National Institute of Environmental Health Sciences
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SRP Progress in Research Webinar Series

Utilizing Innovative Materials Science Approaches to Enhance Bioremediation: Session II - Chlorinated Compounds

Project

Enhancing bioremediation of groundwater co-contaminated by chlorinated volatile organic compounds (CVOCs) and 1,4-dioxane using novel macrocyclic materials

Presenters

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April 29th, 2022

Team

Research team

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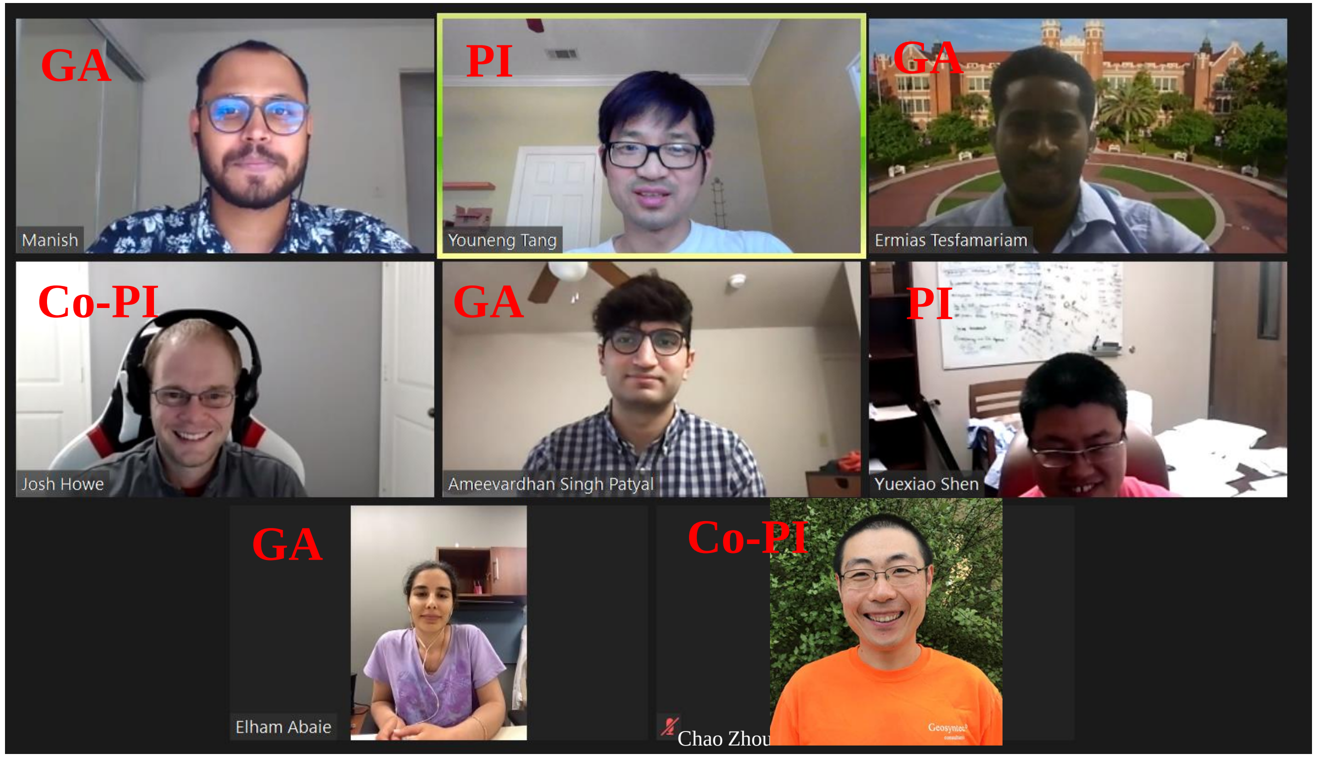


Senior advisors

Neal Durant, Geosyntec

Duane Graves, Geosyntec/ SiREM

Jim Cummings, EPA

Technical support

SiREM

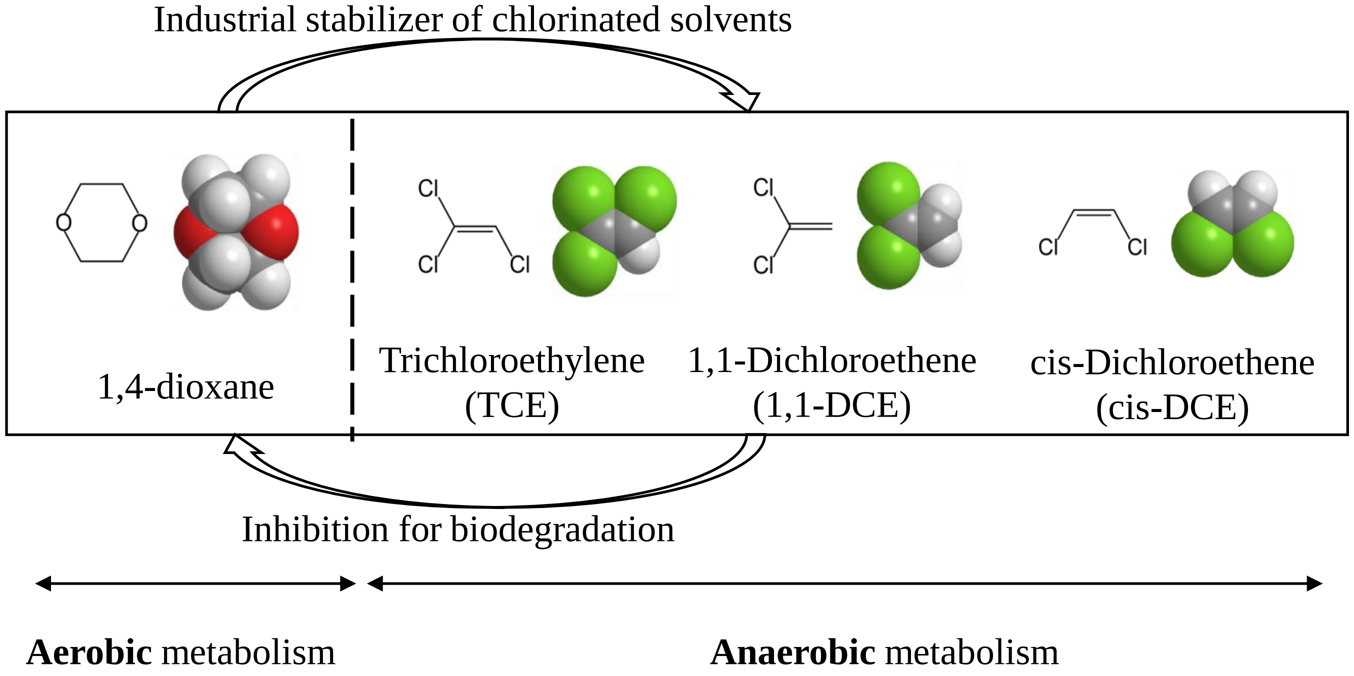
UIC

Problems and Solution

<u>Problem #1</u>: Low 1,4-dioxane concentration

<u>Problem #2</u>: CVOCs inhibit 1,4-dioxane biodegradation

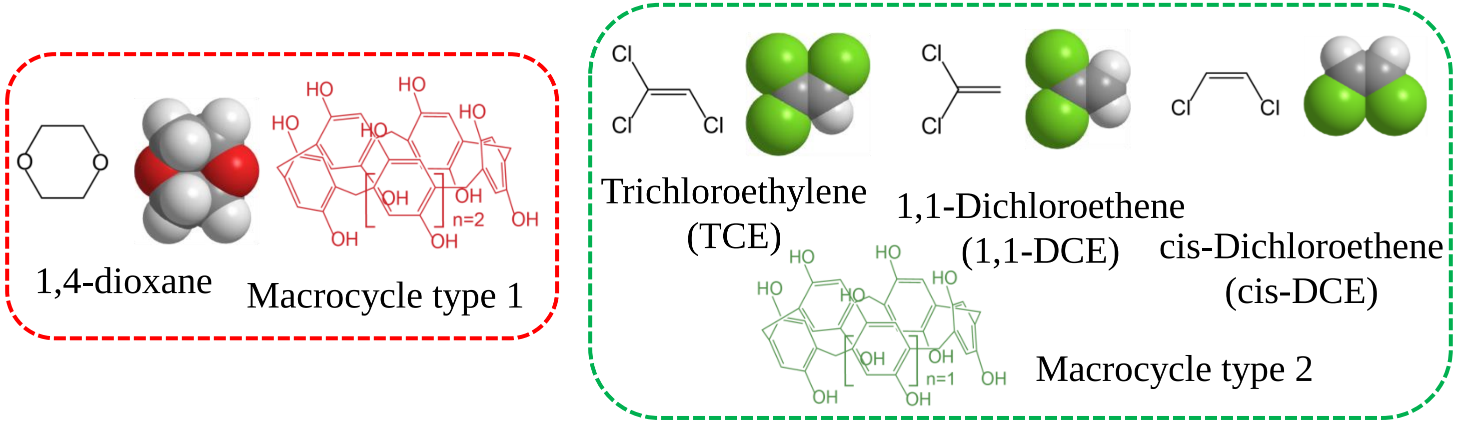
<u>Problem #3:</u> Opposite environmental conditions for biodegradation



Solution:

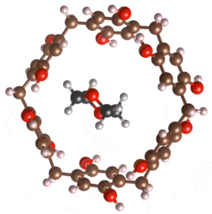
<u>Component #1</u>: Cultures that efficiently degrade 1,4-dioxane at low concentrations

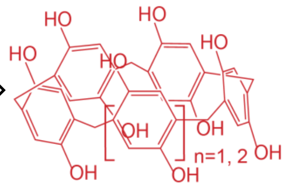
<u>Component #2</u>: Novel macrocyclic sorbents



Technical Approach

Aim #1: Material research (TTU)

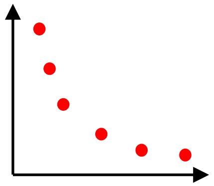




Simulation Synthesis Characterization

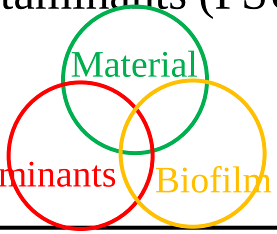
Aim #2: 1,4-Dioxane biodegradation research (FSU)



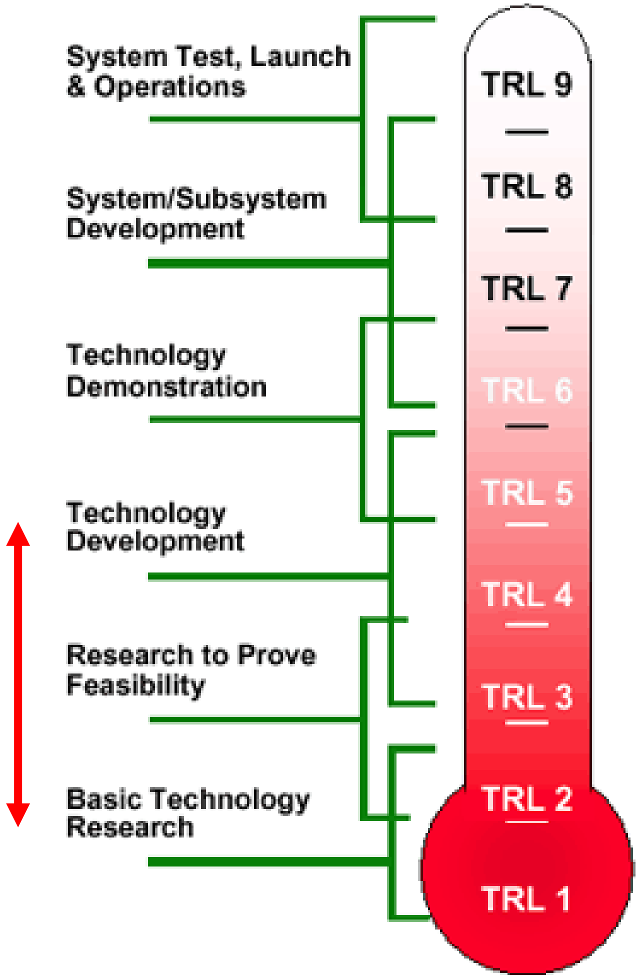


Enrichment Isolation Characterization

Aim #3: Interactions among material, biofilm, and contaminants (FSU & TTU)



Aim #4: Column studies (FSU & Geosyntec)



NASA technology readiness levels

Specific Aim 1: Novel macrocyclic materials modeling, synthesis, optimization and characterization

- Examined conformer stability and statistical distribution at ambient conditions. The most abundant (C7) conformer was used to model Pillar[6]arene (as shown in Table 1 and Figure 1).

- Probed for transport barriers relating to adsorption in different test macrocycles (as shown in Figure 2).

- Analyzed materials based on strength of adsorption CVOCs and 1,4-dioxane on Pillararene-based macrocycles (as shown in Table 2).

Conformer of P6A	C1	C2	C3	C4	C5	C6	C7	C8
Relative Energy to ground state C7 (kJ/mol)	61.8	44	27.4	29.8	15.2	46.8	0	18.8
Population per billion at 298 Kelvin	0	113	41,554	35,463	6,340,558	19	**990,663,288**	2,919,006

Table 1 – Statistical (thermal/energetic) abundance of pristine Pillar[6]arene (P6A) conformers at 298K.

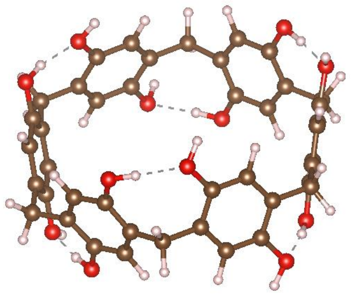


Figure 1 – C7 conformer of Pillar[6]arene (P6A).

Figure 2 – Adsorption path energetics for 1,4-dioxane on a primary amine-substituted Pillar[6]arene (P6A) macrocycle.

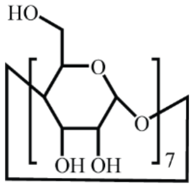
	Pristine P6A	Dimethoxy P6A	P6A with primary amine	P6A with carboxylic acid	P6A with methylbromide
1,1-dichloroethylene	-44.77	-61.74	-66.03	-63.52	-66.97
Cis-dichloroethylene	-48.08	-62.44	-66.54	-68.51	-69.66
Trichloroethylene	-43.15	-69.85	-70.84	-73.52	-74.54
1,4-Dioxane	-59.69	-78.95	-82.30	-81.91	-85.20

Table 2 – Binding Energies (in kJ/mol) for 1:1 adsorption of adsorbate on Pillar[6]arene (P6A)-based macrocyclic materials. (More negative value represents stronger exothermic adsorption)

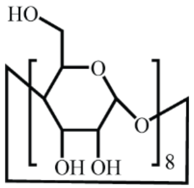
Specific Aim 1: Novel macrocyclic materials modeling, synthesis, optimization and characterization

Monomers:

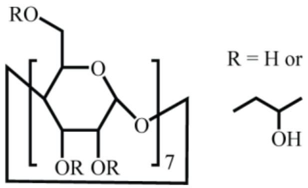
β-Cyclodextrin



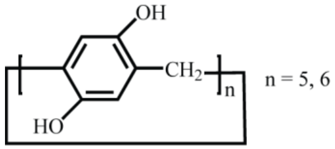
γ-Cyclodextrin



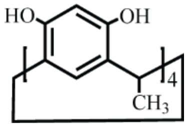
2-Hydropropyl β-Cyclodextrin



Pillar[n]arene

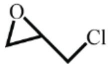


Resorcinarene

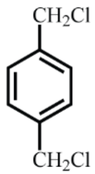


Crosslinkers:

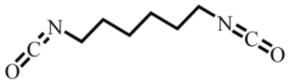
Epichlorohydrin
(EPI)



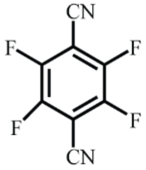
α,ά-Dichloro-p-xylene
(DCX)



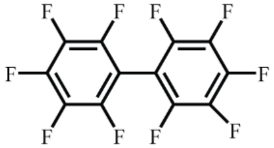
1,6-Hexamethylene diisocayanate
(HDI)



Tetrafluoroterephthalonitrile
(TFN)



Decafluorobiphenyl
(DFB)



Features:

Neutral

Neutral

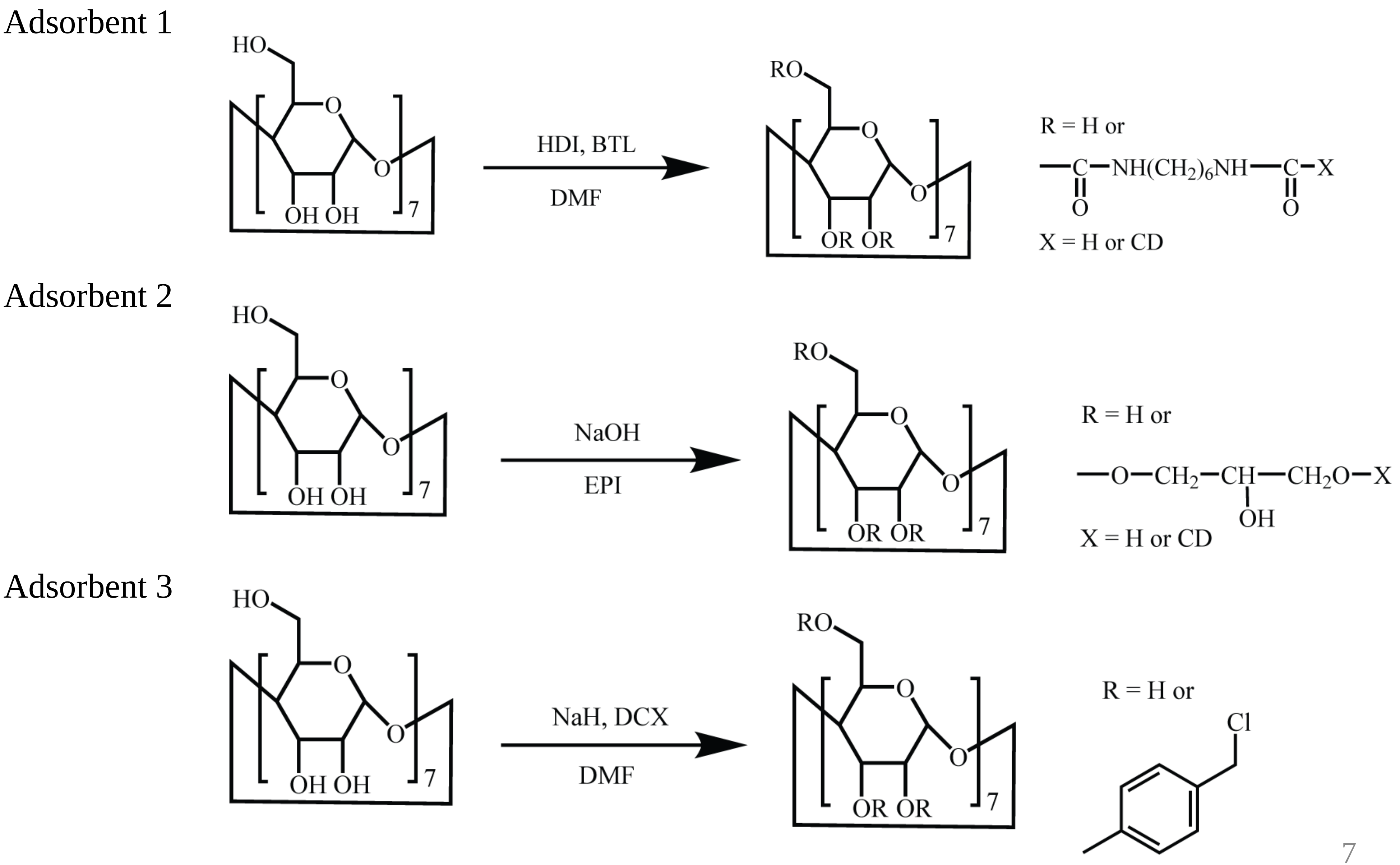
Anionic

Fluorinated

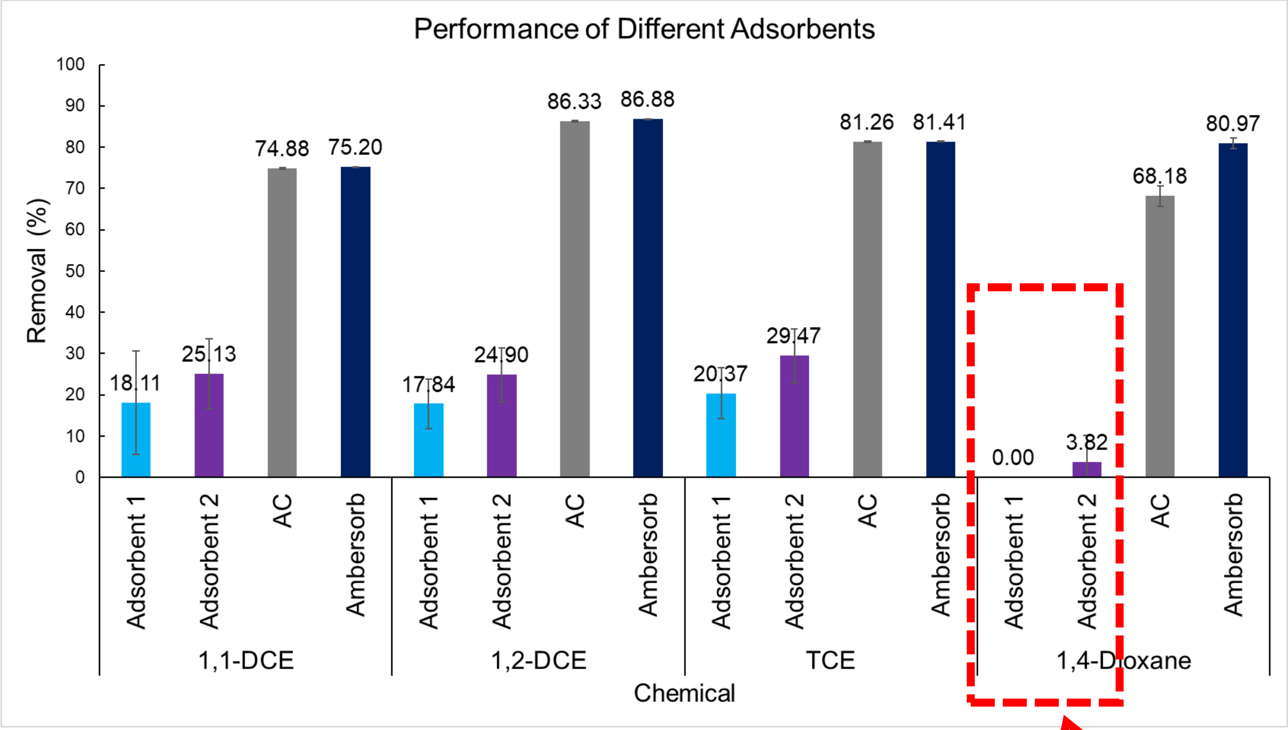
Long-chain fluorinated

Specific Aim 1: Novel macrocyclic materials modeling, synthesis, optimization and characterization

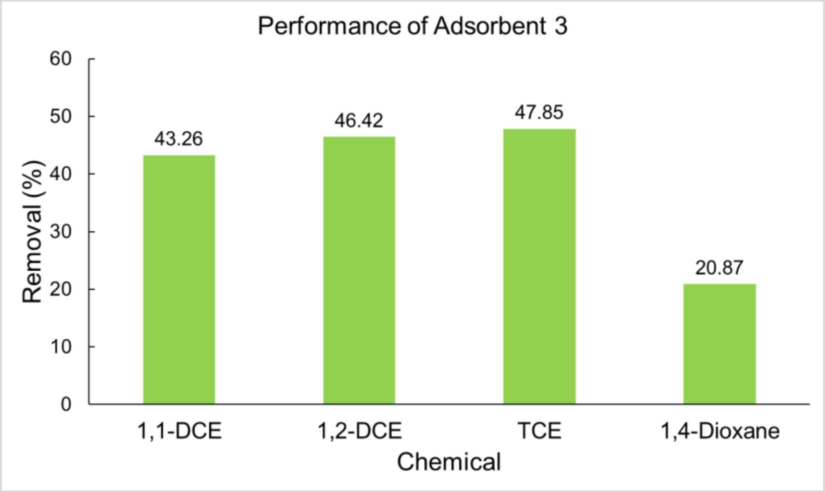
β-Cyclodextrin adsorbents



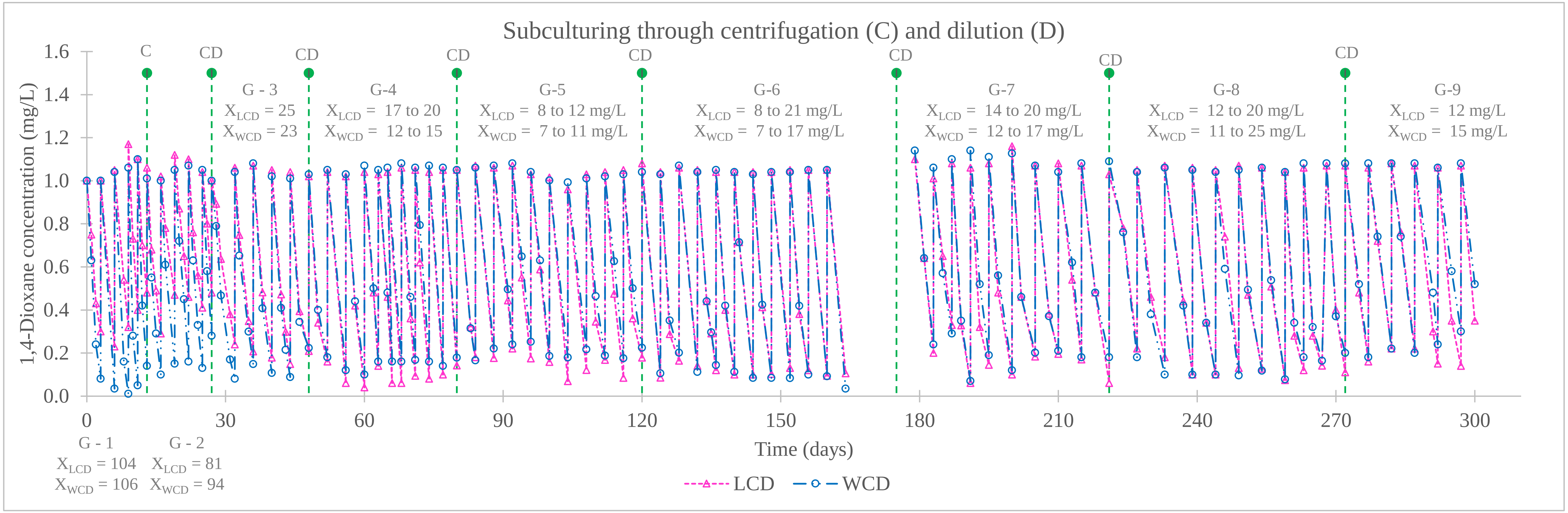
Specific Aim 1: Novel macrocyclic materials modeling, synthesis, optimization and characterization



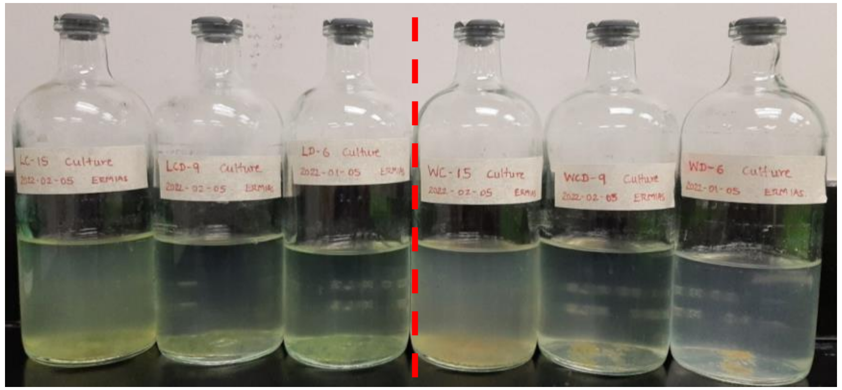
We observed no removal of 1,4-dioxane, meaning selective removal of CVOCs



Specific Aim 2: Mechanistic study of a highly efficient 1,4-dioxane-metabolizing culture: enrichment, isolation, and characterization

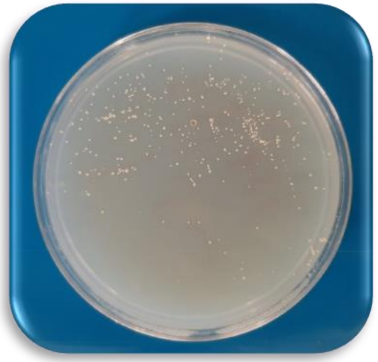


Landfill leachate



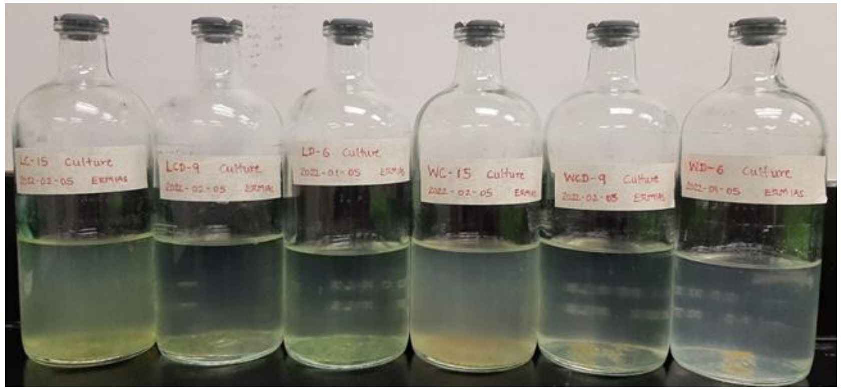
Activated sludge

Specific Aim 2: Mechanistic study of a highly efficient 1,4-dioxane-metabolizing culture: enrichment, isolation, and characterization



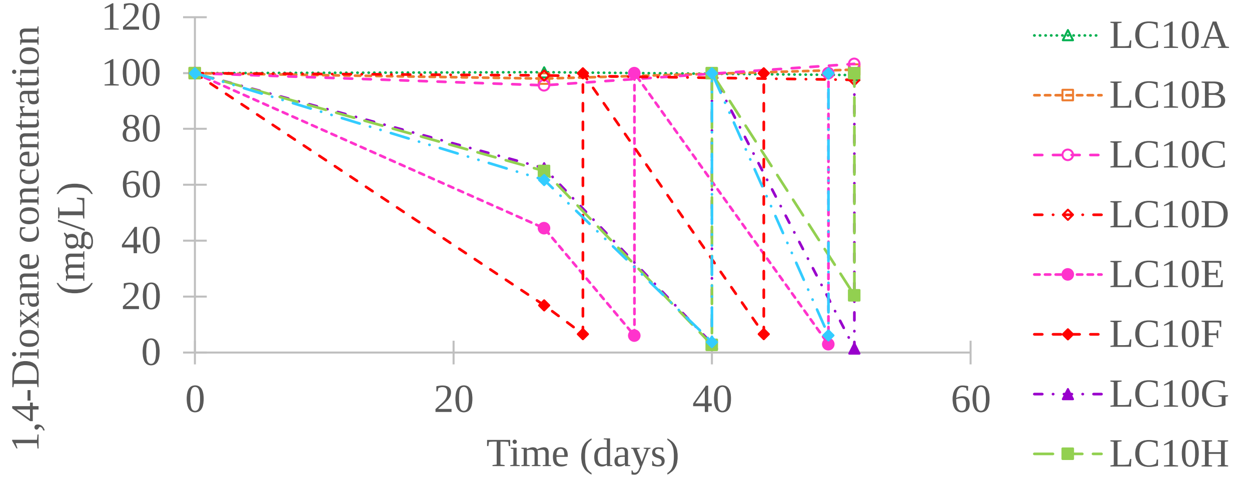
Agar plating
(**18 plates**)

Isolated cultures
(**54 cultures**)



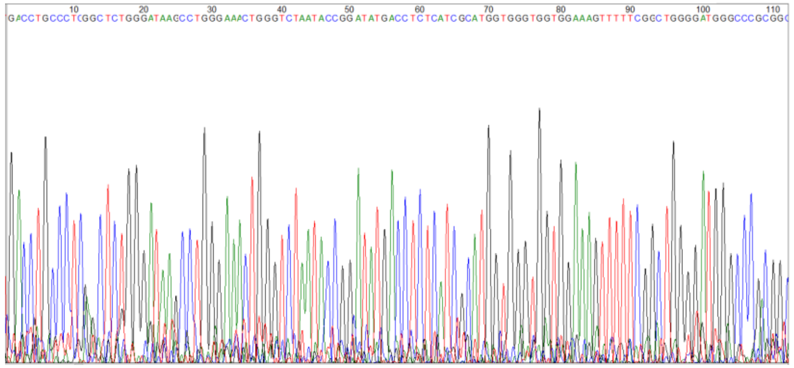
Mixed cultures
(**6 cultures**)

Testing isolated cultures for 1,4-dioxane degradation

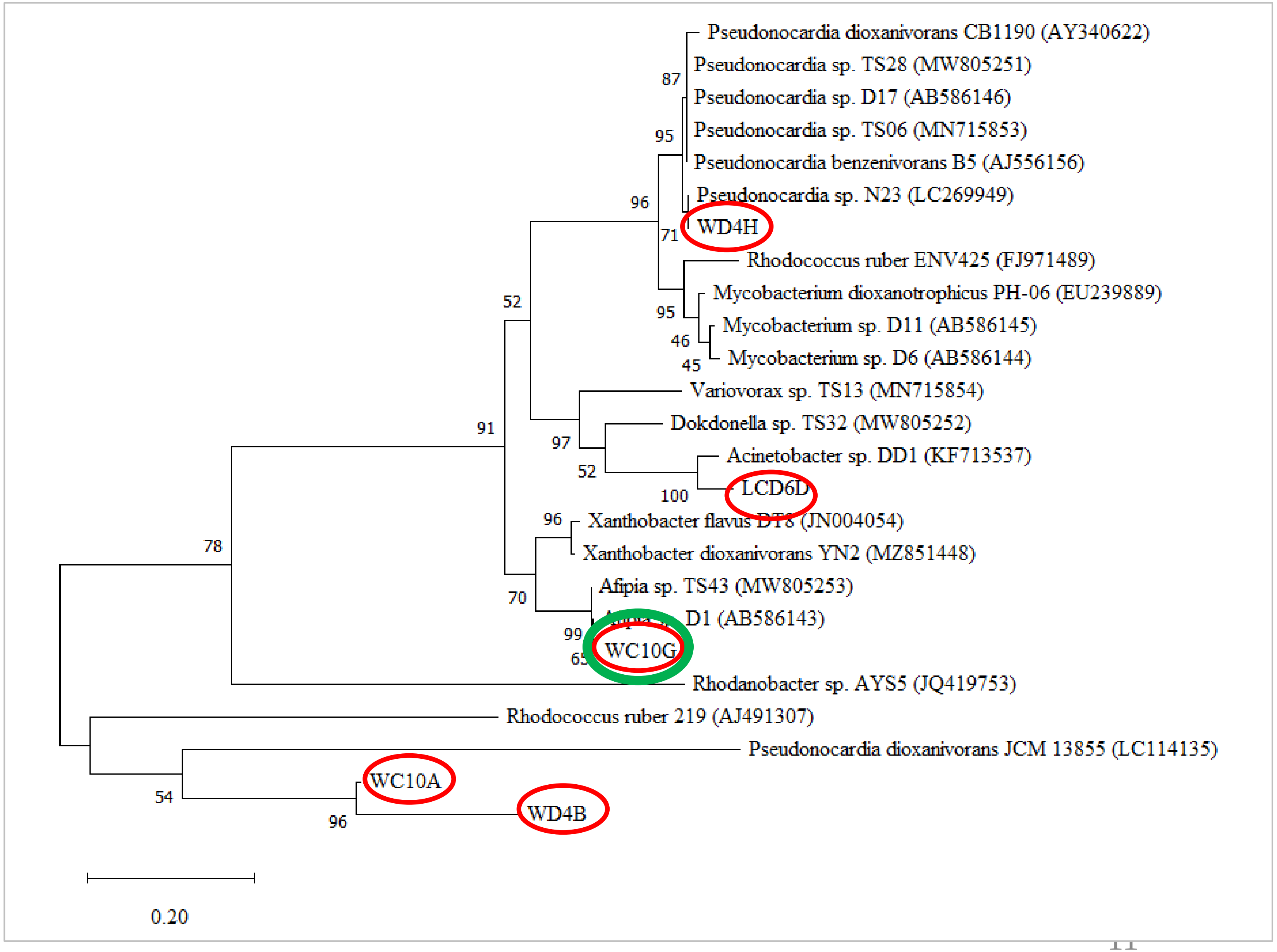


1,4-Dioxane degradation confirmation
(**85%** of the 54 isolated colonies could degrade 1,4-dioxane)

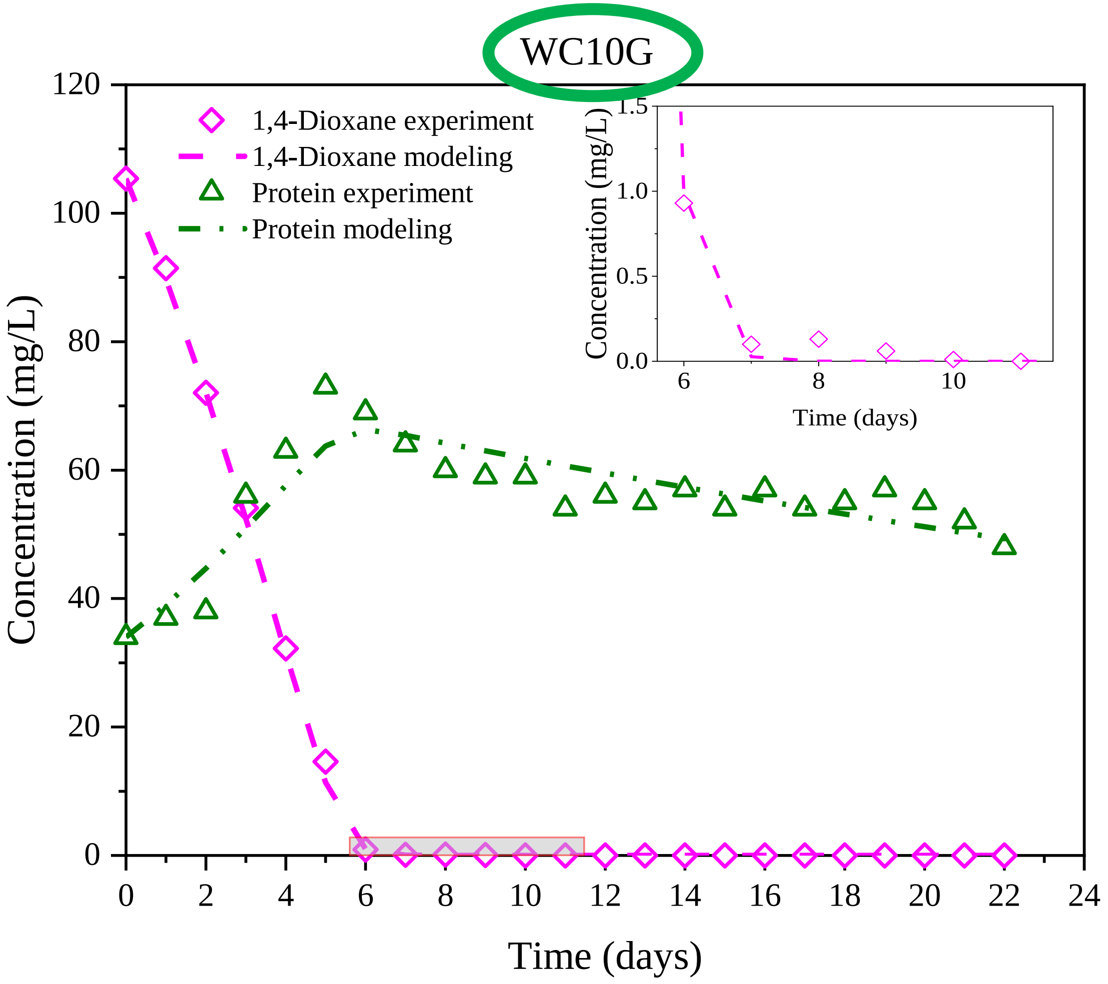
Specific Aim 2: Mechanistic study of a highly efficient 1,4-dioxane-metabolizing culture: enrichment, isolation, and characterization



Pure culture identification
(**5 cultures** identified so far)



Specific Aim 2: Mechanistic study of a highly efficient 1,4-dioxane-metabolizing culture: enrichment, isolation, and characterization



1,4-dioxane degradation:

$$\frac{dS_d}{dt} = -X\, q_d \left[\frac{S_d}{K_s + S_d}\right]$$

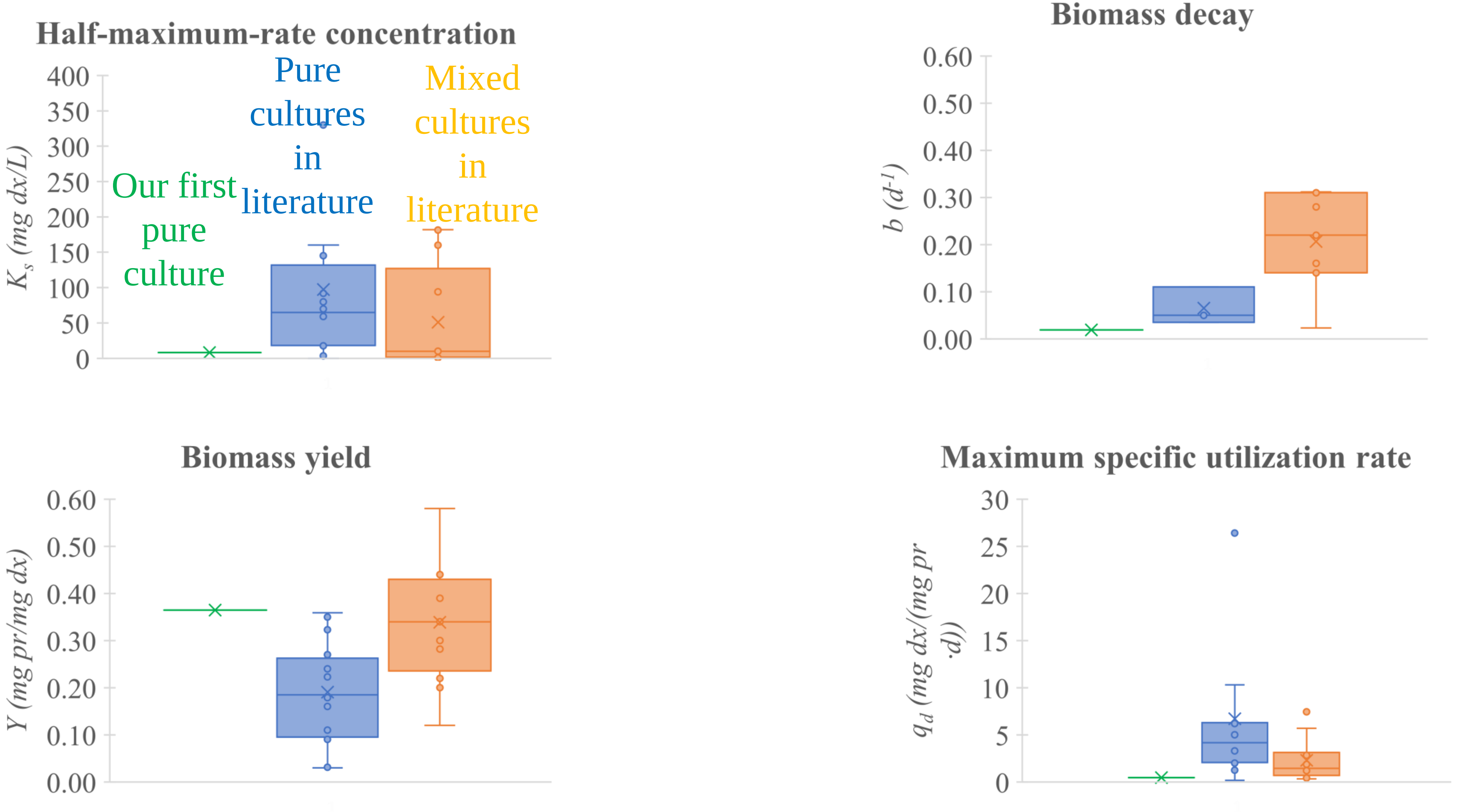
Biomass growth:

$$\frac{dX}{dt} = Y\, X\, q_d \left[\frac{S_d}{K_s + S_d}\right] - b\, X$$

Pure culture	q_d *(mg dx/mg pr · d)*	K_s *(mg dx/L)*	*Y (mg pr/mg dx)*	*b* (d^{-1})
WC10G	0.47	8.24	0.36	0.02

Specific Aim 2: Mechanistic study of a highly efficient 1,4-dioxane-metabolizing culture: enrichment, isolation, and characterization

Comparison with literature



Good fit for 1,4-dioxane degradation at low concentrations

Conclusions

- **Specific Aim 1**
  - **Material modeling**: Macrocycles tend to exist in one conformer that dominates at relevant conditions. Adsorption shows no transport barrier, indicating energetics can drive selectivity. Pillar[6]arene and its variants are predicted to be weakly selective for 1,4-dioxane over CVOCs.
  - **Material synthesis and characterization**: β-Cyclodextrin adsorbents showed strong selectivity towards CVOCs in a mixture of 1,4-dioxane and CVOCs; they adsorbed CVOCs but not 1,4-dioxane.

- **Specific Aim 2**
  - **Microbial enrichment:** We enriched six mixed cultures through feeding 1,4-dioxane at a low concentration.
  - **Microbial isolation and characterization:** We identified five pure cultures and characterized one pure culture (WC10G), which seems a good fit for degrading 1,4-dioxane at low concentrations: low K_s, b, q_d, and high Y.

Progress

Specific Aims

Year 1	Year 2	Year 3	Year 4	Year 5

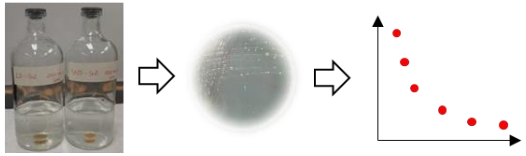
Aim #1: Material research (TTU)

Simulation Synthesis Characterization

Have synthesized material that selectively adsorbs CVOCs

Will synthesize material that selectively adsorbs 1,4-dioxane

Aim #2: 1,4-Dioxane biodegradation research (FSU)

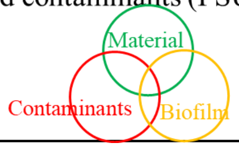


Enrichment Isolation Characterization

Have enriched six mixed cultures, isolated 54 pure cultures, characterized 1 efficient pure culture

Will characterize more pure cultures

Aim #3: Interactions among material, biofilm, and contaminants (FSU & TTU)



Aim #4: Column studies (FSU & Geosyntec)

Acknowledgement

Research reported in this presentation was supported by the National Institute Of Environmental Health Sciences of the National Institutes of Health under **Award Number R01ES032692**. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.