Elucidating mechanisms for enhanced anaerobic bioremediation in the presence of carbonaceous materials using an integrated material science and molecular microbial ecology approach

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Villanova University

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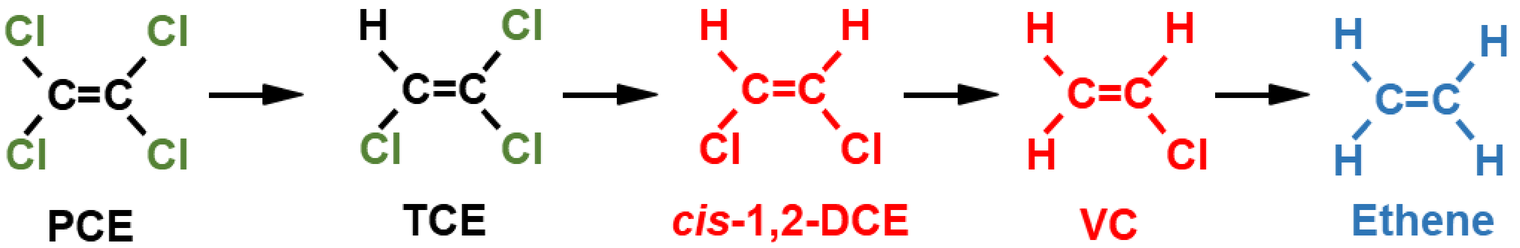
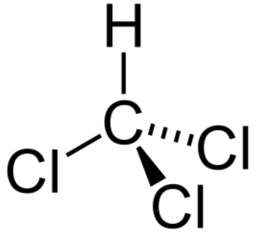
Purpose of this project

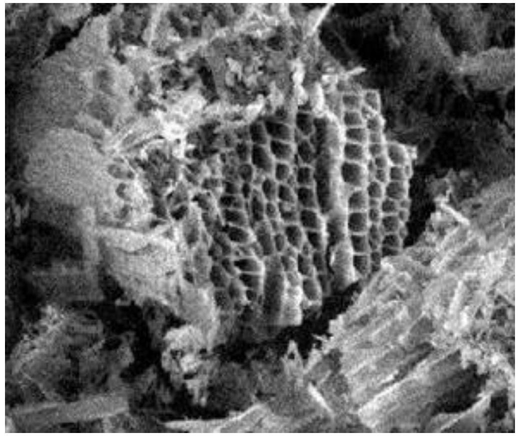
The Problem

- In situ anaerobic bioremediation of halogenated pollutants with organohalide-respiring bacteria (OHRB) has limitations (e.g., cDCE/VC accumulation, toxic co-contaminants)
- Pyrogenic Carbonaceous Matter (PCM) shows promise in promoting OHRB-driven bioremediation; however, how PCM properties shape microbial communities is unclear.





Chloroform



PCM

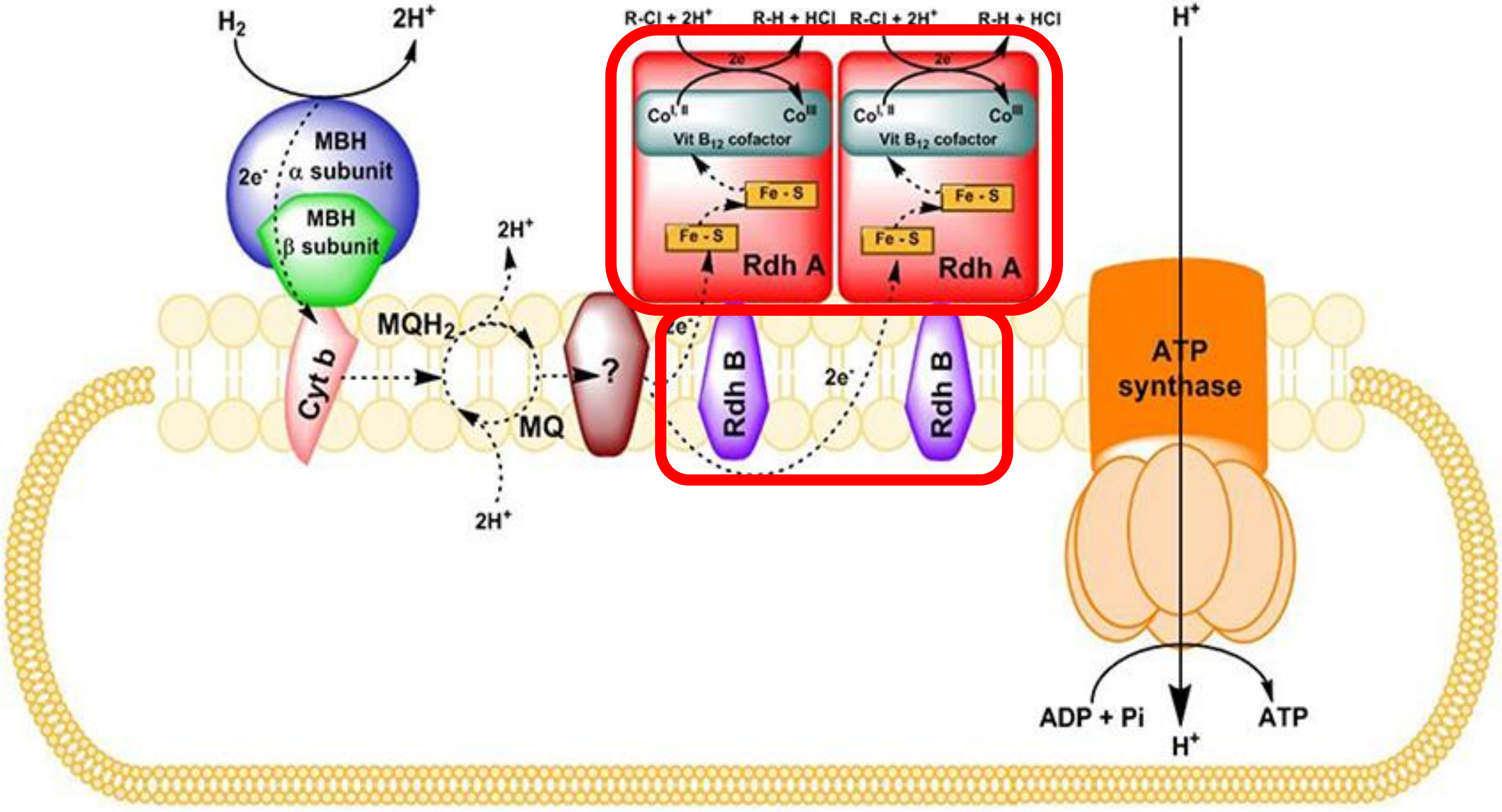
Goal of the project

- Apply trans-disciplinary approaches in molecular microbial ecology and materials science to develop tailored PCM that improves halogenated organic pollutant bioremediation outcomes.

Hypotheses

- Tailored PCM shapes microbial community interaction networks and positively influences reductive dehalogenation processes.
- Tailored PCM sequesters (emerging) pollutants that are recalcitrant or inhibitory to OHRB.

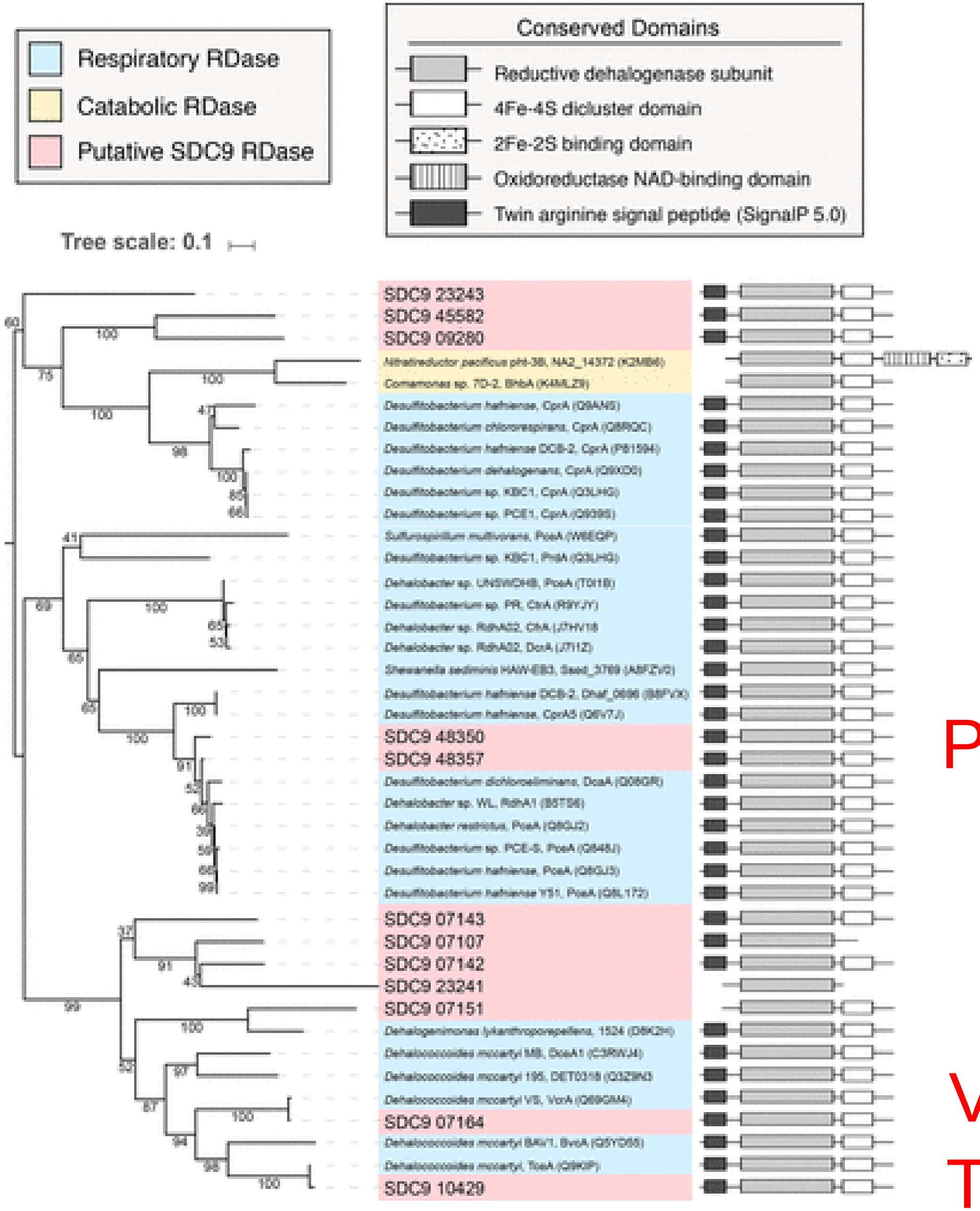
OHRB use reductive dehalogenase enzymes (RDases) to catalyze chlorinated ethene dechlorination



From: Jugder et al. 2016. Front. Microbiol. doi:10.3389/fmicb.2016.00249

RDase	Daughter products formed
PceA	TCE
TceA	cDCE VC ethene
VcrA BvcA	VC ethene

OHRB (with RDases) are present in PCE-dechlorinating culture SDC-9^{TM*}

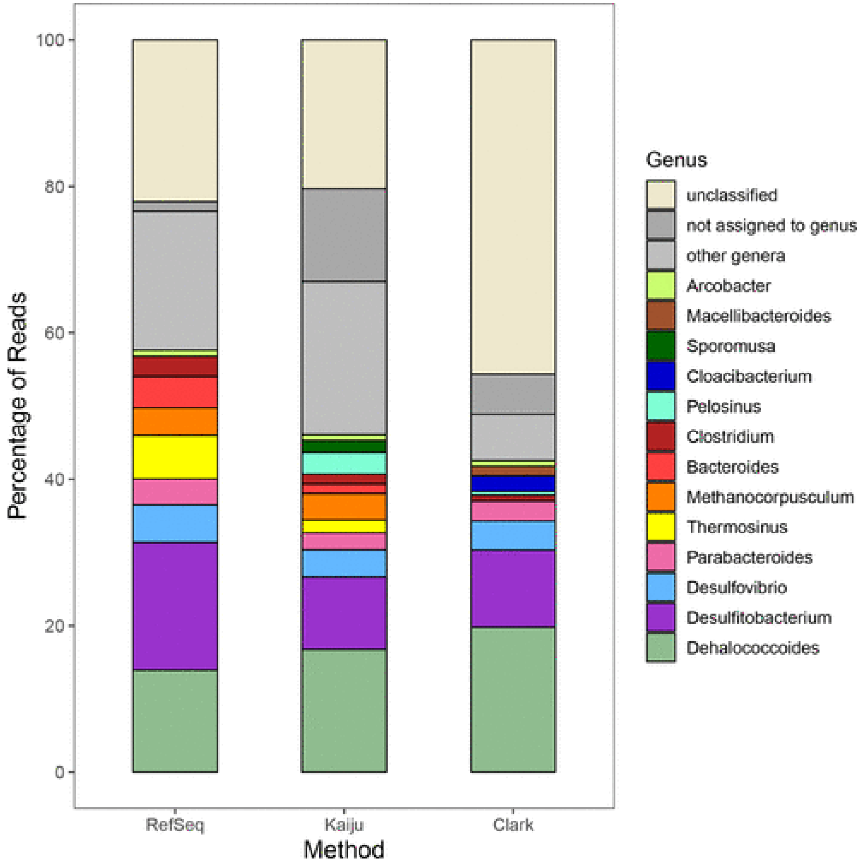


PceA

VcrA

TceA

From: Kucharzyk, et al. 2020. *J. Proteome Res*.
https://doi.org/10.1021/acs.jproteome.0c00072

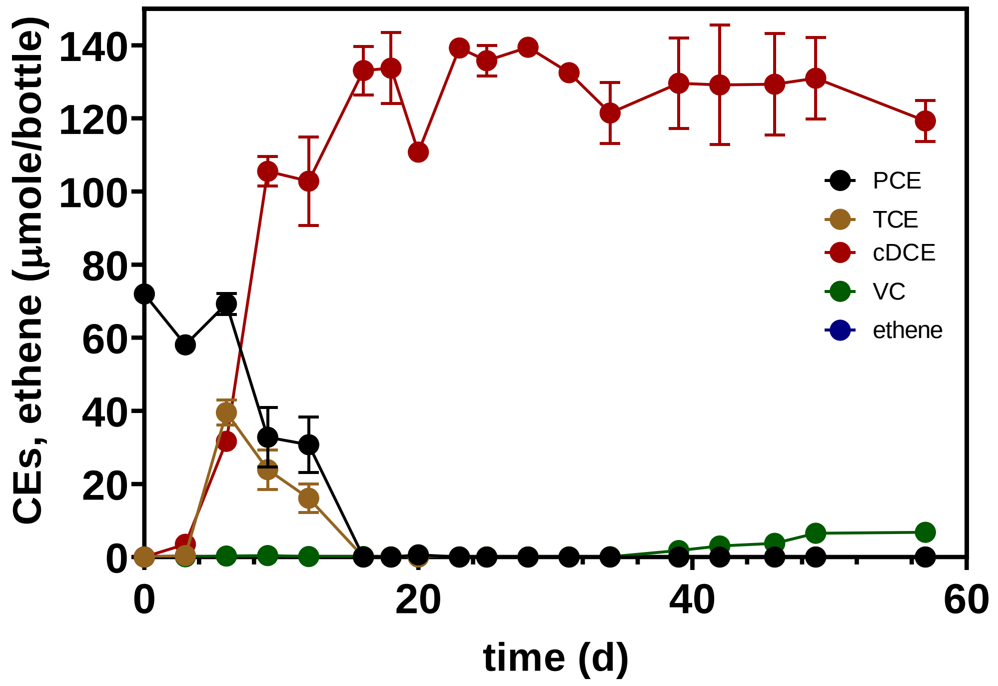


From: Kucharzyk, et al. 2020. *J. Proteome Res*.
https://doi.org/10.1021/acs.jproteome.0c00072

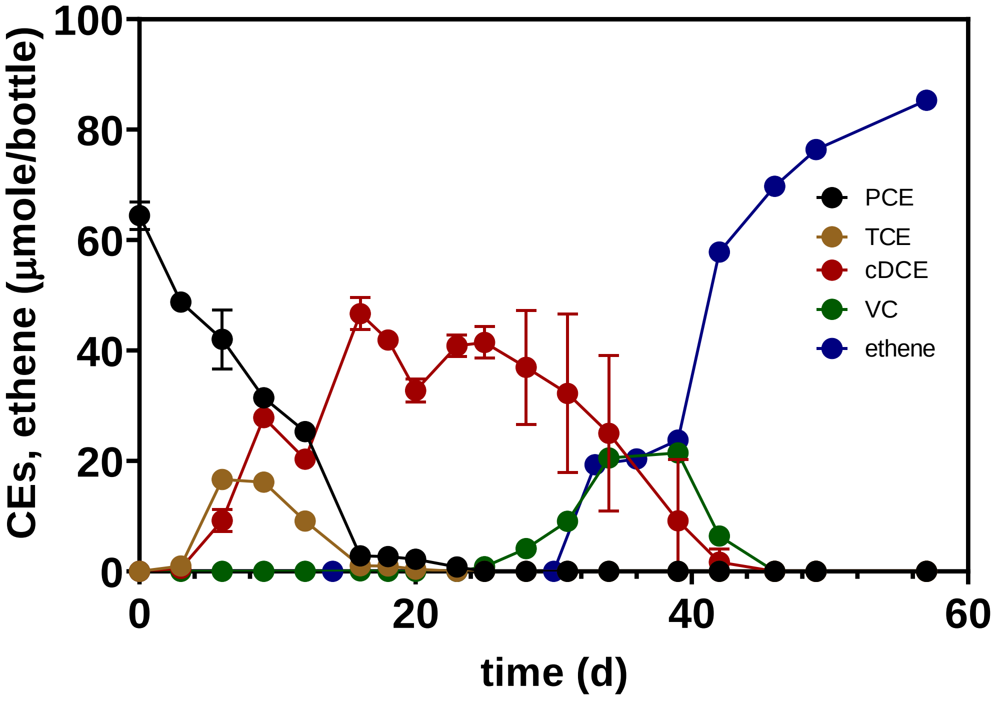
* Commercially available culture for bioaugmentation strategies

Initial findings: PCE-fed dechlorinating culture (SDC-9^{TM}) stalls at cDCE (no biochar) but generates ethene (with poplar biochar)

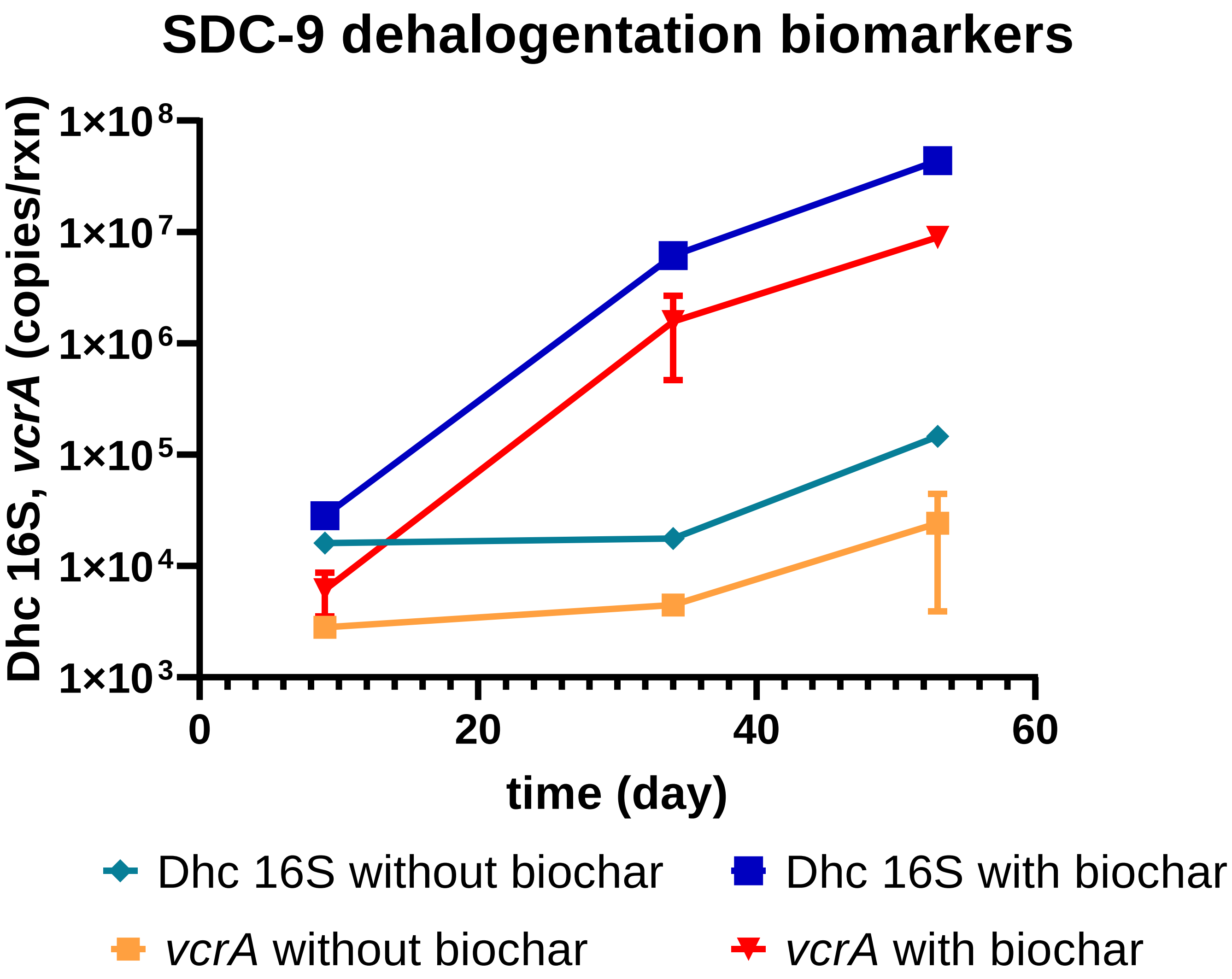
No biochar

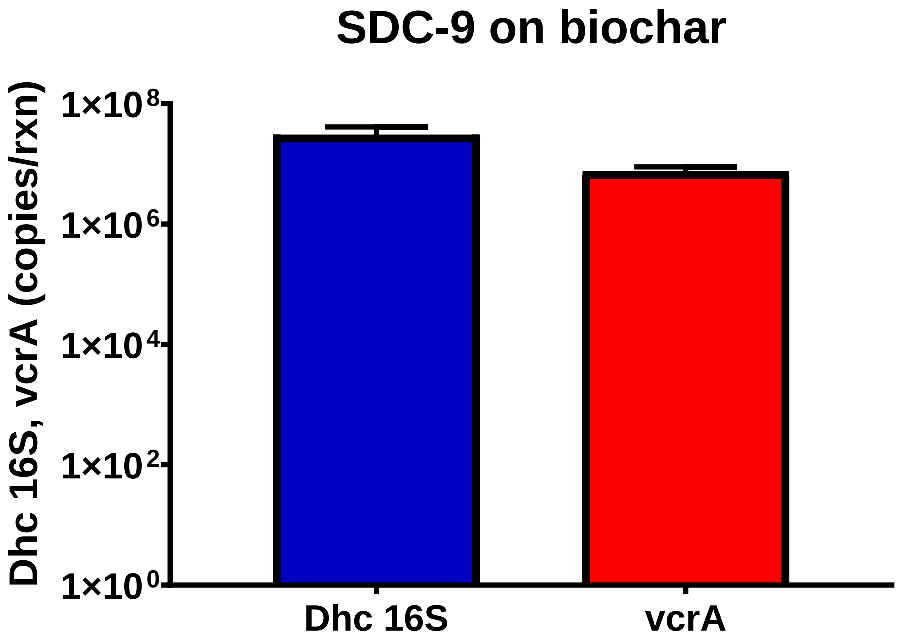


With poplar biochar



Dehalococcoides mccartyii with *vcrA* grow more effectively in bottles with (and attaches to) poplar biochar

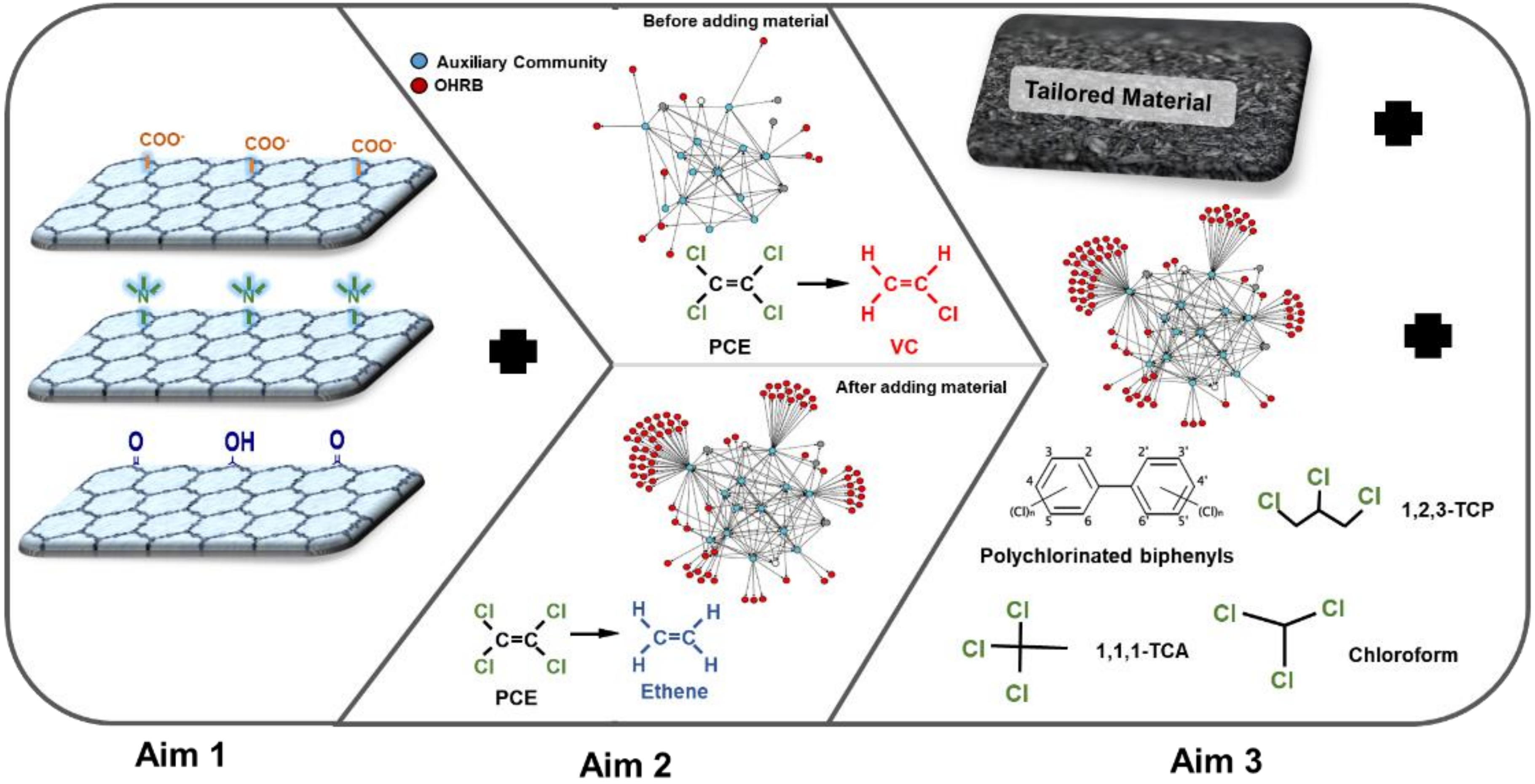




- Increased *vcrA* indicates improved potential to convert cDCE to ethene
- Ratio of *vcrA*/Dhc 16S on biochar = 25.7%

Technical approach

Aim 1. Provide a tunable PCM platform for synthesizing PCM-like polymers where surface charge and redox-active properties can be varied individually.



Aim 2. Quantify the effects of individual PCM surface properties on microbial interaction networks and subsequent performance of an organohalide-respiring mixed culture.

Aim 3. Develop tailored PCM for enhanced OHRB-driven bioremediation, contaminant mixture retention, and validate its performance in microcosms.

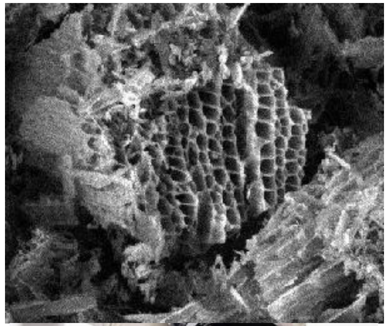
Pyrogenic Carbonaceous Matter (PCM)

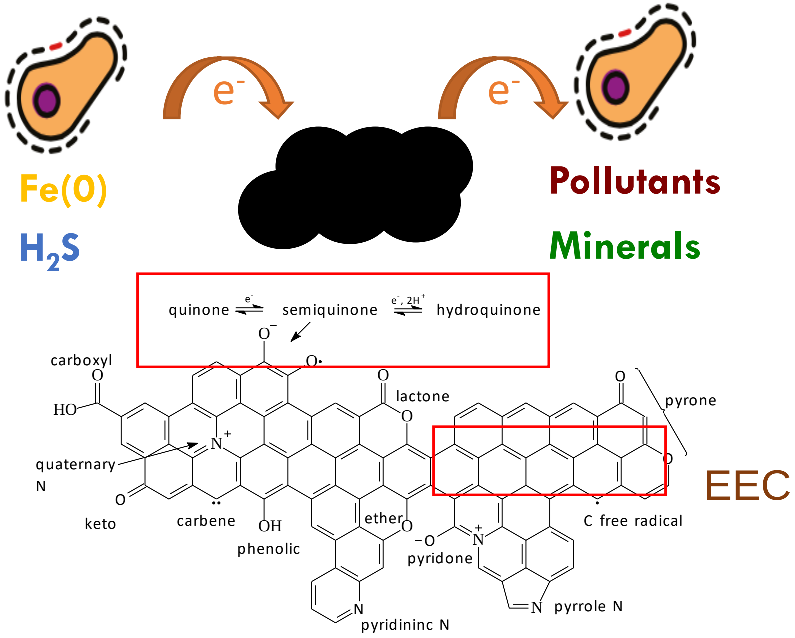
What properties of PCM best supports the microbial network?

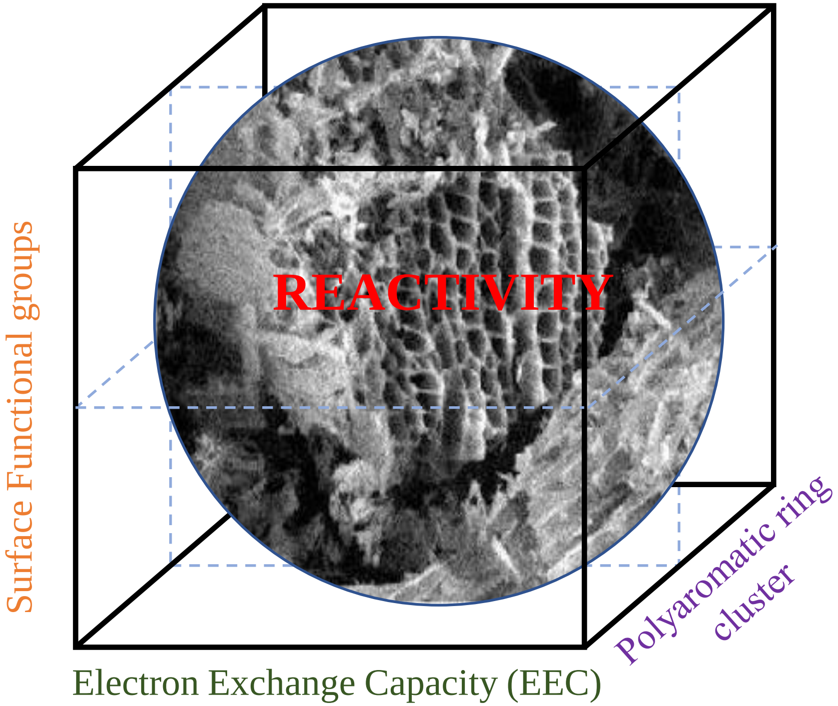
PCM (biochar, activated carbon) as passive adsorbents: large surface area, high pore volume, apolar surface

Recent studies suggest PCM are reactive and promote biotransformation of certain contaminants

Redox status and surface properties of PCM may affect its reactivity and can vary significantly across PCM types







Xu, X., … **Xu, W**. "Black carbon-enhanced transformation of dichloroacetamide safeners: Role of reduced sulfur species, *Science of the Total Environment.,* **2020.** Lawrinenko, M; …Hans van Leeuwen,J. "Macroporous Carbon supported Zerovalent Iron for Remediation of Trichloroethylene." *Sustainable Chemistry & Engineering,* **2017.** Sorengard, M;. " Stabilization of per- and polyfluoroalkyl substances (PFASs) with colloidal activated carbon (PlumeStop®) as a function of soil clay and organic matter content." Journal of Environmental Management, 2019.

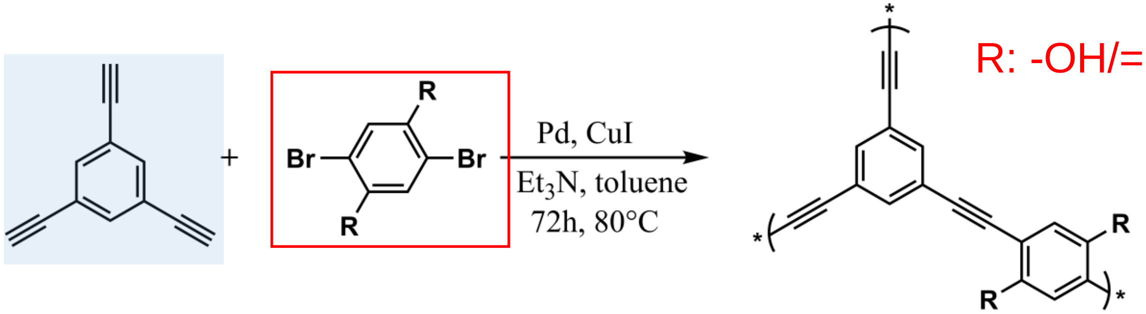
PCM-like Polymers to Elucidate Mechanisms

Like PCM, PCM-like polymers (PLPs) have:

I. large surface area and high microporosity,
II. highly conjugated and amorphous,
III. superior affinity towards apolar organic contaminants.

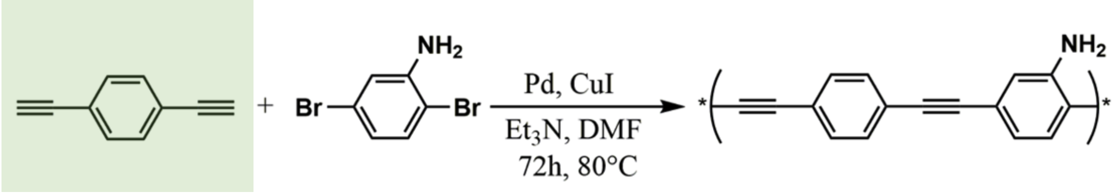
Unlike PCM, the attributes of PLP can be individually tuned and made homogeneously:

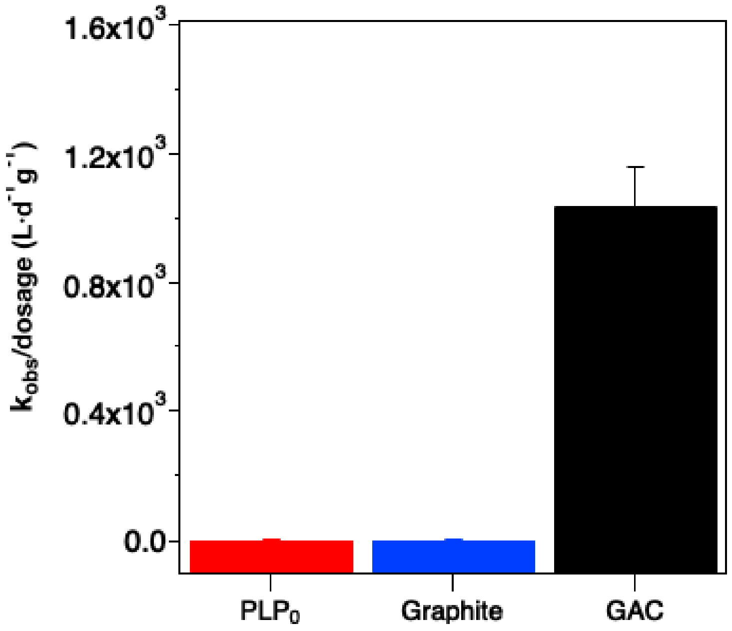
Cross-linked



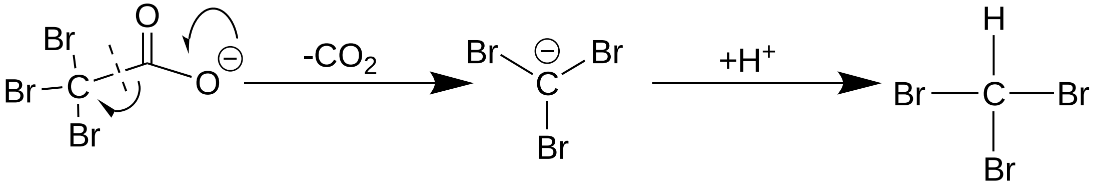
R: -OH/=O

Linear





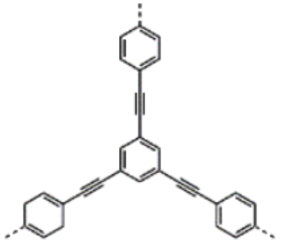
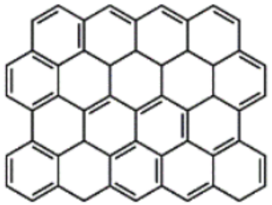
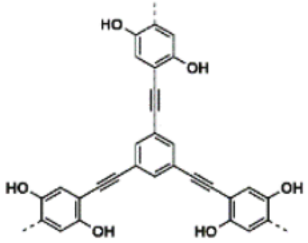
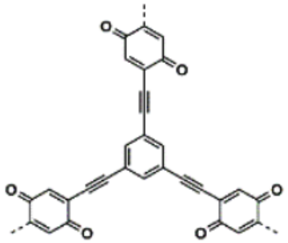
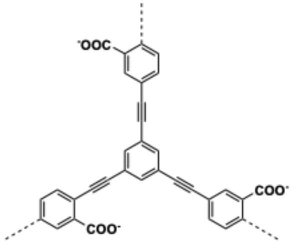
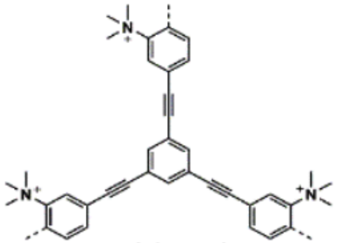
PCM	Degradation rate constants
PLP$_0$	$(2.57 \pm 0.20) \times 10^{-1}$ d^{-1}
Graphite	$(2.02 \pm 0.17) \times 10^{-1}$ d^{-1}
GAC	$(1.04 \pm 0.12) \times 10^{3}$ d^{-1}



Li, Z., … **Xu, W**. "Probing the surface reactivity of pyrogenic carbonaceous materials (PCM) through synthesis of PCM-like conjugated microporous polymers, *Environ. Sci. Technol.,* **2019.**

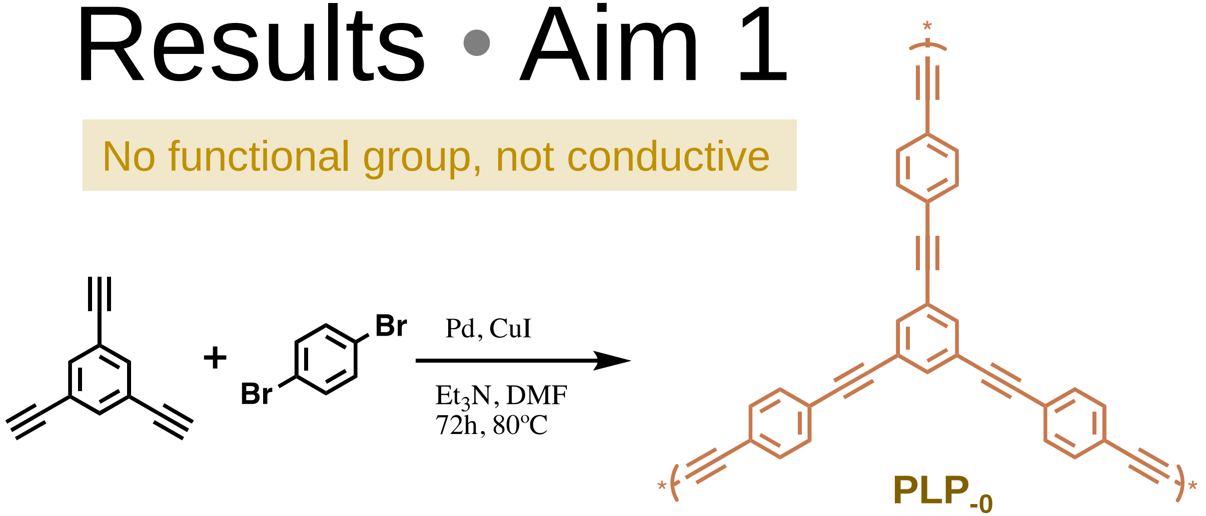
Samonte, P; …**Xu, W**. "Pyrogenic Carbon-Promoted Haloacetic Acid Decarboxylation to Trihalomethanes in Drinking Water." *Water Research,* **2021.**

Technical approach • Aim 1

PCM-like polymer membranes		Expected outcomes	Benchmark for success
 PLP-0	 PLP-0-X (X=400, 600, 700, 800, and 900)	Provides a platform to systematically increase the polyaromatic ring clusters; as T increases, the average size of ring cluster will increase.	Conductivity increases from PCM-0 (not conductive) to PCM-0-900; Characteristic peaks in FTIR, XPS, and solid-state NMR will be used to confirm the structure
 PLP-OH	 PLP=O	Hydroquinone and quinone functional groups are incorporated into the polymer network, which conveys electron exchange capacity.	Both polymers are not conductive; Characteristic peaks in FTIR, XPS, and solid-state NMR will be used to confirm the structure
 PLP-COOH	 PLP-$(CH_3)_3N^+$	Negatively and positively charged polymer networks can be synthesized respectively, which will repel or attract bacteria.	Both polymers are not conductive; Characteristic peaks in FTIR, XPS, and solid-state NMR will be used to confirm the structure

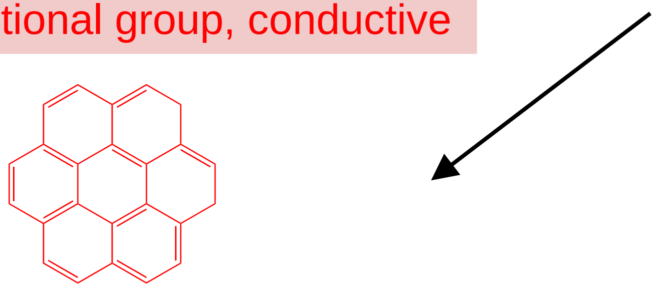
Results • Aim 1

No functional group, not conductive



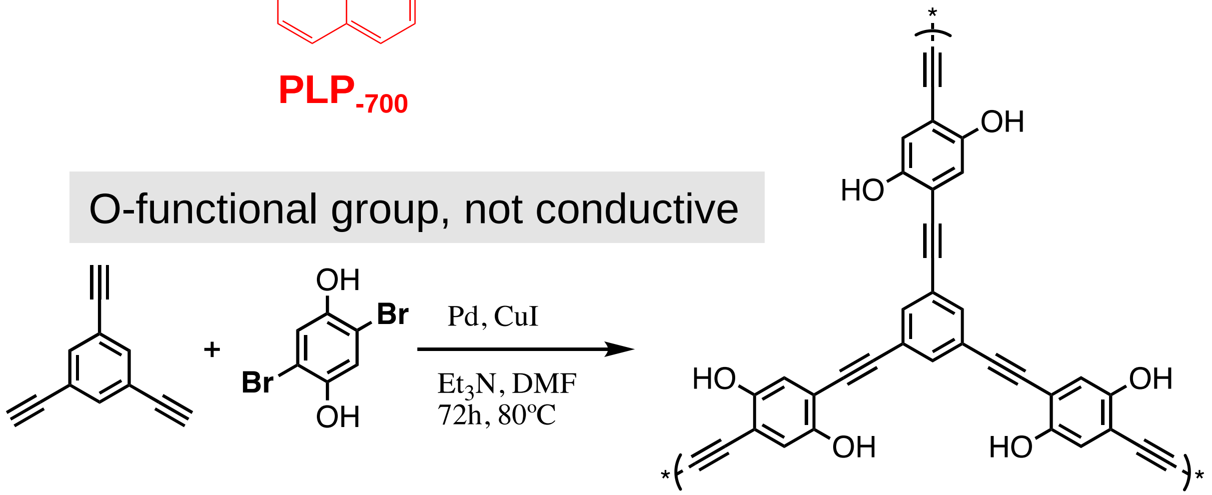
PLP$_{-0}$

No functional group, conductive

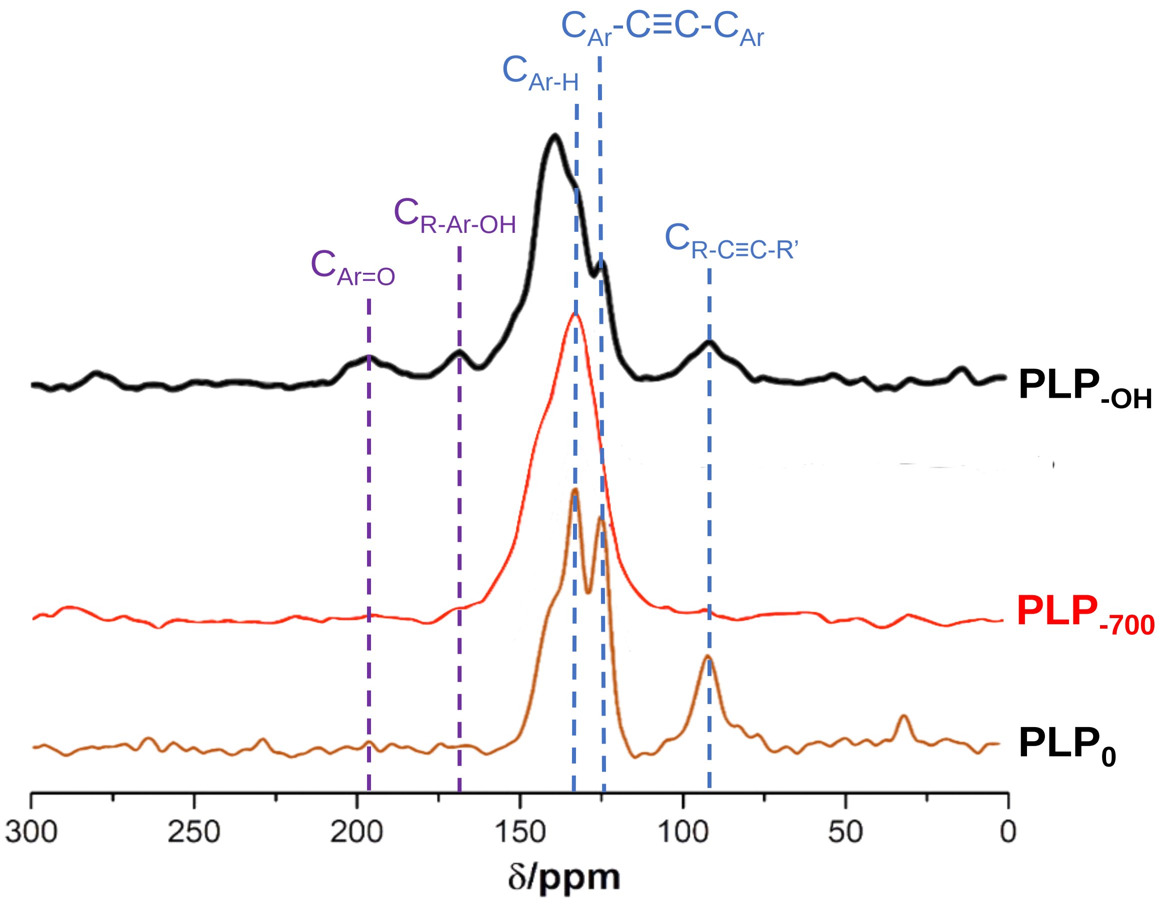


PLP$_{-700}$

O-functional group, not conductive



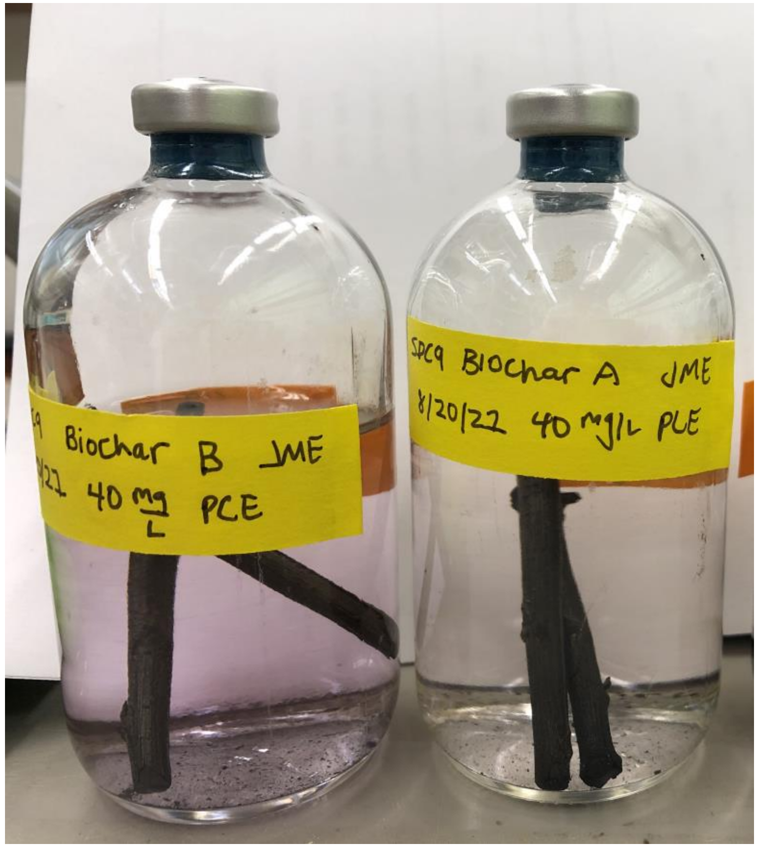
PLP$_{-OH}$



- **Conjugated network**:
  - 131.8 ppm (C_{Ar-H})
  - 123.7 ppm (C_{Ar}-C≡C-C_{Ar})
  - 90.8 ppm ($C_{R-C≡C-R'}$)
- **Quinone and hydroquinone groups**: 167.8 ppm (C_{Ar-OH}) and 196.2 ppm ($C_{Ar=O}$)
- Polyaromatic ring cluster size: PLP$_0$-700 (Θ_b=0.668; σ=0.187 S/m)

Initial experiments with PCE (40 mg/L) and PCM-like polymers (PLP)

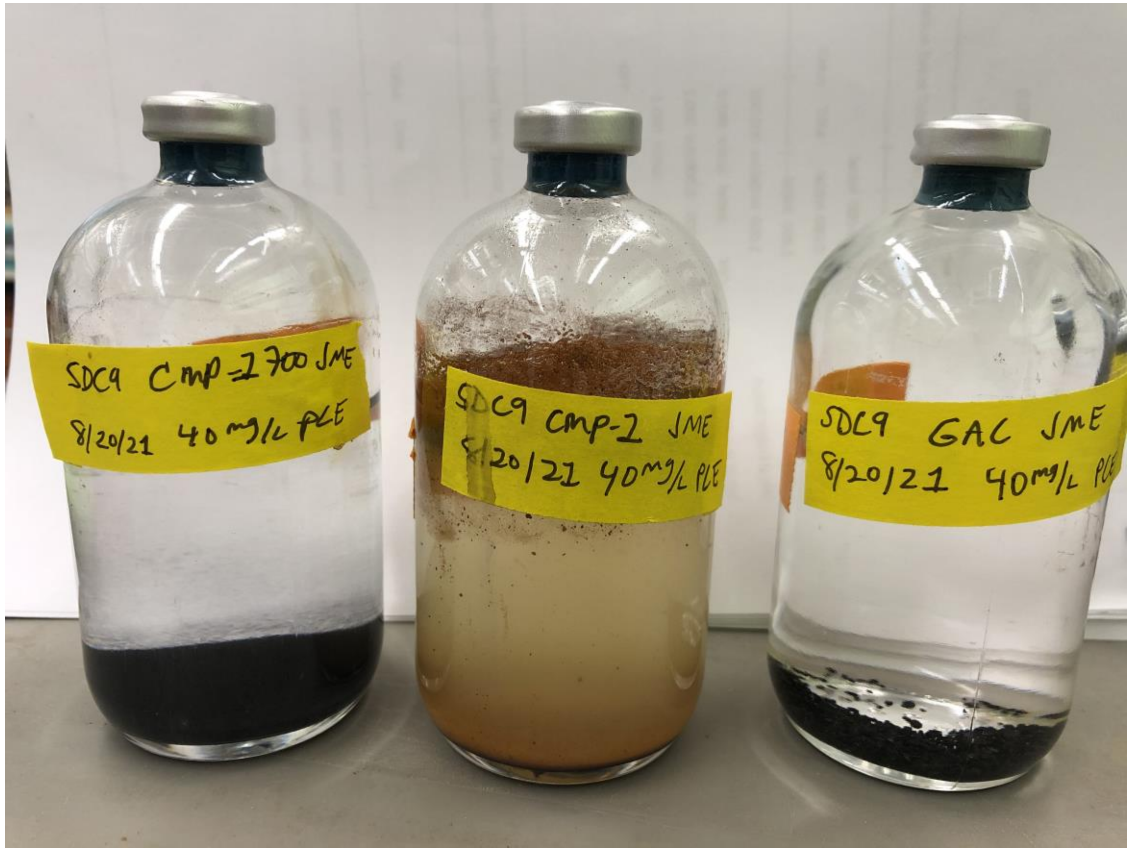
Poplar biochar x2



PLP-0-700

PLP-0

GAC



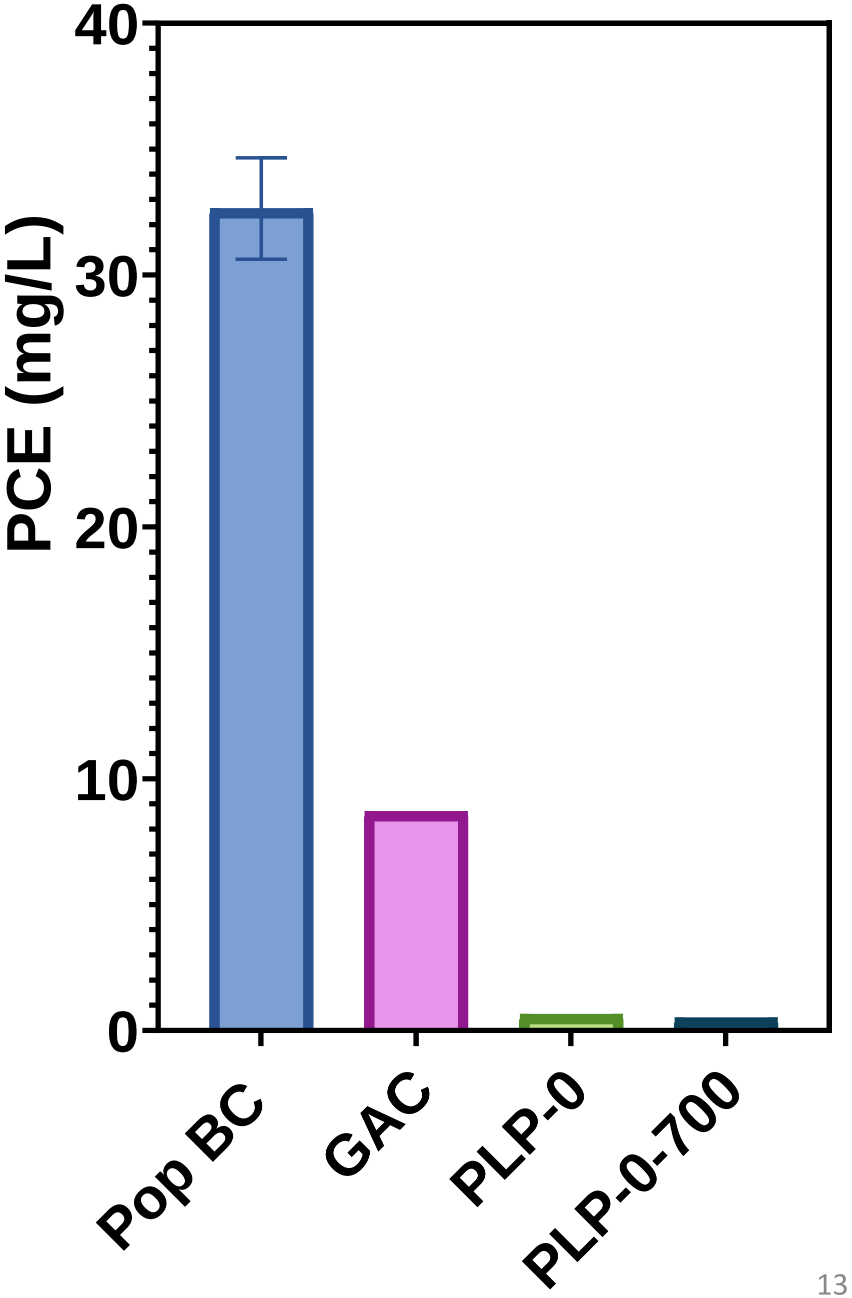
PCM-like polymers (PLP) can be highly sorptive

- PCE sorption with ~1 g loading

PLP > GAC > poplar biochar

- PCE sorption likely to interfere with growth of OHRB and will complicate analyses

<u>Next steps</u>:
- PCE/PLP isotherm experiments
- Experiments with lower PLP loading
- Develop less sorptive PLP



Research Team

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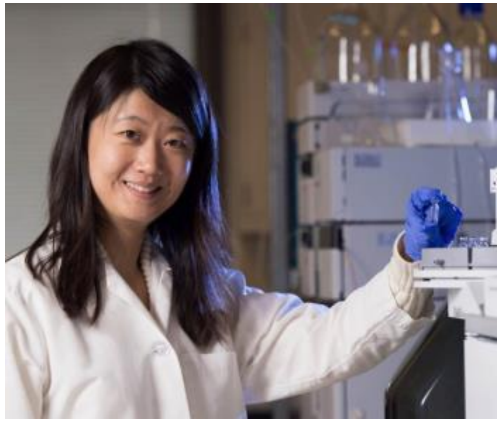

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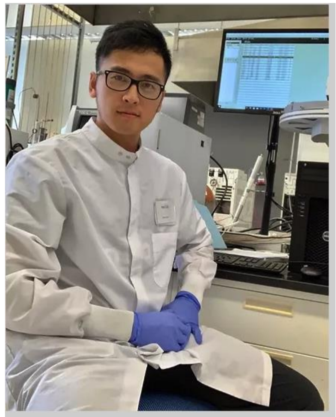

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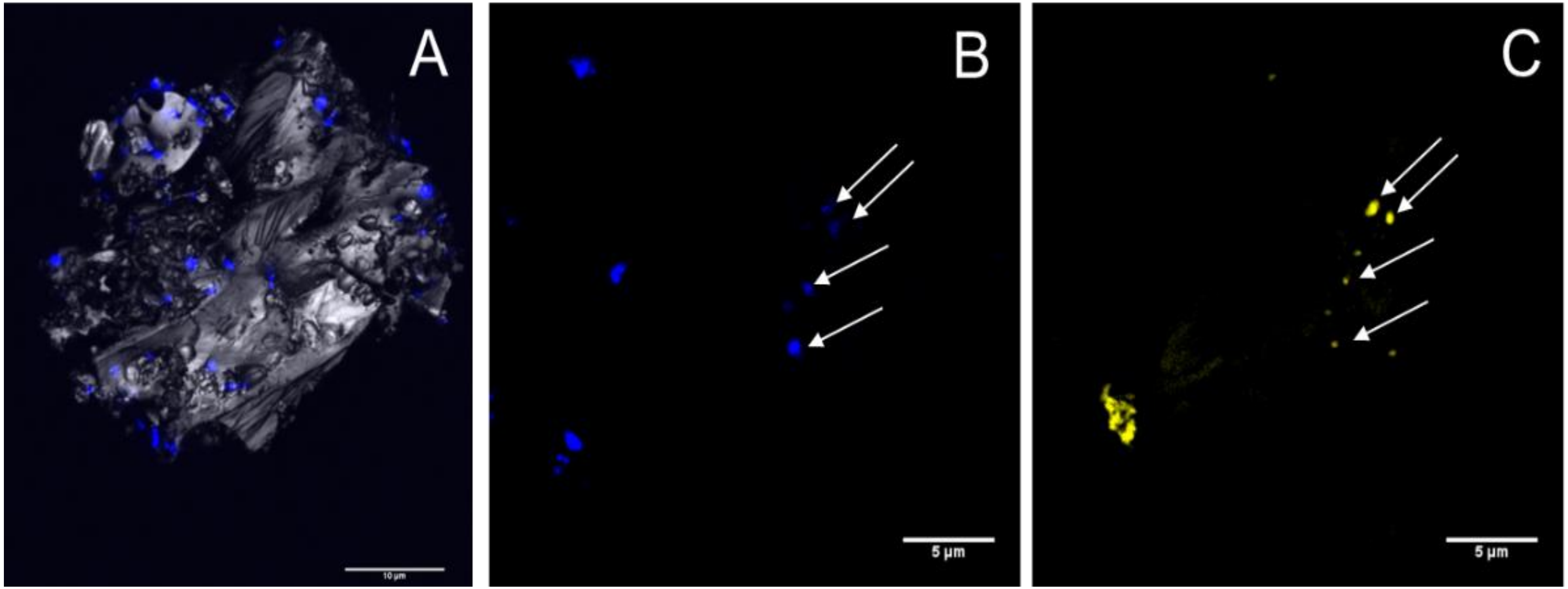
PhD student

<u>Stakeholder Engagement with Remediation Industry</u>
Paul Hatzinger (Aptim), Paul Erickson (Regenesis),
Dimin Fan (Geosyntec)

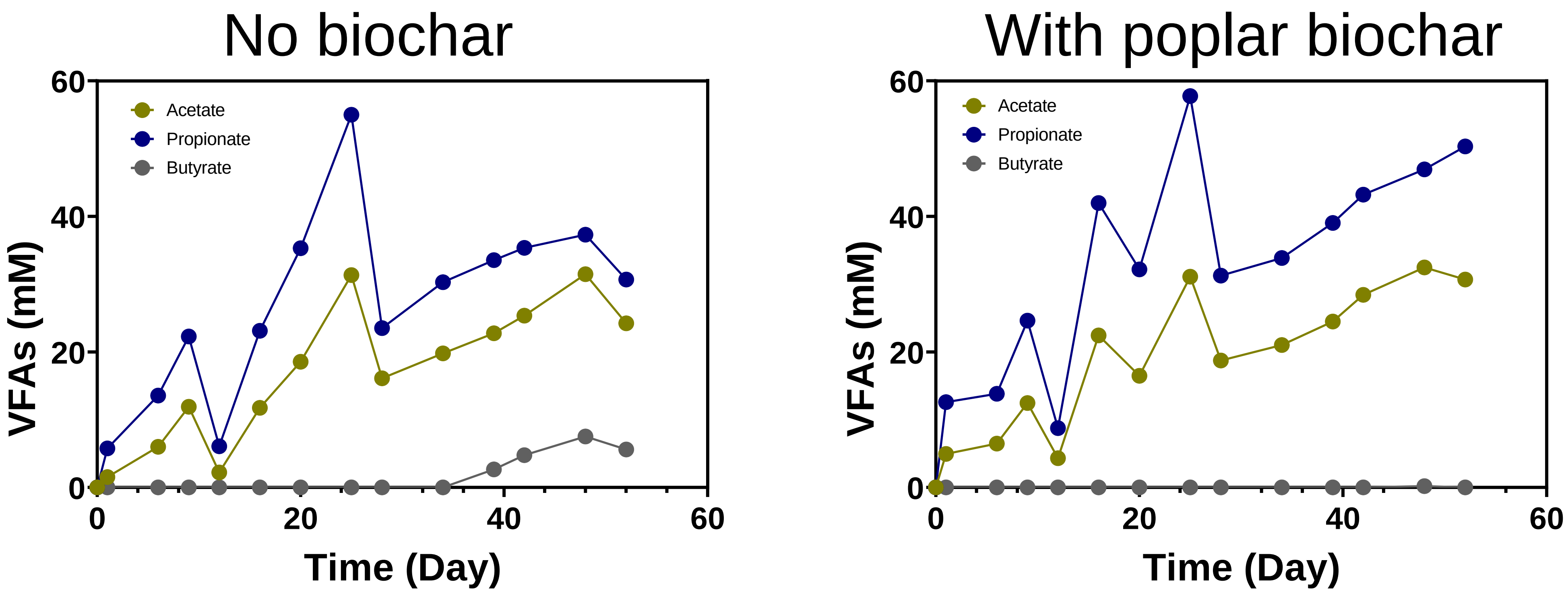
<u>Collaborators</u>
Laura H. Arias Chavez (Tennessee Tech),
Jingdong Mao (Old Dominion)

Backup slides



A) **Biochar sample with biofilm**. DAPI stained cells (blue) are attached to the biochar surface (grey). This is a composite of 27 individual images collected as a stack with a total thickness of 8 µm. B) The entire bacterial community was imaged by non-specific staining with DAPI, and C) *Dehalococcoides* (*Dhc)* cells were detected in the biofilm using fluorescent in situ hybridization (FISH) probes. The arrows indicate several *Dhc* cells detected by both DAPI and FISH.

Butyrate did not accumulate in SDC-9 cultures grown in the presence of poplar biochar

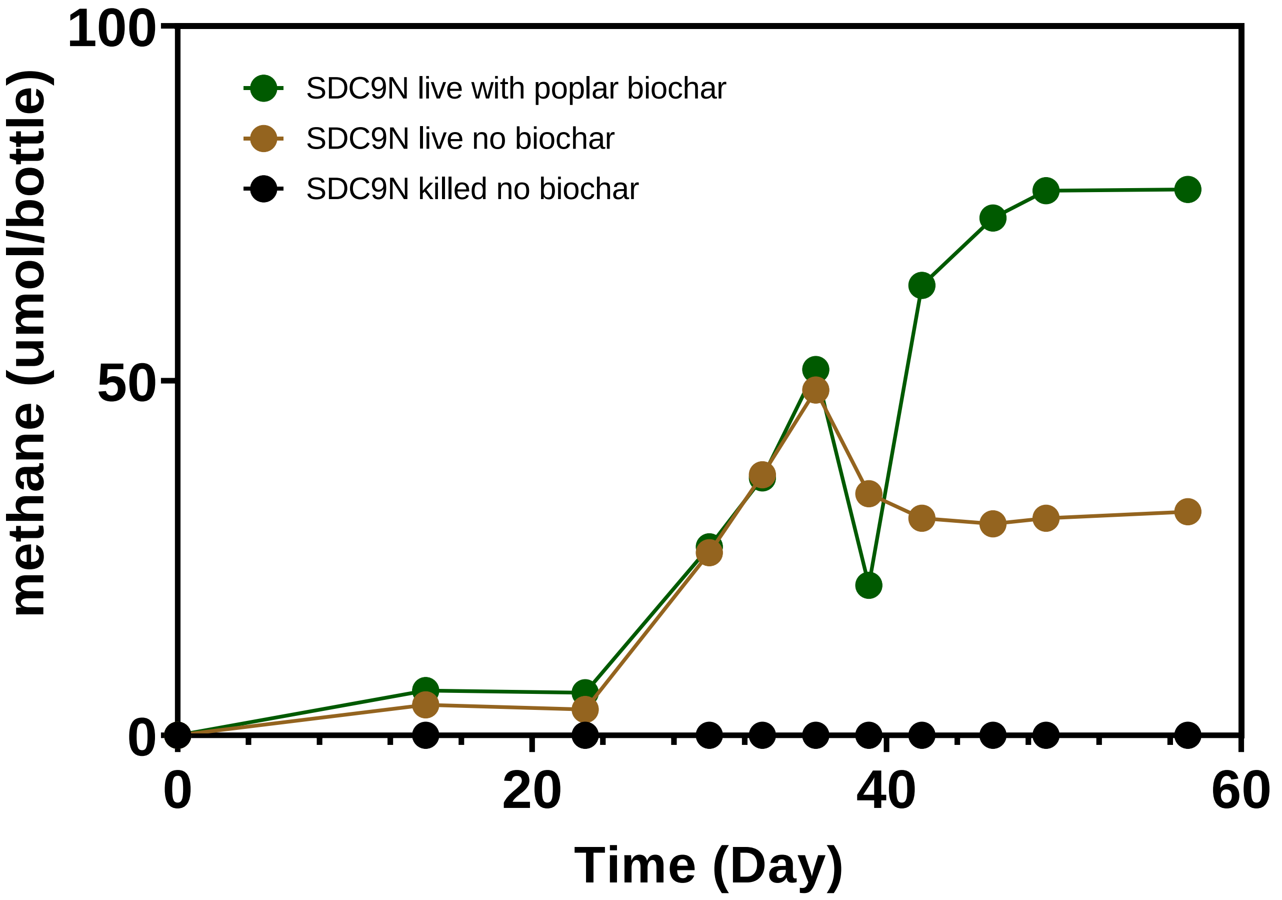


No biochar

With poplar biochar

- Lactate (25 mM) fed twice per week as a source of electron donor for reductive dechlorination (and methanogenesis)

PCE-fed SDC-9 generates more methane in the presence of poplar biochar



Characterization of PLPs

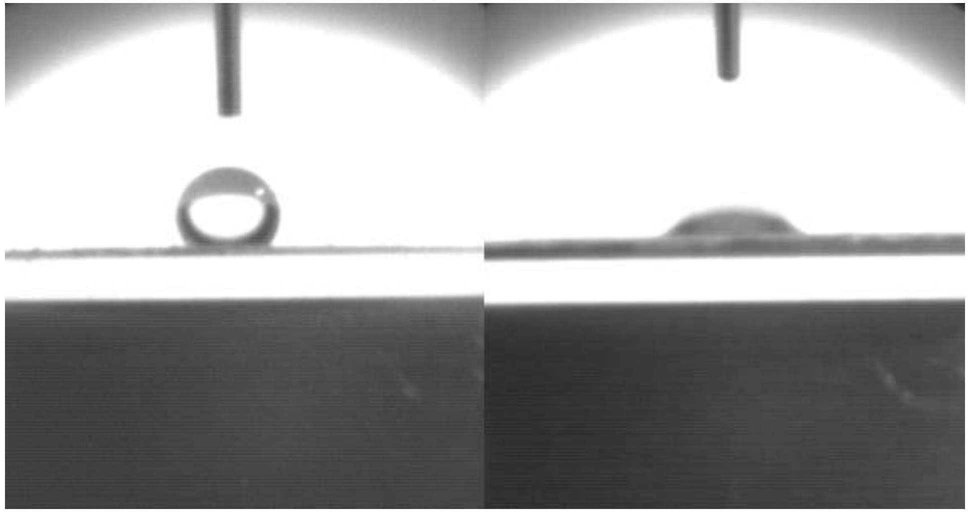
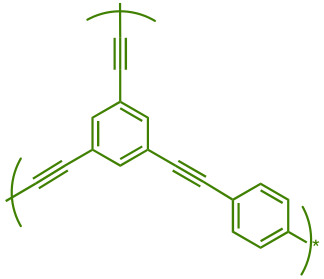


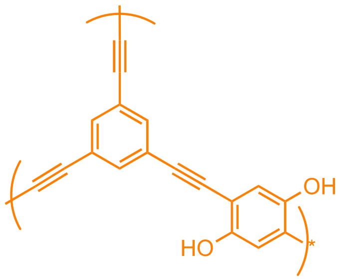
Table 1. Conductivity, surface area and EDC of PLP$_0$, PLP$_{0\text{-}700}$, and PLP$_{-OH}$

Sample	Conductivity, σ (S/m)	Surface area (m^2/g)	EDC ($mmol_{e^-}/g_{PLP}$)
PLP$_0$	1.96×10^{-7}	398.8	N.D.
PLP$_{0\text{-}700}$	0.187	1132.8	1.831 ± 0.137
PLP$_{-OH}$	2.85×10^{-7}	133.3	1.859 ± 0.073

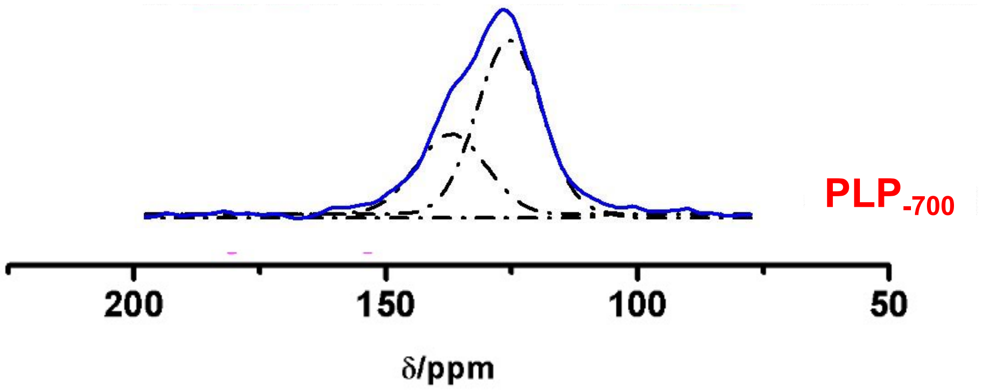
PLP-0



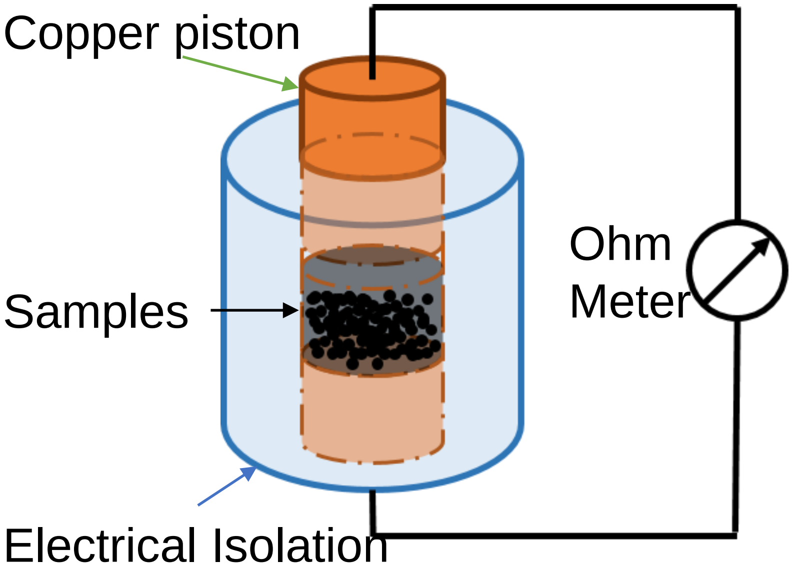
PLP-OH



Characterization of PLPs

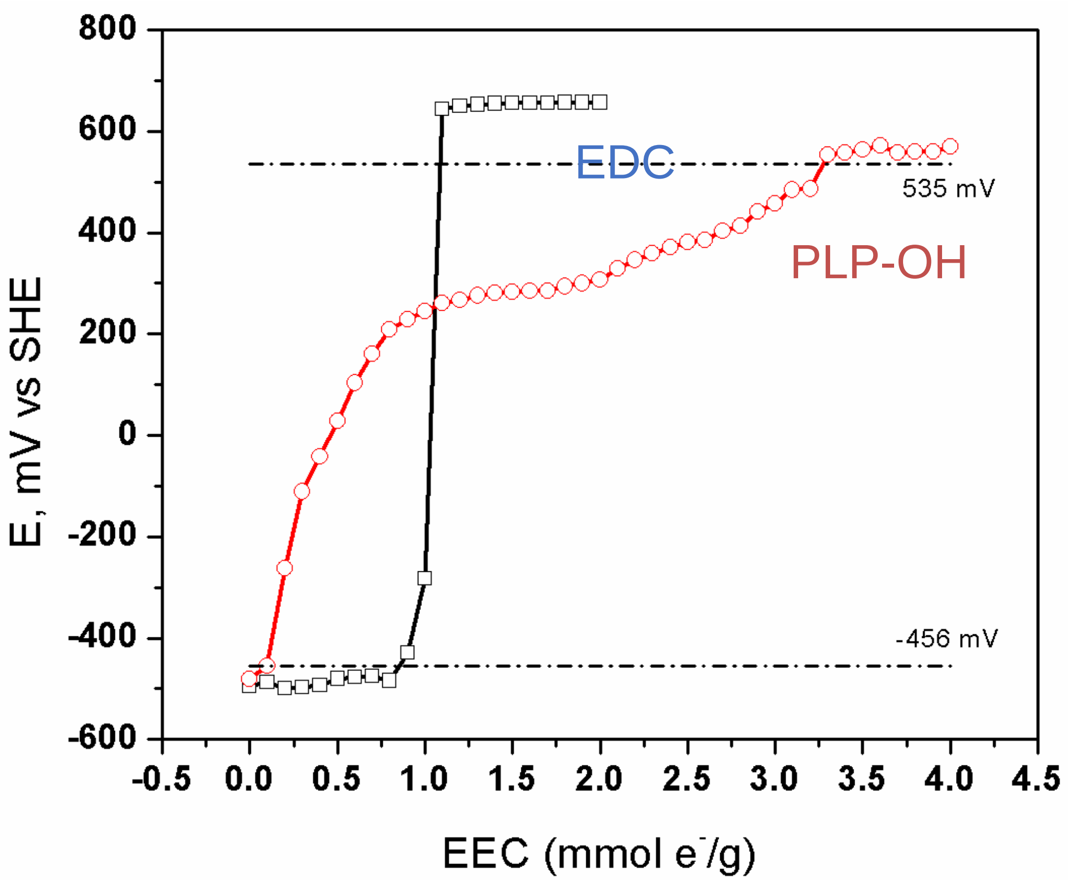


$$\theta_b = \frac{A_{125-135ppm}}{A_{100-165ppm}}$$



	θ_b	Conductivity σ (S/m)
PLP-700	0.668	0.187

Characterization of PLPs



$$EDC\ (mmol\ e^-/g) = C_i V_i * \frac{2e^-}{m_c}$$

Oxidizing agent: 0.05 M I_2

Reducing agent: 0.025 M $NaBH_4$

Sample: 2.5 g/L of PLPs in 0.2 M ammonium buffer at pH 10 (PLP-OH, red)

Blank: 0.2 M ammonium buffer at pH 10 (black)

An excess amount of $NaBH_4$ was added to completely reduce the sample; I_2 was then used to generate an oxidizing titration curve.

EDC was determined by the difference of consumed I_2 between samples and blank