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Sustainable In-Situ Remediation Co-operative Alliance

Using Passive Anode-Cathode Technology to Assess Microbial Happiness and Boost Benzene Biodegradation Rates

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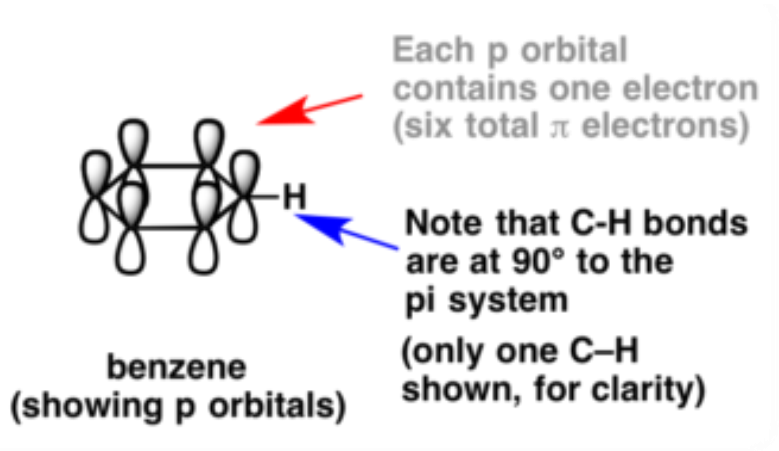
CO-OP

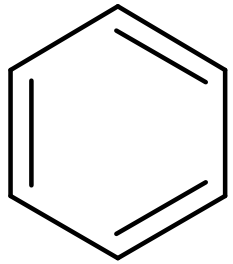
In-situ biostimulant solutions to stimulate anaerobic petroleum hydrocarbon degrading bacteria

Image courtesy of L. Moelhman

Benzene

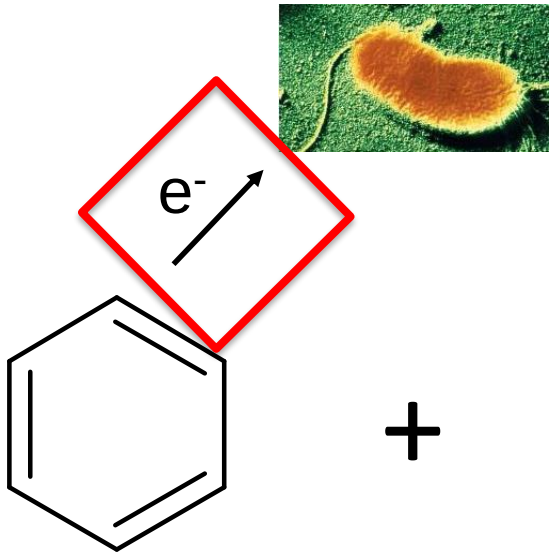
- Nonpolar six carbon aromatic ring (delocalized π bond).
- A highly volatile, colorless liquid.
- Limited to <1% allowable in gasoline due to known carcinogenic effects.





Benzene image from: https://www.masterorganicchemistry.com/2017/02/23/rules-for-aromaticity/

Simplified anaerobic benzene biodegradation



e^-

NO_3^-

SO_4^{2-}

Fe^{3+}

+ → CO_2 +

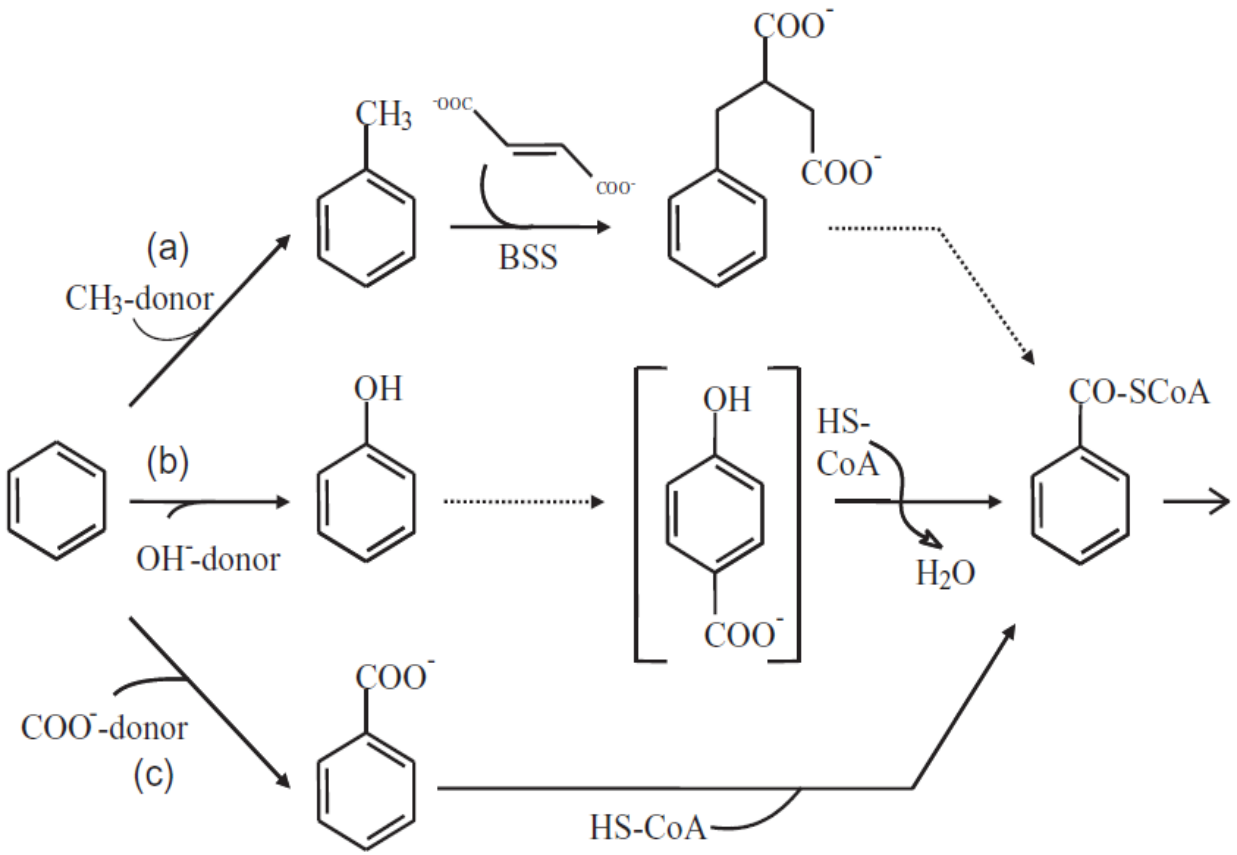
N_2

NO_2^-

H_2S

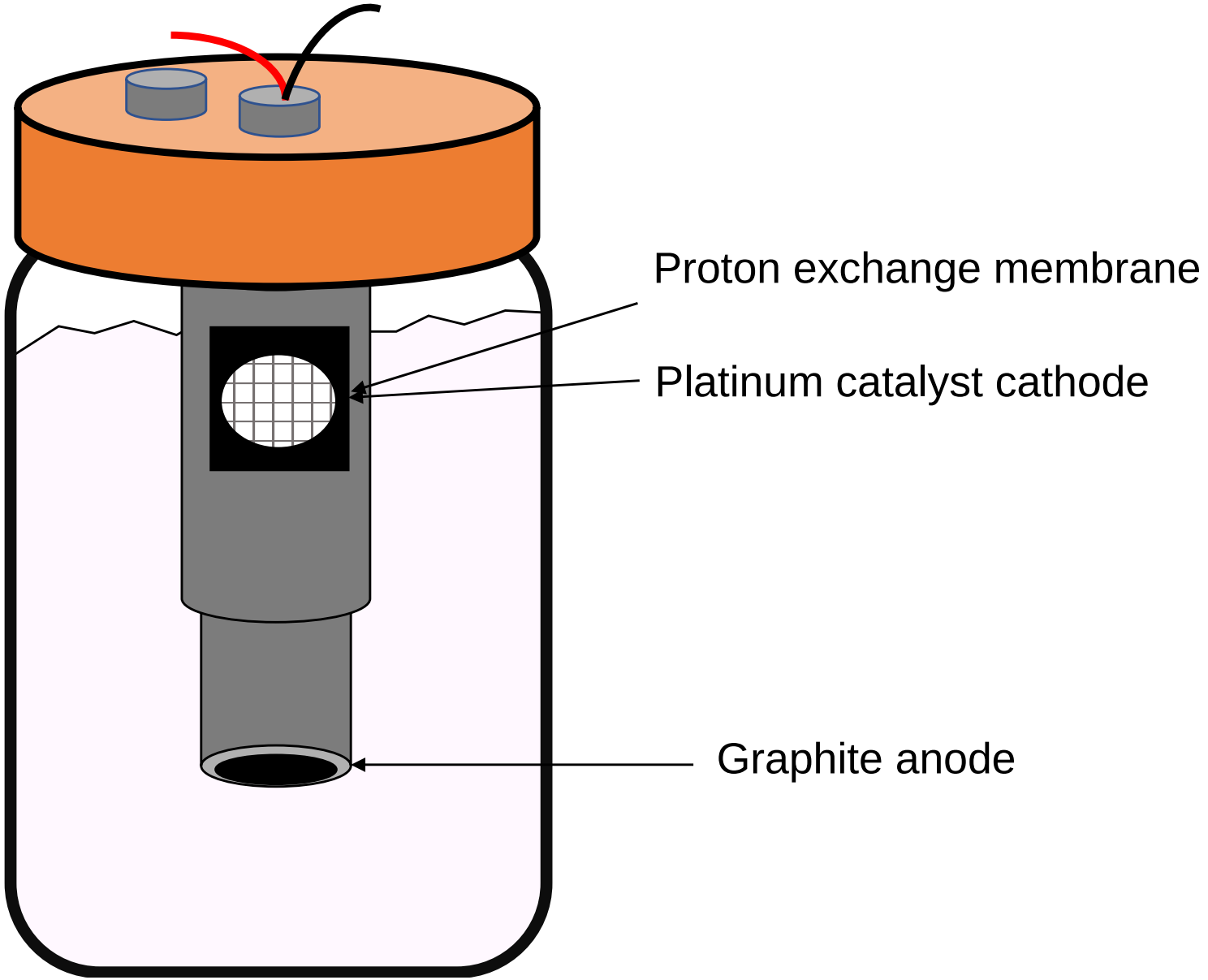
Fe^{2+}

Simplified anaerobic benzene biodegradation

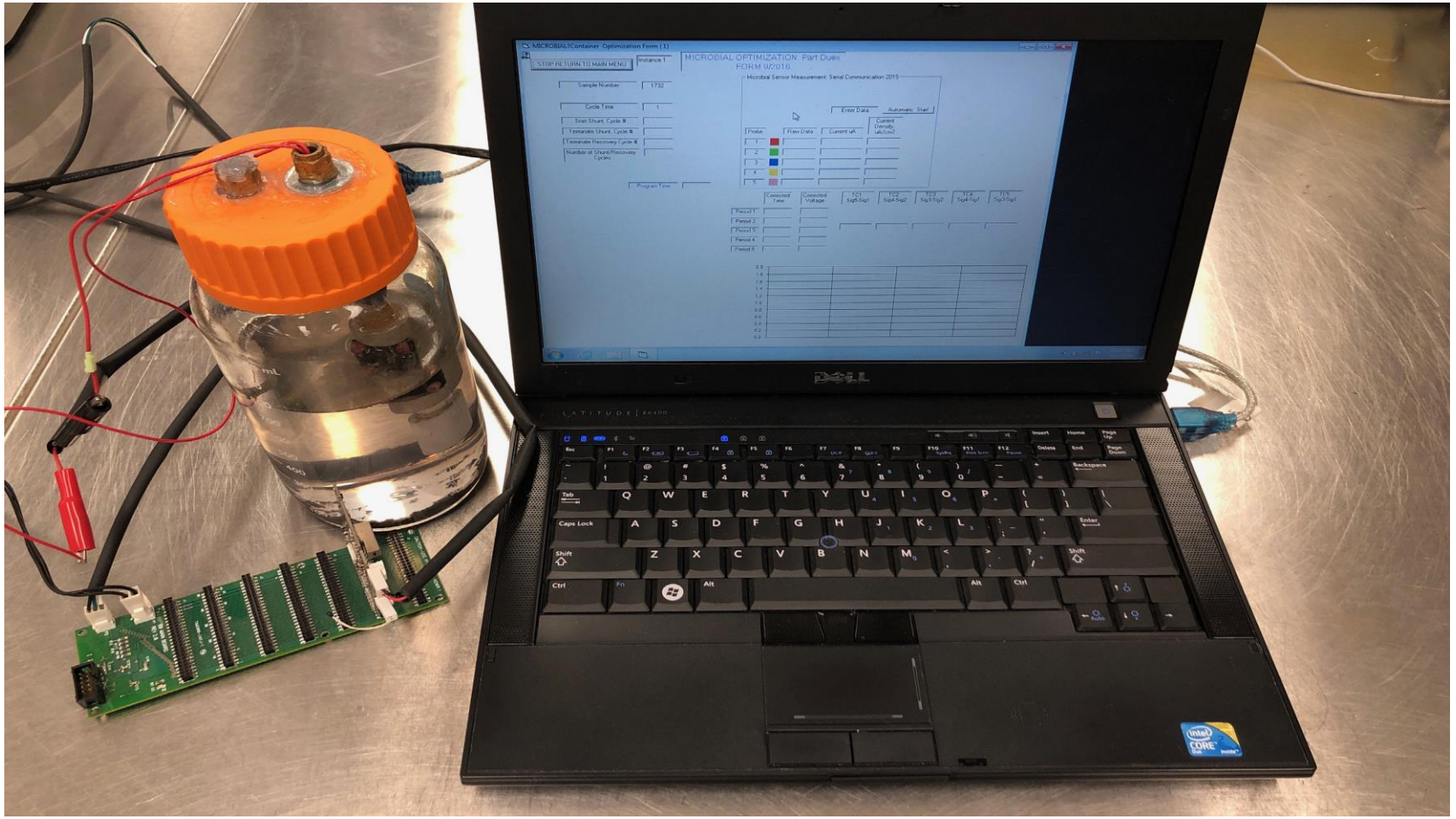


Three plausible pathways for anaerobic benzene degradation via benzoyl-CoA as the central metabolite a) methylation, b) hydroxylation, and c) carboxylation (Modified from Vogt et al., 2011).

Passive Anode-Cathode Technology



Passive Anode-Cathode Technology



Circuit closes to expel deposited electrons and then opens to measure re-deposition in mV

Objectives

i) Determine if voltage gain recorded by PACT indicates microbial respiration and benzene degradation and

ii) If the graphite anode acts as an additional terminal electron acceptors (EA) and/or increases degradation rates.

Experimental Design

Experiment 1: Testing different electron acceptors with PACT (EA)

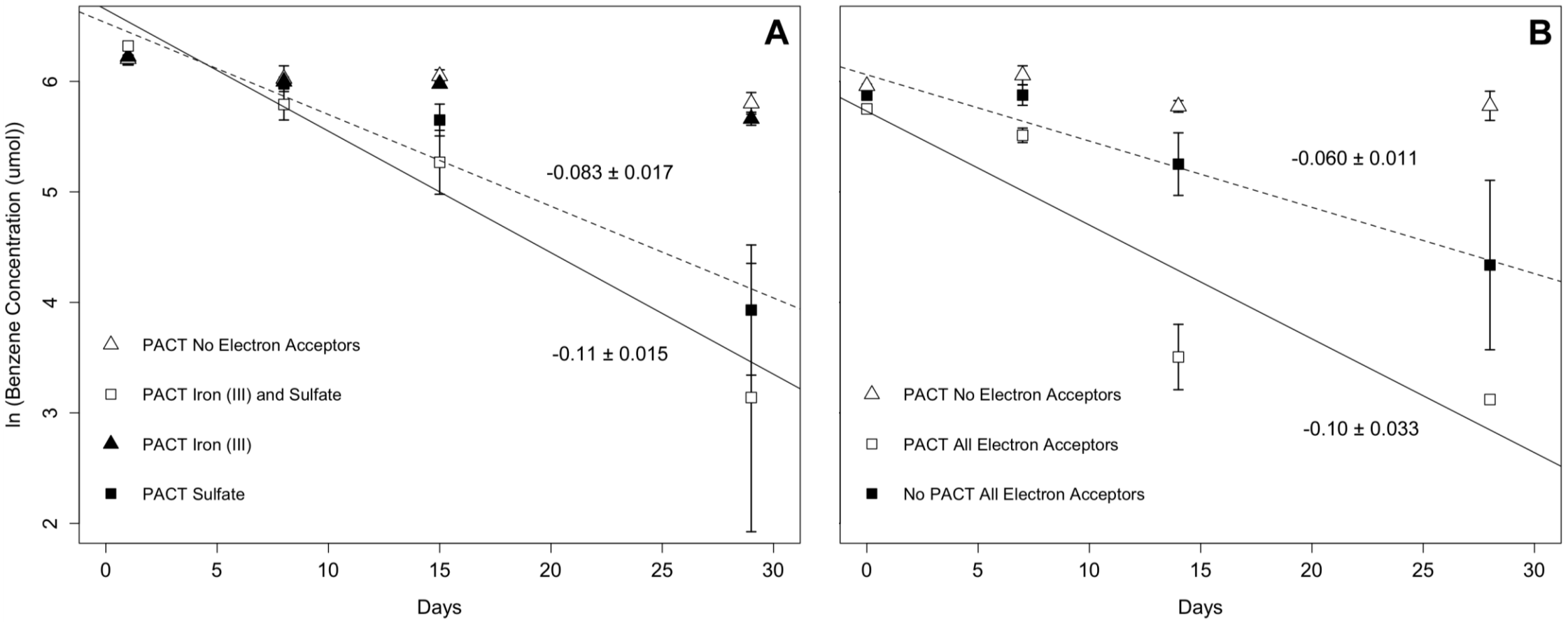
- None
- Fe^{3+}
- SO_4^{2-}
- Fe^{3+} and SO_4^{2-}

Experiment 2: Testing effect of the PACT

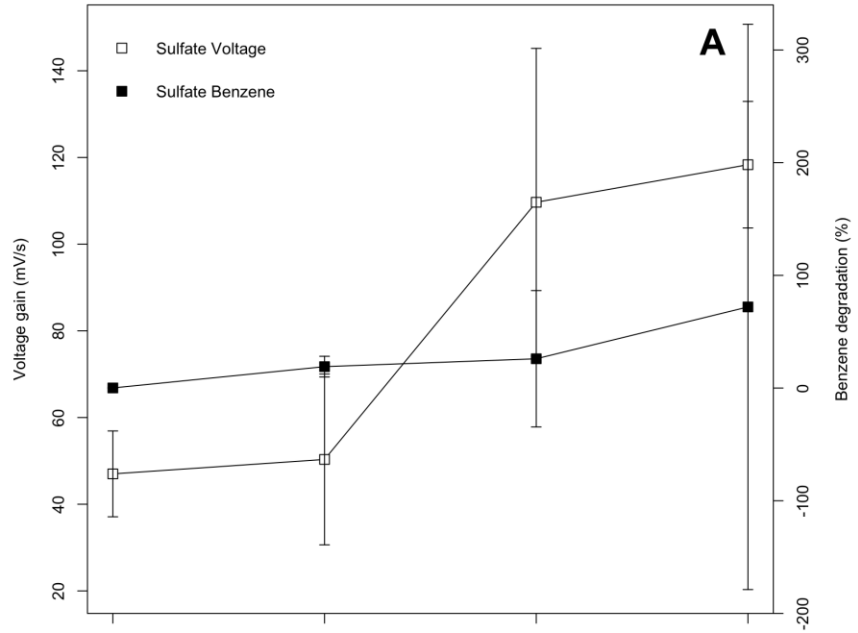
- All EA (NO_3^-, Fe^{3+}, SO_4^{2-}) with and without PACT
- No EA with and without PACT

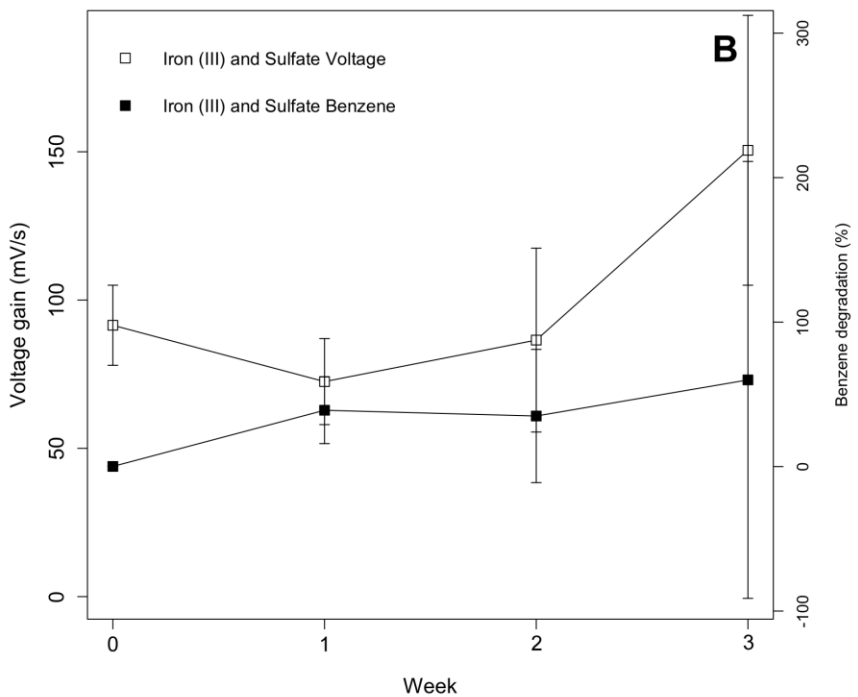
Voltage readings taken and samples were collected for benzene, nutrients, cDNA, and mineralogy each week.

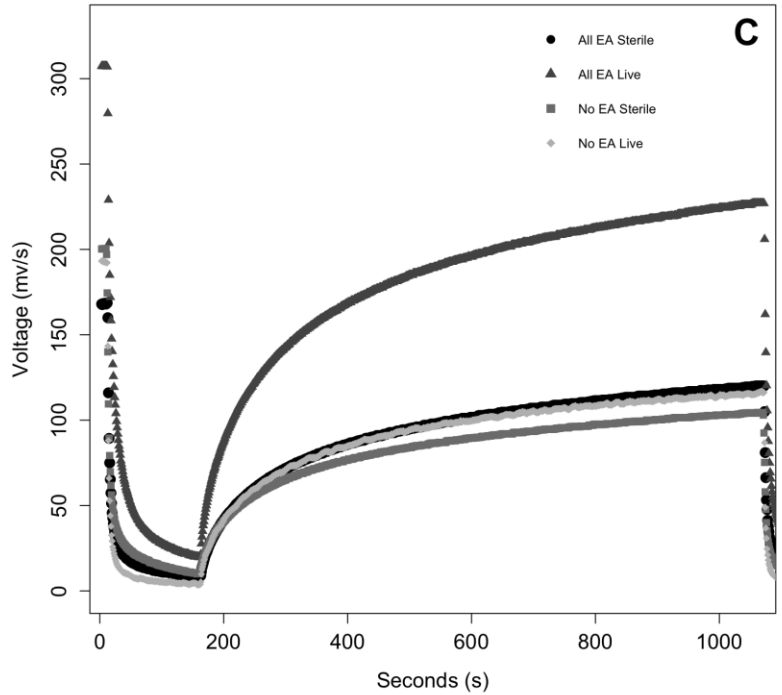
Benzene degradation rates



The greatest degradation rate was observed with Fe^{3+} and SO_4^{2-} with PACT.



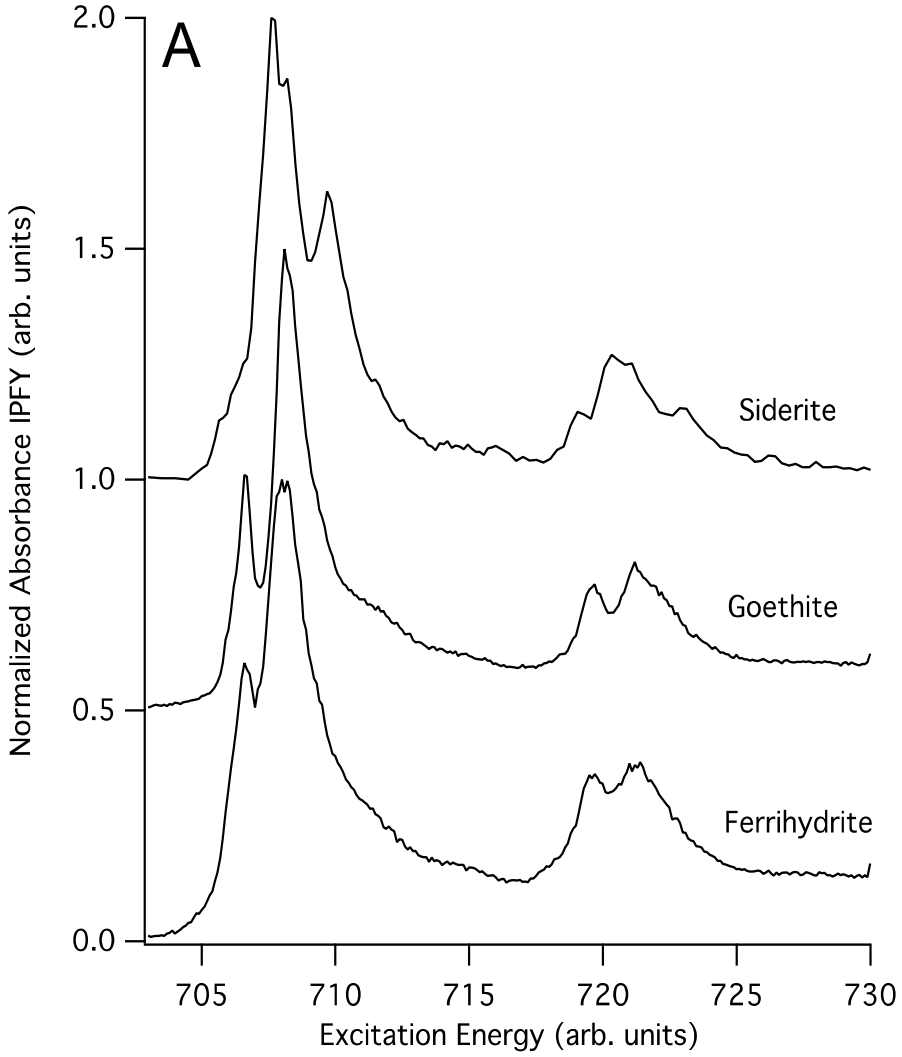


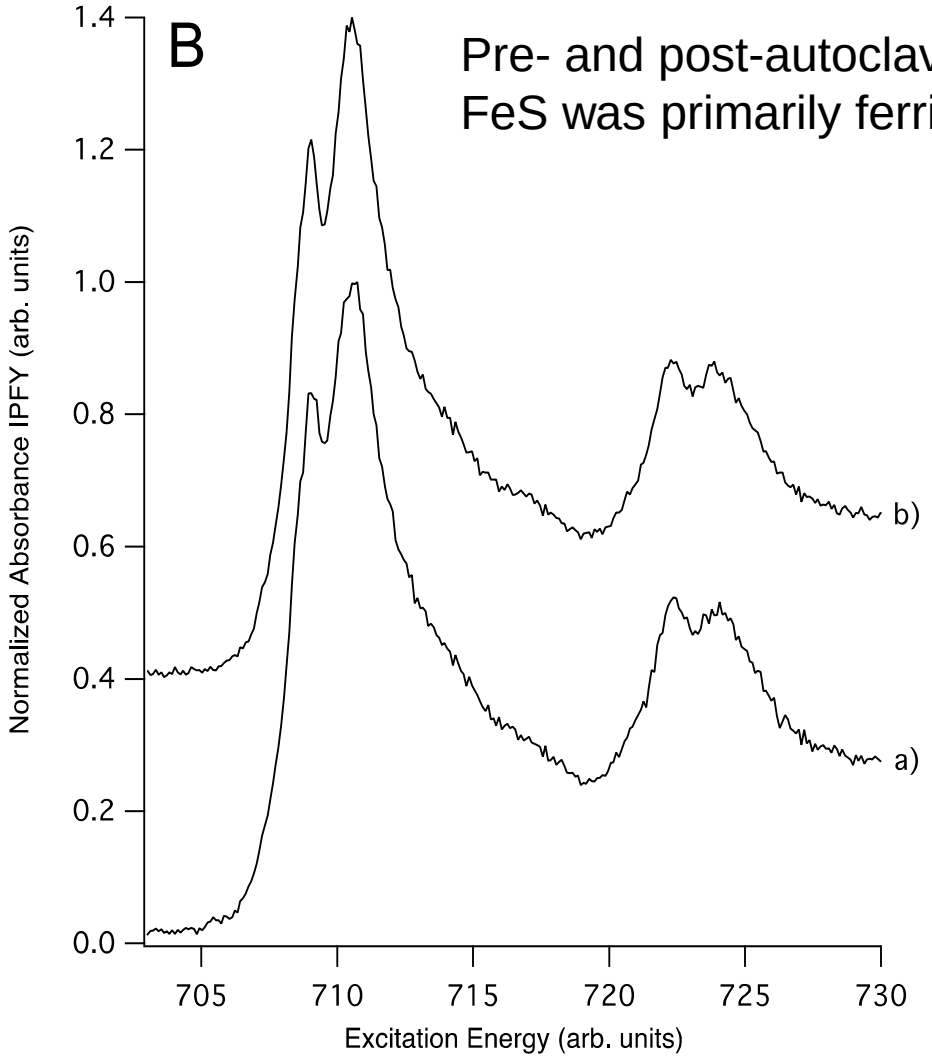


A and B) An increase in voltage gain can be correlated with an increase in percent benzene degraded.

C) Raw voltage data. The All EA treatment shows the greatest increase in mV with electron re-deposition.

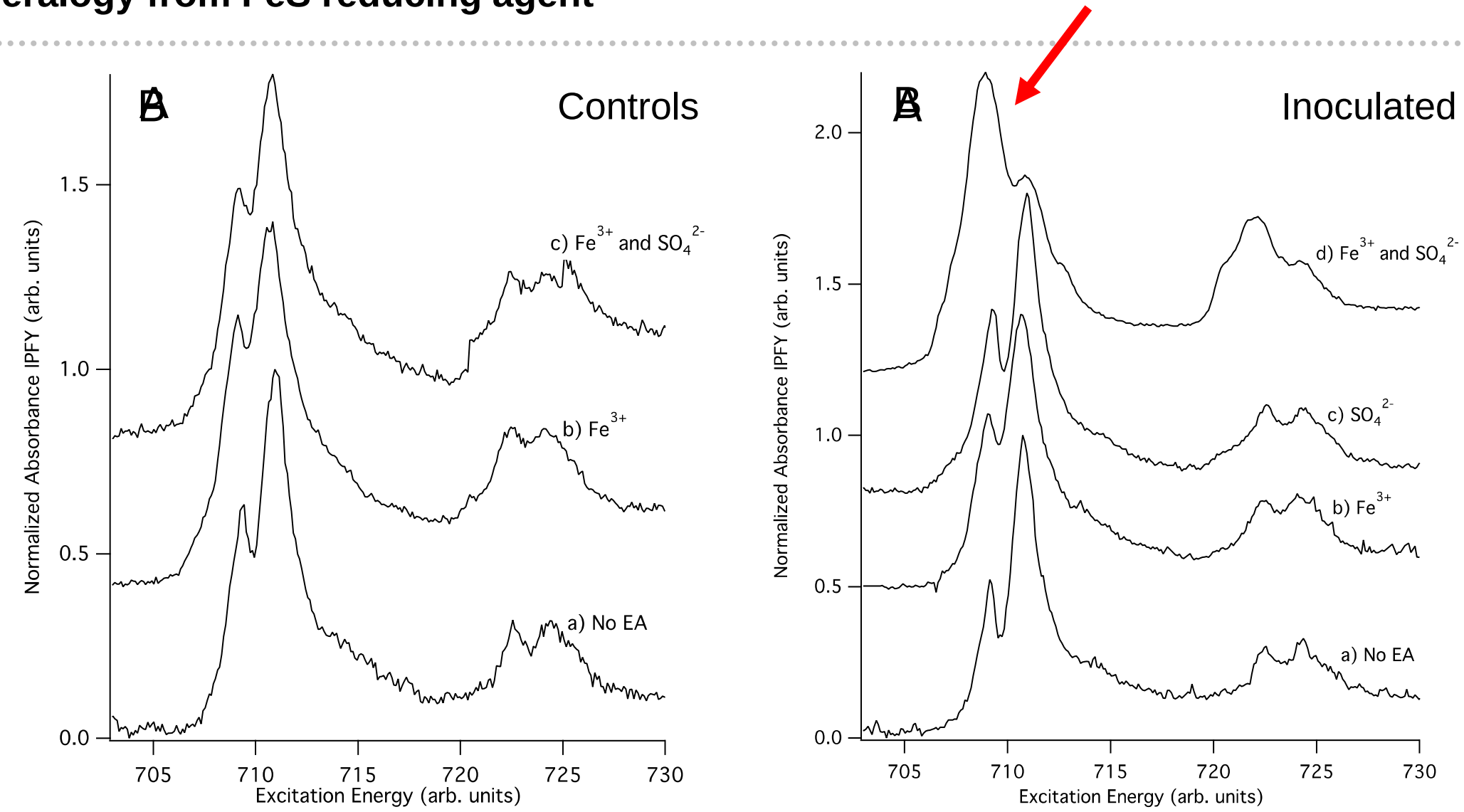
Mineralogy from FeS reducing agent



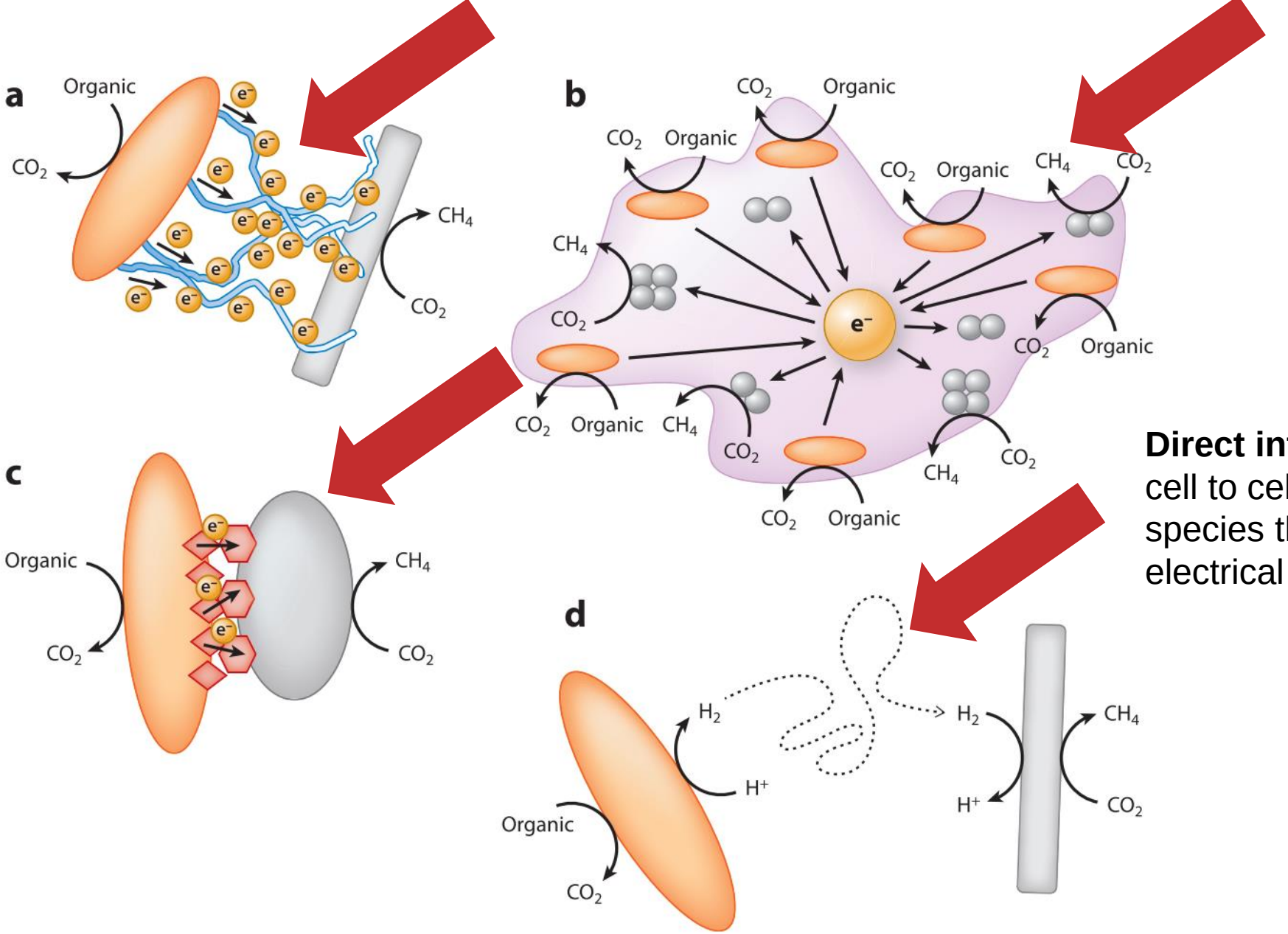


Pre- and post-autoclaving,
FeS was primarily ferrihydrite.

Mineralogy from FeS reducing agent



Inoculated cultures had more crystalline Fe oxyhydroxides.
Fastest degrading treatment has Fe^{2+} rich siderite.



Direct interspecies electron transfer: cell to cell transfer of electrons between species through shared physical electrical connections (Lovley, 2017).

Figure modified from “Syntrophy Goes Electric: Direct Interspecies Electron Transfer” by Derek Lovley (2017)

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PACT graphite anode may have been acting as an alternate EA.

Treatment	Benzene biodegraded	Sulfate produced	Total Solution Iron	Benzene biodegradation rate*
	---------- µmol ----------			µmol day^{-1}
No Electron Acceptors†	49 ± 20^{a}	420 ± 94^{b}	0^{b†}	1.0
Ferric Iron	105 ± 19^{a}	490 ± 90^{b}	62 ± 2.7^{a} (1:1)$^{\#}$	3.5
Sulfate	300 ± 20^{b}	250 ± 250^{a}	0^{b†}	9.3
Ferric Iron and Sulfate	360 ± 20^{b}	530 ± 170^{a}	6.9 ± 15^{b} (1:0.01)^{b}	12

^{a,b} Different letters indicate differences between values within a column at $p < 0.05$.
†Values were below the 1 ∝mol detection limit.
#Benzene:EA ratio.
* Estimated by dividing benzene biodegraded by days incubated.

Benzene:sulfate ratio typical at ~1:4, but degradation rate is much quicker than previously observed.

With PACT, denitrifiers were the active community.
Without PACT, nitrate reducers were the active community.

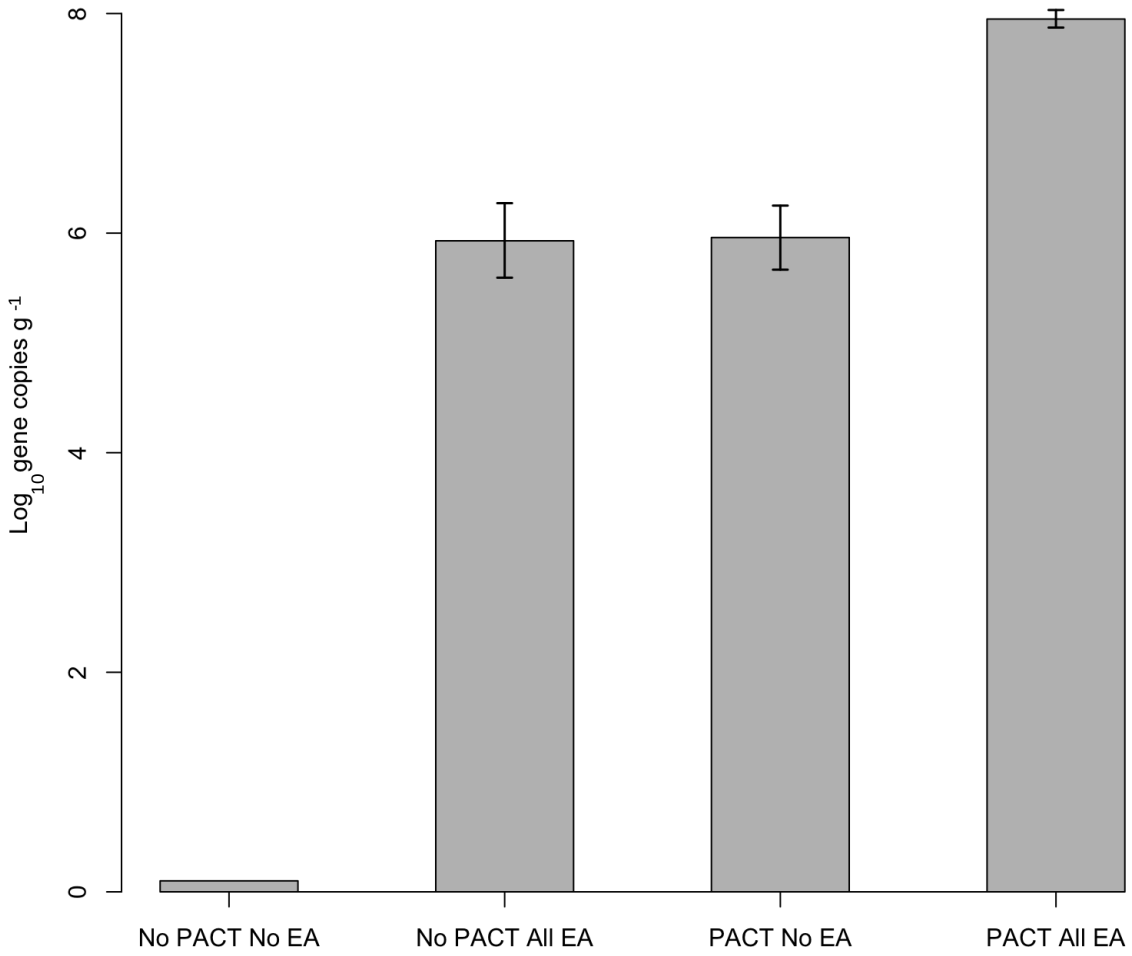
Treatment	Benzene biodegraded	Nitrate reduced	Sulfate produced	Total Iron	Solution	Benzene biodegradation rate†
	---------- µmol ----------					µmol day^{-1}
PACT[a]	260 ± 46	1600 ± 32 (1:6) #	780 ± 60	46 ± 16 (1:0.2)		9.3
No PACT[b]	140 ± 30	1700 ± 44 (1:12)	350 ± 120	65 ± 13 (1:0.5)		5.1

[a,b] Different letters indicate differences between values within a column at $p < 0.05$.

† Estimated by dividing benzene biodegraded by days incubated.

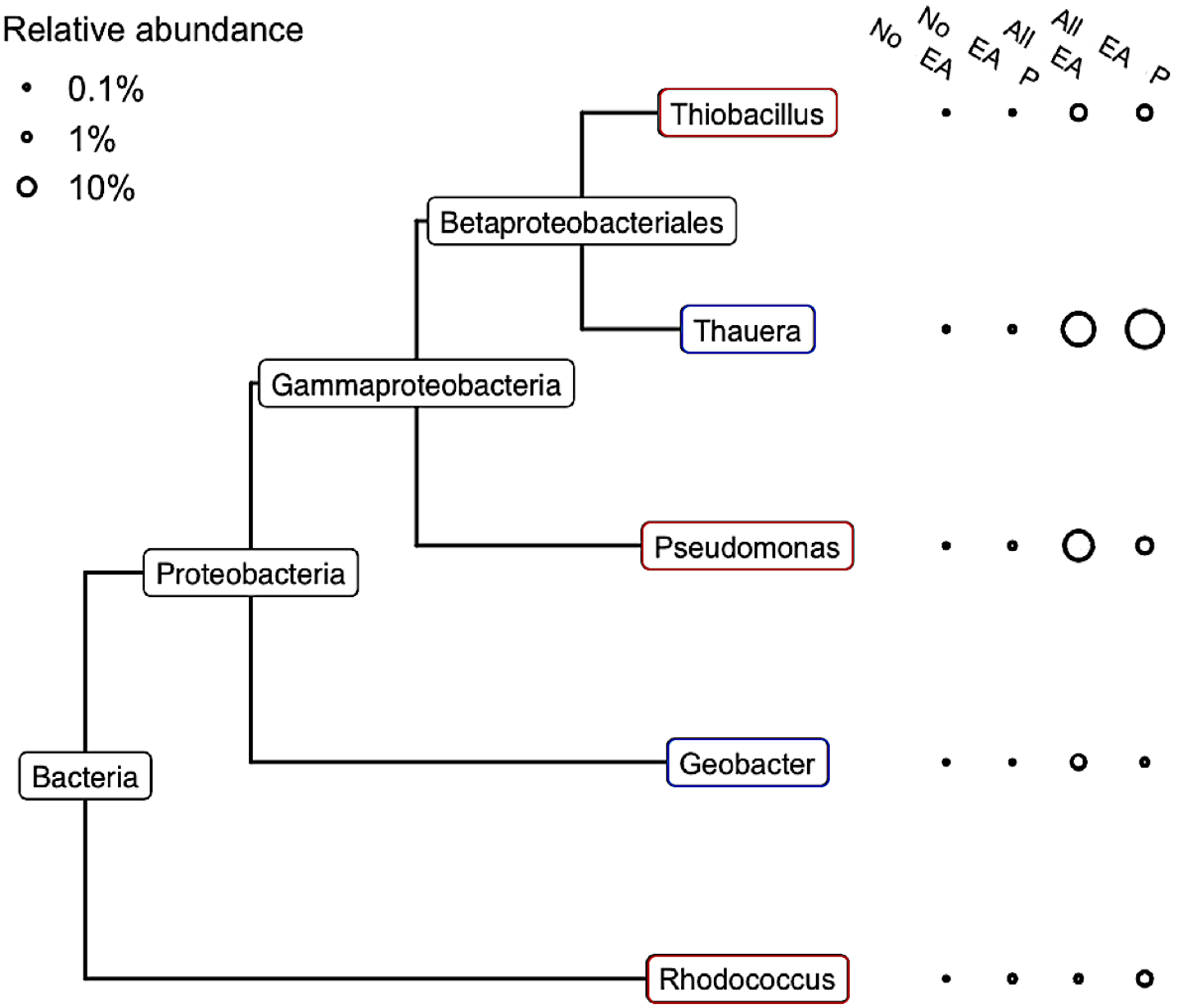
Benzene:EA ratio.

bcrC gene copies (encoding for *Thauera* type bacteria)



Treatments with PACT (all EA and no EA) had *Thauera* type bacteria.

The most biomass was in the PACT All EA treatment.



A microbial community capable of DIET is present in the All EA treatments with and without PACT.

Conclusions

Why it might be direct interspecies electron transfer (DIET):

- There are two surfaces for biofilm formation, the graphite anode and iron oxyhydroxides.
- There was an increase in benzene degradation rates with PACT.
- Unusual stoichiometry suggests alternate electron transfer processes.
- The active microbial community has genera capable of DIET.
- An increase in voltage with benzene degradation indicates an electrically active microbial community.

Applications

The area of influence of the PACT is too small for a field scale…

However, in the case of in-situ biostimulant solutions with alternate EA to stimulate anaerobic bacteria, adding a constituent such as graphite to the mix could help mimic the conditions with the PACT.

Acknowledgments

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Burge Environmental

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Questions?