BIOGEOCHEMICAL MEASUREMENTS TO CHARACTERIZE DUAL ANAEROBIC AND AEROBIC BIODEGRADATION PROCESSES FOR REMEDIATION OF CHLORINATED BENZENES IN A WETLAND

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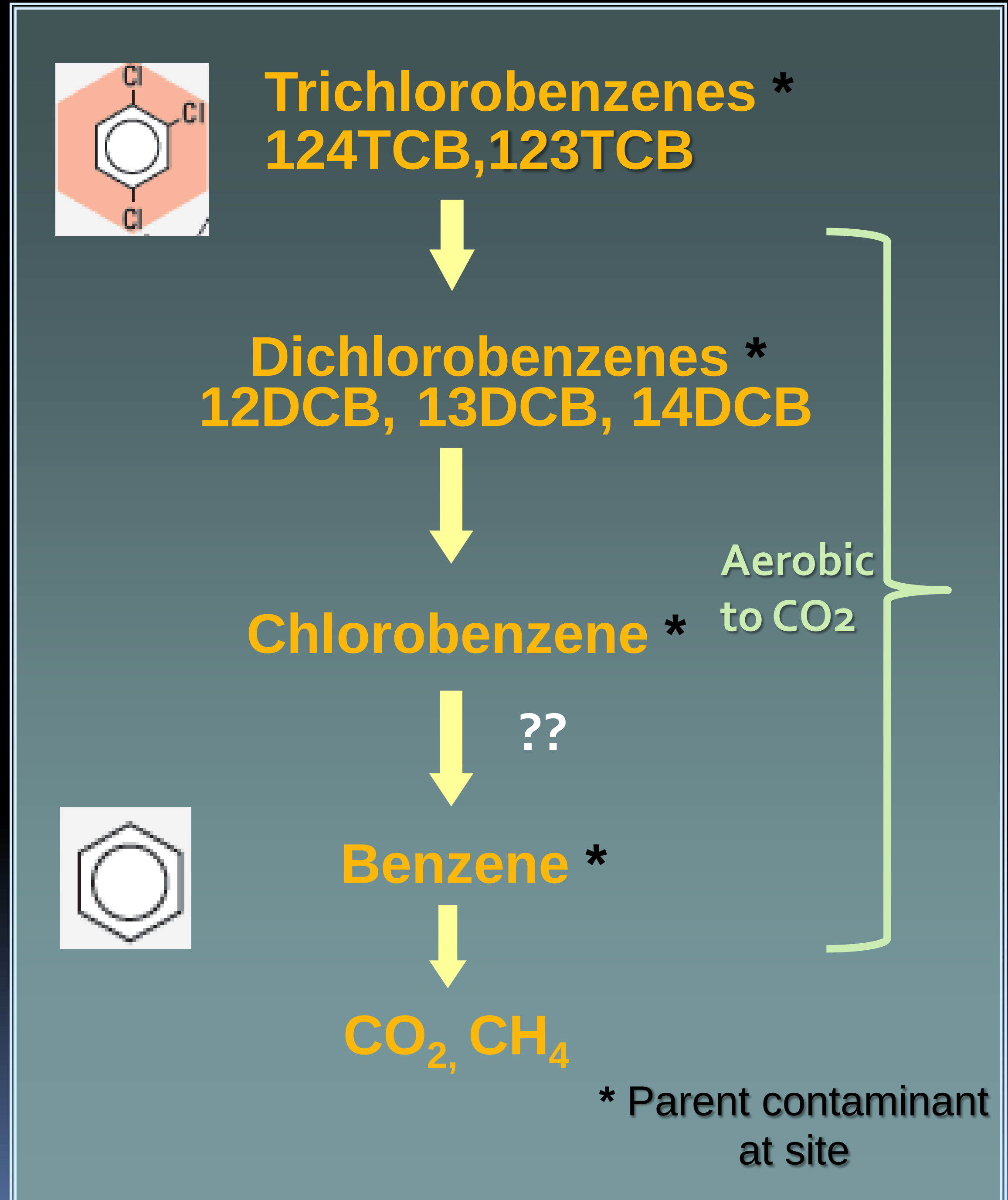
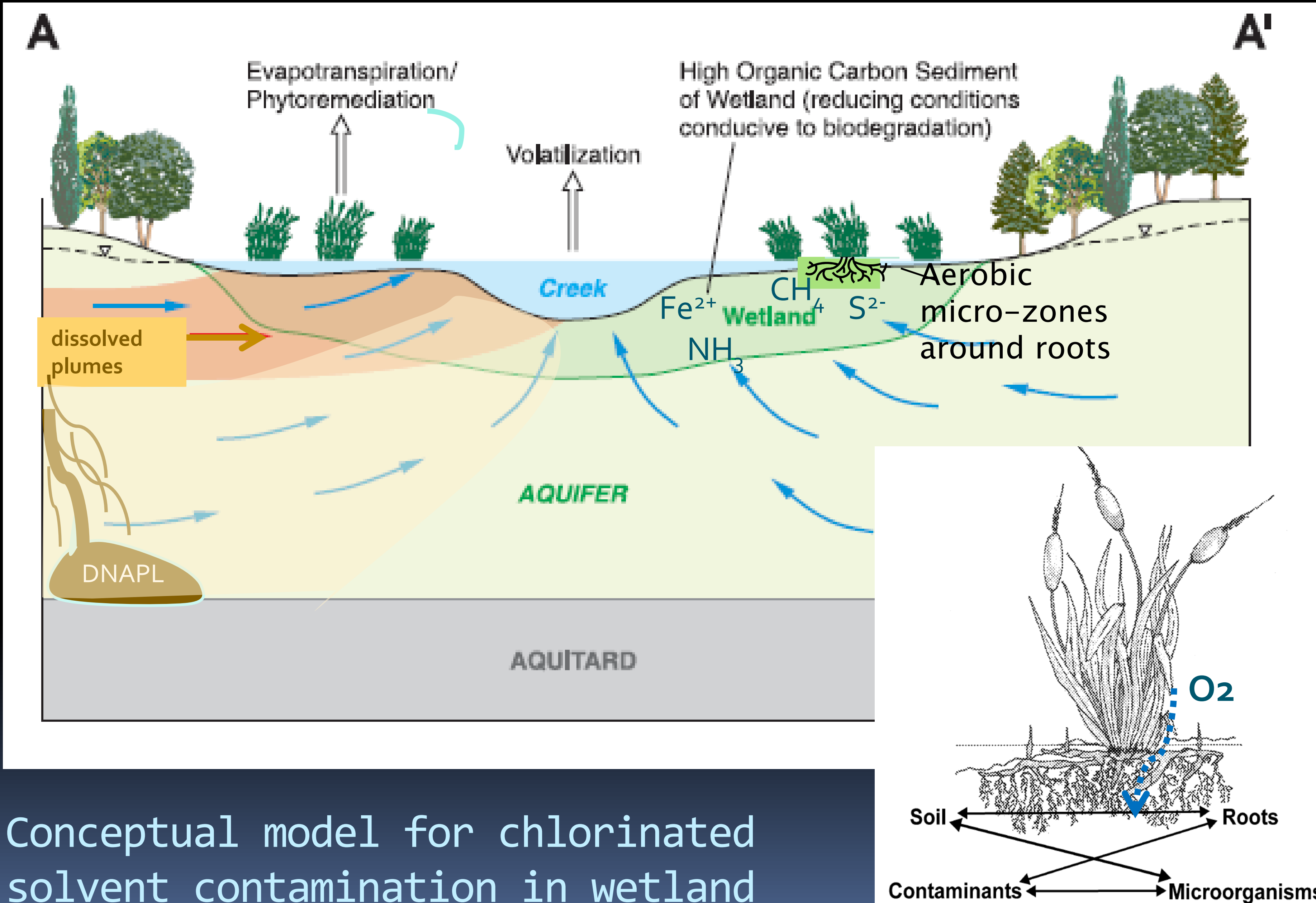
USGS

Biodegradation Pathways

- Anaerobic reductive dechlorination
  - rate decreases with decreasing number of chlorines
- Oxidation reaction pathways
  - typically aerobic
  - rate decreases with increasing number of chlorines





Stottmeister et al.(2003) *Biotech.Advances*

Conceptual model for chlorinated solvent contamination in wetland

(modified from Lorah et al., 2005)

Changing Paradigm

Previous paradigm for chlorinated VOCs:

- Aerobic oxidation requires measurable oxygen
- Anaerobic oxidation responsible for losses of lower VOCs at anaerobic plume fringes

Isolation of an aerobic vinyl chloride oxidizer from anaerobic groundwater

Fullerton et al. 2014

Perils of Categorical Thinking: "Oxic/Anoxic" Conceptual Model in Environmental Remediation

Bradley 2012

Sustained Aerobic Oxidation of Vinyl Chloride at Low Oxygen Concentrations

Gossett 2010

Microbial Mineralization of Dichloroethene and Vinyl Chloride under Hypoxic Conditions

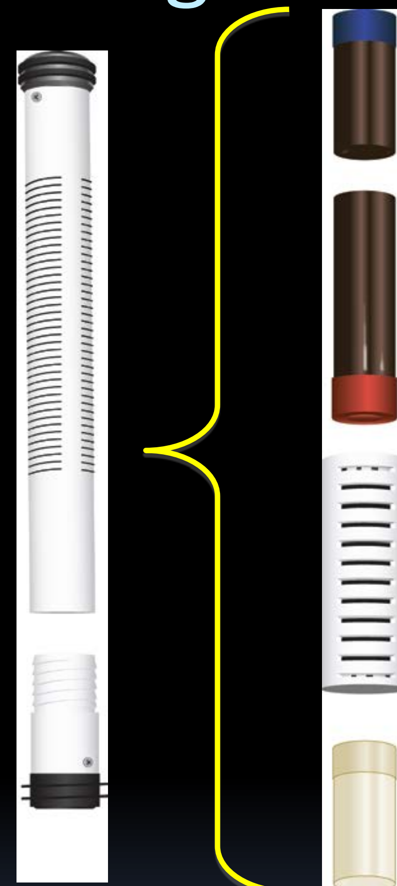
Bradley and Chapelle 2011

Concurrent and Complete Anaerobic Reduction and Microaerophilic Degradation of Mono-, Di-, and Trichlorobenzenes

Burns et al. 2013

Approach to evaluate biodegradation

- *In situ* microcosms with Bio-Traps (Microbial Insights)
  - Stable isotope probing (^{13}C-labeled 14DCB, CB, B)
  - Analysis of species and functional genes for aerobic and anaerobic biodegradation (QuantArray)
- Evaluate biodegradation processes in biofilms
  - Upflow fixed film bioreactors, static bed
  - Robust- can change redox conditions and other parameters more reliably than in batch microcosms
- Enrichment of aerobic degrading bacteria from anaerobic water



GEO
anions, VFAs

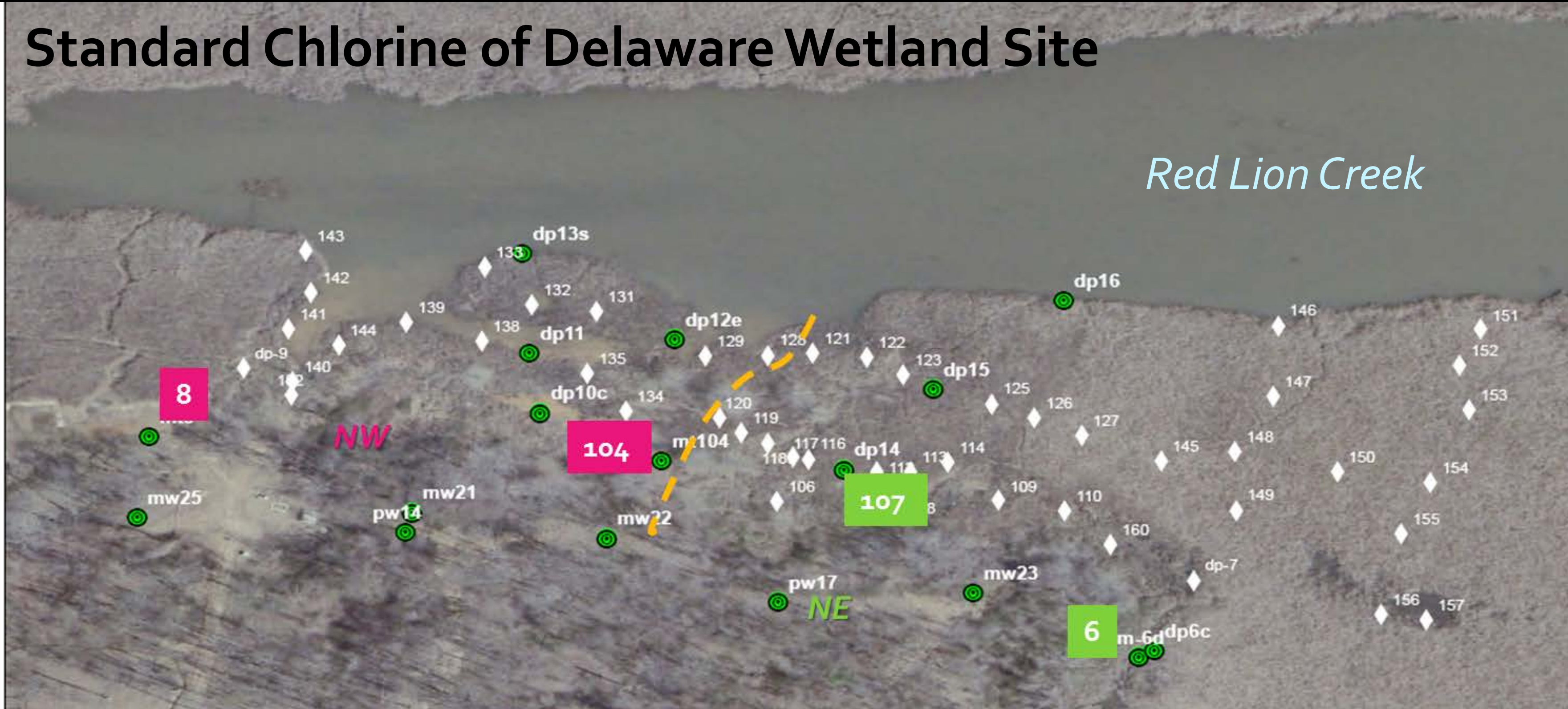
COC
VOCs, redox

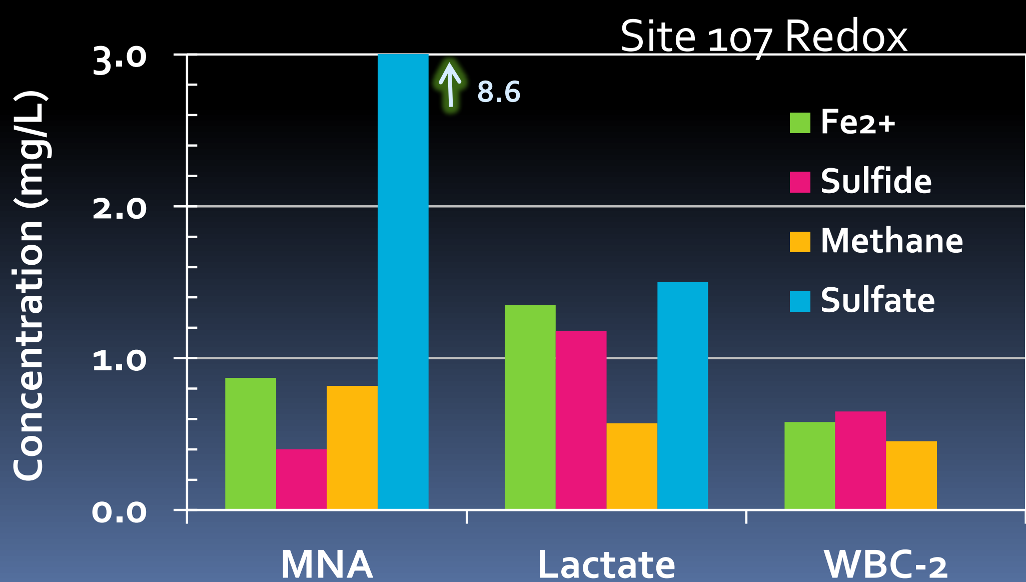
MICRO- Bio-sep beads
Sample microbes
Seed with culture
Load with ^{13}C-VOCs

Amendments
donor

Bioreactor polypropylene support matrix for biofilms

Standard Chlorine of Delaware Wetland Site



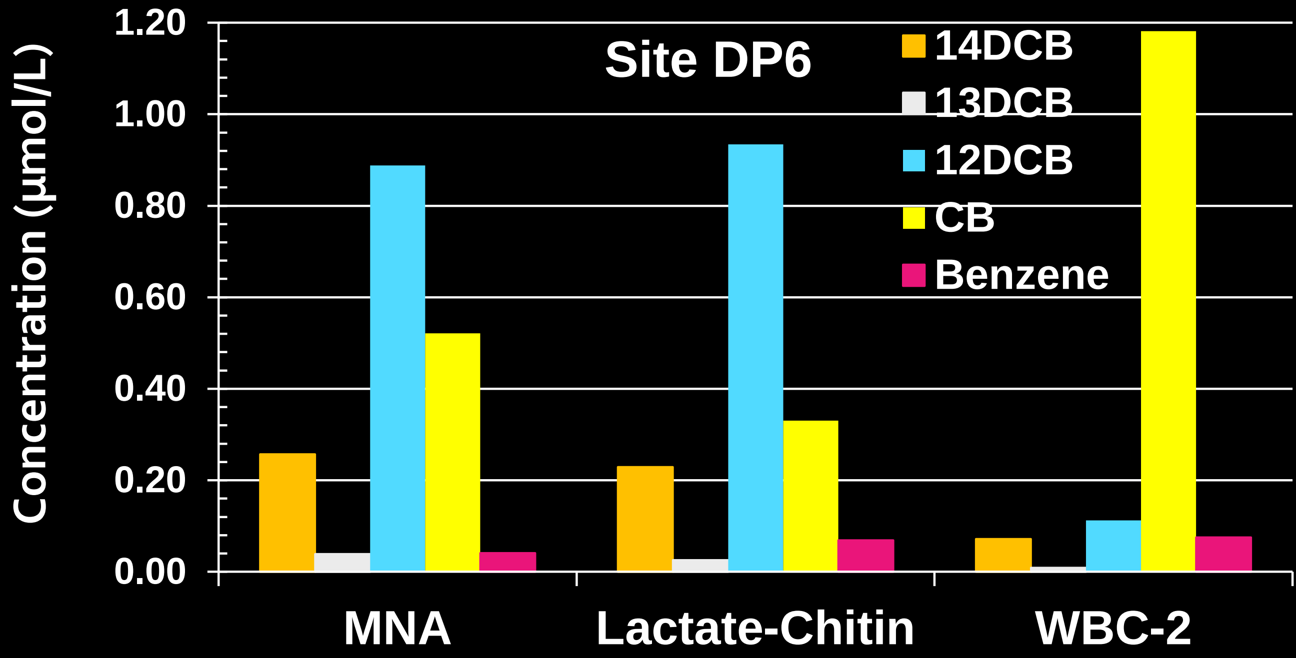


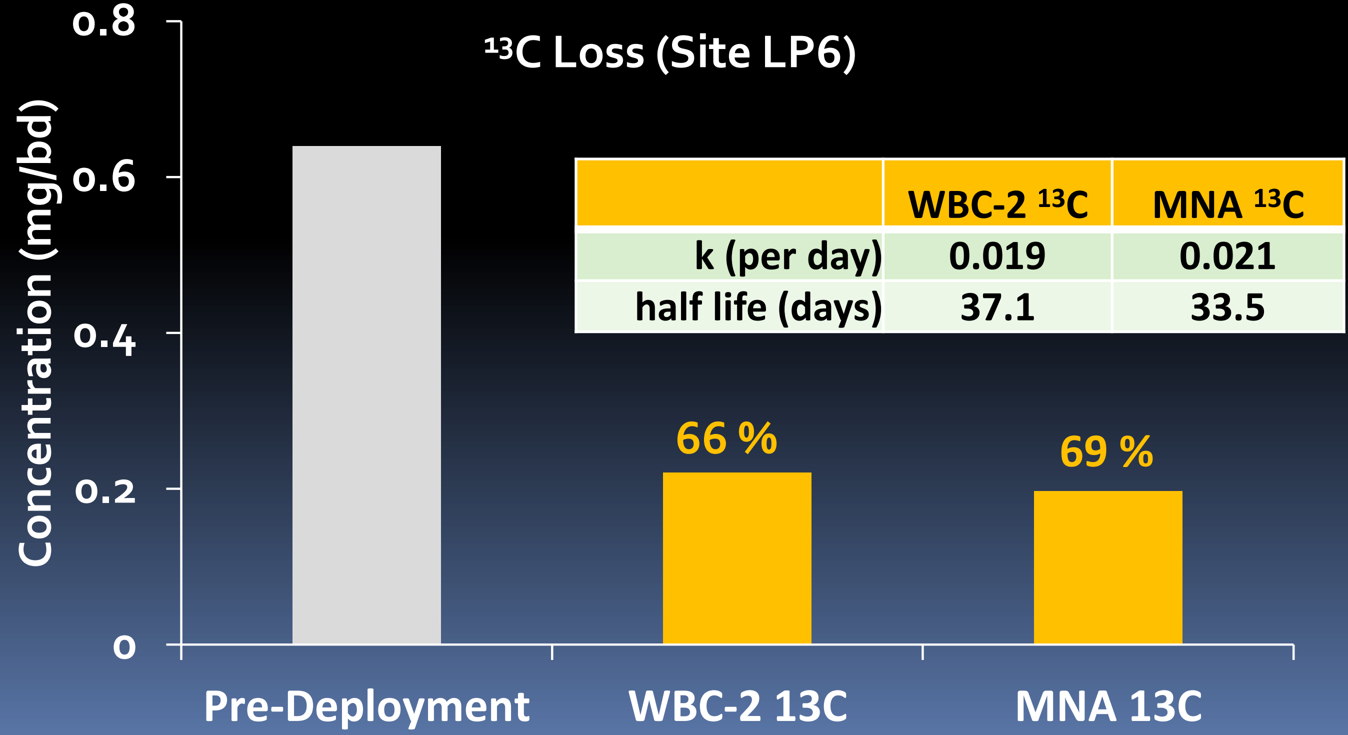
Three treatment types:

- MNA, monitored natural attenuation (no amendments)
- Lactate, biostimulated with lactate +chitin
- WBC-2, bioaugmented

ISM Results:

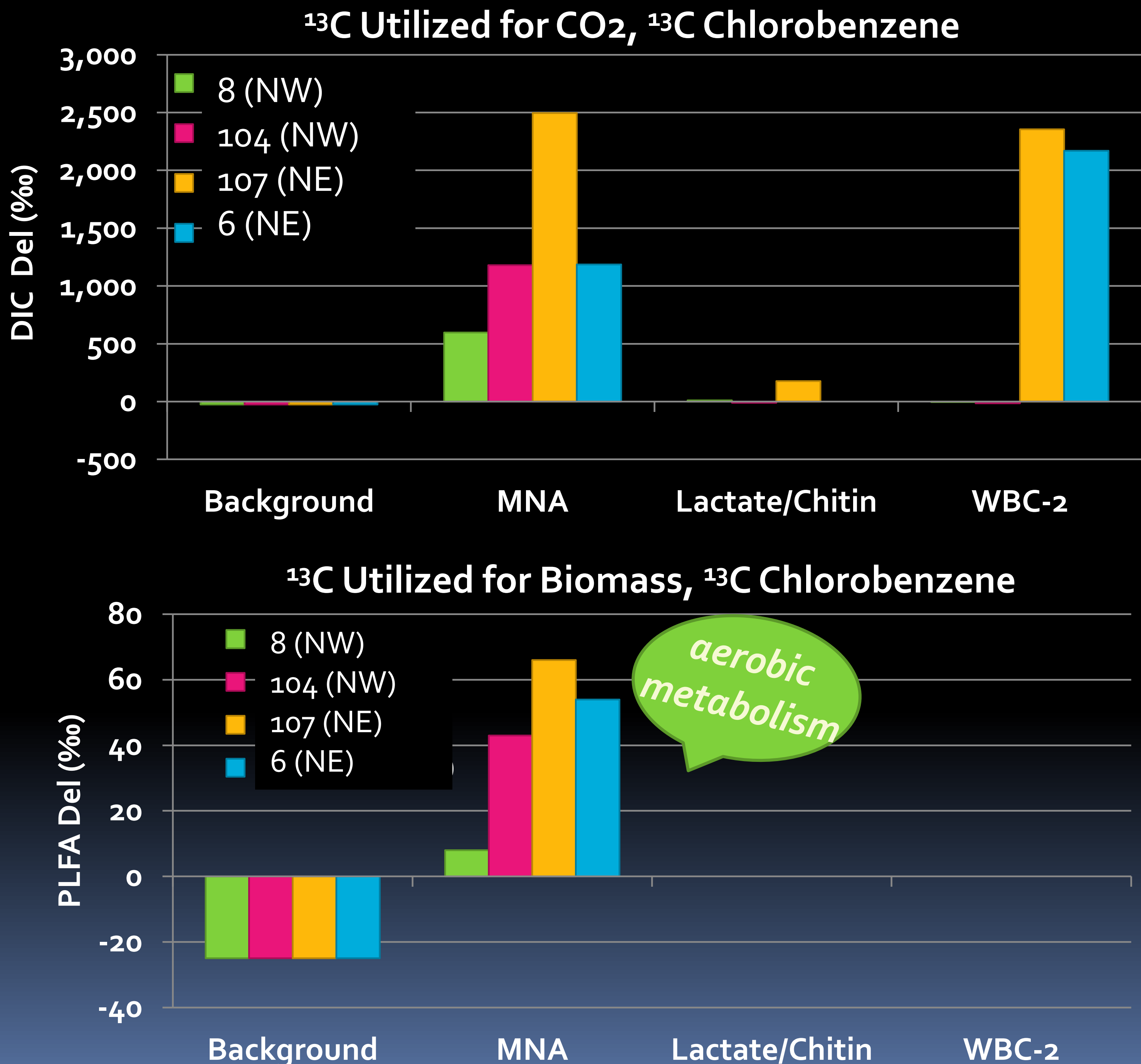
- *Complete degradation of DCBs evident in WBC-2 treatment in standard ISMs*
- *13C-labeled ISMs showed complete degradation of monochloro-benzene in MNA and WBC-2*



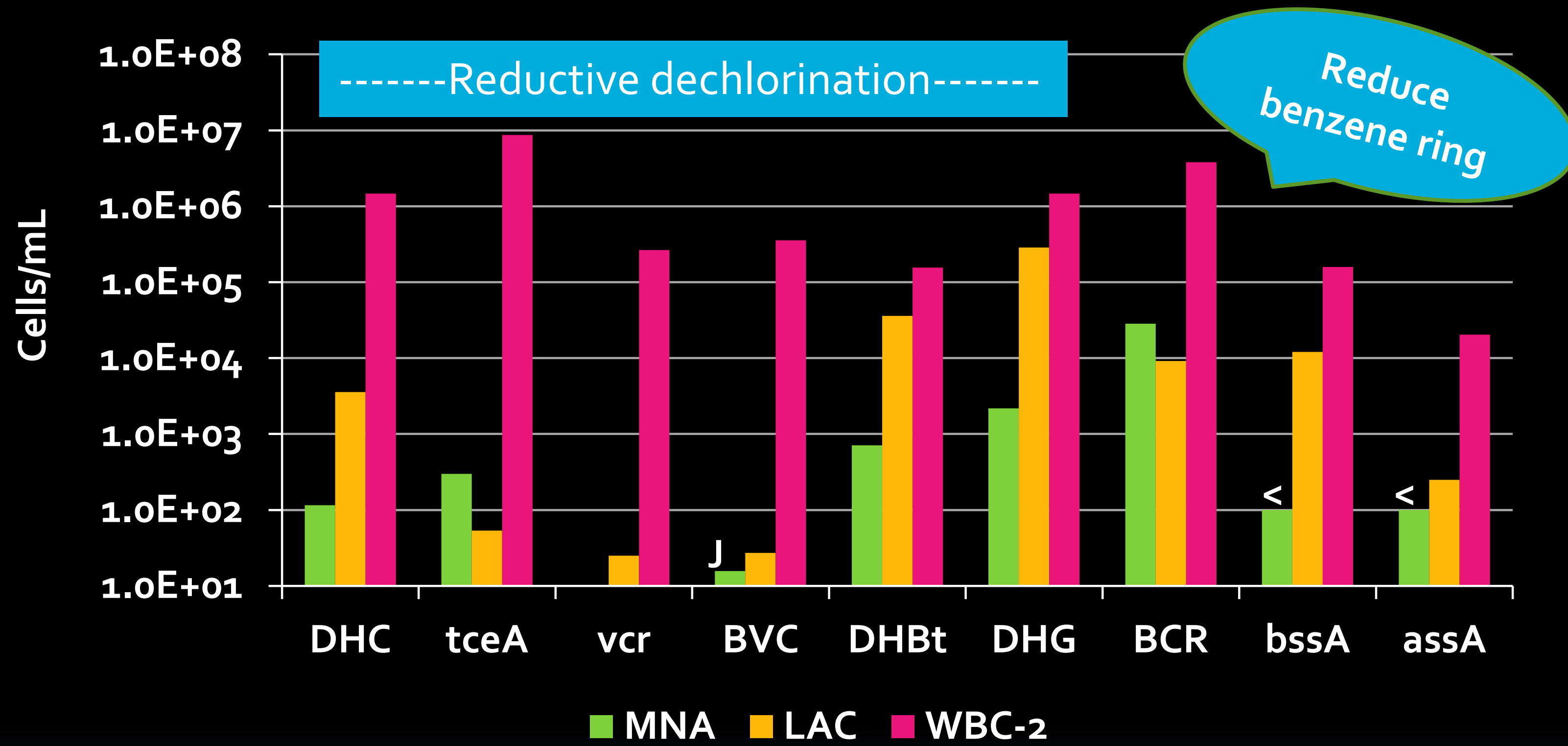


	WBC-2 ^{13}C	MNA ^{13}C
k (per day)	0.019	0.021
half life (days)	37.1	33.5

2010 Bio-Traps: ^{13}C-labeled Chloro-benzene



QuantArray Microbial Analysis- Anaerobic



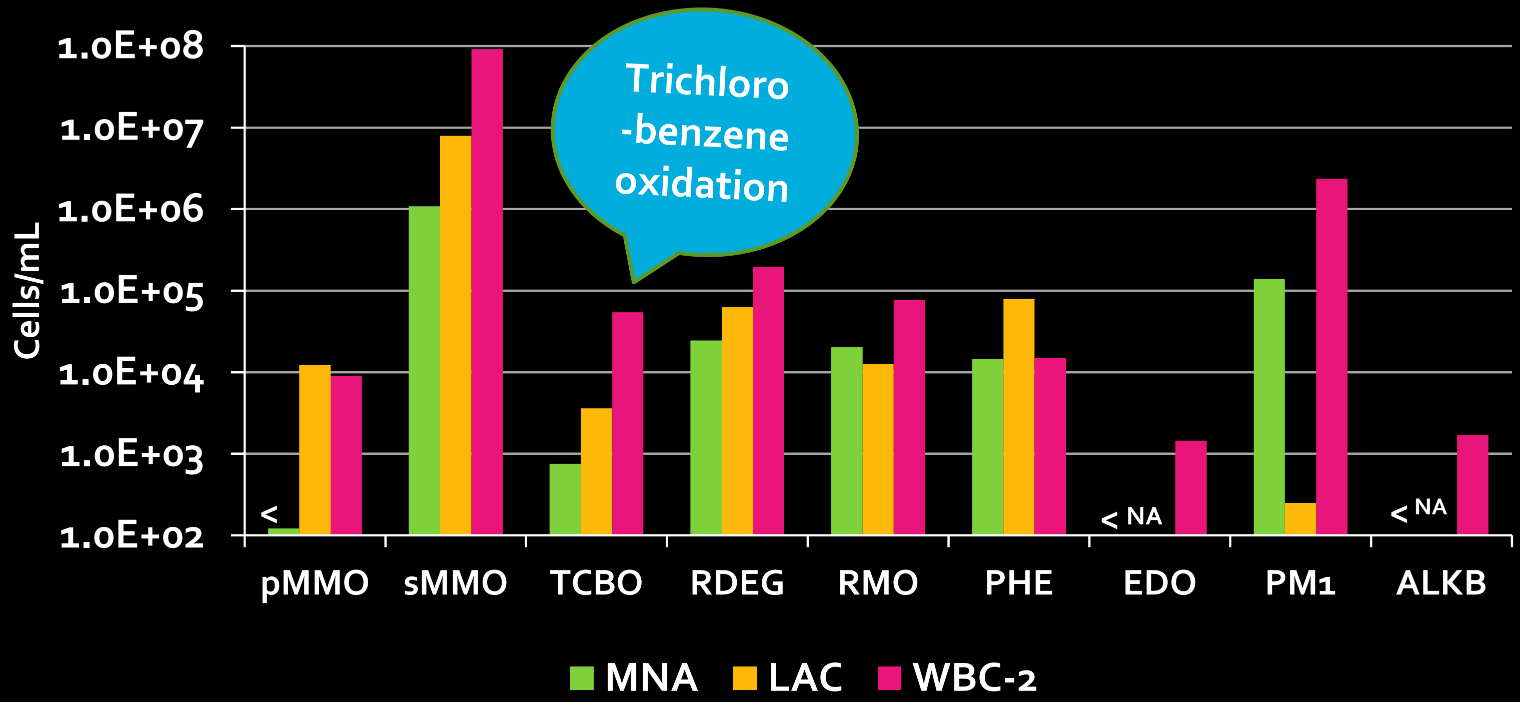
Reductive dechlorination:

DHC , Dehalococcoides spp.
TCE, tceA reductase
VCR, vinyl chloride reductase
BV1 , vinyl chloride reductase
DHBt, Dehalobacter spp.
DHG, Dehalogenimonas spp.

BTEX, PAHs and alkanes:

BCR, Benzoyl coenzyme A reductase
bssA, benzylsuccinate synthase
assA, alkylsuccinate synthase

QuantArray Microbial Analysis- Aerobic



pMMO, particulate methane monooxygenase

sMMO, soluble methane monooxygenase

TCBO, trichlorobenzene dioxygenase

RDEG, toluene monooxygenase 2

RMO, toluene monooxygenase

PHE, phenol hydroxylase

EDO, ethylbenzene/isopropyl-benzene dioxygenase

PM1, Methylibium petroliphilum PM1

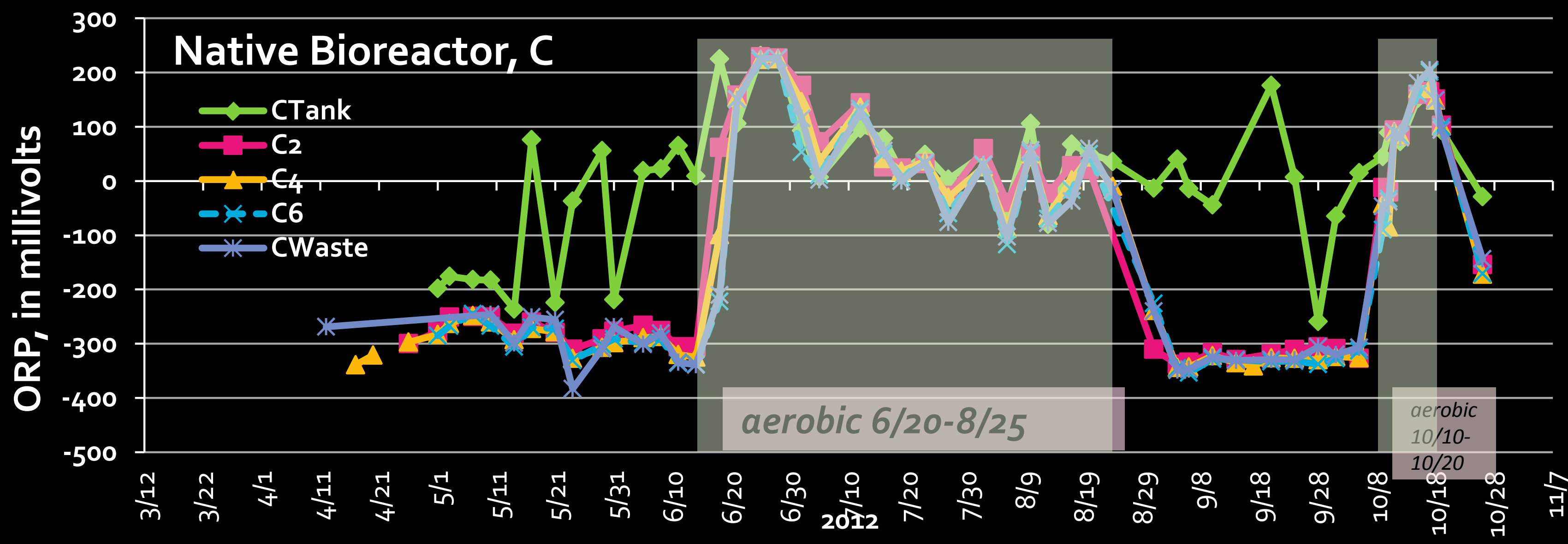
ALKB, alkane monooxygenase

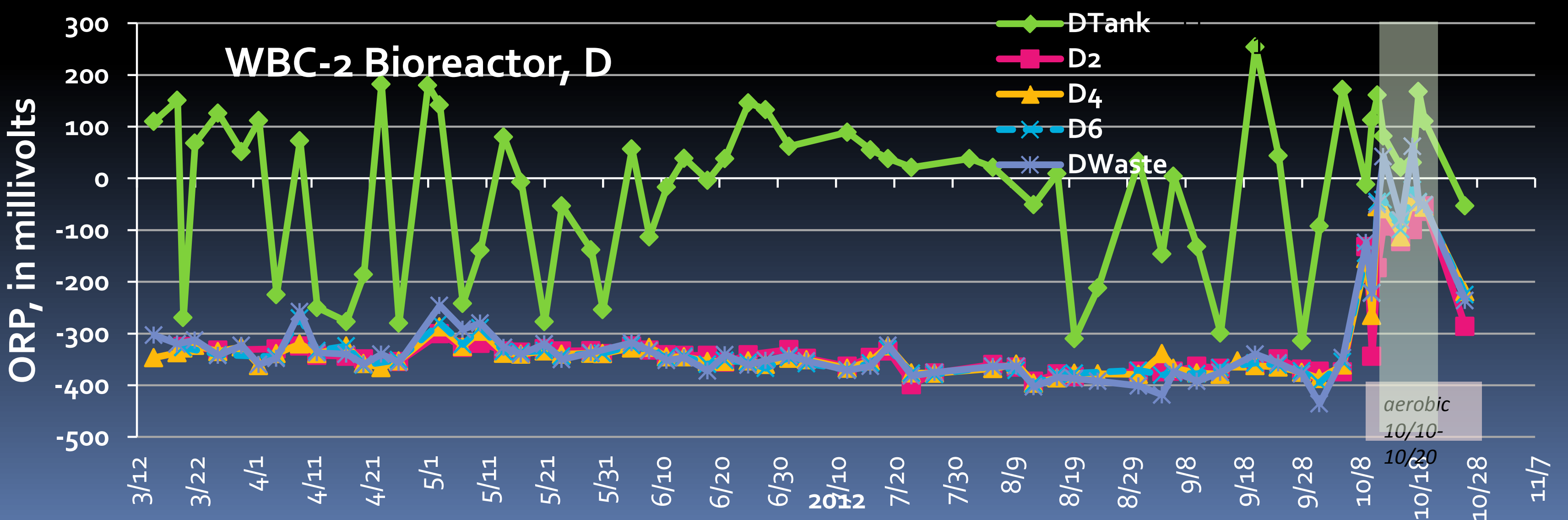
Bioreactors 2012-13

- Native vs. bioaugmented
    - Three vessels (1 liter each) in each bioreactor
    - Native microbe seeded bioreactor (C)
    - Anaerobic dechlorinating culture WBC-2 seeded in two bioreactors (D, E)
    - Varied from anaerobic to aerobic conditions in C

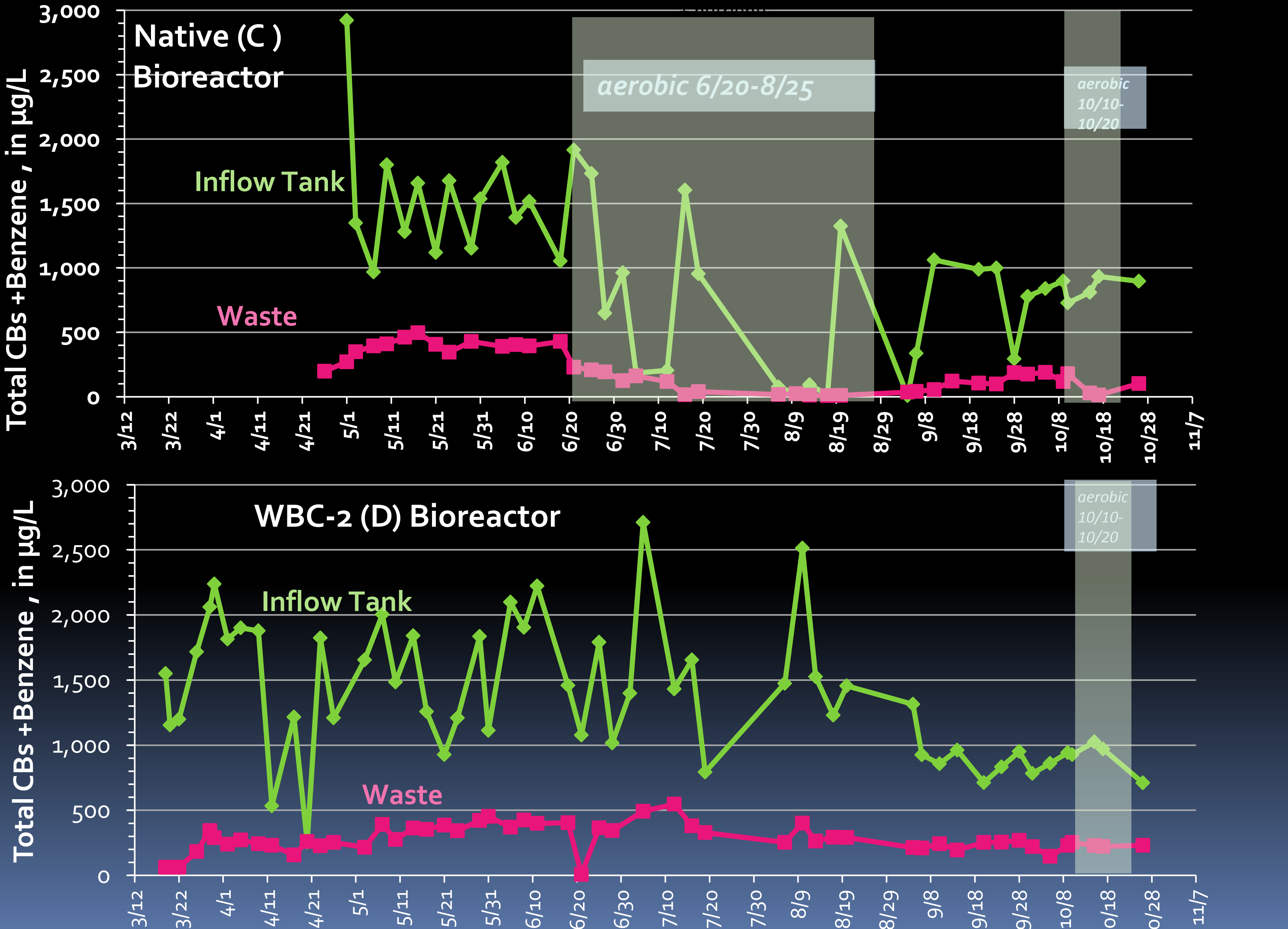
SCD Bioreactors- ORP

(median residence time~ 40 hr; pH~7.0-7.5)

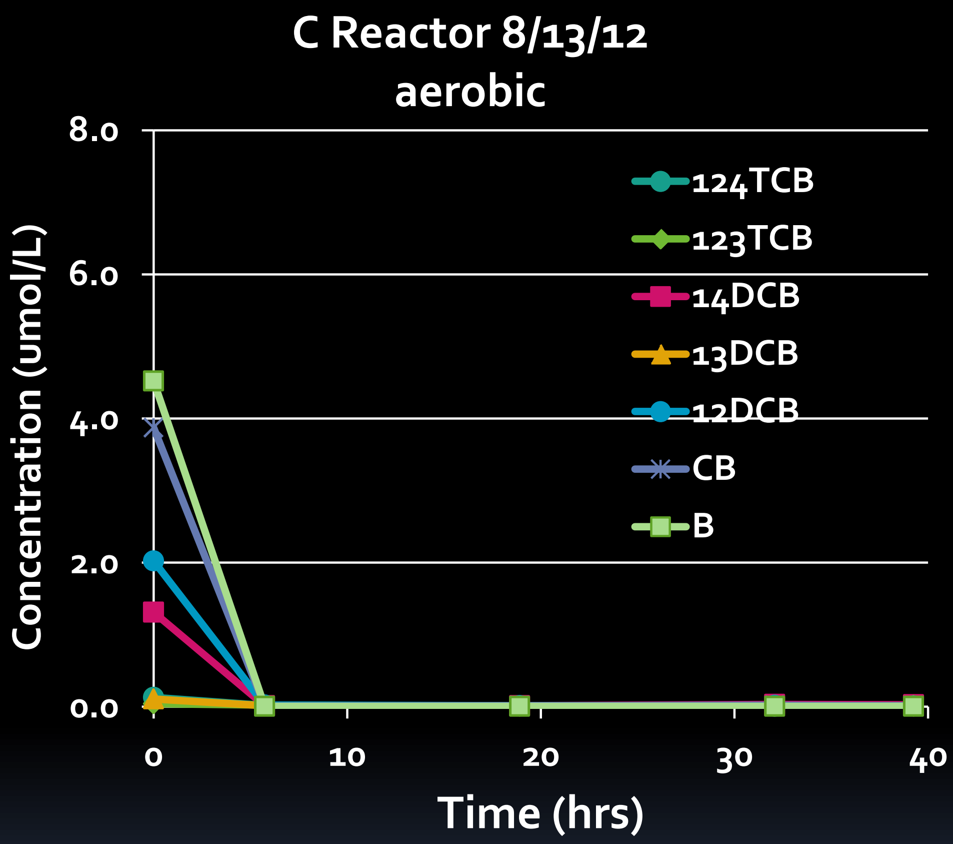


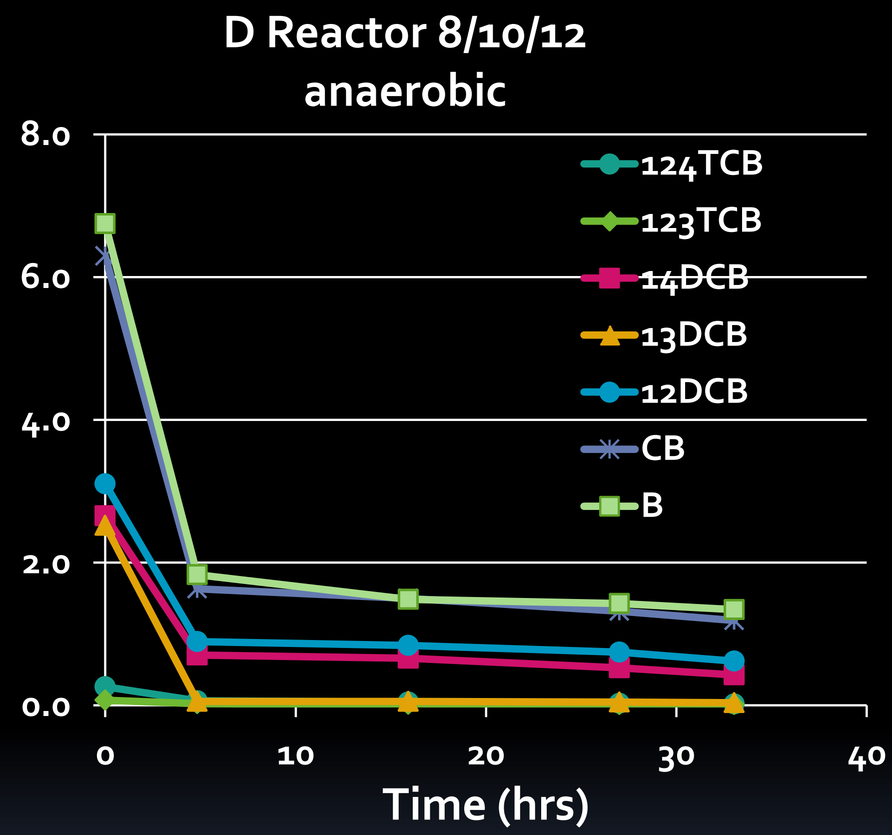


SCD Bioreactors- Total CBs+Benzene



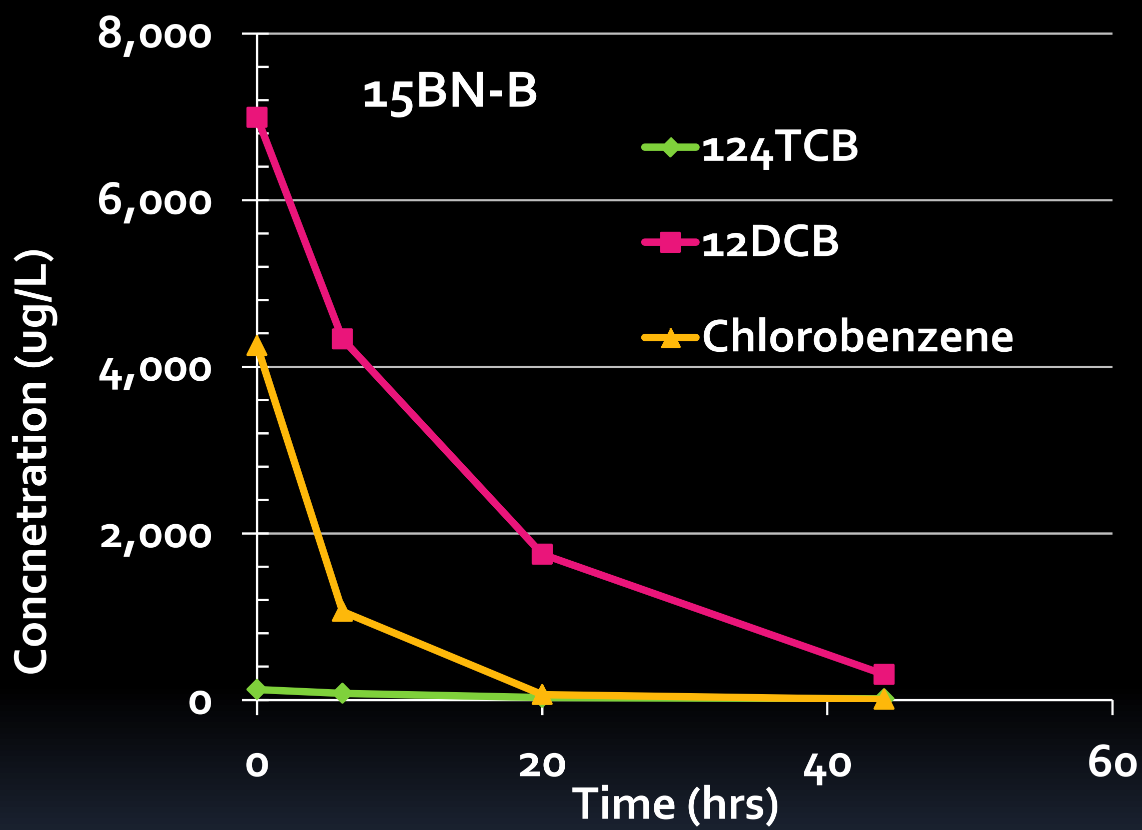
SCD Bioreactors- Aerobic vs. Anaerobic





- *occurrence of aerobic and anaerobic degradation by native bacteria*
- *aerobic degradation much faster than anaerobic biodegradation*
- *WBC-2 able to efficiently degrade chlorinated benzenes and benzene anaerobically*
- *accumulation of daughter products not evident*

Enrichment of Aerobic Native Culture



First order	124TCB	12DCB	CB
k (per hr)	0.051	0.071	0.15
half life (hrs)	13.6	9.8	4.6
r^2	.972	.999	.968

- 8 L of wetland groundwater from 15B filtered (0.2 µm)
- Filtrate placed in tryptone-yeast extract broth (Difco ISP Medium 1)
- Spiked with CB, 12DCB, 14DCB, and 124TCB
- Incubated aerobically on shaker

Conclusion

- Both anaerobic (WBC-2 and native microbial biofilms) and aerobic (native) degradation processes are effective
  - Aerobic degradation fastest but logistically difficult for in situ treatment of wetland sediments and groundwater
  - Concentrations as high as 100 mg/L successfully tested
- Plan to utilize both cultures in a reactive mat with biofilms on GAC. Column tests are underway

Reactive Mat Design and Monitoring