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Reductive Dechlorination of Tetrachloroethylene (PCE) Catalyzed by Cyanocobalamin

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ABSTRACT

A biomimetic system has been developed for the reductive dechlorination of tetrachloroethylene (PCE). PCE was dechlorinated to trichloroethylene (TCE) and 1,2-dichloroethylene (DCE) in the presence of dithiothreitol or Ti (III) citrate and catalytic amounts of cyanocobalamin in both homogeneous reaction mixtures and packed bed reactor systems. In packed bed reactors with Ti (III) citrate as the reductant, PCE (0.18 m*M*) conversion averaged 55% at residence times of 1.75 and 3.5 h. The product distribution was 94% TCE and 6% DCE at the lower residence time. DCE formation increased to 45% at the higher residence time. No reduction of PCE was observed in the absence of cyanocobalamin. This system may be useful as a means of pretreatment of halogenated aliphatic hydrocarbons in advance of biological treatment.

Index Entries: Tetrachloroethylene (PCE); cyanocobalamin; vitamin B12; trichloroethylene (TCE); dichloroethylene (DCE).

INTRODUCTION

Chlorinated hydrocarbons are used as cleaning solvents in industry for metal parts, plastics, and textiles. Their noninflammability is an important property of chlorinated hydrocarbons, which has in part led to their wide use. However, chlorinated hydrocarbons are ozone depleters and suspected carcinogens, and exert a variety of toxic effects in humans (*1*). Therefore, their use is being phased out.

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An alarming account of ground water in the US is contaminated with chlorinated hydrocarbons, and much research has been devoted to the development of both *in situ* and *ex situ* methods of treating this ground water for removal of these compounds. One of the more productive areas of research has been bioremediation. Biodegradation of chlorinated hydrocarbons occurs by one of three mechanisms: hydrolysis, or oxidation or reductive dehalogenation. Several species of bacteria have been isolated by enrichment using dichloromethane (CH_2Cl_2). One species, *Hypomicrobium* sp. DM2, grows on CH_2Cl_2 as a carbon source and removes chloride ions by hydrolysis to form formaldehyde (*2*).

Chlorinated ethenes are inert to hydrolysis, but may be oxidized in a reaction involving molecular oxygen. Pure cultures of aerobic bacteria, such as methane-oxidizing bacteria, have been obtained that will oxidatively dehalogenate several halomethanes, trichloroethylene (TCE), and both isomers of 1,2-dichloroethylene (DCE) (*3,4*). However, there have been no reports of aerobic bacteria that will oxidize tetrachloroethylene (PCE) (*2*).

Two mechanisms of reductive dehalogenation are known in anaerobic bacteria. In one mechanism, vicinal chlorines are eliminated, resulting in the formation of a carbon–carbon bond. For example, TCE would become chloroethyne. In the second mechanism, a chlorine atom is removed as chloride and replaced with a hydrogen atom. Degradation of PCE occurs by this mechanism. For example, the anaerobic isolate DCB-1 will convert PCE to TCE; however, complete reductive dehalogenation is rarely observed (*5,6*).

We describe here an investigation of a biomimetic system for the reductive dehalogenation of PCE using cyanocobalamin. TCE and DCE were obtained as products in homogeneous and packed bed reactor systems.

MATERIALS AND METHODS

Chemicals

Cyanocobalamin (vitamin B12) was purchased from Sigma Chemical Co. (St. Louis, MO). Stock solutions (0.14 m*M*) were prepared in deionized water and stored at room temperature in the dark. The cyanocobalamin concentration was verified from the visible range absorbance spectrum and the molar extinction coefficient at 551 nm.

PCE (HPLC-grade) was obtained from Aldrich Chemical Co. (Milwaukee, WI). Stock solutions of PCE (0.12–0.60 m*M*) were prepared in deionized water by sonication in a Cole-Parmer 8890 ultrasonic bath at about 40°C and used as soon as possible following preparation because of the potential of evaporative losses. Stock solutions of TCE (0.15–0.61 m*M*) and DCE (0.10–0.63 m*M*) were prepared in a similar manner. Both were

obtained from Aldrich Chemical Co. TCE was spectro-grade, and DCE was spectro-grade and a mixture of *E* and *Z* isomers.

Stock solutions of the following reducing agents were also prepared in deionized water: dithiotheoreitol (DTT), 0.25 and 1.0*M*; cysteine, 0.25*M*; and sodium sulfide, 0.25*M*. 2-Mercaptoethanol (Aldrich Chemical Co.) was added as a pure reagent to reaction mixtures. DTT, cysteine, and 2-mercaptoethanol were obtained from Sigma Chemical Co. Sodium sulfide was obtained from Mallinckrodt Chemical Co. (Paris, KY). Titanium (III) citrate was prepared fresh by combining 5 mL of 10 wt% titanium (III) chloride with 50 mL of 0.2*M* sodium citrate in 25 wt% HCl. The final concentration of the citrate complex was 112 m*M* (*7*). Titanium (III) citrate was stored in an inert atmosphere of nitrogen to prevent air oxidation of Ti (III) to Ti (IV).

Reductive Dechlorination of PCE in Batch Reactors

Batch experiments with PCE were conducted in completely filled 15-mL serum bottles sealed with a Mininert valve (Baxter Diagnostics McGaw Park, IL). The reaction mixtures contained 0.009–0.0095 m*M* cyanocobalamin, a reducing agent, and PCE as the substrate. Owing to the limited solubility of PCE in water, initial experiments utilized a two-phase system containing 0.008 mL of pure PCE. Reaction mixtures were sampled until all PCE had dissolved and reacted. This two-phase system was used to facilitate the detection of reaction products.

In subsequent experiments, an aqueous stock solution of PCE was used in formulating homogeneous reaction mixtures in 15-mL serum bottles with Mininert valves where the final PCE concentration was 0.09–0.36 m*M*. Reducing agents used their concentrations in the reaction mixtures were 16 m*M* DTT, 14 m*M* TI (III) citrate, 32 m*M* cysteine, 32 m*M* 2-mercaptoethanol, or 32 m*M* sodium sulfide in 100 m*M* Tris-HCl buffer at pH 9.0. The serum bottles were completely filled to eliminate all gas space that would allow for volatilization of the halogenated hydrocarbons. When pH was the variable of interest, 60 m*M* phosphate buffer was used for pH 7, 100 m*M* potassium hydrogen phthalate (KHP) buffer for pH 5, and 6*N* HCl (added dropwise) for pH 2.3.

With both the two-phase and homogeneous reaction mixtures, the serum bottles were shaken at 160 rpm in a Lab-Line 3526 incubator/shaker at 45°C. Control reactions were conducted in an identical manner, except for the absence of cyanocobalamin. The liquid volume of cyanocobalamin stock solution (1.0 mL) was replaced with a corresponding amount of 100 m*M* Tris-HCl buffer in control reactions.

Immobilization of Cyanocobalamin

After demonstrating the reductive dehalogenation of PCE in the two-phase and homogeneous batch systems, efforts were initiated to immobilize cyanocobalamin on a solid support. DUOLITE S-761 resin (Supelco, Bellefonte, PA) was chosen as an adsorbent, because it tightly bound

cyanocobalamin while reversibly binding a limited amount of PCE. DUOLITE S-761 was 16–50 mesh, had a pore diameter of 600 Å, and a mean surface area of 300 m^2/g. Prior to use, the resin was washed sequentially with 2% NaOH, deionized water, 2% HCl, and deionized water until the pH of the rinse water became neutral.

Cyanocobalamin was immobilized on the resin by incubating the 23°C in a 0.14-m*M* soution of the corrin. The cyanocobalamin solution was replenished until no further reduction in absorbance at 551 nm was observed. The capacity of DUOLITE S-761 for cyanocobalamin was shown to be 0.369 mg/g of resin.

The DUOLITE S-761 resin with bound cyanocobalamin was presaturated with PCE as follows. The resin was packed in a 1.8 cm id × 15 cm L jacketed column (Ace Glass Co., Vineland, NJ) at 23°C. An aqueous PCE solution (0.30 m*M*) was pumped through the column at a flow rate of 1.3 mL/h. For a resin volume of 25 mL (volume of packing, not bed volume), about 14 d were required to saturate all of the binding sites. All PCE-binding sites were assumed to be saturated when the effluent and influent PCE concentration became equal as shown by GC analysis. The cyanocobalamin and PCE-saturated resin were stored in a solution of PCE (0.30 m*M*) at 23°C until needed.

The activity of cyanocobalamin and PCE-saturated resin particles was investigated in completely filled serum bottles. Each reaction mixture contained 0.5 g of DUOLITE presaturated with both cyanocobalamin and PCE as described above. Reaction mixtures contained 16 m*M* DTT or 14 m*M* Ti (III) citrate and 0.093 m*M* PCE in 100 m*M* Tris-HCl buffer. All reactions with DUOLITE were homogeneous with respect to PCE and were conducted at 45°C.

Other experiments were also done to demonstrate that the PCE adsorbed to the DUOLITE was in dynamic equilibrium with the PCE in the aqueous phase. A reaction mixture was prepared using the conditions described above, except that there was no PCE used in the bulk liquid phase. In another experiment, 0.5 g of DUOLITE presaturated with cyanocobalamin and PCE was incubated in 100 m*M* Tris-HCl buffer at 45°C and pH 9.0 in a completely filled serum bottle. The purpose of this experiment was to demonstrate that the resin had no inherent catalytic activity in the dechlorination of PCE.

Reductive Dechlorination of PCE in Continuous Packed Bed Reactor Systems

Two continuous packed bed systems were studied. Both used jacketed condensers packed with DUOLITE S-761 resin presaturated with cyanocobalamin and PCE as described above. Figure 1 shows schematic diagrams of each reactor system. With DTT as the reductant, a 24/40 condenser (1.8 cm id × 14.2 cm L, Ace Glass Co.) was used as the reactor. Water at 45°C was circulated through the condenser from a Brinkman RM6 circu-

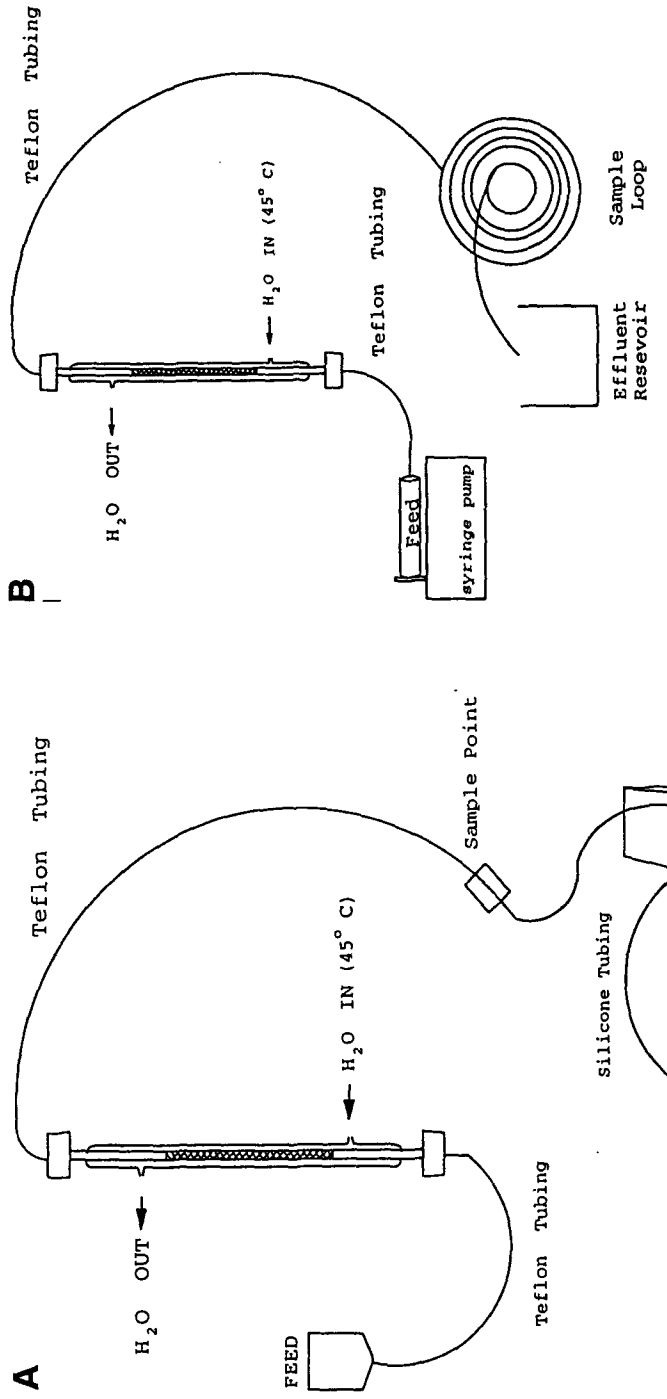

Fig. 1. (A) Schematic diagram of the packed bed reactor system with DTT as the reductant. (B) Schematic diagram of the packed bed reactor system with Ti (III) citrate as the reductant.

lating water bath. The volume of the packed section was 36.9 mL, and the volume of packing measured 25.0 mL by water displacment. The reactor void volume was, therefore, 11.9 mL. A Cole-Parmer Masterflex peristaltic pump equipped with a size 13 Cole-Parmer Masterflex pump head was used to pump feed to the reactor at a flow rate of 1.3 mL/h corresponding to a hydraulic residence time of 9.2 h. PTFE Teflon™ tubing (1/32 in id, Cole-Parmer, Niles, IL) was used from the feed to the sampling point, because the silicone pump tubing adsorbed or allowed the pervaporation of PCE. Prior to starting the DTT reactor feed, the equilibrium between the PCE adsorbed to the DUOLITE resin and the PCE in the aqueous phase was verified at reactor conditions (45°C). A solution of PCE (0.26 m*M*) in 100 m*M* Tris-HCl buffer at pH 9.0 was pumped through the reactor at a flow rate of 1.3 mL/h until the effluent PCE concentration matched the influent PCE concentration as shown by GC analysis. The complete feed typically contained 0.26 m*M*, 52 m*M* DTT, and 0.15*M* Tris-HCl buffer at pH 9.0.

A DTT control reactor was prepared identically in every way, except that the DUOLITE resin was presaturated only with PCE and no cyanocobalamin. The volume of the packed section was 38.2 mL, and the volume of packing was measured to be 28 mL by water displacement. The control reactor void volume was, therefore, 10.2 mL. The reactor was operated at the same flow rate as the reactor containing cyanocobalamin.

With Ti (III) citrate as the reductant, a microscale distillation condenser (7 mm id × 80 mm L, Ace Glass Co.) was used. Water at 45°C was circulated through the jacket by a Techne TE-8A water circulator. The entire citrate reactor system was operated inside a glove box (Vacuum Atmospheres Company, Hawthorne, CA) containing an atmosphere of purified nitrogen to prevent air oxidation of the titanium complexes. The volume of the packed section measured 3.1 mL. The volume of the packing measured 1.0 mL by water displacement, so the void volume of the Ti (III) citrate reactor was 2.1 mL. Because of the small Ti (III) citrate reactor volume, a large coil of PTFE Teflon™ tubing (1/32 in id × 18 m L, Cole-Parmer, Niles, IL) was used to collect the effluent. The effluent was transferred to a sealed vial for removal from the glove box and subsequent analysis.

A syringe pump (Harvard Model 975; Harvard Apparatus, Inc., South Natick, MA) was used to operate the Ti (III) citrate reactor at two flow rates, 1.2 mL/h and 0.6 mL/h. The corresponding hydraulic residence times were 1.75 and 3.5 h, respectively. Prior to starting the Ti (III) citrate reactor feed, the equilibrium between the PCE adsorbed to the DUOLITE resin and the PCE in the aqueous phase was verified at reactor (45°C) conditions. A solution of PCE (0.18 m*M*) in 100 m*M* Tris-HCl buffer at pH 9.0 was pumped through the reactor at a flow rate of 1.2 mL/h until the effluent PCE concentration matched the influent PCE concentration as shown by gas chromatography (GC) analysis. The complete reactor feed

typically contained 0.18 m*M* PCE, 31.4 m*M* Ti (III) citrate, and 0.17*M* Tris-HCl buffer at pH 9.0.

A citrate control reactor was prepared identically in every way, except that the resin was presaturated only with PCE. The volume of the packed section of the control reactor measured 3.1 mL. The volume of the packing measured 1.1 mL by water displacement, so the void volume measured 2.0 mL. The citrate control reactor was operated at the same flow rates.

Analytical

Chlorinated Hydrocarbon Analysis

Samples of the reaction mixture were analyzed by GC with an HP 5890 GC equipped with a flame ionization detector (FID). Samples of 2 μL were withdrawn periodically from the batch experiment through the Mininert valve. For each sampling time, two duplicate analyses were performed. Reaction mixtures were first sampled immediately following mixing of the reagents and subsequently about every 4 h until the reaction was complete. Chlorinated hydrocarbons were quantitated by comparing relative peak areas with calibration curves made from gravimetrically prepared standards for PCE, TCE, and DCE. Product identification was done with an HP 5971 GC/MS equipped with TEKMAR purge and trap. Product identity was confirmed by using standards of various chlorinated hydrocarbons (PCE, TCE, and DCE) and matching retention times with peaks of interest.

Chloride Ion Analysis

Homogeneous batch reactions were analyzed for the presence of free chloride ion (Cl^-) that resulted from the dechlorination of PCE. Disappearance of PCE and formation of TCE were monitored by GC. As product formed, the reaction mixtures were sampled for the presence of free Cl^- using an ORION 701A/digital ion analyzer equipped with a model 94-17B chloride electrode. The initial and final Cl^- concentrations were determined by the known addition method. The chloride standard (0.99 m*M*) was prepared by diluting 28.2 m*M* sodium chloride standard purchased commercially from Orion Research Inc. (Cambridge, MA). The difference in Cl^- concentration between the initial and final samples was assumed to be from the dechlorination reaction.

RESULTS AND DISCUSSION

Reductive Dechlorination of PCE in Batch Reactors

When Ti (III) citrate was the reductant, a definite sensitivity to pH was observed in the two-phase system. Reaction conditions were 45°C, 14 m*M* Ti (III) citrate, and 0.009 m*M* cyanocobalamin in 100 m*M* Tris-HCl

buffer. At pH 2.3, very little product was formed. At pH 5 and 7, similar conversions were obtained that were intermediate between the conversions at pH 2.3 and 9.0. The reaction at pH 9.0 produced complete conversion of PCE to TCE in 47 h. No pH values above 9.0 were investigated, so that may not be the true optimum pH. However, reaction mixtures at pH 9.0 produced the greatest rates of TCE formation and greatest PCE conversion of all pH values studied. It has been previously shown in this laboratory that pH 9.0 is the optimum pH for reduction of nitroaromatics to amines using DTT in a reaction catalyzed by Co-centered porphyrins (*8*). All subsequent work was done at pH 9.0.

A variety of reducing agents were investigated in the batch cyanocobalamin-mediated dechlorination of PCE. DTT was demonstrated to dechlorinate PCE in the presence of cyanocobalamin under the conditions described above. The effectiveness of the other reduced sulfur compounds in dechlorinating PCE in the two-phase system was as follows: cysteine < 2-mercaptoethanol < Na_2S < DTT. Very little dehalogenation of PCE was observed with cysteine and 2-mercaptoethanol as reductants. Complete dechlorination of PCE to TCE was observed with Na_2S in 100 h and with DTT in 75–80 h. Under similar conditions, Ti (III) citrate was shown to be more effective than DTT, giving complete conversion of PCE in about 20 h. Only DTT and Ti (III) citrate were chosen for futher study.

In both the two-phase and homogeneous reaction mixtures, it was shown that neither DTT nor Ti (III) citrate brought about the dechlorination of PCE without the presence of cyanocobalamin. Under homogeneous conditions, the dechlorination reaction was slower and produced lower conversions with DTT than with Ti (III) citrate. Typical results are shown in Figs. 2 and 3. The Ti (III) citrate reaction was about four times faster than the reaction with DTT as the reductant under the same conditions. DTT produced 92% conversion of 0.093 m*M* PCE in 72 h and Ti (III) citrate produced 100% conversion of 0.093 m*M* PCE in 19 h (data not shown).

The reaction products of the reductive dechlorination of PCE were TCE and 1,2-dichloroethylene (DCE) with both *E* and *Z* isomers being identified by mass spectrometry. For all reductants investigated, except Ti (III) citrate, only TCE was formed. For the DTT homogeneous batch reaction (0.093 m*M* PCE) under optimum conditions, including excess DTT, the conversion was 92% after 70 h. Under no conditions studied did the DTT reaction go to completion. There was PCE remaining even after reaction times of >200 h. With Ti (III) citrate as the reductant, under homogeneous conditions, 100% conversion of 0.093 m*M* PCE was obtained in 19 h. For this batch system, both TCE and DCE were formed with TCE predominating in a 95/5% ratio. During the course of the Ti (III) citrate reaction, short-lived intermediates were observed appearing as peaks on both GC and GC/MS chromatograms. They would appear shortly after

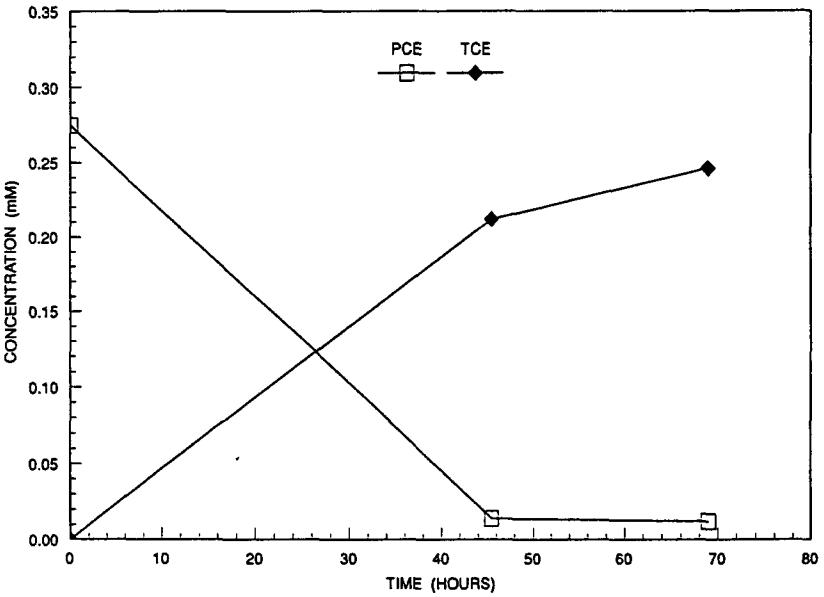

Fig. 2. PCE and TCE concentrations in a homogeneous batch reaction with DTT as the reductant. Reaction conditions: 45°C, pH 9.0, 64 m*M* DTT, 0.0180 m*M* cyanocobalamin in Tris-HCl buffer.

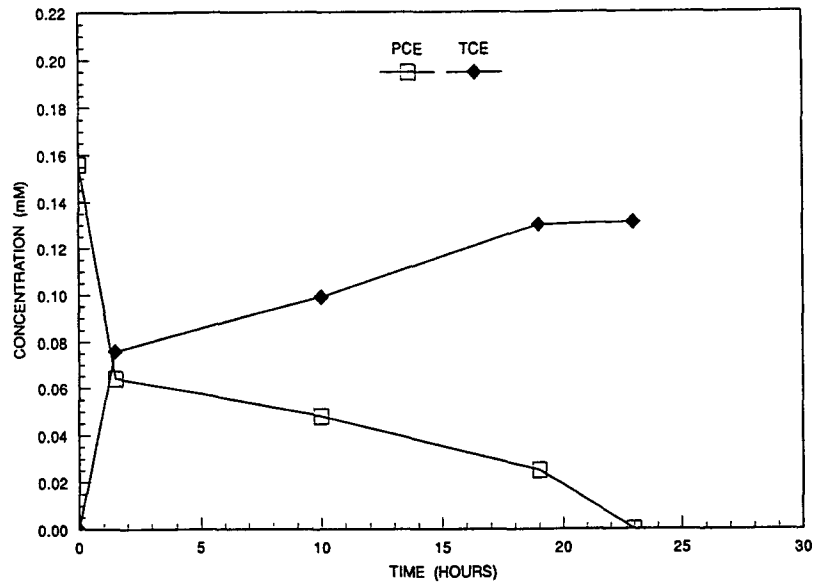

Fig. 3. PCE and TCE concentrations in a homogeneous batch reaction with Ti (III) citrate as the reductant. Reaction conditions: 45°C, pH 9.0, 14 m*M* Ti (III) citrate, 0.0090 m*M* cyanocobalamin in Tris-HC buffer.

the reaction was initiated and would persist for a few hours. These intermediates were all chlorinated derivatives of PCE. These intermediates were observed only in the Ti (III) citrate reaction using the two-phase system with PCE. They were present at much smaller concentrations than PCE, TCE, and DCE and therefore were not observed in the homogeneous batch experiments where a much smaller initial PCE concentration was used. Mass spectrometry identified these intermediates as 1,1,3-4-tetrachloro-1,3-butadiene and 1,4-dichloro-2-butyne.

A chloride ion analysis was conducted on homogeneous batch experiments with DTT as the reductant. The initial chloride concentration was 0.26 m*M* with a final concentration (after 70 h) of 0.42 m*M*. Therefore, 0.16 m*M* was the result of the dechlorination reaction. Reaction conditions were 45°C, pH 9.0, 0.36 m*M* PCE, 64 m*M* DTT, and 0.018 m*M* cyanocobalamin in 100 m*M* Tris-HCl buffer. On the basis of the amount of TCE formed, 0.245 m*M* Cl^- was expected. Therefore, 65% of the expected yield of Cl^- was observed. A duplicate experiment under identical reaction conditons produced similar results. This discrepancy is the result of the demonstrated interference of DTT with the response of the Cl^- electrode. However, production of chloride ion is clearly indicated.

Reductive Dechlorination of PCE in Continuous Packed Bed Reactors

Batch reactions using cyanocobalamin immobilized on DUOLITE S-761 were conducted to demonstrate that the immobilized corrin retained its catalytic properties. It was shown that with Ti (III) citrate and DTT as the reductants, reaction times were comparable to those observed in homogeneous systems with conversion of PCE to TCE. It was also shown that the resin alone had no inherent surface catalytic properties with respect to dechlorination of PCE with either reductant. Finally, it was shown that when DUOLITE S-761, presaturated with both cyanocobalamin and PCE, was incubated in Tris-HCl buffer at pH 9.0, PCE desorbed and was detected in the bulk liquid phase. In the presence of a reductant, the PCE desorbed and was dechlorinated to TCE.

The continuous packed bed reactor system with DTT as the reductant produced conversions of about 22% based on TCE production with a residence time of 9.2 h. No DCE was detected. Figure 4 shows the chlorinated hydrocarbon concentrations in the reactor effluent and feed stream. The rate of the DTT reaction was too slow to produce high conversions within reasonable residence times. The volatility of the halogenated hydrocarbons presented some problems in reactor operation. Carbon balances on the reactor accounted for 66% of the carbon.

A DTT control reactor identical in every way except for the absence of cyanocobalamin was operated at the same residence time under the same conditions. No product formation was observed. The effluent contained

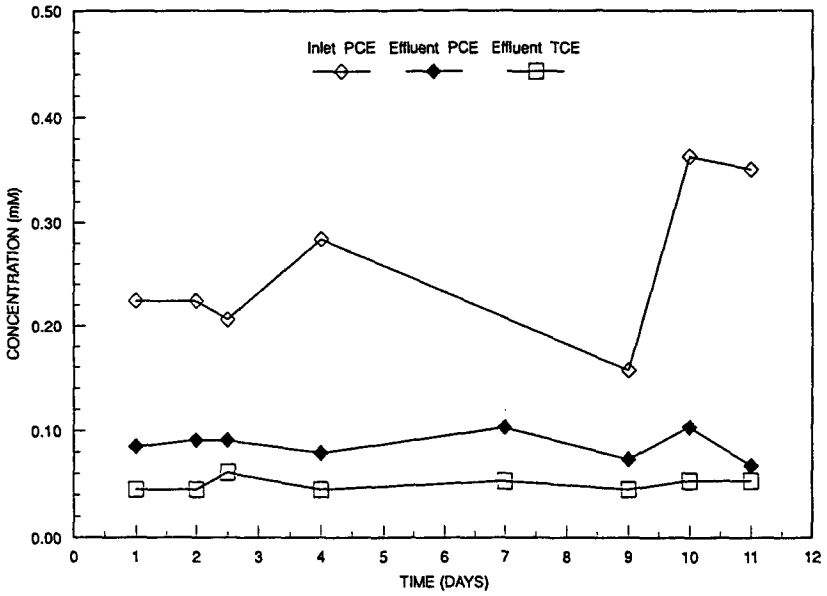

Fig. 4. Inlet and effluent concentrations of chlorinated hydrocarbons in the packed bed reactor system with DTT as the reductant. Cyanocobalamin was immobilized on DUOLITE resin. Operating conditions: 45°C, pH 9.0, residence time 9.2 h, 52 m*M* DTT in Tris-HCl buffer.

only PCE. A carbon balance could account for 79% of the inlet PCE. The remainder was assumed to have been evaporated.

The Ti (III) citrate packed bed reactor system was investigated at two hydraulic residence times. At a residence time of 1.75 h, PCE was converted to TCE and DCE at an overall PCE conversion of 51%. The product distribution in the effluent was typically 94% TCE and 6% DCE. At a residence time of 3.5 h, the overall PCE conversion was 59%. The product distribution at this residence time was 55% TCE and 45% DCE. Reactor conditions were 45°C, 31.4 m*M* Ti (III) citrate in 0.17*M* Tris-HCl buffer. Figures 5 and 6 show the chlorinated hydrocarbon concentrations at the two residence times. The increase in DCE production at longer residence times corresponds to batch experiment findings. It was observed that DCE production did not begin until significant TCE was formed in batch studies. The formation of DCE came from the sequential dechlorination of TCE; it did not come directly from PCE. Other researchers have made similar observations (6). Thus, different residence times yielded a similar overall conversion, but more TCE was converted to DCE.

Carbon balances on the Ti (III) citrate reactor showed 93% of carbon accounted for at a residence time of 1.75 h and 90% at 3.5 h. Losses were much less than the DTT reactor because the residence times were shorter.

A Ti (III) citrate control reactor identical to the test reactor, except for the absence of cyanocobalamin, was operated at residence times of 1.75

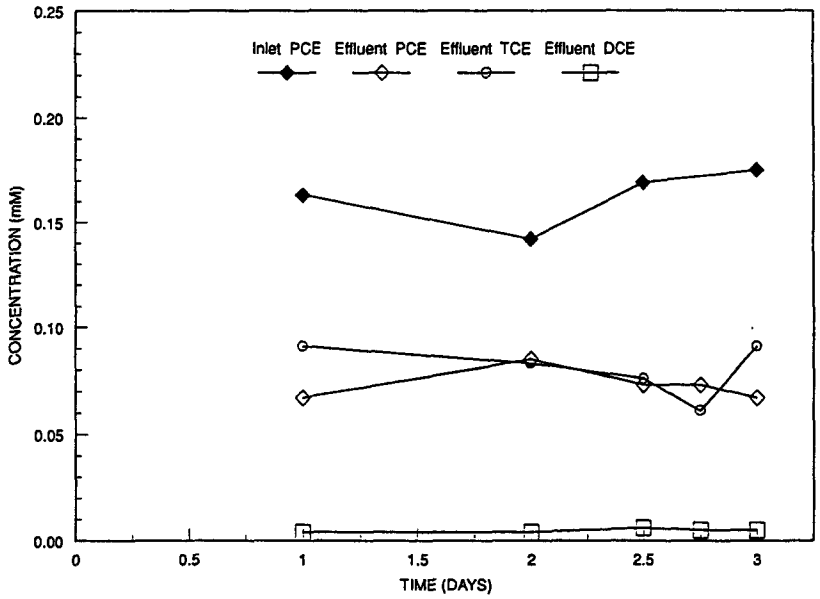

Fig. 5. Inlet and effluent concentrations of chlorinated hydrocarbons in the packed bed reactor system with Ti (III) citrate as the reductant. Cyanocobalamin was immobilized on DUOLITE resin. Operating conditions: 45°C, pH 9.0, residence time 1.75 h, 31.4 m*M* Ti (III) citrate in Tris-HCl buffer.

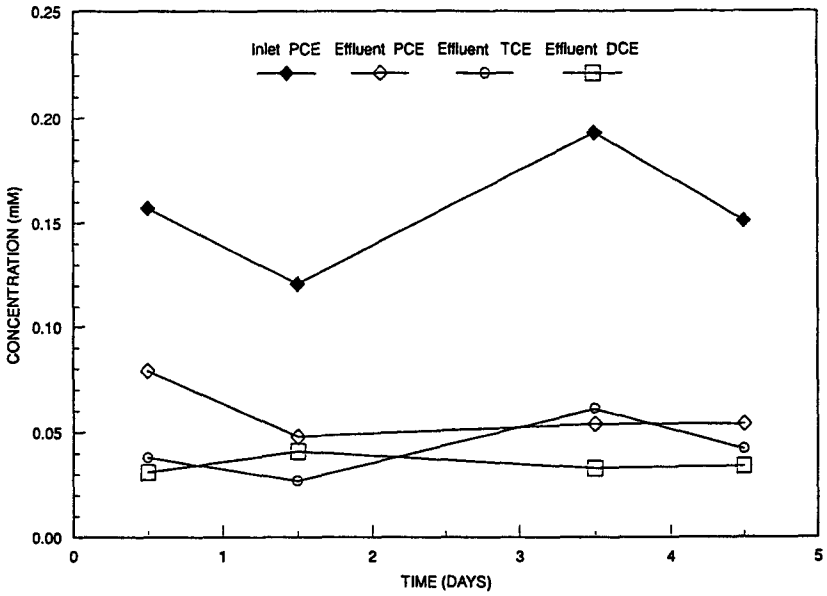

Fig. 6. Inlet and effluent concentrations of chlorinated hydrocarbons in the packed bed reactor system with Ti (III) citrate as the reductant. Cyanocobalamin was immobilized on DUOLITE resin. Operating conditions: 45°C, pH 9.0, residence time 3.5 h, 31.4 m*M* Ti (III) citrate in Tris-HCl buffer.

and 3.5 h under the same conditions. In both cases, no product formation occurred. The effluent contained only PCE.

CONCLUSIONS

A biomimetic system has been developed for the reductive dechlorination of PCE. PCE was dechlorinated to TCE and DCE in the presence of a reducing agent and catalytic amounts of cyanocobalamin in both homogeneous reaction mixtures and packed bed reactors. Similar results have been reported in the reductive dehalogenation of 2,3,4,5-pentachlorobiphenyl and hexachlorobenzene catalyzed by cyanocobalamin (*9*). This system may be useful as a means of pretreatment of halogenated aliphatic hydrocarbons in advance of biological treatment. Alternatively, cyanocobalamin may enhance the degradation of halogenated hydrocarbons directly in anaerobic cultures where the reducing agents are provided by the low redox potential of the culture. This concept is currently being tested.

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