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Novel Organism Deployed for *In Situ* Bioremediation of 1,4-Dioxane in Groundwater

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2024 Chlorinated Conference
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Transformative Biology for 1,4-Dioxane Degradation

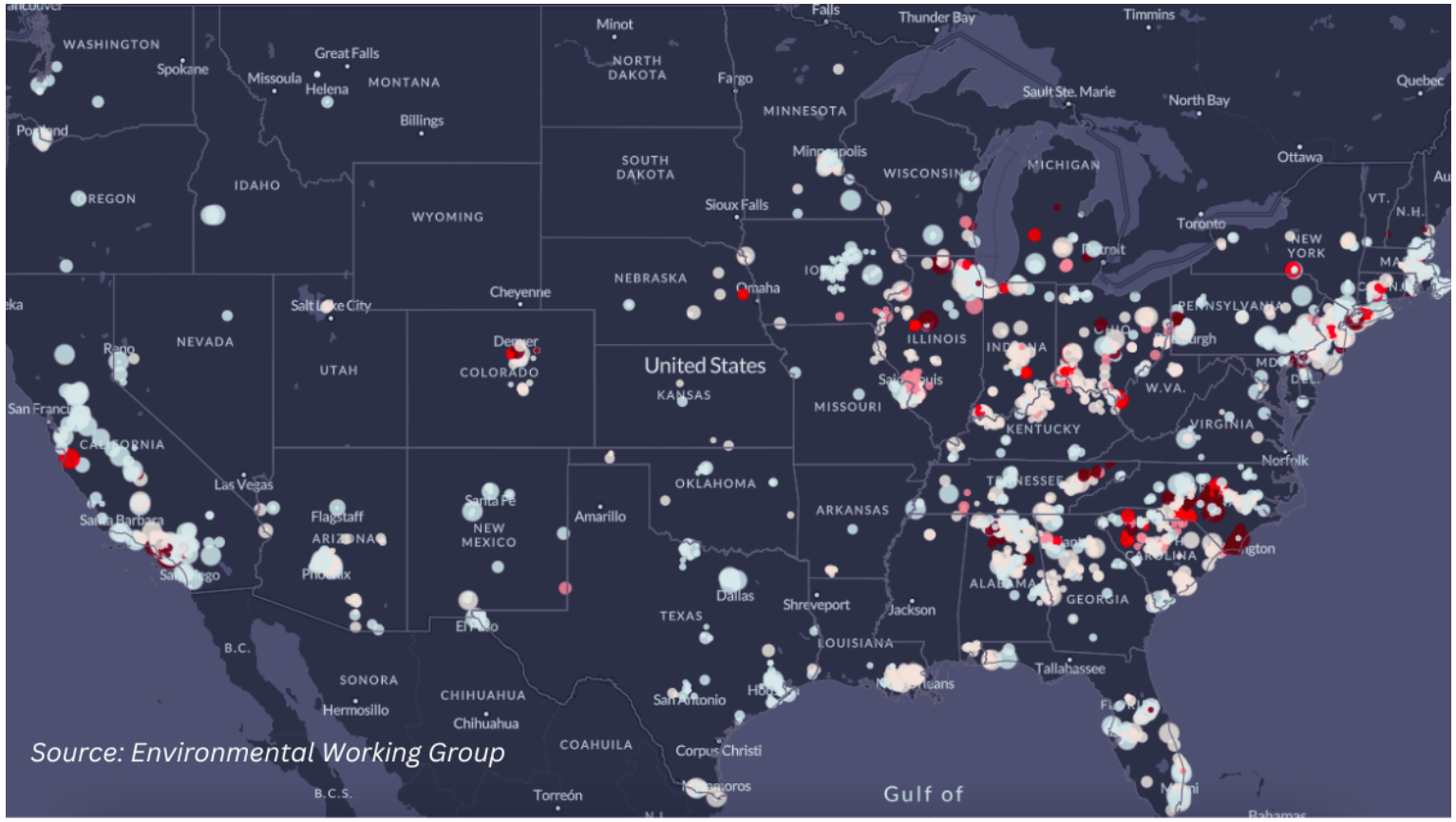
DISCOVER — BIOINFORMATICS

DEVELOP — TRANSFORMATIVE BIOLOGY

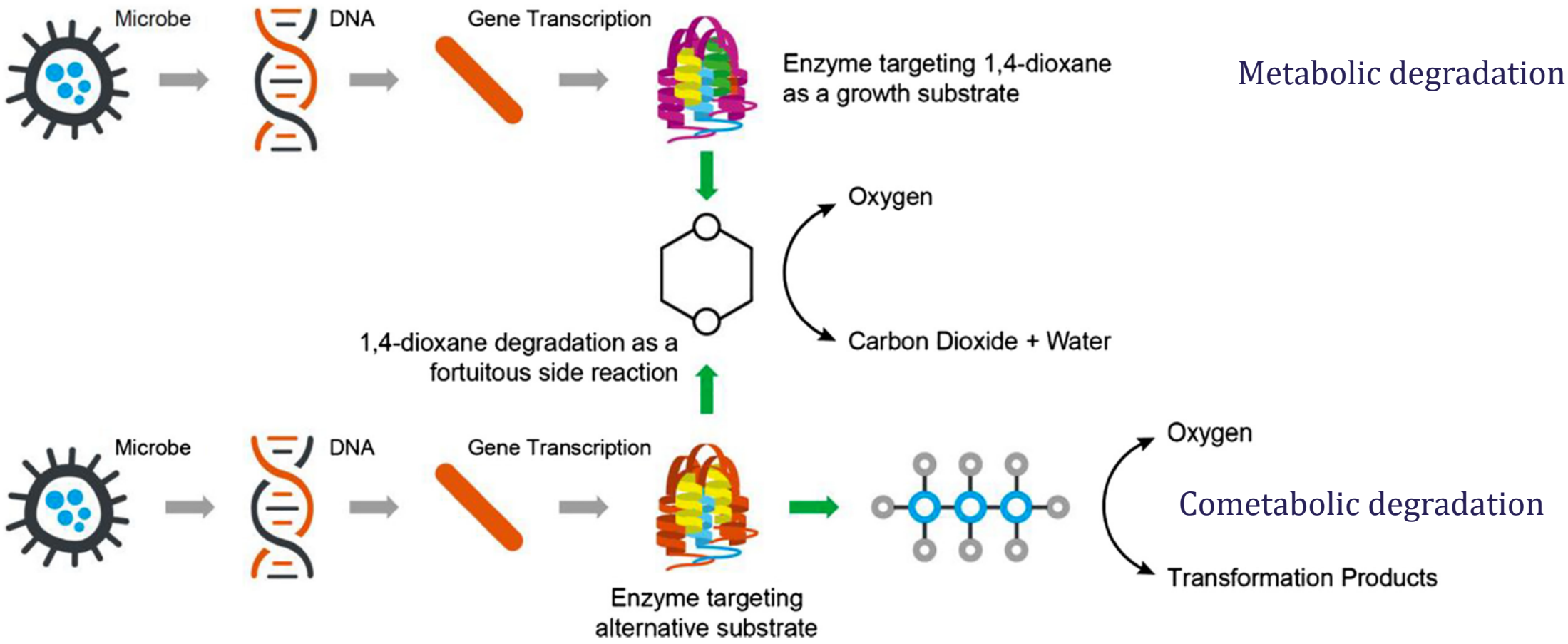
DEPLOY — INTEGRATED SYSTEMS

1,4-Dioxane: An Emerging Contaminant Found in More Than 10% of US Drinking Water

- A chemical that was widely used for industrial chemical processes
- Impacting over 90 million people in the US; >1,900 utilities
- EPA Dioxane Health Reference Level (HRL): 0.35 to 35 µg/L
- Like PFAS, it is slow to degrade, becoming a persistent environmental problem



Biodegradation of 1,4-Dioxane



Horst, J.F., Bell, C.H., Lorenz, A., Heintz, M., Miao, Y., Saling, J., Favero, D. and Mahendra, S. (2019)

Challenges with Cometabolism (and Chemical Oxidation)

In situ mixing

Potentially hazardous substrates

Rapid substrate utilization

Oxygen depletion

Cost

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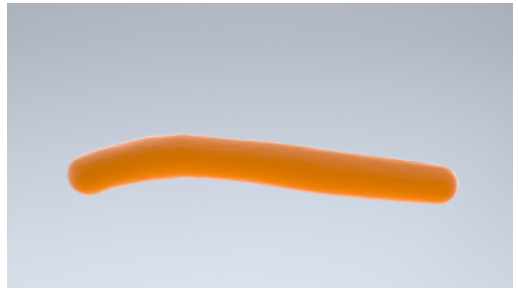
Transformative Biology for 1,4-Dioxane Degradation

DISCOVER

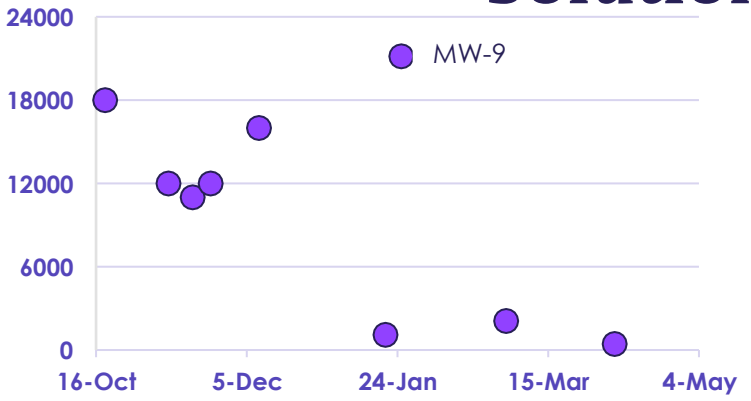
BIOINFORMATICS

1,4 D-Stroy

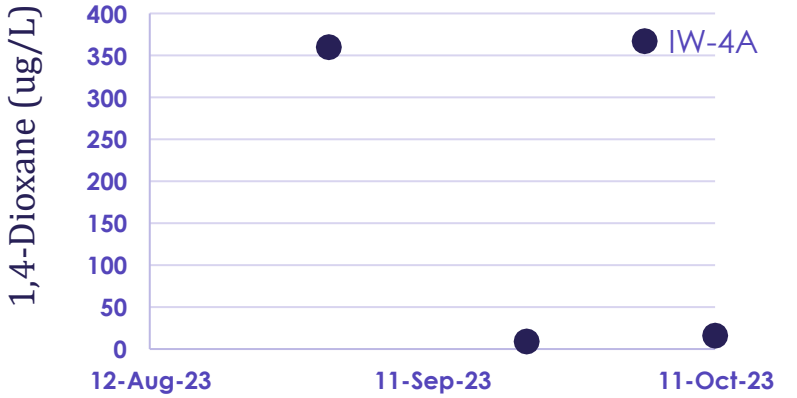


- Gram positive aerobic bacterium
- Naturally occurring and non-sporulating strain
- Easy to cultivate and grows well in nutrient rich medium
- Doesn't need presence of 1,4-Dioxane to maintain degradation phenotype (no activation required)
- Previously shown to degrade 1,4-Dioxane metabolically under lab conditions
- No previously known studies done to show ability of microbe to degrade 1,4-Dioxane in groundwater

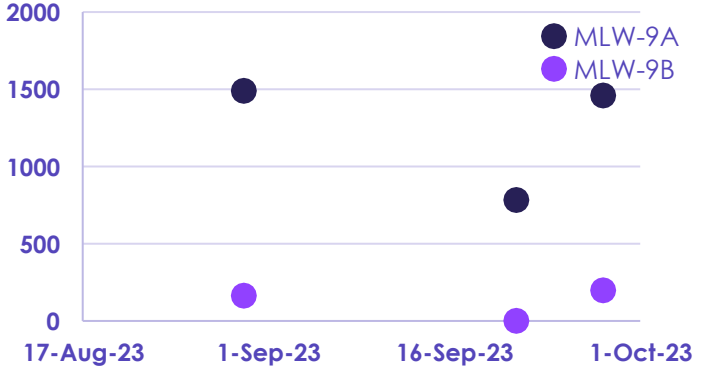
1,4 D-Stroy: Allonnia's first Bio-Based Solution



Southern California(2022)
- First Allonnia Field Trial.
- 97% reduction of 1,4-D.



Pennsylvania (2023)
- Achieved 97.5% reduction of 1,4-D.



South Carolina (2023)
- First field study using Direct Push.
- 50% initial reduction of 1,4-D.

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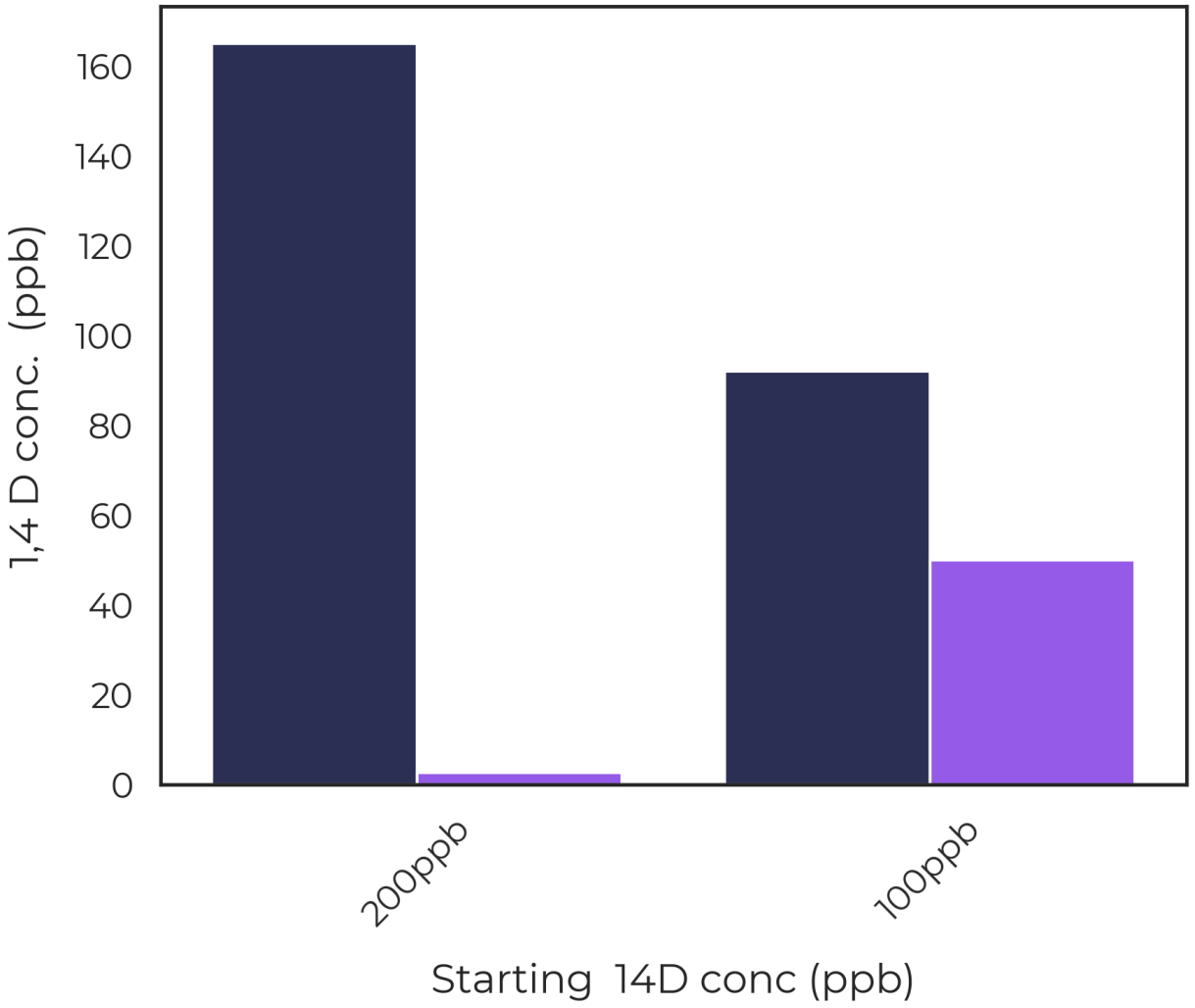
Transformative Biology for 1,4-Dioxane Degradation

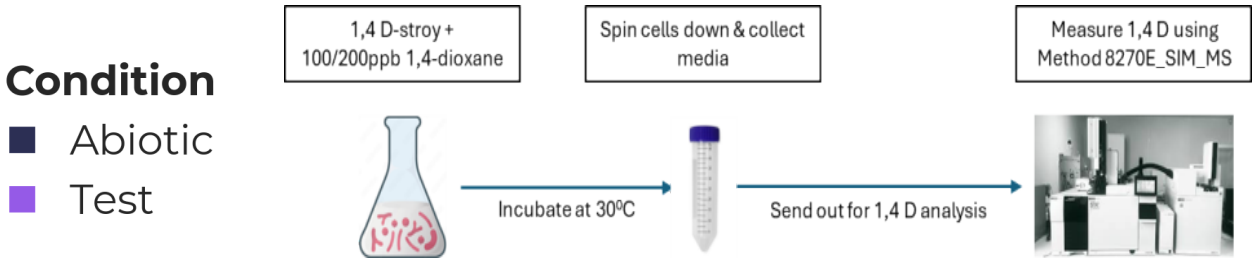
DEVELOP

TRANSFORMATIVE BIOLOGY

Characterization of 1,4 D-Stroy™

1,4 D-Stroy Degrades Low 1,4-Dioxane conc.



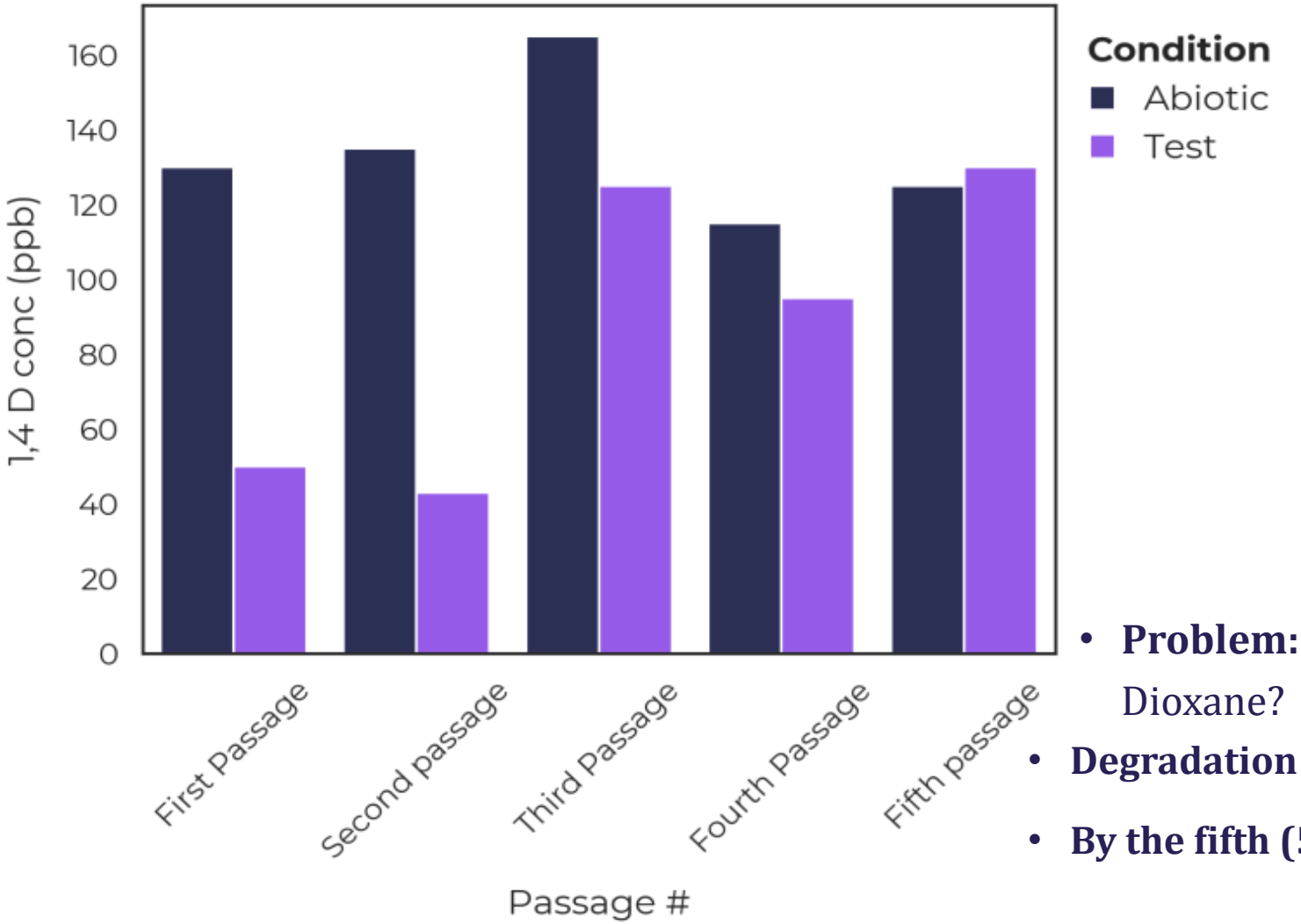


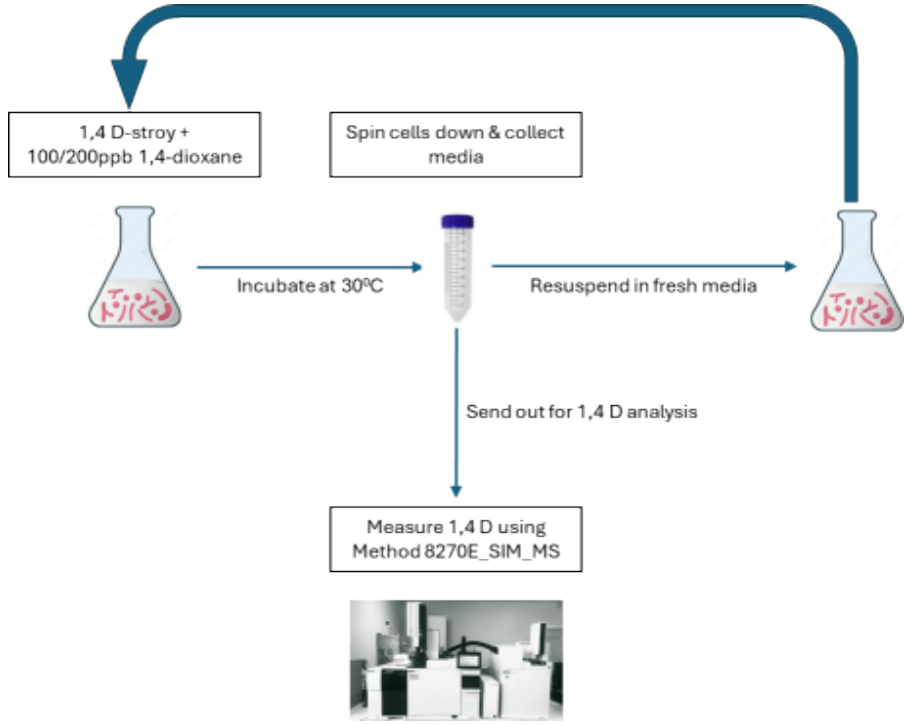
Problem: Can 1, 4 D-Stroy degrade low starting concentration of 1,4 dioxane?

- We tested degradation at 100 & 200ppb starting concentration.

- **1,4 D-Stroy can degrade 1,4-Dioxane at 200ppb starting concentration.**
- **Some challenges in performance at 100ppb 1,4-Dioxane concentration.**

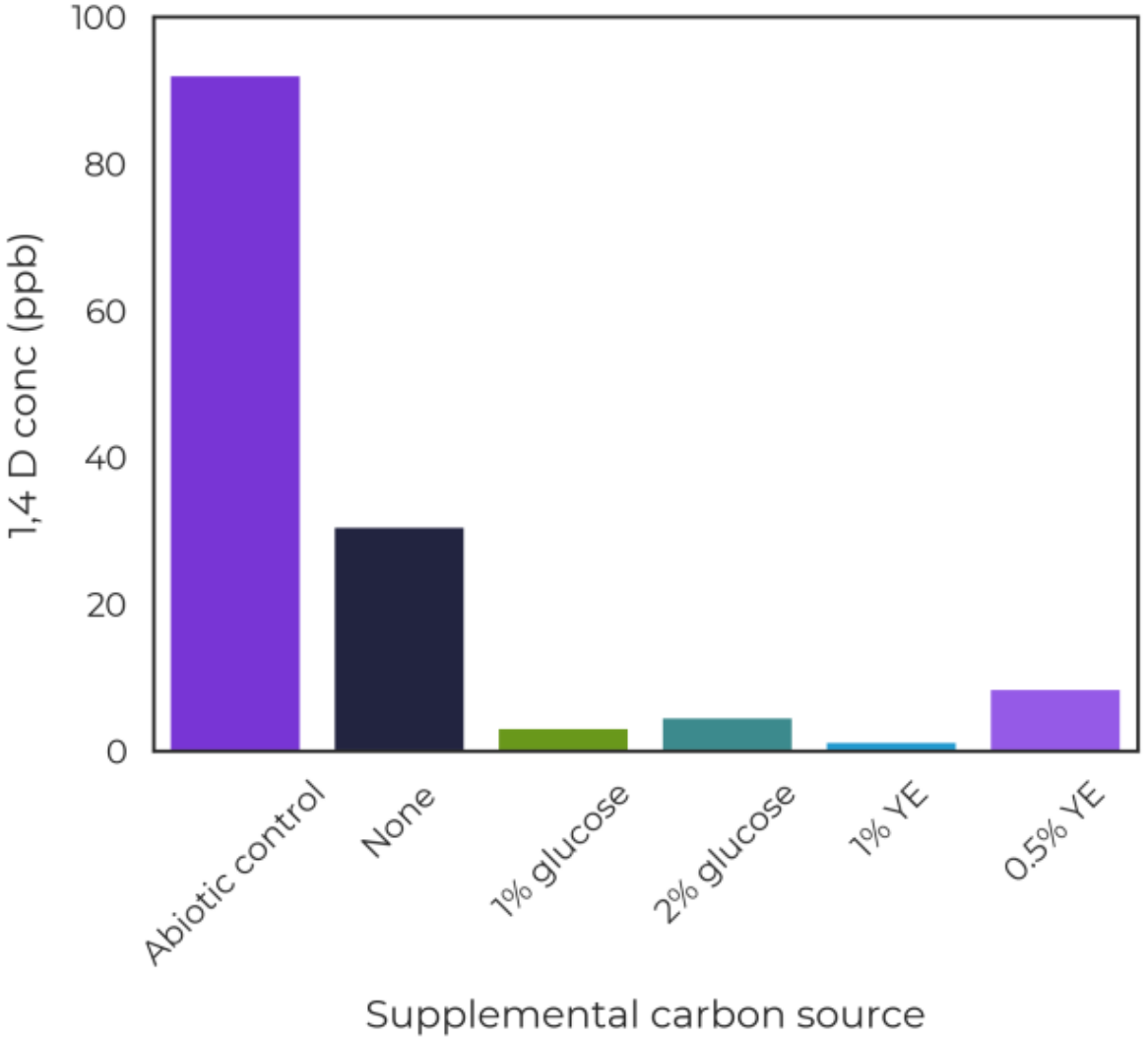
Continuous Exposure to 100ppb 1,4-Dioxane





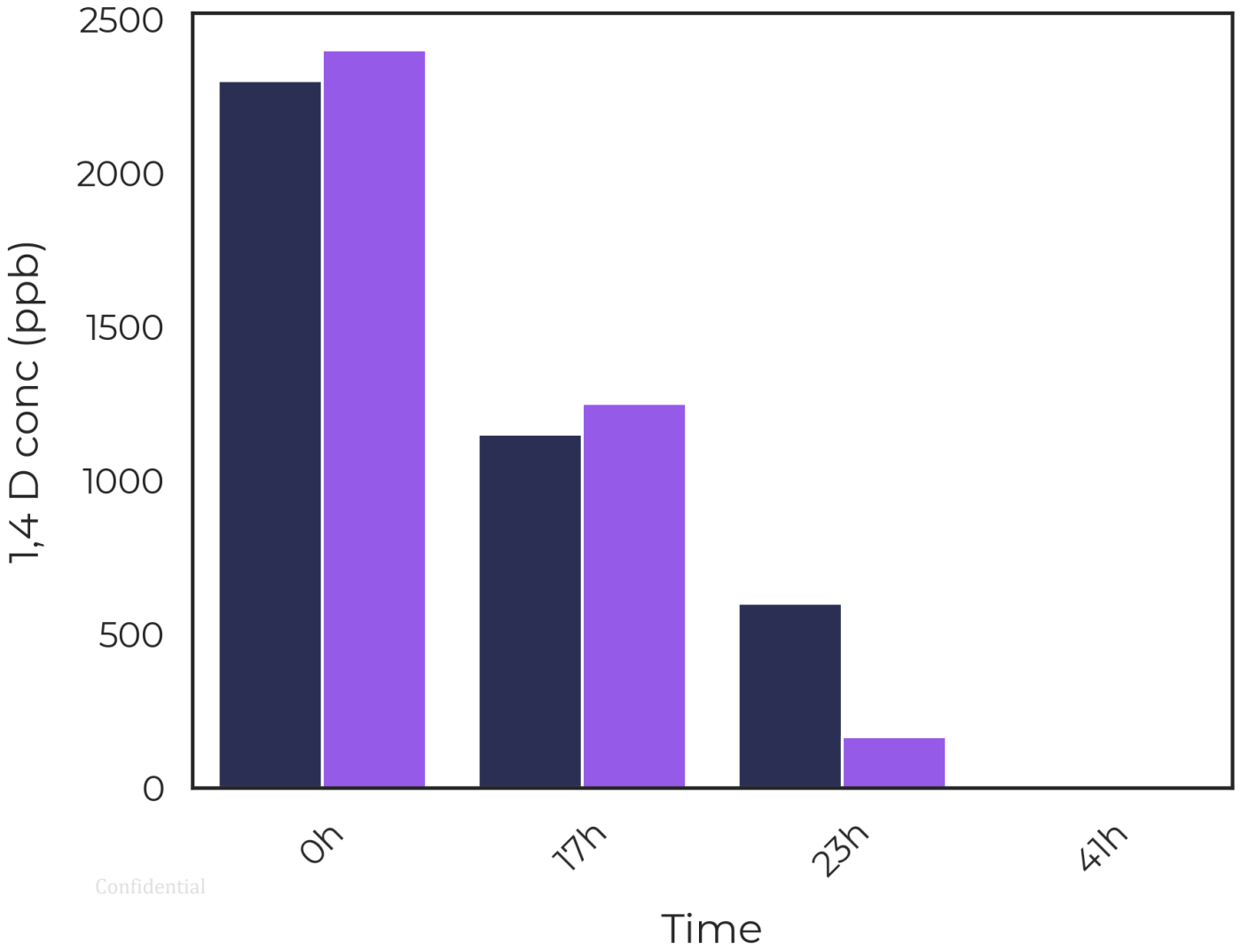
- **Problem:** Can 1,4 D-Stroy persist on low conc. of 1,4-Dioxane?
- **Degradation for two passages and then drop in performance.**
- **By the fifth (5) passage, no degradation was observed**

We Can Improve Degradation by the Addition of Carbon-Sources



- **Problem:** Can we rescue the 1,4-Dioxane degradation phenotype?
- 1,4 D-Stroy cells were grown in minimal media containing the different carbon sources.
  - None
  - 1% or 2% glucose
  - 1% or 0.5% yeast extract
- **The degradation phenotype is rescued by either glucose or yeast extract.**

Increased 1,4-D Degradation when Glucose is Present

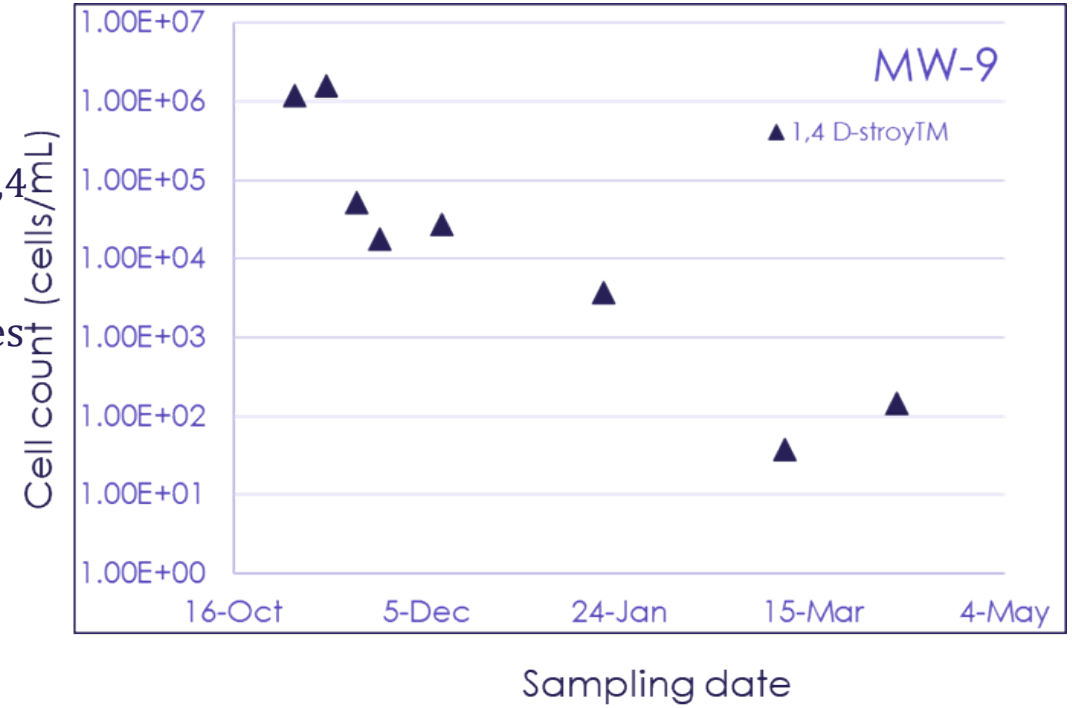


- **Problem:** Can the addition of organic carbon enhance degradation?
- **>92% degradation was observed in 23h when 1,4 D-Stroy was grown with 2% glucose**
- **70% degradation was observed when cells were grown without glucose**

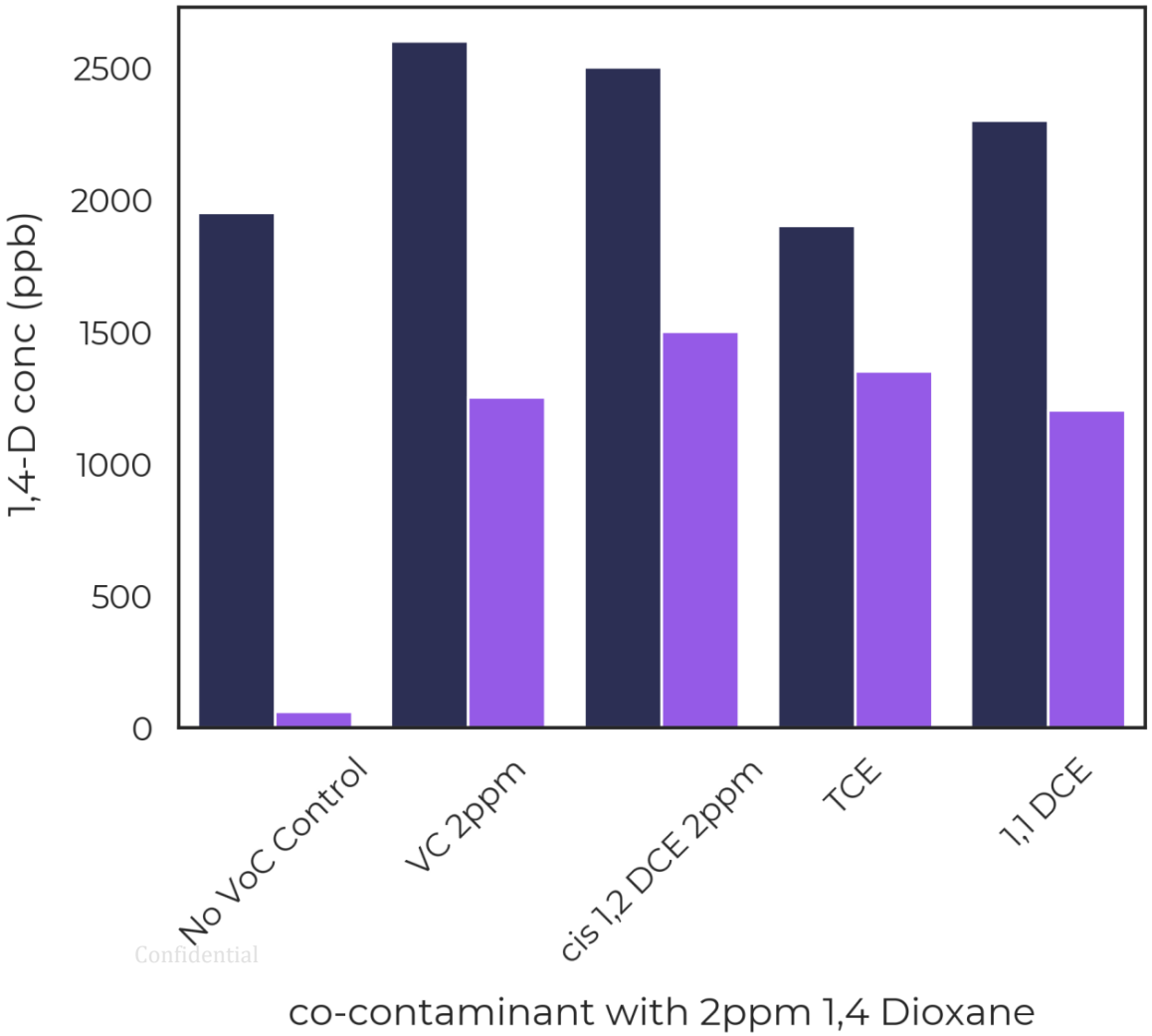
Implications in the Field

- Degradation in the presence of supplemental C-sources is an encouraging sign for *in-situ* applications.

- Results from the Southern California trial suggest the persistence of 1,4 D-Stroy in the GW >6 months post-inoculation.
- This strongly suggests that 1,4 D-Stroy can survive on natural C-sources available in the field while continuing to degrade 1,4 dioxane.



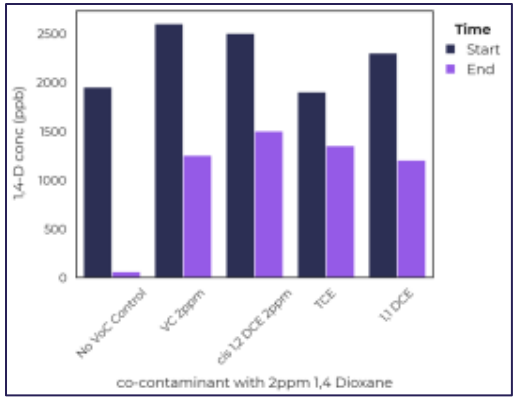
Chlorinated Solvents Affect 1,4-D Degradation



- **Problem:** Can 1,4 D-Stroy degrade 1,4-Dioxane in the presence of chlorinated solvents?
- 1,4 D-Stroy cultures were incubated in the presence of various chlorinated co-contaminants.
- A no-VoC control was set up in parallel.

- **1,4-Dioxane degradation was inhibited in all cultures with co-contaminants. ~50% degradation observed.**

Implications in the Field



- The lab data was generated by using chlorinated solvents in methanol.
- Methanol has bactericidal properties. The VoC results can mean two things:
  - The presence of VOCs inhibits the degradation of 1,4-Dioxane.
  - The bactericidal nature of methanol, kills 1,4 D-Stroy and reduces its effectiveness.
- At the Southern California site, there were a few chlorinated solvents along with 1,4-Dioxane:
  - 1,1 DCE (~1000ppb)
  - TCE (~2500ppb)
  - cis 1,2 DCE (~750ppb)
- In the presence of these chlorinated solvents, 1,4 D-Stroy persists in the groundwater and degrades 1,4-Dioxane.

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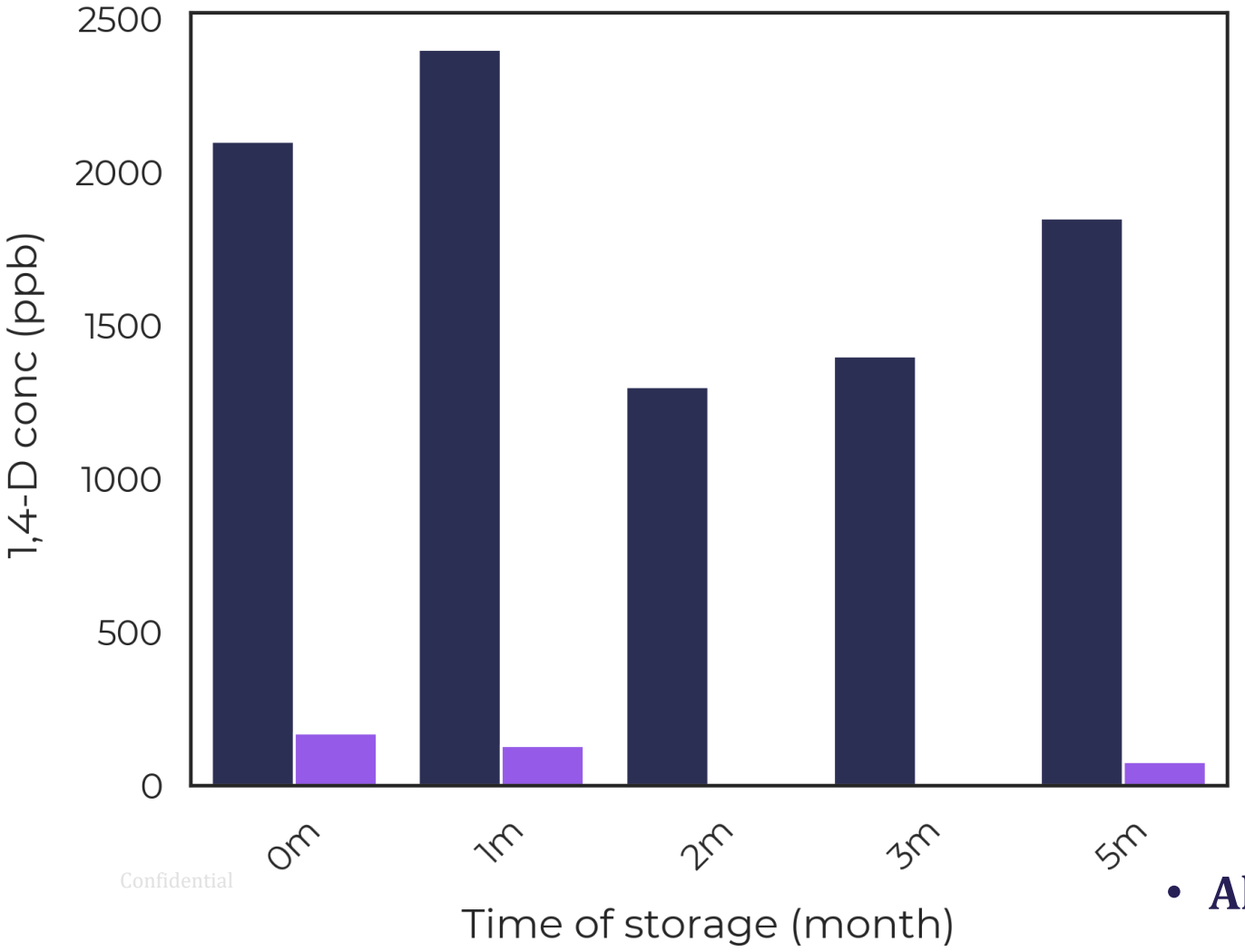
DEPLOY

INTEGRATED SYSTEMS

1,4 D-Stroy Culture is Robust



- **Problem:** Can we store 1,4 D-Stroy in the fridge?
- Samples were stored as pellets at 4^0C for 5 months.
- Cultures tested for 1,4-Dioxane degradation.
- Abiotic controls were assayed in parallel to account for operator errors.

- **All cultures tested degraded 1,4-Dioxane.**

Summary

Three Field Demonstrations

- Field demonstrations in Southern California, Pennsylvania, & South Carolina.
- >97% reduction observed at two out of the three sites.
- Requires aerobic conditions (>1 mg/L DO)

Strain Characterization

- 1,4 D-Stroy can perform in conditions where 100-200ppb 1,4-D is present. Degradation of lower concentrations is likely.
- The addition of simple supplements (Yeast Extract) enhances degradation under low 1,4-D conditions.
- 1,4-Dioxane degradation occurs in the presence of chlorinated solvents.
- 1,4 D-Stroy cultures are highly robust. Active after >6 months of storage at 4^0C.

Easy to Ferment

- Robust fermentation process developed by Allonnia.
- Cultures can be grown in Complex media and then exposed to 1,4-Dioxane without any lag in degradation activity.

Acknowledgement

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Thank You

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