

Reductive Dechlorination of Tetrachloroethene by a High Rate Anaerobic Microbial Consortium

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Tetrachloroethene (PCE) and other chloroethenes are major contaminants in groundwater, and PCE is particularly resistant to attack by aerobes. We have developed an anaerobic enrichment culture that carries out reductive dechlorination of chloroethenes to ethene at high rates, thereby detoxifying them. Although the electron donor added to the culture is methanol, our evidence indicates that H_2 is the electron donor used directly for dechlorination. We have recently obtained a culture from a 10^{-6} dilution of the original methanol/PCE culture that uses H_2 as an electron donor for PCE dechlorination. Because the culture can be transferred indefinitely and the rate of PCE dechlorination increases after inoculation, we suggest that dechlorinating organisms in the culture use the carbon-chlorine bonds in chloroethenes as electron acceptors for energy conservation. — Environ Health Perspect 103(Suppl 5):5-7 (1995)

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Chlorinated ethenes are widely employed as solvents and chemical feedstocks. Not surprisingly, groundwater contamination by chlorinated ethenes has become a serious problem in the industrialized world. A 1984 survey of water supplies in the United States listed tetrachloroethene (PCE), trichloroethene (TCE), and the three dichloroethene (DCE) isomers as the five most frequently found contaminants, other than trihalomethanes (1). These compounds pose a public health concern and are therefore regulated by the 1986 amendments to the Safe Drinking Water Act. The remediation of groundwater contaminated with volatile organic chemicals, including PCE, is typically achieved by groundwater extraction followed by a physical/chemical process to remove the pollutants. This approach can be extremely expensive and inefficient; furthermore, physical/chemical processes (e.g., air-strip-

ping and carbon adsorption) simply transfer the hazard from one part of the environment to another.

In contrast, bioremediation applied *in situ* or in above-ground treatment systems offers the prospect of degrading chlorinated ethenes to environmentally acceptable products in a cost-effective manner. Under aerobic conditions, PCE, which lacks a carbon-hydrogen bond, is generally considered to be nondegradable. TCE is co-metabolized by organisms harboring certain nonspecific oxygenases such as methane monooxygenase and toluene dioxygenase (2). Attempts at stimulating degradation of TCE and less chlorinated ethenes in groundwater aquifers by addition of methane and oxygen have met with some success (3). In general, aerobic biodegradability of chloroethenes is inversely related to their degree of chlorination.

Anaerobic degradation of chlorinated ethenes usually proceeds by a reductive mechanism in which the carbon-chlorine bond undergoes hydrogenolysis (Figure 1). Several investigators have found reductive dechlorination of PCE and TCE by anaerobic, usually methanogenic, habitats and by pure cultures of methanogens and acetogens (2,4). Rates were usually low and dechlorination was incomplete, with vinyl chloride (VC) or more highly chlorinated ethenes persisting. Thus, the net result of

anaerobic metabolism of chlorinated ethenes was to produce VC, a known human carcinogen. Freedman and Gossett (5) were the first to report complete anaerobic reductive dechlorination of chloroethenes to ethene (ETH), which is a plant hormone that is nontoxic to humans. More recently, further reduction of ETH to ethane has been described (6). In general, more highly chlorinated ethenes are more degradable under anaerobic conditions than less chlorinated ones. Since the activities of aerobes and anaerobes against chloroethenes are complementary, there is interest in using sequential anaerobic/aerobic processes to degrade them, as well as other highly chlorinated molecules such as polychlorinated biphenyls.

Catalysis of stepwise reductive dechlorination of PCE to ETH *in vitro* using Ti^{3+} as a reductant was demonstrated using transition metal-containing cofactors such as vitamin B_{12} and the nickel-containing methanogenic cofactor F_{430} (7). The kinetics of dechlorination were first-order for each step, and the rate constant for each step was greater than 10-fold lower than for the previous step so that VC dechlorination to ETH was about 10,000-fold slower than PCE dechlorination to TCE. The results suggest that microorganisms rich in transition metal cofactors, such as methanogens and acetogens, could cat-

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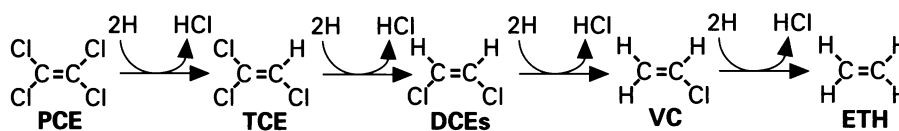


Figure 1. Reductive dechlorination of chloroethenes under anaerobic conditions.

alyze reductive dechlorination by a form of co-metabolism in which reduced forms of these cofactors passively catalyzed the reductions.

The original anaerobic enrichment cultures described by Freedman and Gossett (5) were derived from the anaerobic digester at the Ithaca sewage treatment plant and received nominal PCE doses of 3 $\mu\text{moles/l}$ (about half of this PCE was actually in the headspace); several electron donors appeared to support dechlorination, although methanol (MeOH) appeared to be the most effective. Most of the electron donor was used for methanogenesis, and VC dechlorination to ETH was the rate-limiting step in dechlorination so that considerable amounts of VC persisted.

In a subsequent study (8), we gradually increased the PCE dose to a MeOH-fed culture from 3 $\mu\text{moles/l}$ to 550 $\mu\text{moles/l}$, close to saturation. The MeOH dose was also increased so that it provided approximately twice the electron equivalents needed for complete PCE dechlorination to ETH; a small amount of yeast extract was also added. Increasing the PCE dose actually improved dechlorination such that within 2 days about 80% of the PCE added could be accounted for as ETH with the remainder as VC. Four days of incubation led to 99% conversion to ETH. Furthermore, the culture ceased producing significant amounts of methane, presumably due to inhibition by high PCE concentrations; a fermentation balance showed that the reducing equivalents from methanol not used for PCE reduction (1/3–1/2 of the MeOH added) were used for acetogenesis. The high rates of complete dechlorination of PCE to ETH by the culture were unprecedented as was the high fraction of electron donor used for PCE dechlorination. This culture could be transferred indefinitely, and we have scaled it up to a 6 liter bioreactor (9). We have presented evidence that H_2 is the actual electron donor for dechlorination in the MeOH-fed culture (Figure 2).

More recently (10), we have studied time courses for dechlorination of 550 μmoles PCE added per liter by the MeOH-fed anaerobic enrichment cultures. PCE disappearance was essentially zero-order within the concentration range examined, typically requiring 24 to 36 hr for complete PCE consumption and a specific rate of 4.6 μmoles PCE transformed per milligrams volatile solids per day. Little or no TCE or dichloroethene (DCE) isomers accumulated, and conver-

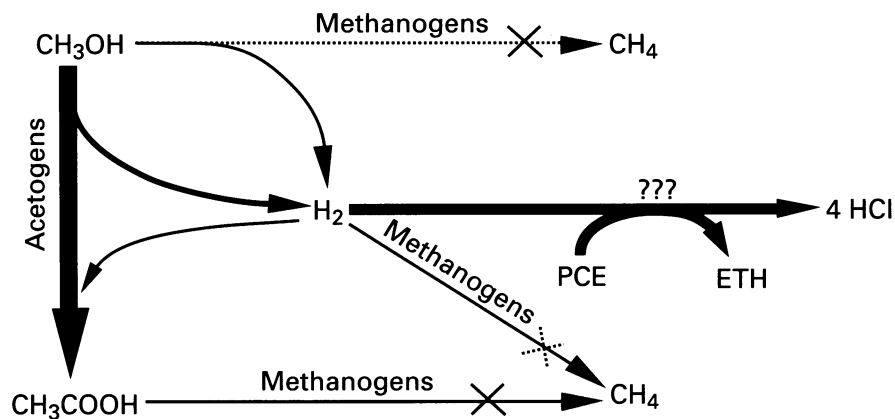


Figure 2. Model for interactions of microbial groups in the MeOH/PCE enrichment culture. Adapted from DiStefano et al. (9).

sion to VC was essentially stoichiometric. Only when most or all of the PCE was consumed were significant amounts of VC converted to ETH. VC conversion to ETH followed first-order kinetics, with a half-life near 17 hr, and ETH was formed stoichiometrically. When VC was added alone to cultures previously fed PCE, it was converted to ETH, with little or no lag, and VC disappearance followed first-order kinetics with a half-life near 17 hr. These results are consistent with inhibition of VC dechlorination by PCE.

This MeOH/PCE culture was capable of dechlorinating all of the other chloroethenes. TCE, *cis*-DCE, and 1,1 DCE were all rapidly and stoichiometrically converted to VC, with subsequent conversion to ETH occurring only when the primary substrate concentrations were low or absent. In contrast, *trans*-DCE disappearance followed first-order kinetics and VC conversion to ETH did not show a lag, suggesting a lack of inhibition of VC dechlorination by *trans*-DCE. It is not clear why substrate utilization followed this pattern, but it should be pointed out that the two chloroethenes for which dechlorination is first-order, VC and *trans*-DCE, lack adjacent chlorines.

We performed a small survey of other haloaliphatics used by the culture and found that the culture could rapidly dehalogenate 1,2-dichloroethane, a common solvent, and 1,2-dibromoethane (ethylene dibromide), a fumigant. In both cases, the primary product was ethene, a typical vicinal reductive dihalo-elimination reaction (4). The culture did not rapidly attack chloromethanes or trichloroethanes, including 1,1,2-trichloroethane, which differs from 1,2-dichloroethane by a single chlorine.

We are presently studying the kinetics of chlorinated ethene utilization more completely, but from these preliminary results, some conclusions can be made. The rates of PCE dechlorination by this mixed culture are three orders of magnitude higher than those obtained for pure cultures of *Methanosarcina* and strain *Desulfomonile tiedjei* (DCB-1) (4), which presumably co-metabolize PCE. The results are also considerably different from those obtained for catalysis by vitamin B_{12} and cofactor F_{430} (7). These results, along with the substrate specificity observed, suggest that PCE dechlorination is a specific, enzymatically catalyzed process rather than one of passive co-metabolism.

The model presented in Figure 2 makes certain predictions about microbial populations present in the MeOH/PCE culture. It predicts a low population of methanogens and a high population of methanol-utilizing acetogens and H_2 -utilizing PCE reducing organisms. We examined the populations in the culture using MPN analysis (V Tandoi et al., unpublished data). We found that there were low numbers (approximately 104/ml) of H_2 - CO_2 -utilizing methanogens and that methanol-utilizing and acetate-utilizing methanogens were essentially absent. This is of interest because, when the PCE concentration was low, the culture was actively methanogenic, and there were greater than 10^7 methanol-utilizing methanogens present. High numbers ($>10^7$ /ml) of methanol-utilizing Gram positive cocci in chains were found, but there were much lower numbers of H_2 -utilizing acetogenic bacteria. There were high numbers of fermentative heterotrophs, presumably growing on the yeast extract added to the medium, and there were relatively high numbers of sulfate reducing

bacteria, presumably using oxidation products of the sulfide added to the medium. It must be borne in mind that DCB-1 is a sulfate reducer (4).

In MPN dilutions provided with MeOH and PCE, PCE reduction products were found only in dilutions of 10^{-3} or less, and even in those tubes, only low levels of products were detected. In contrast, in tubes provided with PCE and H_2 , PCE reduction occurred in dilutions to 10^{-6} ; PCE was completely consumed in those tubes after a lag of 4 weeks (lags were shorter in lower dilutions), as were subsequent PCE additions. The PCE in those tubes was reduced to VC followed by ETH, which was similar to the original MeOH/PCE enrichment. These results are consistent with our hypothesis (9) that H_2 is the primary electron donor for PCE reduction in the MeOH/PCE culture.

We have transferred this purified H_2 /PCE culture several times, and the rate of PCE dechlorination after transfer increases with time, indicating growth. These purified cultures make neither methane nor acetate, even though there is approximately 0.8 atmospheres of H_2 in the headspace, indicating the absence of methanogens and hydrogenotrophic acetogens. This culture still contains yeast extract-utilizing heterotrophs and sulfate reducers, which are presumably contaminants. The culture apparently requires growth factors present in anaerobic sewage sludge supernatant, and after several feedings of PCE, shows signs of limitation. We

are presently studying this nutrient requirement and are attempting isolation of dechlorinating organisms from the culture.

From the evidence we have obtained, we believe that the culture is using the carbon-chlorine bonds in chlorinated ethenes as electron acceptors for energy conservation in a manner similar to the use of chlorobenzoates by DCB-1. The use of reductive dechlorination reactions for energy conservation has been called chlororespiration (or more generally, halorespiration). The E° values for reductive dechlorination of chloroethenes range from +0.36 to +0.58 V, similar to the nitrate/nitrite couple (+0.43 V), and considerably more favorable than the sulfate/sulfide (-0.22 V) or the CO_2/CH_4 (-0.24 V) couples. Thus there is selective pressure for an anaerobe to evolve the ability to use these compounds as electron acceptors in habitats in which they are present in reasonably high concentrations. It should also be mentioned that Holliger (11) have isolated an organism called *Dehalobacter restrictus*, which grows using H_2 and reduces PCE to *cis*-DCE.

Finally, one may question whether these findings have environmental relevance. Evidence that reductive dechlorination of chloroethenes can be stimulated by electron donors added to groundwater aquifers comes from Major et al. (12), who studied a solvent-contaminated site north of Toronto in which solvents, including PCE and MeOH, were transferred to railroad cars and were apparently spilled at the

points of transfer. At one area of the site, there was a plume of PCE with low concentrations of less chlorinated ethenes present. At another area of the site, PCE concentrations are low, but there are high concentrations of *cis*-DCE, VC, and ETH. Also associated with this area is a compact plume of MeOH, suggesting that this solvent was also spilled in that area. Also associated with the MeOH plume are high concentrations of acetate and chloride. These results suggest that an inadvertent addition of MeOH stimulated reductive dechlorination and acetogenesis *in situ*, similar to the culture we have been studying. More recently, researchers at DuPont (unpublished data) have reportedly stimulated reductive dechlorination of chloroethenes at a contaminated site in Texas, using additions of benzoate as the electron donor.

In summary, we have developed an enrichment culture that converts high concentrations of PCE, such as those found in source zones in contaminated groundwater aquifers, to ETH. We have partially purified the organisms responsible for this dechlorination. A better understanding of the physical, chemical, and physiological requirements of this and other dechlorinating cultures is likely to improve dechlorination of chloroethenes in bioreactors and *in situ*. We are presently studying these factors and are particularly interested in the final step of dechlorination of VC to ETH since this represents true detoxification.

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