Battelle

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Bench-Scale Evaluation of 1,4-Dioxane Biodegradation via Alkane Gas-Mediated Cometabolism in the Presence and Absence of 1,1-DCE and 1,1-DCA

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Background

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Background

- Site history
  - Industrial setting
  - 16.6 acres, 16 areas of concern
  - Groundwater extraction and treatment
- Site conditions
  - 1,4-Dioxane ~500 µg/L
  - 1,1-Dichloroethylene (1,1-DCE) ~1,800 µg/L
  - 1,1-Dichloroethane (1,1-DCA) ~1,300 µg/L
  - Uniform, medium to fine sand and silt
- Remedial action objectives
  - Mitigation of contaminated groundwater migration

Background: Cometabolic Bioremediation of 1,4-Dioxane

- 1,4-Dioxane levels not high enough to promote metabolic bioremediation
- Cometabolism of 1,4-Dioxane
  - Better at sub-parts per million (ppm) concentrations
  - Demonstrated using alkane gases as primary growth substrate
    - Methane
    - Propane
    - Ethane
    - Isobutane
  - Many new isolate bacteria cultures
    - *Rhodococcus ruber* ENV425

Biodegradation
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CrossMark
ORIGINAL PAPER

Potential for cometabolic biodegradation of 1,4-dioxane in aquifers with methane or ethane as primary substrates

Paul B. Hatzinger · Rahul Banerjee · Rachael Rezes · Sheryl H. Streger · Kevin McClay · Charles E. Schaefer

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Aerobic biodegradation kinetics for 1,4-dioxane under metabolic and cometabolic conditions

Check for updates

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Experimental Approach

Experimental Approach: Objectives

- Identify a bioremediation approach to achieve cleanup goals:
  - 1,4-Dioxane below 50 µg/L
  - 1,1-DCE below 5 µg/L
  - 1,1-DCA below 5 µg/L
- Evaluate anaerobic biodegradation of 1,1-DCE and 1,1-DCA
  - Enhanced reductive dechlorination (ERD)
  - Vegetable oil/nutrients (Tersus) + bioaugmentation with KB-1 Plus® (SiREM)
- Evaluate aerobic cometabolism of 1,4-dioxane by alkane gases
  - Propane and butane as primary growth substrates
  - Bioaugmentation with propanotroph ENV425 (APTIM) in the presence and absence of 1,1-DCE and 1,1-DCA

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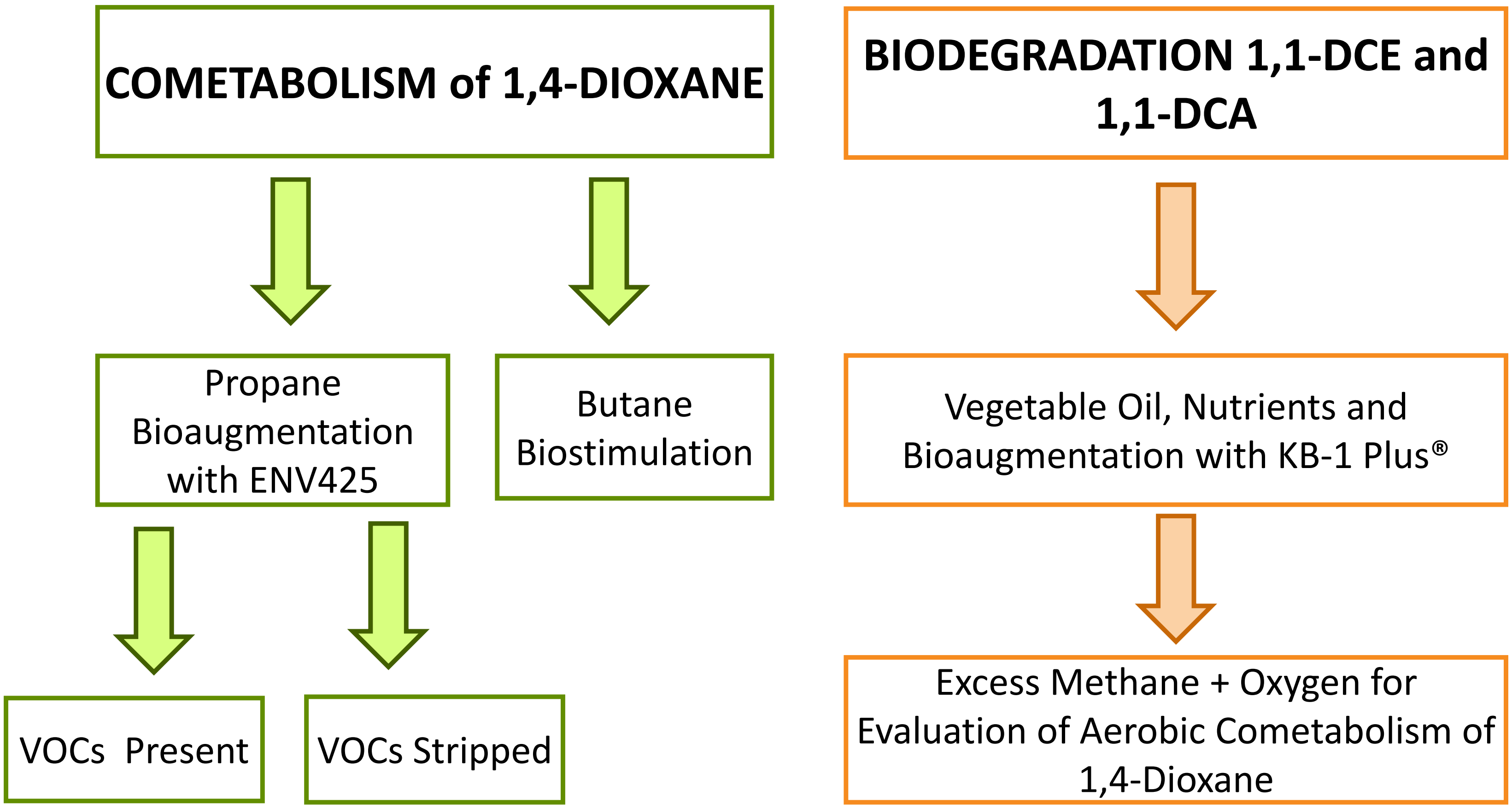
Experimental Approach

- Two sets of microcosms:
  - Aerobic
  - Anaerobic



Experimental Approach: Aerobic Microcosms

#	Treatment/Control	Description
1	Sterile Control	Autoclaved and amended with glutaraldehyde for sterilization
2	Intrinsic Control	Amended (once) with air only
3	Propane Amended and ENV425 Bioaugmented	Amended with air, propane, Rhodococcus ruber ENV425 and nutrients (DAP + NaMo)
4	Propane Amended, ENV425 Bioaugmented, and VOCs Removed	VOCs stripped by sparging microcosms with nitrogen, followed by amendment with air, propane, 2% v/v ENV425 and nutrients (DAP + NaMo)
5	Butane Amended	Amended with air, butane and nutrients (DAP+NaMo) to stimulate the growth of potential indigenous bacteria that could cometabolize 1,4-dioxane

DAP = Diamonium Phosphate
NaMo = Sodium Molybdate Dihydrate

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Experimental Approach: Anaerobic Microcosms

#	Treatment/Control	Description
6	Sterile Control	Autoclaved and amended with glutaraldehyde for sterilization
7	Intrinsic Control	Unamended, prepared in anaerobic chamber
8	Vegetable Oil + KB-1 Plus®	Amended with vegetable oil (Tersus EDS QR/ER), nutrients (Tersus Nutrimens), and 2% v/v bacteria culture KB-1 Plus®

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Experimental Approach

Homogenize Soil and Groundwater →

Assemble Microcosms
- 50 g of site soil
- 120 mL of site groundwater

→

Amendments
- Nutrients
- Alkane gases
- Air
- Bacteria cultures

→

Incubation and Monitoring
- 1,4-Dioxane
- VOCs
- pH/ORP
- Resazurin color
- Microbial analysis

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Results

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Results

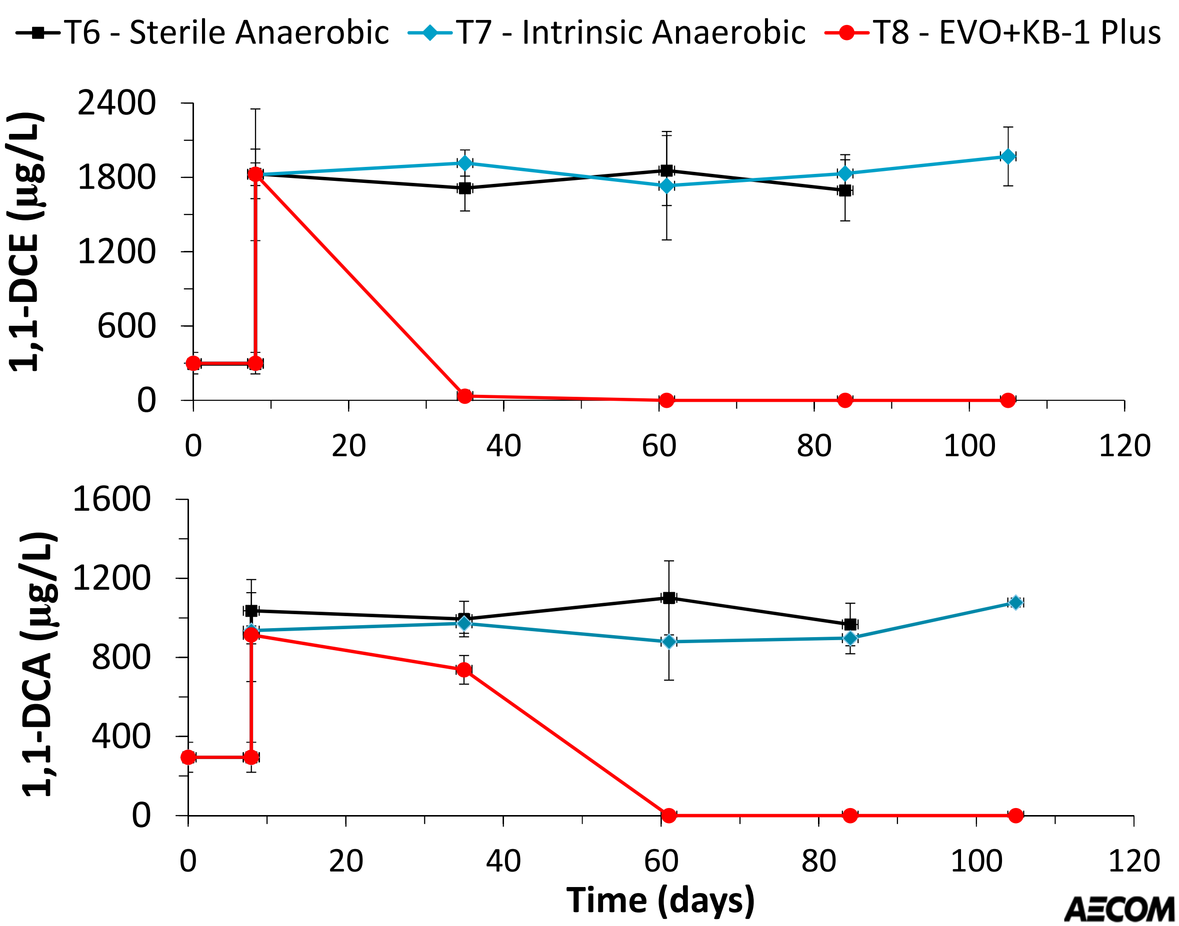
– Groundwater baseline characterization
  - Neutral pH
  - Oxidizing conditions
  - Moderate sulfate levels
  - High iron levels
  - 1,1-DCA and 1,1-DCE lower than expected, so microcosms were spiked

Analyte	Baseline Value
pH	7.2
ORP (mV)	244.3
Alkalinity (mg/L)	319
Chloride (mg/L)	85.1
Sulfate (mg/L)	23
Iron, Total (mg/L)	170
1,1-DCA (µg/L)	**190**
1,1-DCE (µg/L)	**195**
1,2-DCA (µg/L)	19
1,4-Dioxane (µg/L)	**497**
TCE (µg/L)	12
VC (µg/L)	0.59
cis-1,3-DCE (µg/L)	0.94

Results

Reductive Dechlorination Results

- Biodegradation of 1,1-DCE and 1,1-DCA:
  - Bioaugmentation with KB-1 Plus culture and amendment of vegetable oil and nutrients worked
  - 1,1-DCE was preferentially degraded over 1,1-DCA



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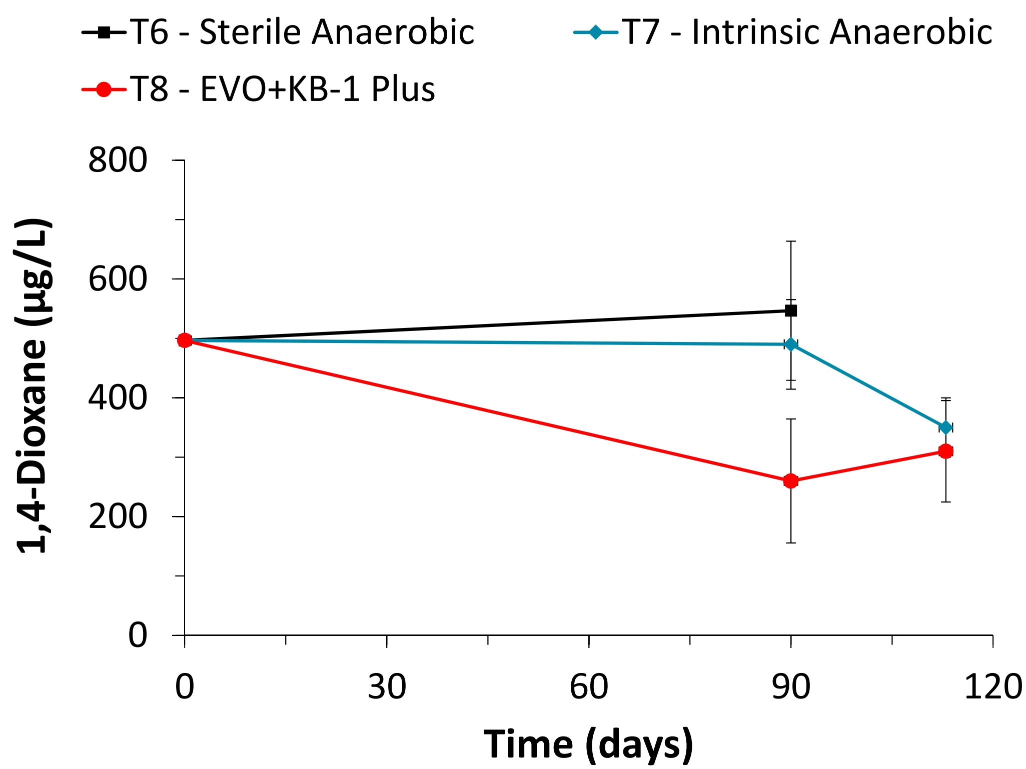
Results

Reductive Dechlorination Results

- 1,4-Dioxane:
  - Partial decrease by day 90
  - Decrease may be due to re-oxidation of reduced iron by oxygen intrusion during sampling which generates hydroxyl radicals (Barajas-Rodriguez, 2016; Sekar & Dichristina, 2014)



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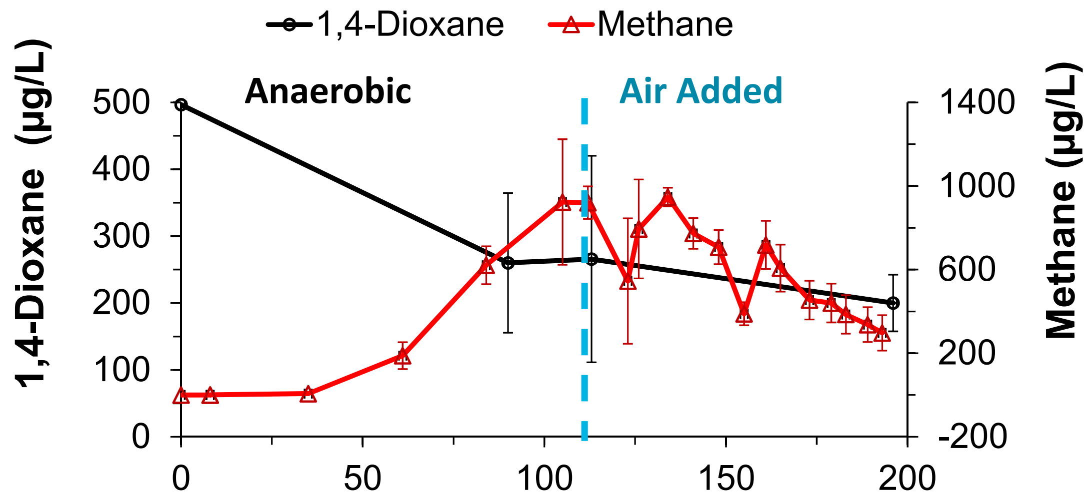
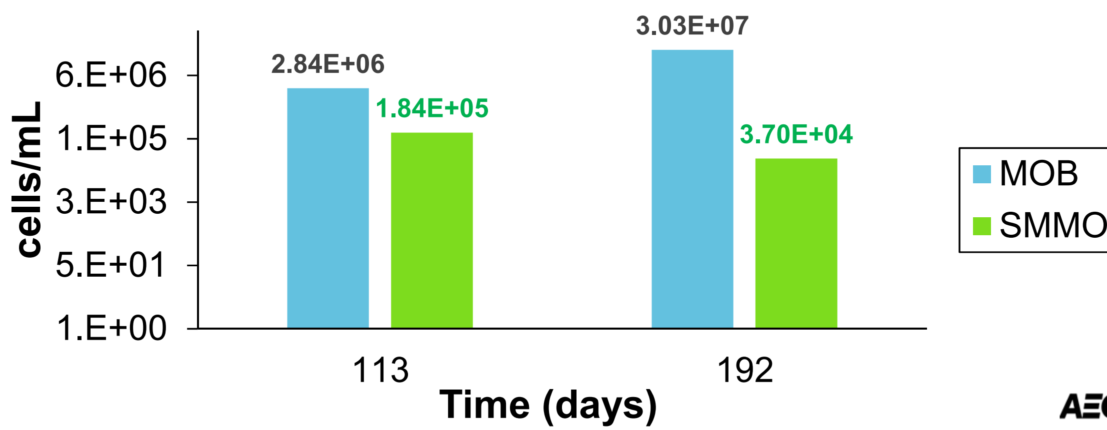
Results

Methane Cometabolism Results

Anaerobic microcosms evaluated for biodegradation of 1,4-dioxane:
- Limited during methane cometabolism
- Methane consumption was achieved after adding oxygen
- Challenge to reverse redox environment

MOB = Methane Oxidizing Bacteria
SMMO = Soluble Methane Monooxygenase





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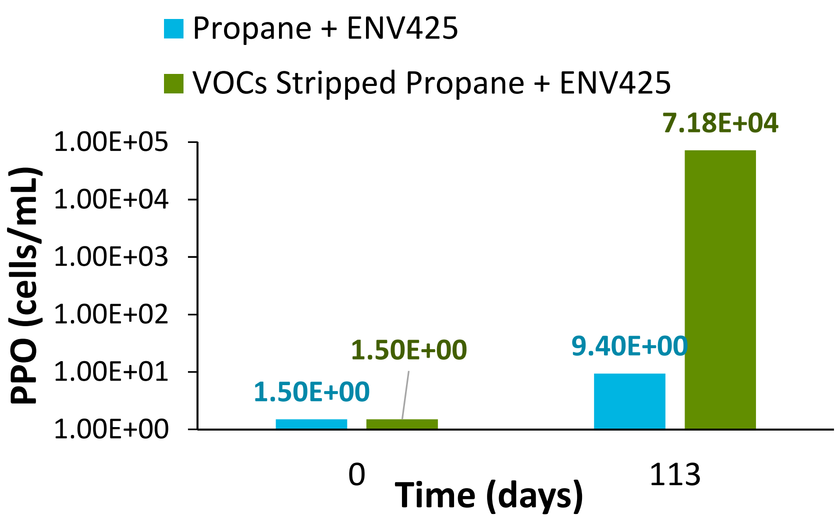
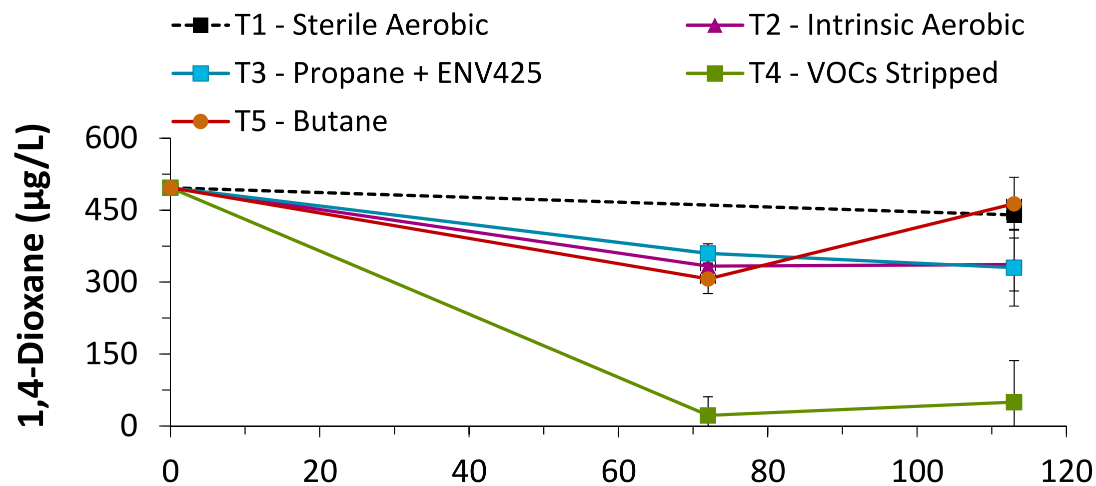
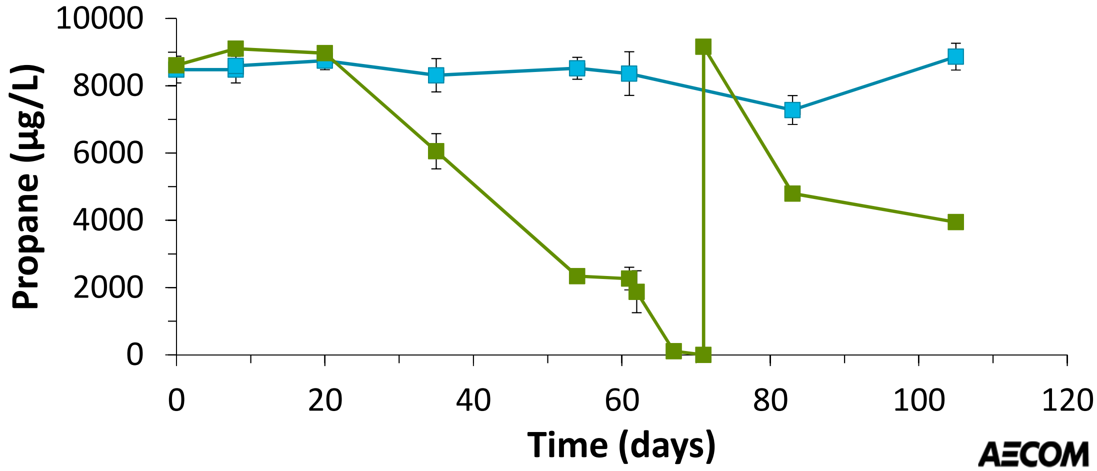
Results

Aerobic Cometabolism Results

- Biodegradation of 1,4-dioxane:
  - 1,1-DCE and 1,1-DCA highly inhibitory
  - VOCs had to be stripped from the groundwater
  - ENV425 culture and propane amended



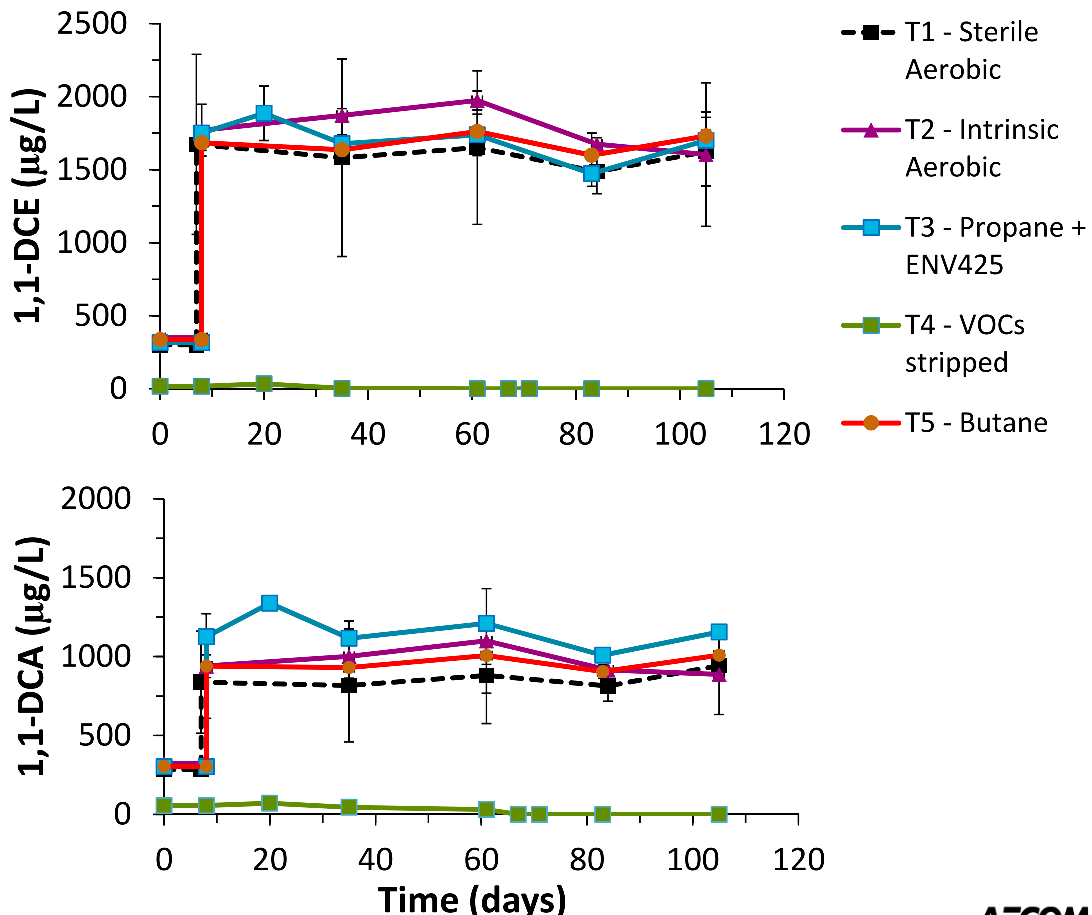




Results

Aerobic Cometabolism Results: VOCs

- Non-stripped bottles
  - No biodegradation
- Stripped bottles
  - 1,1-DCE decreased from 17 µg/L to non-detection
  - 1,1-DCA decreased from 56 µg/L to non-detection



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Conclusions

Conclusions

- Anaerobic microcosms
  - 1,1-DCE preferably degraded over 1,1-DCA
  - Partial degradation of 1,4-dioxane most likely due to reduced iron oxidation
  - Lower ORP (-100 mV) and pH (6.45) in bioaugmented microcosms
- Aerobic propane-biostimulated cometabolism of 1,4-dioxane
  - 1,1-DCE and 1,1-DCA highly inhibitory
  - Propane biostimulation with ENV425 bioaugmentation worked when chlorinated solvents were absent
- Aerobic methane-biostimulated cometabolism of 1,4-dioxane
  - Tested in microcosms that underwent reductive dechlorination
  - Methane consumption demonstrated after adding oxygen
  - Partial 1,4-dioxane cometabolism but oxygen was limiting reagent

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Questions?

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Thank You!

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