Bioremediation Journal, 7(2):81–109 (2003)

ISSN: 1040-8371
DOI: 10.1080/10889860390246141

History and Ecology of Chloroethene Biodegradation: A Review

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Introduction

Biodegradation of chloroethene compounds has been a matter of intense scientific investigation since these compounds were first identified in the late 1970s as common contaminants in groundwater systems. Over this period, perspectives on chloroethene biodegradation and on the role of organochlorine compounds in the environment have changed substantially. Before 1980, chloroethene contaminants were considered recalcitrant to biodegradation. Today, biodegradation is viewed as an essential component of chloroethene plume remediation and several microbial mechanisms for chloroethene transformation have been identified. The purpose of this review is to place these shifting perspectives within historical and ecological contexts, summarize the recent advances in the scientific understanding of reductive and oxidative biodegradation mechanisms, and identify critical needs affecting the application of in situ chloroethene biodegradation to groundwater remediation.

Historical and Ecological Context

The consensus, prior to 1980, that chloroethene compounds were recalcitrant to biodegradation was consistent with the contemporary view that organochlorine compounds were not natural components of the biosphere (Asplund, 1995; Gribble, 1992, 1994). Prior to 1970, fewer than 30 naturally-occurring organochlorine compounds were reported in the scientific literature (Gribble, 1994). In contrast, by the early 1980s, the ecological threat from anthropogenic organochlorines was clearly established by Love Canal and other highly-publicized environmental disasters. Thus, at the time chloroethene compounds were identified as common groundwater contaminants, the general perception was that natural production of organochlorine compounds was negligible.

Equally problematic from the viewpoint of contaminant remediation, shallow aquifer systems generally were perceived as biologically inactive prior to 1980 (Chapelle, 1999). In retrospect, this perception is difficult to reconcile with early reports on microbial activity in deep subsurface environments (Bastin, 1926; Davis, 1967). Nevertheless, perhaps due to the success of non-biological approaches to geochemical characterization of shallow groundwater systems, little importance was assigned to biological processes in shallow ground water before the 1980s (Chapelle, 1999).

The ecological implications of these perceptions were not encouraging. Any organochlorine compounds that were released to the environment through accidents and poor disposal practices would enter a biosphere that lacked indigenous microbial organochlorine-degradation mechanisms. Field evidence appeared to support this view. At Love Canal, chloroethene contamination remained extensive in the 1980s, some 30 years after waste disposal activities had ceased. Hence, the common occurrence of choroethene contamination in groundwater systems was unequivocal confirmation that these compounds could persist in the subsurface for decades and implicit evidence that biological mechanisms for efficient chloroethene degradation were not intrinsic to groundwater systems.

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During the 1980s, accumulation of perchloroethene (PCE) and trichloroethene (TCE) transformation products in anaerobic groundwater systems was widely reported and attributed to microbial reductive dechlorination (Barrio-Lage *et al*., 1987, 1990; Bouwer, 1994; McCarty and Semprini, 1994; Odum *et al*., 1995; Vogel, 1994; Vogel and McCarty, 1985). By the end of the 1980s, the consensus was that microbial reductive dechlorination was essentially ubiquitous in anaerobic, chloroethene-contaminated aquifers (Chapelle, 1996; Bouwer, 1994; Gossett and Zinder, 1996; McCarty, 1996; McCarty and Semprini, 1994; Vogel, 1994). During this period, the number of organochlorine compounds identified as occurring in nature and their recognized environmental sources continued to increase. Gribble's 1992 review estimated that more than 800 natural organochlorine compounds had been identified (Gribble, 1992). Nonetheless organochlorine compounds as a group were still considered fundamentally anthropogenic (Gribble, 1994) and the existence of microbial mechanisms naturally selected for the metabolism of organochlorine compounds was deemed improbable. Consistent with this perception, the best scientific evidence of the time indicated that microbial biotransformation of chloroethene contaminants was a cometabolism, an accidental side-effect of the microbial metabolism of non-chlorinated organic compounds (McCarty and Semprini, 1994).

By the mid-1990s, a proposed ban on the production and use of chlorine prompted several investigations which ultimately challenged the long-held perception that organochlorine compounds as a group were unnatural substances. Today, more than 1500 naturally occurring organochlorine compounds from a variety of biotic and abiotic sources in surface water, ground water, soils and sediments have been identified (Abrahamsson *et al*., 1995a, 1995b; Asplund, 1995; Asplund and Grimvall, 1991; de Jong *et al*., 1995; Gribble, 1992, 1994; Grimvall, 1995; Grøn, 1995; Harper, 1995; Hoekstra *et al*., 1998; Johansson *et al*., 1995; Jonsson *et al*., 1995; Kawano *et al*., 1995; Keppler *et al*., 2002; Manninen and Lauren, 1995; Myneni, 2002), but production and turnover of organochlorines appears to be particularly important in humic-rich aquatic systems and in humic sediments and soils (Asplund, 1995; Asplund and Grimvall, 1991; Keppler *et al*., 2002; Myneni, 2002). Natural production of PCE and TCE in volcanoes (Gribble, 1992, 1994), oceans (Gribble, 1992, 1994) and marine algae (Abrahamsson *et al*., 1995b) and natural production of VC in terrestrial soils (Keppler *et al*., 2002) are now recognized. Thus, while the debate continues over the relative production of organochlorine compounds by natural versus anthropogenic sources and the ecological wisdom of continued anthropogenic production, it is apparent that organochlorine compounds are, in fact, common in nature (Asplund and Grimvall, 1991; Gribble, 1992, 1994; Grimvall, 1995).

The natural occurrence of organochlorine compounds has significant ecological implications for the metabolism of chloroethenes in the environment. Evidence suggests that natural organochlorine compounds are not a modern phenomenon but have occurred for thousands of years (Gribble, 1994; Keppler *et al*., 2002). This history suggests ample opportunity for the natural selection of effective organochlorine-degrading microbial processes. In addition, because many of the naturally-occurring organochlorines are biogenic compounds reputed to function as chemical defense agents (Gribble, 1992, 1994), co-evolution of counter defenses, including biotransformation and biodegradation mechanisms, is probable. Certainly, the energetic potential of chlorinated compounds indicates a role as metabolic substrates for microorganisms inhabiting anaerobic environments (Vogel *et al*., 1987). Such considerations are a compelling argument for the natural selection of microbial mechanisms for organochlorine degradation and transformation.

Consistent with these expectations, a number of reports have provided convincing evidence for the existence of microbial populations capable of degrading chloroethene compounds as primary substrates. The view, that microbial chloroethene degradation was fundamentally a metabolic accident, was largely abandoned by the mid 1990s. The key element in the shift from a predominantly co-metabolic view of chloroethene biodegradation to the concept of chloroethenes as primary substrates for microbial metabolism was the discovery of microorganisms which gain energy from reductive dechlorination (Holliger *et al*., 1993; Krumholz *et al*., 1996; Maymó-Gatell *et al*., 1997; Sharma and McCarty, 1996). Such microorganisms are able to grow using chloroethenes as respiratory terminal electron acceptors, a process termed chlororespiration, and are capable of much higher rates of reductive dechlorination than were previously attributed to cometabolic reductive dechlorination processes (Gerritse *et al*., 1996, 1999; He *et al*., 2002; Holliger *et al*., 1993, 1998; Krumholz, 1997; Krumholz *et al*., 1996; Magnuson *et al*., 1998; Maymo-Gatell *et al*., 1997; Mohn and Tiedje, 1992; Scholz-Muramatsu *et al*., 1995; Sharma and McCarty, 1996; Wild *et al*., 1996). In light of the remarkable impact that the discovery of chlororespiration has had on the perception of chloroethene biodegradation, it is ironic to note that this mechanism was not the

first process by which microorganisms were shown to utilize chloroethenes for growth and energy production. A decade earlier, Hartmans *et al*. (1985) reported oxidative microbial degradation of vinyl chloride (VC) as sole carbon substrate for energy and growth under aerobic conditions (Hartmans *et al*., 1985; Hartmans and deBont, 1992). Because the presence of dichloroethene (DCE) and VC in ground water is generally associated with anaerobic conditions, however, the potential importance of oxidative microbial degradation of chloroethenes under environmental conditions was not realized until microbial oxidation of VC under Fe(III)-reducing conditions was demonstrated in 1996 (Bradley and Chapelle, 1996) and anaerobic microbial oxidation of VC as a primary substrate was reported in 1998 (Bradley *et al*., 1998a). Identification of these reductive and oxidative processes demonstrated that effective mechanisms for microbial degradation of chloroethene contaminants do exist in groundwater systems.

The shift in the perspective on chloroethene biodegradation was, in a sense, inevitable. The demonstrated ability of environmental microorganisms to degrade oil and petroleum hydrocarbons in surface water systems (Atlas and Bartha, 1972; Bartha and Atlas, 1977; Atlas, 1981; Davis, 1967) and the presence of active microorganisms in deep subsurface oil fields (Bastin, 1926), certainly suggested a potential for microbial alteration of organochlorine contamination in ground water. However, the initial scientific approach to chloroethene remediation was conservative and dictated by a strong public mandate for immediate remediation. A conservative approach was reinforced by the apparent long-term persistence of these compounds in some environments and a lack of information on contaminant behavior and microbial activity in shallow groundwater systems. Thus, recognition of chloroethene biotransformation processes in shallow groundwater systems was delayed by the initial focus on highly-contaminated sites. The fact that such sites often exhibit a poor potential for chloroethene biotransformation certainly contributed significantly to the perception that chloroethenes were biorecalcitrant.

Chloroethenes in the Environment

Chloroethene compounds vary from the most chlorinated, PCE, to the monochlorinated vinyl chloride (VC). Due to their widespread use as dry cleaning solvents and as degreasing agents for military and industrial applications, PCE and TCE are the chloroethenes that are found in groundwater systems most frequently and in highest concentration. PCE can also be formed from dichloroelimination of hexachloroethane at mixed contaminant sites. Both PCE and TCE are considered toxic to humans and have an EPA maximum contaminant level (MCL) for drinking water of 5 μg/L. Dichlorinated ethenes (DCEs) occur in groundwater primarily as the result of in situ microbial transformations. *Cis*-DCE and *trans*-DCE are formed from the reduction of TCE. Of the two isomers, *cis*-DCE is the predominant product of TCE reduction under in situ ground-water conditions, while *trans*-DCE is less commonly observed in ground-water. The third DCE isomer, 1,1-DCE is primarily a transformation product of trichloroethane (TCA). *Cis*-DCE and 1,1-DCE currently have EPA drinking water MCLs of 70 μg/L and 7 μg/L, respectively. Of the chlorinated ethenes, VC is the only known carcinogen and is generally considered to be the greatest threat to human health. VC is an EPA priority pollutant and has a drinking water MCL of 2 μg/L. VC contamination of ground-water results primarily from microbial reduction of DCE and TCA under anaerobic conditions. High dissolved concentrations of VC have been reported in ground water, however, as the result of releases from PVC manufacturing operations (Hartmans, 1995).

Depending on the number of chlorine atoms in the molecule, chloroethene compounds display a considerable variability in reduction-oxidation (redox) character. Poly-chlorinated ethenes, such as PCE and TCE are highly oxidized compounds that readily undergo anaerobic reduction reactions in which chlorine substituents are sequentially replaced by hydrogen to yield DCE and vinyl chloride (Vogel, 1994; Vogel *et al*., 1987). However, the tendency of chloroethene compounds to undergo reductive dechlorination appears to decrease with the number of chlorine substituents (Vogel, 1994; Vogel *et al*., 1987). Thus, vinyl chloride is a relatively reduced compound with limited inclination to undergo further reduction. In contrast, the tendency of chlorinated ethenes to undergo oxidation increases with decreasing number of chlorine substituents (Vogel, 1994; Vogel *et al*., 1987) and vinyl chloride has a well documented susceptibility to microbial oxidation (Hartmans and deBont, 1992; Hartmans *et al*., 1985; Vogel, 1994; Vogel *et al*., 1987). For convenience, reductive and oxidative mechanisms for microbial degradation of chloroethene compounds (Figure 1) will be addressed separately in the following discussions.

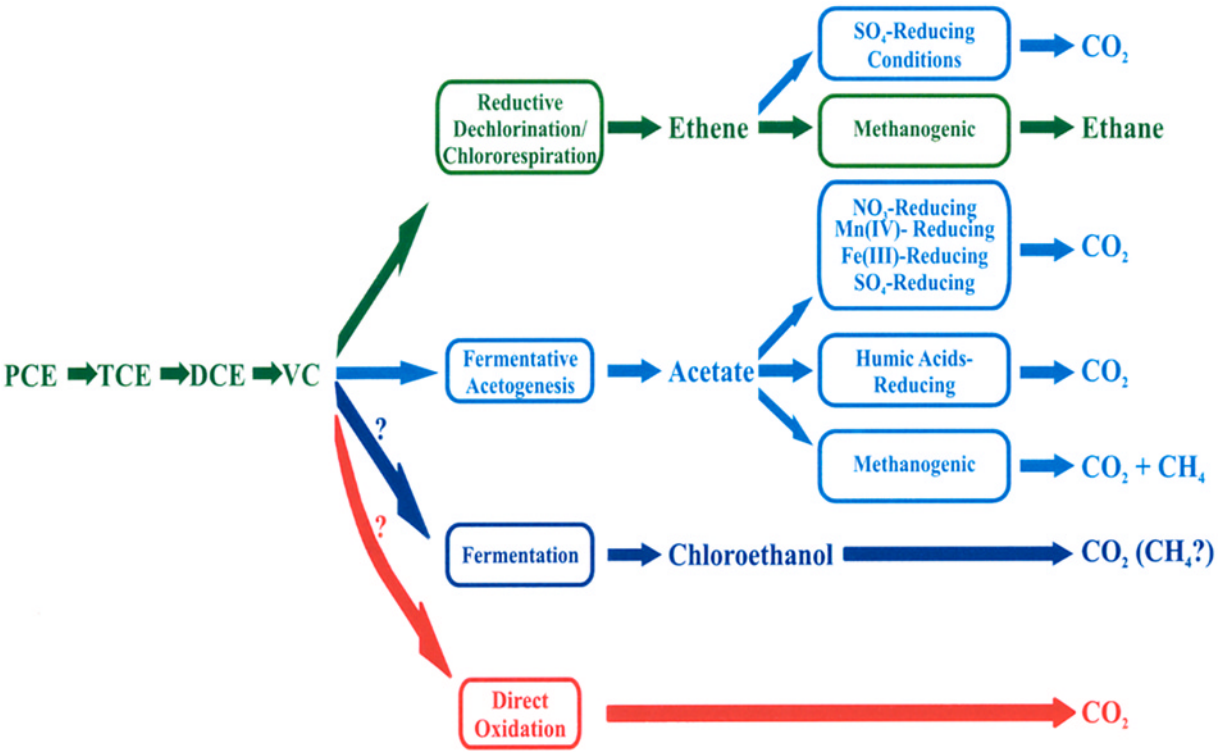

Figure 1. Pathways of anaerobic biodegradation. Potential reaction steps associated with the reductive dechlorination/chlororespiration mechanism of chloroethene biodegradation are shown in green. The remainder are potential reactions attributed to anaerobic microbial oxidations of VC and ethene.

Reductive Microbial Degradation

Reductive Dechlorination Mechanisms

Groundwater site assessments, conducted in the early 1980s in accordance with the mandates of the Resource Conservation and Recovery Act (RCRA) of 1976 and the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) of 1980, demonstrated that chloroethene contaminants were widespread in the environment and that dissolved phase concentrations could remain high decades after their release. These observations fostered the contemporary belief that chloroethene compounds were recalcitrant in the environment. This perceived recalcitrance was consistent with the acknowledged toxicity and presumed xenobiotic character of these compounds. Very quickly, however, follow-up characterization studies aimed at defining the extent and behavior of chloroethene plumes indicated that chloroethene contamination often led to the production and accumulation of reduced daughter products (DCE and VC, see Figure 1). This phenomenon was attributed to a microbiologically catalyzed process of reductive dechlorination (Barrio-Lage *et al*., 1987, 1990; Bouwer, 1994; McCarty and Semprini, 1994; Odum *et al*., 1995; Vogel, 1994; Vogel and McCarty, 1985).

By the early 1980s it had become clear that reductive dechlorination of chloroethene contaminants is common in anaerobic groundwater systems, but that the efficiency of chloroethene reductive dechlorination is highly variable under field conditions and apparently dependent on the number of chlorine substituents present on the chloroethene molecule and on the in situ redox conditions (Bouwer, 1994; McCarty and Semprini, 1994; Vogel, 1994; Vogel *et al*., 1987; Wiedemeier *et al*., 1998). PCE, with its four chlorine atoms, is a stronger oxidant than all of the naturally occurring electron accepting species found in groundwater systems, with the notable exception of O_2 (Vogel *et al*., 1987). Thus, PCE readily undergoes reductive dechlorination to TCE except in aerobic aquifers. Reductive dechlorination of TCE to *cis*-DCE occurs under Fe(III)-reducing conditions and in more strongly reducing environments (Chapelle, 1996). Reductive dechlorination of *cis*-DCE to yield VC appears to be

favored under SO_4-reducing and methanogenic conditions (Chapelle, 1996; Vogel *et al*., 1987). Reductive dechlorination of VC to the nonchlorinated product, ethene, is characteristically slow in situ and generally associated with highly reducing, methanogenic conditions (Ballapragada *et al*., 1995; Barrio-Lage *et al*., 1987, 1990; Bouwer, 1994; Carter and Jewell, 1993; De Bruin *et al*., 1992; DiStefano *et al*., 1991; Fennell *et al*., 1995; Freedman and Gossett, 1989; Maymo-Gatell *et al*., 1995; Odum *et al*., 1995; Vogel and McCarty, 1985; Wu *et al*., 1995). Consequently, reductive dechlorination of chloroethene contaminants is often incomplete in groundwater systems and frequently leads to the accumulation of *cis*-DCE and VC (Haston *et al*., 1994; Kitanidis *et al*., 1993; Major *et al*., 1991; McCarty and Reinhard, 1993; Weaver *et al*., 1995; Wilson *et al*., 1995).

Early efforts to clarify the mechanism of microbial reductive dechlorination concluded that this process was essentially accidental and of no benefit to the responsible organisms. Because the molar concentration of chloroethenes in ground-water systems is generally low in all but the most contaminated source areas, a reasonable assumption has generally been made that these compounds are not sufficiently concentrated to serve as primary substrates to support microbial growth and metabolism under in situ conditions. Consistent with that *a priori* assessment, the first pure cultures shown to be capable of reductive dechlorination were methanogens (Methanosarcina sp.) which did not grow on chloroethenes and apparently did not gain energy from the reaction (Fathepure *et al*., 1987, 1988a, 1988b; Jablonski and Ferry, 1992; El Fantroussi *et al*., 1998). Subsequent investigation demonstrated that reductive dechlorination of PCE to TCE and DCE could be mediated by SO_4-reducing bacteria (Fathepure *et al*., 1987; Freedman and Gossett, 1989; Cole *et al*., 1995; Townsend and Suflita, 1997) and homoacetogens (Terzenbach and Blaut, 1994). Each of these organisms was observed to mediate slow and partial reductive dechlorination of PCE and TCE to yield primarily DCE in a non-specific process attributed to the presence of cellular corrinoids or other metabolic cofactors (El Fantroussi *et al*., 1998; Stupperich, 1993). Based on these observations, reductive dechlorination was originally viewed as an anaerobic cometabolism brought about by the accidental interaction of chloroethenes with enzymes and reduced cofactors produced by the microorganisms for other metabolic purposes (McCarty and Semprini, 1994). Today, this type of cometabolic dechlorinating process is considered ubiquitous in anaerobic systems but generally an inefficient means of affecting complete reduction to non-toxic products like ethene.

In 1984, a SO_4-reducing bacteria, *Desulfonomonile tiedjei* DCB-1, was found capable of coupling the reductive dechlorination of 3-chlorobenzoate to growth (Shelton and Tiedje, 1984). *D. tiedjei* DCB-1 was later shown to reduce PCE to TCE and DCE (Fathepure *et al*., 1987; Deweerd and Suflita, 1990; Cole *et al*., 1995) via an energy conserving, respiratory reductive dechlorination process termed chlororespiration (Mohn and Tiedje, 1992). Subsequent investigations have isolated a number of chlororespiring microbial pure cultures capable of reducing PCE or TCE to DCE, including *Dehalobacter restrictus* (Holliger *et al*., 1993), *Desulfitobacterium sp*. strain PCE-1 (Gerritse *et al*., 1996, 1999; Löffler *et al*., 1999), *Dehalospirillum multivorans* (Scholz-Muramatsu *et al*., 1995), *Desulfuromonas chloroethenica* (Krumholz *et al*., 1996; Krumholz, 1997), *Desulfuromonas sp*. strain BB1 (Löffler *et al*., 2000), *Enterobacter agglomerans* strain MS-1 (Sharma and McCarty, 1996), and *Dehalococcoides ethenogenes* (Maymo-Gatell *et al*., 1997). Among these pure cultures, only *D. ethenogenes* has consistently demonstrated growth and energy production coupled to complete reductive dechlorination of PCE to ethene (Maymo-Gatell *et al*., 1997, 1999).

In addition, a growing number of mixed microbial populations have been described that are capable of efficient reductive dechlorination of the daughter products, DCE and VC (Rosner *et al*., 1997; Löffler *et al*., 1999; Flynn *et al*., 2000). Rosner *et al*. (1997) described DCE and VC dechlorinating activity that appeared to be independent of the corrinoid compounds involved in PCE and TCE reductive dechlorination (Magnuson *et al*., 1998). Löffler *et al*. (1999) reported three acetogenic mixed cultures capable of reductively dechlorinating VC to ethene and concluded, on the basis of energetic calculations and low H_2 consumption thresholds, that this was a respiratory process. In a separate study, two mixed subcultures capable of complete dechlorination of DCE and VC were found incapable of PCE dechlorination (Flynn *et al*., 2000), indicating that the ability of the original cultures to completely dechlorinate PCE to ethene was the result of at least two distinct dechlorinating populations. Consistent with these observations recent reports indicate the existence of distinct *Dehalococcoides* species that, in contrast to *D. ethenogenes*, are capable of efficient dechlorination of *c*-DCE and VC but not of PCE and TCE (Cupples *et al*., 2003; He *et al*., 2003a, 2003b). *D. ethenogenes* is also capable of reductively dechlorinating DCE and VC (Maymó-Gatell *et al*., 1997, 1999), although reductive dechlorination of VC to ethene by *D. ethenogenes* is two orders of magnitude slower than reduction of the other chloroethenes (Magnuson *et al*., 1998), is inhibited by high concentrations of PCE (Maymó-Gatell

et al., 2001), and does not appear to support growth (Maymó-Gatell *et al*., 1999).

A number of observations suggest that reductive dechlorination of chloroethene contaminants in groundwater systems is often attributable to the activities of cooperative consortia of microorganisms rather than to a single species. The relatively large number of pure cultures identified as being capable of chlororespiration of PCE to TCE or DCE (Mohn and Tiedje, 1992; Holliger *et al*., 1993; Gerritse *et al*., 1996, 1999; Löffler *et al*., 1999; Scholz-Muramatsu *et al*., 1995; Krumholz *et al*., 1996; Sharma and McCarty, 1996) and the growing evidence for independent DCE and VC dechlorinating cultures (Rosner *et al*., 1997; Löffler *et al*., 1999; Flynn *et al*., 2000; Cupples *et al*., 2003; He *et al*., 2003a, 2003b) is consistent with this hypothesis. Likewise, a number of mixed cultures capable of growth and complete dechlorination have been identified (Freedman and Gossett, 1989; DiStefano *et al*., 1991; DeBruin *et al*., 1992; Löffler *et al*., 1999; Rosner *et al*., 1997; Flynn *et al*., 2000), while only one pure culture, *D. ethenogenes*, has demonstrated growth and complete reductive dechlorination of chloroethenes (Maymó-Gatell *et al*., 1997). A study of three Michigan river enrichment cultures capable of complete reductive dechlorination of PCE to ethene revealed that two of the three cultures contained distinct microbial populations for PCE and VC dechlorination (Flynn *et al*., 2000). Indeed, the fact that reductive dechlorination of PCE by *D. ethenogenes* appears to involve the sequential production and extracellular release of daughter products, indicates that further reductive dechlorination of these daughter products under field conditions would entail competition with other dechlorinating populations. Thus, it is likely, even in groundwater systems populated by *D. ethenogenes*, that sequential reductive dechlorination results from the interaction of distinct microbial populations (Flynn *et al*., 2000). The fact that VC dechlorination evidently is not sufficient to support growth in *D. ethenogenes* (Maymó-Gatell *et al*., 1999), but apparently is a specialized, highly efficient, and energy conservative process in some mixed microbial cultures (Rosner *et al*., 1997; Löffler *et al*., 1999; Flynn *et al*., 2000; Cupples *et al*., 2003; He *et al*., 2003a, 2003b), further suggests the importance of established microbial consortia in facilitating complete reductive dechlorination of poly-chlorinated chloroethene contaminants. Thus, while the presence of *D. ethenogenes* in chloroethene contaminated ground water suggests the potential for complete reductive dechlorination of PCE or TCE to ethene, the absence of this organism does not appear to preclude complete reductive dechlorination (Löffler *et al*., 1999; Flynn *et al*., 2000; Cupples *et al*., 2003; He *et al*., 2003a, 2003b).

A number of factors have been identified as exerting a significant influence on the effectiveness of reductive dechlorination as an *in situ* mechanism for chloroethene bioremediation. Important factors appear to be the presence of a suitable chlororespiring microbial population, the redox character of the chloroethene contaminant, the presence of alternative terminal electron acceptors, the reduction kinetics of the individual chloroethene compounds, the electron donor supply, and the presence of specific inhibitory compounds.

Distribution of Indigenous Chlororespiring Microorganisms

The presence/absence in the environment of microorganisms capable of in situ chloroethene biotransformation has been a primary concern since chloroethene persistence was first documented in ground water. Historically the presence of reductive dechlorinating bacteria has been inferred from the in situ production and accumulation of reduced daughter products and from laboratory enrichments of environmental samples (Wiedemeier *et al*., 1998). Thus, based on the widely observed occurrence of reductive dechlorination in anaerobic groundwater systems, the microorganisms that catalyze chloroethene reductive dechlorination have been assumed to be widely distributed in the environment. Likewise, the relative infrequency of sites characterized by complete reductive dechlorination of PCE or TCE to ethene has been interpreted as evidence that *in situ* reductive dechlorination was cometabolic and that the microorganisms capable of complete reductive dechlorination are not widely distributed.

In contrast to this inferential approach, recent advances in molecular techniques have allowed direct assessment of the presence of specific chlororespiratory microorganisms in aquifer sediments and groundwater systems. Löffler *et al*. (2000) employed a nested PCR approach to detect the presence of two chlororespiring microbial groups, *Desulfuromonas* and *Dehalococcoides*, in two different chloroethene-contaminated groundwater systems characterized by active dechlorinating activity. In a similar study, *Dehalococcoides* specific 16S rDNA sequences were found in aquifer samples from an actively dechlorinating zone but not from a non-dechlorinating location (Fennell *et al*., 2001). Likewise, the most extensive study completed to date found *Dehalococcoides* specific 16S rDNA sequences at 21of 24 contaminated groundwater sites characterized by active chloroethene dechlorination (Hendrickson *et al*., 2002).

These studies establish the potential utility of PCR-based and other molecular techniques as tools for characterizing dechlorinating microbial populations

in situ and indicate that more comprehensive microbial characterization may be practicable in the immediate future. Although *D. ethenogenes* is the only complete dechlorinator currently in pure culture and, consequently, the most convenient target for molecular characterization techniques, a more comprehensive evaluation would require accurate discrimination between known dechlorinators or targeting of dehalogenase-specific gene sequences (Fennell *et al*., 2001), with a special emphasis on identifying the target sequences of dechlorinators capable of reducing DCE and VC to non-chlorinated products (Flynn *et al*., 2000; Cupples *et al*., 2003; He *et al*., 2003a, 2003b). Because its capacity for PCE chlororespiration is not unique, the most useful characteristic of *D. ethenogenes* as an indicator of chloroethene biodegradation in the field is the fact that *D. ethenogenes* is, currently, the only established molecular target indicative of the *in situ* presence of the capacity for DCE and VC dechlorination. However, DCE and VC dechlorination is cometabolic and inefficient in *D. ethenogenes*. Moreover, while recent reports (Cupples *et al*., 2003; He *et al*., 2003a, 2003b) have identified novel *Dehalococcoides* populations that can efficiently chlororespire *C-dce* and VC, the close similarity in *Dehalococcoides*-type 16S rRNA genes makes discrimination between these VC-respiring strains and strains that mediate the dechlorination of PCE/TCE or other non-chloroethene substrates impractical (He *et al*., 2003a, 2003b). At present, 16S rRNA techniques are insufficient to distinguish between *Dehalococcoides* populations with different dechlorination activities and, consequently, are a poor means of assessing the *in situ* potential for complete reductive dechlorination of chloroethenes to non-toxic products (He *et al*., 2003).

Nevertheless, a number of important conclusions can be made. First, the fact that chlororespiratory microorganisms appear to be common at chloroethene sites, that are characterized by active reductive dechlorination, indicates that the common occurrence of reductive dechlorination activity in anaerobic groundwater systems may be primarily attributable to chlororespiration rather than to cometabolic processes (Löffler *et al*., 1999). This conclusion implies that it may become practical to increase the standing biomass of indigenous reductive dechlorinating microorganisms and, consequently, the *in situ* dechlorinating activity by providing specific chlororespiration substrates. Second, *Dehalococcoides* spp. appear to be common components of the chlororespiratory communities found in chloroethene-contaminated groundwater systems (Hendrickson *et al*., 2002). Third, both dechlorination activity and *Dehalococcoides* spp. appear to be heterogeneously distributed in chloroethene-contaminated groundwater systems (Fennell *et al*., 2001). This conclusion is consistent with the oft-cited hypothesis that incomplete and/or inefficient reductive dechlorination in chloroethene-contaminated groundwater systems is attributable to the lack of sufficient biomass of chlororespiring microorganisms. Fourth, the fact that *D. ethenogenes* was not present in two of three environmental enrichments capable of complete dechlorination of PCE to ethene (Flynn *et al*., 2000), suggests that molecular characterizations based solely on the presence/absence of *D. ethenogenes* may underestimate the indigenous capacity for complete PCE chlororespiration.

The fact that positive PCR results for *Desulfuromonas* and *Dehalococcoides* specific 16S rDNA target sequences were found in sediments from three pristine rivers indicates that chlororespiring organisms also occur in uncontaminated environments in the absence of an apparent anthropogenic substrate for chlororespiration (Löffler *et al*., 2000). Thus, a particularly intriguing question concerns the substrate for these reportedly, obligate PCE degrading organisms in pristine environments (Löffler *et al*., 2000). The sequence divergence of various PCE reductive dehalogenase genes prompted Magnuson *et al*. (2000) to speculate that dehalogenases are ancient enzymes that may have evolved in response to the presence of high PCE or TCE concentrations in early geochemical environments or alternatively that unrecognized, natural substrates for these enzymes exist in the environment. The production and turnover of organochlorine compounds, including VC, in humic-rich sediments and soils (Asplund, 1995; Asplund and Grimvall, 1991; Keppler *et al*., 2002; Myneni, 2002) and the common occurence of organochlorine compounds in remote, apparently uncontaminated surface water bodies receiving humic-enriched runoff and groundwater discharge (Asplund, 1995; Asplund and Grimvall, 1991) are consistent with the hypothesized existence of natural organochlorine substrates for chlororespiration. Whatever the substrate, the existence of chlororespiratory microbial communities in pristine environments provides additional evidence that these organisms are widespread in the environment and raises the possibility that their apparent absence at some sites is more appropriately attributed to unfavorable environmental conditions rather than to environmental scarcity.

Despite the evidence suggesting that chlororespiratory microorganisms are widespread in both pristine and chloroethene-contaminated environments, at least one study indicates that bioaugmentation with known chlororespiratory microorganisms may be useful, in some cases, for enhancing *in situ* bioremediation (Ellis *et al*., 2000). The co-injection of lactate, nutrients and

a TCE dechlorinating enrichment culture into a recirculating, *in situ* groundwater test system resulted in complete dechlorination of TCE and *c*DCE to ethene within 500 days (Ellis *et al*., 2000).

Redox Character and Reduction Kinetics of Chloroethenes

In general, the tendency of chloroethene compounds to undergo reductive dechlorination appears to decrease with the number of chlorine substituents (Vogel, 1994; Vogel *et al*., 1987). From a thermodynamic perspective, however, the potential energy available from sequential chloroethene reduction steps decreases only slightly with decreasing number of chlorine constituents (Vogel, 1994; Vogel *et al*., 1987). The corresponding reduction potential estimates (under standard conditions) for the sequential reductive dechlorinations of PCE to ethene range from approximately +0.58 V for PCE to TCE to +0.36 V and +0.49 V for *c*DCE to VC and VC to ethene, respectively (Vogel *et al*., 1987; Bouwer, 1994). Likewise, the estimated standard Gibbs free energy changes for these sequential reductive dechlorinations of PCE to ethene (assuming H_2 as the ultimate electron donor) decrease only from approximately −164 kJ/mol H_2 for PCE to TCE down to −141 kJ/mol H_2 and −155 kJ/mol H_2 for *c*DCE to VC and VC to ethene, respectively (He *et al*., 2002; Dolfing and Janssen, 1994; Löffler *et al*., 1999; Mazur and Jones, 2001). Thus, assuming that a suitable electron donor is present in the environment in sufficient concentration to realize the above calculated energy potential changes, these thermodynamic considerations indicate that each of these reductive steps is potentially exergonic and a possible source of energy for metabolism and growth (Dolfing and Janssen, 1994; Löffler *et al*., 1999; Mazur and Jones, 2001).

PCE and TCE reductive dechlorination was characterized as rapid in *D. ethenogenes*, while reductive dechlorination of *c*DCE was much slower and reduction of VC was negligible (Maymó-Gatell *et al*., 1999). Consistent with this trend, reductive dechorination of PCE, TCE and *c*DCE supported growth in *D. ethenogenes*. The fact that the kinetics of these three reactions steps were characterized as zero-order with respect to chloroethene concentration indicated that the half-velocity coefficients for these reductive dechlorinations were well below the initial chloroethene concentration of 300 μM in the culture medium (Maymó-Gatell *et al*., 1999). In contrast, reductive dechlorination of VC was not growth supportive in *D. ethenogenes* (Maymó-Gatell *et al*., 1999). This observation combined with the apparent first-order kinetics of the VC reduction step, led the authors to conclude that reductive dechlorination of VC by *D. ethenogenes* is a slow, cometabolic process that requires the presence of a growth substrate, such as PCE, to achieve significant VC reduction (Maymó-Gatell *et al*., 1999, 2001).

The observed decrease in the rate of successive dechlorination steps may be characteristic of the enzymes and the associated corrinoid co-factors that are involved in chloroethene reductive dechlorination in *D. ethenogenes*. Complete reductive dechlorination of PCE to ethene in an enrichment culture containing *D. ethenogenes* appeared to involve two membrane-bound, reductive dehalogenases (RDases) that contained Co(I) corrinoid cofactors (Magnuson *et al*., 1998). *In vitro* studies, using titanium (III) citrate and methyl viologen as reducing agents, indicated that PCE RDase (61 kDa) dechlorinated PCE to TCE at a rate of 20 μmol/min/mg protein (Magnuson *et al*., 1998). The second enzyme, TCE RDase (51 kDa), dechlorinated TCE and *c*DCE at rates of 8–12 μmol/min/mg protein and dechlorinated VC at a rate approximately two orders of magnitude lower (Magnuson *et al*., 1998). The fact that a similar a response pattern is observed when chloroethene compounds are exposed to enzyme-free, super-reduced corrinoid compounds suggests that decreasing rates of reductive dechlorination with decreasing number of chlorine substituents may be characteristic of corrinoid-mediated chloroethene reduction under physiological conditions (Glod *et al*., 1997a, 1997b).

A similar pattern of change in the rate of reductive dechlorination in response to chloroethene concentration was reported by Haston and McCarty (1999), for an anaerobic mixed culture derived from a PCE contaminated aquifer system (Rosner *et al*., 1997; Haston and McCarty, 1999). For each step, chloroethene reductive dechlorination was characterized by Michaelis-Menton kinetics (Haston and McCarty, 1999). The maximum chloroethene dechlorination rates and 95% confidence intervals were reported as 77 ± 5, 59 ± 11, 14 ± 3 and 13 ± 3 μM/day for PCE, TCE, *c*DCE and VC, respectively (Haston and McCarty, 1999). While the pattern of decreasing rate of reductive dechlorination with decreasing chlorine number is similar to that observed in the pure culture of *D. ethenogenes* (Maymó-Gatell *et al*., 1999), it is worth noting that, in contrast to the *D. ethenogenes* study results, the apparent rates of VC dechlorination in the mixed culture study were comparable to those for *c*DCE and within an order of magnitude of those for PCE and TCE (Haston and McCarty, 1999). This observation suggests that more efficient reductive dechlorination of VC may be achievable in mixed culture conditions. Separate investigation of a VC dechlorinating enrichment derived from the same aquifer system confirmed that VC dechlorination in the

mixed culture study was not attributable to *D. ethenogenes* (Rosner *et al*., 1997).

Equally important from an environmental remediation standpoint, are the observed changes in the half-saturation (k_s) coefficients for chloroethene reductive dechlorination in response to changing chlorine number. As noted above, reductive dechlorination of PCE, TCE and *c*DCE by *D. ethenogenes* exhibited saturation kinetics at chloroethene concentrations well below 300 μM, but the kinetics of VC reductive dechlorination remained first order at an initial VC concentration of 300 μM (Maymó-Gatell *et al*., 1999). This observation indicates that the k_s for VC reductive dechlorination by *D. ethenogenes* is substantially higher than 300 μM, while the k_s estimates for the other dechlorinating steps are considerably lower (Maymó-Gatell *et al*., 1999). In the anaerobic mixed culture investigated by Haston and McCarty (1999), the apparent k_s values and 95% confidence intervals for chloroethene reductive dechlorination were 0.11 ± 0.04, 1.4 ± 0.9, 3.3 ± 2.2 and 2.6 ± 1.9 μM for PCE, TCE, *c*DCE and VC, respectively. Both studies indicate that the k_s for reductive dechlorination is at least an order of magnitude higher for VC than for PCE (Haston and McCarty, 1999; Maymó-Gatell *et al*., 1999) and suggest that the substrate affinity of microbial reductive dechlorination mechanisms is relatively poor for VC. Even acknowledging the difficulties in applying laboratory-derived kinetic data to field conditions, these results indicate that *in situ* rates of VC reductive dechlorination are likely to be well below maximum values for a significant portion of the groundwater plume, because *in situ* concentrations of VC often fall below 3.2 μM (200 ppb) and because the MCL for VC is 0.032 μM (2 ppb) (Haston and McCarty, 1999; Maymó-Gatell *et al*., 1999).

Thus, both thermodynamic and kinetic evidence suggest that *in situ* microbial reduction of PCE and TCE is favorable and should be relatively rapid under anaerobic conditions. Microbial reductive dechlorination of VC is dependent on the dissolved VC concentration and likely to be inefficient under relatively oxidized field conditions or at low VC concentrations.

Availability of Electron Donors for Chlororespiration

With few exceptions, H_2 is generally considered to be the ultimate electron donor for respiratory reductive dechlorination (Löffler *et al*., 2000). Of the following chlororespiring microorganisms; *Dehalobacter restrictus* (Holliger *et al*., 1993), *Desulfitobacterium sp*. strain PCE-1 (Gerritse *et al*., 1996, 1999; Löffler *et al*., 1999), *Dehalospirillum multivorans* (Scholz-Muramatsu *et al*., 1995), *Desulfuromonas chloroethenica* (Krumholz *et al*., 1996; Krumholz, 1997), *Desulfuromonas sp*. strain BB1 (Löffler *et al*., 2000), *Enterobacter agglomerans* strain MS-1 (Sharma and McCarty, 1996), and *Dehalococcoides ethenogenes* (Maymo-Gatell *et al*., 1997); only *Desulfuromonas chloroethenica* (Krumholz *et al*., 1996; Krumholz, 1997) and *Desulfuromonas sp*. strain BB1 (Löffler *et al*., 2000) are believed to require an electron donor more complex than H_2 under pure culture conditions. In contrast, *D. chloroethenica* and *Desulfuromonas sp*. strain BB1 reportedly use acetate but not H_2 to support reductive dechlorination (Krumholz *et al*., 1996; Krumholz, 1997; Löffler *et al*., 2000). Based on these observations, H_2 is generally considered the ultimate electron donor for chlororespiration of chloroethene compounds (DiStefano *et al*., 1992; Maymó-Gatell *et al*., 1995; Smatlak *et al*., 1996; Fennell *et al*., 1997; Ballapragada *et al*., 1997; Carr and Hughes, 1998; Yang and McCarty, 1998, 1999; He *et al*., 2002; Gossett and Zinder, 1996; Chapelle, 1996), but acetate and possibly formate may fill this role for a limited number of organisms (Krumholz *et al*., 1996; Krumholz, 1997; Löffler *et al*., 2000).

The apparent restriction of chlororespiratory microorganisms to the use of H_2 and acetate as ultimate electron donors suggests a potential limitation on the utility of chlororespiration as a mechanism for *in situ* chloroethene remediation. Under mixed culture conditions, however, a large number of relatively complex carbon substrates have been shown to support chlororespiration of chloroethene compounds to ethene. Thus, while H_2 has been identified as the ultimate electron donor for *D. ethenogenes*, an aquifer microcosm containing *Dehalococcoides* like microorganisms demonstrated reductive dechlorination of TCE to ethene with a range of electron donor substrates, including butyrate, lactate, lactate/benzoate, and proprionate (Fennell *et al*., 2001). Indeed, the original PCE-dechlorinating mixed cultures from which *D. ethenogenes* was subsequently isolated were shown to utilize diverse carbon substrates, among which were methanol, formate, acetate, lactate, butyrate and glucose (Freedman and Gossett, 1989; DiStefano *et al*., 1991; Maymó-Gatell *et al*., 1995, 1998; Smatlak *et al*., 1996). Likewise, lactate was the initial electron donor for reductive dechlorination in a fluidized bed reactor which transformed PCE to ethene (Ballapragada *et al*., 1997).

A variety of initial electron donors has also been reported in dechlorinating mixed cultures derived from environmental samples. Complete dechlorination of PCE to ethene in an anaerobic river sediment was observed with lactate as electron donor and carbon

substrate (DeBruin *et al*., 1992). A soil-derived mixed culture containing *Desulfitobacterium frappieri* TCE1 utilized H_2, formate, ethanol, lactate and butyrate as initial electron donors for reductive dechlorination of PCE to *c*DCE (Gerritse *et al*., 1999). A mixed culture derived from a PCE-contaminated aquifer utilized propionate and benzoate as initial electron donors for complete reductive dechlorination of PCE to ethene (Yang and McCarty, 1998). In each of these studies, syntrophic microorganisms appeared to ferment the more complex, initial electron donor substrate to H_2 or formate/acetate which were then consumed by chlororespiration as the terminal electron accepting process (Ballapragada *et al*., 1997; Yang and McCarty, 1998, 1999; He *et al*., 2002; Löffler *et al*., 1999; Gerritse *et al*., 1999; Smatlak *et al*., 1996; Maymó-Gatell *et al*., 1995; Fennell *et al*., 2001).

In addition to the ability of syntrophic communities to couple the oxidation of a wide range of carbon substrates to chlororespiration, some individual microbial species appear able to catalyze both the fermentation and the chlororespiration steps. In pure culture, *Dehalospirillum multivorans* has been shown to dechlorinate PCE to *c*DCE using glycerol, pyruvate, lactate, ethanol or formate as the initial electron donor (Schulz-Muramatsu *et al*., 1995) with H_2 apparently serving as the ultimate electron donor for the chlororespiration step (Holliger *et al*., 1998; He *et al*., 2002). In pure culture, *Enterobacter agglomerans* demonstrated dechlorination of PCE to *c*DCE using glucose, pyruvate, formate, lactate, acetate or an amino acid mixture as the initial electron donor (Sharma and McCarty, 1996). Because this microorganism is capable of fermenting a range of carbon substrates to acetate and H_2, the ultimate electron donor for reductive dechlorination of PCE in pure cultured *E. agglomerans* strain MS-1 is presently unclear but may be H_2 (Sharma and McCarty, 1996).

The results of these studies indicate that the efficiency of reductive dechlorination of chloroethene contaminants, under the mixed culture conditions found in groundwater systems, depends on the existence of a ready supply of fermentable carbon substrate(s) and the presence of a microorganism or microbial community capable of fermenting that substrate to H_2 or acetate/formate for subsequent use in chlororespiration. Because hydrogenogenic and acetogenic fermentative microorganisms are common in surface soils and shallow groundwater systems, a complete absence of these microorganisms is unlikely. However, the metabolic activity and standing biomass of the indigenous fermentative community will depend in large part on the magnitude and quality of the *in situ* reservoir of fermentation substrates. Because shallow groundwater systems are often oligotrophic, the *in situ* availability of electron donor has long been considered an important factor limiting the efficiency of microbial reductive dechlorination of chloroethenes to ethene in groundwater systems (Gibson and Sewell, 1992; Bouwer, 1994; McCarty, 1996; McCarty and Semprini, 1994; Gossett and Zinder, 1996; Wiedemeier *et al*., 1998; Vogel, 1994). For this reason, EPA guidelines for establishing natural attenuation as a viable long-term mechanism for *in situ* remediation of chloroethene contamination recommend quantifying the total and dissolved organic carbon (TOC and DOC, respectively) present in the aquifer as a rough indication of the ongoing presence and potential persistence of electron donor capable of supporting *in situ* chloroethene reductive dechlorination (Wiedemeier *et al*., 1998).

Electron donor competition between dechlorinators and other indigenous microorganisms is also expected to be a primary determinant of the efficiency of *in situ* reductive dechlorination in groundwater systems (Yang and McCarty, 1998; Löffler *et al*., 1999). The apparent importance of H_2 as ultimate electron donor for a majority of chlororespiratory microorganisms suggests that, under *in situ* conditions, respiratory dechlorinators potentially must compete with a number of other anaerobic hydrogenotrophs including NO_3-reducers, Mn(IV) reducers, Fe(III)-reducers, SO_4-reducers, autotrophic methanogens and homoacetogens (Yang and McCarty, 1998; Löffler *et al*., 1999). The reduction potential of a terminal electron accepting process defines that process's theoretical H_2 threshold, the minimum H_2 concentration at which the reaction remains exergonic (Cord-Ruwisch *et al*., 1988; Lovley, 1985; Lovley and Goodwin, 1988; Lovley *et al*., 1994). Thus, the theoretical H_2 threshold is inversely related to the Gibbs free energy change of the hydrogen consuming process (Cord-Ruwisch *et al*., 1988; Lovley, 1985; Lovley and Goodwin, 1988; Lovley *et al*., 1994) and is expected to vary as follows (from highest theoretical H_2 threshold to lowest): homoacetogens, autotrophic methanogens, SO_4-reducers, Fe(III)-reducers, Mn(IV) reducers and NO_3-reducers (Yang and McCarty, 1998; Löffler *et al*., 1999). Empirical determinations of H_2 thresholds in sediment systems are consistent with these expectations (Löffler *et al*., 1999; Chapelle *et al*., 1995) and range: $>$300 nM for homoacetogenesis (Breznak, 1994; Cord-Ruwisch *et al*., 1988), 5–95 nM for methanogenesis (Cord-Ruwisch *et al*., 1988; Lovley, 1985; Lovley and Goodwin, 1988; Lovley *et al*., 1994; Conrad, 1996), 1–15 nM for SO_4-reduction (Cord-Ruwisch *et al*., 1988; Lovley, 1985; Lovley and Goodwin, 1988; Lovley *et al*., 1994; Conrad, 1996), 0.1–0.8 nM for Fe(III)-reduction (Lovley, 1985; Lovley and Goodwin, 1988; Lovley *et al*., 1994; Conrad,

1996), and <0.05 nM for Mn(IV)- and NO_3-reduction (Lovley, 1985; Lovley and Goodwin, 1988; Lovley *et al*., 1994). Thermodynamic considerations indicate that the theoretical H_2 threshold for chlororespiration should be similar to that of NO_3-reduction (Yang and McCarty, 1998).

Laboratory estimates of the H_2 threshold associated with chloroethene reductive dechlorination are consistent with the expectation of efficient H_2 consumption by chlororespiratory microorganisms. Smatlak *et al*. (1996) estimated that the H_2 threshold for reductive dechlorination of PCE in a methanogenic mixed culture containing *D. ethenogenes* was less than 2 nM. The H_2 threshold for *c*DCE reductive dechlorination in dechlorinating mixed culture containing dechlorinators, methanogens and homoacetogens was also reported as 2 nM, while the estimated thresholds for methanogenesis and homoacetogenesis were 11 and 400 nM, respectively (Yang and McCarty, 1998). A H_2 threshold of 0.5 nM was attributed to PCE chlororespiration in an estuarine sediment microcosm (Mazur and Jones, 2001). Pure cultures of *Desulfitobacterium* sp. reduced H_2 concentrations to approximately 0.3 nM during PCE dechlorination (Löffler *et al*., 1999). In the same study, H_2 thresholds less than 0.25 nM were associated with reductive dechlorination of PCE, *c*DCE and even VC in anaerobic mixed cultures enriched from pristine river sediments (Löffler *et al*., 1999).

These results suggest that chloroethene chlororespiration remains energetically favorable at dissolved H_2 concentrations well below the H_2 thresholds for methanogens or homoacetogens and, under the electron donor limiting conditions found in many groundwater systems, should generally outcompete both of these metabolic groups (Smatlak *et al*., 1996; Yang and McCarty, 1998; Löffler *et al*., 1999). Likewise the fact, that the H_2 thresholds for chlororespiration of chloroethenes, including VC, appear to be at least as low as 1–2 nM, suggests that chlororespiration also can be significant in groundwater systems under SO_4-reducing conditions (Yang and McCarty, 1998; Löffler *et al*., 1999; Mazur and Jones, 2001) and, perhaps under Fe(III)-reducing conditions (Löffler *et al*., 1999). However, field reports demonstrating ineffective reductive dechlorination of DCE and VC under Fe(III)-reducing and NO_3-reducing conditions (Yager *et al*., 1997; Chapelle, 1996) indicate that, in addition to energetics, other environmental and microbiological factors effect the efficiency of chlororespiration in groundwater systems.

When the *in situ* H_2 concentration is above the H_2 threshold of a number of indigenous microorganisms, interspecies H_2 competition is a function of the species-specific affinity for H_2 uptake and utilization (Smatlak *et al*., 1996; Ballapragada *et al*., 1997). The half saturation coefficient for H_2 uptake, k_s (H_2), by a PCE-dechlorinating enrichment culture was estimated as approximately 100 nM, while the k_s (H_2) for methanogensis in the same culture was approximately 1000 nM (Smatlak *et al*., 1996). In a fluidized bed reactor system, the k_s (H_2) for reductive dechlorination was estimated as 9, 14, 21 and 17 nM for PCE, TCE, *c*DCE and VC, respectively (Ballapragada *et al*., 1997). These reports are consistent with the earlier conclusion based on H_2 thresholds, that, under electron donor limited conditions, dechlorinating microorganisms are expected to hold a significant competitive advantage over methanogens and homoacetogens. However, because the dissolved H_2 concentrations observed in groundwater systems under SO_4-reducing, Fe(III)-reducing, Mn(IV)-reducing and NO_3-reducing conditions are generally less than 2 nM, the rate of H_2 uptake by respiratory reductive dechlorinating microorganisms is expected to be well below optimum under these conditions (Ballapragada *et al*., 1997).

Thus, both thermodynamic and kinetic evidence indicate that efficient uptake and utilization of H_2 by chlororespirers is favored under highly reduced, methanogenic or homoacetogenic conditions and that the competitive character of chlororespiration decreases as the alternative terminal electron accepting processes become more oxidizing (Vogel *et al*., 1987; Ballapragada *et al*., 1997; Löffler *et al*., 1999; Smatlak *et al*., 1996; Yang and McCarty, 1998; Mazur and Jones, 2001). Likewise, both lines of evidence suggest that, under conditions in which the primary alternative terminal electron accepting process is SO_4-reduction, chlororespiratory microorganisms in general should remain competitive (Vogel *et al*., 1987; Ballapragada *et al*., 1997; Löffler *et al*., 1999; Smatlak *et al*., 1996; Yang and McCarty, 1998; Mazur and Jones, 2001) and some chlororespiratory species may be able to sequester the majority of the available electron donor (Vogel *et al*., 1987; Löffler *et al*., 1999; Mazur and Jones, 2001). The ability of chlororespiratory microorganisms to compete for H_2 under Fe(III)-reducing, Mn(IV)-reducing or NO_3-reducing conditions is less clear, but some evidence suggests that reductive dechlorination of the more energetic chloroethene compounds, PCE and TCE, may occur even under conditions in which the primary alternative terminal electron accepting process is Fe(III)-reduction (Chapelle, 1996; Löffler *et al*., 1999; Lorah and Olsen, 1999).

Compounds Inhibitory to Chlororespiration

A number of compounds; including sodium cyanide, sodium azide, sulfite, dithionite, cuprous chloride, zinc

chloride and bromoethane sulfonic acid (BES); capable of inhibiting microbial reductive dechlorination of chloroethene compounds have been reported (Magnuson *et al*., 1998; Miller *et al*., 1997; Gerritse *et al*., 1999; Löffler *et al*., 1997). These compounds are primarily of interest as biochemical tools for clarifying the enzymatic mechanisms involved in microbial reductive dechlorination and, generally, are not expected to have a significant impact on chloroethene reductive dechlorination under *in situ* conditions. However, as discussed above, a number of alternative terminal electron accepting compounds; including NO_3, Mn(IV), Fe(III), SO_4, etc.; have the potential to inhibit microbial reductive dechlorination as the result of electron donor competition (Vogel *et al*., 1987; Townsend and Suflita, 1997; Chapelle, 1996; Ballapragada *et al*., 1997; Löffler *et al*., 1999; Smatlak *et al*., 1996; Yang and McCarty, 1998; Mazur and Jones, 2001) or a facultative metabolic shift toward more energetic electron accepting processes (Sharma and McCarty, 1996).

In addition to these potentially inhibitory factors, microbial reductive dechlorination of chloroethene compounds appears to be inhibited by high concentrations of chlorinated co-contaminant compounds (Townsend and Suflita, 1996; Neumann *et al*., 1996; Maymó-Gatell *et al*., 2001). PCE chlororespiration in a *D. ethenogenes* pure culture was found to be inhibited by chloroform (Maymó-Gatell *et al*., 2001). A similar inhibition of PCE reductive dechlorination by chloroform and chloromethane was reported in *Dehalospirillum multivorans* (Neumann *et al*., 1996). Reductive dechlorination of PCE by *Desulfomonile tiedjei* was shown to be inhibited by the presence of 3-chlorobenzoate (Townsend and Suflita, 1996). The mechanism of inhibition has not yet been clarified, but the fact that these compounds are also relatively oxidized chlorinated solvents suggests competitive inhibition by an alternative electron acceptor. This type of inhibition may be common in groundwater systems that are contaminated with a mixture of chlorinated solvents (Maymó-Gatell *et al*., 2001).

Of particular concern for *in situ* chloroethene bioremediation, however, are reports that reductive dechlorination of VC by *D. ethenogenes* is inhibited by the presence of PCE, TCE or *c*DCE (Tandoi *et al*., 1994; Maymó-Gatell *et al*., 2001). It is not clear whether this inhibition is a direct competition for available enzyme binding sites or electron donor competition by an alternative, more energetically favorable terminal electron acceptor (Maymó-Gatell *et al*., 2001). Nor is it clear whether a similar effect of PCE and TCE concentrations is exerted on reductive dechlorination of *c*DCE. Although complete inhibition of reductive dechlorination of VC by *D. ethenogenes* apparently requires PCE concentrations in excess of 600 μM (100 ppm), the presence of DNAPL PCE and/or TCE in many chloroethene contaminated aquifers and the high aqueous solubilities of PCE, TCE and *c*DCE (each in excess of 100 ppm, McCarty and Semprini, 1994) suggest that complete inhibition of *D. ethenogenes*-mediated reductive dechlorination of VC may occur near the source area and that partial inhibition of this activity may extend well down-gradient (Maymó-Gatell *et al*., 2001). If the pattern observed with *D. ethenogenes* is a general indicator of the interaction of chloroethene contaminants under field conditions, reductive dechlorination of PCE and TCE under anaerobic conditions is expected to result in production and accumulation of DCE and VC in and near the source area as well as concomitant inhibition of the reductive dechlorination of VC and potentially DCE. Thus VC would be expected to move conservatively with ground water until the concentrations of the alternative chloroethenes are significantly diminished along with their associated inhibitory effects. This interaction combined with the reportedly poor kinetics of VC reduction (Haston and McCarty, 1999; Maymó-Gatell *et al*., 1999) under dilute environmental conditions may contribute to the widely reported persistence of VC in chloroethene contaminated groundwater systems. The effect of high PCE and TCE concentrations on the efficiency of VC dechlorination by VC-respiring *Dehalococcoides* strains has not yet been addressed.

Practical Conclusions on Chloroethene Reductive Dechlorination

In summary the following practical conclusions can be made about reductive dechlorination as a mechanism for *in situ* remediation of chloroethene contaminants in groundwater systems. Reductive dechlorination is common in anaerobic groundwater systems and appears to be the primary mechanism for *in situ* biotransformaton of the parent compounds, PCE and TCE. In practice, the efficiency of chloroethene reductive dechlorination appears to decrease with decreasing chlorine number. Reductive dechlorination of chloroethene contaminants to the non-chlorinated product, ethene, can be significant in some groundwater systems, but is often limited *in situ* due to low electron donor supply, high electron donor competition, the presence of alternative terminal electron acceptors for facultative chlororespiring microorgansims, the potential absence or low activity of *c*DCE and VC dechlorinating microorganisms, and the presence of inhibitory substances including more oxidized chloroethene compounds. Thus, production,

accumulation and persistence of the principal daughter products, *c*DCE and VC, are commonly observed in chloroethene contaminated groundwater systems under anaerobic conditions and reductive dechlorination of VC to ethene generally is viewed as inefficient except under highly reducing methanogenic conditions. Although *in situ* production of ethene and its reduction product, ethane, within chloroethene plumes represents compelling evidence of complete chloroethene biodegradation, accumulation of these compounds is often insufficient to explain the decreases in chloroethene concentrations observed in anaerobic groundwater systems (Chapelle, 1996; Bradley *et al*., 1998b; Wiedemeier *et al*., 1996; Ellis *et al*., 1996).

Oxidative Microbial Degradation of Chloroethenes

Aerobic Cometabolic Oxidation

In 1985, Wilson and Wilson reported that methanotrophic bacteria were capable of oxidizing TCE to CO_2 under aerobic conditions (Wilson and Wilson, 1985). Subsequent investigations have identified a wide variety of aerobic microorganisms, that are able to oxidize chloroethene compounds to CO_2 without accumulation of toxic intermediates. These include methane oxidizers (Baek and Jaffé, 1989; Moore *et al*., 1989; Tsien *et al*., 1989; Gerritse *et al*., 1995; Semprini, 1995), methanol oxidizers (Fitch *et al*., 1996), ethene oxidizers (Verce *et al*., 2001), propane oxidizers (Malachlowsky *et al*., 1994), propene oxidizers (Reij *et al*., 1995), aromatic compound oxidizers (Fan and Scow, 1993; Malachowsky *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), ammonium oxidizers (Vannelli *et al*., 1990), isoprene oxidizers (McCarty and Semprini, 1994), and vinyl chloride oxidizers (Verce *et al*., 2002). These processes do not appear to yield energy for microbial growth or metabolism and have been characterized as aerobic cometabolisms (McCarty and Semprini, 1994). The responsible microorganisms contain non-specific oxygenases which fortuitously oxidize chloroethenes to CO_2. Aerobic cometabolism of chloroethenes requires the presence of oxygen and a primary carbon substrate to initiate and maintain the production of a suitable oxygenase (McCarty and Semprini, 1994).

Cometabolic oxidation often is not considered a significant, long-term mechanism for non-engineered bioremediation of chloroethenes in ground water, but has been successfully exploited for engineered remediation of chloroethene-contaminants in ground water (McCarty and Semprini, 1994; Semprini, 1995; Fan and Scow, 1993; Fuller *et al*., 1995; Hopkins and McCarty, 1995; Mars *et al*., 1996). For example, TCE contamination in aerobic aquifers can be biodegraded by methanotrophic microorganisms when methane is supplied to the subsurface in sufficient quantity to stimulate and support methanotrophic activity (McCarty and Semprini, 1994; Semprini, 1995). Because methane and oxygen do not typically co-occur in ground-water systems, however, methanotrophic oxidation of chloroethenes is unlikely under non-engineered circumstances. On the other hand, mixed-waste plumes containing chloroethenes and aromatic compounds are not uncommon and oxidizers of aromatic compounds can effectively cometabolize chloroethenes under aerobic conditions (Fan and Scow, 1993; Fuller *et al*., 1995; Hopkins and McCarty, 1995; Mars *et al*., 1996). Unfortunately, contaminant plumes which contain co-substrates in sufficient concentration to support cometabolic oxidation of chloroethenes are typically anaerobic as the result of respiratory oxygen consumption. Hence, cometabolic oxidation of chloroethenes under non-engineered conditions appears to be primarily a transient phenomenon, poorly suited to long-term contaminant remediation.

Notable exceptions are the potential for aerobic cometabolism of chloroethene contaminants at the fringes and discharge points of chloroethene plumes. The potential for aerobic cometabolic oxidation of chloroethene contaminants exists at the fringe of anaerobic chloroethene plumes if the surrounding aquifer system is oxic or if oxygenated recharge is significant (Dolan and McCarty, 1995). Likewise, the groundwater and surface-water interface is a non-engineered system favoring cometabolic contaminant oxidation. Groundwater contamination is primarily a shallow subsurface phenomenon and plumes of contaminated ground water frequently discharge to nearby surface water bodies. The sediments of surface water systems typically are characterized by geochemical heterogeneity and a variety of cometabolic capabilities including methanotrophy. For example, oxidation of DCE and VC has been reported in stream-bed sediments characterized by mixed aerobic/methanogenic conditions (Bradley and Chapelle, 1997).

Aerobic Oxidation of Chloroethenes as Primary Substrates

The problematic requirement for both oxic conditions and high co-substrate concentrations can be avoided, if the chloroethene contaminant can serve as a primary substrate during biodegradation. The

tendency of chloroethene compounds to undergo oxidation increases with decreasing number of chlorine substituents (Vogel *et al*., 1987). Presumably due to their highly oxidized character, neither PCE nor TCE have been shown to serve as primary substrates for aerobic microbial degradation although aerobic cometabolism of these compounds has been demonstrated (McCarty and Semprini, 1994; Ryoo *et al*., 2000; Shim *et al*., 2001). In contrast, aerobic microbial degradation of DCE and VC as primary substrates has been shown (Hartmans *et al*., 1985, 1992; Hartmans and deBont, 1992; Verce *et al*., 2000; Bradley and Chapelle, 2000a; Coleman *et al*., 2002a, 2002b).

Aerobic VC Oxidation

As the least chlorinated of the chloroethenes, VC has the greatest tendency to undergo oxidation and was the first chloroethene shown to serve as primary substrate for growth and metabolism under aerobic conditions (Hartmans *et al*., 1985, 1992; Hartmans and deBont, 1992; Verce *et al*. 2000). Rapid microbial degradation of vinyl chloride, including mineralization, has been observed in aquifer microcosms under aerobic conditions (Bradley and Chapelle, 1996, 1998a, 1998b; Bradley *et al*., 1998b; Hartmans *et al*., 1985; Davis and Carpenter, 1990; Phelps *et al*., 1991). Hartmans *et al*. (Hartmans *et al*., 1985, 1992; Hartmans and deBont, 1992) isolated several strains of *Mycobacterium aurum* from soil that are capable of growth on VC as a sole source of carbon and energy. *M. aurum* strain L1 is characterized by a relatively high k_s for VC of 3.2 μM and a marked inability to retain its VC degrading capability following a brief absence of VC (Hartmans *et al*., 1985, 1992; Hartmans and deBont, 1992). More recently, *Psuedomonas aeruginosa* strain MF1 has been isolated from an activated sludge enrichment culture and shown to use VC as primary growth substrate (Verce *et al*., 2002). The ability of *P. aeruginosa* MF1 to metabolize VC is stable for extended periods in the absence of VC exposure and is characterized by a low k_s for VC of 0.26 μM (Verce *et al*., 2002). Coleman *et al*. (2002a) reported aerobic VC biodegradation in more than 60% of environmental microcosms and enrichments collected from 22 locations in the U.S. and Europe. From these samples, 11 *Mycobacterium* strains and 1 *Nocardioides* strain capable of aerobic growth on VC as sole carbon substrate were isolated (Coleman *et al*., 2002a). These observations suggest that the potential for non-cometabolic, aerobic biodegradation of VC is widespread in chloroethene-contaminated environments (Coleman *et al*., 2002a).

Aerobic DCE Oxidation

Although co-metabolic oxidation of DCE under aerobic conditions has been demonstrated for a variety of co-substrates including methane, phenol, toluene, and propane (McCarty and Semprini, 1994), several recent studies indicate that microbial oxidation of DCE can also occur aerobically in the absence of an apparent alternative substrate (Bradley and Chapelle, 1998b; Bradley *et al*., 1998b, 1998c; Klier *et al*., 1999). Significant aerobic oxidation of *cis*-1,2-DCE was demonstrated for an organic-rich, stream-bed-sediment (Bradley and Chapelle, 1998b; Bradley *et al*., 1998b, 1998c), organic rich surface soils (Klier *et al*., 1999), and organic-poor aquifer sediments (Bradley and Chapelle, 1998b; Bradley *et al*., 1998b, 1998c). Microorganisms isolated from the sediments of a blackwater stream utilized 1,2-dichloroethene (1,2-DCE) as a sole carbon substrate for aerobic metabolism (Bradley and Chapelle, 2000a). In that study, maximum DCE concentrations were 50 μM, the responsible microorganisms were not identified, and no evidence of growth was observed (Bradley and Chapelle, 2000a). Coleman *et al*. (2002b) recently described an environmental isolate most closely related to *Polaromonas vacuolata*, that is capable of aerobic growth on *c*-DCE as sole source of carbon at maximum dissolved *c*-DCE concentrations of 600–900 μM. These reports suggest that DCE can be degraded with DCE acting as a primary substrate in microbial metabolism, and that this process may contribute to the natural attenuation of DCE even under circumstances where aerobic cometabolism is not favored.

Environmental Importance of Aerobic Oxidation

Unfortunately, aerobic microbial oxidation of DCE and VC is often limited by the absence of oxygen in many chloroethene plumes. In the unusual event that VC is directly released to aerobic aquifers (reported at PVC manufacturing sites, Hartmans, 1995), aerobic mineralization of VC by aquifer microorganisms is assumed to be significant. For the majority of sites, however, the presence of DCE and VC in ground water is associated with reductive dechlorination of PCE and TCE under anaerobic conditions. Thus, under non-engineered conditions, aerobic biodegradation of chloroethenes in ground water is presumably limited to the fringe of the contaminant plume where dissolved oxygen has not been depleted by microbial respiration (Bradley *et al*., 1998b). As was the case for cometabolic oxidation, it is worth noting that microbial oxidation of DCE and VC under aerobic conditions may be important at the interface of ground-water and

surface-water systems (Bradley and Chapelle, 1998a, 1998b).

Anaerobic Oxidation of Chloroethenes in Groundwater Systems

The potential for anaerobic oxidation of chloroethene contaminants was first reported by Vogel and McCarty (1985) for a continuous flow, fixed-film, mixed methanogenic bioreactor amended with [1,2-^{14}C] PCE and acetate (Vogel and McCarty, 1985). Under these conditions, [1,2-^{14}C] PCE was transformed primarily to [1,2-^{14}C] TCE with lesser quantities of [1,2-^{14}C] *c*-DCE and [1,2-^{14}C] VC. Approximately 24% of the [1,2-^{14}C] PCE radiolabel was recovered as $^{14}CO_2$ after four days under steady state flow conditions. The fact that addition of unlabeled VC lowered the recovery of $^{14}CO_2$ by 50% led the authors to hypothesize that [1,2-^{14}C] PCE degradation involved sequential reduction to [1,2-^{14}C] TCE, [1,2-^{14}C] *c*-DCE and [1,2-^{14}C] VC followed by oxidation of [1,2-^{14}C] VC to $^{14}CO_2$ (Vogel and McCarty, 1985). Similar results were recently reported for a lactate-fed, mixed dechlorinating/methanogenic bioreactor (Adamson and Parkin, 2001). Approximately 8% of the initial [1,2-^{14}C] PCE radiolabel was recovered as $^{14}CO_2$, while the remainder was transformed to ^{14}C-TCE, ^{14}C-*c*-DCE, ^{14}C-VC and ^{14}C-ethene (Adamson and Parkin, 2001).

The potential for microbial oxidation of VC to non-toxic products under alternative anaerobic conditions was examined in a 1996 investigation of microbial degradation of VC in Fe(III)-reducing aquifer sediments (Bradley and Chapelle, 1996). Addition of Fe(III) to anaerobic aquifer microcosms resulted in VC mineralization rates comparable to those observed under aerobic conditions. Low but significant VC mineralization was also observed in anaerobic microcosms under ambient Fe(III) conditions (Bradley and Chapelle, 1996; Bradley *et al*., 1998b). These results indicated that vinyl chloride could be mineralized under anaerobic, Fe(III)-reducing conditions and suggested a potential anaerobic alternative to the apparently slow and inefficient reduction of VC to ethene (Bradley and Chapelle, 1996). Subsequent investigation indicated that DCE also is susceptible to microbial oxidation under anaerobic conditions (Bradley *et al*., 1998c).

The ability of aquifer microorganisms to oxidize DCE and VC to non-toxic products under anaerobic conditions has important implications for natural attenuation of chloroethene contaminants in groundwater systems. In anaerobic aquifers, highly oxidized chloroethenes, like PCE and TCE, can be readily transformed to DCE and VC which are susceptible to microbial oxidation under a number of anaerobic redox conditions. Thus, the combination of reductive dechlorination of PCE and TCE under anaerobic conditions followed by anaerobic microbial oxidation of DCE and VC provides a possible microbial pathway for complete degradation of chloroethene contaminants in ground-water systems. Because anaerobic microbial oxidation of DCE or VC yields CO_2 rather than a unique product directly attributable to chloroethene degradation (for example, ethene in the case of complete chloroethene reductive dechlorination), the combination of reductive dechlorination of PCE and TCE followed by anaerobic oxidation of DCE and VC represents an alternative explanation for the common phenomenon of significant DCE or VC production but insignificant accumulation of ethene and ethane in anaerobic, chloroethene contaminated groundwater systems.

Effect of Redox Conditions on Anaerobic Chloroethene Oxidation

The potential effect of redox conditions on DCE and VC oxidation was pursued in microcosm studies using aquifer and stream sediments from a TCE contaminated site (Bradley and Chapelle, 1997, 1998a). Mineralization of DCE and VC to CO_2 decreased under increasingly reducing conditions, but significant mineralization was observed for both sediments under anaerobic conditions, including methanogenic conditions (Bradley and Chapelle, 1997, 1998b). The rate and extent of VC mineralization decreased in the order of aerobic > Fe(III)-reducing > SO_4-reducing > methanogenic conditions. The rate of microbial VC oxidation was greater than microbial oxidation of DCE for each electron-accepting condition. For both sediments, the rate of microbial DCE mineralization under aerobic conditions was at least twice that observed under anaerobic conditions. It is interesting to note that the rate and magnitude of DCE oxidation did not differ significantly between Fe(III)-reducing, SO_4-reducing and methanogenic conditions. Under these conditions, net oxidation of DCE appeared to involve an initial, rate-limiting reduction to VC followed by oxidation of VC to CO_2 (Bradley and Chapelle, 1998b). Based on this and other observations, it was hypothesized that direct oxidation of DCE requires a more powerful oxidant than Fe(III)-oxides. A subsequent investigation demonstrated that aquifer microorganisms can anaerobically oxidize DCE to CO_2 under Mn(IV)-reducing conditions without an apparent initial reduction to VC (Bradley *et al*., 1998c).

The observation that VC could be mineralized to CO_2 by microorganisms under methanogenic

conditions (Bradley and Chapelle, 1997, 1998b) was particularly intriguing considering that significant VC mineralization under methanogenic conditions had been reported only once previously (Vogel and McCarty, 1985) and that a number of laboratory studies concluded that methanogens are not directly involved in VC degradation (Ballapragada *et al*., 1995; DiStefano *et al*., 1991; Maymó-Gatell *et al*., 1995, 1997; Smatlak *et al*., 1996). Prompted by the growing recognition that naturally occurring humic acids compounds can serve as terminal electron acceptors for microbial metabolism, an investigation was initiated to assess the potential contribution of this process to the observed oxidation of VC under methanogenic conditions (Bradley *et al*., 1998b). The fact that addition of 2-bromoethane sulfonic acid (BES) completely inhibited methanogenesis but had no effect on VC oxidation indicated that VC oxidation was not coupled to methanogenesis. Subsequently, 2,6-anthraquinone disulfonate (a model humic acids compound) was used to demonstrate that VC oxidation was coupled to microbial humic acids reduction. Microbial oxidation of DCE under humic acids reducing conditions was also demonstrated. These results indicated that, in the presence of humic acids, efficient mineralization of VC and DCE without accumulation of reduced intermediates can occur even under methanogenic conditions. Because humic acids are abundant in both environments, these results indicate that chloroethene oxidation under humic acids reducing conditions may be important in aquatic sediments and shallow ground-water systems.

Microbial Mechanisms for Chloroethene Oxidation

Identification and clarification of the mechanisms underlying anaerobic microbial oxidation of chloroethene compounds have been hindered by the current lack of pure cultures or even defined mixed cultures capable of net oxidation of DCE or VC to CO_2. Indeed, the evidence gathered thus far suggests that anaerobic chloroethene oxidation generally involves a syntrophic relationship between chloroethene-transforming microorganisms and various respirative microorganisms capable of mineralizing the transformation products to CO_2 or CO_2 and CH_4 (Bradley and Chapelle, 2000b). In this sense, anaerobic chloroethene oxidation resembles the complete reductive dechlorination of PCE and TCE to ethene in anaerobic mixed cultures which lack *D. ethenogenes* (Rosner *et al*., 1997; Löffler *et al*., 1999; Flynn *et al*., 2000). The overall process is recognized but the responsible microorganisms remain unknown and the underlying mechanisms are poorly characterized. Despite these difficulties, four general mechanisms for the net anaerobic oxidation of chloroethene compounds by microorganisms have been proposed: (1) oxidation by a single microorganism to yield CO_2 or CO_2 and CH_4 (Bradley and Chapelle, 1996), (2) net oxidation with chloroethanol as intermediate (Vogel and McCarty, 1985), (3) syntrophic oxidation with acetate as intermediate (Bradley and Chapelle, 1999a, 1999b, 2000b) and (4) syntrophic oxidation with ethene as intermediate (Bradley and Chapelle, 2002).

The potential for direct oxidation of VC by a single microorganism to yield CO_2 as the final product was suggested by Bradley and Chapelle (1996) as a possible explanation for the apparent lack of production and accumulation of intermediate products in aquifer microcosms exhibiting anaerobic oxidation of [1,2-^{14}C] VC to $^{14}CO_2$. The validity of this hypothesis is unclear, however, because subsequent investigations reported a similar lack of accumulation of intermediates was characteristic of readily metabolized intermediate products (Bradley and Chapelle, 1999a, 1999b, 2000b). Thus, in the absence of a pure culture capable of anaerobic oxidation of VC to CO_2, this mechanism remains hypothetical.

Based on an observed production of $^{14}CO_2$ from [1,2-^{14}C] VC, Vogel and McCarty (1985) hypothesized that microbial oxidation of VC may involve a hydration reaction to yield chloroethanol which would then be oxidized to aldehyde and ultimately mineralized to CO_2 and perhaps CH_4 (Vogel and McCarty, 1985). While consistent with the apparent lack of stable intermediate products and the ultimate oxidation of VC to CO_2, none of the postulated intermediates were detected in the study and no alternative evidence for this mechanism was provided (Vogel and McCarty, 1985).

Direct evidence was reported, however, for net anaerobic oxidation of VC to CO_2 via a syntrophic relationship between fermentative acetogens and respiratory microorganisms capable of oxidizing acetate (Bradley and Chapelle, 1999a, 1999b, 2000b). Microcosm studies conducted with stream sediments demonstrated rapid degradation of [1,2-^{14}C] VC and simultaneous production of $^{14}CO_2$ and $^{14}CH_4$ (Bradley and Chapelle, 1999a, 1999b). It was concluded that degradation of VC to CO_2 and CH_4 involved acetotrophic methanogenesis (Bradley and Chapelle, 1999b). Subsequent investigation using radiometric detection liquid chromatography verified the transient accumulation and rapid oxidation of ^{14}C-acetate from [1,2-^{14}C] VC (Bradley and Chapelle, 2000b). This study indicated that the crucial step in the degradation of [1,2-^{14}C] VC to $^{14}CO_2$ and $^{14}CH_4$ was catalyzed by fermentative acetogens (Bradley and Chapelle, 2000b). These results demonstrated that microbial degradation of VC to CH_4 and CO_2 could proceed via oxidative

acetogenesis followed by acetotrophic methanogenesis and suggested that oxidative acetogenesis may also be the initial step in the net oxidation of VC to CO_2 reported previously under Fe(III)-reducing, SO_4-reducing and humic acids-reducing conditions (Bradley and Chapelle, 2000b). Because the underlying mechanism for acetogenic fermentation of VC has not yet been clarified, the mechanism proposed by Vogel and McCarty (1985), in which VC is hydrated to chloroethanol, cannot be ruled out. Microbial fermentation of simple and substituted alcohols to acetate is well known (Eichler and Schink, 1984; Emde and Schink, 1987; Buschhorn *et al*., 1989; Schink, 1984, 1994).

Likewise, compelling evidence exists for the net, anaerobic microbial oxidation of VC to CO_2 with ethene as the major intermediate. From a thermodynamic standpoint, VC oxidation to CO_2 with ethene as an intermediate is favorable and a number of reactions involving ethene as a significant player in the anaerobic oxidation of VC to CO_2 were suggested recently by Dolfing (1999). The potential importance of ethene as an participant in anaerobic microbial oxidation of VC was reinforced by a report of microbial oxidation of ^{14}C-ethene to $^{14}CO_2$ in surface water sediment microcosms under SO_4-reducing conditions (Bradley and Chapelle, 2002). Combined with the growing evidence that respiratory reductive dechlorination of VC can be significant at H_2 concentrations characteristic of SO_4-reducing conditions (Yang and McCarty, 1998; Löffler *et al*., 1999), the potential for microbial ethene oxidation under SO_4-reducing conditions suggests that a net anaerobic oxidation of VC can result from reductive dechlorination of VC to ethene followed by anaerobic oxidation of ethene to CO_2 (Bradley and Chapelle, 2002). Although associated with SO_4-reducing conditions, the mechanism of ethene oxidation and the specific contribution of SO_4-reducing microorganisms have not been clarified (Bradley and Chapelle, 2002). However, published thermodynamic calculations suggest that anaerobic ethene oxidation is favorable provided that an efficient sink for H_2 exists (Dolfing, 1999). Thus, the contribution of SO_4-reducers to anaerobic ethene oxidation may be as a sink for H_2 (Bradley and Chapelle, 2002). This conclusion suggests that anaerobic ethene oxidation also may be significant under a range of alternative electron accepting conditions, including metals-reducing and NO_3-reducing conditions (Bradley and Chapelle, 2002). Alternatively, the process of VC chlororespiration could itself serve as the sink for dissolved H_2 (Löffler *et al*., 1999). Because a similar investigation with surface-water sediments from a different site did not demonstrate ethene mineralization (Bradley, unpublished results), microbial oxidation of VC to CO_2 via ethene does not appear to be ubiquitous.

Kinetics of Anaerobic Microbial Oxidation of Chloroethenes

Data on the kinetic character of anaerobic microbial chloroethene oxidation is presently limited to a single study on anaerobic DCE and VC mineralization in surface-water sediments under methanogenic and Fe(III)-reducing conditions (Bradley and Chapelle, 1997). Under both conditions, anaerobic mineralization of [1,2-^{14}C] VC to $^{14}CO_2$ exhibited Michaelis-Menten kinetics. The V_{max} for anaerobic VC oxidation was four times higher under Fe(III)-reducing conditions than under methanogenic conditions. Consistent with this pattern, the k_s was 1.3 ± 0.5 FM and 7.6 ± 1.7 FM for Fe(III)-reducing and methanogenic conditions, respectively. Because this study was conducted under static conditions specifically selected to yield maximum estimates for k_s, the actual k_s for anaerobic VC mineralization under *in situ* flow conditions was expected to be substantially lower (Bradley and Chapelle, 1997). These results indicate that the mechanism for anaerobic VC oxidation has a relatively high substrate affinity for VC and that the affinity and the maximum rate of VC oxidation increase as the environmental redox conditions become increasingly oxidizing. Moreover, these results indicate that the rates of anaerobic VC oxidation are likely to be saturated with respect to VC even at *in situ* VC concentrations as low 100–200 ppb.

In contrast, under methanogenic and Fe(III)-reducing conditions, anaerobic mineralization of [1,2-^{14}C] DCE to $^{14}CO_2$ exhibited first-order degradation kinetics for dissolved DCE concentrations ranging from 1.2 to 80 μM (0.1 to 7.8 ppm) (Bradley and Chapelle, 1997). The first-order degradation rate constant for DCE mineralization was $0.6 \pm 0.2\%$ d^{-1} for both redox treatments (Bradley and Chapelle, 1997). Detection of trace concentrations of VC, ethene and ethane in both redox treatments, indicated that net anaerobic oxidation of [1,2-^{14}C] DCE involved an initial reductive dechlorination to ^{14}C-VC followed by oxidation to $^{14}CO_2$. Although a separate study demonstrated that microbial oxidation of [1,2-^{14}C] DCE to $^{14}CO_2$ without apparent production of reduced daughter products was possible under Mn(IV)-reducing conditions, the kinetics of the process were not investigated (Bradley *et al*., 1998c). Thus, because the kinetic character of anaerobic DCE oxidation was strikingly different from that of anaerobic VC oxidation and did not differ between methanogenic and Fe(III)-reducing treatments, it was concluded that the initial reduction of DCE to VC controlled the

Table 1
Application of lines of evidence approach to evaluate the contribution of reductive dechlorination or anaerobic oxidation to *in situ* biodegradation of chloroethene contaminants in groundwater

In Situ Biodegradation Lines of Evidence		
Line of Evidence	Reductive Dechlorination	Anaerobic Oxidation
Conducive Conditions:	YES	YES
Red-Ox Evaluation:	Yes	Yes
Microbial Presence:	Some Identified (Promising)	None Identified
Contaminant Loss:	YES	YES
Corroborating Evidence:	$\delta^{13}C$ & $\delta^{37}Cl$ Enrichment (Promising)	$\delta^{13}C$ & $\delta^{37}Cl$ Enrichment (Promising)
Daughter Product Accumulation:	YES DCE VC Ethene (Non-conservative?) Ethane (Non-conservative?) Cl^- (Rarely Conclusive) $\delta^{13}C$ & $\delta^{37}Cl$ Depletion (Promising)	NO CO_2 (Not Diagnostic, Background) Cl^- (Not Diagnostic, Background) $\delta^{13}C$ & $\delta^{37}Cl$ Depletion (Promising)
Microcosm Study:	YES	YES
Experimental Requirments:	Conventional Analytics	Sensitive (Radioisotope) Analytics

overall kinetics of this process (Bradley and Chapelle, 1997).

Practical Conclusions on Microbial Oxidation of Chloroethenes

In summary the following practical conclusions can be made about microbial oxidation mechanisms for *in situ* remediation of chloroethene contaminants in groundwater systems. Aerobic cometabolism and aerobic oxidation of chloroethenes as primary substrates appear to be of limited utility as non-engineered remediation mechanisms within chloroethene contaminant plumes. The potential for these processes can be substantial, however, at the fringe and the discharge point of chloroethene contaminant plumes even under non-engineered conditions. At present, there is no evidence that PCE or TCE are susceptible to anaerobic microbial oxidation processes. Thus reductive dechlorination appears to be the primary mechanism for *in situ* biotransformation of these chloroethene parent compounds under anaerobic conditions. DCE and VC are susceptible to anaerobic microbial oxidation. The potential for anaerobic oxidation is higher for VC than DCE and increases with increasingly oxidizing groundwater conditions. In combination with reductive dechlorination, anaerobic microbial oxidation provides a potential mechanism for complete degradation of chloroethene contaminants.

Assessment of *In Situ* Chloroethene Biodegradation

Lines of Evidence Approach

The EPA protocol (Wiedemeier *et al*., 1998) for evaluating the potential for natural attenuation of chlorinated solvents in groundwater systems recommends multiple lines of evidence (Table 1). Because biodegradation is generally the natural attenuation component with the greatest potential for contaminant destruction, implementation of this approach to chloroethene contaminated groundwater sites typically involves the following: (1) demonstration of the existence of geochemical conditions conducive to known mechanisms of chloroethene biodegradation; (2) demonstration of a distinct trend toward decreasing chloroethene concentrations; (3) demonstration of the production of daughter products indicative of chloroethene biotransformation; and (4) laboratory demonstration of an indigenous mechanism for microbial chloroethene degradation.

Conditions Conducive to Chloroethene Biodegradation

Establishing the existence *in situ* of conditions that are generally considered to be favorable for chloroethene biodegradation typically involves characterization of the *in situ* redox conditions and verification of the

Table 2
The relative efficiency of chloroethene biodegradation via microbial reductive dechlorination (RD) or microbial oxidation (MO) as a function of *in situ* Red-Ox conditions

		Predominant Terminal Electron Accepting Condition				
Contaminant	Mechanism	O_2	Mn(IV)	Fe(III)	SO_4	Methanogenic
PCE	RD	—[a]	Good	Good	Excellent	Excellent
	MO	Fair[b]	—	—	—	—
TCE	RD	—	Fair	Good	Good	Excellent
	MO	Good[b]	—	—	—	—
c-DCE	RD	Poor	Poor	Poor	Fair	Good
	MO	Excellent	Good	Poor	Poor	Poor
VC	RD	Poor	Poor	Poor	Fair	Fair
	MO	Excellent	Excellent	Excellent	Good	Good[c]

[a] — Indicates no evidence for this process under this Red-Ox condition.
[b]Aerobic cometabolism only.
[c]Associated with humic-acids reduction not methanogenic activity.

presence of specific chloroethene degrading microorganisms.

A number of field and laboratory investigations have demonstrated that the mechanisms and the extent of microbial chloroethene degradation are particularly sensitive to the redox character of the groundwater contaminant plume (Vogel *et al*., 1987; Bouwer, 1994; McCarty and Semprini, 1994; Chapelle, 1996; Bradley and Chapelle, 1998b). The relative potential for reductive and oxidative microbial degradation of chloroethene compounds under various redox conditions is summarized in Table 2 on the basis of field and laboratory evidence. Methods for characterization of predominant redox conditions in contaminated groundwater systems are well established (Chapelle *et al*., 1995; Chapelle, 1996; Wiedemeier *et al*., 1998) and readily applicable to investigations of the potential for reductive or oxidative chloroethene biodegradaton.

The presence of chloroethene reducing microorganisms within contaminated groundwater systems can be and historically has been inferred from geochemical evidence of contaminant loss and daughter product formation. Such evidence, however, offers little insight into the relative contribution of cometabolism vs chlororespiration, the potential for complete chloroethene reductive dechlorination to nonchlorinated products, or the potential for reductive dechlorination of chloroethene contaminants in downgradient areas not yet impacted by a contaminant plume. In addressing these questions, an ability to directly assess the presence of chlororespiratory microorganisms is highly desirable. In this regard, ongoing identification of a number of chlororespiratory microorganisms, the discovery of *D. ethenogenes,* and the development of molecular characterization techniques for assessing the *in situ* presence of these microorganisms represent crucial advances in our ability to evaluate the potential for chloroethene biodegradation under field conditions. Because chloroethene reductive dechlorination to ethene can occur in mixed cultures in the apparent absence of *D. ethenogenes*, however, the absence of *D. ethenogenes* under *in situ* conditions does not necessarily preclude efficient or complete reductive dechlorination of chloroethene contaminants to non-chlorinated products. Nevertheless, molecular evidence for the *in situ* presence of chlororespiratory microorganisms is a compelling argument for the potential for reductive chloroethene biotransformation and reenforces the importance of continuing efforts to identify specific DCE and VC dechlorinators and to further characterize dechlorination specific gene sequences.

Unfortunately, molecular techniques for assessing the *in situ* presence of microorganisms capable of oxidative chloroethene degradation under anaerobic conditions are neither presently available nor imminent, as these microorganisms have not yet been identified. The lack of such direct evidence for the presence of anaerobic chloroethene oxidizing microorganisms forces the investigator to infer the presence/absence of such organisms based on field observations of nonconservative chloroethene (particularly DCE and VC) behavior, insufficient accumulation of reduced daughter products that would indicate the sole responsibility of reductive dechlorination, and the existence of relatively oxidizing redox conditions that are known to favor anaerobic chloroethene oxidation under laboratory

conditions. Because such inferences are hardly unequivocal, identification of specific microorganisms capable of anaerobic VC oxidation is an area of active research (Bradley, unpublished results). However, the approximately fifteen year period between the initial field evidence of chloroethene reductive dechlorination and the ultimate isolation of *D. ethenogenes* suggests that identification of anaerobic chloroethene oxidizing microorganisms may be a lengthy process.

Decreasing Chloroethene Concentrations

As evidence of the *in situ* potential for chloroethene biodegradation, a down-gradient decrease in the dissolved concentration of the parent chloroethene compound, generally PCE and/or TCE, is crucial and independent of the degradation mechanism. In theory, such a demonstration is straightforward. In practice, however, the uncertainties surrounding the location of the contaminant plume and the placement and depth of the monitoring apparatus often make such determinations suspect. Consequently, independent corroboration is preferred to ensure that the observed contaminant losses are attributable to biodegradation or other attenuation processes and not to flawed monitoring efforts.

Compound-specific stable carbon and stable chlorine isotope analyses represent promising and potentially powerful tools for deducing the fate of chloroethene compounds in ground water (Sturchio *et al*., 1998; Hunkeler *et al*., 1999, 2002; Bloom *et al*., 2000; Sherwood Lollar *et al*., 2001; Slater *et al*., 2001; Numata *et al*., 2002; Song *et al*., 2002). The characteristic enrichment of residual reactants, with respect to heavy isotopes, during contaminant degradation is useful as a means of distinguishing the loss of contaminants due to degradation mechanisms from those resulting from physical attenuation mechanisms like dilution. This approach holds especial promise for field evaluations of the extent of *in situ* microbial oxidation of VC and ethene, processes for which the accumulation of degradation daughter products is particularly difficult to demonstrate using conventional geochemical indicators (Sturchio *et al*., 1998; Hunkeler *et al*., 1999, 2002; Bloom *et al*., 2000; Sherwood Lollar *et al*., 2001; Slater *et al*., 2001; Numata *et al*., 2002; Song *et al*., 2002).

Accumulation of Daughter Products of Chloroethene Biotransformation

Stoichiometric production and accumulation of DCE and VC along the chloroethene contaminant plume are unequivocal evidence of reductive transformation of the original PCE or TCE contamination in anaerobic groundwater systems. Because DCE and particularly VC are also contaminants of regulatory concern, however, demonstration that biotransformation is sufficient for aquifer remediation requires evidence of chloroethene transformation to non-chlorinated and non-toxic product(s). Such evidence is crucial for regulatory acceptance of reductive dechlorination as a component of *in situ* bioremediation of chloroethene-contaminated ground water.

Dissolved ethene is a product of complete chloroethene reductive dechlorination, but not unique to chloroethene metabolism. Ethene is a primary plant growth hormone, a common product of bacterial metabolism and not uncommon in natural groundwater systems. Consequently, the use of dissolved ethene concentrations as an indicator of chloroethene reduction requires accumulation substantially above background concentrations.

If background concentrations of dissolved ethene are comparatively low, the accumulation of ethene within a chloroethene plume can provide convincing evidence of VC reductive dechlorination (Ellis *et al*., 1996; Wiedemeier *et al*., 1996; Weaver *et al*., 1996). Any effort to utilize ethene accumulation as an indicator of the extent of VC reduction, however, presupposes that ethene persists *in situ* or that the products of ethene degradation are unique and readily detected. Several studies have demonstrated microbial reduction of ethene to ethane under methanogenic conditions (Koene-Cottaar and Schraa, 1998; Oremland, 1981; Oremland *et al*., 1988; Whelan *et al*., 1980) and microbial reduction of chloroethenes to ethene followed by reduction to ethane under methanogenic conditions (De Bruin *et al*., 1992; DiStefano *et al*., 1991; Freedman and Gossett, 1989). Thus, the combined accumulation of ethene and ethane under methanogenic conditions has often been used to assess the extent of microbial reductive dechlorination of VC (De Bruin *et al*., 1992; DiStefano *et al*., 1991; Freedman and Gossett, 1989; Ellis *et al*., 1996; Wiedemeier *et al*., 1996; Weaver *et al*., 1996).

This approach may be problematic, however, because some microbial communities appear to mineralize ethene to CO_2 under anaerobic conditions (Bradley and Chapelle, 2002). Because CO_2 is a common, non-specific product of numerous microbial processes, oxidation of ethene to CO_2 would effectively remove this "fingerprint" of VC reduction. Thus, a lack of ethene accumulation under anaerobic conditions may reflect either insignificant reductive dechlorination of VC or anaerobic mineralization of ethene, VC or DCE to CO_2. Aerobic microbial degradation of ethene is also a potential concern at the fringe or the discharge point of anoxic chloroethene plumes (De Bont, 1975; Bradley and Chapelle, 2002). Reliance on ethene and ethane

accumulation as a quantitative indicator of complete reductive dechlorination of chloroethene contaminants may underestimate the importance of in situ reductive dechlorination (Bradley and Chapelle, 2002).

Likewise, the use of daughter product accumulation as a line of evidence for chloroethene biodegradation is problematic, if anaerobic microbial oxidation of chloroethenes is a significant contributor to biodegradation. The final products of anaerobic microbial oxidation, CO_2 (or CO_2 and CH_4), are common metabolic byproducts and not unique to chloroethene biodegradation. Similar concerns surround the few recognized intermediates of anaerobic chloroethene oxidation. The difficulties associated with ethene quantification under anaerobic conditions have already been discussed. Likewise, acetate is a common microbial product and not unique to anaerobic chloroethene oxidation. Consequently, daughter product accumulation currently is not an informative line of evidence for anaerobic microbial oxidation of chloroethene contaminants.

Similar difficulties beset the use of dissolved chloride concentrations as an indicator of chloroethene biodegradation. Increasing concentrations of dissolved chloride along the groundwater flowpath of a chloroethene plume can provide compelling and, perhaps, quantitative evidence of contaminant dechlorination independent of the underlying degradation mechanism (Wiedemeier *et al*., 1998). In practice, however, the utility of chloride ion accumulation as a line of evidence for chloroethene degradation often is limited by the common occurrence of high background chloride concentrations that effectively mask the chloride released by microbial dechlorination.

Because the primary purpose of this line of evidence is to confirm that observed decreases in chloroethene concentrations are attributable to biodegradation, uncertainty in the origin of the above indicator compounds undermines the utility of this approach. Evaluation of compound-specific stable carbon and stable chlorine isotopes may offer some opportunity to distinguish the sources of potential degradation daughter products in situations where background concentrations mask the contribution of chloroethene biodegradation (Hunkeler *et al*., 1999).

Laboratory Microcosm Studies

Laboratory investigations of the potential for chloroethene reductive dechlorination are generally considered optional, primarily because such studies are resource-intensive and often add little information beyond that provided by other lines of evidence. Thus, if field data on chloroethene disappearance and the production and accumulation of ethene and/or ethane within a chloroethene plume validate *in situ* reductive dechlorination as an acceptable remediation mechanism, laboratory microcosm studies are not required.

In contrast, the difficulty demonstrating the *in situ* potential for microbial oxidation of chloroethenes based solely on the above lines of evidence makes a laboratory microcosm study a particularly important approach for evaluating this biodegradation mechanism. At present, a microcosm study is the only established means of demonstrating anaerobic microbial oxidation of chloroethene contaminants. Likewise, microcosm assessments of chloroethene reductive dechlorination can be worthwhile if oxidative biodegradation of ethene is considered significant or if the potential for chloroethene reductive dechlorination must be evaluated in areas that are not yet contaminated.

The particular utility of the microcosm approach for evaluating the potential for anaerobic microbial oxidation of chloroethenes and the limited information on the kinetics of this process impose significant constraints on the experimental method. To accommodate the relative insensitivity of gas chromatography-based compound disappearance assays, contaminant biotransformation studies are commonly conducted at high substrate concentrations under the assumption of first order degradation kinetics. In contrast, the information available on the kinetics of anaerobic VC oxidation, indicates that under *in situ* flow conditions this process exhibits Michaelis-Menten kinetics with saturation occurring at low dissolved VC concentrations (Bradley and Chapelle, 1997). This observation, in turn, indicates that a first order degradation model is inappropriate under most field conditions and that the potential for anaerobic VC oxidation must be evaluated at *in situ* VC concentrations using sensitive methods, such as radioisotopic techniques, if valid conclusions are to be obtained in the absence of a detailed kinetic examination (Bradley and Chapelle, 1997).

Practical Conclusions on the Lines of Evidence Approach to Natural Attenuation

The lines of evidence approach can provide a comfortingly clear-cut evaluation of biodegradation as a mechanism for *in situ* chloroethene remediation under certain circumstances. For sites at which a decline in chloroethene concentrations to less than action levels occurs up-gradient of the regulatory point of compliance and coincides with direct evidence of contaminant biodegradation (ex. contaminant-associated ethene/ethane accumulation), the lines of evidence approach provides confidence that *in situ* biodegradation is effectively controlling contaminant transport.

Likewise, insignificant chloroethene attenuation or chloroethene concentrations in excess of action levels at the regulatory point of compliance argue for active contaminant interception and remediation.

In contrast, circumstances intermediate to the above examples present substantial challenges to this approach. Particularly problematic are cases in which natural attenuation appears sufficient to prevent contaminant transport to the point of compliance, but the mechanisms of attenuation remain uncertain. A common example is the apparent tendency of *in situ* reductive dechlorination processes to effectively degrade PCE and TCE and then to "stall" at DCE or VC (Vogel, 1994; McCarty and Semprini, 1994). Such incomplete reductive dechlorination patterns are typically attributed to cometabolic reductive dechlorination (Fathepure *et al*., 1987; Fathepure and Boyd, 1988a, 1988b; Jablonski and Ferry, 1992; El Fantroussi *et al*., 1998; Freedman and Gossett, 1989; Cole *et al*., 1995; Townsend and Suflita, 1996; Terzenbach and Blaut, 1994), to the activity of incomplete chlororespiratory microorganisms (Holliger *et al*., 1993; Gerritse *et al*., 1996, 1999; Löffler *et al*., 1999, 2000; Scholz-Muramatsu *et al*., 1995; Krumholz *et al*., 1996; Krumholz, 1997; Sharma and McCarty, 1996), or to the inhibition of subsequent dechlorination steps (Townsend and Suflita, 1996; Neumann *et al*., 1996; Tandoi *et al*., 1994; Maymó-Gatell *et al*., 2001). Equally plausible, however, are the degradation of DCE and VC to non-diagnostic products via anaerobic oxidation reactions (Vogel and McCarty, 1985; Bradley and Chapelle, 1996; Bradley *et al*., 1998b; Adamson and Parkin, 2001) or degradation of DCE and VC by unrecognized mechanisms. In such cases, laboratory microcosm studies can be invaluable in demonstrating the potential for microbial degradation and establishing possible explanations for field observations. Because microcosms are always imperfect aquifer simulators, however, such demonstrations do not constitute definitive evidence. The need to address these shortcomings suggests a number of areas for future research.

Isolation of the microorganisms that have been shown to be capable of VC and DCE chlororespiration in mixed cultures (Cupples *et al*., 2003; He *et al*., 2003) is needed to provide biological models for the study of complete chloroethene reductive dechlorination by microbial consortia. Likewise, isolation of the microorganisms responsible for anaerobic chloroethene oxidation is a priority, in order to clarify the mechanisms underlying this process and to provide molecular targets for the identification of these organisms *in situ*. Indeed, continued development of molecular techniques for *in situ* identification of major chloroethene-degrading microbial groups remains crucial for establishing the potential for non-engineered chloroethene biodegradation in contaminated and non-contaminated ground water. In particular, gene-sequences specific to VC and DCE chlororespiration are needed to complement the growing list of PCE and TCE dehalogenase-specific gene sequences.

Continued development of compound-specific stable carbon and stable chlorine methods or comparable techniques is needed to clarify the mechanism of natural attenuation of chloroethene contaminants in ground water. In addition to differentiating between degradation mechanisms and other physical methods of chloroethene attenuation, refinement of existing techniques to assess the *in situ* isotopic enrichment of residual DCE and VC may allow site investigators to distinguish between incomplete reductive dechlorination to yield DCE or VC versus reductive dechlorination to DCE or VC followed by oxidation to non-diagnostic products like CO_2. Likewise, assessing the extent of compound-specific stable isotopic depletion in potential degradation products, such as ethene, CO_2 and Cl^-, may be useful in distinguishing the sources of these products when background concentrations confound conventional geochemical approaches.

Finally, the clear importance of natural production and turnover of organochlorine compounds suggests that a renewed search for alternative mechanisms of chloroethene biotransformation is appropriate. In light of the evidence that natural production and turnover of chloroethene compounds historically have been important and continue to be significant in the environment, it is probable that a number of biological mechanisms for chloroethene transformation remain unrecognized. To date, much attention has been focused on respiratory degradation processes. Recent evidence of VC biotransformation via oxidative acetogenesis suggests that fermentative processes merit further attention.

Acknowledgments

Sincere thanks to Clifton C. Casey, Francis H. Chapelle, Sandra C. Cooper, and four anonymous reviewers for their critical reviews. This work was supported by the U.S. Geological Survey Toxic Substances Hydrology Program and the Southern Division Naval Facilities Engineering Command.

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