Transport of *Pseudonocardia* through Soil for Bioremediation of 1,4-Dioxane

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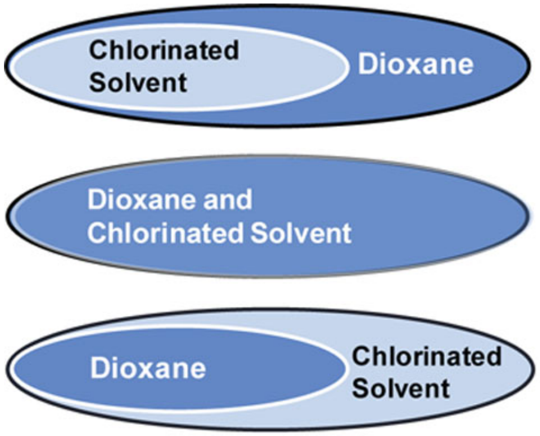
Overview

- **Most 1,4-dioxane plumes seem to be shorter than expected**
- **Finding evidence of natural attenuation is challenging**
- **Some plumes will require active remediation**
- **Bioaugmentation may be required**
- **How well do 1,4-dioxane degrading cultures move through soil?**

Adamson et al. (2014):



Chlorinated Solvent
Dioxane

Dioxane plume is longest = 21%

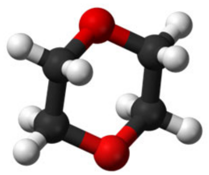
Dioxane and Chlorinated Solvent

Dioxane plume is same length = 17%

Dioxane
Chlorinated Solvent

Chlorinated solvent plume is longest = 62%

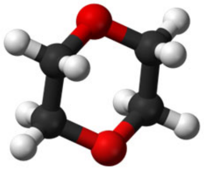
n = 103 sites where dioxane and chlorinated solvents co-occur



Overview

1,4-Dioxane Aerboic Cultures for Bioagumentation

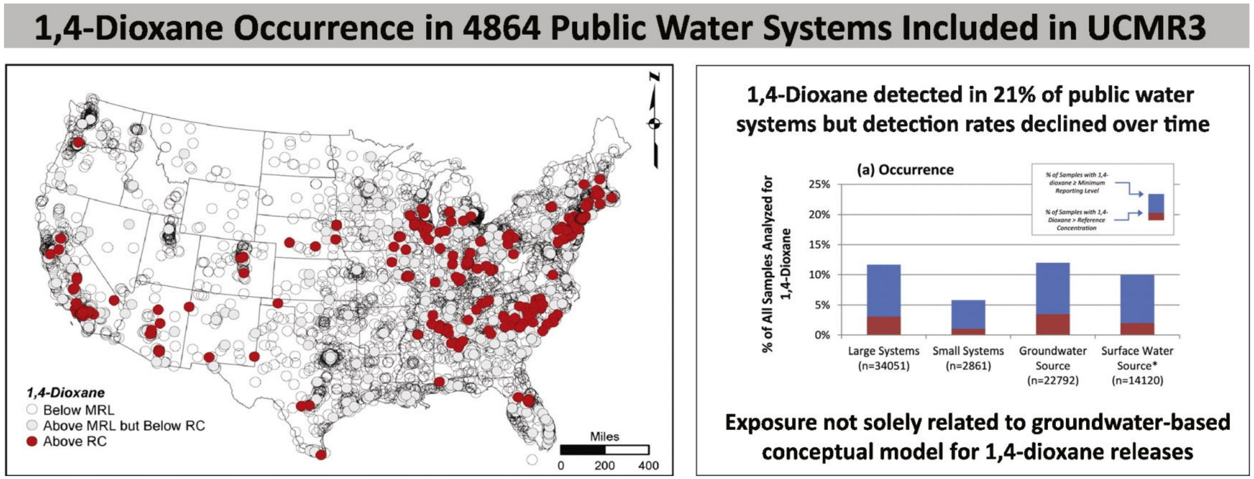
- Use 1,4-dioxane as a growth substrate
  - Best known is *Pseudonocardia dioxivorans* CB1190
  - Isolated from activated sludge
  - Tendancy to clump when grown in the lab; raises questions about movement through soil for bioaugmentation applications
- Cometabolize 1,4-D following growth on a primary substrate
  - Various isolates that grow on various substrate (methane, propane, butane, etc.)
  - Less propensity to clump?



Objectives

- **Enrich and isolate 1,4-D degraders from 2 aquifers**
  - **Industrial site in the southeastern US**
  - **Industrial site in Europe**
- **Compare one of the aquifer isolates to CB1190 in movement through porous media**
  - **Sand**
  - **Silt**

Adamson et al. (2017):



1,4-Dioxane Occurrence in 4864 Public Water Systems Included in UCMR3

1,4-Dioxane detected in 21% of public water systems but detection rates declined over time

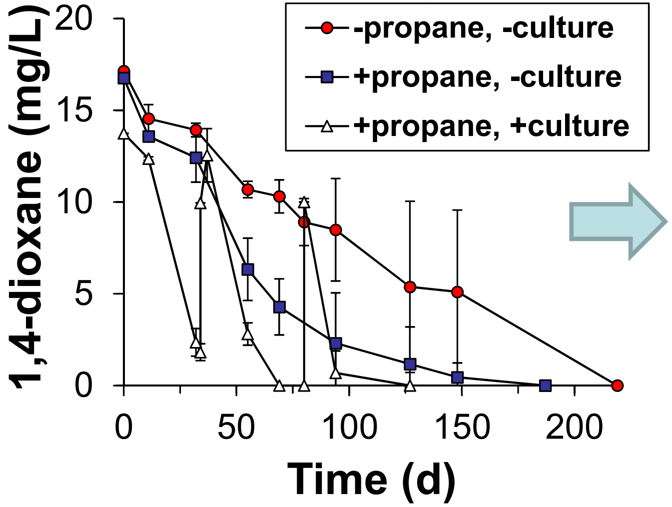
Exposure not solely related to groundwater-based conceptual model for 1,4-dioxane releases

1,4-D Isolates

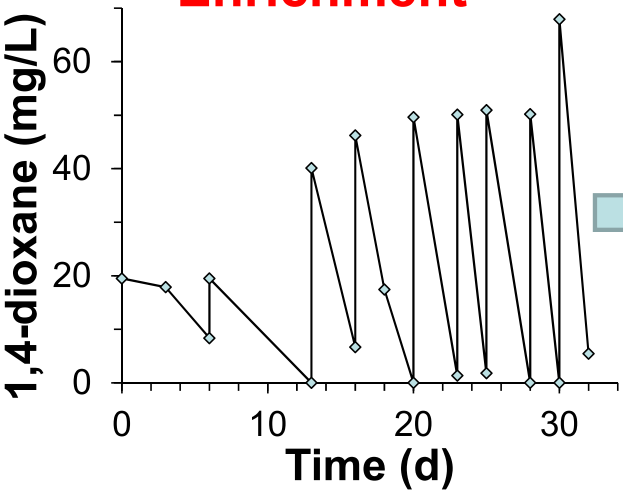
- Microcosms prepared with soil (20 g) + GW (50 mL)
  - Treatments evaluated natural attenuation, propane addition, and propane + propanotrophic culture
- Enriched microcosms with only 1,4-D and transfers to ammonium mineral salts medium (AMSM)
- Isolates obtained by repeated steaking on Noble agar + AMSM plates with 50 mg/L 1,4-dioxane
- Whole genome sequencing performed at the UNC-Chapel Hill Microbial Core Facility
- *Pseudonocardia dioxanivorans* strain CB1190 provided courtesy of Dr. Shaily Mahendra, UCLA

Isolate #1

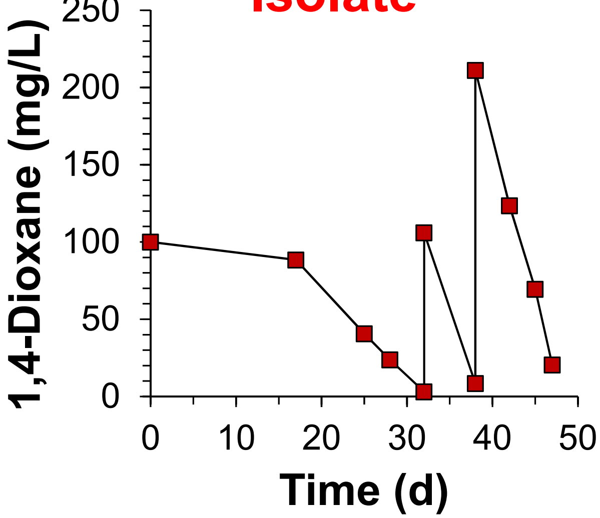
Microcosms, Site I

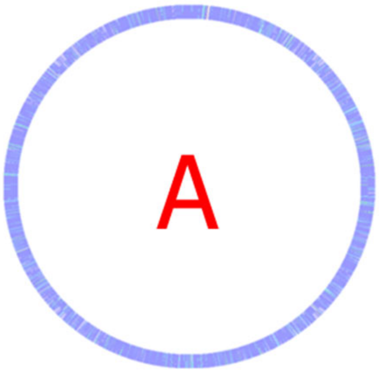


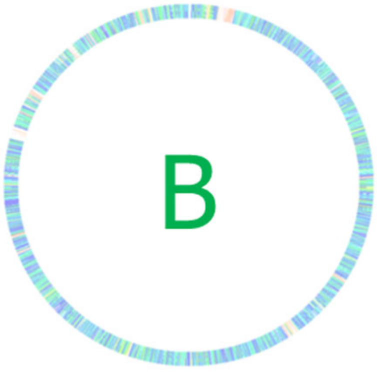
Enrichment



Isolate







- **16S rRNA sequence: same as CB1190**
- **Gene-to-gene comparison**

A = Positive control, CB1190 from literature vs. CB1190 at Clemson → nearly identical

B = CB1190 vs. isolate #1 → different strains; isolate named "BERK-1"

Ramos-Garcia, et al. 2018. Draft genome sequence of the 1,4-dioxane-degrading bacterium *Pseudonocardia dioxanivorans BERK-1. Genome Announcements 6* (14), e00207-18.

Isolate #1

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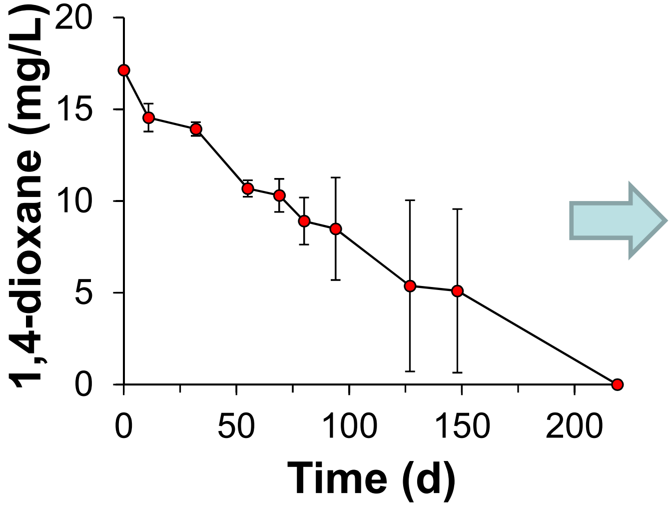
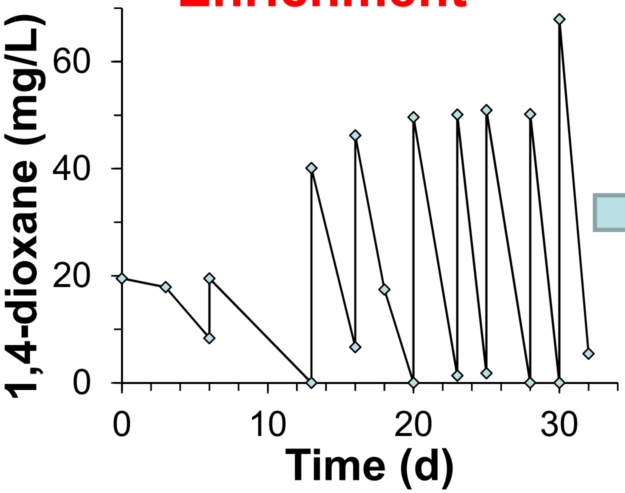
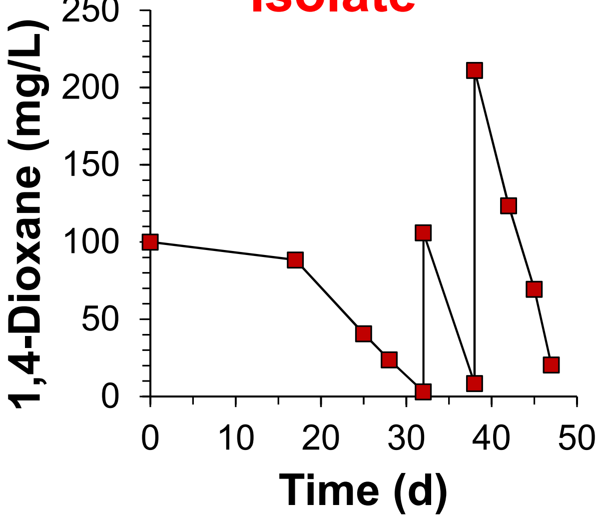
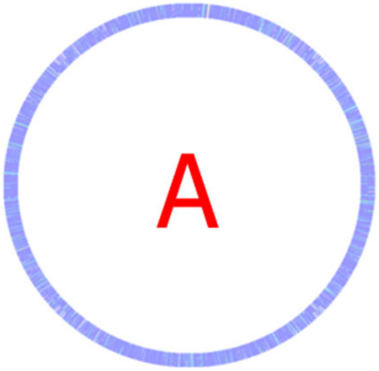


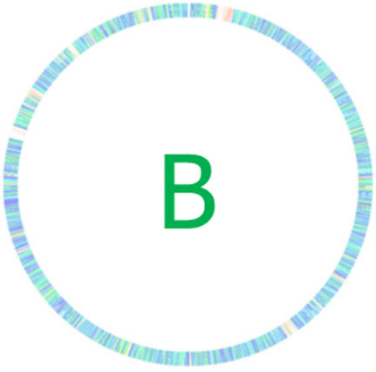
Enrichment



Isolate







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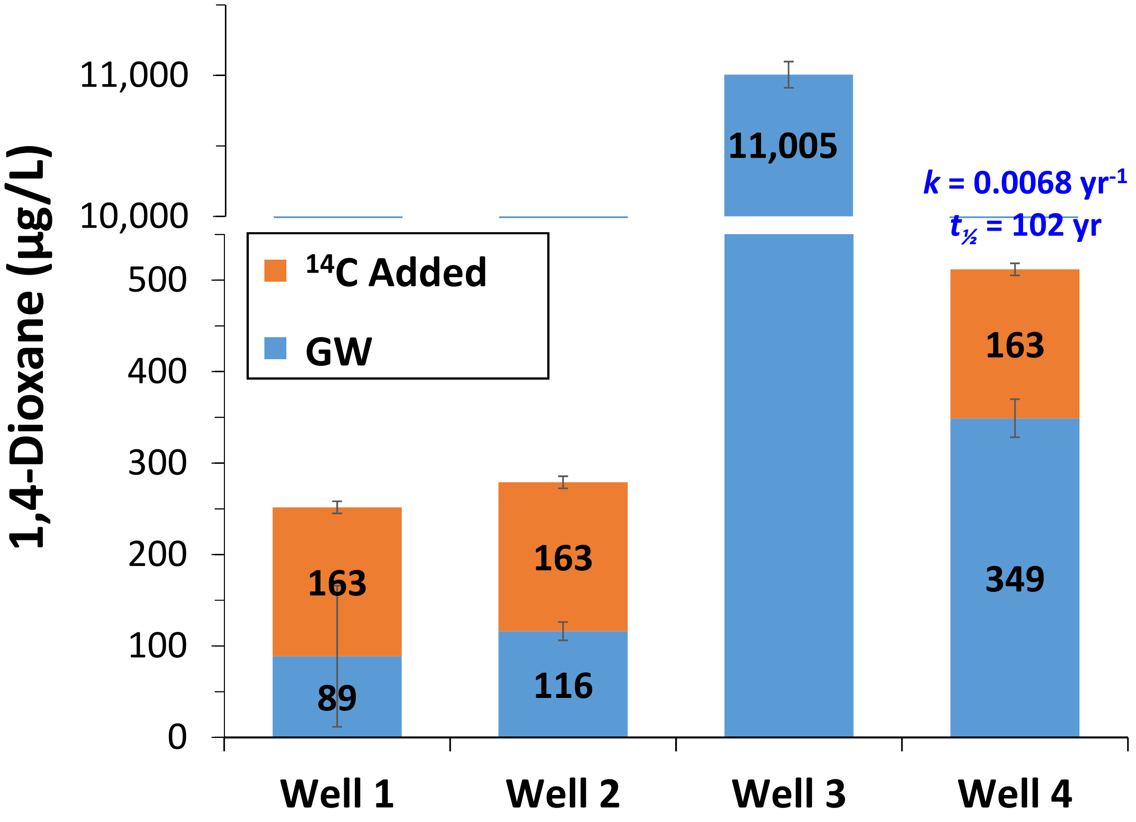
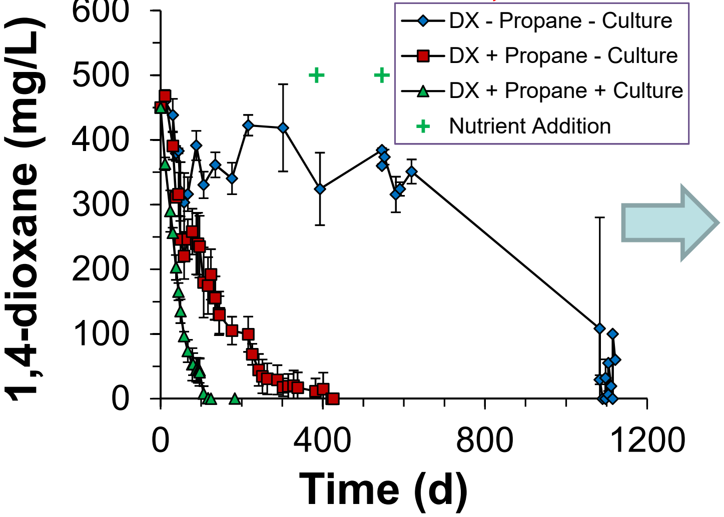
^{14}C Confirmation

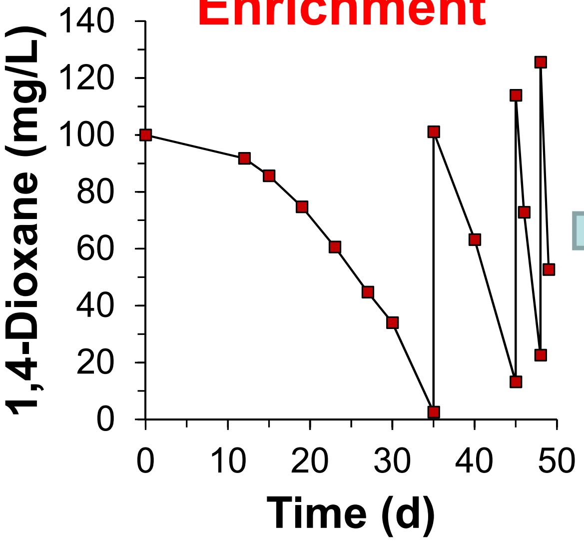


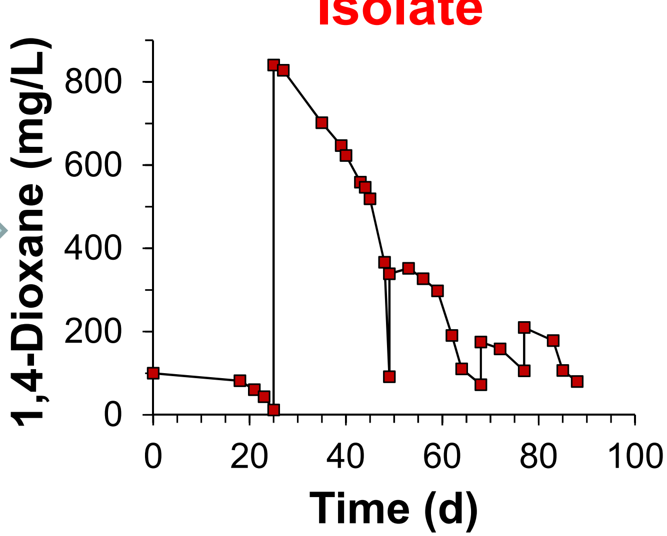
New samples of GW from site I collected

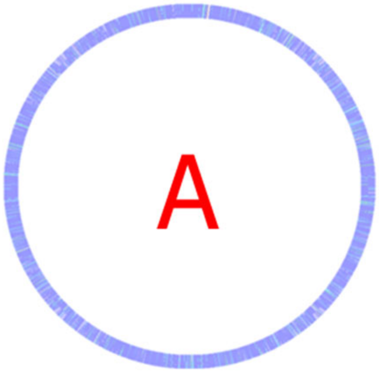
- **Part of ESTCP Project ER-201730: *Development of a Quantitative Framework for Evaluating Natural Attenuation of 1,1,1-TCA, 1,1-DCA, 1,1-DCE, and 1,4-Dioxane in Groundwater***
- **Biodegradation of 1,4-D observed in samples from one well**
- **Low rate constant is consistent with a slow rate of degradation in original microcosms**
- **VOC levels are low, non-inhibitory**

Isolate #2


Microcosms, Site II


Enrichment


Isolate



- **16S rRNA sequence: same as CB1190**
- **Gene-to-gene comparison:**
  A = BERK-1 versus EUR-1 → nearly identical; same strain
  B = EUR-1 versus CB1190→ different strains

Isolates

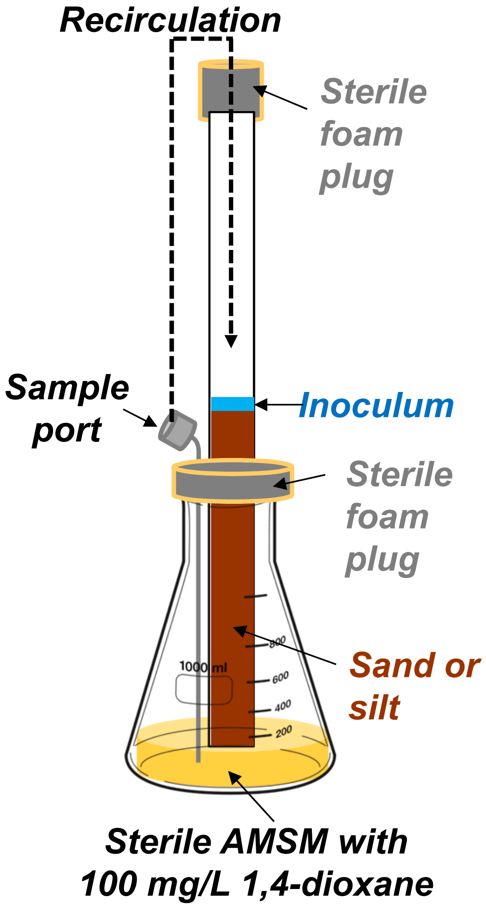
CB1190

BERK-1
from site II

BERK-1
from site I

- Significantly greater clumping and attachment by CB1190 versuses BERK-1
- Is this related to the origin of the isolates, i.e., activated sludge vs. aquifers?
- Hypothesis: BERK-1 will move through porous medium at a higher rate since it is less “sticky”

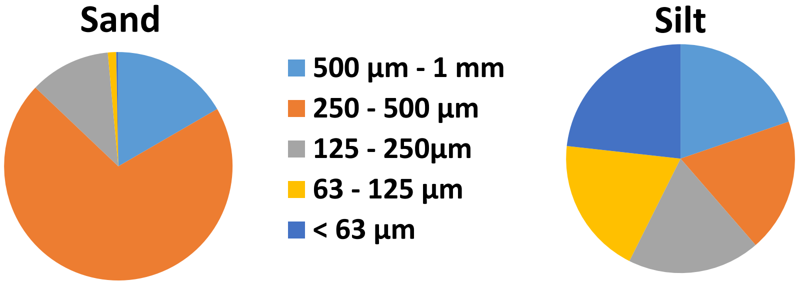
Movement of Isolates



- Glass tube: 0.87" inner diameter x 11.8" long; 5" filled with sand or silt
- Glass wool plug at base retained the sand or silt
- Porosity:

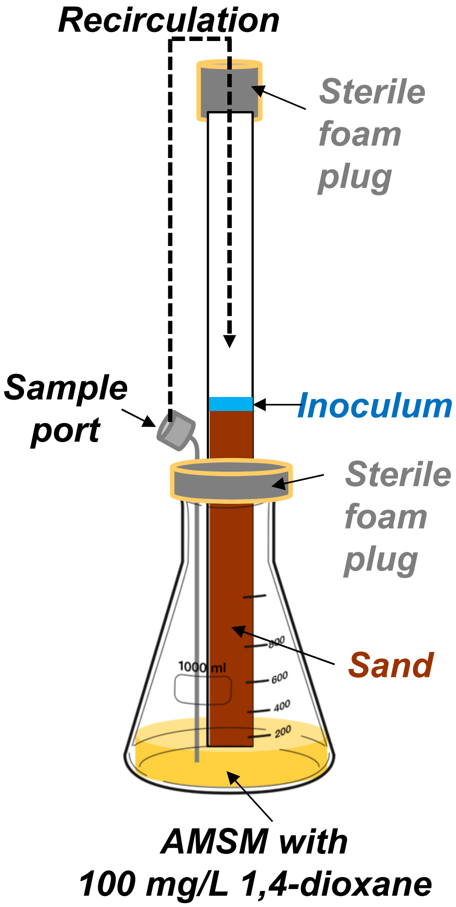
  sand ~0.4

  silt ~0.3



- Inocula:
  - 6 mL of CB1190 or BERK-1 grown on 1,4-dioxane;
  - ~0.13 mg protein/mL (~10^8 cells/mL)
- Positive control: directly inoculate the MSM

Movement of Isolates: Sand



Recirculation

Sterile foam plug

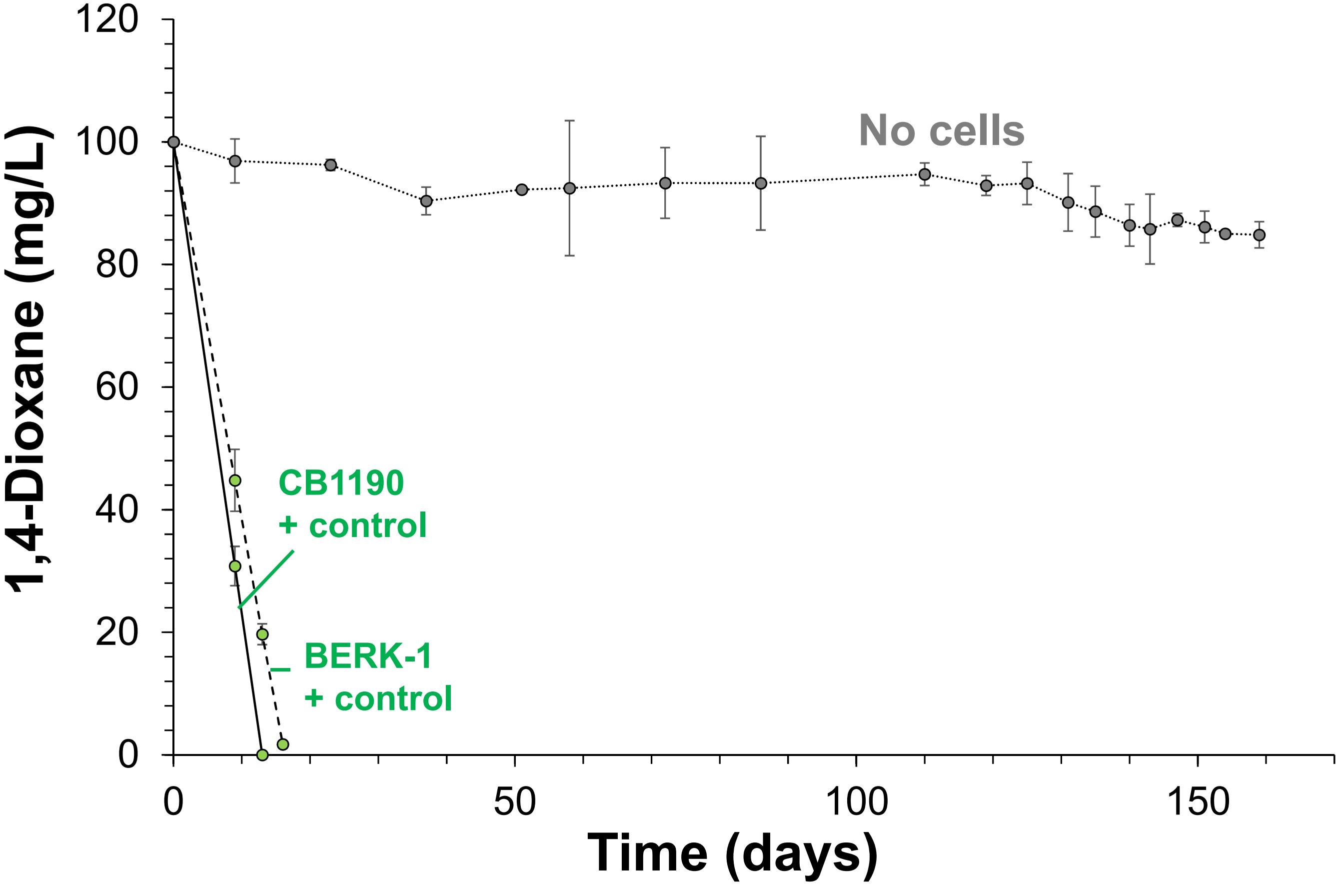
Sample port

Inoculum

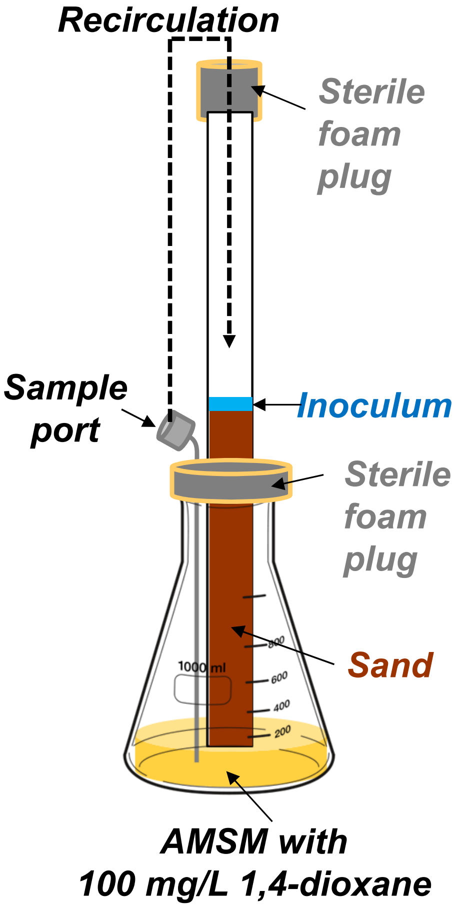
Sterile foam plug

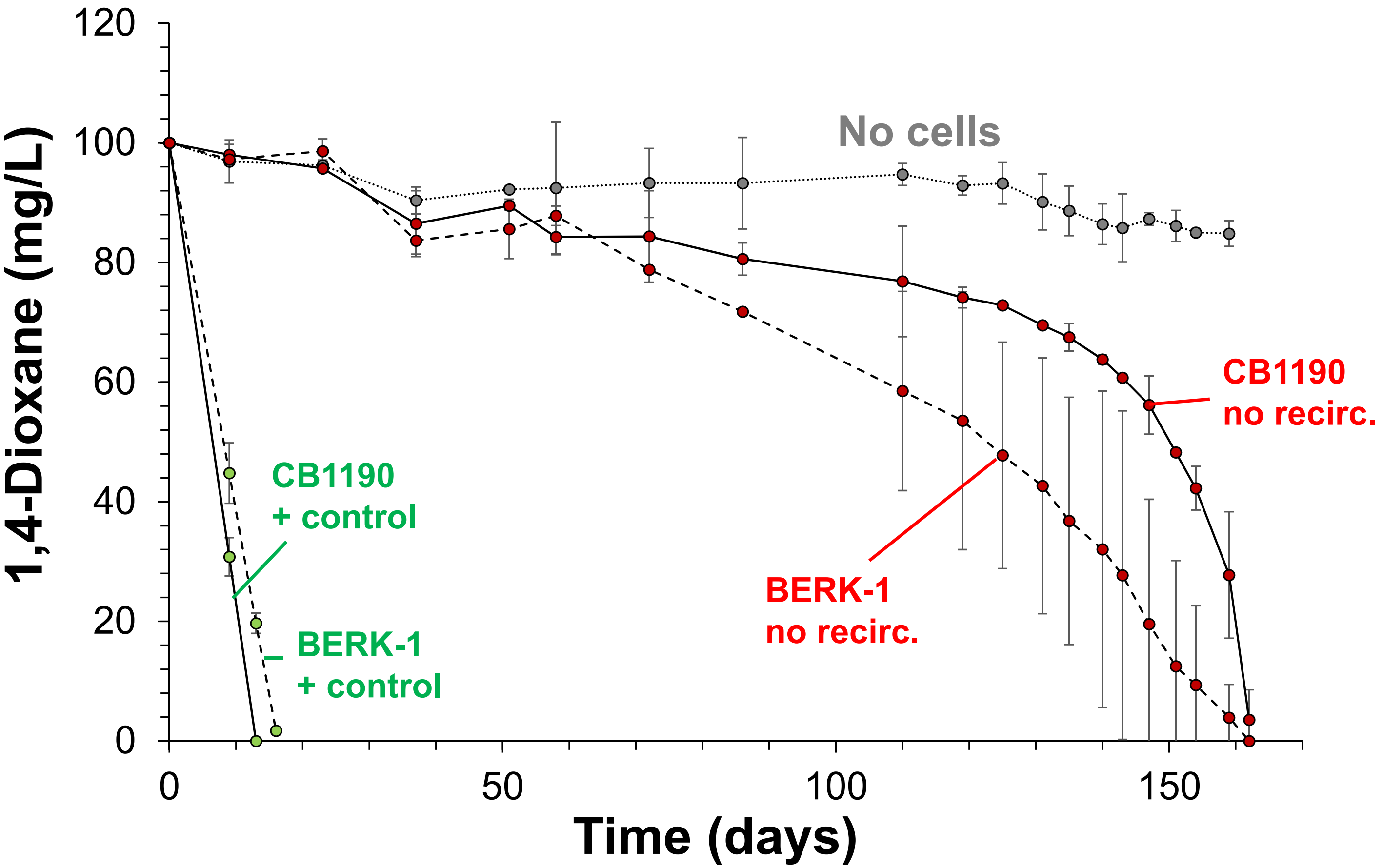
Sand

AMSM with 100 mg/L 1,4-dioxane

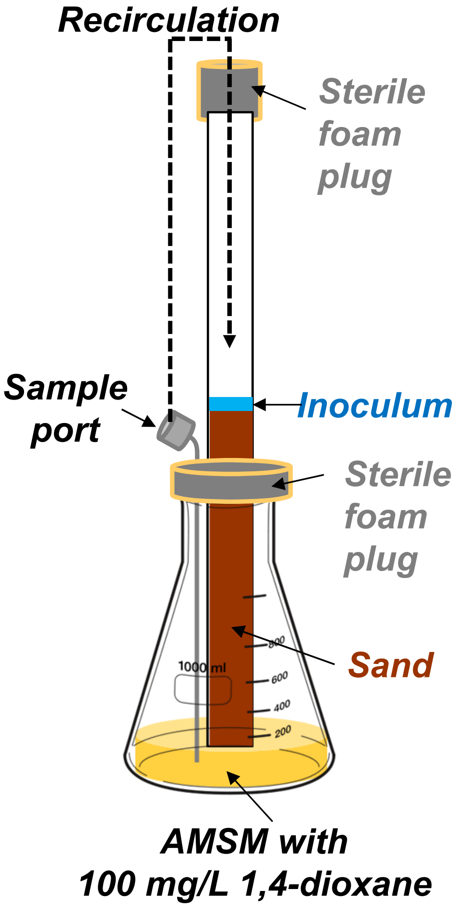


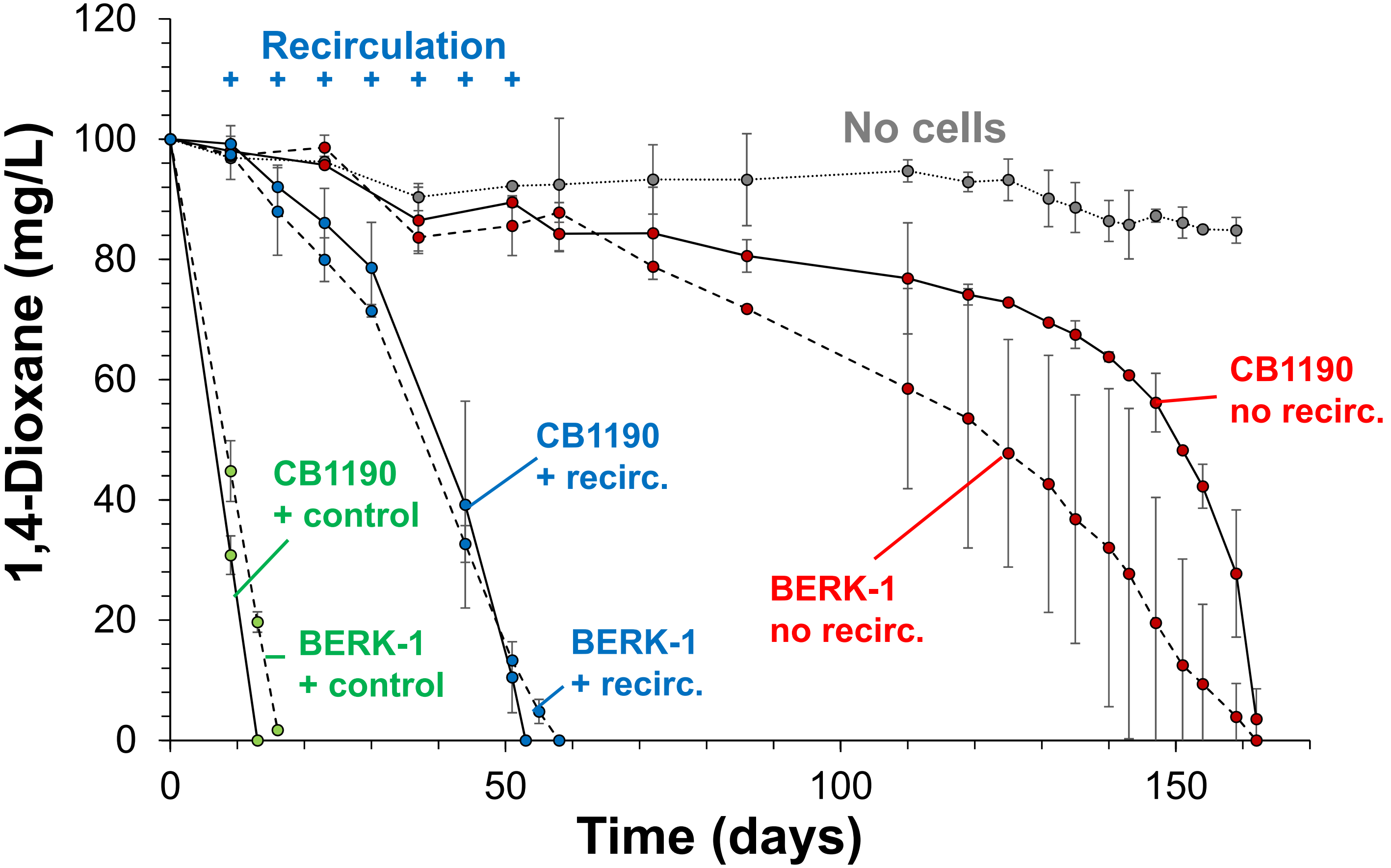
Movement of Isolates: Sand



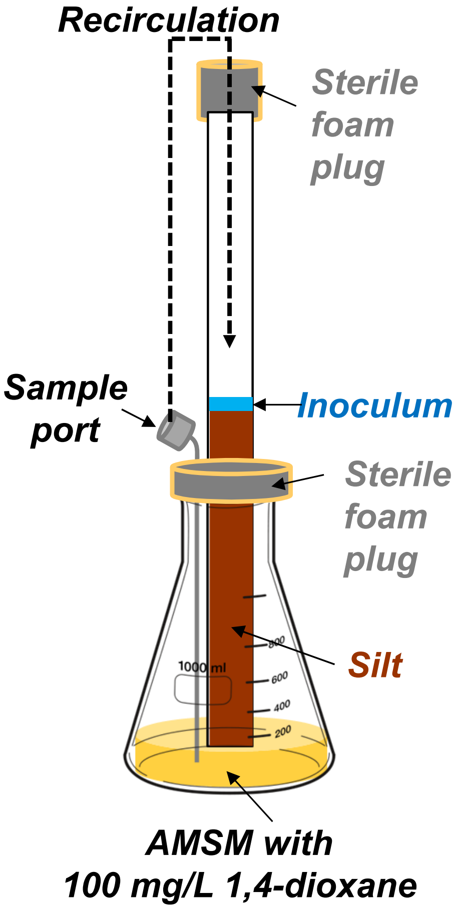


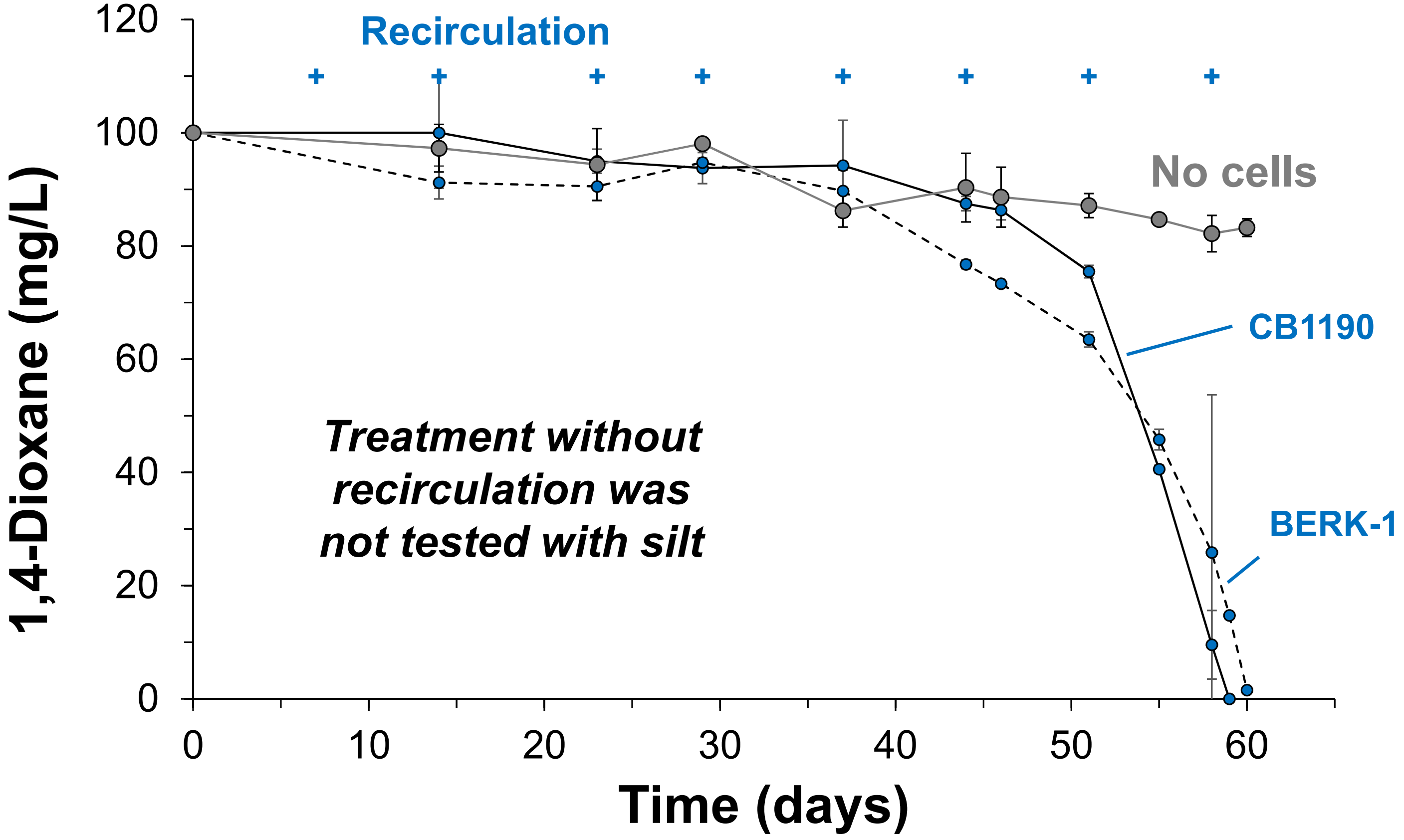
Movement of Isolates: Sand





Movement of Isolates: Silt





Treatment without recirculation was not tested with silt

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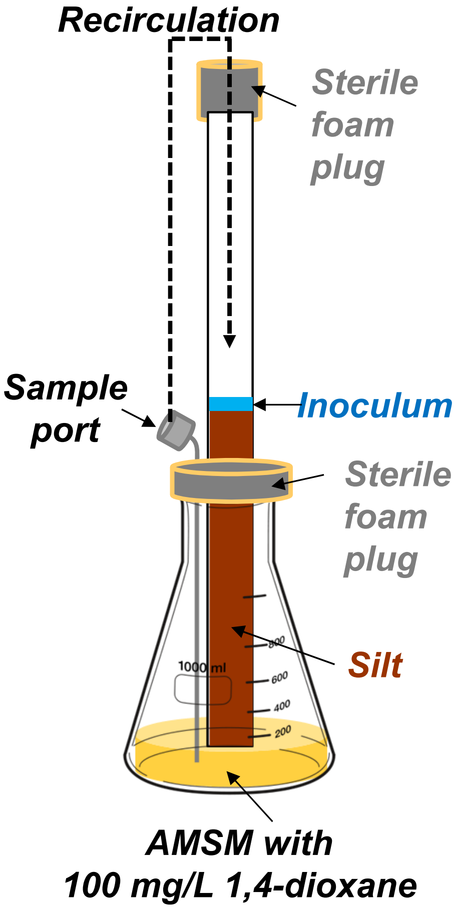
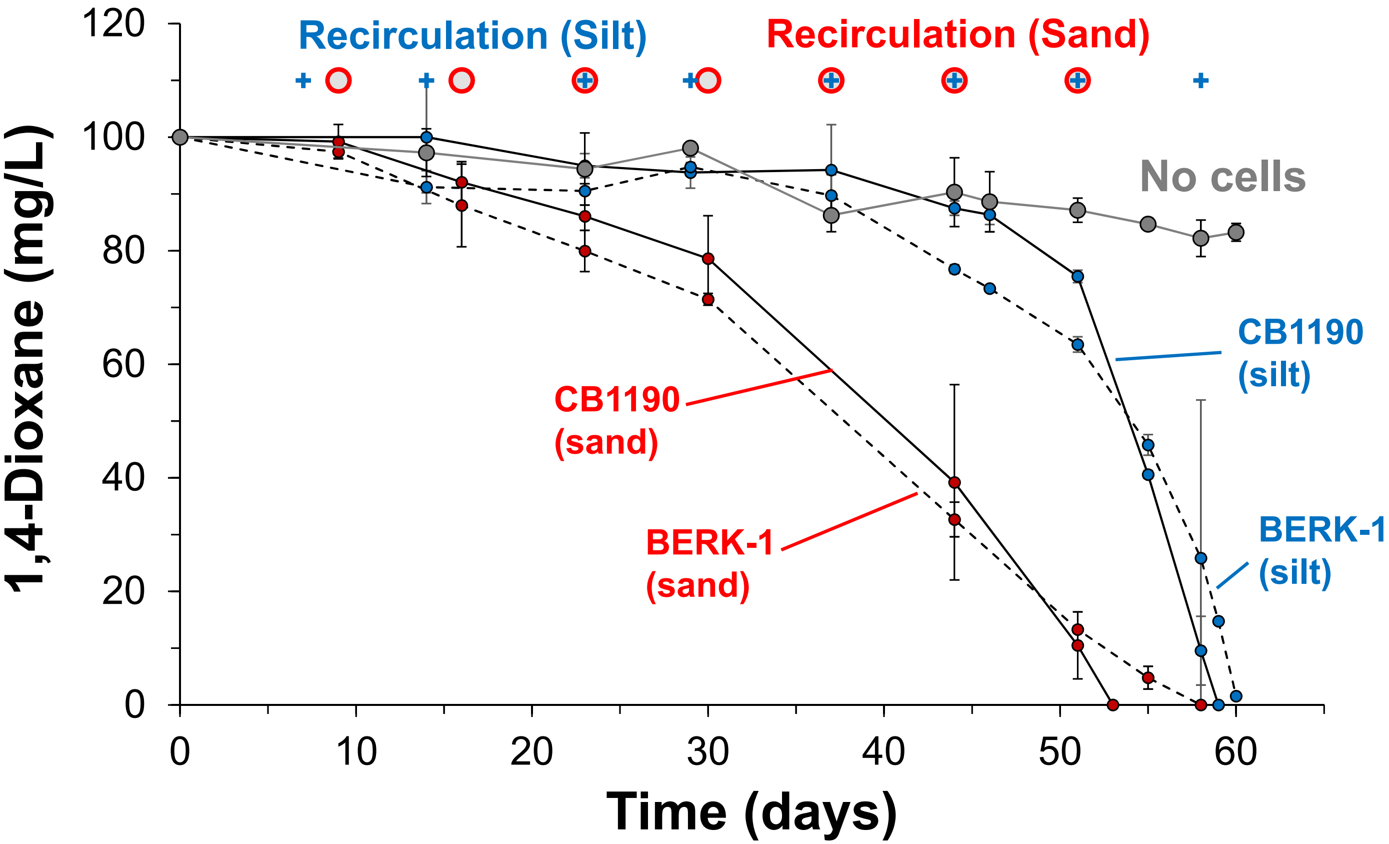
Movement of Isolates: Sand vs. Silt

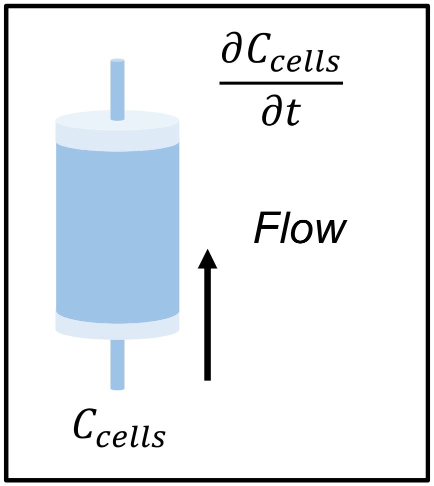




Conclusions

- 2 isolates obtained from aquifers; same genus and species as *Pseudonocardia dioxivorans*, different strain
- Likely presence of BERK-1 at southeastern US site confirmed with a ^{14}C assay; low 1st order rate consistent with microcosms
    - *In situ* 1,4-D degraders probably limited by low oxygen and/or nutrients
- CB-1190 initially moved more slowly than BERK-1 through sand and silt, but made up for this by a higher rate of degradation
- Clumping behavior of cultures grown on rich medium is not necessarily a good predictor of transport *in situ*
    - Transport may be facilitated by conditioning the cultures, e.g., starvation response to reduce size and clumping
- Need to perform continuous flow column tests and develop a predictive model for microbial transport

Modeling Approach for Transport of Microbes through Soil



Perform column studies to determine parameters

Microbes

Mechanical Dispersion

Molecular Diffusion

Darcy's velocity

$$\frac{\partial X}{\partial t} - \left(D_{D,X} + D_{F,X}\right)\frac{\partial^2 X}{\partial x^2} + (v_P)\frac{\partial X}{\partial x} + \frac{\partial X_S'}{\partial t}$$

$$=$$

$$\mu_X \cdot (X + X_S')$$

Specific Growth Rate (Monod)

Attachment and Detachment

Questions?