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From engineering iconic skyscrapers, to using microbes to clean up hazardous waste - and everything in between.

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Generating Definitive Data for Biodegradation:

Case Studies on Practical Use of Stable Isotope Probing

*Authors: Matthew Burns – WSP Boston
Christine Warford – WSP Minneapolis
Judy Andrews – WSP Minneapolis
Daniel Liwicki – WSP Denver*

Stable Isotope Probing (SIP)

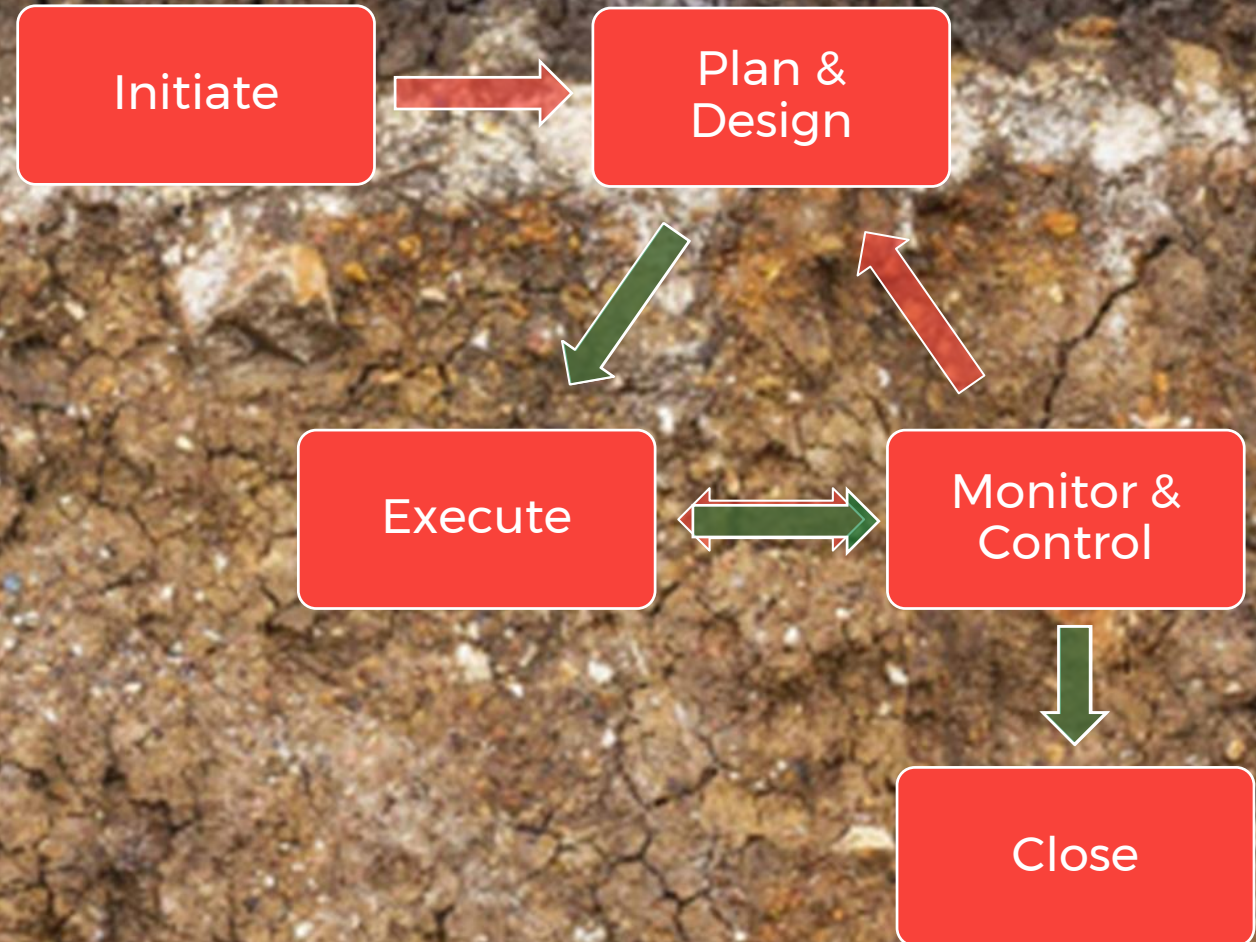
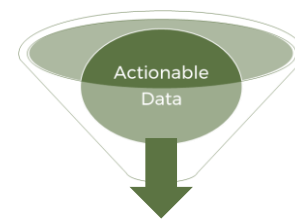
Why use SIP

How does it work

When to use - 3 practical examples

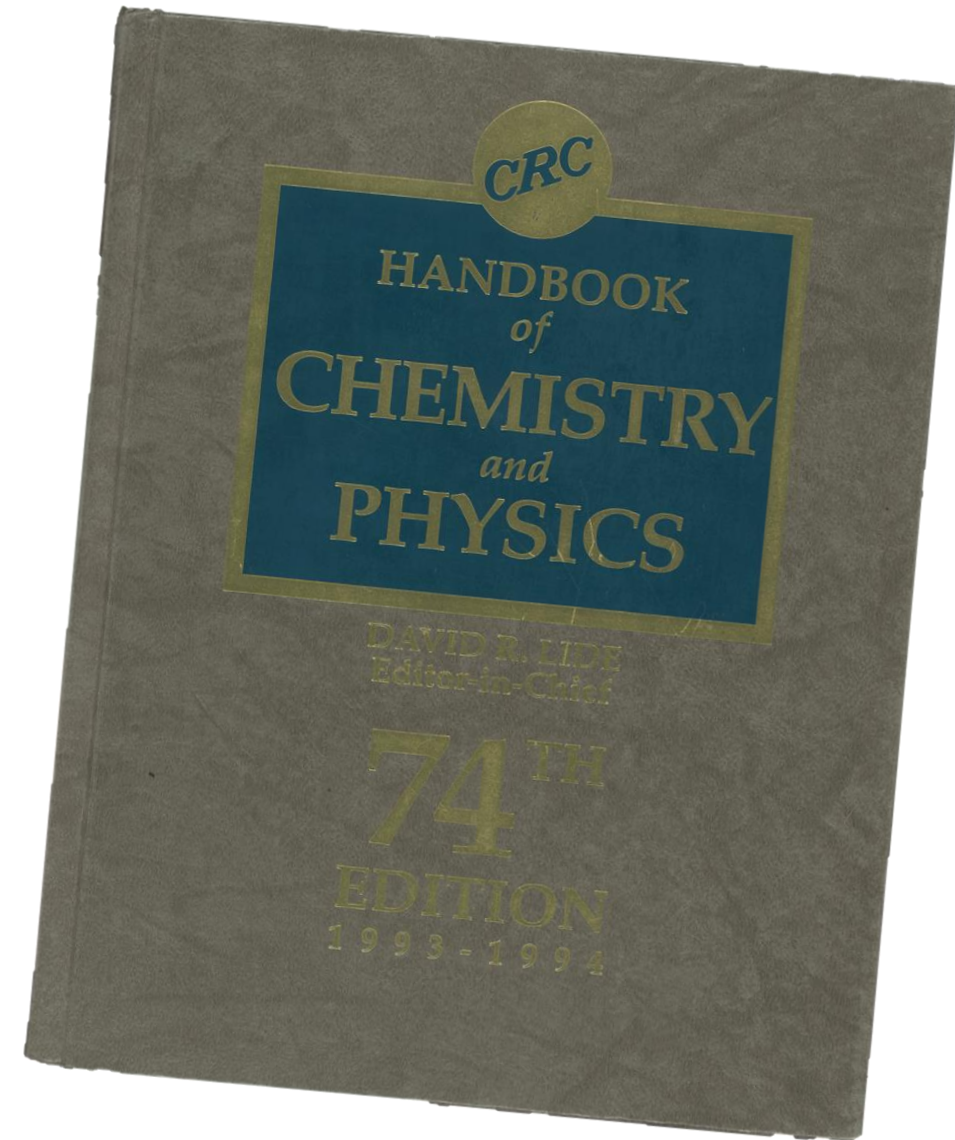
WHY

Actionable Data:



Actionable Data:

4



Actionable Data:

5

What Matt Calls Fail Small/Win Big

Definition: Actionable data that defines efficacy and/or mechanism of contemplated treatment strategy performed using site media at sub full-scale

Bench-Scale: *Ex situ*

- Matt typically uses for abiotic (ISCO/ISCR) treatment options
- Estimate amendment dose
- Confirm stability across varying conditions
- Identify undesirable intermediates
- Check for full-scale safety concerns (e.g., heat, off gassing, etc)

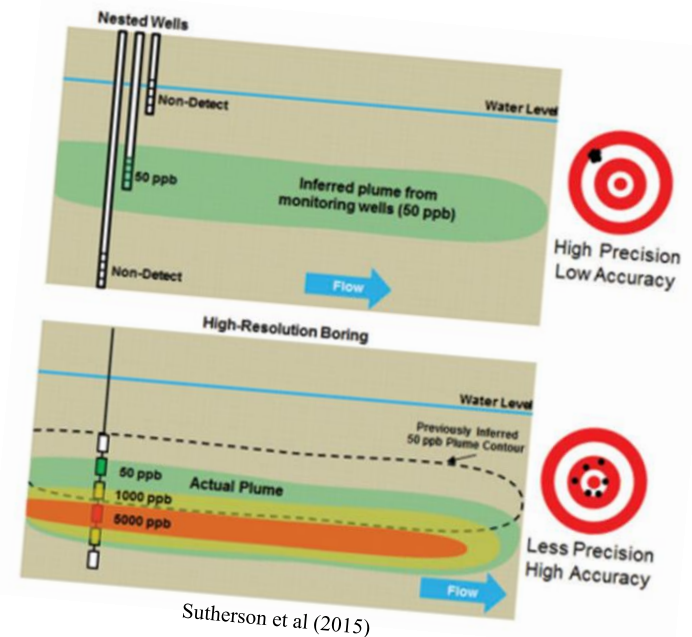


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Actionable Data

Definition: Any data collection activity specifically planned to define project direction

Example:



7-5

HOW

Stable Isotope Probing

stable isotope probing (SIP):

not applicable for contaminants used
as electron acceptor
(other tools are available)



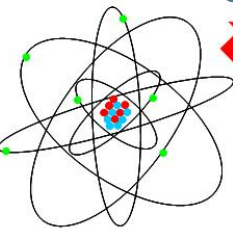
The SIP Label

HELLO
my name is

^{13}C enriched
(~ 10 – 100 %)
manufactured
contaminant

^{13}C enriched contaminant
loaded on a bio-trap
in situ microcosm

incubate in site
well (30 to 90 days)



^{13}C Stable Isotope
Natural Abundance ~ 1%



CSIA used to track ^{13}C enrichment in
biomass and dissolved inorganic carbon

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WHEN

Groundwater Treatment Case Studies

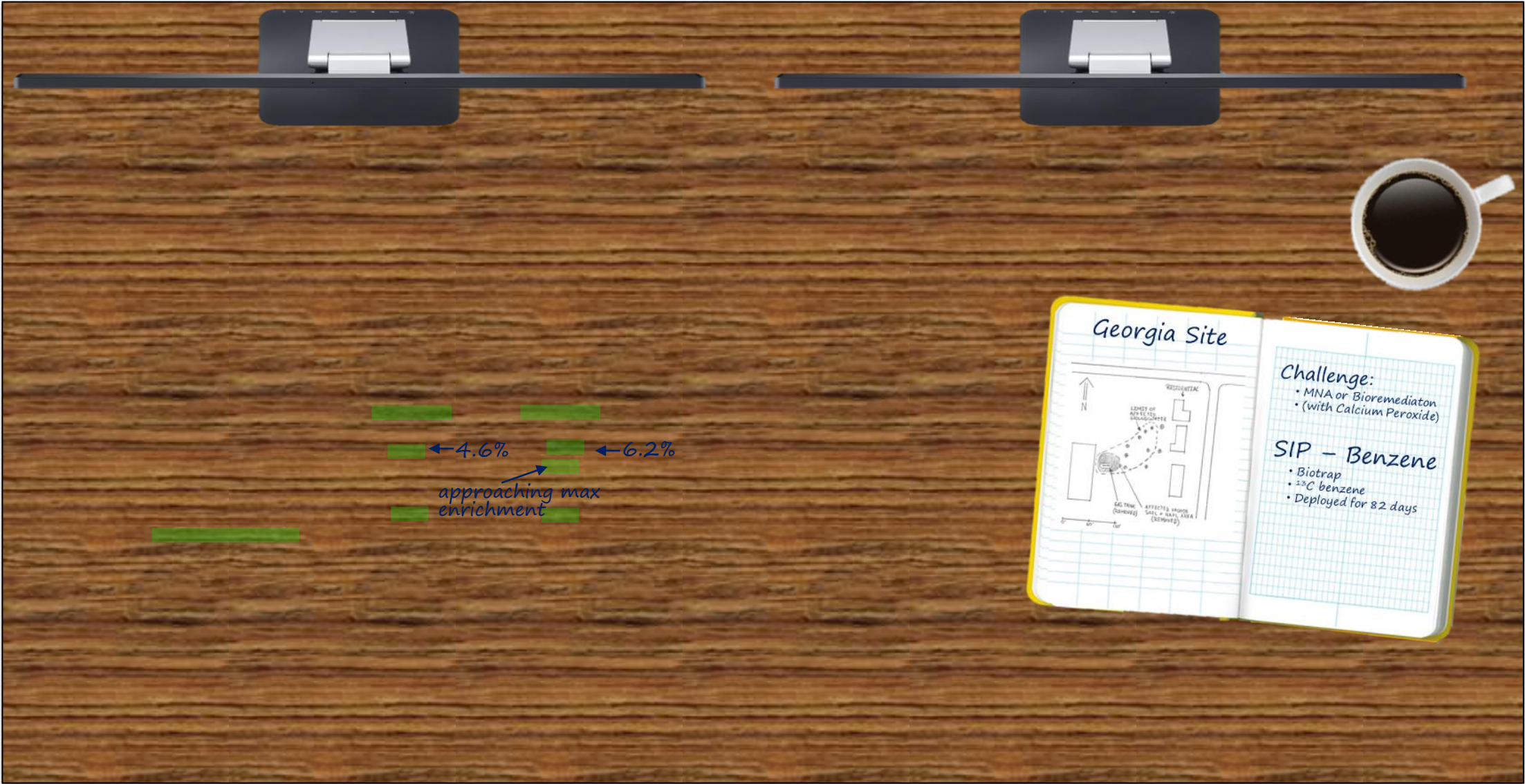
Case Study 1 – MNA vs Enhanced Aerobic Treatment of Benzene

Case Study 2 – Bio Contribution of a Chem/Bio Treatment Train of Benzene

Case Study 3 – Stimulating Multiple Degradation Pathways for the Treatment of Chlorobenzenes

Case Study 1: MNA vs Enhanced Aerobic Treatment of Benzene

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Case Study 1: MNA vs Enhanced Aerobic Treatment of Benzene

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Results

Table 1. Summary of document.

Sample Name
Contaminant Loss
¹³ C Benzene Pre-deploy
¹³ C Benzene Post-deploy
Biomass & ¹³ C Incorporation
Total Biomass (Cells/bd)
¹³ C Enriched Biomass (C)
Average PLFA Del (%)
Maximum PLFA Del (%)
¹³ C Mineralization
DIC Del (%)
% ¹³ C
Community Structure
Firmicutes (TerBrSats)
Proteobacteria (Monos)
Anaerobic metal reducers
Actinomycetes (MidBrSat)
General (Nsats)
Eukaryotes (Polyenols)
Physiological Status (Pro)
Slowed Growth
Decreased Permeability

Figure 1

PFLA-SIP Monoenoic Biomarker ¹³C Enrichment Trends

pmol/bead

PFLA Monoenoic Biomarkers

increase in ¹³C abundance:

- 15:1w6c,
- 17:1w8c,
- CY17:0,
- 18:w9c
- Cy19:0

Georgia Site

Challenge:

- MNA or Bioremediation
- (with Calcium Peroxide)

SIP – Benzene

- Biotrap
- ¹³C benzene
- Deployed for 82 days

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Case Study 1: MNA vs Enhanced Aerobic Treatment of Benzene

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MEMO

TO: Georgia Site Project Team

FROM: Matt Burns

SUBJECT: SIP Results

DATE: May 24, 2017

The stabile isotope probing (SIP) study for the Georgia site provided the information listed below:

Both Remedial Options Are Viable

- ✓ ¹³C-benzene loss from both the MNA and stimulated biotrap
- ✓ ¹³C enriched biomass in both biotrap
- ✓ ¹³C enriched dissolved organic carbon (DIC) in both biotrap
- ✓ High abundance of proteobacteria, a phylum containing benzene-degrading bacteria

Aerobic Microbes More Efficient

- ✓ Greater incorporation of ¹³C into biomass and DIC
- ✓ Biomass approaching greatest possible enrichment (given the initial enrichment of 10%)
- ✓ Five proteobacteria biomarkers showed significant relative increase in ¹³C abundance (compared to MNA)

marker ds

increase in ¹³C abundance:

- 15:1w6c,
- 17:1w8c,
- CY17:0,
- 18:w9c
- Cy19:0

Challenge:

- MNA or Bioremediation
- (with Calcium Peroxide)

SIP – Benzene

- Biotrap
- ¹³C benzene
- Deployed for 82 days

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Boston, MA 02116
wsp.com

Matt Burns
Technical Fellow

Case Study 2: Bio Contribution of a Chem/Bio Treatment Train of Benzene

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Plan to Assess Bio Component

- Basic: benzene concentration trends
 - ISCO relatively quick – attribute degradation within first month
 - Post 1 month = bio
 - Correlate bio to decrease in sulfate concentrations
- Advanced Diagnostic – SIP
 - Apply amendment
 - Monitor for indicators that chemical oxidation complete (ORP & Sulfate)
 - When ORP negative, deploy ^{13}C benzene bated biotrap
 - Incubate for one month

Injection Area

Google earth
12/2013 Google

Legend

- 25-ft Radius
- Pilot Test Injection Point
- Temporary Monitoring Well
- UMW-6

UMW-6

PI-1

PTW-2

PTW-3

PTW-4

PI-2

PTW-1

60 ft

Site: Oklahoma

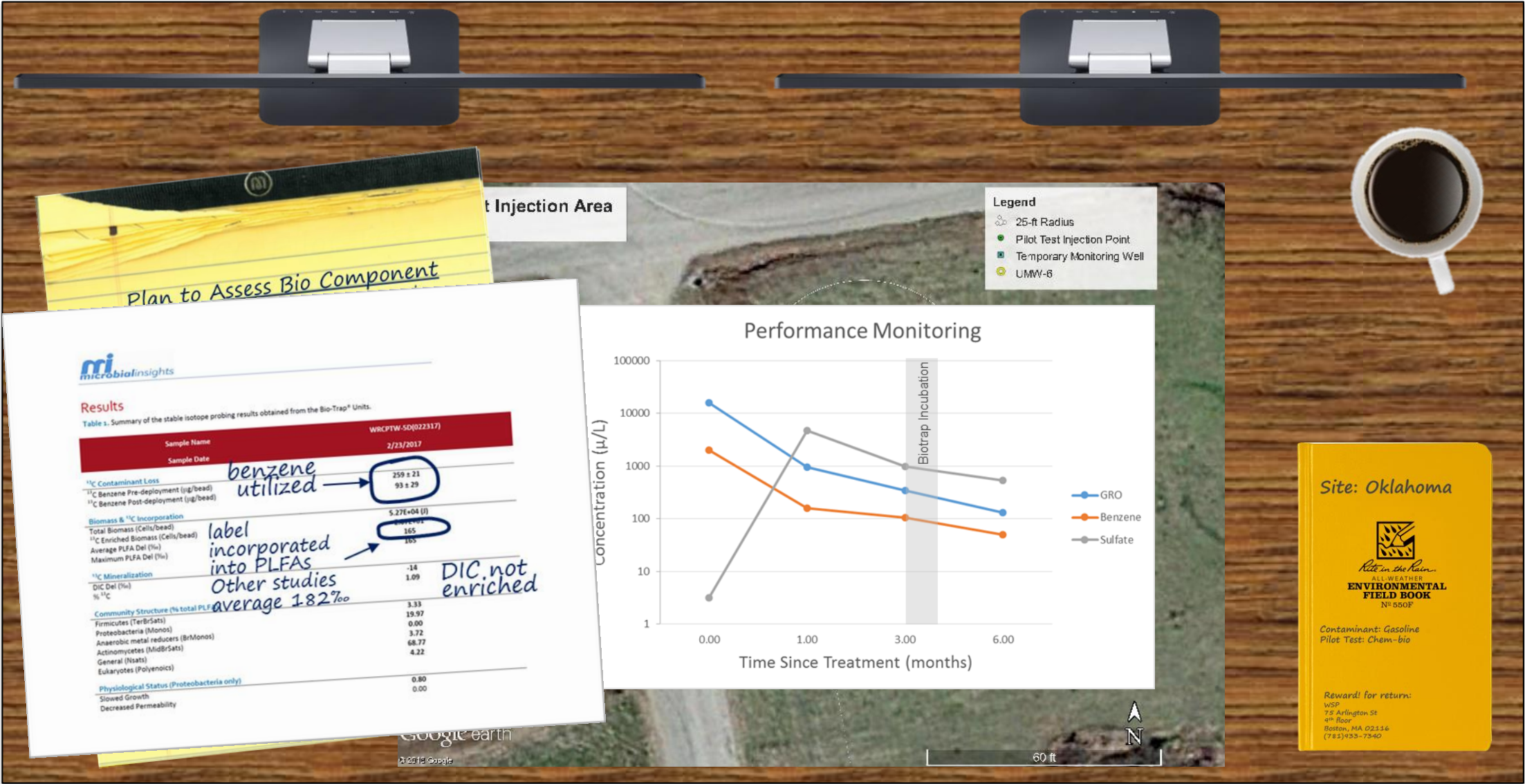


Contaminant: Gasoline
Pilot Test: Chem-bio

Reward! for return:
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Boston, MA 02216
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Case Study 2: Bio Contribution of a Chem/Bio Treatment Train of Benzene

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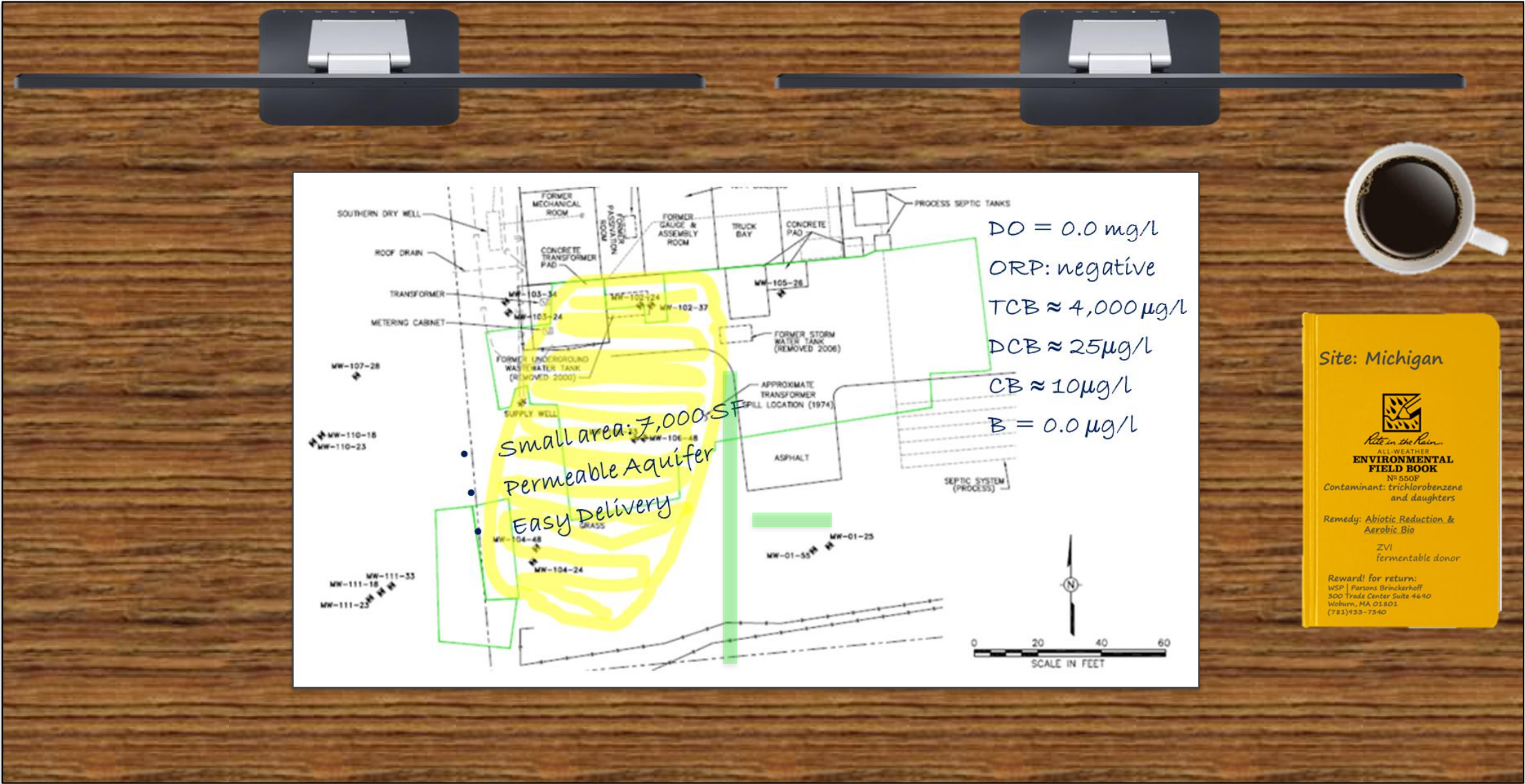


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Case Study 3: stimulating Multiple Degradation Pathways for the Treatment of Chlorobenzenes

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Case Study 3: stimulating Multiple Degradation Pathways for the Treatment of Chlorobenzenes

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- Combined reductive and anaerobic oxidation
- ✓ Repeat SIP study but trace label to DNA to identify attenuating microbes
 - ✓ Use 100% ^{13}C Chlorobenzene
 - ✓ Deploy 126 Days

small a
permea
easy!

Sensitive Detection of Anaerobic Monochlorobenzene Degradation Using Stable Isotope Tracers

IVONNE KILIANHUIS,^{1,*}
NICOLE STELZER,¹
MATTHIAS KÄSTNER,² AND
HANS-H. RICHNOM²

¹Department of Isotope Biogeochemistry (IGB) and
Biomineralization (BIOBM), UFT-Helmholtz Centre
Environmental Research, Postfach 101553,
50159 Leipzig, Germany

Microbial degradation of monochlorobenzenes under anaerobic conditions was investigated using isotope tracers under in situ conditions. Microcosms were incubated directly in an aquifer and carbon derived from ^{13}C -MCB was incorporated into the microbial biomass. In all microcosms, amended with ^{13}C -MCB, anaerobic mineralization of MCB was indicated by the ^{13}C recovery of the ^{13}C -label in the acids confirmed the assimilation of MCB-derived carbon into the microbial biomass. The described approach of concern using other organic groundwater of concern using carbon (^{13}C) as well as other isotope tracers, such as nitrogen (^{15}N), allows sensitive detection of biodegradation.

Introduction
Monochlorobenzene (MCB) is a widespread groundwater contaminant found at many former chlorobenzene chemical sites, particularly where chlorinated pesticides have been produced (www.who.int/whs). The Bitterfeld-Wolfen region in Germany is a former production site for Isodure (hexachlorocyclopentadiene) where today MCB is present in an ubiquitous concentration throughout the entire aquifer with concentrations up to 30 mg L^{-1} (1–3). While MCB contamination may be the result of a spillage event, it can also be produced in situ by sequential biodegradation or during the biodegradation of higher chlorinated benzenes (4–6). MCB is the most mobile chlorobenzene due to its relatively high solubility and low sorption potential to the aquifer matrix and thus, it is the most persistent, indicating its high persistence in anaerobic aquifers. Detaching water lines for MCB in the United States are currently set at $<0.1 \text{ mg L}^{-1}$ below the state site (groundwater) (1 $\mu\text{g L}^{-1}$), consistent with European Union standards. MCB has been well studied for a review see van Aartsen et al. (8). Complete anaerobic degradation and mineralization of MCB has been well studied for a review see van Aartsen et al. (8). Complete anaerobic degradation and mineralization of MCB has been well studied for a review see van Aartsen et al. (8). Complete anaerobic degradation and mineralization of MCB has been well studied for a review see van Aartsen et al. (8).

Anaerobic
Oxidation of
chlorobenzene
(Michigan Site?)

$\text{D}_2\text{O} = 0.0 \text{ mg/L}$
 DRP: negative
 $\text{TCB} \approx 4,000 \mu\text{g/L}$
 $\text{PCB} \approx 25 \mu\text{g/L}$
 $\text{CB} \approx 10 \mu\text{g/L}$
 $\text{B} = 0.0 \mu\text{g/L}$

Site: Michigan



ALL-WEATHER
ENVIRONMENTAL
FIELD BOOK

Nº 550F

Contaminant: trichlorobenzene
and daughters

Remedy: Abiotic Reduction &
Aerobic Bio

ZVI

fermentable donor

Reward! for return:
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300 Trade Center Suite 440
Woburn, MA 01801
(781) 933-7340

Case Study 3: stimulating Multiple Degradation Pathways for the Treatment of Chlorobenzenes

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- Combined reductive and anaerobic oxidation
- ✓ Repeat SIP study but trace label to DNA to identify attenuating microbes
 - ✓ Use 100% ^{13}C Chlorobenzene
 - ✓ Deploy 126 Days

Sensitive Detection of Anaerobic Monochlorobenzene Degradation Using Stable Isotope Tracers

YVONNE KILLENHUIS,^{1,2} NICOLE STELZER,¹ MATTHIAS KÄSTNER,¹ AND HANS-H. RICHNOW¹

¹Department of Isotope Biogeochemistry (IGB) and ²Environmental Geochemistry (IGEG), UFZ Helmholtz Center for Environmental Research, Leipzig, Germany

LAB REPORT

Site: Michigan Chlorbenzenes
Sample: MW-102
Procedure: SIP with 100% ^{13}C Chlorobenzene with DGGE

Band	Similar Genus	Similarity Index
^{13}C Natural Abundance Bands		
1.1	Bacteroides spp.	0.851
1.2	Geobacter spp.	0.811
1.3	Marinilabiaceae (family)	0.938
1.4	Acinetobacter spp.	0.986
1.5	Trichococcus spp.	0.912
1.6	Geobacter spp.	0.881
1.7	Trichococcus spp.	0.947
1.8	Bacteroidales (order)	0.887
^{13}C Enriched Bands		
2.1	Acinetobacter spp.	0.886
2.2	Trichococcus spp.	0.946

Similarity Index:
> 0.9 = excellent
0.7 - 0.8 = good
< 0.6 = unique sequences

Acinetobacter:
aerobic bacteria known to mineralize aromatic compounds.

Trichococcus:
Aerotolerant/facultative fermentative bacteria.

In recent years, stable isotope approaches have been developed to investigate natural attenuation of contaminants. Stable isotope fractionation analysis may be used to identify the degradation pathways of pollutants (1,2). Previously, evidence for the biodegradation of chlorobenzene was provided by the detection of stable isotope tracers (3,4). Recently, in situ microcosm (ISCT) studies of stable isotope tracers, were developed to investigate degradation of BTEX compounds in the subsurface (5).

Concurrent and Complete Anaerobic Reduction and Microaerophilic Degradation of Mono-, Di-, and Trichlorobenzenes

Matt Burns
Kerry L. Sublette
James Sobieraj
Dora Ogles
Stephen Koenigsberg

Two ^{13}C -labeled in situ microcosm studies were conducted to evaluate EAC-MP simulated degradation of mono-, di-, and trichlorobenzenes in anaerobic groundwater at a site in Michigan. The data show that the EAC-MP amendment stimulated an overall increase in microbial activity and a shift in the microbial community structure, indicating more reduced conditions. Stable isotope separation and characterization of the ^{13}C - and ^{15}N -labeled tracers, and subsequent analysis of the ^{13}C - and ^{15}N -labeled tracers, were used to identify the attenuating microbes. These data clearly show the participation of an obligate anaerobe in the chlorobenzene biodegradation process.

Due to the thermodynamically favorable reducing conditions stimulated by EAC-MP, the mechanisms of degradation of the trichlorobenzenes is presumed to be reductive dehalogenation. However, on the strength of the DGT-based analysis of microbial community structure, concurrent microaerophilic degradation of chlorobenzene or its metabolites was definitively demonstrated and cannot be ruled out for the other chlorobenzenes. © 2013 Wiley Periodicals, Inc.

INTRODUCTION

Mono-, di-, and trichlorobenzenes are derivatives of benzene with one, two, or three chlorine atoms substituted for hydrogen, respectively, which have a wide range of industrial, agricultural, and commercial applications. Accordingly, sites with chlorinated benzene-contaminated soil and groundwater are diverse and numerous with releases from equipment containing oils, lubricants, and degreasing fluid being among the most frequent releases. The US Environmental Protection Agency (USEPA) primary drinking water standards for chlorobenzenes vary by degree of chlorine substitution and configuration. Among the lower molecular weight chlorobenzenes, the drinking water maximum contaminant levels (MCLs) vary, with the 1,2,4-trichlorobenzene (70 micrograms/liter [pg/L]), 1,4-dichlorobenzene (75 pg/L), and chlorobenzene (100 pg/L) having the lowest criteria for each level of chloro substitution. In addition to human health concerns, chlorobenzenes have been shown to bioaccumulate (Makino et al., 2004).



Site: Michigan



ENVIRONMENTAL FIELD BOOK

No. 550F
Contaminant: trichlorobenzene and daughters

Remedy: Abiotic Reduction & Aerobic Bio

ZVI
fermentable donor

Reward! for return:
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(781) 933-7340

Case Study 3: stimulating Multiple Degradation Pathways for the Treatment of Chlorobenzenes

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Combine anaerobi
✓ Repea
label t
attenu
✓ Use 1
Chlor
✓ Deplo

wsp

MEMO

TO: Michigan Site Project Team
FROM: Matt Burns
SUBJECT: SIP Results
DATE: May 24, 2017

The stable isotope probing (SIP) study for the Michigan site provided the information listed below:

Chlorobenzene Microaerophilic Oxidation

- ✓ ^{13}C -chlorobenzene loss from the biotrap
- ✓ ^{13}C enriched DNA in biomass
- ✓ DGGE separation and sequencing identify attenuating organism as *Acinetobacter*
- ✓ *Acinetobacter*: aerobic bacteria known to mineralize

Concurrent Degradation of Mono-, Di-, and Trichlorobenzenes

- ✓ Microcosm amended with ZVI and fermentable donor which stimulated reduction of more chloro-substituted chlorobenzenes
- ✓ Concurrent microaerophilic oxidation of monochlorobenzene
- ✓ Similar to vinyl chloride oxidation postulated by Gossett

Matt Burns
Technical Fellow

Sensitive Detection of Anaerobic Monochlorobenzene Degradation Using Stable Isotope Tracers

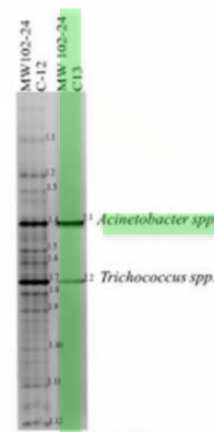
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²Department of Earth and Atmospheric Sciences, University of Toronto, 127 St. George Street, Toronto, Ontario M5S 1A5, Canada

ABSTRACT

INTRODUCTION

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a known to mineralize
ounds.

reducing/fermentative
bacteria.

Concurrent and Complete Anaerobic Reduction and Microaerophilic Degradation of Mono-, Di-, and Trichlorobenzenes

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Two ^{13}C -labeled in situ microcosm studies were conducted to evaluate ^{13}C -AMP stimulated degradation of mono-, di-, and trichlorobenzenes in anaerobic groundwater at a site in Michigan. The data show that the ^{13}C -AMP amendment stimulated an overall increase in microbial activity and a shift in the microbial community structure, indicating more reduced conditions. Stable isotope probing with ^{13}C -chlorobenzene demonstrated attenuation of chlorobenzene and subsequent use to identify the attenuating microbes. The ^{13}C - and ^{13}C -chlorobenzene, acid (CPA) factors were used to identify the attenuating microbes. These data clearly show the participation of an obligate anaerobe in the chlorobenzene biodegradation process.

Due to the chemically favorable conditions, anaerobic biodegradation of chlorobenzene is a common mechanism of degradation of the trichlorobenzenes as measured by ^{13}C -AMP. The microaerophilic degradation of chlorobenzene or its metabolites was definitively demonstrated and cannot be ruled out for the other chlorobenzenes. © 2013 Wiley Periodicals, Inc.

INTRODUCTION

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Site: Michigan



ENVIRONMENTAL
FIELD BOOK

Contaminant: trichlorobenzene
and daughters

Remedy: Abiotic Reduction &
Aerobic Bio

ZVI
fermentable donor

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Conclusions

- ▶ Collect actionable data early in the project management cycle
- ▶ Focus on actionable data that provides a fail small/win big opportunity