
## Welcome to the CLU-IN Internet Seminar

# Contaminated Sediments: New Tools and Approaches for in-situ Remediation - Session IV

Sponsored by: National Institute of Environmental Health Sciences, Superfund Research Program

February 14, 2011, 2:00 PM - 4:00 PM, EST (19:00-21:00 GMT)

*Instructors:*

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*Dr. Harold D. May, Professor, Microbiology and Immunology, Medical University of South Carolina (mayh@musc.edu)*

*Moderator:*

*Steve Mangion, U.S. EPA Region 1 (mangion.steve@epa.gov)*

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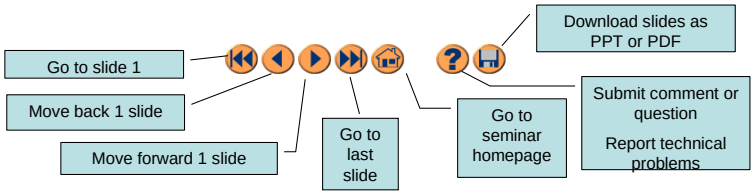
- Please mute your phone lines, Do NOT put this call on hold
- Q&A
- Turn off any pop-up blockers
- Move through slides using # links on left or buttons

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# *Integrating Microbial Biostimulation and Electrolytic Aeration to Degrade POPs*

**Harold D. May, Kevin R. Sowers**
**Chanlan Chun and Ray Payne**

**Medical University of South Carolina**
**And**
**University of Maryland**

**February 14, 2011**

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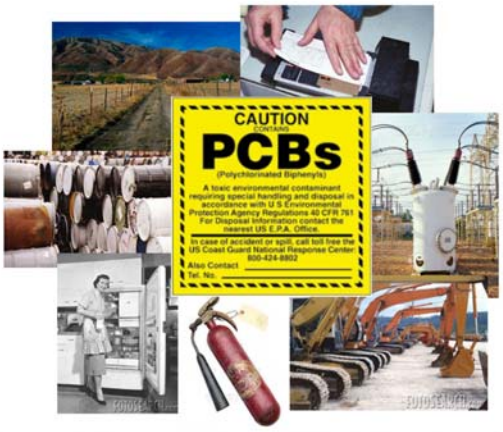
## *Persistent Organic Pollutant: Polychlorinated Biphenyls*




Environmental Legacy: Estimated 0.6-1.2 billion kg worldwide

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***Goal:***

***Microbial Degradation of PCBs in Aquatic Sediment***

***Approach:***

***1.Identify PCB Dechlorinating Bacteria***

***2.Develop in situ Monitoring Tools***

***3.Develop Bioaugmentation***

***4.Test Bioelectrochemical Stimulation***

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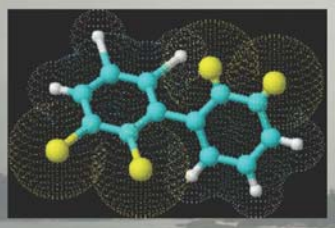
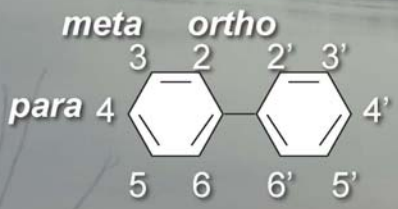
## Polychlorinated Biphenyls (PCBs)






- 209 congeners
- Very stable
- Bioaccumulate
- Toxicity concern
- Anoxic sediments are sinks

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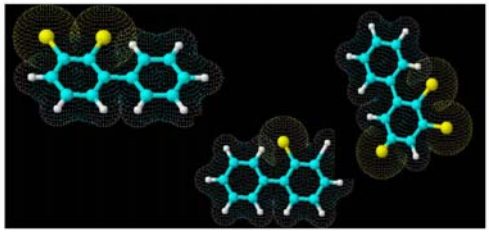
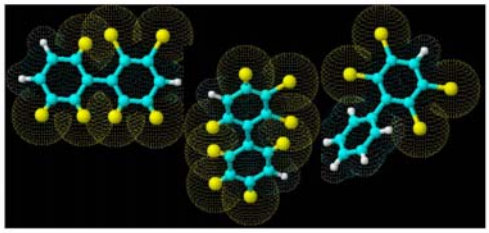
# *Microbial PCB Degradation*





Complementary processes

✓ Aerobic biodegradation
Generally with < 4-6 Cl

✓ Anaerobic dechlorination
generally with ≥ 3 Cl

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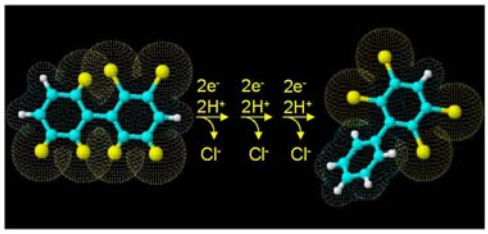
# *Reductive Dechlorination of PCBs*




✓ Performed by anaerobes, e.g. members of the Chlorofexi such as strains DF1, SF1 and *Dehalococcoides* strain CBDB1

✓ Theoretically can produce biphenyl but rarely do. Ordinarily leave mono, di and trichlorobiphenyls behind

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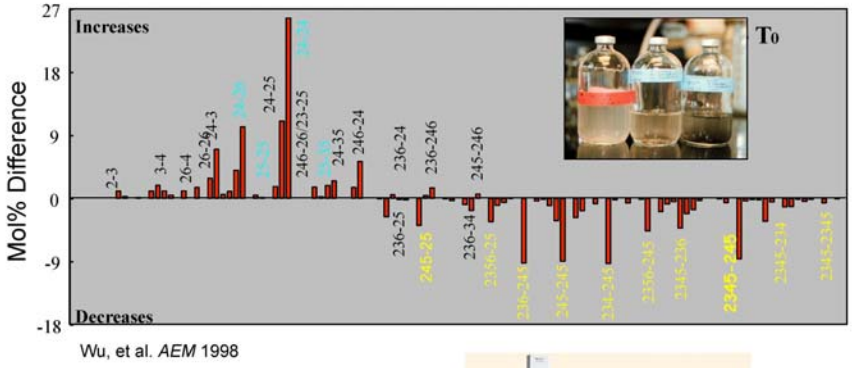
# Dechlorination of Aroclor 1260




Wu, et al. *AEM* 1998

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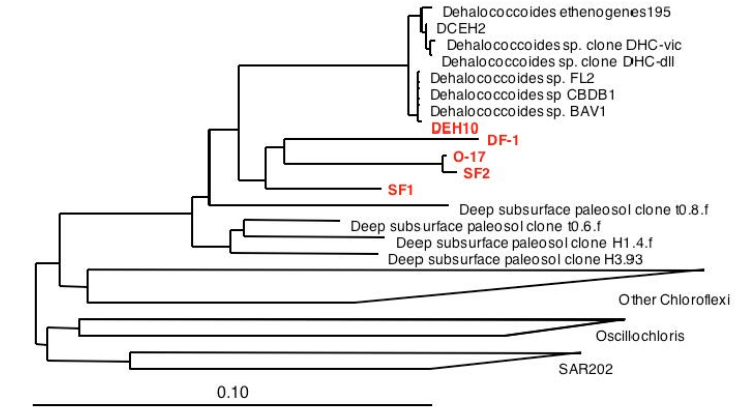
# PCB Dechlorinating Bacteria




Based on 16S rRNA gene sequences (1500+ bp)

Cutter et al. *Environ. Microbiol.* 2001, Wu et al., *AEM* 2002, Fagervold et al. *AEM* 2006 &2007, Kjellerup et al. *AEM* 2008

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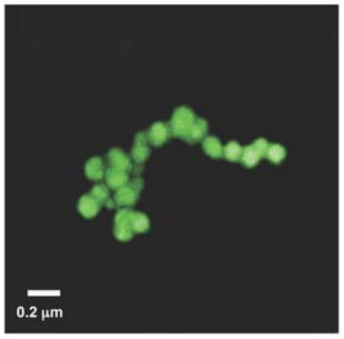
## *Detecting PCB Dechlorinating Bacteria*




- ~~Optical density~~
- ~~Plating~~
- ~~Direct cell counts~~
- ~~FISH~~

May et al, 2008

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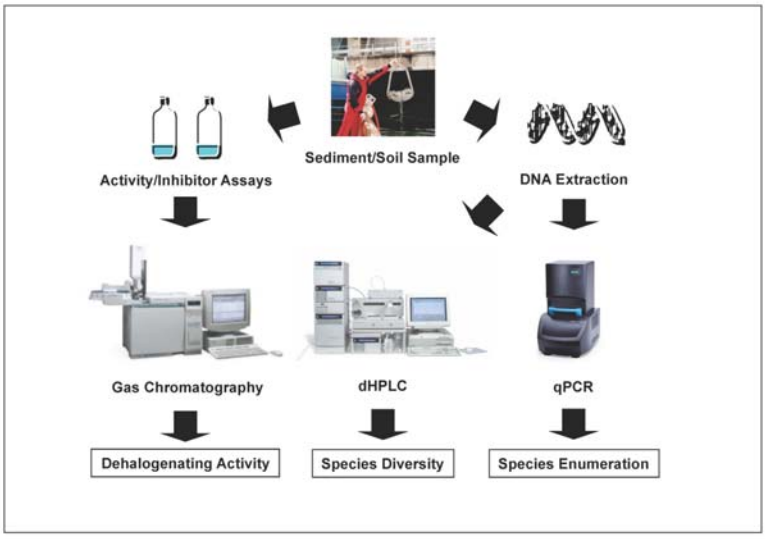
## *High Throughput Microbial Analysis/Monitoring*

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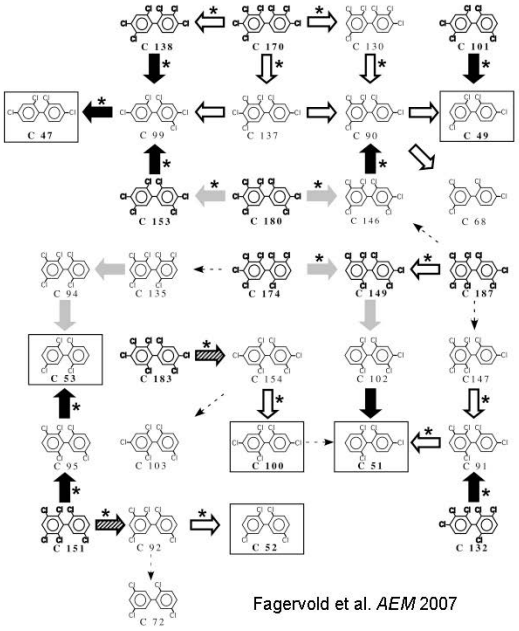
## Dechlorination of Congeners Predominant in Aroclor 1260

***Only 3 Phylotypes Required***

- DEH10
- SF1
- DEH10 & SF1
- O-17




Fagervold et al. *AEM* 2007

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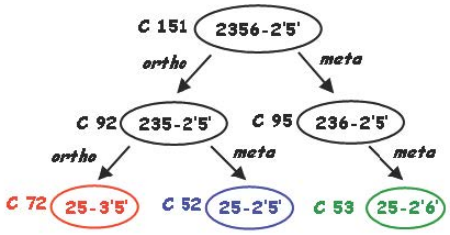
## *Bioaugmentation with PCB Dechlorinators*




| | | | |
|---|---|---|---|
| o-17,DF1,SF1,DH10 | 14 | 46 | 40 |
| o-17,DF1 | 54 | 21 | 24 |
| SF1,DH10 | | | 100 |
| No augmentation | 50 | | 50 |

Fagervold, submitted

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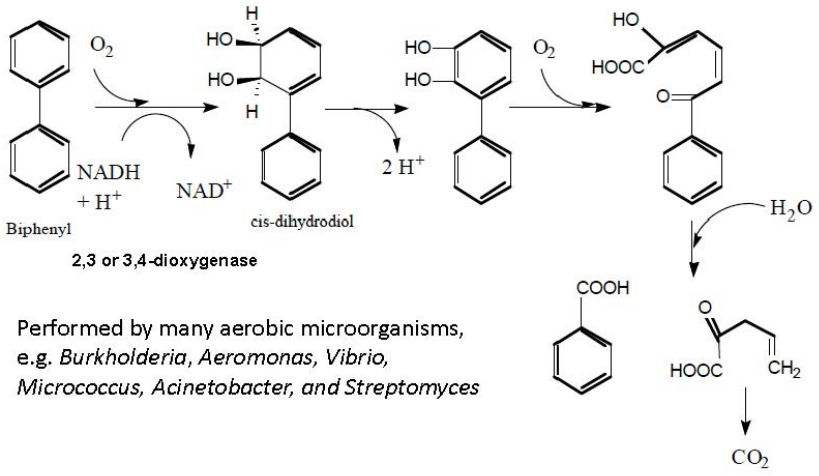
# Aerobic Biodegradation of Biphenyl




2,3 or 3,4-dioxygenase

Performed by many aerobic microorganisms, e.g. *Burkholderia*, *Aeromonas*, *Vibrio*, *Micrococcus*, *Acinetobacter*, and *Streptomyces*

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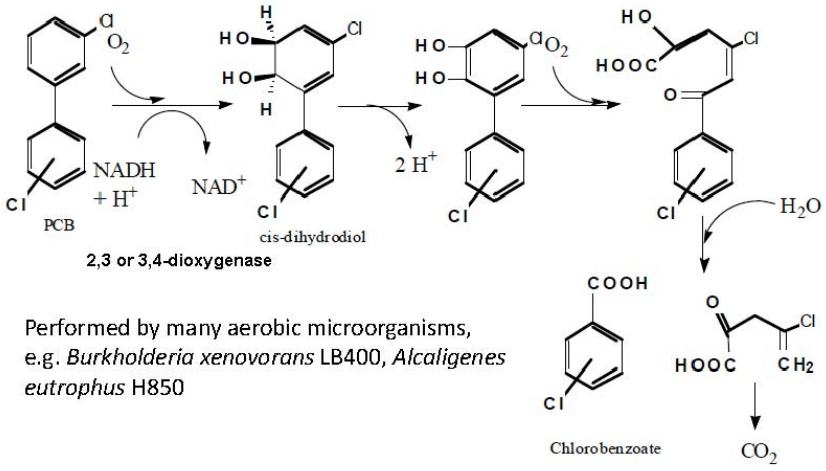
# Aerobic Biodegradation of PCBs




Performed by many aerobic microorganisms, e.g. *Burkholderia xenovorans* LB400, *Alcaligenes eutrophus* H850

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## *Bioaugmentation of weathered Aroclors*

Baltimore Harbor Sediment: 5-10 ppm weathered PCBs

- 1 liter sediment incubated at 20°C in dark
- indigenous water with no additional nutrients
- PCB dechlorinating anaerobe DF1
  and/or PCB degrading aerobe LB400

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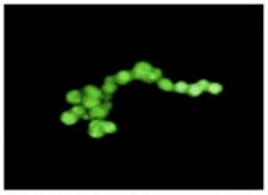
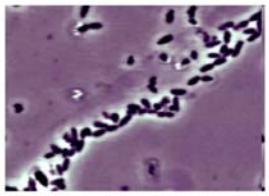
## *Bioaugmentation of weathered Aroclors*



***Dehalohalobium chlorocoercia* DF-1**



***Burkholderia xenovorans* LB400**

- each mesocosm was inoculated with 5 x $10^5$ cells
- inoculation by direct injection or on solid substrate
- lactate added as carbon source when indicated

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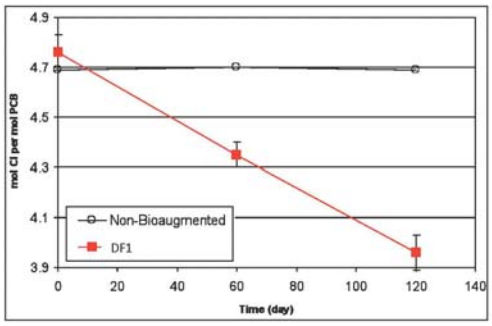
## *Bioaugmentation of Baltimore Harbor Sediment with DF1*




DF1 removed about 0.9 chlorines per mol PCB in 120 days.

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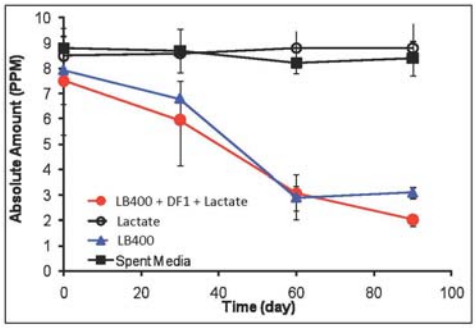
## *Bioaugmentation with DF-1 + LB400 Results in Degradation of Weathered PCBs*




About 75% of total PCBs by mass degraded through oxidative processes and reductive dechlorination in 90 days. Experiment ongoing.

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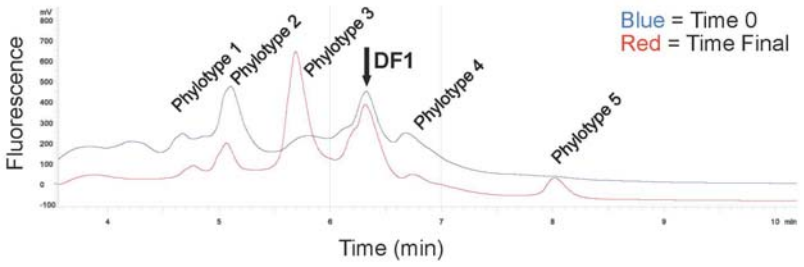
## Dechlorinator phylotypes in mesocosm amended with DF1

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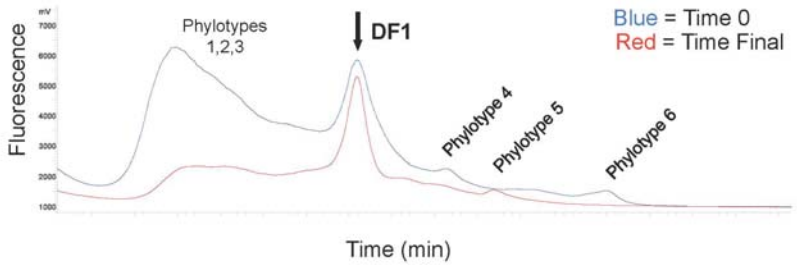
## Dechlorinator phylotypes in mesocosm amended with DF1 & LB400




- DF-1 detected by dHPLC at similar levels on days 0 and 90
- Fate of LB400 currently being determined

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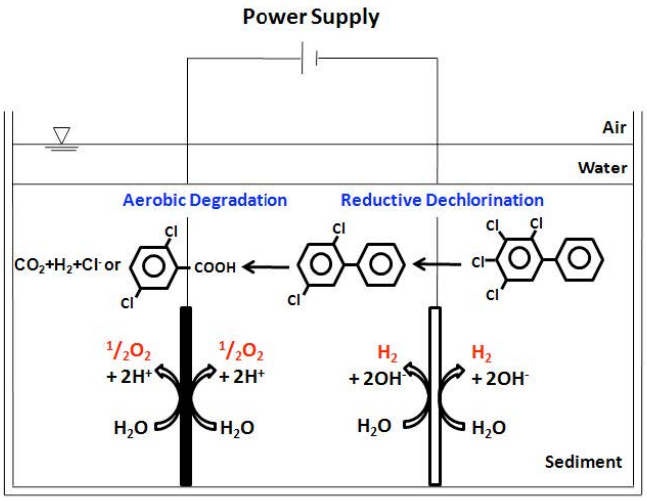
## *Bioelectrochemical Approach: Hypothesis*

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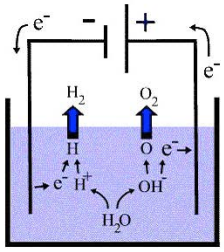
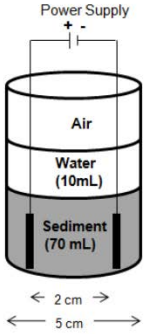
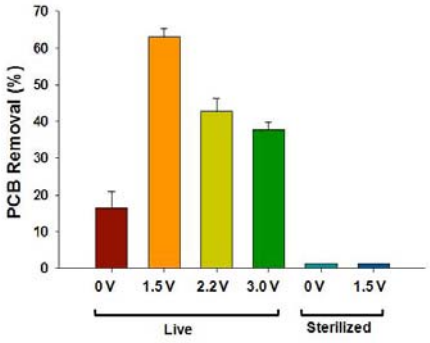
## Sediment Bioelectrochemical Reactors (SBRs)

- Microcosm-SBRs
  - Aroclor 1242 impacted sediment (~20 ppm) from Fox River, WI
  - Electrode: Ti sheet (surface area: 12.5 $cm^2$)
  - Applied voltage: 0, 1.5, 2.2, and 3 V



## Sediment Bioelectrochemical Reactors (SBRs)




Up to 65% of total weathered PCBs by mass degraded through oxidative processes in 88 days without bioaugmentation. Increasing chlorobenzoates and benzoate detected with all voltages. Analysis continues.

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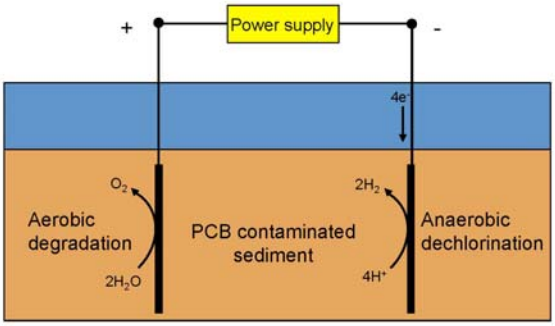
## *Application of Voltage to Aquatic Sediment*




pH problem?

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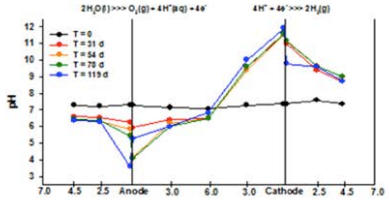
# *Problem with pH*

## *(Redox, $O_2$ and $H_2$ gradients follow suit)*

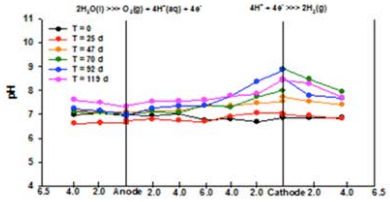
**High current density : 0.067 mA/cm² (3.8-4.4 V, 15.2-17.6 mW)**

**Electrode potential vs. Ag/AgCl**
**Cathode: -1.6 V ($H_2$ generation)**
**Anode : 2.5 V ($O_2$ generation)**




**Low current density : 0.003 mA/cm² (2.0-2.5 V, 0.036-0.045 mW)**

**Electrode potential vs. Ag/AgCl**
**Cathode: -1.1 V ($H_2$ generation)**
**Anode : 1.4 V (no or slow $O_2$ generation)**

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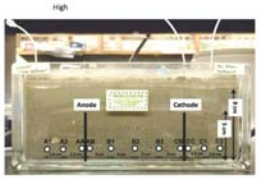
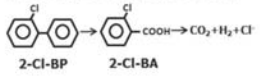
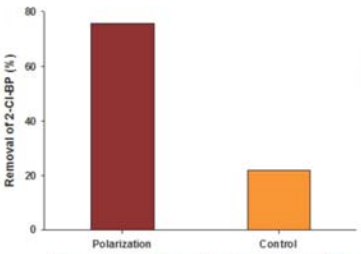
# SBR-Polarization

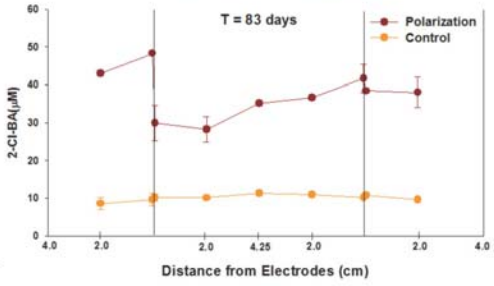
- Mesocosm-SBR
  - PCB-impacted sediment from Fox River, WI
  - Electrodes: Ti sheet (surface area: 55cm$^2$)
  - Constant Current: 0.014 mA/cm$^2$, 2.5-3.8 V
  - Model PCB added: 2-Cl-BP




2-Cl-BP 2-Cl-BA




Repeated polarization (cycling of the potential) avoids pH shifts and supports PCB degradation throughout the mesocosm. Weathered PCBs to be tested.

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## *Conclusions*

- Bioaugmentation results in 75% PCB degradation in 90 days
- Application of 1.5V results in 65% PCB degradation
- Anaerobic oxidation of PCBs is hypothesized
- Repeated polarization is successful and avoids pH problems

## *Future Goals*

- Determine full extent of effect of bioaugmentation and electrochemical stimulation
- Determine extent of repeated polarization
- Combine bioaugmentation and electrochemical stimulation
- Test *in situ*


THE UNIVERSITY OF
TEXAS
AT AUSTIN
Georgia Tech
Carnegie Mellon University
Danny Reible
Environmental and Water Resources
University of Texas
FUNNEL AND GATE APPROACH FOR ACTIVE SEDIMENT CAPS
CO-INVESTIGATORS:
G. LOWRY, K. GREGORY, CARNEGIE-MELLON
J. HUGHES, GA TECH
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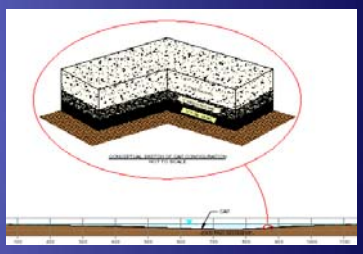
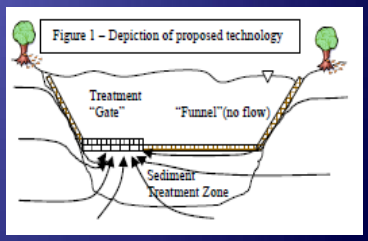
# Sediment In-situ Capping/Treatment

- Reduce risk by:
  - Stabilizing sediments
  - Physically isolating sediment contaminants
  - Reducing contaminant flux to benthos and water column
- Sand effective for strongly solid associated contaminants
- "Active caps" for other situations (w/amendments)
- Funnel and Gate approach to maximize ability to place/replace amendments

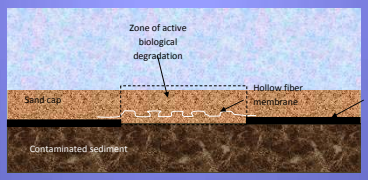


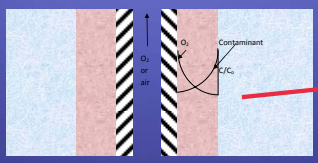


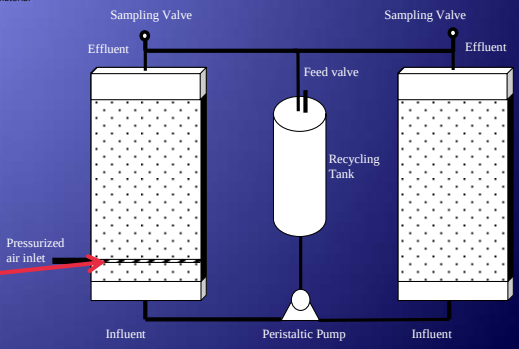
# Project Objectives and Scope

- Can a funneling cap effectively contain contaminants and effectively channel interstitial fluids (water or NAPL) to a collection or treatment gate
- Can treatment gates composed of reactive materials effectively manage residual contamination from the in-situ treatment zone
- Scope
  - Preliminary studies with sorbents (e.g. organoclays and activated carbon)
  - Hollow fiber membrane to introduce oxygen in reduced environment
  - Low voltage electrodes to encourage development of appropriate redox conditions

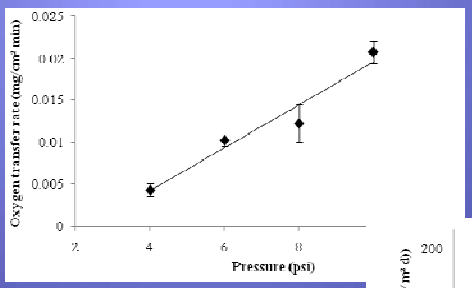
# Hollow fiber membrane to introduce oxygen & encourage aerobic degradation



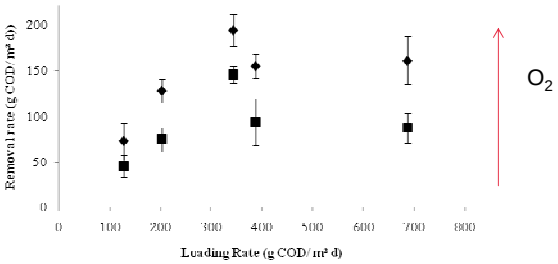



# Hollow Fiber Membrane Induced Oxidation




Initially reduced conditions without oxygen introduction via pressurized air

Minimal membrane fouling




$O_2$

Acetate oxidation

# The idea of electro-reactive capping

- Functions:
  - Provide redox control
  - Direct reduction and oxidation on electrode surface
  - Deliver electron donor and acceptor for further degradation
- Advantages:
  - Real time and site-specific control
  - complete mineralization of contaminants through sequential reduction and oxidation

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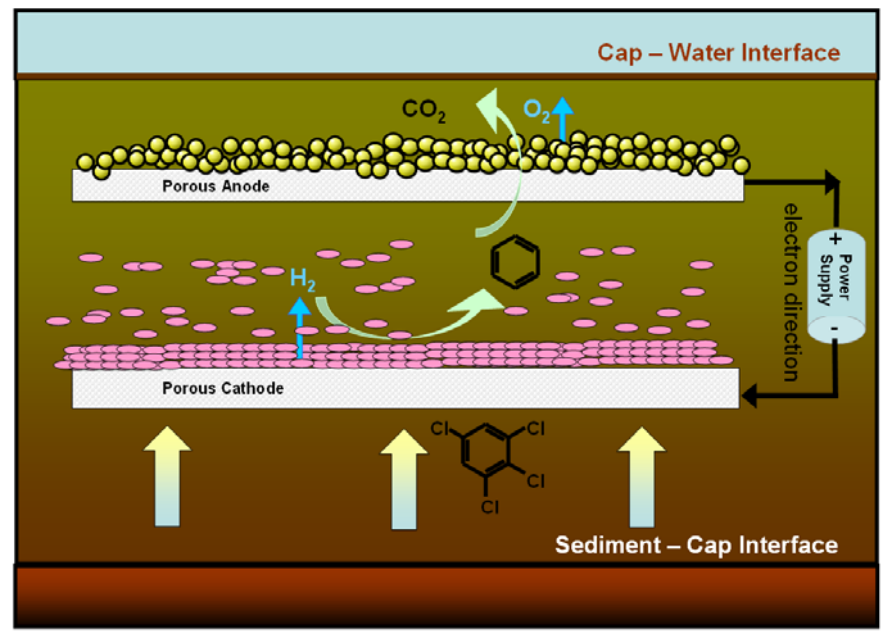
- Functions:
  - Provide redox control
  - Direct reduction and oxidation on electrode surface
  - Deliver electron donor and acceptor for further degradation
- Advantages:
  - Real time and site-specific control
  - complete mineralization of contaminants through sequential reduction and oxidation

# Conceptual model- Electrode cap

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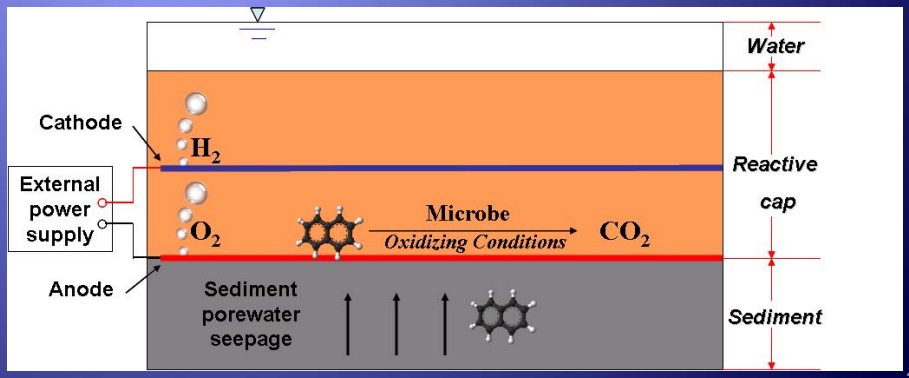
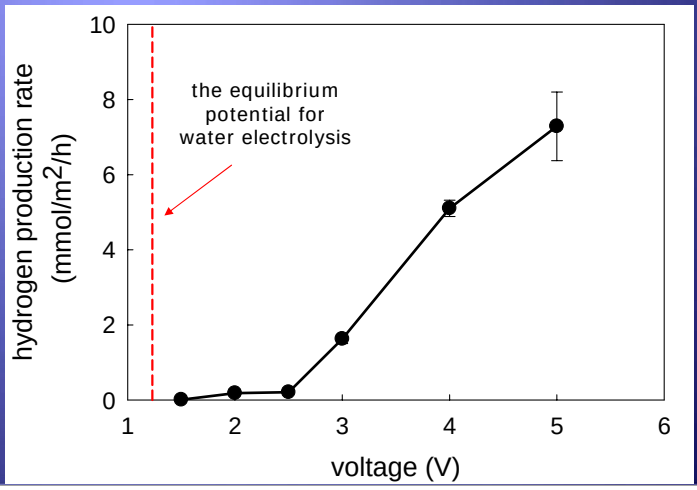
# Configuration for PAH oxidation

- Anode at depth to encourage oxidizing conditions where normally strongly reducing conditions exist



# Influence of voltage

- Real-time control of electron donor production in the cap by adjusting voltage

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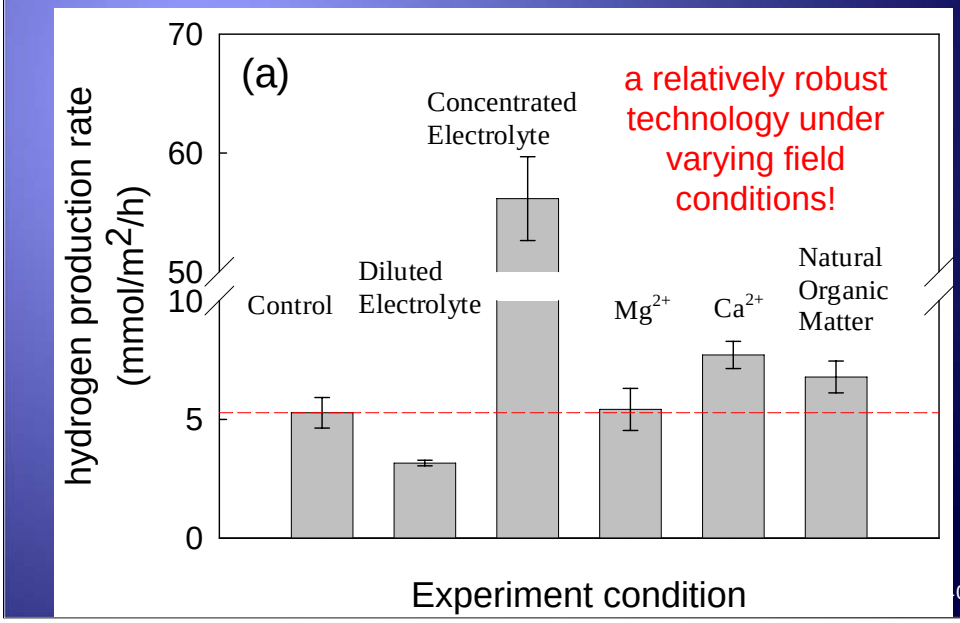
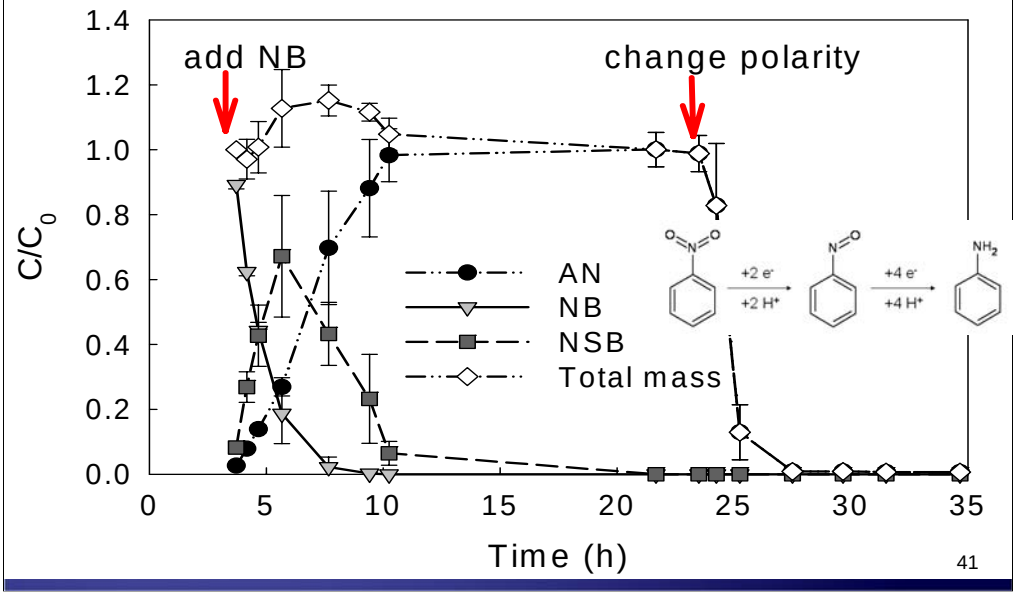
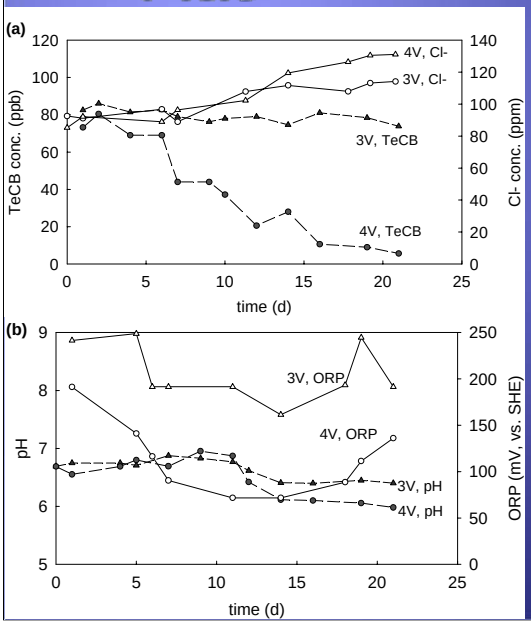
# Influence of aqueous chemical species



# NB sequential reduction-oxidation



# Electrode stimulated degradation of 1,2,3,5- Tetrachlorobenzene



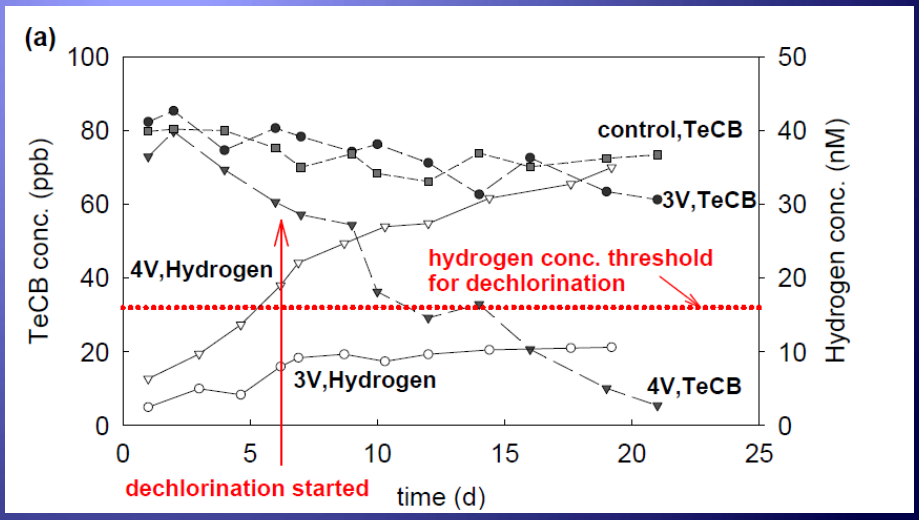

3v insufficient

4v removed ~90% TeCB

ORP: from 200mv to 70mv

pH changes: less 1 unit

# Electrode induced dechlorination

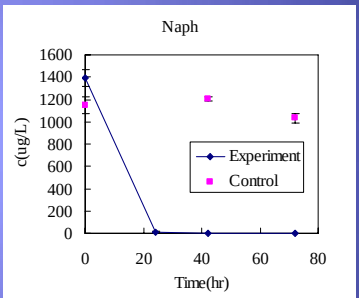


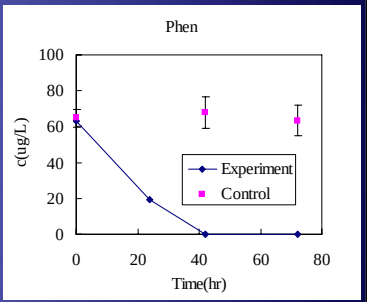
# PAH biodegradation in slurry

- PAH degradation under aerobic and nitrate reducing condition
  - To verify PAH biodegradation potential by indigenous microbe in sediment
  - To prove that biodegradation rate is faster in more oxidizing condition (aerobic condition)
- Electrode enhanced degradation of PAH in slurry
  - To examine the feasibility of electrode enhanced biodegradation of PAH in slurry phase

# PAH biodegradation under aerobic conditions

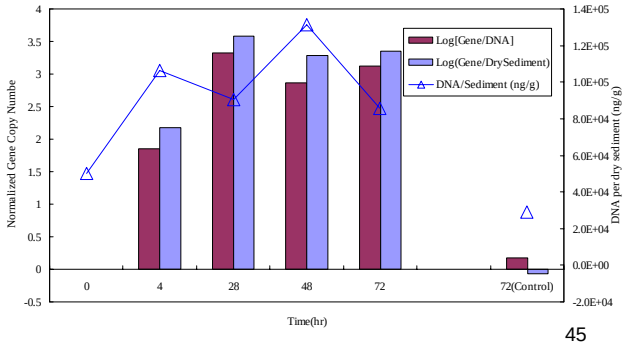
**Naphthalene/ Phenanthrene Concentration**



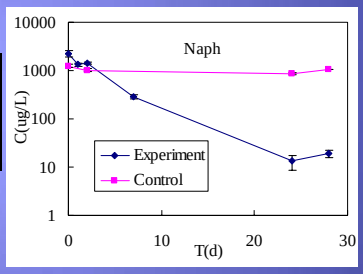

**PAH degrading genes**

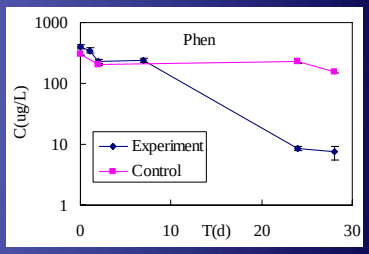
Quantitative polymerase chain reaction (qPCR) using primers that target PAH ring-hydroxylating dioxygenase (PAH-RHD) genes



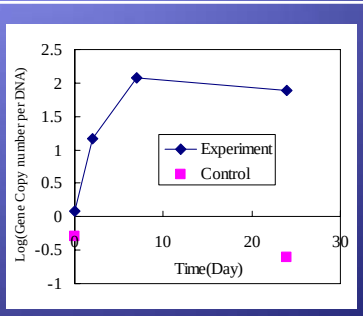
# PAH biodegradation under nitrate reducing condition

**Naphthalene/ Phenanthrene Concentration**



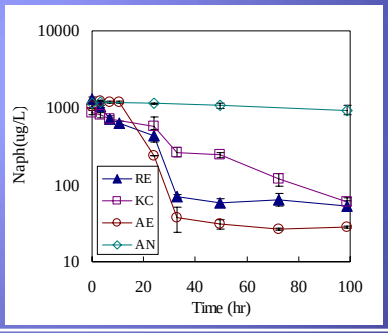

**PAH degrading genes**

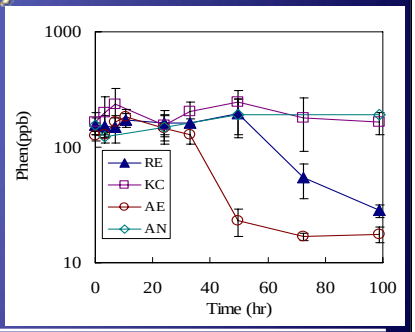


# Electrode enhanced biodegradation of PAH in sediment slurry

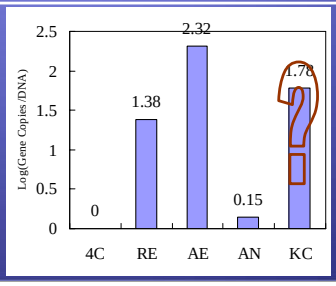
Initially anaerobic slurries

Except fully aerobic slurry




**PAH degrading genes**




Constant condition slurries

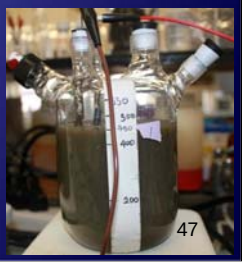
AE: Aerobic condition

AN: Anaerobic condition

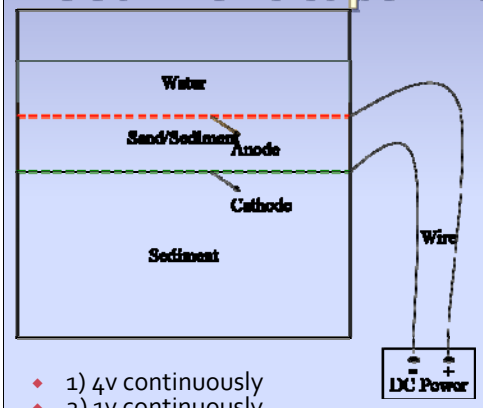
Electrode induced aerobic conditions

RE: Electrode biodegradation

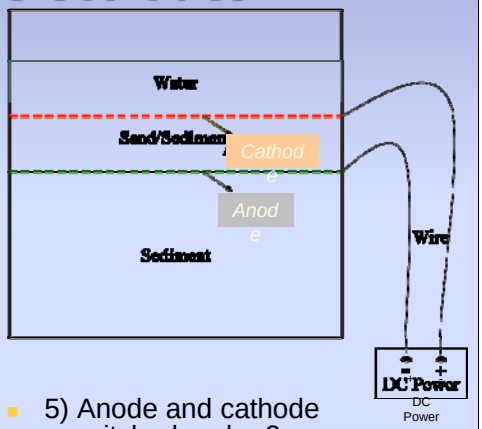
KC: Killed control



# Redox control and pH changes in sediment caps with electrodes

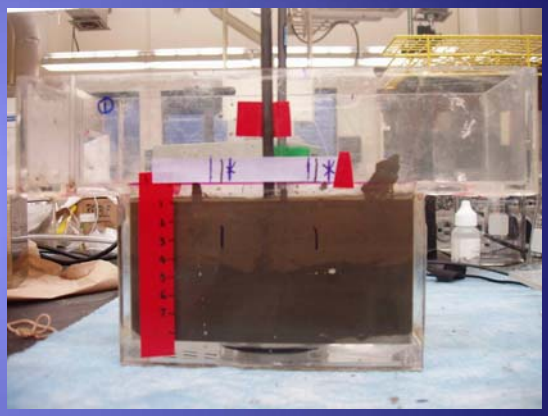



- 1) 4v continuously
- 2) 1v continuously
- 3) 2v continuously
- 4) 4v intermittently (1-3 hours per day)



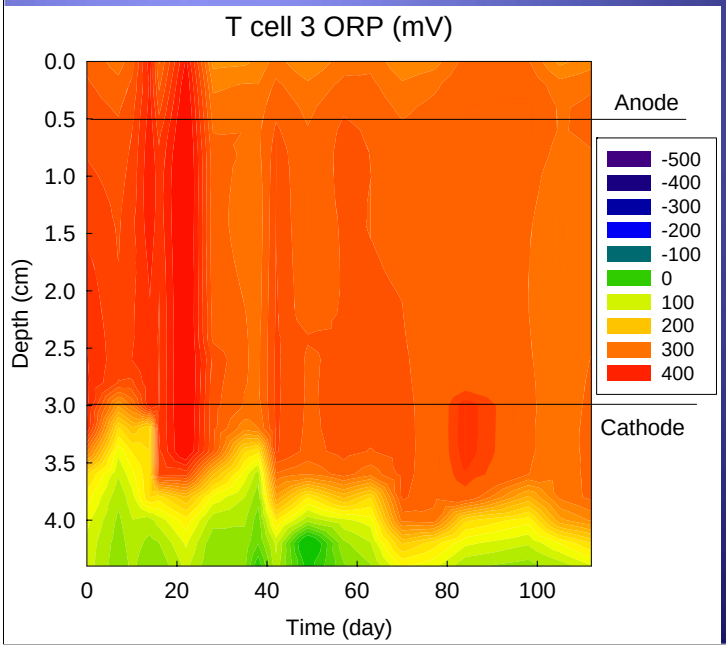

- 5) Anode and cathode switched under 2v

- Side View



- Top View

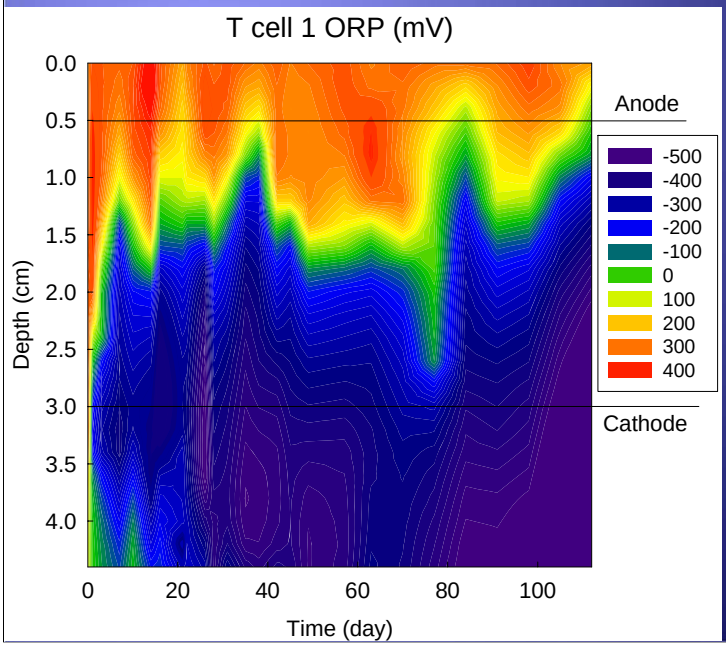
# Redox control




Unpowered control

50

# Redox control




Continuously connected

to 4V DC power supply for~100 days

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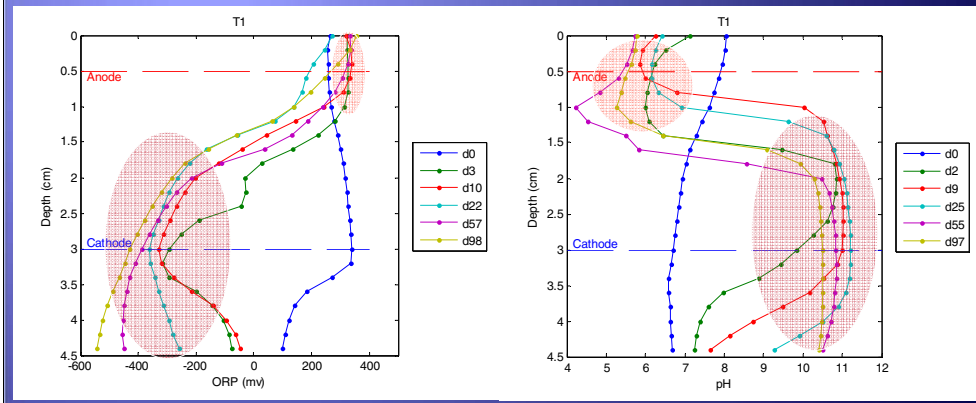
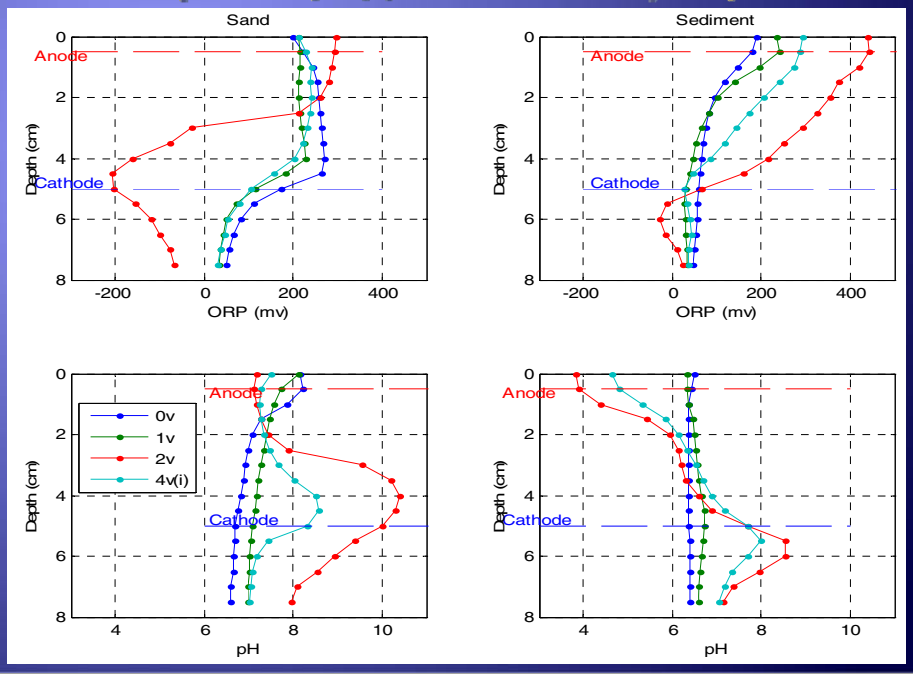
# Redox and pH in 4v experiment




Oxidized zone <-> acidic zone

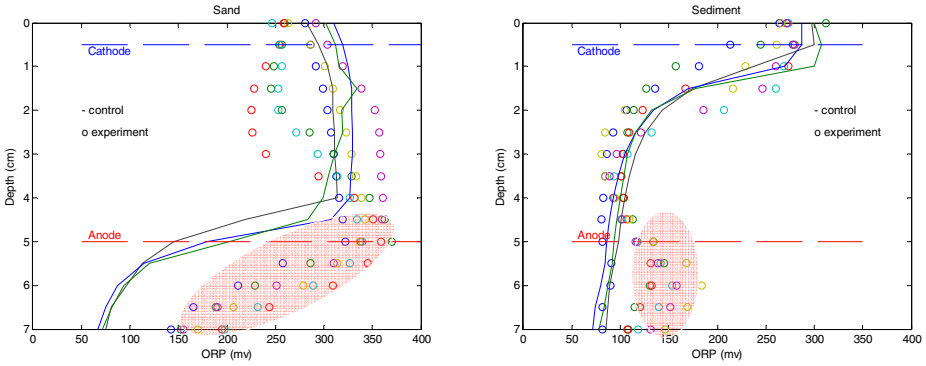
Reduced zone <-> basic zone

# Redox and pH in 1v,2v,4v(intermittently) experiment



# Redox in *deep oxidation mode*

- Anode at depth (sediment-cap interface)
- PAH degradation mode



# Redox control by electrodes

- Proof of concept was successful
- Electrodes could establish oxidizing conditions at the anode
  - Currently studying degradation and increases in PAH degrading genes as per slurry experiments
- pH changes were always associated with redox changes
  - Currently studying use of siderite (iron carbonate) as buffer material to control pH

# Intermediate Scale Demonsration

- Large flume demonstrations in cooperation with Bayani Cardenas (UT Geosciences)
- 2 dimensional flow (upwelling, river flow, hyporheic exchange)

# Conclusions

- Funnel and gate approach allows significantly more complex and sophisticated capping
  - Regular replacement of finite capacity sorbents
  - Sustainable biodegradation
- Hollow fiber membranes to introduce air/oxygen and encourage aerobic degradation
  - Enhanced degradation shown
- Low power electrodes for encouragement of reduction/oxidation at different layers in sediments
  - Enhanced degradation shown in idealized systems
  - In-sediment studies ongoing
  - Intermediate scale demonstrations planned

# Resources & Feedback

- To view a complete list of resources for this seminar, please visit the **Additional Resources**
- Please complete the **Feedback Form** to help ensure events like this are offered in the future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