SIRCA

Sustainable In-Situ Remediation
Co-operative Alliance

Does Surface Matter? Bacterial Response to Amendments and Benzene Adsorbed to Fe Oxides

Kathlyne Hyde, Derek Peak, and Steven D. Siciliano

Presenting Members

Battelle's Fourth International Symposium on
Bioremediation and Sustainable Environmental Technologies
May 22-25, 2017 | Miami, Florida

University of Saskatchewan

CO-OP

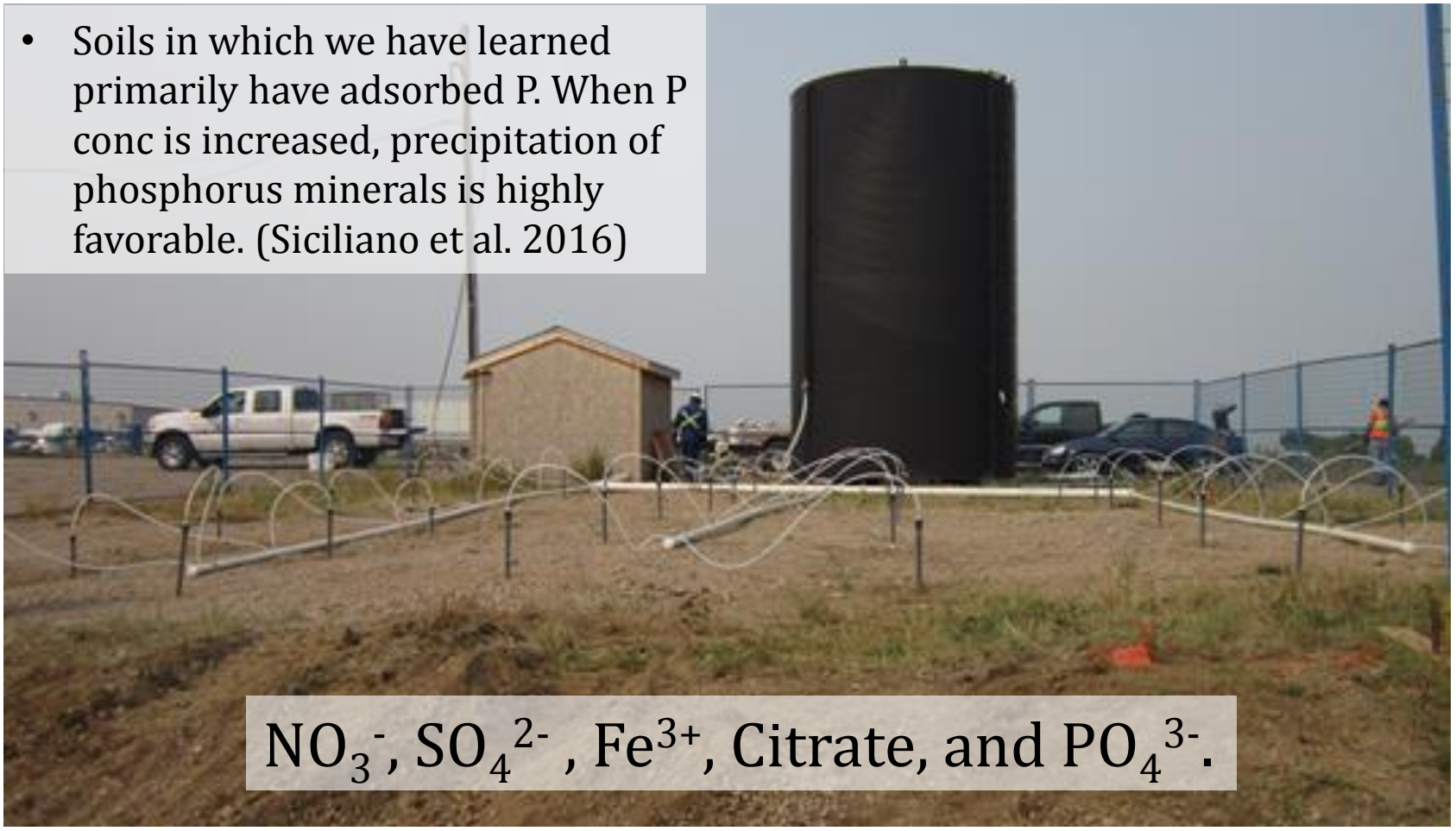
Background

Western Canadian Soils

- Glacial till
- Highly heterogeneous
- Calcareous
- High clay content
- Undergo freezing

What we know about our PHC contaminated sites...

- Soils in which we have learned primarily have adsorbed P. When P conc is increased, precipitation of phosphorus minerals is highly favorable. (Siciliano et al. 2016)



NO_3^-, SO_4^{2-} , Fe^{3+}, Citrate, and PO_4^{3-}.

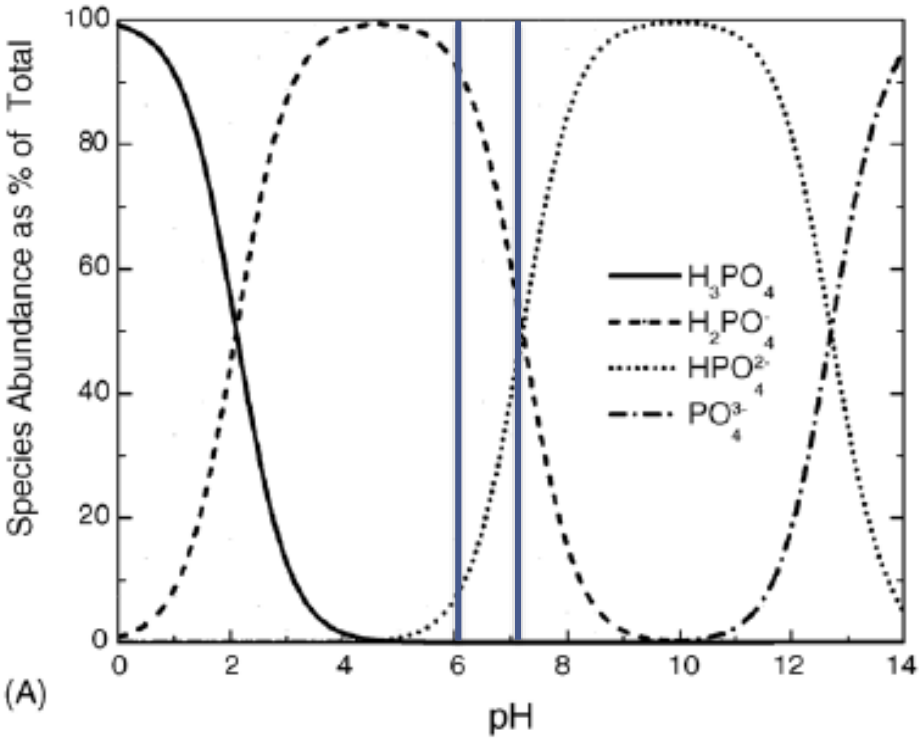
Image courtesy of L. Moelhman

Linking soil mineralogy and microbiology

What happens when we inject these solutions? How can we understand our sites better to cater the amendment for the best possible outcome?

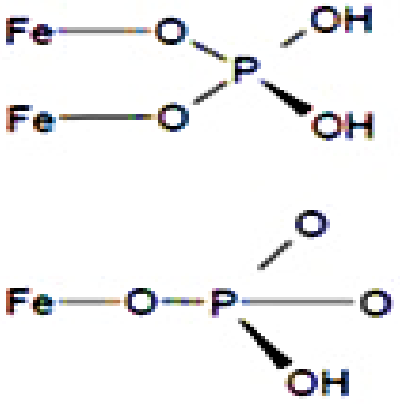
Orthophosphate

- Limiting nutrient for microbial growth
- Highly reactive in soils
- Dominate forms are HPO_4^{2-} and $H_2PO_4^-$



Adsorption complexes

- Outer-sphere
- Inner-sphere



Bidentate mononuclear and monodentate mononuclear bonding of orthophosphate to hematite surface groups

(Hanrahan et al., 2015)

Representative Minerals

- **Reactive Surface: Hematite**
- Iron oxide α-Fe_2O_3
- Mostly singly coordinated oxygen in the hexagonal orientation
- PZC ~ 8.2 = positively charge surface at neutral pH

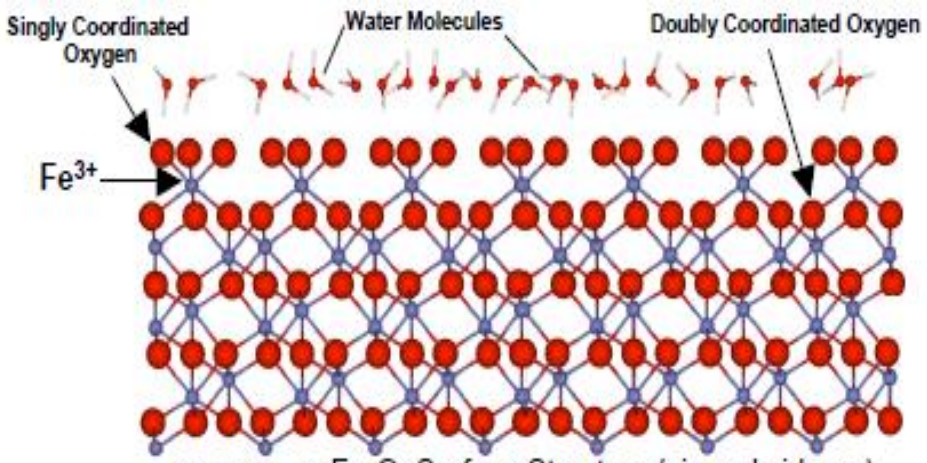


Figure 2 - α-Fe_2O_3 Surface Structure (viewed side-on)

(Sparks, 2013; Trainor et al., 2004)

Representative Minerals

- **Semi-Reactive Surface: Corundum**
- Aluminum oxide α-Al_2O_3
- Doubly coordinated oxygen in the hexagonal orientation
- PZC 4-6 or 8-10

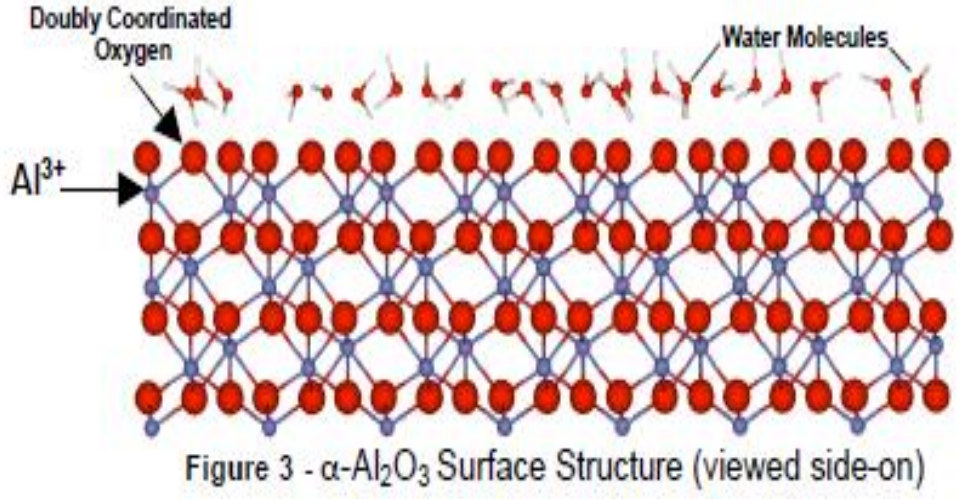
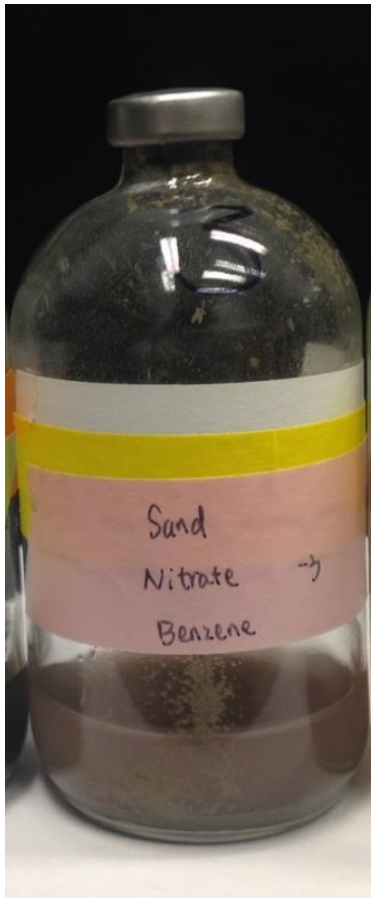


Figure 3 - α-Al_2O_3 Surface Structure (viewed side-on)

(Trainor et al., 2004)

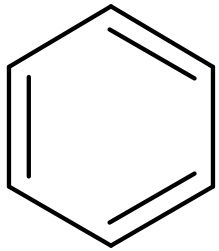
Enrichment Culture: The Ulrich Culture



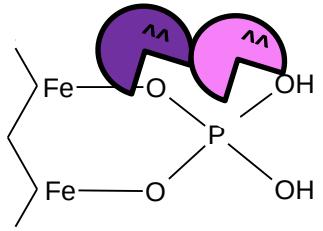
- Oil sands process affected water
- Mixed culture
- Nitrate – reducing benzene – degraders
  - Common Genera: *Azoarcuz* & *Thauera*

Possible benzene degradation pathways

1) Methylation
2) Hydroxylation
3) Carboxylation



Hypotheses



1) Hematite (reactive surface – inner-sphere) and corundum (partially reactive surface – outer-sphere) will have similar capacities for adsorbing orthophosphate.

1) Benzene degradation rates will increase in the presence of hematite due to hematite's unique ability as a reactive surface.

2) Orthophosphate adsorbed to mineral surfaces via inner-sphere and outer-sphere complexes is accessible to bacteria – specifically hydrocarbon degraders.

3) The orientation of a benzene molecule differs between aqueous and adsorbed phases, thus making it more accessible for bacteria to use as a carbon source.

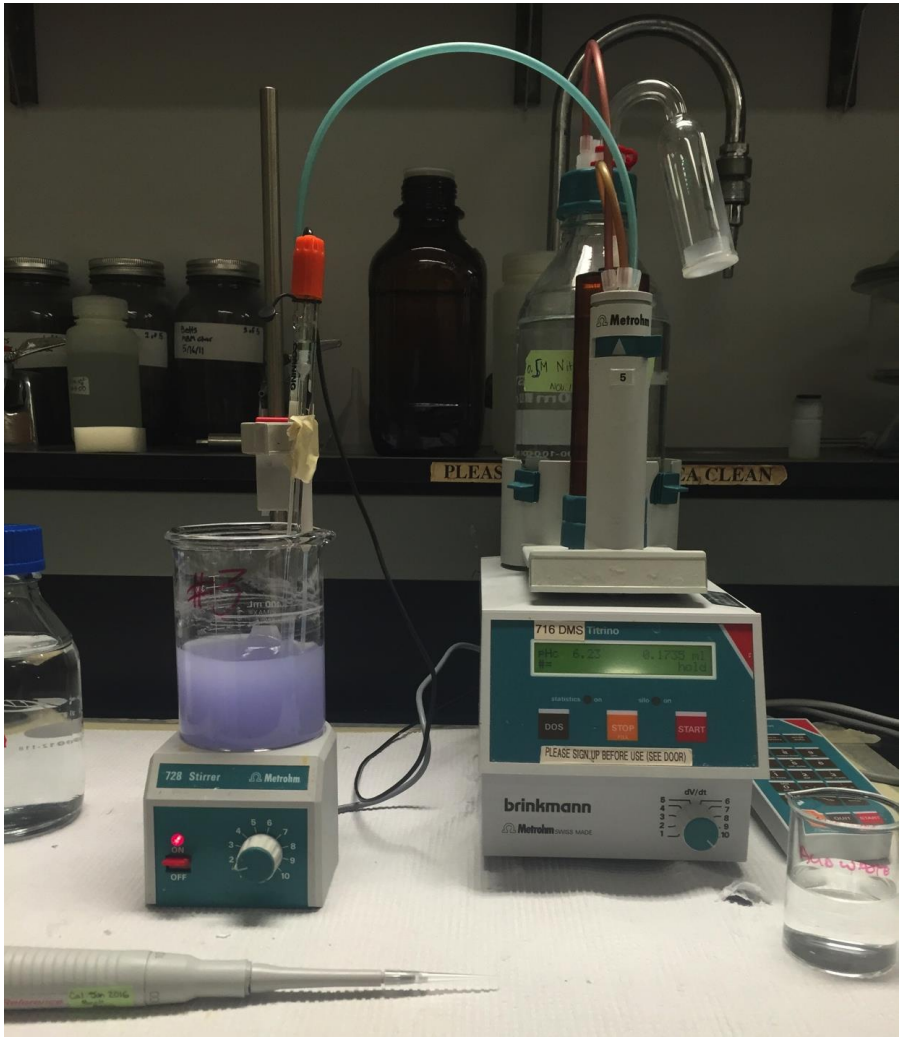
Laboratory Experiments

1) Adsorption isotherms to determine surface coverage of orthophosphate.

2) Incubate benzene degrading cultures under nitrate reducing conditions.

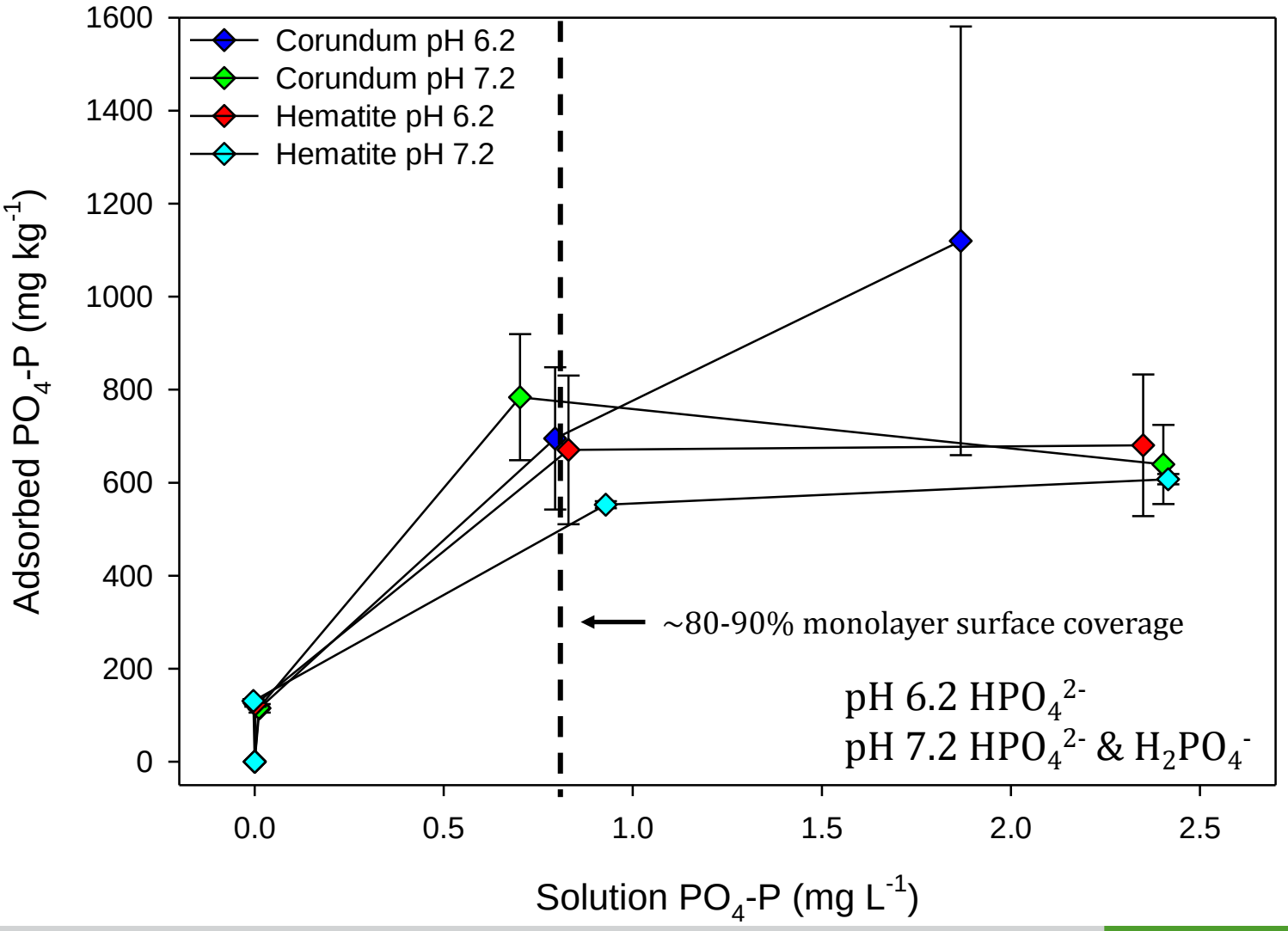
3) ATR-FTIR spectroscopy to investigate benzene adsorption on hematite.

Adsorption isotherms



- 1 g L^{-1} mineral
- Increasing the orthophosphate concentration and subsampling
- Constant pH
- Measure solution P via colorimetric techniques

Adsorption Isotherms (25˚C)



Culture set up

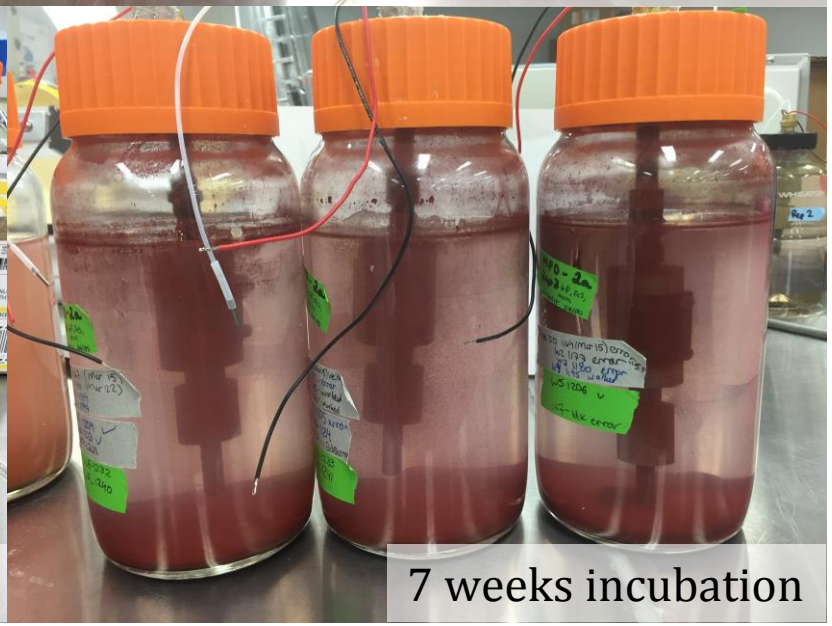
- 3 Controls Media Only
- 4 Inoculated Media Only
- 3 Controls with Hematite
- 4 Inoculated with high P + Hematite
- 4 Inoculated with low P + Hematite

- 3% v/v inoculant

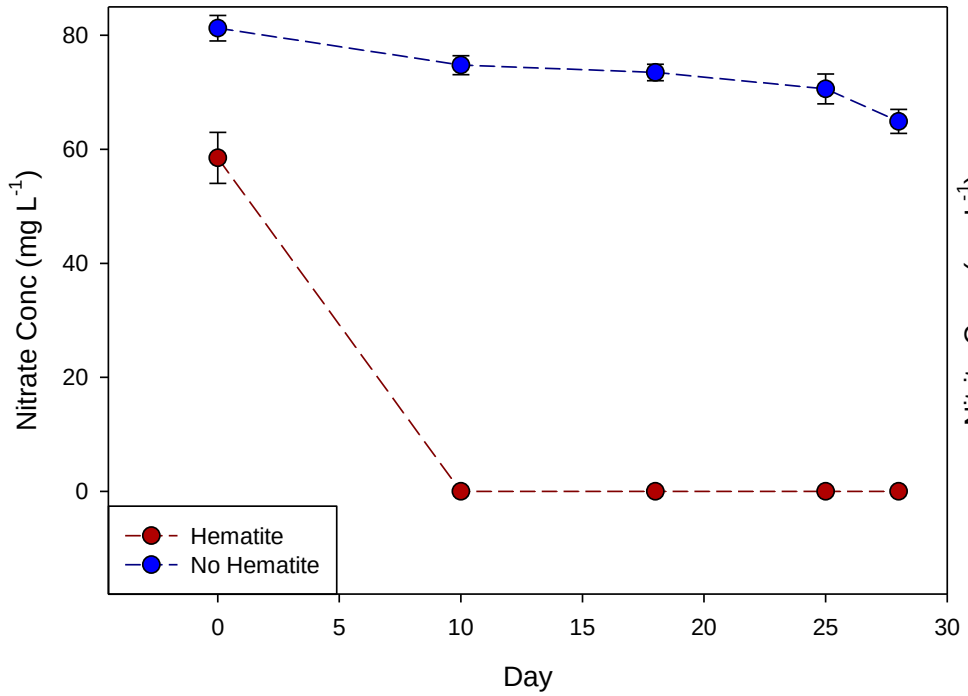
- Original media (high P)
  - 4 mM P
  - FeS
- Low P media
  - 400 µM (~80% monolayer coverage)
- 10 g L^{-1} hematite

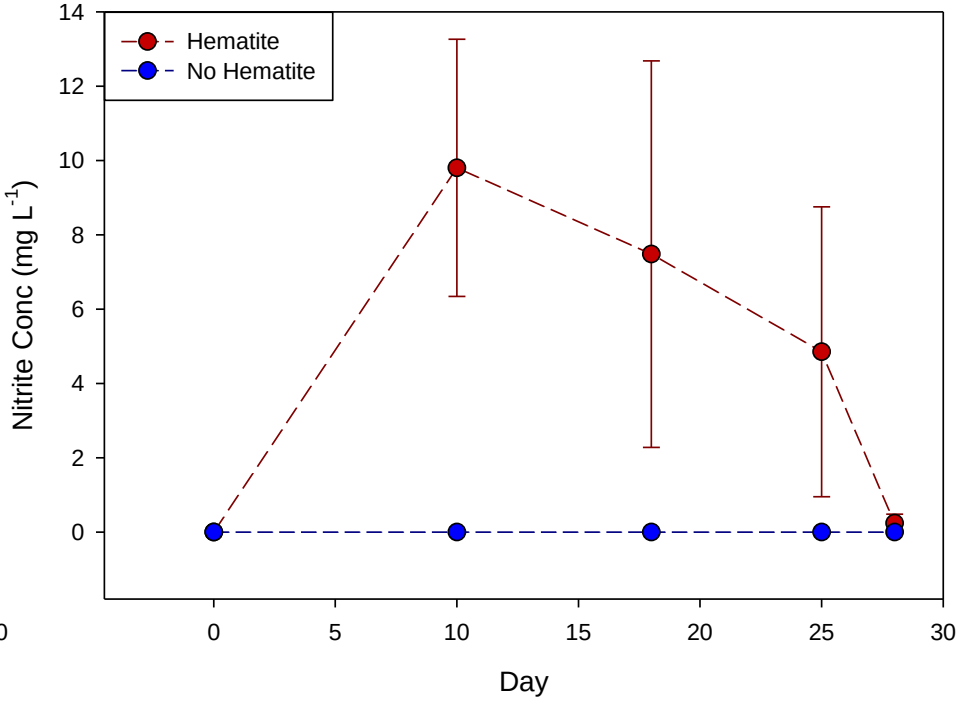

1 week incubation


3 weeks incubation

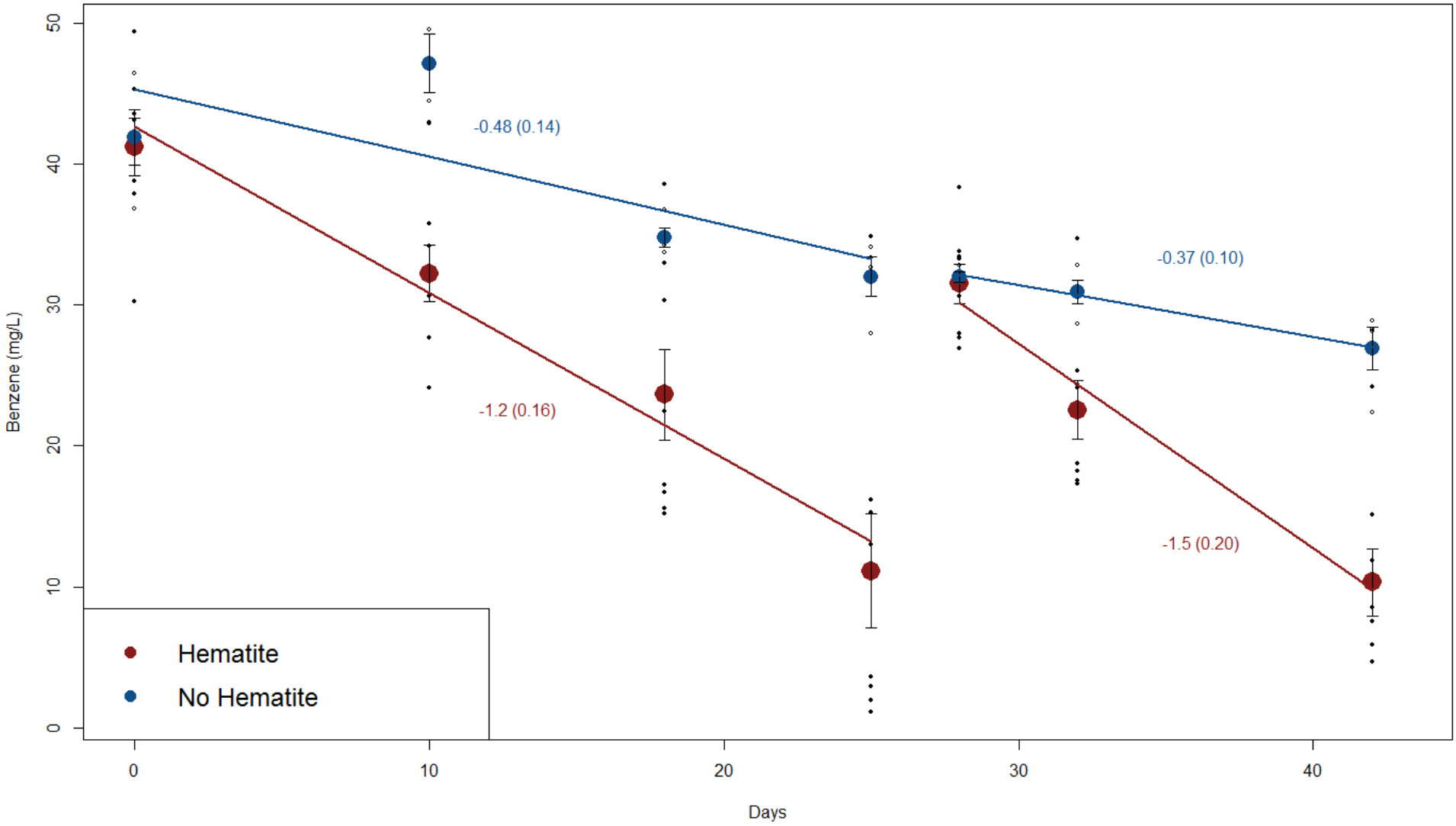

7 weeks incubation

Nitrate and Nitrite Concentrations – Evidence of Active Denitrifiers





Benzene Degradation



ATR-FTIR Theory

- Natural vibrations of molecules
  - Stretching, bending, twisting, etc.

- The vibrations have an electrical field, when the infrared radiation electrical field matches that of the molecule, it increases the amplitude of the vibration.

- IR active vibrations (peaks in a spectrum) indicates there is a change in the dipole moment (unequally shared electrons)

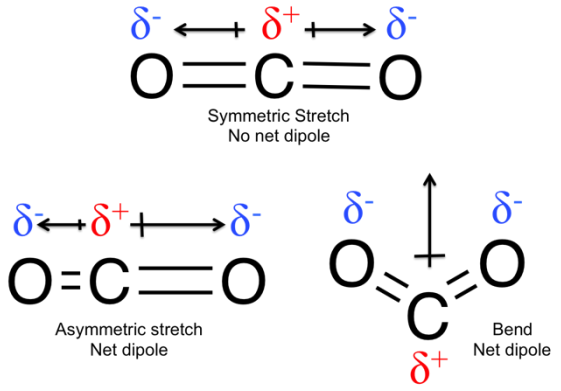
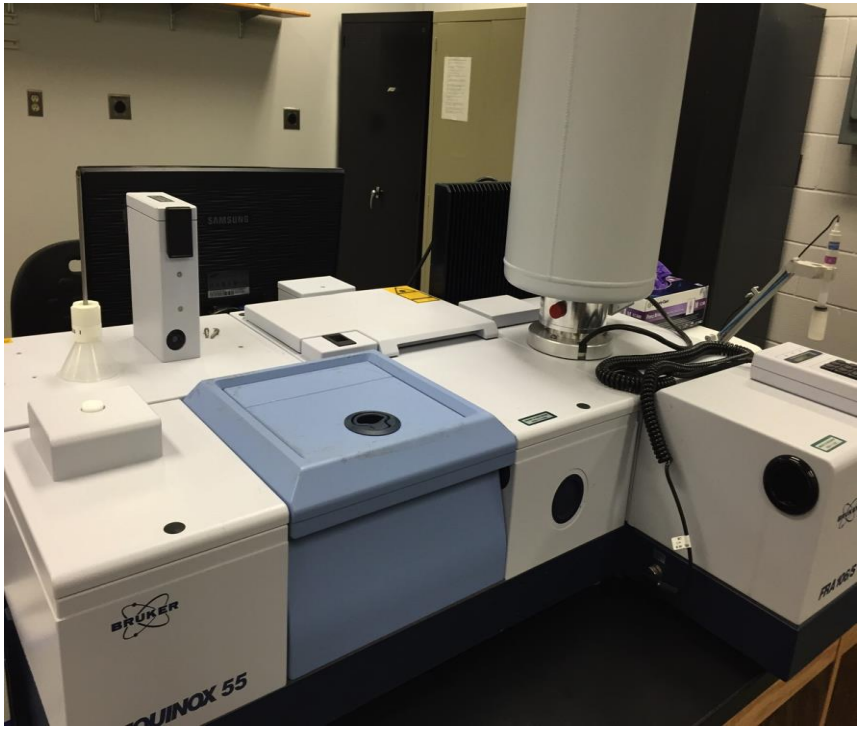
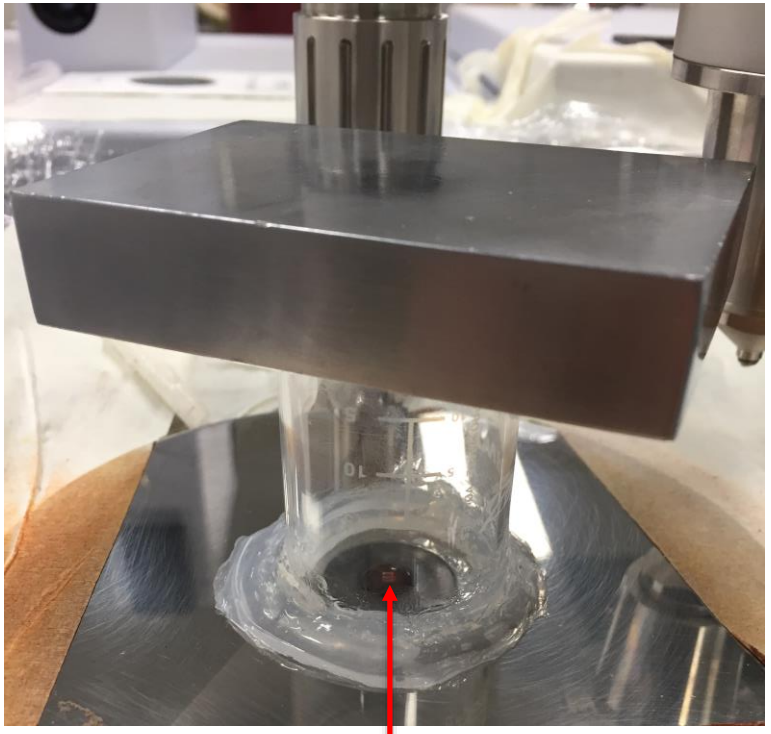


Figure 1-2. Molecular vibrations of carbon dioxide.

(Phillips, 2015)

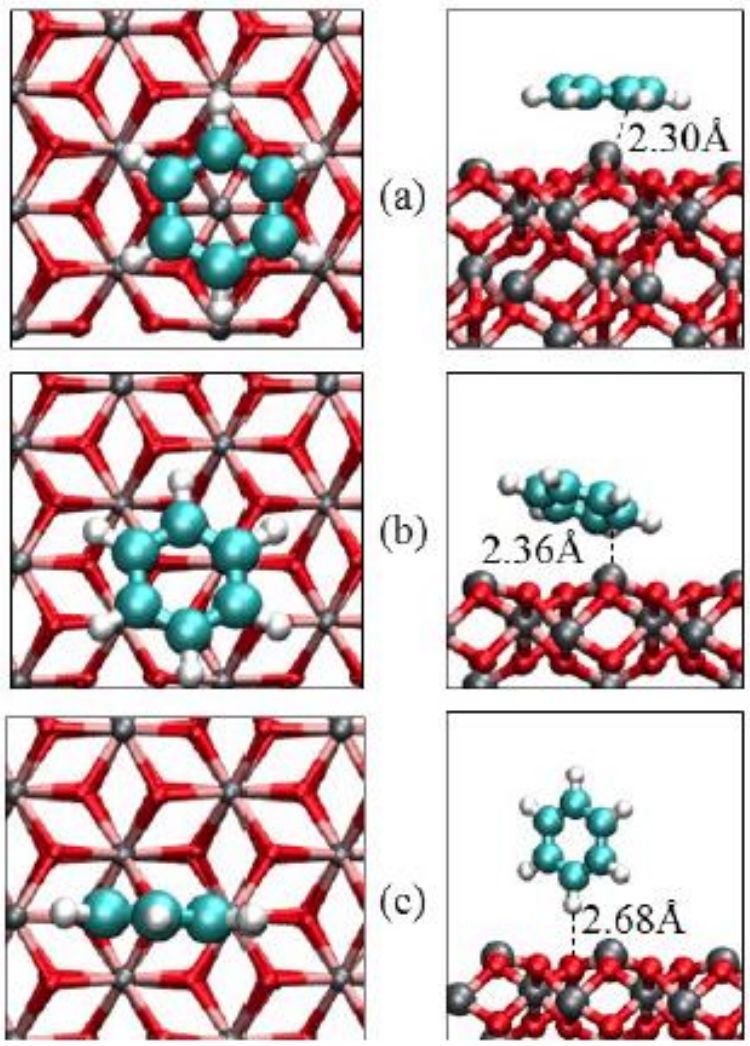
ATR-FTIR set up





Hematite deposit on diamond crystal with aqueous benzene solution.

Predicting relaxed orientations of benzene on hematite (Dzade, Roldan, and Leeuw, 2014)



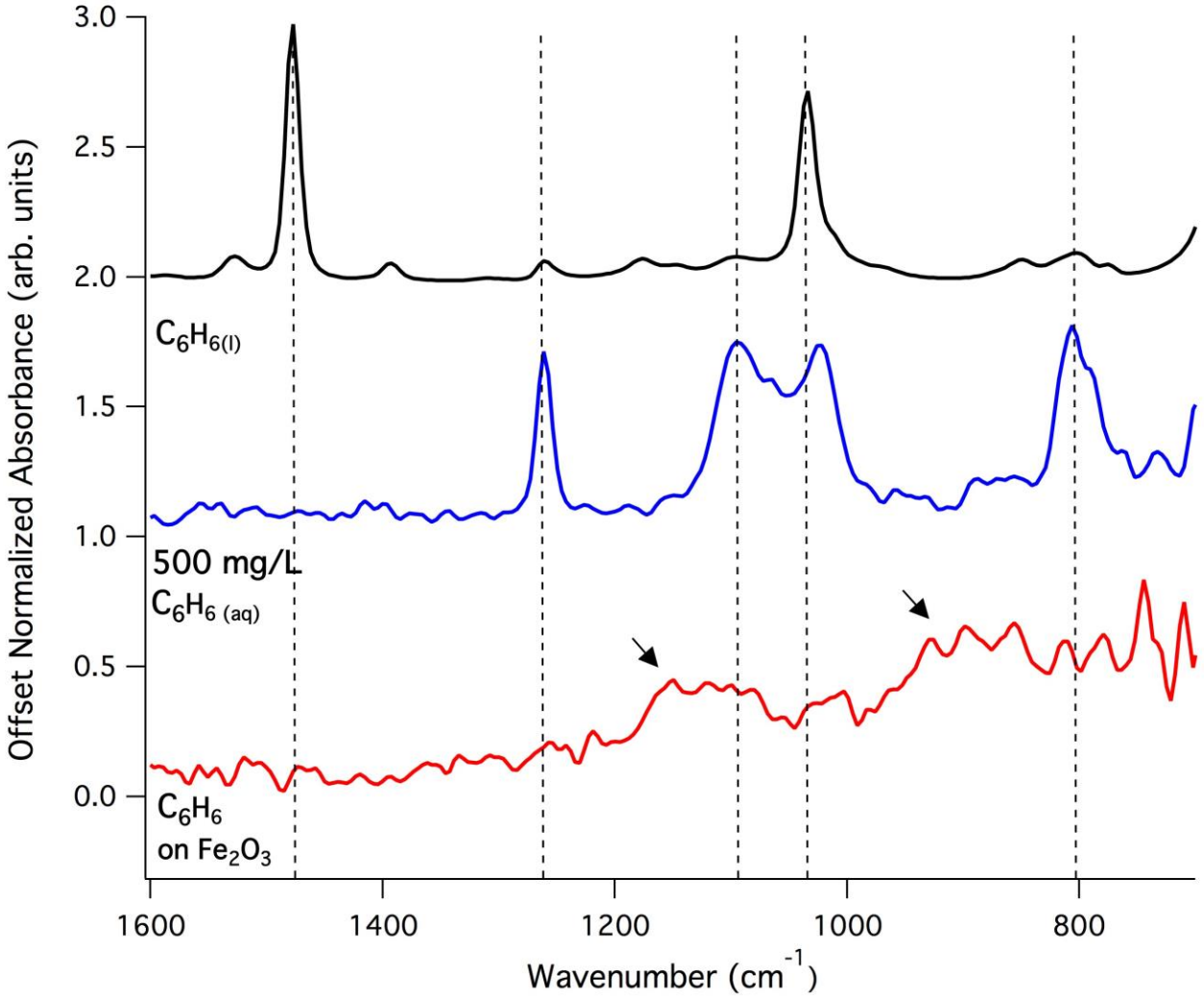
Parallel

Slant

Vertical

*Hydrogen bonding to oxygen's in the hematite structure

ATR-FTIR Spectra: Benzene



Conclusions

- The orthophosphate adsorption capacities of hematite (inner-sphere complex) and corundum (outer-sphere complex) are not significantly different.

- The presence of hematite enhanced microbial benzene degradation (likely by denitrifying bacteria) when compared to media controls with no hematite.

- Degradation still continuing in Low P cultures after 63 days – it is likely that the microbes can access orthophosphate bound to the surface via inner-sphere adsorption.

- Benzene's dipole moments differ between pure state (l), in water (aq), and when adsorbed to hematite. It is possible that degradation was increased by an increase in bioavailability of benzene adsorbed to the hematite surface.

Acknowledgments

SIRCA

Sustainable In-Situ Remediation Co-operative Alliance

<u>Supervisors</u>
Dr. Steven Siciliano
Dr. Derek Peak

<u>Lab Technician</u>
Alix Schebel

<u>Undergraduate Summer Student</u>
Samantha Chomyshen

Environmental Toxicology Lab Group
Environmental Chemistry Lab Group

NSERC CRSNG

CO-OP Federated Co-operatives Limited

University of Saskatchewan