Environ. Sci. Technol. **1997**, *31*, 253−260

Corrinoid-Mediated Reduction of Tetrachloroethene, Trichloroethene, and Trichlorofluoroethene in Homogeneous Aqueous Solution: Reaction Kinetics and Reaction Mechanisms

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It is shown that in homogeneous aqueous solution containing titanium(III) citrate or titanium(III)−NTA as bulk electron donor, cobalamin, cobinamide, and cobamide are effective electron transfer mediators for the reduction of tetrachloroethene (PCE), trichloroethene (TCE), and trichlorofluoroethene (TCFE). For a given chlorinated ethene, the reaction rate varied only slightly with pH and type of corrinoid present and was about 5 and 50 times faster for PCE as compared to TCFE and TCE, respectively. Evidence is presented that the first and rate-limiting step of the reduction of PCE, TCE, and TCFE by super-reduced corrinoids is a dissociative one-electron transfer yielding the corresponding vinyl radicals. Furthermore, the elimination of a chloride radical from the 1,1-dichlorovinyl radical yielding chloroacetylene and subsequently acetylene is proposed to account for the direct formation of acetylene out of TCE. Finally, it is demonstrated that at higher reduction potentials the corrinoid mediators may be blocked by the formation of addition products.

Introduction

Reductive dehalogenation reactions are presently of great interest, primarily because of their potential use in the treatment of halogenated solvent wastes as well as in remediation approaches to removing such chemicals from contaminated soils and aquifers (*1−3*).

It has been shown that chlorinated ethenes including tetrachloroethene (perchloroethene, PCE) and trichloroethene (TCE) can be reduced microbially under methanogenic, acetogenic, and sulfate-reducing conditions to dichloroethenes (mainly *cis*-1,2-dichloroethene), vinyl chloride, ethene, and ethane (*4−7*). Enzymes containing tetrapyrrole cofactors like iron porphyrins (*8, 9*), cobalamins (*10*), and factor F_{430} (*11, 12*) are thought to be involved in the dehalogenation reactions. Recently, it has been demonstrated that certain bacteria can even use PCE and TCE as electron acceptors by reducing it to *cis*-dichloroethene (*13−15*). The PCE reductase, the enzyme catalyzing this reduction, probably contains a corrinoid (*16, 17*).

In a variety of studies, it has been shown that reduced corrinoids are potent reductants for chlorinated compounds in homogeneous aqueous solution (*18−25*). Several groups have demonstrated that such reduction reactions may also be carried out with immobilized cobalt corrins (*26−28*) or in bicontinuous microemulsions containing Co(I) macrocycles (*29*). However, rather little is known about the mechanism(s) and rate-limiting step(s) of the reaction between reduced corrinoids and halogenated compounds, particularly with halogenated ethenes. Such knowledge is important from both a fundamental scientific and a practical point of view, because corrinoids could be very useful electron transfer mediators for the abiotic treatment of halogenated solvent wastes (*28*).

Super-reduced corrinoids are very strong nucleophiles (*30*) that may react by a nucleophilic substitution, addition, or electron transfer mechanism with halogenated compounds (*31*). Any remediation or waste treatment technique using corrinoids as electron mediators should guarantee the rapid regeneration of the reactive corrinoid species without significant loss of activity over long time periods. Furthermore, it has to be assured that no toxic products accumulate. It is therefore necessary to understand the influence of important factors such as redox potential, pH, and structure of the corrinoid on the reaction kinetics as well as their effect on the product distribution. The reduction of PCE and TCE by corrinoids has been reported to occur via sequential hydrogenolysis yielding vinyl chloride as an intermediate (*22*). This compound is of great concern as a drinking water contaminant because of its high toxicity (*32*). In addition, it has been postulated that radicals may be formed as intermediates in dehalogenation reactions (*24, 25, 33*) that may lead to the formation of a variety of products, because such radicals may react with any nucleophile present (*24*).

In the study presented in this paper, we have investigated the effect of redox potential and pH on the cobalamin-mediated reduction of PCE, TCE, and trichlorofluoroethene (TCFE) in homogeneous aqueous solution using titanium(III) citrate or titanium(III)−NTA (Ti(III)−NTA) as bulk electron donors. In addition, experiments were conducted with cobinamide and cobamide as mediators. The major goals of this study were to evaluate the effect of the redox potential on reduction rates and product distributions and to get hints on the reaction mechanism(s).

Experimental Section

Chemicals and Stock Solutions. Tetrachloroethene (perchloroethene, PCE) (IR spectroscopy grade), trichloroethene (TCE) (puriss), *cis*-dichloroethene (cis-DCE) (purum), ethanol (puriss), trisodium citrate dihydrate (MicroSelect), nitrilotriacetic acid (NTA) (puriss), hexane (Burdick & Jackson), pentane (Burdick & Jackson), vitamin B_{12} (cyanocobalamin) (>98%), piperazine-*N*,*N*′-bis(2-ethanesulfonic acid) (PIPES) for pH 7 solutions and glycine for pH 9 solutions were from Fluka AG (Buchs, Switzerland). Trichlorofluoroethene (TCFE) (Fluorochem LTD, United Kingdom) was obtained from Chemie Brunschwig AG (Basel, Switzerland). 1-Chloro-3-fluorobenzene (99%) and *trans*-dichloroethene (trans-DCE) were purchased at Aldrich Chemie, Switzerland. 2-Amino-2-(hydroxymethyl)-1,3-propanediol (Tris) for pH 8 solution, $TiCl_3$ (15% in 10% HCl), and Na_2CO_3 were from Merck AG (Dietikon, Switzerland). A gas mixture containing methane, acetylene, ethene, ethane, carbon monoxide, and carbon dioxide (1% in N_2) (Scott Specialty Gases) was purchased from Supelco. d_7-Isopropyl alcohol (99.5%) and D_2O were from Armag AG (Döttingen, Switzerland). Cobinamide dicyanide

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S0013-936X(96)00386-0 CCC: $14.00

(approximately 95%) was from Sigma Chemie, Switzerland. Deuterochloroform ($CDCl_3$) was from Dr. Glaser AG (Basel, Switzerland). *p*-Cresolyl cobamide was isolated from *Sporomusa ovata* by a procedure similar to the one described in ref 34. All chemicals were used as received.

Stock solutions of the halogenated ethenes (0.1 M) were prepared in ethanol and were found to be stable for at least 10 days. Stock solutions of the corrinoids were prepared in water at a concentration of about 5 mM and stored at 4 °C in the dark. The exact concentration of the corrinoids was determined spectrophotometrically as dicyano complexes using the ϵ-value of 30 500 M^{-1} cm^{-1} (*31*).

Solutions of 100 mM buffer were titrated using HCl or NaOH to the desired pH at 35 °C. Stock solutions of 100 mM titanium(III) citrate were prepared in an anaerobic glovebox containing 96% N_2 and 4% H_2 according to the procedure described in ref 35. The final concentration of citrate was 300 mM. Ti(III)−NTA stock solutions of 100 mM were prepared as described in ref 36. The pH was adjusted with Na_2CO_3. Stock solutions of Ti(III) were kept in the glovebox at room temperature.

Experimental Procedures. *The kinetic experiments* were conducted in 59-mL serum flasks sealed with a Viton stopper (Maagtechnik, Dübendorf, Switzerland) and an aluminum crimp cap. All experiments were done in triplicate unless otherwise stated or as indicated by omission of standard deviations in the figures. The reaction mixtures were prepared in the glovebox. The pH buffer concentration was 90 mM, and the titanium(III) citrate or Ti(III)−NTA concentrations were 10 mM. The concentrations of corrinoids were ajusted by adding the appropriate aliquot from the stock solution. The concentration of the corrinoid was in the range of 0.4−8.0 μM for the experiments with PCE, 20−75 μM for TCE, and 2−3 μM for TCFE and was always smaller than the concentration of the halogenated compound. The headspace of the reaction mixture was less than 2 mL.

The experiment was started by adding 57 μL of an anaerobic ethanolic stock solution of the halogenated ethene with a gas-tight Hamilton syringe through the Viton stopper. The flasks were incubated at 35 °C in the dark. This temperature was chosen because it is the optimal temperature for many bacteria. At appropriate time intervals, 100-μL samples were withdrawn with a gas-tight syringe and extracted with 1 mL of hexane containing 10^{-4} M 1-chloro-3-fluorobenzene as the standard. The samples containing TCFE were extracted with 0.8 mL of pentane containing cis-DCE as the standard.

The product studies of the reduction of TCE and TCFE were carried out in 8-mL sealed serum flasks containing 2 mL of reaction mixture. The preparation of the reaction mixture was identical to the kinetic experiments. The concentration of cobalamin was 20 or 100 μM for TCE and 10 or 100 μM for TCFE experiments. The reaction was started by adding 10 μL of the ethanolic stock solution of the compound to the mixture. The stock solution contained pentane as the internal standard. The vials were incubated at 35 °C in the dark; 200-μL samples of the headspace were taken at appropriate times with a gas-tight syringe during more than 3 half-lives.

$D^\bullet$ abstraction experiments were conducted in duplicate in 8-mL sealed serum flasks containing 4 mL of the reaction mixture. Experiments with PCE and TCE were carried out at pH 9.0, those with TCFE were carried out at pH 8.0. Cobalamin was the mediator used for all abstraction experiments. Different volumes of d_7-isopropyl alcohol (0.1, 0.5, or 2% of the total volume of the solution) containing the appropriate amount of PCE, TCE, or TCFE to yield the same final concentration of the halogenated ethene in all bottles were added to the reaction mixture. After about 3 half-lives, 0.1 mL of the PCE reaction mixture was extracted with hexane and analyzed by GC/MS. Head space samples (200 μL) were taken when TCE or TCFE were the reactants and analyzed by GC/MS.

Experiments in D_2O. The 100 mM stock solutions of titanium(III) citrate (pH 9) and the 100 mM glycine buffer solution were lyophylized and then resuspended in D_2O. Cobalamin stock solutions were prepared in D_2O. The experimental setup was the same as for the experiments with d_7-isopropyl alcohol. A total of 20 μL of an anaerobic ethanolic stock solution of PCE, TCE, or TCFE, respectively, was added to start the reaction. After 3 half-lives, the products were analyzed by GC/MS.

Analytical Procedures. The hexane and pentane extracts were analyzed by GC on a Carlo Erba HRGC 5160 equipped with an electron capture detector (Carlo Erba ECD 400 with a Ni-63 source) or on a Carlo Erba MFC Mega Series GC equipped with a QMD 1000 mass spectrometer. The column used was a 30-m fused-silica DB-624 from J&W Scientific. As mentioned above, pentane or hexane contained 1-chloro-3-fluorobenzene or cis-DCE as standards, and concentrations were calculated by comparison with a calibration curve.

Head space samples were analyzed by GC on a Carlo Erba HRGC 5160 equipped with a flame ionization detector (FID-40, Carlo Erba) or on a Fisons Instruments GC 8165 equipped with a Fisons MD 800 mass spectrometer. The column was a 30-m megabore GS-Q from J&W Scientific. Quantification was done with pentane as the standard and by comparing with a calibration curve established at 35 °C.

The relative amounts of the deuterated versus non-deuterated products were calculated from the intensity ratios of the respective molecular ions, i.e., *m/z* 131 and 130 for TCE, m/z 97 and 96 for cis- and trans-DCE, and *m/z* 115 and 114 for the products of TCFE reduction.

For the quantification of the relative amounts of the dehalogenation products of TCFE, the reaction solution was extracted with CCl_3D, and ^1H-NMR spectra were recorded on an ASX-400 NMR spectrometer from Bruker at 400 MHz because no standards of the products were available.

Spectroscopic Measurements. Spectroscopic measurements were conducted to study the change of the oxidation state of the cobalt-center of the corrinoid during the reaction.

EPR Measurements. Two separate solutions were prepared, one containing 1 mM PCE, 1 mM titanium(III) citrate, and 95 mM glycine buffer (pH 9), and the other containing 1 mM cobalamin, 2.5 mM titanium(III) citrate, and 95 mM glycine (pH 9). An argon-purged 500-μL syringe was first filled with 200 μL of the PCE solution followed by 200 μL of the cobalamin solution. The solution was then transferred to an anaerobic EPR tube where mixing occurred and was frozen immediately in liquid nitrogen. Continous-wave EPR spectra were obtained with a Bruker ESP 300 spectrometer at 77 K (microwave frequency, 9.4225 GHz; modulation frequency, 100 kHz; modulation amplitude, 10 G; microwave power, 12.5 mW).

Spectrophotometric Studies. A total of 10 μL of a 0.01 M oxygen-free ethanol solution of PCE was added to a 1-cm quartz cuvette with a gas-tight syringe. The cuvette was sealed with a butyl rubber stopper and filled with 1 mL of a solution containing 35 μM cobalamin, 90 μM titanium(III) citrate, and 100 mM glycine buffer at pH 9. Spectra were recorded at room temperature with a Hitachi U 2000 spectrophotometer.

The influence of TCE and acetylene on cobalamin spectra was studied in 1-cm quartz cuvettes sealed with a Teflon or butyl rubber stopper, respectively, containing solutions made of 1.6 mL of 25 μM cobalamin, 10 mM titanium(III) citrate, and 90 mM PIPES buffer (pH 7). In one experiment, 10 μL of a 0.03 M oxygen-free ethanol solution of TCE was added to the cuvette in a glovebox. In another experiment, 30 μL of pure acetylene was added to the cuvette through the stopper

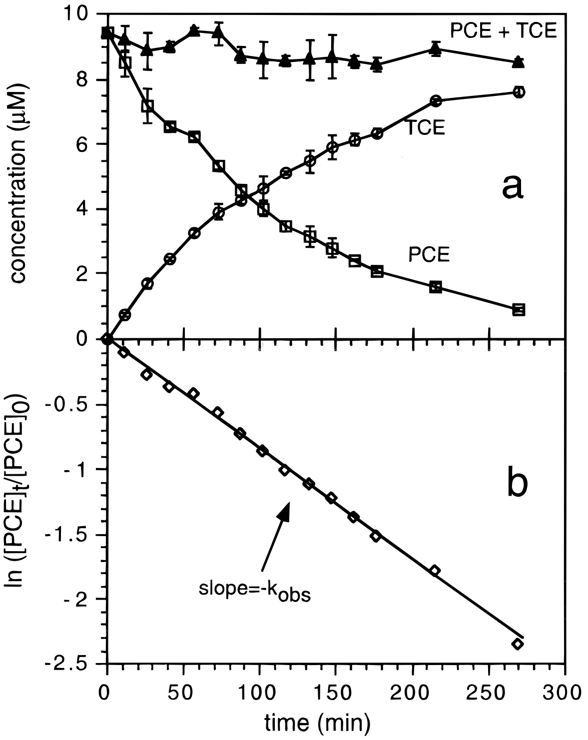


FIGURE 1. Reduction of 9.5 μM tetrachloroethene (PCE) (□) in 1 μM cobalamin and 10 mM titanium(III) citrate at pH 9.0 and 35 °C. (a) Mass balance (▲): The only product observed was trichloroethene (TCE) (○). (b) Plot of ln ([PCE]$_t$/[PCE]$_0$) (◇) versus time.

with a gas-tight Hamilton syringe. Spectra were recorded on a Kontron Uvikon 810 spectrophotometer at 12 °C.

Results and Discussion

Reaction Kinetics. With cobalamin present as a mediator at various concentrations, the reduction kinetics of PCE, TCFE, and TCE were studied at pH 7, pH 8, and pH 9 in 10 mM aqueous titanium(III) citrate and at pH 8 and pH 9 in 10 mM aqueous Ti(III)−NTA. In addition, experiments were carried out with cobinamide or cobamide at pH 9 in 10 mM aqueous titanium(III) citrate. Except for the reaction of TCE at pH 8 in aqueous Ti(III)−NTA, the rate of disappearance of a given chlorinated ethene (CE) could be described by a pseudo-first-order rate law:

$$-\frac{\mathrm{d[CE]}}{\mathrm{d}t} = k_{\mathrm{obs}}[\mathrm{CE}] \qquad (1)$$

and, thus

$$\ln\frac{[\mathrm{CE}]_t}{[\mathrm{CE}]_0} = -k_{\mathrm{obs}}t \qquad (2)$$

where $[\mathrm{CE}]_t$ and $[\mathrm{CE}]_0$ are the concentrations of the chlorinated ethene at time t and time zero, respectively. This is illustrated in Figure 1 for the reaction of PCE in 10 mM titanium(III) citrate at pH 9.0 in the presence of 1 μM cobalamin. Note that the pseudo-first-order rate constant, k_{obs}, derived from this type of experiment is a composite of the reaction of the CE with the mediator and with the bulk reductant, i.e., titanium(III) citrate or Ti(III)−NTA, respectively. For all compounds and conditions investigated, the contribution of the reaction with the Ti(III) bulk reductant to the overall reaction rate was found to be less than 10% and was accounted for by eq 3:

$$k'_{\mathrm{obs}} = k_{\mathrm{obs}} - k_{\mathrm{obs,bulk}} \qquad (3)$$

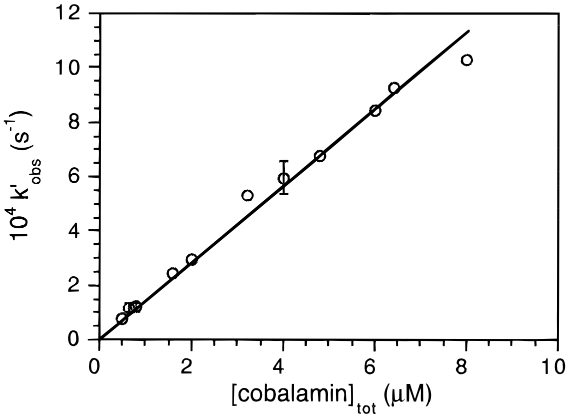


FIGURE 2. Pseudo-first-order rate constants k'_{obs} (○) obtained from eq 3 as a function of initial cobalamin concentration; [PCE]$_0$ = 10 μM; [titanium(III) citrate] = 10 mM; pH 9.0; 35 °C.

where k'_{obs} is the pseudo-first-order rate constant for the disappearance of the CE due to the reaction with the mediator.

The fact that, for a given mediator concentration, k'_{obs} was independent of the CE concentration even at very high CE to mediator concentration ratios (e.g., 200:1, data not shown) indicates that the reactive corrinoid was always present at a constant steady-state concentration. Furthermore, as is illustrated by Figure 2, k'_{obs} was linearly dependent on the total mediator concentration:

$$k'_{\mathrm{obs}} = k_2[\mathrm{mediator}]_{\mathrm{tot}} \qquad (4)$$

where k_2 is the apparent second-order rate constant of a given CE for the reaction with the reduced corrinoid. Based on the UV/VIS spectra, it can be assumed that more than 90% of the corrinoid was always present in the super-reduced form [i.e., as cobalt(I) corrinoid] before the addition of the CE to the reaction solution. Thus, k_2 values were determined for the various compounds and solution conditions using eq 4. They are summarized in Table 1. Considering the reaction with cobalamin as mediator, it can be seen that, in general, PCE reacted about 3−4 times faster than TCFE and about 50−80 times faster than TCE. The latter result is consistent with the findings of Gantzer and Wackett (*22*). Furthermore, with titanium(III) citrate as the bulk reductant, somewhat smaller k_2 values were obtained at pH 7 as compared to pH 8 and pH 9. Finally, at a given pH, the k_2 values were smaller if Ti(III)−NTA was used as the bulk reductant instead of titanium(III) citrate.

The most likely cause for the differences found in the k_2 values of a given compound are the different redox conditions prevailing in the various experiments. Although, as mentioned above, the cobalamin was initially completely reduced in all cases, its regeneration rate may have become a limiting factor at higher reduction potentials. In the pH range of the experiments, the reduction potential of the Ti(IV)/Ti(III) couple is strongly pH-dependent [i.e., $\Delta E/\Delta\mathrm{pH} = -60$ mV at 25 °C(*37*)]. It is therefore possible that in the case of titanium(III) citrate at pH 7, in contrast to pH 8 and pH 9, the steady-state concentration of the reactive cob(I)alamin during the reaction was smaller than the total cobalamin concentration, thus yielding a smaller apparent k_2 value. The reduction potential of the cobalamin itself is not pH-dependent in this pH range (*38*). The same arguments can be used for the experiments with Ti(III)−NTA, which has a higher reduction potential than titanium(III) citrate. In fact, at pH 7, as indicated by the UV/VIS spectrum, the cobalamin was not completely reduced in 10 mM Ti(III)−NTA. Furthermore, the above explanation is supported by the findings that the reduction of cob(II)alamin and cob(III)alamin occured at slower rates at pH 7 than pH 8 and pH 9 in titanium(III)

TABLE 1. Second-Order Rate Constants k_2 ($M^{-1}\ s^{-1}$)[a] of PCE, TCFE, and TCE for Reaction with Cobalamin

	titanium(III) citrate			Ti(III)−NTA	
	pH[b] 7.05	pH 8.05	pH 9.0	pH 8.05	pH 9.0
PCE	125 ± 7	179 ± 10	155 ± 21 (200 ± 21)[c] (154 ± 5)[d]	102 ± 12	120 ± 15
TCFE	28 ± 1	41 ± 1	49 ± 5 (98 ± 1)[c] (nd)[d]	28 ± 1	37 ± 2
TCE	2.4 ± 0.2	3 ± 0.1	2.7 ± 0.2 (5.4 ± 0.2)[c] (nd)[d]	(1.2 ± 0.1)[e]	1.5 ± 0.1

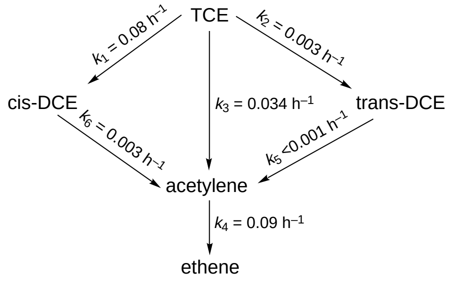
[a] The intervals given are standard deviations. [b] The error range of the pH is 0.05 in all the reactions. [c] Rate constants for the reaction with cobinamide. [d] Rate constants for the reaction with cobamide. [e] Initial reaction rate constant. nd, not determined.

citrate. Similarly, at a given pH (i.e., pH 8 and pH 9), the reduction was slower in Ti(III)−NTA than in titanium(III) citrate (data not shown). Finally, similar effects of the redox conditions on the cobalamin-mediated reduction of halogenated compounds have been observed in other studies (*20, 23, 33*).

To evaluate the influence of the coordinating base on the reactivity of the corrinoid, the reaction of PCE with cobinamide and cobamide was studied at pH 9 in 10 mM titanium(III) citrate. Cobinamides can be obtained from cobalamins by hydrolytic cleavage of the nucleotide side chain at the phosphate group. Thus, their side chain has lost the coordinating ability toward the cobalt atom. *p*-Cresolyl cobamide is a corrinoid that has been isolated from *Sporomusa ovata*, a homoacetogenic bacterium, which has been shown to reduce PCE cometabolically *(39)*. In this corrinoid the base 5,6-dimethylbenzimidazole of cobalamin is replaced by *p*-cresol that cannot coordinate the cobalt. The results summarized in Table 1 show that only minor differences were found between the k_2 values obtained for the three mediators, which indicates that the effect of the axial ligand on the reactivity of the corrinoid is not very pronounced. This is in accordance with the results of Lewis (*24*), who also observed only a slightly faster reduction rate of CCl_4 with cobinamide as compared to cobalamin.

Initial Product Formation. As illustrated in Figure 1a, in all experiments summarized in Table 1, initially PCE was converted quantitatively (i.e., ≥ 95%) to TCE. No other products could be detected after 3 reaction half-lives with the analytical techniques used. These findings are consistent with the results of Gantzer and Wackett (*22*). Particularly, no evidence was obtained that PCE also reacted by β-elimination to yield dichloroacetylene and subsequently acetylene, as has been proposed for its reduction by zero-valent metals (*3*).

Figure 3 shows the time course of the initial product formation when reducing 220 nmol of TCE by cobalamin at pH 9 in titanium(III) citrate. As is indicated by the mass balance, under these conditions, all TCE initially added could be recovered in the detected products after 30 h: cis-DCE (63%), trans-DCE (5%), acetylene (7%), and ethene (25%). The time courses of the concentrations of the various compounds shown in Figure 3 can be modeled by using the following simple reaction scheme:



(5)

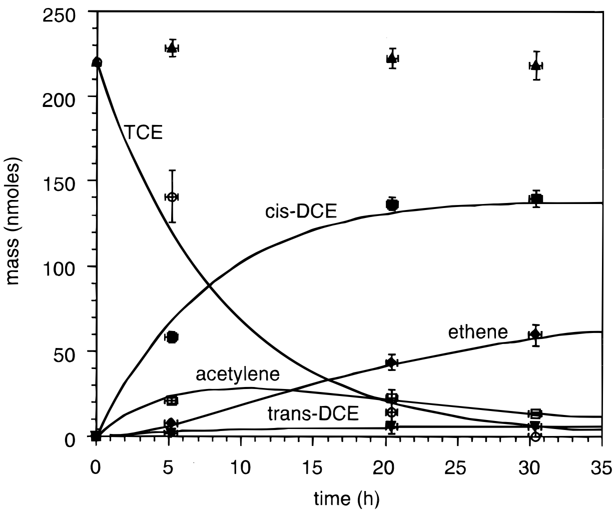
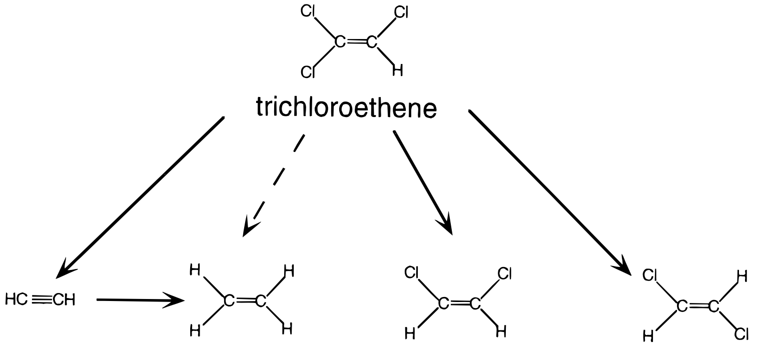


FIGURE 3. Reduction of 220 nmol of trichloroethene (TCE) (○) in 20 μM cobalamin and 10 mM titanium(III) citrate at pH 9.0 at 35 °C. Time courses of the masses of TCE (○) and its transformation products. The products observed were *cis*-dichloroethene (cis-DCE) (■), *trans*-dichloroethene (trans-DCE) (▼), acetylene (□), and ethene (◆); mass balance (▲). The lines are the best fits using the model described by eq 5.

The indicated pseudo-first-order rate constants were determined by using the simulation tool AQUASIM (*49*). Figure 3 shows that the experimental data could be modeled quite well (solid lines) with this reaction scheme. Note that in this scheme (eq 5), it is assumed that all of the cis- or trans-DCE is converted to acetylene. However, as is indicated by the rate constants, even under these assumptions, no significant fraction of the acetylene was formed by the reduction of cis- or trans-DCE, thus suggesting that acetylene was produced by another pathway. Furthermore, this is supported by the finding that cis-DCE and trans-DCE are indeed reduced very slowly by cobalamin under the given conditions (data not shown). The data also suggest that ethene was primarily formed by the reduction of acetylene, which is in agreement with the finding that acetylene was readily reduced to ethene under the same reaction conditions (data not shown).

Figure 4 shows that with decreasing pH and thus with increasing redox potential, the amount of acetylene, and particularly of ethene produced, decreased and that the mass balance became less complete. A similar product distribution as found for pH 7 and titanium(III) citrate (Figure 4) was obtained at pH 8 using Ti(III)−NTA as the bulk reductant. Obviously, with increasing redox potential, part of the reduction products of TCE was present as nonvolatile product(s) as no other volatile products could be detected with the analytical setup used. With cobinamide as the mediator at pH 9 in 10 mM titanium(III) citrate, the amount of DCEs



	acetylene	ethene	cis-DCE	trans-DCE	Σ
pH 7	4 %	2 %	72 %	6 %	84%
pH 8	5 %	10 %	70 %	4 %	89%
pH 9	7 %	25 %	63 %	5 %	100%

FIGURE 4. Product distribution of the reduction of trichloroethene (TCE) at pH 7, pH 8, and pH 9 in 10 mM titanium(III) citrate and 20 μM cobalamin.

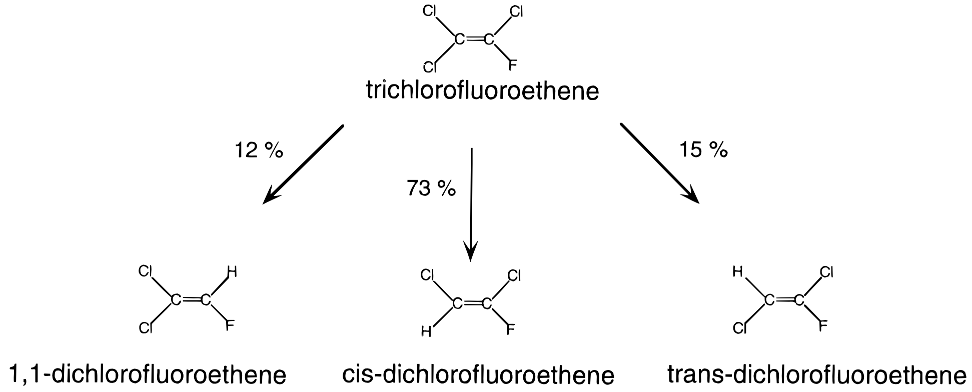


FIGURE 5. Product distribution of the reduction of trichlorofluoroethene (TCFE) at pH 7, pH 8, and pH 9 in 10 mM titanium(III) citrate and 3 μM cobalamin.

produced did not change while the recovery of TCE transformation products was just about 90% and less acetylene and ethene were detected (data not shown).

For TCFE, after 3 half-lives, only the three hydrogenolysis products could be detected with *cis*-dichlorofluoroethene (DCFE) being the major product formed (see Figure 5). At all pH values, the same product distribution was found.

Reaction Mechanisms and Reaction Pathways. Several different reaction mechanisms have been postulated for the reaction of Co(I) corrinoids with halogenated compounds. For many halogenated alkanes, nucleophilic substitution is generally thought to be the initial reaction step (*20, 25, 30*), while addition to double bonds has been proposed for the reaction with halogenated ethenes (*40*). However, in many cases it is difficult to distinguish clearly between these two mechanisms and a pure electron transfer mechanism that may also occur (*41, 31*).

In order to gain insight into the mechanism(s) of the reactions of PCE, TCFE, and TCE with the reduced cobalamin, the reactions were followed by UV/VIS spectroscopy under conditions where re-reduction of the cobalamin was not possible. Figure 6 shows that upon the addition of 100 μM PCE from an anaerobic stock solution to 35 μM cobalamin, which had been reduced with 90 μM titanium(III) citrate in 100 mM glycine (pH 9), the large peak at 390 nm (characteristic of cob(I)alamin), disappeared and maxima around 310 and 470 nm (typical of cob(II)alamin) appeared. The same observations were made at pH 7 and pH 8 and after the addition of TCE or TCFE instead of PCE. When a pure anaerobic solution of ethanol was added to the cobalamin as a control, cob(I)alamin did not react and the spectrum remained constant. Note, however, that similar spectra as the ones shown in Figure 6 are also obtained for methylaquocobinamide (*42*) and other alkylcorrinoids (*20*). This led Gantzer and Wackett (*22*) to postulate from similar results that a carbon–cobalt bond formed between cobinamide and tetrachloroethene.

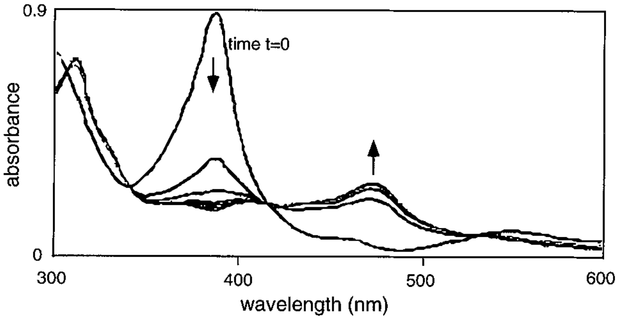


FIGURE 6. UV/VIS spectra of cobalamin (35 μM) in 90 μM titanium(III) citrate solution at pH 9.0 at room temperature before and after the addition of 10 μL of 10 mM tetrachloroethene(PCE). The maximum at 390 nm characteristic of cob(I)alamin is disappearing, and a new maximum at 470 nm characteristic of cob(II)alamin appears.

As cobalamin is paramagnetic in the cob(II)alamin state, EPR measurements were carried out to verify whether really cob(II)alamin was formed. EPR spectra were recorded upon

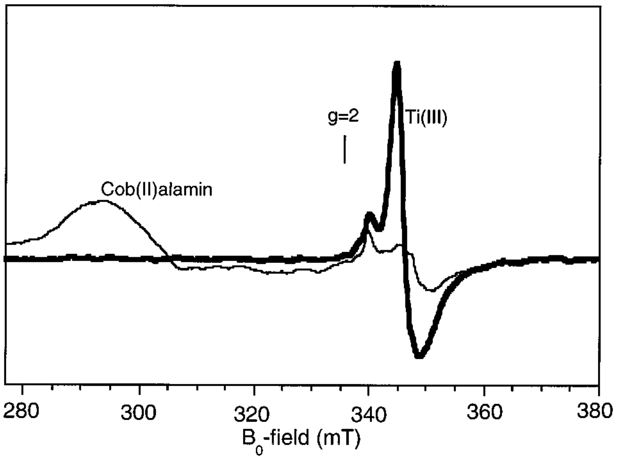
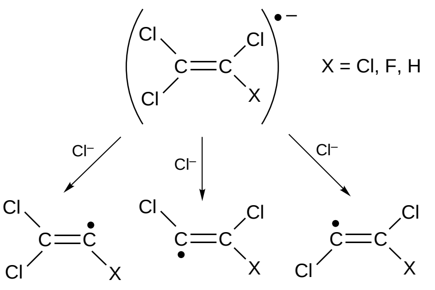


FIGURE 7. EPR spectra at 77 K of 0.5 mM cobalamin in 1.7 mM titanium(III) citrate at pH 9.0 upon the addition of an anaerobic solution of ethanol (thick line) and upon the addition of an anaerobic solution of 1 mM tetrachloroethene (PCE) (thin line).

addition of PCE to a titanium(III) citrate-reduced cobalamin solution. As shown in Figure 7, cobalamin exhibited a EPR signal attributable to cob(II)alamin as indicated by the *g* values (g_{xy} is approximately 2.23 and g_z is 2.00). The decrease of the signal of Ti(III) (g_{xy} is approximately 1.94 and g_z is 1.98) upon addition of PCE showed that titanium(III) was oxidized when PCE was added to the solution. Extraction of the reaction mixture afterwards revealed the presence of TCE, meaning that PCE was reduced to TCE with concurrent formation of cob(II)alamin. The addition of pure ethanol instead of PCE gave no Co(II) signal (Figure 7), indicating that oxygen contamination was not an inherent problem. These observations suggest that the first step of the reduction of PCE, TCFE, and TCE is a one-electron transfer of cob(I)alamin to the halogenated ethene with formation of cob(II)alamin.

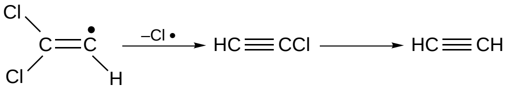
It is likely that, after the proposed one-electron transfer, Cl^- is eliminated, thus forming one (when X = Cl) or three (when X = F, H) possible radical species:



(6)

Unfortunately, spin-trapping or spin-labeling agents could not be used to demonstrate the formation of these radicals, because these agents reacted readily with cob(I)alamin and titanium(III) citrate. Nevertheless, good indications for the formation of the above postulated radicals were obtained from experiments carried out in the presence of different amounts of d_7-isopropyl alcohol, which is a good D•-donor. Table 2 shows that with increasing amounts of d_7-isopropyl alcohol, the amount of the deuterated products of PCE, TCE, and TCFE increased. In the case of TCFE, the three deuterated products indicated that all three expected radicals were formed, while evidence for only two radicals, namely, the two 1,2-dichlorovinyl radicals, was found for TCE because no 1,1-dichloroethene was detected. The relative abundances of the deuterated isomers and of the non-deuterated isomers formed were identical.

In analogy to TCFE, the formation of all three radicals could have been expected for TCE as well. A possible explanation for the observed difference is that, because of the low stability of the 1,1-dichlorovinyl-radical, the elimination of a chloride radical yielding chloroacetylene and subsequently acetylene is much faster than the abstraction of a D•:



(7)

The proportion of acetylene and ethene formed relative to the DCEs was unaffected when increasing the amount of d_7-isopropyl alcohol in solution, suggesting that the formation of acetylene was unaffected by the presence of a good D•-donor. Furthermore, when 100 μM cobalamin was used instead of 20 μM, the product distribution of TCE did not change. A recombination of the radicals with cobalamin to form a Co−C bond, a process that has been postulated for many Co(I) substitutions of alkyl halides (*31*), can therefore be excluded. The cleavage of the resulting Co−C bond would lead to acetylene, and thus, an increase of the amount of acetylene formed would be expected with increasing concentration of cobalamin.

Using a similar method, Chiu and Reinhard (*23*) showed that the reduction of CCl_4 proceeded via the formation of radicals. It should be pointed out that the formation of radicals would also be expected if cob(I)alamin added to the double bond and the resulting Co−C bond was cleaved homolytically. However, other studies have shown (*43, 44*) that in protic solvents the heterolytic cleavage of the Co−C bond is favored, leading to cob(III)alamin and the anion. The reduction of Co(III) to Co(II) in the experiment shown in Figure 6 would proceed slowly as the reductant Ti(III) is limiting. Thus, the rapid formation of cob(II)alamin and the formation of radical intermediates support the postulated electron transfer mechanism.

Table 2 shows that the less halogenated radicals, i.e., the radicals formed from the reduction of TCE abstract a D• much more efficiently from d_7-isopropyl alcohol than the radicals formed from PCE and TCFE. For the radicals, an alternative reaction pathway is reduction by the bulk reductant (that is present in large excess) to the anion with subsequent protonation. This pathway was verified by conducting the reaction in deuterated water. For PCE and TCFE, over 90% of the reaction products were deuterated, while TCE only yielded about 60% deuterated cis- and trans-DCE, which is consistent with the results of the D• abstraction experiments (Table 2). Note that in neither case was there any indication of the elimination of a Cl^- from the anionic intermediate, which would have yielded the corresponding acetylene. This is in agreement with observations made by Houser (*45*), who found that the elimination of chloride from the trichloroethene anion is a slow process. These findings support the hypothesis that the formation of acetylene and ethene from the reduction of TCE (Figure 4) occurred primarily through the 1,1-dichlorovinyl radical (eq 7). The preferential formation of cis isomers was found by Kopchik and Kampmeier for other ethenes, and they concluded that the cis/trans ratio was kinetically controlled (*48*).

Figure 5 shows that with decreasing pH both the amount of acetylene and ethene formed from TCE and the recovery of TCE in volatile products decreased. A spectrophotometric analysis of the reaction mixtures of the TCE experiments at pH 7 and pH 8, where the mass balance was incomplete, revealed that the cobalamin was not completely present as cob(I)alamin. The mixture showed a weak absorption maximum around 520 nm (spectra not shown), which is typical for alkylcobalamins (*23*). When adding 300 μM TCE to 25 μM cobalamin that had been reduced with 10 mM titanium(III) citrate at pH 7 at 12 °C, the maximum at 390 nm disappeared slowly and a maximum appeared at 520 nm,

TABLE 2. Ratio of Deuterated and Non-Deuterated Products of PCE, TCE, and TCFE as a Function of the Amount of d_7-Isopropyl Alcohol Present in Solution

amount of d_7-isopropyl alcohol (in % of total vol)	% of deuterated TCE, the products of PCE	% of deuterated DCEs, the products of TCE	% of deuterated DCFEs, the products of TCFE
2 (1 for PCE)	10.4 ± 0.9	31 ± 3	5.2 ± 0.3
0.5	8.5 ± 0.6	14.5 ± 2	4.6 ± 0.02
0.1	6.5 ± 0.5	7.5 ± 1	4.4 ± 0.01

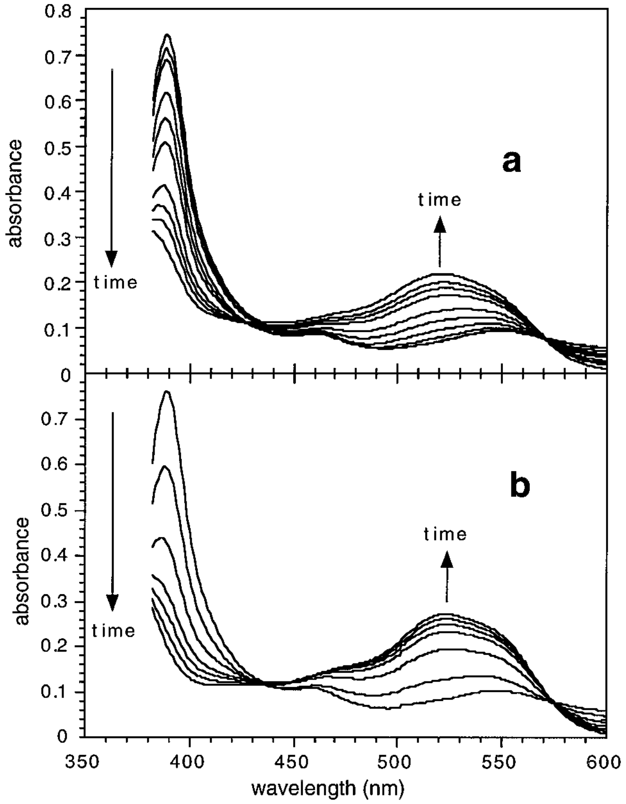


FIGURE 8. UV/VIS spectra of cobalamin in 10 mM titanium(III) citrate solution at pH 7.05 at 12 °C. (a) Spectra before and after the addition of 300 μM trichloroethene (TCE). (b) Spectra before and after the addition of acetylene.

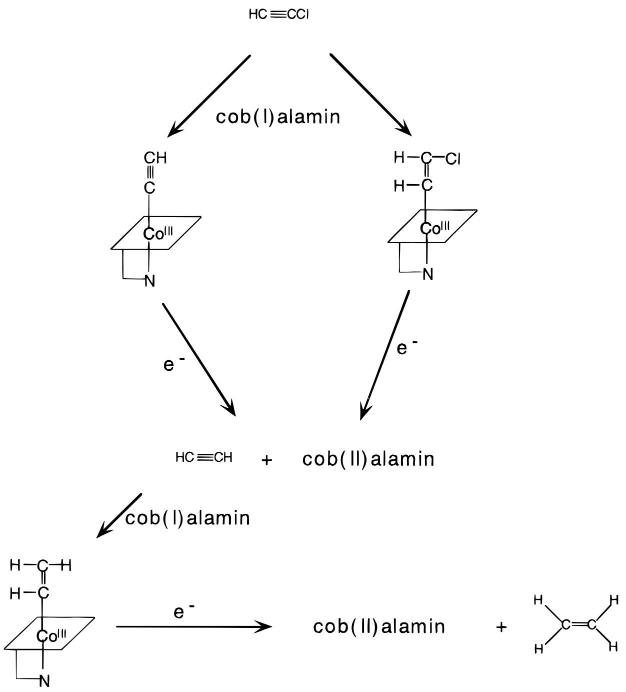


FIGURE 9. Possible reaction pathways of chloroacetylene to ethene in the presence of cob(I)alamin.

indicating the formation of an alkylcobalamin (Figure 8a). After the disappearance of cob(I)alamin, the reaction mixture was extracted, and about 70 μM cis-DCE could be detected. Thus, significantly more cis-DCE was formed than cob(I)-alamin had disappeared. As shown before, the formation of cis-DCE did not proceed via an alkylcobalamin. However, another fraction of the TCE reacted to give an alkylcobalamin and blocked the regeneration of the reactive cobalamin species. Part of the unrecovered TCE at low pHs or with Ti(III)−NTA as bulk electron donor may, therefore, have been present as alkylcobalamins. These were most likely the products of the reaction of cobalamin with chloroacetylene (Figure 9) that was formed from 1,1-dichlorovinyl radical (eq 7). The reaction of acetylene in 25 μM cobalamin that had been reduced with 10 mM titanium(III) citrate at pH 7 at 12 °C gave a series of spectra very similar to the one found with TCE (Figure 8b). It is known that acetylene and bromoacetylene (*40*) form addition products with cobalamin. Johnson (*46*) demonstrated that acetylene reacts with cobalamin to form vinylcobalamin while bromoacetylene yields ethynylcobalamin and bromovinylcobalamin. The spectra of vinylcobalamin and bromovinylcobalamin were identical, and the spectra of ethynyl-, vinyl-, and bromovinylcobalamin differ only by their most intense bands (γ-bands) in the 350−370-nm region. If one assumes that bromovinylcobalamin and chlorovinylcobalamin exhibit the same spectrum, and because titanium(III) citrate itself absorbs in the region of the γ-bands, it cannot be concluded from our spectra which of the alkylcobalamins were present in the solution. Currently work is in progress to determine the structure of the alkylcobalamin(s) formed from the reduction of TCE.

Zhou et al. (*44*) showed that electron-withdrawing groups at the Co-bound C-atom shift the reduction potential of the corrinoid to more positive values. So, it is likely that adducts like vinyl- and ethynylcobalamin are more difficult to reduce (*44*), thus slowing down or even blocking the regeneration of the reactive Co(I) species. In fact, Figure 10 shows that, at pH 8 with Ti(III)−NTA as the reductant, the reaction of 100 μM TCE with 3 μM cobalamin as a mediator did not follow pseudo-first-order kinetics; it proceeded more slowly than anticipated after a few hours. The regeneration of the reactive cobalamin became probably rate-limiting. Hence, if corrinoids are to be used for remediation purposes, the redox potential of the reaction medium has to be kept low enough to allow quick regeneration of the reactive Co(I) species.

In conclusion, the results of this study suggest that the first and rate-limiting step of the reduction of PCE, TCE, and TCFE by super-reduced corrinoids is a dissociative one-electron transfer yielding the corresponding vinyl radicals. No evidence for a reductive β-elimination mechanism leading to the direct formation of chloroacetylenes and finally acetylene, as has been proposed for the reduction of chlorinated ethenes by zero-valent metals (*3*), has been found. The significant amounts of acetylene and ethene formed from the reduction of TCE can be attributed to a dissociation reaction of the 1,1-dichlorovinyl radical yielding chloroacetylene. As compared to the two 1,2-dichlorovinyl radicals, the 1,1-dichlorovinyl radical seems to be very unstable in

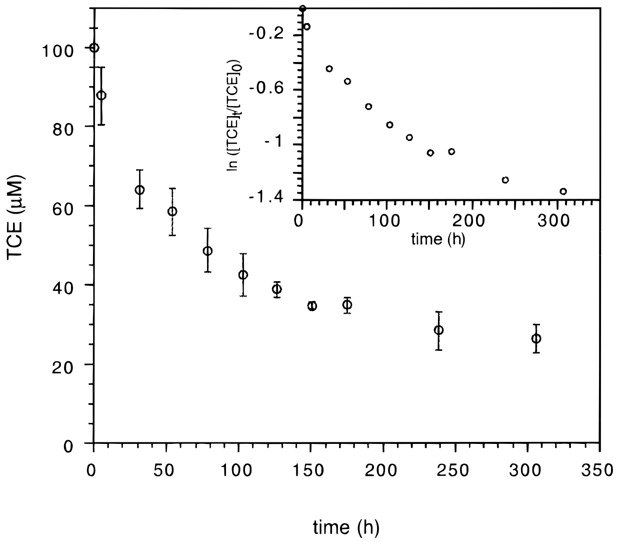


FIGURE 10. Reduction of trichloroethene (TCE) (○) in 3 μM cobalamin and 10 mM Ti(III)−NTA at pH 8.05 at 35 °C. Inset: Plot of ln ($[TCE]_t$ / $[TCE]_0$) (○) versus time.

aqueous solution, which may explain why 1,1-dichloroethene has never been found in abiotic or microbially mediated reductions of TCE (*4, 13, 15, 22*). To our knowledge this is the first report demonstrating a direct pathway for acetylene and ethene formation from the reduction of TCE by super-reduced corrinoids.

From a practical point of view, the results of this study are important for several reasons. First, we have shown that at a low enough redox potential, independent of pH, several corrinoids are effective electron transfer mediators that could be used for the reductive dehalogenation of PCE, TCE, and TCFE. Furthermore, we have demonstrated that TCE may be transformed by a pathway that does not lead to the formation of vinyl chloride. For waste treatment or remediation schemes, a further evaluation of this pathway should be of great interest. Finally, we have also shown that, at higher reduction potentials, the regeneration of the super-reduced corrinoid species may become rate-limiting and that these species may even be blocked by addition products. The formation of such addition products, particularly during the reduction of dichloroethenes and vinyl chloride, is presently under investigation in our laboratory.

Acknowledgments

We thank Thomas Bucheli for help with GC/MS measurements, Roland Hany for the NMR measurements, Paul Schosseler for the EPR measurements, and Francisco Vazquez for technical assistance. We are indepted to Jörg Klausen, who performed the simulations shown in Figure 3. Johanna Buschmann, Rick Devlin, Jörg Klausen, Klaus Pecher, Anja Sinke, and Wolfram Schumacher are kindly acknowledged for reviewing the manuscript.

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Received for review May 1, 1996. Revised manuscript received August 27, 1996. Accepted August 28, 1996.[⊗]

ES9603867

[⊗] Abstract published in *Advance ACS Abstracts*, November 1, 1996.