



PDHonline Course C175 (3 PDH)

Hazardous and Toxic Waste Treatment of Soils & Sludges

Instructor: John Poullain, P.E.

2012

PDH Online | PDH Center

5272 Meadow Estates Drive
Fairfax, VA 22030-6658
Phone & Fax: 703-988-0088
www.PDHonline.org
www.PDHcenter.com

An Approved Continuing Education Provider

2.4.2 Anaerobic Reductive Dechlorination

Under anaerobic conditions, reductive dechlorination mechanisms can effectively biodegrade CAHs. Reductive dechlorination generally involves the sequential replacement of a chlorine atom on a CAH with a hydrogen atom (that is, converting PCE to TCE to DCE, and so on) and has been observed to occur both directly and cometabolically. In anaerobic reductive dechlorination (direct), the mediating bacteria use the CAH directly as an electron acceptor in energy-producing redox reactions. Anaerobic reductive dechlorination (cometabolic) occurs when bacteria incidentally dechlorinate a CAH in the process of using another electron acceptor to generate energy. Reductive dechlorination theoretically is expected to occur under most anaerobic conditions, but has been observed to be most effective under sulfate-reducing and **methanogenic** conditions (EPA 1998). As in the case of aerobic oxidation, the direct mechanisms may biodegrade CAHs faster than cometabolic mechanisms (McCarty and Semprini, 1994) (refer to the following case studies in Appendix A: *Enhanced Bioremediation at the Texas Gulf Coast Site, Houston, Texas*[Texas Gulf Coast]; *Molasses Injection at the Avco Lycoming Superfund Site, Willimasport, Pennsylvania*[Avco Lycoming]; *Anaerobic In Situ Reactive Zone at an Abandoned Manufacturing Facility, Emeryville, California*[Emeryville]; *Sequential Anaerobic/Aerobic Biodegradation of PCE at Watertown, Massachusetts*[Watertown]; and *Bioaugmentation (Accelerated Anaerobic Bioremediation) at Area 6 of the Dover Air Force Base, Dover, Delaware* [Dover Area 6]).

Anaerobic Reductive Dechlorination (Direct)

Anaerobic reductive dechlorination (direct) is a biodegradation reaction in which bacteria gain energy and grow as one or more chlorine atoms on a chlorinated hydrocarbon are replaced with hydrogen (McCarty 1997b; Fennel and others 1997; Mayo-Gatell and others 1997; Gerritse and others 1999). In that reaction, the chlorinated compound serves as the electron acceptor, and hydrogen serves as the direct electron donor (Fennel and others 1997). Hydrogen used in the reaction typically is supplied indirectly through the fermentation of organic substrates. The reaction is also referred to as *halorespiration* or *dehalorespiration* (Gossett and Zinder 1997).

Anaerobic reductive dechlorination (direct) has been observed in anaerobic systems in which PCE, TCE, DCE, VC, and DCA are used directly by a microorganism as an electron acceptor in their energy-producing redox reactions (Neumann and others 1994; Scholz-Muramatsu and others 1995; Freedman and Gossett 1989; Yagi and others 1994; Hollinger and Schumacher 1994; Major and others 1991; McCarty 1997b; Gossett and Zinder 1996; Gerritse and others 1996; DeBruin and others 1992; Maymo-Gatell and others 1997; Sharma and McCarty 1996; Hollinger 1993; Smatlak 1996; Tandol 1994; Yager and others 1997). The mechanism generally results in the sequential reduction of a chlorinated ethene or chlorinated ethane to ethene or ethane. Exhibit 2-13 shows the step-by-step dechlorination of PCE.

The anaerobic reductive dechlorination of the more chlorinated CAHs (PCE and TCE) occurs more readily than the dechlorination of CAHs that already are somewhat reduced (DCE and VC); for that reason, DCE and VC may accumulate in anaerobic environments. It also has been observed that, while VC can be effectively dechlorinated, the presence of PCE in groundwater may inhibit the anaerobic reductive dechlorination of VC (Tandol and others 1994).

VC is more commonly remediated using aerobic mechanisms than anaerobic mechanisms. In anaerobic environments in which VC accumulates, enhanced aerobic bioremediation can be implemented to degrade the VC. Recent studies have demonstrated significant anaerobic oxidation of VC to carbon dioxide under Fe(III)-reducing conditions (Bradley and Chapelle 1998b) and of DCE to VC and VC to carbon dioxide under humic acid-reducing conditions (Bradley and Chapelle 1998a). These studies suggest the possibility of alternative biotransformation mechanisms under anaerobic conditions.

Exhibit 2-13: Anaerobic Reductive Dechlorination of PCE

	$\text{H}_2 \xrightarrow{\quad} \text{HCl}$ PCE → TCE	$\text{H}_2 \xrightarrow{\quad} \text{HCl}$ TCE → DCE	$\text{H}_2 \xrightarrow{\quad} \text{HCl}$ DCE → VC	$\text{H}_2 \xrightarrow{\quad} \text{HCl}$ VC → Ethene	
Carbon oxidation state	+ 2	+ 1	0	- 1	- 2
	Most oxidized \longrightarrow Most reduced				

Source: Modified from DeStephano and others 1992

Hydrogen has been observed to be an important electron donor in anaerobic reductive dechlorination (Fennell and others 1997). The presence of hydrogen establishes a competition between the bacteria that mediate the anaerobic reductive dechlorination (such as *Dehalococcus ethenogenes* and *Dehalospirillum multivorans*) and methanogenic bacteria that also use hydrogen as an electron donor (ITRC 2000). However, it has been observed that the dechlorinating bacteria can survive at a partial pressure of hydrogen ten times lower than that at which the methanogenic bacteria can survive (Smatlak and others 1996), thus providing an opportunity to support the dechlorinating bacteria by providing hydrogen at a slow rate. (Hydrogen addition at a slow rate has been demonstrated with the fermentation of butyric or propanoic acid) (Fennell and others 1997). In addition, in some subsurface environments, competition from nitrate or sulfate-reducing bacteria may limit both methanogenic activity and the extent of anaerobic reductive dechlorination (RTDF 1997).

Studies have shown that anaerobic reduction of CAHs can occur by reductive dechlorination in a variety of environmental conditions (Beeman and others 1994; Semprini and others 1995). A review of the transformation of halogenated compounds has shown that the theoretical maximum redox potential for transformation of PCE to TCE is +580 millivolts and for TCE to DCE is +490 millivolts (Vogel and others 1987). Therefore, the anaerobic reductive dechlorination of the compounds is thermodynamically possible under manganese- or iron-reducing conditions. No peer-reviewed reports of the transformation of PCE to TCE under aerobic conditions were identified. However, the efficiency of the anaerobic dechlorination processes at high redox potential values is limited; efficiency improves as the redox potential decreases.

Pilot studies have been conducted at a variety of sites to examine the feasibility of stimulating *in situ* anaerobic reductive dechlorination by providing to the subsurface simple organic substrates, such as lactate, butyrate, methanol, ethanol, and benzoate (Harkness and others 1999; Freedman and Gossett 1989; Gibson and Sewell 1992; Buchanan and others 1997; Becvar and others 1997; Sewell and others 1998; Litherland and Anderson 1997; Spuij and others 1997).

Anaerobic Reductive Dechlorination (Cometabolic)

Anaerobic reductive dechlorination (cometabolic) is a biodegradation reaction in which a chlorinated hydrocarbon is fortuitously degraded by an enzyme or cofactor produced during microbial metabolism of another compound. In such a case, biodegradation of the chlorinated compound does not appear to yield any energy or growth benefit for the microorganism mediating the reaction (Gossett and Zinder 1997).

Several CAHs have been observed to be reductively dechlorinated by cometabolic mechanisms. In those instances, the enzymes that are intended to mediate the electron-accepting reaction “accidentally” reduce and dehalogenate the CAH. Anaerobic reductive dechlorination (cometabolic) has been observed for PCE, TCE, DCE, VC, DCA, and CT under anaerobic conditions (Fathepure 1987; Workman 1997; Yager and others 1997).

In pilot- and full-scale applications, it is generally difficult to distinguish between direct and cometabolic anaerobic reductive dechlorination reactions. Both biodegradation mechanisms are referred to more generally as anaerobic reductive dechlorination. In laboratory-scale applications, direct and cometabolic anaerobic reductive dechlorination reactions can be distinguished. The role played by anaerobic reductive dechlorination (cometabolic) in relation to anaerobic reductive dechlorination (direct) remains under study.

Anaerobic Reductive Dechlorination Combined with Aerobic Oxidation

Several investigators have suggested that the most efficient bioremediation of CAHs will occur in aquifers that are characterized by an upgradient anaerobic zone and a downgradient aerobic zone (Bouwer 1994; Carter and Jewell 1993; Gerritse and others 1995; Fathepure and others 1987). In the upgradient aerobic zone, anaerobic reductive dechlorination of PCE might degrade to TCE, and eventually to VC. VC could then be degraded by aerobic oxidation (direct) downgradient in the aerobic zone of the CAH plume (the leading-edge fringe of the plume). Stratified redox conditions in the field may provide the best opportunities, other than engineered remedies, for intrinsic biodegradation of CAHs.

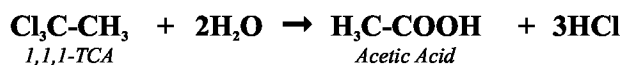
Generally, the substrate requirement for direct metabolism is relatively less than that for cometabolism. In cometabolism, often the amount of primary substrate required is a factor of 100 to 1,000 times the amount of the CAH. In direct metabolism (respiration with only the chlorinated solvent as the electron acceptor), the stoichiometry is much more favorable, and a much smaller amount of supplemental chemical is required (Bouwer 1994).

2.4.3 Abiotic Degradation Mechanisms

Abiotic degradation mechanisms involve chemical reactions to treat CAHs without biological processes. These mechanisms include hydrolysis, elimination, and abiotic reductive dechlorination. In general, the rates of abiotic degradation may be slow relative to biological mechanisms. However, the abiotic mechanisms may play a significant role in the overall remediation of a site at which CAH contamination is present, depending on the specific site conditions (for example, a site at which the contaminant plume is moving slowly) (EPA 1998). Hydrolysis and elimination reactions are generally independent of redox conditions, while abiotic reductive dechlorination is highly dependent on redox conditions.

Hydrolysis is a substitution reaction in which a CAH may react with water to substitute a chlorine atom with a hydroxyl group, producing organic alcohols, acids, or diols, such as the formation of acetic acid from 1,1,1-TCA (Exhibit 2-14). Generally, less chlorinated CAHs are more susceptible to degradation by hydrolysis. Hydrolysis rates have been reported that have half-lives ranging from days for monochlorinated alkanes to thousands of years for tetrachloromethane.

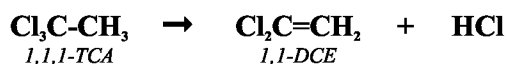
Exhibit 2-14: Example of Hydrolysis Reaction



Hydrolysis is a common transformation mechanism for 1,1,1-TCA, chloroethane, and chloromethane, producing acetate, ethanol, and methanol, respectively (Vogel and McCarty 1987).

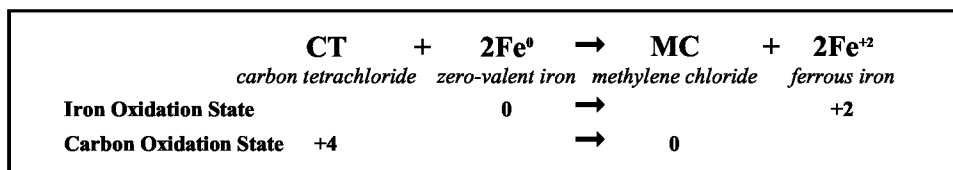
Elimination reactions involve the removal of a hydrogen and a chlorine atom (sometimes referred to as dehydrohalogenation) from a chlorinated alkane, with the formation of the corresponding alkene (Exhibit 2-15). In contrast to hydrolysis reactions, elimination reactions become more effective as the CAHs become more chlorinated. Assuming that elimination rates for monochlorinated CAHs are negligible, the abiotic conversion of TCA to DCE at 20°C has been reported to exhibit relatively rapid first-order kinetics, with a rate constant of approximately $0.04 \pm 0.003 \text{ year}^{-1}$ (Vogel and McCarty 1987).

Exhibit 2-15: Example of Elimination Reaction



Abiotic reductive dechlorination of several CAHs also has been observed (Reinhard and others 1990; Gillham and O'Hannesin 1994; Workman and others 1997). Abiotic reductive dechlorination occurs in the presence of an extremely strong reductant (for example, zero-valent iron or reduced vitamin B₁₂). When the reductant present is sufficiently strong, the more chlorinated (and, therefore, more oxidized) of the CAHs (PCE, TCE, CT, and CF) can be reduced to less chlorinated species without the mediation of bacteria. As in the case of biologically mediated reductive dechlorination, abiotic reductive dechlorination becomes less effective or ineffective for the less chlorinated CAHs (which already are reduced somewhat). Exhibit 2-16 shows the general mechanism of abiotic reductive dechlorination (using zero-valent iron as the reducing agent).

Exhibit 2-16: Example of Abiotic Reductive Dechlorination



3.0 *IN SITU* BIOREMEDIATION TECHNOLOGIES

In situ bioremediation technologies are used to enhance the mechanisms that degrade CAHs in contaminated soil and groundwater (discussed in Section 2). Technologies include **bioaugmentation** and the addition of **nutrients**, **electron donors** (substrates such as toluene, propane, and methane), and **electron acceptors** (such as oxygen). Design configurations of *in situ* bioremediation systems include direct injection, groundwater recirculation, installation of permeable reactive barriers (PRBs), and bioventing.

3.1 TECHNOLOGIES

Generally, *in situ* bioremediation technologies employ engineered systems to heighten the effects of naturally occurring **degradation** mechanisms. The engineered systems are designed to include one or more of the following general classes of technologies: the addition of bacteria (bioaugmentation), the addition of nutrients, the addition of electron donors, or the addition of electron acceptors. Each of the technologies is discussed below in more detail. Exhibit 3-1 presents a summary of information about each technology, including an example of how each may be applied, a discussion of the biodegradation mechanisms generally supported by each, a discussion of the typical CAHs targeted through the use of each technology, and a summary of how the enhancement technologies have been applied at the case study sites included in Appendix A of this report.

Bioaugmentation – involves the addition of supplemental microbes to the subsurface where organisms able to degrade specific contaminants are deficient. Microbes may be “seeded” from populations already present at a site and grown in aboveground reactors or from specially cultivated strains of bacteria known to degrade specific contaminants. The application of bioaugmentation technology is highly site-specific and highly dependent on the microbial ecology and physiology of the subsurface (EPA 1998).

Nutrient addition – involves the addition of key biological building blocks, such as nitrogen and phosphorus and other trace nutrients necessary for cell growth. Addition of nutrients generally is applied as a supplement to bioaugmentation or addition of electron donors or electron acceptors, so that concentrations of nutrients in the subsurface do not become a limiting factor for an *in situ* bioremediation application.

Electron donor addition – involves the addition of a substrate that acts as a reductant in the redox reaction used by the CAH-degrading microbe to produce energy. A substrate such as toluene, propane, or methane may be added to act as a cometabolic oxidant, when the CAH also is oxidized. A substrate such as hydrogen, a source of hydrogen, or a hydrogen release compound may be added to act as a direct reductant, when the CAH is reduced.

Electron acceptor addition – involves the addition of oxygen (for **aerobic** mechanisms) or an **anaerobic** oxidant such as nitrate (for anaerobic mechanisms), which is used by the CAH-degrading microbes present in the subsurface.

As Exhibit 3-1 shows, one or more of the technologies were used at several of the case study sites. For example, bioaugmentation was used at *Dover Area 6*, while addition of nutrients was used at *SRS, Texas Gulf Coast, Watertown, and Dover Area 6*. Addition of electron donors, such as toluene, propane, or methane, and an electron acceptor (oxygen) for aerobic cometabolic oxidation were used at the following five sites: *Moffett Field, Edwards AFB, SRS, Watertown, and Dover Building 719*. Addition of an electron donor in the form of a hydrogen source, such as methanol, molasses, or lactate, for anaerobic

Exhibit 3-1: Components of *In Situ* Bioremediation Technology

Component	Example	Biodegradation Mechanisms Supported	Targeted CAHs	Case Study Sites ¹
Bioaugmentation	Seed the subsurface with non-native, CAH-degrading bacteria	Aerobic oxidation (cometabolic and direct)	TCE, DCE, TCA, DCA, CA, CT, CF	None
		Anaerobic reductive dechlorination (cometabolic and direct)	TCA, DCA, CA, CT, CF, CM	Dover Area 6
Addition of Nutrients	Add nitrogen, phosphorus, or other growth factors that may be deficient in the subsurface	Aerobic oxidation (cometabolic and direct)	TCE, DCE, TCA, DCA, CA, CT, CF	SRS; Watertown
		Anaerobic reductive dechlorination (cometabolic and direct)	TCA, DCA, CA, CT, CF, CM	Texas Gulf; Dover Area 6
Addition of Electron Donors	Add a substrate, such as toluene, propane, or methane	Aerobic oxidation (cometabolic)	TCE, DCE, TCA, CF, MC	Moffett Field; Edwards AFB; SRS; Watertown; Dover Building 719
	Add hydrogen, a hydrogen source, or a hydrogen release compound	Anaerobic reductive dechlorination (cometabolic and direct)	PCE, TCE, DCE, VC, TCA, DCA, CA, CT, CF, MC	Texas Gulf; Avco Lycoming; Emeryville; Watertown; Dover Area 6
Addition of Electron Acceptors	Add oxygen by bioventing, biosparging, or adding an oxygen source such as hydrogen peroxide	Aerobic oxidation (direct)	TCE, DCE, VC, TCA, DCA, CA, CE, MC, CM	Moffett Field; Edwards AFB; SRS; Watertown; Dover Building 719
	Add an anaerobic reductant such as nitrate	Anaerobic reductive dechlorination (cometabolic)	PCE, TCE, DCE, VC, DCA, CT	None

Source: ITRC, 1998; Leeson, 1999; Sewell, 1998; U.S. Air Force, 1998

¹ Case studies provided in Appendix A of this report are cited as examples of each of the technologies.