Leveraging the chemo-physical interaction of halorespiring bacteria with solid surfaces To enhance halogenated organic compounds bioremediation

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Background

The Problem:

- Chloroethenes and polychlorinated biphenyls contaminate water resources
- Microbial dechlorination feasible, but confounded by sorption to geosorbents
- Poor understanding of how dechlorination is impacted by surfaces

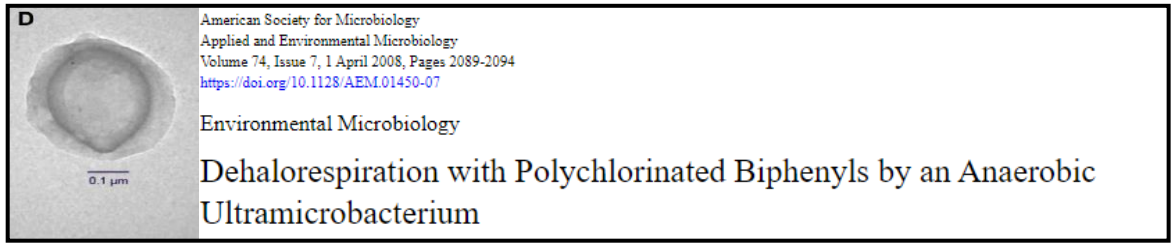
Specific Aims:

- Quantitatively evaluate the effect of material properties on microbial dechlorination
- Evaluate the effect of materials on localized bacterial populations and develop innovative materials for enhanced bioremediation
- Predict field-scale performance of innovative bioamendment materials through advanced site models

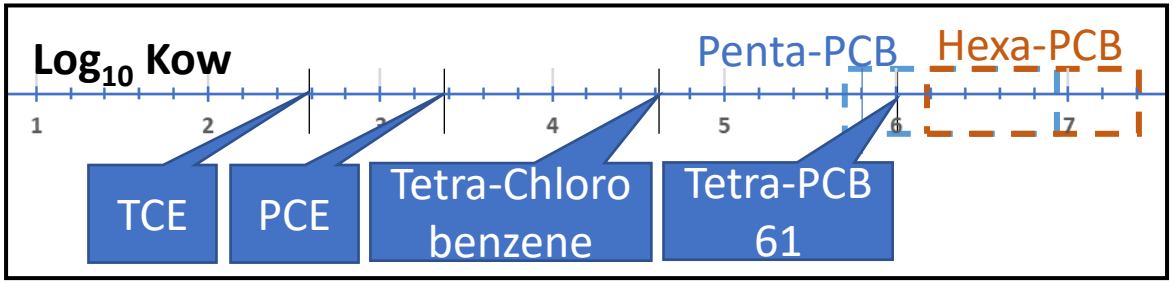
Bacterial Model

***Dehalobium Chlorocoercia* DF-1**

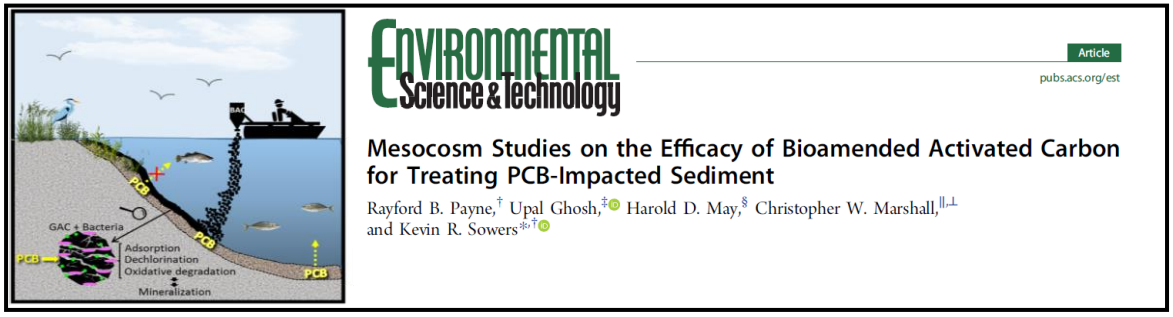
Isolated in pure culture since 2008
(May et al., 2008)



Dehalogenation wide range of chlorinated compounds
(Wu et al, 2002 a,b)



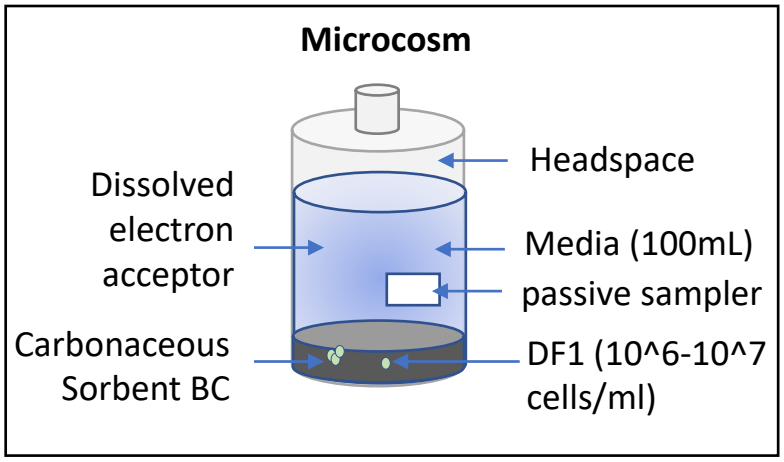
Already used for in situ bioremediation of PCB contaminated sites
(Payne et al., 2019)

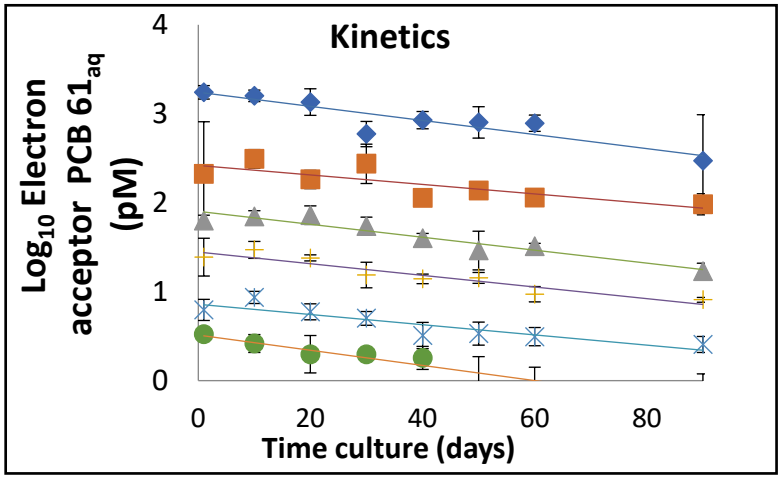


Specificities

- Strictly anaerobic
- Slow growing (G=2-4 days)
- Low cell density (10^6 - 10^7 cells/mL)
- Requires Desulfovibrio sp. (DSV) coculture or DSV extract

Study design





PCE and PCB dechlorination kinetics accuracy, reproducibility in absence of BC

Precise DF1 cells enumeration in aqueous and solid phases

Media transition from Estuarine (E) to Freshwater (F) media

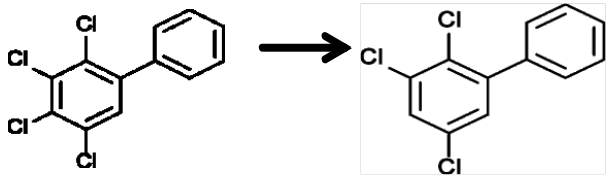
Black Carbon (BC) sorption capacity and physico chemical properties

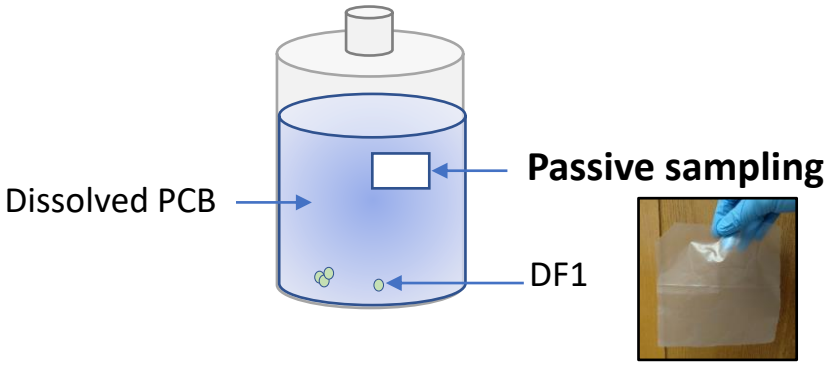
Model True kinetics in presence of solids

Link BC properties to dechlorination kinetic changes

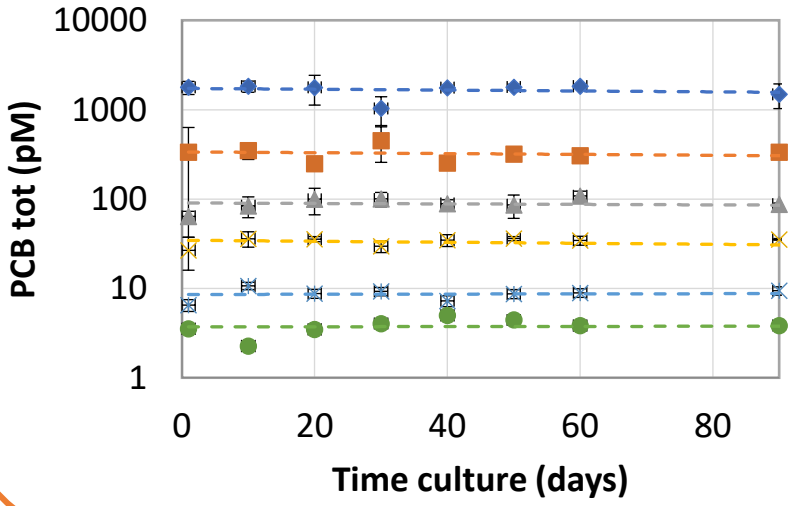
PCB and PCE monitoring and mass balance

PCB 61 → PCB 23

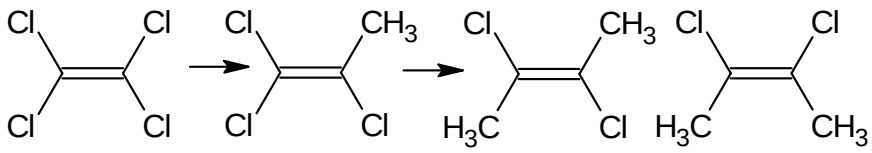


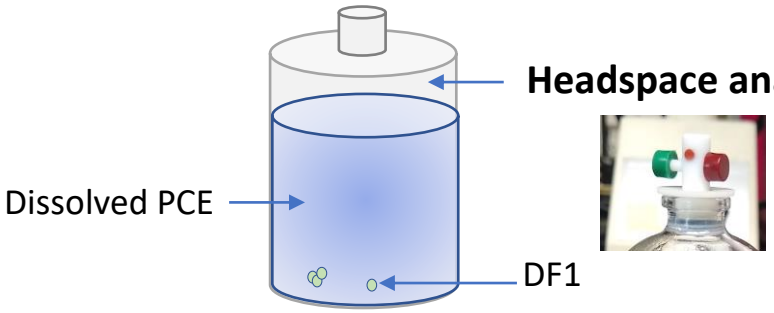


Total PCBs over time

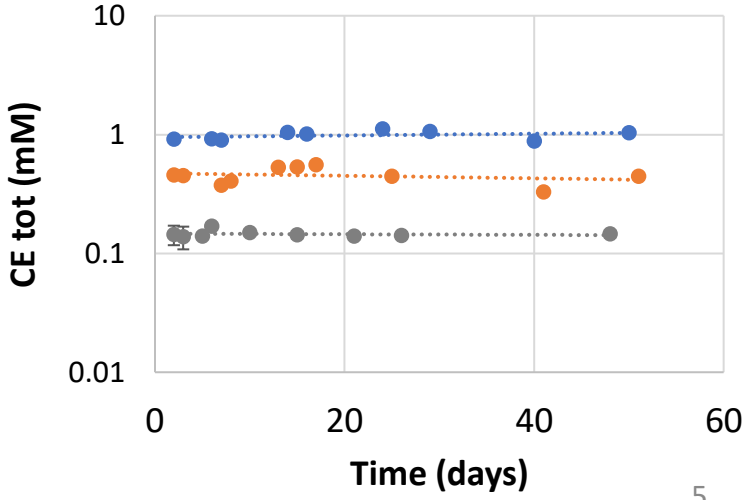


PCE → TCE → Cis, trans DCE





Chlorinated Ethenes over time



BC selection and Sorption isotherms (1/2)

Carbonaceous material	Manufacturer	C(%)	Surface area (g/cm^2)	Skeletal density (g/cm^3)	Bulk density (g/cm^3)	Electron accepting cap. (mole/g)
Pine dust biochar	Biochar Eng Corp.	22.1 ± 0.5	109 ± 2	0.98	0.43	3.08
Coconut GAC	Calgon Corp.	90.8 ± 2.7	1305 ± 11	1.45	0.57	3.16
Coal GAC	Calgon Corp.	80.9 ± 1.4	1116 ± 25	1.61	0.64	3.62

Method

- GAC sterilised and anaerobic
- 500 ml F- media(pH=6.9±0.1).
- Neat PCE is then spiked for C_0 0.77 mM . Agitation 170rpm

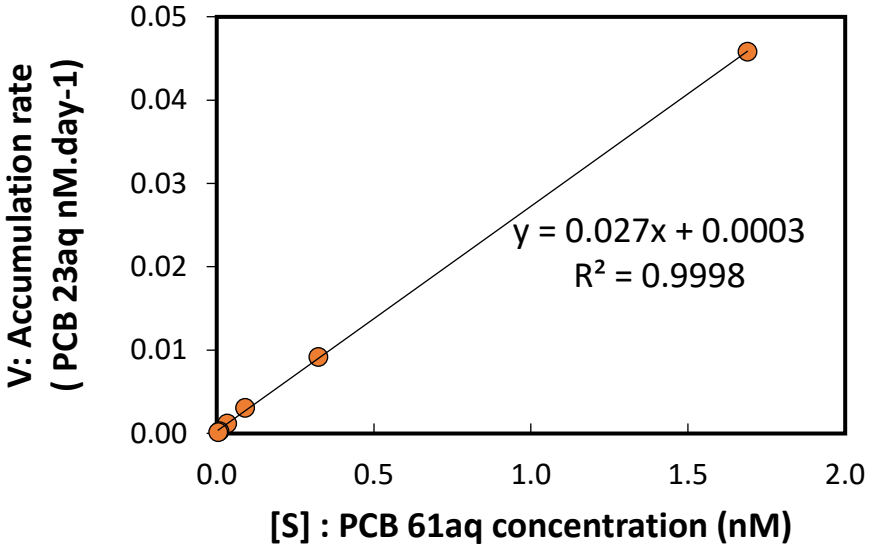
$$Total\ PCE = C_0V_{aq} = C_gV_g + C_{aq}V_{aq} + Q_eM_{BC} = \frac{HC_{aq}}{RT} \times V_g + C_{aq}V_{aq} + Q_eM_{BC}$$

$$Q_e = (TOTPCE - \frac{HC_{aq}}{RT} \times V_g - C_{aq}V_{aq})/M_{BC}$$

PCB and PCE dechlorination kinetics

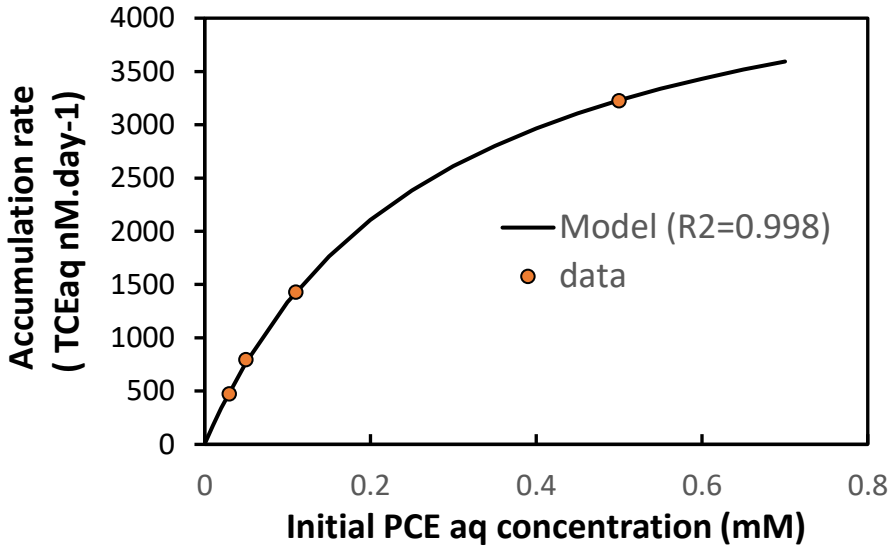
	PCB $61_{(sat.)}$ → PCB 23	$PCE_{(<0.2mM)}$ → TCE
Estuarine media 0.14M NaCl	-0.016 +/-0.0046 day^{-1} (n=15)	-0.041+/- 0.0094 day^{-1} (n=3)
Freshwater media 0.05M NaCl	-0.010 day^{-1} (n=1)	-0.018 +/- 0.0063 day^{-1}, (n=4)

First order dechlorination kinetics
Up to PCB 61 saturating level



Lombard et al., 2014

PCE dechlorination kinetics
approaching saturation conc.

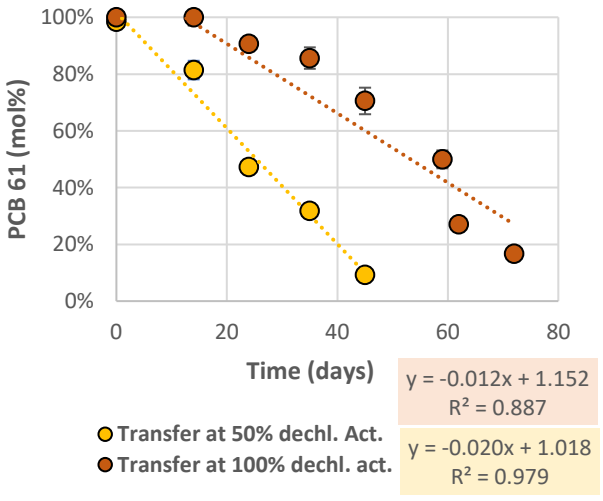


Modeling ongoing

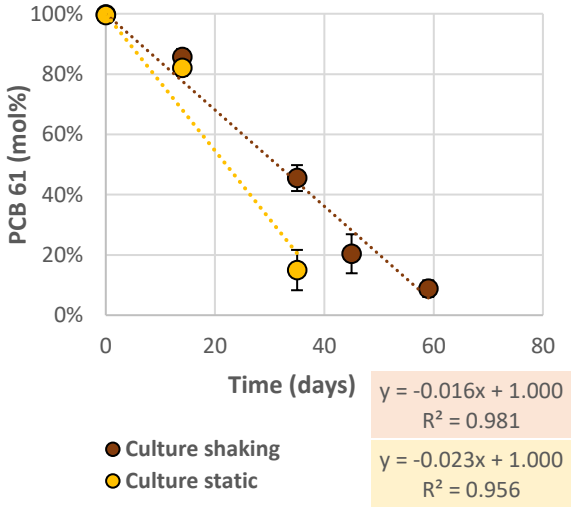
PCB and PCE dechlorination kinetics

	PCB 61$_{(sat.)}$ → PCB 23	PCE$_{(<0.2mM)}$ → TCE
Estuarine media 0.14M NaCl	-0.016 +/-0.0046 day^{-1} (n=15)	
Freshwater media 0.05M NaCl		-

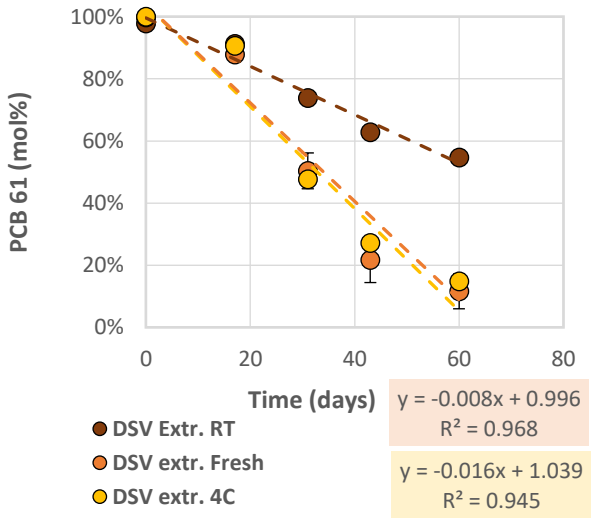
Physiological state inoculum



Culture condition



DSV extract quality



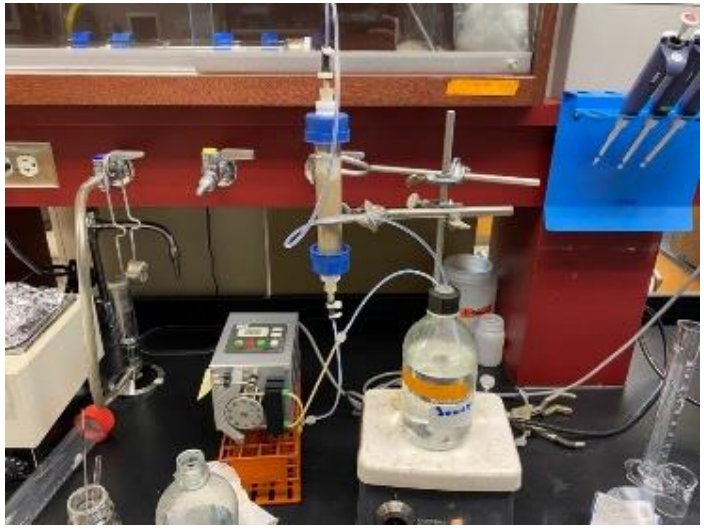
Variability of dechlorination kinetics linked to culture conditions

Selected optimal parameters for future experiments

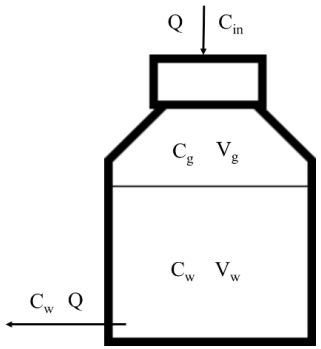
Freely dissolved PCE generating columns

Motivation

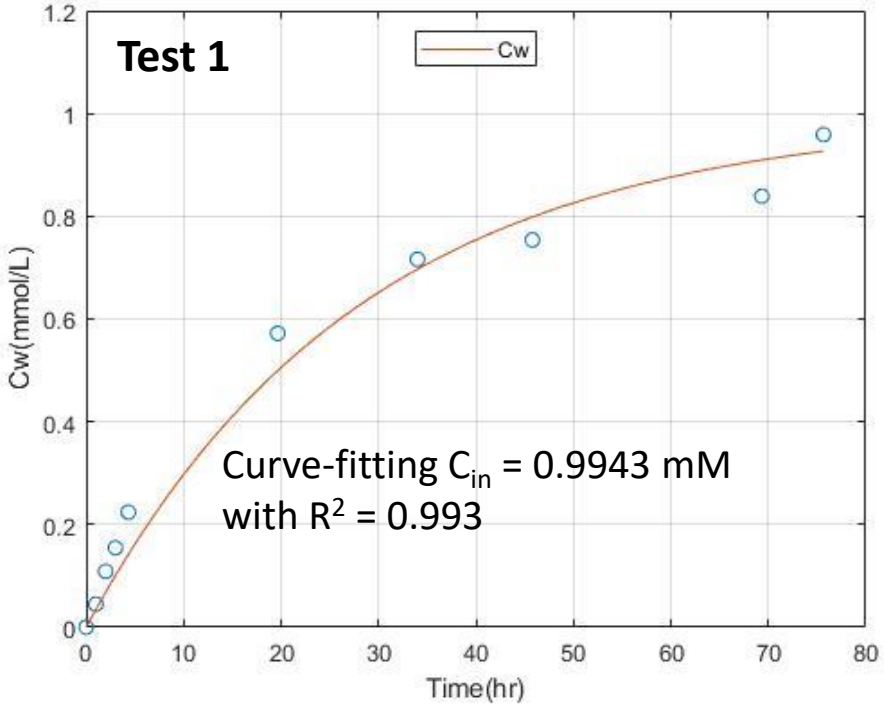
- **Improving maximum cell density** >10^7 cells/ ml.
- **Steady state concentration PCE** in culture (continuous reactor)



Testing the PCE profile in medium without cells



$$\frac{dC_w}{dt} = \frac{Q}{\left(\frac{HV_g}{RT} + V_w\right)}(C_{in} - C_w)$$



Cell growth monitoring

DNA extraction

Instagene Matrix modified

✓ Higher recoveries (2X)

✓ Higher precision (RSD: 5-9%)

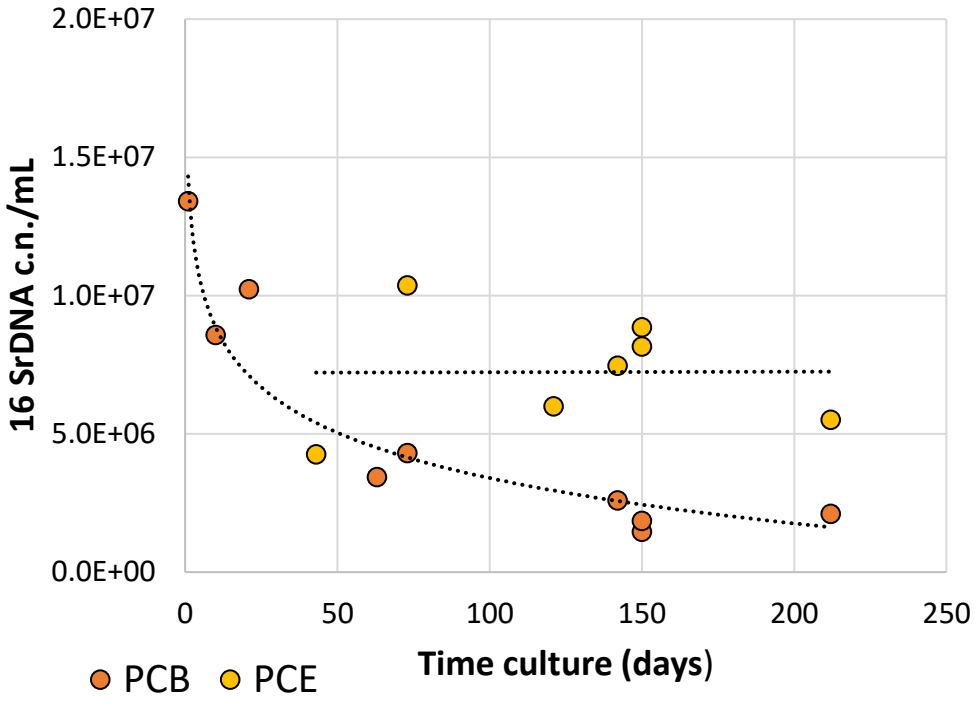
Versus Powersoil DNA isolation kit

qPCR

348F/884R targeting

Chloroflexi phylum

(Fagervold et al. 2006)



Results:

Variability between individual sampling linked to

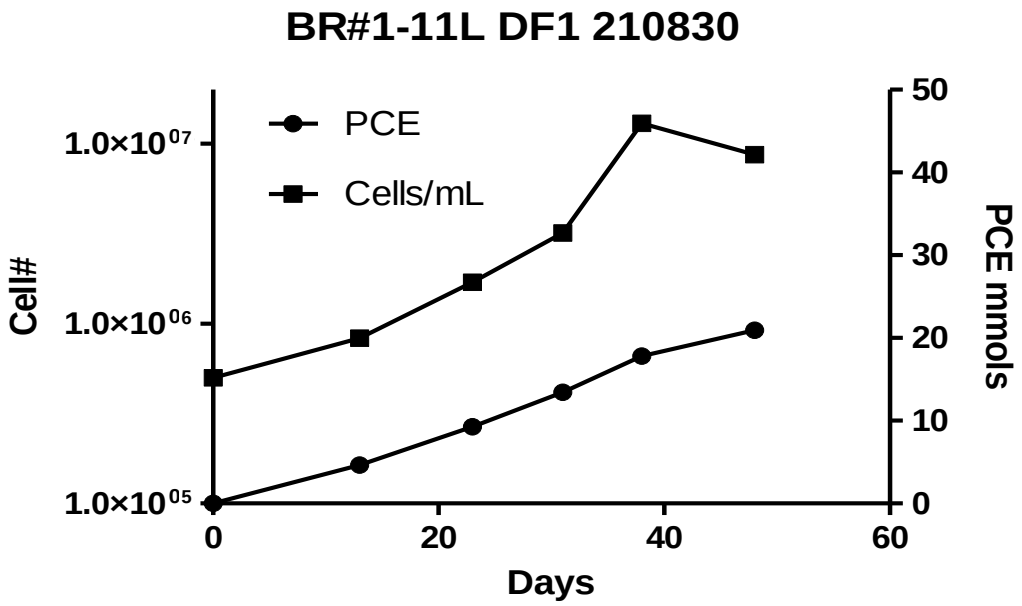
- PCE: DF1 sustained at ~1E7 cells/mL
- PCB: DF1 decrease to ~1E6 cells/mL

Similar findings for Dehalococcoides (Wang et al. 2014)

Implications:

Adjusting cell density inoculum for kinetic study based on electron acceptor

Anaerobic bioreactor (Scale up)



- Scale-up culture maintained at 1×10^7 cells/mL
- Semi-continuous scale up provides inocula for kinetic experiments

Main takeaways and next steps

Summary:

- Experimental conditions (media, cell growth, PCE and PCB monitoring tools) optimized for the kinetic study
- Similar intrinsic dechlorination kinetics observed for chlorinated compound with different hydrophobicity ($\log_{10} K_{ow}$ 3-6)
- A range of BC materials being prepared and characterized for the study

Next steps:

- Tailor Black Carbon for specific properties
- Measure Tailored BC on dechlorinating kinetics
- Relate dichlorination kinetics to BC physicochemical properties
- Explore native associations with natural BC particles in sediments
- Translate using advanced site models

UMBC

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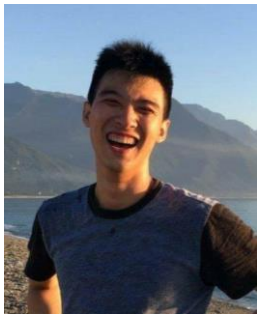

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