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Reductive Dehalogenation of Organic Contaminants in Soils and Ground Water

Judith L. Sims, Joseph M. Suflita, and Hugh H. Russell

The Regional Superfund Ground-Water Forum is a group of ground-water scientists, representing EPA's Regional Superfund Offices, organized to exchange up-to-date information related to ground-water remediation of superfund sites. One of the major issues of concern to the Forum is the transport and fate of contaminants in soil and ground water as related to subsurface remediation. Process which influence the behavior of contaminants in the subsurface must be considered both in evaluating the potential for movement as well as in designing remediation activities at hazardous waste sites. Such factors not only tend to regulate the mobility of contaminants, but also their form and stability. Reductive dehalogenation is a process which may prove to be of paramount importance in dealing with a particularly persistent class of contaminants often found in soil and ground water at superfund sites. This paper summarizes concepts associated with reductive dehalogenation and describes applications and limitations to its use as a remediation technology.

For further information contact Dr. Hugh Russell, FTS 743-2444 at RSKERL-Ada.

Abstract

Introduction and large scale production of synthetic halogenated organic chemicals over the last 50 years has resulted in a group of contaminants which tend to persist in the environment and resist both biotic and abiotic degradation. The low solubility of these types of contaminants, along with their toxicity and tendency to accumulate in food chains, make them particularly relevant targets for remediation activities.

Although the processes involved in dechlorination of many of these organic compounds are well understood in the fields of chemistry and microbiology, technological applications of these processes to environmental remediation are relatively new--particularly at pilot or field scale. It is well established, however, that there are several mechanisms which result in dehalogenation of some classes of organic contaminants, often rendering them less offensive environmentally. These include: stimulation of metabolic sequences through introduction of electron donor and acceptor combinations; addition of nutrients to meet the needs of dehalogenating microorganisms; possible use of engineered micro-organisms; and use of enzyme systems capable of catalyzing reductive dehalogenation.

The current state of research and development in the area of reductive dehalogenation is discussed along with possible technological applications of relevant processes and mechanisms to the remediation of soil and ground water contaminated with chlorinated organics. In addition, an overview of research needs is suggested which might be of interest for development of in situ systems to reduce the mass of halogenated organic contaminants in soil and ground water.

Introduction

Large scale production of synthetic halogenated organic compounds, which are often resistant to both biotic and abiotic degradation, has occurred only in the last few decades (Hutzinger and Verkamp 1981). However, naturally occurring halogenated organic compounds have existed in marine systems for perhaps

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Robert S. Kerr Environmental Research Laboratory
Ada, OK

millions of years. These compounds, including aliphatic and aromatic compounds containing chlorine, bromine, or iodine, are produced by macroalgae and invertebrates. The presence of these natural compounds, at potentially high concentrations, may have resulted in populations of bacteria that are effective dehalogenators (King 1988). Microorganisms exposed to halogenated compounds in soil and ground water may also have developed enzymatic capabilities similar to those in marine environments. Enzyme systems that have evolved to degrade nonchlorinated compounds may also be specific enough to degrade those that are chlorinated. (Tiedje and Stevens 1987).

Many halogenated organic compounds are not very soluble and tend to be highly lipophilic, therefore having the potential to bioaccumulate in some food chains. These chemical properties, along with their toxicity and resistance to degradation, present the potential for adverse health effects and ecosystem perturbations upon exposure (Rochkind et al. 1986).

Recent research findings indicate that anaerobic processes that remove halogens from these compounds produce dehalogenated compounds that are generally less toxic, less likely to bioaccumulate, and more susceptible to further microbial attack, especially by aerobic microorganisms utilizing oxidative biodegradative processes. Both aromatic and nonaromatic organic compounds are subject to these dehalogenation processes. Technological applications of these processes for remediation of contaminated soils and ground waters is of a relatively new concept.

Recent research also has shown that anaerobic dehalogenation reactions specifically involving reductive processes can effectively degrade a wide variety of halogenated contaminants in soil and ground water (Vogel et al. 1987, Kuhn and Suflita 1989a). Organic compounds generally represent reduced forms of carbon, making degradation by oxidation energetically favorable. However, halogenated organic compounds are relatively oxidized by the presence of halogen substituents, which are highly electronegative and thus more susceptible to reduction. A compound with more halogen substituents is therefore more oxidized and more susceptible to reduction. Thus, with increased halogenation, reduction becomes more likely than does oxidation (Vogel et al. 1987).

An organic compound is considered to be reduced if a reaction leads to an increase in its hydrogen content or a decrease in its oxygen content; however, many reduction reactions (e.g., the vicinal reduction process) do not involve changes in the hydrogen or oxygen content of a compound. Oxidation and reduction reactions are more precisely defined in terms of electron transfers. An organic chemical is said to be reduced if it undergoes a net gain of electrons as the result of a chemical reaction (electron acceptor), and is said to be oxidized if it undergoes a net loss of electrons (electron donor). Under environmental conditions, oxygen commonly acts as the electron acceptor when present. When oxygen is not present (anoxic conditions), microorganisms can use organic chemicals or inorganic anions as alternate electron acceptors under metabolic conditions referred to as fermentative, denitrifying, sulfate-reducing or methanogenic. Generally, organic compounds present at a contaminated site represent potential electron donors to support microbial metabolism. However, halogenated compounds can act as electron acceptors, and thus become reduced in the reductive dehalogenation process. Specifically, dehalogenation by reduction is the replacement of a halogen such as chloride, bromide, fluoride, or iodide on an organic molecule by a hydrogen atom. Vicinal reduction occurs when two halogens are released while two electrons are incorporated into the compound.

An organic chemical would be expected to be reduced if the electrode potential of the specific soil or ground-water system, in which the chemical is present, is less than that of the organic chemical (Dragun 1988). The electrode potential is described by the oxidation-reduction (redox) status of the system, refering to potential for the transfer of electrons to a reducible material. The electron (e^-) participates in chemical reactions in soil and ground water similar to the hydrogen ion (H^+) in that electrons are donated from a reduced compound to an oxididized. Redox potential (Eh) is usually reported in volts and is measured using a reference electrode in combination with a metallic electrode, such as platinum, which is sensitive and reversible to oxidation-reduction conditions.

The redox potential of a soil system is complex. The oxidation state of each soil constituent, such as organic compounds, humus, iron, manganese, and sulfur, contributes to the measured redox potential. The contribution of each constituent in a system varies with such factors as soil water content, oxygen activity, and pH. Well-oxidized soils have redox potentials of 0.4 to 0.8 V, while extremely reduced soils may have potentials of -0.1 to -0.5 V (Dragun 1988).

The potential for anaerobic biological processes to reductively dehalogenate organic compounds may be important in the bioremediation of soils and aquifers contaminated with these compounds. These environments often become anaerobic due to depletion of oxygen by the microbial degradation of more easily degradable organic matter. When compounds can be degraded under anaerobic conditions, the cost associated with the maintenance of an aerobic environment by providing air, ozone, or hydrogen peroxide would be eliminated (Suflita et al. 1988).

While anaerobic biological mediated reductive dehalogenation mechanisms were demonstrated as early as 1983 (Allan, 1955), the utilization of this process as a remedial alternative to reduce the overall mass of halogenated organic compounds from soil and ground water is a new concept and still subject to field demonstrations.

For this reason research is currently underway to better define the basic mechanisms of reductive dehalogenation reactions. Such approaches may include: (1) stimulation of desirable metabolic sequences in soil and ground water through the intentional introduction of suitable electron donor and acceptor combinations (Suflita et al. 1988); (2) addition of adequate nutrients to meet the nutritional requirements of dehalogenating microorganisms (Palmer et al. 1989); (3) use of engineered microorganisms with optimum dehalogenating activity (Palmer et al. 1989); and (4) addition of cell-free enzymes capable of

catalyzing reductive dehalogenation reactions (DeWeerd and Suflita 1989).

Dehalogenation Mechanisms

Anaerobic reductive dehalogenation is only one of the mechanisms available to remove halogens from organic compounds. Other anaerobic dehalogenation processes are identified in Figure 1 (Kuhn and Suflita 1989a). The reactions are classified according to the type of compound undergoing dehalogenation, i.e., aromatic or nonaromatic.

Dehalogenation of Aromatic Compounds

Two mechanisms of dehalogenation for aromatic compounds under anaerobic conditions have been observed: reduction and hydrolysis. Reductive mechanisms are recognized as the predominant pathway for removal of halogens from homocyclic aromatic rings under anaerobic conditions, while hydrolytic dehalogenation (including both chemically and enzymatically mediated reactions) is the preferred mechanism for heterocyclic aromatic compounds (Suflita et al. 1982; Kuhn and Suflita 1989a). However, Adrian and Suflita (1989) have recently demonstrated reductive debromination of the herbicide bromacil under methanogenic conditions. This is the first report of reductive dehalogenation of a heterocyclic aromatic compound.

Reductive Dehalogenation of Aromatic Compounds

Many classes of halogenated aromatic compounds have been shown to be degraded by reductive dehalogenation processes (Table 1). Evidence for the involvement of microorganisms in aryl or aromatic reductive dehalogenation reactions include: (1) the specificity of the reductive reaction; (2) characteristic lag periods required before significant dehalogenation is observed; (3) the absence of activity in autoclaved controls; and (4) the isolation of aryl dehalogenating bacteria.

Reductive dehalogenation is rare in well-aerated environments. Methanogenic conditions, in which the typical redox potential is -0.3 V, the preferred electron acceptor is carbon dioxide, and the

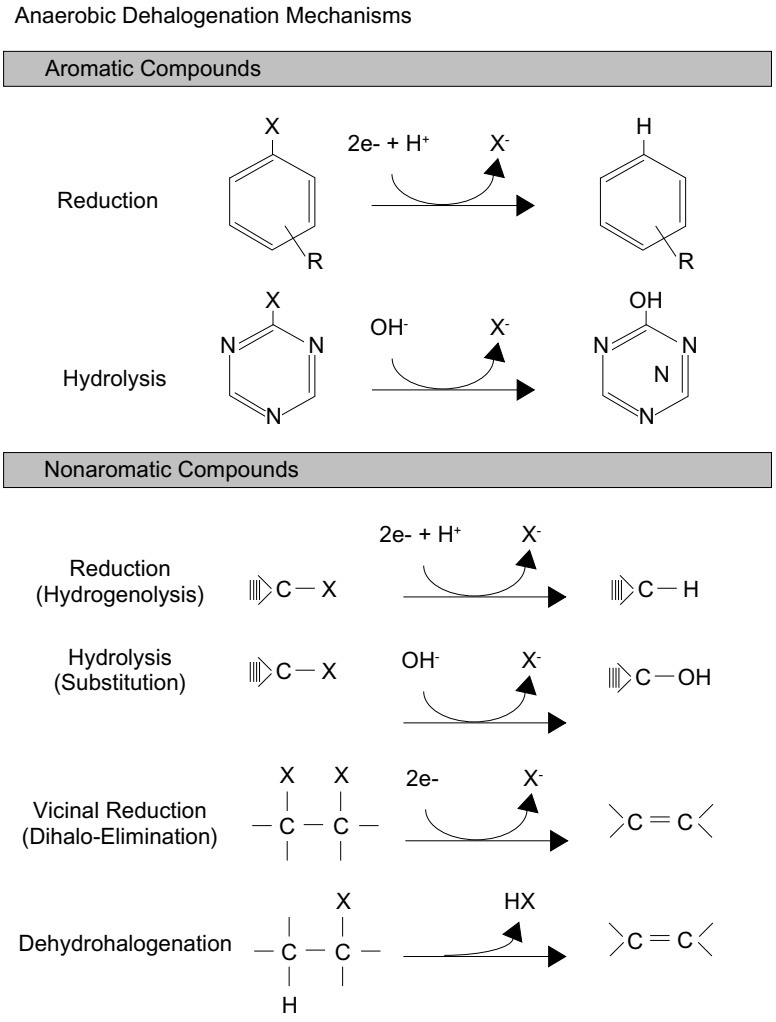

Figure 1. Examples of anaerobic dehalogenation mechanisms for aromatic and nonaromatic pesticides (Kuhn and Suflita, 1989a)

product is methane (Dragun 1988), appear to be optimal for this type of biotransformation. Genthner et al. (1989), have recently investigated dehalogenation of monochlorophenols and monochlorobenzoates under four anaerobic enrichment conditions: methanogenic, nitrate-reducing, sulfate-reducing, and bromoethane sulfonic acid (BESA)-amended. BESA is a potent inhibitor of methanogenesis and was used to promote reductive dechlorination as a terminal electron process.

Aquatic sediments used as inocula were collected from a salinity gradient that included both freshwater and estuarine environments and varying degrees of exposure to industrial effluents. Degradation was observed least often in enrichments with nitrate or sulfate, and most often when amended with 1 mM BESA. In contrast to 1mM BESA, 10mM BESA prevented or delayed the degradation of several of the chloroaromatic compounds, suggesting inhibition of methanogenesis or inhibition of reductive dechlorination by BESA. Other sulfur oxyanions also have been shown to inhibit anaerobic dehalogenation reactions where sulfate is present as an inorganic contaminant (DeWeerd et al. 1986, Gibson and Suflita 1986, Suflita et al. 1988, Kuhn and Suflita 1989b). Additional research is being conducted in environments where sulfate occurs naturally. King (1988) showed that sulfate-reducing bacteria did not participate in dehalogenation of 2,4-dibromophenol (DBP), a naturally occurring halogenated organic compound in some marine sediments, but did appear to degrade phenol, a metabolic product of DBP dehalogenation.

The reductive dehalogenation of chlorinated compounds, as shown in Table 1, is characterized by their specificity for compounds within a particular chemical class, for example benzoates, phenols, or phenoxyacetates (Suflita et al. 1982, Gibson and Suflita 1986, Suflita and Miller 1985, Kuhn and Suflita 1989a). Recently, however, research has shown that cross-acclimation between compound classes can occur. Struijs and Rogers (1989) demonstrated the reductive dehalogenation of dichloroanilines by anaerobic microorganisms in pond sediments acclimated to dehalogenate dichlorophenols. Since both hydroxyl and amino groups have a tendency to donate electrons, the authors hypothesized that organisms that were capable of dechlorinating dichlorophenols, which have been shown to be relatively non-persistent in the environment, could possibly dechlorinate the more persistant dichloroanilines. The monochloroanilines produced by dechlorination of the dichloroanilines were stable under anaerobic conditions, but have been shown previously to be readily degraded under aerobic conditions (Zeyer and Kearney 1982, Zeyer et al. 1985).

The specificity of dehalogenation also is dependent on the position of halogens on the aromatic ring within a class of compounds. For example, chlorinated benzoates are generally more readily dehalogenated at the *meta* position, followed by the *ortho* and *para* positions (Suflita et al. 1982, Genthner et al. 1989). Hydroxy, alkoxy, and nitrogen-substituted aromatic compounds generally are dehalogenated faster at *ortho* and *para* halogens (Kuhn and Suflita 1989a, 1989b), however, Genthner et al. (1989) recently have shown that the order of degradability of monochlorophenols was *meta* > *ortho* > *para*..

Mikesell and Boyd (1986) have shown that three groups of acclimated microorganisms can act in concert to completely

Class of Halogenated Aromatic Compounds	Examples of Specific Compounds
Carboxylated Benzenes	Amiben Dicamba 2,3,6-trichlorobenzoate
Oxygen-Substituted Benzenes	Pentachlorophenol Chlorinated phenoxyacetates (e.g., 2,4-D, 2,4,5-T Halogenated diphenyl ether herbicides (e.g., chloronitrofen)
Nitrogen-Substituted Benzenes	3,4-Dihalogenated aromatic compounds (diuron, DCPU, linuron, DCIPC, propanil) Pentachloronitrobenzene
Cyano-Substituted Benzenes	2,4,5,6-tetrachloroisophthalonitrile (TPN)
Methylene-substituted Benzenes	Benthiocarb
Chlorinated Benzenes	Hexachlorobenzene
Polychlorinated biphenyls	Araclors (commercial PCB products)

Table 1. Classes of halogenated aromatic compounds demonstrated to be susceptible to degradation by reductive dehalogenation processes (Kuhn and Suflita 1989a).

dehalogenate pentachlorophenol (PCP) to form phenol, a substrate that was labile under the methanogenic conditions of their experiments. Each type of microorganism, acclimated to one of three monochlorophenol isomers, transformed PCP by removal of halogens from the same relative ring positions at which they dehalogenated the monochlorophenol substrates. The 2-chlorophenol adapted cells dehalogenated PCP at the two *ortho* positions as well as from the *para* position. Similarly, 4-chlorophenol adapted cells cleaved the *para* chlorine of PCP in addition to the two *ortho* substituents. In contrast, the 3-chlorophenol adapted cells exclusively dehalogenated the *meta* position.

Other studies of PCP degradation have shown accumulation of tri- and tetrachlorophenol intermediates, which indicates that higher halogenated phenols tend to be more readily dehalogenated than their lesser halogenated congeners. Similarly, dehalogenation of chlorinated anilines shows shorter lag periods and faster dehalogenation rates with multi-halogenated compounds compared to di- and monohalogenated anilines. Dehalogenation of aromatic amines occurs predominately at the *ortho* and *para* positions as has been demonstrated with the dechlorination of anilines (Kuhn and Suflita 1989b), though removal of *meta* halogens from this group of compounds has also been demonstrated.

Reductive dehalogenation may require the induction of enzymes responsible for dehalogenation. DeWeerd and Suflita (1990) have demonstrated reductive dehalogenation of 3-chlorobenzoate using cell-free extracts of an anaerobic bacterium. The extracts exhibited the same substrate specificity as whole cells. Rapid dehalogenation activity was found only in extracts of cells cultured in the presence of the halogenated molecule, indicating that the enzymes responsible required induction. Dehalogenation was inhibited by sulfite, thiosulfate, and sulfide. Dehalogenation activity was associated with the membrane fraction and required a low potential electron donor. These results suggest that a specific enzyme is made by the cells for dehalogenation of selected halogenated substrates. Research into the use of enzymes as a potential amendment to enhance bioremediation should be encouraged.

Further evidence that reductive dehalogenation may depend upon the induction of enzymes has been presented by Linkfield et al. (1989). Acclimation periods prior to detectable dehalogenation of halogenated benzoates in anaerobic lake sediments ranged from 3 weeks to 6 months. These periods were reproducible over time and among sampling sites and characteristic of the specific benzoate compound tested. The lengthy acclimation period appeared to represent an induction phase in which little or no aryl dehalogenation was observed. This was followed by an exponential increase in activity typical of an enrichment response. Extremely low activities during the early days of acclimation, coupled with the fact that dehalogenation yields no carbon to support microbial growth, suggests that slow continuous growth from time of the first exposure of the chemical was not responsible for the acclimation period. The characteristic acclimation period for each chemical also argues against nutritional deficiency, inhibitory environmental conditions, or predation by protozoa or other microbial grazers as the cause of the acclimation period. The reproducibility of the findings with time and space and among replicates argues against genetic changes as the explanation.

The removal of chloride or bromide from an aromatic molecule proceeds easier when the ring also is substituted with electron destabilizing groups, such as carboxy, hydroxy, or cyano groups (Kuhn and Suflita 1989a). Other chemical groups attached to the aromatic ring by nitrogen or oxygen bonds may have the same effect on the reductive dehalogenation reaction. However, recent research has shown that even highly chlorinated, poorly water soluble aromatic hydrocarbons that do not contain polar functional groups can also undergo reductive dehalogenation. Hexachlorobenzene (HCB) has generally been considered recalcitrant to microbial attack, particularly in the absence of oxygen (Bouwer and McCarty 1984, Kuhn et al. 1985); however, HCB was shown to degrade to tri- and dichlorobenzenes by Fathepure et al. (1988). Brown et al. (1987) performed standard thermochemical calculations of free-energy changes associated with the oxidation of organic compounds (in this case, glucose) coupled with the reduction of chlorobenzene compounds. The reactions involving HCB and monochlorobenzene offered more energy to anaerobic bacteria than the reduction of compounds available naturally in anaerobic environments, such as sulfate and carbon dioxide (Table 2). Also, more energy could be obtained from the dehalogenation of hexachlorobenzene to benzene than the dehalogenation of monochlorobenzene, indicating that dehalogenation reactions are more likely to occur with aromatic compounds containing many chloro groups since they are more highly oxidized and more electronegative than those containing fewer chloro groups.

Polychlorinated biphenyls (PCBs), commonly thought to be resistant to biodegradative processes, have also been shown to be susceptible to degradation by reductive dehalogenation (Brown et al. 1987, Quensen et al. 1988). Brown et al. (1987) suggest that dehalogenated products formed were less toxic than the original PCB congeners and may possible be more susceptible to oxidative biodegradation by aerobic bacteria.

Hydrolytic Dehalogenation of Aromatic Compounds

Hydrolytic dehalogenation represents a substitution reaction in which a hydroxyl group replaces a halogen on an organic molecule (Figure 1). In general, the anaerobic hydrolytic removal of halogen substituents from homocyclic aromatic compounds is rare (Kuhn and Suflita 1989a), but has been observed under aerobic conditions. Also, the enzymes involved have been shown to be active in reduced media, and some were inhibited by oxygen (Marks et al. 1984, Thiele et al. 1988). This transformation has been observed in anaerobic soil for a single herbicide, flamprop-methyl; however no anaerobic bacteria were isolated with the ability to catalyze this type of dehalogenation. A hydrolytic defluorination product of the herbicide was identified in anaerobic soil incubation studies (Roberts and Standen 1978).

Heterocyclic chloroaromatic compounds, such as chlorinated triazine herbicides, tend to react more readily with hydroxy, amino, or sulfhydryl groups than their homocyclic chemical

Oxidant	Reduced Product	ΔG (kcal/mol)
Molecular oxygen (O_2)	Water (H_2O)	-676.10
Hexachlorobenzene (C_6Cl_6)	Benzene (C_6H_6)	-410.16
Monochlorobenzene (C_6H_5Cl)	Benzene (C_6H_6)	-369.50
Sulfate (SO_4^{2-})	Reduced Sulfur	-131.78
Carbon dioxide (CO_2)	Methane (CH_4)	- 95.63

Table 2. Standard free-energy changes for the oxidation of glucose to CO_2 and H_2O using various oxidants (Brown et al. 1987).

counterparts. Hydrolytic dehalogenation is, therefore, the preferred mechanism for removing halogens from hetero-cyclic aromatic compounds under anaerobic conditions (Adrian and Suflita 1989).

The hydrolysis of triazine herbicides to form dehalogenated and less phytotoxic products has been known for many years (Paris and Lewis 1973). However, there has been controversy over the involvement of microorganisms in this process. Reactions with reactive soil surfaces, such as clays and organic matter, appear to be significant with regard to the rate of hydrolysis (Kuhn and Suflita 1989a). Dechlorination of *s* -triazines has been shown to be catalyzed by microorganisms. This was demonstrated by Cook and Huetter (1984, 1986). The organisms studied were aerobic, but biotransformation of the herbicides did not require molecular O_2 and was functional under anaerobic conditions.

Dehalogenation of Nonaromatic Compounds

Dehalogenation of nonaromatic compounds, particularly halogenated aliphatic chemicals, is generally better understood than aryl dehalogenation reactions. The reductive processes of hydrolysis and dehydrohalogenation have been identified as anaerobic dehalogenation mechanisms (Figure 1).

In general, biologically mediated anaerobic dehalogenation of nonaromatic compounds tends to be faster than dehalogenation of aromatic compounds, does not require long adaptation times, and does not exhibit a high degree of substrate specificity. Some of these reactions also are not too sensitive to the presence of oxygen and have been observed in aerobic incubation systems. The greater variety of reaction mechanisms potentially available to metabolize nonaromatic halogenated compounds in general results in rendering these compounds more susceptible to biodegradation than the haloaromatic compounds (Vogel et al. 1987, Kuhn and Suflita 1989a).

Dehalogenation has been demonstrated with many bacterial species representing diverse genera. Mesophilic and thermophilic methanogenic bacteria as well as some thermophilic clostridial species may catalyze dehalogenation of some aliphatic compounds. For example, metabolism of hexachlorocyclohexanes by thermophilic clostridia was reported by Sethunathan (1973). Dehalogenation reactions are also sometimes heat resistant, suggesting that some reactions may not be enzymatically mediated, and therefore not dependent on intact microorganisms or microbial consortia. The dehalogenation of nonaromatic compounds can be catalyzed by transition metal complexes with or without the involvement of enzymes (Kuhn and Suflita 1989a).

Reductive and Vicinal Dehalogenation of Nonaromatic Compounds

If a nonaromatic carbon atom in a synthetic molecule is highly halogenated, dehalogenation is more easily accomplished by reductive, vicinal reductive or elimination reactions (Vogel et al. 1987). Compounds that have been demonstrated to be degraded by reduction or vicinal reduction mechanisms are listed in Table 3.

Reductive and vicinal dehalogenation reactions are dependent on the redox potential of the electron donor and acceptor. To be thermodynamically feasible, the E_h of the electron accepting reactant (dehalogenation) must be higher than that of the electron donating reactant. This requirement can limit the number of available electron donors for dehalogenation of some compounds (Castro et al. 1985, Vogel et al. 1987, Kuhn and Suflita 1989a). For example, free ferrous iron (Fe(II)) has a redox potential of + 0.77 V; but most of the halogenated alkanes and alkenes with lower redox potentials will not react with this transition metal. However, when Fe(II) is in a complexed form, such as a porphyrin or as ferredoxin, the redox potential is dramatically lowered, and the reaction is possible (Kuhn and Suflita 1989a). As examples, Fe(II)deuteroporphin IX and cytochrome P-450 have redox potentials of 0.00 V and -0.17 V, respectively.

Active transition metal complexes, which include complexes of iron (Fe), cobalt (Co), nickel (Ni), and perhaps chromium (Cr) and zinc (Zn), have redox potentials less than zero and can be as low as -0.8 V for the cobalt complexed vitamin B_12. The low redox potentials of these electron donors allow for their reduction

Class of Halogenated Nonaromatic Compound	Examples of Specific Compounds
Aliphatic Compounds	Tetrachloromethane (carbon tetrachloride) Trichloromethane (chloroform) Dichloromethane (methylene chloride) Chloromethane (methyl chloride) Bromomethane (methyl bromide) Trichloronitromethane (chloropicrin) Hexachloroethane Tetrachloroethene (perchloroethylene) 1,1,1-Trichloroethane Trichlorethene (trichloroethylene) 1,2-Dichloroethane (ethylene dichloride, EDC) 1,2-dibromoethane (ethylene dibromide, EDB) 1,2-dibromo-3-chloropropane (DBCP) 1,1,1-trichloro-2,2-bis (p-chlorophenyl)ethane (DDT) (aliphatic portion)
Alicyclic Compounds	Toxaphene Mirex Heptachlor
Hexahalocyclohexanes	Lindane and its isomers

Table 3. Classes of halogenated nonaromatic compounds demonstrated to be susceptible to degradation by reductive dehalogenation processes (Kuhn and Suflita 1989a).

to be coupled with dehalogenation of many nonaromatic compounds having redox potentials which range from 0 to 1.2 V (Vogel et al. 1987).

Highly halogenated aliphatic compounds have higher reduction potentials than their lesser halogenated analogues; therefore, more energy is released by their dehalogenation, indicating a greater driving force for these reactions. In general, reductive dehalogenation of tetra- and tri-halogenated carbon atoms is easier than di- or monohalogenated congeners (Vogel et al. 1987).

In natural environments, Fe(II) porphyrins (e.g., cytochromes), Co complexes (e.g., vitamin B_12), and Ni complexes (e.g., F-430) are likely to be dominant in the reductive dehalogenation process. Dead cells can release these stable transition metal complexes which are then more available for participation in the dehalogenation process. Such complexes are also active in living cells, as was demonstrated with *Pseudomonas putida* by Castro et al. (1985). *Pseudomonas putida* contains Fe(II)porphyrin bound to the cytochrome P-450 complex, but movement of halogenated compounds across the bacterial membrane and diffusion to the active iron center can limit the rate of dehalogenation.

Another potential reductant available for dehalogenation of haloaliphatic compounds in natural environments is the flavin/flavoprotein complex, which has been shown to mediate many of the known reductive reactions of xenobiotic compounds in laboratory studies (Esaac and Matsumura 1980). To date, no studies have clearly demonstrated the environmental significance of this reductant. Relative to other dehalogenation reaction mechanisms, dehalogenation by vicinal reduction appears to be more tolerant of oxidized conditions and may even be independent of transition metals or metallo-organic complexes (Kuhn and Suflita 1989a).

Dehydrohalogenation of Nonaromatic Compounds

Dehydrohalogenation is an elimination reaction in which two groups are lost from adjacent carbon atoms so that a double bond is formed, resulting in the release of a halogen and a proton (HX) and the formation of an alkene (Figure 1). The rate of dehalogenation is higher when additional chloride ions are bonded to the carbon atom that loses its chloride ion substituent (Vogel et al. 1987). Bromine atoms rather than chlorine atoms are generally more readily eliminated by this reaction. Elimination reactions can proceed spontaneously (1,1,1-trichloroethane; 1,2-dibromoethane) or can be catalyzed by microbial enzymes such as the dechlorinase enzyme which is responsible for the conversion of DDT to DDE--a dechlorination reaction involving the aliphatic portion of the DDT molecule (Kuhn and Suflita 1989a).

Hydrolytic Dehalogenation

Hydrolysis, a substitution reaction in which one substituent on a molecule is replaced by another, has been demonstrated with

many aliphatic compounds. Hydrolysis is favored for carbon atoms with only one or two halogens; however, hydrolytic dehalogenation has been shown with higher chlorinated compounds, such as 1,1,1-trichloroethane. This transformation can be chemically or biologically catalyzed by methanogenic mixed cultures and by a number of aerobic bacterial isolates. Bromine loss tends to be favored compared to the corresponding chlorinated compounds (Kuhn and Suflita 1989a).

Applications And Limitations of Reductive Dehalogenation of Organic Halogenated Pollutants

The degradation of trichloroethylene (TCE), as shown in Figure 2, may be used to illustrate the potential effectiveness of the reductive dehalogenation process to remove common pollutants from the environment, as well as to present some of the cautions that should be observed (Dragun 1988). TCE is an industrial solvent used extensively for degreasing metal as well as in dry-cleaning operations, organic synthesis, refrigerants, and fumigants. Most septic tank cleaning fluids contain TCE (Craun 1984).

Also illustrated in Figure 2 are possible degradative pathways of tetrachloroethylene (PCE) and 1,1,1-trichloroethane (1,1,1-TCA). PCE is a solvent widely used in dry cleaning and degreasing operations; 1,1,1-TCA is used extensively as an industrial cleaner and degreaser of metals, spot remover, adhesive, and vapor pressure depressant (Craun 1984). These compounds have relatively high water solubility (e.g., 1000 mg/l for TCE) and are highly mobile in soils and aquifer materials and often are found in ground waters. Since they are suspected carcinogens (Infante and Tsongas 1982), they represent a threat to human health.

The degradative pathway for TCE (Dragun, 1988) can be described as follows:

(1) TCE can undergo reductive dehalogenation, i.e., the removal of one chloride atom (Cl) and the addition of one hydrogen (H) atom. Three possible reaction products can be formed: 1,1-dichloroethylene (1,1-DCE), cis-1,2-dichloroethylene (c-1,2-DCE), and/or trans-1,2-dichloroethylene (t-1,2-DCE).

(2) 1,1-DCE can undergo reductive dehalogenation to form vinyl chloride, or its carbon-carbon double bond can be reduced to form 1,1-dichloroethane (1,1-DCA).

(3) The two dichloroethylene compounds, c-1,2-DCE and t-1,2-DCE can undergo reductive dehalogenation to form vinyl chloride. Their carbon-carbon double bonds can be reduced to form 1,2-dichloroethane (1,2-DCA).

(4) 1,1-DCA and 1,2-DCA can undergo dehydrohalogenation to form vinyl chloride. These two chemicals can also undergo reductive dehalogenation to form chloroethane.

The degradation pathway of a single compound, TCE, can lead to the production of six chlorinated volatile hydrocarbons. The degradation of PCE can lead to the production of seven chlorinated

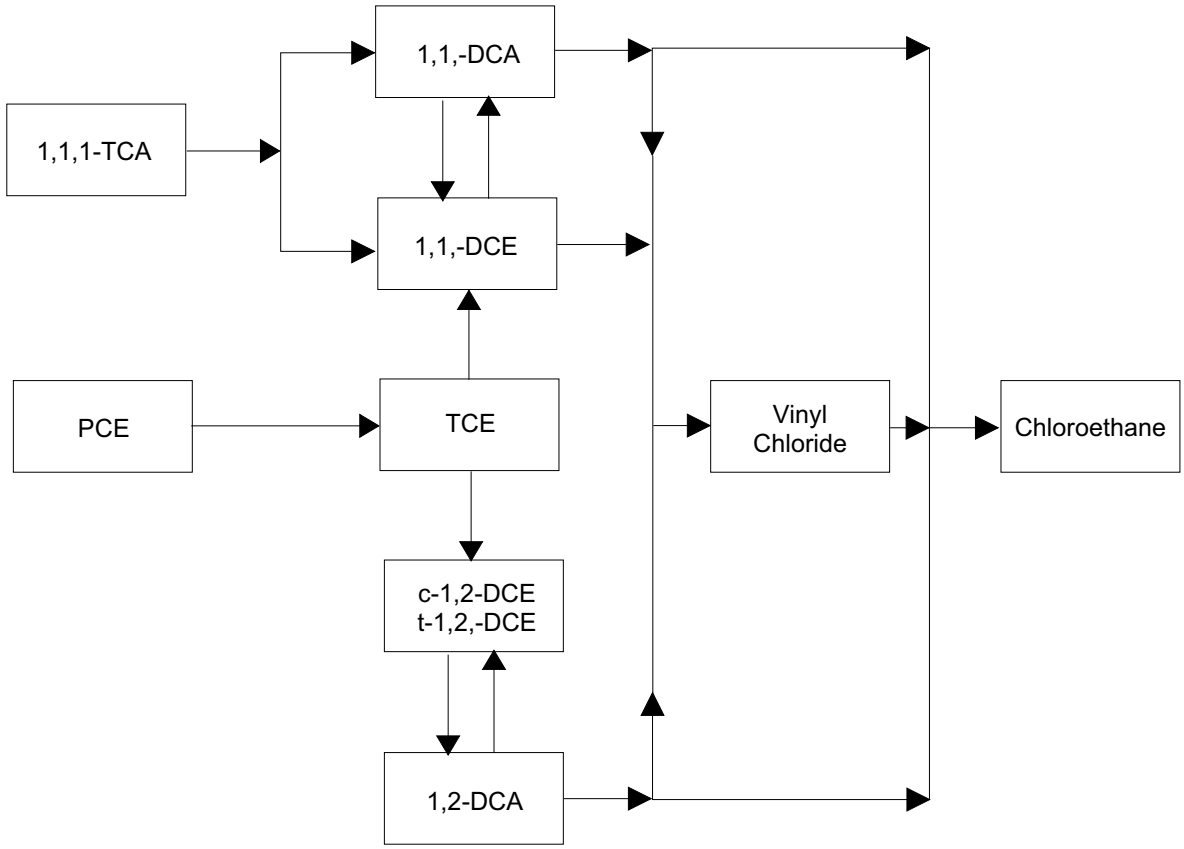

Figure 2. Transformation pathways for various chlorinated volatile hydrocarbons in soil systems (Drugun 1988).

volatile hydrocarbons, while the degradation of 1,1,1-TCA can lead to the production of four chlorinated hydrocarbons. Two of the metabolic products formed, vinyl chloride and 1,1-DCA, have been classified as a carcinogen and a probable carcinogen, respectively (Vogel et al. 1987). The dichloroethylene products, c-1,2-DCE and t-1,2-DCE, and vinyl chloride are also regulated under the 1986 Safe Drinking Water Amendments (Freedman and Gossett 1989). Vinyl chloride is the most persistent of the compounds under anaerobic conditions, but can be rapidly degraded under aerobic conditions (Hartsmans et al. 1985, Fogel et al. 1986).

Management of a bioremediation system to accomplish treatment of these compounds in a manner to protect human health and the environment should incorporate considerations of detoxification as well as disappearance of the parent compounds. Disappearance is not synonymous, however, with mineralization to inorganic salts, carbon dioxide, and water. Partial degradation of organic substrates can result in the production of metabolic products that generate their own environmental and health consequences. Such contaminants may be of more toxicological concern than the parent compounds (Suflita et al. 1988).

Fathepure and Boyd (1988) recently suggested that in situ dechlorination of PCE to TCE could be enhanced by stimulating methanogenesis. They found that reductive dechlorination of PCE occurred only during methanogenesis, and no dechlorination was seen when methane production ceased. There was a clear dependence of the extent of PCE dechlorination on the amount of methanogenic substrate (methanol) consumed. Methanogenic bacteria are present in a diversity of environmental habitats, including those where chloroethylenes are commonly found as contaminants (e.g., soils, ground waters, and aquifers near landfills).

A bioremediation system for chlorinated ethylenes and ethanes could consist of maintenance of an anaerobic environment, followed by aeration to complete the degradation process after anaerobic degradative processes have reduced the parent compounds to acceptable levels. Recent research, however, by Freedman and Gossett (1989) has shown that PCE and TCE can be degraded to ethylene, a non-chlorinated environmentally acceptable biotransformation product, under anaerobic methanogenic conditions if an adequate supply of electron donors was supplied to a mixed anaerobic enrichment culture. Methanol was the most effective electron donor, although hydrogen, formate, acetate, and glucose also served.

Ethylene is sparingly soluble in water and has not been associated with any long-term toxicological problems (Autian 1980). It is also a naturally occurring plant hormone. Since complete conversion of VC to ethylene was not observed in the study, the authors suggested that further research is required to determine the concentration of electron donors required to complete the conversion.

A major operational cost of this method of enhanced anaerobic bioremediation will be the supply of electron donors. Alternatively, means to channel more of the donors into the reductive dechlorination process and less into methane production should be investigated.

As proposed by Fathepure et al. (1988), a similar potential for the use of an anaerobic environment followed by an aerobic environment, for mineralization and detoxification of halogenated organic pollutants, is illustrated by the degradation of hexachlorobenzene (HCB) (Figure 3). HCB is a fungicide used as a seed coating for cereal crops. Two pathways of dechlorination were proposed: (1) a major pathway in which 1,3,5-trichlorobenzene (1,3,5-TCB) is formed via pentachlorobenzene and 1,2,3,5-tetrachlorobenzene (1,2,3,5-TTCB); and (2) a minor pathway in which dichlorobenzenes are formed via 1,2,4,5-TTCB and 1,2,4-TCB.

The authors presented explanations for the existence of two pathways. One is that there were two populations, each using a different pathway. The other is that the products reflect the distribution of reactive ring intermediates in which a chlorine, between two other chlorines, was lost most readily and dechlorination ceased when there are no adjacent chlorines as with 1,3,5-TCB.

Reductive dechlorination appeared to occur in a stepwise fashion until lower chlorinated compounds accumulated. Most of the added HCB accumulated as 1,3,5-TCB, which remained unchanged. Although metabolic products identified in this study were not further utilized by the anaerobic sludge populations used to elucidate the metabolic pathways, it is likely that they would be degraded by aerobic organisms (Reineke and Knackmuss 1984, deBont et al. 1986, Schraa et al. 1986, Spain and Nishino 1987) or by facultative anaerobes possessing dechlorinating activity (Tsuchiya and Yamaha 1984).

The U.S. Environmental Protection Agency is presently sponsoring research to develop engineered microorganisms capable of anaerobic reductive dehalogenation of organic halogenated compounds (Palmer et al. 1989). *Desulfomonile tiedjei* (DeWeerd et al, 1990), formerly known as DCB-1, is the first obligate anaerobe known to accomplish reductive dehalogenation. Results using this organism indicated that no plasmid genes responsible for dehalogenating activity could be detected. Therefore, in order to clone the gene or genes responsible for the activity, a genomic library of the bacterial chromosome is being constructed to isolate the dehalogenase gene. The isolation of the gene would be greatly facilitated by the isolation and characterization of the requisite dehalogenase.

Summary

Bioremediation of soils and ground waters contaminated with organic pollutants involves management of the contaminated system to control and enhance biodegradation of the pollutants present (Sims et al. 1989, Thomas and Ward 1989). Reductive dehalogenation appears to be a potentially powerful process for achieving bioremediation of a site contaminated with organic halogenated pollutants, if mechanisms and pathways of degradation are known and can be managed to achieve removal of the compounds of interest as well as potentially toxic metabolic degradation products.

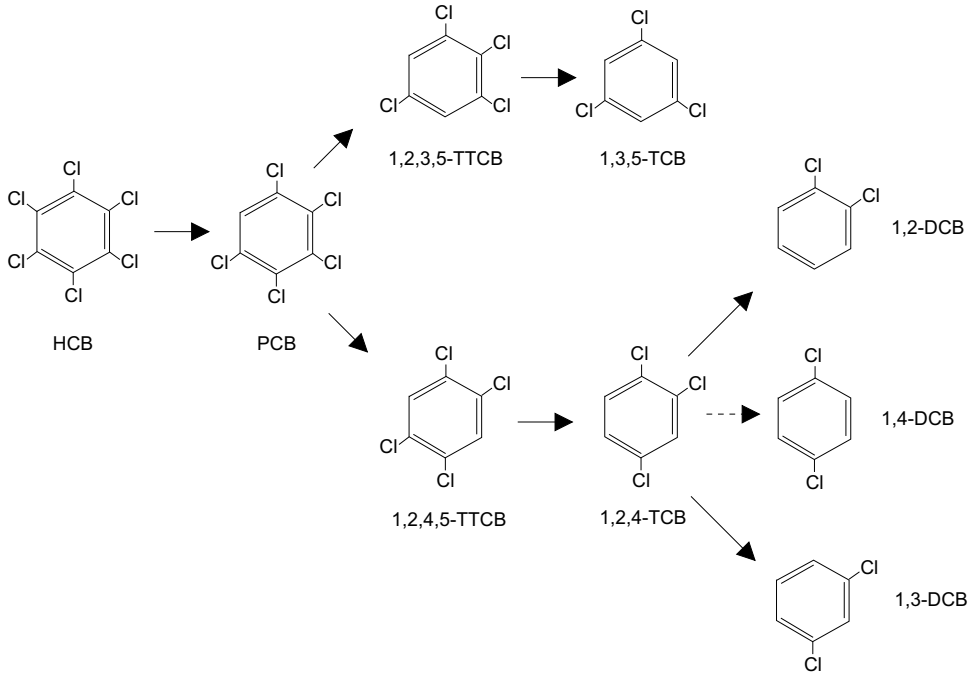

Figure 3. Proposed pathway for HCB dechlorination by an anerobic microbial community (Fathepure et al. 1988).

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