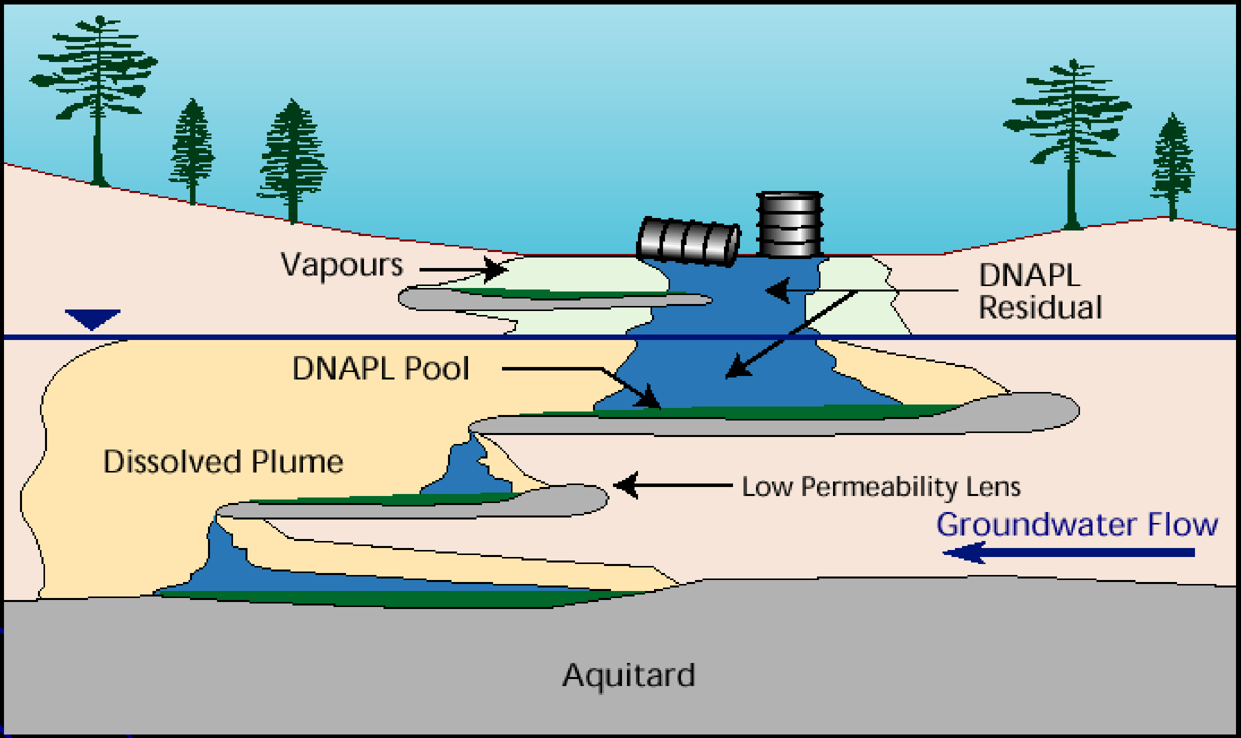
Biodegradation of Chlorinated Solvents



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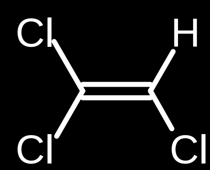
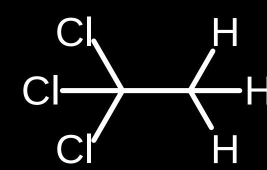
Importance of Chlorinated Solvents

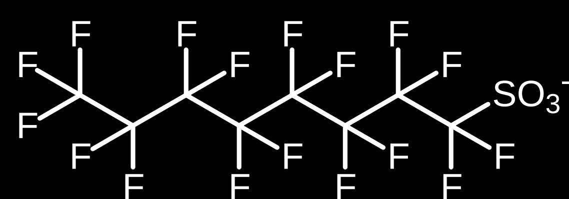
- **Historical Contamination:** Chlorinated solvent plumes still persist today from past use at semiconductor industries

TCE  1,1,1-TCA 

- **Examples of Famous Chlorinated Plume Sites:**

Motorola 52nd Street Superfund Site, Phoenix Az	TCE 1,1,1-TCA, freon
Fairchild Semiconductor, Mountain View, Ca	1,1,1-TCA, freon, PCE

- **Clues on biodegradation of perfluorinated compounds**

PFOS 

Literature Review

Reviews in Environmental Science & Bio/Technology (2004) 3: 185–254

Biodegradability of chlorinated solvents and related chlorinated aliphatic compounds

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Key words: biodegradation, chloroacetic acids, chlorobutadienes, chloroethanes, chloroethenes, CFC, chlorofluorocarbons, chlorinated aliphatic compounds, chloromethanes, chloropropanes, chloropropenes, epichlorohydrin, halorespiration, microbial dechlorination, PCE, TCE.

Abstract

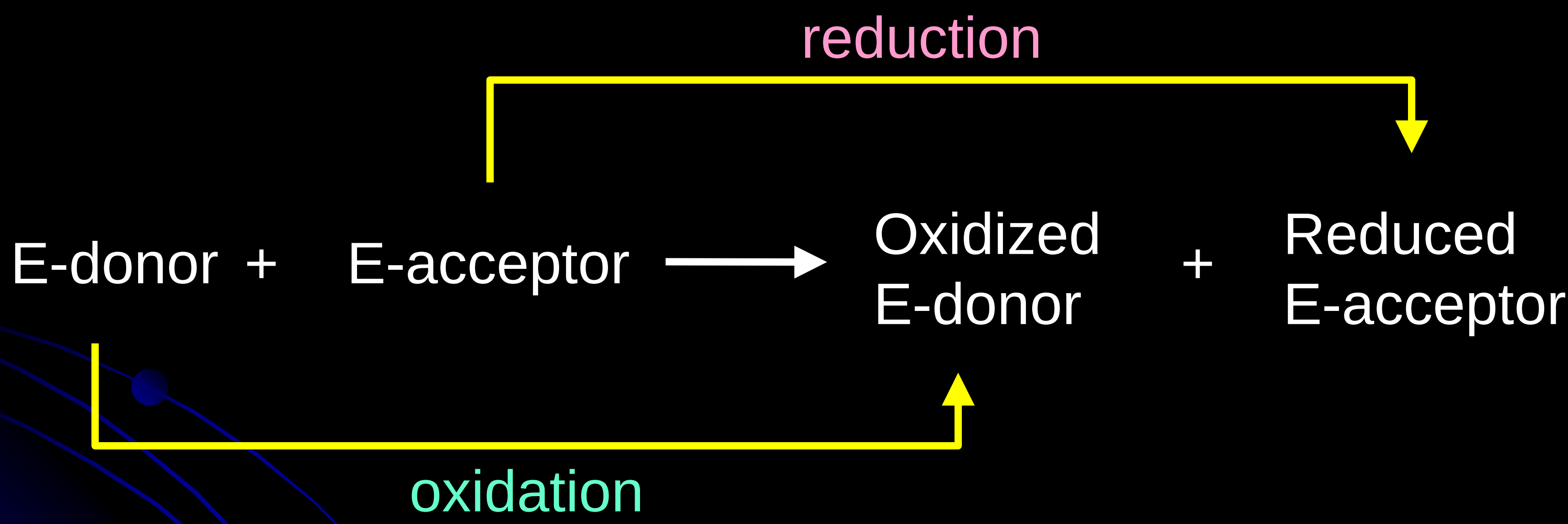
The biodegradability of chlorinated methanes, chlorinated ethanes, chlorinated ethenes, chlorofluorocarbons (CFCs), chlorinated acetic acids, chlorinated propanoids and chlorinated butadienes was evaluated based on literature data. Evidence for the biodegradation of compounds in all of the compound categories evaluated has been reported. A broad range of chlorinated aliphatic structures are susceptible to biodegradation under a variety of physiological and redox conditions. Microbial biodegradation of a wide variety of chlorinated aliphatic compounds was shown to occur under five physiological conditions. However, any given physiological condition could only act upon a subset of the chlorinated compounds. Firstly, chlorinated compounds are used as an electron donor and carbon source under aerobic conditions. Secondly, chlorinated compounds are cometabolized under aerobic conditions while the microorganisms are growing (or otherwise already have grown) on another primary substrate. Thirdly, chlorinated compounds are also degraded under anaerobic conditions in which they are utilized as an electron donor and carbon source. Fourthly, chlorinated compounds can serve as an electron acceptor to support respiration of anaerobic microorganisms utilizing simple electron donating substrates. Lastly chlorinated compounds are subject to anaerobic cometabolism becoming biotransformed while the microorganisms grow on other primary substrate or electron acceptor. The literature survey demonstrates that, in many cases, chlorinated compounds are completely mineralised to benign end products. Additionally, biodegradation can occur rapidly. Growth rates exceeding 1 d^{-1} were observed for many compounds. Most compound categories include chlorinated structures that are used to support microbial growth. Growth can be due to the use of the chlorinated compound as an electron donor or alternatively to the use of the chlorinated compound as an electron acceptor (halorespiration). Biodegradation linked to growth is important, since under such conditions, rates of degradation will increase as the microbial population (biocatalyst) increases. Combinations of redox conditions are favorable for the biodegradation of highly chlorinated structures that are recalcitrant to degradation under aerobic conditions. However, under anaerobic conditions, highly chlorinated structures are partially dehalogenated to lower chlorinated counterparts. The lower chlorinated compounds are subsequently more readily mineralized under aerobic conditions.

Abbreviations: A – ethane; BTEX – benzene–toluene–ethyl benzene–xylene; CA – chloroethane; CAA – chloroacetic acid; 2-CBD – 2-chloro-1,3-butadiene; cDCE – *cis*-dichloroethene; CF – chloroform; CFC – chlorofluorocarbons (*see* Table *15 for CFC-nomenclature)*; Cl – inorganic chloride; CM – chloromethane;

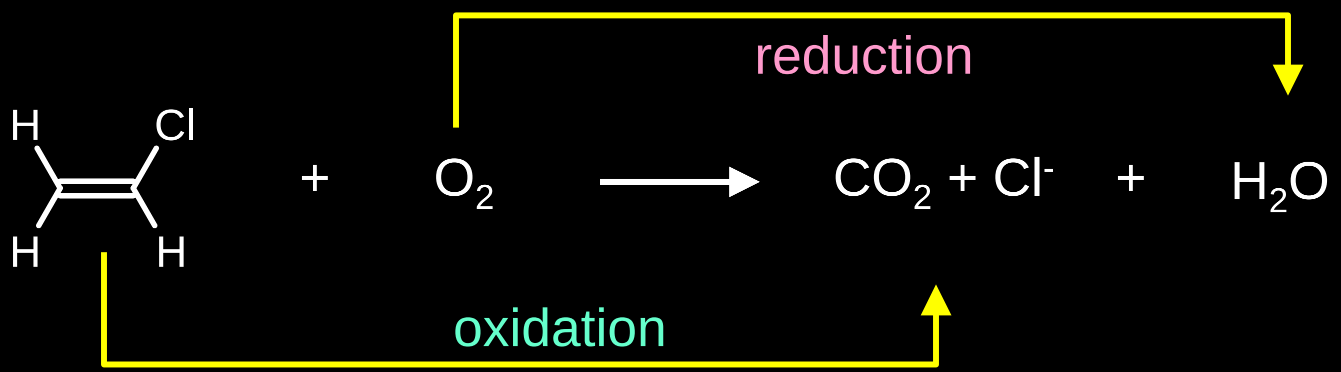
Field, J.A. & R. Sierra-Alvarez (2004) Biodegradability of chlorinated solvents and related chlorinated aliphatic compounds. *Reviews in Environmental Science & Bio/Technology* **3**:185-254.

Biodegradation Reaction

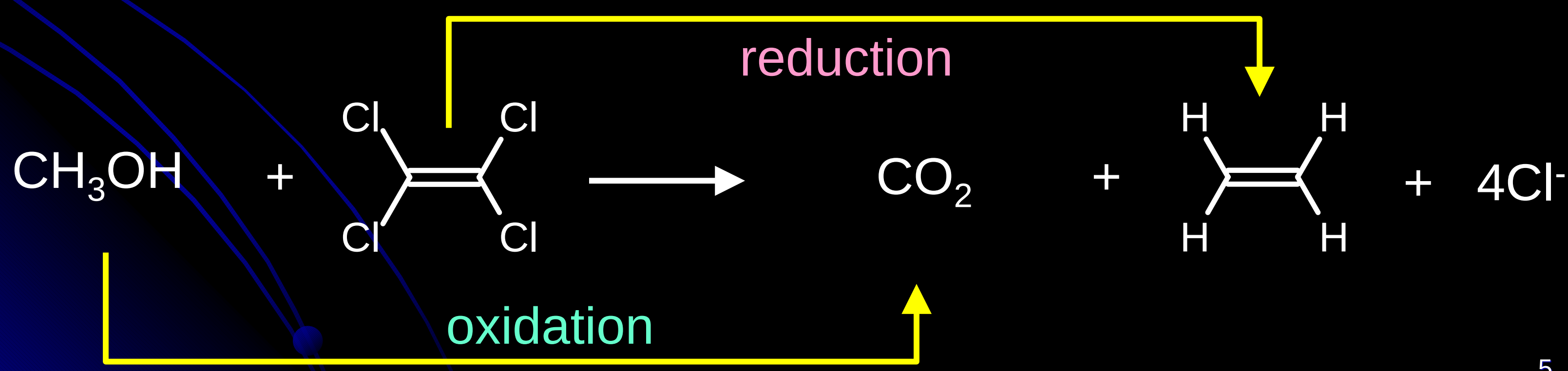
- **Biodegradation = Redox Reactions**



- **Example:** vinyl chloride as electron donor



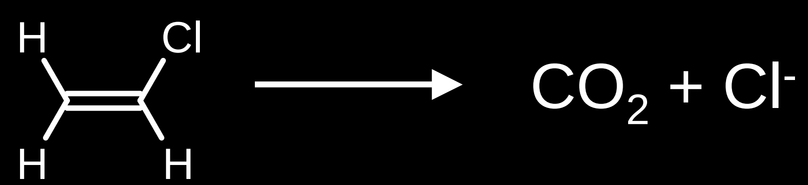
- **Example:** perchloroethylene as electron acceptor



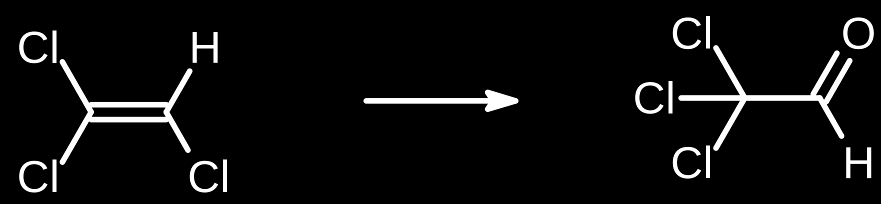
Definitions Biodegradation

- **Biodegradation:** biologically catalyzed transformation of chemical resulting in simpler forms

- **Mineralization:** Conversion of organics to mineral products

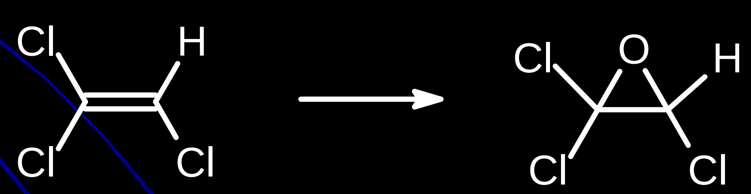


- **Biotransformation:** Transformation of pollutant by a biological process



Definitions Biodegradation

- **Growth Substrate, Primary Metabolism:** Pollutant (substrate) used as the primary energy and carbon source for microbial growth
  - as pollutant is degraded biocatalyst concentration increases
- **Cometabolism:** Accidental conversion of pollutant by enzymes and cofactors used for the metabolism of a primary substrate



MMO

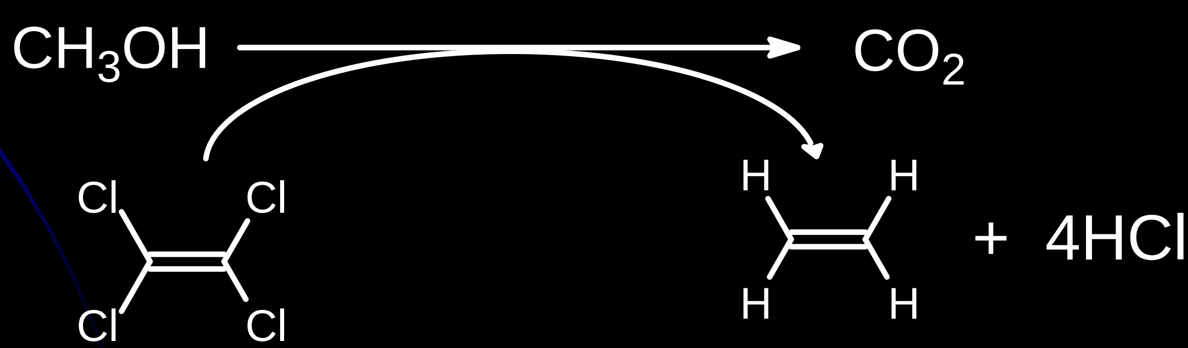
$$CH_4 \longrightarrow CH_3OH \longrightarrow \longrightarrow \longrightarrow CO_2$$

Definitions Biodegradation

- **Reductive Dehalogenation:** Microbially catalyzed replacement of a halogen atom on an organic compound with a hydrogen atom

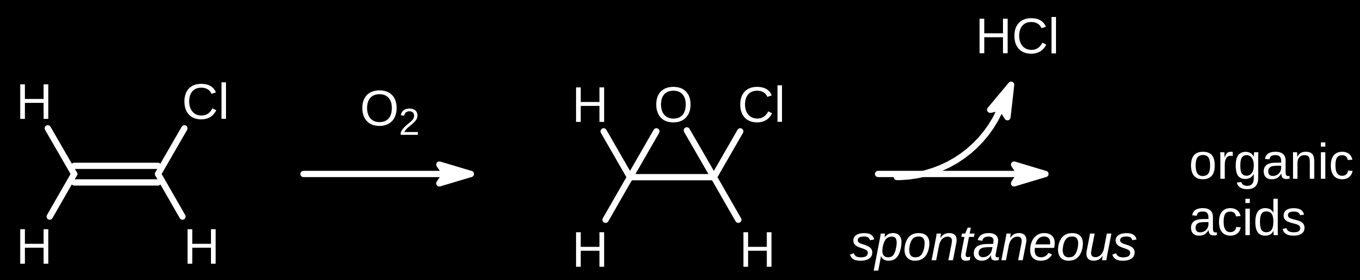
$$R\text{-}Cl + 2e^{-} + 2H^{+} \longrightarrow R\text{-}H + HCl$$

- **Halorespiration:** An organohalogen is used as an electron acceptor in an energy yielding metabolism
  - as pollutant is degraded biocatalyst concentration increases

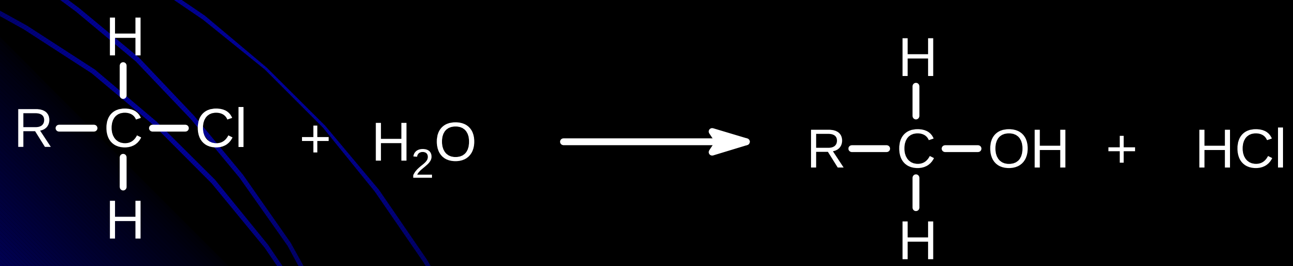


Mechanisms of Dechlorination

- **Oxygenolytic:**

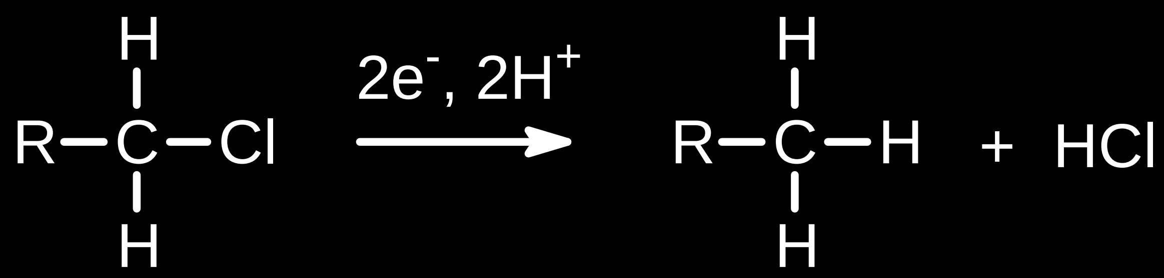


- **Hydrolytic:**

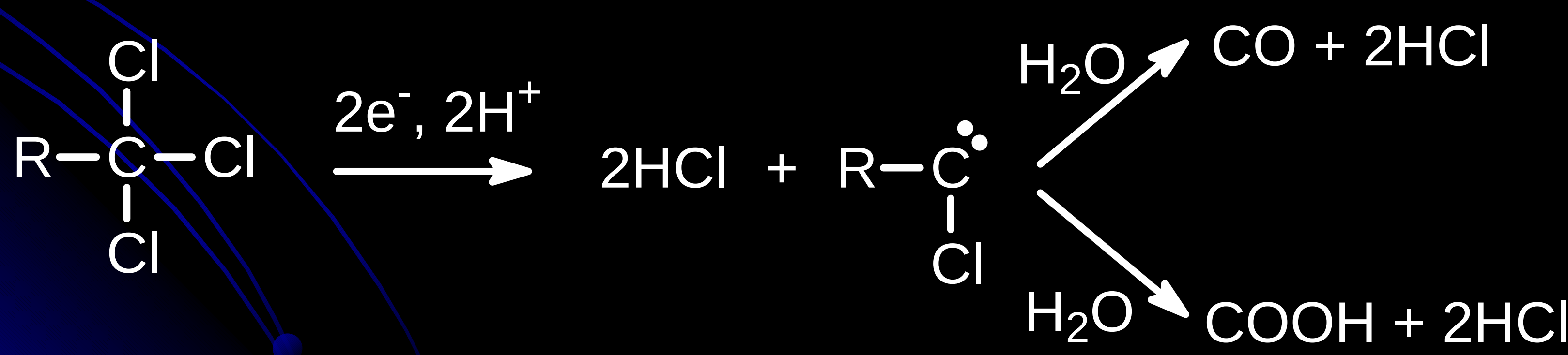


Mechanisms of Dechlorination

- **Reductive Hydrogenolysis:**

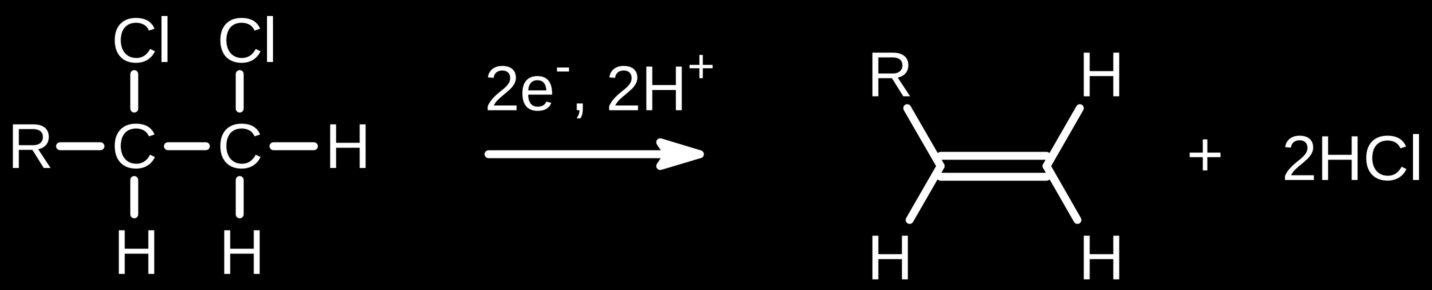


- **Hydrolytic Reduction:**



Mechanisms of Dechlorination

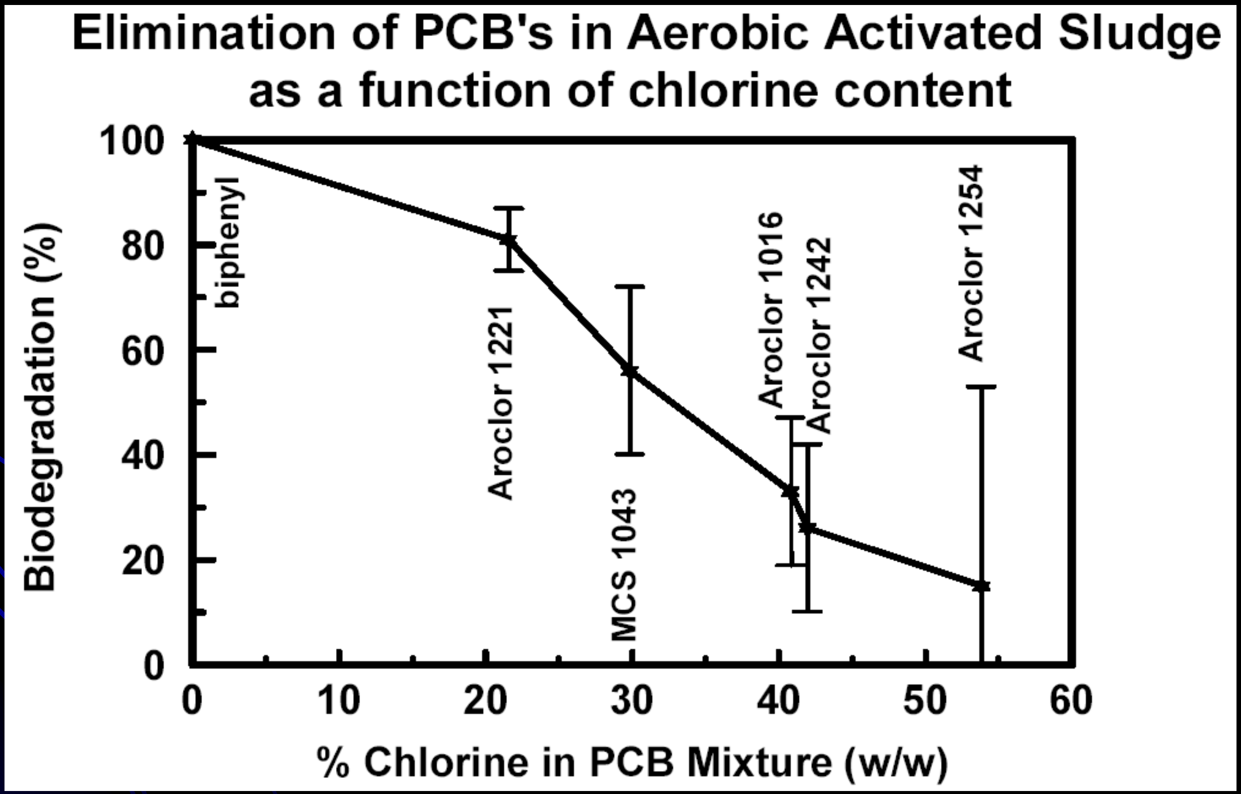
- **Reductive Dichloroelimination:**



Important Trends

- **Aerobic Degradation**

Chlorine # increases ↑ Biodegradation decreases ↓

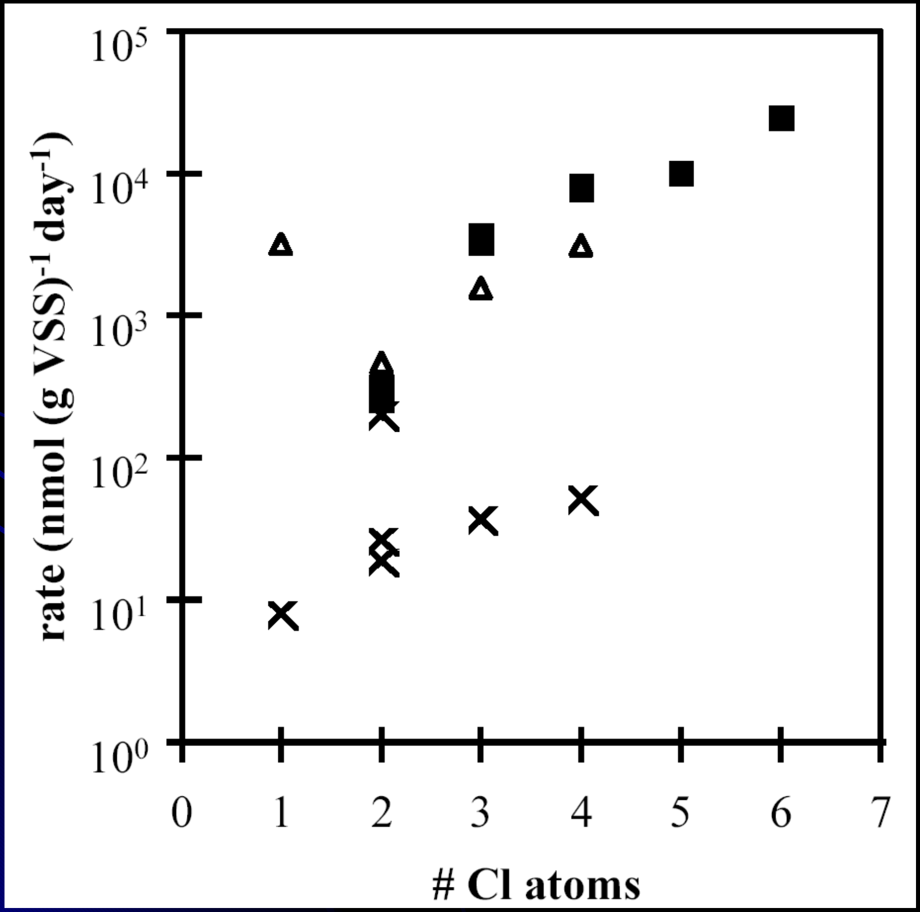


Tucker et al. 1975. *Bull. Environ. Contam. Toxicol.* **14**, 705-713

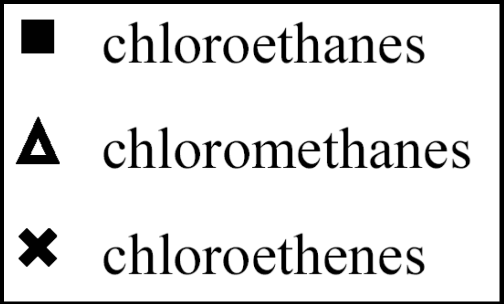
Important Trends

- **Anaerobic Degradation**

Chlorine # increases ↑ Biotransformation increases ↑



Cometabolism of chlorinated solvents by anaerobic sludge

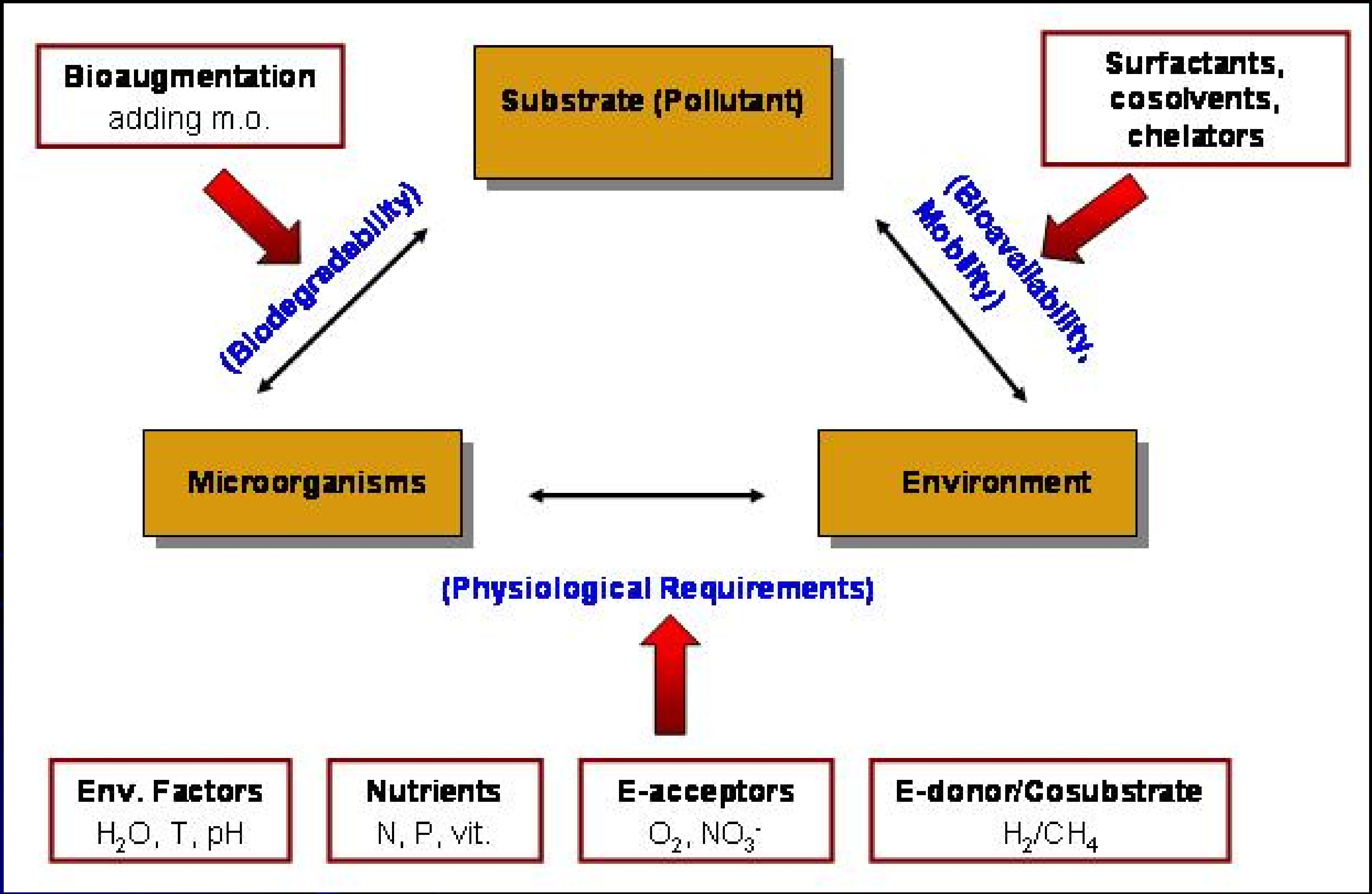


Van Eekert 1999. PhD Dissertation (no. 2638). Wageningen University, The Netherlands, p. 129.

Five Physiological Roles

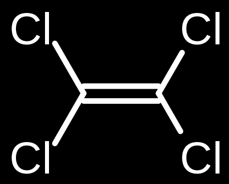
- **1st:** aerobic carbon and energy source — ED-A
- **2nd:** aerobic cometabolism (cooxidation) — CoM-A
- **3rd:** anaerobic carbon and energy source — ED-AN
- **4th:** anaerobic electron acceptor (halorespiration) — EA-AN
- **5th:** anaerobic cometabolism (reduced cofactors) — CoM-AN

Strategies of Bioremediation

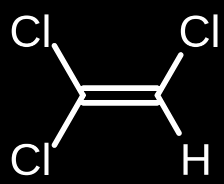


Modified from: Tiedje, J. M. 1993. Bioremediation from an ecological perspective. In: *In Situ Bioremediation: When Does it Work?* National Academy of Sciences, Washington DC, pp. 110-120.

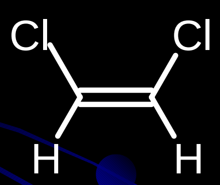
Abbreviations Chloroethenes



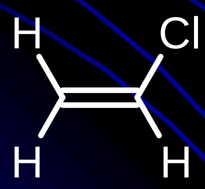
Perchloroethylene (PCE)



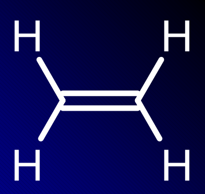
Trichloroethene (TCE)



cis Dichloroethene (cDCE)



Vinyl chloride (VC)

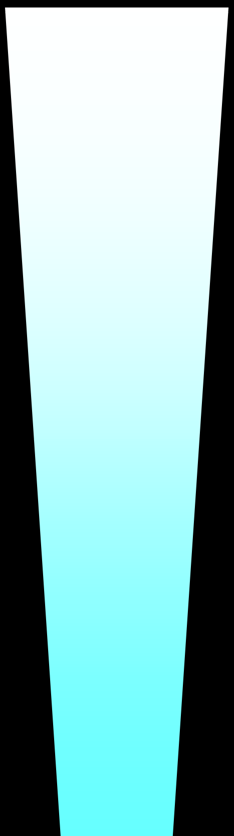


Ethene (E)

Biodegradation Chloroethenes

- **ED-A** **VC;** cDCE
- **CoM-A** **VC; cDCE; TCE**
- **ED-AN** VC (?), cDCE (?)
- **EA-AN** VC; cDCE; **TCE; PCE**
- **CoM-AN** VC; cDCE; TCE; PCE

faster

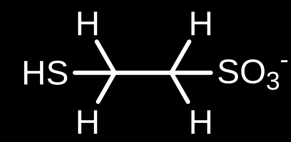


slower

Chloroethenes ED-A

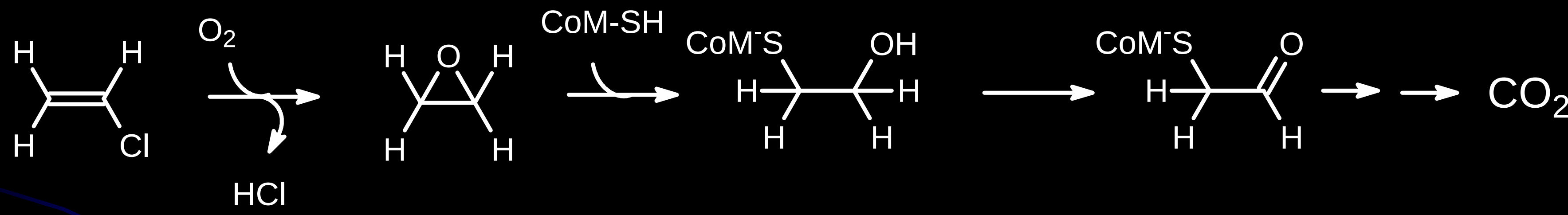
- **Microorganisms Involved:** *Mycobacterium*, *Nocardioides*, *Pseudomonas*

- **Pathway**



Coenzyme M

monooxygenase epoxyalkane:CoM transferase



Coleman & Spain 2003 JB 185:5536

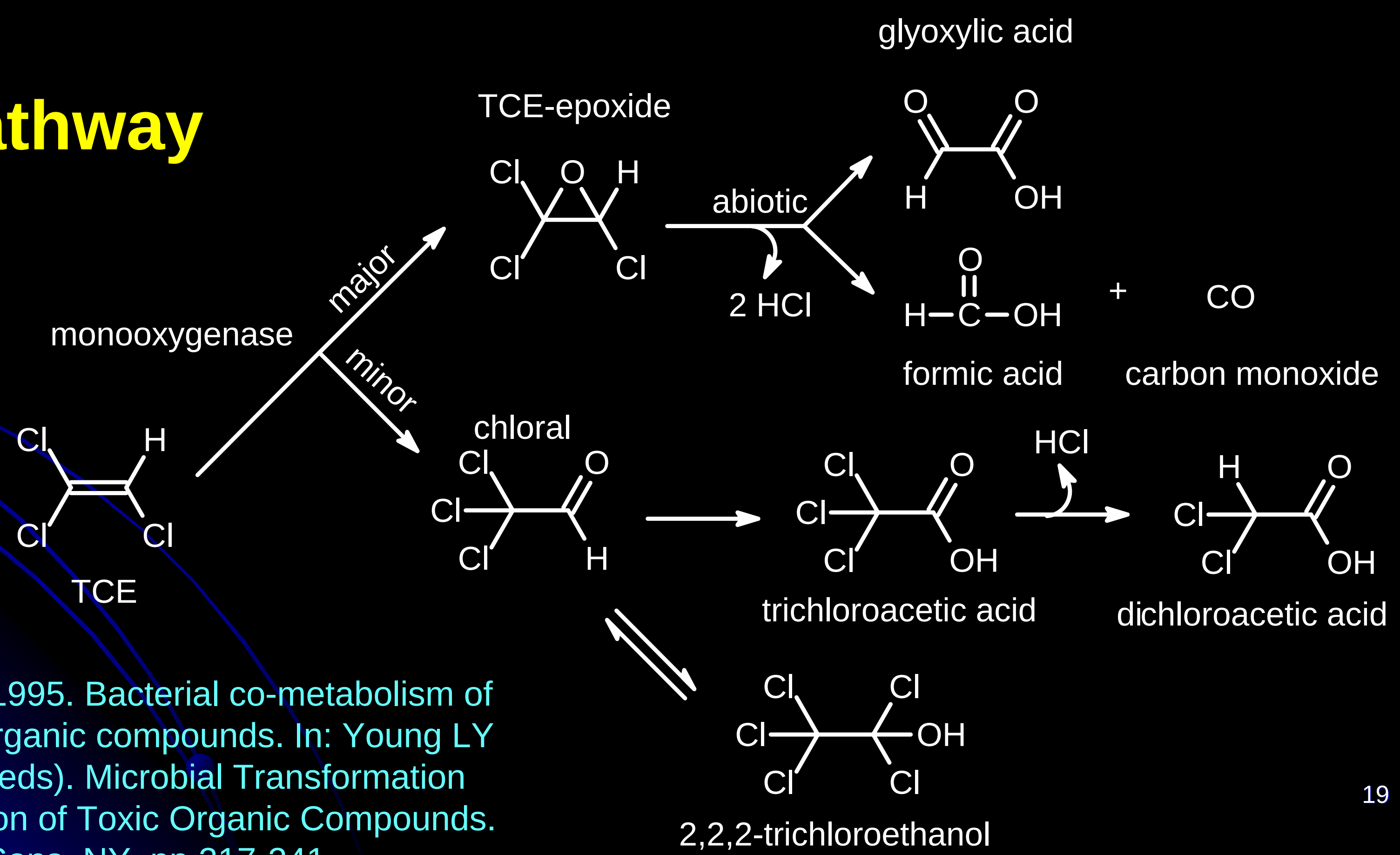
- **Kinetics**

Growth rates	0.05 to 0.96 d^{-1}
Activity	226 to 4950 mg g^{-1} dwt d^{-1}
K_m or K_s	0.07 to 0.70 mg l^{-1}

Chloroethenes CoM-A (cooxidation)

- **Microorganisms Involved:** *Methylosinus*, *Peudomonas*, *Burkholderia*, *Nitrosomonas*, *Mycobacterium*, *Rhodococcus*, *Alcaligenes*

- **Pathway**



Wackett L. P. 1995. Bacterial co-metabolism of halogenated organic compounds. In: Young LY & Cerniglia C (eds). Microbial Transformation and Degradation of Toxic Organic Compounds. John Wiley & Sons, NY, pp 217-241.

Chloroethenes CoM-A (cooxidation)

- **Primary Substrates Supporting Cooxidation:** methane, toluene, phenol, ammonium, ethane, ethene, propane *etc*
  - Substrates for which monooxygenases are utilized for biodegradation

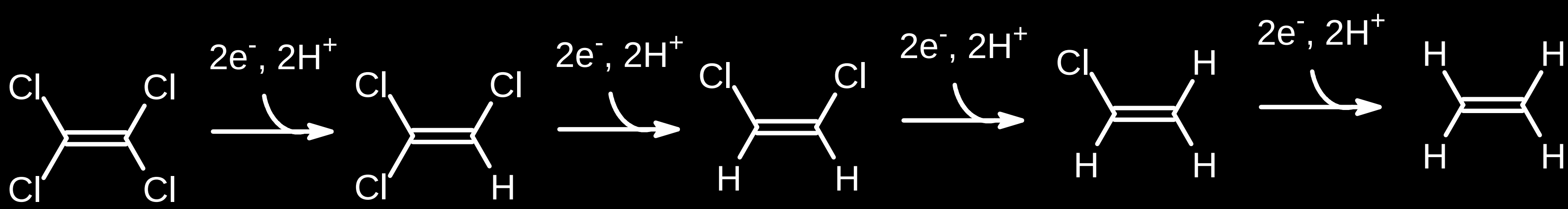
- **Kinetics**

Activity	57 to 55,000 mg g^{-1} dwt d^{-1}
Transformation Capacity	86 to 150 mg TCE g^{-1}dwt
K_m	0.4 to 29.6 mg l^{-1}

Chloroethenes EA-AN (Halorespiration)

Pathway

Successive Steps of Reductive Hydrogenolysis



PCE TCE cDCE VC E

High Biodiversity

7 genera from 4 major bacterial phyla

Low Biodiversity

1 genus

Chloroethenes EA-AN (Halorespiration)

- **Microorganisms Involved: PCE to cDCE**

Low G+C gram +	*Desulfitobacterium*	H_2, lactate, formate, etoh
	Clostridium	YE, glucose
	Dehalobacter	H_2
δ Proteobacteria	*Desulfuromonas*	acetate,pyruvate
ε Proteobacteria	*Dehalospirillim*	H_2, lacate, formate, etoh
	Sulfurospirillum	lactate
Green non-sulfur	*Dehalococcoides*	H_2

- **Microorganisms Involved: cDCE to E**

Green non-sulfur	*Dehalococcoides*	H_2

Chloroethenes EA-AN (Halorespiration)

- **Biochemistry**
  - Reactions catalyzed by specific reductive dehalogenases
    - All contain vitamin B12
    - Most are membrane bound enzymes

- **Kinetics: PCE to TCE and/or cDCE**

Growth rates	0.23 to 6.65 d^{-1}
Activity	856 to 37,312 mg g^{-1} dwt d^{-1}

- **Kinetics: VC to E**

Growth rates	0.32 to 0.40 d^{-1}
Activity	3047 to 6030 mg g^{-1} dwt d^{-1}
K_m or K_s	0.16 to 0.31 mg l^{-1}

Chloroethenes EA-AN (Halorespiration)

- **Hypothetical Example**

Assumptions: t_0 = 1 bacterium per m^3

1 bacterium = 1×10^{-12} g

Ideal conditions for growth

Kinetic data:	*Dehalosprillium multivorans*	*Dehalococcoides* strain VS
Growth rate (d^{-1})	6.65	0.40
Activity (mg g^{-1} dwt d^{-1})	5970	3047

Growth Equation:

$$C_{xt} = C_{x0} e^{ut}$$

C_{x0} & C_{xt} = cell biomass conc. at time 0 & t (g dwt l^{-1})

μ = growth rate (d^{-1}), t = time (d)

Chloroethenes EA-AN (Halorespiration)

- **Hypothetical Example** (*continued*)

Question: How long will it take for a bioconversion rate of 10 mg l^{-1} chloroethenes per day?

Initial Biomass: 1×10^{-15} g dwt l^{-1}

Final Biomass: $10/5970 = 1.675 \times 10^{-3}$ g dwt l^{-1} *Dehalosprillium*

$10/3047 = 3.282 \times 10^{-3}$ g dwt l^{-1} *Dehalococcoides*

Time:

$$t = \frac{\ln\left(\frac{C_{xt}}{C_{x0}}\right)}{\mu}$$

4.2	days	*Dehalosprillium*
72.1	days	*Dehalococcoides*

Chloroethenes CoM-AN

- **Microorganisms Involved:** Methanogens, Acetogens
- **Pathway** Successive Steps of Reductive Hydrogenolysis
  - Reactions catalyzed by reduced enzyme cofactors
    - Cobalt containing vitamin B12; Nickel containing Factor 430
- **Kinetics: PCE to TCE and/or cDCE**

  Activity 0.006 to 20 mg g^{-1} dwt d^{-1}
- **Kinetics: cDCE or VC to E**

  Activity 0.001 to 0.366 mg g^{-1} dwt d^{-1}

Chloroethenes Bioremediation

- **Anaerobic - Aerobic**

First: Rapid reductive dehalogenation to TCE & cDCE

Second: Rapid cooxidation of TCE and cDCE to CO_2 & Cl^-

$$\text{PCE} \xrightarrow[\text{+ electron donor}]{\text{anaerobic}} \text{cDCE} \xrightarrow[\text{+ } O_2]{\text{aerobic}} CO_2, Cl^-$$

+ primary substrate

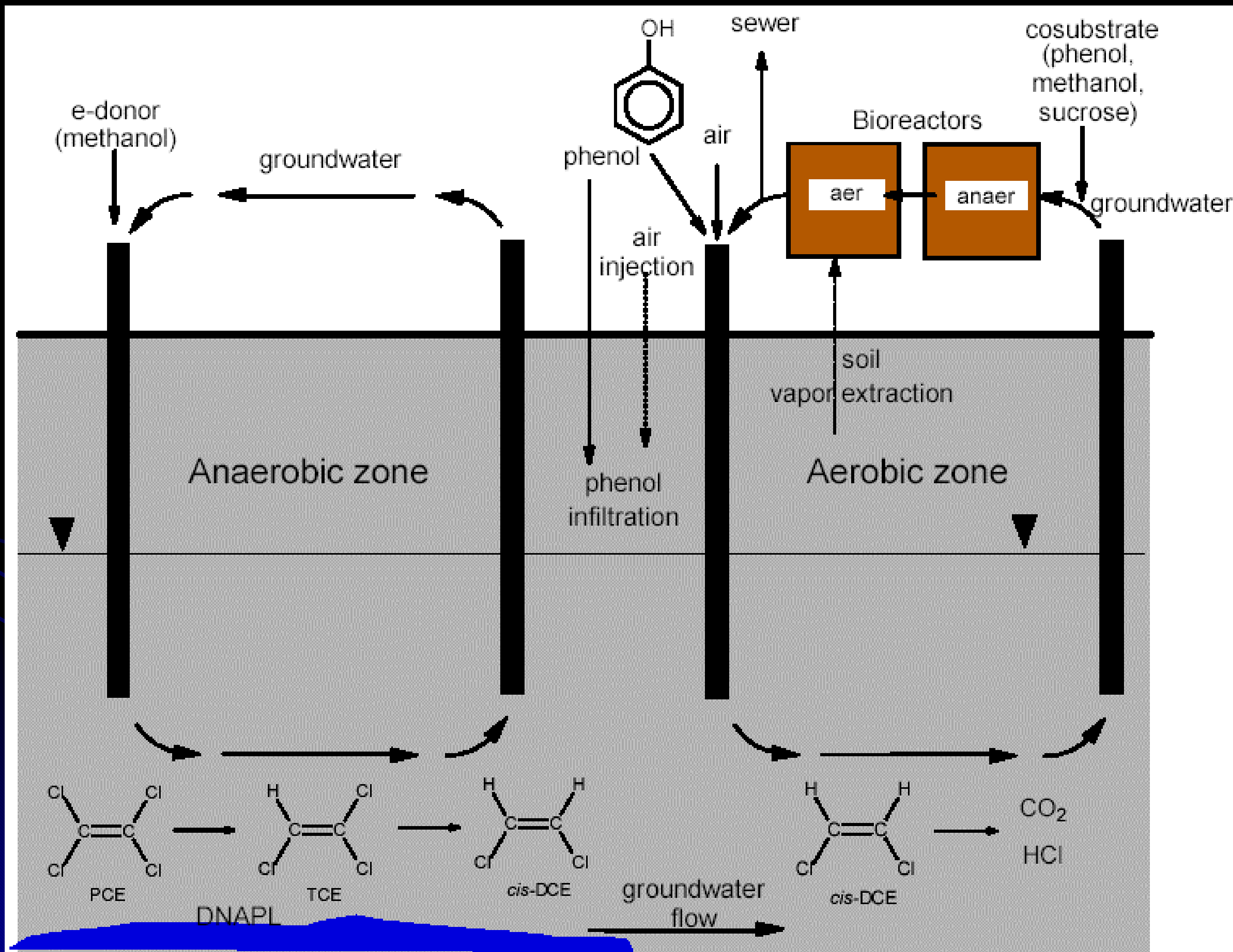
- **Anaerobic with *Dehalococcoides***

Promote complete halorespiration to ethene

$$\text{PCE} \xrightarrow[\text{+ electron donor}]{\text{anaerobic}} \text{E}$$

+ *Dehalococcoides* (if absent)

Bioremediation Breda (Holland)



Bioremediation Breda (Holland)

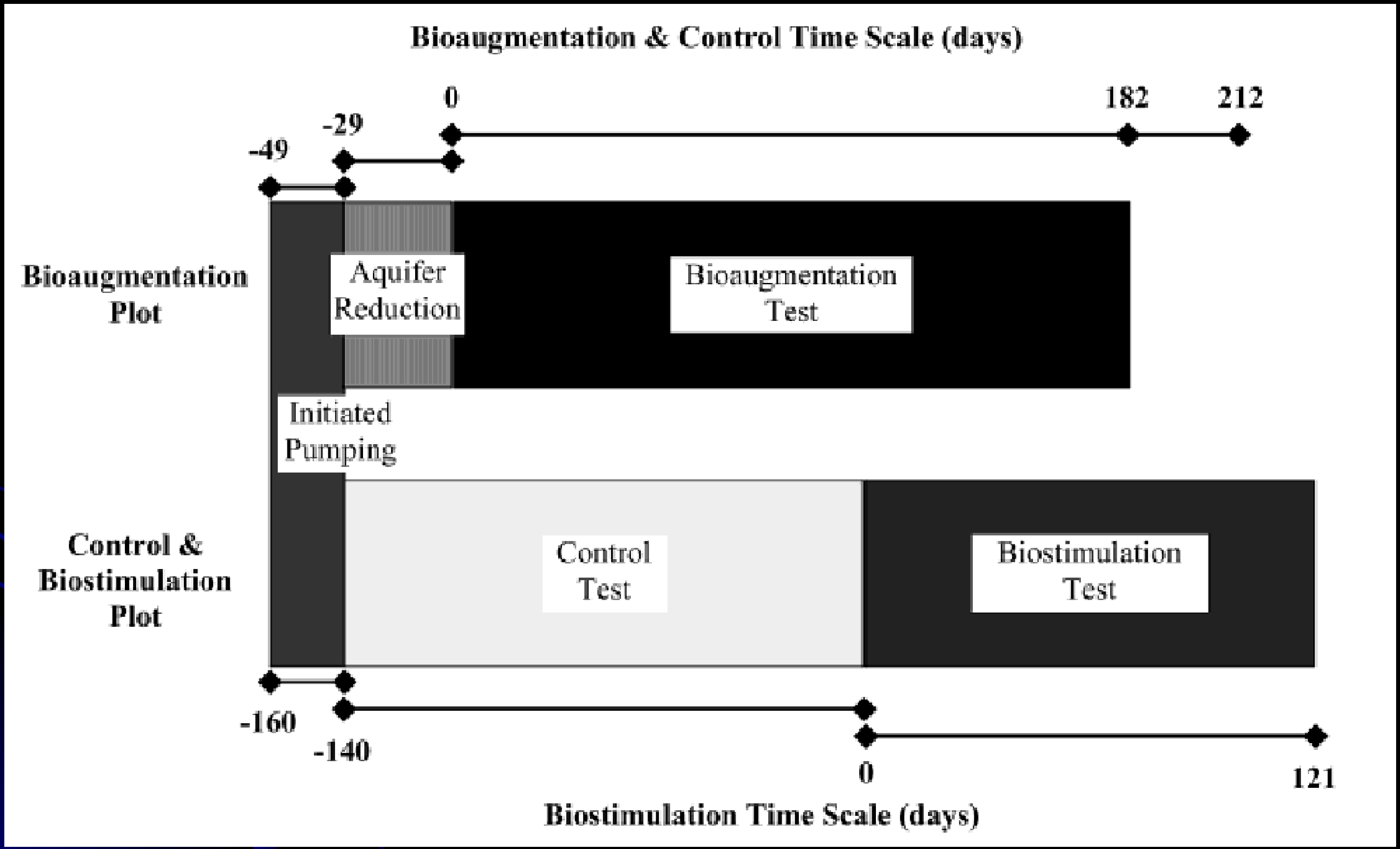
Facts about Full-Scale Bioremediation

- 85% removal of PCE in situ within 6 months
- Inorganic chloride concentration in anaerobic zone increased from 1 to 6 mM
- In the aerobic zone all of the cDCE and VC as well as injected phenol was removed
- After one year the total mass of chloroethenes decreased from 1500 to 550 mol

Spuij et al. 1997. Full-scale application of in situ bioremediation of PCE-contaminated soil. *4th Int. In Situ and On Site Bioremediation Symp., New Orleans, LA*, Columbus, OH: Battelle Vol 5, pp. 431–37.

Bioremediation (Bachman Rd, Mi)

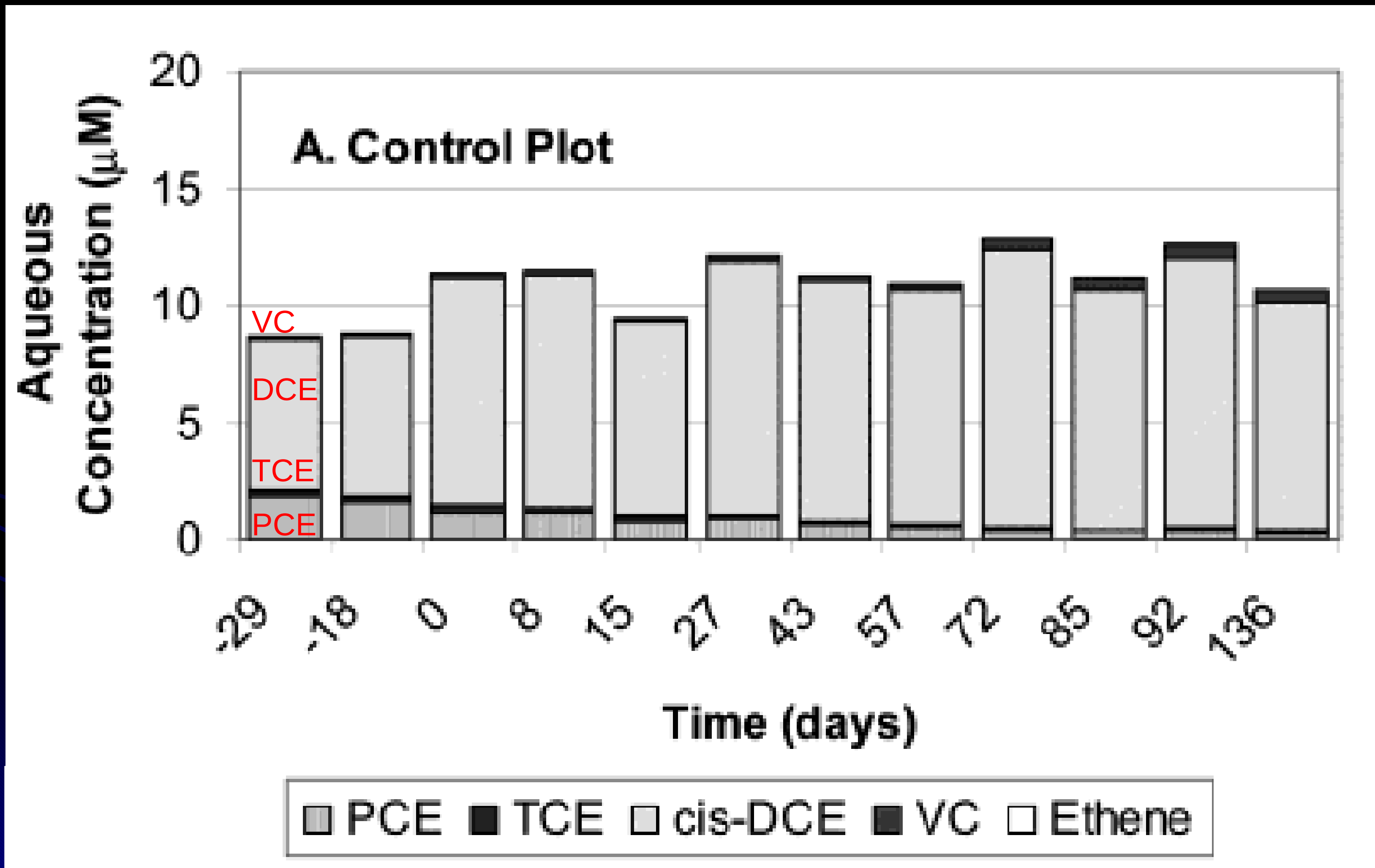
- **Comparison Bioaugmentation vs Biostimulation**



Lendvay et al. 2003. Environ. Sci. Technol. 37:1422

Bioremediation (Bachman Rd, Mi)

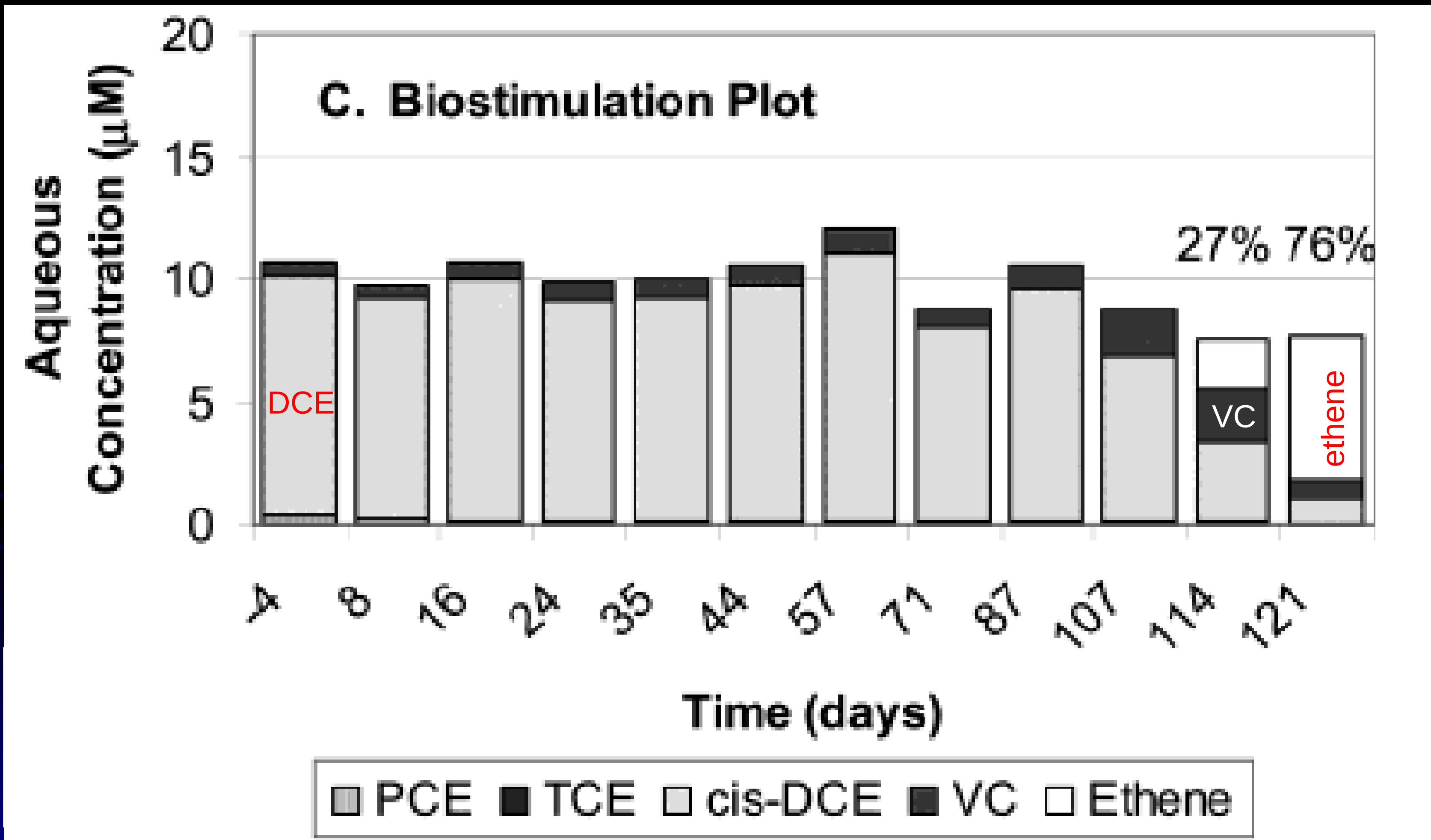
Control Experiment at Bachman Road Site (Michigan)



Lendvay et al. 2003. Environ. Sci. Technol. 37:1422

Bioremediation (Bachman Rd, Mi)

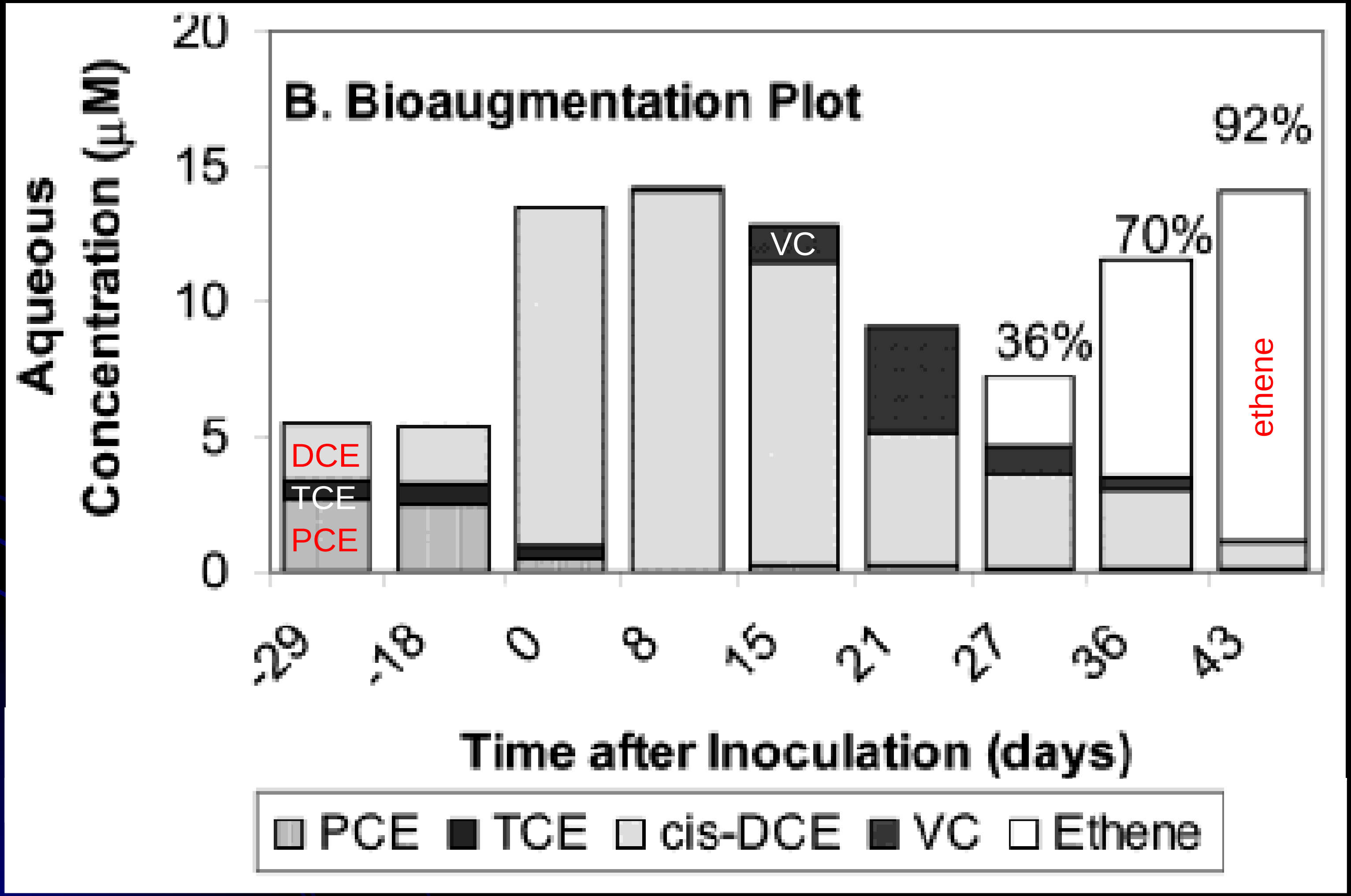
Biostimulation Plot: Day 0 = lactate addition



Lendvay et al. 2003. Environ. Sci. Technol. 37:1422

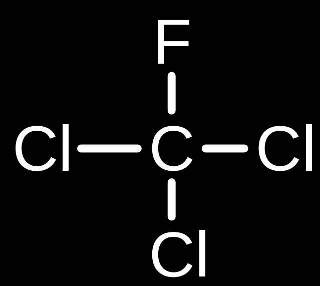
Bioremediation (Bachman Rd, Mi)

Bioaugmentation Plot: Day –29 = lactate addition; Day 0 = *Dehaloccocoides* addition

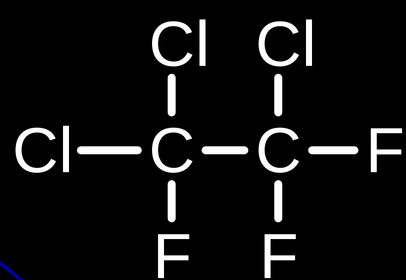


Lendvay et al. 2003. Environ. Sci. Technol. 37:1422

Chlorofluorocarbons



CFC-11



CFC-113

Chlorofluorocarbons

- **Microorganisms Involved CoM-An:**

Methanogens, fermentative bacteria

Activity 0.005 to 165 mg g^{-1} dwt d^{-1}

Pathways: Reactions catalyzed by reduced enzyme cofactors

- **Microorganisms Involved CoM-A:**

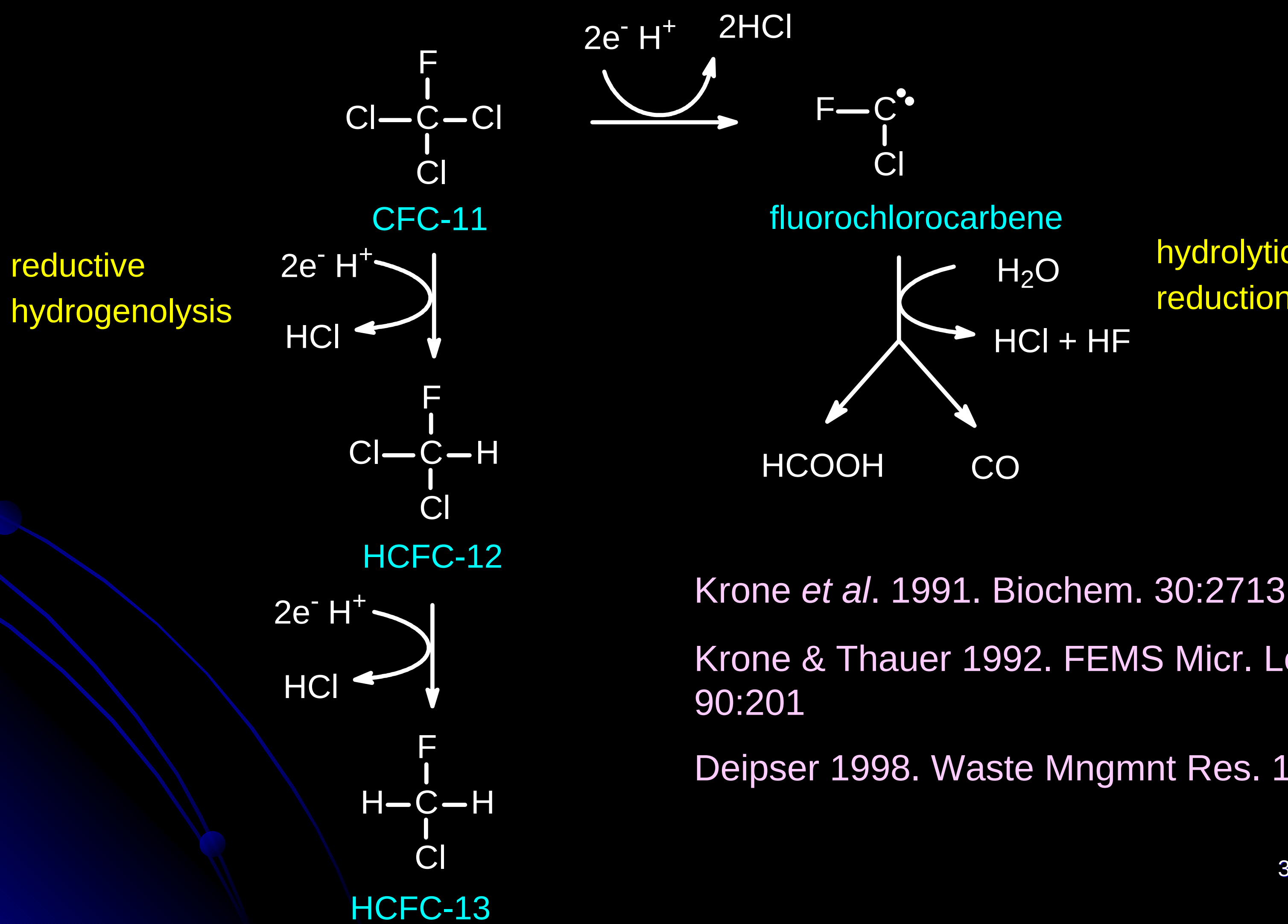
Methane oxidizing bacteria

Activity 119 to 2374 mg g^{-1} dwt d^{-1}

Pathways: methane monooxygenase

Chlorofluorocarbons

Anaerobic Pathways CFC-11 Biodegradation



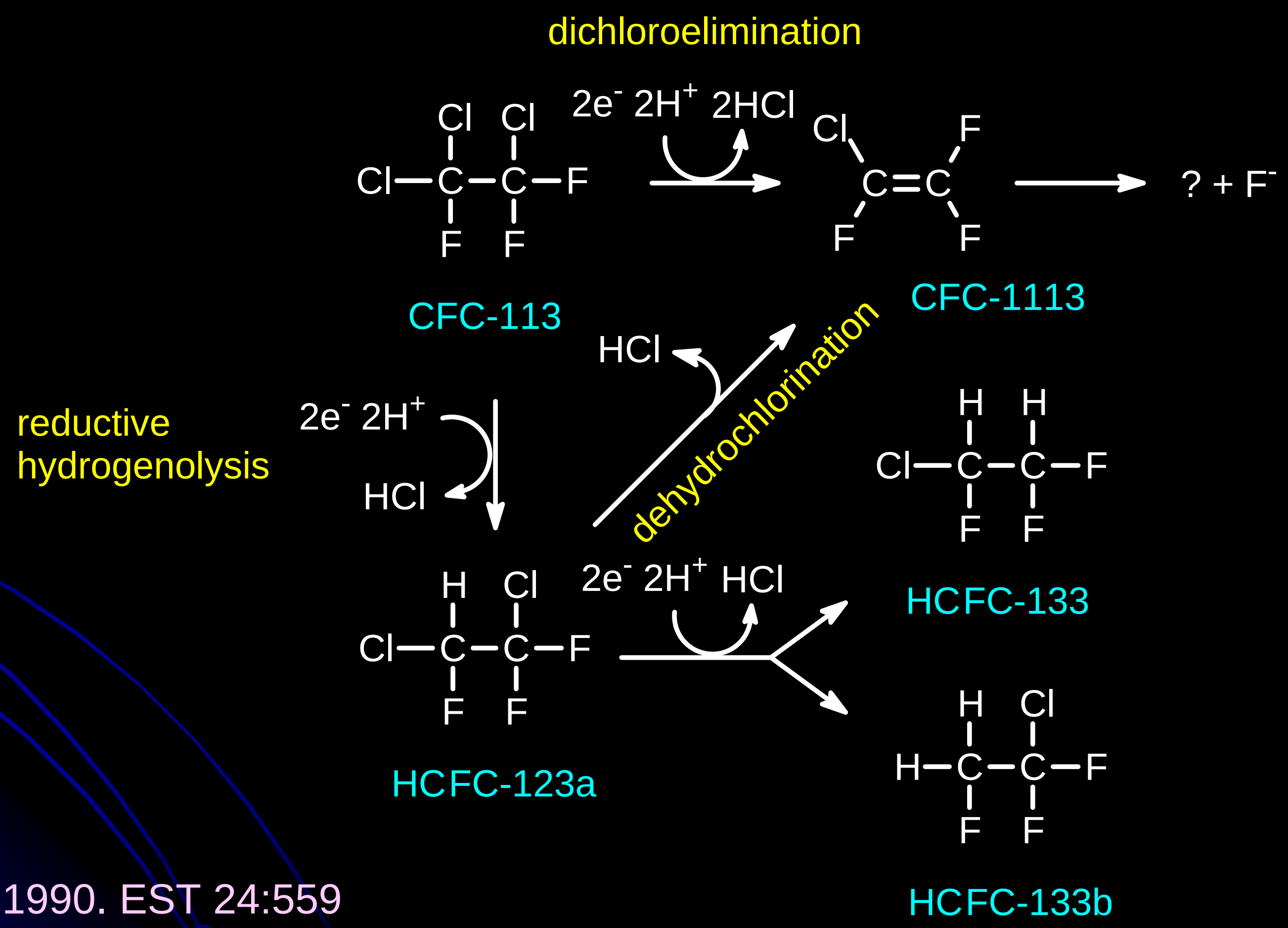
Krone *et al*. 1991. Biochem. 30:2713

Krone & Thauer 1992. FEMS Micr. Lett 90:201

Deipser 1998. Waste Mngmnt Res. 16:330

Chlorofluorocarbons

Anaerobic Pathways CFC-113 Biodegradation



Lesage *et al.* 1990. EST 24:559

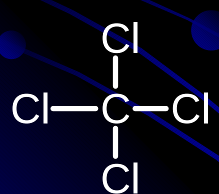
Lesage *et al.* 1992. Chemosphere 24:1225

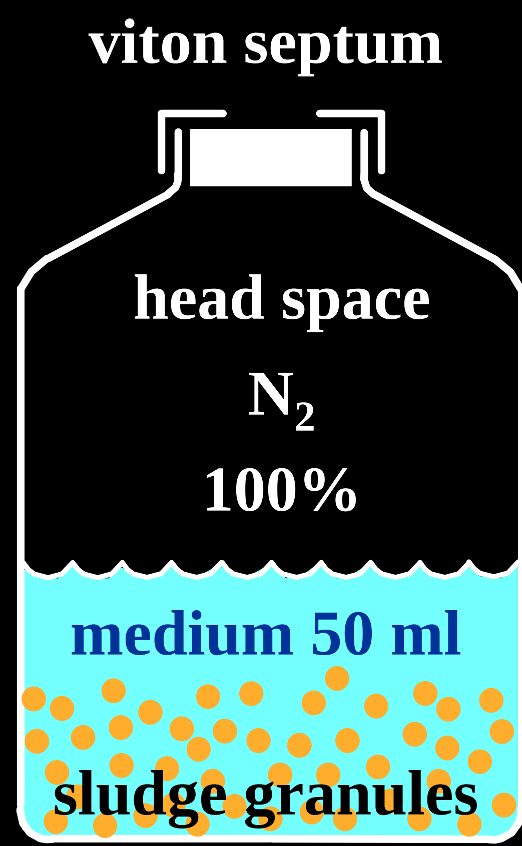
Carbon Tetrachloride CoM-AN

Effect Redox Mediators

Experimental

- Phosphate buffer pH 7.0
- mineral medium (Cl^- free)
- methanogenic sludge (0.5 g VSS/L)
- VFA mixture (0.25 g COD/L)
- CT or CF (100 uM)
- Redox Mediators (10 uM)

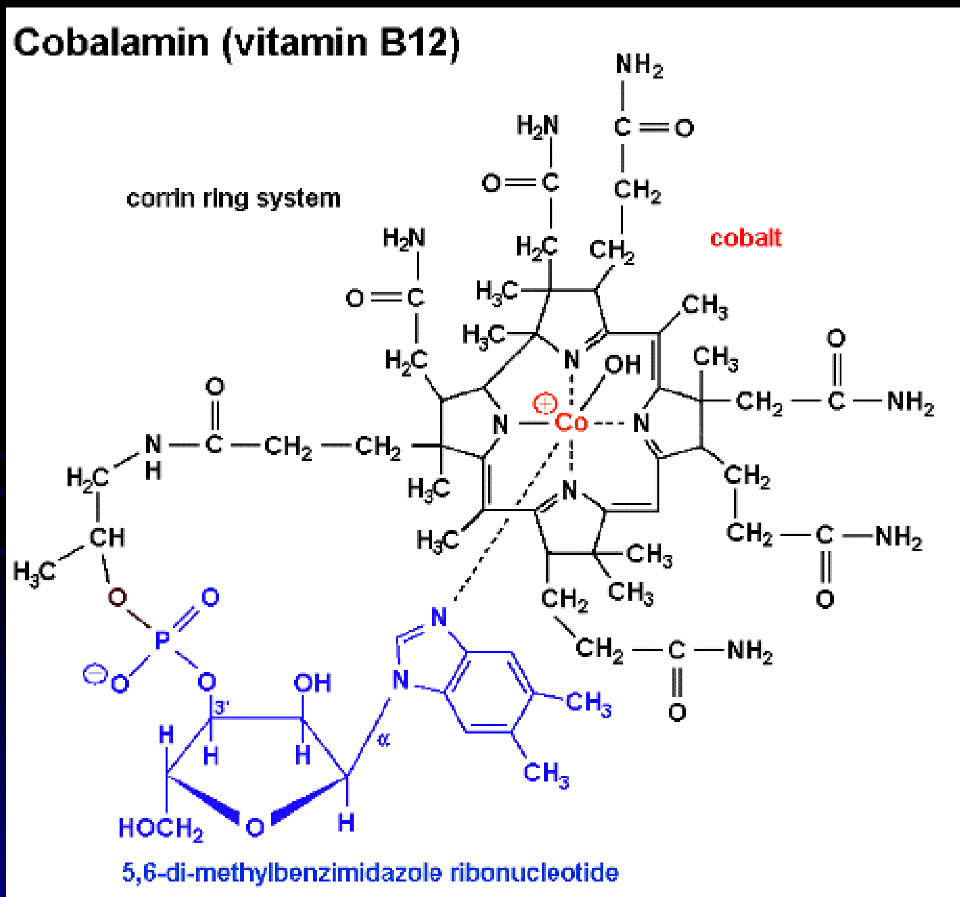




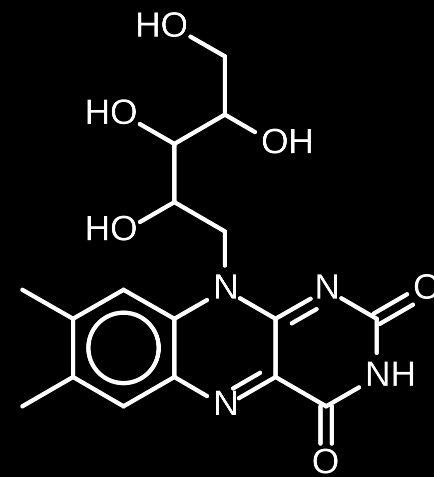
Guerrero-Barrajas & Field. 2005. Enhancement of anaerobic carbon tetrachloride biotransformation in methanogenic sludge with redox active vitamins. Biodegradation 16: 215–228.

Carbon Tetrachloride CoM-AN

- **Effect Redox Mediators**

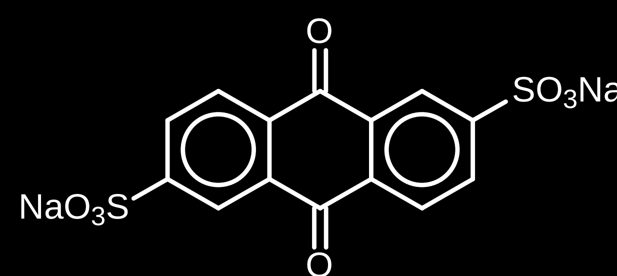


(B12)



Riboflavin

(RF)

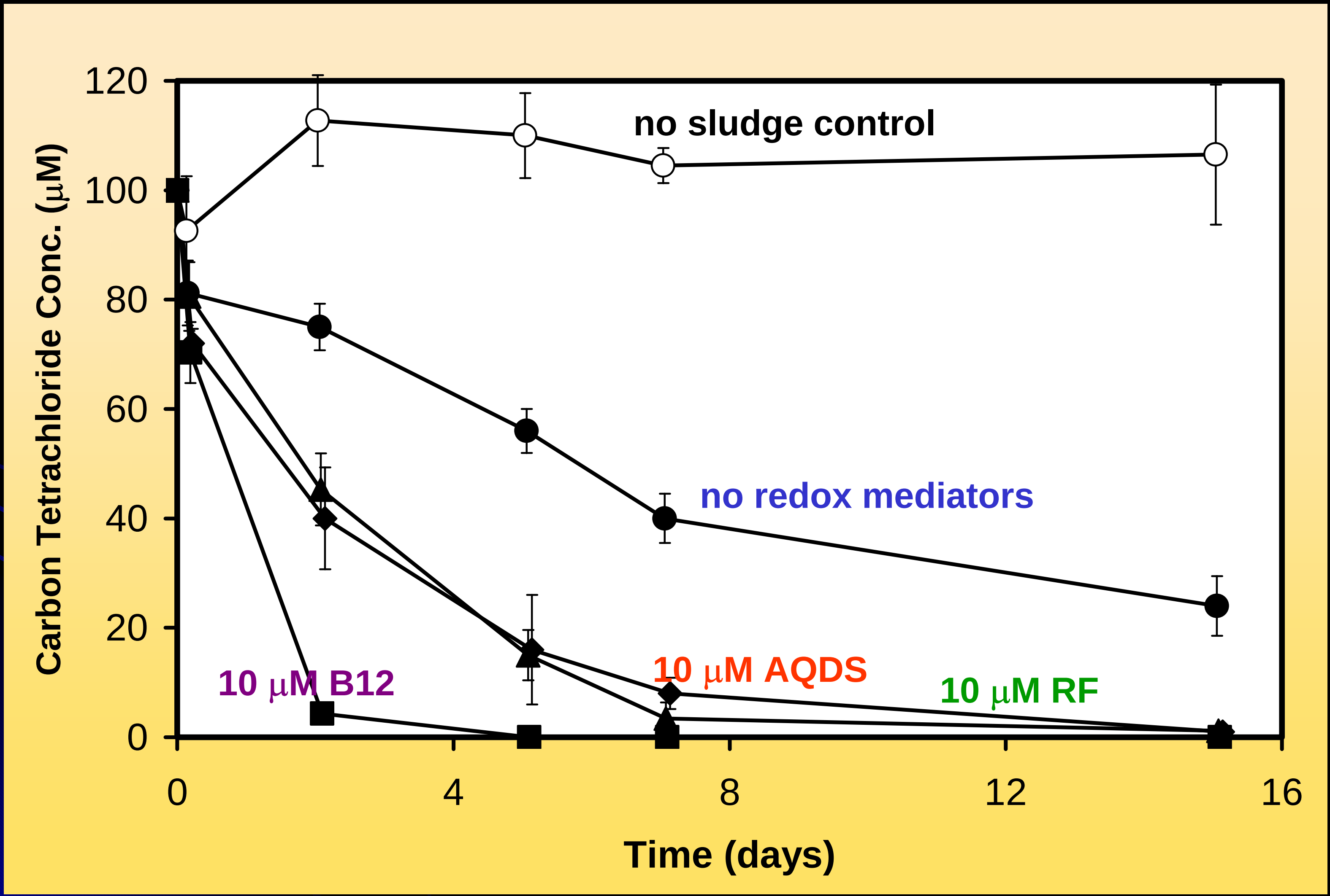


Anthraquinone Disulfonate (AQDS)

(AQDS)

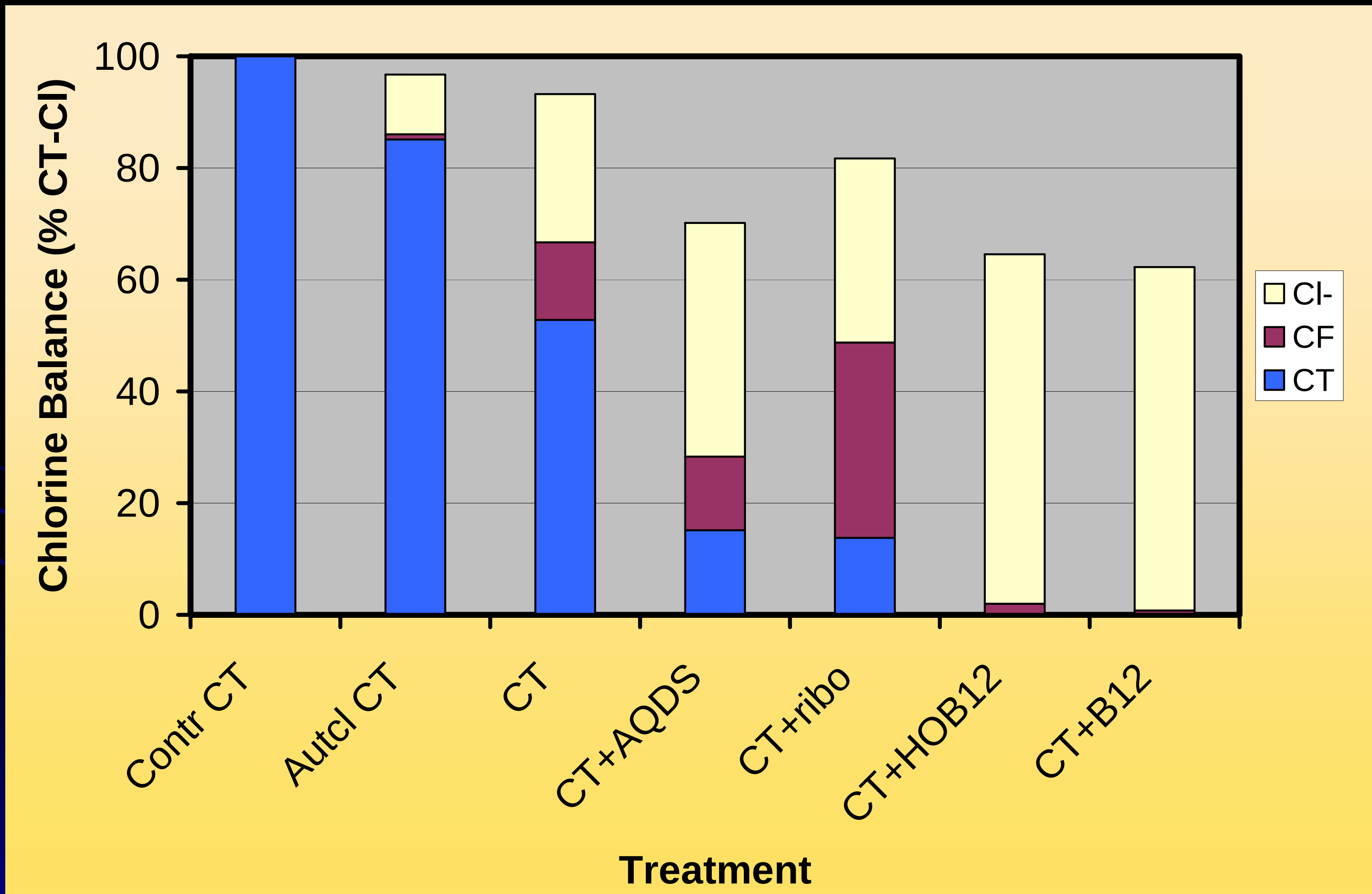
Carbon Tetrachloride CoM-AN

- **Effect Redox Mediators:** CT Concentration



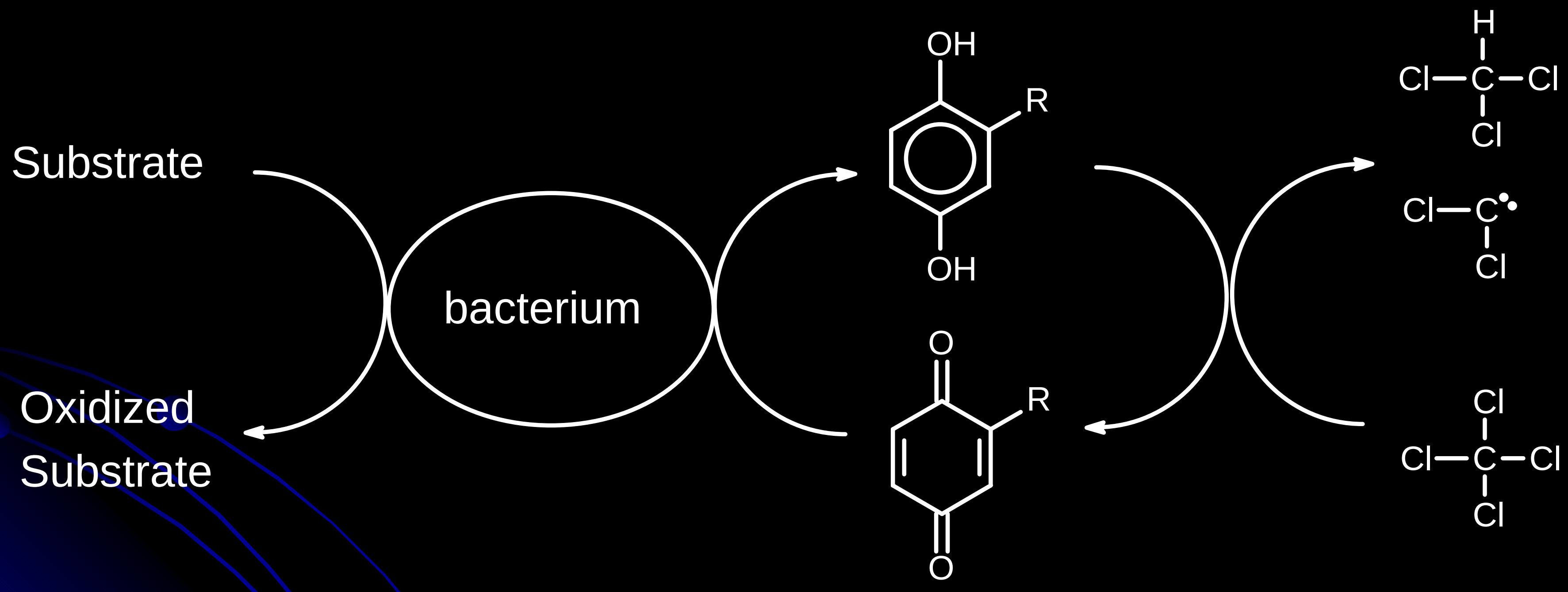
Carbon Tetrachloride CoM-AN

- **Effect Redox Mediators**: Chlorine Balance day 5



Carbon Tetrachloride CoM-AN

- **Role Redox Mediators:**



Conclusions 1

Biodegradation Chloroethenes

- High biodiversity for rapid halorespiration PCE to cDCE; halorespiration cDCE to E restricted to one genus, *Dehaloccocoides*
- Slow anaerobic cometabolism of PCE, TCE, cDCE and VC (dominant process reductive hydrogenolysis)
- Rapid aerobic cooxidation of VC, cDCE, TCE feasible
- Aerobic biodegradation of VC (and cDCE) as growth substrates feasible with newly discovered bacterial strains

Bioremediation Chloroethenes

- Anaerobic halorespiration (PCE $\rightarrow$ cDCE) followed by aerobic cooxidation (cDCE $\rightarrow$ CO_2, Cl^-)
- Complete reductive dechlorination with *Dehaloccocoides* (PCE $\rightarrow$ E)

Conclusions 2

Biodegradation Chlorofluorohydrocarbons

- Aerobic cooxidation feasible
- Anaerobic cometabolism of CFC-11

  1) hydrolytic reduction to CO and HCOOH

  2) reductive hydrogenolysis to HCFC of lower chlorine number

Biodegradation Carbon Tetrachloride

- Biodegradation of CT only feasible by anaerobic cometabolism
- Redox mediators can greatly enhance anaerobic biotransformation CT, CF
- Vit. B12 stimulates hydrolytic reduction (> formation Cl^-)