

**Two Birds One Stone:**  
**Concurrent Biodegradation of 1,4-Dioxane and**  
**1,1-DCE by a Gram-Negative Propanotroph**  
***Azoarcus* sp. DD4**

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Department of Chemistry & Environmental Science  
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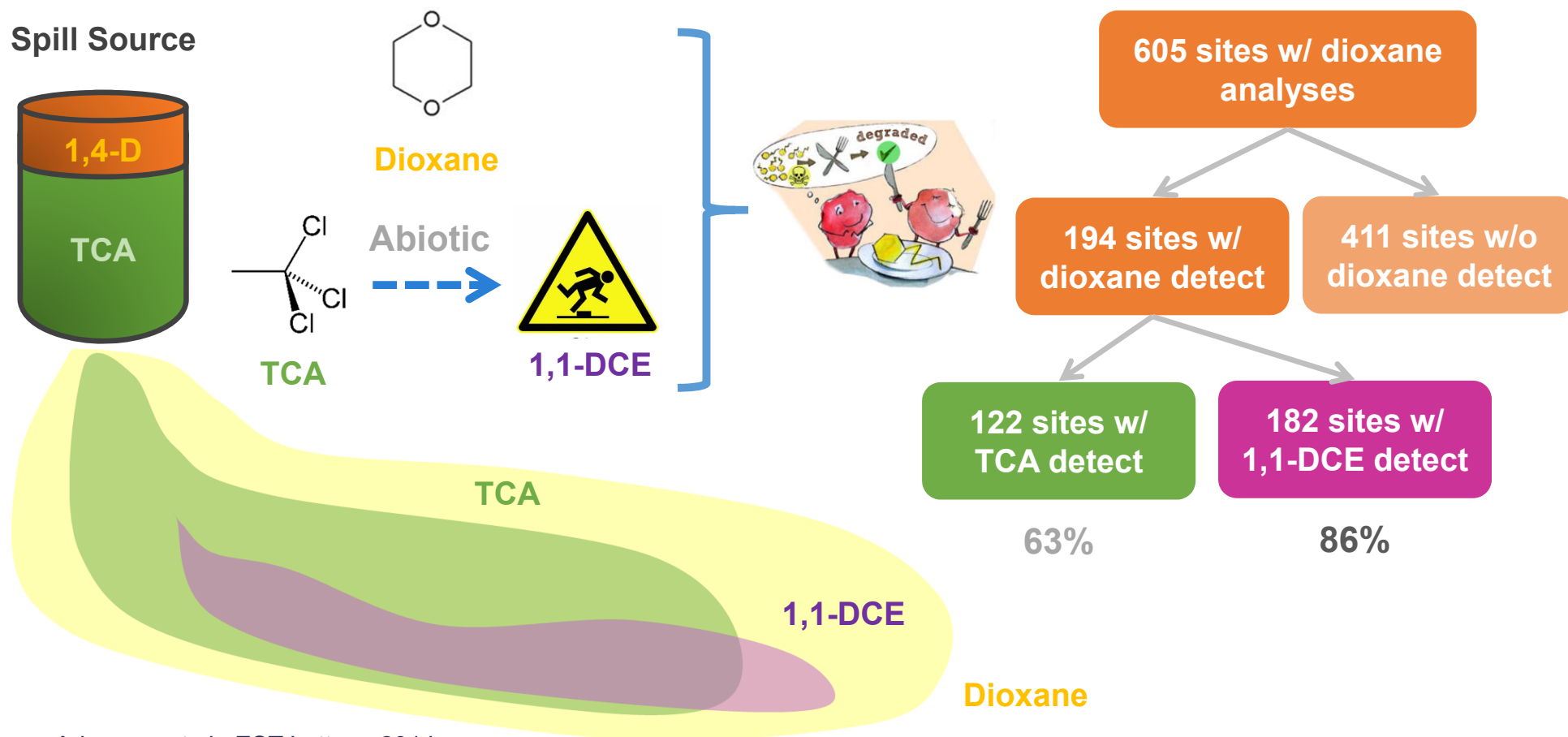
5<sup>th</sup> International Symposium on Bioremediation and Sustainable  
Environmental Technologies

Baltimore, MD

April 17<sup>th</sup>, 2019



# Prevalent Co-contamination of Dioxane and 1,1-DCE

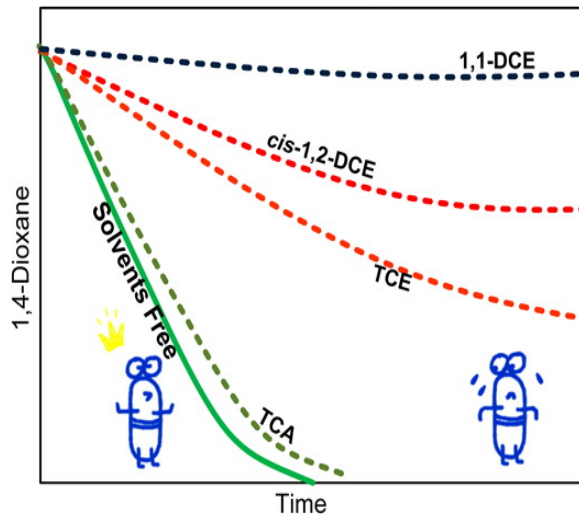


Adamson et al., EST Letters, 2014.

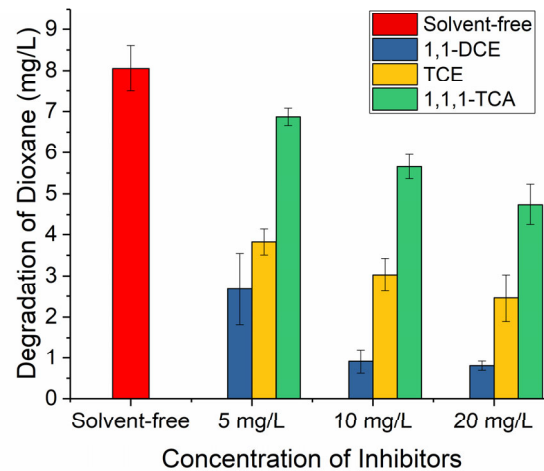
# 1,1-DCE: a Stumbling Block for Dioxane Bioremediation

Significant Inhibition of 1,1-DCE to Type Strains That Metabolize Dioxane

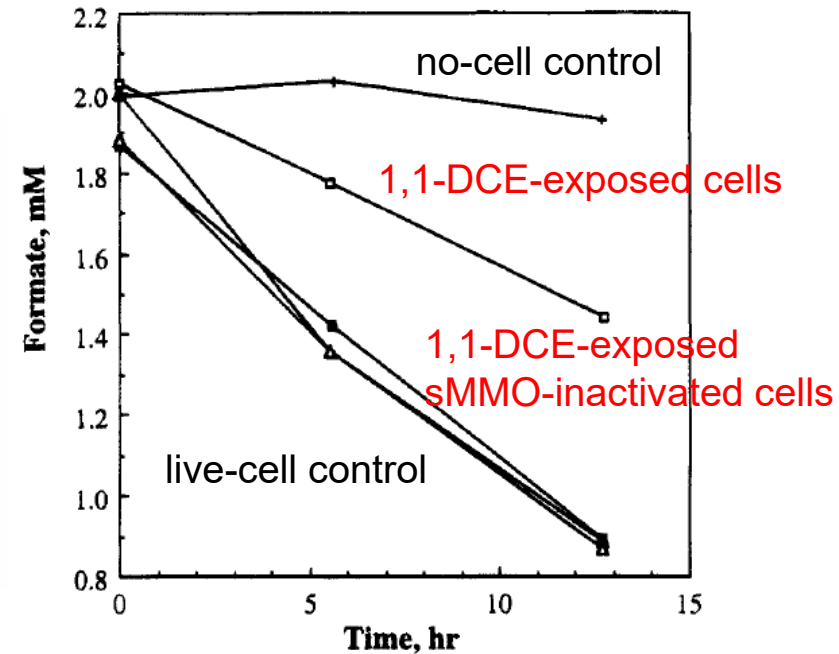
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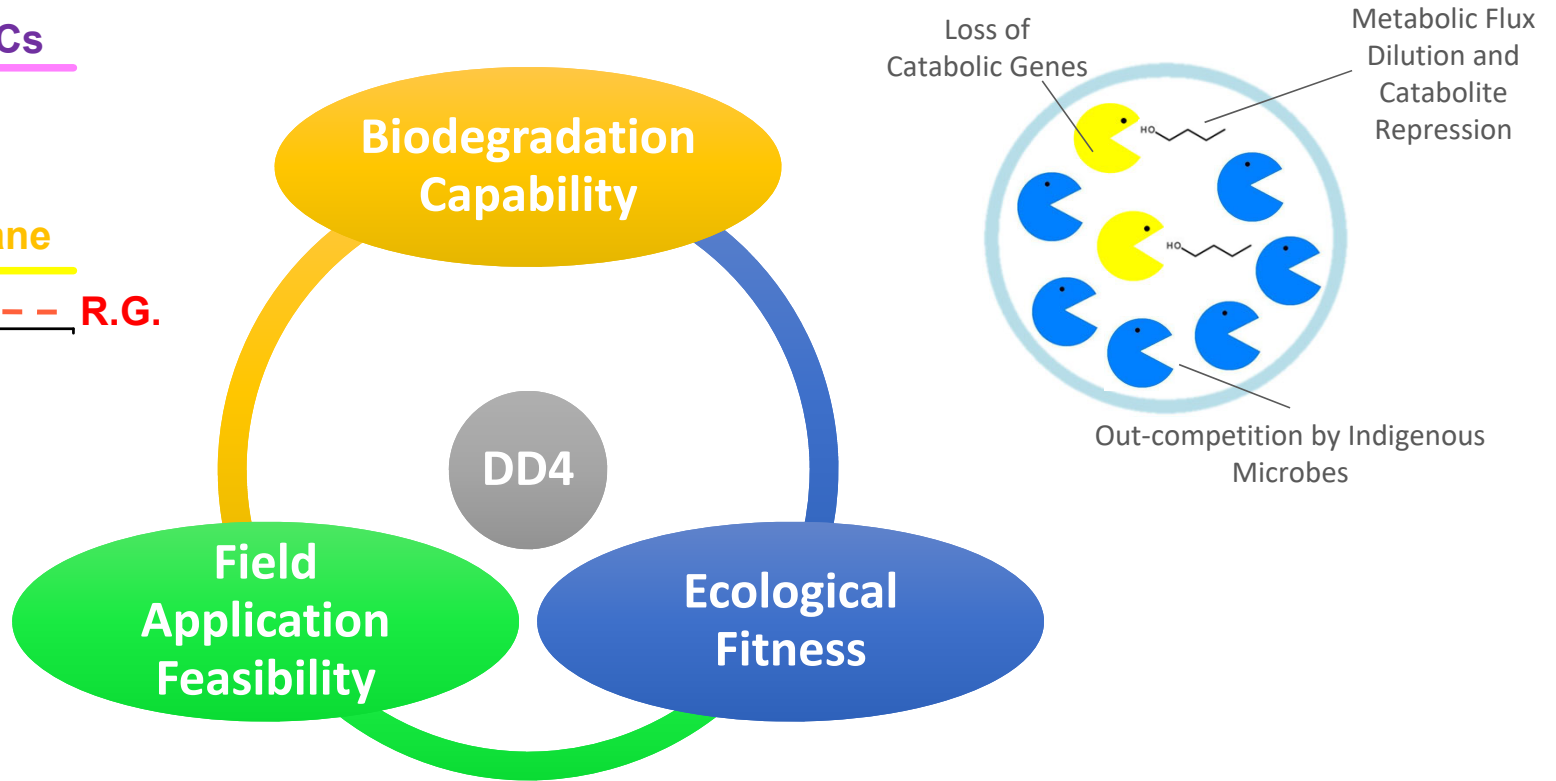
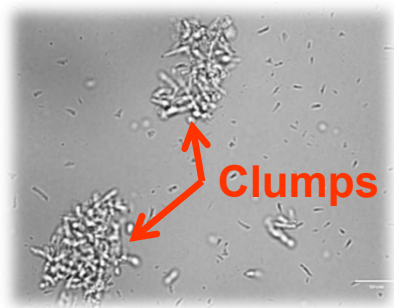
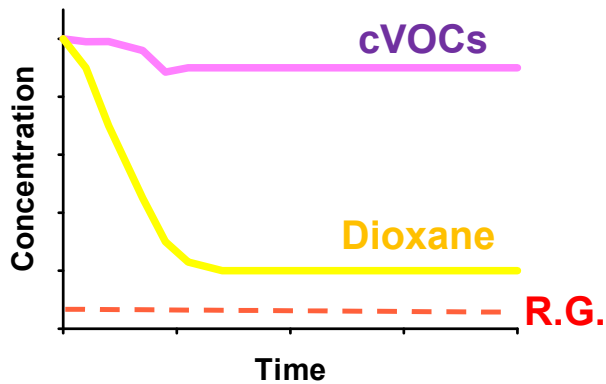
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Potent Toxicity of 1,1-DCE Transformation Products to a Mixed Methanotrophic Culture



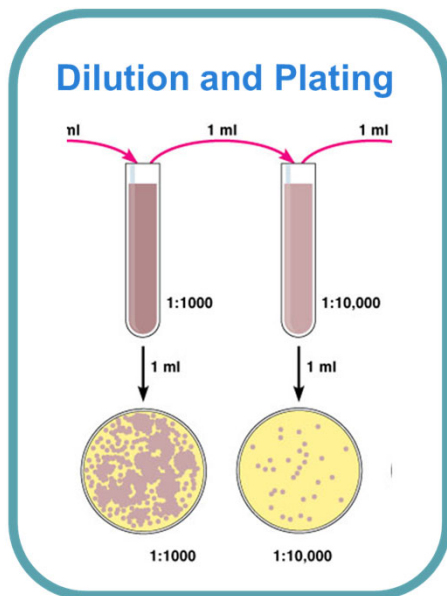
# What makes a robust inoculum for *in situ* bioaugmentation?



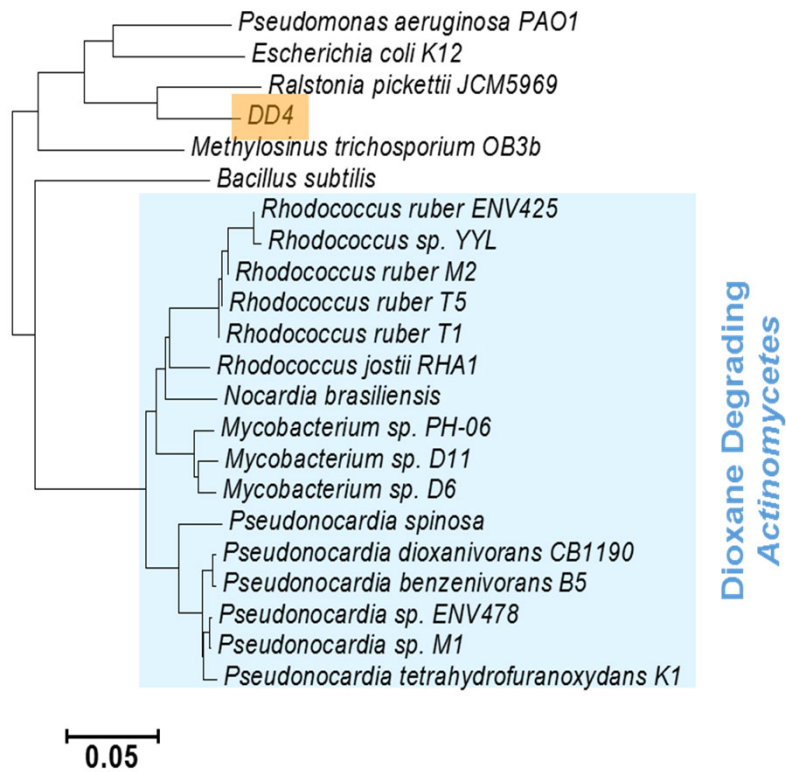
Barajas-Rodriguez and Freedman, J Hazard Mater, 2018; Li et al., Water Res, 2017.

# Objective

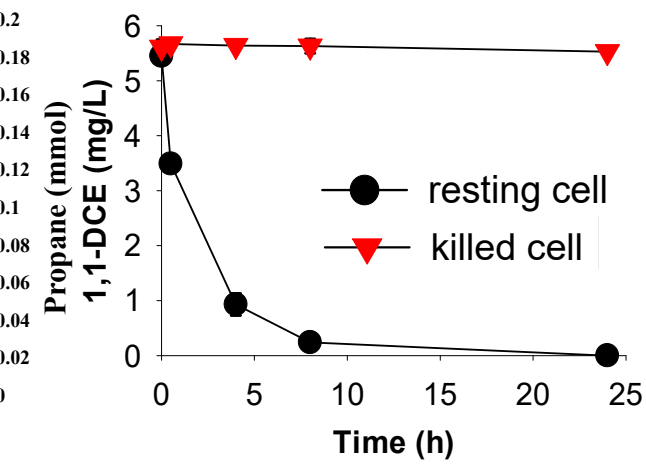
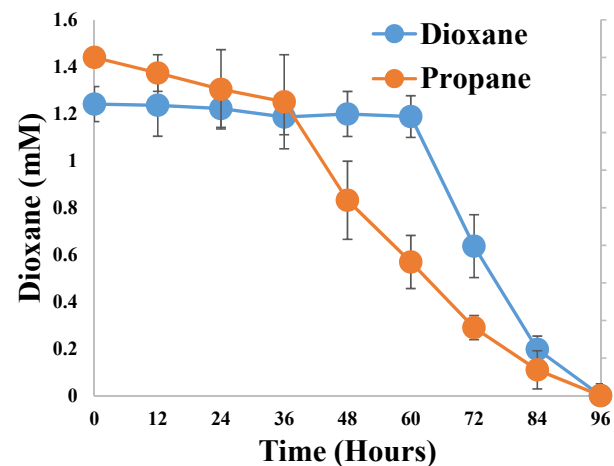
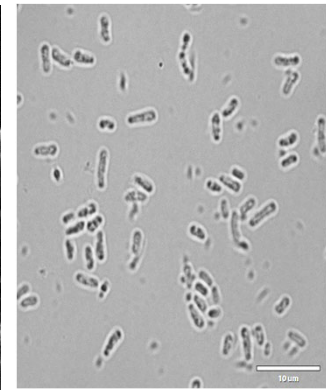
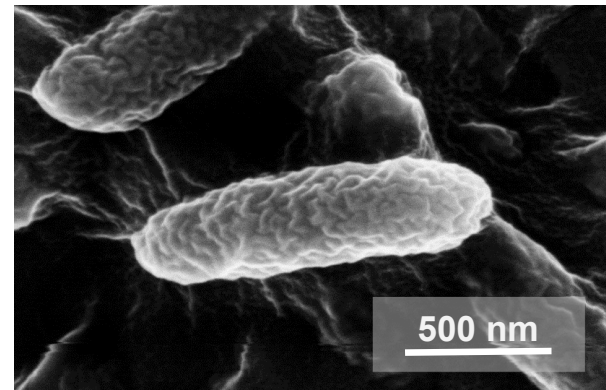
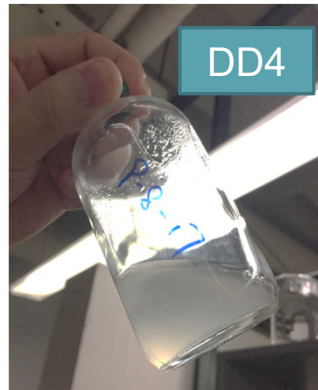
- ▶ To *isolate and characterize microbes with robust dioxane and cVOCs degradation capabilities and superior potential for in situ bioaugmentation* to mitigate commingled solvent-stabilizer contamination near the source zone.



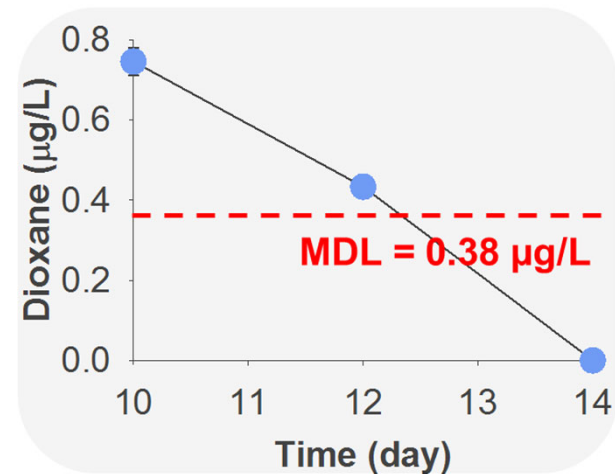
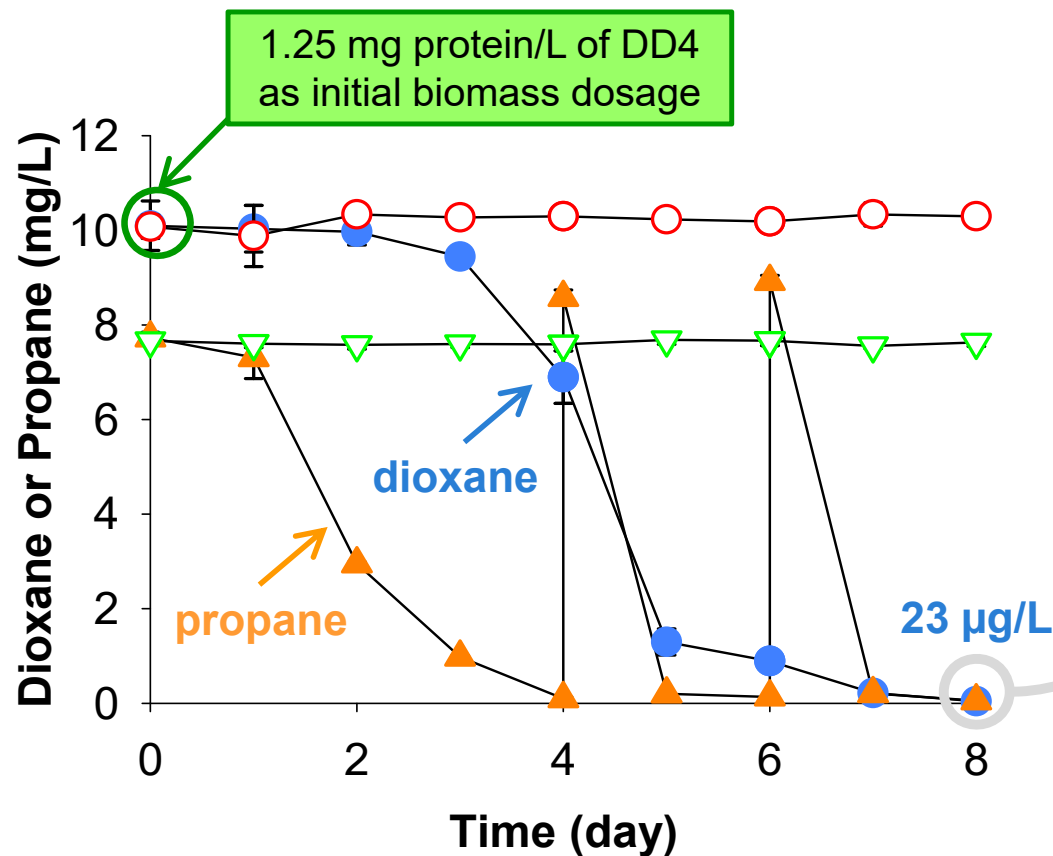
# Novel Gram- Isolate DD4 Can Cometabolize Dioxane and 1,1-DCE with Propane



$$Y = 0.25 \pm 0.003 \text{ mg protein mg propane}^{-1}$$



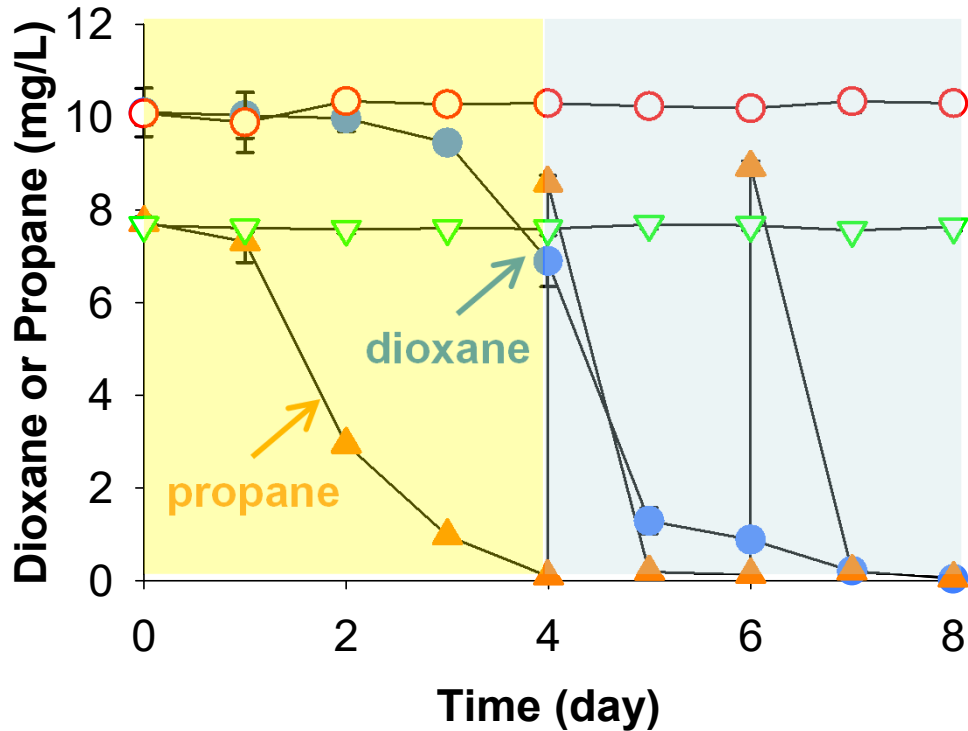
# Microcosm Assay Demonstrated DD4 as an Effective Bioaugmentation Inoculum to Remove Dioxane



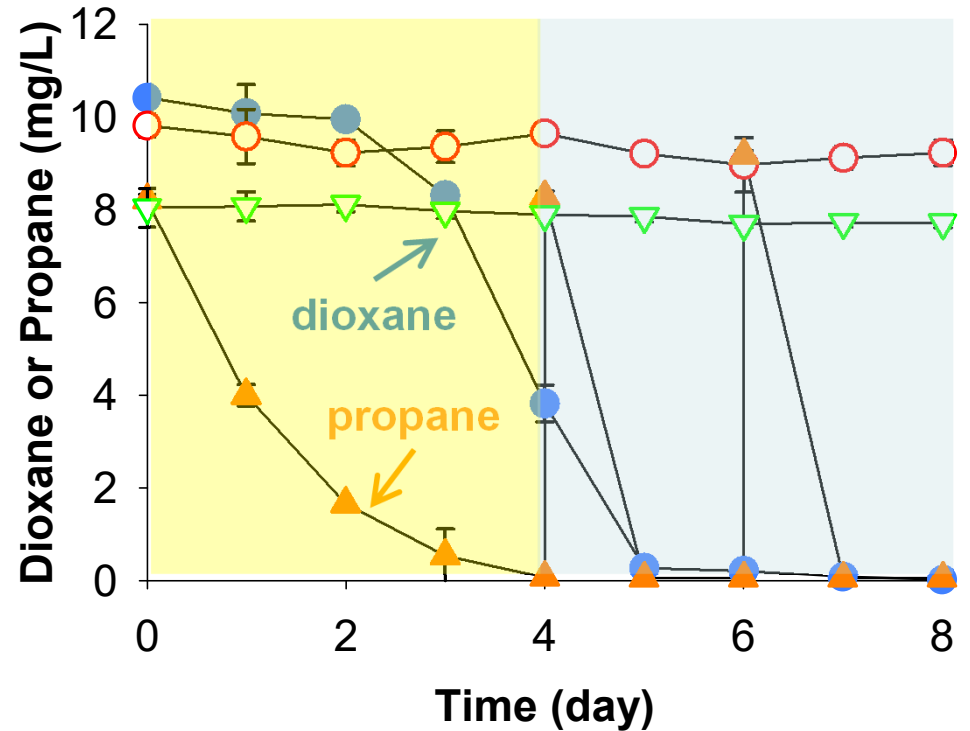
State	Guidance Level (µg/L)
CA	1
NJ	0.4
MA	0.3

# DD4 Can Overcome Field Detriments and Sustain Its Degradation Activities

(A) Groundwater from a CA Site



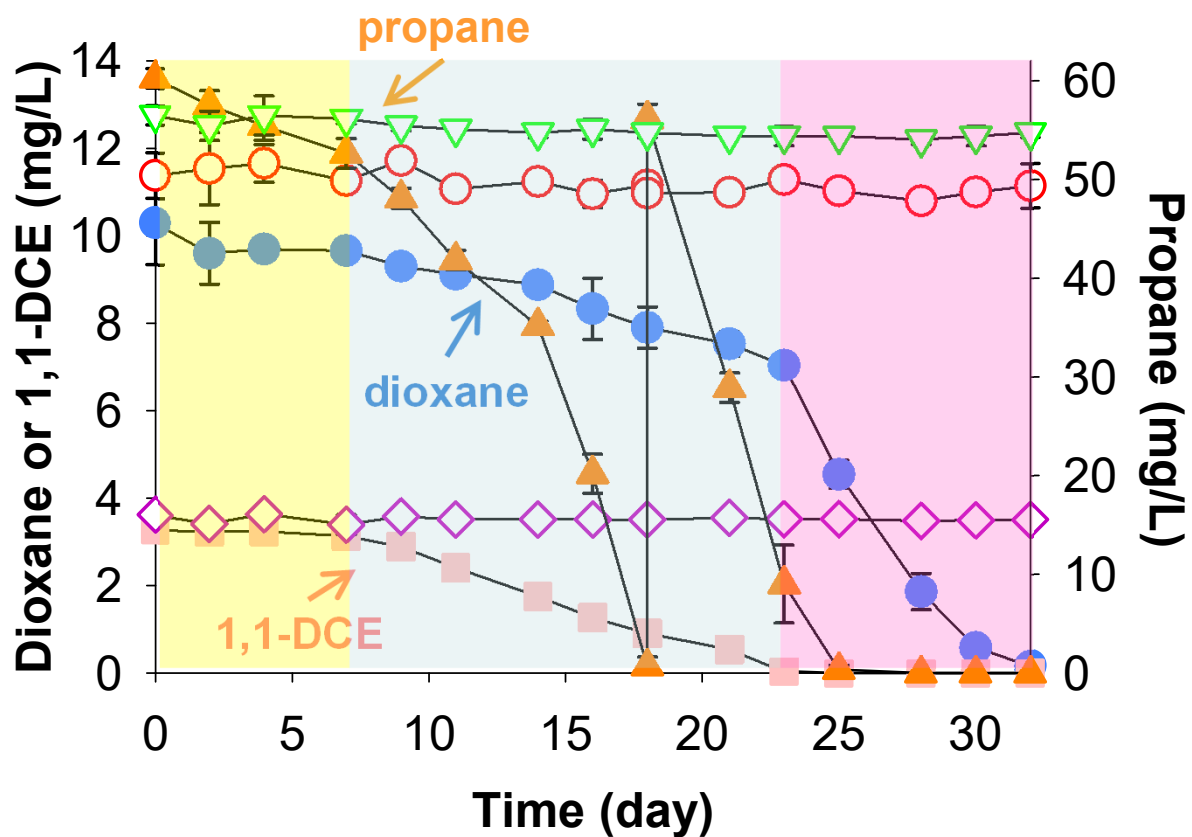
(B) Dioxane-dosed NMS Medium



① Inhibition → ② Recovery

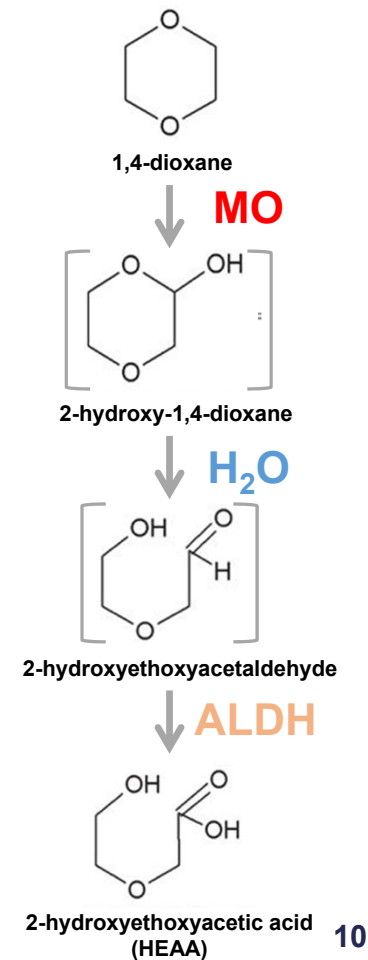
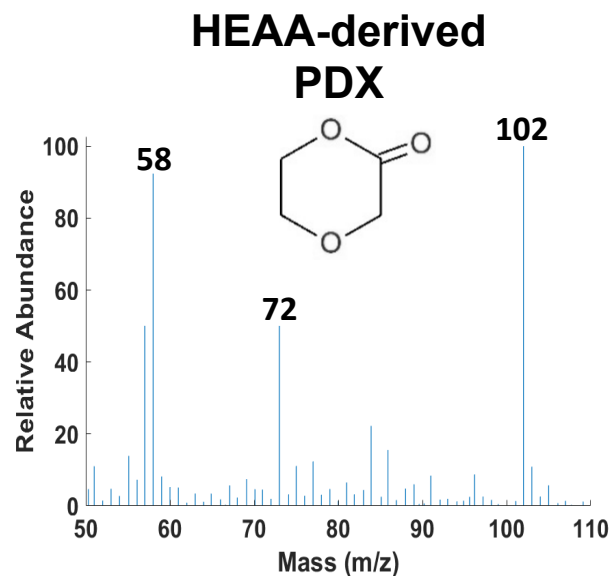
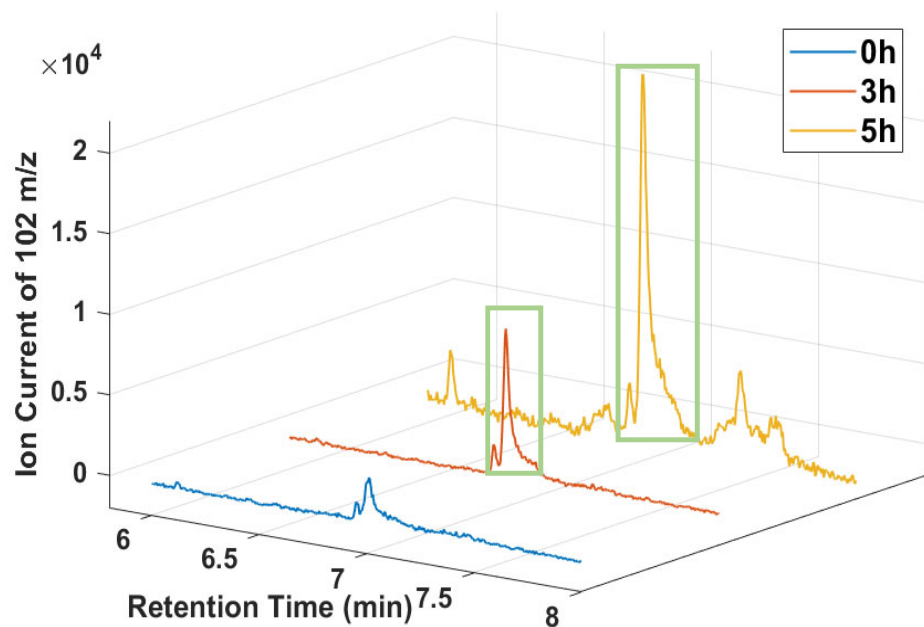


# Concurrent Removal of Dioxane and 1,1-DCE by DD4

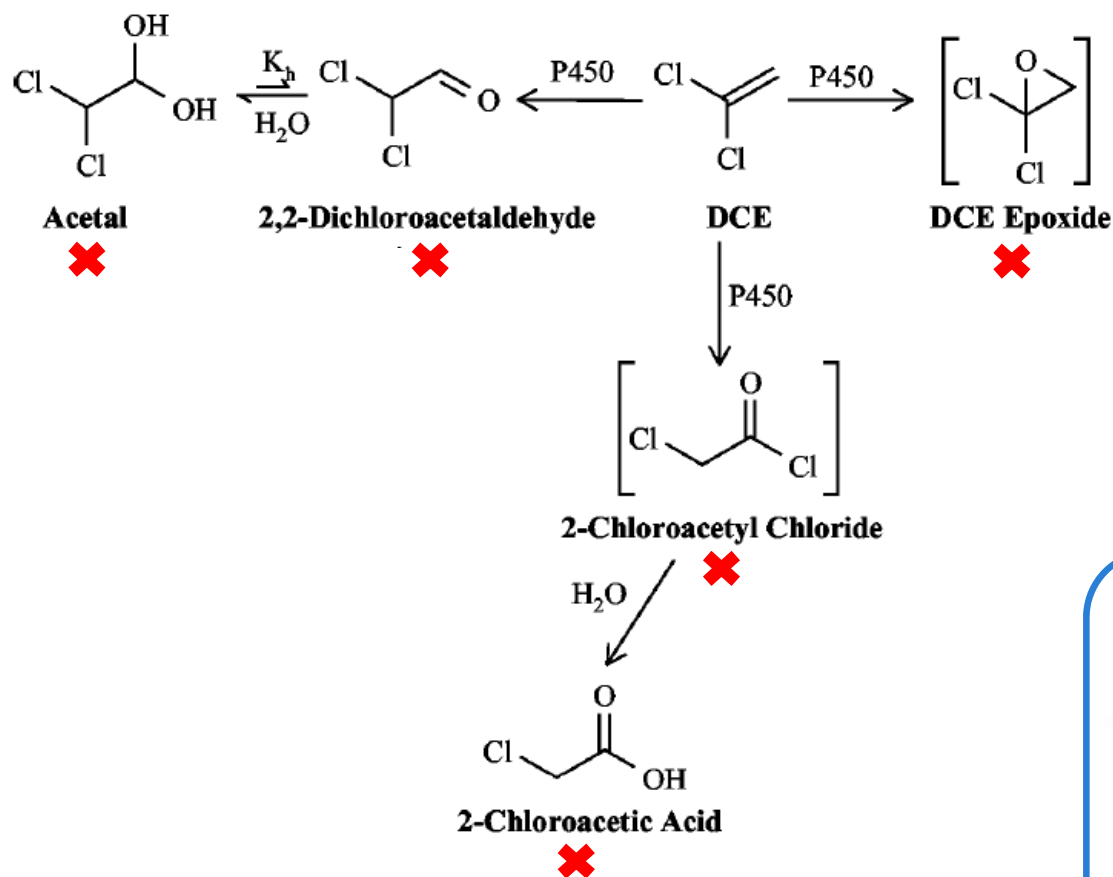


Stage	Propane	1,1-DCE	Dioxane
①	+	-	-

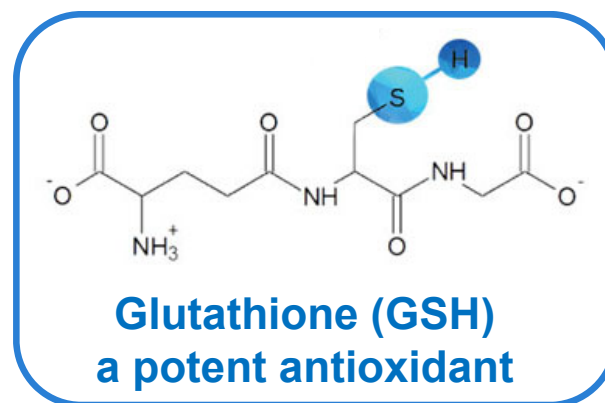
# GC/MS Coupled with Acidification Revealed HEAA as the Dioxane Oxidation Product in DD4



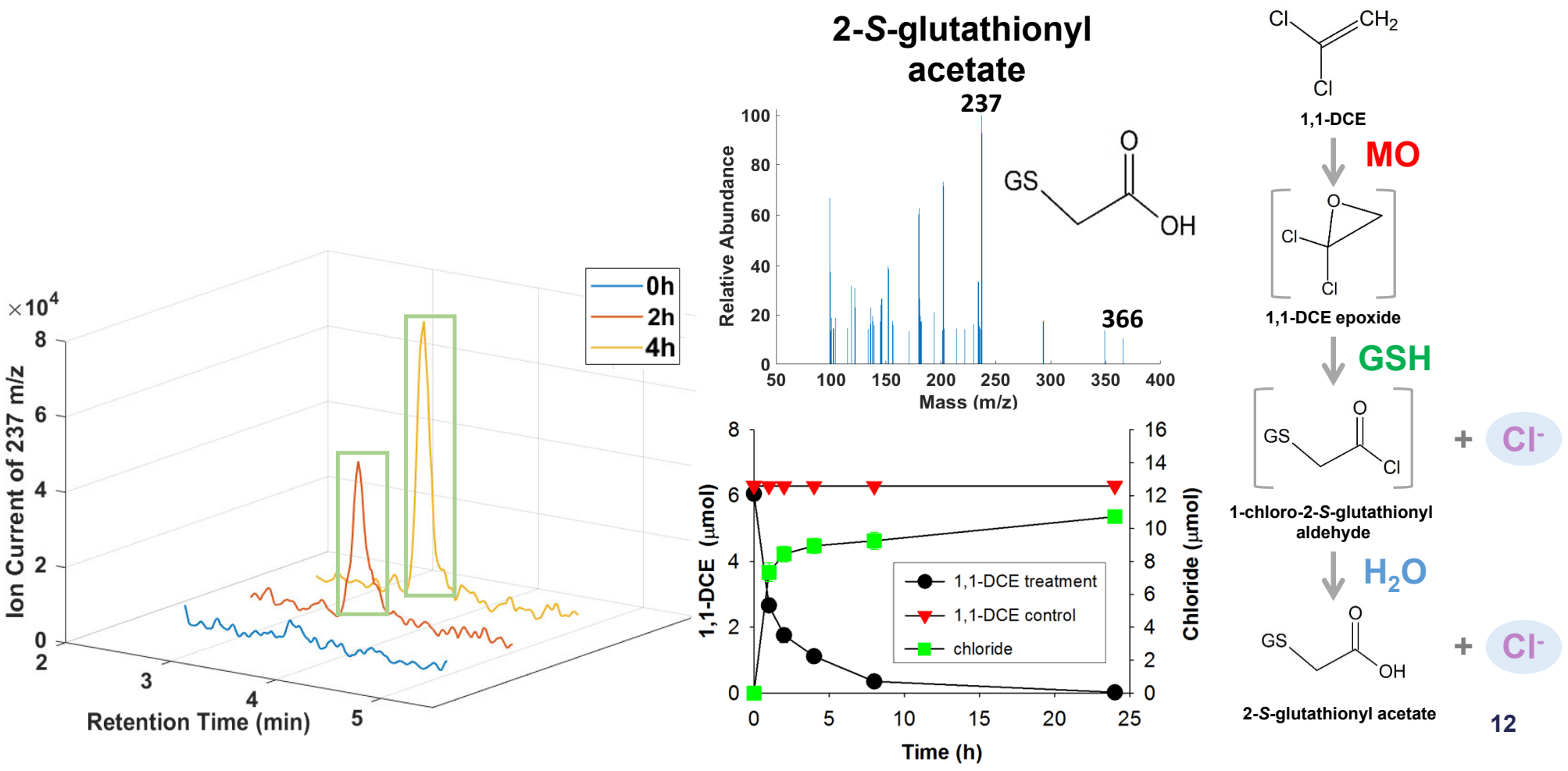
# Possible Oxidation Metabolites of 1,1-DCE



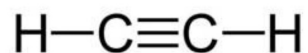
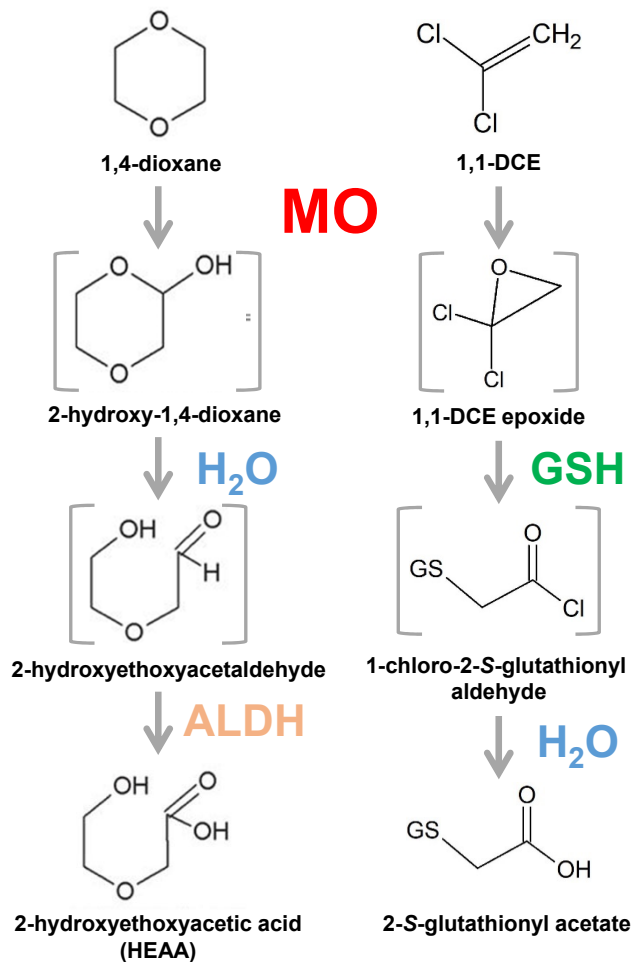
Simmonds et al., Drug Metab Dispos, 2004.



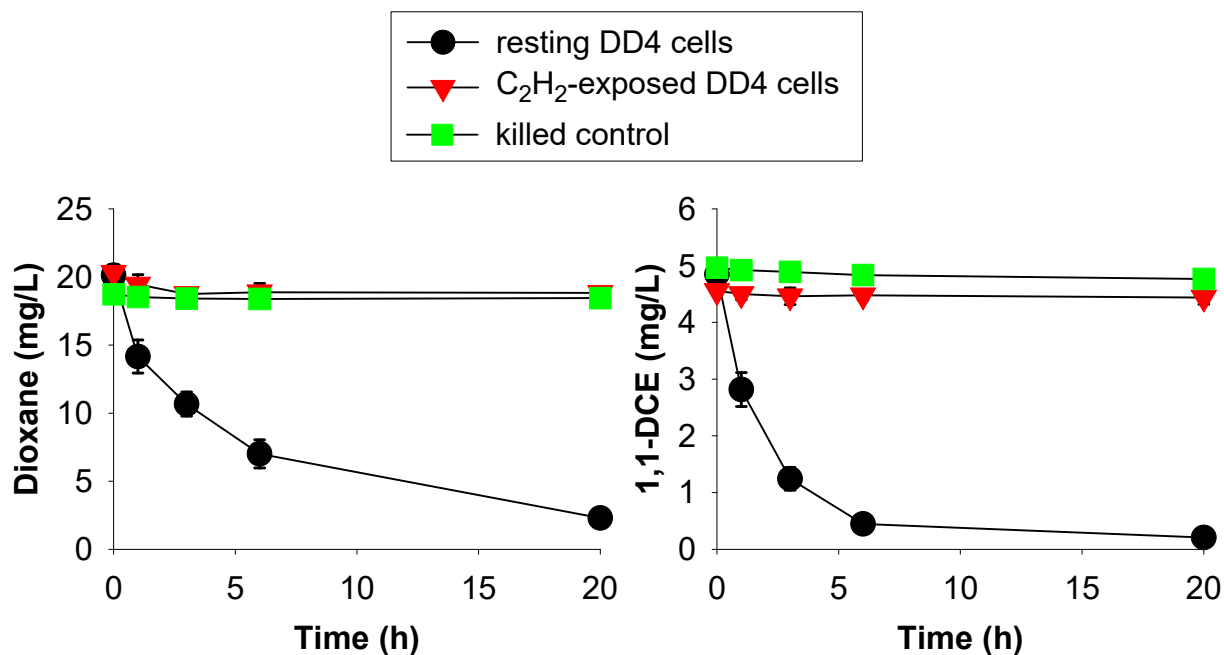
# LC/MS/MS Detected GSH-Conjugated Metabolite of 1,1-DCE Oxidation



# Bacterial Monooxygenase (MO) Is In Charge of Dioxane and 1,1-DCE Oxidation

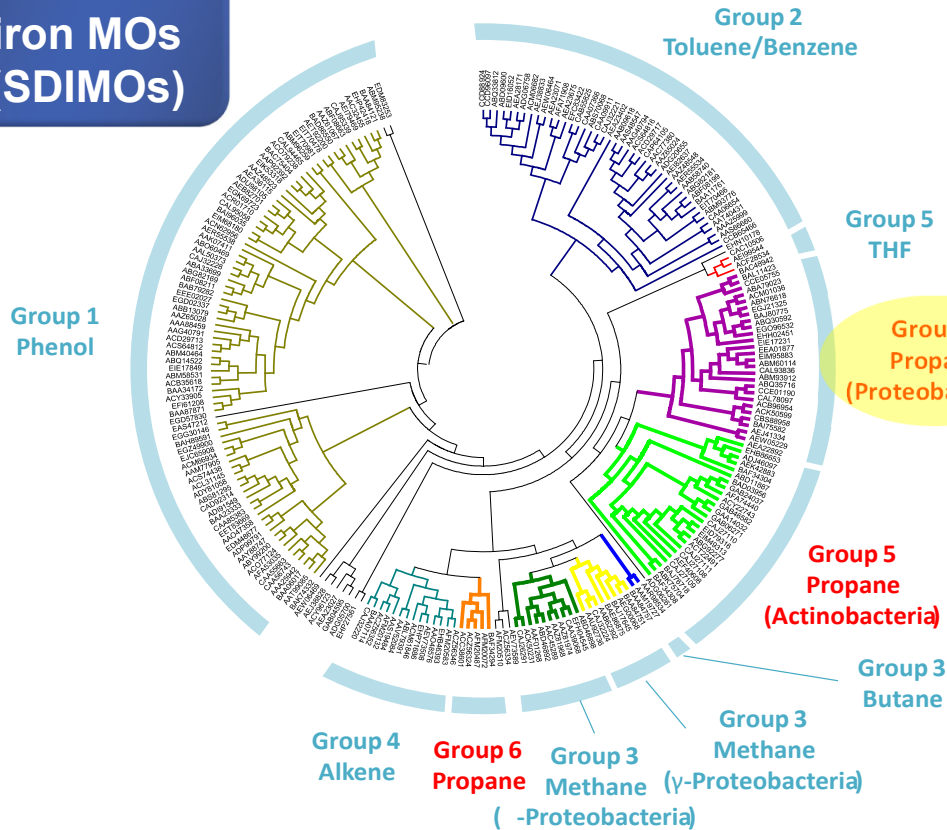


Acetylene ( $\text{C}_2\text{H}_2$ )  
an irreversible suicide  
substrate for MOs



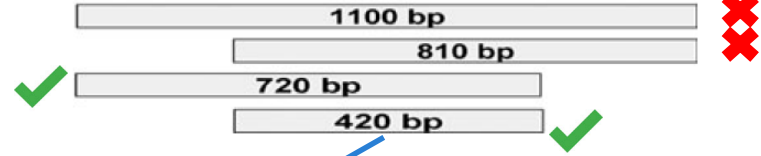
# A Putative Propane MO Is Uncovered in DD4

Soluble Di-iron MOs (SDIMOs)



Four SDIMO Degenerate Primers

SDIMO group	JS60 aa #	NVC65 122	NVC57 221	NVC66 352	NVC58 483
①	Py2 XamoA	:GMLDETRH	QTDEARHA	WLNQKYPNW	FNYRWAG
②	G4 TomA3	:OSIDELRH	QSDESRRH	WLSEKYPET	FDPEGSE
	OB3b MmoX	:OVLDEIRH	ETDELRRH	WFEANYPGW	WTLEDIK
③	Bath MmoX	:OVLDEIRH	ETDELRRH	WFEANYPGW	WTLDDIK
	Ps. but BmoX	:OVLDEIRH	ETDELRRH	WFEANYPGW	WTLDDLK
	JS60 EtnC	:OMLDEVRH	QSDESRRH	WFENHYPGW	WTLDDIR
	JS614 EtnC	:OMLDEVRH	QSDESRRH	WFENHYPGW	WTLDDIR
④	B276 AmoC	:OMLDEVRH	QSDEARRH	WFENHYPGW	WTLDDIR
	M156 PmoC	:OMLDEVRH	QSDESRRH	WFENNYPGW	WTLDDIR
	TY5 PrmA	:OMIDEVRH	QSDESRIH	WFEKYPGW	WTLDDVR
	Blr 3677	:QMVDEFRH	QSDESRIH	WFEHKYPGW	WTLDDVR
⑤	Rsp_2792	:QMVDEFRH	QSDESRIH	WFEQKYPGW	WTLDDVR
	Rge1 2823	:QMVDEFRH	QSDESRIH	WFEHKYPGW	WTLDHVR
	K1 ThmA	:QQLDEQRH	QSDEARRH	WFEISKYPGW	WTLDDLK

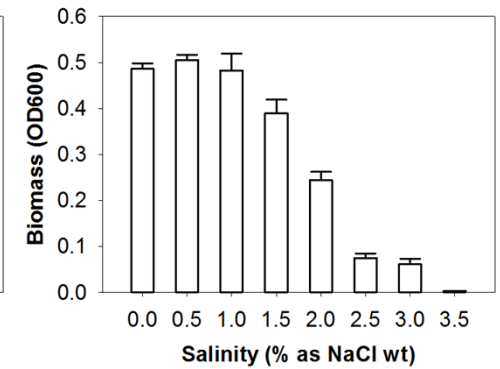
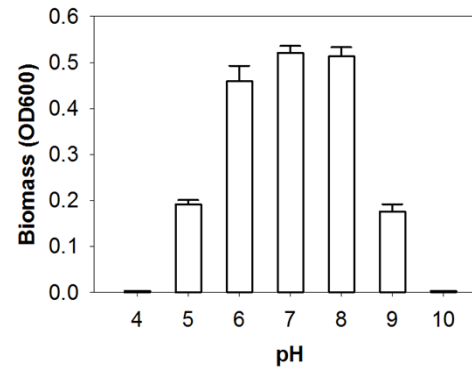
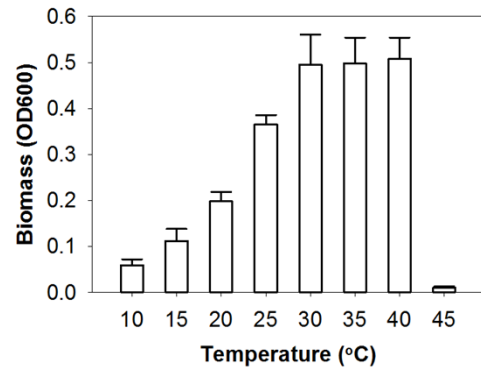


The deduced protein sequence is highly identical to those in Gram-negative *Burkholderia* sp. H160 (79%) and Gram-positive *Rhodococcus* sp. RHA1 (58%).

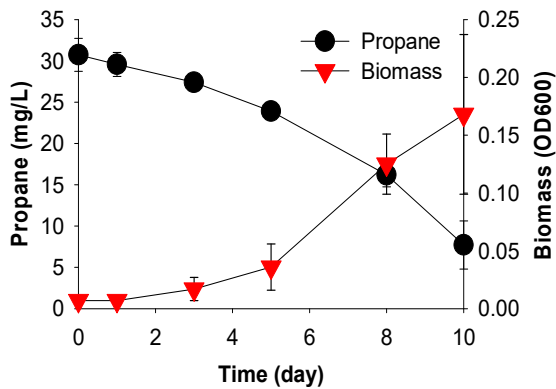
Notomista *et al.*, J. Mol. Evol., 2003; Coleman *et al.*, EM, 2006; Hand *et al.*, Sci. Total Environ. 2015.

# Superior Physiological and Ecological Properties of DD4 for Field Application

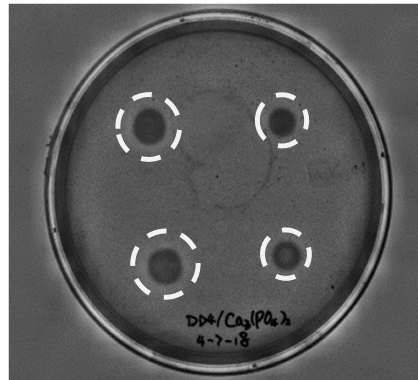
## Adaptation to Aquifer Conditions



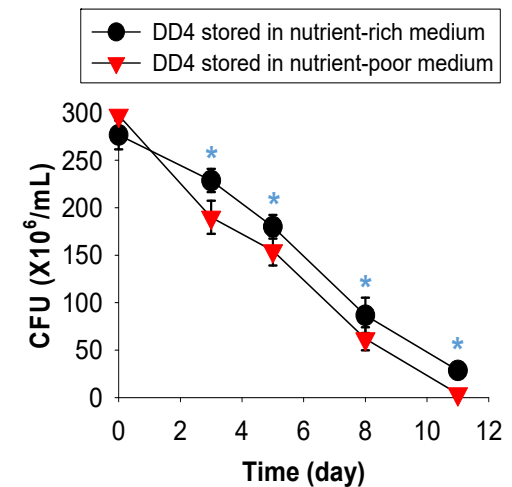
## N Fixation



## P Solubilization



## Durable Viability

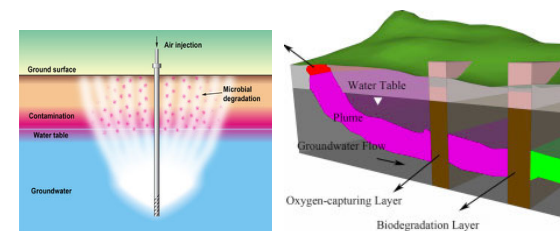


## Synchronic Biotransformation of 1,4-Dioxane and 1,1-Dichloroethylene by a Gram-Negative Propanotroph *Azoarcus* sp. DD4

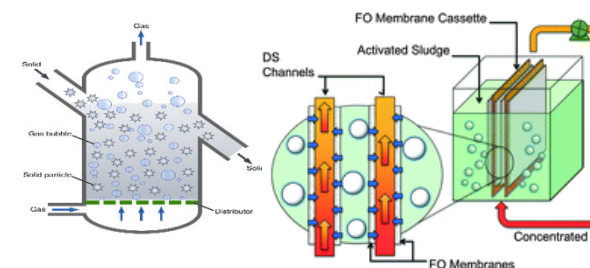
Daiyong Deng, Fei Li, Chen Wu, and Mengyan Li\*<sup>ORCID</sup>

Department of Chemistry and Environmental Science, New Jersey Institute of Technology, Newark, New Jersey 07102, United States

*in situ*



*ex situ*



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CHEMICAL & ENGINEERING NEWS

POLLUTION

## Persistent pollutant broken down by sludge microbe

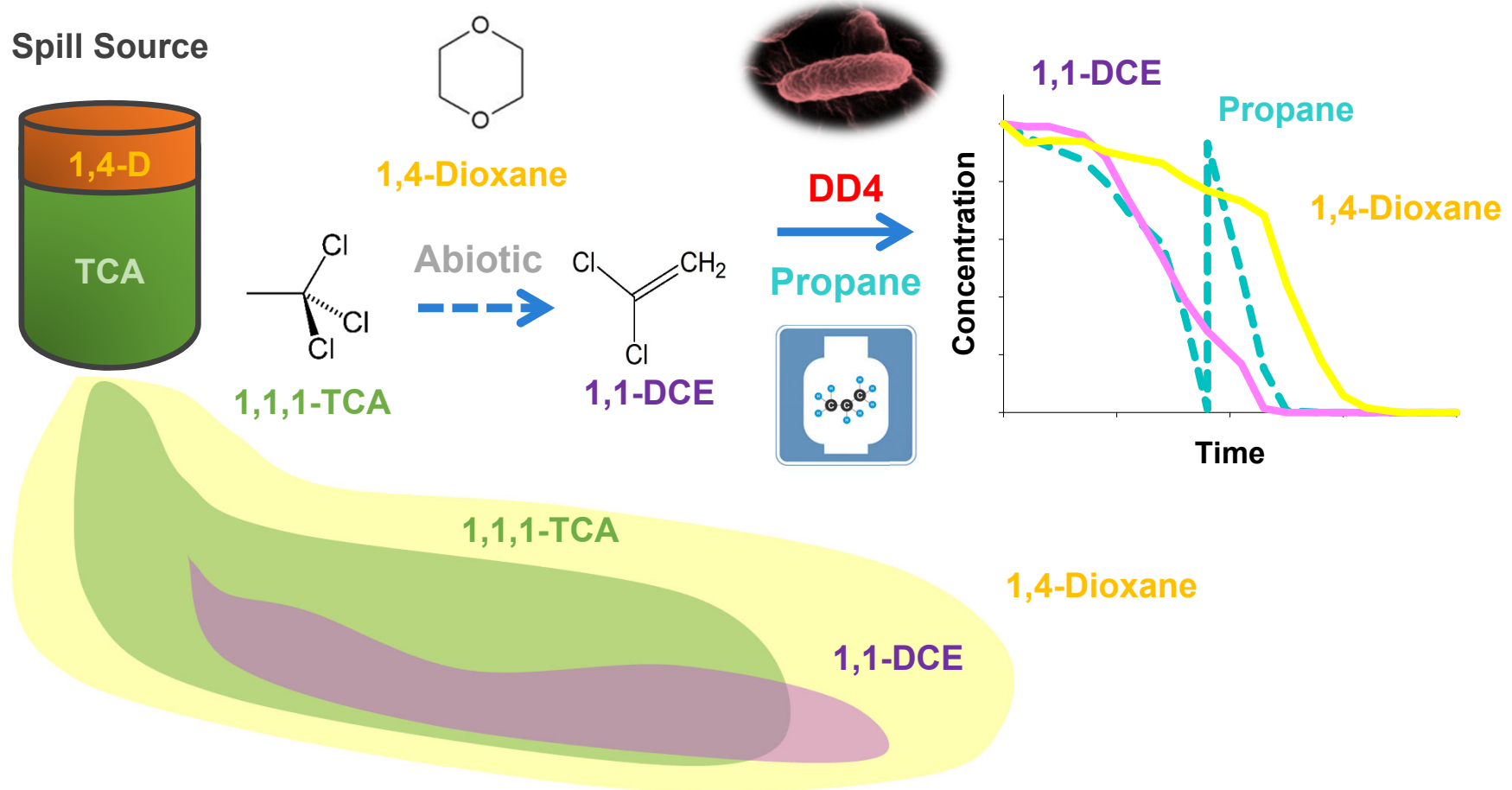
Pilot demonstrations at sites contaminated with dioxane could happen as early as next year

by *Cici Zhang*

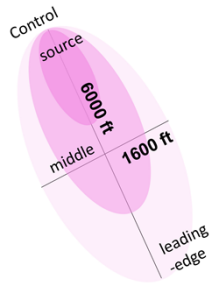
AUGUST 2, 2018 | APPEARED IN VOLUME 96, ISSUE 32



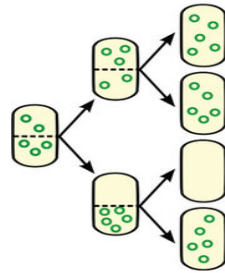
# Take Home Message: DD4 is a Superior Microbe Suited for Mitigating Dioxane and 1,1-DCE Co-Contamination



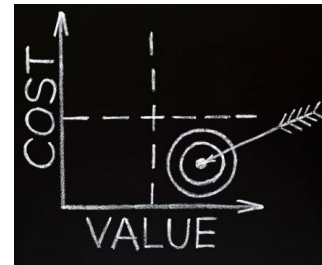
# Advantages of Bioremediation with DD4



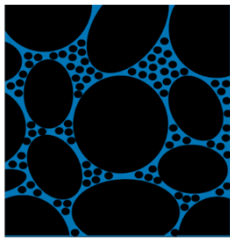
**Effective Decontamination**



**Sustained Activity**



**Cost Efficiency**



**Ease of Implementation**

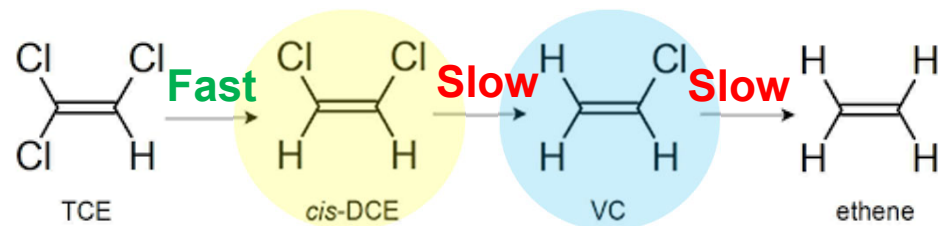
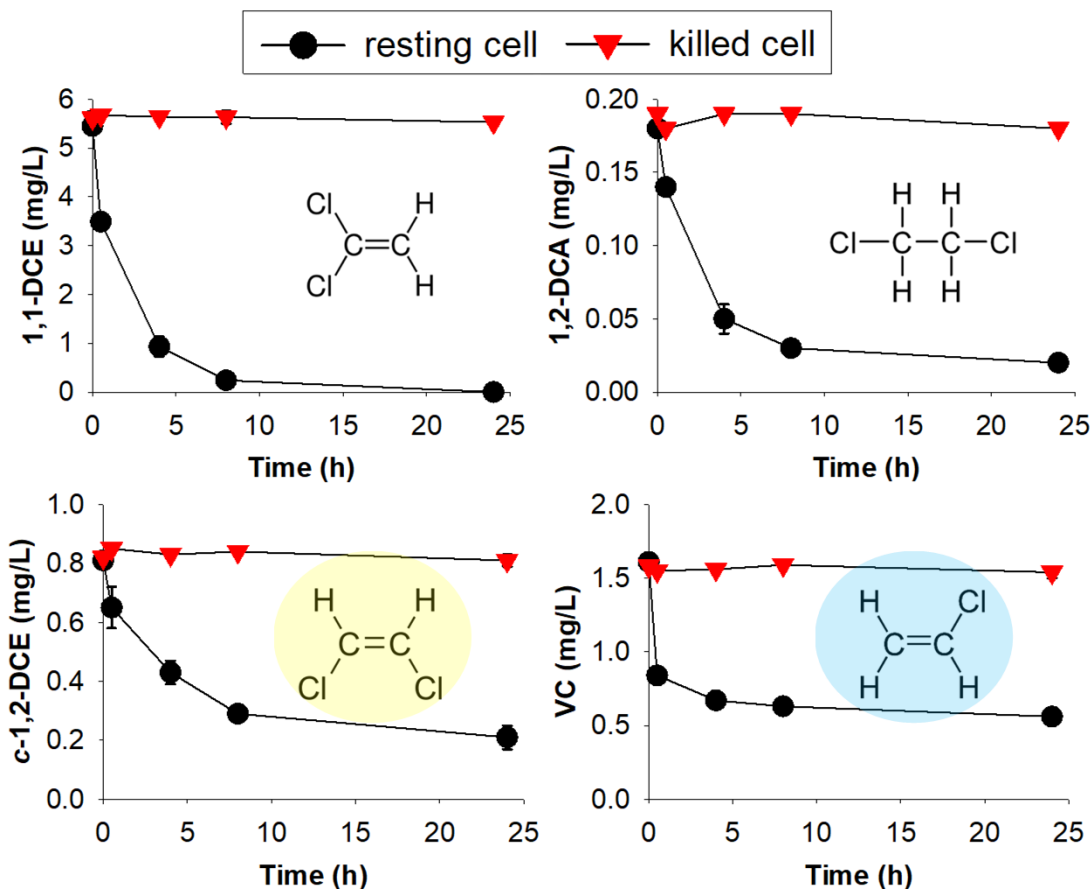


**Ecological Friendliness**



**Environmental Safety**

# DD4 Degrades a Variety of Co-occurring Chlorinated Compounds



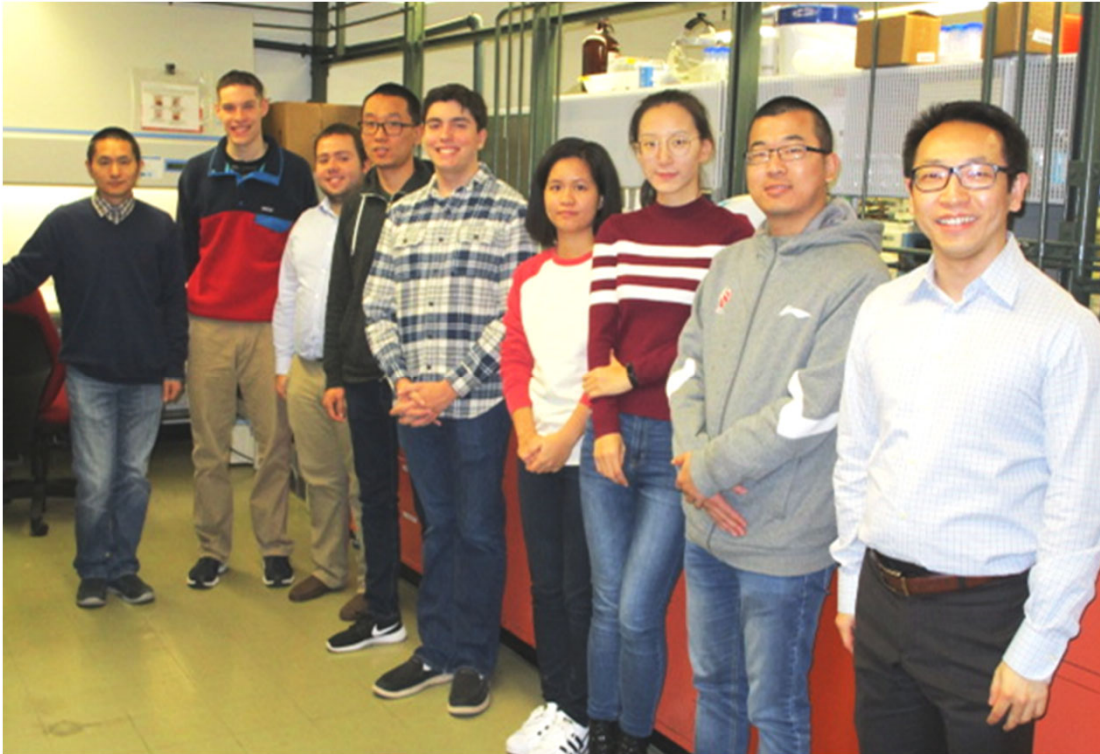
DD4 can be used to remove *cis*-DCE and VC that commonly accumulate with reductive dechlorination of TCE.

Deng et al, in preparation; Li et al., in preparation.

# Welcome to Our Platforms/Posters

Title	Presenter	Location and Time
Concurrent Biodegradation of 1,4-Dioxane and 1,1-Dichloroethylene by a Gram-Negative Propanotroph <i>Azoarcus</i> sp. DD4.	Mengyan Li NJIT	Holiday Ballroom 5 Wednesday 1:50 pm.
Effective Removal of Contaminants of Emerging Concern by Biologically-Active Filters.	Mengyan Li NJIT	Holiday Ballroom 3 Thursday 1:00 pm.
Comparison of Catalytic Behaviors between Two 1,4-DioxaneDegrading Monooxygenases.	Fei Li NJIT	Holiday Ballroom 5 Wednesday 10:30 am.
1,4-Dioxane Bioaugmentation during and after Anaerobic Degradation.	Lingke Zeng LANGAN	Holiday Ballroom 5 Wednesday 2:50 pm.
1,4-Dioxane Contamination Survey at River Estuaries and Wastewater Treatment Plants in Northern NJ.	Fei Li NJIT	Exhibit Hall No.70 Monday/Tuesday
Substrate-Mediated Biotransformation and Biodefluorination of 6:2 FTOH by <i>Mycobacterium</i> and <i>Rhodococcus</i> Species.	Chen Wu NJIT	Exhibit Hall No.8 Monday/Tuesday

# Acknowledgements



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