Laboratory Evaluation of Alternative Substrates for Enhancing the Cometabolic Biodegradation of 1,4-Dioxane and Tetrahydrofuran

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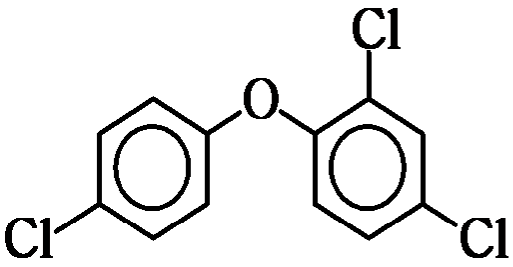
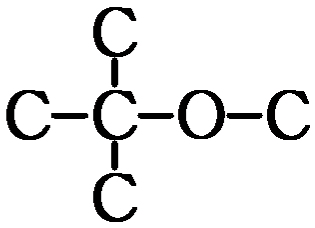
CB&I

Co-Authors: Paul Hatzinger, Sheryl Streger and Rachael Rezes (CB&I Federal Services), Andrew Madison and Timothy Richards (Golder Associates)

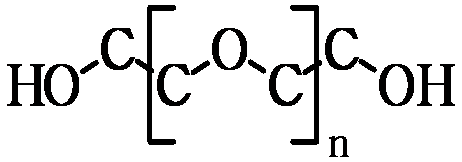
Examples of Widely Used Ethers



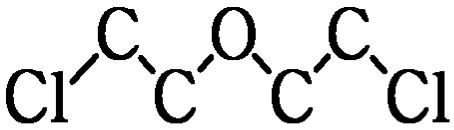
Triclosan



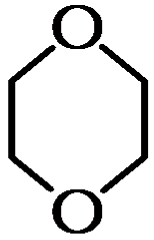
MTBE



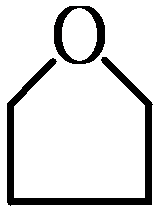
Polyethylene glycol



bis-2-chloroethylether



1,4-Dioxane



Tetrahydrofuran

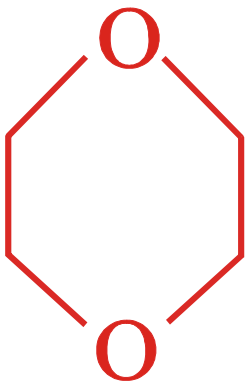
Typical Uses

- **Stabilizer in 1,1,1-TCA and other solvents**

Characteristics of 1,4-Dioxane

Chem/Phys/Tox

- Cyclic Ether
- High Miscibility in Water
- Low Henry's Law Coefficient → 4.9×10^{-6} atm/m^3/mol
- Low Partitioning Coefficient → K_{oc}= 1.23
- Probable Human Carcinogen



The Result

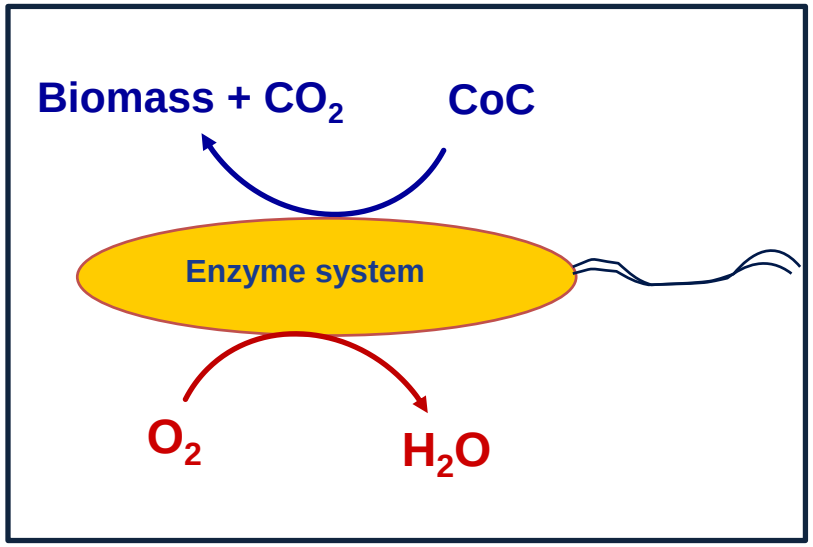
- Chemically Stable
- Low Odor and Taste Threshold
- Difficult to Remove by Air Stripping or Carbon Sorption
- Very Mobile in Groundwater
- Recently Identified as a Contaminant of Concern

Regulatory Drivers

- No Federal MCL established
- State Standards (EPA Fact Sheet, 2014)
  - Colorado: Interim groundwater quality cleanup standard of 0.35 µg/L
  - Massachusetts: Drinking water guideline level of 0.3 µg/L
  - New Hampshire: Reporting limit of 0.25 µg/L for public water supplies
  - California: Notification level for drinking water of 1 µg/L
- Found at many federal facilities

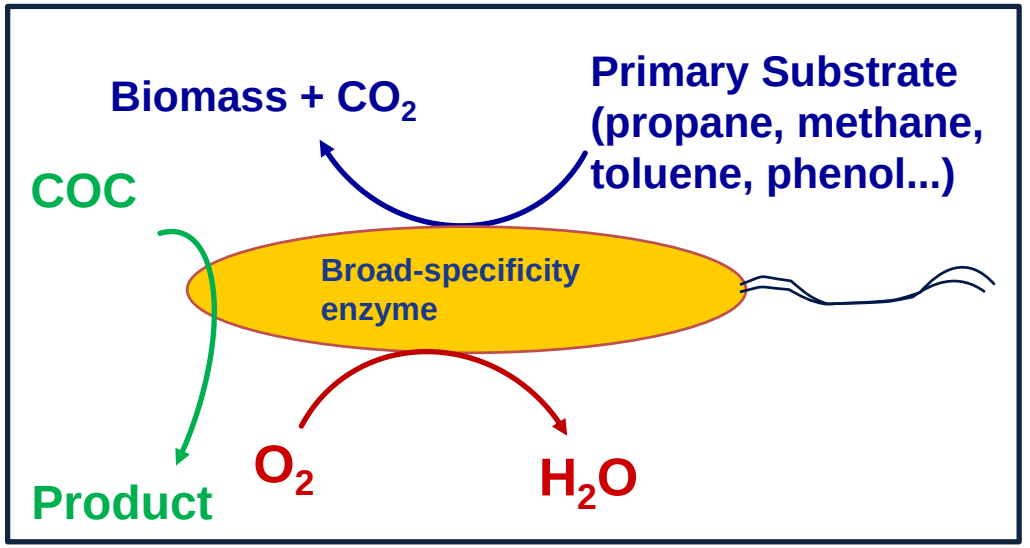
Background

Growth-Linked Metabolism



Cells gain energy and carbon from CoC

Cometabolism



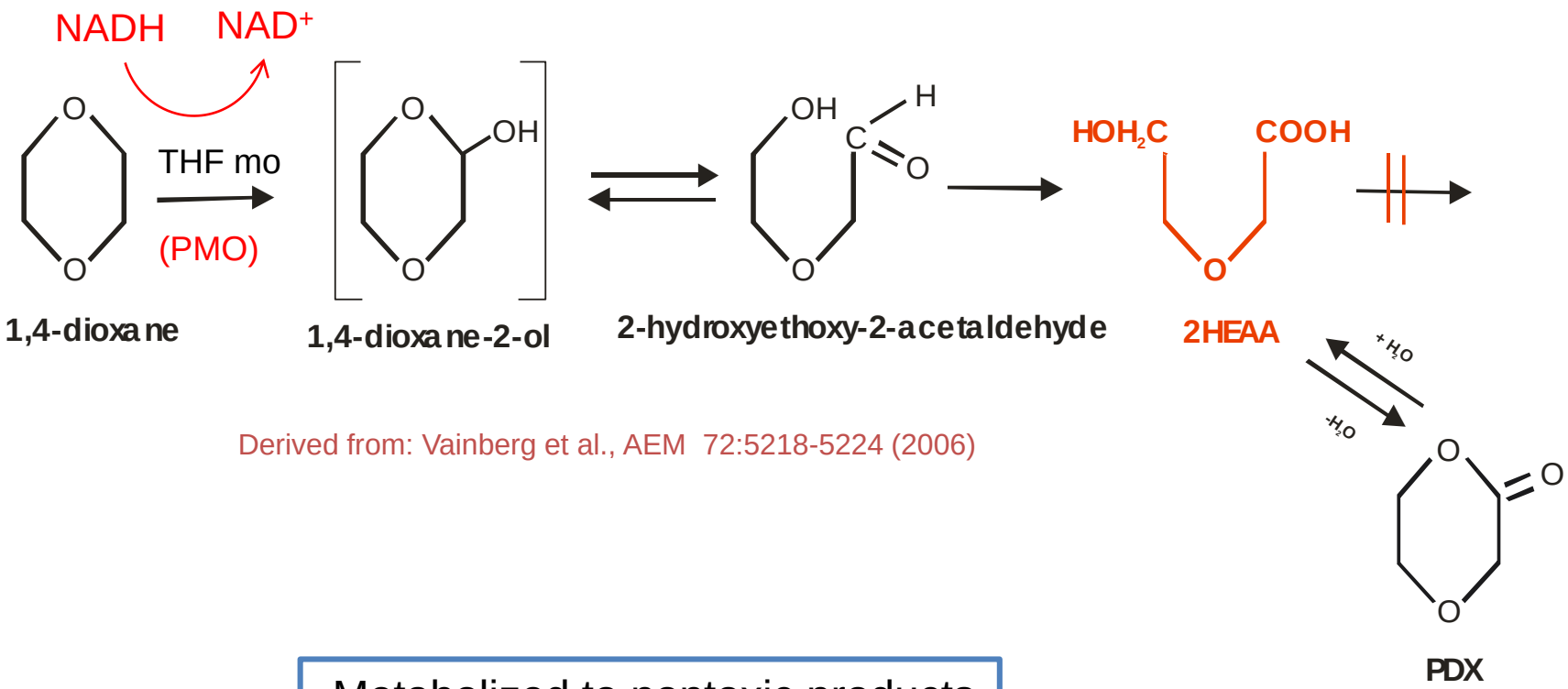
Cells gain energy and carbon from primary substrate

"Cometabolism"

Transformation of an organic compound by a microorganism that is unable to use the substrate as a source of energy or one of its constituent elements (Alexander, 1967)

ENV478 1,4-Dioxane Biodegradation Pathway Analysis

Needed:
-Enzyme – THFmo, PMO, etc.
-Energy – NADH

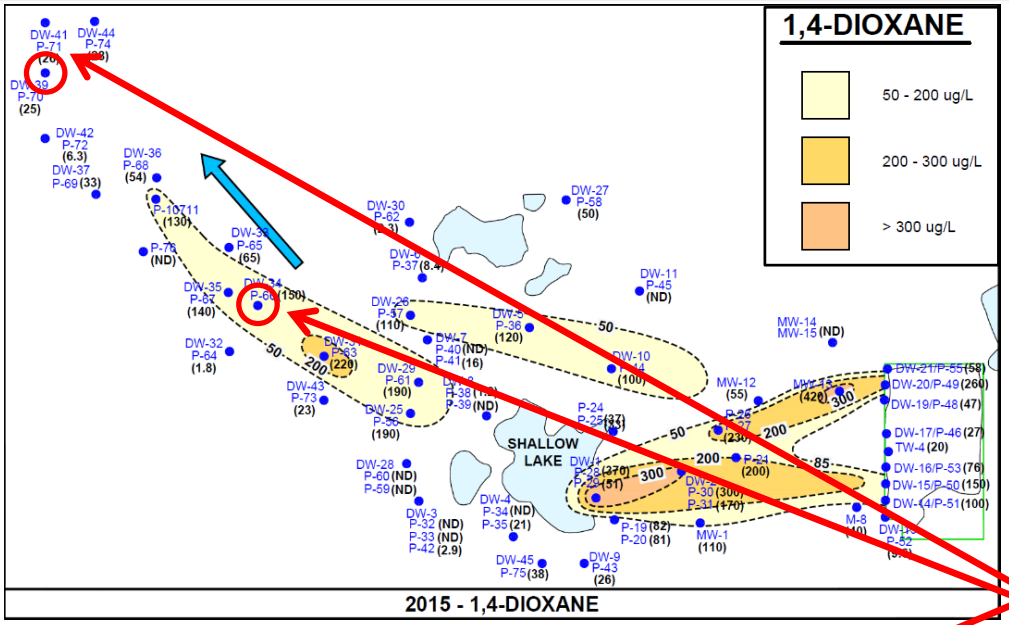


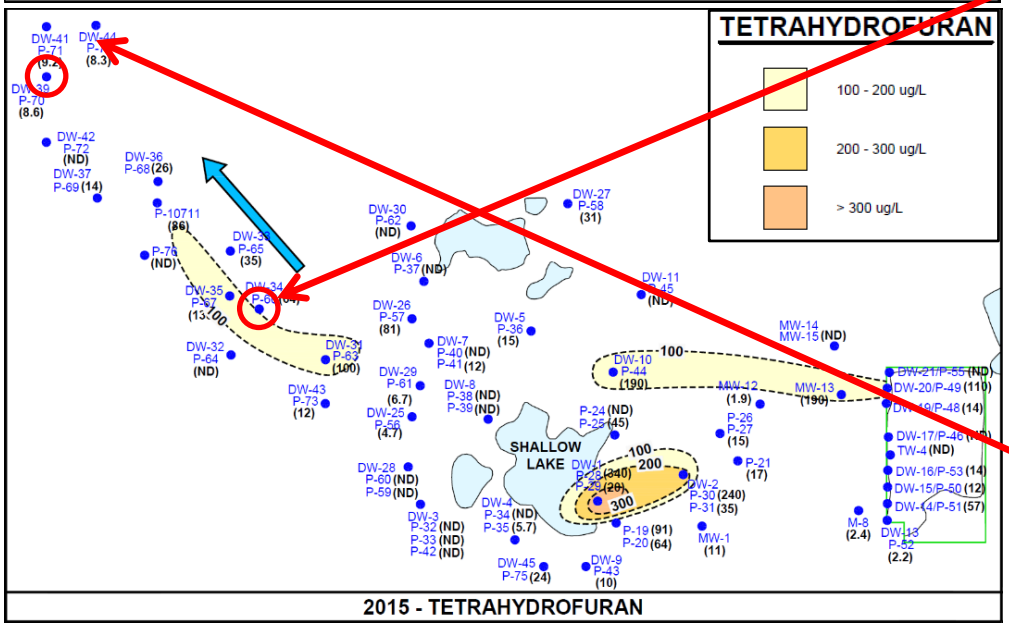
Derived from: Vainberg et al., AEM 72:5218-5224 (2006)

-Metabolized to nontoxic products
-Ultimately mineralized to CO_2

Mahendra et al., 2007

Study Site





- Upper Midwest
- Unconsolidated Aquifer
- Large Dilute Plume

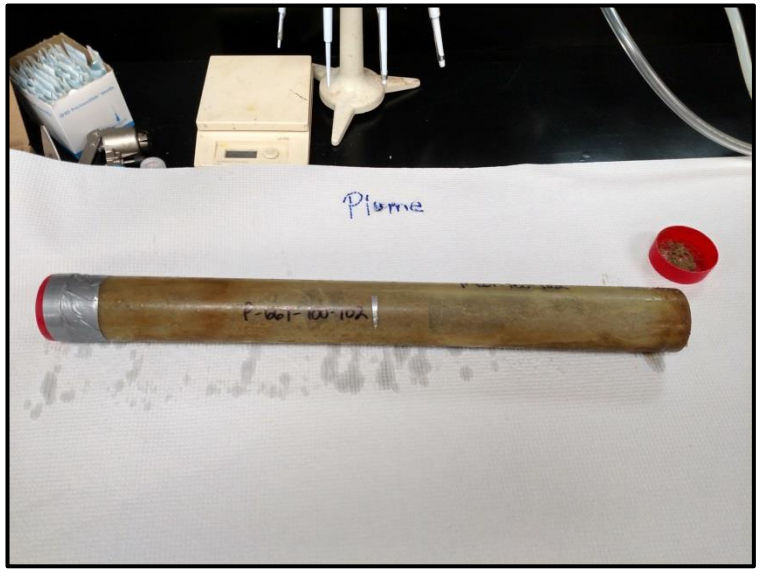
P-66 Study Area

- Anaerobic (<1 mg/L DO)
- 1,4-D: ~125 µg/L
- THF: ~50 µg/L
- Methane: ~450 µg/L

P-70 Study Area

- Mildly Aerobic (>1 mg/L DO)
- 1,4-D: ~20 µg/L
- THF: ~7 µg/L
- Methane: ~ 3 µg/L

Microcosm Setup



Soil

- Saturated soil cores
- Soil homogenization
  - Aerobic
- 160 mL serum bottles
  - Triplicate + 1
- 30 grams of soil

Microcosm Setup

Groundwater

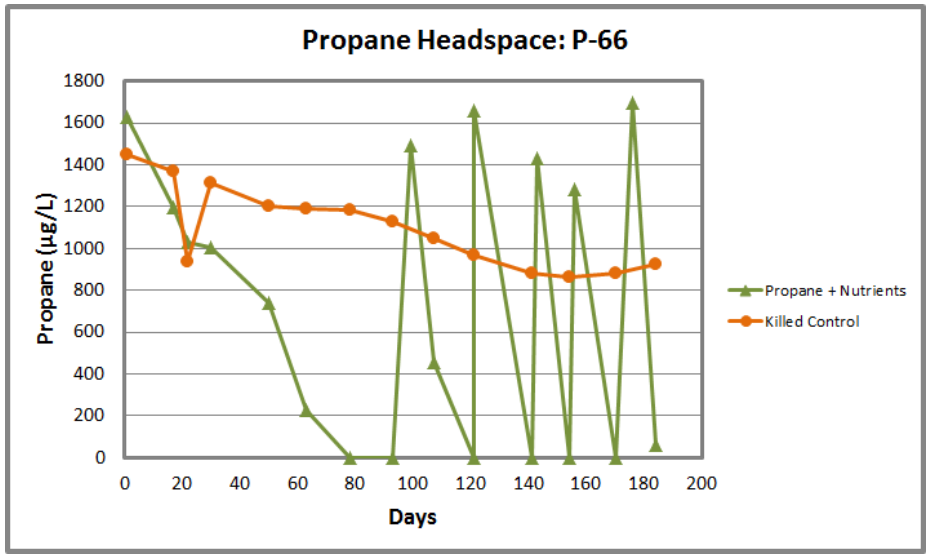
- Collected from local wells
- Groundwater homogenization
- 100 mL per bottle
- 40 mL headspace

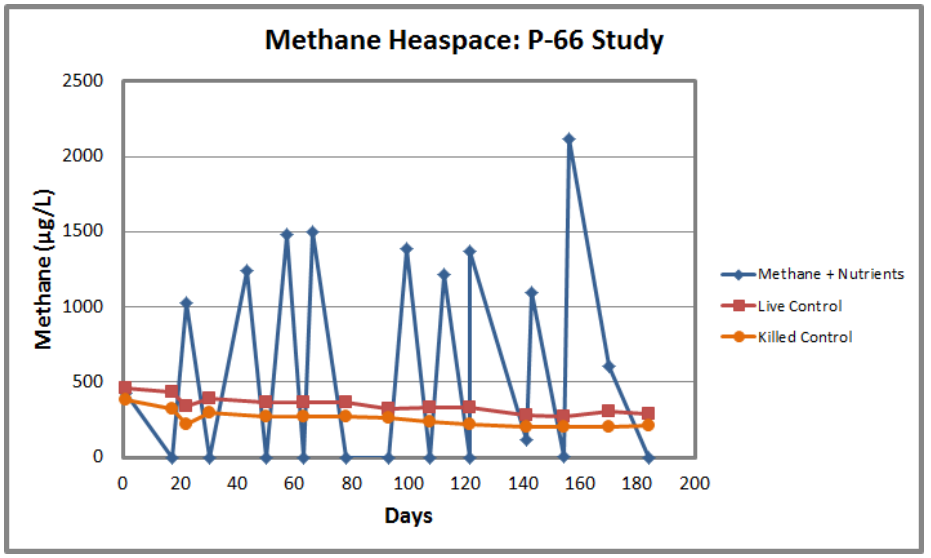
Microcosm Treatments & Sampling

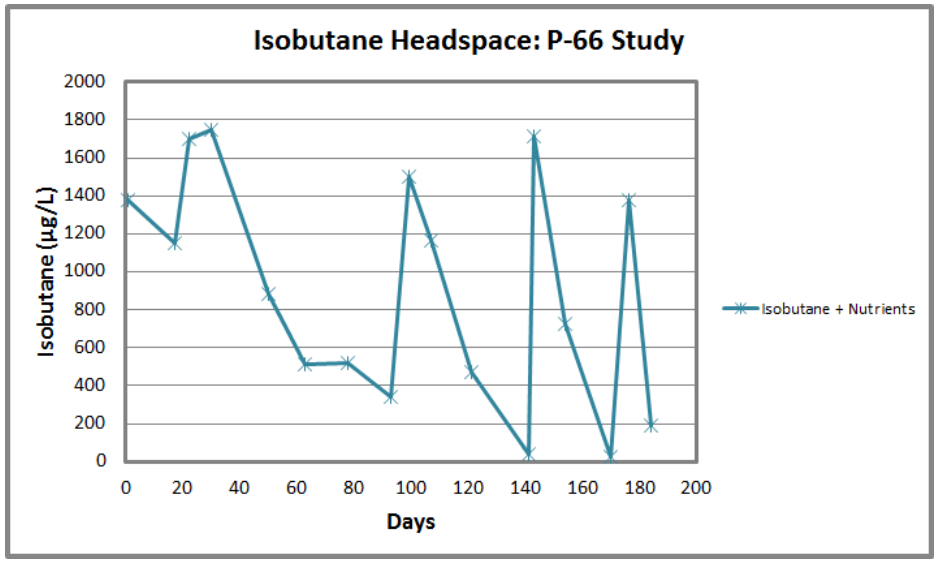
Treatment	P-66	P-70	Headspace	Alkane Gas	Nutrients
Live Control	X		100% Nitrogen	None	None
Live Control		X	Air	None	None
Killed Control	X	X	Air	2.3% Propane	50 mg/L DAP
O_2 + DAP	X	X	Air	None	50 mg/L DAP
O_2 Only	X		Air	None	None
Ethane + DAP	X	X	Air	2.5% Ethane	50 mg/L DAP
Propane + DAP	X	X	Air	2.3% Propane	50 mg/L DAP
Propane Only	X	X	Air	2.3% Propane	None
Methane + N&P	X	X	Air	6.7% Methane	20 mg/L KNO_3 & KH_2PO_4
Isobutane + DAP	X	X	Air	2.2% Isobutane	50 mg/L DAP

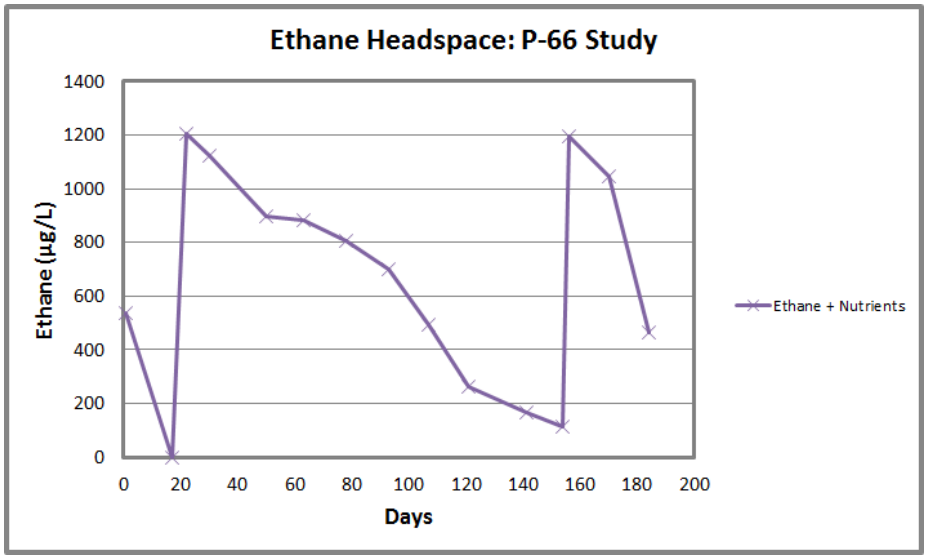
- Oxygen headspace → weekly
- Alkane gas headspace → bi-weekly
- 1,4-dioxane and THF → monthly
  - EPA Method 8260/SIM (heated purge & trap)

P-66 Alkane Gas Consumption

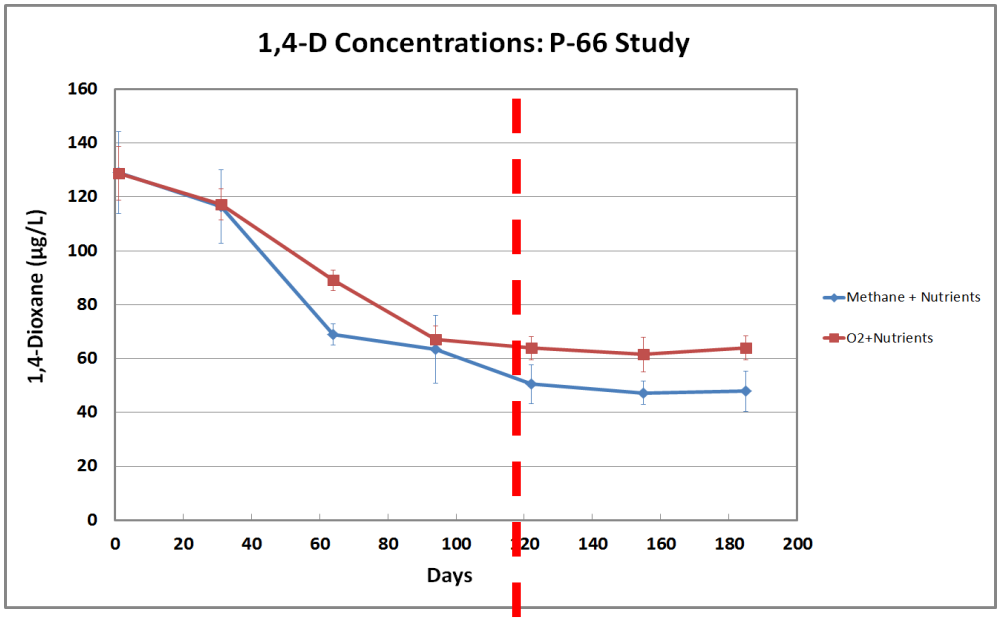


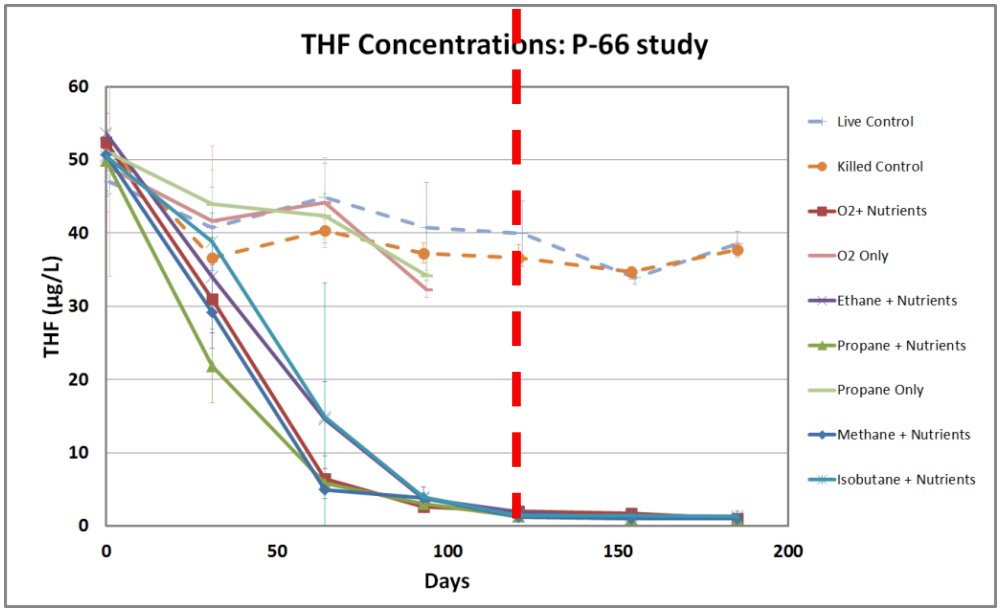






P-66 Microcosm Results





1,4-Dioxane

- Propane + Nutrients → <3 µg/L at 3 months
- Isobutane + Nutrients → 89% decrease
- Ethane + Nutrients → 88% decrease
- Nutrients critical
- Methane + Nutrients: Rate & magnitude of reduction

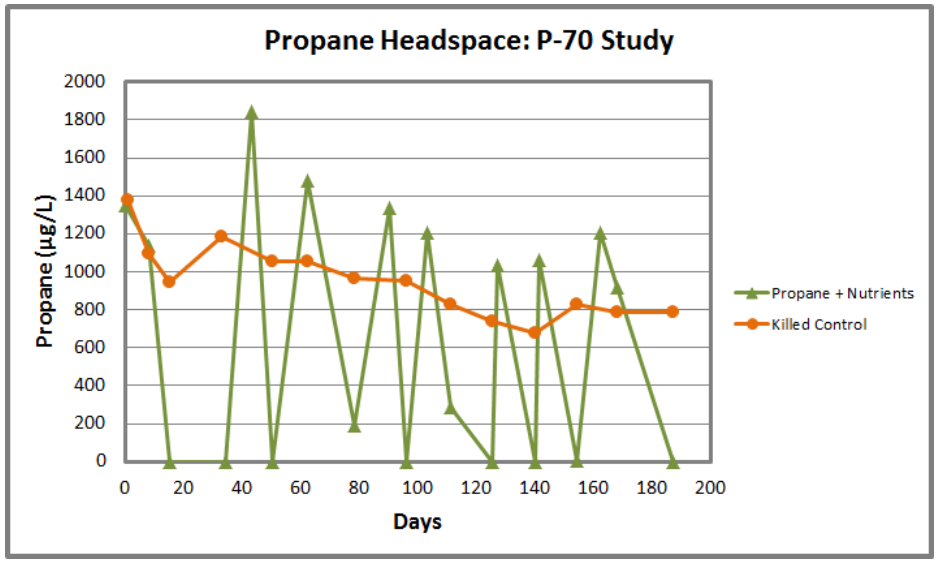
~ the same as O_2 + Nutrients

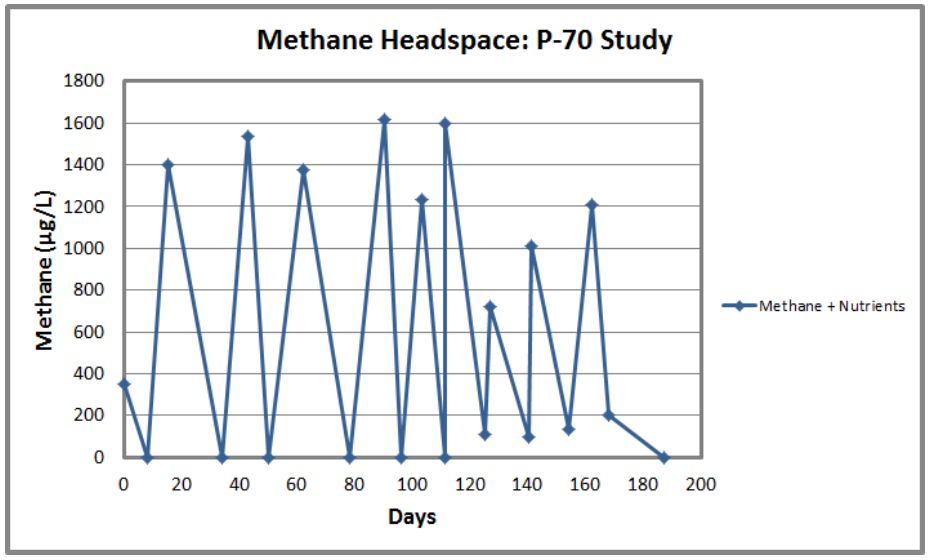
- Suggests methane did not stimulate degradation
- Degradation stalled when THF degraded

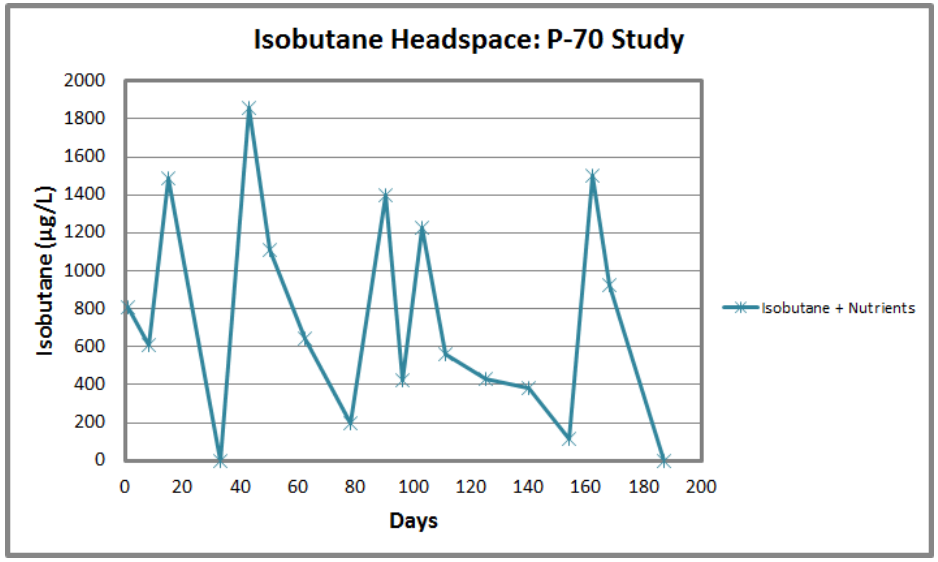
THF

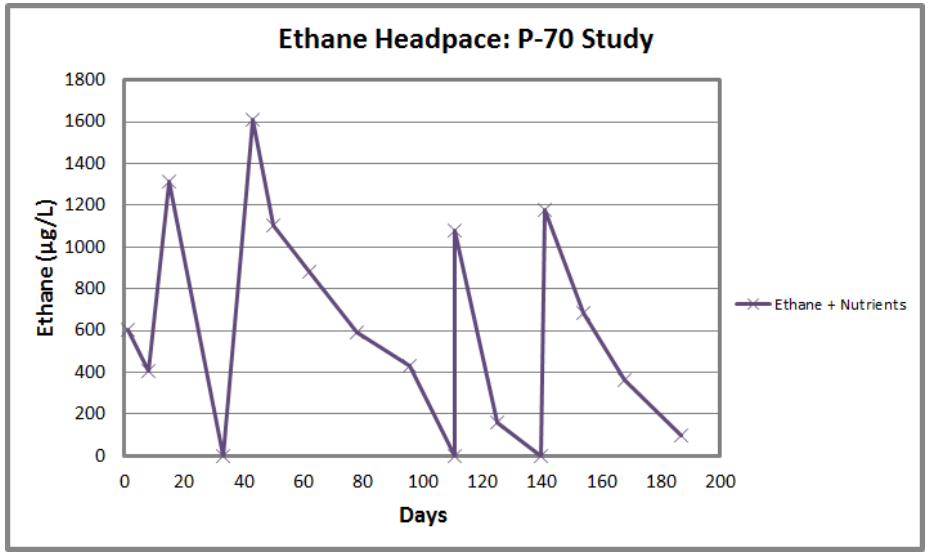
- Nutrients critical
- At or near detection level at 3 months

P-70 Alkane Gas Consumption

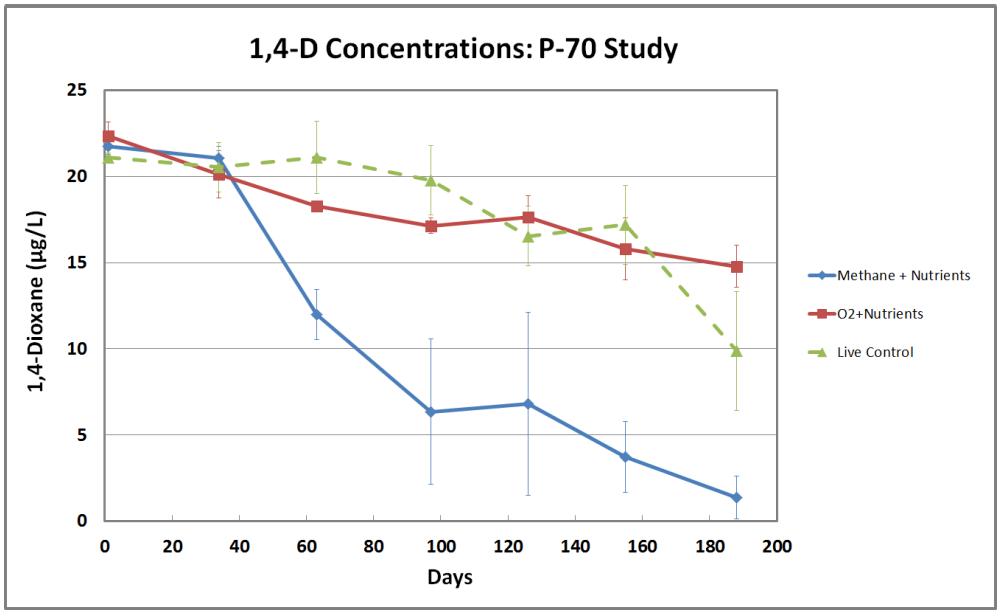


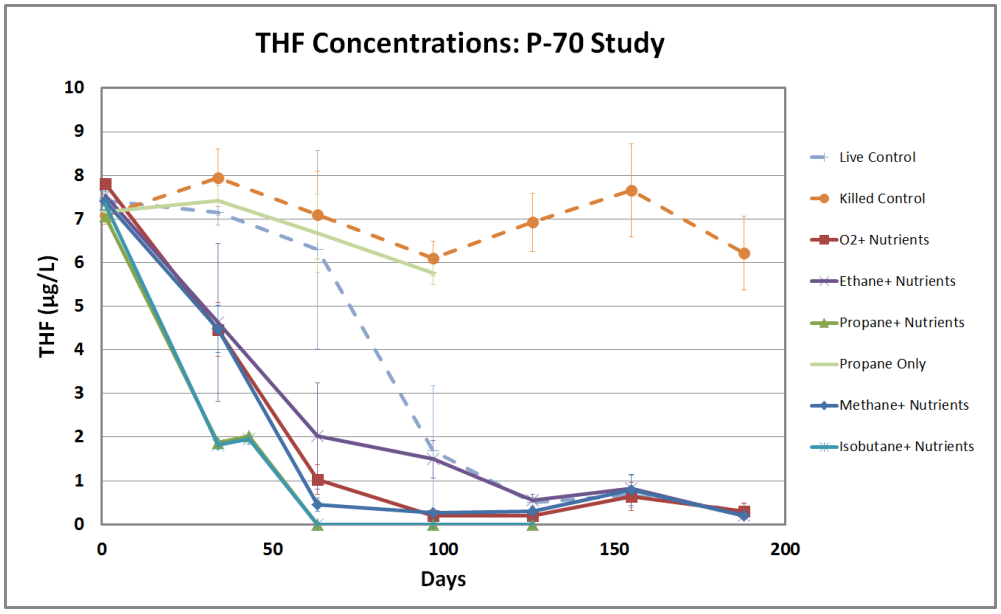






P-70 Microcosm Results



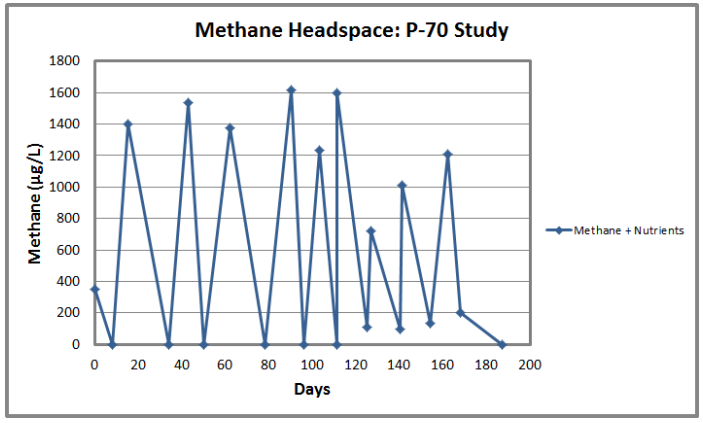


1,4-Dioxane

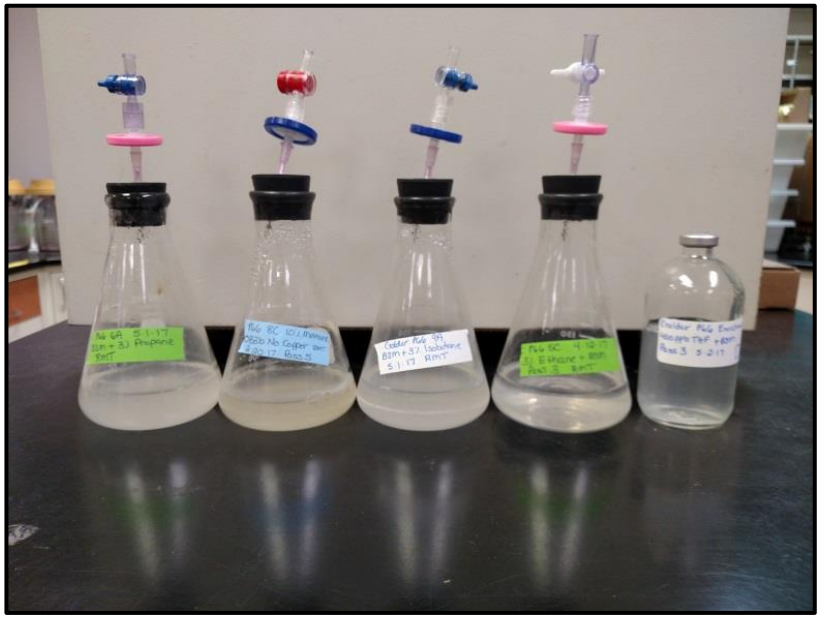
- Propane + Nutrients and Isobutane + Nutrients → <2 µg/L at 34 days
- Ethane + Nutrients → 76% decrease
- Nutrients critical
- Methane + Nutrients → 94% decrease (~20 µg/L)
- Slow degradation rate considering degree of methane consumption
  - sMMO or another enzyme?
  - Biomass acting as carbon source?
  - Enrichments did not degrade 1,4-D

THF

- Nutrients important - not as critical
- Most at or near detection level at 2-3 months

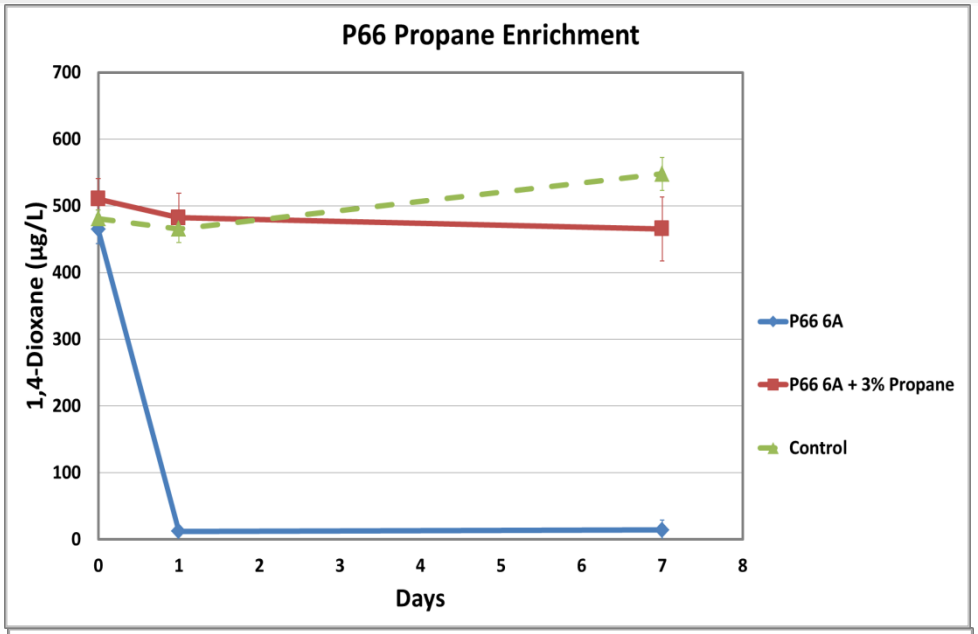


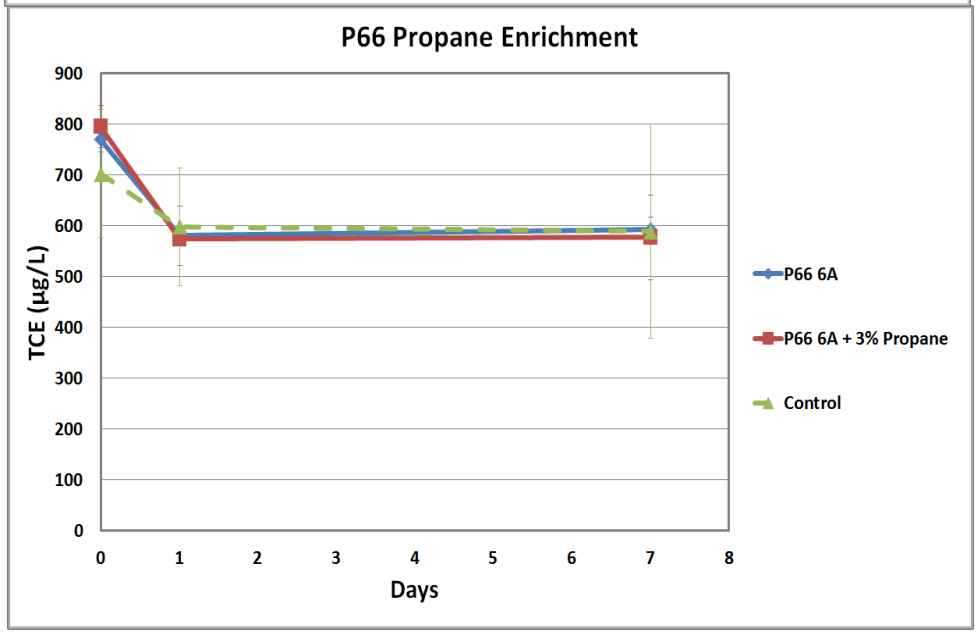
Enrichments



- Samples from microcosm bottles
- Grown in media with respective alkane gases
- Passed 3 times (most cases)
- Tested with and without alkane gas
- Tested for 1,4-dioxane degradation
- TCE added in one treatment to confirm activity

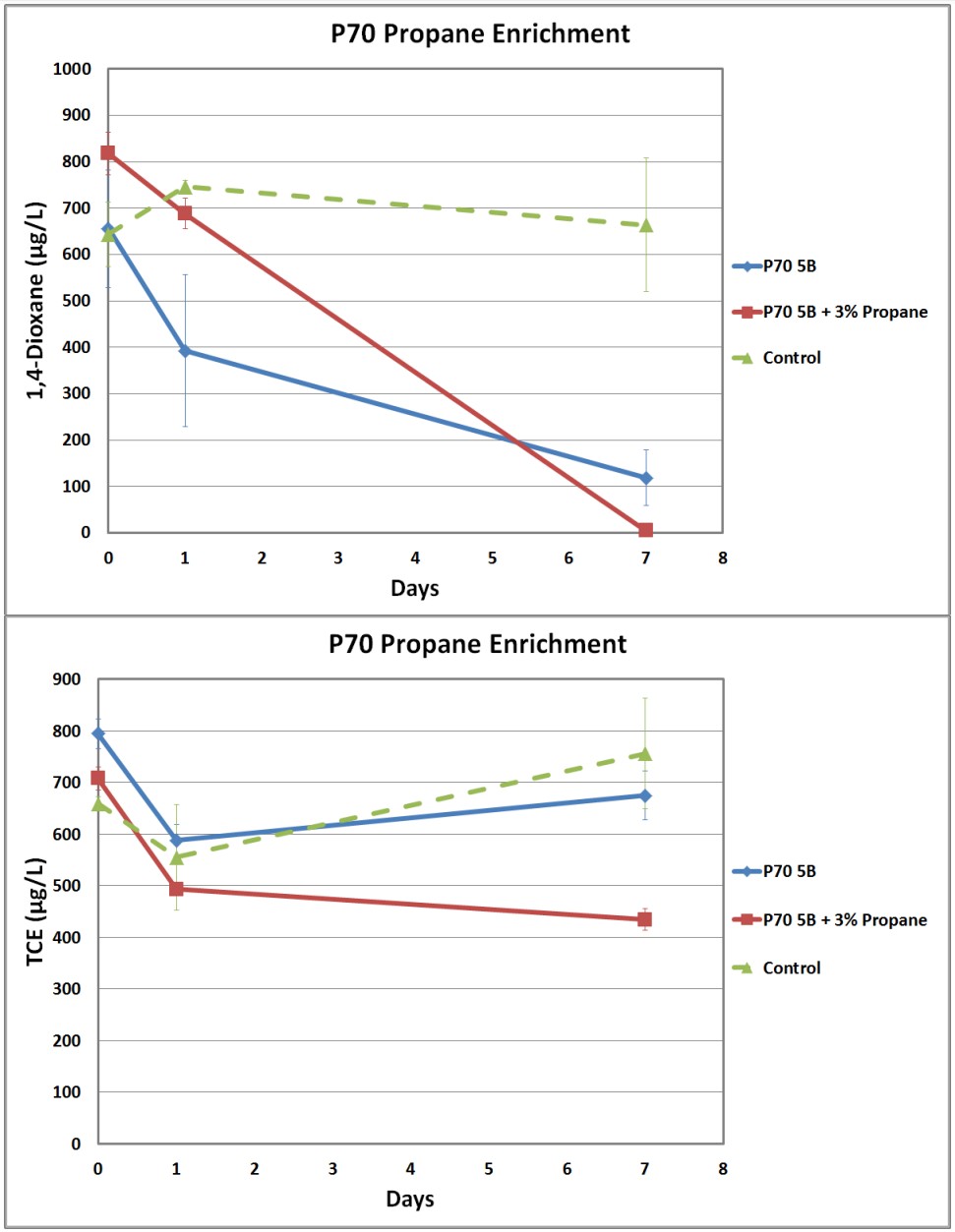
P-66 Propane Enrichments





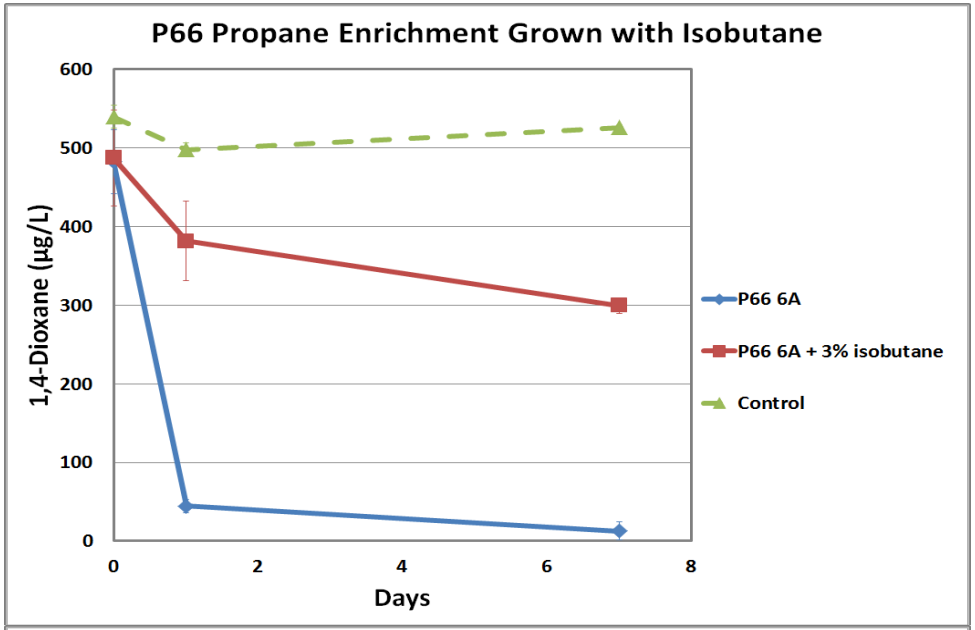
- 1,4-D quickly degraded in absence of propane
- Propane Inhibition
- Did not degrade TCE
  - Very uncommon

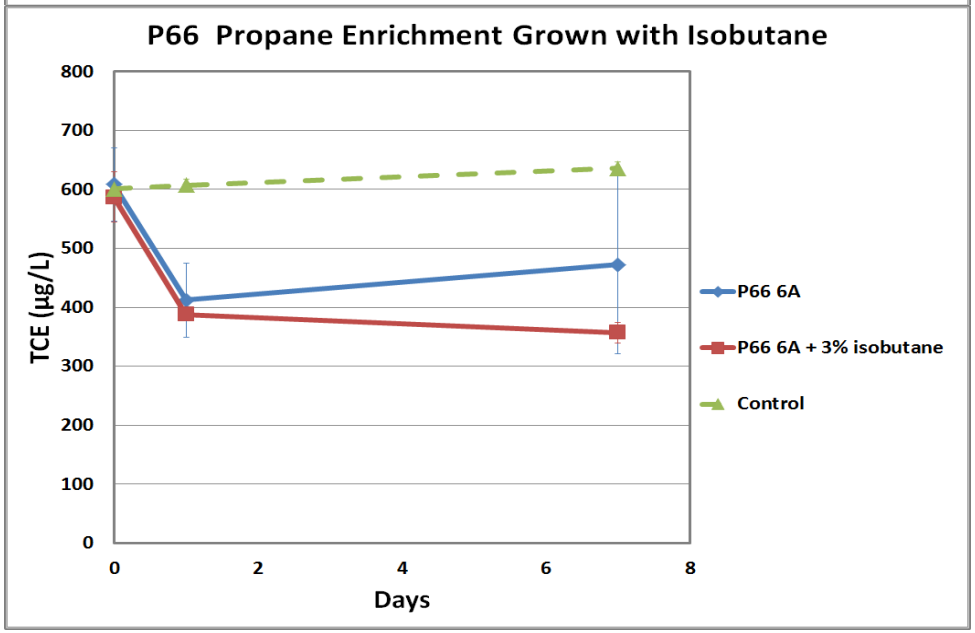
P-70 Propane Enrichments



- Minor propane inhibition
- Did not significantly degrade TCE

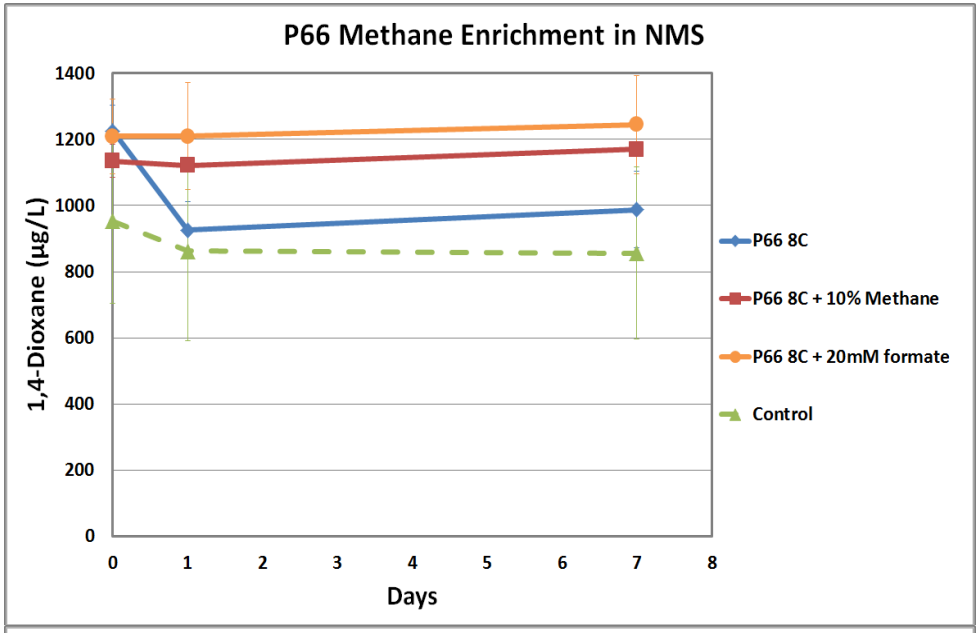
P-66 Propane Enrichments Grown on Isobutane

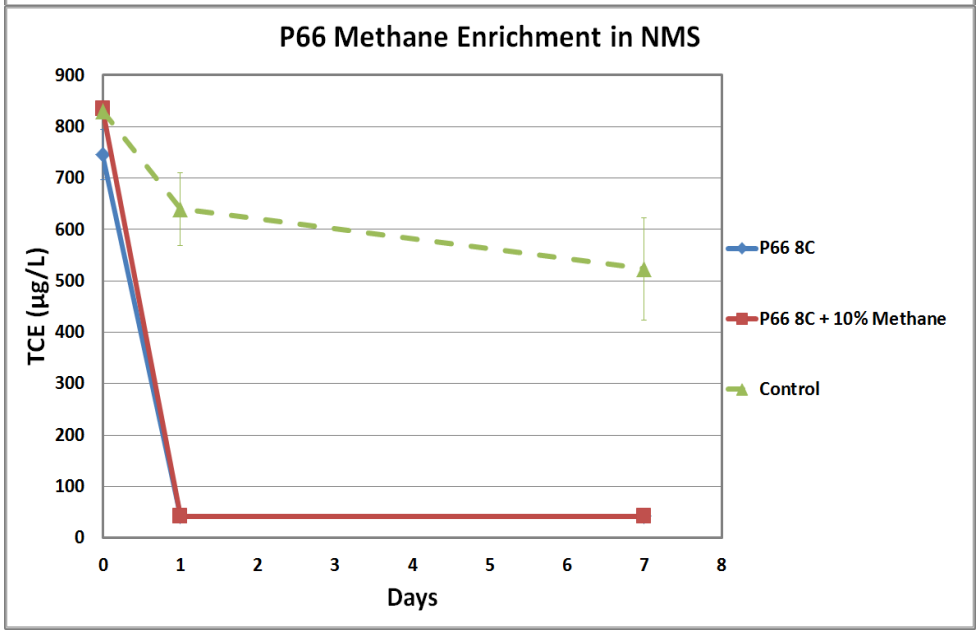




- Passed propanotroph culture
- Fed 3% isobutane - Did not pass before testing
- 1,4-D quickly degraded in absence of propane
- Propane Inhibition
- No significant TCE degradation
  - Dr. Michael Hyman (NCSU) and Dr. Lewis Semprini (OSU) –SERDP Project ER-2303

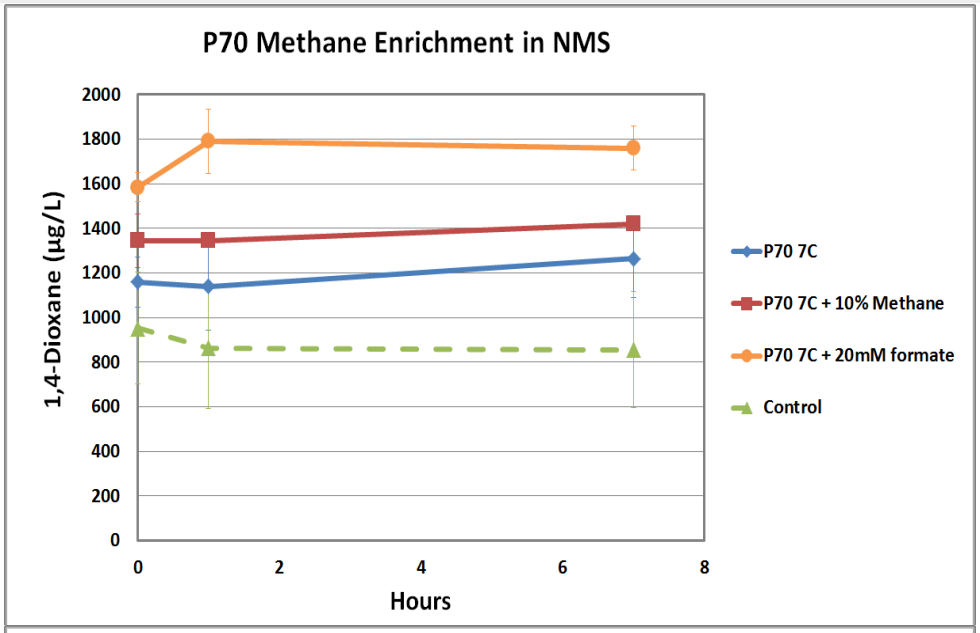
P-66 Methane Enrichments (NMS media)

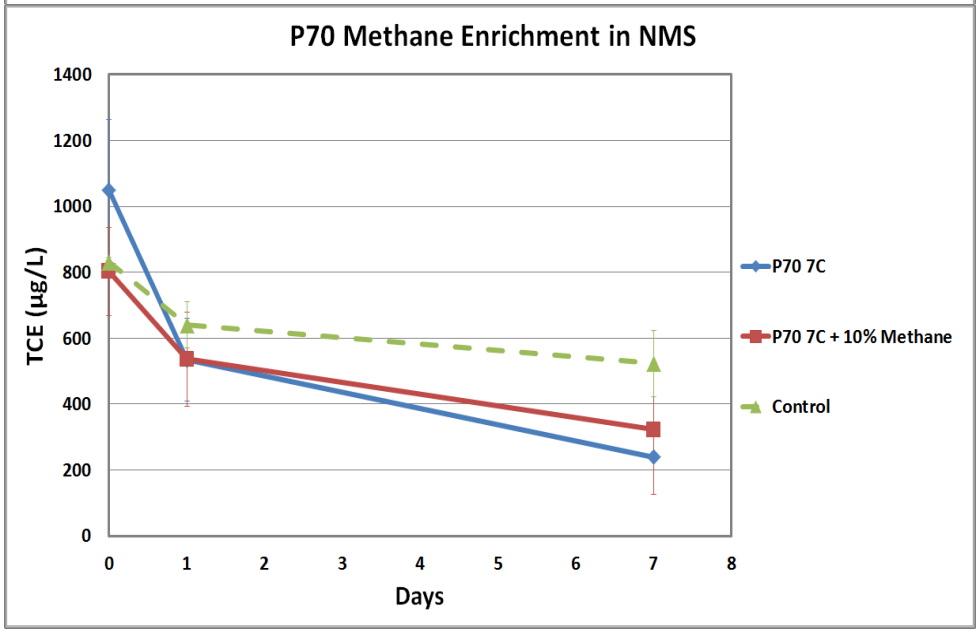




- No 1,4-D degradation
- Quickly degraded TCE

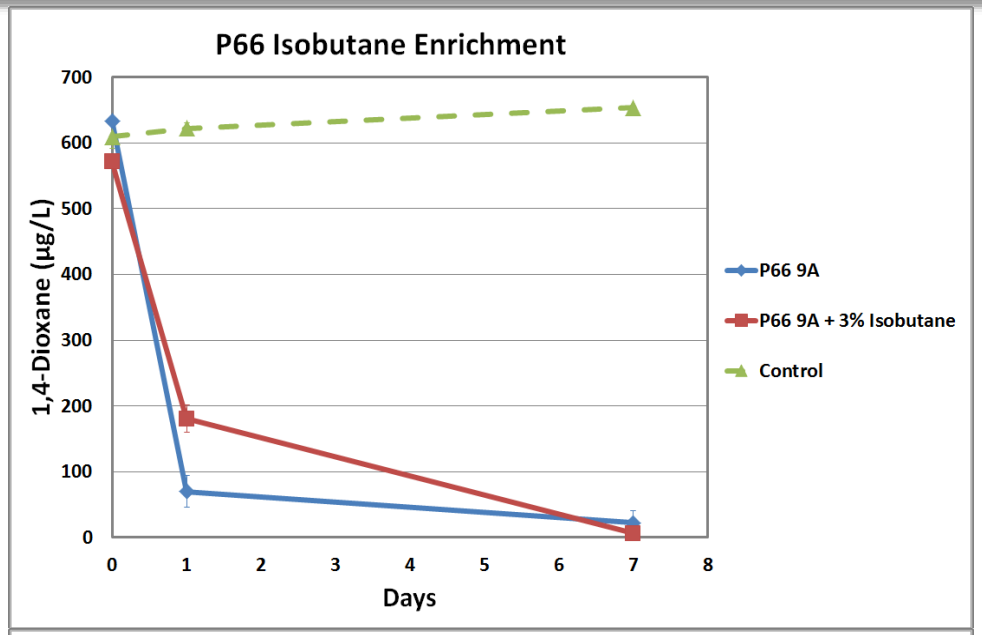
P-70 Methane Enrichments (NMS media)

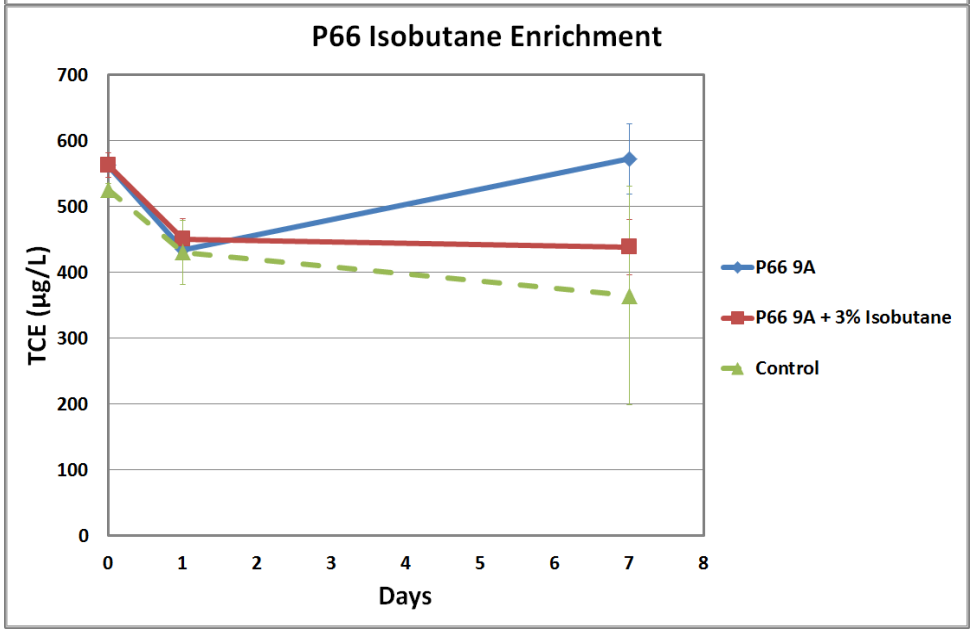




- No 1,4-D degradation
- Some TCE degradation

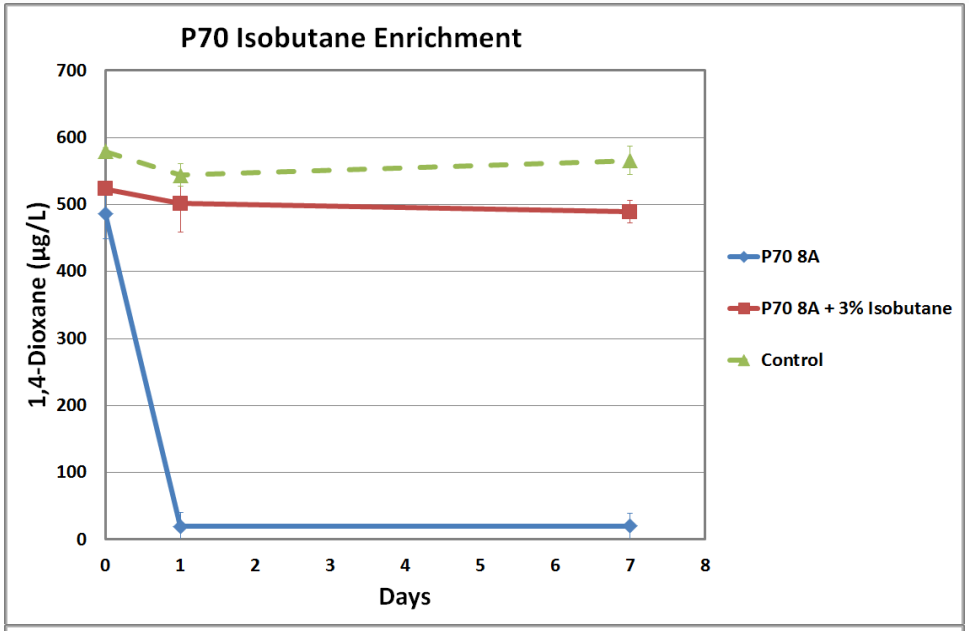
P-66 Isobutane Enrichments

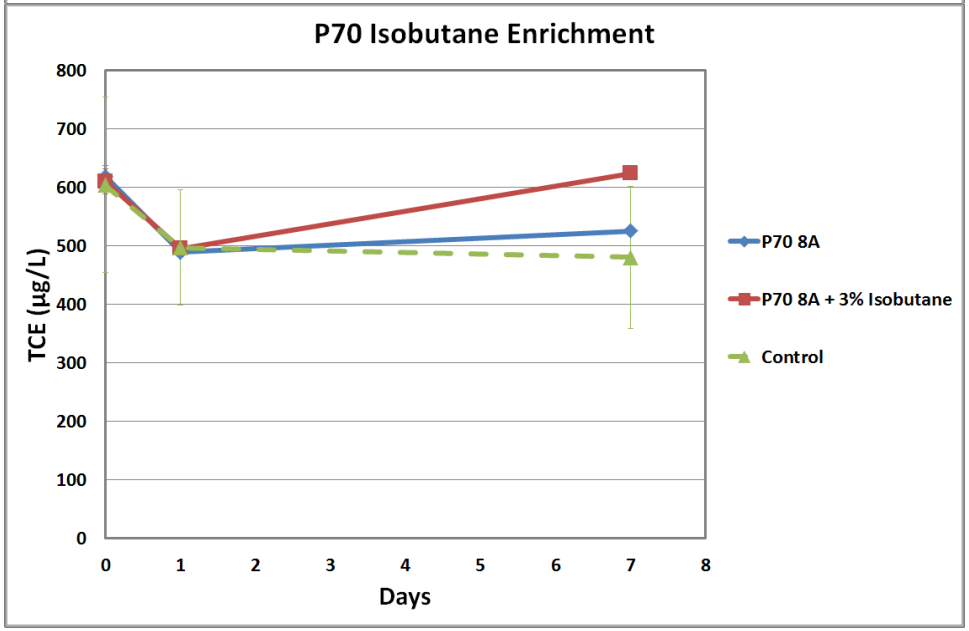




- 1,4-D quickly degraded
- No significant isobutane Inhibition
- Did not degrade TCE
  - Common for isobutane cultures

P-70 Isobutane Enrichments





- 1,4-D quickly degraded in absence of isobutane
- Isobutane Inhibition
- Did not degrade TCE

Conclusions

- Propane was the most effective substrate in stimulating 1,4-D biodegradation in both studies
- Isobutane was also an effective substrate. However, regulatory target (7 µg/L) not achieved in the P-66 study
- Significant biodegradation of 1,4-D was not observed in any treatments microcosms that were not amended with inorganic nutrients
- Microcosm data, in conjunction with enrichment testing data, suggest that methane is, at best, a very poor cometabolic substrate for degradation of 1,4-D at this site
- In the presence of nutrients and oxygen, THF was biodegraded in both studies, irrespective of the presence of alkane gases
- The addition of propane, in conjunction with oxygen and nutrients, is a viable approach for in situ degradation of 1,4-D and THF at this site…even in the anaerobic portion of the plume

Acknowledgements

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Questions?

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