Können mikrobiologische Untersuchungen helfen das Bioremediations-Potential von Chlorethen-kontaminierten Standorten abzuschätzen ?

Prof. Christof Holliger, Dr. Julien Maillard,
Noam Shani, and Elsa Lacroix

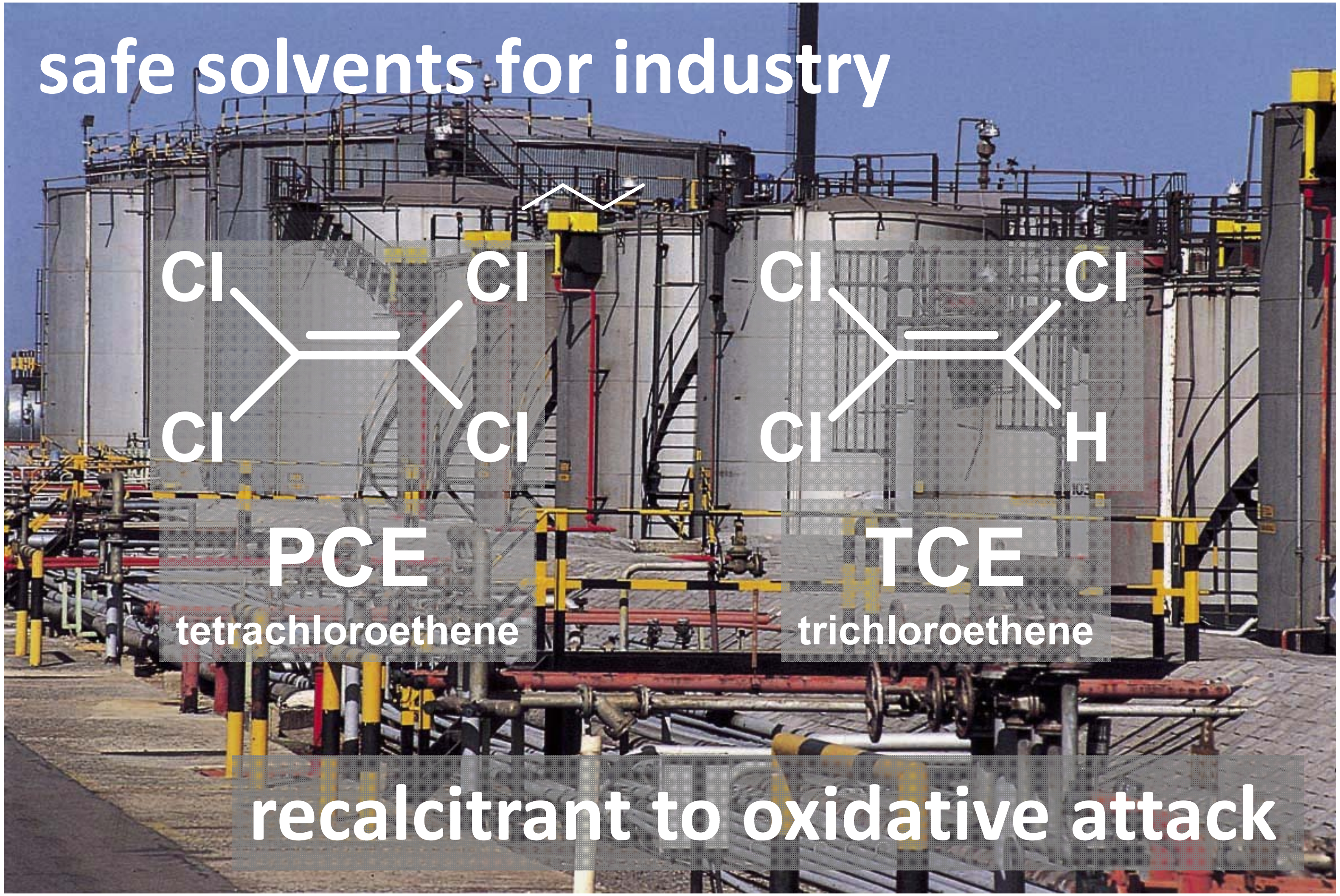
Laboratory for Environmental Biotechnology (LBE)

Dr. Pierre Rossi

Central Environmental Molecular Biology
Laboratory (CEMBL)

ÉCOLE POLYTECHNIQUE
FÉDÉRALE DE LAUSANNE

Chlorinated ethenes



Isolates of chloroethene-respiring bacteria

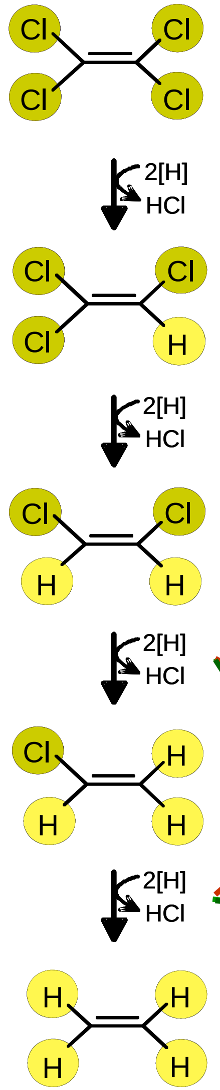
tetrachloroethene (PCE)

trichloroethene (TCE)

***cis*-1,2-dichloroethene (*cis*-1,2-DCE)**

vinyl chloride (VC)

ethene



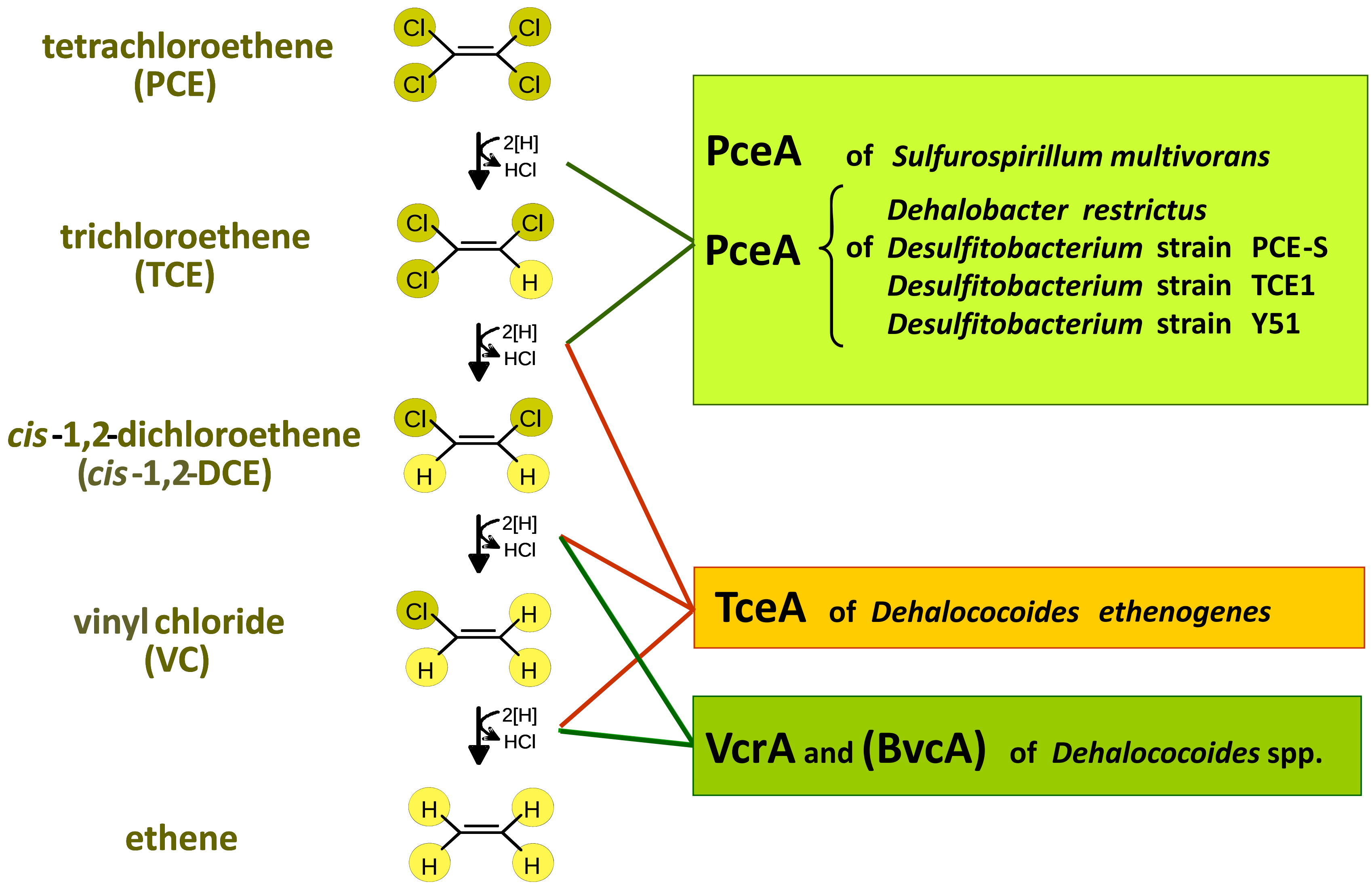
***Desulfitobacterium* strain PCE1**

Dehalobacter restrictus
***Desulfitobacterium* strain PCE -S**
***Desulfitobacterium* strain TCE1**
***Desulfitobacterium* strain Y51**
Desulforomonas chloroethenica
***Geobacter lovleyi* strain SZ**
Sulfurospirillum multivorans
Sulfurospirillum halorespirans

Dehalococoides ethenogenes

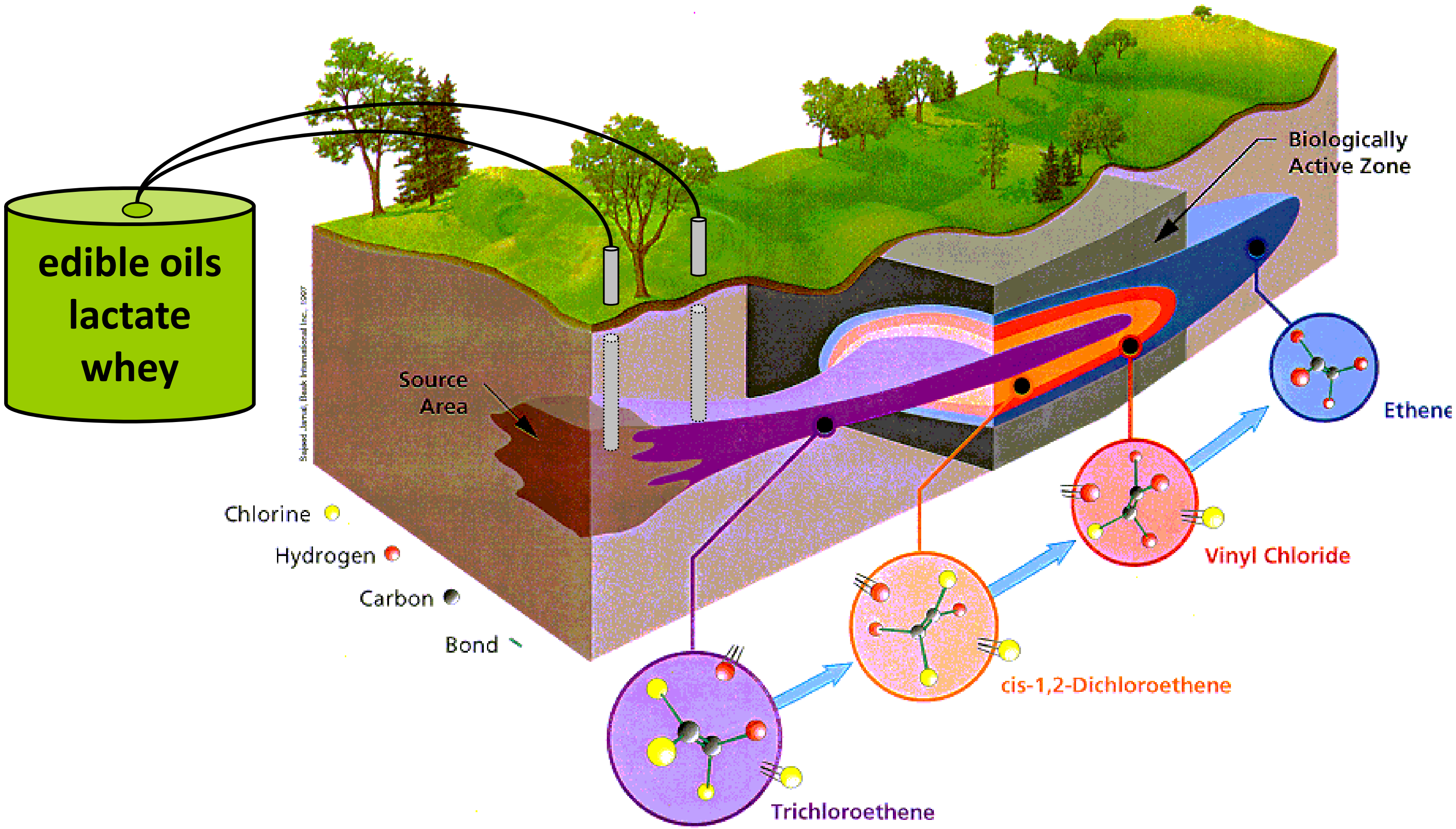
***Dehalococoides* sp . strain BAV1**
***Dehalococoides* sp. strain VS**

Chloroethene reductive dehalogenases



Their application in remediation

(enhanced) natural attenuation

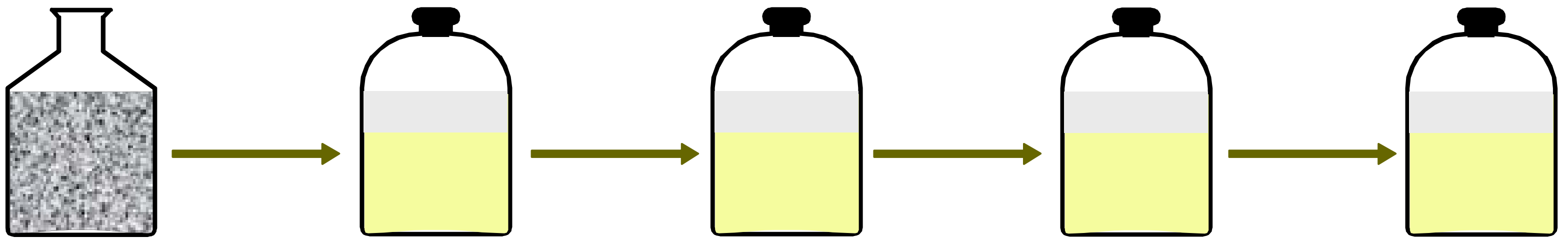


Challenges for (enhanced) natural attenuation of chloroethene contamination

- Assure that vinyl chloride is not end product
  - Is the biochemical potential present for complete chloroethene dechlorination ?
  - If yes, why does vinyl chloride accumulate ?
  - For enhanced NA which electron donors sustain best complete dechlorination ?
- Understand the fate of toxic dechlorination products
  - Is dechlorination the only transformation process responsible for VC removal ?

Experimental set-up of microcosms

Samples **Enrichments**



- sludges
- sediments
- ...

- PCE
- TCE
- DCEs
- VC

+ substrates

7 days

21 days

n days

ANALYSIS

- Chlor-o-counter
- GC
- ...

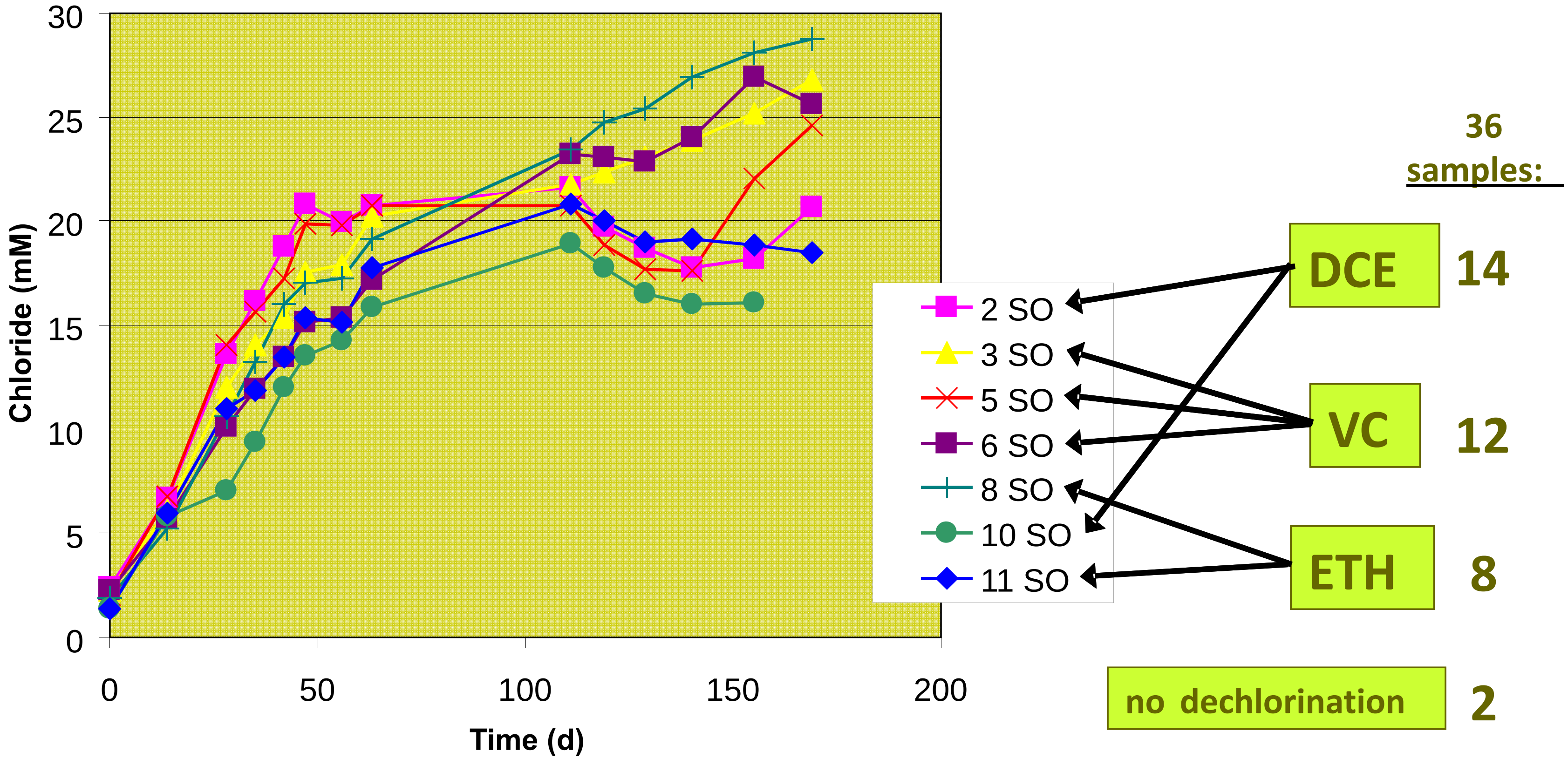
+

Isolation of DNA and RNA from samples and enrichments

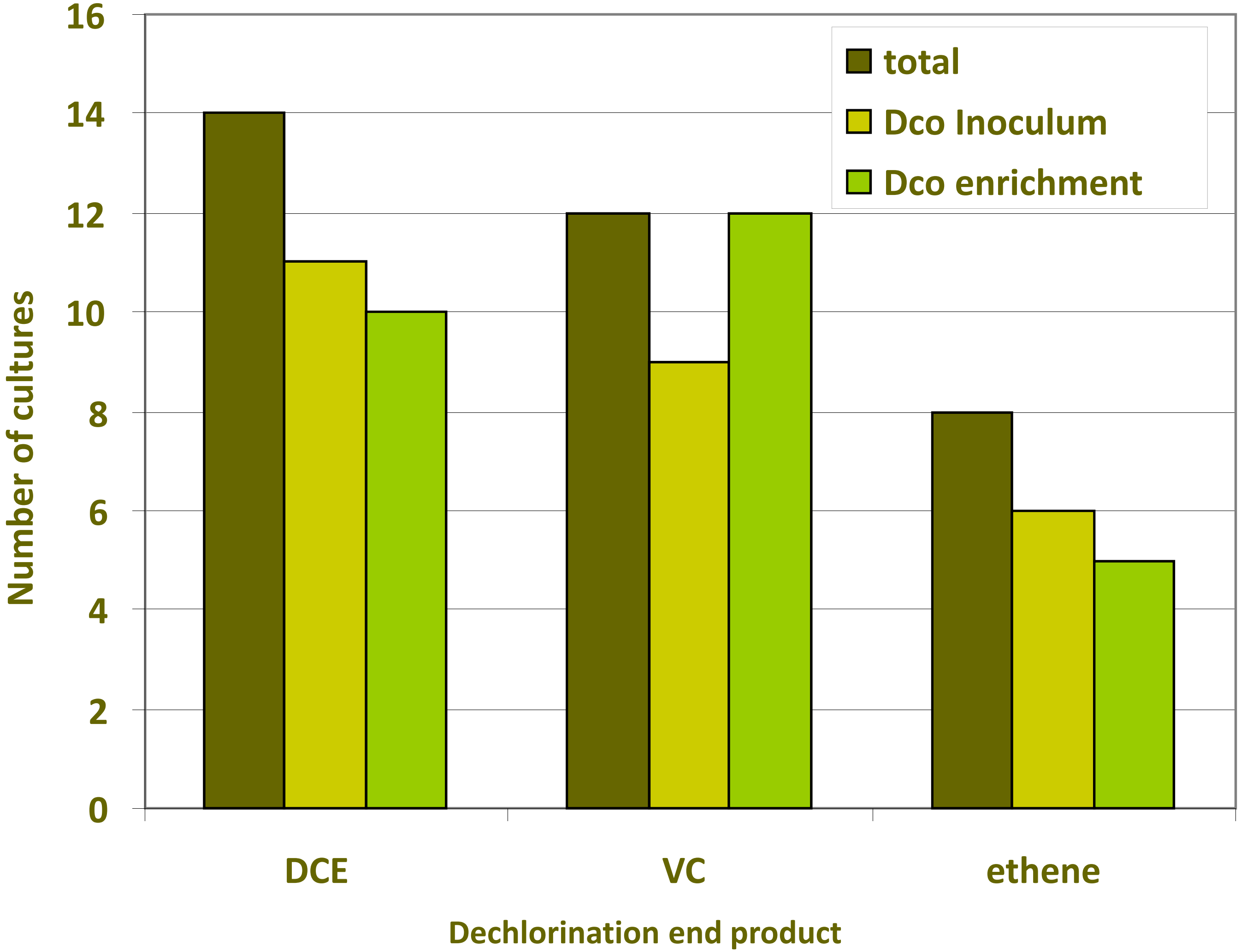
Different substrate mixtures added
(1-2 mM per week)

- **acetate, formate, lactate, pyruvate, propionate, butyrate, ethanol**
- **acetate + formate**
- **propionate, butyrate, ethanol**
- **lactate + pyruvate**

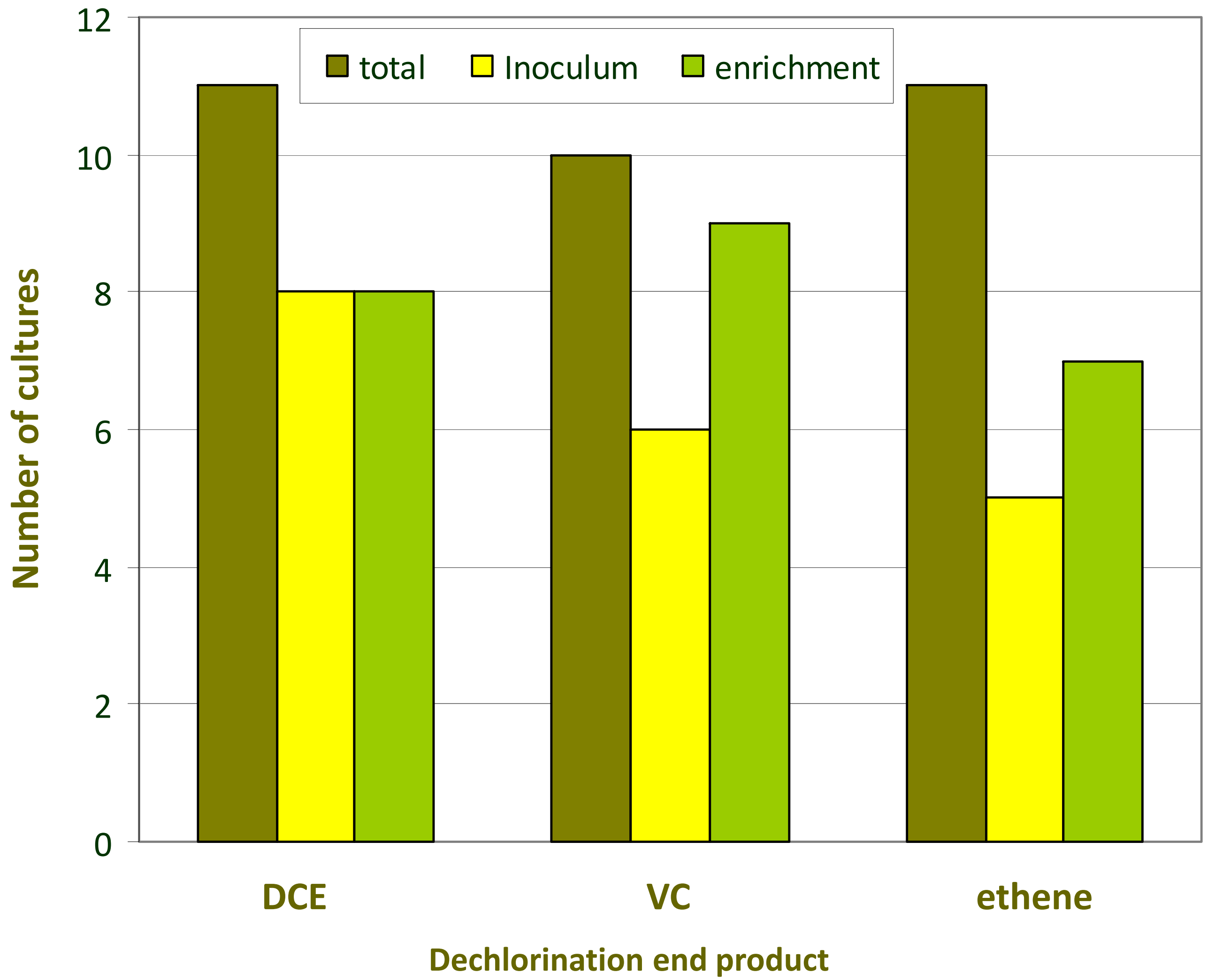
Dechlorination in enrichment cultures



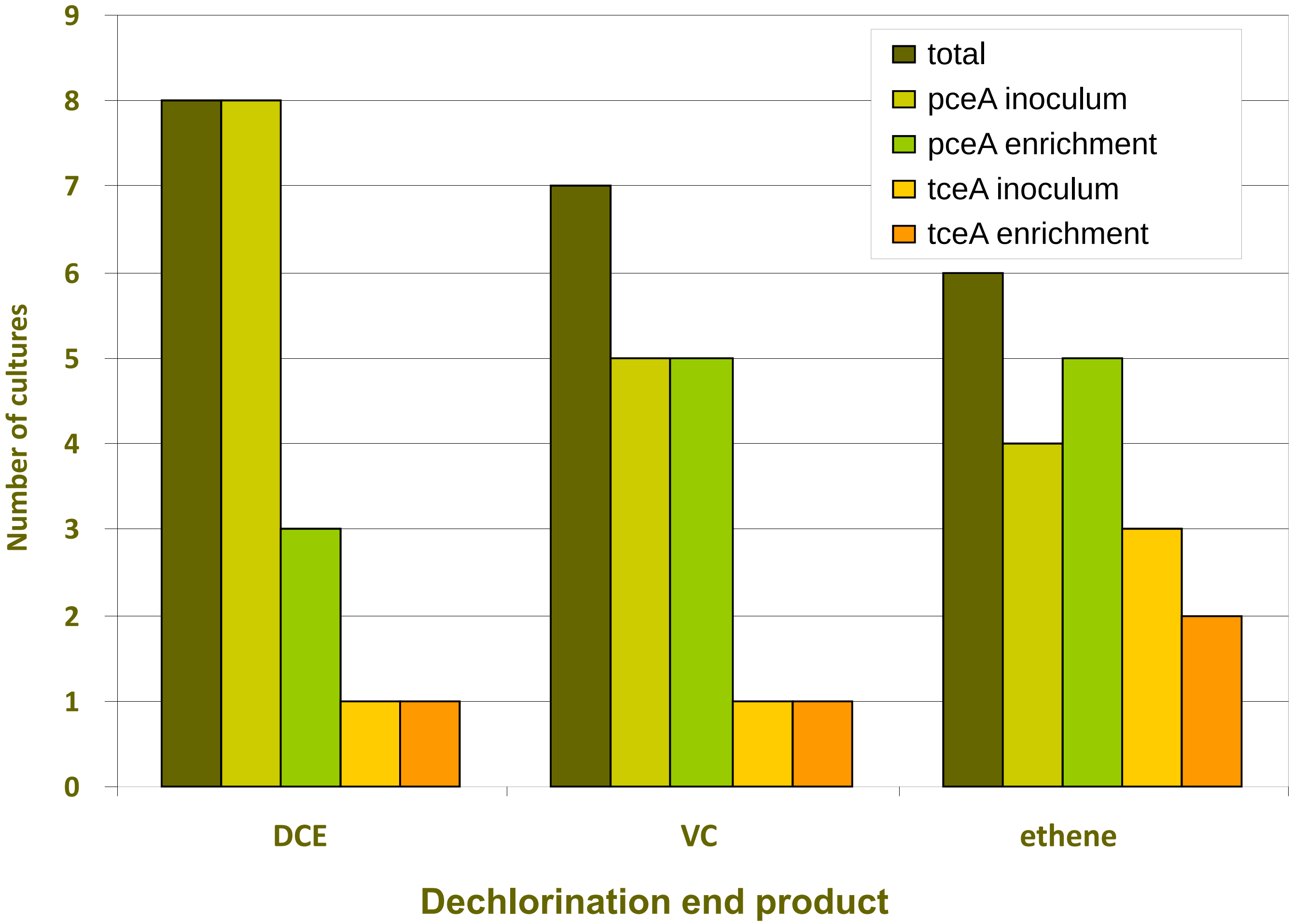
Detection of *Dehalococcoides* spp. in 36 environmental samples



Detection of *Dehalococcoides* spp. together with *Desulfitobacterium* spp. in 24/32 samples



Detection of *pceA* et *tceA* in environmental samples

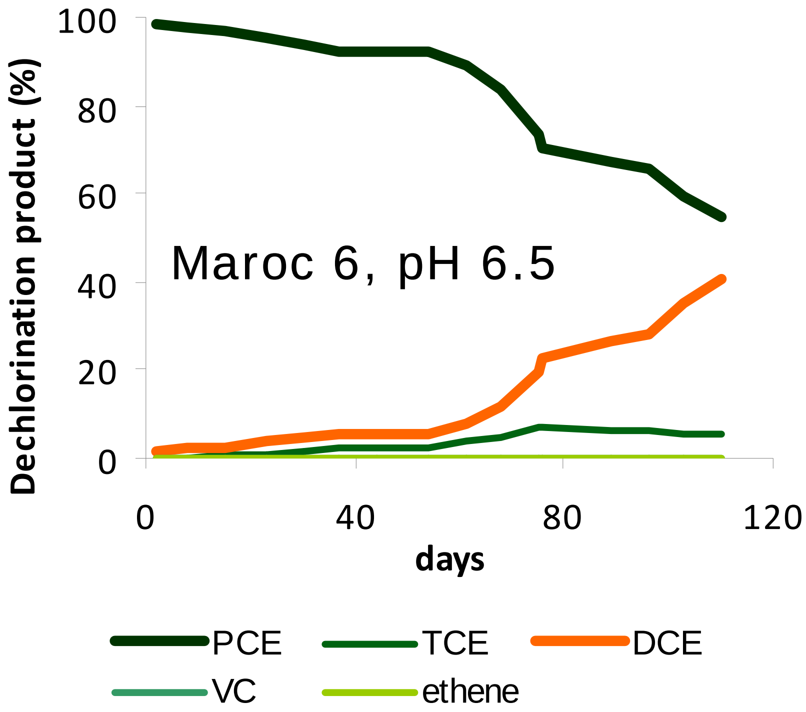


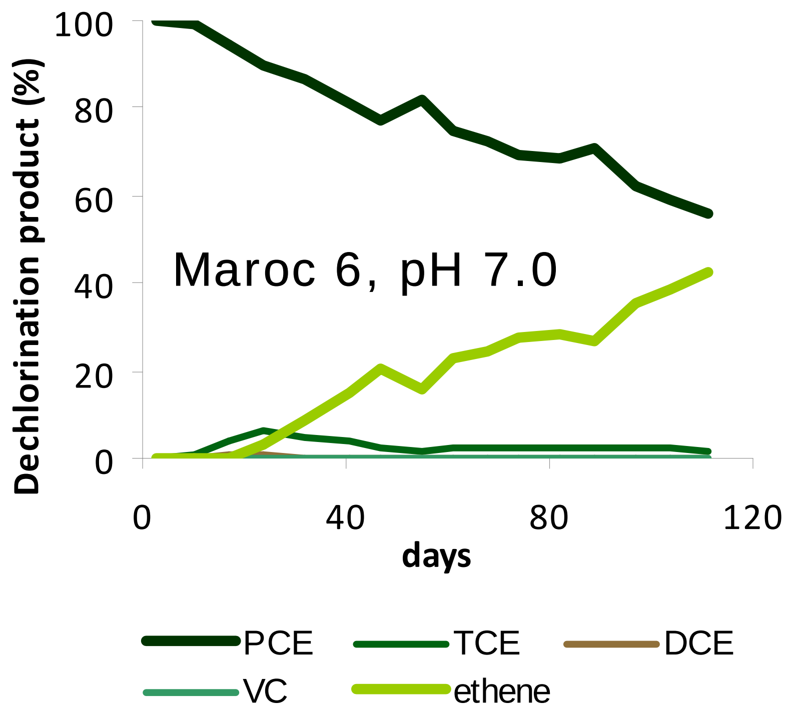
Inoculum: nested PCR; enrichment: direct PCR

Stepwise dechlorination by different organohalide-respiring bacteria

- PCE → DCE → ethene:
  - *Desulfitobacterium spp.* → *Dehalococcoides spp.*
- PCE → DCE → ethene:
  - *Sulfurospirillum spp.* → *Dehalococcoides spp.*
- PCE → DCE → ethene:
  - *Dehalobacter spp.* → *Dehalococcoides spp.*
  - **no vcrA and bvcA detected**
- DCE → VC → ethene:
  - *Desulfitobacterium spp.* → *Dehalococcoides spp.*
  - **no vcrA and bvcA detected**

The *Dehalobacter-Dehalococcoides* culture



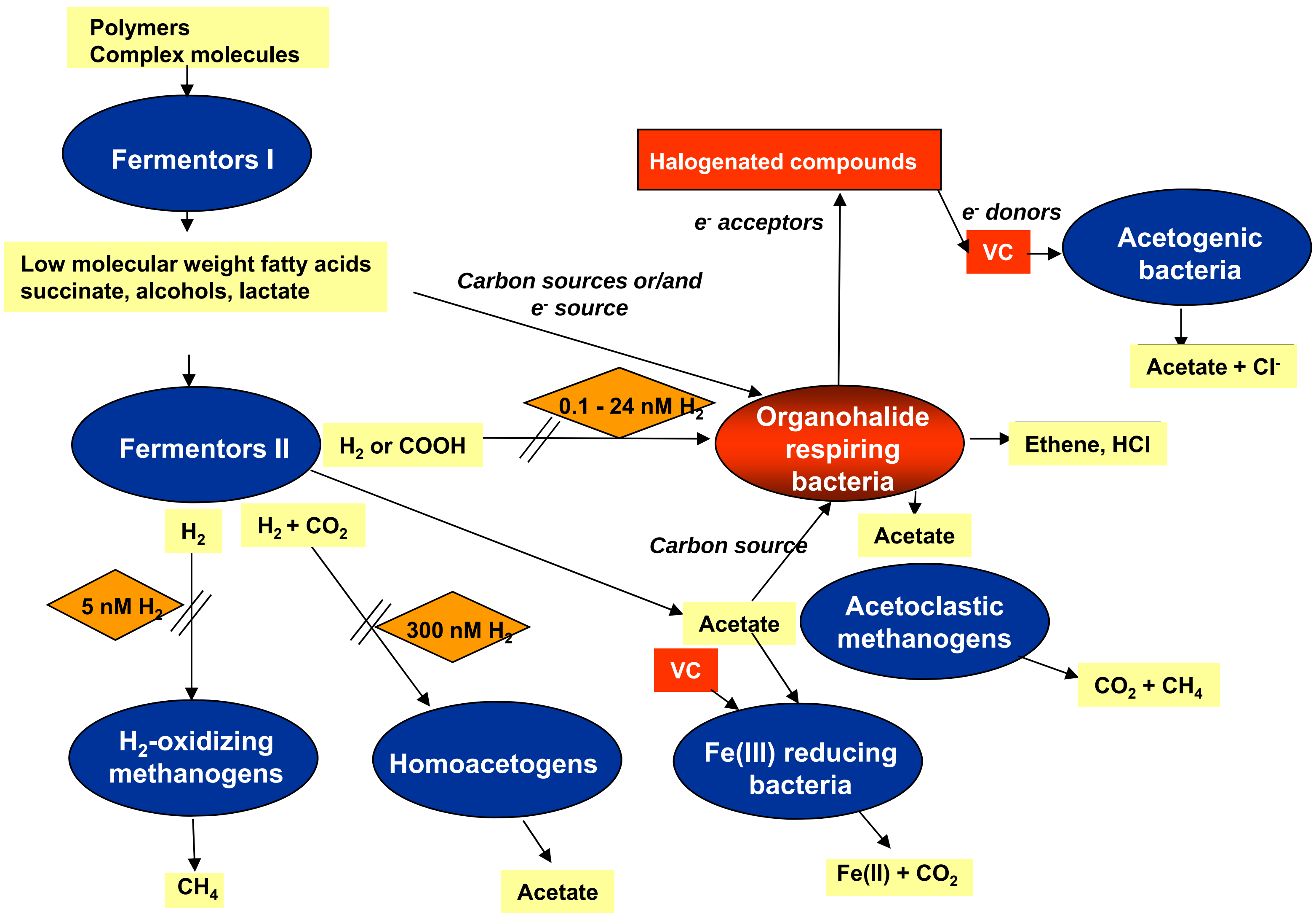


- very slow dechlorination
- production of DCE with low accumulation of TCE at pH 6.5
- production of ethene with low accumulation of TCE and no accumulation of DCE and VC at pH 7.0

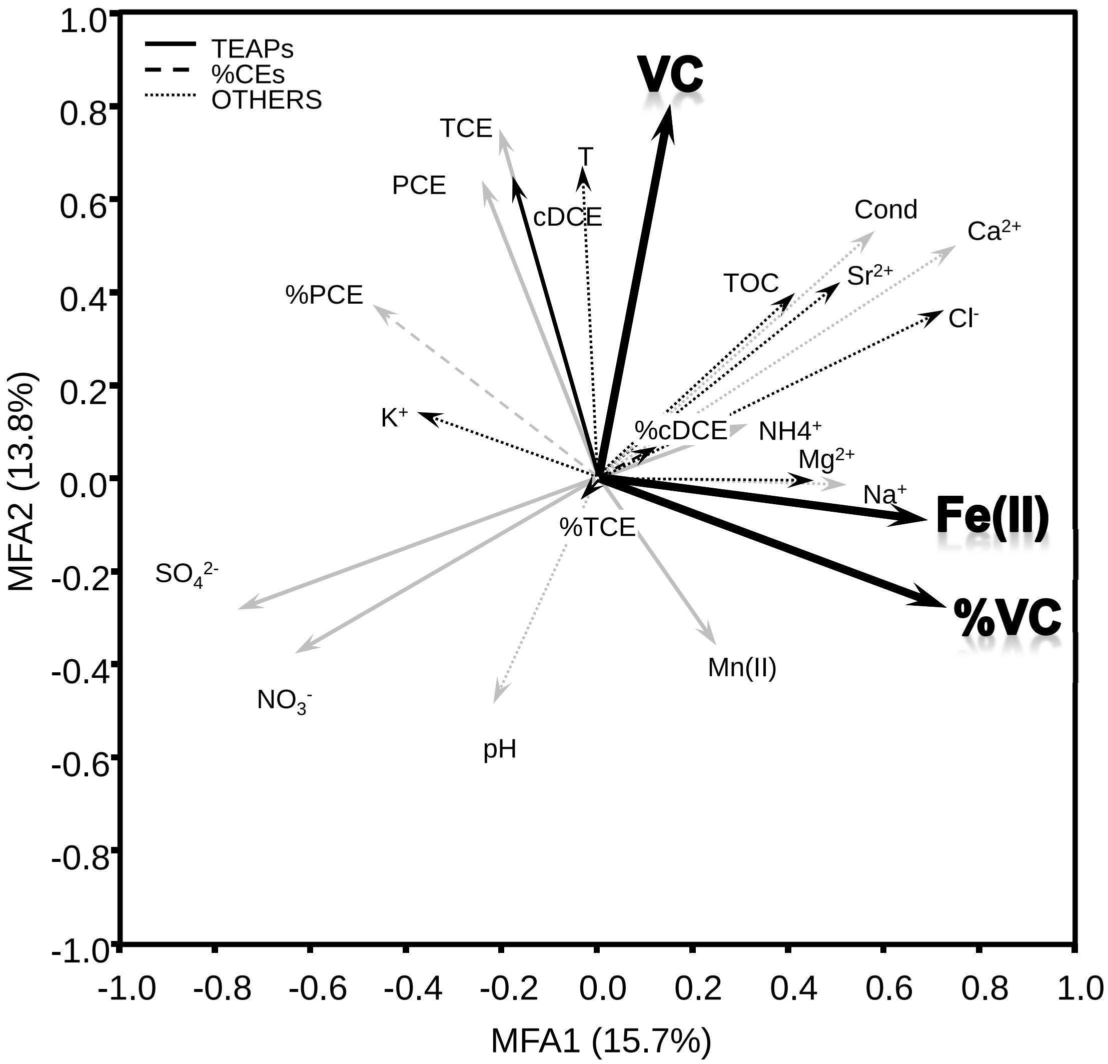
Challenges for (enhanced) natural attenuation of chloroethene contamination

- Assure that vinyl chloride is not end product
  - Is the biochemical potential present for complete chloroethene dechlorination ?
  - If yes, why does vinyl chloride accumulate ?
  - For enhanced NA which electron donors sustain best complete dechlorination ?
- Understand the fate of toxic dechlorination products
  - Is dechlorination the only transformation process responsible for VC removal ?

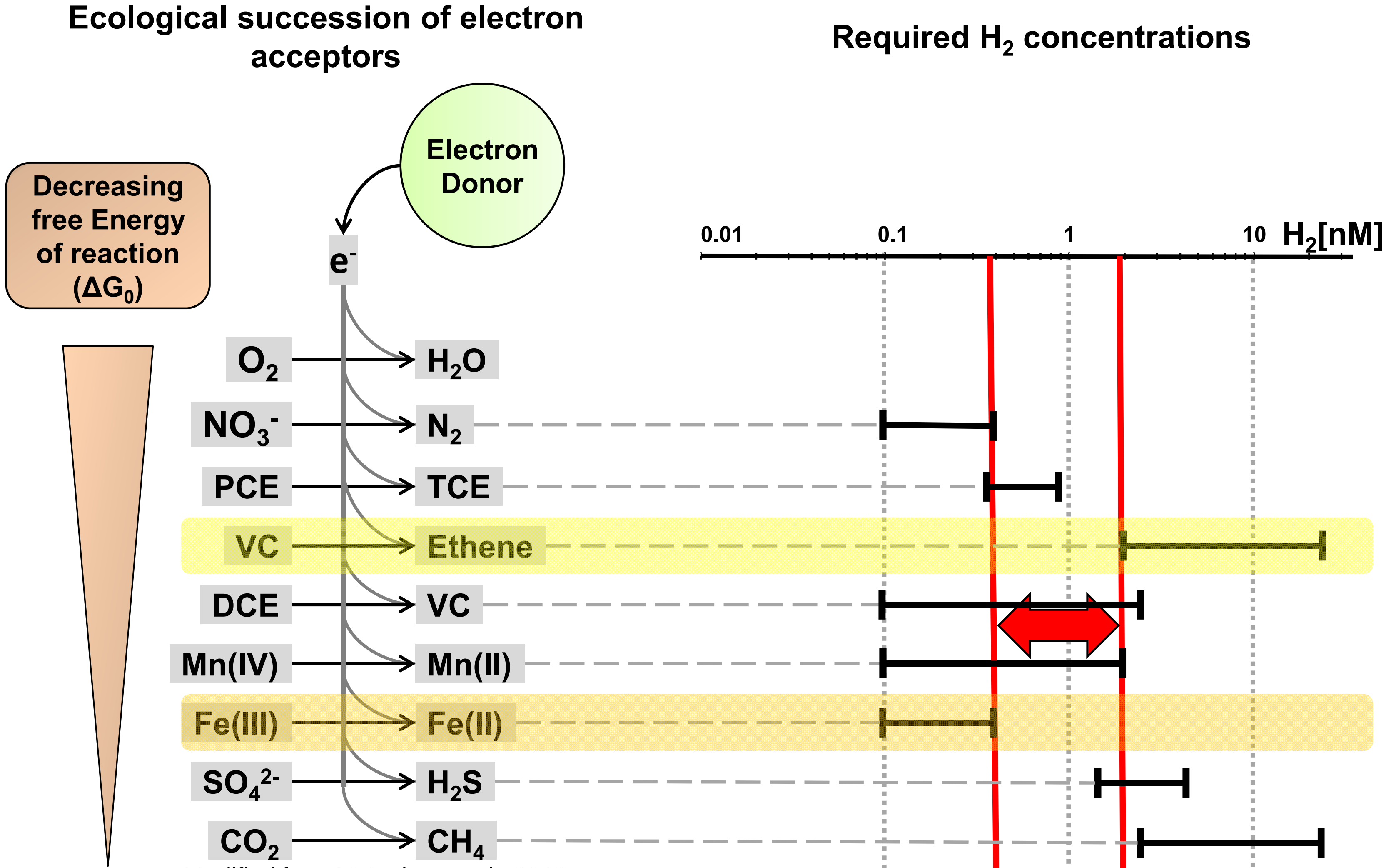
Bacterial guilds involved in organohalide respiration



Competition between iron-respiring and organohalide-respiring bacteria



Competition between iron-respiring and organohalide-respiring bacteria



Modified from McMahon et al., 2008

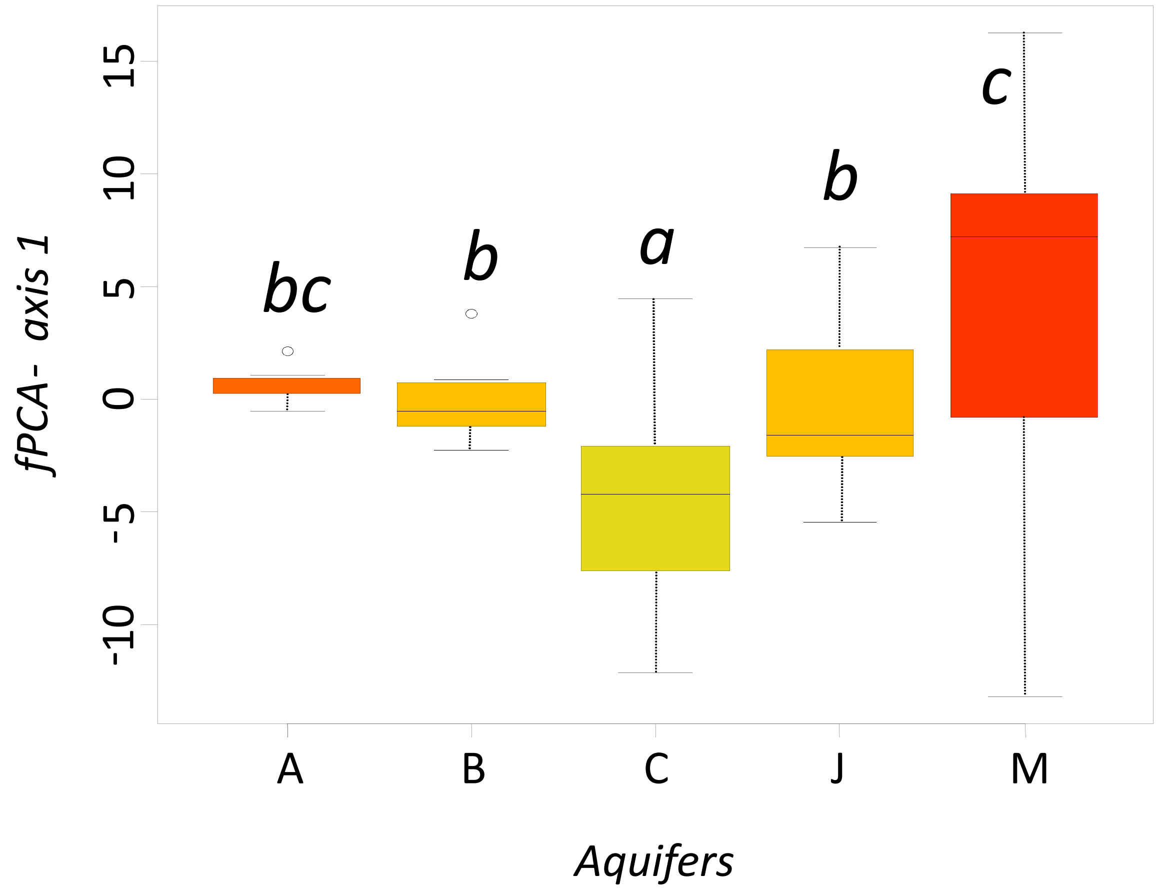
Challenges for (enhanced) natural attenuation of chloroethene contamination

- Assure that vinyl chloride is not end product
  - Is the biochemical potential present for complete chloroethene dechlorination ?
  - If yes, why does vinyl chloride accumulate ?
  - For enhanced NA which electron donors sustain best complete dechlorination ?
- Understand the fate of toxic dechlorination products
  - Is dechlorination the only transformation process responsible for VC removal ?

Electron donors for complete dechlorination

Inoculum	Electron donor mixture leading to ethene formation
Anaerobic digester sludge	Acetate - formate
PCE bioreactor sludge	Propionate – butyrate - ethanol
Aquifer I	Propionate – butyrate - ethanol
Aqufer II	Propionate – butyrate - ethanol

Bacterial diversity and microbial community composition varies according to aquifers



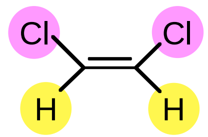
Coordinates of record calculated on the first ordination axis of the fPCA, grouped by aquifers. Different letters indicate significant differences between the microbial communities of the five aquifers (P≤ 0.05 Tukey's HSD).

Challenges for (enhanced) natural attenuation of chloroethene contamination

- Assure that vinyl chloride is not end product
  - Is the biochemical potential present for complete chloroethene dechlorination ?
  - If yes, why does vinyl chloride accumulate ?
  - For enhanced NA which electron donors sustain best complete dechlorination ?
- Understand the fate of toxic dechlorination products
  - Is dechlorination the only transformation process responsible for VC removal ?

Anaerobic oxidation of chloroethenes

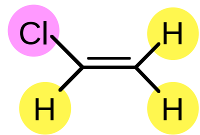
cis-1,2-dichloroethene
(*cis*-1,2-DCE)



$\longrightarrow \longrightarrow \longrightarrow$ CO_2

Fe(III)-reducing
conditions

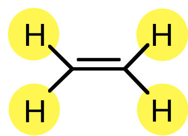
vinyl chloride
(VC)



$\longrightarrow \longrightarrow \longrightarrow$ CO_2

Fe(III)- or Mn(IV)-reducing
conditions

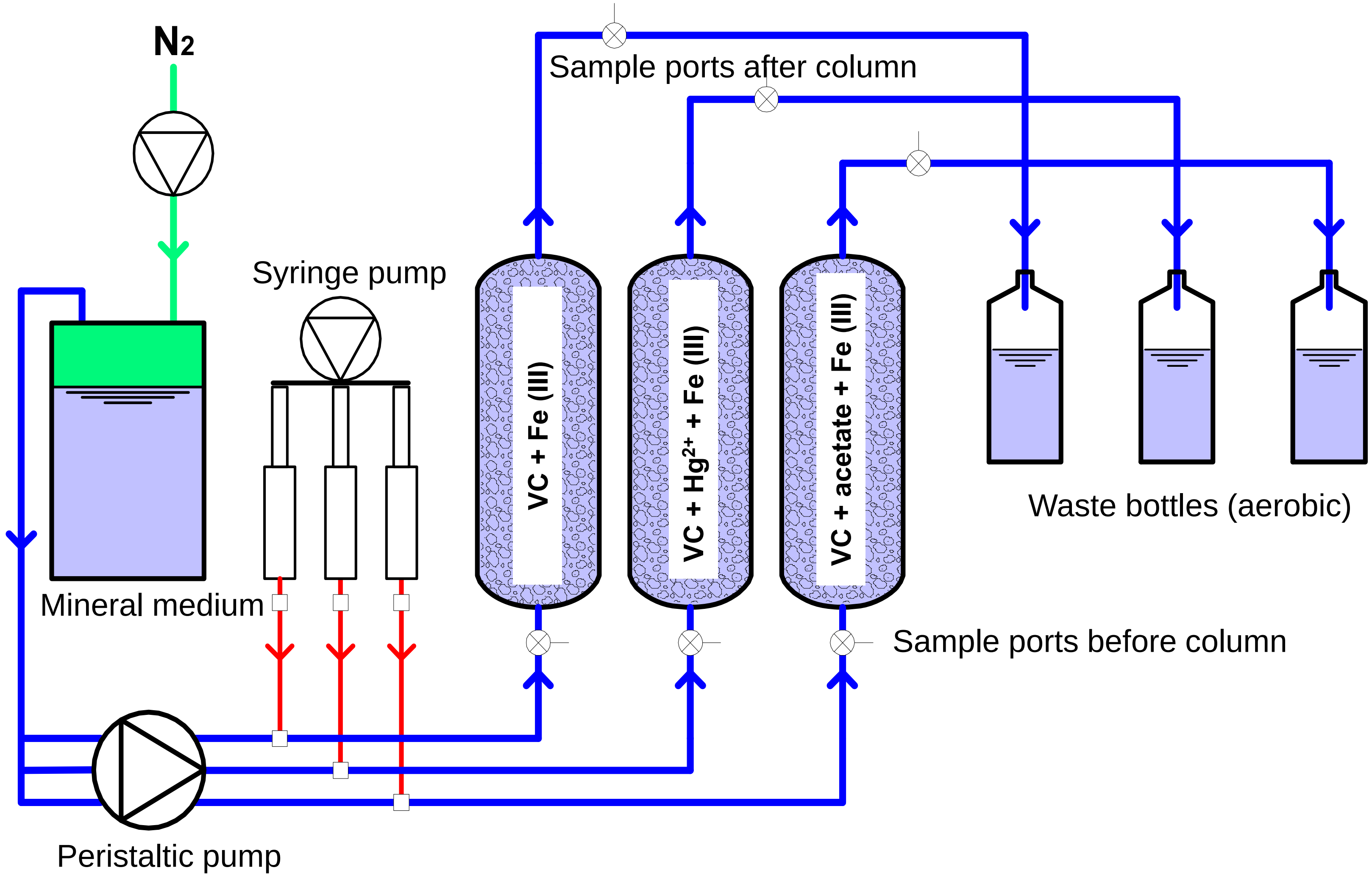
ethene



$\longrightarrow \longrightarrow \longrightarrow$ CO_2

SO_4^{2-}-reducing
conditions

Scheme of experimental system



Stable isotopes indicated anaerobic oxidation of VC

$$\delta^{13}C_{out} = \delta^{13}C_{in} + \varepsilon \ln(C_{out}/C_{in})$$

ε = isotope enrichment factor

		ε
→ **VC + Fe (III)** →	0-20% VC no ethene some Fe(II)	**- (3 – 5)**
→ **VC + acetate + Fe (III)** →	20-50% VC ethene Fe(II)	**- (11 – 18)***

* literature data for reductive dechlorination ε = - (20-25)

Conclusions

- Reductive dechlorination of chloroethenes is a promising microbial process for aquifer bioremediation
- Organohalide-respiring bacteria seem to be widespread but not all biochemical potential seems to be everywhere
- We do not know yet all the microbiology and enzymology involved in complete dechlorination to ethene
- VC accumulation can be the result of missing biochemical potential or a consequence of loosing competiton for substrate
- VC can also be transformed by processes other than reductive dechlorination
- There is no general concept that can be applied to all sites, each site has its own characteristics and individual solutions have to be found

Final Conclusion

- The bioremediation potential can be assessed with microbial investigations and a combination of molecular ecology, microcosms, and numerical ecology is the optimal approach to predict qualitatively the fate of chloroethenes during (enhanced) natural attenuation