Bioremediation of Chlorinated Solvents and Mixtures

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Engineering Progress

Chlorinated Ethenes Often Found with Co-Contaminants

Chloroethenes:

- perchloroethene (PCE)
- trichloroethene (TCE)
- *cis*-1,2-dichloroethene (cDCE)
- 1,1-dichloroethene (1,1-DCE)
- vinyl chloride (VC)

And others:

- 1,4-dioxane
- Chloromethanes
- Chloroethanes (e.g., 1,1,1-TCA)
- Chloropropanes

Co-Occurrence of 1,4-Dioxane with Trichloroethylene in Chlorinated Solvent Groundwater Plumes at US Air Force Installations: Fact or Fiction

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Environmental Science & Technology Letters

Letter

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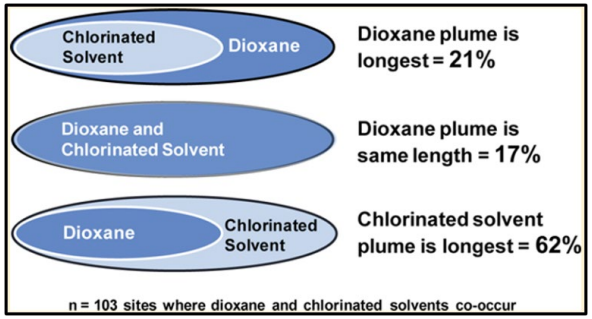
A Multisite Survey To Identify the Scale of the 1,4-Dioxane Problem at Contaminated Groundwater Sites

David T. Adamson,*,† Shaily Mahendra,‡ Kenneth L. Walker, Jr.,† Sharon R. Rauch,† Shayak Sengupta,§ and Charles J. Newell†

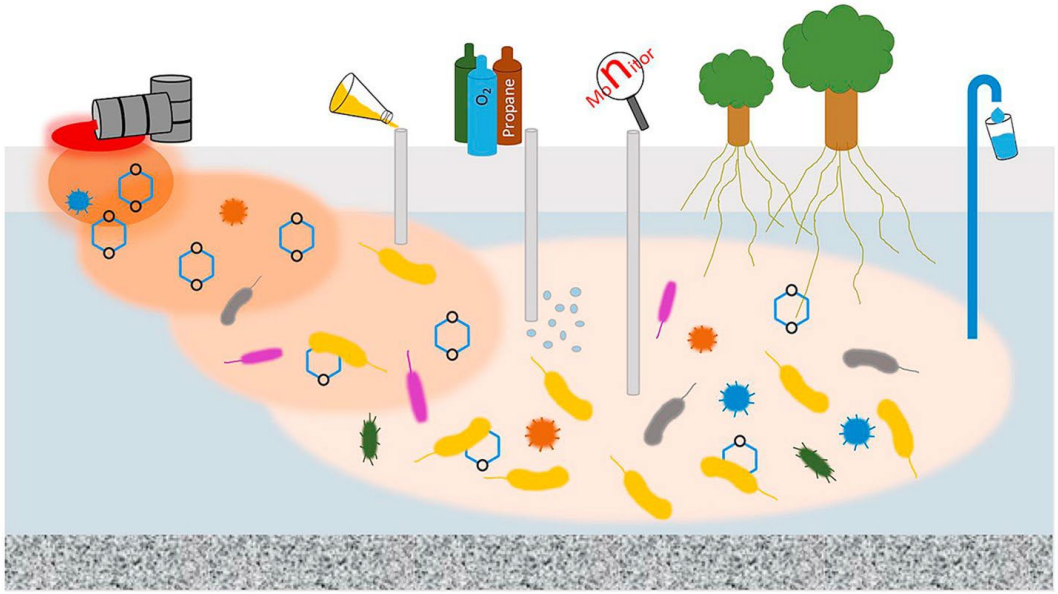
Environmental Science & Technology

Evidence of 1,4-Dioxane Attenuation at Groundwater Sites Contaminated with Chlorinated Solvents and 1,4-Dioxane

David T. Adamson,*,† R. Hunter Anderson,‡ Shaily Mahendra,§ and Charles J. Newell†



Luckily, Microbes Can Biodegrade 1,4-Dioxane & Chlorinated Ethenes

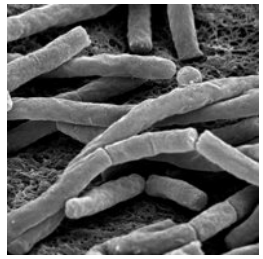


Pseudonocardia dioxanivorans CB1190

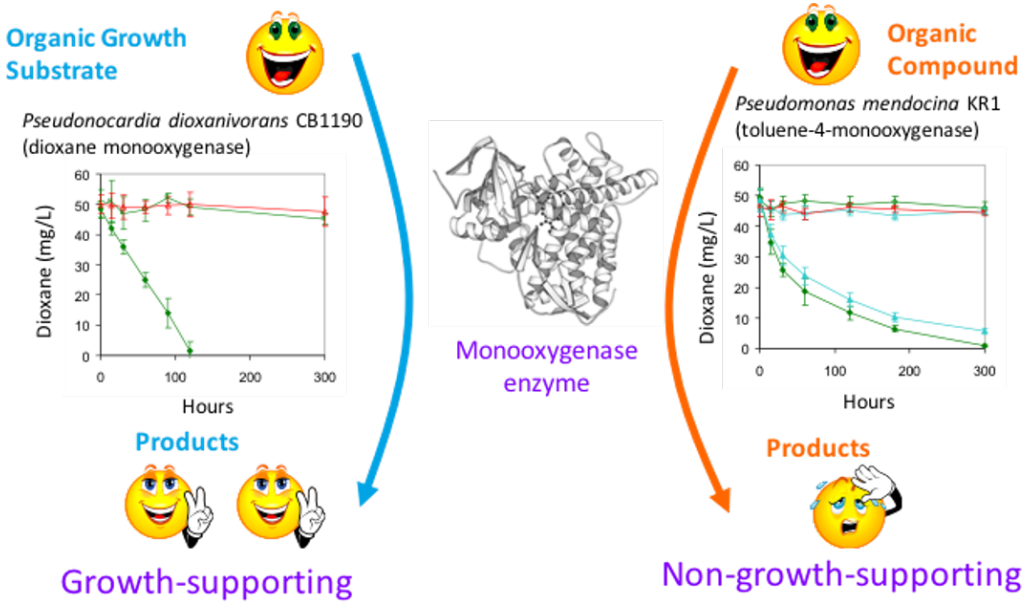
Rhodococcus ruber ENV425

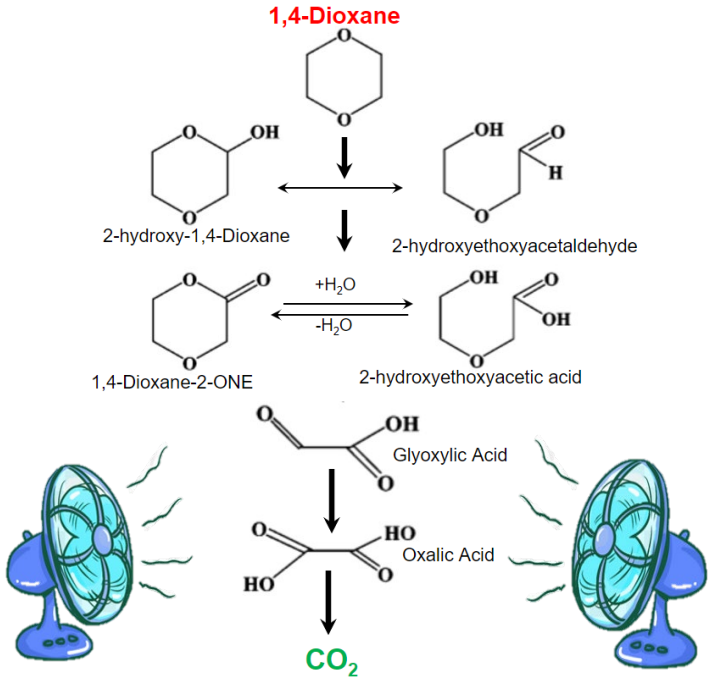
Methylosinus trichosporium OB3b

CB1190 Aerobically Biodegrades 1,4-Dioxane



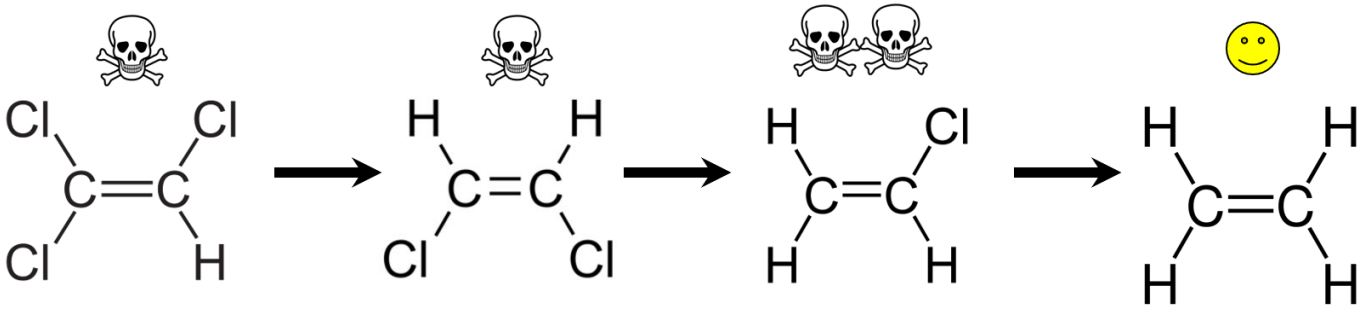
- **Metabolism:** microbe **gains energy** and carbon from contaminant
- **Co-metabolism:** microbe produces an enzyme to metabolize a primary substrate; the enzyme will also transform the contaminant of concern



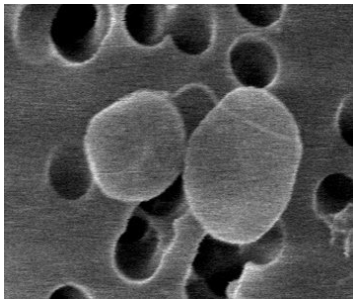


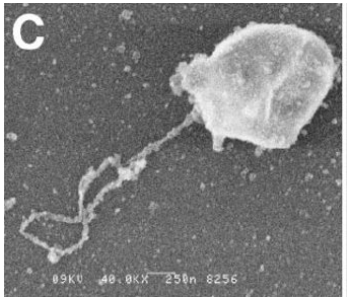
Mahendra & Alvarez-Cohen, IJSEM, 2005; Mahendra et al. ES&T. 2006; Grostern et al. ES&T. 2012

Dehalococcoides (Dhc) Anaerobically Biodegrades TCE



Dehalococcoides strain BAV1 *Dehalococcoides* strain 195 *Dehalococcoides* strain GT





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

He et al. *Nature*. 2003; Vogel and McCarty. *Environ. Microbiol.* 1985; Sung et al. AEM, 2006; Yan et al. ISME J., 2017; Mao et al., AEM, 2017

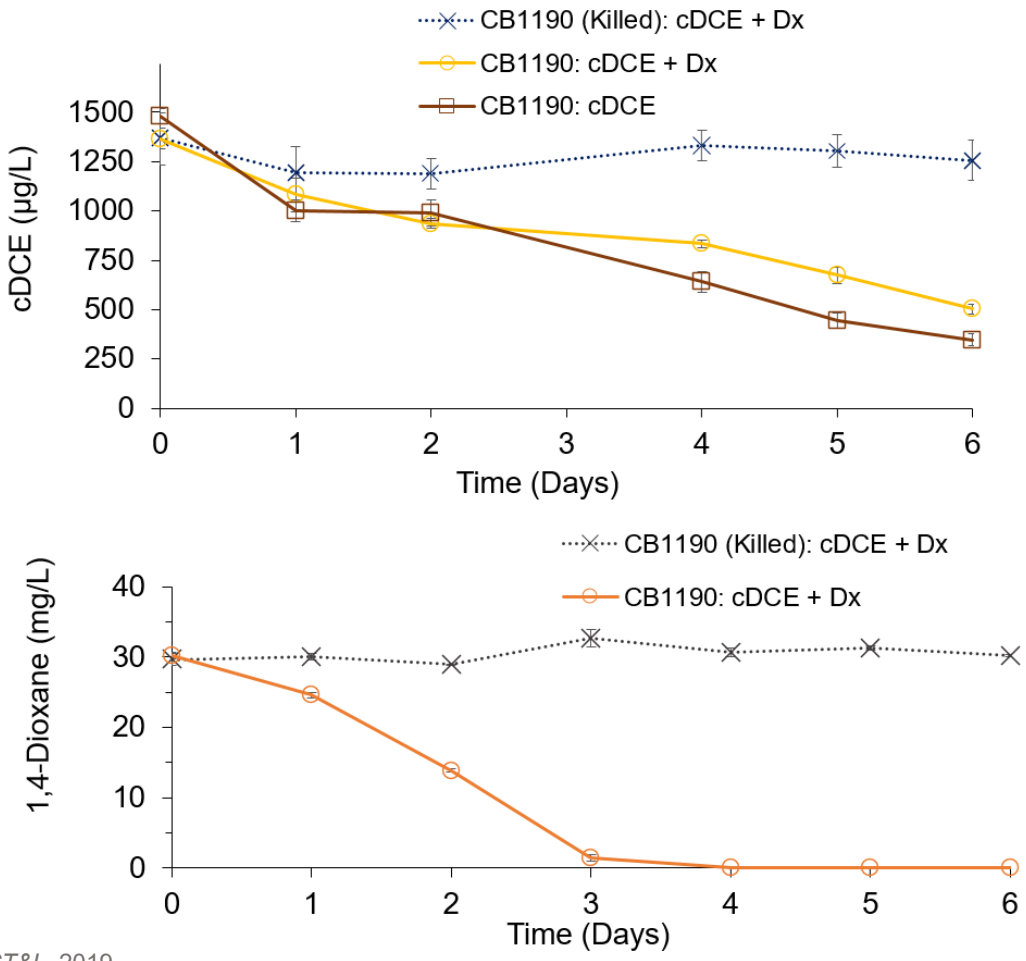
Good News/Bad News

- Good News: TCE and 1,4-Dioxane Biodegradable
- Bad News: Need Opposing Redox Conditions

Good News/Good News

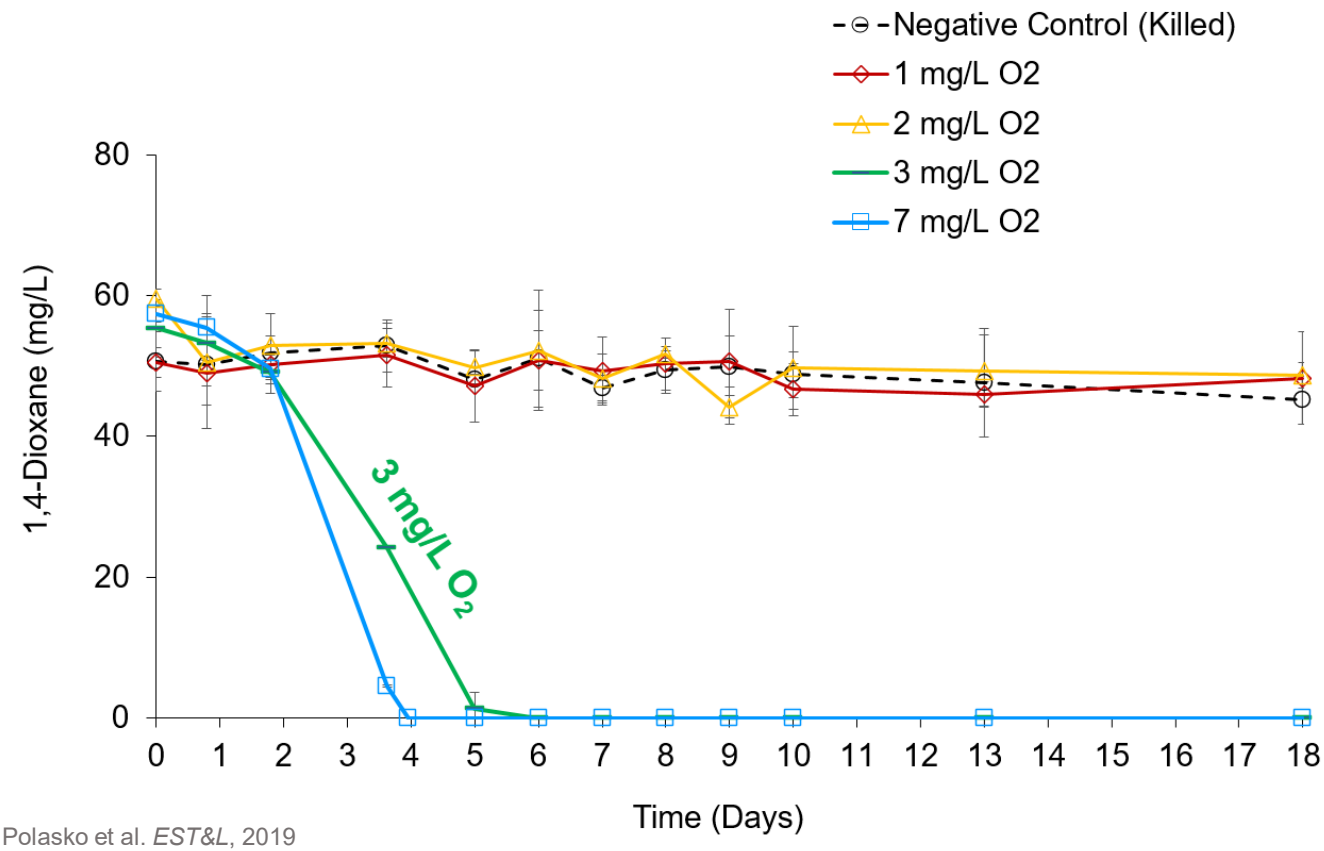
- Good News: TCE and 1,4-Dioxane Biodegradable
- ~~Bad~~ Good News: Combine Anaerobes and Aerobes

CB1190 Degrades cDCE AND 1,4-Dioxane (Dx)



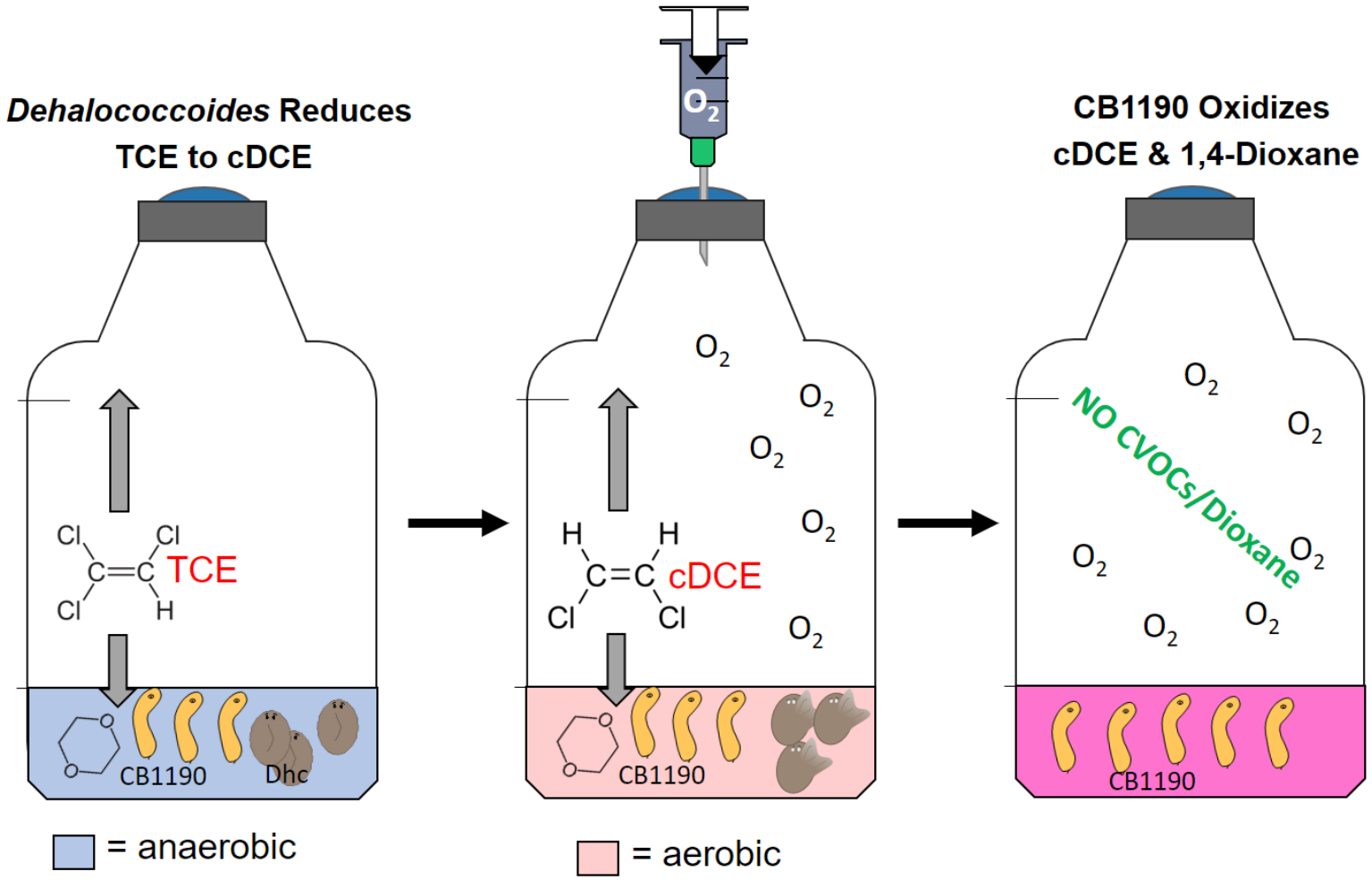
Polasko et al. *EST&L*, 2019

CB1190 Degrades 1,4-Dioxane with 3 mg/L Dissolved Oxygen

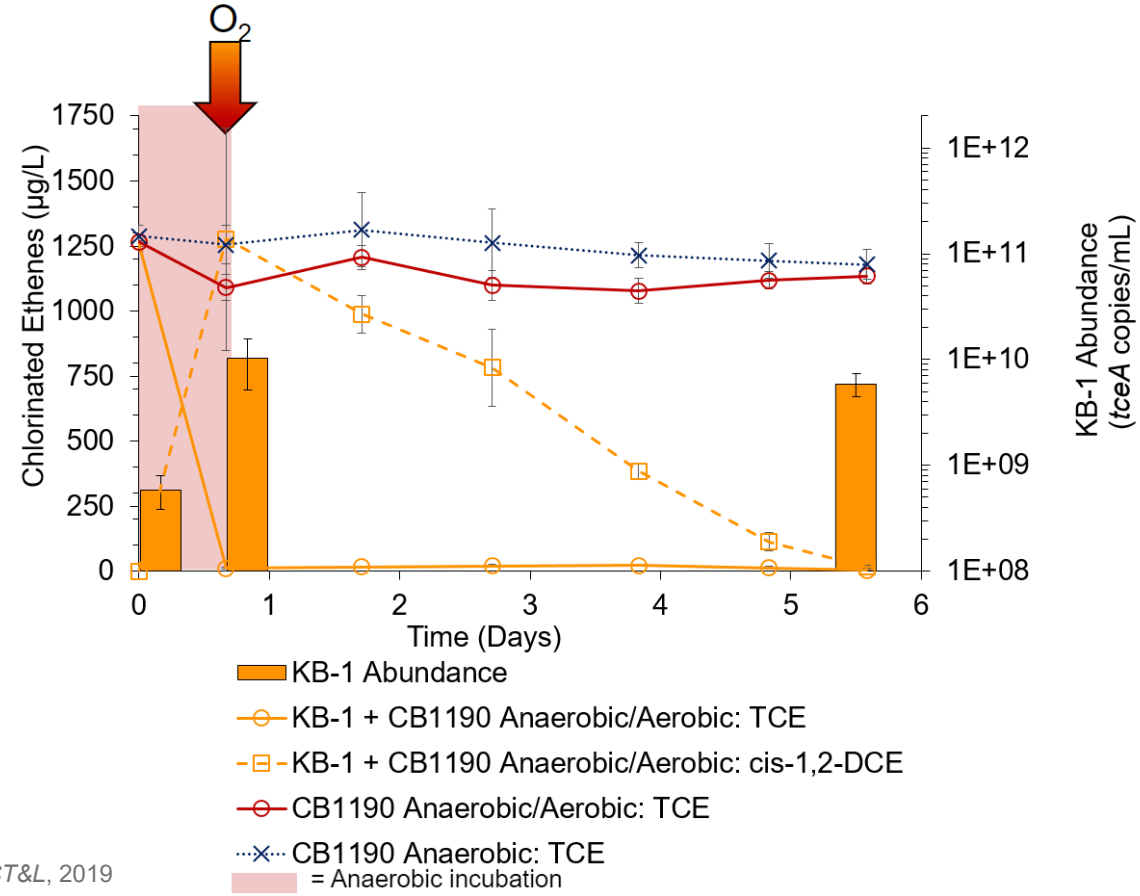


Polasko et al. *EST&L*, 2019

Engineered Microbial Community

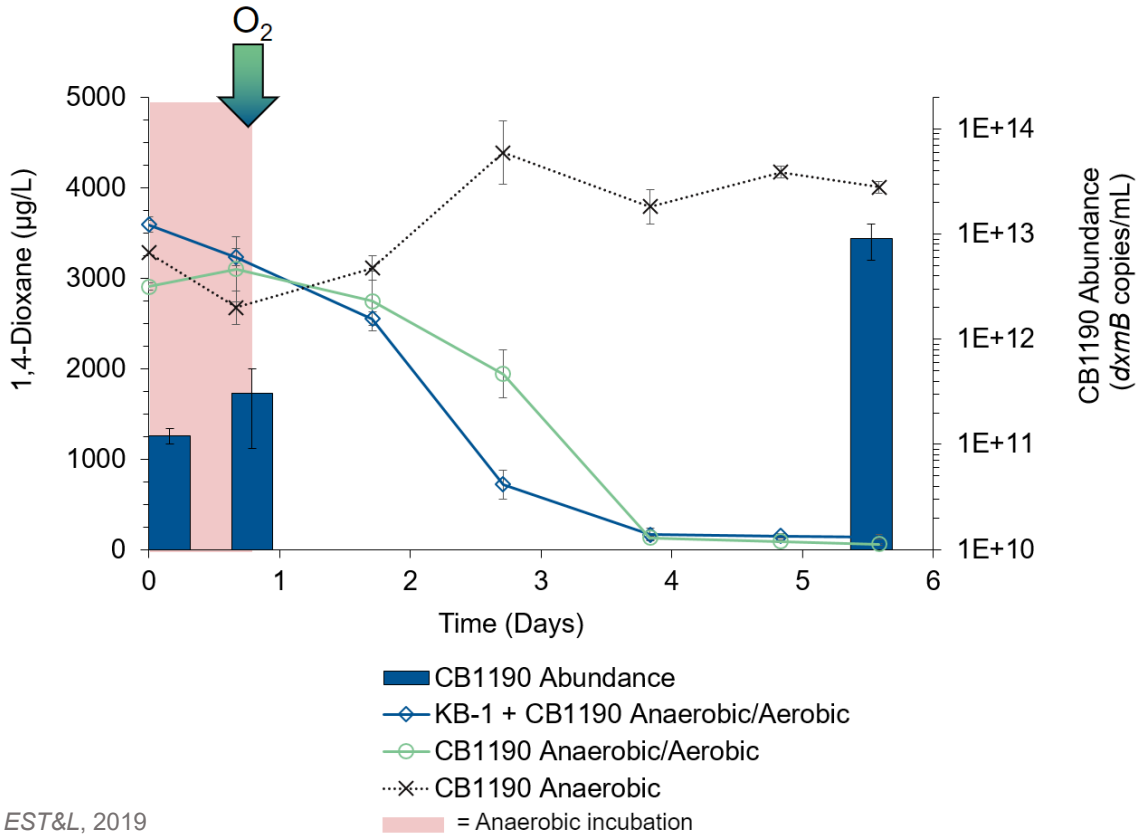


Dhc Degrades TCE → cDCE; CB1190 Degrades cDCE



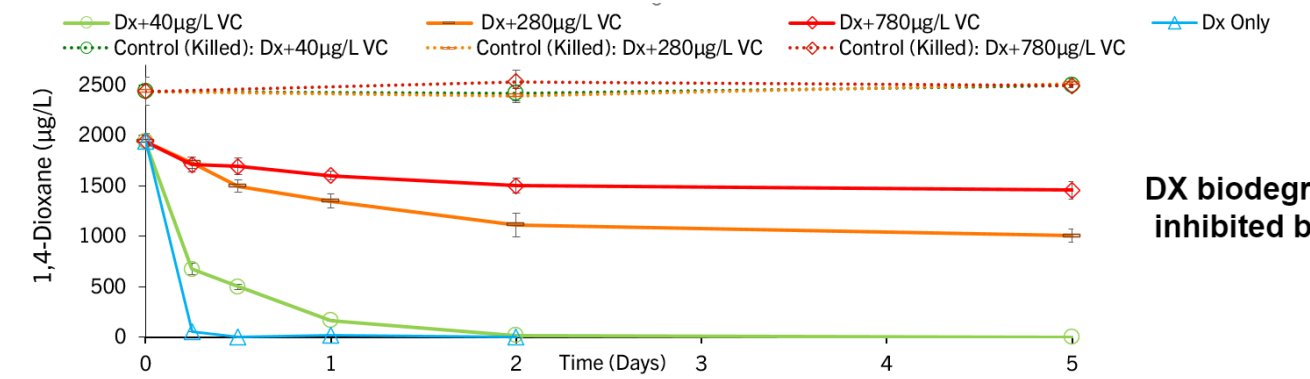
Polasko et al. *EST&L*, 2019

Mixed Culture & Strain CB1190 Degrades 1,4-Dioxane

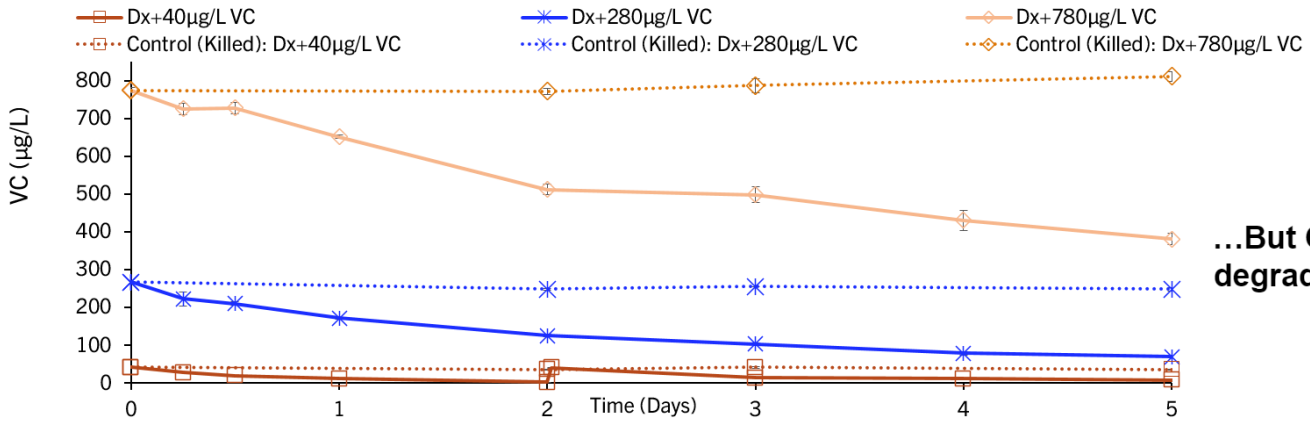


Polasko et al. *EST&L*, 2019

CB1190 Biodegrades VC & 1,4-Dioxane Simultaneously



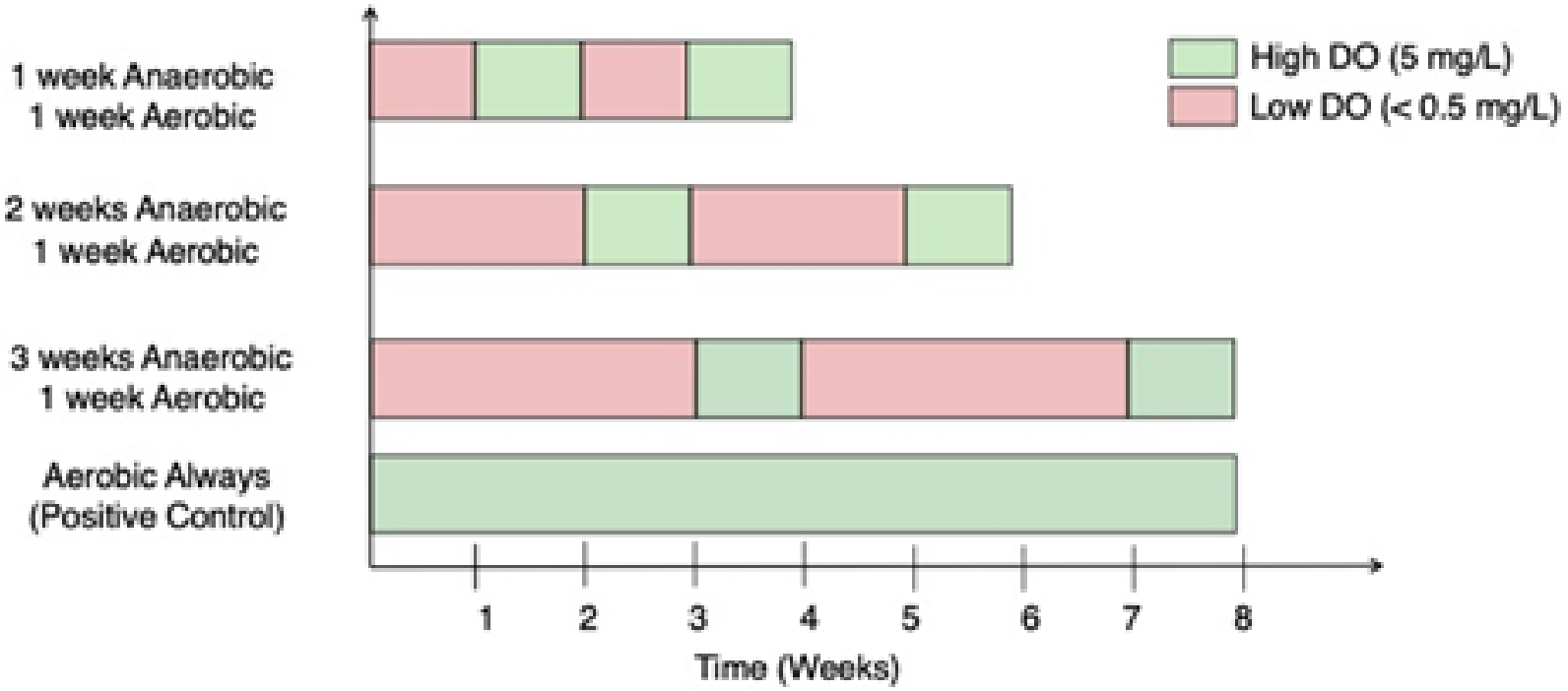
DX biodegradation inhibited by VC…



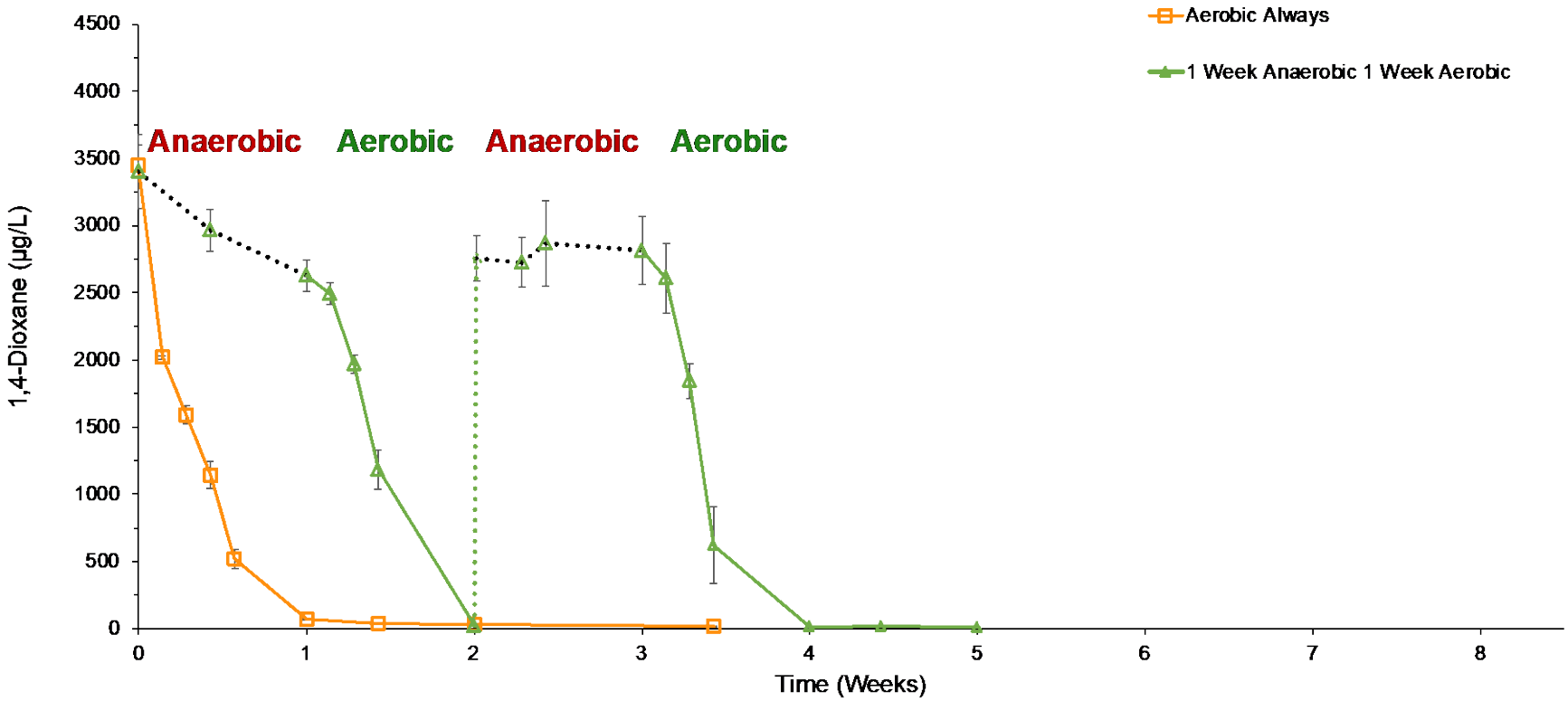
…But CB1190 can degrade inhibitor!

How Long Can CB1190 Survive Without Oxygen?

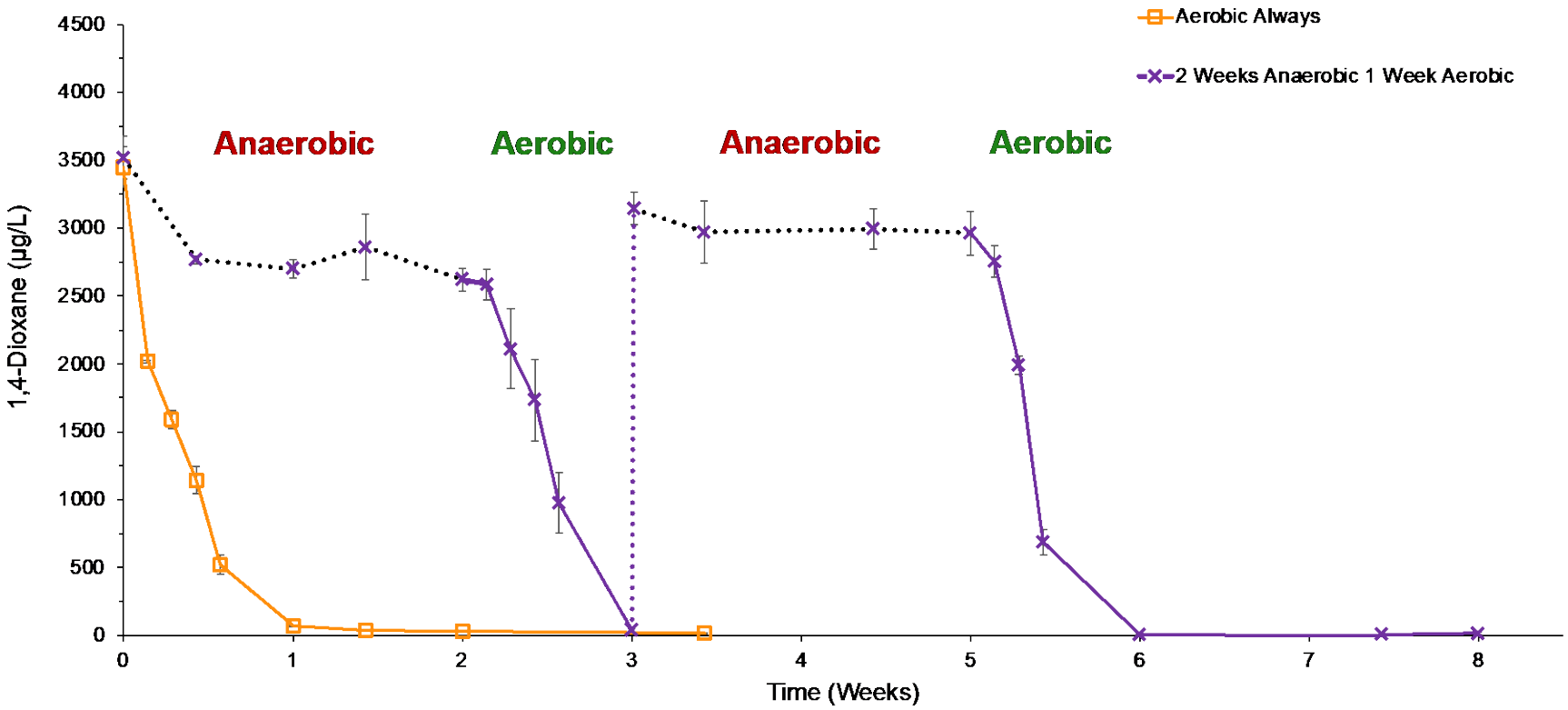
Can CB1190 Degrade 1,4-Dioxane After Multiple Low Dissolved Oxygen Periods?



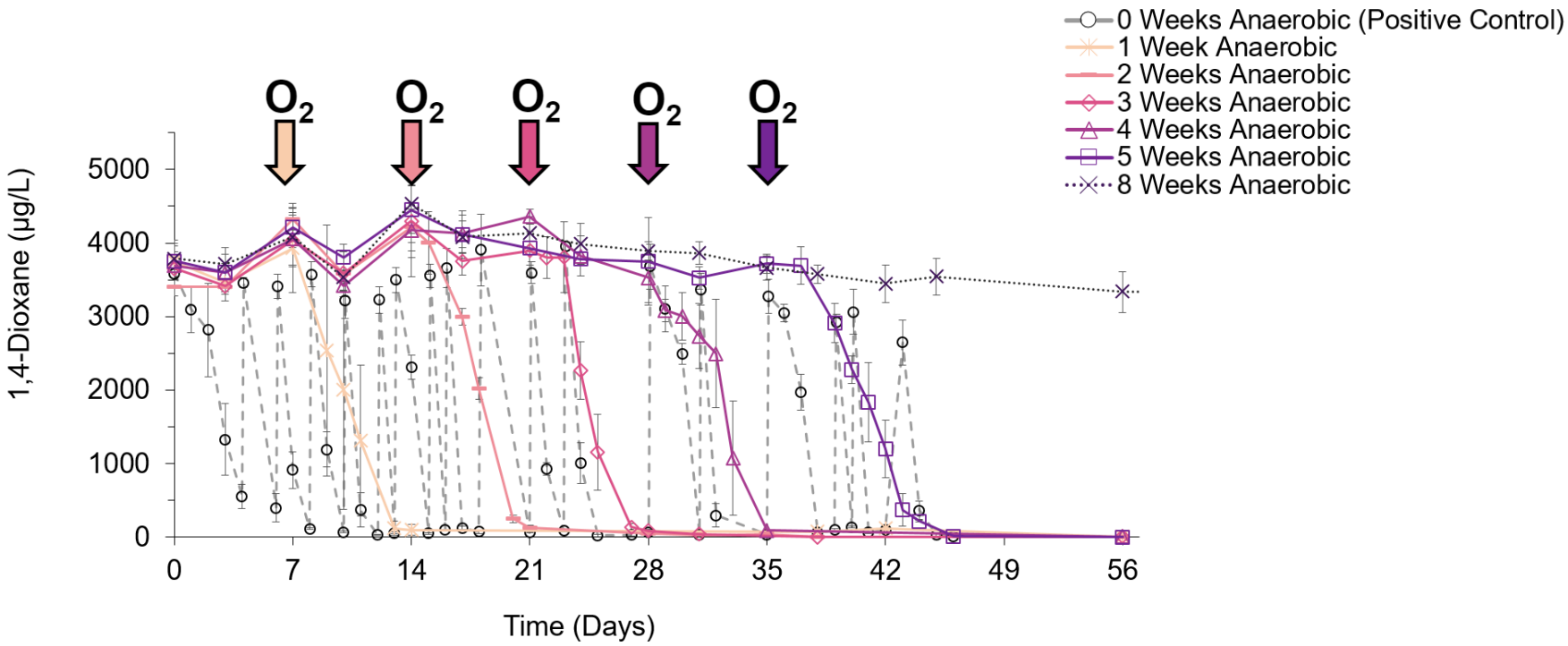
CB1190 Degrades 1,4-Dioxane After ONE Week Anaerobic Cycles



CB1190 Degrades 1,4-Dioxane After TWO Week Anaerobic Cycles

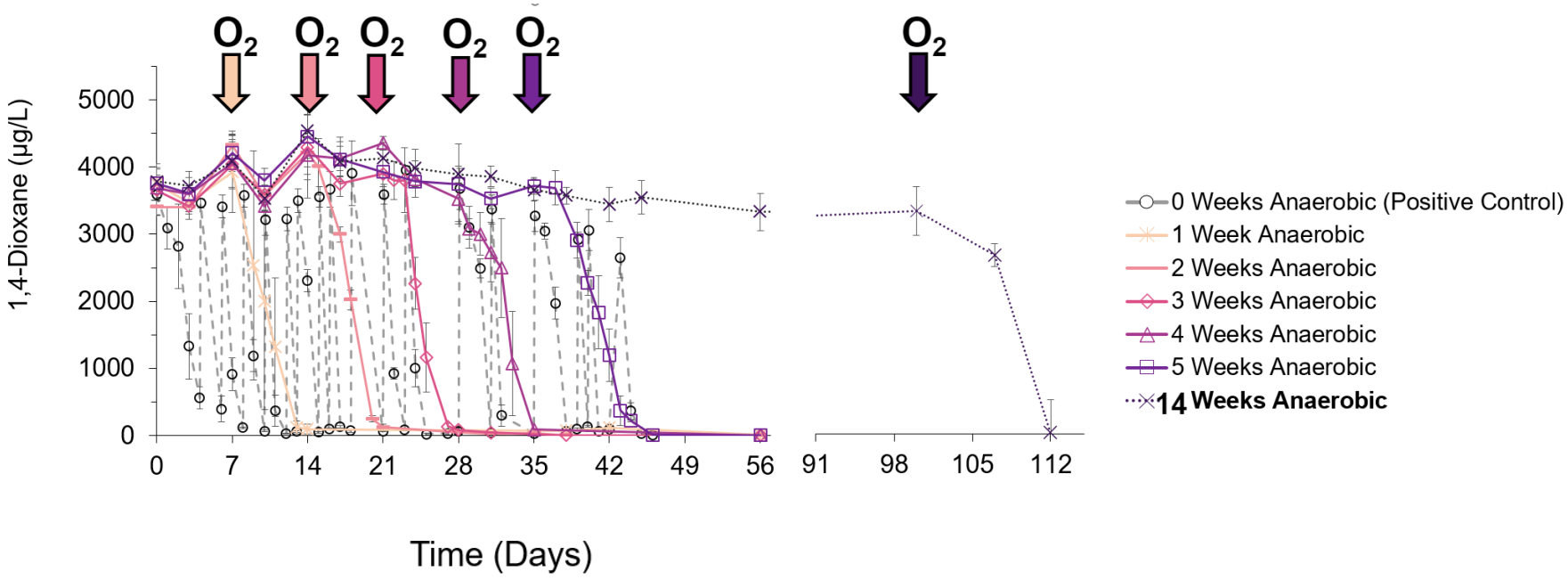


1,4-Dioxane Not Degraded in Anaerobic Bottles



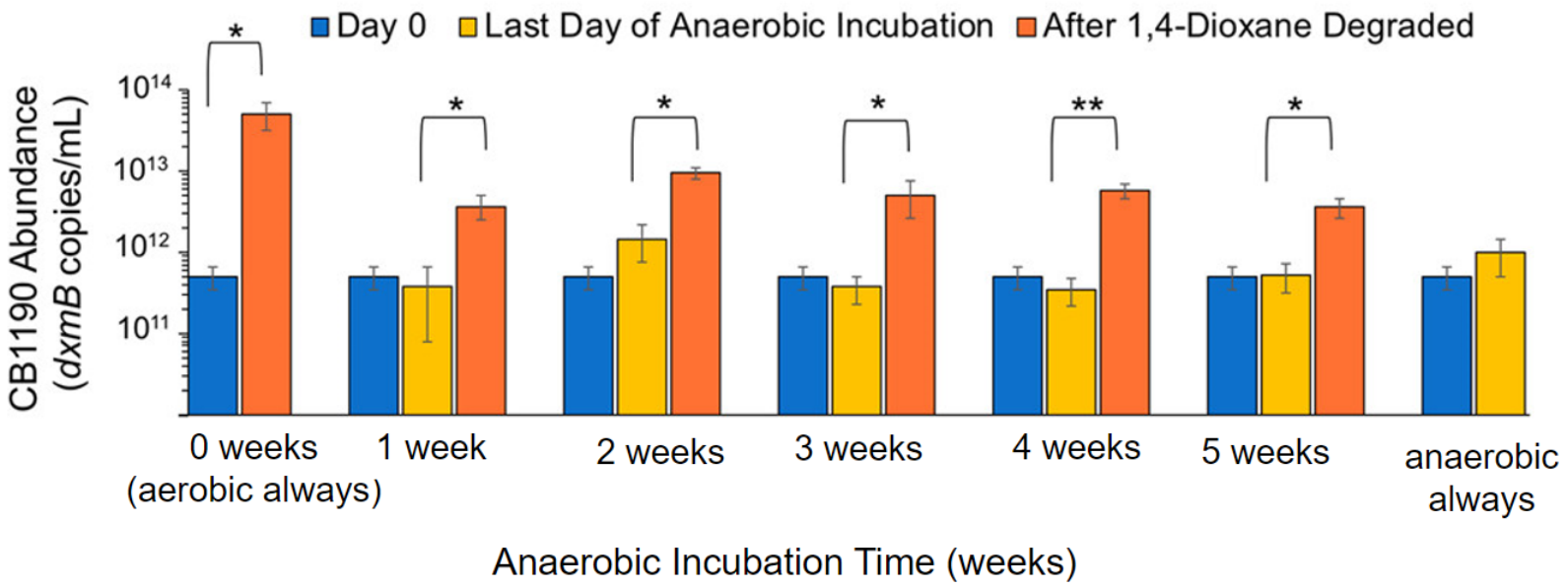
Polasko et al. *EST&L*, 2019

1,4-Dioxane Degraded After 100 Days Without Oxygen!



Polasko et al. *EST&L*, 2019

Significant Growth After Oxygen Amendments



* p-value < 0.05;** p-value < 0.01

Polasko et al. *EST&L*, 2019

Significance of Bench-Scale Tests

- CB1190 aerobically biodegrades cDCE without VC generating potential
- CB1190 aerobically biodegrades VC
- CB1190 can withstand 100 days of anaerobic incubation when conditions turn aerobic
- CB1190 biodegrades 1,4-dioxane with 3 mg/L O_2
- Monooxygenase enzymes induced in the CB1190 + KB-1® culture can biodegrade 1,4-dioxane with minimal lag

- Significance: **Engineered microbial communities can subsist under changing redox conditions and degrade contaminant mixtures**

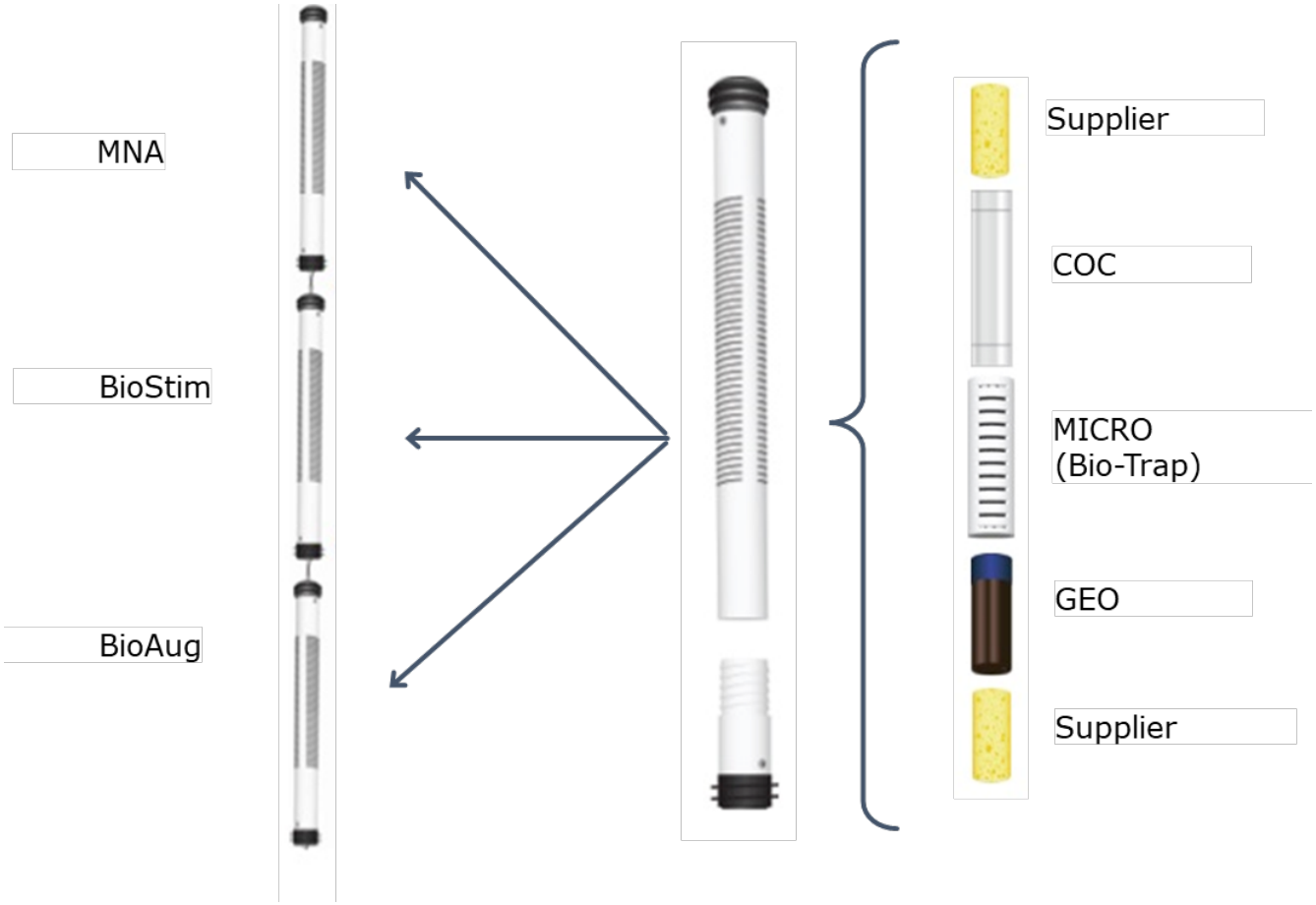
Site Background Information

- Manufacturing company in Eastern USA – >50 years ago
  - Used a variety of chlorinated solvents at facility
- Several processing areas- used chlorinated solvents as degreasing agents
- Two separate plumes: east and west
  - Eastern plume has very low or non-detectable 1,4-dioxane concentrations
  - Western plume has elevated 1,4-dioxane concentrations
- Shallow, unconfined aquifer

Groundwater Chlorinated Ethene and 1,4-Dioxane Data

Sample Location	Sample Date	PCE	TCE	cis-1,2-DCE	trans-1,2-DCE	Vinyl Chloride	1,1-DCE	1,4-Dioxane
Screen	Micrograms per Liter							
Standards		5	5	70	100	2	7	32
MW-31	11/19/2015	3,000	4,900	1,400	5.8	36	2,800	5,400
	6/10/2016	2,100	3,300	860	4.3	20	1,700	4,300
MW-32	11/19/2015	3,000	3,900	3,100	6.9	110	2,300	1,800
	6/10/2016	2,000	2,400	2,100	9.1	55	1,400	1,900

In Situ Microcosm Study



Bio-Trap Testing for 1,4-Dioxane Key Genes

Client Sample ID:	MW-30	MW-31	MW-32
Dioxane Monooxygenase DXMO	<5.10E+00	1.00E-01 (J)	<5.00E+00
Aldehyde Dehydrogenase ALDH	<5.10E+00	<5.10E+00	<5.00E+00

CSIA Results in Flow Path Wells

- No significant change in CSIA values down gradient
  - No clear indication of 1,4-dioxane degradation
  - Plume appears fairly uniform

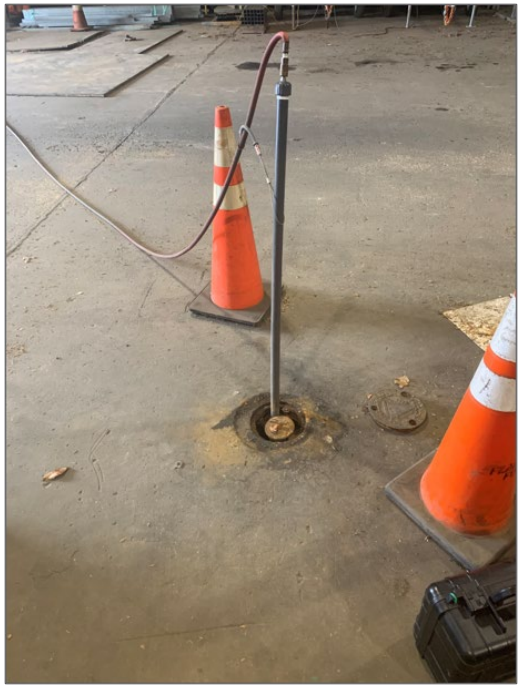
Isotope	Monitoring Well		
	MW-30	MW-31	MW-32
$\delta^{13}C$ (‰, VPDB)	-31.1	-30.8	-30.6
$\delta^{2}H$ (‰, VSMOW)	-48	-51	-47

In Situ Microcosm Bio-Trap Results

Client Sample ID:	Units	MW-32 MNA	MW-31 BioAug	MW-30 BioAug+ORC+Osmo	MW-30 BioAug+Osmo
CSIA of 1,4-dioxane Carbon	$\delta^{13}C$ (‰, VPDB)	-30.6	-29.3	-26.4	-23.8

Gene Targets	Units	MW-32 MNA	MW-31 BioAug	MW-30 BioAug+ORC+Osmo	MW-30 BioAug+Osmo
Dioxane Monooxygenase (DXMO)	Cells/bead	<2.5E+02	1.71E+05	1.53E+04	3.39E+05
Aldehyde Dehydrogenase (ALDH)	Cells/bead	<2.5E+02	1.36E+05	1.14E+04	2.27E+05

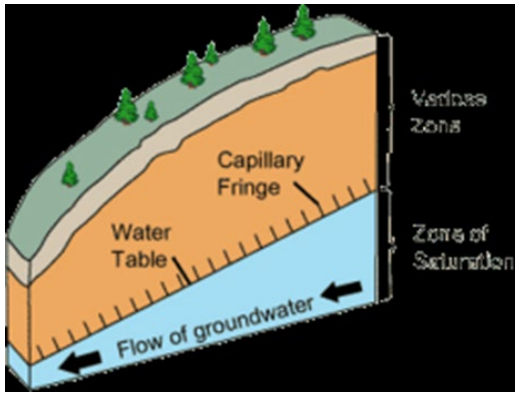
Let’s Go To The Field!



CB1190

Site Hydrogeology

- Two distinct water-bearing zones, separated by siltstone and shale layers
- Groundwater in the shallow, unconfined aquifer occurs from 4 to 9 feet below ground surface (bgs)
- Second water-bearing zone at depth of approximately 118 to 152 feet bgs
- Contaminants identified in shallow groundwater aquifer are NOT observed in deep aquifer monitoring wells



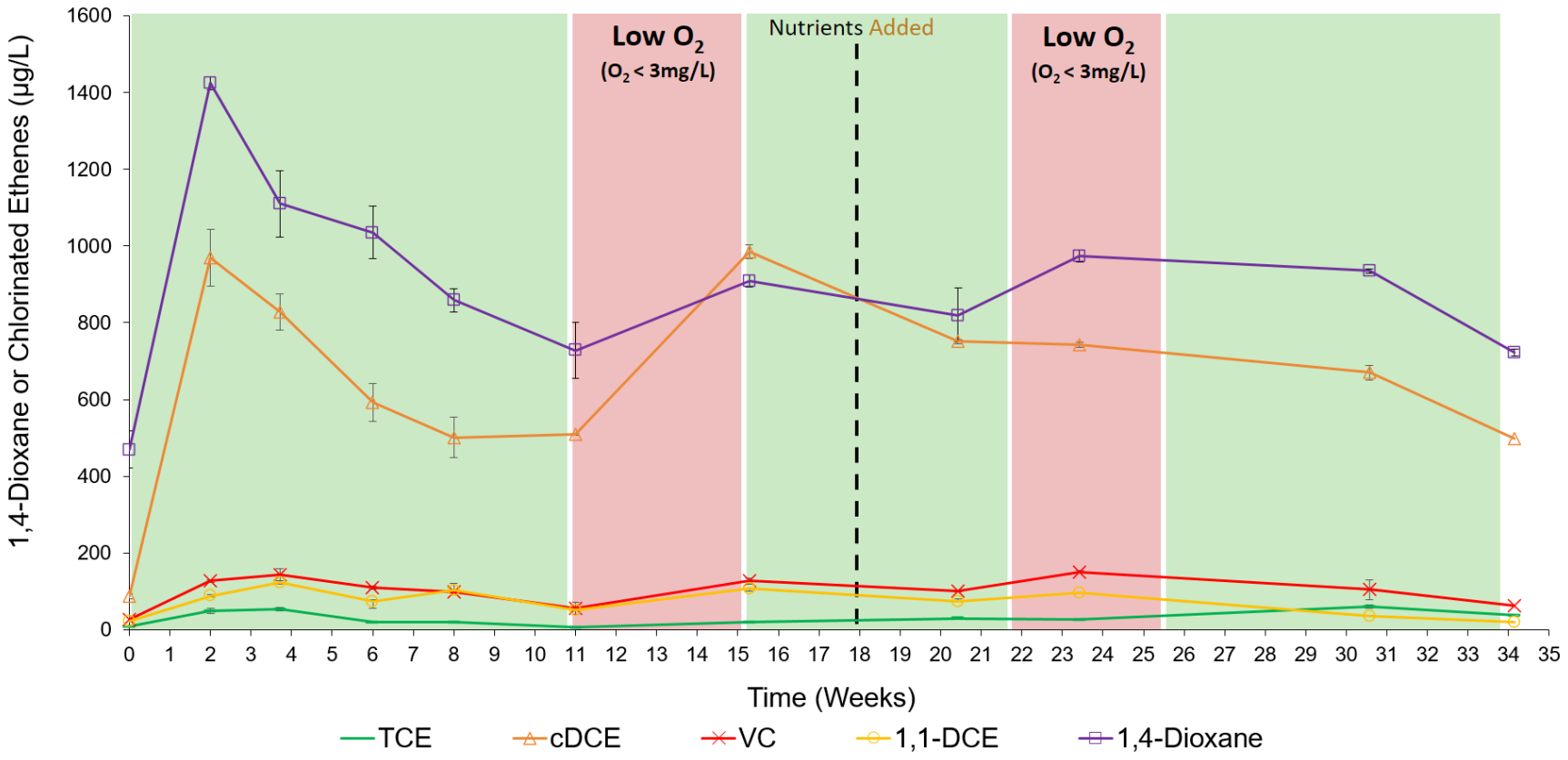
Field Site: Electron Acceptor & Nutrient Data

- Groundwater Data:
  - Dissolved Oxygen (DO): < 0.5 – 4.3 milligrams per liter (mg/L)
  - Nitrate: 0.6 – 1.9 mg/L
  - Nitrite: < 0.03 mg/L
  - Sulfate: 23 – 32 mg/L
  - TOC: 0.3 – 0.9 mg/L
  - Total Kjeldahl Nitrogen: ≤ 0.1 mg/L
  - Ammonia: 0.08 – 0.1 mg/L
  - Phosphorus: 0.1 – 0.2 mg/L

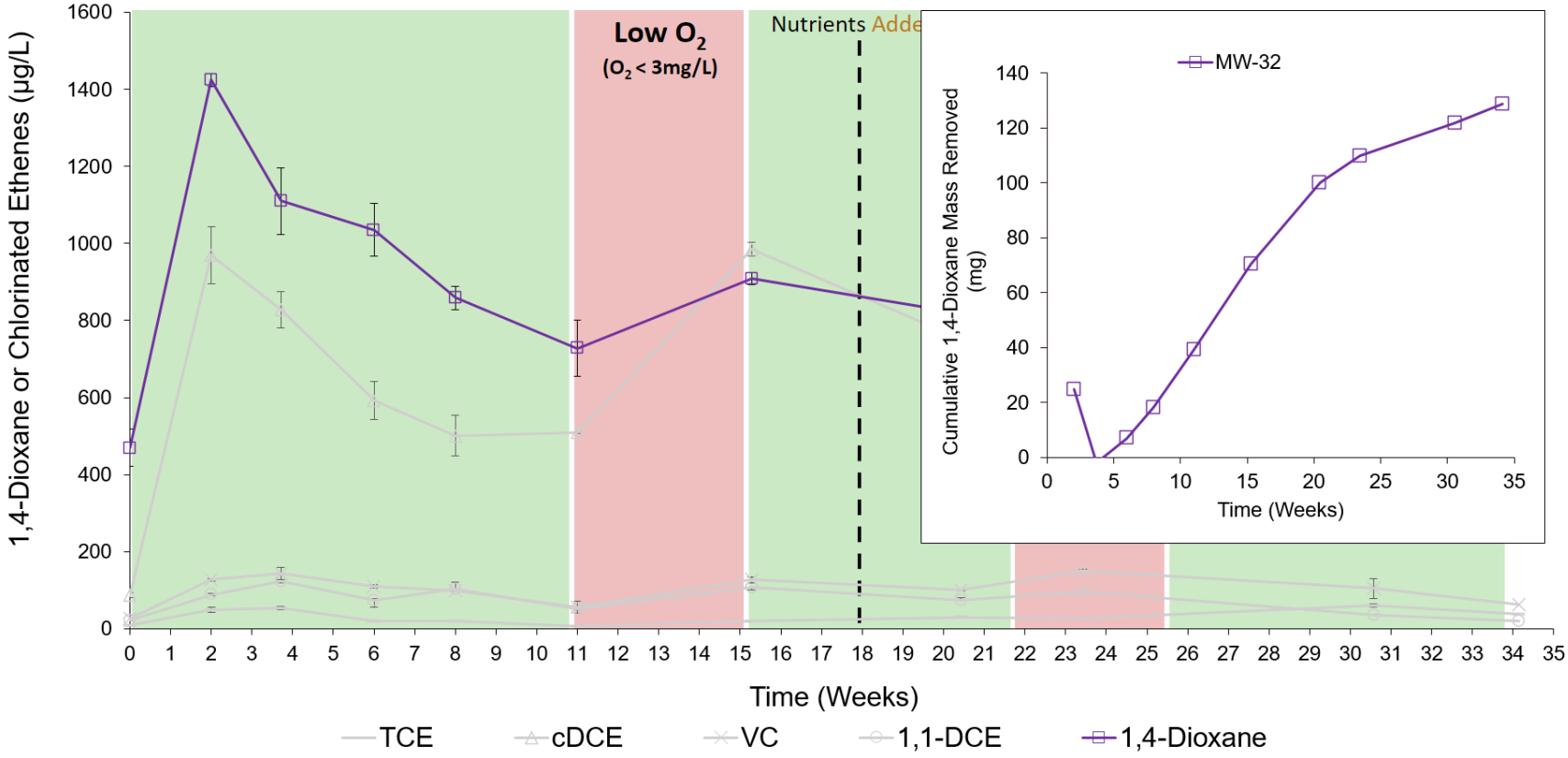
Field Site: Chlorinated Volatile Organic Compounds & 1,4-Dioxane Data Post ERD & Excavation

Sample Location	Sample Date	1,1,1-TCA	1,1-DCE	PCE	TCE	cis-1,2-DCE	Trans-1,2-DCE	Vinyl Chloride	1,4-Dioxane
Screen	Micrograms per Liter								
Standard		200	7	5	5	70	100	2	32
MW-31	2nd Quarter 2016	190	1,700	2,100	3,300	860	4.3	20	4,300
MW-31	1st Quarter 2020	29	200	72	170	4,100	13	240	1,400
MW-32	2nd Quarter 2016	600	1,400	2,000	2,400	2,100	9.1	55	1,900
MW-32	1st Quarter 2020	200	1,100	210	150	5,300	8.4	840	1,600

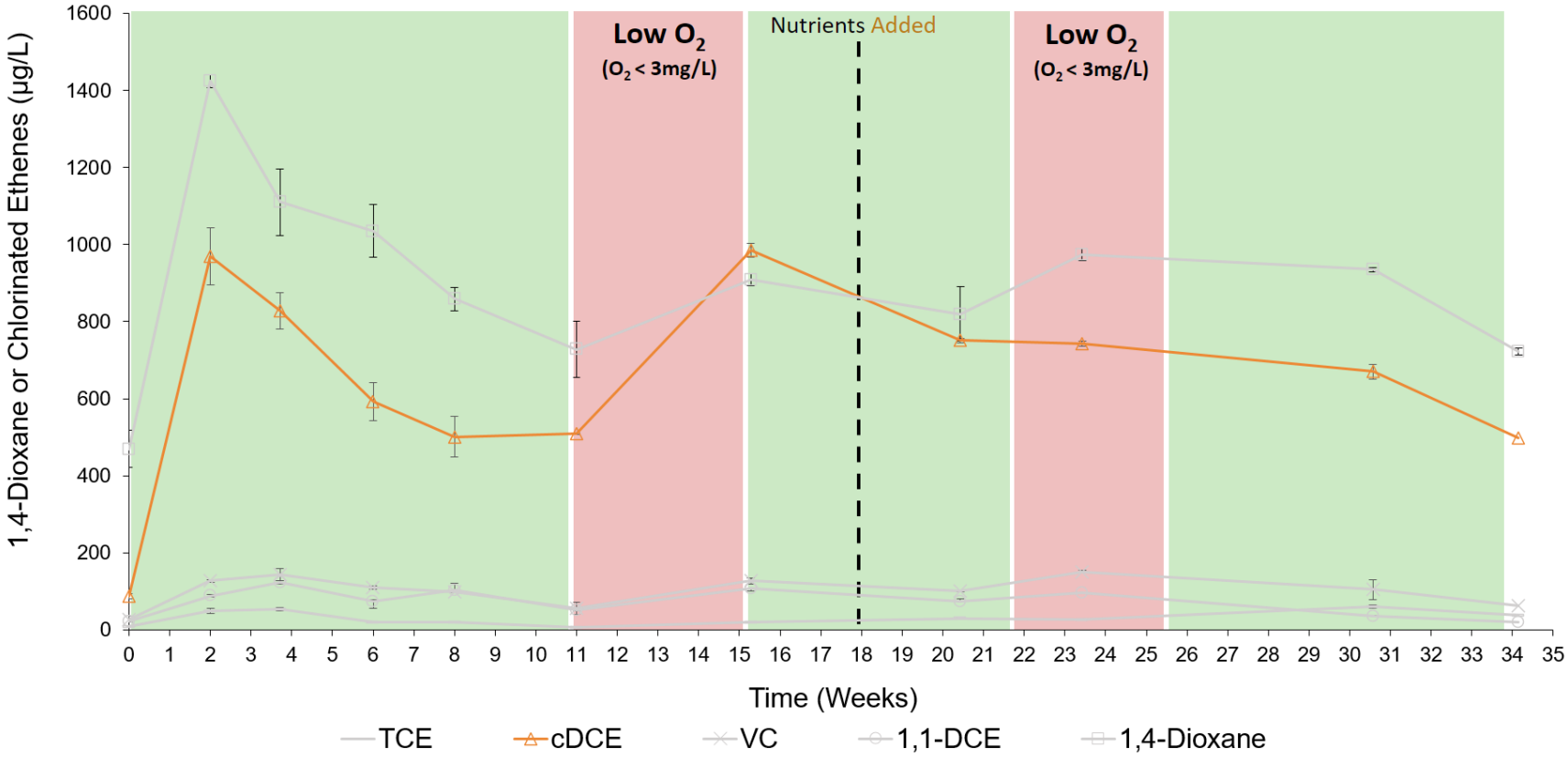
MW-32 CB1190 Bioaugmented: Biodegradation Driven by O_2 & Nutrients



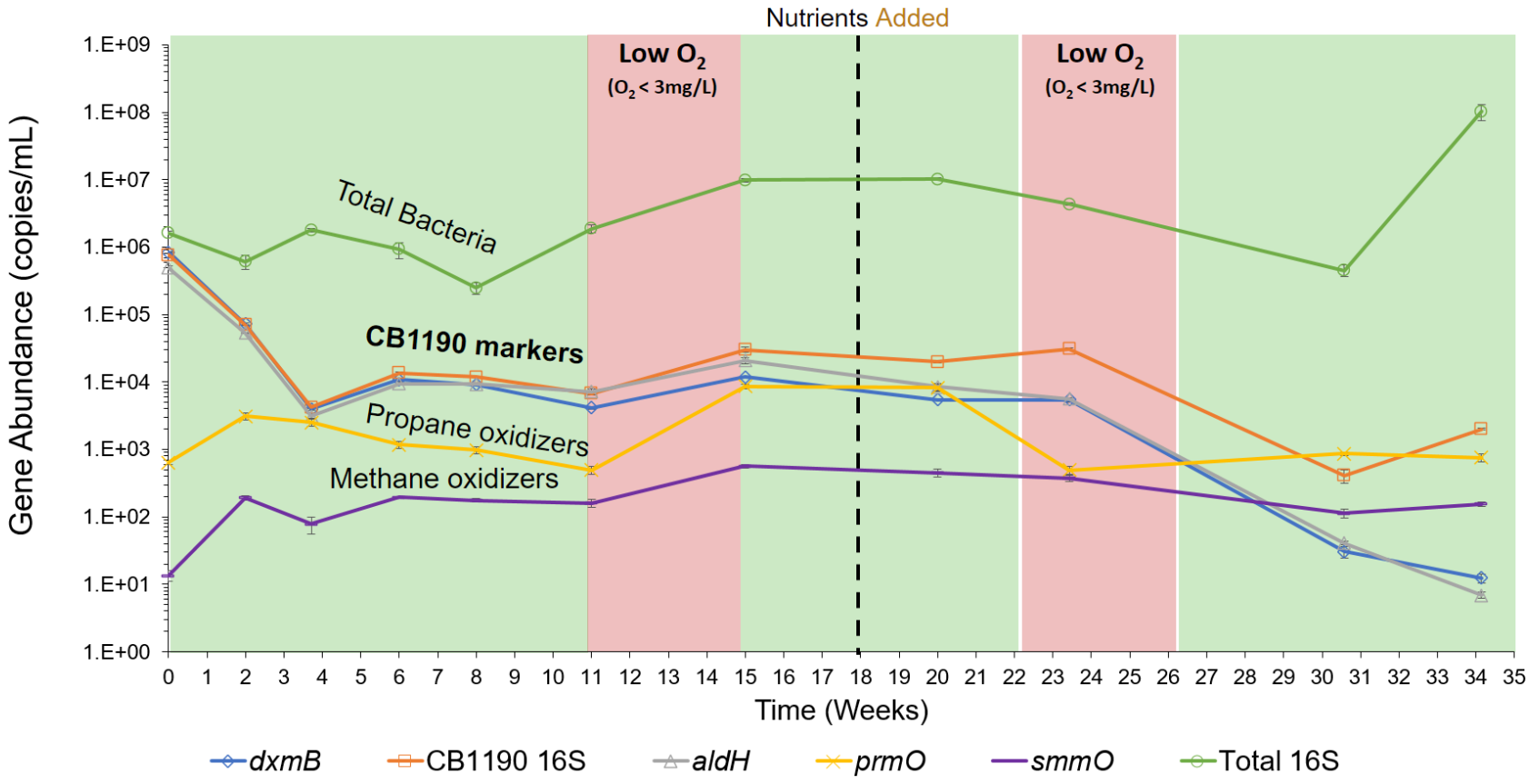
MW-32: 1,4-Dioxane Biodegradation Driven by O_2 & Nutrients



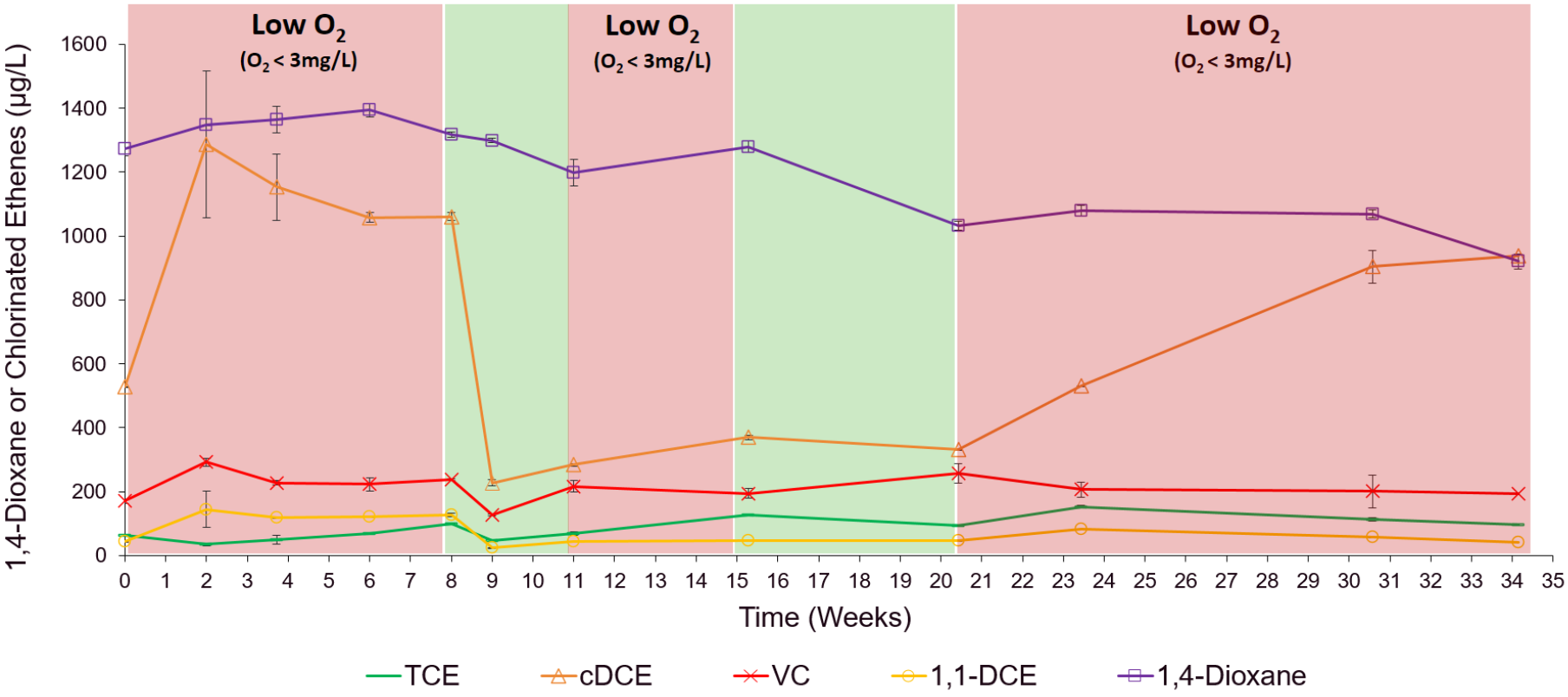
MW-32: cDCE Biodegradation Driven by O_2 & Nutrients



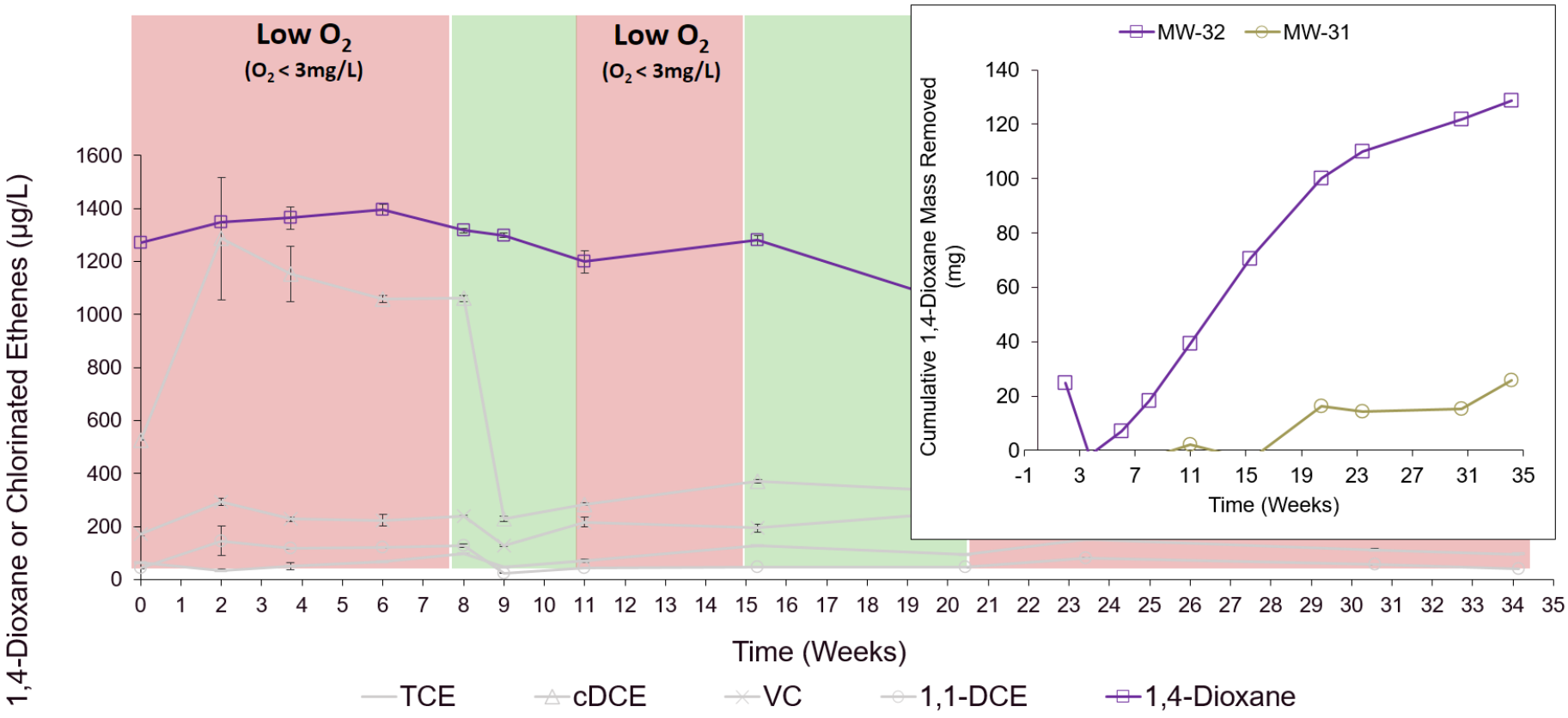
MW-32: *prm*O and *smm*O Biomarkers Present, but Below CB1190 Markers



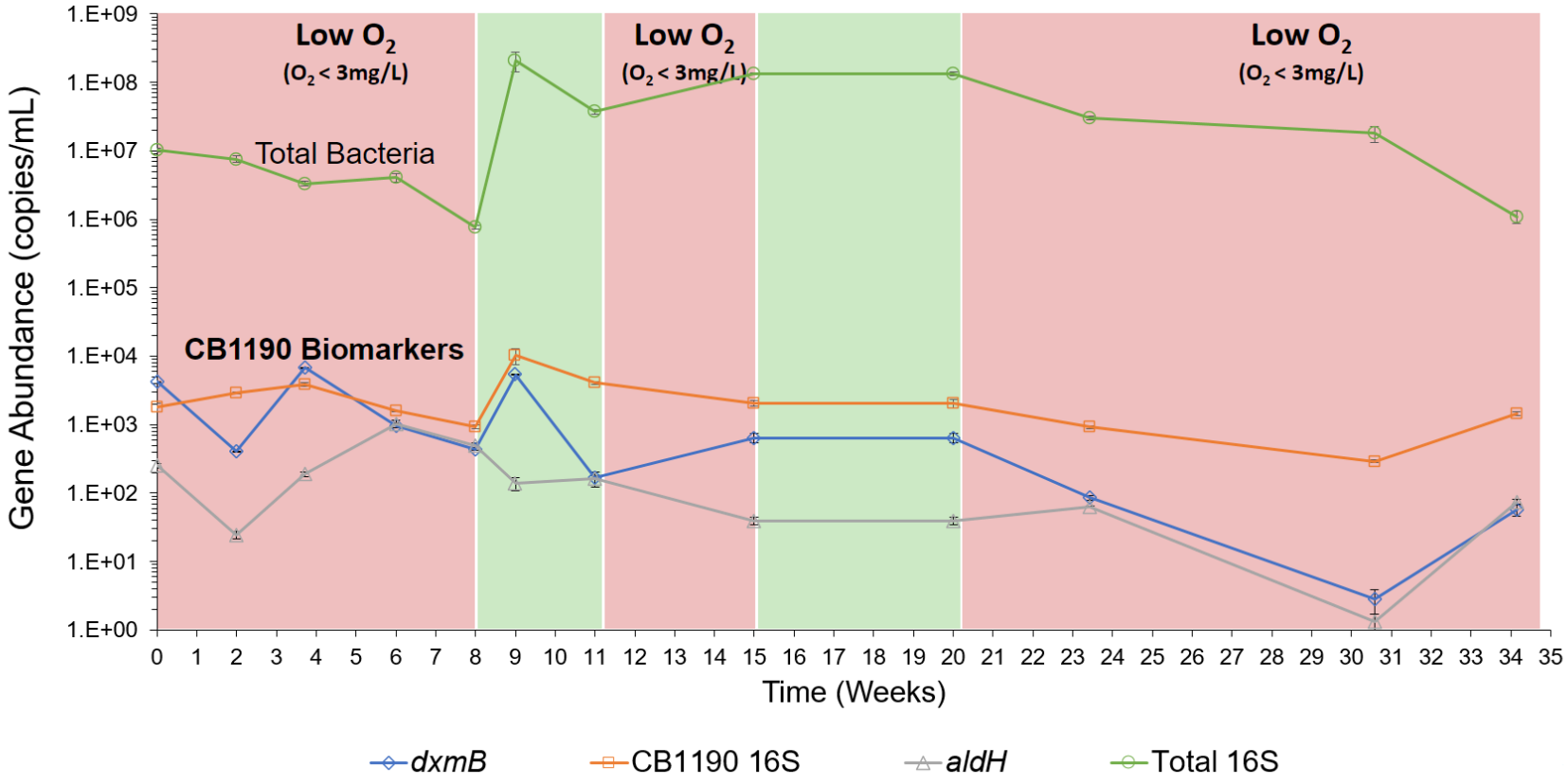
MW-31 (Unaugmented Well): Sparging Drives CVOC Changes



MW-31: Dioxane Decreased Over Time, But Not as Much as MW-32



MW-31: Sparging System Increases Community's Abundance



Post CB1190 Bioaugmentation & Biostimulation in MW-31 & MW-32 Areas

Sample Location	Sample Date	1,1-DCE	PCE	TCE	Cis-1,2-DCE	Trans-1,2-DCE	Vinyl Chloride	1,4-Dioxane
Screen	Micrograms per Liter							
Standard		7	5	5	70	100	2	32
MW-31	1st Quarter 2020	200	72	170	4,100	13	240	1,400
	3rd Quarter 2022	330	38	270	3,400	18	270	240
MW-32	1st Quarter 2020	1,100	210	150	5,300	8.4	840	1,600
	3rd Quarter 2022	36	2.2	15	350	2.7	23	280

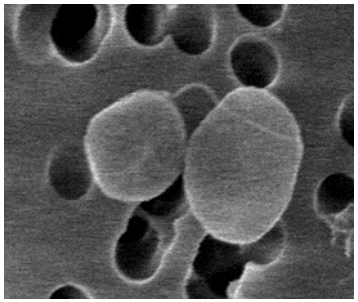
Summary and Significance

SEM image
CB1190

- Less than one year post biostimulation and bioaugmentation, 1,4-dioxane and cis-1,2-DCE decreased by 49% in MW-32.
- After the 2nd CB1190 injection, there was more than an 80% reduction in 1,4-dioxane and more than a 90% reduction in cis-1,2-DCE and VC in MW-32.
- In MW-32, dissolved O_2 and nutrients appeared to be driving factors for biodegradation of CVOCs & 1,4-dioxane.
- Significance: **CB1190 can be an efficient microbe at removing 1,4-dioxane & less chlorinated CVOCs *in situ*!**

Mahendra & Alvarez-Cohen, 2005, *IJSEM*

Thank You & Questions



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