# PUSHING THE PERFORMANCE BOUNDARIES OF DECHLORINATING BACTERIA

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SAMUEL GINN
COLLEGE OF ENGINEERING

# Research Team and Acknowledgements


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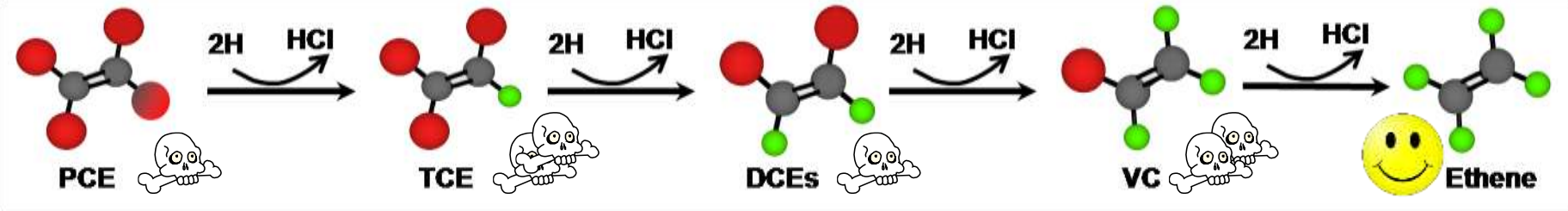
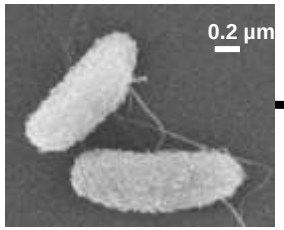
Special thanks to SiREM Labs for providing the KB-1® culture.

# Reductive Dechlorination of Chloroethenes




*Dehalococcoides mccartyi* (*Dhc*) strains

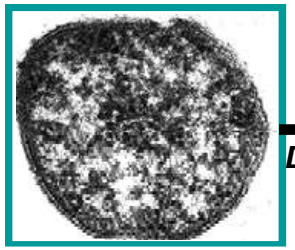



PCE to DCE

***Geobacter lovleyi***

***Sulfurospirillum, Desulfitobacterium, Dehalobacter, Clostridium, Desulfuromonas***

STOP

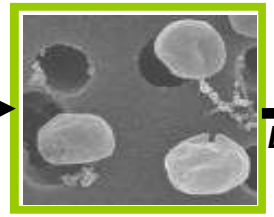


PCE to VC

*tceA*

***Dehalococcoides mccartyi* strain 195**

YIELD



TCE to VC

*tceA*

***Dehalococcoides mccartyi* strain FL2**

YIELD

Function has been assigned to three genes-- Reductive Dehalogenase (RDase) Genes

***Dehalococcoides mccartyi* strain BAV1**

*bvcA*

DCE/VC to ethene


TCE to ethene

*vcrA*

***Dehalococcoides mccartyi* strains GT and VS**

**Microbial consortia with 3 *Dhc* strains used in studies**

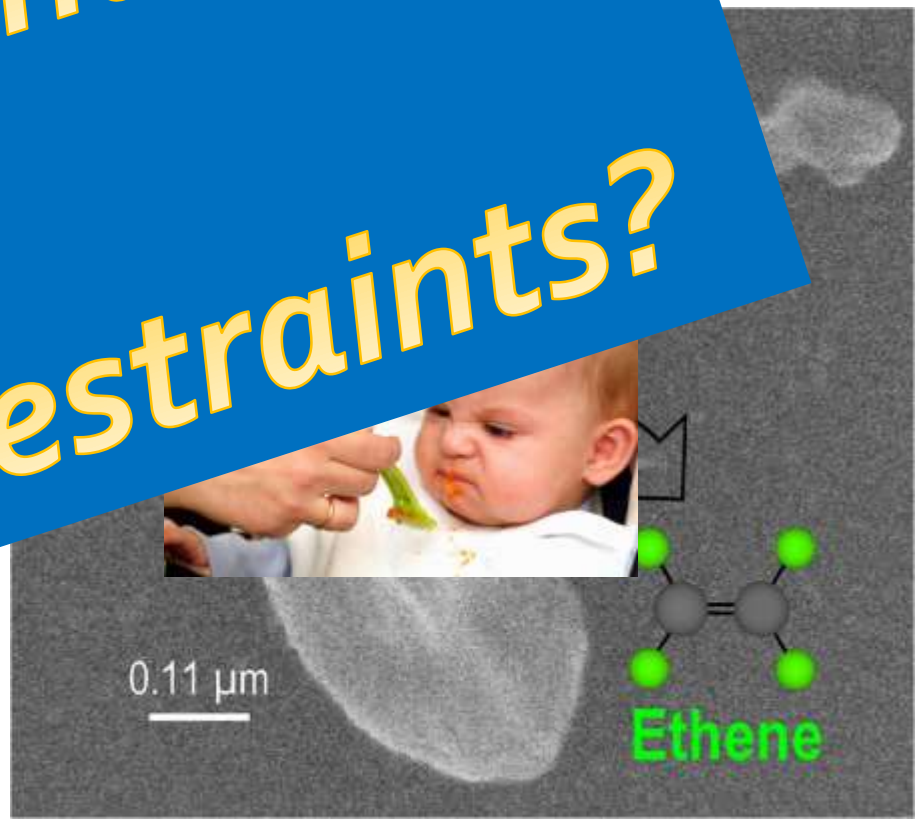
# *Dehalococcoides :* The *Goldilocks* of D[illegible]nation

Electron Acceptor –some chlorinated compounds
Electron Donor
Carbon Source (e.g., ac[illegible]
Strictly Anoxic
ORP [illegible]

- [illegible]
- [illegible]

[illegible]temperature between 25 and 30 °C.

**Can we optimize conditions or overcome these restraints?**


Löffler et al. 2013, IJSEM. 63: 625-635
Yan et al. 2012, Appl. Environ. Microbiol. 78:6630-6636
Yan et al. 2013. Phil. Trans. R. Soc. B. 368 (1616):20120320

# Influence of Biogeochemistry on Microbial Reductive Dechlorination: Impacts of low pH

- Biostimulation can also lower groundwater pH through:
  - formation of organic acids and $CO_2$
  - releases of hydrochloric acid (HCl) during microbially-catalyzed dechlorination
- Activity of ***Dehalococcoides mccartyi (Dhc)*** is reduced at pH= 6.5 or no growth occurs when pH drops below 6.0
- Counteracting acidification by adding buffer (e.g., bicarbonate) is costly
- **How ubiquitous are bacteria that can dechlorinate under low pH conditions?**
- **Will the activity of dechlorinating microbes rebound following low pH exposure ?**

# Screening of Existing Dechlorinators

- Literature pH values for optimal growth of dechlorinating bacteria

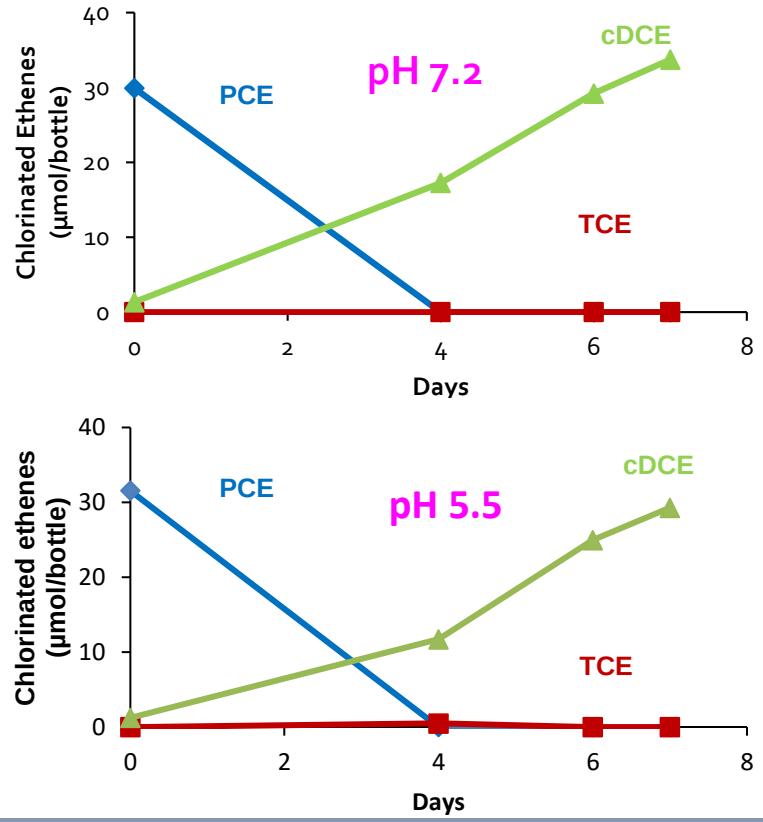
| Bacteria | Optimal pH |
|---|---|
| *Geobacter lovleyi* SZ | 6.5~7.5 |
| *Desulfitobacterium* sp. Y51 | 6.5~7.5 |
| *Desulfuromonas chloroethenica* TT4B | 6.5~7.4 |
| *Desulfuromonas michiganensis* BB1 | 6.8~8 |
| *Sulfurospirillum multivorans* | 7~7.5 |
| *Dehalococcoides mccartyi (Dhc)* | 6.5~8 |

- Screening of existing PCE dechlorinators

| Dechlorinators | pH 7.2 |
|---|---|
| Lab Consortium (multiple *Dhc* strains) | Ethene |
| *Geobacter lovleyi* SZ | cDCE |
| *Desulfuromonas michiganensis* BB1 | cDCE |
| *Sulfurospirillum multivorans* | cDCE |
| *Desulfitobacterium* sp. JH1 | cDCE |
| *Desulfitobacterium* sp. Viet1 | TCE |

Note: X--no degradation detected within monitoring period.

*Sulfurospirillum multivorans*




Defined, synthetic medium: @pH5.5 (MES buffer); @pH6 (MES buffer); @pH7 (Bicarbonate buffer); ED: Lactate or Acetate + $H_2$; EA: PCE

Yang, Y., Cápiro, N. L., Marcet, T. F., Yan, J., Pennell, K. D., & Löffler, F. E. (2017). Organohalide respiration with chlorinated ethenes under low pH conditions. *Environmental science & technology*, *51*(15), 8579-8588.

# Enrichment of Low pH Dechlorinators

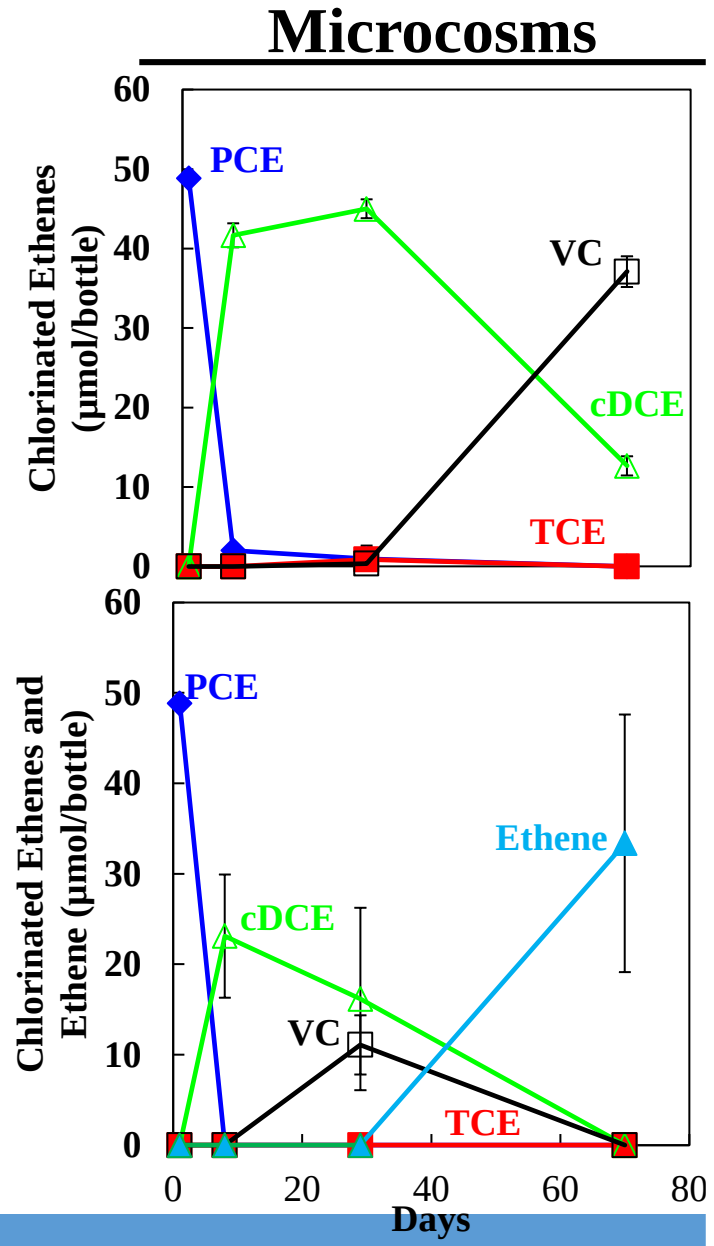
Soil/groundwater samples and test conditions

| # | Sample sites ID | Locations | Sample Type | Carbon Source Electron Donor | Electron Acceptor | PCE Degradation End Product | |
|---|---|---|---|---|---|---|---|
| | | | | | | pH 5.5 | pH 7.2 |
| 1 | Ft. Pierce | USA | Soil | Lactate + $H_2$ | PCE | X | X |
| 2 | PNNL | USA | Soil | Lactate + $H_2$ | PCE | X | X |
| 3 | Contaminated Site | CA, USA | Soil | Lactate + $H_2$ | PCE | X | X |
| 4 | - | Brazil | Soil | Lactate + $H_2$ | PCE | X | X |
| 5 | Third Creek | TN, USA | Sediment | Lactate + $H_2$ | PCE | Ethene | Ethene |
| 6 | Neckar River | Germany | Sediment | Lactate + $H_2$ | PCE | Ethene | Ethene |
| 7 | Rotenberg Trester | Germany | Soil | Lactate + $H_2$ | PCE | VC, Ethene | VC, Ethene |
| 8 | Rotenberg Creek | Germany | Soil | Lactate + $H_2$ | PCE | X | X |
| 9 | McGuire AFB | USA | Soil, GW | Lactate + $H_2$ | PCE | X | X |
| 10 | - | USA | Soil, GW | Lactate + $H_2$ | PCE | X | X |
| 11 | - | USA | Soil, GW | Lactate + $H_2$ | PCE | Ethene | Ethene |
| 12 | Shady Valley | TN, USA | Soil, Sediment | Lactate + $H_2$ | PCE | *c*DCE | *c*DCE |
| 13 | Axton Cross | USA | Soil, GW | Lactate + $H_2$ | PCE | VC | Ethene |
| 14 | - | USA | Soil | Lactate + $H_2$ | PCE | X | X |
| 15 | Tidal Flat | Korea | Soil | Lactate + $H_2$ | PCE | X | TCE |
| 16 | Elkhart Rail Yard | USA | Soil, GW | Lactate + $H_2$ | PCE | Ethene | Ethene |

# Axton Cross Microcosms and Transfers

**pH 5.5**

**pH 7.2**

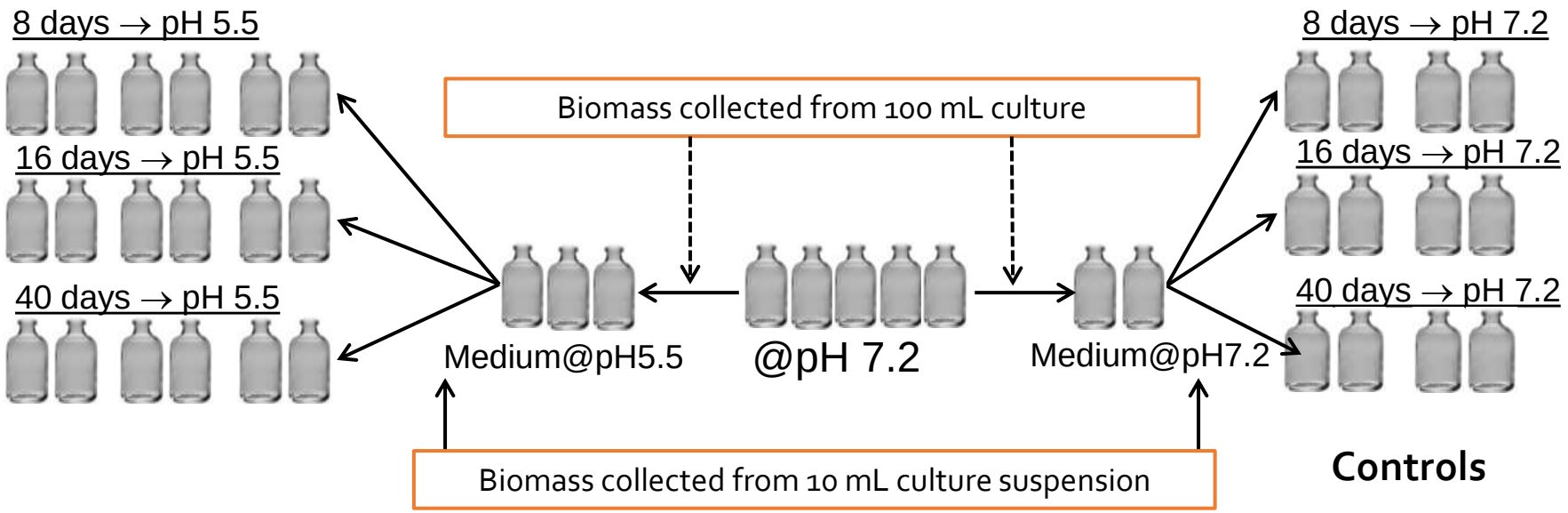


# pH-Induced Microbial Community Changes

Illumina MiSeq 16S rRNA gene amplicon sequencing on pH 5.5 and pH 7.2 enrichment cultures derived from Axton Cross soil sample

| Major Genera (%) | pH 7.2 | pH 5.5 |
|---|---|---|
| *Dehalococcoides* | **22.6** | **0.0** |
| *Acetobacterium* | **57.6** | **0.0** |
| *Spirochaetaceae* Uncultured | **4.6** | **0.1** |
| *Caldisericum* | 4.2 | 0.1 |
| *Desulfuromonadales* BVA18 | 2.6 | 0.0 |
| vadinBC27 | 1.1 | 0.0 |
| *Desulfovibrio* | **0.1** | **33.0** |
| *Sulfurospirillum* | **0.2** | **25.2** |
| *Megasphaera* | **0.0** | **19.9** |
| *Propionibacterium* | 0.0 | 1.5 |
| *Pelosinus* | 0.0 | 1.00 |
| Others | 7.0 | 19.2 |
| Total | 100.0 | 100.0 |

# Resilience of Dechlorinating Bacteria to low pH Exposure




→ Laboratory Consortium (contains multiple *Dhc* strains) biomass exposed to pH 5.5 for 8, 16, 40 days

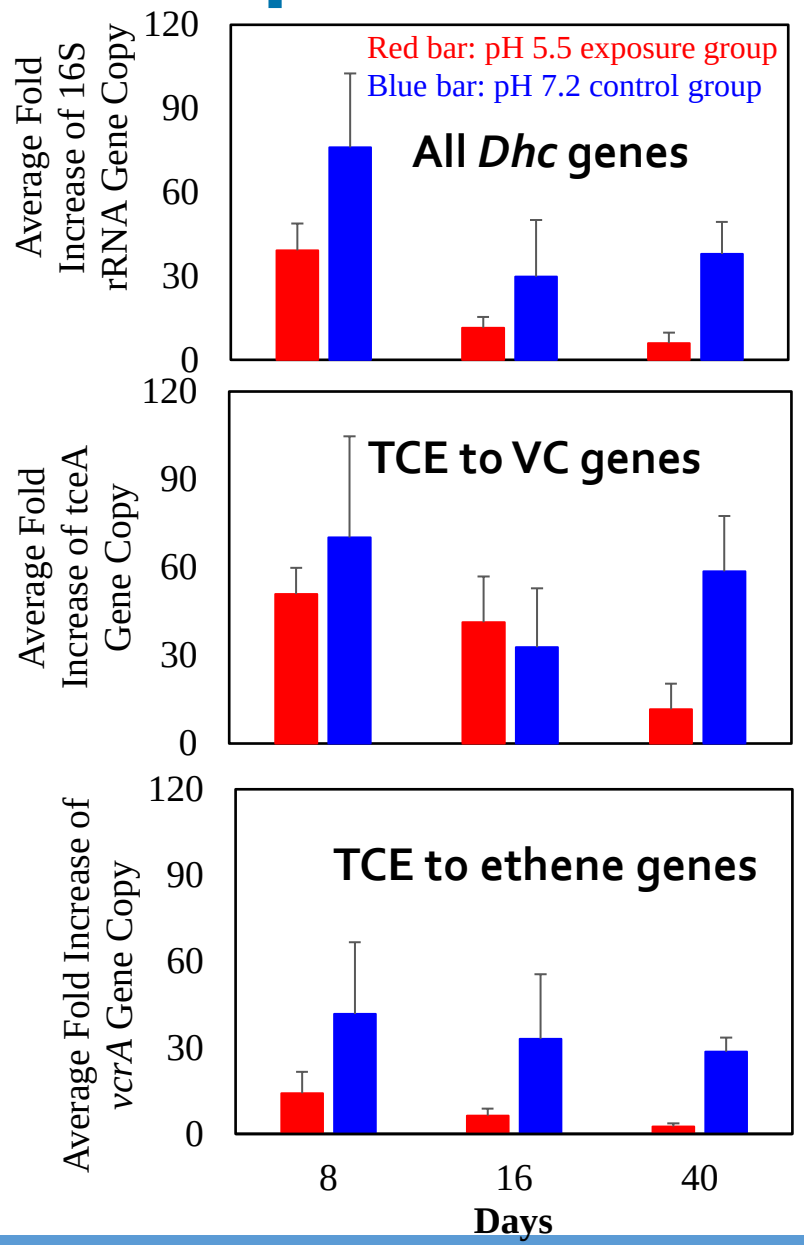
→ Biomass collected, transferred and incubated @ pH 7.2 with ED and PCE for 40 days

→ Dechlorination and gene copy growth monitored and compared to pH 7.2 controls

Yi Yang, Natalie L. Cápiro, Jun Yan, Tyler F. Marcet, Kurt D. Pennell, Frank E. Löffler, Resilience and recovery of *Dehalococcoides mccartyi* following low pH exposure, *FEMS Microbiology Ecology*, Volume 93, Issue 12, December 2017, fix130, https://doi.org/10.1093/femsec/fix130

# pH Tolerance and Resilience

Biomass measured from transfer cultures after returning to pH 7.2 for a 40-day incubation period




- Longer low pH exposure time resulted in longer recovery time of dechlorinators

- *Dhc* recovery is **strain-specific**. *Dhc* strain GT carrying the VC RDase gene, ***vcrA* (TCE to ethene)** was most susceptible to pH stress
  - Consistent with impaired ethene formation following 8- and 16-day exposure durations and
  - no ethene after 40-days of exposure, even upon transfer to pH 7.2 for + 200 days

# pH Work Conclusions

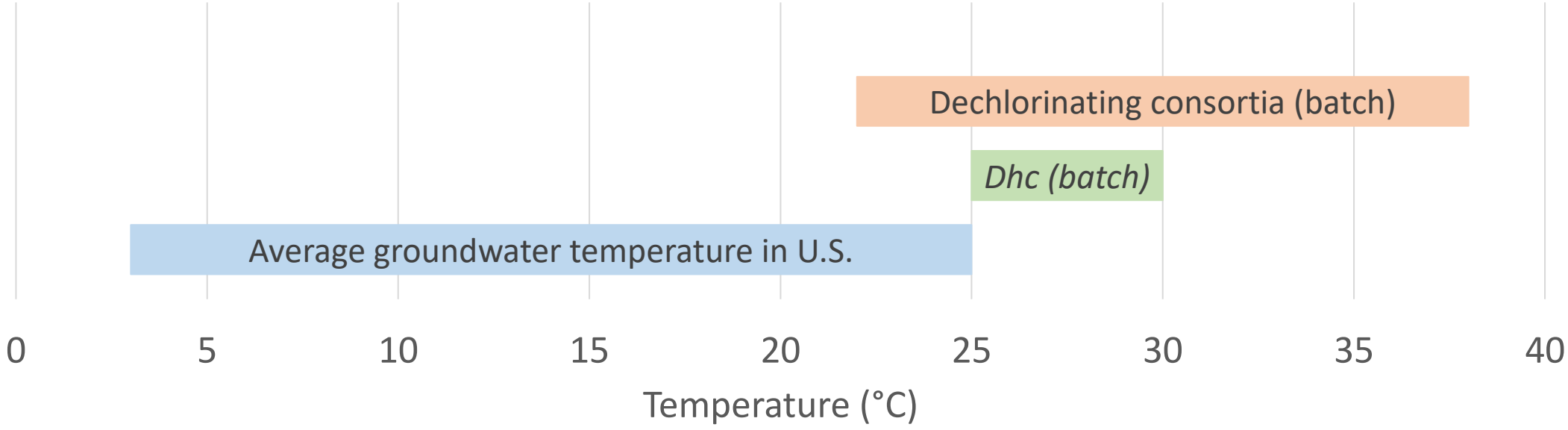
- The screening efforts suggest that microbes capable of dechlorination at pH 5.5 are uncommon.
  - *Sulfurospirillum (specific strains)* are critical at low pH sites, members of the genus may hold promise for other chemical transformations under unhospitable conditions
- The presence of certain solids and cell attachment enabled dechlorination of PCE to ethene at pH 5.5
- Impacts to low pH exposure are *Dhc* strain specific
  - Despite the same enzyme system for VC-reduction and cDCE-reduction.

# Benefits of Coupling Thermal Treatment and Bioremediation

| | Electrical resistance heating | Enhanced microbial reductive dechlorination |
|---|---|---|
| Primary target | Source zone | Plume |
| Duration | 3 – 12 months | Years |
| Cost* | $120-300 (median $161)/yd$^2$ | $30-180(median $99)/yd$^2$ |

*McGuire et al. *ESTCP ER-201120 final report* (2016)

- Elimination of competing microorganisms
- Improved redox conditions and substrate availability (Marcet et al. 2018 ES&T)
- **Direct temperature stimulation of dechlorinating bacteria** (Marcet et al. 2018 WR)



# Low-Temperature Heating Experimental Design

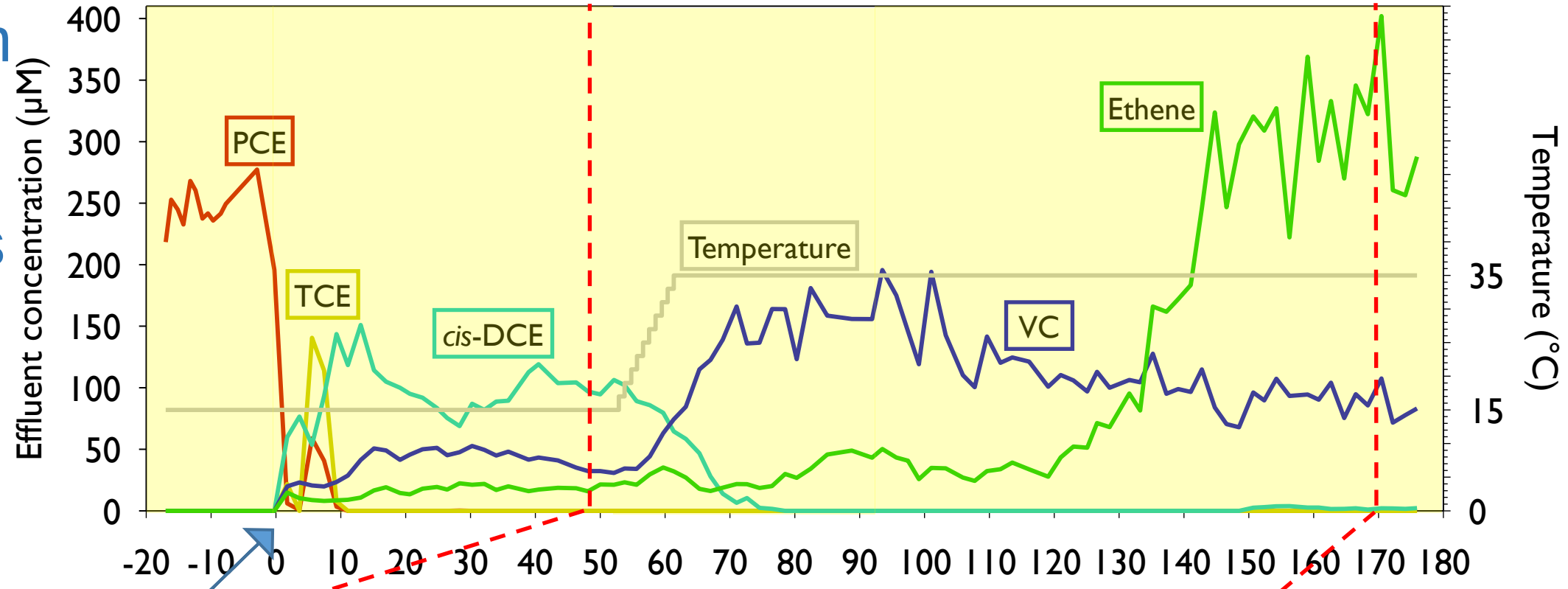
- Parallel columns (15 cm *l* × 2.5 cm *ID*) packed with Federal Fine Ottawa sand
- Influent synthetic groundwater solution, introduced at $v_s$ = 15 cm/day:
  - PCE-electron acceptor
  - Lactate-electron donor and carbon source
- External wrap to raise Column B to 35 °C and cool Column A to 15 °C
- Bioaugmented with KB-1® dechlorinating culture
- PCE and daughter products, *Dhc 16S rRNA* and *RDase* genes monitored

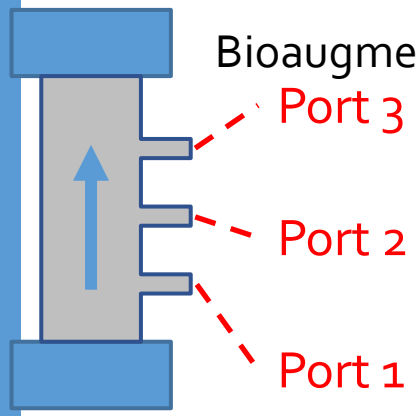
Marcet, T. F., Cápiro, N. L., Yang, Y., Löffler, F. E., & Pennell, K. D. (2018). Impacts of low-temperature thermal treatment on microbial detoxification of tetrachloroethene under continuous flow conditions. *Water research*, *145*, 21-29.

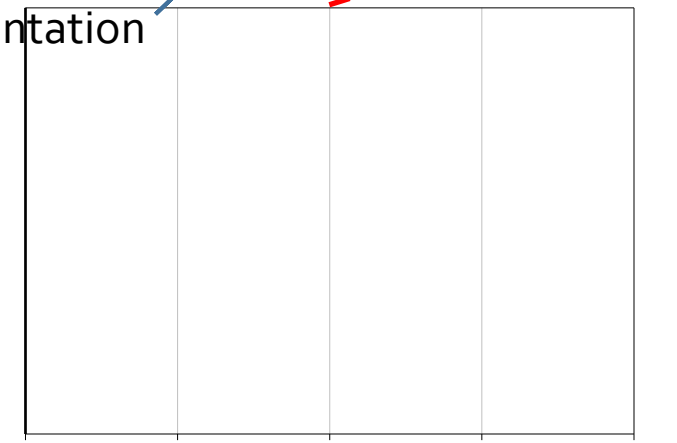
# Stepwise Column Heating

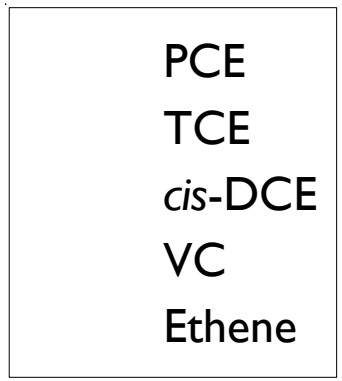
| | Intended to assess: | Action: |
|---|---|---|
| **Phase I** (0 – 52 PV) | Dechlorination activity at ambient versus elevated groundwater temperature | Column A = 15 °C<br>Column B = 35 °C |

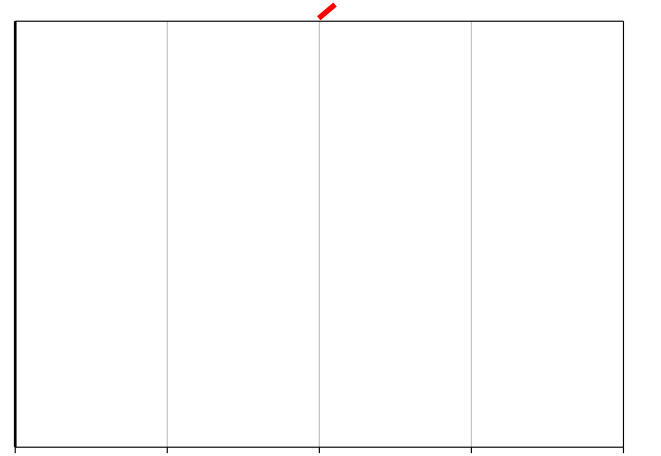
# Column A VOC Results



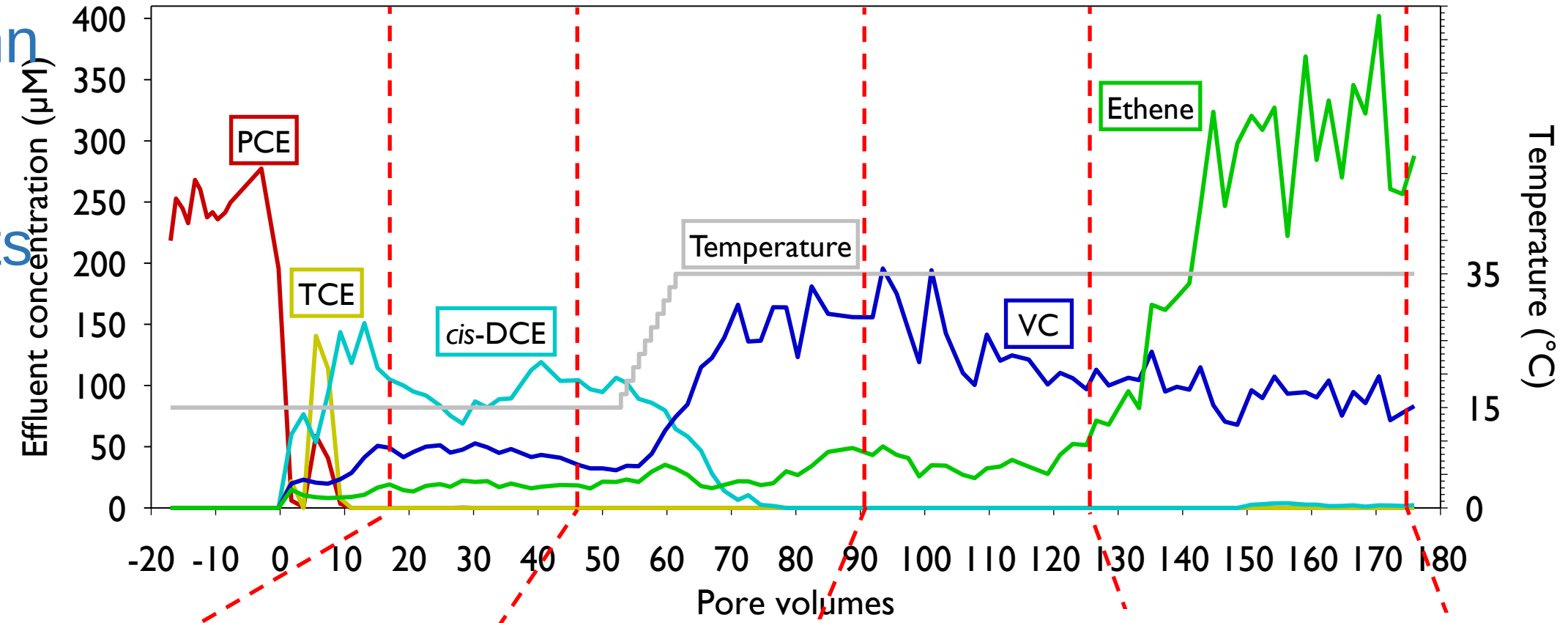



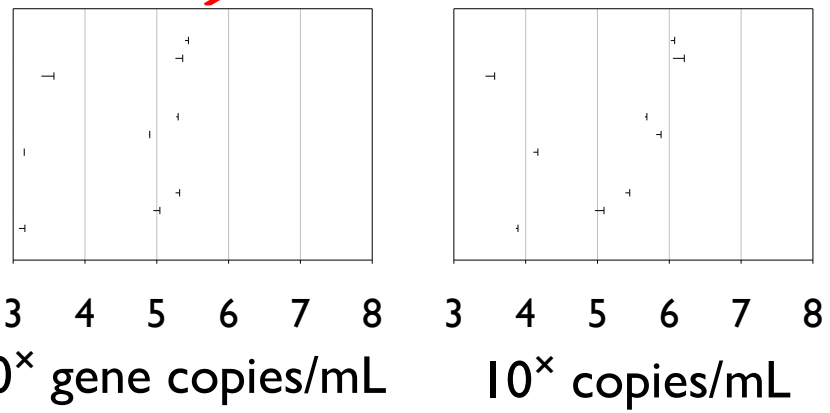



# Column A *Dhc* Results





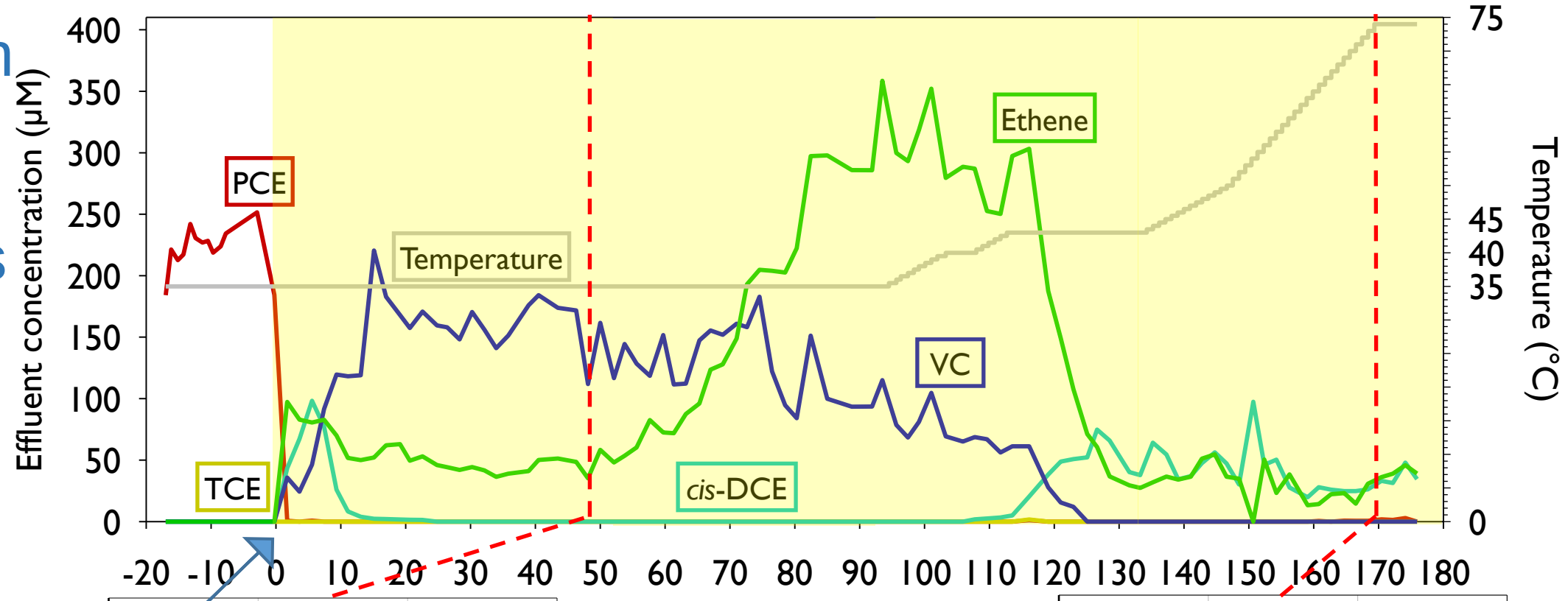


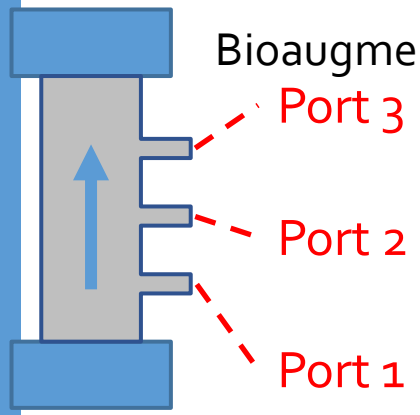

Represents all *Dhc* genes

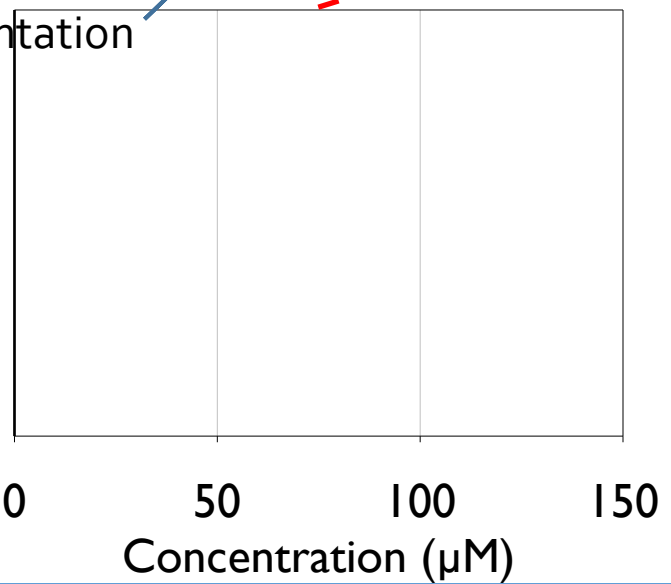
*Dhc* *vcrA* *bvcA*

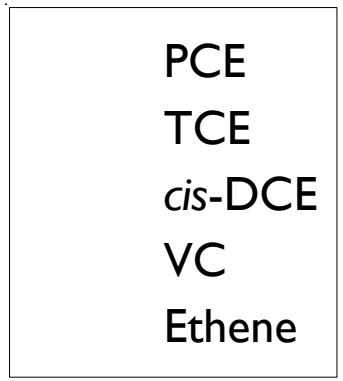
Both genes associated with transformation to ethene

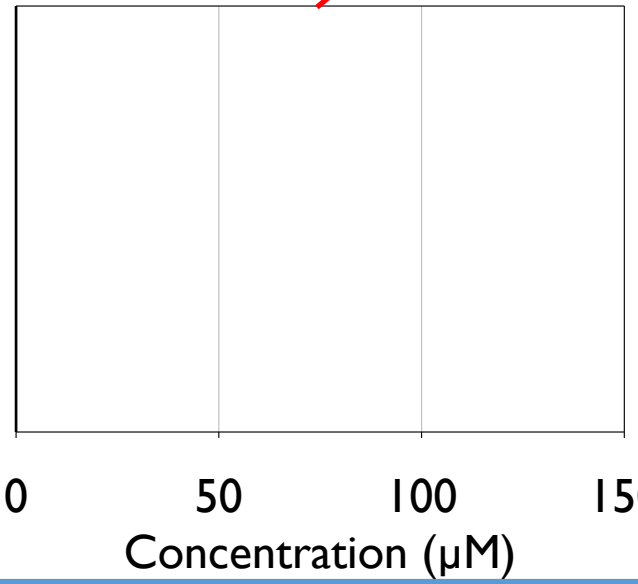
# Column B VOC Results



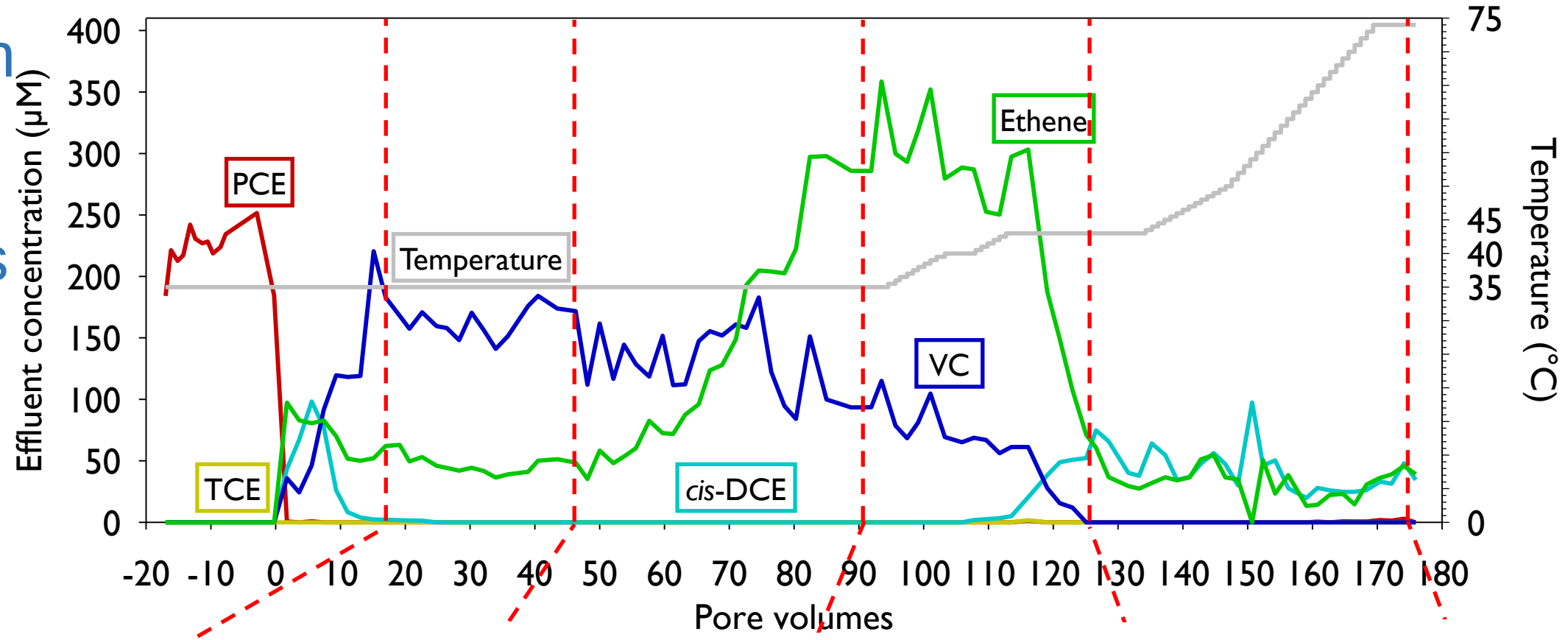



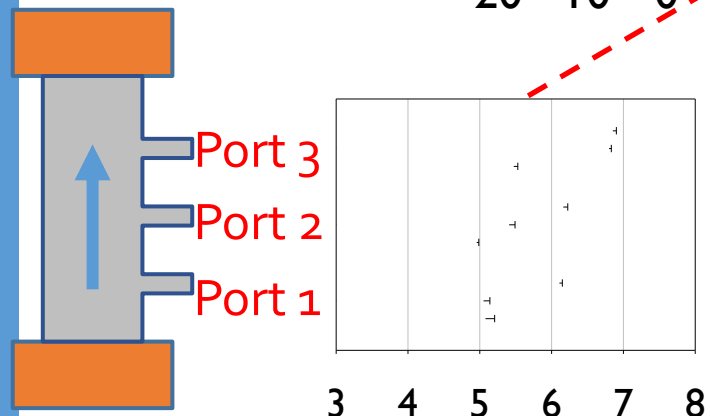



# Column B *Dhc* Results







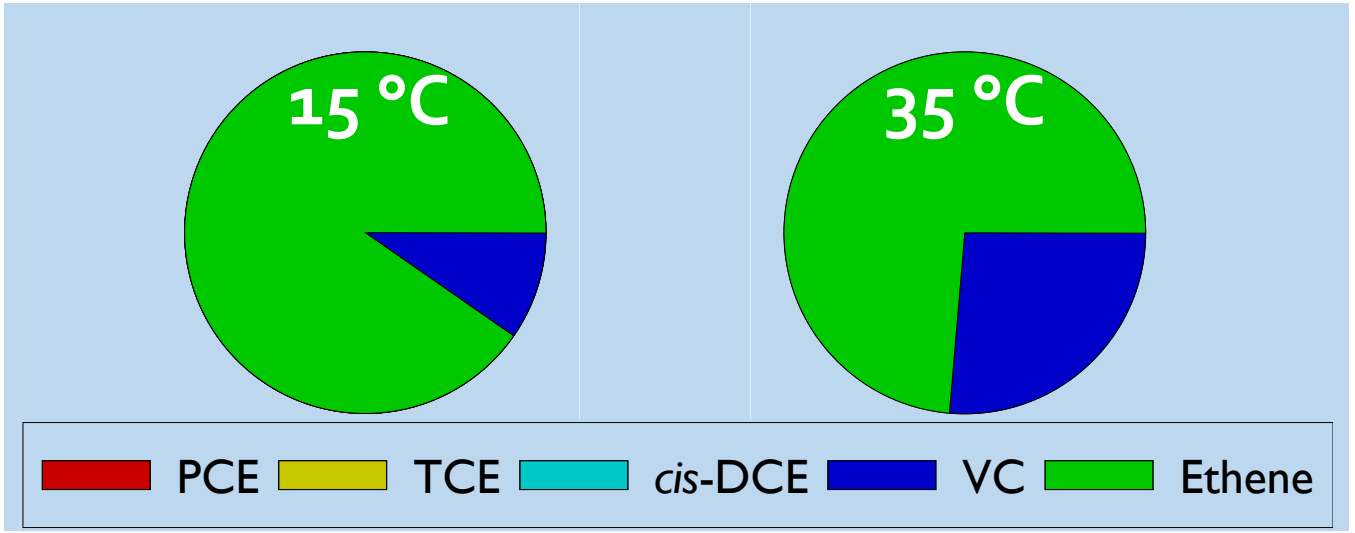
Represents all *Dhc* genes

*Dhc* *vcrA* *bvcA*

Both genes associated with transformation to ethene

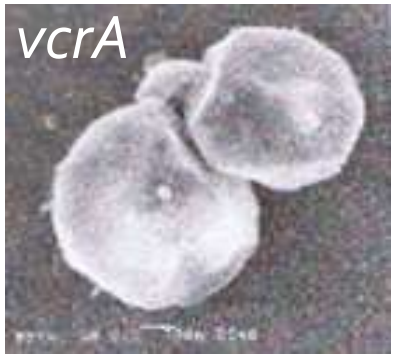
# Temperature Work-Conclusions

1) Heating increased dechlorination activity in a 1-D flow system, but did not impact all steps equally (e.g., lagged VC transformation).

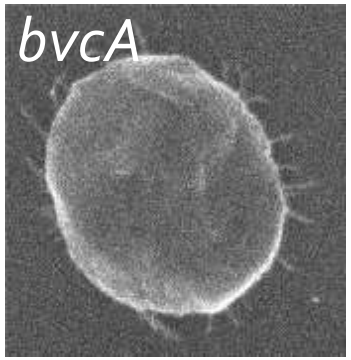



2) The optimal temperature for *Dhc* activity was approximately 35-43 °C, at least 5 °C greater than previously determined in batch reactor studies**…scale is critical for determining microbial thresholds and potential!**

3) The relative importance of *microbial* strains contributing contaminant degradation shifted with temperature…**important for monitoring of microbes in the field!**




✔ **15 – 35 °C**



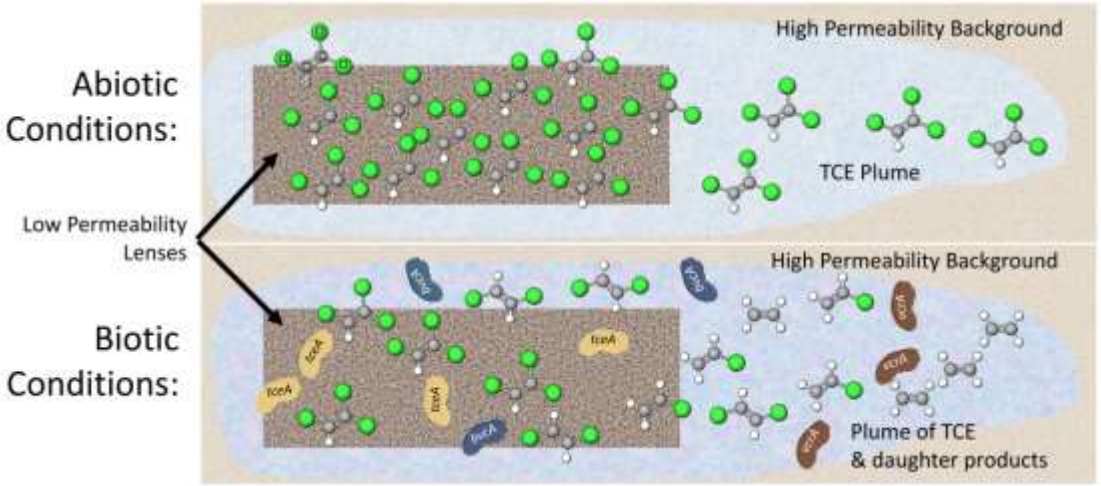

✔ **35 – 43 °C**

✗ **> 43 °C**

# Bioenhanced Mass Transfer in Heterogenous Aquifers

## Bioenhanced Dissolution

- Biological activity near DNAPL source zones increases rate of mass removal is well documented
  - Up to 14-fold enhancement reported
- Expect similar enhancement of **DIFFUSION** and **DESORPTION** processes

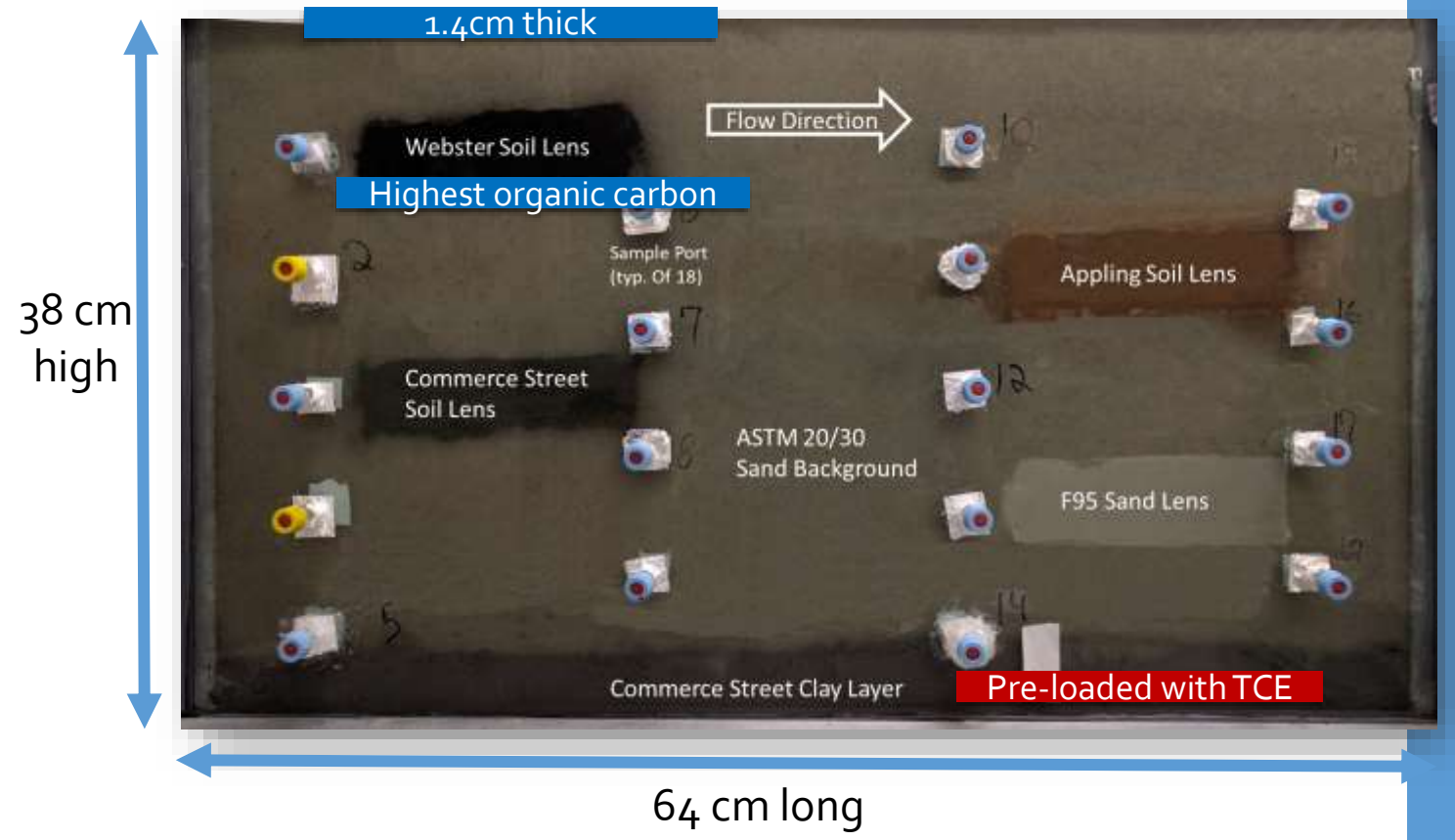



- Knowledge Gaps
  - No systematic study of fine-scale heterogeneity on biologically-enhanced back diffusion and *Dhc* strain abundance
- Objectives
  - Quantify enhancement of back-diffusion
  - Examine the distribution of RDase genes (*Dhc* strains)

# Back Diffusion Aquifer Cell Setup

- Four 5 cm x 14 cm lenses
- Underlain with 3cm of TCE-saturated clay

| Material | Hydraulic Conductivity | Organic Carbon |
|---|---|---|
| Commerce Street Clay | 0.08 m/day | 0.3% |
| ASTM 20/30 | 200 m/day | 0.01% |
| Webster | 0.86 m/day | 3.33% |
| Appling | 10.2 m/day | 0.75% |
| Commerce Street Soil | 4.0 m/day | 0.09% |
| F95 Sand | 2.5 m/day | 0.01% |

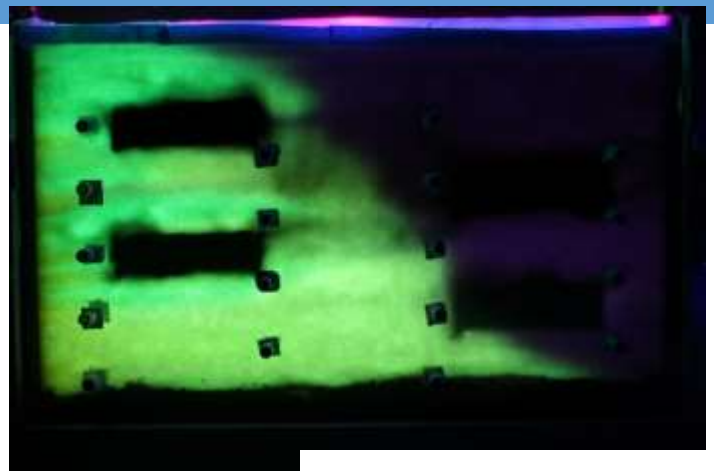



Hnatko, Jason P., Lurong Yang, Kurt D. Pennell, Linda M. Abriola, and Natalie L. Cápiro. "Bioenhanced back diffusion and population dynamics of *Dehalococcoides mccartyi* strains in heterogeneous porous media." *Chemosphere* (2020): 126842. https://doi.org/10.1016/j.chemosphere.2020.126842

# Abiotic Results

## Tracer – fluorescein and bromide

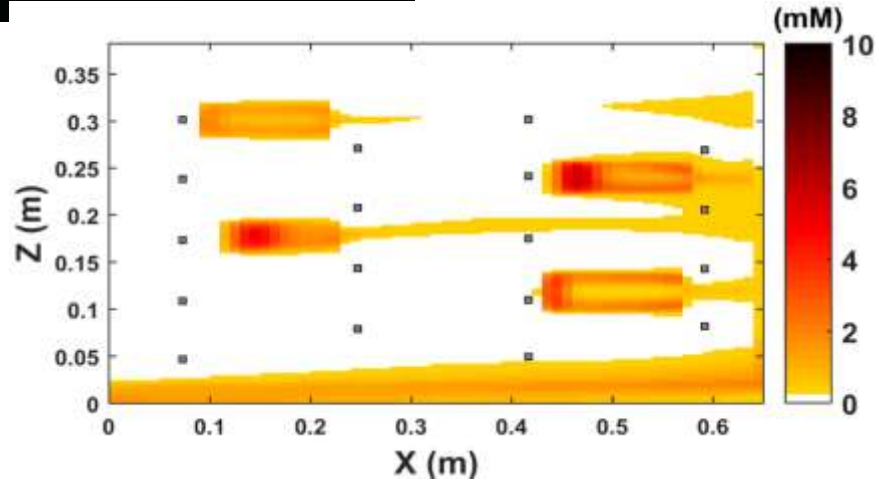
- Used to calibrate flow model parameters



## Abiotic Experiment

- Used to calibrate sorption parameters
- 48.5% to 97.4% TCE removal (sorbing)
- 98.1% to 99.7% bromide removal (non-sorbing)
- Most retention of TCE in Webster soil (high OC)

**Model of Back-Diffusion**



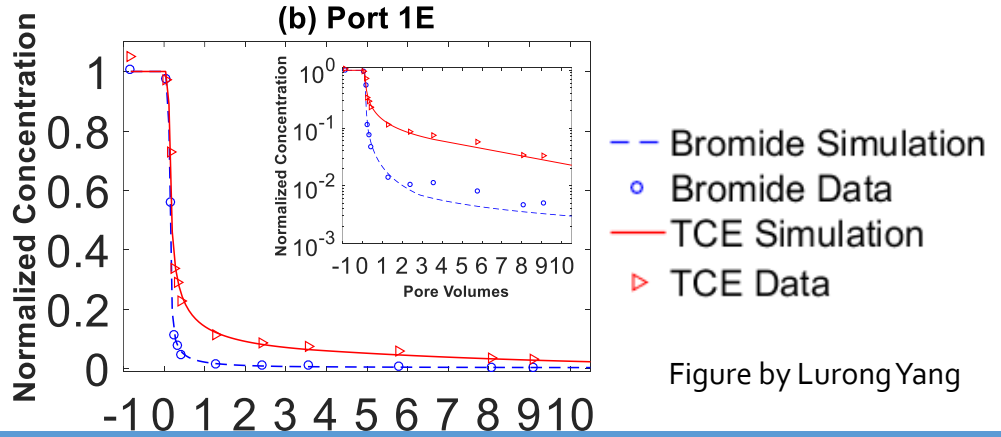
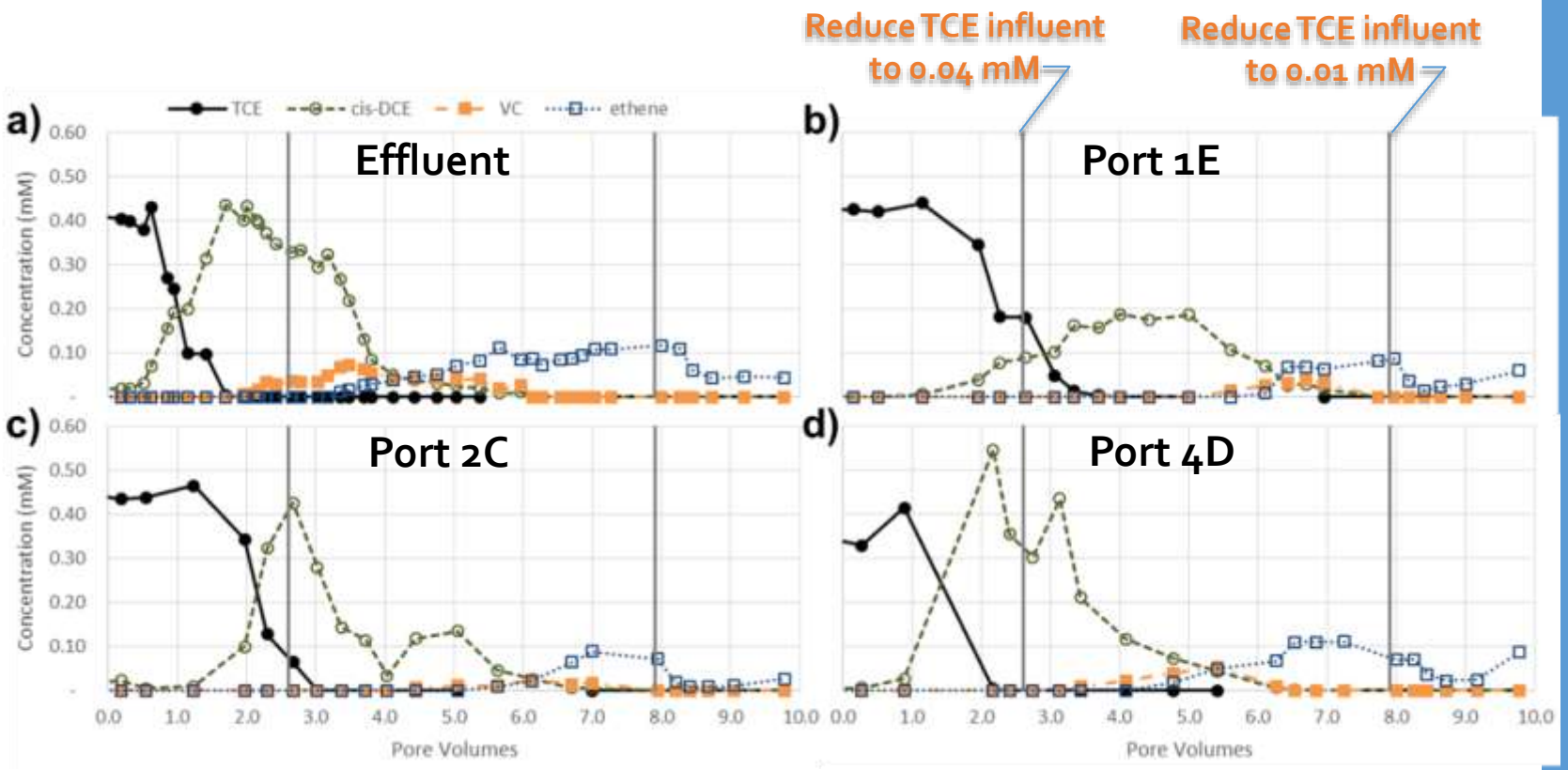
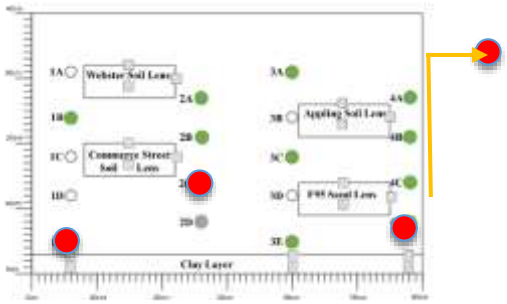




Figure by Lurong Yang

# VOCs during Biotic Experiment w/ KB-1

- Rapid biotransformation to *cis*-DCE
- Transformation to VC and ethene after lowering input concentration
- Sustained ethene *(2-4 times higher than influent TCE)* at late times: diffusion/desorption
  - Highest above clay



# Bioenhanced Back Diffusion

- Modeled abiotic TCE (red) closely matched measured total chlorinated ethene and ethene (blue) trends
- Higher chlorinated ethenes and ethene measured due to bioenhanced back diffusion/desorption from low prem. zones vs. abiotic
- *6% to 53% greater (beyond abiotic alone) mass removal during microbial reductive dechlorination*

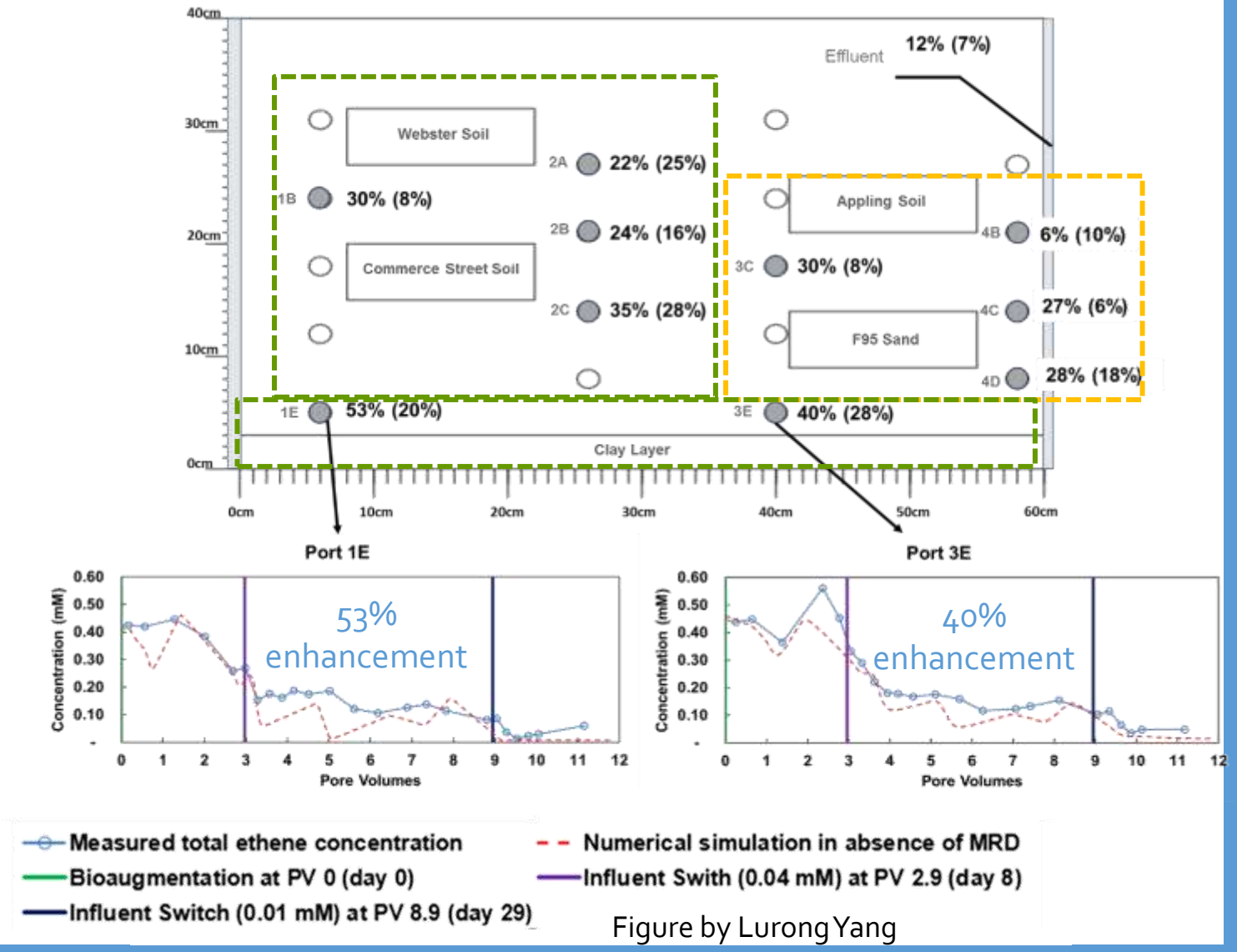
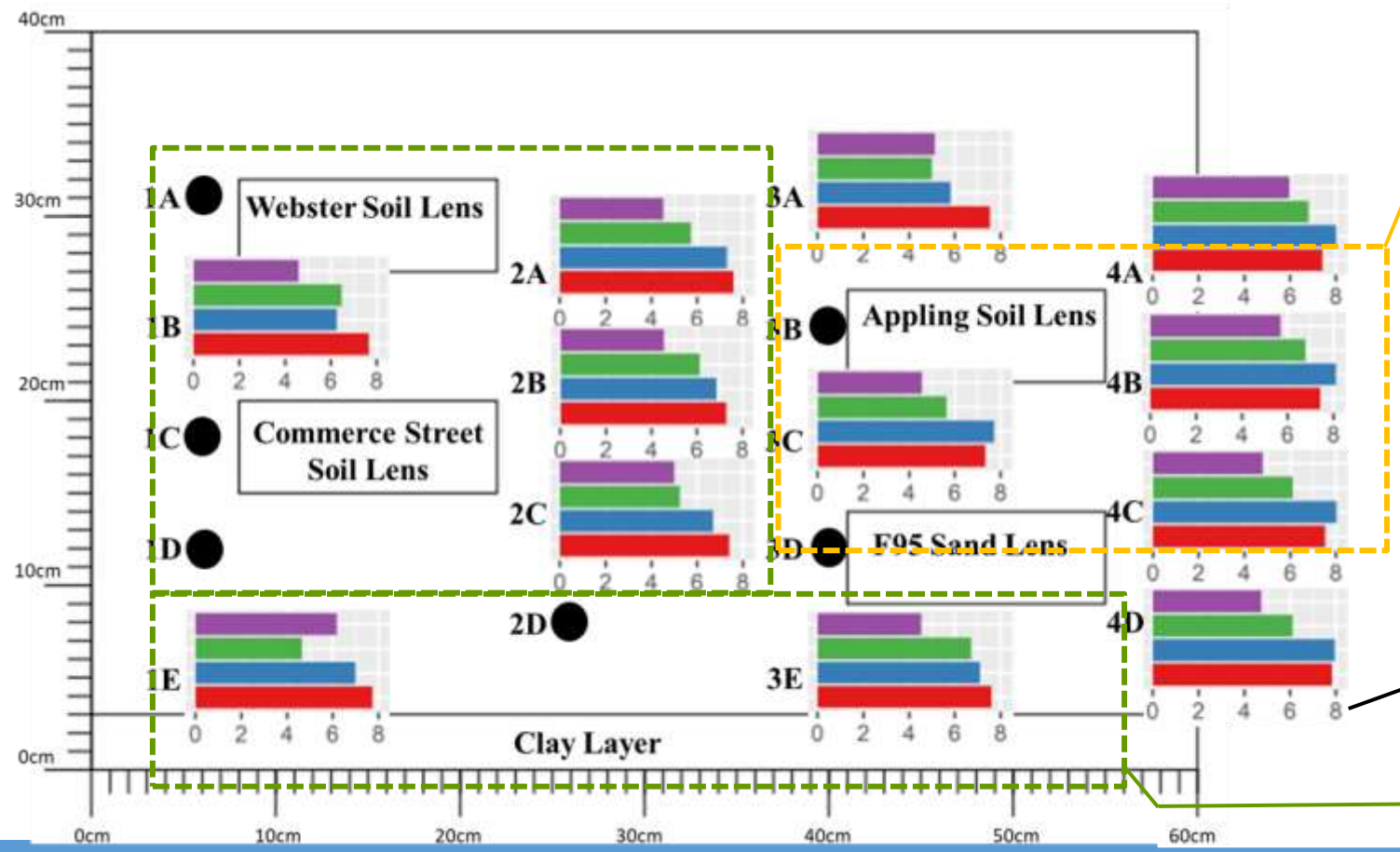



Figure by Lurong Yang

# Unattached *Dhc* Cell Growth

- Minor growth differences through domain, slight increases near low perm zones w/ contam.
- Growth of two orders of magnitude: $10^5$ to $10^7$ cells/mL




Region of Lower Bioenhancement (*Dhc* abundance decrease at end)

**Days after bioaugmentation**

| | |
|---|---|
| 2 | (2 days of 0.5 mM TCE) |
| 8 | (8 days of 0.5 mM TCE) |
| 16 | (8 days of 0.04 mM TCE) |
| 32 | (8 days of 0.01 mM TCE) |

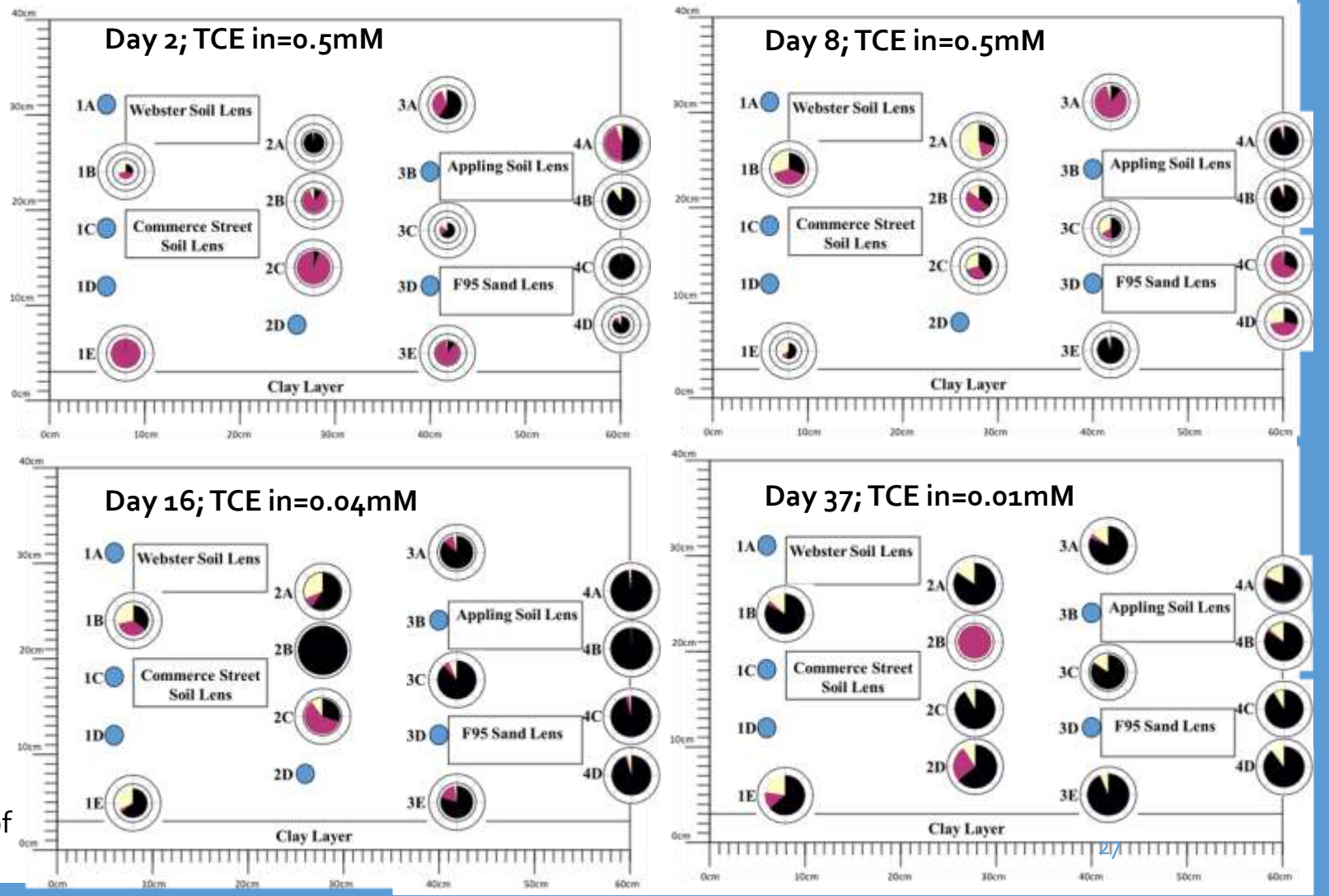
x-axis=log cells/mL

Region of Higher Bioenhancement (no decrease in *Dhc* abundance)

# *Dhc* Strain Spatial and Temporal Variability

## Aqueous RDases

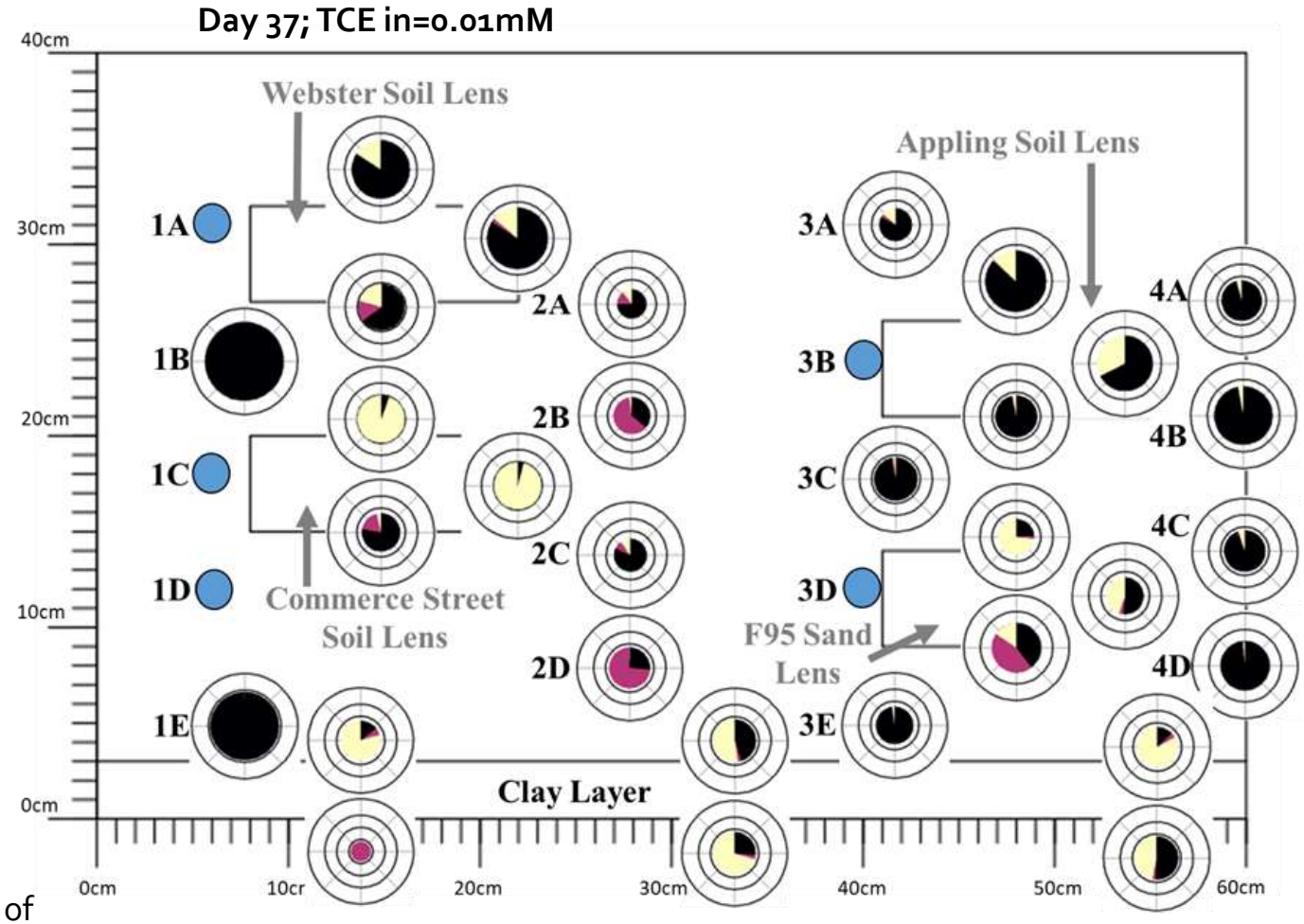
- *tceA* gene detected in locations where TCE likely to be present
- Proportion of *bvcA* gene elevated in locations where *cis*-DCE likely
- vcrA gene present throughout

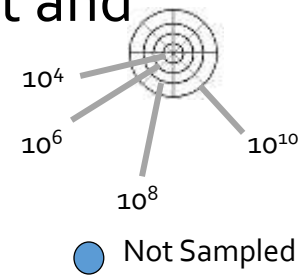


# Attached *Dhc* Strain Distribution

## Soil RDases

- *tceA* gene in center and downgradient of lenses, above clay, where TCE persisted
- *bvcA* gene primarily in locations where electron acceptors continued to diffuse (edges of lenses, in the clay), and lower OC & perm regions
- *vcrA* gene near TCE input and higher OC regions




Circle Size Proportional to log of total RDase Genes

Gene
bvcA (DCE to ethene)
tceA (TCE to VC)
vcrA (TCE to ethene)

# Bioenhanced Diffusion Conclusions

- **Dechlorinating microbes enhance mass transfer of chlorinated ethenes from low permeability regions over abiotic processes alone**
  - Incorporating enhancement will improve accuracy of predicted cleanup times, especially during MNA
- *Dhc* cells capable of penetrating low permeability porous media, including clays


- Distribution of cells harboring specific RDase genes influenced by the availability of electron acceptors within and near soils of differing physical properties
  - Important to **maintain diverse community**
  - Different species and strains will grow where conditions are most favorable

# Take Home Messages

- Microbial growth constraints/conditions determined in stagnate systems without a solid phase vs. those with solids, especially with flow, may differ
- Physiochemical-biological processes/technologies synergies can help to improve efficiency, shortcomings and/or reduce cost of standalone approaches
- Microbes can influence contaminant mass transfer in heterogeneous geologies leading to improved remediation
- Monitoring specific *Dhc* strains (sometimes in both phases) could be necessary for a complete understanding of microbial metabolism

# Questions?

***Course code: ANLC***

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