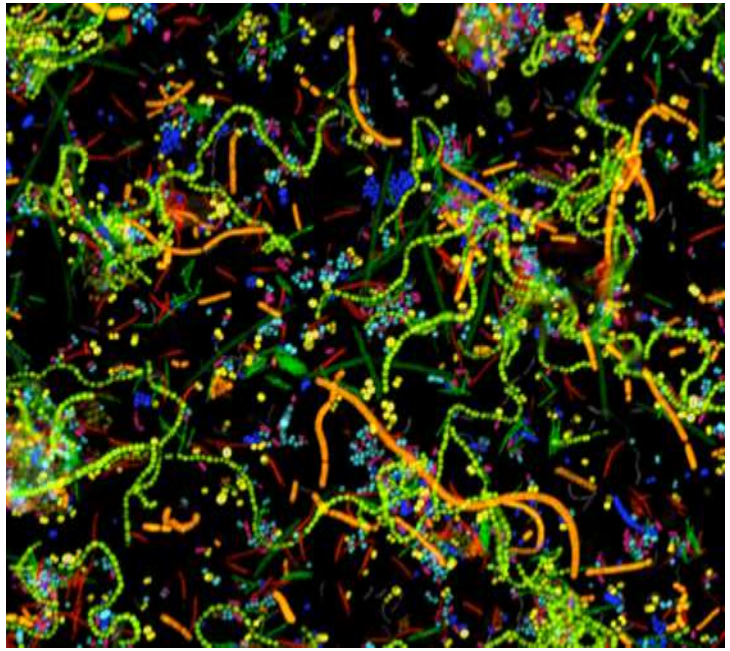
Microbial Dechlorinating Consortia & Brief Introduction to Metagenomics



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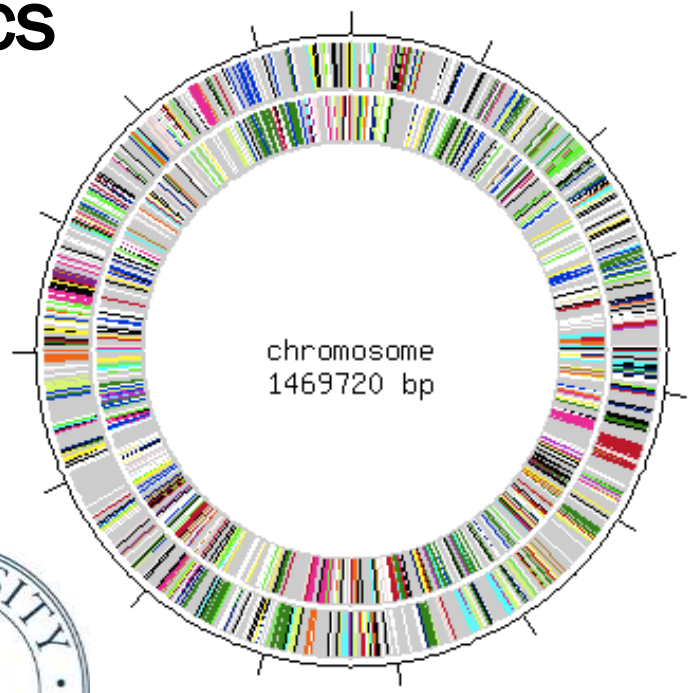

BioZone

Centre for Applied Bioengineering Research

Co-Authors and Acknowledgments

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And many more

Sandra Dworatzek
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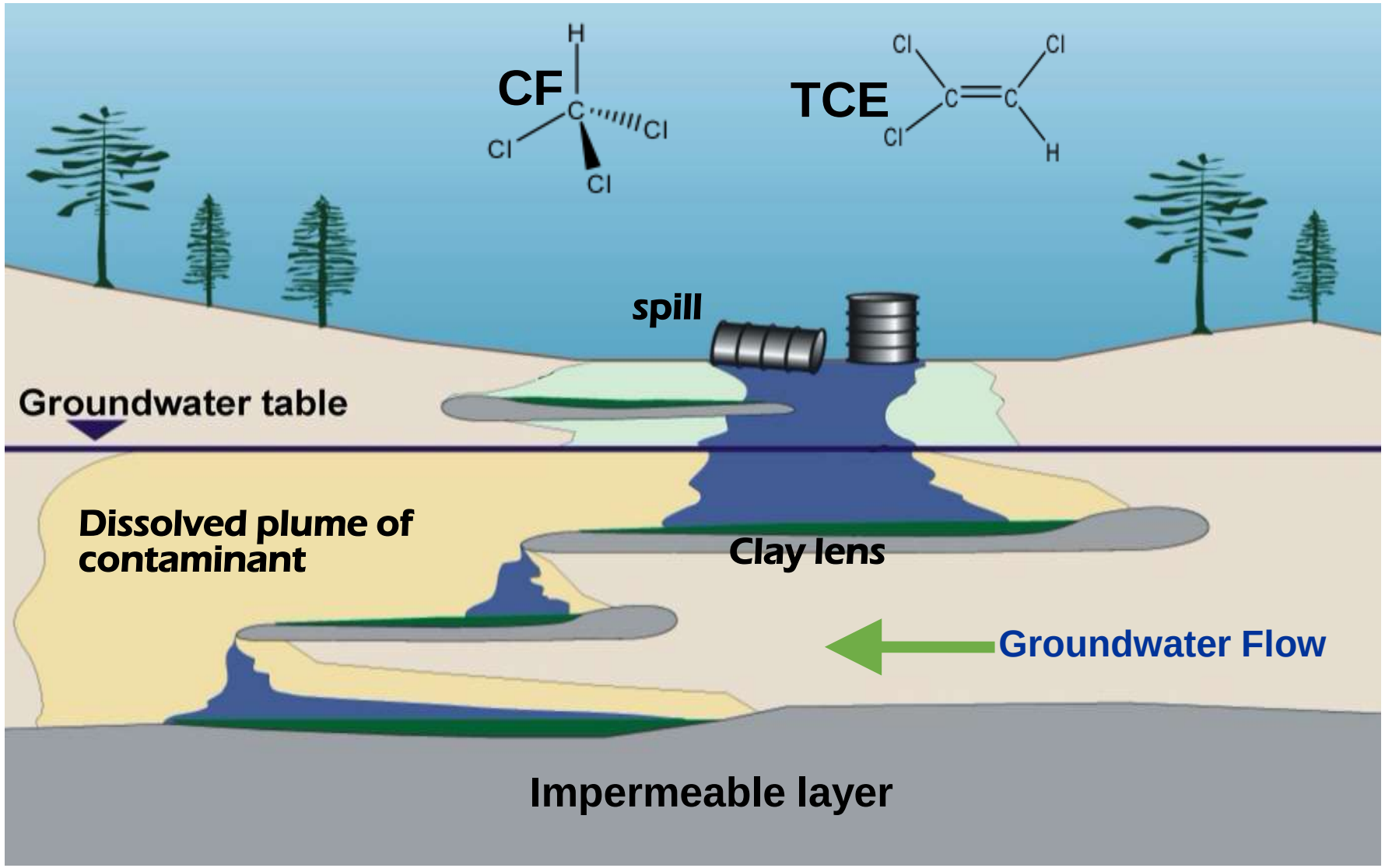
Alfred Spormann (Stanford)
Ruth Richardson & Stephen Zinder (Cornell)
Lorenz Adrian (UFZ); Craig Criddle (Stanford)

Fate of contaminants in the environment: role of Biology

Typically oxygen becomes depleted

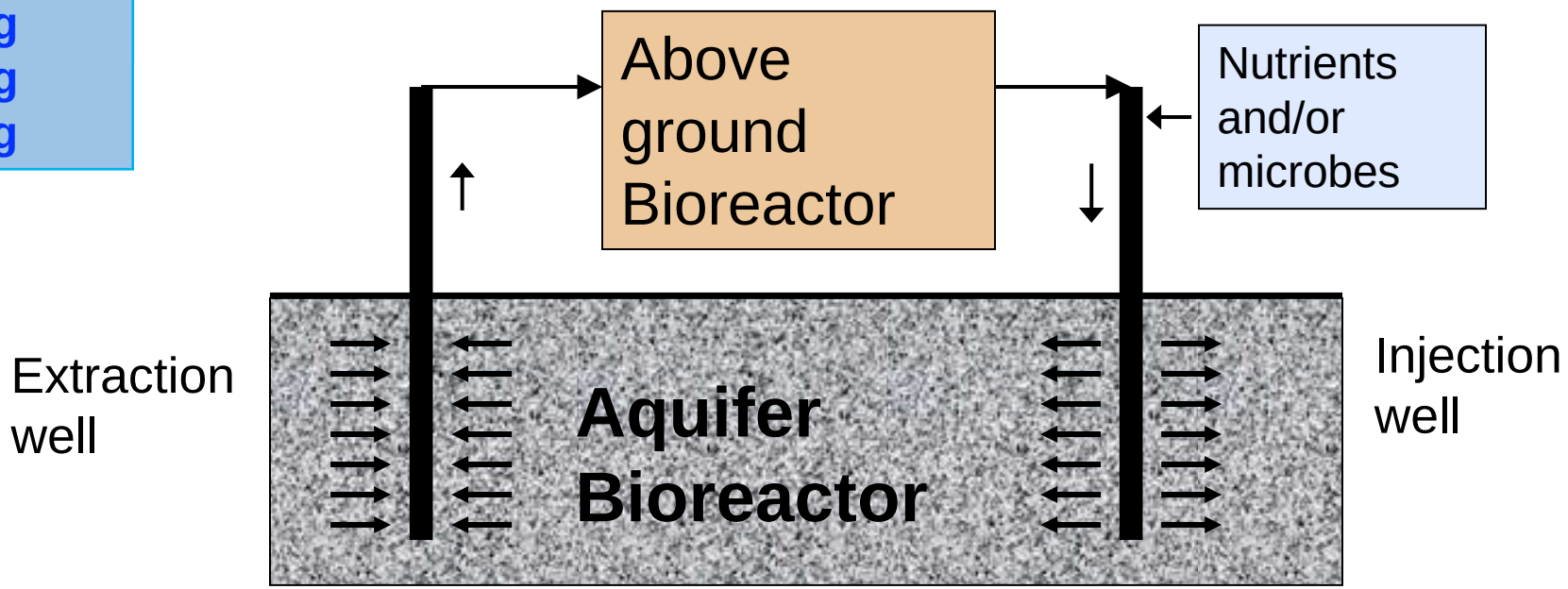


Bioremediation

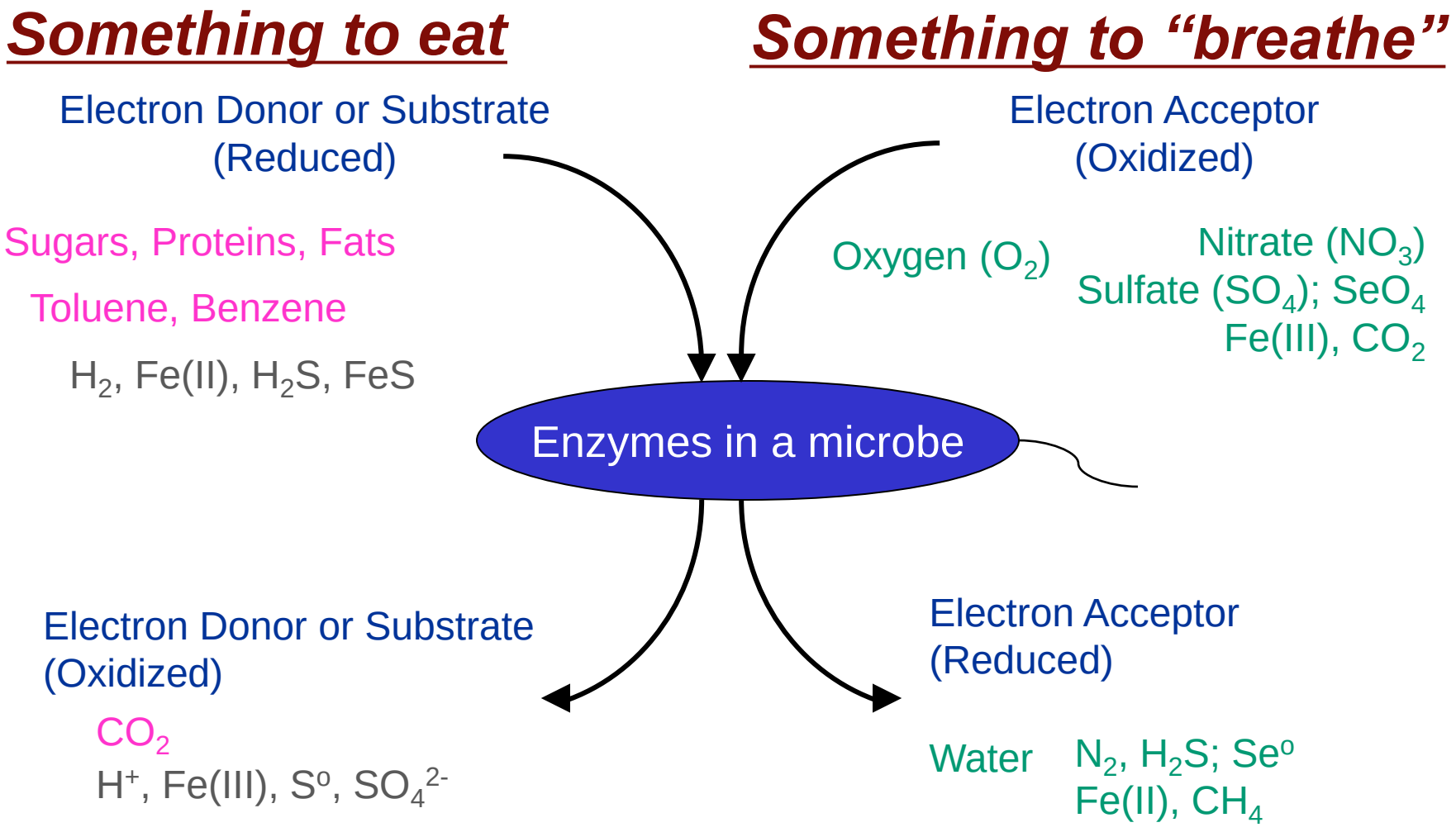
Bioremediation: the remediation (clean up) of contaminated sites (soil, sediment, groundwater) using microorganisms in an engineered system

- *ex situ* (on-site): in above-ground bioreactors
- *in situ* (in-place): the subsurface is the bioreactor
- Biostimulation *vs.* Bioaugmentation

Three challenges:
1) Mixing
2) Mixing
3) Mixing

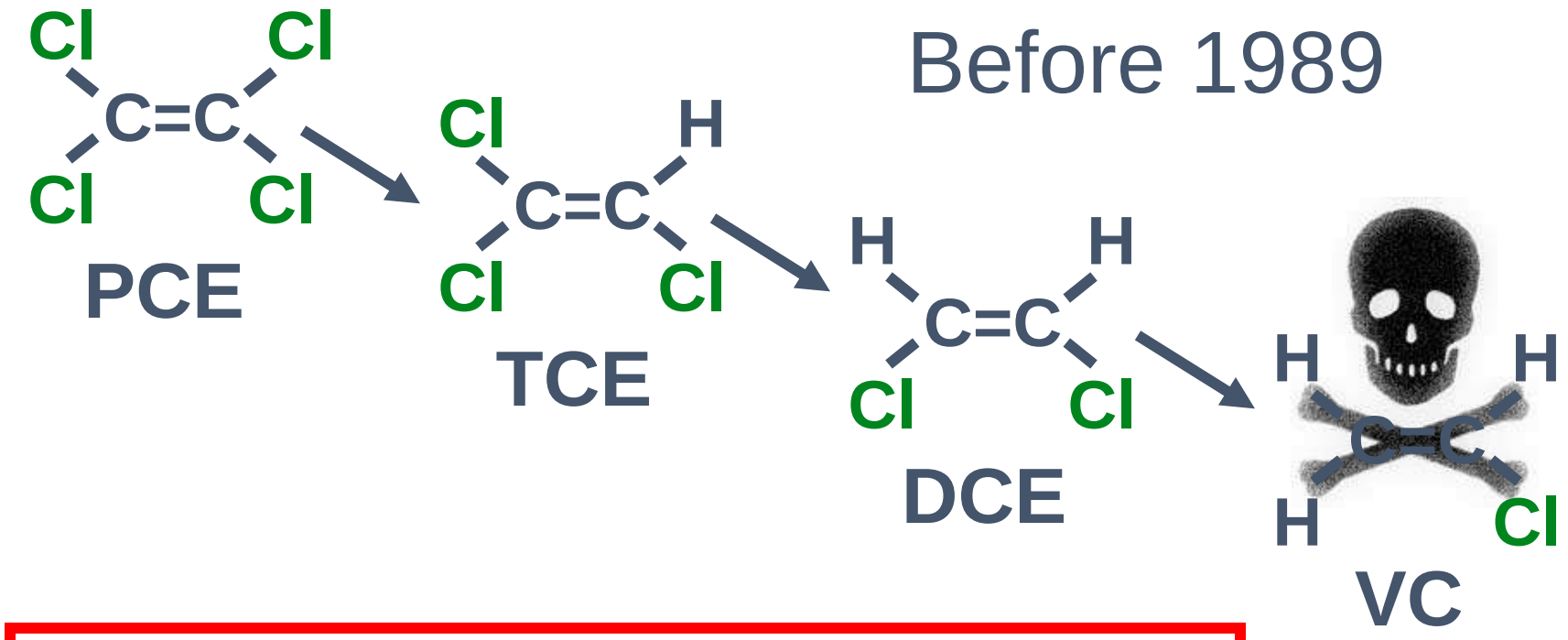


Overview of Microbial Metabolism



If energy is released (DeltaG<0) then microbe can grow

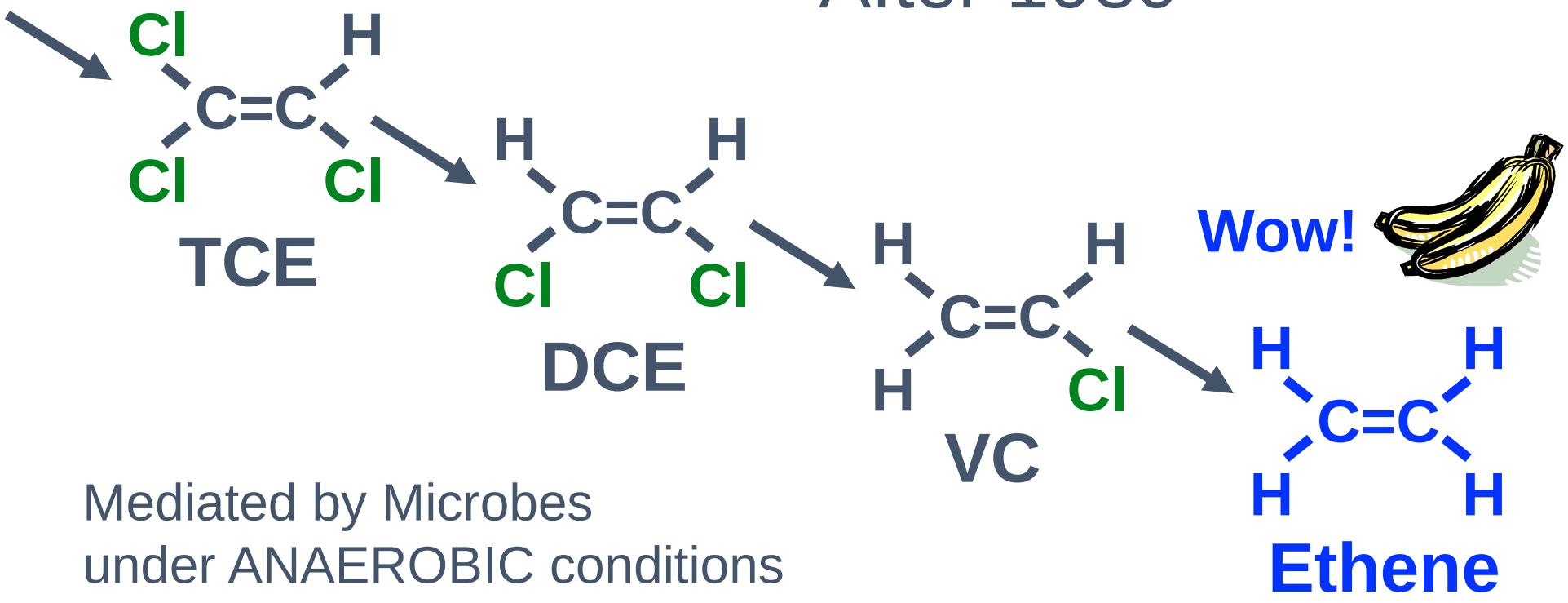
Anaerobic PCE Dechlorination



Made a bad situation worse!

Complete Reductive Dechlorination

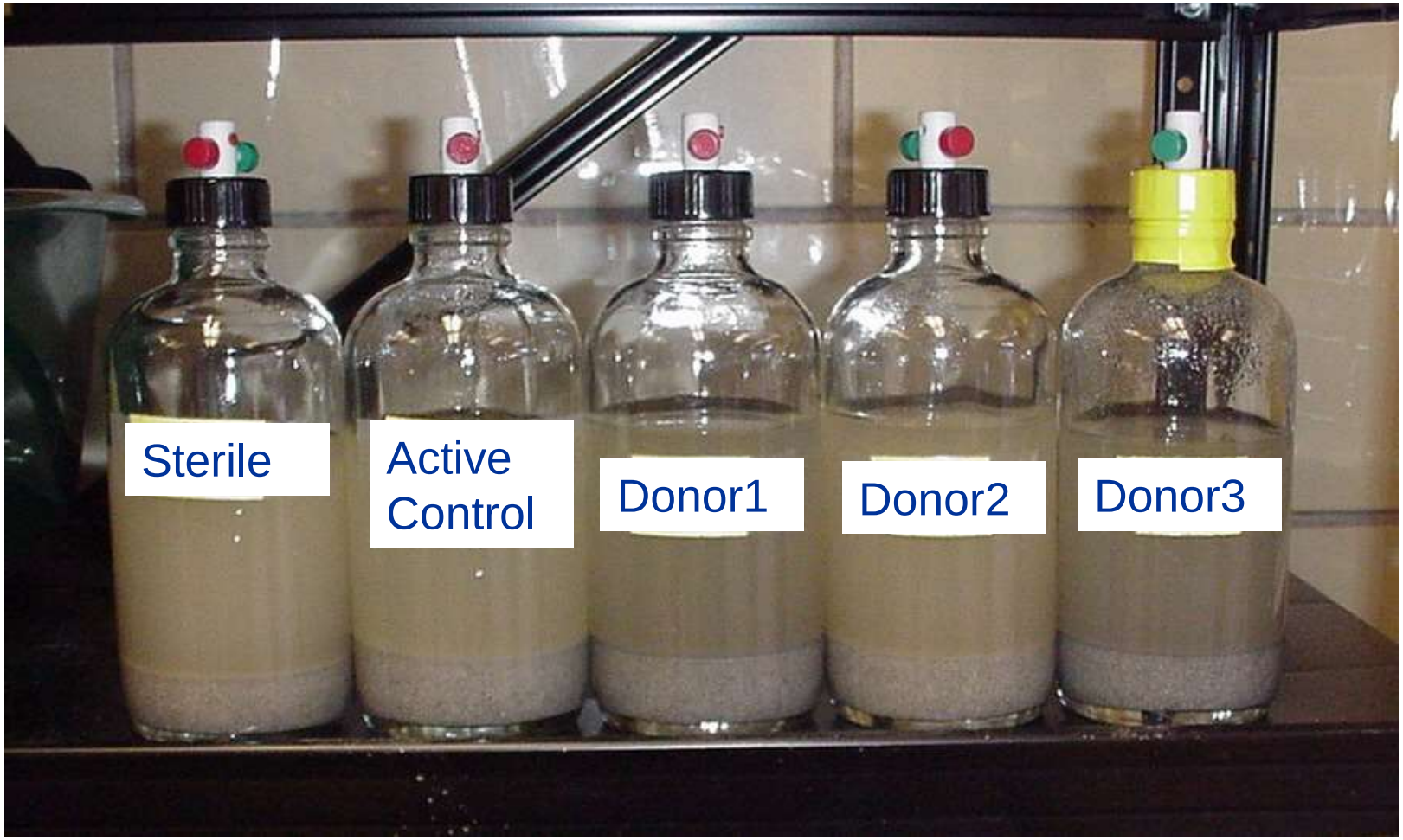
After 1989



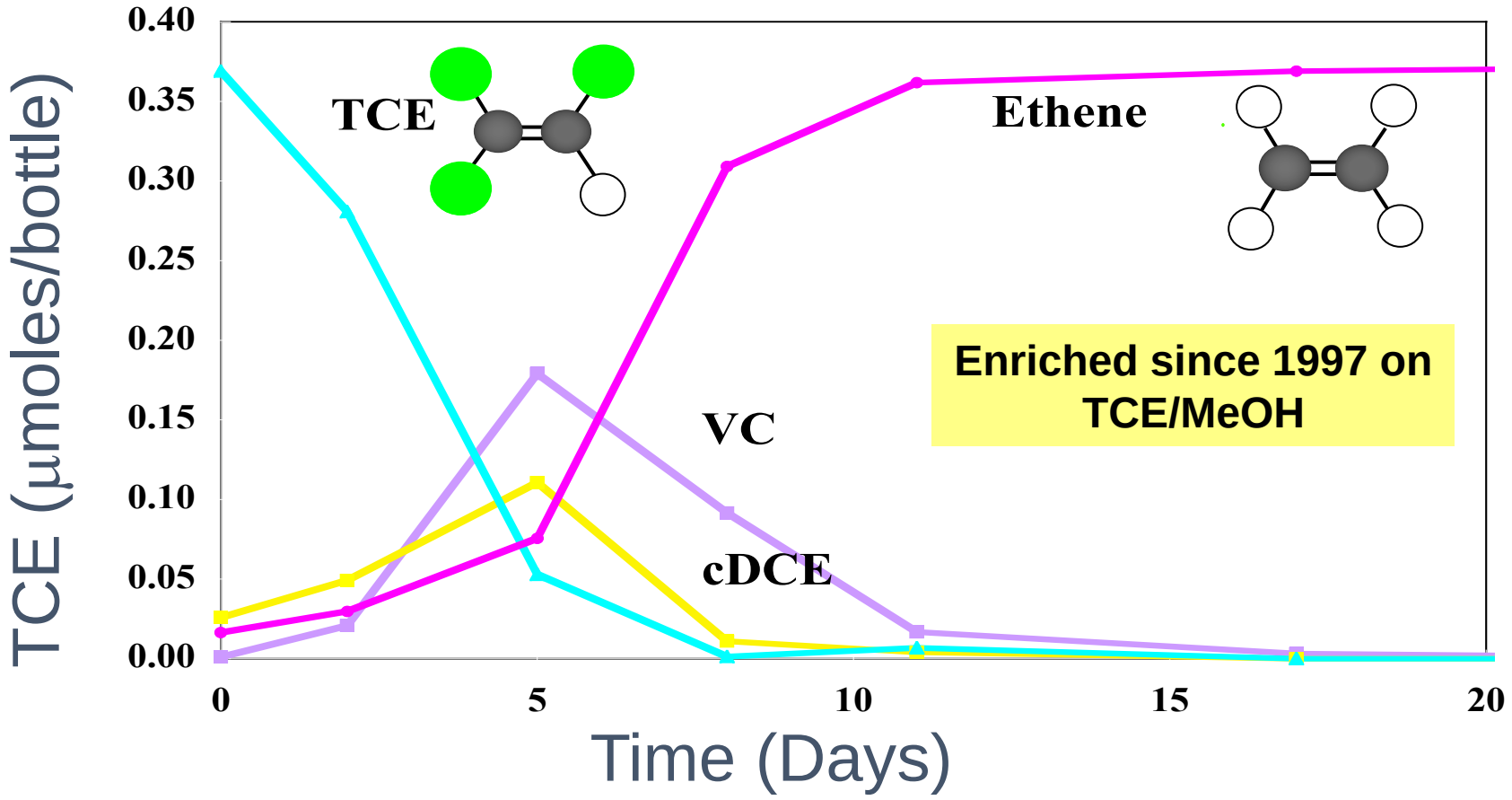
Mediated by Microbes
under ANAEROBIC conditions
Requires electron donor

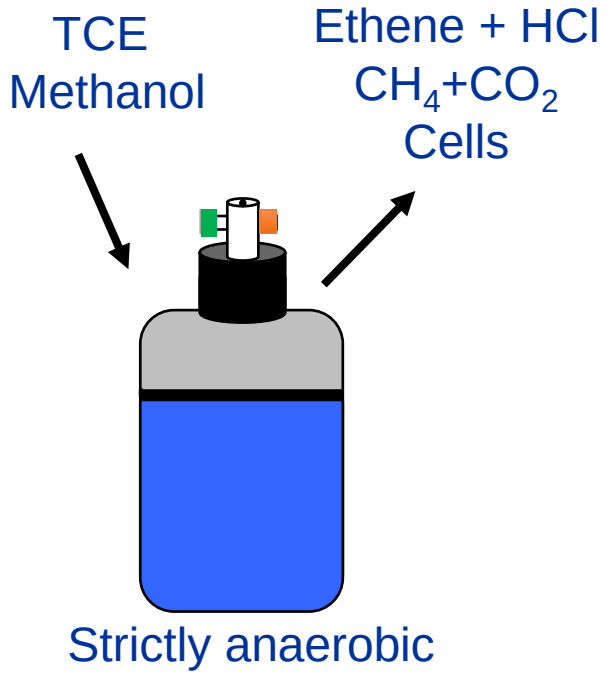
Gossett and Freedman, 1989 - Cornell

Anaerobic Microcosms from Site where lots of ethene was detected (1995)



TCE Dechlorination to Ethene by the "KB-1" Consortium (circa 1998)





1995 – 1999: Enrichment of KB-1

UNIVERSITY OF TORONTO

Sediment/Groundwater Microcosms from Ontario site - Produced lots of Ethene

Sediment Free Culture in defined Anaerobic Media

Growth scale-up to several liters

Dave Major & Evan Cox, Geosyntec

Phil Denis, Sandra Dworatzek, Jeff Roberts and many other fabulous staff

2002: Founding of

SiREM

KB-1®

1995 – 1999: Enrichment of KB-1

UNIVERSITY OF TORONTO

oundwater Microcosms from
Produced lots of Ethene

Sediment Free Culture in
defined Anaerobic Media

Growth scale-up to several liters

2002: Founding of

SiREM

KB-1®

Field Demonstration of Successful Bioaugmentation To Achieve Dechlorination of Tetrachloroethene To Ethene

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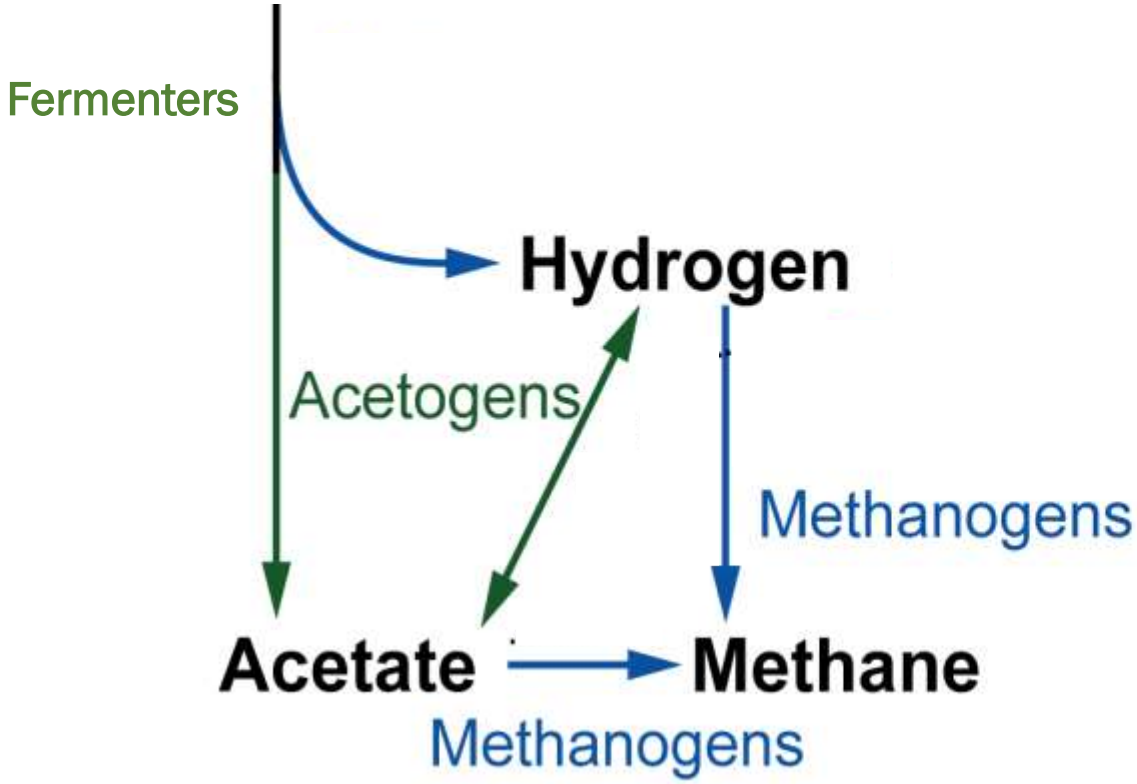
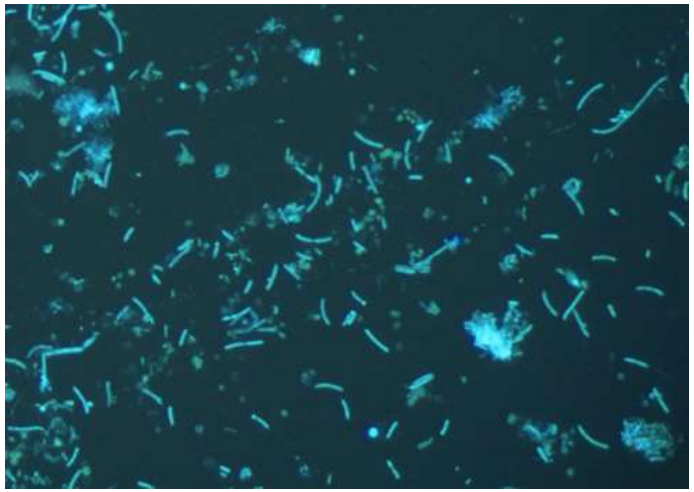
Environ. Sci. Technol. **2002**, *36*, 5106−5116

Dechlorinators occupy a similar niche to methanogens

Fermentable substrate
(ethanol, lactate, methanol)





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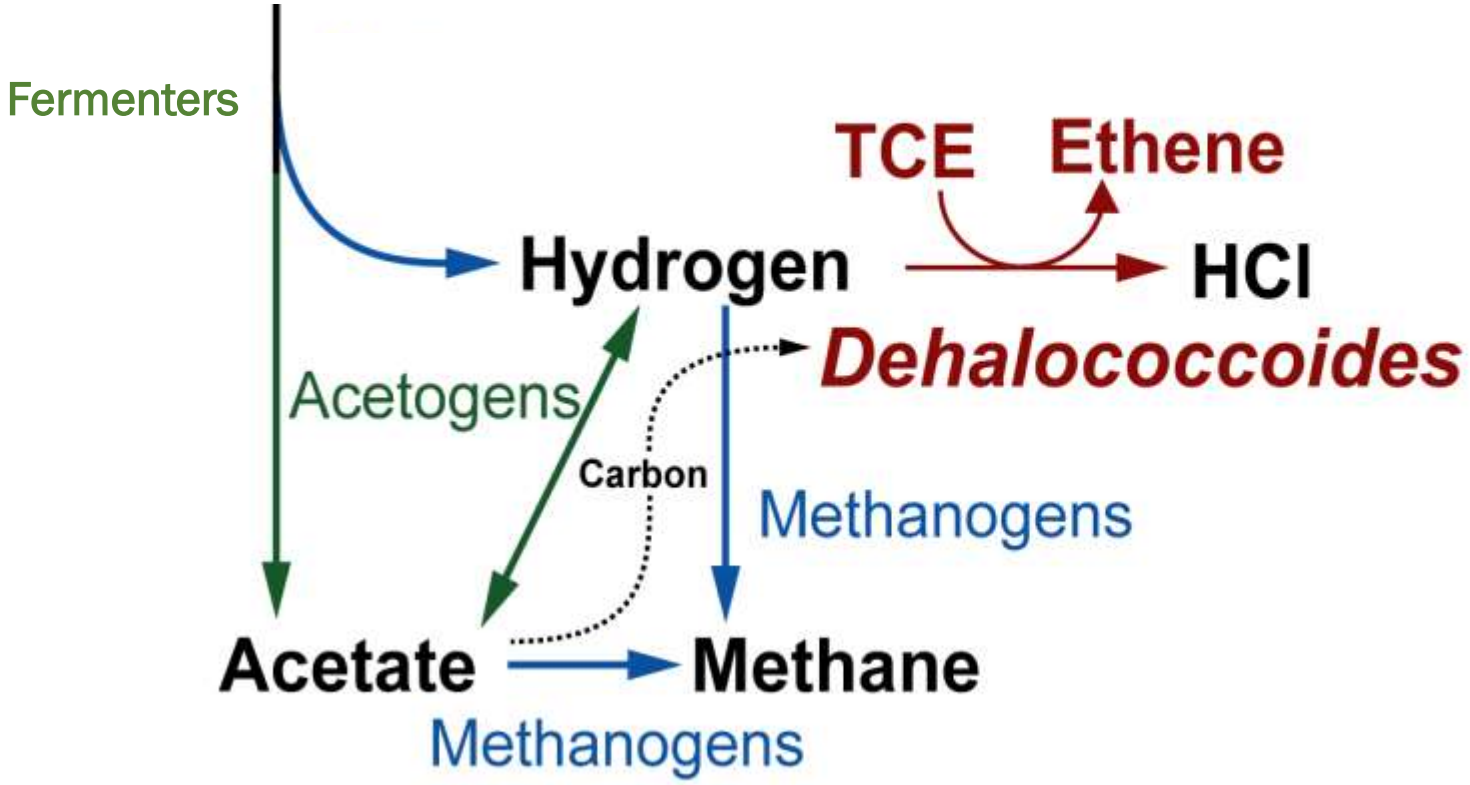
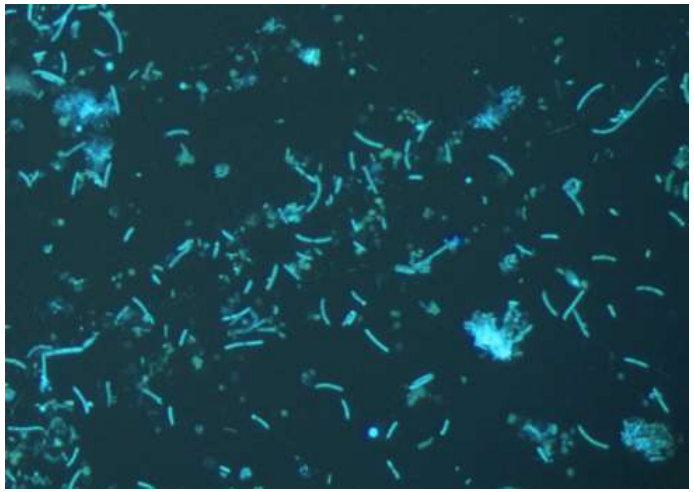
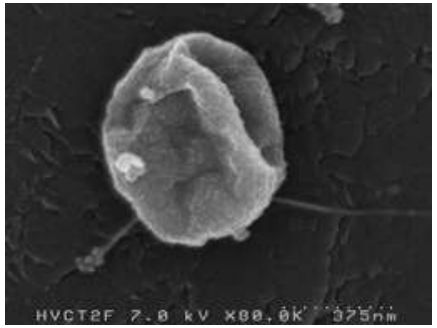
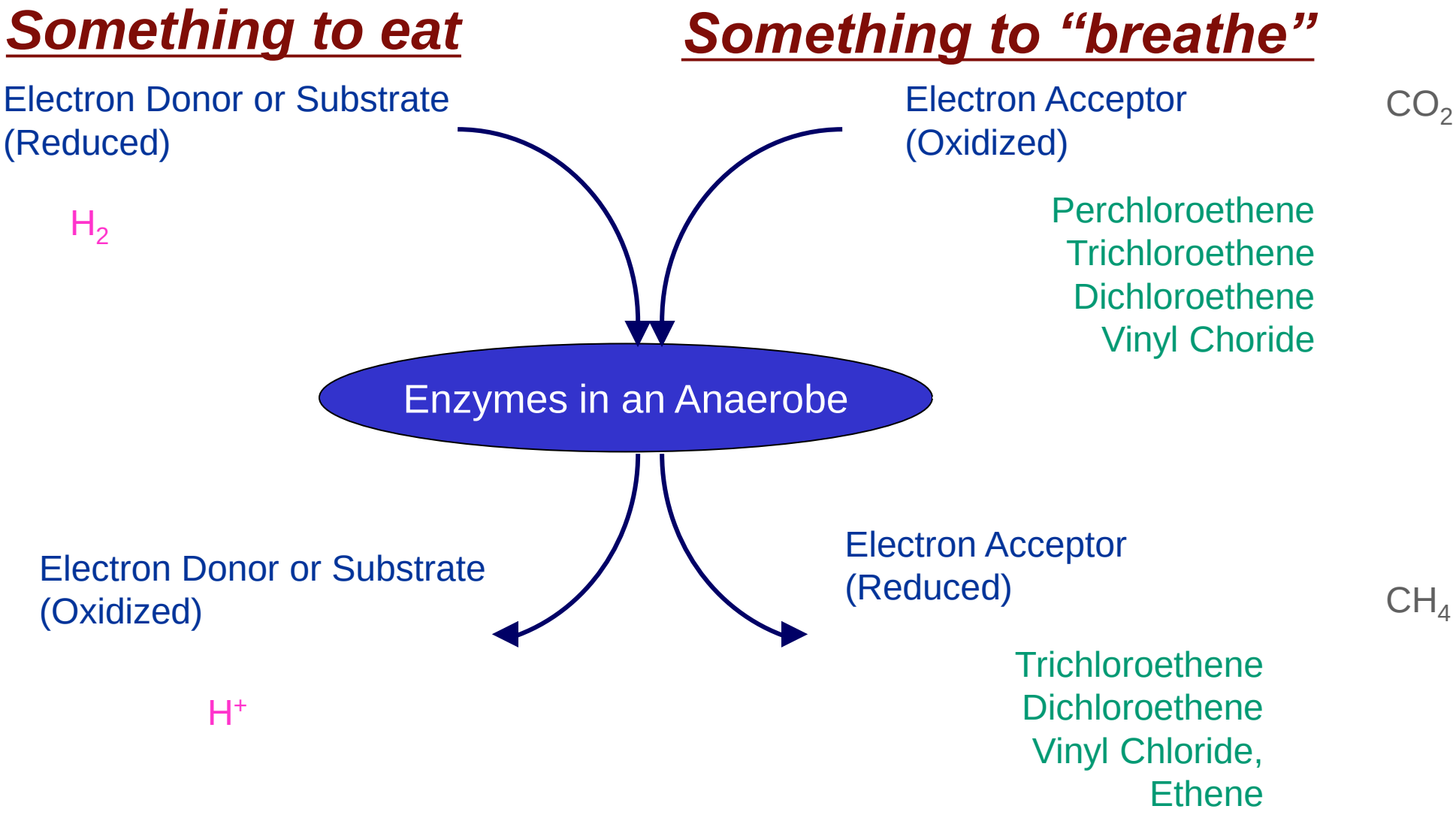






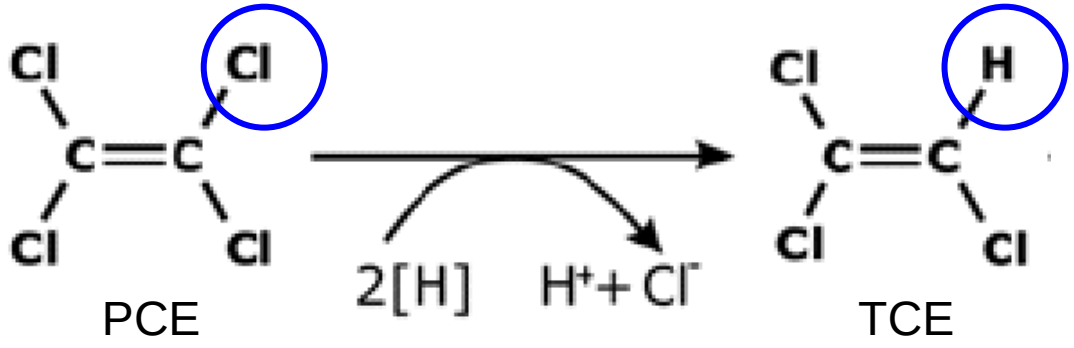
Dehalococcoides KB-1/VC (SEM)

Organohalide Respiration



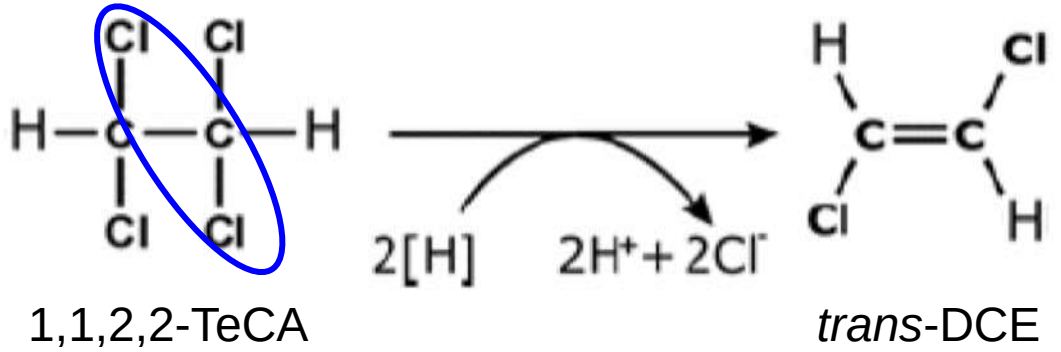
Organohalide-respiring bacteria use these reactions as electron sinks

Hydrogenolysis

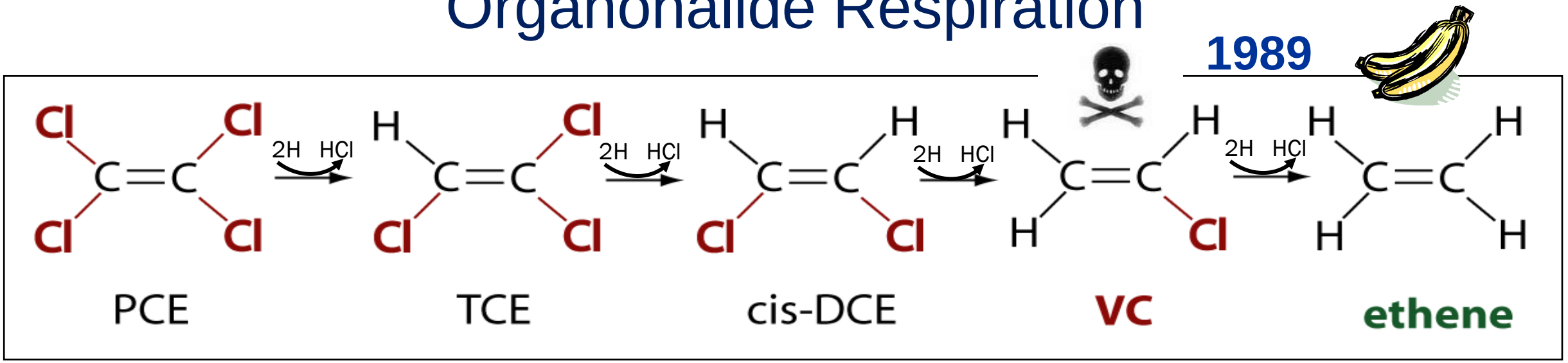


Catalyzed by reductive dehalogenases

Dihaloelimination

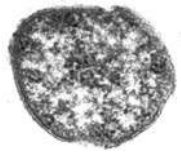


Research on Reductive Dechlorination and Organohalide Respiration



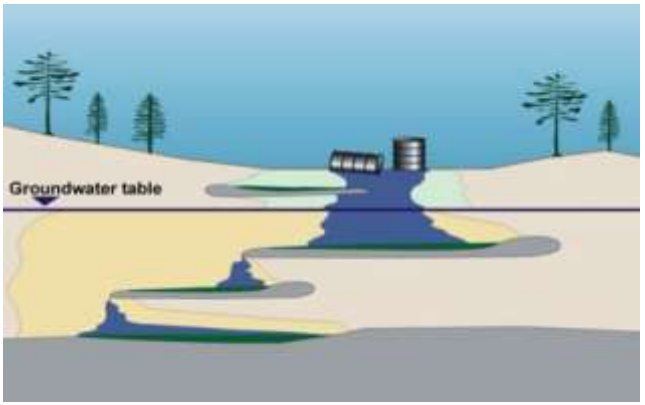
1993

Dehalobacter
Dehalospirillum
Desulfitobacterium
Desulfuromonas
+Dehalococcoides

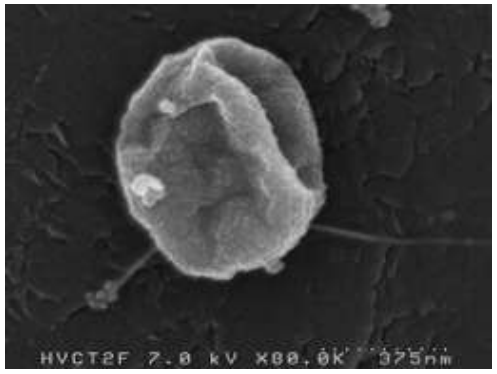


Dehalococcoides **1997**
(Only some strains)
Dehalogenimonas **2015**

Ethene is non-toxic, produced by many fruit to stimulate ripening



What followed since ~2001



- **Practical Application for Bioremediation**
  - How to scale up & grow faster
  - Regulatory approvals
  - How to deliver to subsurface
  - How to tracking organisms
  - Other contaminants
  - Modelling fate and transport
  - How much culture to add?
  - What are inhibitors?

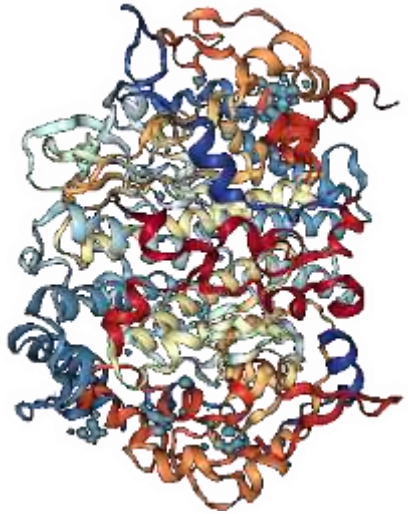
- **Fundamental Science**
  - Organisms that dechlorinate?
  - Microbial ecology, physiology, evolution
  - Growth kinetics
  - Enzymes involved
  - Mechanisms and substrates
  - Microbial community interactions
  - Omics….

Fundamental Science

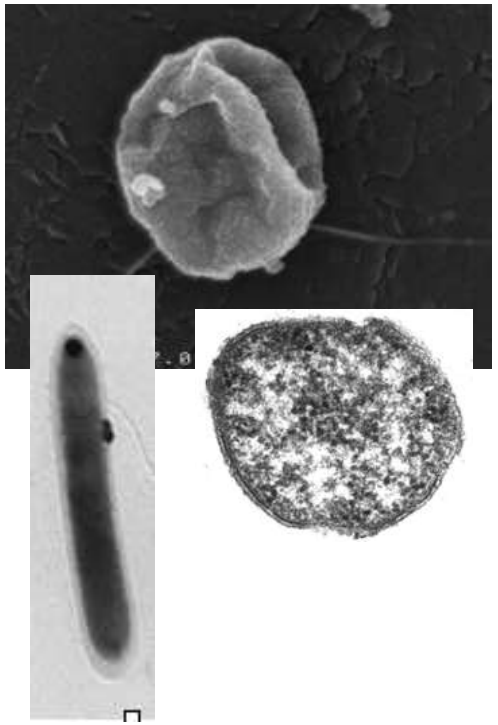
KEY ORGANISMS

(*Dehalococcoides*, *Dehalobacter*, *Dehalogenimonas*)

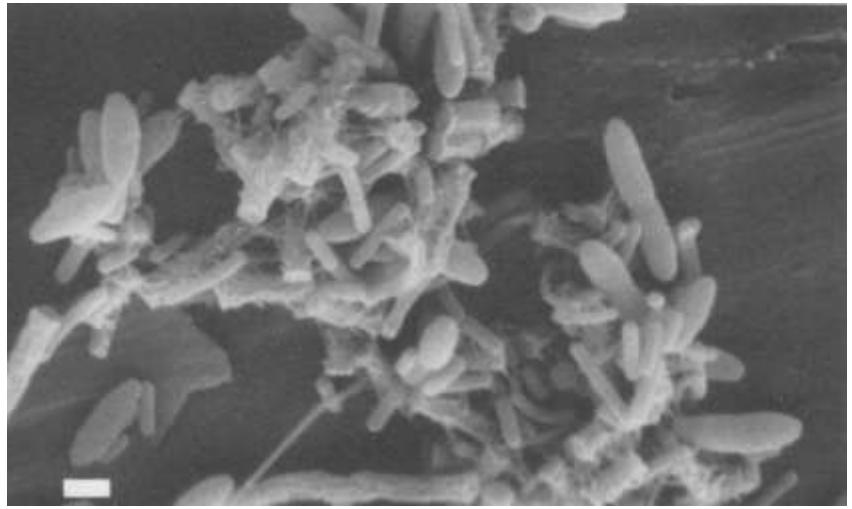
ENZYMES



Bommer, M., *et al*. Structural Basis for Organohalide Respiration. *Science.* **2014**, *346:*455–458.



MICROBIAL COMMUNITY



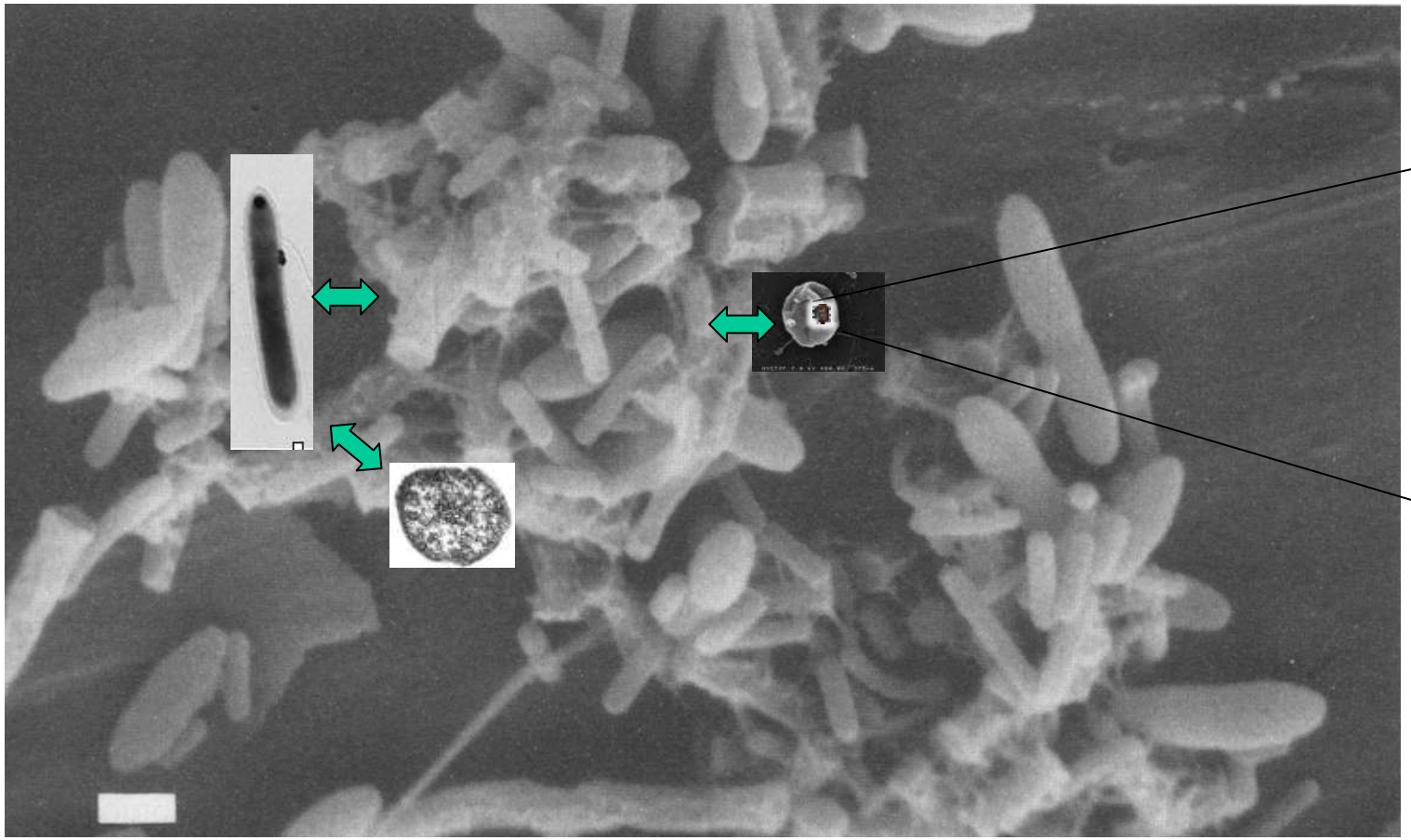
Fundamental Science

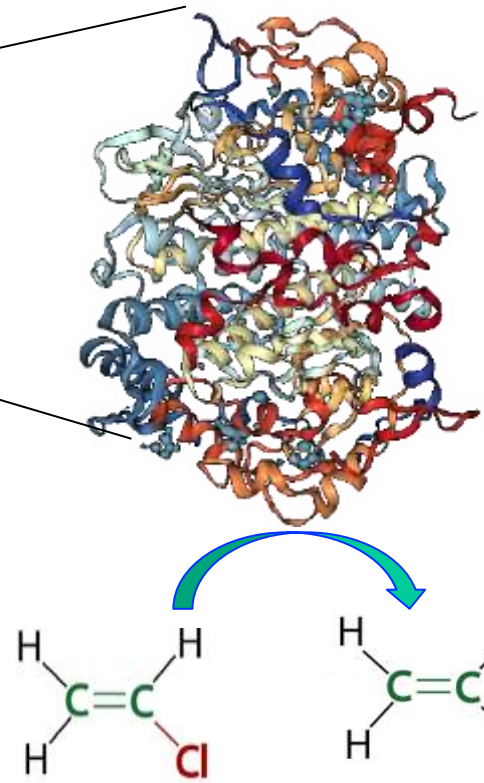
MICROBIAL COMMUNITY

KEY ORGANISMS

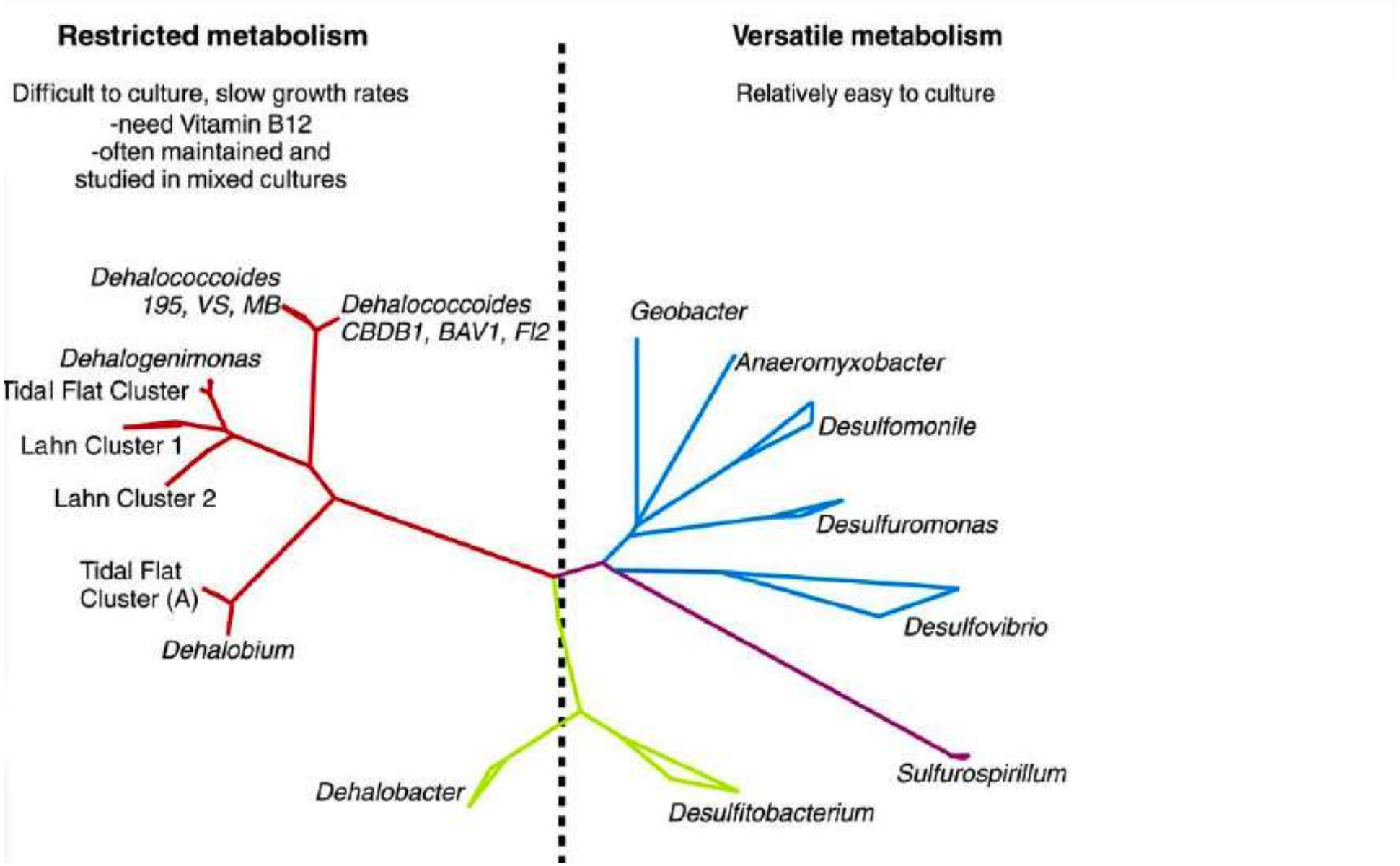
(specific dechlorinating bacteria)

ENZYMES (a protein)





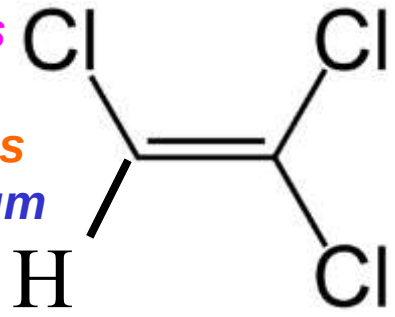
Two types of organohalide-respiring bacteria



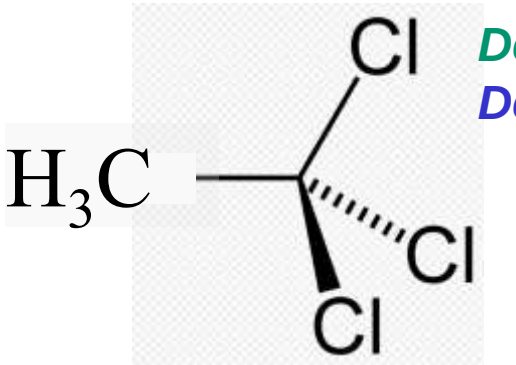
Maphosa, de Vos and Smidt, Trends in Biotechnology Vol.28 No.6, 2010

Some Good Substrates for Dechlorinators

Dehalococcoides
Dehalobacter
Dehalogenimonas
Desulfitobacterium
Sulfurospirillum
Geobacter

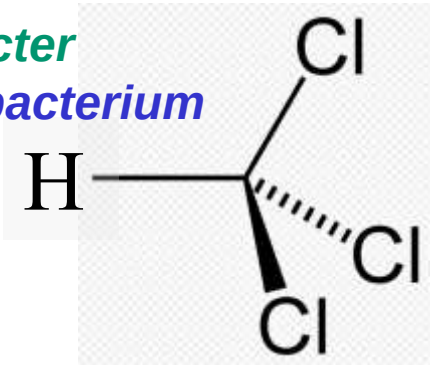


Trichloroethene (TCE)



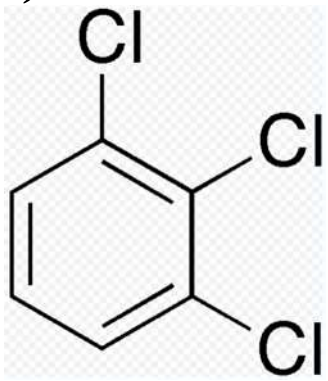
1,1,1-Trichloroethane (1,1,1-TCA)

Dehalobacter
Desulfitobacterium



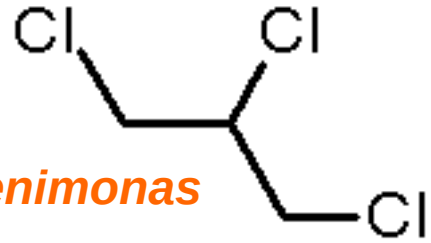
Chloroform (CF)

Dehalococcoides
Dehalobacter
Dehalogenimonas



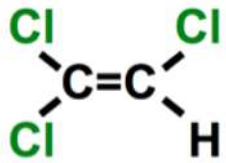
Trichlorobenzene

Dehalogenimonas

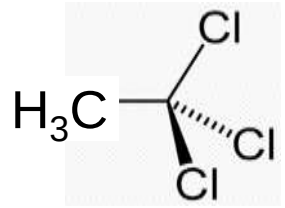


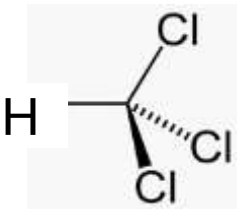
Trichloropropane

Two cultures:
No overlap in substrates

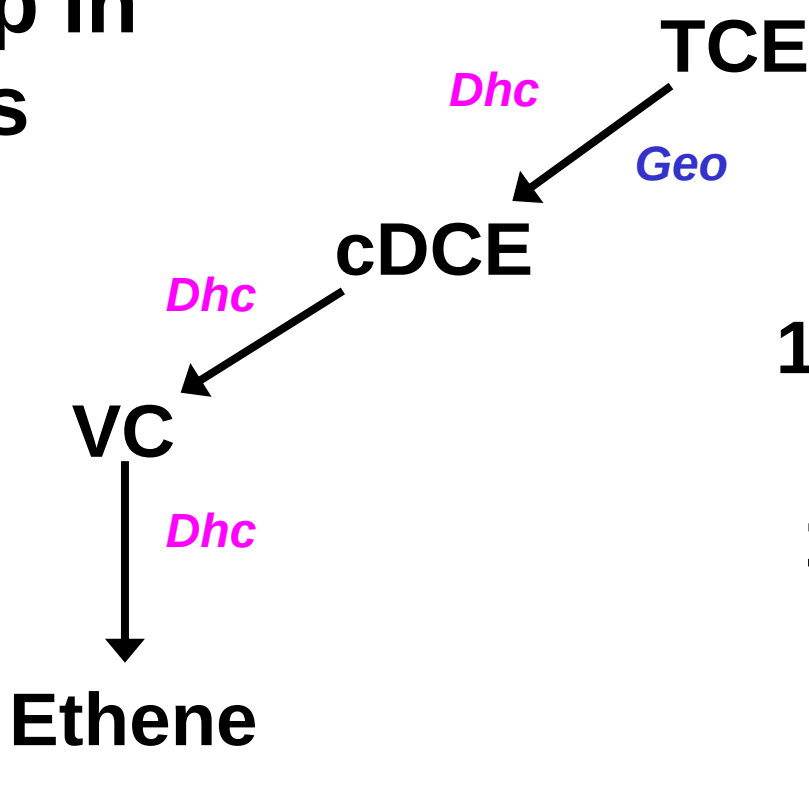


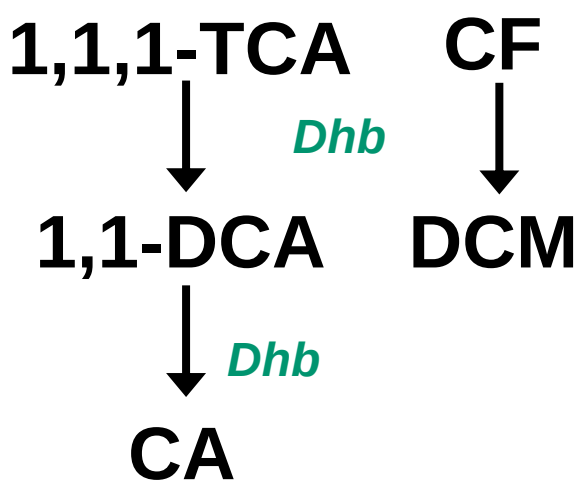
KB-1 Culture





ACT-3 Culture

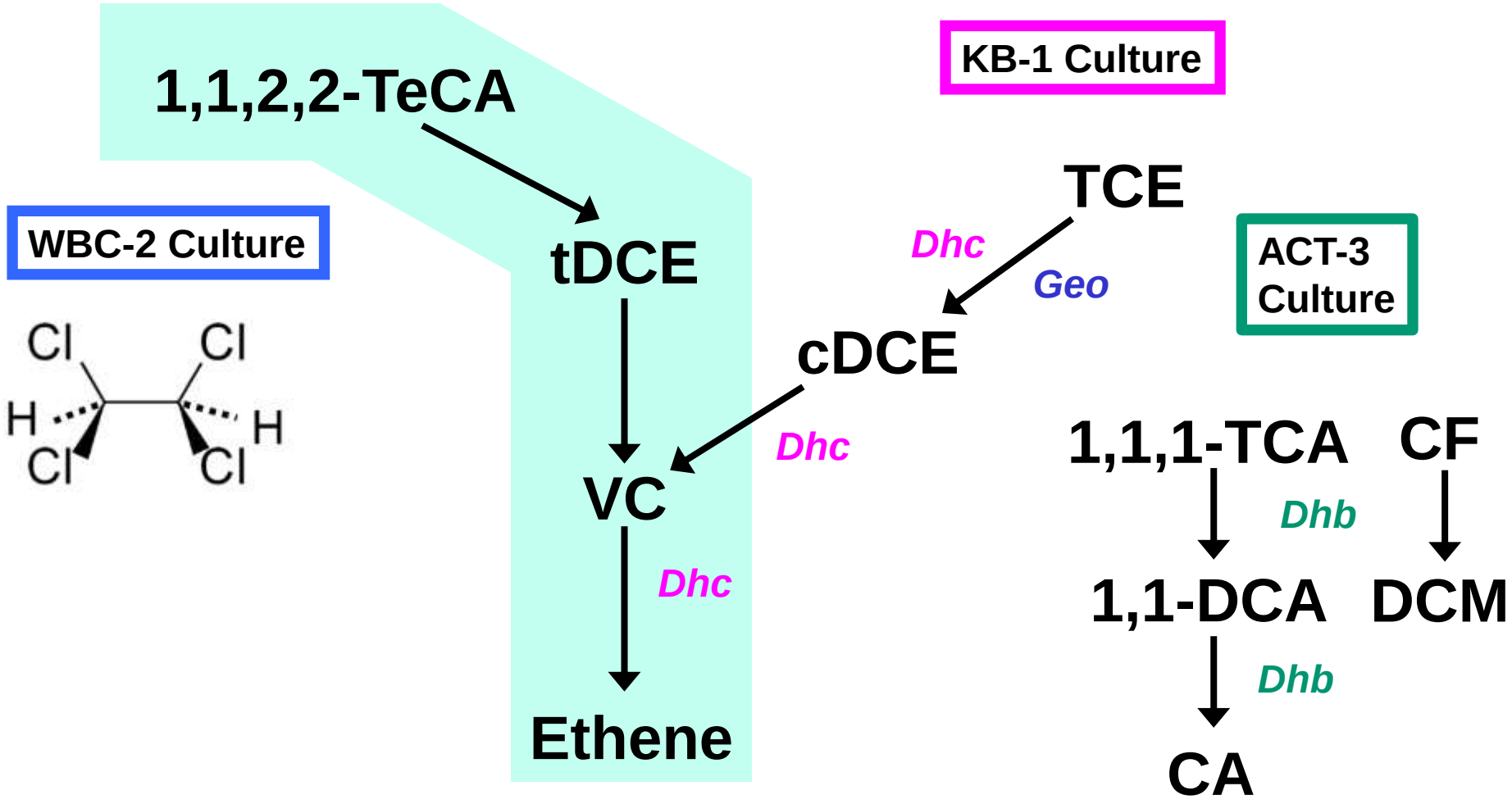




Dhc = Dehalococcoides
Dhb = Dehalobacter

Geo = Geobacter

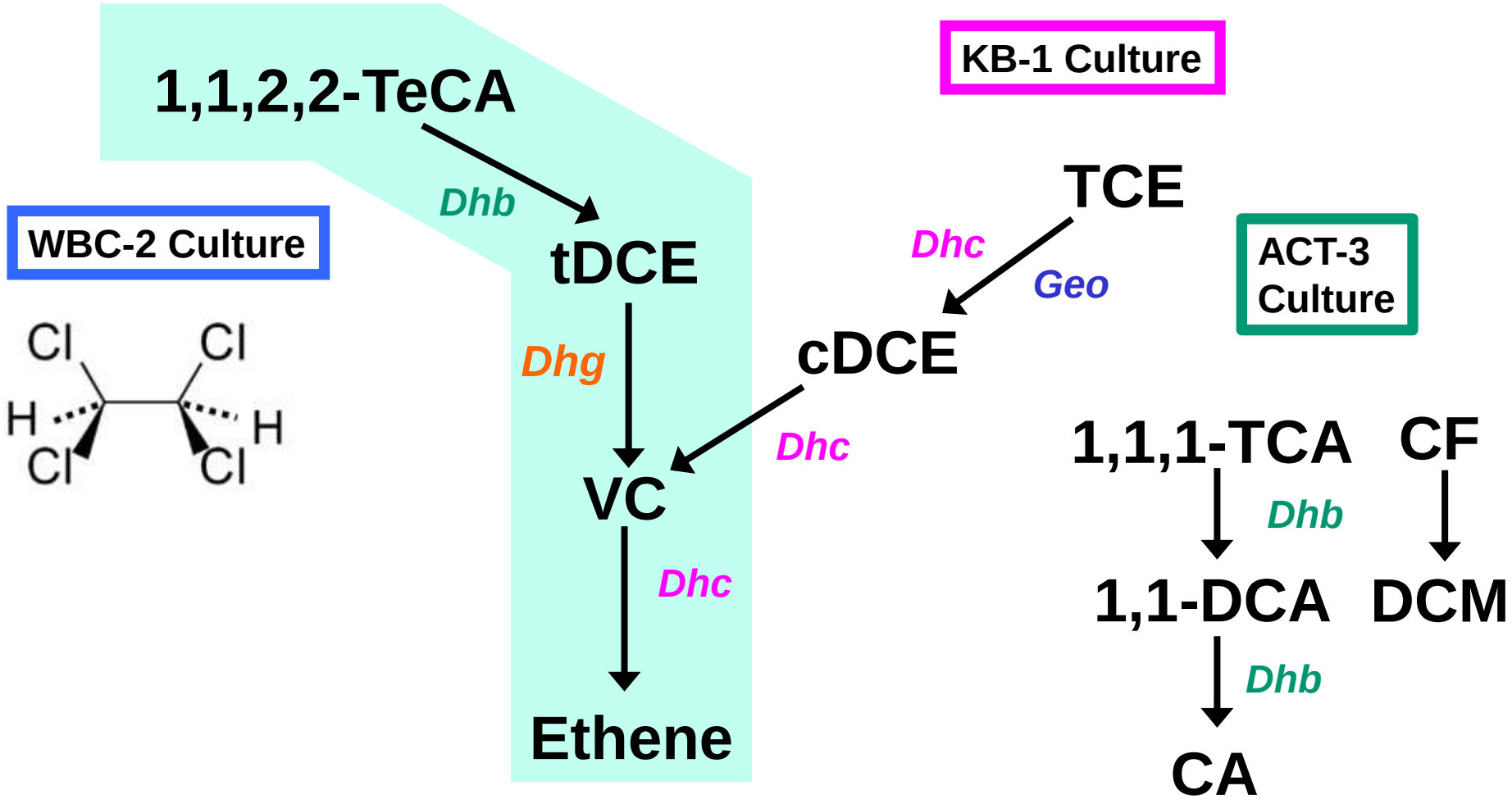
WBC-2 and KB-1 Pathway Convergence



Dhc = Dehalococcoides
Dhb = Dehalobacter

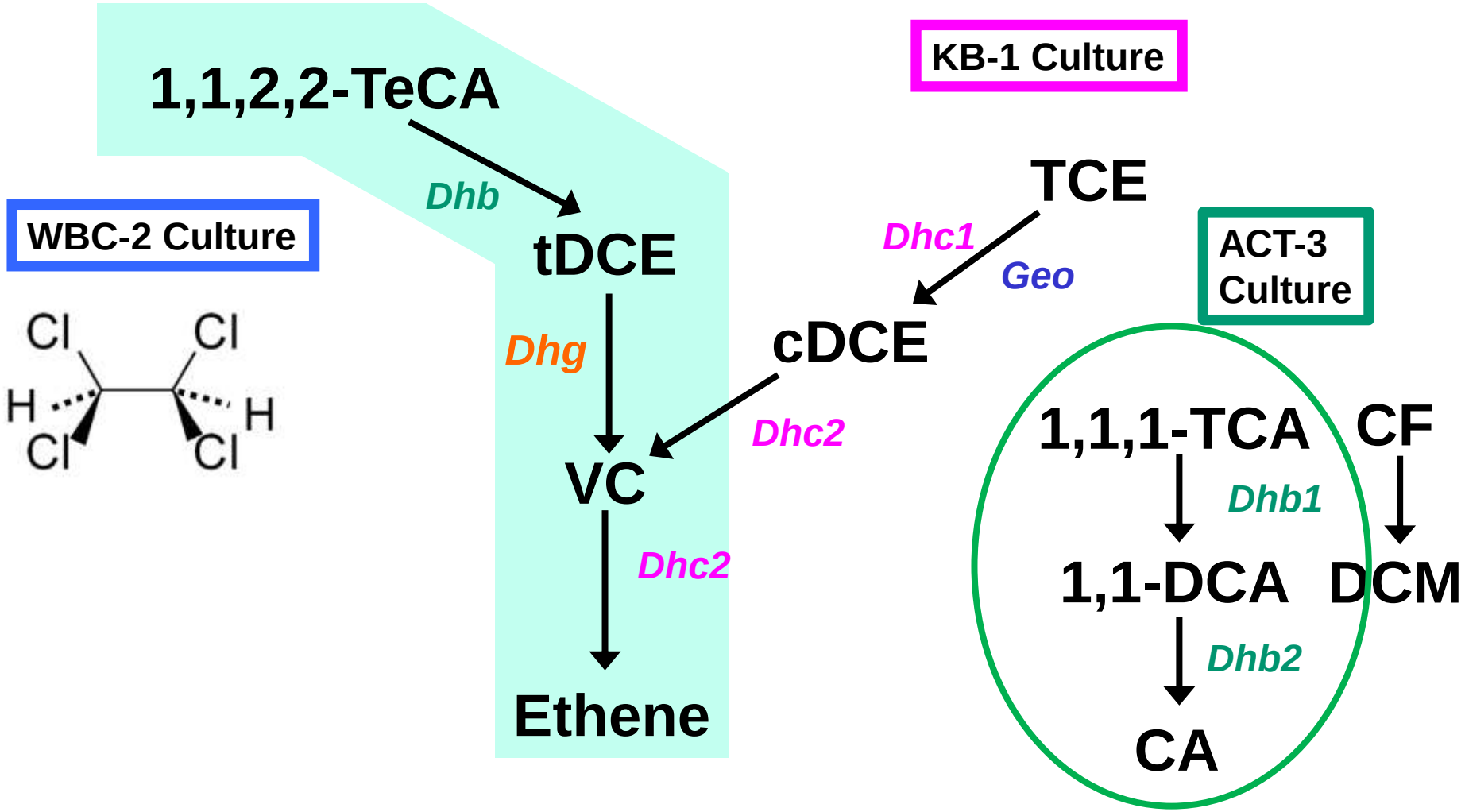
Geo = Geobacter

Dehalogenimonas dechlorinates tDCE



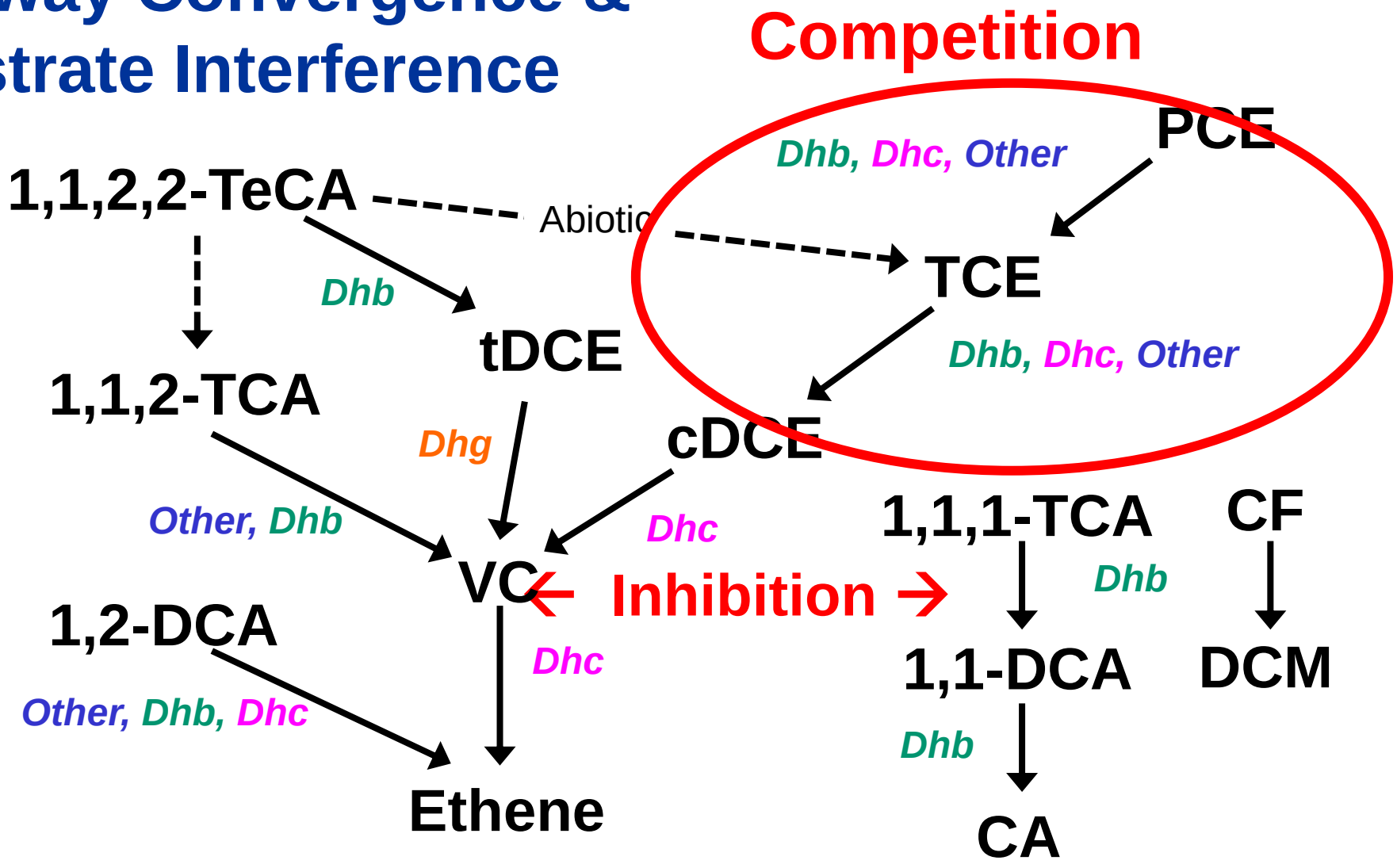
Dhc = Dehalococcoides *Dhb = Dehalobacter* *Dhg = Dehalogenimonas*
Geo = Geobacter

Dehalogenimonas dechlorinates tDCE



Dhc = Dehalococcoides *Dhb = Dehalobacter* *Dhg = Dehalogenimonas*
Geo = Geobacter

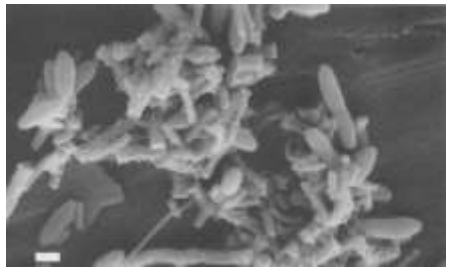
Pathway Convergence & Substrate Interference



Dhc = Dehalococcoides
Dhb = Dehalobacter
Dhg = Dehalogenimonas
Other = Desulfitobacterium, Sulfurospirillum, Geobacter

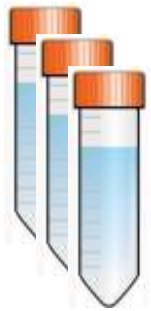
Enzyme Assays to explore impact of inhibition

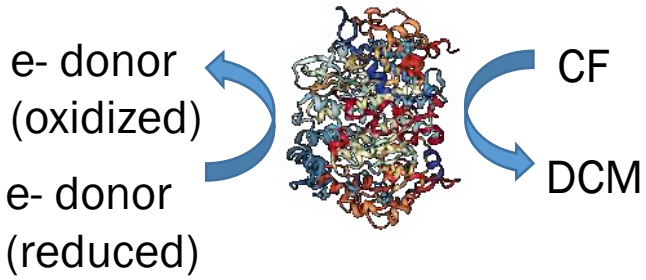
CF $\xrightarrow{Dhb1}$ DCM



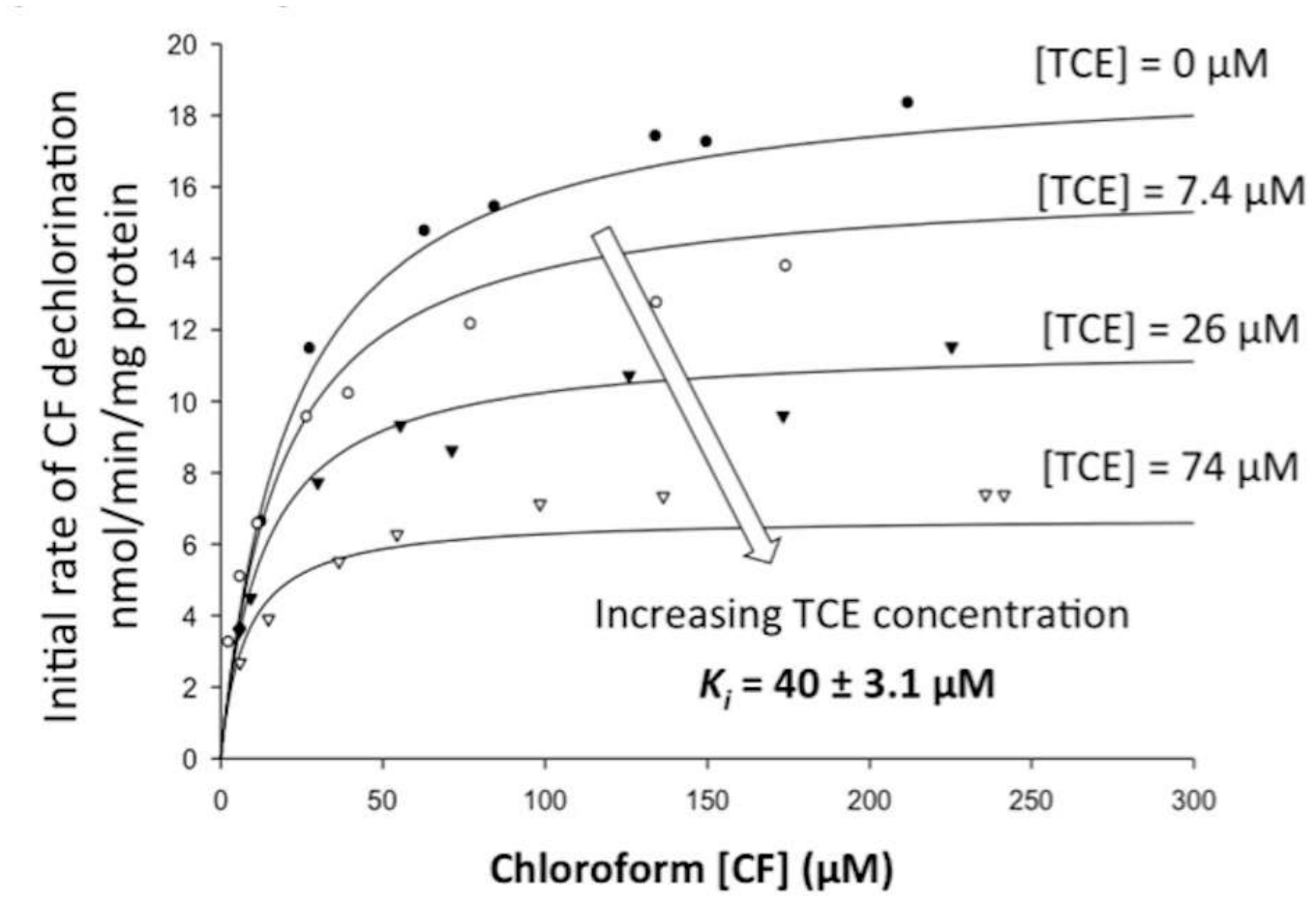
Lyse all cells to release all proteins (including dehalogenases)

Assay enzymes directly
Adding CF+ artificial electron donor in reducing buffer





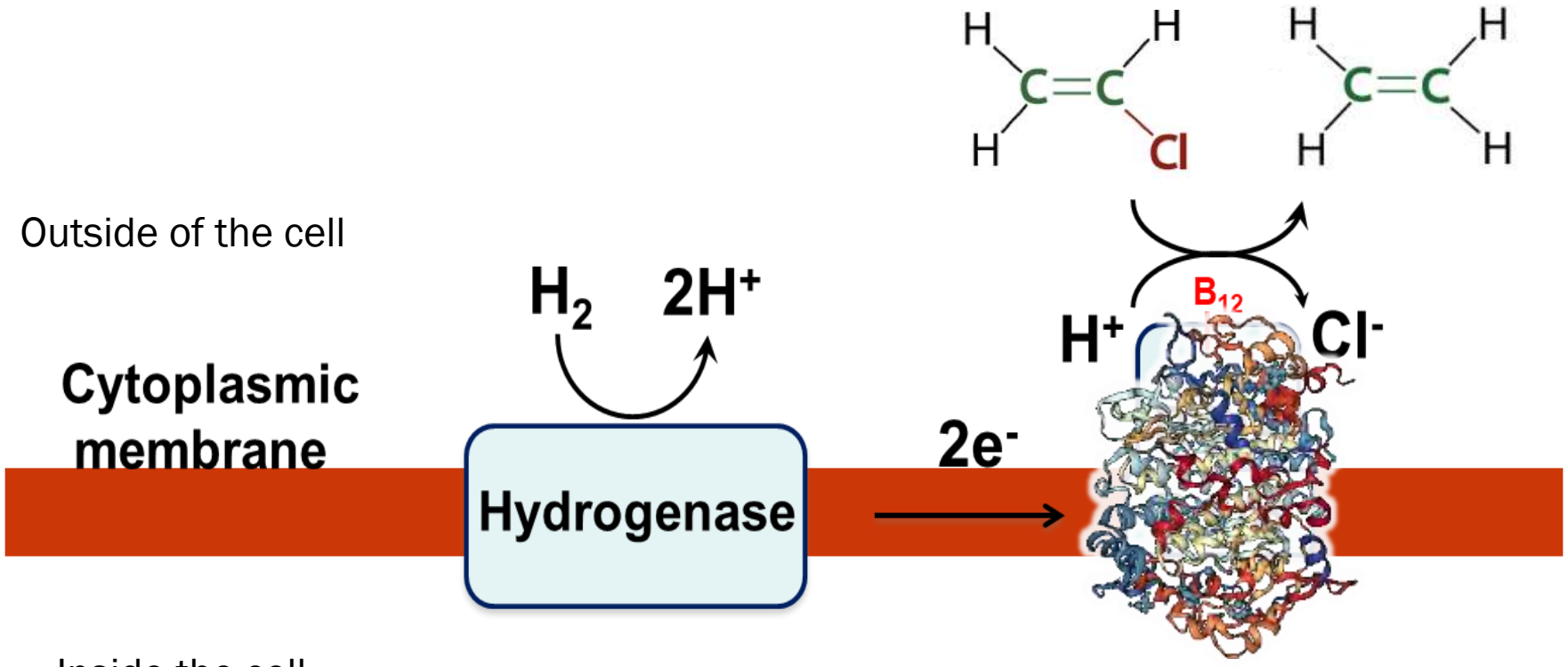
Data from "Cell-Free" extracts of a *Dehalobacter* mixed culture



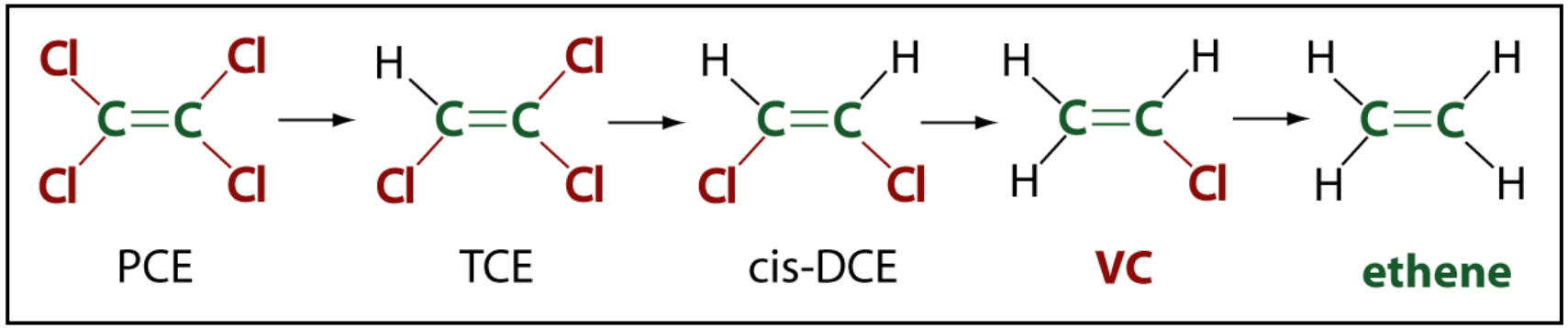
Genomes of Organohalide-Respiring Bacteria (OHRB) (Sequence DNA)

	Organohalide-respiring Bacteria	Mbp	*rdhA* genes
	Versatile metabolism (facultative OHRB):		
Halogenated and non halogenated electron acceptors	*Desulfitobacterium hafniense Y51*	5.73	4
	Desulfitobacterium hafniense DCB-2	5.28	7
	Anaeromyxobacter dehalogenans 2CP-C	5.01	2
	Geobacter lovleyi SZ	3.87	2
	Restricted metabolism (Obligate OHRB):		
Only halogenated electron acceptors	*Dehalobacter restrictus*	2.94	25
	Dehalobacter strain CF	2.91	20
	Dehalogenimonas lykanthroporepellens	1.66	19
	Dehalococcoides mccartyi strain 195	1.47	17
	Dehalococcoides mccartyi strain VS	1.41	36
	Dehalococcoides mccartyi strain CBDB1	1.4	32
	Dehalococcoides mccartyi strain KBVC1	1.39	22

Organohalide respiration and reductive dehalogenases (RDases) with cobamide (B_{12}) as the cofactor



Enzyme Characterization



pceA

Sulfurospirillum, Desulfitobacterium, Dehalobacter, Clostridium, Geobacter

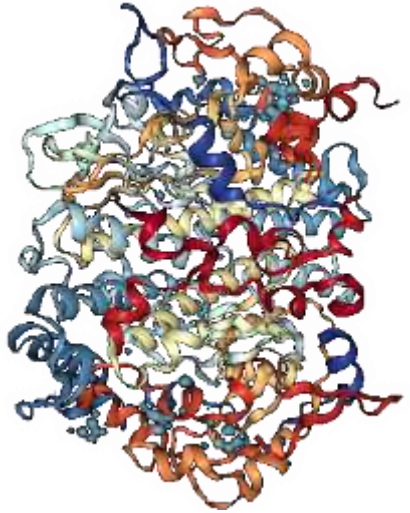
Multiple ***Dehalococcoides*** *strains*

tceA

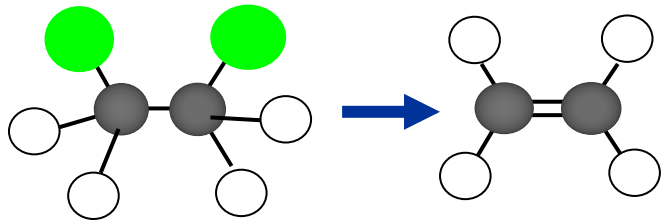
vcrA

Certain ***Dehalococcoides***

bvcA

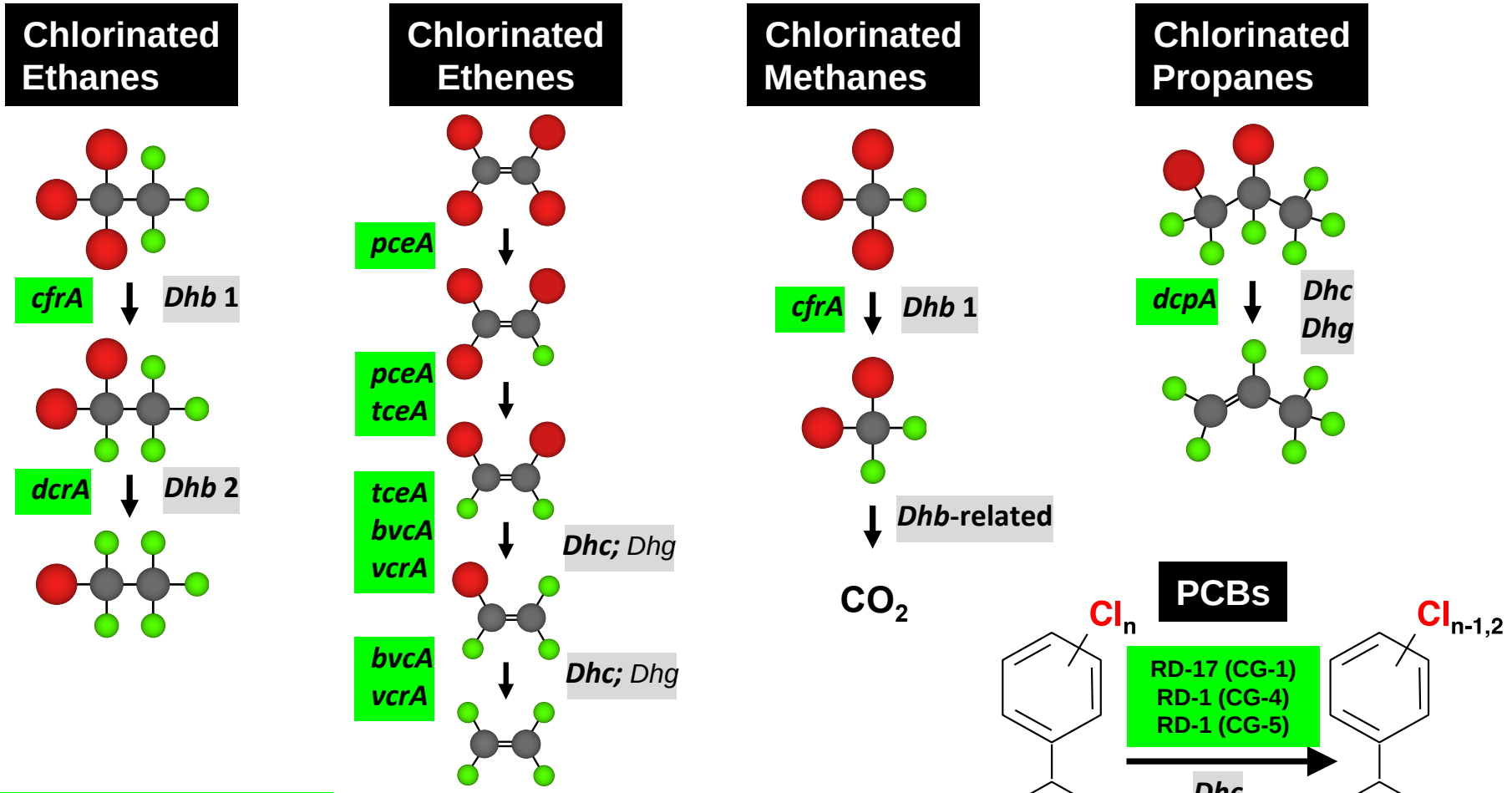


1,2-Dichloroethane



Process- and Organism-Specific Biomarkers

Quantitative PCR based tests to detect DNA in Environmental Samples



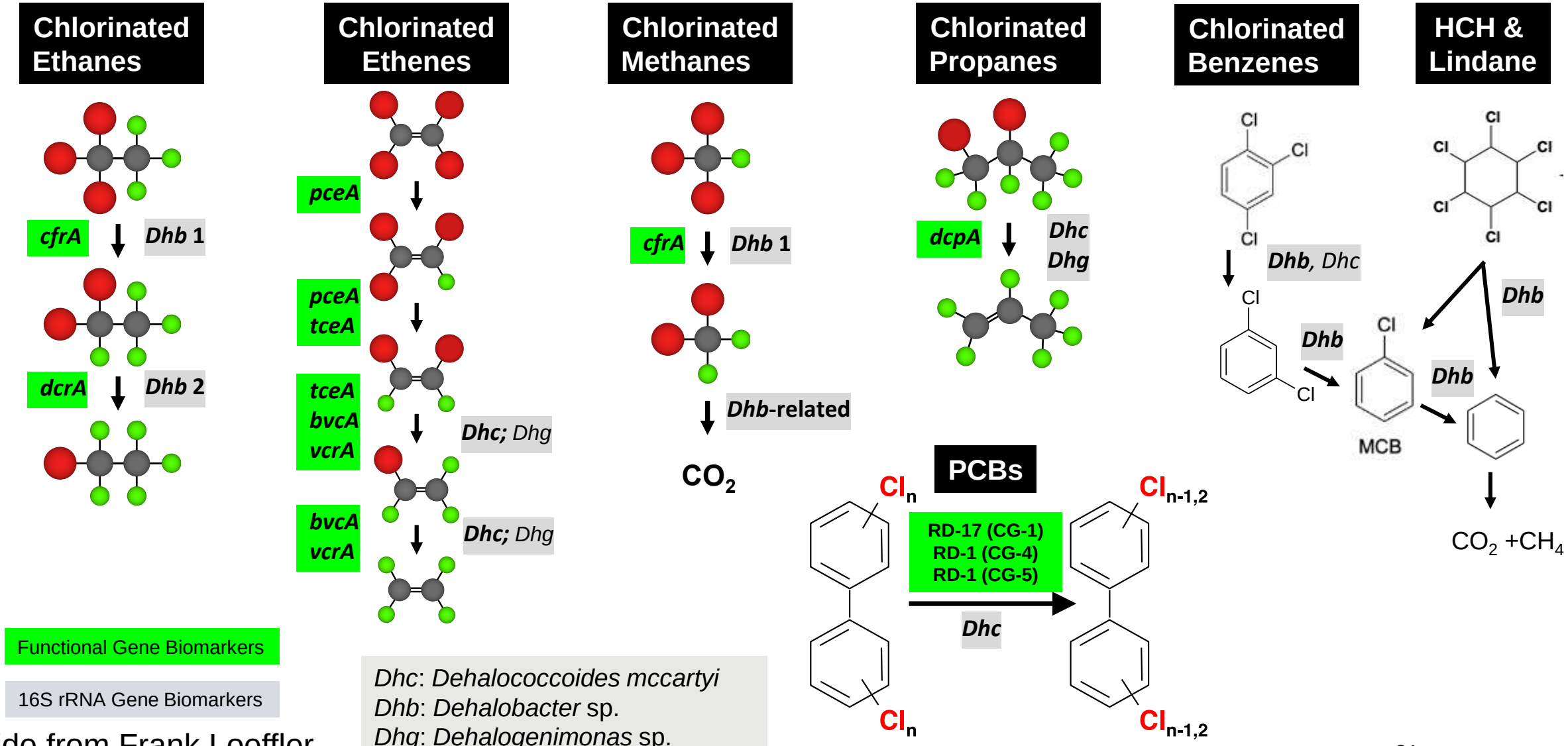
Functional Gene Biomarkers

16S rRNA Gene Biomarkers

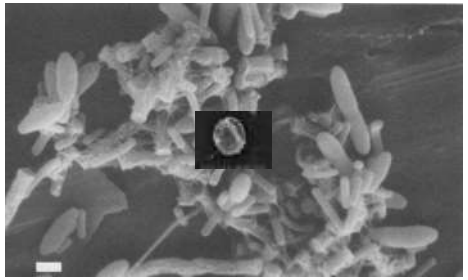
Dhc: *Dehalococcoides mccartyi*
Dhb: *Dehalobacter* sp.
Dhg: *Dehalogenimonas* sp.

Slide from Frank Loeffler

Process- and Organism-Specific Biomarkers

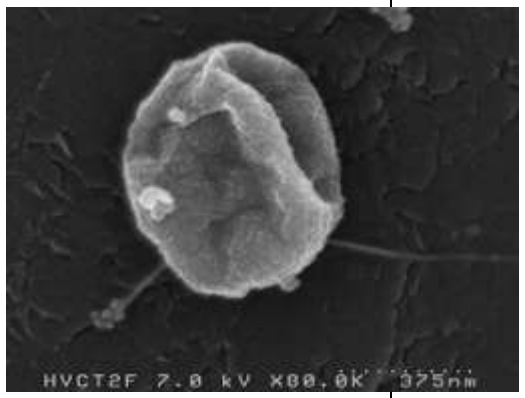


Slide from Frank Loeffler



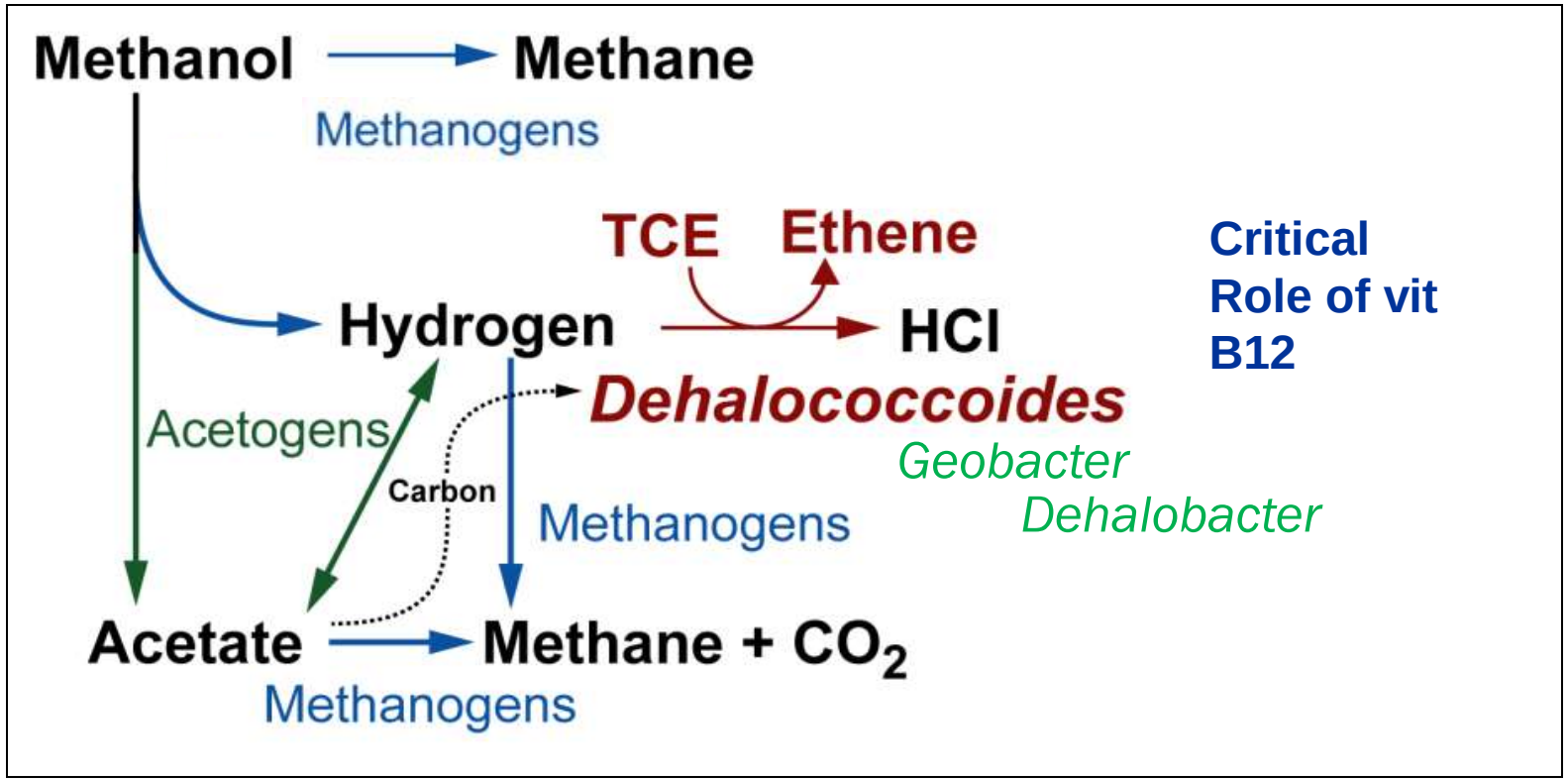
Role of the Community: *Dehalococcoides* isolates grow slowly

KB-1 Consortium



Community Diversity

KB-1 Consortium



Critical Role of vit B12

Dhb: Encode genes to make B12

Dhc: Encode genes to salvage B12

Methanogens and acetogens provide essential nutrients and enhance dechlorination

Case Study: Field site in Toronto

Objective:

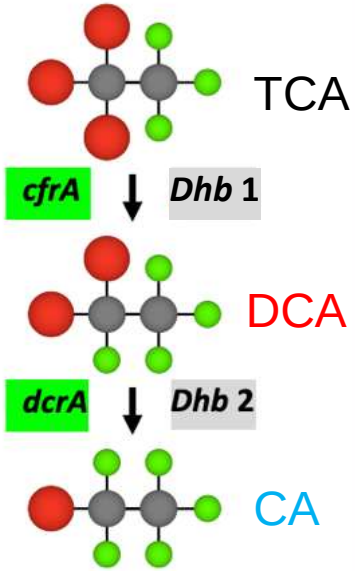
- Assess the impact of ZVI on indigenous dechlorinating microbial communities

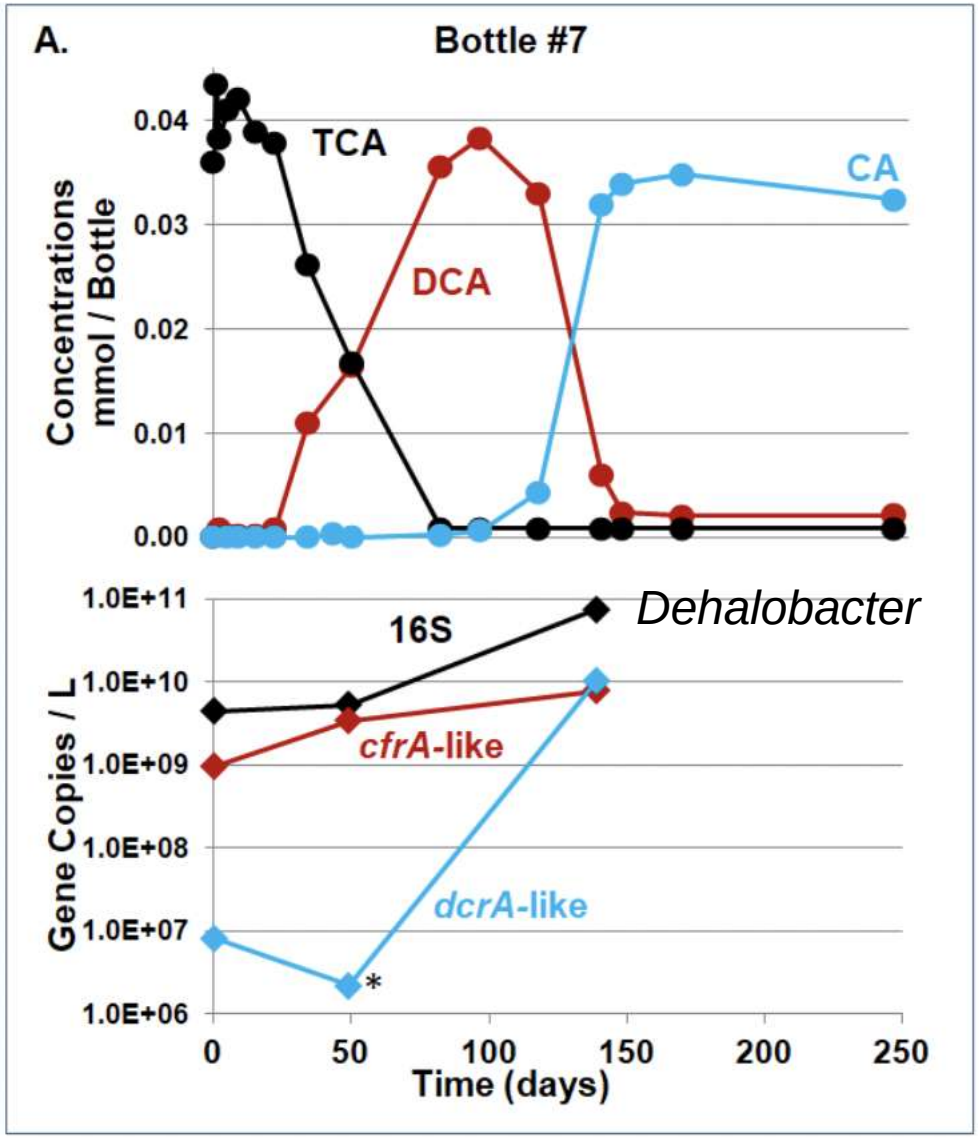
Approach:

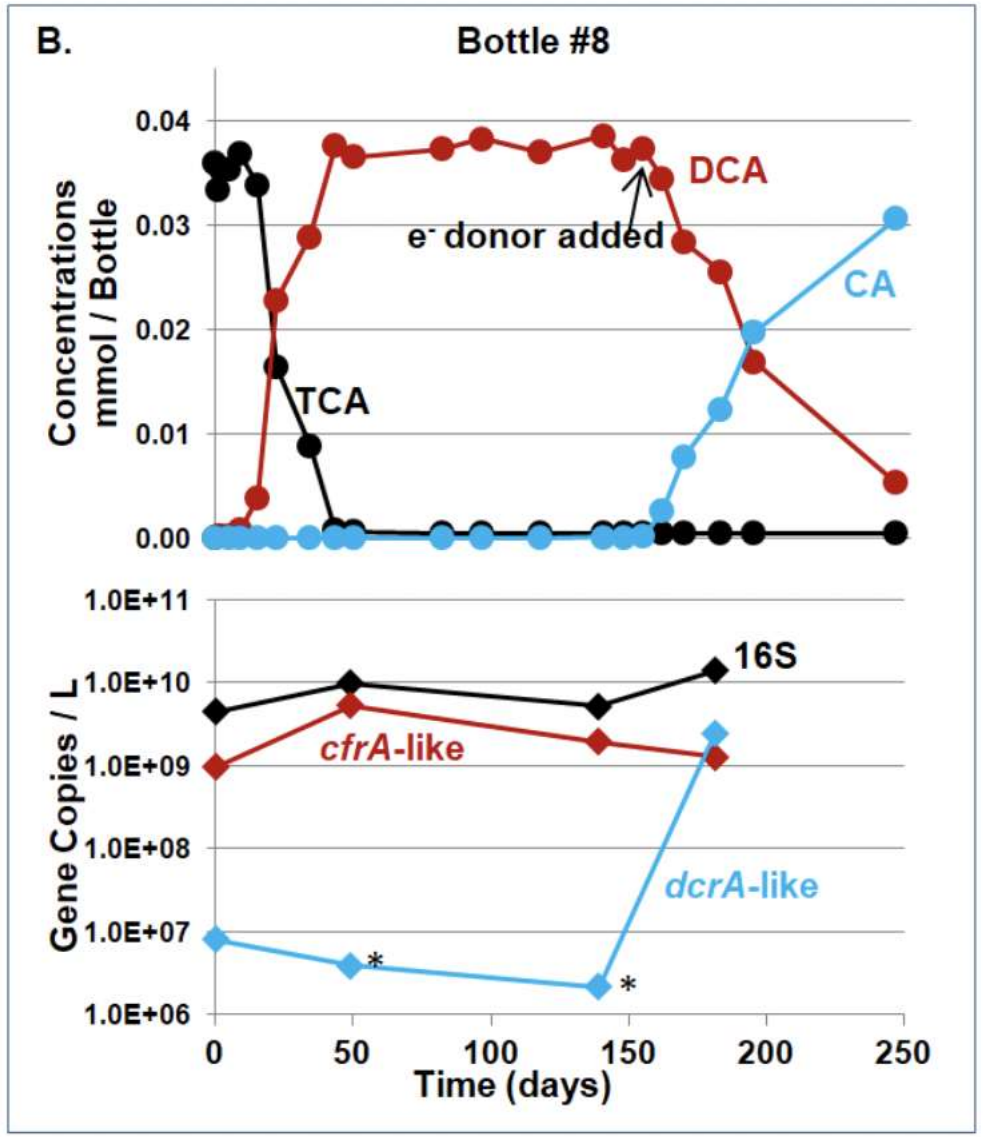
- Carried out a microcosm study with Site groundwater (+/- ZVI+Guar Gum)
- Sampled and analyzed groundwater from the field site during remediation

Groundwater Microcosm Data

Chlorinated Ethanes

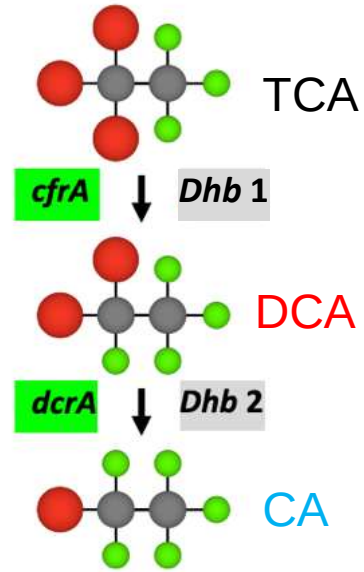


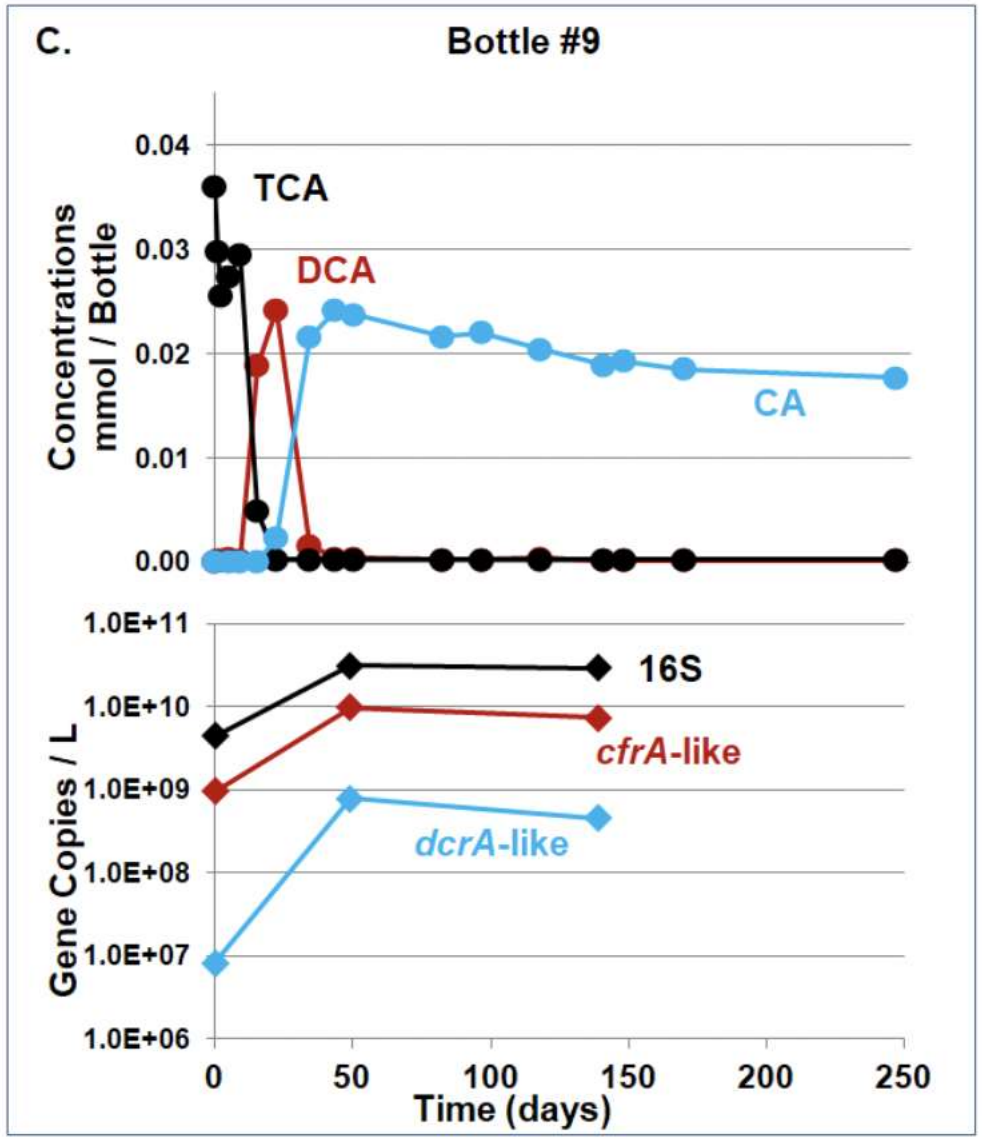


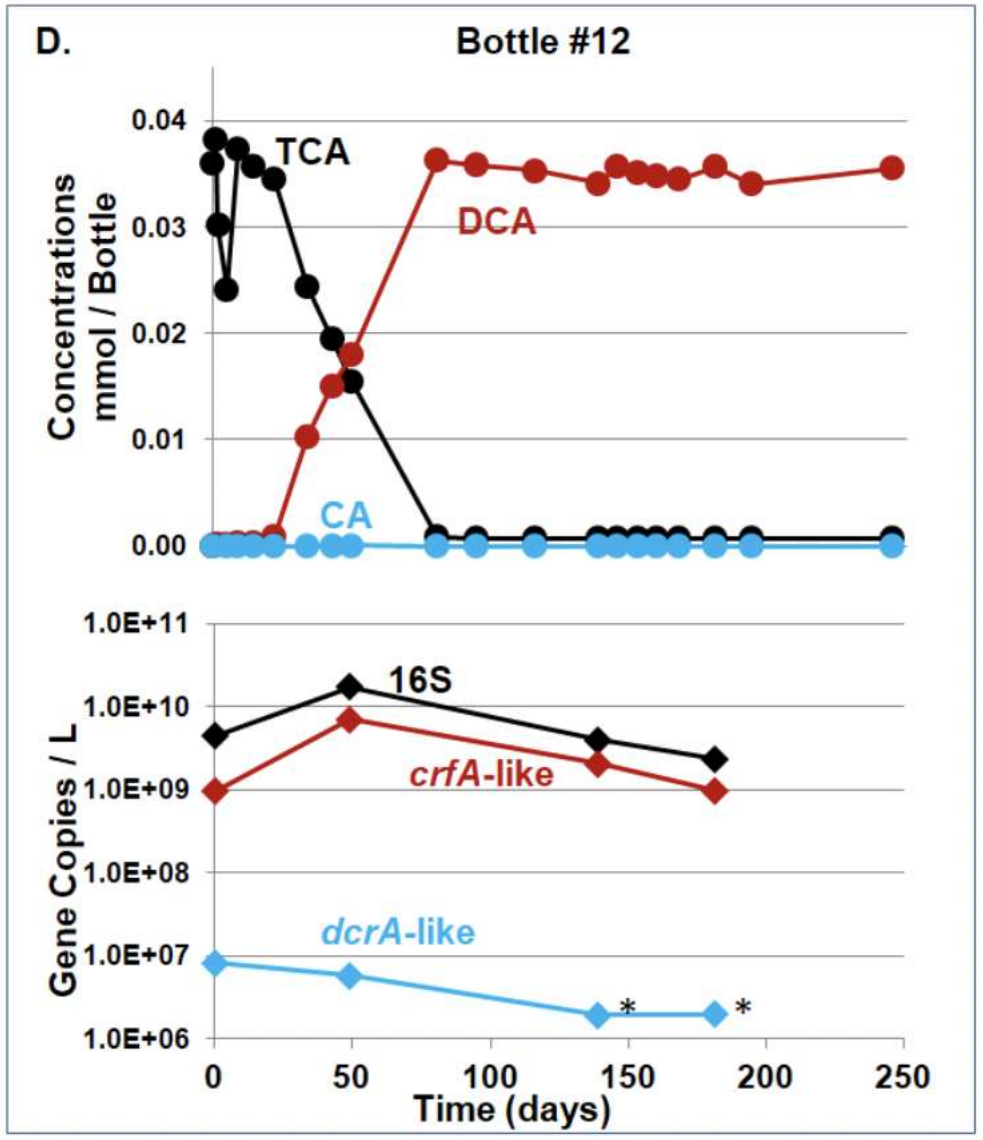


Groundwater Microcosm Data

Chlorinated Ethanes

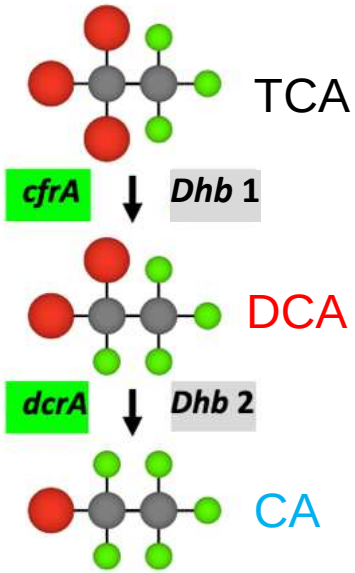


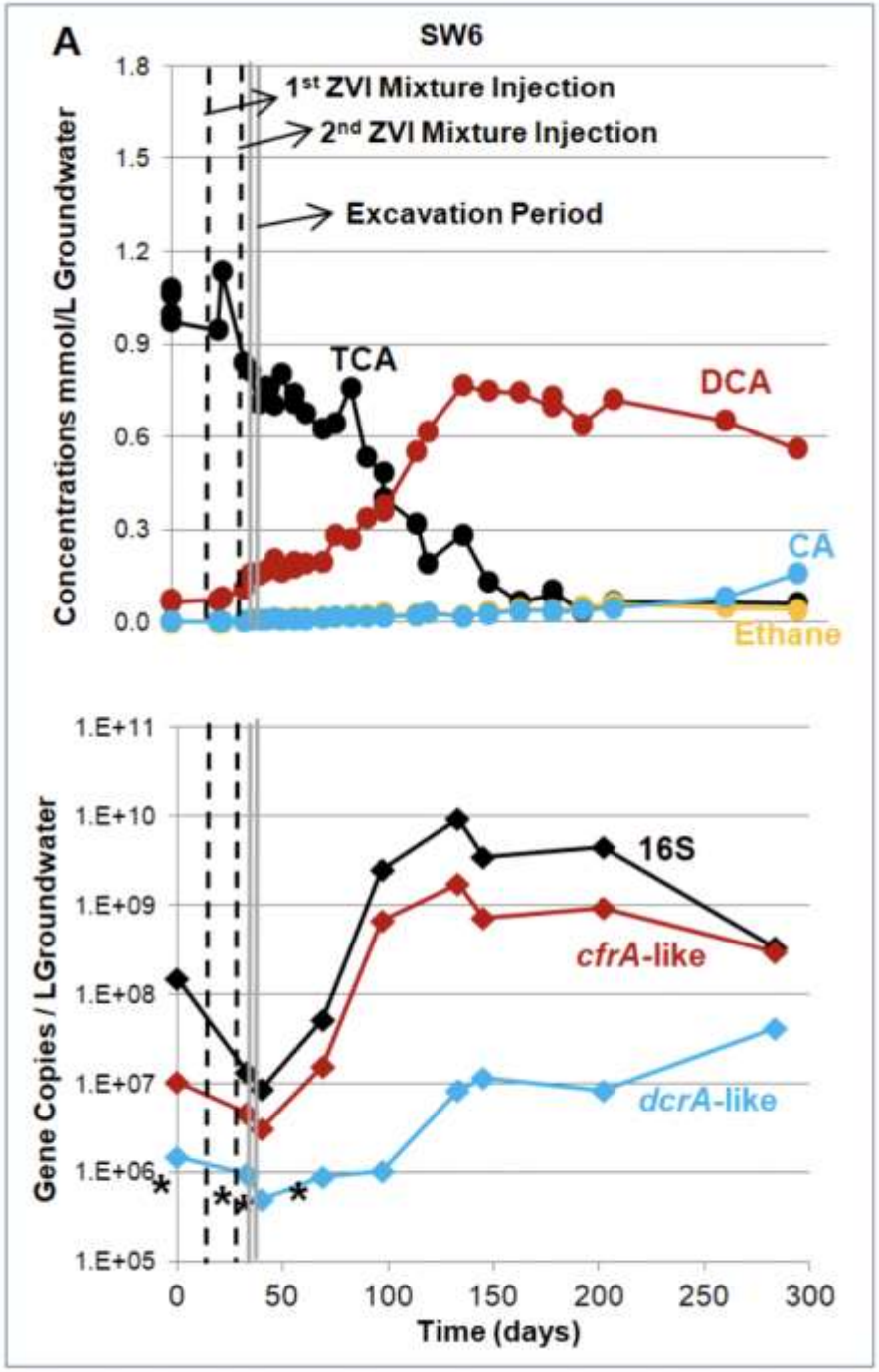




Results from the Field

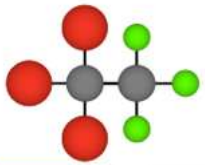
Chlorinated Ethanes





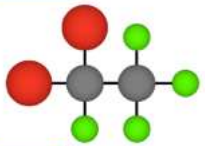
Results from the Field

Chlorinated Ethanes



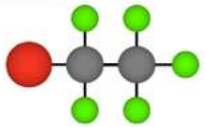
TCA

cfrA → *Dhb* 1

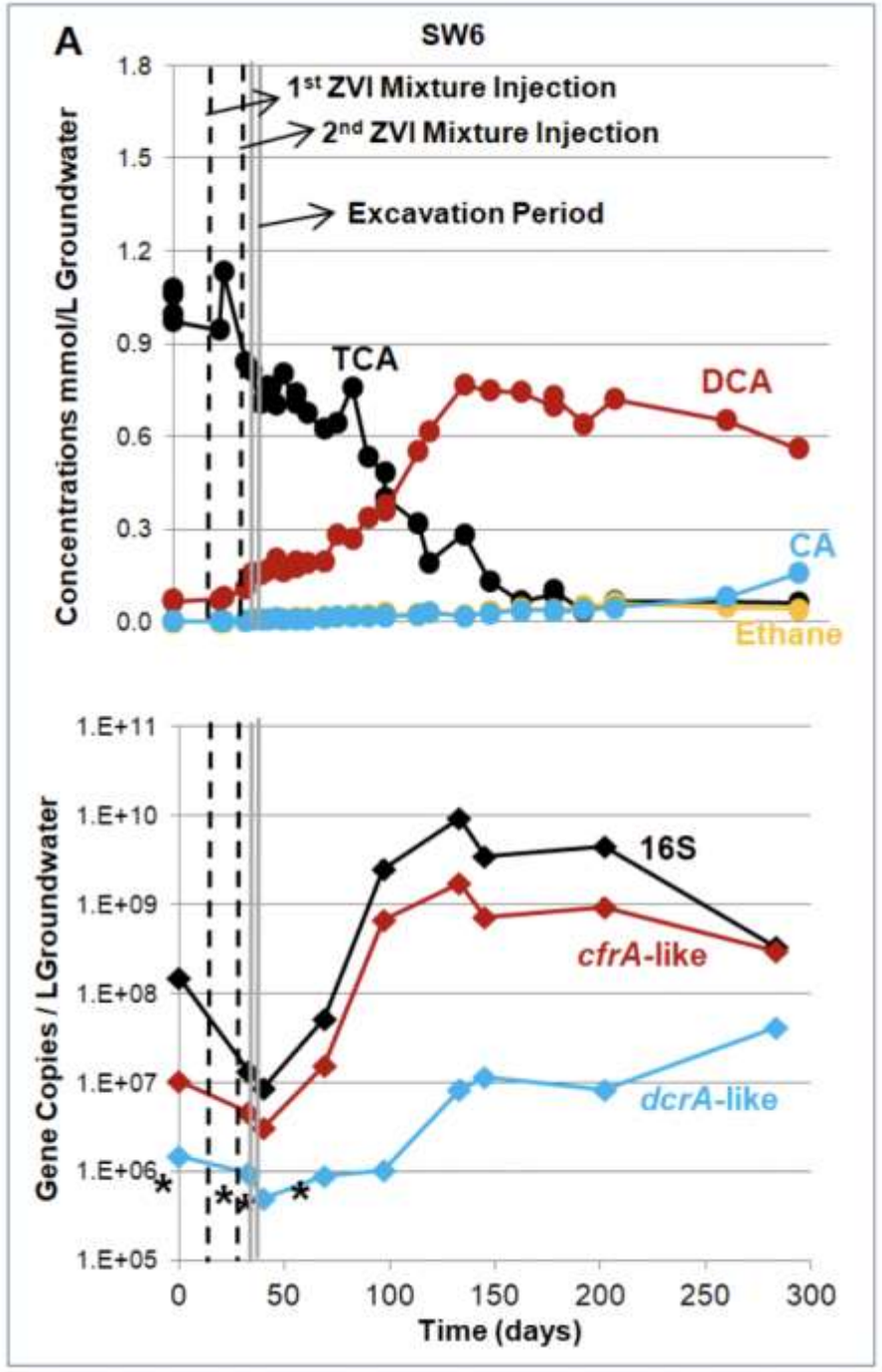


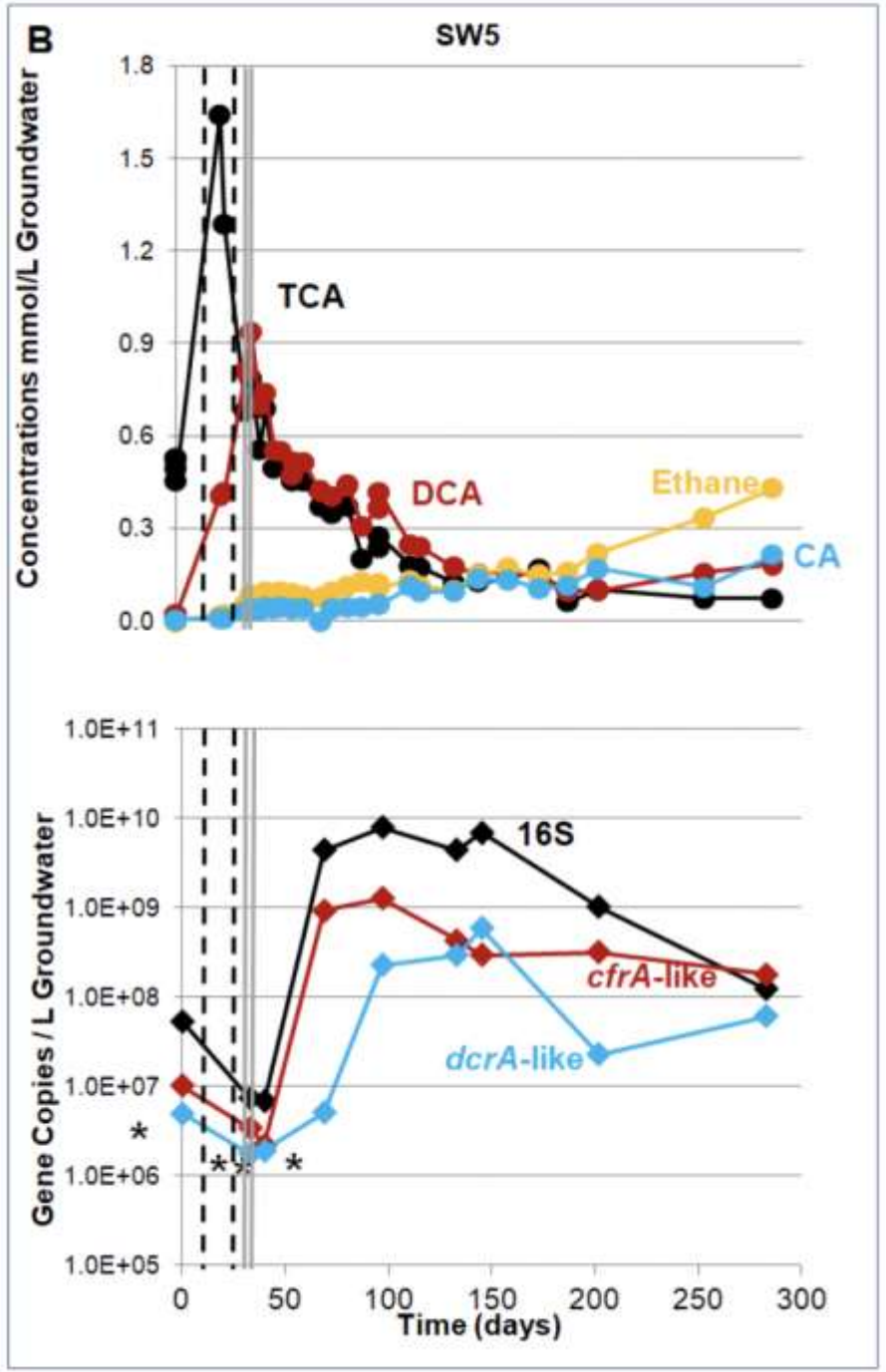
DCA

dcrA → *Dhb* 2



CA

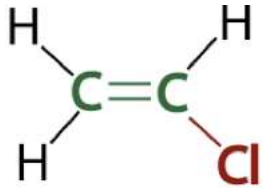


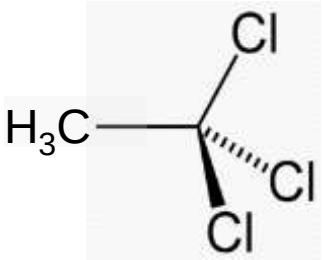


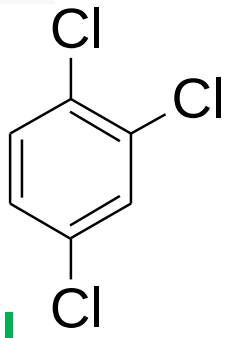
Microcosms and the Need for Activity-Based Tests

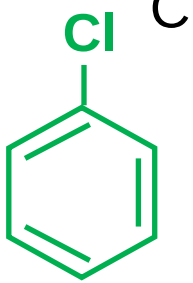
- Microcosm studies and other direct measurements of microbial activity are the best and most definitive way to understand complex processes at a given site
  - More time-consuming but more informative than simple measurements of chemical & biological markers
- Activity-driven assays do not rely on pre-existing knowledge of microbial identity.
- Microcosms are essential to enriching novel microbes.
- Microcosms can more readily detect combined abiotic and biotic processes, cometabolism, presence of inhibitors, and substrate interactions and interferences.

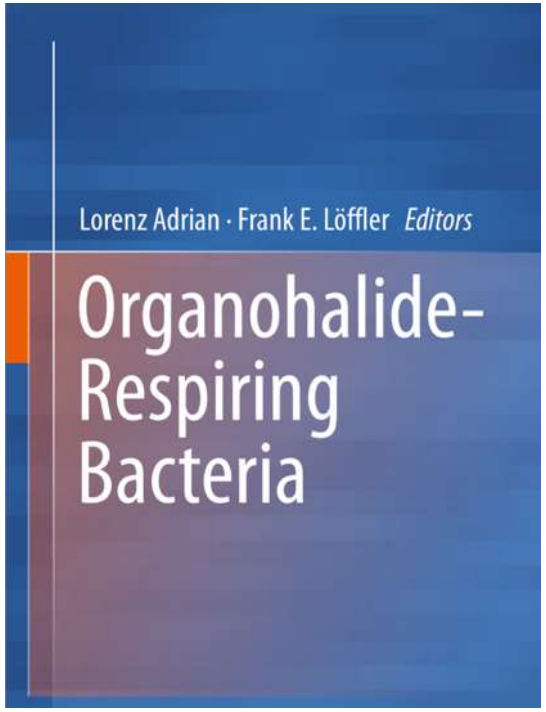
From Hug et al., Chapter 11; Bioaugmentation for Groundwater Remediation. Springer 2013











Springer, 2016

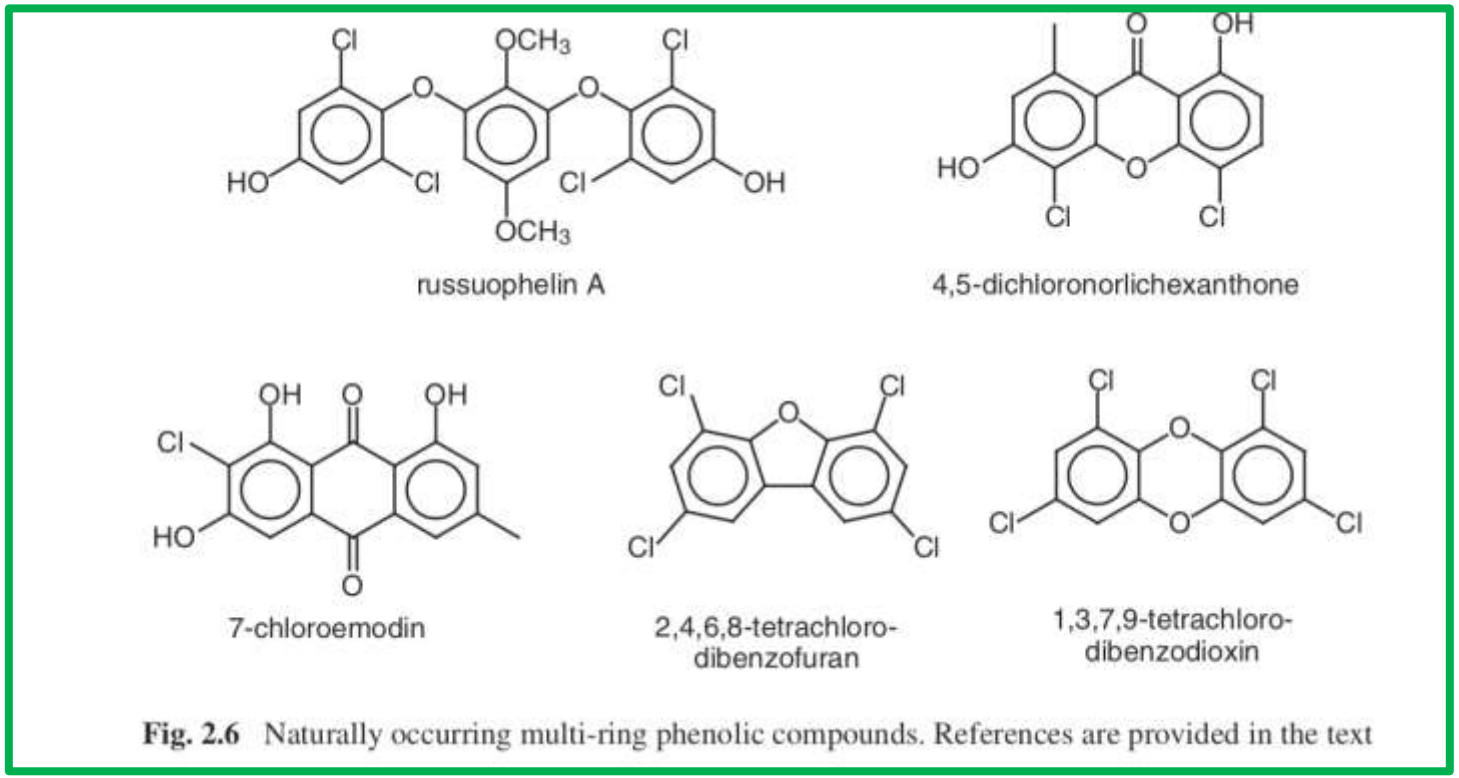
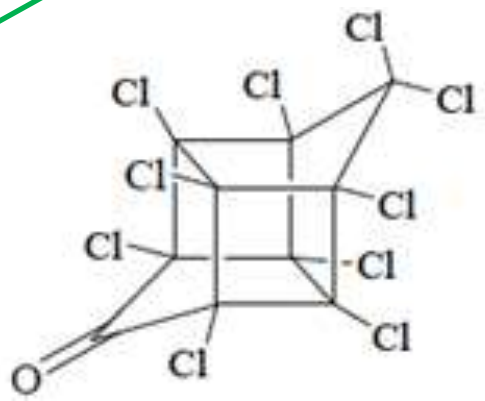
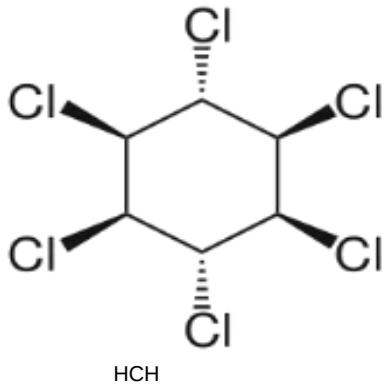


Fig. 2.6 Naturally occurring multi-ring phenolic compounds. References are provided in the text

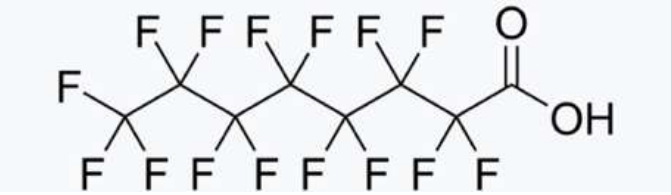


Chlordecone



HCH

Hexachlorocychlohexane
Lindane (γ-HCH)



????

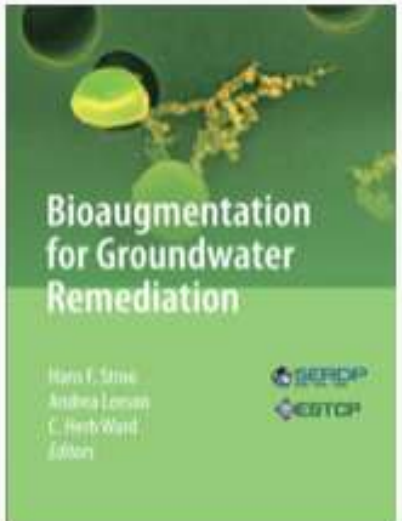
Take Home Messages

- Many different types of organohalide respiring bacteria (OHRB) exist. They are often highly specialized
- A diverse array of enzymes exists that are selective in their substrates
- OHRB live within a community of fermenting organisms exchanging substrates, vitamins, and other nutrients
- Multiple closely related strains co-exist in the field and in enrichment cultures
- Need to study as a community, not individuals

FIG 1 Microbes at a communal table depict interactions and handoffs occurring in the environment.

Hug, L. A. and Co, R. 2018. It Takes a Village: Microbial Communities Thrive through Interactions and Metabolic Handoffs. *MSystems* 3 (4).

GOOD RESOURCES

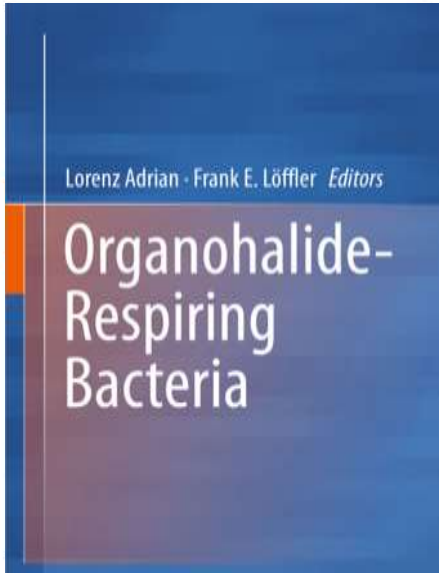


Bioaugmentation for groundwater remediation

Stroo, Hans F et al.

Springer

2013



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Organohalide-Respiring Bacteria

Editors: **Adrian**, Lorenz, **Löffler**, Frank E. (Eds.)

Thank You
&
Questions