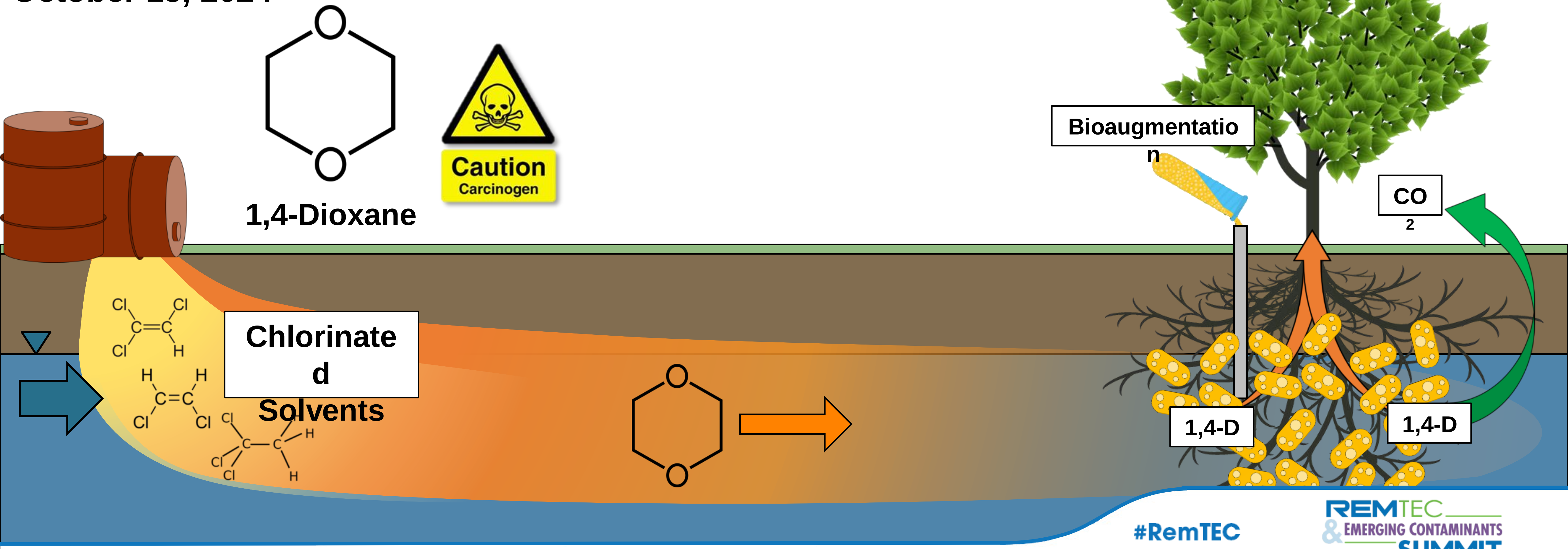
IOWA | IIHR—Hydroscience and Engineering
SERDP ER-2719
ESTCP ER21-5096

Bioaugmented Phytoremediation to Treat 1,4-Dioxane Contaminated Groundwater

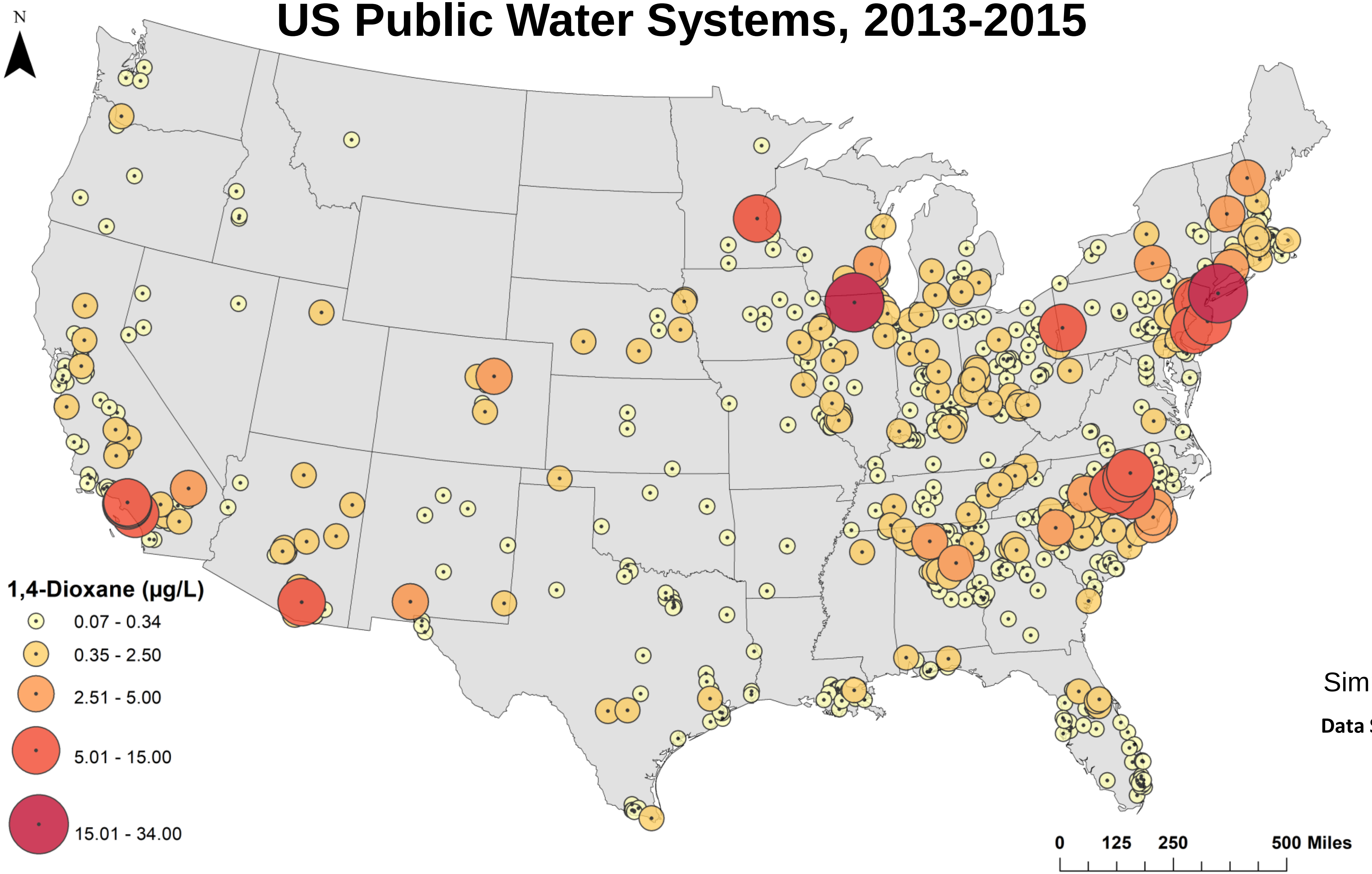
Reid Simmer, Emily Jansen, Joel Burken, Lou Licht, Tim Mattes, and Jerry Schnoor

RemTEC & Emerging Contaminants Summit

October 15, 2024



UCMR3: 1,4-Dioxane Contamination in Finished Drinking Water
US Public Water Systems, 2013-2015

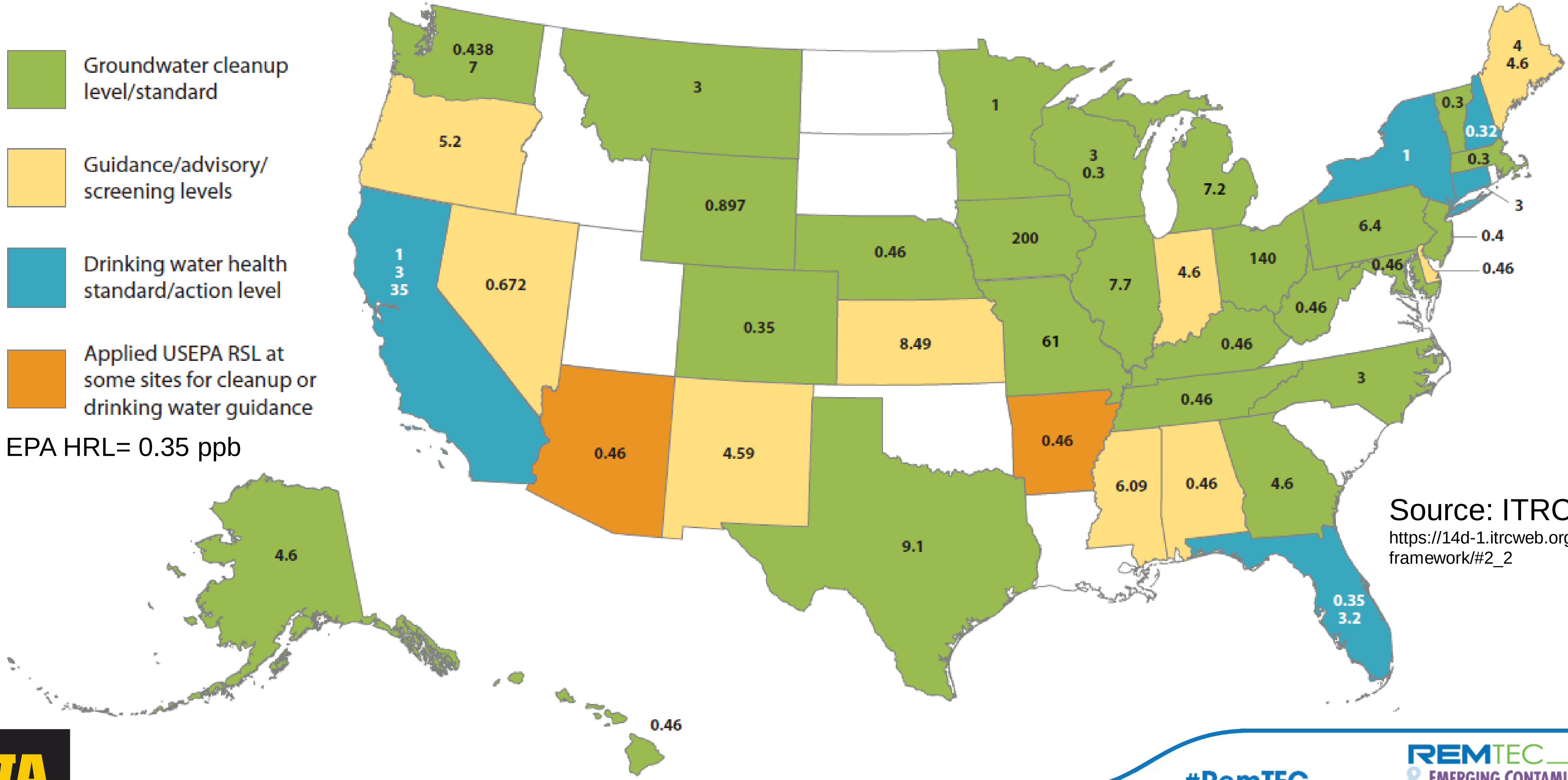


Simmer et al., 2020

Data Source: EPA UCMR3

“1,4-Dioxane in drinking water: emerging for 40 years and still unregulated”

-McElroy, Hyman and Knappe, *Current Opinion in Environmental Science & Health*, 2019



EPA HRL= 0.35 ppb

Source: ITRC

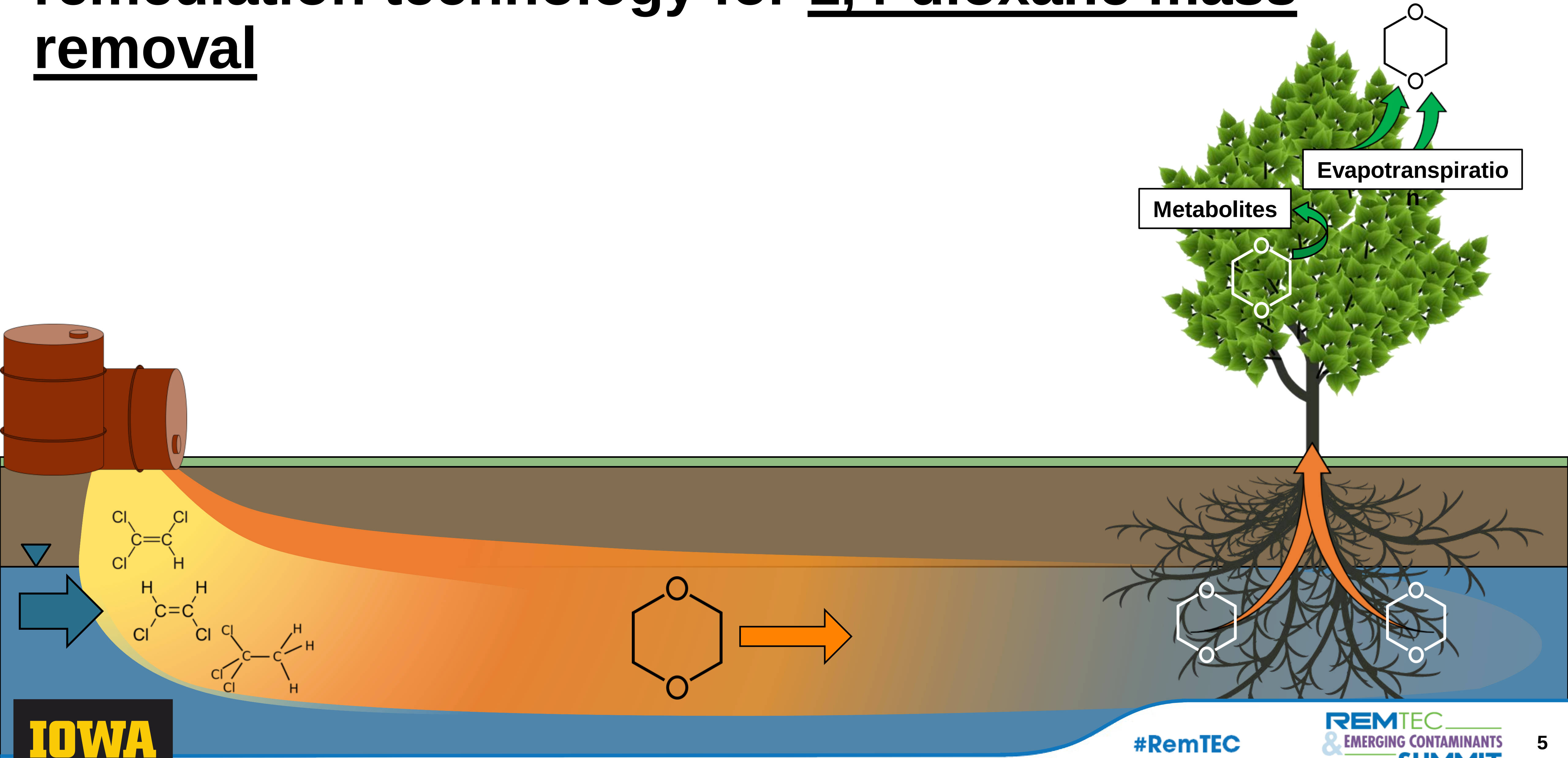
https://14d-1.itrcweb.org/regulatory-framework/#2_2

Common remediation strategies are costly

Advanced Oxidation Facility for Dioxane Treatment, Tucson, AZ

Advanced Oxidation Facility for Dioxane Treatment, Arden Hills, MN

Phytoremediation is a low cost, green remediation technology for <u>1,4-dioxane mass removal</u>



st, green 4-dioxane

water ENVIRONMENT RESEARCH
Water Environment Federation the water quality people®

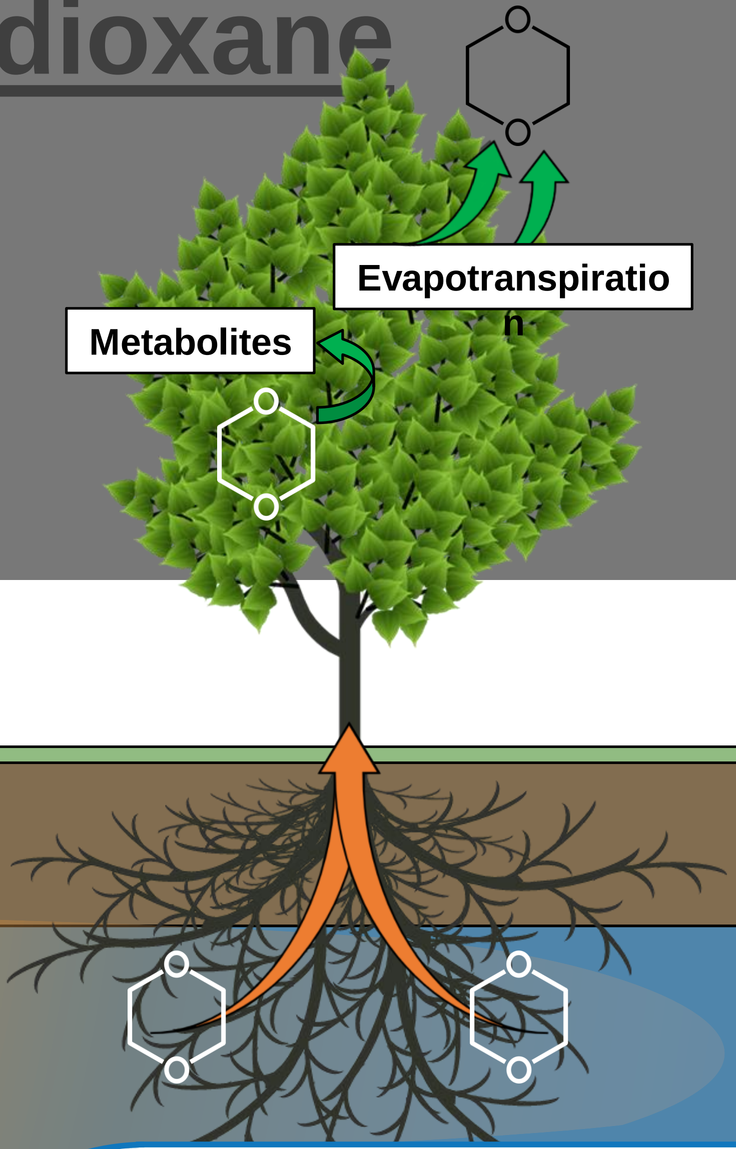
Research Paper | Full Access

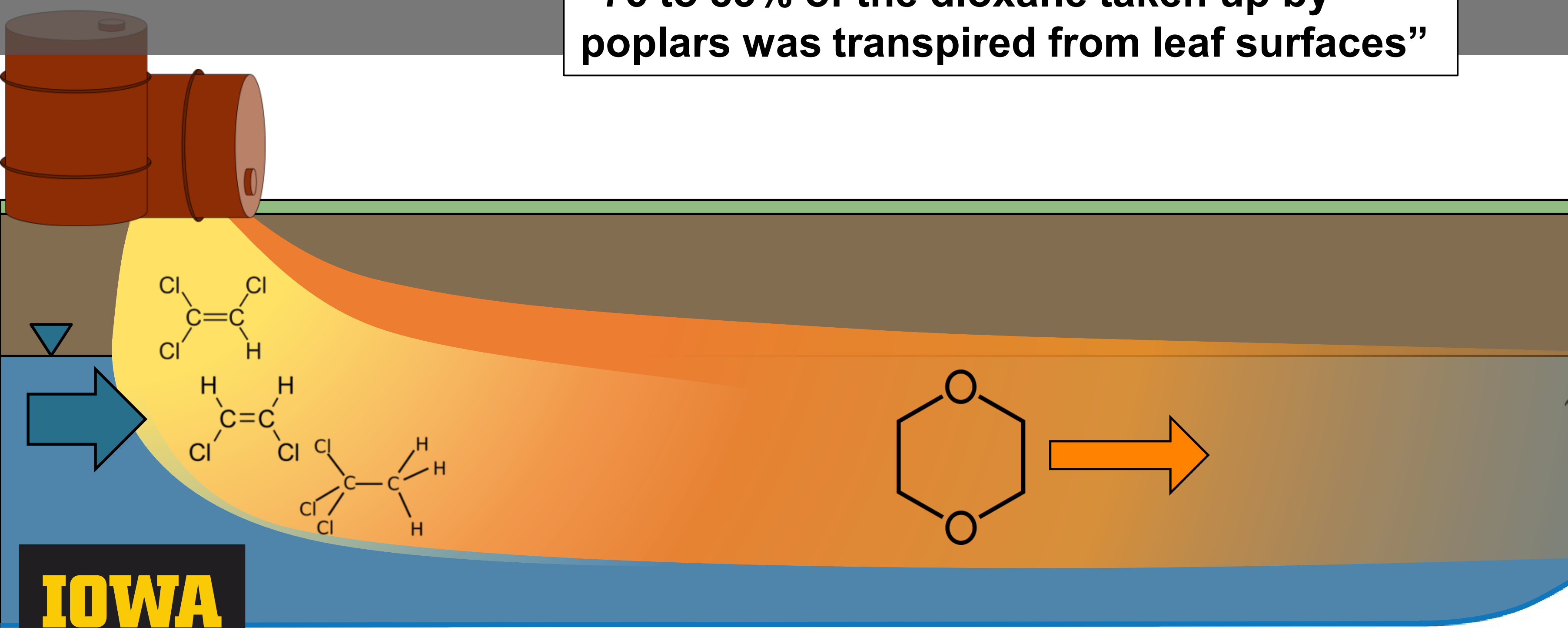
Phytoremediation of 1,4-Dioxane by Hybrid Poplar Trees

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

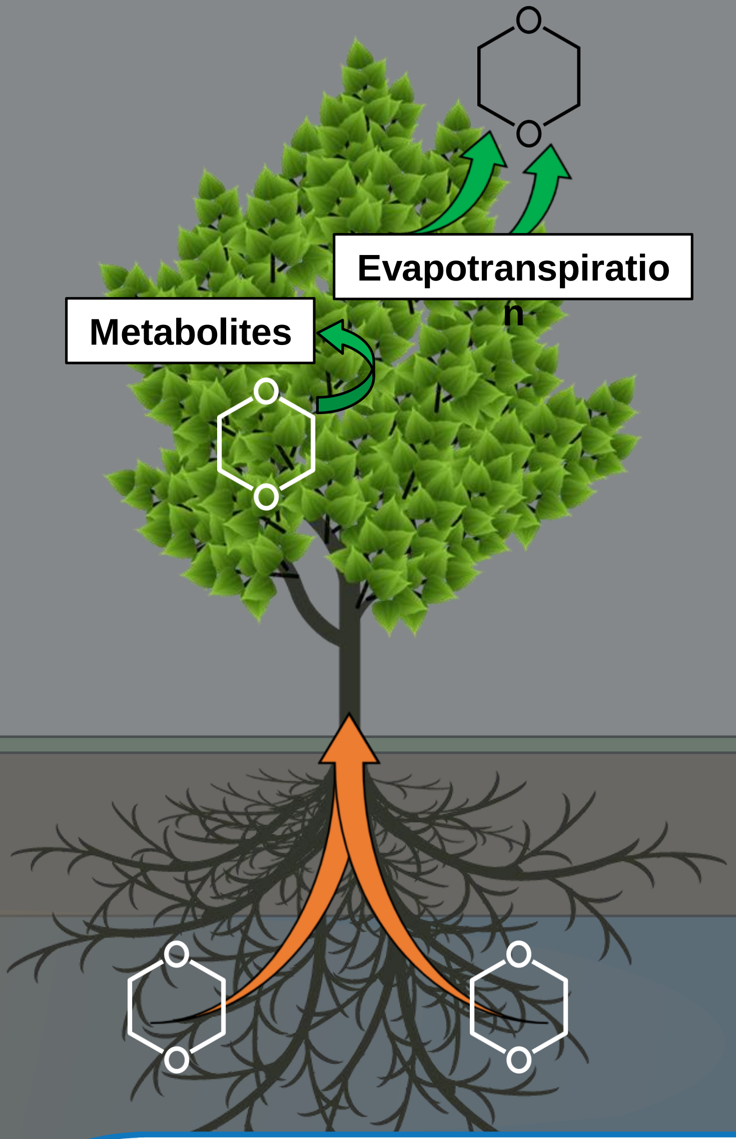
“76 to 83% of the dioxane taken up by poplars was transpired from leaf surfaces”



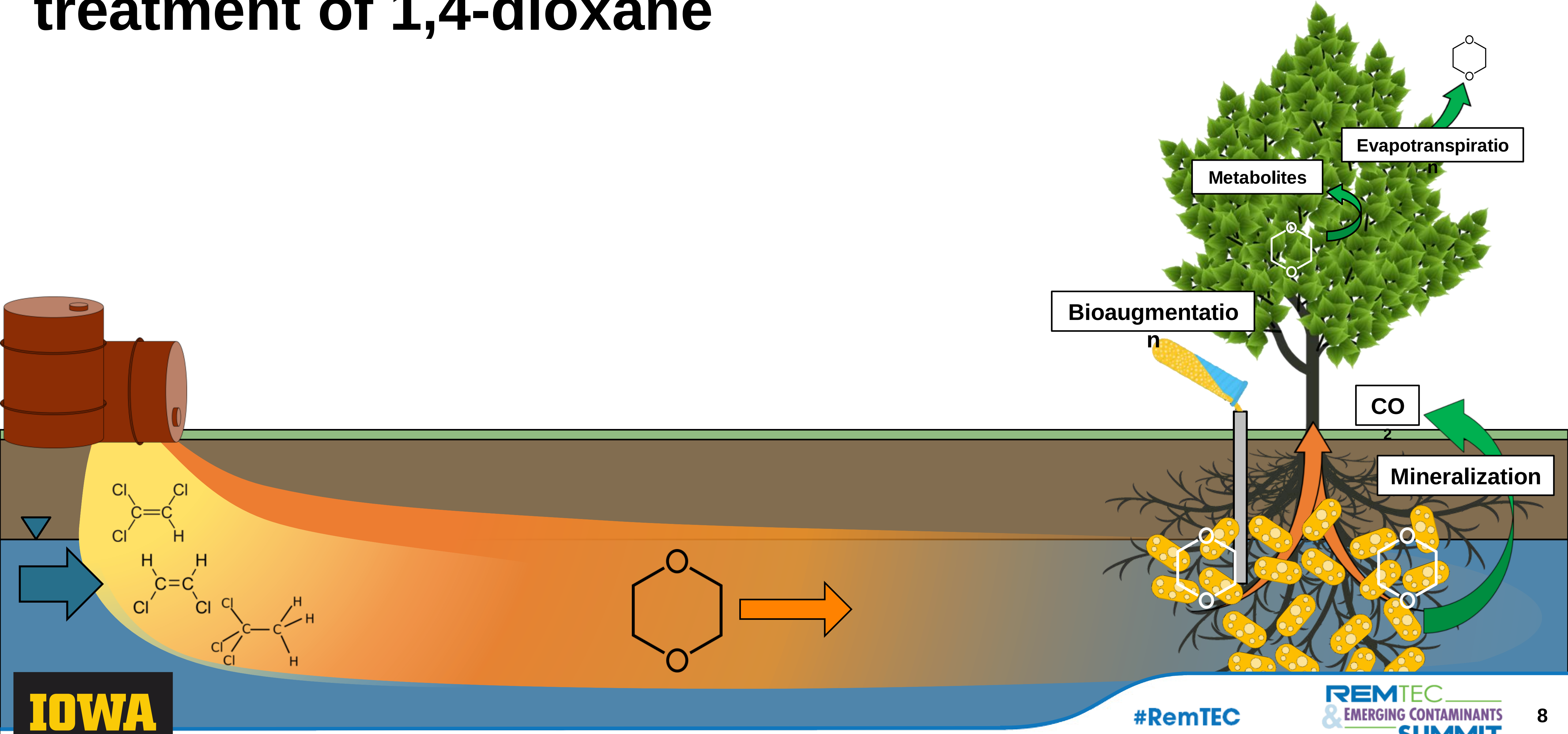


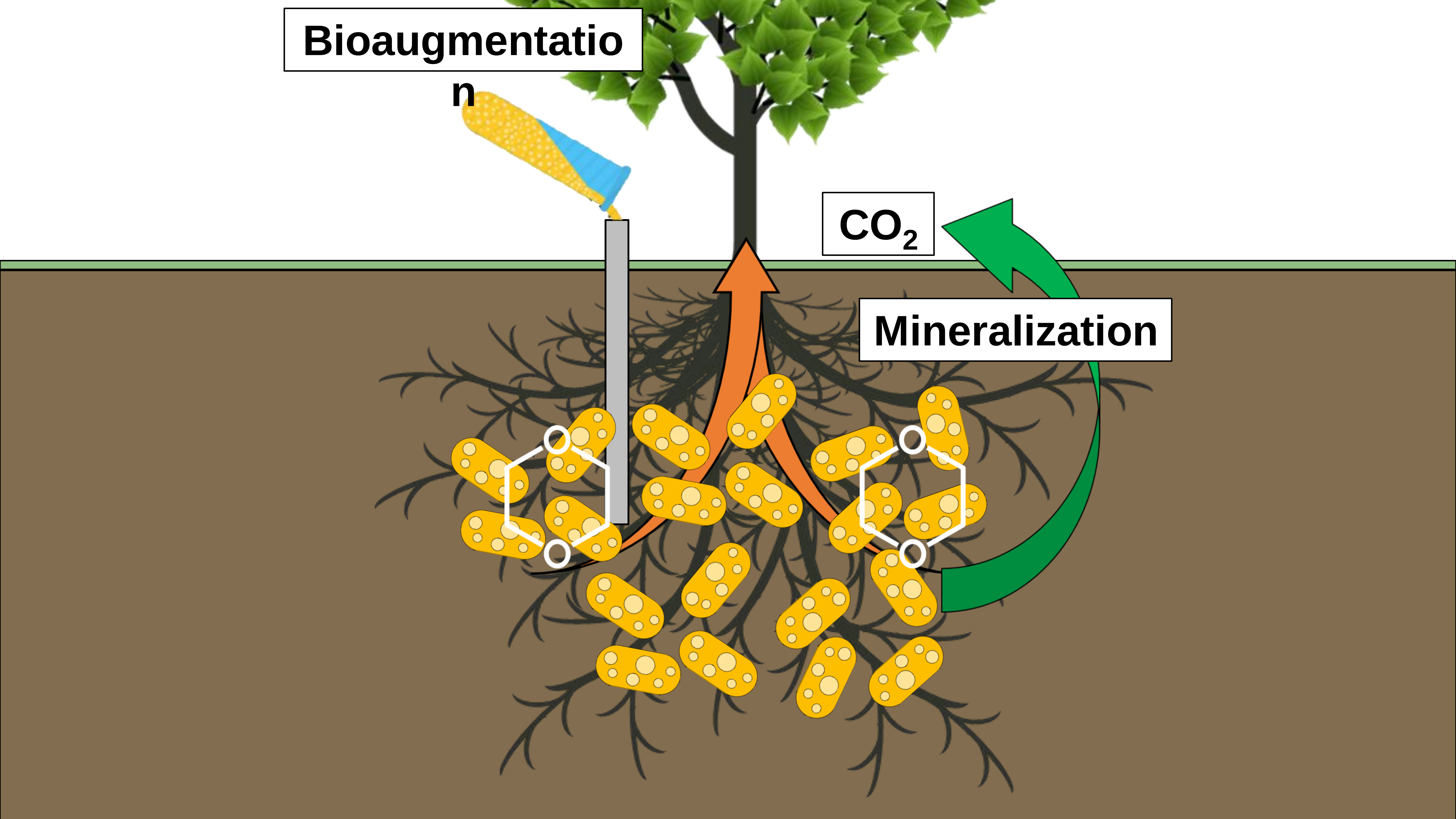
Remediation/ Treatment Technology	Development status		Effectiveness
	GW	VZS	
Monitored natural attenuation (includes physical, chemical, and biological mechanisms)	E	NA	May be effective at reducing 1,4-D at lower starting concentrations (e.g., <500 µg/L), depending on the time available and relevant 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

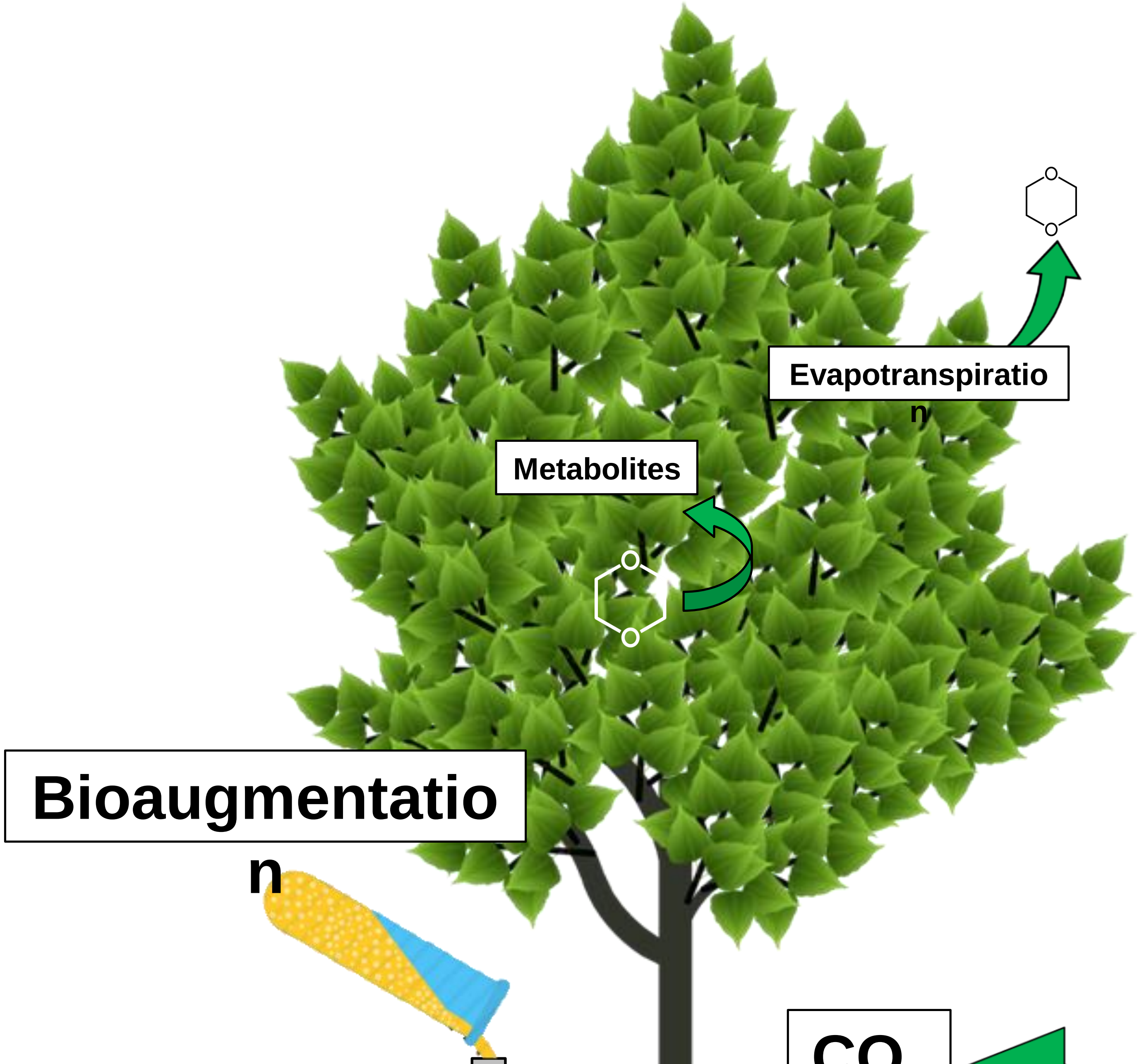
Interstate Technology and Regulatory Council, 2021



Bioaugmented phytoremediation speeds treatment of 1,4-dioxane




Bioaugmentatio
n
CO_2
Mineralization

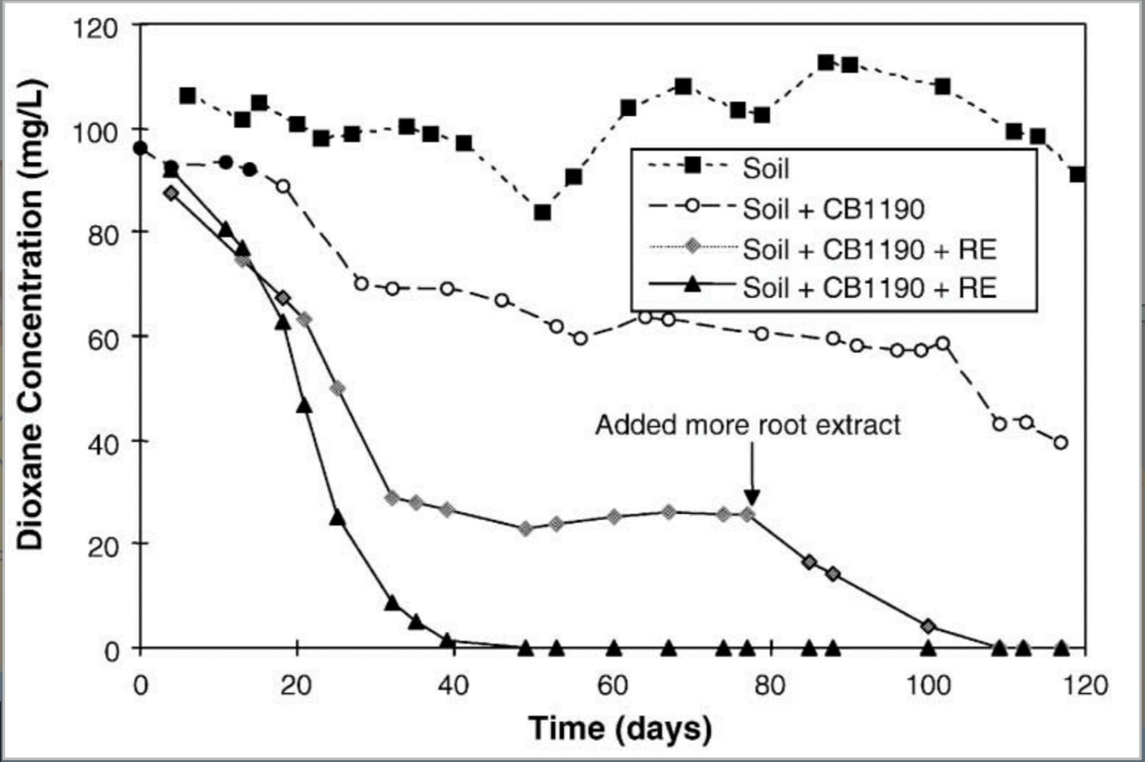


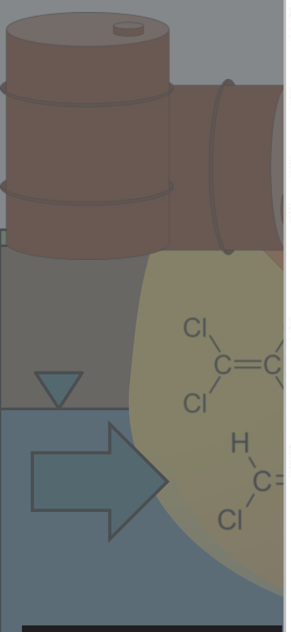
Evapotranspiratio
n
Metabolites
Bioaugmentatio
n
CO

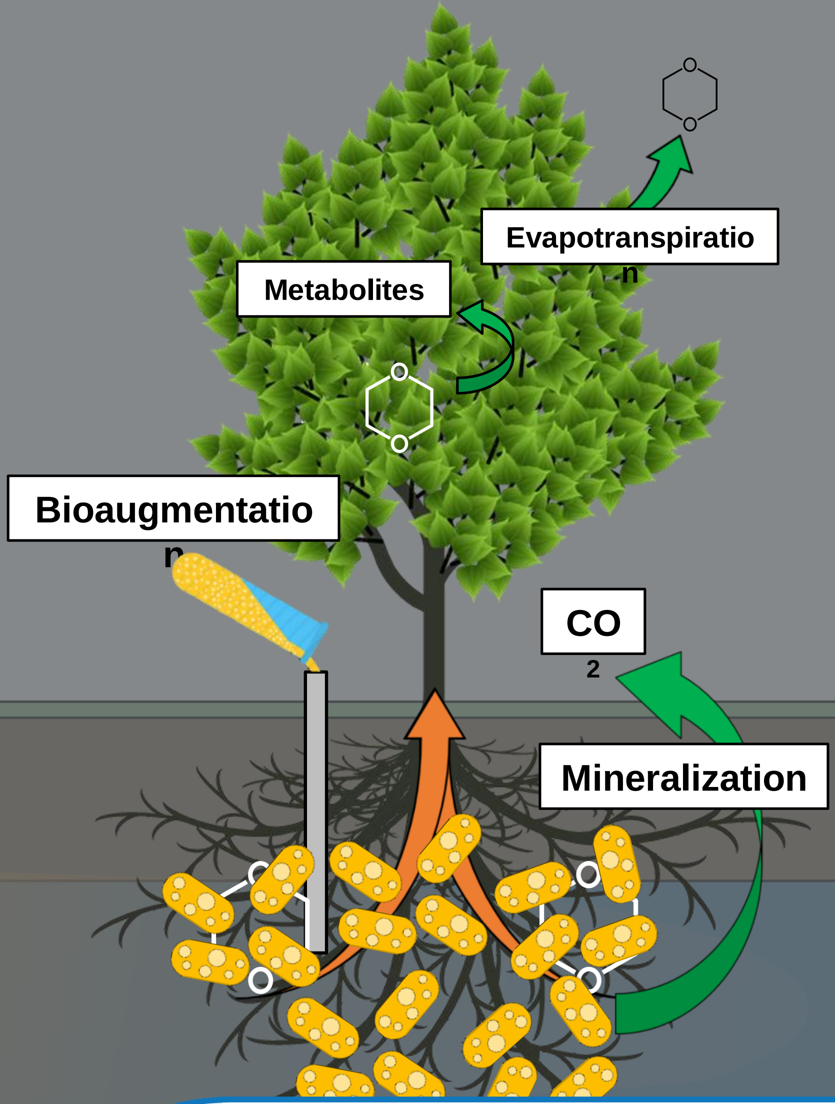
Water Research
Volume 35, Issue 16, November 2001, Pages 3791-3800

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

Sara L. Kelley [1], Eric W. Aitchison [1], Milind Deshpande [2], Jerald L. Schnoor [1], Pedro J.J. Alvarez [1]







ELSEVIER

Water Research

Volume 35, Issue 16, November 2001, Pages 3791-3800

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

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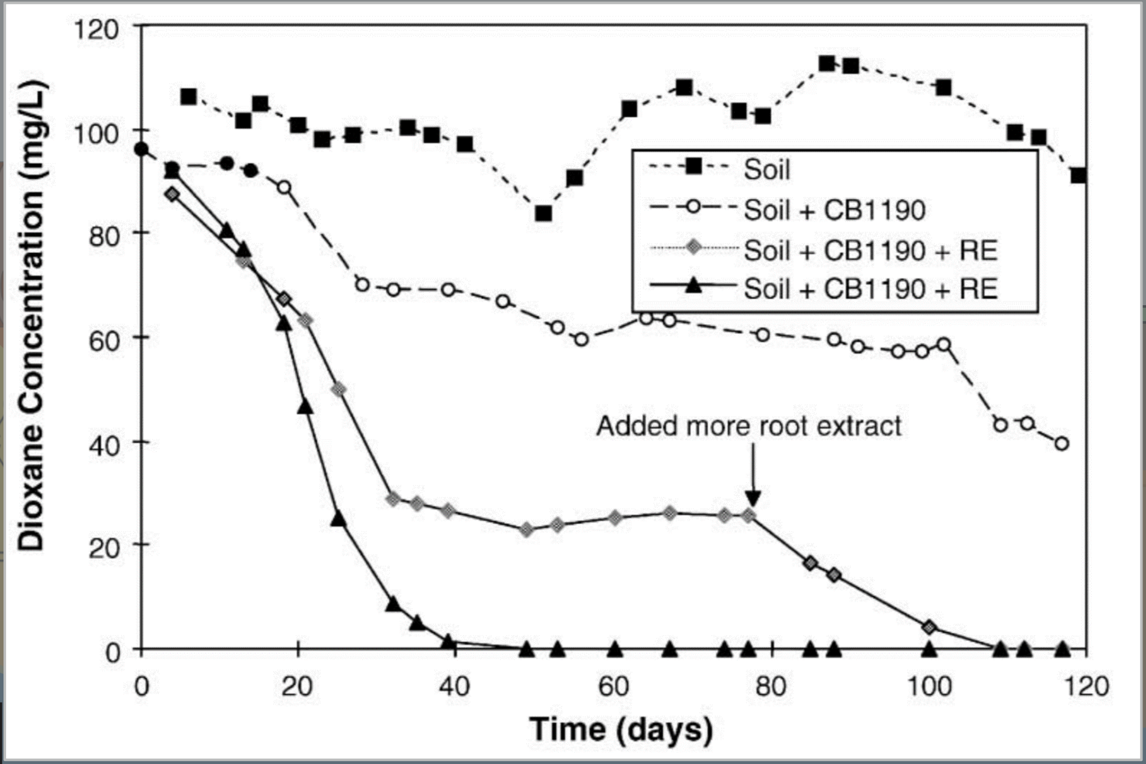
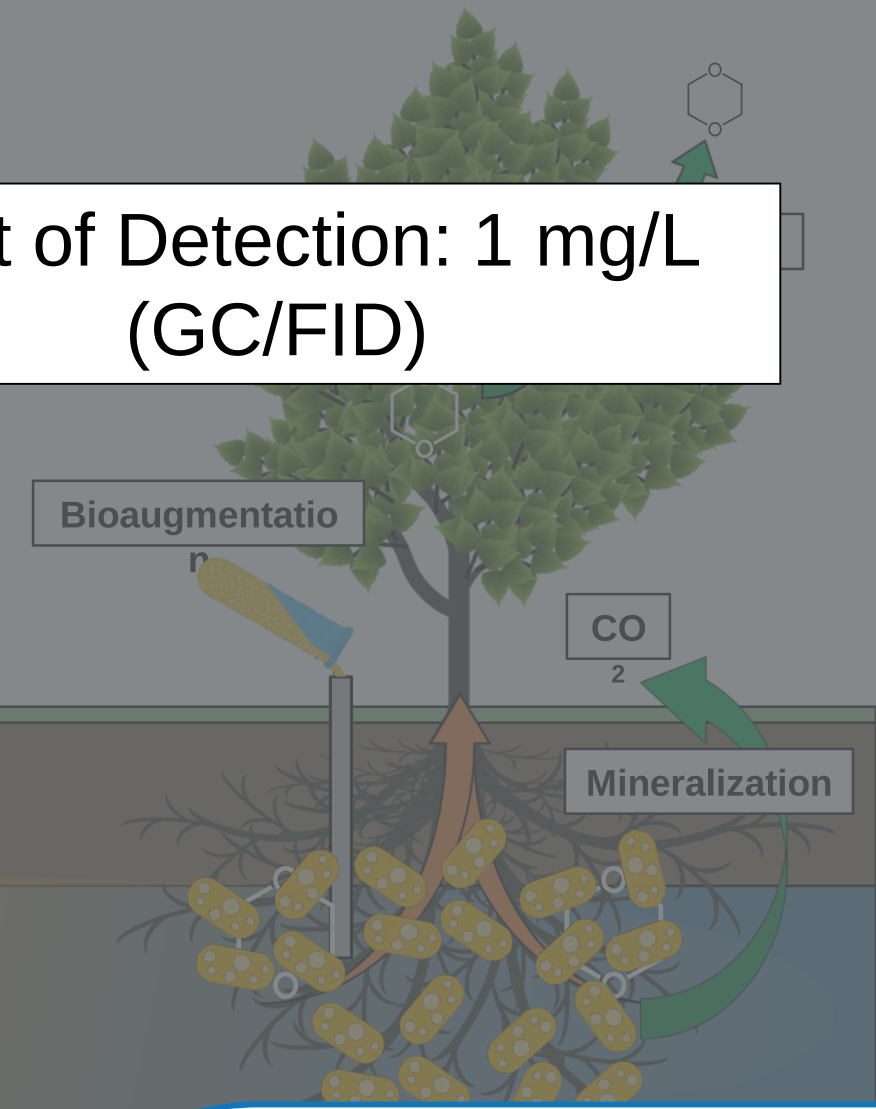
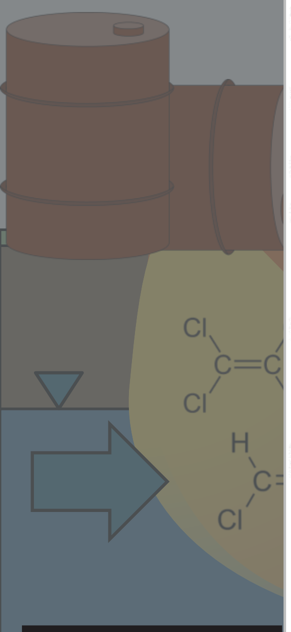


Limit of Detection: 1 mg/L (GC/FID)





Science of the Total Environment 744 (2020) 140823

Contents lists available at ScienceDirect

Science of the Total Environment

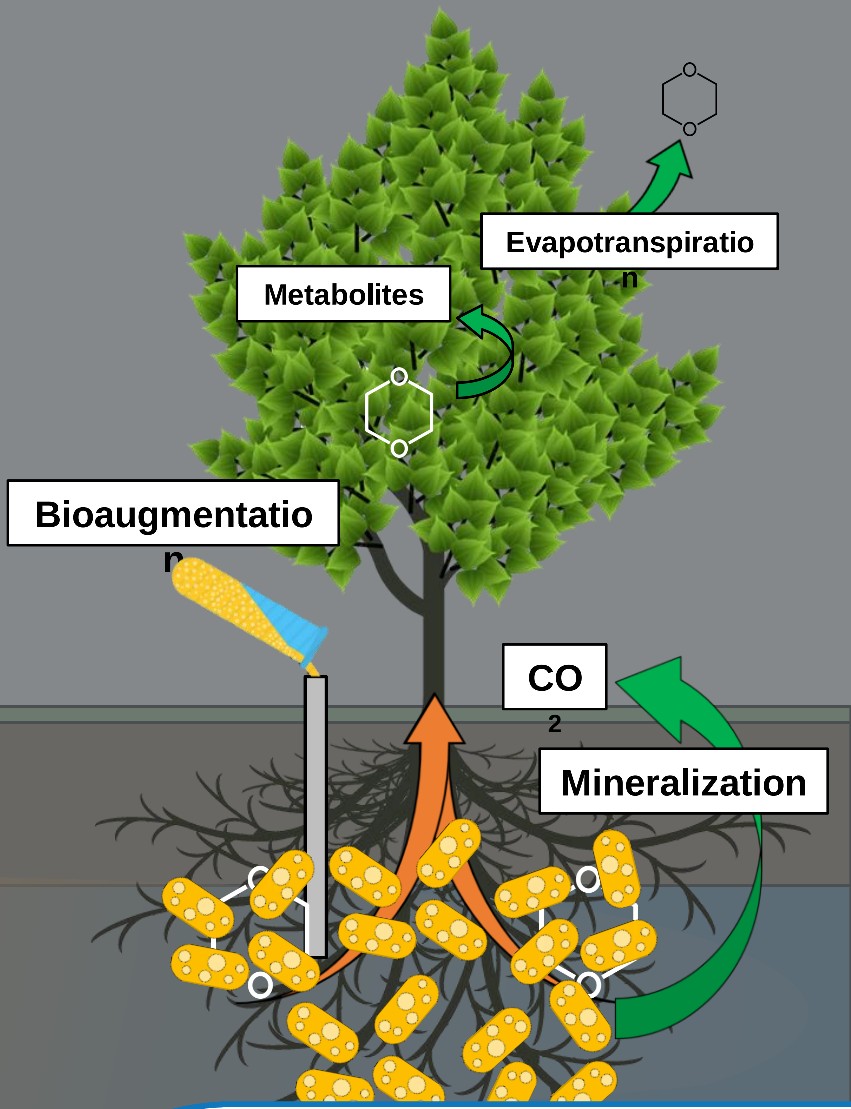
journal homepage: www.elsevier.com/locate/scitotenv

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

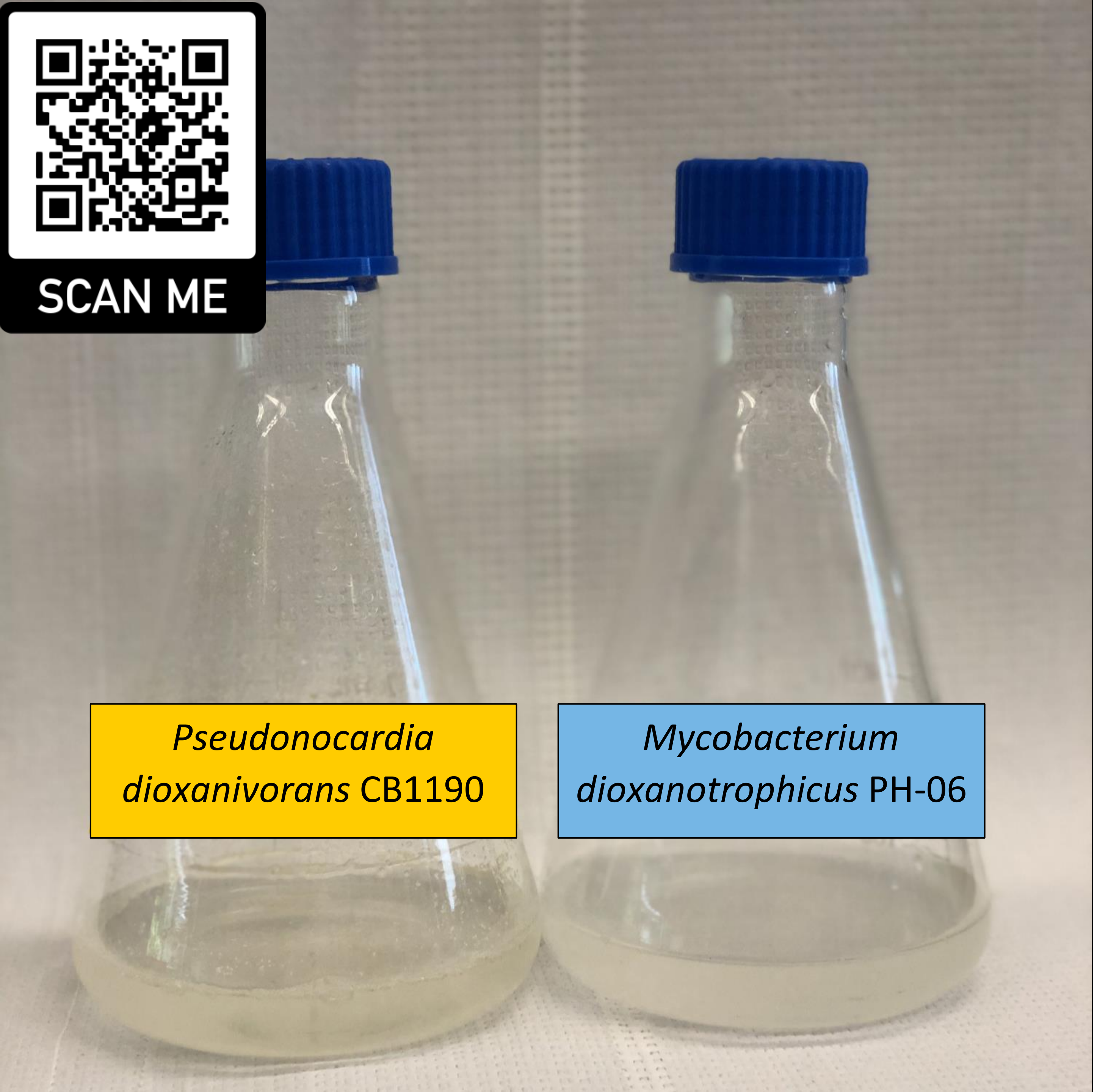
SCAN ME

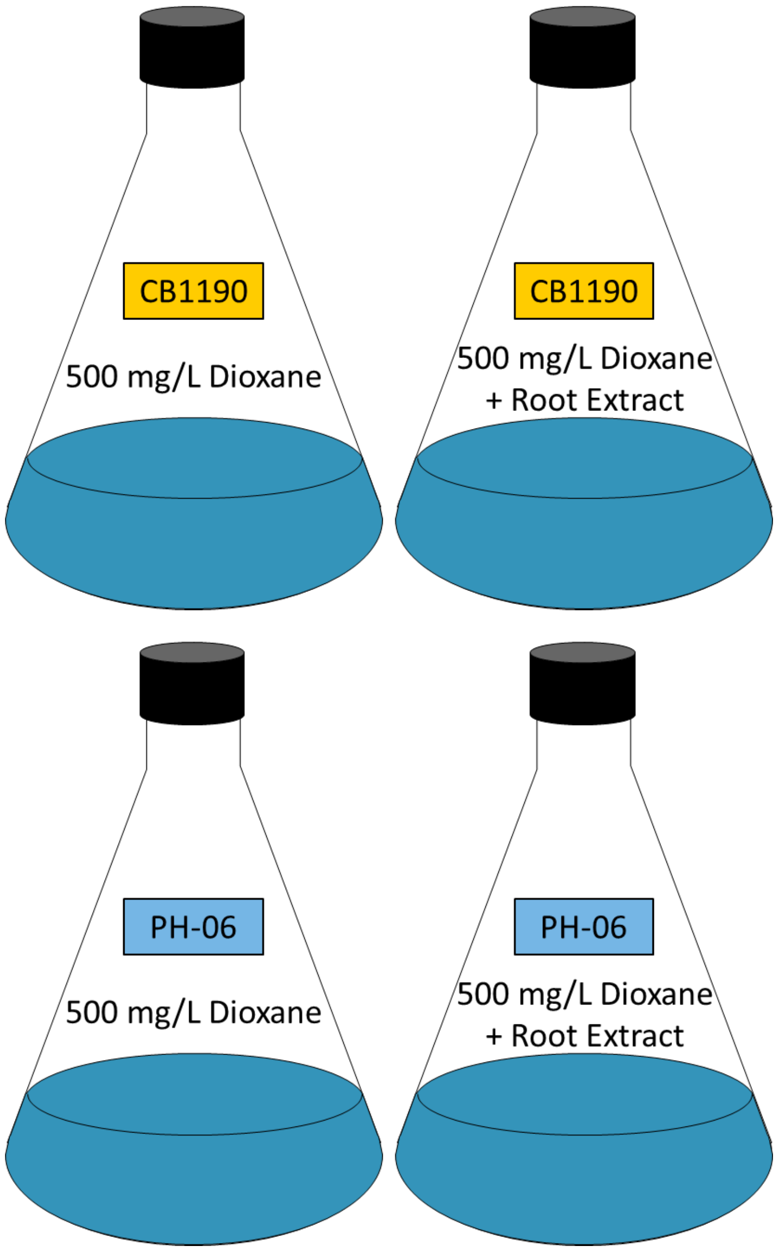


Does *Mycobacterium sp.* PH-06 utilize root exudates as a supplementary carbon source?

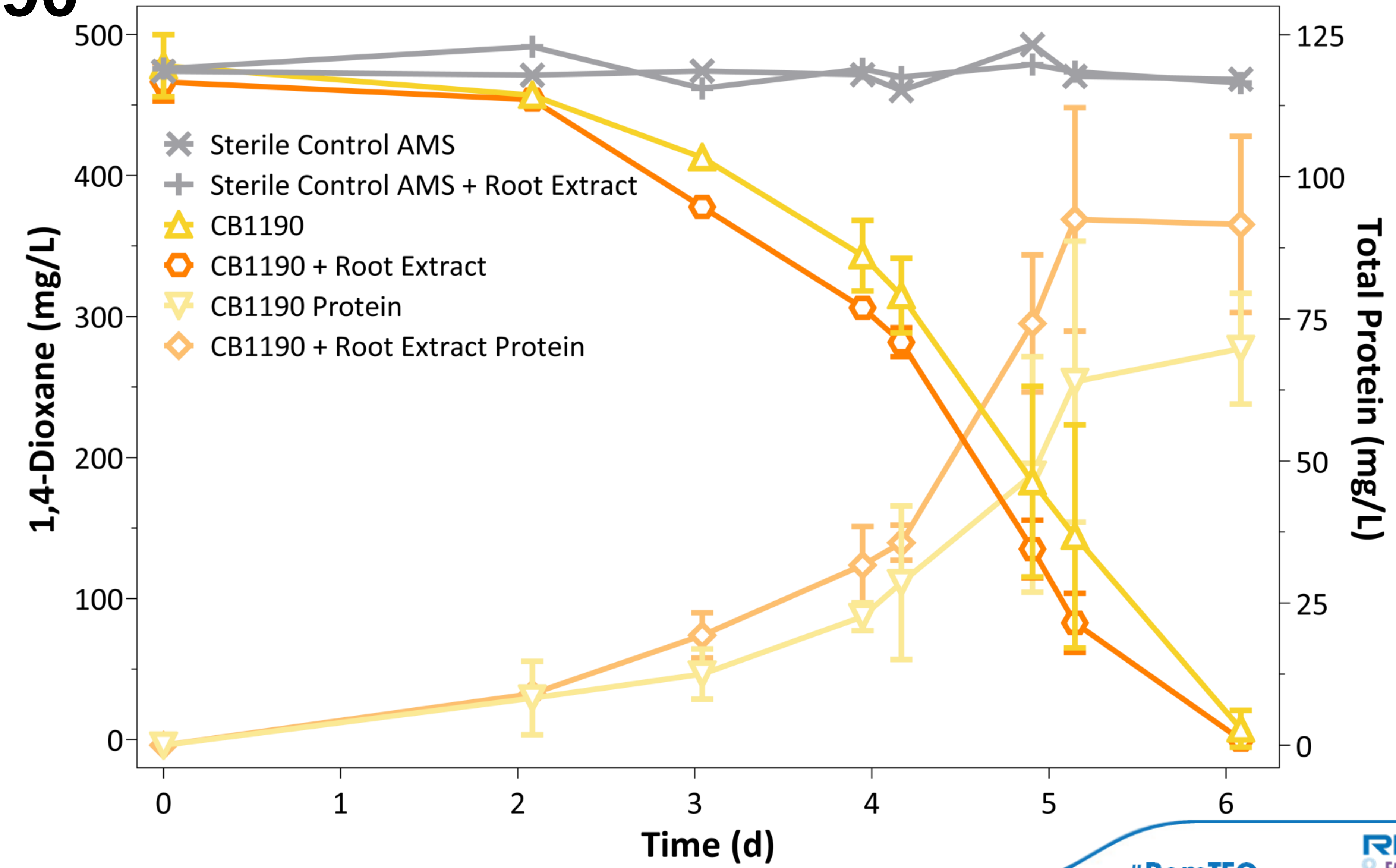
Can bioaugmentation with PH-06 remove 1,4-dioxane faster than CB1190?

SCAN ME


SCAN ME
Pseudonocardia dioxanivorans CB1190
Mycobacterium dioxanotrophicus PH-06

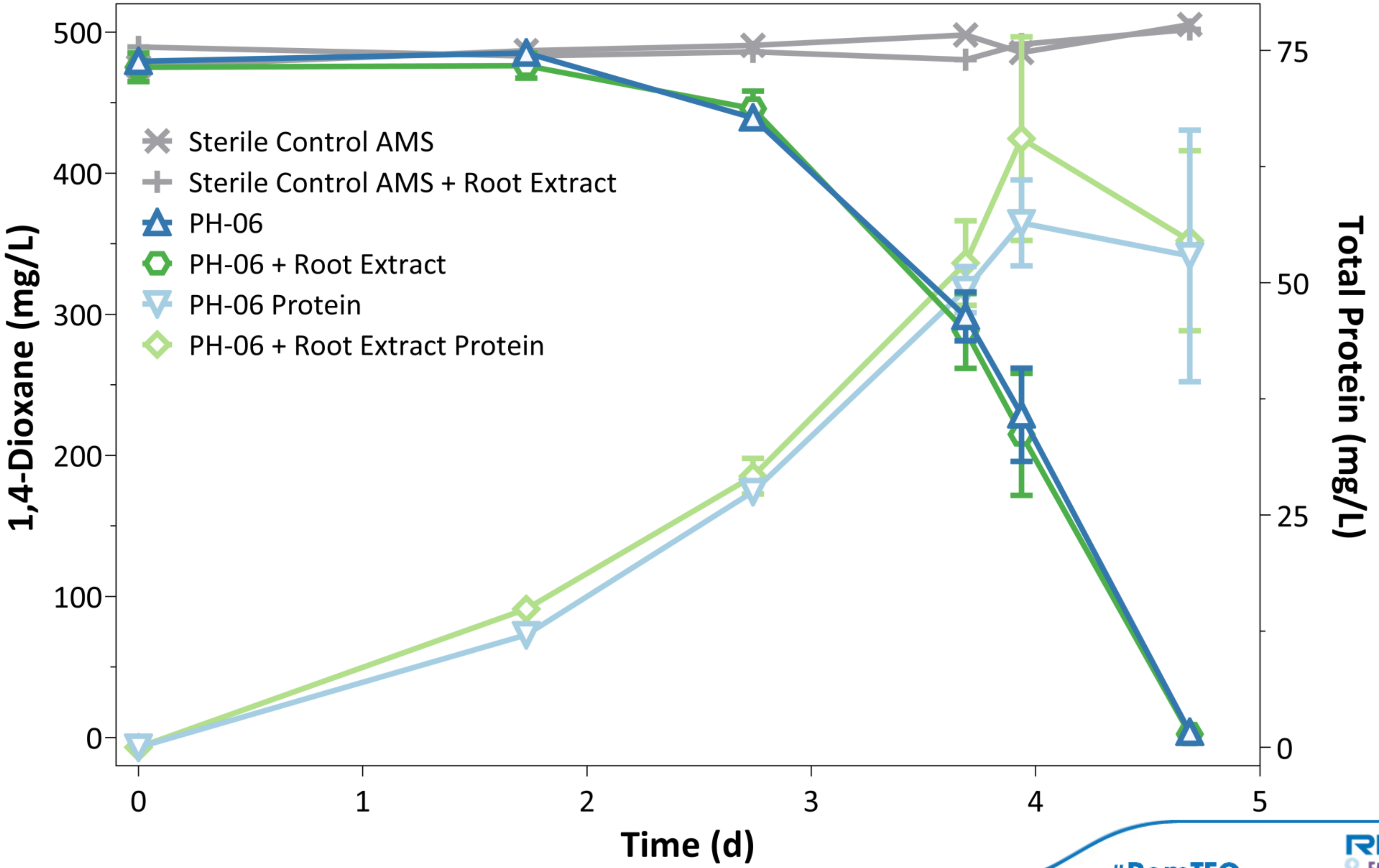

CB1190
500 mg/L Dioxane
CB1190
500 mg/L Dioxane
+ Root Extract
PH-06
500 mg/L Dioxane
PH-06
500 mg/L Dioxane
+ Root Extract

Root extract acted as auxiliary supplement for CB1190



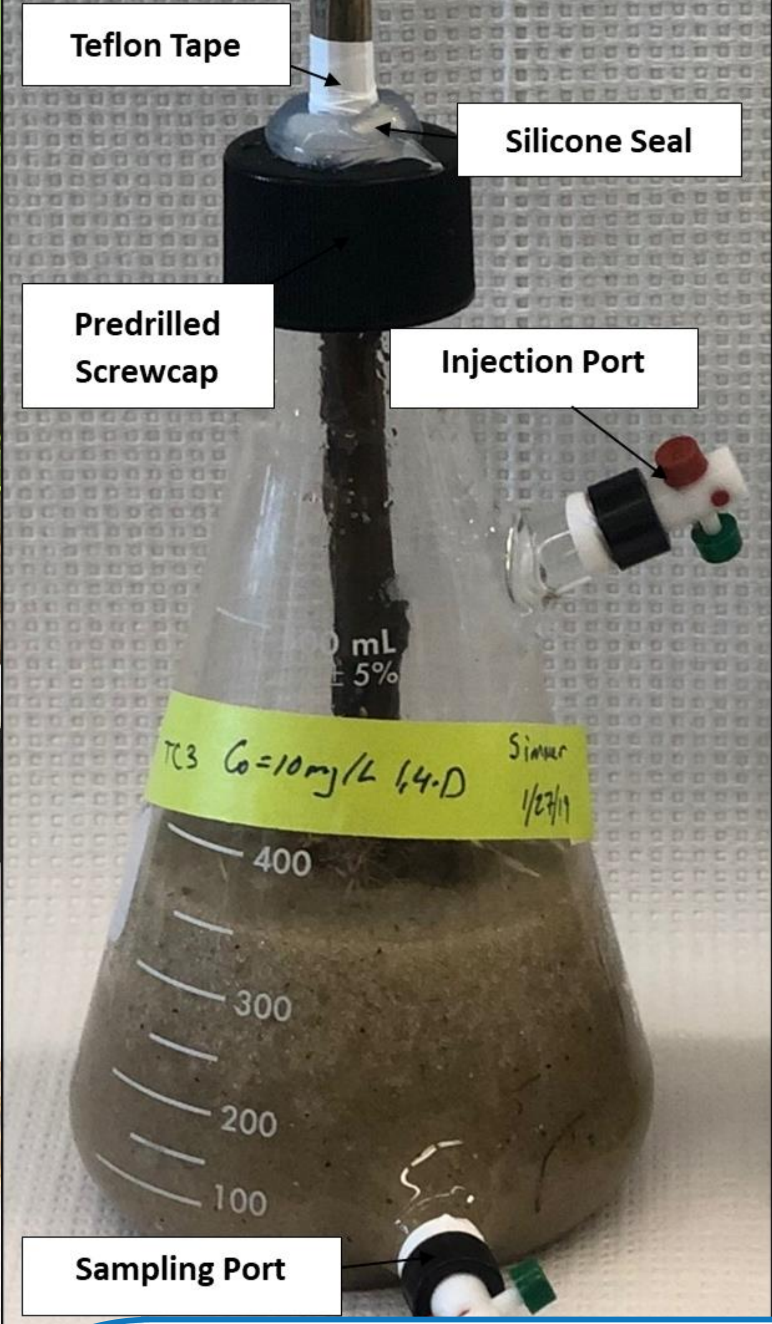
SCAN ME

Root extract did not increase growth of PH-06

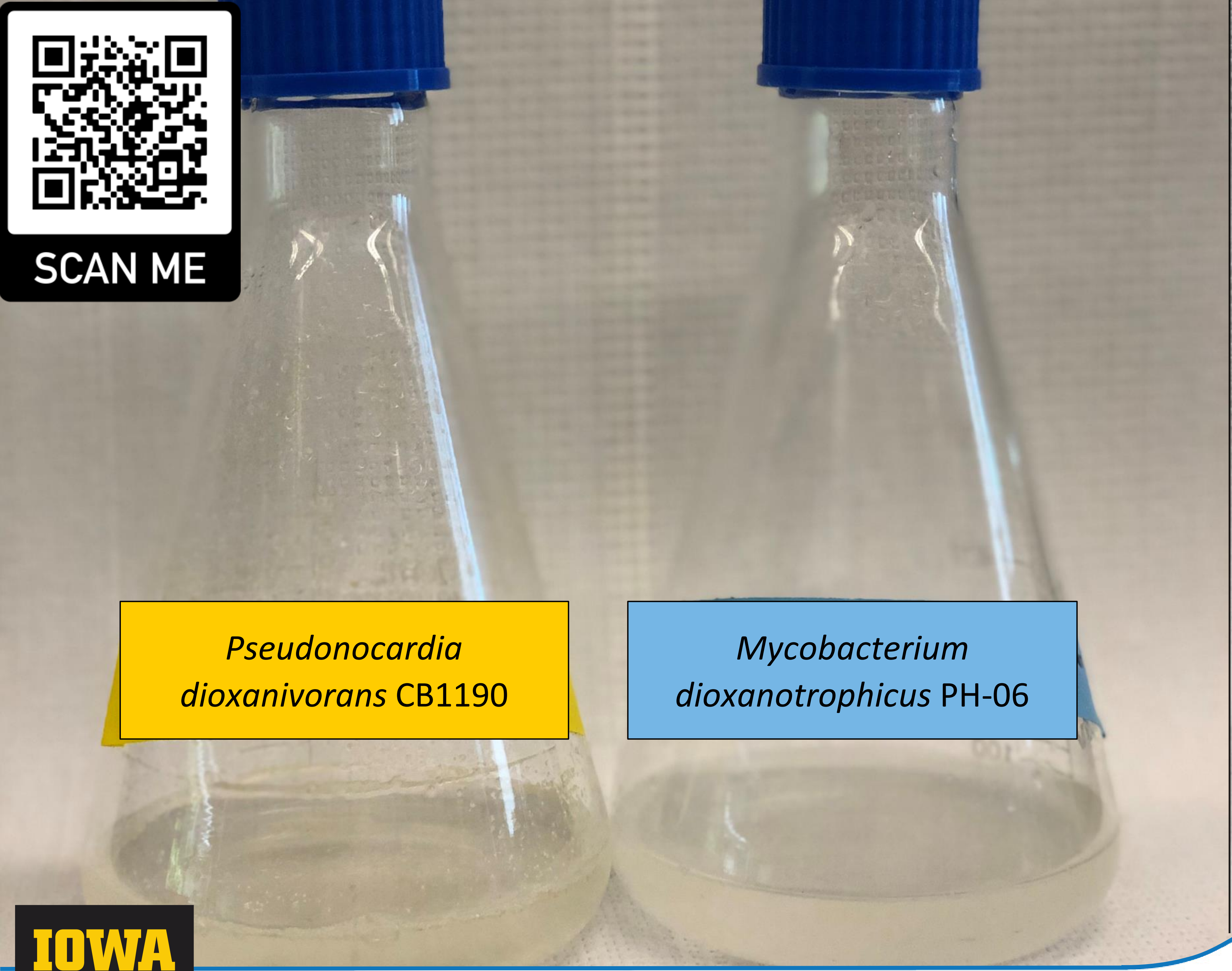


SCAN ME

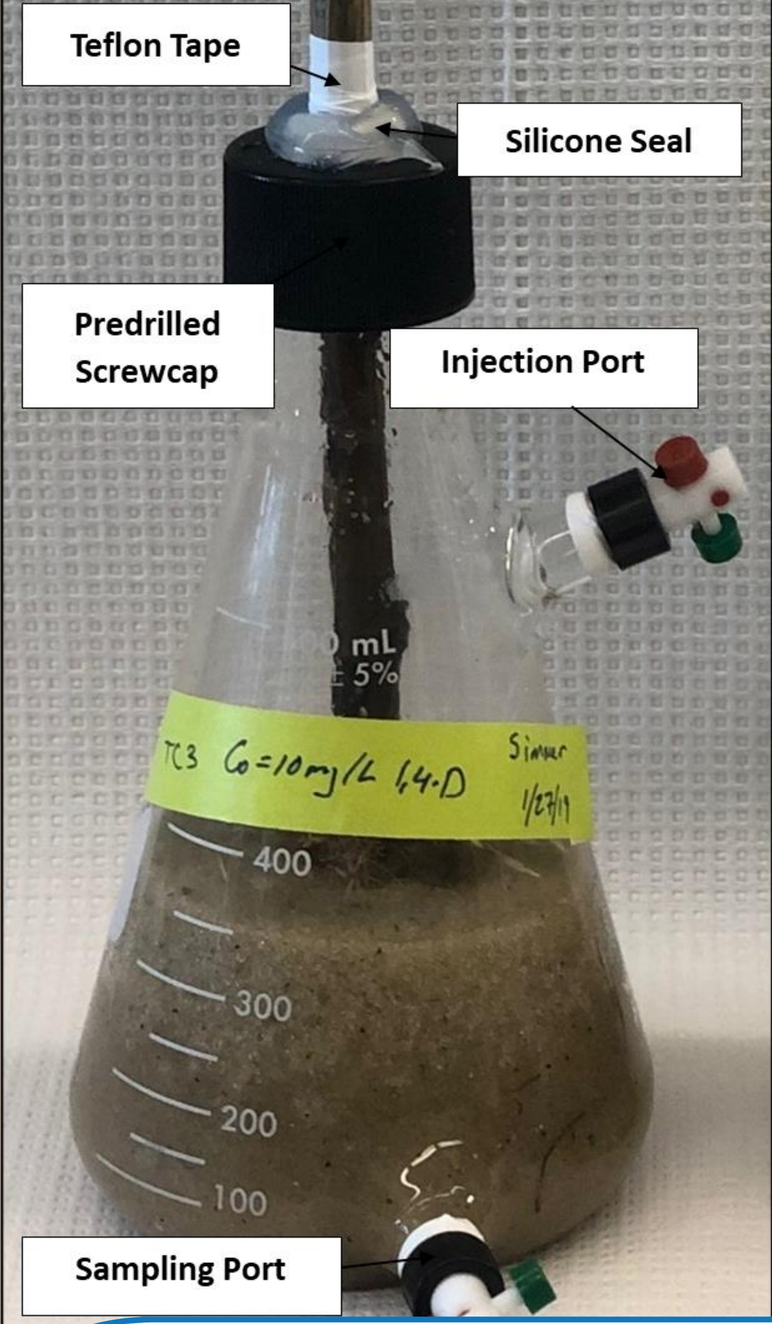
SCAN ME


Teflon Tape
Silicone Seal
Predrilled Screwcap
Injection Port
Sampling Port
400
300
200
100

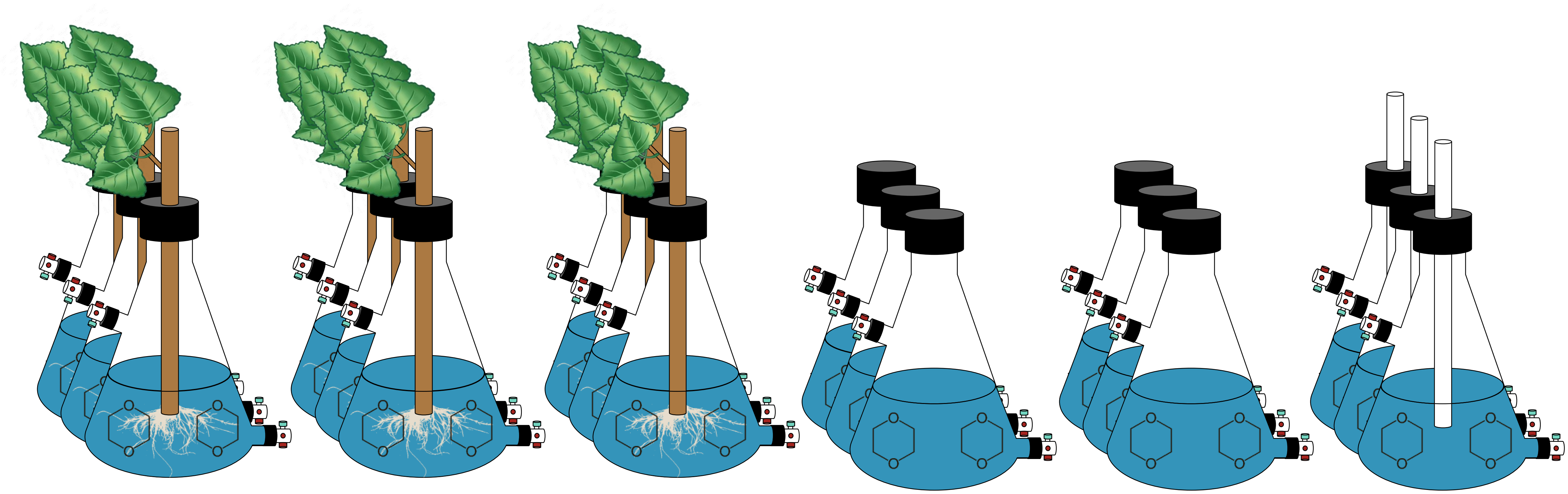
SCAN ME



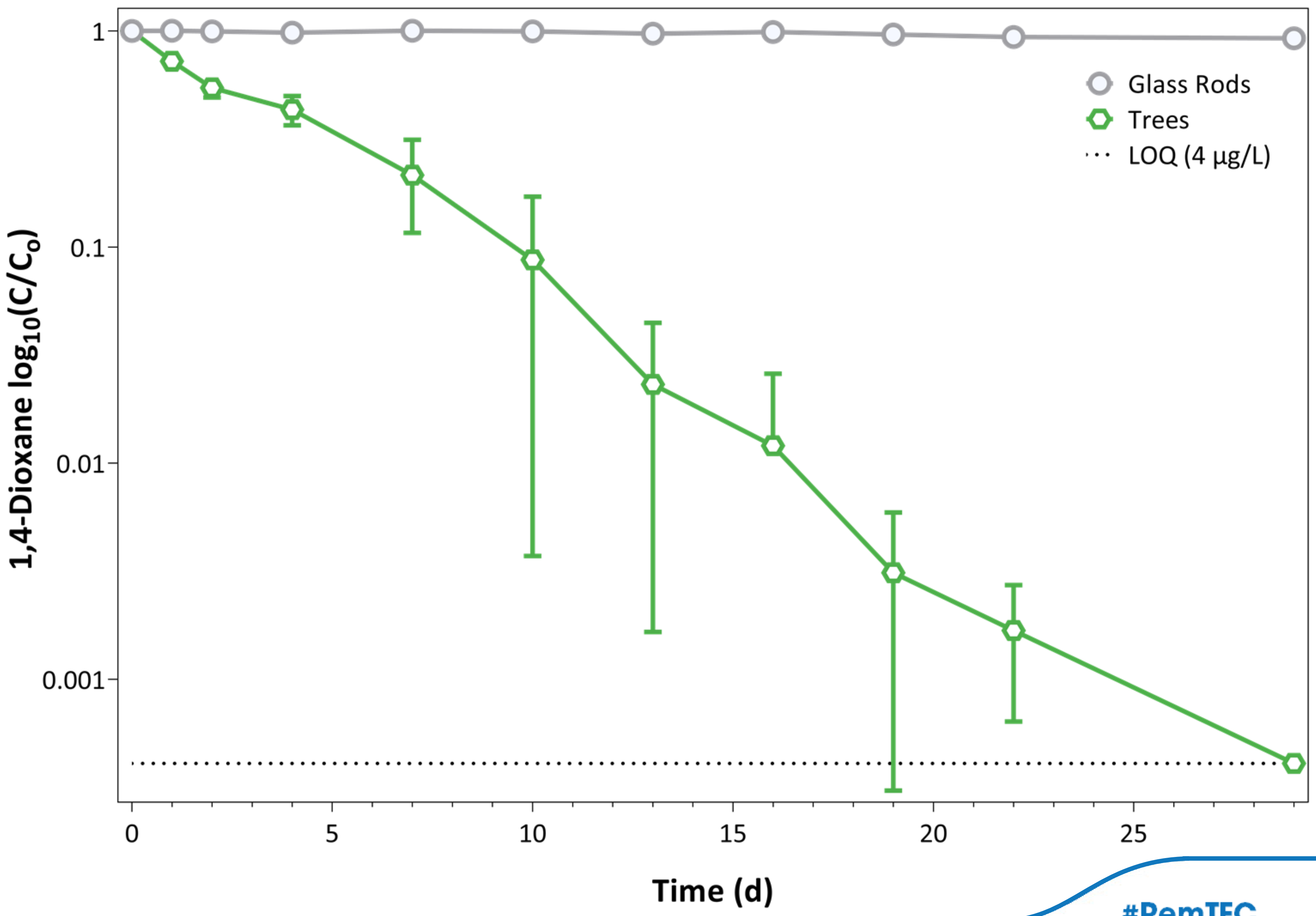
Pseudonocardia dioxanivorans CB1190
Mycobacterium dioxanotrophicus PH-06



Teflon Tape
Silicone Seal
Predrilled Screwcap
Injection Port
400
300
200
100
Sampling Port

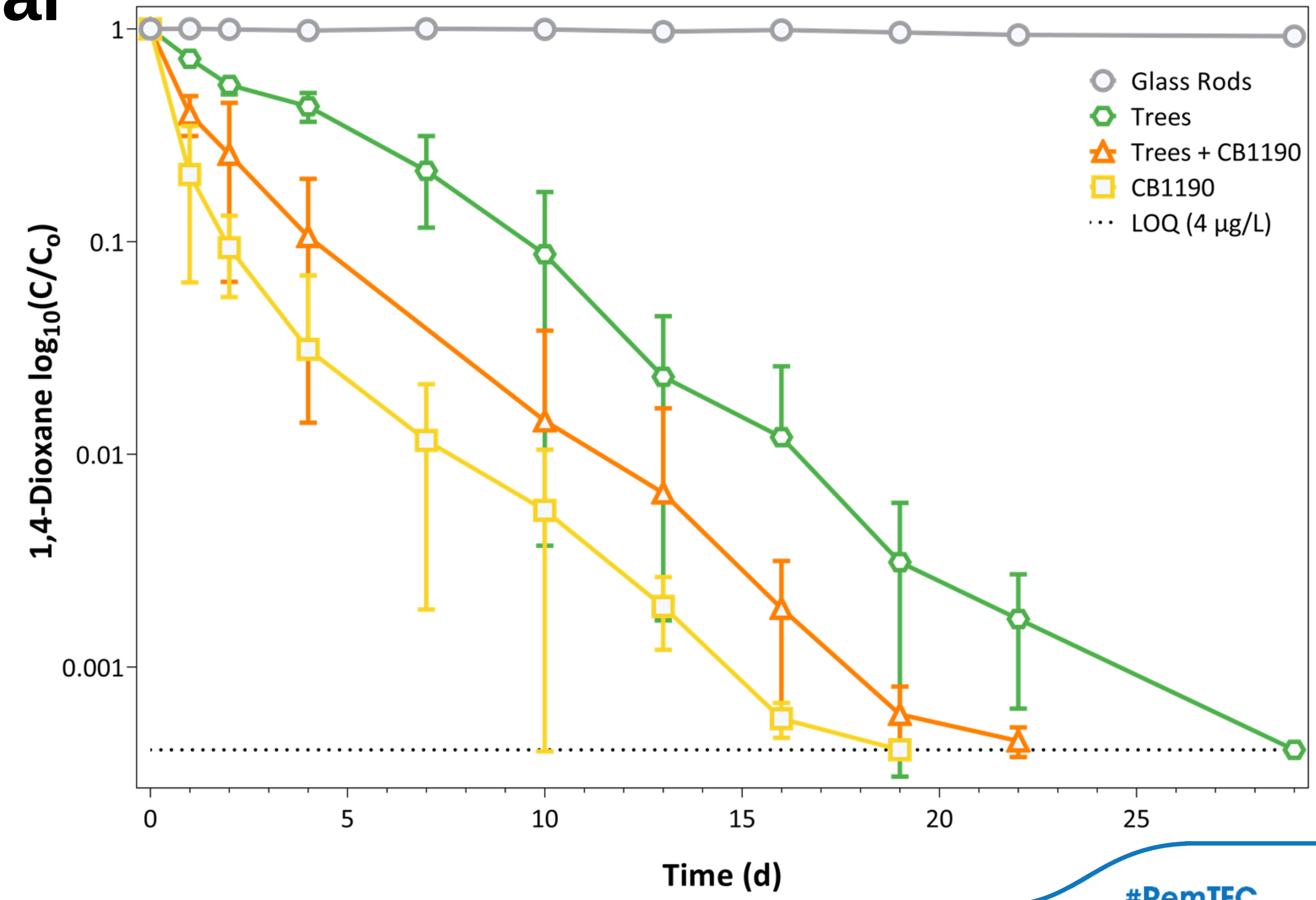

Trees only
Trees + CB1190
Trees + PH-06
CB1190 only
PH-06 only
Sterile Glass Rod Control

Poplar trees readily removed dioxane to LOQ

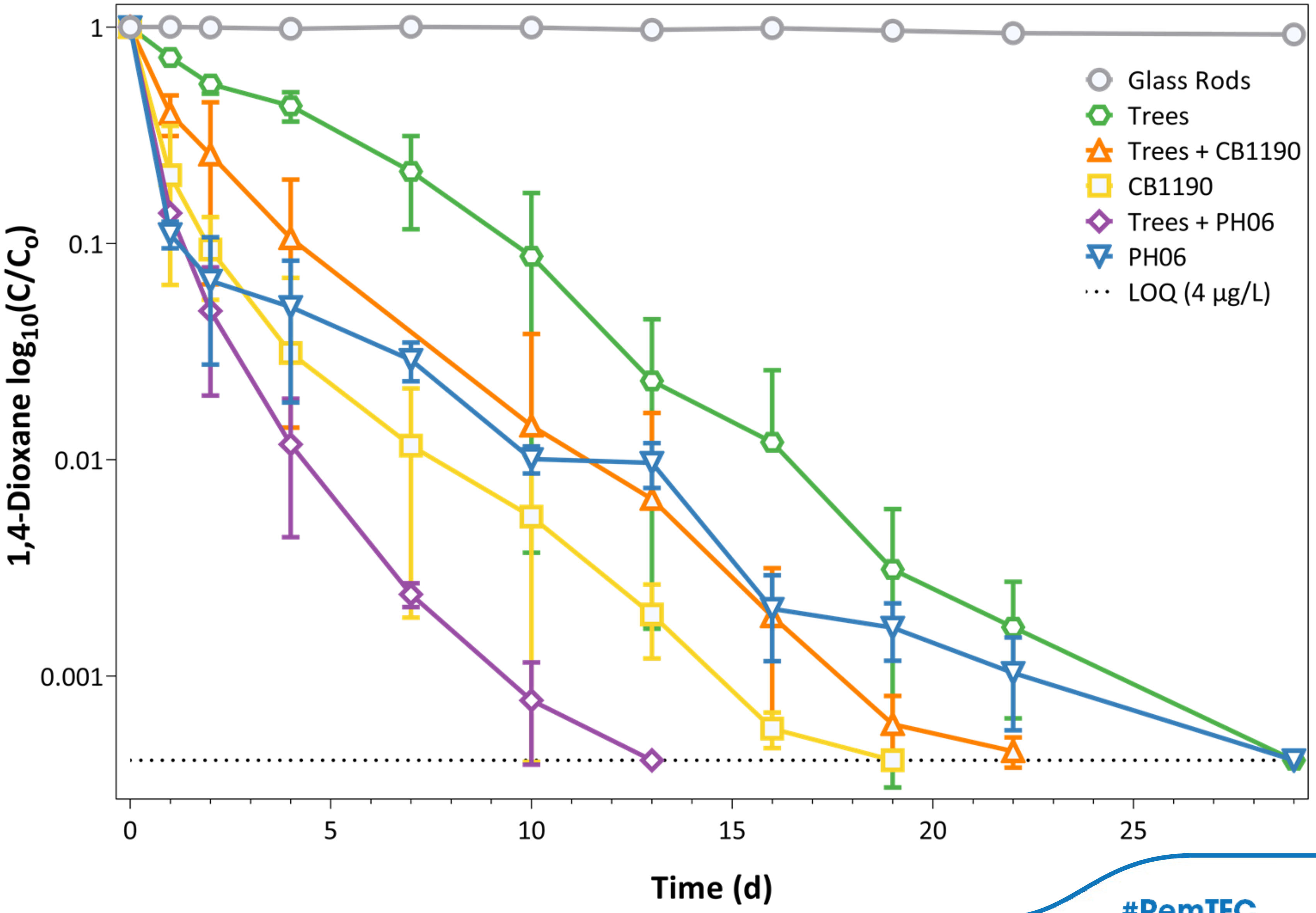


SCAN ME

Bioaugmenting trees with CB1190 accelerated removal



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

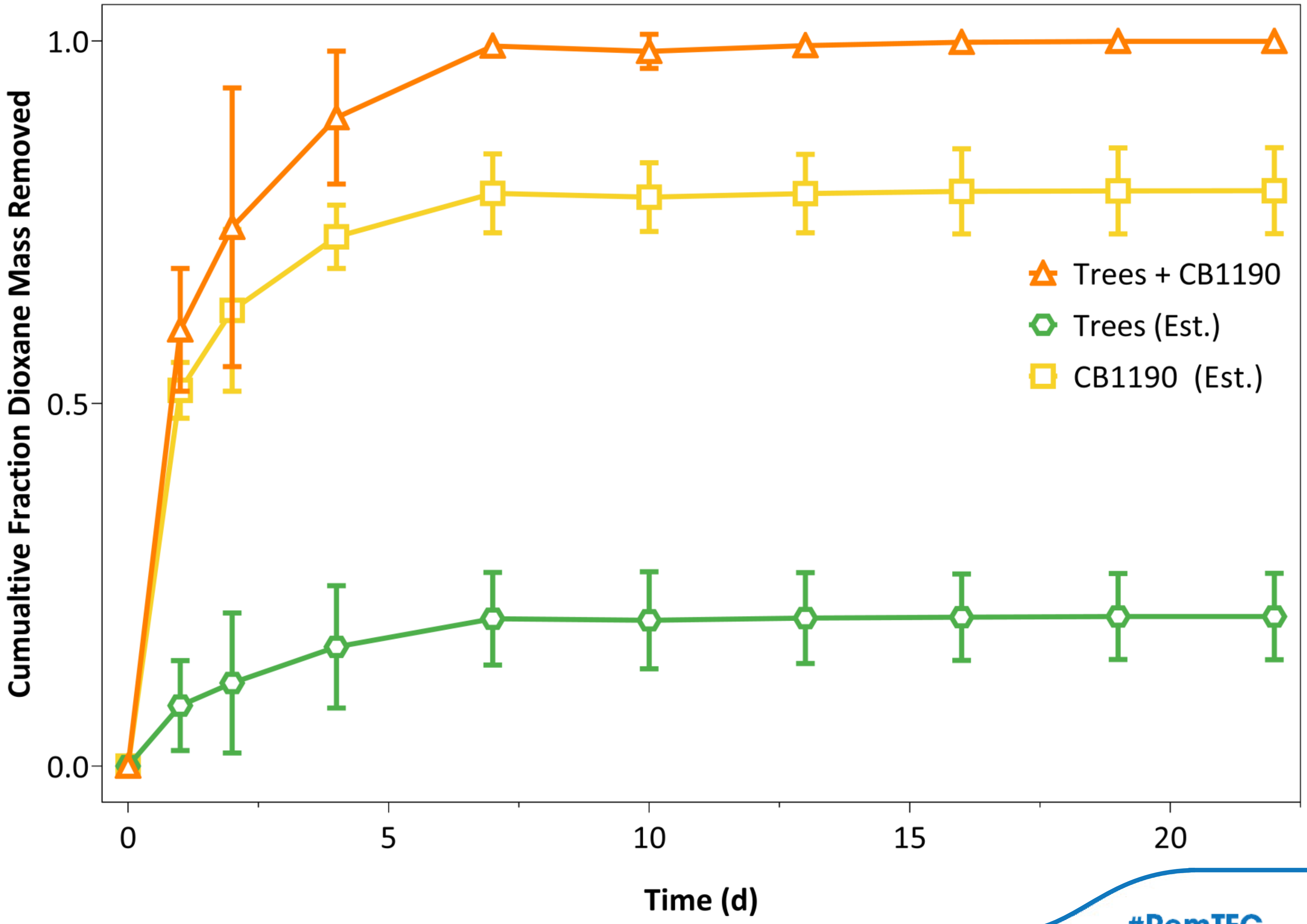


SCAN ME

Trees + PH-06 outpaced all other treatments

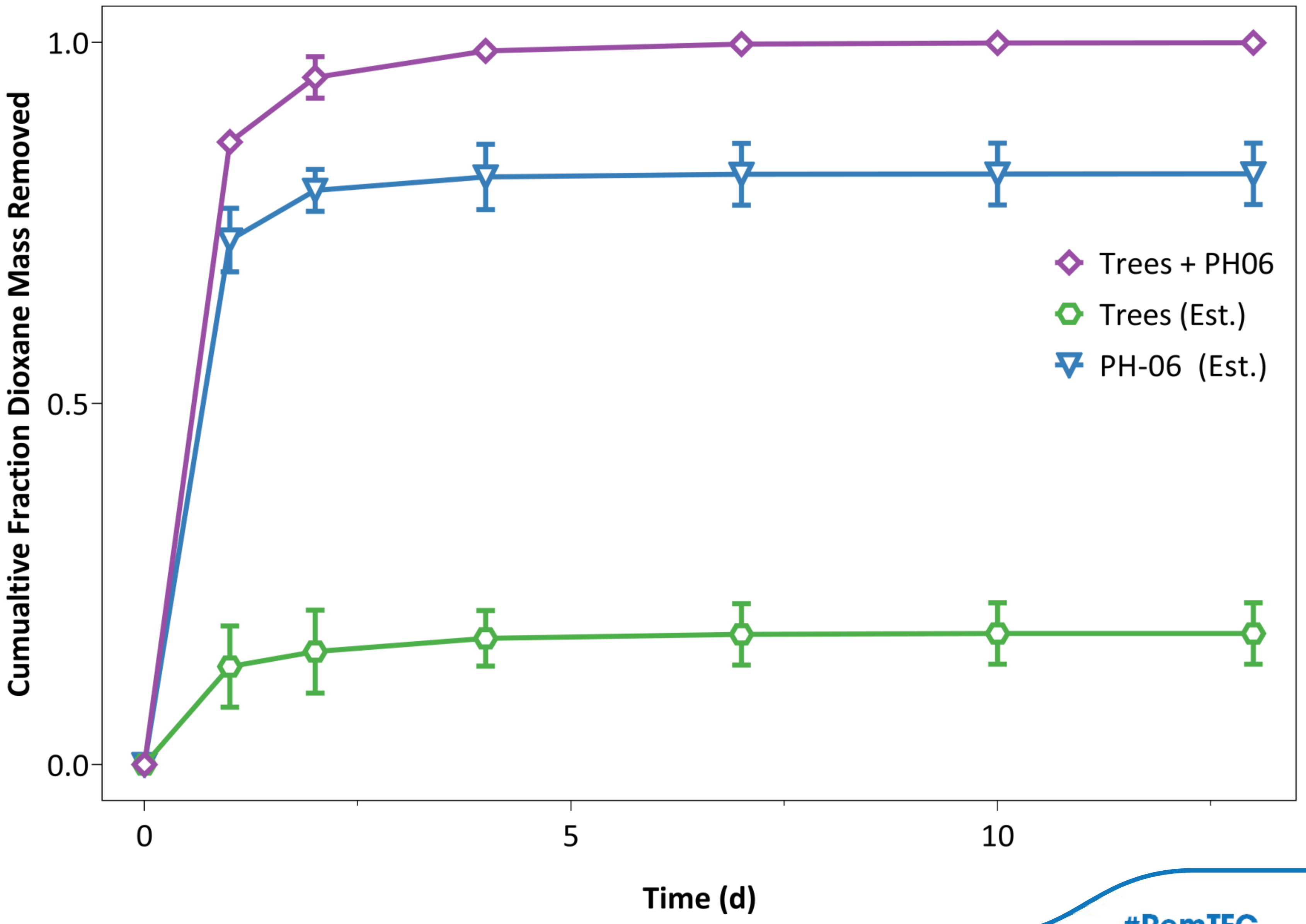
Treatment	Days to reach LOQ (4 ppb)	Degradation Rate Constant (day^{-1})	Transpiration Rate (mL day^{-1})
Trees Only	29	0.29 ± 0.013	32.81 ± 2.53
CB1190	22	0.37 ± 0.034	N/A
Trees + CB1190	19	0.34 ± 0.031	25.72 ± 9.64
PH-06	29	0.23 ± 0.015	N/A
Trees + PH-06	**13**	**0.56 ± 0.046**	**27.87 ± 3.50**

Bioaugmentation with CB1190 Removed ~79% of Initial 1,4-Dioxane



SCAN ME

Bioaugmentation with PH06 Removed ~81% of Initial 1,4-Dioxane



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} (µg 1,4-dioxane /L)	Reference
Pseudonocardia dioxanivorans CB1190	1.65 ± 0.05	**6.32 ± 0.22**	0.45 ± 0.09	**487.14 ± 173.45[a]**	Barajas-Rodriguez et al. (2018)
Mycobacterium dioxanotrophicus PH-06	Not reported	**78.00 ± 10.00**	0.16	Not reported	He et al. (2018)

Can novel metabolic dioxane-degrading strains treat low-level 1,4-dioxane ($\leq$100 µg/L) to below health advisory levels?

Do vitamin supplements enhance metabolism of dioxane?

Environmental Science & Technology Letters

pubs.acs.org/journal/estlcu

Letter

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

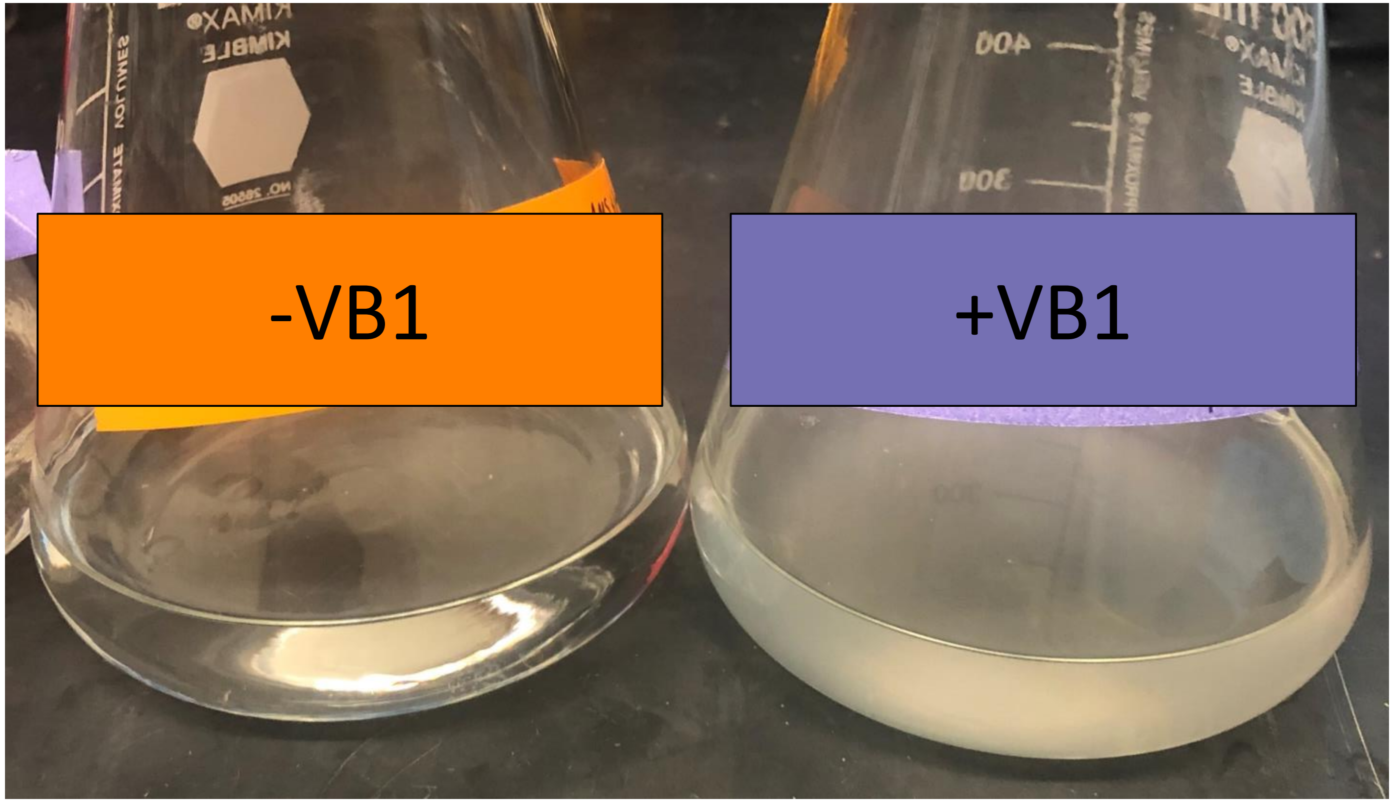
Reid A. Simmer,* Patrick M. Richards, Jessica M. Ewald, Cory Schwarz, Marcio L. B. da Silva, Jacques Mathieu, Pedro J. J. Alvarez, and Jerald L. Schnoor

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

Read Online

OPEN ACCESS

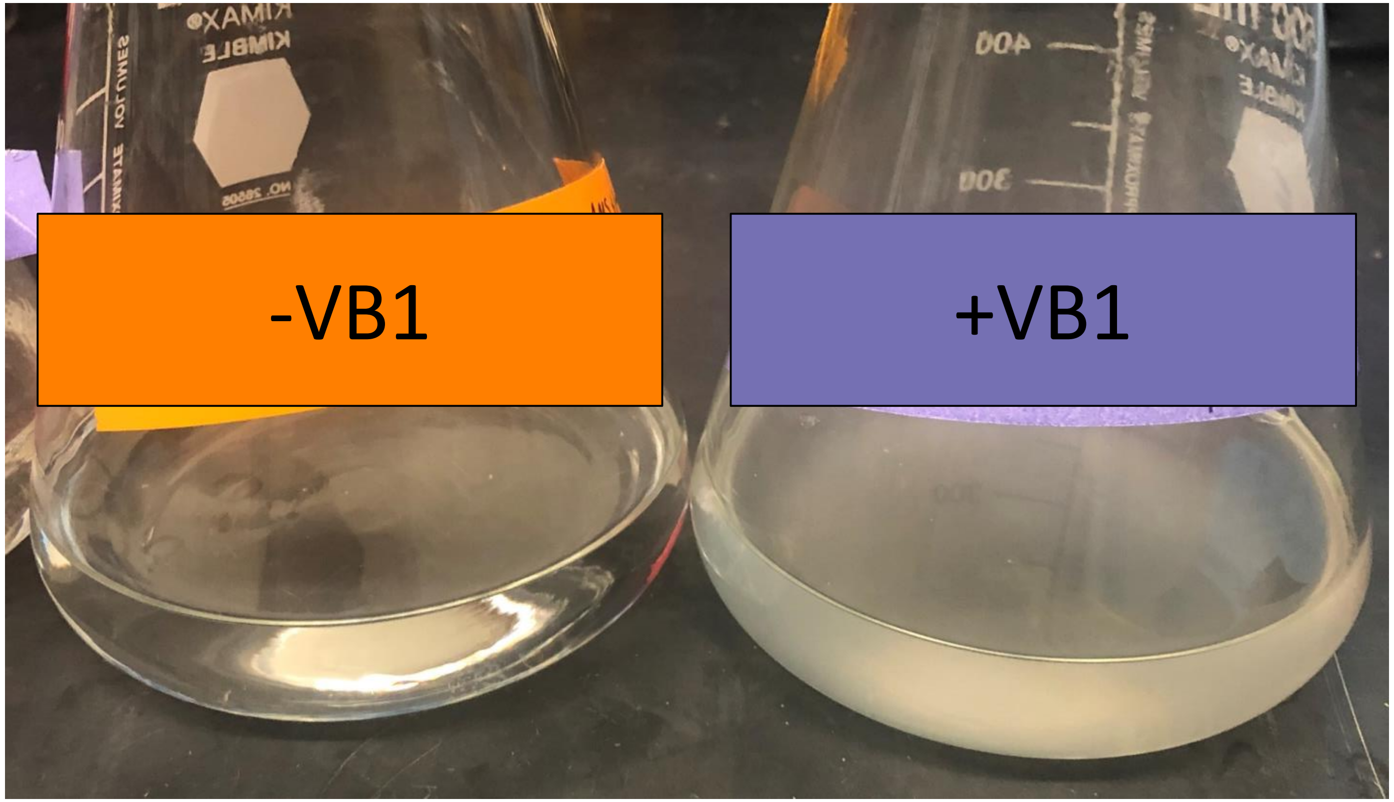
Vitamin B1 (thiamine) supplements accelerate growth in *Rhodococcus ruber* 219



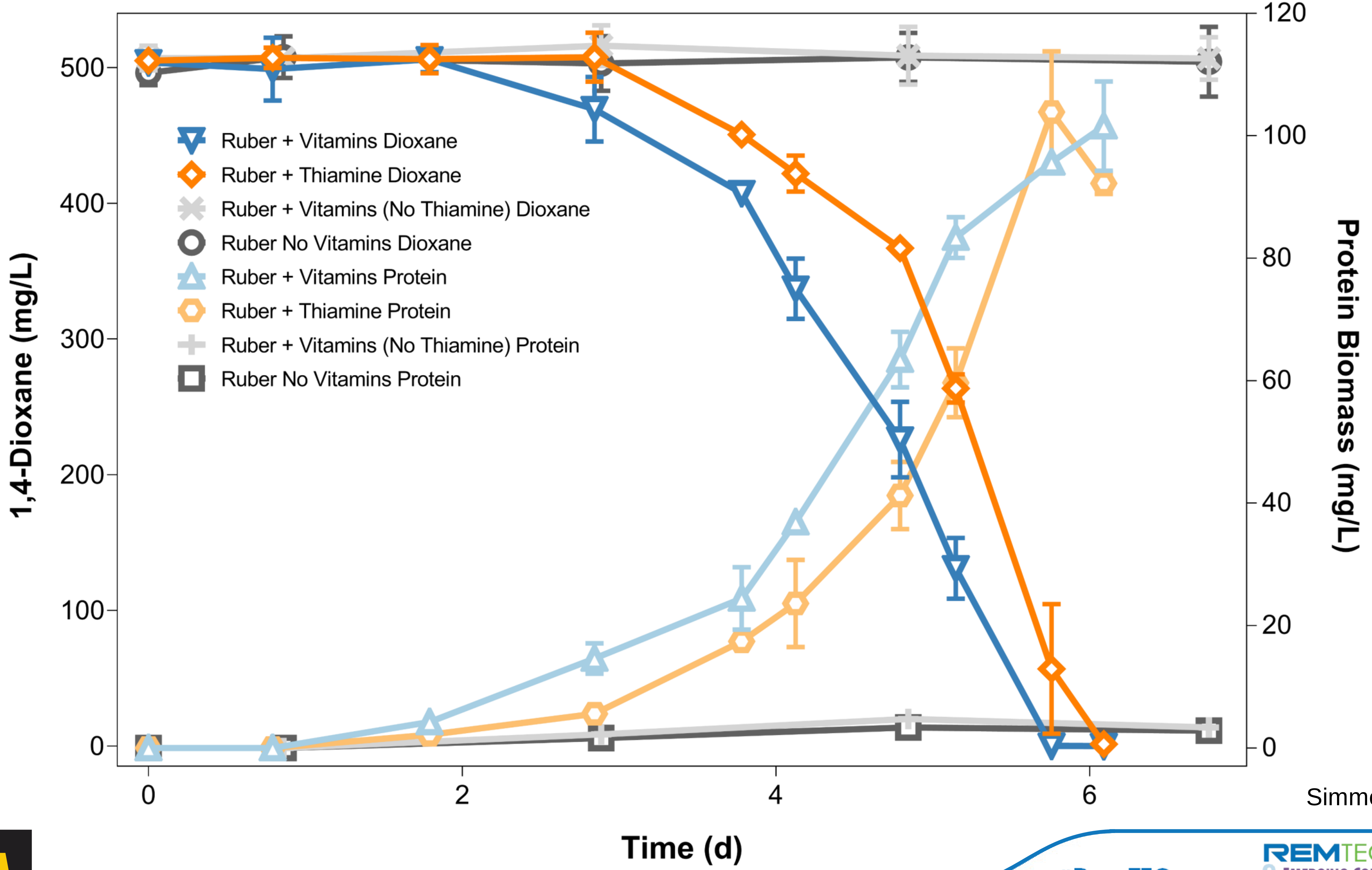
Simmer et al., 2021

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

OPEN ACCESS

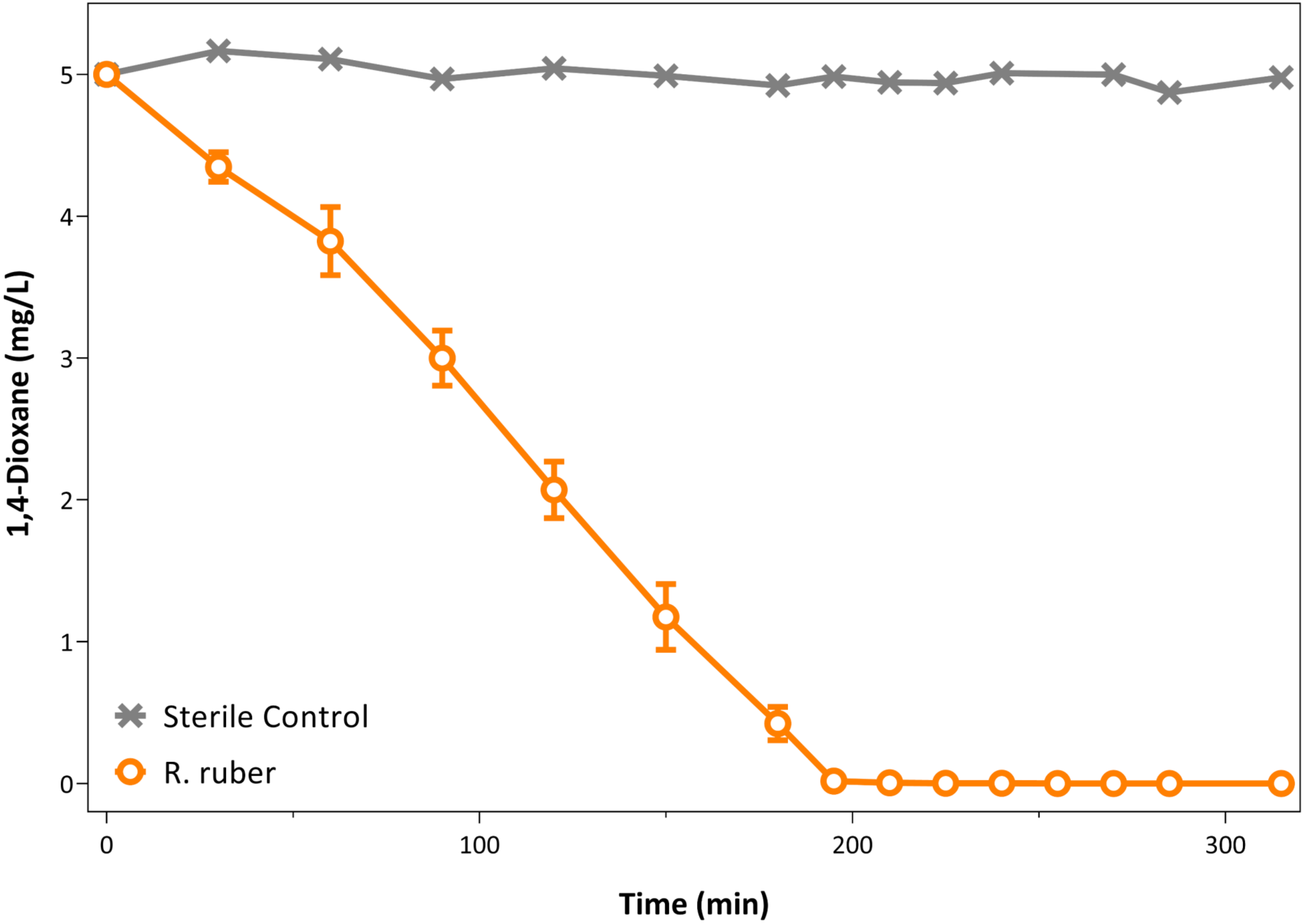


Simmer et al., 2021



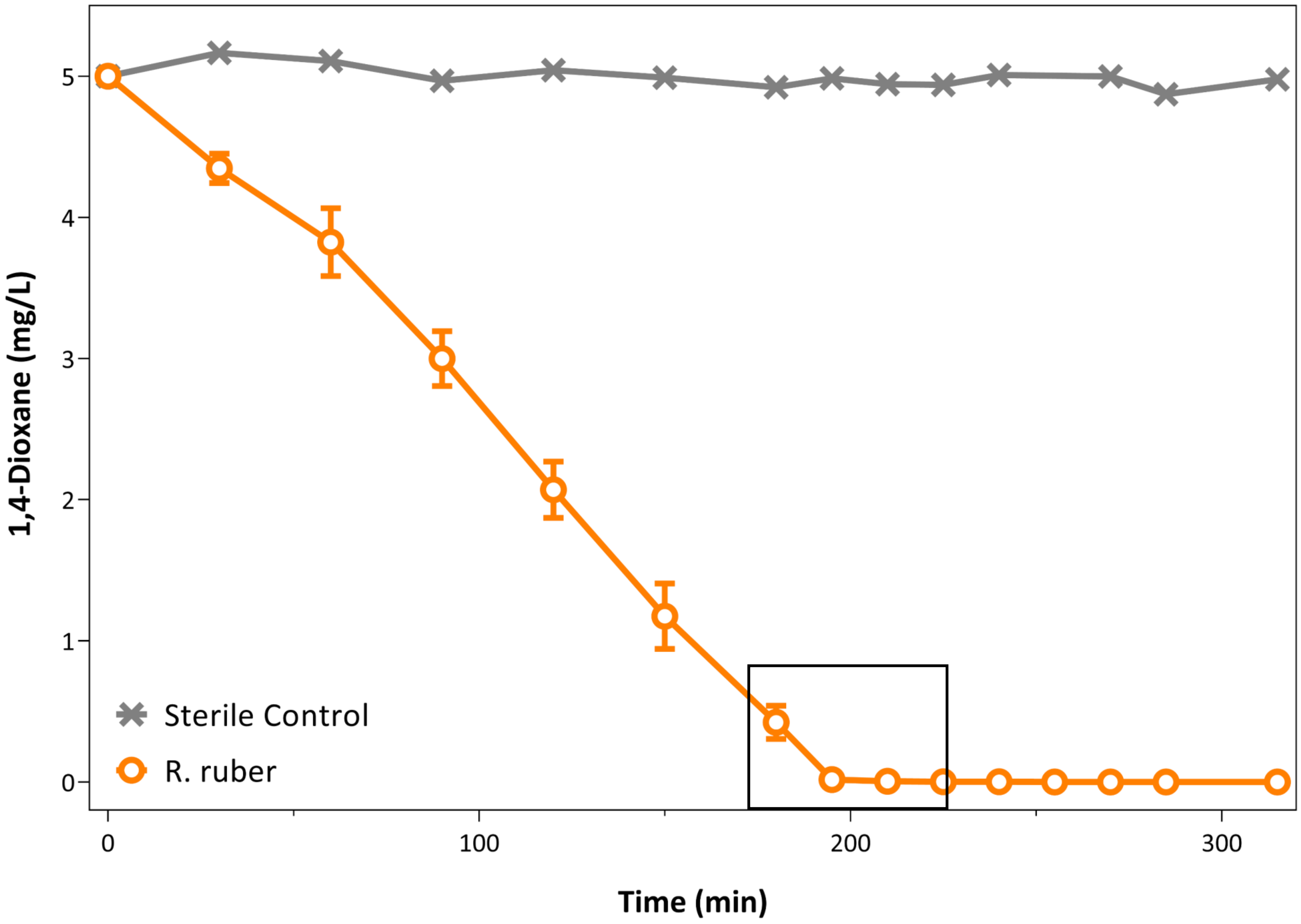
OPEN ACCESS

Simmer et al., 2021



OPEN ACCESS

Simmer et al., 2021



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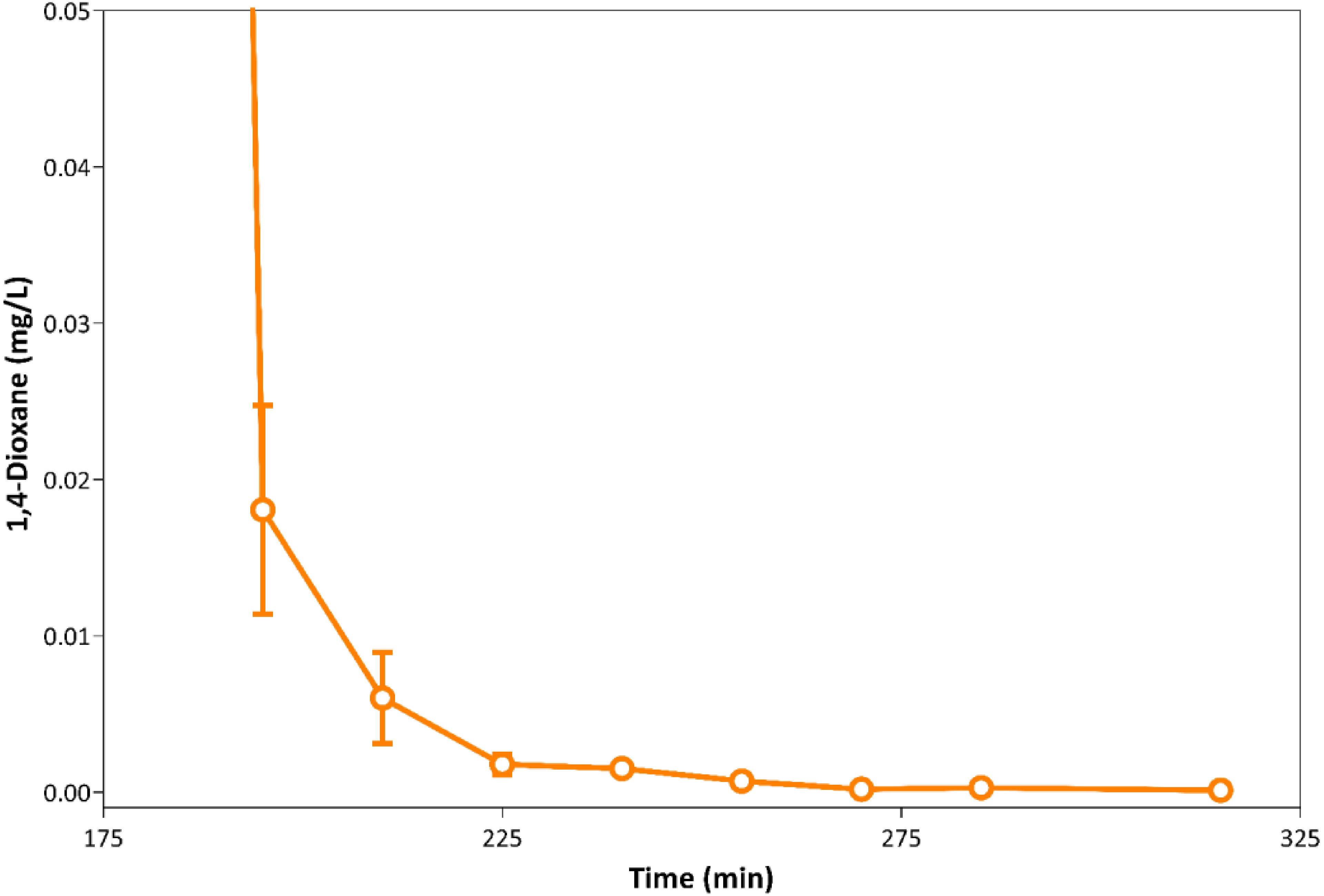
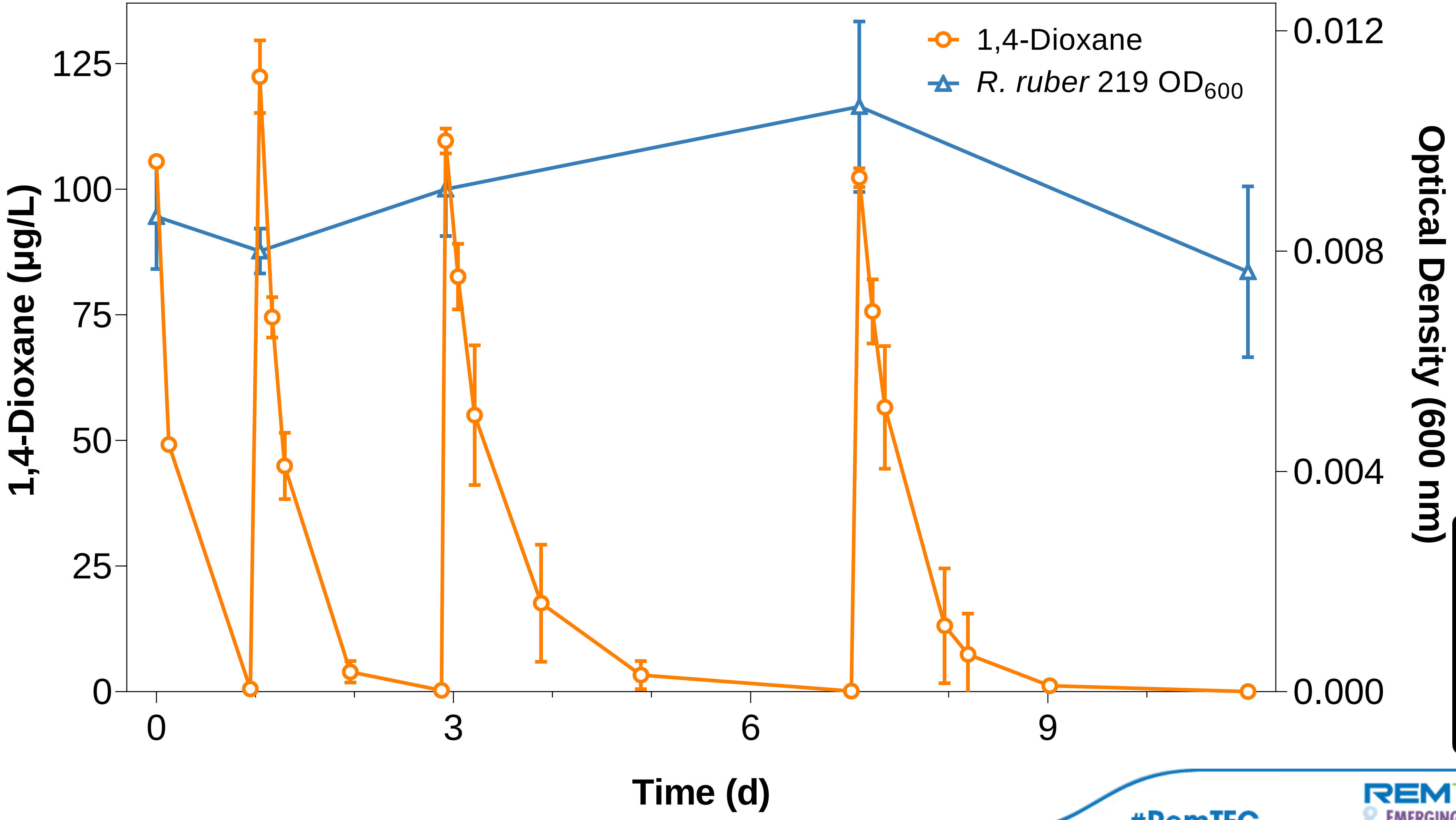
Simmer et al., 2021



OPEN ACCESS

Simmer et al., 2021

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



OPEN ACCESS

Rhodococcus ruber 219's Monod kinetics make it a strong candidate for field-scale bioaugmentation

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
Pseudonocardia dioxanivorans CB1190	1.65 ± 0.05	6.32 ± 0.22	0.45 ± 0.09	487.14 ± 173.45[a]	Barajas-Rodriguez et al. (2018)
Mycobacterium dioxanotrophicus PH-06	Not reported	78.00 ± 10.00	0.16	Not reported	He et al. (2018)
***Rhodococcus ruber* 219**	**5.00 ± 0.24**	**0.015 ± 0.03**	**0.24 ± 0.02**	**0.49 ± 1.16**	**Simmer et al. (2021)**

OPEN ACCESS

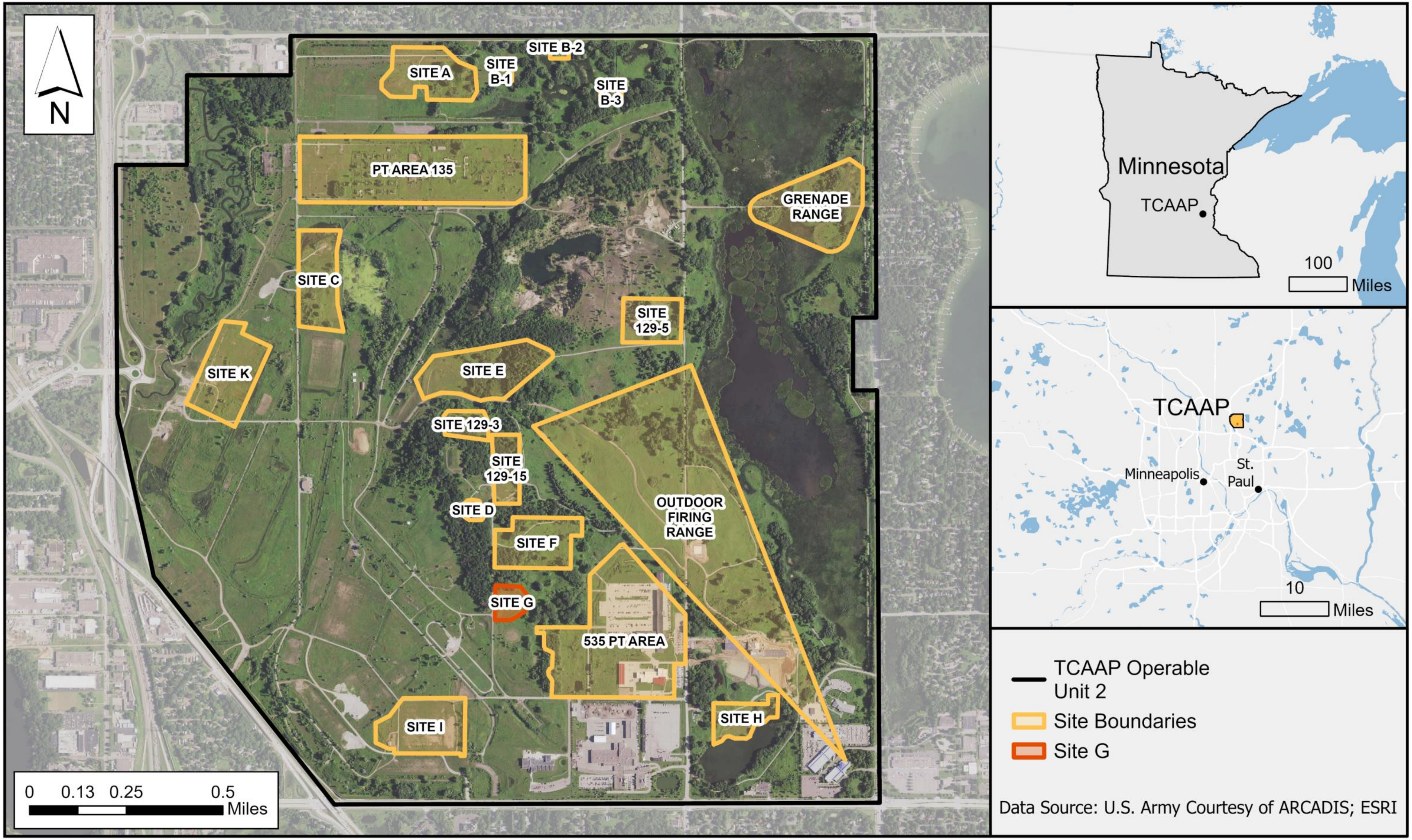
Simmer et al., 2021

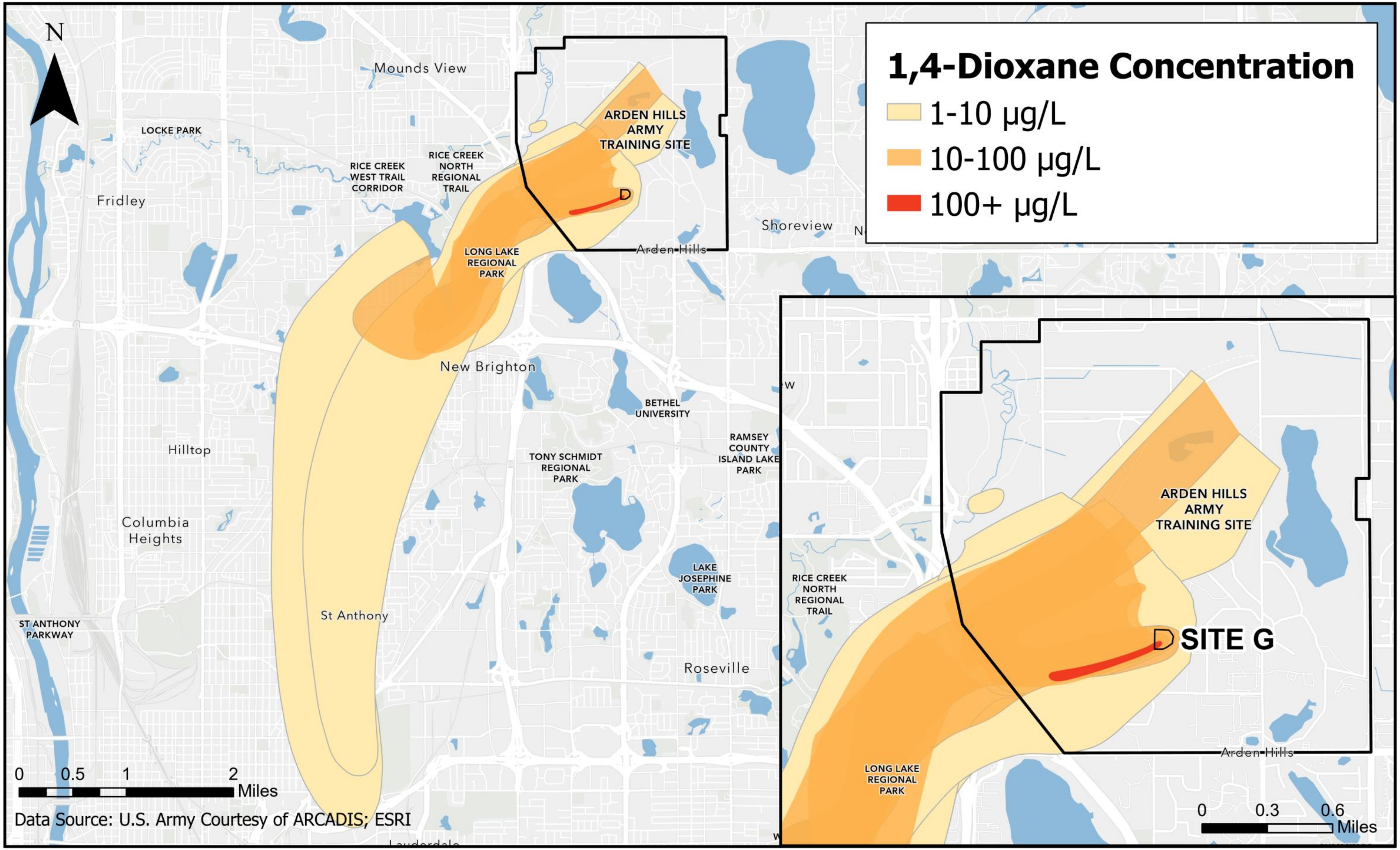
Can phytoremediation + bioaugmentation with *R. ruber* 219 treat 1,4-dioxane-contaminated water at the pilot scale?

Can phytoremediation + bioaugmentation with *R. ruber* 219 treat 1,4-dioxane-contaminated water at the pilot scale?

ESTCP Demonstration at former Twin Cities Army Ammunition Plant (TCAAP)
Arden Hills, MN

ER21-5096

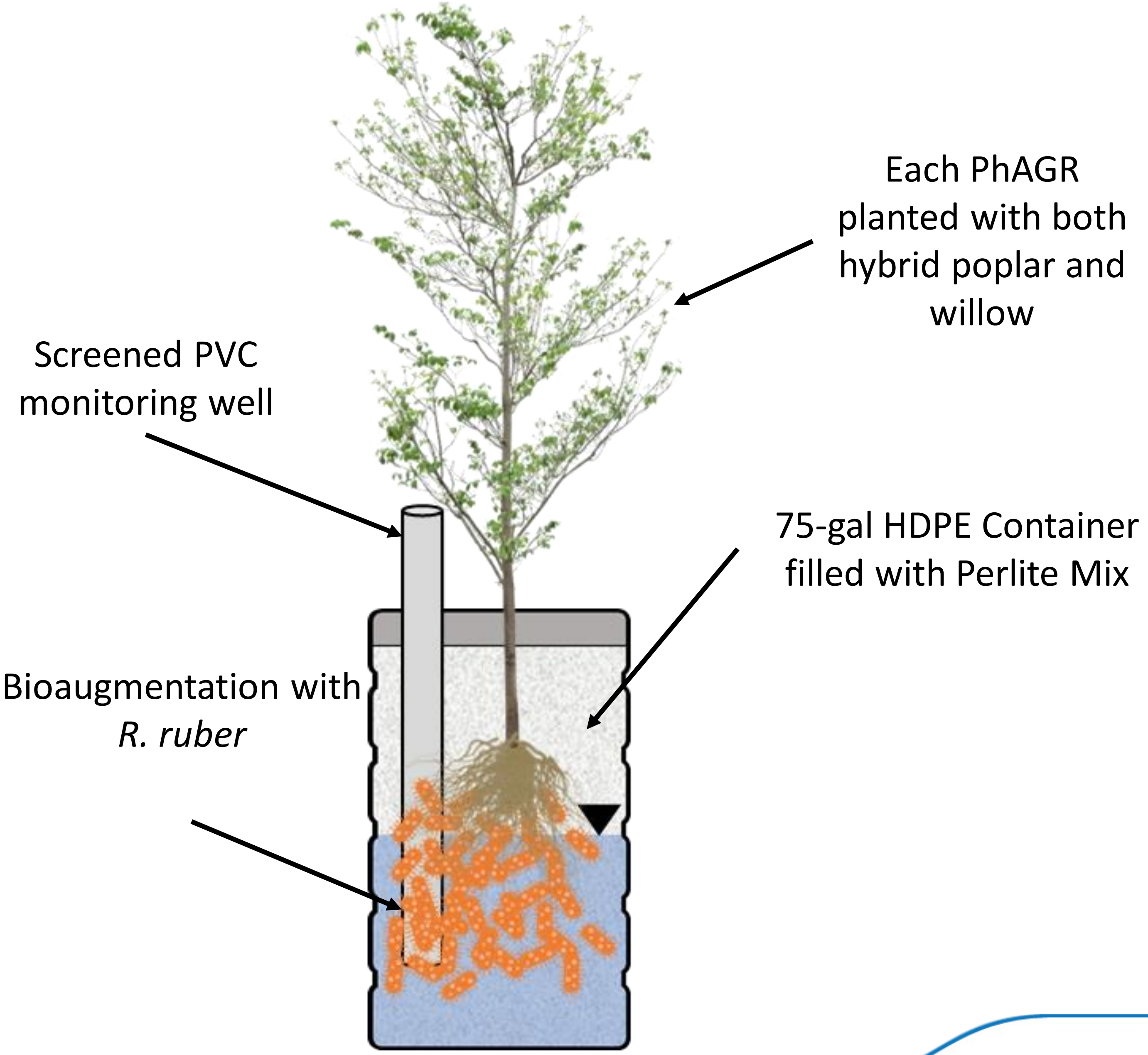

N
SITE A
SITE B-1
SITE B-2
SITE B-3
PT AREA 135
GRENADE RANGE
SITE C
SITE 129-5
SITE K
SITE E
SITE 129-3
SITE 129-15
OUTDOOR FIRING RANGE
SITE D
SITE F
SITE G
535 PT AREA
SITE I
SITE H
0 0.13 0.25 0.5 Miles
Minnesota
TCAAP
100 Miles
TCAAP
Minneapolis
St. Paul
10 Miles
TCAAP Operable Unit 2
Site Boundaries
Site G
Data Source: U.S. Army Courtesy of ARCADIS; ESRI

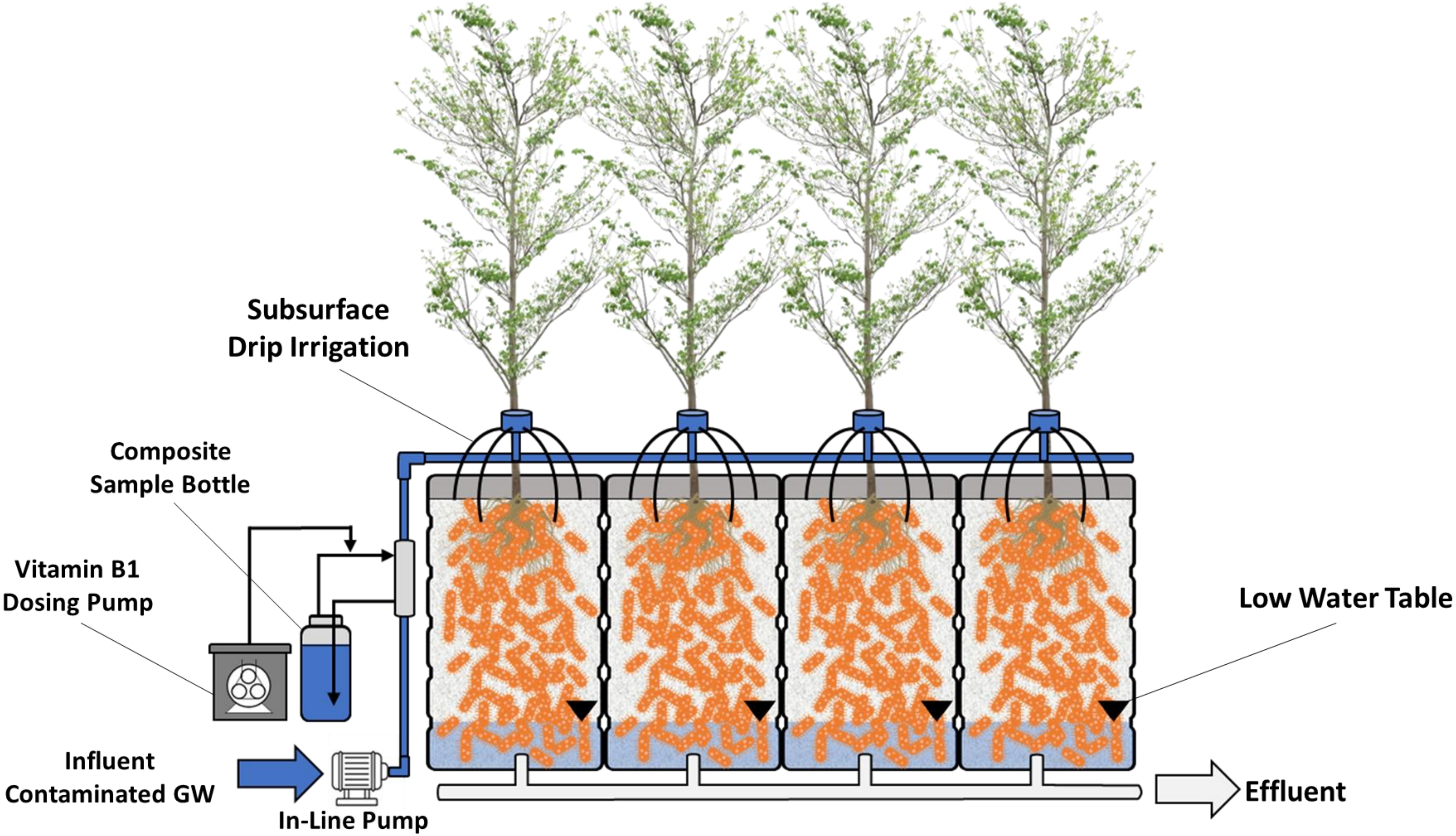


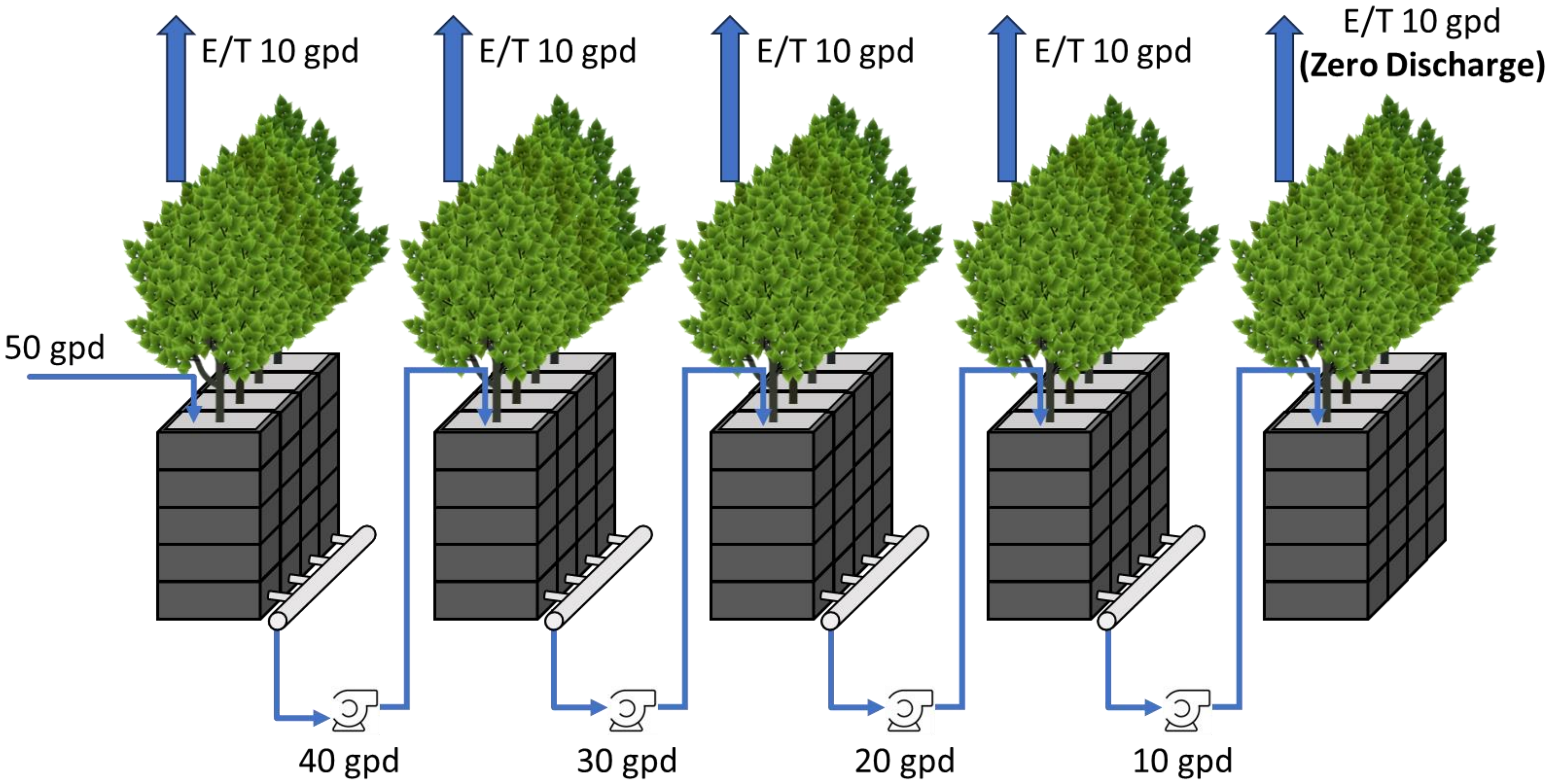
1,4-Dioxane Concentration
1-10 µg/L
10-100 µg/L
100+ µg/L
N
Mounds View
LOCKE PARK
RICE CREEK WEST TRAIL CORRIDOR
RICE CREEK NORTH REGIONAL TRAIL
ARDEN HILLS ARMY TRAINING SITE
Fridley
Shoreview
Arden Hills
LONG LAKE REGIONAL PARK
New Brighton
BETHEL UNIVERSITY
RAMSEY COUNTY ISLAND LAKE PARK
Hilltop
TONY SCHMIDT REGIONAL PARK
Columbia Heights
LAKE JOSEPHINE PARK
St Anthony
ST ANTHONY PARKWAY
Roseville
0 0.5 1 2 Miles
Data Source: U.S. Army Courtesy of ARCADIS; ESRI
SITE G
0 0.3 0.6 Miles

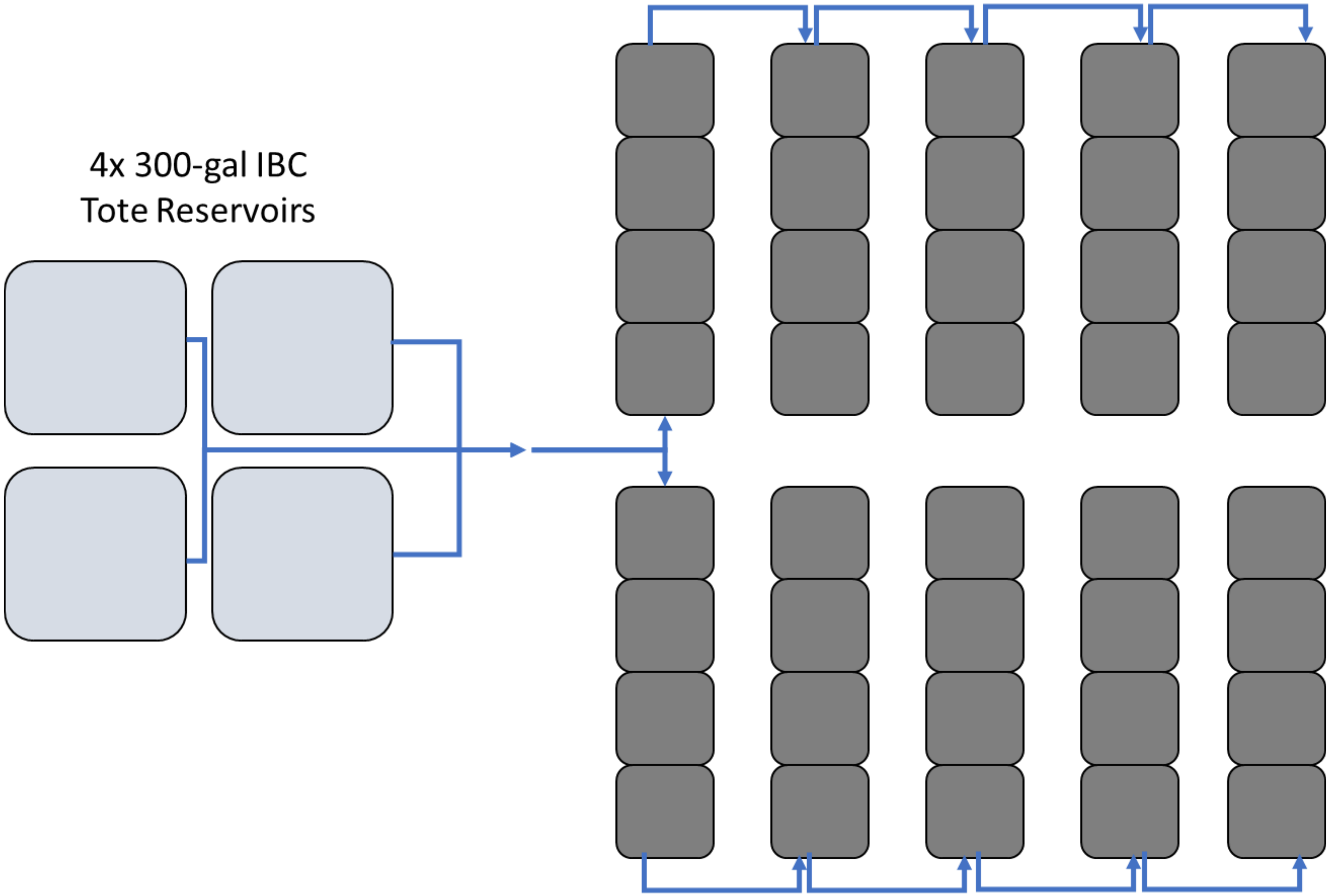

N
Old Pumphouse
Site G Source Control Pumphouse
ESTCP Demonstratio n
SITE G
0.04
Miles
0 15 30 60 Feet
Data Source: U.S. Army Courtesy of ARCADIS; ESRI

Phyto-Attached Growth Reactors (PhAGR®) Ecolotree Inc.

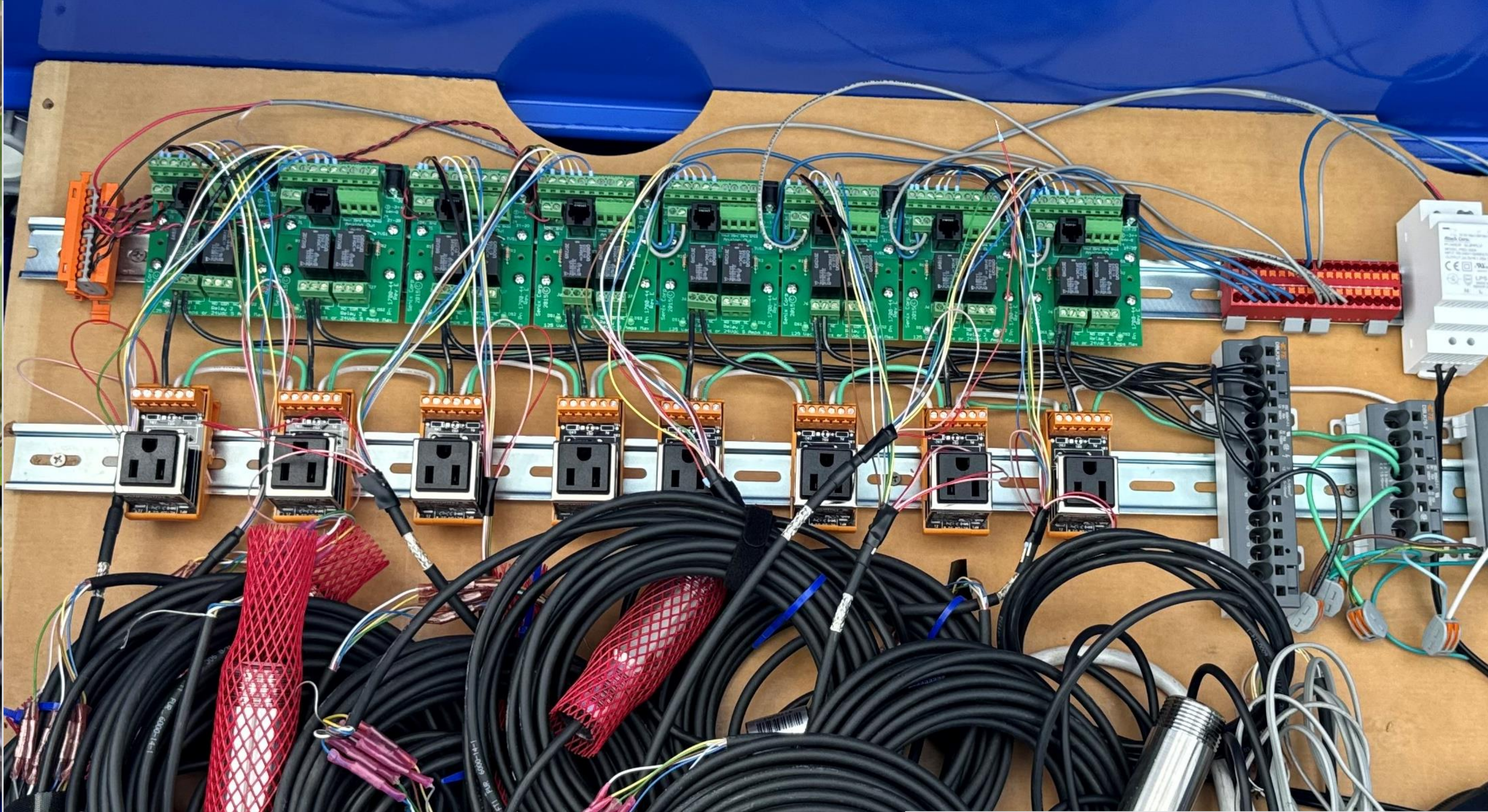

Each PhAGR planted with both hybrid poplar and willow
Screened PVC monitoring well
75-gal HDPE Container filled with Perlite Mix
Bioaugmentation with *R. ruber*


Subsurface
Drip Irrigation
Composite
Sample Bottle
Vitamin B1
Dosing Pump
Low Water Table
Influent
Contaminated GW
In-Line Pump
Effluent


E/T 10 gpd
E/T 10 gpd
E/T 10 gpd
E/T 10 gpd
E/T 10 gpd
(Zero Discharge)
50 gpd
40 gpd
30 gpd
20 gpd
10 gpd


4x 300-gal IBC
Tote Reservoirs


BALT




KOBALT

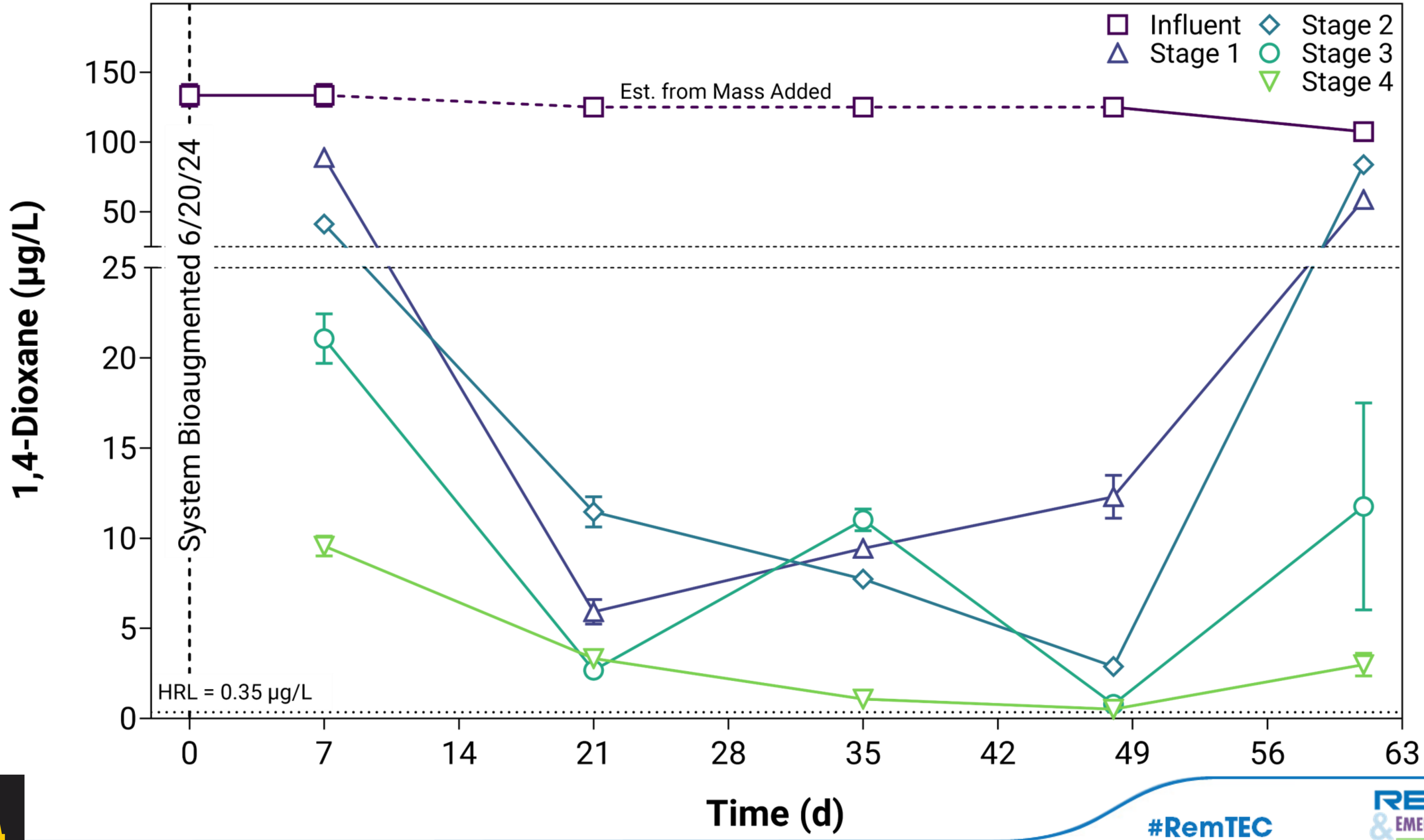

PR 450
3/4" PVC 1120 SCH 40 PR 480 PSI
CHARLOTTE PIPE


Lab - Please return to 4256 SC!


geopump
geotech

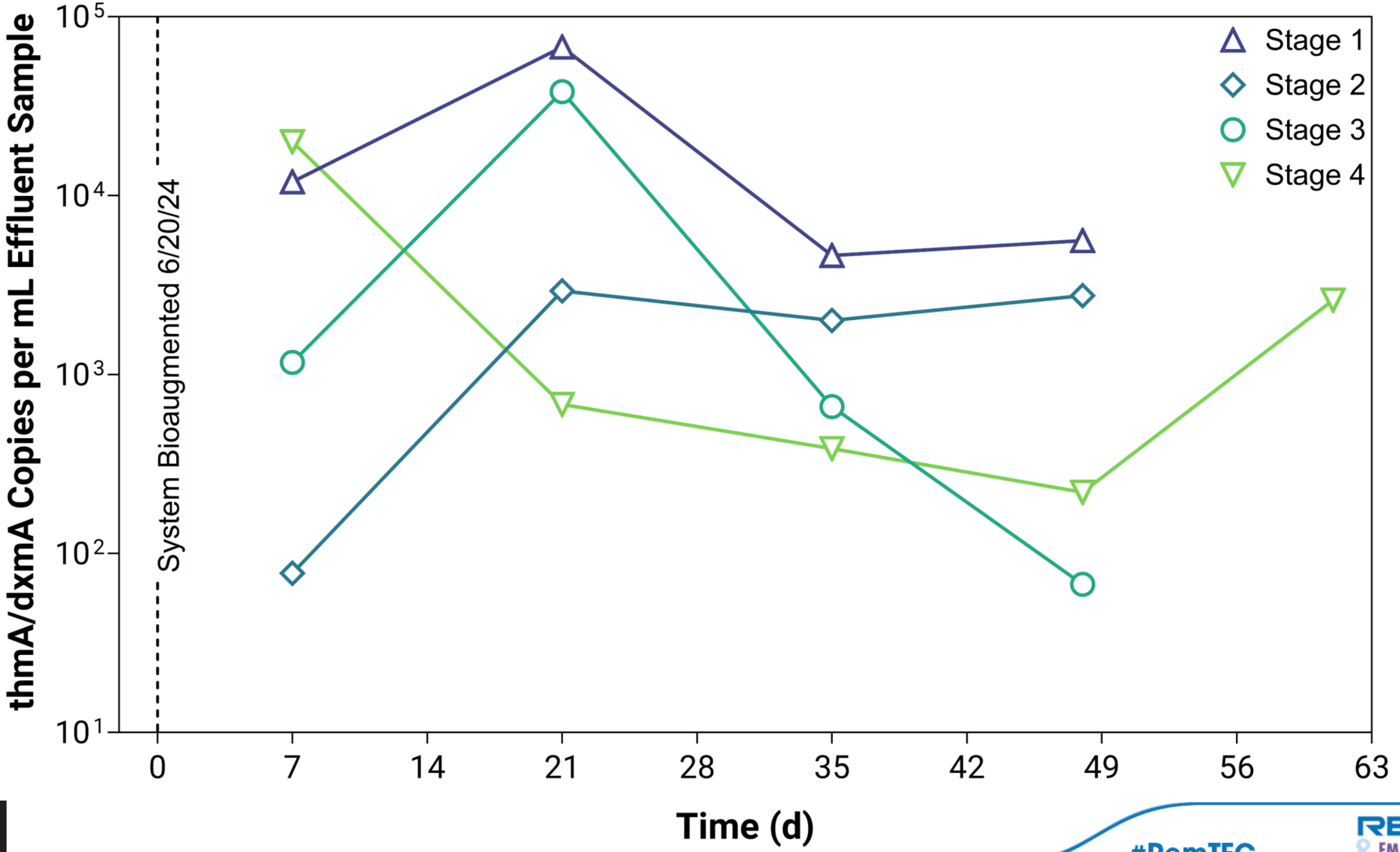
Agilent Technologies
7693 Autosampler
University of Iowa
Agilent Technologies
7000C GC/MS Triple Quad
Agilent Technologies
Intuvo
9000 GC System
Maintenance Log
Run Log
System Log
Sequence Log
Run History Log
Run
Not Ready
Attention
RESTEK
RESTEK
AQUATek
LUMIN
TO CONCENTRATOR
SYSTEM PRESSURE

In-series phyto + bio system effectively treated influent dioxane for 9 weeks to as low 0.5 µg/L



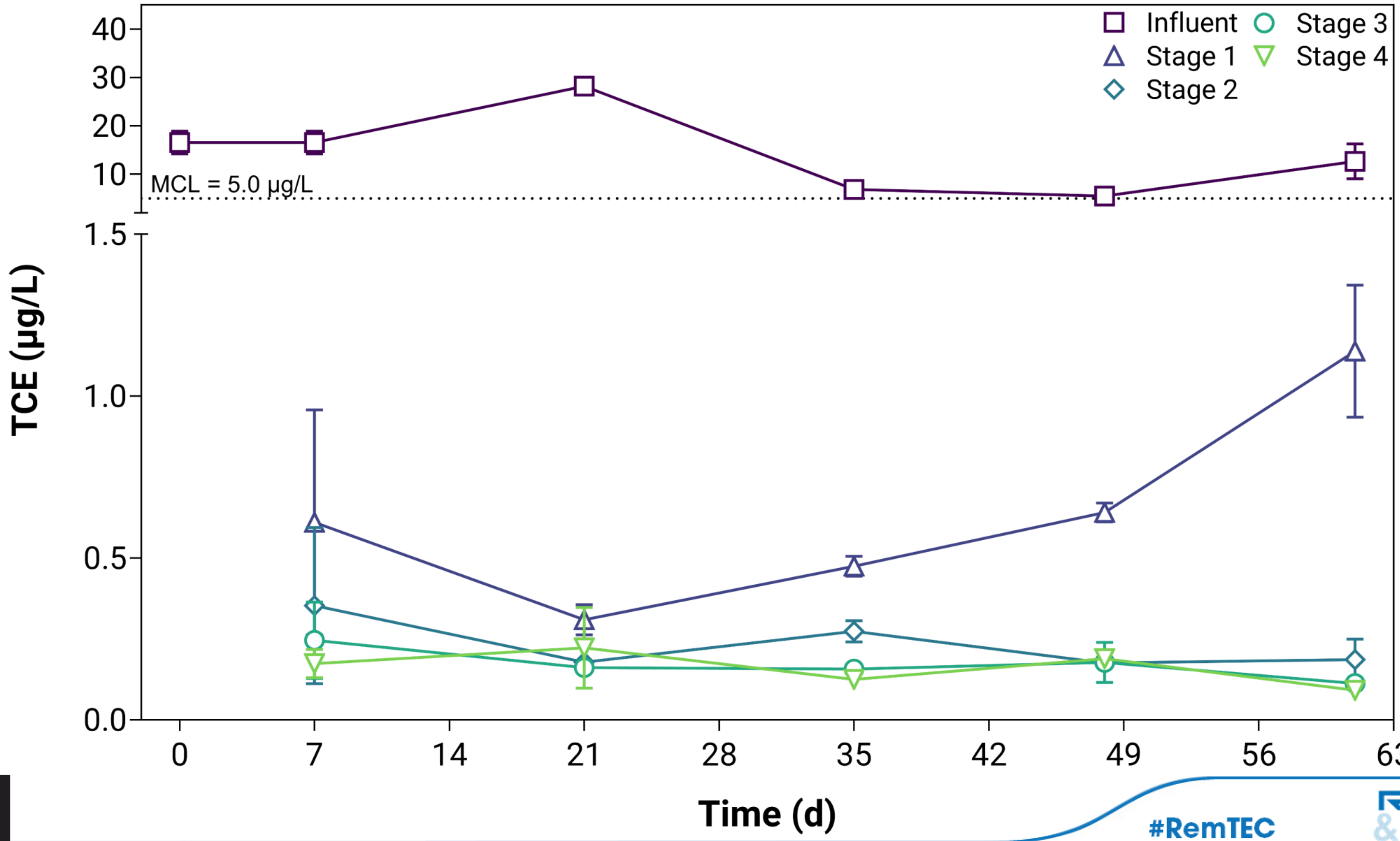
Simmer et al., Manuscript in progress

qPCR analyses suggest sustained *R. ruber* population over 9 weeks



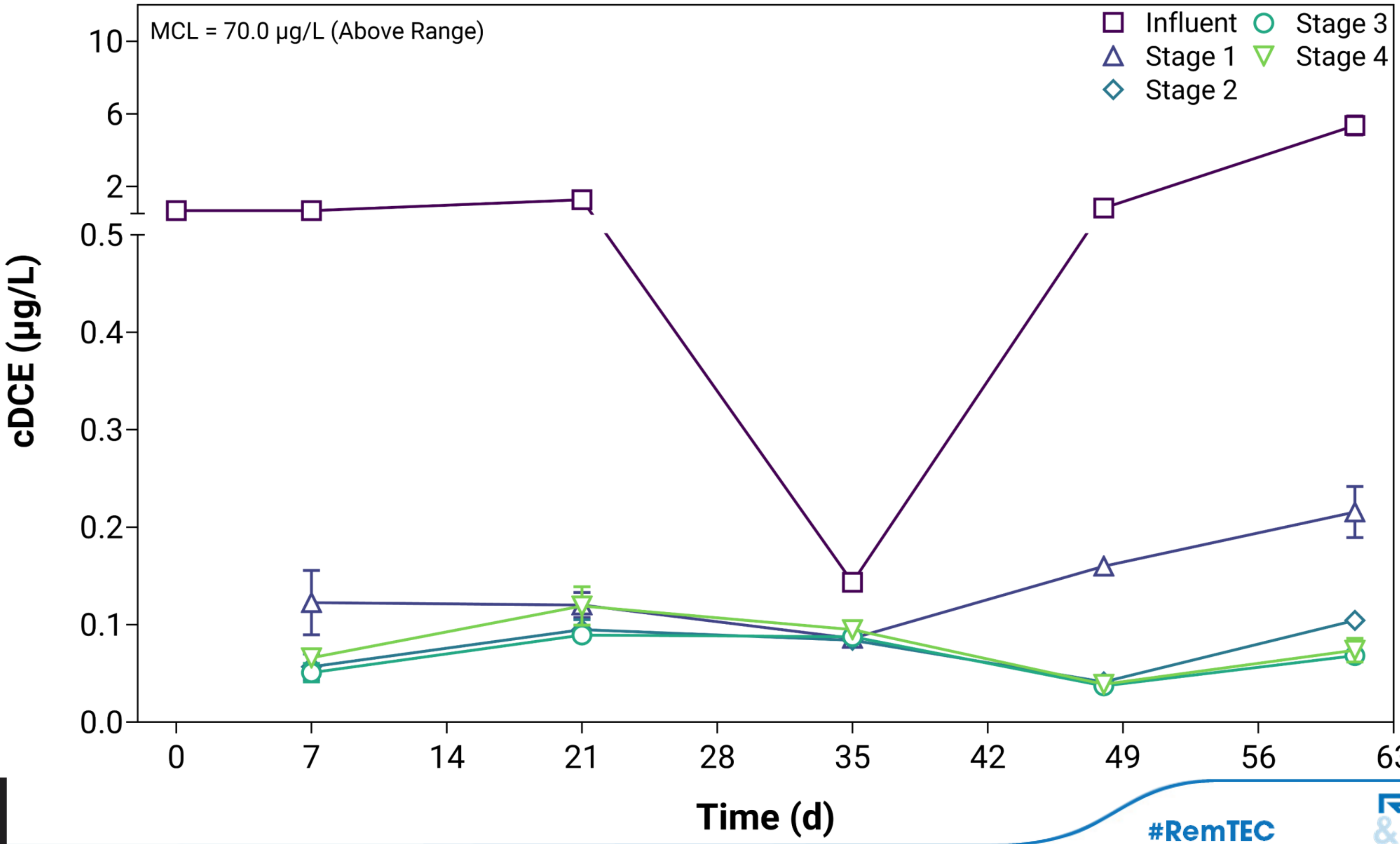
Simmer et al., Manuscript in progress

Bioaugmented phytoremediation effectively treated TCE to ≤0.2 µg/L



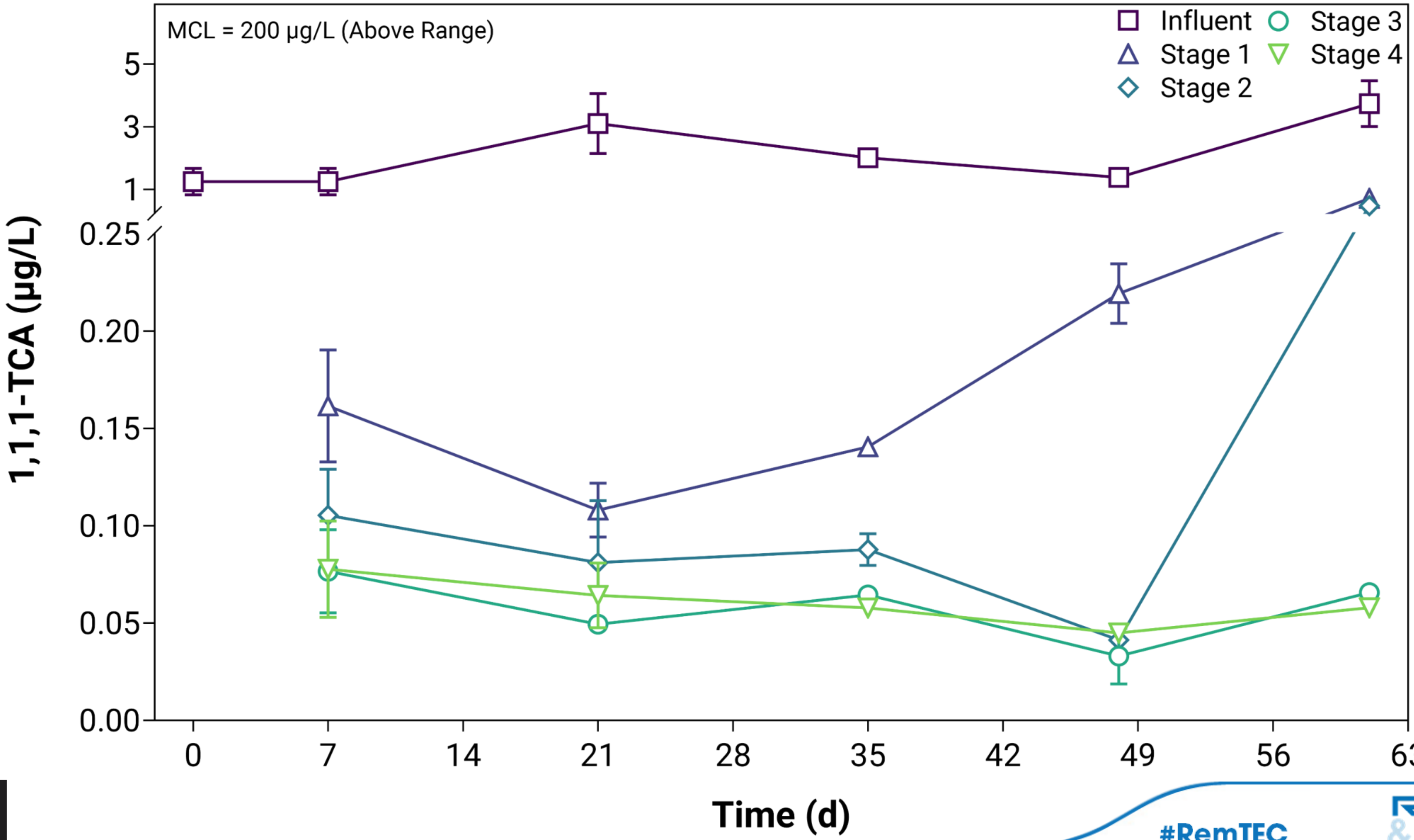
Simmer et al., Manuscript in progress

Bioaugmented phytoremediation effectively treated cDCE to ≤0.1 μg/L



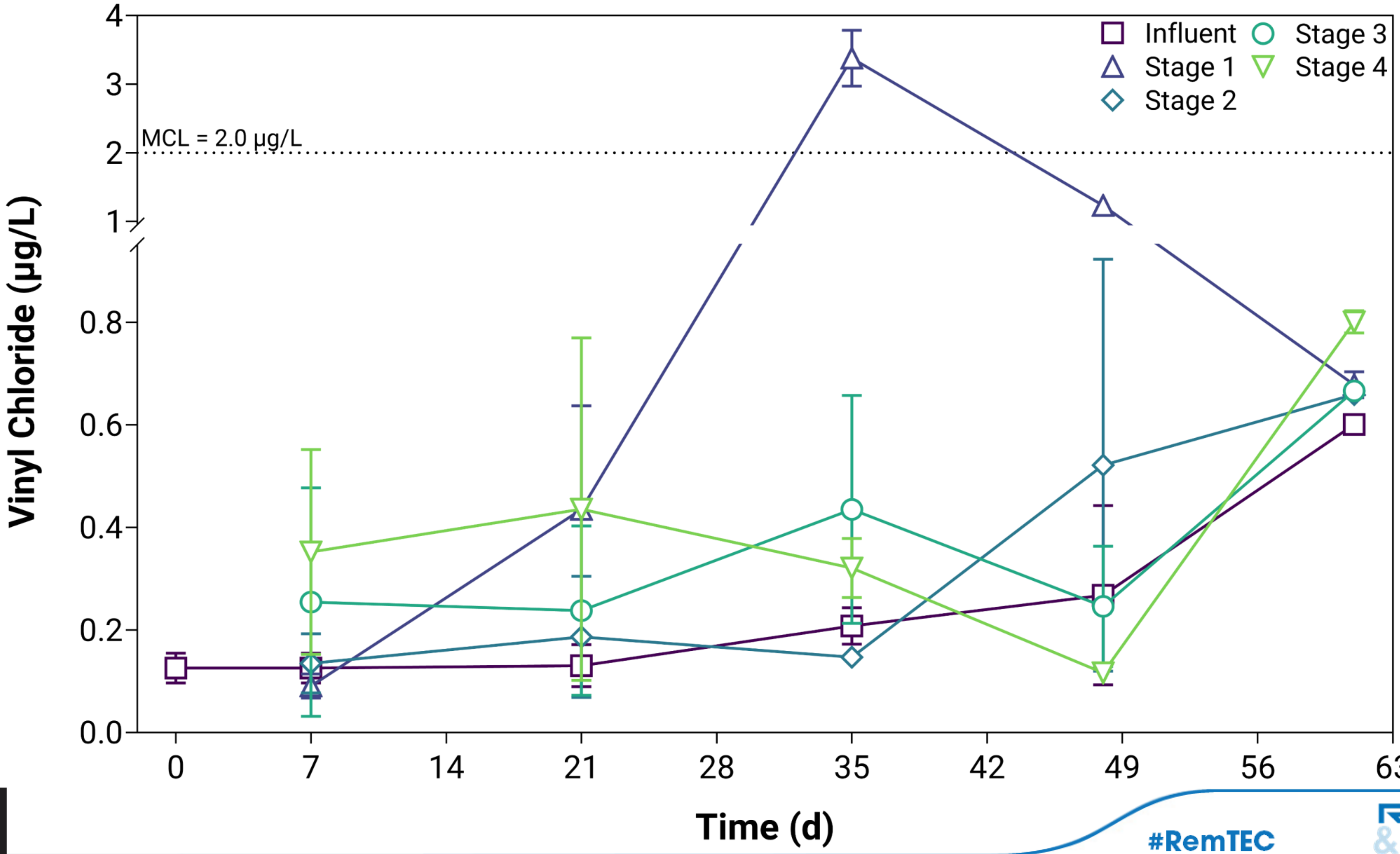
Simmer et al., Manuscript in progress

Bioaugmented phytoremediation effectively treated 1,1,1-TCA <0.1 µg/L



Simmer et al., Manuscript in progress

Minor vinyl chloride (<1.0 µg/L) formation observed in effluent



Simmer et al., Manuscript in progress

Major Takeaways

- ✓Phytoremediation system bioaugmented with *R. ruber* 219 capable of sustained remediation of dilute ($\leq$100 µg/L) 1,4-dioxane to as low as 0.5 µg/L
- ✓*R. ruber* effluent thmA concentrations stable for 9 weeks, highest in 1st treatment stage
- ✓Repeated bioaugmentation likely needed for best results
- ✓Poplar and willow capable of complete transpiration (zero discharge), dioxane mass removal
- ✓Chlorinated solvents effectively treated to below MCLs

Next Steps

- Core sample DNA analysis
- Phytoforenics (uptake of dioxane and CVOCs by poplar + willow)
- Dioxane metabolite analyses
- **Full-scale implementation?**

SERDP ER23-3717: Optimizing Bioaugmentation with *Rhodococcus ruber* for Cost-Effective Bioremediation of Dilute 1,4-Dioxane Plumes

- Develop and evaluate practical strategies to improve survival of *R. ruber*

Bioaugmented phytoremediation offers a cost effective, green solution for 1,4-dioxane

New Brighton, MN

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- Center for Global & Regional Environmental Research (CGRER)

IOWA | IIHR—Hydroscience and Engineering

ESTCP

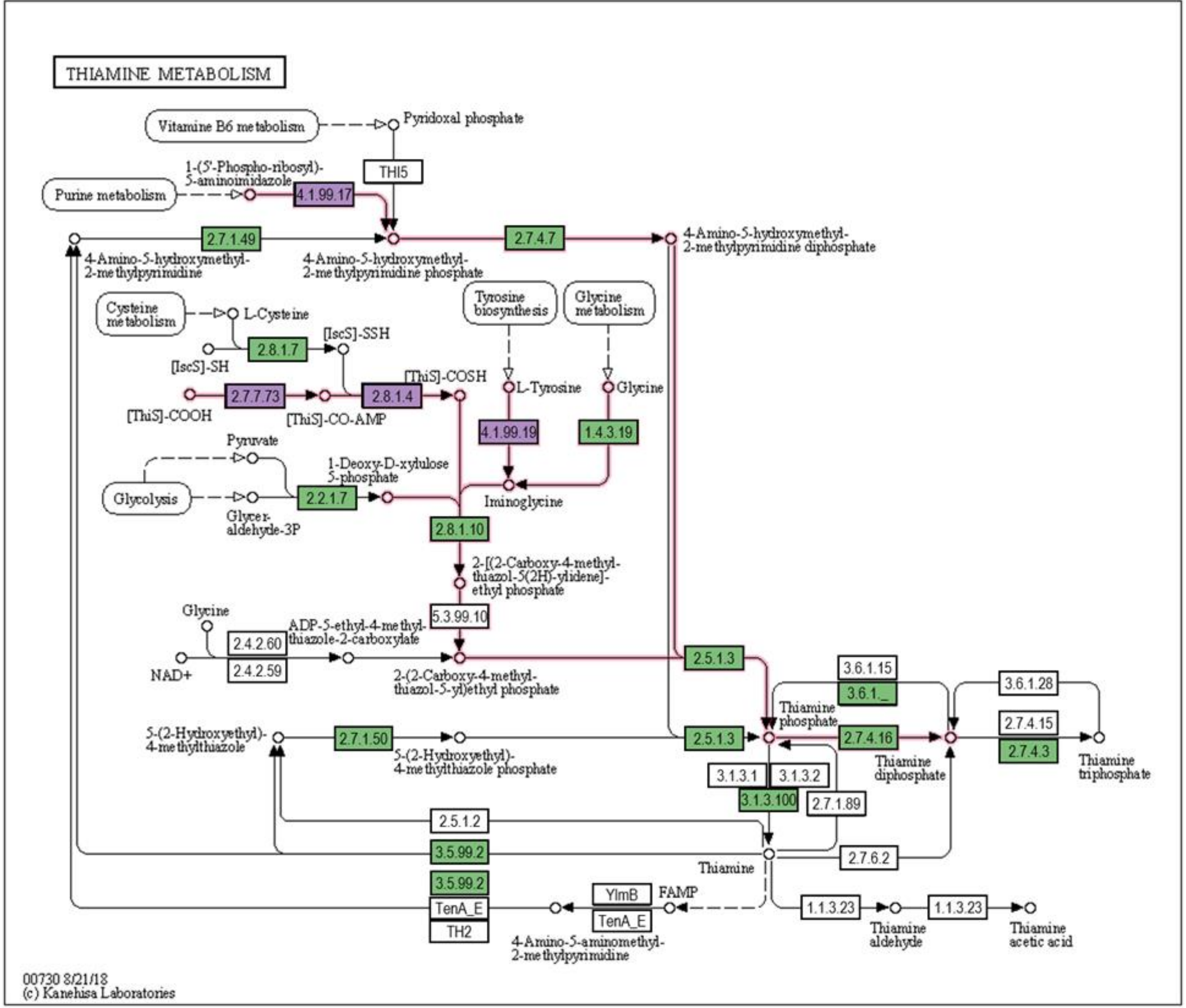
SERDP DOD · EPA · DOE

NSF

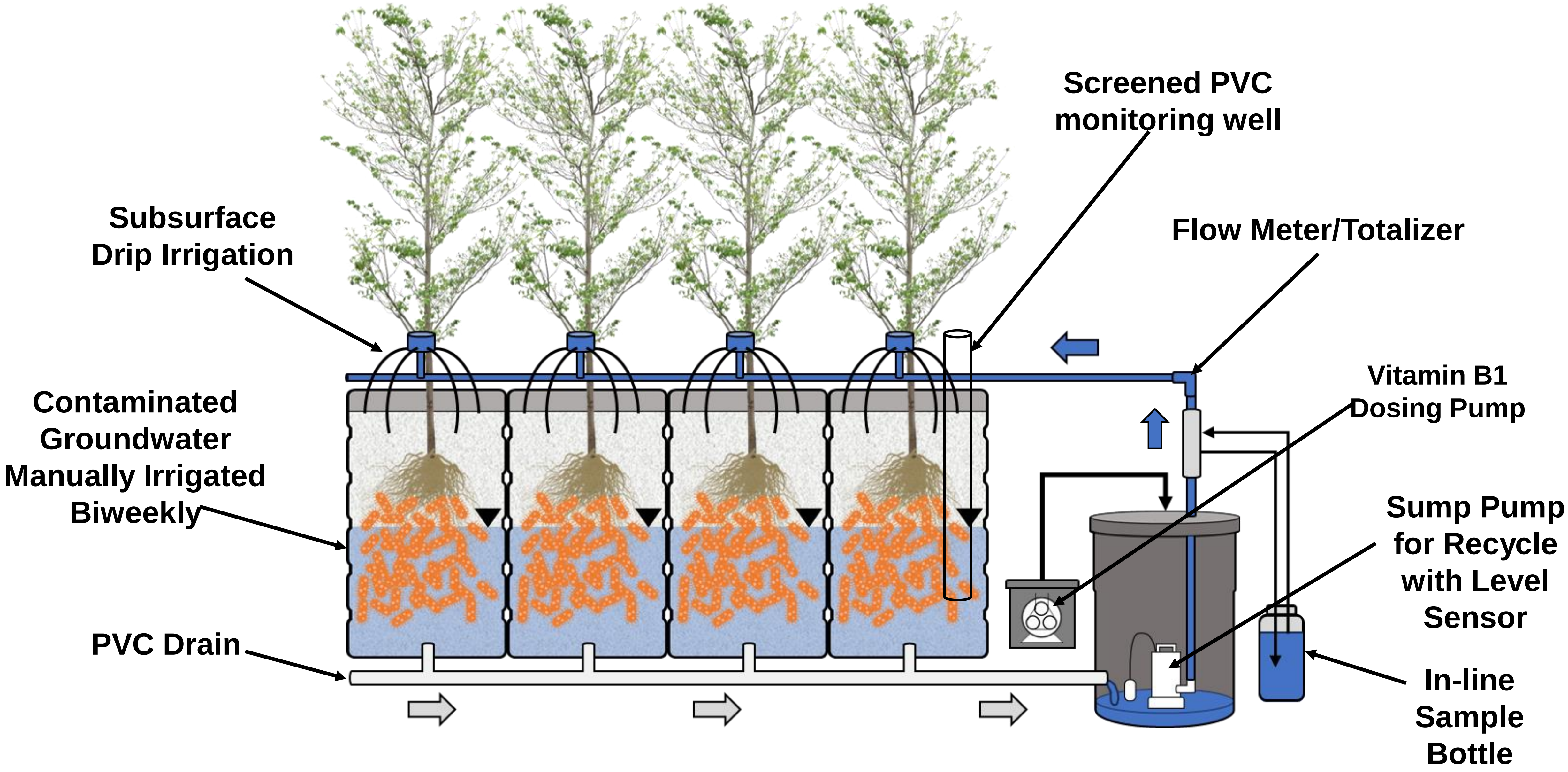
GEEMaP Geoinformatics for Environmental and Energy Modeling and Prediction

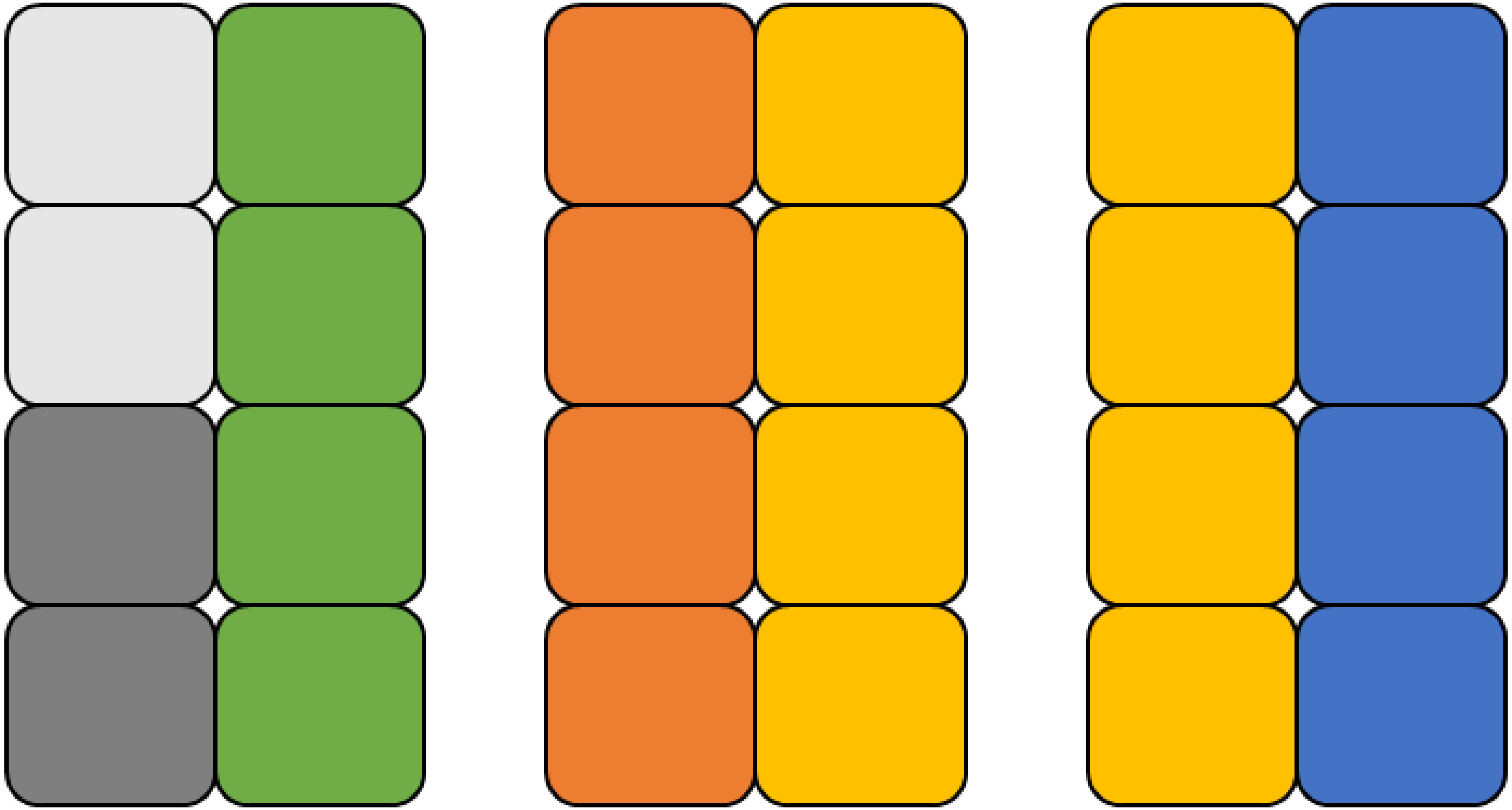
CGRER

Backup Slides



THIAMINE METABOLISM
Vitamine B6 metabolism
Pyridoxal phosphate
THI5
Purine metabolism
1-(5'-Phospho-ribosyl)-
5-aminoimidazole
4.1.99.17
2.7.1.49
2.7.4.7
4-Amino-5-hydroxymethyl-
2-methylpyrimidine diphosphate
4-Amino-5-hydroxymethyl-
2-methylpyrimidine
4-Amino-5-hydroxymethyl-
2-methylpyrimidine phosphate
Cysteine metabolism
L-Cysteine
Tyrosine biosynthesis
Glycine metabolism
[IscS]-SSH
2.8.1.7
[IscS]-SH
[ThiS]-COSH
2.7.7.73
2.8.1.4
L-Tyrosine
Glycine
[ThiS]-COOH
[ThiS]-CO-AMP
4.1.99.19
1.4.3.19
Pyruvate
1-Deoxy-D-xylulose
5-phosphate
Glycolysis
2.2.1.7
Iminoglycine
Glycer-
aldehyde-3P
2.8.1.10
2-[(2-Carboxy-4-methyl-
thiazol-5(2H)-ylidene]-
ethyl phosphate
Glycine
5.3.99.10
ADP-5-ethyl-4-methyl-
thiazole-2-carboxylate
2.4.2.60
NAD+
2.4.2.59
2.5.1.3
2-(2-Carboxy-4-methyl-
thiazol-5-yl)ethyl phosphate
3.6.1.15
3.6.1.28
3.6.1._
Thiamine
phosphate
2.7.4.15
5-(2-Hydroxyethyl)-
4-methylthiazole
2.7.1.50
2.5.1.3
2.7.4.16
2.7.4.3
5-(2-Hydroxyethyl)-
4-methylthiazole phosphate
Thiamine
diphosphate
Thiamine
triphosphate
3.1.3.1
3.1.3.2
3.1.3.100
2.7.1.89
2.5.1.2
3.5.99.2
2.7.6.2
Thiamine
3.5.99.2
YlmB
FAMP
TenA_E
TenA_E
1.1.3.23
1.1.3.23
TH2
Thiamine
aldehyde
Thiamine
acetic acid
4-Amino-5-aminomethyl-
2-methylpyrimidine
00730 8/21/18
(c) Kanehisa Laboratories


Screened PVC
monitoring well
Subsurface
Drip Irrigation
Flow Meter/Totalizer
Vitamin B1
Dosing Pump
Contaminated
Groundwater
Manually Irrigated
Biweekly
Sump Pump
for Recycle
with Level
Sensor
PVC Drain
In-line
Sample
Bottle



Perlite Only Control

Perlite + Biochar Control

Trees in Perlite

R. ruber 219 in Perlite

Trees with R. ruber 219 in Perlite

Trees with R. ruber 219 in Perlite + Biochar

IOWA

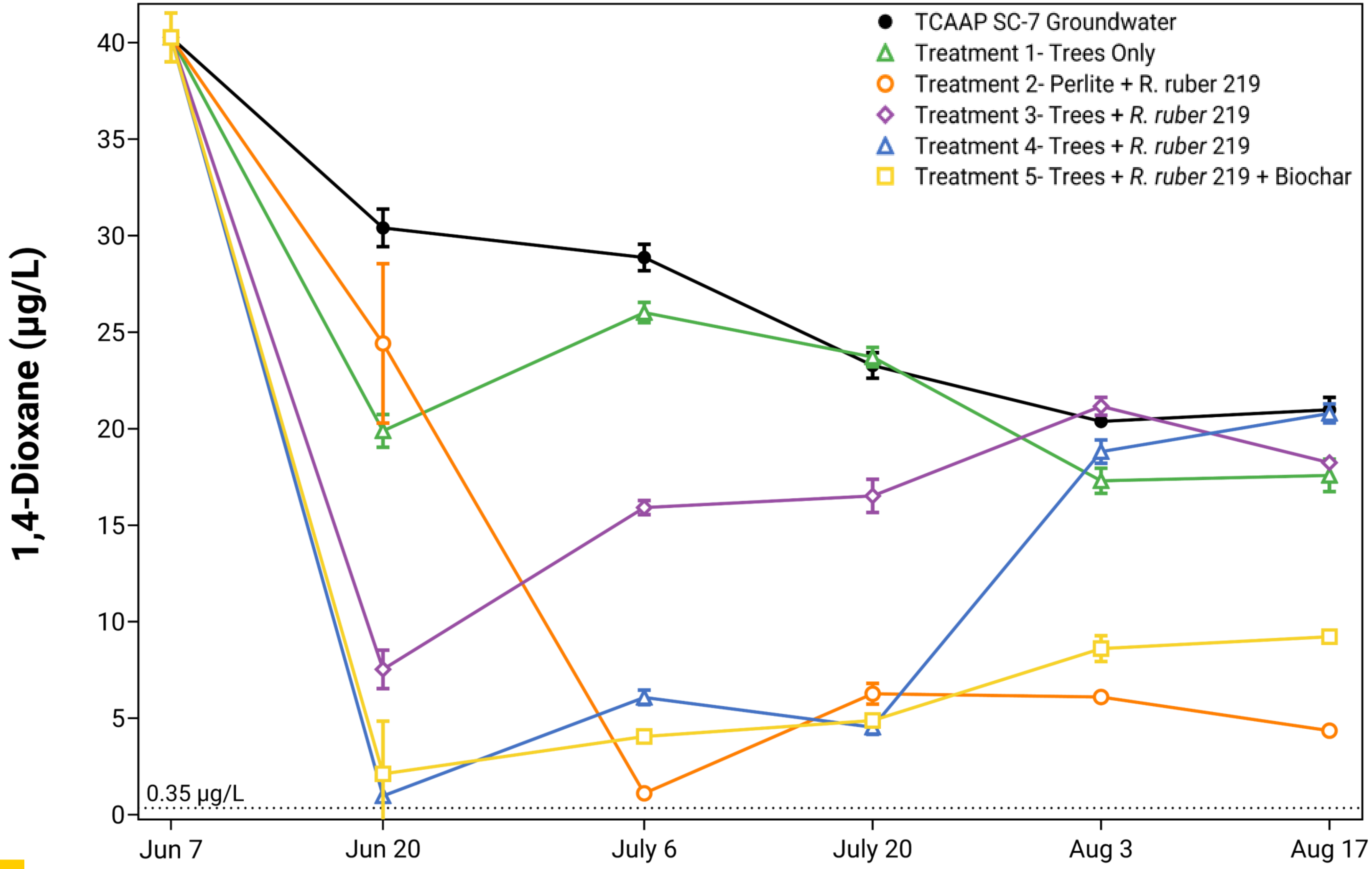

MADE IN U.S.A.05/23/2318:35 EXT. #13R


18 PACK
IGLOO

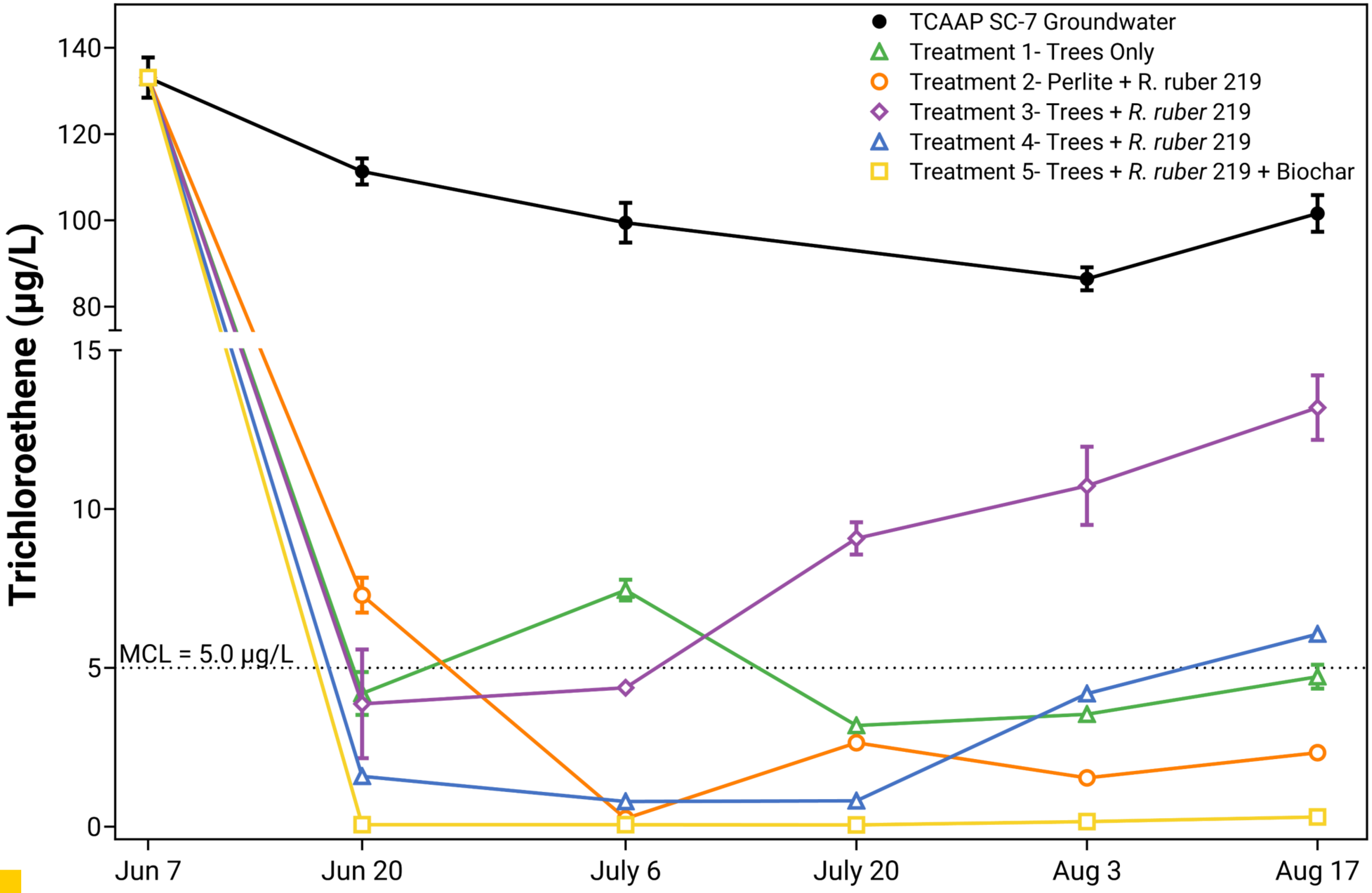

geopump
geotech
1000 mL
900
800
700
600
500
400
100


Pro DSS
07/20/23 12:30:19PM
Run
Log One Sample
23.6 °C
735.9 mmHg
46.4 DO %
3.92
1042
7.11 pH
System
Probe
Calibration
File
Cal
Esc
ENTER

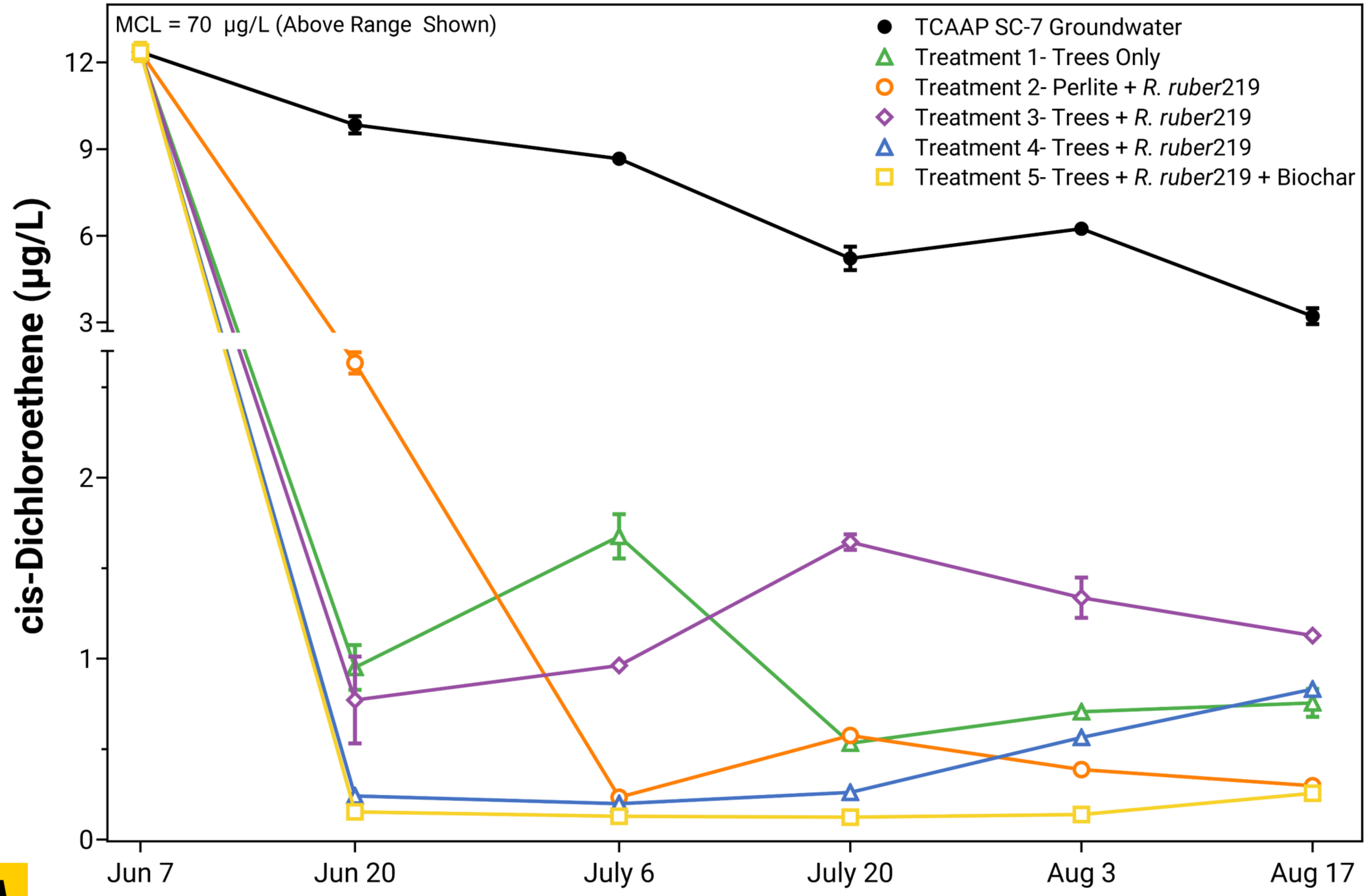

Sample
Date/Time



TCAAP SC-7 Groundwater
Treatment 1- Trees Only
Treatment 2- Perlite + R. ruber 219
Treatment 3- Trees + *R. ruber* 219
Treatment 4- Trees + *R. ruber* 219
Treatment 5- Trees + *R. ruber* 219 + Biochar
1,4-Dioxane (µg/L)
0
5
10
15
20
25
30
35
40
0.35 µg/L
Jun 7
Jun 20
July 6
July 20
Aug 3
Aug 17

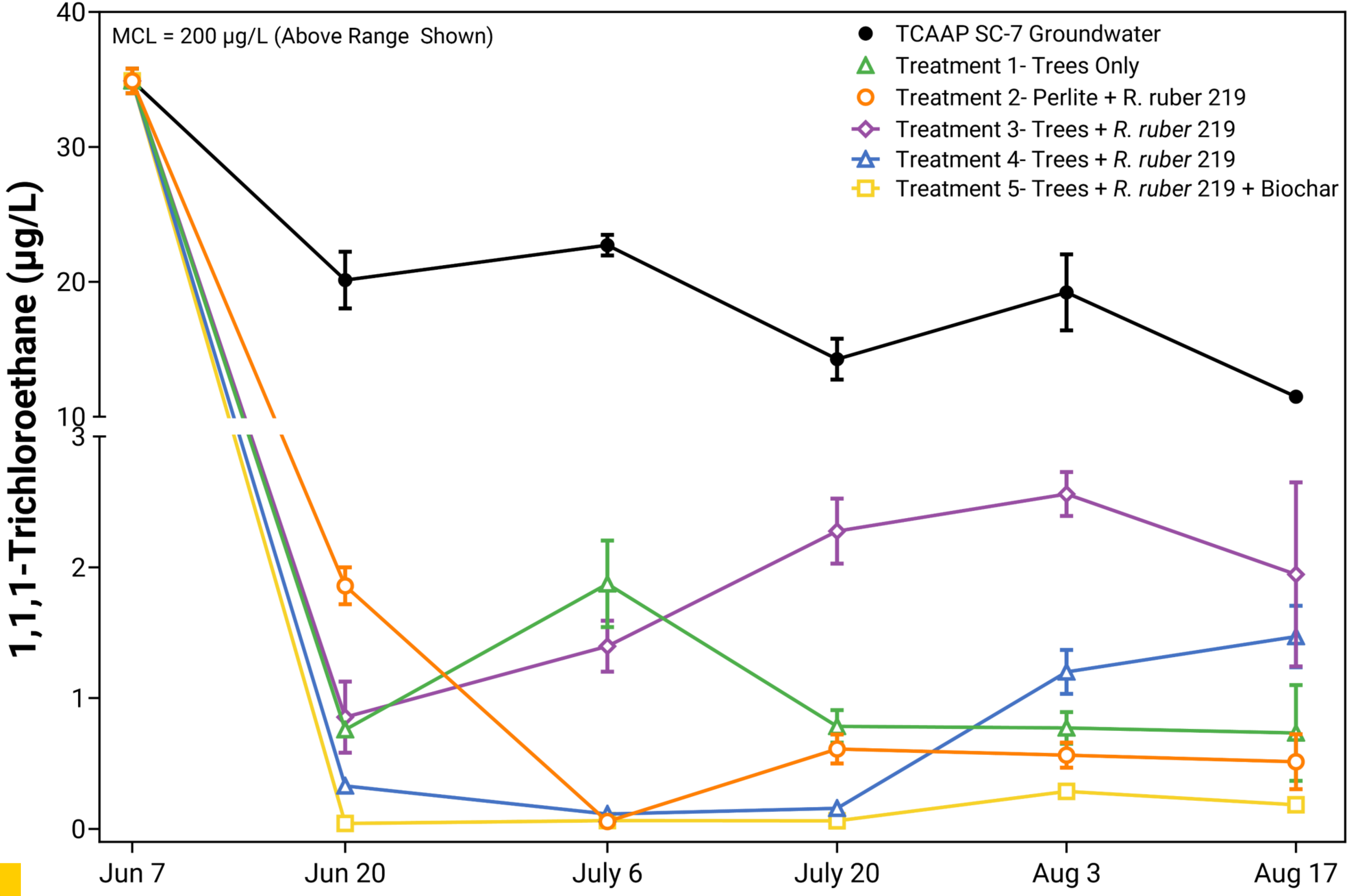

TCAAP SC-7 Groundwater
Treatment 1- Trees Only
Treatment 2- Perlite + R. ruber 219
Treatment 3- Trees + *R. ruber* 219
Treatment 4- Trees + *R. ruber* 219
Treatment 5- Trees + *R. ruber* 219 + Biochar
Trichloroethene (µg/L)
140
120
100
80
15
10
5
0
MCL = 5.0 µg/L
Jun 7
Jun 20
July 6
July 20
Aug 3
Aug 17

IOWA



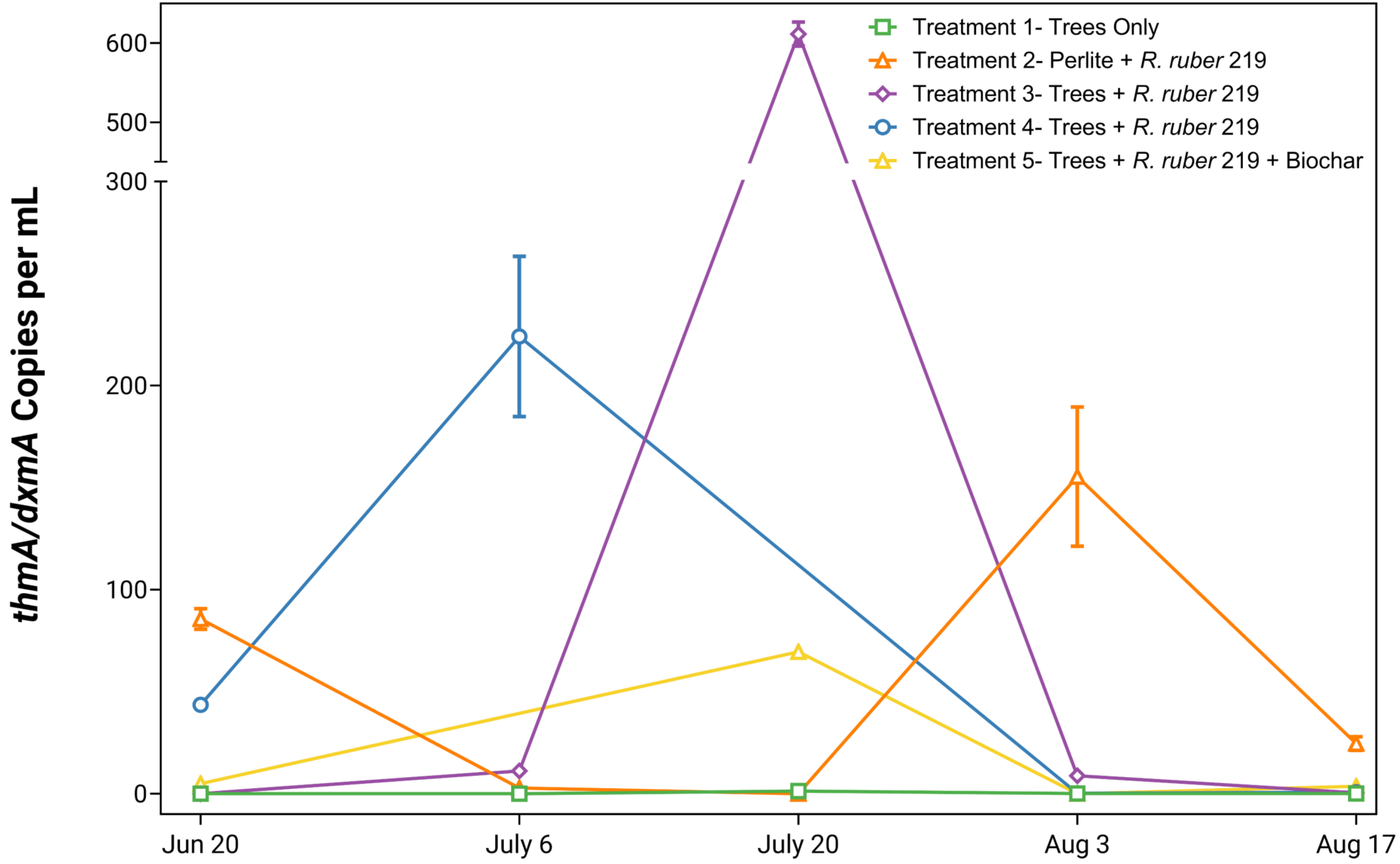
MCL = 70 µg/L (Above Range Shown)
TCAAP SC-7 Groundwater
Treatment 1- Trees Only
Treatment 2- Perlite + R. ruber219
Treatment 3- Trees + R. ruber219
Treatment 4- Trees + R. ruber219
Treatment 5- Trees + R. ruber219 + Biochar
cis-Dichloroethene (µg/L)
12
9
6
3
2
1
0
Jun 7
Jun 20
July 6
July 20
Aug 3
Aug 17

IOWA



MCL = 200 µg/L (Above Range Shown)
TCAAP SC-7 Groundwater
Treatment 1- Trees Only
Treatment 2- Perlite + R. ruber 219
Treatment 3- Trees + *R. ruber* 219
Treatment 4- Trees + *R. ruber* 219
Treatment 5- Trees + *R. ruber* 219 + Biochar
1,1,1-Trichloroethane (µg/L)
40
30
20
10
3
2
1
0
Jun 7
Jun 20
July 6
July 20
Aug 3
Aug 17

IOWA



thmA/dxmA Copies per mL
600
500
300
200
100
0
Jun 20
July 6
July 20
Aug 3
Aug 17
Treatment 1- Trees Only
Treatment 2- Perlite + R. ruber 219
Treatment 3- Trees + R. ruber 219
Treatment 4- Trees + R. ruber 219
Treatment 5- Trees + R. ruber 219 + Biochar

IOWA

Major Takeaways 2023

- ✓Bioaugmented phytoremediation with *R. ruber* 219 accelerates treatment dioxane concentrations to as low as 0.18 µg/L
- ✓Sustained treatment (>50%) of dilute dioxane by *R. ruber* 219 for >2 months
- ✓Trees capable of complete transpiration (zero discharge), dioxane mass removal
- ✓Root zone may provide *R. ruber* with vitamin B1
- ✓CVOCs effectively removed in all treatments, volatilization due to pumping likely played an important role