Supplementary Information to:

Co-encapsulation of slow release compounds and *Rhodococcus Rhodochrous* strain ATCC 21198 in gellan gum beads to promote the long-term cometabolic transformation of 1,1,1-trichloroethane, *cis*-1,2-dichloroethene and 1,4-dioxane

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S1. Alginate SRC Encapsulation

The methods used to encapsulate LNAPL SRCs in alginate were adapted from Soliman et al. who developed an emulsification based method to encapsulate essential oils at mass loadings as high as 30% (w/w) in alginate microspheres, ~500um in diameter [93]. The final method followed the same approach to make 2% (w/v) alginate pre-gel solution as section 3.4.1 above. Alginate was added to heated Nanopure and mixed for 30 minutes to complete hydration, and was then autoclaved. After removing the alginate pre-gel solution from the autoclave a known amount, typically around 40-50mL, was transferred to a 125mL wide mouth glass vial and allowed to cool to ~70°C.

After the solution cooled, an addition of Span-80 emulsifier was made to achieve a concentration of 0.1% (v/v). A known volume of LNAPL SRC was then added to the pre-gel solution and the mixture was emulsified using an IKA RW 20 digital overhead impeller mixer at 2500rpm for 10 minutes. Following emulsification, the pre-gel solution was transferred to a 50mL Falcon tube and allowed to cool to ~45°C. The pH was then adjusted to seven by addition of pH-7 phosphate buffer to achieve a final concentration of 4mMol total phosphate. If co-encapsulation of cells was desired a known volume of concentrated cell slurry suspended in 50mMol phosphate buffer was added here to the SRC containing alginate pre-gel solution and vortexed for 30 seconds. Following the addition of cells or just after pH adjustment, the emulsified pre-gel solution was transferred to 5-20mL liquid syringes fitted with a 25 gauge x 1 inch needle (Figure S1). The pre-gel solution was then extruded into 900mL of 0.25% (w/v) $CaCl_2$ solution from a distance of 2-5cm above liquid surface. $CaCl_2$ solution was continuously mixed with a magnetic stir plate at 150-250rpm. The beads were allowed to crosslink for a total of 60 minutes measured from the time that the last bead was formed. Figure S2 shows the uniform size of the beads that were formed.

Soliman, E.A., El-Moghazy, A.Y., El-Din, M.S.M., and Massoud, M.A. (2013). Microencapsulation of Essential Oils within Alginate: Formulation and Evaluation of Antifungal Activity. J. Encapsulation Adsorpt. Sci. 03, 48–55.

Figure S1. Calcium-alginate macrobead formation by extrusion into crosslinking solution.

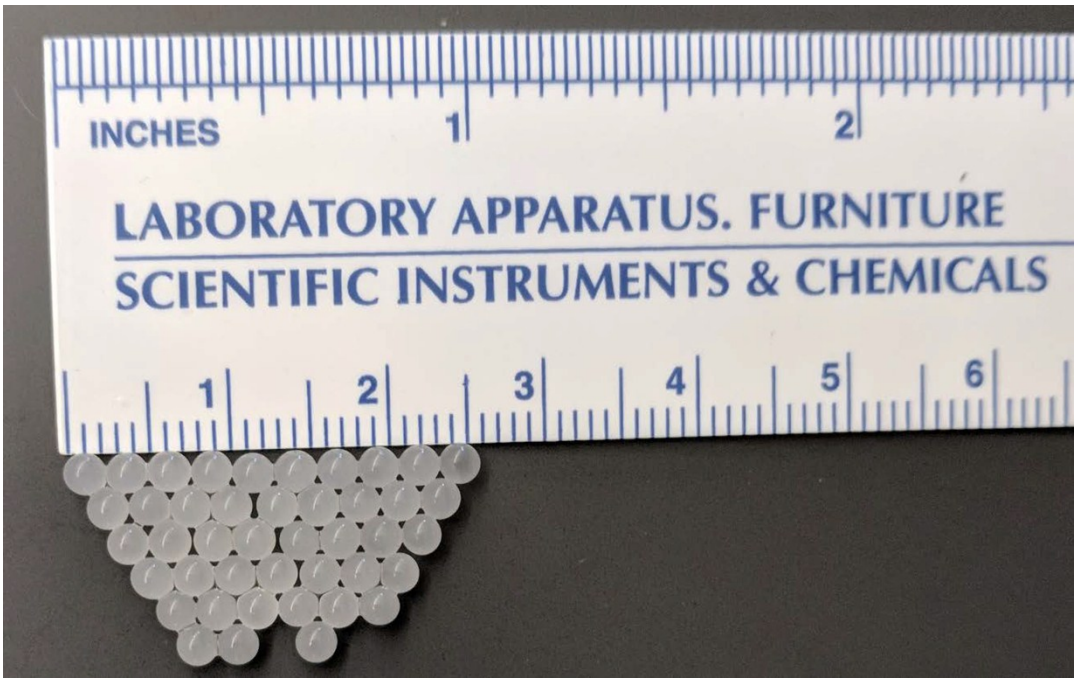


Figure S2. Gelated alginate macrobeads with a VWR ruler for scale.

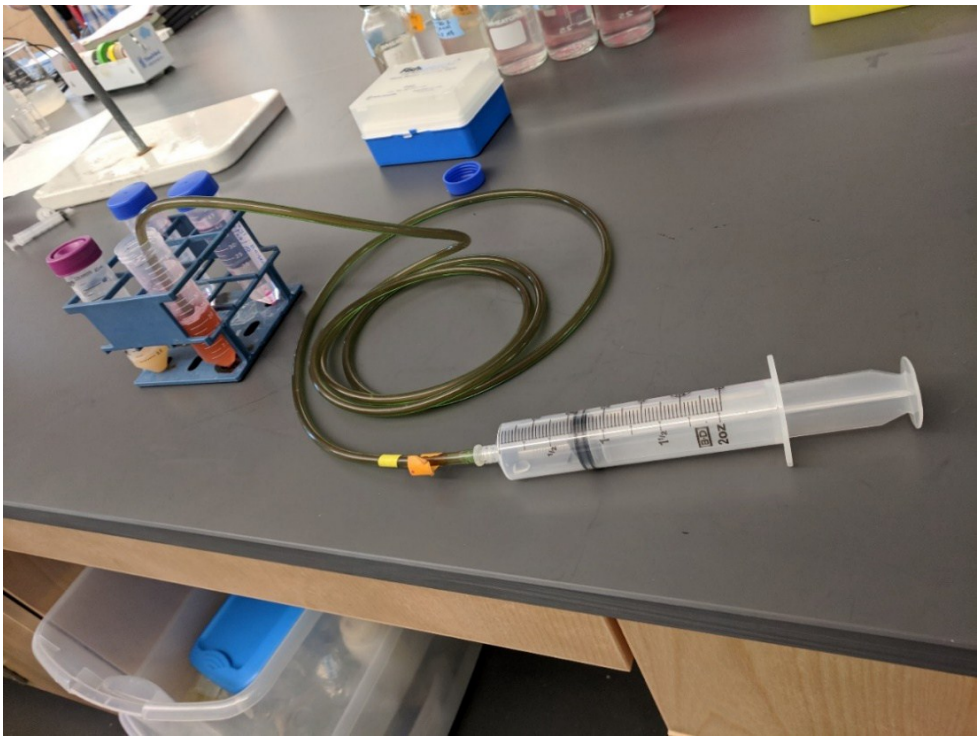


Figure S3. Gellan gum macrobead generation step 1. Warm gellan gum pre-gel solution is drawn into the green tubing with a 60mL syringe. For better visualization GG pre-gel solution was dyed orange.

Figure S4. Extrusion of solidified gellan gum cylindrical sections from rubber tubing onto Parafilm. For better visualization GG pre-gel solution was dyed orange.

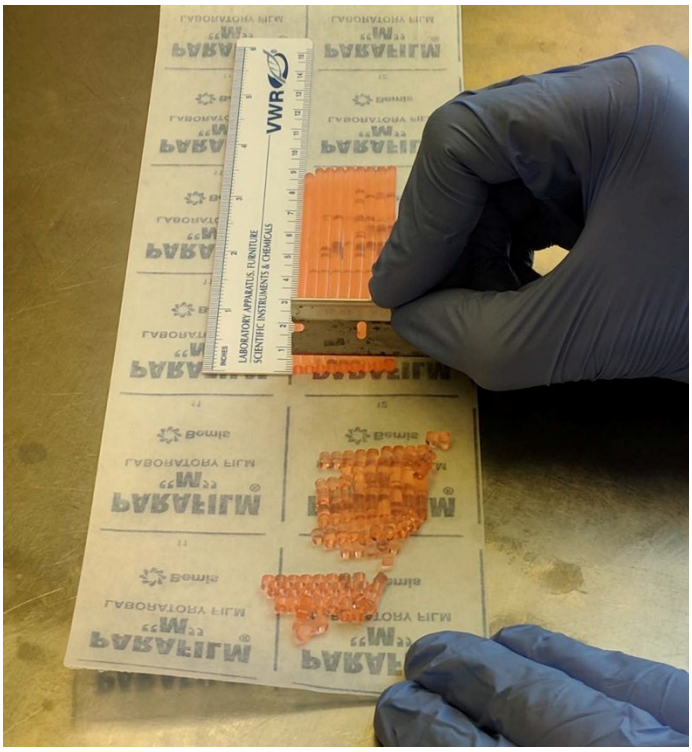


Figure S5. Manual cutting of gellan gum strands into 2mm right cylinders for experimental use. For better visualization GG pre-gel solution was dyed orange.

Figure S6. Gellan gum beads with ATCC 21198 and TBOS co-encapsulated 2 mm in diameter by 2 to 3 mm in length.

Table S1 Encapsulated cell viability assessment. Percent difference is calculated as the percent change from suspended cell utilization rates to encapsualted cell utilization rates.

Encapsulation Matrix/Method	**Benchmark Suspended Cell Utilization Rate (µmol/day/mg)**	**Encapsulated Cell Utilization Rate (µmol/day/mg)**	**Percent Difference**
Alginate Macro-bead	15.78	14.19	-10.1%
Gellan Gum Macro-bead	13.33	13.25	-0.6%
Gellan Gum Micro-bead	11.11	11.03	-0.7%

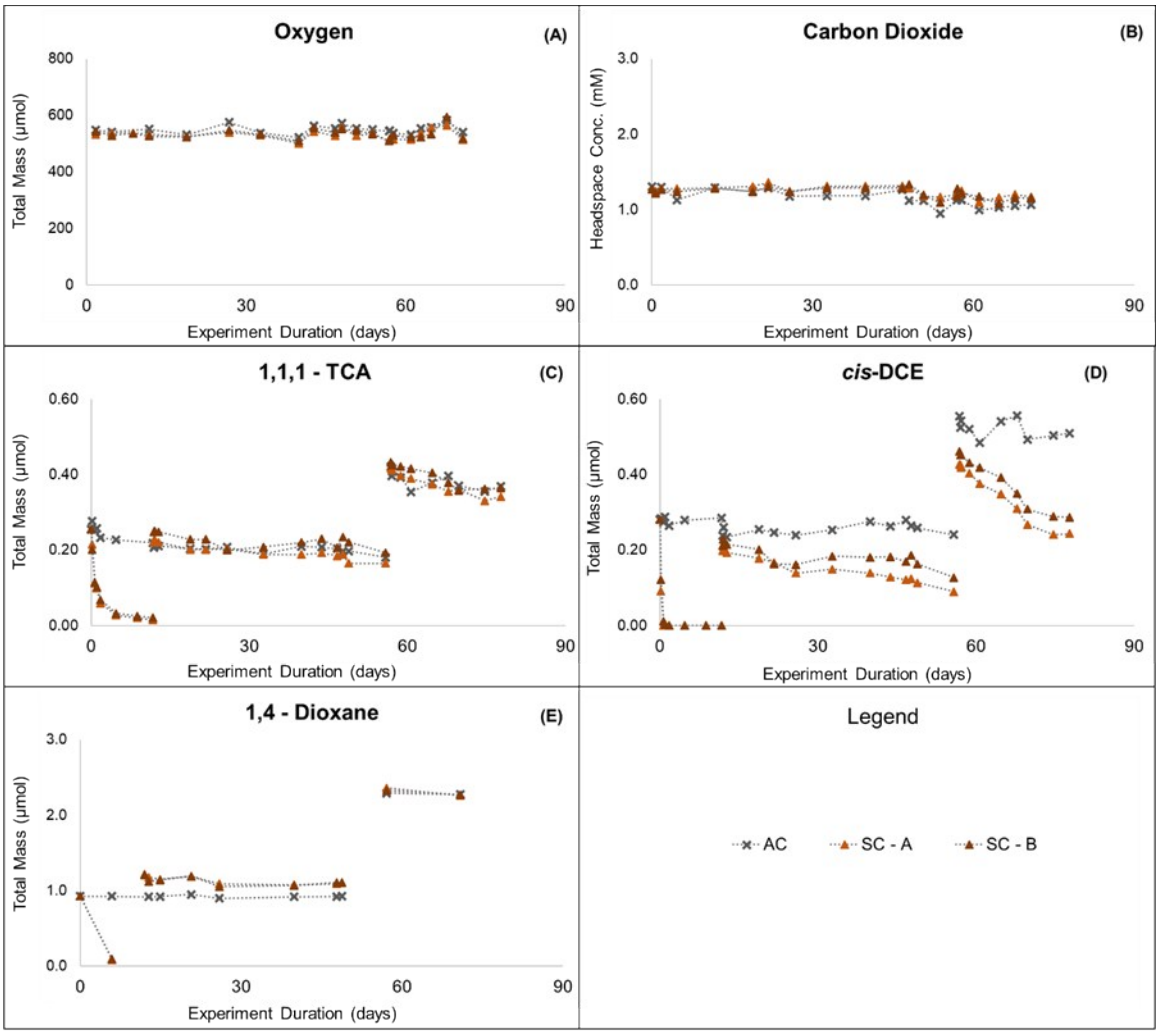


Figure S7. Suspended Cell Control Reactor respiration data (A and B) and contaminant data (C-E). (AC) – Abiotic control. (SC) – Suspended cell control reactors. Alphabetical designations (A,B,C) are used to signify replicate reactors. AC has a single reactor, SC are duplicate reactors with suspended cells.

Table S2. Rates of oxygen consumption in the CET reactors.

O2 Addition	Method	Time of Addition (days)	Estimated O2 Utilization Rate (umol/day)	
			CET - A	CET - B
0	Initial O2	0	6.0	9.2
1	Pure O2 Addition 1	40	18.6	38.1
2	Pure O2 Addition 2	47	23.1	40.5
3	Atmosphere Headspace Equilibration	57	18.3	34.5

Table S3. Rates of oxygen consumption in the CET_2 reactors.

Treatment	Abbreviation	O2 Utilization Rate (umol/day)
Abiotic Control	AC	0.04 ± N/A
Suspended Cell Remediation Control	SCRC	0.09 ± 0.02
C-encapsulated T2BOS/ATCC 21198	CET2	0.48 ± 0.003

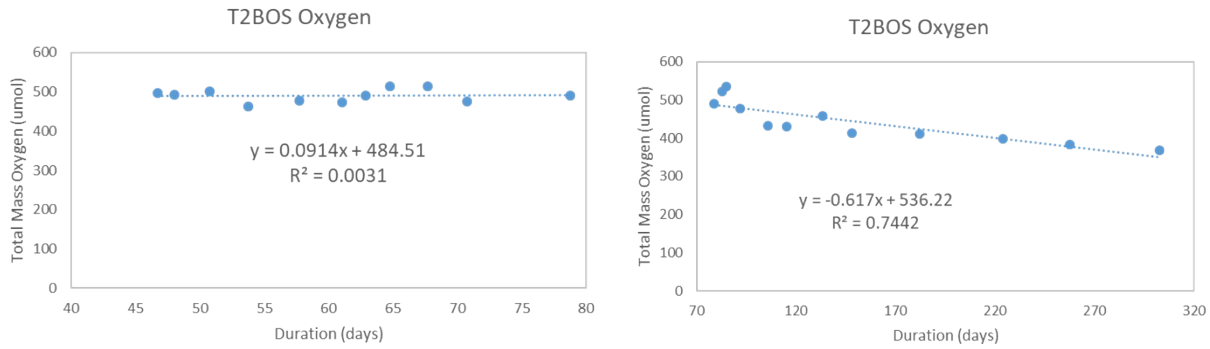


Figure S8. Rates of O_2 consumption in the CET_2 reactors with GG beads co-encapsulated the ATCC 2198 and T2BOS. Rates were determined over the early and later time periods of the incubation.

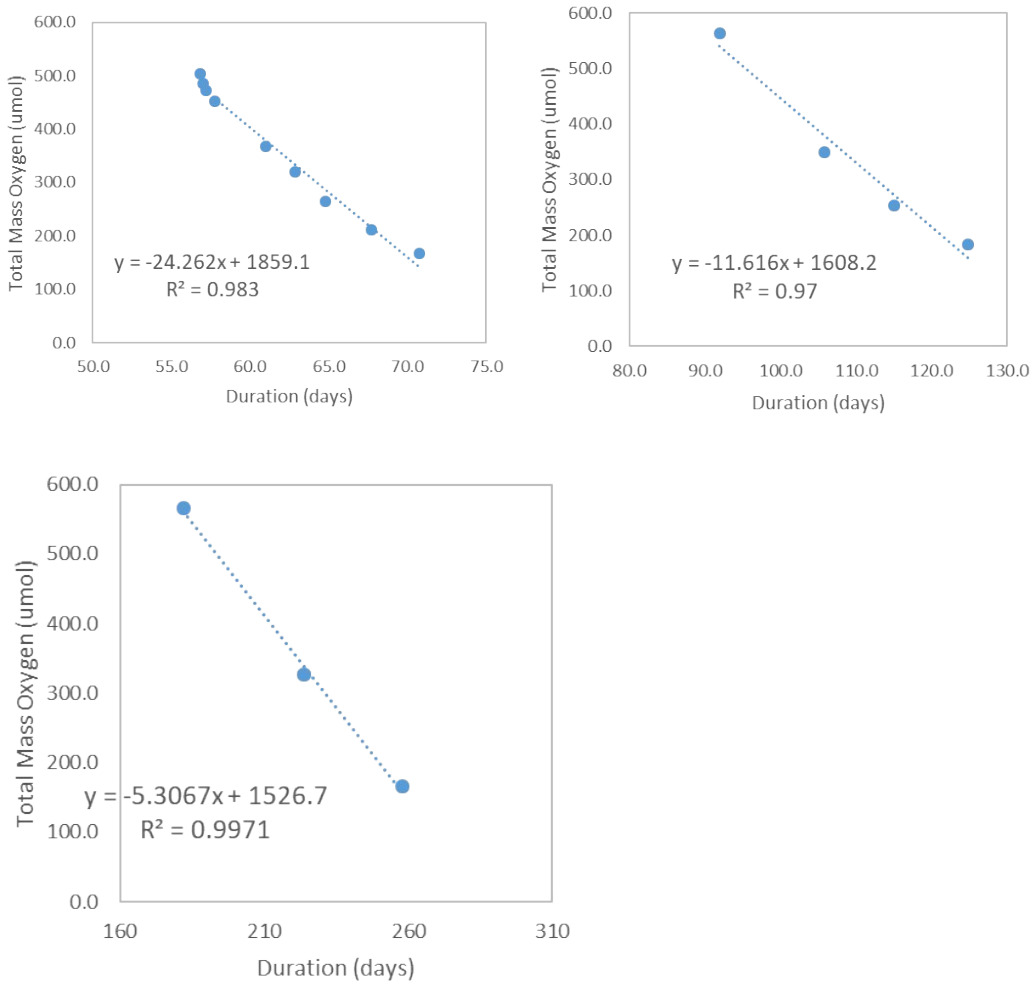


Figure S9. Rates of O_2 consumption in the CET reactors. Rates were determined for different periods of 300 day incubation and show a gradual slowing in the rate of O_2 consumption.

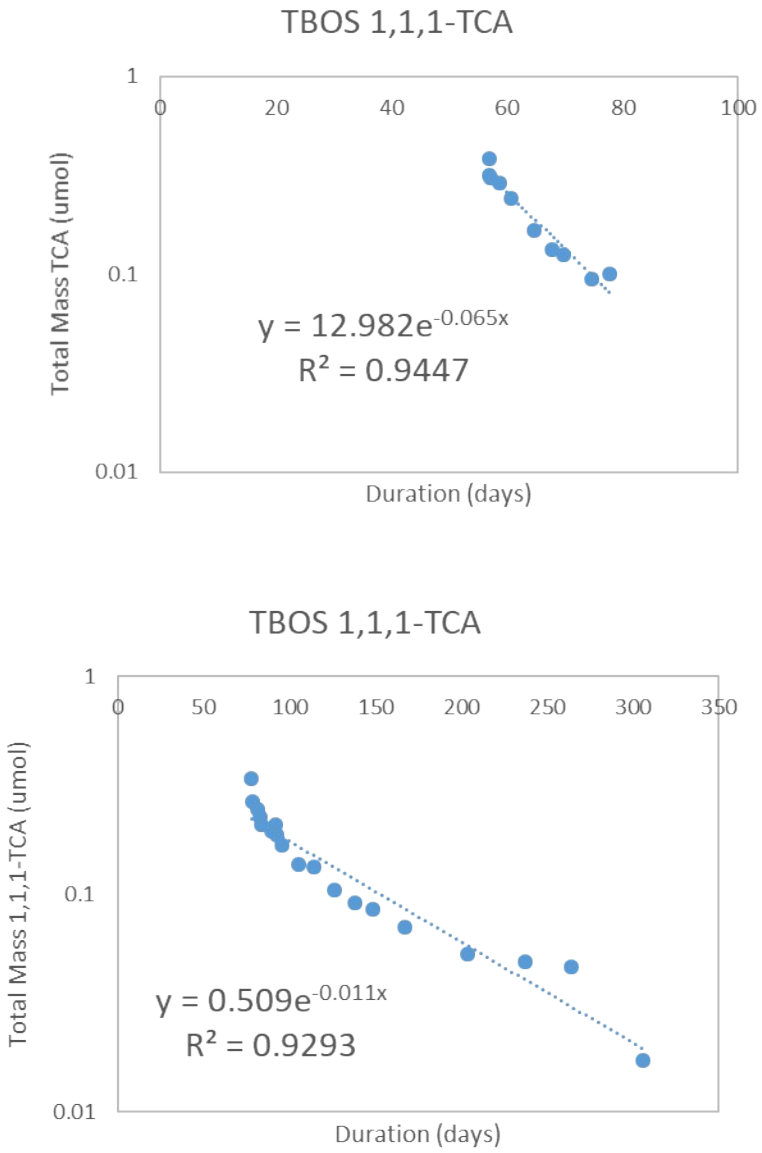
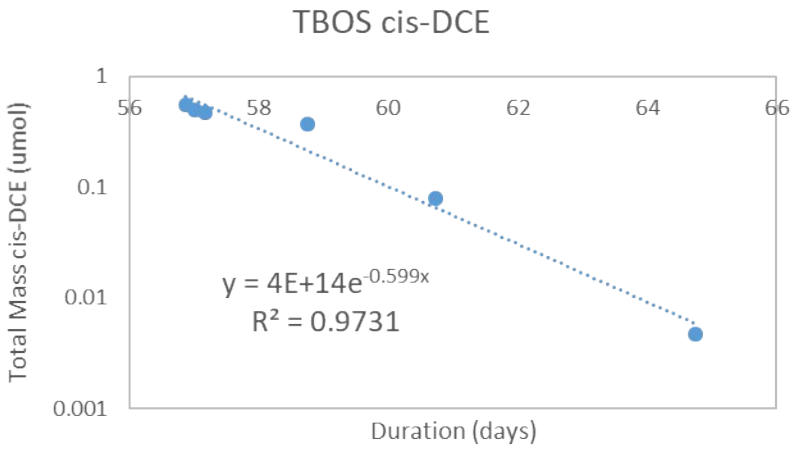


Figure S10. First-order plots of 1,1,1-TCA concentration versus time in a CET reactor with GG beads co-encapsulated the ATCC 2198 and TBOS. Rates were determined for different periods of 300 day incubation and show a slowing in the rate of 1,1,1-TCA transformation with time.



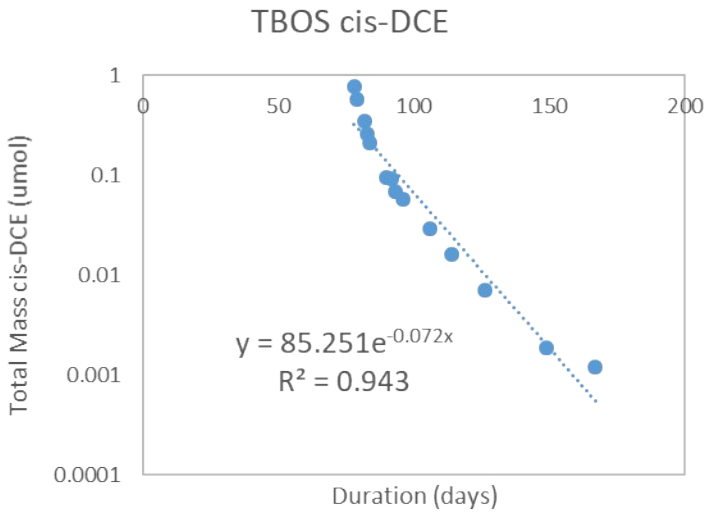


Figure S11. First-order plots of cis-DCE concentration versus time in a CET reactor with GG beads co-encapsulated the ATCC 2198 and TBOS. Rates were determined for different periods of 300 day incubation and show a slowing in the rate of *cis*-CCE transformation with time.

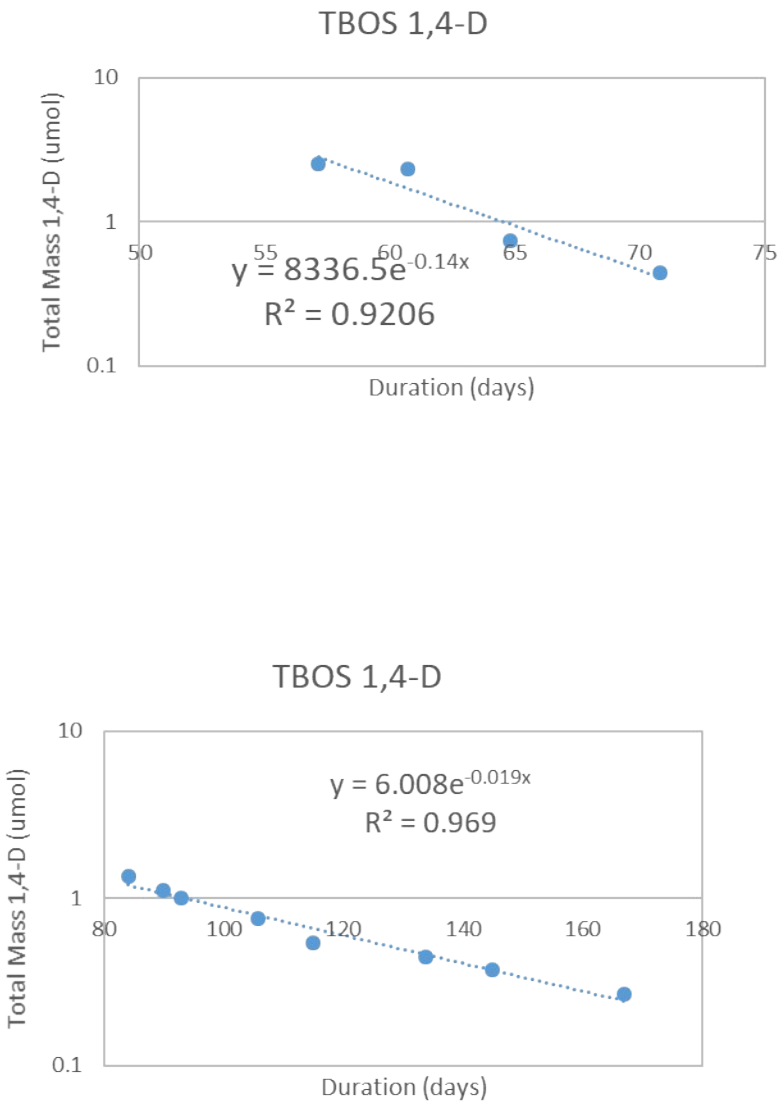


Figure S12. First-order plots of 1,4-D concentration versus time in a CET reactor with GG beads co-encapsulated the ATCC 2198 and TBOS. Rates were determined for different periods of 300 day incubation and show a slowing in the rate of 1,4-D transformation with time.

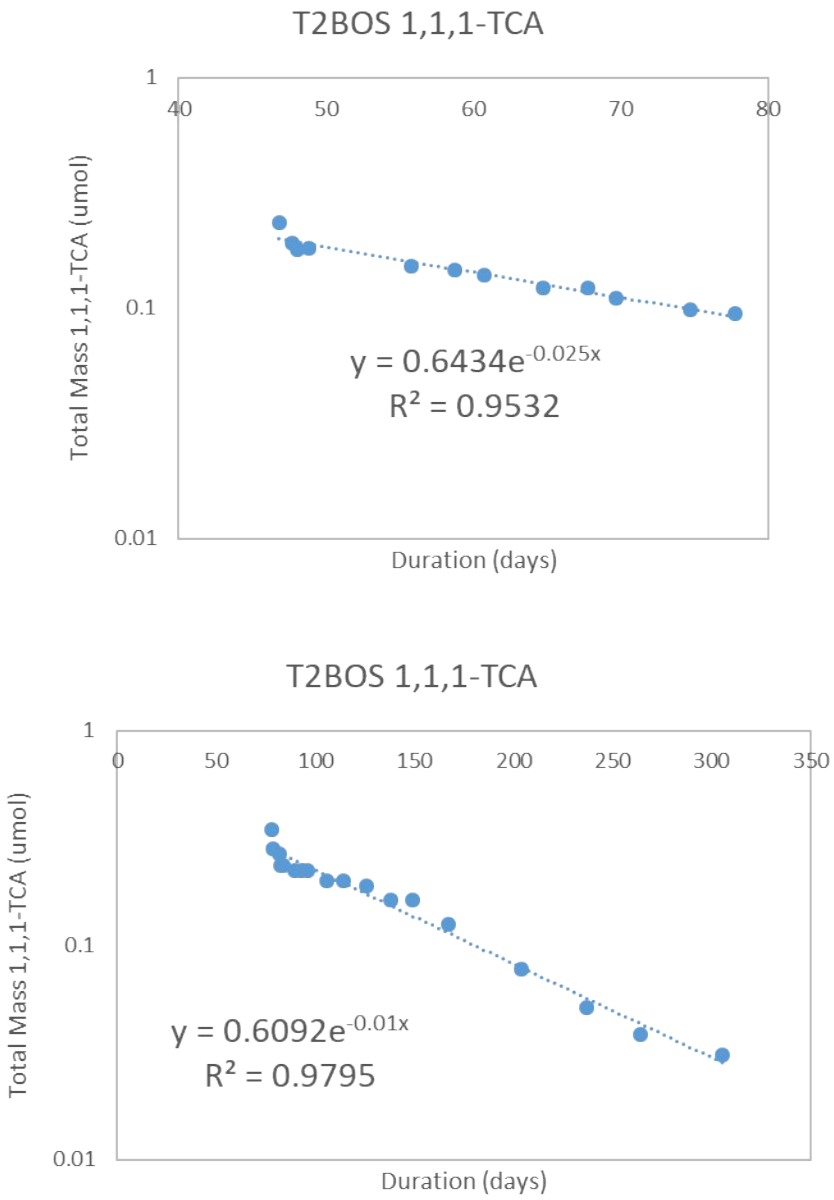
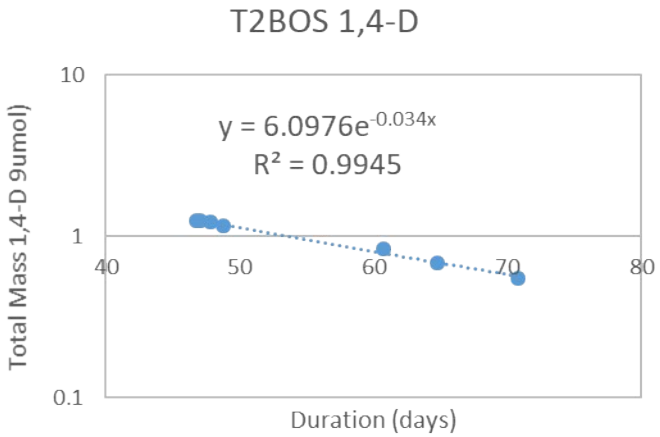


Figure S13. First-order plots of 1,1,1-TCA concentration versus time in a CET_2 reactor with GG beads co-encapsulated the ATCC 2198 and T2BOS. Rates were determined for different periods of 300 day incubation and show a slowing in the rate of 1,1,1-TCA transformation with time.



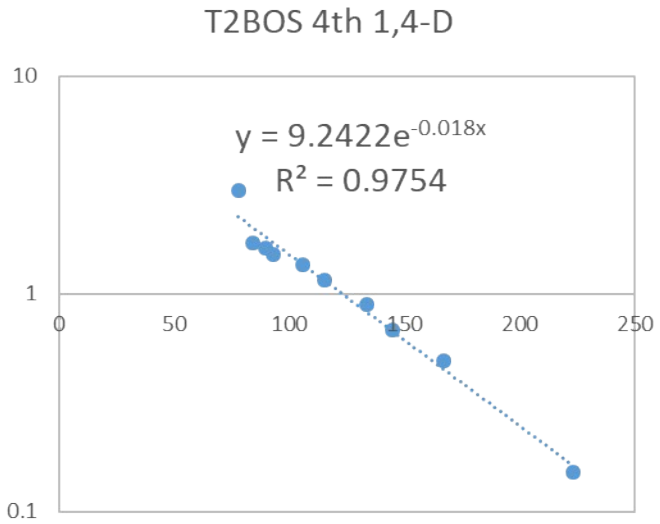


Figure S14. First-order plots of 1,4-D concentration versus time in a CET2 reactor with GG beads co-encapsulated the ATCC 2198 and T2BOS. Rates were determined for different periods of 300 day incubation and show a slowing in the rate of 1,4-D transformation with time.

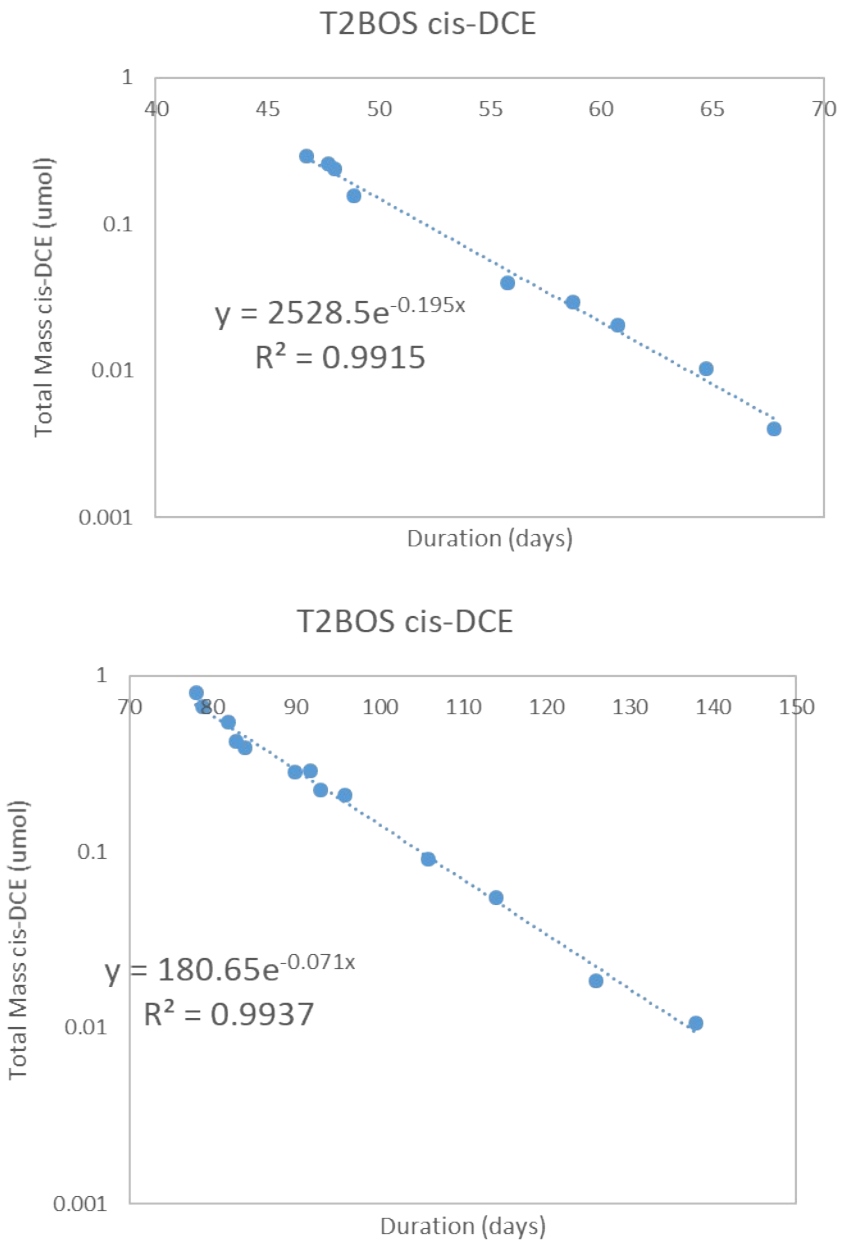
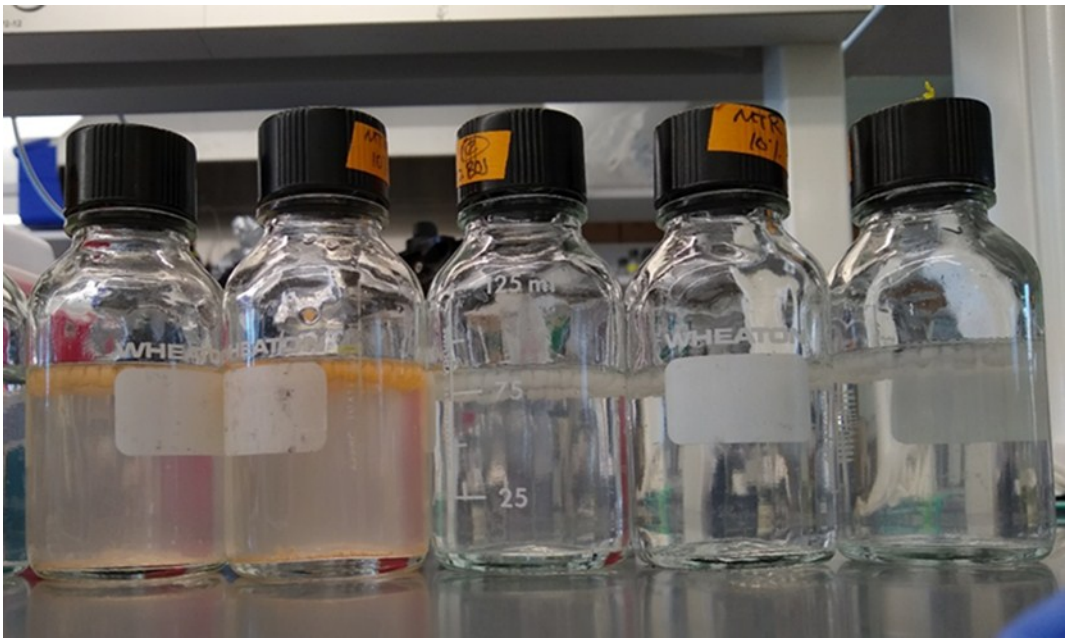


Figure S15. First-order plots of *cis*-DCE concentration versus time in a CET_2 reactor with GG beads co-encapsulated the ATCC 2198 and T2BOS. Rates were determined for different periods of 300 day incubation and show a slowing in the rate of 1,4-D transformation with time.



TBOS/21198 (CET) Reactors T2BOS/21198 (CET2) Reactors

Figure S16. Two CET reactors with the TBOS/21198 GG beads (on the left) and three CET2 reactors T2BOS/21198 GG beads (CET_2 reactors on the right) after 232 days of incubation on a shaker table operating at 20˚ C and 100 rpm.

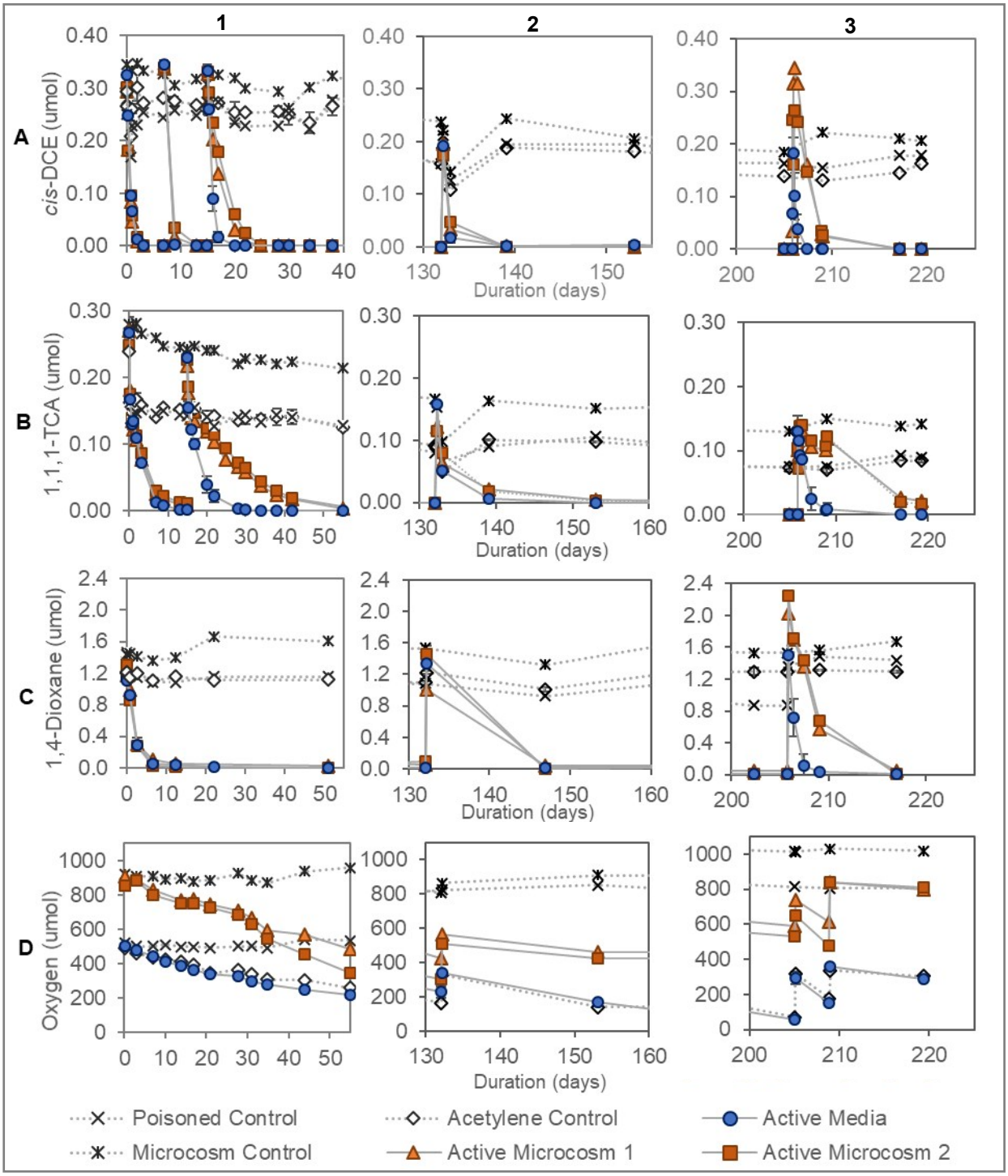
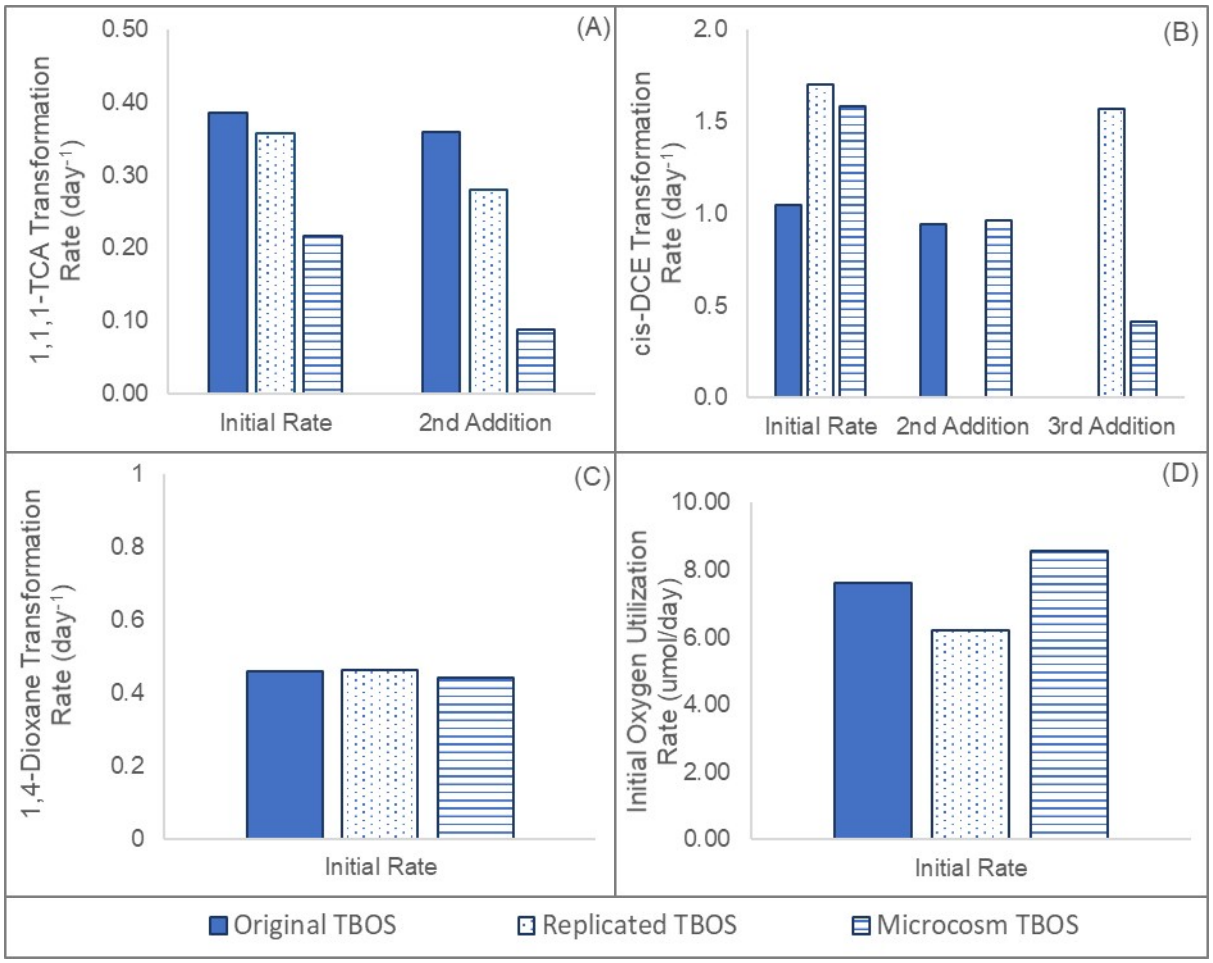


Figure S17. Longer-term incubation of groundwater microcosms and media with TBOS and ATCC 21198 in GG beads. 2 grams of GG beads containing 8% TBOS by WT were added

to the microcosm and media batch reactors. The panel on the left presents results presented in Figure 9.



S18. Pseudo-first order rate comparison for 1,1,1-TCA, cis-DCE and 1,4-dioxane between gellan gum coencapsulated beads containing 8% TBOS by WT. (Original TBOS) – Coencapsulated cells with 8% (wt/wt) TBOS, the data which is presented in Figure 4. (Replicated TBOS) - Coencapsulated cells with 8% (wt/wt) TBOS that were made separately to those used in Figure 4 to test replicability. (Microcosm TBOS) - Coencapsulated cells with 8% (wt/wt) TBOS in a microcosm system. Time of contaminant additions are approximately the same between treatments. Missing rate data for some additions of cis-DCE is due to inability to calculate a rate from the amount of data collected.