Bioremediation Approaches and Tools for Anaerobic Benzene Remediation

Phil Dennis
SiREM

SMART Remediation
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Bioremediation Approaches and Tools for Anaerobic Benzene Remediation

Phil Dennis, Jeff Roberts, Sandra Dworatzek and Peter Dollar, SiREM Guelph, Ontario
Elizabeth Edwards and Fei Luo, University of Toronto, Toronto, Ontario

Presented by:
Sandra Dworatzek, SiREM

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Introduction to SiREM

- Founded in 2001 to provide laboratory testing services and products for site remediation
- Located in the University of Guelph Research Park
- More information www.siremlab.com

University of Guelph Research Park

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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,

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)

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Bioremediation

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

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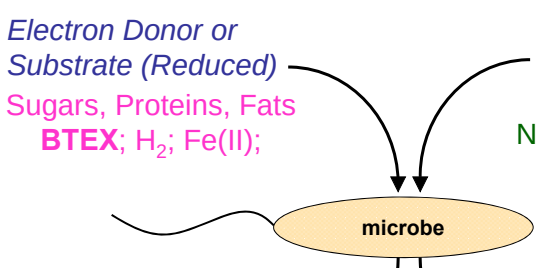
Overview of Microbial Metabolism



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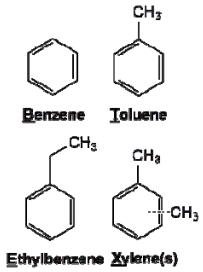
BTEX Bioremediation

- 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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BTEX Bioremediation continued…

- Biodegradation of BTEX occurs under anaerobic conditions
  - Methanogenic
  - Nitrate reducing
  - Sulfate reducing
- Microbial populations – may be present at low concentration but growth is slow
- Benzene is biggest challenge due to its unsubstituted ring structure

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Anaerobic Benzene Degradation

- Advantages of sulfate/anaerobic processes for benzene remediation
  - Sulfate highly soluble
  - Sulfate naturally present in many aquifers
  - Sulfate easier subsurface application than O_2 (e.g., Tersus Nutrisulfate®-LT)
  - Anaerobic bio less inclined towards biofouling
- Bioaugmentation culture likely required at many sites as the organisms that anaerobically degrade benzene are less ubiquitous and slow growing

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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

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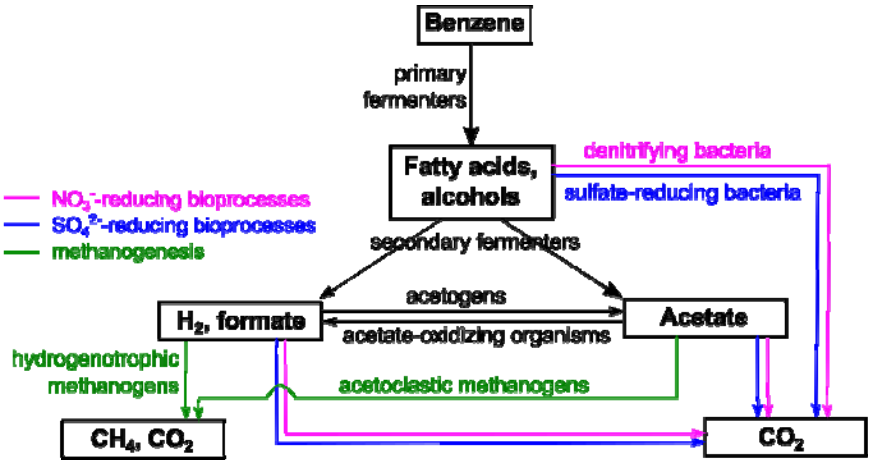
Anaerobic Benzene Degradation



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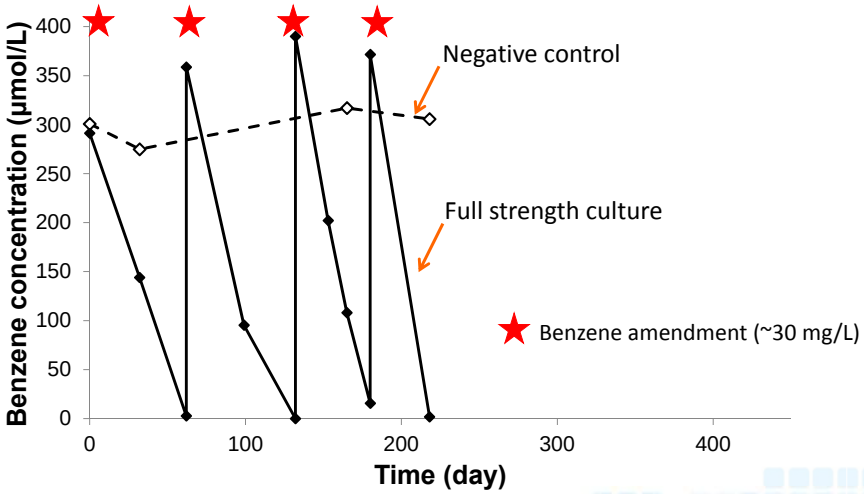
DGG-1 Anaerobic Benzene Bioaugmentation Culture



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Characterization of Benzene Culture

Identification of key microbes in degradation pathways

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

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Gene-Trac® Testing

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

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Gene-Trac® Tests Offered by SiREM

Gene-Trac® Test	Target	Relevance
Dhc	*Dehalococcoides*	Dechlorination of chlorinated ethenes, 1,2-DCA, PCB's, TCB/DCB
FGA (*vcrA/bvcA/tceA*)	VC-reductases/ TCE-reductase	*Dhc* functional genes: convert TCE, to cDCE and cDCE & VC to ethene
Dhb	*Dehalobacter*	Chlorinated methane & ethane degradation
cfrA	*cfrA* and *dcrA* reductive dehalogenases	*Dhb* functional genes: dechlorinate CF, 1,1,1-TCA & 1,1-DCA
Dhg	*Dehalogenimonas*	Dechlorinates chlorinated propanes, tDCE & 1,2-DCA
Dsb	*Desulfitobacterium*	Dechlorinates PCP, TCP/DCP, PCE/TCE, 1,2-DCA
Geo	*Geobacter*	Dechlorinates PCE & TCE / biogeochemical reduction
Polaromonas	*Polaromonas* Isocitrate Lyase	Aerobic cDCE/VC degraders (JS-666)
etn	*etnC/etnE*	Aerobic VC degradation
1,4-Dioxane	*dxmB* - dioxane monooxygenase & ALDH aldehyde dehydrogenase	Aerobic 1,4-Dioxane degradation
ORM-2	Deltaproteobacterium ORM-2	Anaerobic benzene degradation
Anammox	Planctomycetes	Ammonium and nitrite removal
SRB	Sulfate reducing bacteria	Anaerobic hydrocarbon oxidation/ biogeochemical reduction
Arch	Archaea	Methanogenic microorganisms
Universal	All Bacteria	For determining total bacterial biomass
NGS	Bacteria and Archaea	Next generation sequencing for overall microbial community composition

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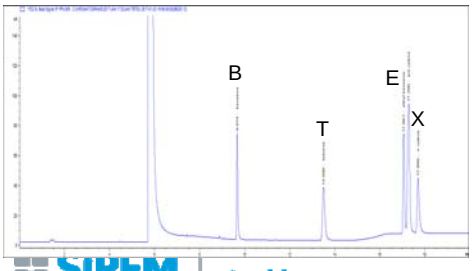
Anaerobic Biotreatability Studies

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

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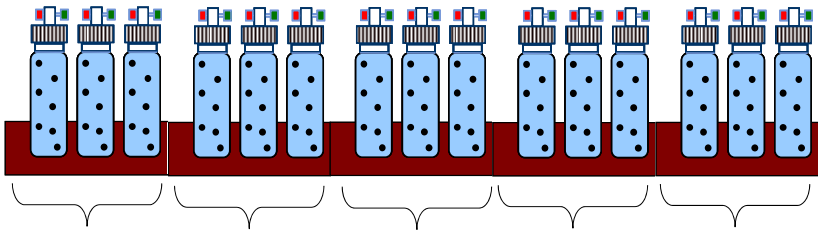
Batch Treatability Study Design Features



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

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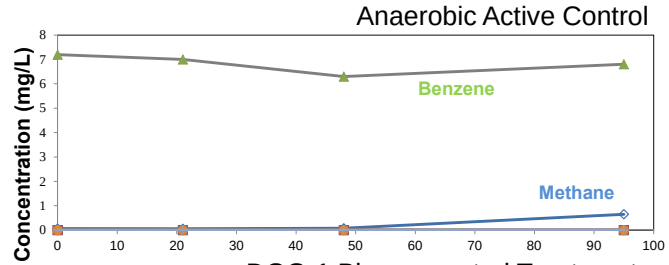
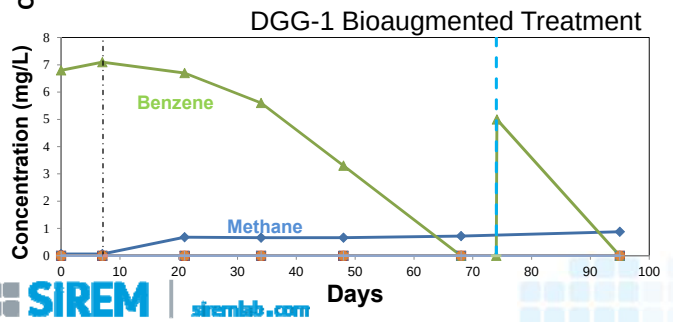
Biotreatability Testing Results – ON Site





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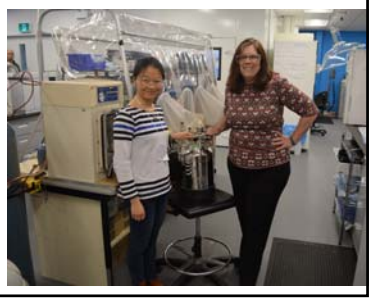
Anaerobic Benzene Research

- SiREM collaborator on 3 year $750K 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!



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Ontario

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Thank you for attending!

Further Information

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1-866-251-1747
sdworatzek@Siremlab.com

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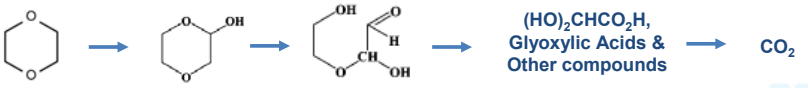
Webinar

Presenter: Dr. Shaily Mahendra
UCLA
Topic: 1,4-Dioxane
Date: 23 February 2016
Time: 12:00 – 1:00 pm EST



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