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A Sustainable Bioremediation Approach for BTEX-Contaminated Groundwater Under Methanogenic and Sulfate-Reducing Conditions

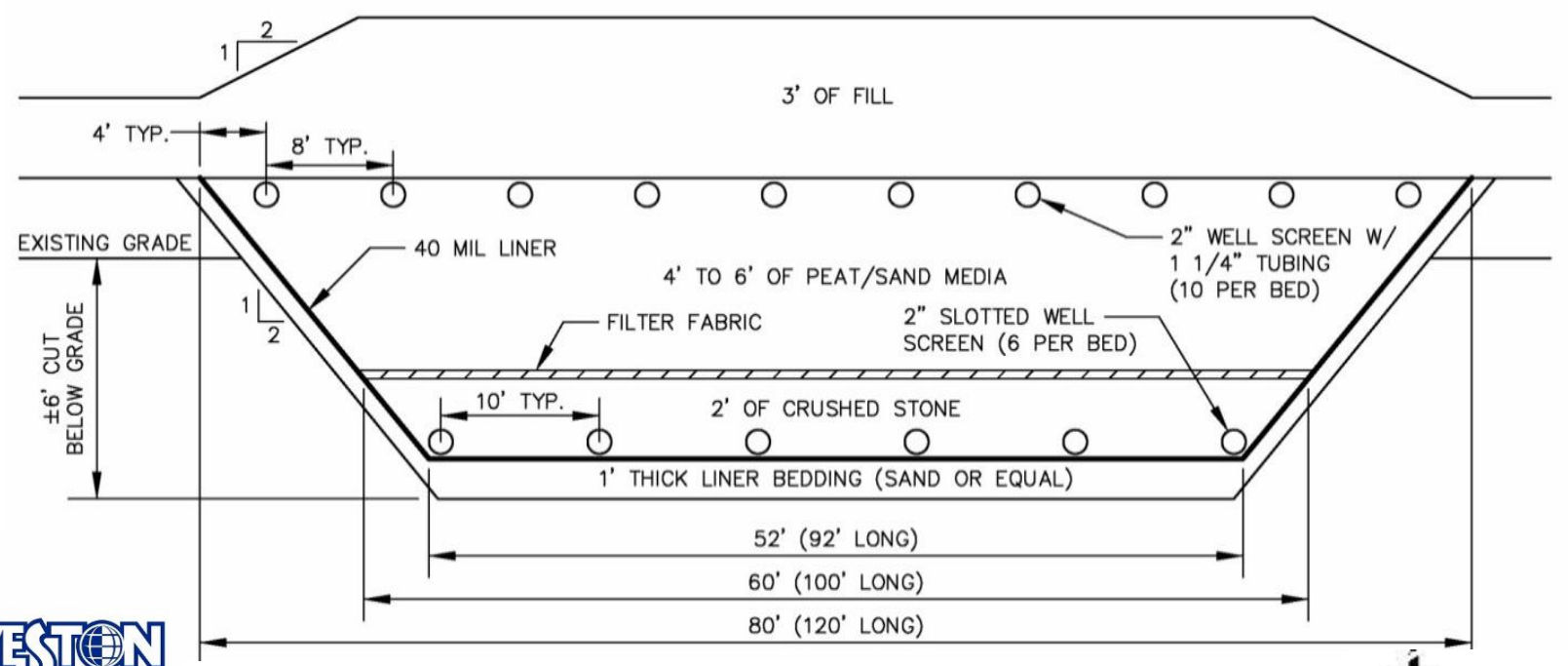
Background: What are ABRs?

Anaerobic Bioreactors

- Porous media, flow-through beds for treatment of various contaminants
- Built without vegetation but with highly organic soils
- Used to replace physical/chemical treatment processes
- VOC sorption on organic matter increases residence time to accomplish biodegradation in smaller volume

Background (cont.)

- Bioremediation Benefits
  - Cost-effective and sustainable
  - Accelerates natural metabolic processes
  - Used in both aerobic & anaerobic environments



Cross-sectional view of Anaerobic Bioreactors Design (Re-Solve, Inc. Superfund Site, 2009).

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Overall study hypotheses

- Bioremediation of organic compounds can be accomplished in scalable porous media reactors in volumes that are practical at field scale
- A variety of terminal electron acceptor processes can be established in these reactors by altering the geochemistry of the porous media and feedwater
- Microbial community structure changes as the geochemistry changes in ways that can enhance biodegradation of the target compounds

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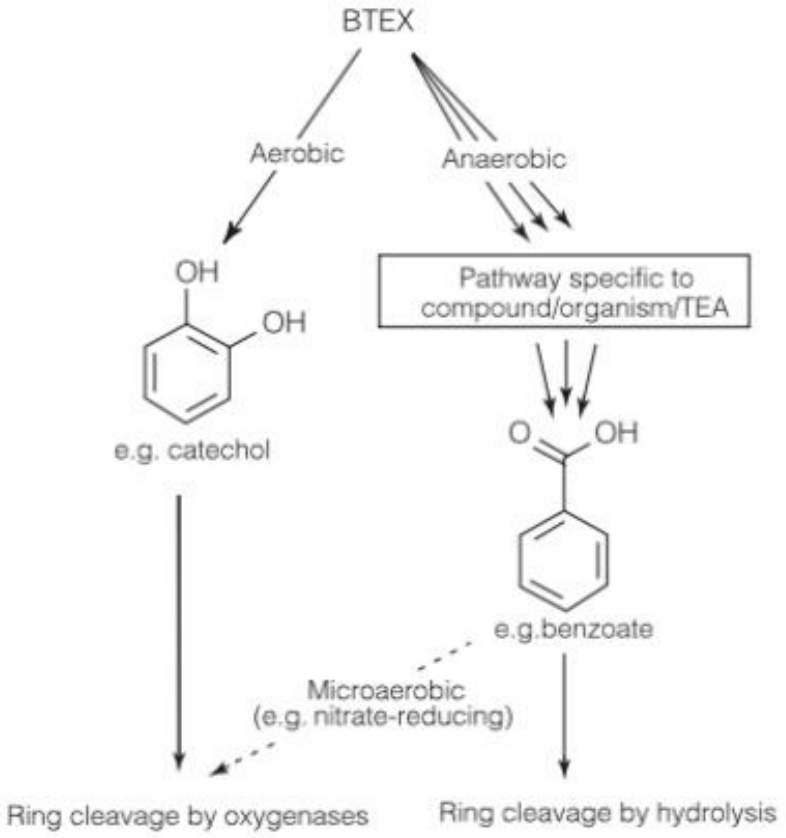
Research Objective

- **Objective: BTEX Study**

Assessment of enhanced bioremediation of benzene, toluene, ethylbenzene, and xylenes (BTEX) in anaerobic bioreactor (ABRs) systems and the evaluation of varying anaerobic terminal electron acceptor (TEA) processes on the rate and extent of biodegradation.

Background



Generalized BTEX biodegradation pathways (Johnson *et al.*, 2003).

- BTEX biodegradation pathways
- Compounds must overcome aromatic carbon ring system
- Can biodegrade under aerobic and anaerobic conditions
- Benzene is known to be more recalcitrant under anaerobic conditions

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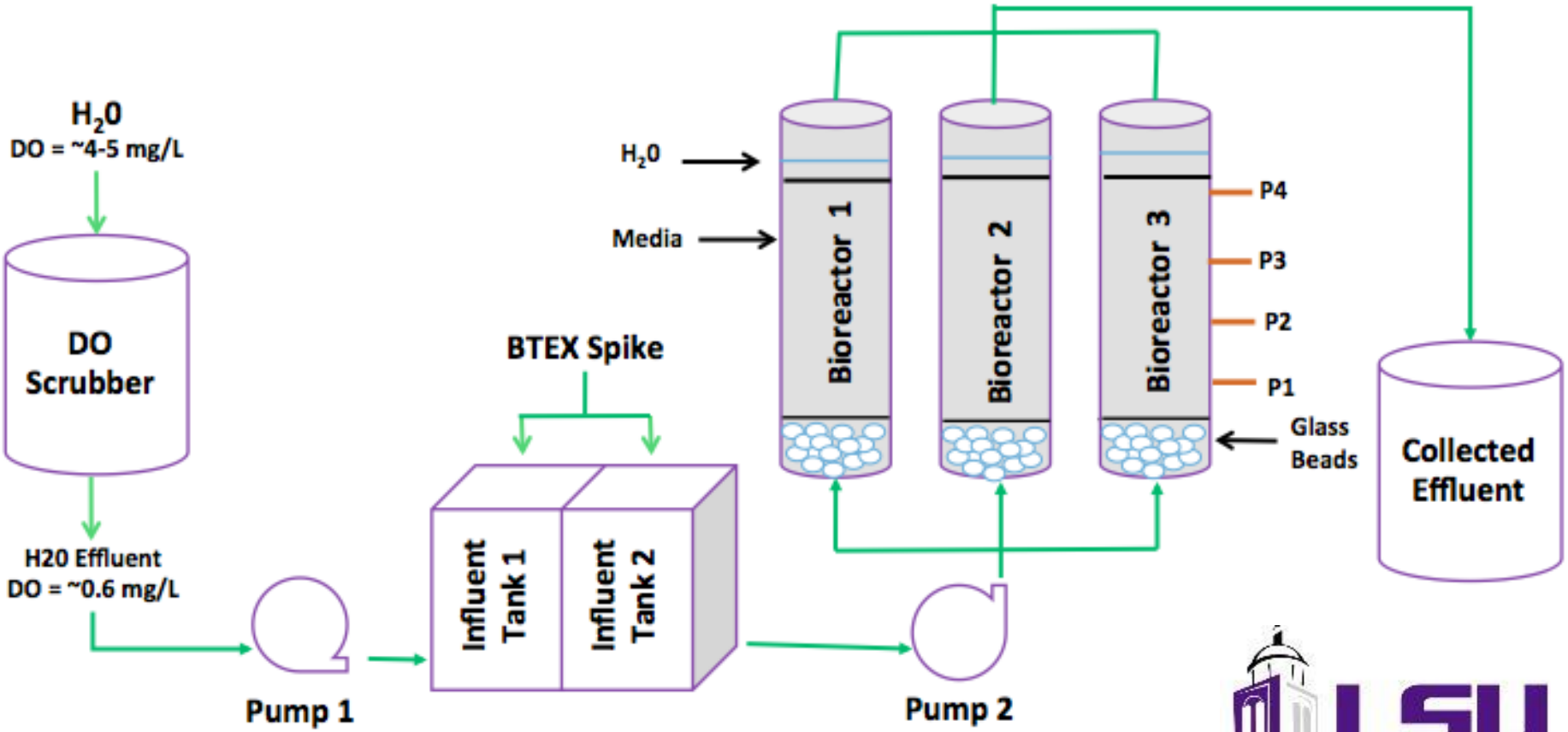
Research Objective: BTEX Study

Materials & Methods: ABR Experimental Design

- Continuously fed ~10mg/L of BTEX
- Loading rate of 1.93 g BTEX/m^2/day
- Pump flow rate of 1.1 mL/min
  - ~1.6 L/day
- Retention time ~1.6 days
- Evaluation of BTEX degradation
  - Phase 1: No addition of exogenous electron acceptors (termed "methanogenic" later)
  - Phase 2: Amended with 500 mg/L of SO_4^{2-}



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Research Objective 1: BTEX Study

Materials & Methods: Chemical Analysis

BTEX aqueous analysis

- 25 mL liquid sample collected
- Placed in 30 mL glass serum bottle, and immediately crimp-sealed with a Teflon coated septum
- Samples inverted at least 3-hours prior to analysis
- Analyze 1.5 mL of headspace using a gas-tight syringe
- Analysis using a GC-FID via direct injection

Anion Analysis

- 2 mL of liquid sample collected
- Placed in screw-cap sampling vials
- Analyze SO_4^{2-}
- Analysis using a SmartChem 170 Discrete Analyzer

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Research Objective 1: BTEX Study

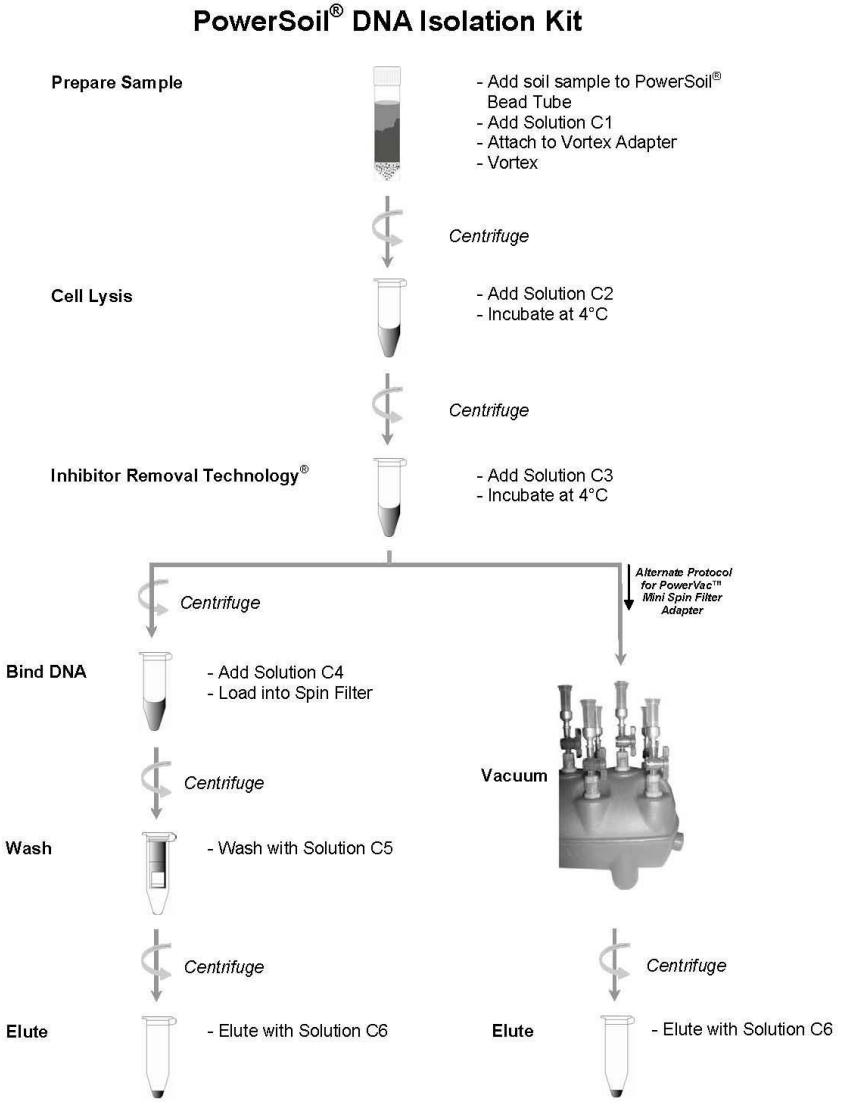
Materials & Methods: Microbial Analysis

DNA Extraction Method:

- Extracted media samples via coring horizontally through sampling ports at various depths in the ABRs
- ~ 1 g soil sample collected
- PowerSoil DNA Isolation Kit, MoBio Laboratories, Inc.

DNA Sequencing:

- The use of next generation sequencing (NGS) of 16S rRNA to characterize microbial populations
- Bioinformatics completed using Mothur® software program



MoBio Laboratories, Inc. PowerSoil® DNA Isolation Kit; Instruction Manual; 2013

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Next Generation Sequencing: Bioinformatics

Mothur			
Align Sequences	Pair-end reads were combined and then screened	Sequences aligned against SILVA database	Chimeric sequences removed
Sequences Assigned OTUs	RDP classifier at 50% cutoff		
Species Distribution			
Comparative Analysis	Observations of richness and diversity	Population Change during varying TEA processes	Mining of sequences for specific anaerobic hydrocarbon-degrading genera

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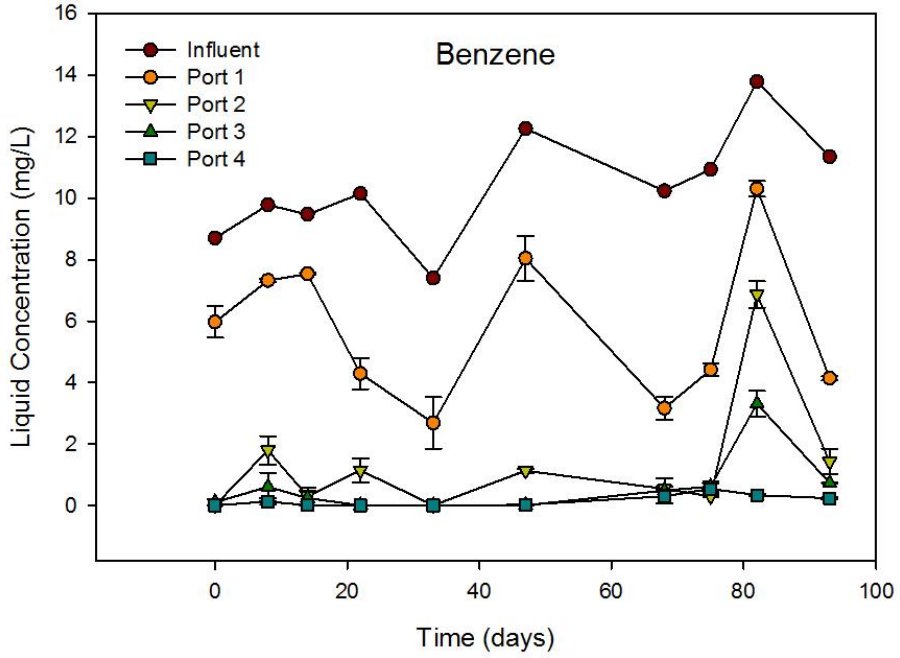
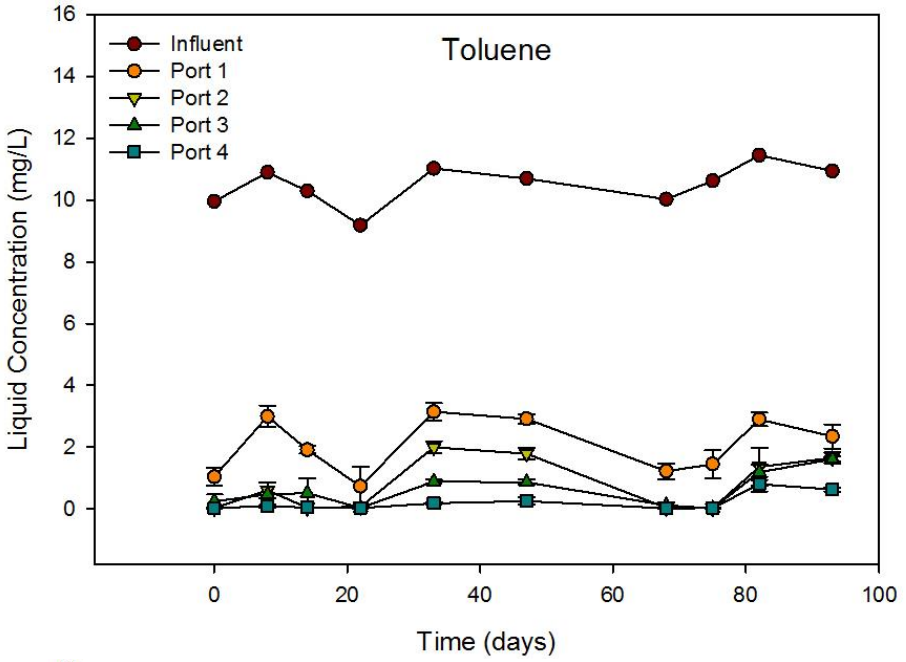
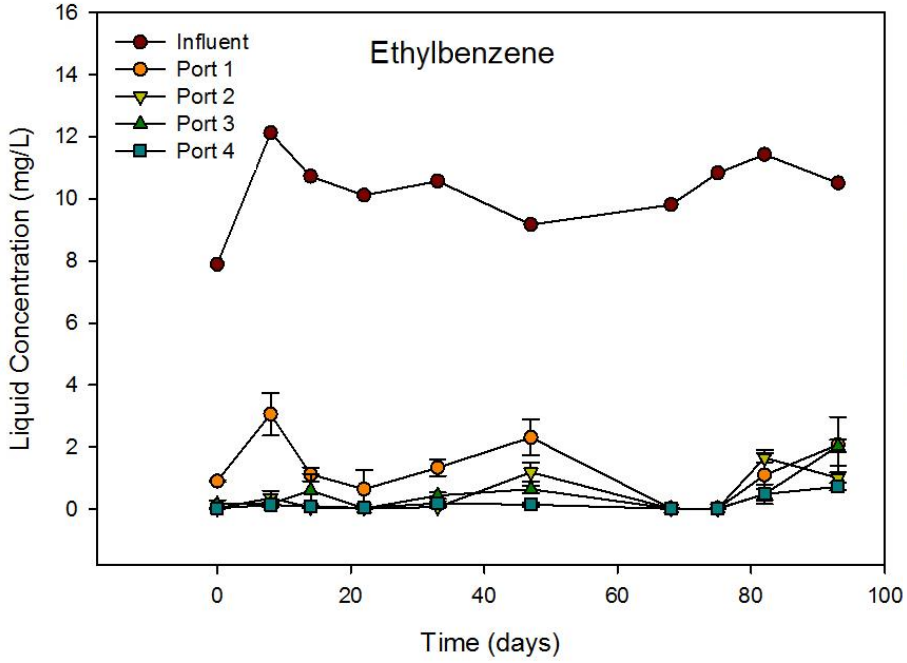
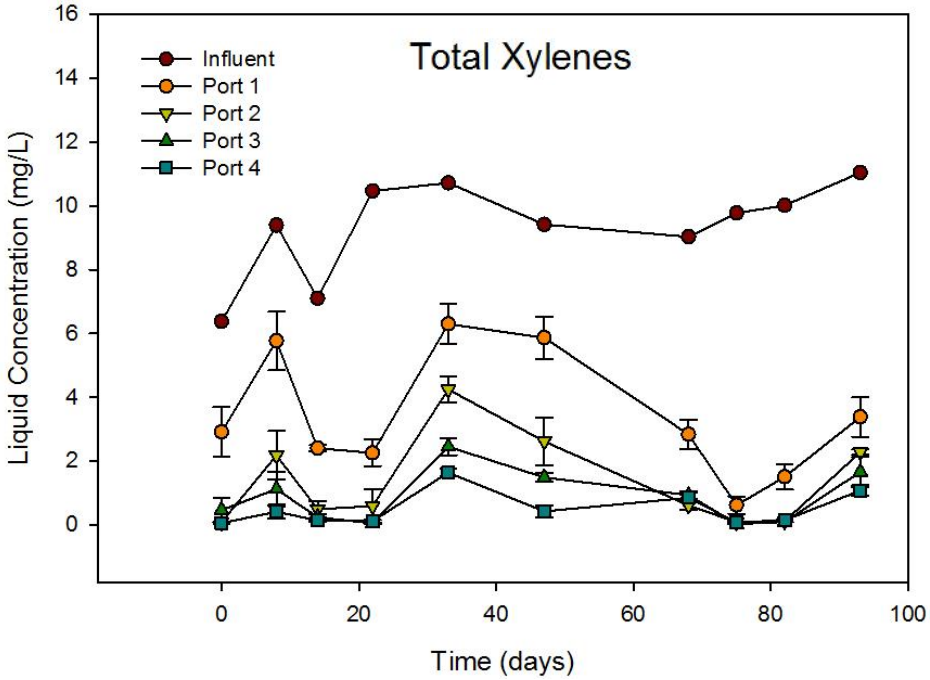
Results: Methanogenic Conditions



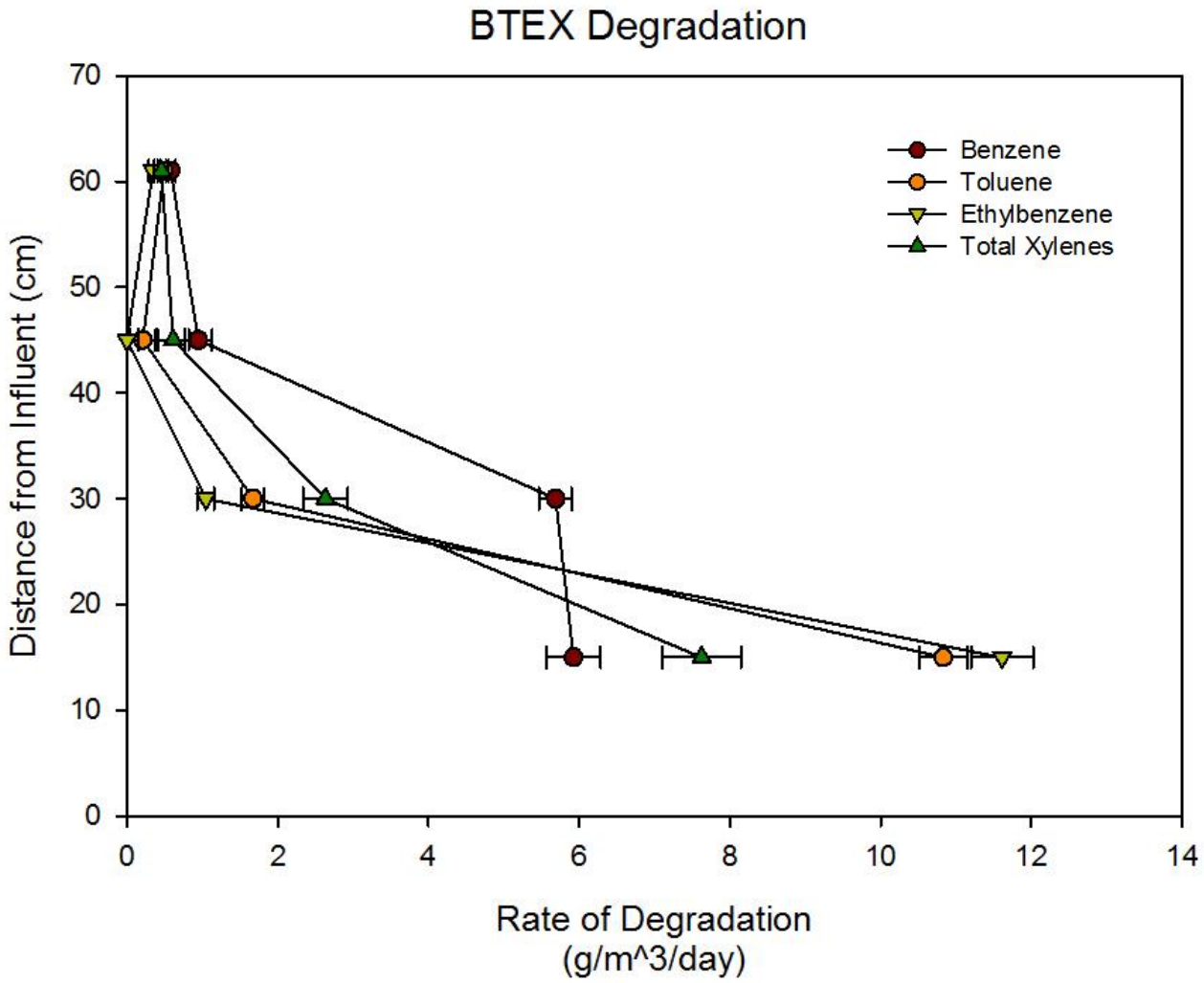






Research Objective 1: BTEX Study

Results: Methanogenic Conditions

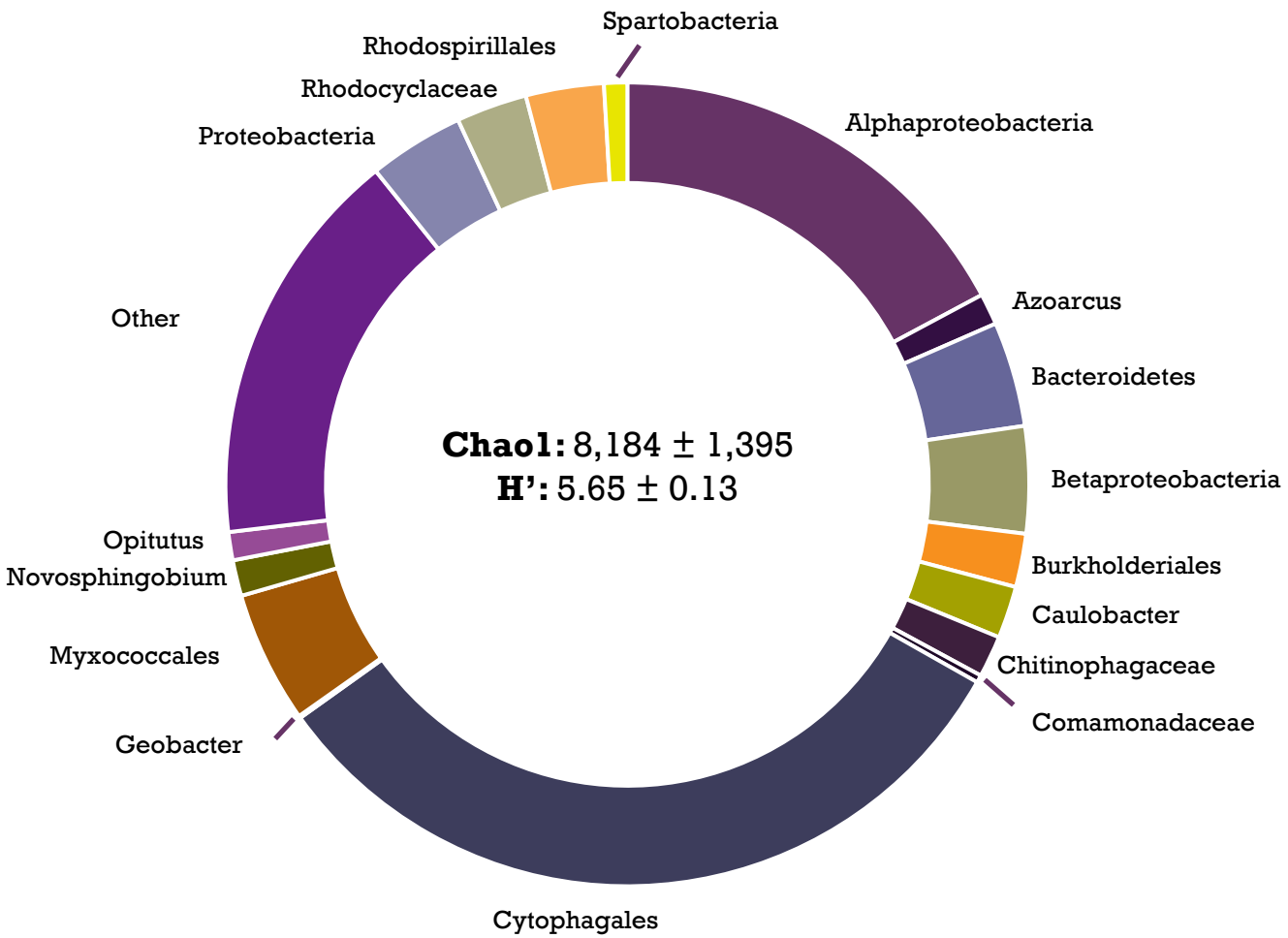


Rate of degradation of BTEX versus distance from the influent.

Research Objective: BTEX Study

Results: Methanogenic Conditions

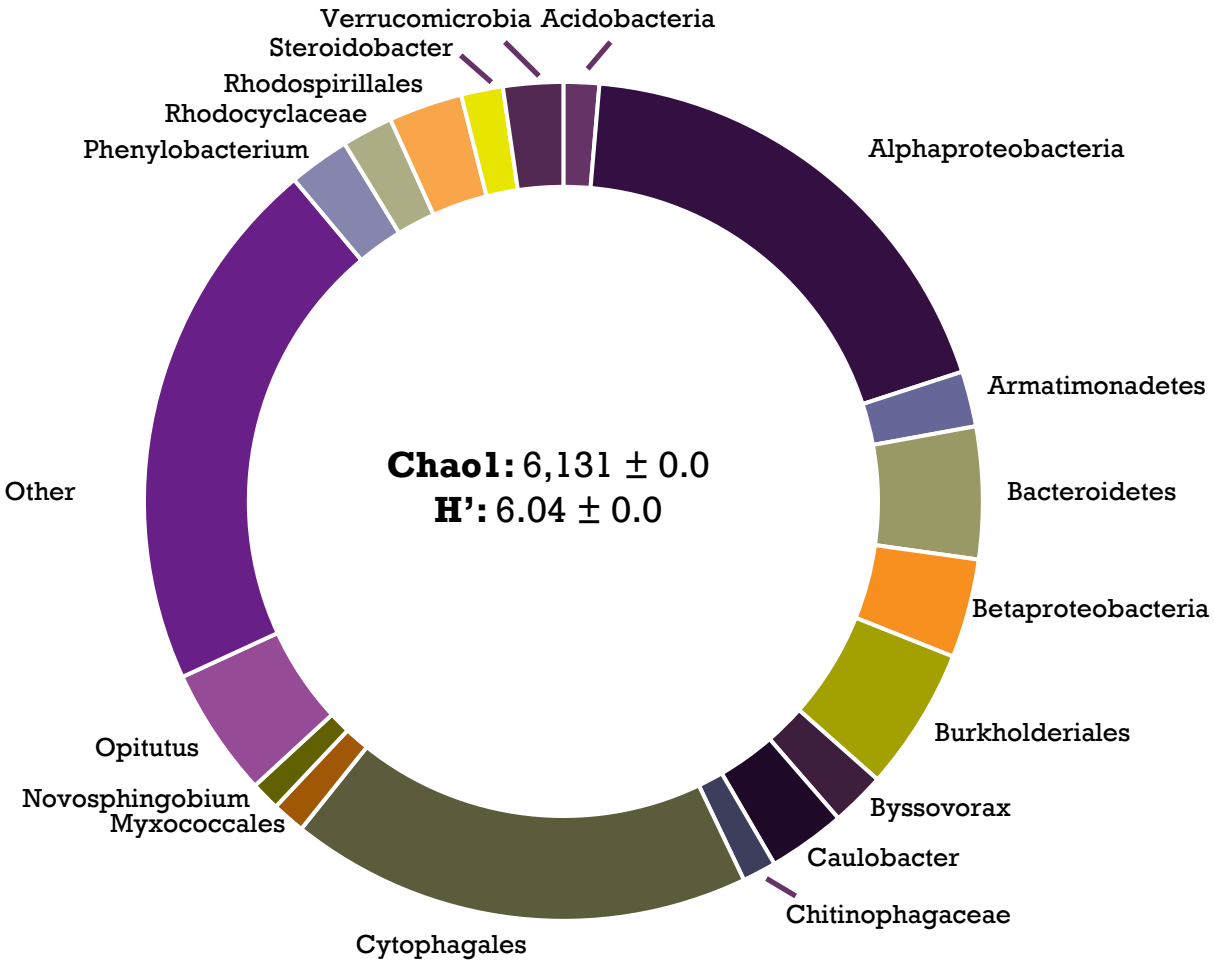
Zone 1 - Methanogenic Conditions: Attached Bacteria



Research Objective: BTEX Study

Results: Methanogenic Conditions

Zone 2 - Methanogenic Conditions: Attached Bacteria

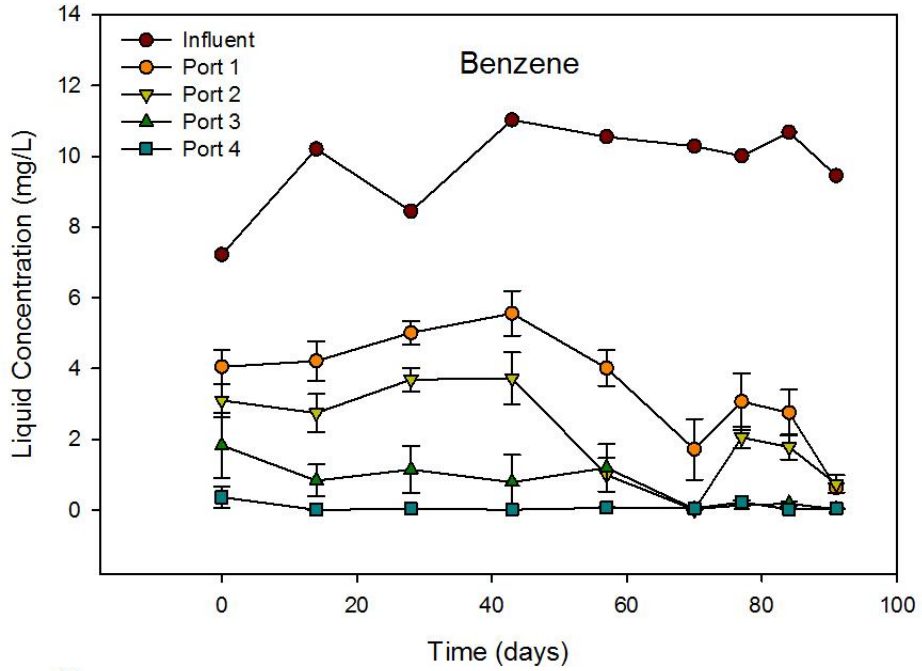


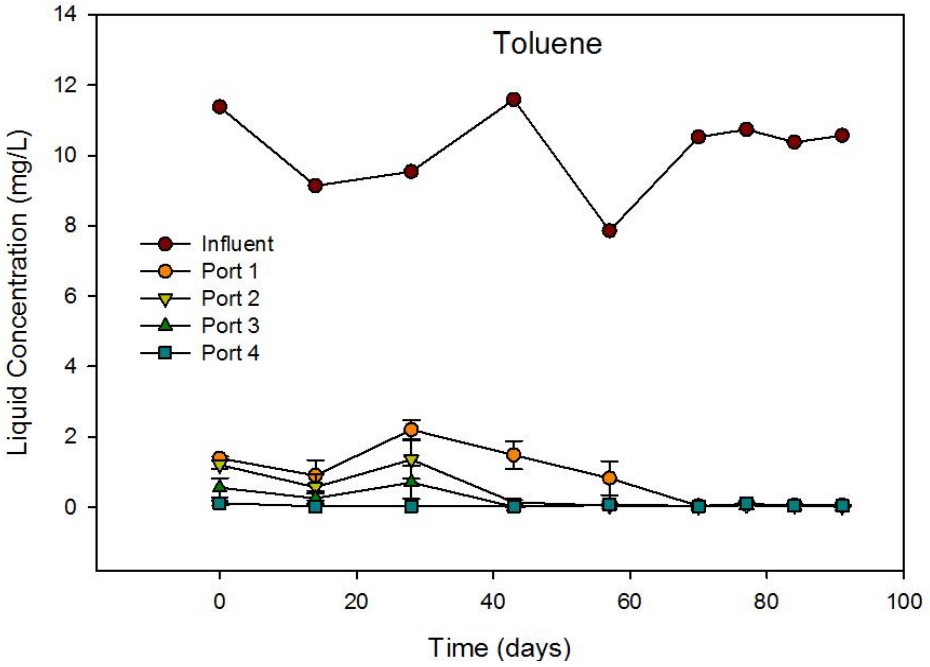
Research Objective: BTEX Study

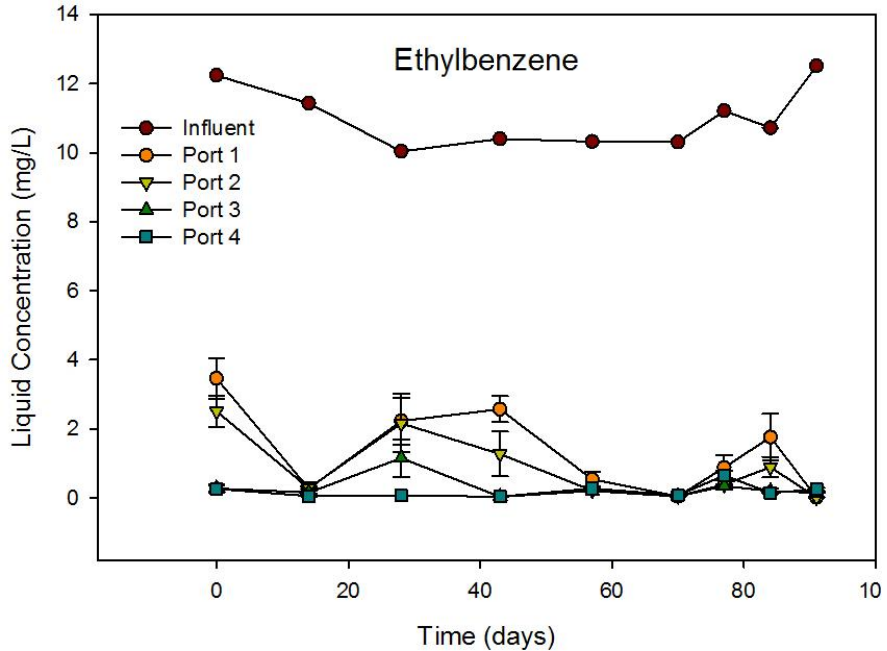
Example Bacteria

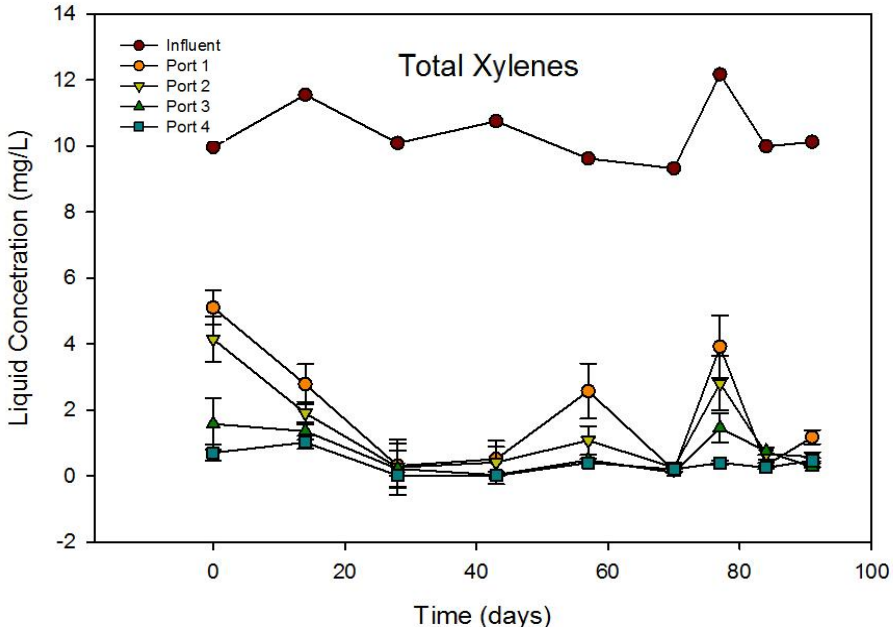
Organism	Classification	Metabolism
Azoarcus	Anaerobic	Denitrification
Opitutus	Anaerobic	Fermentation
Geobacter	Anaerobic	Iron reducer
Cytophagales	Facultatively anaerobic or aerobic	Various

Results: Sulfate-Reducing Conditions



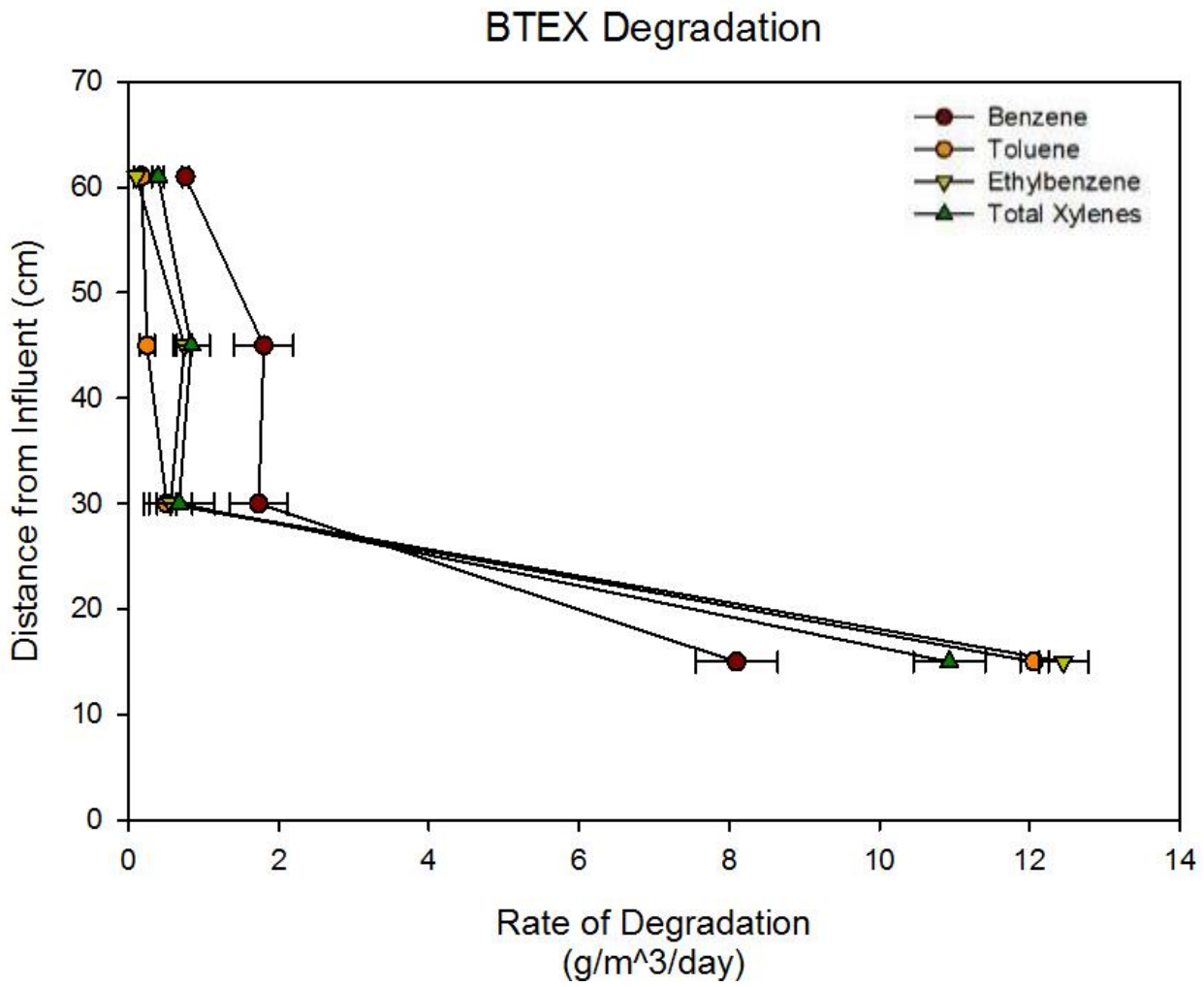






Research Objective: BTEX Study

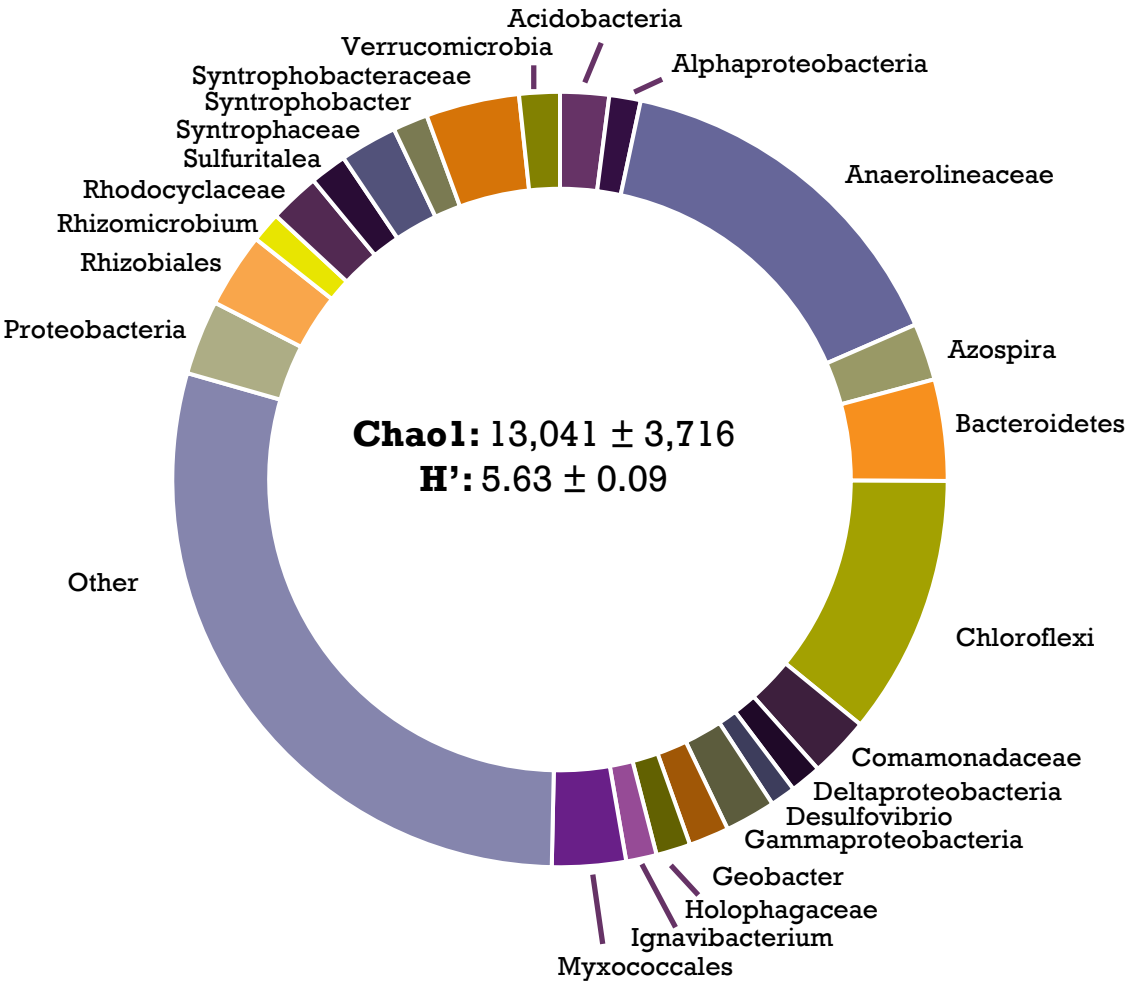
Results: Sulfate-Reducing Conditions



Rate of degradation of BTEX versus distance from the influent.

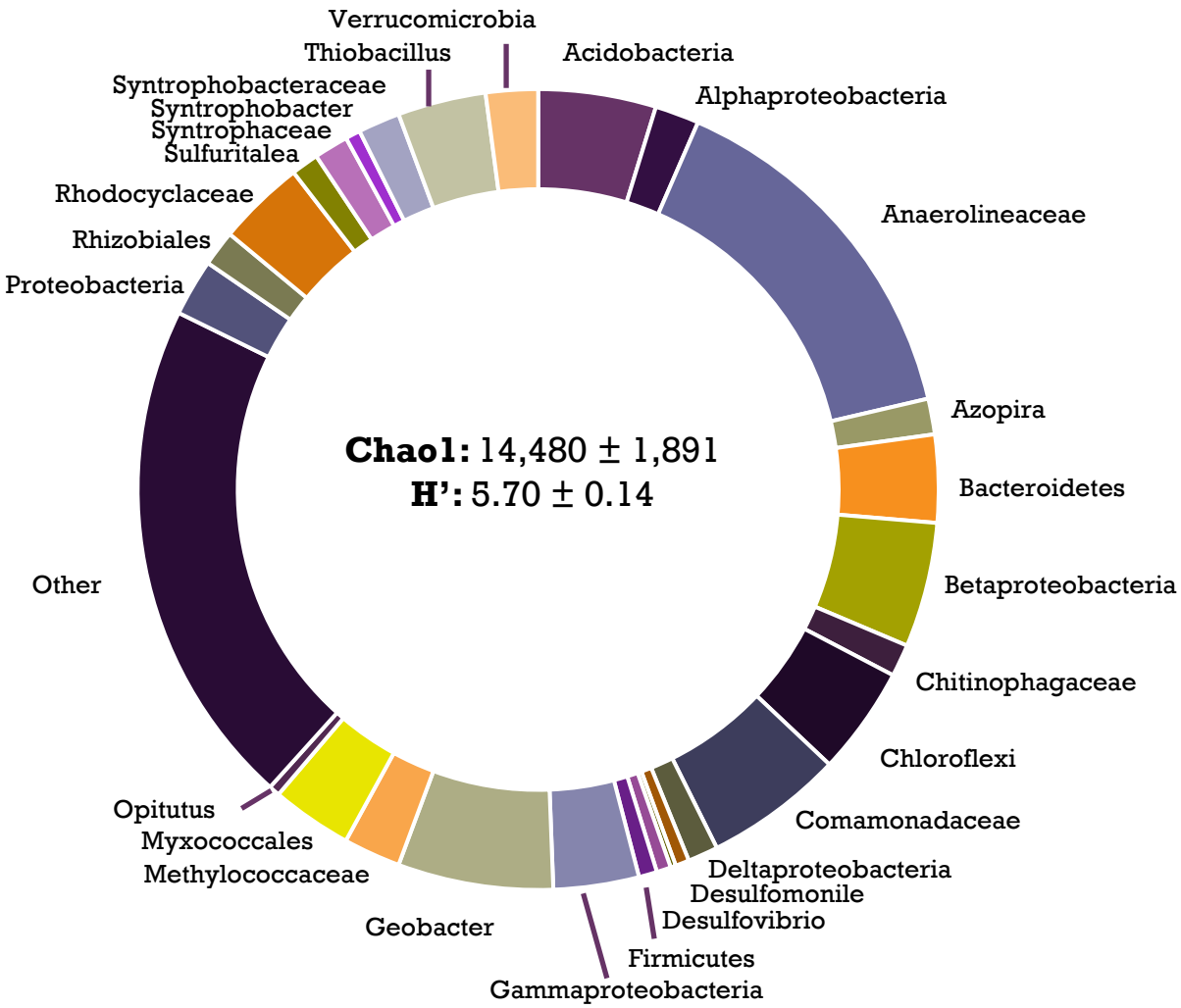
Results: Sulfate-Reducing Conditions

Zone 1 - Sulfate Reducing Conditions: Attached Bacteria



Results: Sulfate-Reducing Conditions

Zone 2 - Sulfate Reducing Conditions: Attached Bacteria



Example Bacteria

Organism	Classification	Metabolism
Sulfuritalea	Facultative anaerobes	Sulfate-reducer
Family Syntrophobacteraceae	Anaerobic	Sulfate-reducers
Geobacter	Anaerobic	various
Family Anaerolineaceae	Obligate anaerobes	Sulfate-reducers

Where are the BTEX degraders?

- Sequences of *Geobacter, Azoarcus,* and *Sulfuritalea* detected in attached populations using Illumina Mi-Seq
- *Proteobacteria* represent 45.5% of sequences in Phase 1 and 37.6% in Phase 2. Genera include *Rhodocyclaceae, Comamonadaceae,* and *Anaerolineaceae.*
- Known anaerobic hydrocarbon degraders account for 4-7% in the methanogenic phase
- Known sulfate-reducers represent ~24% in the sulfate-reducing phase

Results: Statistical Analysis of Microbial Communities between Treatment Phases

Zone 1 Comparisons of bacteria

Zone 1 Comparison			Methanogenic Conditions	Sulfate-Reducing Conditions	p-value
Average Values	N_{seqs}		14,398 ± 3,365	68,017 ± 21,583	**0.0238***
	0.03	S_{obs}	2,351 ± 340	4,539 ± 1,039	**0.0496***
		Chao1	8,184 ± 1,395	13,041 ± 3,716	0.1846
		H'	5.65 ± 0.13	5.63 ± 0.09	0.8660
	0.05	S_{obs}	1,662 ± 203	3,035 ± 619	**0.0416***
		Chao1	4,394 ± 707	7,324 ± 1,794	0.1121
		H'	5.32 ± 0.13	5.31 ± 0.09	0.9665

Zone 2 Comparisons of bacteria communities

Zone 2 Comparison			Methanogenic Conditions	Sulfate-Reducing Conditions	p-value
Average Values	N_{seqs}		11,027 ± 0.0	79,443 ± 13,818	**0.0248***
	0.03	S_{obs}	2,105 ± 0.0	4,949 ± 657	**0.0379***
		Chao1	6,131 ± 0.0	14,480 ± 1,891	**0.0354***
		H'	6.04 ± 0.0	5.70 ± 0.14	0.1557
	0.05	S_{obs}	1,546 ± 0.0	3,341 ± 405	**0.0349***
		Chao1	3,728 ± 0.0	8,503 ± 904	**0.0202***
		H'	5.67 ± 0.0	5.40 ± 0.13	0.2198

Research Objective: BTEX Study

Design Implications: Conclusions

- VOC sorption on organic matter increases residence time to accomplish biodegradation in smaller volume
- Benzene not degraded under methanogenic conditions in Zone 1, until the addition of sulfate
- Through addition of 500 mg/L of SO_4^{2-}, into the same ABR columns, immediate (~90 days) change to the microbial population and the performance pattern of the system

Questions?

Thank you!