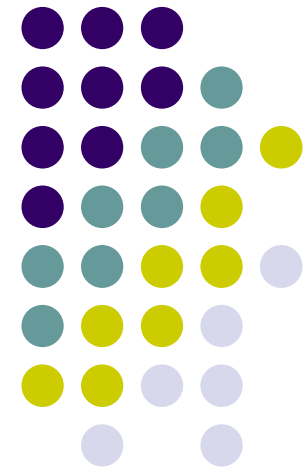


Biodegradation of MTBE and TBA by three distinct anaerobic microbial cultures

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Outline



- Introduction
- Background & Objectives
- Material and Method
- Result & Discussion
- Conclusion

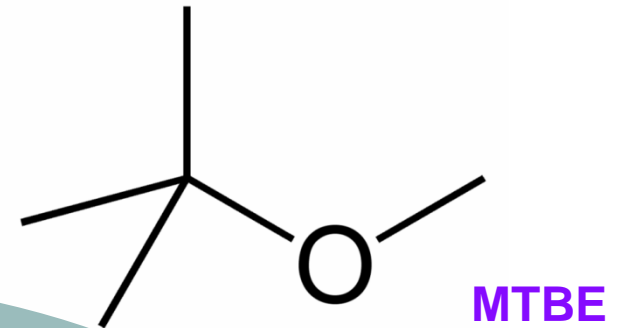
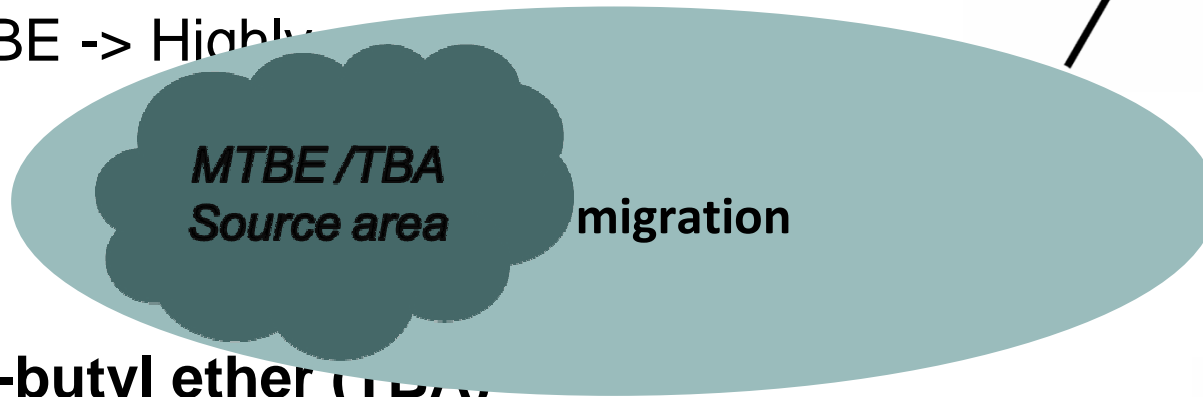
Introduction



➤ **Methyl *tert* butyl ether (MTBE)**

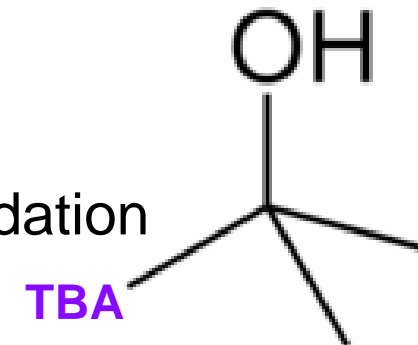
Major fuel oxygenate compound

- MTBE -> Highly



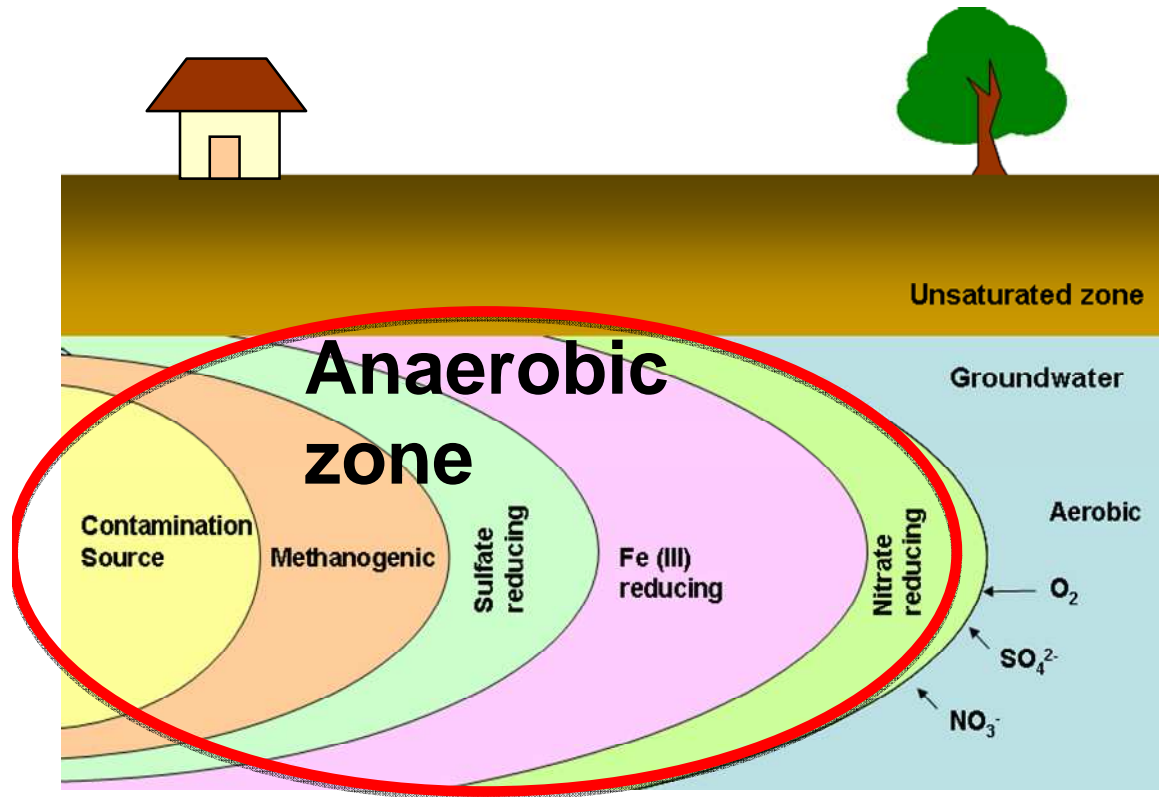
➤ ***Tert*-butyl ether (TBA)**

- TBA -> Metabolic by product of MTBE
- Chemical structure resistant to microbial degradation



Background

- Why anaerobic condition?



Distribution of dominant terminal electron accepting processes (TEAPs) within the anaerobic source area

How can we remove the contaminant in the aspect of cost effectiveness?

-> **In situ remediation** applicable in subsurface environment

Objectives



- **Mechanism of MTBE & TBA biodegradation** in **anaerobic** condition to provide stronger strategy for encompassing diverse source area
- Investigate the **effect of shifting biogeochemical condition** in MTBE & TBA degradation
- Analyze the **change of microbial community** as electron acceptor shifts in the system

Research overview



- Sample collected from contaminated aquifer
- Find appropriate electron acceptor

❖ **Mimic in situ conditions to assess MTBE/TBA biodegradation under different redox conditions**

- 14C MTBE/TBA amended for tracking mineralization percentage



❖ **Select and enrich anaerobic MTBE/TBA degradations to investigate**

- Kinetics & pathway of anaerobic MTBE/TBA biodegradation under different TEAPs
- Factors affecting the degradation process
- Biochemistry aspects: related genes, proteins, etc

❖ **Microbial community study with various biogeochemical conditions**

- via molecular biology tool

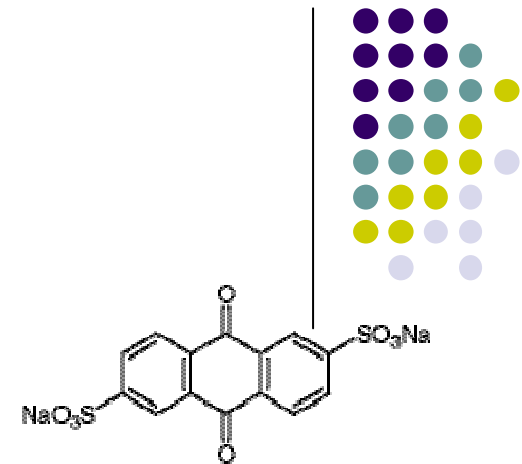




Culture development

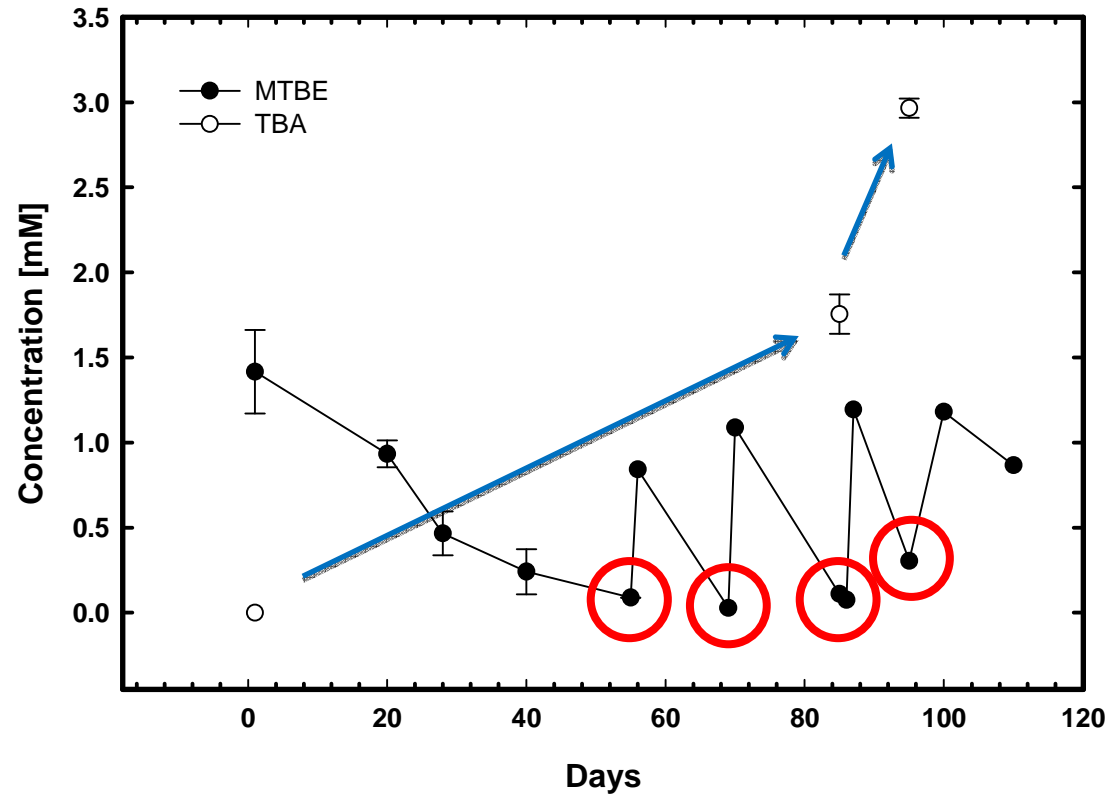
Electron acceptor in each culture

- Anthraquinone-2,6-disulfonate [AQDS]
Fe(III) reducing microorganism use this.



- Sulfate [SO₄²⁻]
Widely reported as a significant electron acceptor for in situ, anaerobic MTBE and TBA transformation.
- Fumarate [C₄H₂O₄²⁻]
Fumarate reduced to succinate. Key carbon compound in central metabolism. Relatively non toxic and soluble.

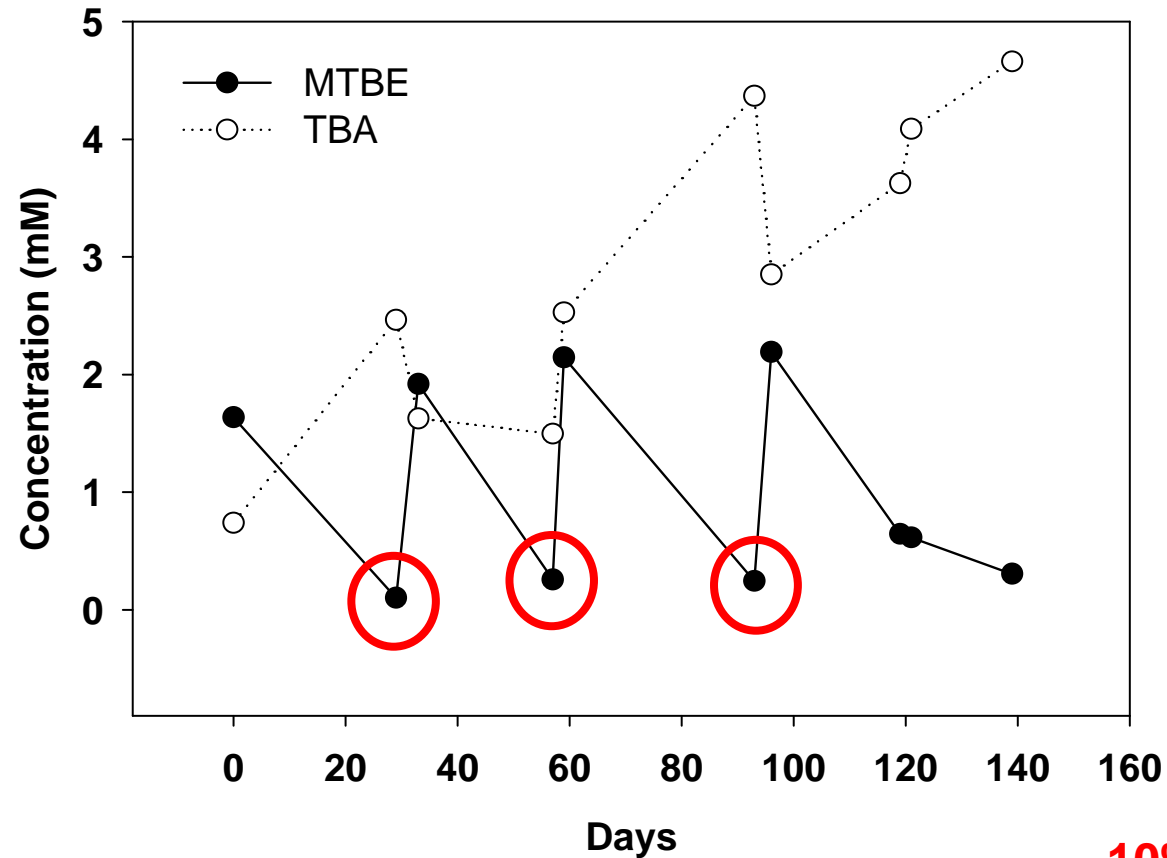
MTBE degrading cultures



10% transfer

NW 1 – Liquid anaerobic culture with 5 mM **AQDS** as terminal electron acceptor. Initial concentration of MTBE amended was 1mM.

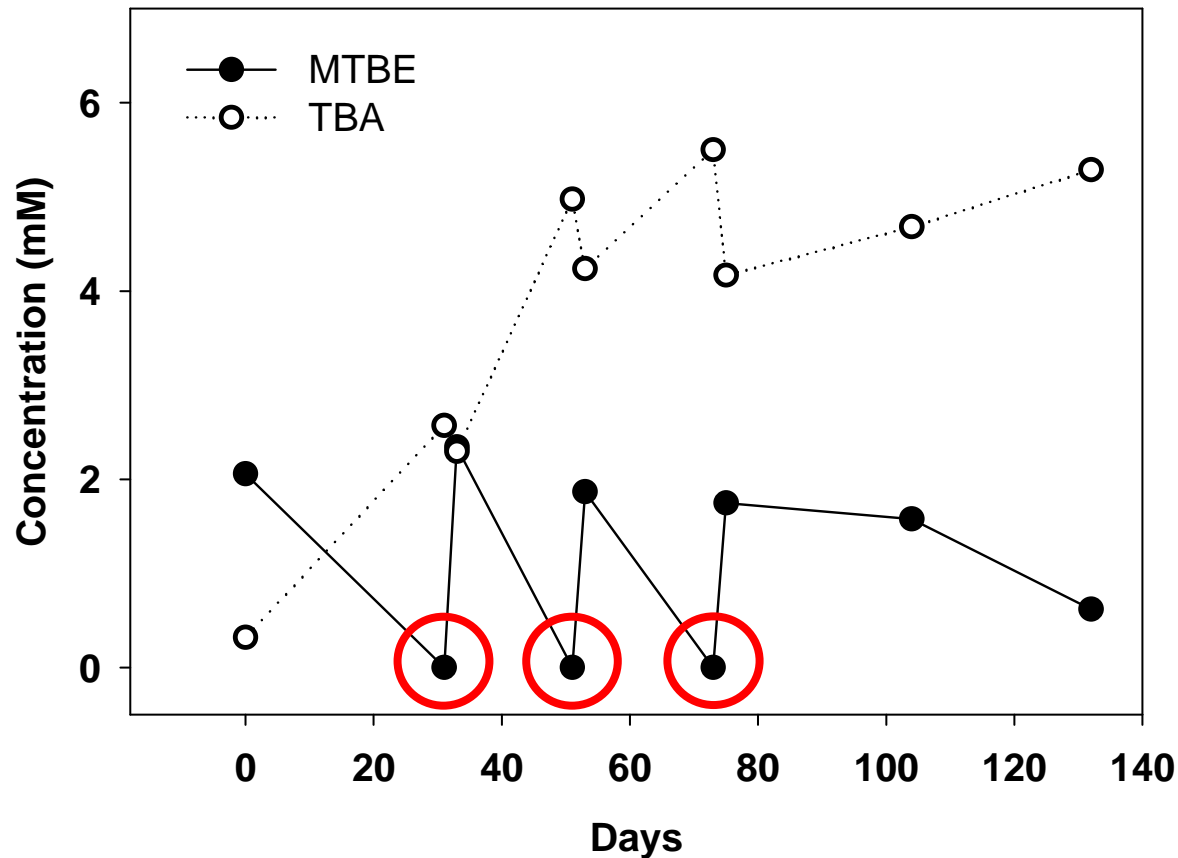
MTBE degrading cultures



10% transfer

NW 2 – Liquid anaerobic culture with 10 mM **sulfate** as terminal electron acceptor. Initial concentration of MTBE amended was 1mM.

MTBE degrading cultures



10% transfer

NW 3 – Liquid anaerobic culture with
10 mM **fumarate** as terminal electron acceptor.
Initial concentration of MTBE amended was 2mM.



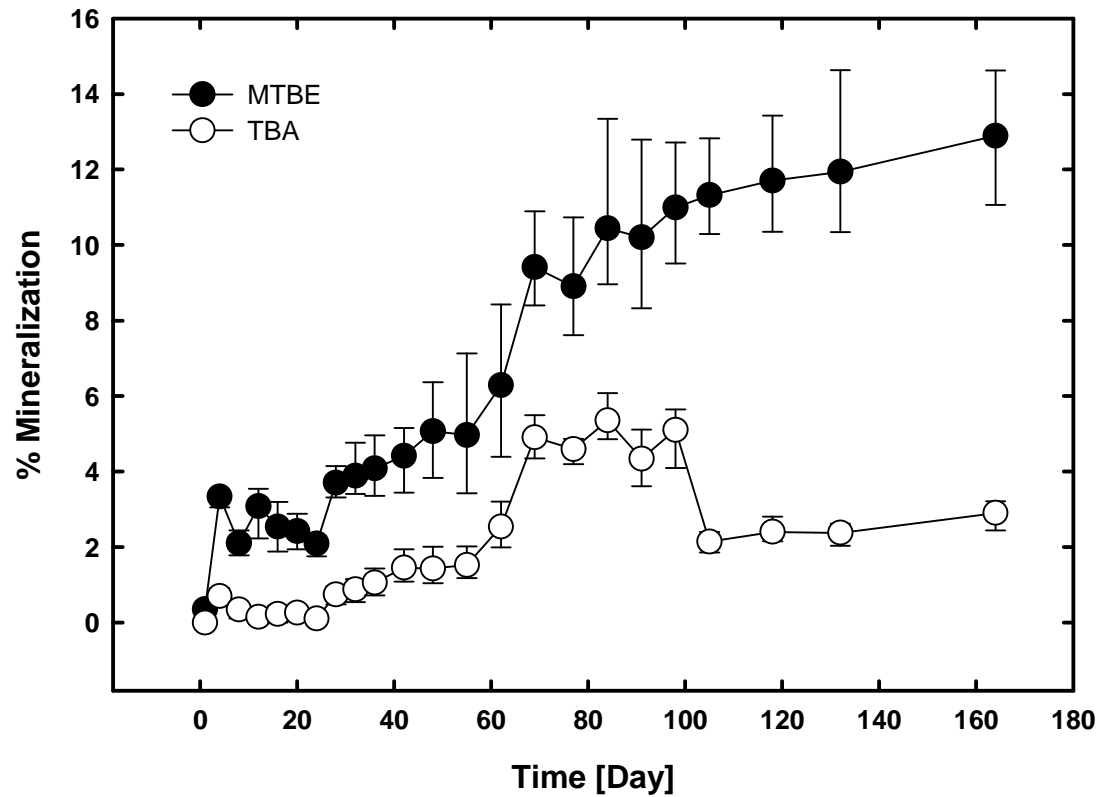
Microcosm Study

Material and Method



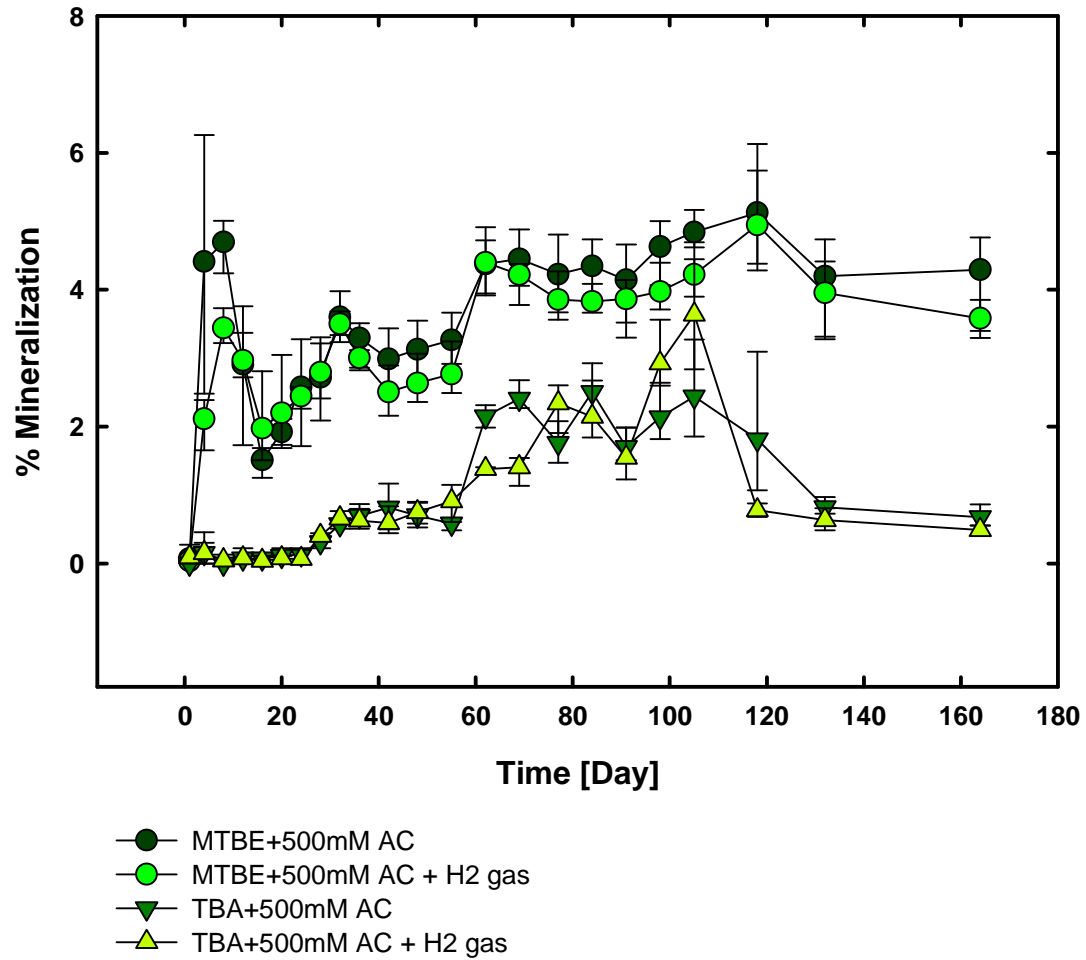
- **Native sediment**
 - Collected from petroleum-contaminated aquifer in California
- **Mineralization analysis**
 - ^{14}C radiolabeled MTBE/TBA
 - **GC-GPC** (Gas chromatography – Gas proportional counting)
 $^{14}\text{CO}_2$ measured with Gas Chromatography equipped with a Gas Proportional Counter
- **Sampling technique (aqueous MTBE/TBA analysis)**
 - **GC-FID**
(Gas chromatography – Flame ionization detector)
 - **GC-MS**
(Gas chromatography – Mass spectrometry)

Microcosm study



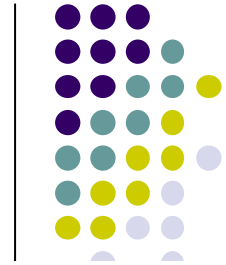
Percent of mineralization of
[¹⁴C]-MTBE/TBA to ¹⁴CO₂ in **sulfate** amended sediment

Microcosm study



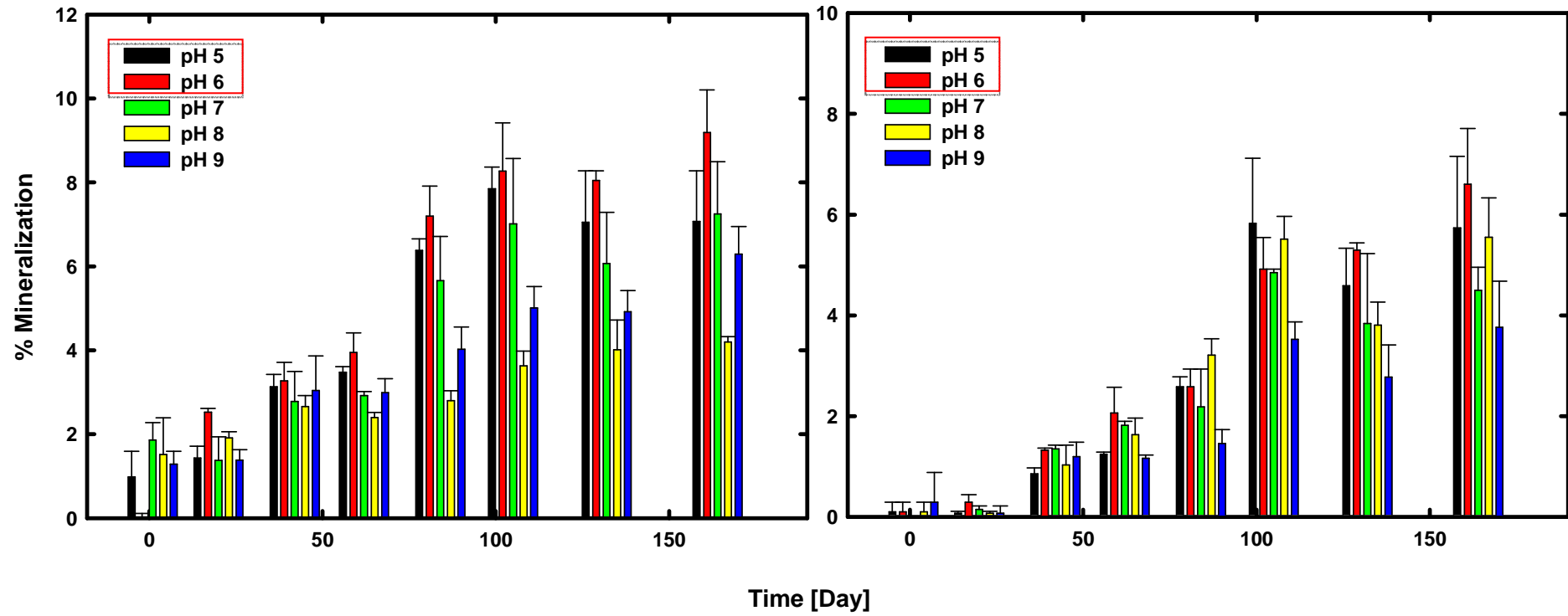
Acetate amended as **alternate carbon**

Microcosm study



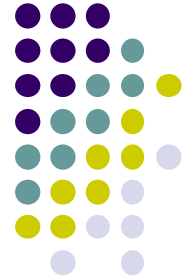
MTBE

TBA



pH variation

Conclusion

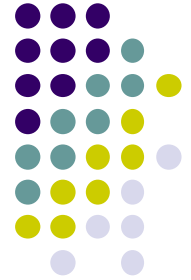


- **Anaerobic MTBE degradation is feasible** when appropriate electron acceptor is provided.
- **Stable anaerobic MTBE degrading cultures** are developed
- In these cultures, **TBA builds up** as MTBE degrades in **stoichiometric level**

Acknowledgements

- Dr. Kevin T. Finneran
- Finneran Lab Group
- British Petroleum (BP)





Thank you !

Q & A

Session