Groundwater Remediation and Dual-Biofilm Barrier for Treatment of Chlorobenzenes

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NIEHS Webinar

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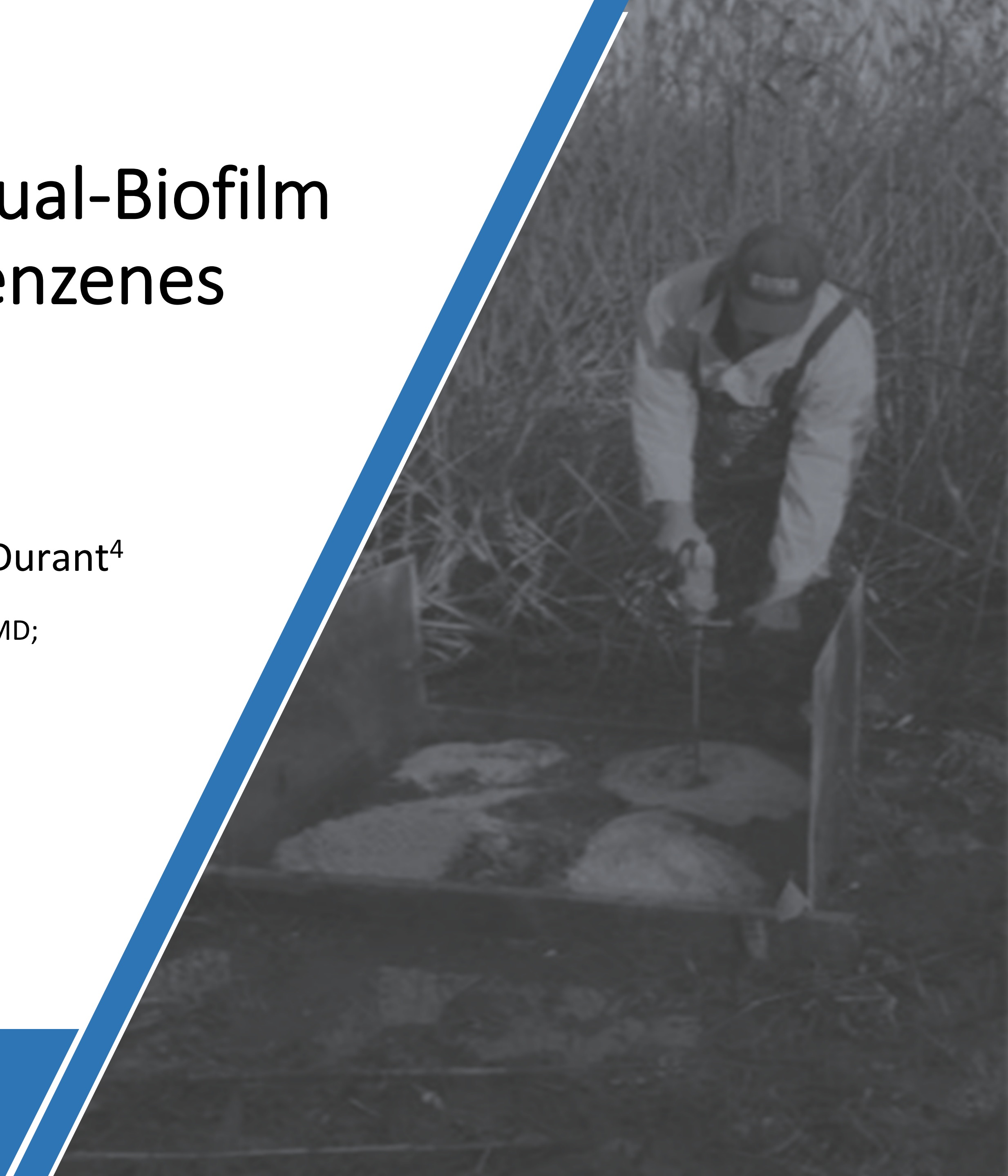

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Groundwater remediation background

- Over the past 30 years, some progress made on hazardous waste site remediation
  - 360 of 1,723 (21%) National Priorities List sites have been “cleaned up”
  - 70% of the 3,747 sites regulated under RCRA have “control of human exposure”
  - Closure of over 1.7 million underground chemical storage tanks since 1984
- Complete restoration of contaminated groundwater is difficult, not likely to be achieved in less than 100 years at many sites
- Difficult sites to remediate: large size, heterogeneous hydrogeology, and multiple (and recalcitrant) contaminant
- Over 126,000 sites remain in the U.S. with residual contamination
- Estimated cost to complete: **$110-$127 billion**

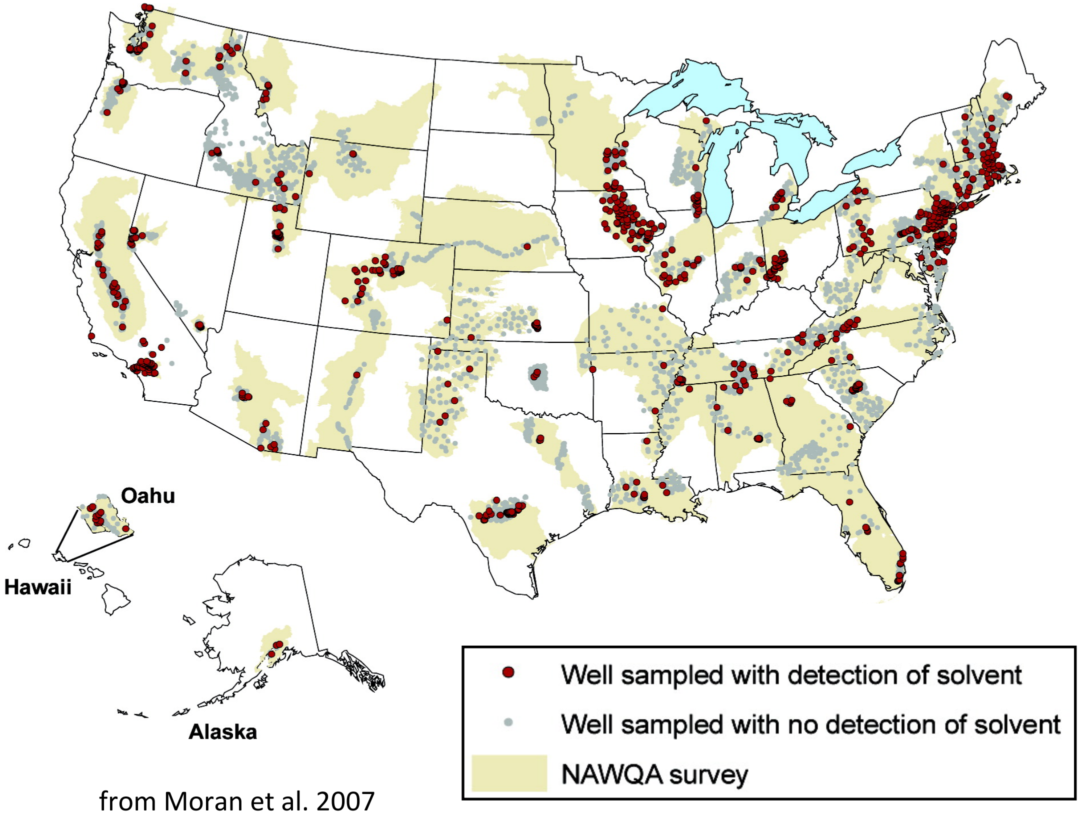
National Research Council. *Alternatives for Managing the Nation’s Complex Contaminated Groundwater Sites*. (The National Academies Press, 2013).

Bioremediation

- Requires appropriate organisms and favorable biogeochemistry
- Natural Attenuation
- Enhanced in-situ Bioremediation
  - Treatment of contaminated source zones and groundwater plumes
  - **Biostimulation**: delivery of electron donors, electron acceptors, or other growth factors (e.g., nutrients)
  - **Bioaugmentation**: amendment of the subsurface with certain microbes
- Used as the remedy at approx. 25-30% of Superfund sites
- Common applications for chlorinated solvents, petroleum hydrocarbons (BTEX, PAHs), PCBs, and pesticides
- Both aerobic and anaerobic processes

Chlorinated solvents – a continuing legacy

- Versatile uses – dry cleaning solvents, coolants, degreasers, deodorizers, herbicides, chemical intermediates
- Common contaminants – TCE, PCE, PCBs, CBs
- 881 of 5,068 (17%) National Water Quality Assessment wells tested positive for chlorinated solvents (1985 – 2002)
- 8% of EPA National Priority List sites contaminated with chlorobenzenes (CBs) (1990 estimate)



from Moran et al. 2007

Moran et al. 2007. Environmental Science and Technology; Russell et al. 1991. *Hazardous Waste Remediation: The Task Ahead;* ATSDR. 1990. Toxicological Profile for Chlorobenzene.

Contaminant profile - chlorobenzenes

- Sparingly soluble, semi-volatile dense nonaqueous phase liquids (DNAPLs)

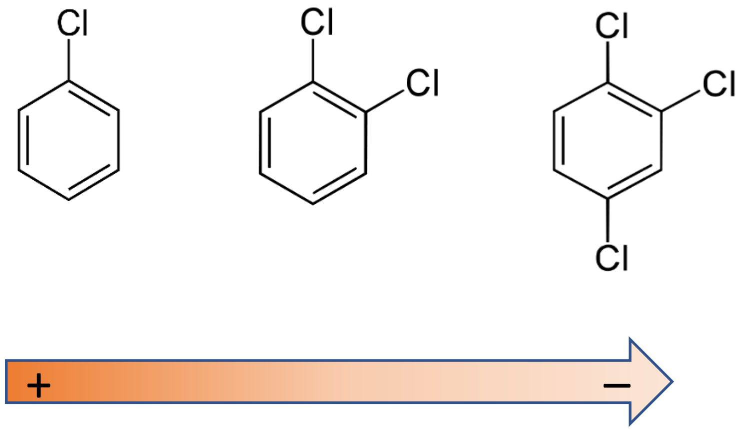
- Chronic low-dose exposure
    - Allergic sensitivity
    - Respiratory inflammation
    - Oxidative stress
    - Suspected carcinogenesis

- EPA drinking water max concentration limits
    - 1 µg/L (HCB)
    - 600 µg/L (1,2-DCB)
    - 8 CBs + benzene on EPA priority contaminant list

Physical properties of select chlorobenzenes

	Mono- (MCB)	**Di- (DCB)**	**Tri- (TCB)**
Aq. Solubility [mg/L]	450	130	17
Vapor P* [Pa]	1665	197	45
K_{OC}* [mg/mg]	466	987	2670

*at 25° C



Solubility, volatility, mobility

Locating and Estimating Sources of Chlorobenzene, US EPA 1994; Fields and Sierra-Alvarez, *Biodegradation* 2008, US EPA, O. Table of Regulated Drinking Water Contaminants. ATSDR Registry., Toxicological Profile for Chlorobenzene. In Service, U. S. P. H., Ed. 1990.

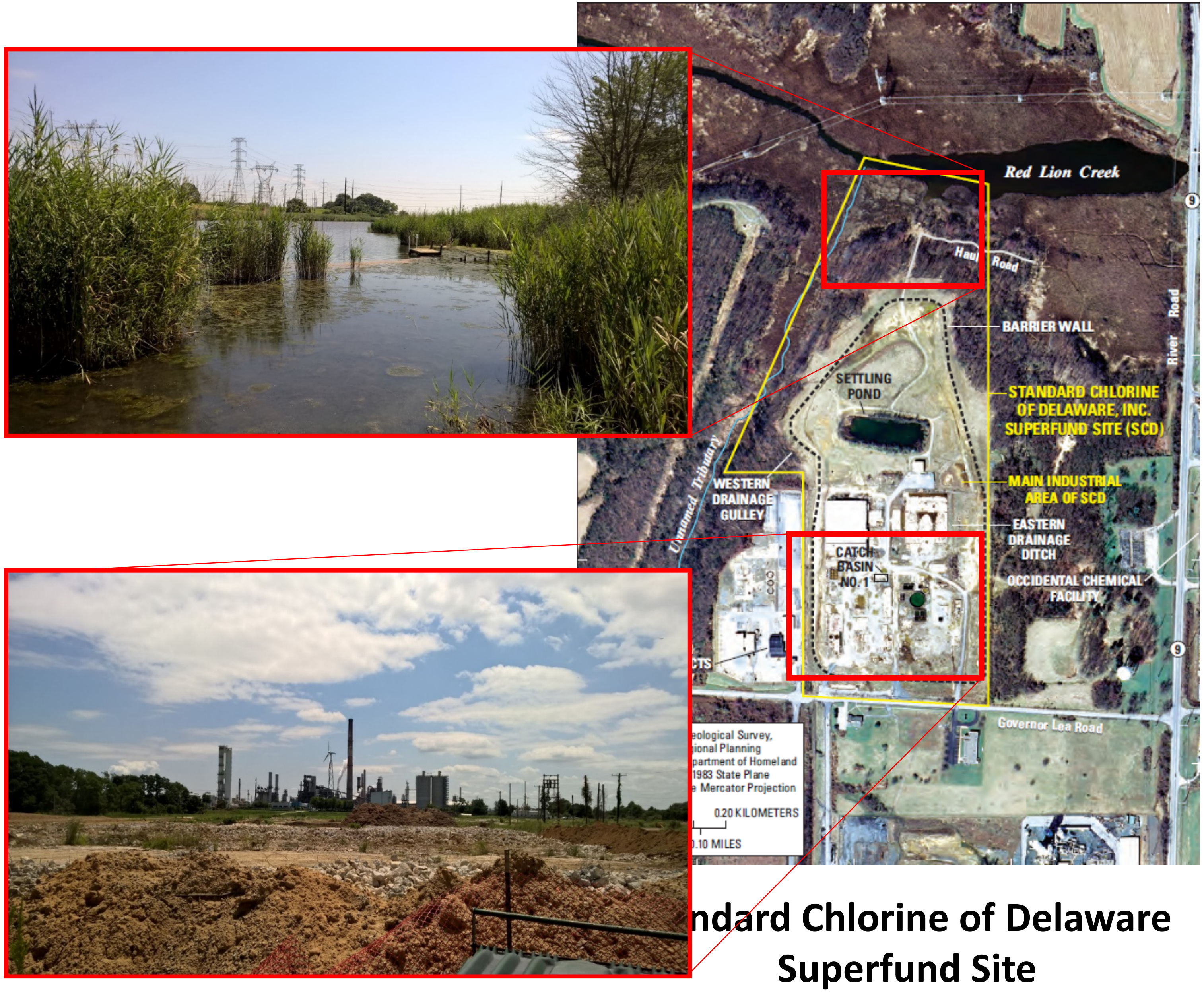
Site Overview

Standard Chlorine Superfund Site

- 2,000,000 L of mixed mono-, di- and tri-chlorobenzenes (CBs) released from tanks and containment pond

- Extensive remediation at industrial site (excavation, barrier wall, pump and treat)

- Adjacent wetland remains highly contaminated with DNAPL concentrations



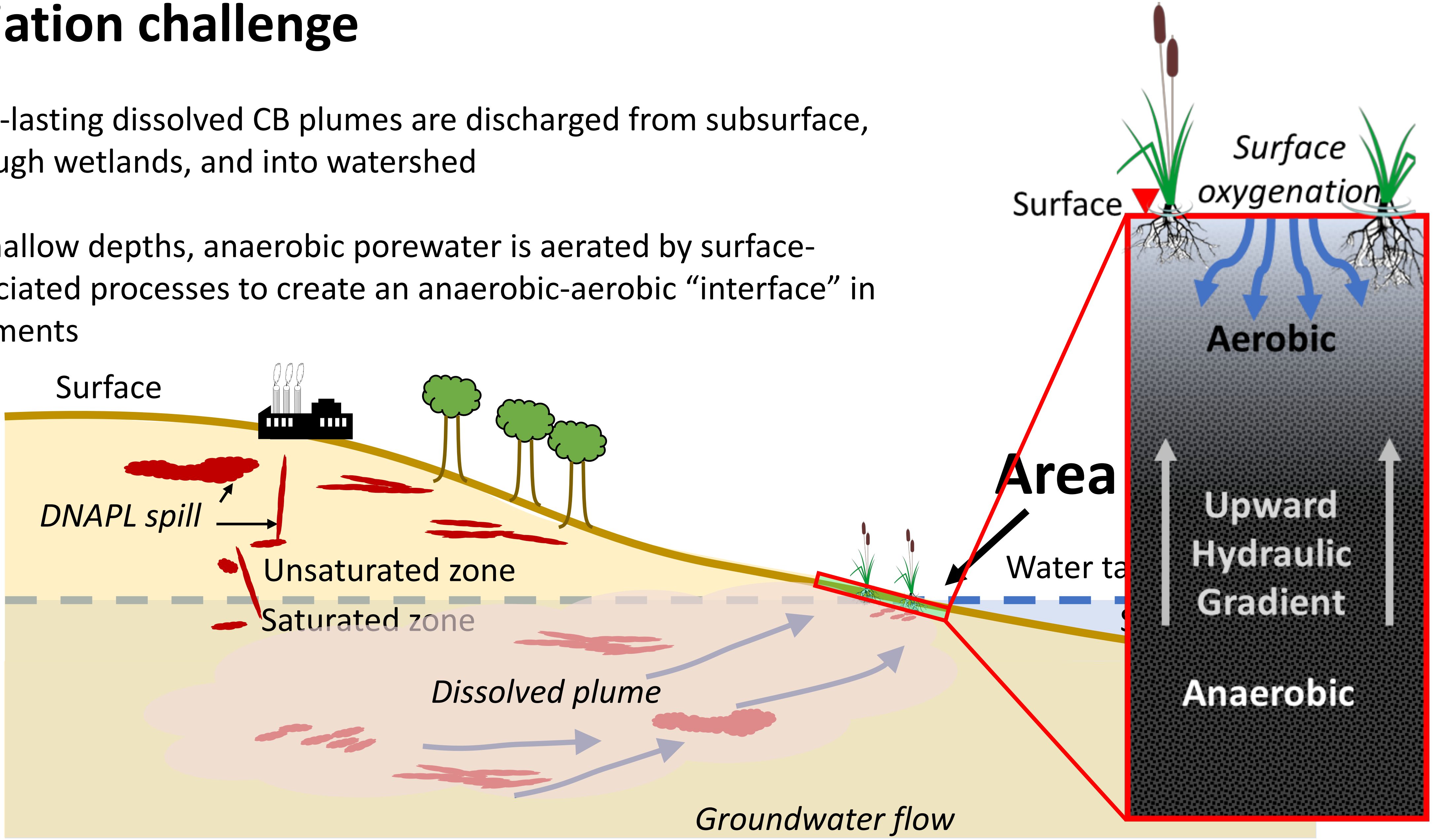
ndard Chlorine of Delaware Superfund Site

Lorah et al. 2014. USGS

ATSDR. 1990. Toxicological Profile for Chlorobenzene. http://www.epa.gov/reg3hscd/npl/DED041212473.htm

Remediation challenge

- Long-lasting dissolved CB plumes are discharged from subsurface, through wetlands, and into watershed
- At shallow depths, anaerobic porewater is aerated by surface-associated processes to create an anaerobic-aerobic "interface" in sediments



Lorah et al. 2014. USGS

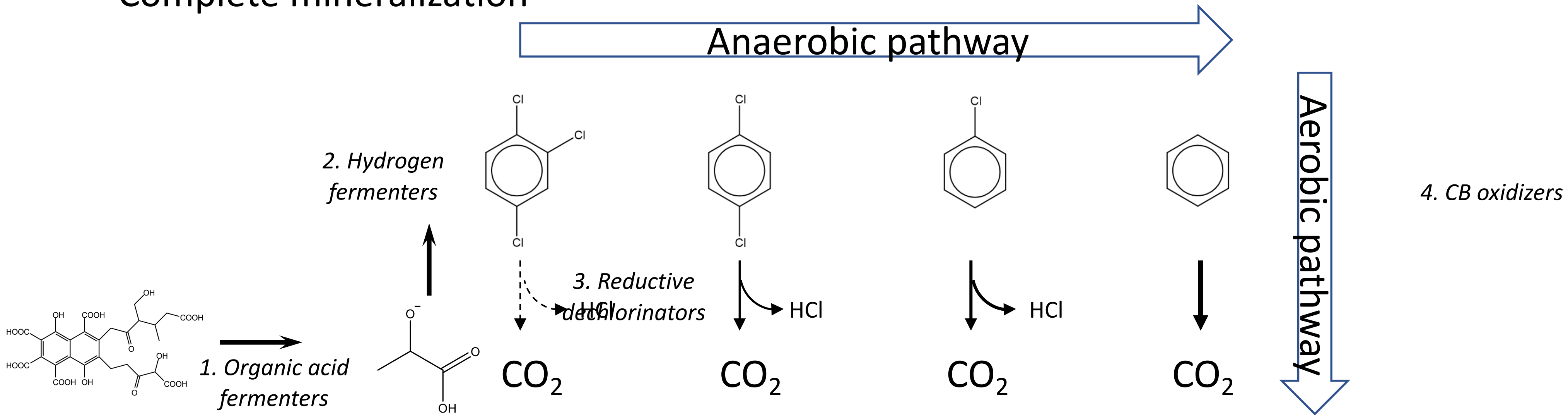
Reactive barrier concept

- Deploy as a mat near surface of “gaining” hydraulic systems
- Options for matrix composition
  - High-permeability sand
  - Degrading microbial inocula
  - Sorptive activated carbon
  - Complex electron donor (chitin, peat, mulch, etc)
- Benefits
  - Low capital costs (digging, materials)
  - Low maintenance (substrate replacement, pumping)
  - Minimal disturbance below surface layer
  - Sequestration + degradation potential

Lorah et al. 2014. USGS

Coupled anaerobic – aerobic biodegradation

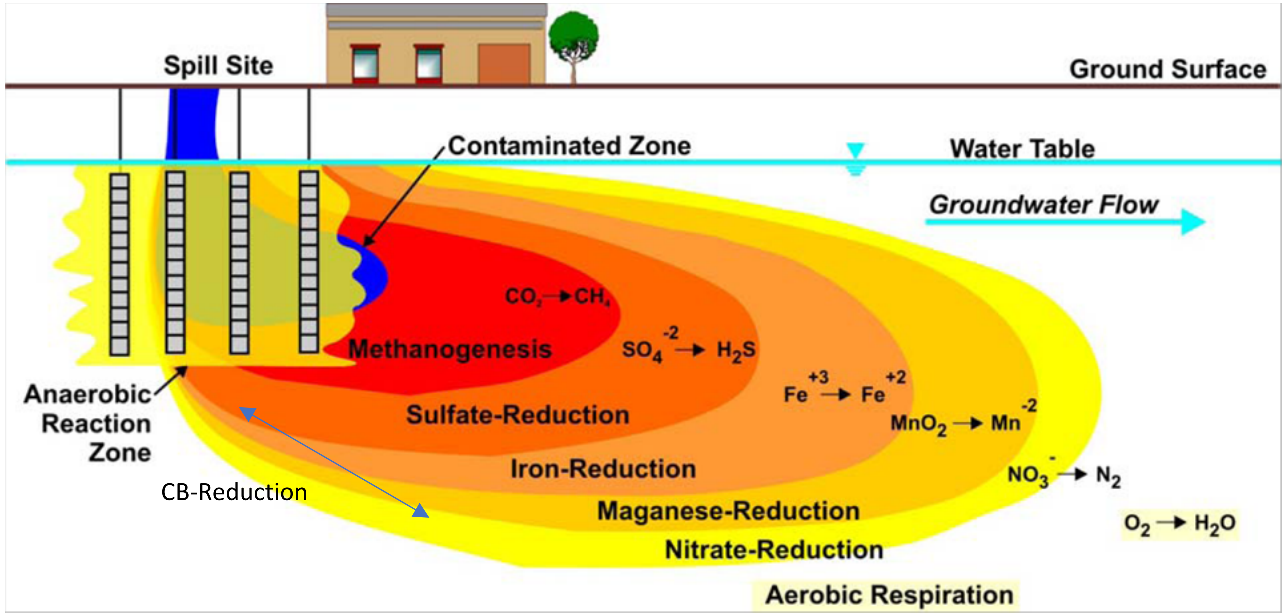
1. **Anaerobic:** reduce highly-chlorinated (highly oxidized) compounds to less-chlorinated products
    - External substrate + CB e^- acceptor
    - Toxic daughter products remain
    - Mineralization possible, but MCB stall common
2. **Aerobic:** oxidize less-chlorinated CBs to innocuous products
    - CB substrate + O_2 e^- acceptor
    - Complete mineralization



Demonstrated with CBs[1], PCBs[2], chloroethenes and chloroethanes[3], azo dyes[4], and others

1. Fathepure and Vogel 1991, *AEM*; 2. Payne et al. 2013, 2016, *ES&T*; 3. Tartakovski et al. 2003, *ES&T*; 4. van der Zee and Villaverde 2005, *Water Research*;

- Redox conditions can be temporally and spatially heterogeneous at sites
- Other externalities (chemical spills, flooding, seasonality) introduce even more perturbation
- SCD site survey
  - Average 14-56 mg/L DOC
  - 0.42 – 1090 mg/L sulfate



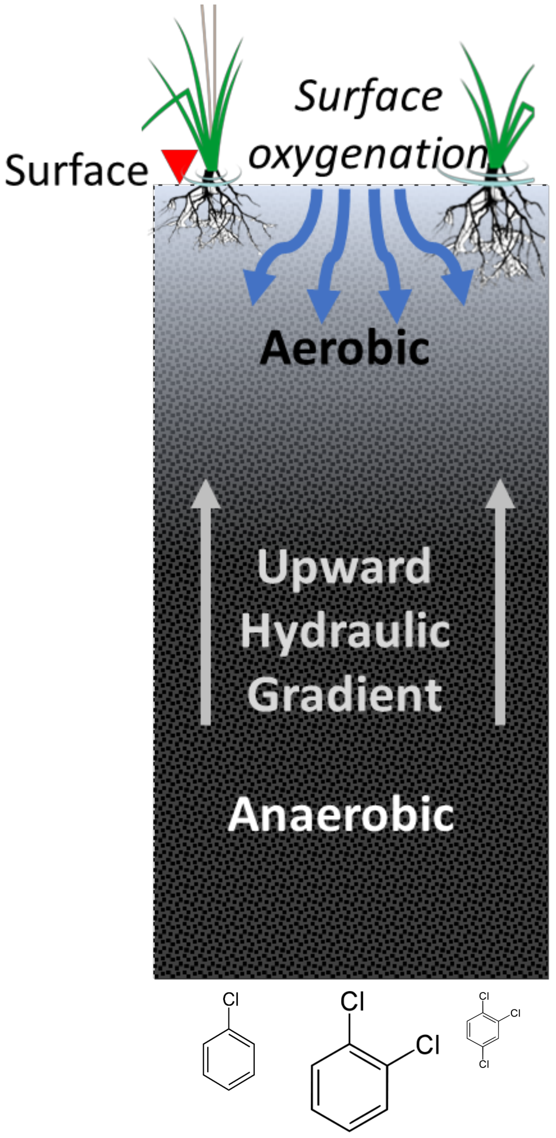
(CB-Oxidation)

Research questions

- **What is the potential for CB biodegradation at anaerobic-aerobic interfaces?**
- **How do natural geochemical conditions affect the dynamics of the degradation processes?**
  - **e^- donor availability**
  - **Alternative e^- acceptor availability**

Parsons. 2004. Principles and Practices of Enhanced Anaerobic Bioremediation of Chlorinated Solvents. AFCEE, NFEC, ESTCP 457 pp, August 2004

Simulating the interface



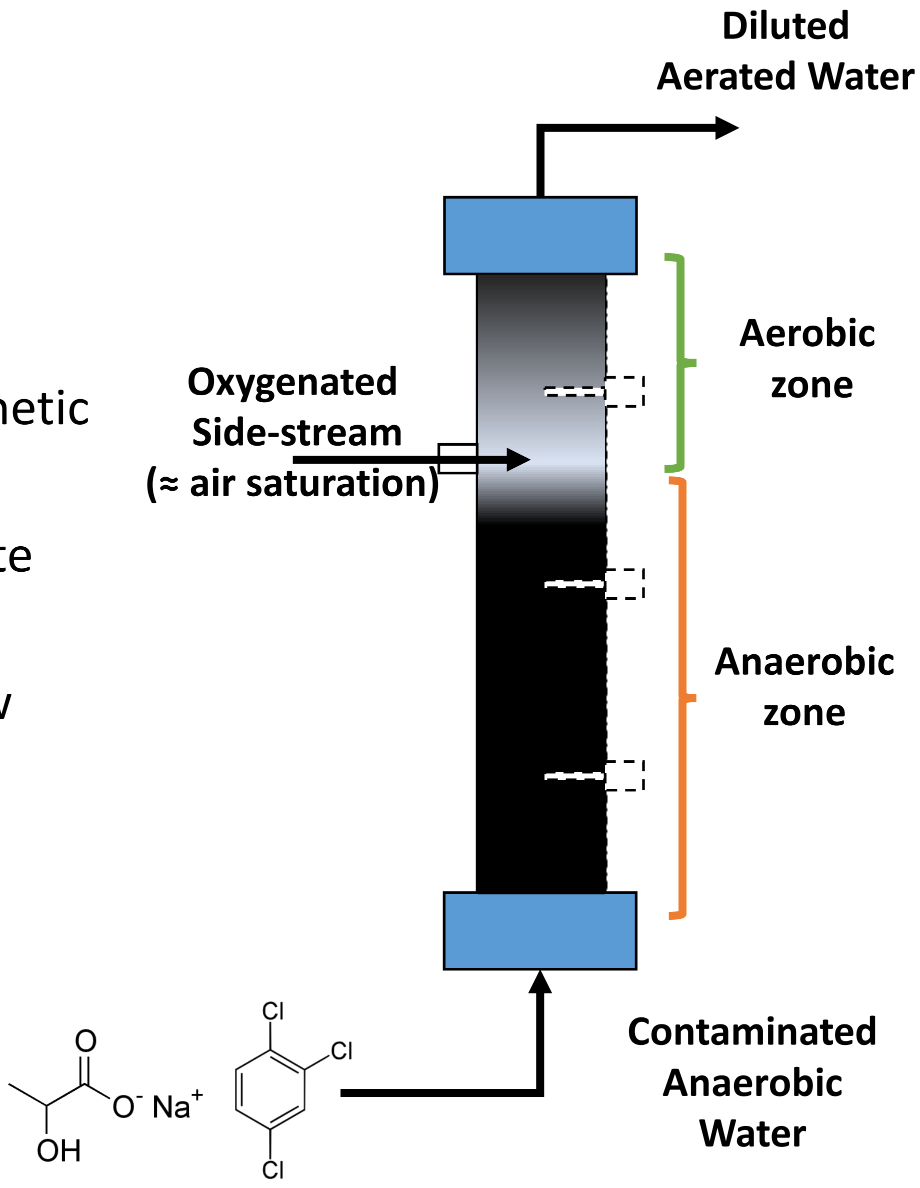
Conceptual model

Simplifications

Natural water → Defined synthetic media

Complex DOC source → Sodium lactate model donor

Variable flow and oxygen flux → Constant-flow system

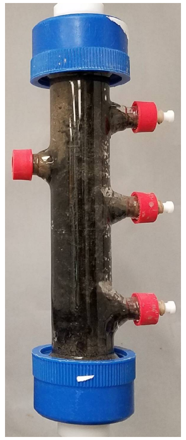


Experimental design

Simulating the interface

Packed columns

1. Filter Sand



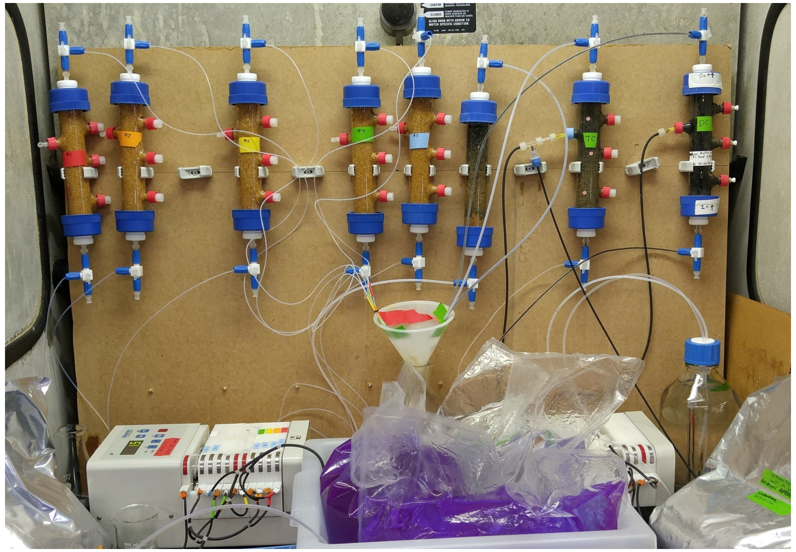
2. Site Sediment + Filter Sand

Bioaugmentation cultures

Anaerobic degrader culture (WBC-2, SiREM Labs)

Aerobic degrader enrichment

Upflow simulated groundwater system

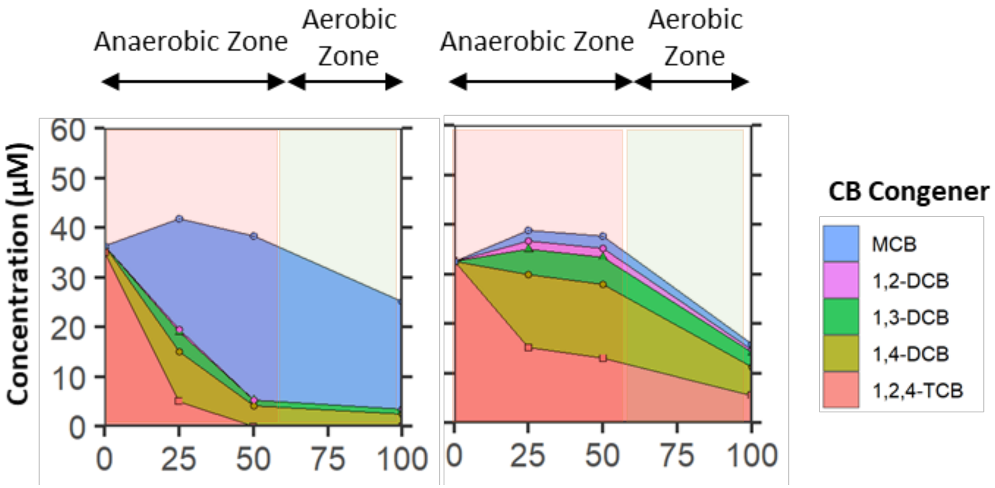


- 300-day continuous flow study
- Low-sulfate, sterilized simulated media
- Excess 6-7 mg/L 1,2,4-TCB contaminant
- Aeration to ~ 7 mg/L O_2 in aerobic zone

Proof of concept

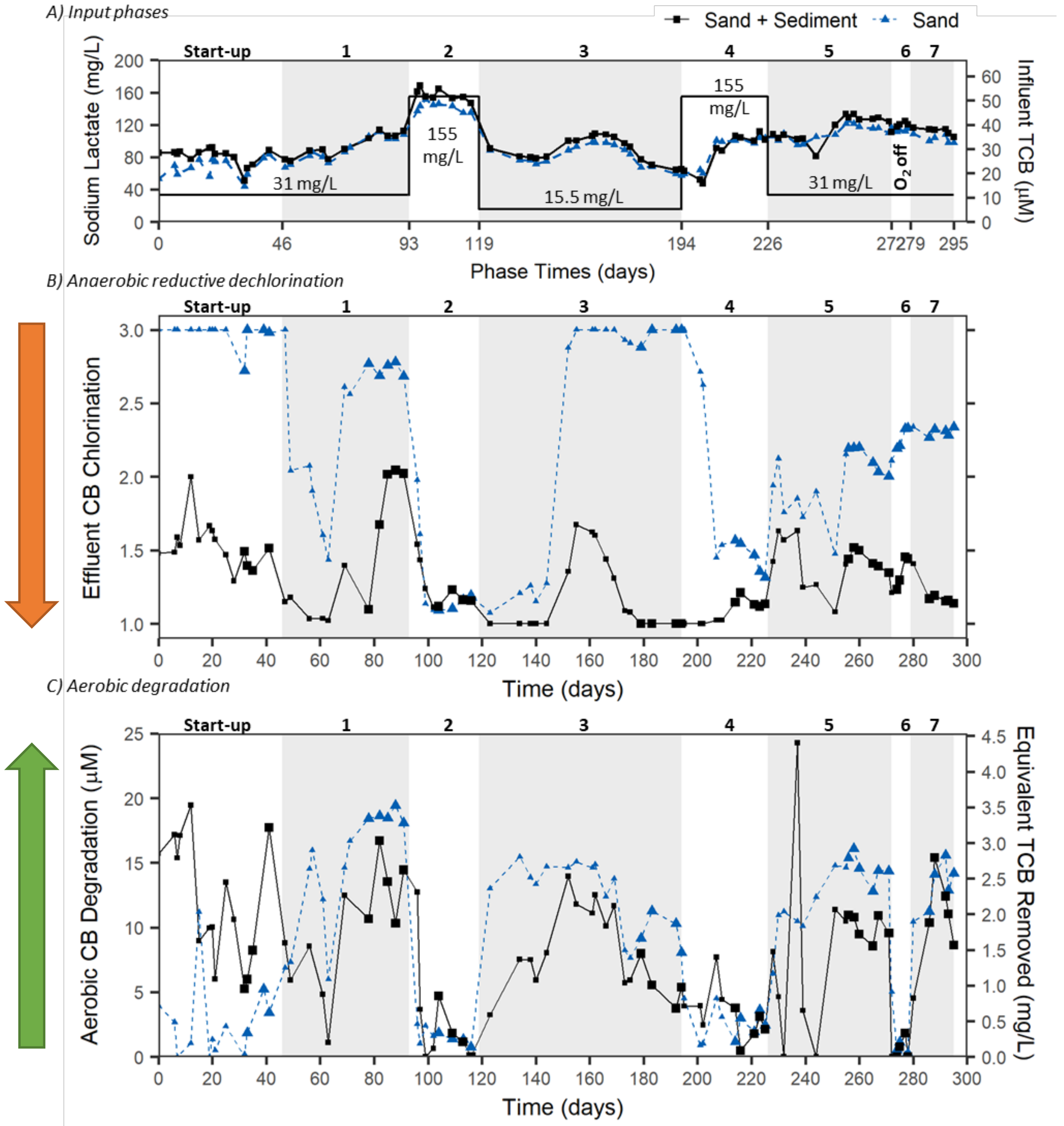
Cycled 15.5, 31, and 155 mg/L sodium lactate (NaLac) influent e$^-$ donor doses (5-50 mg/L DOC)

- Sustained anaerobic and aerobic CB degradation over time
- Dechlorination pathway: 1,2,4-TCB → 13/14-DCB → MCB
- Degradation pathways spatially separated across interface

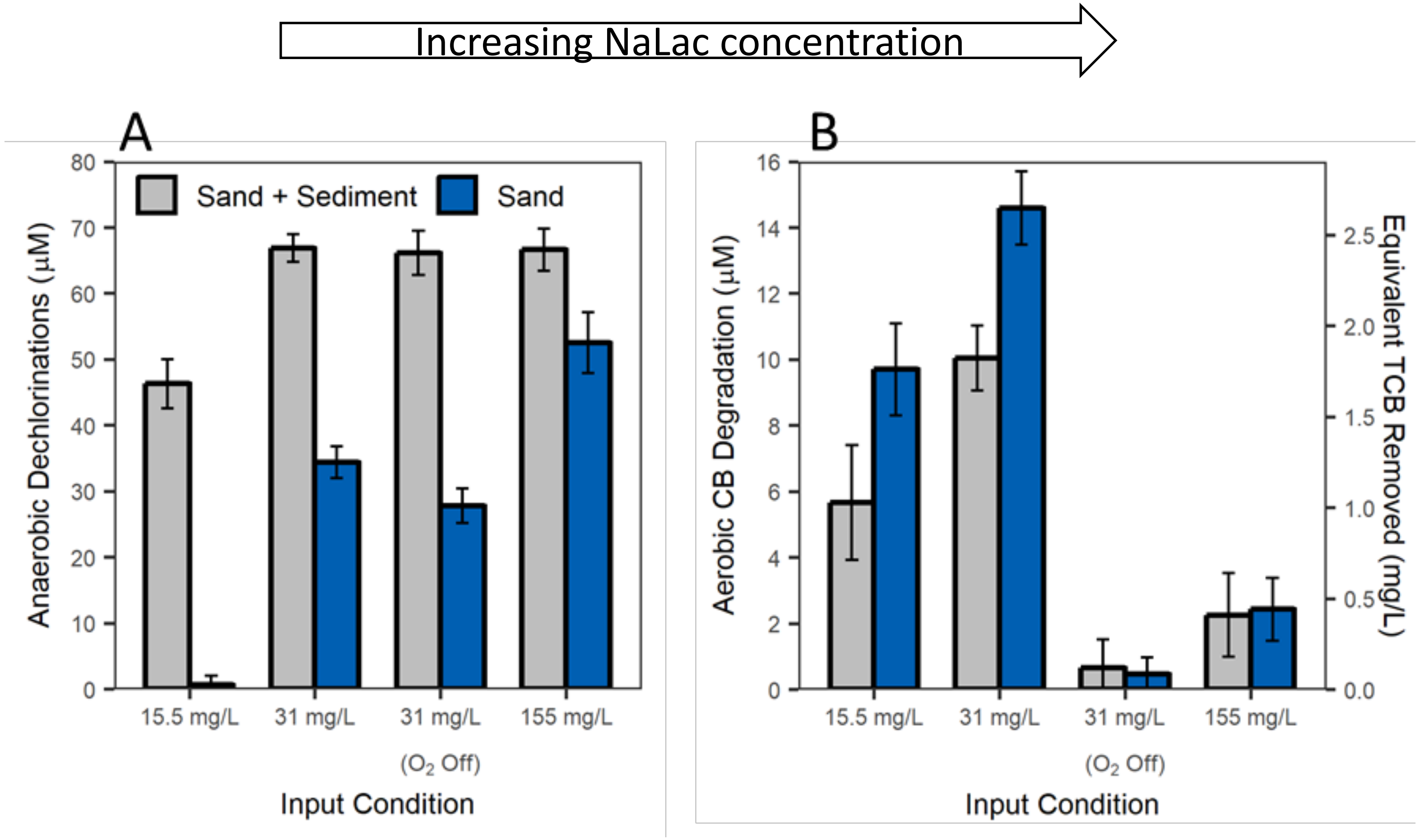


Sediment + Sand *Filter Sand*

Column Flow →



Influence of electron donor concentration



↑NaLac

- Enhanced reductive dechlorination
- Minimal addition (31 mg/L) enhanced aerobic degradation
- Above threshold (155 mg/L), inhibition of aerobic degradation – residual organic acids and sulfides depleted O_2

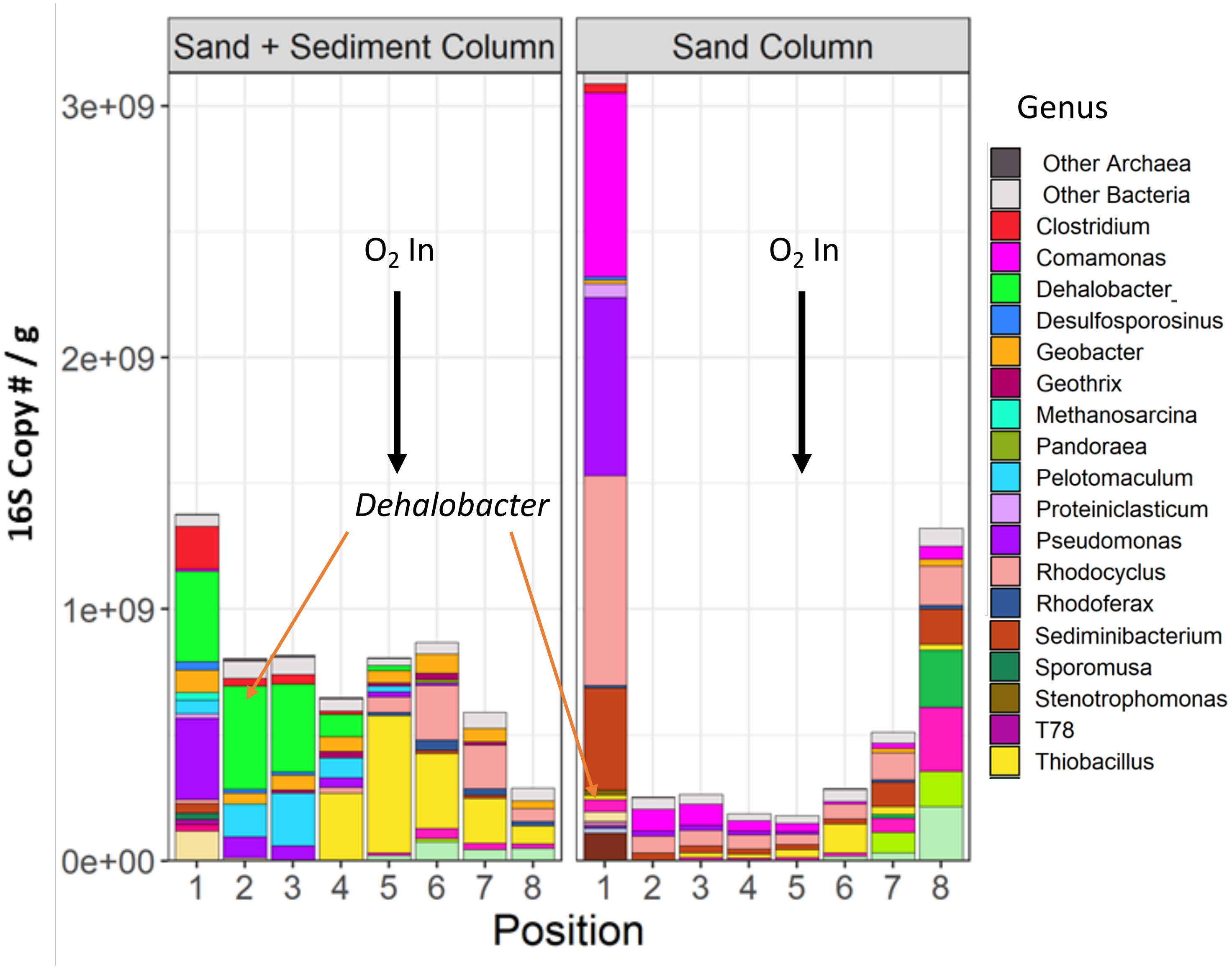
Sand matrix

- Sensitive to NaLac dose
- Greatest observed mineralization

Sediment addition

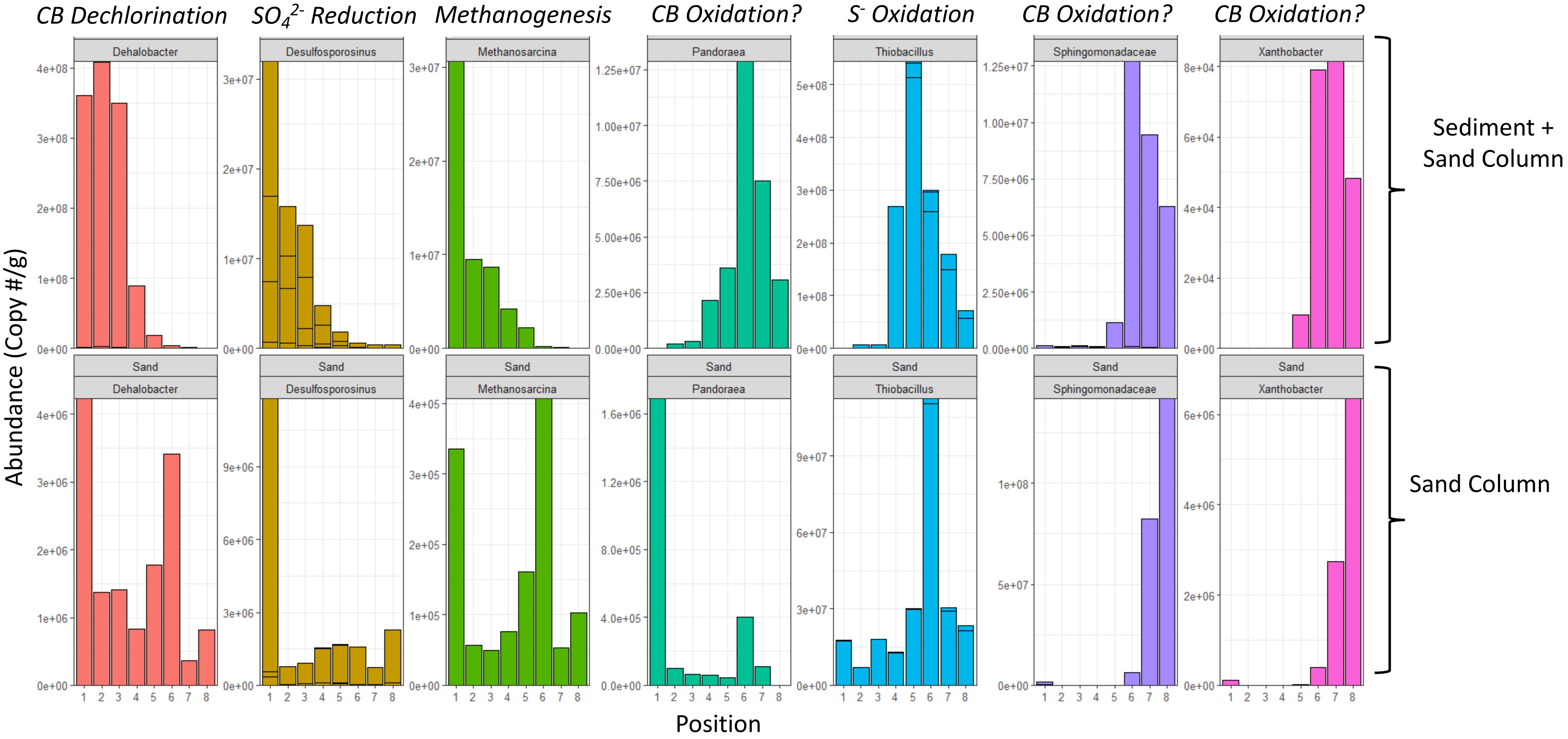
- Stable, enhanced dechlorination at all inputs

Microbial community profile



- Populations highest at influent and at anaerobic-aerobic interface
- *Dehalobacter* enriched in biofilm as anaerobic dechlorinator (shift from *Dhc* and *Dhg* in WBC-2)
- High enrichment in sediment column (up to 50% of community)
- Low enrichment (<1%) in sand column
  - More sensitive to lower concentrations, but same order of magnitude degradation
- Sediment column enriched with functional bacteria
  - *Desulfosporisinus* (sulfate reduction)
  - *Methanosarcina* (methanogenesis)
  - *Thiobacillus* (sulfur oxidation)
- Sand enriched with functionally ambiguous biofilm-forming bacteria *(Comamonas, Pseudomonas*)
- Diverse aerobic generalists – difficult to determine aerobic bacteria

Functionally-relevant bacteria

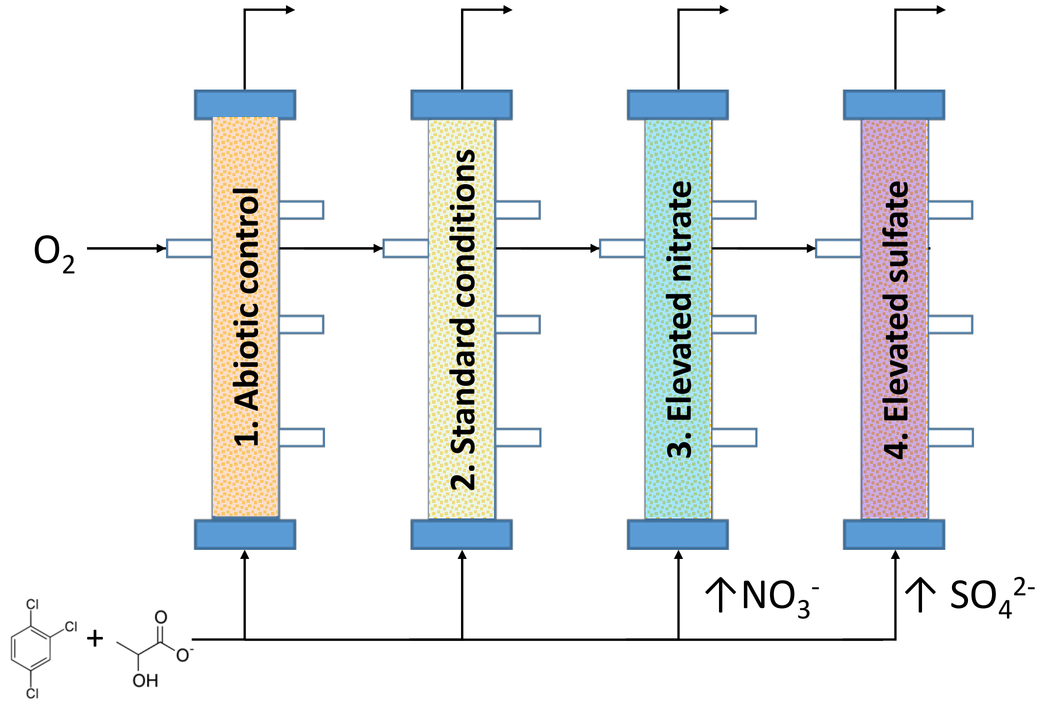


Influence of electron acceptor dose

- 300-day parallel column study
- Simple sand matrix system
- Vary nitrate and sulfate doses over time

Stepped e^- acceptor concentrations in experiment phases

Phase	Time (d)	NO_3^-		SO_4^{2-}		n
		mM	mg/L	mM	mg/L	
I	60	0	0	0.15	14	7
II	60	0.15	9.3	0.5	48	5
III	58	0.5	31	2.5	240	6
IV	103	2.5	160	10	960	3



Influence of electron acceptor dose

↑ Nitrate

- ↓ Reductive dechlorination
- ↑ Aerobic degradation
- Significant change >= .5 mM

↑ Sulfate

- ↓ Reductive dechlorination
- ↓ Aerobic degradation
- Significant change >=2.5 m

Stepped e^- acceptor concentrations in experiment phases

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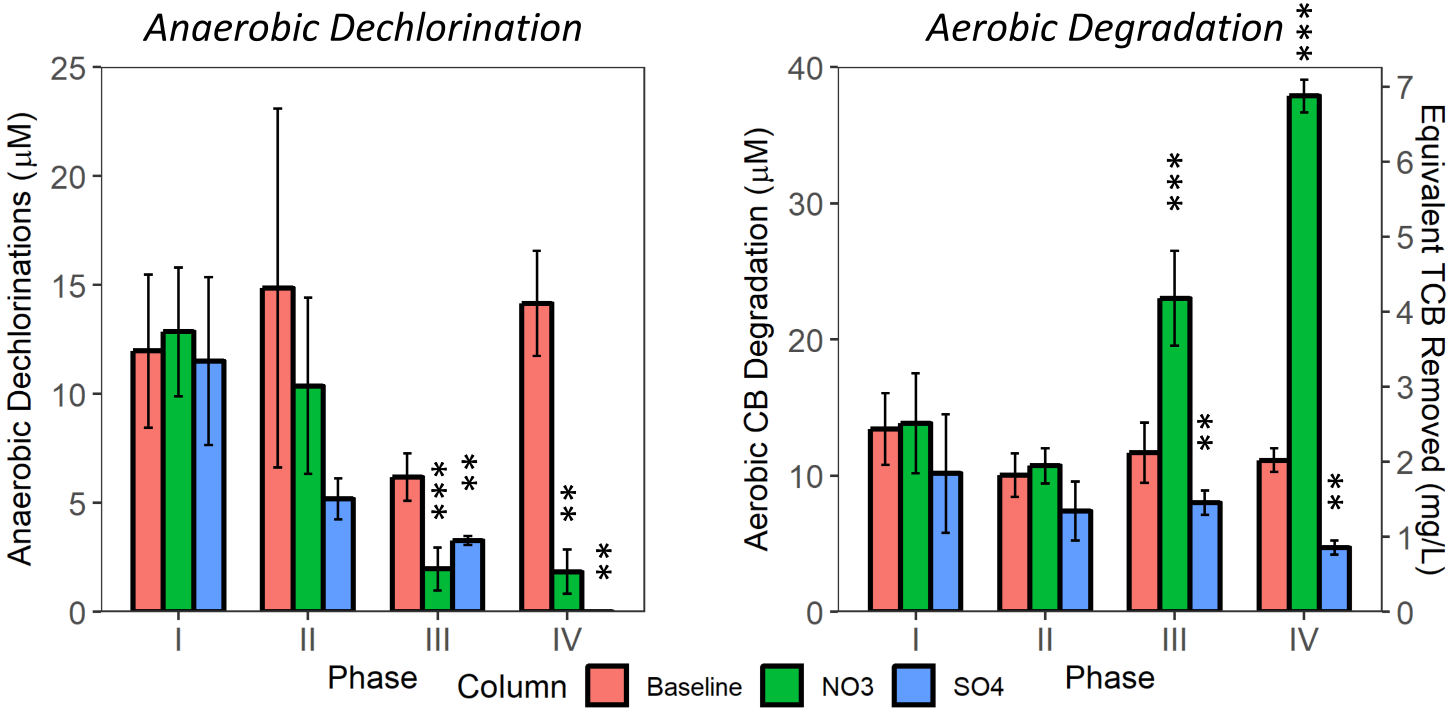
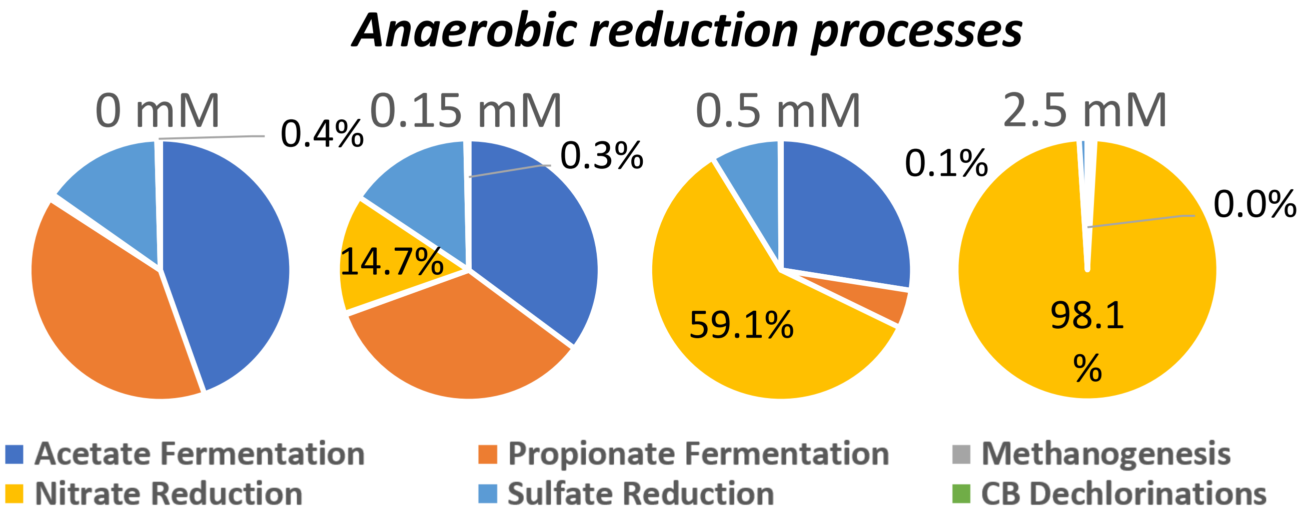
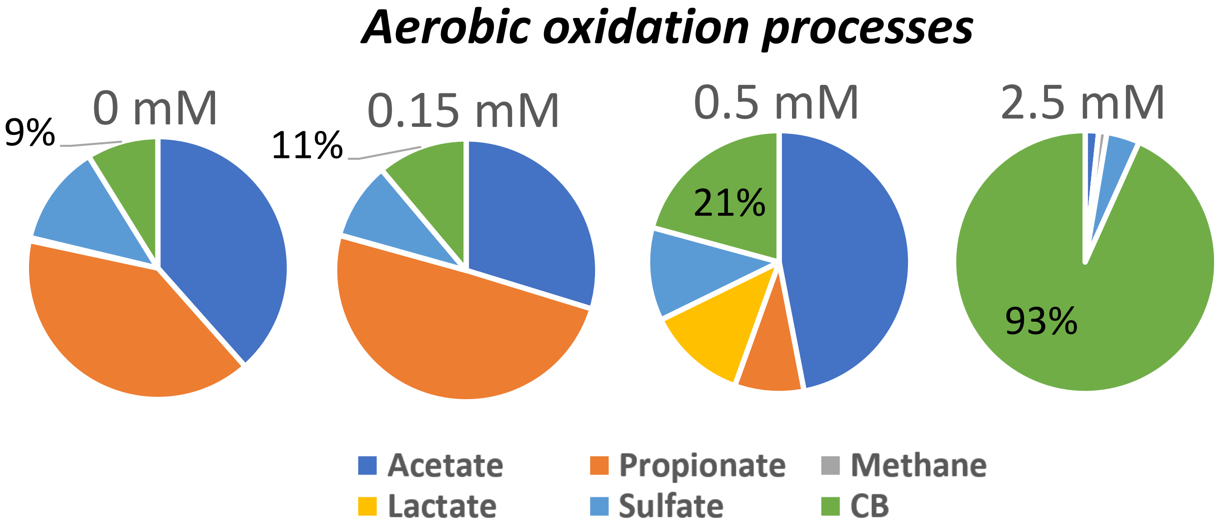


Significance vs baseline ** *p < .01* *** *p < .001*

Nitrate effect on electron donor / acceptor utilization


Anaerobic reduction processes

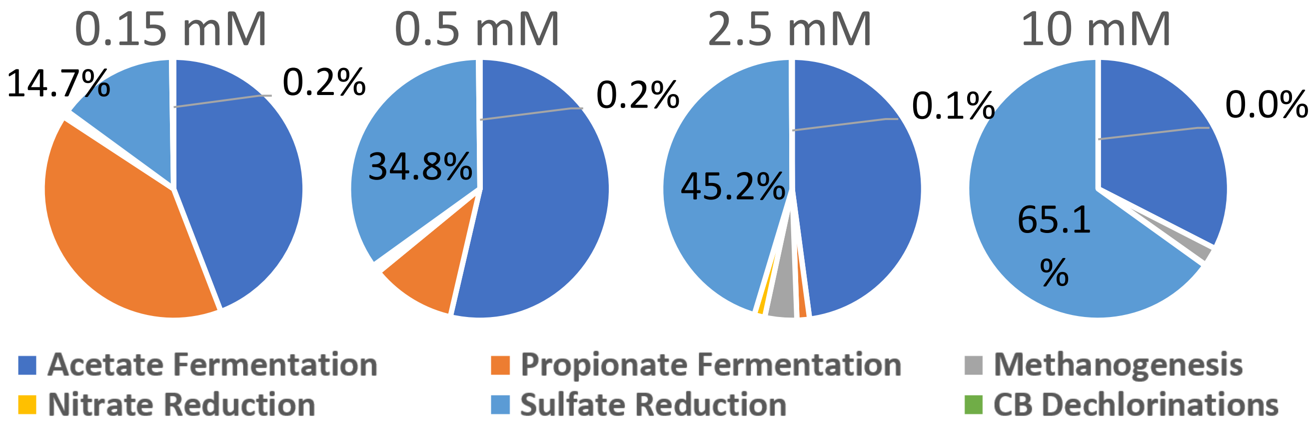

Aerobic oxidation processes

- $\uparrow NO_3^-$
  - Nitrate reduction outcompetes other anaerobic processes, forming permanent e^- donor sink
  - CB dechlorination inhibited
  - Depletes residual organic acids within anaerobic zone
- Majority of e^- donor (>99.5%) not used for CB dechlorination (observed in all columns and conditions)

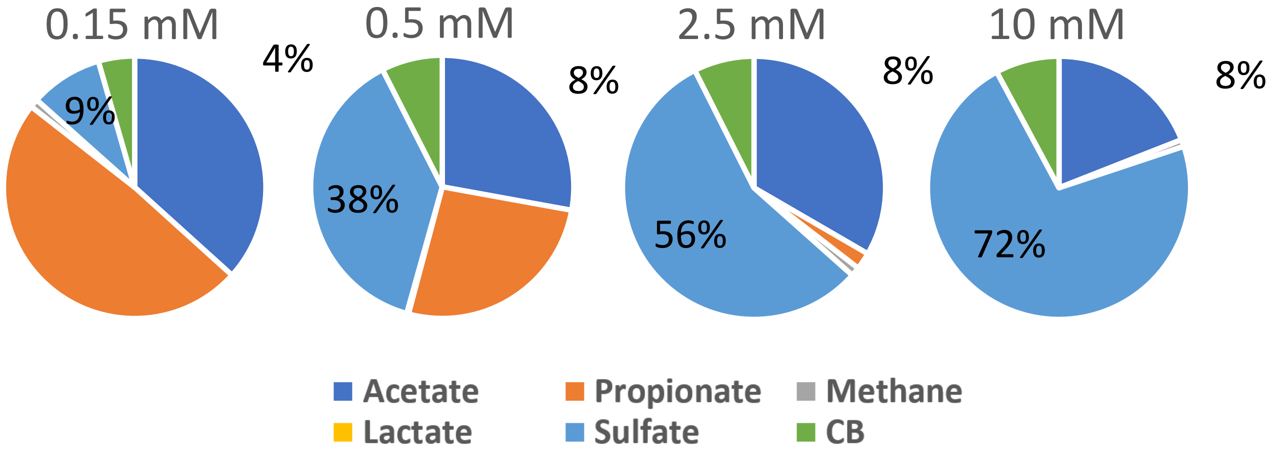
- $\uparrow NO_3^-$
  - Inhibited organic acid and sulfide production minimizes competition for O_2
  - CB oxidation dominates
- No NO_3^- reduction in aerobic zone, so NO_3^- not utilized as supplemental e^- acceptor for CB degradation

Sulfate effect on electron donor / acceptor utilization

Anaerobic reduction processes



Aerobic oxidation processes



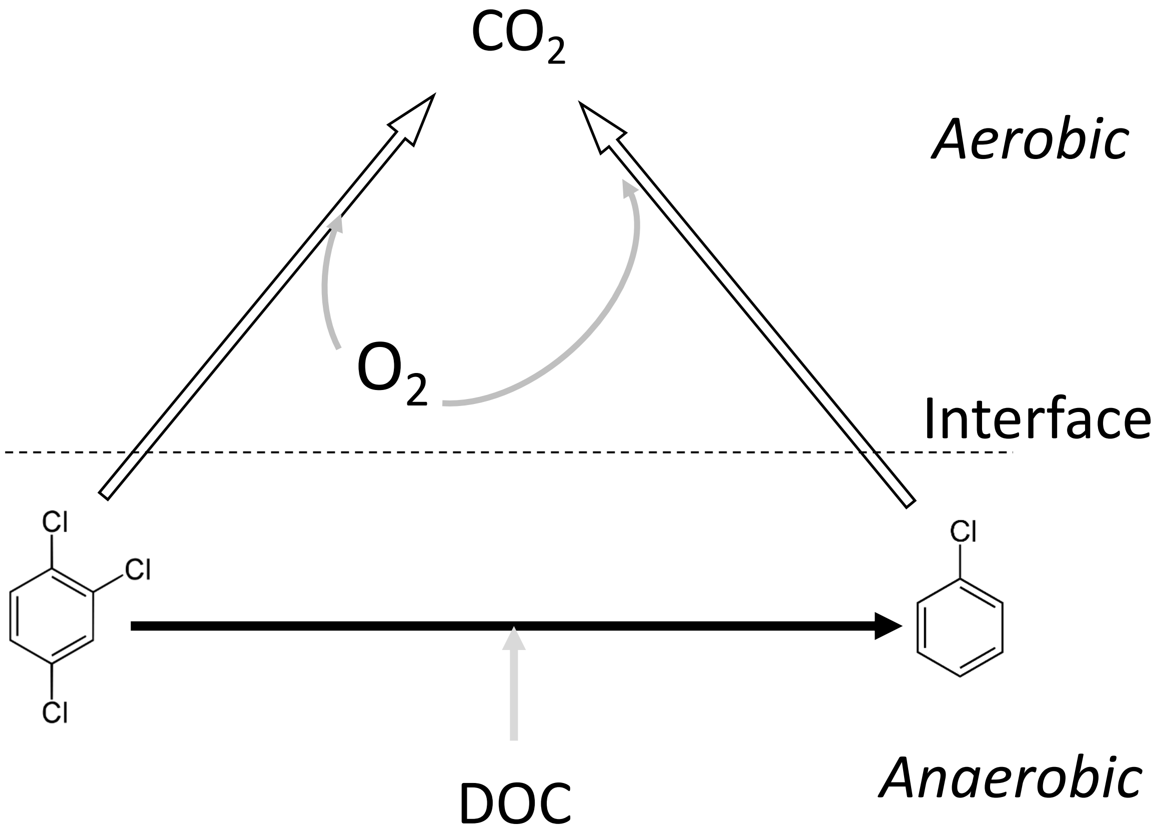
- $\uparrow SO_4^{2-}$
  - Increased sulfate reduction
  - Propionate formation and CB dechlorination inhibited.
  - Methanogenesis and acetate fermentation persist
  - Residual organic acids remain

- $\uparrow SO_4^{2-}$ -
  - Increased competition for O_2 by reduced sulfides, limiting aerobic CB degradation
  - Aerobic CB degradation persists
- Unlike NO_3^-, reduced sulfur easily re-oxidized by aerobes

Sulfur detrimental to both anaerobic and aerobic CB degradation processes, wasting donor/acceptor as intermediate between lactate and O_2

Key points

- **Both anaerobic and aerobic pathways sustained in model anaerobic-aerobic interface**
  - However, necessity for reductive dechlorination to facilitate aerobic degradation not demonstrated with 1,2,4-TCB. Aerobic degradation potential may be congener, site, and community-dependent
- DOC had stimulatory effect on both aerobic and anaerobic degradation processes, but above certain threshold (50 mg/L DOC) increased O_2 demand inhibited aerobic degradation
- Sediment amendment facilitated enhanced anaerobic processes
- 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



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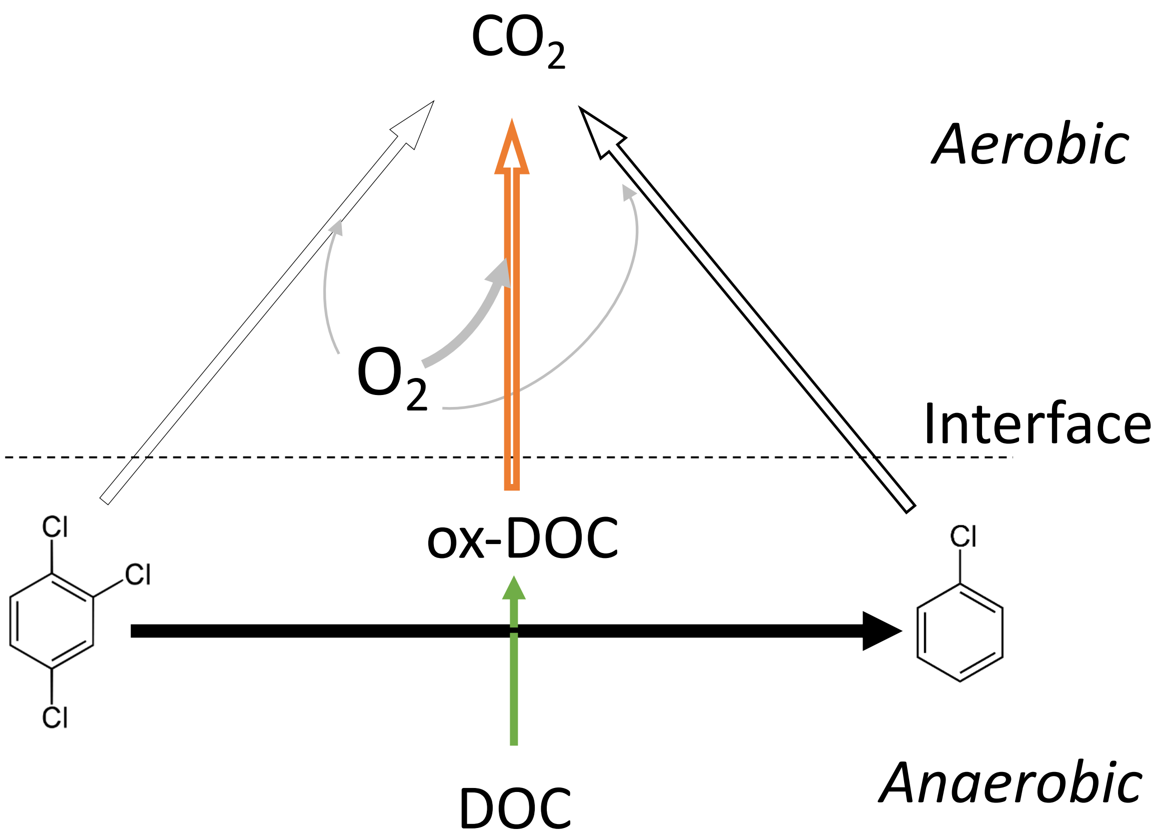

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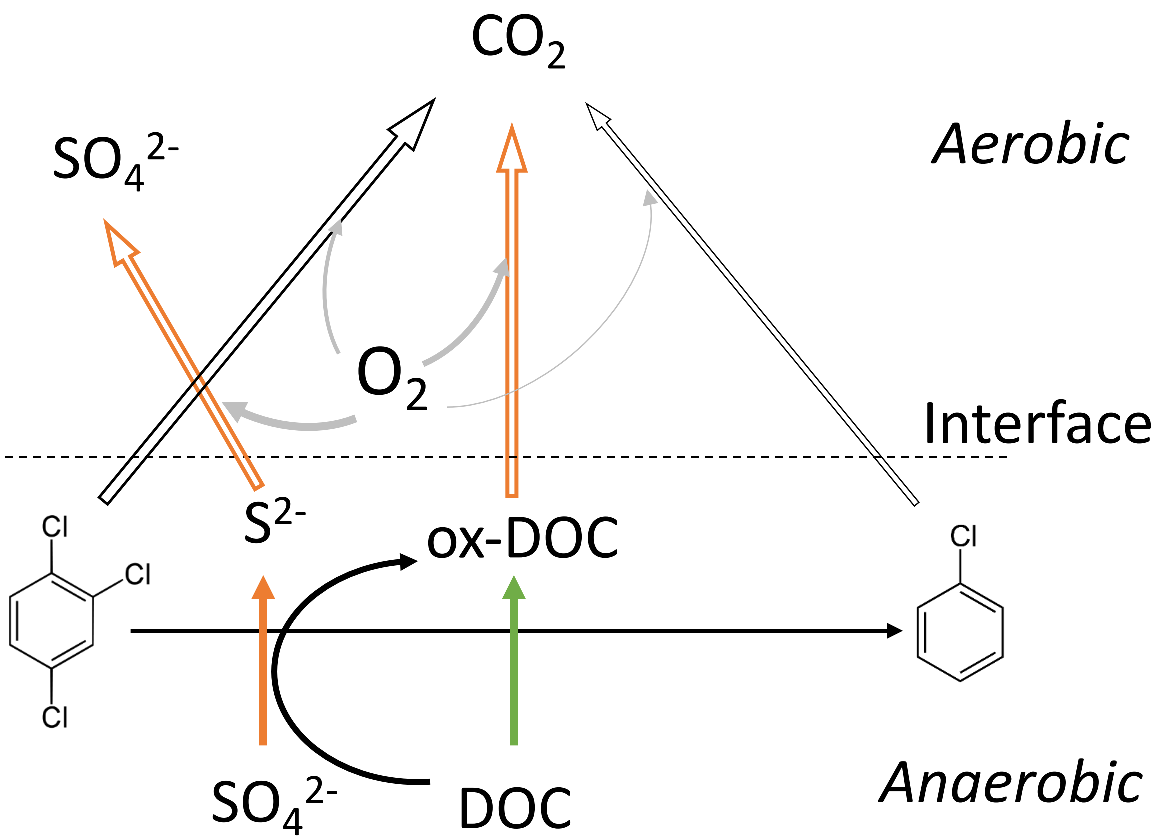

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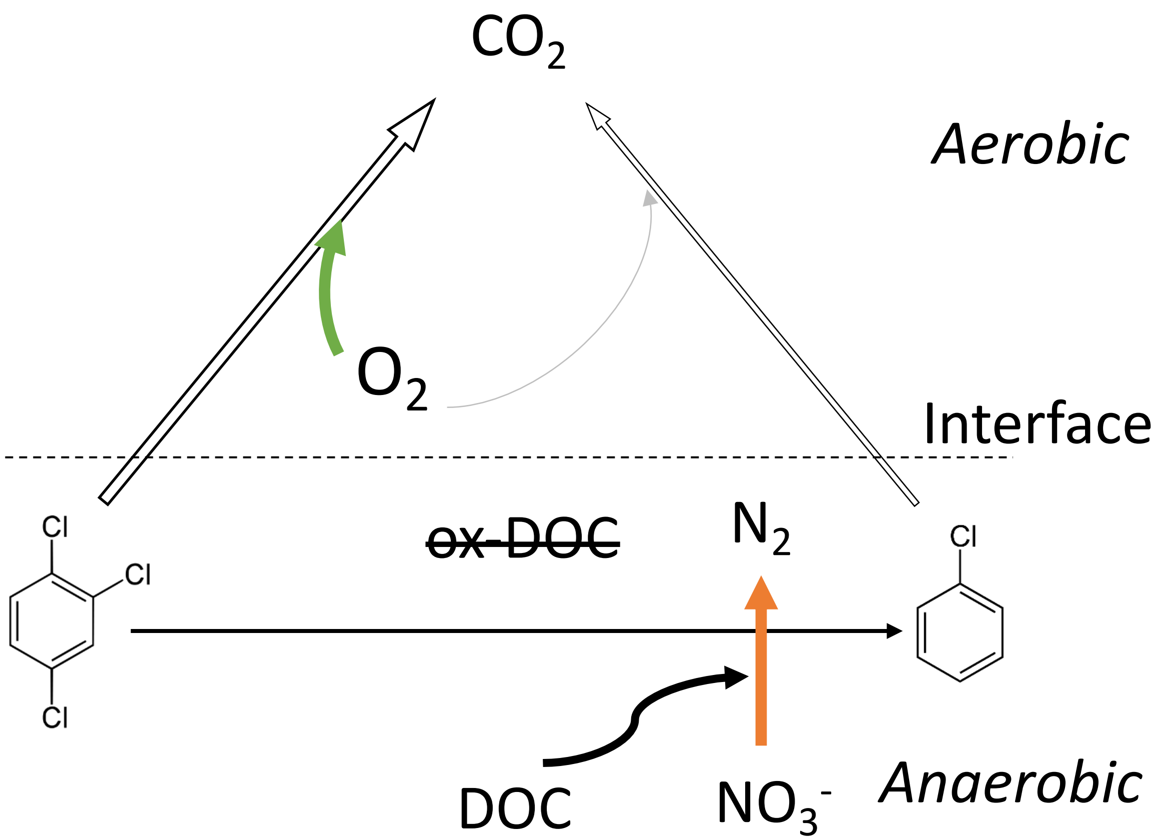

Field-scale testing

- Field tests by collaborators at US Geological Survey
- 1 x 1 m^2 test plots at contaminated SCD Superfund wetland
- Sand mixed with GAC, chitin, and bacteria cultures mixed with site sediment
- 2 pilot sites with distinct geochemical conditions (Sites 8, 135)
- Monitor total VOCs and geochemical conditions through time and compared to control plot

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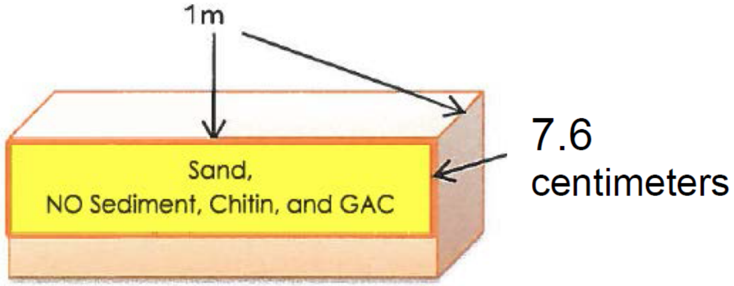

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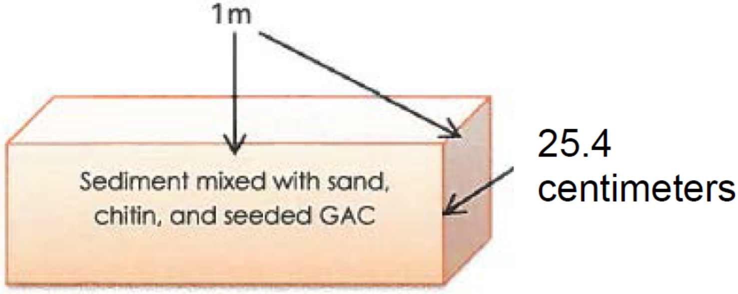

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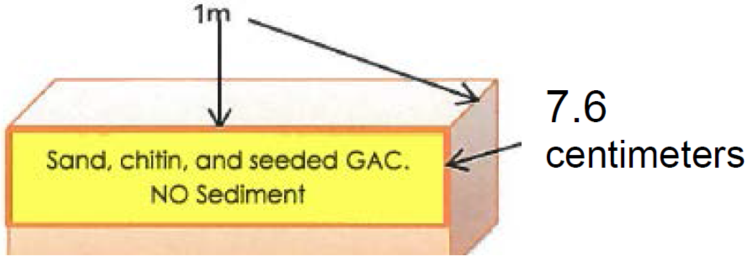
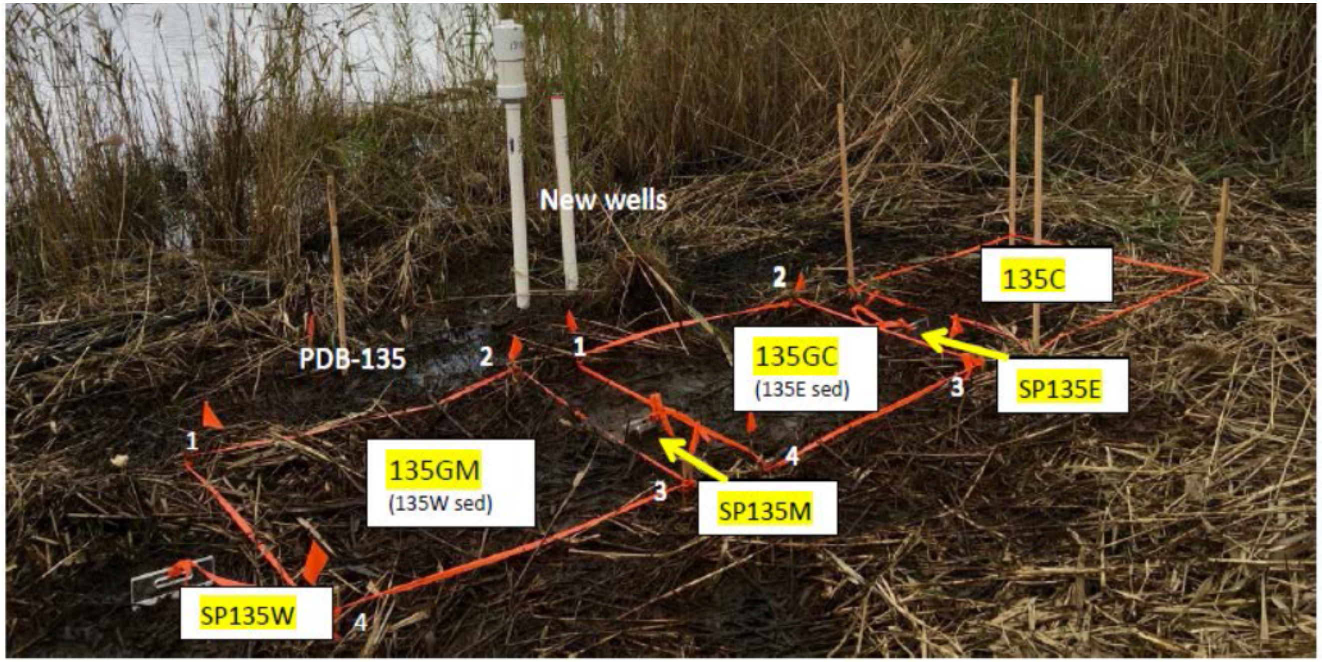
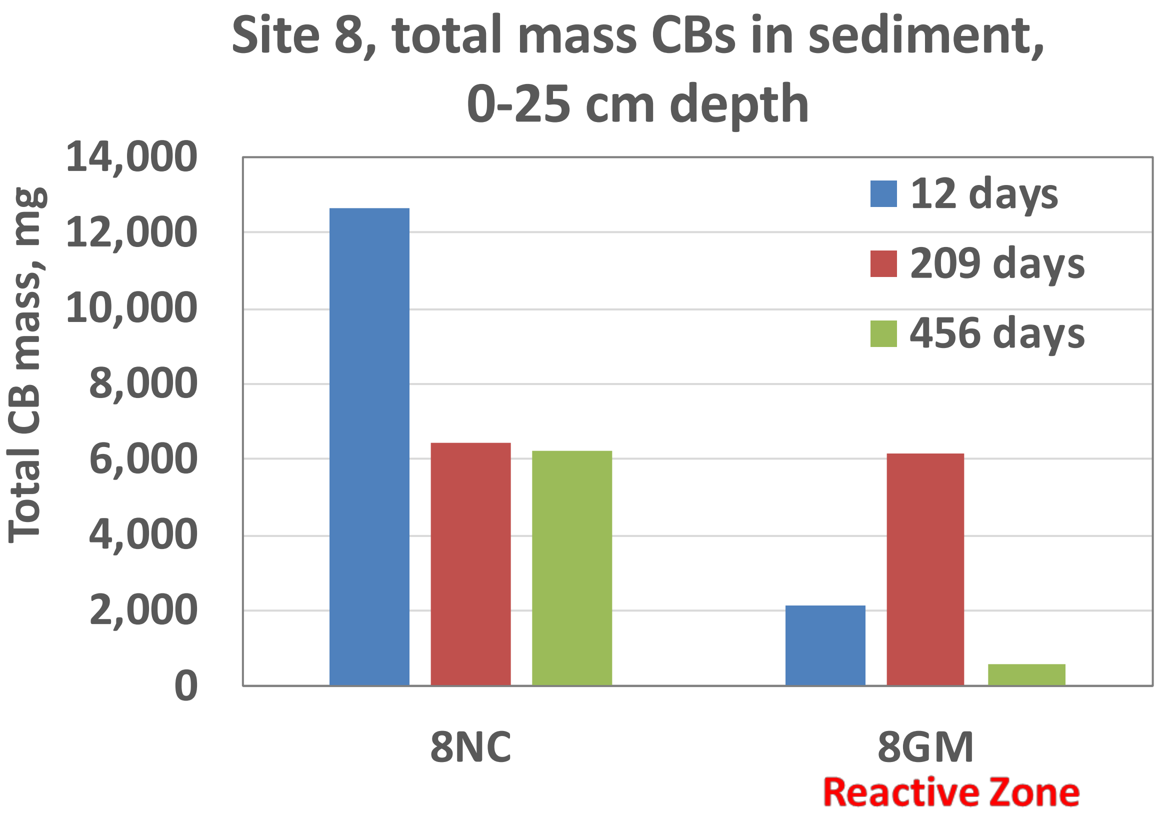
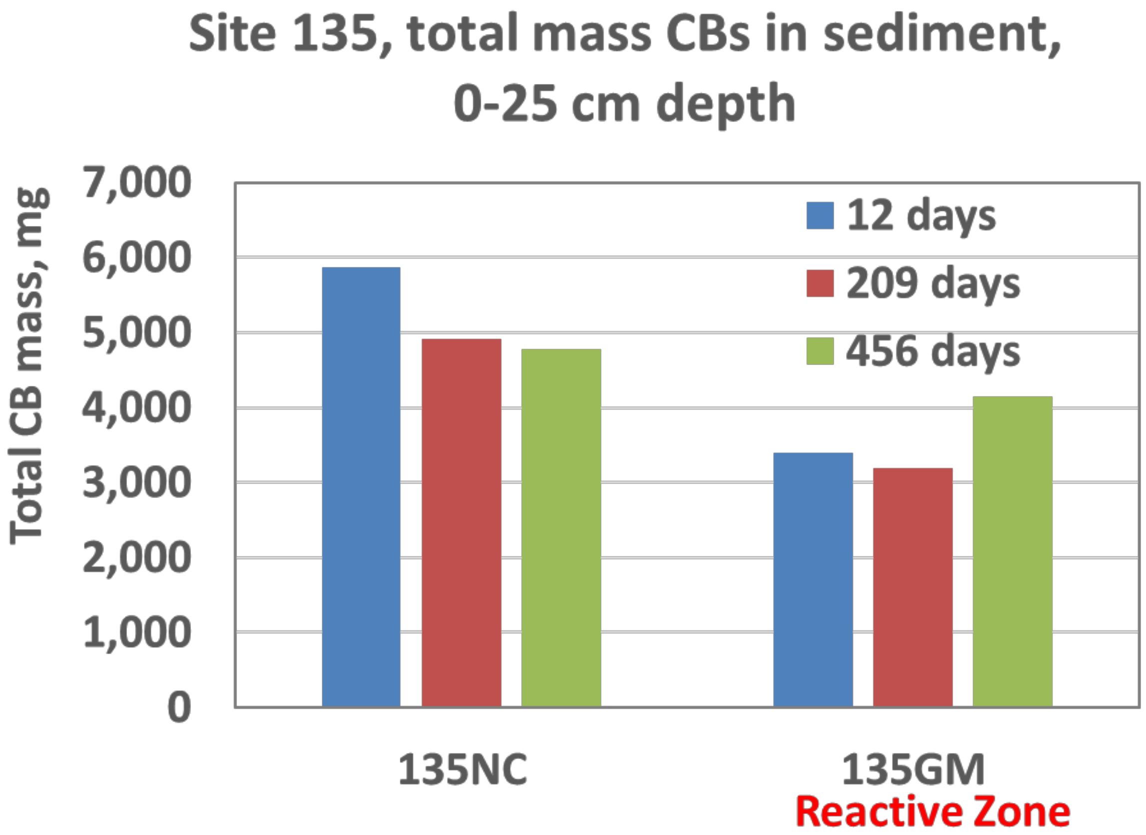




Sediment contaminant mass

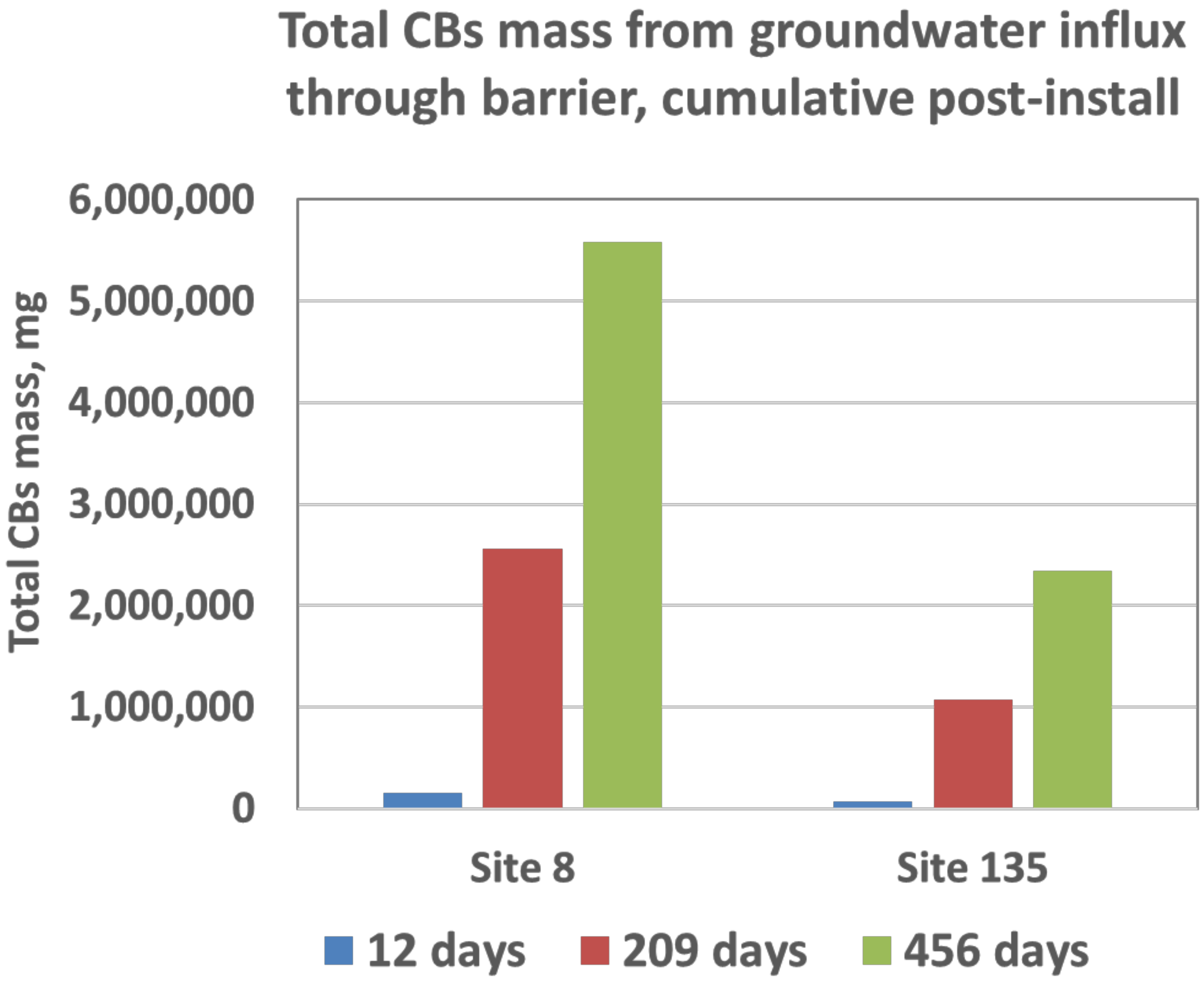
All data in this presentation are provisional.


Site 8, total mass CBs in sediment, 0-25 cm depth


Site 135, total mass CBs in sediment, 0-25 cm depth

- 44 to 74% decrease in sediment mass of CBs in first 12 days compared to controls
- After 12 days, there is still a consistent decrease in total CBs within the reactive barrier zone on each sampling date, but sediment total mass no longer changes significantly over time

Groundwater contaminant mass



Specific discharge, q = 0.25 m/day

- Mass contribution from groundwater influx is **1,000x** greater than sediment mass
- An increase in sediment mass of CBs would have been clearly evident if only sorption to GAC accounted for the removal of CBs from the groundwater
- Because groundwater concentrations exiting the reactive barriers at surface were non-detect:

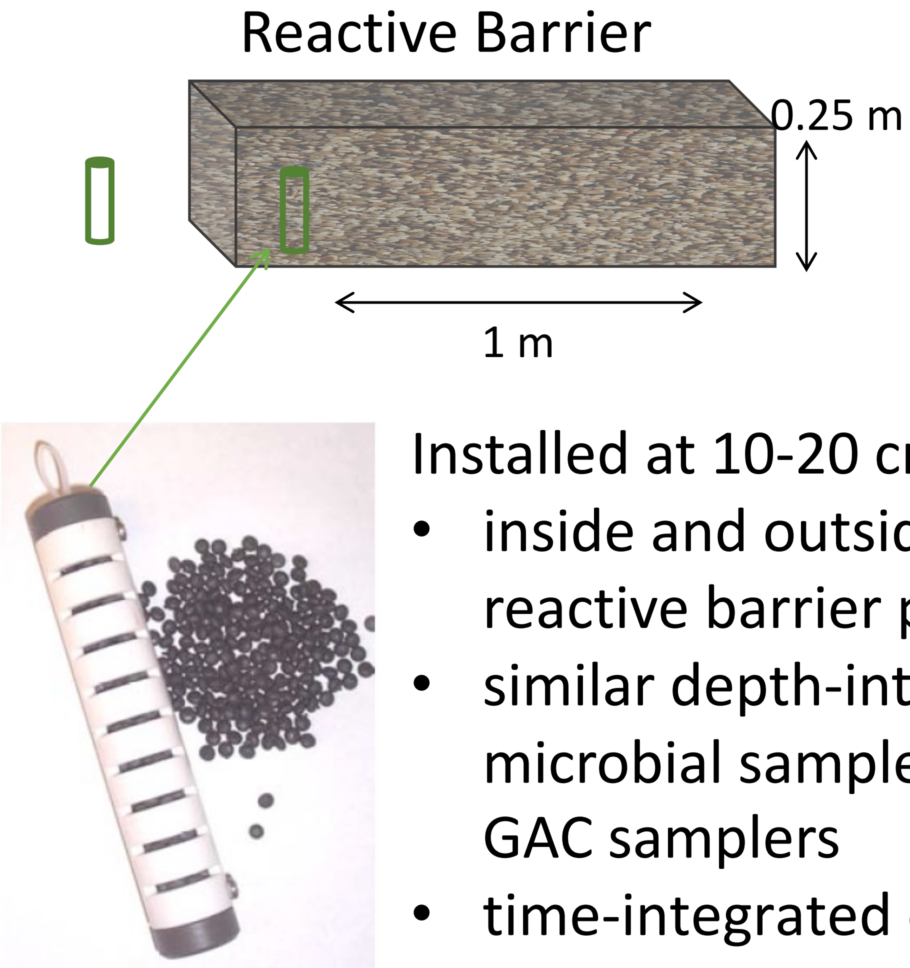
groundwater influx mass = mass removed from water

All data in this presentation are provisional.

In situ Microcosms

Bio-Traps (Microbial Insights) used to conduct in situ microcosms, with and without Biosep beads that pre-loaded with ^{13}C-labeled monochlorobenzene.

- Concurrent microbial and isotopic data to verify biodegradation activity.
- Measure incorporation of ^{13}C in CO2 and PLFA.
- Analysis of functional genes to relate microbial presence to degradation ability.

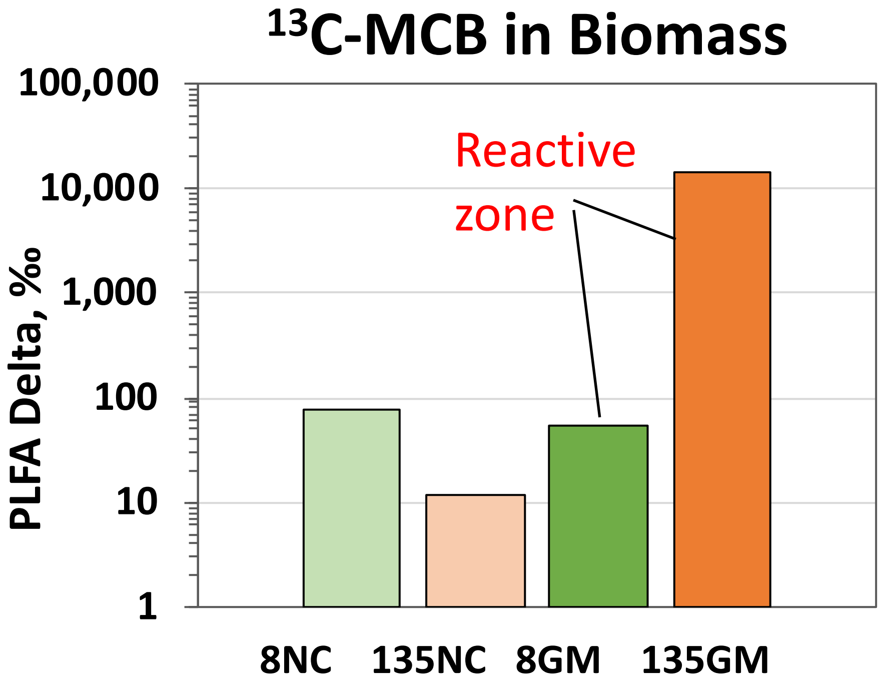


Installed at 10-20 cm bls

- 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?

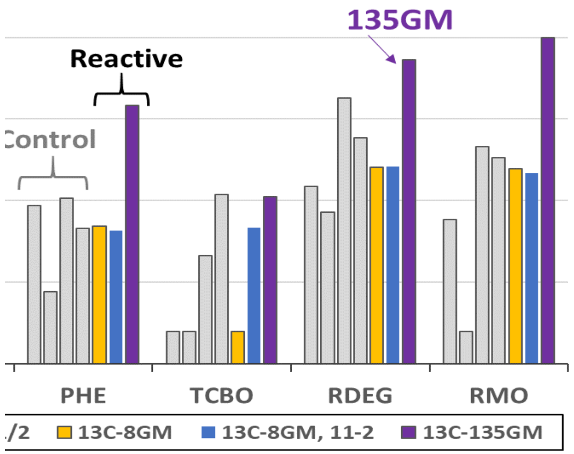
^{13}C-Monochlorobenzene in Biomass in Bio-Traps

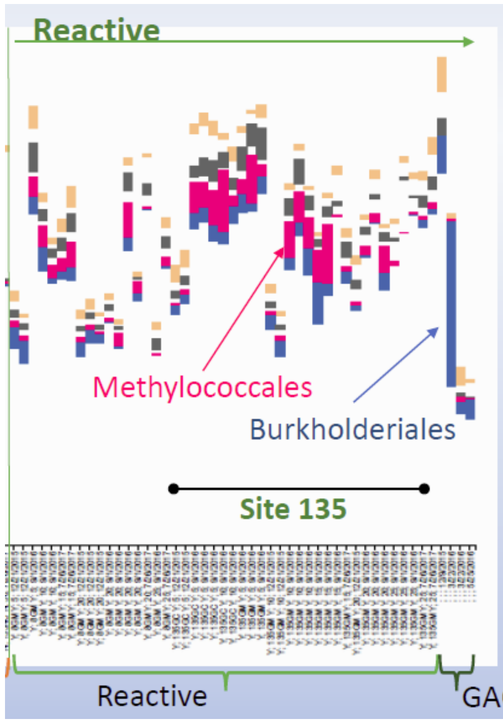


1,000 High

100 Moderate

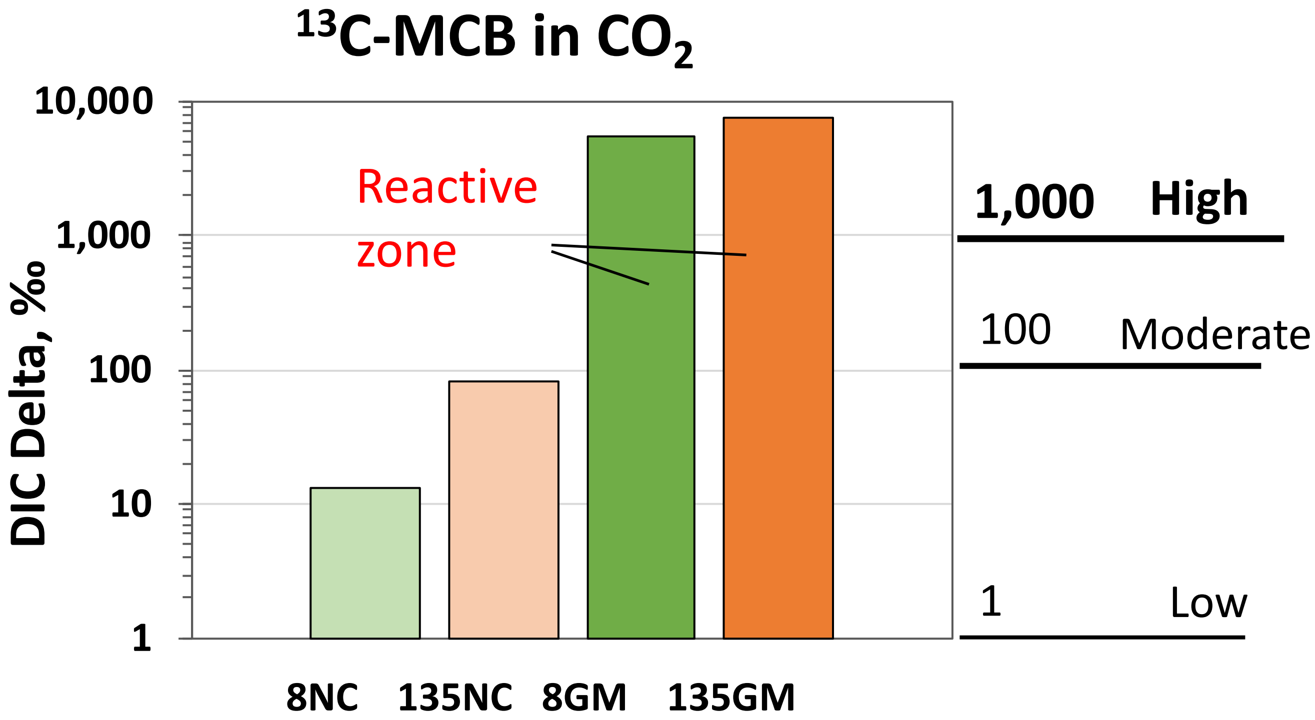
1 Low





- **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.

^{13}C-Monochlorobenzene in Bio-Traps



- 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.

Questions?

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Geosyntec consultants

Remediation implications

- High potential for natural site matrices to degrade CBs anaerobically and aerobically
- Under site-simulated conditions...
  - 1.8-6.9 mg/L 1,2,4-TCB continuously degraded aerobically (rates > 1.6 mg/L-hr^{-1}) across simulated interface
  - 1.5 kg/m^{2}-year^{-1} dechlorinating capacity
  - 0.32 kg/m^{2}-year^{-1} mineralization capacity
- Sites with high sulfate or other re-oxidizable electron acceptors (Fe, Mn, etc.) may suppress anaerobic and aerobic bioremediation efforts
- 16S amplicon sequencing useful tool to ID anaerobic functional potential; less clear aerobic potential

Research needs

- Characterize shifts in microbial communities and functionally-relevant organisms under varied redox conditions (in progress)
- Develop specific tools (shotgun metagenomics, qPCR assays) targeting aerobic functional potential in metabolic generalists
- Development of commercial *Dehalobacter*-based enrichments for CB dechlorination (potential for anaerobic mineralization?)
- Determine impacts of sorption on biodegradation at anaerobic-aerobic interfaces
  - Longer CB retention may possibly facilitate anaerobic mineralization via dechlorination to benzene?