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

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MATRIXNEWORLD
Engineering Progress

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

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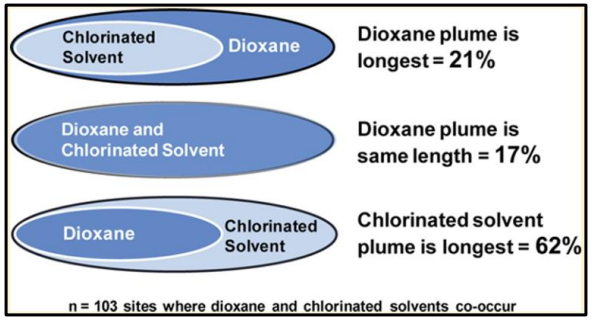
A Multisite Survey To Identify the Scale of the 1,4-Dioxane Problem at Contaminated Groundwater Sites

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

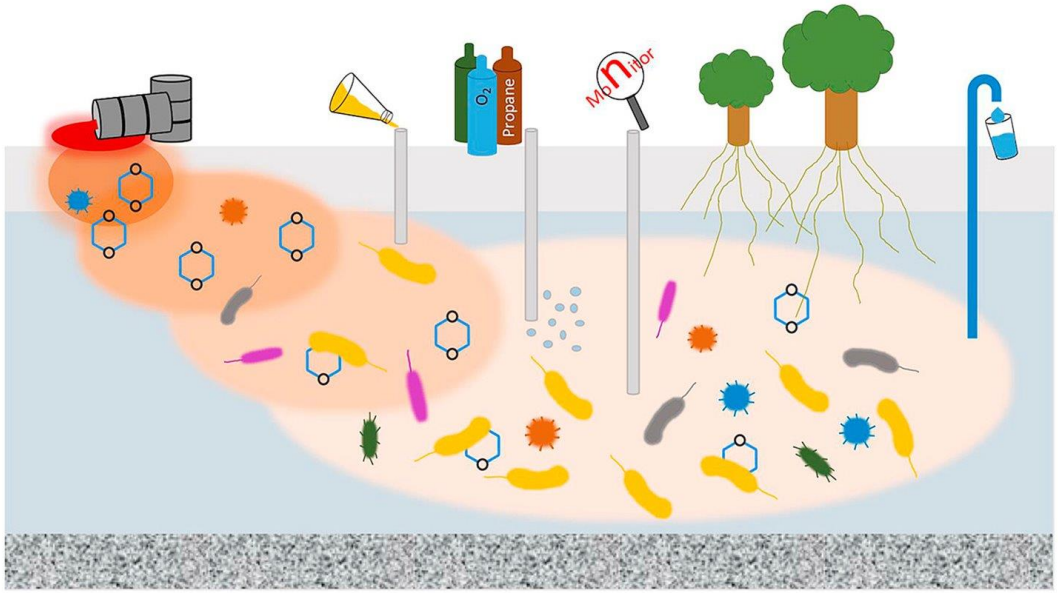
Environmental Science & Technology

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†



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

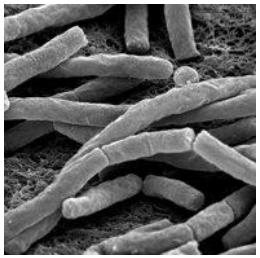


Pseudonocardia dioxanivorans CB1190

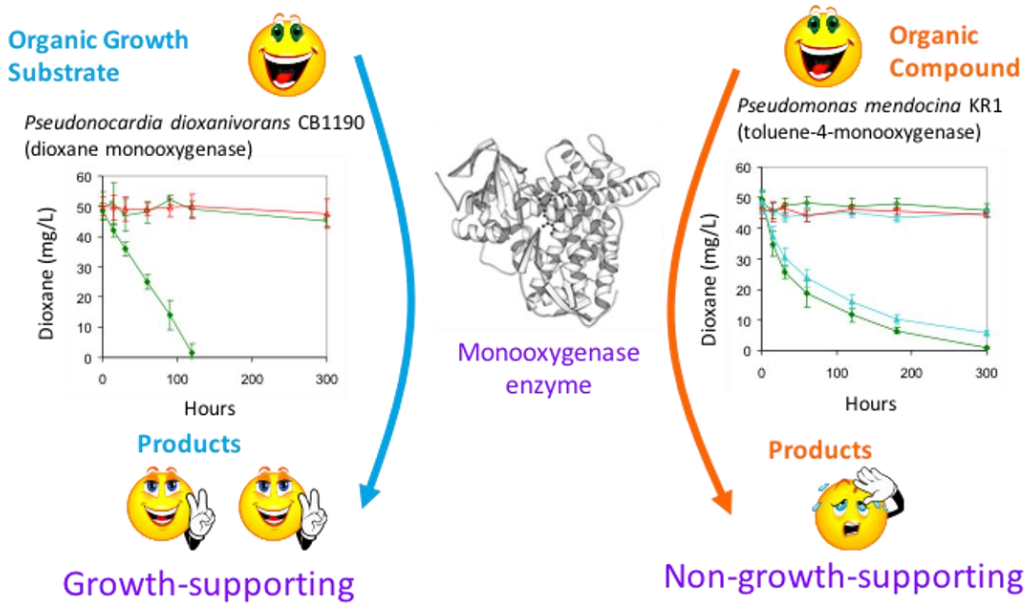
Rhodococcus ruber ENV425

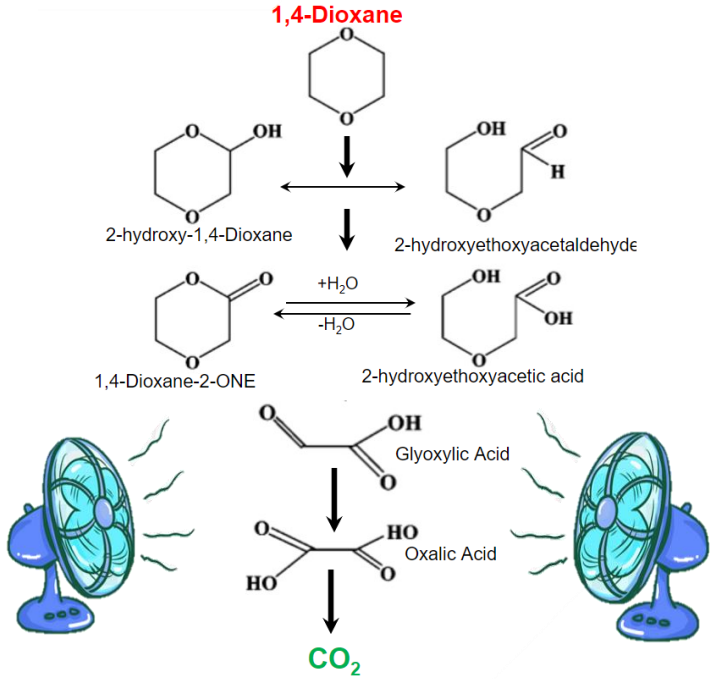
Methylosinus trichosporium OB3b

CB1190 Aerobically Biodegrades 1,4-Dioxane



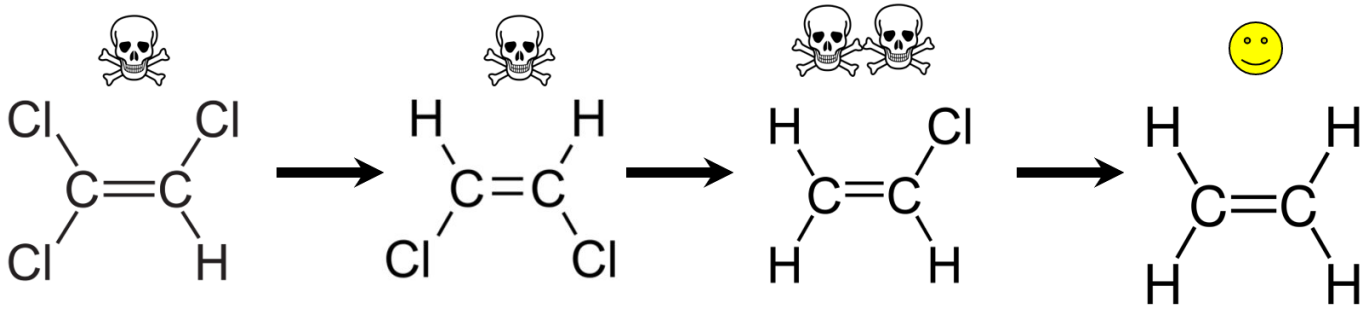
- **Metabolism**: microbe **gains energy** and carbon from contaminant
- **Co-metabolism**: microbe produces an enzyme to metabolize a primary substrate; the enzyme will also transform the contaminant of concern





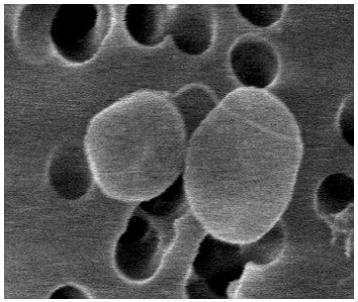
Mahendra & Alvarez-Cohen, IJSEM, 2005; Mahendra et al. ES&T. 2006; Grostern et al. ES&T. 2012

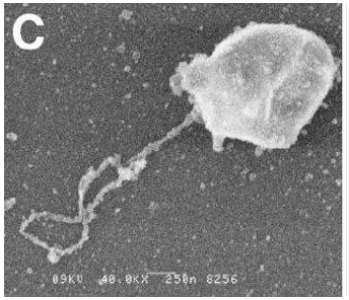
Dehalococcoides (Dhc) Anaerobically Biodegrades TCE



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

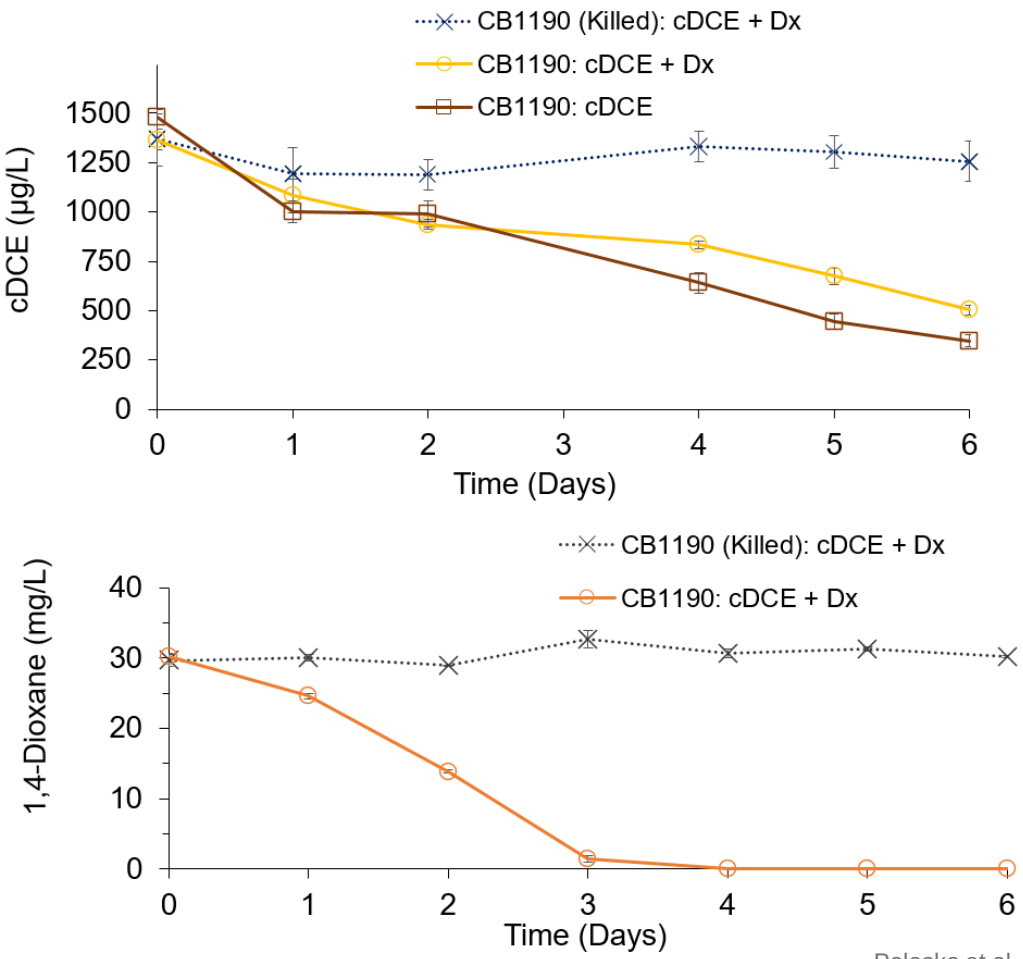
Good News / Bad News

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

Good News / Good News

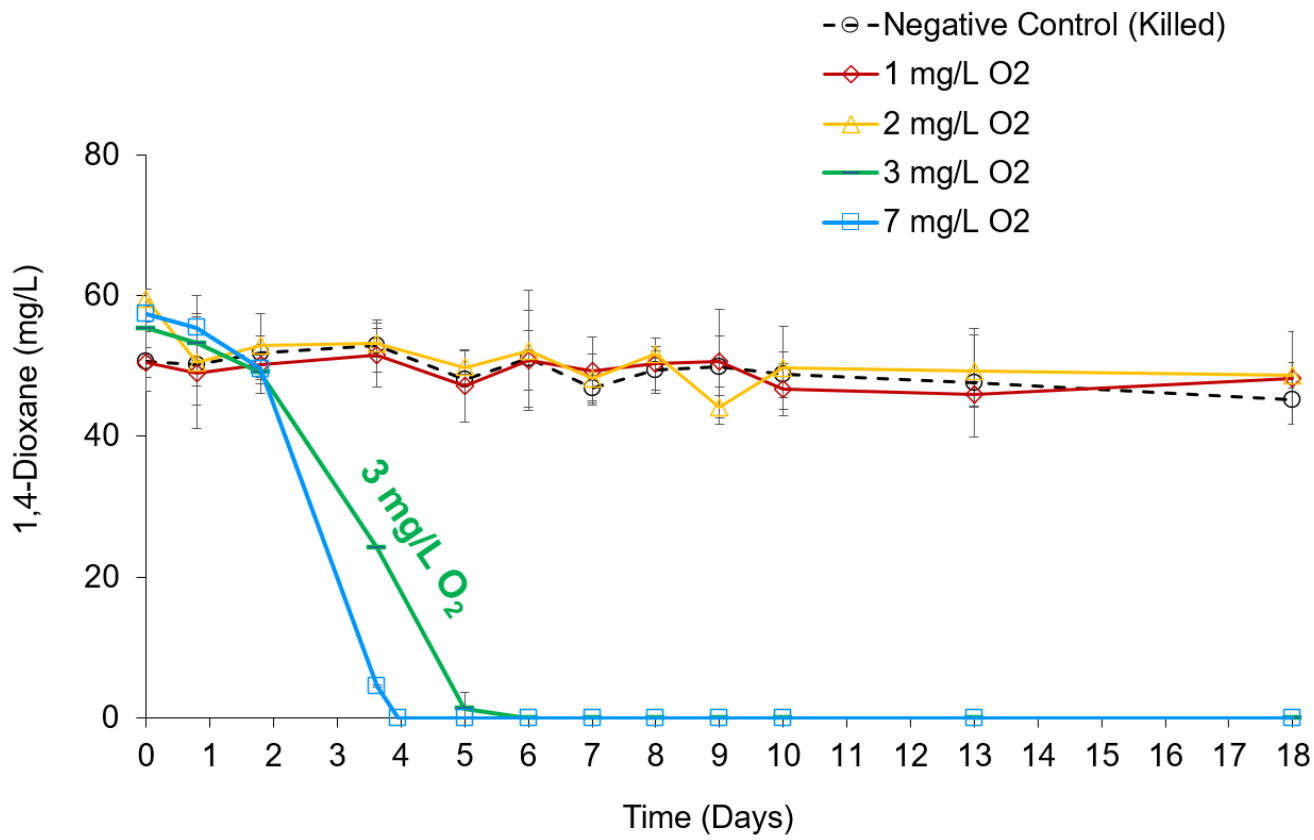
- Good News: TCE and 1,4-Dioxane are Biodegradable
- ~~Bad~~ Good News: Can Combine Anaerobes and Aerobes

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



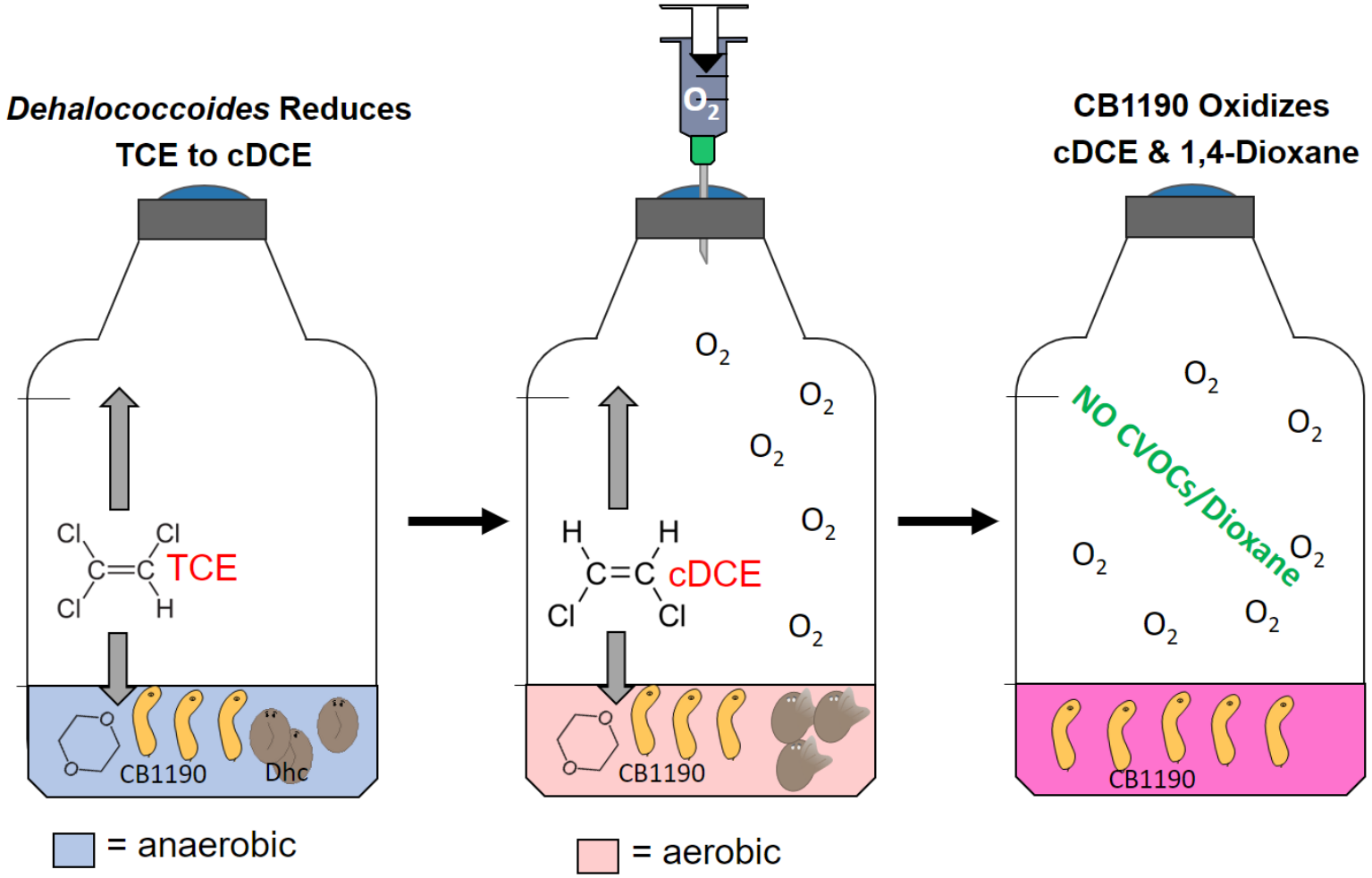
Polasko et al. *EST&L*, 2019

CB1190 Can Degrade 1,4-Dioxane with as Little as 3 mg/L Oxygen (O_2)

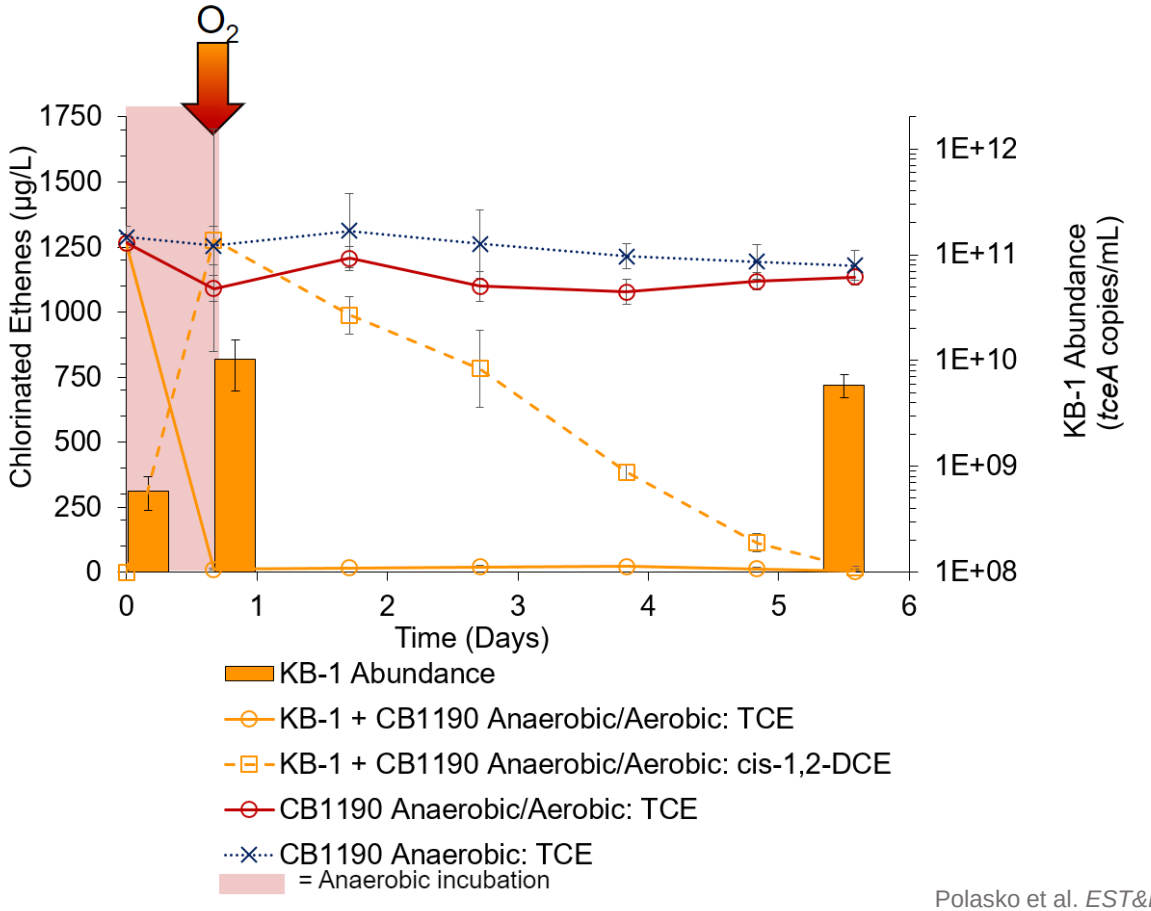


Polasko et al. *EST&L*, 2019

Engineered Microbial Community

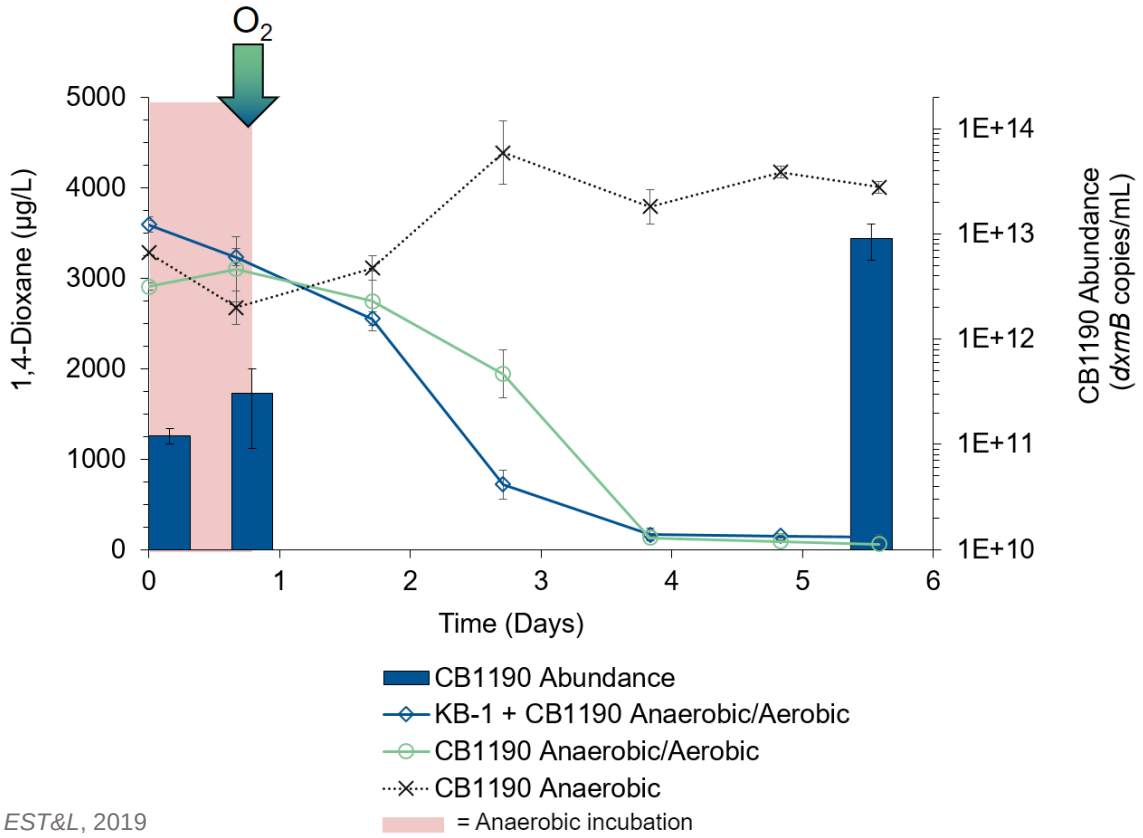


Dhc Degrades TCE → cDCE; then CB1190 Degrades cDCE



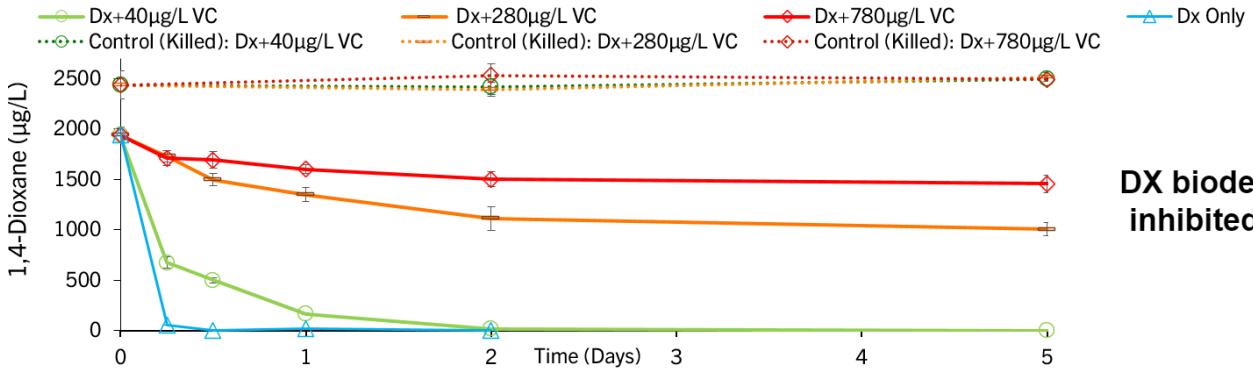
Polasko et al. *EST&L*, 2019

Mixed Culture & Strain CB1190 Degrade 1,4-Dioxane

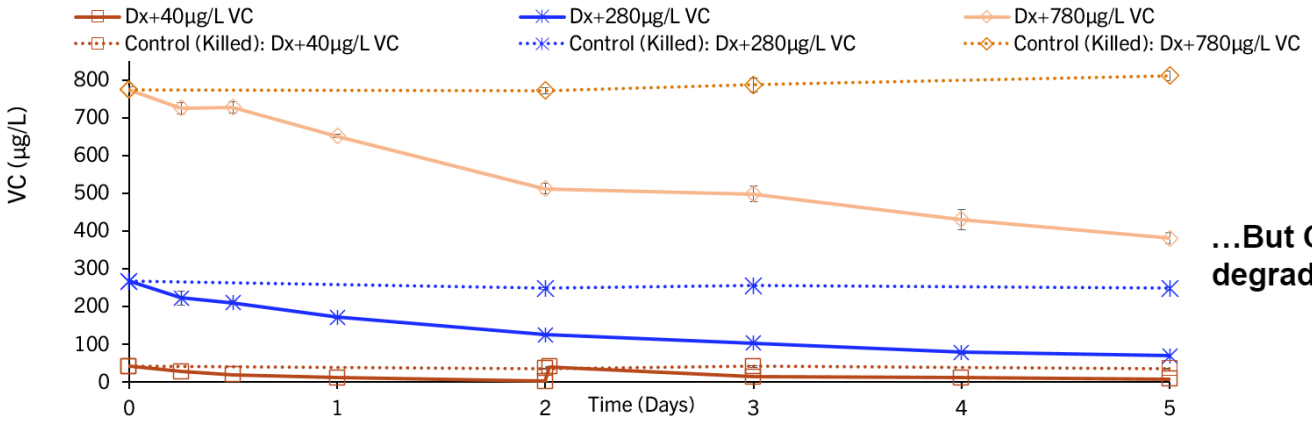


Polasko et al. *EST&L*, 2019

CB1190 Biodegrades VC and Dioxane Simultaneously



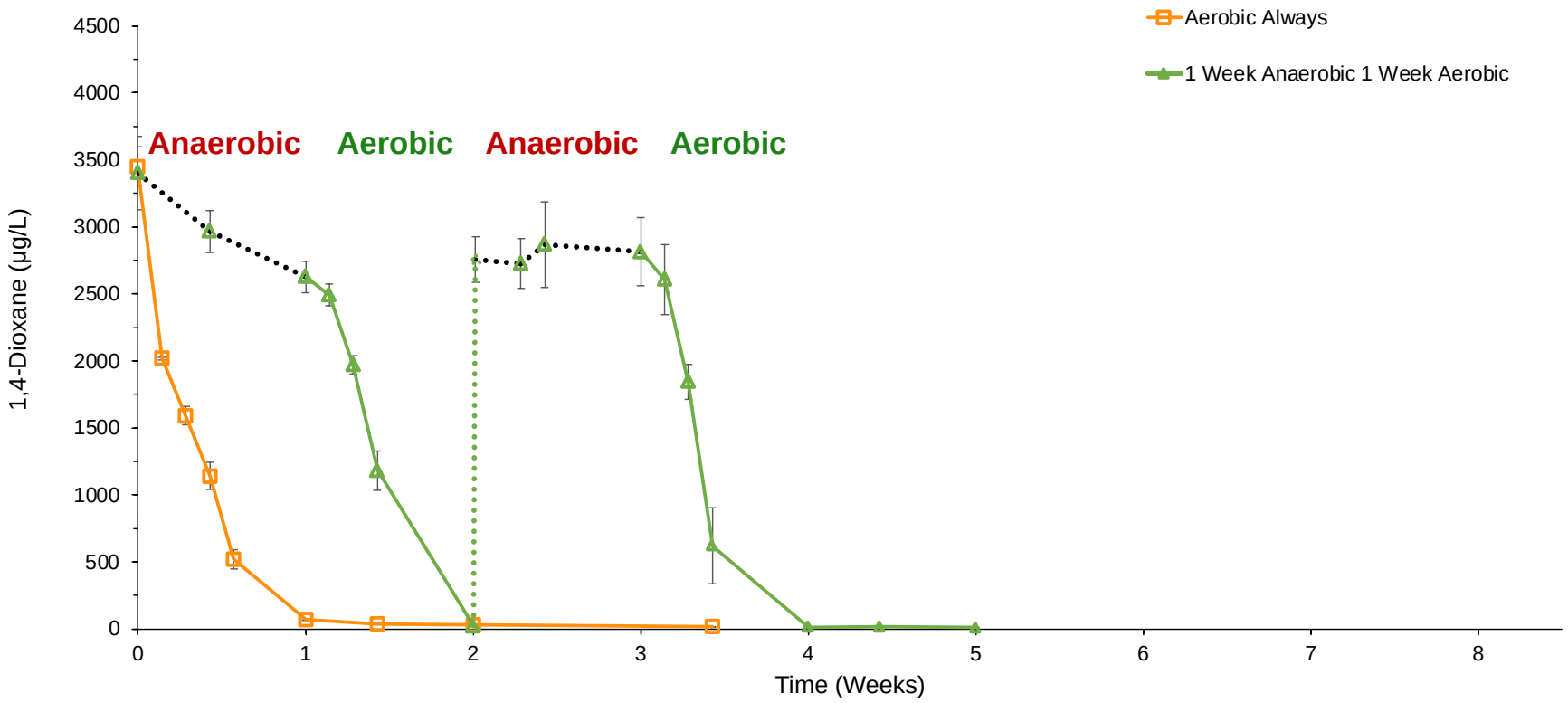
DX biodegradation inhibited by VC…



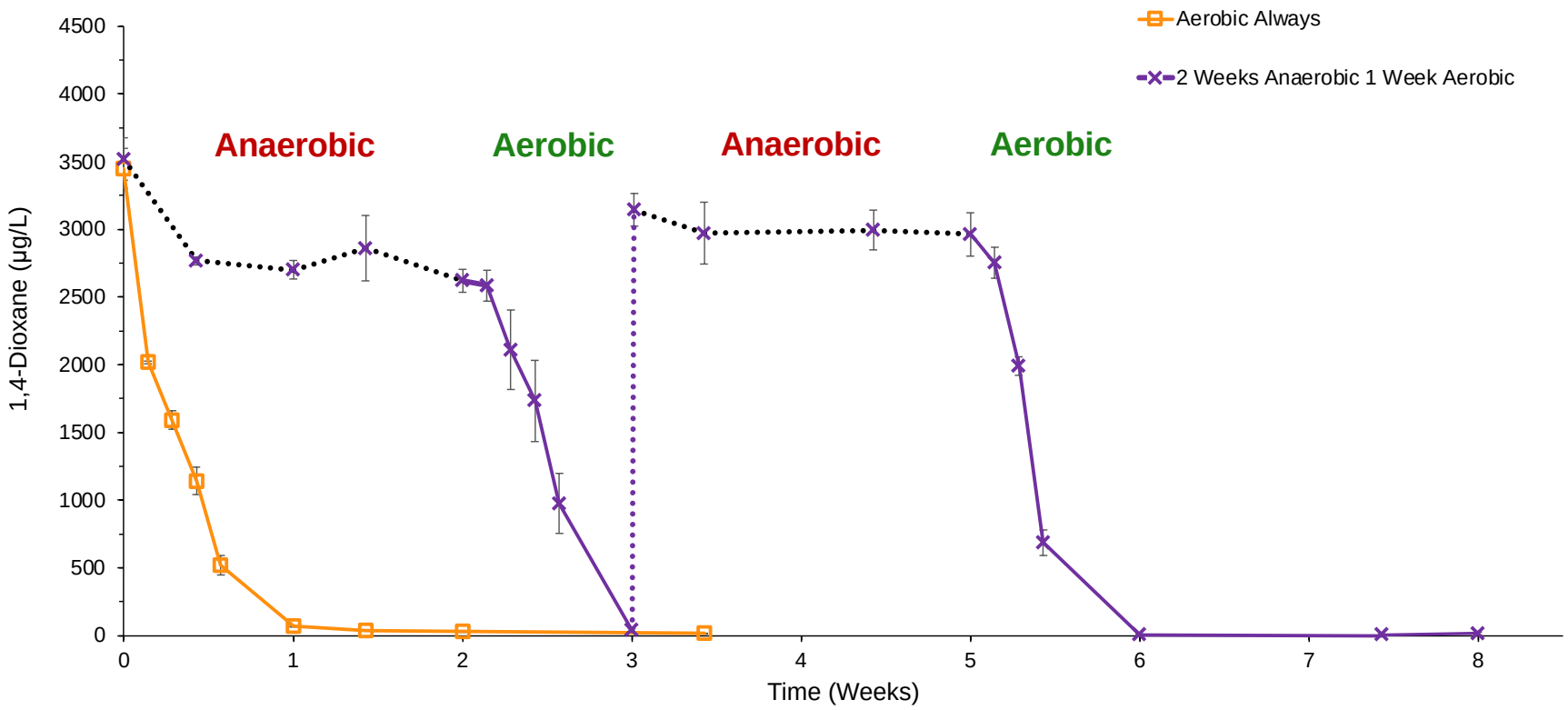
…But CB1190 can degrade inhibitor!

How Long Can CB1190 (Aerobe) Survive Without Oxygen?

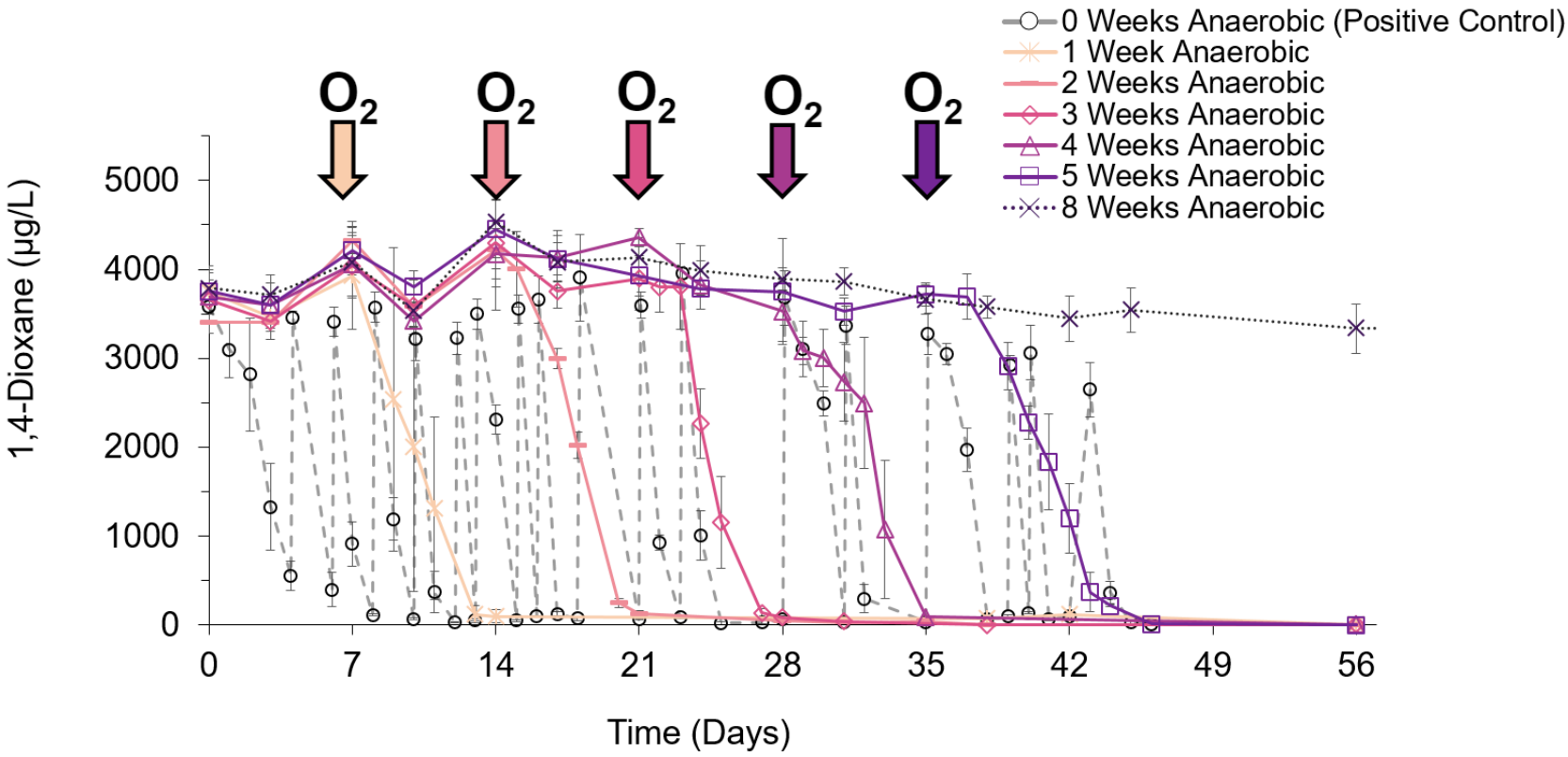
CB1190 Degrades 1,4-Dioxane After ONE-Week Anaerobic Cycles



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

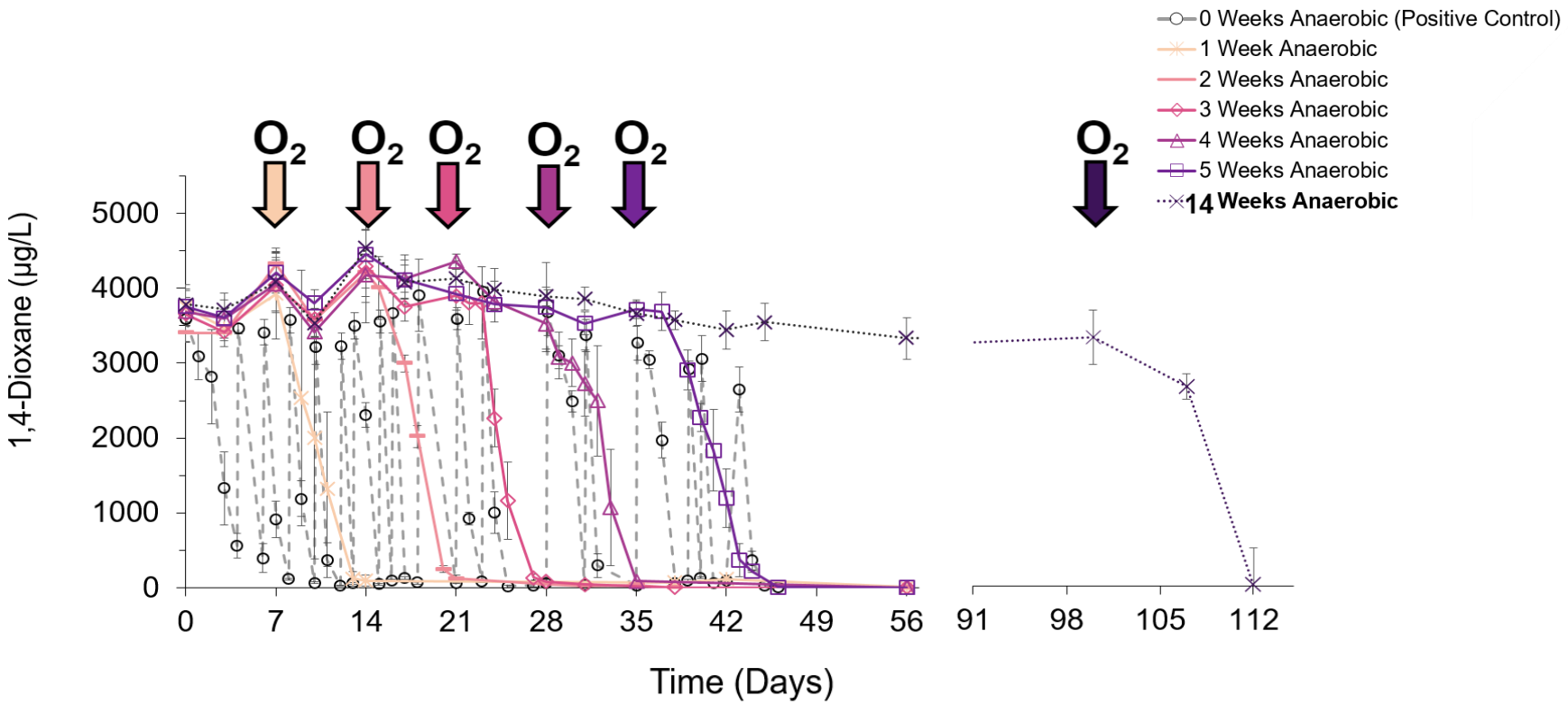


1,4-Dioxane Was Not Degraded in Anaerobic Bottles



Polasko et al. *EST&L*, 2019

CB1190 Degrades 1,4-Dioxane After 14 Weeks (100 Days) Without O_2!



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 can biodegrade 1,4-dioxane with as little as 3 mg/L O2
- Monooxygenase enzymes in 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!***

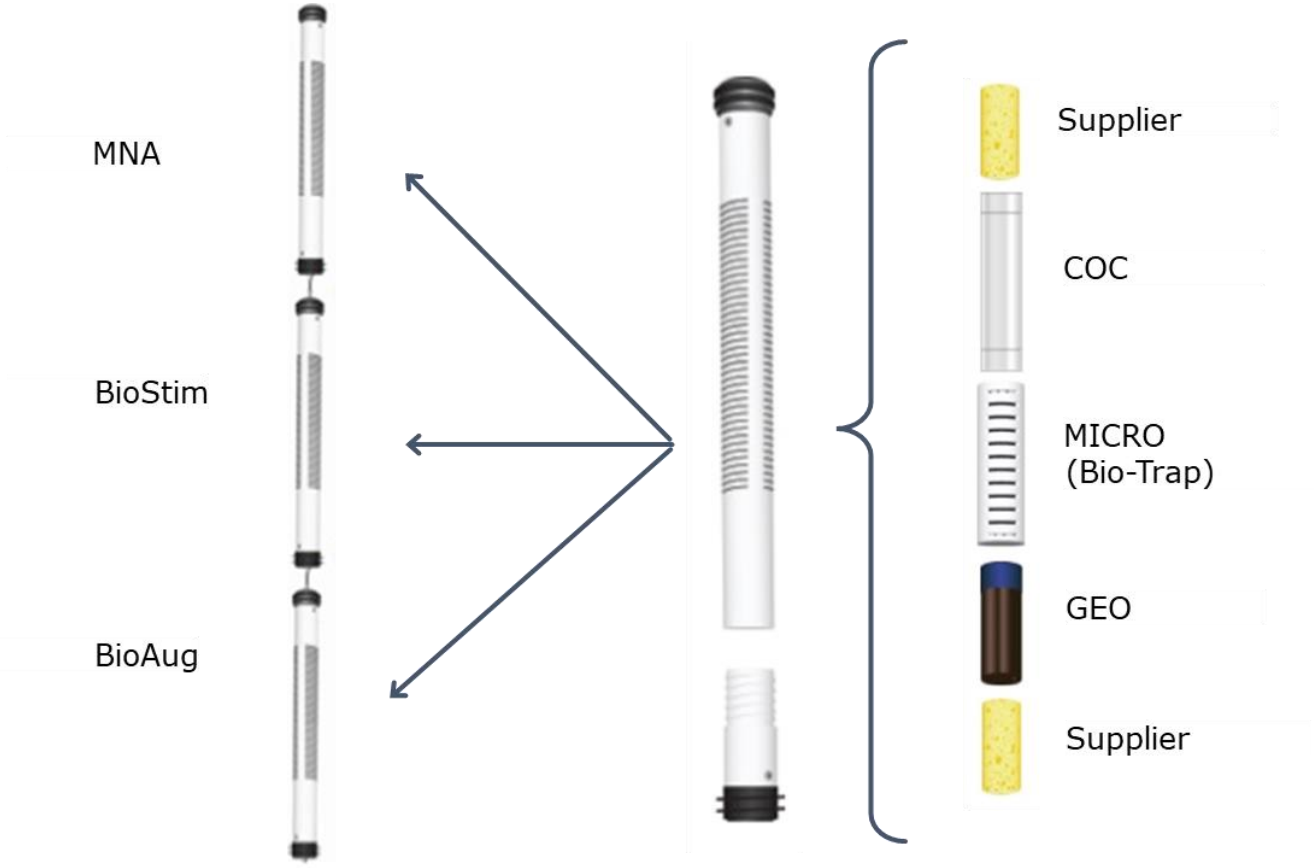
Site Background Information

- Manufacturing company in Eastern USA (>50 years ago)
- 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 (Screen Depth)	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
Feet bgs	Micrograms per Liter									
MCL		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	12,472
(15-40)	6/10/2016	190	1700	860	2100	4.3	3300	20	4300	12,864
MW-32	11/19/2015	880	2300	3100	3000	6.9	3900	110	1800	13,297
(15-40)	6/10/2016	600	1400	2100	2000	9.1	2400	55	1900	10,174

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

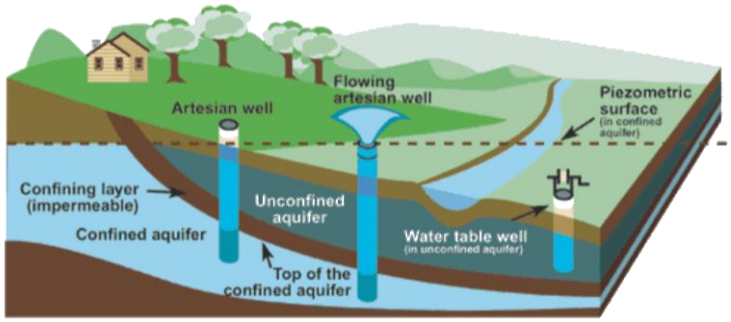
Client Sample ID:	Units	MW-32 MNA	MW-31 BioAug	MW-30 BioAug+ORC+Osmo	MW-30 BioAug+Osmo
CSIA of 1,4-dioxane Carbon	$\delta^{13}C$ (‰, VPDB)	-30.6	-29.3	-26.4	-23.8

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!

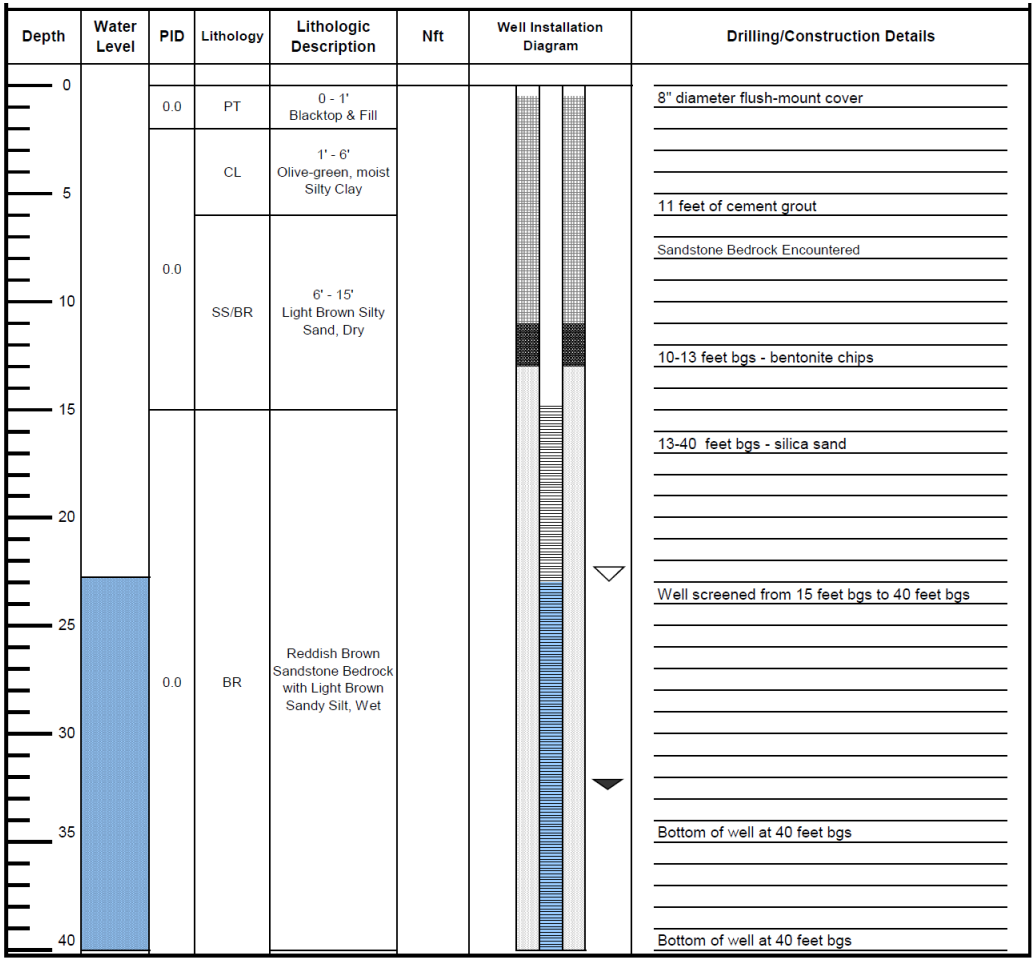
CB1190

Site Hydrogeology



- Two distinct water-bearing zones, separated by siltstone and shale layers
- Groundwater in the shallow, unconfined aquifer occurs from 4 to 30 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



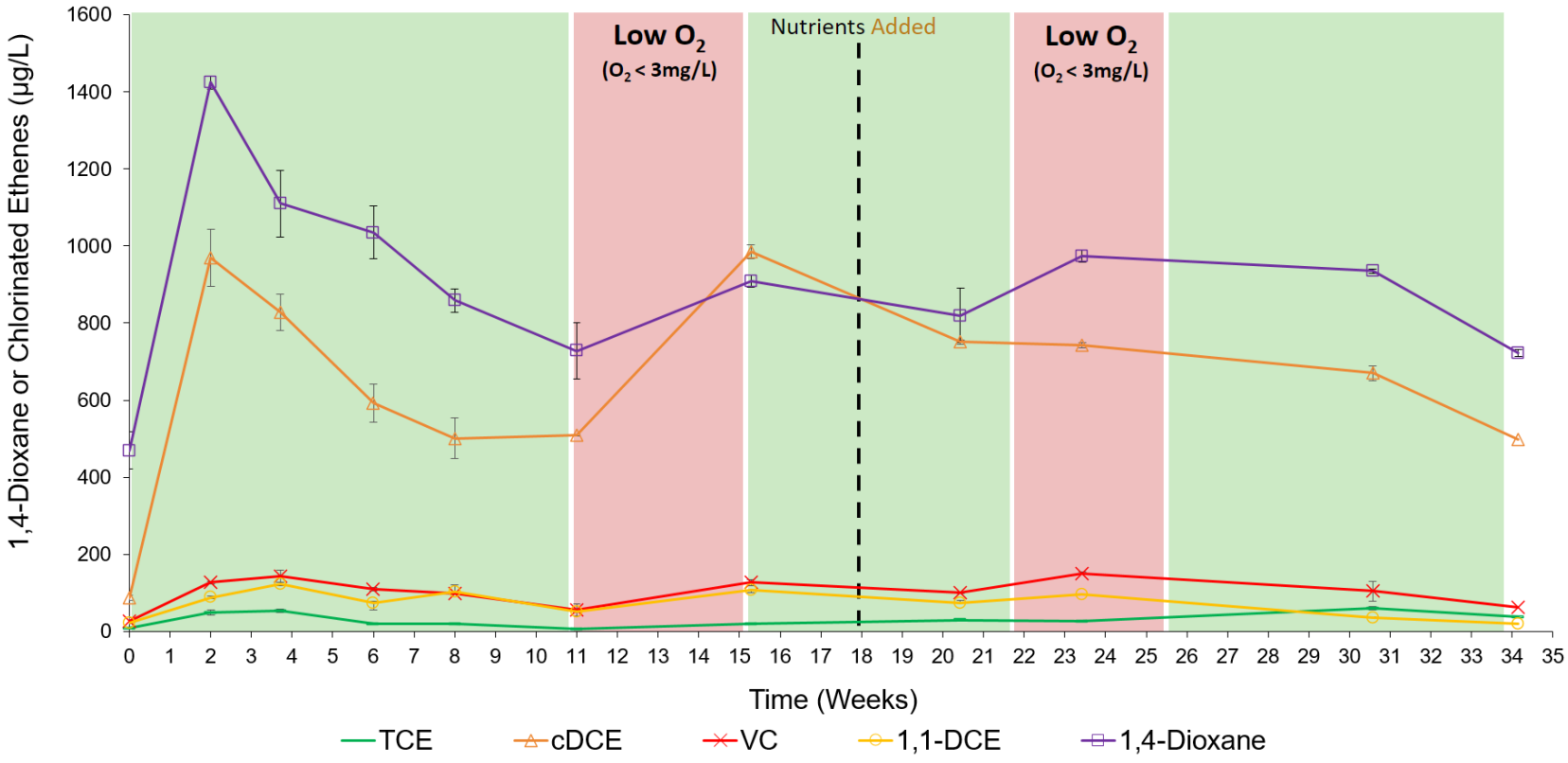
CVOC & 1,4-Dioxane Data, Post-Enhanced Anaerobic Biodegradation

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
	Micrograms per Liter								
MCL		200	7	5	5	70	100	2	32
MW-30	2016 QTR 2	430	1,000	2,300	2,300	510	<4.5	18	3,700
	2019 QTR 4	67	330	660	890	340	1.2	17	65
MW-31	2016 QTR 2	190	1,700	2,100	3,300	860	4.3	20	4,300
	2020 QTR 1	29	200	72	170	4,100	13	240	1,400
MW-32	2016 QTR 2	600	1,400	3,000	3,900	3,100	6.9	110	1,800
	2020 QTR 1	200	1,100	210	150	5,300	8.4	840	1,600

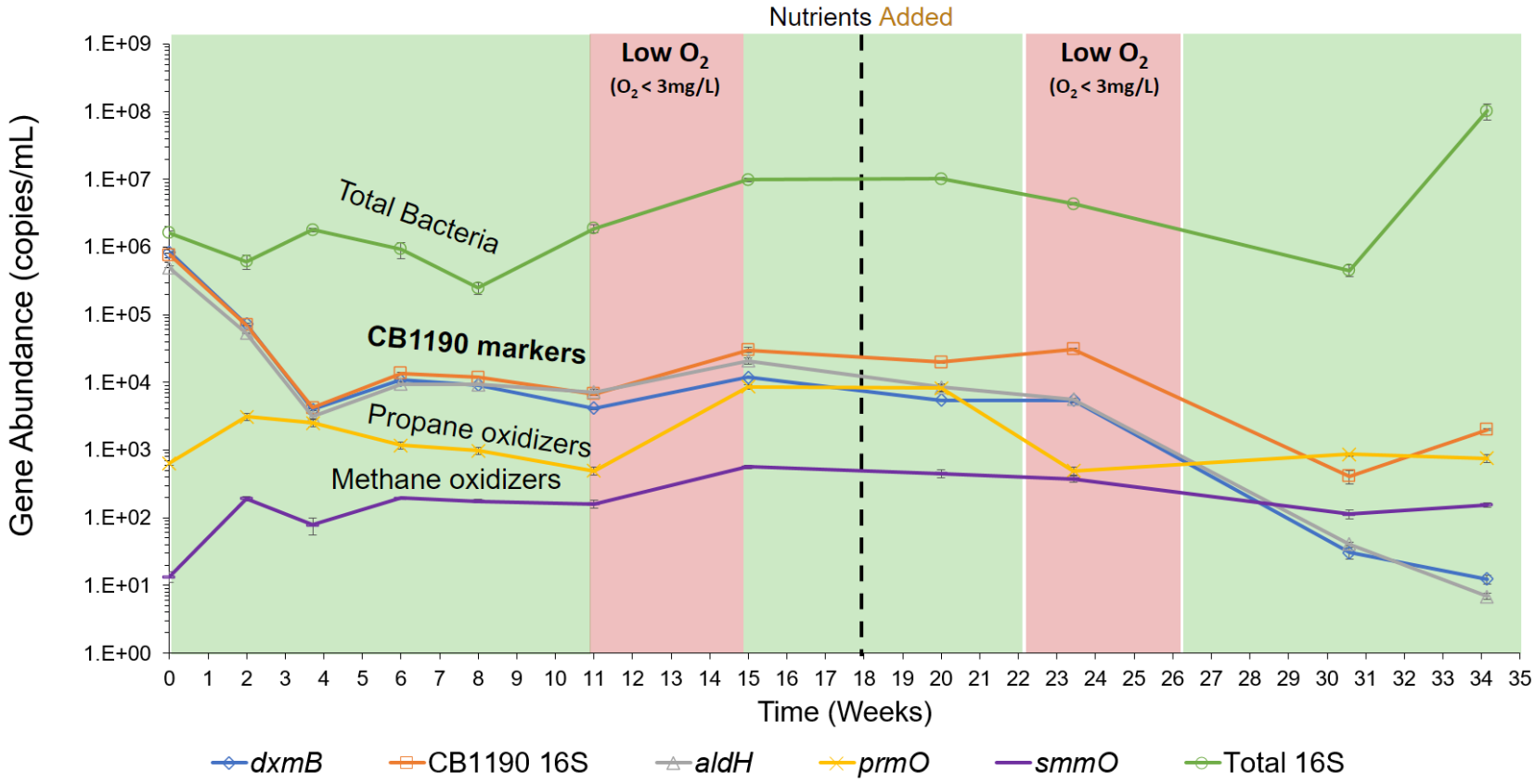
Field Site: Electron Acceptor & Nutrient Groundwater Data

- 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

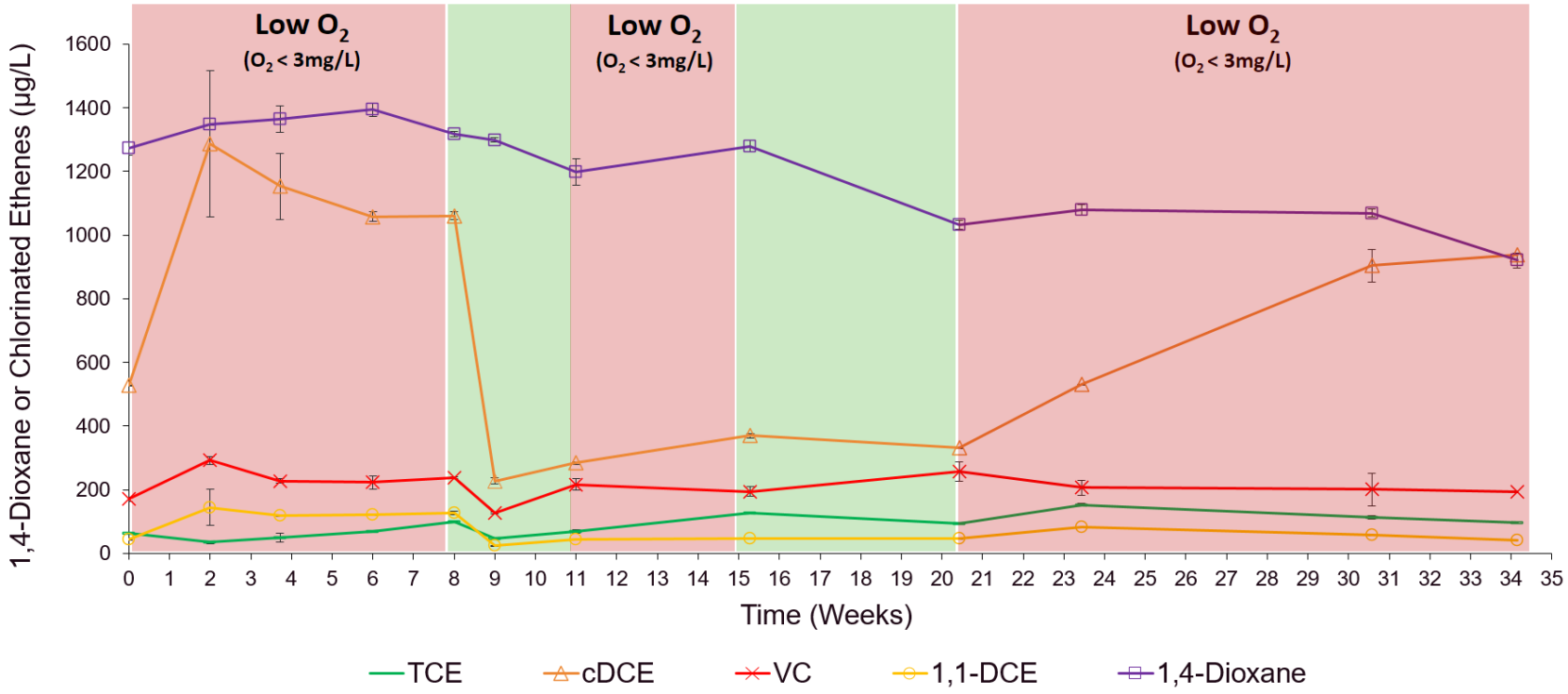
MW-32 Bioaugmented: Biodegradation Driven by O_2 & Nutrients



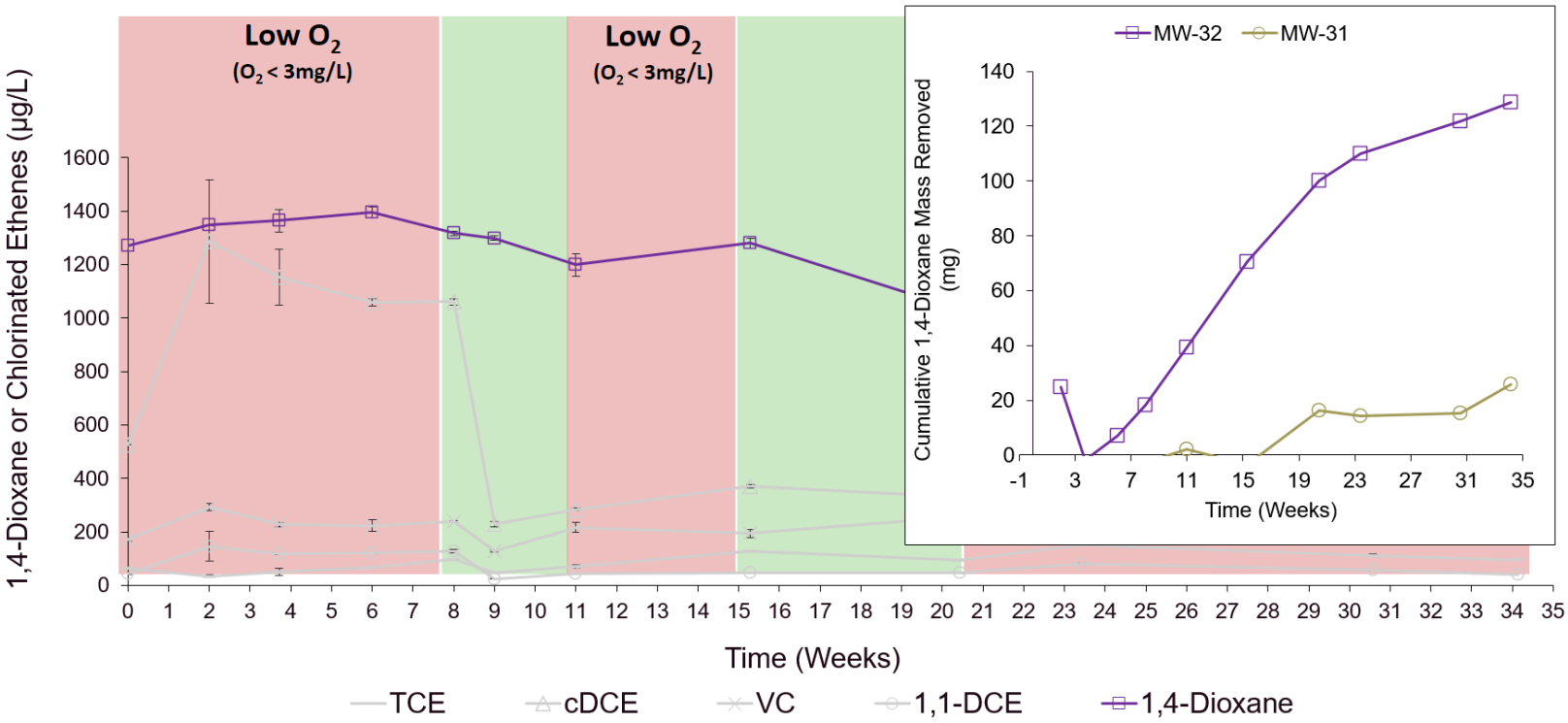
MW-32: *prm*O and *smm*O Biomarkers Present, but Remain Below CB1190 Markers



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



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

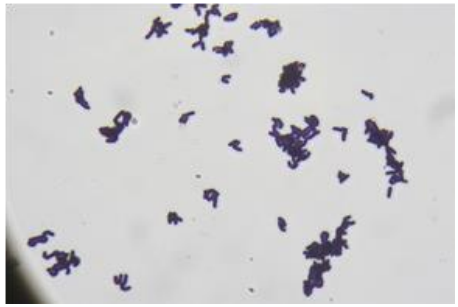


Post CB1190 Bioaugmentation Results

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
	Micrograms per Liter								
MCL		200	7	5	5	70	100	2	32
MW-31	2020 QTR1	29	200	72	170	4,100	13	240	1,400
	2022 QTR3	5.0	330	38	270	3,400	18	270	240
MW-32	2020 QTR1	200	1,100	210	150	5,300	8.4	840	1,600
	2022 QTR3	7.8	36	2.2	15	350	2.7	23	280

Alternative Microbe: 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 1,4-Dioxane to maintain degradation phenotype
- Metabolically degrades 1,4-Dioxane in aerobic environments
- Provided by Allonnia

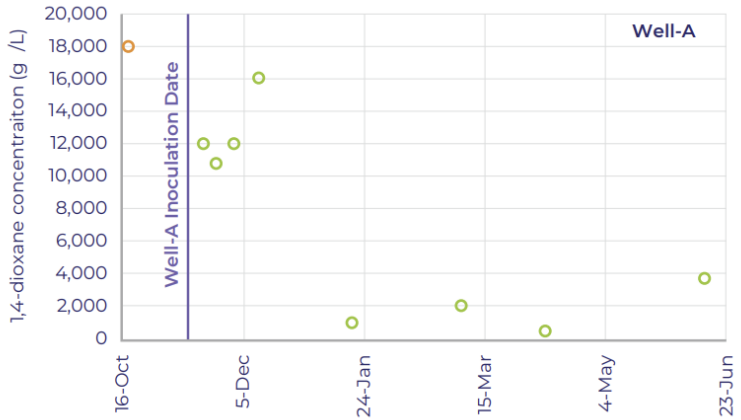


Figures provided by Allonnia

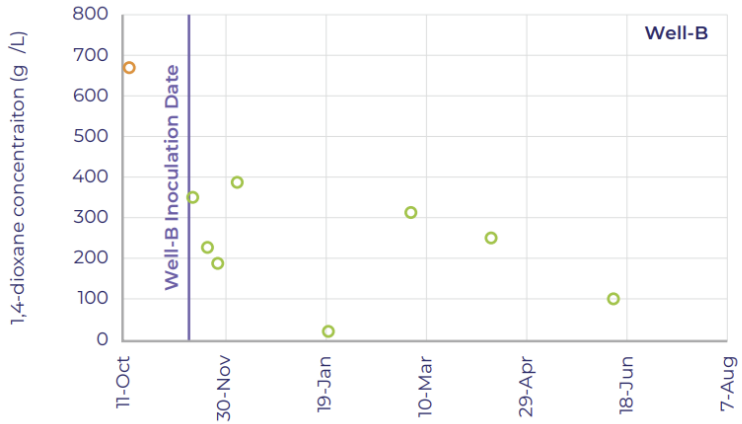
Alternative Microbe: 1,4 D-Stroy™

- 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

Constituent:	MW-32*			MW-31*			IW-3**		
	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
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
cis-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
trans-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-Stroy;
All results are reported in micrograms per liter (µg/L).

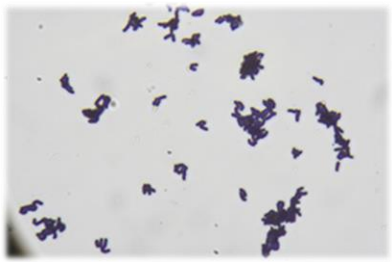
< = Less than. Value is the analytical reporting limit for that constituent.

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

Comparison of CB1190 & 1,4 D-Stroy™

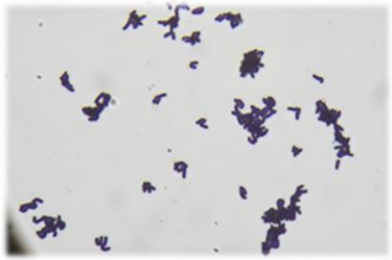
Microbial Enumeration Results Pre and Post Bioaugmentation (cells/milliliter)

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™

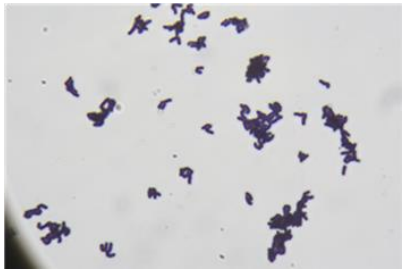
Constituent:	MW-32*			MW-31*			IW-3**		
	QTR2	QTR3	QTR4	QTR2	QTR3	QTR4	QTR2	QTR3	QTR4
	23-Apr	23-Sep	23-Dec	23-Apr	23-Sep	23-Dec	23-Apr	23-Sep	23-Dec
Bioaugmentation Injection	Pre	Post	Post	Pre	Post	Post	Pre	Post	Post
1,4-Dioxane (μg/L)	510	620	510	280	190	85	210	9	31
CB1190 (cells/ml)	12.2	68.8		4.6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



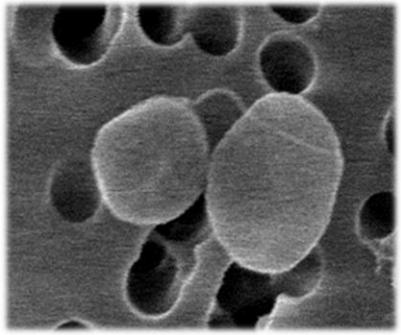
Note: * CB1190 injected: ** 1,4-D Story injected

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



Thank You! Any Questions?



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