In Situ Bioremediation of Chlorinated Solvents and 1,4-Dioxane in Groundwater

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1,4-Dioxane Often Found with Chlorinated Solvents

Chloroethenes:

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

And others:

- 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

Richard H Anderson,† Janet K Anderson,† and Paul A Bower‡*

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‡Contractor, Air Force Center for Engineering and the Environment, Environmental Restoration Branch (AFCEE/ERD), Lackland AFB, Texas, USA

Environmental Science & Technology LETTERS

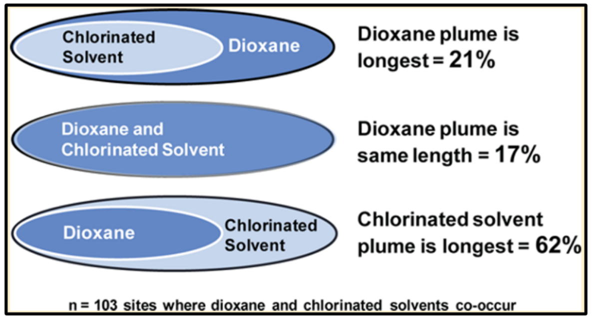
A Multisite Survey To Identify the Scale of the 1,4-Dioxane Problem at Contaminated Groundwater Sites

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

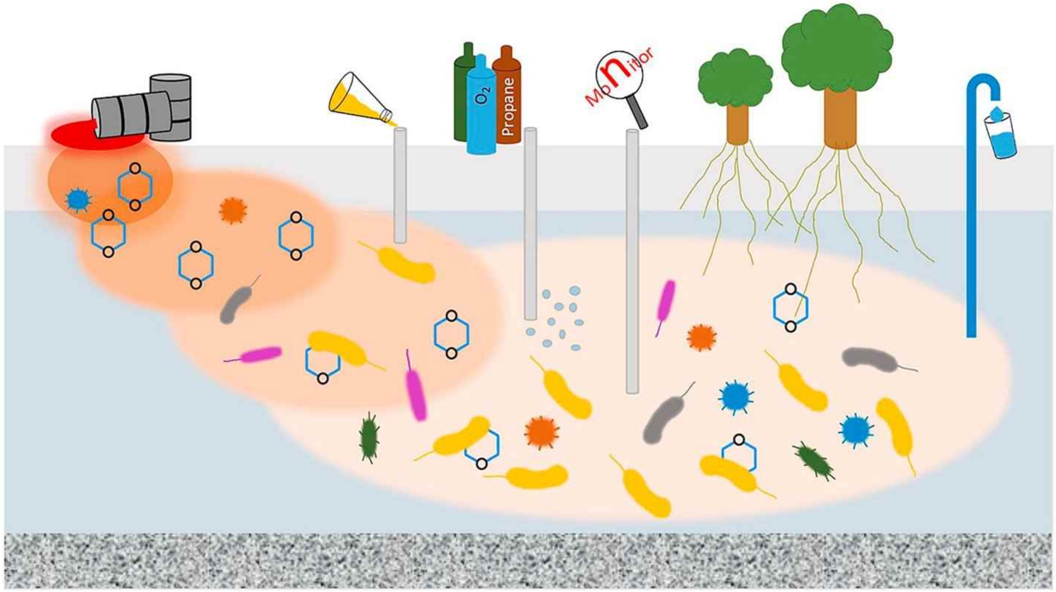
Environmental Science & Technology

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

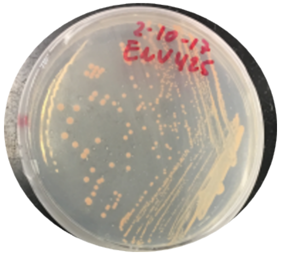
David T. Adamson,[*,†] R. Hunter Anderson,[‡] Shaily Mahendra,[§] and Charles J. Newell[†]



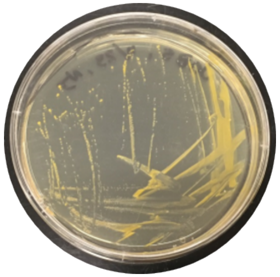
Luckily, Microbes Can Biodegrade 1,4-Dioxane & Chlorinated Ethenes



Pseudonocardia dioxanivorans CB1190

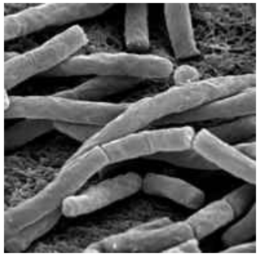


Rhodococcus ruber ENV425

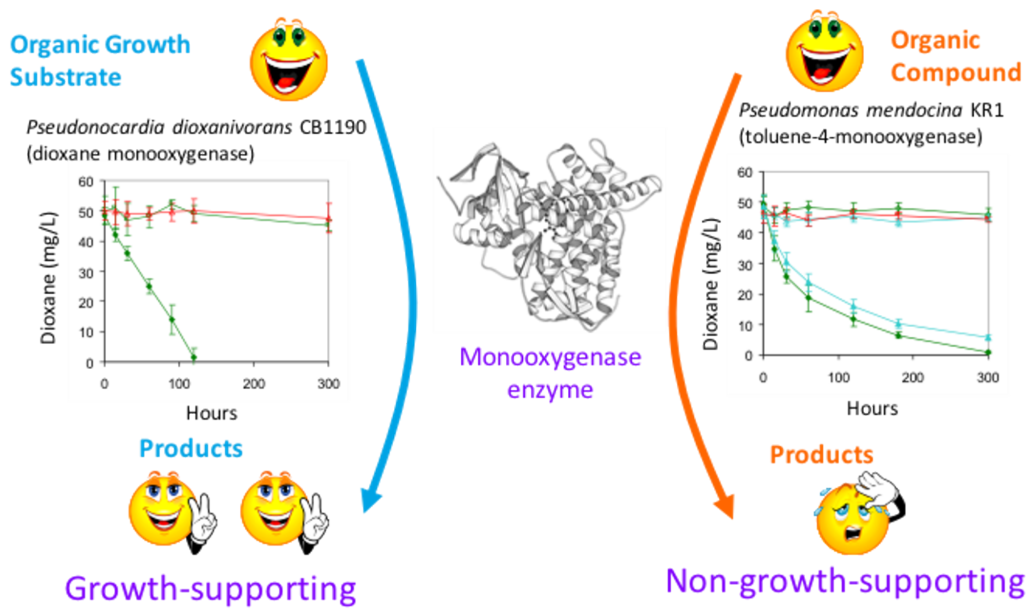


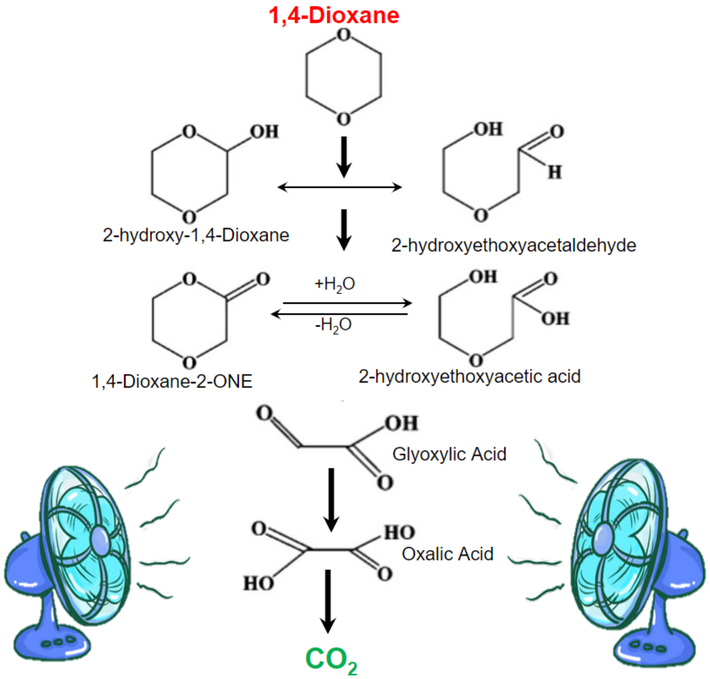
Methylosinus trichosporium OB3b

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

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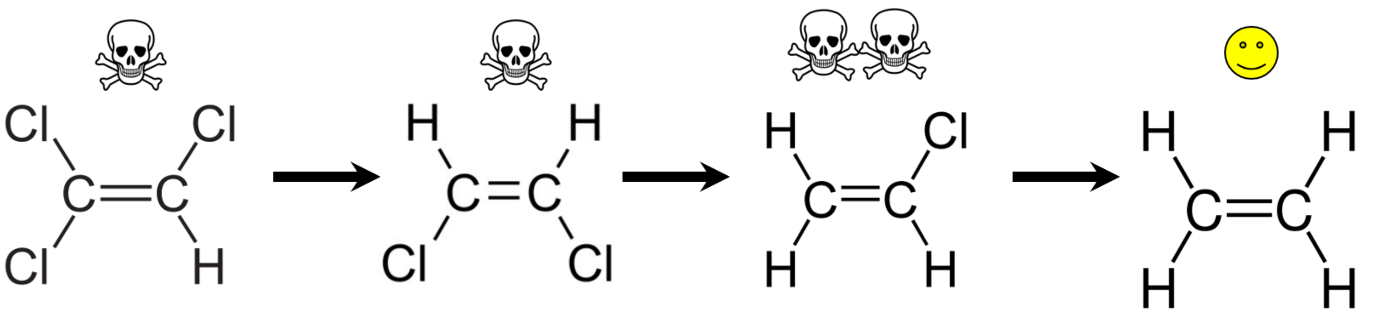
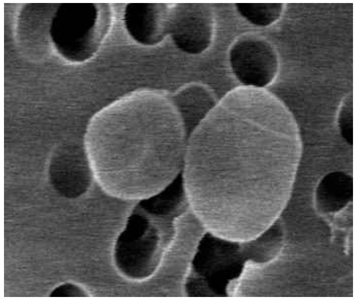
Dehalococcoides (Dhc) <u>Anaerobically</u> Biodegrades TCE

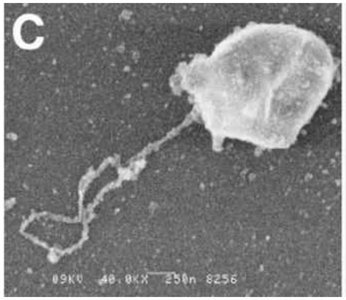


He et al. *Nature*. 2003; Vogel and McCarty. *Environ. Microbiol.* 1985

trichloroethene (TCE) → **cis-1,2-dichloroethene (cDCE)** → **vinyl chloride (VC)** → **ethene**

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





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

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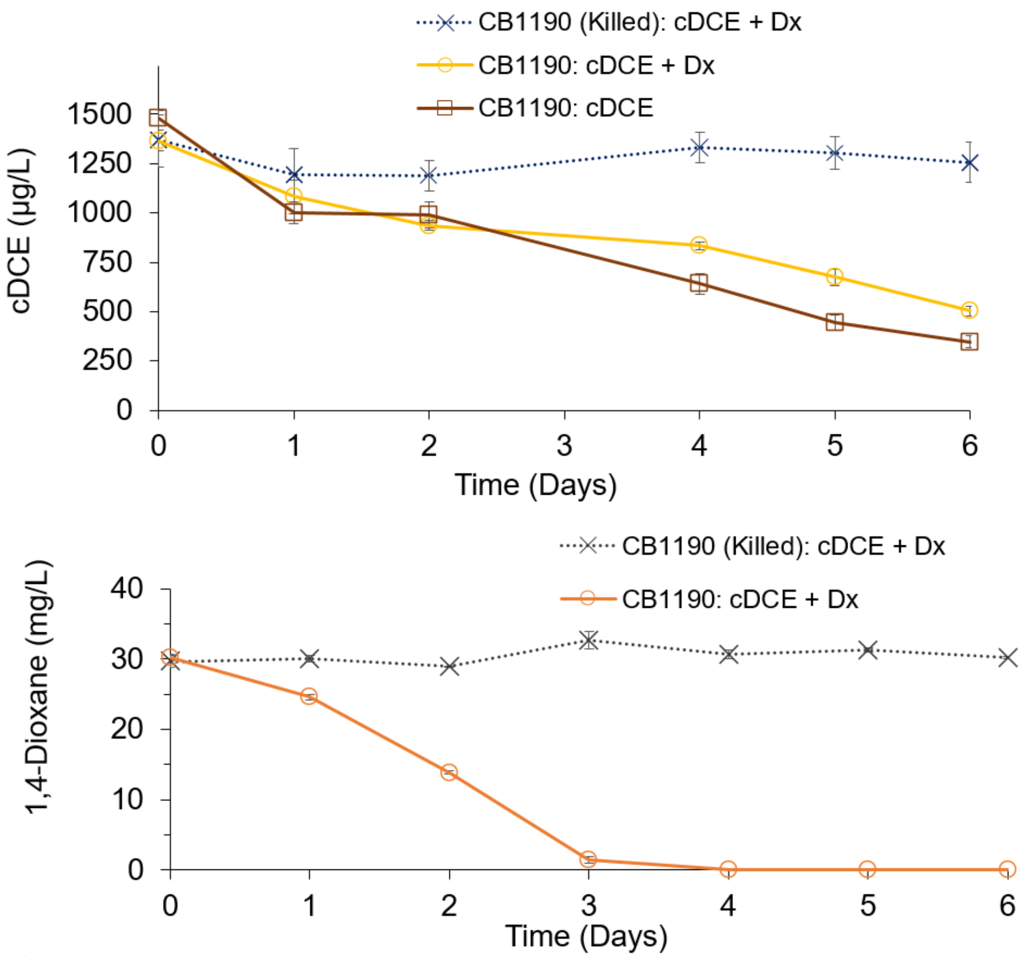
Good News/Bad News

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

Good News/Good News

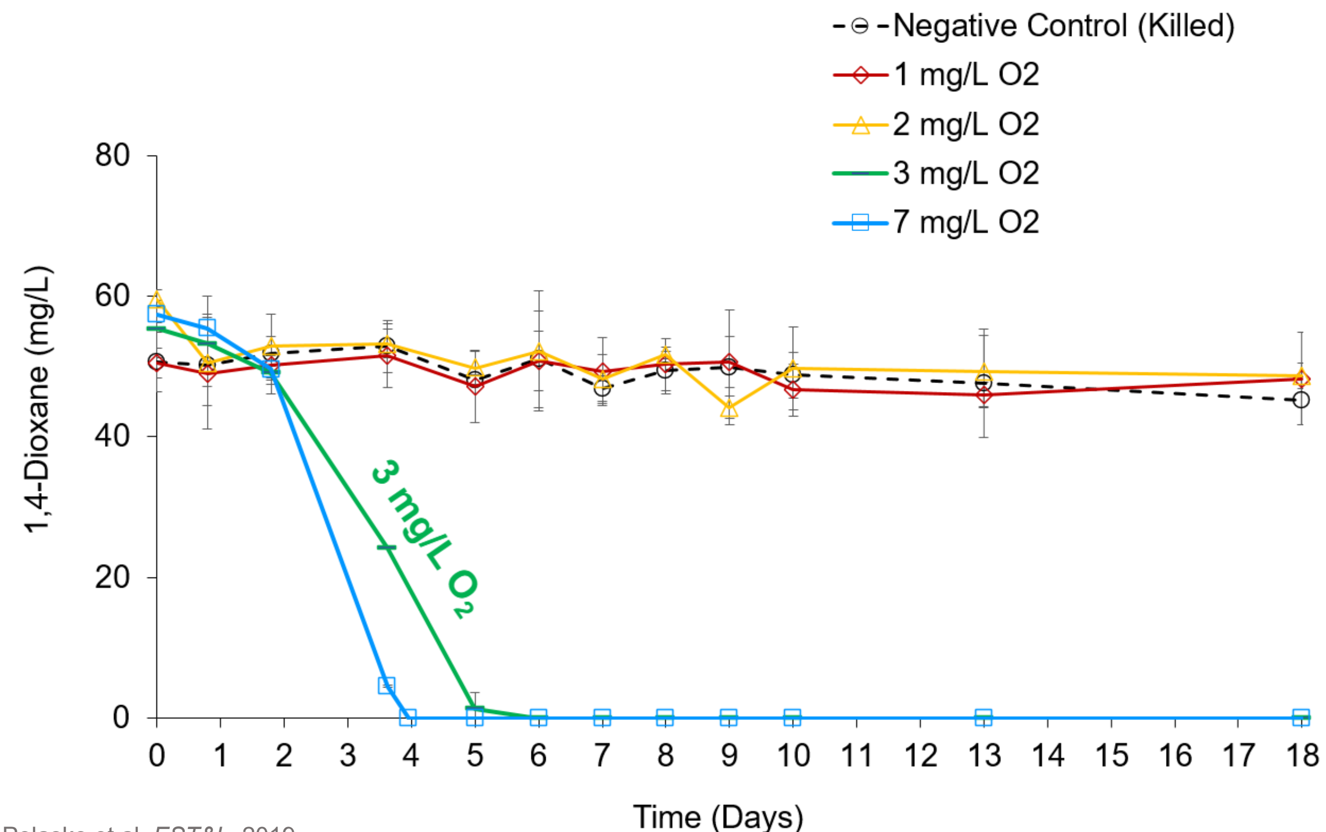
- Good News: TCE and 1,4-Dioxane Biodegradable
- ~~Bad~~ Good News: Combine Anaerobes and Aerobes

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



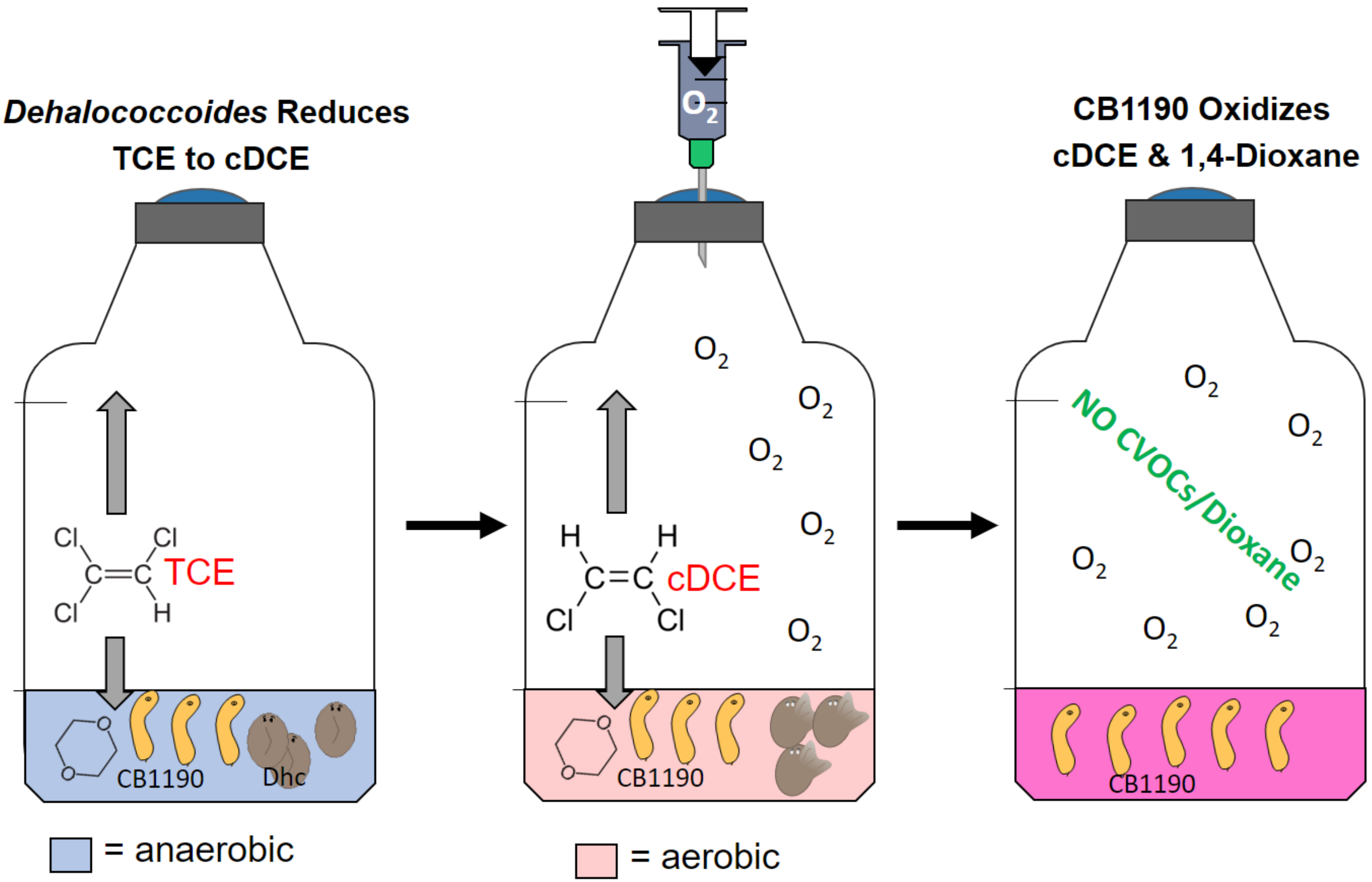
Polasko et al. *EST&L*, 2019

CB1190 Degrades 1,4-Dioxane with 3 mg/L O2

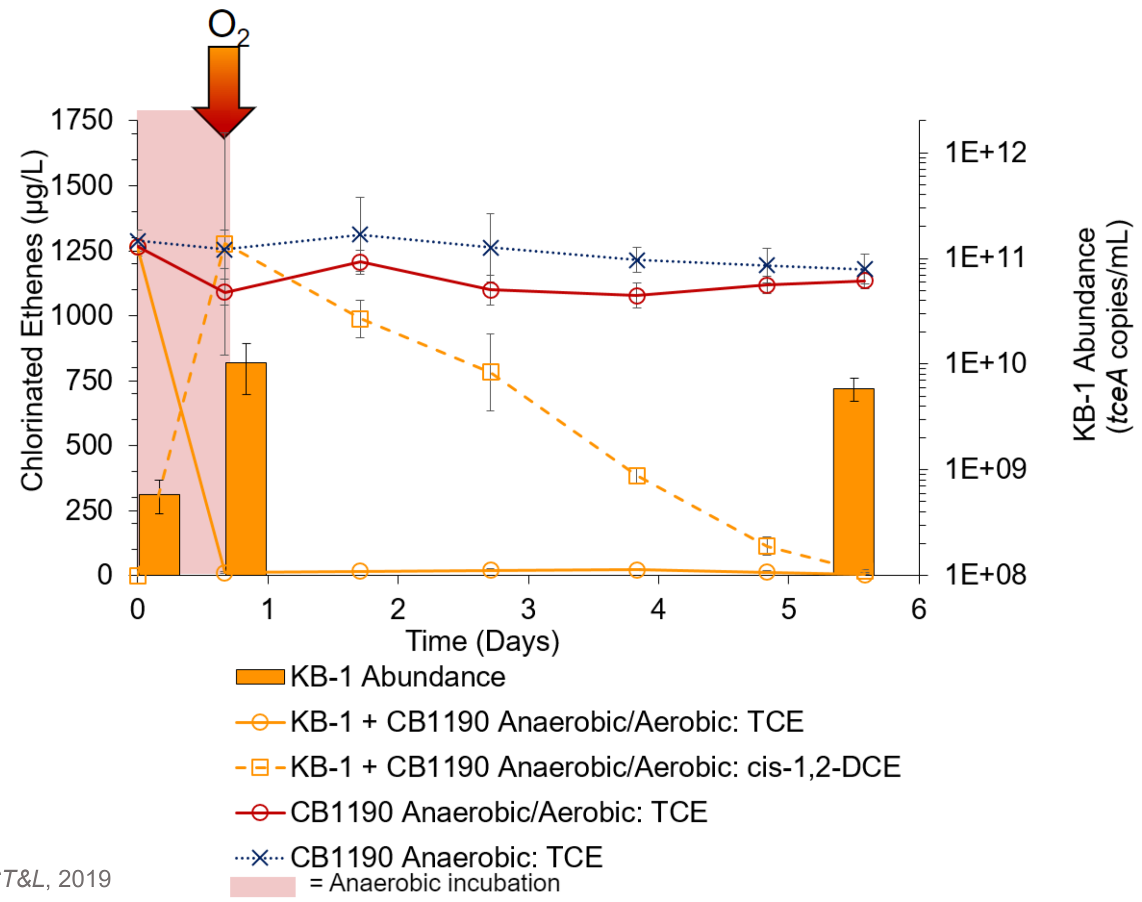


Polasko et al. *EST&L*, 2019

Engineered Microbial Community



Dhc Degrades TCE → cDCE; CB1190 Degrades cDCE



Polasko et al. *EST&L*, 2019

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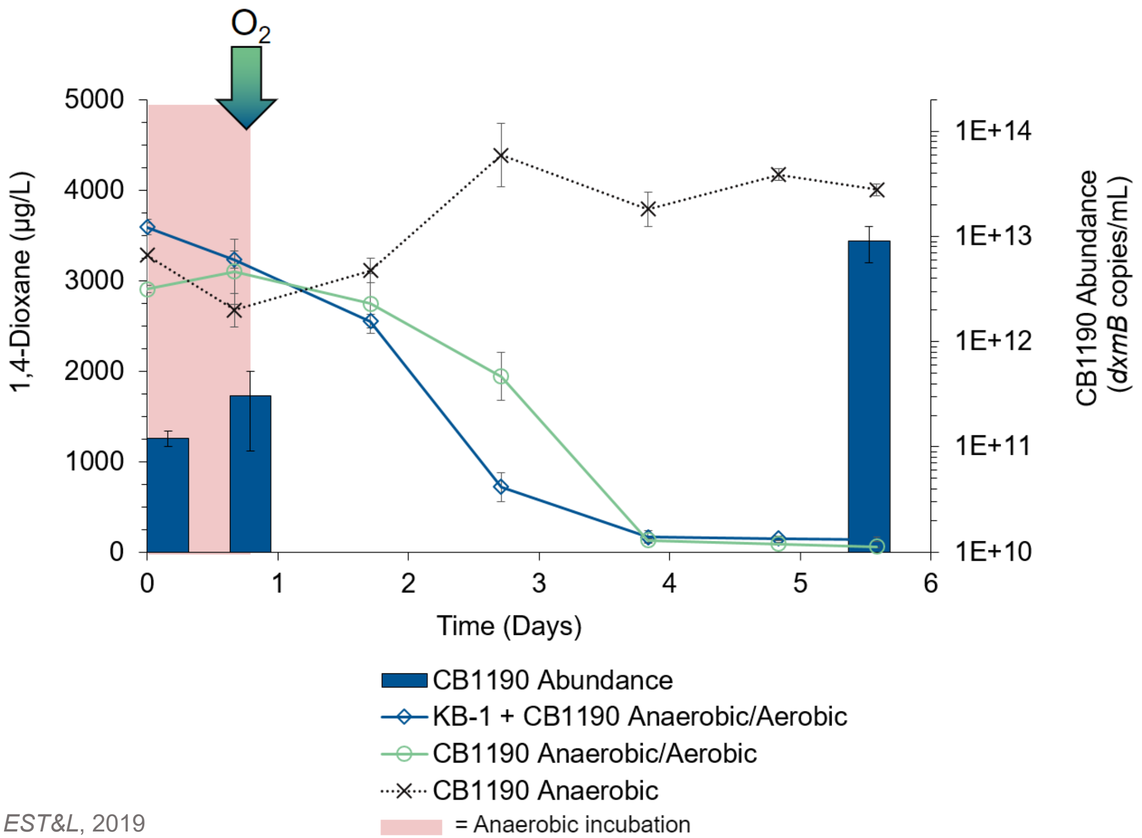
Mixed Culture & Strain CB1190 Degrades 1,4-Dioxane



Polasko et al. *EST&L*, 2019

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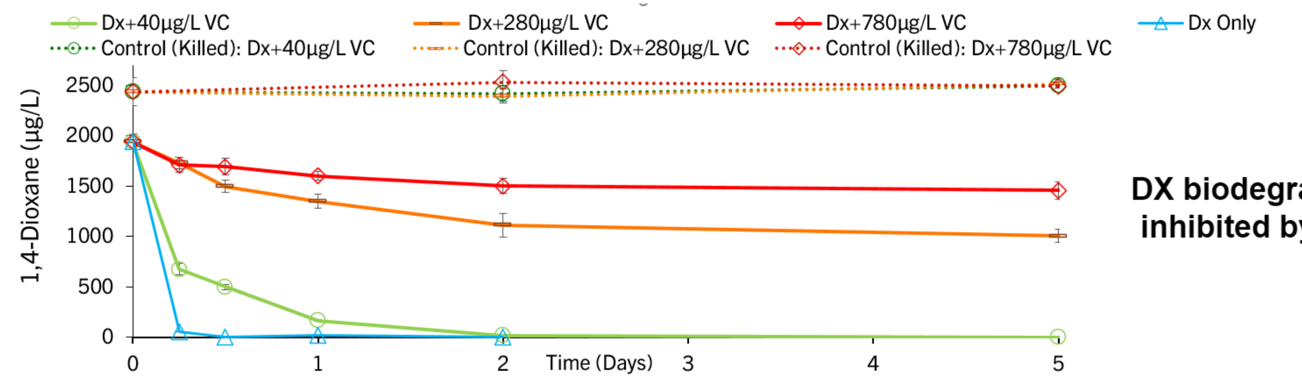
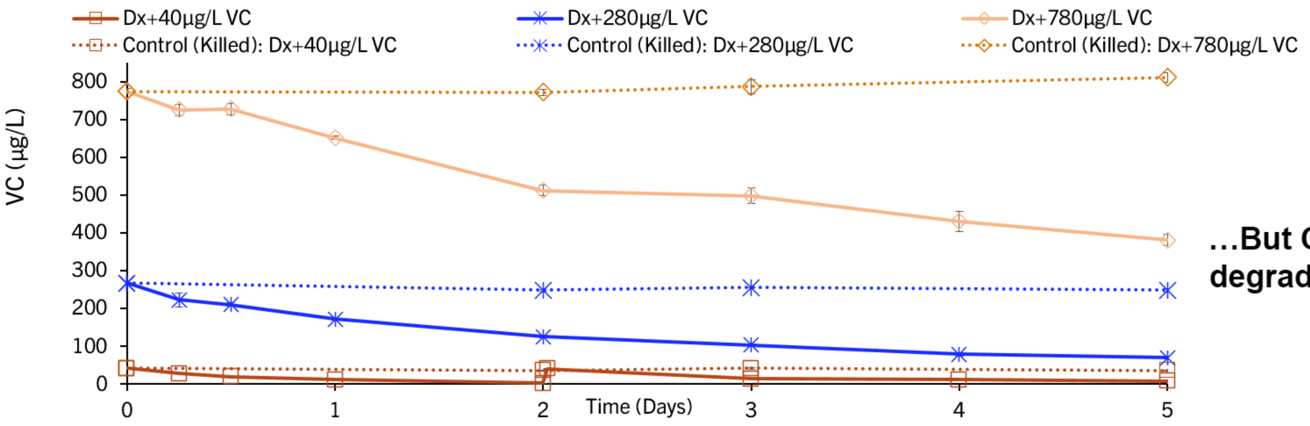
CB1190 Biodegrades VC AND Dioxane Simultaneously



DX biodegradation inhibited by VC…



…But CB1190 can degrade inhibitor!

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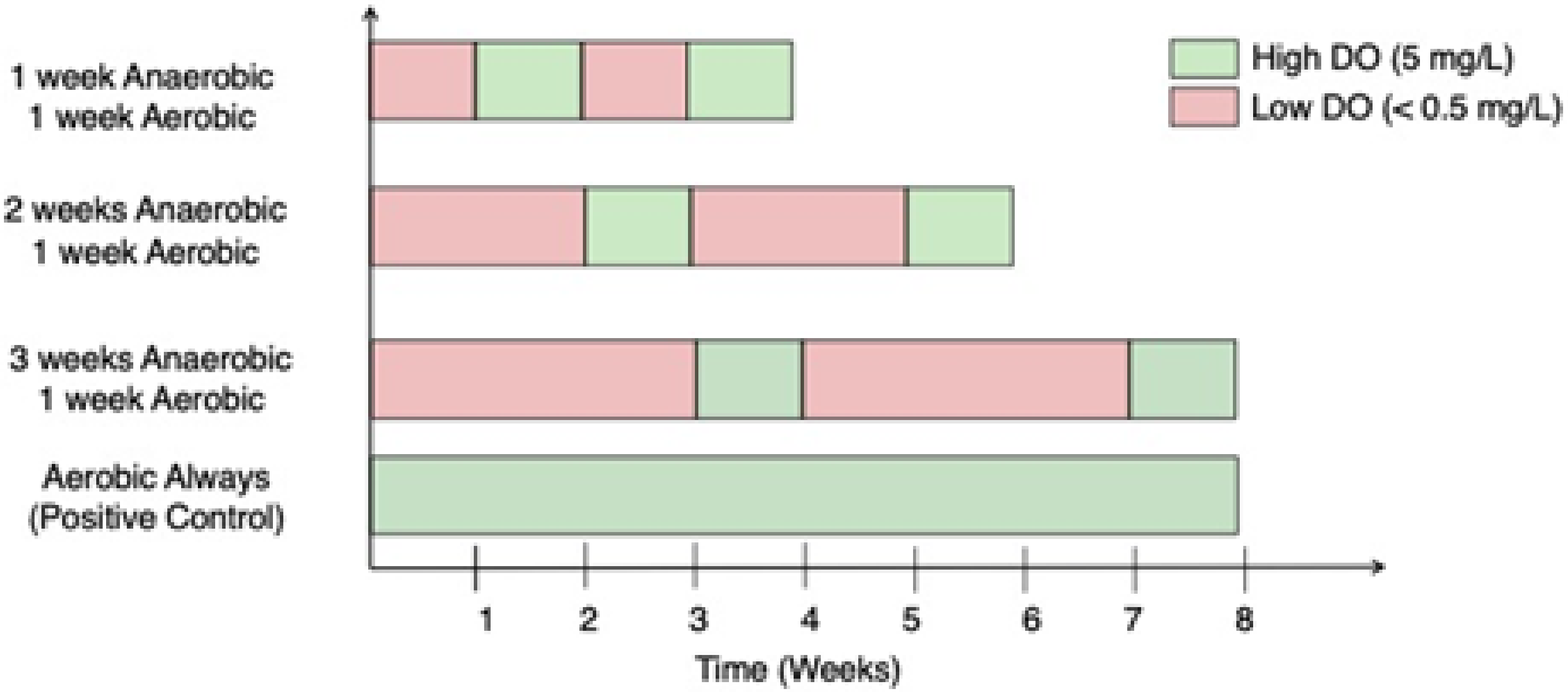
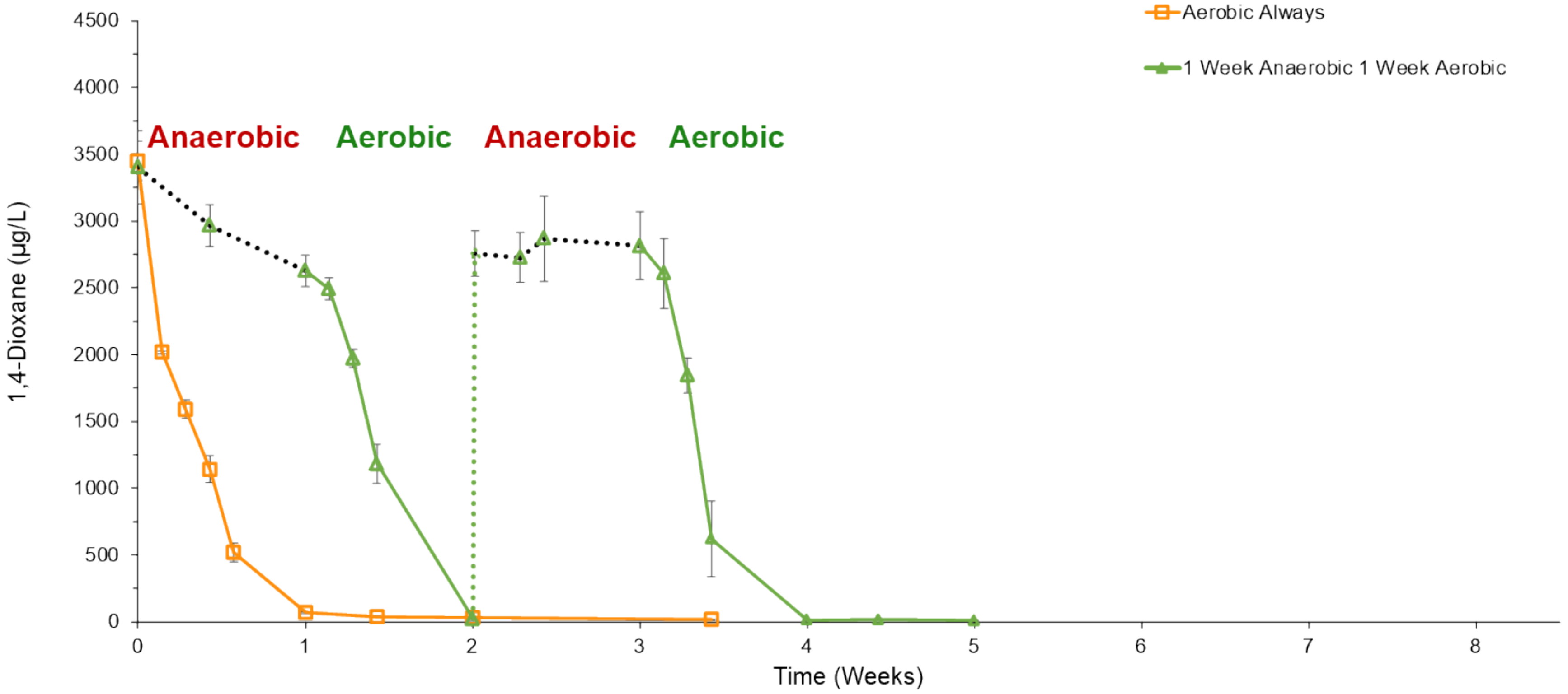
How Long Can CB1190 Survive Without Oxygen?

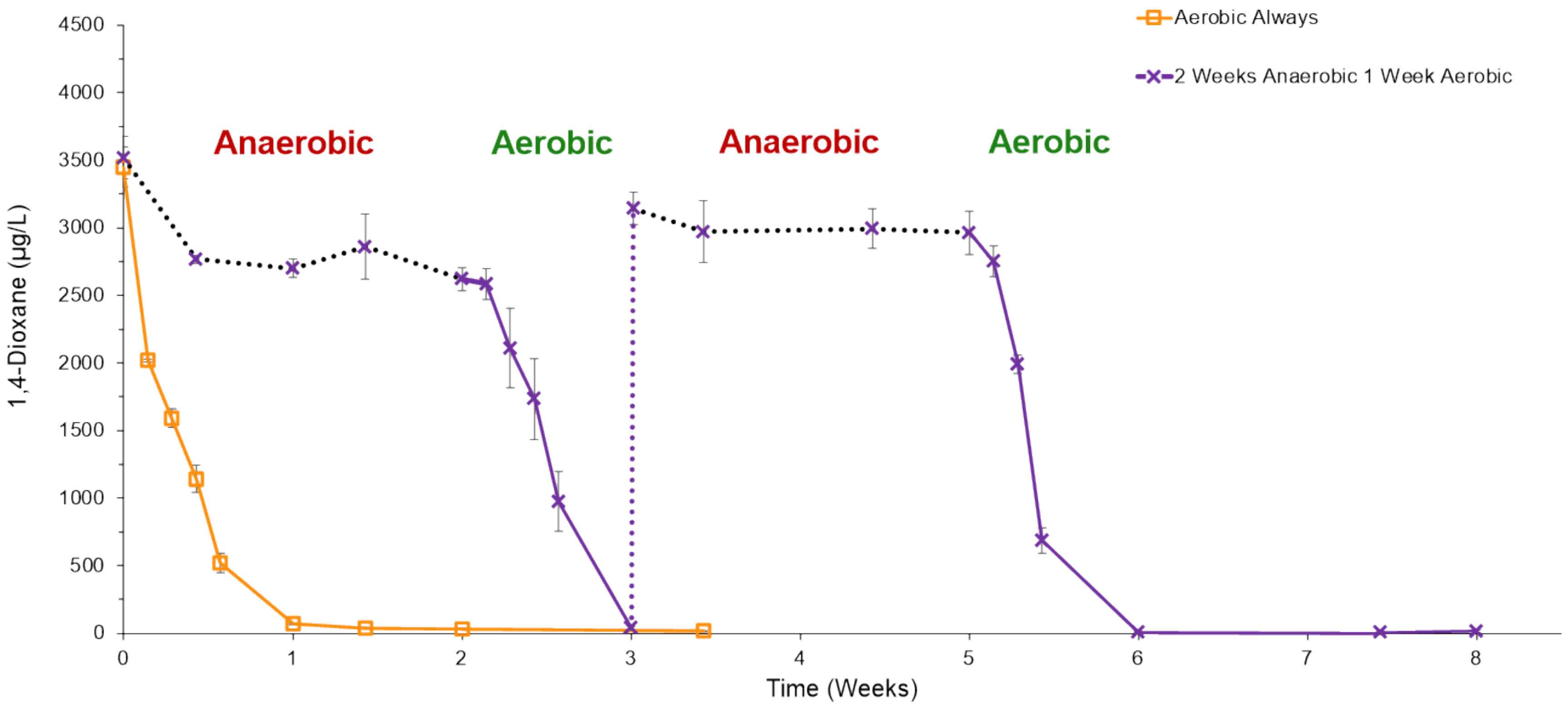
Can CB1190 Degrade Dioxane After Multiple Low Dissolved Oxygen Periods?



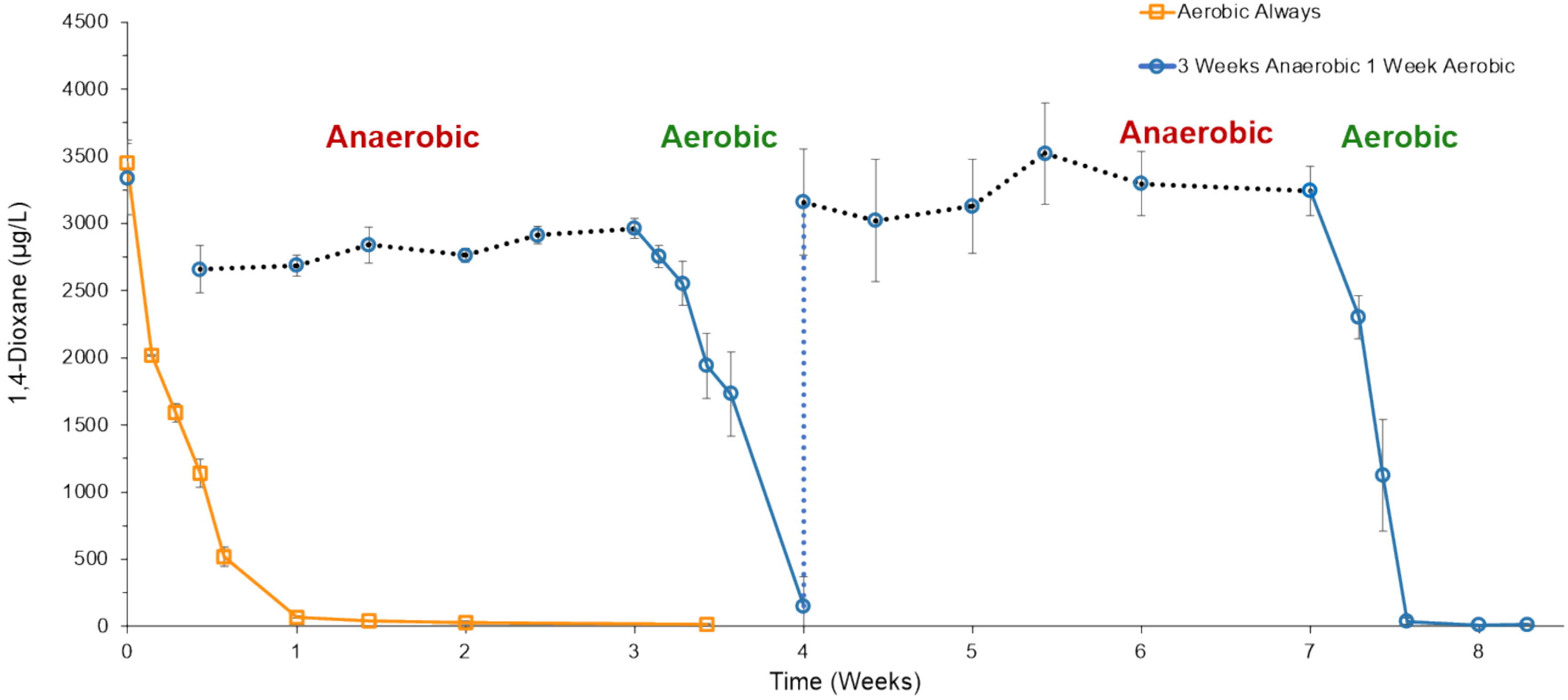
CB1190 Degrades Dioxane After ONE Week Anaerobic Cycles



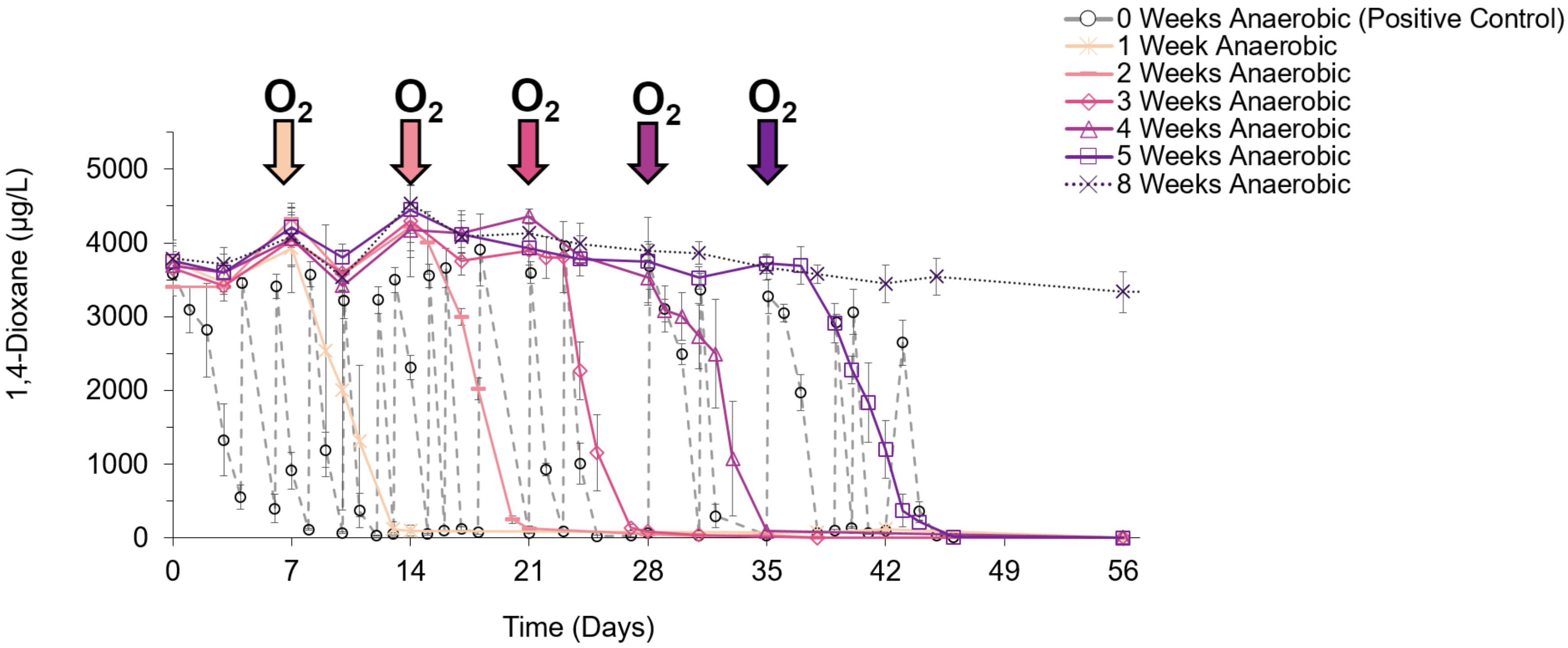
CB1190 Degrades Dioxane After TWO Week Anaerobic Cycles



CB1190 Degrades Dioxane After THREE Week Anaerobic Cycles

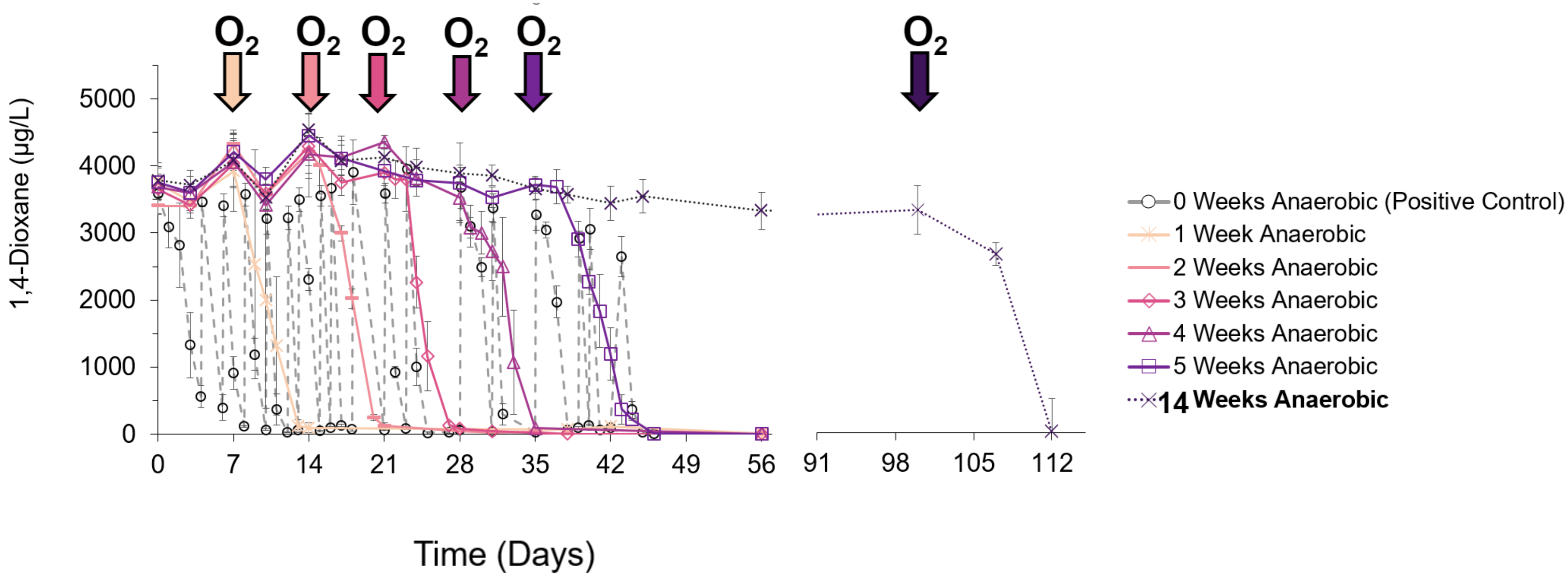


1,4-Dioxane Not Degraded in Anaerobic Bottles



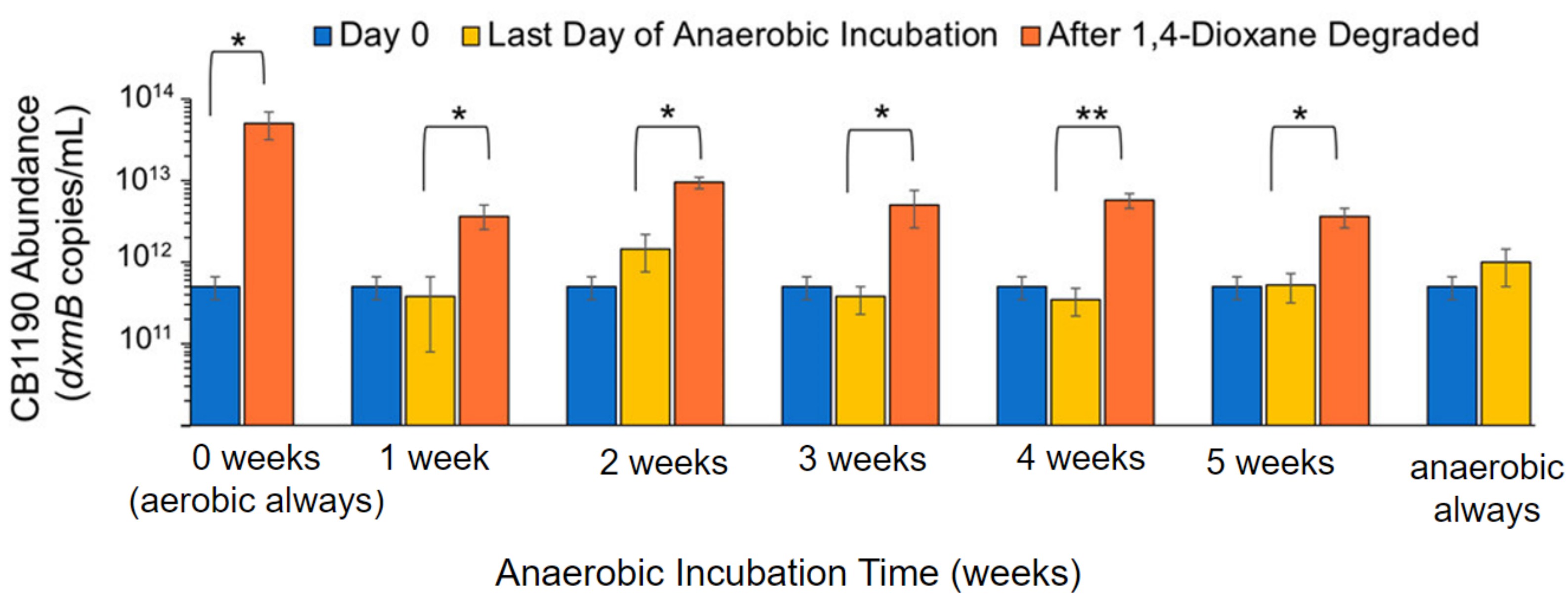
Polasko et al. *EST&L*, 2019

1,4-Dioxane Degraded After 100 Days Without O2!



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
- 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**

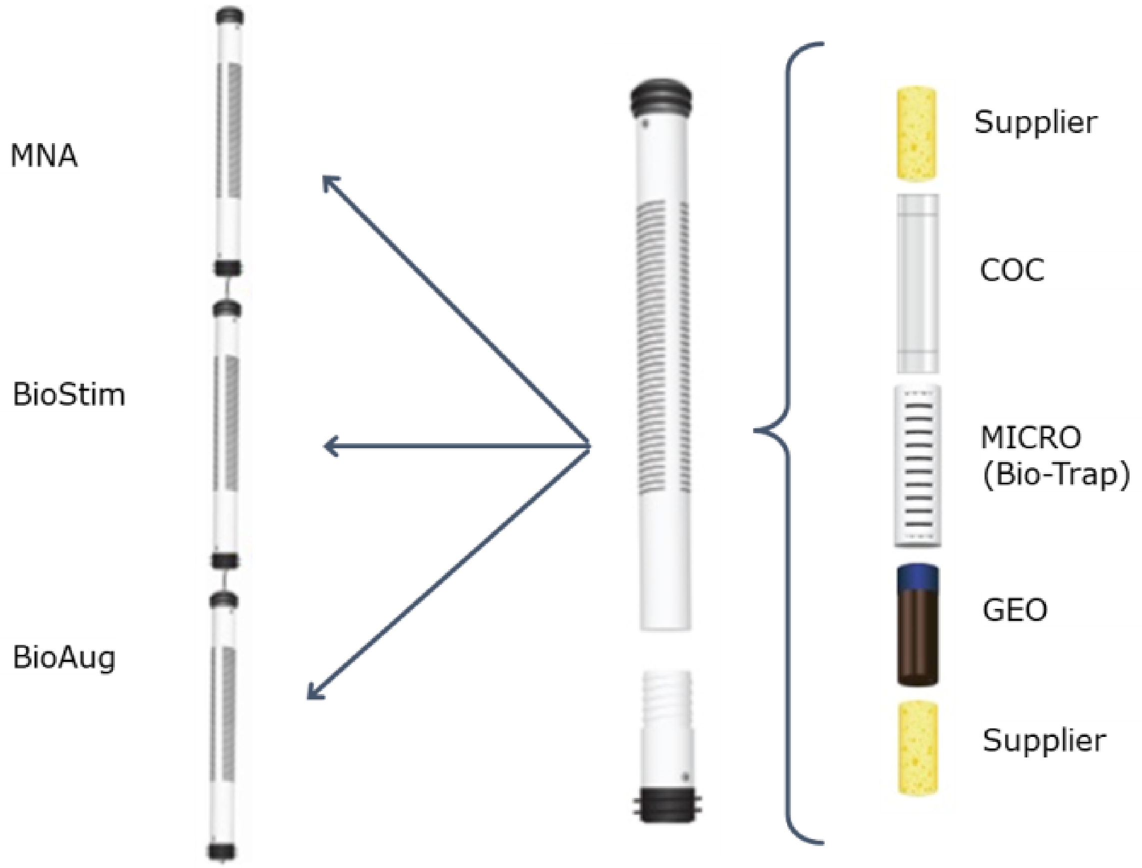
Site Background Information

- 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 CVOC and 1,4-Dioxane Data

Sample Location	Sample Date	1,1,1-TCA	1,1-DCE	cis-1,2-DCE	PCE	trans-1,2-DCE	TCE	Vinyl Chloride	1,4-Dioxane	Total
Screen	Micrograms per Liter									
Standards		200	7	70	5	100	5	2	32	
MW-30	11/19/2015	840	1400	570	3200	<4.5	3500	17	4500	9,527
(15-40)	6/10/2016	430	1000	510	2300	2.5	2300	18	3700	10,260
MW-31	11/19/2015	330	2800	1400	3000	5.8	4900	36	5400	12472
(15-40)	6/10/2016	190	1700	860	2100	4.3	3300	20	4300	12864
MW-32	11/19/2015	880	2300	3100	3000	6.9	3900	110	1800	13297
(15-40)	6/10/2016	600	1400	2100	2000	9.1	2400	55	1900	10174

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

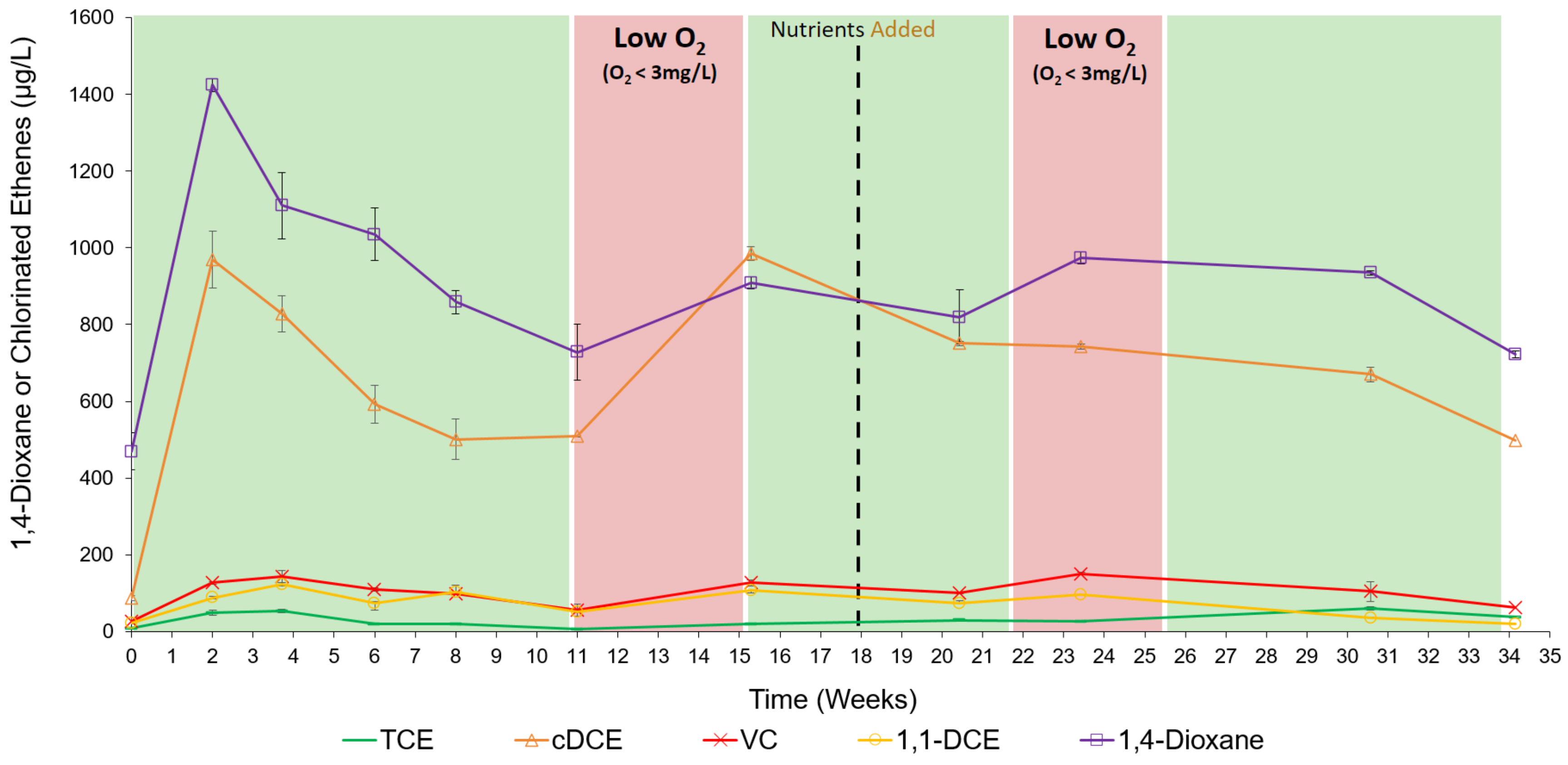
Field Site: CVOC & 1,4-Dioxane Data

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-30	4th Quarter 2019	67	330	660	890	340	1.2	17	65
MW-31	1st Quarter 2020	29	200	72	170	4,100	13	240	1,400
MW-32	1st Quarter 2020	200	1,100	210	150	5,300	8.4	840	1,600

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

MW-32 Bioaugmented: Biodegradation Driven by O_2 & Nutrients



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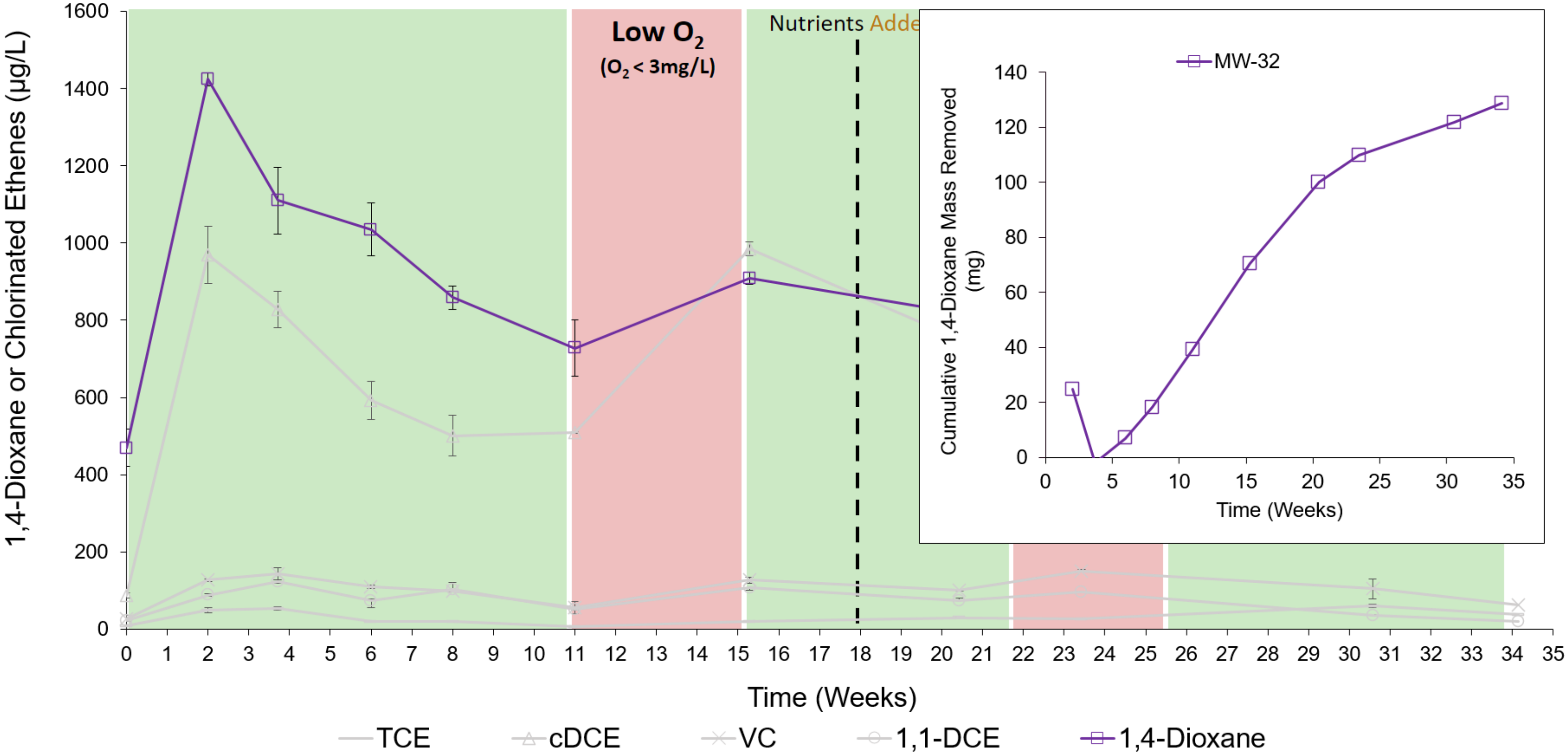
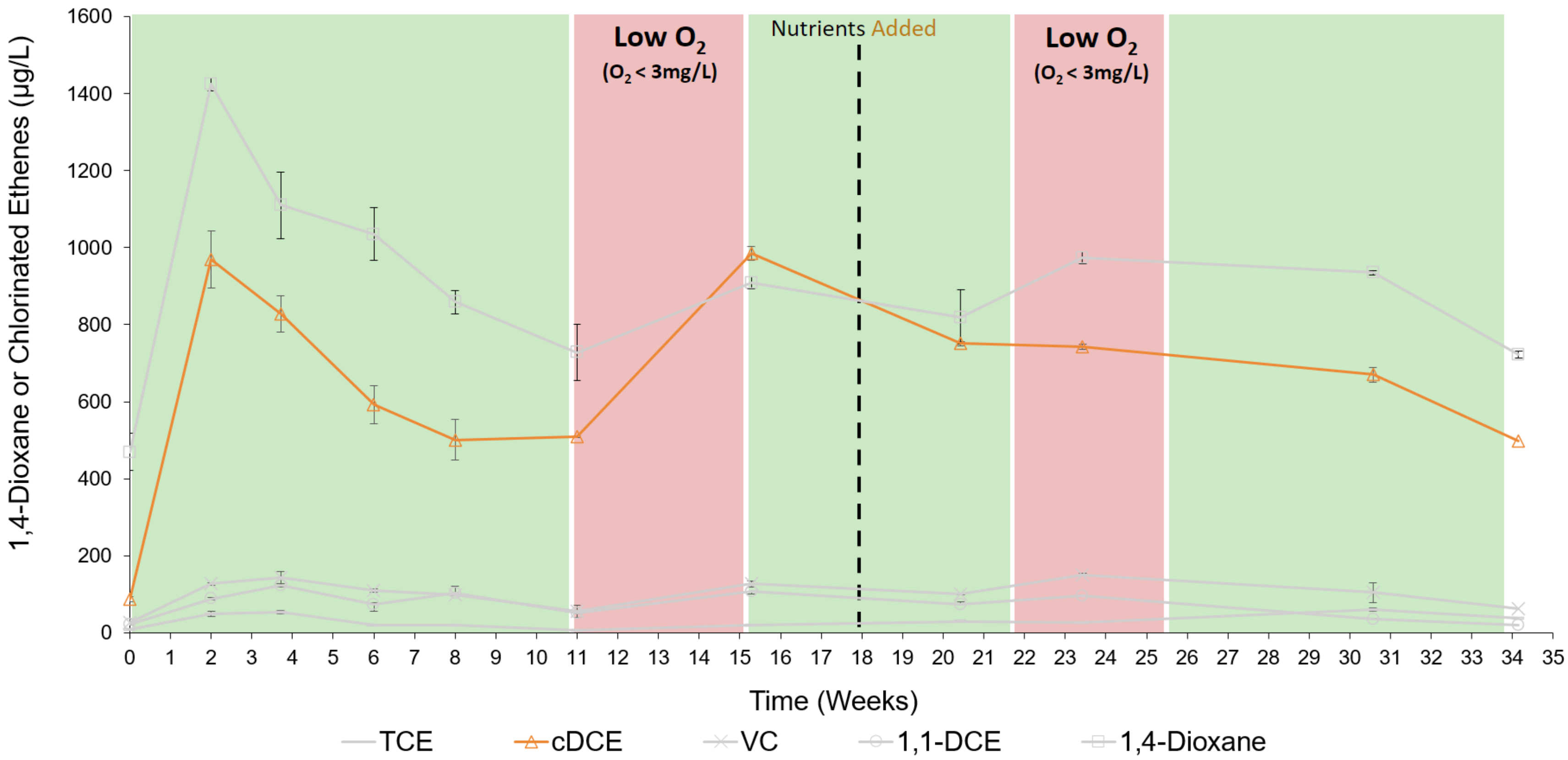
MW-32: 1,4-Dioxane Biodegradation Driven by O_2 & Nutrients



MW-32: cDCE Biodegradation Driven by O_2 & Nutrients



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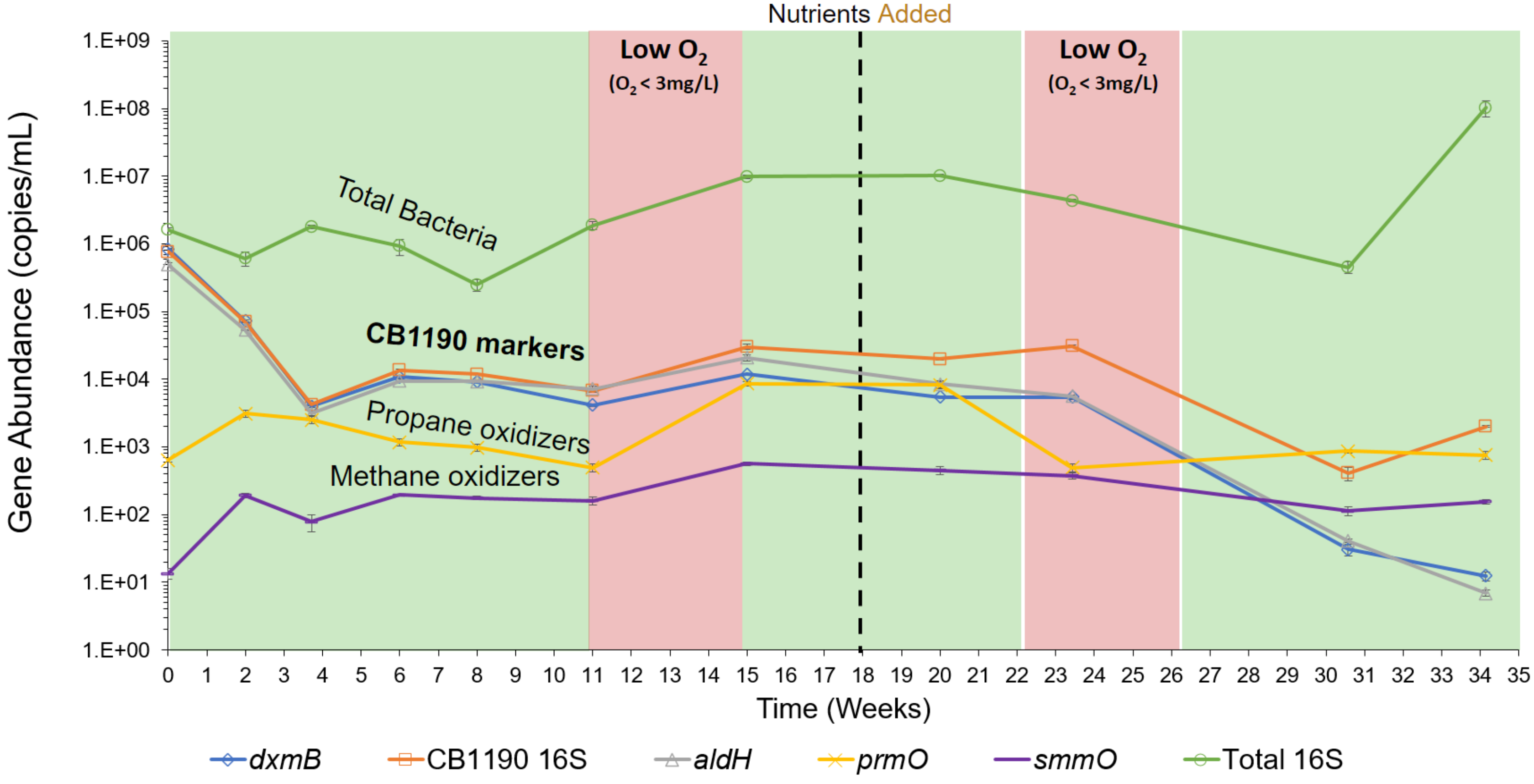
MW-32: *prm*O and *smm*O Biomarkers Present, but Remain Below CB1190 Markers



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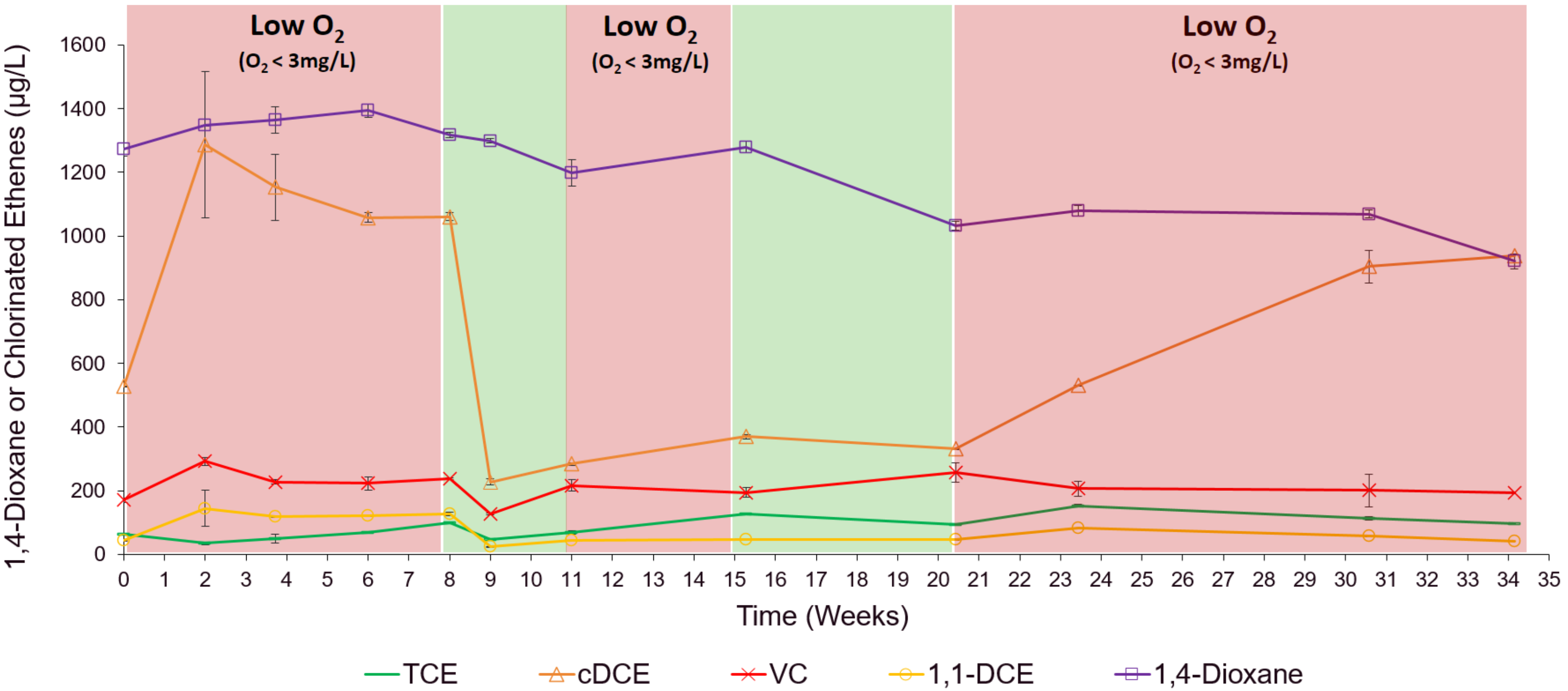
MW-31 (Unaugmented Well): Sparging Drives CVOC Changes



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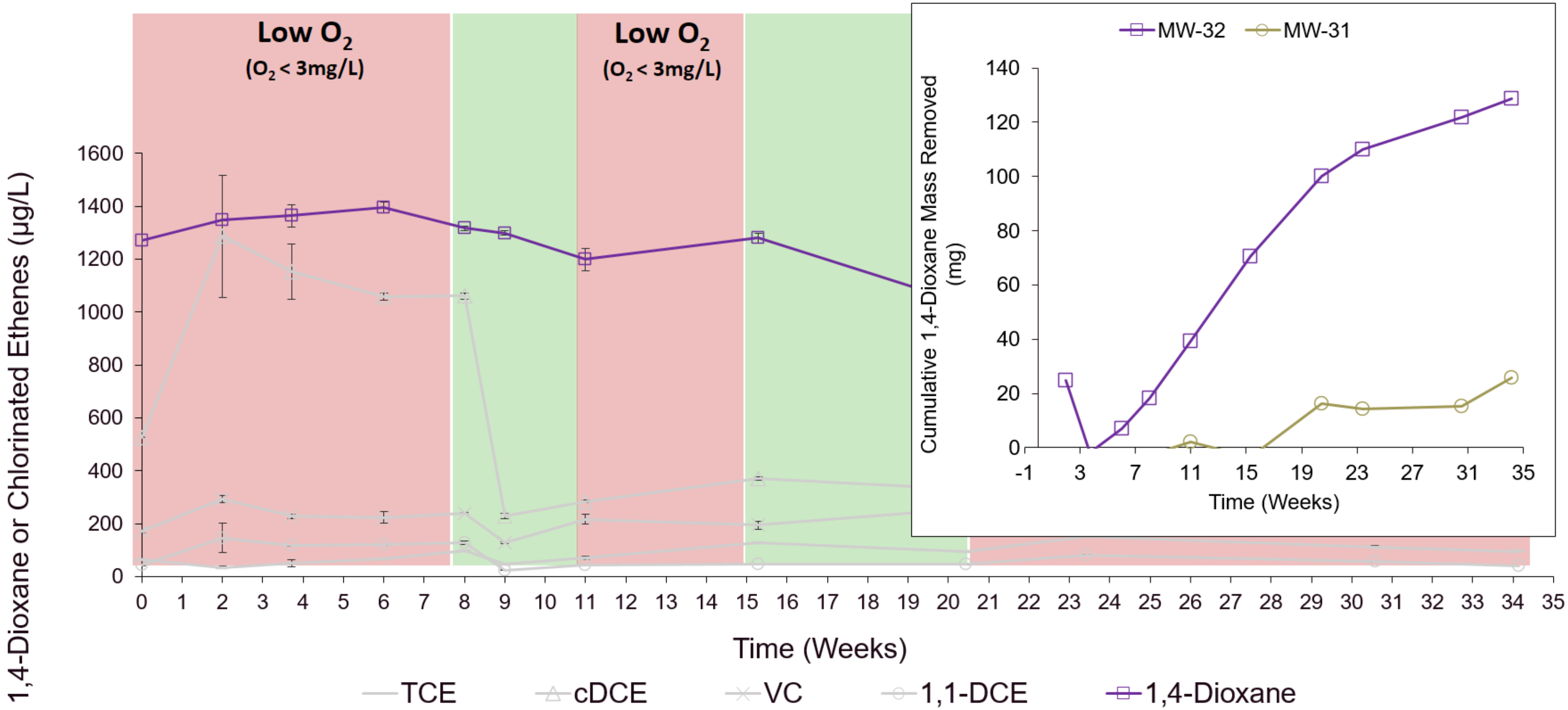
MW-31: Dioxane Decreased Over Time, But Not as Much as MW-32



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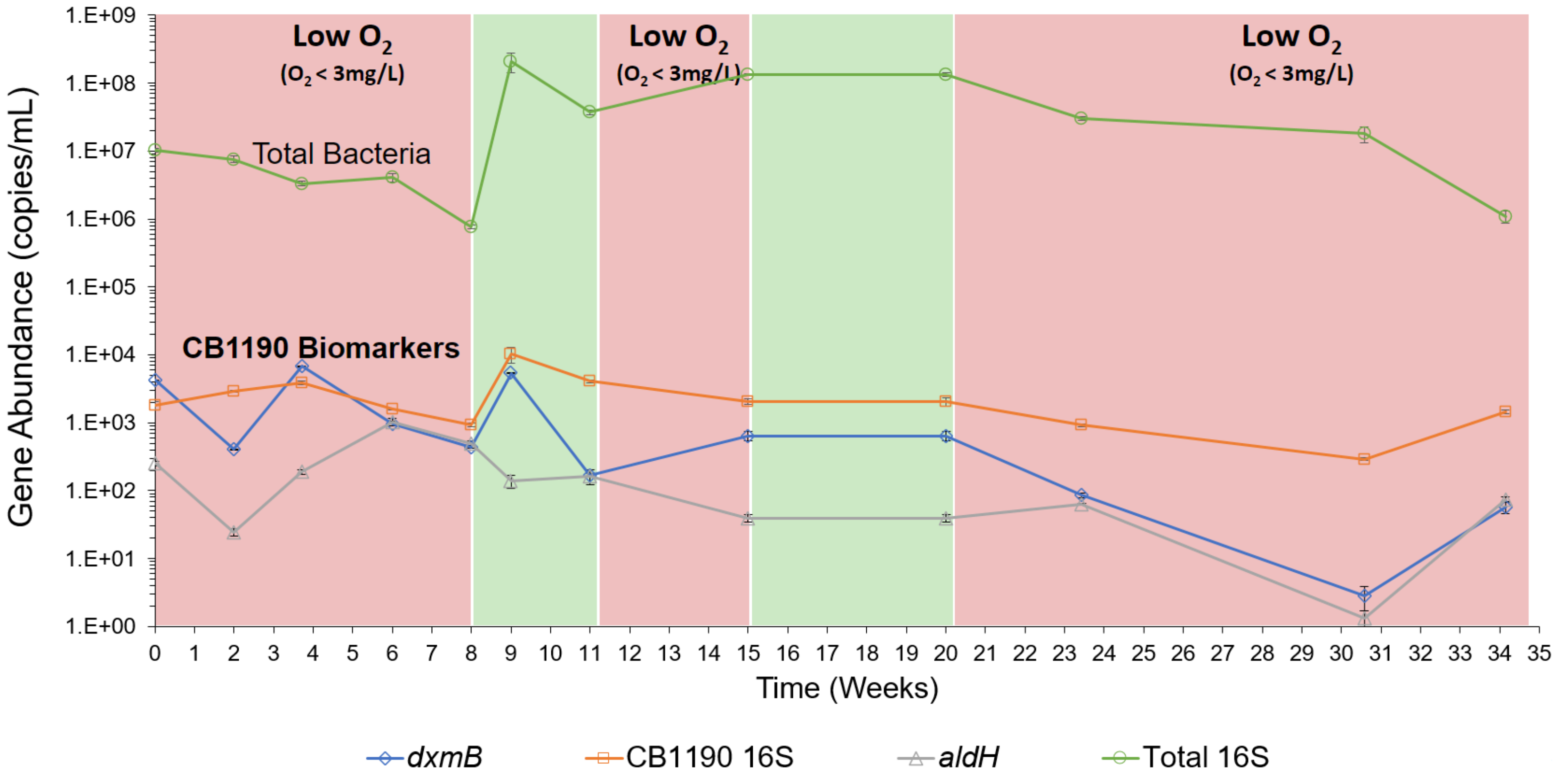
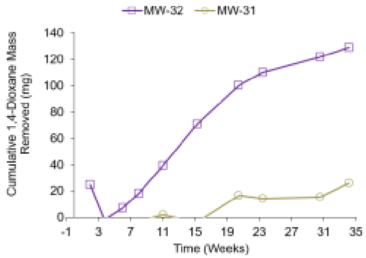
MW-31: Sparging System Increases Community's Abundance



Post CB1190 Bioaugmentation Results

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	1st Quarter 2020	29	200	72	170	4,100	13	240	1,400
	1st Quarter 2023	<2.4	190	51	150	2,100	12	290	400
	Percent Change	-92%	-5%	-29%	-12%	-49%	-8%	+21%	-71%
MW-32	1st Quarter 2020	200	1,100	210	150	5,300	8.4	840	1,600
	1st Quarter 2023	12	58	11	27	570	2.6	21	500
	Percent Change	-94%	-95%	-95%	-82%	-89%	-69%	-98%	-69%

Summary and Significance



- In MW-32, 1,4-dioxane & cDCE decreased by ~50% during initial biostimulation and bioaugmentation.

- 2nd CB1190 injection in source area IW-3 and MW-32. Current 1,4-dioxane concentrations in MW-31 & MW-32 ~ 400-500 μg/L, 70% reduction in 1,4-dioxane and a 49% and 89% reduction in cis-1,2-DCE in MW-31 and MW-32, respectively.

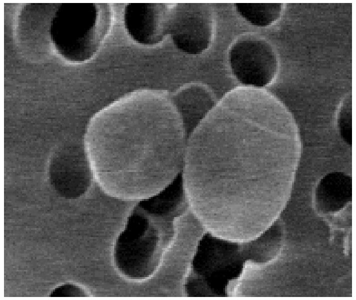
- In MW-32, dissolved O_2 appeared to be a driving factor for biodegradation of CVOCs & 1,4-dioxane.

- Significance: **CB1190 can be an efficient microbe at removing 1,4-dioxane & less chlorinated CVOCs *in situ*!**

Mahendra & Alvarez-Cohen, 2005, *IJSEM*

SEM image CB1190

Thank You & Questions



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