Thank you for signing in early

The webinar will begin promptly at
12:00 pm ET, 9:00 am PT

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SERDP and ESTCP Webinar Series

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    - https://www.youtube.com/user/SERDPESTCP
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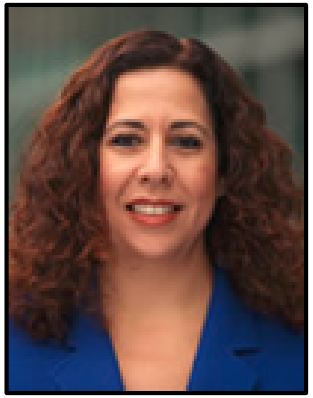
Managing Chlorinated Solvents in Groundwater Using Biological Treatment

February 25, 2021

SERDP DOD • EPA • DOE | ESTCP

Welcome and Introductions



Rula A. Deeb, Ph.D.
Webinar Coordinator

SERDP DOD • EPA • DOE | ESTCP

Webinar Agenda

- **Webinar Logistics** (5 minutes)
  Dr. Rula Deeb, Geosyntec Consultants
- **Overview of SERDP and ESTCP** (5 minutes)
  Dr. Andrea Leeson, SERDP and ESTCP
- **Aerobic and Anaerobic Bacteria Team Up to Biodegrade CVOCs and 1,4-Dioxane** (25 minutes + Q&A)
  Dr. Shaily Mahendra, University of California, Los Angeles
- **Cometabolic Biobarriers: A Sustainable Approach to Treat Large Dilute CVOC Plumes** (25 minutes + Q&A)
  Mr. David Lippincott, APTIM Federal Services, LLC
- **Final Q&A session**

Zoom Instructions

- Download Zoom
  - https://zoom.us/download
- If you cannot download Zoom, you can view the slides using an internet browser
  - Create a free Zoom account (https://zoom.us/signup)
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Zoom Instructions (Cont'd)

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- We will also be live streaming the webinar on the SERDP and ESTCP YouTube channel
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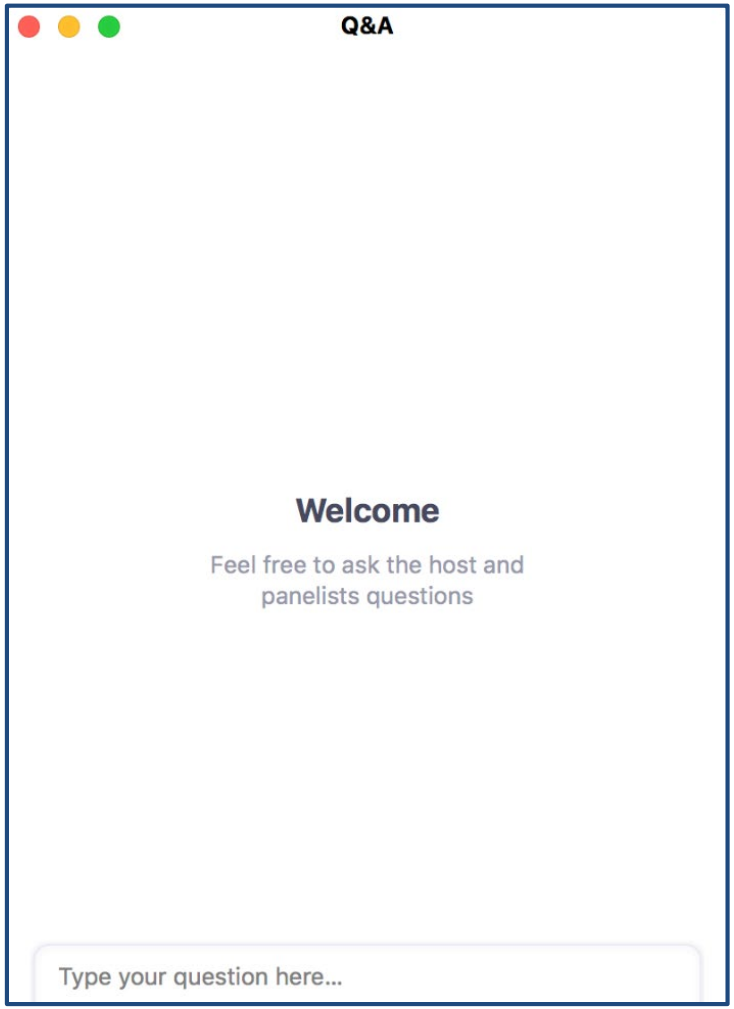
How to Ask Questions

- Find the Q&A button on your control bar and type in your question(s)
- Make sure to add your organization name at the end of your question so that we can identify you during the Q&A sessions



SERDP and ESTCP Overview

Andrea Leeson, Ph.D.
SERDP and ESTCP

SERDP DOD • EPA • DOE | ESTCP

DoD's Environmental Technology Programs

Science and Technology

- Statutory program established 1991
- DoD, DOE, EPA partnership
  - Advanced technology development to address near-term needs
  - Fundamental research to impact real world environmental management

Demonstration and Validation

- Demonstrate innovative cost-effective environmental and energy technologies
  - Transition technology out of the lab
  - Establish cost and performance
  - Partner with end user and regulator
  - Technology transfer
    - Accelerate commercialization or broader adoption
    - Direct technology insertion

Environmental Drivers

Sustaining Ranges, Facilities and Operations

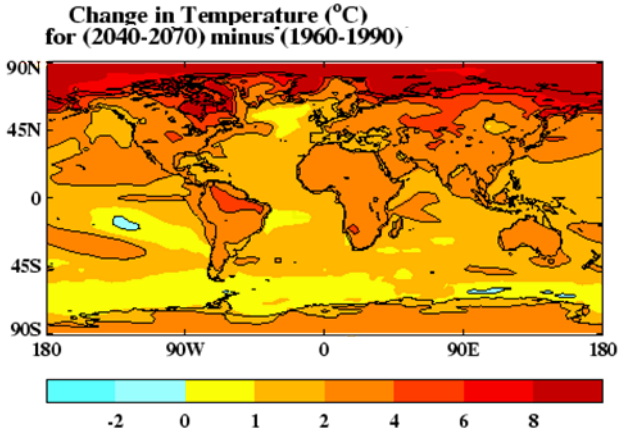
Maritime Sustainability
Threatened and Endangered Species

Toxic Air Emissions and Dust

UXO and Munitions Constituents

Noise

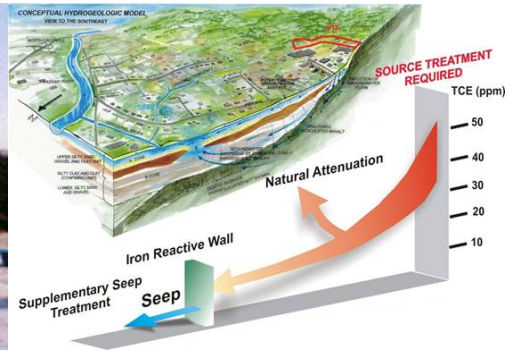
Sustainable FOB



Environmental Drivers

Reducing Current and Future Liability

Contamination from Past Practices



- Groundwater, soils and sediments
- Large UXO liability
- Emerging contaminants

Pollution Prevention to Control Life Cycle Costs

- Elimination of pollutants and hazardous materials in manufacturing, maintenance, and operations
- Achieve compliance through pollution prevention

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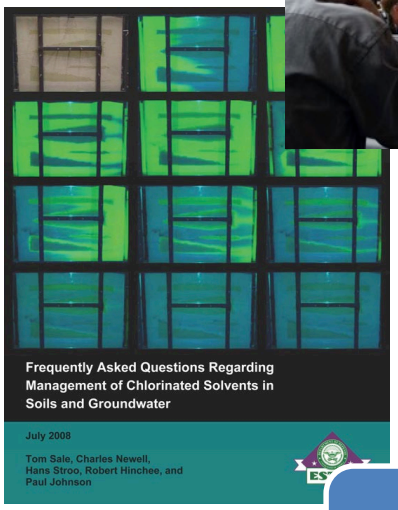


Webinars

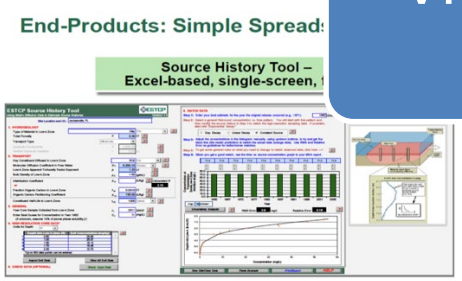
In Person Training

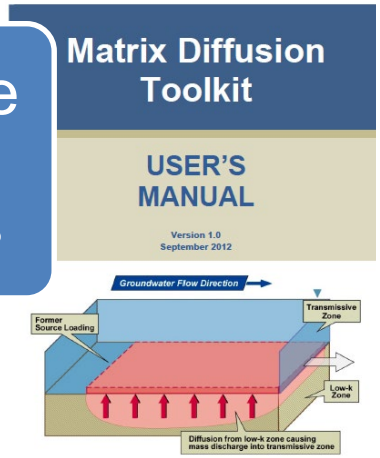
EnviroWiki

Technology Transfer

Videos

Guidance and Manuals





SERDP and ESTCP Webinar Series

Date	Topic
March 11, 2021	New Resource Conservation Paradigms on DoD Lands
March 25, 2021	Safer Alternatives for Surface Engineering and Structural Materials in Weapons Systems and Platforms: A Fred Lafferman Tribute Webinar
April 8, 2021	Advances in Understanding PFAS Ecological Risks
April 22, 2021	Innovative Approaches to Monitor and Survey At-Risk Species on DoD Lands
May 6, 2021	Fate, Transport and Treatment of Munitions Constituents in Soil and Groundwater
May 20, 2021	Advancing Microgrid Solutions
June 3, 2021	PFAS Analytical Challenges and Opportunities
June 17, 2021	Changes in Pathogen Exposure Pathways under Non-Stationary Conditions
July 8, 2021	Energetic Materials and Munitions

For upcoming webinars, please visit

http://serdp-estcp.org/Tools-and-Training/Webinar-Series

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Aerobic and Anaerobic Bacteria Team Up to Biodegrade CVOCs and 1,4-Dioxane

Shaily Mahendra, Ph.D.
University of California, Los Angeles

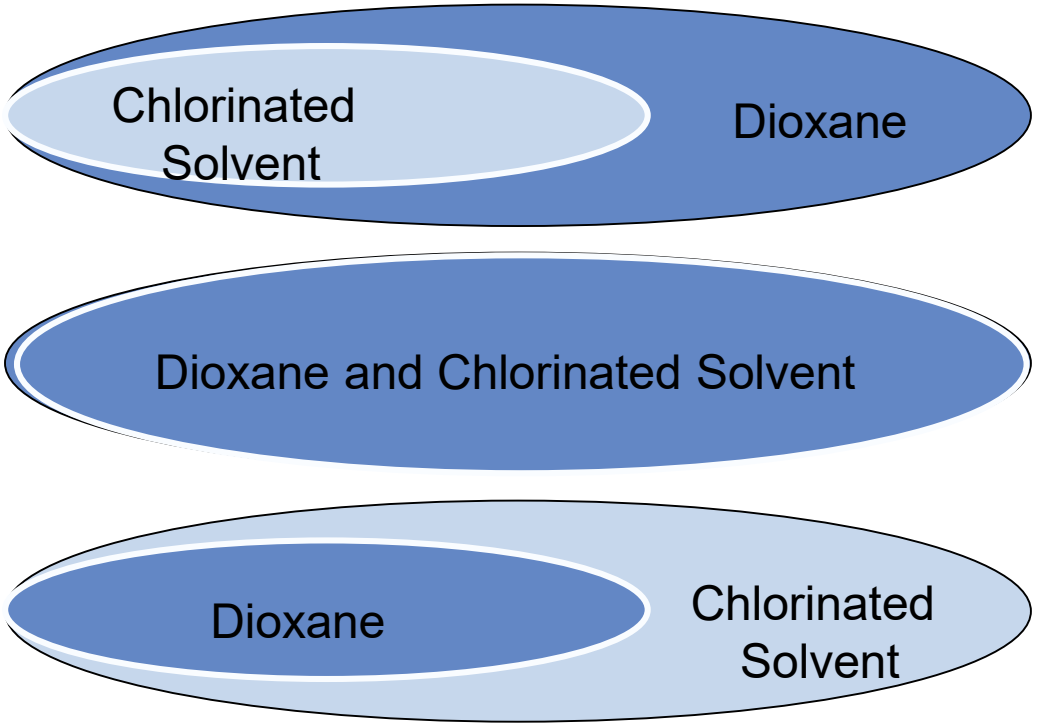
SERDP DOD • EPA • DOE | ESTCP

Agenda

- The problem
- Technical objectives
- Anaerobic-aerobic microbial communities
- Oxygen dosing and timing
- In-well Bio-Trap studies
- Conclusions
- Benefits to DoD

Comingled Contaminant Plumes

- ~95% of 1,4-dioxane (dioxane) plumes are comingled with one or more chlorinated volatile organic contaminants (CVOCs)



Dioxane plume is longest = 21%

Dioxane plume is same length = 17%

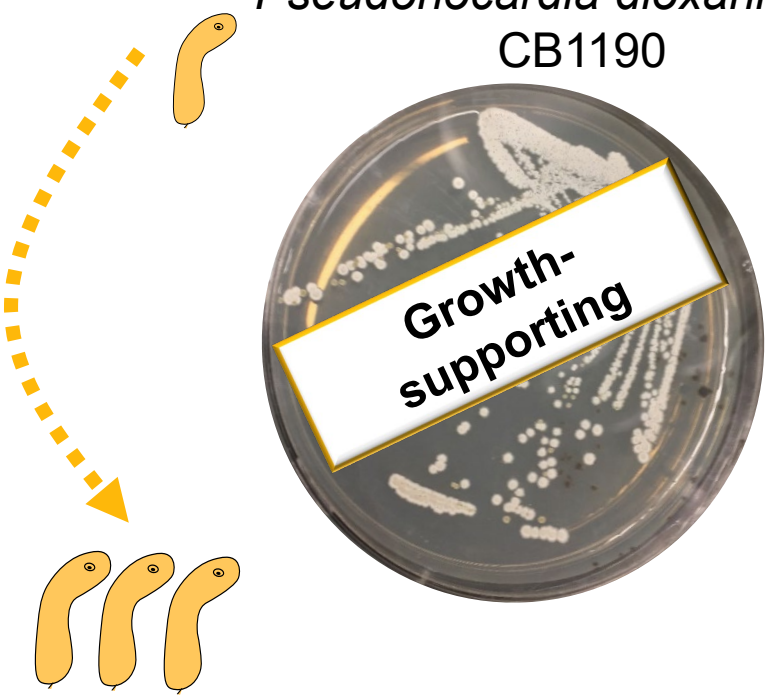
Chlorinated solvent plume is longest = 62%

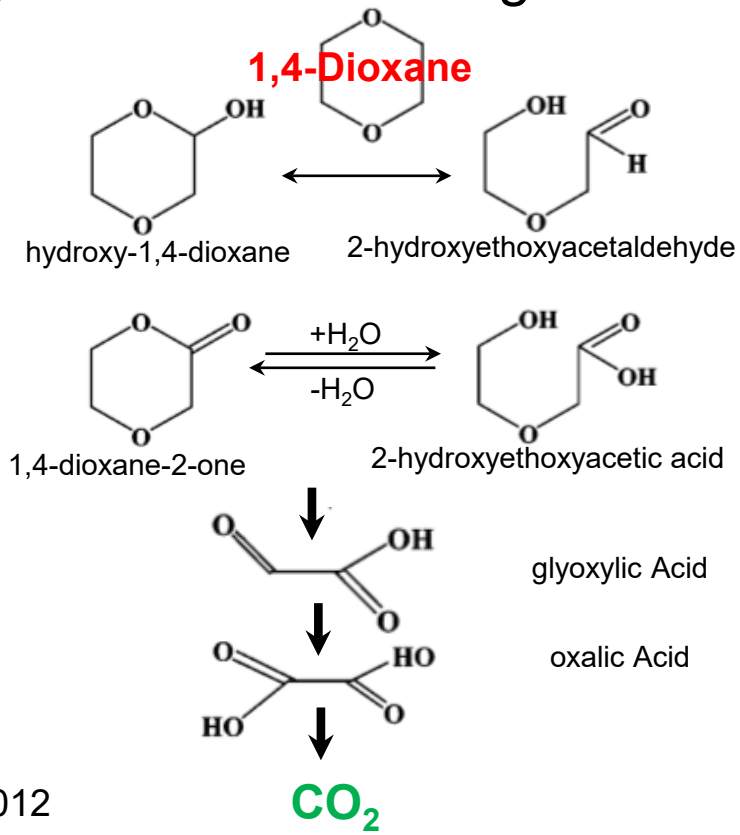
Source: Adamson et al. *ES&TL.* 2014

1,4-Dioxane is Metabolized Aerobically

Biodegradation is often a viable option for each pollutant, but opposing redox conditions favored by 1,4-dioxane and CVOC-degrading microbes pose a challenge for concurrent degradation

Pseudonocardia dioxanivorans
CB1190

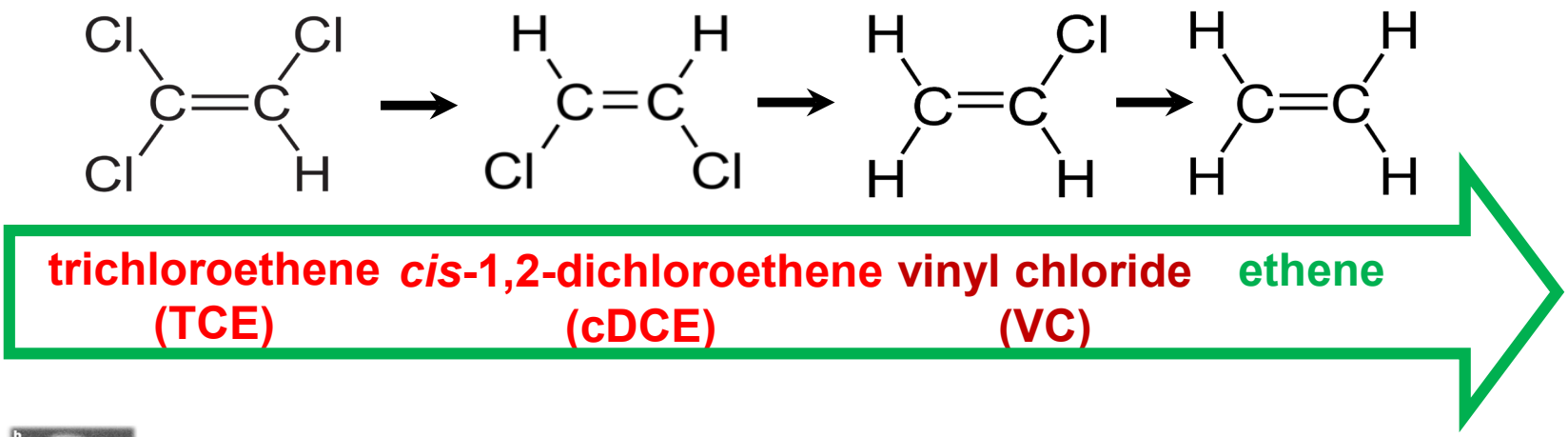




Source: Mahendra et al. *ES&T*. 2007; Grostern et al. *ES&T*. 2012

Chlorinated Ethenes Degraded Anaerobically

Biodegradation is often a viable option for each pollutant, but opposing redox conditions favored by 1,4-dioxane and CVOC-degrading microbes pose a challenge for concurrent degradation

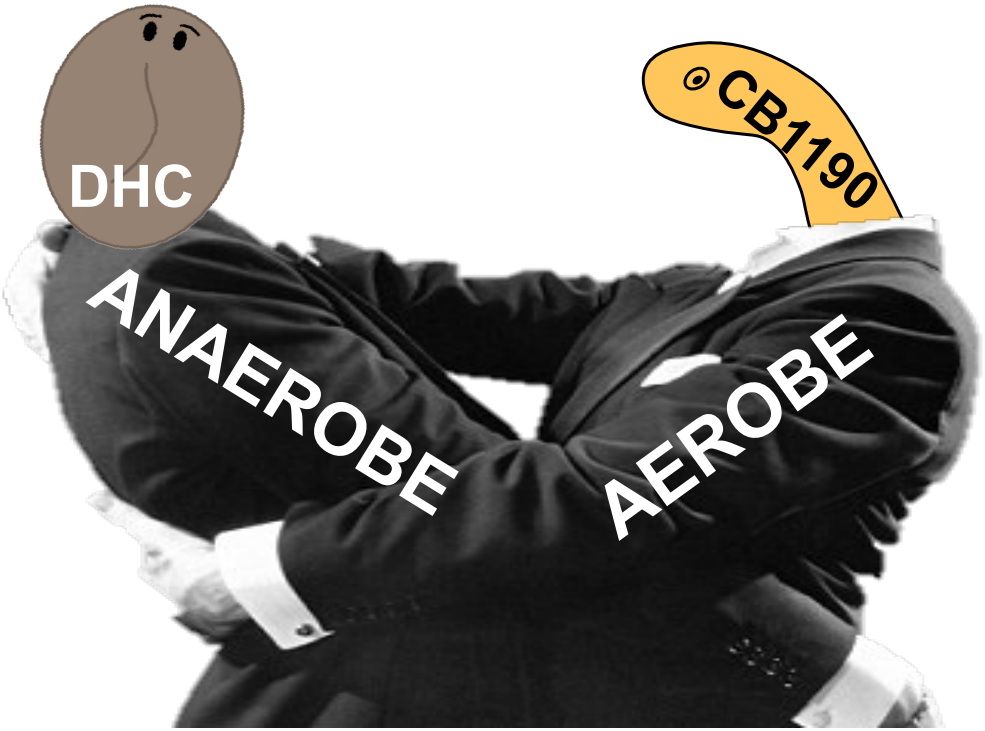


TCE = electron acceptor; H_2 = electron donor; Carbon source needed (e.g., acetate); Reductive dehalogenating bacteria are strict anaerobes (e.g., *Dehalococcoides* (DHC), KB1)

Source: He et al. *Nature*. 2003; Vogel and McCarty. *Environ. Microbiol.* 1985

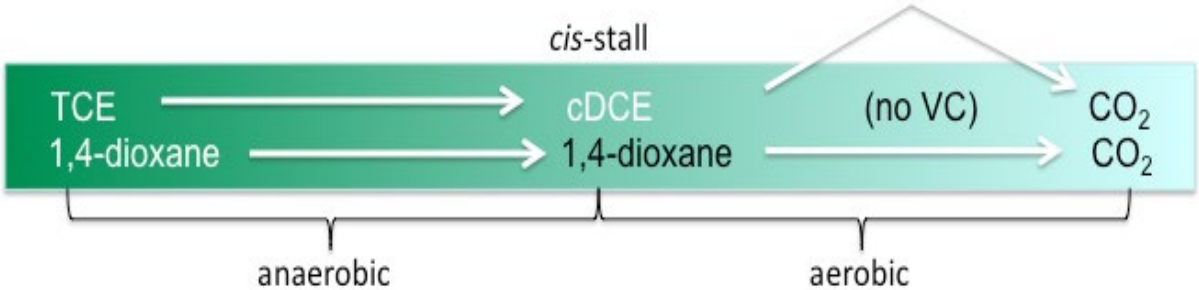
Contaminant Degrading Microbes Favor Opposing Redox Conditions

Microbial Communities Needed to Work Across Changing Redox Conditions



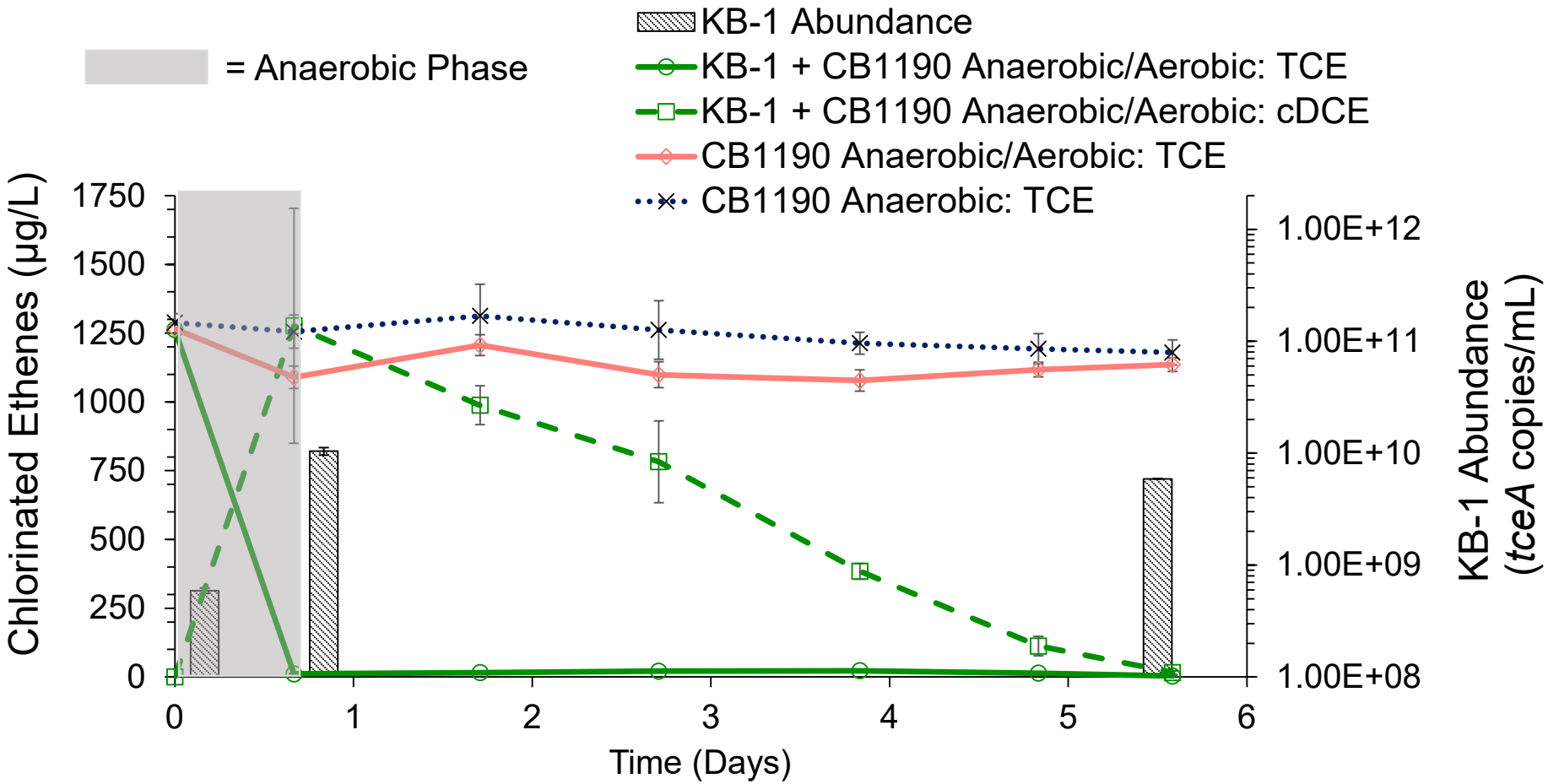
Technical Objectives

1. Design and test microbial communities composed of previously identified anaerobic and aerobic contaminant-degrading bacteria to simultaneously or sequentially degrade CVOCs and dioxane

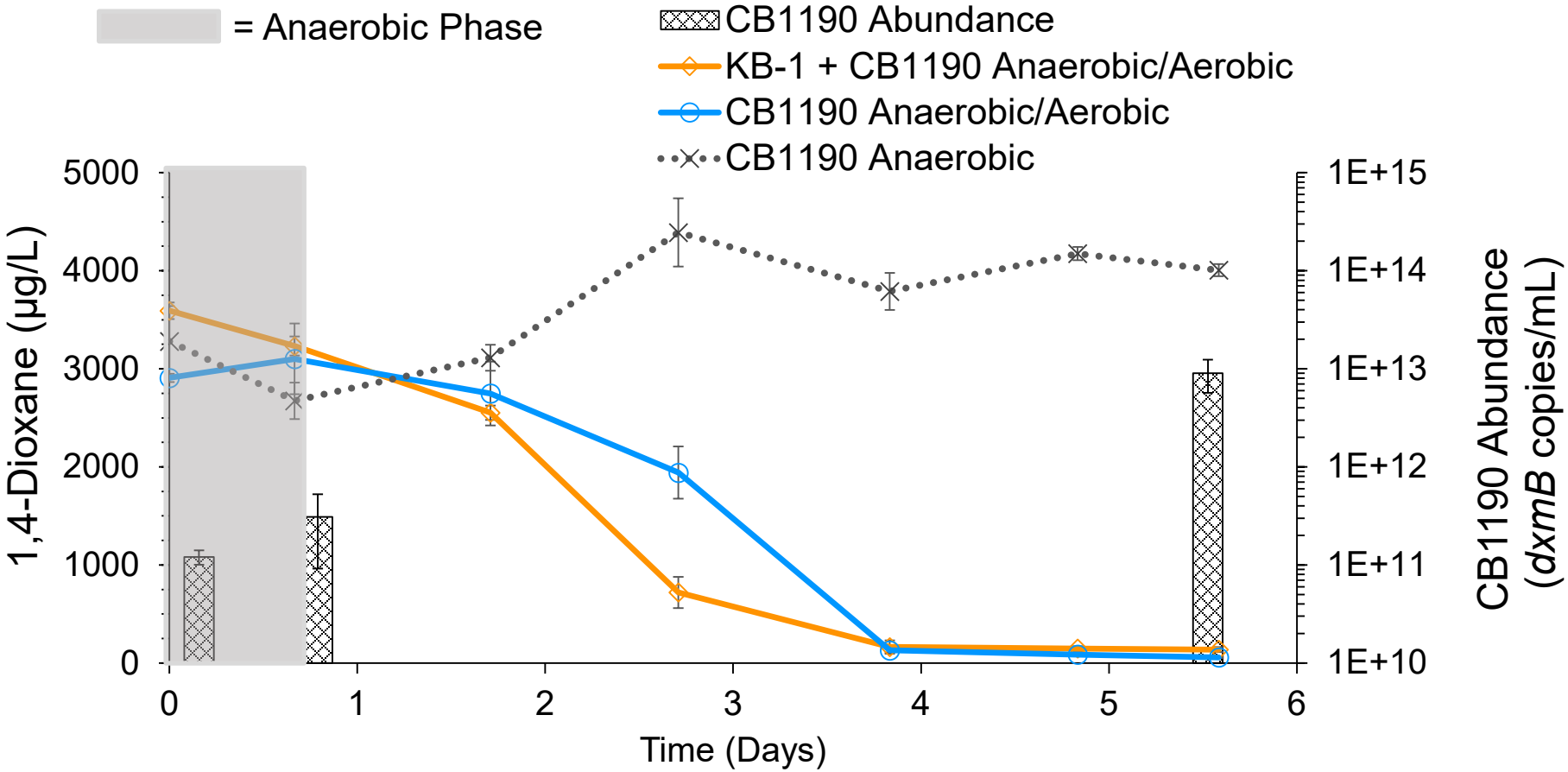


2. Elucidate the influence of O_2 dose and timing on the individual biodegradation rates and overall contaminant mass removal by the formulated microbial community
3. Determine the taxonomic abundance and functional activity of the formulated microbial community biodegrading mixed contaminants using molecular biomarkers

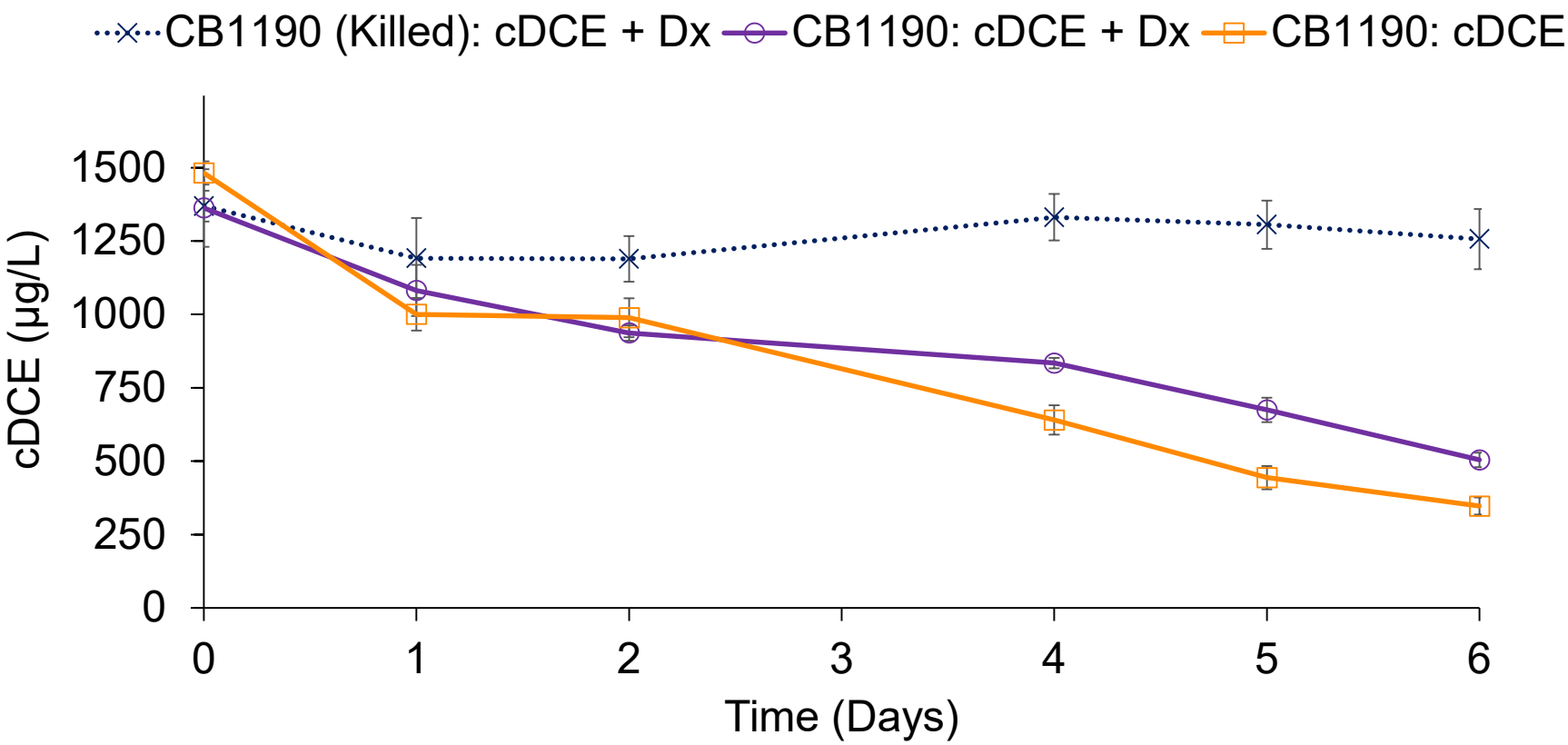
KB-1 Reduces TCE; CB1190 Oxidizes cDCE



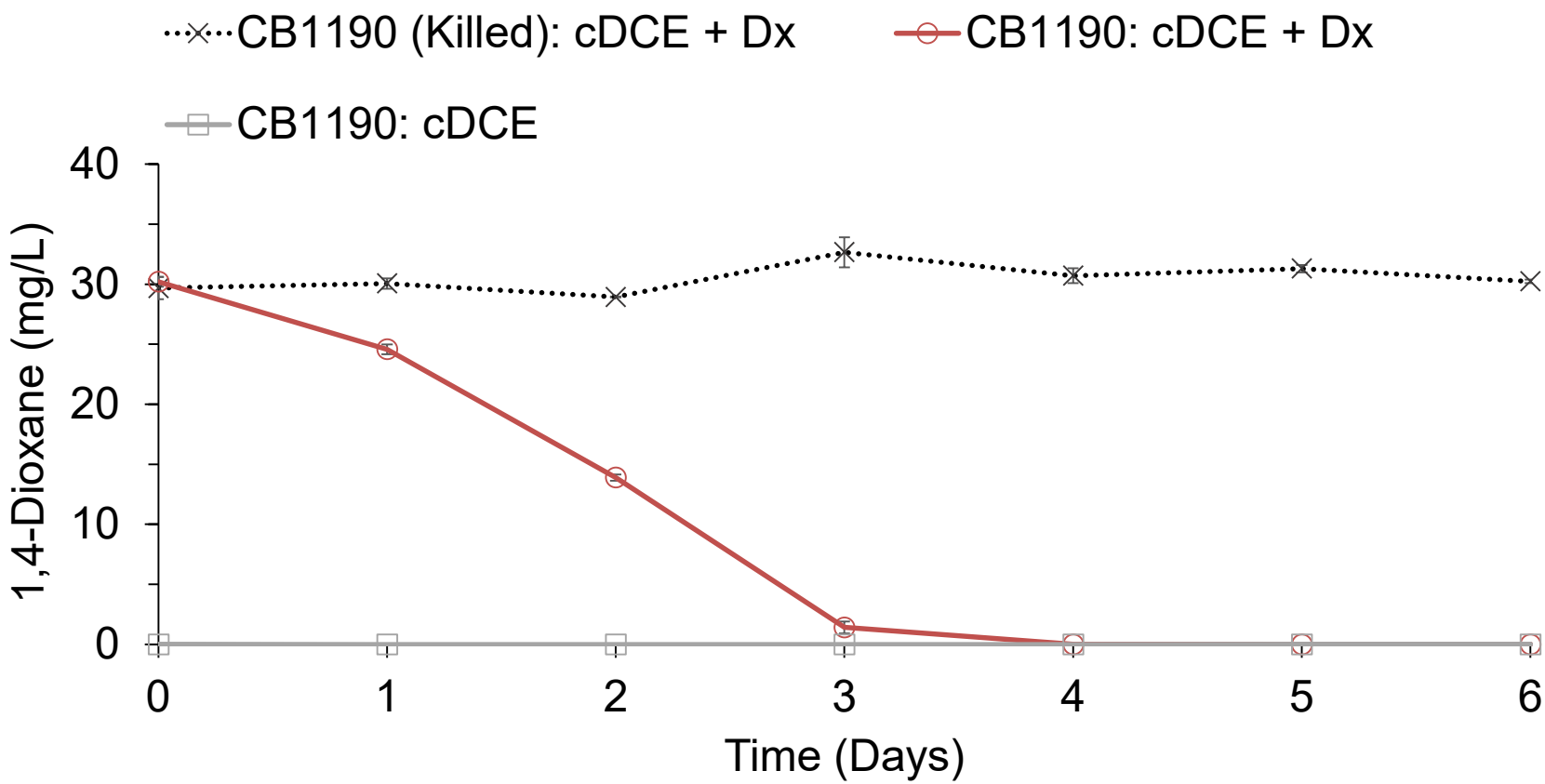
Mixed Community and Pure CB1190 Degrade Dioxane After Anaerobic Incubation



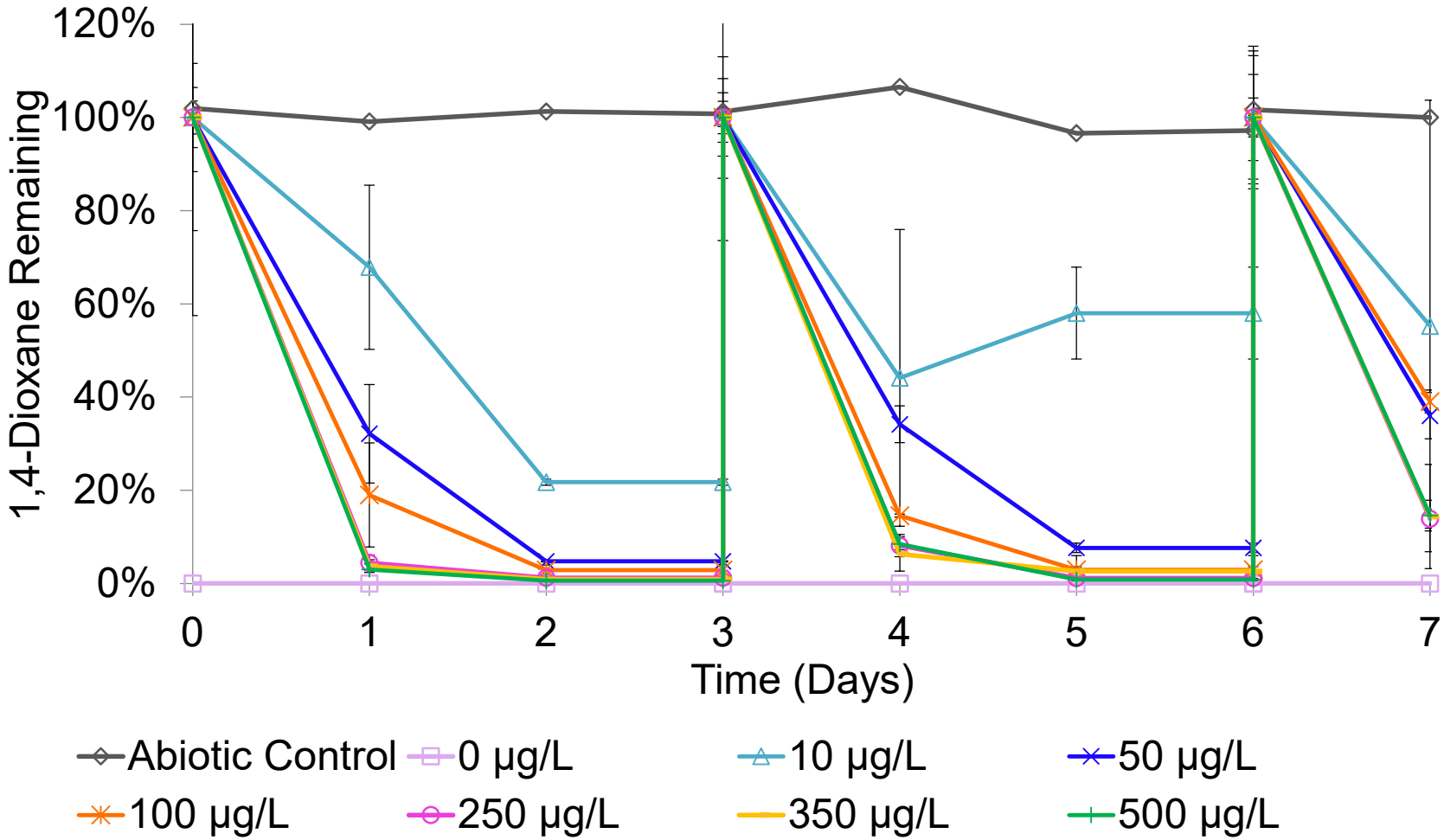
First Report of Aerobic cDCE Degradation by CB1190



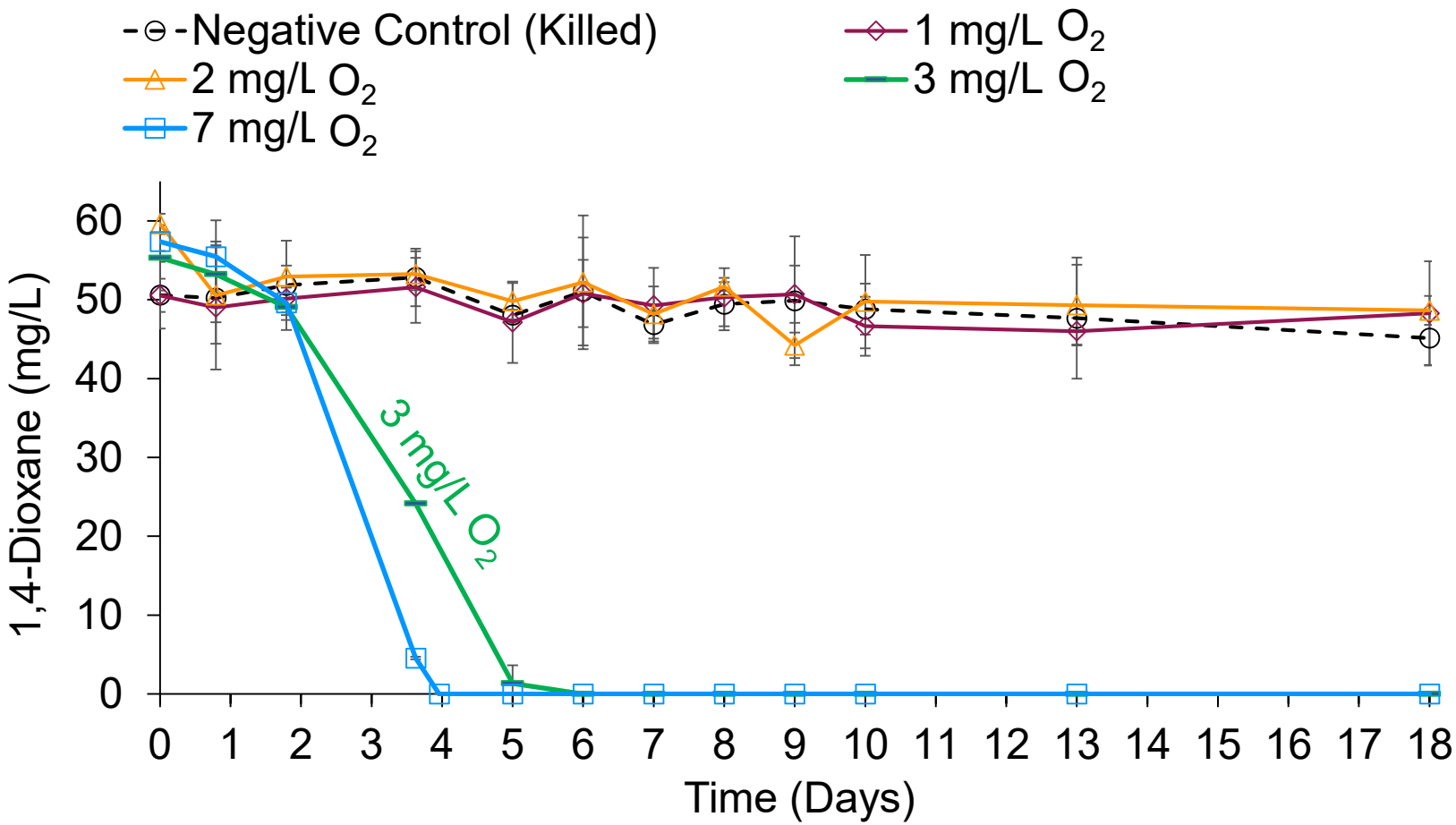
Dioxane and cDCE Simultaneously Degraded



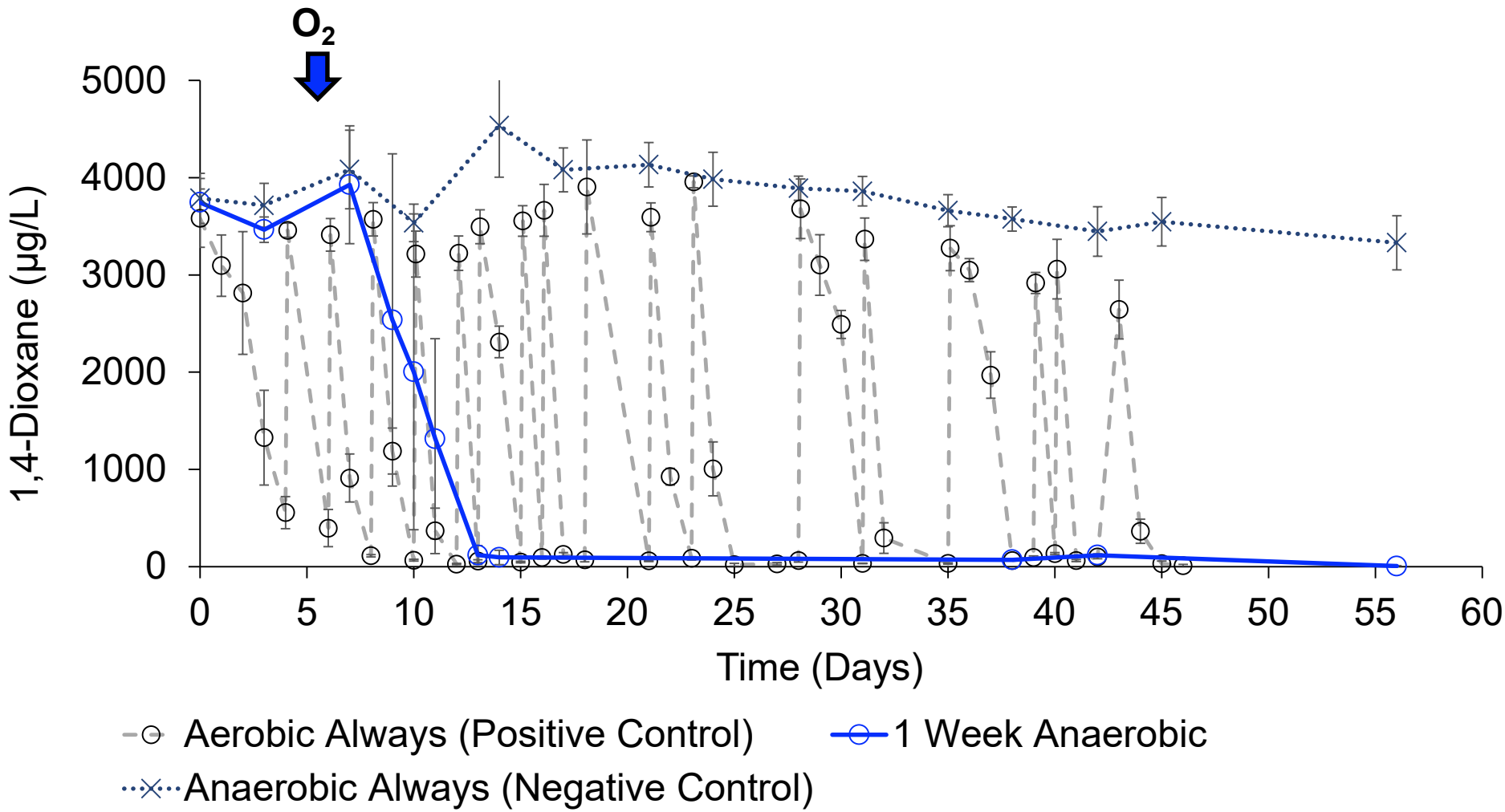
CB1190 Biodegrades Environmentally Relevant Concentrations of Dioxane



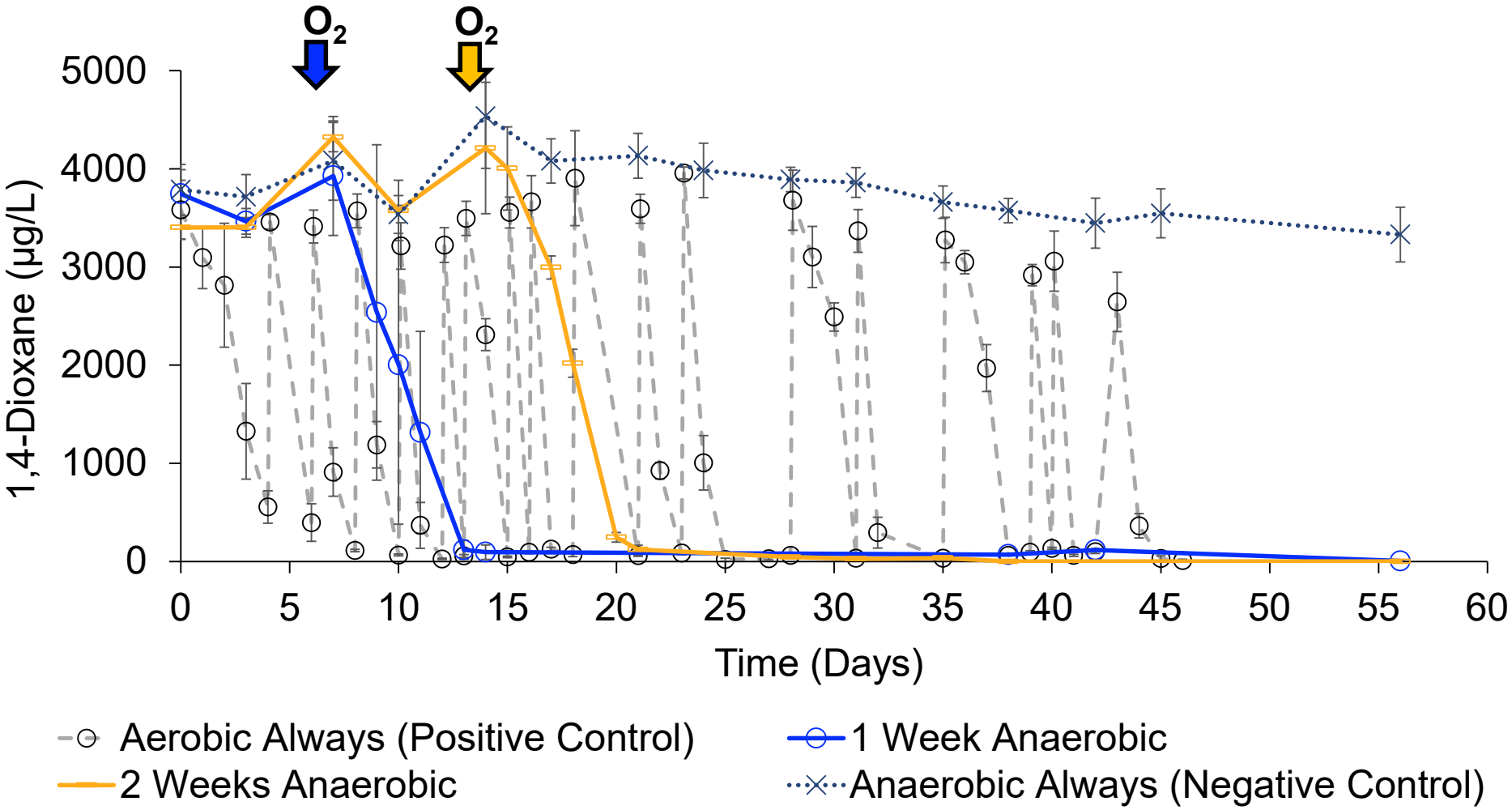
CB1190 Degrades Dioxane with ~3 mg/L O_2



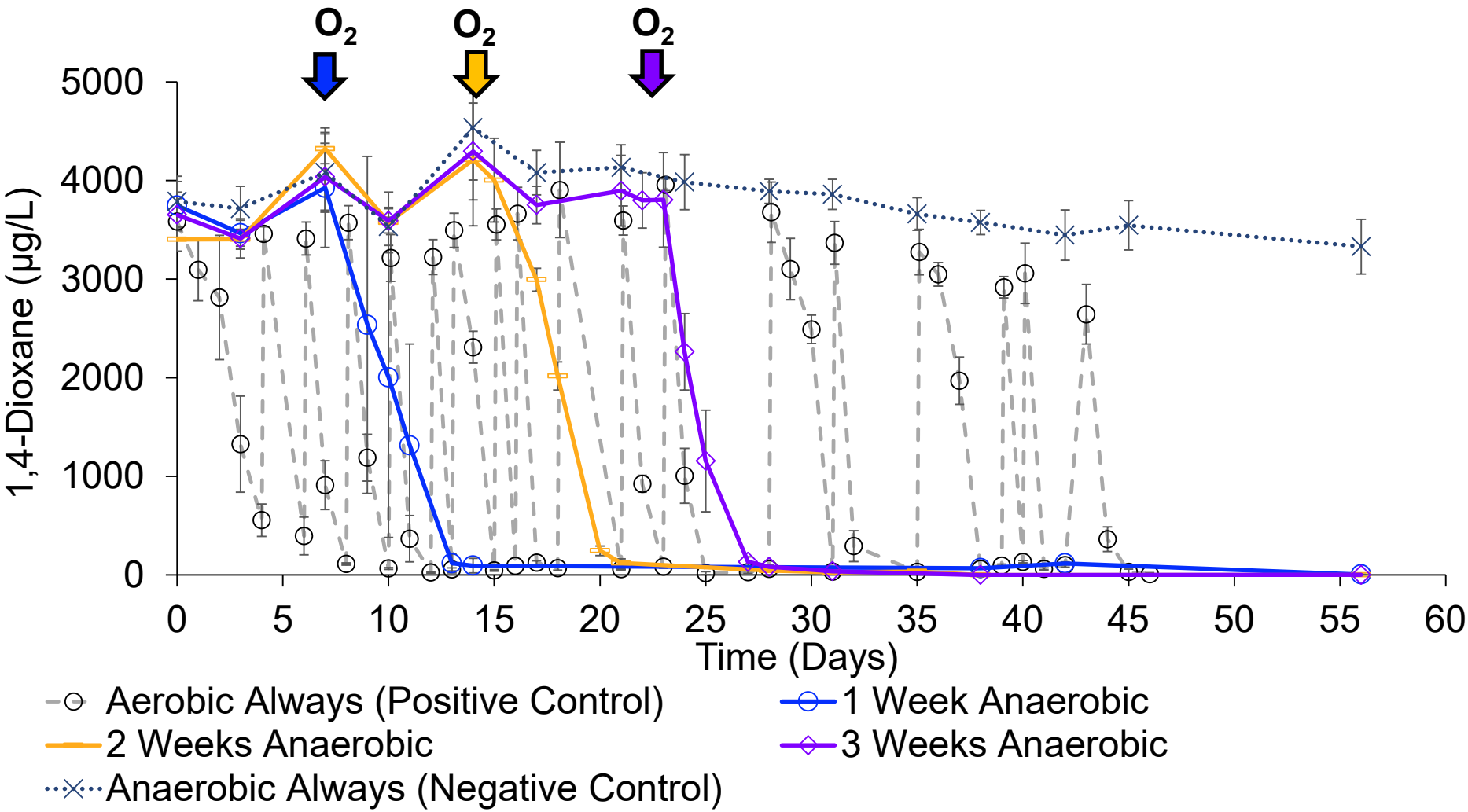
CB1190 Degrades Dioxane After 1 Week of Anaerobic Incubation



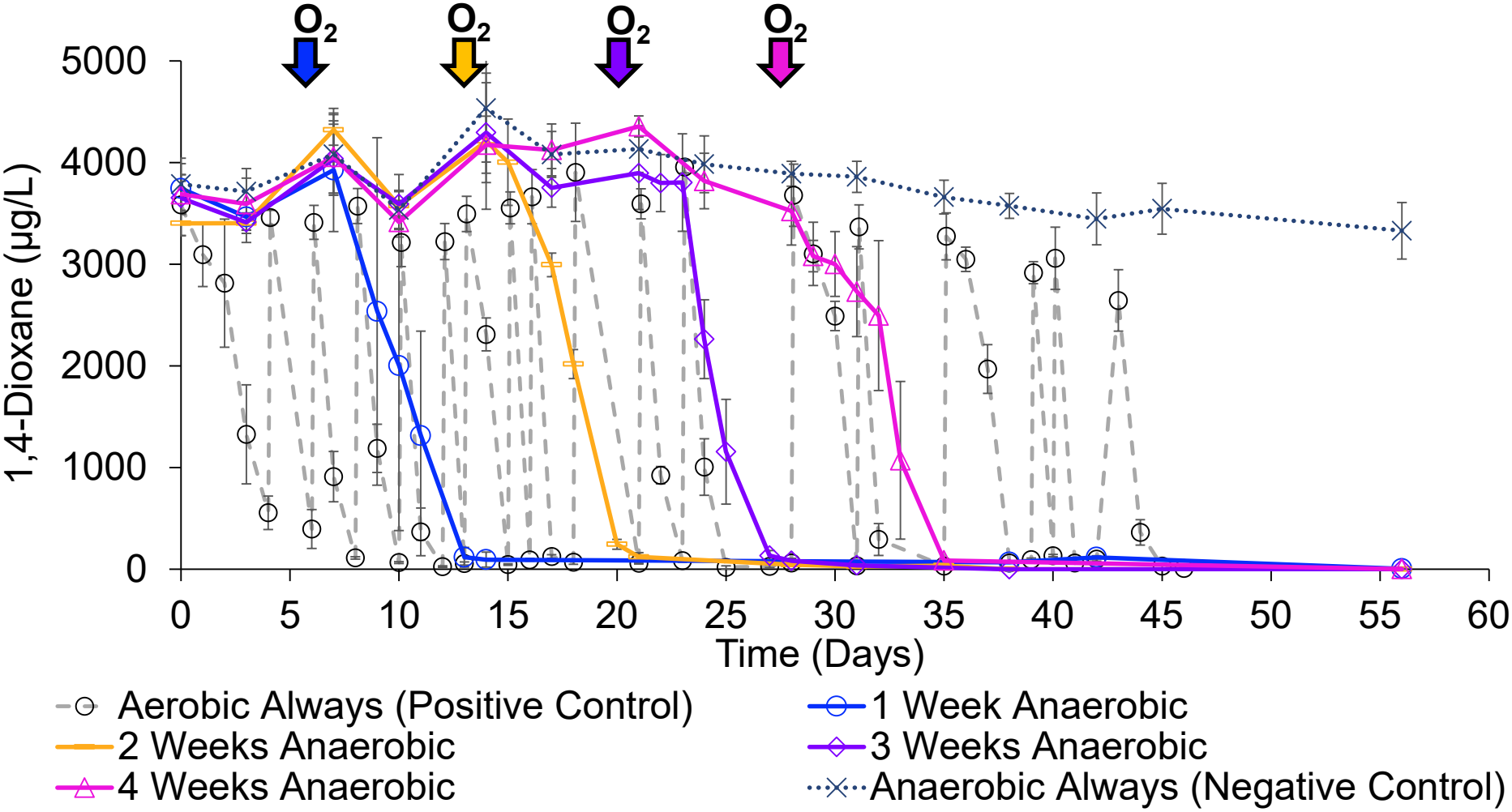
CB1190 Degrades Dioxane After 2 Weeks of Anaerobic Incubation



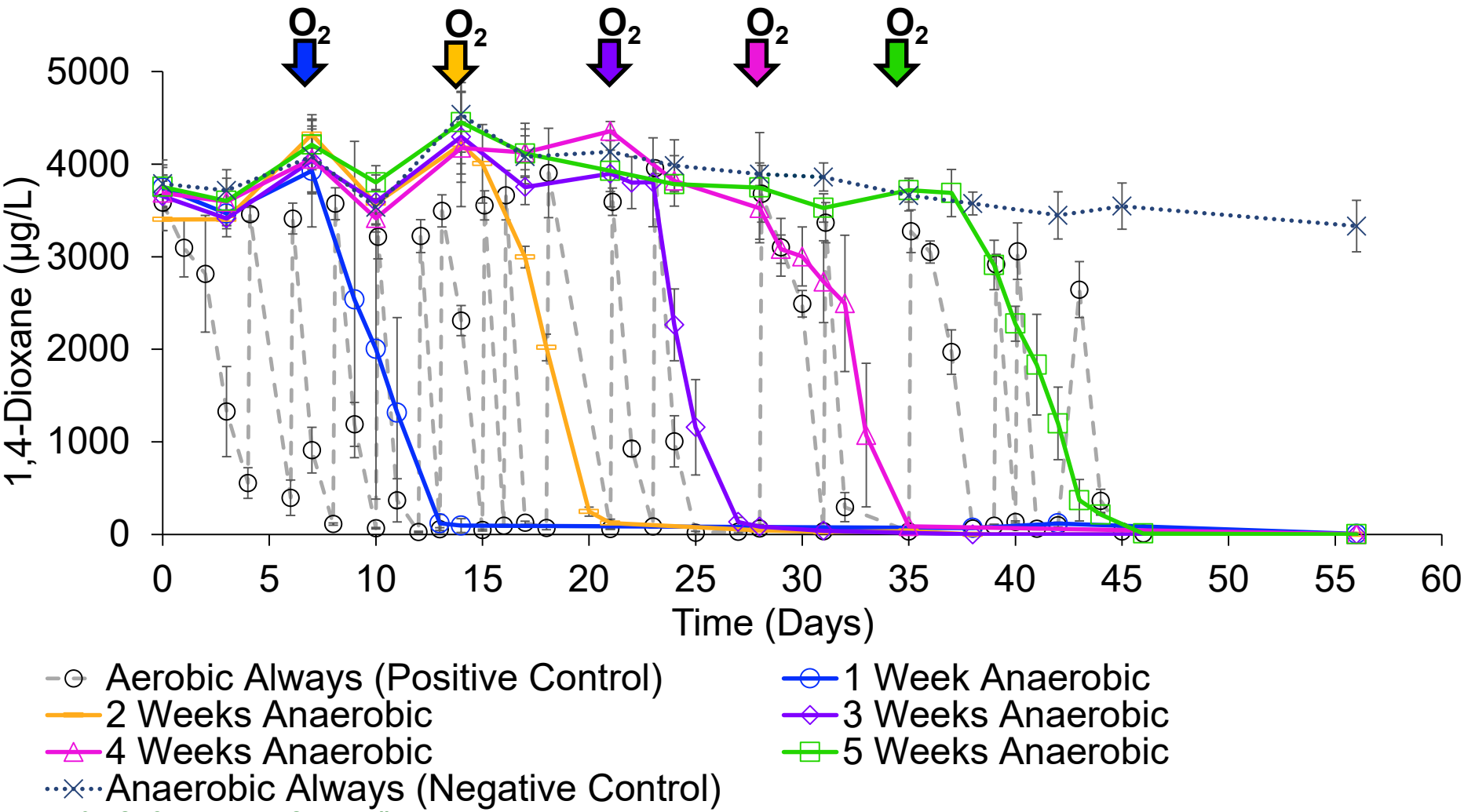
CB1190 Degrades Dioxane After 3 Weeks of Anaerobic Incubation



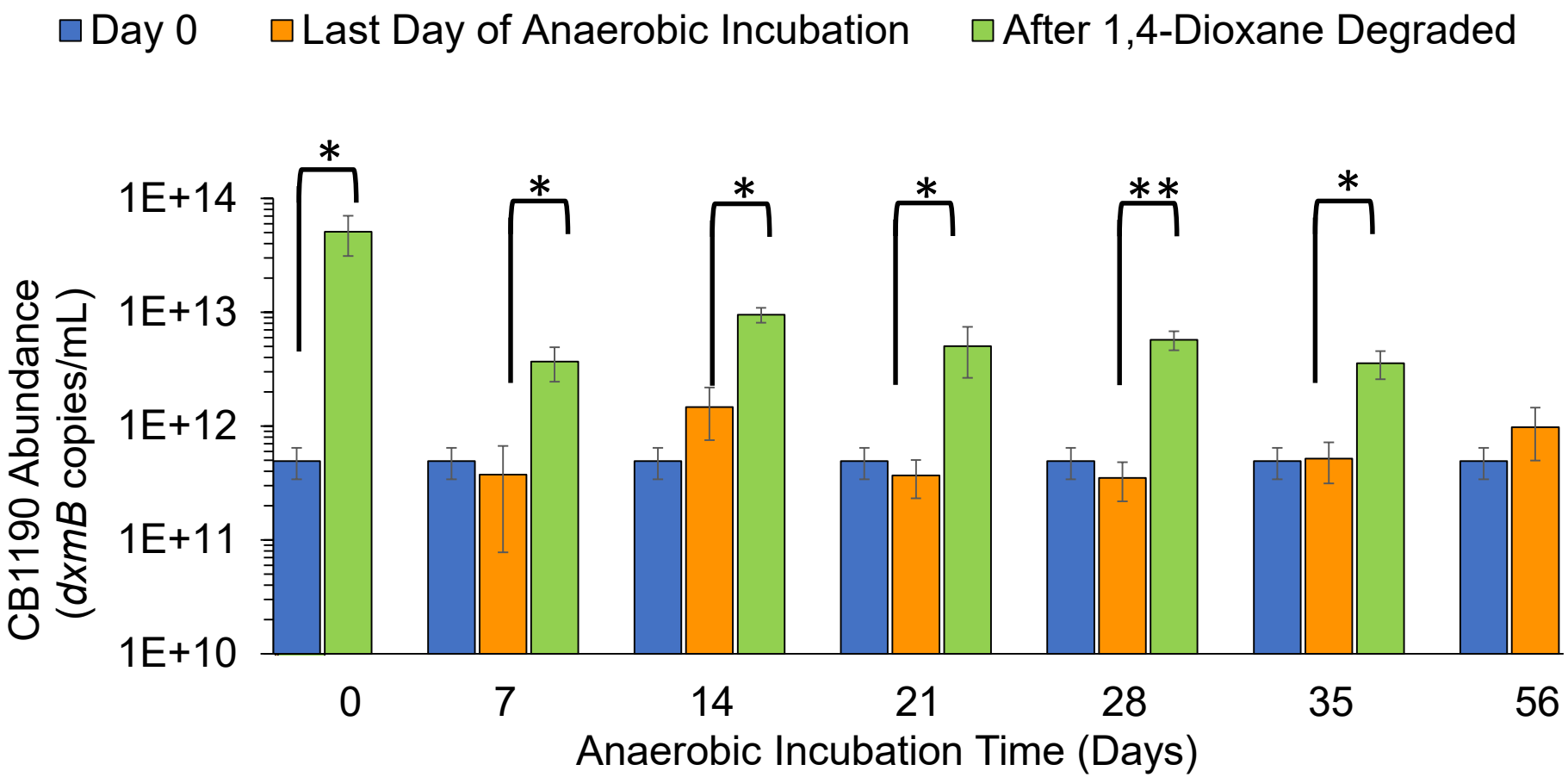
CB1190 Degrades Dioxane After 4 Weeks of Anaerobic Incubation



CB1190 Degrades Dioxane After 5 Weeks of Anaerobic Incubation

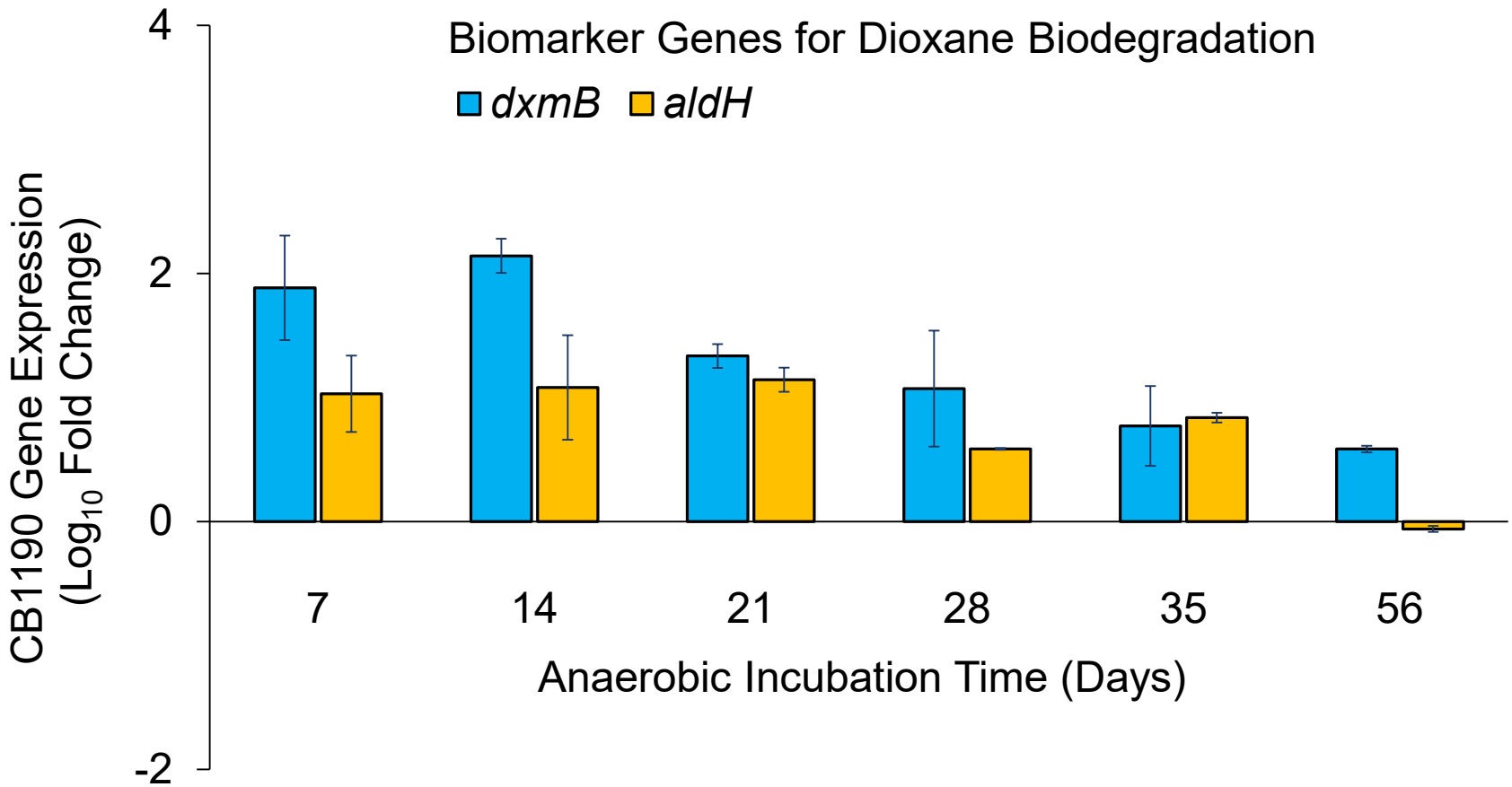


CB1190 Subsists Under Anaerobic Conditions and Grows in the Presence of Oxygen



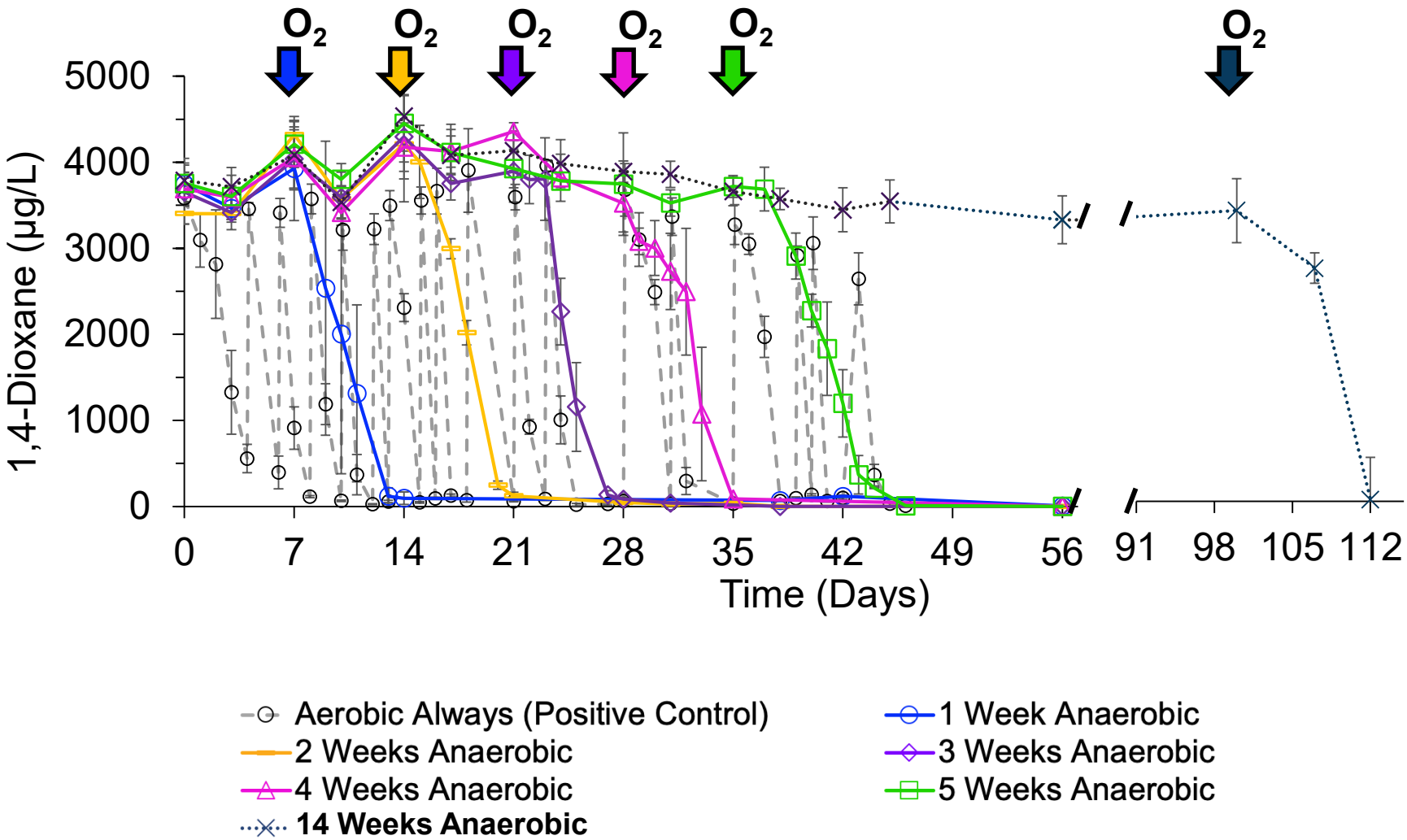
Notes: * p value < 0.05,** p value < 0.01

Biomarker Genes Upregulated After Oxygen Addition

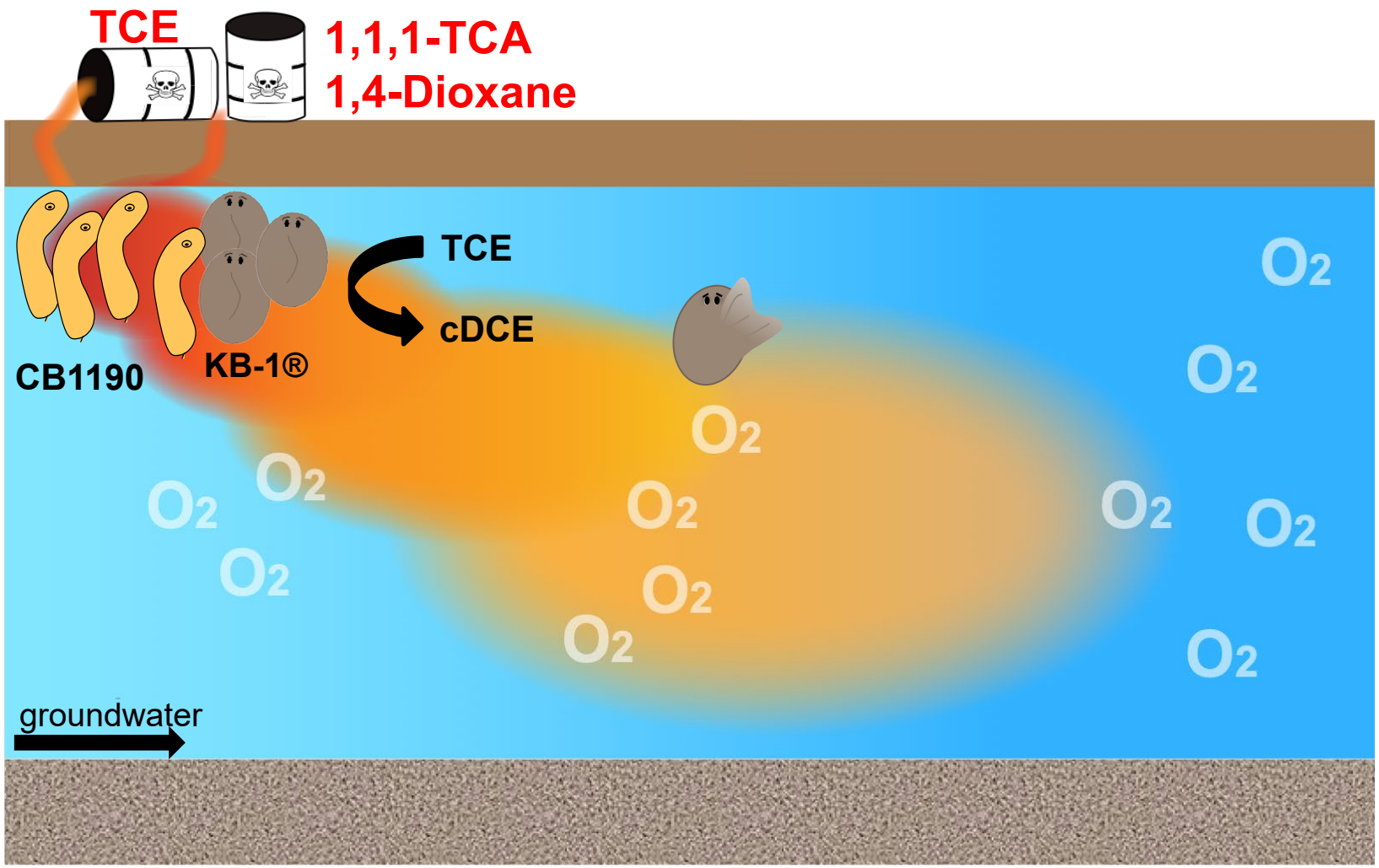


How Long Can CB1190 Survive Without Oxygen?

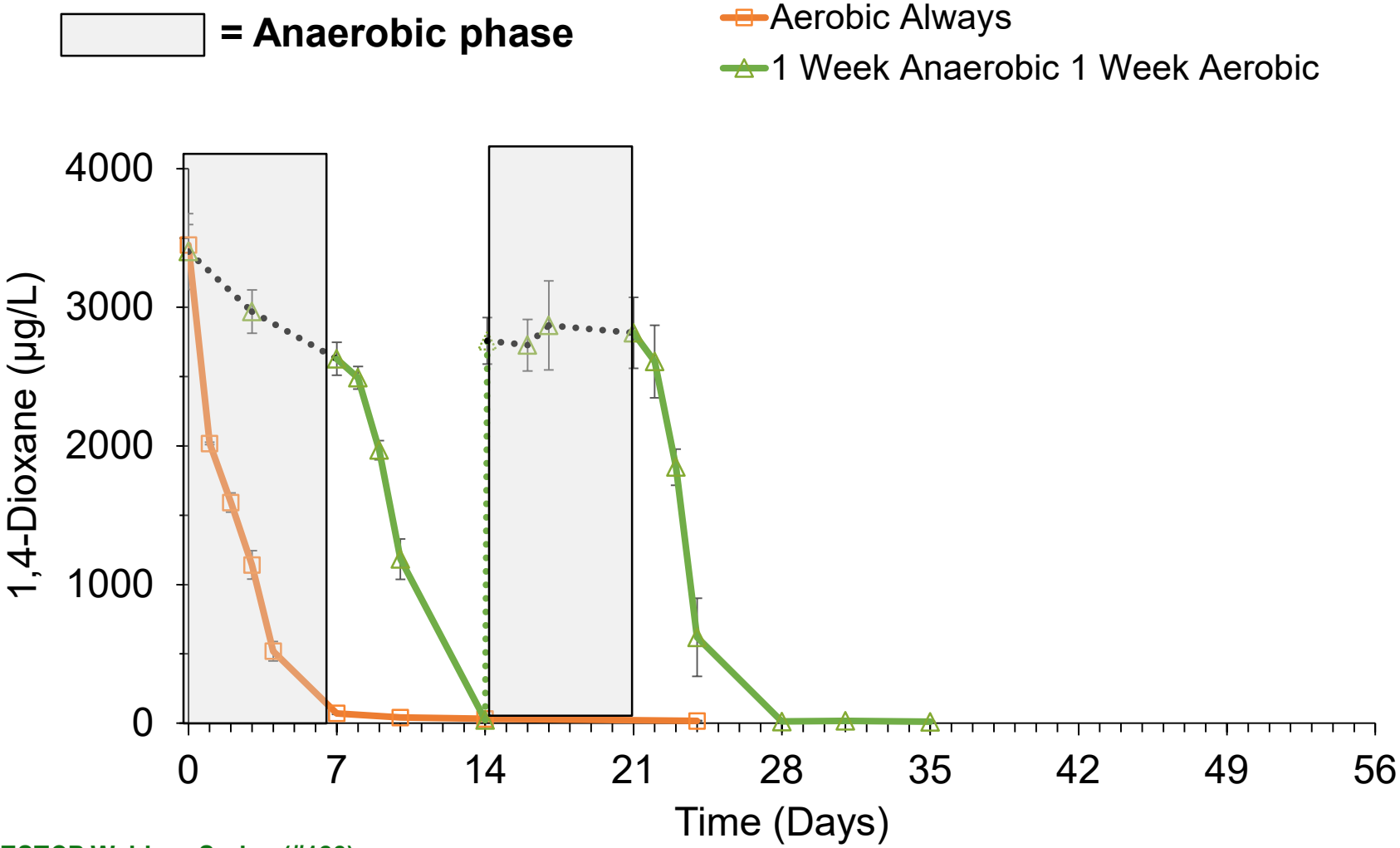
CB1190 Degrades Dioxane After 100 Days Anaerobic Incubation!



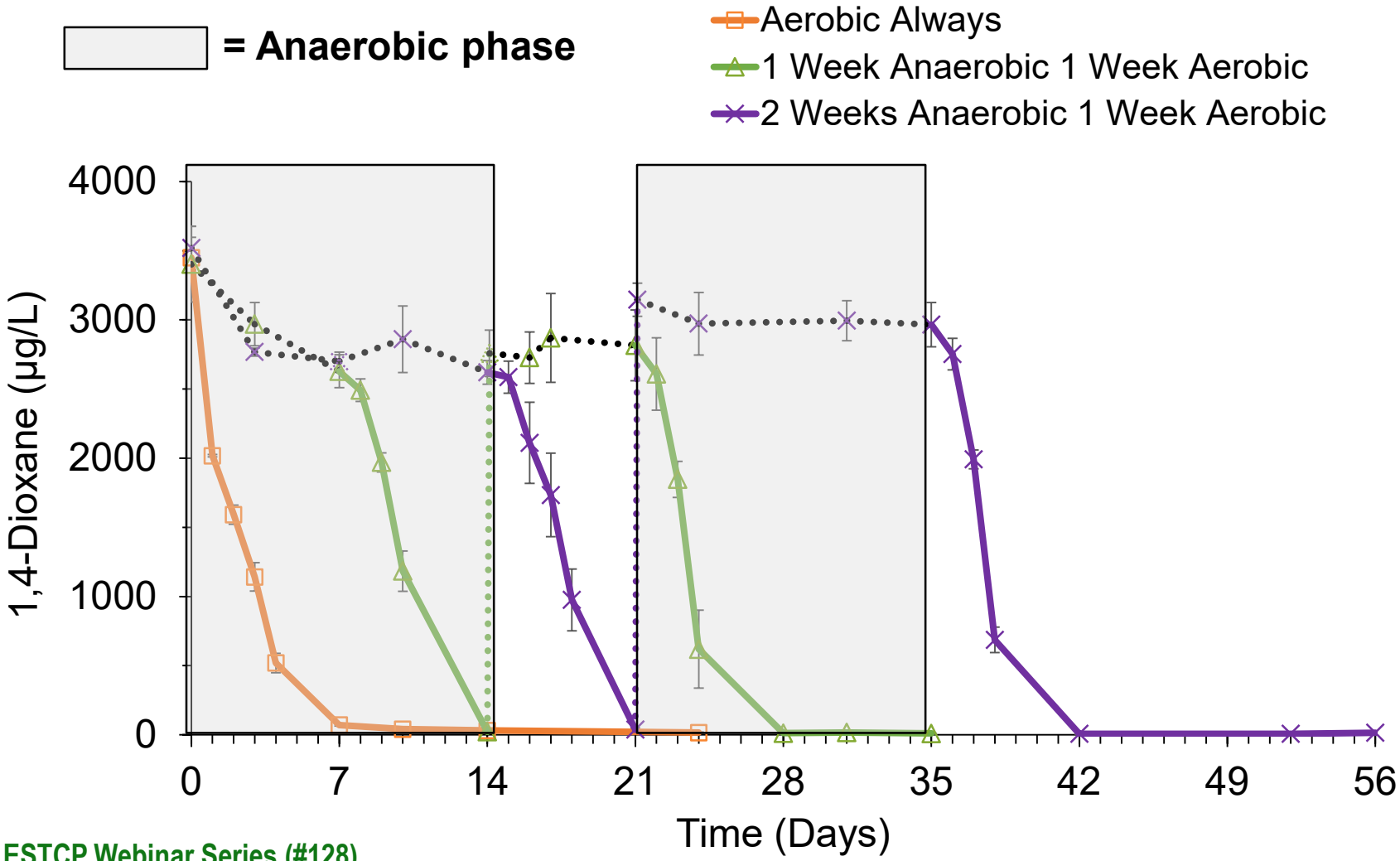
Toggling Redox Environments in the Presence of Contaminant Mixtures



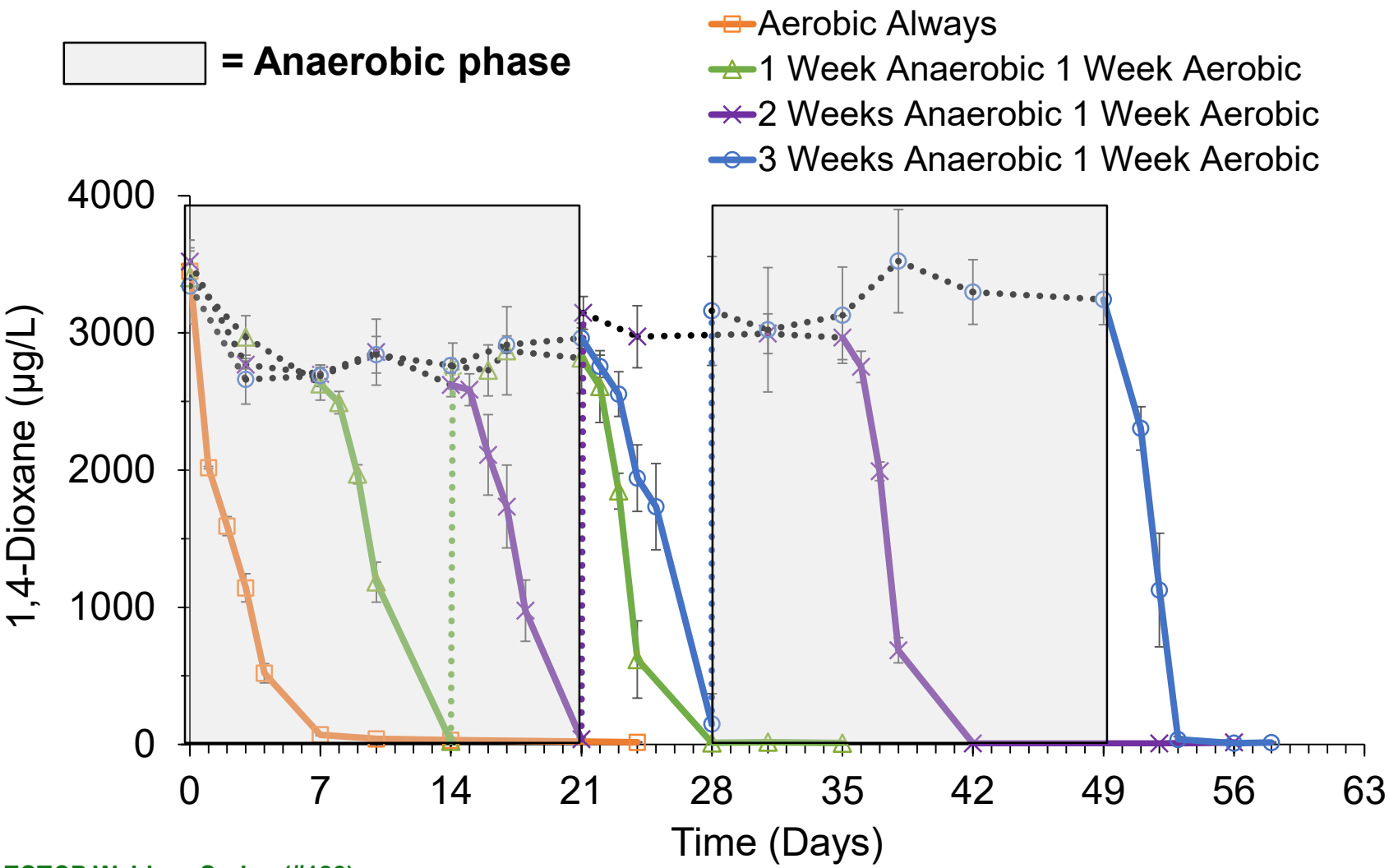
CB1190 Degrades Dioxane After ONE Week Anaerobic Cycles



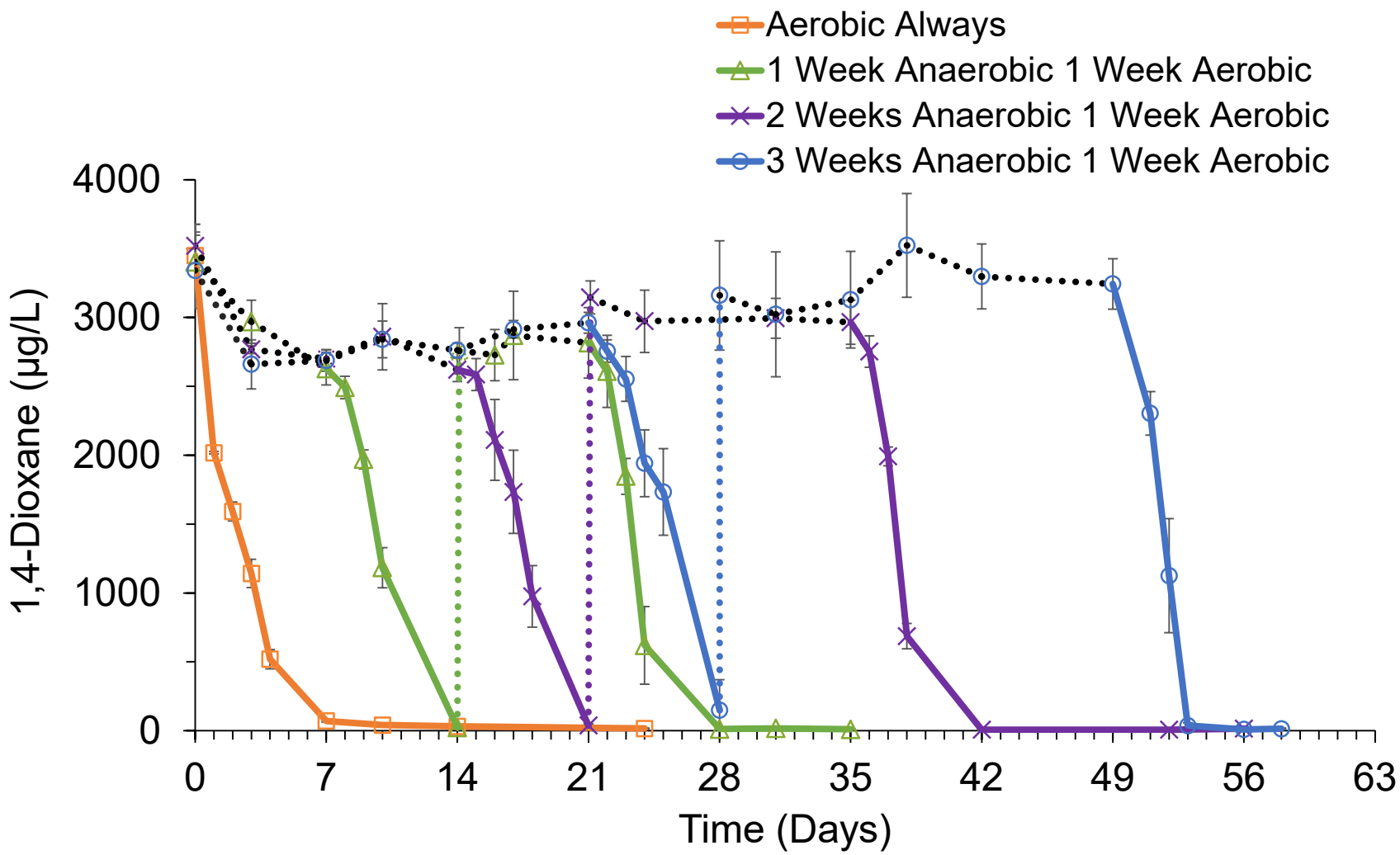
CB1190 Degrades Dioxane After TWO Week Anaerobic Cycles



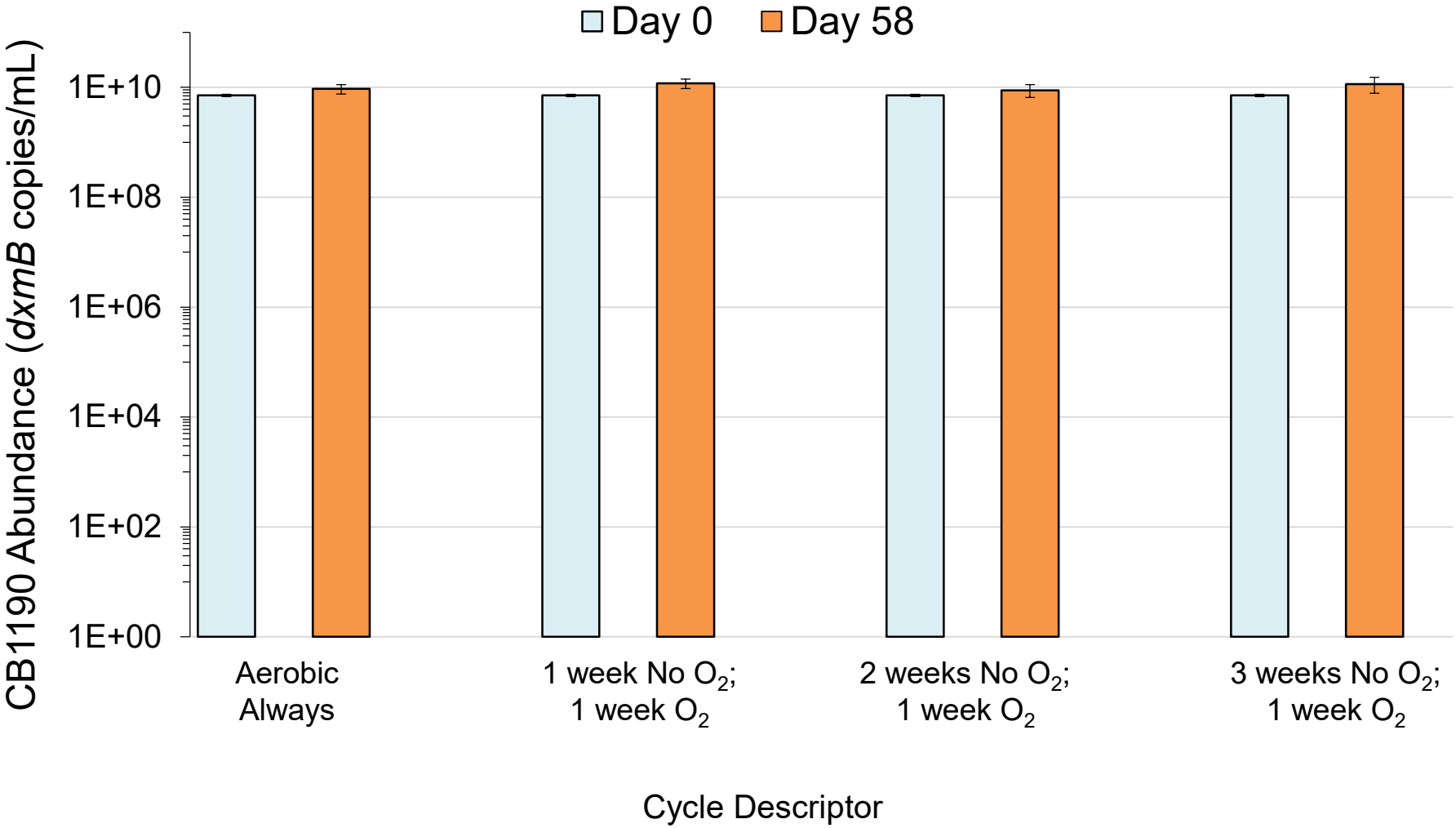
CB1190 Degrades Dioxane After THREE Week Anaerobic Cycles



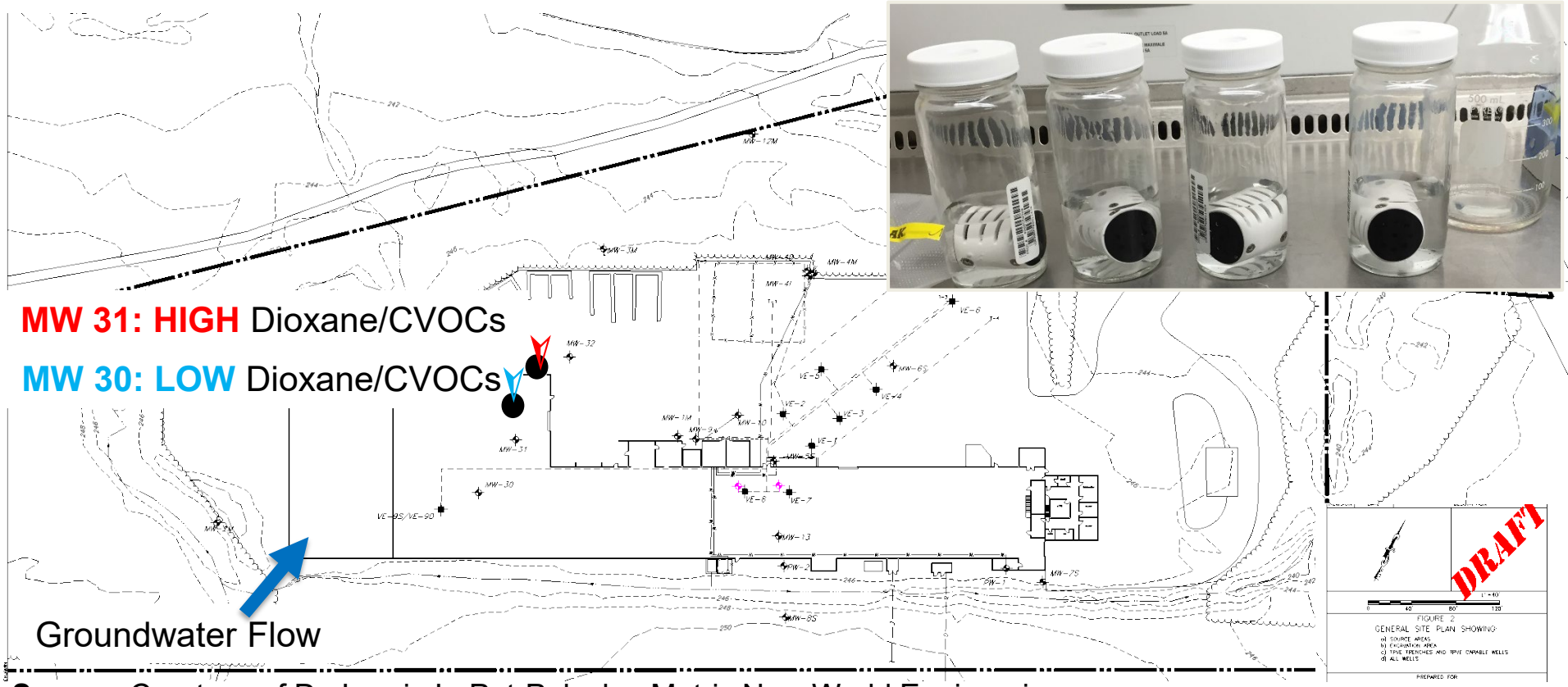
CB1190 Degrades Dioxane After THREE Week Anaerobic Cycles



CB1190 Abundance Maintained After Anaerobic-Aerobic Toggle



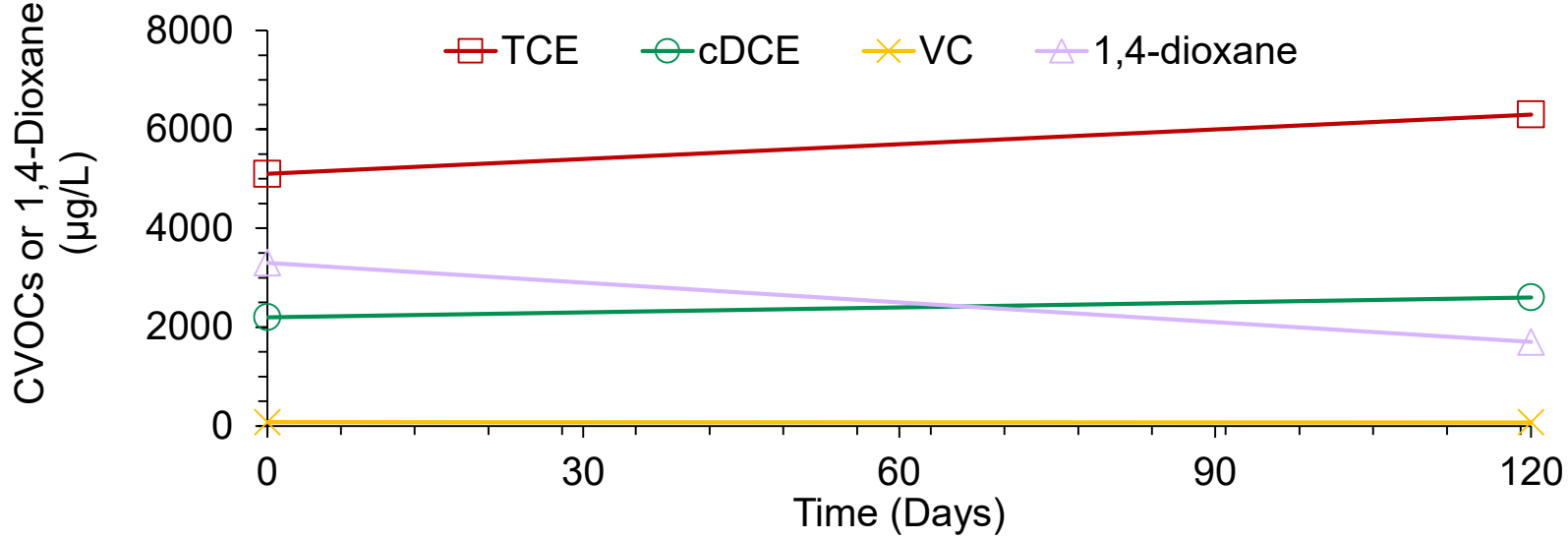
CB1190-Augmented Bio-Traps Suspended in Wells at a Site Previously Treated by ERD

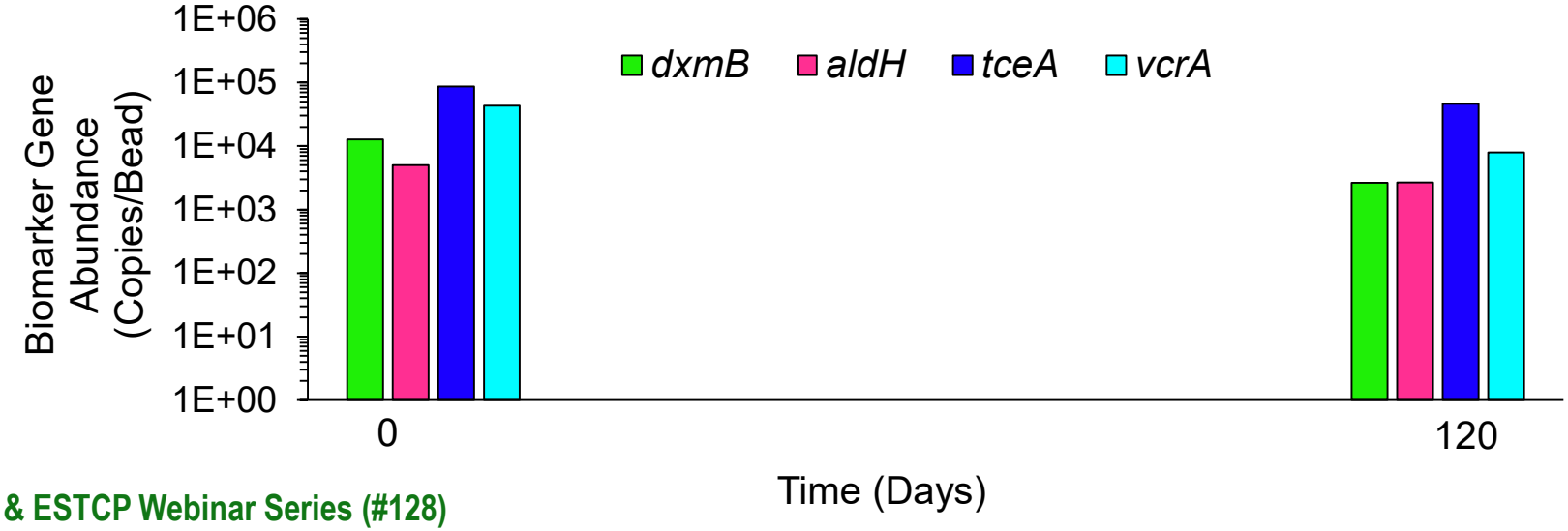


Source: Courtesy of Dr. Laurie LaPat-Polasko, Matrix New World Engineering

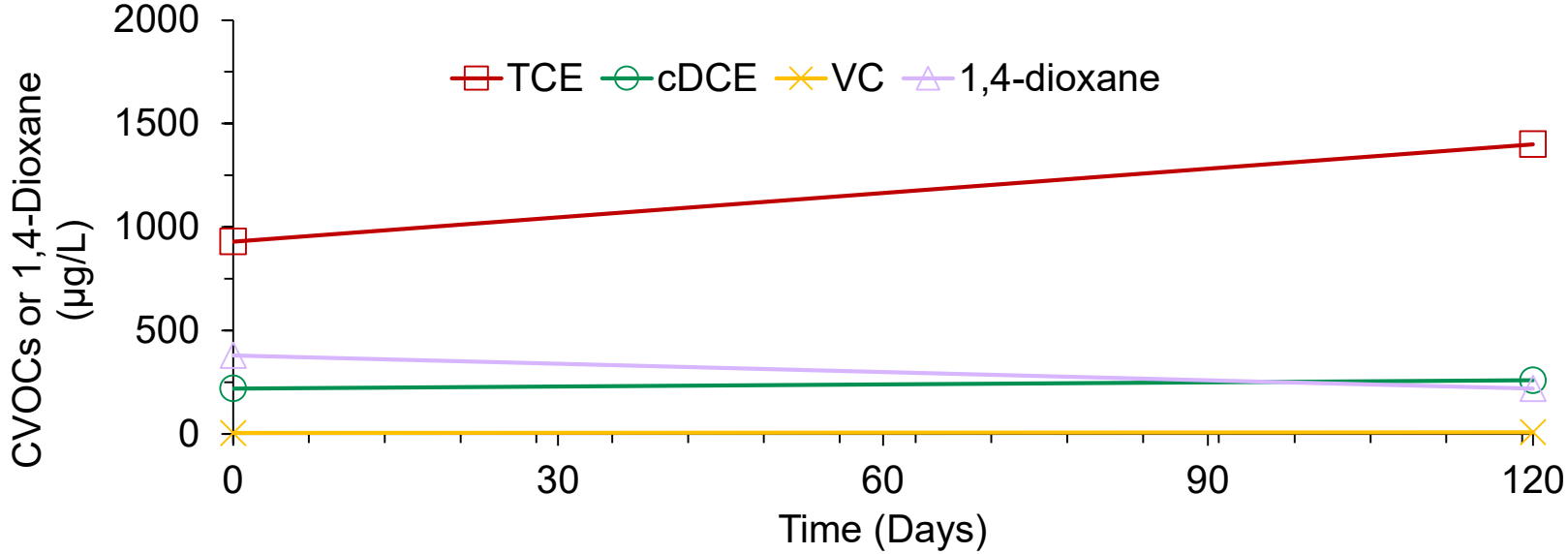
Note: ERD = Enhanced Reductive Dechlorination

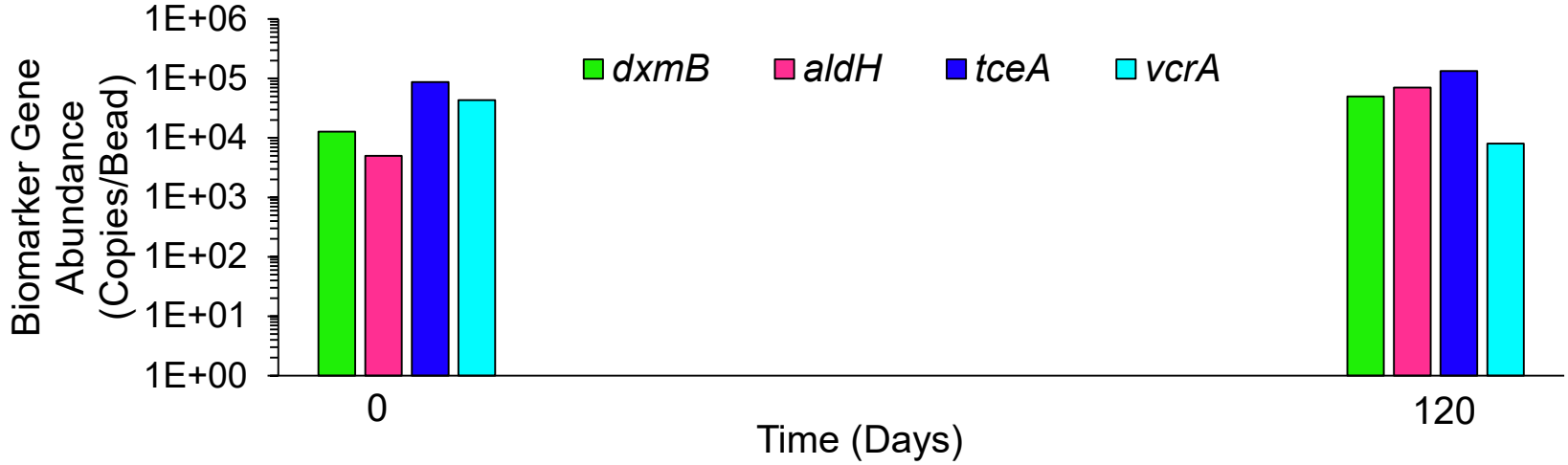
CB1190 on BioSep Beads Aerobically Degraded >1500 ppb Dioxane





CB1190 Grew Aerobically and Degraded Dioxane at a Former ERD-Treated Site





Conclusions

- A microbial community was assembled to survive changing redox conditions and efficiently degrade contaminant mixtures
- CB1190 aerobically biodegrades cis-DCE without generating VC
- CB1190 can withstand 100 days of anerobic incubation and still grow when conditions turn aerobic
- 1,4-Dioxane biodegradation occurs at dissolved oxygen levels as low as 3 mg/L
- CB1190 remains viable and active at sites previously treated with ERD

Benefits to DoD

- Aerobic-anaerobic microbial communities formulated to biodegrade CVOCs and 1,4-dioxane without the accumulation of toxic intermediates like VC
- Limit ERD timeframe or replace ERD at some CVOC sites
- Potentially lower cost of in-situ bioremediation by decreasing injection of microbes, carbon substrates, and nutrient amendments
- Better understanding of adaptations of environmentally-relevant microbes for remediation and monitoring
- Inform design and select bioaugmentation and biostimulation strategies for fluctuating dissolved oxygen levels and wide range of contaminant concentrations and mixtures

Additional Resources

- Adamson, D.T., S. Mahendra, K.L. Walker, S.R. Rauch, S. Sengupta, and C.J. Newell. A Multisite Survey to Identify the Scale of the 1,4-Dioxane Problem at Contaminated Groundwater Sites. *Environ. Sci. & Technol. Lett.*, **2014**, 1, 254-258.
- Mahendra, S., C.J. Petzold, E.E. Baidoo, J.D. Keasling, and L. Alvarez-Cohen. Identification of the Intermediates of in Vivo Oxidation of 1,4-Dioxane by Monooxygenase-Containing Bacteria. *Environ. Sci. & Technol.*, **2007**, 41, 7330-7336.
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Q&A Session 1

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Cometabolic Biobarriers: A Sustainable Approach to Treat Large Dilute CVOC Plumes

David Lippincott, P.G.
APTIM

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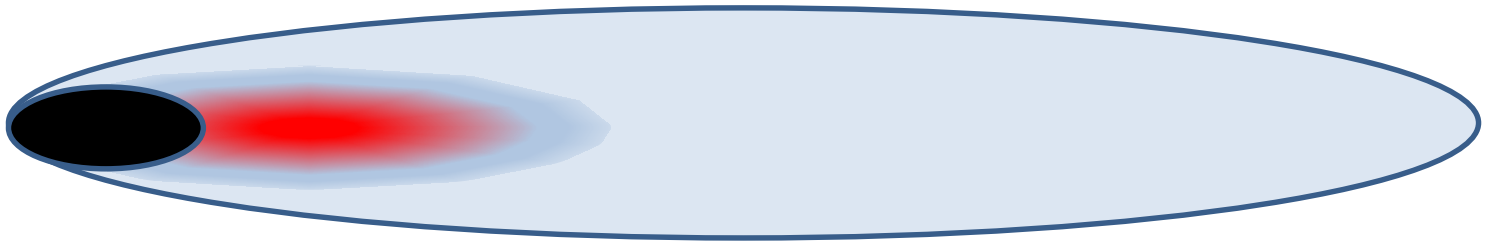
Agenda

- The problem
- Overall objectives
- What is cometabolism?
- ESTCP biobarrier field demonstration
- Conclusions
- Benefits to DoD

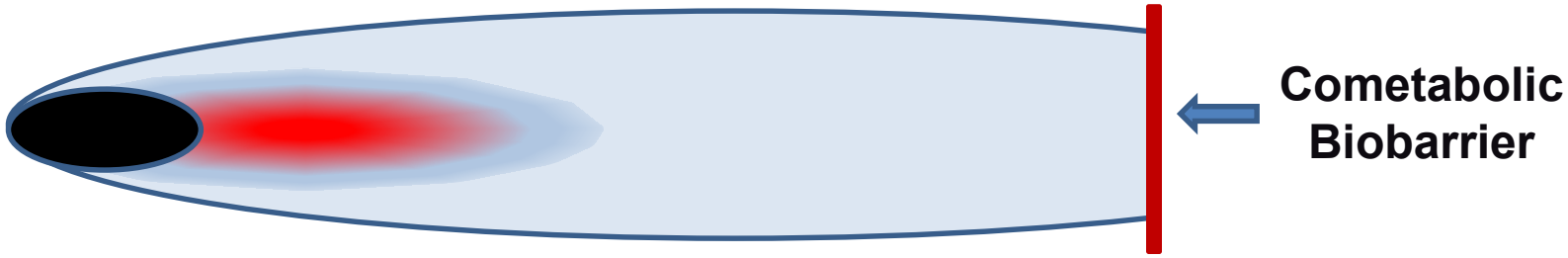
The Large, Dilute Plume Problem

- Treatment to regulatory levels is often impractical or cost prohibitive
- Plumes are sustained by:
  - Matrix diffusion / mass transfer
  - Slow natural attenuation processes



Technical Objectives

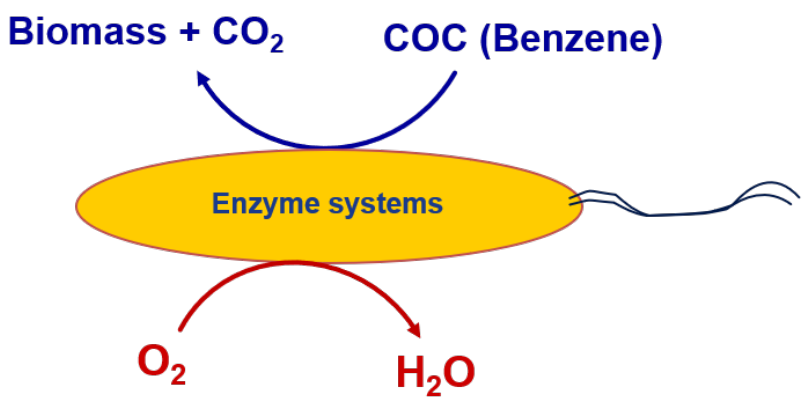
- Cometabolic treatment of a large, dilute CVOC plume
  - Sustainable
  - Semi-passive
  - Achieve regulatory limits (MCLs = maximum contaminant limits)
  - Cost effective



What is Cometabolism?

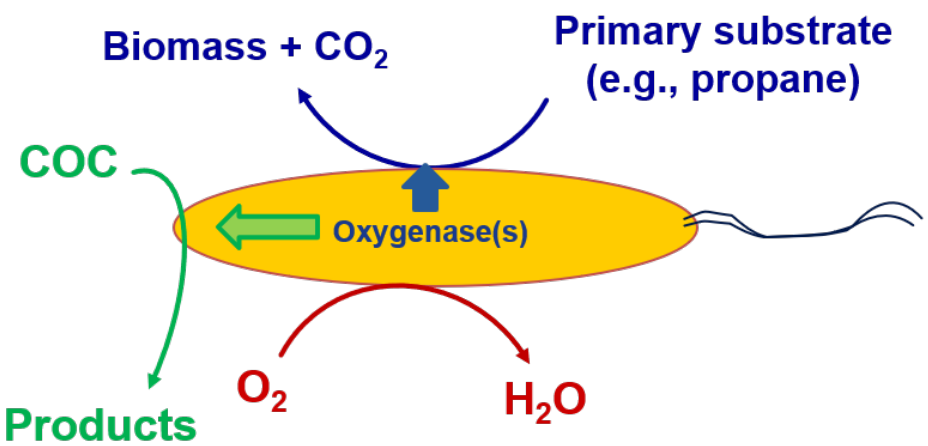
Transformation of an organic compound by a microorganism that is unable to use the substrate as a source of energy or one of its constituent elements

Growth-Linked Biodegradation



Cells gain energy and carbon from COC **itself**

Cometabolic Biodegradation

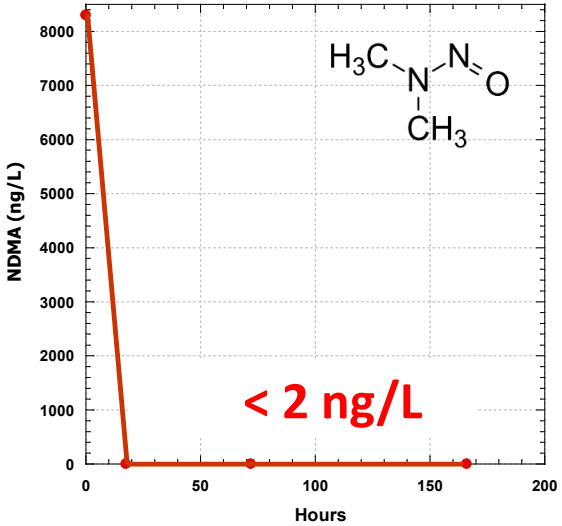


Cells gain energy and carbon from the primary substrate and degrade COC fortuitously

Note: COC = contaminant of concern

Advantages of Cometabolism

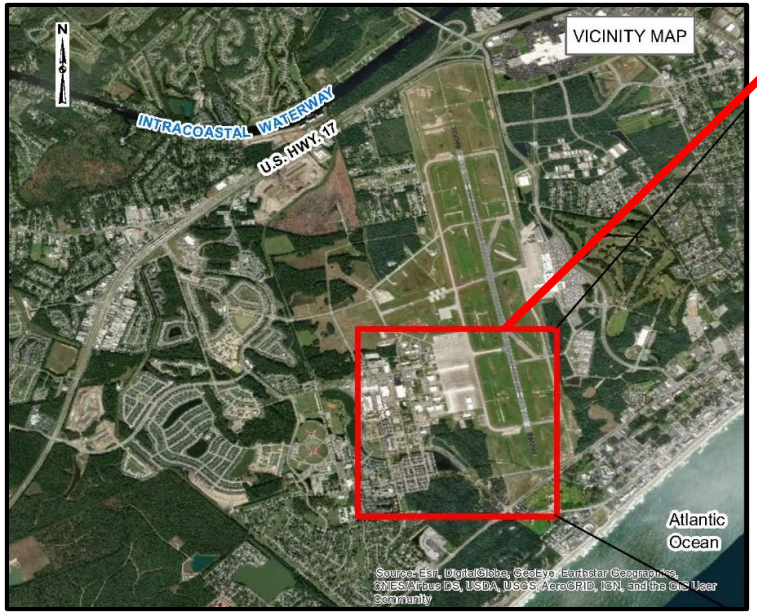
- Growth is on an abundant primary supplied substrate
  - Propane, methane, isobutane
  - High biomass
- Low contaminant concentrations achievable
- Cometabolic organisms are widely distributed
- Typically no toxic daughter products (ex. TCE→CO_2)

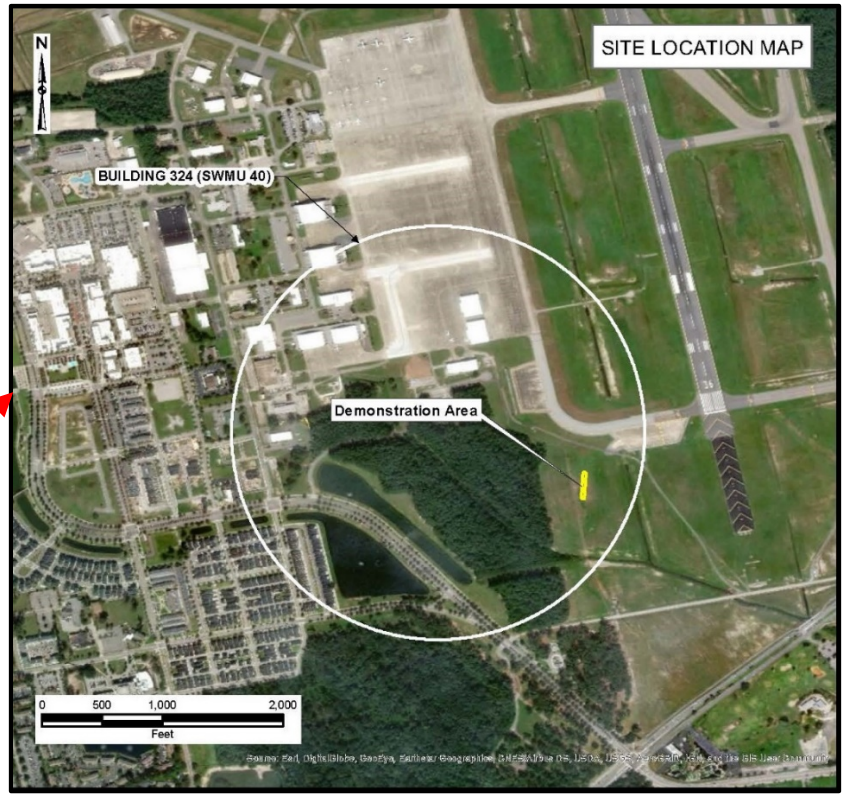


Biodegradation of NDMA by propane-grown *Rhodococcus ruber* ENV425

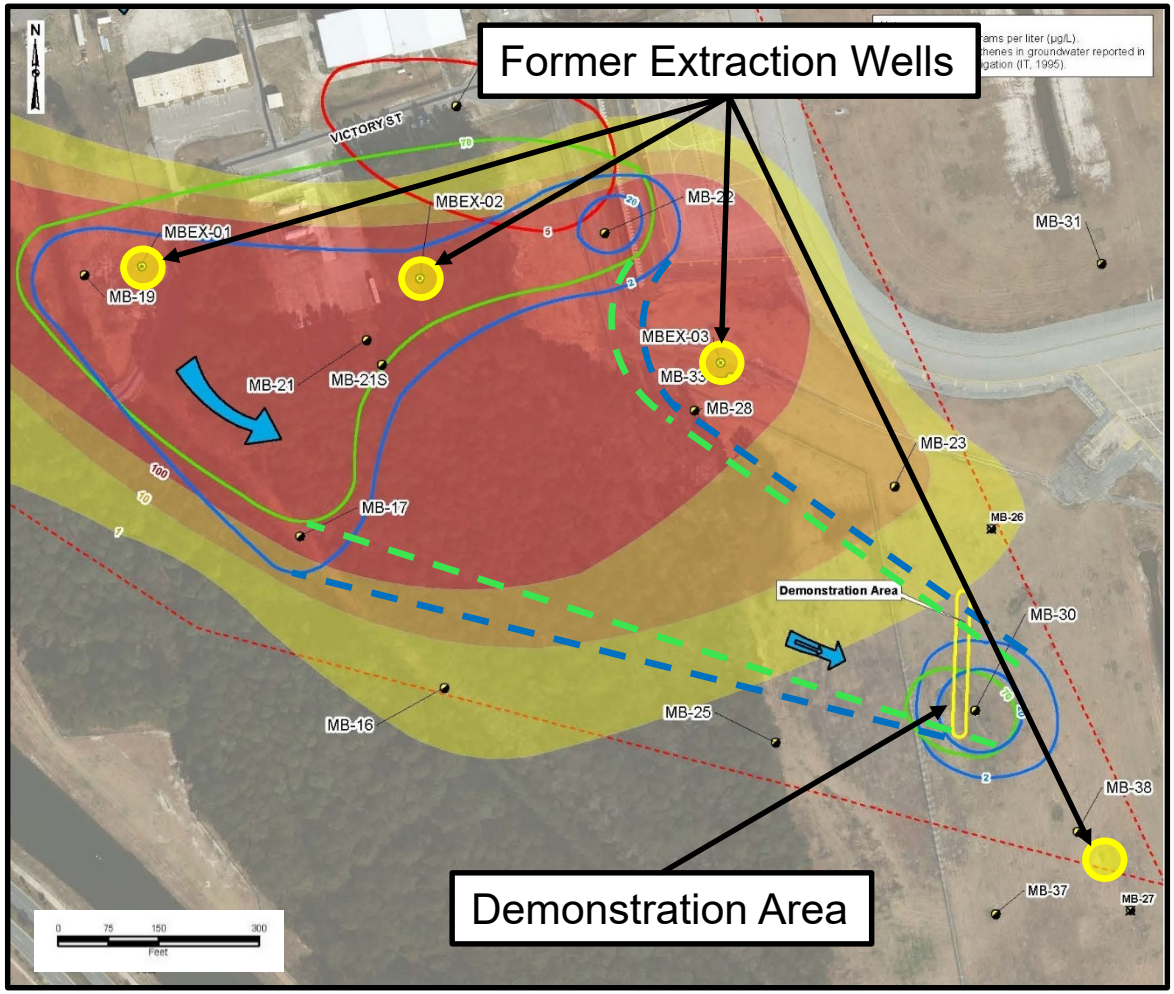
Cometabolic Biobarrier Field Demo

- ESTCP ER-201629
- Former Myrtle Beach AFB, SC
- Building 324 Plume

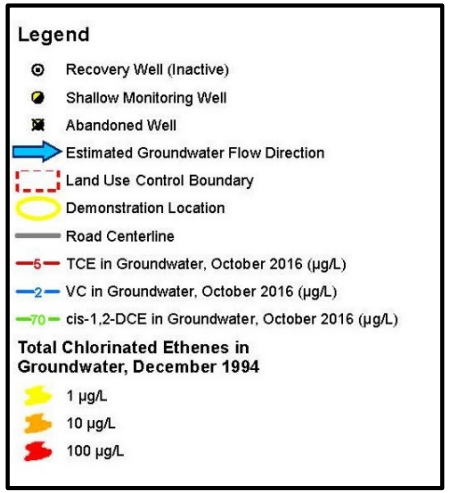




Cometabolic Biobarrier Field Demo

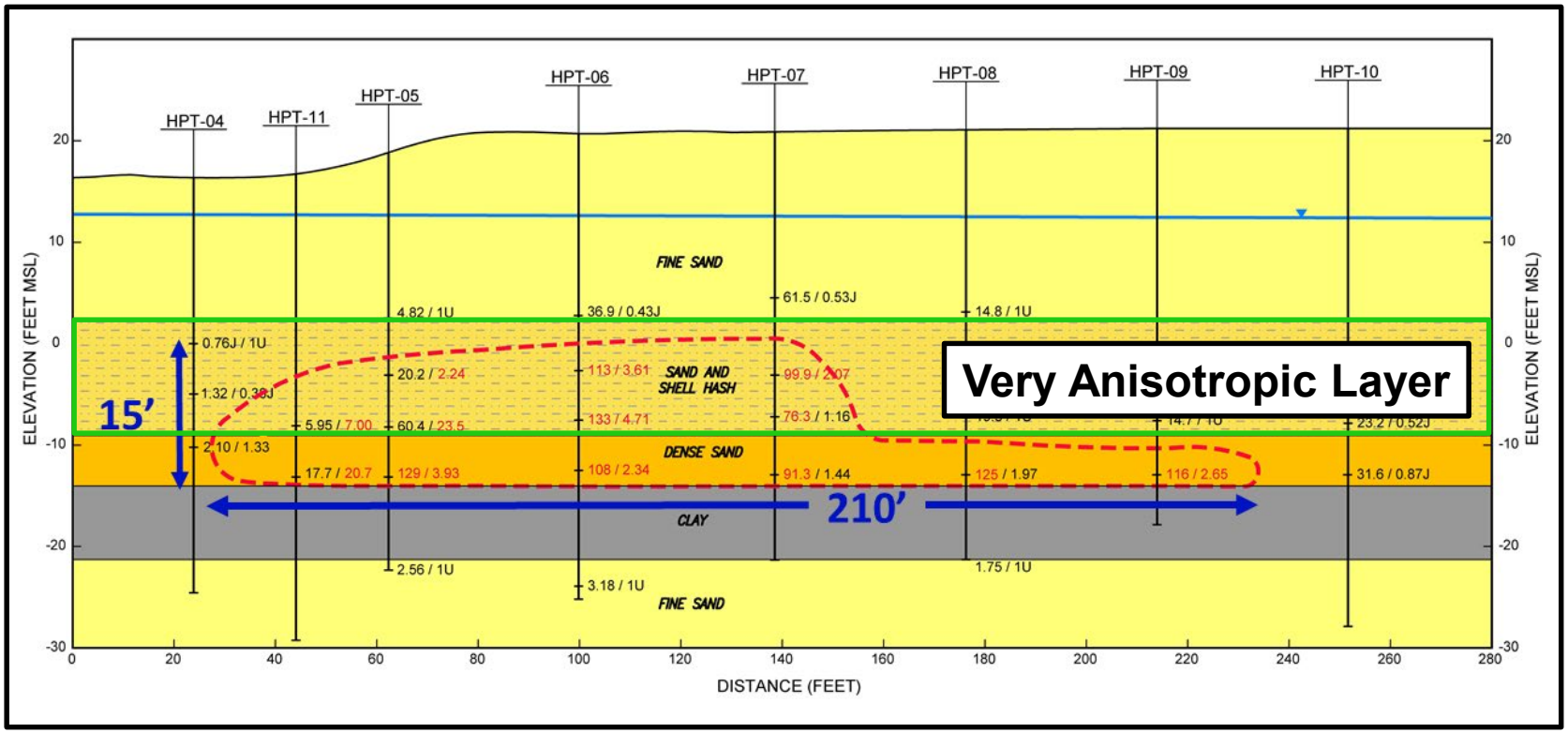


- TCE, cis-DCE, and VC above MCLs
- 1994: Plume >2,000' long and 1,000' wide
- Pump and treat remedy from 1995 to 2006 (140 gpm)
- Current plume dimensions less defined



Note: TCE = trichloroethylene; cis-DCE = cis-1,2-dichloroethylene; VC = vinyl chloride

Conceptual Site Model



MCLs

cis-DCE → 70 µg/L

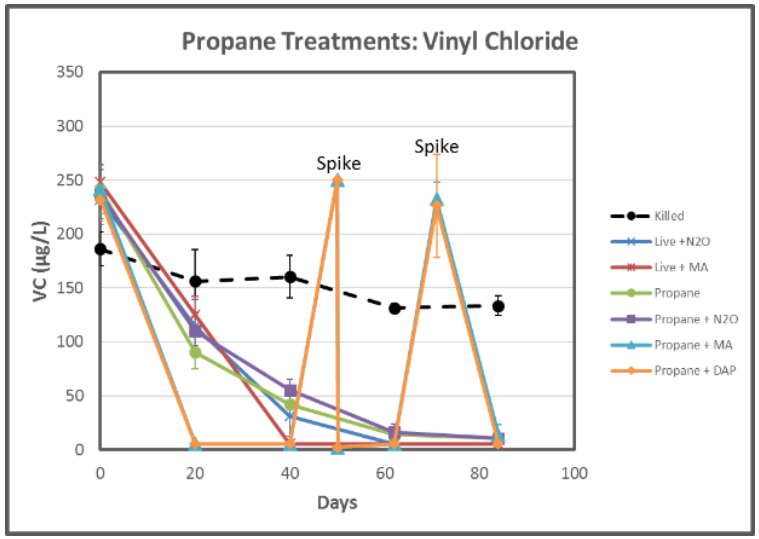
VC → 2 µg/L

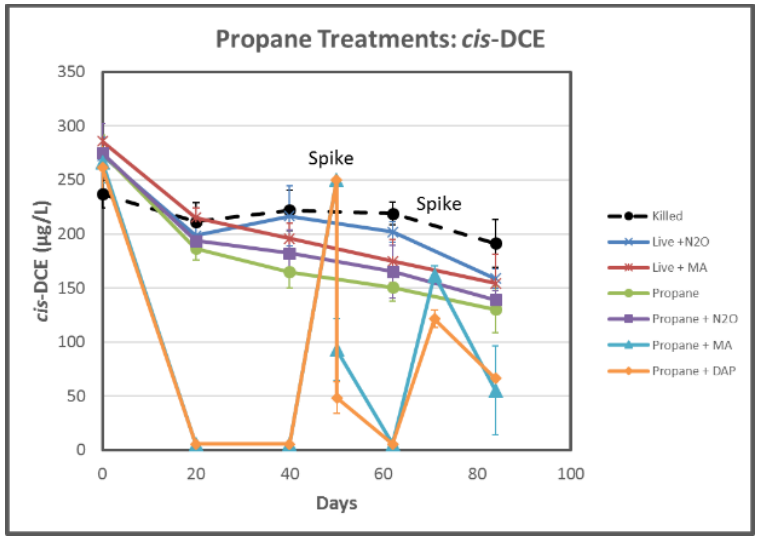
LEGEND:

113 / 3.61	cis-1,2-DCE CONCENTRATION / VC CONCENTRATION (ug/L)
- - -	LINE ENCLOSING LOCATIONS WITH CONCENTRATIONS EXCEEDING MCL.
91.3	RED FONT INDICATES CONCENTRATION EXCEEDS MCL.
U	UNDETECTED AT INDICATED CONCENTRATION
J	ESTIMATED RESULT BELOW DETECTION LIMIT

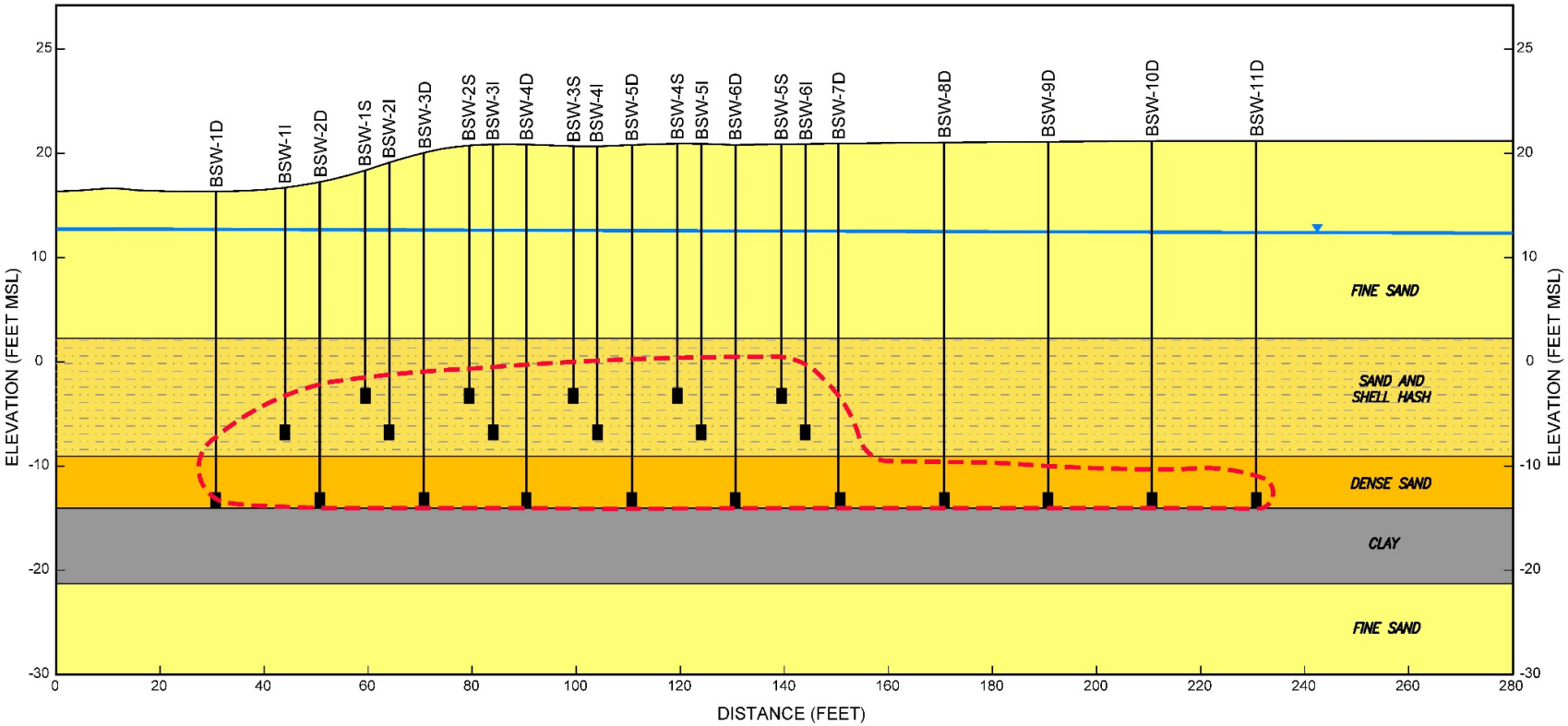
Microcosm Results

- Complete degradation of VC observed in most treatments
- cis-DCE degradation in propane and ethene treatments
- Degradation continued for >1 month in absence of amendment addition
- Nutrient (N) addition critical

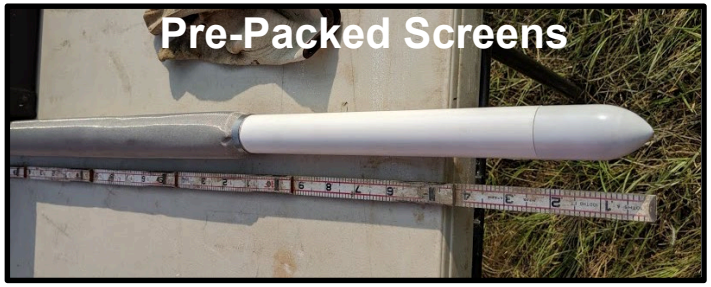




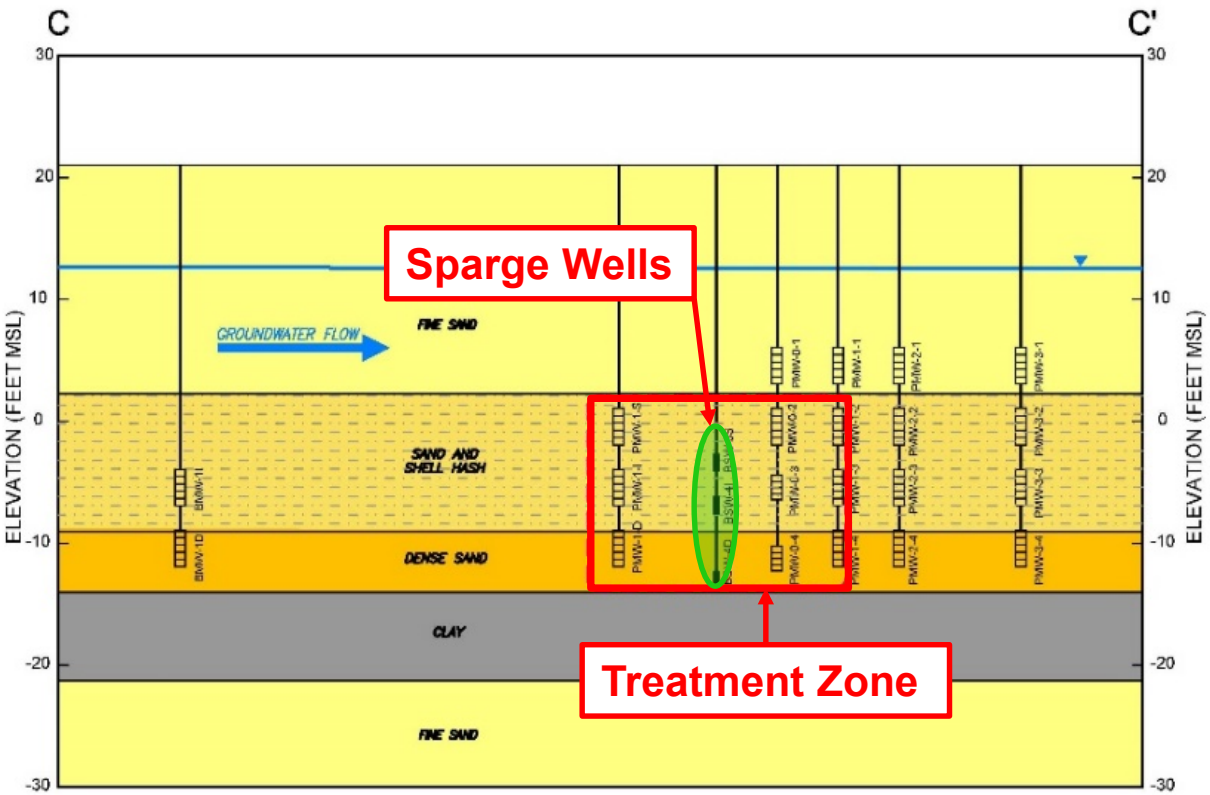
Sparge Well Layout



22 sparge wells
20’ spacing
3 intervals



Monitoring Network



- 27 monitoring wells
- 6 vapor probes

Well Installations

System Design and Installation

- Compressed oxygen and nitrogen
- Liquified propane and anhydrous ammonia
- Solar power (off grid)
- Remote access/control

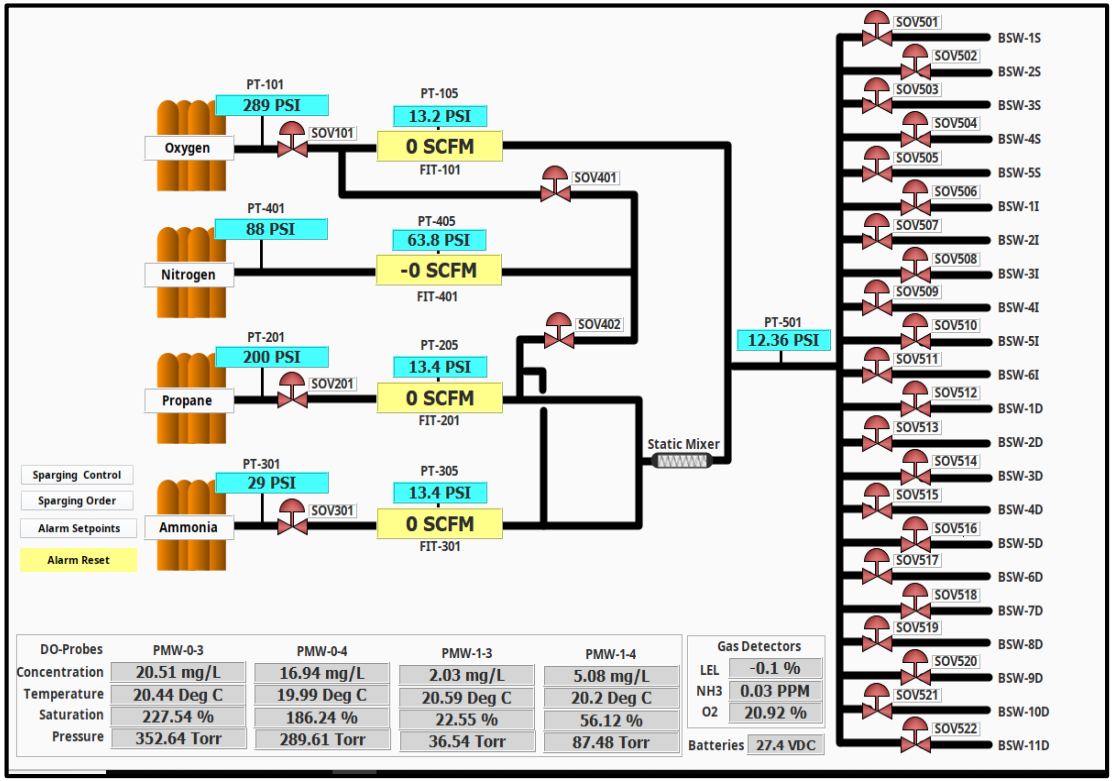
Gas Distribution System

Off-the-Grid Cometabolic Sparging System

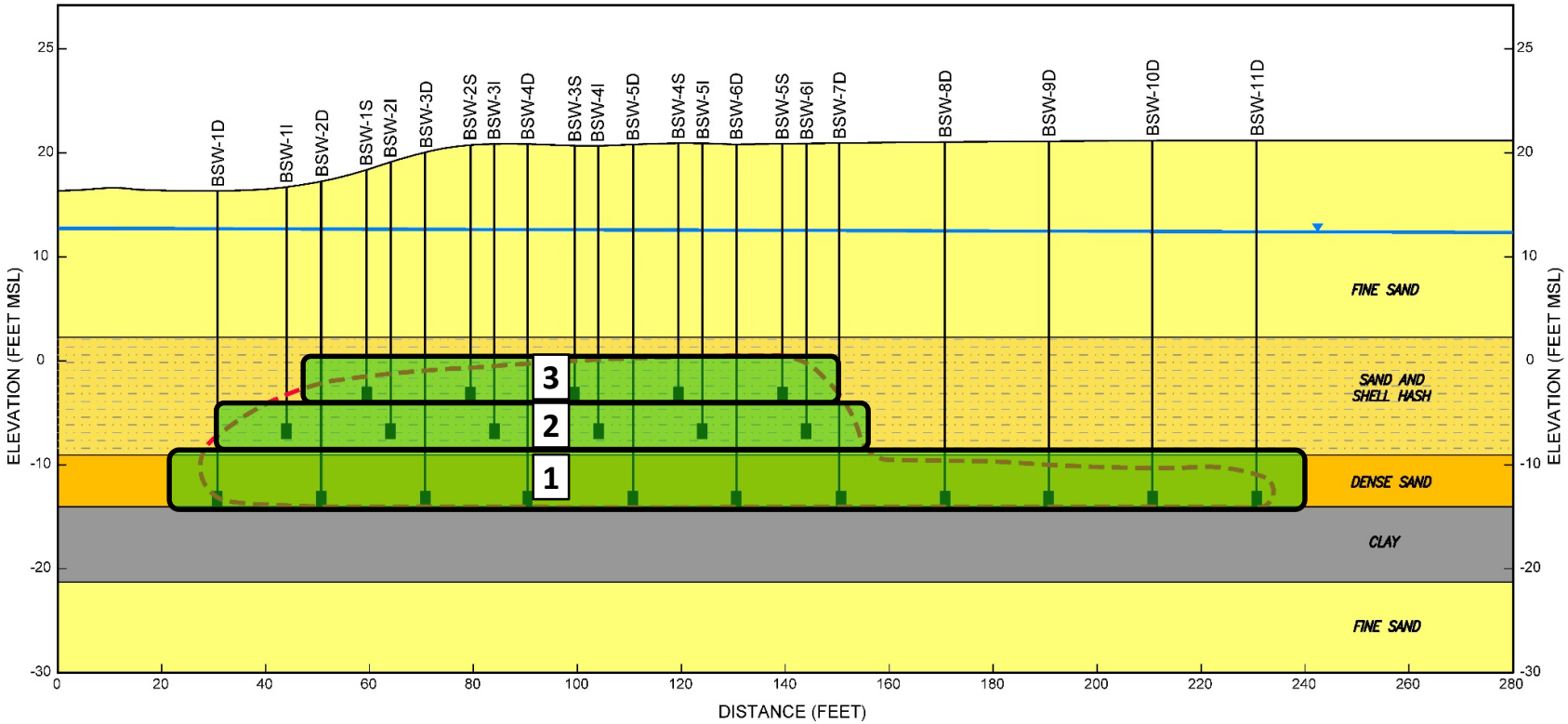
System Operation

- 9 weeks of oxygen-only sparging
- 12 months of cometabolic biosparging

System Interface Screen



System Operation

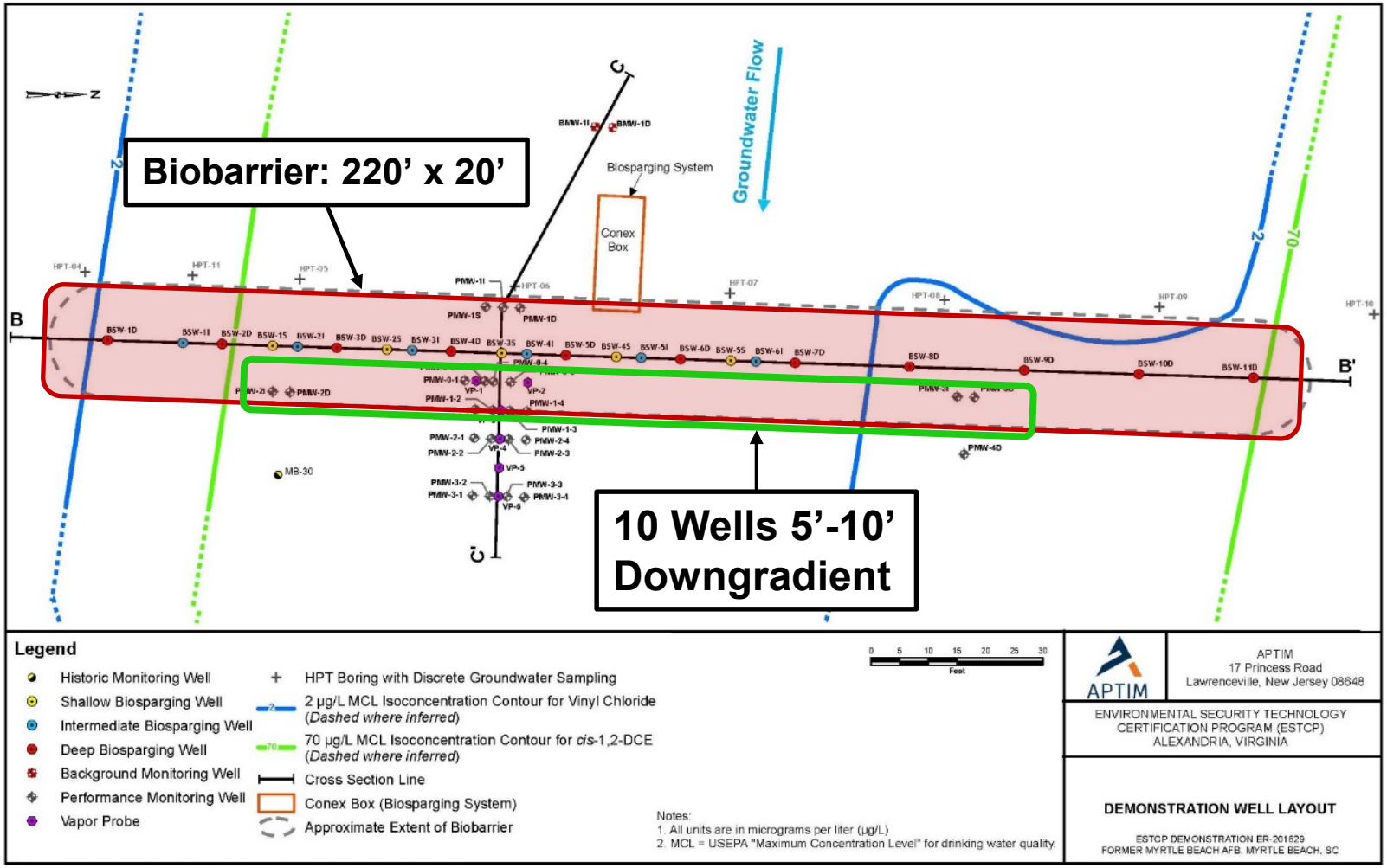


- 10-12 SCFM
- 1 well at a time, bottom up
- Oxygen ~1-2 times per week
- Propane and ammonia ~2-4 times per month

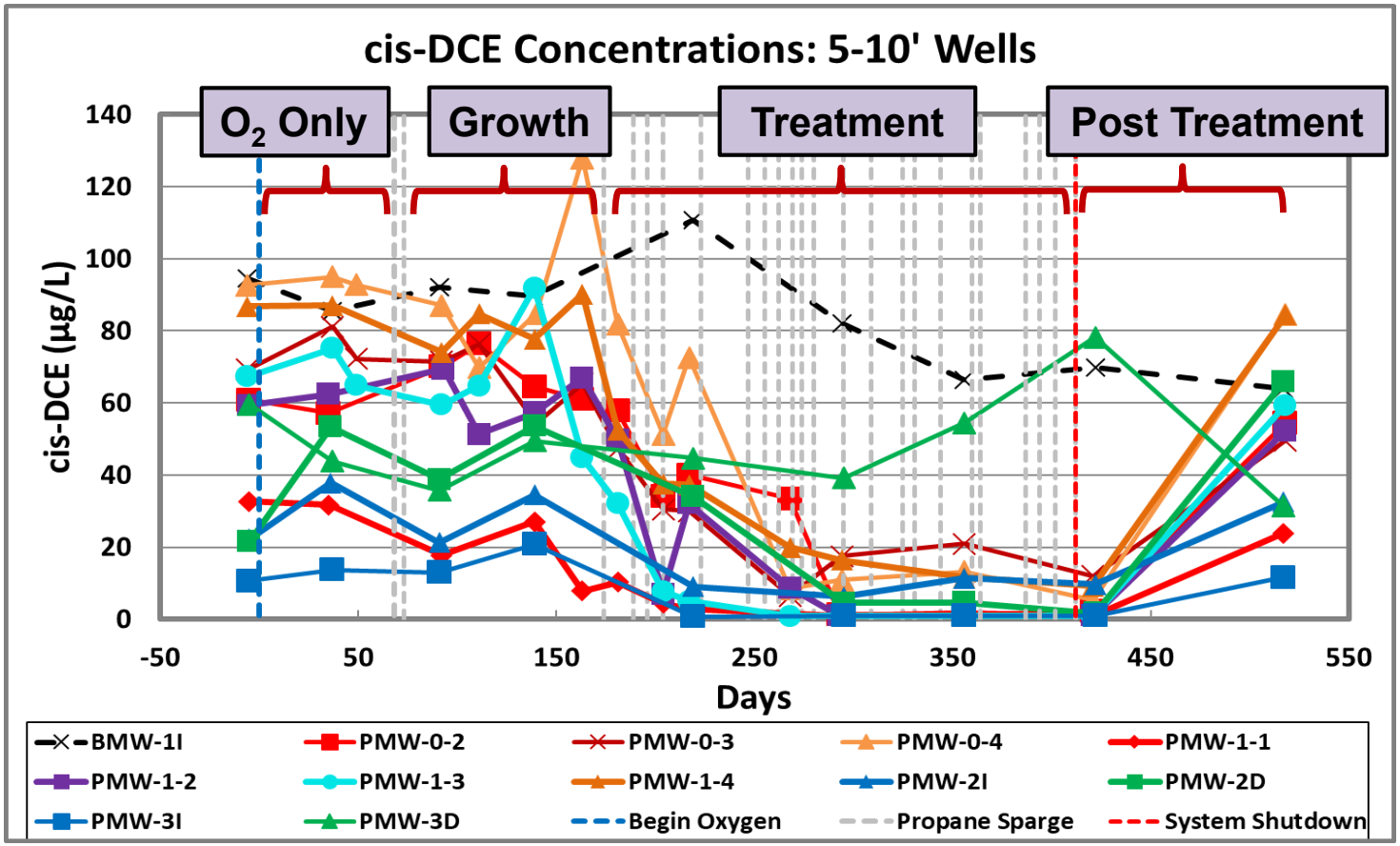
System Operation

- Oxygen
  - ~20 lbs/day
  - ~5 cylinders/week
- Propane
  - ~45 lbs/month
  - ~1/2 cylinder/month
- Ammonia
  - 7.5 lbs/month
  - 1/8 cylinder/month

Performance Monitoring

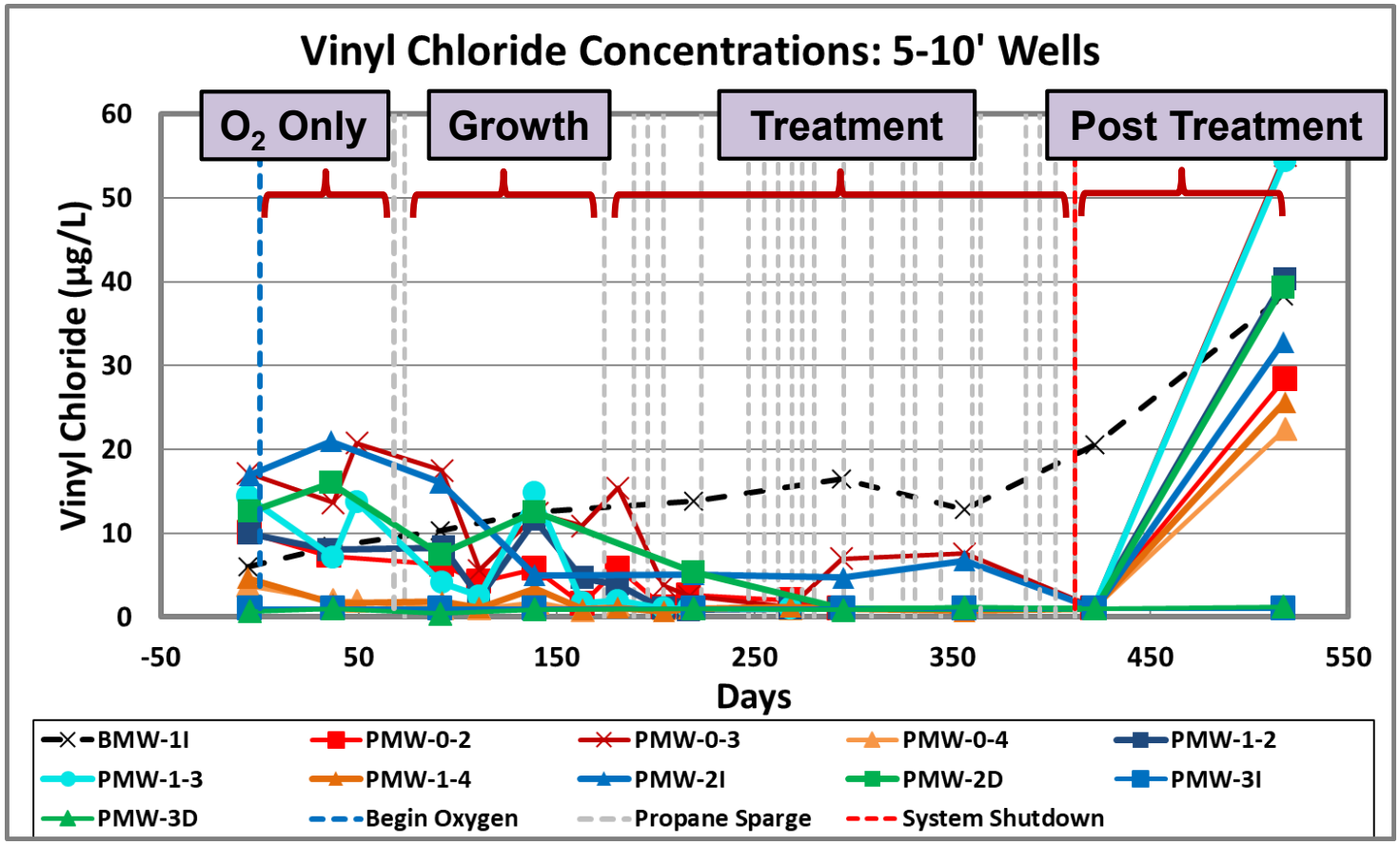


Treatment Performance



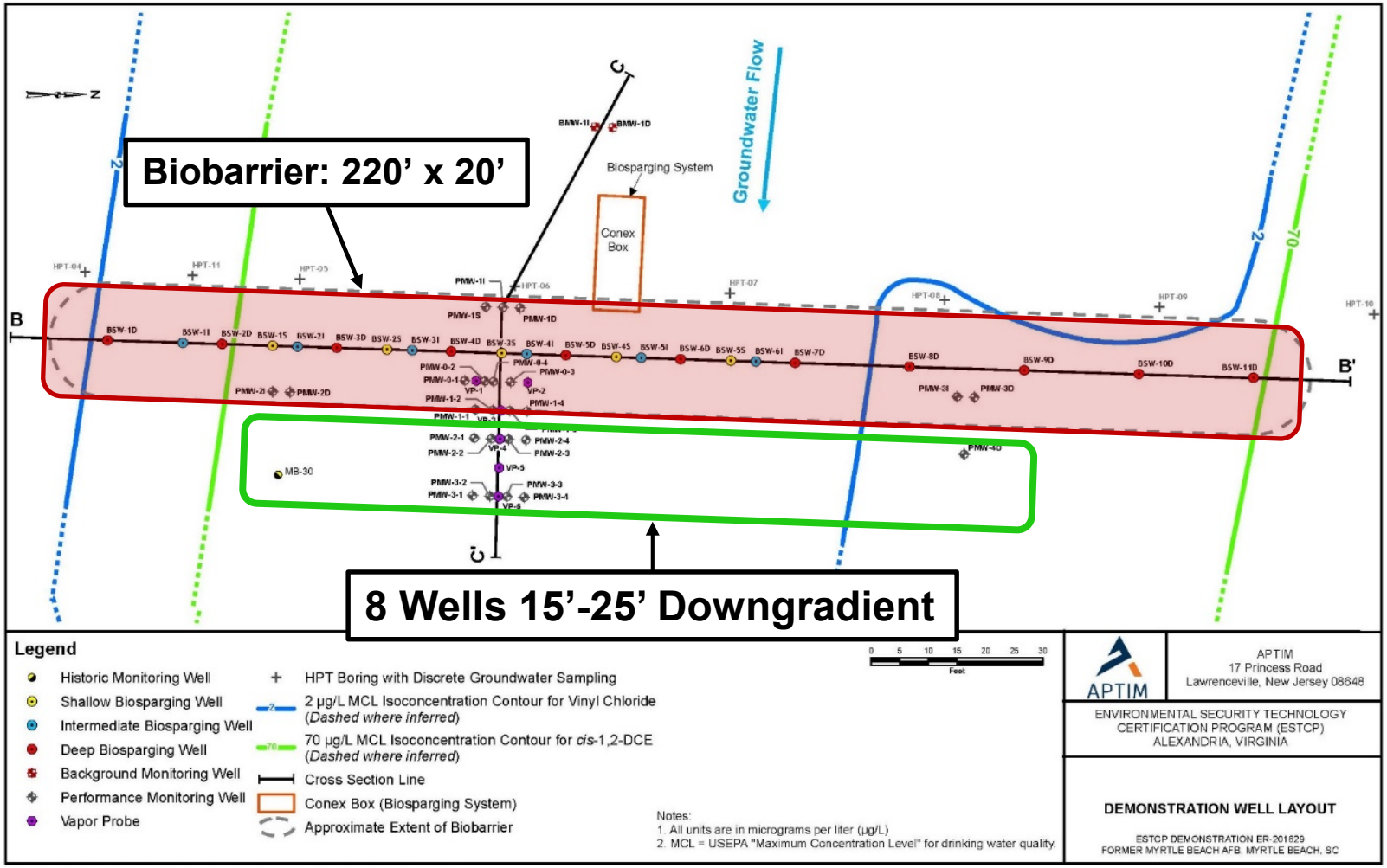
cis-DCE below MCL at all 10 wells

Treatment Performance

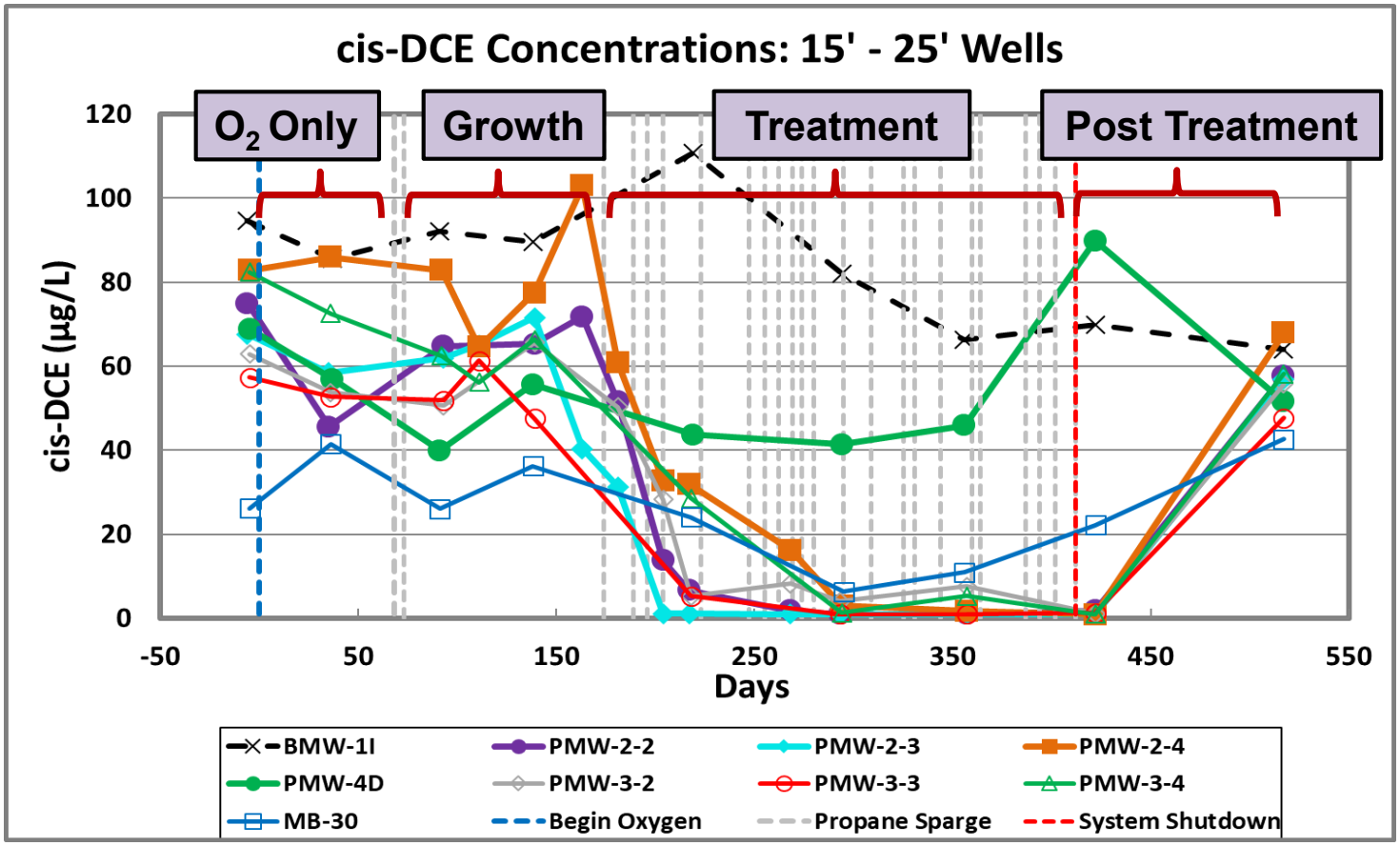


Vinyl chloride below MCL at all 10 wells

Treatment Performance

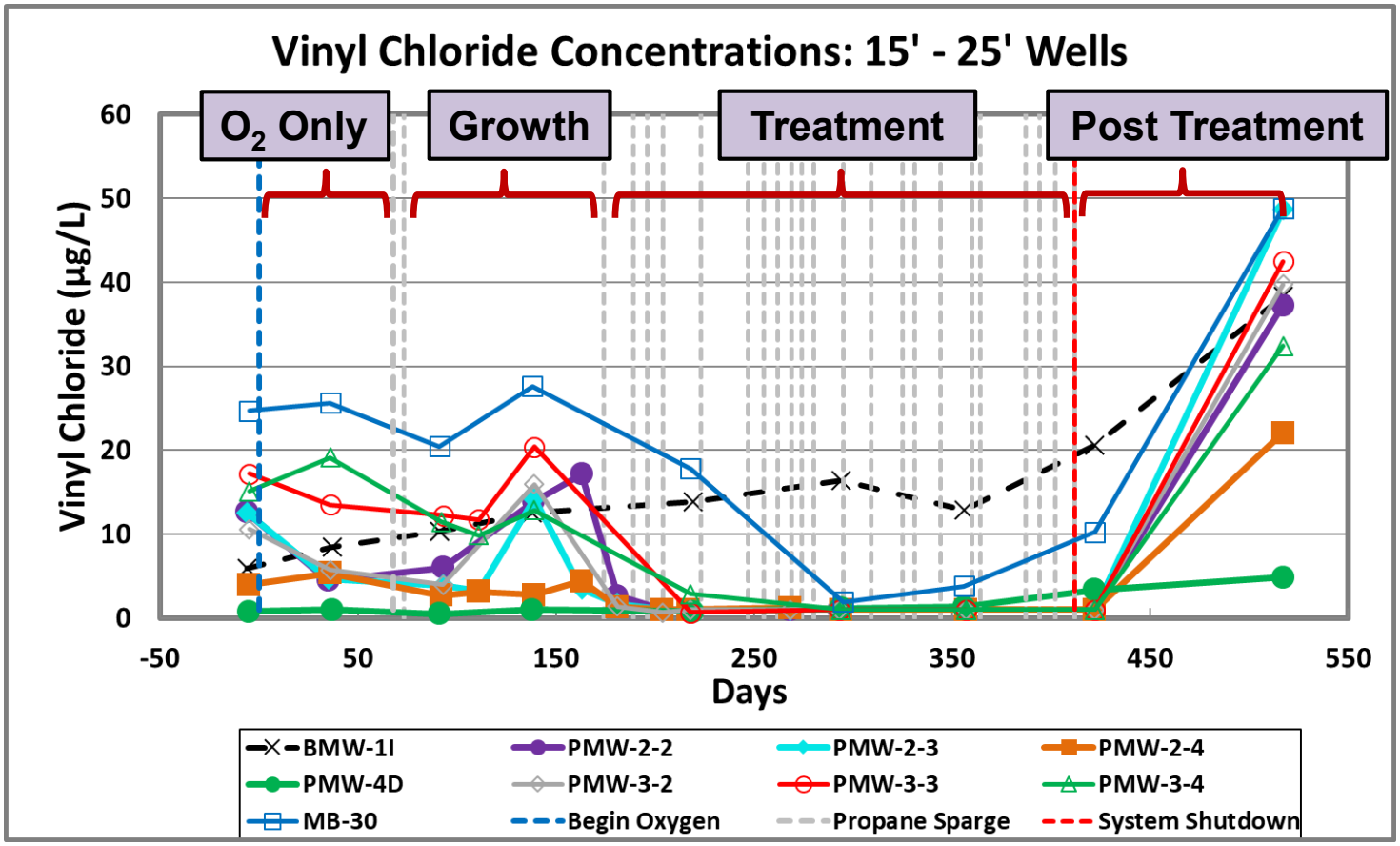


Treatment Performance



cis-DCE below MCL at all 8 wells

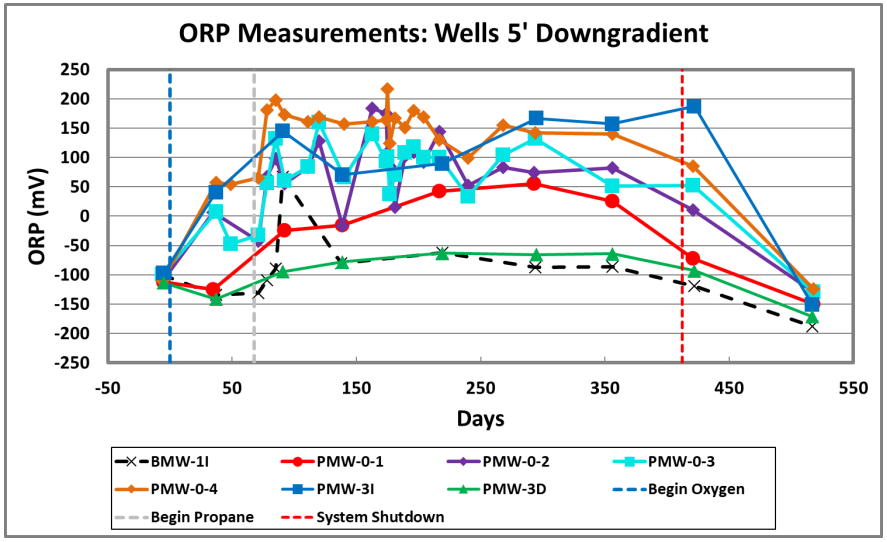
Treatment Performance

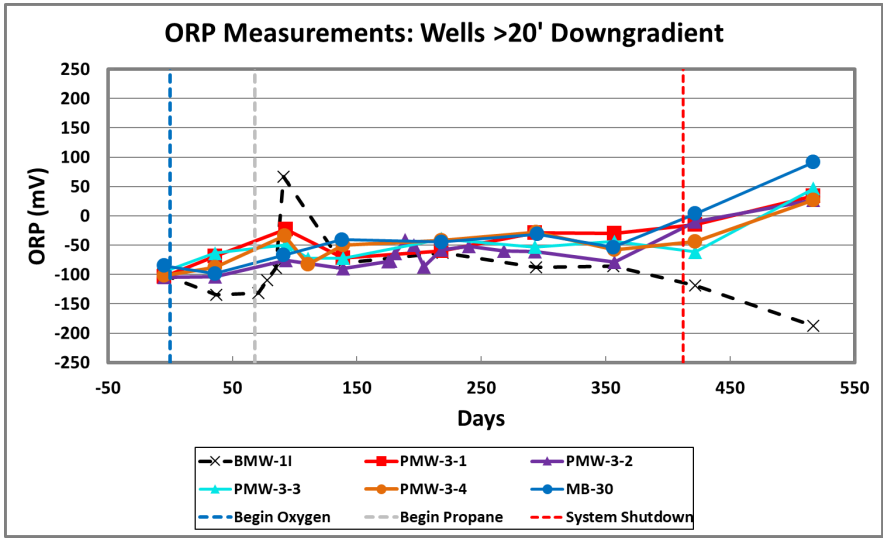


Vinyl chloride below MCL at 7 of the 8 wells

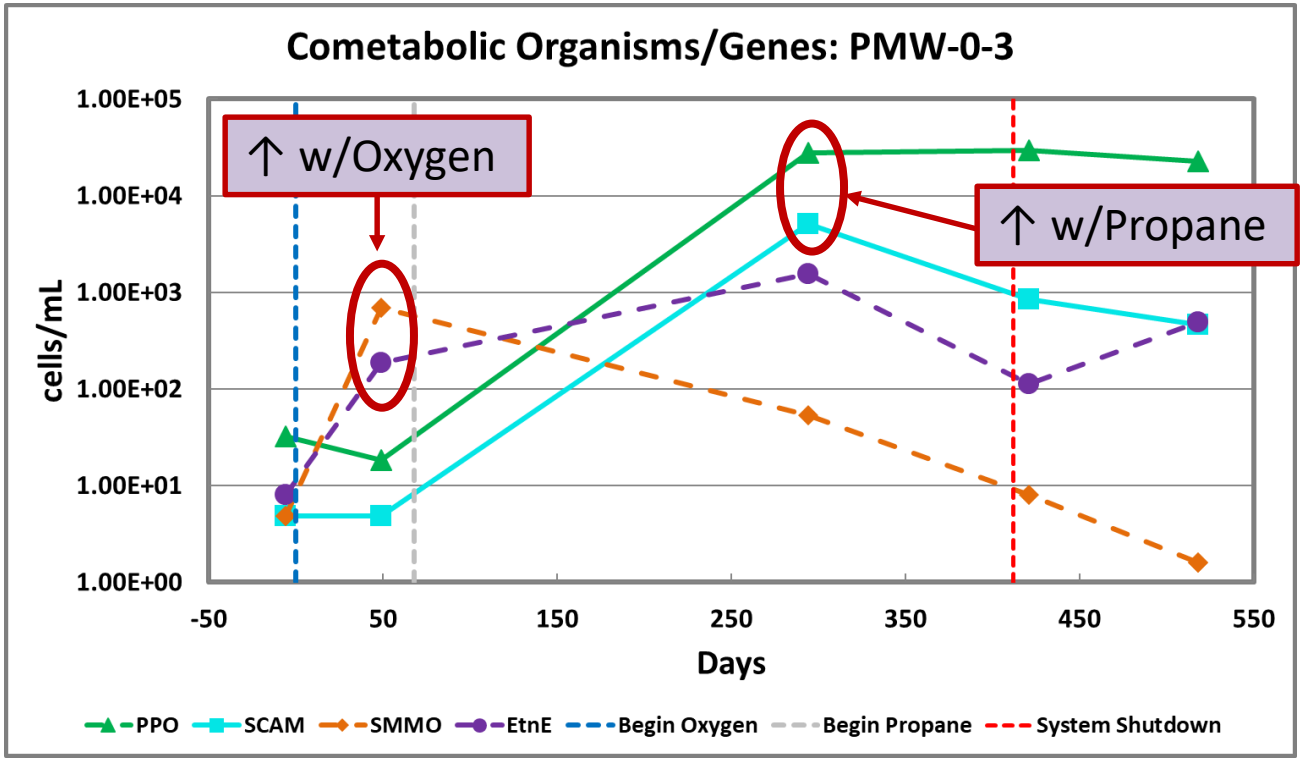
Treatment Performance

- ORP results
  - Oxidizing conditions within the biobarrier
  - Consistently reducing >10' downgradient
  - Aquifer "oxygen buffering capacity"





Cometabolic Organisms/Genes



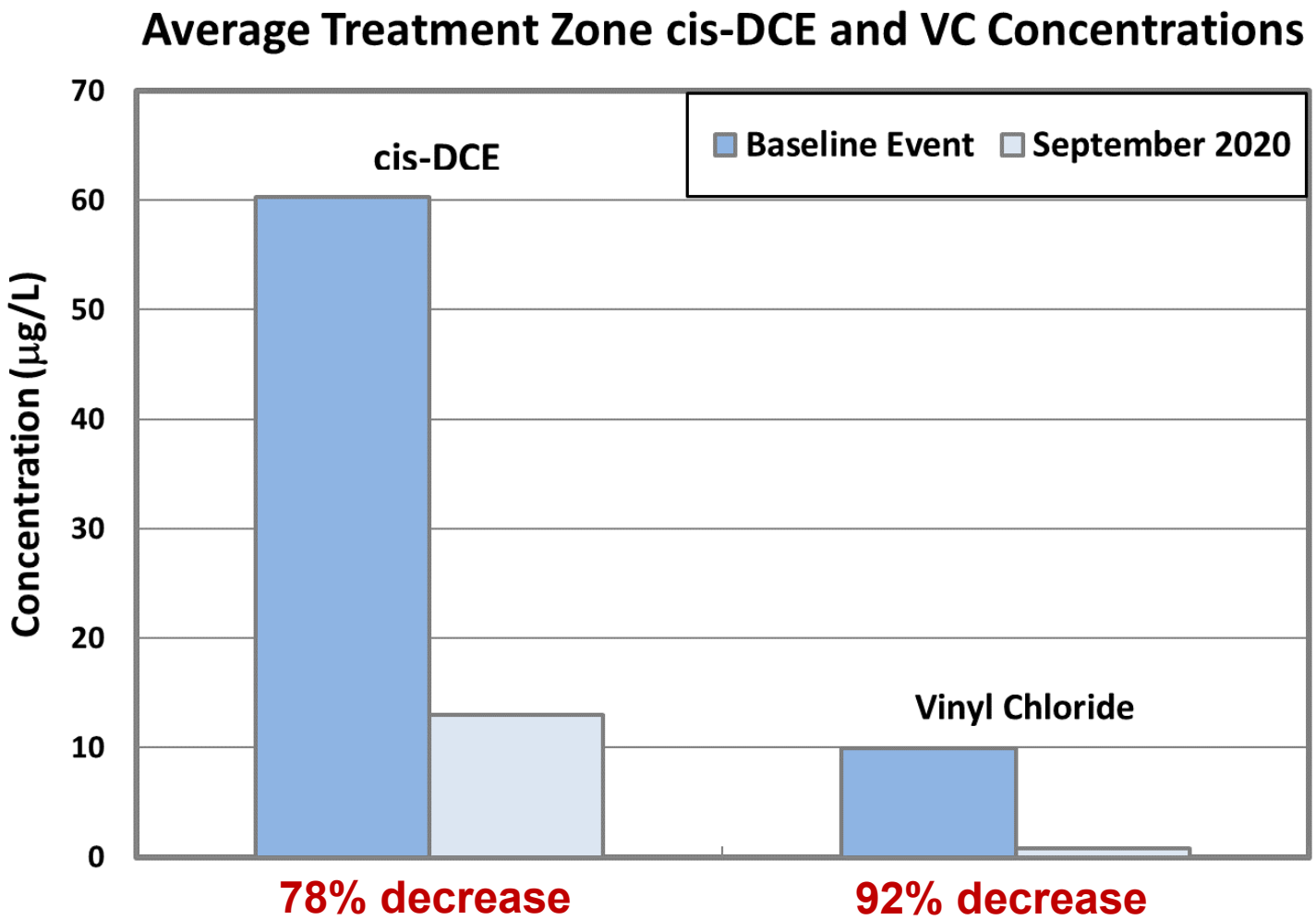
PPO = *Propane Monooxygenase*

SCAM = *Short Chain Alkane Monooxygenase*

sMMO = *Soluble Methane Monooxygenase*

EtnE = *Epoxyalkane transferase (ethene)*

Big Picture



Note: *Average concentrations from 18 wells

Conclusions

- Achieved significant reductions of COCs
  - Most wells below MCLs
- System required minimal operation and maintenance
- No negative impacts to aquifer quality
  - No mobilization of metals
  - No generation of daughter products
- It's not as difficult as you might think

Benefits to DoD

- Alternative to more active/expensive treatment approaches
- A sustainable, semi-passive, cost-effective technology (off the grid)
- Applicability for emerging contaminants
  - 1,4-dioxane (1,4-D)
  - Ethylene dibromide (EDB)
  - N-nitrosodimethylamine (NDMA)
  - 1,2,3-Trichloropropane (TCP)
  - Bis(2-chloroethyle)ether (BCEE)
  - Methyl tertiary-butyl ether (MTBE)
- Applicability for mixed plumes (CVOCs +)

For additional information, please visit

https://www.serdp-estcp.org/Program-Areas/Environmental-Restoration/Contaminated-Groundwater/Persistent-Contamination/ER-201629

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Q&A Session 2

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The next webinar is on March 11, 2021

New Resource Conservation Paradigms on DoD Lands

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Please take a moment to complete the survey that will pop up on your screen when the webinar ends

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