EOS Remediation is now a Redox Tech company.

Carbon + Nutrients = Stronger Bacteria = Faster Remediation

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- 17 years of remediation design & implementation experience
  - 13 in consulting
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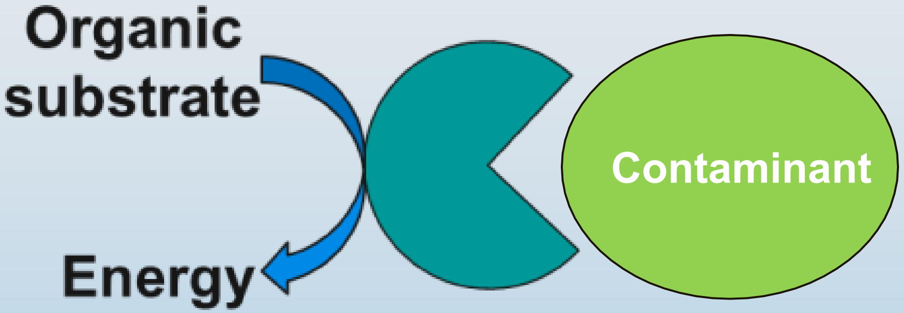
- Two Dogs and a Cat – All Rescues
- Super "Auntie LaLa" to my niblings
- Chef
- Artist
- Volunteer
  - www.comfortzonecamp.org
  - www.gwpnc.org

Joule Unit

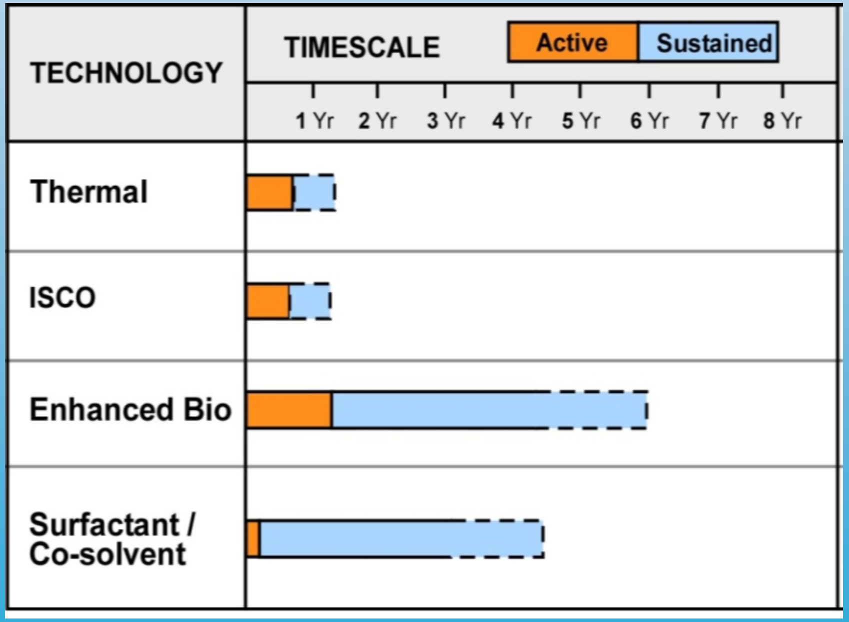
Dali Parton

Tesla Coil

Why bioremediation?

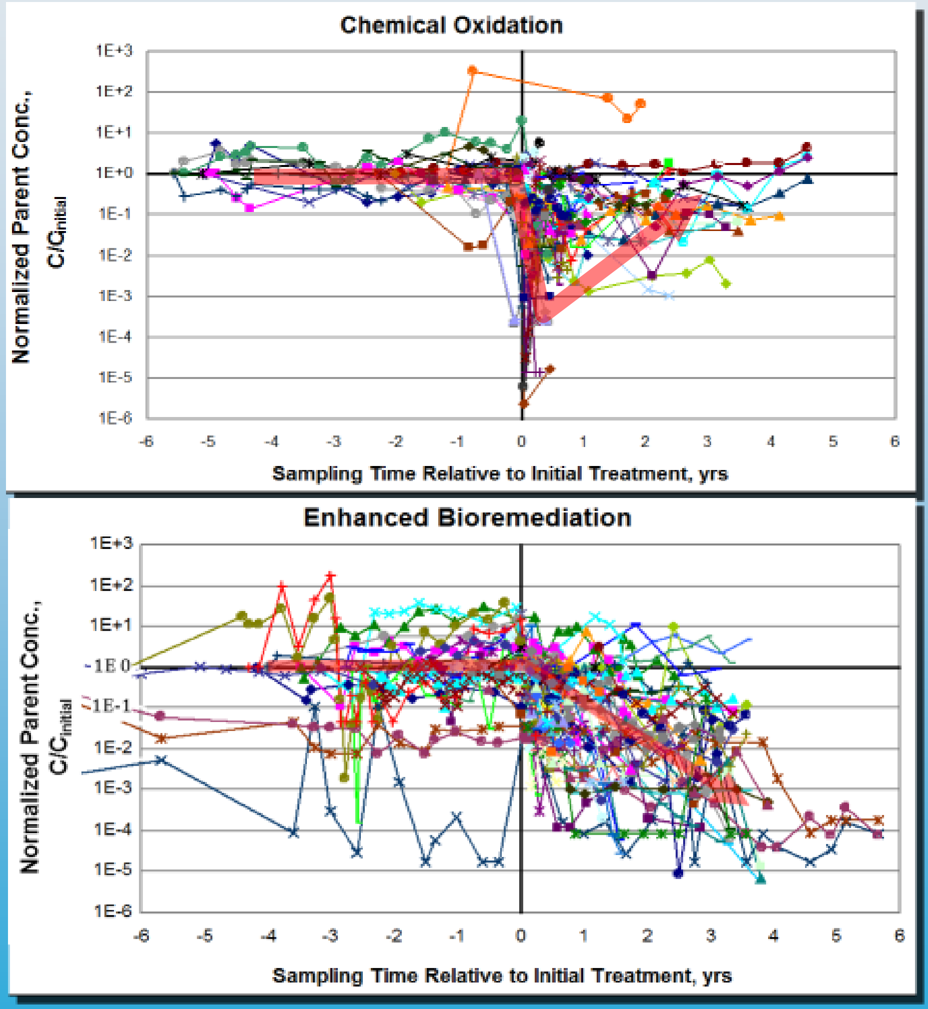


Long Lasting Technology



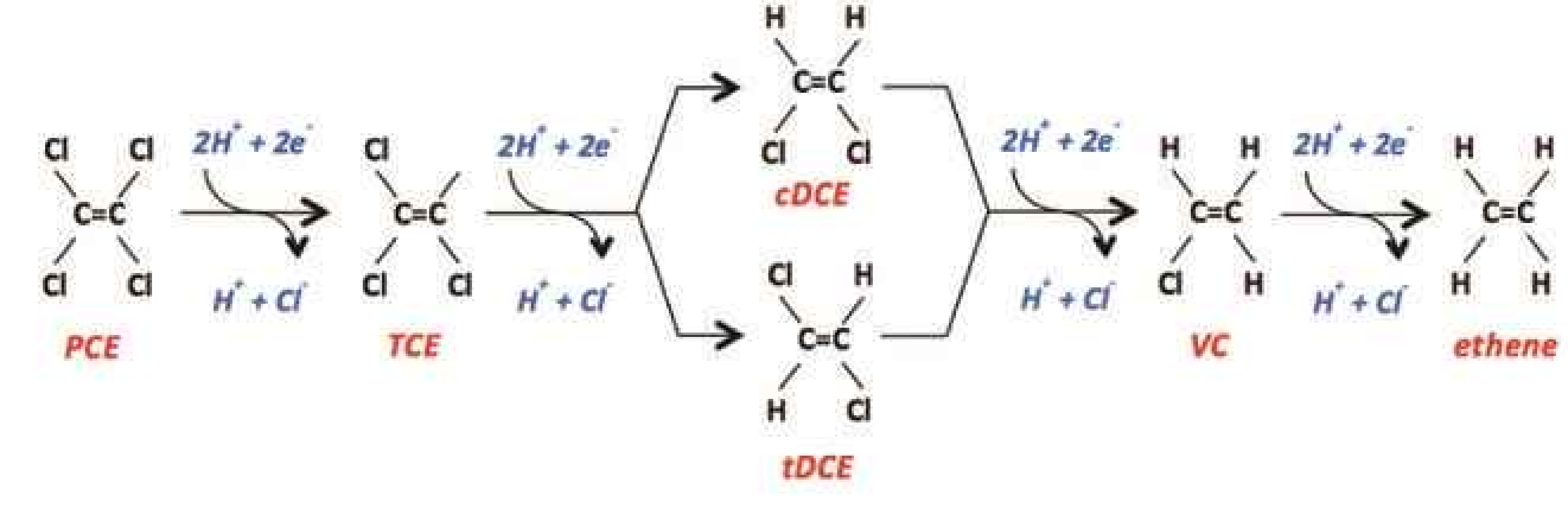
Adamson et. al. 2011

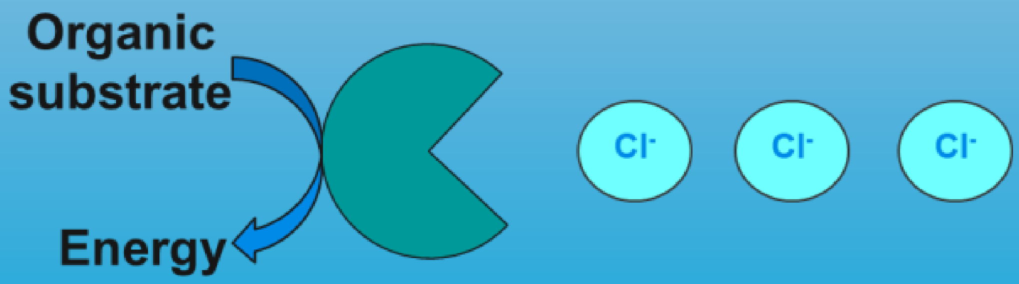
Rebound vs. Sustained Treatment



McGuire et al. 2006

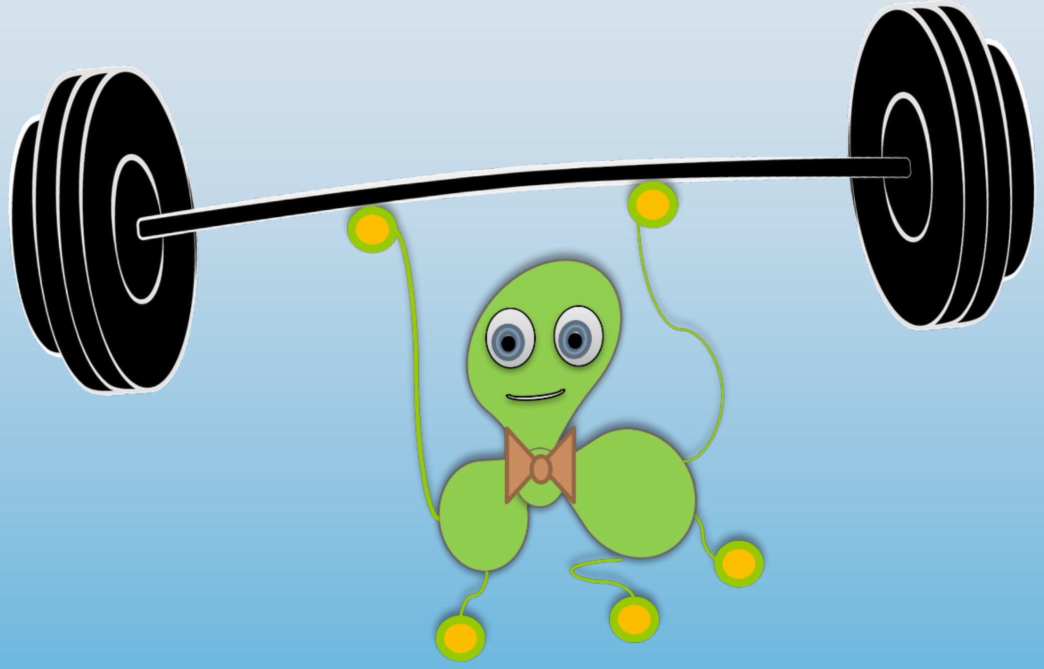
Bioremediation of chlorinated compounds:
Enhanced reductive dechlorination (ERD)





Conditions for rapid biological activity

- Adequate bacterial concentrations
  - (~10^7 cells/mL)
- pH close to neutral
  - (6.5 – 7.0)
- No inhibitors or competing reactions
- Good substrate distribution
- **Enough nutrients**



Importance of nutrients

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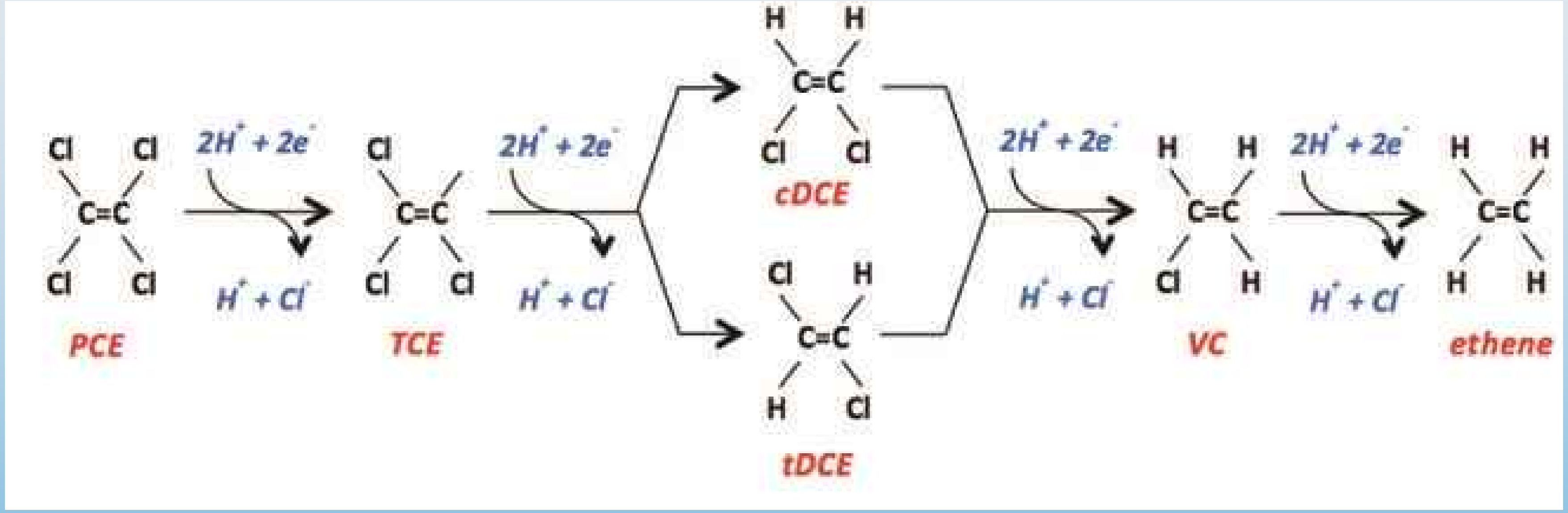
- Essential elements for growth (carbohydrates, proteins, lipid membranes, DNA, etc.):
  - Carbon
  - Nitrogen
  - Phosphorus
  - Oxygen
  - Hydrogen
  - Sulfur
  - Magnesium
  - Calcium

- Trace elements required for specific processes (e.g. enzymatic processes, redox control):
  - Iron
  - Cobalt
  - Manganese
  - Copper
  - Zinc
  - Molybdenum

The proportion of elements depend on type of microorganism and conditions and are often empirical

- E.g. Redfield ratio, C:N:P = 106:16:1

Bioremediation of chlorinated compounds:
Enhanced reductive dechlorination (ERD)



Multiple Organisms

Dehalobacter, Desulfuromonas, Sulfurospirillum, Geobacter, Desulfitobacterium

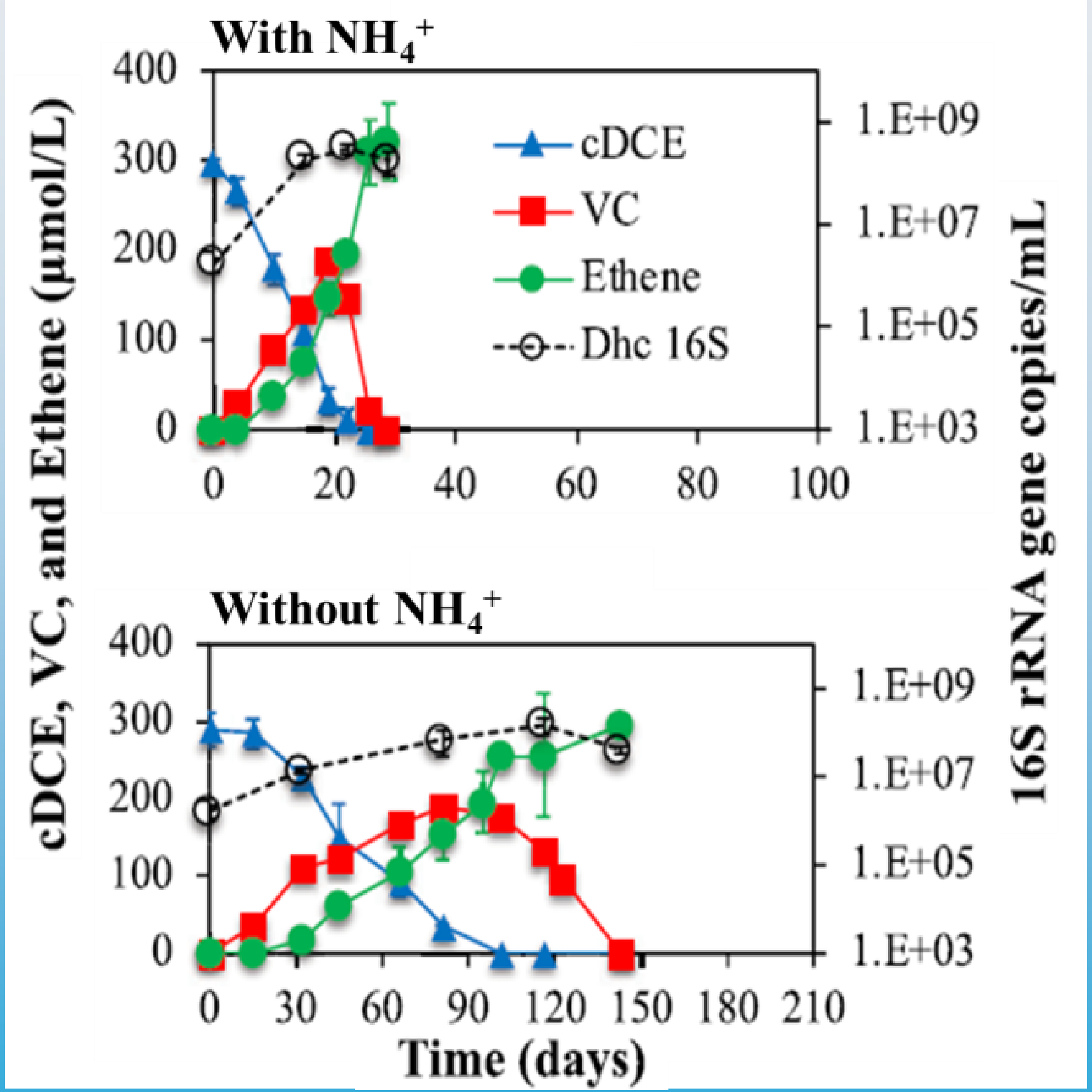
- Grow rapidly
- Use H_2 or Acetate
- Utilize either N_2 or NH_4^+
- Can produce B_{12}

Dehalococcoides spp. (DHC)

- Grows slowly
- Use only H_2
- Requires NH_4^+
- Needs external source of B_{12}

Importance of nitrogen for ERD

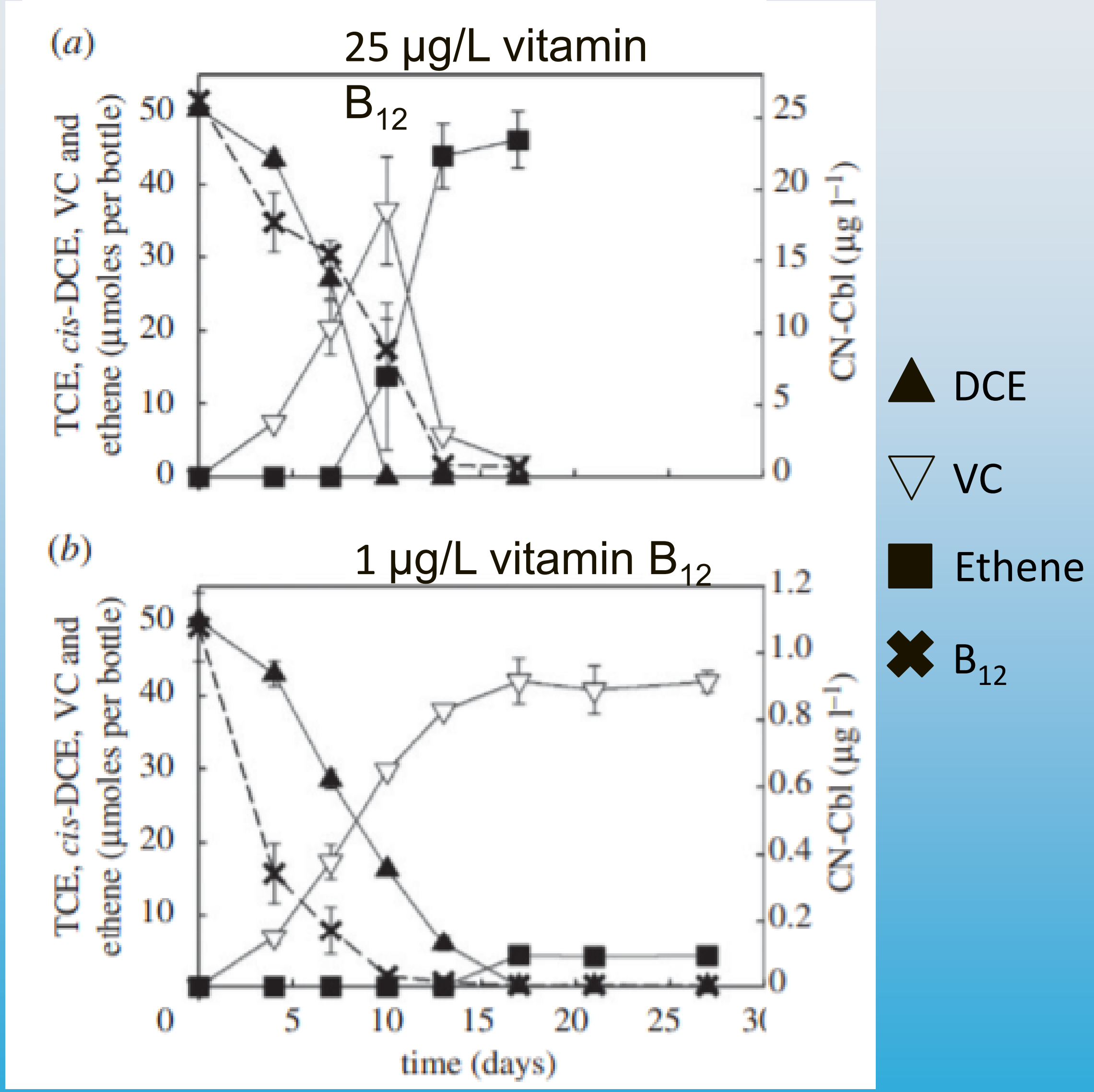
- Nitrogen atoms (N) are mainly needed for proteins and nucleic acids
- Nitrogen can be assimilated from atmospheric nitrogen (N_2) or from fixed molecules (e.g. NH_4^+,NO_3^-).
- While N_2 fixation can provide N to DHC (from other organisms) during ERD, addition of external N results in higher dechlorination rates.



Kaya et al., 2019

Importance of vitamin B_{12} for ERD

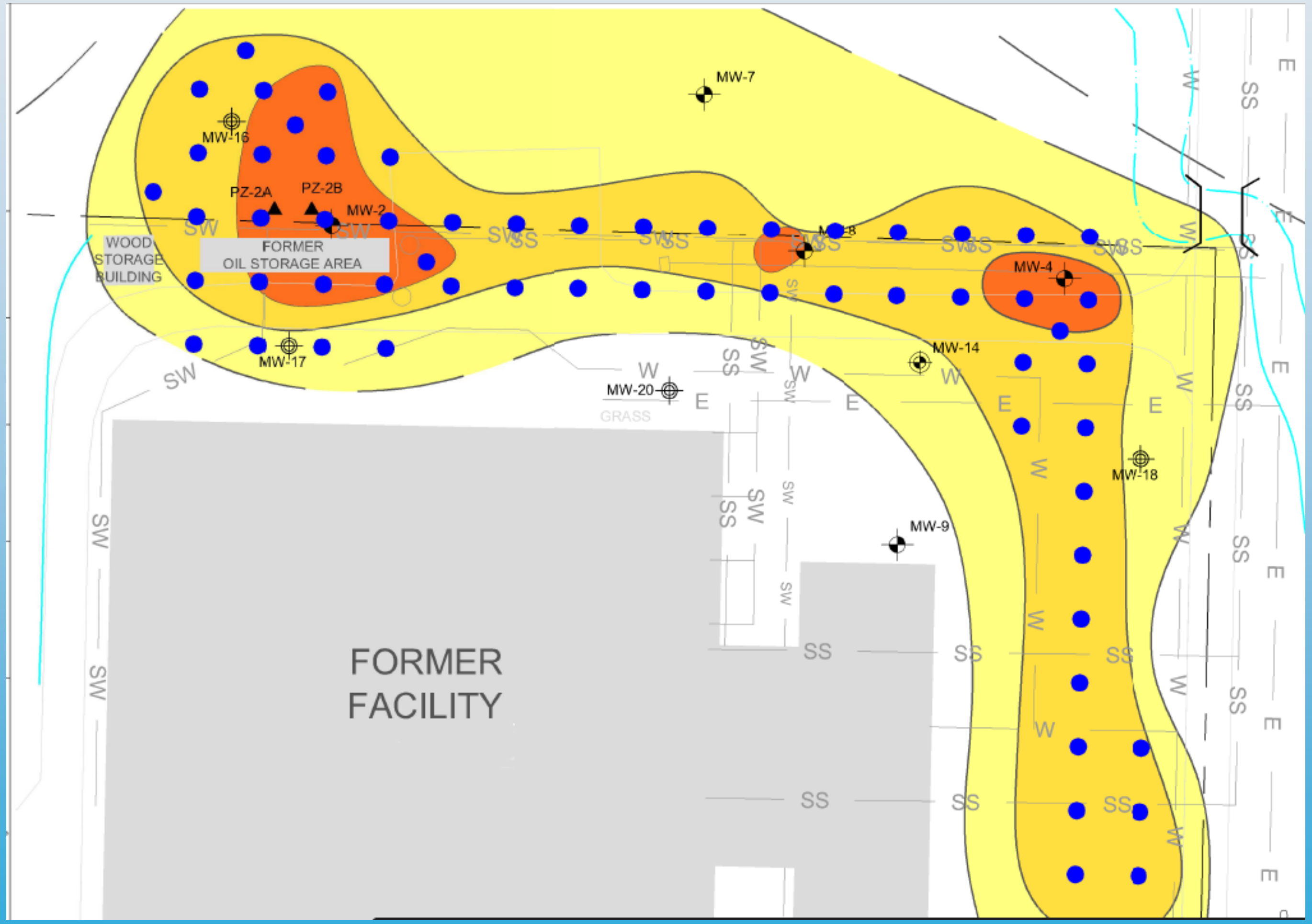
- Cyanocobalamin (Vitamin B_{12}) is needed to synthesize cofactor for reductive dehalogenase enzymes.
- As DHC cannot synthesize B_{12}, it must be provided by other microorganisms or present on site.
- Adding B_{12} increases complete dechlorination rates



Yan et al. 2013

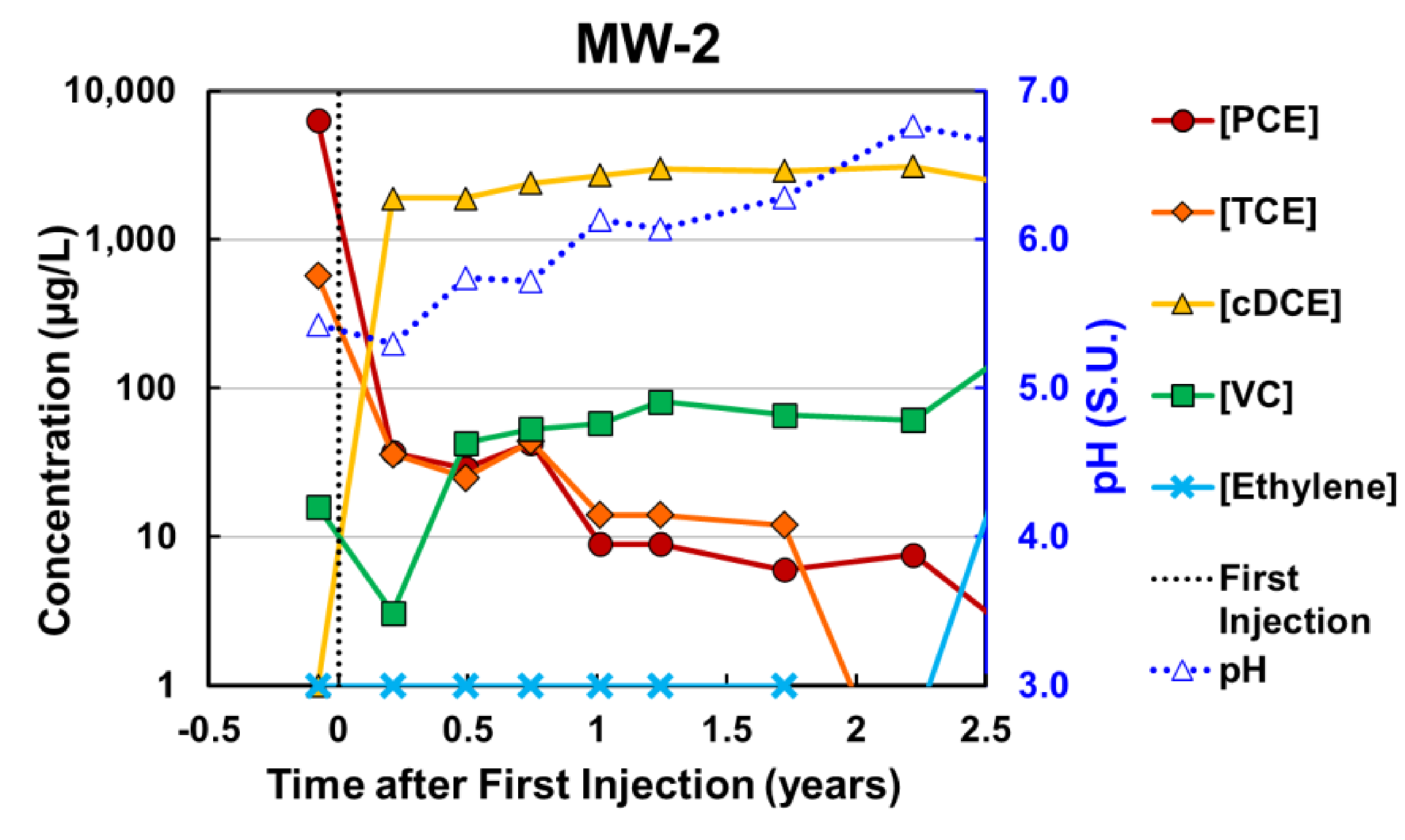
Bioremediation of a challenging site

- Former manufacturing site in North Carolina.
- High concentrations of chlorinated compounds detected in 2008:
  - PCE: >10,000 µg/L
  - TCE: >1,000 µg/L
- pH ~5
- High concentrations of sulfate
  - >3,000 mg/L
- <10 *Dehalococcoides* (DHC) cells/mL



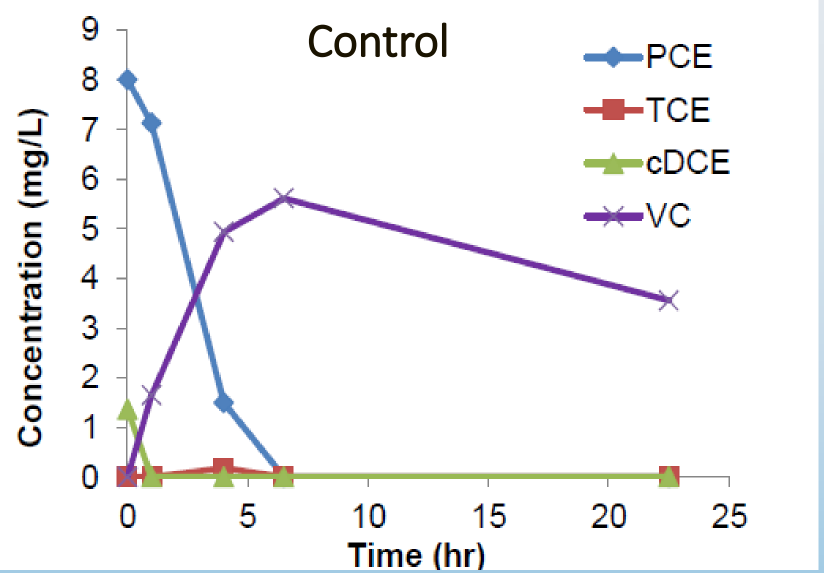
Initial bioaugmentation

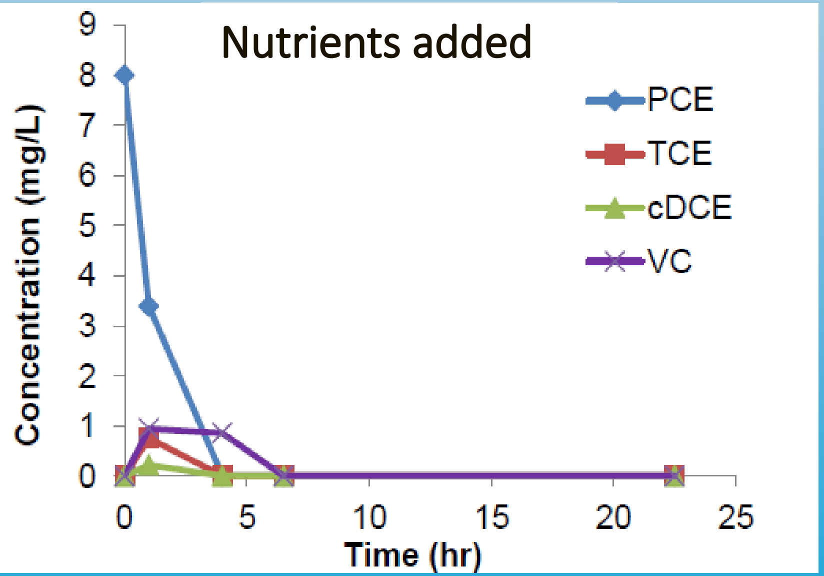
- Bioaugmentation culture + emulsified vegetable oil (EVO) + colloidal buffer (CoBupH) injected.
- Increase in DHC population (~2 x 10^4 cells/mL)
- Excellent PCE and TCE removal
- High cDCE and VC
- No Ethylene detected



Pilot test to optimize bioremediation

- Nitrogen <0.15 mg/L TKN.
- A solution of nutrients (PLUS) was added to groundwater samples.
- Growth of DHC was stimulated. cDCE and VC were degraded at a faster rate than controls.





Second injection event

- Nutrients were supplemented along with substrate to optimize bioremediation.

BAC-9 + EOS PRO + CoBupH

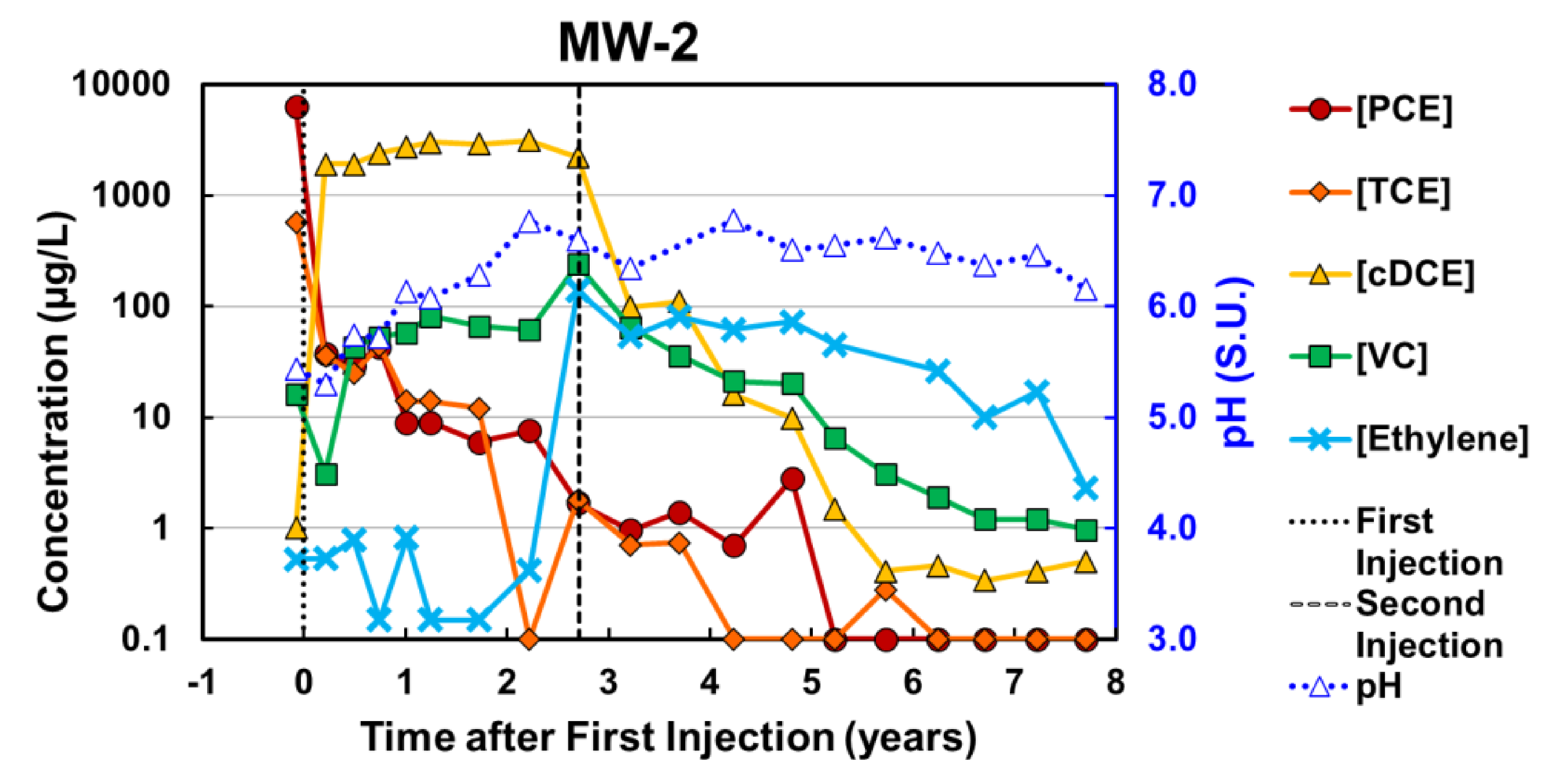
Bioaugmentation culture

Emulsified vegetable oil substrate with nutrients

Colloidal buffer for pH control

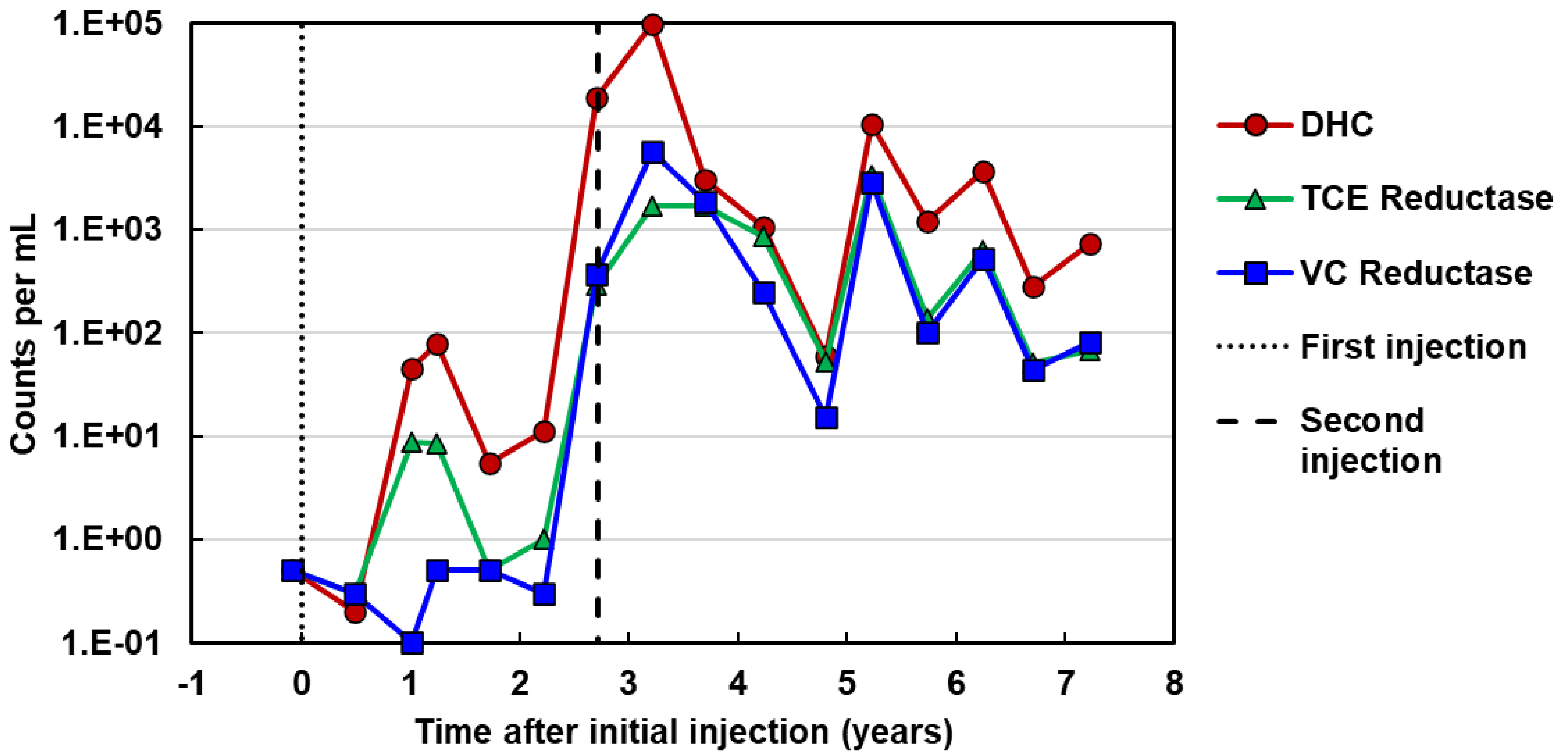
Performance after second injection

- DCE and VC decreased (>3-fold reduction in ~2 years).
- Ethylene was produced.
- pH has been stable for years



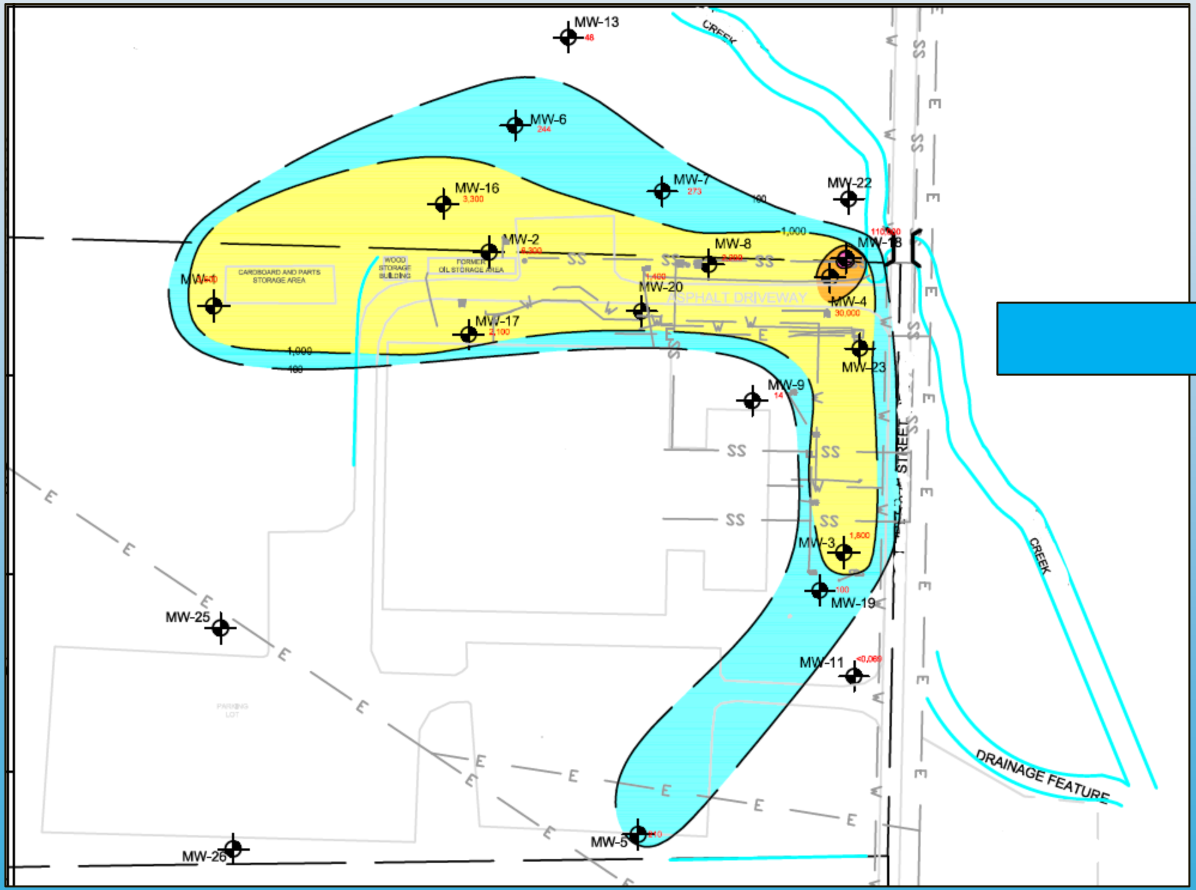
Performance after second injection

- Growth of dechlorinating bacteria due to amendments

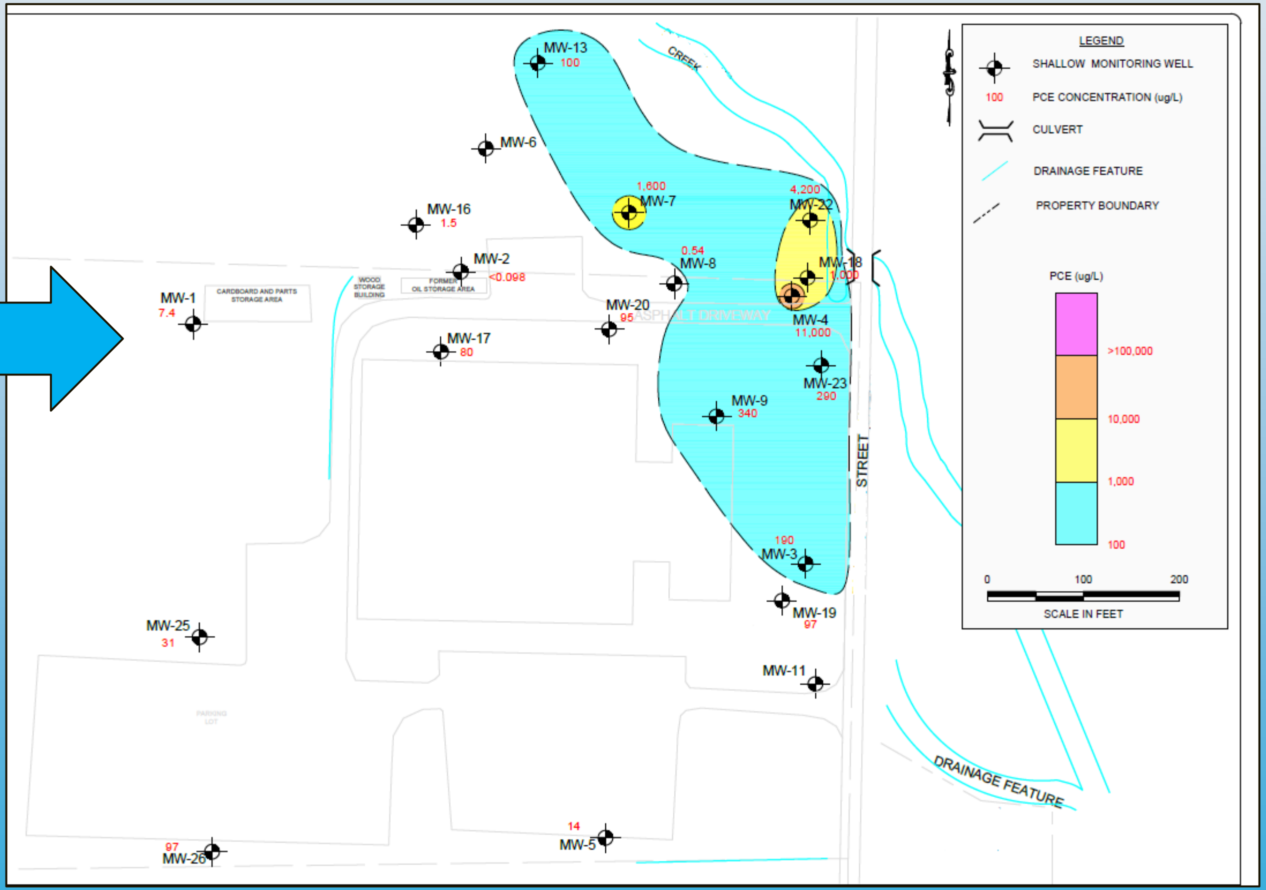


Performance after second injection event

2013



2021



Lessons Learned

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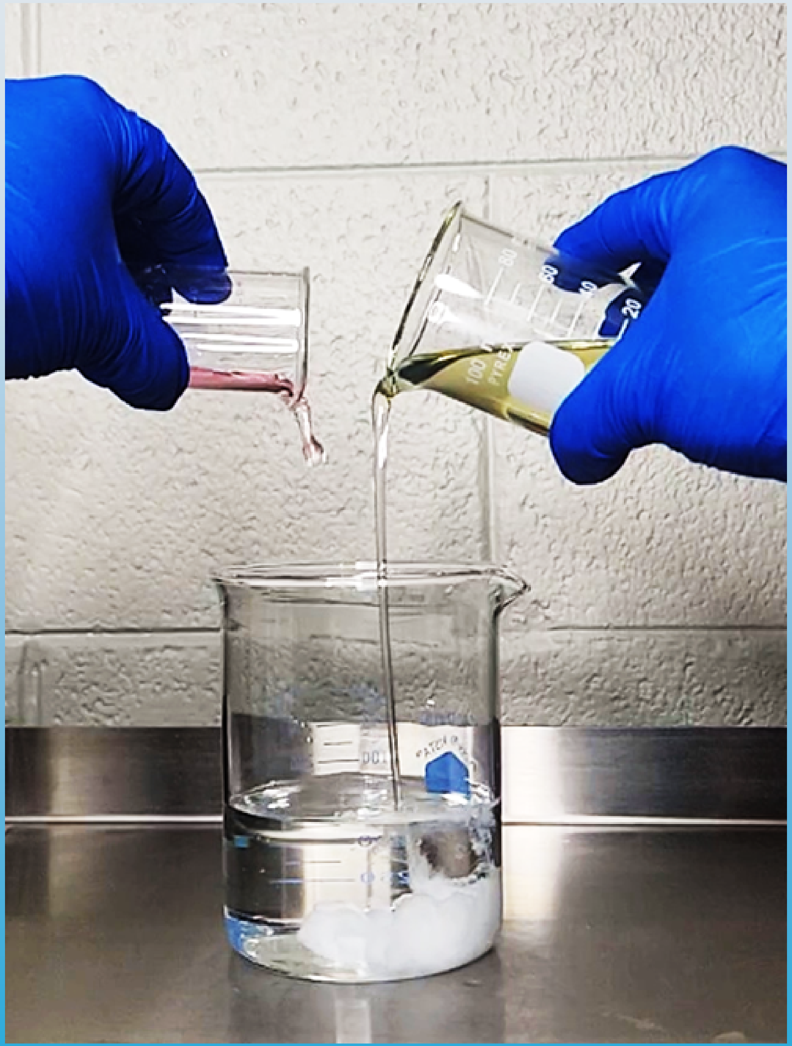
- Nutrients are an essential part of bioremediation.
- Adding appropriate nutrients (e.g. Nitrogen and vitamin B_{12}) can accelerate removal rates.
- Adding nutrients helps to overcome 'DCE stall' and achieve complete dechlorination.
- EOS Remediation products are designed to optimize nutrients for ERD.

EOS PRO   PLUS



EOS Remediation is now a Redox Tech company.

EOS + REDOX TECH

Thank you!

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Supplementary slides

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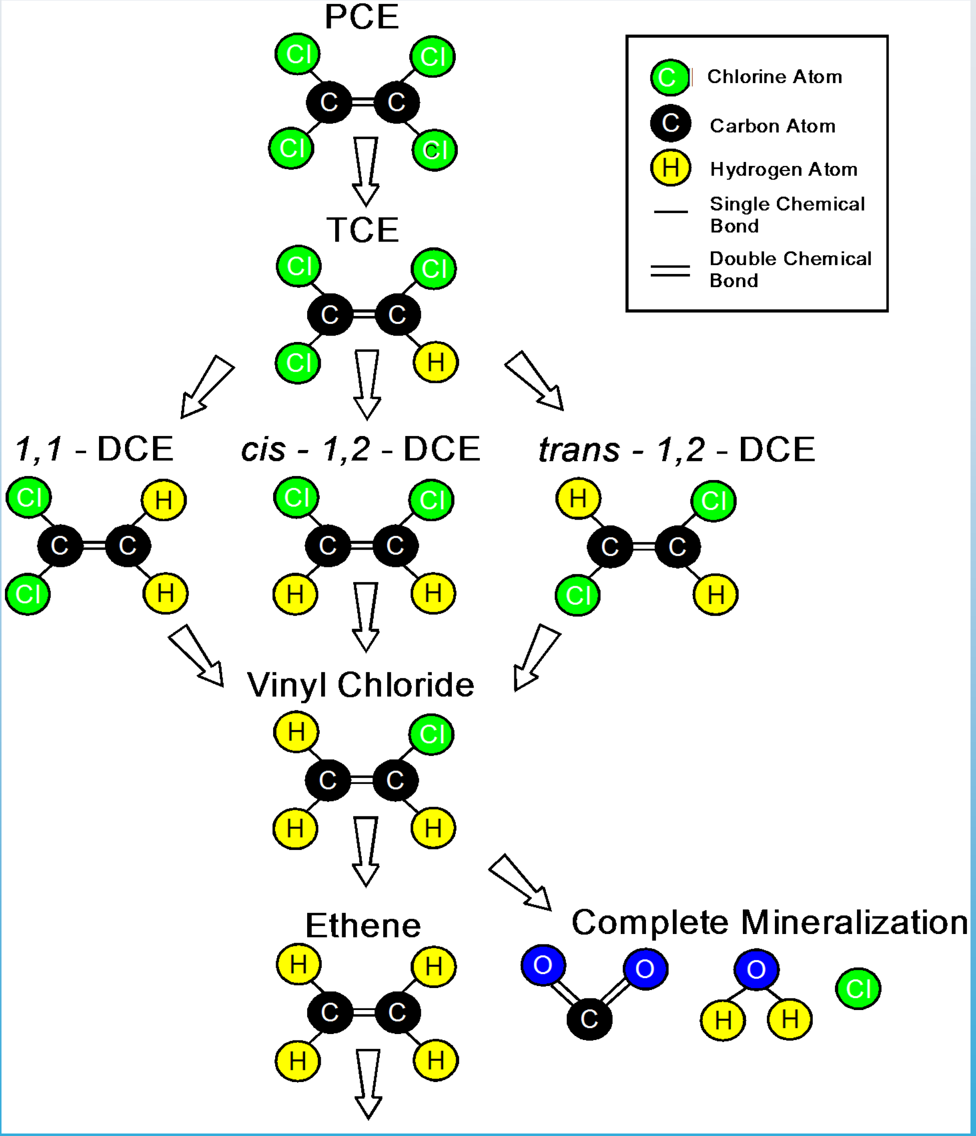
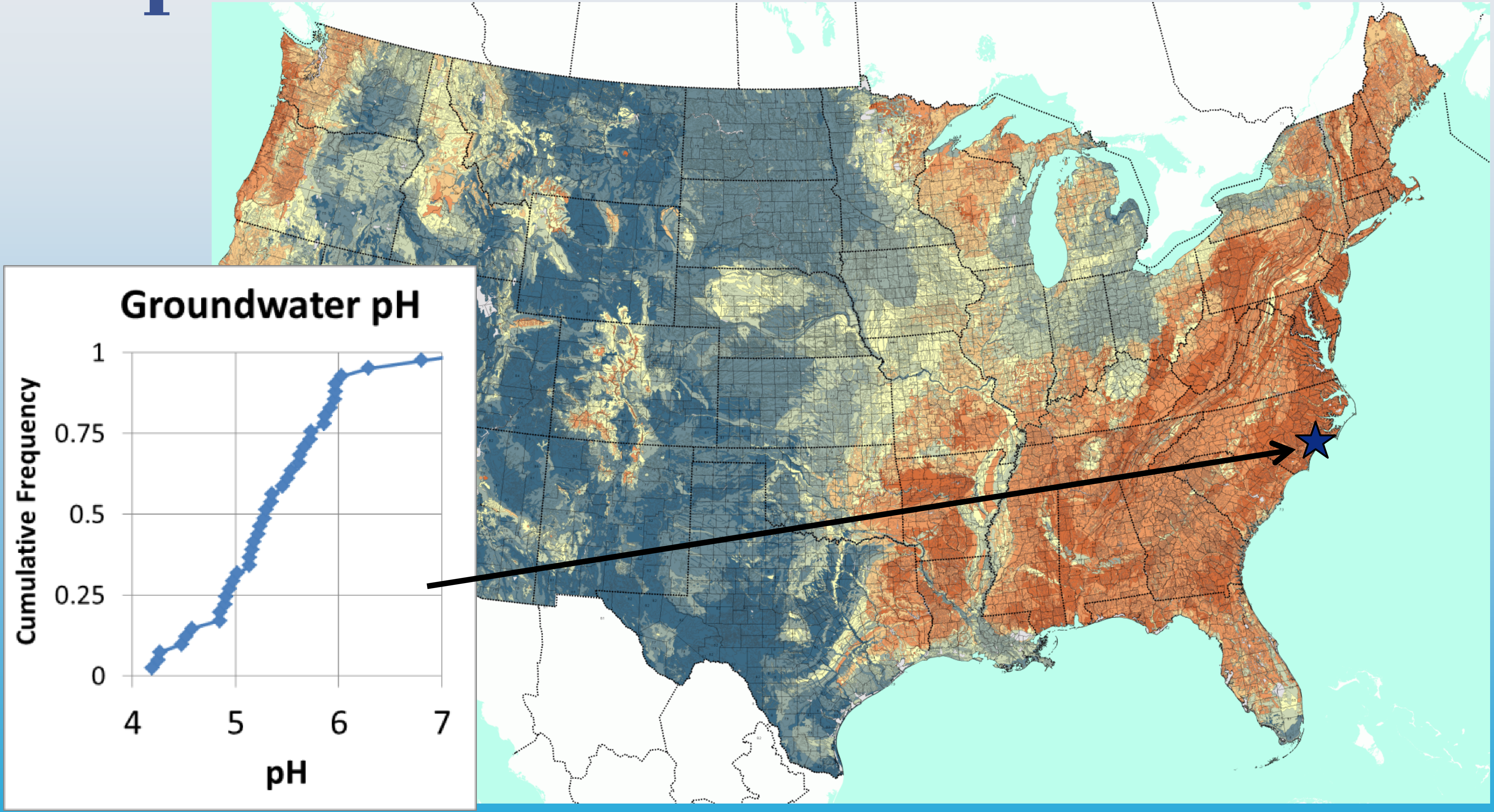
Target Contaminants for ERD

- Chlorinated Solvents
  - Ethenes (PCE, TCE)
  - Ethanes (TCA)
  - Methanes (CT)
- Explosives (TNT, RDX, HMX)
- Nitrate (NO_3^-)
- Perchlorate (ClO_4^-)
- Chromate (CrO_4^{-2})
- Radionuclides (TcO_4^-, UO_2^{+2})
- Acid Mine Drainage



Soil pH



Biota of North America, 2012 http://www.bonap.org/

Sources of low pH: Substrate fermentation

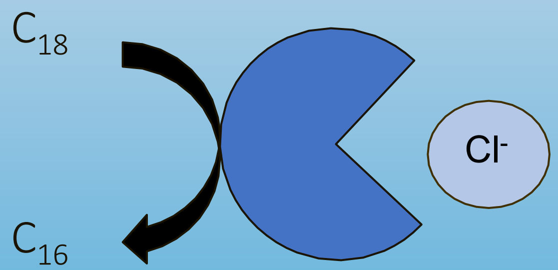
a REDOX TECH company

Vegetable Oil Fermentation

- Natural fats → Triglycerides
  - Glycerol
  - Three long chain fatty acids (LCFA)
  - Bacteria hydrolyze ester linkages releasing glycerol and LCFA

- Glycerol (Very soluble, Easily biodegraded)

- Beta Oxidation of LCFA (e.g. linoleic acid or $C_{18}H_{32}O_2$)



$$C_{18}H_{32}O_2 + 2H_2O \rightarrow C_{16}H_{30}O_2 + C_2H_4O_2 + H_2$$
$$C_{16}H_{30}O_2 + 2H_2O \rightarrow C_{14}H_{28}O_2 + C_2H_4O_2 + H_2$$
$$C_{14}H_{28}O_2 + 2H_2O \rightarrow C_{12}H_{26}O_2 + C_2H_4O_2 + H_2$$

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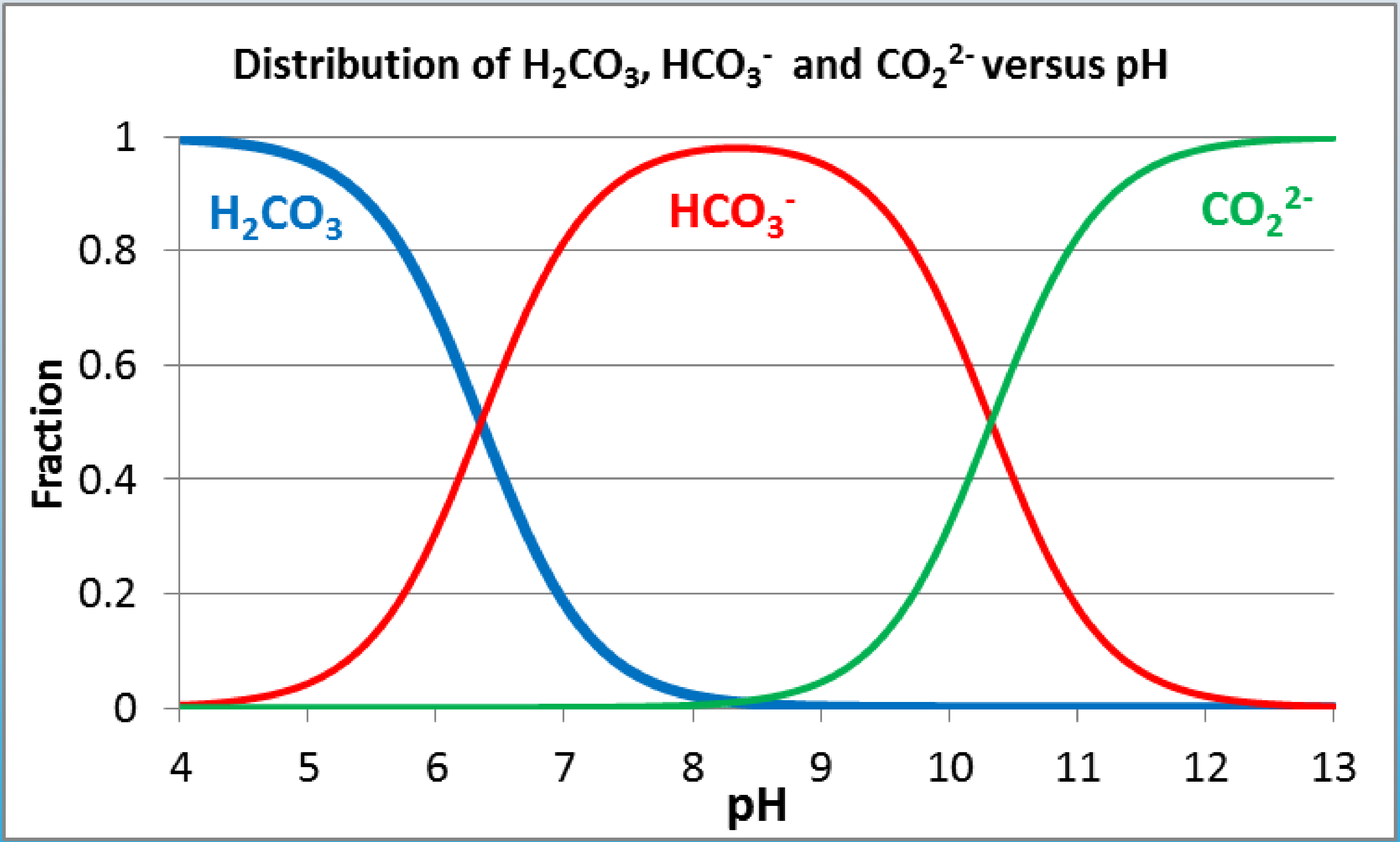
$$C_{18}H_{32}O_2 + 16H_2O \rightarrow 9C_2H_4O_2 + 14H_2$$

Acetic Acid and Hydrogen

Carbonate System and pH

$$CO_2(g) + H_2O \Leftrightarrow H_2CO_3 \Leftrightarrow H^+ + HCO_3^- \Leftrightarrow 2H^+ + CO_3^{2-}$$

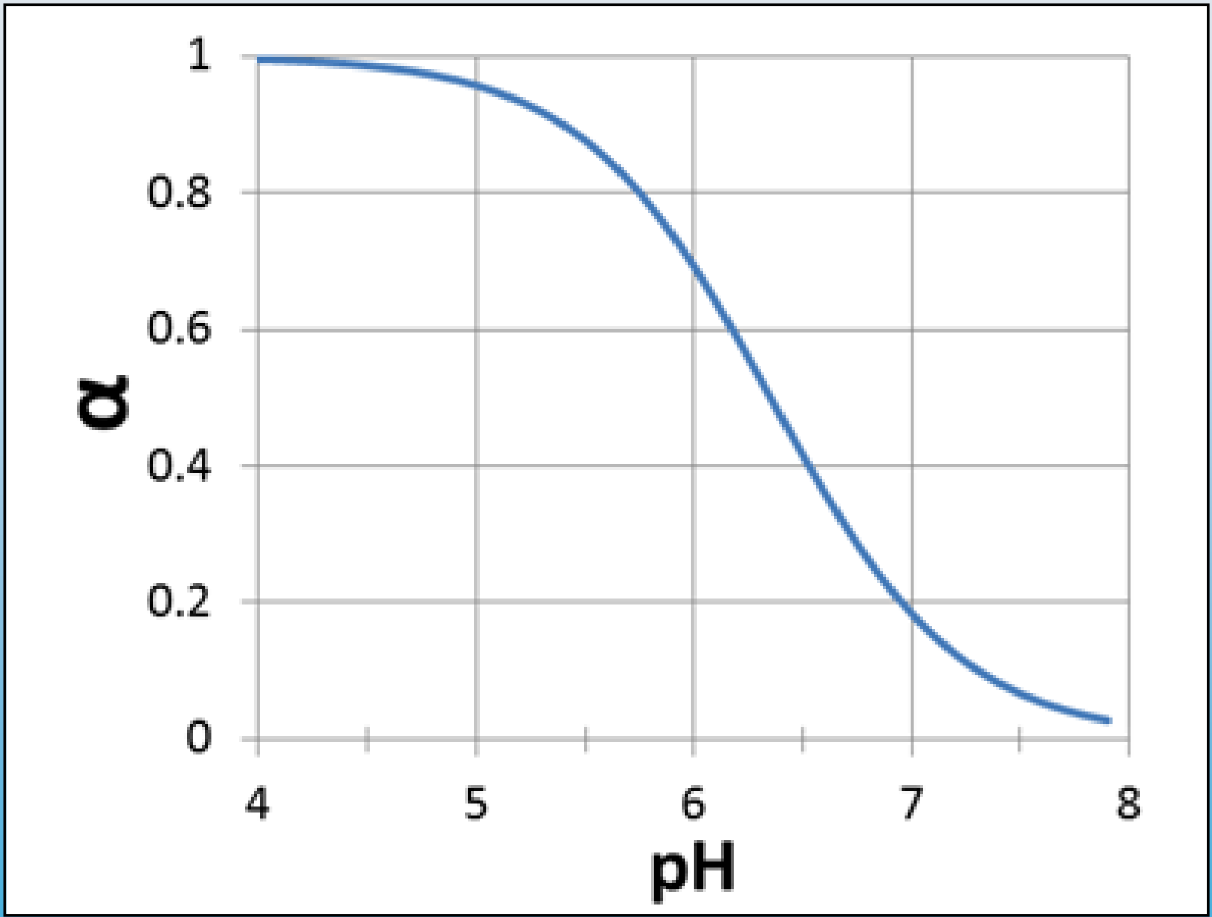


Acid (H^+) Release from CO_2 Production below Water Table

$$CO_2 + H_2O \Leftrightarrow H_2CO_3$$

$$H_2CO_3 \Leftrightarrow H^+ + HCO_3^-$$

$$K_{eq} = \frac{[H^+]\,[HCO_3^-]}{[H_2CO_3]}$$

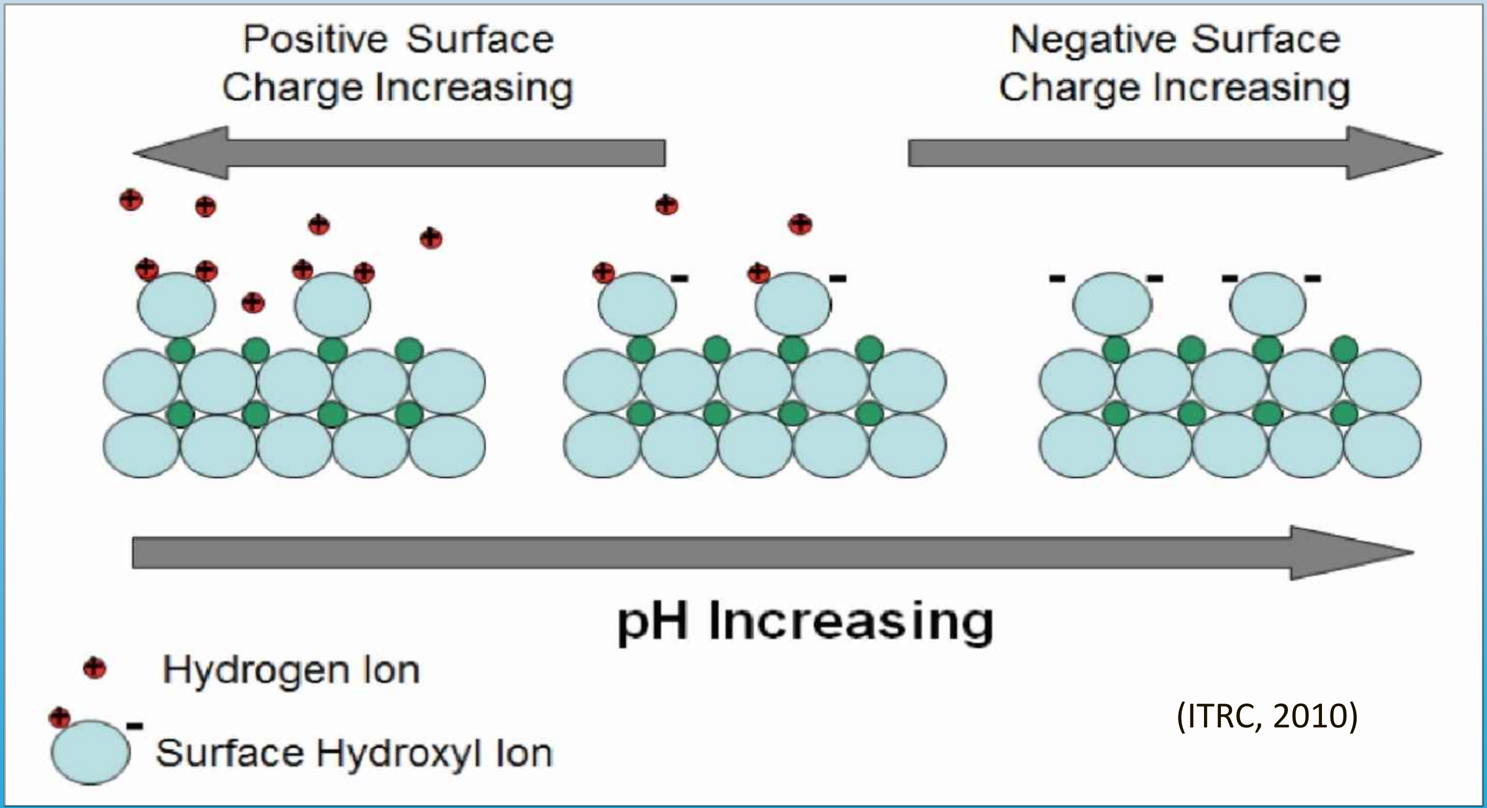


$$CO_2 + H_2O \rightarrow \alpha H_2CO_3 + (1-\alpha)H^+ + (1-\alpha)HCO_3^-$$

Aquifer Buffering Capacity

Surface Complexation and Ion Exchange

- H^+ and OH^- exchange on Fe and Al oxide surfaces and clay minerals
- Strong buffer, reduce the pH decline → adsorbing H^+
- Increase required base amount to increase aquifer pH → adsorbing OH^-



(ITRC, 2010)

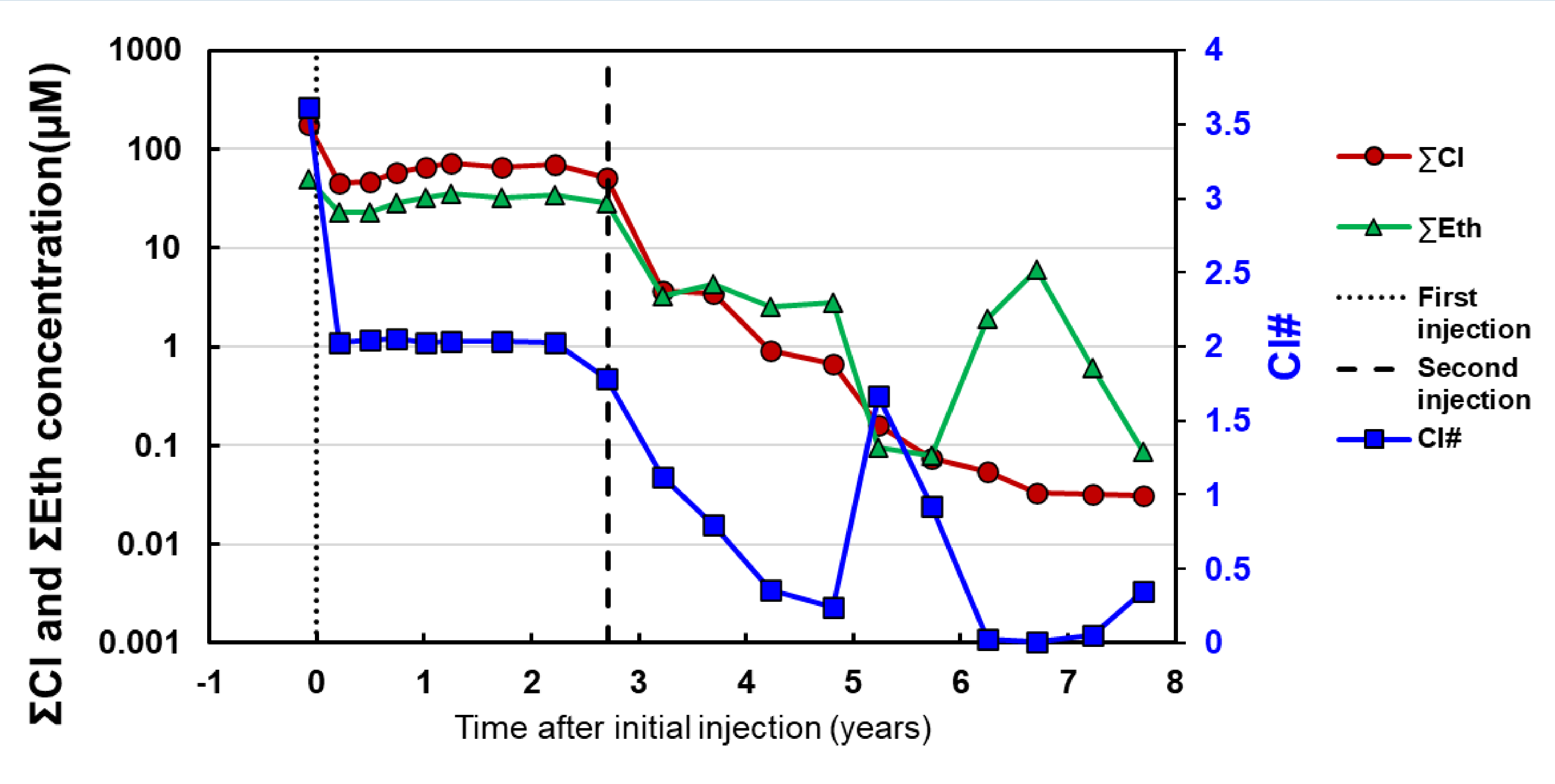
Performance after second injection

ΣEth = [PCE] + [TCE] + [DCE] + [VC] + [ethene]

ΣCl = 4[PCE] + 3[TCE] + 2[DCE] + [VC]

Cl# = ΣCl / ΣEth

[ ] → concentration in mole/L or µmole/L



MW-18 Source Area Treatment

- 2018 Injection
  - Double normal EOS Pro loading
  - Extra N + P + B_{12} + yeast extract
  - Massive amount of $Mg(OH)_2$ + $KHCO_3$