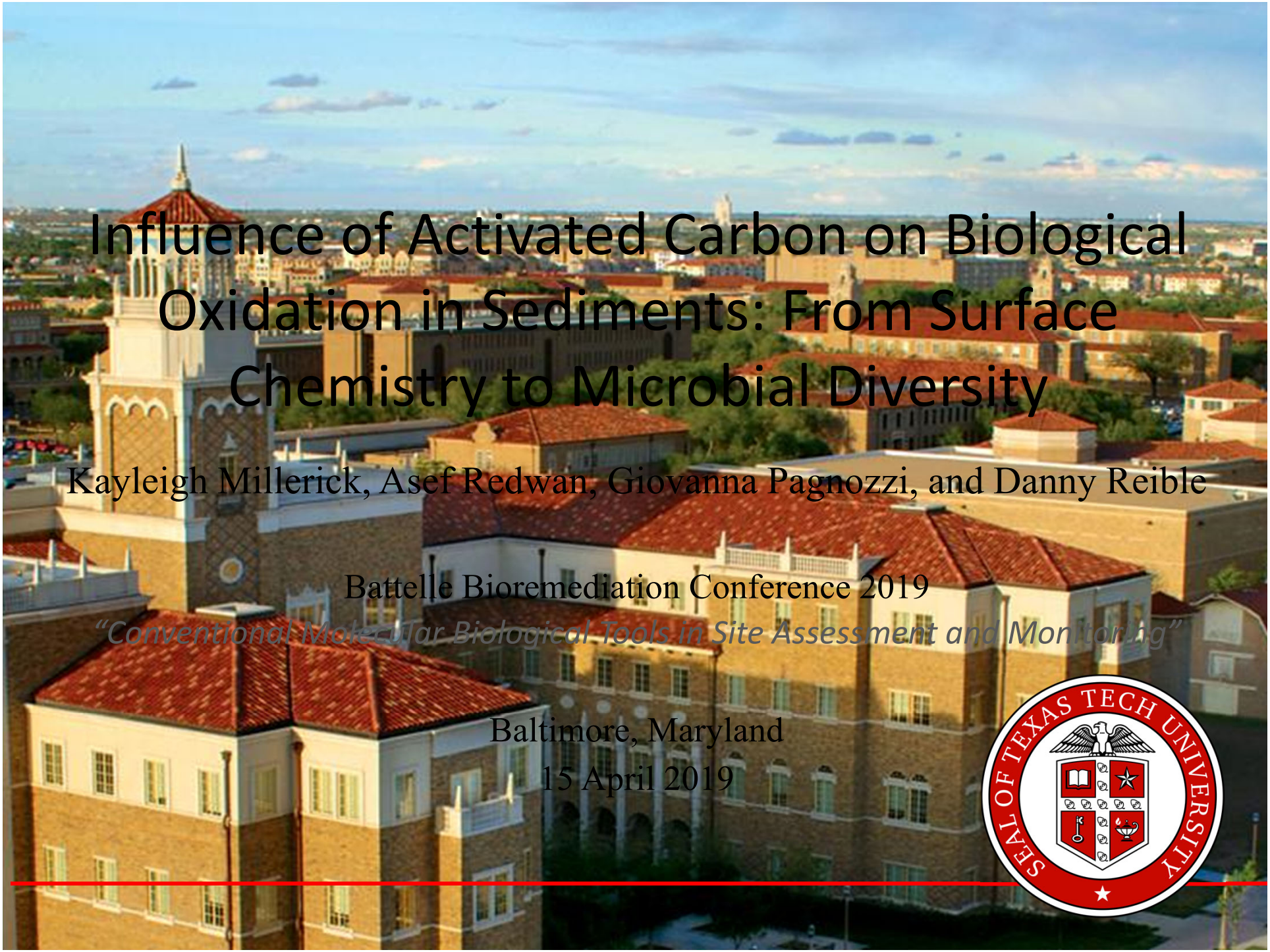


Influence of Activated Carbon on Biological Oxidation in Sediments: From Surface Chemistry to Microbial Diversity

Kayleigh Millerick, Asef Redwan, Giovanna Pagnozzi, and Danny Reible

Battelle Bioremediation Conference 2019

"Conventional Molecular Biological Tools in Site Assessment and Monitoring"

Baltimore, Maryland

15 April 2019

Biostimulation: Increasing the presence and/or activity of microorganisms of interest [1]

Desired microorganisms are **already present in the contaminated subsurface** but at concentrations too low to be effective.

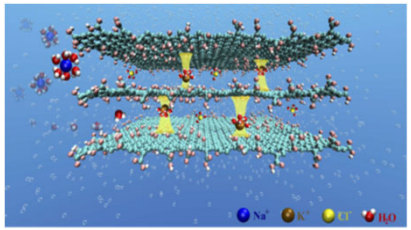
Aqueous vitamins, nutrients, and/or preferred electron donors/acceptors can be amended, **promoting growth**.

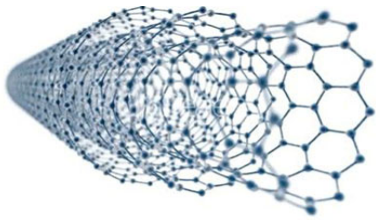
Amendments are often **non-specific** or are **dependent upon subsurface transport**, reducing their efficiency and utility.

The factor most likely to promote growth of microorganisms with requisite contaminant-degrading genes **is the contaminant itself**, which cannot be amended.

[1] …hopefully.

Carbon Amendments: Traditionally for Adsorption





Mechanisms for Immobilization: "Adsorption"

- Electrostatic binding on the surface
- Entrapment in porous matrix
- Encapsulation (chitosan-alginate composites)

Key Physical and Chemical Properties

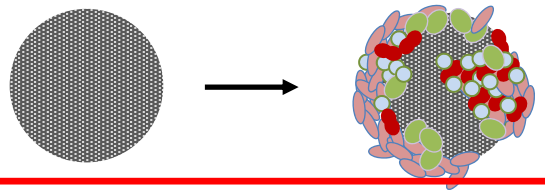
- High Surface Areas (400–2,500 m^2/g),
- Semi- to high conductivity (5–50 S/cm)
- Highly concentrated surface functionality

Broadly, applications include: Contaminant adsorption, catalysis, gas storage, and electrochemical energy storage.

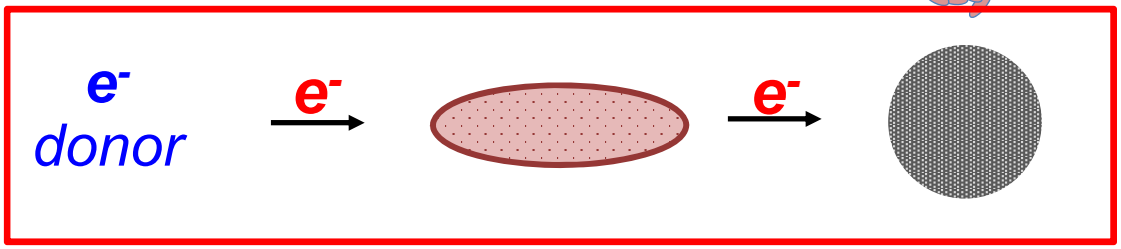
Properties of CMs are **tailored** to maximize their utility in abiotic applications

Carbon Amendments: Applications in Bioremediation

Approaches:



in situ placement



contaminant reduction

For stim se:

- It can
- It can tor

Possible

- Chan logy, metal
- Bacte

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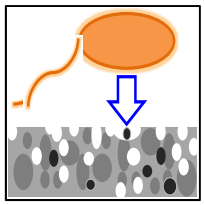
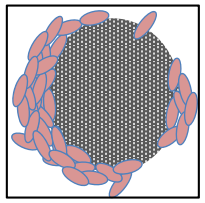
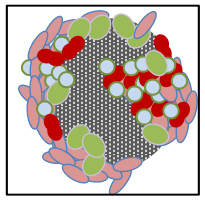
Assessment of activated carbon-microbe interactions in systems of increasing complexity



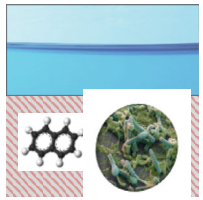
1. In a **pure culture, static system** under non-growth conditions containing GAC



2. In a **pure culture, growth system** containing GAC

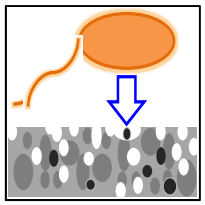


3. In a **mixed culture, growth system** containing GAC



4. In a **mixed culture, growth system,** comparing media in the presence of a model contaminant

Assessment of activated carbon-microbe interactions in systems of increasing complexity



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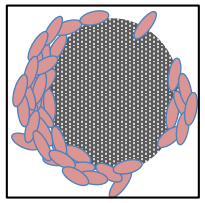

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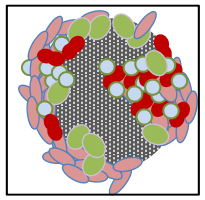

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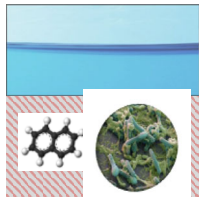

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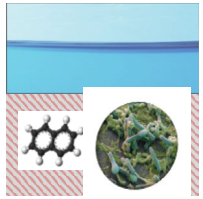
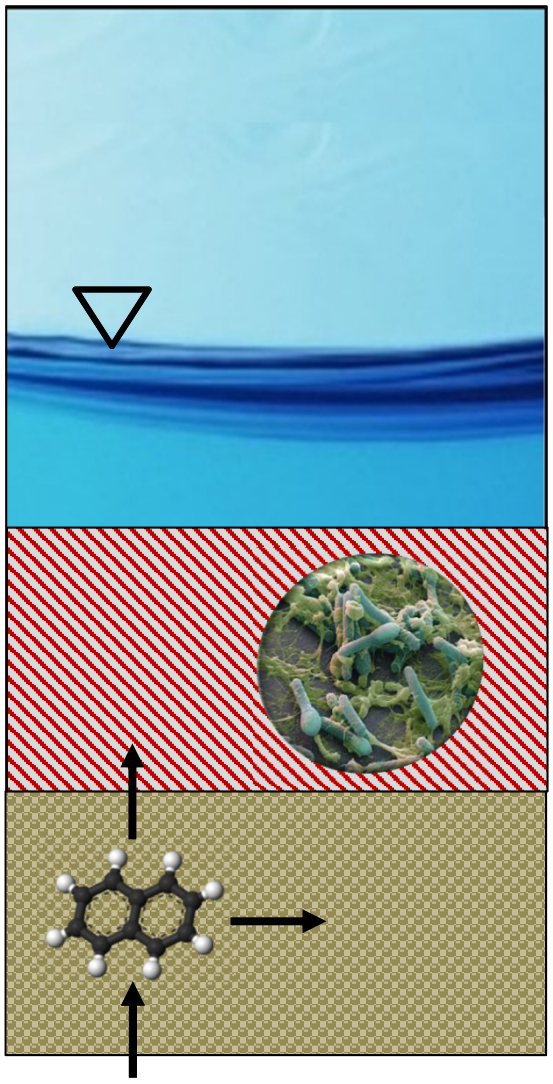




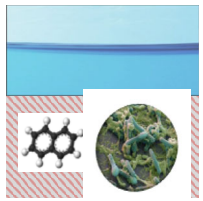
Scenario of Interest: PAH degradation in contaminated sediments under **highly reducing conditions**

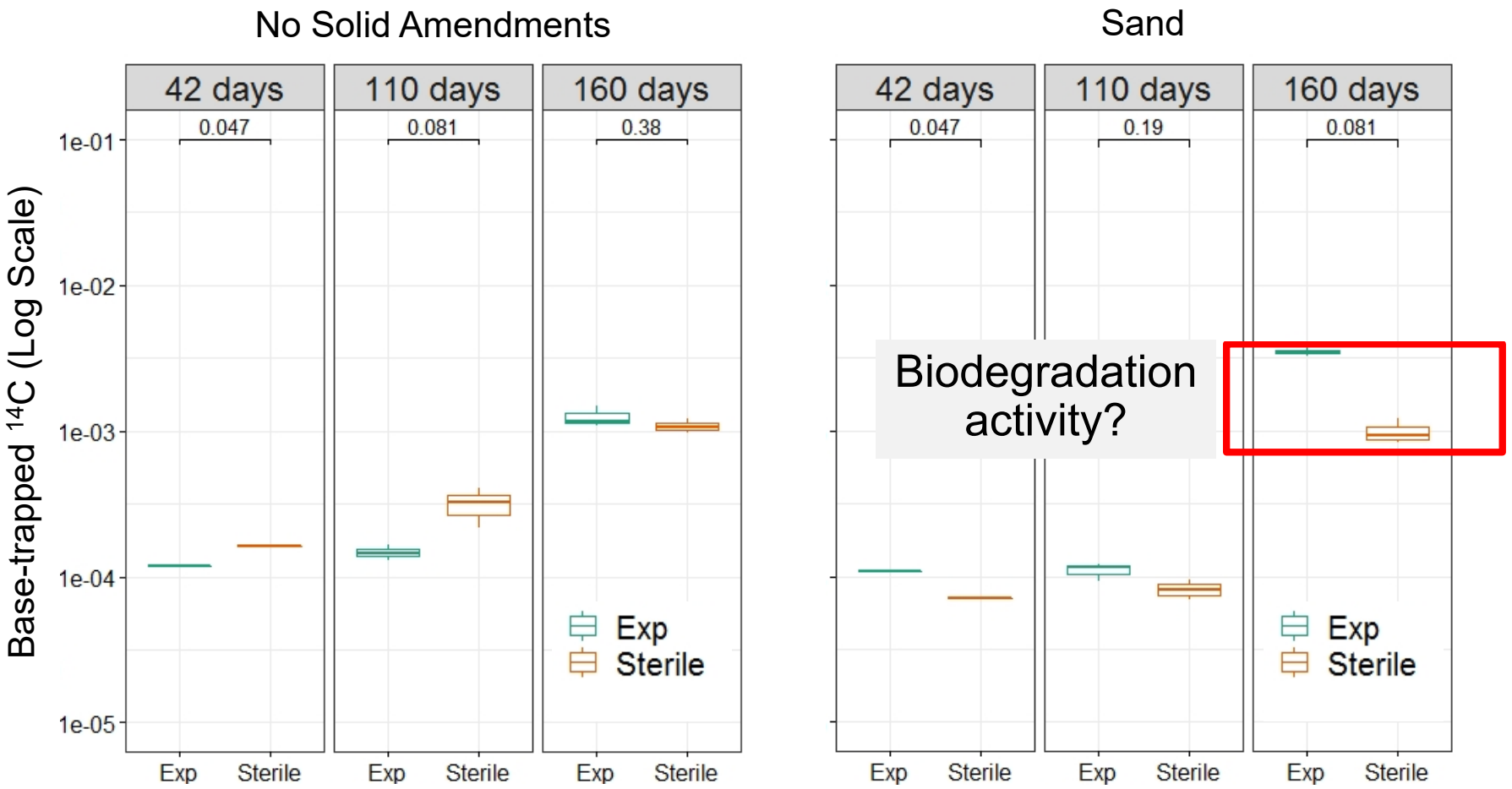
Naphthalene (^{14}C labeled) selected as a model contaminant

Inoculated with a **sulfate reducing enrichment** or 2.5% NaN_3; sulfate serves as the sole (aqueous) acceptor

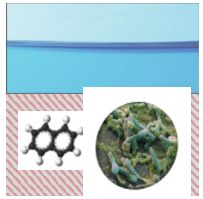
500-day experiment conducted **sand, GAC** or purely aqueous system

“Passive” media promotes anaerobic degradation of naphthalene

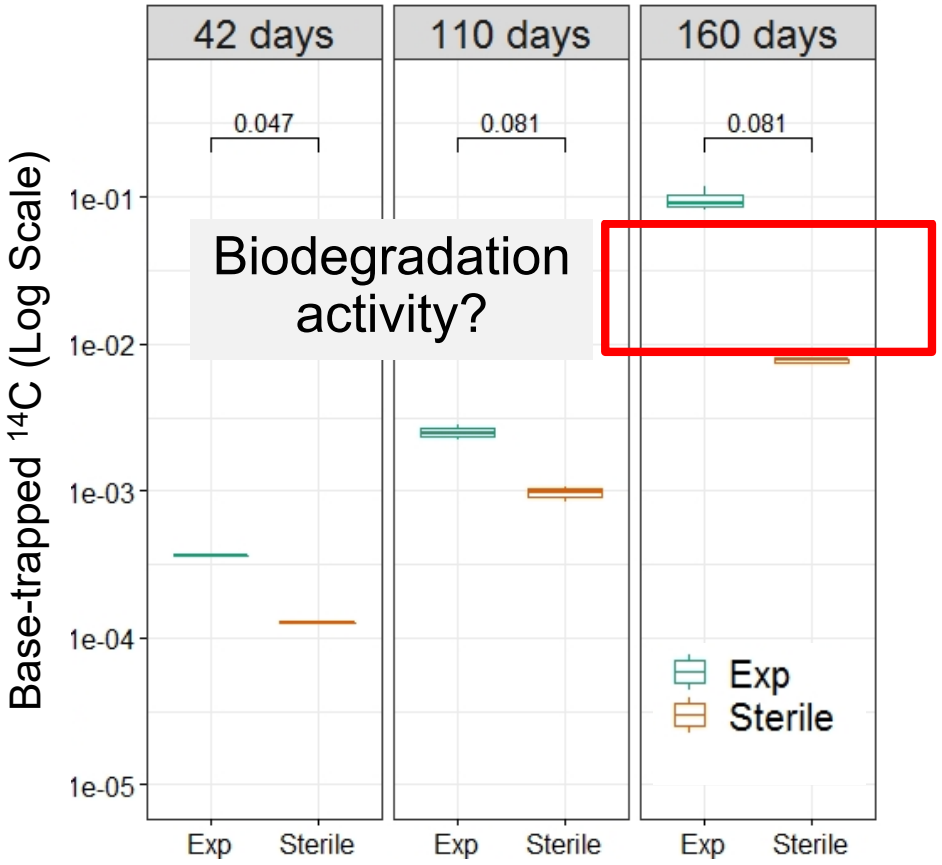




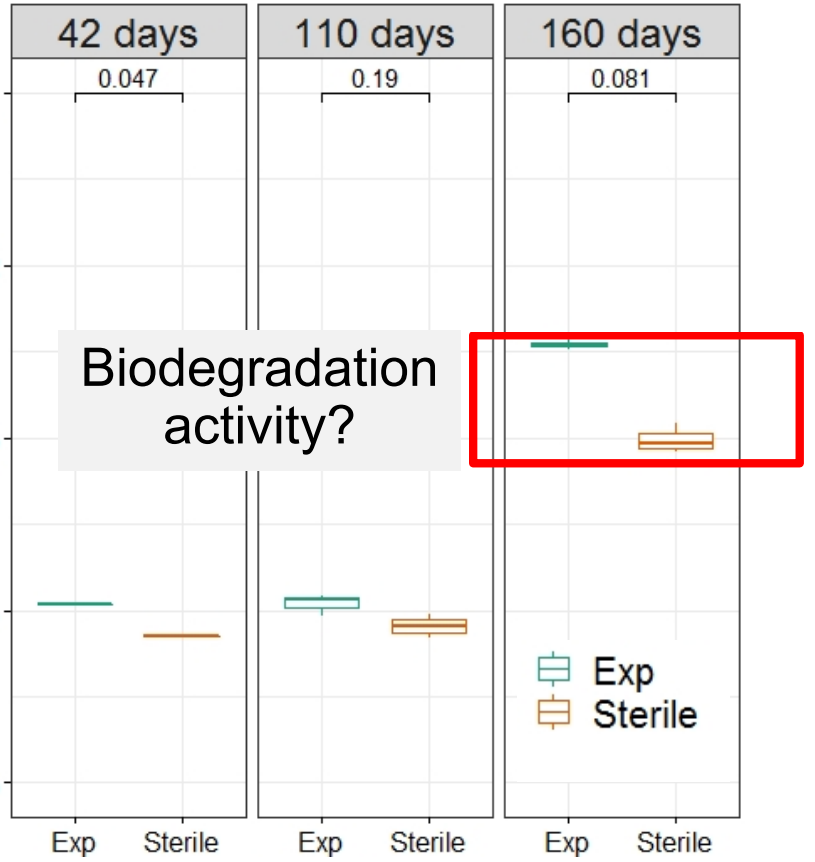
…in a mixed culture, growth system, comparing media in the presence of a model contaminant



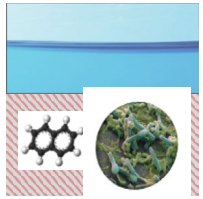
Crushed GAC



Sand



Correct selection and application of media in caps can promote biodegradation activity



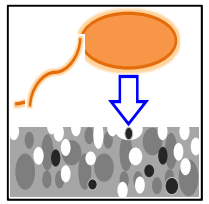
Data supporting naphthalene biodegradation includes:

- Sulfide production, possible $Na_2{}^{13}CO_3$ enrichment, and decreases in total naphthalene within biological systems
- Behavior similar to aerobic experiments (Battelle 2017)

GAC systems may increase bioactivity because:

- Unique physical/chemical properties of the material itself promotes biological behavior
- Interface and desorption of naphthalene may create a microenvironment primed for biological activity

…in a pure culture, static system under non-growth conditions containing GAC

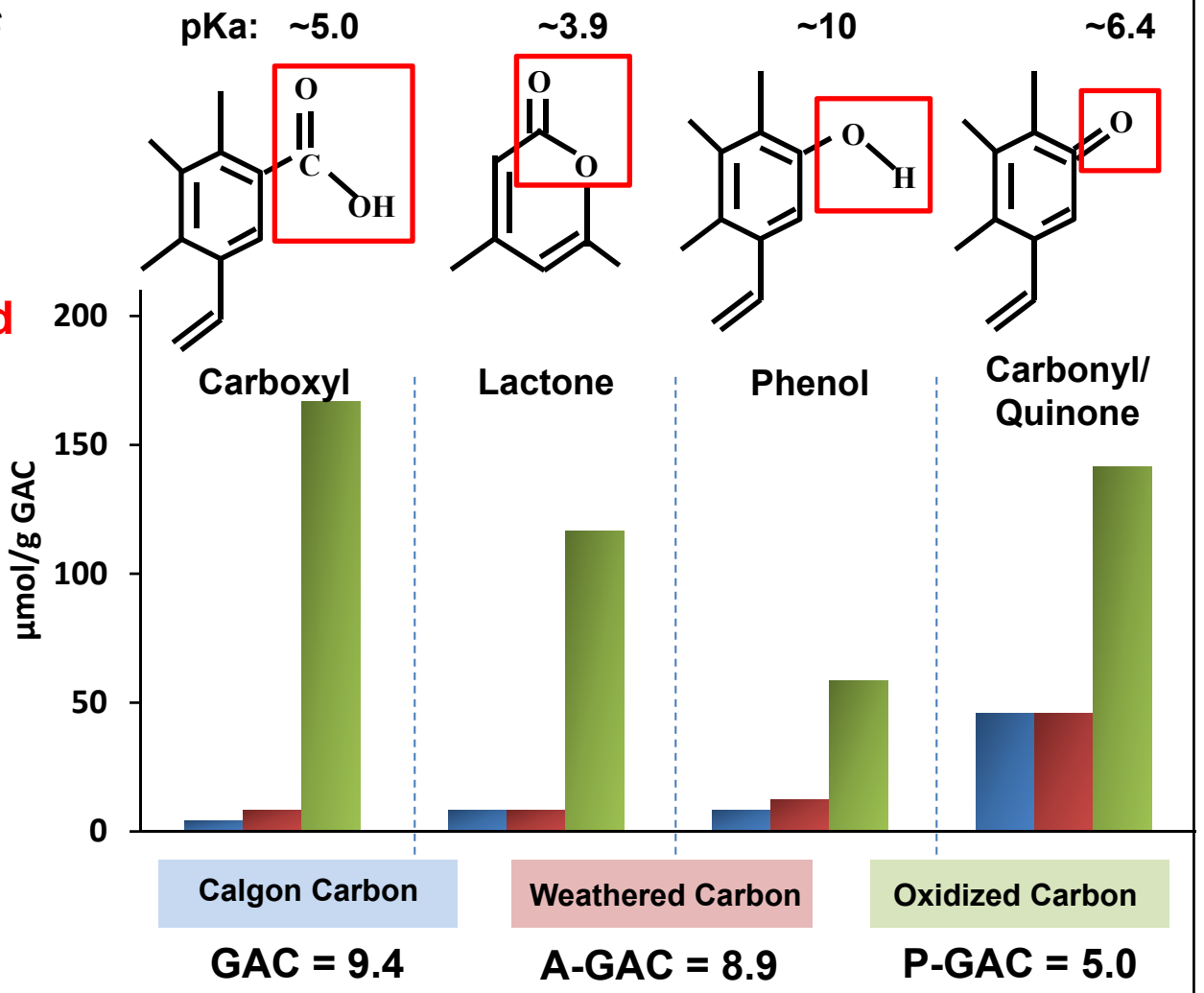


Geobacter sulfurreducens strain PCA inoculated at a final concentration of **10^8 cells/mL**.

Systems **heavily buffered** at respective pH values **but contain no other amendments**, which minimizes growth.

Systems contain **1.5 g/L acetate** as the electron donor (in excess)

Three types of **GAC** examined



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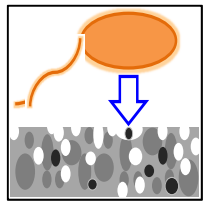
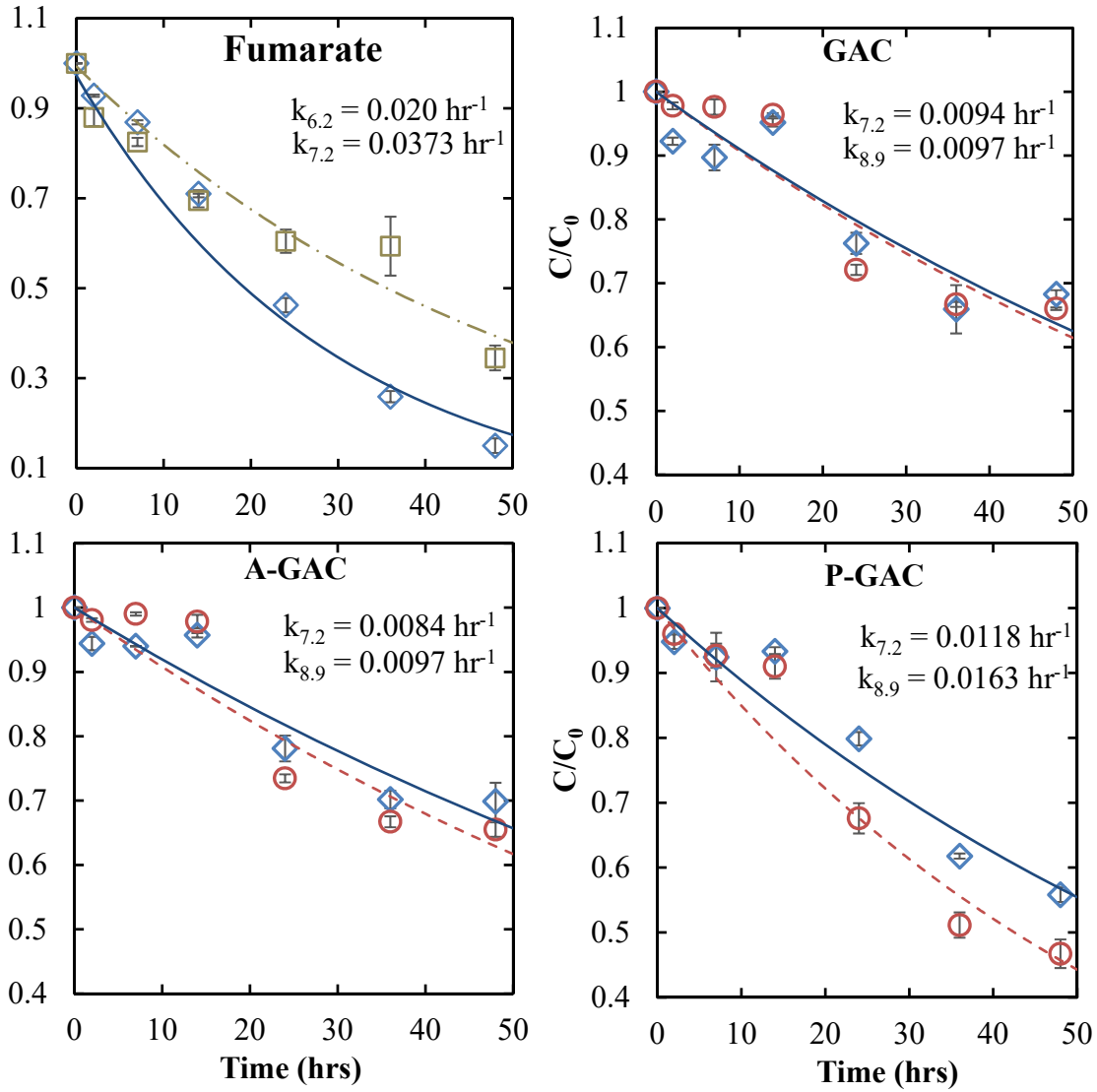




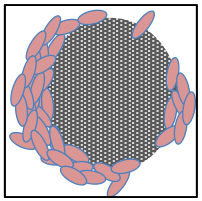
Fumarate as acceptor:

- No activity at pH = 8.9
- Activity most pronounced when pH is circumneutral

AC as acceptor:

- No activity at pH = 6.2
- Activity most pronounced at pH = 8.9

…in a pure culture, growth system containing GAC

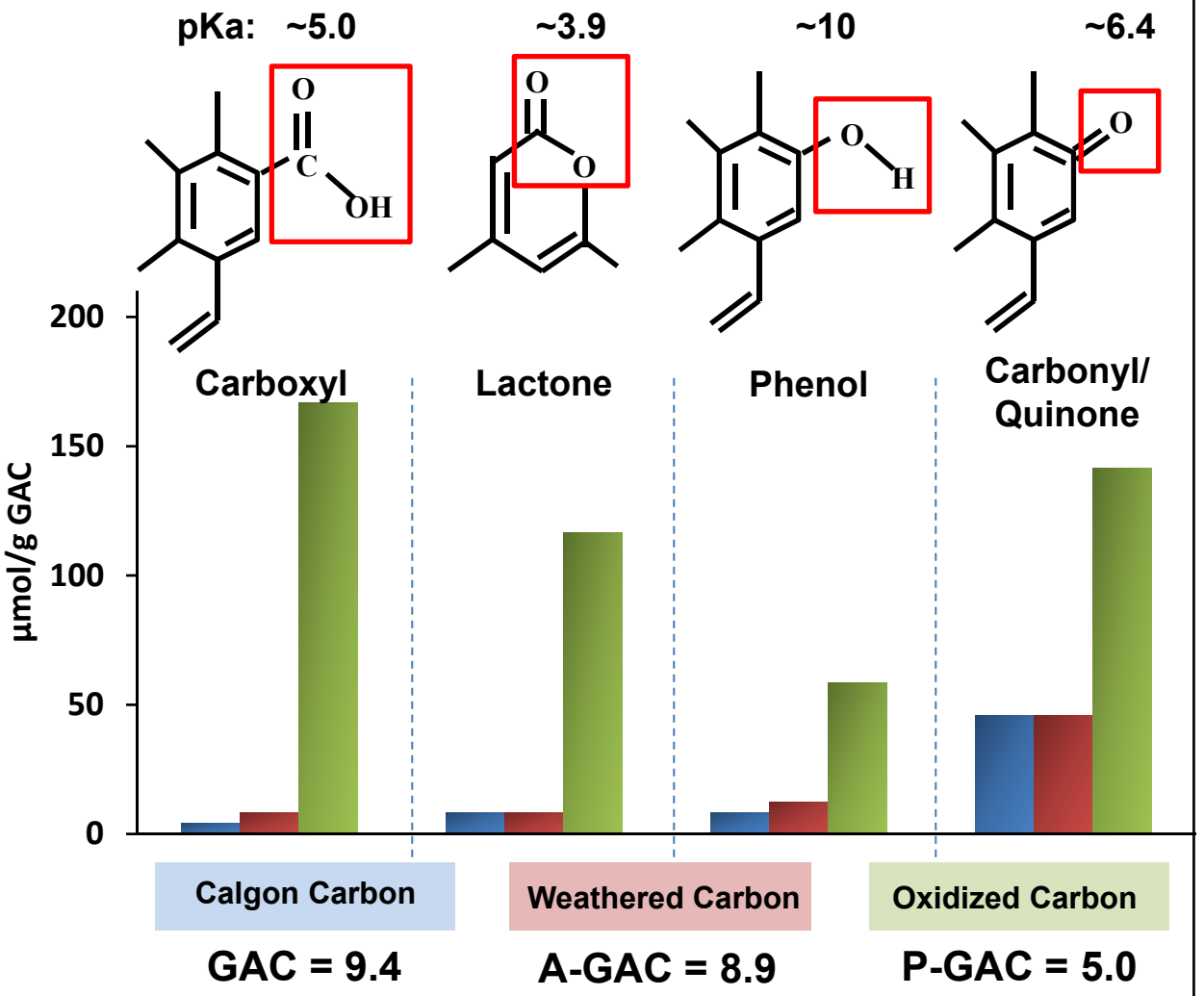


G. sulfurreducens strain PCA inoculated as a **low volume transfer**.

Systems examined at pH = 7.2 **but contains media optimized for cell growth**

Systems contain **1.5 g/L acetate** as the electron donor (in excess)

Three types of **GAC** examined



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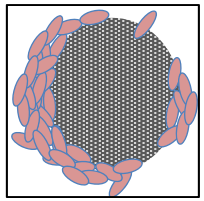
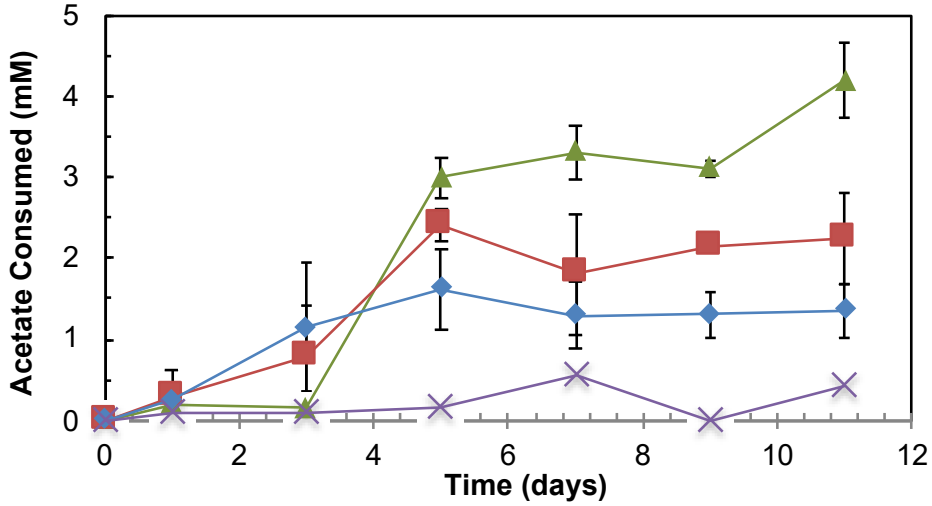
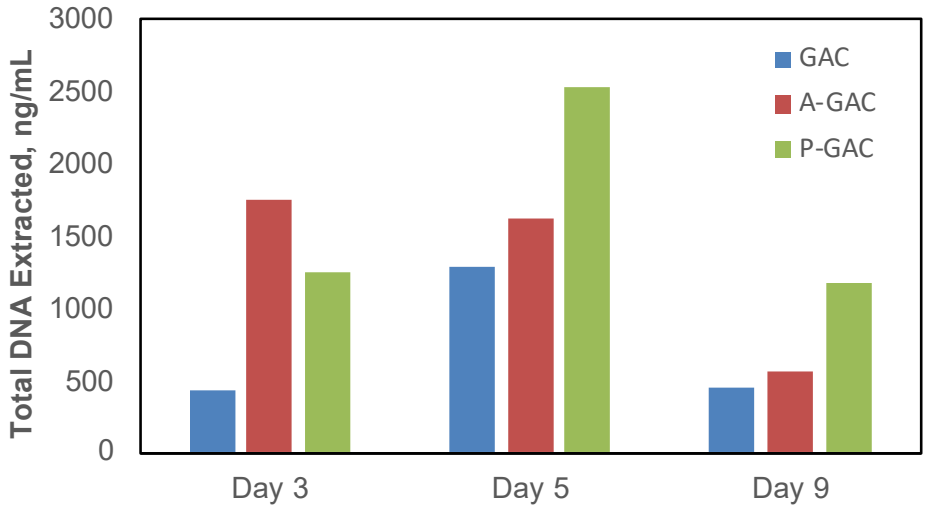
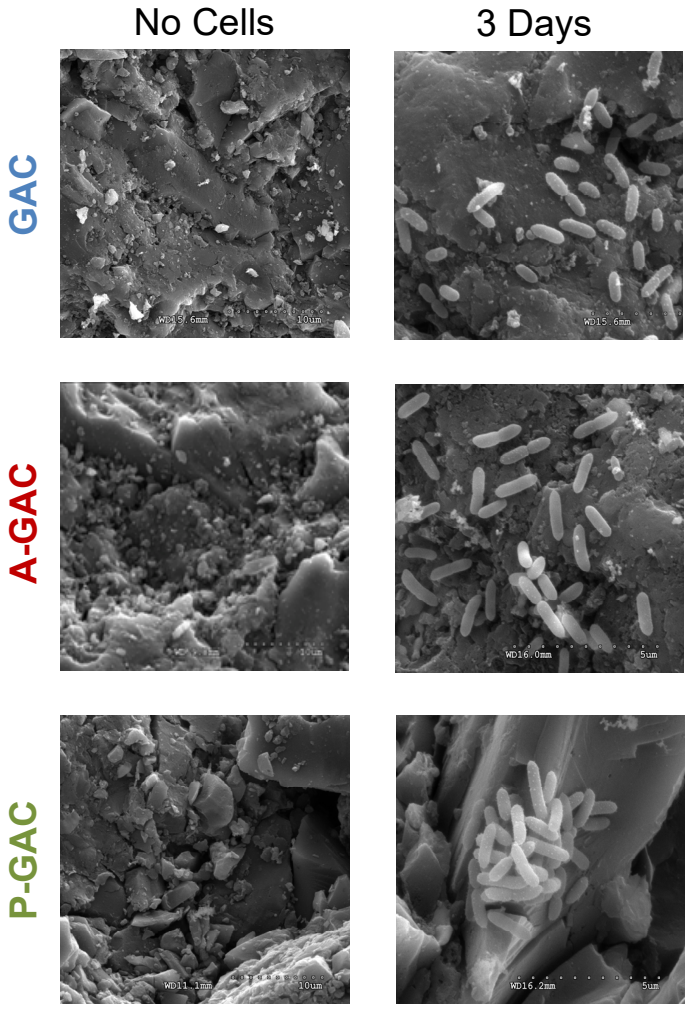


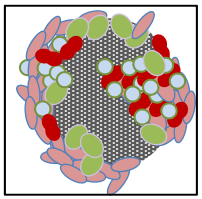






SEM images are 10 μm by 10 μm

…in a **mixed culture, growth system** containing GAC

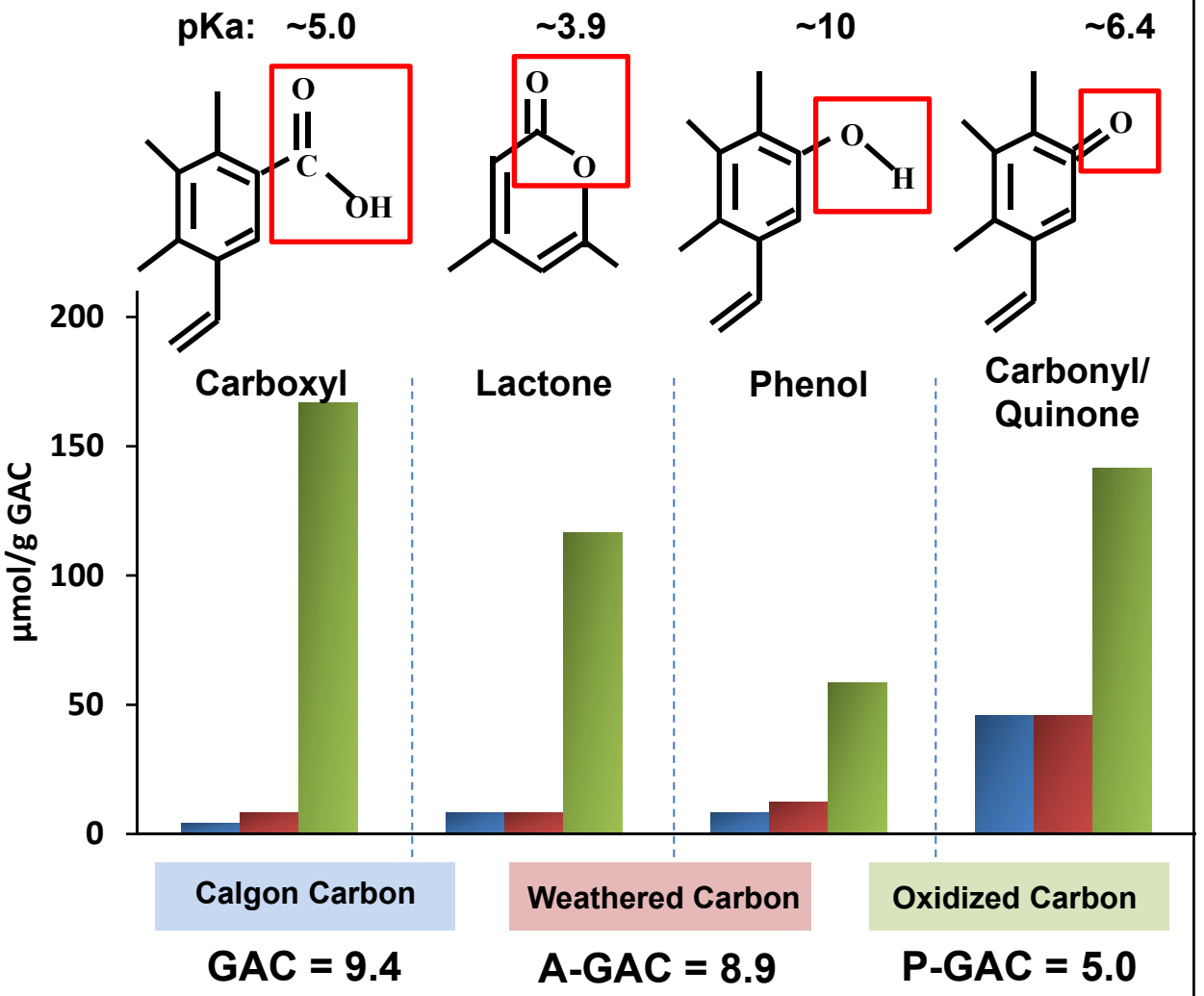


Sulfate reducing enrichment transferred at **low volume transfer**.

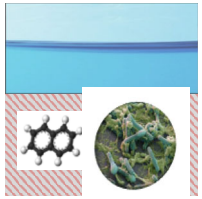
Systems examined at pH = 7.2 **but contains media optimized for cell growth**

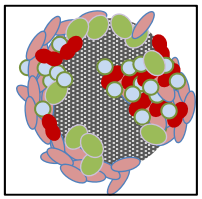
Systems contain **1.5 g/L acetate** as the electron donor (in excess)

Three types of **GAC** examined

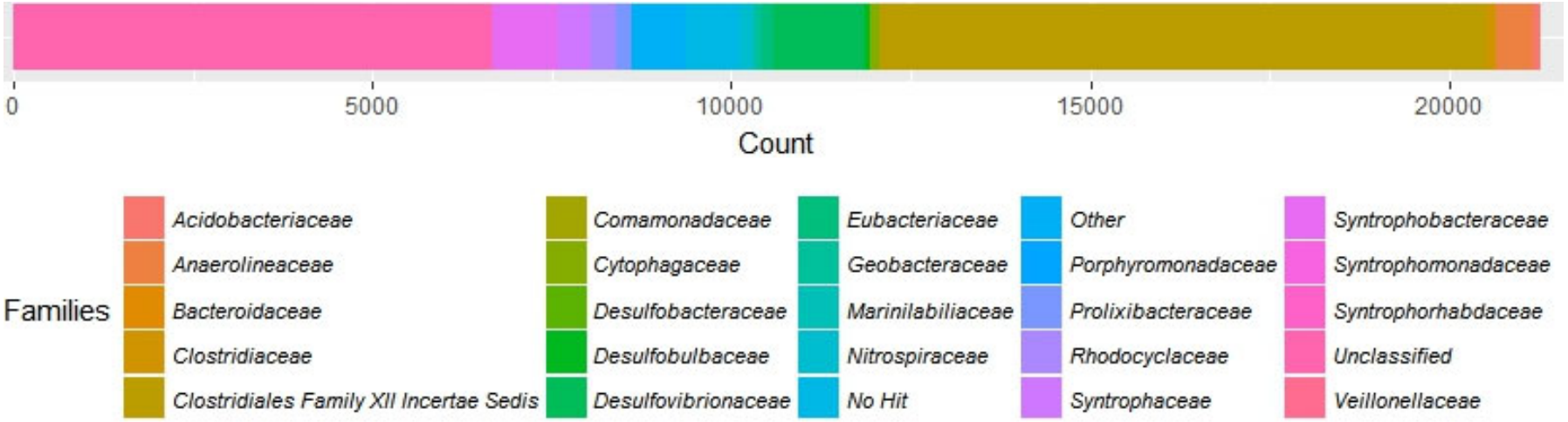


Biological growth on activated carbon:





Community Analysis before Activated Carbon



Community Analysis after Activated Carbon: No Amplification

Solid Media Extraction Techniques
Soil Extraction Kits (*e.g.* PowerSoil)

Common DNA extraction procedures may be insufficient for low biomass/GAC systems

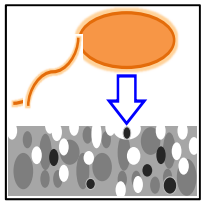
Extraction Techniques	Mechanism	Potential Application to GAC Systems?
Extraction Kits	Lysis via surfactant	Surfactants are (mostly) ionic
Sonication	Lysis via waves	Needs large sample volumes
Phenol-Chloroform	DNA partitions into organics	Improves efficiency of extraction kits
Chelation	Suspends adsorbed cells	Minimal effectiveness

Most DNA extraction protocols for GAC were developed based on **biological drinking water filters**, which are very different than the systems described here

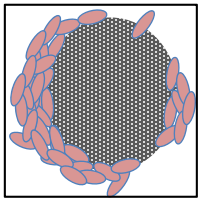
Issues with extractions when **functionalized surfaces (clay)** are present are well understood; GAC represents a similar (but challenging) system

We have optimized an extraction method based on pulsed probe sonication. **It is effective, but time-intensive.**

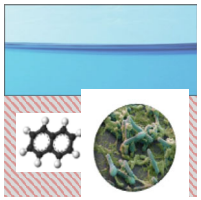
Take Home Messages:



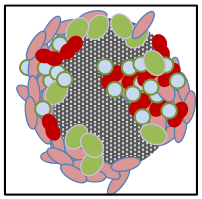
In a static system, use of GAC as a electron acceptor mimics iron. Activity is **most pronounced at elevated pH** (untrue for soluble acceptors).



Increased GAC functionality leads to **more rapid utilization of GAC** as an electron acceptor, and possibly more localized biofilm development



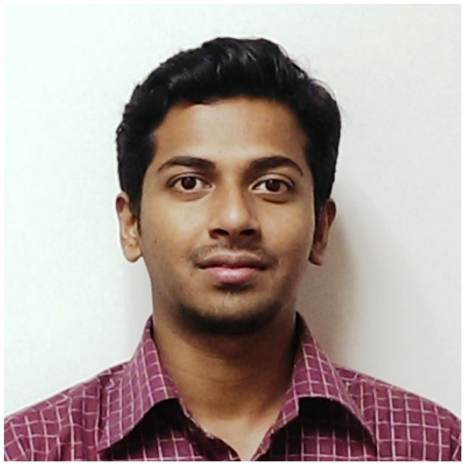
GAC can increase the biodegradation rate constants of **recalcitrant compounds,** even under unfavorable electron accepting conditions



Most methods for DNA (and EPS) extraction are based on systems with **high biomass yields**

We still have much to learn about molecular biology tools in systems containing carbonaceous material and microbes

Acknowledgements



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The Institute of Environmental and Human Health
Haley and Aldrich, Inc.
Electric Power Research Institute
Jenny Qiu, TTU Mechanical Engineering

QUESTIONS?