Aerobic Biodegradation of Aromatic, Chlorinated Aliphatic, and Ether Contaminants by *Pseudonocardia sp.* strain ENV478 and Native Populations

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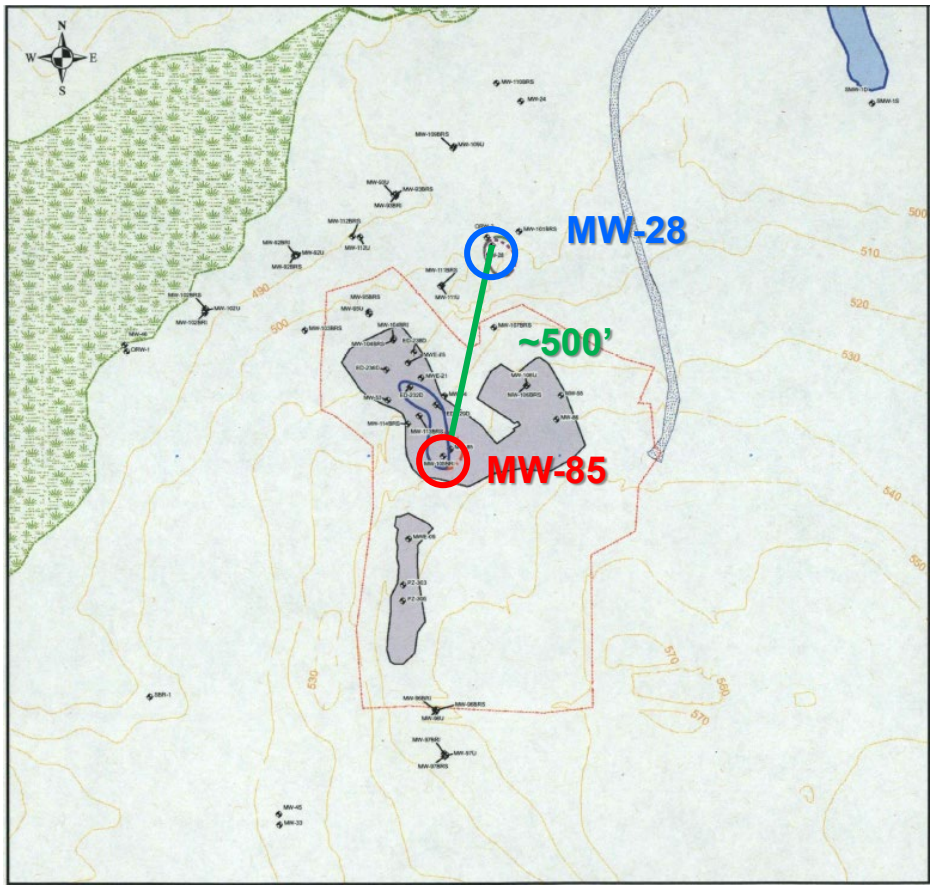
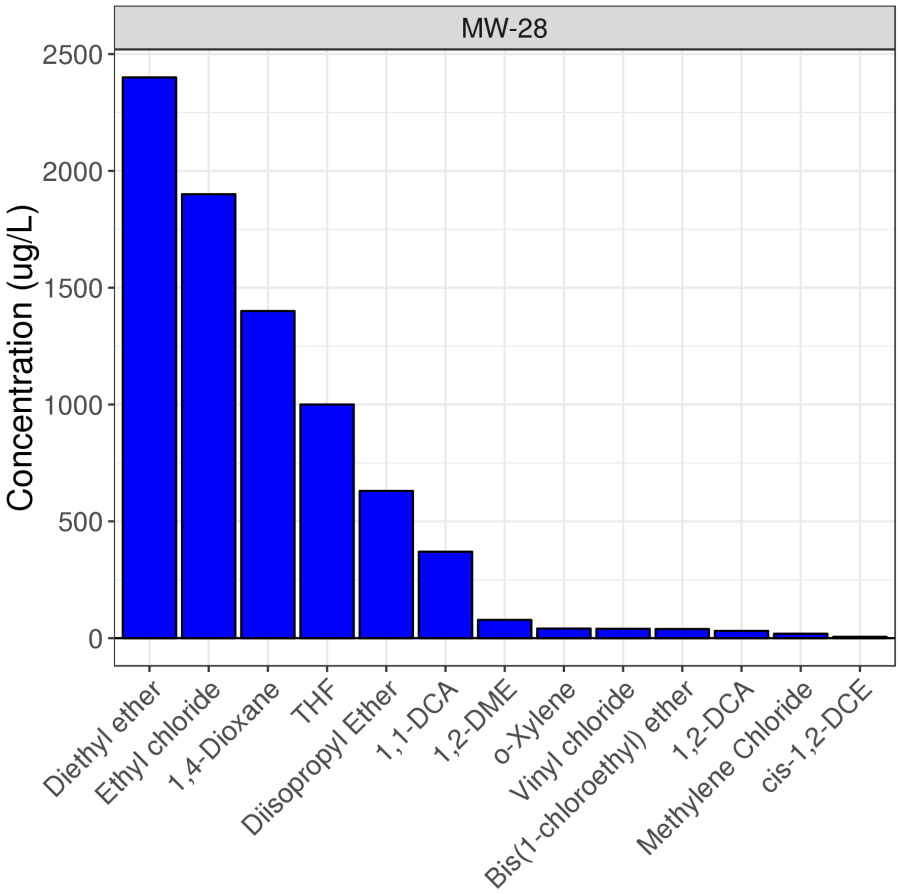
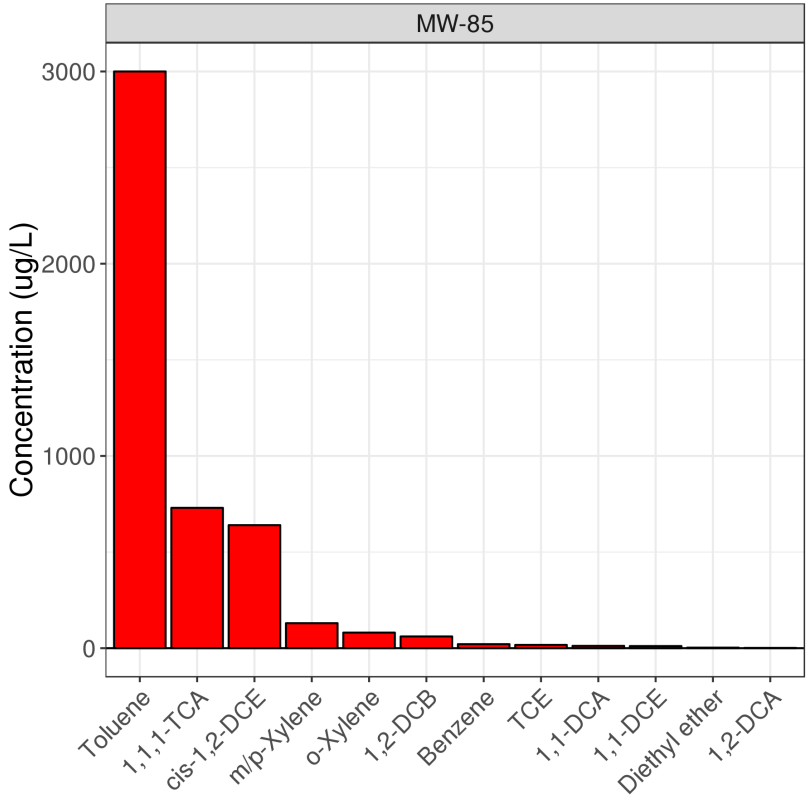
Background

- Superfund site in NE US
- Decades of remedial efforts
  - ppt – low ppm aromatics, cVOCs, ethers remaining
- Geology
  - MW-28 –competent bedrock (32-42 ft bgs)
  - MW-85 - overburden (25-29 ft bgs), weathered bedrock (29-30 ft bgs)
  - Wells are not hydraulically connected
- Geochemistry
  - pH: 5.8 – 6
  - DO: 0.24 – 0.5 mg/L
  - ORP: -36.6m to -78 mV



COCs of interest





COCs differ between MW-28 and MW-85

Objectives

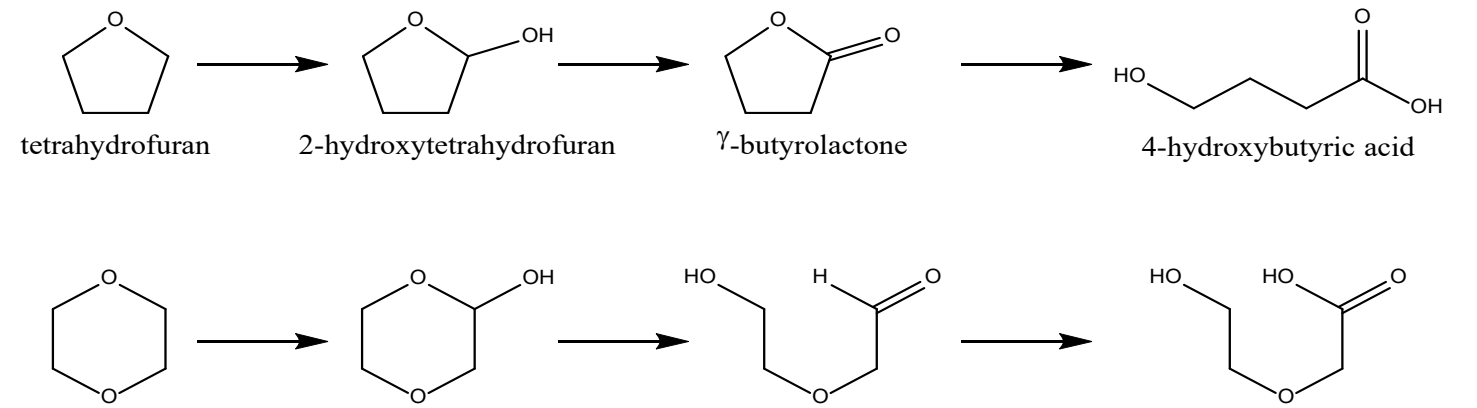
Evaluate potential bioremediation options

- Aerobic biostimulation & bioaugmentation
- THF present as growth substrate for 1,4-DX degradation

Evaluate biodegradation of site COCs using *Pseudonocardia sp.* strain **ENV478**

- Co-metabolic ether degrader (1,4-DX, BCEE, MTBE)
- Degradation of **aromatics and cVOCs *not* previously tested**

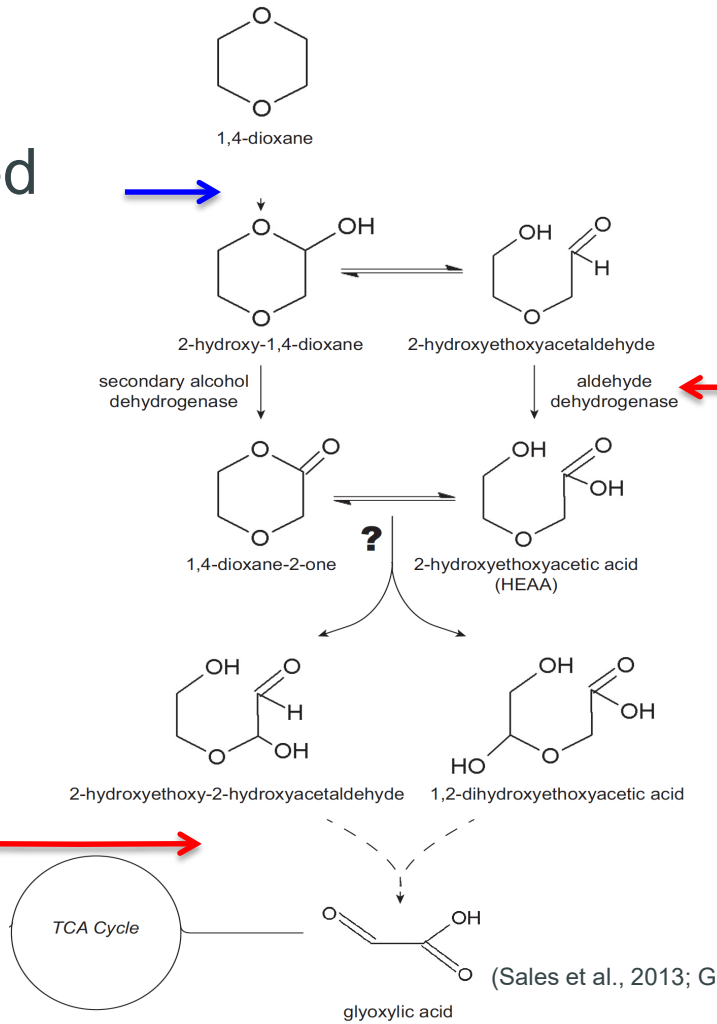
Proposed Degradation Pathways



Appl. Environ. Microbiol. **2006,** *72* (8), 5218-24.

Pathways

MO catalyzed reaction



Potential ALDH catalyzed reaction

Potential ALDH catalyzed reaction

(Sales et al., 2013; Grostern et al., 2012; Mahendra et al., 2007)

CENSUS (qPCR) Data
MW-28

Functional Gene	Abbreviation	cells/mL	Percentile (%)
Total Eubacteria	**EBAC**	**1.83×10^{6}**	**70**
Dioxane/THF Monooxygenase	*DXMO*	*6.00×10^{-1} (J)*	---
Aldehyde Dehydrogenase	ALDH	<5.00	---
Propane Monooxygenase	PPO	7.17×10^{1}	---
Soluble Methane Monooxygenase	**SMMO**	**9.86×10^{2}**	**38**
Particulate Methane Monooxygenase	PMMO	<5.00	---
Phenol Hydroxylase	**PHE**	**3.71×10^{3}**	**72**
Toluene-2-Monooxygenase	**RDEG**	**4.93×10^{3}**	**82**
Toluene-3,4-Monooxygenase	**RMO**	**2.27×10^{3}**	**75**

- High levels of bacteria and functional gene expression measured
- Estimable amounts of DXMO suggest some dioxane/THF degradation may be ongoing

CENSUS (qPCR) Data

MW-85

Analyte	Abbreviation	cells/mL	Percentile (%)
Total Eubacteria	**EBAC**	**1.32×10^6**	**65**
Dioxane/THF Monooxygenase	DXMO	<4.70	---
Aldehyde Dehydrogenase	ALDH	<4.70	---
Propane Monooxygenase	PPO	9.40	---
Soluble Methane Monooxygenase	**SMMO**	**9.86×10^2**	**22**
Particulate Methane Monooxygenase	PMMO	<5.00	---
Phenol Hydroxylase	**PHE**	**3.71×10^3**	**65**
Toluene-2-Monooxygenase	**RDEG**	**4.93×10^3**	**62**
Toluene-3,4-Monooxygenase	**RMO**	**2.27×10^3**	**66**

- High levels of bacteria and functional gene expression were measured

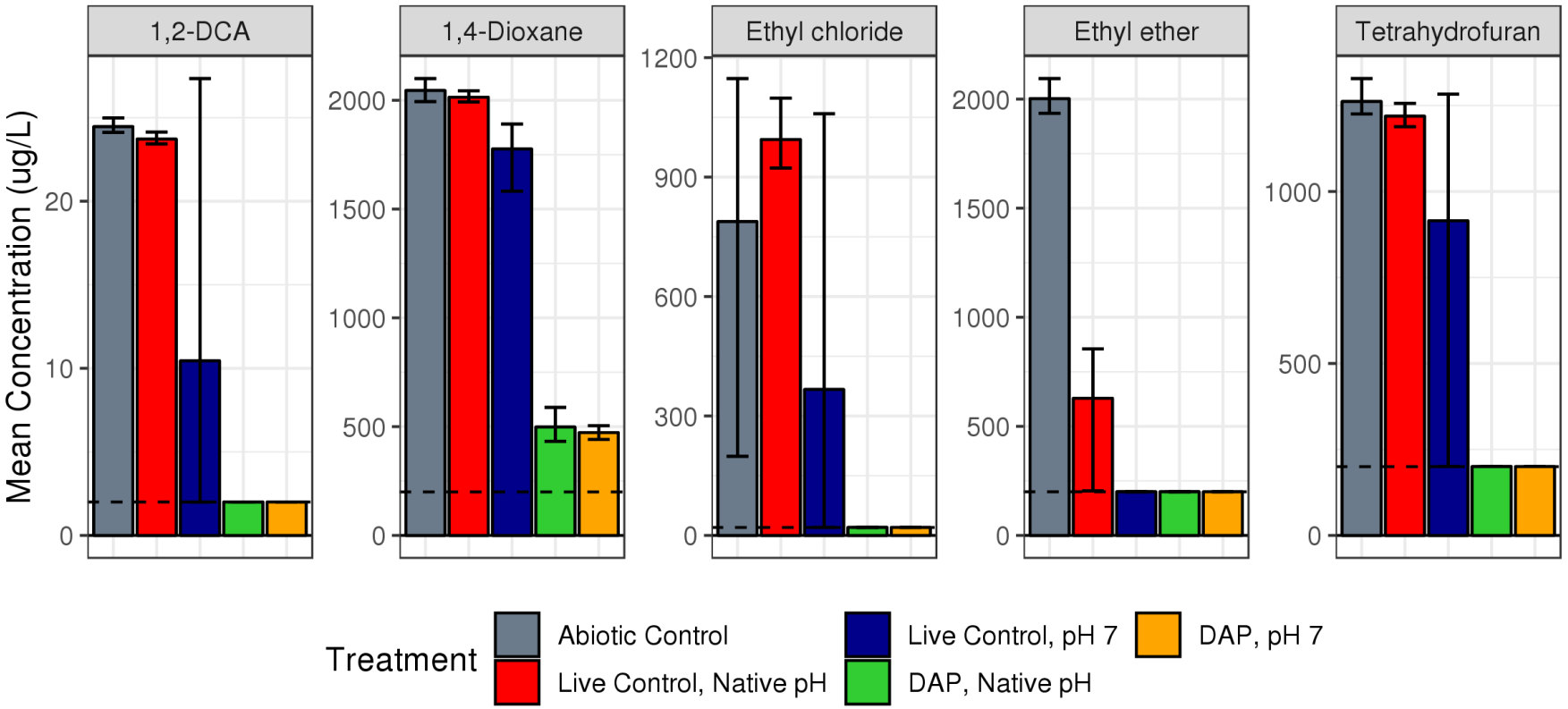
Treatments

Treatment	Amendment	Amendment Amount	pH
Biostimulation			
Abiotic Control	$HgCl_2$	250 mg/L	Native
Live Control	---	---	Native, 7
DAP	Diammonium phosphate*	9.6 mg/L	Native,7
DAP + propane	Diammonium phosphate* Propane	9.6 mg/L 3 mL @ 1 atm	Native,7
Bioaugmentation			
Groundwater	ENV478	5 mL	Native
Basal Salt Medium (BSM)	ENV478 + COCs	5 mL	7

Microcosms prepared in triplicate
All treatments except BSM contain 125 mL groundwater from MW-28 or MW-85
*Diammonium phosphate (DAP) based on C estimate from COCs
BSM treatments to differentiate degradation by ENV478 from native organisms

Biostimulation: *MW-28*

Native pH = 6.0

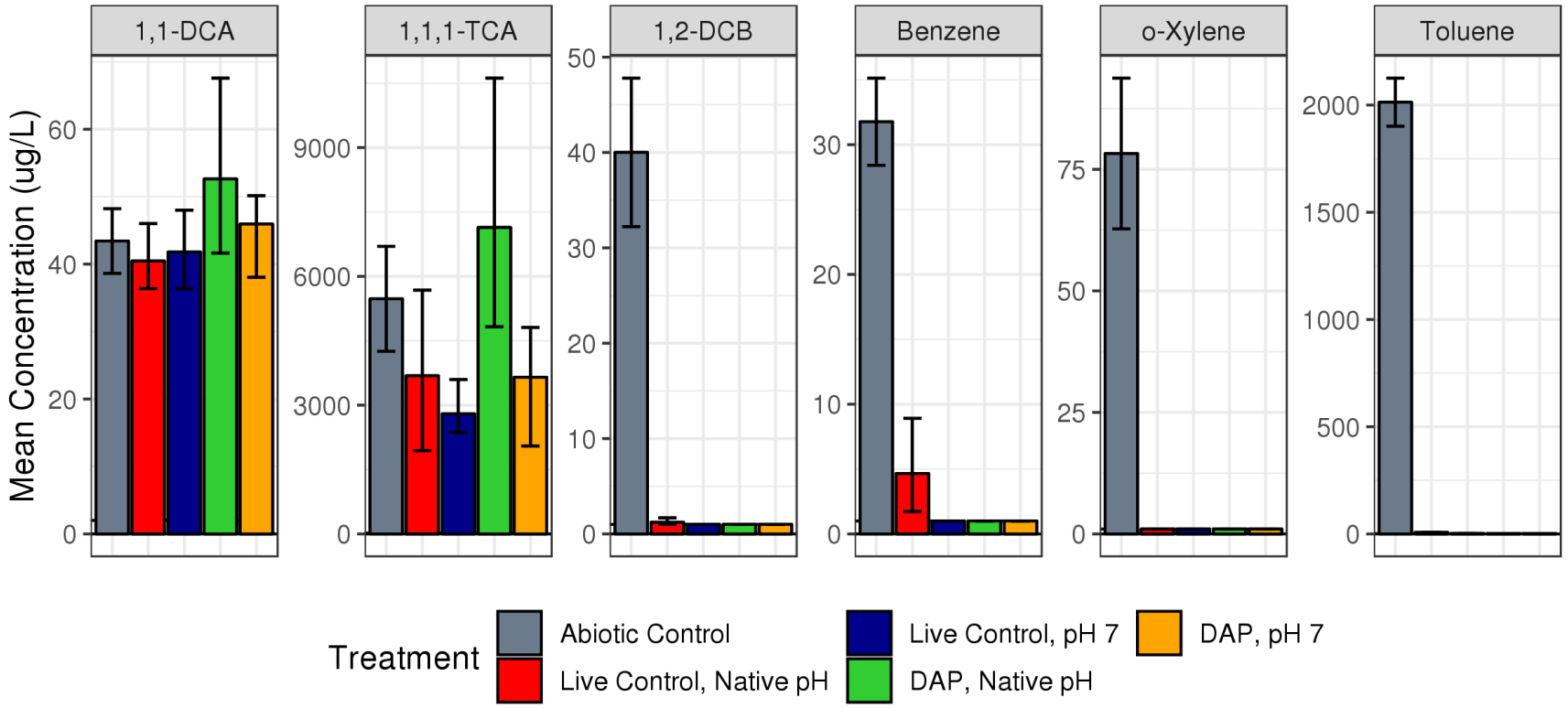


Bars represent the range of replicate data. Data collected at 180 d.

- **Oxygenation of groundwater is not sufficient for degradation**
- **Addition of N&P stimulates biodegradation**
- **Increasing pH helped accelerate removal**
- **1,4-DX degradation stalled**

Biostimulation: *MW-85*

Native pH = 5.8

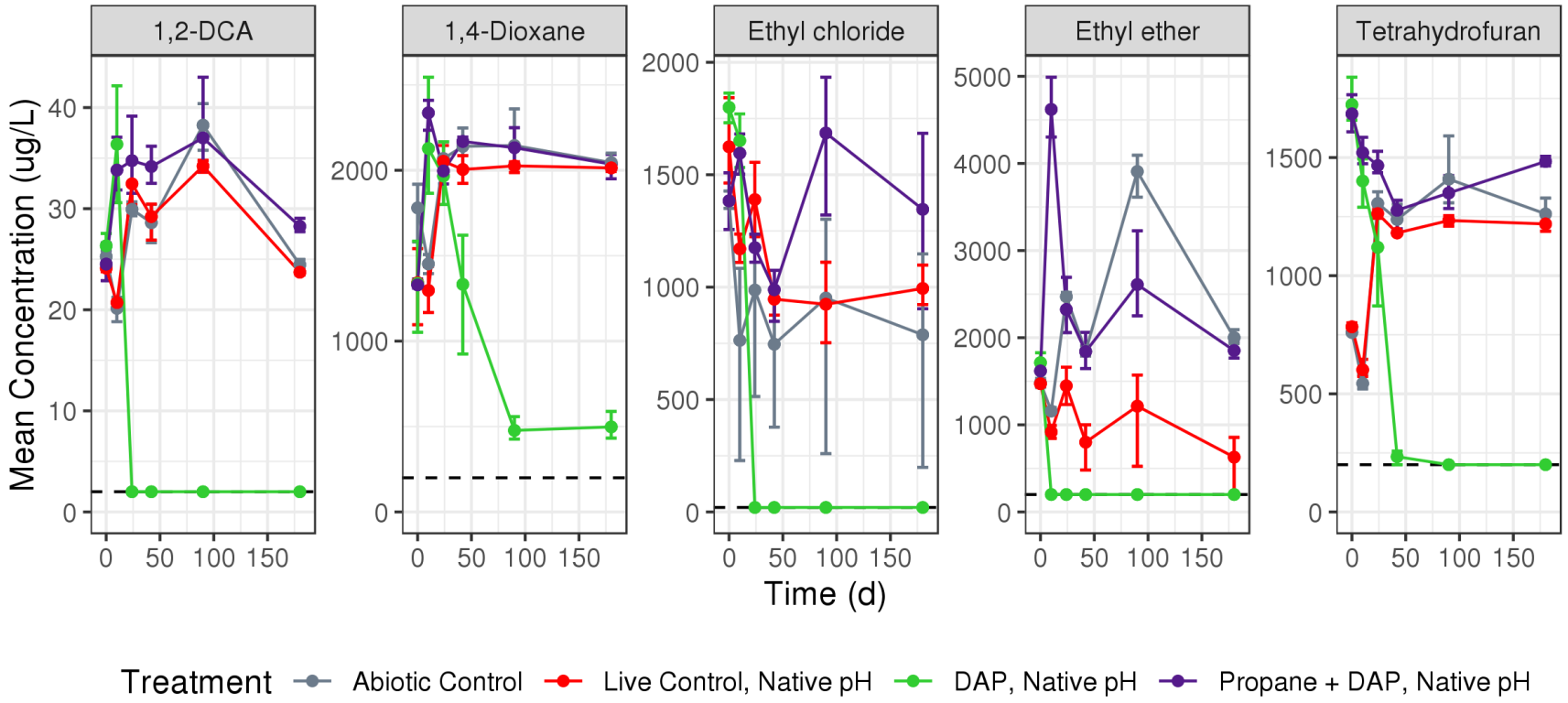


Bars represent the range of replicate data. Data collected at 180 d.

- **Oxygenation of groundwater was sufficient for degradation of aromatics**
- **Amendment with nutrients and pH adjustment accelerated degradation**
- **No significant degradation of 1,1-DCA and 1,1,1-TCA**

Biostimulation: Effect of Propane

Native pH = 6.0
MW-28

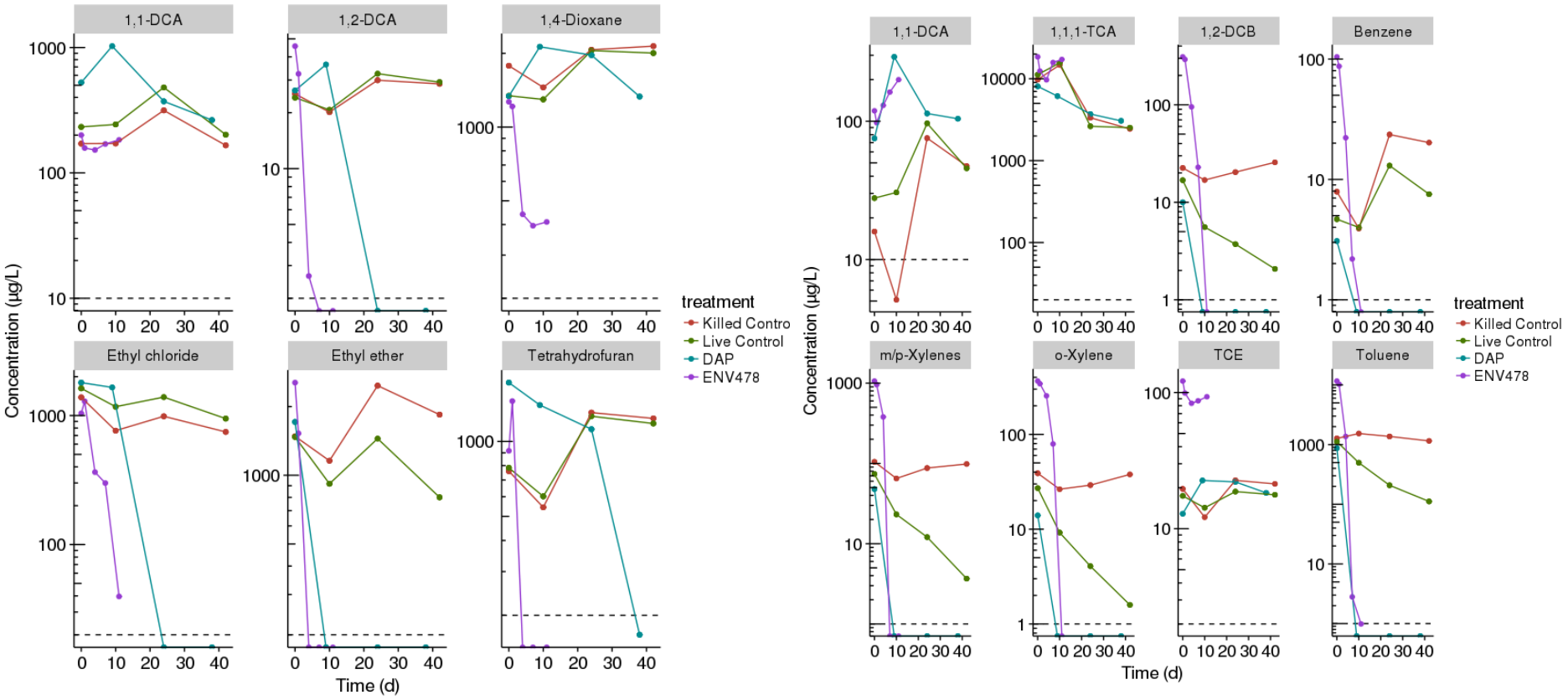


Bars represent the range of replicate data.

Propane amendment inhibited degradation of COCs
→ preferential C source?

Bioaugmentation Experiments

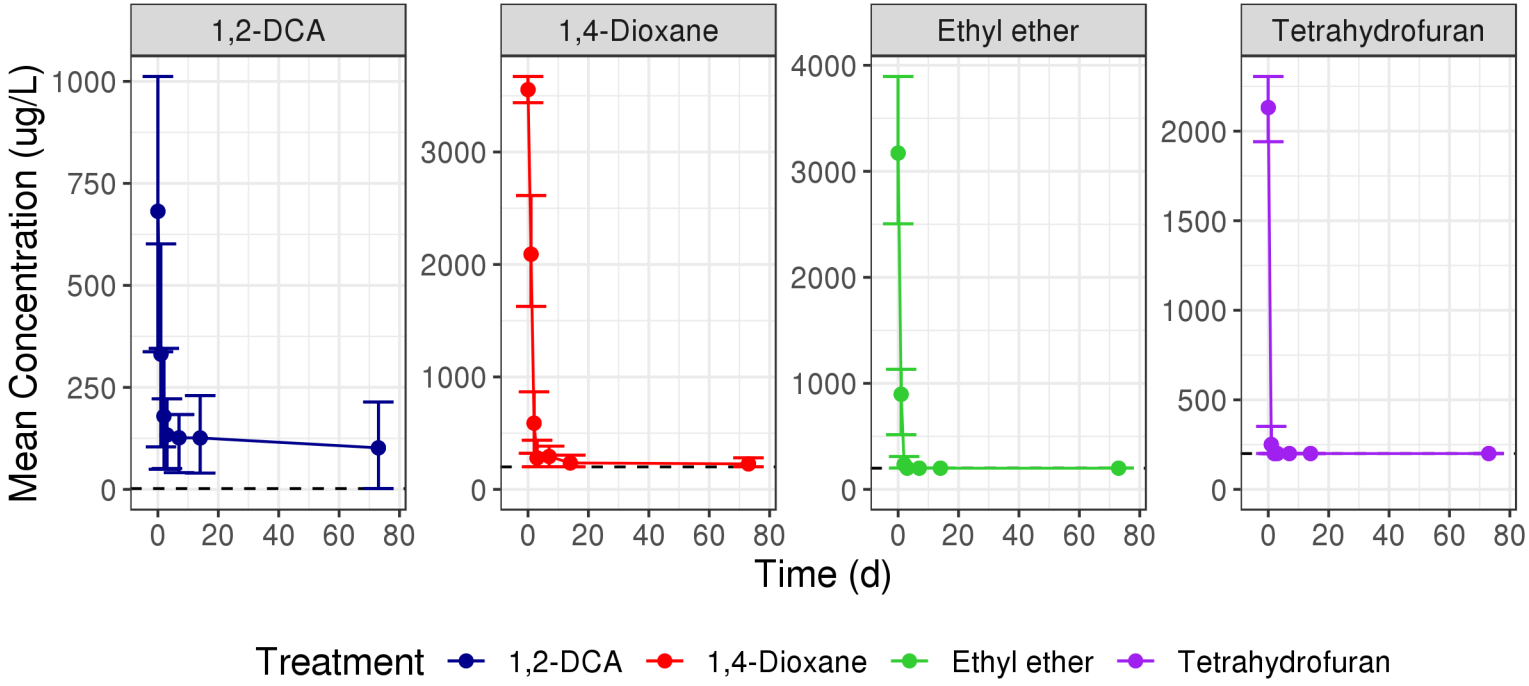
MW-28 *Native pH* **MW-85**



- Addition of **ENV478** resulted in faster degradation of ethers, ethyl chloride & 1,2-DCA than DAP; aromatics showed similar removal to DAP
- **No significant degradation of 1,1-DCA & 1,1,1-TCA observed**

Bioaugmentation with ENV478

MW-28 Simulated Sample (BSM)

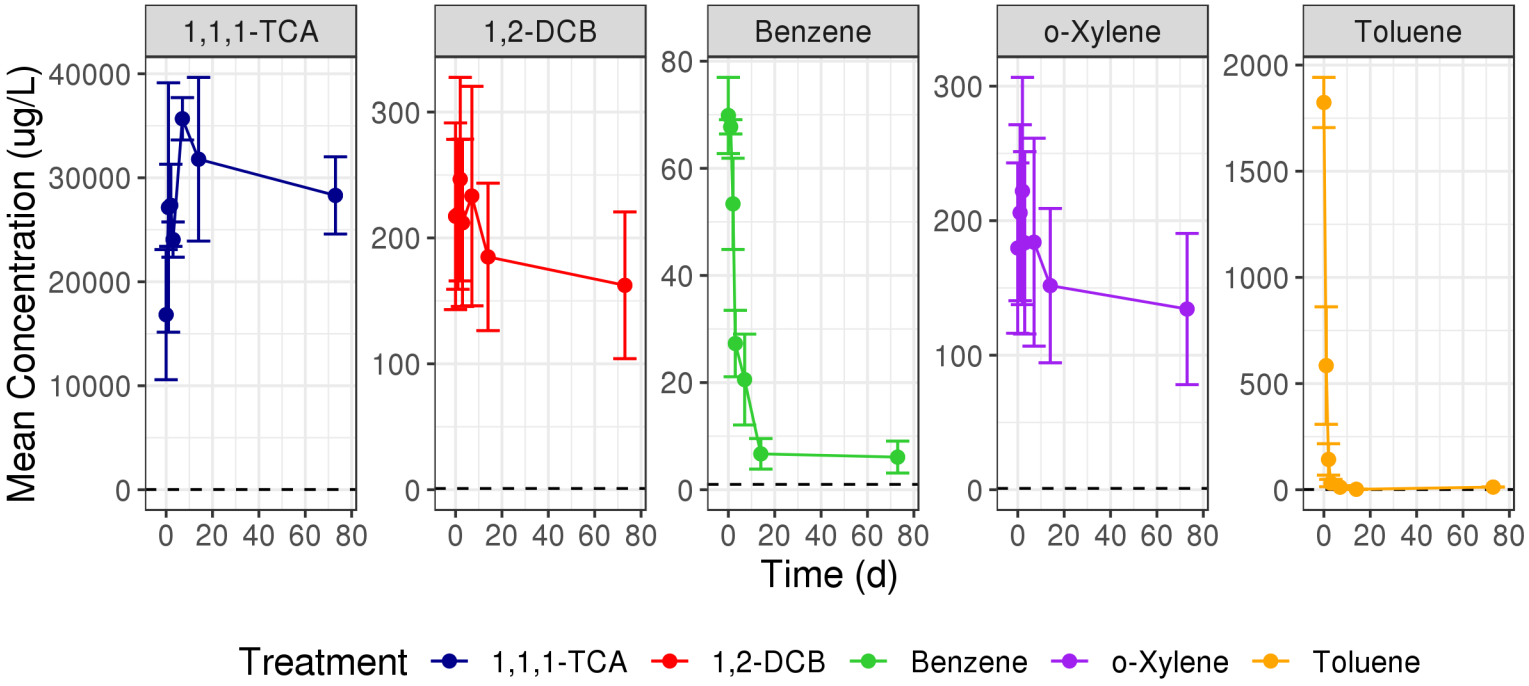


Bars represent the range of replicate data.

- **Rapid degradation of all ethers observed**
- **Initial rapid degradation of 1,2-DCA stalled after consumption of ethers → co-metabolic?**

Bioaugmentation with ENV478

MW-85 Simulated Sample (BSM)



Bars represent the range of replicate data.

- **ENV478 rapidly degraded benzene and toluene**
- **No significant degradation for other COCs**

Summary

- Lack of nutrients & low pH are limiting aerobic biodegradation
- >95% removal of THF, diethyl ether, ethyl chloride and 1,2-DCA after biostimulation in less than 50 days while 1,4-DX showed about 75% removal
- Similarly non-detectable levels of benzene, toluene, xylene, and 1,2-DCB were observed under biostimulating conditions
- Treatments with propane to induce native co-metabolic degradation were unsuccessful
- ENV478 rapidly degraded all ethers within 2 weeks
- ENV478 also degraded benzene, toluene to ND & 1,2 DCA by ~ 85%
- 1,1-DCA & 1,1,1-TCA were persistent under all conditions tested

Thank You

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