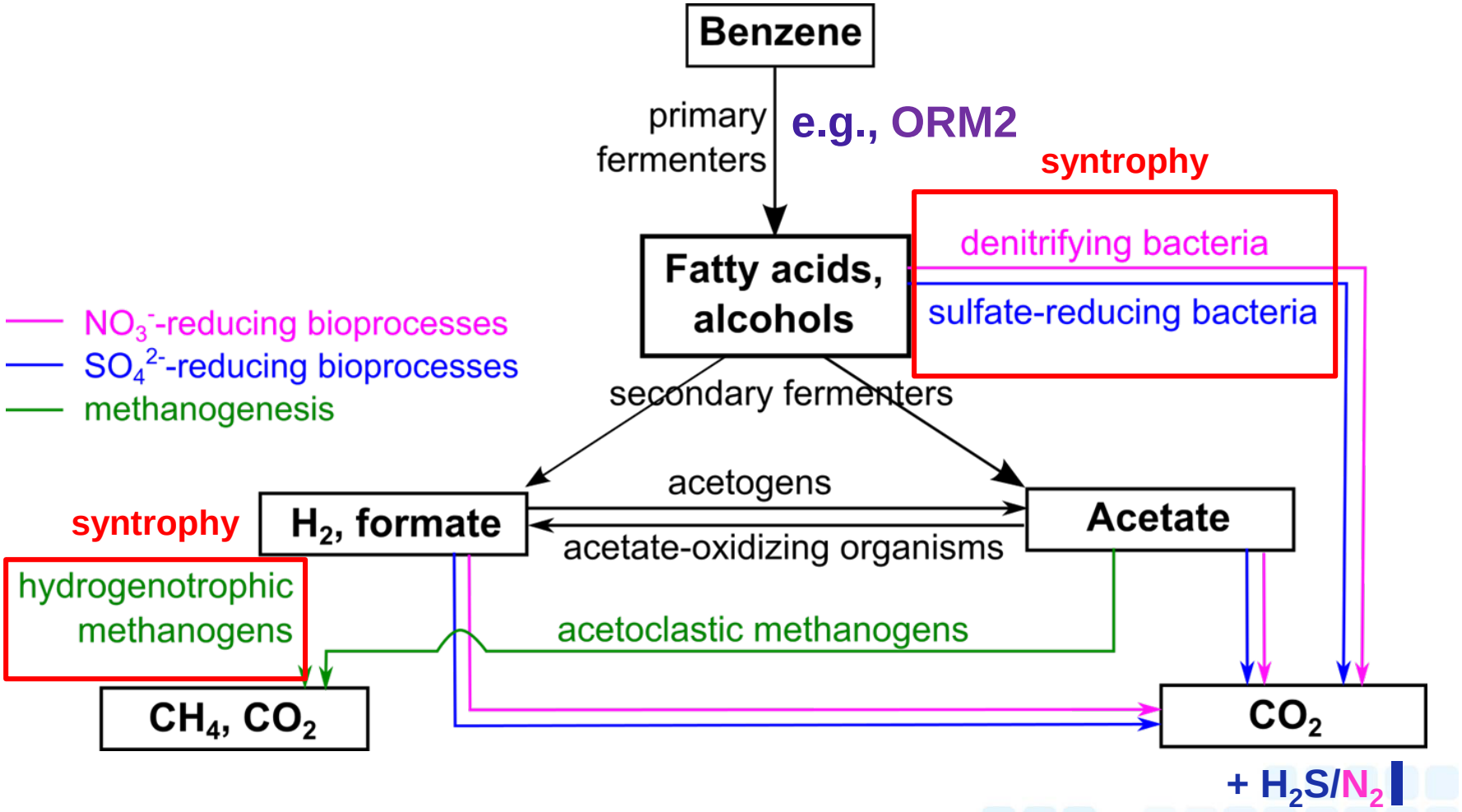
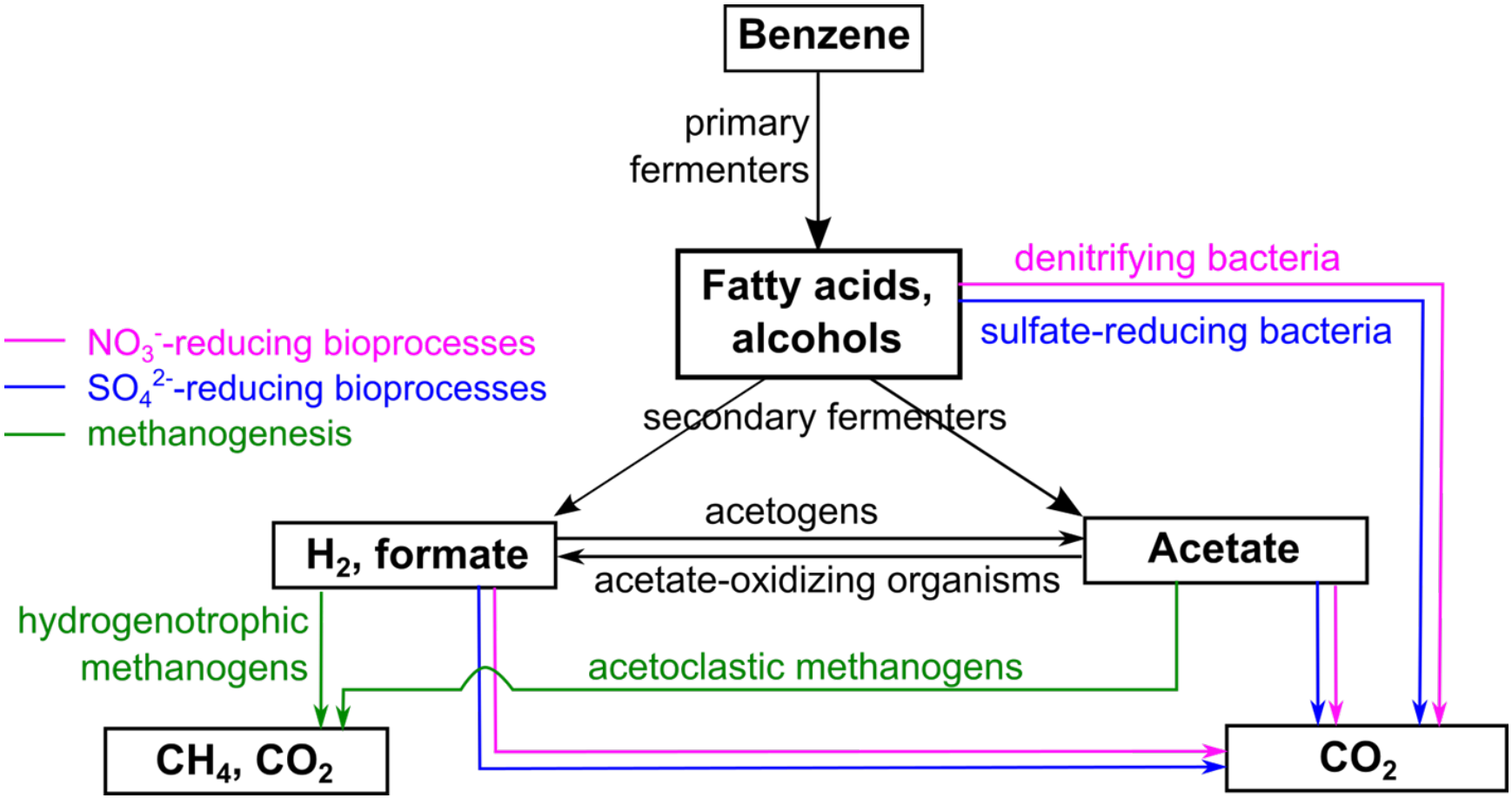
Anaerobic BTEX Bioremediation

- 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 can becomes a bottleneck = need for bioaugmentation
- Benzene is biggest challenge due to its unsubstituted ring structure = need for bioaugmentation

Anaerobic Benzene Degradation



Anaerobic Benzene Degradation



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Anaerobic Benzene Culture – DGG-1

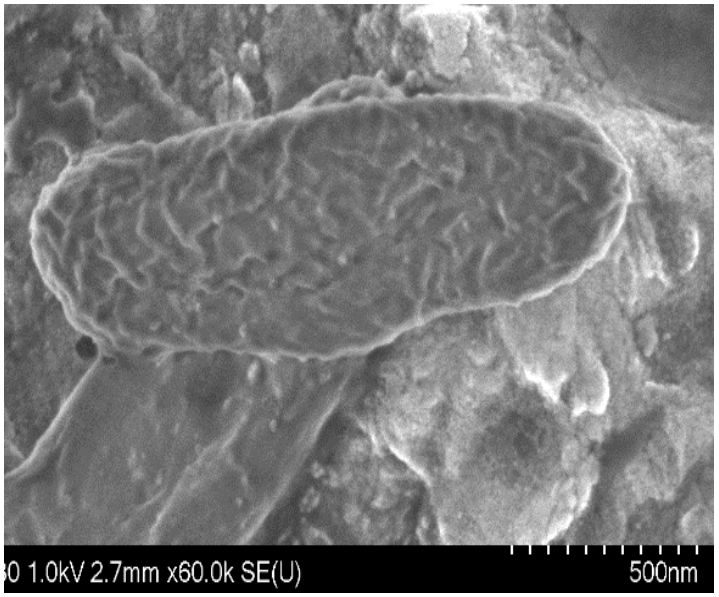
Currently Scaling up to Field Scale volumes

Anaerobic benzene seed culture (above) benzene fermenter is ORM2 (right)

Photos Courtesy of University of Toronto

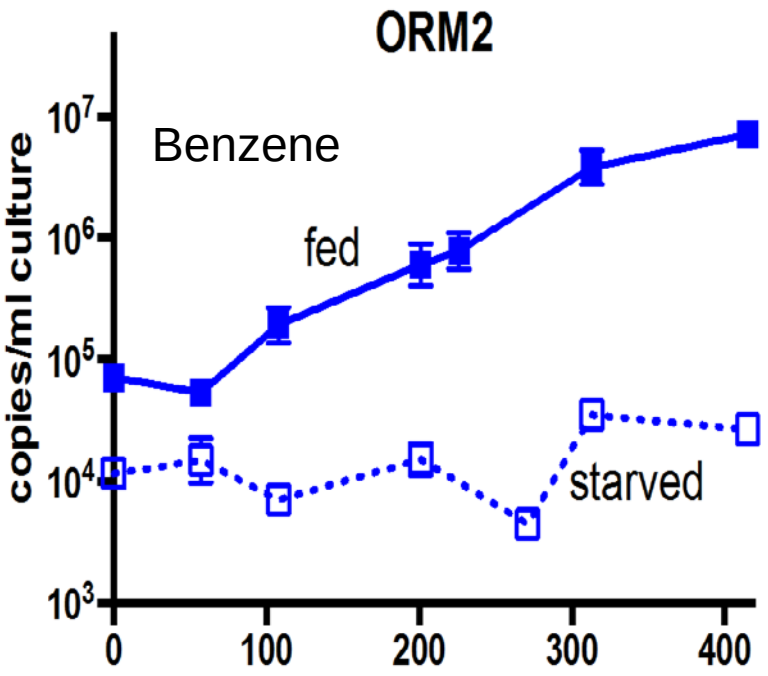
Edwards and Grbic-Galic, 1992

Introducing ORM2



- Benzene specialist derived from an oil refinery site in 2003
- Deltaproteobacterium not yet isolated in pure culture
- Slow growing ~ 30 day doubling time
- Has enzymes that ferment benzene
- Observed to degrade 40 mg/L

- Can ORM2 be used to bioaugment benzene sites?



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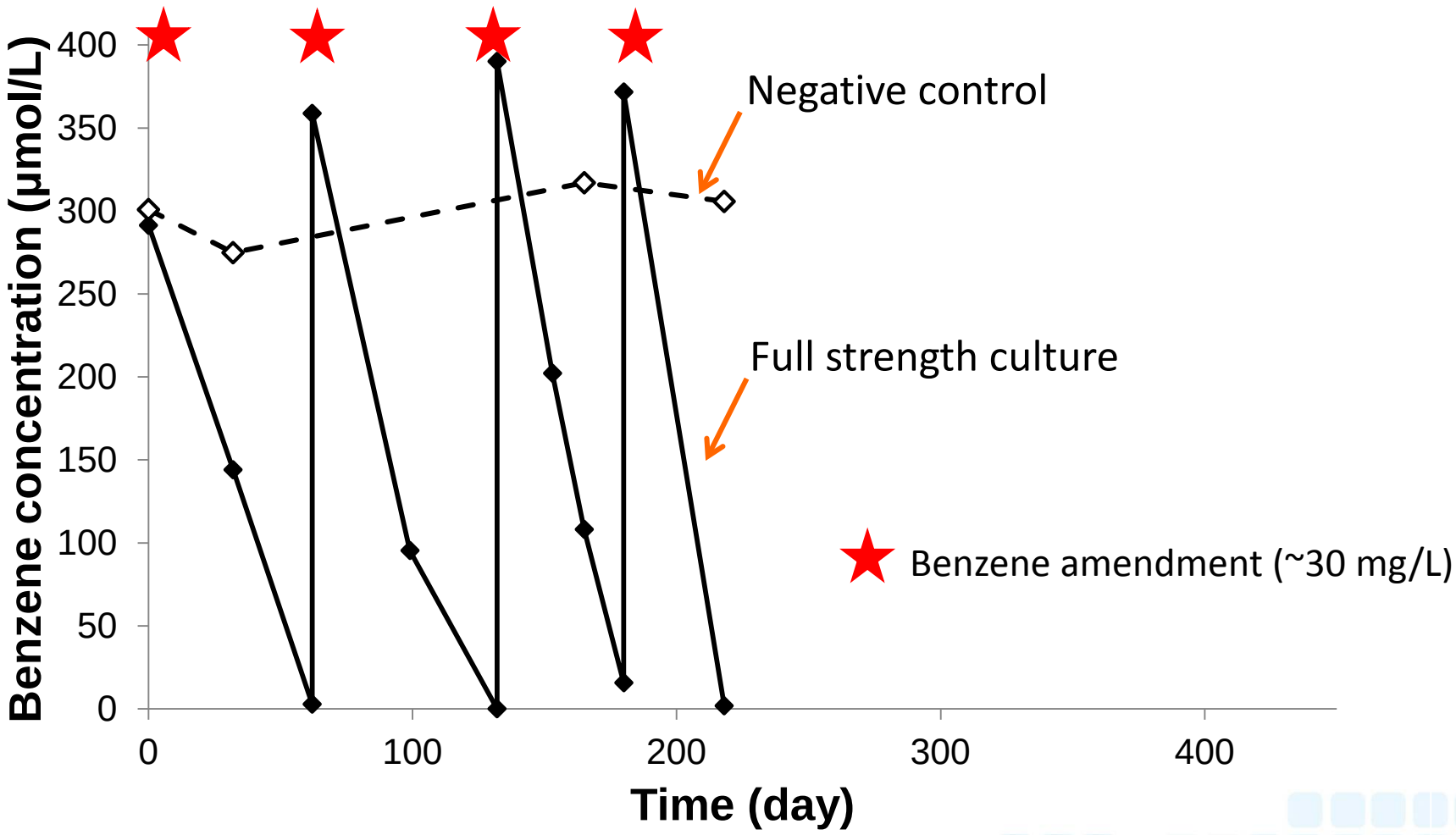
DGG-1 Anaerobic Benzene Bioaugmentation Culture



DGG-1 Microbial Characterization

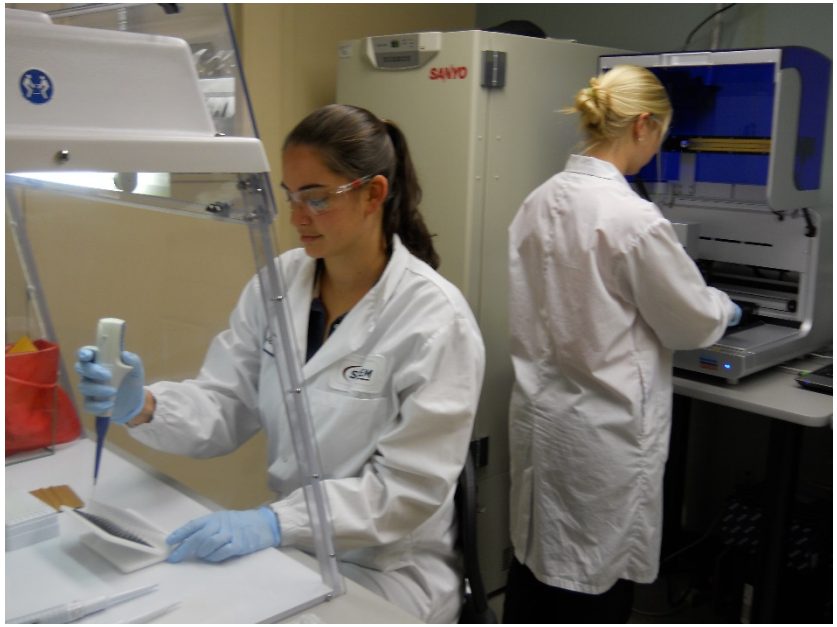
Identification of key microbes in degradation pathways

- Allows identification by qPCR analysis
  - Anaerobic Benzene – ORM-2
  - Sulfate degrading bacteria - SRB

Molecular Tools: Anaerobic BTEX Degradation

Gene-Trac® Tests:

- **ORM2** – benzene degrader (sulfate reducing/methanogenic conditions)
- **SRB** - sulfate reducing bacteria
- **Peptococcaceae**- benzene degrader (under nitrate reducing conditions)
- ***abcA*** - (Peptococcaceae functional gene benzene carboxylase

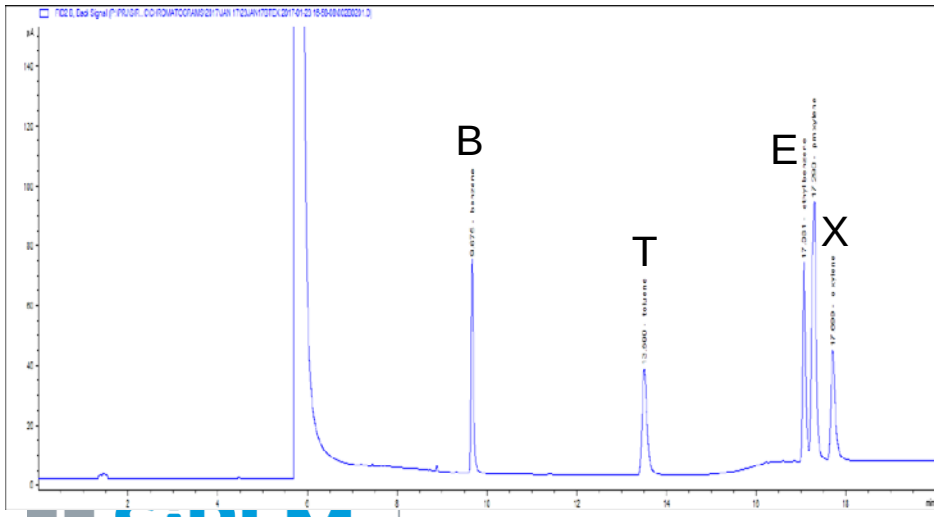


Biomarker Testing

- Are the required microorganisms indigenous to the site?
- Is bioaugmentation required?
- Impact of site amendments?
- Growth and spread of organisms in enhanced bioremediation?

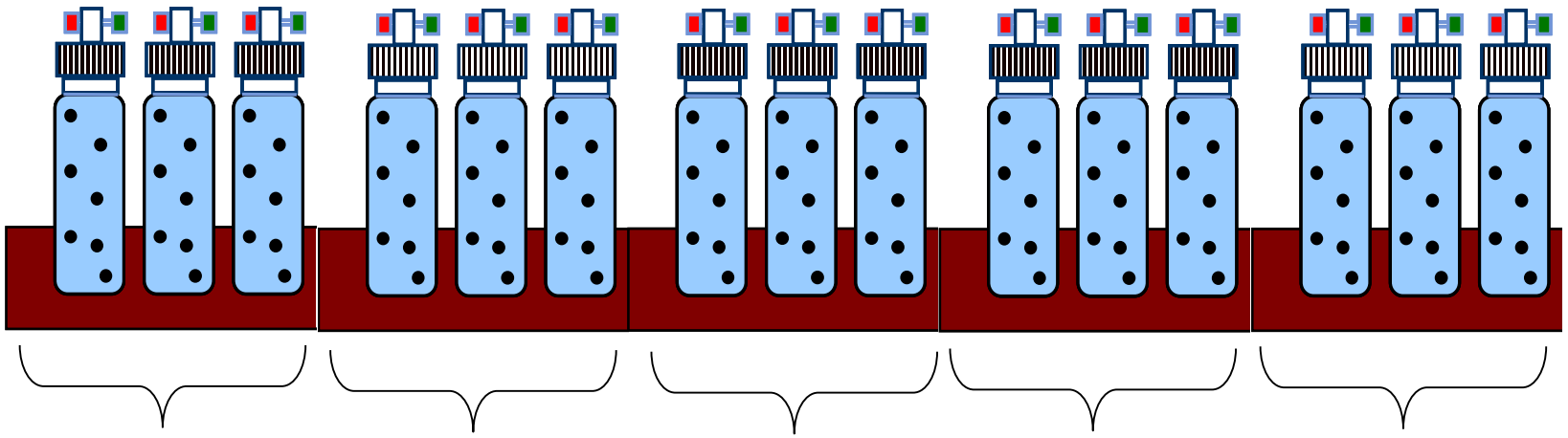
Anaerobic Biotreatability Studies

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



Degradation of BTEX monitored by GC under various conditions

Batch Treatability Study Design Features



Sterile Control
autoclaved and poisoned to inhibit microbes measure possible abiotic losses

Active Control
unamended

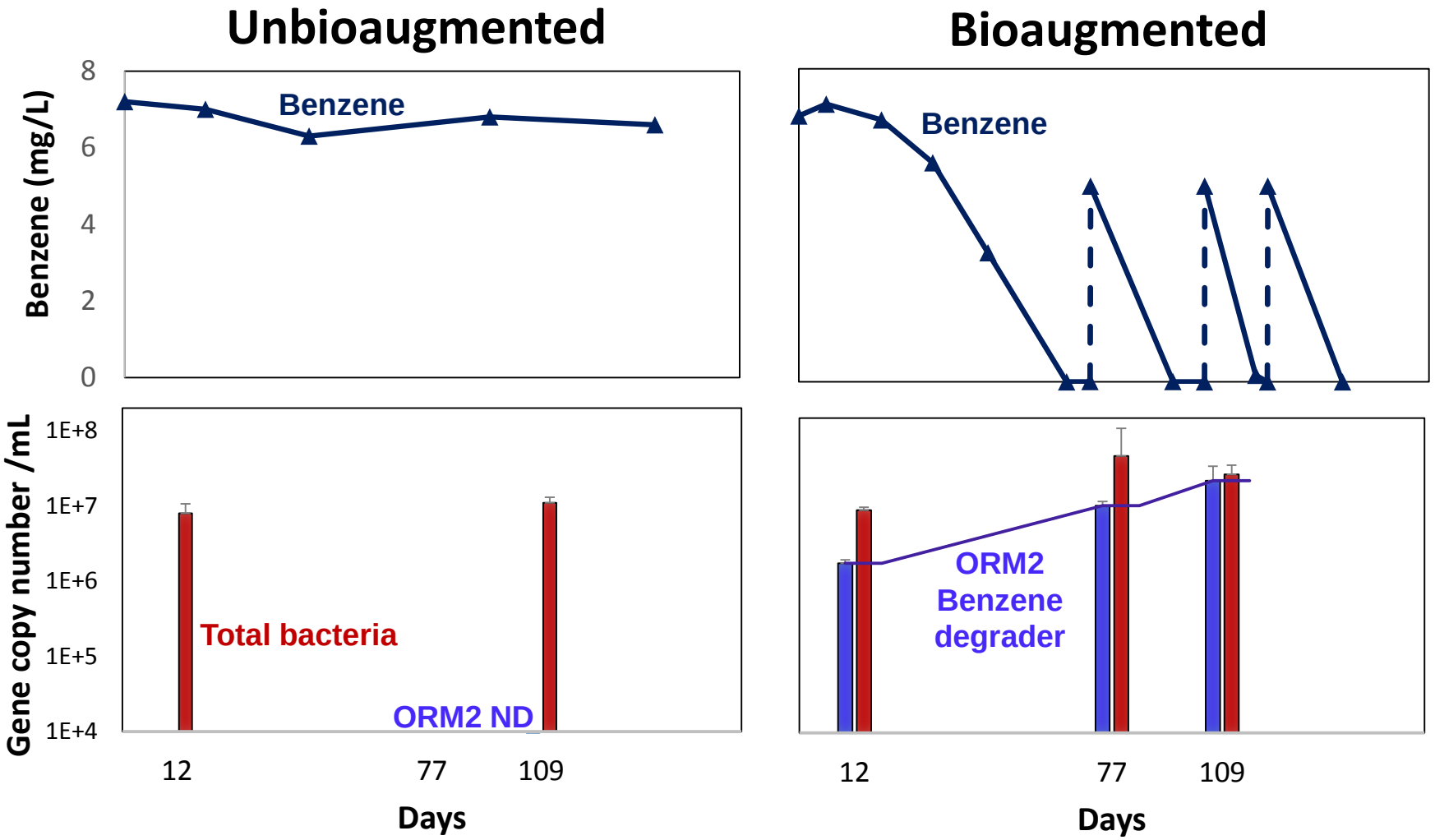
Biostimulation
addition of organic electron donors

Bioaugmentation+ Biostimulation
addition of known degrading populations e.g., KB-1

Gas Addition
H_2 /O_2 addition etc. To measure impact of gas infusion /cometabolic processes e.g. propane addition

Treatability studies are custom designed for each site

Ontario Site Treatability Study



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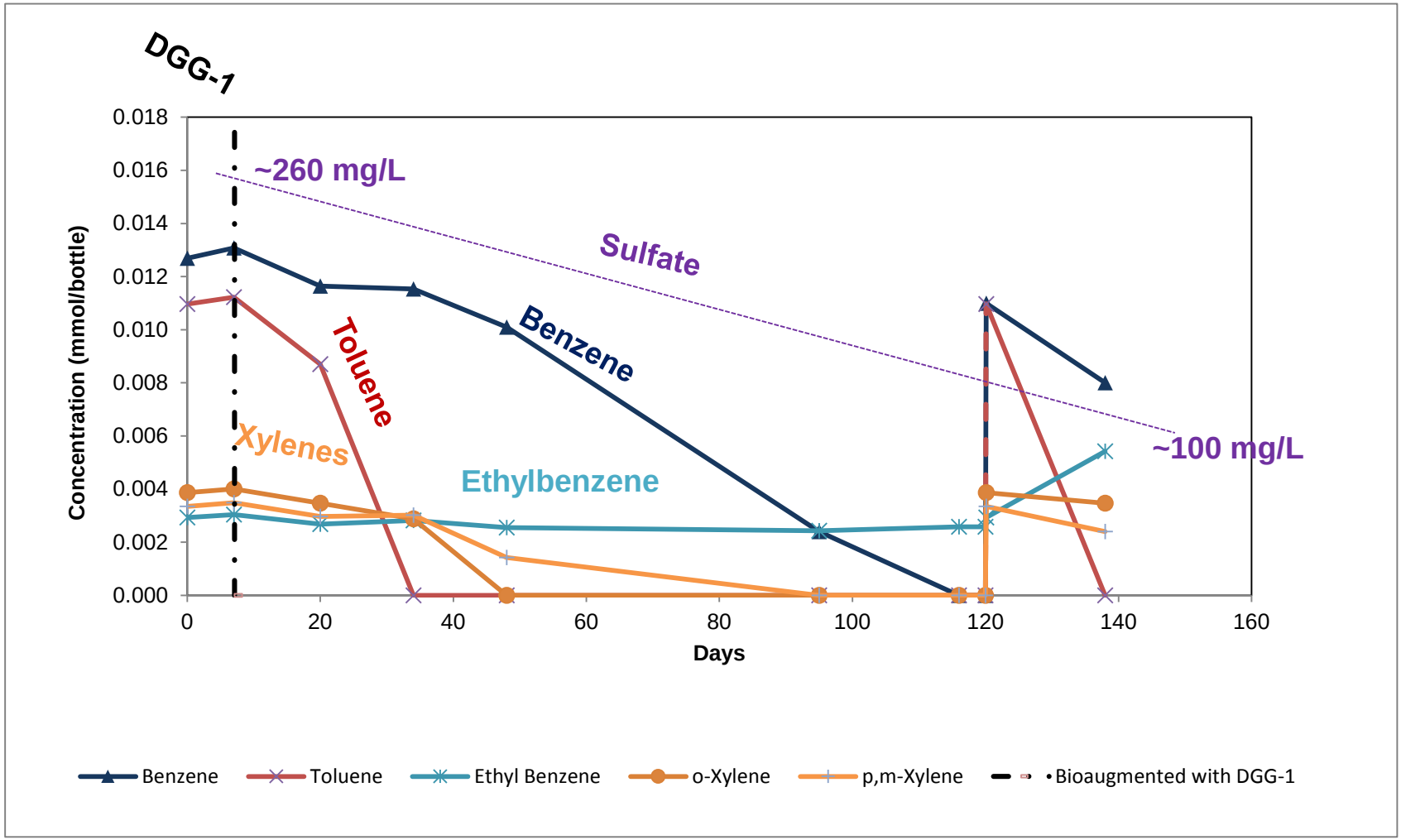
BTEX Degrade under Sulfate Reducing Conditions – Ontario Site Microcosms



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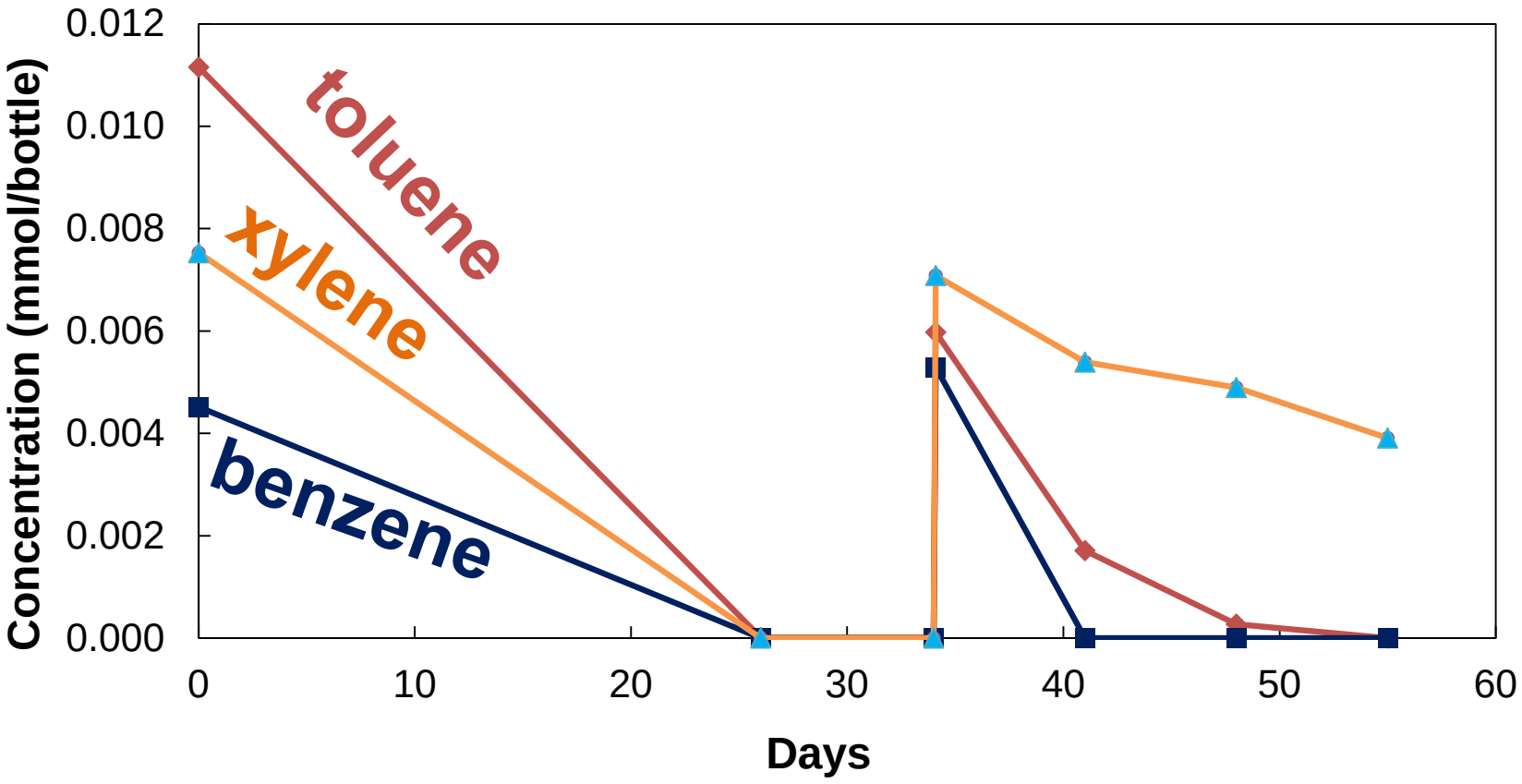
We are developing Cultures Can Degrade Multiple Substrates Simultaneously



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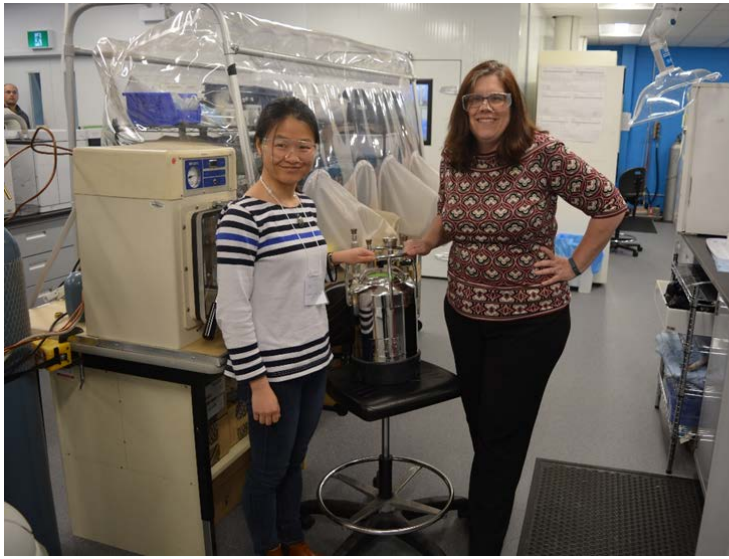
Anaerobic Benzene Research

- SiREM collaborator on 3 year grant with University of Toronto (Elizabeth Edwards) and Federated Co-operatives Limited
  Project Goals:
- Bioaugmentation culture scale-up
- Treatability Testing
- Develop molecular genetic tests to track key organisms
- Data for regulatory approvals (safety/performance)
- Field pilot testing (Co-op site)

Do you have a benzene site? Please let us know!



Conclusions con’t and Future Work

- Scale up to 100L of anaerobic benzene culture for field scale applications-in progress
- Lab treatability studies underway for 4 sites- indicate the DGG-1 culture is effective under simulated site conditions
- Molecular tools to quantify key microorganisms/functional genes have been test under development
- Cultures for the TEX compounds being tested can be combined
- Field testing is planned for 2018

Acknowledgements

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Ontario

MITACS

Thank you for attending!

Further Information

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