Microbiology of chloroethene degradation in groundwater

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Industrial development, population growth and urbanisation have all contributed to an increase in the release of chemical pollutants into the environment. Consequently, many natural resources show some degree of anthropogenic impact, including the widespread contamination of groundwater aquifers by hazardous wastes[1]. This is particularly significant because groundwater represents about 98% of the available freshwater on the planet. The fact that we are already using approximately 50% of readily available freshwater makes groundwater protection and clean-up of paramount importance. Increasing incidences of aquifer contamination by chloroethene solvents is of current concern throughout Australia. Further, due to the adverse effects of chloroethene contaminants to environmental and human well-being, it is of upmost importance to understand the potential for the natural microbial population within the groundwater to degrade the chloroethene to innocuous byproducts.

Introduction to chlorinated hydrocarbons

Chlorinated ethenes or chloroethene are common groundwater pollutants; in 1995 trichloroethene (TCE) was found to be the second most common hazardous waste in the US[2]. TCE does not occur in the environment naturally, it is an anthropogenic chemical[3]. It was widely used as a metal degreaser, in paints, dry cleaning, paint strippers and carpet shampoos. Improper handling and disposal of these compounds has led to severe soil and groundwater contamination[4]. TCE is a clear liquid, denser than water, which allows it to sink forming a dense-non-aqueous-phase-liquid (DNAPL). Since the partition coefficient (Log Kow) is 2.61, TCE is hydrophobic and so is only slightly soluble in water, aiding the formation of the DNAPL.

TCE has been found to be a carcinogen in model animals such as rats and mice, causing kidney, lung and liver cancers[5]. It has been found to strongly correlate with a higher incidence of renal cancer in humans when exposed to TCE as metal degreasers[6]. People exposed to TCE due to their occupation were also found to experience an increase in the prevalence of non-Hodgkin's lymphoma[6].

Environmental fate and prevalence of chlorinated solvents

TCEs degrade slowly with half-lives in the natural environment of 80–800 days[7], and due to the formation of DNAPLs at the bottom of aquifers, TCE exhibits sustained release over time into groundwater[8]. TCE was found in breast milk in Arizona, USA at concentrations of 6 µg/L, which is above the EPA maximum contaminant level (MCL) of 5 µg/L. The MCL for cis-DCE and VC are 70 µg/L and 1.2 µg/L respectively. TCE had also been found in foods such as decaffeinated coffee and eggs, demonstrating the scope of products that can lead to TCE ingestion[9].

Chloroethene contaminant detoxification: the microbiology

The discovery of microorganisms in the mid-1990s that gain energy from the process called reductive dechlorination of chloroethene

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led to a turning point from a predominantly co-metabolic view of chloroethene biodegradation to the concept of chloroethenes serving as primary substrates for microbial metabolism[10]. Most of the chlorinated compounds have a synthetic origin and have not been in contact with microorganisms through evolutionary periods of time[11]. As a result, chlorinated solvents are not frequently metabolised by indigenous organisms. Nevertheless, several biotransformation mechanisms have been identified that could be exploited for degradation.

The main biotransformation pathways for chlorinated ethenes are explained below[12]:

(1) Aerobic oxidation: the pollutant serves as the primary substrate for growth. Oxygen serves as the electron acceptor. Aerobic metabolism is limited to the less chlorinated compounds such as chloromethane, dichloromethane, chloroethane, 1, 2-DCA and VC.
(2) Aerobic co-metabolism: in addition to oxygen, an electron donor must also be present. In general, the fewer the Cl atoms, the better the co-metabolic process will work. Toluene, methane, propane, butane and phenol have all been used as primary substrates to support such co-metabolic transformation.
(3) Anaerobic oxidation: in this mechanism, the chlorinated organic serves as an electron donor for growth. Only a few chlorinated aliphatics are amenable to this treatment (i.e. dichloromethane; 1, 2-dichloroethene; cis- and trans-DCE and VC). Nitrate and sulphate can serve as electron acceptors in such cases and dichloromethane can also be fermented. Nevertheless, degradation rates are relatively slow and this process has not yet been demonstrated or exploited for site remediation.
(4) Anaerobic reductive dechlorination: in this process, the compound serves as an electron acceptor. All chlorinated aliphatics are susceptible to anaerobic, co-metabolic, reductive dechlorination. This requires a suitable electron donor and it works mainly under sulphate-reducing or methanogenic conditions. An exception is carbon tetrachloride, which can also be dechlorinated under denitrifying conditions.

Current research activity is focused on dehalorespiration, where PCE, TCE, DCE and VC serve as terminal electron acceptors in support of microorganism growth. There are two reductive dehalogenation mechanisms. The first is hydrogenolysis (hydrodehalogenation), which involves replacing halogen atoms such as Cl, Br and F by a hydrogen atom. This is illustrated in Figure 1 for the stepwise reduction of TCE via DCE to VC and ultimately to ethene. The other reductive dehalogenation mechanisms are dihaloelimination, which involves the simultaneous removal of two halogen atoms after two electrons are transferred. Reductive dechlorination generally decreases the toxicity and enhances the solubility (bioavailability) of the pollutant, but there are exceptions where the toxicity can be accentuated (e.g. TCE reduction to VC). Reductive dechlorination is often a co-metabolic reaction since the microorganisms that catalyse it cannot harvest the energy released by the redox process. Recently however, many bacterial strains have been found that can utilise PCE and TCE as a terminal electron acceptor during respiration using H_2, formate, acetate and pyruvate as electron donor. This process is known as halorespiration and it can be mediated by species such as *Desulfomonile tiedjei*, *Dehalobacter restrictus*, *Desulfitobacterium* and *Dehalococcoidese thenogenes*[11]. PCE and TCE readily undergo reductive dechlorination but the efficiency of the reaction decreases with decreasing degree of chlorination. Some dechlorinators sequentially dechlorinate PCE to TCE, some preferentially to cis-DCE and some to VC. However, the conversion of DCE and VC as electron acceptor to non-toxic ethene is principally mediated by *Dehalococcoides* species-affiliated bacteria. Conversely, the tendency for aerobic oxidation of chlorinated ethenes increases with decreasing number of chlorine atoms of the molecule. Both metabolic and cometabolic oxidation of lower chlorinated ethenes have been reported. However, mineralisation of DCE and VC tends to increase with higher reduction potential.

Dehalorespiring bacteria

All of the known dehalorespiring microorganisms are bacteria and their dehalogenation capacities are highly strain dependent[13]. Anaerobic bacteria that grow with chloroethenes as final electron acceptors include *Dehalobacter, Dehalococcoides, Desulfitobacterium, Desulfuromonas, Geaobacter* and *Sulfurospirillum*. The well-studied organisms, *Sulfurospirillum multivorans* and *Dehalobacter restrictus* PER-K23 dechlorinate PCE to *cis*-DCE[11]. *S. multivorans* is a Gram-negative anaerobic spirilum, which belongs to the ε- subdivision of Proteobacteria. The *Dehalobacter* genus belongs to *Firmicutes* and is allied with the genus *Desulfitobacterium.* However, dehalorespiration is the sole system of energy production in the genus *Dehalobacter.* Although these strains can utilise PCE or TCE as electron acceptors, they cannot completely dechlorinated cis-DCE or VC to ethene. One genus of particular interest for such bioremediation is '*Dehalococcoides*' *(Dhc)*, obligate anaerobes that cannot use oxygen, nitrate or sulphate as electron acceptors. They are Gram-positive, coccoid cells closely related to a member of the *Chloreflexi* phylum (green non-sulphur bacteria), which possess diverse dehalogenation ability, grow robustly in mixed cultures and are present globally in microbial populations[14]. *Dhc* species are of particular interest as members of the genus are the only known bacteria capable of complete reduction of chlorinated ethenes (PCE and TCE) to ethene (Figure 1). *Dehalococcoides ethenogenes* 195 and *Dhc*sp.FL2 respectively dechlorinated PCE and TCE to ethene[15]. However, these two strains are unable to use VC as an electron acceptor. Thus, the slow dechlorination of VC to ethene is considered to proceed in a co-metabolic fashion uncoupled to energy production[16]. In contrast, four other *Dhc* strains, BAV1, VS, GT and KB1/VC can use VC as the electron acceptor in their dehalorespiration and can dechlorinated VC to ethene efficiently[17]. In the genus *Dhc*, dehalorespiration is solely an energy preservation system. These isolates exhibit a metabolic specialisation, using only H_2 as an electron donor and chlorinated compounds as electron acceptors to support growth.

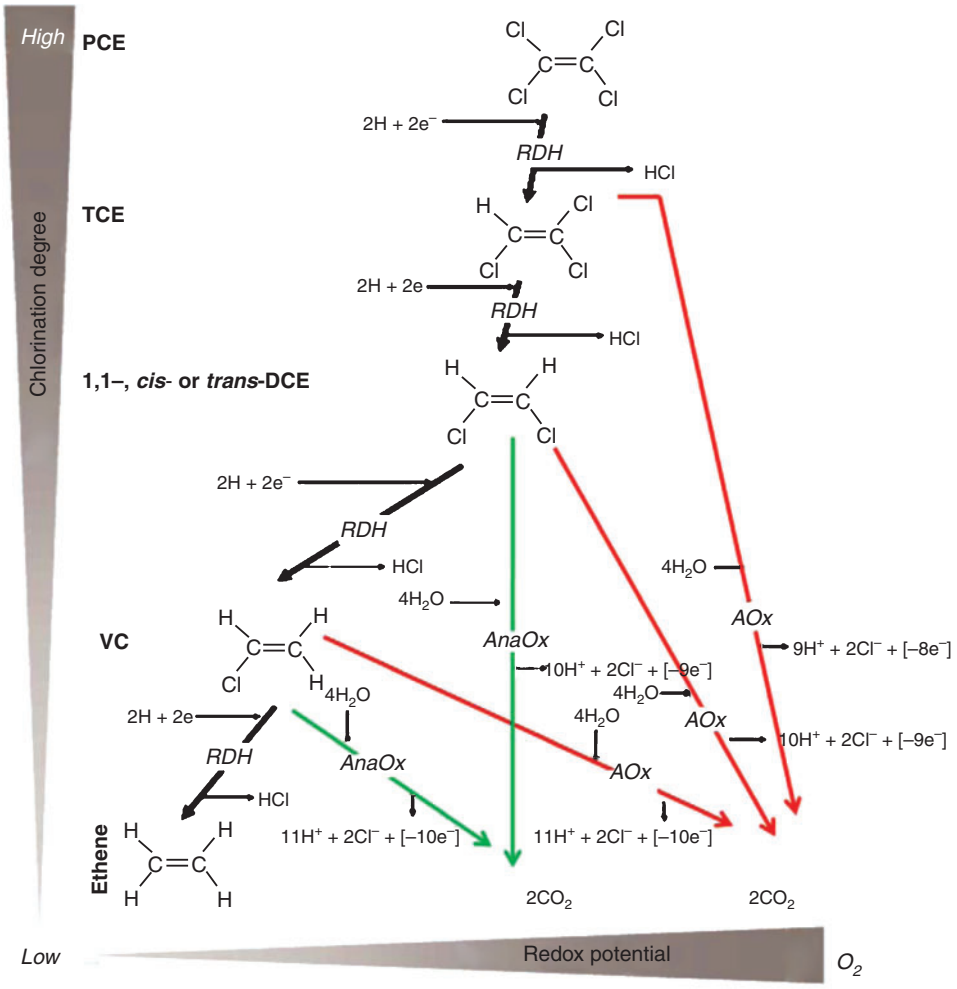

Figure 1. Pathways for the degradation of chlorinated ethene. Black, green and red arrows indicate different pathways such as reductive dechlorination (RDH); anaerobic oxidation (AnaOx) and aerobic oxidation (AOx) respectively[12].

Reductive dehalogenases (RDases)

Reductive dechlorination reactions are catalysed by the reductive dehalogenases (RDases). RDases are a class of enzymes found mostly in *Dhc* species and other dechlorinating organisms that catalyse the following reaction[13]:

$$R{-}Cl + 2[H] \rightarrow R{-}H^{+}H^{+} + Cl^{-}$$

Hydrogenases are a crucial part of the reaction mechanism because they supply electrons to the reaction from H_2. In anoxic environments, the above reaction is thermodynamically favourable and chlorinated compounds can act as electron acceptors. However, it has been observed previously that hydrogenases are oxygen sensitive, whereas RDases may retain some activity following exposure to oxygen[18]. 'Dehalorespiration' is defined as the process whereby energy from the above reaction is conserved and coupled to ATP synthesis in a chemo-osmotic mechanism[11]. Dechlorinating organisms obtain energy from the process and in many cases dechlorination activity can be linked to growth[19]. The hydrogenases spilt hydrogen into protons, driving the proton gradient that is utilised for ATP synthesis; and electrons (e^-) are carried through the electron transport chain to the dechlorination reaction, where the chlorinated substrate acts as a terminal electron acceptor. Reactions are proposed to take place with a coronoid co-factor and 2 Fe-S clusters.

Although many putative dehalogenases exist, few have been purified and characterised. Those relevant to TCE dechlorination are listed in Table 1 along with their distribution among *Dhc* isolates. A TCE dehalogenase, encoded by the *tceA* gene was first discovered in *Dehalococcoides ethenogenes* strain 195 and is thought to be co-transcribed with the *tceB* gene encoding a small membrane anchor. This gene has a wide distribution among a range of environmental samples and those that contain *tceA* can degrade TCE, although not all TCE-degrading organisms contain *tceA*. Two VC-RDases have been discovered, originating from two different isolates –*vcrA* from strain VS and *bvcA* from strain BAV1[20]. These are believed to be the distinguishing feature of *Dhc* from other dechlorinating organisms.

In conclusion, chloroethenes represent a serious threat to both human health and the environment. Microbial communities naturally present in contaminated groundwater have been found that are

Table 1. Distribution of characterised RDases involved in the dechlorination of TCE to ethene in Dhc isolates[19].

Dhc strains	Known expression of RDase genes	Reaction catalysed	Molecular mass (Da)
195[A]	*pceA*	PCE → TCE	50,800
	tceA	TCE → VC	57,700
VS	*vcrA*	DCEs, VC → ethene	53,100
BAV1[B]	*bvcA*	VC → ethene	52,800
FL2	*tceA*	TCE → VC	–
CBDB1	*pceA*	None, respires other chlorinated compounds	–
GT	–	TCE, cDCE, VC	–

[A]Dhc strain 195 co-metabolises VC at a slow rate.
[B]BAV1 only co-metabolises PCE and TCE in the presence of DCE and VC.

capable of the complete mineralisation of chloroethenes using a variety of mechanisms of which anaerobic reductive dechlorination represents the most ecologically significant process. Recent advances in molecular microbial ecology have led to greater understanding of the mechanisms underpinning the degradation process. This will lead to improvements in the management and remediation of contaminated groundwater.

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Biographies

Dr Sayali Patil received her PhD degree in Environmental Biotechnology from Flinders University in South Australia, Adelaide. Dr Patil has produced publications in the field of Environmental Microbiology and Biotechnology and given proffered presentations at several international conferences. Dr Patil has worked as a Project Manager at the South Australian Research and Development Institute (SARDI) for two years.

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The biography for **Professor Ball** is on page 182.