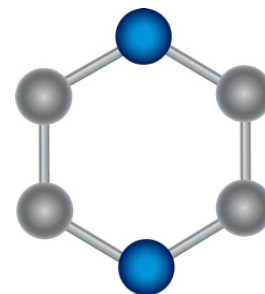


# Stable Isotope Probing Demonstrates In Situ Biodegradation of 1,4-Dioxane

Dora Ogles (Microbial Insights)

James Hatton (CH2MHILL)

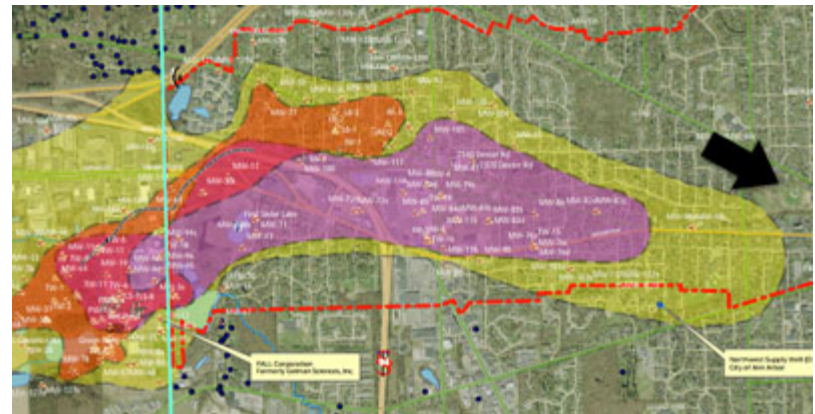
## 1,4-Dioxane Sources



- Stabilizer for chlorinated solvents (1,1,1-TCA)
- Solvent for paper, cotton, and textile processing
- Inadvertent byproduct in surfactant production

# Physical/Chemical Properties

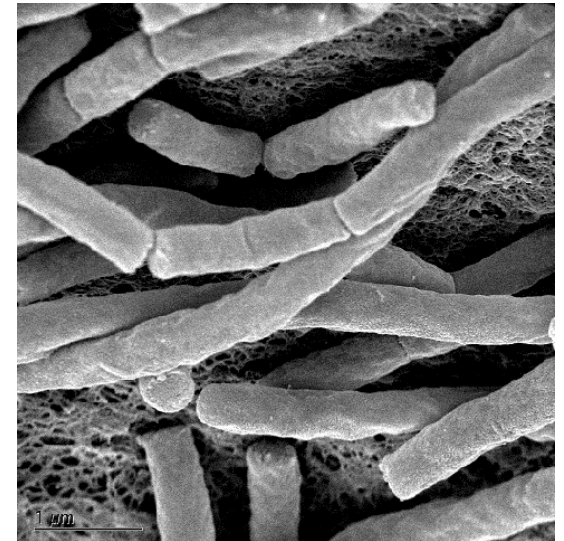
- Probable human carcinogen (0.35  $\mu\text{g}/\text{L}$ )
- Miscible with water
- Low sorption
- Relatively low volatilization



Department of Public Health, Washtenaw County, Michigan

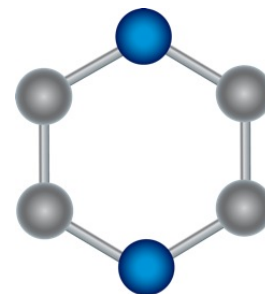
# Biodegradation

- Aerobic Growth Supporting
  - *Cordyceps sinensis* (fungus)
  - *Mycobacterium* sp. PH-06
  - *Pseudonocardia dioxanivorans* CB1190

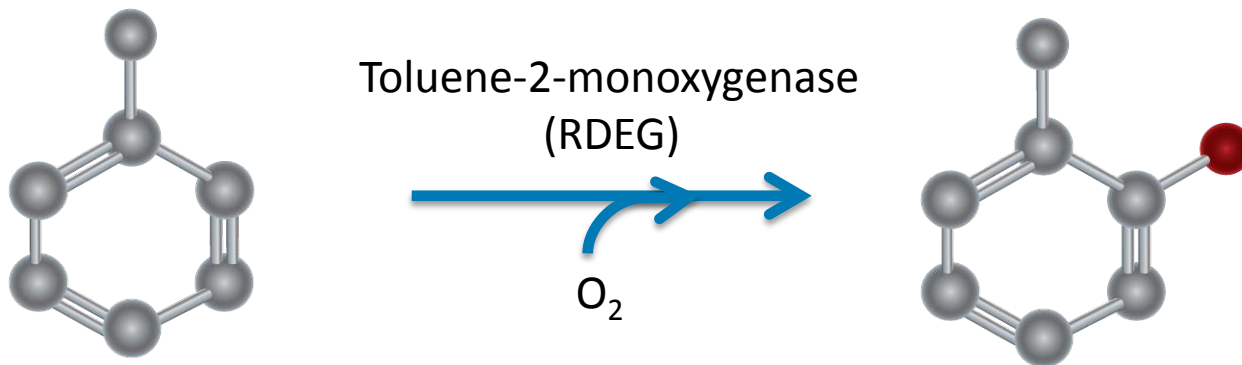


(Mahendra & Alvarez-Cohen, IJSEM, 2005)

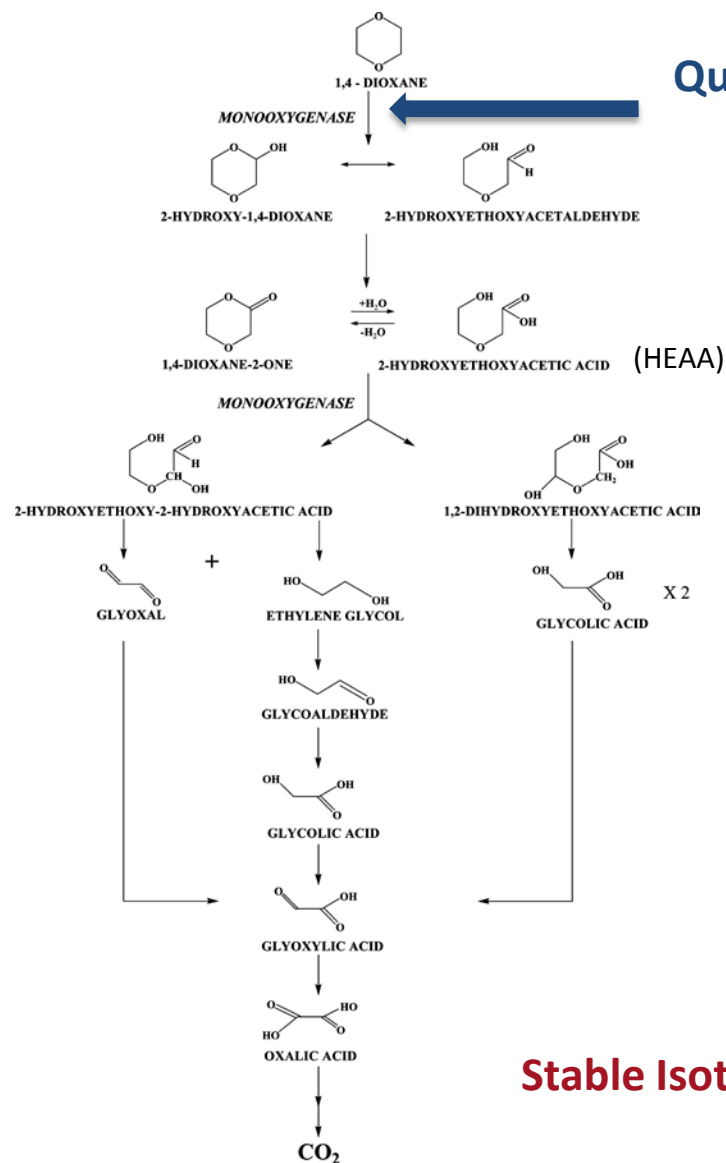
# Biodegradation



- Aerobic Growth Supporting
- Aerobic Co-oxidation
  - Ring hydroxylating toluene monooxygenases (RMO,RDEG,PHE)
  - Soluble methane monooxygenase (sMMO)
  - Propane, alkenes, tetrahydrofuran (THF), etc.



# Pathway



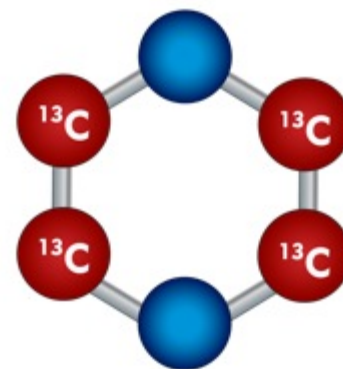
Quantitative Polymerase  
Chain Reaction  
(qPCR)

Mahendra et al. 2007  
ES&T 41(21): 7330-7336

Stable Isotope Probing (SIP)

# Stable Isotope Probing (SIP)

- Specially produced “heavy” compounds which are composed of 99%  $^{13}\text{C}$ 
  - Similar characteristics as  $^{12}\text{C}$  contaminant
  - Similar environmental behavior
- Quantify the  $^{13}\text{C}$  “tracer” in end-products of degradation (biomass and  $\text{CO}_2$ )



# How do Bio-Traps work?

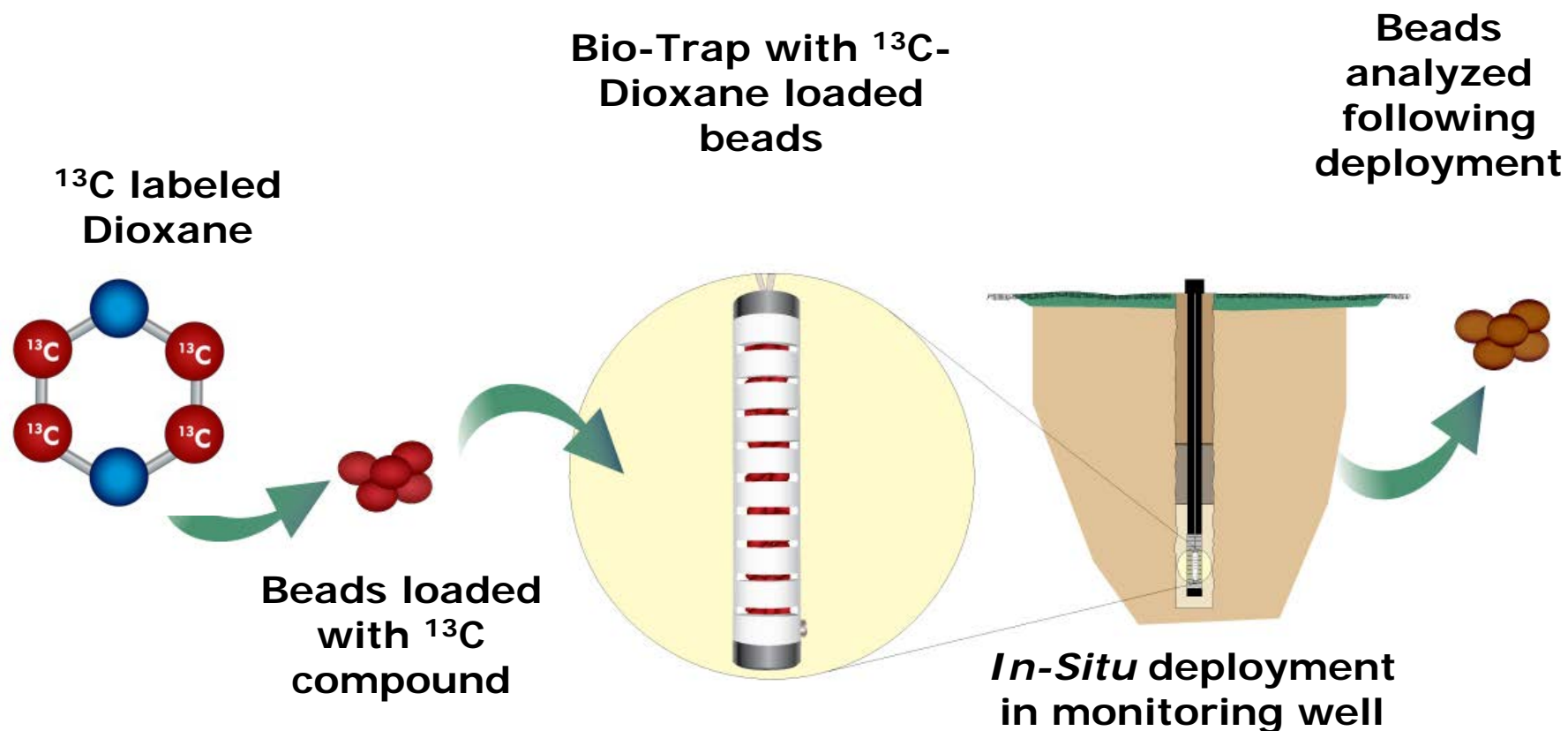
## Properties of Bio-Sep Beads

- 25% Nomex & **75% PAC**
- 3-4 mm in diameter
- 74% porosity
- 600 m<sup>2</sup> of surface area/g
- Heat sterilized 300°C



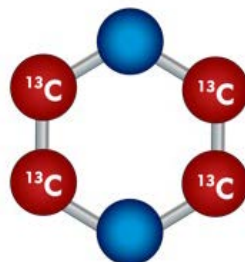
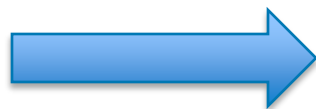


# Overview of SIP Approach



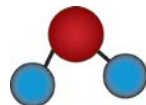
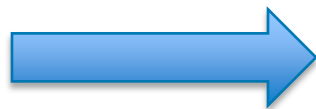
# Bio-Trap SIP Analysis

Residual  $^{13}\text{C}$ -Compound



Contaminant Loss

$^{13}\text{C}/^{12}\text{C}$  Dissolved Inorganic Carbon (DIC)



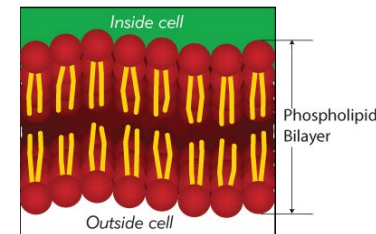
Mineralization

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



PLFA  
DNA  
RNA

Biomass



# Site Management Options

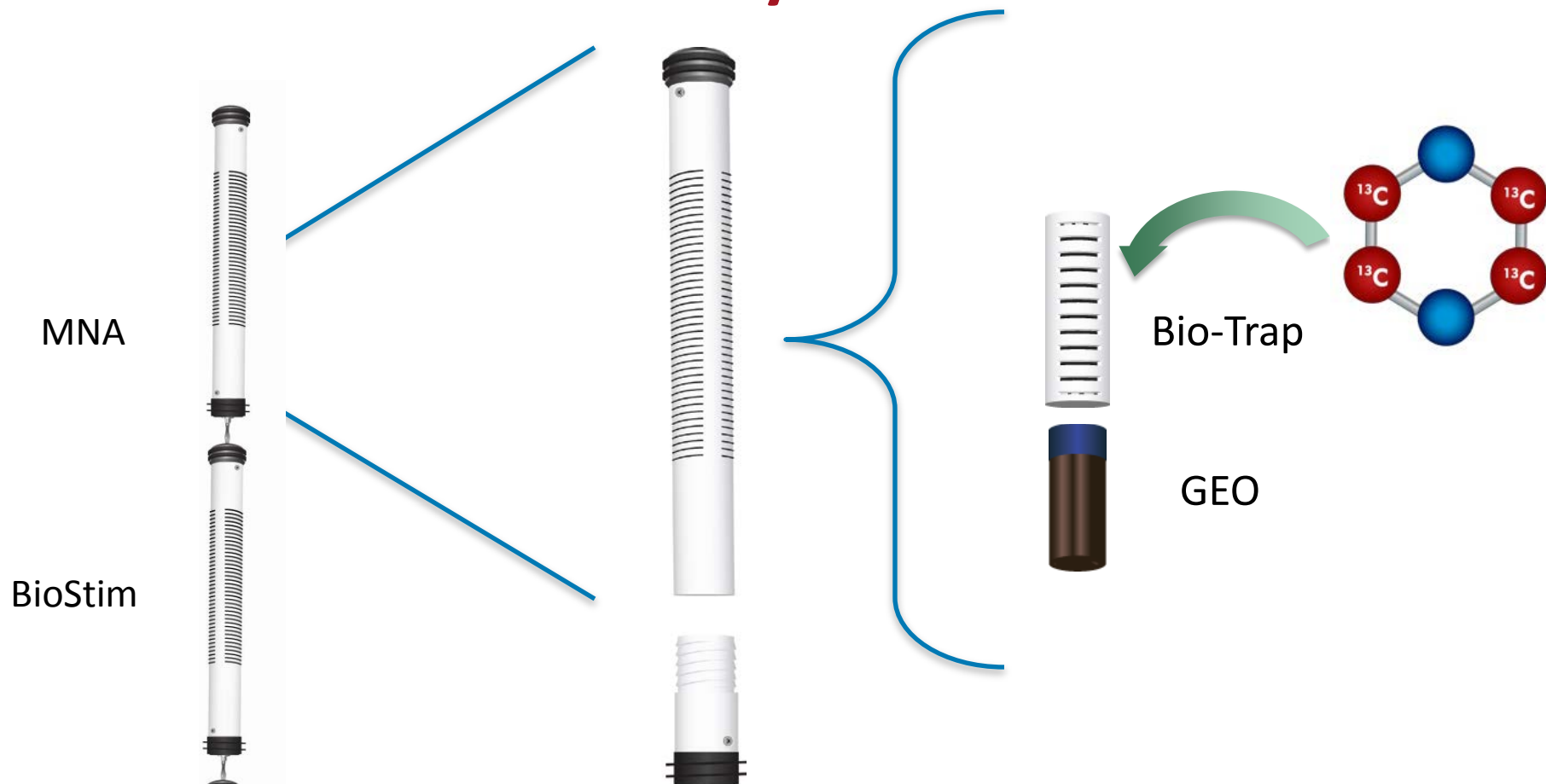
- **Monitored Natural Attenuation (MNA)**
- **Biostimulation (BioStim)**
  - Butanol addition
  - Kelley et al. (2001)
    - Bioaugmented phytoremediation
    - Butanol addition stimulated growth of CB1190

MNA

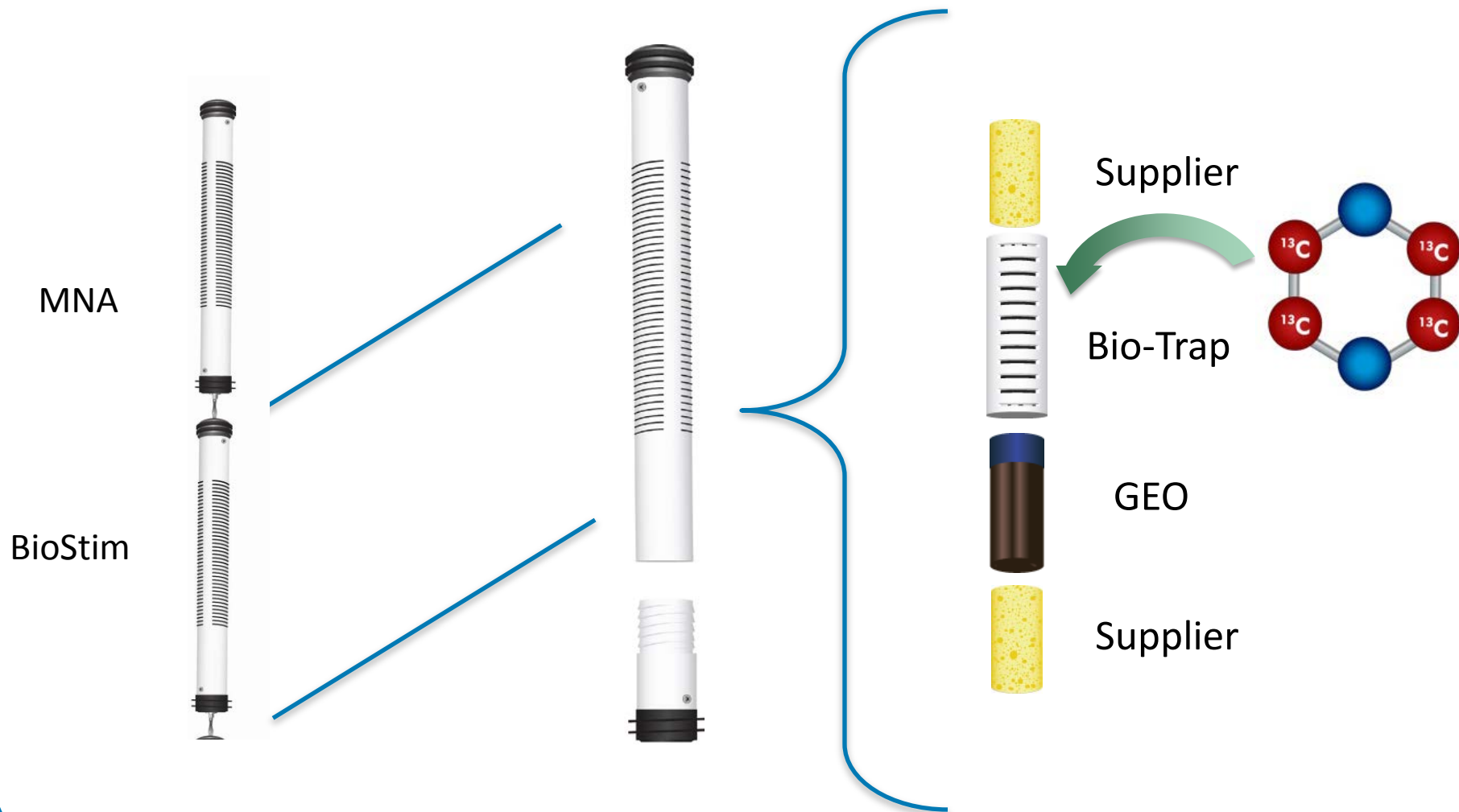
BioStim



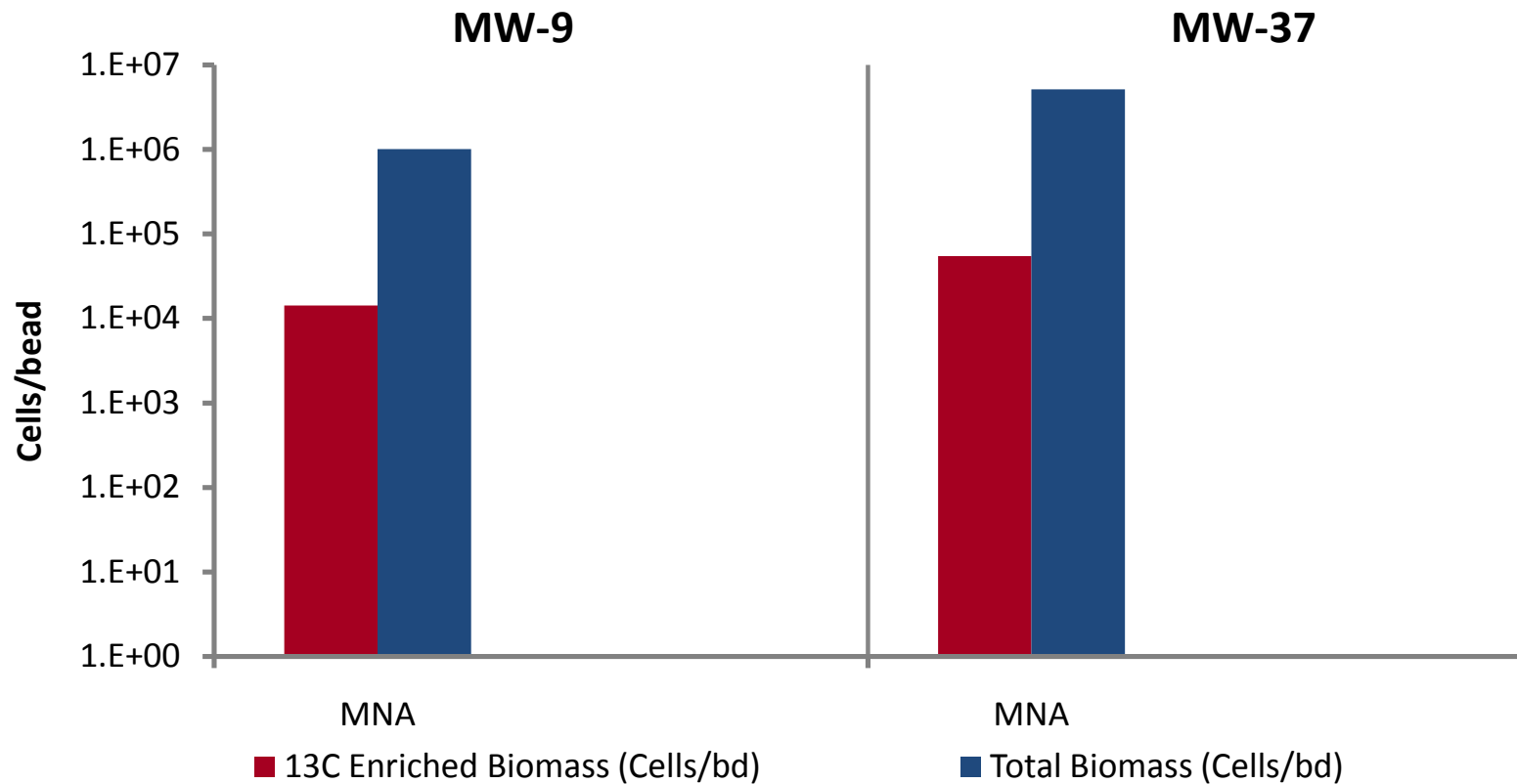
# *In Situ* Microcosm Study – MNA Unit



# *In Situ* Microcosm – BioStim Unit



# $^{13}\text{C}$ Incorporation into Biomass



## Unit of measure

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

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

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

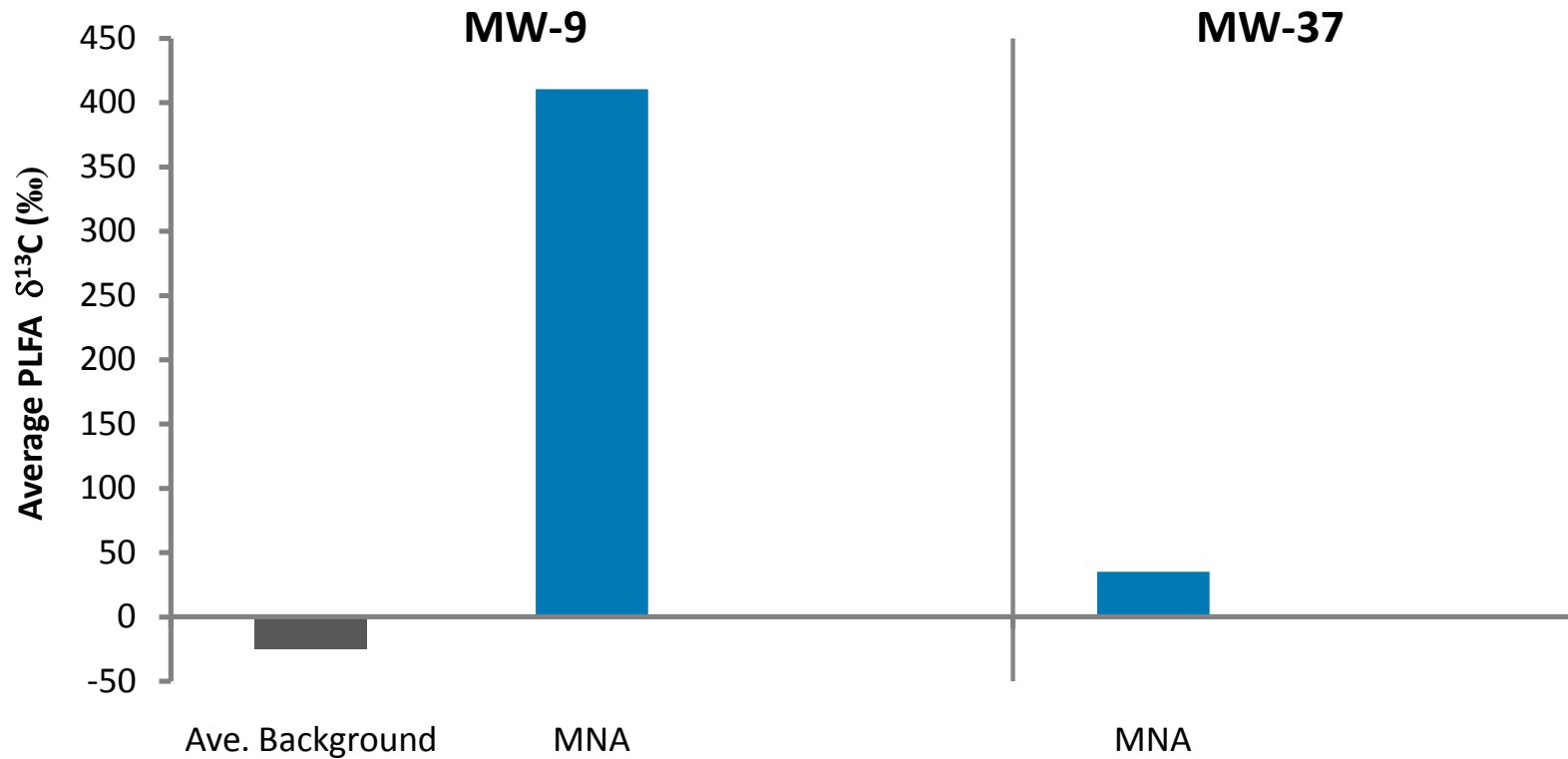
**Units of  $\delta^{13}\text{C}$  are ‰ or “per mill”**

# $^{13}\text{C}$ Incorporation into DIC

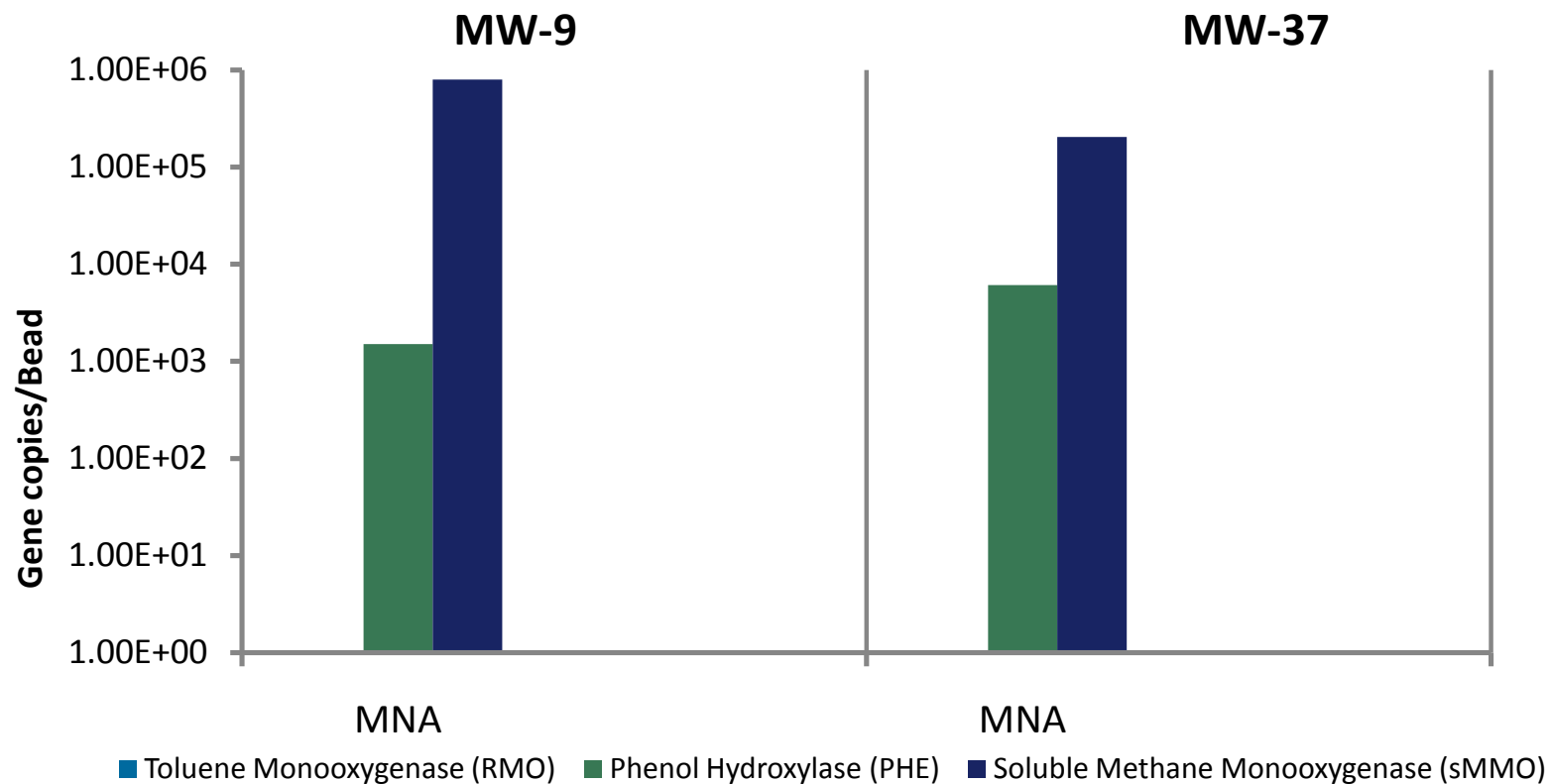




# $^{13}\text{C}$ Incorporation into Biomass



# Co-oxidation Potential



# Conclusions – MNA Units

## qPCR

Moderate abundances of genes encoding oxygenases capable of co-oxidation of dioxane

## Incorporation into Biomass

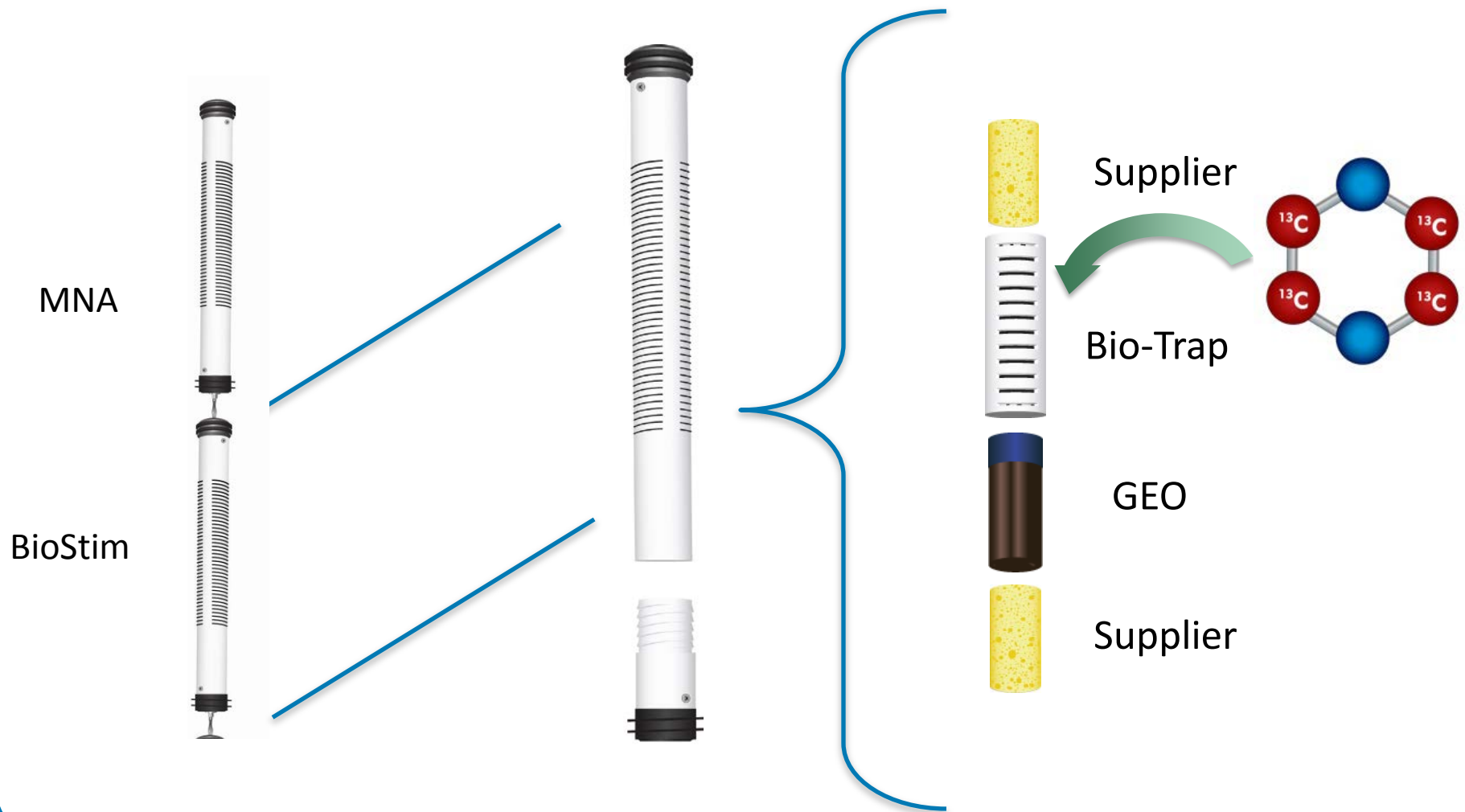
Detection of  $^{13}\text{C}$  enriched PLFA demonstrated that dioxane biodegradation occurred within the passive microbial sampler

## Incorporation into DIC

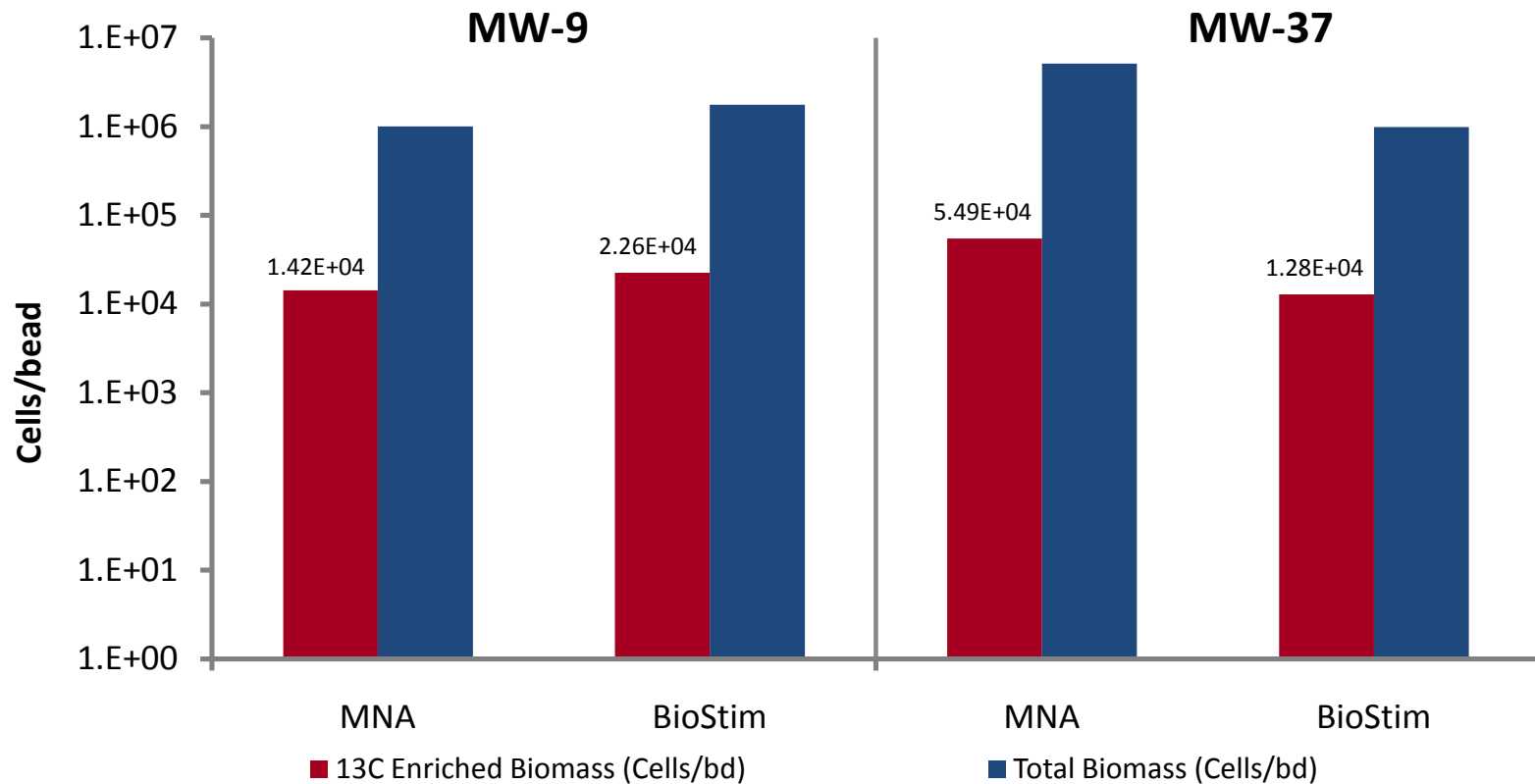
Although low,  $^{13}\text{C}$  enriched DIC was detected indicating dioxane mineralization had occurred



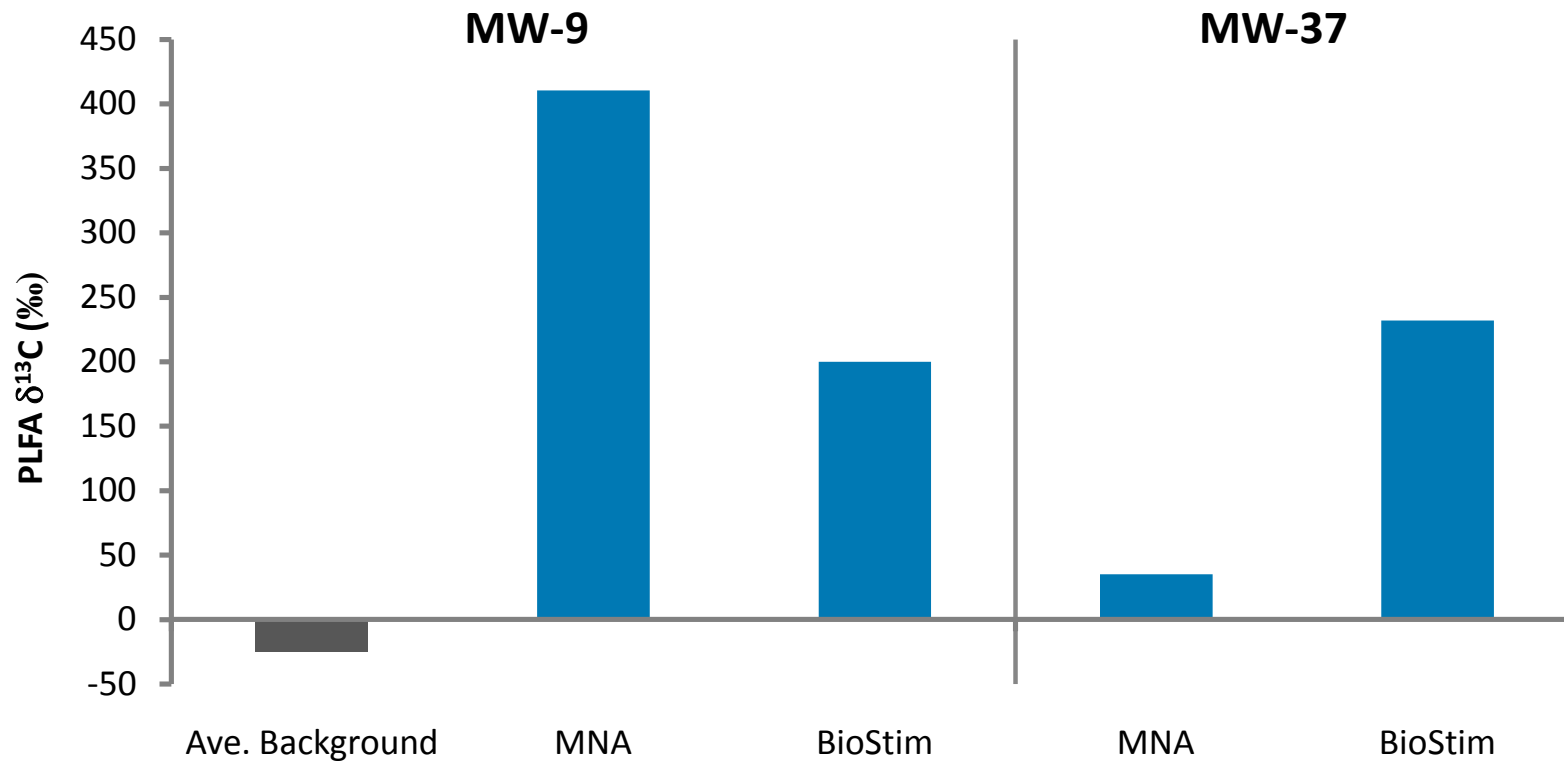
# *In Situ* Microcosm – BioStim Unit



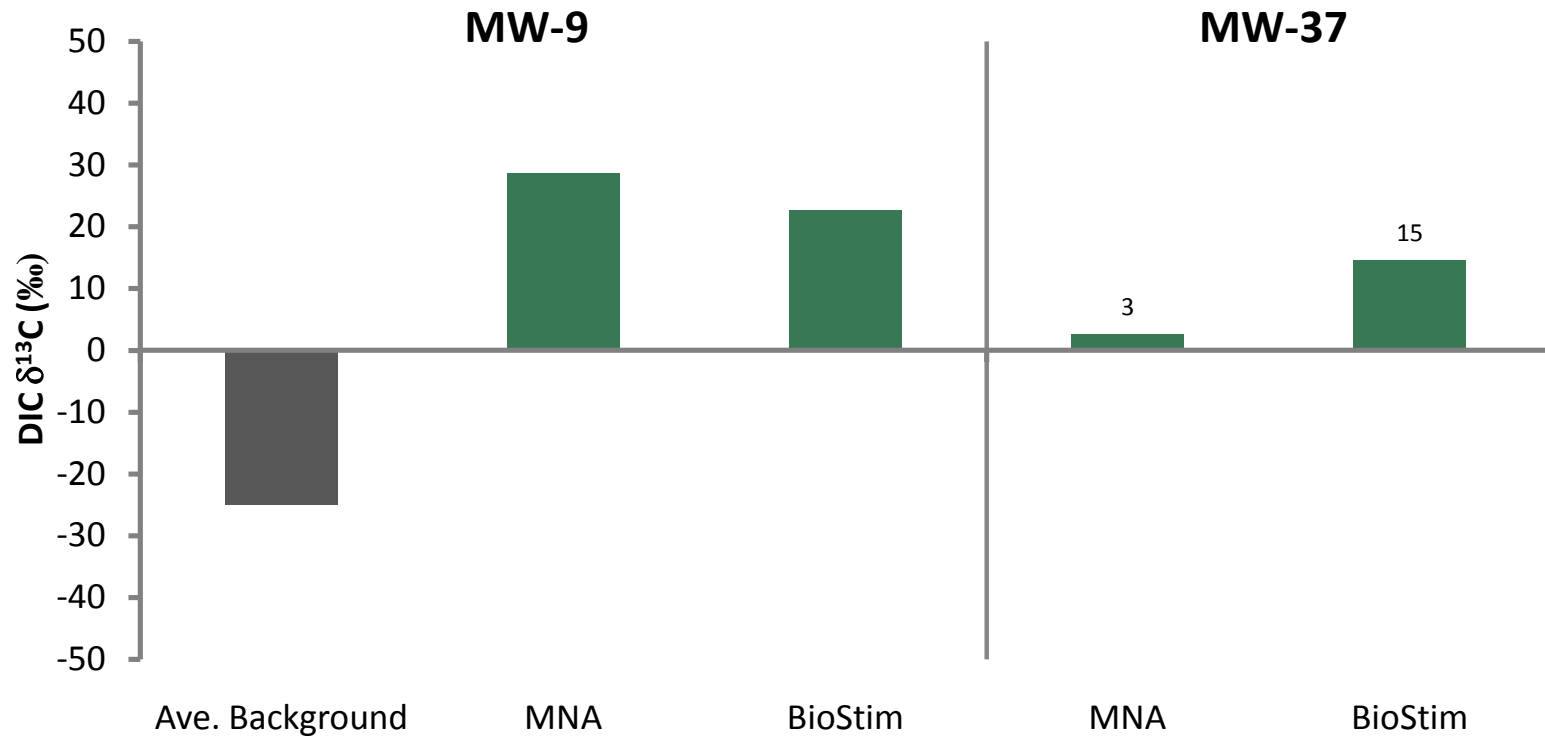
# $^{13}\text{C}$ PLFA – MNA vs BioStim



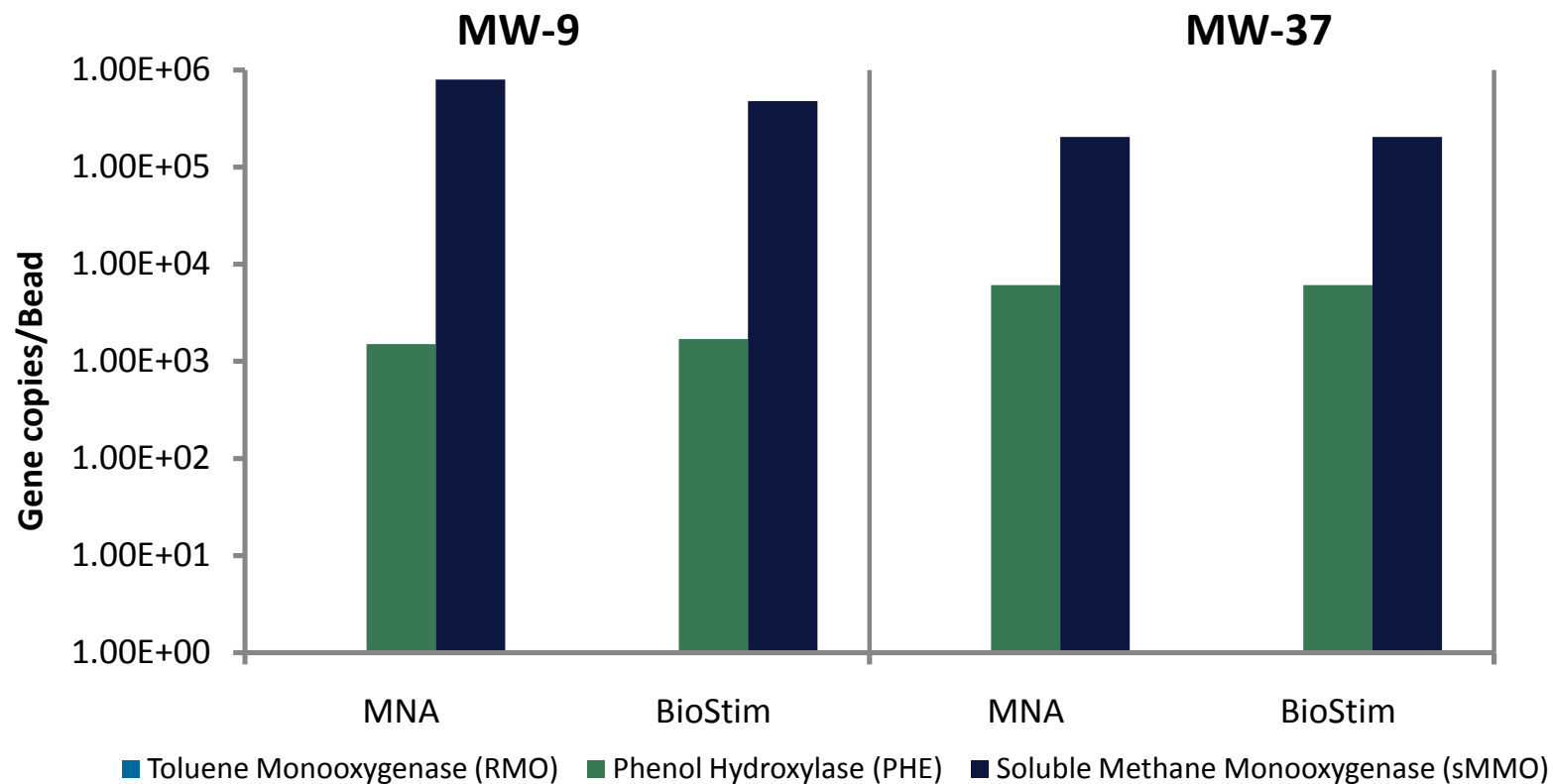
## $^{13}\text{C}$ PLFA – MNA vs BioStim



# $^{13}\text{C}$ Incorporation into DIC



# Co-oxidation Potential





# Conclusions – BioStim Units

## qPCR

Confirmed potential for co-oxidation

## Stable Isotope Probing

Demonstrated that  $^{13}\text{C}$  dioxane biodegradation and mineralization occurred in situ

## BioStimulation (butanol addition)

Overall results did not conclusively demonstrate enhanced biodegradation vs MNA



# Conclusions

- Dioxane biodegradation occurred under existing site conditions at both locations
- Bacteria harboring oxygenase genes capable of dioxane co-oxidation were present
- Butanol addition may not have appreciably enhanced dioxane biodegradation

# SIP Advantages

- Relatively conclusive evidence of contaminant biodegradation in situ
- No knowledge of biodegradation pathway or organisms responsible is needed.
- Applicable to a wide variety of common contaminants



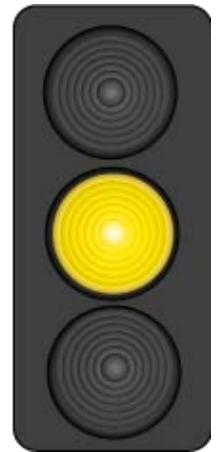
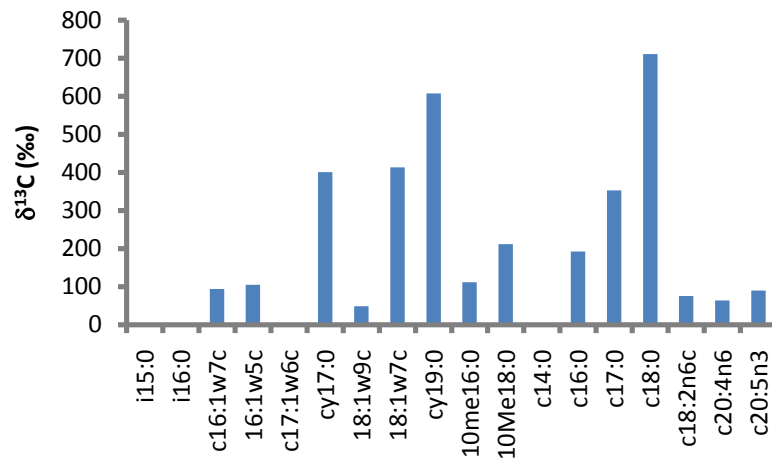
# SIP Limitations

- Greater contaminant concentrations in sampler
- Potential desorption (dioxane)
- Some  $^{13}\text{C}$  compounds can be expensive to synthesize



# SIP Limitations

- Generally not appropriate for compounds used as electron acceptors
- SIP-PLFA cannot identify degraders



**Questions ???**