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Novel organism deployed for *In situ* Bioremediation of 1,4-Dioxane in Groundwater

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Transformative biology for 1,4-Dioxane degradation

DISCOVER — DEVELOP — DEPLOY

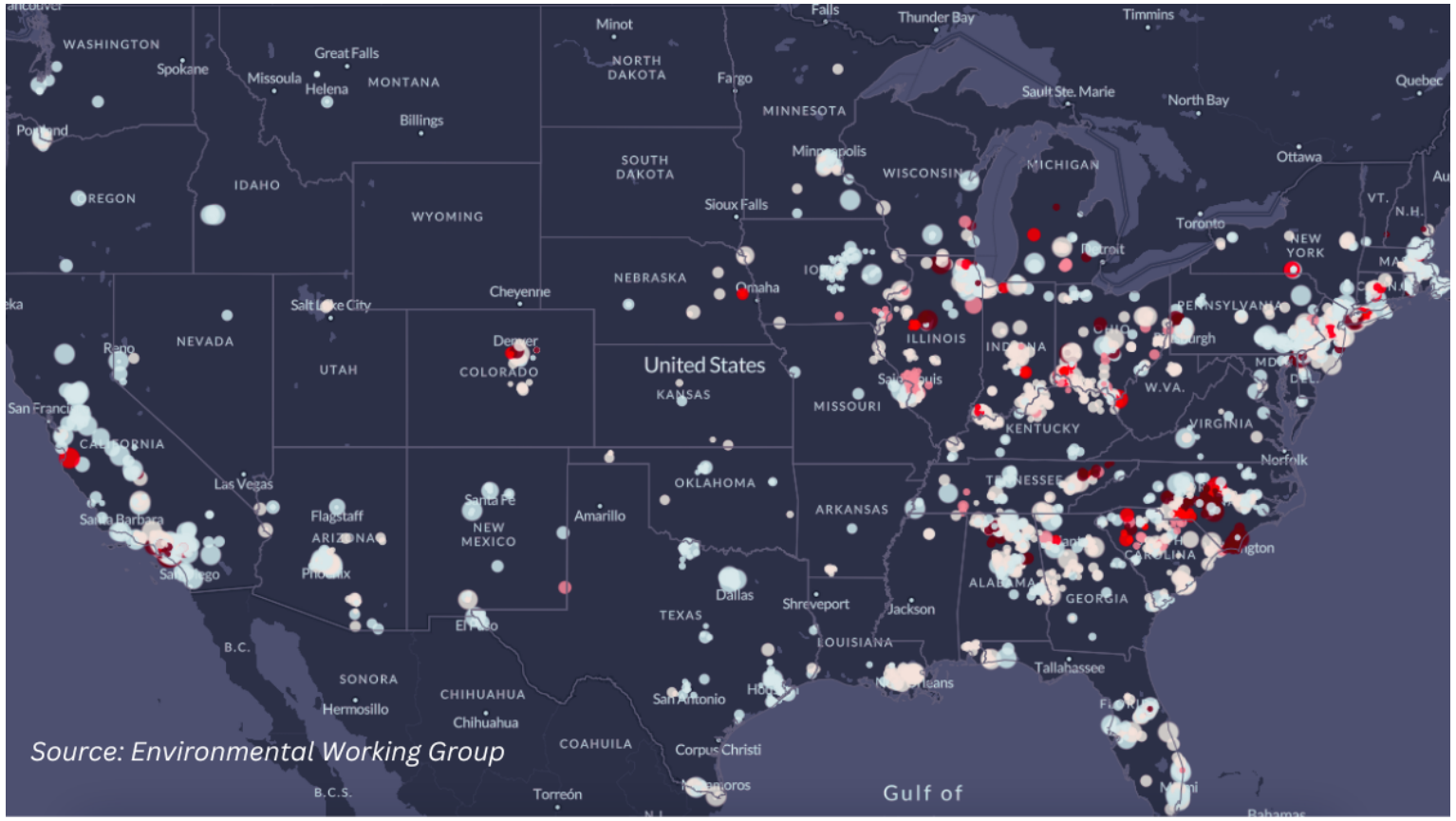
BIOINFORMATICS

TRANSFORMATIVE BIOLOGY

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



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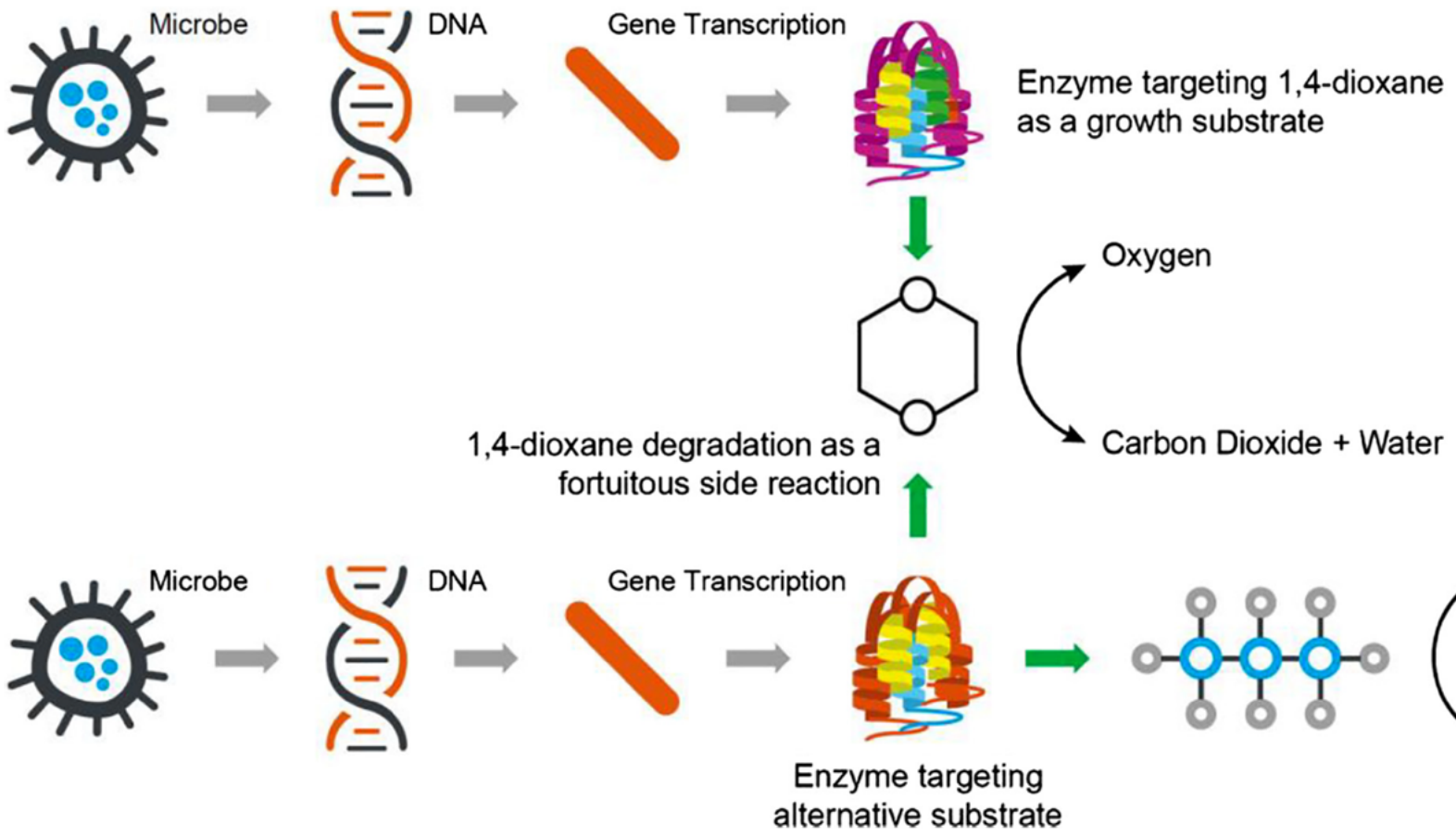
Biodegradation of 1,4-dioxane



Metabolic degradation

Cometabolic degradation

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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

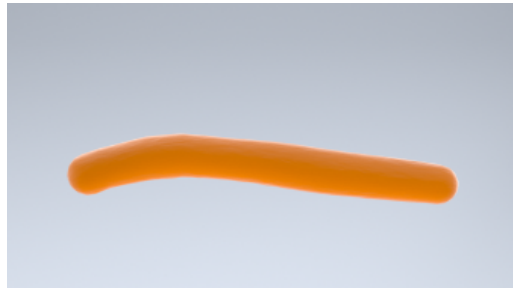
- Potentially hazardous substrates

- Rapid substrate utilization

- Oxygen depletion

- Cost

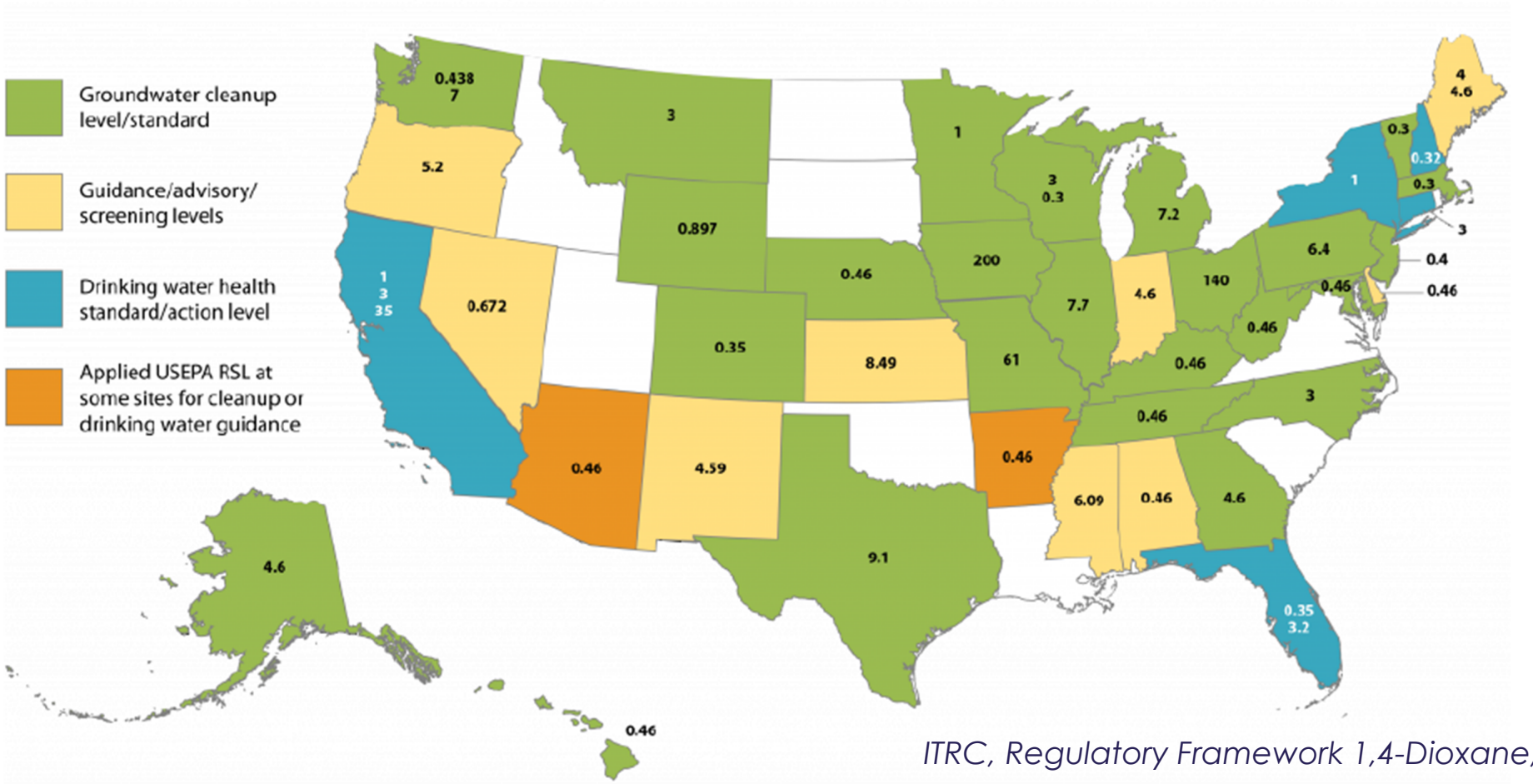
ALL22_0001



- 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

State Regulations for 1,4-D

- Half of US states have a groundwater cleanup standard for 1,4-dioxane



ITRC, Regulatory Framework 1,4-Dioxane, 2021

Laboratory experiments

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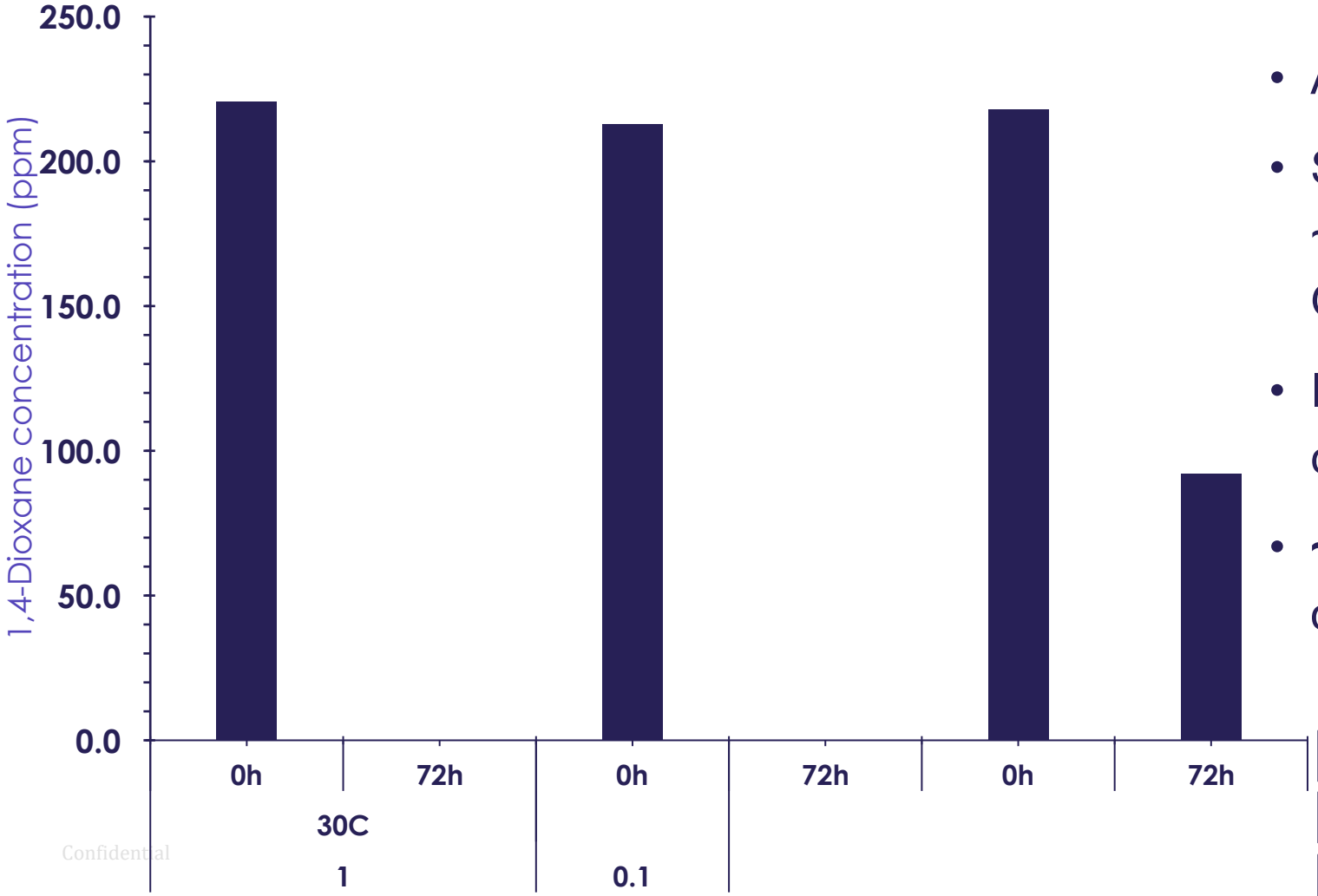
Lab degradation of 1,4-Dioxane



- ALL22_0001 grown in nutrient media.
- Synthetic minimal media containing ~200 ppm of 1,4-Dioxane inoculated at OD 0f 1 & 0.1
- Incubated for 72h and 1,4-Dioxane concentration measured.
- **~100%** degradation of 1,4-Dioxane observed.

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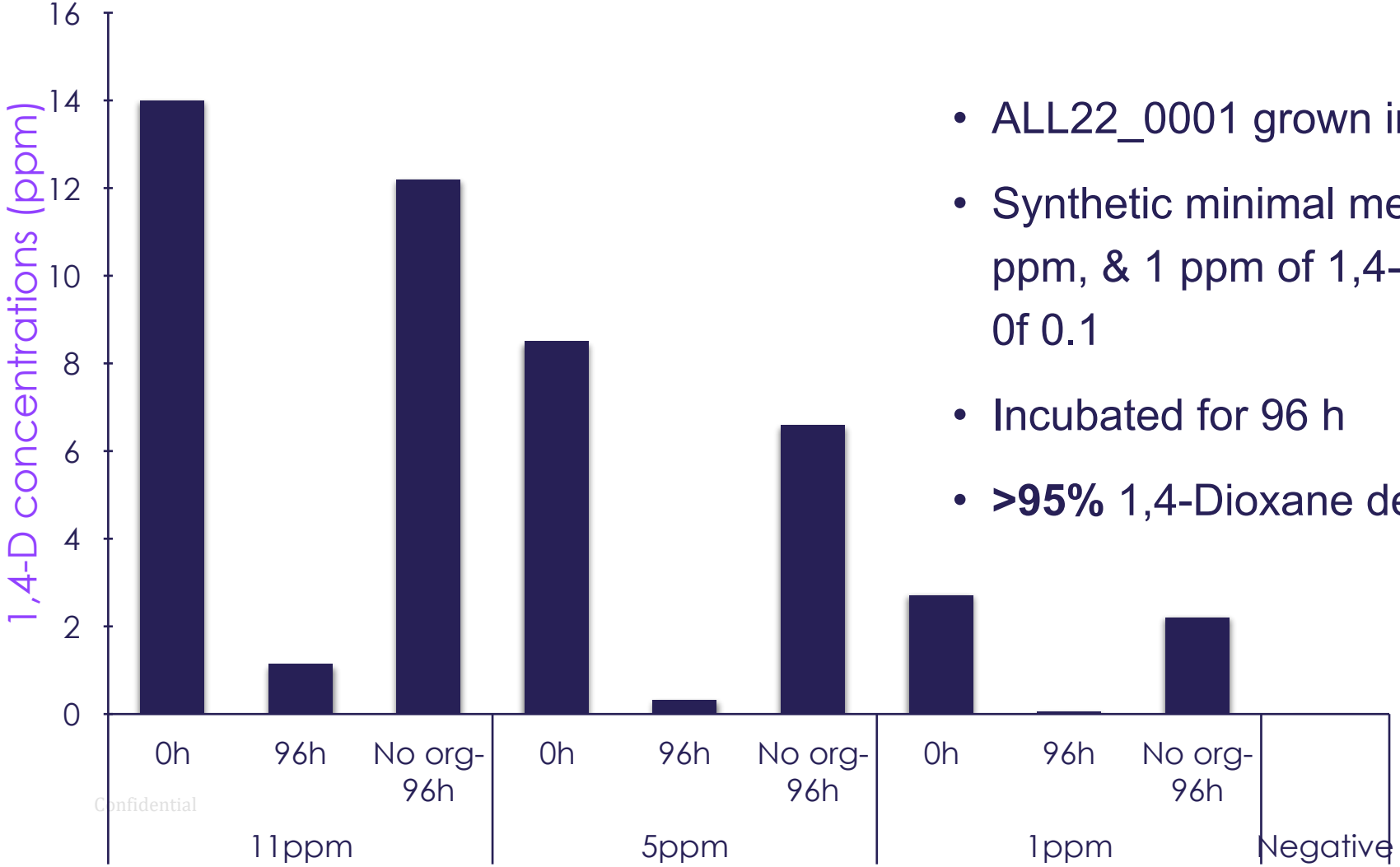
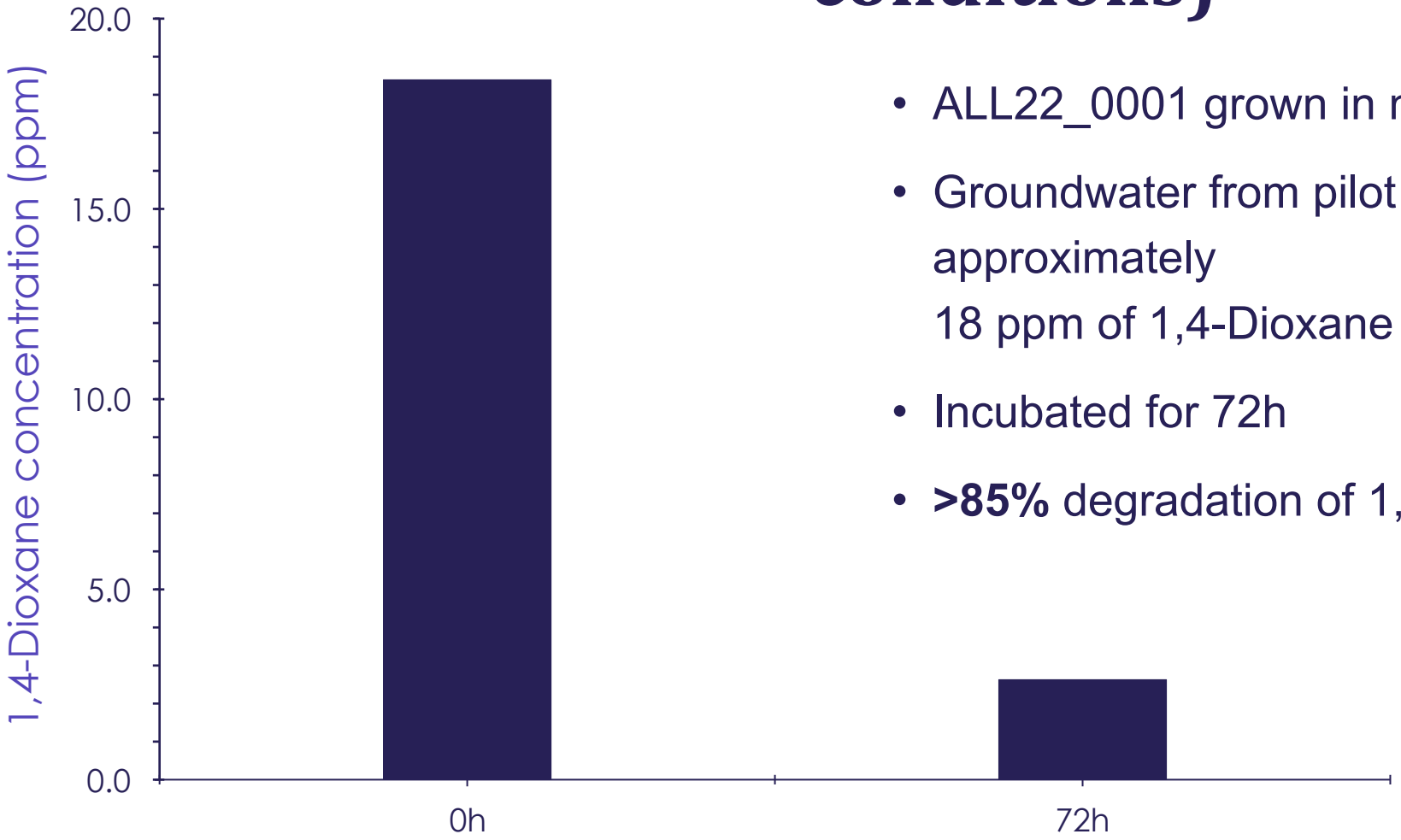
1,4-Dioxane degradation at low concentrations



- ALL22_0001 grown in nutrient media
- Synthetic minimal media containing ~11 ppm, 5 ppm, & 1 ppm of 1,4-Dioxane inoculated at OD 0f 0.1
- Incubated for 96 h
- **>95%** 1,4-Dioxane degradation observed

1,4-Dioxane degradation in groundwater (Lab conditions)



- ALL22_0001 grown in nutrient media
- Groundwater from pilot site containing approximately
  18 ppm of 1,4-Dioxane inoculated at OD_{600} of 0.1
- Incubated for 72h
- **>85%** degradation of 1,4-Dioxane

First *In Situ* Application of ALL22_0001

Location: Southern California

Inoculation Date: November 2022

Client: Confidential Site

Site Type: Industrial, Groundwater Plume

Application: In Situ Groundwater Pilot Study

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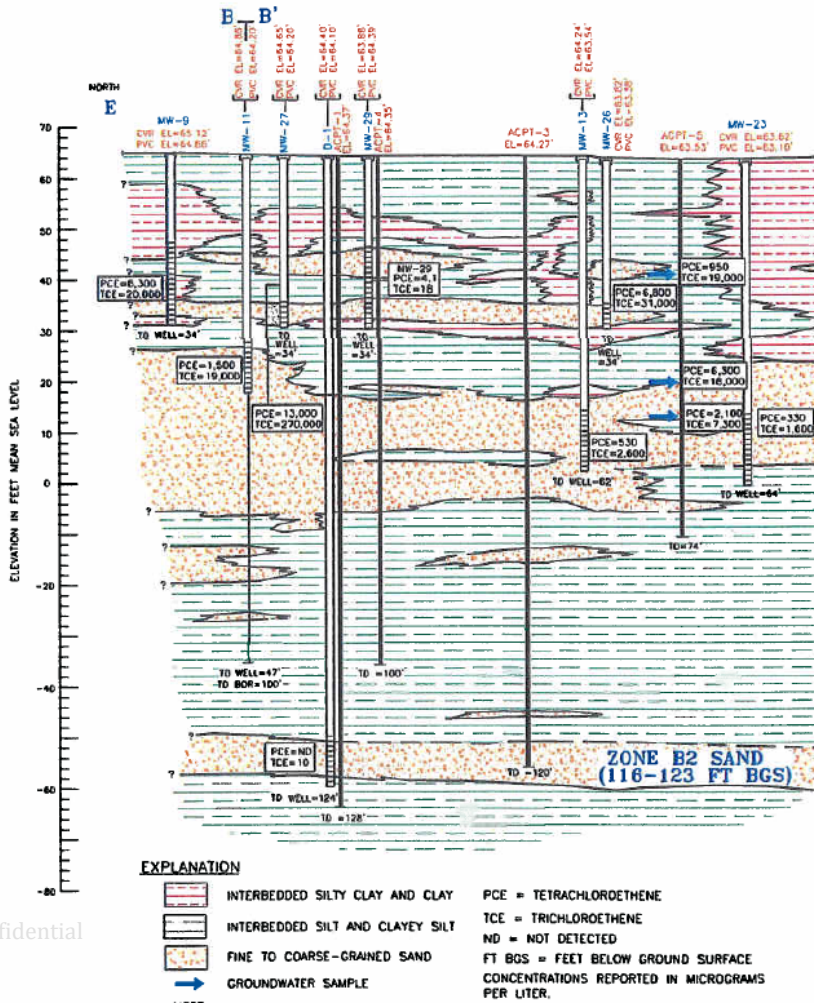
Existing conditions



- CVOC plume due to industrial contamination post-ISCO
- Significant remaining 1,4-dioxane, not originally targeted
- Two plumes at different depths in separate confined sandy transmissive aquifers
  - Well A: shallow water bearing zone (17-32ft bgs); 18,000 ppb 1,4-dioxane
  - Well B: deep water bearing zone (49-59ft bgs); 670 ppb 1,4-dioxane

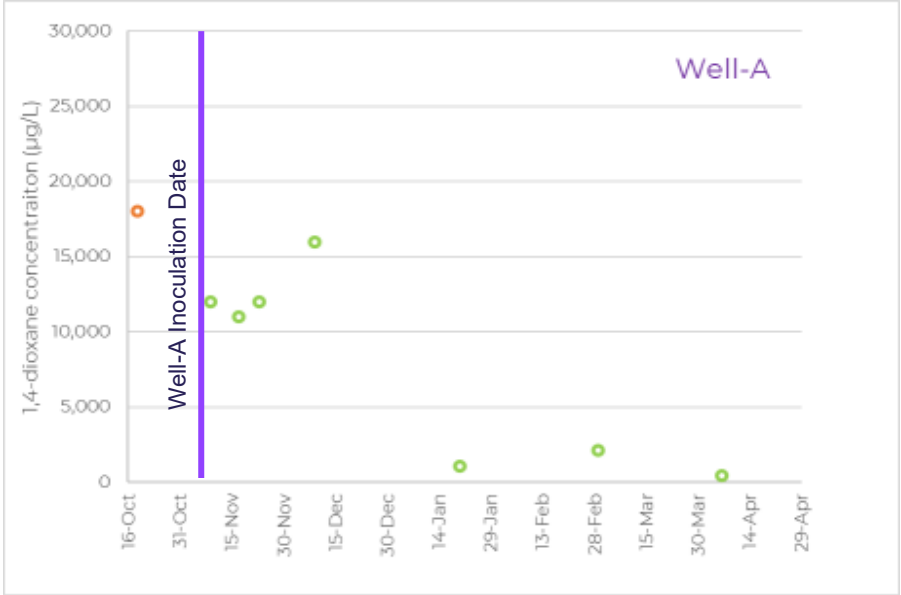
Experimental conditions

- First *in-situ* application of ALL22_0001
- Bioaugmentation with live culture and nutrient mixture of two wells
- Periodic down-well air sparging due to anaerobic conditions in the aquifer
- Five-month monitoring period with no additional injection of organisms or nutrients

Results

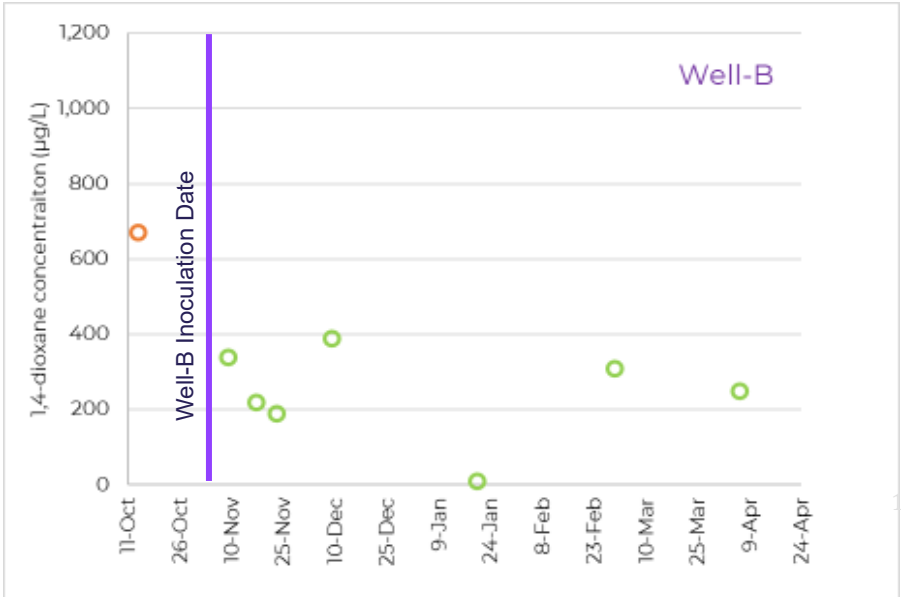
Well-A

- **Up to 97% reduction** of 1,4-dioxane (18,000-440 ug/L in 5 months)
- Negligible rebound observed in the well
- 33% reduction after just one week



Well-B

- **Up to 98% reduction** of 1,4-dioxane (670-12 µg/L in 2.5 months)
- 63% pilot study endpoint reduction
- 49% reduction after just one week



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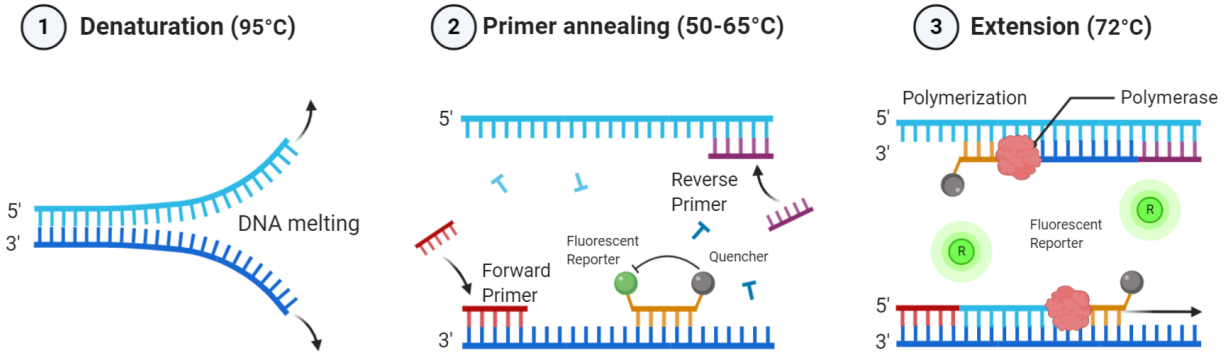
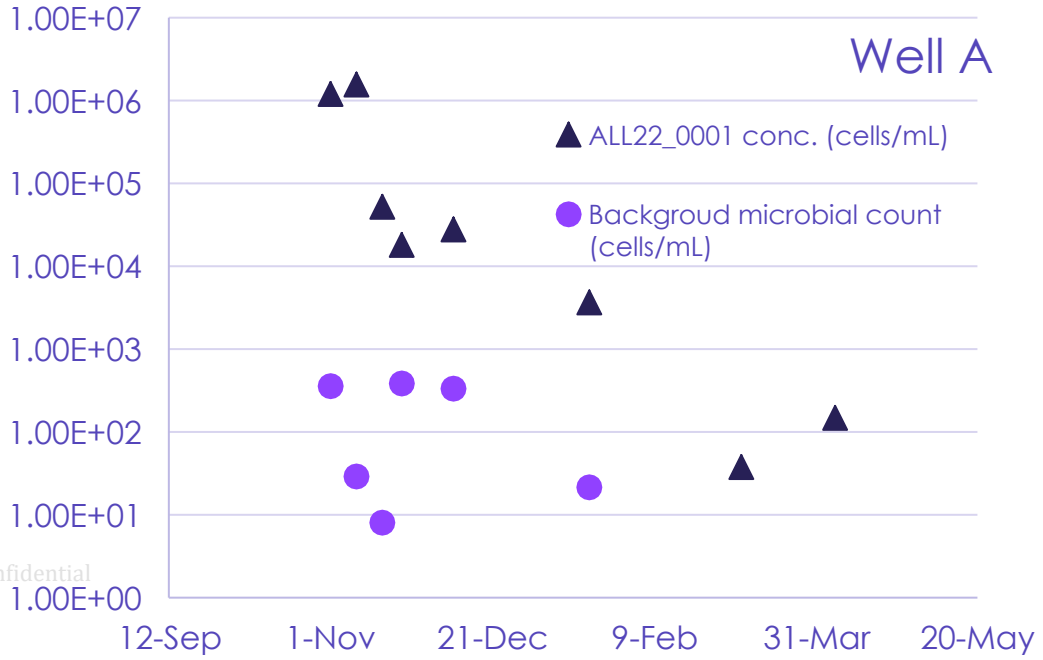
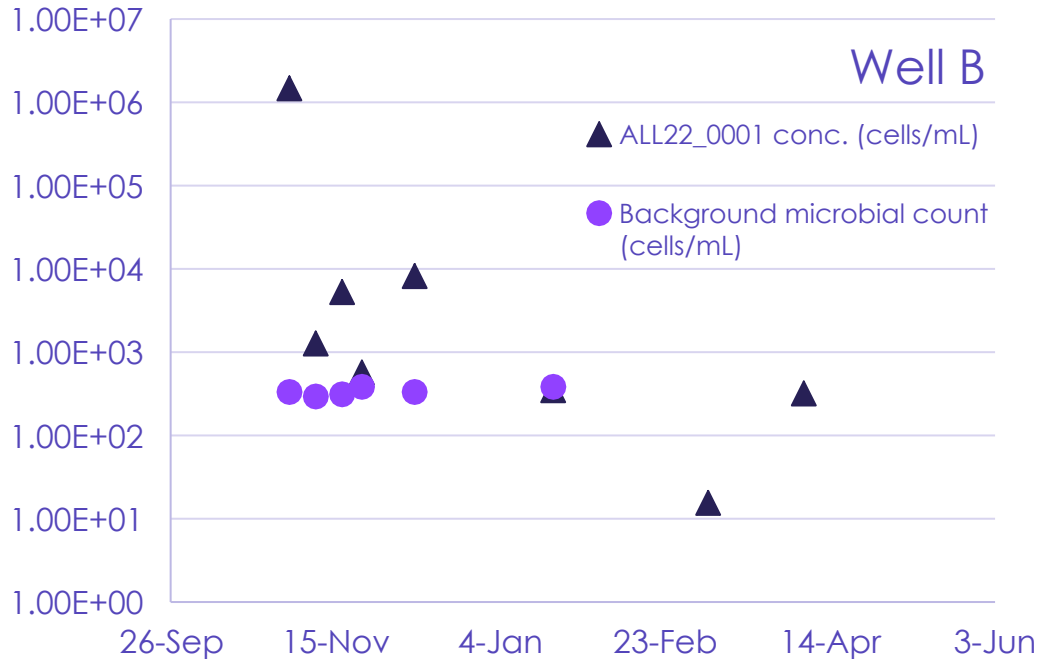
qPCR analysis for microbial tracking







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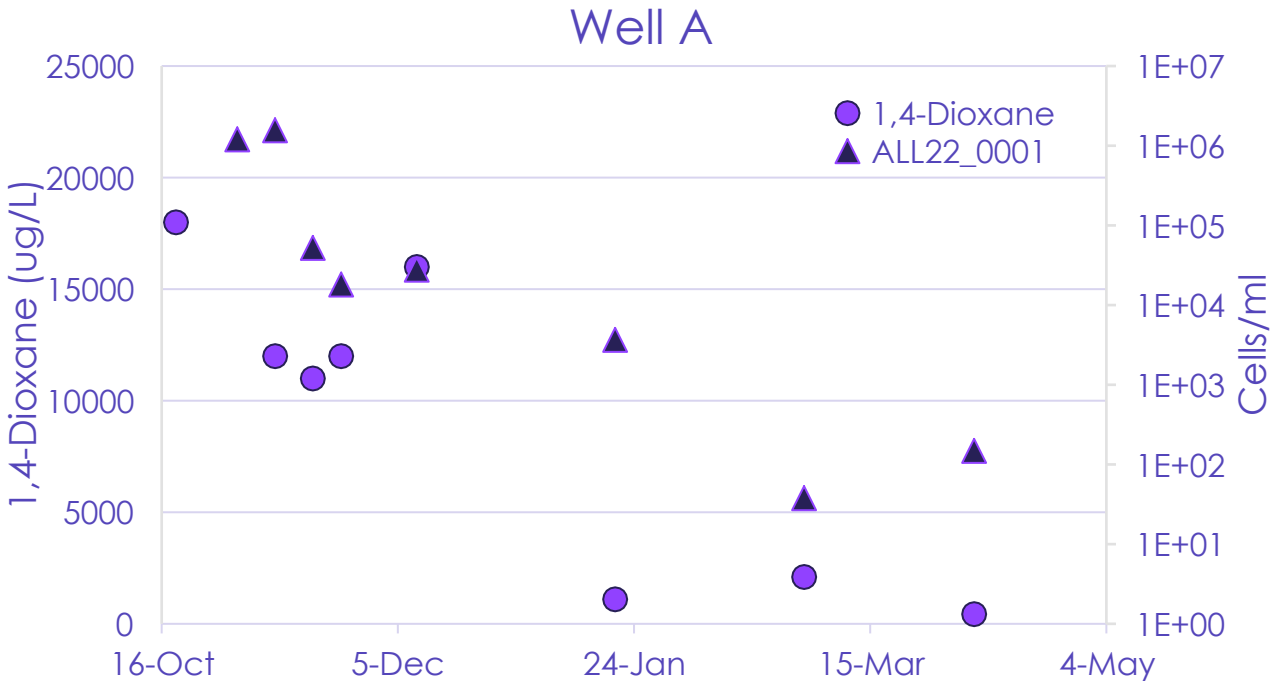
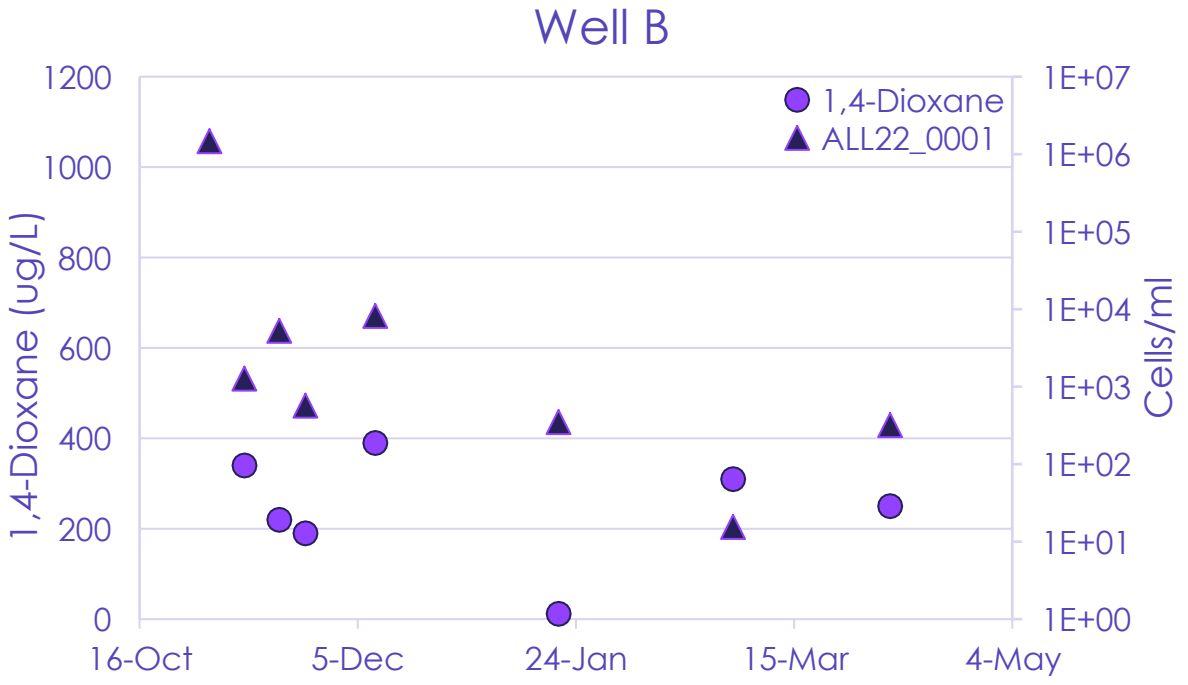
1,4-Dioxane degradation in relation to cell number





- Cell concentration in the range of 500 cells/ml is enough to sustain in-situ degradation.
- Culture can sustain post-inoculation in-situ for at least 5 months without supplementing carbon/nutrients/substantial oxygen

Summary

First in situ demonstration

of ALL22_0001 to degrade 1,4-Dioxane

>95% reduction

in 1,4-Dioxane concentration observed in both wells

Negligible rebound

in Well 1 1,4-Dioxane concentrations

63% reduction

in Well 2 1,4-Dioxane concentrations at the end of trial

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Microbial Insights

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Thank you

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