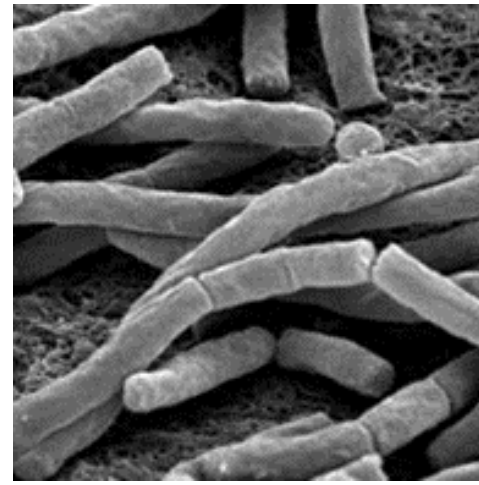
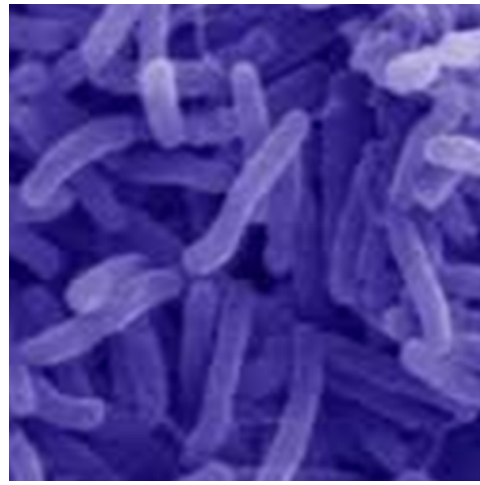


Enhanced Aerobic Biodegradation of Chlorinated Solvents and 1,4-Dioxane

Laurie LaPat-Polasko, PhD, QEP, Matrix New World Engineering

Dr. Alexandra L. Polasko, Stanford University and Dr. Shaily Mahendra, UCLA



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)

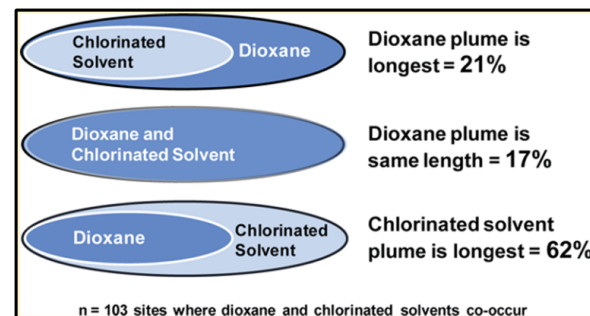
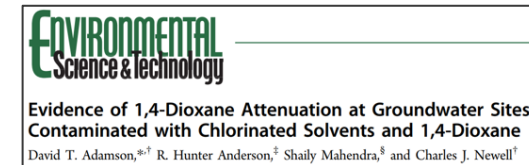
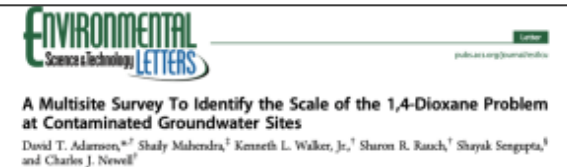
And others:

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

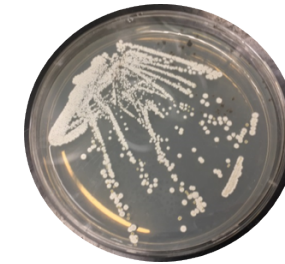
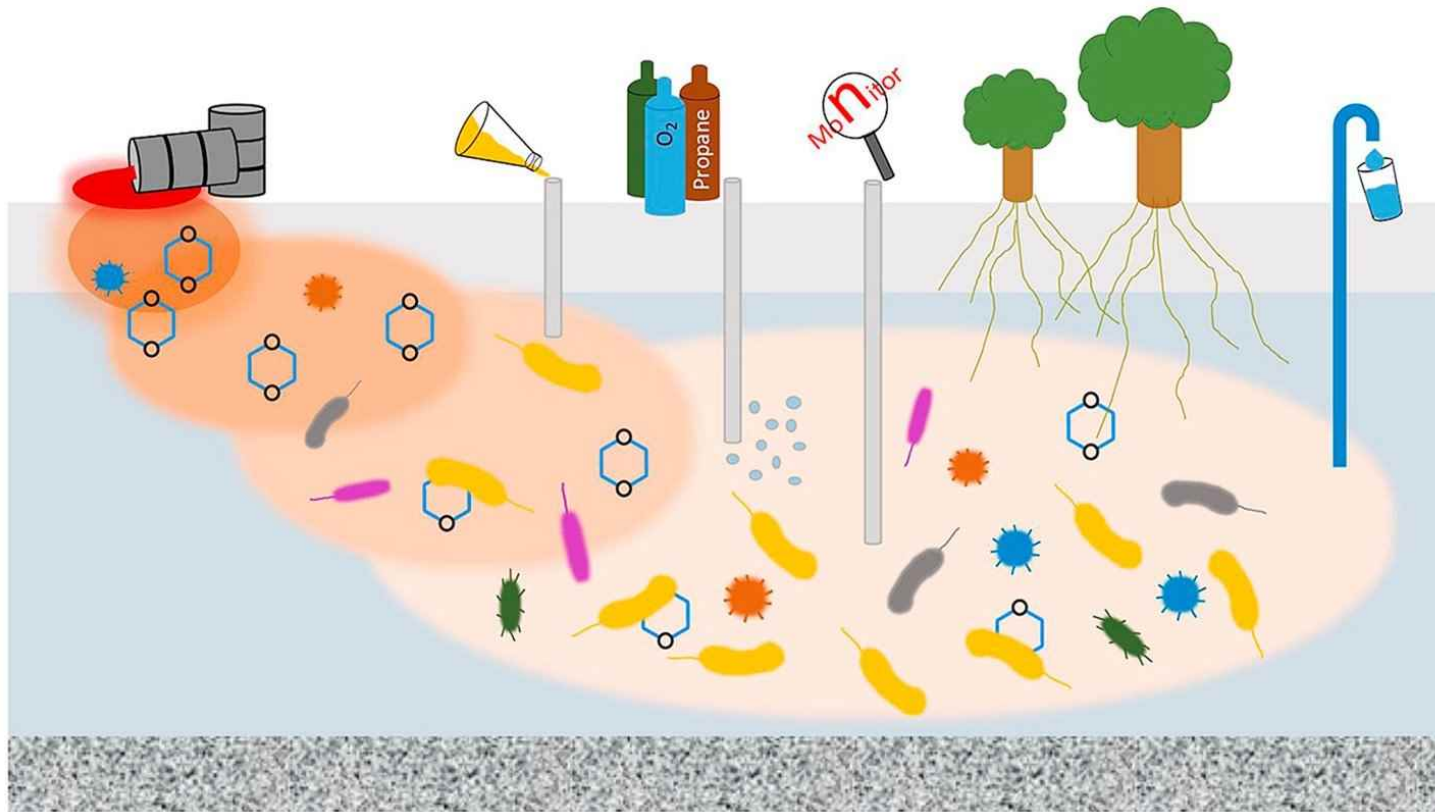
Co-Occurrence of 1,4-Dioxane with Trichloroethylene in Chlorinated Solvent Groundwater Plumes at US Air Force Installations: Fact or Fiction

Richard H. Anderson,^{*,†} Janet K. Anderson,[‡] and Paul A. Bower,[‡]

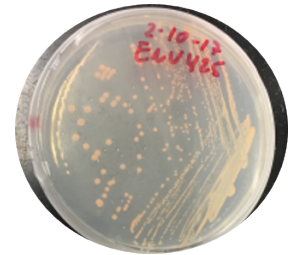
^{*}Air Force Center for Engineering and the Environment, Technical Support Division (AFCEE/TDV), 2261 Hughes, Site 155, Lackland AFB, Texas 78236, USA
[†]Contractor, Air Force Center for Engineering and the Environment, Environmental Restoration Branch (AFCEE/ERB), Lackland AFB, Texas, USA



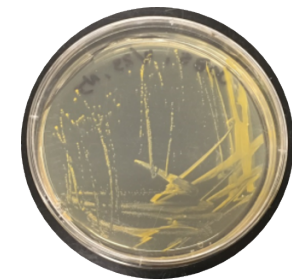
Luckily, Microbes Can Biodegrade 1,4-Dioxane & Chlorinated Ethenes



*Pseudonocardia
dioxanivorans* CB1190

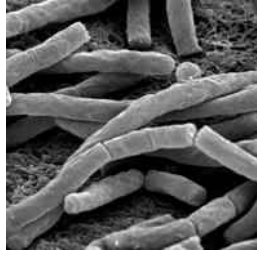


Rhodococcus ruber
ENV425

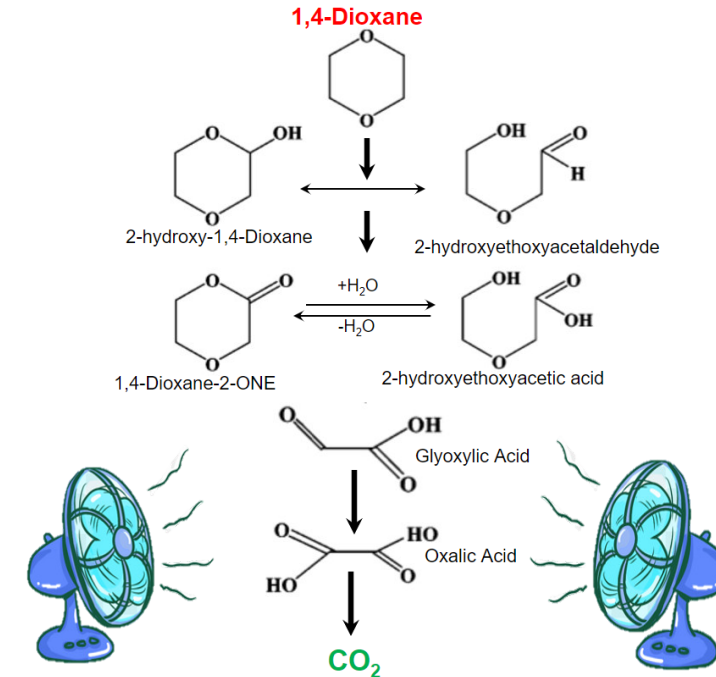
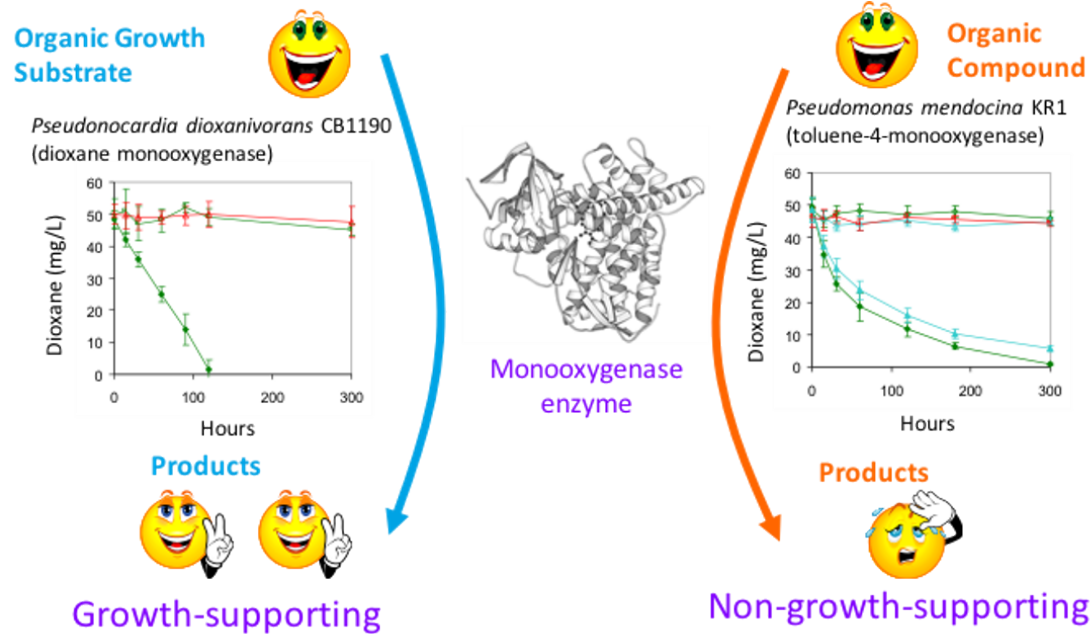


Methylosinus trichosporium
OB3b

CB1190 Aerobically Biodegrades 1,4-Dioxane

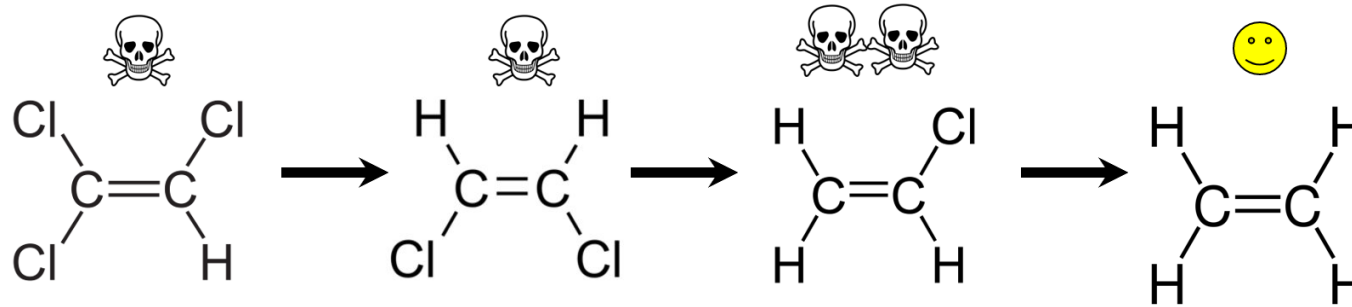


- **Metabolism:** microbe **gains energy** and carbon from contaminant
- **Co-metabolism:** microbe produces an enzyme to metabolize a primary substrate; the enzyme will also transform the contaminant of concern



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

Dehalococcoides (Dhc) Anaerobically Biodegrades TCE



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

trichloroethene
(TCE)

cis-1,2-
dichloroethene
(cDCE)

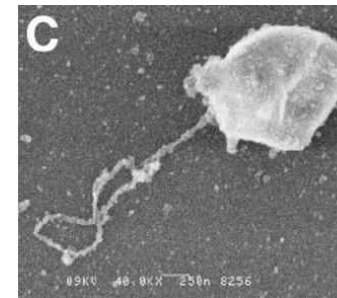
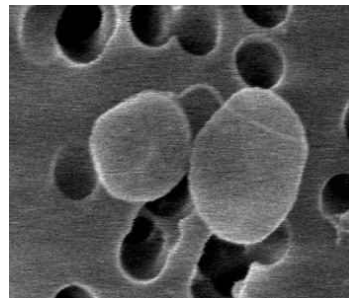
vinyl chloride
(VC)

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

Good News/Bad News

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

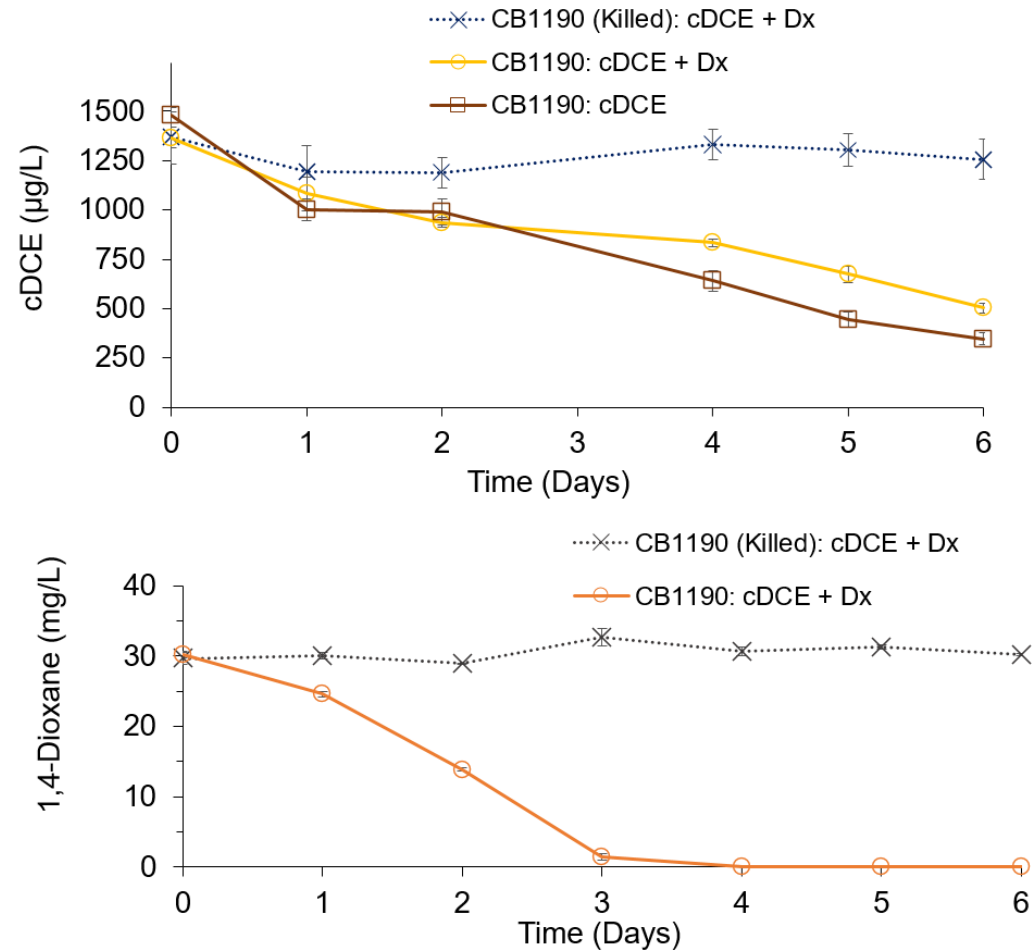


Good News/Good News

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

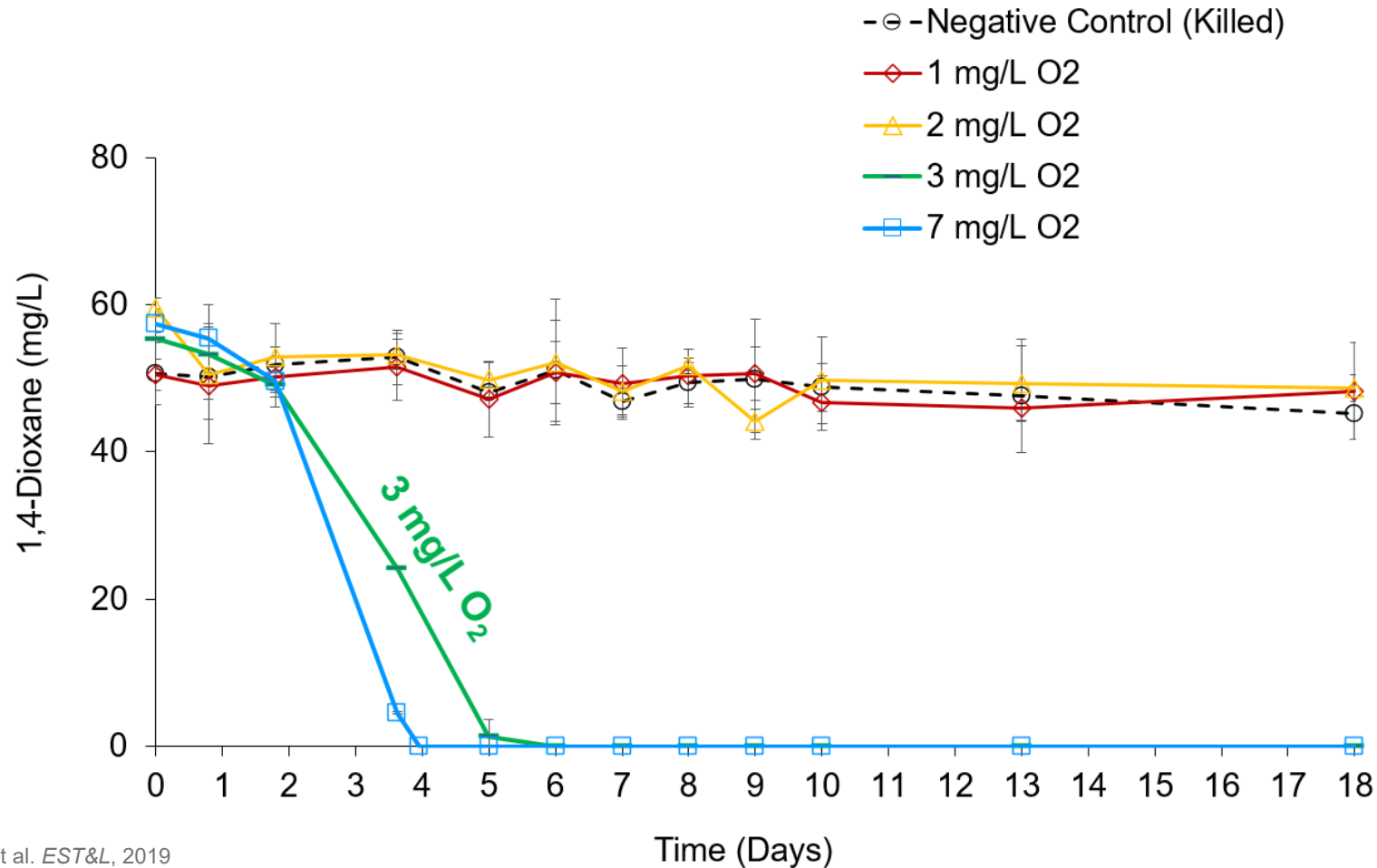


CB1190 Degrades cDCE AND 1,4-Dioxane (Dx)



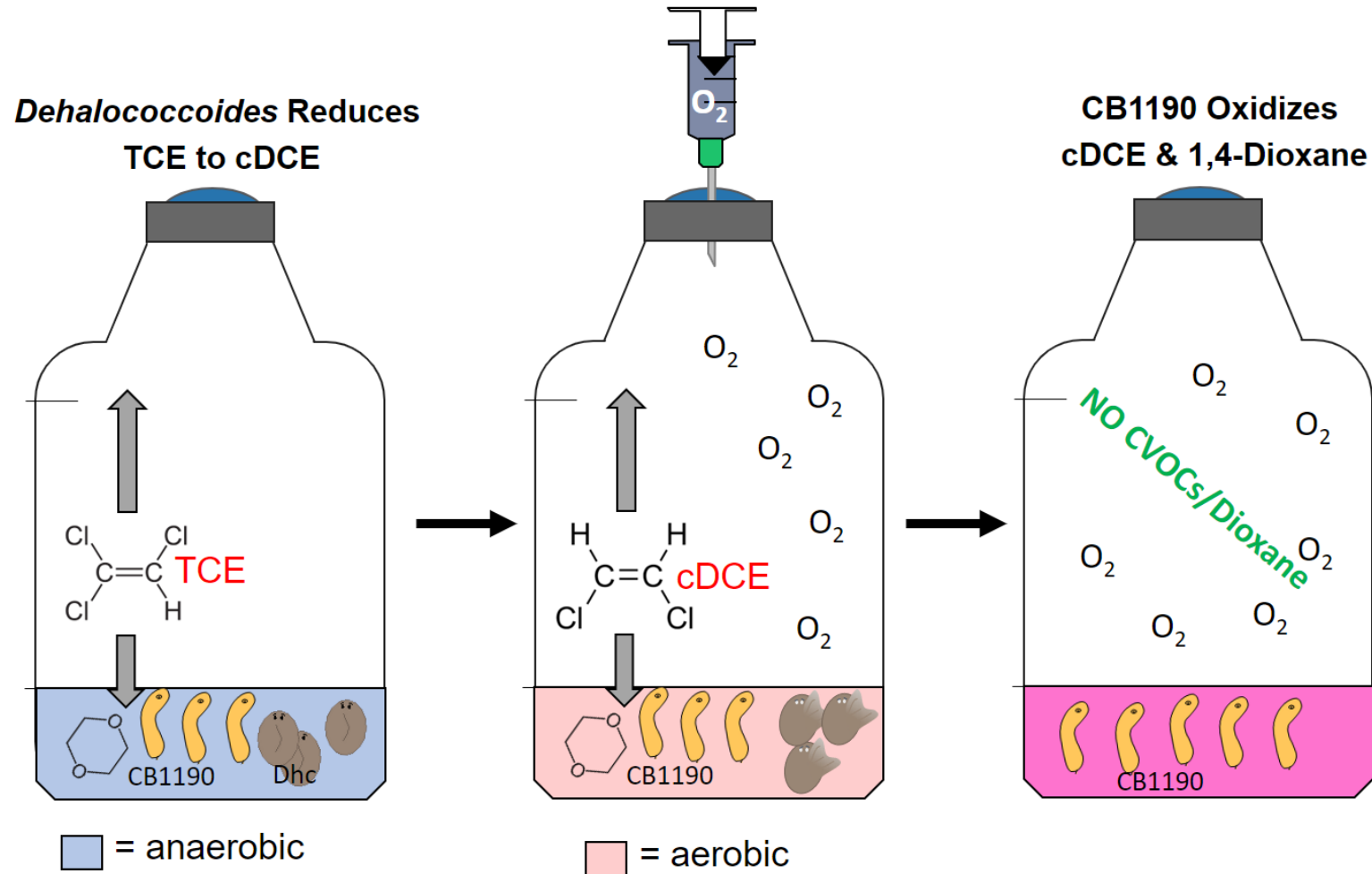
Polasko et al. *EST&L*, 2019

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

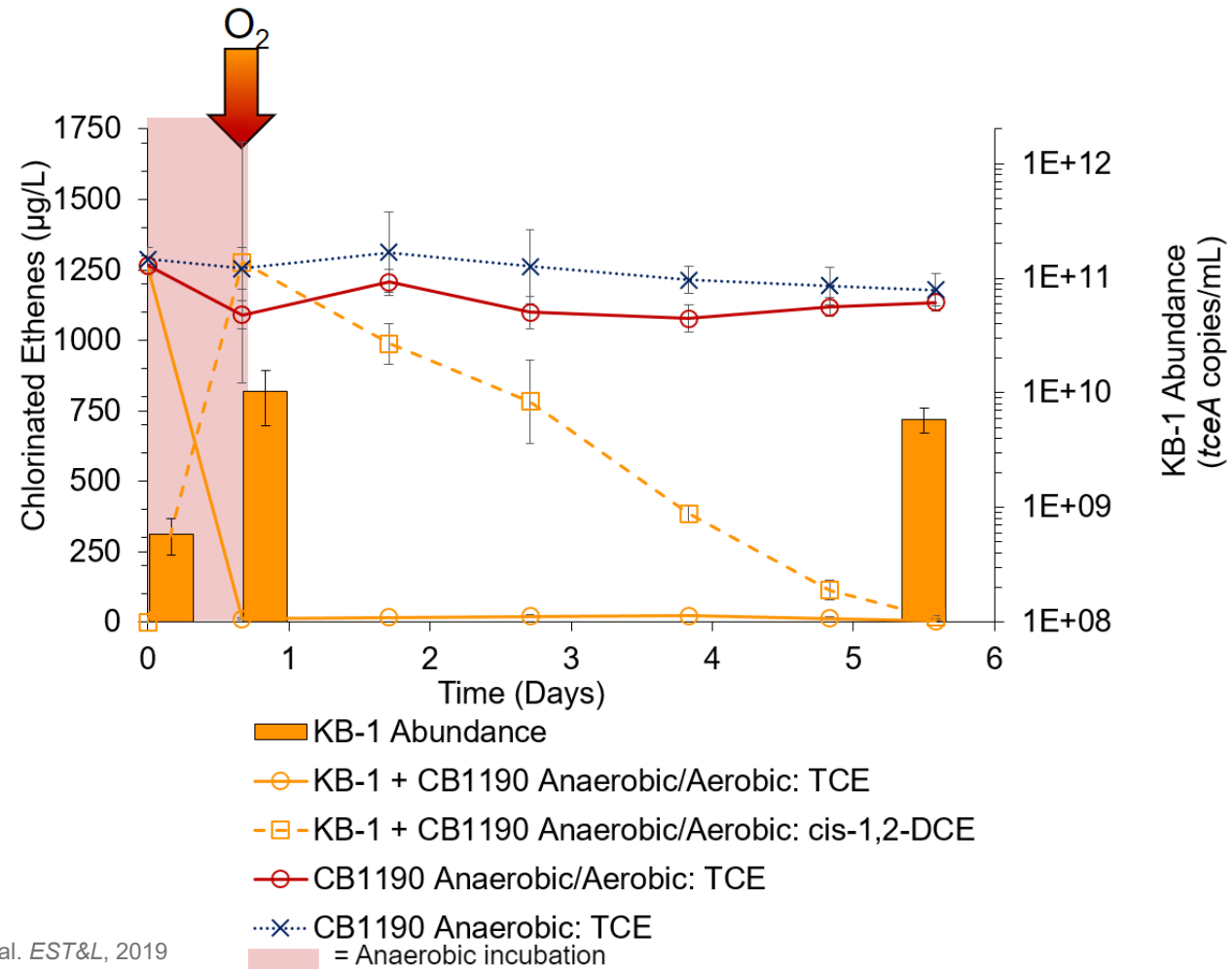


Polasko et al. *EST&L*, 2019

Engineered Microbial Community

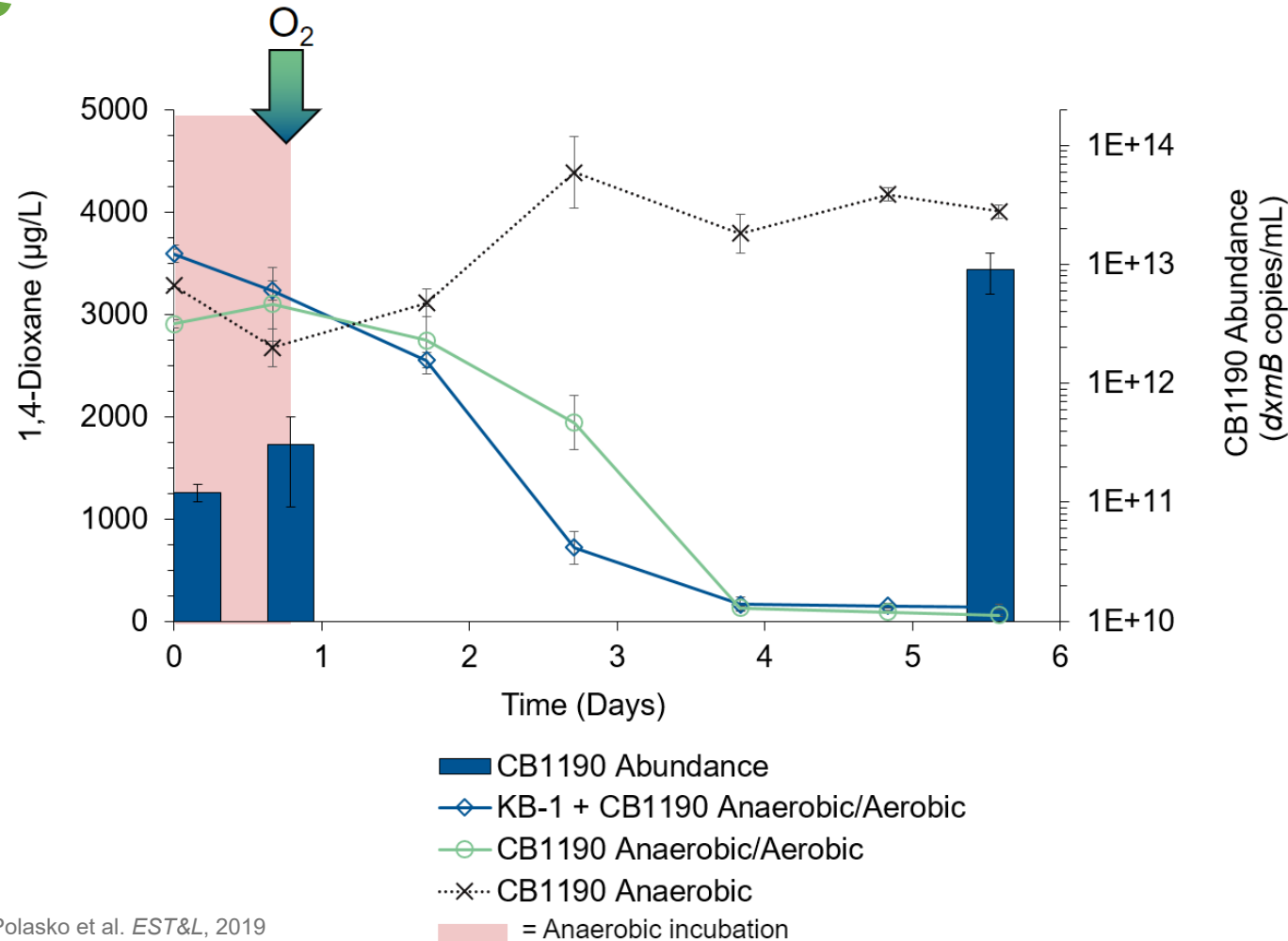


Dhc Degrades TCE → cDCE; CB1190 Degrades cDCE



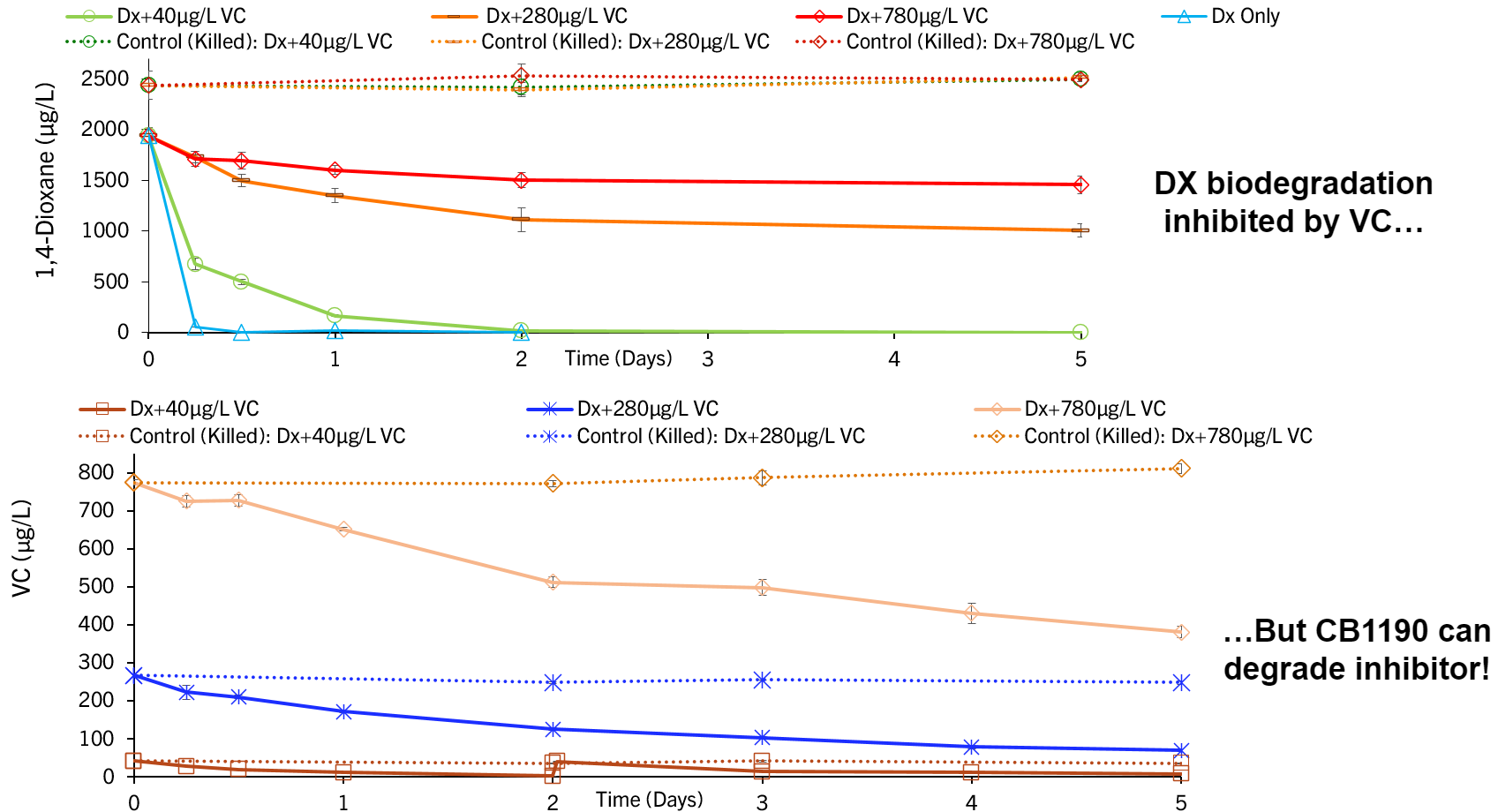
Polasko et al. *EST&L*, 2019

Mixed Culture & Strain CB1190 Degrades 1,4-Dioxane



Polasko et al. *EST&L*, 2019

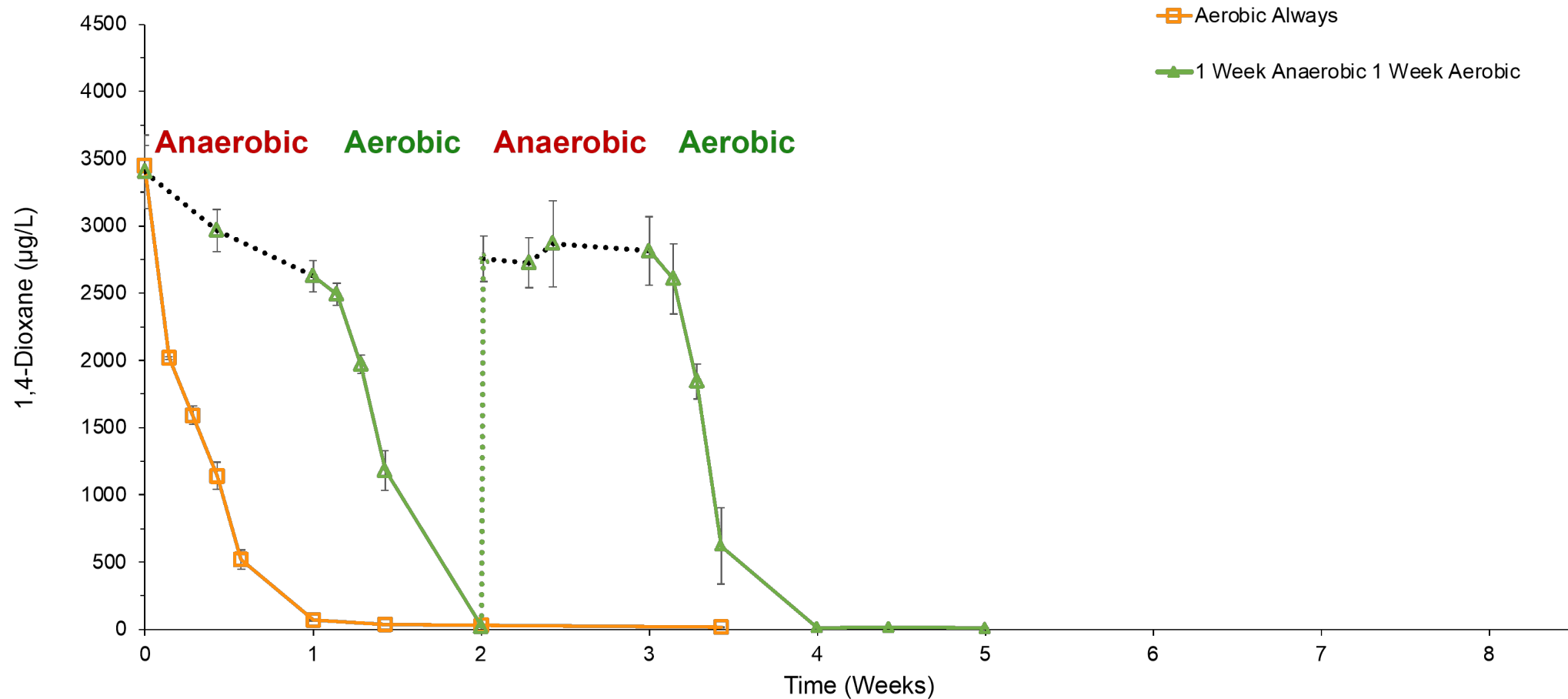
CB1190 Biodegrades VC AND Dioxane Simultaneously



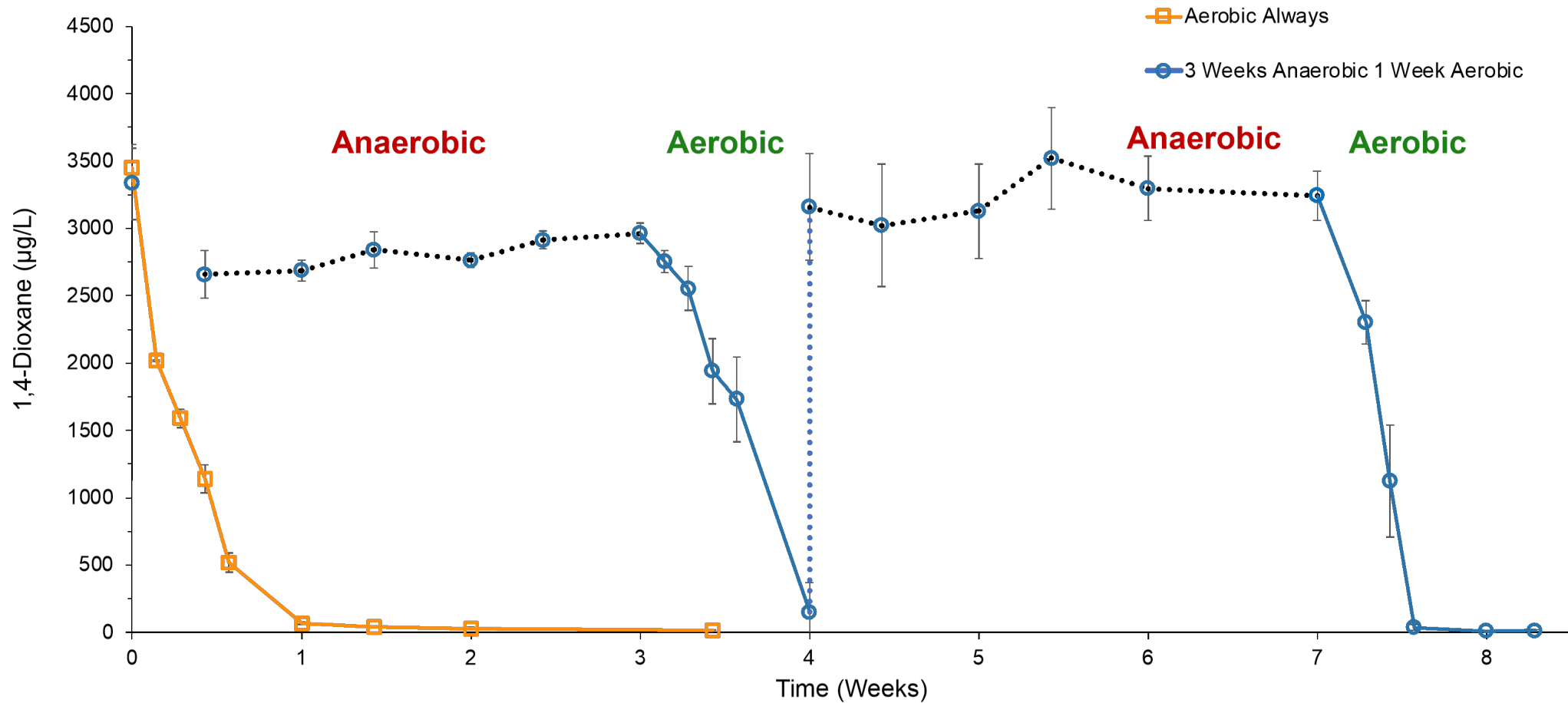
How Long Can CB1190 Survive Without Oxygen?



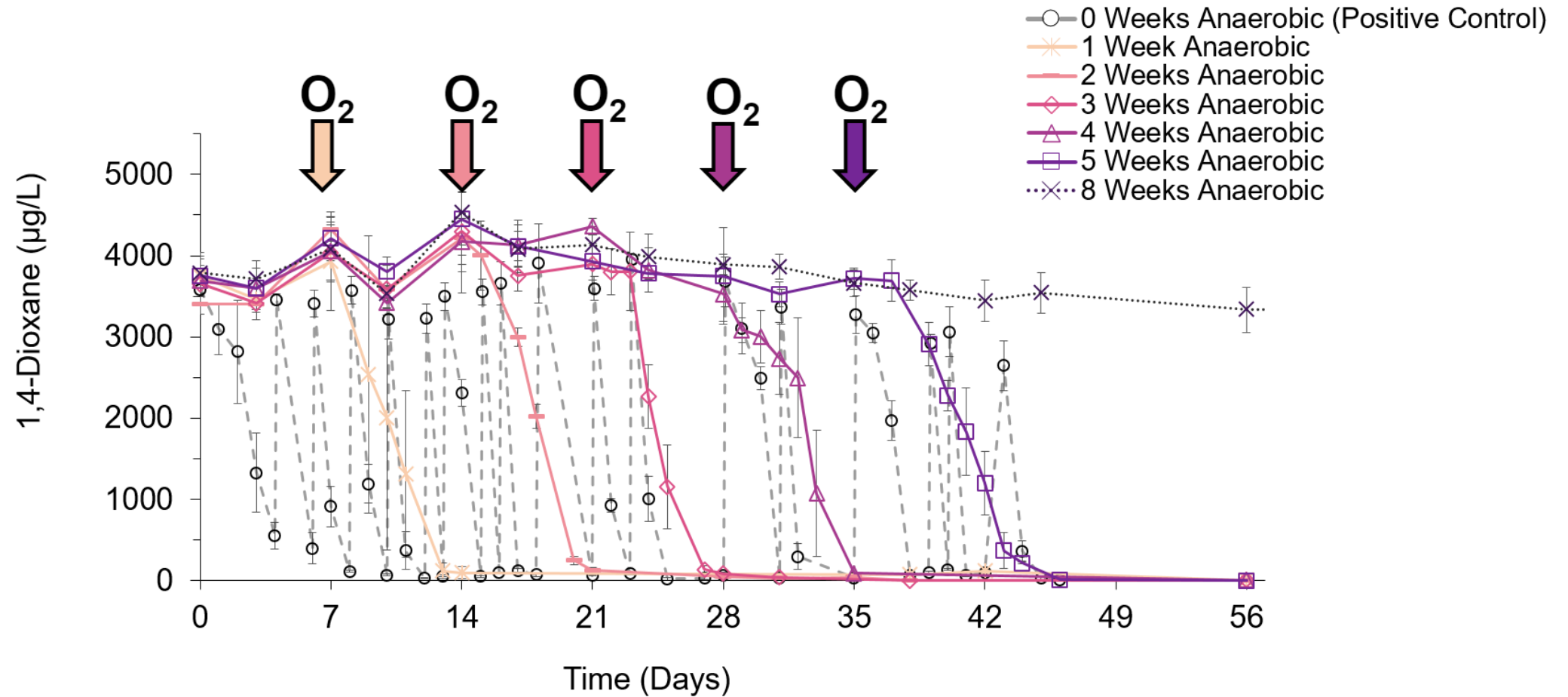
CB1190 Degrades Dioxane After ONE Week Anaerobic Cycles



CB1190 Degrades Dioxane After THREE Week Anaerobic Cycles

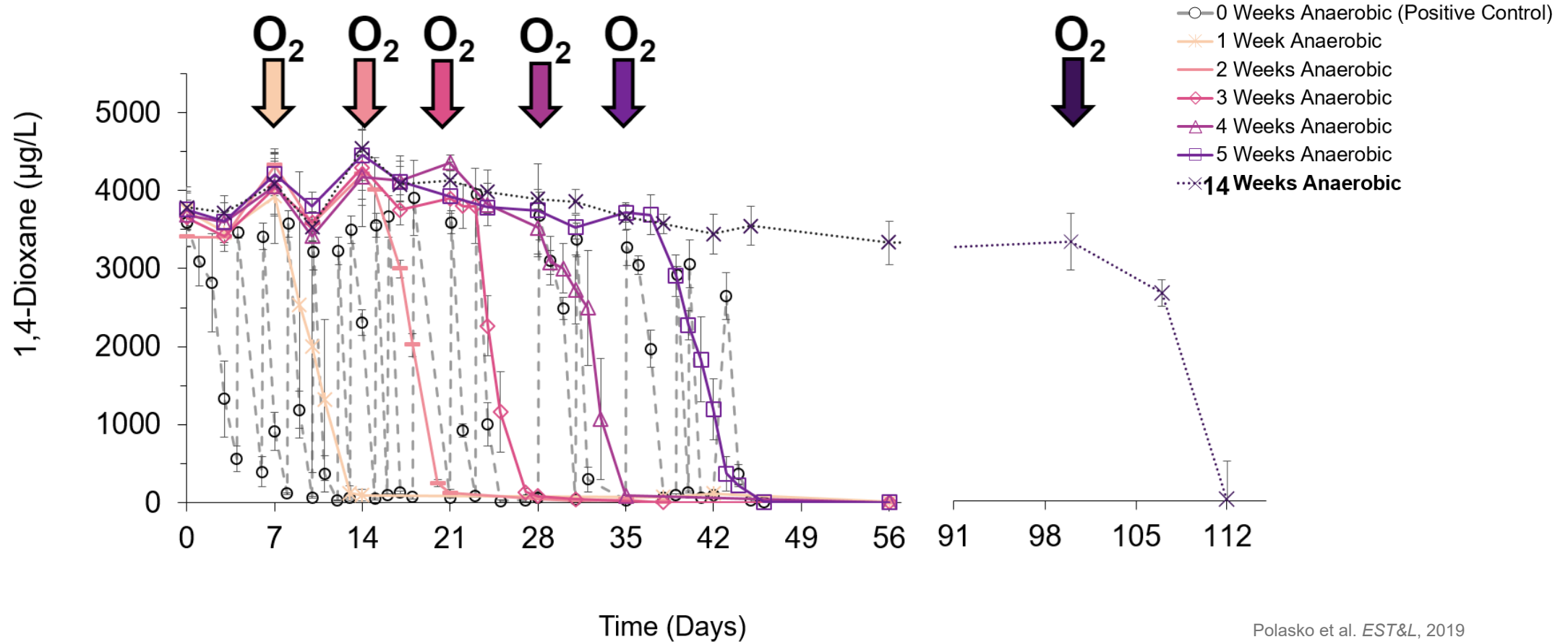


1,4-Dioxane Not Degraded in Anaerobic Bottles



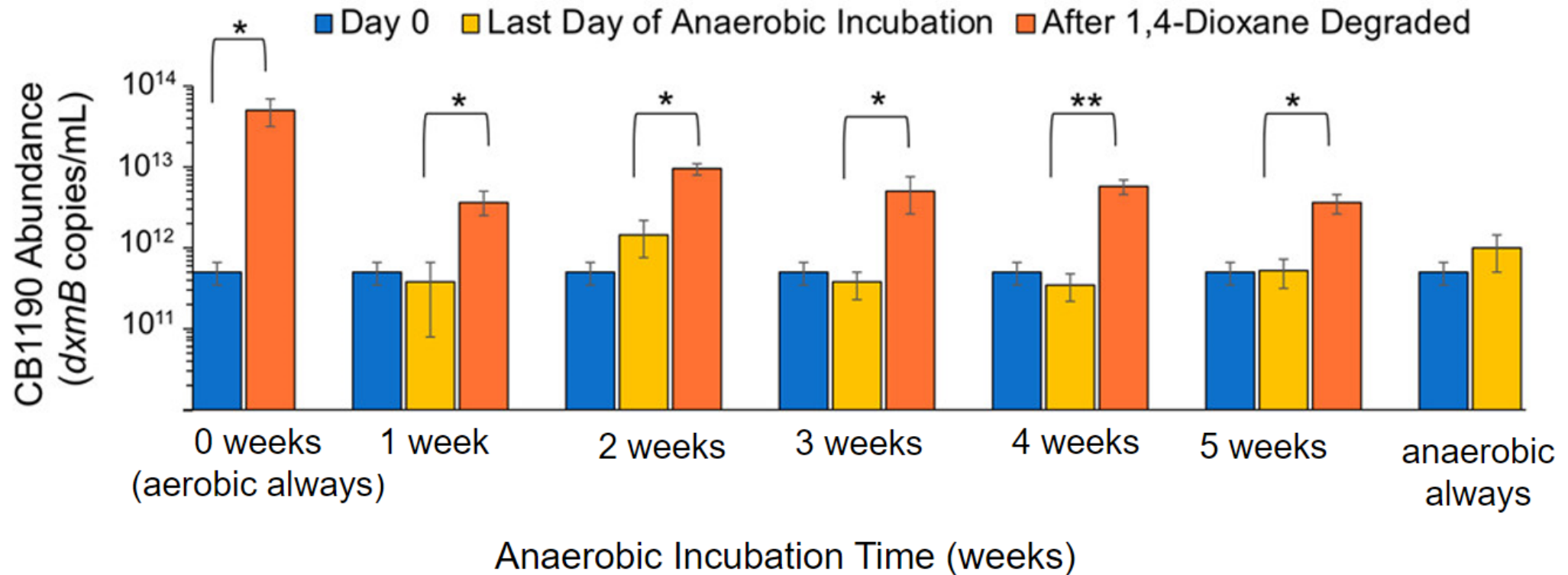
Polasko et al. *EST&L*, 2019

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



Polasko et al. *EST&L*, 2019

Significant Growth After Oxygen Amendments



Polasko et al. *EST&L*, 2019

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

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 O₂
- 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**

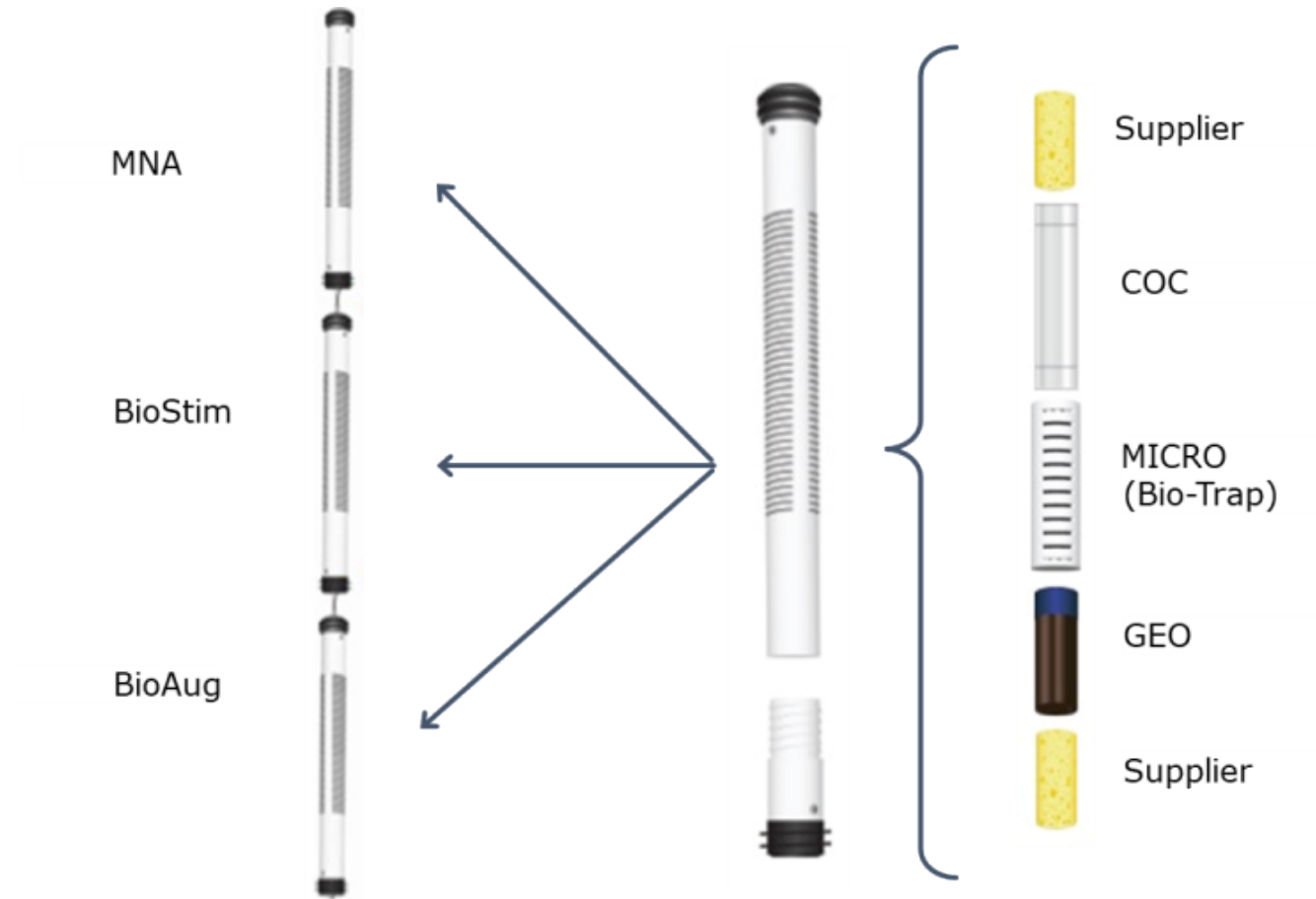
Site Background Information

- Manufacturing company in Eastern USA – >50 years ago
 - Used a variety of chlorinated solvents at the 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

In Situ Microcosm Bio-Trap Results

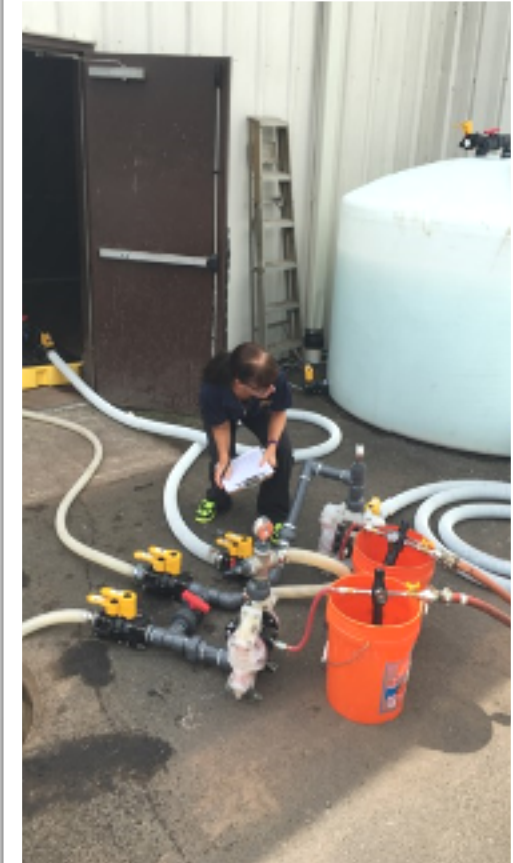
Client Sample ID:	Units	MW-32 MNA	MW-31 BioAug	MW-30 BioAug+ORC+Osmo	MW-30 BioAug+Osmo
CSIA of 1,4-dioxane Carbon	$\delta^{13}\text{C}$ (‰, VPDB)	-30.6	-29.3	-26.4	-23.8

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!

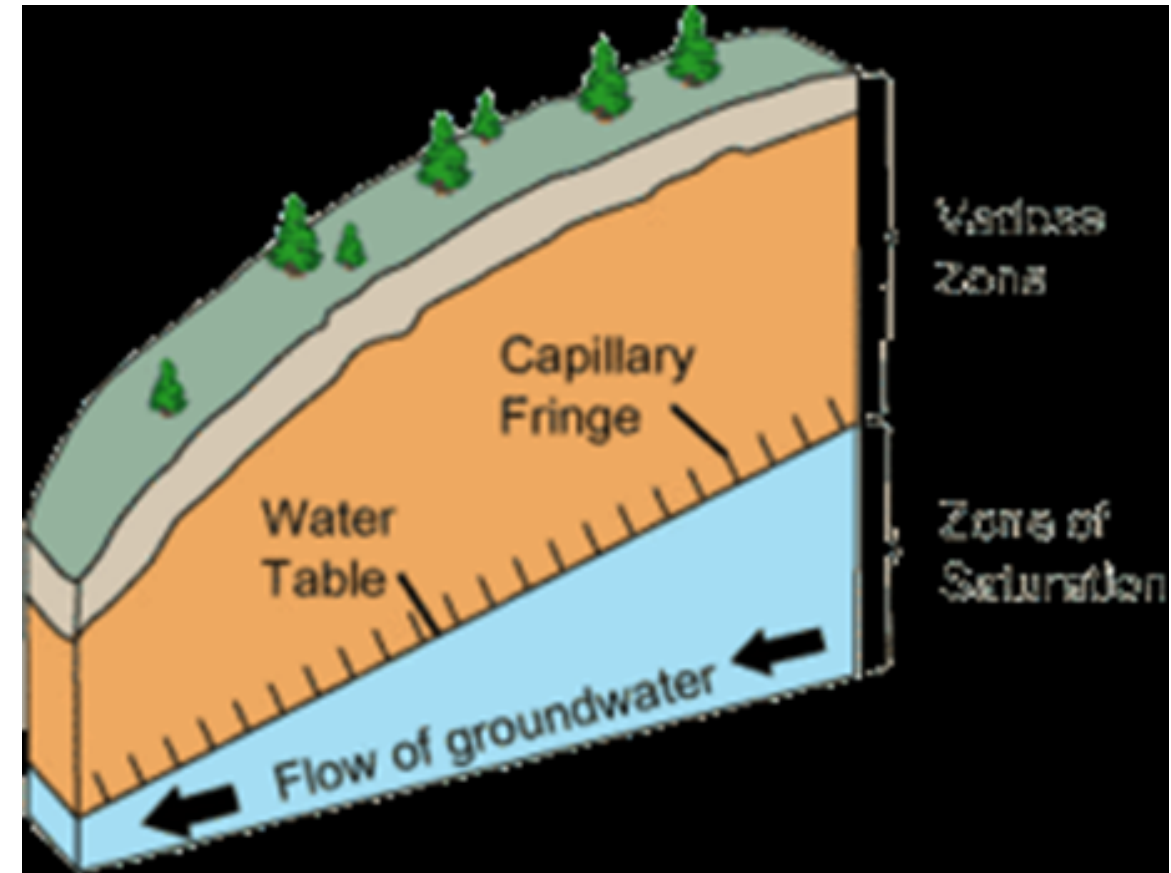


CB1190

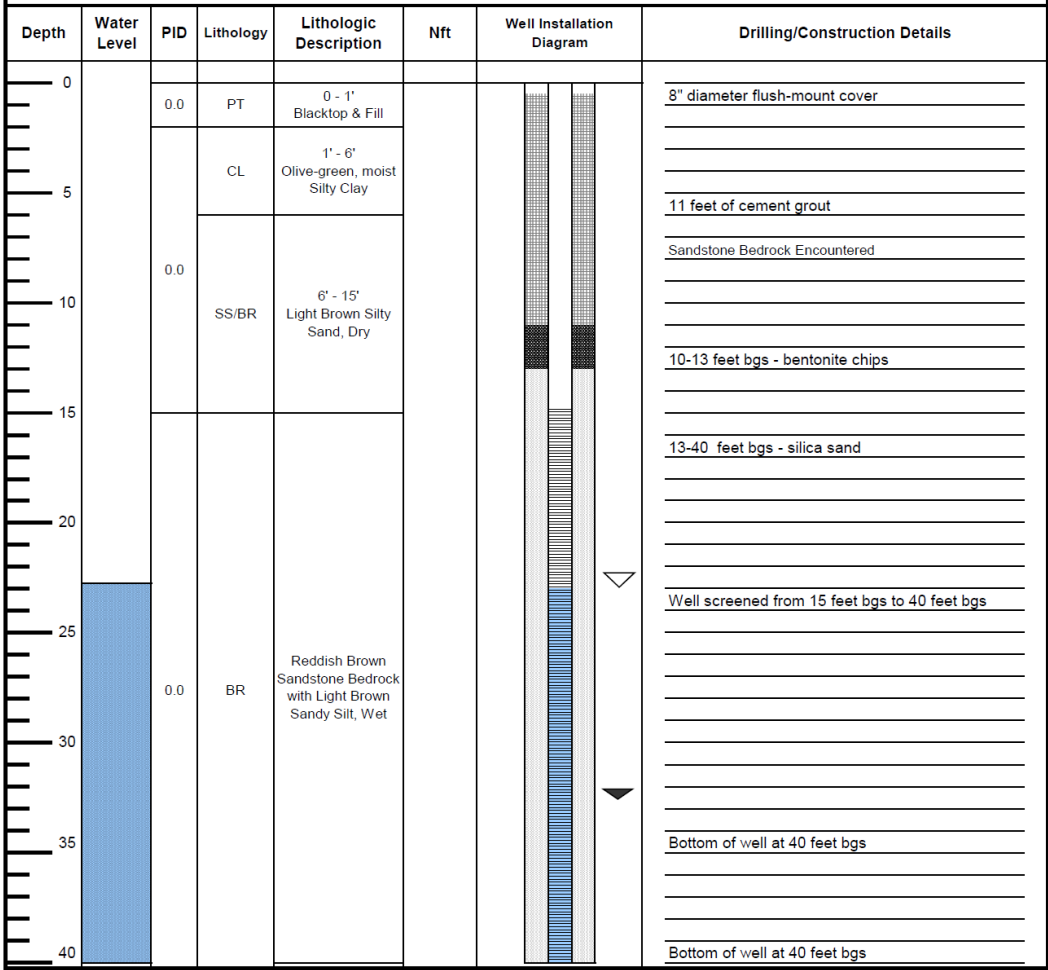


Site Hydrogeology

- 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



Well Construction



CVOC & 1,4-Dioxane Data Post Enhanced Anaerobic Biodegradation

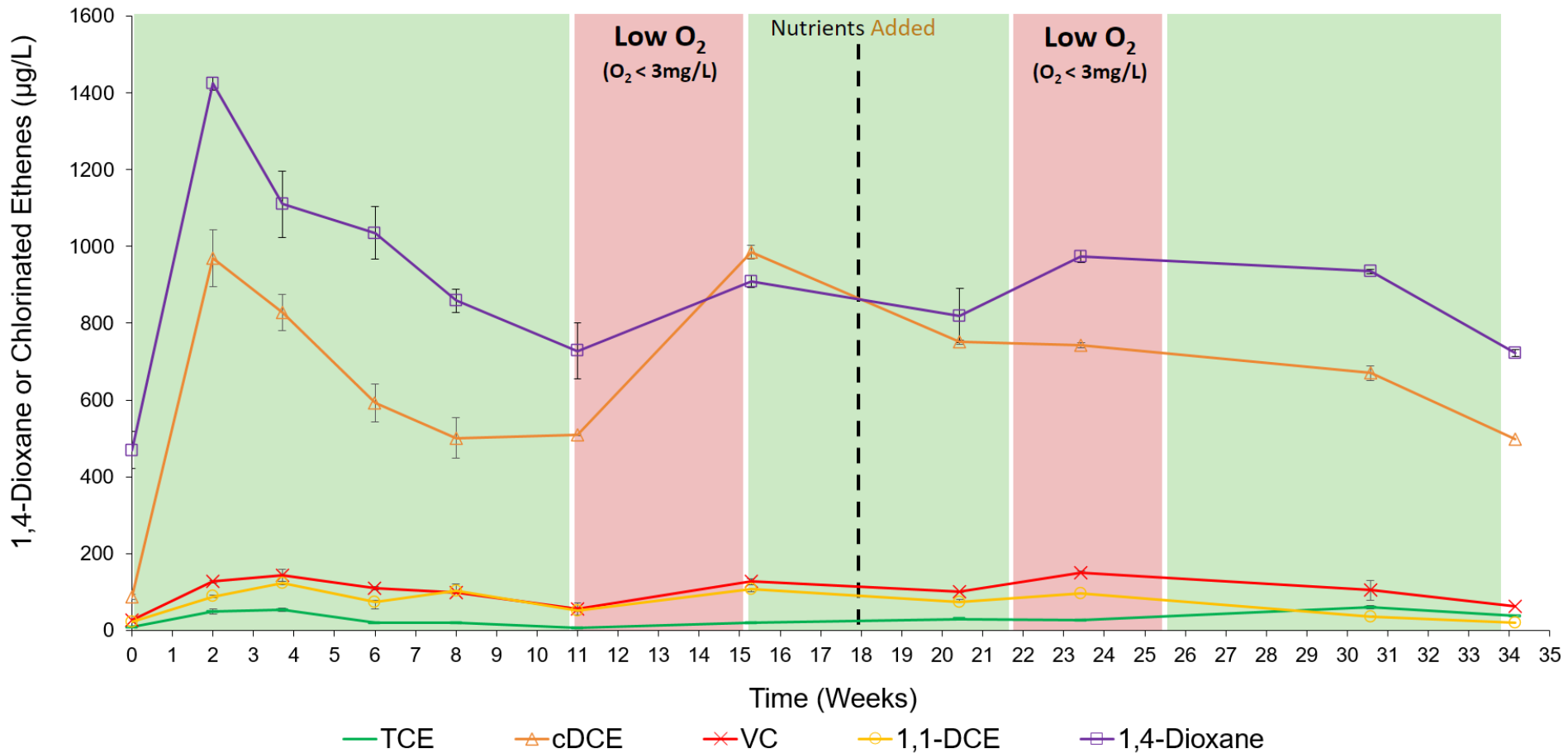
Sample Location	Sample Date	1,1,1-TCA	1,1-DCE	PCE	TCE	cis-1,2-DCE	Trans-1,2-DCE	Vinyl Chloride	1,4-Dioxane
Screen	Micrograms per Liter								
Standard		200	7	5	5	70	100	2	32
MW-30	4th Quarter 2019	67	330	660	890	340	1.2	17	65
MW-31	1st Quarter 2020	29	200	72	170	4,100	13	240	1,400
MW-32	1st Quarter 2020	200	1,100	210	150	5,300	8.4	840	1,600

Field Site: Electron Acceptor & Nutrient Data

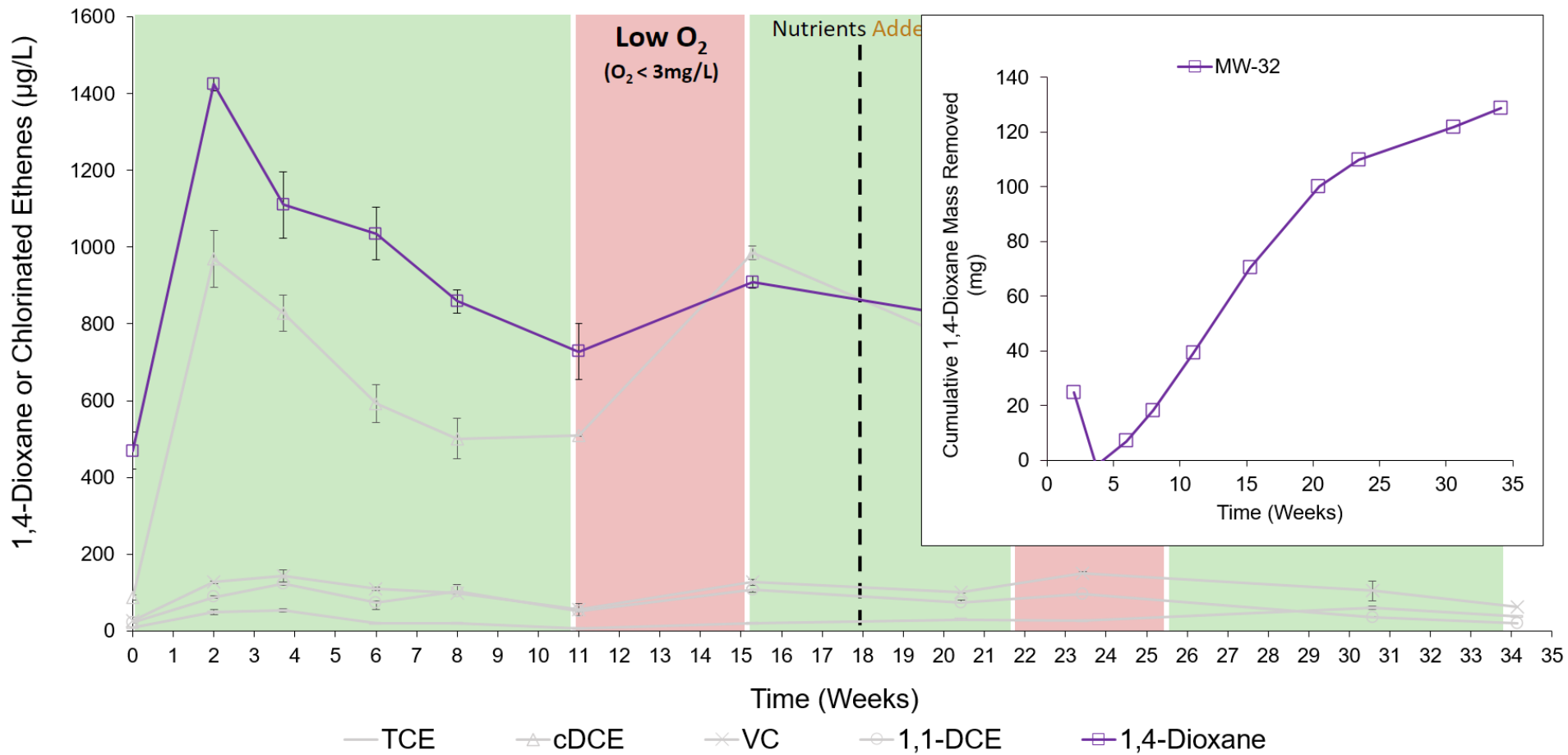
- Groundwater Data:
 - Dissolved Oxygen (DO): $< 0.5 - 4.3$ milligrams per liter (mg/L)
 - Nitrate: $0.6 - 1.9$ mg/L
 - Nitrite: < 0.03 mg/L
 - Sulfate: $23 - 32$ mg/L
 - TOC: $0.3 - 0.9$ mg/L
 - Total Kjeldahl Nitrogen: ≤ 0.1 mg/L
 - Ammonia: $0.08 - 0.1$ mg/L
 - Phosphorus: $0.1 - 0.2$ mg/L



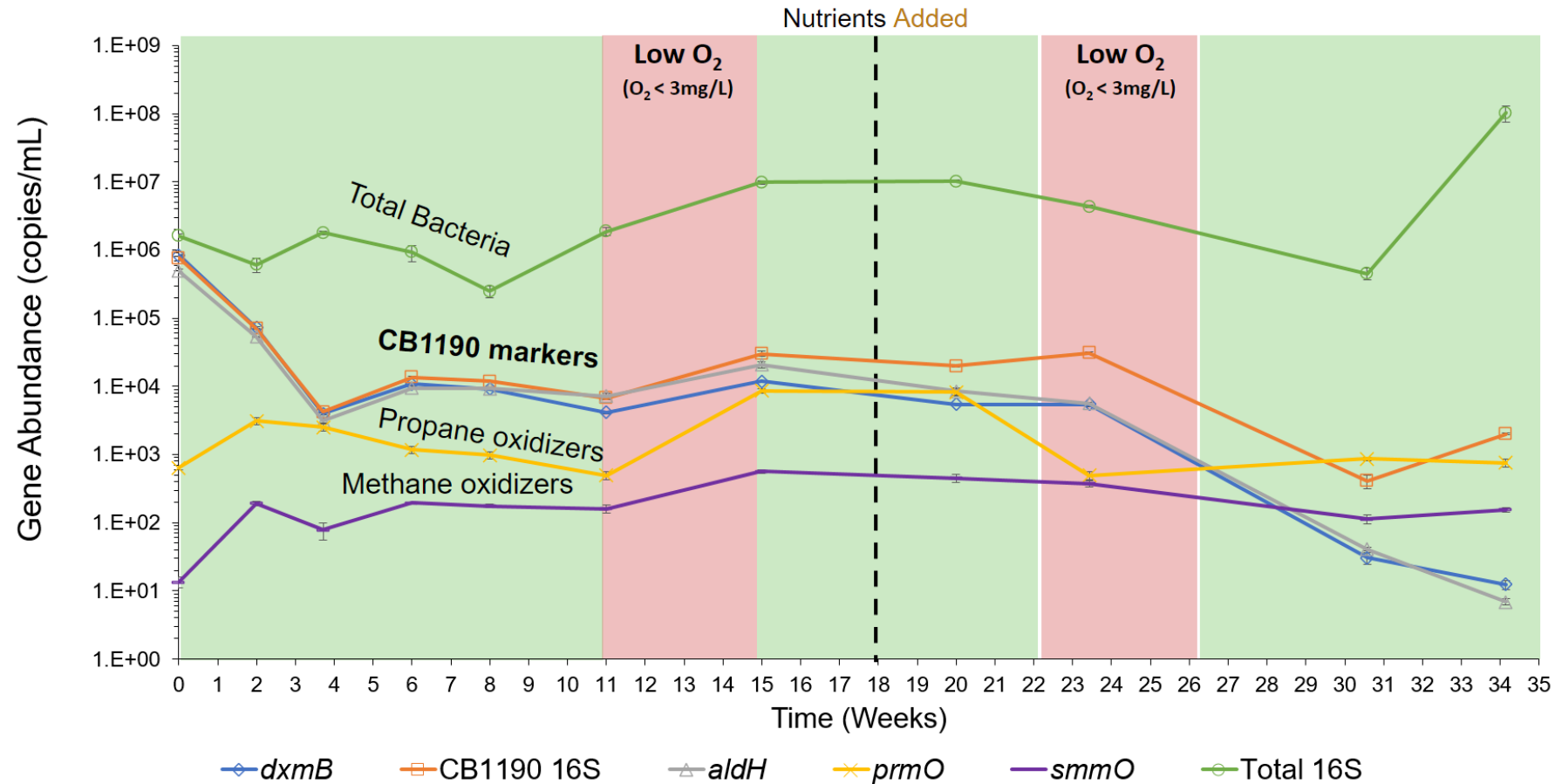
MW-32 Bioaugmented: Biodegradation Driven by O₂ & Nutrients



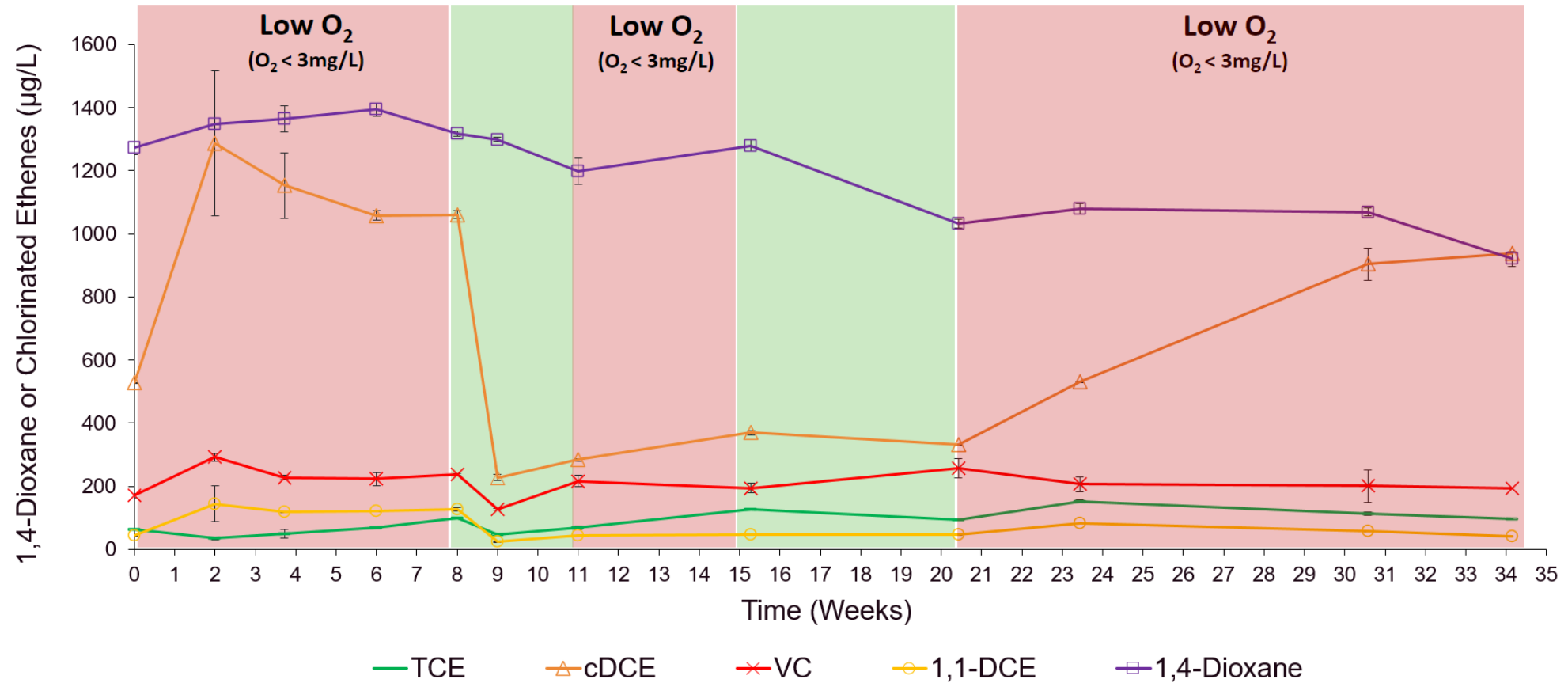
MW-32: 1,4-Dioxane Biodegradation Driven by O₂ & Nutrients



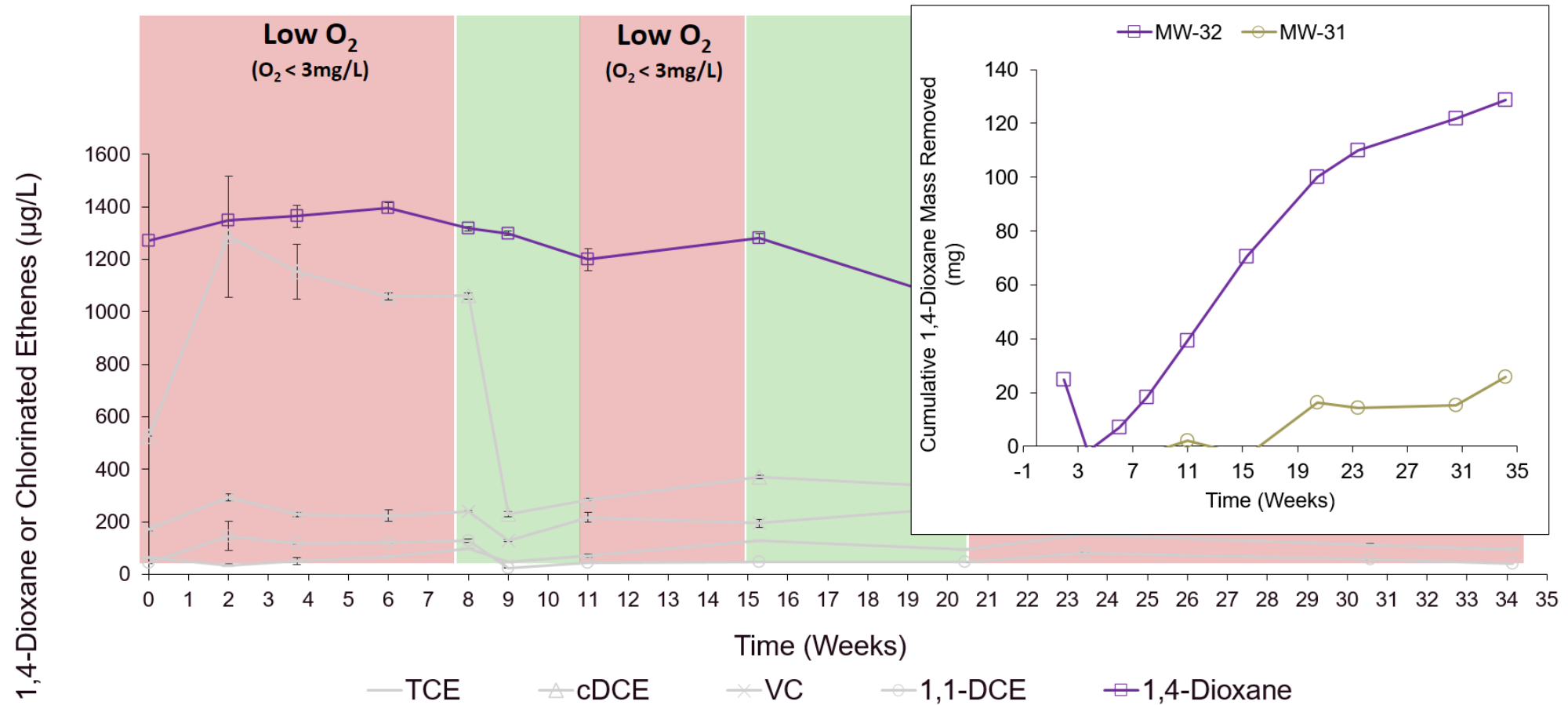
MW-32: *prmO* and *smmO* Biomarkers Present, but Remain Below CB1190 Markers



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



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

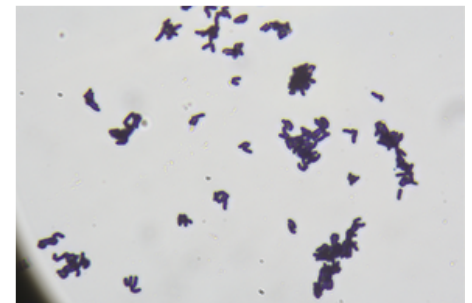


Post CB1190 Bioaugmentation Results

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
	3rd Quarter 2022	5.0	330	38	270	3,400	18	270	240
MW-32	1st Quarter 2020	200	1,100	210	150	5,300	8.4	840	1,600
	3rd Quarter 2022	7.8	36	2.2	15	350	2.7	23	280

Alternative Microbe: 1,4 D-Stroy™

- Information on 1,4 D-Stroy™
 - *Rhodococcus* sp.
 - Gram-positive aerobic bacterium
 - Naturally occurring and non-sporulating strain
 - Easy to cultivate and grows well in nutrient rich medium
 - Doesn't need the presence of 1,4-Dioxane to maintain degradation phenotype
 - Metabolically degrades 1,4-Dioxane in an aerobic environment
 - Provided by Allonnia



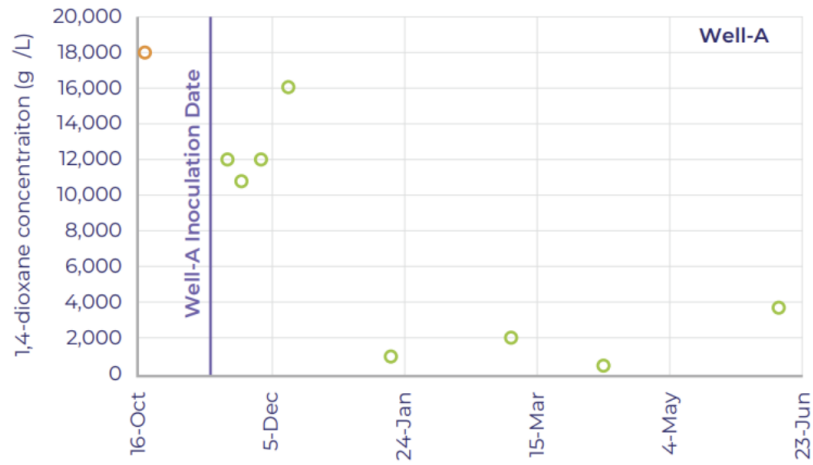
Figures provided by Allonnia

Alternative Microbe: 1,4 D-Stroy™

- Results for 1,4-Dioxane site using 1,4 D-Stroy™
- Able to biodegrade high concentrations of 1,4-dioxane

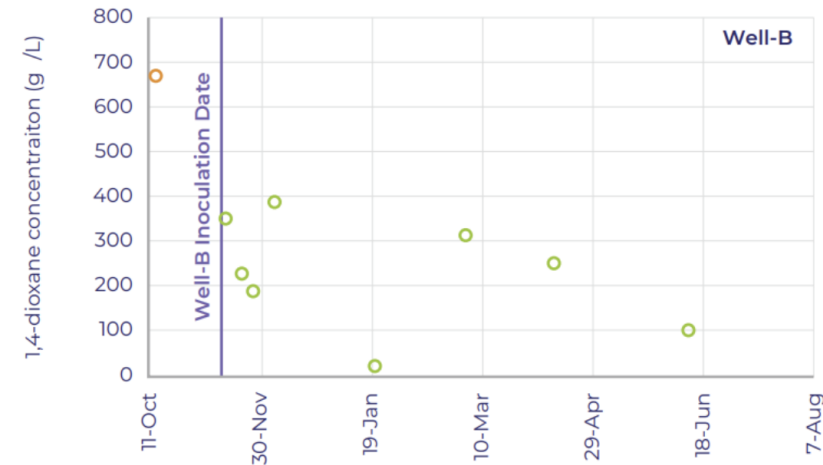
WELL-A:

- Up to 97% reduction of 1,4-dioxane (18,000-440 µg/L in 5 months)
- Negligible rebound observed in the well
- 33% reduction after just one week



WELL-B:

- Up to 98% reduction of 1,4-dioxane (670-12 µg/L in 2.5 months)
- 63% pilot study endpoint reduction after rebound
- 49% reduction after just one week



Figures provided
by Allonnia

Comparison of CVOC and 1,4-Dioxane Results

Table 1 Concentrations of CVOCs and 1,4-Dioxane Over Time									
	MW-32*			MW-31*			IW-3**		
Constituent:	QTR2 23-Apr	QTR3 23-Sep	QTR4 23-Dec	QTR2 23-Apr	QTR3 23-Sep	QTR4 23-Dec	QTR2 23-Apr	QTR3 23-Sep	QTR4 23-Dec
1,1,1-Trichloroethane	24	1	6.8	<2.4	<0.24	<0.24	<0.24	<0.24	<0.24
1,1-Dichloroethene	130	4	39	240	1.7	<0.26	1.3	<0.26	0.93
<i>cis</i> -1,2-Dichloroethene	1,000	110	270	2,500	21	4	4.7	0.31	1
Tetrachloroethene	37	1.2	14	47	1.2	<0.25	0.46	0.43	0.26J
<i>trans</i> -1,2-Dichloroethene	5.2	0.25	2.4	14	<0.24	<0.24	0.37	<0.24	<0.35J
Trichloroethene	44	3.7	17	130	3.1	0.44J	0.51	0.32	<0.31
Vinyl Chloride	62	1.9	28	450	1.7	0.26J	2.9	0.28	3.1
Total VOC Concentration	1302.2	122.05	377.2	3381	28.7	4.7	10.24	1.34	5.64
1,4-Dioxane	510	620	510	280	190	85	210	9	31

Note:

* Bioaugmented with CB1190; ** Bioaugmented with 1,4 D-Story; All results are reported in micrograms per liter (µg/L).

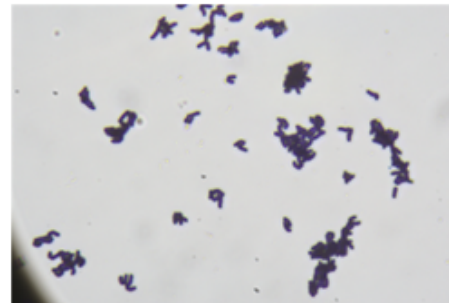
< = Less than. Value is the analytical reporting limit for that constituent.

Bold = Analytical result is greater than or equal to the Maximum Contaminant Level (MCL).

Comparison of CB1190 & 1,4 D-Stroy™

Microbial Enumeration Results Pre and Post Bioaugmentation (cells/milliliter)

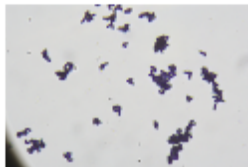
Well ID	Microbe	7/25/23	7/26/23	8/4/23	8/16/23	8/31/23	9/14/23	9/21/23
MW-31	CB1190	8.50E+00		2.39E+03	4.60E+00		4.64E+01	1.30E+00
MW-32	CB1190	<4.7E+00	6.60E+00		1.22E+01		6.88E+01	2.0E+00
IW-3	1,4 D-Stroy™					<1.60E+01	3.16E+04	6.34E+03



Comparison of CB1190 & 1,4 D-Stroy™

	MW-32*			MW-31*			IW-3**		
Constituent:	QTR2	QTR3	QTR4	QTR2	QTR3	QTR4	QTR2	QTR3	QTR4
	23-Apr	23-Sep	23-Dec	23-Apr	23-Sep	23-Dec	23-Apr	23-Sep	23-Dec
Bioaugmentation Injection	Pre	Post	Post	Pre	Post	Post	Pre	Post	Post
1,4-Dioxane (mg/L)	510	620	510	280	190	85	210	9	31
CB1190 (cells/ml)	12.2	68.8		4.6	1.3				
1,4-D Stroy™ (cells/ml)							1.6	6.34E+03	
1,4-Dioxane mg/L removed per cells/ml of 1,4-Dioxane degrader		-1.9			27.3			3.2E-02	

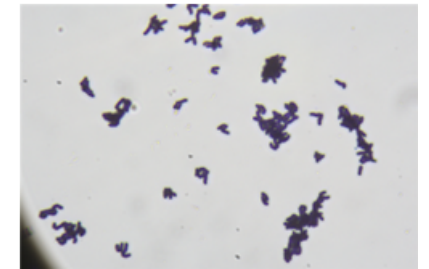
Note: * CB1190 injected: ** 1,4-D Story injected

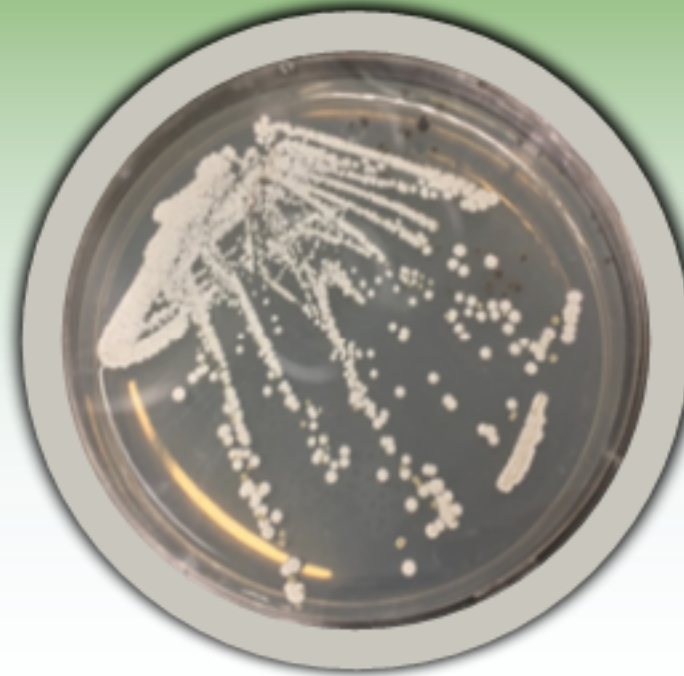
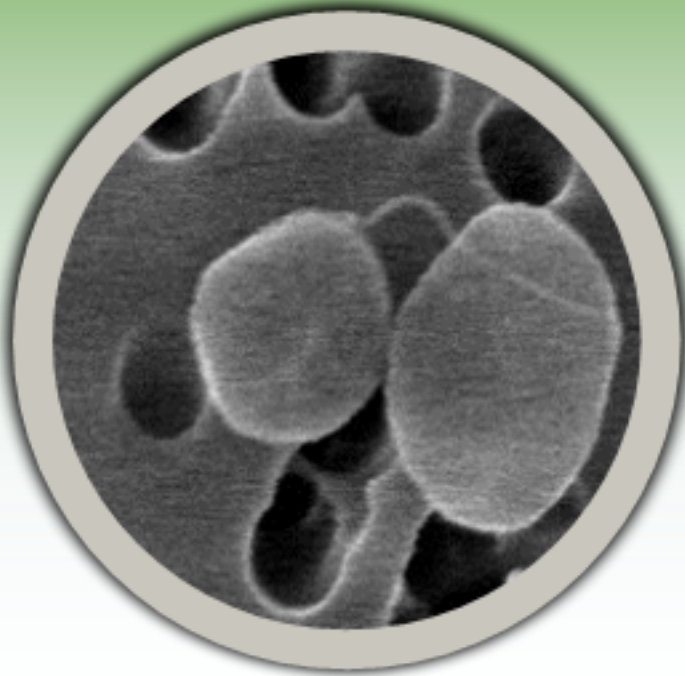


Conclusions



- Both CB1190 and 1,4 D-Stroy™ can biodegrade 1,4-Dioxane
- 1,4 D-Stroy™ showed higher level of 1,4-Dioxane degradation during 4-month time period
- However, CB1190 showed higher degradation of 1,4-dioxane per cells/ml
- CB1190 shown to degrade cis-1,2-DCE and VC
- Additional bioaugmentation in 2024





Questions?

LLapat@mnwe.com