

Using DNA Stable Isotope Probing to Identify MTBE and TBA Degraders *In Situ*

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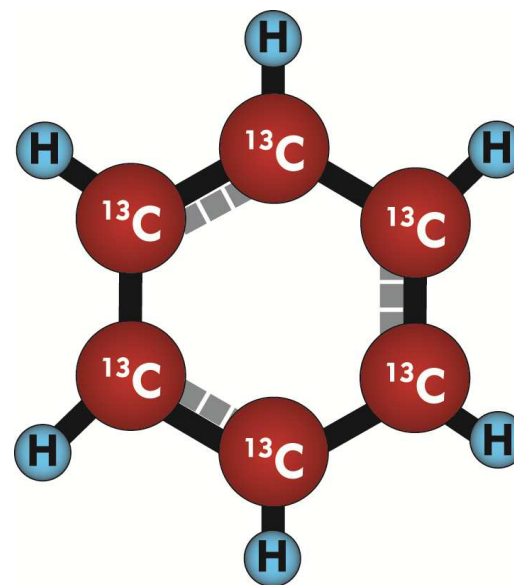
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Stable Isotope Compounds

- Specially produced “heavy” compounds which are composed of 99+% ^{13}C
 - Natural compounds are 1.1% ^{13}C
 - Very similar chemical and biological characteristics as original compound
- Used as “tracers” to increase our understanding of contaminant fate



How do we do stable isotope probing?

- Obtain a ^{13}C -labeled compound from a vendor
- Introduce the compound to the environment of interest and incubate
 - Soil
 - Groundwater
 - Microcosm
- Recover ^{13}C -labeled material and look for ^{13}C (usually involves GC-IRMS)
 - Residual compound
 - To assess biodegradation
 - Biomolecules of interest
 - DNA or RNA
 - Phospholipids
 - Proteins (^{13}C or ^{15}N)
 - Biodegradation products
 - Carbon dioxide or methane

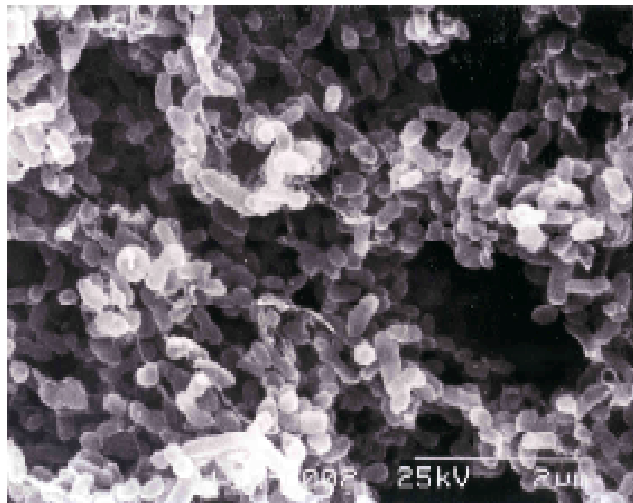
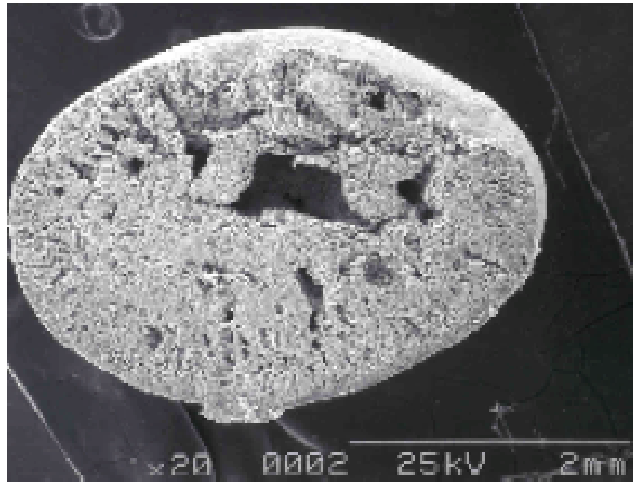
What do we learn from stable isotope probing?

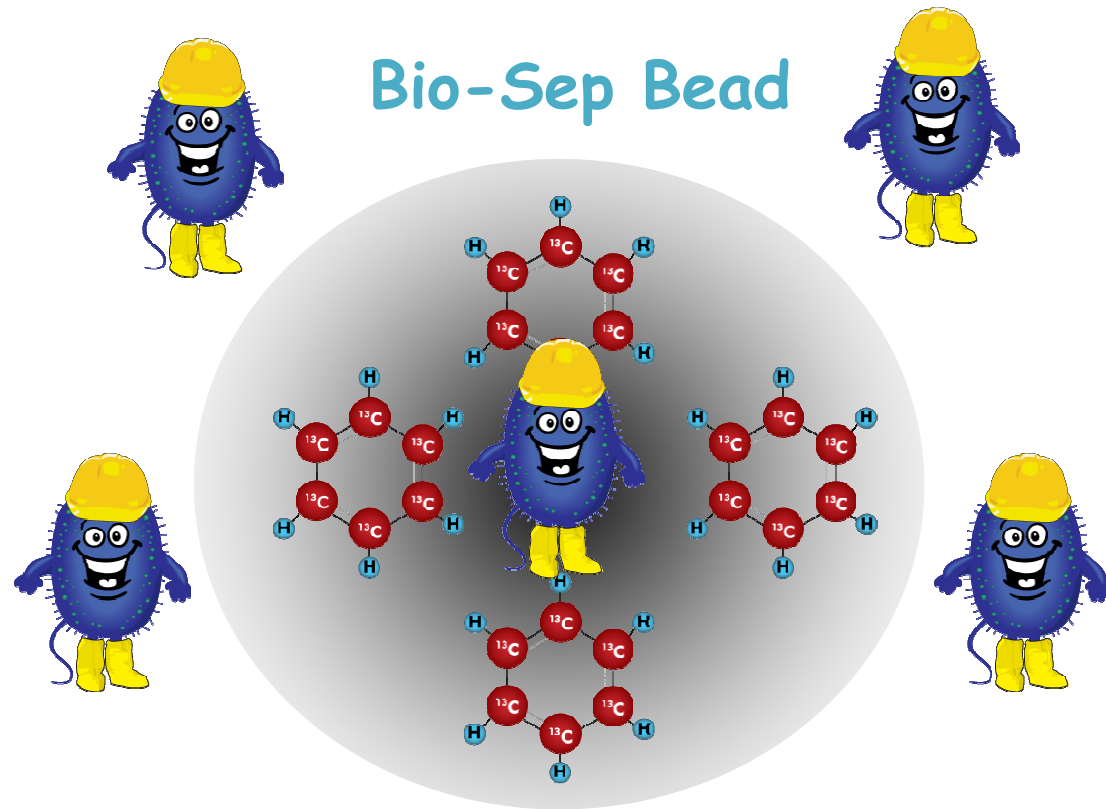
- Finding ^{13}C in biomass (usually membrane lipids) or biodegradation products indicates that biodegradation potential for the labeled compound exists in the sampled environment and that potential was expressed under *in situ* conditions (soil and groundwater)
- What organisms are involved in processing a ^{13}C -labeled compound?
 - DNA
 - ^{13}C - and ^{12}C -DNA separated by ultracentrifugation
 - Sequencing of ^{13}C -labeled 16SrDNA genes can identify organisms involved in processing the ^{13}C -labeled compound
 - Differential labeling of a compound can even help identify microbes involved in processing different functional groups

Anchoring the ^{13}C -labeled compound

- Limiting dilution of the stable isotope during incubation in soil or groundwater greatly increases sensitivity of the method
 - Adsorption to activated carbon
 - Entrapment
- Bio-Sep[®] beads
 - 3-4 mm in diameter
 - 25% Nomex and 75% powdered activated carbon
 - 600 m² of surface area
 - Heat sterilized
 - Colonized by actively growing microorganisms

Bio-Sep[®] beads and Bio-traps

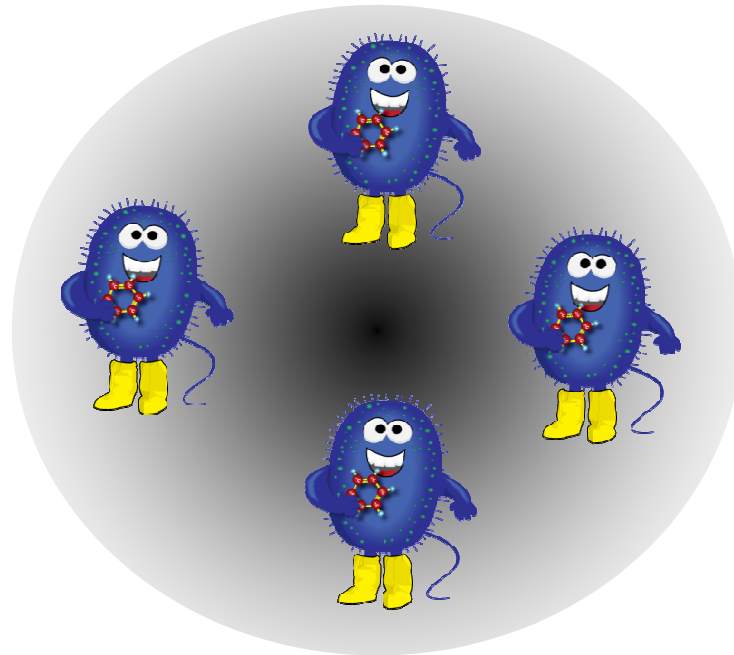




**^{13}C -labeled compounds sorbed to Bio-Sep[®] beads
Bio-Trap colonized by indigenous microorganisms**

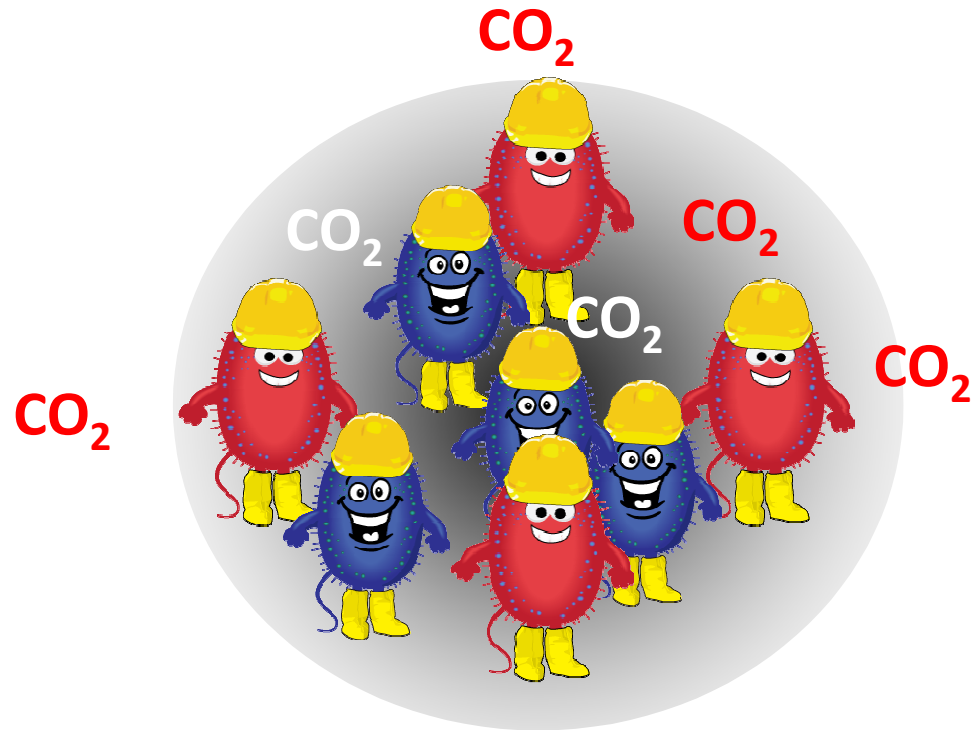
Microbes utilize target compound

Bio-Sep Bead



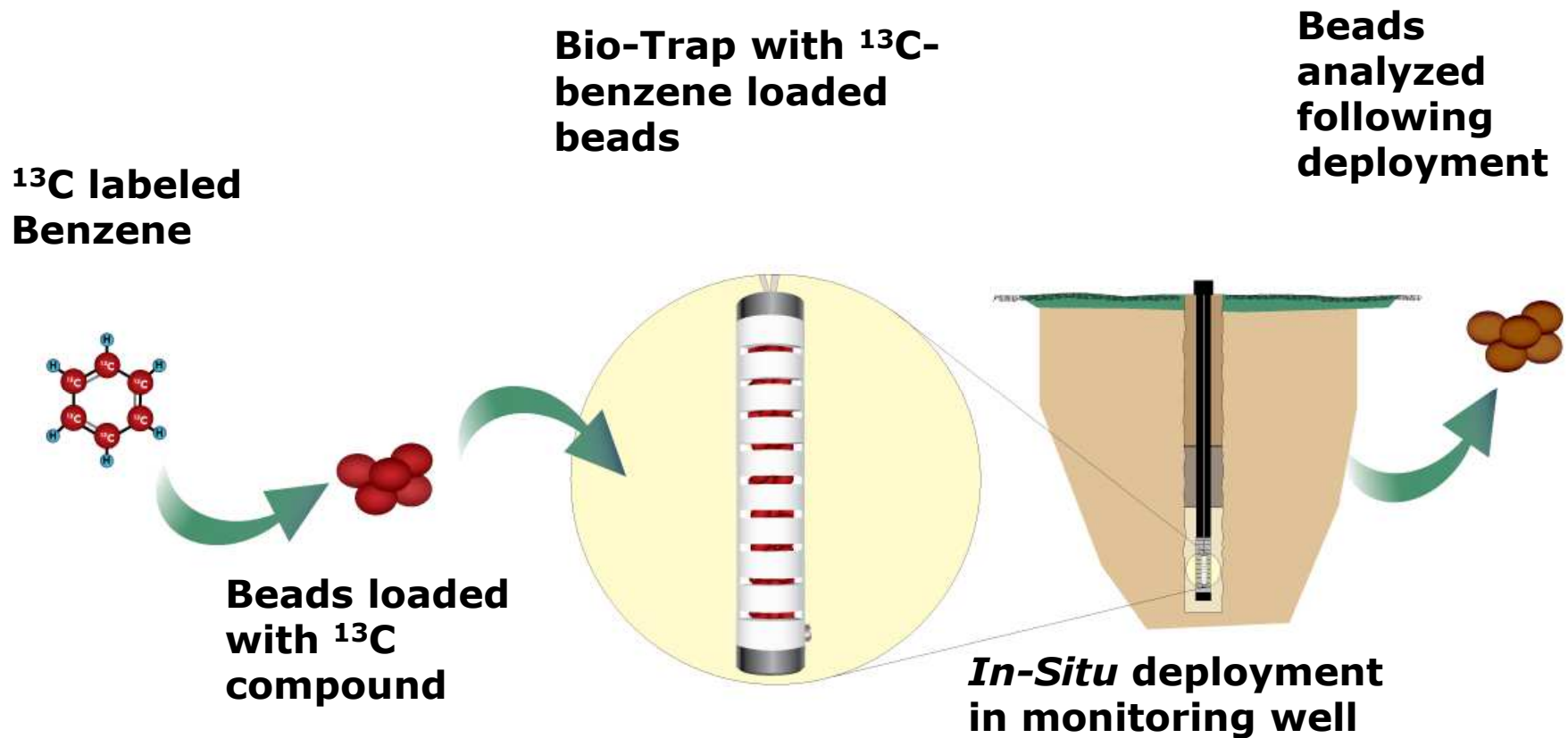
Some microbes that colonized the Bio-Sep[®] bead can utilize ^{13}C labeled target compound.

^{13}C incorporation into biomass and CO_2



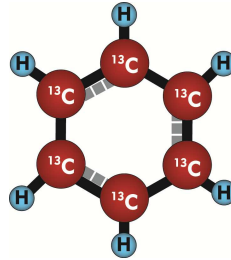
^{13}C is incorporated into new cells growing in the beads and in CO_2

Overview of Bio-Trap SIP Approach

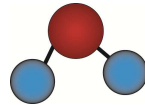


Bio-Trap SIP Analysis

Residual ^{13}C -Compound



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



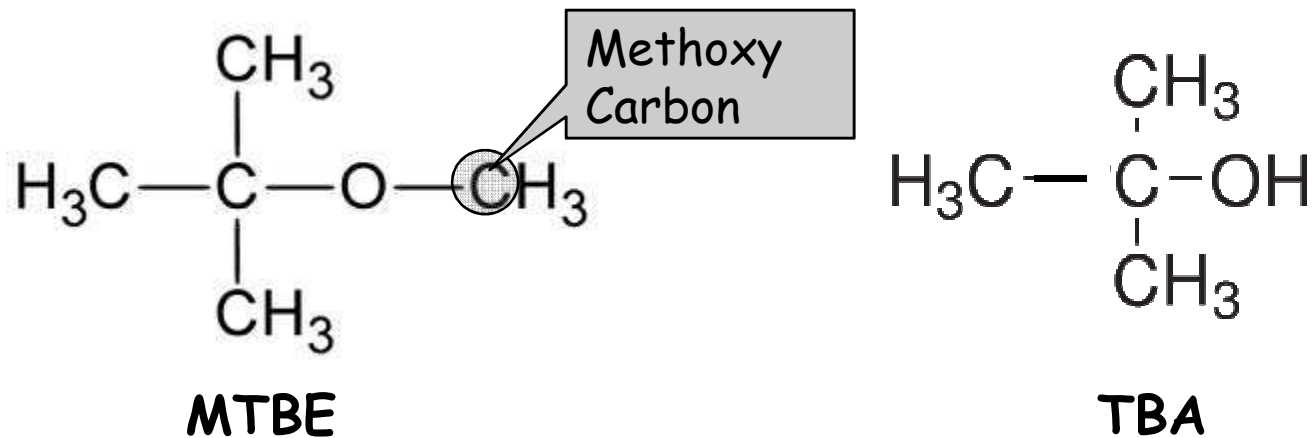
$^{13}\text{C}/^{12}\text{C}$ of Biomarkers



PLFA
DNA
RNA

Anaerobic MTBE and TBA Biodegradation

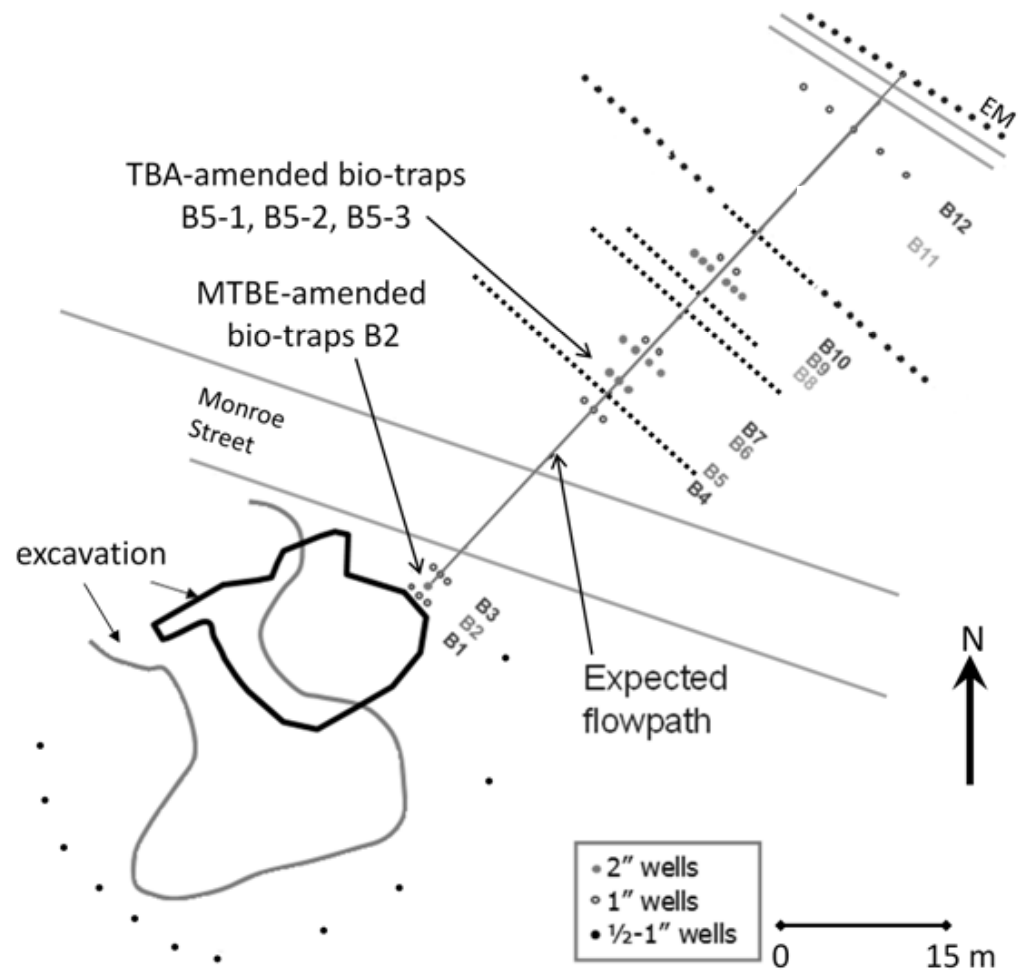
- No microorganisms capable of degrading MTBE or TBA anaerobically have been isolated
- O-demethylation thought to be first step in anaerobic MTBE degradation (acetogens)



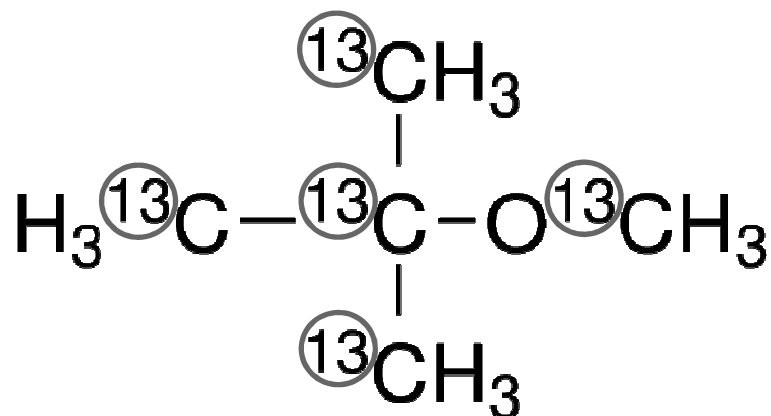
- TBA accumulation commonly observed

Vandenberg Air Force Base, Site 60

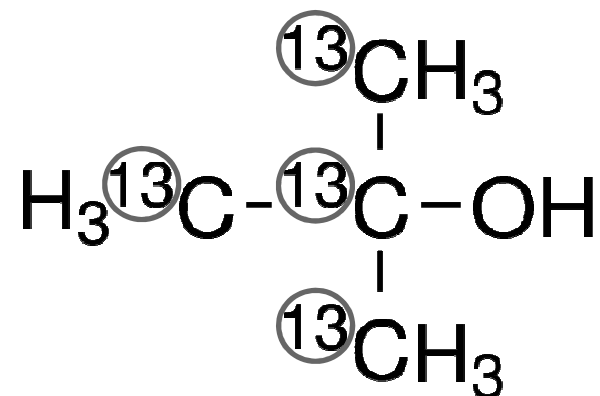
- 572 gal gasoline leak in 1994
- Source area excavation in 1995 and 2007
- Within 10 years BTEX had been degraded, but MTBE and TBA persisted
- Anaerobic, sulfate-reducing aquifer



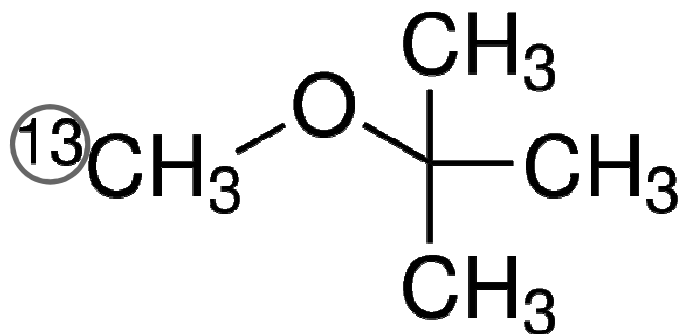
Contaminants used for DNA-SIP



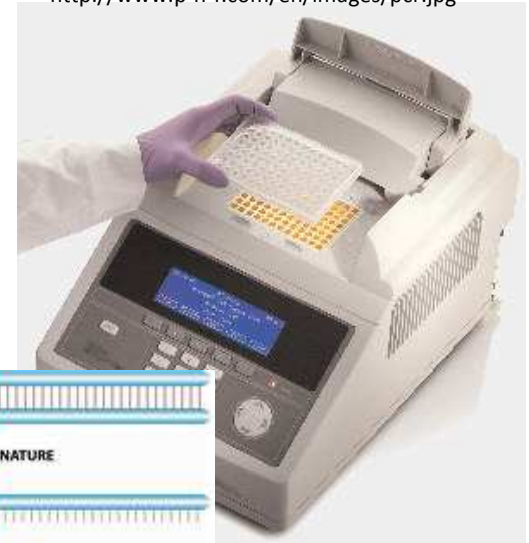
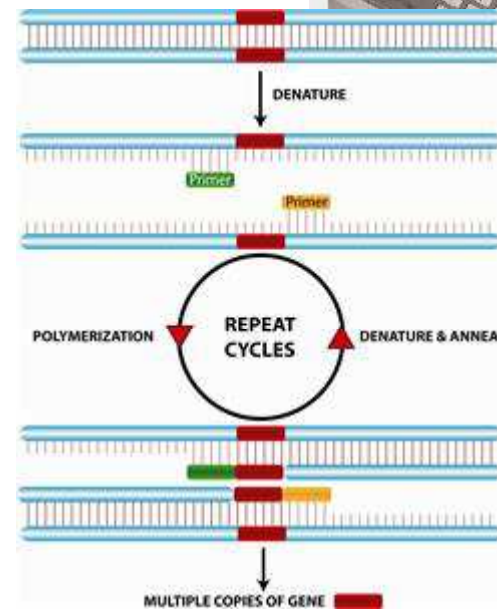
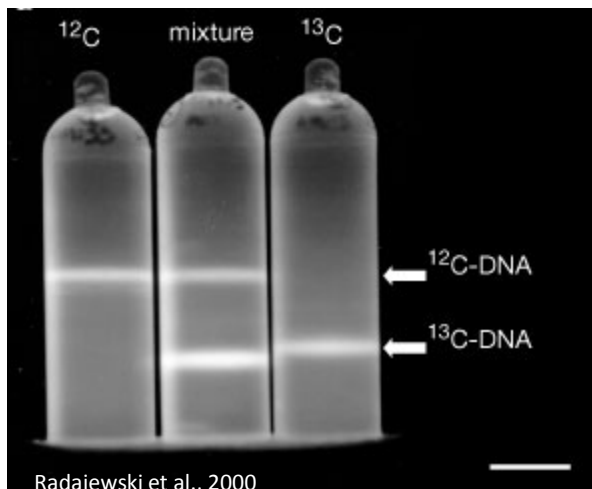
$^{13}\text{C}_5$ -MTBE:
13MALL and 12MALL clone
libraries



$^{13}\text{C}_4$ -TBA:
13TBA and 12TBA clone
libraries



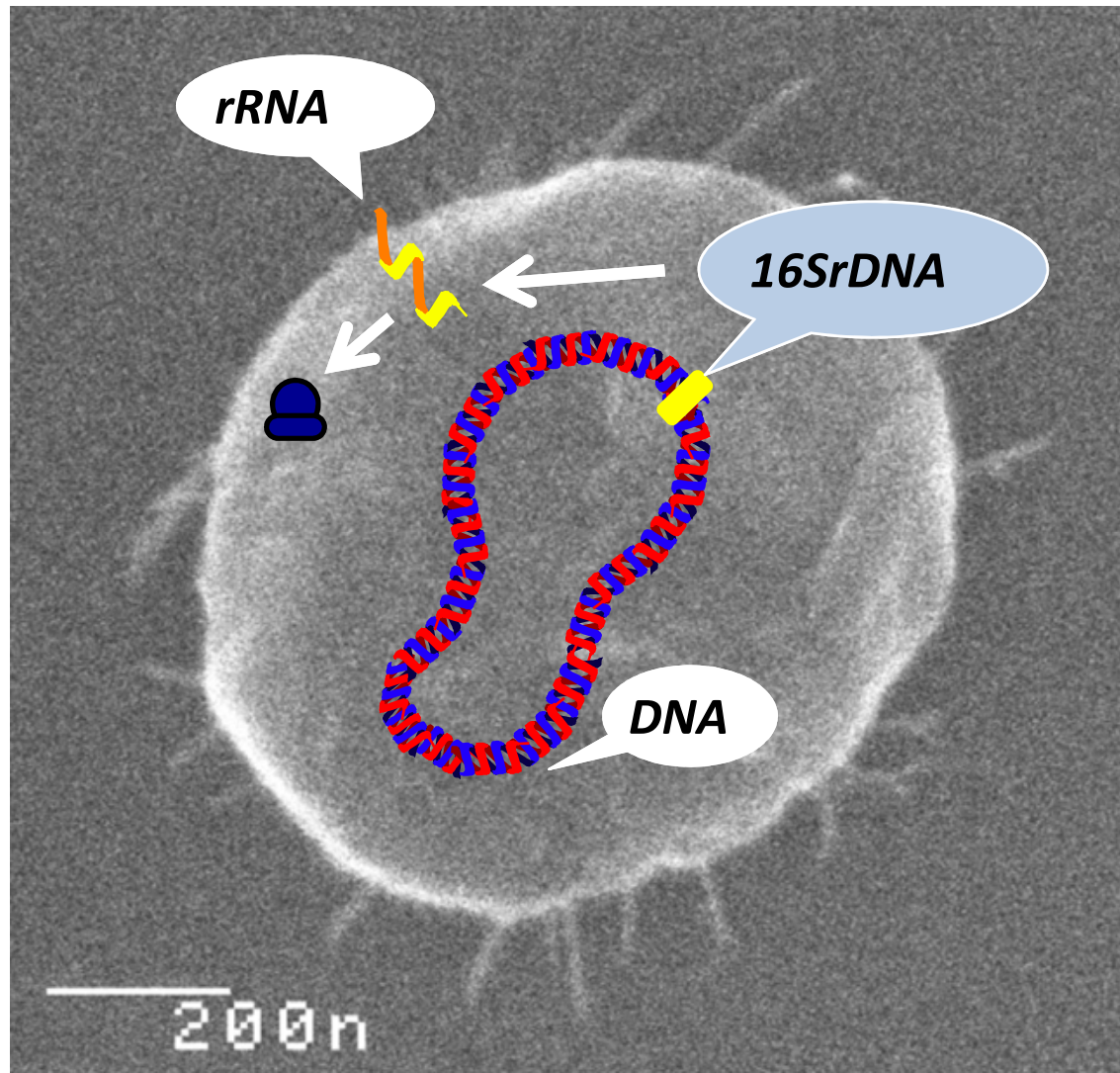
$^{13}\text{C}_1$ -MTBE:
13MTBE and 12MTBE clone libraries



DNA was extracted from Bio-Sep[®] beads and ^{12}C - and ^{13}C -DNA are separated by CsCl density gradient centrifugation

16S rRNA gene sequence of purified ^{12}C - and ^{13}C -DNA was amplified by PCR

16SrDNA is one of many genes in a microbe's DNA



The 16SrDNA gene is like an organisms
business card

mi
microbialinsights

Microbe Man

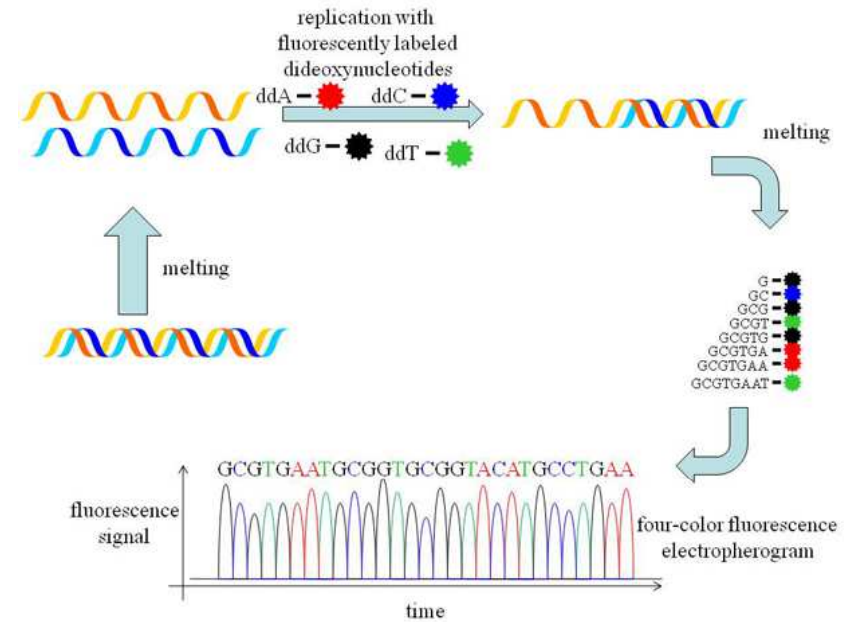
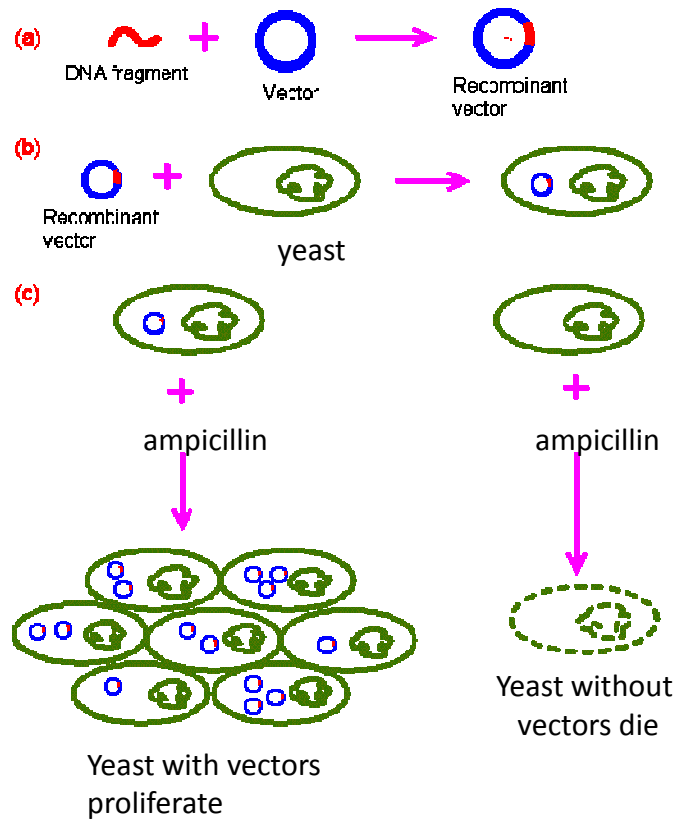
Diesel Degrading Specialist

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Rockford, TN 37853-3044

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microbe.com



<http://www.kirbyresearch.com/images/etc/textbook/mae76.jpg>

(d) Isolation of recombinant DNA clones

<http://www.web-books.com/MoBio/Free/images/Ch9A1.gif>

Clone libraries were created from the PCR products and sequenced

Sequence Analysis Summary

- 205 sequences were grouped into 60 OTUs
- Jaccard's Similarity Index indicated all clone libraries were dissimilar from each other

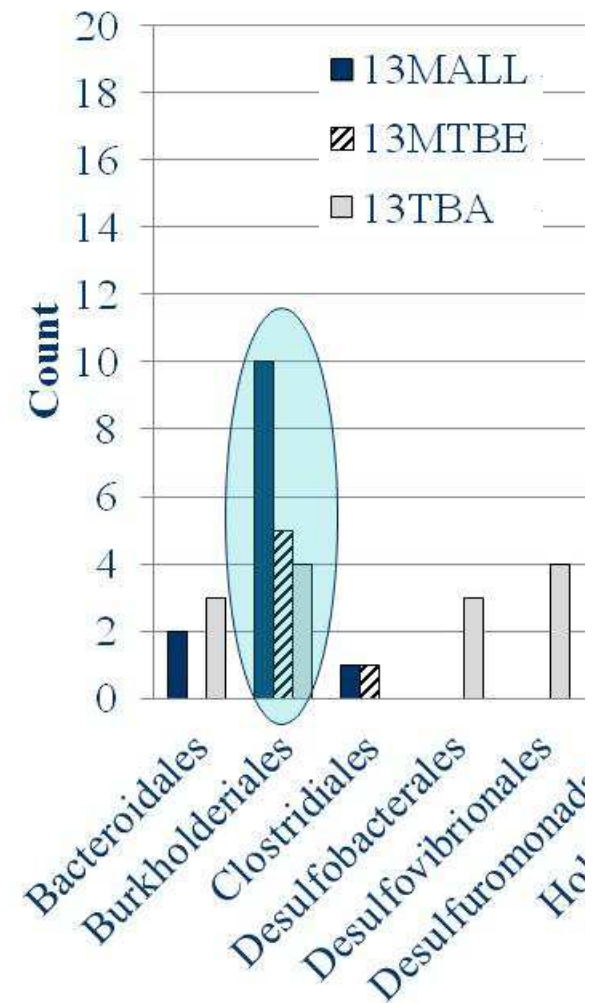
	#	#	%
	Sequences	OTUs*	Coverage**
13MTBE	41	7	93%
13MALL	42	20	69%
13TBA	42	23	69%
12MTBE	21	10	76%
12MALL	33	20	58%
12TBA	26	14	77%

*Operational taxonomic units based on 97% sequence similarity

**Good's method

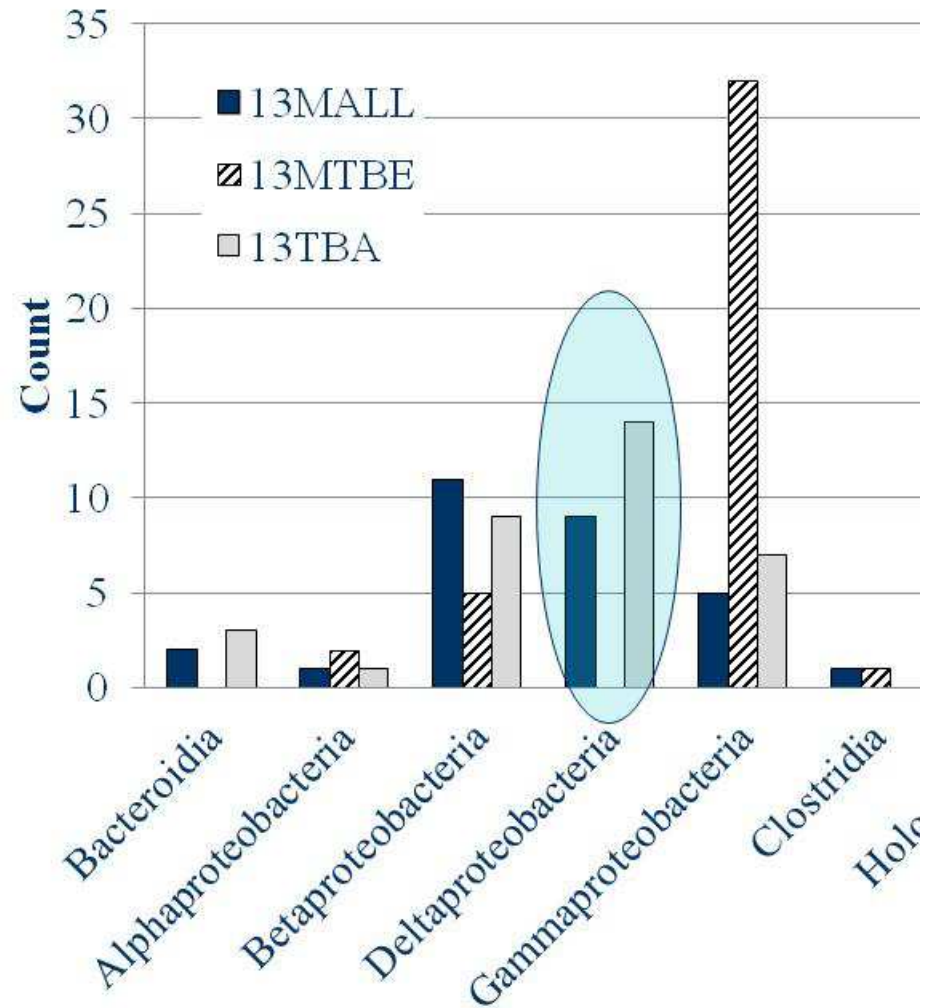
Sequences related to known MTBE and TBA degraders

- Many known MTBE and TBA degraders belong to the Burkholderiales order of the Betaproteobacteria, including PM1
- Members of *Sphingomonas* genus have been detected in anaerobic microcosms of MTBE-degrading cultures
- 1 *Sphingomonas* sequence in 13TBA and 2 in 13MTBE
- Likely to be primary degraders



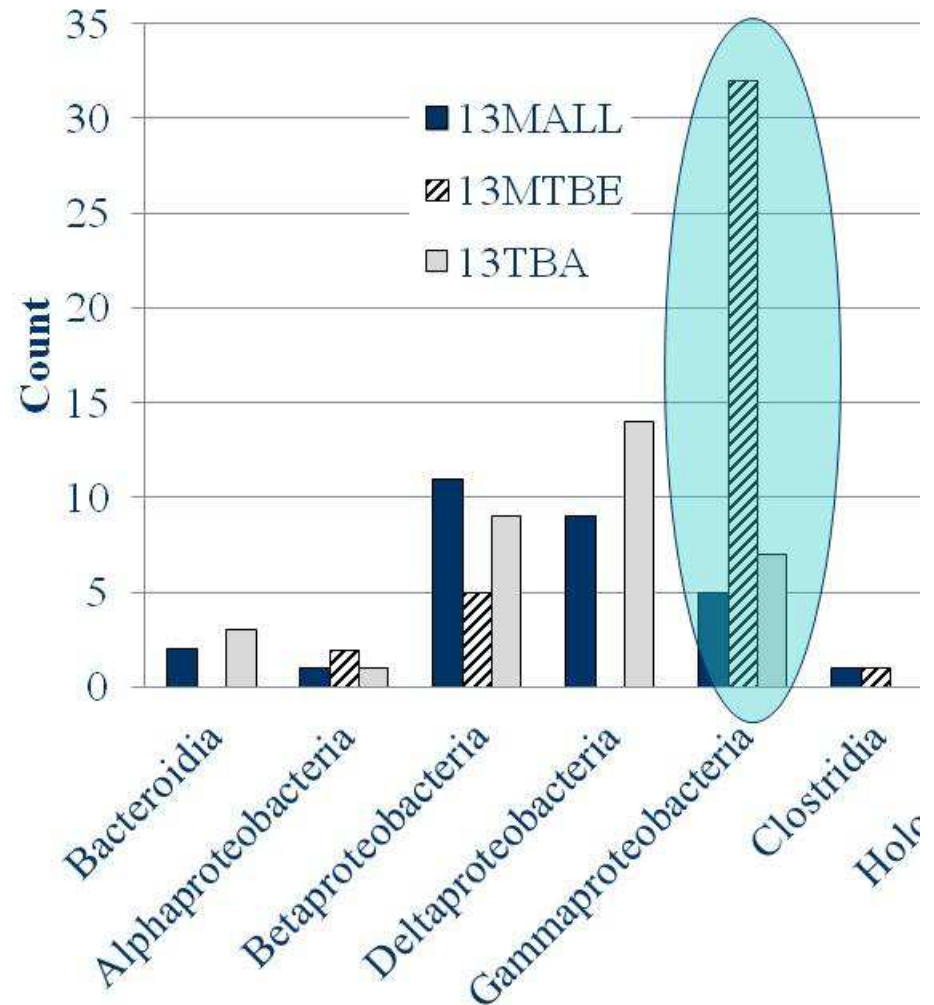
Deltaproteobacteria in 13MALL and 13TBA libraries

- Sulfate- and iron-reducers likely involved in processing the *tert*-butyl group
- *Desulfobulbus*, *Desulfovibrio*, *Desulfuromonas*, and *Geobacter* belong to Deltaproteobacteria
- *Geothrix* also detected in 13TBA



Gammaproteobacteria predominate in 13MTBE library

- 19 sequences similar to *Pseudomonas fluorescens*
- May have cross-fed on ^{13}C -acetate
- *P. fluorescens* likely a secondary degrader



Conclusions

- Microorganisms degraded the ^{13}C -labeled MTBE and TBA *in situ*, and ^{13}C was incorporated into DNA
- OTUs belonging to order Burkholderiales and genus *Sphingomonas* were related to known MTBE and TBA degraders and most likely primary degraders
- Sulfate- and iron-reducers were likely involved in processing the *tert*-butyl group
- *P. fluorescens* detected in ^{13}C -MTBE (only methoxy carbon labeled) was likely a secondary degrader, cross-feeding on metabolites, likely acetate

Questions?



Unit of measure

Amount of ^{13}C relative to ^{12}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 Belemnite (PDB)

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