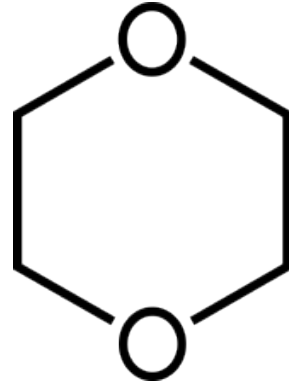


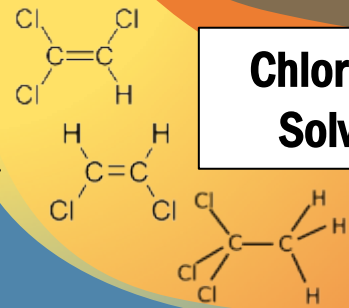
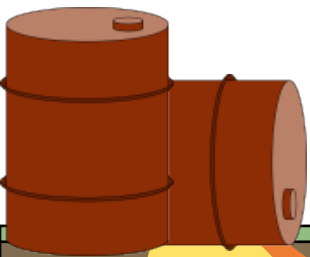
Bioaugmented Phytoremediation to Treat 1,4-Dioxane Contaminated Groundwater

Reid Simmer, Phillip Dixon, Timothy Mattes, and Jerald Schnoor

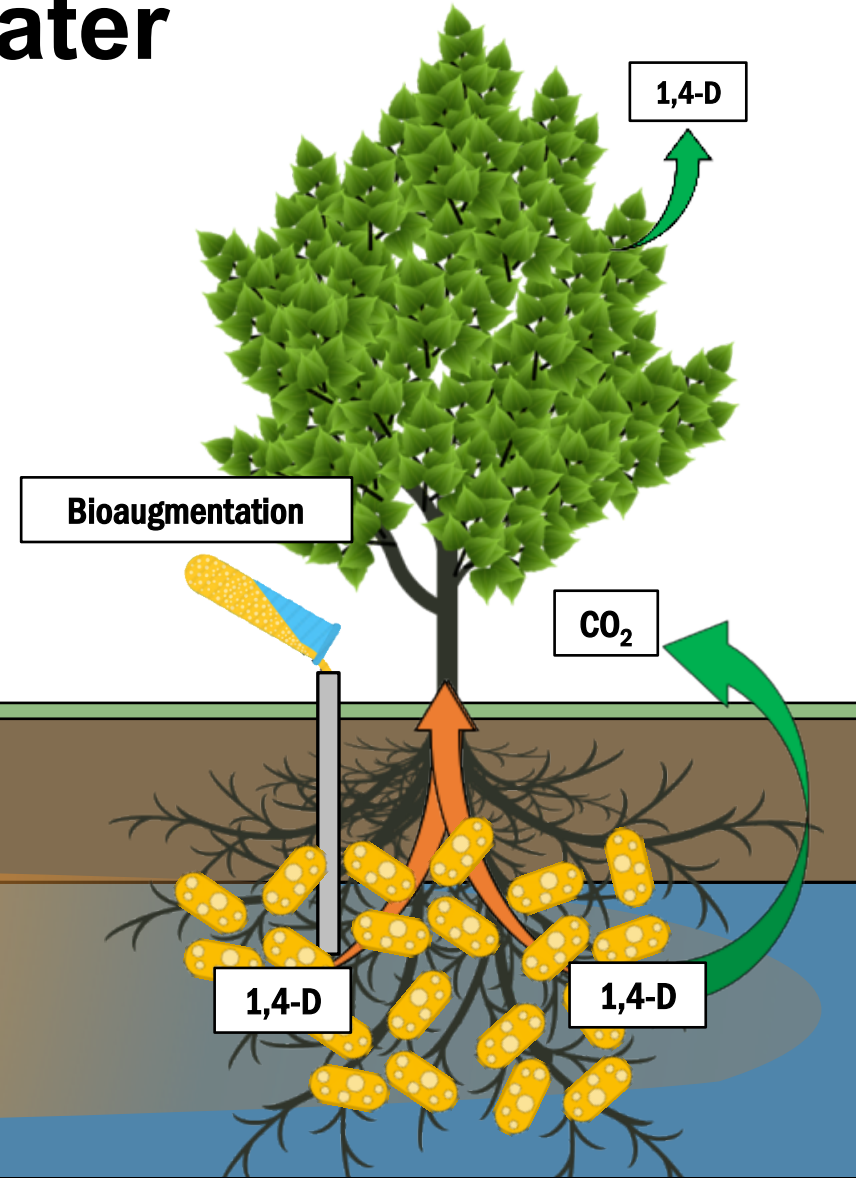
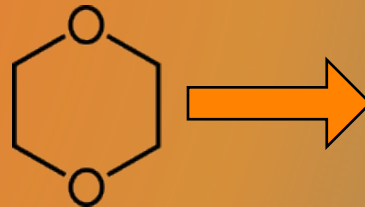
May 9th, 2023



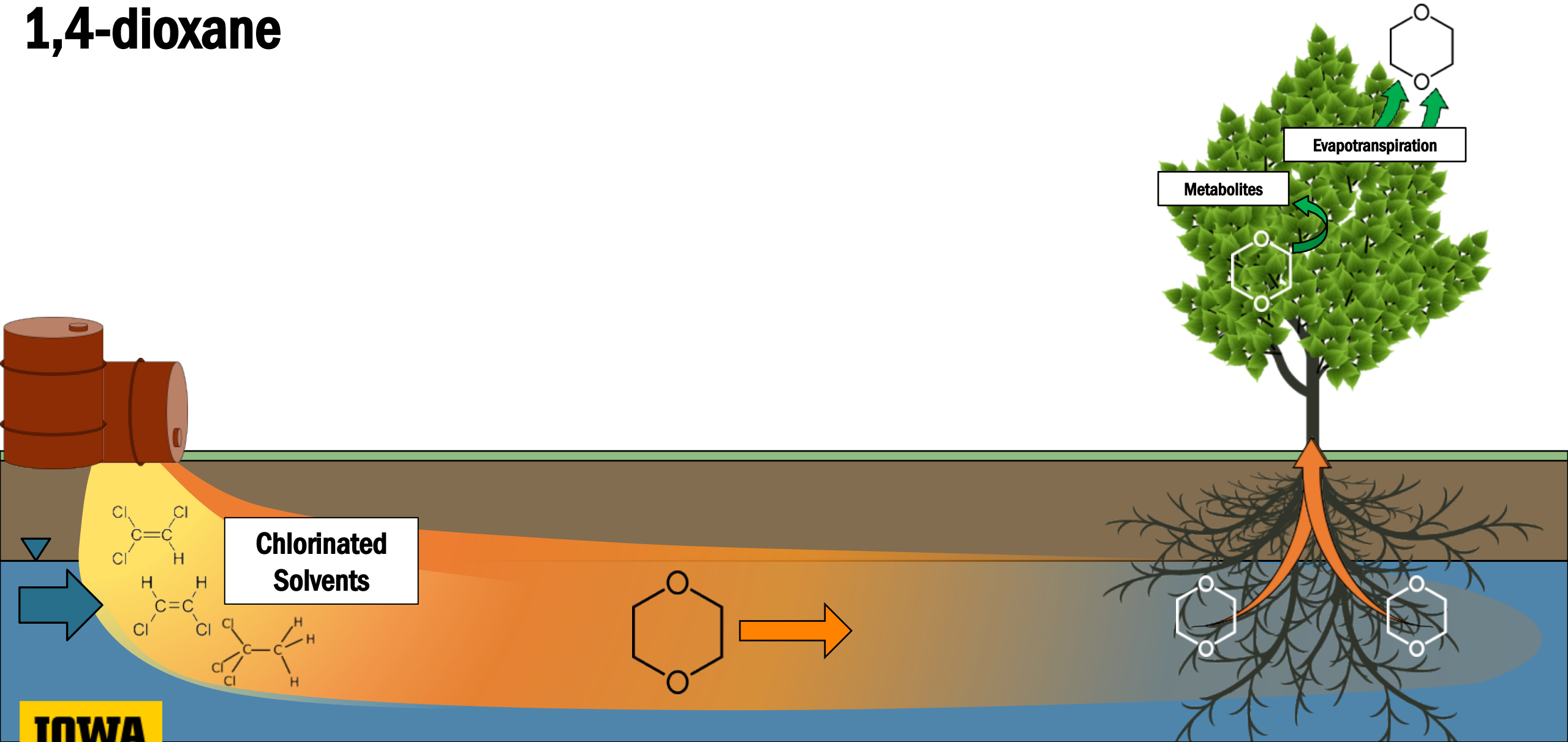
1,4-Dioxane




Chlorinated Solvents



Phytoremediation is a low cost, green remediation technology for 1,4-dioxane



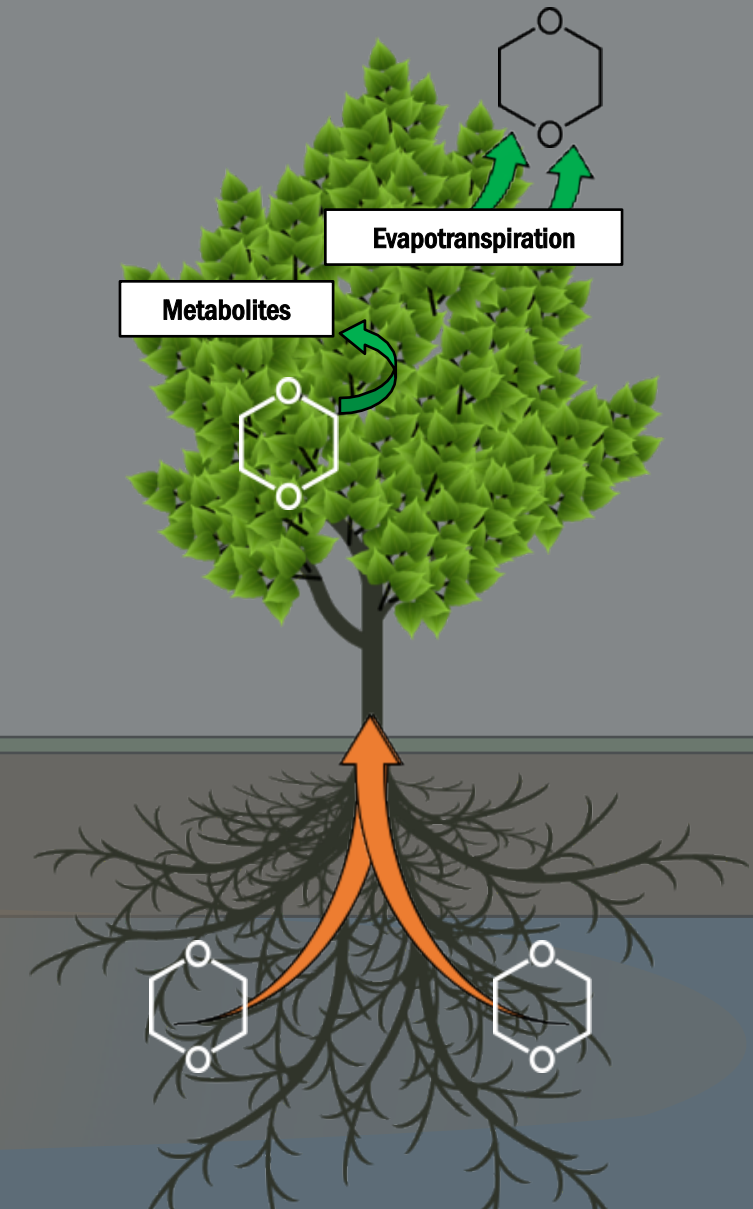
Research Paper |  Full Access

Phytoremediation of 1,4-Dioxane by Hybrid Poplar Trees

Eric W. Aitchison, Sara L. Kelley, Pedro J.J. Alvarez, Jerald L. Schnoor

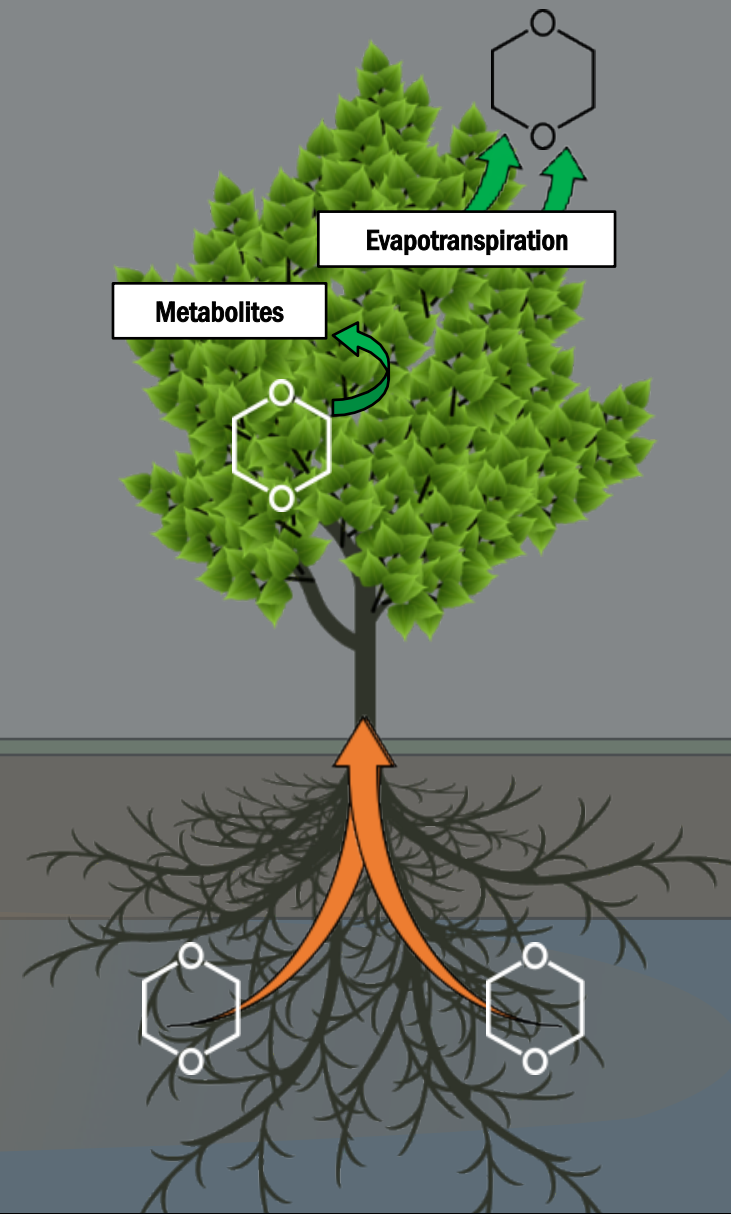
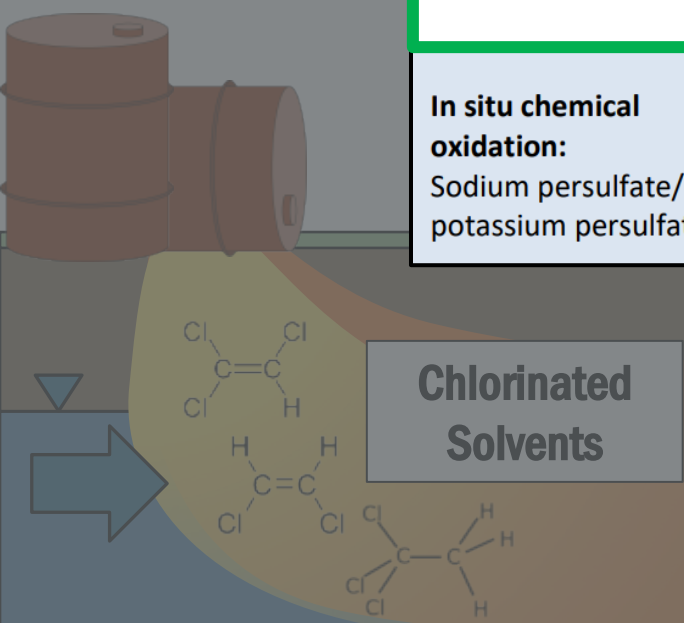
First published: 01 May 2000 | <https://doi.org/10.2175/106143000X137536> | Citations: 72

“76 to 83% of the dioxane taken up by poplars was transpired from leaf surfaces”

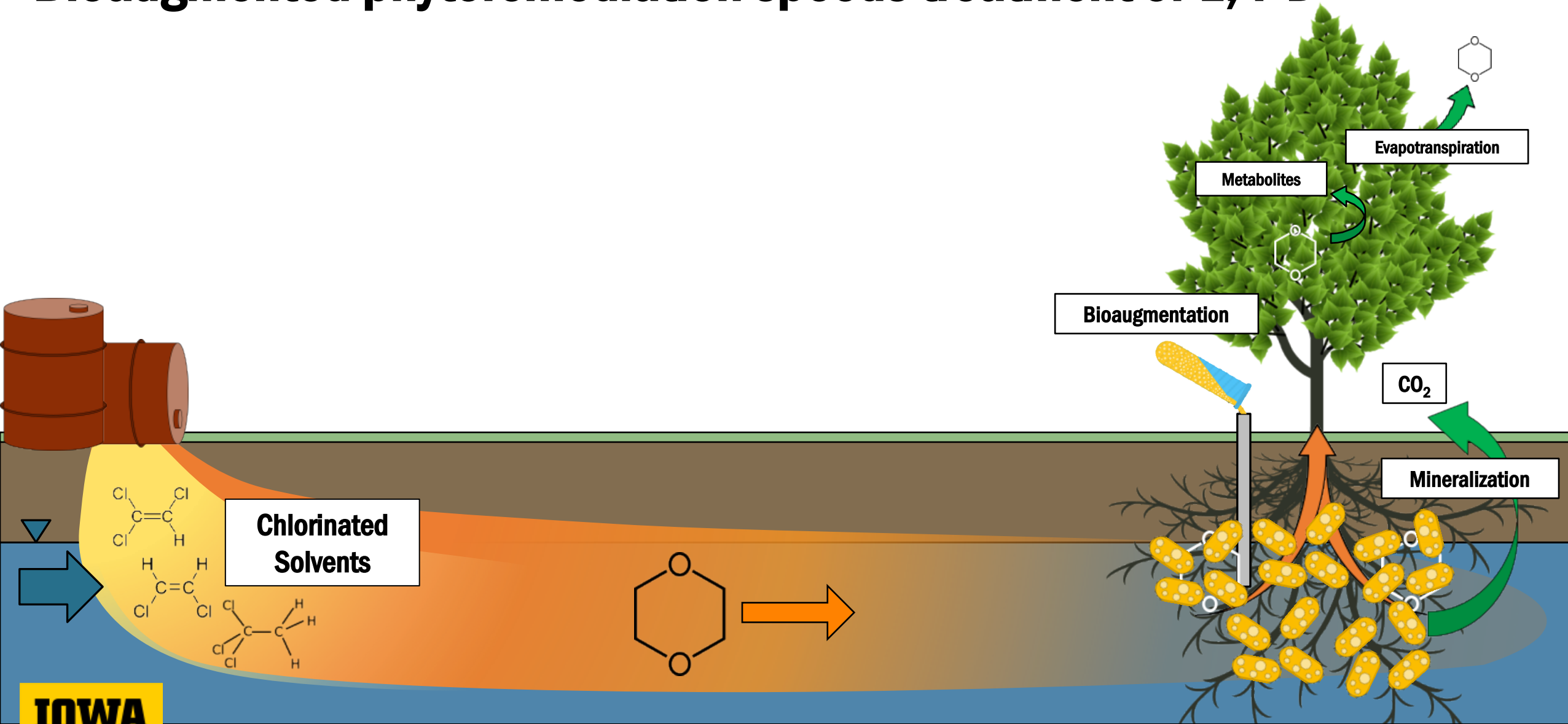


Remediation/ Treatment Technology	Development status		Effectiveness
	GW	VZS	
Monitored natural attenuation (includes physical, chemical, and biological mechanisms)	E	NA	May be effective at reducing 1,4-D at lower starting concentrations (e.g., <500 µg/L), depending on the time available and relevant attenuation mechanisms
Phytoremediation	F	F	Effective for a range of starting concentrations (up to >2,500 µg/L)
In situ chemical oxidation: Sodium persulfate/ potassium persulfate	F	E	Effective at oxidizing 1,4-D to <1 µg/L for high starting concentrations (500 to >2,500 µg/L), depending on proper design and implementation



ITRC,
2021

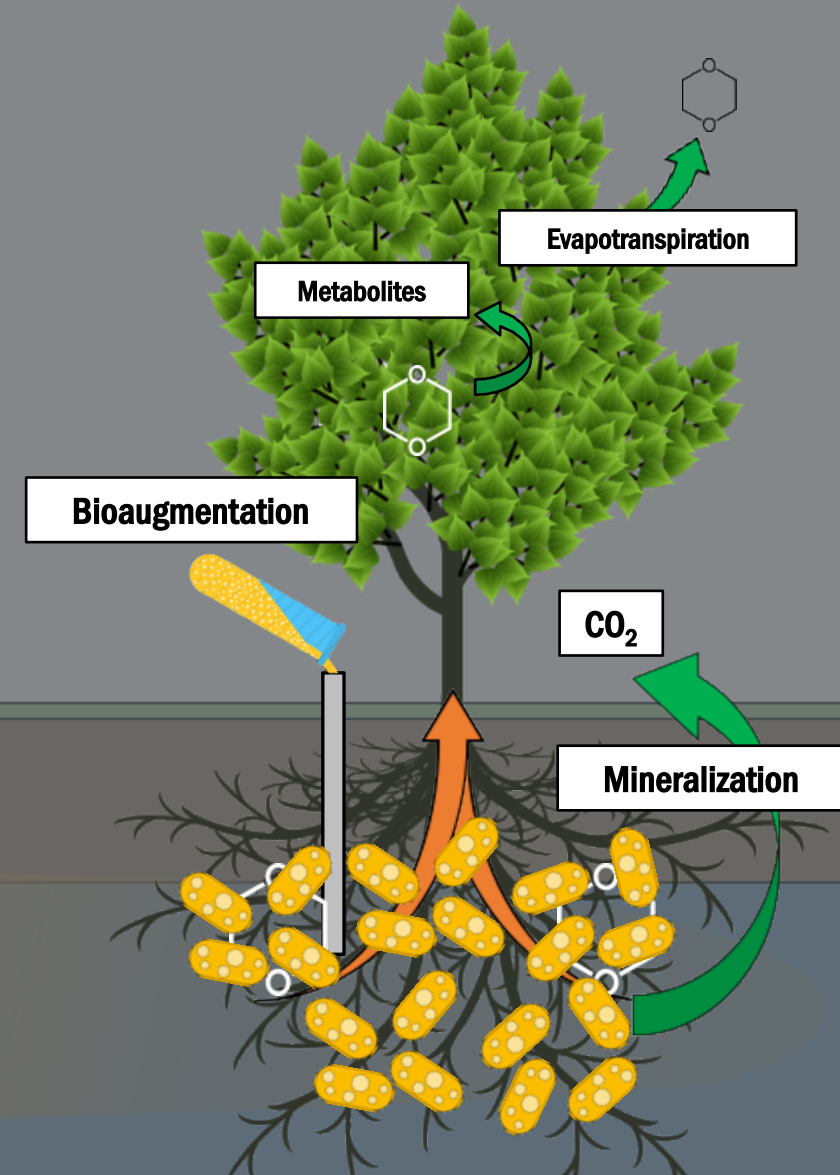
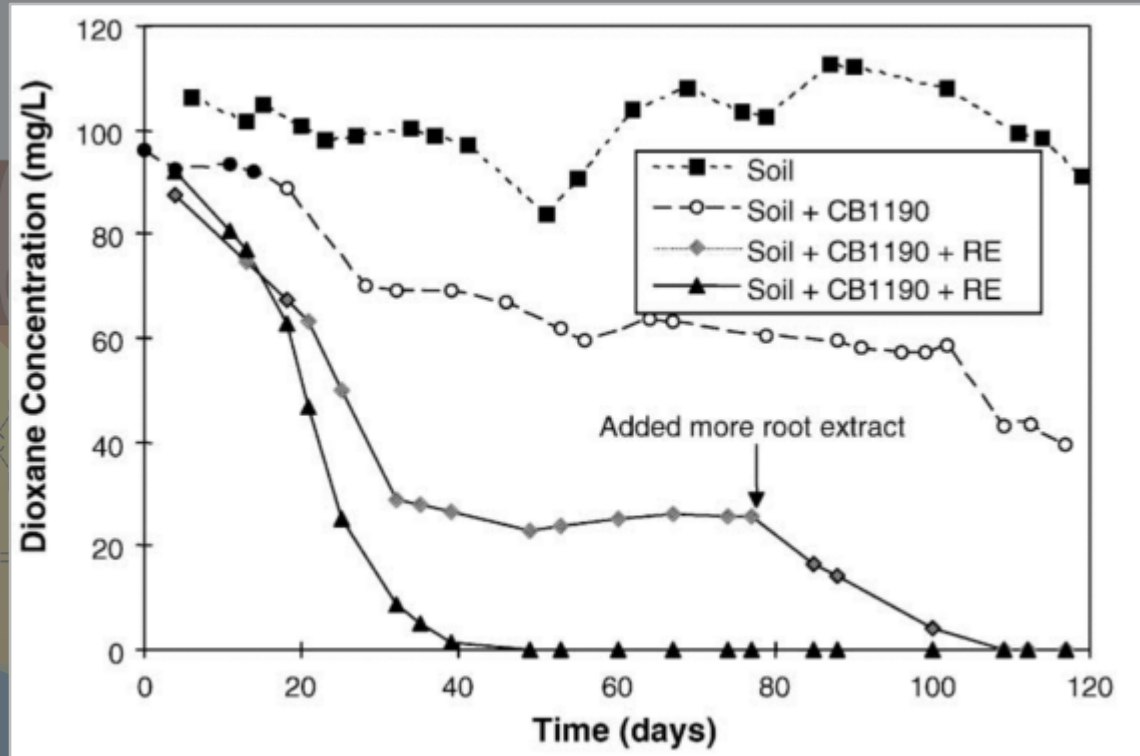


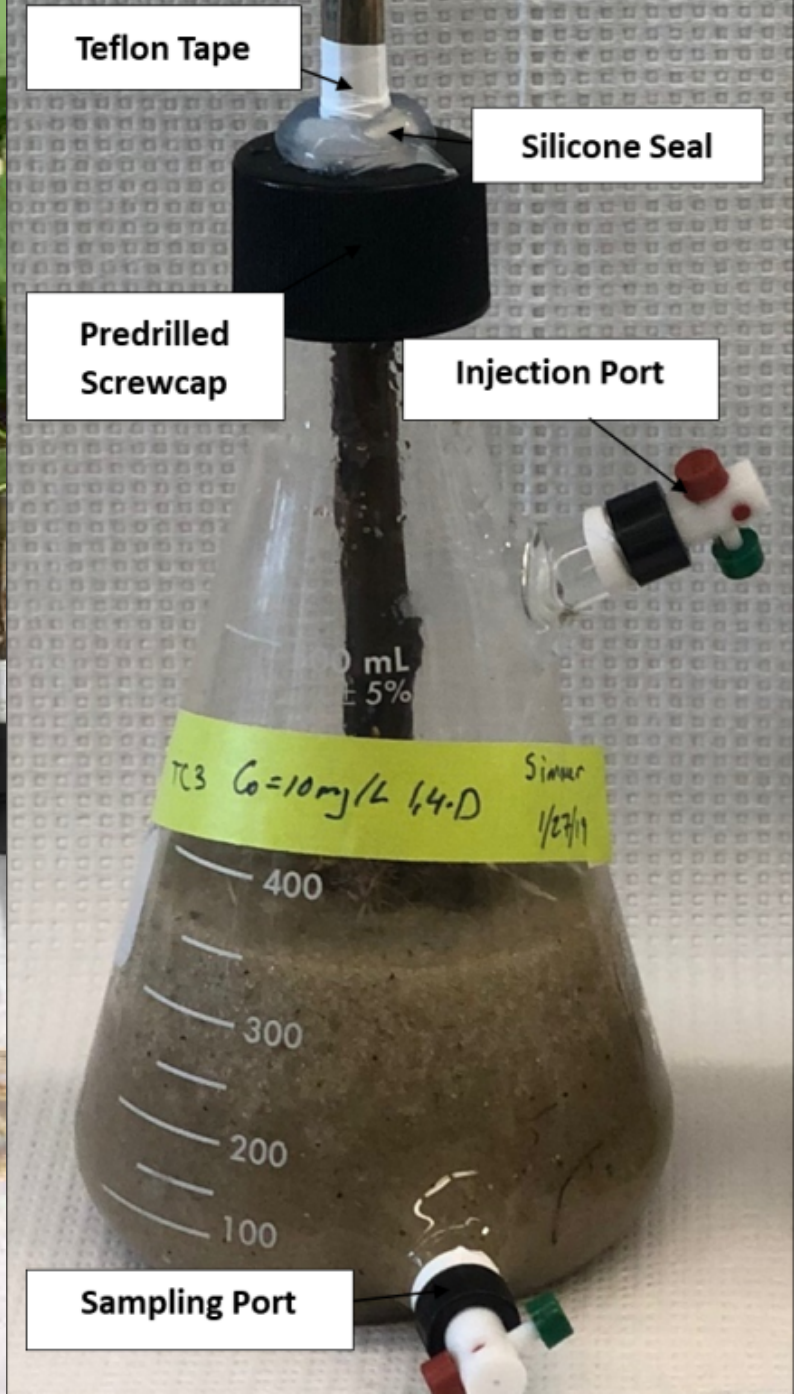
Bioaugmented phytoremediation speeds treatment of 1,4-D



Biodegradation of 1,4-dioxane in planted and unplanted soil: effect of bioaugmentation with *amycolata* sp. CB1190

Sara L. Kelley¹, Eric W. Aitchison¹, Milind Deshpande², Jerald L. Schnoor¹,
Pedro J.J. Alvarez¹  





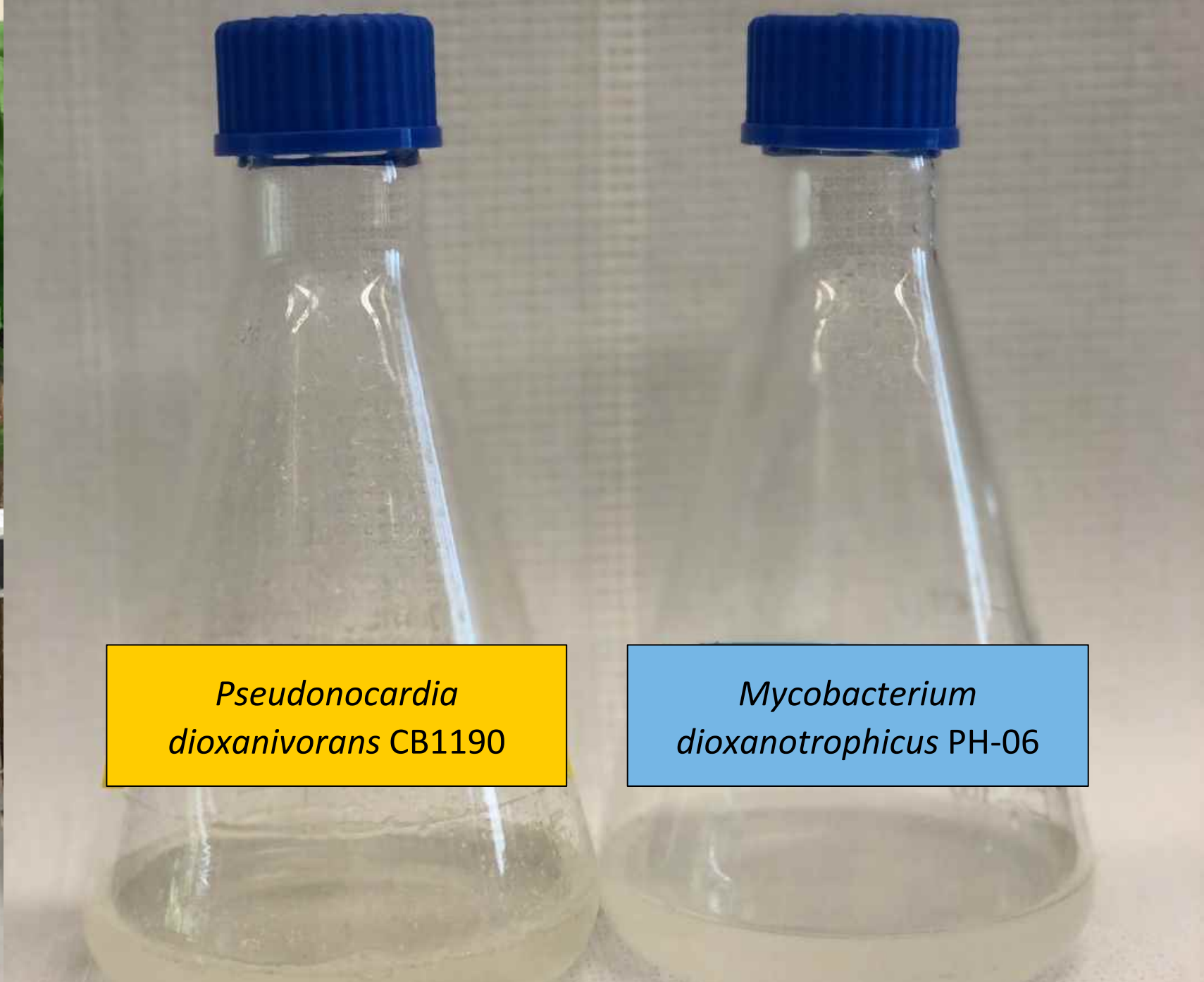
Teflon Tape

Silicone Seal

Predrilled Screwcap

Injection Port

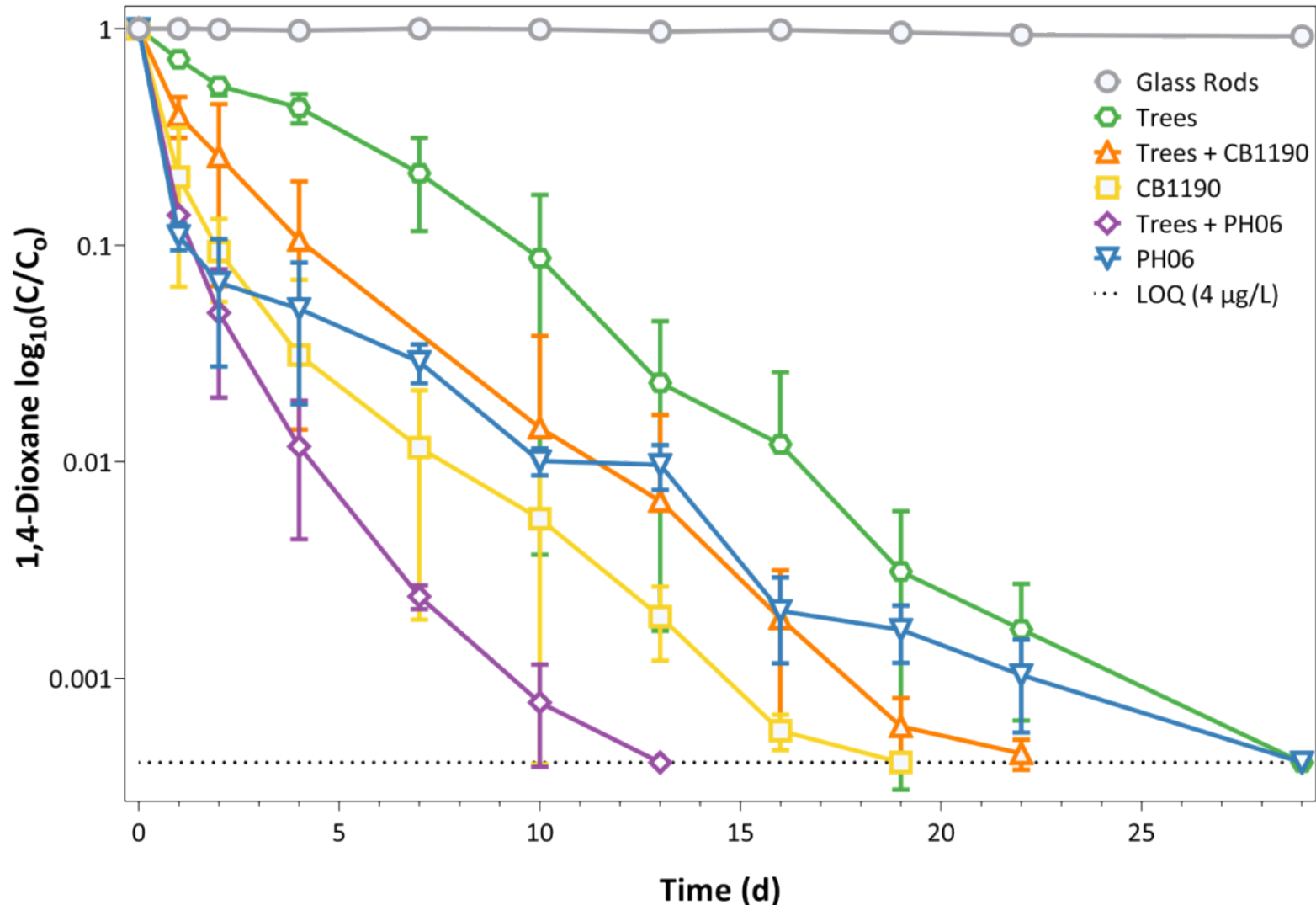
Sampling Port



*Pseudonocardia
dioxanivorans* CB1190

*Mycobacterium
dioxanotrophicus* PH-06

Trees bioaugmented with *Mycobacterium sp.* PH-06 speed treatment of 1,4-D to ~4 ug/L





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Bioaugmenting the poplar rhizosphere to enhance treatment of 1,4-dioxane

Reid Simmer^{a,*}, Jacques Mathieu^b, Marcio L.B. da Silva^b, Philip Lashmit^c, Sridhar Gopishetty^c, Pedro J.J. Alvarez^b, Jerald L. Schnoor^a

^a Department of Civil and Environmental Engineering, College of Engineering, The University of Iowa, Iowa City, IA, USA

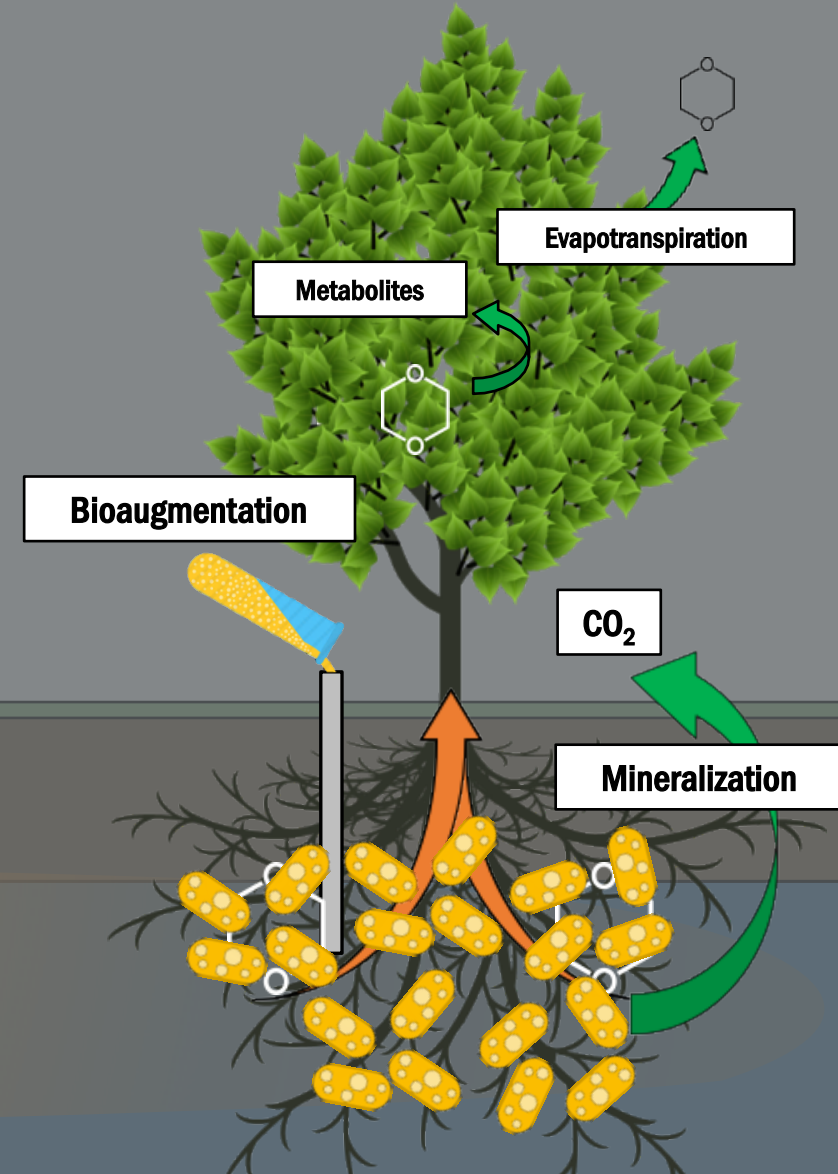
^b Department of Civil and Environmental Engineering, College of Engineering, Rice University, Houston, TX, USA

^c Center for Biocatalysis and Bioprocessing, Office for the Vice President for Research and Economic Development, University of Iowa Research Park, The University of Iowa, Coralville, IA, USA



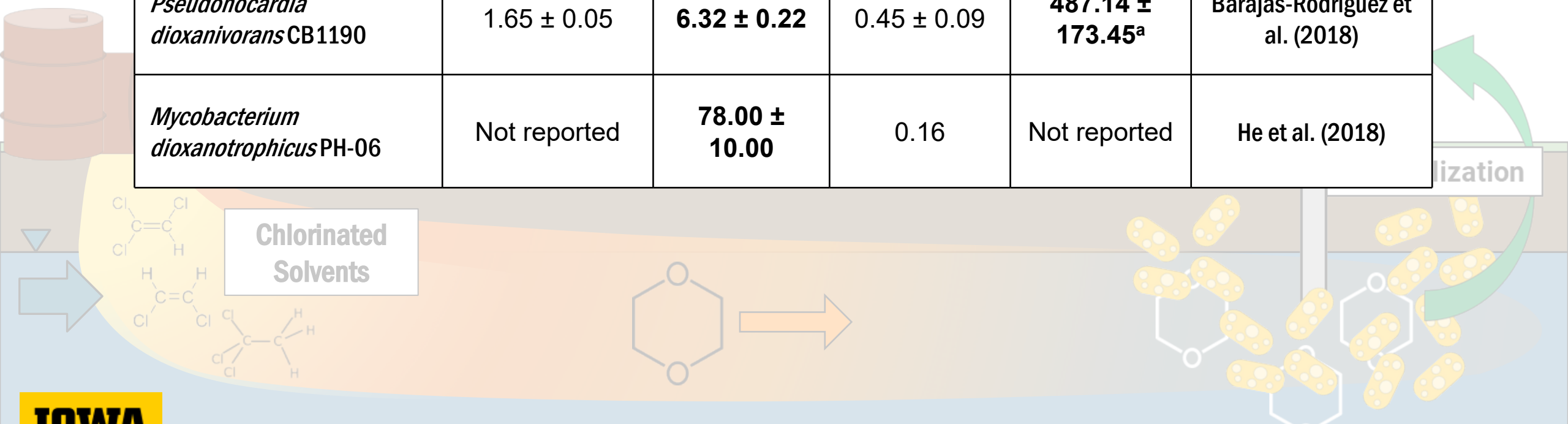
SCAN ME

IOWA

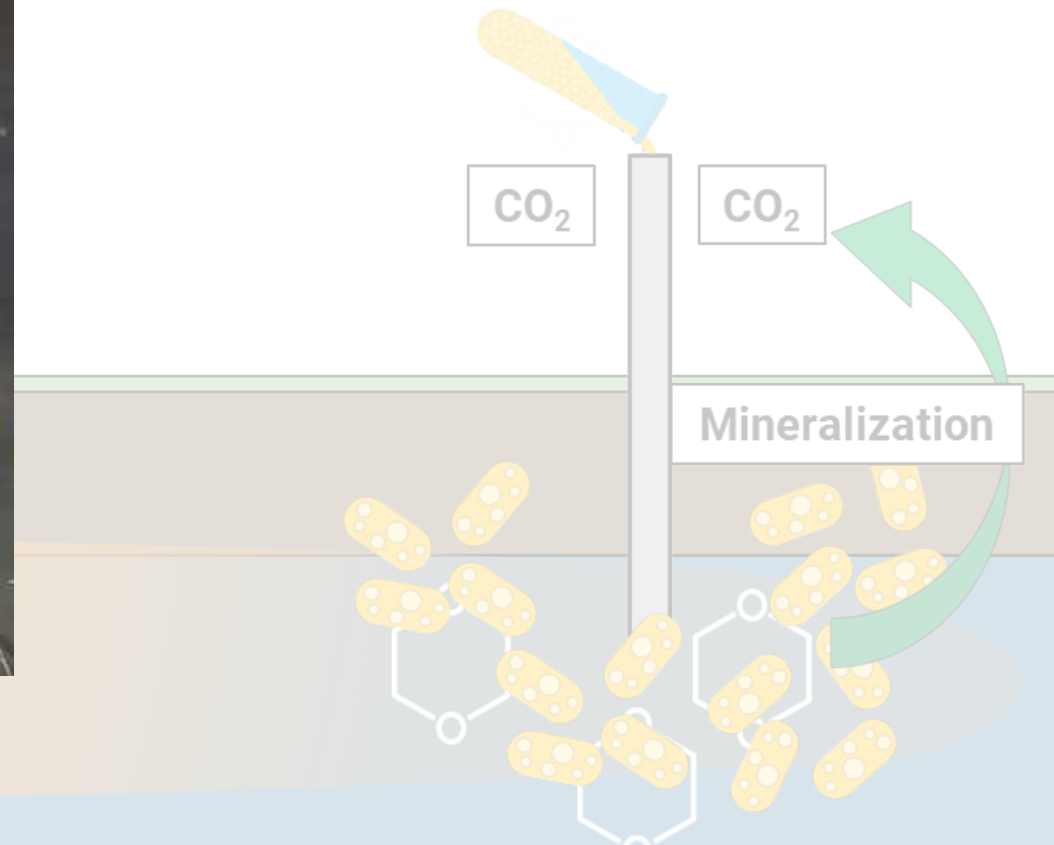
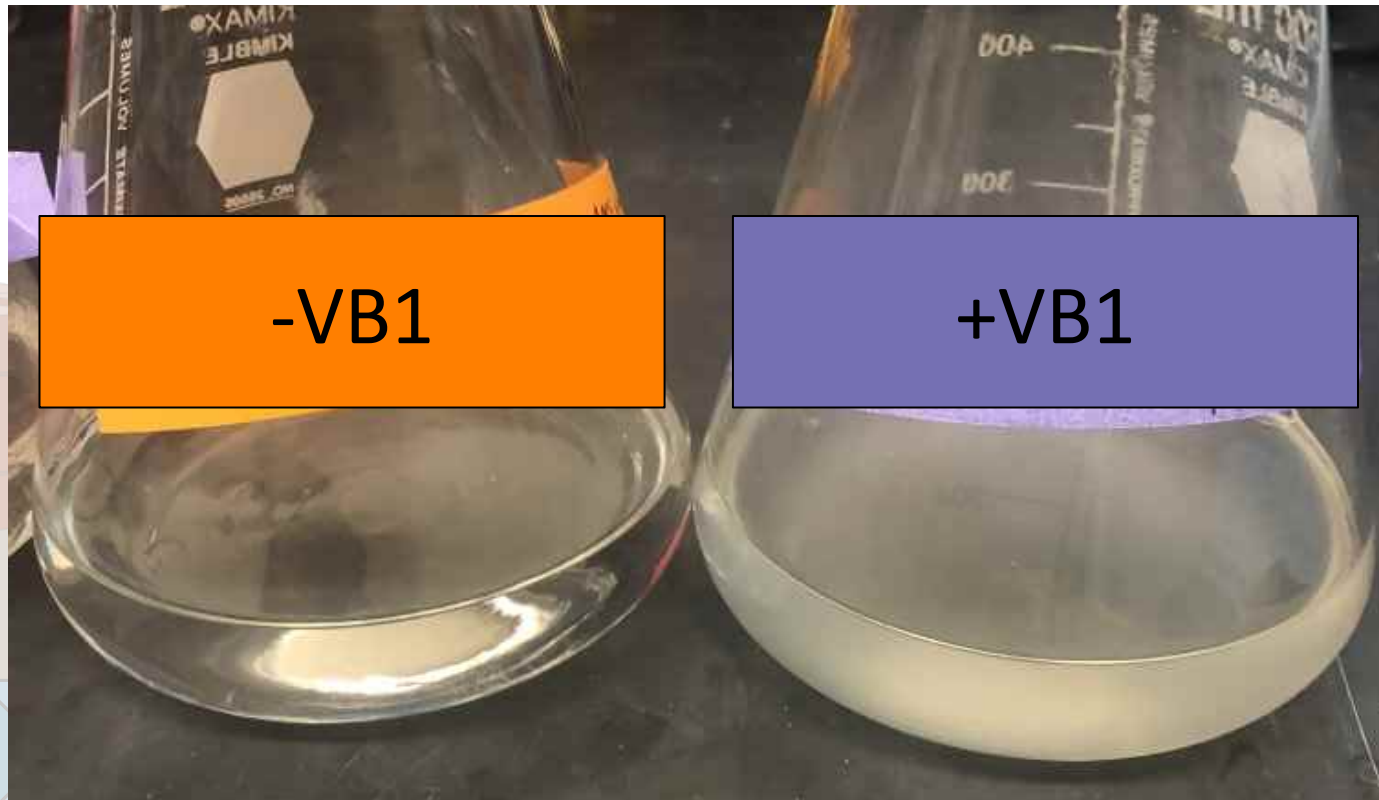


However, metabolic degraders have significant kinetic limitations

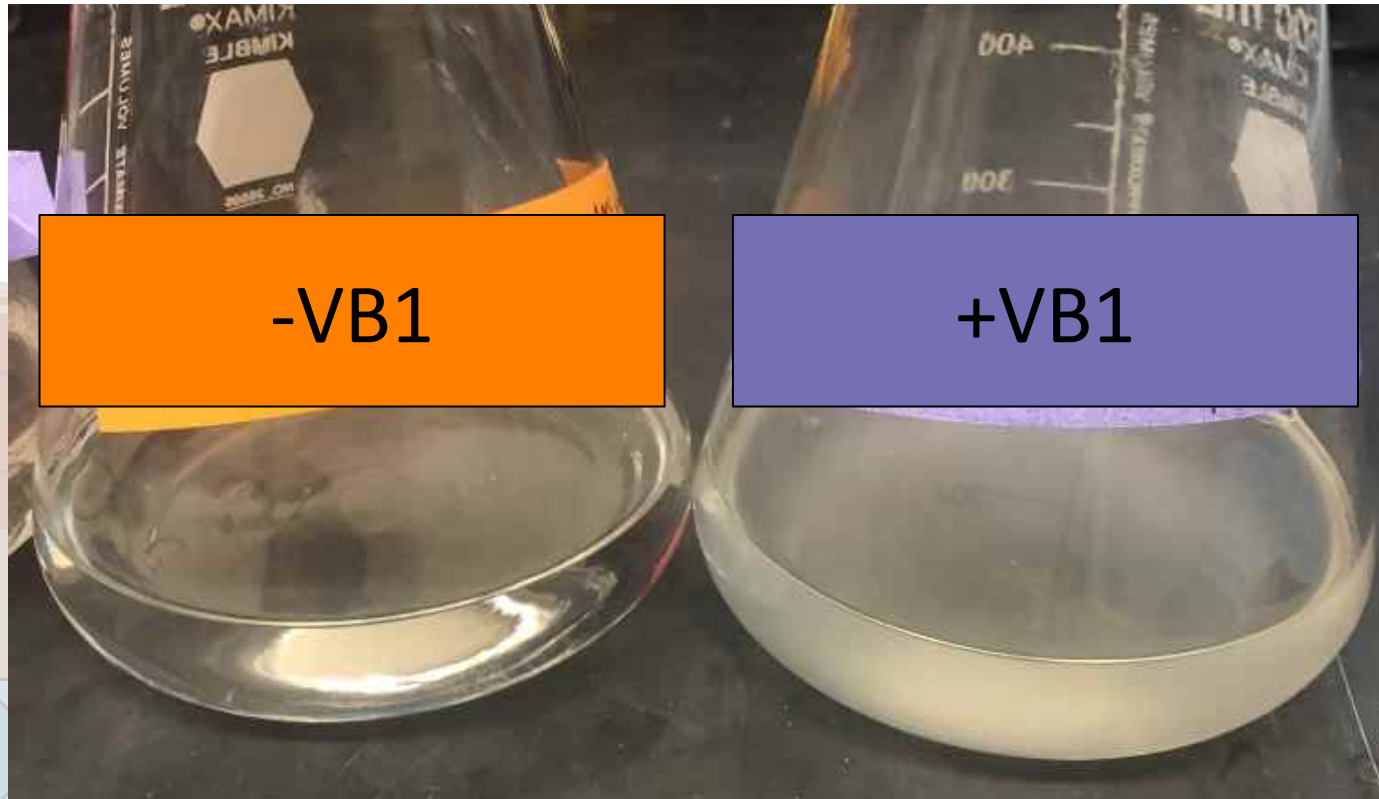
Strain	q_{\max} (mg 1,4-dioxane/mg protein/day)	K_s (mg 1,4-dioxane /L)	Yield (mg protein/ mg dioxane)	S_{\min} (μ g 1,4-dioxane /L)	Reference
<i>Pseudonocardia dioxanivorans</i> CB1190	1.65 ± 0.05	6.32 ± 0.22	0.45 ± 0.09	487.14 ± 173.45^a	Barajas-Rodriguez et al. (2018)
<i>Mycobacterium dioxanotrophicus</i> PH-06	Not reported	78.00 ± 10.00	0.16	Not reported	He et al. (2018)



Vitamin B1 (thiamine) supplements accelerate growth in *Rhodococcus ruber*219



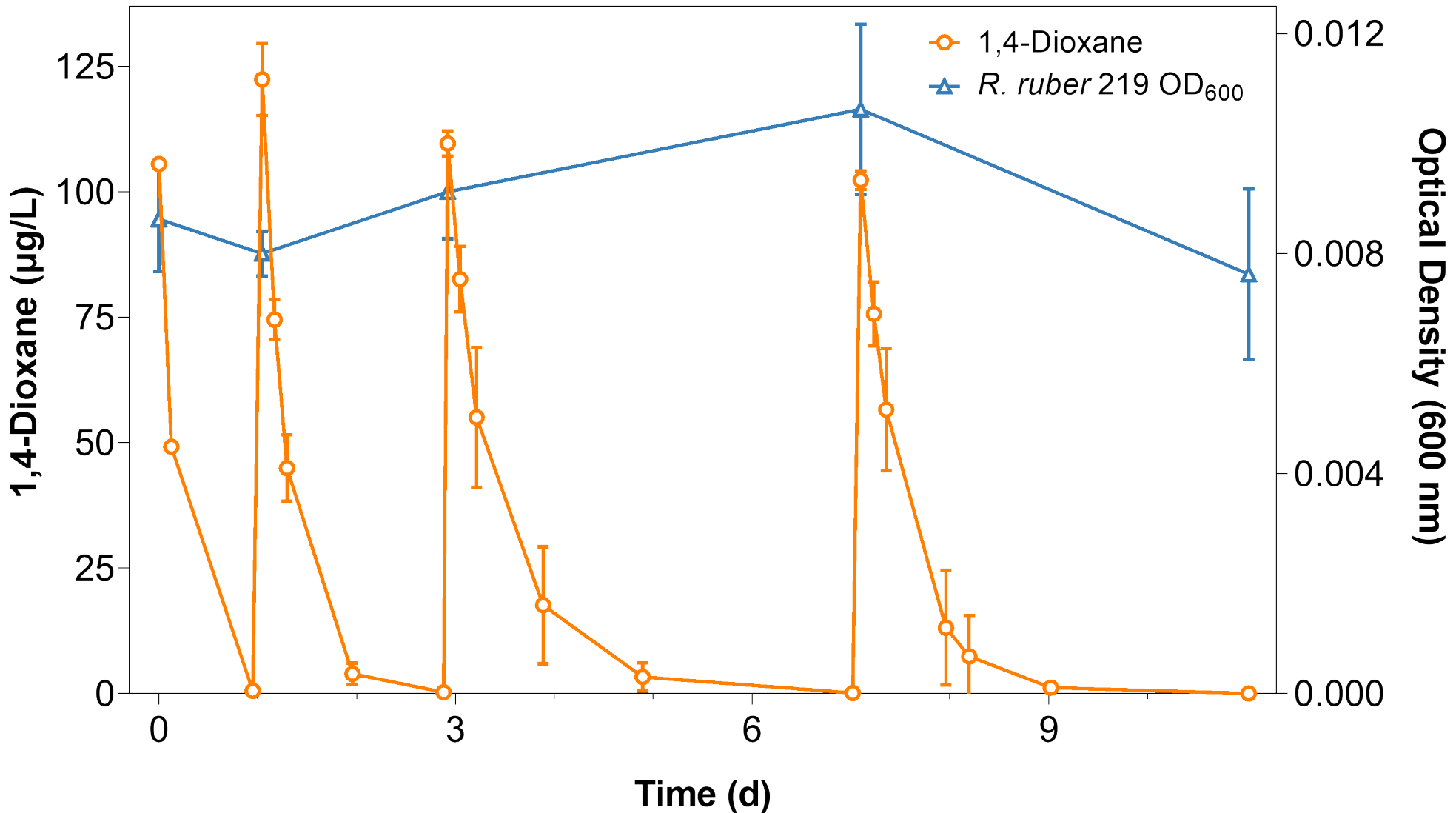
Vitamin B1 (thiamine) supplements accelerate growth in *Rhodococcus ruber*219



***Rhodococcus ruber* 219**, when supplemented with Vitamin B1, has the **lowest K_s and S_{min}** reported for dioxane-metabolizing bacteria to date, **degrading 100 $\mu\text{g/L}$ dioxane to below 0.1 $\mu\text{g/L}$.**

Strain	q_{max} (mg 1,4-dioxane/mg protein/day)	K_s (mg 1,4-dioxane /L)	Yield (mg protein/ mg dioxane)	S_{min} (μg 1,4-dioxane /L)	Reference
<i>Pseudonocardia dioxanivorans</i> CB1190	1.65 \pm 0.05	6.32 \pm 0.22	0.45 \pm 0.09	487.14 \pm 173.45 ^a	Barajas-Rodriguez et al. (2018)
<i>Mycobacterium dioxanotrophicus</i> PH-06	Not reported	78.00 \pm 10.00	0.16	Not reported	He et al. (2018)
<i>Rhodococcus ruber</i> 219	5.00 \pm 0.24	0.015 \pm 0.03	0.24 \pm 0.02	0.49 \pm 1.16	Simmer et al. (2021)

R. ruber 219 repeatedly degraded ~100 µg/L dioxane to <0.35 µg/L



Rapid Metabolism of 1,4-Dioxane to below Health Advisory Levels by Thiamine-Amended *Rhodococcus ruber* Strain 219

Reid A. Simmer,* Patrick M. Richards, Jessica M. Ewald, Cory Schwarz, Marcio L. B. da Silva, Jacques Mathieu, Pedro J. J. Alvarez, and Jerald L. Schnoor



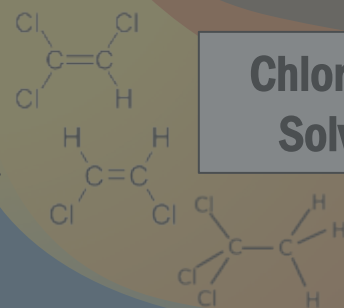
Cite This: *Environ. Sci. Technol. Lett.* 2021, 8, 975–980



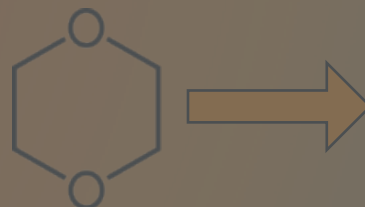
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OPEN
ACCESS



Chlorinated
Solvents



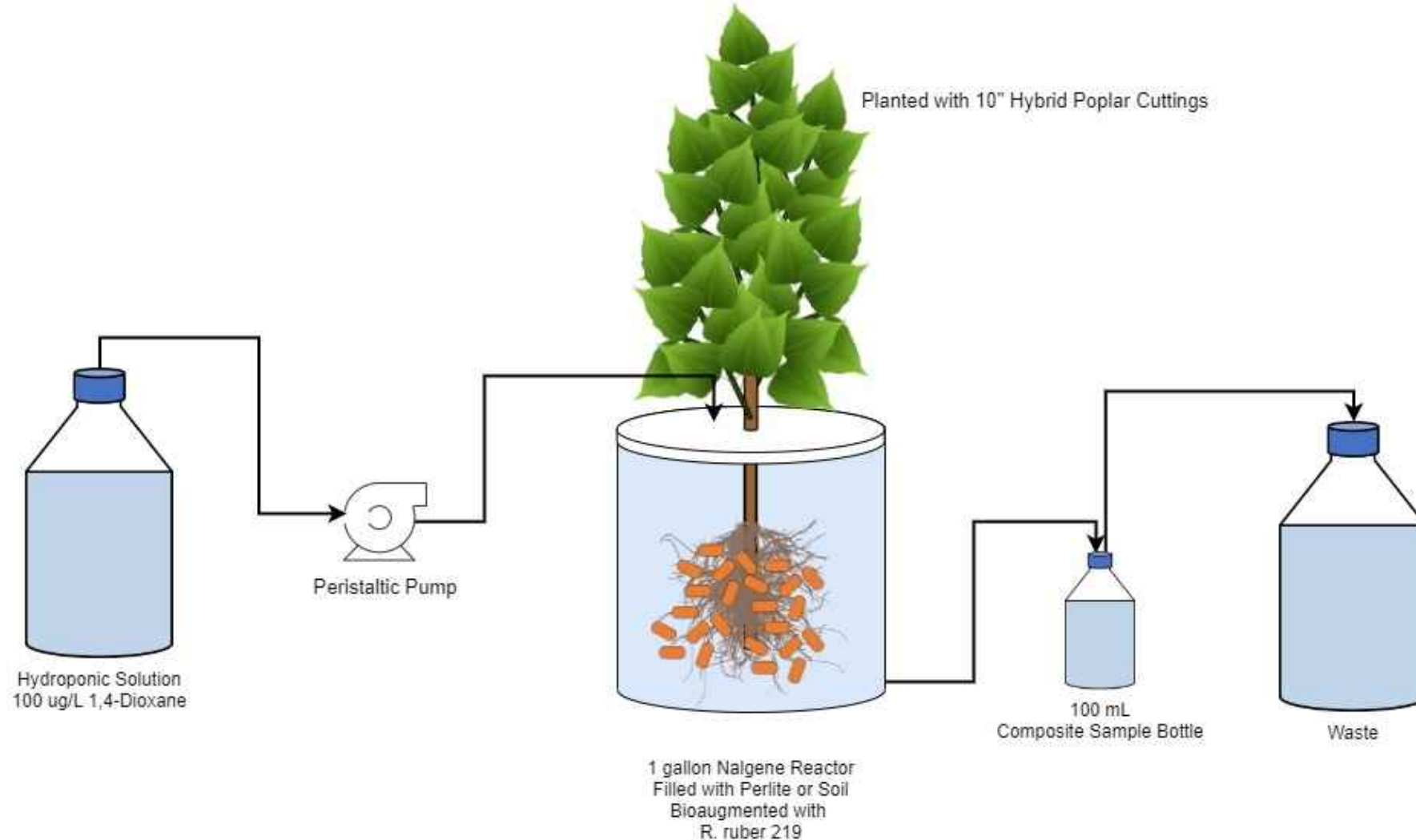
Mineralization

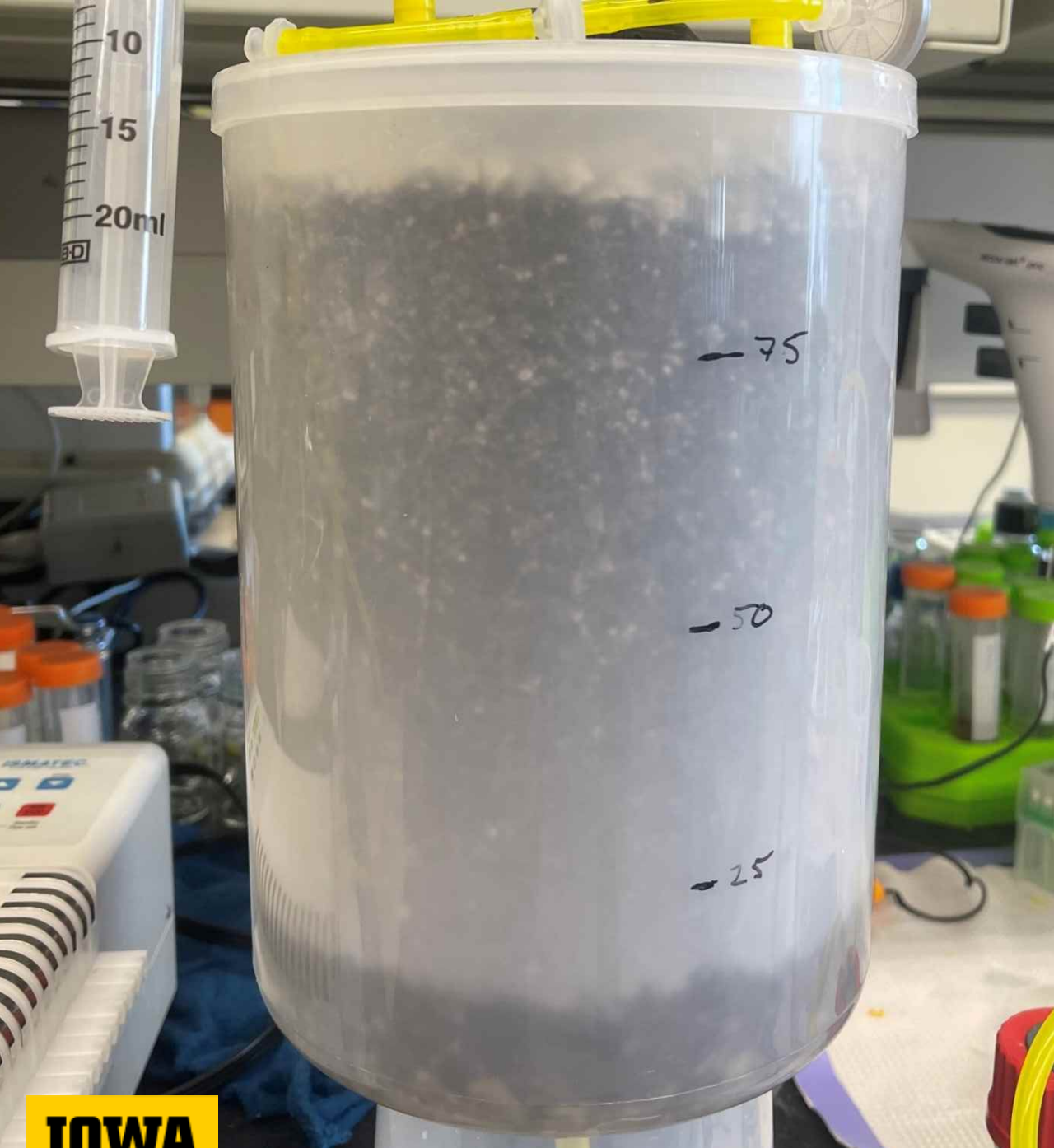
Current Work

- Does bioaugmented phytoremediation with *R. ruber*219 accelerate treatment of 1,4-D to $<1 \mu\text{g/L}$?
- Can treatment of 1,4-D be sustained in long term flow-through experiments?



Flow-through experiments were used to emulate environmentally relevant field-scale conditions





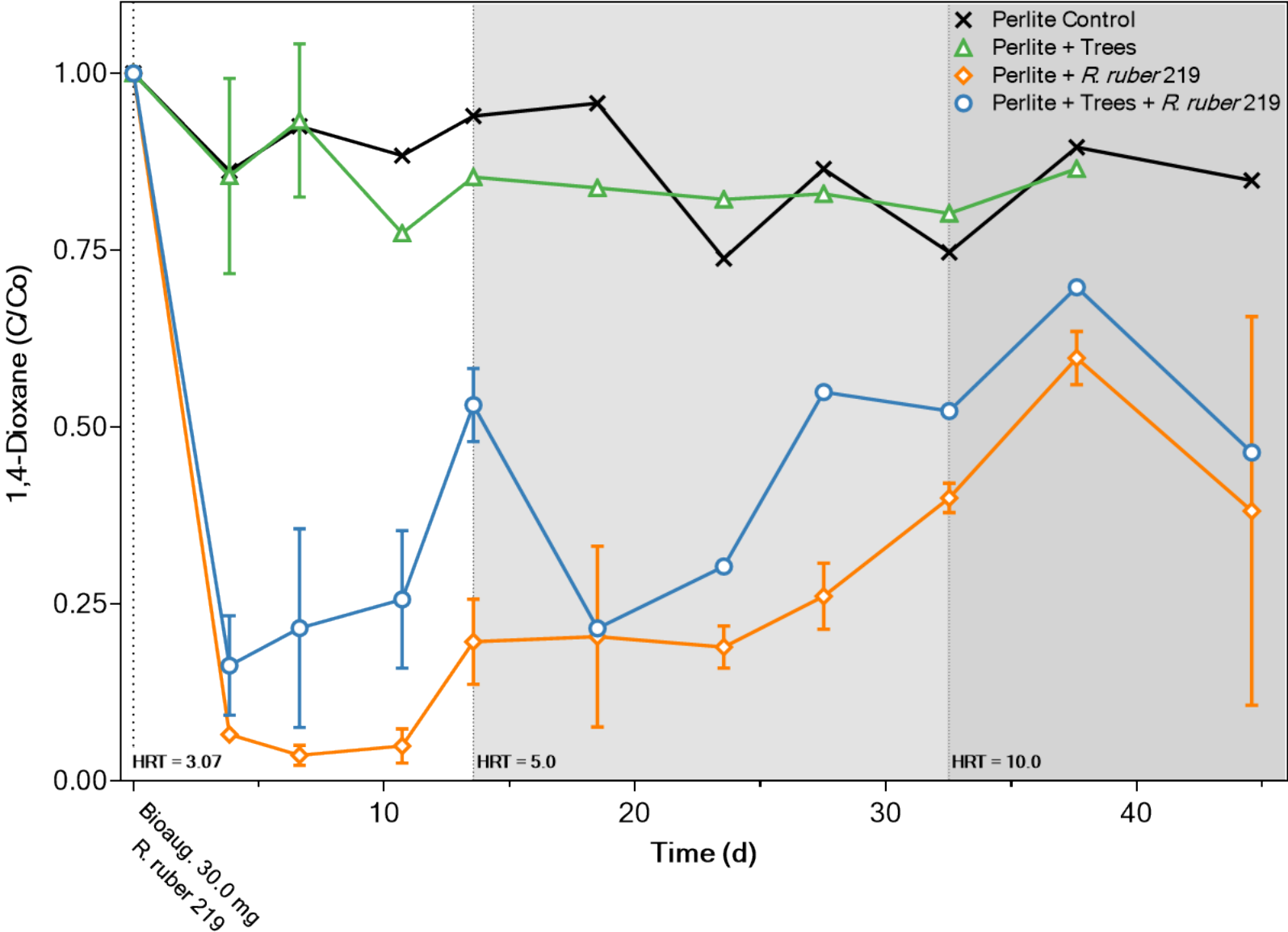
Treatment	Soil Substrate	Replicates
Glass Rod Control	Perlite	1
Tree Only	Perlite	2
<i>R. ruber</i> 219 only	Perlite	2
Tree + <i>R. ruber</i> 219	Perlite	3
Glass Rod Control	Soil	1
Tree Only	Soil	2
<i>R. ruber</i> 219 only	Soil	2
Tree + <i>R. ruber</i> 219	Soil	3
		Total: 16



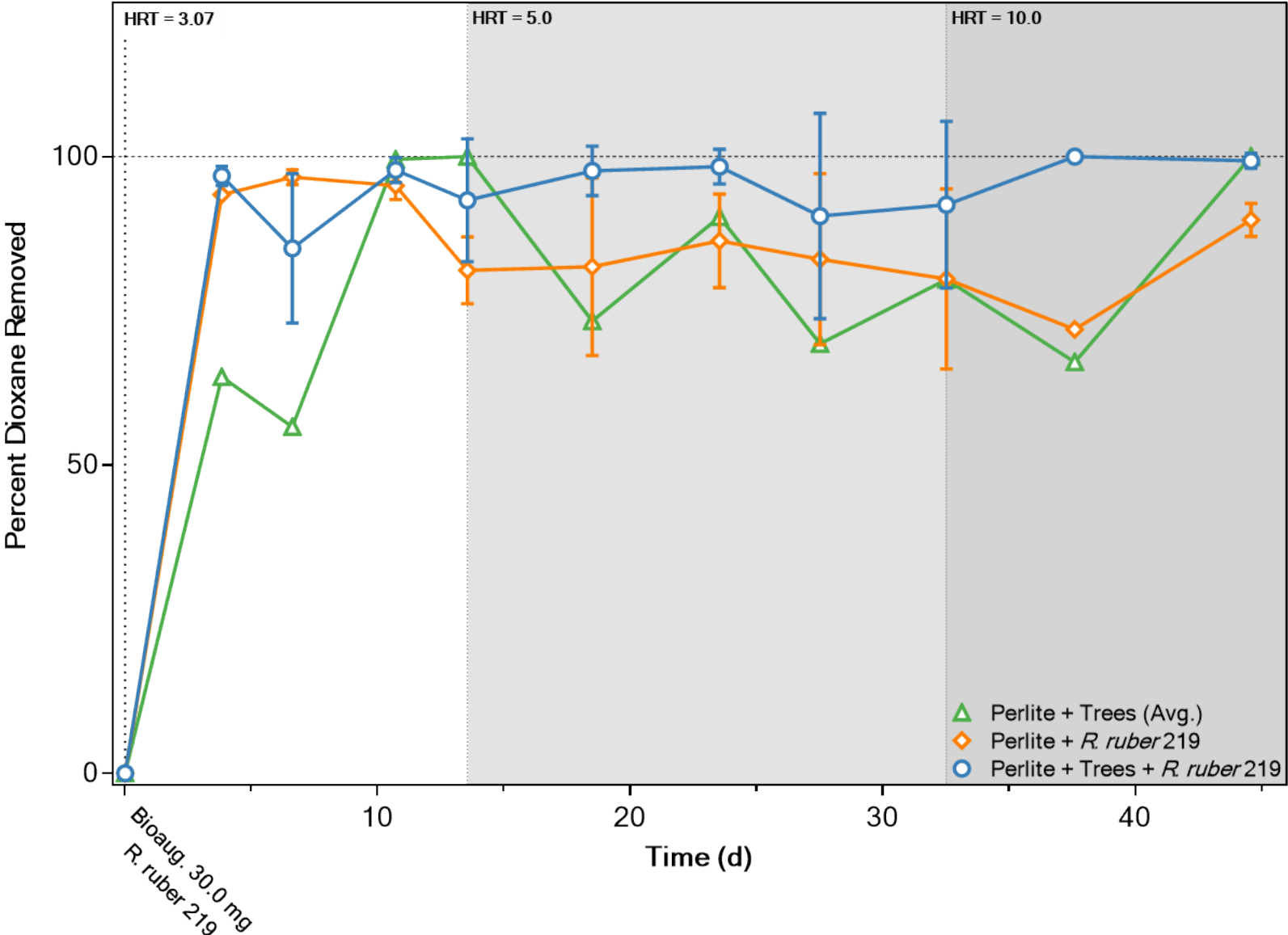




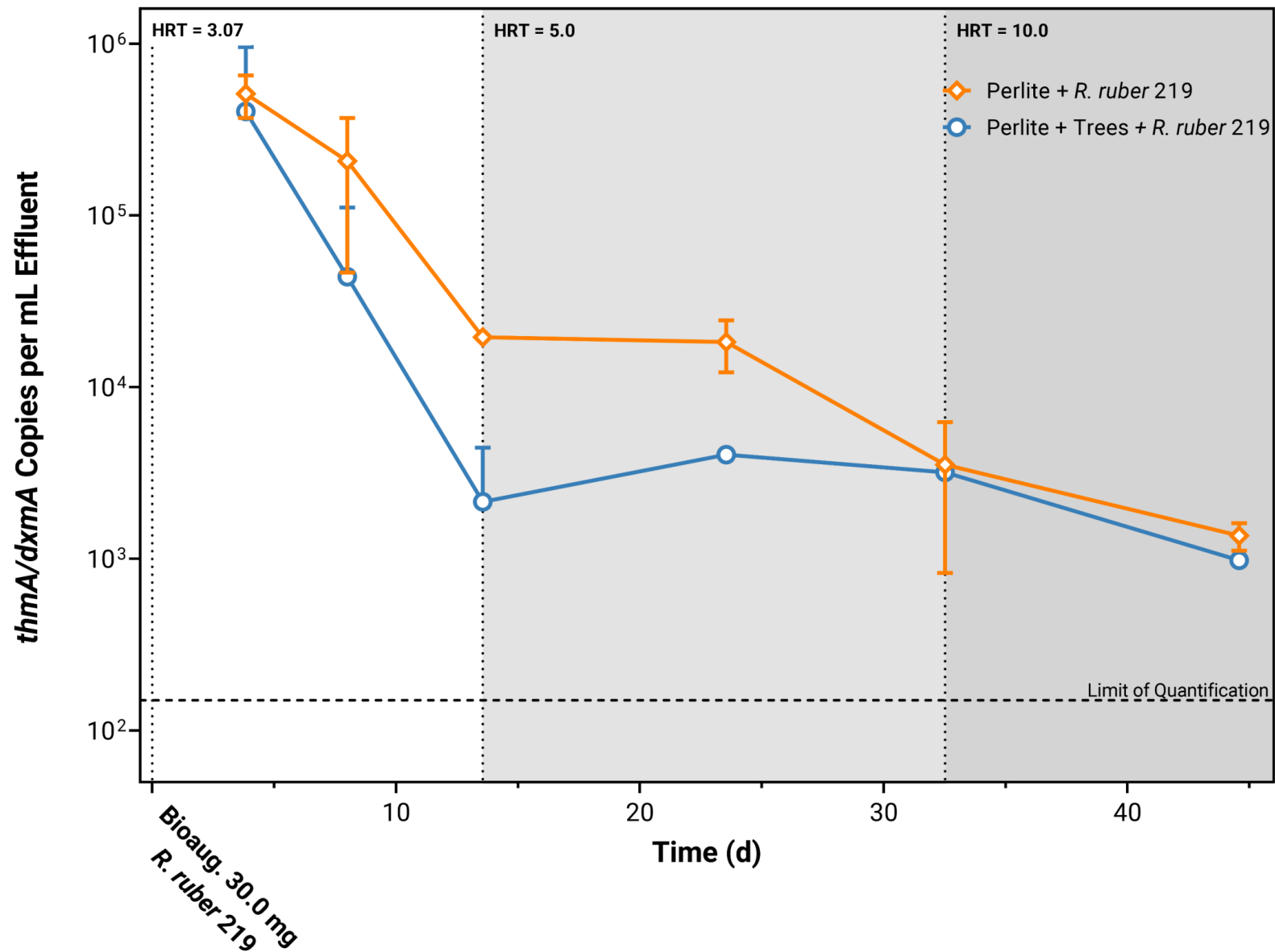
Perlite reactors bioaugmented with *R. ruber* achieved the lowest effluent concentrations



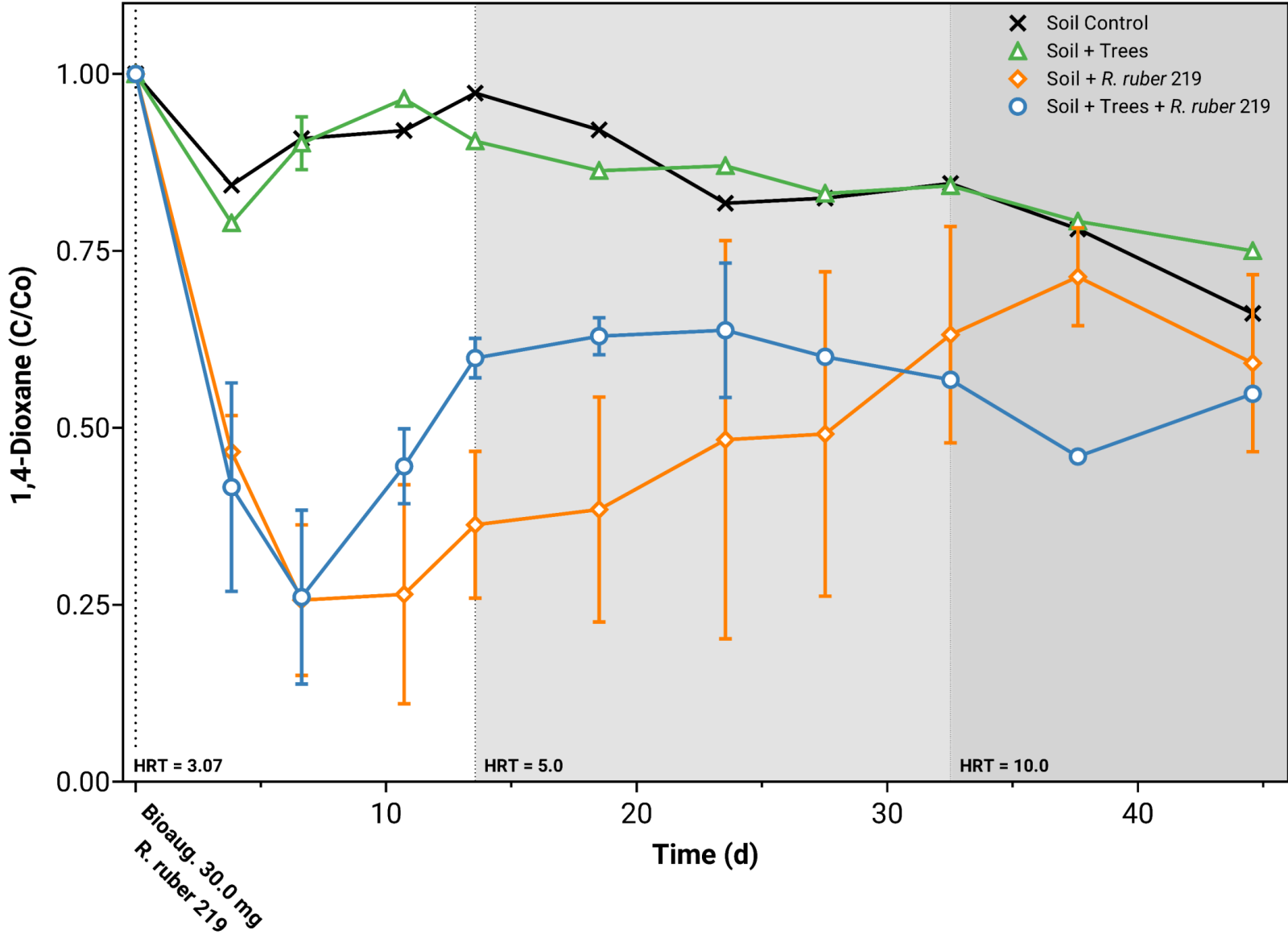
However, trees with *R. ruber*219 was best on a mass removal basis (biodegradation + phytoextraction/evapotranspiration)



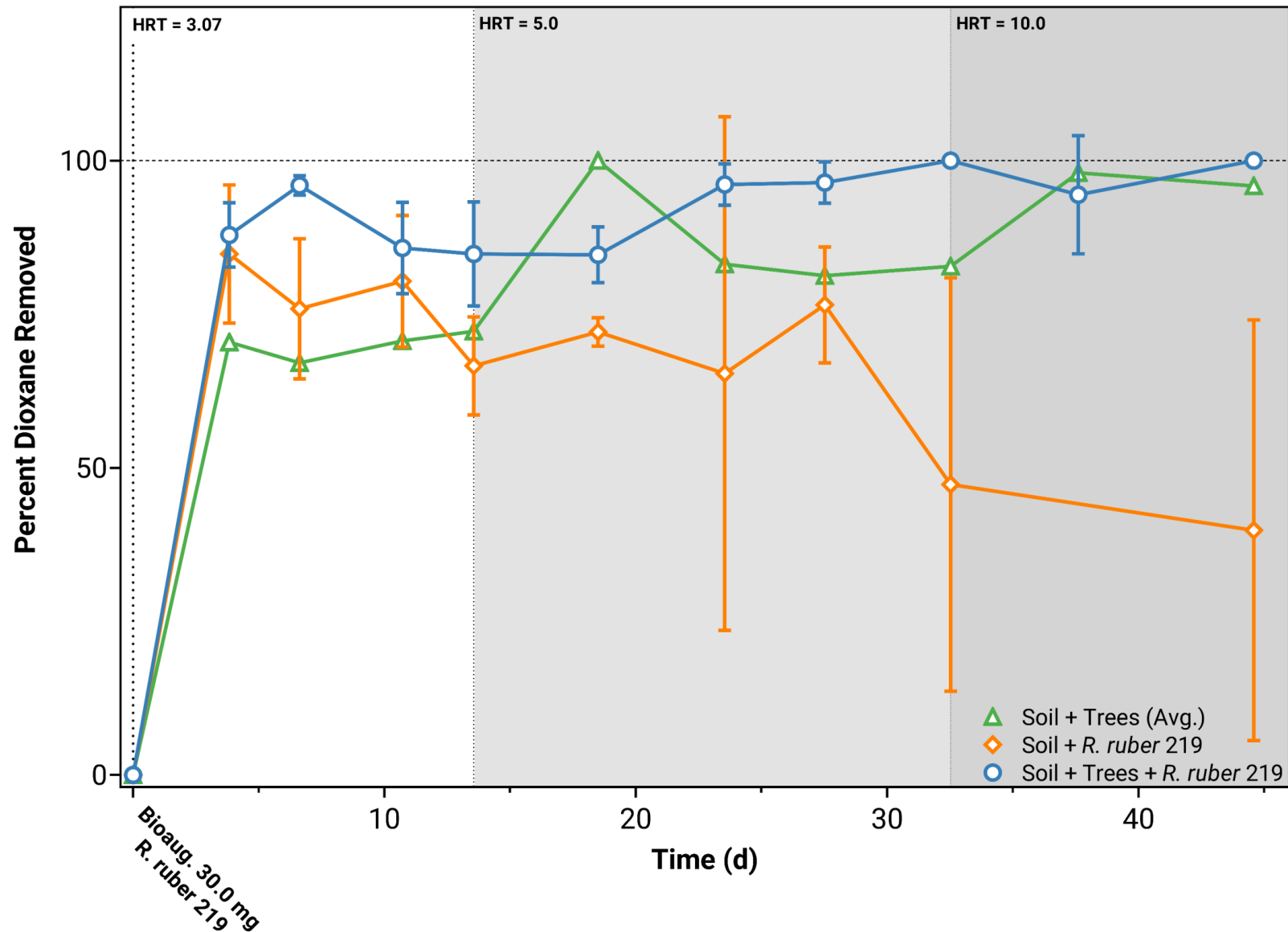
qPCR Results Show Reduced Washout of *R. ruber*219 Gene Copies in Planted Reactors



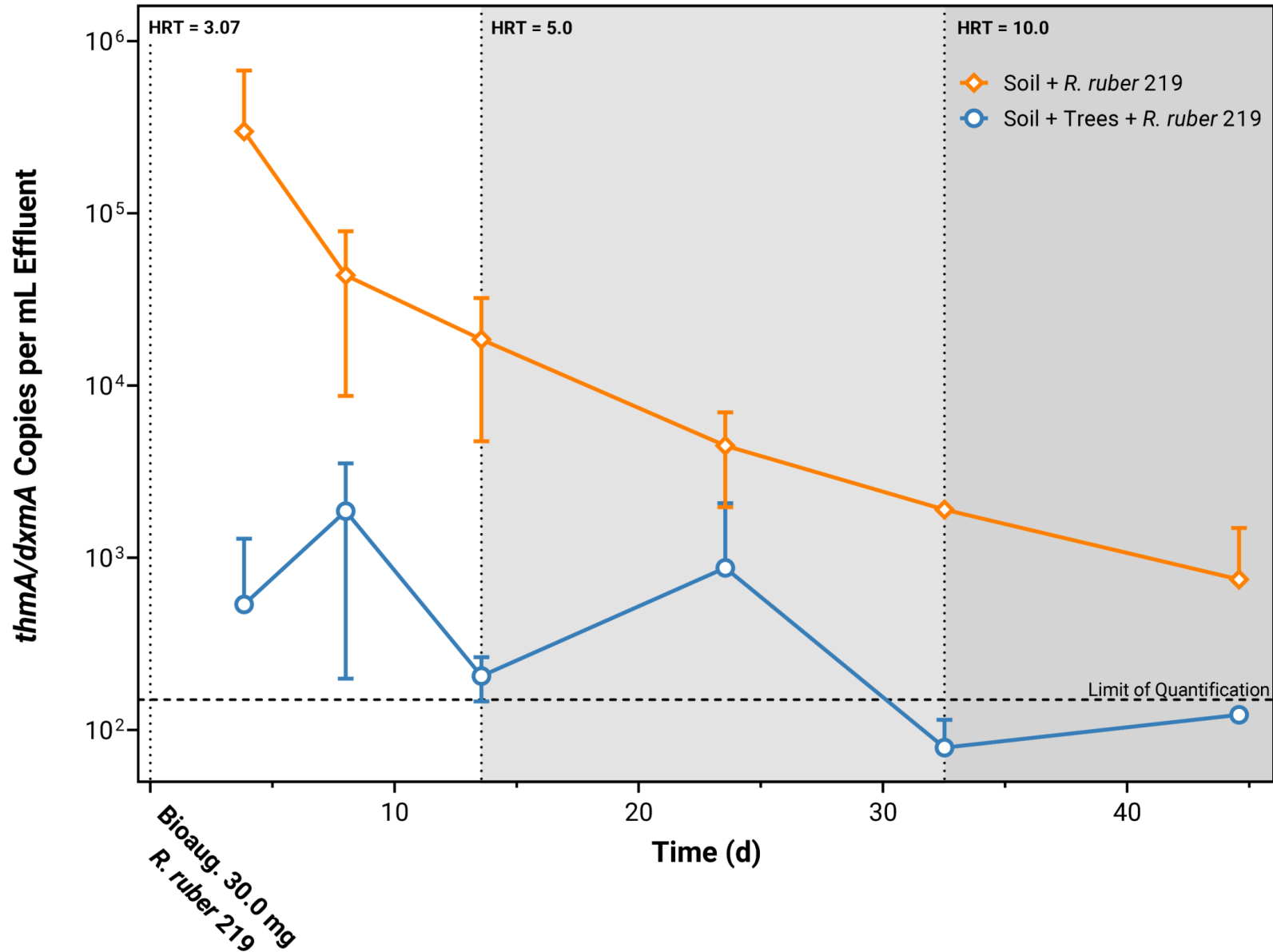
Dioxane effluent concentrations were somewhat higher in soil compared to perlite media



Mass removal by trees with *R. ruber*219 in soil was significantly higher than bio alone



Significantly lower washout of *R. ruber*219 gene copies in Planted Soil Reactors vs. Unplanted (tree roots help)



Major Takeaways

- ✓ Bioaugmented phytoremediation with *R. ruber*219 accelerates treatment of 100 µg/L influent dioxane concentrations to near 1 µg/L
- ✓ Sustained treatment ($\geq 50\%$ degradation) for 44 days

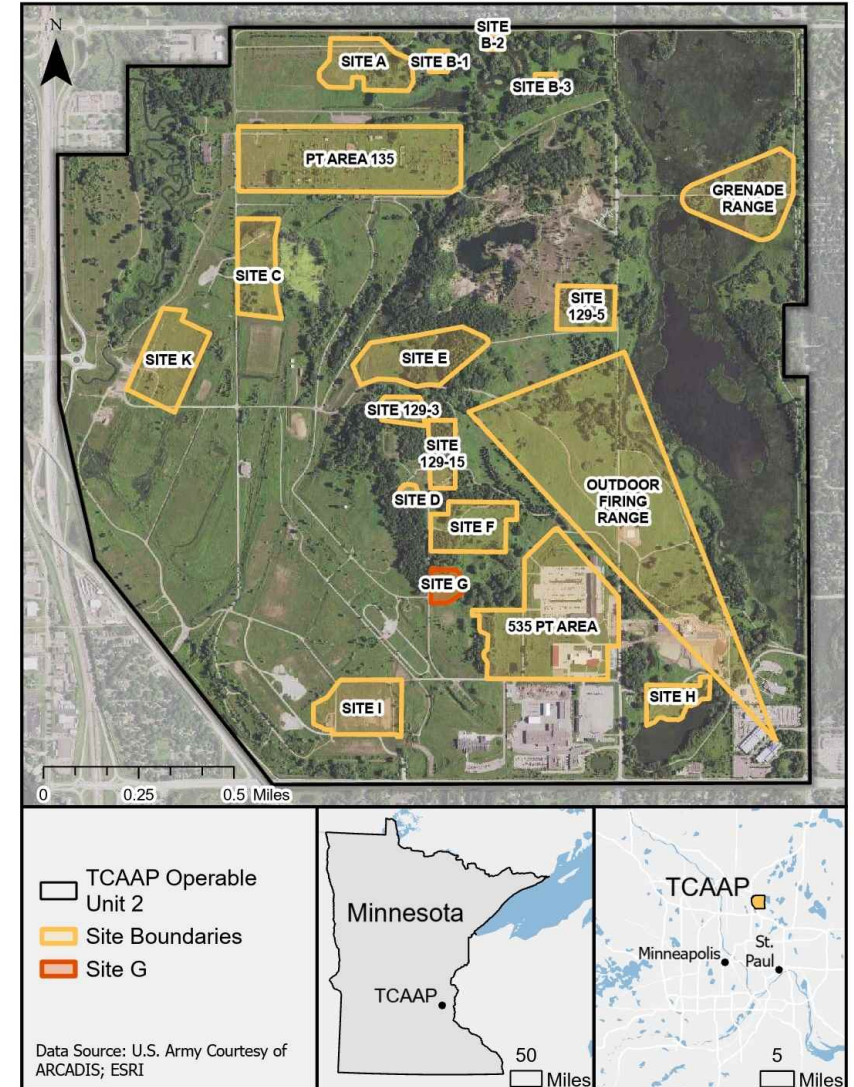
Other observations

- Trees reduce washout of bioaugmented organisms
- Extended HRT improves bioremediation
- Trees capable of complete transpiration (zero discharge)
- Alternative treatment schemes may improve treatment (in-series)
- Repeated bioaugmentation may be necessary

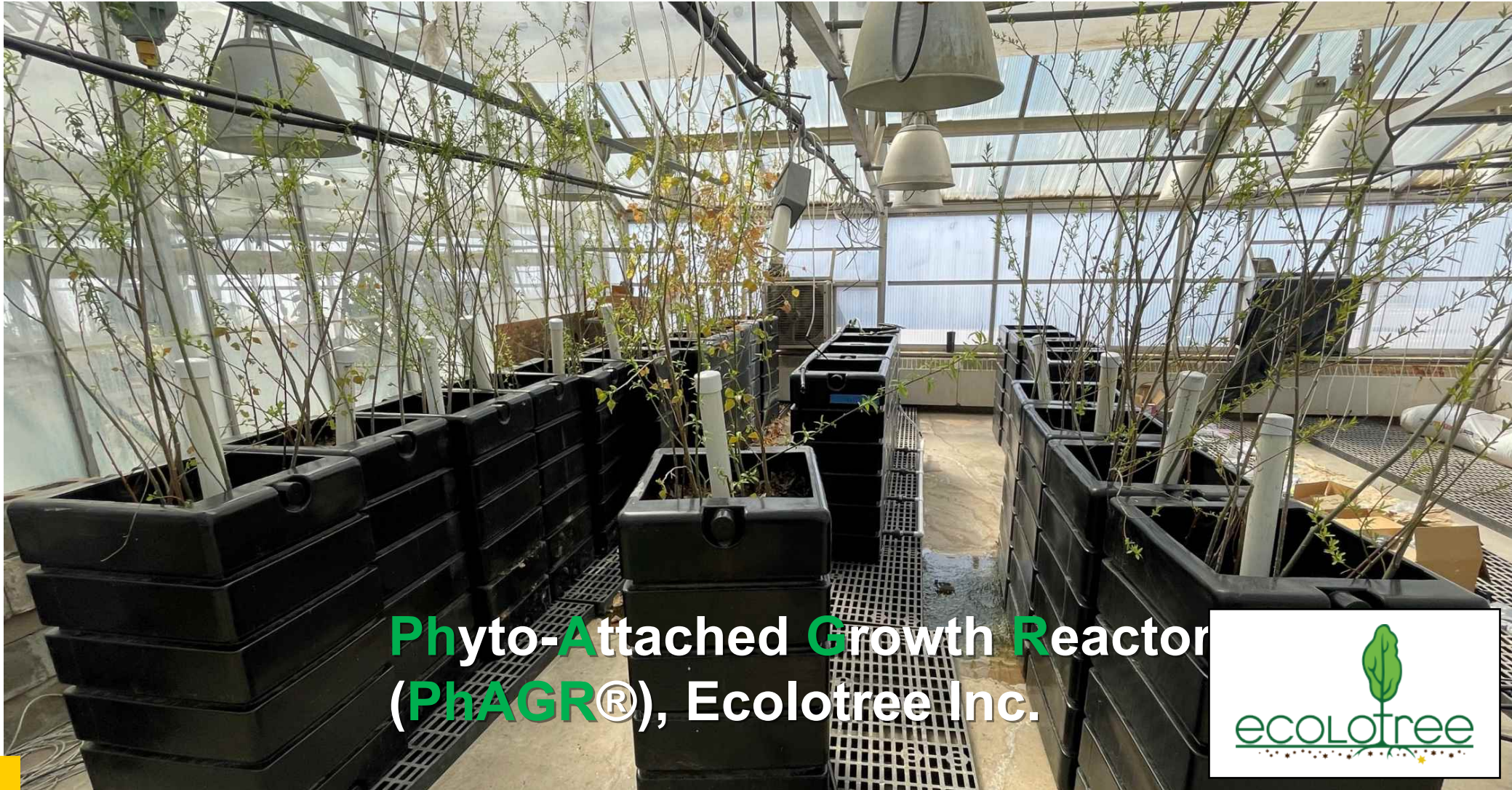
Bioaugmented phytoremediation offers a **green** solution



ESTCP Demonstration at former Twin Cities Army Ammunition Plant, 2023-2025



ESTCP Demonstration at former Twin Cities Army Ammunition Plant, 2023-2025



Acknowledgements

- Emily Jansen, Kyle Patterson, Chris Knutson (Ulowa)
- Lou Licht – Ecolotree, Inc.
- Joel Burken – MS&T
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- SERDP Project ER-2719
- ESTCP Project ER21-5096
- NSF Integrative Graduate Education Research Traineeship (IGERT) [Grant # 0966130]

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and Engineering



ESTCP

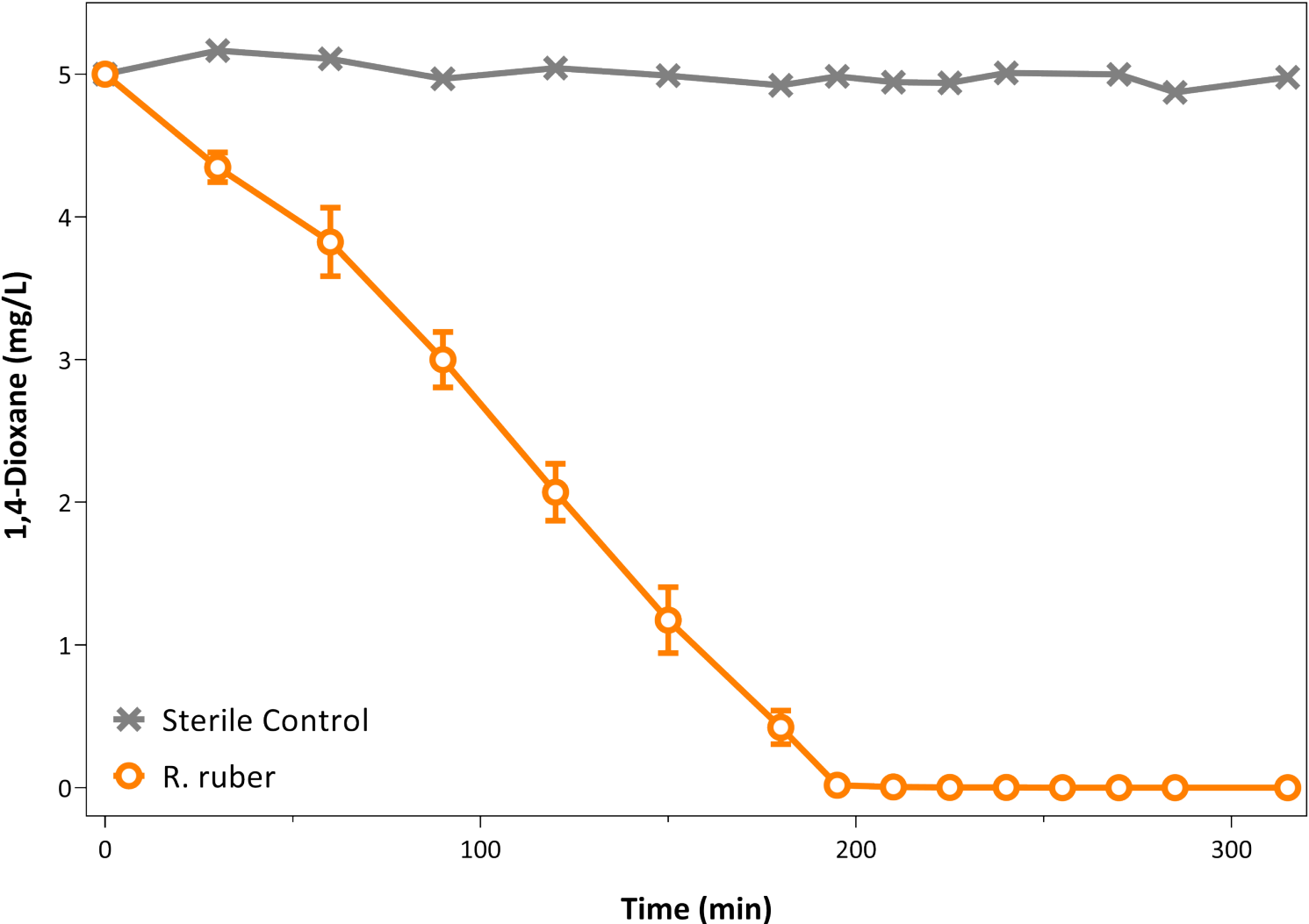


SERDP
DOD • EPA • DOE

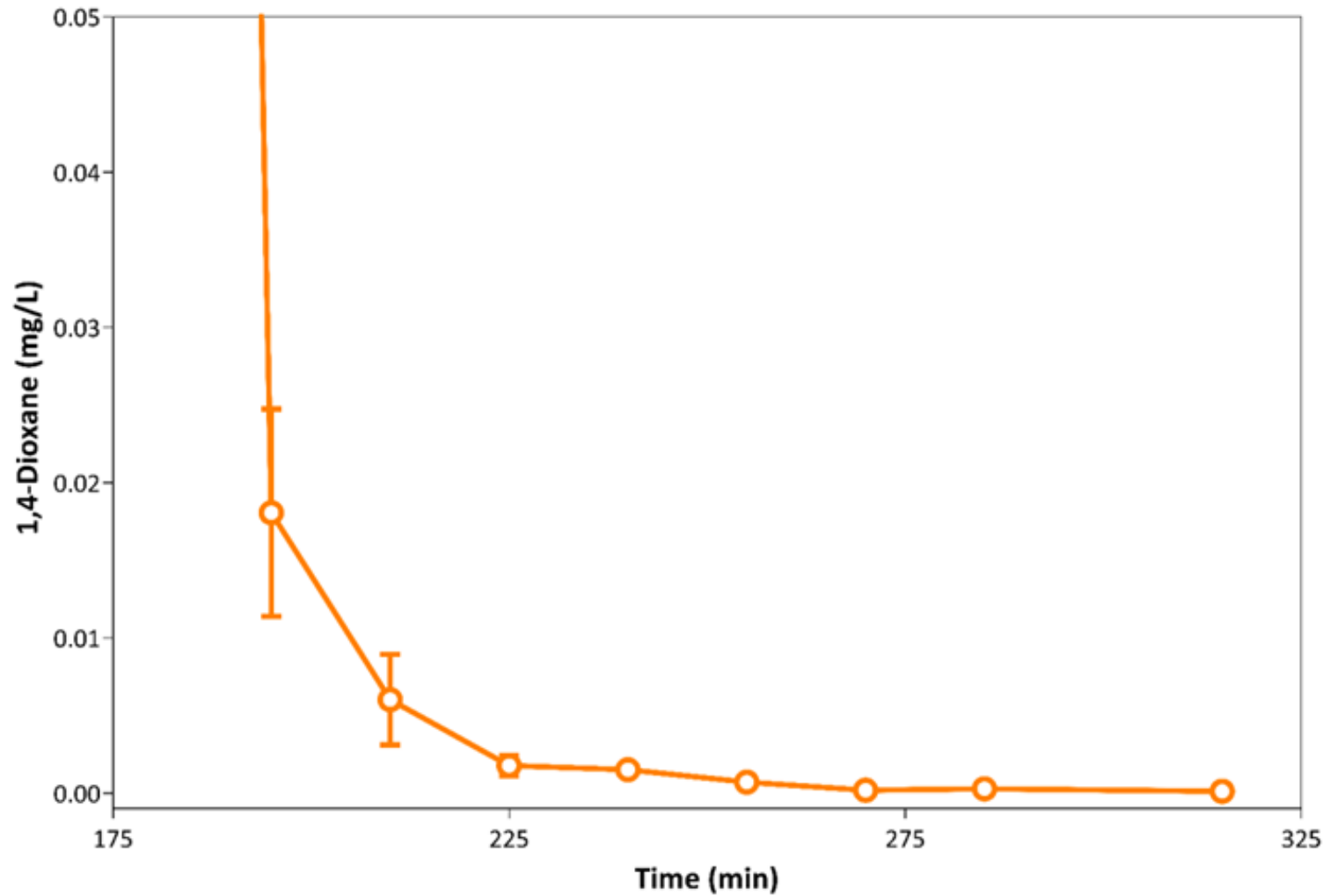


Backup Slides

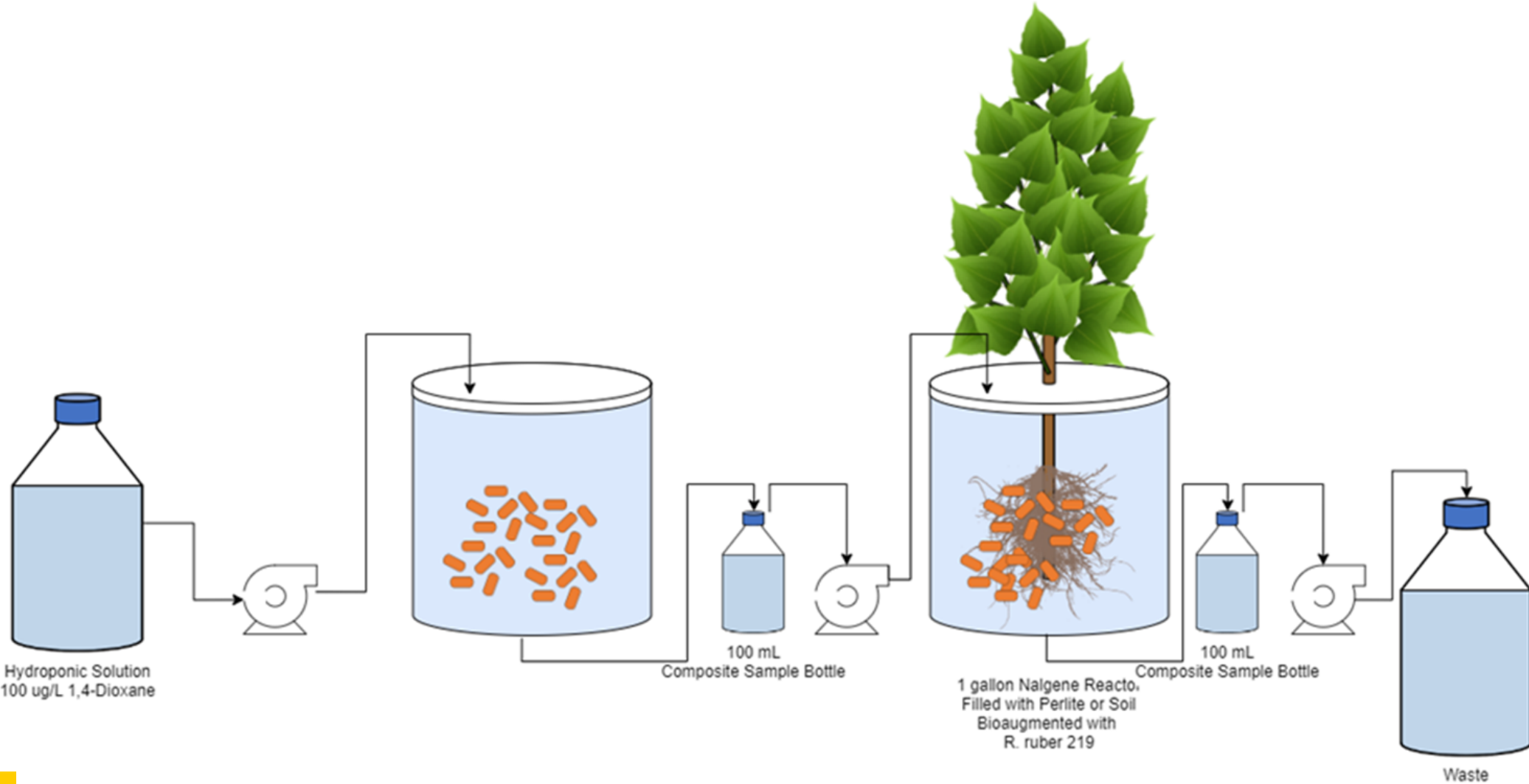
*R. ruber*219 rapidly degrades 5 mg/L dioxane to <1µg/L



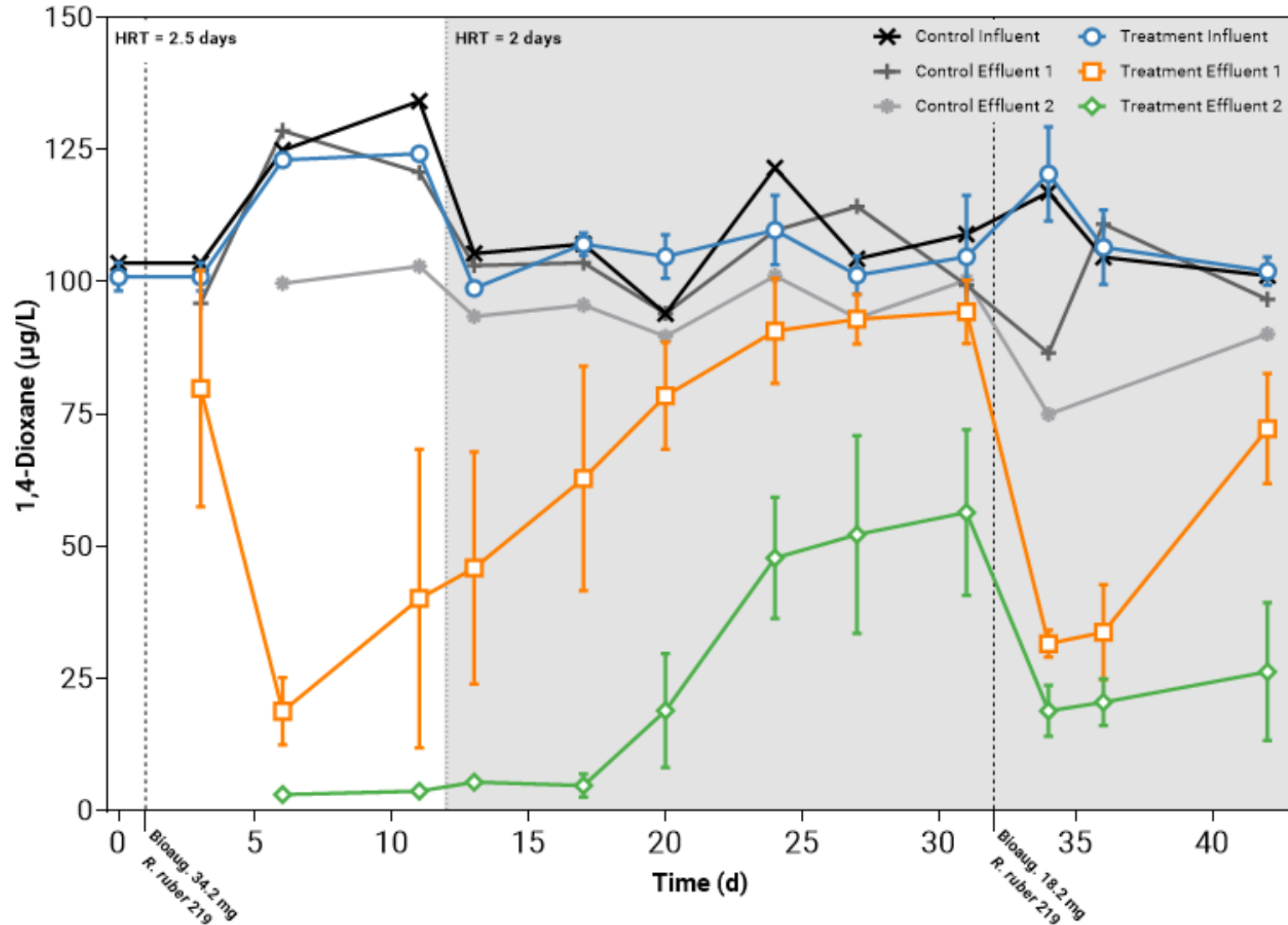
*R. ruber*219 rapidly degrades 5 mg/L dioxane to <1μg/L



In-series arrangement tested to improve long-term treatment



In-series arrangement significantly improves effluent concentration from second reactor



Essential nutrient thiamine (50 ppb) was absent from effluent likely due to sorption

