Case Studies of Advances in Bioremediation of Organics: Part 2

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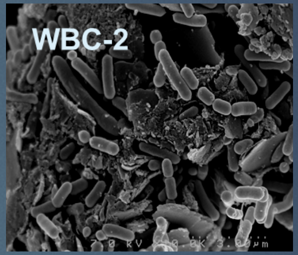
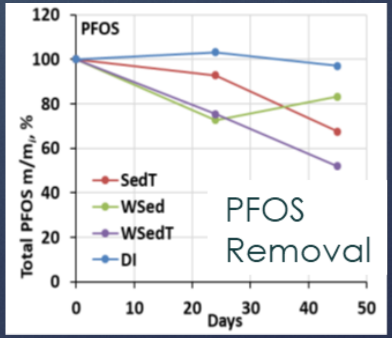
USGS

FRTR Spring 2020 Webinar

4 Case Studies

- Combined anaerobic and aerobic bioaugmentation
  - groundwater-surface-water interface
  - bioaugmentation on activated carbon
- TCE in fractured rock— permeable units
  - microbial community analyses to track bioaugmentation effects, DCE/VC stall
- TCE in fractured rock— rock matrix
  - borehole test to quantify diffusion and degradation
- Anaerobic PFAS biodegradation with chlorinated solvent co-contaminants



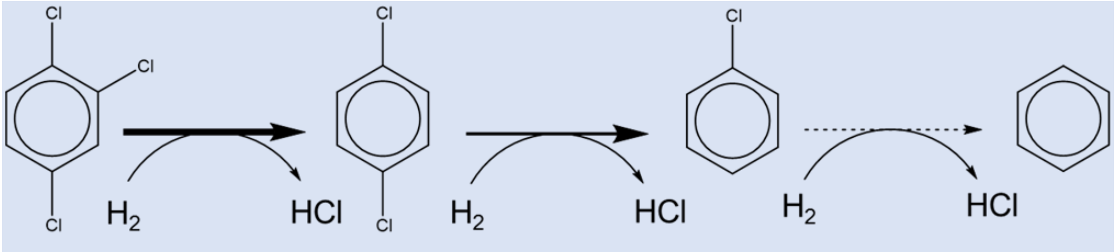


1. Coupled Anaerobic – Aerobic Biodegradation

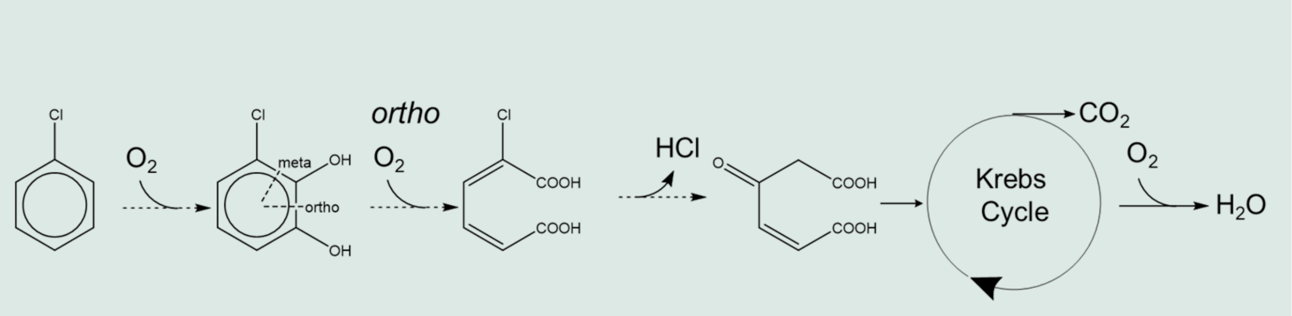
Dual-Biofilm Reactive Barrier for Treatment of Chlorinated Benzenes at Anaerobic-Aerobic Interfaces (NIEHS R01ES024279 & EPA Region III)

- Anaerobic reductive dechlorination
  - Highly chlorinated CBs thermodynamically favorable
  - Toxic daughter products remain
  - Mineralization possible, but MCB stall common
- Aerobic oxidation– oxygen-mediated process
  - Less chlorinated CBs favorable thermodynamically (<= TeCB)
  - Complete mineralization

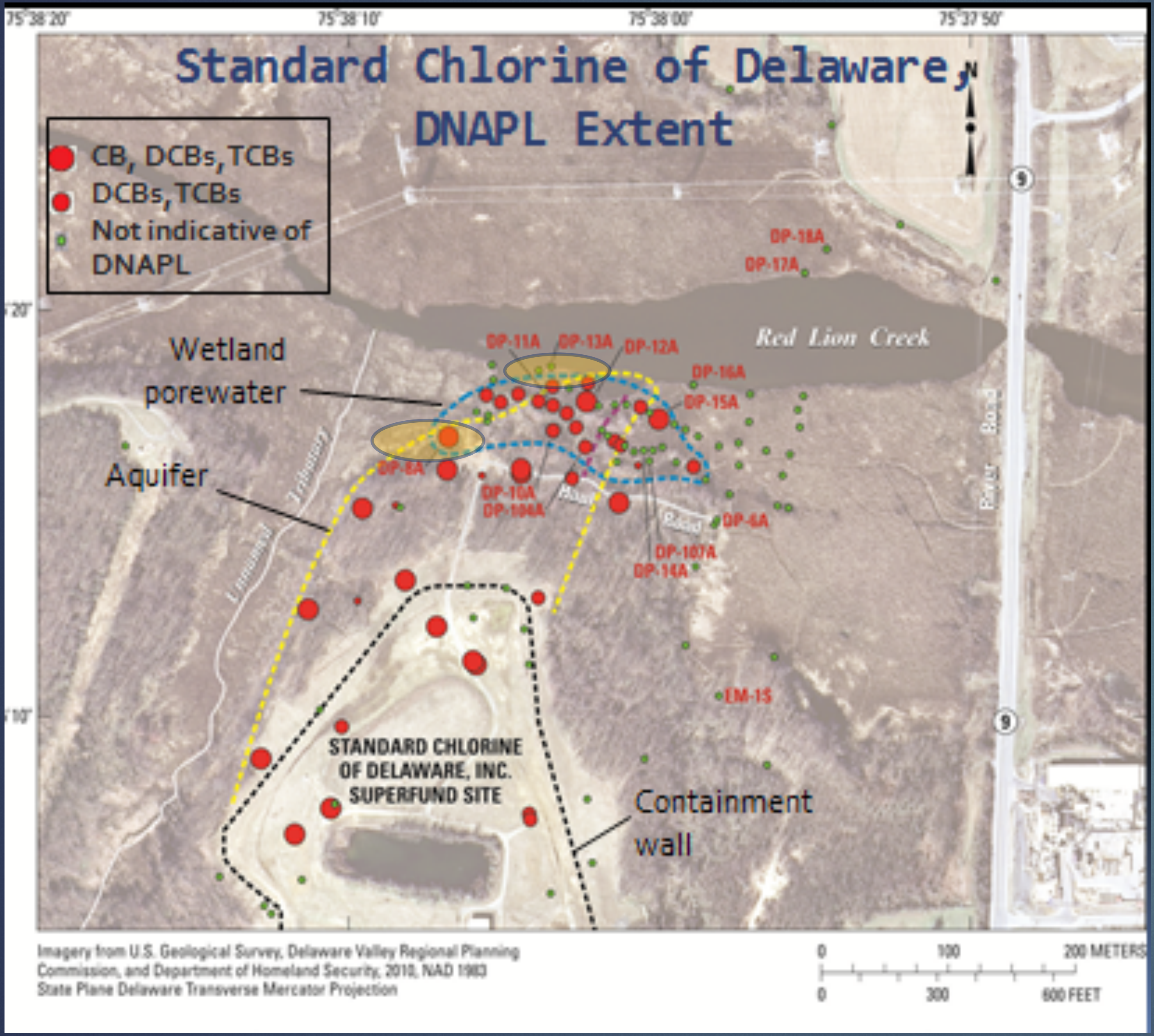
Anaerobic reductive dechlorination



Aerobic oxidation



Fields and Sierra-Alvarez. 2008. Biodegradation

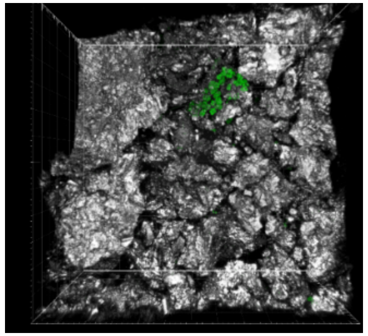


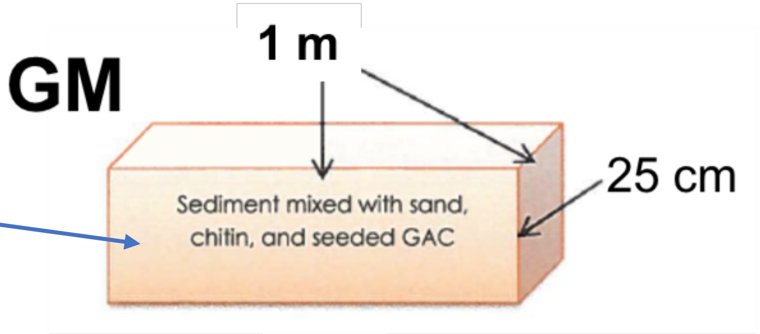
- Chemical plant 1966-2002
- 1986 tank failure released >500,000 gal 14DCB and TCBs that flowed over wetland
- Superfund site 1987; EPA 2002
- Underlying DNAPL-contaminated aquifer
- Abuts Red Lion Creek, part of Delaware River watershed

Permeable Reactive Bio-barrier

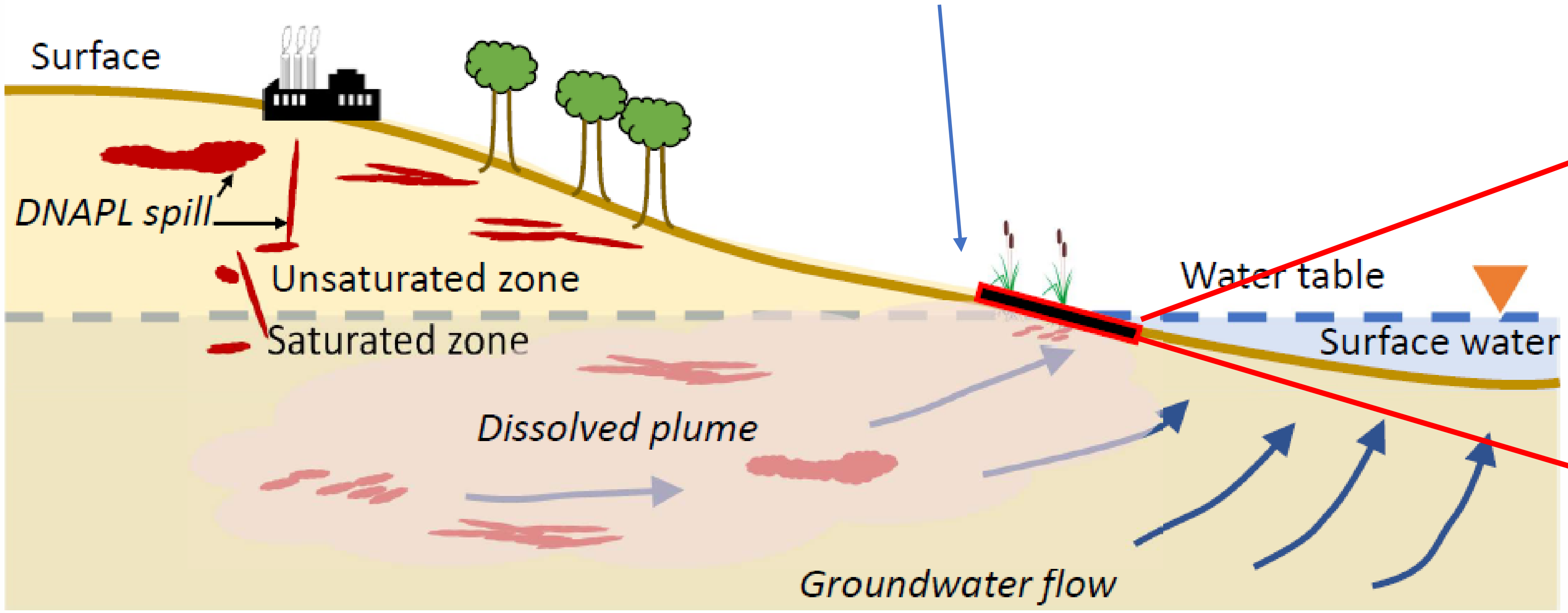
- Intercept discharge and treat passively
- Use GAC to add cultures and favorable growth matrix
- Sorb with GAC and allow more time for biodegradation
- Leverage natural mixed anaerobic-aerobic conditions

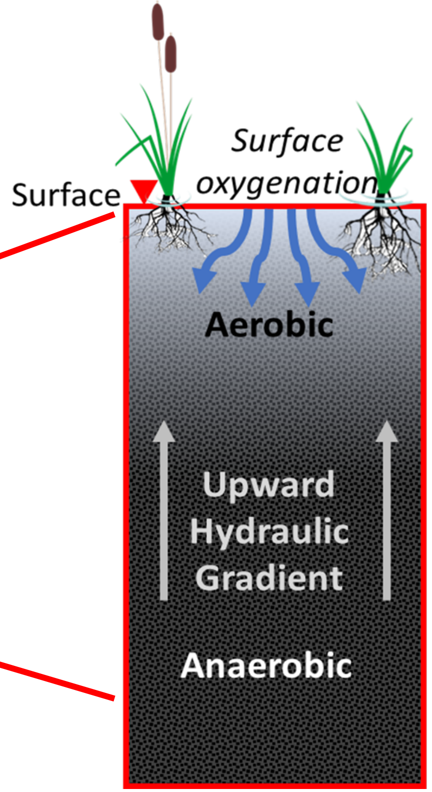
Anaerobic and aerobic cultures bioaugmented on granular activated carbon (GAC)



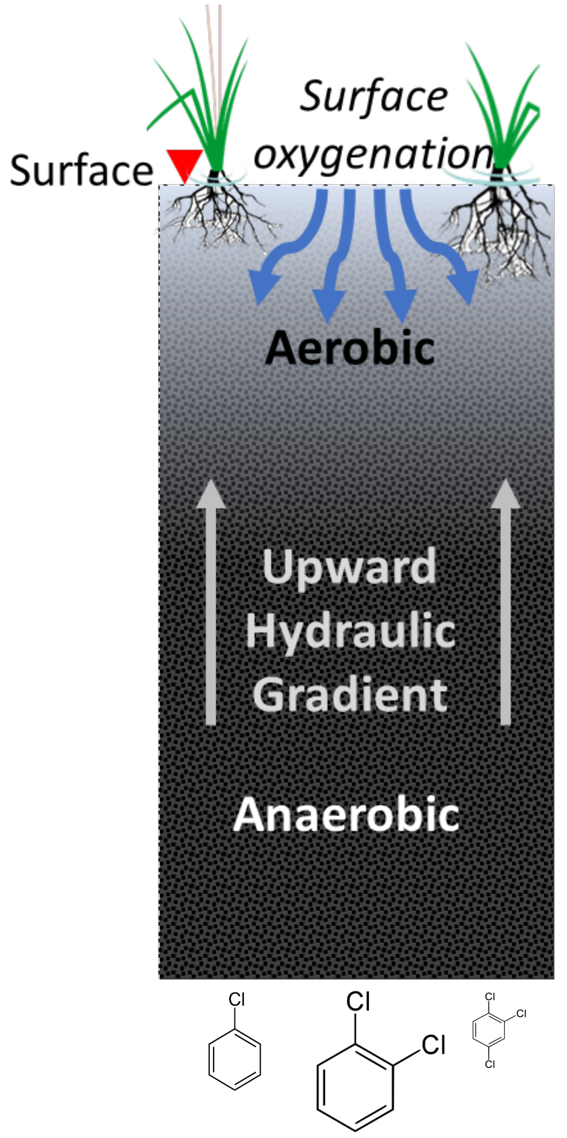


Use granular activated carbon (GAC) for bioaugmentation





Bench-scale column studies: Simulate anoxic-oxic interface

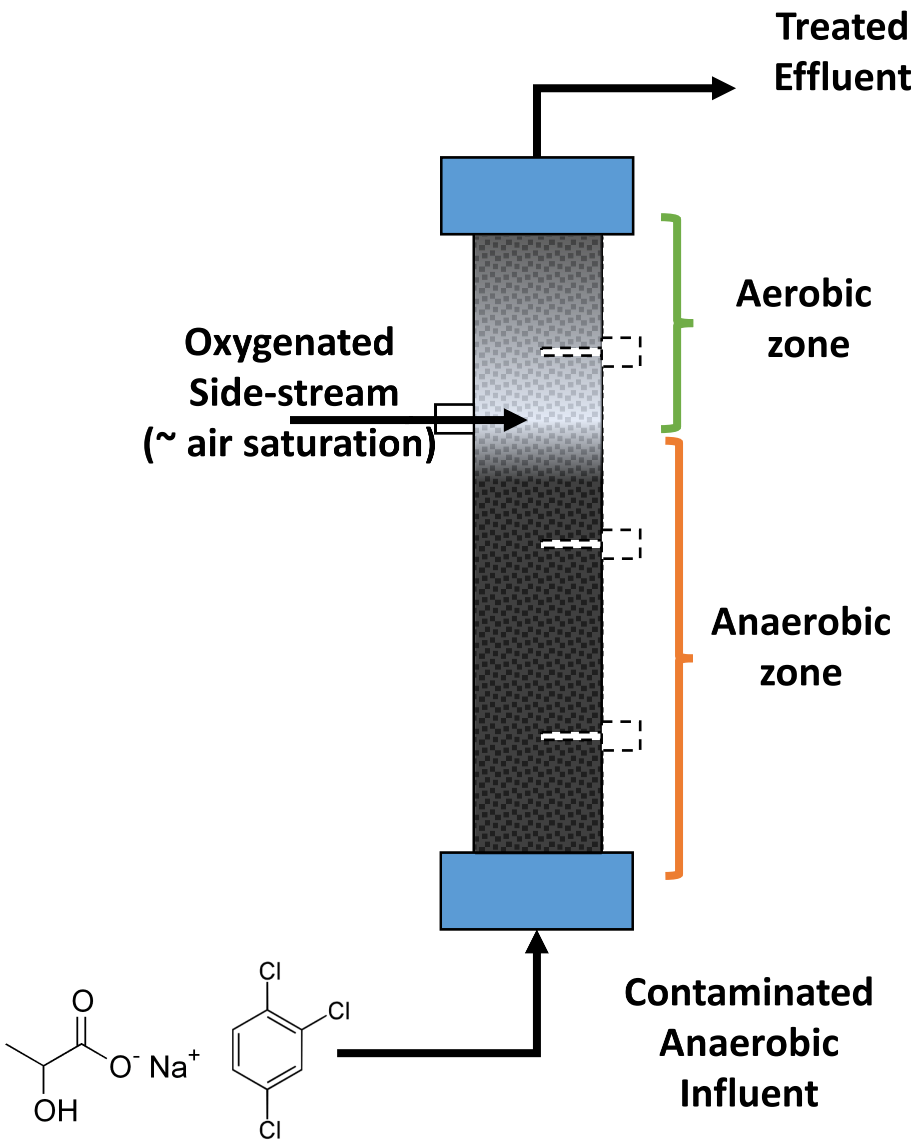


Conceptual model

Simplifications

- Isolate biodegradation activity – no GAC amendment
- Defined, constant media composition and flux
- Inoculate with microbial enrichments:
  - Anaerobic – WBC-2
  - Aerobic – enrichment from site water
- Model contaminant:

  1,2,4-trichlorobenzene

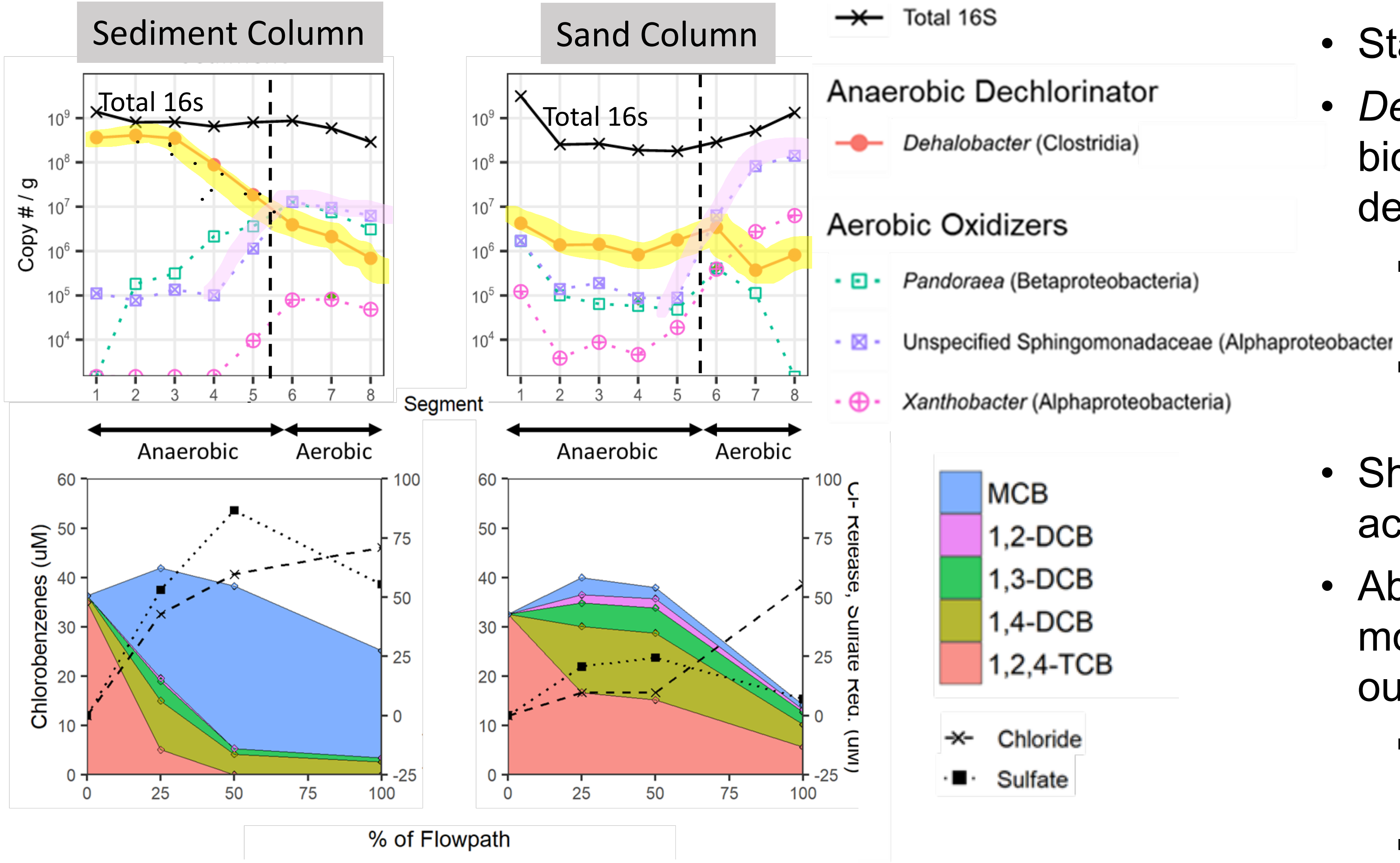


Experimental design

(Chow et al., 2020, J Contaminant Hydrology, https://doi.org/10.1016/j.jconhyd.2020.103639)

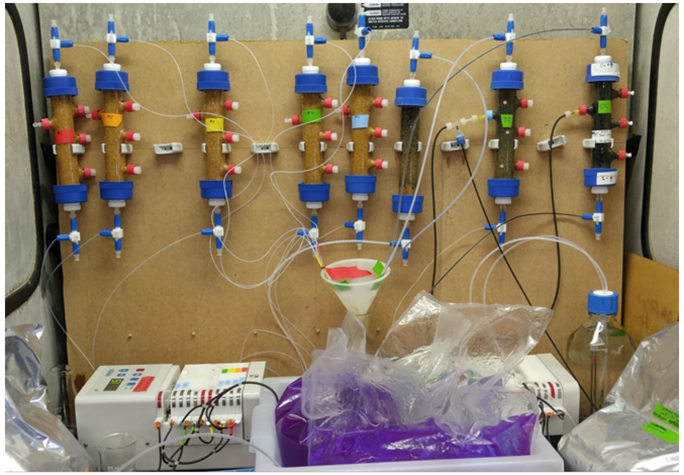
Stratification of Putative CB Degraders along oxic-anoxic interface

Next generation 16s rRNA sequencing [Relative abundance × total 16S gene copy #]

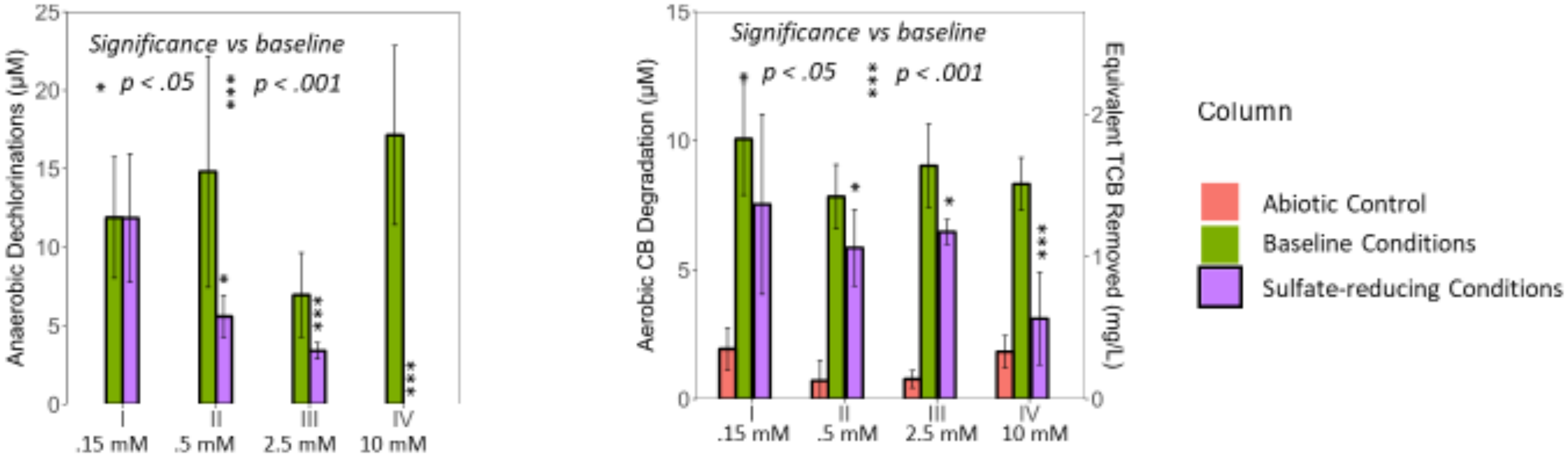


- Stable cell density in sediment.
- *Dehalobacter* enriched in biofilm as anaerobic dechlorinator
  - High enrichment (up to 50%) in sediment column
  - Low enrichment (<1%) in sand column
- Shift in degrading populations across interface
- Abundances corresponded to more favorable degradation outcomes
  - Sediment – reductive dechlorination
  - Sand – aerobic degradation

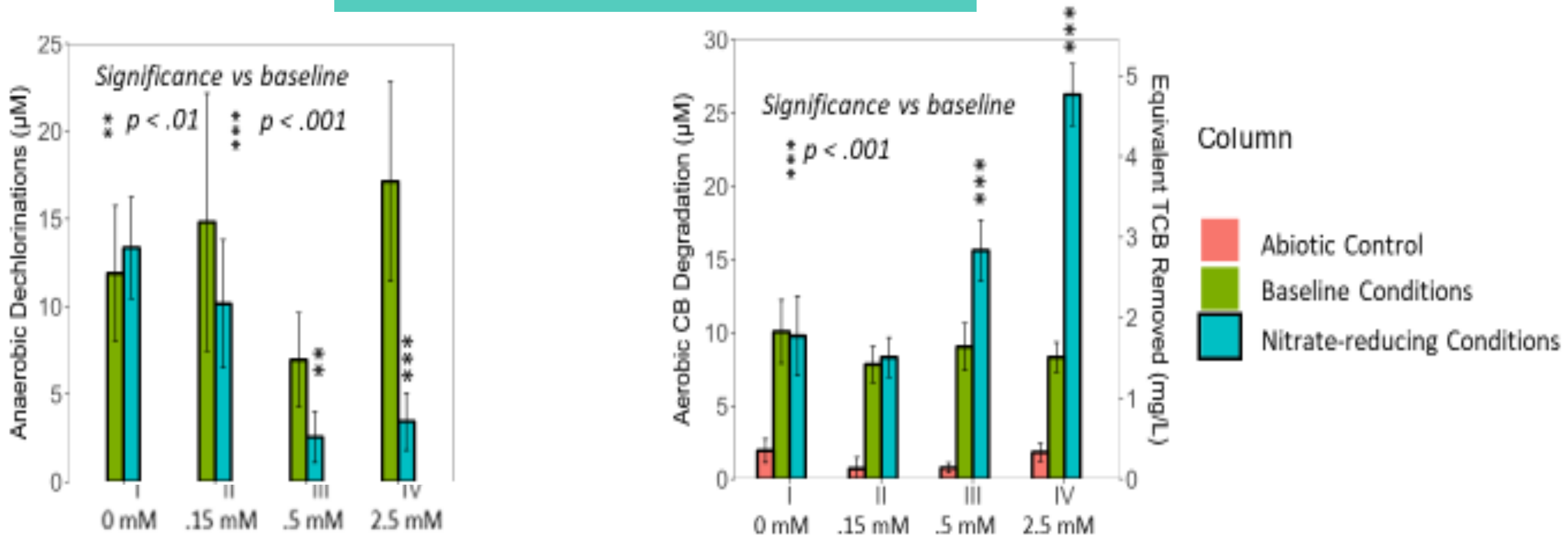
Alternative Redox Conditions- year-long experiment



Sulfate-reducing conditions



Nitrate-reducing conditions

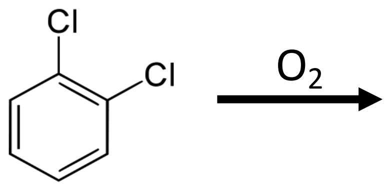


Reductive Dechlorination **Aerobic Oxidation**

- SO_4^{2-} negatively impacted reductive dechlorination; reduced S^- downgradient negatively impacts aerobic degradation

- NO_3^- negatively impacted reductive dechlorination; enhanced aerobic degradation, serving as sink for competing e^- donors

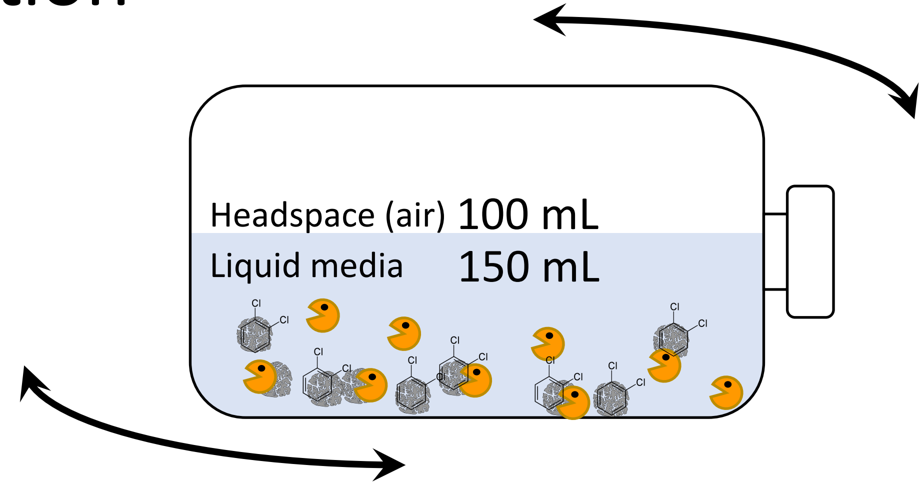
GAC Sorption Effect on Aerobic Biodegradation

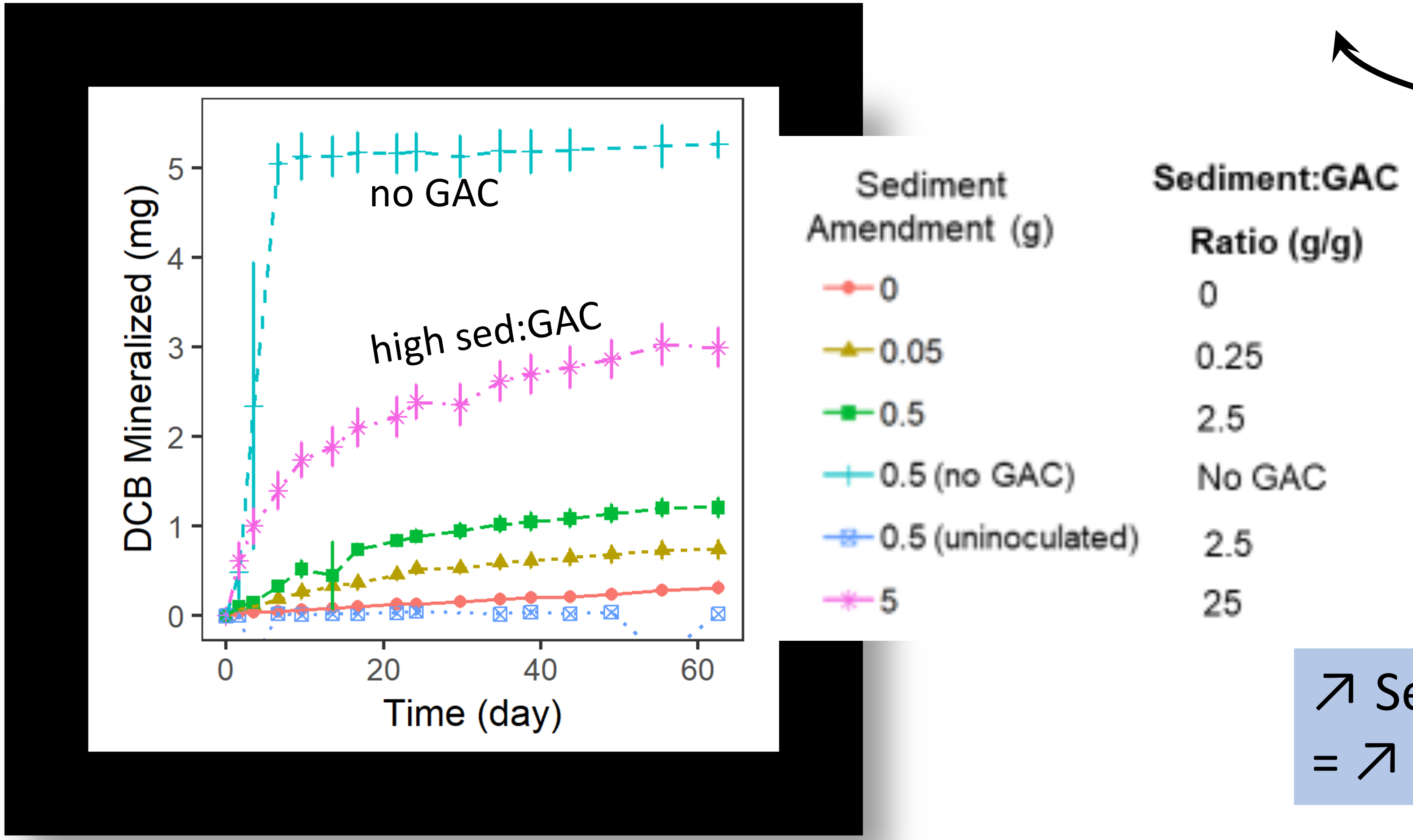


$2\ H^+$

$\mathbf{2\ Cl^-}$

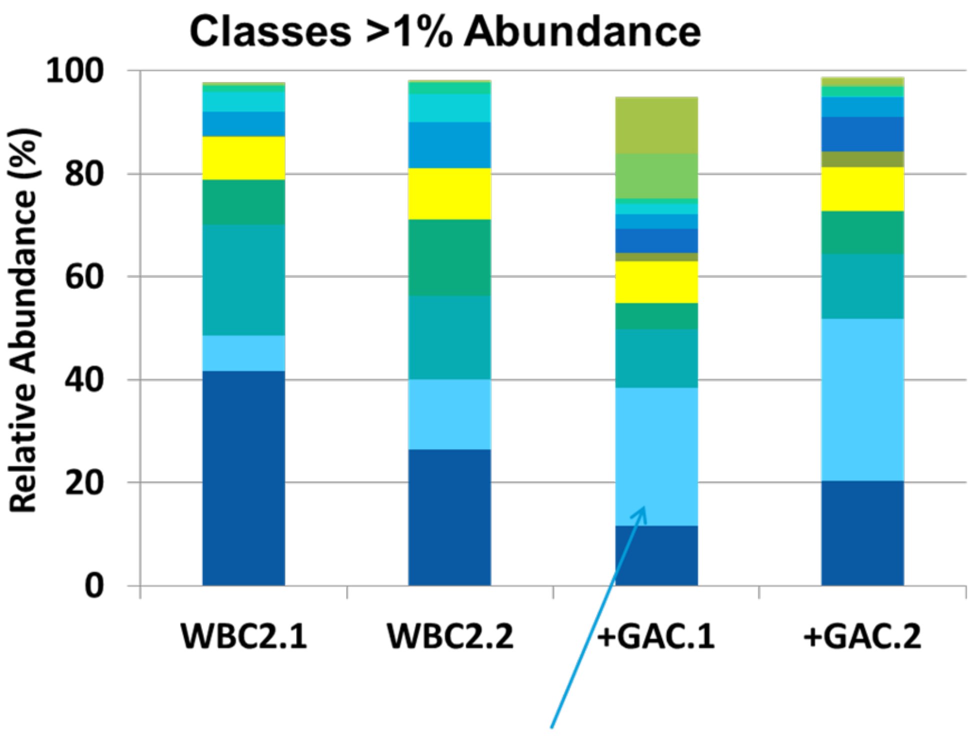
Biodegradation = ½ $\Delta\ Cl^-_{aq}$





↗ Sediment:GAC
= ↗ Biodegradation Extent + Rate

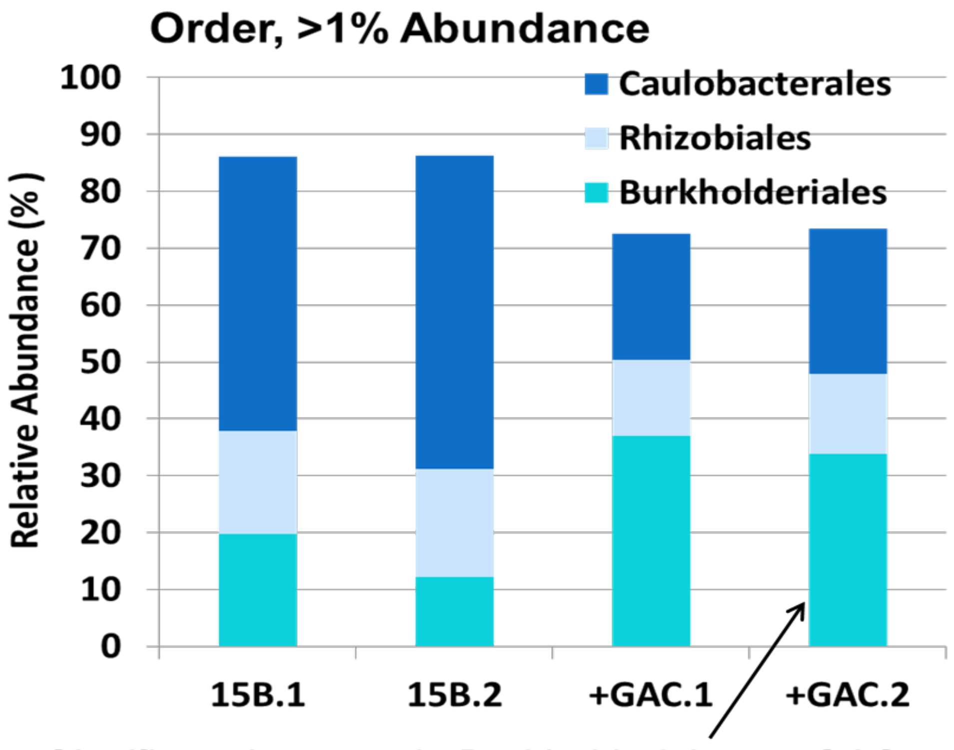
Pilot Test Preparation: GAC Effect on Culture Growth



Significant increase in Dehalococcoidales on GAC.

f__Dehalococcoidaceae;g__Dehalococcoides
f__Dehalococcoidaceae;g__Dehalogenimonas

Anaerobic Culture WBC-2



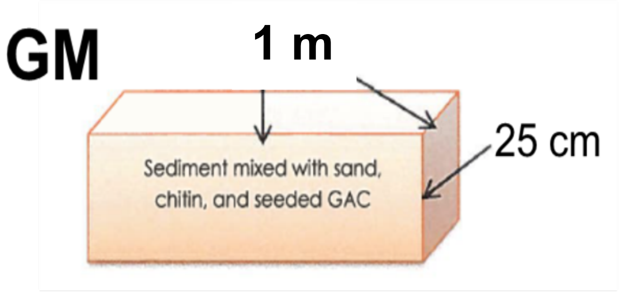
Significant increase in Burkholderiales on GAC.

f__Alcaligenaceae;Other
f__Alcaligenaceae;g__
f__Comamonadaceae;g__Comamonas

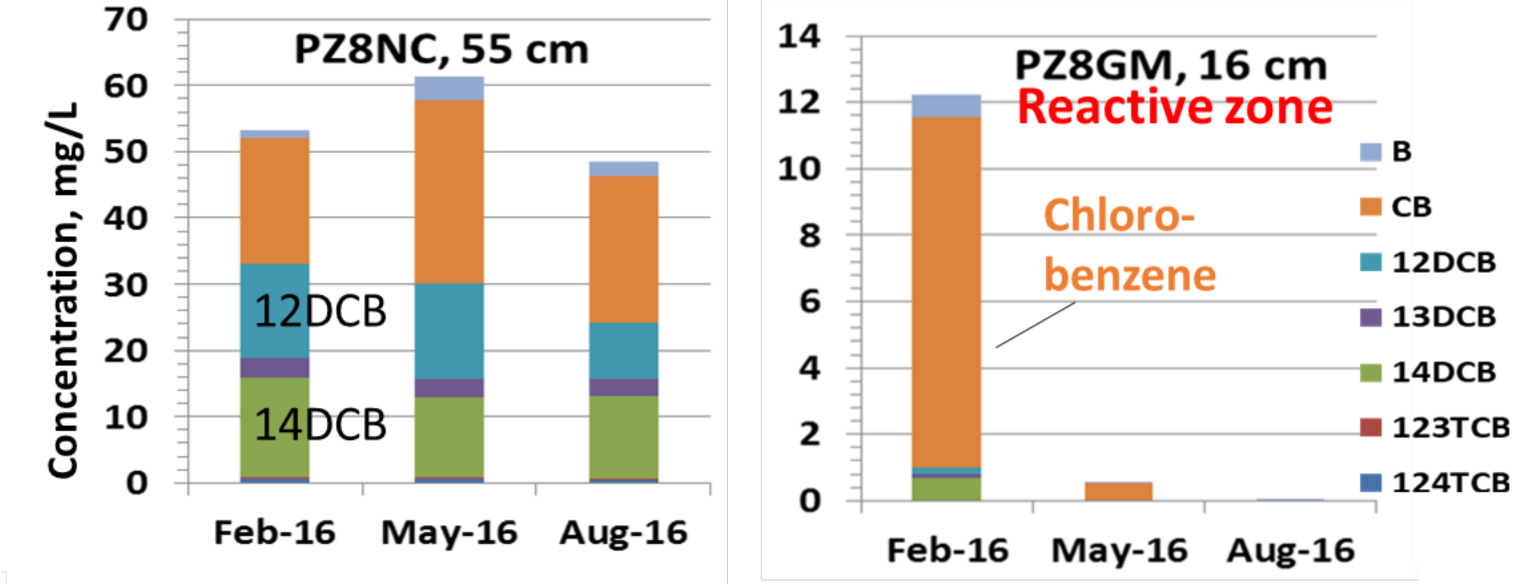
Aerobic Culture (Native, 15B)

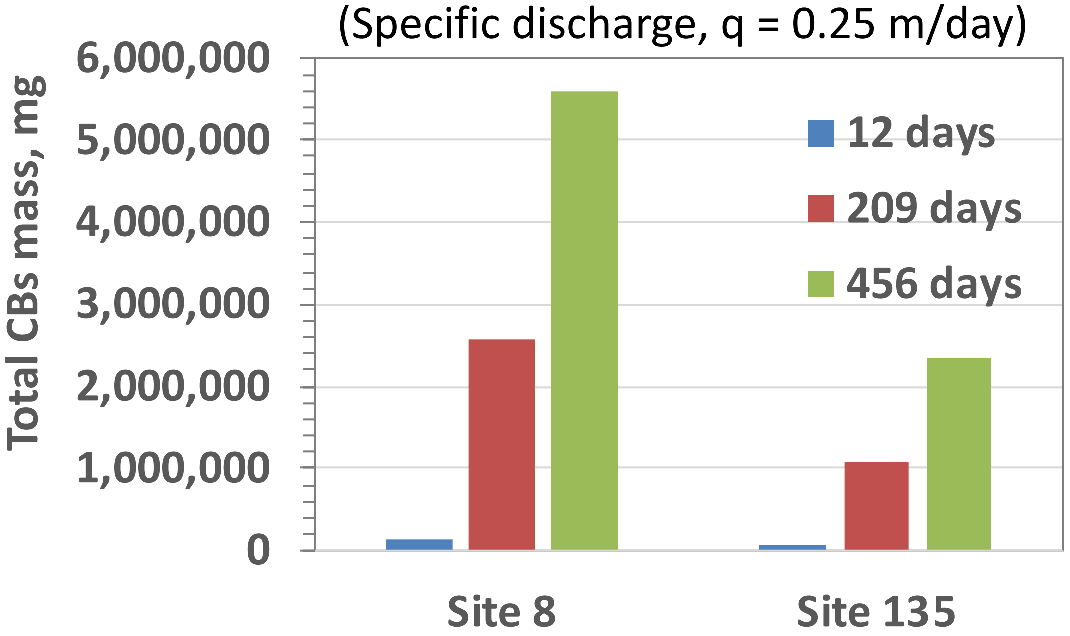
Field Pilot Test

Vol %	GAC	Chiten	Sand
GM	5	3	12



Total CB groundwater concentrations



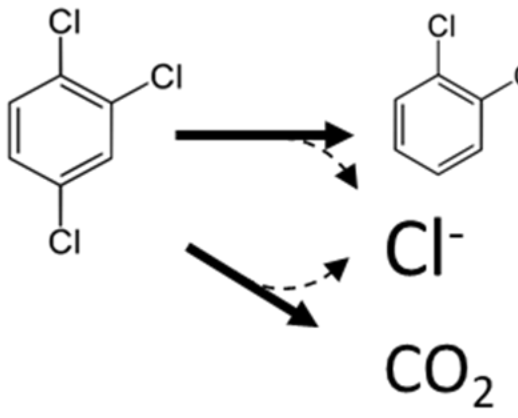


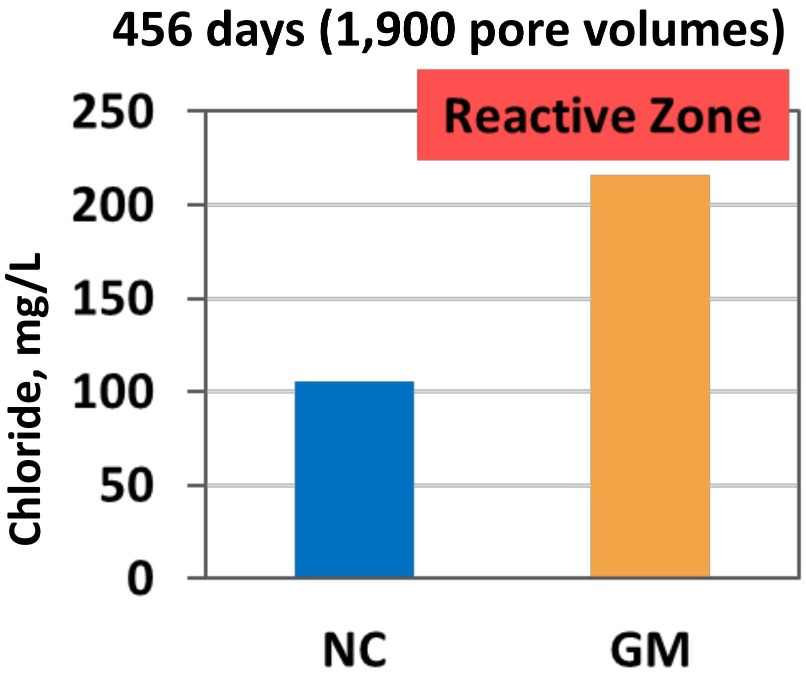
Cumulative CB mass influx

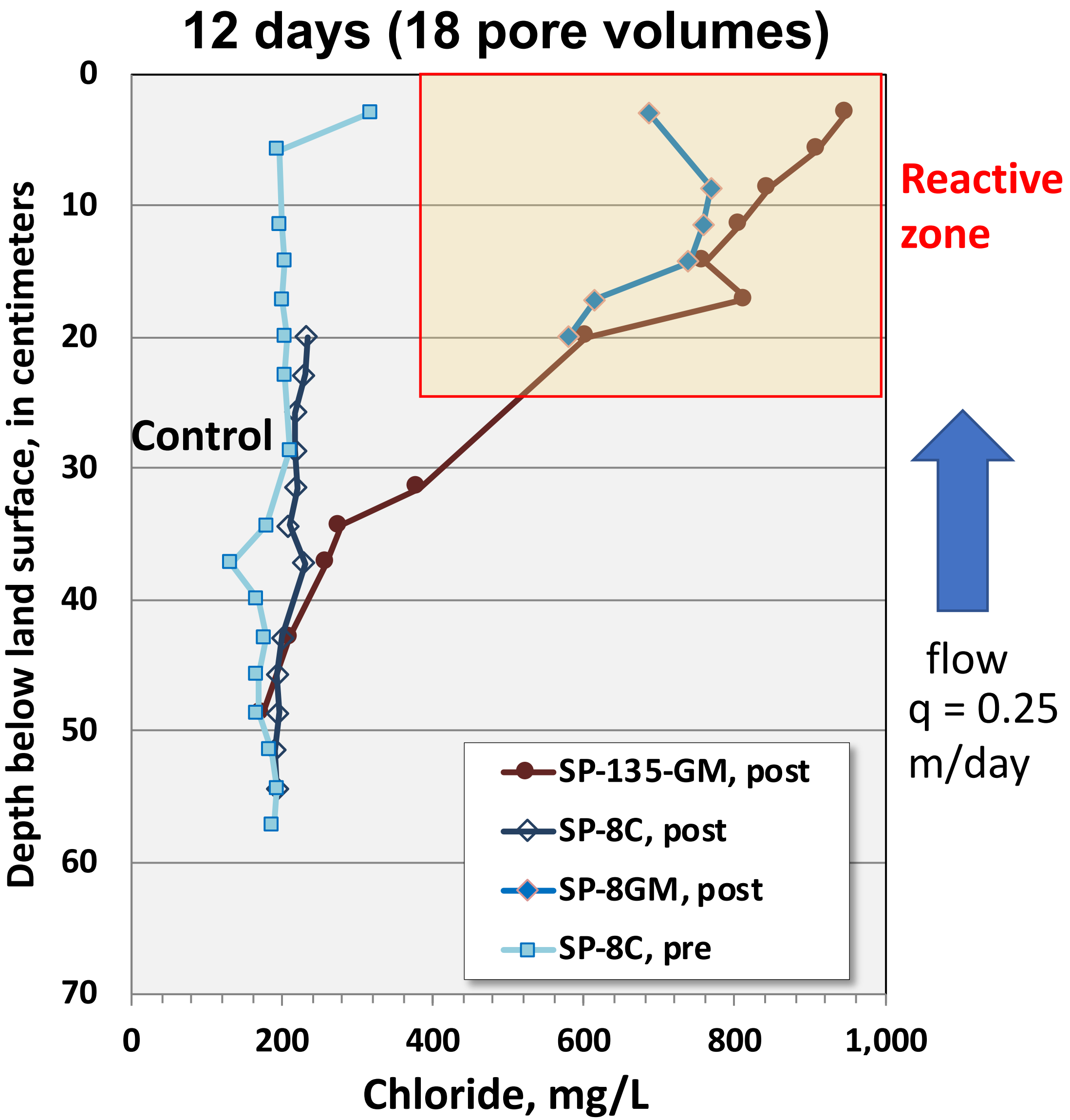
- mass CBs contributed from groundwater influx is **1,000x** greater than sediment mass
- non-detect near surface of the reactive barriers
- biodegradation rate is at least equal to groundwater flow rate

Pilot Test: Chloride

Chloride increased by **factor of 2 to 5** in reactive zone, showing CB degradation was a major removal mechanism.

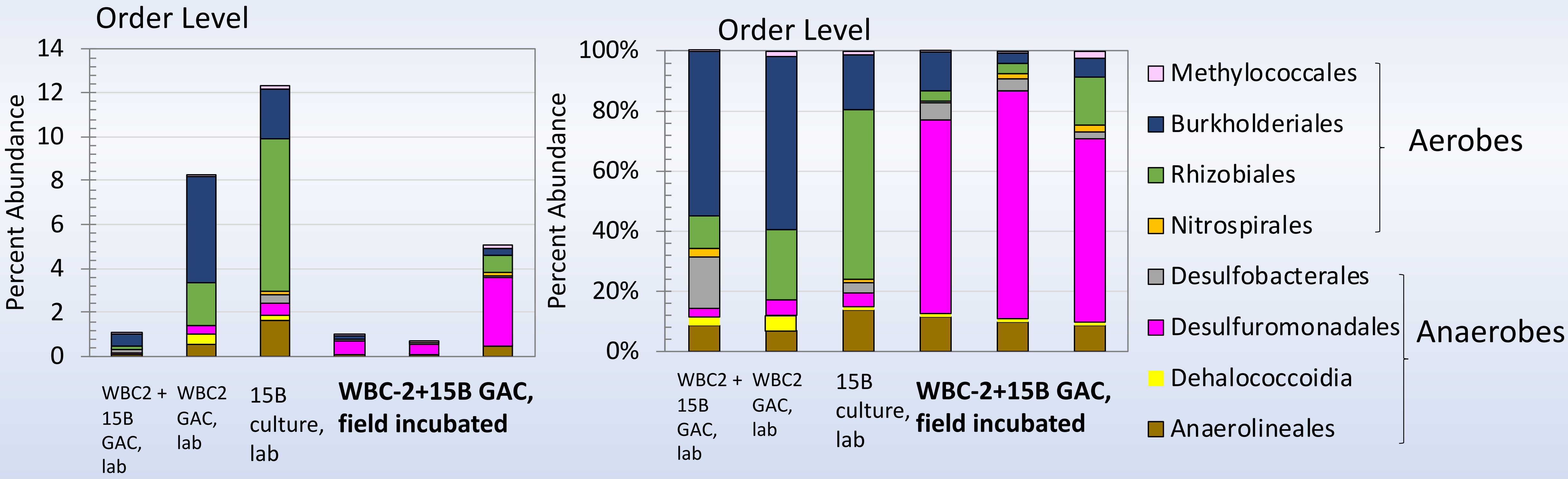






All data are provisional.

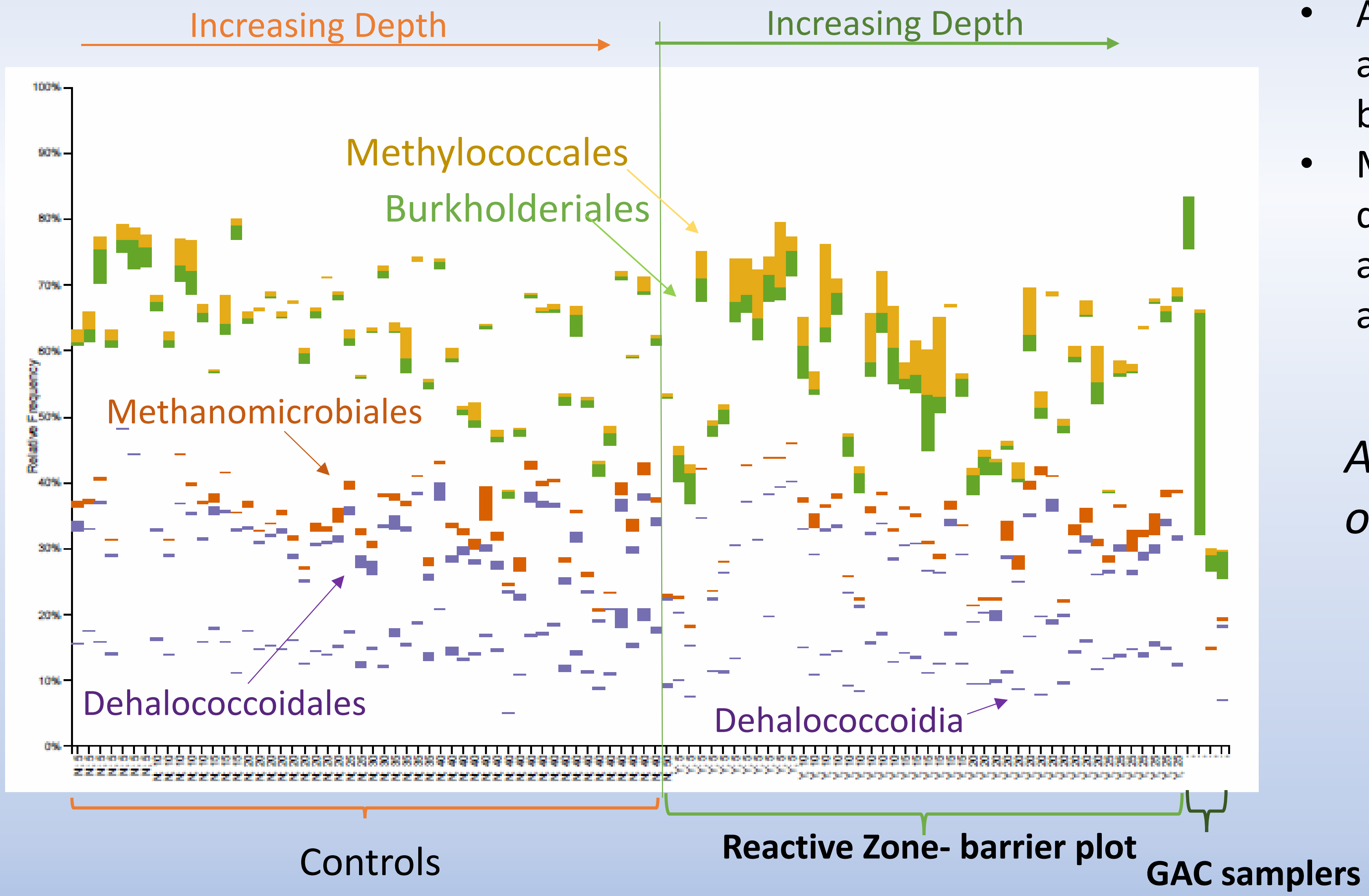
Field-Incubated and Lab-Incubated GAC: Microbial Community



- Burkholderiales- aerobic degraders in 15B culture and in GAC in lab and field
- Desulfuromonadales- anaerobes in WBC-2 culture that increased in abundance on field incubated GAC
- Dehalococcoidia- consistently present in low percent abundance in field incubated GAC

All data are provisional.

Sediment Samples Taxonomy – Percent abundance by depth for select orders (control vs. **reactive** plots)



- Aerobes remained relatively abundant at depth in the reactive barriers compared to the controls.
- Methanogens and anaerobic dechlorinators increased in abundance with depth in control and reactive barrier plots.

Anaerobes and aerobes overlap at all depths.

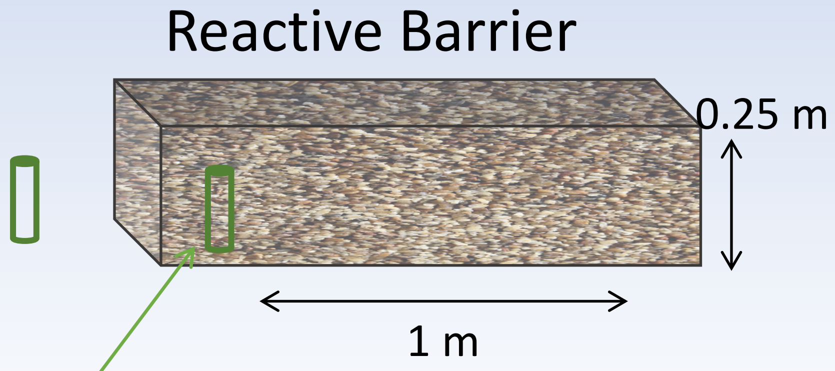
All data are provisional.

In Situ Microcosms

Bio-Traps (Microbial Insights), with and without Biosep beads pre-loaded with ^{13}C-labeled monochlorobenzene.

- Measure incorporation of ^{13}C in CO_2 and PLFA
- Analysis of anaerobic and aerobic functional genes by advanced qPCR to relate microbial presence to degradation ability

Concurrent microbial and isotopic data to verify biodegradation activity.

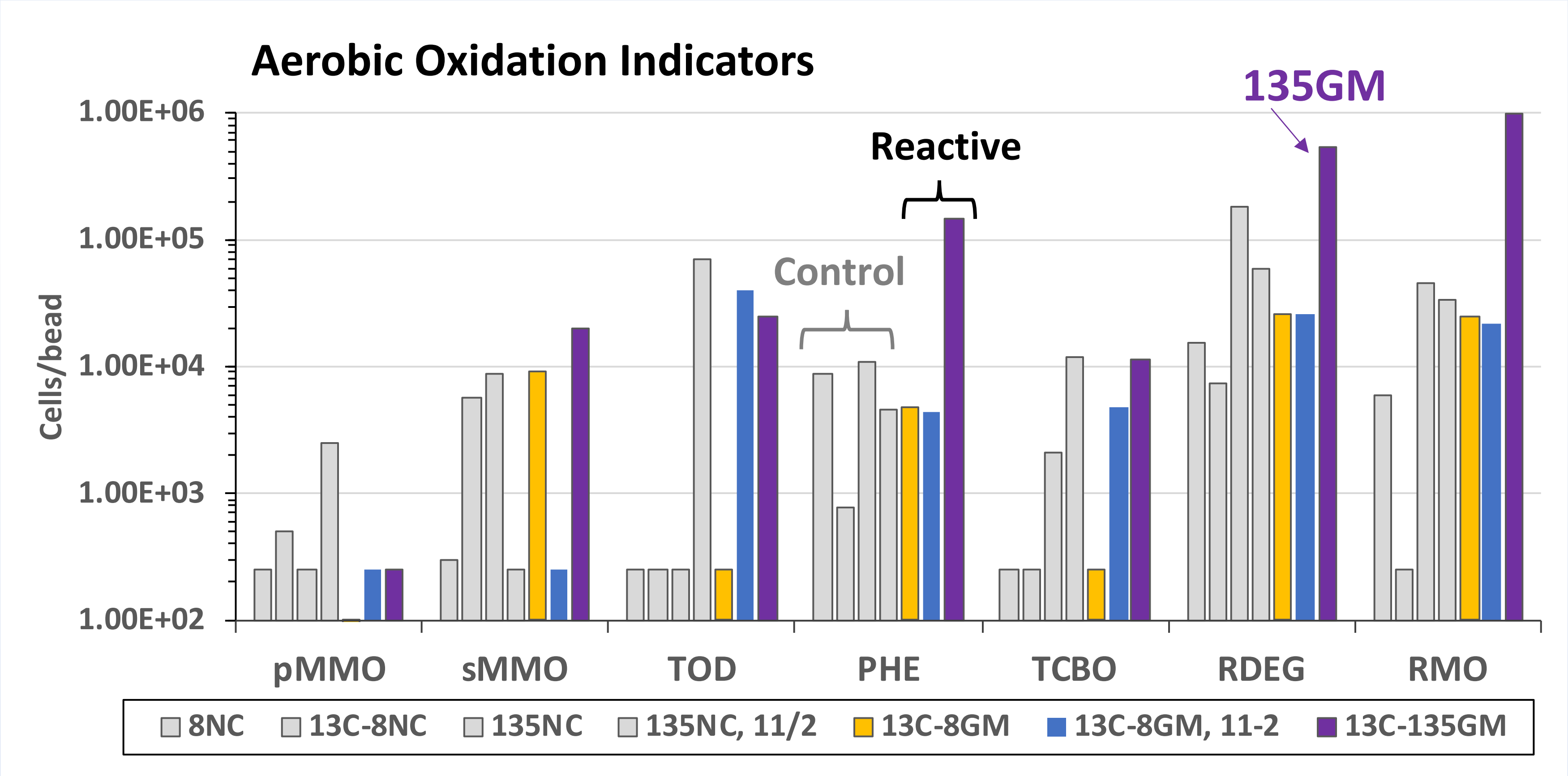


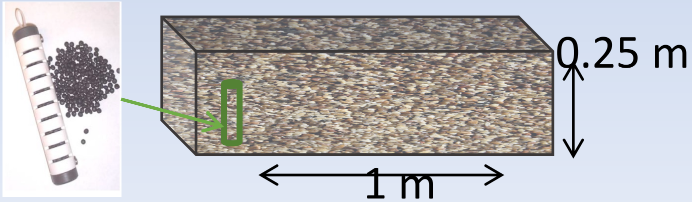
Installed at 10-20 cm bls

- inside and outside reactive barrier plots
- similar depth-integrated microbial sample as the GAC samplers
- time-integrated over 50-day incubation.

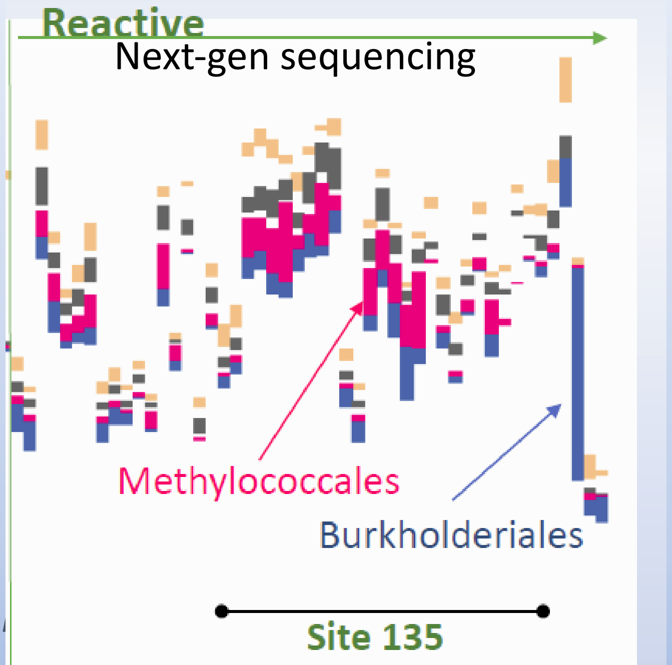
Is biodegradation in the reactive barriers enhanced compared to the control sediment areas, and does aerobic and anaerobic biodegradation co-occur?

In Situ Microcosms and Advanced qPCR: Aerobes





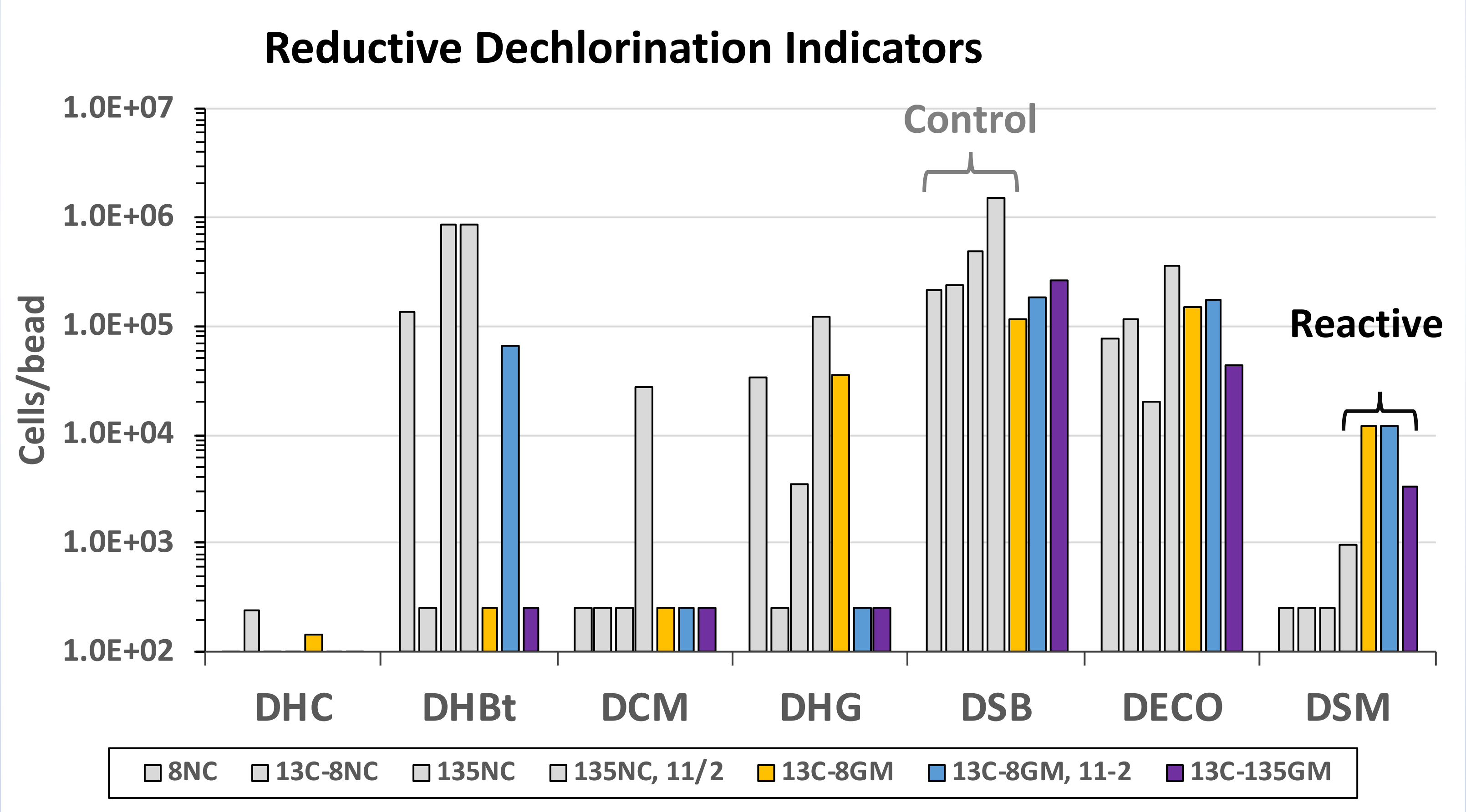
pMMO	particulate methane monooxygenase
sMMO	soluble methane monooxygenase
TOD	toluene dioxygenase
PHE	phenol hydroxylase; benzene monooxygenase
TCBO	trichlorobenzene

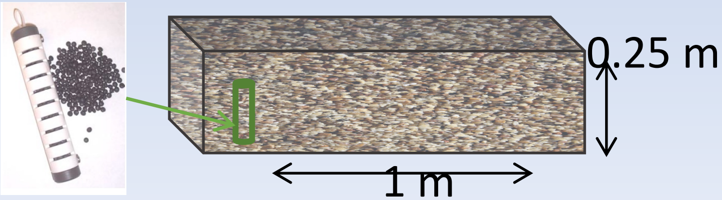


- Aerobic oxidation indicators higher in reactive barrier at site 135 compared to site 8 reactive barrier or controls.
- Agrees with next generation sequencing results.

All data

In Situ Microcosms and Advanced qPCR: Anaerobes





DHC ***Dehalococcoides***

DHBt ***Dehalobacter* spp.**

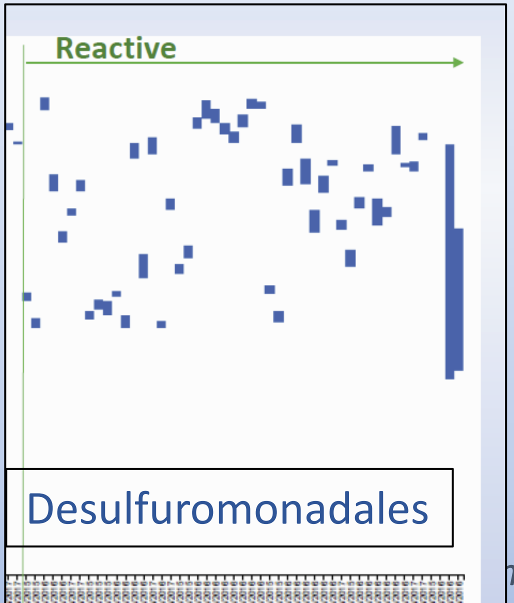
DCM *Dehalobacter* DCM

DHG *Dehalogenimonas* spp.

DSB *Desulfitobacterium* spp.

DECO ***Dehalobium chlorocoercia***

DSM *Desulfuromonas* spp.



- DSM (*Desulfuromonas*) was the only reductive dechlorination indicator that was consistently higher in the reactive barrier plots than controls.
- Indicators of both aerobic oxidation activity and anaerobic reduction present in all ISMs.

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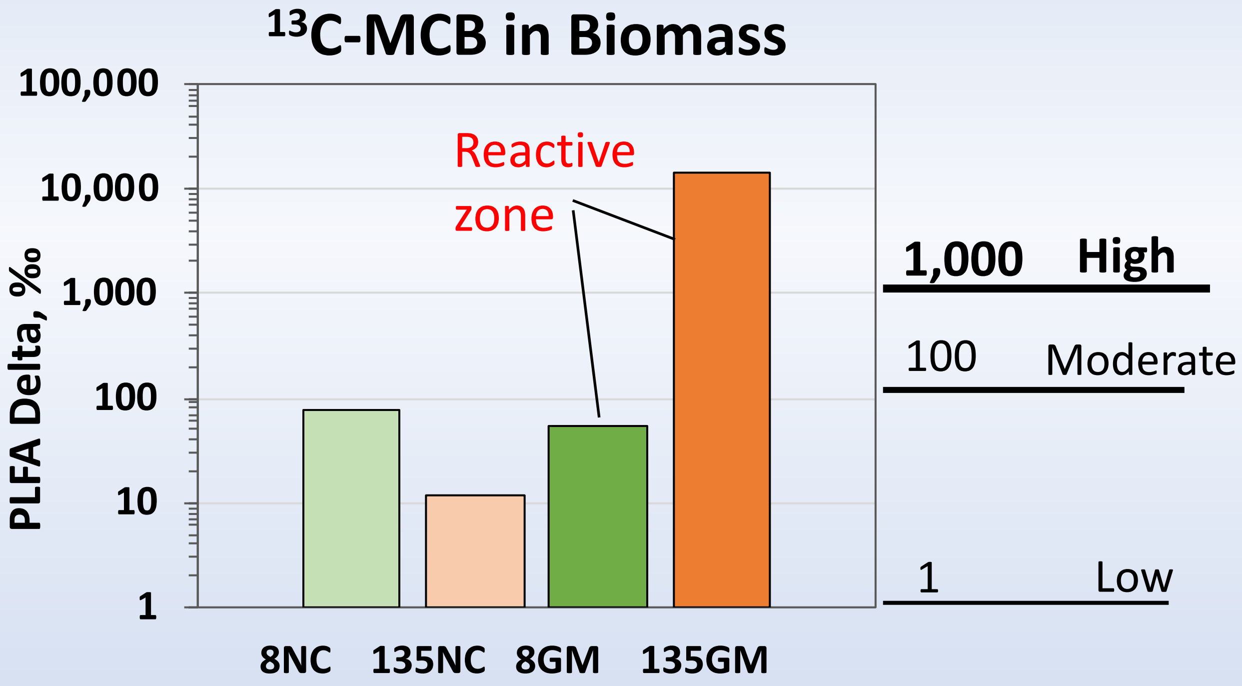
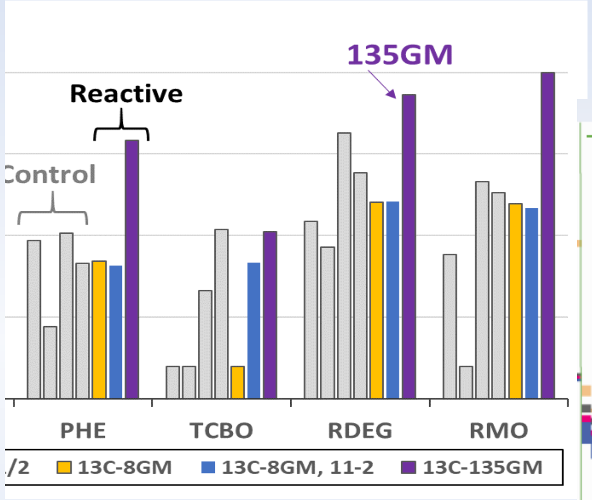
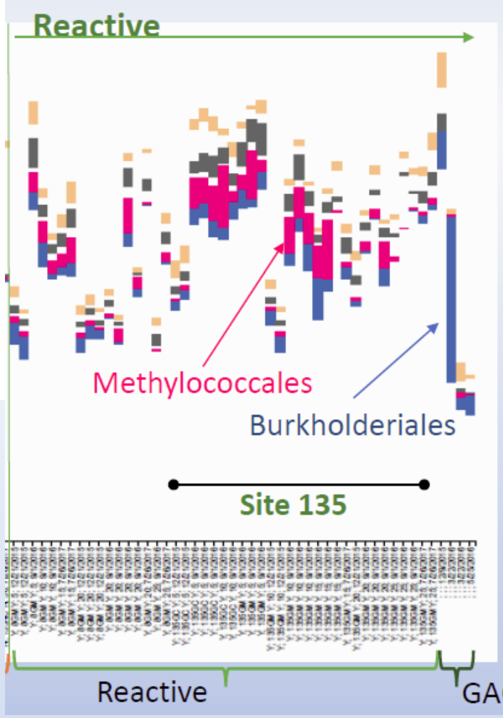
In Situ Microcosms: ^{13}C from Labeled MCB in Biomass

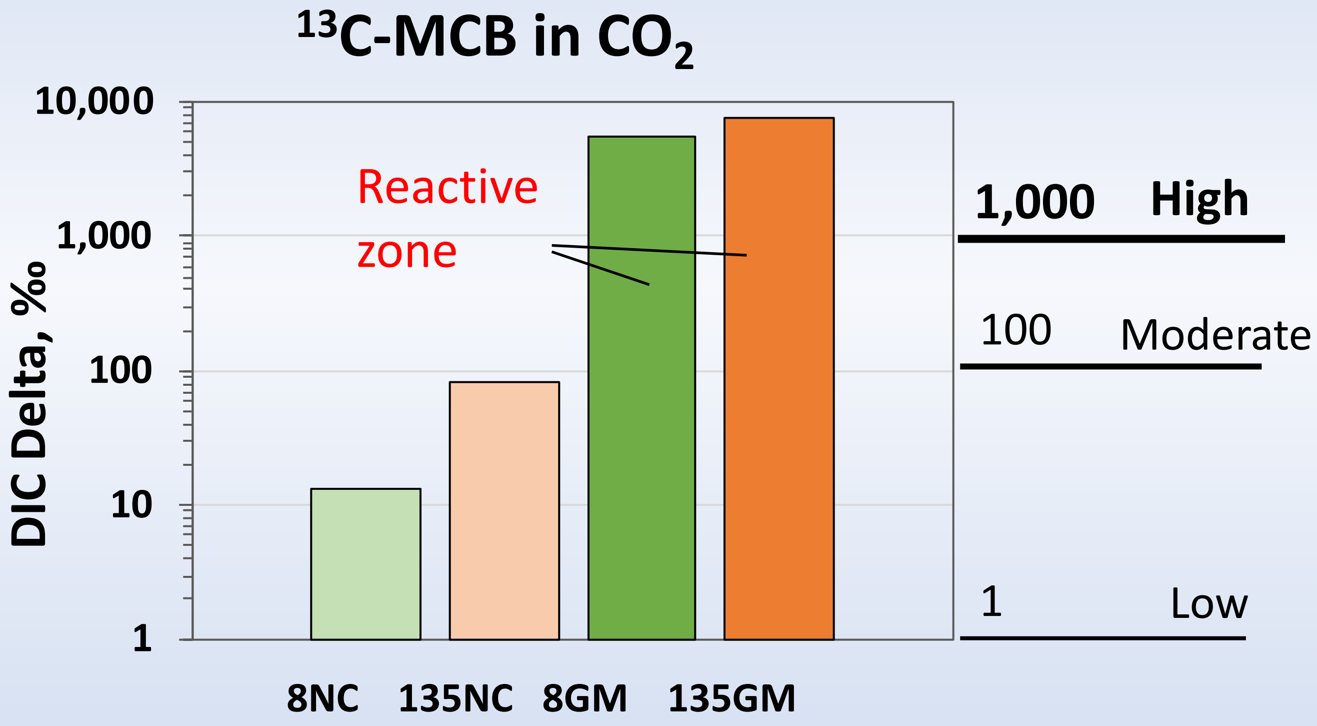






- **High ^{13}C uptake in biomass** (PLFA) in the reactive barrier at site 135 indicates high aerobic oxidation of MCB.
- Agrees with the observed higher abundance of aerobic oxidizers and functional genes at site 135 compared to site 8.

In Situ Microcosms: ^{13}C from Labeled MCB in CO_2



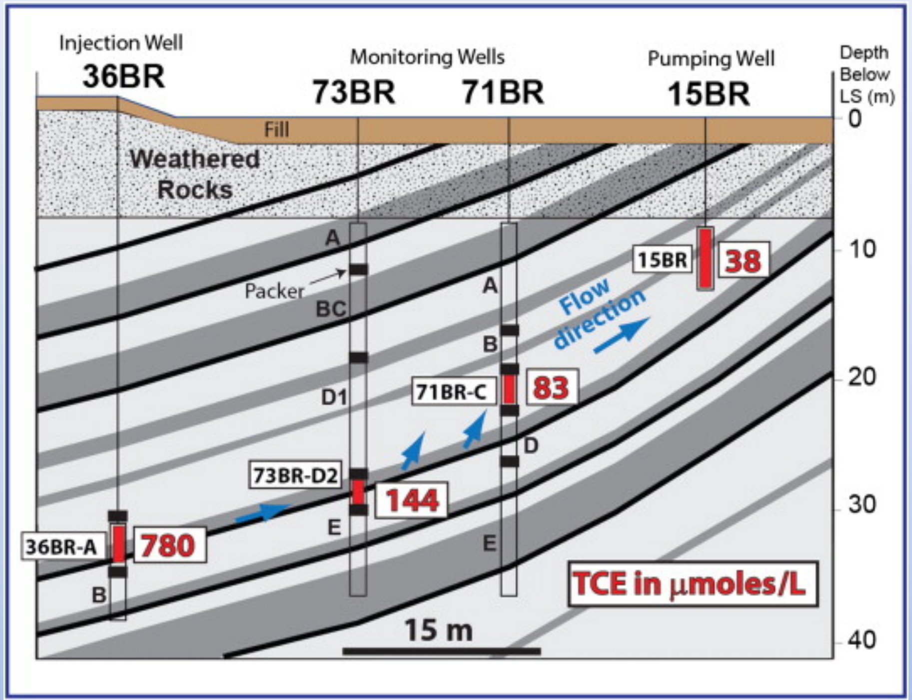
- Incorporation of ^{13}C in CO_2 was **high in both reactive barriers** and low in the controls, verifying complete enhanced biodegradation in the reactive barriers.
- Complete degradation to CO_2 is ~ equal in the two reactive barriers, despite the lower use of MCB as growth substrate at site 8. Indicates a combination of anaerobic (^{13}C for energy) and aerobic biodegradation processes in the reactive barrier.

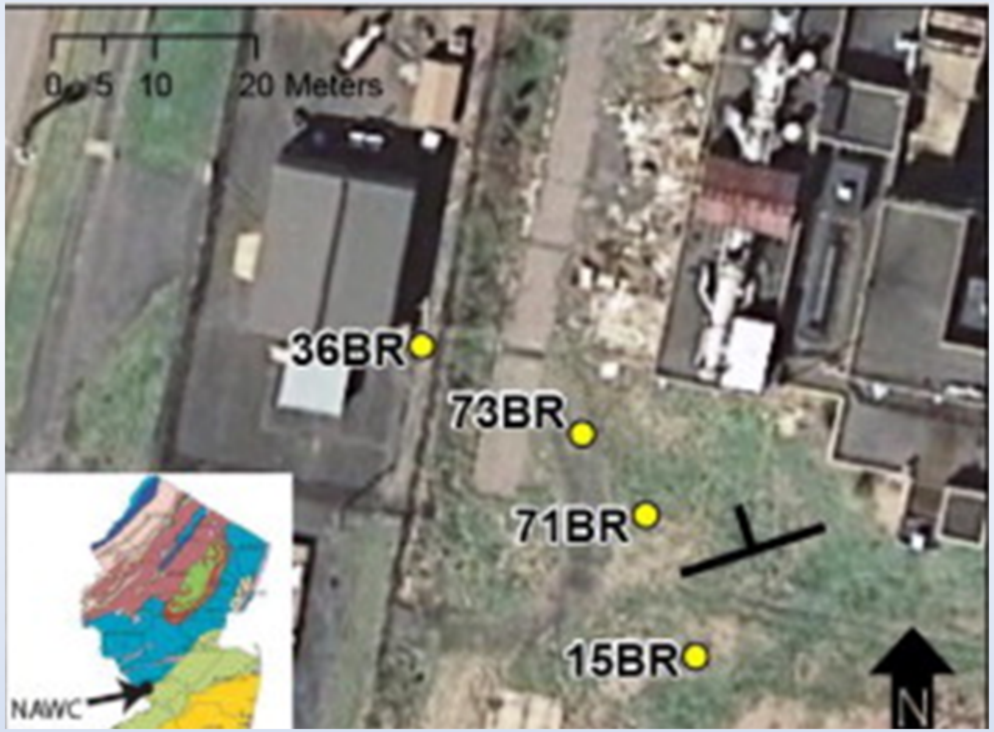
2. TCE in Fractured Rock- Permeable Units

Microbial community dynamics with next-generation sequencing to monitor bioremediation

- Examined archived DNA from prior bioaugmentation pilot (SERDP ER-1555) with next generation sequencing
- Metagenomics to better understand bioremediation effects and "DCE stall"

- *One-time injection of EOS and KB-1 consortium*
- *2008-2015*

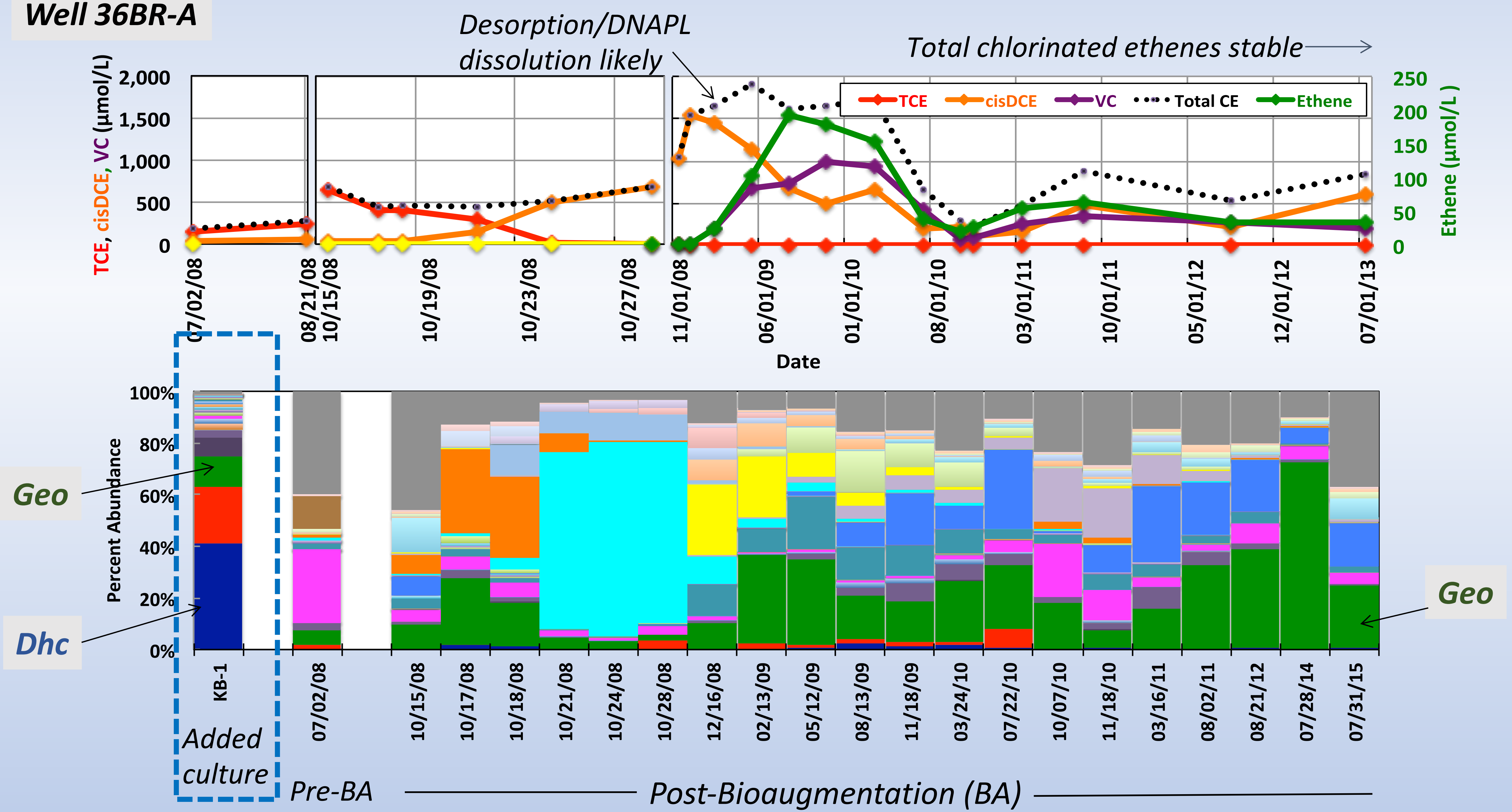




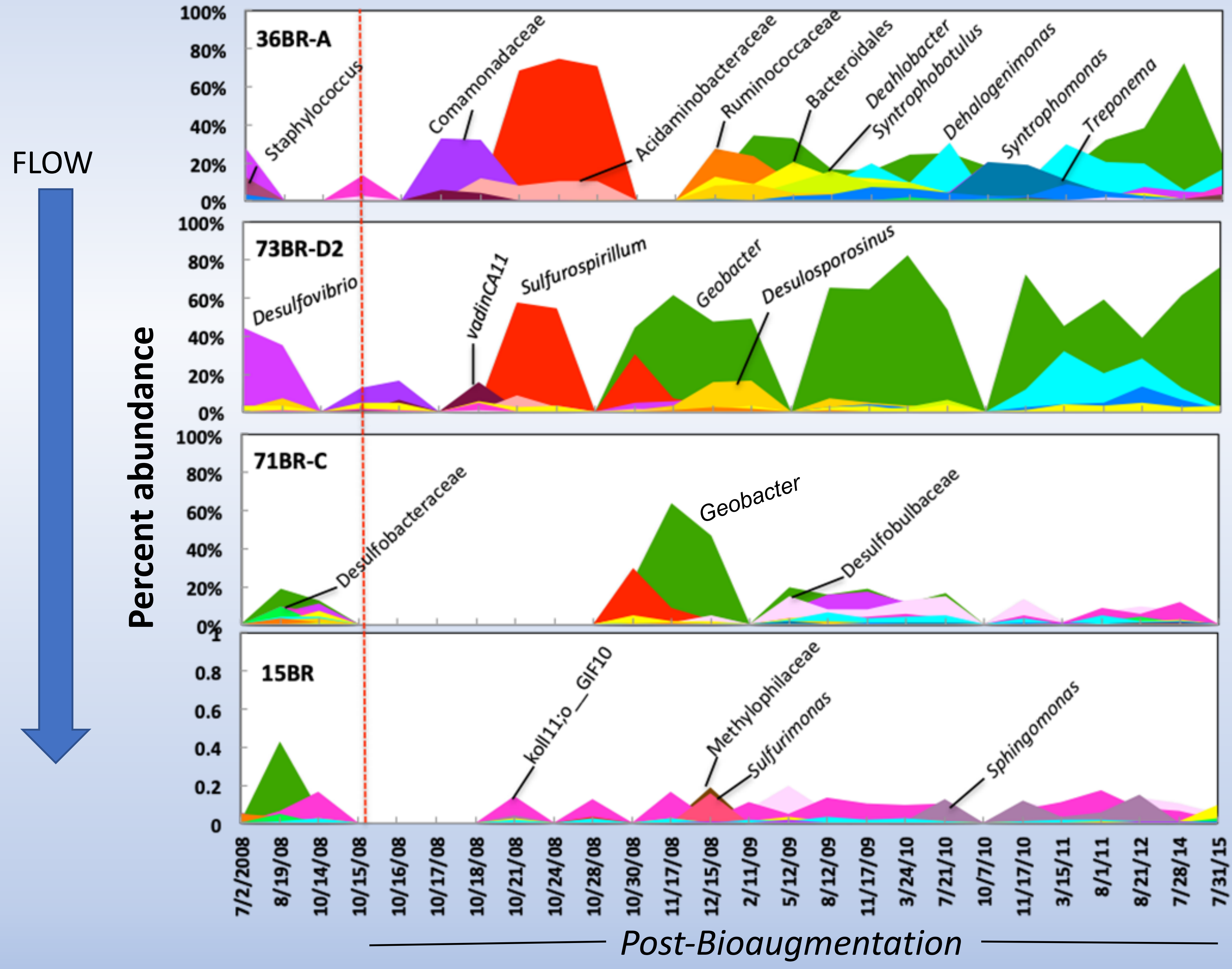
Former Naval Air Warfare Center (NAWC) West Trenton, NJ

- Focus site for USGS research on fractured-rock contamination
- Several SERDP and ESTCP studies

Well 36BR-A



Desorption/DNAPL dissolution likely
Total chlorinated ethenes stable
TCE, cisDCE, VC (µmol/L)
Ethene (µmol/L)
TCE
cisDCE
VC
Total CE
Ethene
Date
Percent Abundance
Geo
Dhc
Geo
KB-1
Added culture
Pre-BA
Post-Bioaugmentation (BA)



Predominant taxa with time along flowpath

- Microbial shifts were observed in downgradient wells 73BR-D2 and 71BR-C, but not in the pumping well, 15BR.
- An increased predominance of *Geobacter* spp. (partial dechlorinators) marked the microbial effects of bioaugmentation.

Shared OTUs

Dehalococcoides

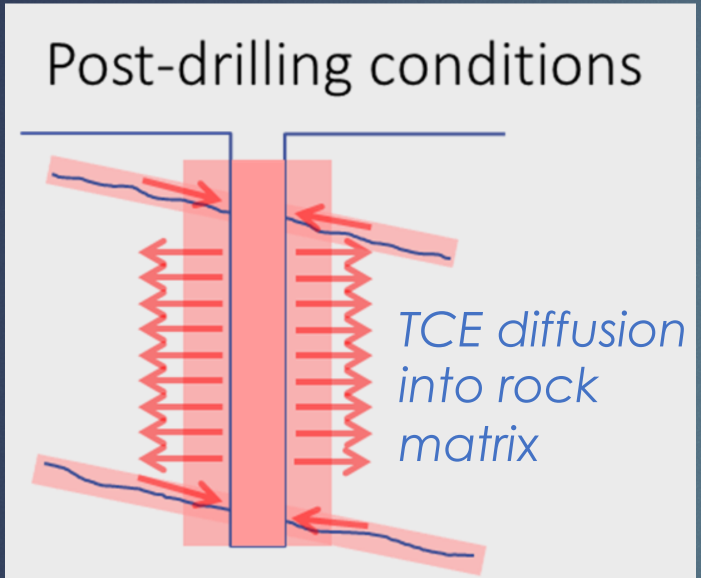
Dehalococcoides OTUs		Pre Bioaugmentation					Post BA (Max OTUs)			
		36BR-A	73BR-D2	71BR-C	15BR	KB-1	36BR-A	73BR-D2	71BR-C	15BR
Pre Bioaugmentation	36BR-A	6	2	4	2	0	4	4	4	3
	73BR-D2	2	36	16	3	1	20	16	18	9
	71BR-C	4	16	245	5	2	71	60	72	29
	15BR	2	3	5	7	0	6	7	5	3
	KB-1	0	1	2	0	534	2	2	2	1
Post BA (Max OTUs)	36BR-A	4	20	71	6	2	405	117	58	14
	73BR-D2	4	16	60	7	2	117	335	54	10
	71BR-C	4	18	72	5	2	58	54	231	32
	15BR	3	9	29	3	1	14	10	32	108

Geobacter

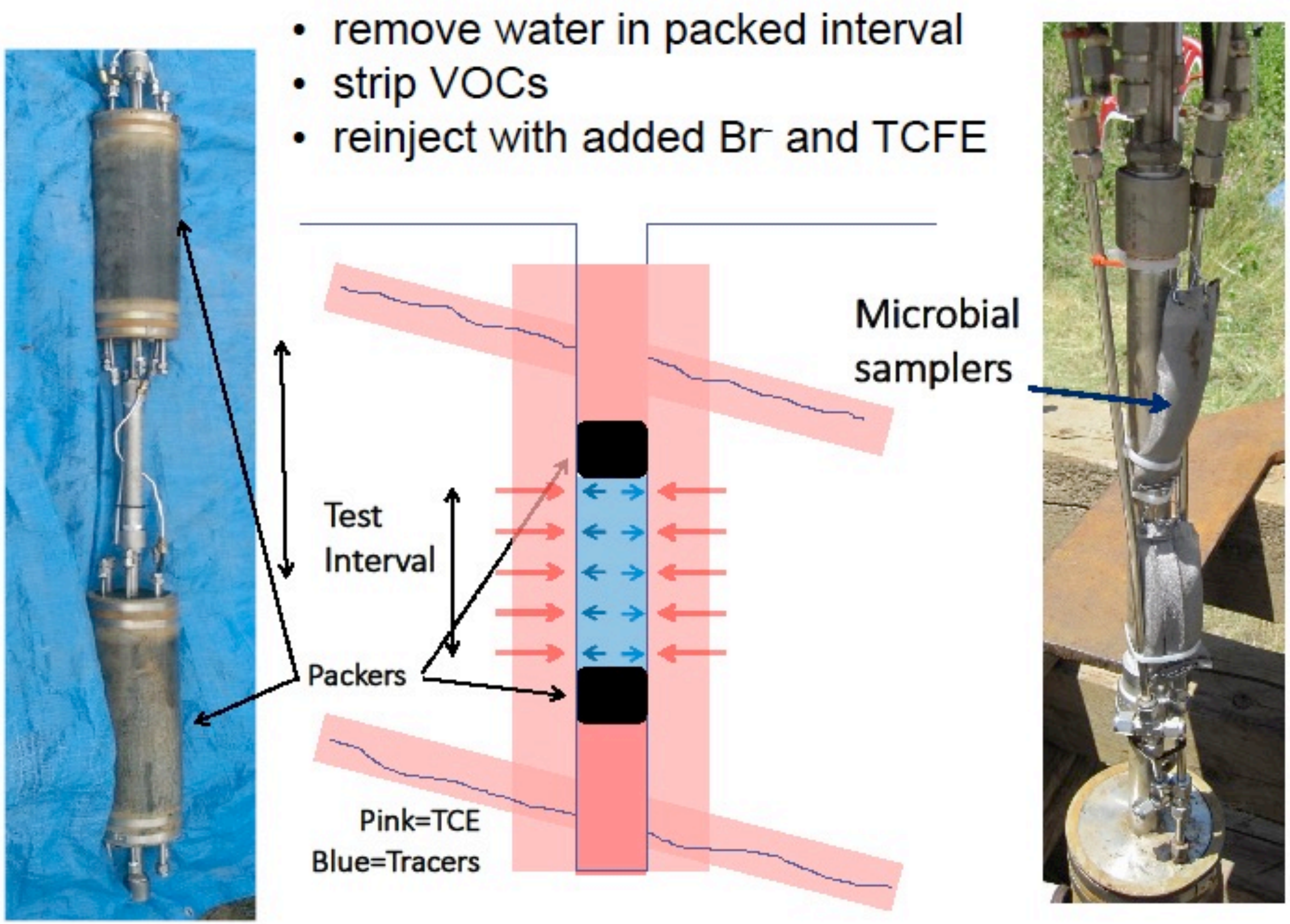
Geobacter OTUs		Pre Bioaugmentation					Post BA (Max OTUs)			
		36BR-A	73BR-D2	71BR-C	15BR	KB-1	36BR-A	73BR-D2	71BR-C	15BR
Pre Bioaugmentation	36BR-A	17	15	15	4	0	15	16	16	11
	73BR-D2	15	126	40	5	0	41	50	41	22
	71BR-C	15	40	198	6	0	49	34	52	34
	15BR	4	5	6	8	0	7	5	5	4
	KB-1	0	0	0	0	32	0	0	0	1
Post BA (Max OTUs)	36BR-A	15	41	49	7	0	188	49	54	29
	73BR-D2	16	50	34	5	0	49	175	43	19
	71BR-C	16	41	52	5	0	54	43	189	33
	15BR	11	22	34	4	1	29	19	33	77

Bioaugmented populations out-competed by native bacteria

3. TCE in Rock Matrix-Borehole Test (NAWC)



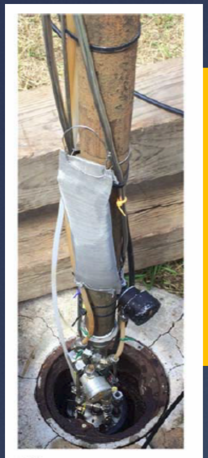
- Permeable fractures feed TCE into wellbore water
- TCE diffuses into low-permeability matrix post-drilling
- Concentration history of TCE, DCE, VC used as boundary condition



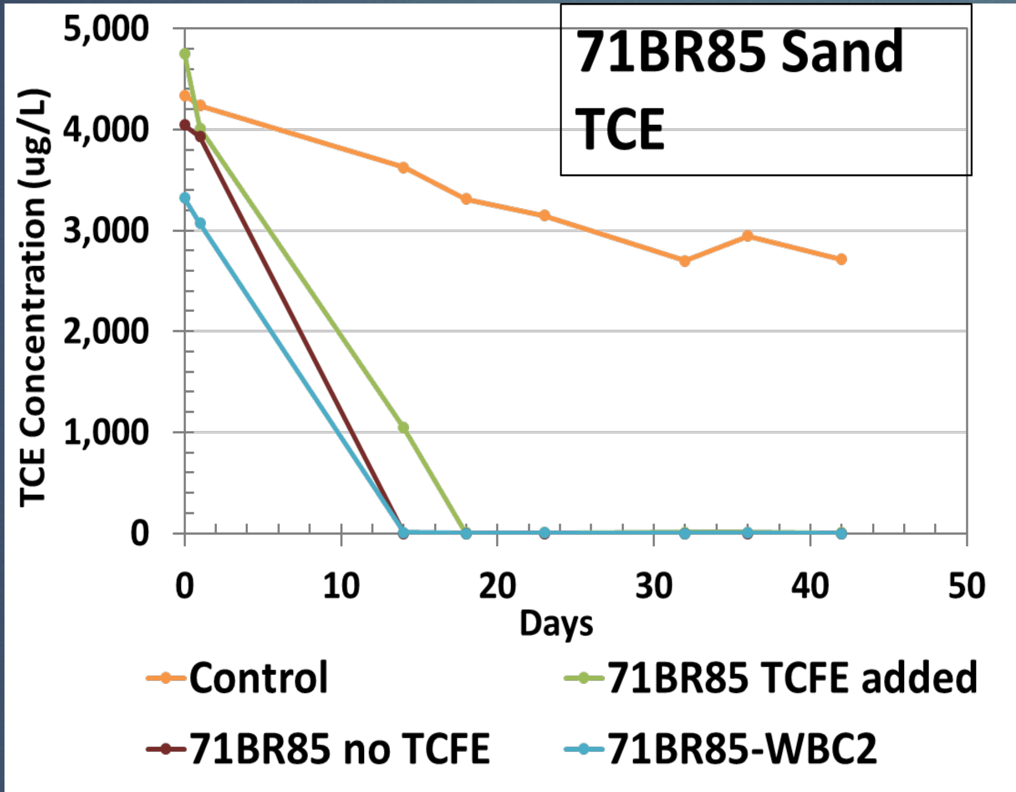
field method for diffusion & sorption coefficients & cVOC reaction rates in low-permeability strata

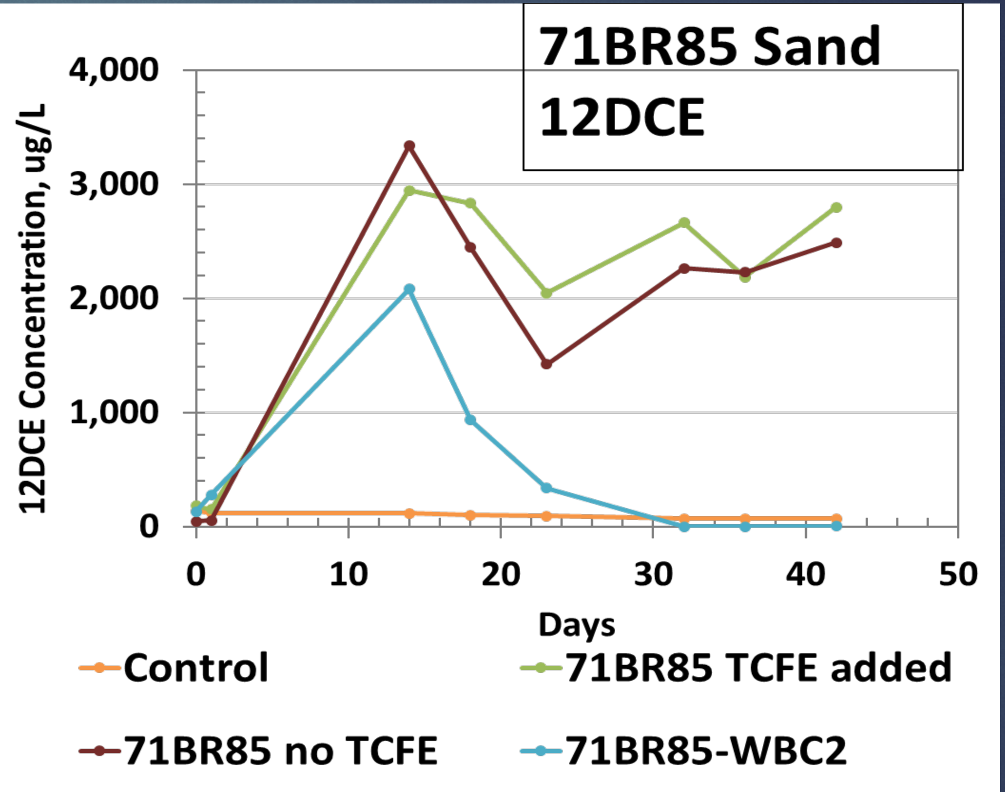
SERDP ER-2533 & USGS Toxic Substances Hydrology Program

TCE Degradation in Microcosms-
Borehole water and *in situ* incubated sand



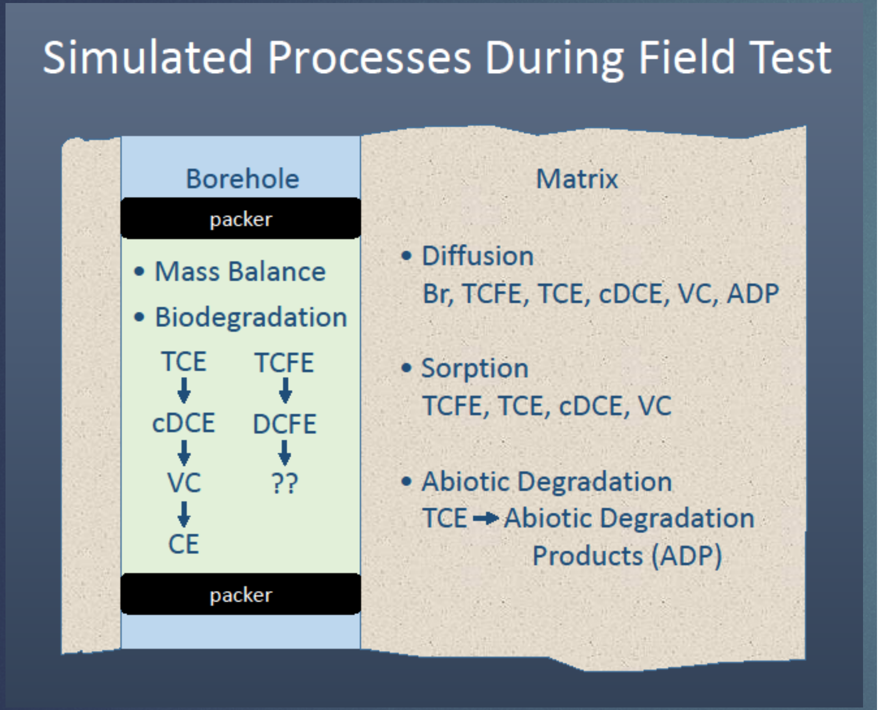
Microbial samplers, made with clean Ottawa sand inside stainless steel mesh, placed **in test interval** between packers and left for duration of tracer test.

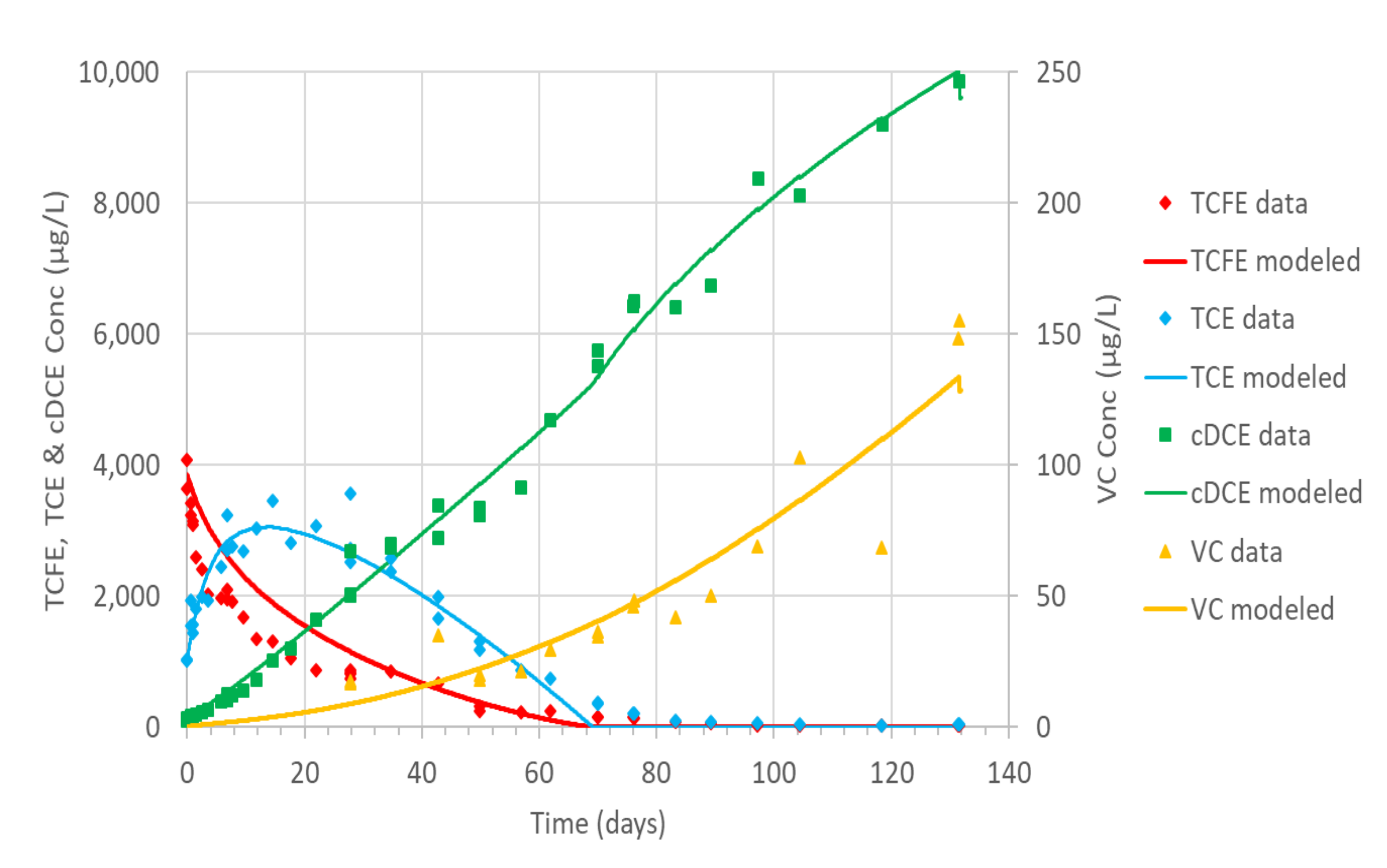




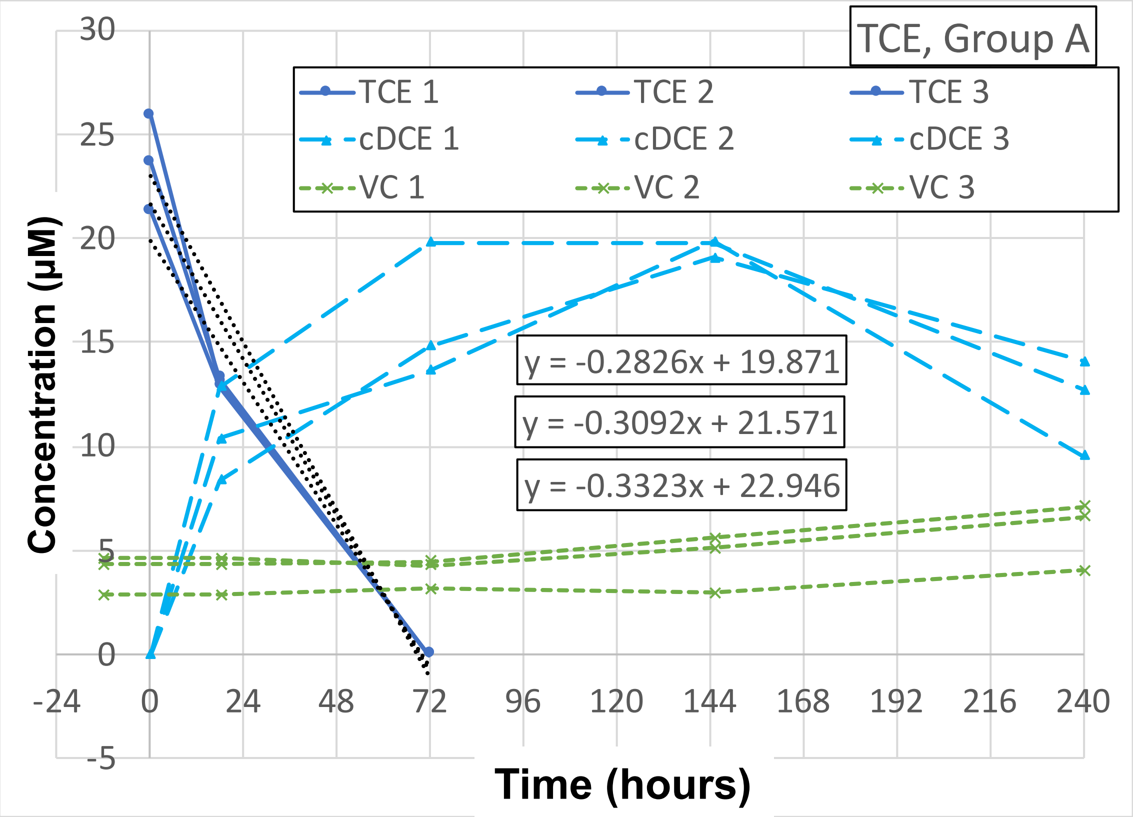
- **TCE degradation rates same with and without WBC-2 addition**
- Addition of WBC-2 did result in 12DCE and VC degradation; indicates lack of necessary microbial species/densities for complete dechlorination.

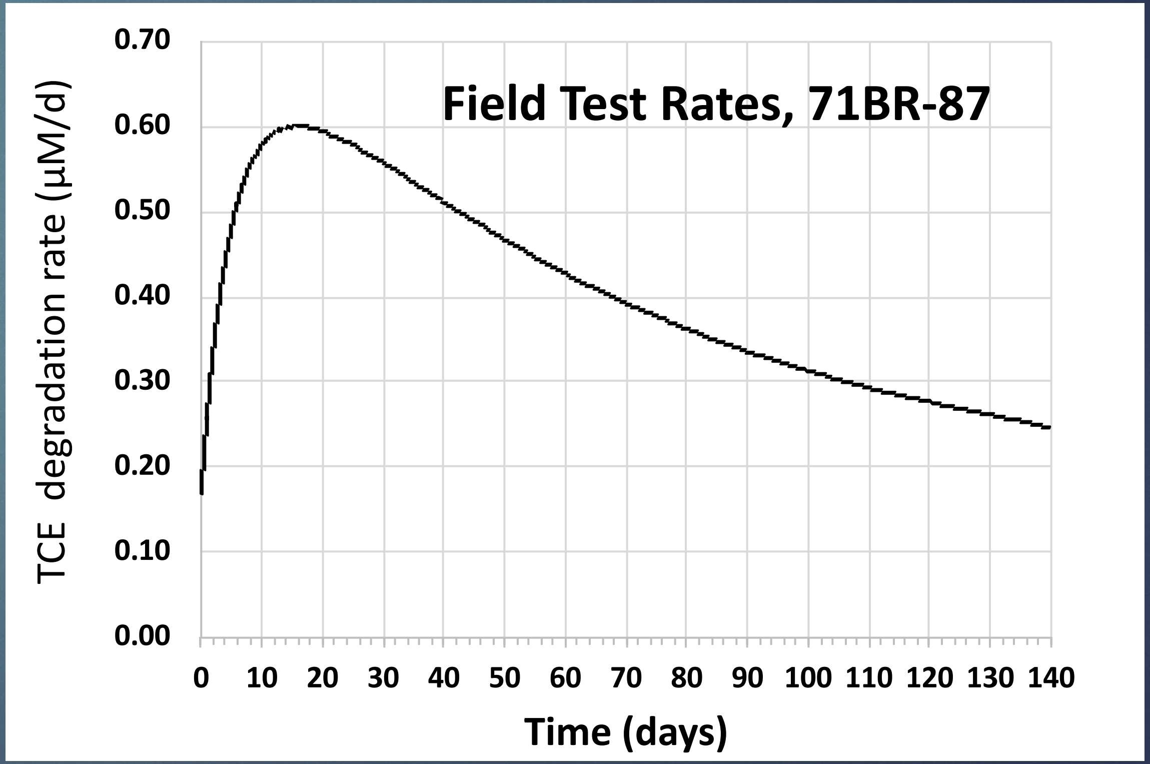
Borehole Test- Measured and Simulated cVOC Concentrations





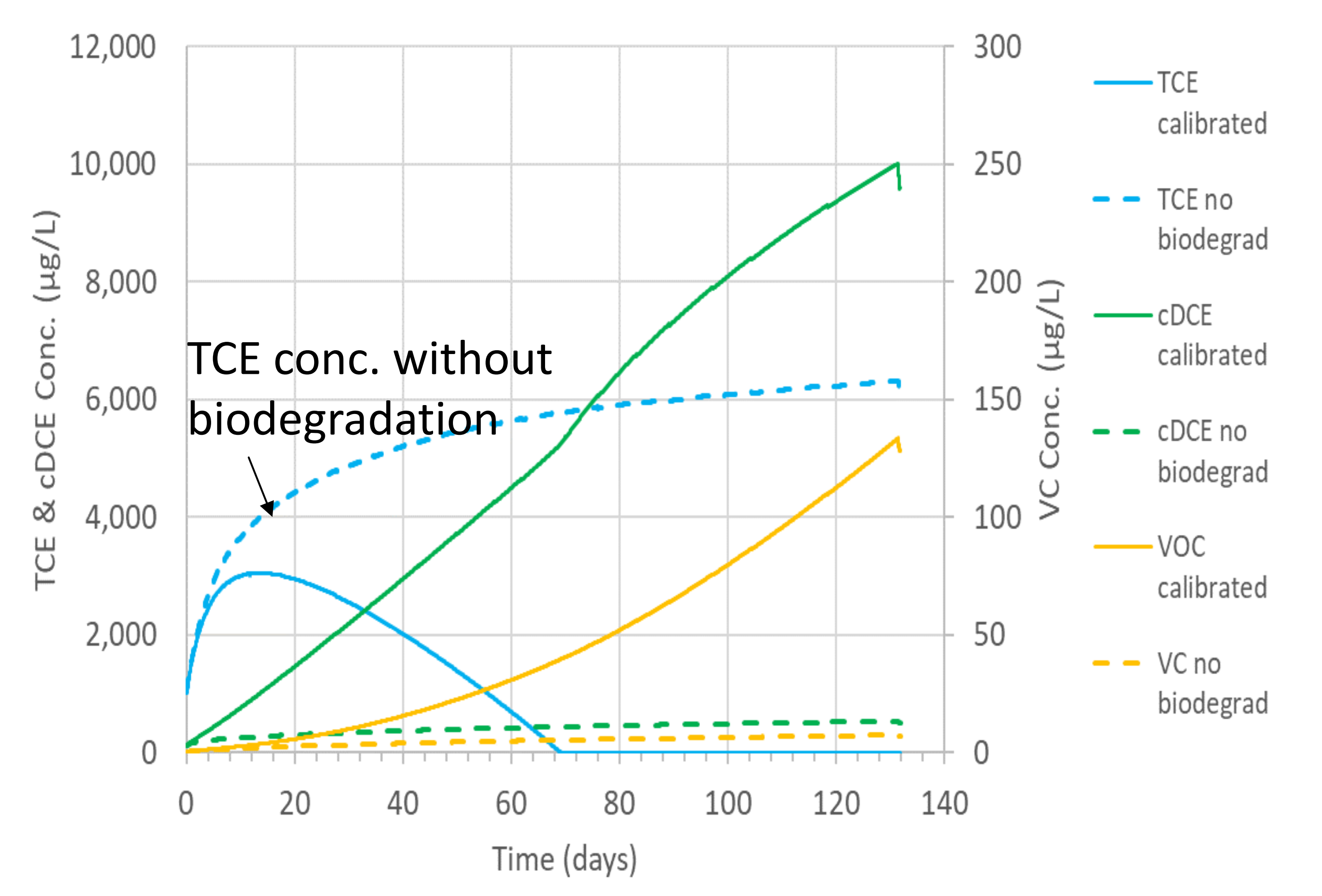
Comparison of Lab and Field Test Rates





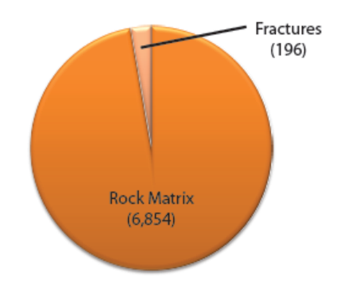
Max zero order rate, uM/day	
	TCE
Lab- USGS initial	3.4
Lab- respike	7.9
Field test	0.6

Impact of Biodegradation in Borehole



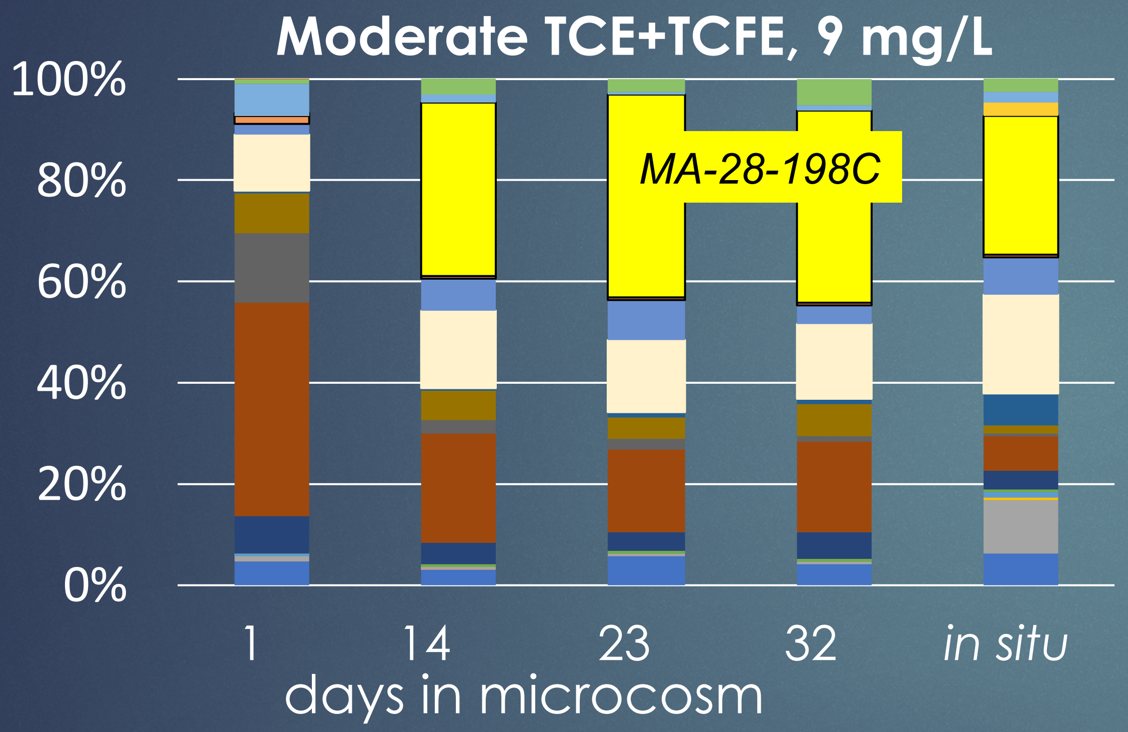
- Biodegradation of TCE to cDCE in borehole is significant under experiment (no flow) conditions
  - maintains low TCE concentration compared to matrix
  - biodegradation occurs at the interface, not in the matrix
  - cDCE to VC degradation rate increased in later part of test
- Sorption is significant
  - cVOC mass stored mostly within a few cm's of the borehole wall.
- Abiotic degradation not detected

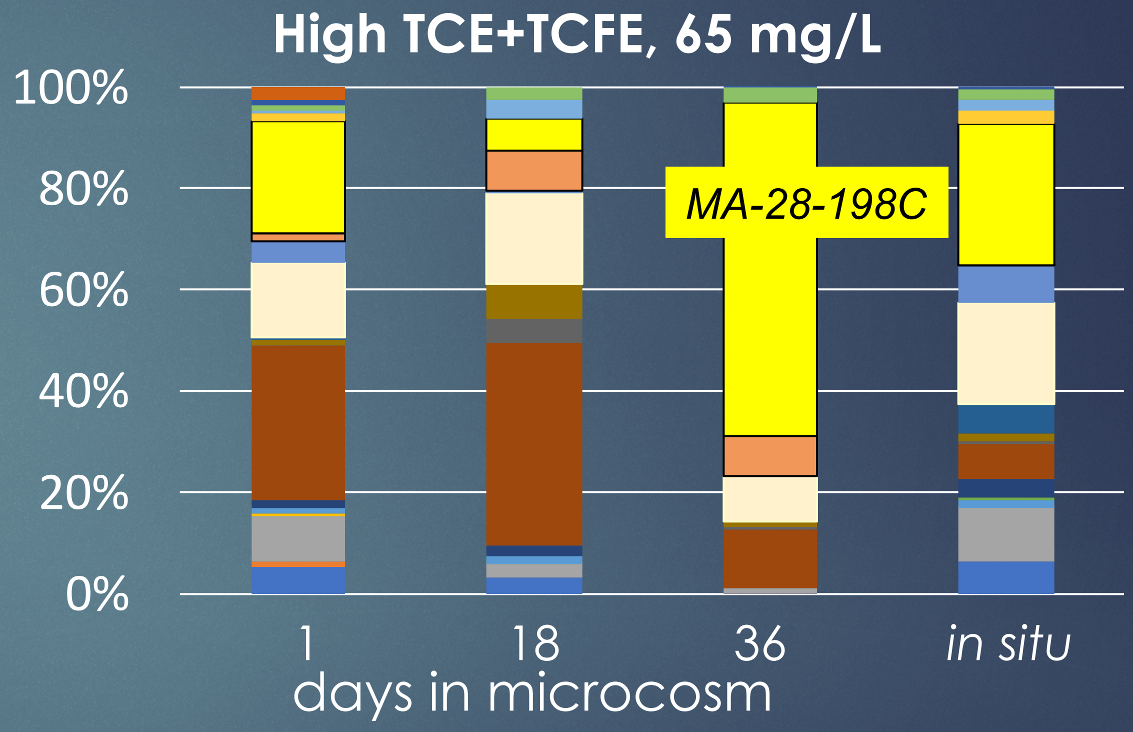
Previous study showed 97 % of TCE mass is in rock matrix (Goode et al. 2014)



Family level in microcosms (sand sampler from borehole)

- *MA-28-198C in Desulfuromonadales order, close to Geobacter*
- *Increase in abundance delayed at high conc.*

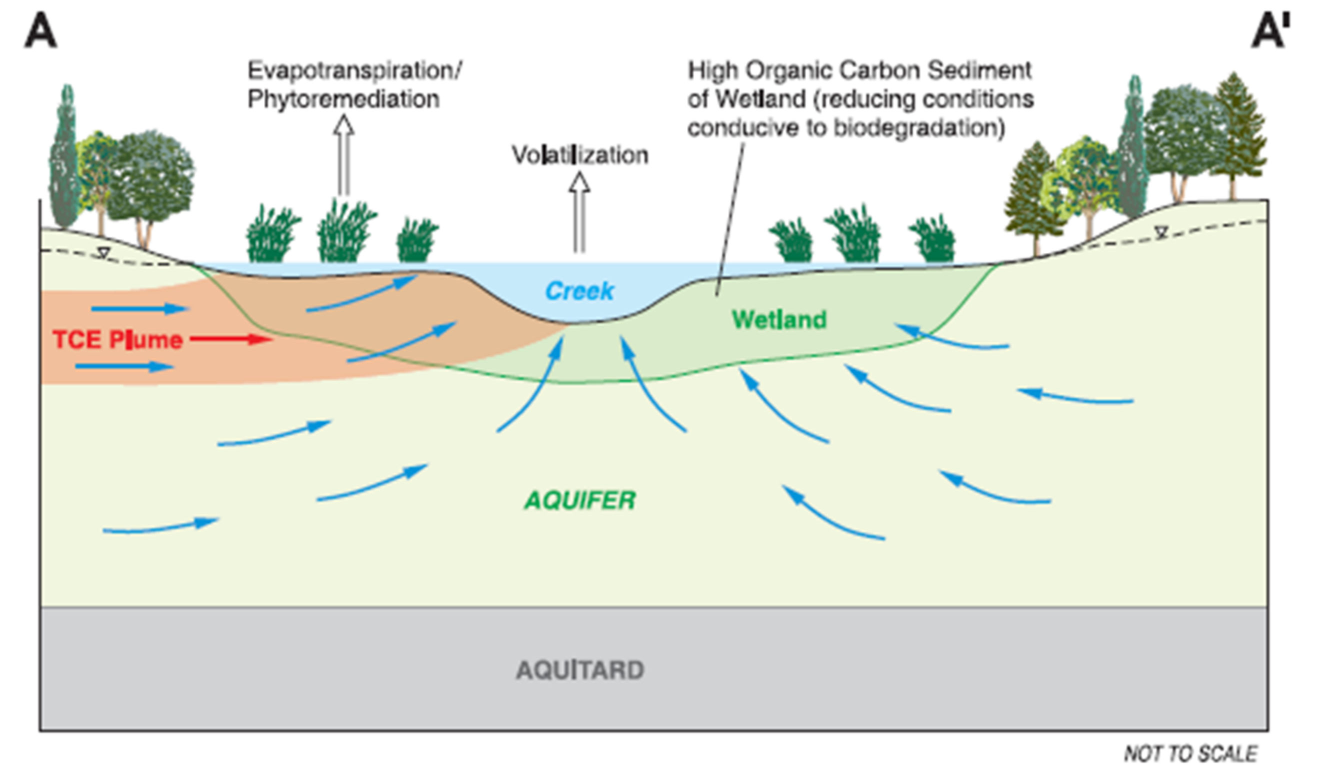




- WCHB1-32_fa
- Cloacimonetes_Incertae_Sedis_Unknown_Order_Unknown_Family
- Rhodobacteraceae
- Rhodocyclaceae
- Desulfomicrobiaceae
- Syntrophobacteraceae
- Synergistaceae
- Candidatus_Berkelbacteria_fa
- Peptococcaceae
- Comamonadaceae
- Desulfobacteraceae
- Geobacteraceae
- Helicobacteraceae
- Kosmotogaceae
- Anaerolineaceae
- Veillonellaceae
- Methylophilaceae
- Desulfobulbaceae
- MA-28-I98C
- Spirochaetaceae

4. Anaerobic PFAS Biodegradation

- Biodegradation studies using sediment in groundwater discharge areas- diversity of microbial species
- Initial focus on co-contaminant effects with cVOCs and added organohalide-respiring bacteria
- Sediment samples for initial test collected at Ft. Drum, NY (by US Army Corps of Engineers, Baltimore)



(modified from Lorah et al., 2005)

Overlap in species capable of reductive dechlorination and reductive defluorination?

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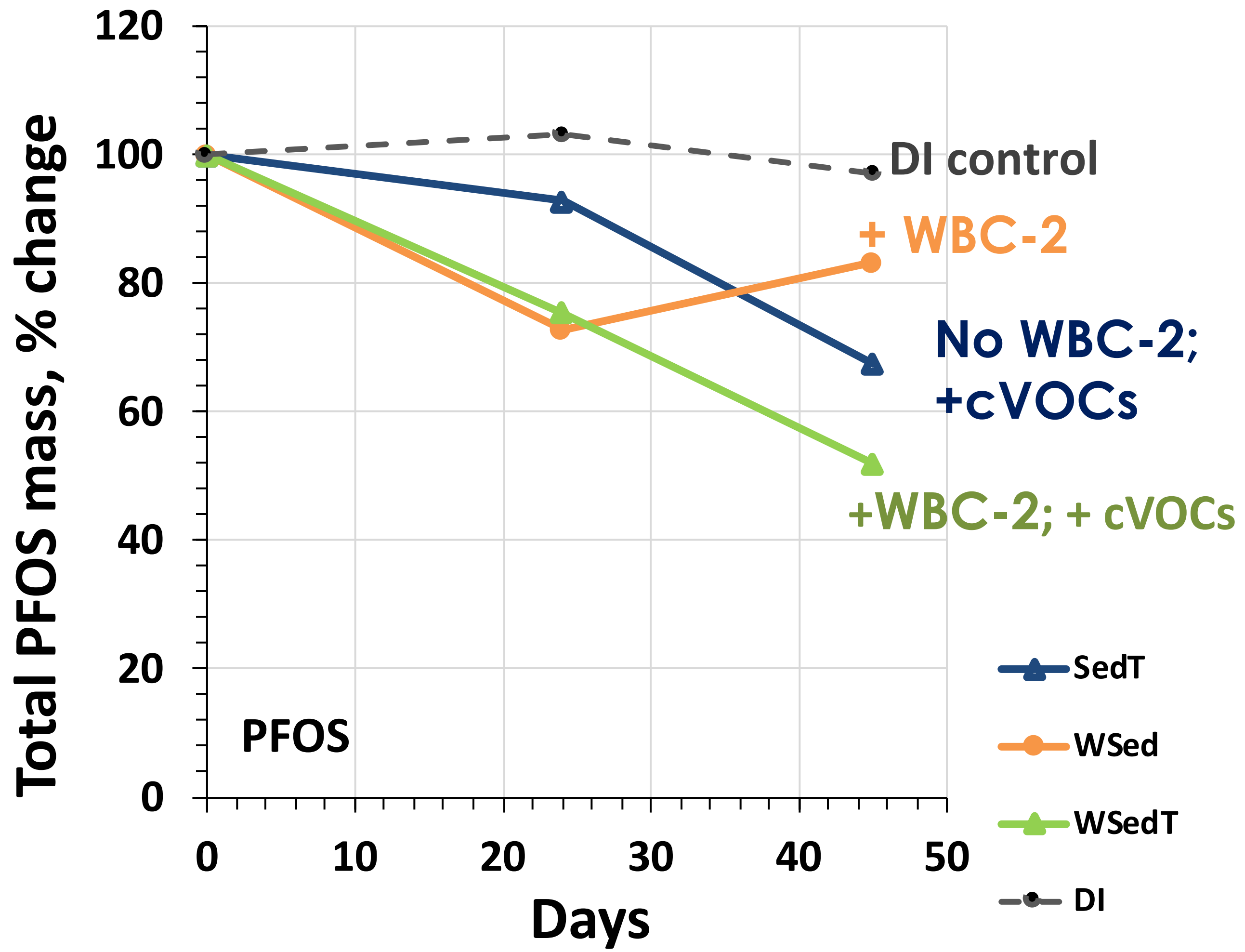
Microbial Reductive Defluorination

- Generally believed not possible for the perfluorinated compounds, although is thermodynamically feasible.
- Highly chlorinated and brominated organics that initially were believed to be recalcitrant are now known to undergo reductive dehalogenation.
- An association between Chloroflexi abundance and PFAS contamination was shown in soil (Chen et al, 2019) and river sediments (Bao et al., 2018; PFOS in particular)
- Recent identification by Huang and Jaffé (2019) of defluorination by *Acidimicrobium* sp. strain A6, a natural microbe that also dechlorinates PCE, TCE.

PFAS Microcosms—PFOS Removal

- PFOS removal in two treatments with added cVOCs— site sediment with and without WBC-2
- PFOS removal greatest with added WBC-2 and cVOCs
- **25 and 45% removal** (after account for loss in control)
- PFOA and 6:2 FtS added in the same microcosms did not show removal



Amended PFOS concentration at day 0 = 100 μg/L. 2:1 ratio of sediment to simulated groundwater.

All data are provisional.

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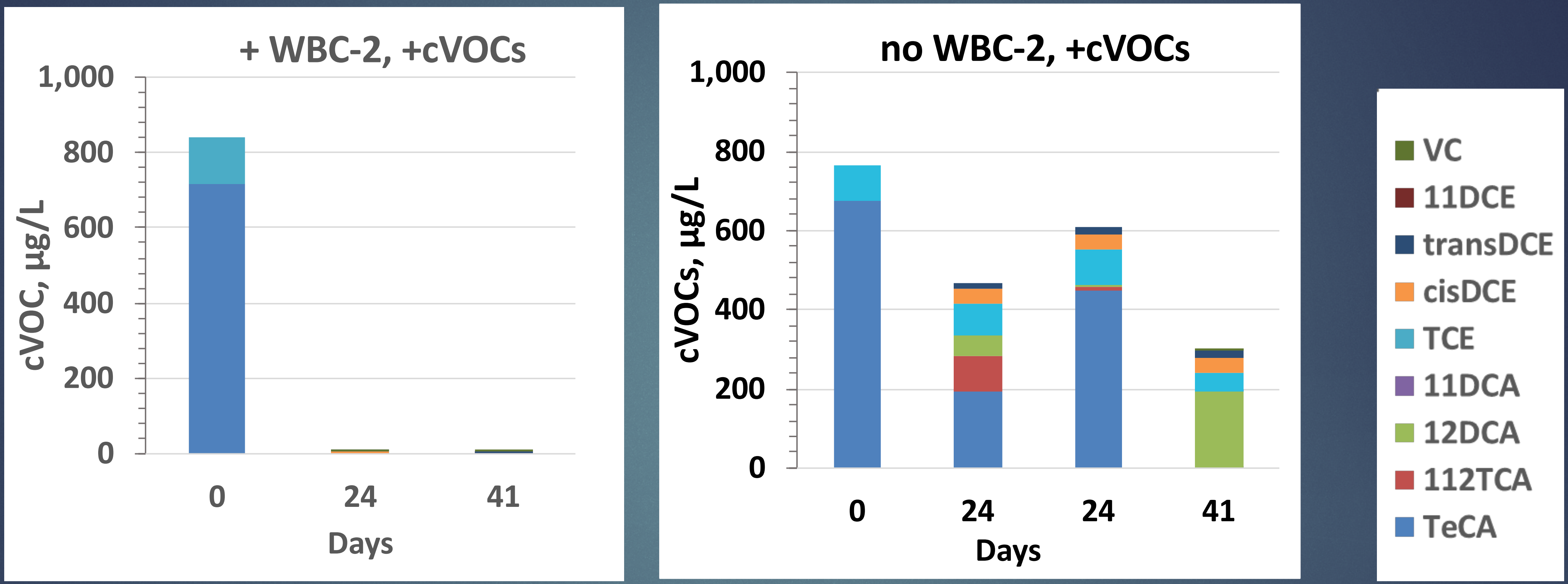
PFAS Micrososm: cVOC degradation

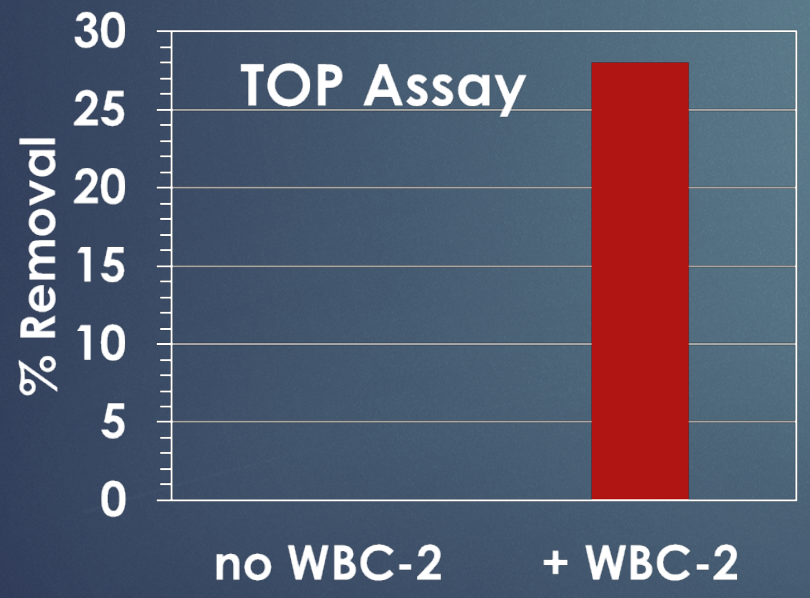


- Fast cVOC degradation in WBC-2 bioaugmented microcosm and low daughter product accumulation.
- Slower cVOC degradation and greater daughter product detections in the non-bioaugmeted site sediment.

All data are provisional.

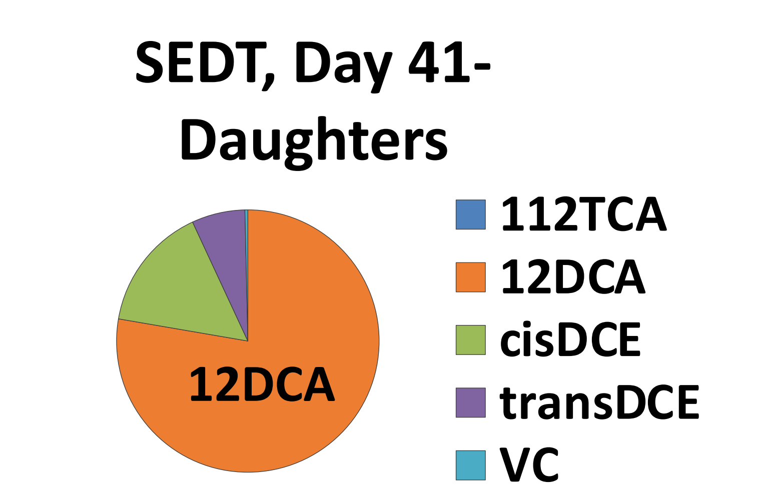
PFOS and cVOCs Removal Rates

Half-life, Days			
	PFOS	TeCA	TCE
Site sed +cVOCs	50	4	46
+WBC-2, +cVOCs	27	2	5



Removal of total organofluorine compounds, measured by Total Oxidizable Precursor (TOP) Assay on day 1 and day 45 microcosm samples.

- Link indicated between cVOC degraders and PFOS degraders.
- Microbes involved in PFOS transformation in native sediment possibly associated with alkane degradation pathway?



All data are provisional.

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Acknowledgements

1. Coupled Anaerobic – Aerobic Biodegradation

- NIEHS Grant R01ES024279
- EPA Region III
- Ed Bouwer (Johns Hopkins Univ., deceased)
- **Steven Chow** (Johns Hopkins Univ)
- Neal Durant (Geosyntec, Columbia, MD)
- Amar Wadhawan (Arcadis, Hanover, MD)
- Denise Akob (USGS, Reston, VA)
- USGS Baltimore FAB team

2. TCE in Fractured Rock- Permeable

- SERDP ER-1555
- US Navy
- USGS Toxic Substances Hydrology Program
- **Jennifer Underwood**, Ronald Harvey (USGS, Boulder, CO)
- Denise Akob (USGS, Reston, VA)
- Thomas Imbrigiotta (USGS, NJ)
- USGS Baltimore FAB team

3. TCE in Fractured Rock- Matrix

- SERDP Project no. ER-2533
- US Navy
- USGS Toxic Substances Hydrology Program
- USGS New Jersey WSC Hydrologic Research & Devel. Program
- **Richelle Allen-King**, (Univ. of Buffalo, SUNY) and students:
  - Rebecca Kiekhaefer, Jonathan Brotsch, Rory Dishman, Matthew Buzzeo, **Hannah Annunziata**
- Dan Goode (USGS, PA)
- **Paul Hsieh**, Claire Tiedeman (USGS, CA)
- Tom Imbrigiotta, Alex Fiore, Pierre Lacombe (USGS, NJ)
- Denise Akob, Allen Shapiro, Karl Haase (USGS, Reston)

4. PFAS Biodegradation

- US Army and US Army Corps of Engineers, Baltimore, Brian Shedd
- USGS Toxic Substances Hydrology Program
- Denise Akob (USGS, Reston, VA)
- Lee Blaney, Ke He (Univ of Maryland Baltimore County)
- Andrea Tokranov (USGS, MA)
- USGS Baltimore FAB team

in bold: contributed figures or slides

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