Culture Testing for a Dual-Biofilm Reactive Barrier for Treatment of Chlorinated Benzenes in Wetland Groundwater and Sediment

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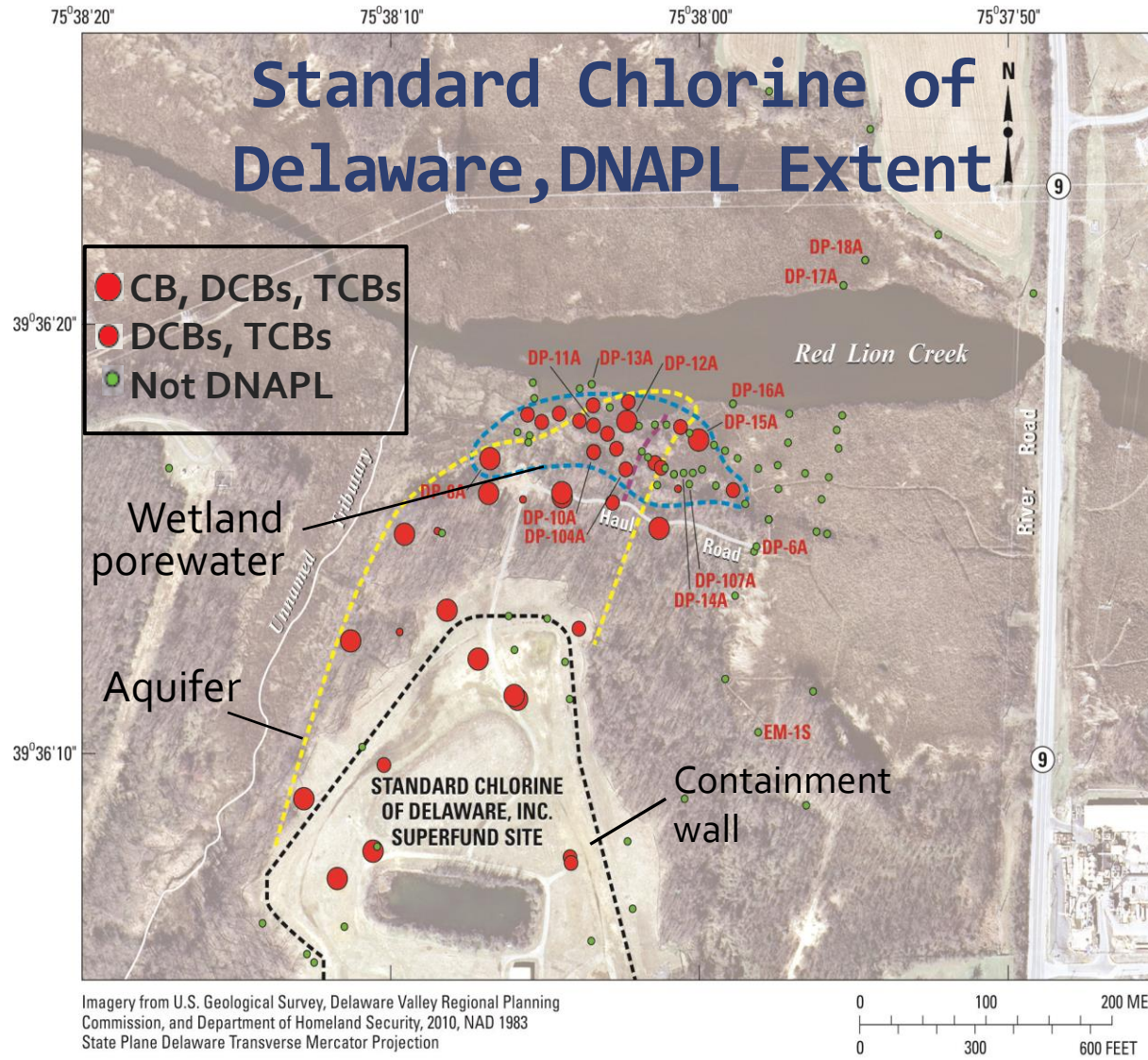
in cooperation with

USEPA, Region III and
NIEHS (National Institute of Environmental Health Sciences)

Background

- Chemical plant 1966-2002; EPA Superfund site since 2002
- USGS wetland study with EPA since 2009 to characterize wetland and evaluate bioremediation
- NIEHS research study started Oct 2014 in collaboration with JHU and Geosyntec



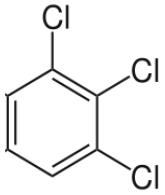
Biodegradation Pathways

Anaerobic (reductive dechlorination)

- CB serves as terminal electron acceptor
- Separate e- donor required
- rate decreases with decreasing number Cl

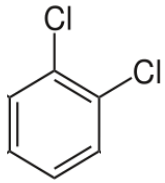
Aerobic (oxidation)

- O_2 required as electron acceptor
- CBs utilized as C and e^- donor
- rate increases with decreasing number Cl
- Short-lived intermediates



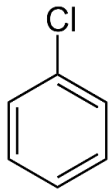
70 (124TCB)

Trichlorobenzenes*
135TCB,124TCB, 123TCB



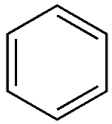
75 (14DCB)

Dichlorobenzenes*
14DCB, 13DCB, 12DCB



100

Chlorobenzene*



5

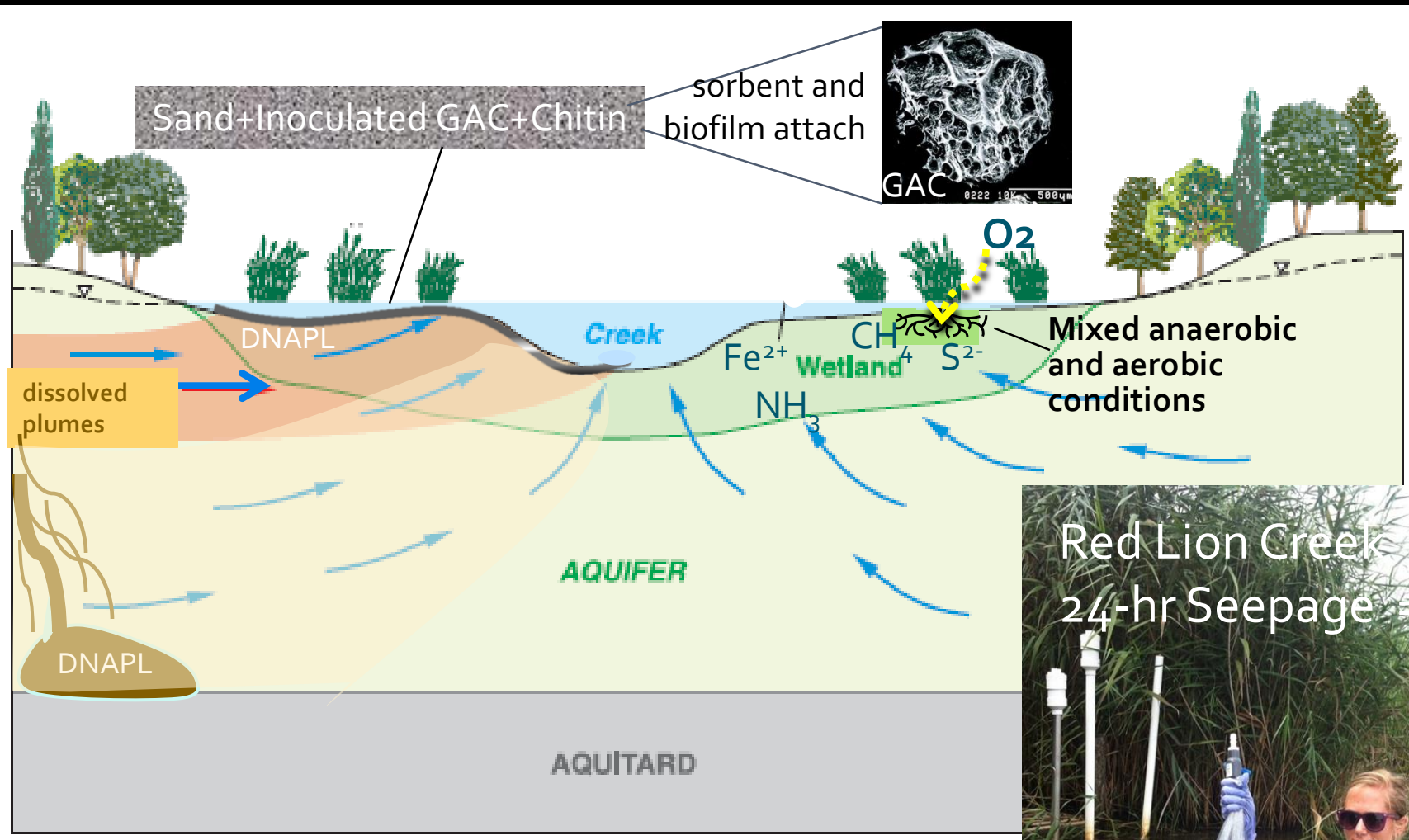
Benzene *

CO_2, CH_4

Drinking Water MCL µg/L

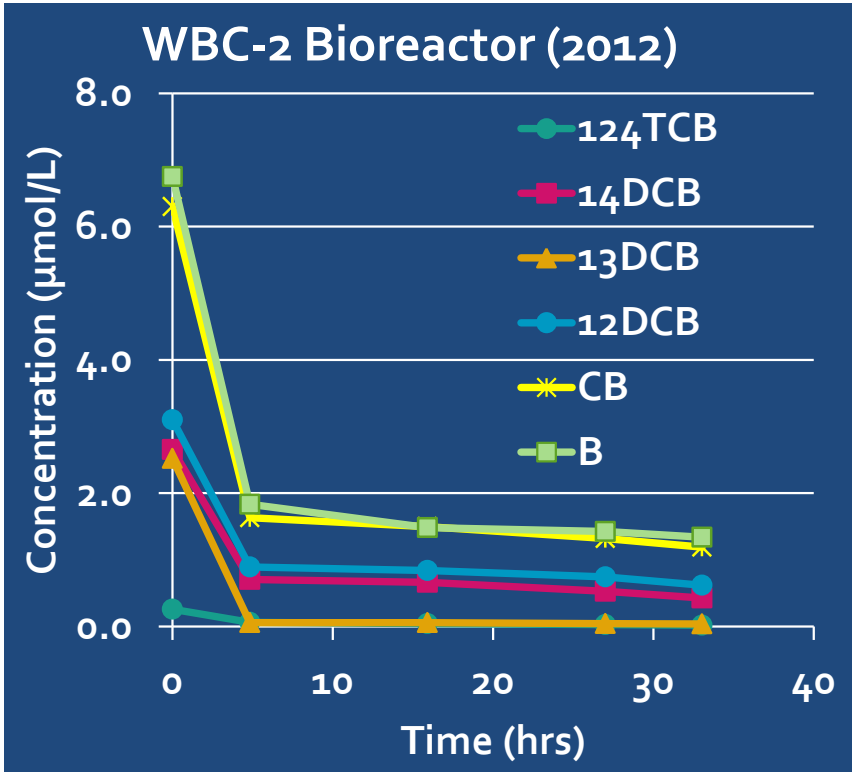
Aerobic CO2, HCl

* Parent contaminant



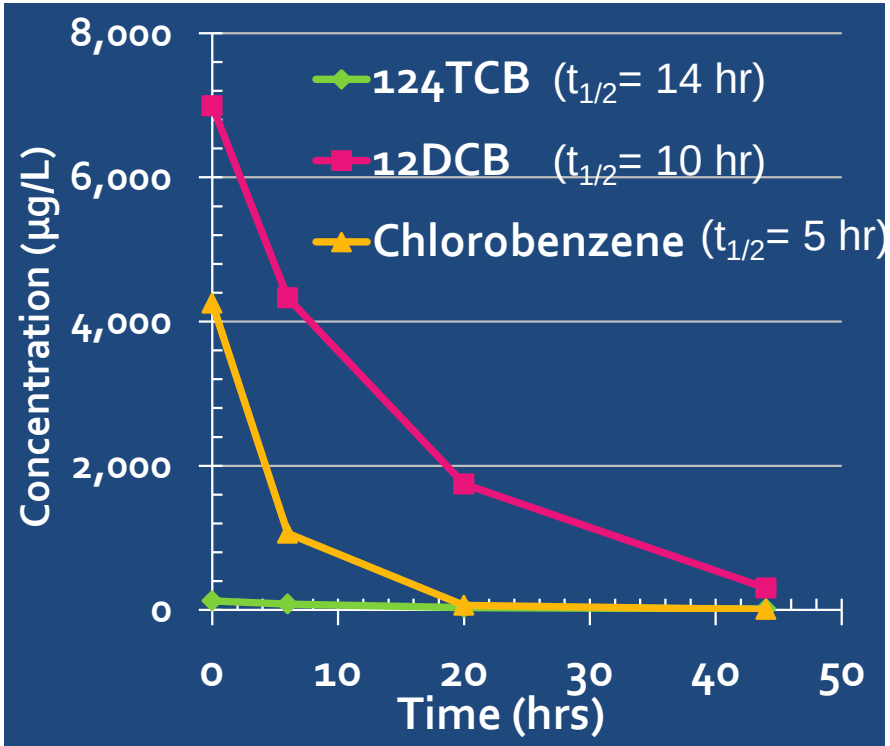
Conceptual model for contamination and dual-biofilm reactive barrier in wetland

Anaerobic Culture WBC-2



- Enriched from wetland sediment at APG to degrade chlorinated ethanes and ethenes
- Sediment-free culture since 2002
- Readily available in large quantities

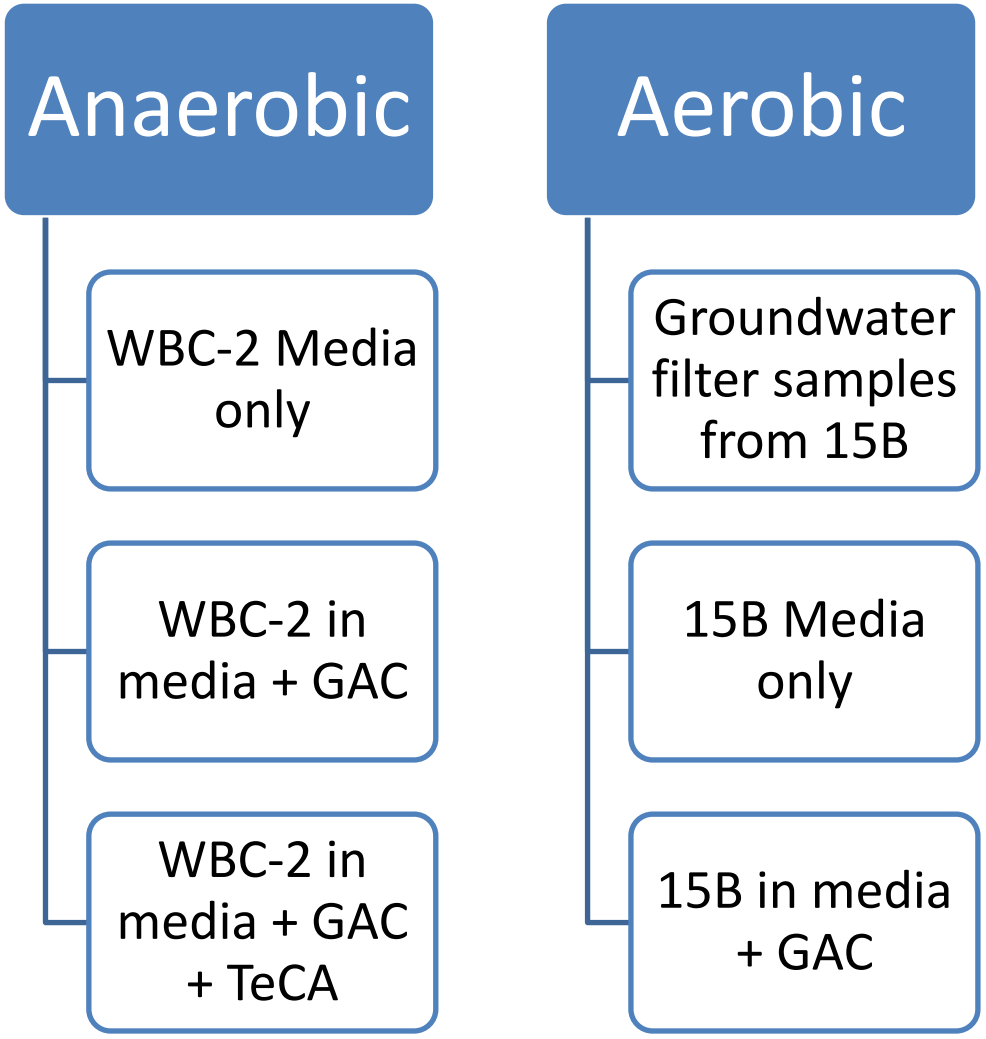
Aerobic Culture 15B



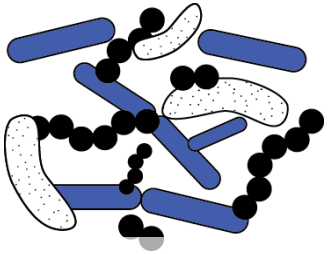
- Wetland groundwater from DP-15B at SCD
- Tryptone-yeast extract media
- Fed with CB, 12DCB, 14DCB, and 124TCB
- Incubated aerobically on shaker

Methods: GAC Seeding

- Treatments in duplicate
- 10 g of GAC (Calgon Carbon Corp. Filtrasorb 600-Unsorted) for each
- 10 mL of media or culture in media
- Soaked GAC for 3 days for seeding (anaerobic glove box for WBC-2)
- DNA extracted and frozen until genetic analysis

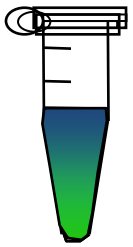


Workflow for Characterizing Microbial Communities

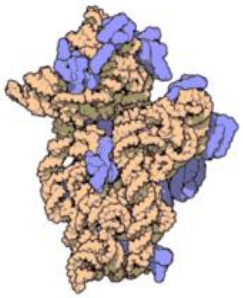


Microbial Community

Extract DNA



Amplify 16S rRNA gene

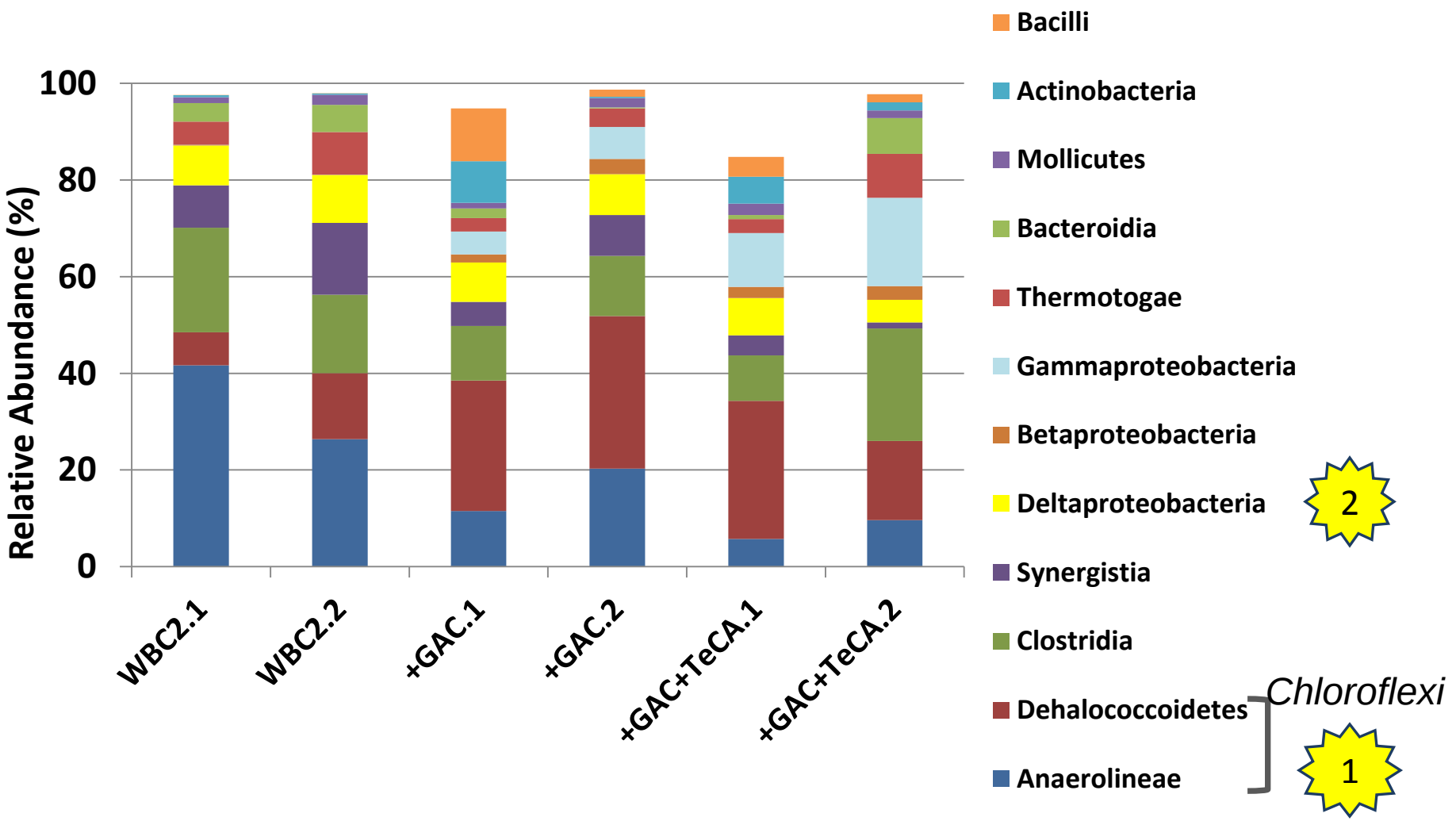


- Gold standard taxonomic gene for microbiology

Illumina iTag Sequencing

- Characterize community composition—diversity, abundance, & identity of organisms

GAC with WBC-2: Classes >1% Abundance



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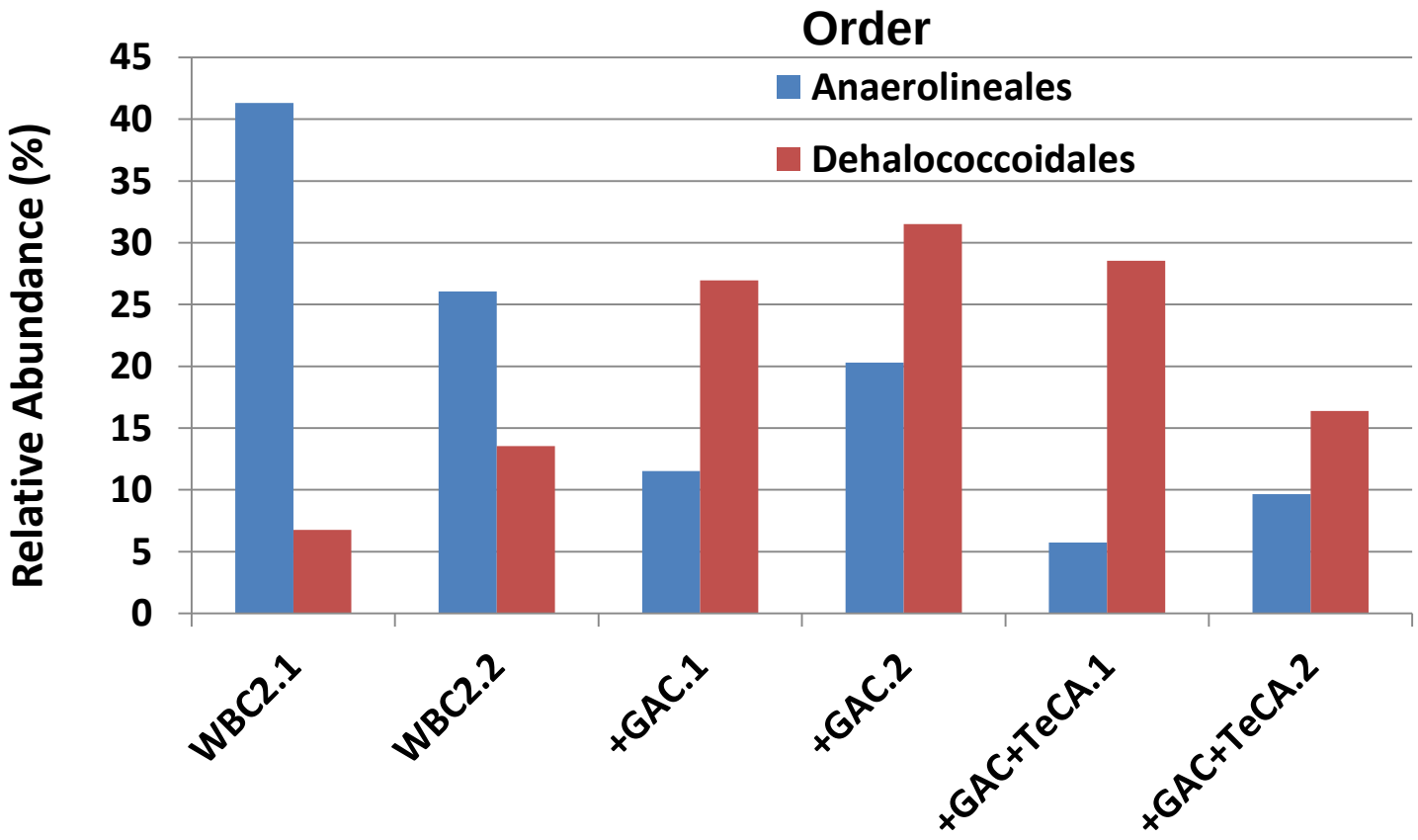
GAC with WBC-2: Chloroflexi, Order



- *Significant increase in Dehalococcoidales on GAC.*
- *Decrease in Anaerolineales on GAC.*

GAC with WBC-2: Genus

1

Anaerolineae

- f__Anaerolinaceae;Other
- f__Anaerolinaceae;g__C1_B004
- f__Anaerolinaceae;g__T78
- f__Anaerolinaceae;g__WCHB1-05

Genus T78 and Dehalococcoides were predominant in both the culture and the WBC-2-seeded GAC

1

Dehalococcoidetes

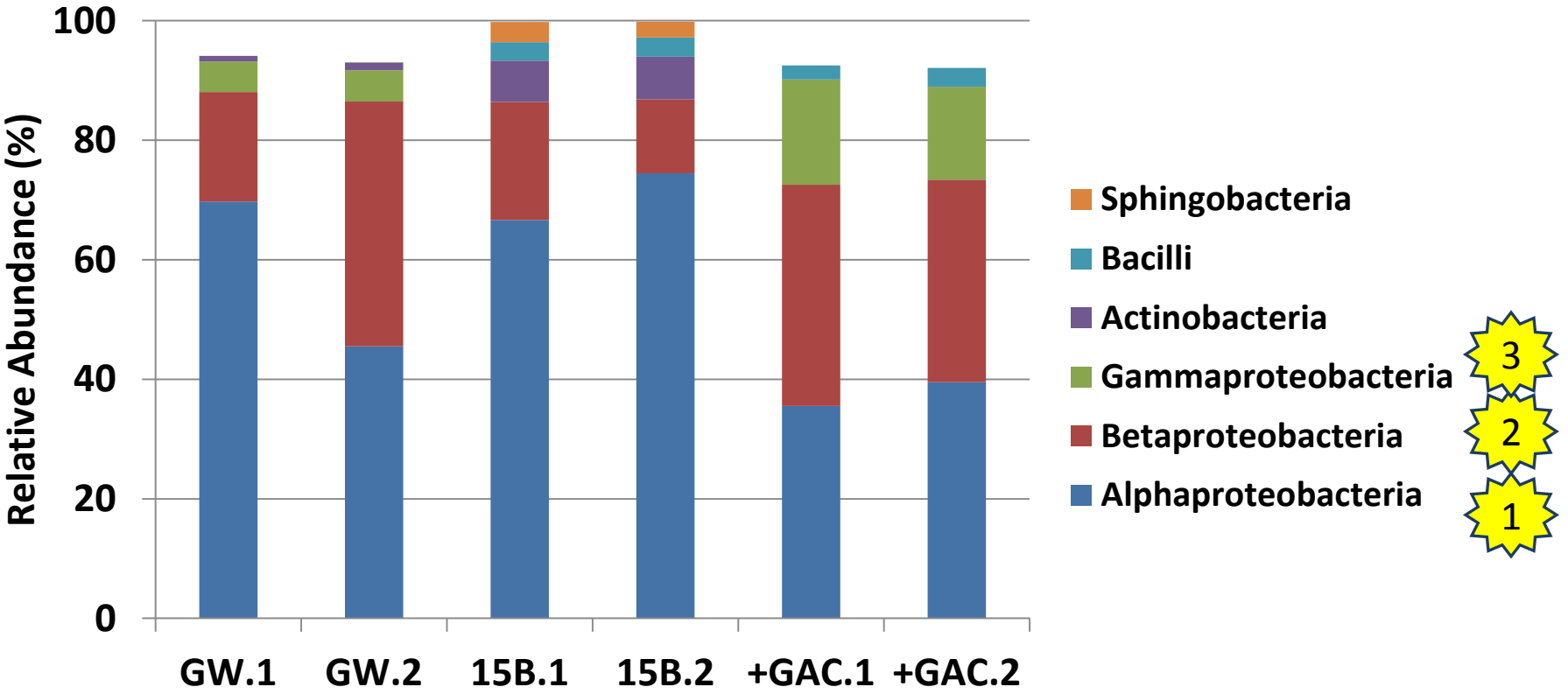
- f__Dehalococcoidaceae;g__Dehalococcoides
- f__Dehalococcoidaceae;g__Dehalogenimonas

Deltaproteobacteria

2

- f__Geobacteraceae;Other
- f__Geobacteraceae;g__
- f__Geobacteraceae;g__Geobacter

GAC with 15B: Classes >1% Abundance



GAC with 15B: Proteobacteria, Order

- *Significant increase in the Betaproteobacteria group Burkholderiales on GAC.*
- *Moderate decrease in the Alphaproteobacteria group Rhizobiales on GAC; greater decrease in Caulobacterales.*

GAC with 15B: Proteobacteria, Genus

1

Alphaproteobacteria (Orders Caulobacterales, Rhizobiales)

- f__Caulobacteraceae;Other
- f__Caulobacteraceae;g__Brevundimonas
- f__Brucellaceae;g__Ochrobactrum
- f__Phyllobacteriaceae;Other
- f__Phyllobacteriaceae;g__
- f__Rhizobiaceae;Other
- f__Rhizobiaceae;g__
- o__Rhizobiales;Other;Other

cited by others, i.e. Vogt et al., 2002, "Two Pilot Plant Reactors Designed for the In Situ Bioremediation of Chlorobenzene-contaminated Ground Water…"

2

Betaproteobacteria (Order Burkholderia

- f__Alcaligenaceae;Other
- f__Alcaligenaceae;g__
- f__Comamonadaceae;g__Comamonas

3

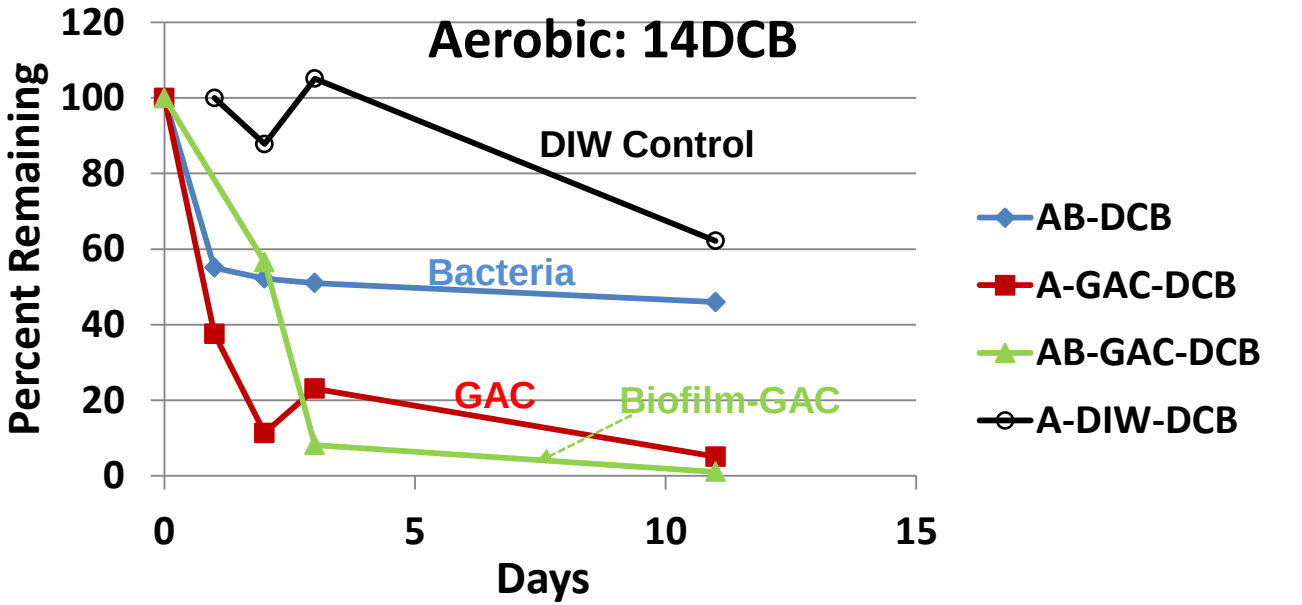
Gammaproteobacteria

- f__Pseudomonadaceae;g__Pseudomonas
- f__Xanthomonadaceae;g__Stenotrophomonas

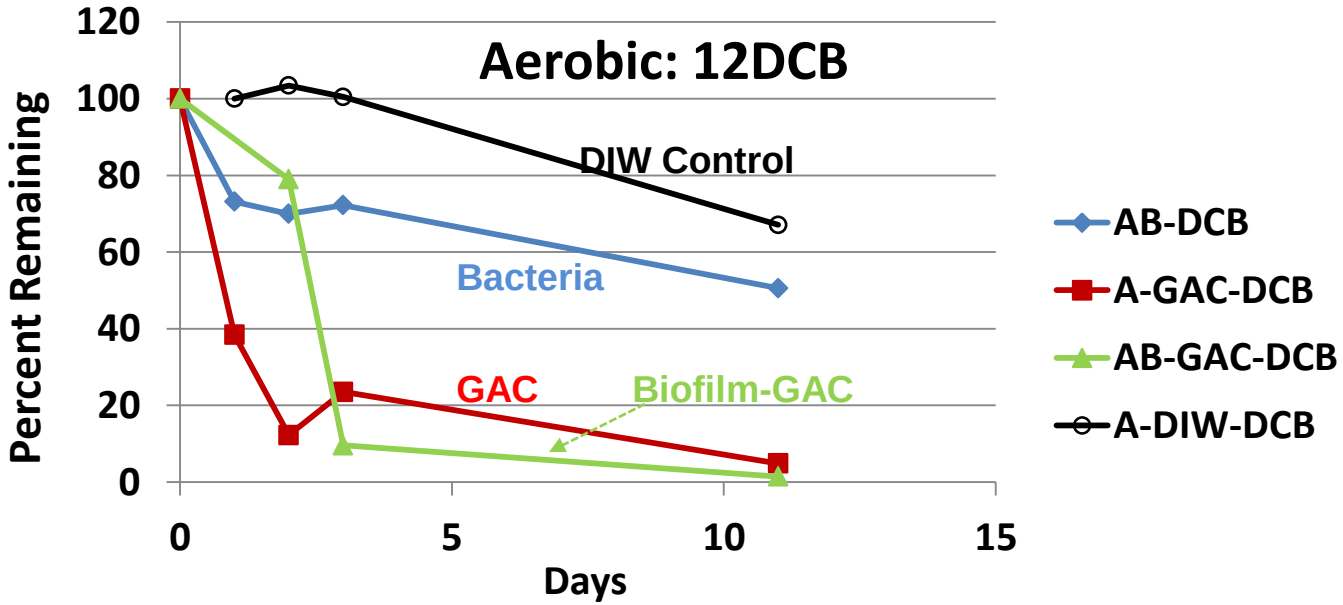
15B Isolate 16S rRNA Identification
(Spiked with mixture of 124TCB, 14DCB, 12DCB, CB)

Isolate Name	Isolated From	Top GenBank BlastN Hit	Score
15B-YJ-3	Full 15B in 10% media	Pseudomonas genticula	1724
15B-YJ-1	10% media plate isolate, directly from plate	Pseudomonas genticula	1818
15B-YJ-5	10% media plate isolate transferred to liquid media	Pseudomonas genticula	1724
15B-YJ-6	10% media plate isolate transferred to liquid media	Pseudomonas genticula	1740
15B-YJ-2	1% media plate isolate, directly from plate	Pseudomonas genticula	1803
YJ-15B-1	100% media plate isolate with DNAPL CBs	Pseudomonas aereginosa	2217
YJ-15B-2	100% media plate isolate with DNAPL CBs	Pseudomonas aereginosa	2584
YJ-15B-3	100% media plate isolate with DNAPL CBs	Pseudomonas aereginosa	2547

Microcosm Results: Aerobic 15B Seeded on GAC

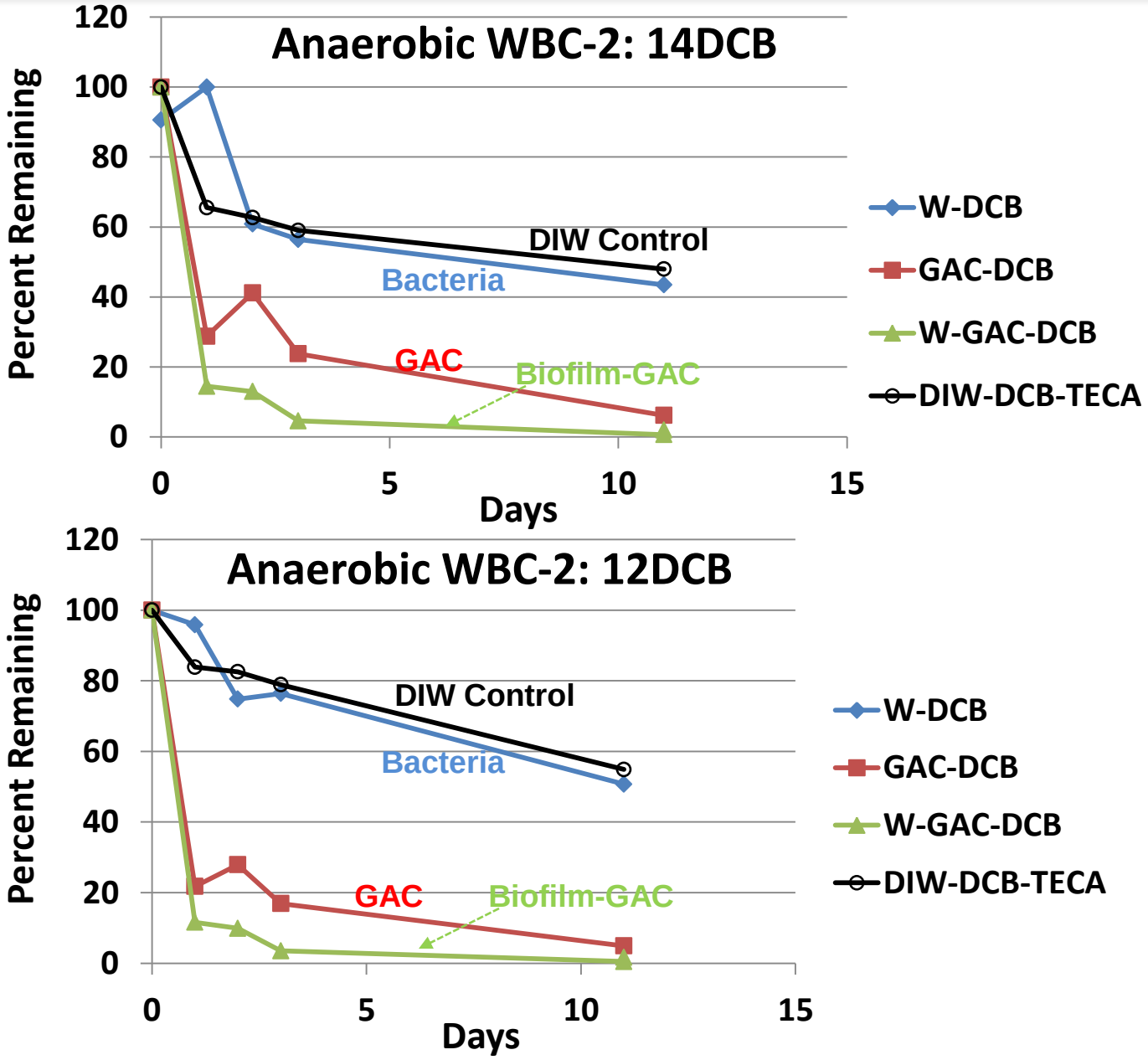


- Distinct decrease in CBs with culture in mineral media compared to DIW
- Delay in sorption to GAC with aerobic biofilm
- Slightly faster overall CB removal in biofilm-GAC



Microcosm Results: Anaerobic WBC-2 Biofilm on GAC

- Slight decrease in CBs with culture in mineral media compared to DIW Rapid sorption to GAC with and without anaerobic biofilm
- Distinctly faster overall CB removal in biofilm-GAC



Conclusions

- GAC provides an effective growth matrix for the anaerobic WBC-2 and aerobic 15B cultures
- GAC enhanced the abundance of microbial groups/species involved in biodegradation; thus a good delivery and support matrix for the cultures
  - Dehalococcoides significantly increased (~doubled) in WBC-2 seeded GAC
  - Burkholderiales group doubled in 15B seeded GAC
  - Pseudomodales in 15B-seeded GAC community
- Biodegradation occurred in conjunction with sorption by GAC seeded with the cultures

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Feasibility evaluation

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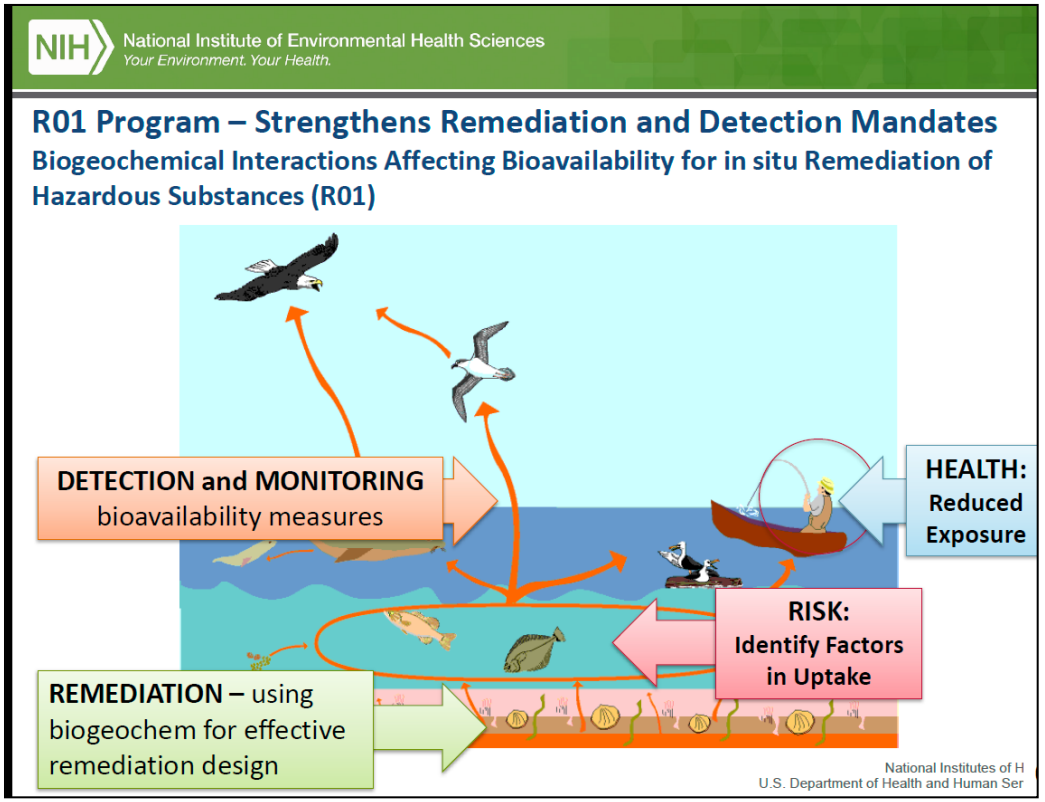

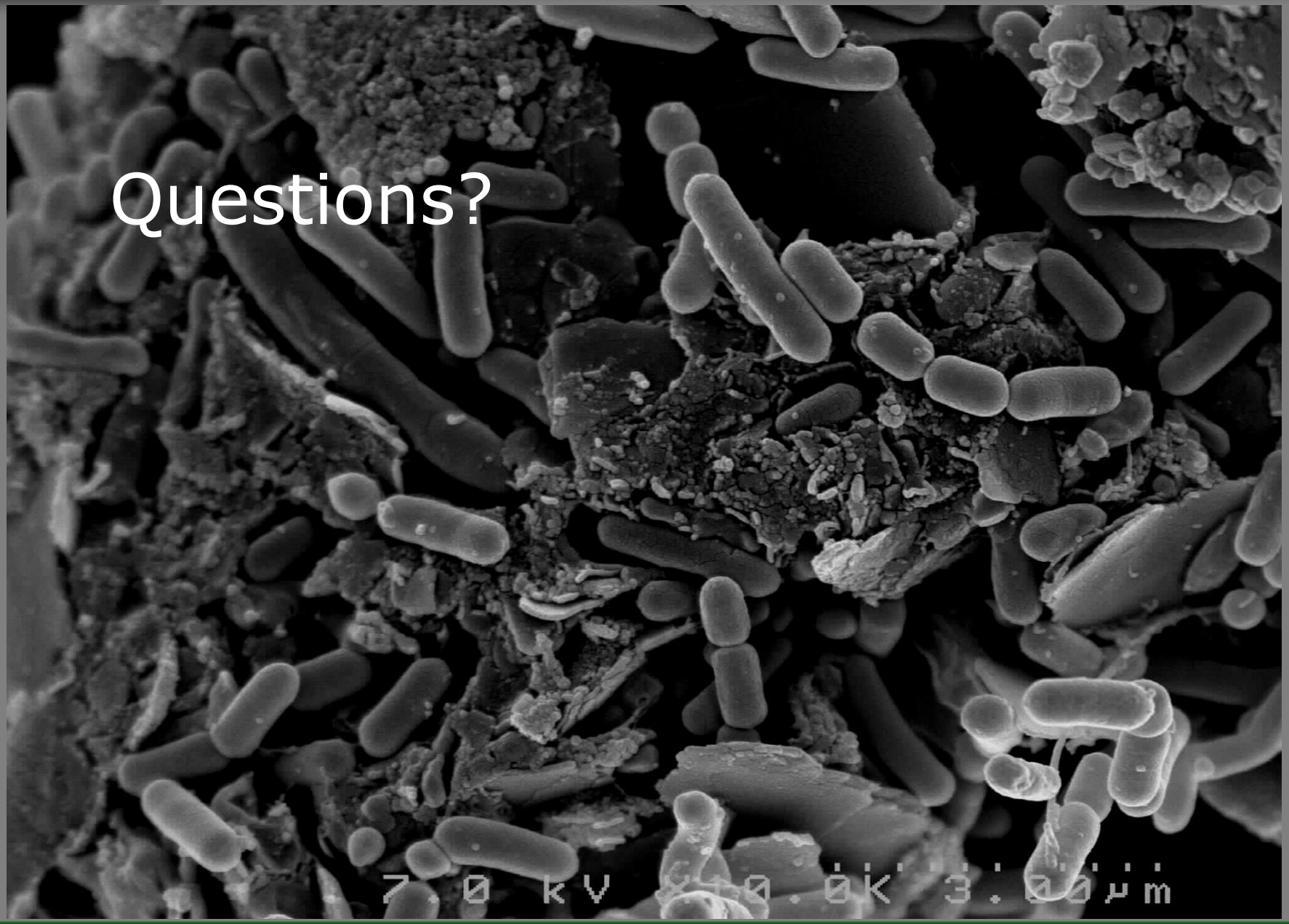
Questions?