Stable Isotope Probing and QuantArray: Evaluate MNA of Petroleum Hydrocarbons & Emerging Contaminants

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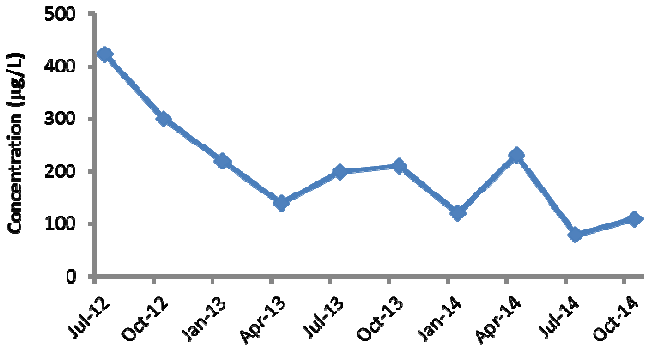
MNA Assessment

- Chemistry



- Geochemistry

$$C_6H_6 + 7.5O_2 \rightarrow 6CO_2 + 3H_2O$$

- Microbiology

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Microbiology

Is biodegradation occurring?

- Stable Isotope Probing

What is the concentration of contaminant degraders?

- qPCR
- QuantArray

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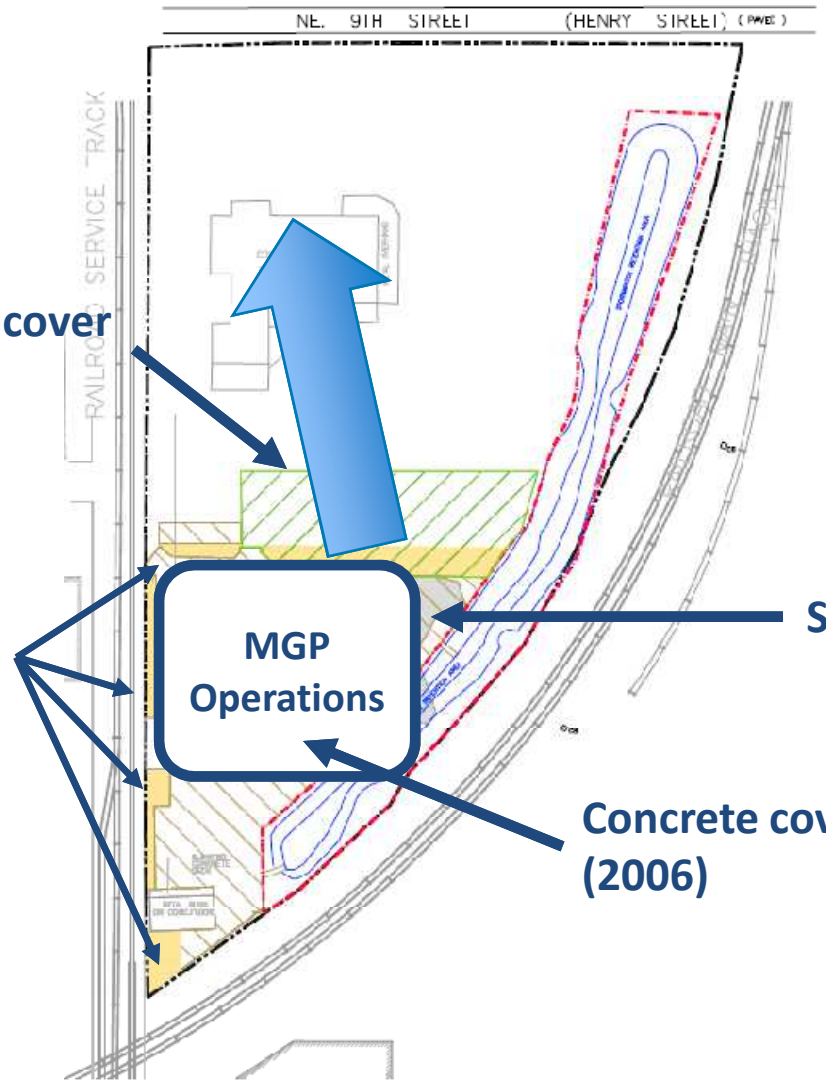
Site Background

- Former manufactured gas plant (MGP)
- Operated from 1890s until 1953
- NAPL present
- Benzene, ethylbenzene
- Naphthalene, methylnaphthalenes, and other PAHs

Site Map



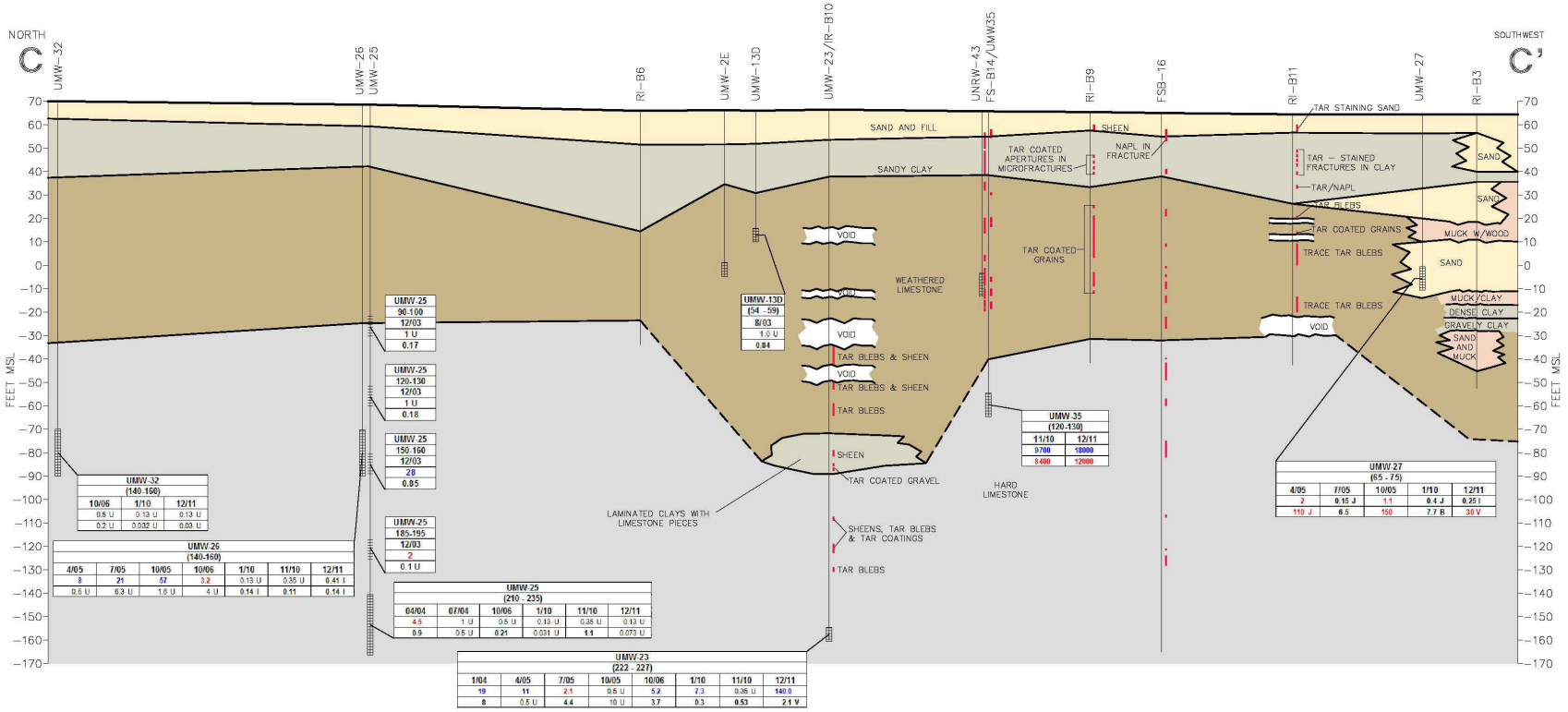
Concrete cover (2011)

Surface soil removals (2008)

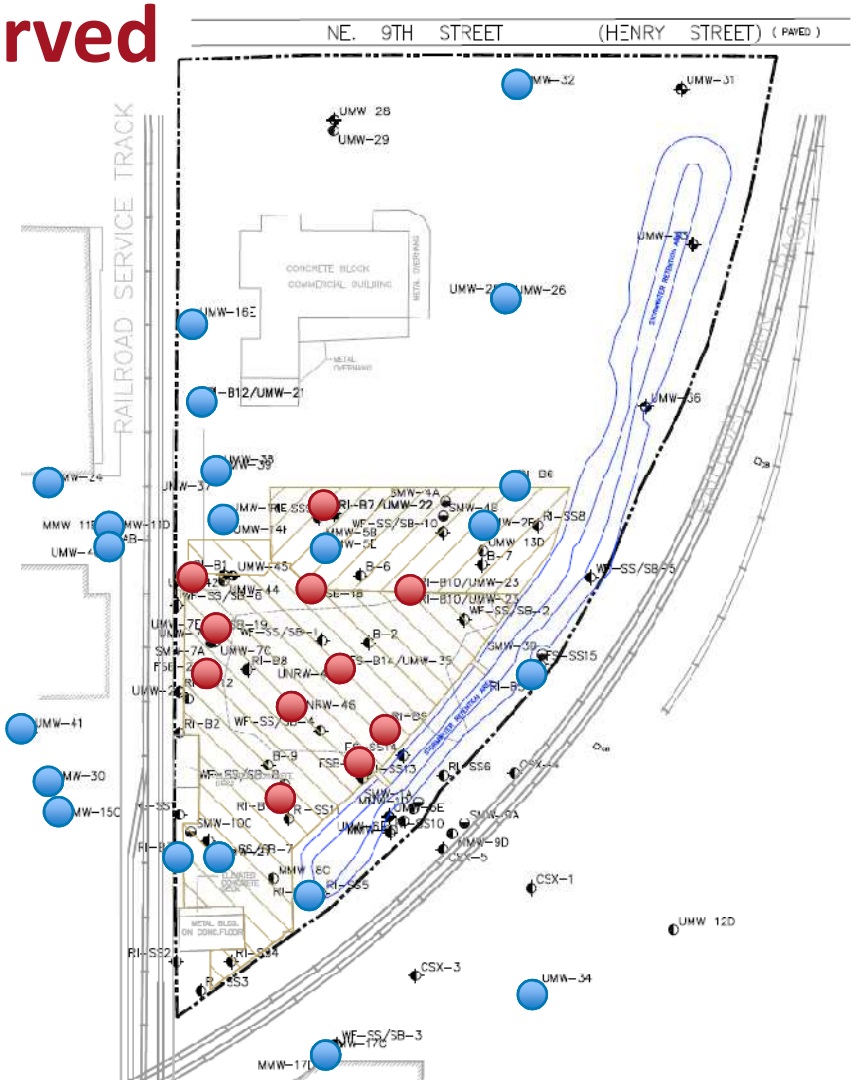
Soil excavation (1990)

Concrete cover (2006)

Geology and NAPL staining, sheens, blebs



LNAPL Observed



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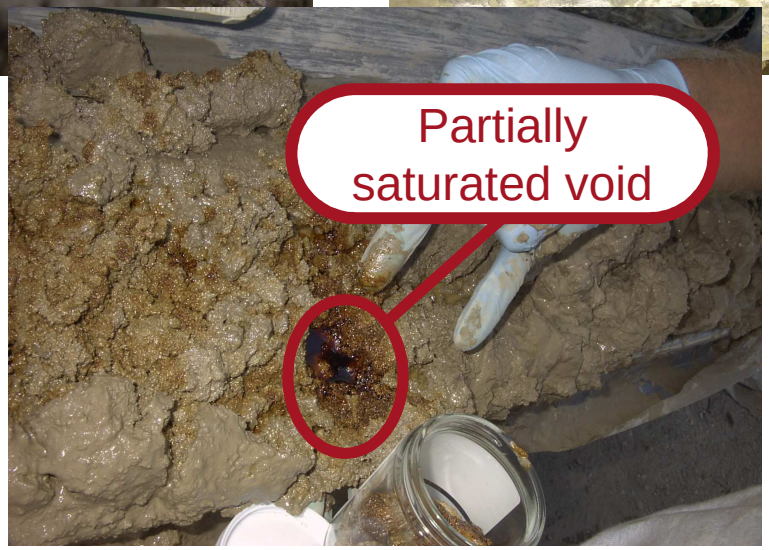
Sandy clay unit - NAPL

- Tar staining at discrete intervals in aperatures
- Vertical migration

Weathered limestone unit - NAPL



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Groundwater Impacts - PAHs

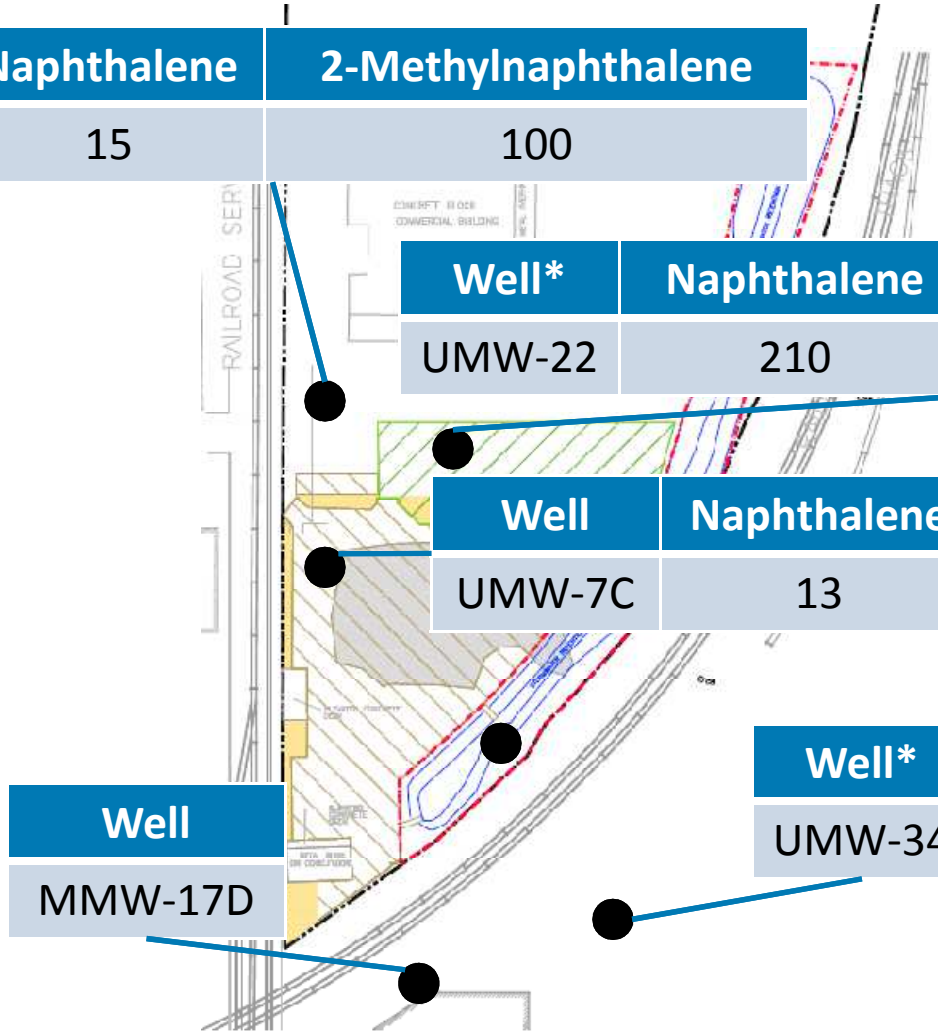
Well	Naphthalene	2-Methylnaphthalene
UMW-44	15	100

Well*	Naphthalene	2-Methylnaphthalene
UMW-22	210	1,700

Well	Naphthalene	2-Methylnaphthalene
UMW-7C	13	1,000

Well*
UMW-34

Well
MMW-17D



*** Hard limestone wells**

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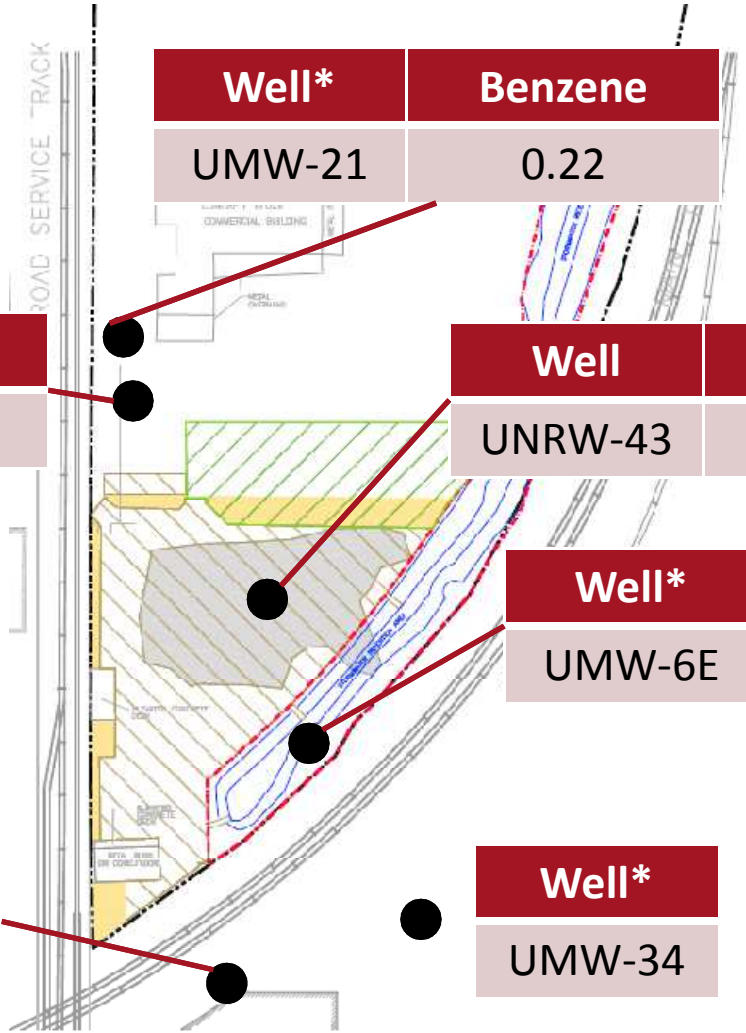
Groundwater Impacts – Benzene



Well*	Benzene
UMW-21	0.22

Well	Benzene
UMW-37	<0.2

Well	Benzene
UNRW-43	20,000

Well*	Benzene
UMW-6E	200

Well
MMW-17D

Well*
UMW-34

*** Hard limestone wells**

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Contaminant Concentrations

Monitoring Well	Benzene Trend	Naphthalene Trend
UMW-7C	No Trend	Decreasing
UMW-44	No Trend	No Trend
UMW-37	No Trend	Near DL
UMW-6E	Decreasing	Decreasing
UMW-21	Decreasing	No Trend
UMW-22	No Trend	Decreasing

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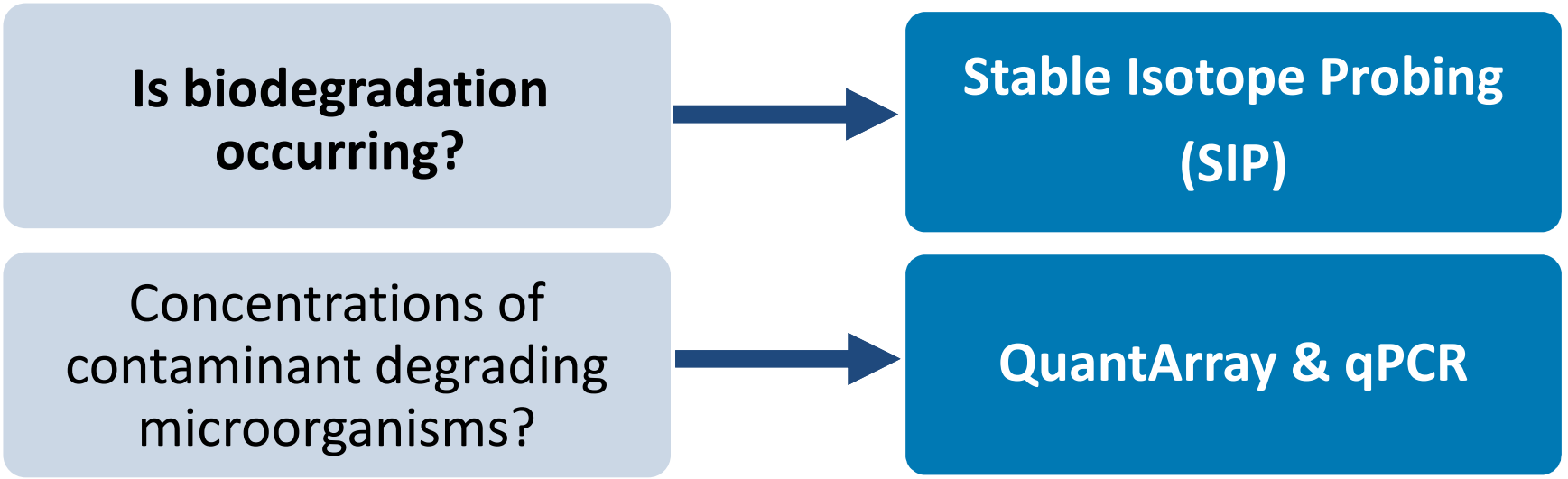
MNA Assessment

- ✓ Contaminant concentrations
- ✓ Geochemistry
- • Molecular Biological Tools



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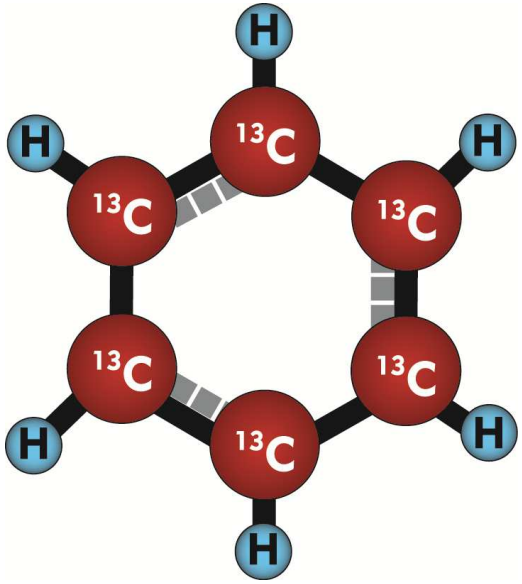
Stable Isotope Compounds

- **Specially produced "heavy" compounds which are composed of 99+% ^{13}C**
  - Natural compounds are 99% ^{12}C
  - Same characteristics & behavior as original compound
- **^{13}C label used as a "tracer" to determine if biodegradation of the compound occurred**
- **Incorporation of the ^{13}C label into biomass and CO_2 demonstrates biodegradation**



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Overview of Bio-Trap SIP Approach

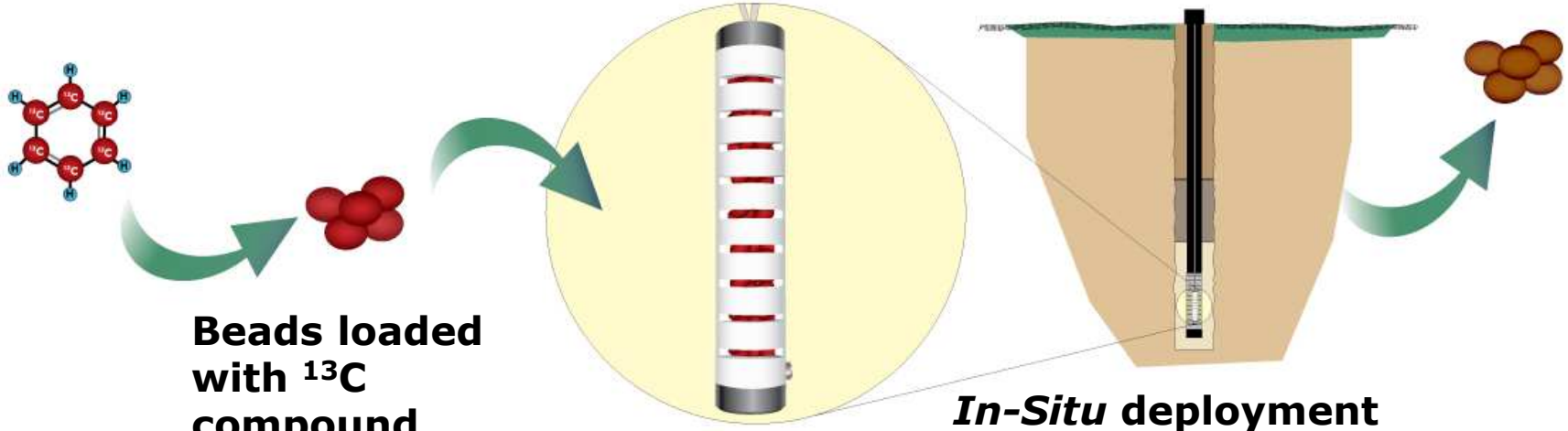
^{13}C labeled Benzene

Beads loaded with ^{13}C compound

Bio-Trap with ^{13}C-benzene loaded beads

***In-Situ* deployment in monitoring well**

Beads analyzed following deployment



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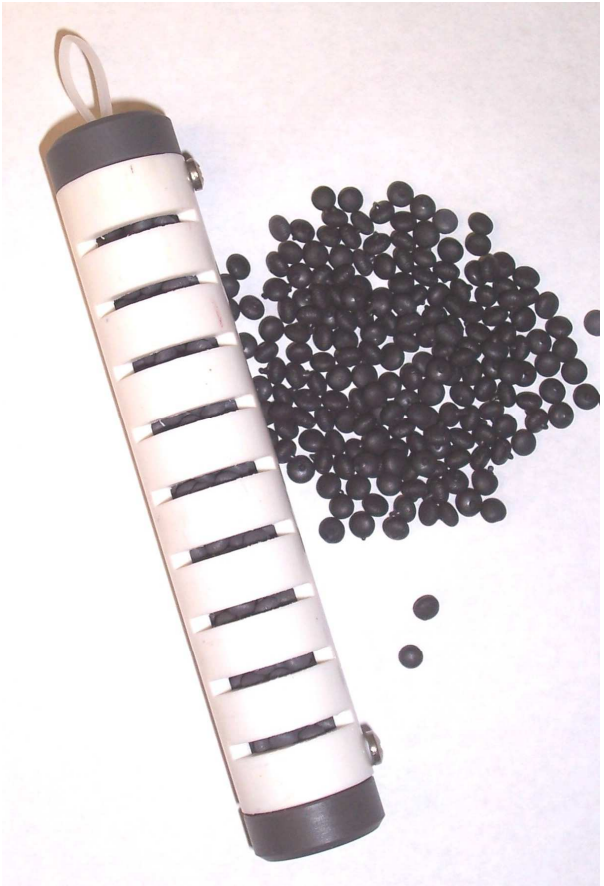
What Are Bio-Trap® Samplers?

- Passive microbial sampling tool
- Colonized by active microbes
- 25% Nomex and 75% PAC
- Used in conjunction with
  - Stable isotope probing
  - qPCR and QuantArray
  - Other MBTs



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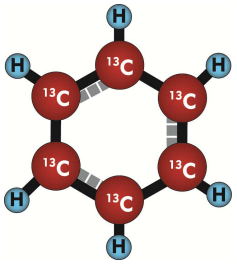
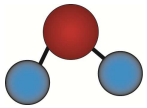
Bio-Trap SIP Analysis

Residual ^{13}C-Compound



Contaminant Loss

$^{13}C/^{12}C$ Dissolved Inorganic Carbon



Mineralization
(C for energy)

$^{13}C/^{12}C$ of Biomarkers

PLFA
DNA
RNA

Metabolism
(C for growth)

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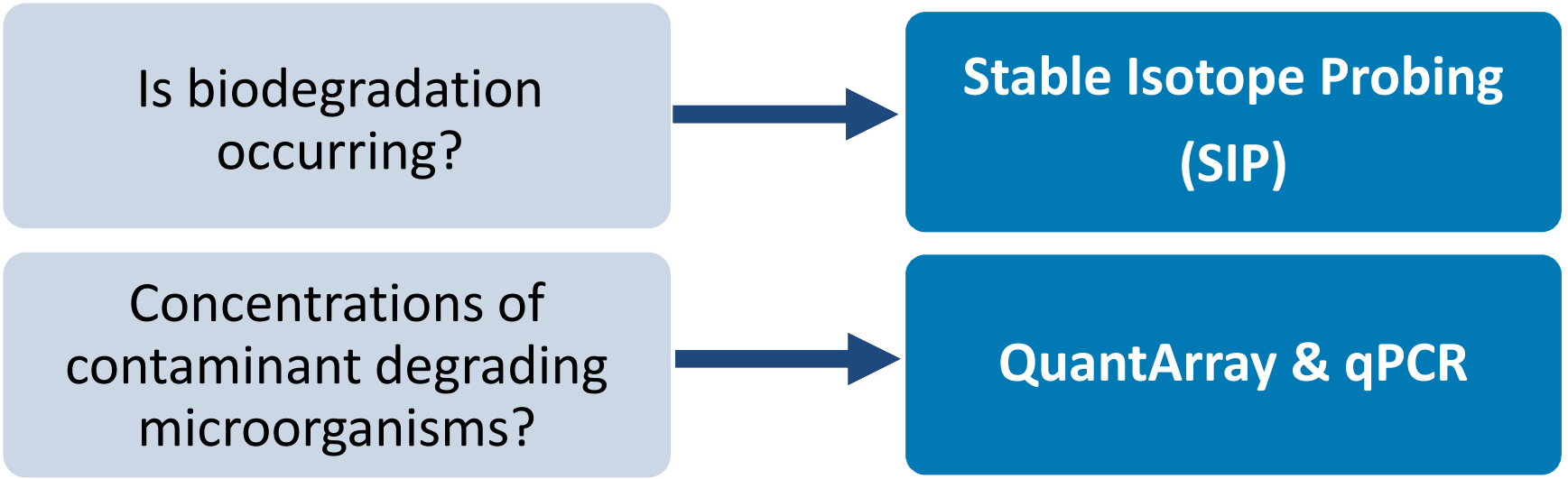
MNA Assessment

- ✓ Contaminant concentrations
- ✓ Geochemistry
- Molecular Biological Tools



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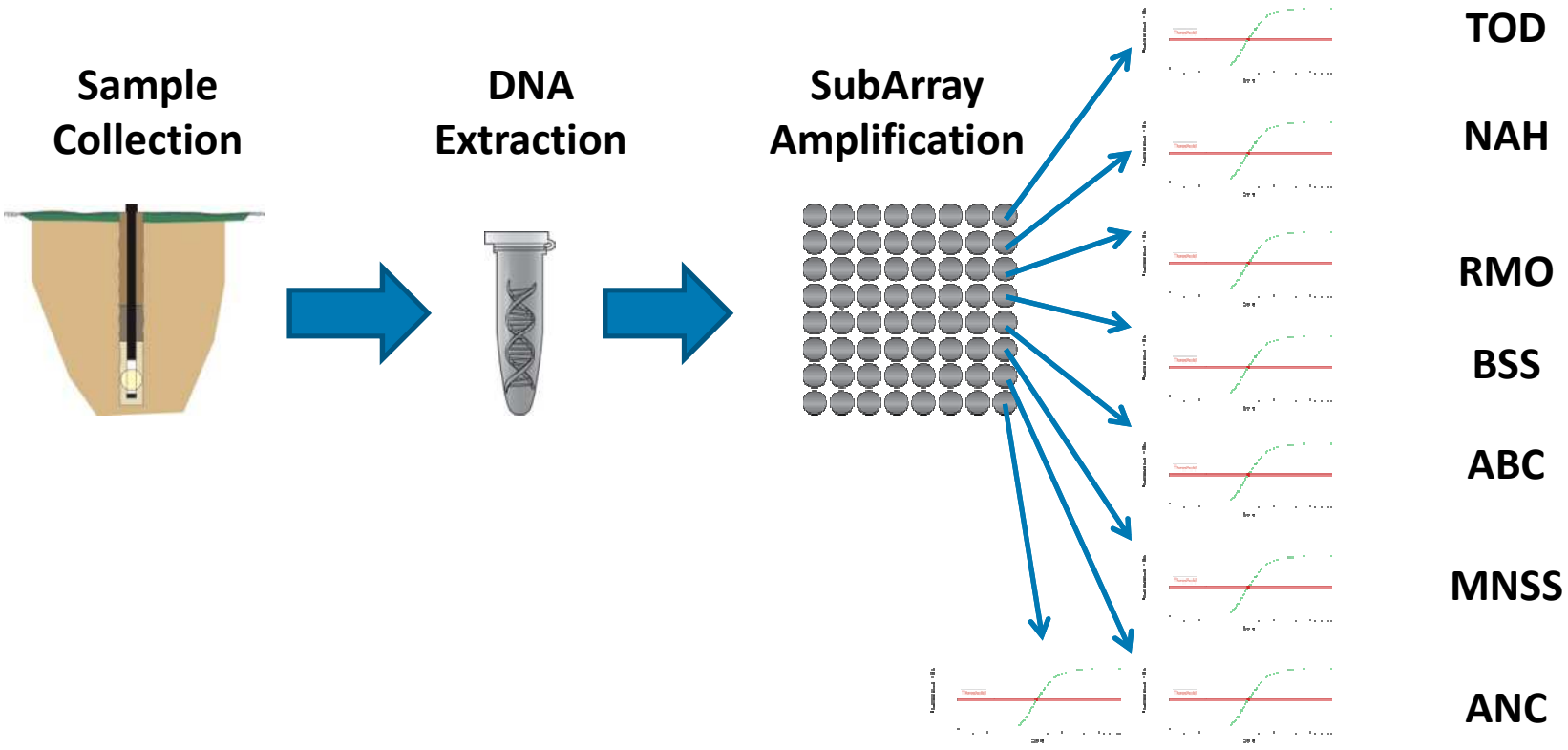
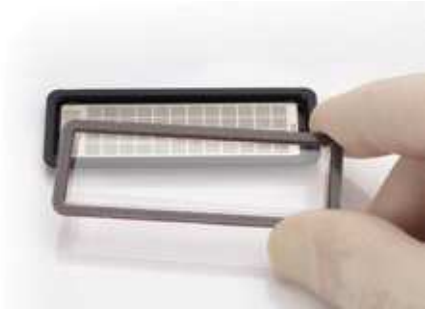
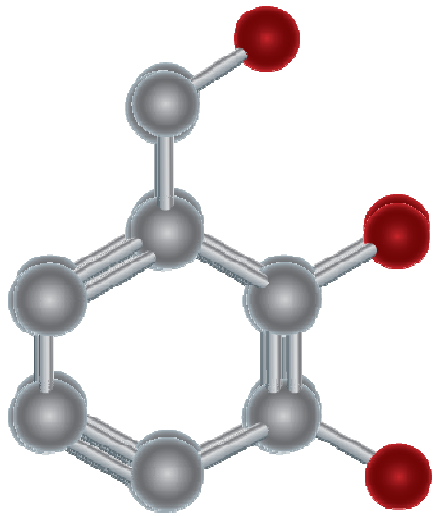
QuantArray


Sample Collection
DNA Extraction
SubArray Amplification
TOD
NAH
RMO
BSS
ABC
MNSS
ANC

QuantArray-Petro





Aerobic BTEX and MTBE (cells/mL)

Toluene 3- and 4-Monooxygenases (RMO)
Toluene 2 Monooxygenase (RDEG)
Phenol Hydroxylase (PHE)
Toluene/Benzene Dioxygenase (TOD)
Xylene/Toluene Monooxygenase (TOL)
Ethylbenzene/Isopropylbenzene Dioxygenase (EDO)
Biphenyl/Isopropylbenzene Dioxygenase (BPH4)
Methylibium petroliphilum PM1 (PM1)
TBA Monooxygenase (TBA)

Aerobic PAHs and Alkanes (cells/mL)

Naphthalene Dioxygenase (NAH)
Phenanthrene Dioxygenase (PHN)
Alkane Monooxygenase (ALK)

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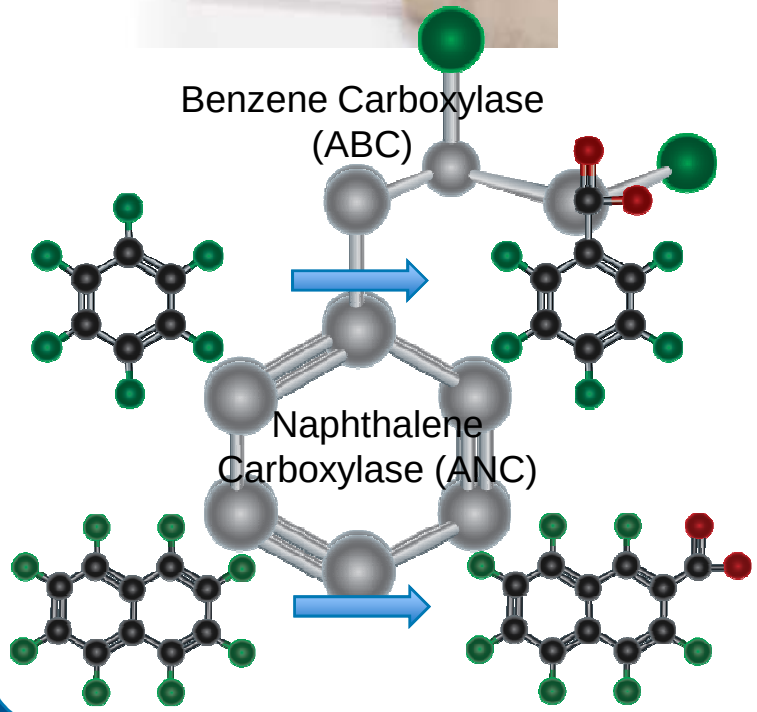
QuantArray-Petro



Anaerobic BTEX (cells/mL)

Benzoyl Coenzyme A Reductase (BCR)
Benzylsuccinate synthase (BSS)
Benzene Carboxylase (ABC)

Anaerobic PAHs and Alkanes (cells/mL)

Benzoyl Coenzyme A Reductase (BCR)
Naphthylmethylsuccinate Synthase (NMS)
Naphthalene Carboxylase (ANC)
Alklysuccinate Synthase (ASSA)

Other (cells/bead)

Total Eubacteria (EBAC)
Sulfate Reducing Bacteria (APS)

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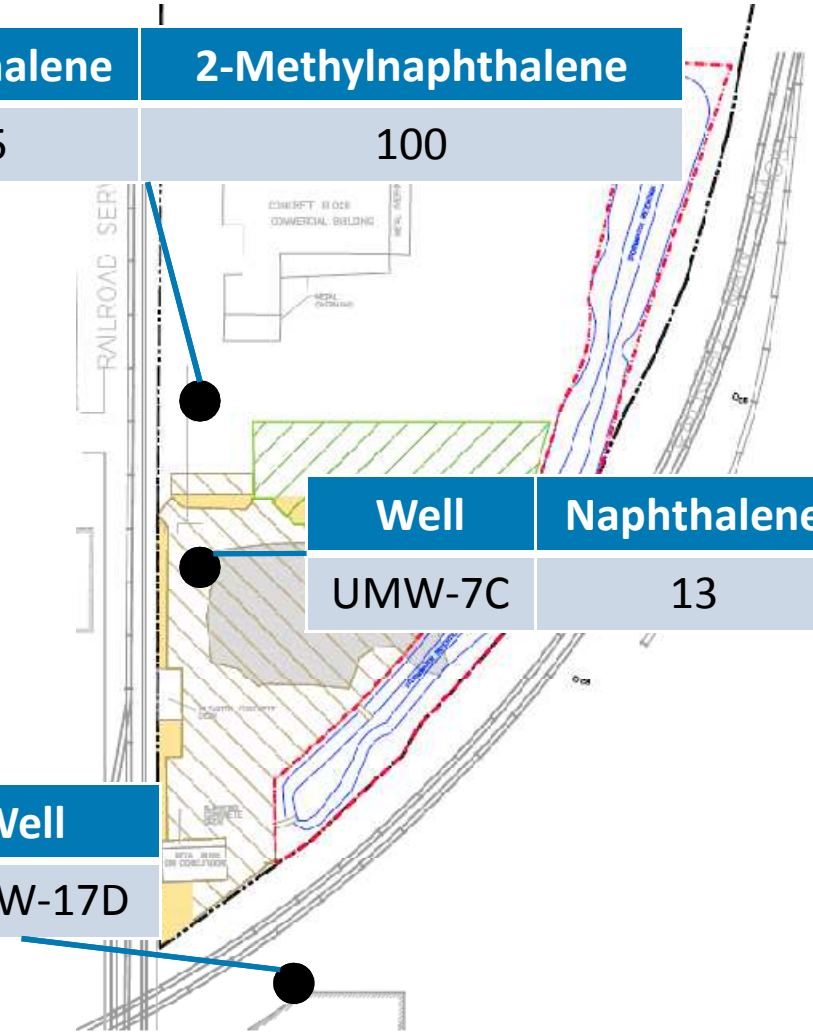
Study Wells – Weathered Limestone

Well	Naphthalene	2-Methylnaphthalene
UMW-44	15	100



Well	Naphthalene	2-Methylnaphthalene
UMW-7C	13	1,000

Well
MMW-17D

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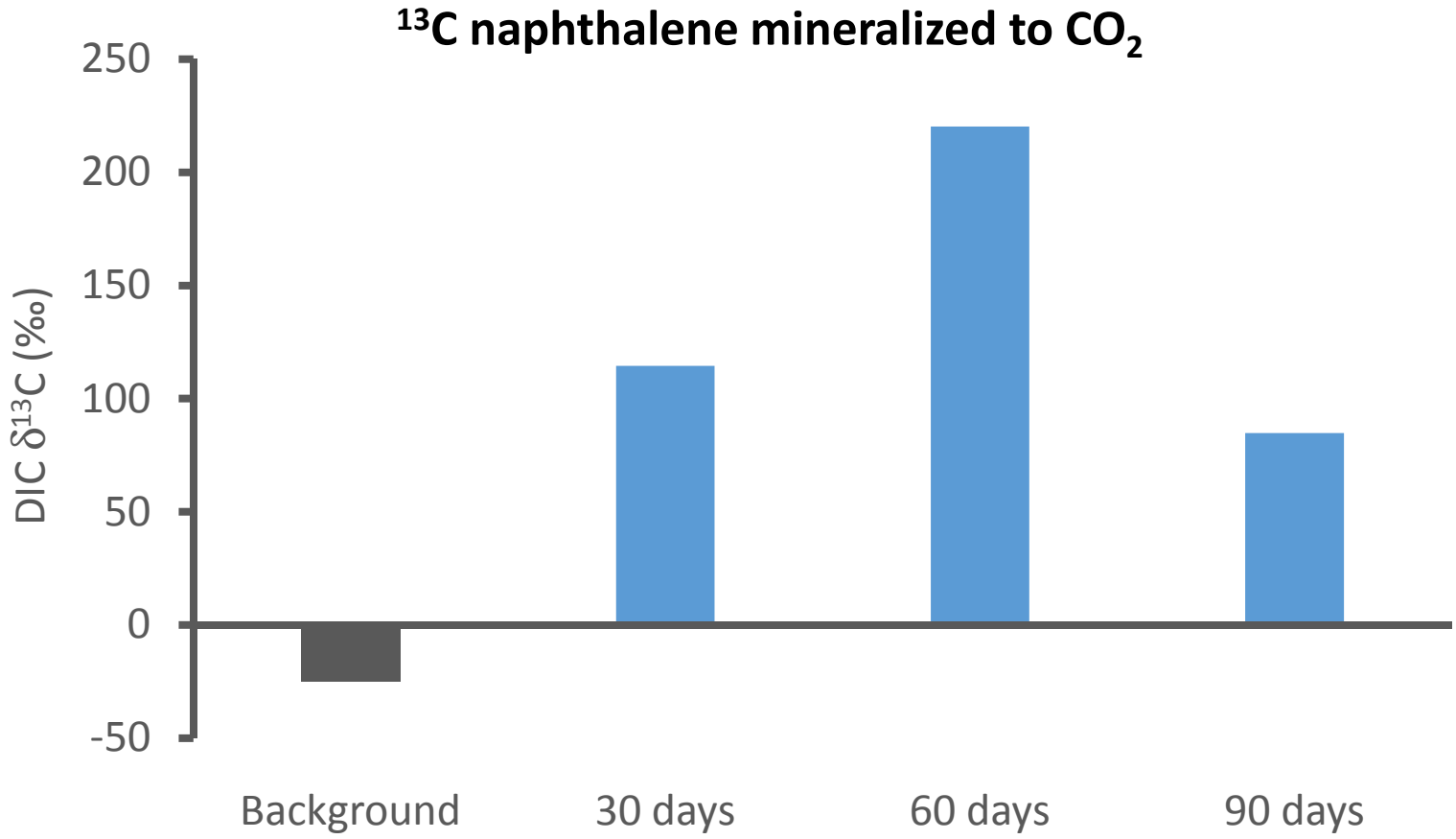
Is naphthalene biodegradation occurring?



UMW-7C

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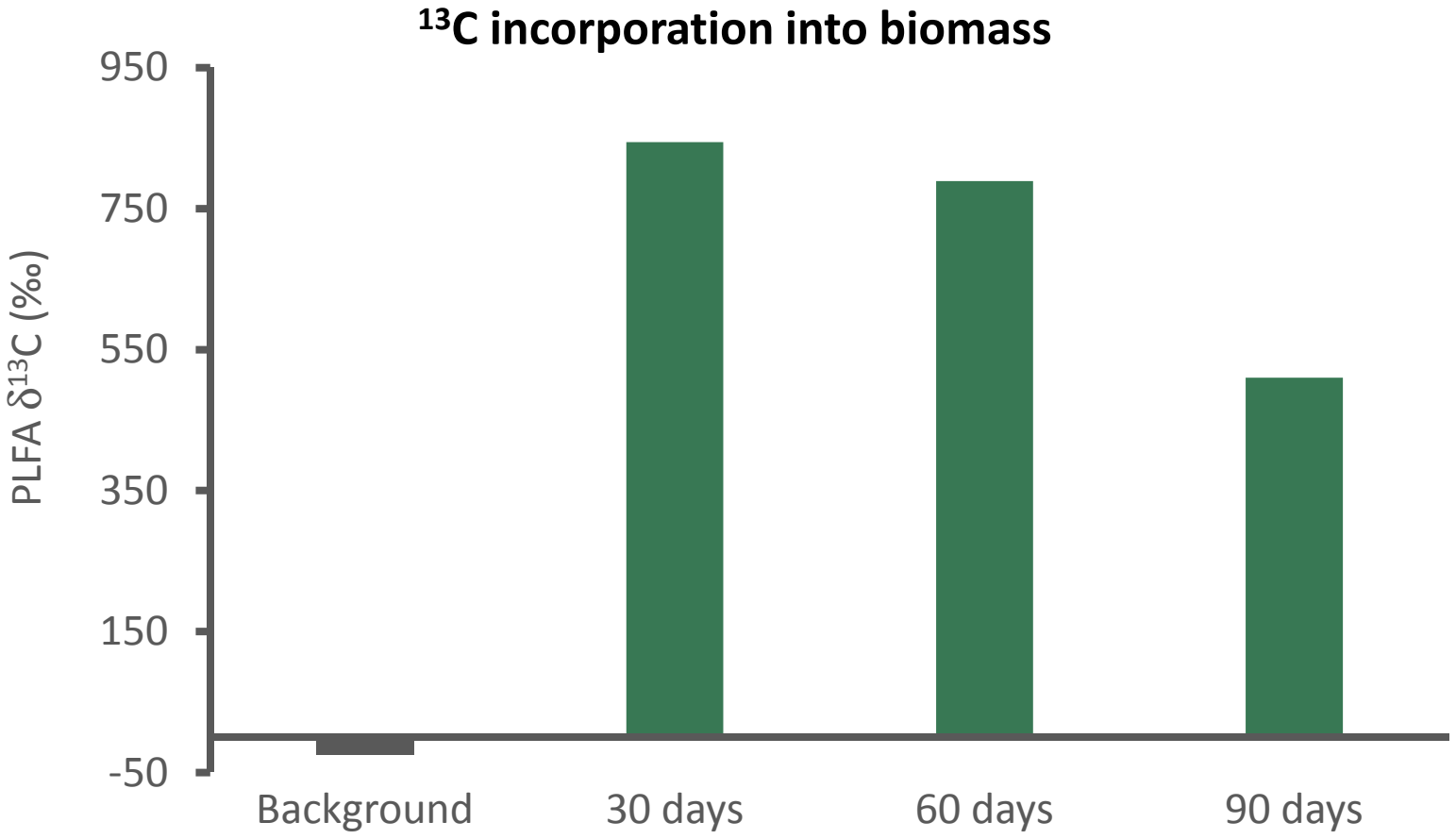
Is naphthalene biodegradation occurring?



UMW-7C

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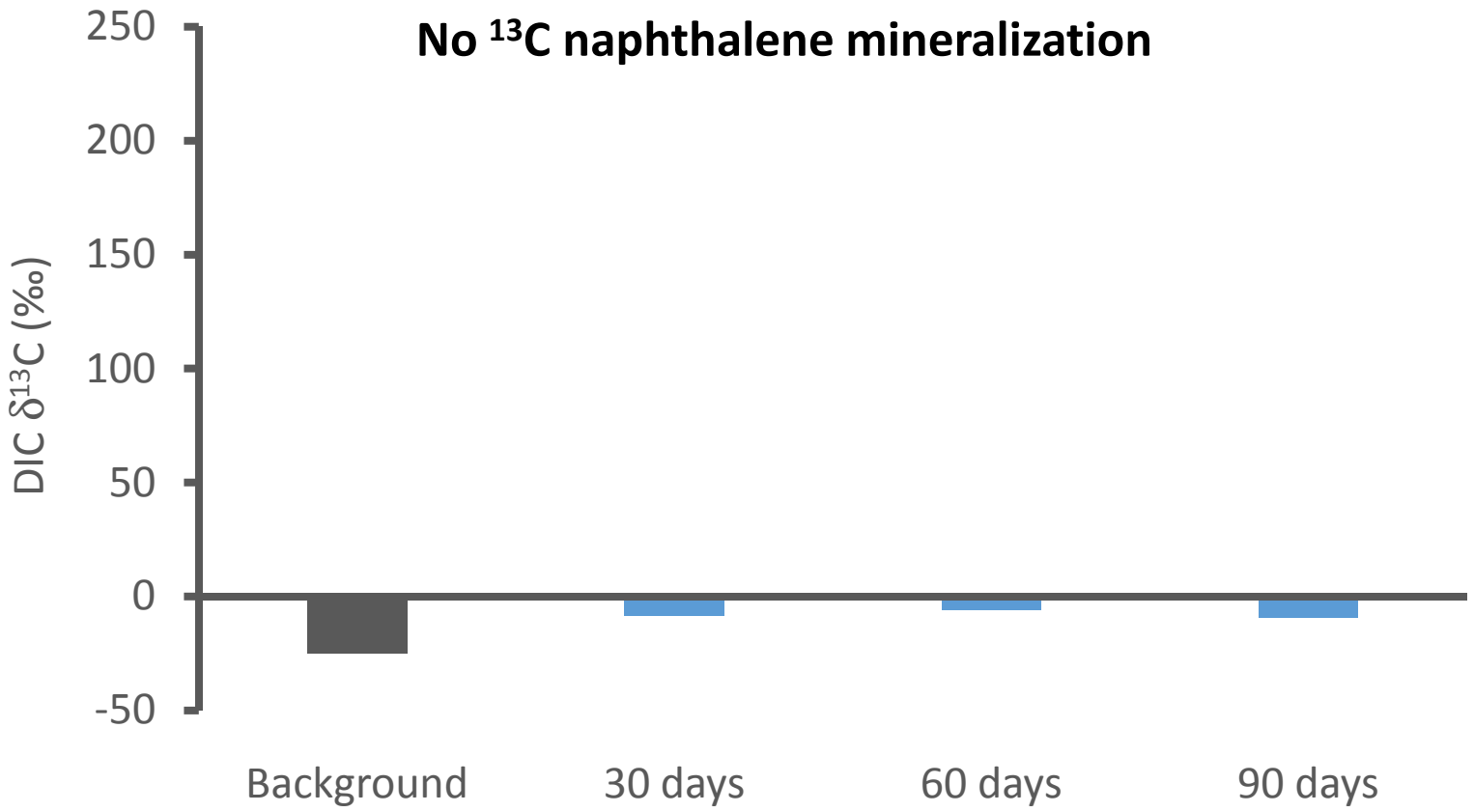
Naphthalene biodegradation downgradient?



UMW-44

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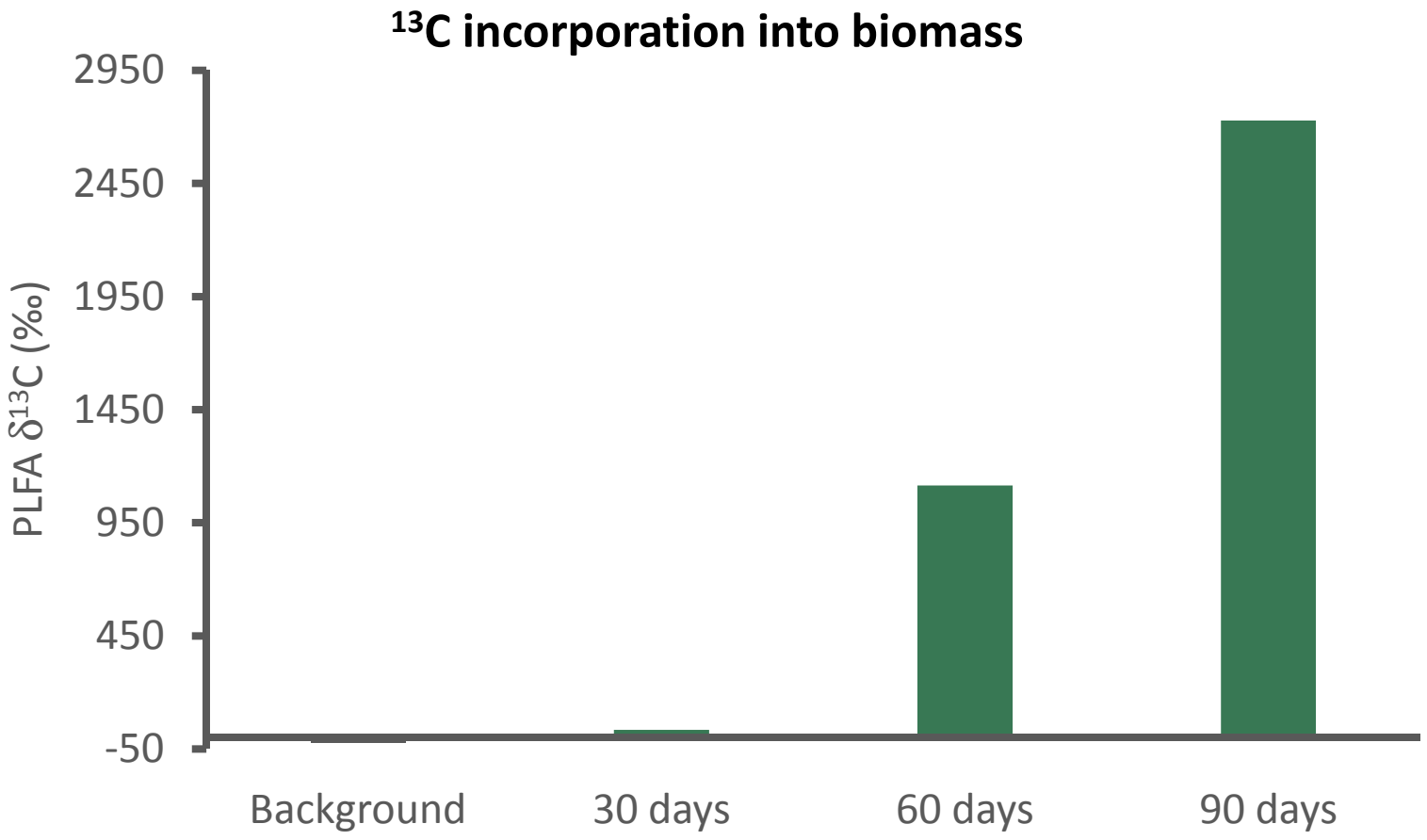
Naphthalene biodegradation downgradient?



UMW-44

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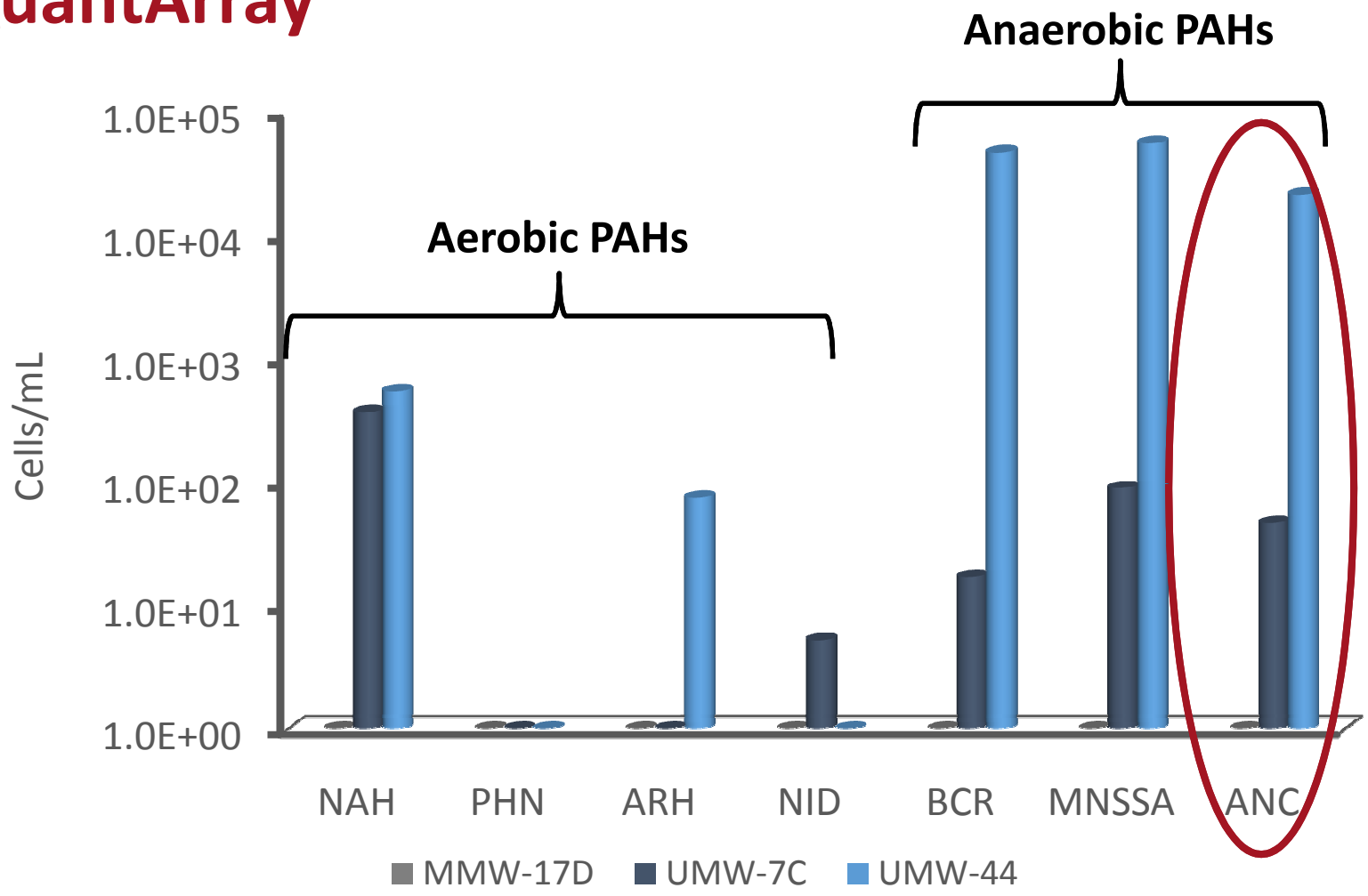
QuantArray



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MNA Assessment

	Chemical	Microbiological	
	Decreasing contaminant concentration?	**Stable Isotope Probing** Did biodegradation occur?	**QuantArray** Concentrations of contaminant degraders?
Naphthalene			
Benzene			

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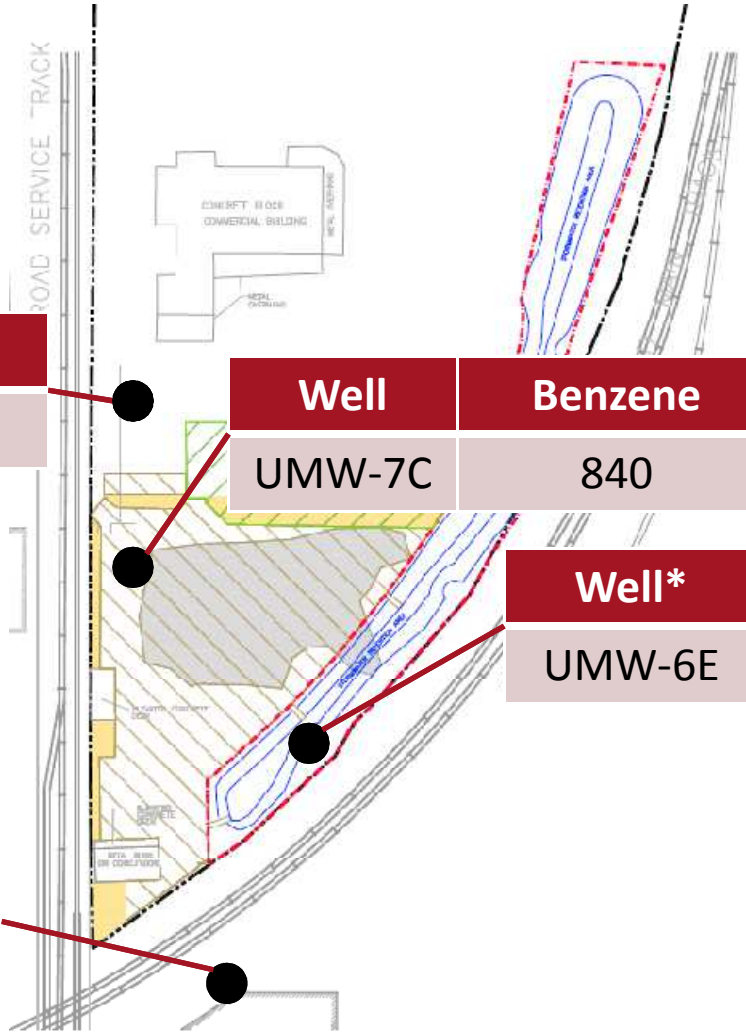
Groundwater Impacts – Benzene



Well	Benzene
UMW-37	<0.2

Well	Benzene
UMW-7C	840

Well*	Benzene
UMW-6E	200

Well
MMW-17D

*** Hard limestone wells**

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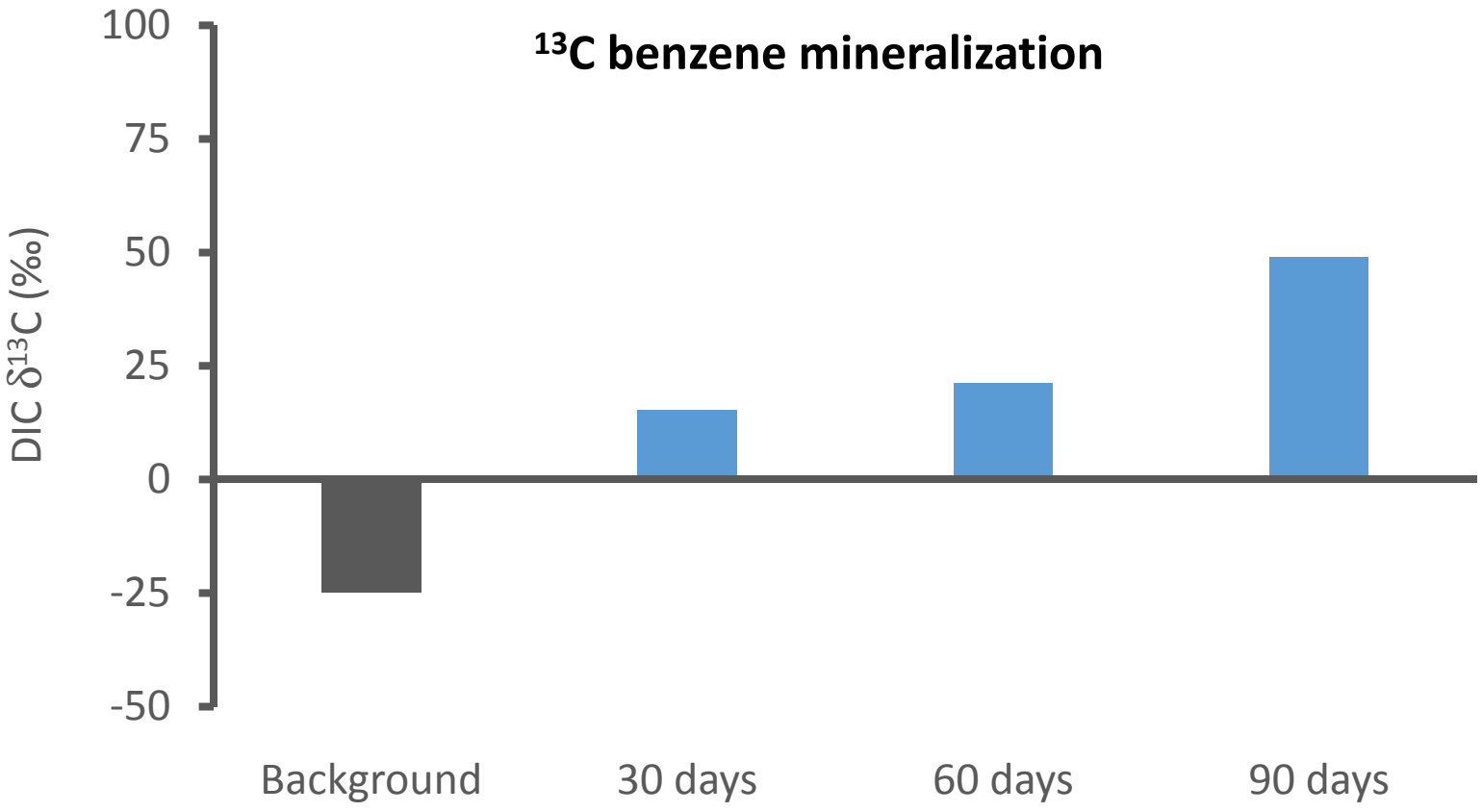
Is benzene biodegradation occurring?



UMW-6E*

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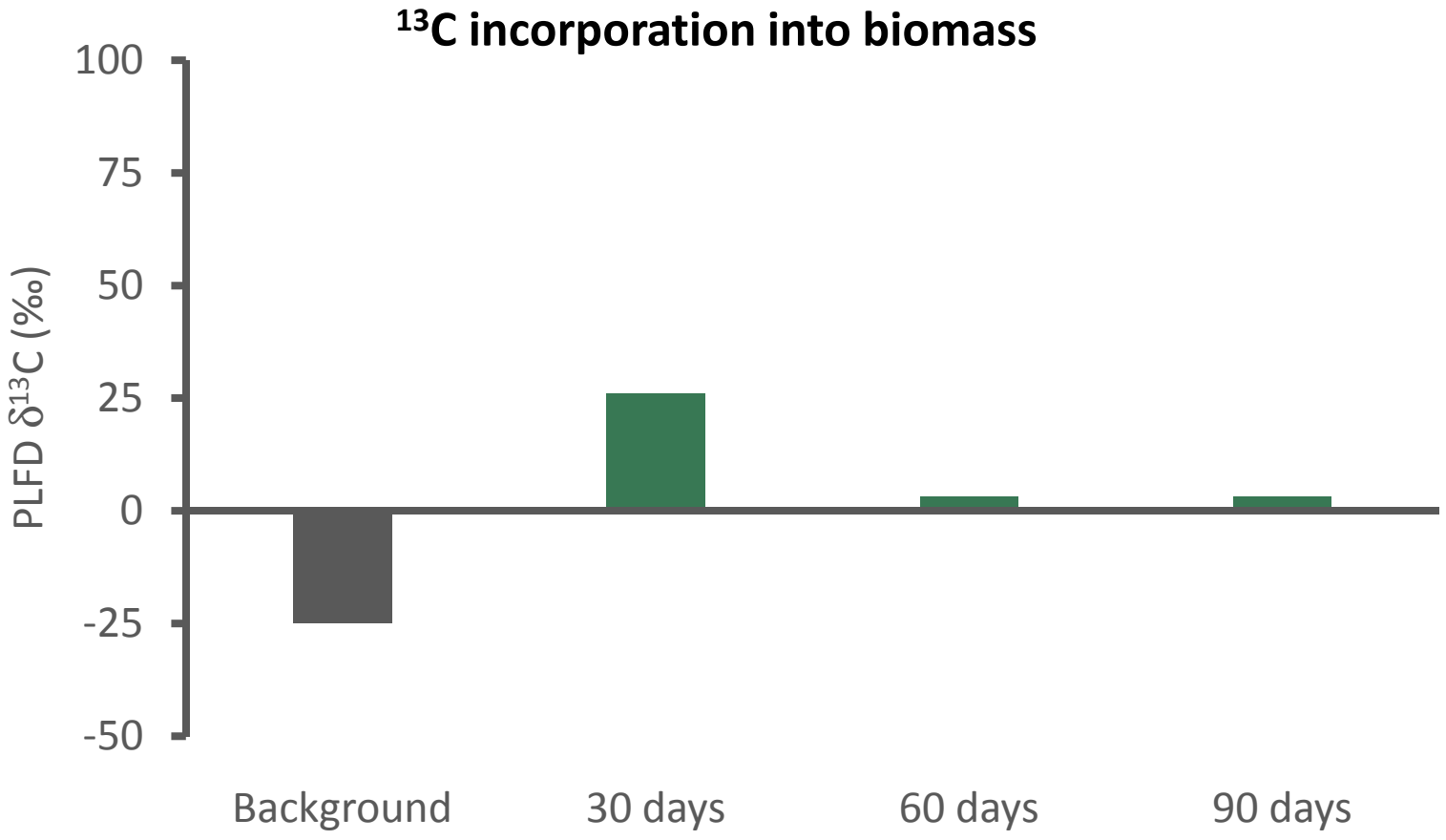
Is benzene biodegradation occurring?



UMW-6E*

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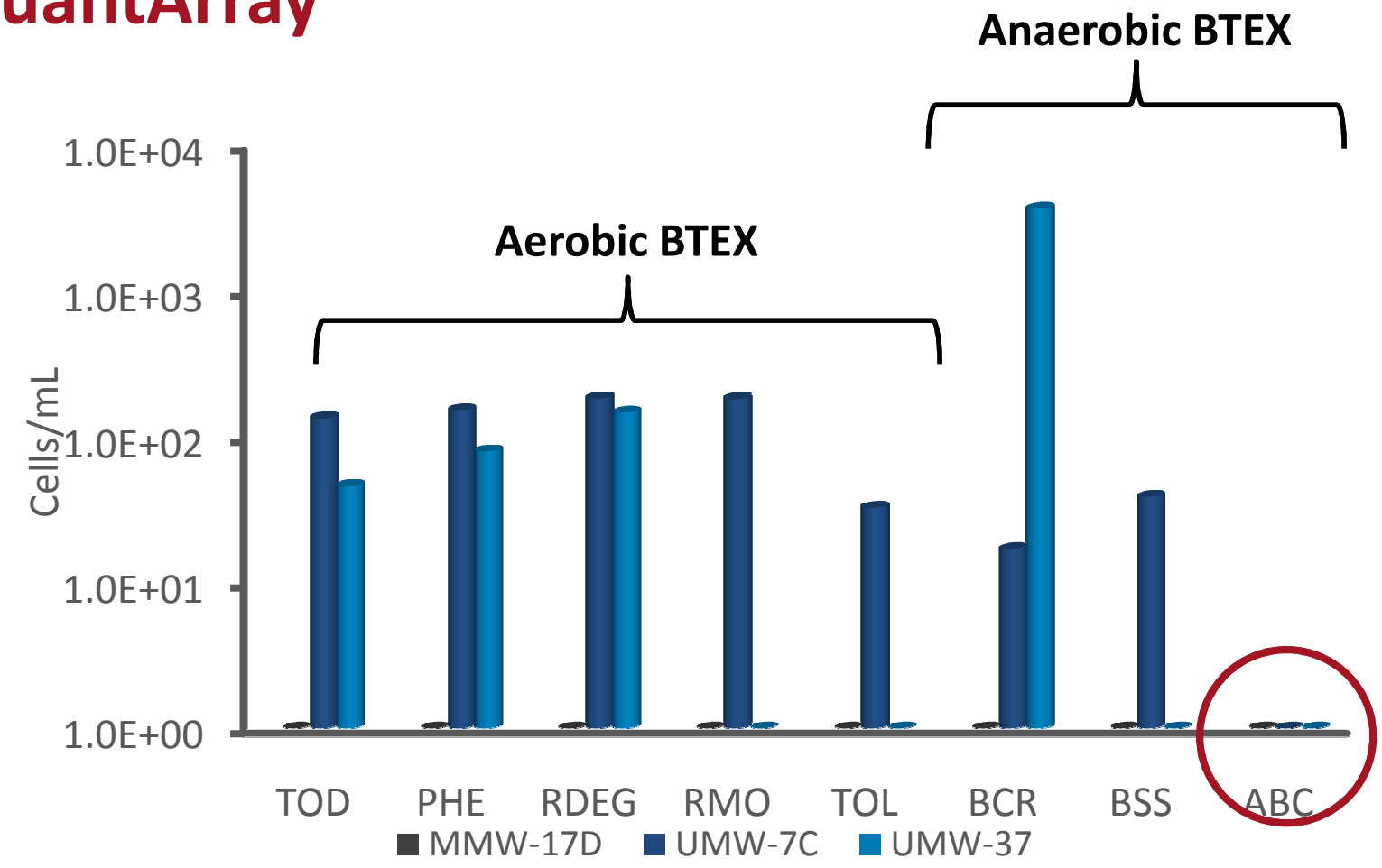
QuantArray



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MNA Assessment

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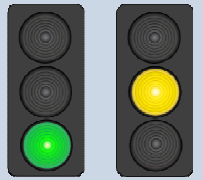
Benzene			

Applicability and Advantages of SIP

- Conclusive evidence of biodegradation
- Contaminants used as carbon and energy sources
  - Naphthalene and & PAHs
  - BTEX, chlorobenzene
  - MTBE, TBA
- Independent of pathway or conditions
  - Aerobic or anaerobic
  - Pathway unknown
  - Emerging contaminants

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1,4-Dioxane

- Emerging contaminant
  - Stabilizer for chlorinated solvents (1,1,1-TCA)
  - Solvent for paper, cotton, and textile processing

- Physical properties of dioxane often make traditional treatment technologies impractical
  - Miscible in water
  - Low sorption
  - Relatively low volatility

Biodegradation of 1,4-Dioxane

- Aerobic metabolism (carbon and energy source)
  - *Pseudonocardia dioxanivorans* CB1190 and others
  - Dioxane monooxygenase and aldehyde dehydrogenase
- Aerobic co-oxidation
  - Ring hydroxylating toluene monooxygenases
  - Soluble methane monooxygenase
  - Alkane monooxygenases

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Biodegradation of 1,4-Dioxane

- Metabolism (carbon and energy source)

Stable Isotope Probing

- Aerobic co-oxidation

qPCR

(RMO, RDEG, PHE, sMMO)

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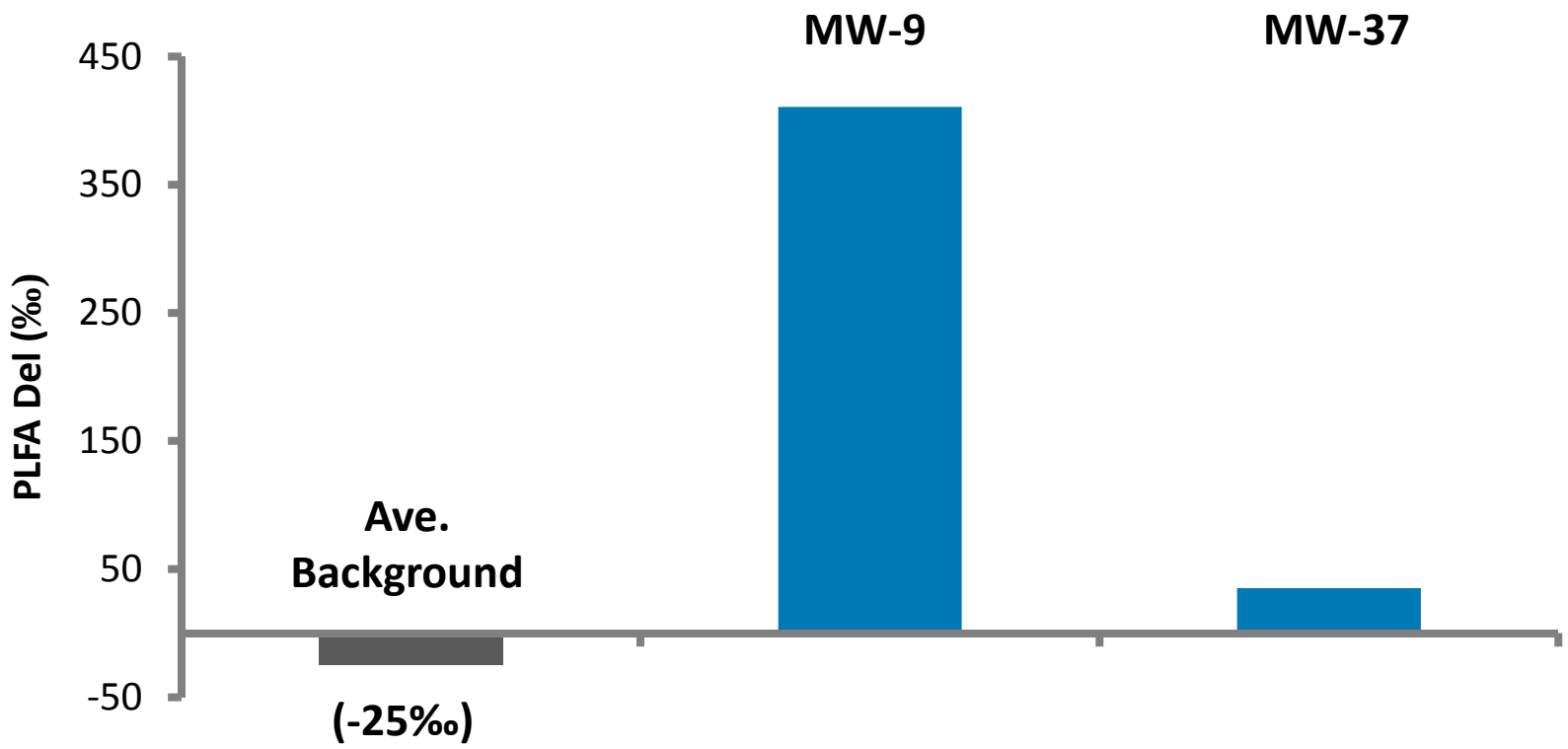
^{13}C Incorporation into Biomass



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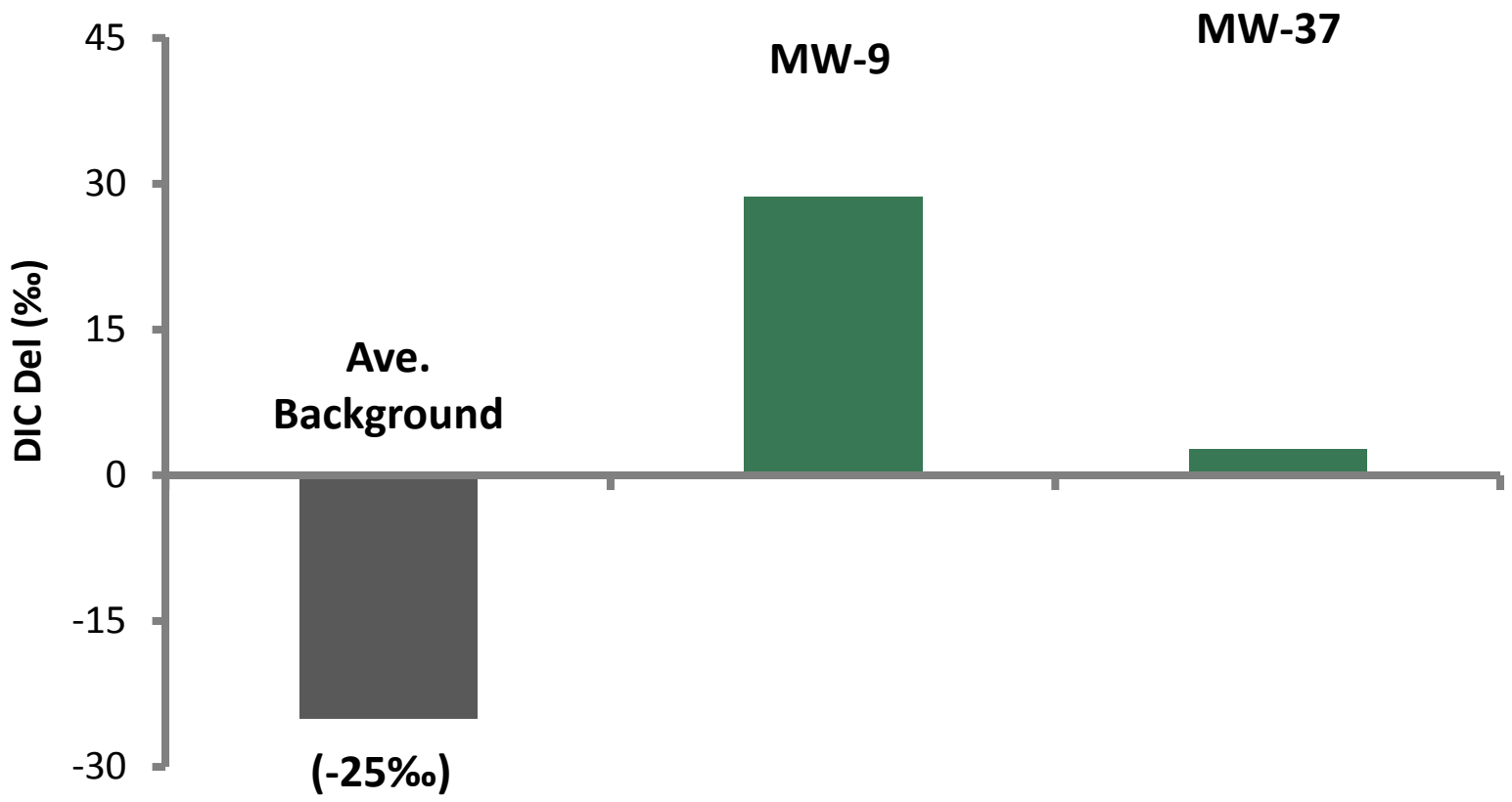
^{13}C Incorporation into DIC



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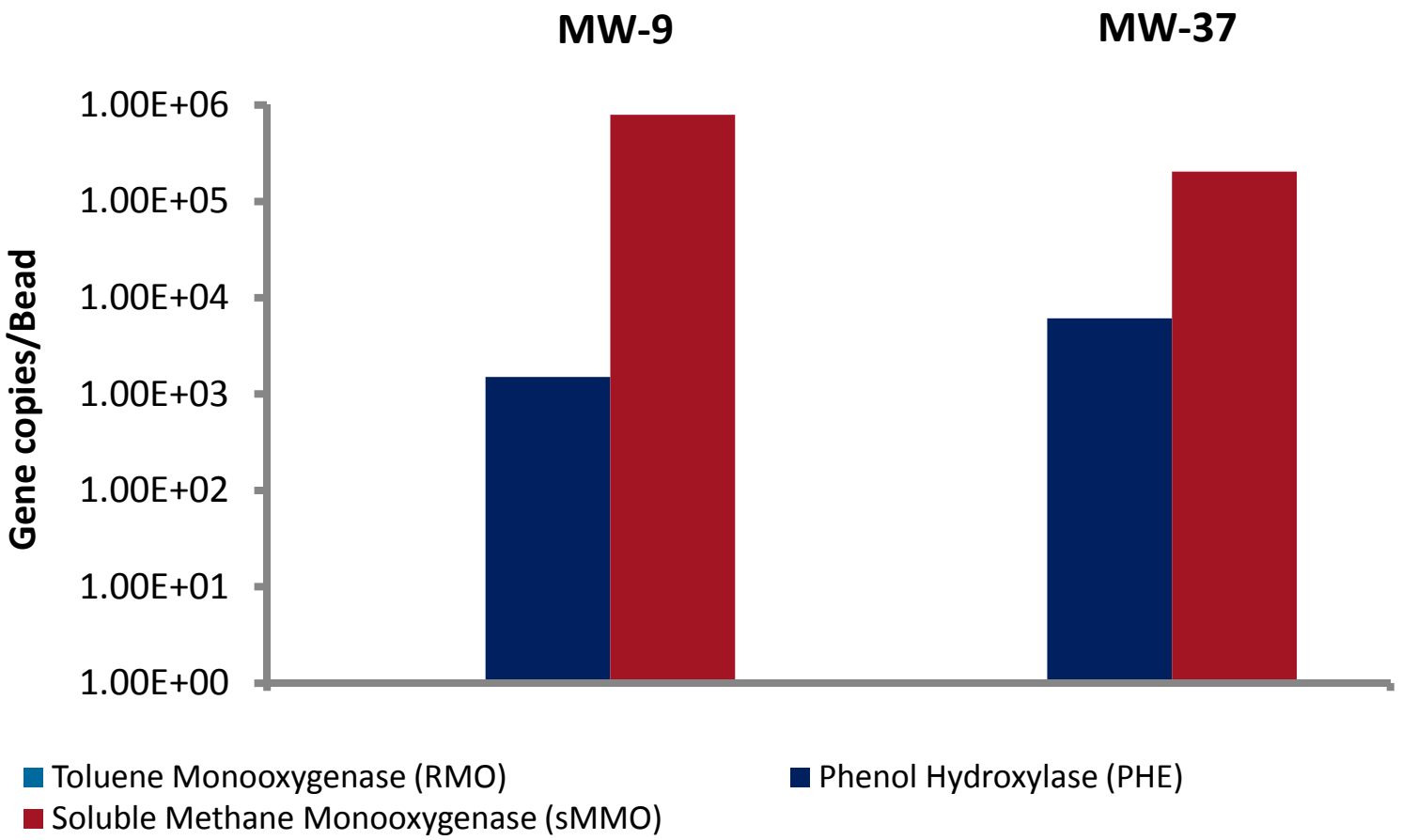
Co-oxidation Potential



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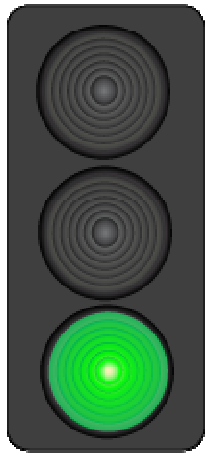
Did Dioxane Biodegradation Occur?

Incorporation into Biomass

Detection of ^{13}C enriched PLFA demonstrated that dioxane biodegradation occurred within the passive microbial sampler



Incorporation into DIC

Although low, ^{13}C enriched DIC was detected indicating dioxane mineralization had occurred

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Assessing Biodegradation of 1,4-Dioxane

- Aerobic metabolism (carbon and energy source)

Stable Isotope Probing

qPCR (DMXO & ALDH)

New

- Aerobic co-oxidation

qPCR (RMO, RDEG, PHE, sMMO)

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Questions???

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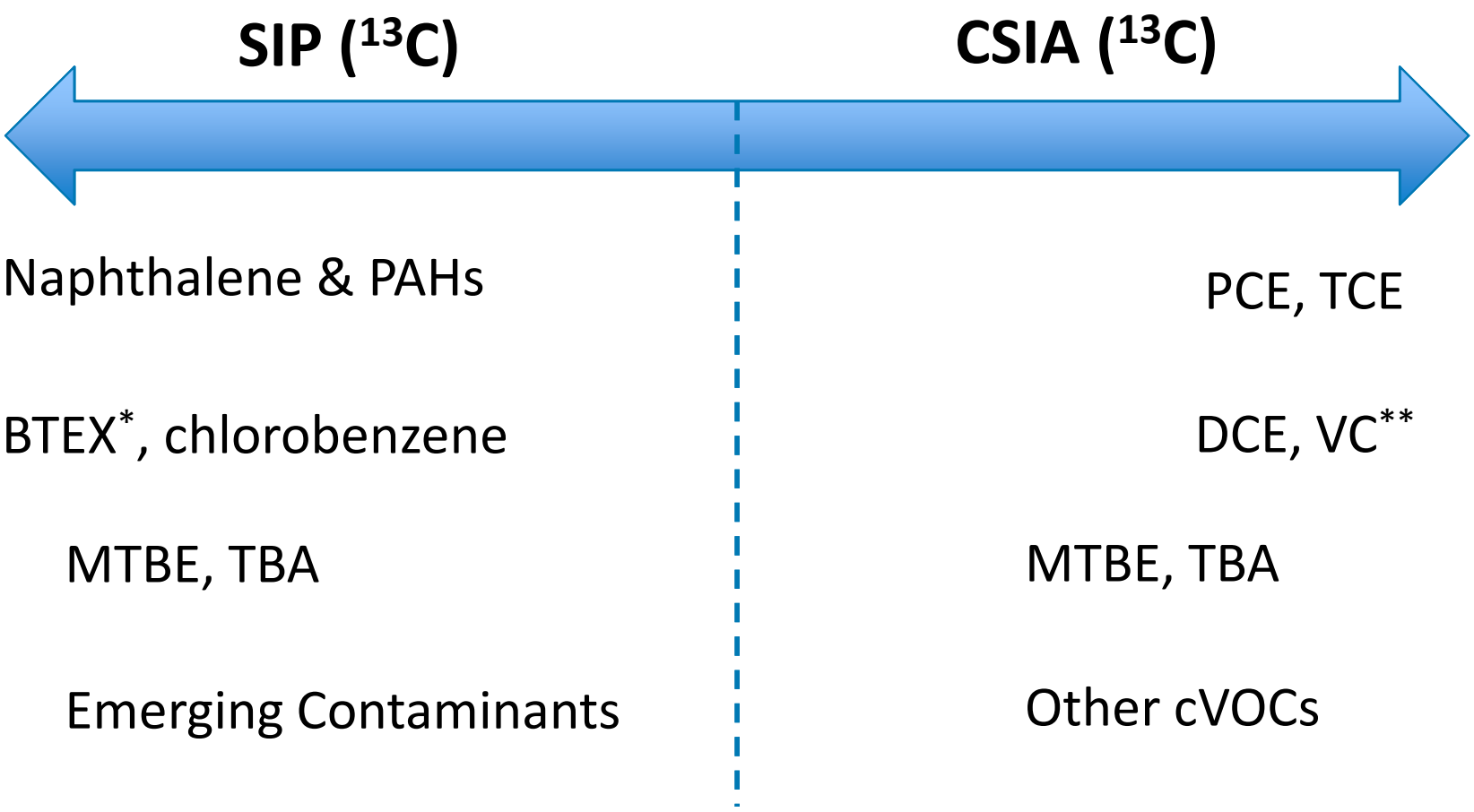
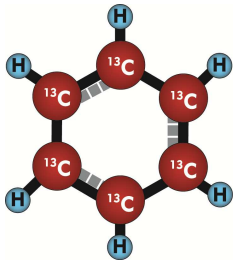
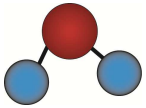
Applicability of SIP vs CSIA



Differences between SIP and CSIA

Stable Isotope Probing	Compound Specific Isotope Analysis
	**Dissolved contaminant**
^{13}C Enriched CO_2  **Mineralization**	$^{13}C/^{12}C$ of Contaminant **Compare over time or space**
^{13}C Enriched of Biomass **PLFA** DNA RNA **Biomass Growth**	$^{13}C/^{12}C$ of Daughters **Compare over time or space**

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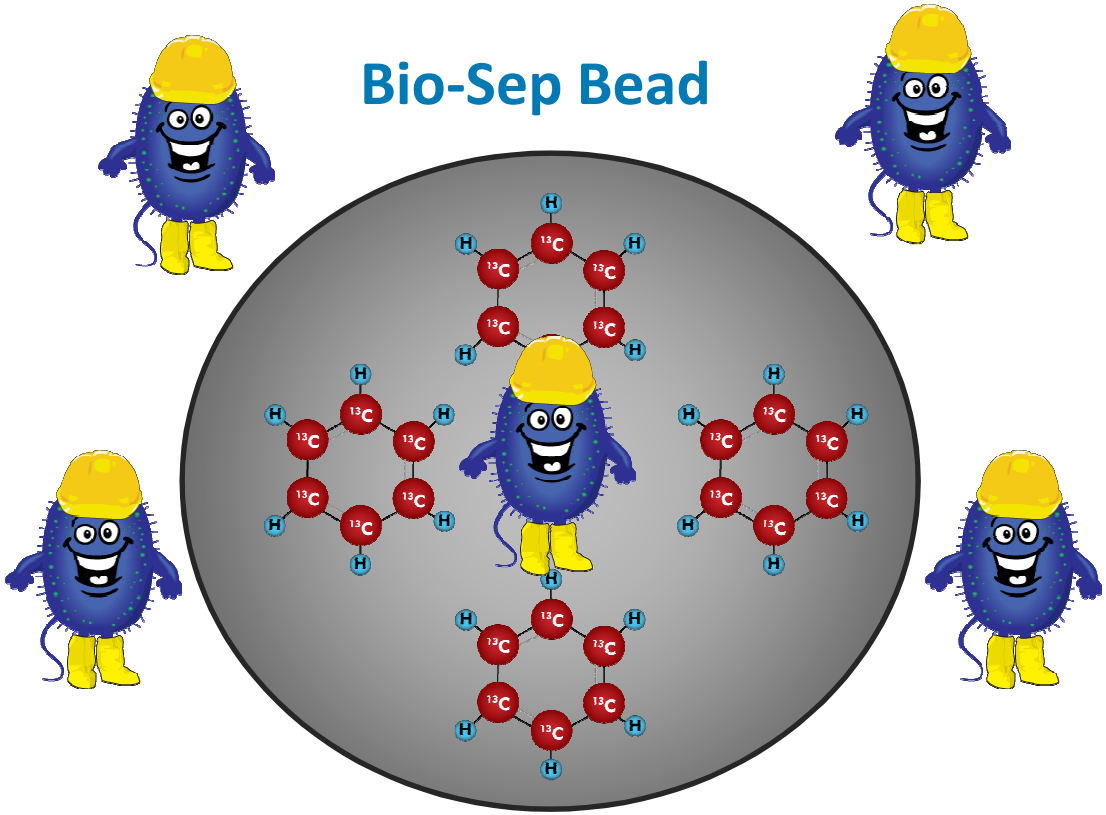
Microbes colonize beads



^{13}C-labeled compounds sorbed to Bio-Sep® beads

Bio-Trap colonized by indigenous microorganisms

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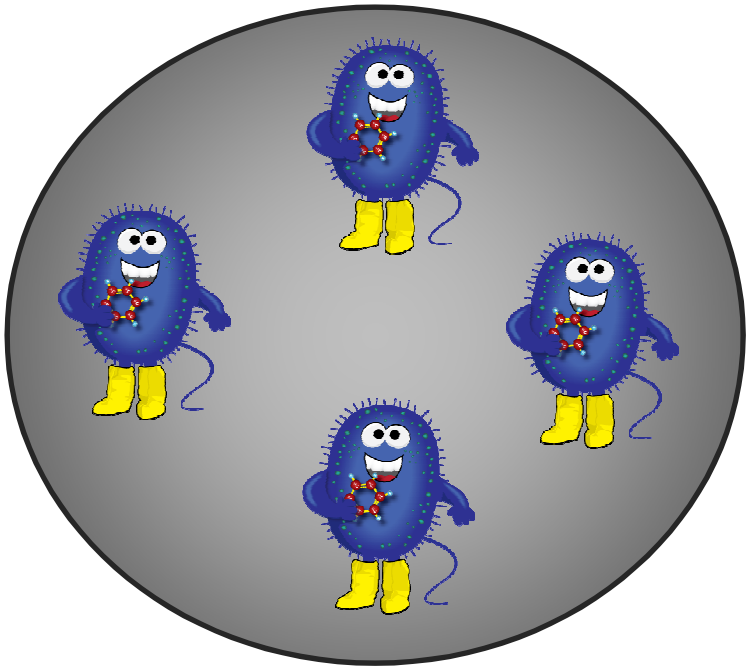
Microbes utilize target compound

Bio-Sep Bead



Some microbes that colonized the Bio-Sep® bead can utilize ^{13}C labeled target compound.

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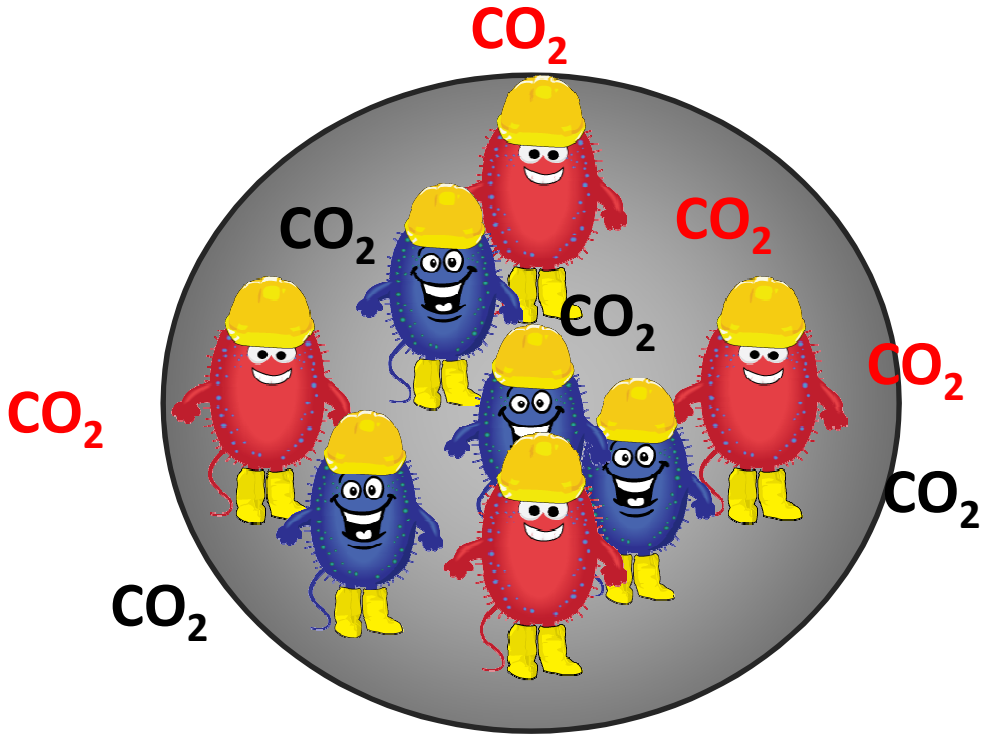
^{13}C Incorporation into biomass and CO_2



^{13}C is incorporated into new cells growing in the beads and in CO_2

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Unit of measure

Amount of ^{13}C relative to ^{12}C is expressed by the $\delta^{13}C$ notation

$$\delta^{13}C\,[‰] = \left(\frac{(^{13}C/^{12}C)_{\text{Sample}}}{(^{13}C/^{12}C)_{\text{Standard}}} - 1 \right) \cdot 1000$$

The standard is a specific carbon-containing mineral from a specific location: Pee Dee Belimnite (PDB)

Units of $\delta^{13}C$ are $^{o}/_{oo}$ or "per mill"

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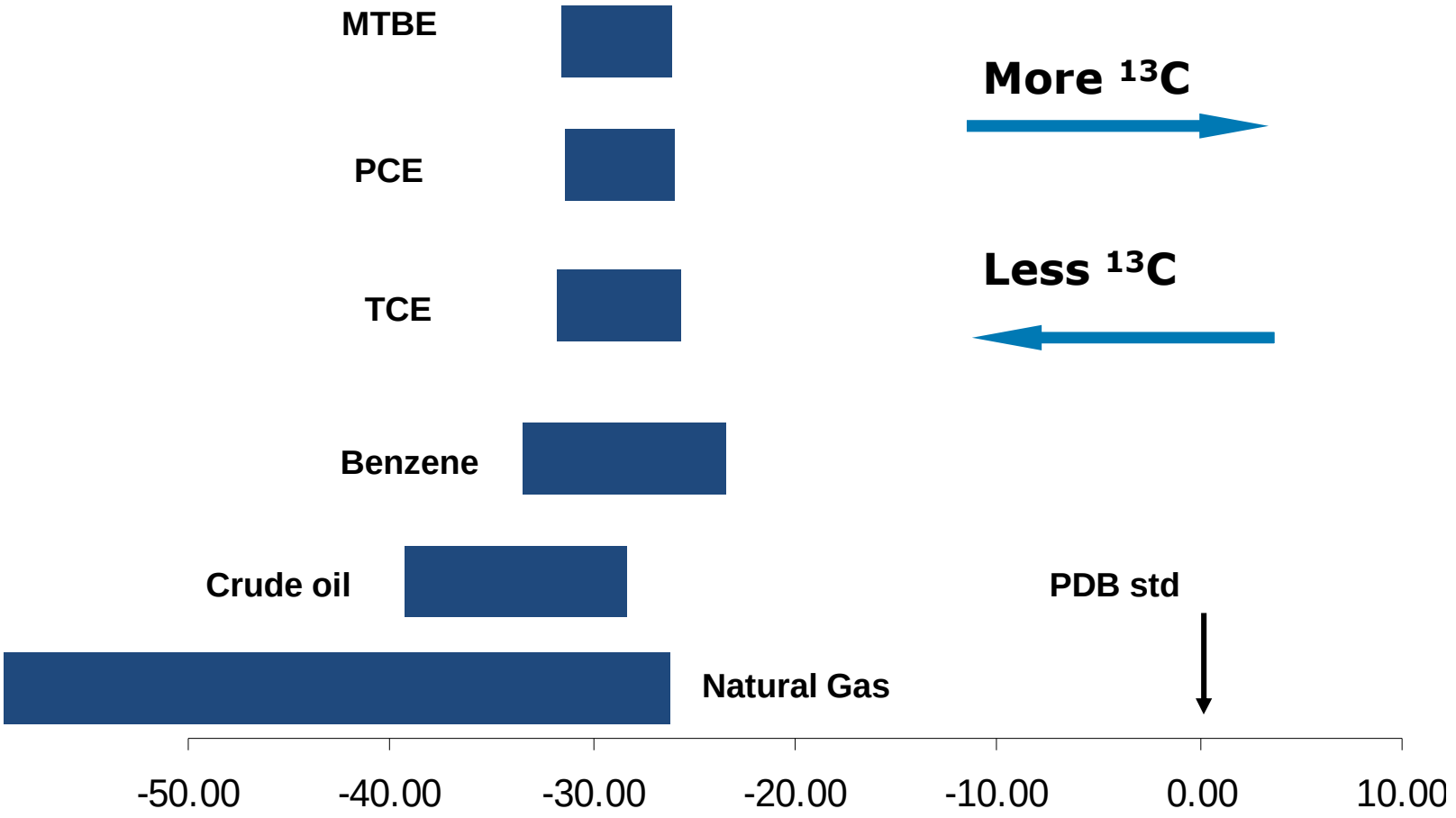
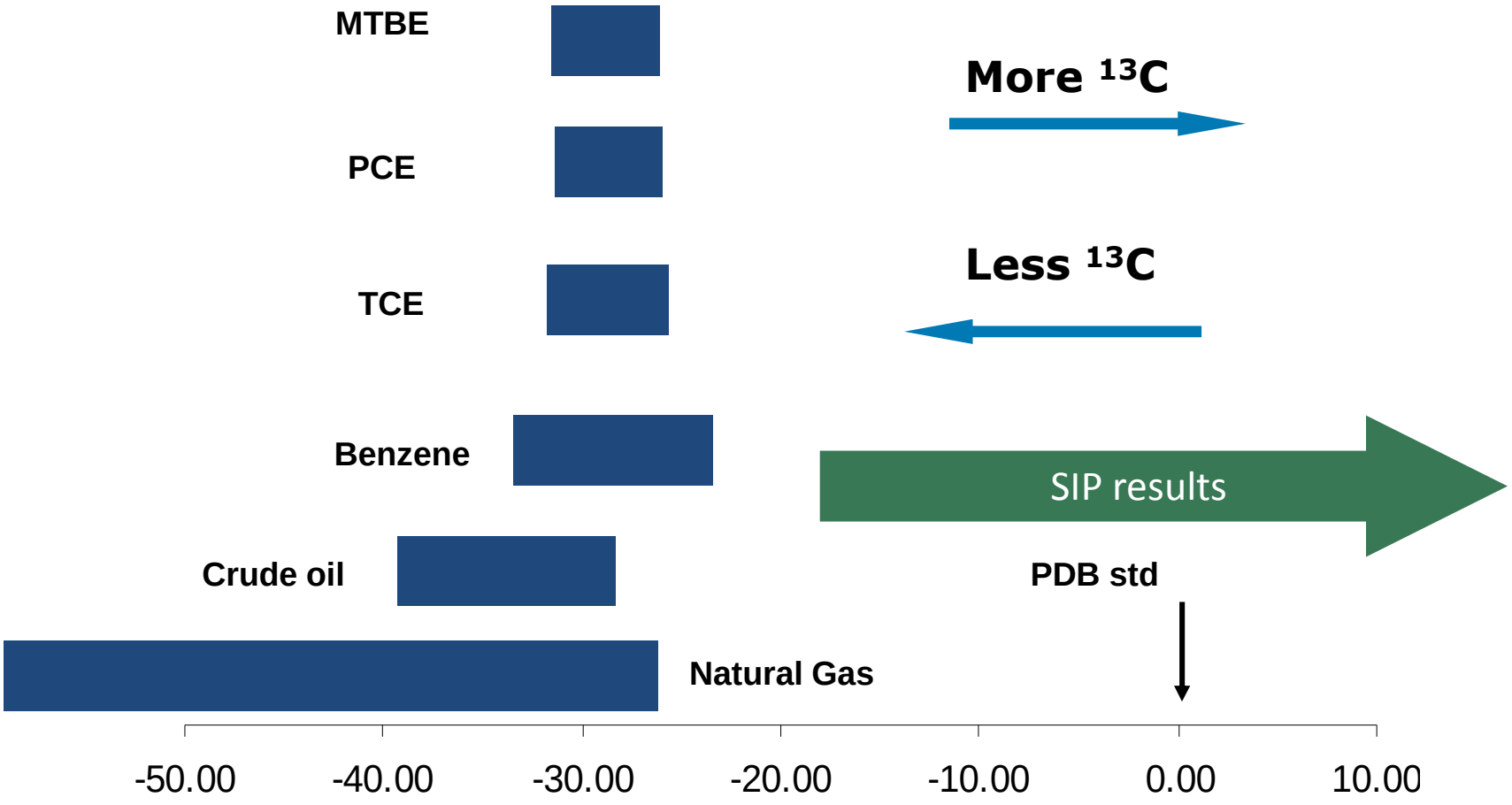
$\delta^{13}C$ of COCs



$\delta^{13}C$ of COCs



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