MECHANISM, CHEMISTRY, AND KINETICS OF ANAEROBIC BIODEGRADATION OF *cis*-DICHLOROETHENE AND VINYL CHLORIDE

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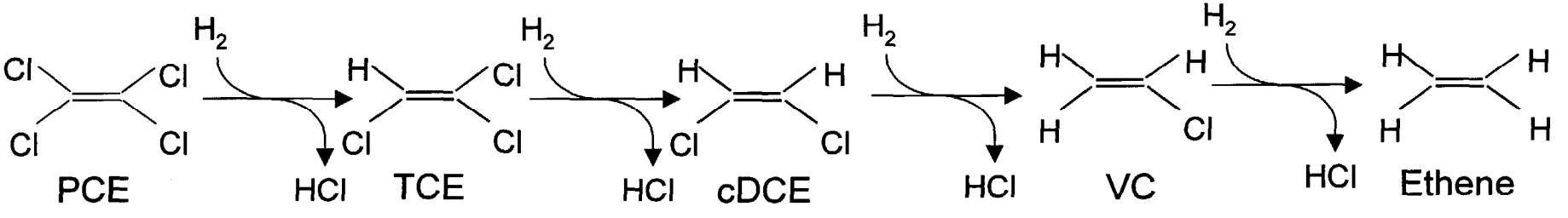
Introduction

Chlorinated aliphatic hydrocarbons such as tetrachloroethene (PCE) and trichloroethene (TCE) are among the most frequent groundwater contaminants in the United States. Anaerobic microorganisms can transform PCE and TCE to ethene via reductive dehalogenation using hydrogen as the electron donor.



The accumulation of *cis*-dichloroethene(cDCE) and vinyl chloride (VC) is often observed during reductive PCE dehalogenation. In contrast to detailed mechanistic studies on PCE dehalogenation to cDCE, much less is known about the reductive dehalogenation of cDCE and VC to ethene.

Competition for hydrogen within a chlorinated solvent degrading anaerobic mixed culture

Competition for Hydrogen In Anaerobic Processes

Complex Organic Materials

↓ Fermentation

Hydrogen

↓

			H_2 threshold conc. (nM) (Lovley et al.)
Carbon Dioxide	Acetogenesis →	Acetate	400
Carbon Dioxide	Methanogenesis →	Methane	7 - 10
Sulfate	Sulfate Reduction →	Sulfide	1 - 1.5
Iron (III)	Fe (III) Reduction →	Iron (II)	0.2
Nitrate	Nitrate Reduction →	Nitrite	0.05

Reductive Dehalogenation

PCE → TCE → *cis*-DCE → VC → Ethene ?

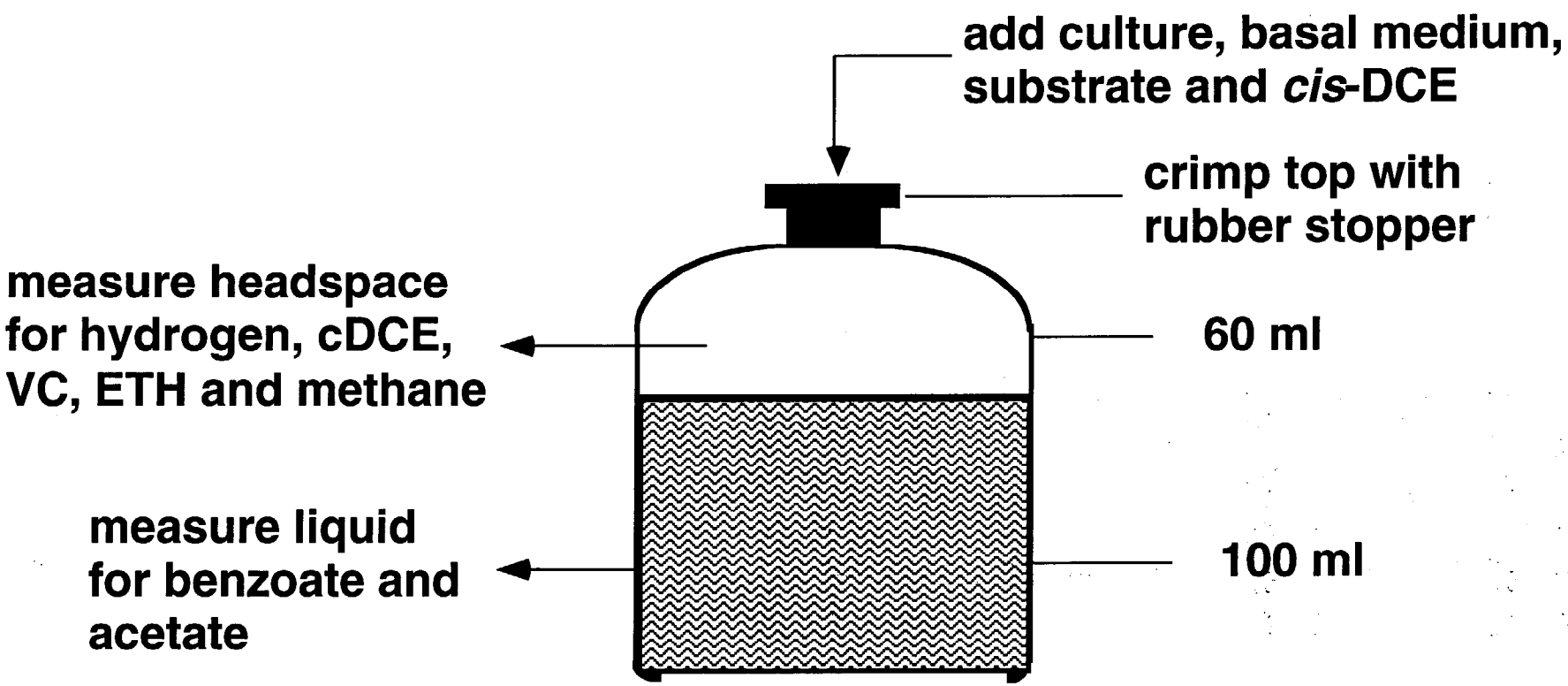
Objectives

- To understand the nature of the competition for hydrogen between dehalogenating and other hydrogen-utilizing microorganisms.
- To explore approaches for increasing the competitive advantage for dehalogenating microorganisms.

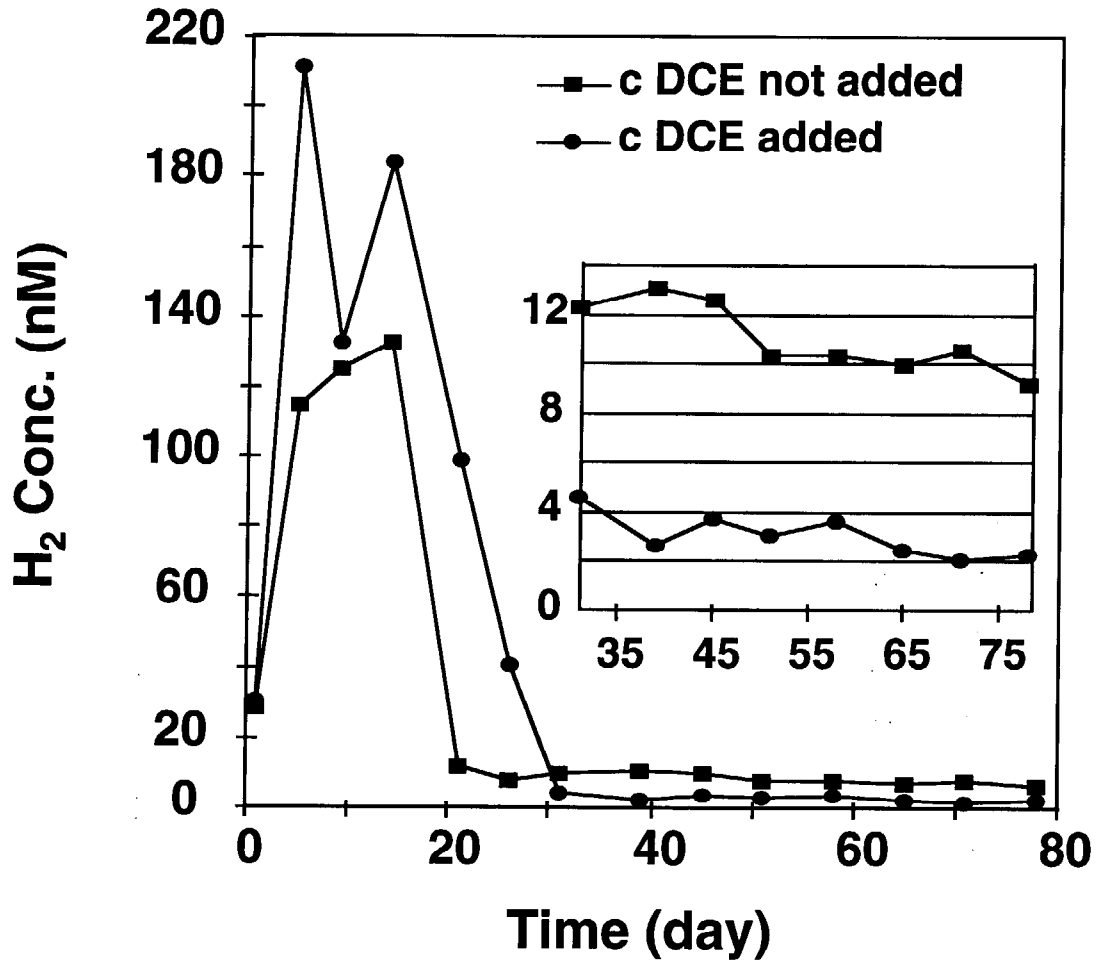
Conclusions

- For *cis*-DCE and VC dehalogenation, the threshold H_2 concentration is about 2 nM.
- For efficient use of H_2 for dehalogenation, H_2 concentration should be maintained between 2 and 11 nM.
- One strategy is to select appropriate substrates that produce constant but low H_2 level.
- Another strategy is to simulate a CSTR reactor, which can maintain H_2 conc. at 2 to 4 nM.

Batch Reactor Setup



Threshold H_2 Conc. – Methanogenesis & Dehalogenation



10. 9 ±3. 3 nM

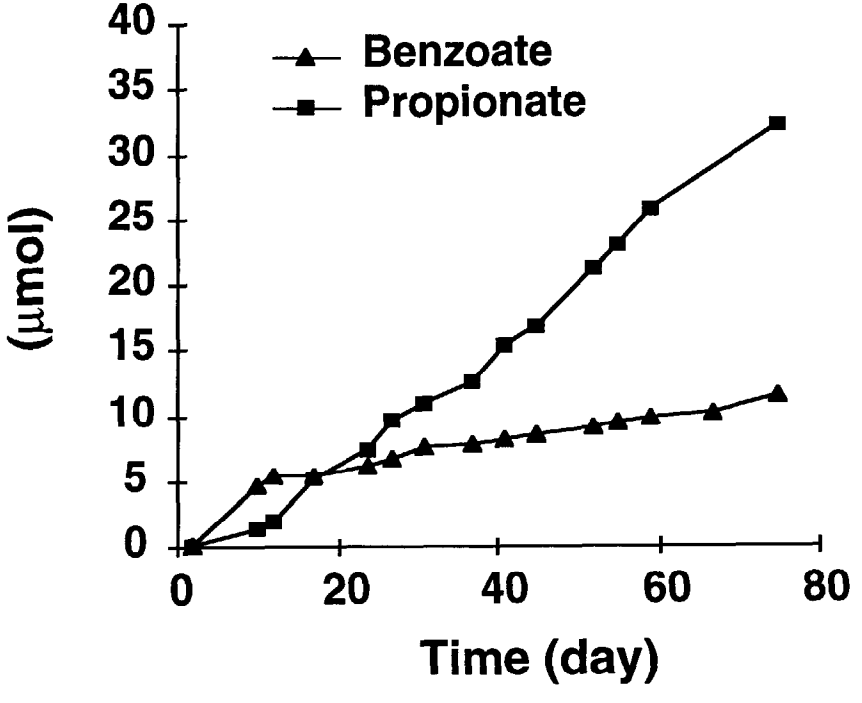
2. 2 ±0. 9 nM

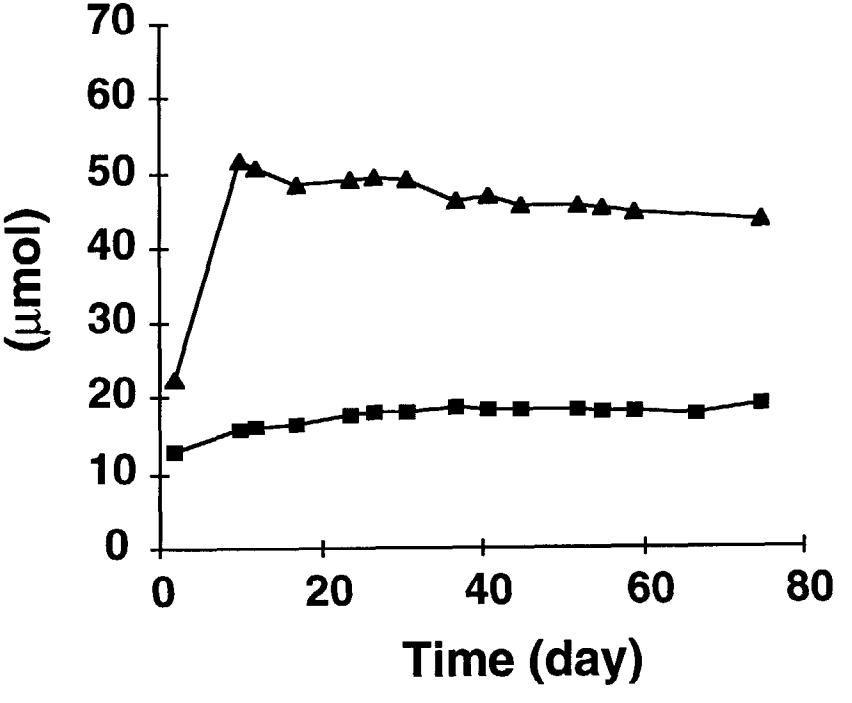
- **Initial burst of hydrogen resulted from fermentation of electron donors added, benzoate in this case, and a small amount of yeast extract added as a necessary nutrient.**

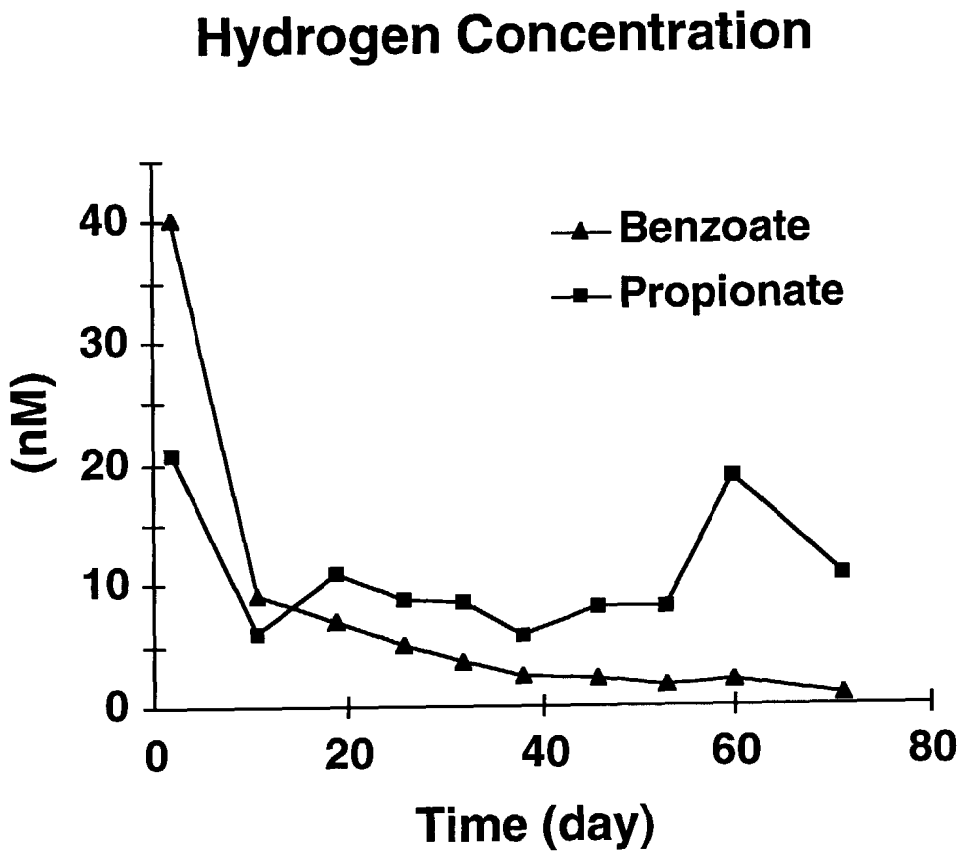
- **Subsequently, hydrogen concentration stabilized at about 2 nM in the presence of *cis*-DCE (the dehalogenation threshold) and 11 nM in the absence of *cis*-DCE (the methanogenic threshold).**

- **Similar threshold hydrogen concentrations were obtained with other substrates such as hydrogen, acetate, and yeast extract alone. The statistical average values for all substrates are 10.9 $\pm$ 3.3 nM (n=40) and 2.2 $\pm$ 0.9 nM (n=32) in the absence and presence of *cis*-DCE, respectively.**

Dehalogenation of *cis*-DCE with Benzoate & Propionate





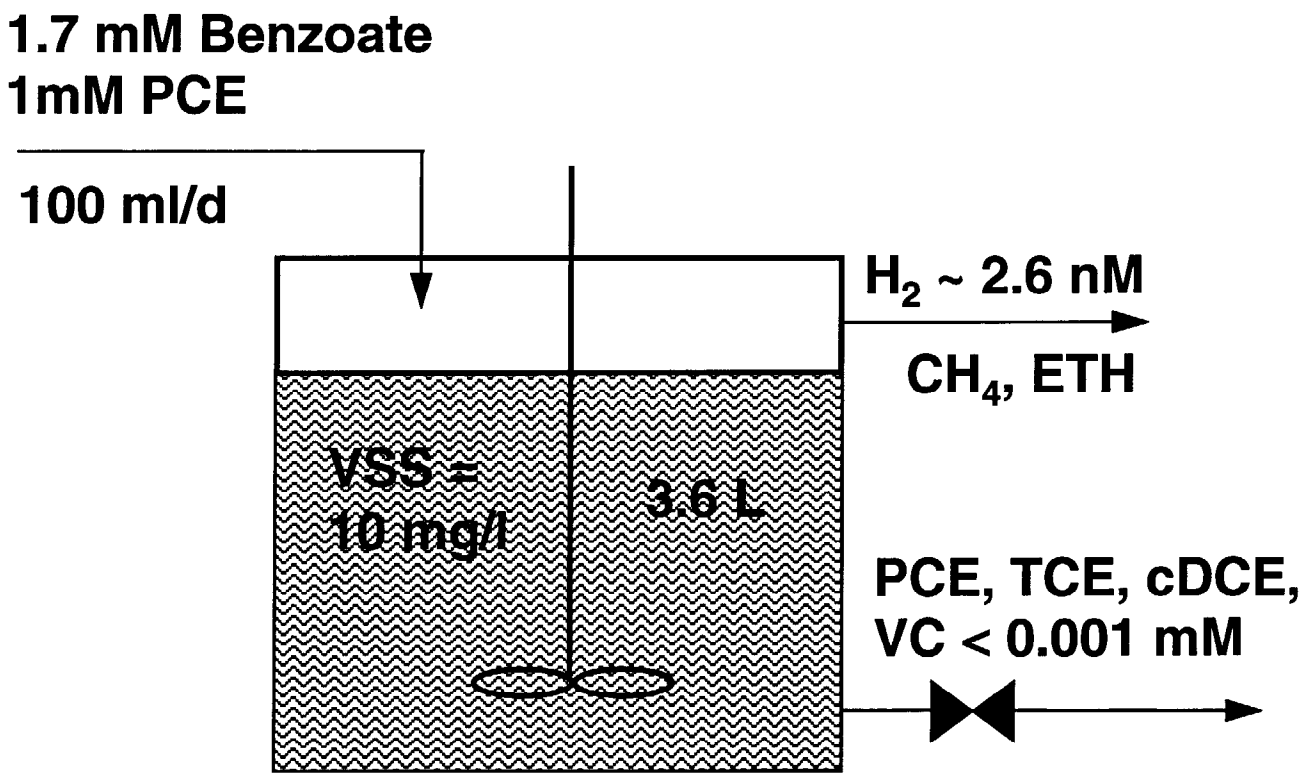

Hydrogen Concentration
Benzoate
Propionate
(nM)
40
30
20
10
0
0
20
40
60
80
Time (day)

- **Within 80 days, three times more ethene was produced from dehalogenation of *cis*-DCE with propionate as electron donor rather than benzoate, while benzoate produced three times more methane than propionate.**

- **With benzoate, rapid fermentation and thus rapid hydrogen production occurred initially, with most hydrogen utilized for methane formation. This resulted in a lower hydrogen level and dehalogenation rate.**

- **In contrast, slower continuous propionate fermentation resulted in a higher long-term hydrogen production rate that produced a persistent hydrogen level just below the threshold for methanogens. This not only limited methanogenesis, but also resulted in a higher rate of dehalogenation.**

Semi-continuous CSTR Reactor



Two-Day Mass Balance for CSTR

Parameter	e^- equivalents consumed (μeq)	e^- equivalents formed (μeq)	% available H_2
Benzoate	- 9690		
Yeast extract	- 700		
Acetate		7200	
Biomass		360	
Methane		1288	58
Ethene		912	42
Total	- 10390	9760	100

- By delivering benzoate in a continuous manner, hydrogen remained at a low steady-state concentration of 2.6 nM, which is near the dehalogenation threshold.

- Of the hydrogen produced from benzoate fermentation, about 58% was used for methane production and 42% for dehalogenation. In batch studies, the corresponding efficiency of hydrogen utilization for dehalogenation was about 10%.

Biochemistry of vinyl chloride and *cis*-DCE dehalogenation

Objective

- To characterize the VC dehalogenating enzyme activity in cell-free extracts of an anaerobic mixed culture.

Conclusions

- Reductive VC dehalogenation is enzyme-catalyzed and can be studied *in vitro*.
- The VC dehalogenating enzyme activity is membrane-associated.
- Based on inhibition experiments with propyl iodide, the VC dehalogenating activity appears to be independent of corrinoid coenzymes and may therefore be different from previously described dehalogenases.
- cDCE is dehalogenated at approximately the same rate as VC. TCE and PCE were dehalogenated at significantly lower rates.

Growth and ethene formation from vinyl chloride in an anaerobic mixed culture

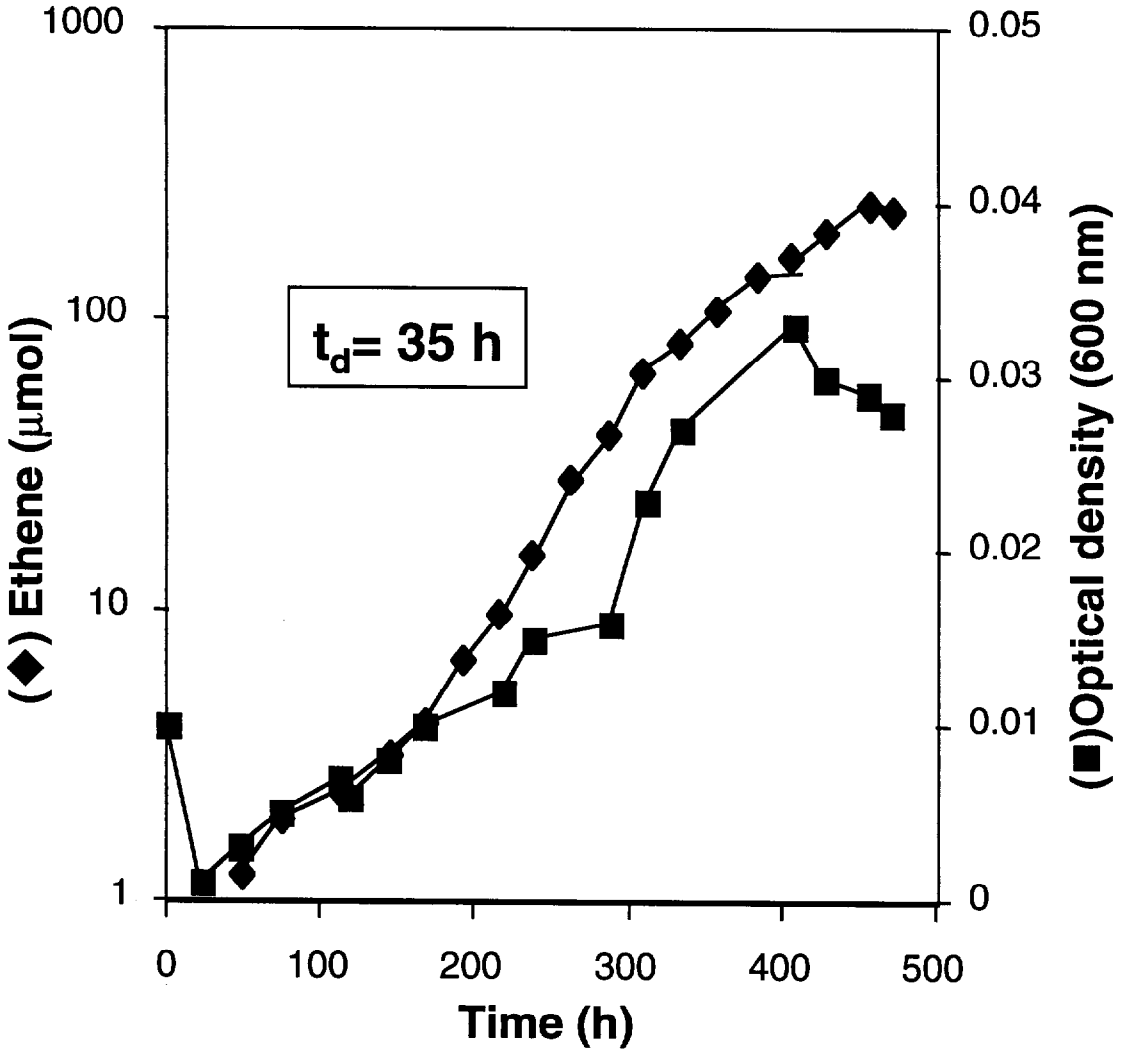
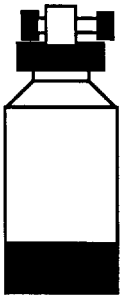


Fig.1. Optical density and ethene formation in a 1-Liter culture grown in the presence of 300 µmol VC. Note the log-scale for ethene formation.

- Ethene formation increased exponentially at a rate of 0.02 h^{-1}, corresponding to an apparent doubling time of 35 h.
- The specific in vivo ethene formation rate was about 30 $nmol \cdot min^{-1} \cdot mg\ protein^{-1}$.

Reductive VC dehalogenation catalyzed by cell-free extracts



***In vitro* assay**

100 mM Tris-HCl, pH 8.5
1 mM titanium(III) citrate
1 mM methyl viologen
cell-free extract (15 - 100 µg protein)
vinyl chloride (0.4 mM)

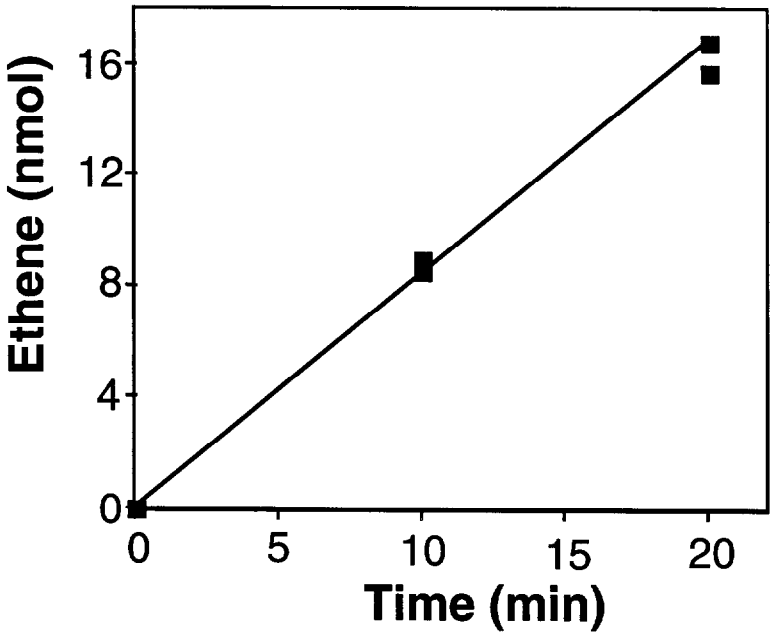


Fig.2. *In vitro* ethene formation from VC. Rates are calculated from the linear increase in the amount of ethene.

- Cell-free extracts catalyzed the reductive dehalogenation of VC at a specific rate of about 20 $nmol \cdot min^{-1} \cdot mg\ protein^{-1}$.

- No ethene formation was detected in heat-treated cell-free extracts or in the absence of VC.

Characteristics of the VC dehalogenating activity

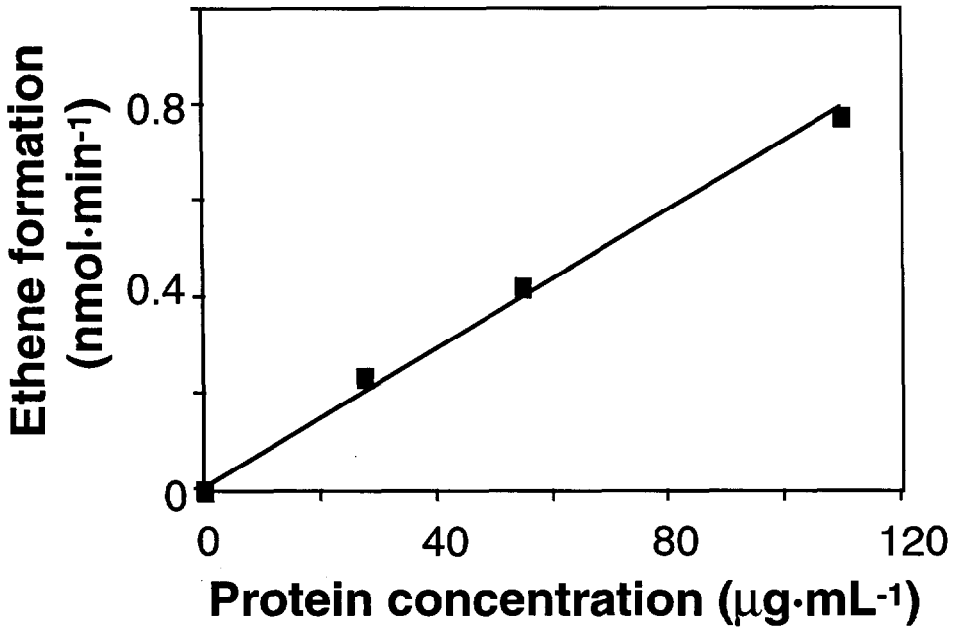


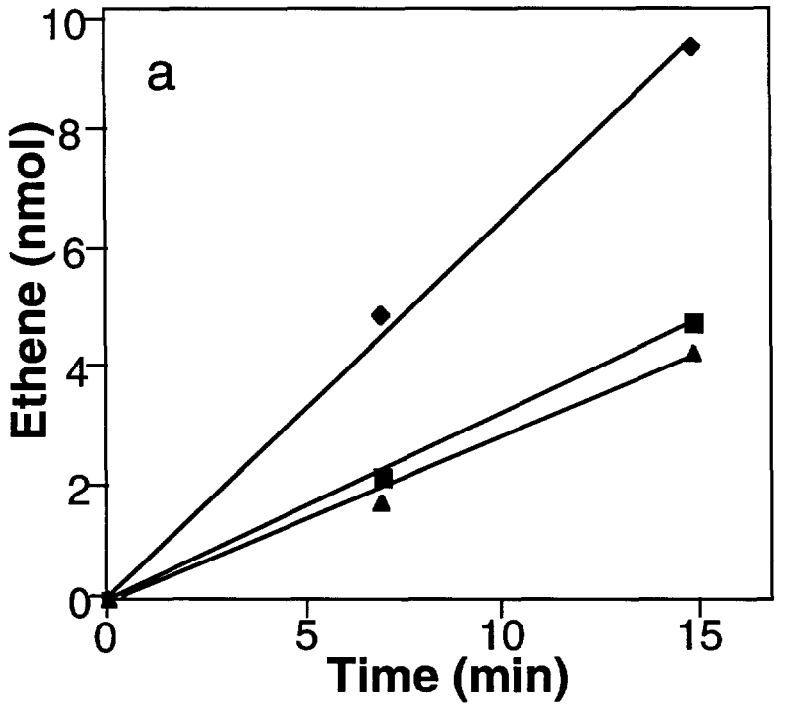
Fig.3. Protein dependence of reductive VC dehalogenation.

- The rate of VC dehalogenation was linearly dependent on the protein concentration in the assay. This suggests that not more than one diffusible cellular component is involved in the dehalogenation reaction.

- The apparent K_m-value for VC was determined to be about 76 μM, V_{max} was about 28 nmol·min^{-1}·mg protein $^{-1}$.

- After separating the cell-free extract into the cytoplasmic and the membrane fraction, the VC dehalogenating activity was found to be membrane-associated.

Effect of propyl iodide on reductive VC dehalogenation



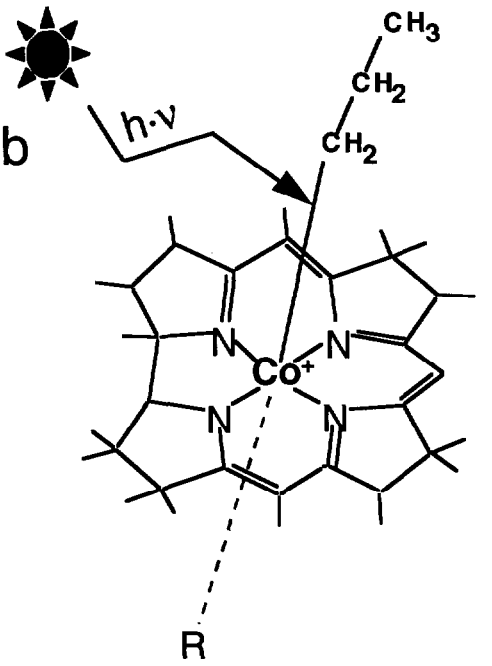


Fig.4a. Ethene formation from VC in the absence of inhibitor (◆), after incubation of cell-free extract with 100 uM propyl iodide (■), and after incubation with propyl iodide and subsequent illumination (▲).
Fig. 4b. Propyl iodide is a specific inhibitor of corrinoid-catalyzed reactions.

- Propyl iodide reduced the VC dehalogenating activity by about 60%. However, the inhibition could not be reversed by illumination of the assay mixture which suggests that a corrinoid is not involved in reductive VC dehalogenation.

Reductive cDCE dehalogenation catalyzed by cell-free extracts

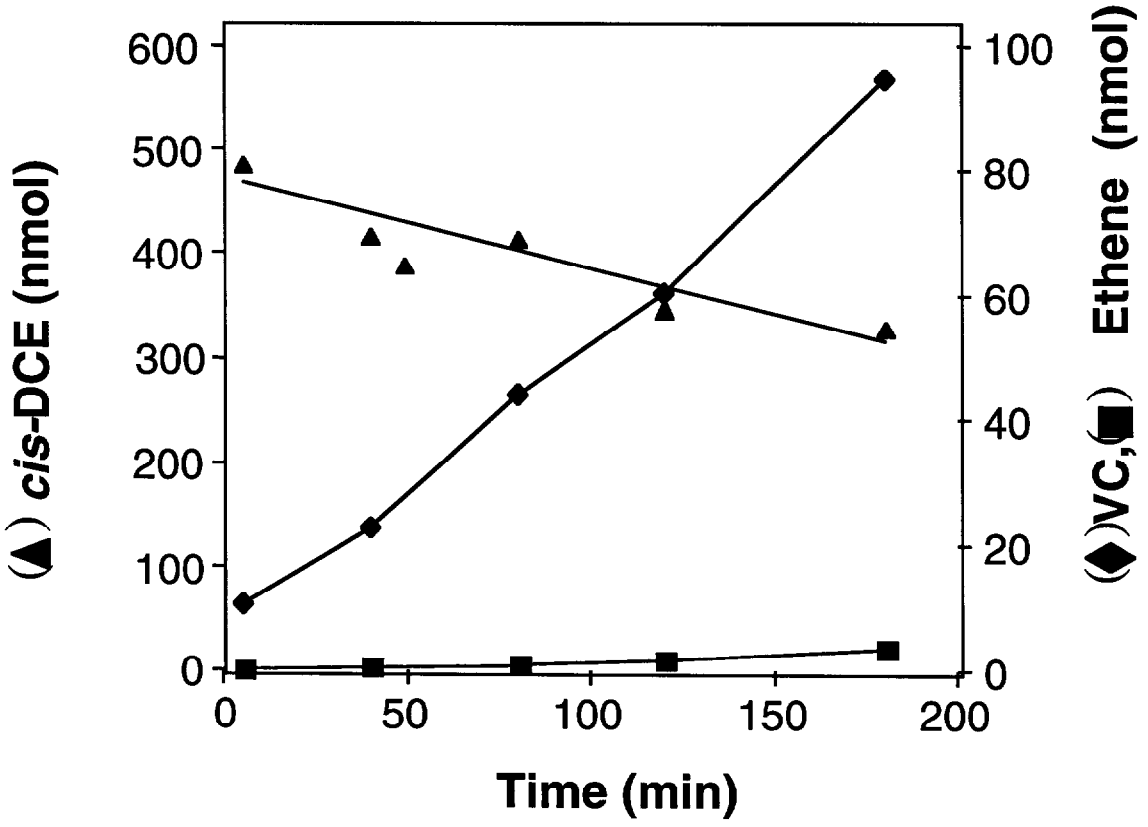


Fig.5. Reductive dehalogenation of cDCE (▲) to VC(◆) and ethene(■).

- cDCE was dehalogenated to VC and ethene. The apparent K_m-value for cDCE was about 190 μM, the apparent V_{max} was about 26 nmol·min^{-1}·mg protein^{-1}.

- TCE was transformed to cDCE, VC, and ethene at a low rate. The transformation ceased after about one hour. The PCE dehalogenation rate was too low to be measurable.

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