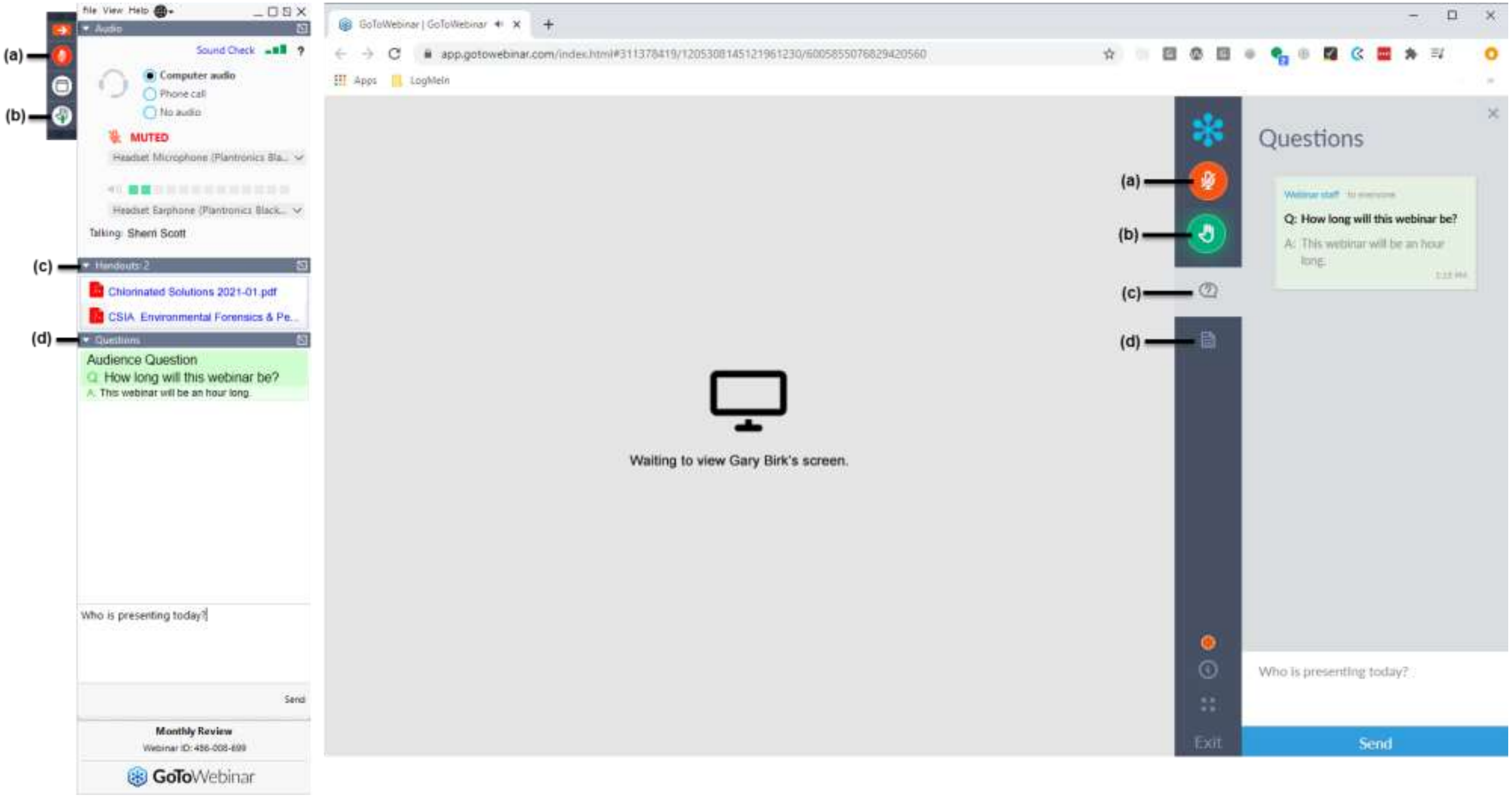
Remediation Seminar

# Volatile Fatty Acids: Key Markers for Electron Donor Optimization in Bioremediation Systems

Thu, Jan 28, 2021 1:00 PM - 2:00 PM EST

# User Dashboard




Remediation Seminar

# Today's Speakers

- Gary M. Birk, P.E.
- Managing Partner
- Tersus Environmental, Wake Forest, NC

Remediation Seminar

# Today's Speakers

- Brent G. Pautler, Ph.D.
- Customer Service Coordinator
- SiREM, Guelph, ON

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# How Does Bioremediation Work?

Electron Donor (Food)

+

Electron Acceptor (something to breathe) [$O_2$, $NO_3$, $SO_4$, TCE, etc.]

→

Waste Products [$CO_2$, $N_2$, $FeS_2$, $Cl^-$]

+

Energy

(Drawing Modified from AFCEE and Wiedemeier)

# What is needed?

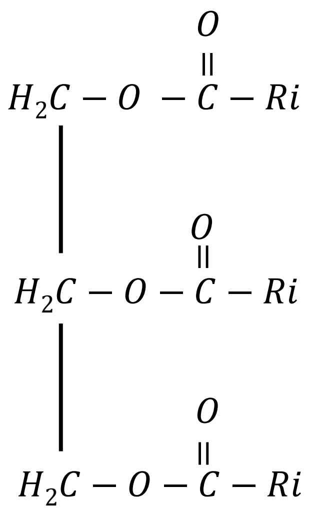
- Organic substrates that ferment to:
  - Acetate
  - Hydrogen
- Strong reducing conditions
- Right halorespiring bacteria
- Nutrients

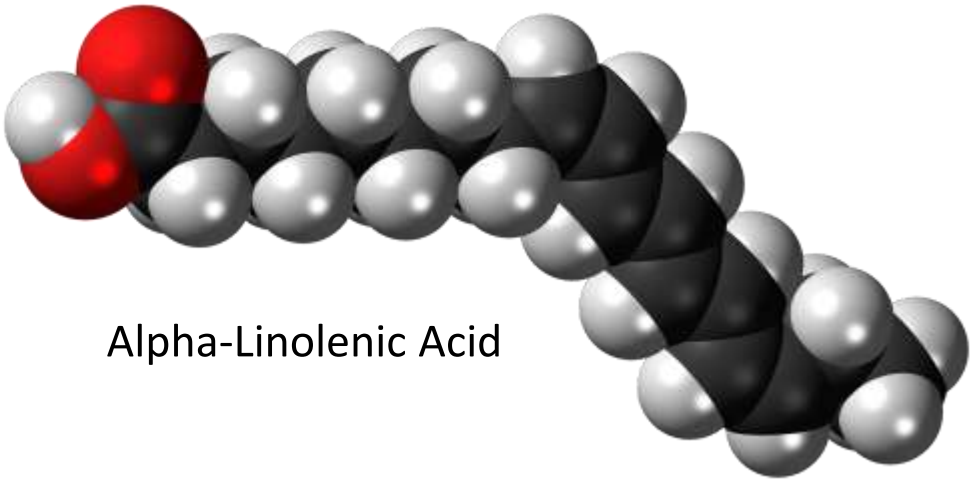
# Anaerobic Fermentation

**Soybean oil ferments to acetic acid and hydrogen**

# Soybean Fatty Acid Distribution

| Fatty Acid | | Percent |
|---|---|---|
| C-16:0 | Palmitic | 11.0 % |
| C-18:0 | Stearic | 4.0 % |
| C-18:1 | Oleic | 24.0 % |
| C-18:2 | Linoleic | 54.0 % |
| C-18:3 | Linolenic | 7.0 % |

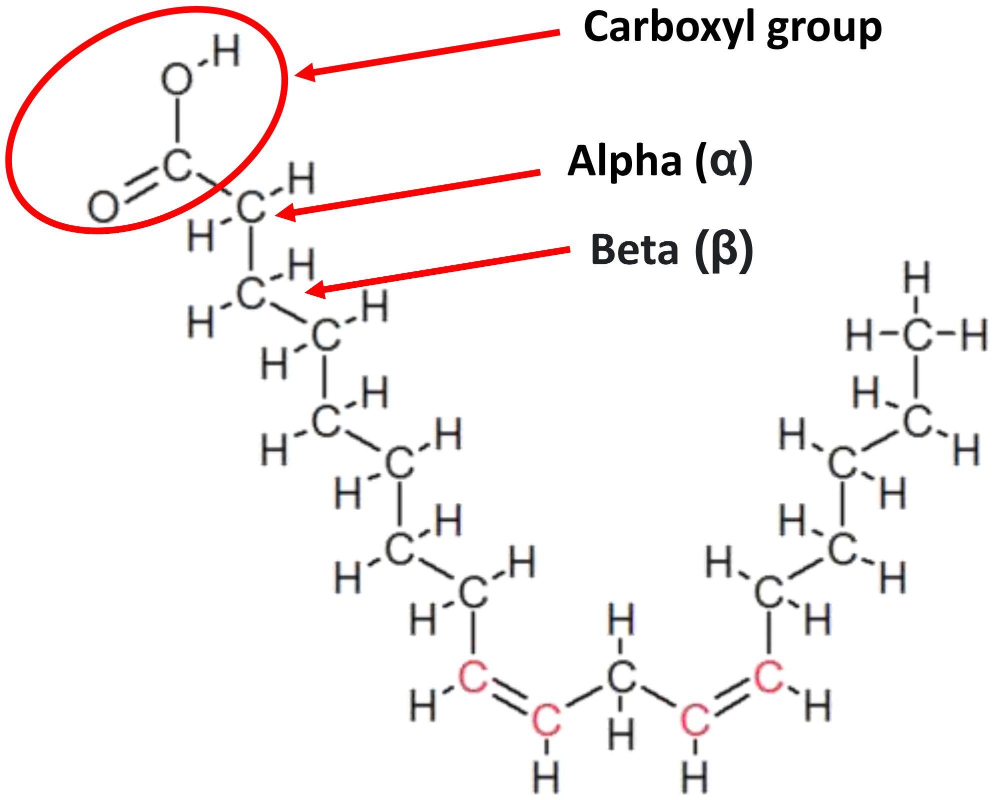


Alpha-Linolenic Acid

# Fatty Acid Oxidation

**Linoleic Acid**




**Multiple step metabolic process**

$$C_nH_{2n}O_2 + 2\ H_2O \Rightarrow C_{n-2}H_{2n-4}O_2 + 2\ H_2 + C_2H_4O_2$$

- Removes two carbons from the chain
- Releases:
  - Four hydrogen atoms (H)
  - Acetic Acid ($C_2H_4O_2$)

# Distribution of the correct type of fatty acids is essential

## Acetate

- Slow consumption
- Will migrate downgradient
- Stimulates PCE -> TCE -> cDCE
- Will not stimulate cDCE -> VC -> ethene

## Hydrogen ($H_2$)

**Produced from linolenic acid, propionate, butyrate, etc.**

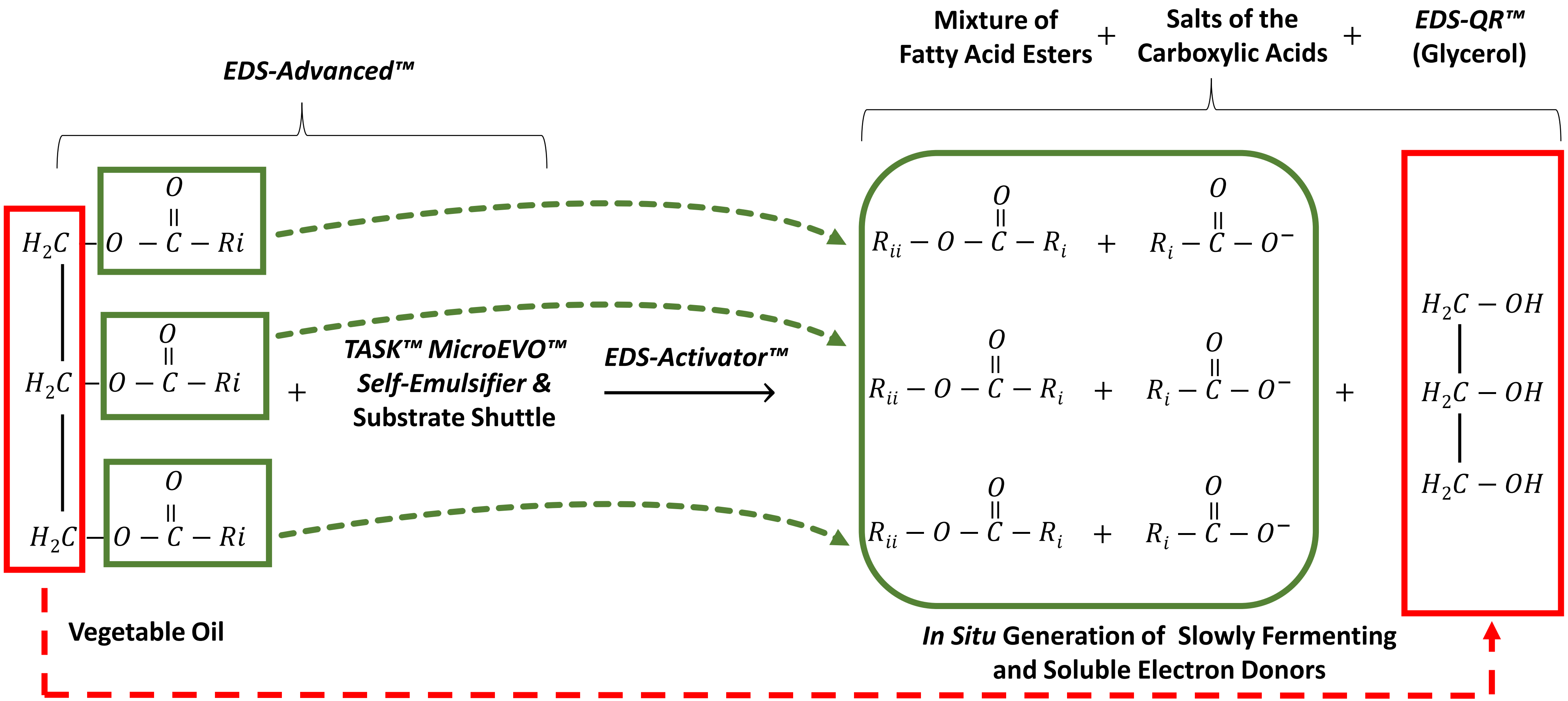
- Rapid consumption
- Does not migrate beyond injection zone
- Required for cDCE -> VC -> ethene

# pH Plays a Key Role in VFA Production

Systems under alkaline conditions

- Enhances the activity of fatty acid-producing bacteria
- Inhibits methanogens
- Increases production of VFAs

# Anaerobic Bioremediation Deploying Electron Donor Via *In Situ* Alcoholysis



SiREM
Leading Science · Lasting Solutions

# Volatile Fatty Acids: Key Markers for Electron Donor Optimization in Bioremediation Systems

Brent G. Pautler, Ph.D.
Customer Service Coordinator

# Introduction to SiREM

Founded in 2002 in Guelph, ON
Expanded to Knoxville, TN in 2020

Provide products and testing services to support and improve site remediation

Further information: siremlab.com

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# The Basics of Enhanced Bioremediation

- **Biostimulation:** The addition of nutrients to stimulate microbial activity (e.g., electron donors)
- **Bioaugmentation:** The addition of beneficial microorganisms to improve the rate or extent of biodegradation
- **SiREM bioaugmentation cultures:** for remediation of chlorinated volatile organic chemicals and benzene

*Injection of KB-1® each liter has ~100 billion Dhc cells*

KB-1® KB-1 plus® DGG B™

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# Key Components for Bioremediation

## Electron donor

A compound that donates electrons (becomes oxidized)

- Example: simple organic compounds such as sugars, alcohols, or methane can be oxidized to carbon dioxide ($CO_2$)

## Electron acceptor

A compound that accepts electrons (becomes reduced)

- Example: inorganic compounds like oxygen, nitrate, sulfate, oxidized metals, or $CO_2$ can be reduced to water, dinitrogen gas, hydrogen sulfide, dissolved metals, or methane, respectively

# Key Microbial Biodegradation Processes

## Oxidative Biodegradation

Microorganisms use the contaminant as a food source (electron donor)

- Need an electron acceptor (e.g., oxygen)

## Reductive Biodegradation

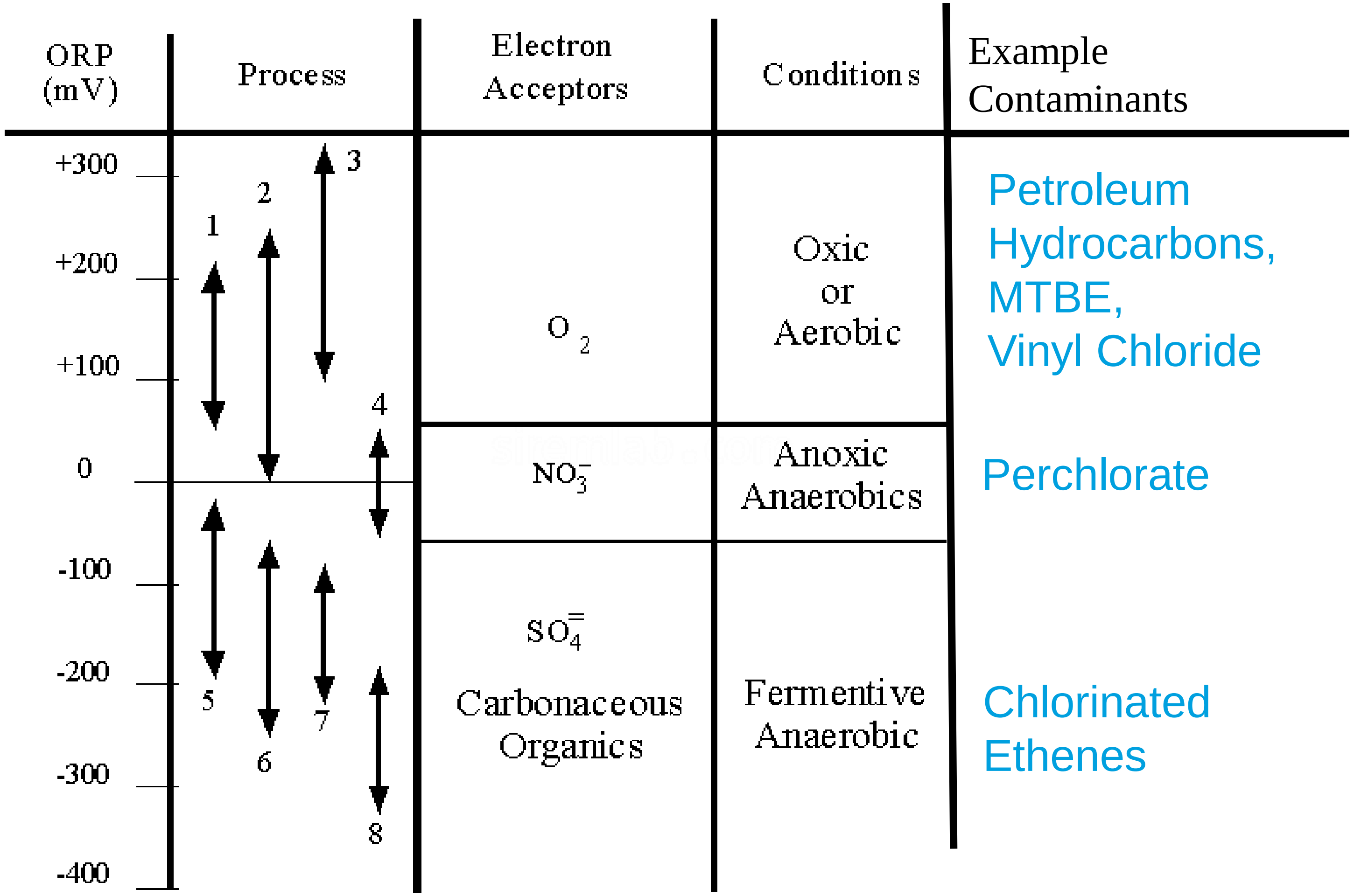
Microorganisms use the contaminant as an electron acceptor

- Need a food source (electron donor)

## Co-metabolism

Microorganisms break down contaminant w/o using it as a growth substrate (e.g., by enzyme secretion)

# Importance of Oxidation-Reduction Potential




1- Organic Carbon Oxidation
2- Polyphosphate Development
3- Nitrification
4- Denitrification
5- Polyphosphate Breakdown
6- Sulfide Formation
7- Acid Formation
8- Methane Formation

ORP Probe

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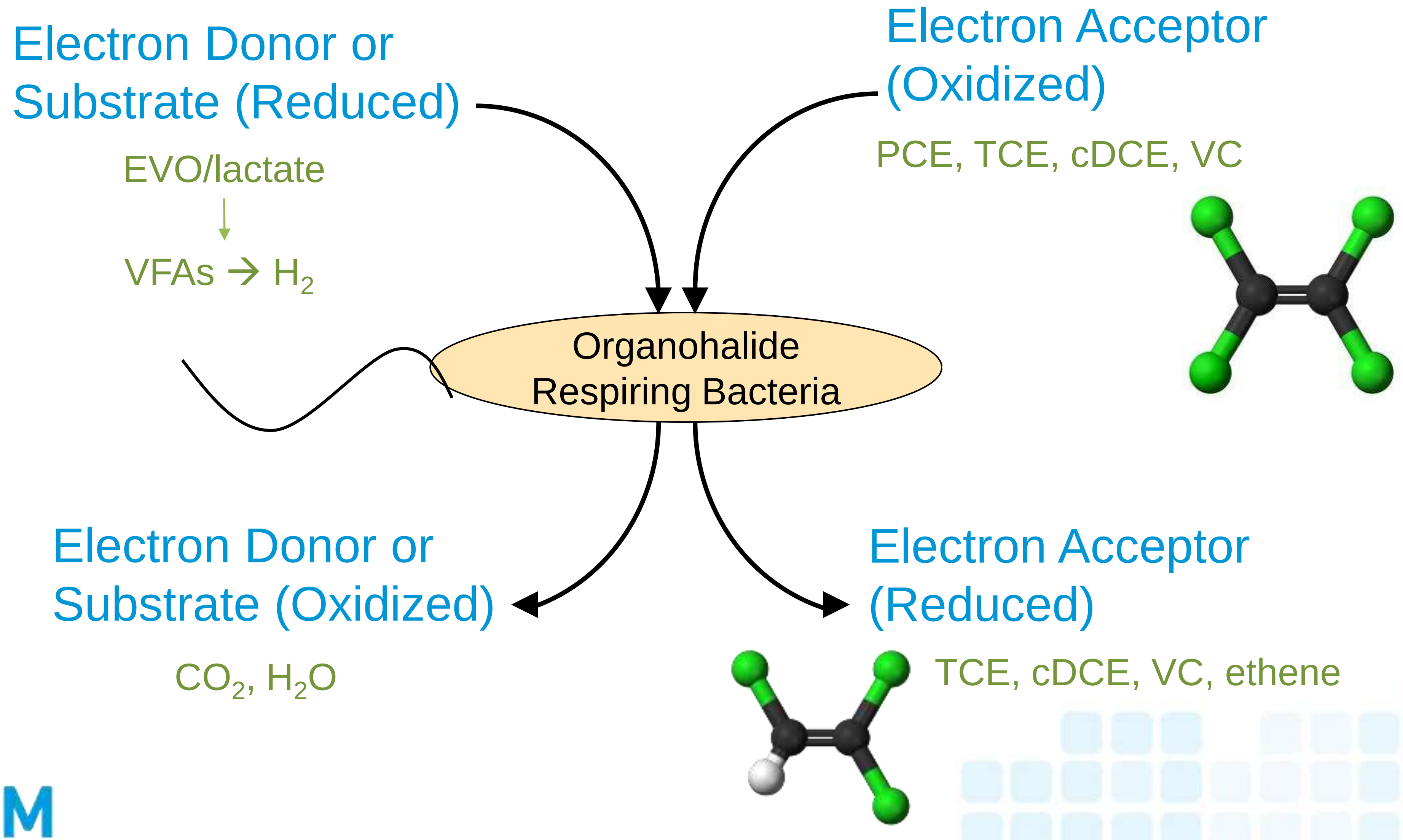
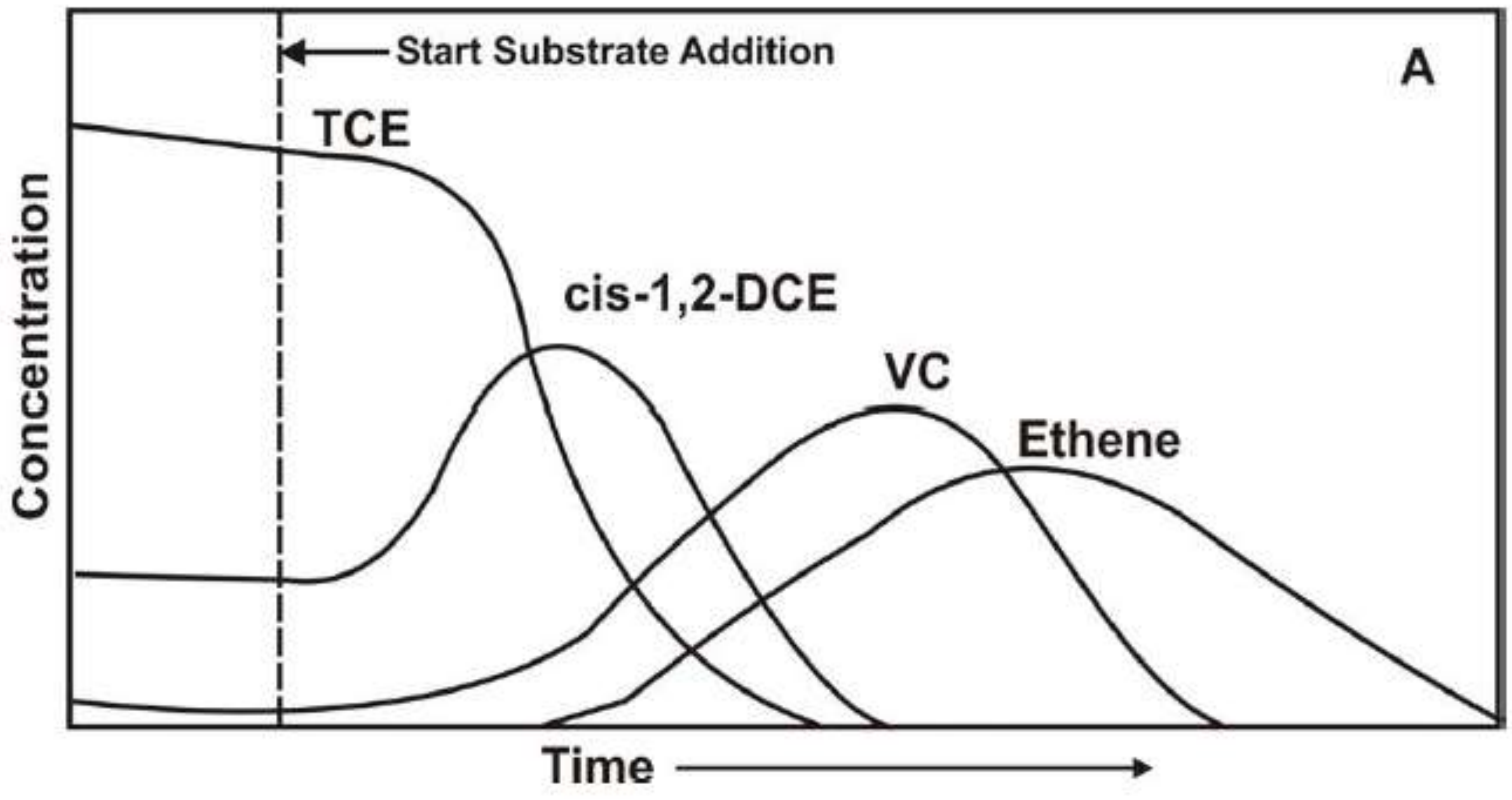
# Chlorinated VOCs and Molecular Metabolism



# Conceptual Bioremediation of Chlorinated VOCs

- Pre- and post-substrate contaminant mass from bioremediation

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Conceptual Changes in Contaminant Molar Concentration over Time with Sequential Anaerobic Dechlorination

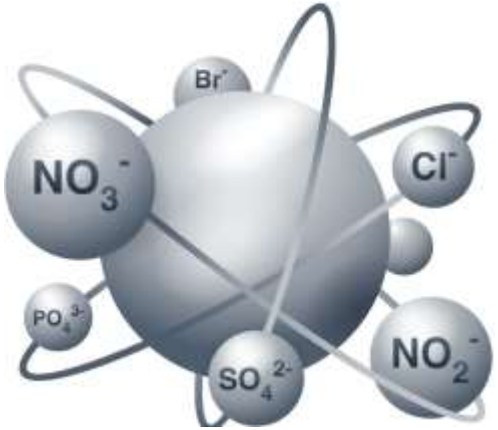
# Bioremediation Monitoring

gene trac

- Quantify microbial biodegraders (qPCR)
- Determine impact of site amendments including electron donor/acceptors on microbial community
- Monitor progress and validate performance of bioremediation

## Anions Analysis

- Monitor $Cl^-$ released during reductive dechlorination
- Monitor competing electron acceptors, e.g., nitrate/sulfate




## Volatile Fatty Acids

- Confirm fermentation of slow release and soluble electron donors
- Map fermentation pathways
- Determine the need for additional electron donor

## Dissolved Hydrocarbon Gases

- Confirm complete dechlorination of chlorinated ethenes, ethanes and propanes
- Quantify methanogenesis
- Quantify gases used in co-metabolic remediation

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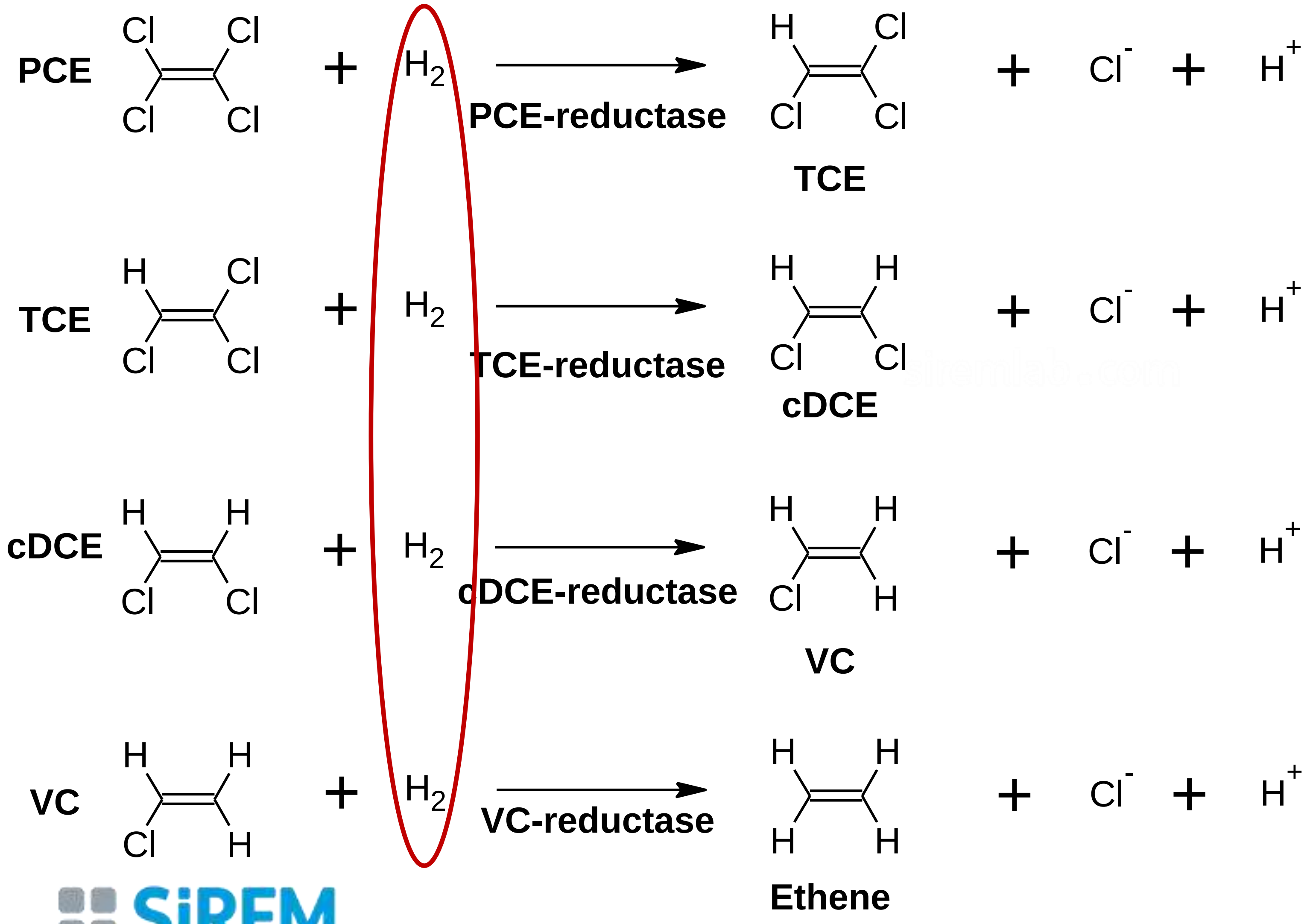
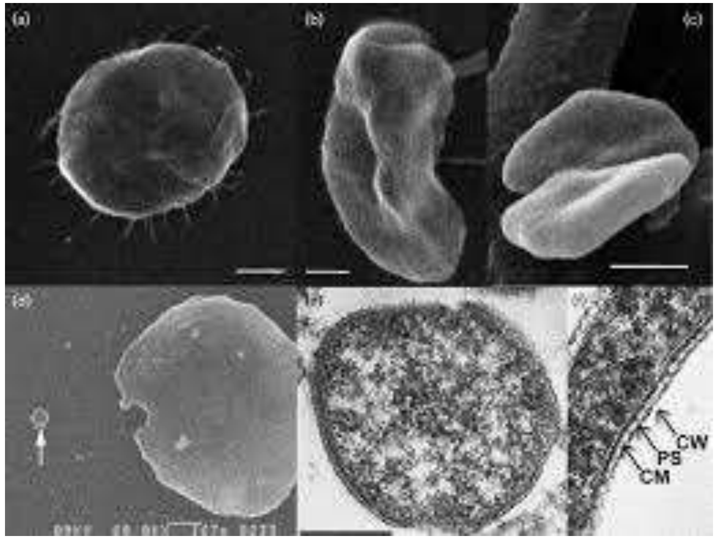
# Reductive Dechlorination by *Dhc*




## $H_2$ as Direct Electron Donor

- Produced by fermentation organic substances
  - Carbohydrates
  - Alcohols
  - Fatty Acids (VFAs)
- Consumed quickly

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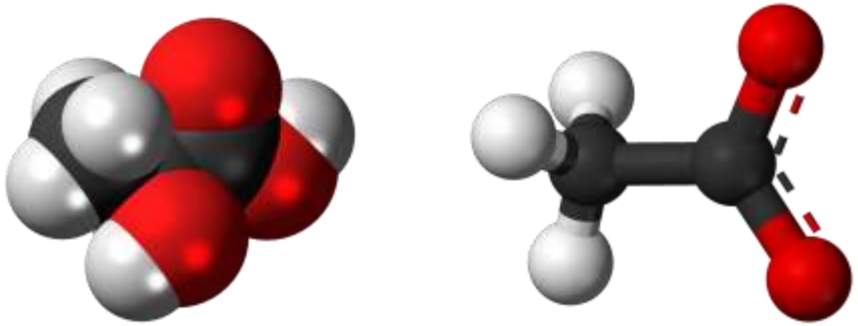
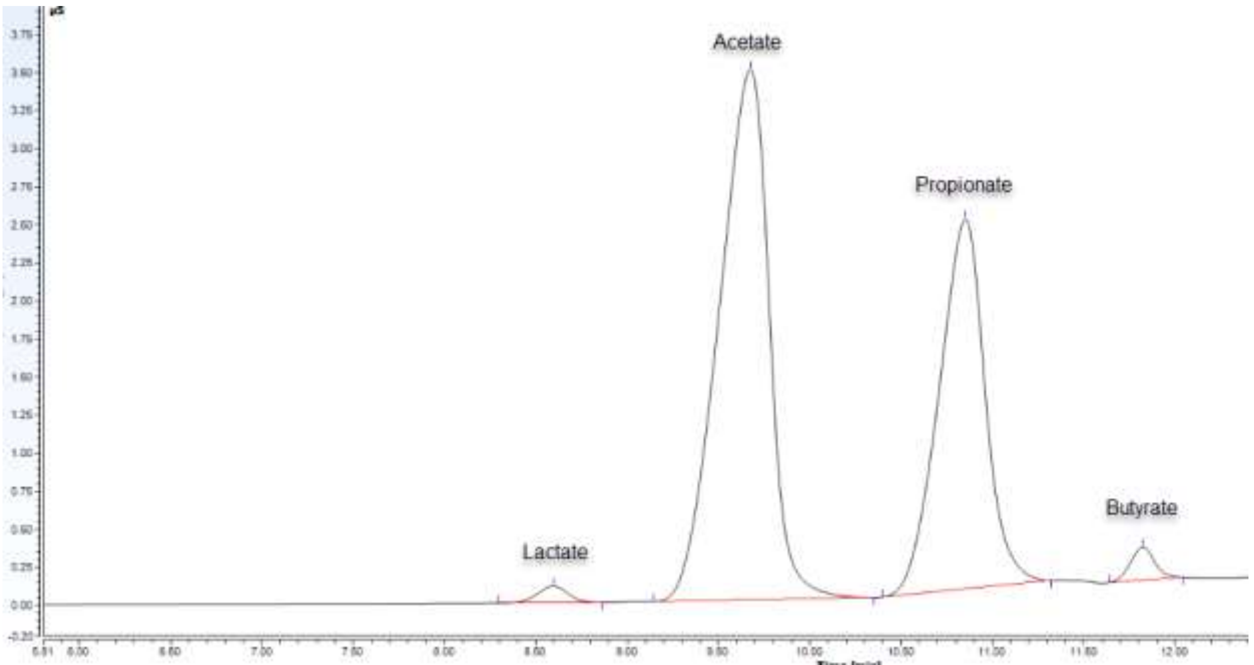
# Specialty Chemical Analytical Services for Remediation

## Volatile Fatty Acids (VFAs)

- Quantification used to assess electron donor status in bioremediation systems and fermentation
- Simple sampling procedures and laboratory analysis
- Typically done with a standard IC Method

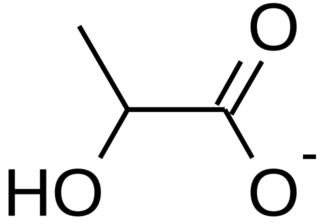




# Electron Donors for Anaerobic Bioremediation

- TOC as a proxy for energy source for bioremediation?
  - Not all TOC the same and available/ferments to $H_2$
    - VFAs not quantified by many TOC methods
  - Many competing processes and electron acceptors
- Choose the most appropriate based on the site characteristics

**EVO**




**Lactate**


**Alcohol(s)**

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# Electron Donors - Reactions

- Fermentation
  - Redox reaction where different portions of a single substrate are oxidized and reduced → Energy

$$C_{56}H_{100}O_6 \textit{ (soybean oil)} + 50H_2O \longrightarrow 28CH_3COOH + 44H_2$$

| Electron Donor | Electron-Donor (Oxidation) Reaction |
|---|---|
| Ethanol | $C_2H_6O + H_20 \Rightarrow C_2H_3O_2^- + H^+ + 2H_2$<br>*ethanol fermentation to acetate* |
| Methanol | $CH_4O + 2H_20 \Rightarrow CO_2^- + H_2O + 3H_2$<br>*methanol fermentation* |
| Acetate | $C_2H_3O_2^- + 4H_20 \Rightarrow 2CO_2^- + 2H_2O + 4H_2$<br>*acetate fermentation* |
| Butyrate | $C_4H_7O_2^- + 2H_20 \Rightarrow 2C_2H_3O_2^- + H^+ + 2H_2$<br>*butyrate fermentation to acetate* |
| Propionate | $C_3H_5O_2^- + 3H_20 \Rightarrow C_2H_3O_2^- + CO_2^- + H_2O + 3H_2$<br>*propionate fermentation to acetate* |
| Lactate | $C_3H_5O_3^- + 2H_2O + \Rightarrow C_2H_3O_2^- + CO2^- + H_2O + 2H_2$<br>*lactate fermentation to acetate* |

Fennell & Gossett, (1998) *ES&T, 32: 2450-2460*

He *et al*, (2002) *ES&T, 36: 3945-3952*

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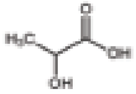
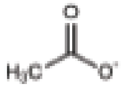
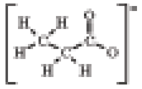
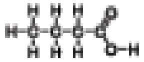
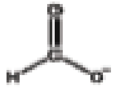
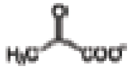
# Sources and Roles of Specific VFAs

| VFA | Structure | Formula (Ion) | Source | Role |
|---|---|---|---|---|
| Lactate |  | $C_3H_6O_3^-$ | Common primary amendment/component of EVO mixtures | Rapidly fermented to propionate and acetate producing hydrogen for dechlorination (Aulenta et al., 2007) |
| Acetate |  | $C_2H_2O_2^-$ | From fermentation of lactate/EVO/sugars | Electron donor for some (incomplete) dechlorination reactions (e.g., Krumholtz et al., 1996) Carbon source for *Dhc* (Cupples et al., 1993) |
| Propionate |  | $CH_3CH_2COO^-$ | From fermentation of lactate/EVO/alcohols | Fermented to produce hydrogen and formate |
| Butyrate |  | $CH_3CH_2CH_2COO^-$ | From fermentation of EVO/alcohols | Fermented to produce hydrogen and acetate |
| Formate |  | $CH_2O_2^-$ | From fermentation of propionate | Fermented to produce hydrogen and bicarbonate |
| Pyruvate |  | $C_3H_4O_3^-$ | From fermentation of sugars | Fermented to propionate and acetate with hydrogen production (Cope and Hughes, 2001) |

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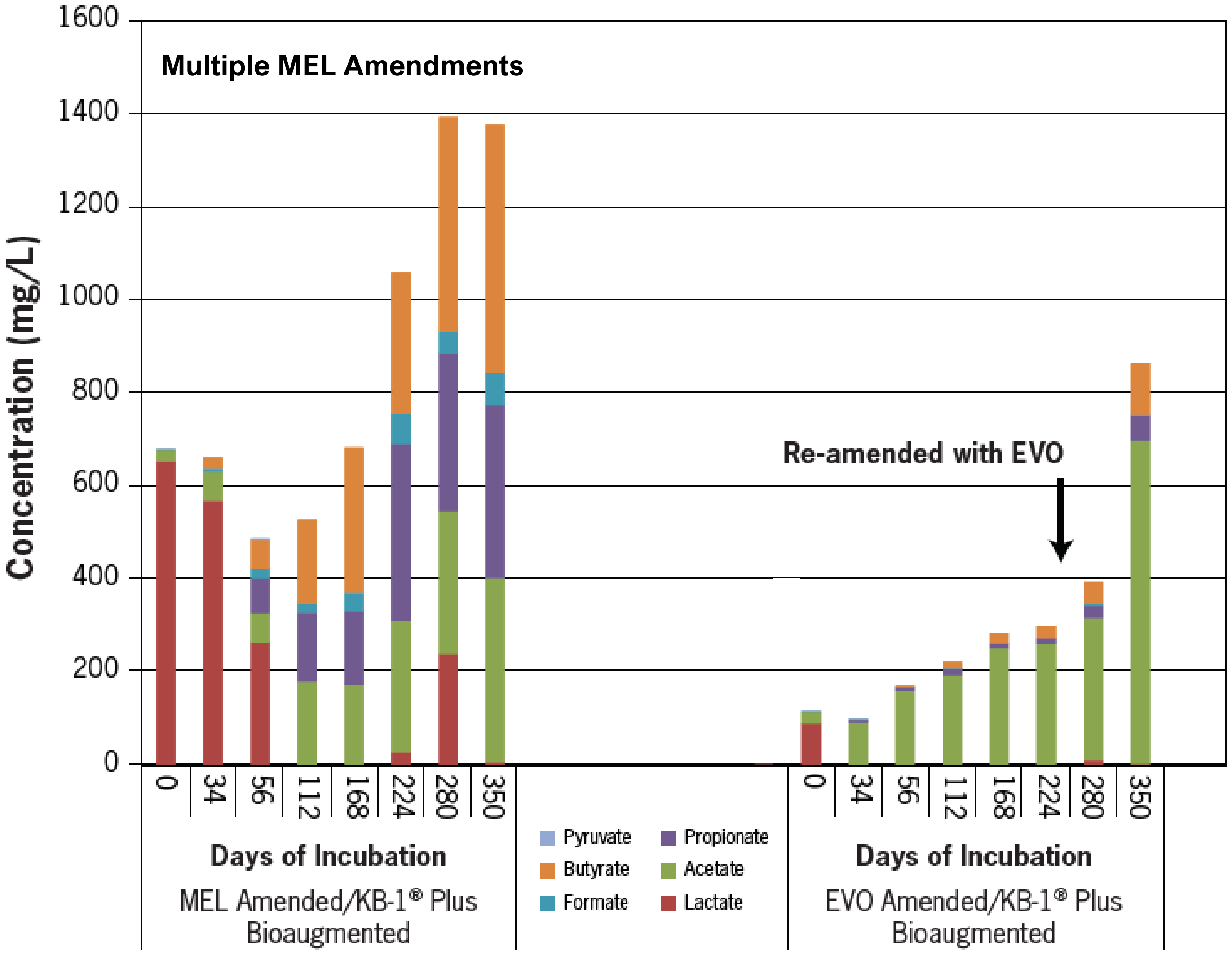
# VFAs as an Electron Donor Status Indicator



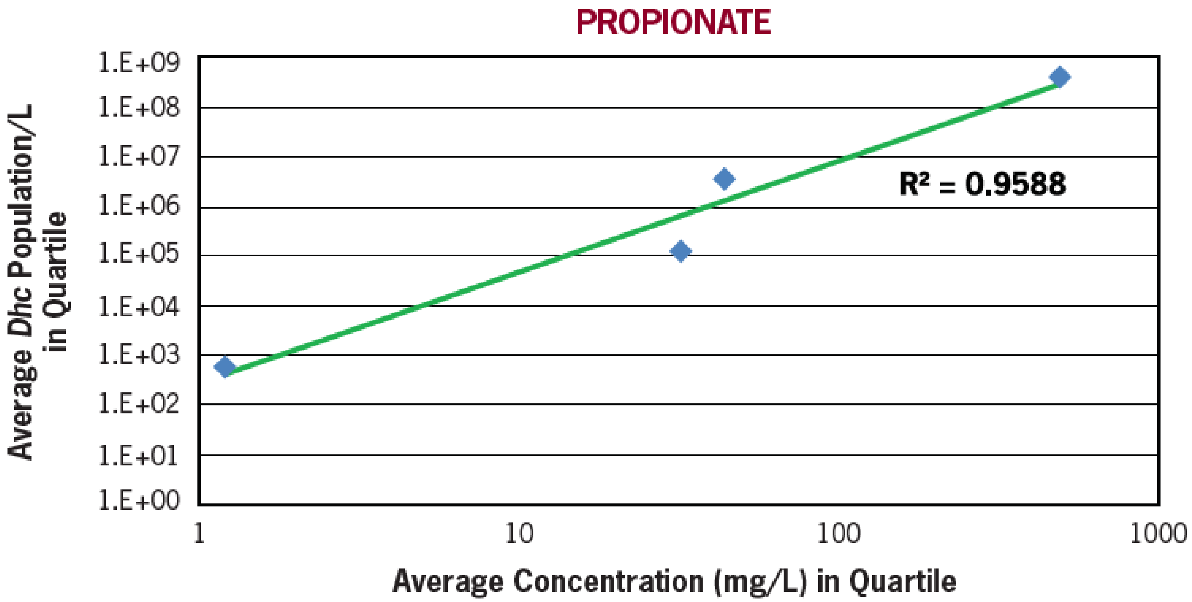

## During Active Fermentation

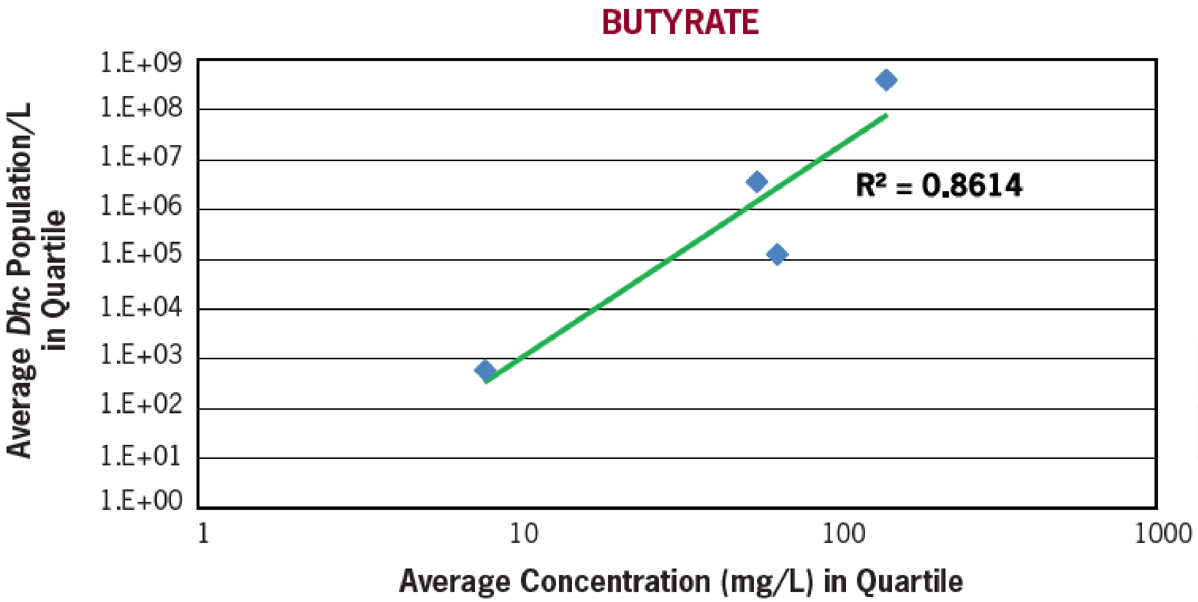
- Acetate generally increases over time
- Stabilization of acetate over time in absence of other VFAs may indicate exhaustion of electron donor supply
  - Longer lasting when compared to other VFAs with low energy yield
  - Mobile: will migrate downgradient
  - Tends to encourage acetoclastic methanogenesis
  - Will not stimulate cDCE → VC → Ethene

# VFAs Composition and *Dhc* Populations

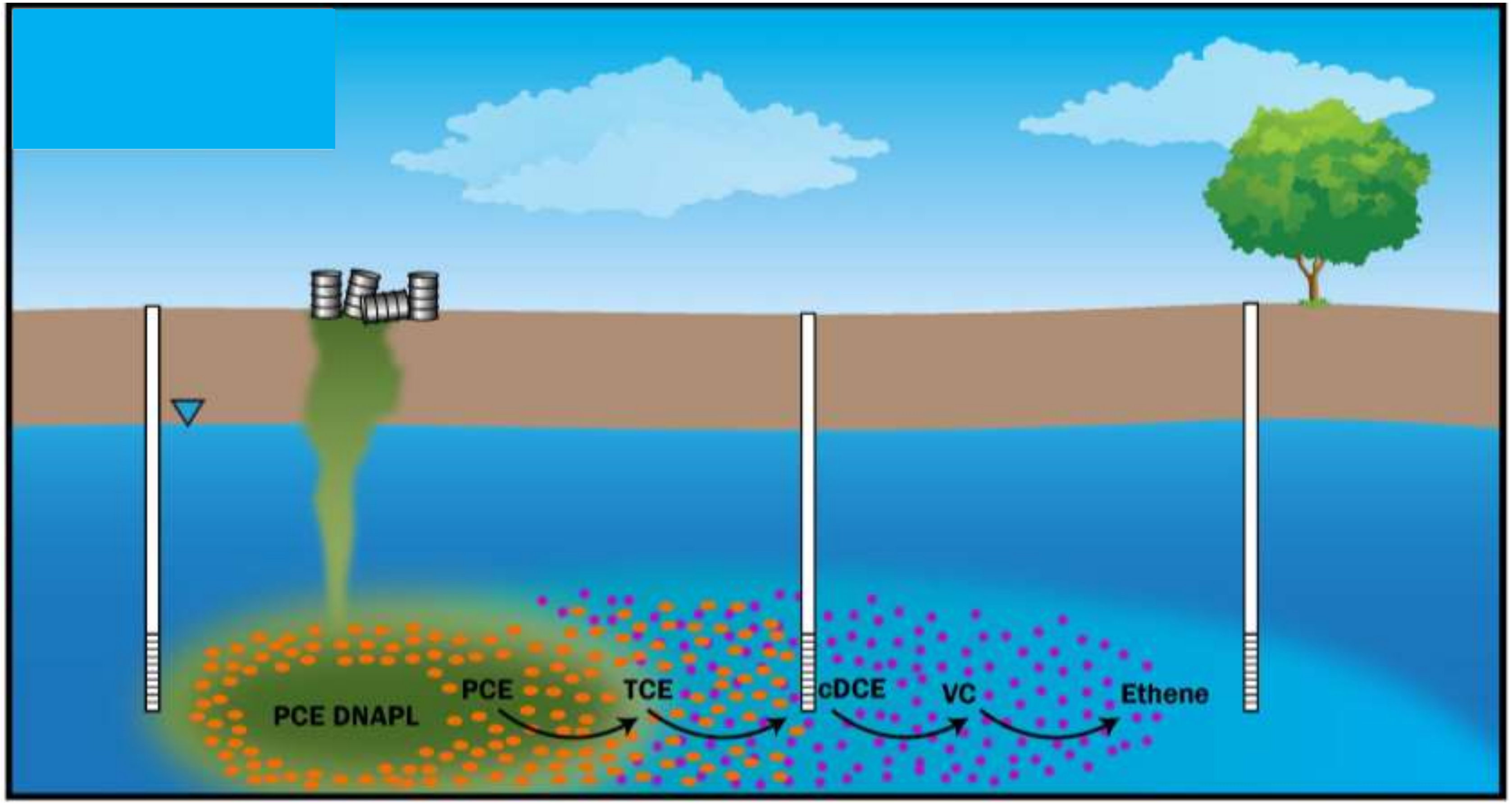
## VFA composition positively impacts growth of *Dhc* and dechlorination

- Propionate and butyrate showed strongest correlation
- Lesser extent formate and acetate
    - Acetate formation linked to production of propionate (from lactate fermentation) or related to previous fermentation
    - Acetate in the absence of other VFAs → No positive relationship with *Dhc* population
- Lactate and pyruvate were not positively correlated



# VFAs & Source Zone Bioremediation Challenges




**DNALP**

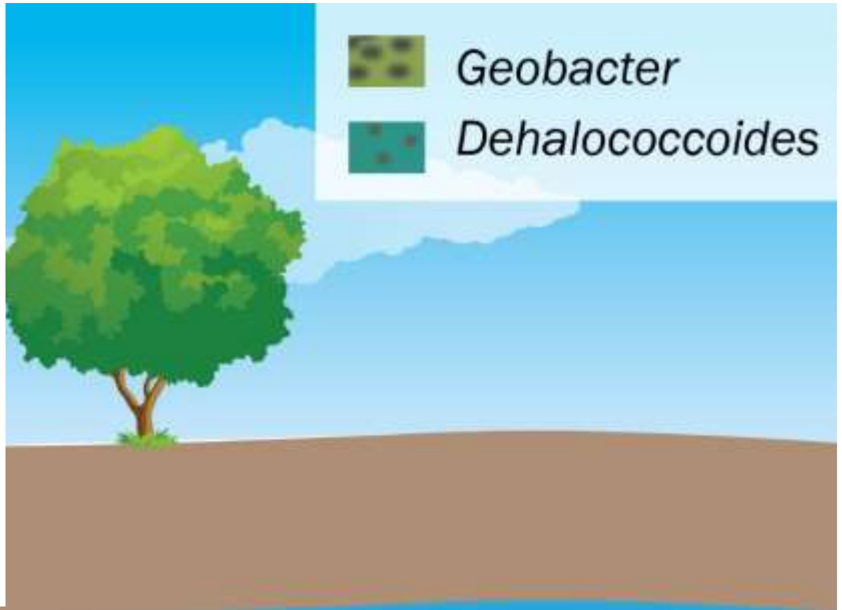
- Toxic concentrations/pH
- Absence of proper microbes
- Electron donor and micronutrients are in high demand and limiting
- Delivery challenges

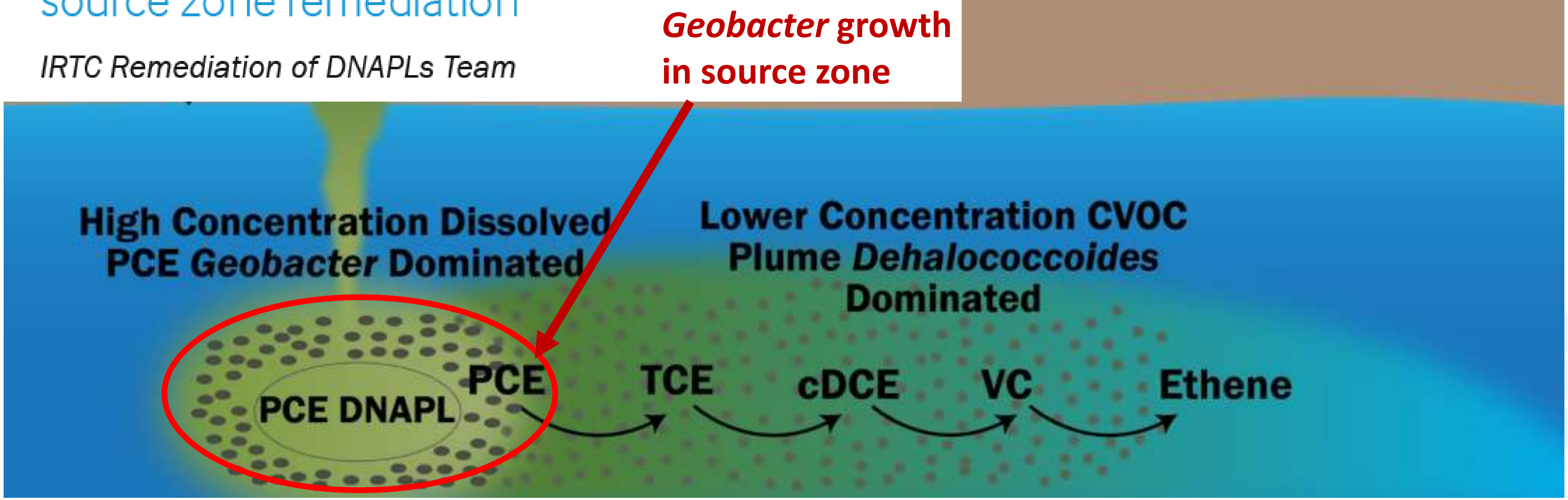
Can overcome source zone bioremediation challenges and optimize remediation systems

# Importance of *Geobacter to DNAPL*

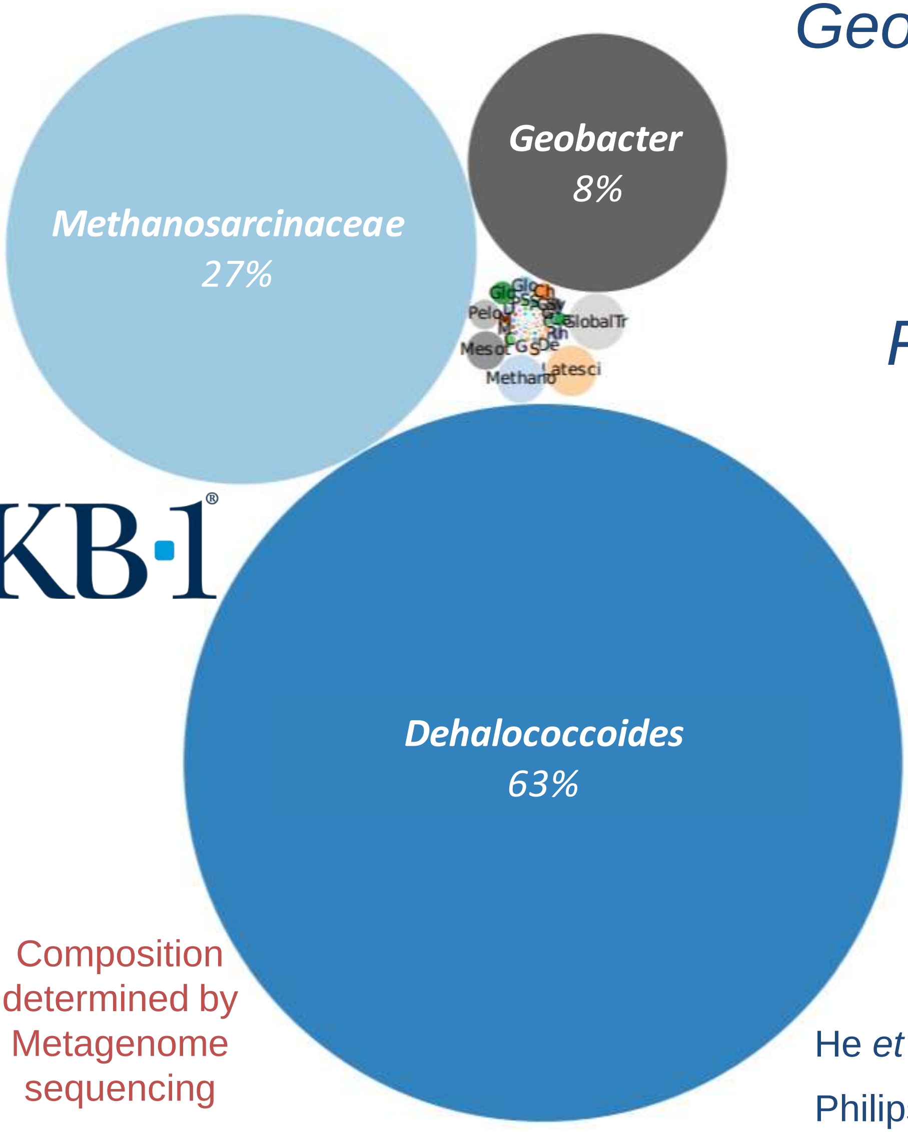
"A potential advantage of bioremediation technology is that microorganisms-which can attack the contaminant at or near the DNAPL water interface, may provide an effective, efficient, and less costly approach to DNAPL source zone remediation"

*IRTC Remediation of DNAPLs Team*



# Composition of KB-1® Bioaugmentation Culture




Composition determined by Metagenome sequencing

*Geobacter uses* ***Acetate*** *to dechlorinate PCE/TCE to cDCE*

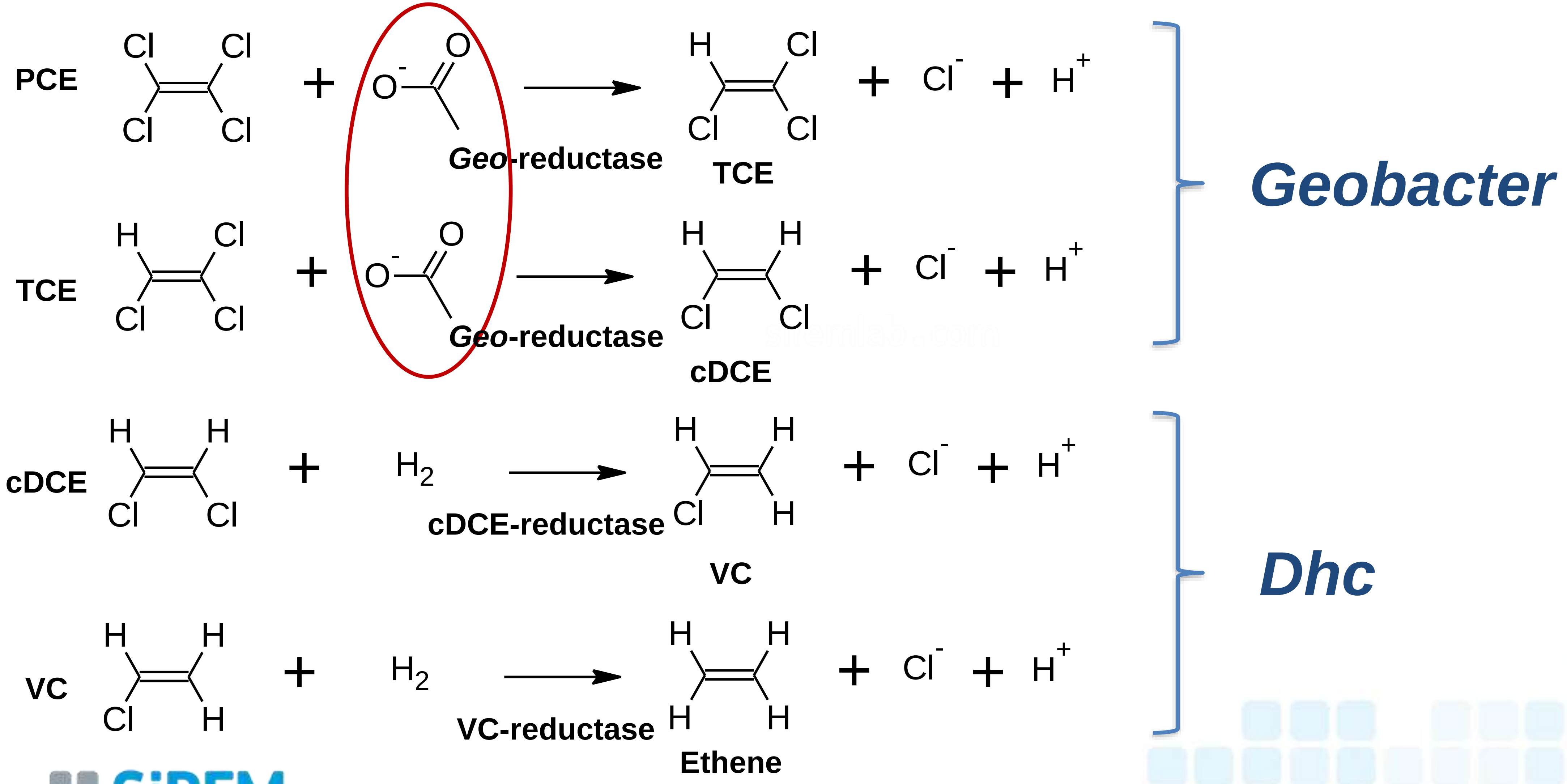
*Ferment e- donor to $H_2$ +* ***Acetate***

*Dhc uses $H_2$ to dechlorinate cDCE to* ***ethene***


He *et al.*, (2002) *ES&T, 36: 3945-3952*

Philips *et al.*, (2012) *FEMS Microbiol, 81: 636-647*

# Reductive Dechlorination - *Geobacter*

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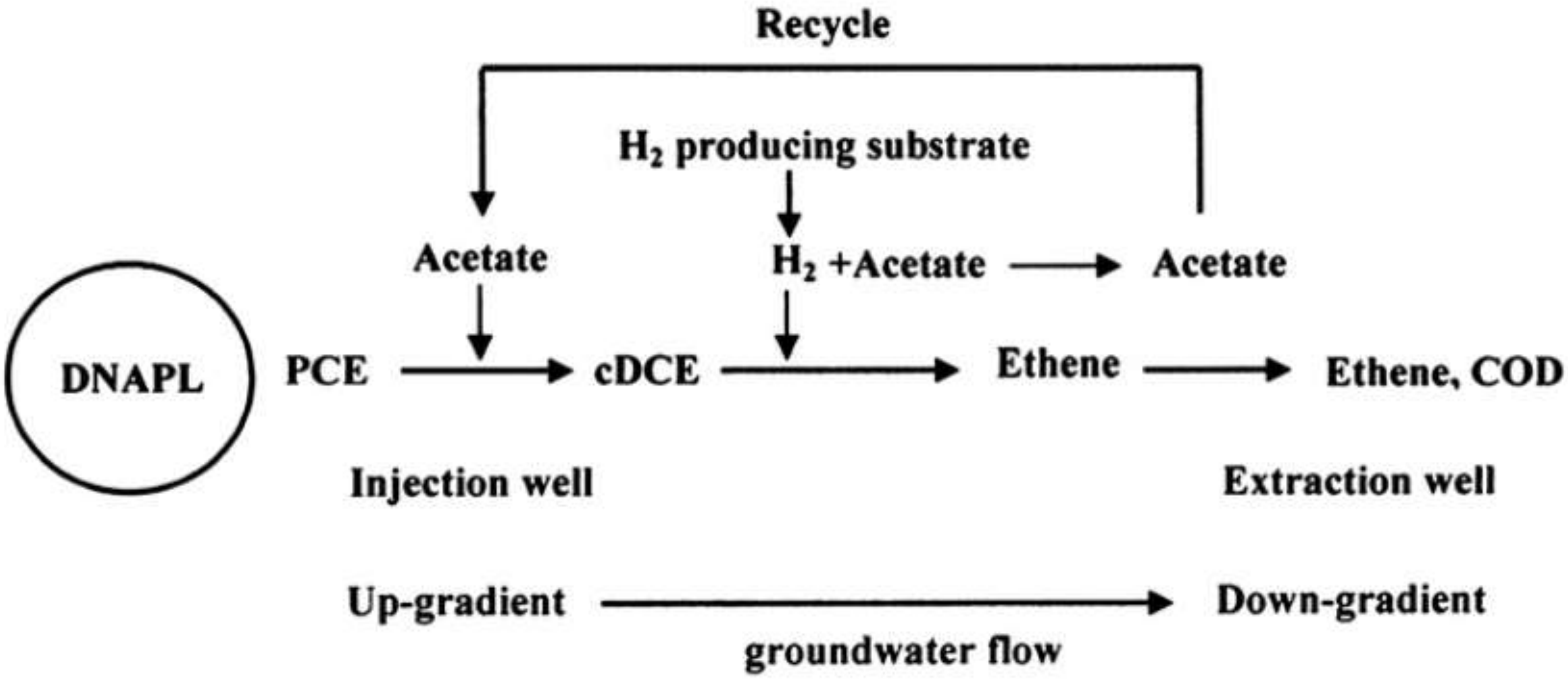
# Proposed DNAPL Bioremediation Strategy



# Case Study: Biotreatability New York Site

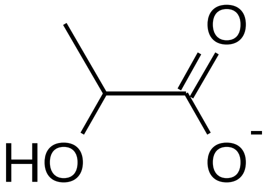
treatability studies

**Mixed chlorinated ethenes, chlorinated ethanes and CFC-113**

- CFC-113 (1.5 mg/L)
- 1,1,1-TCA (1 mg/L)
- 1,1,-DCA (0.5 mg/L)
- VC (0.15 mg/L)


**Treatments**

- Sterile and Active Controls
- Lactate Amended
- Lactate Amended & KB-1® Plus Bioaugmented





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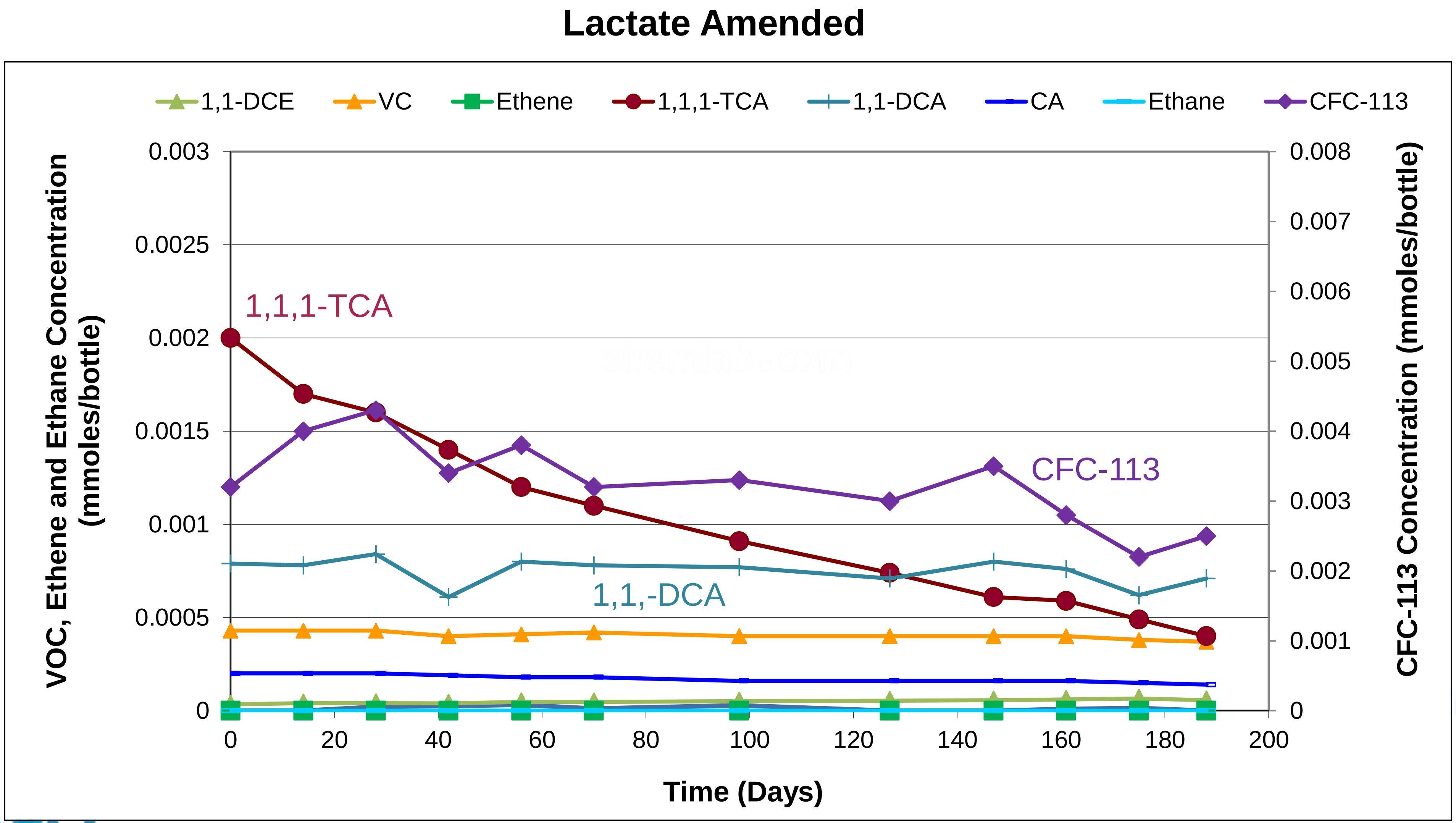
# Case Study: Biotreatability New York Site

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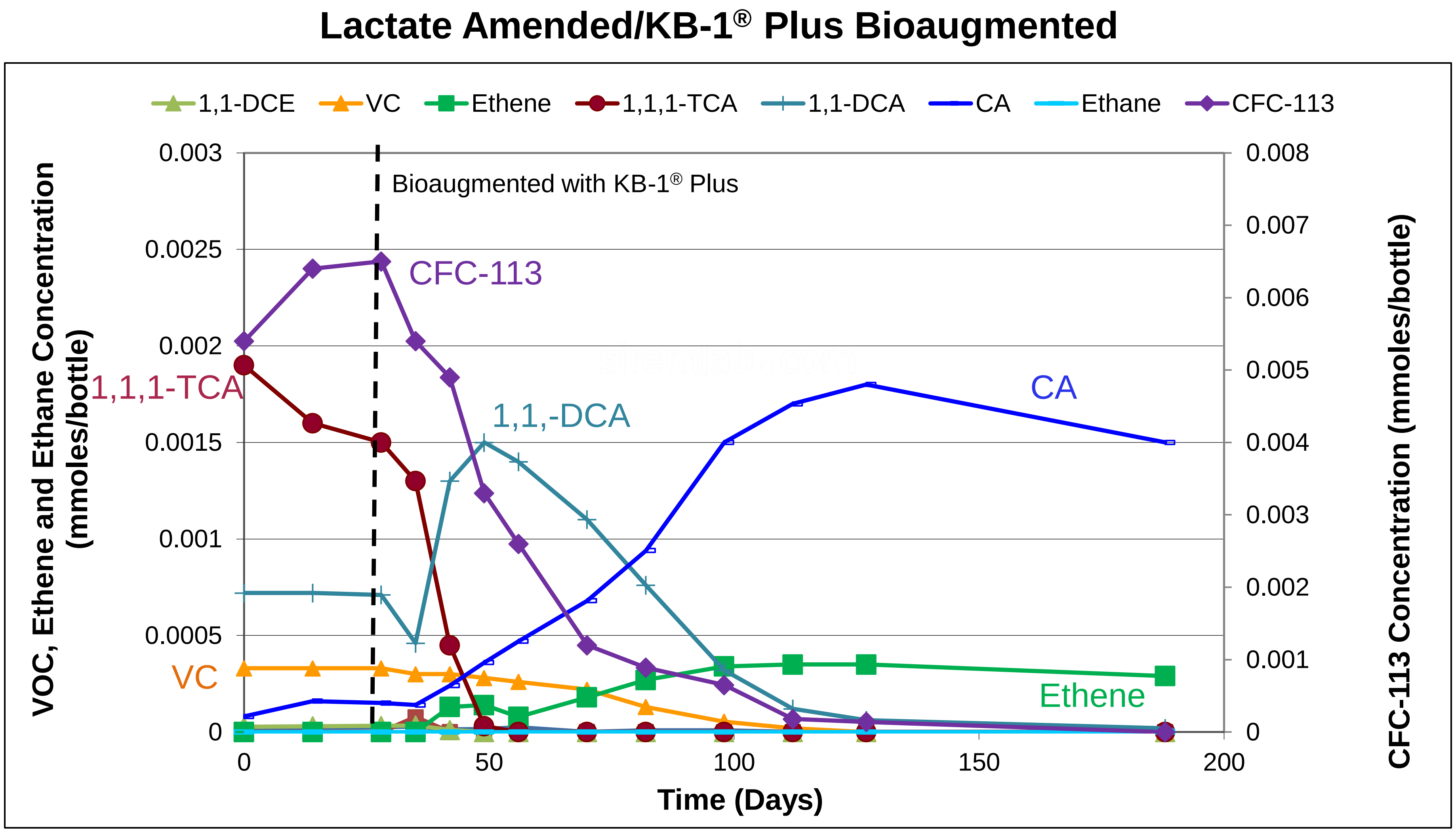
# Case Study: Biotreatability New York Site

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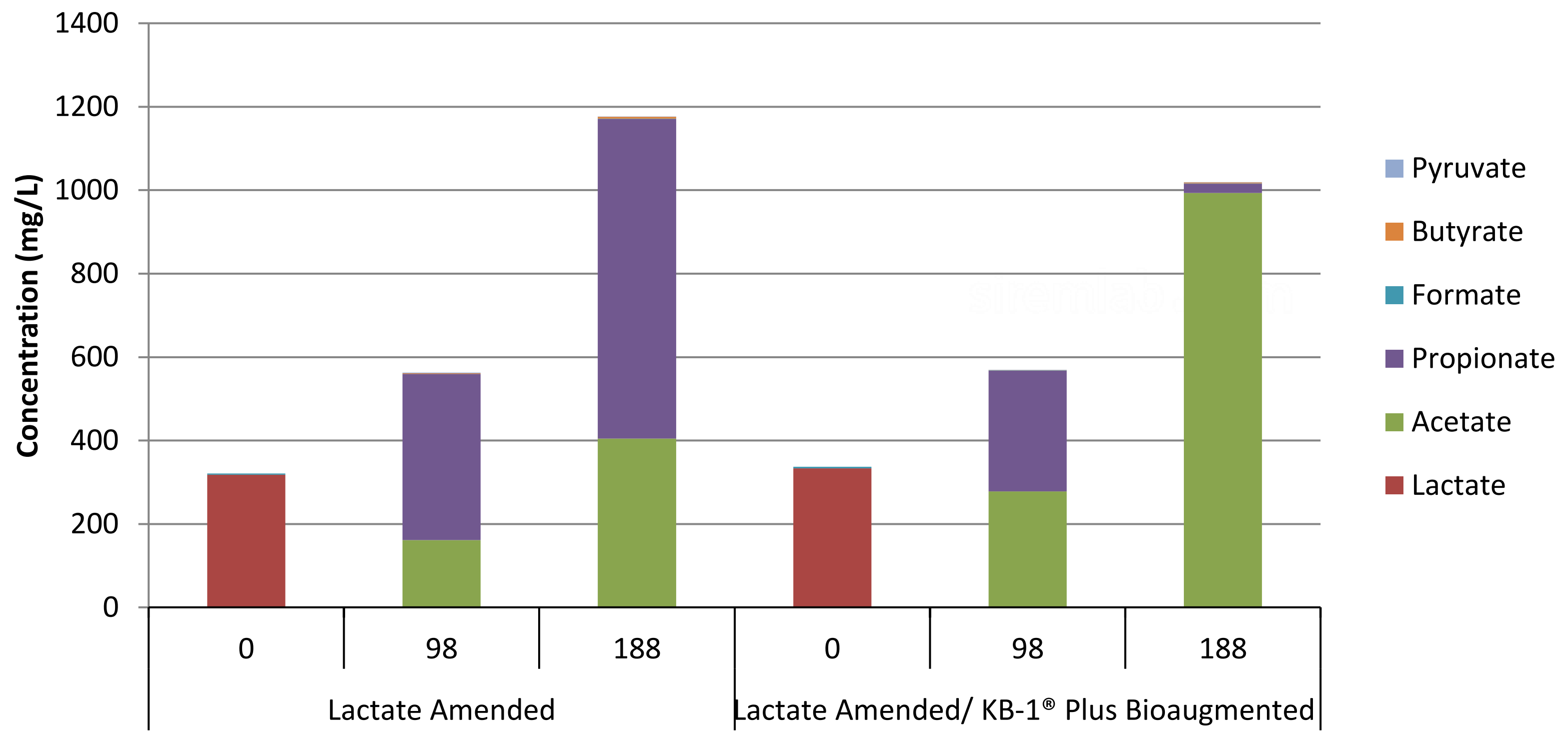
# Case Study: Biotreatability New York Site

## Volatile Fatty Acids




KB-1 plus®

**Contains Fermenters**

- More robust fermentation

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# Case Study: Bioaugmentation, California Site



**EVO (EDS-ER™) & soluble donor**


## Chlorinated ethenes

- TCE
- PCE
- DCE
- VC

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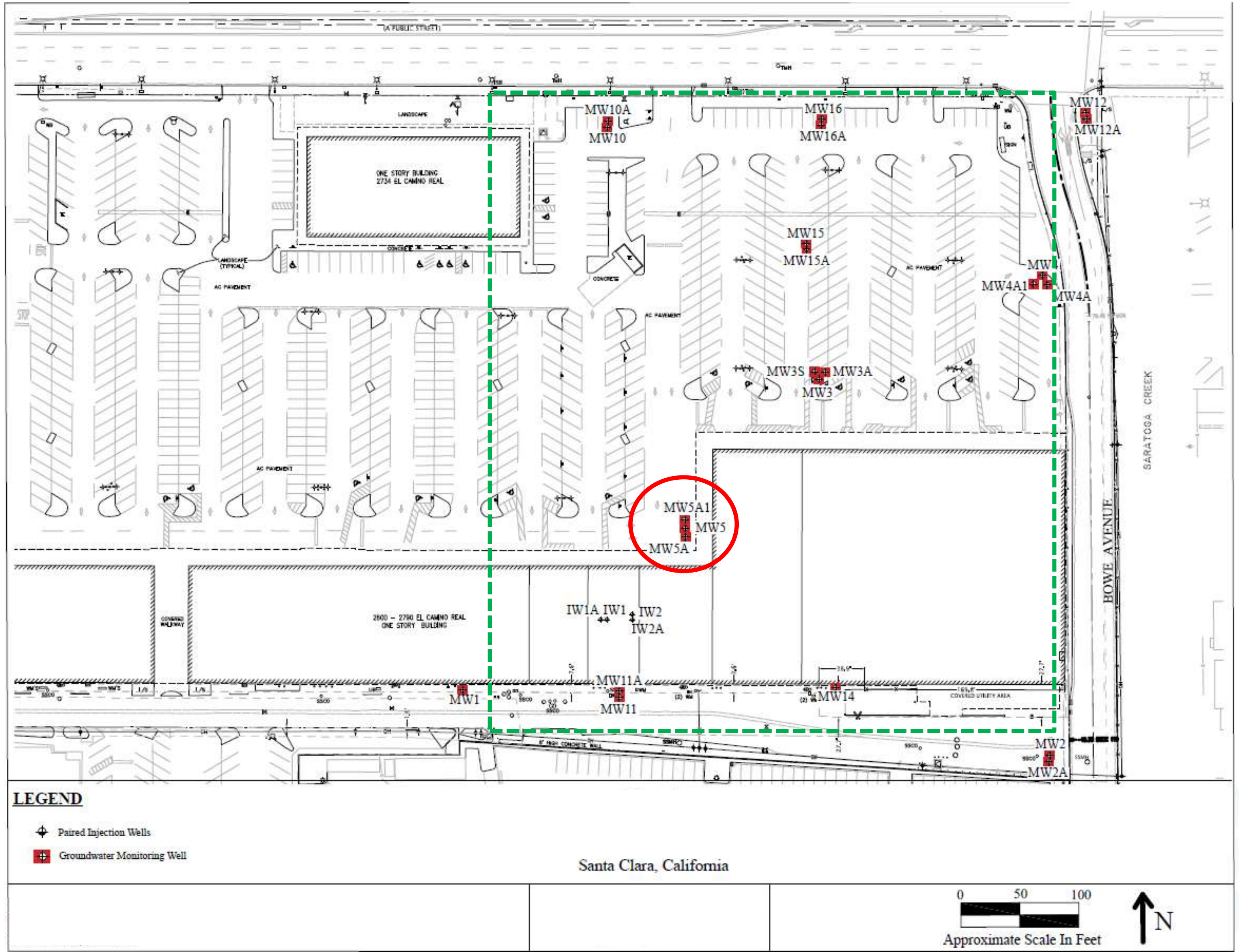
KB-1®

KB-1® PRIMER

# Case Study: Bioaugmentation, California Site

**140 Injection Points Total**

- Quarterly Monitoring

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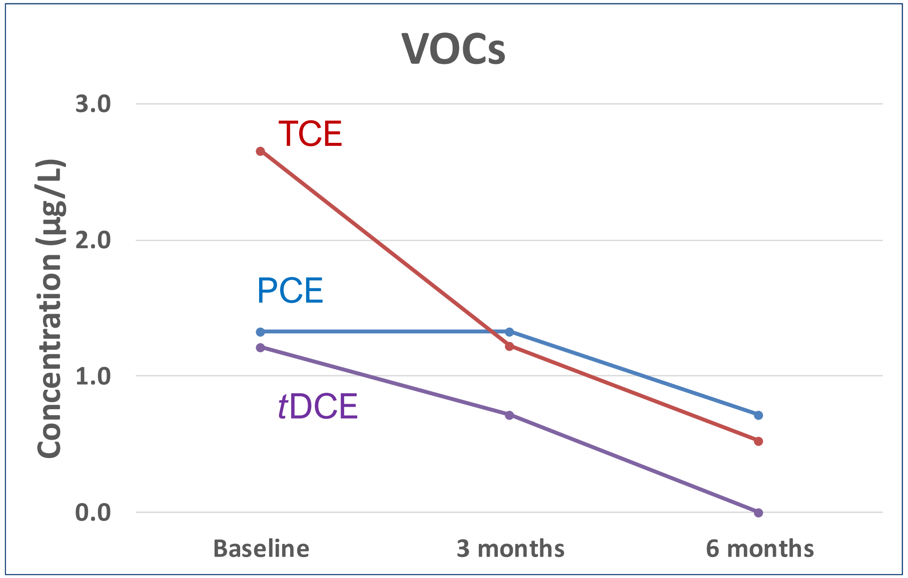
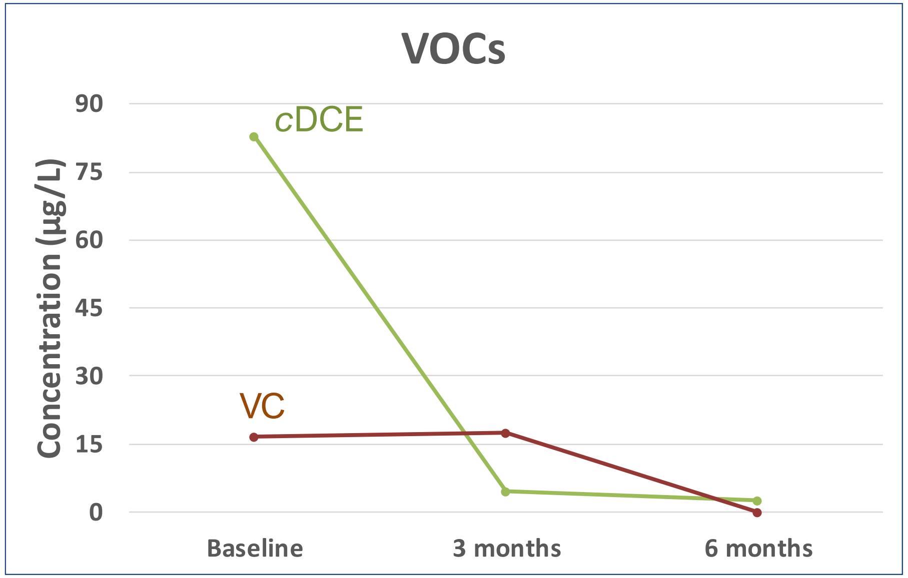
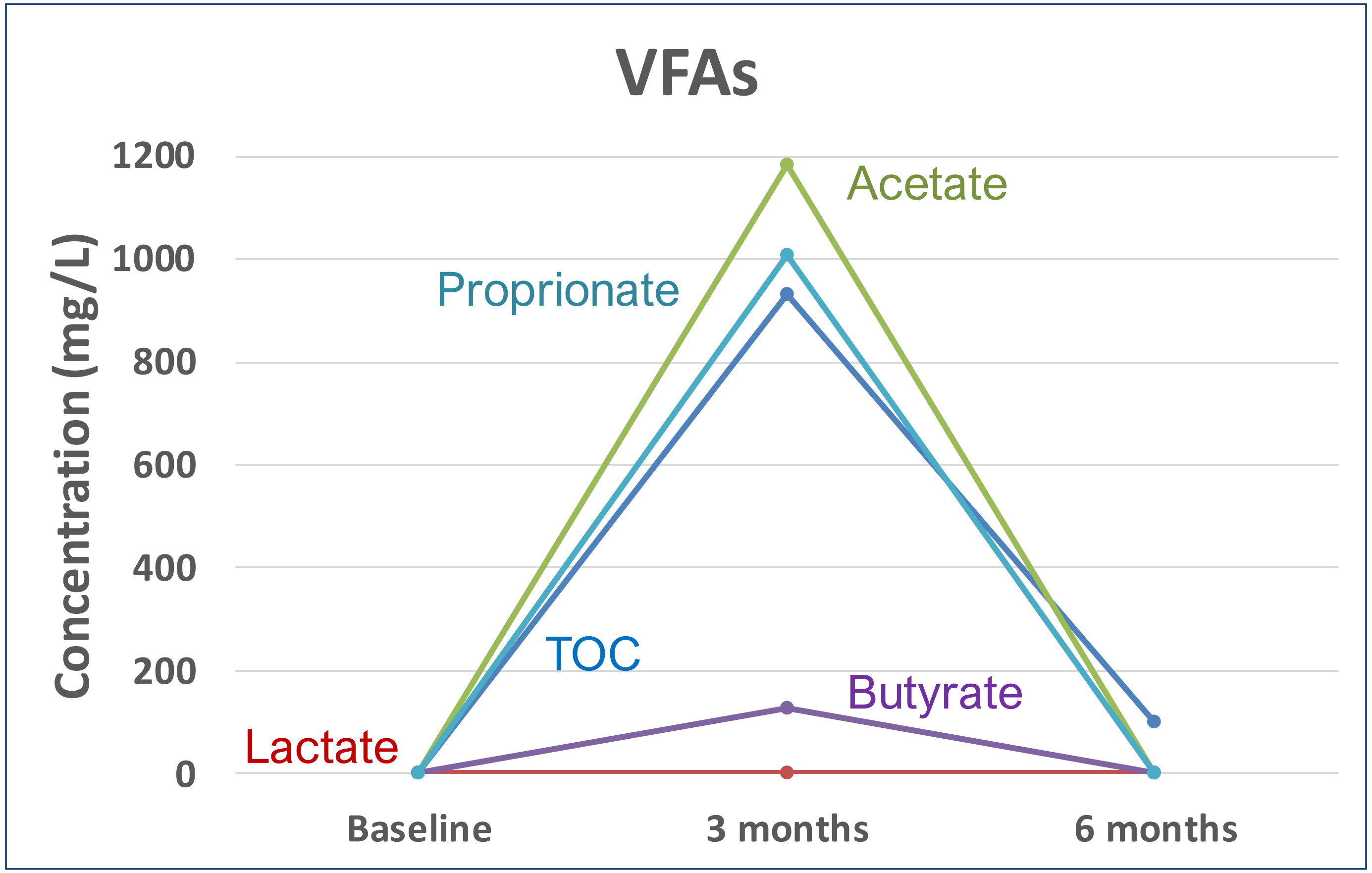
# Case Study: Bioaugmentation, California Site




**Formate & Pyruvate N.D**

# Sample Collection and VFA Analytical Method

## Sample Collection

- Purge sampling points
- Collect samples in 40 mL VOA vials (duplicate)
    - Unpreserved
    - Filled with no headspace
- Ship and store at 4ºC




## Ion Chromatography (IC) – Electrical Conductivity Detector (ECD)

- Modified EPA Method 300.0
- Calibrated with external standards
- MDLs (mg/L)
    - Lactate (0.40)
    - Acetate (0.54)
    - Propionate (0.31)
    - Formate (0.23)
    - Butyrate (0.41)
    - Pyruvate (0.69)

# Summary

## Volatile Fatty Acids

- Maximizing total VFAs is beneficial, optimal amount is greater than 100 mg/L
- If VFAs are predominantly acetate, additional electron donor may be required
- VFA composition (propionate) is positively associated with high *Dhc* concentrations
- Acetate is consumed by *Geobacter and* can be utilized in DNAPL bioremediation

***Affordable IC VFA analyses provide valuable data for monitoring, managing and optimizing bioremediation systems***

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# Questions?

**http://www.siremlab.com/**

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