Coupled Biodegradation of Chlorinated Benzenes at Anaerobic-Aerobic Interfaces

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Environmental Health
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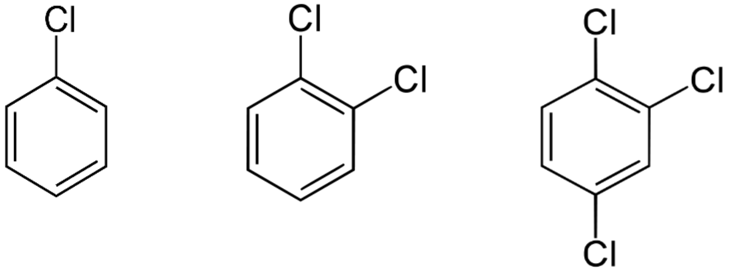
Background

Chlorobenzenes

- Legacy contaminants, present in over 8% of EPA National Priority List sites (1990 estimate)
- Unique physical and chemical properties: sparingly soluble, semi-volatile, aromatic, chlorinated solvents
- Known health and ecotoxicity risks, regulated in drinking water (1-600 µg/L)
- Large spill sites provide challenges for remediation, but also fertile test-beds for fundamental research and new technologies



Standard Chlorine of Delaware Superfund Site

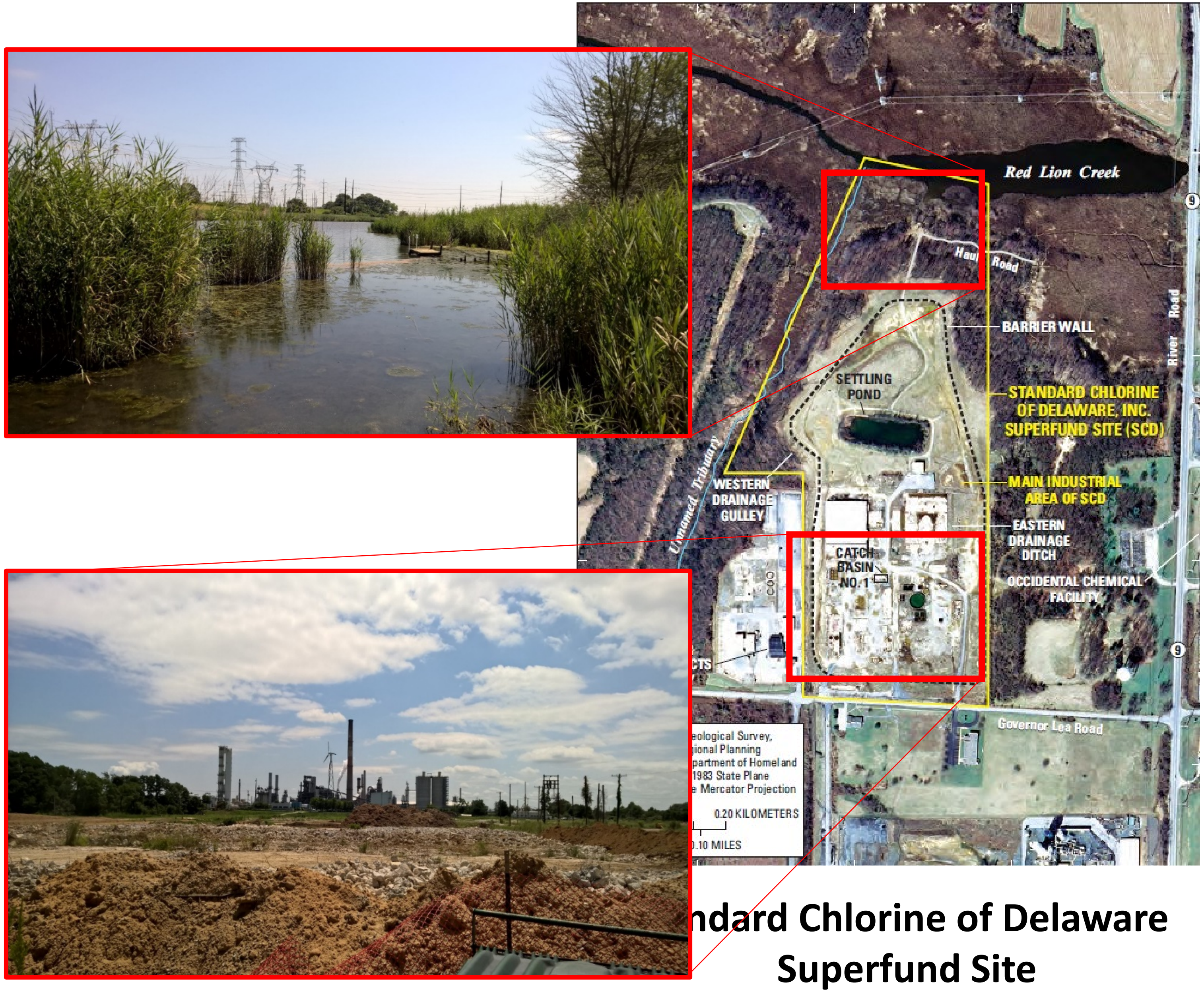
Lorah et al. 2014. USGS

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

Background

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



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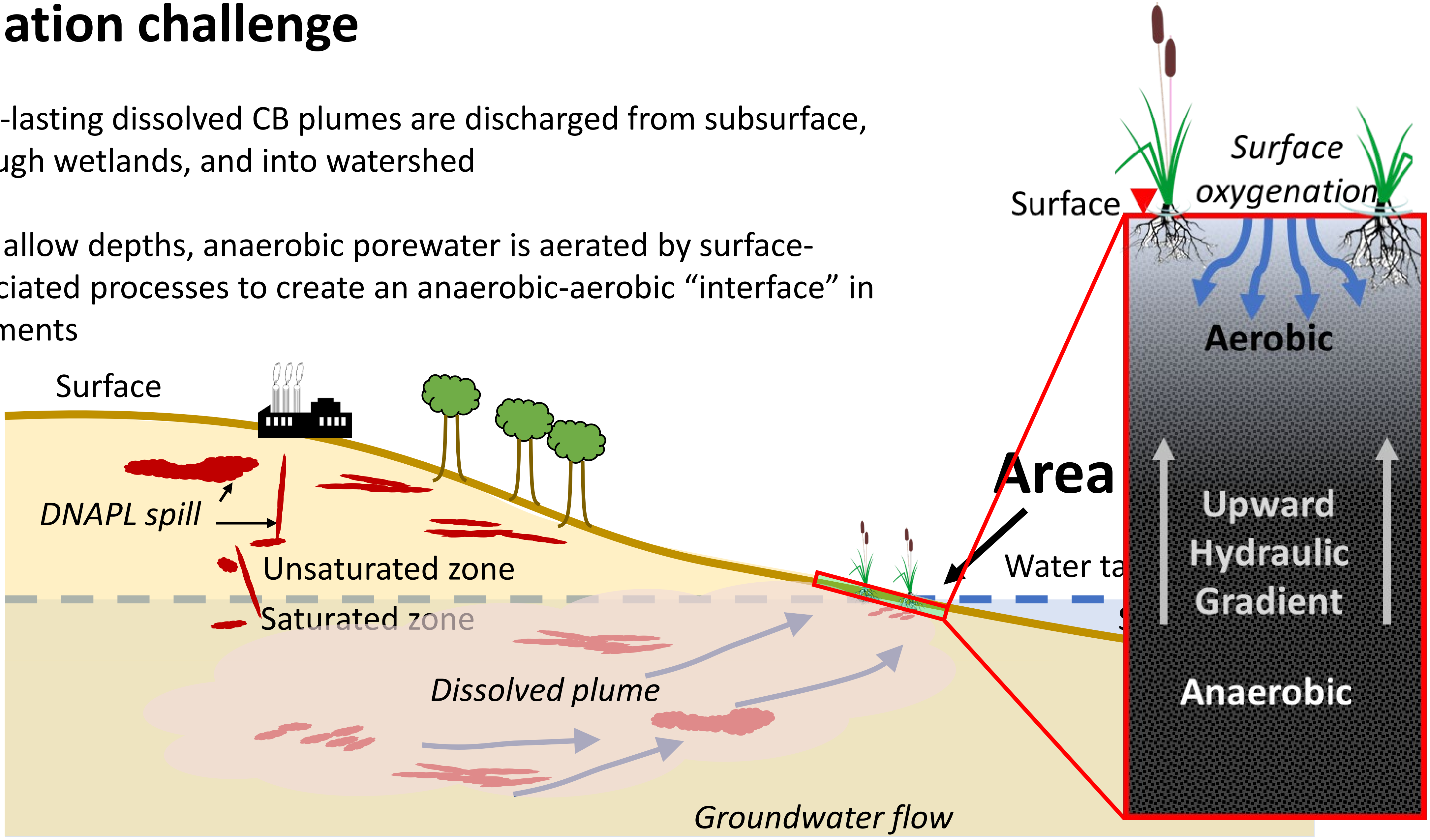
Remediation challenge

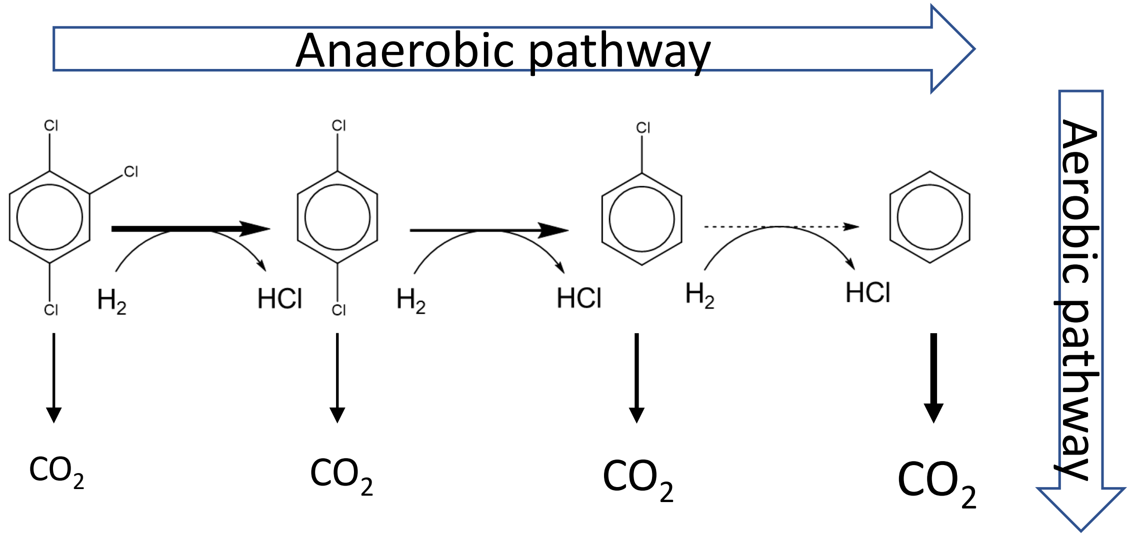
- Long-lasting dissolved CB plumes are discharged from subsurface, through wetlands, and into watershed
- At shallow depths, anaerobic porewater is aerated by surface-associated processes to create an anaerobic-aerobic “interface” in sediments



Lorah et al. 2014. USGS

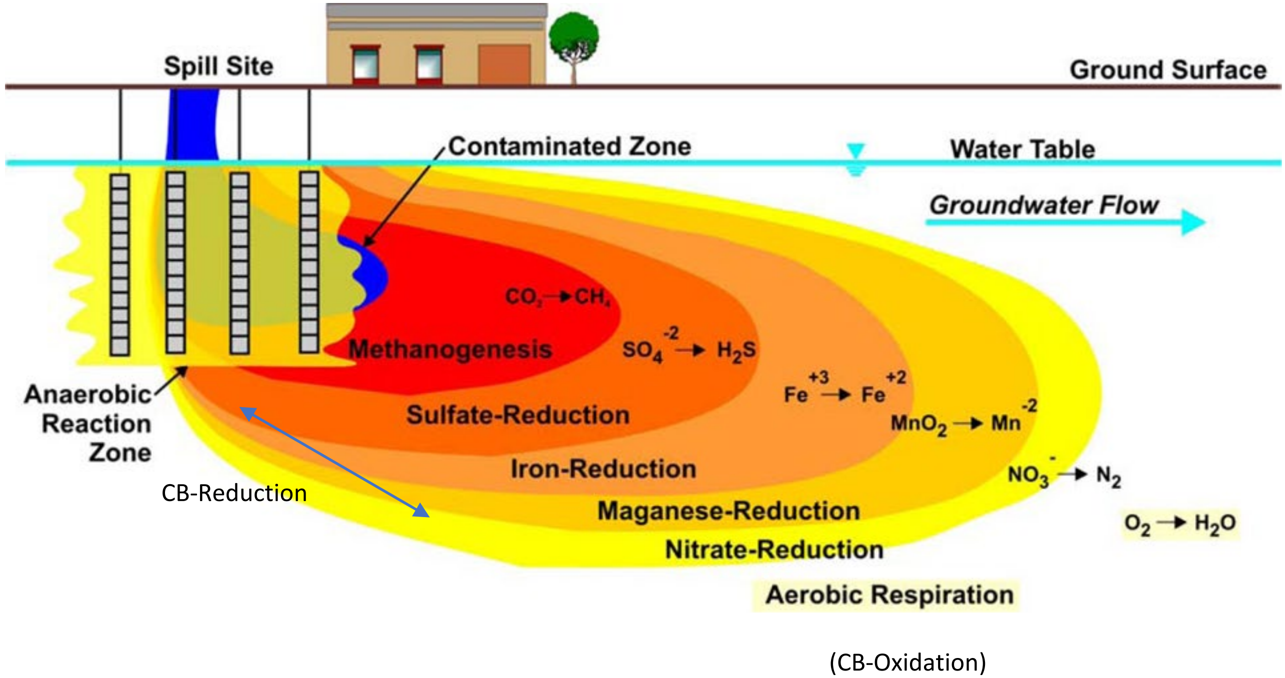
CB biodegradation pathways

- 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 thermodynamically favorable (<= TeCB)
    - Complete mineralization



Research questions

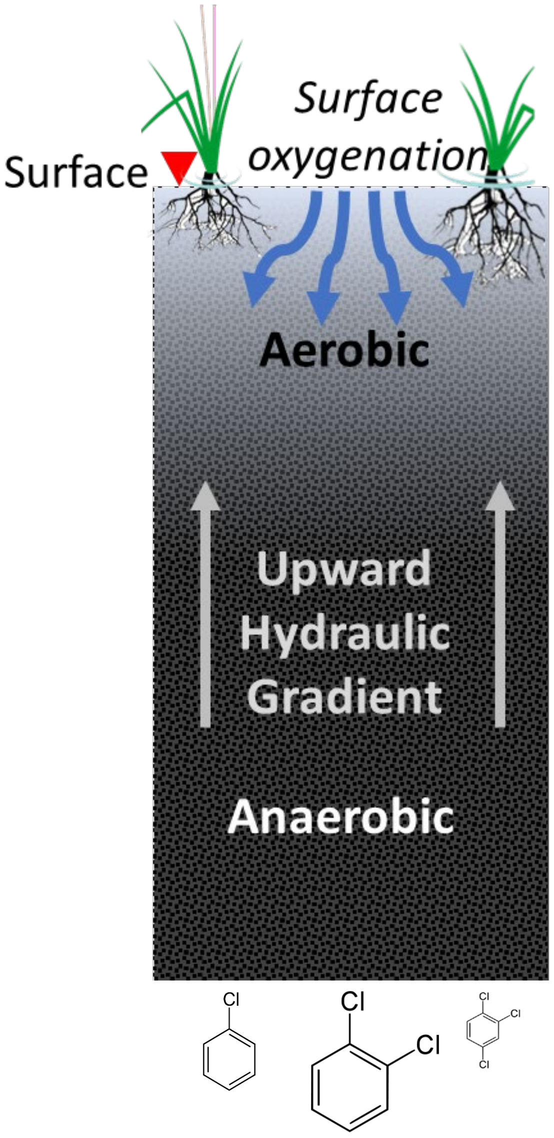
- Redox conditions can be temporally and spatially heterogeneous at sites
- Other externalities (chemical spills, flooding, seasonality) introduce even more perturbation
- SCD site survey
    - Average 14-56 mg/L DOC
    - 0.42 – 1090 mg/L sulfate



- **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



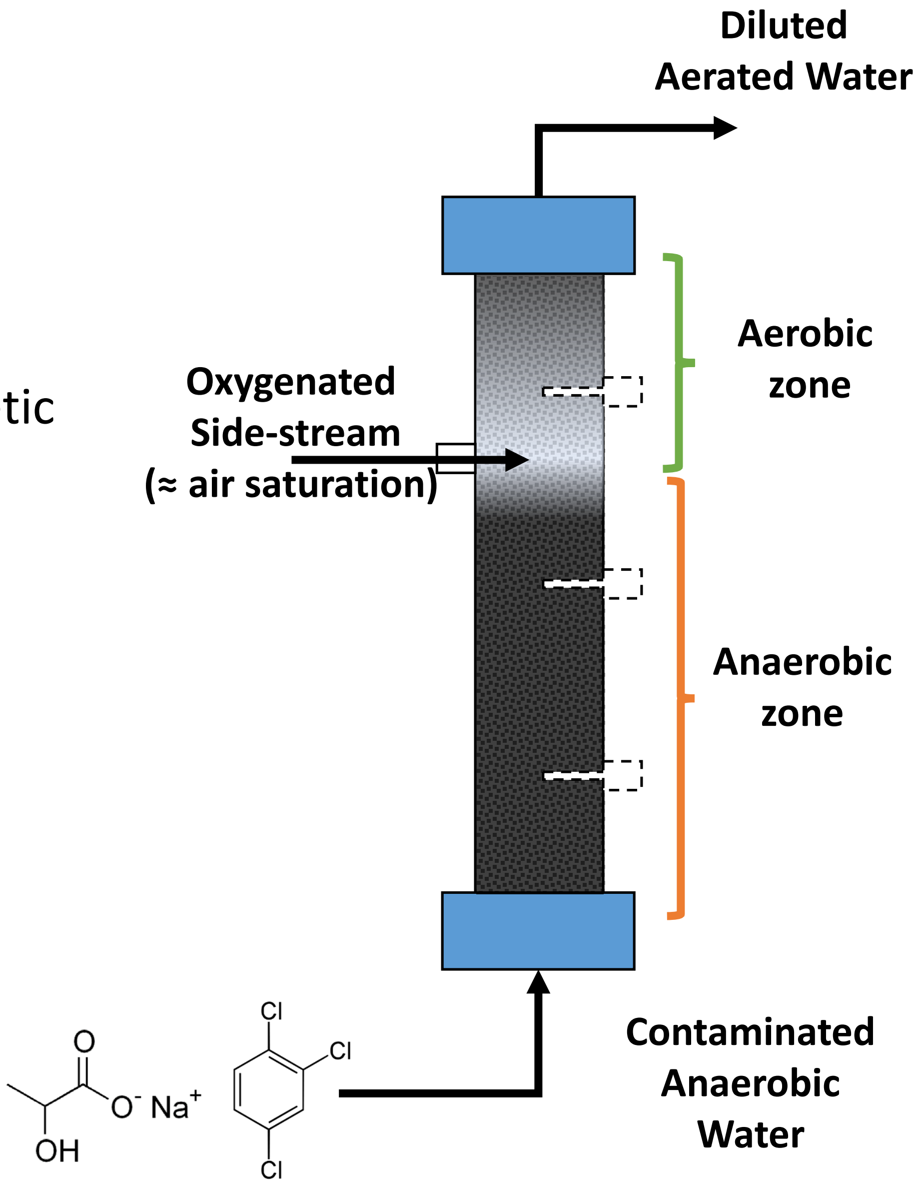
Conceptual model

Simplifications

Natural water → Defined synthetic media

Complex DOC source → Sodium lactate model donor

Variable flow and oxygen flux → Constant-flow system

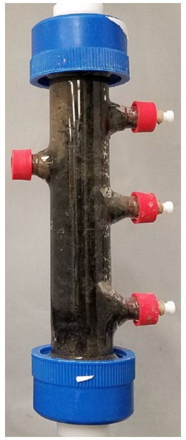


Experimental design

Simulating the interface

Packed columns

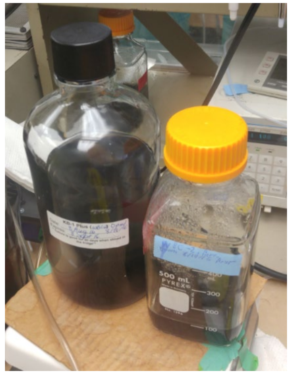
1. Filter Sand



2. Site Sediment + Filter Sand

+

Bioaugmentation cultures

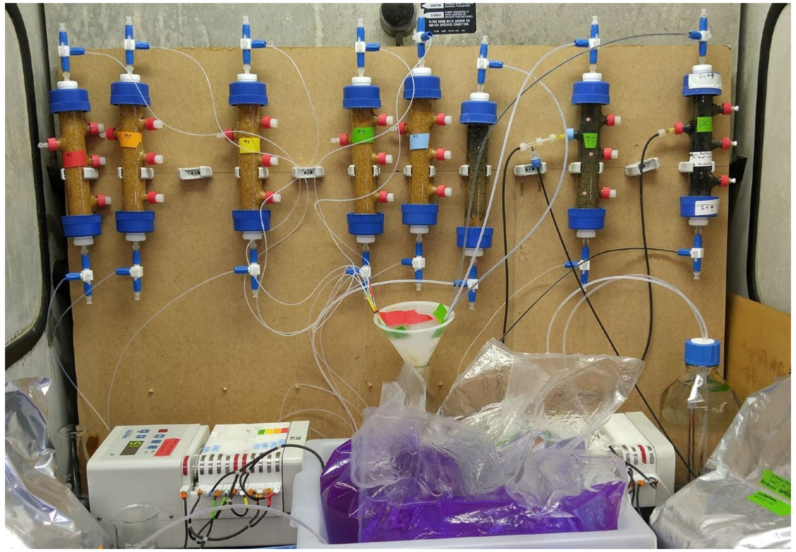


Anaerobic degrader culture (WBC-2, SiREM Labs)

Aerobic degrader enrichment

→

Upflow simulated groundwater system

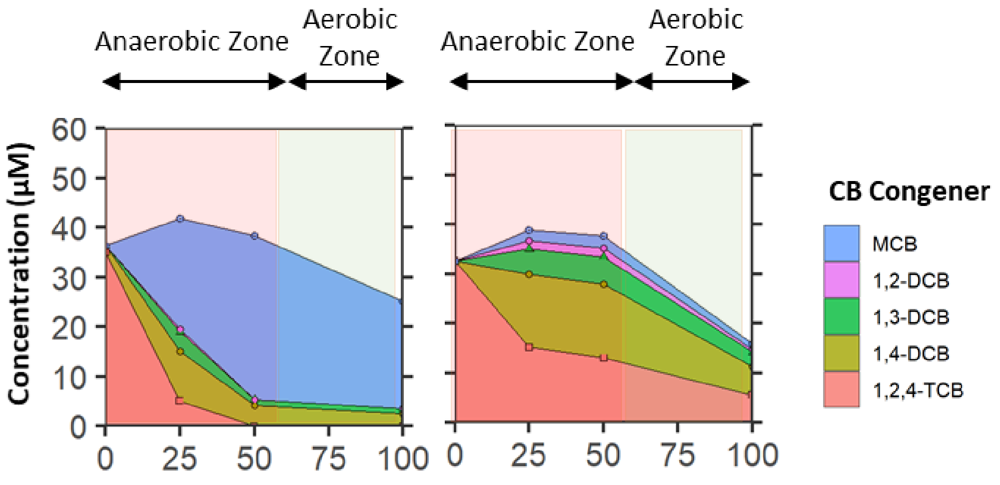


- 300-day continuous flow study
- Low-sulfate, sterilized simulated media
- Aeration to ~ 7 mg/L O_2 in aerobic zone

Proof of concept

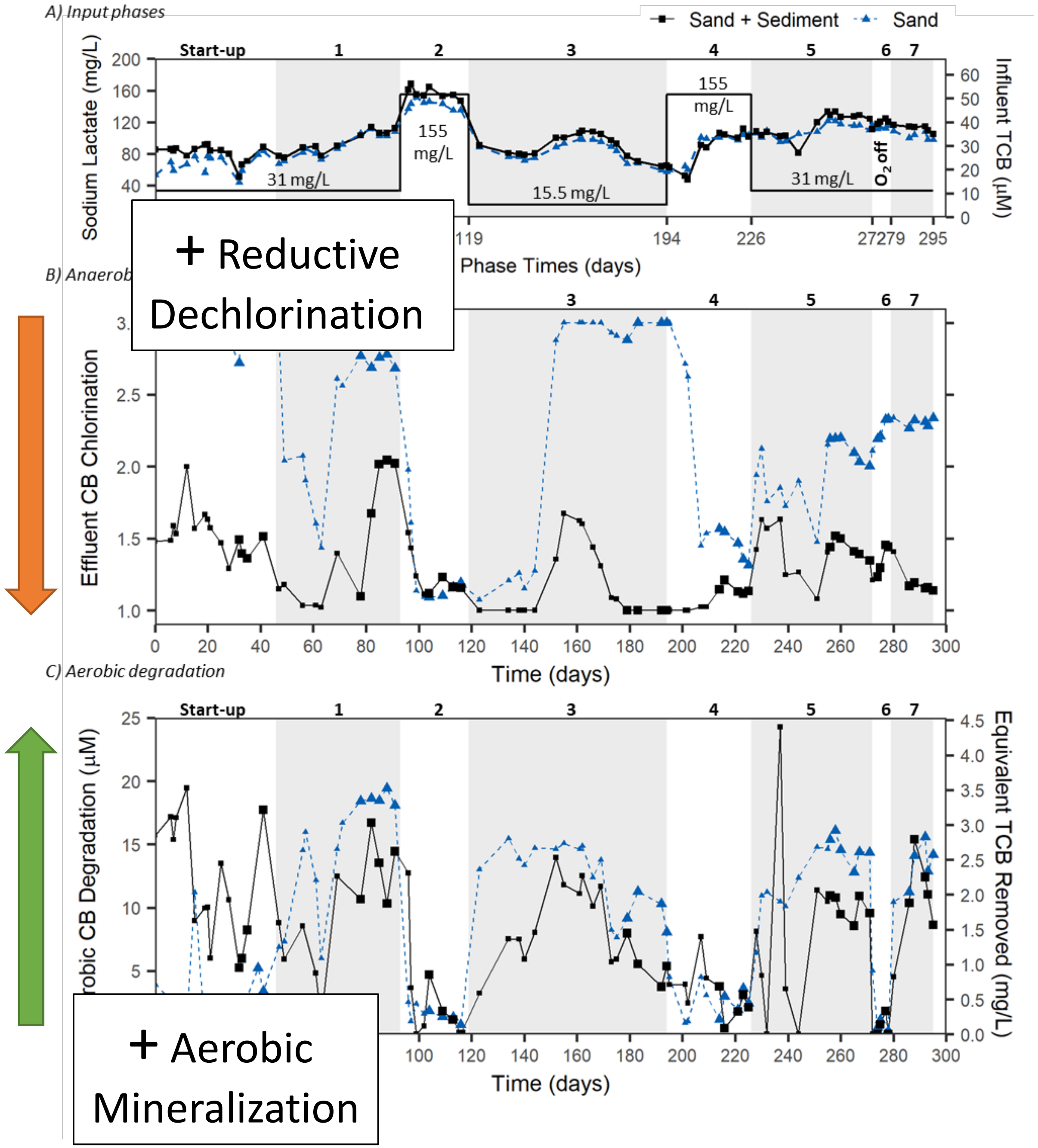
Cycled 15.5, 31, and 155 mg/L sodium lactate (NaLac) influent e^- donor doses (5-50 mg/L DOC)

- 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*

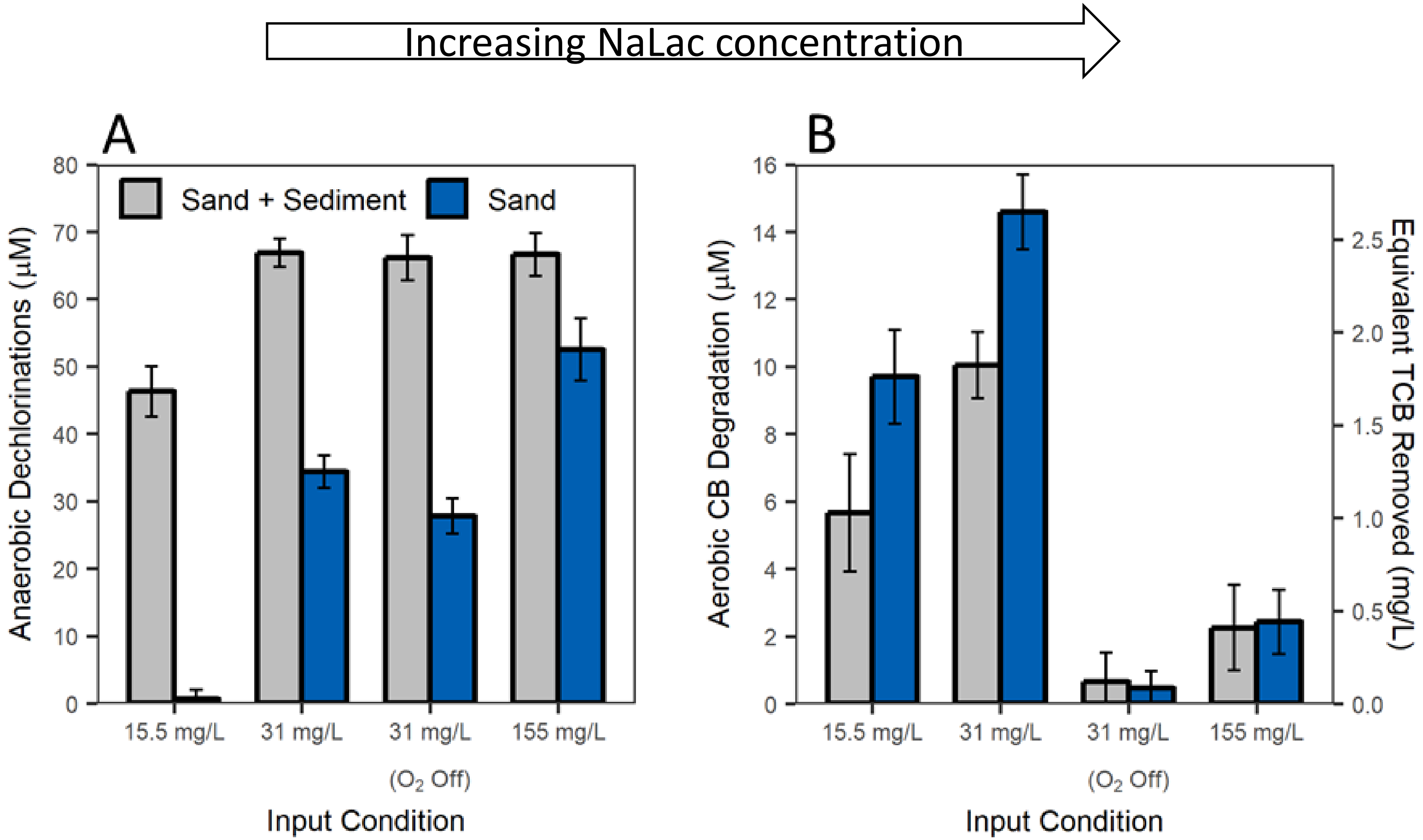
Column Flow →



+ Reductive Dechlorination

+ Aerobic Mineralization

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

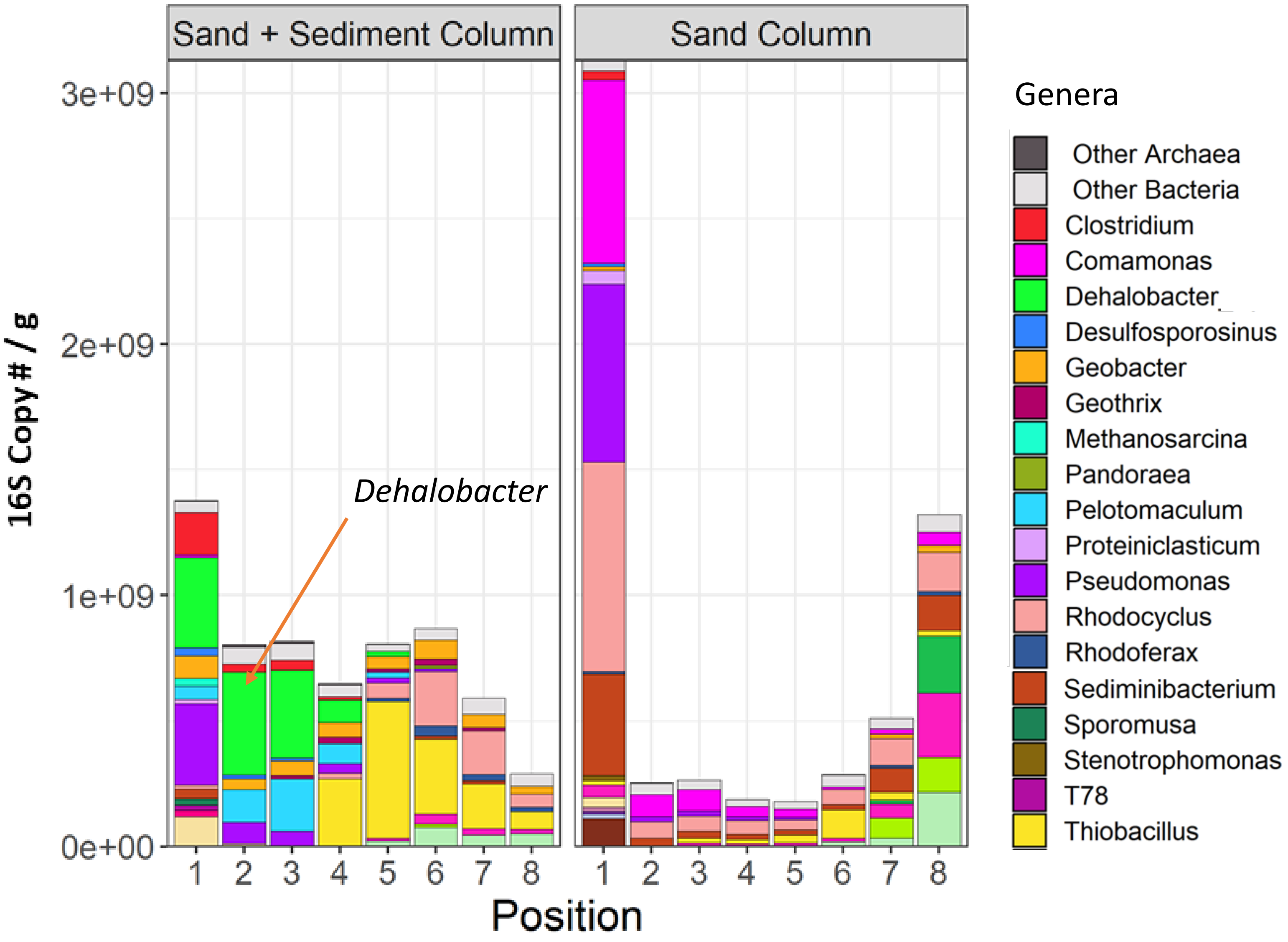
Sand matrix

- Sensitive to NaLac dose
- Greatest observed mineralization

Sediment addition

- Stable, enhanced dechlorination at all inputs

Microbial community profile



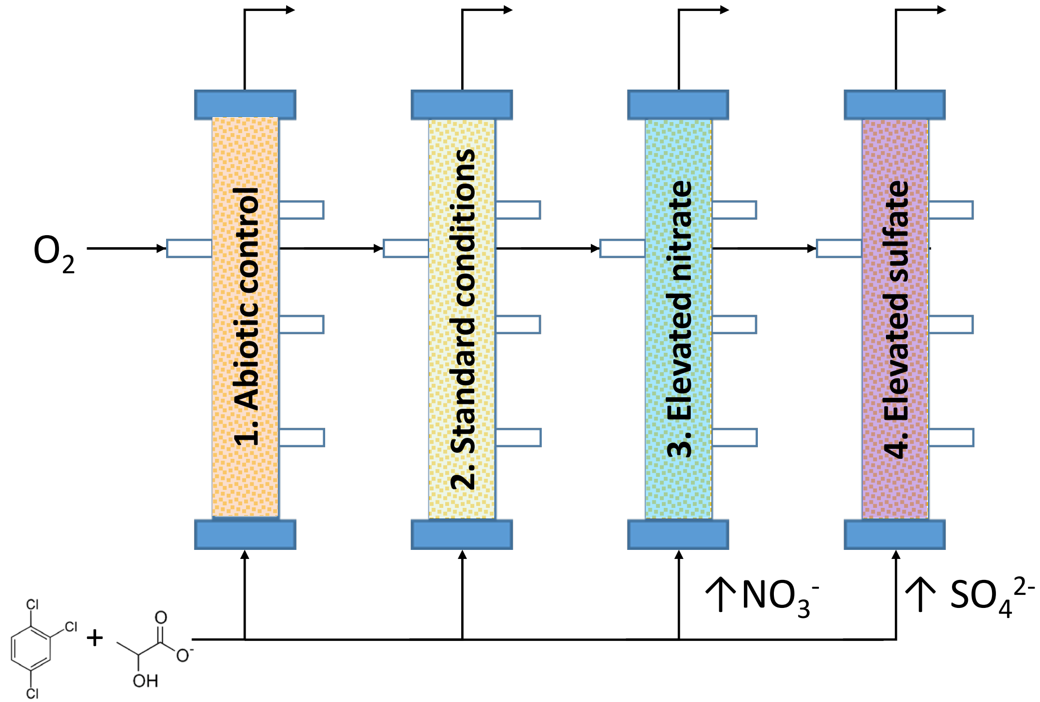
- *Dehalobacter* enriched in biofilm as anaerobic dechlorinator
- High enrichment in sediment column (up to 50% of community)
- Low enrichment (<1%) in sand column
  - More sensitive to lower concentrations, but same order of magnitude degradation
- Sediment column enriched with functional bacteria
  - *Desulfosporisinus*
  - *Methanosarcina*
  - *Thiobacillus*
- Sand enriched with functionally ambiguous biofilm-forming bacteria (*Comamonas, Pseudomonas*)
- Diverse aerobic diversity – difficult to determine aerobic bacteria

Influence of electron and 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 and acceptor dose

- ↑ Nitrate
  - ↓ Reductive dechlorination
  - ↑ Aerobic degradation
  - Significant change >= .5 mM
- ↑ Sulfate
  - ↓ Reductive dechlorination
  - ↓ Aerobic degradation
  - Significant change >=2.5 mM

Stepped e^- acceptor concentrations in experiment phases

Phase	Time (d)	NO_3^- mM	NO_3^- mg/L	SO_4^{2-} mM	SO_4^{2-} mg/L	n
I	60	0	0	0.15	14	7
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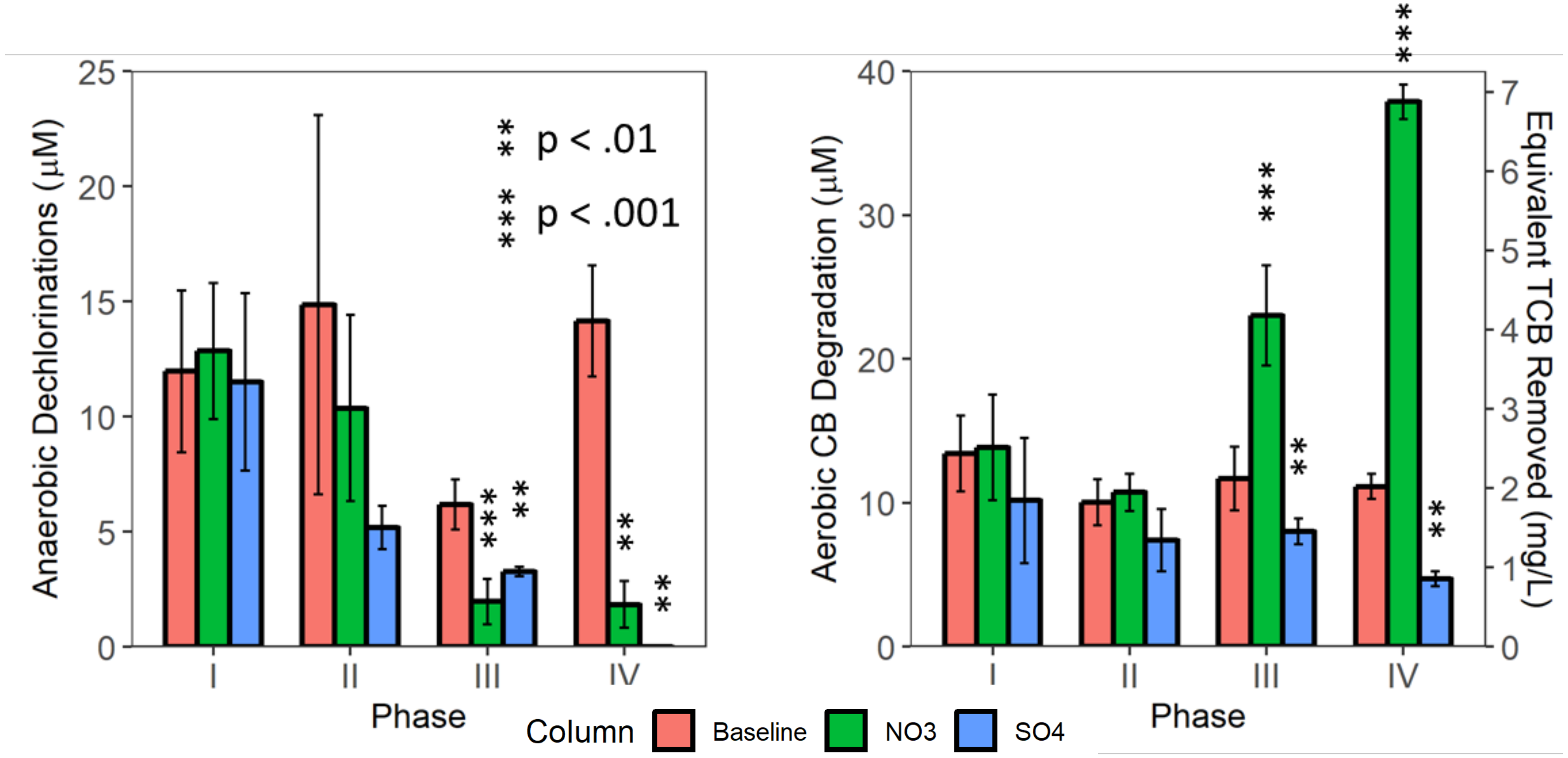
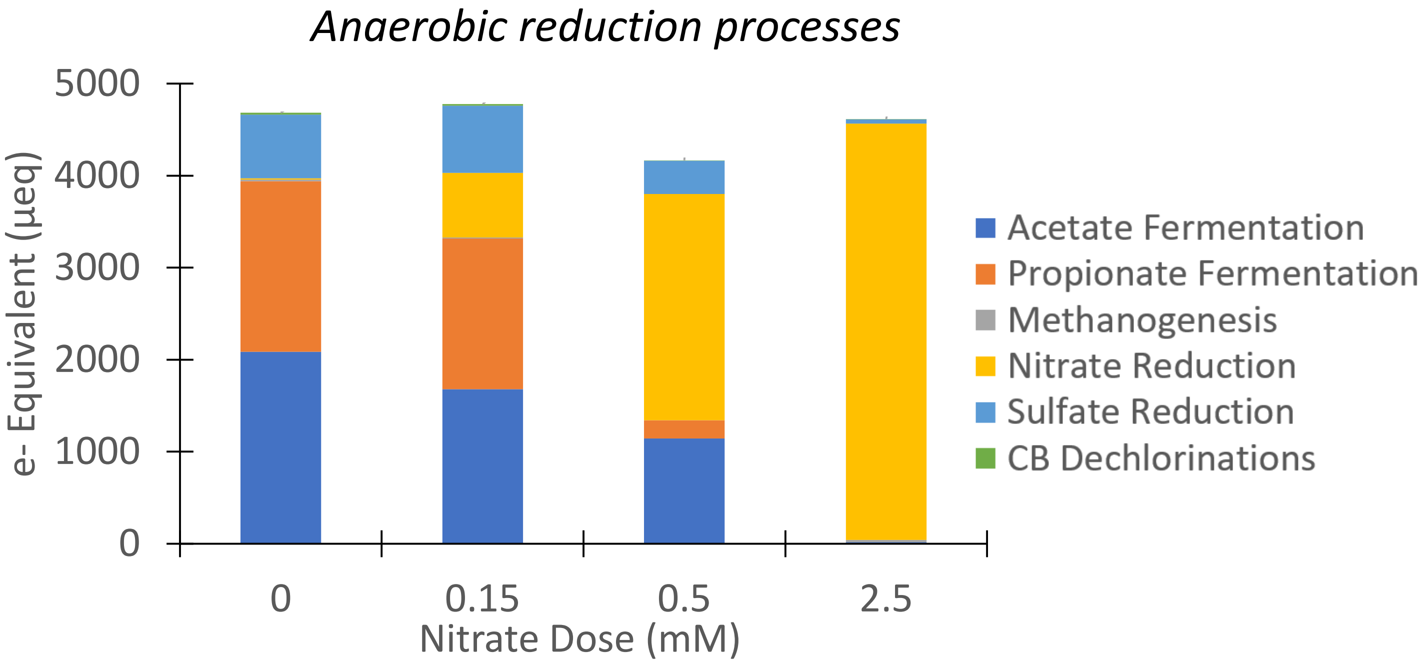
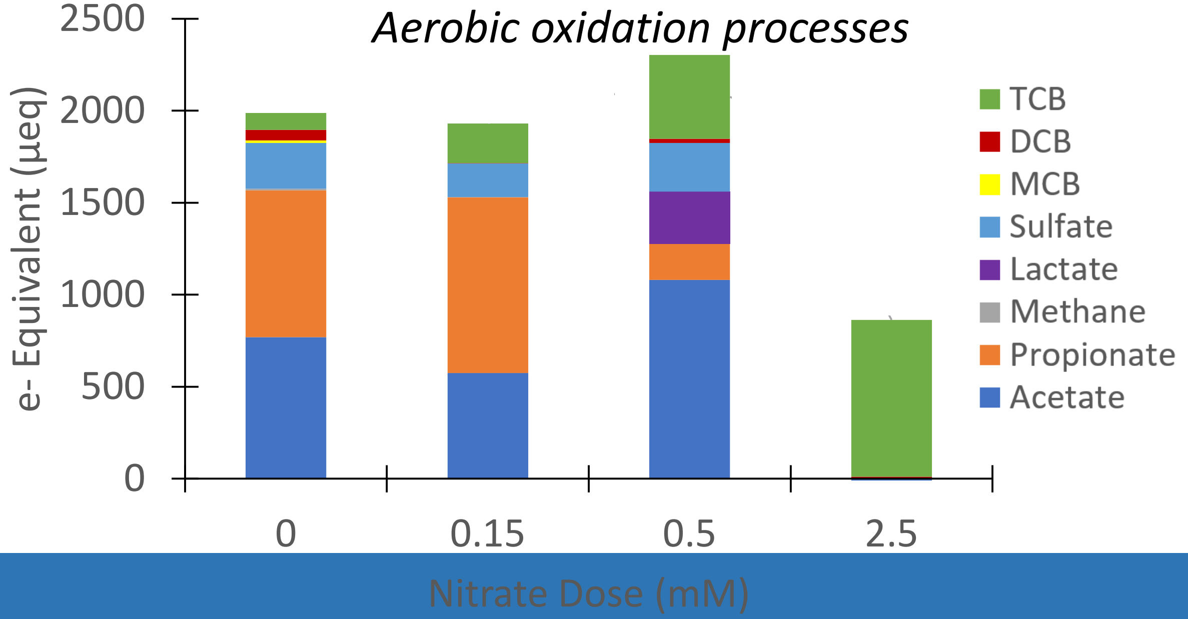


Nitrate effect on electron donor / acceptor utilization



Anaerobic reduction processes



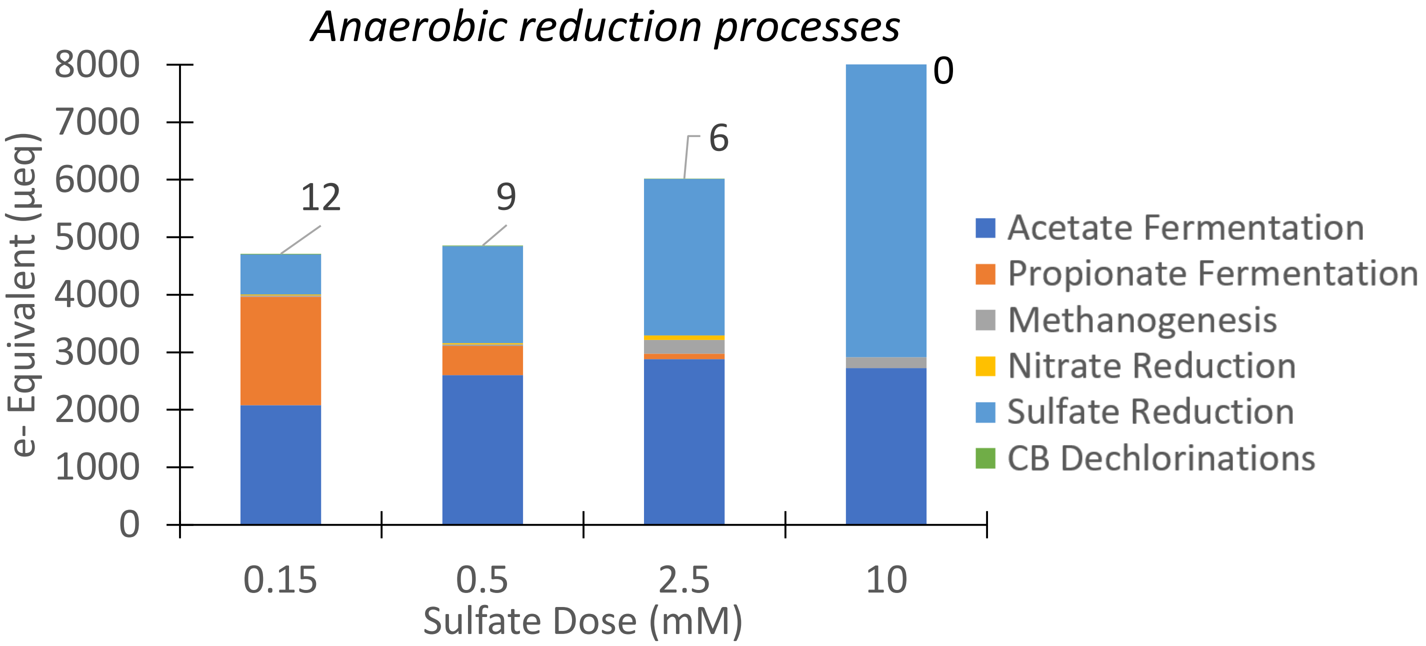
Aerobic oxidation processes

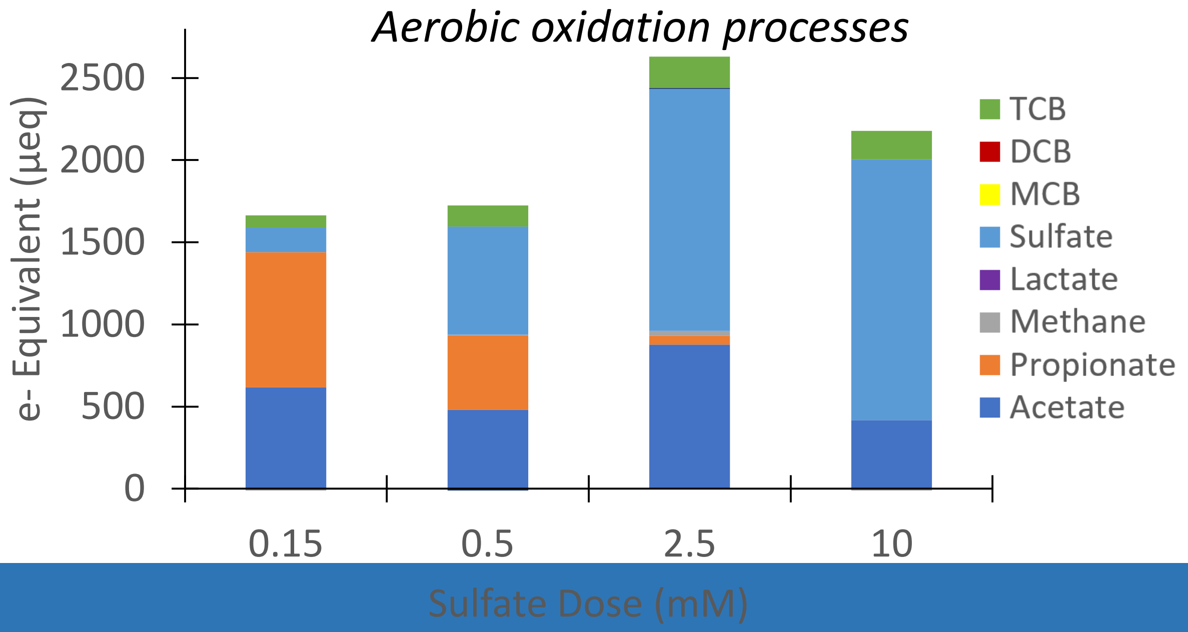
- Majority of e^- donor (>99.5%) not used for CB dechlorination (all columns)
- ↑NO_3^-
  - Nitrate reduction outcompetes other anaerobic processes, forming permanent e^- donor sink
  - Depletes residual organic acids within anaerobic zone

- ↓ NO_3^- - competition for limited O_2 limits CB oxidation
- ↑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 used as supplemental e^- acceptor for CB degradation

Sulfate effect on electron donor / acceptor utilization

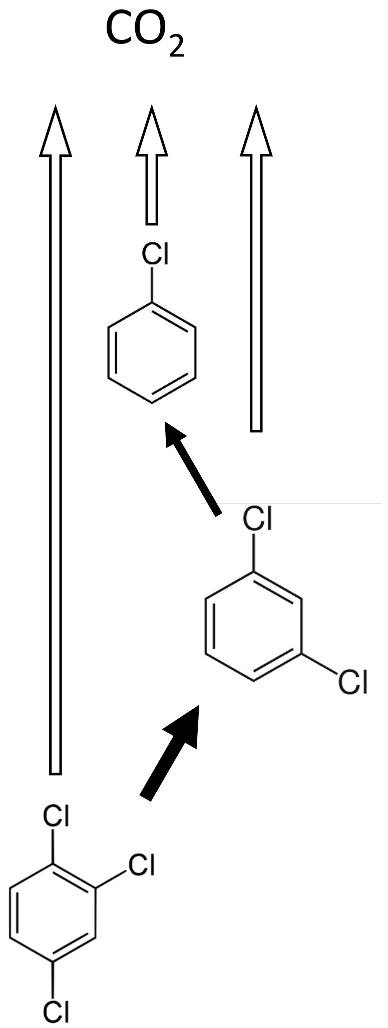




- $\uparrow SO_4^{2-}$ increases sulfate reduction, suppressing propionate formation and CB reduction. Methanogenesis and acetate fermentation persist
- Unlike NO_3^-, reduced sulfur easily re-oxidized by aerobes
- $\uparrow SO_4^{2-}$ - increased competition for O_2 by reduced sulfides, limiting aerobic degradation
- Sulfur detrimental to both anaerobic and aerobic CB degradation processes, essentially 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. Degradation potential of native and bioaugmented cultures needs to be determined
- Sediment amendment facilitated enhanced anaerobic processes
- DOC had stimulatory effect on both aerobic and anaerobic degradation processes, but above certain threshold inhibited aerobic degradation by increasing O_2 demand
- NO_3^- and SO_4^{2-} negatively impact reductive dechlorination and compete for e^- donor
- NO_3^- enhanced aerobic degradation, serving as sink for competing e^- donors



Remediation implications

- High potential for natural site matrices to facilitate passive remediation of porewater CBs
- 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
- *Dehalobacter* isolated from WBC-2 culture dominated sediment-associated anaerobic communities biofilm, facilitating highly efficient reductive dechlorination
- Experiments demonstrated robust ability of microbial communities to recover functionally from shifting redox conditions
- Sites with high sulfate may be particularly difficult to treat with anaerobic and aerobic degradation due to significant redox cycling
- Additional remediation strategies (e.g. reactive barrier) have potential to further enhance degradation based on site characteristics

Further studies

- Characterize shifts in microbial communities under varied redox conditions (in progress)
- Evaluate reactive barrier treatment on degradation at interface (sorption and non-steady state effects) (M. Lorah)

Questions?

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