Development of an Amendment Recipe and Identification of Benzene Degraders for Anaerobic Benzene Bioremediation

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Outline

1 Introduction

2 Objectives

3 Materials and Methods

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1 Introduction

- Petroleum hydrocarbons (PHC) release into soil and groundwater occur at every stage of oil extraction, refinement, storage, transportation, and disposal.
- Benzene contamination induced by PHC release is a major concern.
  - Highly water soluble (i.e., solubility of 1,791 mg/L at 25°C)
  - Volatile
  - Group A human carcinogen
  - A maximum acceptable concentration of 5 µg/L for benzene in drinking water

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1 Introduction

- Benzene is readily biodegradable under aerobic conditions
- Anaerobic subsurface environmental development
  - Fast oxygen consumption rate
  - Slow oxygen supply rate
- Anaerobic reducing conditions typically predominate
- Anaerobic intrinsic benzene biodegradation
  - Occurs very slowly
  - Incomplete
  - Long lag time

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1 Introduction

- Enhanced anaerobic bioremediation (EAB) is a practical and cost effective PHC remediation method
  - Biostimulation: addition of nutrients and electron acceptors
  - Bioaugmentation: addition of microorganisms
- Success of biostimulation
  - An effective configuration of nutrients and electron acceptors
  - A community structure of dominant microbes
- Understanding the indigenous microbial community is also critical for bioaugmentation

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2 Objectives

- To establish optimal chemical composition of nutrients and electron acceptors
- To identify dominant benzene-degrading consortium using molecular approaches

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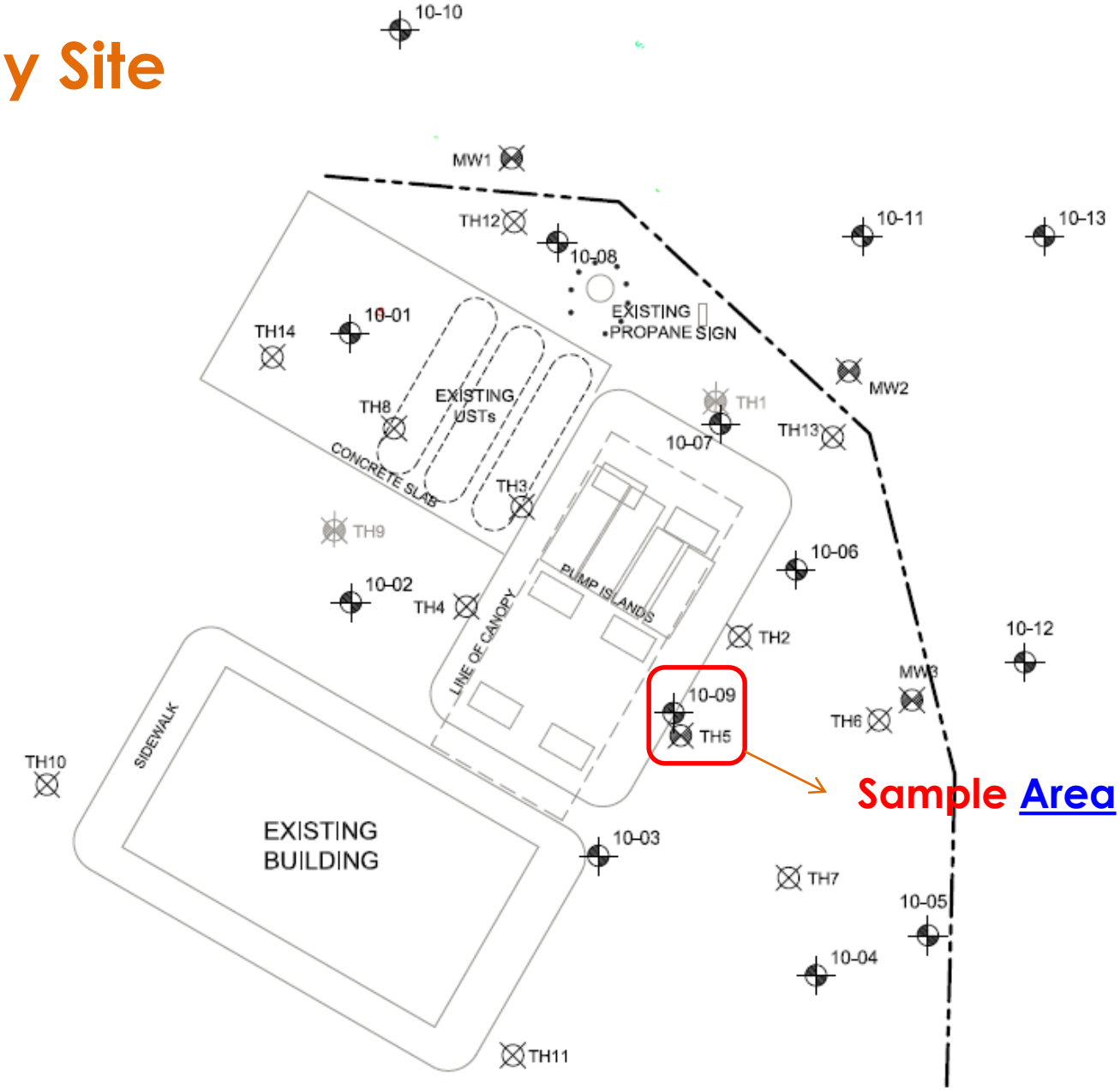
Study Site



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Soil Characteristics

Parameters	Value
Benzene (mg/kg)	**50**
Toluene (mg/kg)	10.3
Ethylbenzene (mg/kg)	251
Xylenes (mg/kg)	1080
Petroleum Hydrocarbon F1 (mg/kg)	9680
Petroleum Hydrocarbon F2 (mg/kg)	2990
Petroleum Hydrocarbon F3 (mg/kg)	120

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Groundwater Characteristics

Parameters	Value
Temperature (°C)	11.13
pH	6.59
Electrical Conductivity (µs/cm)	8.13
Oxidation Reduction Potential (mV)	– 40
Dissolved Oxygen (mg/L)	0.56
Nitrate (mg/L)	1.24
Phosphate (mg/L)	0.02
Sulphate (mg/L)	14.8
Benzene (mg/L)	**10.7**
Toluene (mg/L)	1.12
Ethylbenzene (mg/L)	3.18
Xylenes (mg/L)	3.1
Petroleum Hydrocarbon F1 (mg/L)	15
Petroleum Hydrocarbon F2 (mg/L)	4.72
Petroleum Hydrocarbon F3 (mg/L)	0.051

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Amendment Recipes

Recipe	Media Composition (each litre)	Benzene Concentration (mg/L)
NP	3.5 g $NaNO_3$, 0.3 g K_2HPO_4, 1.49 mg $FeCl_2 \cdot 4H_2O$	25
Coates	4.0 g Na_2SO_4, 1.36 g sodium acetate, 20 g NaCl, 0.5 g KCl, 0.2 g KH_2PO_4, 0.25 g NH_4Cl, 0.15 g $CaCl_2 \cdot 2H_2O$, 3 g $MgCl_2 \cdot H_2O$, 1.49 mg $FeCl_2 \cdot 4H_2O$	25
Kazumi	2.84 g Na_2SO_4, 1.3 g KCl, 0.2 g KH_2PO_4, 23 g NaCl, 0.5 g NH_4Cl, 0.1 g $CaCl_2 \cdot 2H_2O$, 1 g $MgCl_2 \cdot H_2O$, 2.5 g $NaHCO_3$, 1.49 mg $FeCl_2 \cdot 4H_2O$	17
SA	2.7 g K_2SO_4, 0.5 g KNO_3, 1 g KH_2PO_4, 0.5 g NH_4Cl, 0.14 g $CaCl_2.2H_2O$, 1 g $MgCl_2.6H_2O$, 2.5 g $NaHCO_3$, 2 mg $FeCl_2.4H_2O$	17
SA+T	10 mg toluene 2.7 g K_2SO_4, 0.5 g KNO_3, 1 g KH_2PO_4, 0.5 g NH_4Cl, 0.14 g $CaCl_2.2H_2O$, 1 g $MgCl_2.6H_2O$, 2.5 g $NaHCO_3$, 2 mg $FeCl_2.4H_2O$	17
SA+BP	25 mg benzoate, 35 mg phenol 2.7 g K_2SO_4, 0.5 g KNO_3, 1 g KH_2PO_4, 0.5 g NH_4Cl, 0.14 g $CaCl_2.2H_2O$, 1 g $MgCl_2.6H_2O$, 2.5 g $NaHCO_3$, 2 mg $FeCl_2.4H_2O$	17

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Microbial Analysis

- BioSep BioTrap pre-amended with [^{13}C] benzene
- Soil, groundwater, BioSep BioTrap was incubated with recipe SA+T
- Analysis of [^{13}C] of dissolved inorganic carbon
- Analysis of fatty acid methyl esters (FAMEs)
- Analysis of real-time quantitative polymer chain reaction (qPCR)
- Analyses of denaturing gradient gel electrophoresis (DGGE) and 16S rRNA

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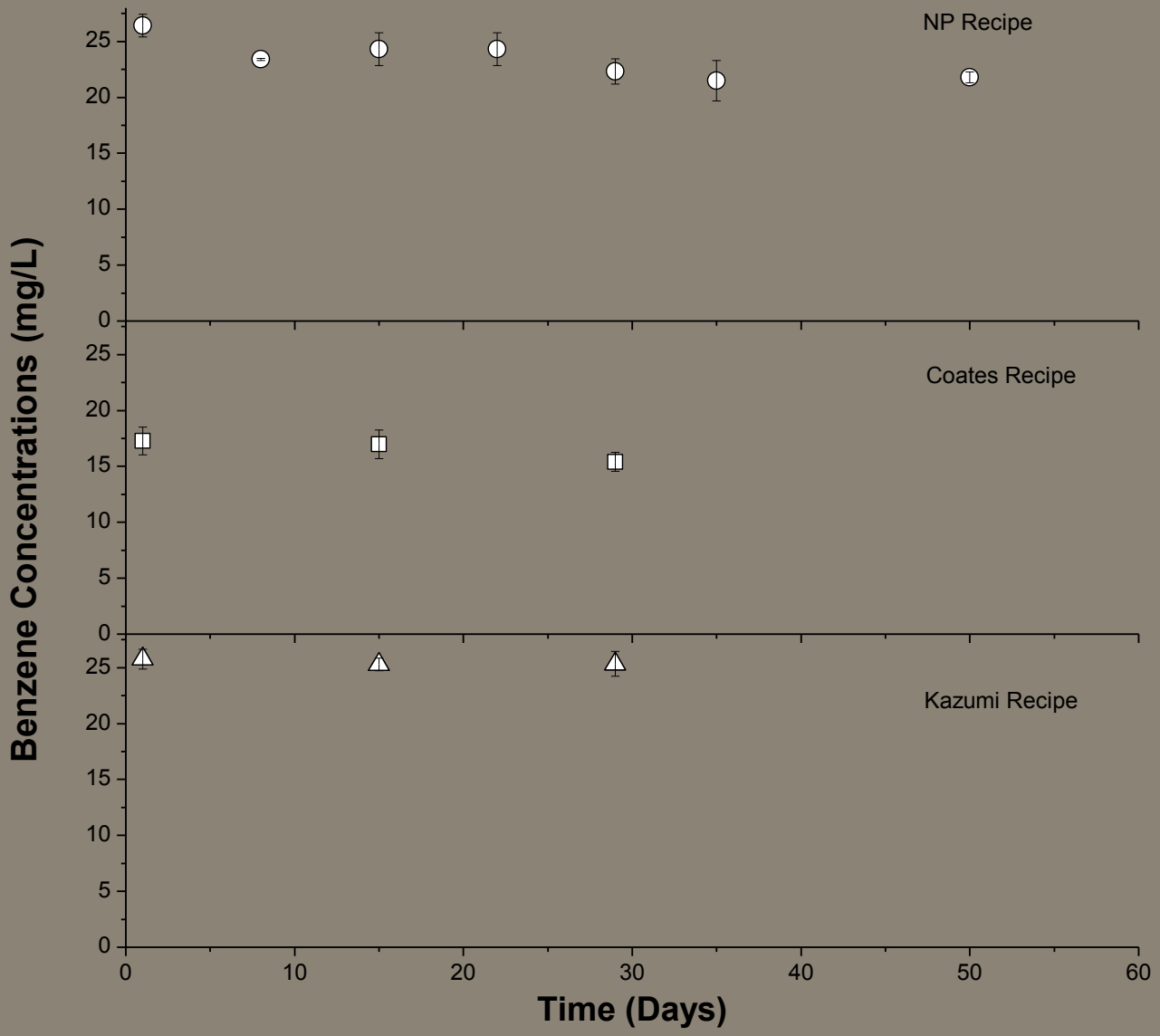
4 Results and Discussion

Recipes NP, Coates, and Kazumi



- Benzene was degraded only slightly in microcosms incubated with Recipe NP, Coates, and Kazumi.
- Salinity, high initial benzene concentration, alternative carbon source, and an extended lag time may result in the low benzene degradation rate.

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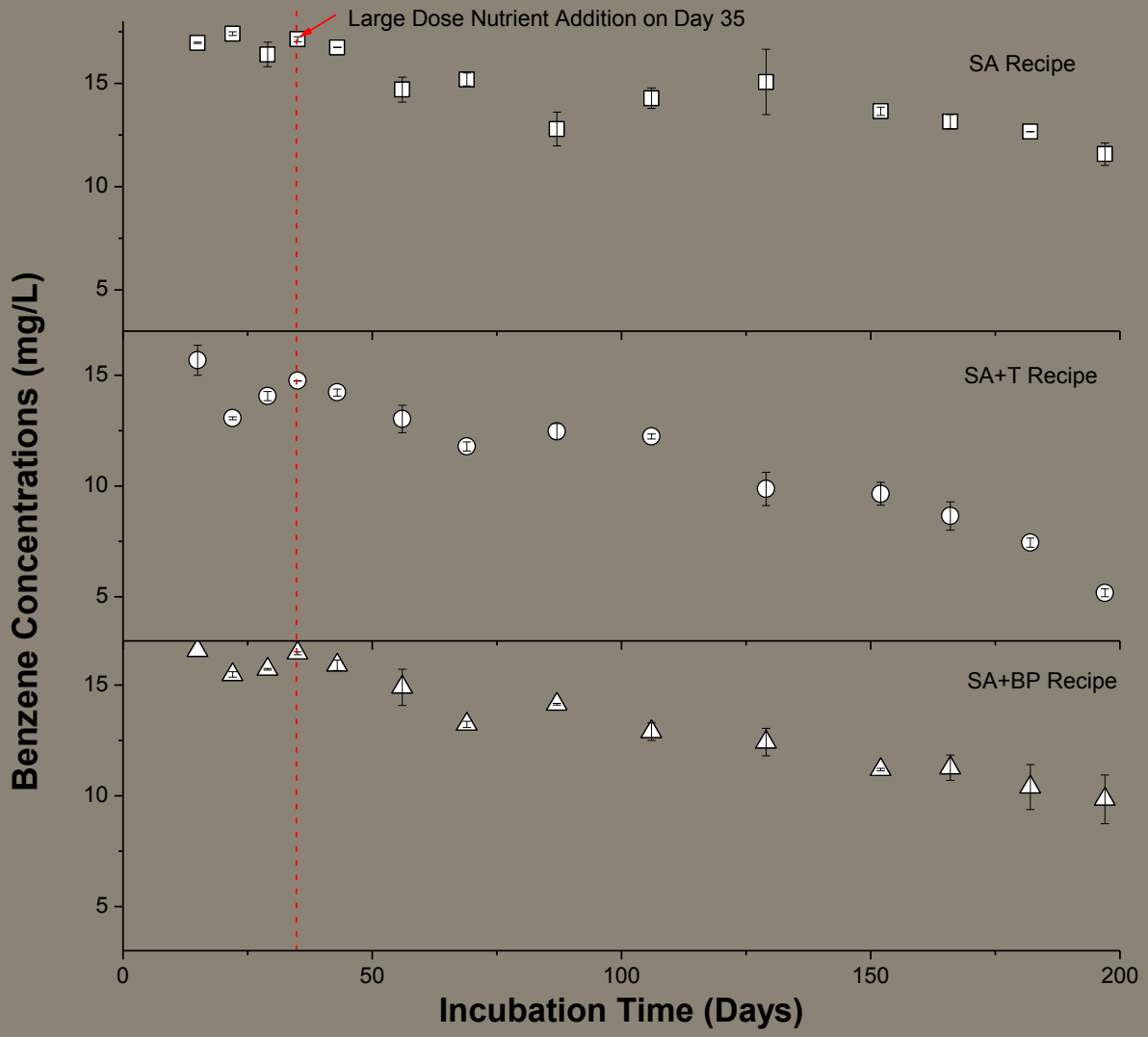
4 Results and Discussion

Recipes SA, SA+T, SA+BP



- The lag time was overcome by the large-dose addition of the amendments.
- Recipe SA+T with toluene amendment achieved the highest benzene degradation rate.

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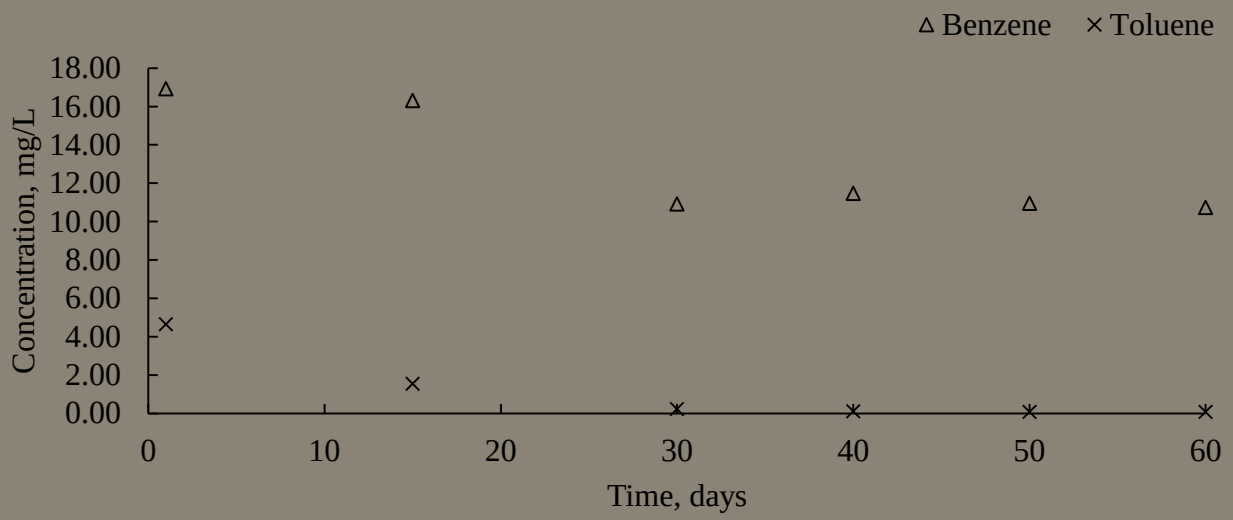
4 Results and Discussion

Toluene's Role on Benzene Biodegradation



- Both toluene and benzene were degraded effectively in a rapid manner during the first 30 days.
- After 30 days, benzene degradation stopped with the toluene depletion.

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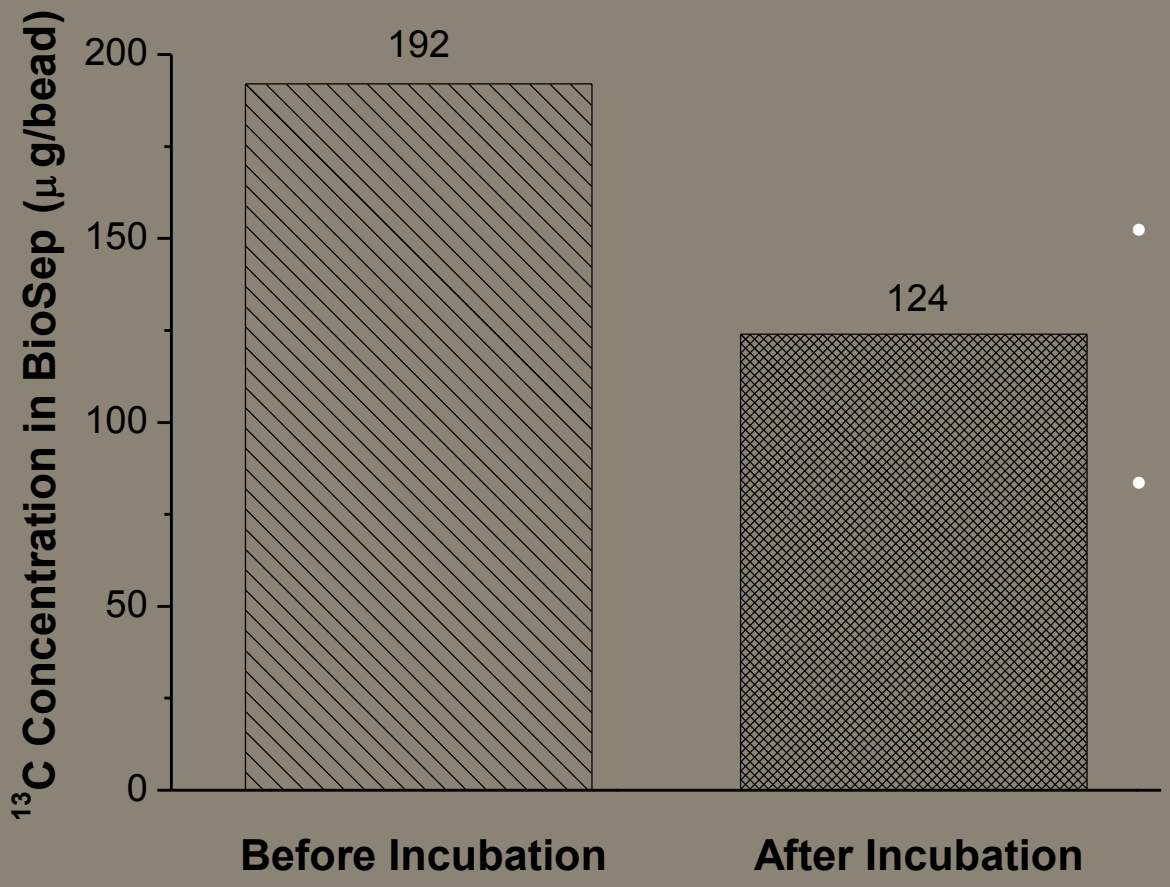
4 Results and Discussion

[^{13}C] Concentrations on BioSep Beads



- Degradation rate of [^{13}C] benzene was calculated to be 35.42%.
- BioSep beads could support microorganism growth.

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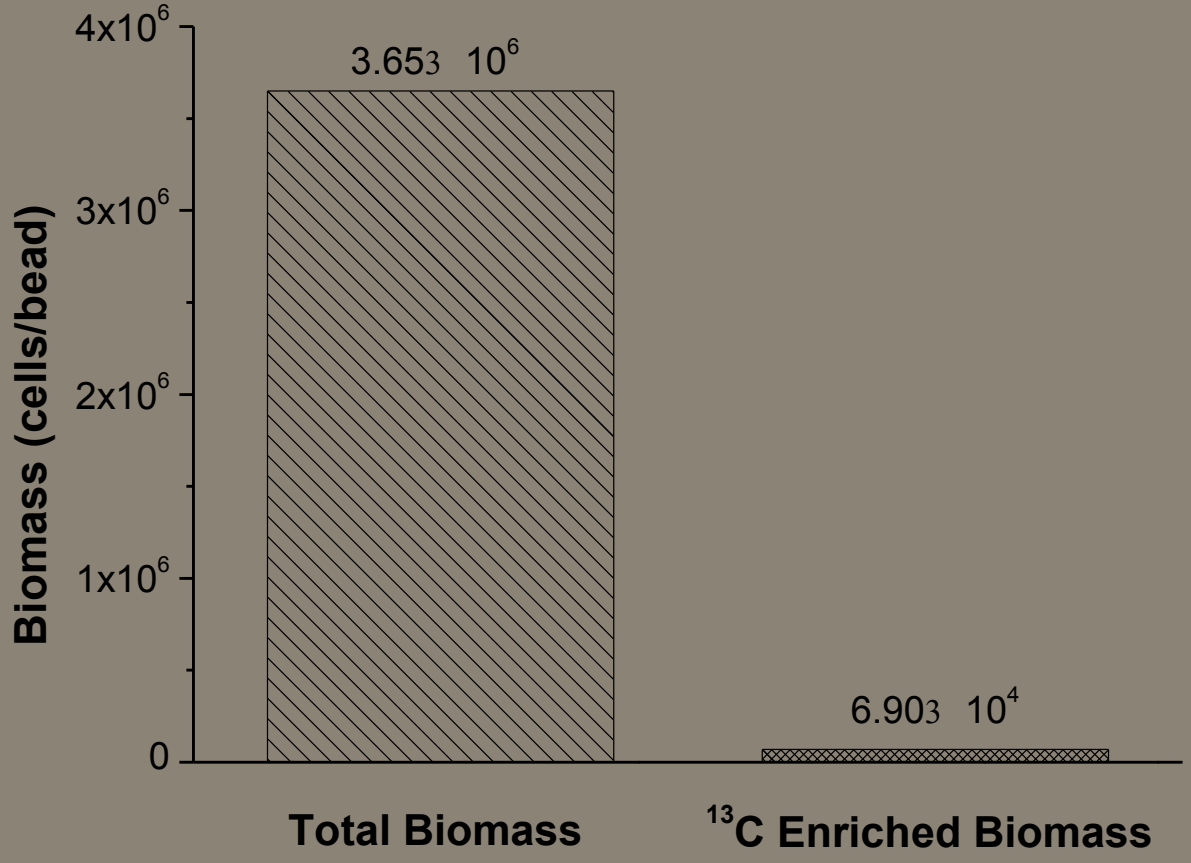
4 Results and Discussion

Total and [^{13}C]-enriched biomass



- [^{13}C] benzene incorporation into biomass
- Biodegradation of [^{13}C]-benzene

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4 Results and Discussion

Microbial Community Structure

Community Structure	% of Total PLFA
Proteobacteria (Monos)	63.1
General (Nsats)	32.9
Firmucutes (TerBrSats)	2.8
Anaerobic metal reducers (BrMonos)	1.1
Actinomycetes (MidBrSats)	0.2
Eukaryotes (Polyenoics)	0.0

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4 Results and Discussion

qPCR Analysis

Functional Genes	Gene Copy Numbers
Benzene carboxylase (abcA)	<50
Benzylsuccinate synthase (bssA)	<50
nirS	2.01×10^{6}
nirK	3.05×10^{7}
Phylogenetic Group	
APS	5.13×10^{2}

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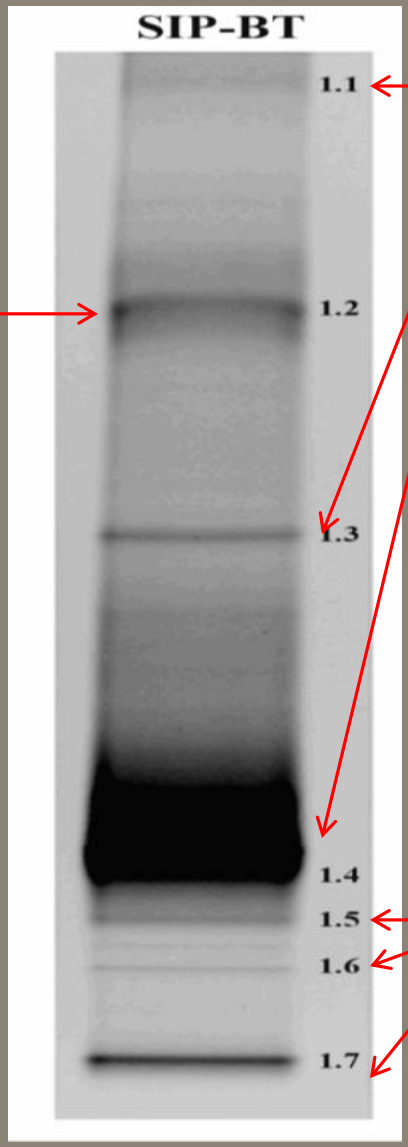
4 Results and Discussion

DGGE Image



Pusillimonas spp.

Advenella spp.

Dokdonella spp.

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4 Results and Discussion

16S rRNA Analysis

Band	Similar Genus	Similarity Index	Affiliation	GenBank Accession Number
1.1	*Pusillimonas* spp.	0.926	Betaproteobacteria; Alcaligenaceae	FN667020.1
1.2	*Advenella* spp.	0.880	Betaproteobacteria; Alcaligenaceae	KC464861.1; KC207092.1; JQ799008.1
1.3	*Pusillimonas* spp.	0.990	Betaproteobacteria; Alcaligenaceae	KC464818.1; HQ326782.1; FN667020.1
1.4	*Pusillimonas* spp.	0.923	Betaproteobacteria; Alcaligenaceae	FM956659; FJ791048; GQ246953
1.5	*Dokdonella* spp.	0.980	Gammaproteobacteria; Xanthomonadaceae	JQ726695.1; JQ726692.1; JQ726691.1
1.6	*Dokdonella* spp.	0.970	Gammaproteobacteria; Xanthomonadaceae	JQ726695.1; JQ726692.1; JQ726691.1
1.7	*Dokdonella* spp.	0.876	Gammaproteobacteria; Xanthomonadaceae	AY921834.1

5 Lessons Leaned / Conclusions

- Salinity and a high initial concentration of benzene were detrimental for benzene biodegradation.
- A large dose of amendment can shorten the lag time for benzene biodegradation.
- Toluene was an essential co-substance for promoting benzene biodegradation.
- Incorporation of ^{13}C-benzene into microbes provided direct evidence for benzene biodegradation.
- Dominant mechanism for benzene biodegradation was nitrate reduction in this study.
- *Dokdonella* spp., *Pusillimonas* spp., and *Advenella* spp. were predominant in the microbial community and involved in anaerobic benzene bioremediation.

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Thank you

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