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# A review: Advances in microbial remediation of trichloroethylene (TCE)

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**Abstract**

Research works in the recent past have revealed three major biodegradation processes leading to the degradation of trichloroethylene. Reductive dechlorination is an anaerobic process in which chlorinated ethenes are used as electron acceptors. On the other hand, cometabolism requires oxygen for enzymatic degradation of chlorinated ethenes, which however yields no benefit for the bacteria involved. The third process is direct oxidation under aerobic conditions whereby chlorinated ethenes are directly used as electron donors by microorganisms. This review presented the current research trend in understanding biodegradation mechanisms with regard to their field applications. All the techniques used are evaluated, with the focus being on various factors that influence the process and the outcome.

**Key words**: biodegradation; trichloroethylene; reductive dechlorination; cometabolism; direct oxidation

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## Introduction

Trichloroethylene (TCE) is a halogenated volatile organic compound (VOC), which is believed to be carcinogenic and mutagenic (USEPA, 1997). It is considered a primary pollutant with 5 μg/L maximum contamination level allowed in drinking water (ASTDR, 1997; Spitz and Moreno, 1996). Most of the hazardous-waste sites in the United States listed in the National Priority List (NPL) are contaminated with VOC, including TCE (Zachritz et al., 1996). It is quite alarming that almost all the states in the USA have problems related to TCE and/or other chlorinated ethenes in soil and groundwater (Moran et al., 2007; http://www.epa.gov/superfund/sites/npl). Most of these TCE contaminants in the NPL sites originated from usage of chlorinated degreasing solvents during nuclear reactor operations.

TCE is also widely used in textile processing, refrigeration, lubricants and adhesives, along with the production of vinyl chloride, pharmaceuticals, and insecticides (Schettler et al., 1999). Due to inappropriate disposal methods that are carried out, soil and groundwater contamination by TCE has become widespread (Doucette et al., 2007; Riley and Zachara, 1992). Reports are also available documenting contamination of the surface water as a result of TCE release from the groundwater sources (Brewer and Fogle, 2001; Brigmon et al., 2001). The toxic effects associated with TCE have raised a serious public concern over the extensive contamination problems, and knowledge of its effective remedial process will be highly valuable.

Several methods are available for remediation of TCE pollution. Physical processes, such as soil excavation and venting, and ground water extraction techniques, all of which have been used in remediation of TCE pollution in the subsurface environments, have been found relatively slow, costly, and inefficient, along with being environmentally disruptive in nature (Bankston, 2002). The complex nature of aquifers, contaminant sorption onto solid media, and entrapment of contaminants in the porous media also attribute to the limited effectiveness of "pump and treat" technologies in groundwater remediation (Beeman and Bleckmann, 2002). Several other chemical and physical pollution control methods have been found to require toxic chemicals or are expensive (Han et al., 2002). In this context "Monitored Natural Attenuation (MNA)" has emerged as a promising technique for TCE pollution remediation. The term MNA refers to the reliance on natural processes to achieve site-specific cleanup objectives within a reasonable time frame (USEPA, 1997). These *in-situ* natural processes include biodegradation, dispersion, dilution, sorption, volatilization, and chemical or biological stabilization, transformation, or destruction of contaminants (USEPA, 1997). Of these processes, bioremediation technology is preferred over other technologies because it allows for complete mineralization of TCE to harmless chemical forms, including carbon dioxide, water and chlorine (Russell et al., 1992). Other processes, such as chemical reaction, sorption, dispersion, and volatilization, are either slow or unable to degrade TCE, thus not resulting in a net degradation of TCE (Lorah et al., 1997).

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This article reviews the types of microbial processes that are available for TCE pollution remediation, and evaluates different biological and physical factors that may affect the successful biodegradations of TCE in the environment. This review also reports other compounds, including dichloroethylene (DCE) and vinyl chloride (VC), which are often considered as the daughter products of TCE biodegradation.

## 1 Physical and chemical aspects of TCE

TCE and its daughter products, such as DCE and VC, are double bonded between two carbons, and thus are grouped together with other chlorinated alkenes (Fig. 1). TCE is a colorless, volatile liquid with a sweet smell similar to that of chloroform (Jacoby et al., 1998). TCE is relatively insoluble in water and thus commonly categorized as a hydrophobic compound. Table 1 lists a few important physico-chemical parameters pertaining to its biodegradation potential.

While all the characteristics are important in understanding the TCE behavior, its density plays a crucial role in groundwater contamination, even when the discharge of TCE occurs at the surface. Agarwal and Singh (2004) have experimentally determined the density of TCE as 1.4557 g/mL at 298.15 K (25°C), which compares well with an earlier reported value of 1.4514 g/mL (Venkatesulu et al., 1997). Jacoby et al. (1998) also reported a similar value: 1.46 g/mL at 25°C. Given the fact that water has a density of 0.9970479 g/mL (calculated) at the same temperature, it is clear that TCE is much denser than water and thus it infiltrates easily into the aquifer where it forms a dense non-aqueous liquid phase (DNAPL) at the bottom (Bourg et al., 1992). Furthermore, the diffusion rate of TCE in water has been cited as $9.0\times10^{-5}$ $m^2$/day by Chiao et al. (1994), based on their study with the molar volume of 107 $cm^3$/mol at 25°C. The lower rate of TCE diffusivity in water can reduce the mobility, and hence its transport in a slow flowing groundwater system. Parker et al. (2004) conducted a simulation study on TCE diffusion in aquifer, and reported that TCE migration is diffusion-dominated within aquifer. Further, adsorption of TCE to both organic and mineral components in groundwater matrix such as in sediments and rocks may also retard the movement of TCE in groundwater. Extensive research has already been done to establish a phenomenon of TCE adsorption with respect to various minerals and organic carbon (Aggarwal et al., 2006; Li and Werth, 2001; Lin et al., 1994; Smith et al., 1990). In general, the adsorption coefficient ($K_d$), which is a measure of TCE partitioning between water and sediment/soil phases, has been reported to increase with organic carbon content in the soil or sediment (Lee et al., 2007; Poulsen et al., 2000). Adsorption not only hinders the mobility of TCE, it also influences the process of TCE biodegradation by affecting TCE availability in aqueous phase. Studies by Alvarez-Cohen et al. (1993) and Sheremata et al. (2000) have indicated that organic contaminants, such as TCE, are not available to bacteria for degradation when they are adsorbed in soil or sediments. Similarly, as shown in Table 1, TCE also has a higher Henry's law constant (ability to volatilize) resulting from its high vapor pressure and low solubility in water (Marrin and Kerfoot, 1988). As such, some TCE in water phase may volatilize into head space or inter-particular-space that are available in unsaturated soil above the water table, thus becoming less bioavailable for bacterial degradation. The same scenario can be expected in case of unsaturated zone or surface soil polluted by TCE. Although volatilization of TCE is not a predominant process in groundwater, it certainly is in the case of surface waters, as documented by Pant et al. (2007), Rathbun (2000), and Stocking and Kavanaugh (2000), who have reported an increase in TCE volatilization from surface waters with the increase of velocity of water and wind.

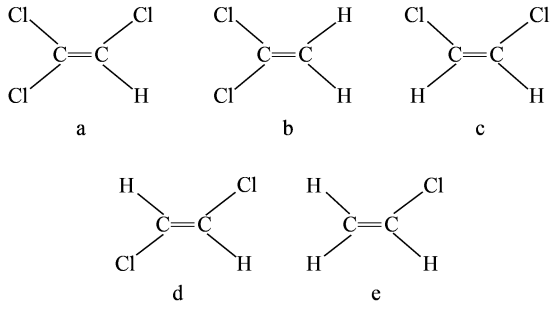


**Fig. 1** Molecular structures. (a) TCE with three chlorine atoms and one hydrogen atom bonded to carbon atoms; (b) 1,1-DCE; (c) *cis*-1,2-DCE; (d) *trans*-1,2-DCE; (e) vinyl chloride.

**Table 1** Chemical and physical properties of TCE

| Description | Symbol | Common value |
|---|---|---|
| Molecular weight (g/mol)[a] | MW | 131.39 |
| Liquid density at 25°C (g/mL)[a] | | 1.46 |
| Water solubility (mg/L)[c] | S | 1000 |
| Melting point (°C)[a] | $T_m$ | –86.5 |
| Boiling point (°C)[a] | $T_b$ | 87.3 |
| Vapor pressure (Pa)[b] | VP | 9700 |
| Octanol-water partition coefficient[c] | $K_{ow}$ | 320 |
| Sorption coefficient (organic carbon)[c] | $K_{oc}$ | 2.42 |
| Henry's law constant ((Pa·m$^3$)/mol)[b] | $H$ | 890 |
| Diffusion coefficient in pure air ($m^2$/day)[b] | $D_{air}$ | 0.68 |
| Diffusion coefficient in pure water ($m^2$/day)[b] | $D_{water}$ | $9.00\times10^{-5}$ |

[a] Jacoby et al., 1998 ; [b] Chiao et al., 1994; [c] Russell et al., 1992.

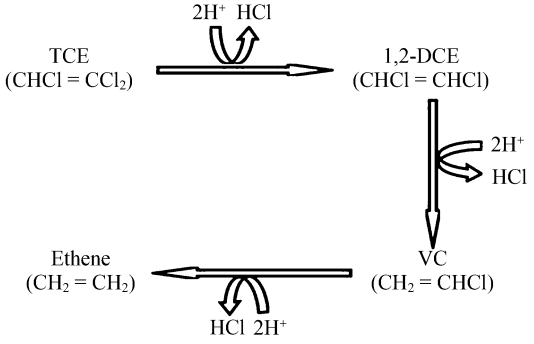


**Fig. 2** Reductive dechlorination mechanisms (modified from DeBruin et al., 1992; Freedman and Gossett, 1989; Maymo-Gatell et al., 1999; Mohn and Tiedje, 1992).

## 2 TCE biodegradation processes and mechanisms

Over the past two decades, several laboratory and field studies have shown that subsurface microorganisms can degrade a variety of hydrocarbons including chlorinated solvents. The works of Bouwer et al. (1981), Harker and Kim (1990), Hartmans and de Bont (1992), and Wilson and Wilson (1985), for example, have indicated the importance of microorganisms in the attenuation process of organic contaminants. In line with these findings, the biodegradation potential of TCE and its intermediate products have been thoroughly investigated. Studies have revealed that three types of metabolic processes are generally involved in the biological degradation of chlorinated ethenes. McCarty (1994) has provided an overview of the reductive dechlorination process for chlorinated solvents. This is an anaerobic process in which chlorinated ethenes are used as electron acceptors. Similarly, Alvarez-Cohen and McCarty (1991) and Hanson and Brusseau (1994) have reported another biodegradation method known as co-metabolism. In this process chlorinated ethenes are anaerobically degraded as a result of fortuitous biochemical interactions, which yield no benefit to bacteria. The third method is direct oxidation, an aerobic or anaerobic process in which sparsely chlorinated ethenes are used as electron donors (Bradley and Chapelle, 1996; McCarty and Semprini, 1994). Any of these processes could be occurring at a given site depending on the form of chlorinated ethenes and the site environmental characteristics. There is a decrease in the rate of reductive dechlorination process as the number of chlorine atoms in the chlorinated molecules decreases (Bouwer 1994; Mohn and Tiedje, 1992; Vogel and McCarty, 1985). This might be the reason that several studies have indicated the accumulation of dichloroethylene and vinyl chloride at various TCE contaminated sites that has anaerobic condition (Freedman and Gossett, 1989; Griffin et al., 2004; Mohn and Tiedje, 1992; Murray and Richardson, 1993). However, aerobic environment favors oxidative biodegradation of less chlorinated ethenes. Aerobic biodegradation goes more rapid than reductive dechlorination.

### 2.1 Reductive dechlorination of TCE

DeBruin et al. (1992), Freedman and Gossett (1989), and Maymo-Gatell et al. (1999) have pointed out that TCE undergoes complete sequential reductive dechlorination under anaerobic conditions, yielding ethene or ethane as final products. According to Mohn and Tiedje (1992), chlorine atom is replaced by a hydrogen atom at each reaction step during reductive dechlorination, producing hydrochloric acid (HCl) as byproducts (Fig. 2). The various intermediates have been reported as *cis*-1,2-dichloroethene (cis-DCE), *trans*-1,2-dichloroethene (*trans*-DCE), 1,1-dichloroethene (1,1-DCE) and vinyl chloride. According to Bouwer (1994), *cis*-DCE is a more prevalent intermediate than *trans*-DCE, and 1,1-DCE is the least prevalent intermediate during reductive dechlorination.

Although the reductive dechlorination option is available, environmental conditions become important for the initiation and termination of this process. McCarty (1997) has reported that the major environmental requirement in aquifers of concern is the presence of sufficient concentrations of other organics, which can act as electron donors for energy metabolism. Also, sufficient amount of organic co-contaminants, other than TCE or its intermediate products, is required to reduce other electron acceptors. Further, the presence of other electron acceptors can negatively impact TCE dechlorination. Byl and Williams (2000) have provided a list of such electron acceptors, which could interfere in or delay the process of dechlorination. Those electron acceptors that interfere in TCE dechlorination are oxygen, nitrate/insoluble manganese, insoluble ferric iron, sulfate and carbon dioxide, respectively, in the order of bacterial preference. Noell (2009) also reported the presence of competing electron acceptors such as dissolved oxygen, nitrate, ferrous iron, and sulfate, during TCE bioremediation pilot test conducted in contaminated groundwater at the Naval Weapons Industrial Reserve Plant in Dallas, Texas.

### 2.2 Co-metabolism of TCE

The possibility of aerobic co-metabolism of TCE became clear from the work of Wilson and Wilson (1985). They reported that aerobic biodegradation of TCE is carried out by methanotrophic bacteria in soil pre-exposed to methane. Later, the study by Little et al. (1988) and Alvarez-Cohen and McCarty (1991) made it clear that it is the methane monooxygenase (MMO) produced by methanotrophs that was actually responsible for the aerobic degradation of TCE. Little et al. (1988) also reported that 3.4% to 4.0% of the final products were cell bound materials, 40.1% to 42.7% was carbon dioxide, and the remaining 53.5% to 56.2% was water-soluble products. Similar findings were made available by Henry and Grbic-Galic (1994), who proposed a mechanism of TCE degradation by methanotrophs. According to these researchers, MMO converts TCE into a TCE epoxide, which breaks down into carbon monoxide, formate, glyoxylate, and chlorinated acids in aqueous environment. These compounds are subsequently metabolized by methanotophs and heterotrophs either collectively or individually, forming the final products (i.e., carbon dioxide and cell mass). It is not only TCE, but also its intermediates (DCE and VC) produced from reductive dechlorination that may be co-metabolized.

Besides methane-oxidizing bacteria, other bacteria, such as toluene oxidizers and phenol oxidizers, have also received tremendous attention. These bacteria produce oxygenases in response to phenol or toluene, which initiate the oxidative degradation and mineralization of TCE. Fan and Scow (1993) in their study with TCE biodegradation by indigenous soil microbial populations reported that 60%–75% of TCE was degraded when both TCE and toluene were present at 1 μg/mL and 20 μg/mL, respectively. The ratio of TCE and co-metabolites, such as toluene or phenol, has been found to control co-metabolism process by influencing microbial growth. Mu and Scow (1994) reported an increase in population of TCE/toluene degraders

by more than 4 orders of magnitude when 20 μg of toluene and 1 μg of TCE were added to 1 mL of soil slurry. However, a retarding effect on the microbial population size and TCE degradation was reported for increasing concentrations of TCE (> 1 μg/mL). Also, under stagnant conditions, Ely et al. (1997) have reported a substrate competition (e.g., TCE vs. DCE) for microbial utilization, together with substrate toxicity leading to inhibition and inactivation of enzymes that are responsible for degrading TCE or other chlorinated VOCs. Competition for the active site on the oxygenases among natural substrates (for example, $CH_4$, $NH_3$ or toluene) and chlorinated solvents has been documented by Semprini et al. (1991).

Other environmental factors, such as temperature and soil moisture, also influence the co-metabolism activities. Fan and Scow (1993) reported an increase in lag period in microbial activities with decreasing temperature, although the initial rates of degradation were similar for toluene and TCE. They also reported the role of moisture contents in the soil in TCE degradation. The degradation was found to be below detection when moisture content was 2.5%–5%, while the degradation was observed at 16%–30% soil moisture contents. Additionally, contact time contact between the microorganisms and co-solvent has been found to play an important role in expression of oxygenase. Han et al. (2007) studied TCE co-metabolism in a slurry microcosm by supplementing toluene as a co-solvent. They reported a decreased degradation constant (0.5 $day^{-1}$) for TCE with only 46% biodegradation efficiency, which was very low as compared to over 90% efficiency observed during an *in-situ* pilot study. According to these researchers, toluene in the microcosm was detectable for only a day, while in case of *in-situ* experiments toluene was detected for 3 days.

### 2.3 Direct oxidation of TCE

With regard to direct oxidation of TCE, studies have indicated that the less chlorinated reductive dechlorination products can serve as electron donors (primary substrates) for some respiratory bacteria. McCarty and Semprini (1994) have reported that VC can serve as a primary substrate for some microorganisms under aerobic conditions. During the direct oxidation process, microorganisms obtain energy and organic carbon from the chlorinated ethenes that undergo oxidative biodegradation. In another study with VC conducted in iron-reducing aquifer sediments, Bradley and Chapelle (1996, 1997) noted a complete mineralization of VC in the presence of Fe(III), which was attributed to the direct oxidation of chlorinated ethenes. Apart from environmental factors, the types of substrates have also been found to play an important role in direct oxidations of chlorinated ethenes. Olaniran et al. (2008) found that the bacterial growth pattern was greatly influenced by the substrate types. The peak bacterial cell densities ranged from $5.1 \times 10^6$ to $2.2 \times 10^7$ colony forming units (CFUs)/mL in the presence of *cis*-DCE, while $8.9 \times 10^6$ to $2.6 \times 10^7$ CFUs/mL in the presence of *trans*-DCE. According to the same authors, the bacterial isolates were able to degrade 69.24%–75.00% of *cis*-DCE but only 68.88%–71.83% of *trans*-DCE within seven days of incubation. When both DCE isomers were presented in a mixture, *trans*-DCE showed a greater degradation (45.74%–63.63%) than *cis*-DCE (32.93%–46.45%) over the same incubation period, indicating a microbial preference towards *trans*-DCE during direct oxidation processes. The authors reported that *trans*-DCE is more readily utilized as sole carbon source than *cis*-DCE, an observation also reported by other researchers (Federle et al., 1990; Hopkins et al., 1993). *cis*-DCE is more toxic than *trans*-DCE (Olaniran et al., 2008). It remains to be determined if the difference in oxidative degradation and supporting bacterial growth between *cis*-DCE and *trans*-DCE is attributable to their toxicity difference.

## 3 Microbiology of TCE degradation

The reductive dechlorination also depends on the microbial populations. The microorganisms that are present in TCE contaminated sites can differ significantly in their ability and potential to dechlorinate TCE. It has been found that not all TCE dechlorinating organisms can degrade TCE completely. Maymo-Gatell et al. (1997) reported *Dehalococcoides ethenogenes* strain 195 that is capable of reducing TCE to ethane. Bacteria related to *D. ethenogenes* were found in sites that showed complete TCE dechlorination (Fennel et al., 2001; Hendrickson et al., 2002). However, other bacteria only partially dechlroinate TCE, producing intermediate product, such as *cis*-DCE. For example, *Dehalospirillum multivorans* (Neuman et al., 1994), *Enterobacter agglomerans* (Sharma and McCarty, 1996) and *Desulfitobacterium* sp. strain PCE1 (Gerritse et al., 1996) only dechlorinate tetrachloroethylene (PCE) and TCE to *cis*-DCE.

There are several aerobic bacteria that can co-metabolize TCE using their oxygenases, thus giving rise to complete mineralization of TCE. Lau et al. (1994) and Wackett and Gibson (1988) reported that toluene-oxidizing bacteria (e.g., *Pseudomonas putida* F1) can produce toluene a dioxygenase that mediates co-metabolization of TCE. Radway et al. (1998) and Takami et al. (2000) have also reported co-metabolization of TCE by toluene-oxidizing bacteria and recombinant cells. In an earlier study, Winter et al. (1989) reported the production of a toluene para-monooxygenase by *Pseudomonas mendocina* KR1 when vapor-phase toluene is supplied as a sole carbon source. According to this study, *P. mendocina* KR1 can degrade TCE completely with monooxygenase initiating the oxidative process. Sun and Wood (1996) carried out an extensive study to examine the TCE degrading capability of *Pseudomonas cepacia* G4, *Pseudomonas cepacia* G4PR1, *Pseudmonas mendocina* KR1, *Pseudomonas putida* F1, and *Methylosinus trichosporium* OB3b. Their results indicated that, although differing in degradation rates, all the microorganisms were capable of degrading TCE. Just as in the case with *Methylosinus trichosporium* OB3b mentioned above, which is a methanotroph oxidizing methane, Chang and Alvarez-Cohen (1997) noted that a mixture of methanotrophic bacteria in a methanotrophic

bioreactor which was seeded with landfill soil could also co-metabolize TCE.

As discussed earlier, along with reductive dechlorination and cometabolism of TCE, direct oxidation of TCE has also been noted, but yielded complete mineralization of TCE. However, this process requires microorganisms that are capable of utilizing chlorinated ethenes as their sole source of carbon. Kitayama (1997) has reported *Pseudomonas aeruginosa* JI104 as a potential microbe that can use TCE as a sole carbon source and degrade it aerobically. On the other hand, Olaniran et al. (2008) reported seven bacterial isolates that were able to degrade *cis*- and *trans*-DCE directly by using them as their energy source. According to these authors, two of the isolates were closely related to strains of *Acinetobacter* species. Other two isolates belonged to *Bacillus* species, mainly *Bacillus subtilis* and *Bacillus cereus*. Other three isolates each were similar to the strains of *Achromobacter xylosoxidans*, *Klebsiella* and *Pseudomonas aeruginosa*, respectively. These bacterial strains had been isolated from the contaminated sites in Nigeria and South Africa.

## 4 Microbial bioaugmentation for TCE remediation

Many contaminated sites undergoing natural remediation under the influence of indigenous microorganisms have been found to accumulate *cis*-DCE and VC, which can be attributed to the lack of microbial community that is capable of complete dechlorination of TCE either through reductive or oxidative pathways. In this regard, bioaugmentation technique, which involves the introduction of specific microorganisms in the contaminated aquifer to enhance the biodegradation of chlorinated ethenes, has been found very successful (Ellis et al., 2000; Major et al., 2002; Lendvay et al., 2003). Bioaugmentation can be used to treat a range of TCE concentrations in groundwater, including source areas where high concentration of TCE is often seen as advantage in reducing the competitive role of other electron donors (DiStefano et al., 1991; Yang and McCarty, 2000). Over the years many bacterial strains have been isolated and studied for their efficacy in biodegradation of TCE, most of them have been found capable of dechlorinating only specific molecules (TCE, DCE or VC), while only one bacterial strain (*Dehalococcoides* sp.) has been reported to dechlorinate TCE to ethene/ethane completely (Cupples et al., 2003; Sung et al., 2006). Accordingly, bioaugmentation for TCE remediation may utilize the combination of different bacterial strains or just a population of *Dehalococcoides* species. However, most of the bioaugmentation efforts have relied on mixed cultures of bacteria including *Dehalococcoides* sp.

Fathepure et al. (2005) studied *Mycobacterium* sp. for its potential role in bio-augmenting TCE biodegradation in aquifer materials. They isolated *Mycobacterium* sp. strain TRW-2 from a chloroethene-degrading enrichment culture. This strain was found to utilize VC or ethene as the sole carbon source. According to the authors, bioaugmentation of *Mycobacterium* sp. yielded a higher rate of VC degradation in the mesocosm filled with aquifer materials, in comparison to the native microbial community. Also, the maximum degradation of VC was achieved between 30 and 37°C. Schaefer et al. (2009) in a separate study evaluated *Dehalococcoides* sp. for its application during the bioaugmentation for chlorinated ethenes. They performed batch and column experiments using groundwater and soil that were obtained from the TCE contaminated area at US Air Force Facility in Ft. Worth, Texas. The transport and the growth of *Dehalococcoides* bacteria in saturated porous media, together with dechlorination efficiency were measured. The study showed higher concentrations of *Dehalococcoides* bacteria in the aqueous phase compared to that of the soil phase, which suggested a greater degree of bacterial mobility in porous media. This was confirmed with the finding that the dechlorination did occur throughout the length of the column in this study. Additionally, it was observed that the aqueous phase concentrations of *Dehalococcoides* bacteria increased uniformly across the column with respect to the time after the injection but not in relation to the distance from the column influent. It was also observed that the batch experiments indeed underestimated the outcomes from the column studies, thus the authors expected a higher rate of dechlorination in field conditions than that suggested through the laboratory batch experiments. In this study, the dechlorination activity was reported almost 200-times greater for the columns than for the batch experiments.

Hood et al. (2008), in their laboratory study and a pilot test at Launch Complex 34, Cape Canaveral Air Force Center, observed a significant increase in the rate of dechlorination after bioaugmentation with a dechlorinating culture (KB-1; SiREM, Guelph, Ontario, Canada). KB-1 is a natural microbial culture containing *Dehalococcoides* organisms that was proven to be highly efficient in dechlorination of TCE and other chlorinated ethenes. The authors utilized the bioaugmentation technique in conjunction with the amendments (methanol, ethanol, acetate, and lactate) in microcosm that contained indigenous microorganisms. They noted a half life ($t_{1/2}$) as short as 1 day for TCE biodegradation, which was significantly shorter than that in those microcosms with indigenous microorganisms that were either amended ($t_{1/2}$ = 17 day) or unamended ($t_{1/2}$ = 92 day) without bioaugmentation. Similar trend was observed during a pilot study, in which TCE concentration declined almost to the limit of detection (20 μg/L) after bioaugmentation. However, the accumulated VC was ultimately dechlorinated to ethene only after a 5-months lag period. It was observed that the ethene concentrations in groundwater increased significantly to an average value of 67 mg/L at the end of the demonstration (249 day for bioaugmentation). Kane et al. (2005) also reported a successful use of KB-1 culture in TCE bioremediation. They utilized KB-1 culture that included *Dehalococcoides ethenogenes* for bioaugmentation in the groundwater areas near the TCE source zone that had high TCE concentration (approx. 700,000 μg/L). The contaminated aquifer was located in the Caldwell Trucking Superfund Site in Essex County, NJ. Also, Duhamel et al. (2002, 2004) in

their studies with microcosms have reported a complete dechlorination of chlorinated ethenes when bioaugmented with KB-1 culture.

## 5 Sequential anaerobic-aerobic processes for TCE remediation

As discussed earlier, when the number of chlorine atoms in the chlorinated molecules decreases, the reductive dechlorination process slows down; and aerobic environment favors the oxidative biodegradation of the resulting less chlorinated ethenes. Therefore, the oxidation rates for 1,2-DCE and VC are relatively faster compared to that of TCE (Pfaender, 1990). Researchers have utilized these concepts in developing sequential anaerobic-aerobic processes in order to biodegrade TCE more efficiently. Lorah et al. (1997) reported both anaerobic and aerobic biodegradation of TCE and other chlorinated ethenes occurring in a fresh water tidal wetland in Maryland. In that study, the contaminated groundwater discharged into the wetland passed through the lower sediment layer first, which had iron-reducing conditions, followed by an upper layer of peat, which had methanogenic conditions. They noted a tremendous decrease in the concentration of TCE in the groundwater after being transported upward through these two layers with different redox potentials. The original groundwater TCE concentration of 100–2000 μg/L declined to a very low or undetectable concentration in the recipient wetland water, while 1,2-DCE and VC were detected in the same recipient water. According to these researchers, 1,2-DCE and VC concentrations became undetectable near the surface in the wetland, which was attributed to the aerobic biodegradation of these low molecular chloroethenes. The microcosm experiments conducted using the wetland sediments and water further confirmed the aerobic biodegradation process in near surface regions of the wetland that showed methanotrophic activities. In a similar study, Bankston et al. (2002) also investigated the possibility of anaerobic/aerobic biodegradation of TCE in wetland environment. Based on their findings from the wetland microcosm in which the mineralization of TCE occurred in concurrence with the oxidation of methane, they suggested a role of methanotrophs in TCE mineralization. Further, they revealed the importance of wetland vegetation in aerating the root zone.

In a separate study, Noell (2009) has reported anaerobic biodegradation of chlorinated ethenes in the upgradient portion of the aquifer followed by aerobic biodegradation in the downgradient segment of the aquifer. These observations were made during a pilot test that was conducted in a section of a larger TCE plume located in the groundwater at the Naval Weapons Industrial Reserve Plant in Dallas, Texas. The pilot test constituted biostimulation of indigenous microbes with amendments (sodium acetate and ammonium phosphate) for 163 days to anaerobically biodegrade chlorinated ethenes in the upgradient areas of the aquifer, followed by air-sparging of the downgradient aquifer for another 69 days in order to stimulate aerobic biodegradation of the remaining chlorinated compounds. Before the onset of biostimulation process, the groundwater was anaerobic in nature and had iron reducing conditions, which transformed into sulfate reducing conditions after biostimulation. It was observed that the biostimulation enhanced the reductive dechlorination of TCE, cDCE, and VC, and there was an accumulations of ethane, which implied that there were a complete sequence of anaerobic biodechlorination of TCE occurring in the groundwater that was biostimulated. However, owing to the fact that the rate of degradation of TCE is faster than its daughter products under anaerobic environment, accumulations of cDCE and VC were observed. Thus, when biosparging was conducted to create nitrate-reducing conditions in the groundwater downgradient from the biostimulation site, there was an enhancement of biodegradation of cDCE and VC, however TCE degradation was not observed. It has also been reported that the rate of biodegradation for cDCE and VC during aerobic conditions were greater than that under anaerobic conditions. Similarly, it was also noticed that the biodegradation of VC was much faster than that of cDCE under aerobic conditions. It was noted that there was an increase in the concentrations of TCE during the aerobic conditions, which was attributed to the processes like desorption and leaching from soils/sediments that were less permeable earlier. In another study with groundwater, Devlin et al. (2004) reported different kinds of *in-situ* anaerobic-aerobic sequential biodegradation of TCE. In this study, instead of water flowing through an anaerobic and aerobic environment, a section of aquifer (Borden aquifer, Canada) was isolated and maintained anaerobically with the supply of nutrient solution until the daughter products of anaerobic dechlorination of TCE were observed; later on the same groundwater was biosparged with oxygen to stimulate aerobic biodegradation. Both the processes were found to enhance microbial biodegradation of chlorinated ethenes and their daughter products.

Reports are also available on anaerobic-aerobic sequential TCE degradation in engineered systems like reactors. Tartakovsky et al. (2003) studied TCE degradation under sequential anaerobic-aerobic conditions using a biofilm reactor that was fed with ethanol and was oxygenated using hydrogen peroxide. In order to seed the reactor, microbial consortium from an anaerobic digester was used as the inoculums. During operational phase of the reactor, two major groups of microorganisms, methanogenic and methanotrophic, were observed. These microorganisms were able to degrade TCE almost completely into ethenes/ethanes. In a similar study with reactors, Guiot et al. (2008) used a single-stage laboratory scale reactor by coupling anaerobic/aerobic conditions that favored both reductive dechlorination and oxidative mineralization of chlorinated ethenes such as PCE, TCE and other daughter products. A unique feature of this reactor was the use of electrolysis-generated hydrogen as an electron donor for reductive dechlorination as well as for the formation of methane. The authors have reported over 98% reductive dechlorination of PCE to DCE, with TCE as intermediate product. Similarly, a maximum oxidative DCE mineraliza-

tion to $CO_2$ was reported to be 89%. The values reported above were based on the experimental study utilizing 6 days of hydraulic retention time and loading rate of 4.3 μmol PCE/L of reactor per day.

## 6 Case studies

### 6.1 Bitterfeld, Germany

Imfeld et al. (2008) studied a contaminated site located in the city of Bitterfeld in eastern Germany. This site covering an area of approximately 2 $km^2$ was originally contaminated with PCE and TCE. However, *cis*-DCE and VC were also found later in the same site and were believed to be products of biodegradation of PCE and TCE. The authors used carbon stable isotope technique and biomarkers to confirm the occurrence of biodegradation and the presence of microbial consortia likely involved in the process of chloro-ethene biodegradation. The study revealed the occurrence of biodegradation and also the presence of *Dehalococcoides* species that is capable of degrading TCE to ethene. According to the authors, the presence of *cis*-DCE in the aquifer was suggestive of the premise that a reductive dechlorination process was occurring at the site. Also, the isotopic data and the results from microbial profiling using taxon-specific molecular analyses supported that notion. The chlorinated ethene concentrations were reported to influence the bacterial community composition. The authors also observed a positive relationship between the presence of *Dehalococcoides*-like bacteria and the ethane concentration in some of the wells studied.

### 6.2 Connecticut, USA

Chapman et al. (2007) investigated a TCE plume, which originates from a DNAPL source zone in a shallow sand aquifer and its discharges into a river, at a former industrial facility in Connecticut. Earlier, this site was used for manufacturing metal related articles. TCE was used as a primary solvent for degreasing and was stored in underground tanks that probably leaked forming a DNAPL into the bottom of the aquifer and contaminating even the underlained aquitard. The authors found minimal degradation of TCE within the distance of 280 m from the source, as indicated by the low concentration of *cis*-DCE and the undetectable VC. However, *cis*-DCE was observed further downstream, with a maximum *cis*-DCE concentration reaching 500 μg/L. It was also reported that in most of the samples the *cis*-DCE concentration exceeded that of TCE. The presence of *cis*-DCE at high concentrations in groundwater, along with carbon isotopic results and hydrogeochemical information available for the site have all confirmed the existence of natural attenuation process at the site. The authors also maintained that dilution and volatilization might also accounted greatly for the TCE reduction observed in the groundwater.

### 6.3 Duluth, USA

Semer and Banerjee (2001) conducted a study on anaerobic bioremediation of TCE in groundwater at a former landfill site near the Duluth International Airport. This area was earlier used by the US Air Force Air Combat Command (ACC) for disposing of different types of waste materials that included aircraft parts, empty drums, and drums containing chemicals. As the groundwater recharge in the site was primarily through infiltration from the surface, this could have easily aided in contamination of the groundwater over the years. The bioremediation study focused on enhancing the dechlorination of TCE, DCE, and VC by injecting polylactate ester (HRC®) that released lactic acid. The authors reported a significant reduction (> 99.5%) in TCE concentration over a period of nine months. The decrease in TCE concentration accompanied an increase in anaerobic biodegradation products, with concentrations of cis-1,2-DCE and VC increasing from 50 to 750 μg/L and 10 to 20 μg/L, respectively. Over the same time period, TCE decreased from 400 μg/L to less than 2 μg/L, thus corroborating the fact that the degradation products present in groundwater were derived from reductive dechlorination of TCE.

### 6.4 Savannah River Site, South Carolina, USA

The Savannah River Site (SRS) that belongs to the US Department of Energy is located in South Carolina. This is one of the Superfund Sites and is included in the National Priority List. Brigmon et al. (1999) conducted a TCE bioremediation study on a Sanitary Landfill (SLF) in the SRS area. This site had been used to dispose of materials contaminated with TCE and PCE, including sanitary waste, construction material, and disposable batteries. Due to leaching process, TCE migrated into groundwater beneath the SLF. A project was initiated for the biodegradation of TCE by injecting gaseous nutrients to stimulate microbial populations that would degrade TCE and the products of TCE. The nutrient injection mix contained methane (4%), nitrous oxide (0.07%) and phosphate (Triethyl phosphate, 0.007 to 0.01%) and was blended with air. Helium was also added to the nutrient mix to a final concentration of 0.1% as a tracer. The results revealed an increase in methanotrophic bacterial population in groundwater, which was attributed to selective biostimulation during the nutrient injection test. Concurrent with the increasing methanotrophic counts, concentrations of chlorinated hydrocarbons decreased. The authors maintained that the processes involved in TCE and *cis*-DCE biodegradation could be of cometabolic nature, but metabolic evidence was not available from their study.

## 7 Concluding remarks

Studies have shown various microbial machineries that are capable of degrading TCE and its intermediate forms. Given the fact that there are hundreds of sites contaminated

with TCE and other chlorinated ethenes in the United States alone, microbial remediation techniques serve as a great promise in dealing with these contaminants. These techniques are relatively inexpensive compared to other engineering solutions, and they are also more efficient as complete mineralization of TCE can be achieved with the proper knowledge of biodegradation mechanism and its requirements. It was also noted during the review that both reductive dechlorination and cometabolism require either a carbon source or a co-solvent. Whereas, direct oxidation of TCE has a potential to directly utilize TCE (or DCE and VC). However, more research in this area is deemed necessary, especially field-scale studies. Additionally, aerobic TCE biodegradation is much faster than anaerobic counterpart, and the TCE degradation products are environmentally safe, thus aerobic biodegradation may be of more value in situations where rapid remediation of TCE is required. The rate enhancing technique for biodegradation, such as bioaugmentation, can be very useful in remediating groundwater areas that lack natural capacity for the remediation. Based on the discussions presented in this paper, the rate of TCE degradation into DCE and VC is favored by anaerobic biodegradation, however there is a progressive reduction of reaction rates for DCE to VC, and VC to ethane under anaerobic environment. Reports also suggested that DCE and VC are more rapidly degraded under aerobic conditions, provided appropriate microorganisms are made available. Thus, sequential anaerobic-aerobic biodegradation of TCE and its daughter products have also received recent attention.

In spite of the fact that microbial remediation of TCE and its anaerobic daughter products have a very high potential, most of the reported works are laboratory based in comparison to *in-situ* applications of this method. In order to facilitate wider applications of *in-situ* microbial remediation of chlorinated ethenes, it will be highly beneficial to have a collaboration among microbiologists, chemists, geologists, and engineers, so that the issues pertaining to fundamentals of the above mentioned fields in concern to the microbial remediation efforts can be addressed collectively. While field scale studies should make the full use of experimental studies that are conducted under the laboratory conditions, it is also essential that more laboratory studies may be conducted under simulated field environment, so that it is easier to translate laboratory scale studies into field-scale studies. As stated earlier, the direct oxidation of chlorinated ethenes looks fairly inexpensive and effective, as it neither requires co-solvent nor does it produce any toxic by-product. Hence, further research in this area is required to harness this technology which may also involve bacterial genomic research.

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