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Review

Chloroethenes contaminants in the environment: Still a cause for concern

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Prior to 1980, chloroethenes were generally believed to be recalcitrant to degradation. Efforts to remediate sites polluted by this group of compounds were thus limited to the ineffective and tedious pump-and-treat method. The environmental significance of chloroethenes and the absence of effective alternative physical remediation methods have warranted intense scientific investigation into the role of microorganisms for detoxifying these environmentally important compounds to benign products. Several microbial mechanisms have therefore been identified to significantly degrade these compounds in soil and ground water polluted systems. This paper provides a mini-review of such mechanisms with the view of understanding the biodegradation processes for better exploitation for bioremediation of chloroethene contaminants, which continue to be of serious environmental challenges.

Key words: Bioremediation, chloroethenes, cometabolism, oxidation, reductive dechlorination.

INTRODUCTION

A large volume of chlorinated compounds are produced yearly for a variety of industrial, agricultural and commercial uses, including solvents, lubricants, intermediates in chemical industry, pesticides, pharmaceuticals, and medical equipment (Hileman, 1993). Although, a number of chlorinated compounds are produced naturally in soil and marine environments (Gribble, 1996), the vast majority is anthropogenic in origin. A large number of these compounds persists and bioaccumulate in the environment because of their resistance to both chemical and biological attack, causing considerable environmental pollution and human health problems (Bradley *et* al., 1997; Squillace et al., 1999). For example, some chlorinated ethanes, chlorinated ethenes, and propenes are reported to be carcinogenic and/or genotoxic (Henschler, 1994). Besides the local and regional contamination of soils and groundwater, long-range transport through water and air helps the compounds to spread even further across the earth (Connell et al., 1999). The extensive use of chloroethenes, in particular, has resulted in widespread environmental contamination, which is of serious concern due to their persistence, toxicity and carcinogenicity. They are therefore widely distributed in the environment and are found in many sediments, soils, groundwater aquifers, and subsurface environment worldwide. Prior to

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Table 1. Physical and chemical properties of chloroethene compounds.

Compound	Melting point (^{o}C)	Density (g/cc)	Log K_{ow}	Log K_{oc}	Water solubility (mg/L)	Vapour Pressure (mm Hg)	Henry' law constant (atm-m^3 mol)	Maximum Contaminant level (µg/L)
Tetrachloro-ethene(PCE)	-22.7	1.625	3.14	2.82	1.5 E+02	1.40 E+01	2.27 E-02	5
Trichloro-ethene (TCE)	-87	1.462	2.42	2.10	1.0 E+03	5.87 E+01	8.92 E-03	5
Cis-1,2-dichloroethene	-81	1.284	1.86	1.50	3.5 E+03	2.0 E+02	7.5 E-03	70
trans- 1,2-dichloroethene	-50	1.257	2.09	1.77	6.3 E+03	2.65 E+02	6.6 E-03	100
1,1-dichloro-ethene	-97.4	1.175	1.79	1.48	5.5 E+03	1.82 E+02	5.7 E-03	7
Vinyl Chloride	-157	0.9121	0.60	0.91	1.1 E+03	2.30 E+03	6.95 E-01	2

1980, chloroethenes were generally believed to be recalcitrant to biodegradation. During the 1980s, there was a wide report of accumulation of tetrachloroethene (PCE) and trichloroethene (TCE) transformation products, which was attributed to microbial reductive dechlorination (Vogel and McCarty, 1985; Barrio-Lage et al., 1987, 1990; Bouwer, 1994; McCarty and Semprini, 1994; Vogel, 1994; Odum et al., 1995). Microbial degradation of vinyl chloride (VC) as the sole carbon substrate for energy and growth under aerobic conditions was also reported by Hartmans et al. (1985). The discovery of microorganisms by the mid 1990s which gain energy from reductive dechlorination of chloroethene led to a turning point from a predominantly co-metabolic view of chloroethene biodegradation to the concept of chloroethenes serving as primary substrates for microbial metabolism (Holliger et al., 1993; Krumholtz et al., 1996; Sharma and McCarty, 1996; Maymó-Gatelle et al., 1997). Subsequently, microbial oxidation of VC under Fe(III)-reducing conditions was demonstrated (Bradley and Chapelle, 1996) and anaerobic microbial oxidation of VC as a primary substrate also reported (Bradley et al., 1998a). These observations therefore led to the conclusion that effective mechanisms exist for microbial degradation of chloroethene contaminants in groundwater systems and this has been a matter of intense scientific investigation.

Today, several microbial mechanisms have been identified for effective and significant transformation of chloroethenes in soil and groundwater systems. Chloroethene organics still continue to be among the most common contaminants frequently observed in groundwater systems (Bouwer, 1994; McCarty and Semprini, 1994; Vogel, 1994). Most of the members of this group of chlorinated organic compounds are considered toxic and/or carcinogenic to humans and are grouped under the list of priority pollutants of the Environmental Protection Agency (EPA) and they continue to be of public and regulatory concern. This paper is therefore aimed at providing a brief overview of such mechanisms for a proper understanding of the role of microorganisms in the biotransformation of chloroethenes in the environment. This is particularly important since most environments in the African continent also suffer serious pollution by this group of chlorinated organics without appropriate regulatory effort to control the discharge of such pollutants into the environment.

CHLOROETHENES: TYPE, USES, PROPERTIES AND DISTRIBUTION IN THE ENVIRONMENT

Chloroethene compounds vary from the monochlorinated vinyl chloride (VC) to the most chlorinated tetrachloroethenes (PCE). Other members include; trichloroethene (TCE), cis 1,2-dichloroethene (cDCE), trans 1,2-dichloroethene (tDCE), and 1,1-dichloroethene (1,1-DCE). Both PCE and TCE are detected with the greatest frequency and highest concentration in groundwater systems worldwide, owing to their widespread industrial use as degreasing agents and dry-cleaning solvents (Hopkins et al., 1993; Canter and Sabatini, 1994; McCarty, 1997). cDCE and tDCE occur primarily as a result of in situ microbial transformations of TCE, with cDCE being the predominant daughter product and tDCE less commonly observed in groundwater (Bradley, 2000). 1,1-DCE is formed primarily from the transformation of trichloroethane (TCA) while VC contamination results primarily from anaerobic microbial reduction of DCE and TCA. Table 1 shows the chemical and physical properties of chloroethenes, which is important for their ultimate fate and transport in the environment. The octanol-water partition coefficient (K_{OW}) is a measure of the tendency of the compounds to partition into lipids and it is used to estimate the bioconcentration of the chlorinated organics. The Koc is the adsorption coefficient of a compound in a particular environmental compartment and is a function of the

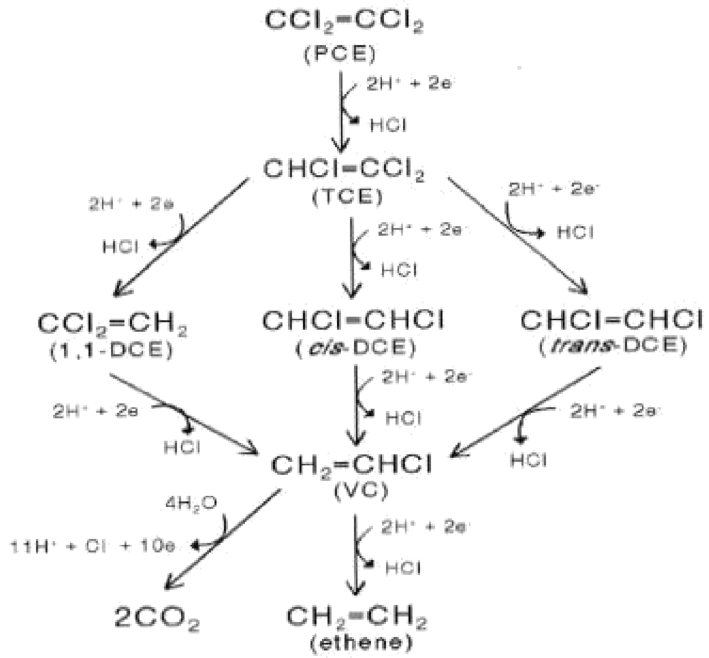

Figure 1. Proposed pathway for biological transformation of chlorinated ethenes (Maymo-Gatelle et al., 1997).

organic content and other properties of the environment compartment as well as properties of the organic compound. The environmental fate of chloroethene compounds is impacted primarily by their physical and chemical properties and by their point of entry into the environment (Lee and Davis, 2000). The maximum contaminant level (MCL) of these compounds for drinking water set by EPA is also included. Vinyl chloride is considered the greatest threat to human health because of its carcinogenic property and has a drinking water MCL of 2 mg/L.

MICROBIAL TRANSFORMATION OF CHLOROETHENES

Previous efforts to remediate chloroethene-contaminated sites were limited to largely unsuccessful, ineffective and costly pump -and-treat methods (Biswas, 1992; Beeman et al., 1994; Löffler et al., 2000). However, laboratory and field evidence now accumulates that microorganisms can transform chloroethenes to non-toxic products under a variety of environmental conditions (Bolesch et al., 1997; DeWeerd et al., 1998; Holliger et al., 1998; Lee et al., 1998; Nielsen and Keasling, 1999; Ellis et al., 2000).

REDUCTIVE DECHLORINATION OF CHLORO-ETHENES

The observed production and accumulation of reduced daughter products (DCE and VC) was attributed to microbiologically catalyzed process of reductive dechlorination (Vogel and McCarty, 1985; Barrio-Lage et al., 1987, 1990; Bouwer, 1994; McCarty and Semprini, 1994; Vogel, 1994; Odum et al., 1995). Chloroethenes are readily oxidized compounds and can act as electron acceptors owing to the presence of electronegative chlorine substituents (Vogel et al., 1987). The efficiency of reductive dechlorination of chloroethenes, however, depends on the number of chloro-moieties attached to the carbon atoms, the in situ redox condition, acetate condition, pH and temperature (Vogel, 1994; Chapelle, 1996; Gosset and Zinder, 1996; Stuart et al., 1999). The potential for reductive dechlorination of chloroethene compounds increases with increasing number of chlorine atoms present on the molecule (Bouwer, 1994; Wiedemeir et al., 1998). Of all the chloroethene compounds, PCE is the strongest oxidant, with four chlorine atoms and it can readily undergo reductive dechlorination to TCE under highly reduced anaerobic conditions in the absence of oxygen, nitrate, sulphate etc., which are more thermodynamically favourable to serve as electron acceptors than chlorinated solvents. However, reductive dechlorination of PCE and TCE under aerobic conditions has been reported (Enzien et al., 1994; Shim et al., 2001) and a facultative bacterium, *Enterobacter agglomerans* that transform PCE to *cis*-DCE under aerobic condition has been described (Sharma and McCarty, 1996). On the other hand, reductive dechlorination of TCE to *cis*-DCE in a more strongly reducing environment such as under Fe (III)-reducing condition (Chapelle, 1996). Reductive dechlorination of *cis*-DCE to VC requires SO_4- reducing conditions, at least (Vogel et al., 1987; Chapelle, 1996), while those of VC to the non-chlorinated product, ethene is relatively very slow and only observed under highly reducing methanogenic conditions (Maymo-Gatelle et al., 1985; Barrio-lage et al., 1987, 1990; Distefano et al., 1991; De Bruin et al., 1992; Carter and Jewell, 1993; Bouwer, 1994; Odum et al., 1995; Wu et al., 1995). The proposed pathway for the sequential reductive dechlorination of PCE through the daughter products to the non-chlorinated ethenes as proposed by Maymo-Gatelle et al. (1997) is as shown in Figure 1. However, reductive dechlorination of PCE and TCE often leads to accumulation of daughter products, mainly *cis*-DCE and VC in subsurface and groundwater systems (Major et al., 1991; Pill et al., 1991; Kitanidis et al., 1993; McCarty and Reinhard, 1993; Haston et al., 1994; Weaver et al., 1995; Wilson et al., 1995). The detection of VC (a known human carcinogen) and on the list of the Environmental Protection Agency’s priority pollutants has increased regulatory concern over chloroethene contamination over the years, thus slowing down early exploitation of microbial reductive dechlorination for chloroethene remediation. Subsequently, effort has been directed towards the use of physical remediation method, such as

Table 2. Electron acceptors for chloroethenes biodegradation and their degradation products.

Compound	Electron acceptor	Products	References
Tetrachloroethene (PCE)	SO_4	TCE, cDCE	Bagley and Gossett, 1989
	CO_2	TCE, cDCE, VC, ethene carbon dioxide	Vogel and McCarty, 1985 De Bruin et al., 1992
Trichloroethene (TCE)	O_2	Not identified	Leahy et al., 1996
	O_3 + CoM	TCE-epoxide, CO, glyoxylic acid, trichloroethanol, trichloroacetate, trichloroacetaldehyde, glyoxylate, dichloroacetate, formic acid, carbon-dioxide	Semprini et al., 1992, Dolfing et al., 1993
	NO_3	Not identified	Leahy et al., 1996
	SO_4	cDCE	Vogel and McCarty, 1985
	CO_2	cDCE, tDCE, VC, carbon dioxide	Cox et al., 1994
	CO	cDCE, tDCE, 1,1-DCE, VC, ethene	Jablonski and Ferry, 1992
cis-1,2-dichloroethene (cDCE)	O_2	carbon dioxide	Davis and Carpenter, 1990, 2000 Klier et al., 1999
	O_2+CoM	cDCE-epoxide, tDCE epoxide	Hopkins and McCarty, 1995
	Fe^{3+}	Carbon dioxide	Bradley and Chapelle, 1997
	CO_2	VC, ethene, ethane	Komatsu et al., 1994
	Humic acid	Carbon dioxide	Bradley et al., 1998
trans-1,2-dichloroethene (tDCE)	O_2+CoM	tDCE-epoxide	Hopkins and McCarty, 1995
	O_2	Carbon-dioxide	Klier et al., 1999
	CO_2	VC	Wilson et al., 1986
1,1-dichloroethene (1,1-DCE)	O_2+CoM	Not identified	Hopkins and McCarty, 1995
	O_2	Not identified	Klier et al., 1999
	CO_2	VC	McCarty et al., 1986 Wilson et al., 1986
Vinyl chloride (VC)	O_2	Carbon dioxide	Davis and Carpenter, 1990
	O_2+CoM	Not identified	Bradley and Chapelle, 1998
	Fe^{3+}	Carbon dioxide	Dolan and McCarty, 1994 Bradley and Chapelle, 1996
	CO_2	Ethene, ethane, carbondioxide, methane, chloromethane	Barrio-Lage et al., 1990 DeBruin et al., 1992
	Humic acid	Carbon dioxide	Bradley and Chapelle,1997 Bradley et al., 1998a

[a] O_2=aerobic; CO_2= methanogenic; CoM= cometabolic substrate; Fe^{3+}= iron reducing; NO_3= nitrate reducing; SO_4= sulfate reducing.

pump-and-treat for remediating groundwater contaminated with chloroethenes. These methods have however, been shown to be generally slow and ineffective (Bouwer, 1994).

The increasing understanding of various microbial mechanisms for chloroethene degradation coupled with the absence of effective alternative methods for remediating these compounds has led to the re-evaluation of bioremediation of chloroethenes in contaminated systems. Now, a lot of reaction sequences leading to complete dechlorination has been observed (Cabirol et al., 1998). For example, anaerobic transformation of PCE through a sequential reductive dechlorination to environmentally acceptable product, ethane, has been reported (Barrio-Lage et al., 1986; Freedman and Gossett, 1989; Holliger et al., 1993). Other chloroethenes, such as TCE, DCEs and VC have also been shown to be sequentially dechlorinated by microorganisms via reductive dechlorination under anaerobic conditions (Freedman and Gosstett, 1989; Distefano et al., 1991; Rosner et al., 1997). Also, reductive dechlorination of PCE with mixed anaerobic consortia has been conducted in many laboratories and several strains of dehalogenating bacteria capable of transforming PCE to ethane have been isolated (Maymo-Gatelle et al., 1997). Substantial information on bacteria that use polychlorinated ethenes (PCE and TCE) as metabolic electron acceptors now accumulates (Holliger et al., 1998; Loffler et al., 2003). This is shown in Table 2. Recently, a novel bacterium, *Dehalococcoides* sp strain BAV1 that used VC as growth-supporting electron acceptor, thereby transforming this carcinogenic compound to the benign products ethene and inorganic chloride was isolated (He et al., 2003b). This organism was reported to also be able to utilize some other chloroethenes, such as cis-DCE, trans-DCE, and 1,1-DCE as growth-supporting electron acceptors (He et al., 2003b).

Investigations from 1993 has pointed out that microorganisms capable of utilizing chloroethene compounds as terminal electron acceptors and gaining energy from the process (called halorespires) can carry out reductive dechlorination of chloroethenes at a much higher rate. However, only one bacterium, *Dehalococcus ethenogenes* strain 195, has been shown to completely dechlorinate PCE to ethene, in spite of several reports on the relative abundance of halorespires capable of reducing PCE or TCE to DCE (Maymo-Gatelle et al., 1997). The presence of Dehalococcus- like organisms in chloroethene-contaminated sites has therefore been reported to result into enhanced reductive dechlorination in such sites (Hendrickson et al., 2002). Several distinct *Dehalococcoide* species, capable of efficient dechlorination of cis-DCE and VC but not PCE and TCE have also been reported recently (Cupples et al., 2003; He et al., 2003a, 2003b). Similarly, a number of mixed microbial populations capable of efficient reductive dechlorination of DCE and VC have been described (Rosner et al., 1997; Loffler et al., 1999; Flynn et al., 2000). Reductive dechlorination of chloroethene cntaminants in groundwater systems is often attributable to the activities of cooperative consortia of microorganisms rather than to a single species (Bradley, 2003). Most but not all halorespiring microorganisms can use hydrogen as electron donor. Maymo-Gatelle et al. (1995) suggested that in anaerobic mixed cultures, methanogens and acetogens provide the hydrogen necessary for respiratory dehalogenating bacteria through the transformation of other electron donors. This explains why inhibition of acetogens and methanogens often affects PCE dechlorination (Distefano et al., 1992; Freedman and Gossett, 1989), although these bacteria are not directly responsible for dechlorination.

Although, reductive dechlorination in subsurface environment and groundwater systems is ecotoxicologically important, the effectiveness of this method for chloroethenes' bioremediation has been affected by certain factors as mentioned previously. Besides, it often leads to the accumulation of daughter products, which are even more toxic than the parent compounds. The use of reductive dechlorination together with other recently described effective microbial oxidation of DCEs and VC under both anaerobic and aerobic conditions will lead to the successful bioremediation of sites contaminated with chloroethenes.

MICROBIAL OXIDATION OF CHLOROETHENES

One of the methods involving oxidation of chloroethenes by microorganisms is aerobic cometabolic oxidation. This process requires the presence of oxygen and another carbon source as a primary substrate to induce the production of non-specific oxygenases by the organisms involved (McCarty and Semprini, 1994). Methanogenic bacteria capable of aerobic oxidation of TCE to CO_2 were described by Wilson and Wilson (1985) . Subsequently, a wide variety of microorganisms able to oxidize; methane (Baek and Jaffe, 1989; Moore et al., 1989; Tsien et al., 1989; Gerritse et al., 1995; Semprini, 1995), propane (Malachlowsky et al., 1994), propene (Reij et al., 1995), methanol (Fitch et al., 1996), ammonium (Vannelli et al., 1990), isopropene (McCarty and Semprini, 1994), vinyl chloride (Verce et al., 2002), and aromatic compounds (Fan and Scow, 1993; Malachlowsky et al., 1994; Fuller et al., 1995; Hopkins and McCarty, 1995; Semprini, 1995; Mars et al., 1996; Ryoo et al., 2000; Shim et al., 2001) have been reported to oxidize chloroethenes to CO_2 with no detectable accumulation of toxic intermediates. Cometabolic oxidation of chloroethenes has been successfully applied for engineered remediation by simply adding the required primary substrate to the contaminated groundwater systems (Semprini et al., 1991; McCarty and Semprini, 1994; Semprini, 1995). Also, cometabolic oxidation of DCE has also been described using various co-substrates including, phenol, toluene, propane and methane (McCarty and Semprini, 1994). This method is considered to be of long-time significance for the transformation of chloroethenes.

Despite the observed microbial oxidative cometabolism of chloroethenes, aerobic oxidation of chloroethene contaminants in the absence of any co-substrates has also been reported. The tendency for aerobic oxidation of chloroethenes increases with decreasing number of chlorine atoms attached to the carbon atoms (Vogel et al., 1987). Several laboratory and field studies have shown that VC can be easily degraded under aerobic conditions (Hartmans et al., 1985; Davis and Capenter, 1990; Phelps et al., 1991; Bradley and Chapelle, 1996, 1998; Coleman et al., 2002a). Vinyl chloride has also been shown to serve as the main carbon source for microbial growth (Hartmans et al., 1985; Hartmans and deBont, 1992) . Recently, DCE has also been shown to be aerobically oxidized and mineralized to CO_2 without adding any co-substrate (Bradley and Chapell, 1998b; Bradley et al., 1998b, 1998c, Klier et al., 1999). Similarly, *cis*-DCE has been shown to be utilized as the sole carbon source by some microorganisms (Bradley and Chapelle, 2000a, Coleman et al., 2002b; Olaniran et al., 2004). Although, cometabolism of PCE and TCE has been demonstrated under aerobic conditions, none of the two compounds has been shown to serve as primary substrate for aerobic microbial degradation. Since, aerobic oxidation of chloroethenes is influenced by the presence of dissolved oxygen; it is worth mentioning that

Table 3. Half reactions of reductive transformations of chloroethene compounds and conventional electron acceptors.

Electron Acceptor	Half-reaction of reductive transformation	$\Delta G^{0'}$/electron (kJ)[a]	E_0' (mV)[b]
PCE[c]	$C_2Cl_4 + H^+ + 2e^- \longrightarrow C_2HCl_3 + Cl^-$	-55.3	574
TCE[c]	$C_2HCl_3 + H^+ + 2e^- \longrightarrow$ cis-$C_2H_2Cl_2 + Cl^-$	-53.0	550
TCE	$C_2HCl_3 + H^+ + 2e^- \longrightarrow$ trans-$C_2H_2Cl_2 + Cl^-$	-50.9	528
TCE	$C_2HCl_3 + H^+ + 2e^- \longrightarrow$ 1,1-$C_2H_2Cl_2 + Cl^-$	-50.8	527
cis-1,2-DCE[c]	cis-$C_2H_2Cl_2 + H^+ + 2e^- \longrightarrow C_2H_3Cl + Cl^-$	-38.3	397
trans-1,2DCE	trans-$C_2H_2Cl_2 + H^+ + 2e^- \longrightarrow C_2H_3Cl + Cl^-$	-40.4	419
1,1-DCE	(1,1-) $C_2H_2Cl_2 + H^+ + 2e^- \longrightarrow C_2H_3Cl + Cl^-$	-40.5	420

[a] Calculated based on data from Thauer et al. (1977), Vogel et al. (1987) and Dolfing and Janssen (1994), assuming that $H^+ = 10^{-7}$ M and $Cl^- = 10^{-3}$ M.

[b] The redox potentials were calculated from $\Delta G^{0'}$ according to Thauer et al. (1977) with the equation $E_0' = \Delta G^{0'}/-nF$; where n= number of electron equivalents transferred (= 1) and F = Faraday's constant (96.487 kJ/volt-equivalent).

[c] ATP-producing reactions in pure cultures of known anaerobic dechlorinating bacteria, as so far reported in literature.

this process will be applicable at the surface of the water systems. This process could help overcome the problems associated with the oxidative cometabolic oxidation which requires both oxic and co-substrates.

Microorganisms capable of oxidizing VC to CO_2 under anaerobic conditions were reported by Bradley and Chapelle (1996). This led to the increasing acceptance of in situ bioremediation as a way of remediating chloroethene contaminated sites. Anaerobic oxidation of VC occurs in the presence of strong oxidant such as Fe(III)-oxides. In a recent investigation, Bradley and Chapelle (1998b) described the effect of redox conditions on DCE and VC oxidation in microcosms. Significant mineralization of DCE and VC was observed and the mineralization decreases with increasing reducing conditions. The general order of VC mineralization observed was; aerobic > Fe(III) z- reducing > SO_4-reducing > methanogenic conditions. Anaerobic oxidation of DCE to CO_2 under Mn(IV)-reducing conditions has also been observed in aquifers without VC formation (Bradley et al., 1998c). The presence of humic acids has also been shown to lead to the efficient mineralization of cis-DCE and VC (Bradley et al., 1998). Microbial oxidation of DCE and VC under anaerobic conditions therefore provides a potential anaerobic alternative to the slow and inefficient reductive dechlorination of VC to ethene.

Overall, the combined effort of microbial reductive dechlorination of higher chlorinated ethenes (PCE and TCE) under anaerobic conditions to the daughter products (DCEs and VC) and the microbial oxidation of the daughter products to ethene and CO_2 under both aerobic and anaerobic conditions is important for significant and complete removal of chloroethene contaminants in both soil and groundwater systems.

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