Attenuation of the vinyl chloride in the vadose zone

Study of VC degradation in the unsaturated zone at different scales

Investing in Opportunities
This project has received European Regional Development Funding through INTERREG IV B.
INTERREG IVB

Summary

The work carried out and presented here in this report concerns the attenuation of vinyl chloride in the vadose zone.

The first step of the study includes the gathering and processing of field data and information available in the literature and bibliography. Works consisting of soil gas monitoring have been also implemented in the same time, including measures of cis-1,2 DCE and vinyl chloride (VC) soil gas concentration. VC was detected in only one area in soil gas, near a suspected contamination source. The sinking behavior of the chlorine solvents and the presence of strong adsorbent (peat) could limit the extension of the plume of cis 1,2-DCE in the upper part of the aquifer.
The result of this step was the set up of a protocol for lab work with microcosms of soil and for complementary in situ surveys.

Due to the large extension of a concrete slab (with an active factory) which covers nearly all the area, only limited complementary investigations could be done. A soil gas survey focuses on one major source of contamination with canister. Volumetric soil moisture measurements with TDR give also hints concerning the role of the capillary fringe in the area of ten meters in the downgradient of the groundwater plume of TCD/cis-1,2-DCE. The capillary fringe in the upper part of the aquifer (clayed silty loam) limits soil air diffusion and allows a slow aerobic degradation in the vadose zone.

In the second step, lab work was done, to evaluate the kinetics of aerobic biodegradation in soil microcosms. These tests conducted with replicates and controls reveal an aerobic biotic slow to median degradation process, which need a lag period, probably for the adaptation of the bacteria. The biodegradation kinetics were compared with other published data. Similar velocities were found for the process of aerobic degradation in the vadose zone and in the saturated soils.

These results were obtained by INERIS with the collaboration of the CHYN and the funds of the Interreg IV B, of the Picardy region and ADEME.

Key words : VC, attenuation, vadose, microcosms, aerobic oxidation, biodegradation

Table of content

1 Introduction

1.1 CityChlor and the integrated approach

Space is scarce in Europe. Even in the subsurface it is getting busier. Large-scale soil and groundwater contamination with chlorinated solvents are often an obstruction for urban developments. The traditional way of dealing with polluted soil and groundwater does not work in all cases and is not economically and sustainable feasible. In urban environments multiple contaminations with chlorinated solvents are often mixed with each other and spread underneath buildings. This not only leads to technical problems for remediation, but also to liability and financial discussions and hence has an impact on society. An integrated approach and area-oriented approach is needed to tackle the problems. The CityChlor project has demonstrated that remediation and sustainable development can evolve on a parallel timescale.

An integrated approach combines all aspects that are relevant to tackle the problems that pollution with VOC in urban environment causes. Depending on area, site and context different aspects together or parallel to each other can be used. Not only technical solutions are included, but also socio-economical aspects as urban development, communication, financial and legal aspects, time, space, environment and actors (active & passive) have to be handled.

CityChlor did not remain at single case remediation, but looked at the area as a whole in a bigger context: the area-oriented approach. A technical approach that makes it possible to remediate, monitor and control multiple groundwater sources and plumes within a fixed area.

1.2 CityChlor and technical innovations

The managing of knowledge and technical innovations are one of the key to achieve a sustainable city development. A development project has to cope with loads of information coming from different disciplines in different (technical) languages and with different uncertainties. With chlorinated solvents, the knowledge about the pollution will always have a certain uncertainty that can have an impact on the course and the costs of the remediation. An efficient 'managing of knowledge' will try to decrease this degree of uncertainty.

CityChlor therefore also worked on the technical aspects of characterization and remediation. The conventional techniques that are applied for investigation and remediation have their limitations dealing with chlorinated solvents. Promising innovative techniques exist, but do not easily find their way to current application. This barrier is often caused by lack of knowledge on different levels. Experts and contractors do not always have the means to invest in experiments with new techniques, authorities are reluctant to accept techniques of which the results may be uncertain and clients aren't eager to pay for experimental techniques.

Dissemination of knowledge can break this deadlock. CityChlor therefore collected experiences from field application of innovative techniques and implemented itself a number of techniques in pilot projects. For the detailed outcomes, the reader is referred to the specific reports.

CityChlor - “new solutions for complex pollutions” **http://www.citychlor.eu/**

2 Aim of this study and context

2.1 Goals

A specific knowledge gap in the modelling of the transfer from groundwater to indoor air concerns the fate of vinylchlorid (VC) in the unsaturated soil. VC is the most toxic and mobile degradation compound of the chlorinated solvents. It is often the limiting compound in a remediation based on risk. But curiously, VC is almost never detected in soil air, even when measured at high concentrations in groundwater. Current models do not reproduce this particularity. One main explanation is that VC biodegrades in soil air.

In order to assess the importance of aerobic biodegradation of vinyl chloride in the vadose zone, specific protocols concerning the use of gas wells and soil microcosms were tested with in situ and lab measurements.

The final goal is a more realistic and robust risk assessment, and ultimately a better management of the contaminated sites in urban area.

2.2 Context

The chlorinated ethenes with high molecular weight (PCE, TCE) found in the environment comes from leakage and poor removal devices inside the sites where these compounds are produced and used. In contrast, the chlorinated ethenes of lower molecular weight (DCE and VC) are usually derived from reductive biotransformations of PCE, TCE and chlorinated ethanes, provided by anaerobic bacteria (Lorah & Olsen, 1999; Lorah & Voytek, 2004).

Different bacterial populations are involved in the biodegradation of chlorinated ethenes (Mattes, 2010). These can be classified into four groups according to their metabolism: anaerobic reductive dechlorination, anaerobic oxidation, aerobic oxidation and aerobic cometabolism and assimilation. These four types of biodegradation, characterized by specific metabolic pathways are described below.

3 Methodology

3.1 Combination of in situ survey and microcosm studies

Degradation of the chlorinated solvents was studied at several scales, at the scale of the plume in soil gas and in groundwater, at the scale of the multilevel gas probe and at the laboratory microcosm scale. The field data coming from two surveys of a network of 30 gas probe to study the plume of chlorinated compounds in the vadose zone over a year was also used.

The laboratory study was conducted from monitored triplicate soil microcosms of the Citychlor site n°4, incubated at different temperatures, and with soil abiotic controls. This particular study was conducted in collaboration with the Center of Hydrogeology and Geothermics, University of Neuchâtel (CHYN).

3.2 Description of the Citychlor pilot site “Ile de France”

3.2.1 Geological and hydrogeological context

The site is located in a flood plain, with very little relief. The site area is approximately 7000 m2 with a built largely in urban areas. The site is located to the right of workshops using chlorinated solvents even today.

From top to bottom we find these geological layers:
various backfills, with a thickness which reachs usually 0.5 to 1 m. These embankments have a varied texture;
3 meters of clay/sandy clays, beginning to 1 m deep, and extending deeper in the form of sandy clay and finally fine sand to 10 m deep, These layers consist of recent/old alluvium and were attached to the Würm glacial formations. These layers are above the “marnes & Caillasses” Lutetian horizon, reached at 10 m depth. Recognitions were mainly made in the first 10 meters.

A shallow aquifer exists in the alluvium. It is related to the deeper water limestones of the Eocene (Lutetian and Ypresian). The static water level was reached generally at 2 m depth (locally reached 1.5 m depth). The wall of the shallow aquifer is about 10 m deep.

The flow direction was not well known until the end of 2011: it was generally described with a direction N-NW-S-SE, with a variation depending on water regime. Relations between aquifer and rivers were also discussed, but beyond the site boundaries.

3.3 Chemical contamination of the soils and groundwater

3.3.1 Site investigations

The site has been the subject of several characterization campaigns in order to measure and delineate the chemical contamination on soil, water and soil gas.

Concerning the chlorinated solvents, the goal was to discriminate between different sources of contamination and possibly to observe natural attenuation.

Various types of investigations were carried out on the first geological layers and superficial aquifer:

- laboratory analyzes of groundwater coming from the pumping and sampling of the entire water column (piezometer) and from the entire soil gas well;
- some CPT / BAT surveys on soil/water pollutants, in order to locate the chlorinated solvents contamination ;
- multilevels sampling of soil gas and groundwater with dedicated piezometers near the piezometers Pz 3 (“flute de pan” device) and simple, duplicate / triplicate soil gas wells with two layers targets screened intervals.

Groundwater sampling was done on 8 piezometers (Pz1 to Pz8), including five piezometer located on the same site (Pz1 to Pz5). Monitoring (water table level measurements and sampling) was done on these 8 piezometers and occasionally four private wells noted, PsB PSL, PsP and PST.

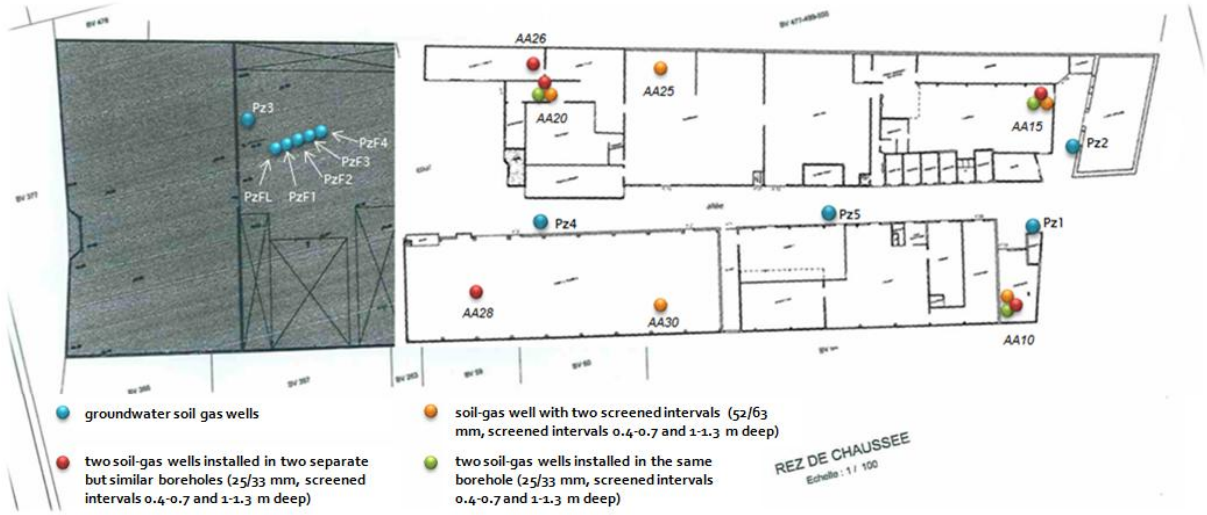

Figure 1 : Description of the site and of the location of the piezometer (Pz1 – Pz5) and of the locations of the soil gas wells.

3.3.2 Contamination

TCE, PCE and degradation products, hydrocarbons and metals (chromium, copper and nickel) were encountered on-site in the soils and groundwater.

Due to the presence of a concrete slab and ongoing factory workshops, the contamination recognitions was mainly done with piezometers and soil gas wells between 2011 and 2013.
If we made a focus on groundwater contamination, two sources of contamination (PCE, TCE) were suspected on the site: the multi-source problem is often common in dense urban environments.

Groundwater samples were collected by pumping at a low flow in the shallow superficial aquifer which was probably impacted mainly by TCE sources.

Considering PCE + TCE, the highest concentrations are found in the groundwater samples coming from the piezometers Pz 2 and Pz 3. These levels of contamination reached 8 - 21 mg /l.

Concerning the degradation products, vinyl chloride groundwater concentration higher points vary between 28 and 4 100 microg / l for piezometer Pz 3 and between 8 and 60 microg / l for the groundwater coming from the piezometer Pz 4. The groundwater concentrations from other piezometers were lower (less than 5 to 10 microg / l).

If we focus on vinyl chloride (VC), VC ground concentration reached strong concentration at only one point, the piezometer Pz3, in 2007, to a level of 870 microg / l (and a concentration of TCE and cis 1,2-DCE, respectively, of 8.1 and 6.4 mg / l). This Piezometer has a special behavior because of its high content of cis-1,2-DCE, found in smaller amounts in Pz 1 (730 microg / l) and very low quantity Pz 2 (44 microg / l).

The redox potential of groundwater is of the order of 300-370 mV / ENH at the base of the site. During periods of high water table (2011), there is a nitrate concentration gradient across the site (0.5 mg / l in the West, 50 mg / l in the East), which indicates probably a recharge by the North - West by groundwater with low nitrates concentration.

For the same period we have observed lower levels of sulphate at the piezometer PZ4 (330 mg / l), Pz5 (510 mg / l) and PZ1 - Pz2 (respectively 280 and 350 mg / l), whereas the levels have reached 770 mg at Pz 3. Relatively high redox potential observed from the groundwater did not allow a natural attenuation under reducing conditions in the aquifer.

3.3.3 Focus on vadose zone contamination characterization

The characterization of the vadose zone was made by the use of 30 soil gas wells and by surveys with CPB/BAT and an auger.

Soil characterization

Soil samples were analyzed in order to estimate the texture of the soil and the first geological layers below the concrete slab.
Three layers were identified :

- surface layer, just below the concrete slab;
- sub surface layer;
- layer just above the water table, which contents the capillary fringe.

The general content of carbonates was high (average of 325 g/kg Dry Weight (DW)) and the bulk density reachs an average of 1,65 kg/dm^3.

Soil gas characterization of the site

On-site measurements showed highly variable VC and c-1,2-DCE gas concentrations in the vicinity of one of the possible source of the TCE.

The network of soil gas wells was used mainly to test different combination of multi-level soil gas sampling with two depth of the screened interval (0.4 – 0.7 meter below ground (mBG) and 1.0 – 1.3 mBG).

Several soil gas surveys were realized in 2012 with different type of gas sampling and laboratory analyzes.

Levels of TCE and cis-1,2-DCE could reached on the hot spots very high values (of respectively 2 200 and 950 mg/m3 for the area AA20, Pza 20, March 2012), while the PCE values reached up to 30 mg/m3(Pza 15, March 2012). But these levels of contamination were highly variable during the same week survey. These variations, due to the proximity of the water table (1.5 – 2 mBG), the heating/non heating of the factory and other parameters make more complex the comparisons of the designs of multi level sampling gas well and the use of different type of adsorbent of the tasks 6.3/6.4 of the project Citychlor.

Due to the major amount of cis-1,2 DCE found in groundwater (compared to trans 1,2 dichloroethene and other metabolites), the anaerobic reductive dechlorination in very thin layer of the aquifer could be an explanation, due to the silty clayed loam texture in the upper part of the aquifer (with the possible presence of peat).

But even with levels of 0,18 mg/l of VC (Pz_F1, October 2011), VC was not found in the soil gas near the area of the Pz 3.

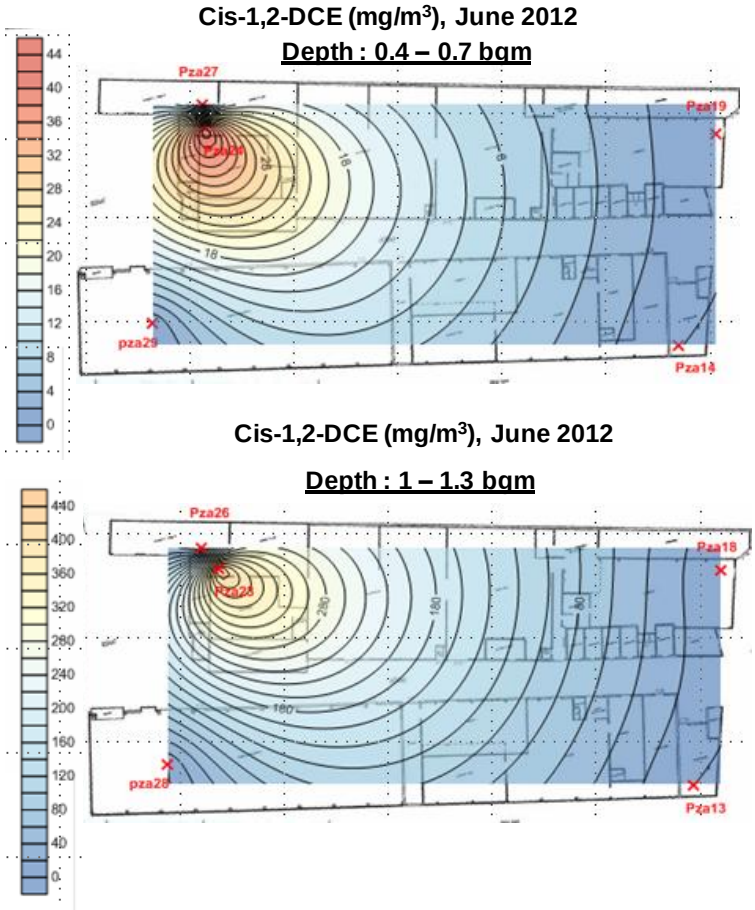

Figure 2 :Cis-1,2-DCE gas soil concentration by active sampling at two depth (June 2012)

If we plot the cis-1,2-DCE gas soil concentration obtained in june 2012 with charcoal tube (ORBO 32) at the depth of 0.4- 0.7 mBG and 1.0 – 1.3 mBG, we observed a “hot spot” of the contamination near the Pza 24/25, in the AA20 area. These data were obtained with two different design of gas well (simple slotted tube in independent drilling hole for the depth of 1 – 1.3 mBG, double slotted tube for the depth of 0.4 - 0.7 mBG). The gas soil concentration obtained from of the upper part of the double slotted tube could be higher, probably due to the migration of soil gas in the extrados area (thin area around the perimeter of the tube) of the tube.

Considering VC, VC was generally not quantified during these laboratories analyses : VC concentrations did not reached the limit of quantification of the sampling method used (charcoal and other adsorbent tubes, Tedlar® bag). The concentration of VC was detected only at a level of 0.5 mg/m^3 from the sample coming from Pza 23 (March 2012), with the use of Tedlar® bag (detection limit : 0.1 mg/m3). For the adsorbent tubes, the limit of detection/quantification of the VC was generally between 0.4 and 1 mg/m^3.

Also, canisters were used for specific measurements near the sources of contamination (soil gas well of the area AA20), in order to detect and quantify the contamination. These results were presented in the section 6.

4 Bibliography of the degradation of the chlorinated ethane in the vadose zone

4.1 Anaerobic reductive dechlorination

The ethenes and chlorinated ethanes with more than three chlorine atoms are highly oxidized compounds, which makes them less attractive as energy sources for microorganisms. This is confirmed by the absence of microorganisms which can grow on TCE or TCA as a source of carbon and energy (Mattes, 2010). In contrast, ethenes and ethanes tri-and tetra-chlorinated were efficiently metabolized by many bacteria as electron acceptors during reductive dechlorination process or dihaloelimination (Vogel & al., 1987).

Thus, the bacteria metabolizing chlorinated ethenes under anaerobic conditions appear to be promising agents for bioremediation of contaminated sites. Indeed, they seem to be widespread, are active in anoxic conditions typically encountered in the basement, and some can catalyze the complete reduction of PCE and TCE to ethene (Freedman & Gossett 1989; Maymo- Gatell & al., 1997; Parkin, 1999; Bradley, 2003).

Anaerobic reductive dechlorination has been widely described in the literature (Bradley, 2003; Cupples, 2008; Futagami & al., 2008).

To summarize, many anaerobic microorganisms: Dehalobacter restrictus (Holliger & al., 1998) Dehalospirillum multivorans (Neumann & al., 1994; Scholz-Muramatsu & al., 1995) and Geobacter lovleyi (Sung & al., 2006), can reduce the PCE and TCE cDCE (Figure 1), but only one member of the Dehalococcoides are known to reduce PCE or TCE beyond cDCE.

The 195 type strain bacterial Dehalococcoides ethenogenes is the only known bacterium which can completely reduce PCE to ethene. In this case and according Maymo-Gatell & al. (1997), the first three steps (Figure 1) are producing energy and thus related to bacterial growth, while the final reaction (CV => ethene) is performed by cometabolism and therefore not related to growth.

In contrast, Dehalococcoides strain BAV1 generates a particular interest through its ability to reduce the CV to ethene for growth (He & al., 2003), and strain FL2 can reduce TCE to CV for growth (He & al., 2005). The strain GT can reduce TCE to ethene for growth (Sung & al., 2006b). The amplification of the 16S RNA of bacteria of the genus Dehalococcoides from soil samples from contaminated sites has revealed that these bacteria were observed when the CV or ethene was present as a product of PCE dechlorination or TCE, but absent of contaminated sites where the final product of dechlorination was c-1,2-DCE (Hendrickson & al., 2002).

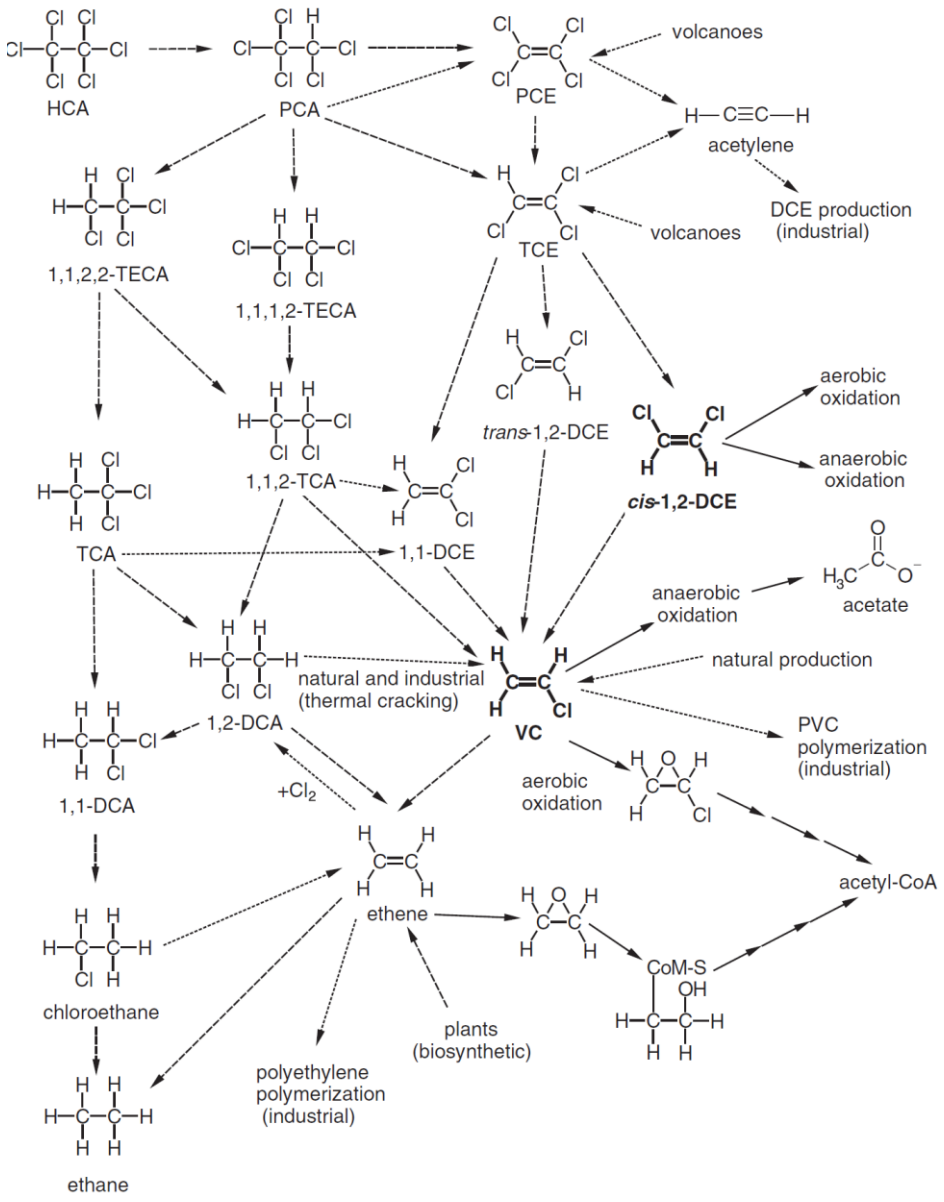

Figure 3 : Metabolic degradation of chlorinated ethanes and ethenes by Mattes (2010). The arrows indicate the oxidative biological reaction, the dashed lines indicate reductive biological reactions, and dotted lines indicate abiotic reactions

Incomplete dechlorination of chlorinated ethanes and ethenes lead to an accumulation of c-1,2-DCE and VC on many sites (Edwards & Cox, 1997; Lorah & Olsen, 1999; Lorah & Voytek, 2004). This may be due to the lack of suitable microorganisms (ie Dehalococcoides spp.) or of unfavorable conditions limiting the activity of microorganisms capable of complete dechlorination. Bioaugmentation or biostimulation processes could give a solution for the treatment of polluted sites (Major & al., 2002; Macbeth & al., 2004; Scheutz & al., 2008).

4.2 « Anaerobic oxidation » of VC and c-1,2-DCE observed by Bradley & Chapelle

This reaction was observed during the reduction of iron (III) (Bradley & Chapelle, 1996), humic acids (Bradley & al., 1998a), Mn (IV) (Bradley & al., 1998b) or SO42- (Bradley & Chapelle, 1998a), and during methanogenic conditions (Bradley & Chapelle, 1999, 2000b). CV and c-1,2-DCE are then electron donors and Fe (III), organic matter, Mn (IV), or SO42-or methane are electron acceptors. In many studies, evidence of anaerobic microbial oxidation is the production of 14CO2 from labeled with 14C c-1,2-DCE or VC (Bradley, 2003).

The first observation of the anaerobic oxidation of VC was performed by Bradley & Chapelle (1996) in sediments under reducing conditions Fe (III). In this case study, an amendment of chelated Iron (III) stimulated activity, suggesting that the bioavailability of iron (III) is important for the oxidation process. Since this experiment, the 14C labeled acetate was observed as an intermediate in the anaerobic oxidation of VC. This process has been described as an oxidative acetogenesis Bradley and Chapelle (2000b).

The anaerobic oxidation of c-1,2-DCE was first reported in microcosms under reducing conditions of Mn (IV) (Bradley & al., 1998a, b). In this case, the addition of iron (III) caused an inhibition of the activity, suggesting that different microbial populations were involved.

The anaerobic oxidation of c-1,2-DCE and VC was also observed in microcosms coming from landfill leachate sediments, with a release of chloride in the absence of production of ethene or ethane (Hata & al., 2003). The anaerobic oxidation could be transferred in successive media but curiously this activity was not directly related to the reduction of iron (III) and could occur even in the absence of added electron acceptors. In contrast, the oxidation of c-1,2-DCE and VC was absolutely dependent on the addition of glucose, thus indicating a phenomenon of co-metabolism rather than a process related to growth.

Similarly, Hata & al. (2004) and Kim & al. (2006) studied respectively two strains of Clostridium (DC1 and KYT-1) capable of degrading VC and c-1,2 -DCE but having two different behaviors. Strain DC1 degrades VC and c-1,2-DCE during the stationary phase, while the KYT-1 metabolizes during the exponential phase. Metabolic pathways and enzymes for degradation of chlorinated ethenes of these strains remain poorly understood.

A recent study by Darlington & al. (2008) also suggests a possible abiotic degradation of c-1,2 -DCE in organic acids, a phenomenon explained by the presence of reactive iron ore (eg pyrite & fougerite) in a fractured sandstone aquifer. Abiotic degradation of c-1,2-DCE produce organic acids, then used by local populations for bacterial growth. This study highlights the importance of understanding the geochemical and biological components in contaminated sites in order to predict and accurately manage in situ bioremediation of chloroethene.

The microbial anaerobic oxidation of c-1,2 DCE and CV seems to be present on many sites and the identified microorganisms seem to accept a large variety of final electron acceptors. These studies suggest that the process of anaerobic oxidation of VC and c-1,2-DCE are involved in the natural attenuation of chloroethenes, and could be stimulated to enhance bioremediation of

polluted sites. However, the complexity of biotic-abiotic interactions, different geochemistries depending on the sites studied, as well as the lack of knowledge concerning the mechanisms of anaerobic oxidation of chloroethene make difficult to use today these mechanisms in situ. Clearly, further research is needed in this area (Mattes, 2010).

4.3 Oxidation by cometabolism

Aerobic bacteria growing on hydrocarbons produce dioxygenase monooxygenase enzymes allowing conversion of these substrates to phenols, alcohols, or epoxides. Many of these enzymes have a wide range of substrates and can oxidize chloroethene to produce chlorinated epoxides which decompose spontaneously. The chlorinated ethenes aerobic cometabolism was investigated through various types of hydrocarbon-oxidizing bacteria, including those growing on phenol, toluene, methane, ethene, ethane, propane, propene or ammonia (Semprini, 2001)

Oxidation technologies using aerobic cometabolism have been implemented as part of the bioremediation of polluted sites with chloroethene (McCarty & al., 1998). These techniques have the advantage of a broad distribution of bacteria needed and of high specific activities oxygenases potential. However, these approaches have several drawbacks such as the need of "growth" substrate to support cell growth and oxygenase activity. In addition, the growth substrate and co-substrate (vinyl chloride) are competing for the active site of the oxygenase and epoxide products are not metabolized, which can lead to toxicity (Cai & Guengerich, 2000). For example, chlorooxirane (VC epoxide) can form covalent bonds with DNA (Guengerich & al., 1999), making it a mutagen. TCE epoxides rapidly decomposes to a variety of chemical species (formic acid, trichloroacetaldehyde, dichloroacetic acid ... (Li & Wackett, 1992; Newman & Wackett, 1997; Cai & Guengerich, 2000) and can induce toxic effects for bacteria themselves (Cai and Guengerich, 2000; Yeager & al., 2001. Halsey & al., 2005).

A strategy to minimize these toxic effects is the use of assimilative ethylene bacteria to attack VC and c-1,2-DCE (Verce & al., 2001; Jin and Mattes, 2008). Indeed, epoxyethane (oxirane) is a metabolic intermediate of the ethene degradation pathway and these bacteria are able to use effectively chlorinated oxiranes (Coleman & Spain, 2003). Assimilating cultures of VC, known to degrade c-1,2-DCE, the t-1,2-DCE, and 1,1-DCE (Hartmans and de Bont, 1992; Freedman & al., 2001; Verce & al., 2002), could also be useful for cometabolism strategies.

In the same spirit, a line of research to improve these cometabolism mechanisms is to create bacterial strains with increased enzyme activities and / or other epoxide detoxification systems. For example, Rui & al. (2004) have created a strain of Escherichia coli expressing monooxygenase combining high activity towards chloroethenes and c-1,2-DCE epoxides hydrolase. This strain has a five times higher efficiency in converting c-1,2-DCE to chloride ions than its parent strain without metabolic system for epoxides.

4.4 Aerobic oxidation of VC and c-1,2-DCE

Studies in microcosms from samples of contaminated sites showed that the aerobic oxidation of VC is common but it is rare for c-1,2-DCE (Coleman & al., 2002a, b; Abe & al., 2009b). Natural attenuation would be more appropriate for VC than for c-1,2-DCE in the "aerobic" sites.

The first evidence of the oxidation of VC is due to Davis & Carpenter in 1990 on samples of groundwater from a shallow aquifer, in the absence of co-substrate and in the absence of latency phase. This study showed that microorganisms growing on VC were likely to be active with in situ conditions of contaminated sites. Similarly, Witt & al. (2002) measured a drastic reduction in VC during the passage of groundwater from an anaerobic zone to an aerobic zone at a contaminated site and deduced an aerobic oxidation activity. Bradley & Chapelle (1998b) confirmed the oxidation of c-1,2-DCE and VC by a study in microcosms. However, if 57 mM VC was mineralized to 100% in 2 days, c-1,2-DCE was mineralized to only about 75% at a concentration of 1.4 to 7.6 mM and 30% for 15-80 mM. Other studies subsequently confirmed the oxidation of c-1,2-DCE in situ in freshwater sediments (Abe & al., 2009b), and deep aquifer (Broholm & al., 2005; Schmidt & Tiehm, 2008), and in soils rich in organic matter (Klier & al., 1999), although none of these studies have identified metabolites or micro-organisms responsible for the biodegradation of c-1,2-DCE.

The aerobic microorganisms capable of biodegrading the lighter chlorinated ethenes are present on site as well, but their distribution seems to be uneven and their activities appear to be variable. Therefore, the aerobic biodegradation may be an important factor in the process of natural attenuation of c-1,2-DCE and CV, at least for some sites. Thus, linking micro-organisms, the genes and metabolites with in situ activities remains a major area of research for the future (Mattes, 2010).

4.5 Oxidation at low oxygen concentration

As emphasized Gossett & al. (2010), the balance not observed in some anaerobic zones between the 'parents' compounds and the expected products of reductive dechlorination has induced many research teams to evaluate the possibility of oxidative mechanisms occurring under anaerobic conditions in the presence of 'electron acceptors' (nitrate, sulfate, iron (III), Mn (IV), humic substances) as described in chapter 2. However, although many studies (chapter 2) have shown a link between the addition of electron acceptors and the disappearance of VC and / or c-1,2-DCE their anaerobic oxidation is not formally proven. Thus, Gossett et al. (2010) have shown that very low concentrations of dissolved oxygen (0.02 to 0.1 mg/L) allow aerobic degradation of VC. These authors have built soil microcosms (extract from anaerobic zones) in saturated condition (groundwater), and highlighted a VC aerobic degradation by controlling dissolved oxygen through a system of permeation tubes (Figure 2). This study shows that the disappearance of VC in zones called anaerobic may be due to aerobic mechanisms that occur in the presence of very low concentrations of oxygen, coming for example by diffusion mechanisms from aerobic zones.

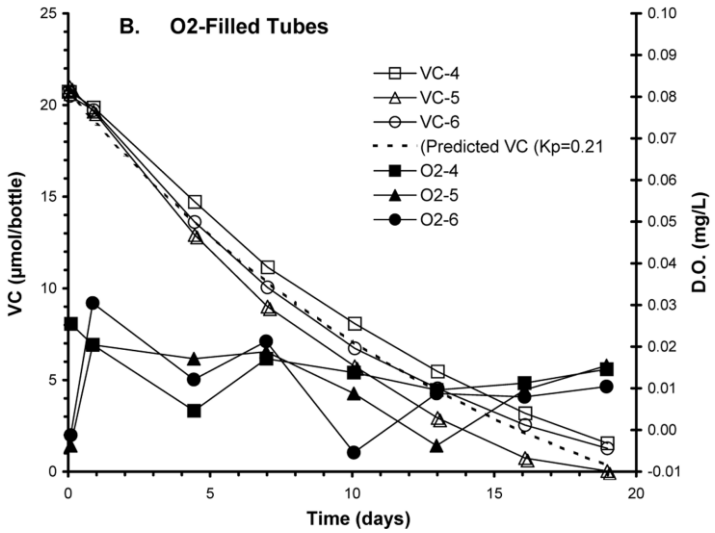

Figure 4 : On the left, VC degradation with very low concentrations of dissolved oxygen (Gossett & al., 2010), and on the right is an example of a microcosm saturated with permeation tube.

4.6 Summary

Chloroethene plumes are often anaerobic and allow reductive dechlorination but they generate an accumulation of VC and / or c-1,2-DCE (DiStefano & al., 1991; Distefano, 1999). VC and c-1,2-DCE can then migrate to aerobic areas with aerobic treatment (possibility of bioaugmentation). Thus, the coupling of anaerobic and aerobic biodegradation in situ would be an elegant solution to the incomplete reduction of chloroethene and to the variability of redox conditions on the ground, but this remains to be confirmed (Mattes, 2010).

Aerobic degradation of c-1,2-DCE and VC coupled with growth has several theoretical advantages over degradation by cometabolism: no addition of carbon or energy sources, no problems of competition between the substrate on the enzyme site, resulting in fewer toxic metabolites. Although this pattern of action seems promising, it is assumed that the appropriate microorganisms are present or that the bio-augmented strains are robust, and that the site conditions are suitable for microbial activity. In all cases, the sampling and measurement of biological and geochemical parameters are essential to demonstrate that the removal of pollutants is the result of microbial activity, and not abiotic processes.

5 Set up of a protocol

5.1 How to evaluate microbial activity ?

Chloroethene biodegradation by aerobic microorganisms is more complex and more difficult to evaluate than anaerobic dechlorination (eg Dehalococcoides spp.) Due to a lack of correlation between metabolic capacity and phylogeny of the strains involved (Mattes, 2010). Indeed, aerobic microorganisms are not necessarily dependent of the metabolism of chlorinated compounds. This fact implies that they have a wide spread distribution, including uncontaminated sites (Coleman & al., 2002b; Fathepure & al., 2005; Elango & al., 2006). Various bacterial genera are thus able to grow on VC: Pseudomonas, Mycobacterium, Nocardioides, Ralstonia and Ochrobactrum (Verce & al., 2001, Coleman & al., 2002b; Danko & al., 2004; Elango & al., 2006). These strains, isolated from various media (soil, sediment, groundwater, activated sludge ...) raises questions about the type of active microorganisms in a specific environment. Factors, such as competition between microorganisms, the presence of multi-source contaminated site or physico-chemical characteristics of the site (Eh, pH, availability of inorganic elements ...), could have an effect on the species present and active on a specific site.

In this spirit, Abe & al. (2009) conducted a comparative analysis of chemical and biological data on a Canadian site contaminated with PCE. This study provided the distribution of aerobic and anaerobic microorganisms involved in the degradation of chloroethene. The reductive dechlorinations to ethene or ethane were well correlated with redox potentials equivalent to the reduction of sulfates or lower. The presence of DNA of Dehalococcoides (detected by PCR) was correlated with significant amounts of organic materials (H2 production). The potential for aerobic degradation of VC was established on the site through microcosm studies and Etne (epoxyalkane coenzymeMtransferase or EaCoMT) gene amplification, even for deep anoxic samples.
However, the gene detection by PCR does not indicate the degree of in situ activity. Thus, future studies could focus on determining the microbial degradation through quantitative molecular biology techniques (Q-PCR DNA and RNA). However, at present discriminate between degradation activities of ethene and VC is not possible with only the detection of sequences.

Similarly, the development of proteomic analyzes can target peptides related to the degradation activity of chloroethene. Chang & al. (2010) has observed in microcosms (VC contaminated groundwater enriched with ethene) the presence of peptides EtnC et EtnE concomitant with the presence of genes EtnC et EtnE amplified by PCR. Proteomic analysis reveals the presence of a biodegradation activity and not just the detection of biodegradation potential as does the PCR amplification. In this, proteomic analysis joined quantitative molecular biology technologies.

The quantitative (Cupples, 2008) and proteomics (Morris &al., 2006, 2007) molecular biology technologies could be an important component of the monitoring of bioremediation techniques based on natural attenuation or bioaugmentation and / or biostimulation (Mattes, 2010). The monitoring of the Dehalococcoides spp genus presence accompanied by reductive dechlorination activity measurements during a process of anaerobic bioaugmentation could be an example.

5.1.1 Isotopic fractionation

Although isotopic fractionation does not directly identify microbial species or genes, it provides an idea of the nature of the microbial groups and the metabolic pathways involved. Indeed, the isotopic fractionation is based on the fact that the enzymatic activities tend to promote the use, for the same compound, of connections between light isotopes, causing an isotopic enrichment in heavy compounds. A bond involving a heavy isotope is stronger than that between two light isotopes, and therefore has a higher activation energy (Chu & al., 2004. Abe & al., 2009b).
Isotopic fractionation provide hints of actual organic pollutants degradation (biotic or abiotic) due to the fact that the purely physical processes (adsorption, volatilization, dilution ...) have a very low isotopic fractionation (Bill & al., 2001, Chu & al ., 2004; Chartrand & al., 2005; Tiehm & al., 2008).

13C enrichment enabled to differentiate between the reductive dechlorination and aerobic oxidation of VC. Enrichments measured during reductive dechlorination microcosms of VC in the presence of Dehalococcoides range between - 21.5 ‰ to - 31.1 ‰ (Hunkeler & al., 2002; Chartrand & al., 2005, Lee & al., 2007; Tiehm & al., 2008, Abe & al., 2009b).
For the aerobic oxidation of VC, the 13C enrichments are lower, reflecting a difference in reaction mechanism: - 5.7 ‰ to - 8.2 ‰ for aerobic oxidation coupled to growth and - 3.2 ‰ to - 4.8 ‰ for aerobic oxidation by cometabolism (Chu & al., 2004; Chartrand & al., 2005, Abe & al., 2009b).

The weak point of 13C enrichment monitoring is the small difference between the oxidation and oxidation by cometabolism of VC. This could be a problem for in situ monitoring where the two mechanisms can occur simultaneously.

Thus, the simultaneous analysis of isotope ratios of two elements provides correlation characteristics of specific mechanisms, independent of abiotic changes (Elsner & al., 2005, Abe & al., 2009b). For example, a reaction in which a C-Cl bond is broken, should show a greater preference for molecules without 37Cl than a reaction that breaks first one C = C bond with an adjacent Cl. The dual isotope approach has been used by Abe & al. (2009b) to study the anaerobic dechlorination and aerobic oxidation of c-1,2-DCE and VC. These authors highlighted a significant difference between the □13C and δ37Cl isotopic ratios of aerobic oxidation and reductive dechlorination for the c-1,2-DCE and VC. This study showed that it was possible to distinguish between these two processes for in situ monitoring.

5.1.2 In situ specific measurements

The diffusion of VC in soil air depends of the water saturation of the soil void. One major blocking layer for soil air diffusion was probably the capillary fringe.
Due to the presence of the backfill below the concrete slab, we intend to encounter large lateral variation of the mean grain size of the soil for the surface and sub surface soil, but a reduced variation for the silty loam layer which was observed at a depth of more 0.7 m.

Time Domain Reflectometry (TDR) was used to quantify the volumetric soil moisture content, in order to give hints of the extent of the capillary rise.

Due to the presence of the concrete slab, the soil humidity below this slab was directly linked with the depth of the water table and with the soil grain size. The TDR probe (Trime –IPH, SDEC

France) was calibrated in factory and the precision was approximately 2 – 3 % in the range 0 – 70 % volumetric moisture.

During field operations, two soil locations, near the location of the gas probe, were investigated. Two specific TDR tube (1 m length) allow to give a good estimate of the soil humidity. The length of the TDR probe (18 cm) allows a volumetric soil moisture measure for a diameter of the sphere of influence of approximately 15 cm (3 liter soil volume).

Figure 5 : Specific tube used for the TDR measurements

Concerning the measurements of VC in soil air, canisters were used during July 2012 survey, in conjunction with laboratory analyzes (thermodesorption, followed by an injection on a CPG/SM for the quantification of BTEX and chlorine compounds).

5.1.3 Laboratory biodegradation experiments

Two samples of soil were collected at 0.5 m below the ground surface. The site of the soil sampling is near the locations of the monitoring wells Pz3. The soil consists in this location of a first horizon of backfill with a soil texture comparable to a sand, with an average content of organic matter (foc = 1.42%). The next horizon below the backfill was a sandy clay loam layer with chalk debris, which contents the first aquifer.
The location of the soil sample is situated at the end of the plume of chlorinated compounds encountered in groundwater. The two soil gas surveys done in soil gas well do not find VC in this location. Also the soil could not be considered as contaminated by the vapor from the plume. This fact could explain that an incubation lag occurred during the laboratory biodegradation experiments (with VC and c-1,2-DCE added in soil microcosms).

Due to the rather coarse grain size, the humidity of the sample was chosen as average. The capillary fringe was observed in situ in the layer of clay loam below the horizon of backfill.

The experimental plan was established in order to use duplicate of soil, of pollutants (CV/CV + c-1,2-DCE) and conditions of the partial pressure of oxygen (100% N2/20% O2) with also a abiotic control (see Figure 6).

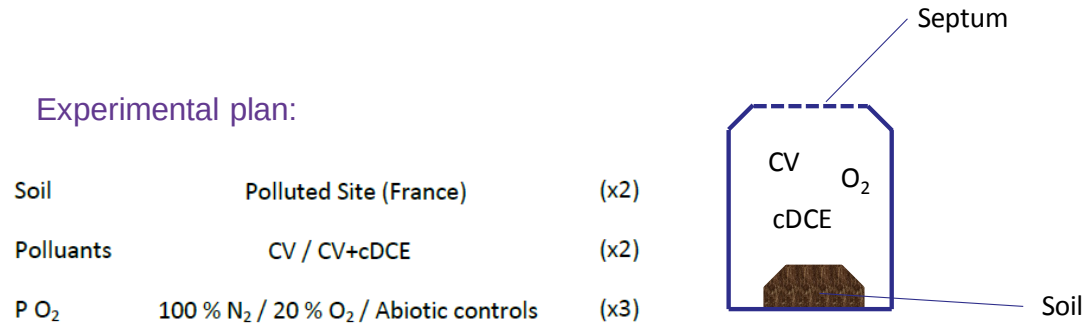

Figure 6 : Experimental plan used for the laboratory microcosms

In order to reach a concentration of 0.5% of VC in the gas volume of the bottle used for the microcosm (100 ml of the volume of the bottle was available), a volume of 0.5 ml of pure VC was injected with a gas-tight syringue (1 mL) through the septum of the bottle. VC (Sigma Aldrich) was used from a regulation valve on a Fluka pressure tin (purity > 99.5 %, 250 ml, 360 bar).
A purge of the sampling syringe was realized with N2 gas before each sampling of gas. Sampling and injection were performed under laboratory hood.

Figure 7 : Bottle used for microcosm with/without the substrate (soil of pilote site ”Ile de France”.

6 Results

6.1 In situ measurements

TDR measurements

Below the concrete slab, we could observe a real increase of the soil humidity, which reach a value of 27%. This increasing humidity shows the proximity of the capillary fringe and the presence of fine grained material.

Due to the length of the probe, the value of the humidity observed was an average value. A fast transition (near 0.75 - 0.85 m below ground surface) was observed on the field with a soil auger between the texture of a soil with residues of organic matter and backfill and a loamy silty sand / silty clayed loam.

The porosity was estimated by an average value of the saturation by water of a sample of soil and a calculus with the real density of 4 fractions. The average porosities vary between 33 % and 43% for the deeper soil layer investigated.

At 1 m depth, if we compare the volumetric moisture and the porosity, we reach a saturation of 64 – 82% of the soil voids, which could be considered a rather high level of saturation and could explain the low velocity of the gas diffusion in this soil level.

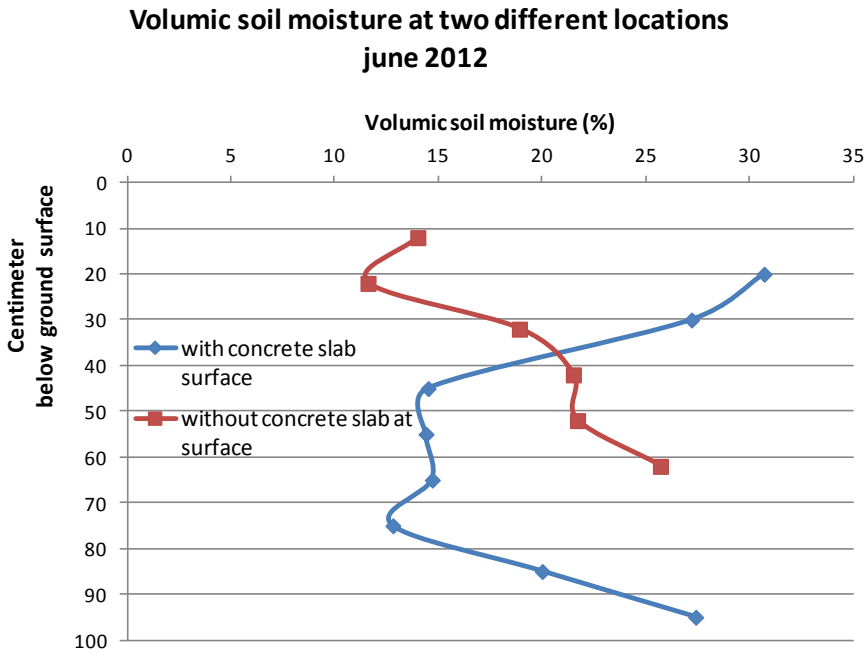

Figure 8 : Volumetric soil moisture for two locations (near the piezometer Pz 3)

The soil was investigated on 11 locations, near the gas probe locations. The 3 deepest layers give an uniform distribution of the grain size in a semi logarithmic grain size graph, with an average grain size (d50) varying between 0.03 mm and 0.2 mm.

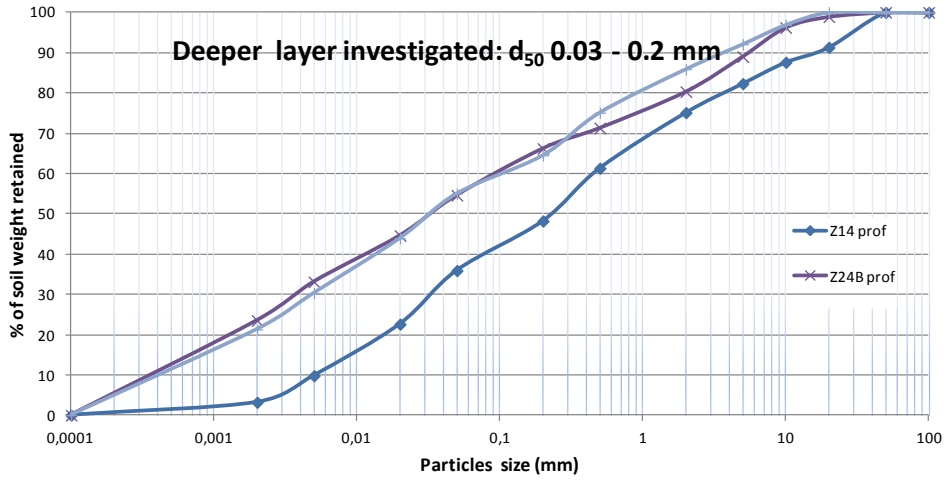

Figure 9 : Uniform distribution of the particles sizes of the deeper layer investigated by auger

The rise of the capillary fringe above the water table could be estimated. Depending of the relation used (capillary rise in a tube of the diameter specified, or Fetter & al, 1994), the length of the rise reachs several decimeters or meters above the water table for the silty loam.

The presence of a silty loam just above the water table (1.5 bgm) and the sinking behavior of the chlorine solvents at the contamination sources could explain the rather low concentration of soil air contamination observed in the locations which were not near the contamination sources.

Characterization of soil air near the contamination source

VC soil gas concentration has varied between 70 and 500 microg/m3 in early February 2012 (with a low external temperature) in the network of soil gas wells Pza 20 - Pza 24 (area AA20 of the Figure 1). The soil gas concentration of TCE and c-1,2-DCE were too high to be measured.

Table 1 : Soil gas concentration coming from the soil gas well network Pza 20 Pza 24 (February 2012)

Reference	Pza 24 µg/m3	Pza 23 µg/m3	Pza 22 µg/m3	Pza 21 µg/m3	Pza 20, 1.3 mbg µg/m3	Pza 20 0.7 mbg µg/m3
VC	106	499	73.6	149	462	237
1,1 DCE	122	437	75.9	177	235	232
t-1,2 DCE	154	1 650	152	782	262	876
c-1,2 DCE	. > 20 400	. > 20 400	. > 20 400	. > 20 400	. > 20 400	. > 20 400
TCE	. > 27 700	. > 27 700	. > 27 700	. > 27 700	2 590	. > 27 700
PCE	154	85.1	363	193	98.9	416

6.2 Biotic degradation in microcosm

The concentration of VC and c-1,2-DCE could be compared to the initial concentration and the concentration of the control batches and plot as a relative concentration of VC and c-1,2-DCE and as a function of the time since the beginning of the experiments in microcosms. The incubation was firstly conducted during 38 days and then extended, for a total period of 186 days.
These experiments were conducted by INERIS and by the CHYN in order to observe the evolution for a longer period, which was more representative of in situ conditions.

Nevertheless, we have observed that in the case of the pilot site “Ile de France”, the variations of chlorine solvent concentration in the groundwater could reach more than one order of magnitude. This fact could be explained by the variation of the groundwater flow direction (in the first aquifer). Also, the duration of the laboratory experiments (186 days) was representative of VC diffusion for a half year period (of high/low water table)

Measured relative concentration (in %, with triplicate microcosms) of VC for the microcosm experiments could be observed in the Figure 10. A low decrease was observed in the first 38 days, but the real decrease was observed after 50 days for the microcosm incubated at 25°C. For the microcosm incubated at 15°C, the duration of the first period seems to be more important and we observe a real decrease after 78 days.

The use of the controls (microcosm incubated with N2 only) allows to verify the absence of VC concentration decrease. The mechanisms involved need oxygen, for VC degradation with or without the presence of c-1,2-DCE.

Only slight variations of the concentrations were observed. The sampling at the head space of the vials and the analyze of the VC and c-1,2-DCE could give some artifacts and variations. The same type of variations was observed on all the samples, and especially the controls. As mentioned before, no real evolution was observed with microcosm amended only with N2 (see Figure 12). These results allow to modify the concentration of the other microcosms, in order to reduce the

artifacts of sampling. In the Figure 11, the curve corrections with controls allow to observe the two parts of the decrease of the VC relative concentration for the vials incubated at 25°C.

In the first part of the curve, the slope was rather low and we can observe more easily the increase of this slope after incubation duration of 50 days. The presence of c-1,2 DCE gives a very low modification of the VC degradation curve : the first period of incubation seems only a bit longer.

Depending of the points chosen for the calculus, the half life of the VC vary between 70 – 150 days for the first part of the curve and could reach approximately 4 days for the second part of the curve, for the microcosms incubated at 25°C.

For the microcosms incubated at 15°C, half lives vary between 70 days and 600 days, with an average of approximately 100 days for the first part of the curve.

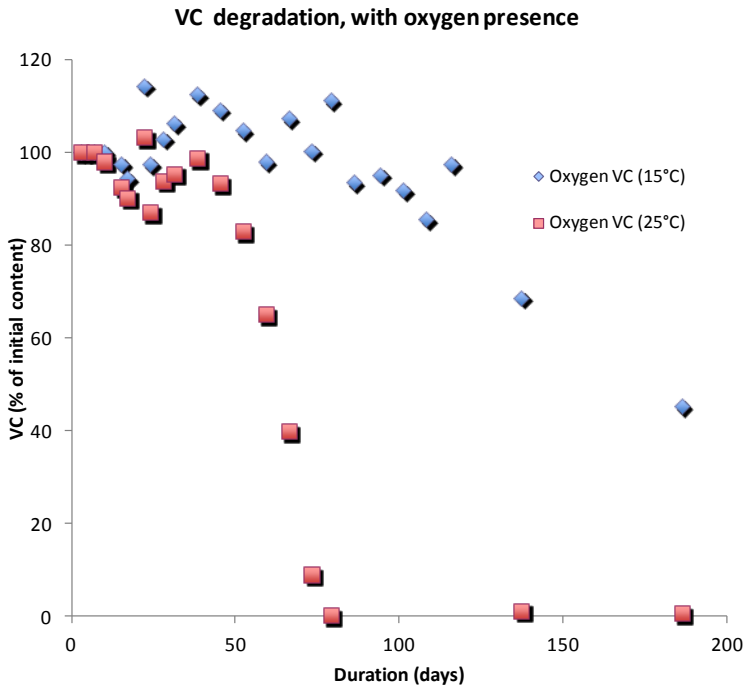

Figure 10 : Degradation of VC observed during 186 days for the microcosm incubated at 25°C and 15°C with oxygen presence

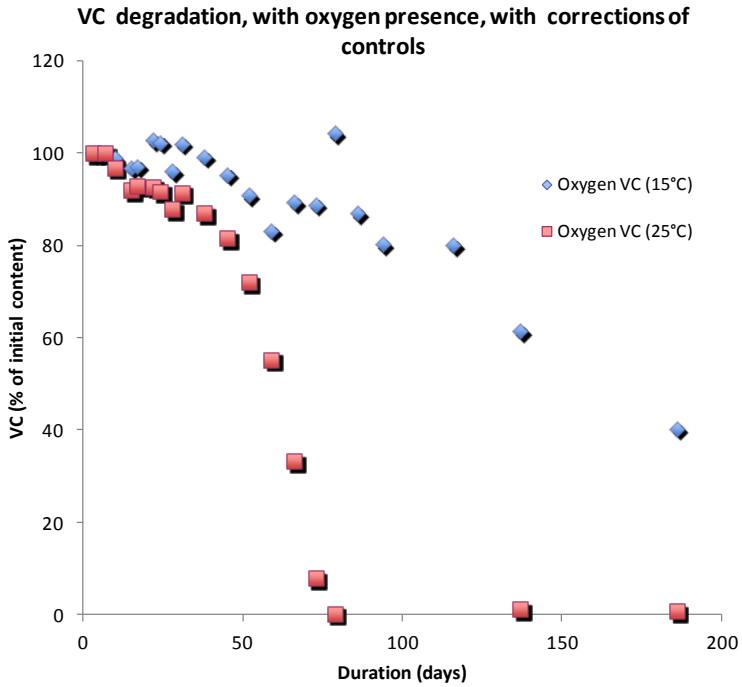

Figure 11 : Degradation of VC observed during 186 days for the microcosm incubated at 25°C and 15°C with oxygen presence, with controls corrections.

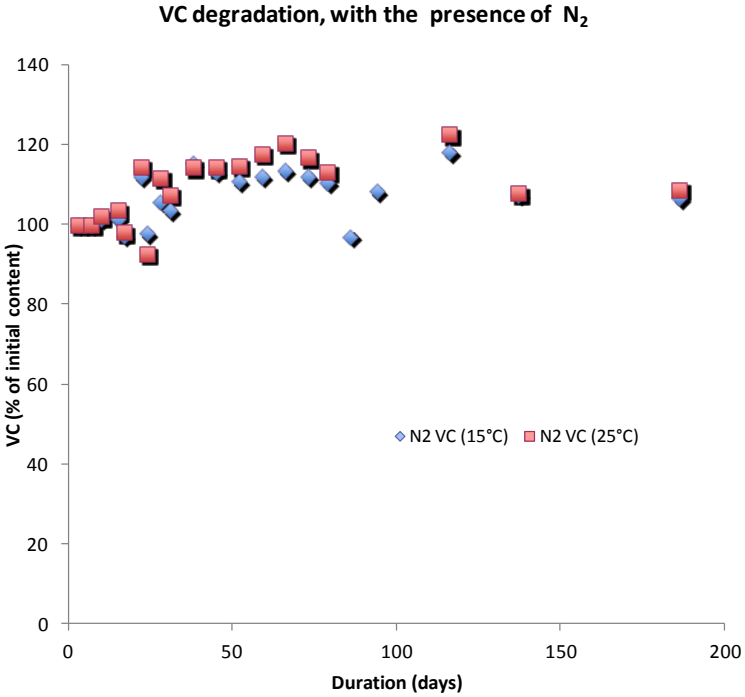

Figure 12 : Degradation of VC observed during 186 days for the microcosm incubated at 25°C and 15°C with N2

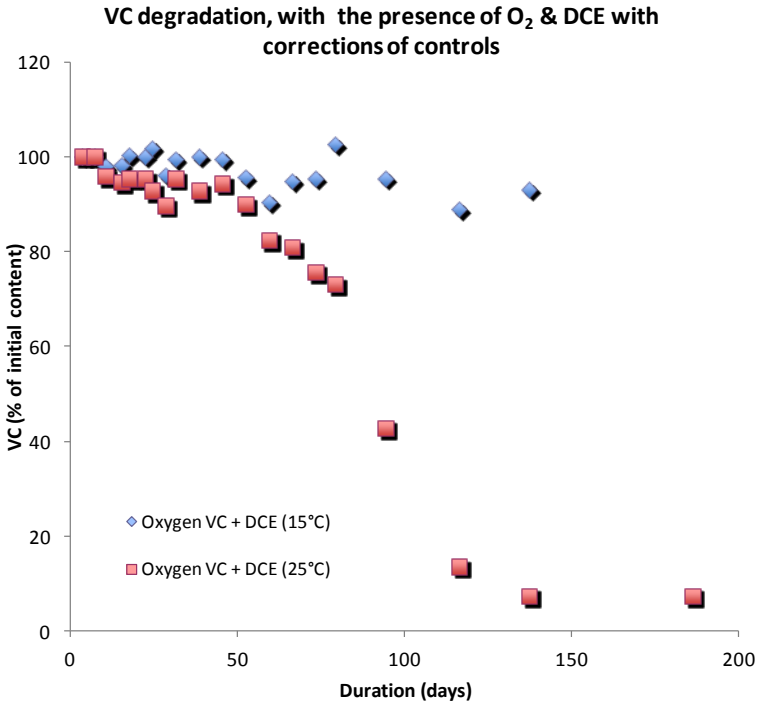

Figure 13 : Degradation of VC observed during 186 days for the microcosm incubated at 25°C and 15°C with N2 and c-1,2-DCE

This rate of decrease could be compared to other results published before. These results could be extracted from the on line data file Sorp-bio, released by INERIS during the TRANSPOL Project. An extraction of the VC data published produced 20 values of half life of VC with the description of the different parameters (redox condition, mechanism, ..) in the Table 2.

The values of VC aerobic degradation half life obtained from laboratory microcosm (L_Microcosm for soil in the Table 2) reach 70 and 103 days (depending of the average soil temperature considered of respectively 25°C and 15°C) and even lower half life for the second part of the curve.

The texture of the soil used was similar to a sand. These results are not very different from previous measurements done with a medium combining soil and water. It seems that the saturation of the soil by the water is not the main parameter. Nevertheless, the redox condition and the mechanism were not sufficient to define half life range of the of VC degradation: in anaerobic condition half life of VC degradation vary between 8 days and 1406 days (see the Figure 14).

Table 2 : Half life of VC degradation for a selection of parameters (soil type, redox,..)

Medium	Redox	Mechanism	Type	Granulometry	T 1/2 (days)	L (1/day)
Soil-water	Aerobic		L_Microcosm		58	0,012
Soil-water			L (Laboratory)		60	0,0115
Aquifer	Anaerobic		T (In situ)		8	0,0866
Aquifer	Anaerobic	NO3 Reduction	T (In situ)		8	0,0866
Aquifer	Anaerobic	SO4 Reduction	T (In situ)		116	0,006
Aquifer	Anaerobic	Fe(III) Reduction	T (In situ)	sand	116	0,006
Aquifer	Aerobic		T (In situ)		347	0,002
Aquifer			T (In situ)		845	0,0008
Aquifer			T (In situ)		444	0,0016
Aquifer	Anaerobic	Methanogenic	T (In situ)	sand	218	0,0032
Aquifer	Anaerobic	SO4 Reduction	T (In situ)	sand	218	0,0032
Aquifer	Anaerobic	Fe(III) Reduction	T (In situ)	sand	218	0,0032
Aquifer	Anaerobic	NO3 Reduction	T (In situ)	sand	218	0,0032
Aquifer	Anaerobic	SO4 Reduction	T (In situ)	sand	287	0,0024
Aquifer	Anaerobic	Fe(III) Reduction	T (In situ)	sand	287	0,0024
Aquifer	Anaerobic	Fe(III) Reduction	T (In situ)		347	0,002
Aquifer	Anaerobic	Fe(III) Reduction	T (In situ)		372	0,0019
Aquifer	Anaerobic	SO4 Reduction	T (In situ)	sand	1406	0,0005
Aquifer	Anaerobic	Fe(III) Reduction	T (In situ)	sand	1406	0,0005
Soil	Anaerobic	Fe(III) Reduction	L_Batch	sand	12,6	0,055
Soil	Aerobic		L_Microcosm	approx sand	70	0,0099
Soil	Aérobic		L_Microcosm	approx sand	103	0,00673

The variation between the temperature of the laboratory experiments and in situ soil conditions (more or less an average of 10 °C usually in European countries) could also explain only a fraction of the variation of the half life.

The variation of ten Celsius degree (15 C and 25 °C) used for the laboratory experiments could also simulates a boost of the degradation by the mixing of groundwater (with heated ground water coming from energy storage). These conditions could perhaps be reached with the “bio washing machine” scheme. In these conditions, the half life of VC degradation was reduced from 103 days to 70 days in the vadose zone.

We have not investigated the type of the bacterial strains involved in this aerobic degradation. The efficiency of the degradation was generally linked with the type of strains and the lag between the first contact with the new substrate (VC and VC and c-1,2-DCE).

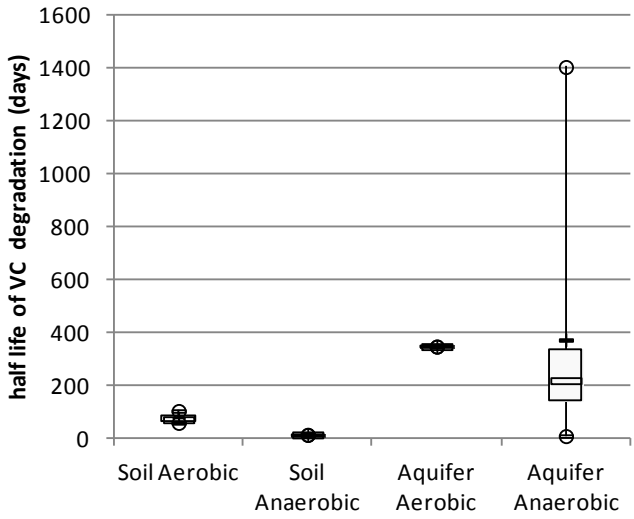

Figure 14 : Box plot of half life VC degradation for the 2 types of soil and 2 types of redox conditions

7 Conclusions and perspectives

Several explanations are provided concerning the limited presence of vinyl chloride (VC) in the soil gas plume in the vadose zone, in the down gradient of a groundwater plume of TCE / cis-1,2 DCE.

The in situ surveys highlight the sinking behavior of the chlorine solvents which increase the distance of the plume core and the water table in the down gradient.

In the specific case of the pilote site « Ile de France » the role of the capillary fringe was also important: the width of the vadose zone (1.5 - 2 m BG) was even reduced by the development of the capillary fringe in the silty clayed loam to a width of approximately 1 meter. In this case the soil gas was very sensible of the movement of the water table, and the presence of a concrete slab at the surface expands the pumping/compressing role of the water table for the soil gas.

In this context of potential large soil gas migration due to soil gas convective motion, the average time of residence of the soil gas in the vadose zone was rather difficult to assess.

The laboratory works, which was conducted with triplicate microcosms, demonstrate the presence of the aerobic degradation process. Monitored laboratory microcosms showed an aerobic biotic mechanism of a slow and median degradation of VC and DCE under conditions close to the vadose zone (15°C). The higher temperatures used to boost the slow kinetics of reactions (25°C) could simulate the anthropogenic heating of the groundwater.

The kinetics obtained in insaturated conditions were similar with kinetics obtained with saturated soils : the diffusion or convection of soil gas were the majors factors which could reduced the aerobic degradation of the plume before soil vapor intrusion in a building.

The aerobic biodegradation of VC in vadose zone gives hope to the possible inclusion of this biodegradation term in a qualitative mass flux balances of chlorine solvent and metabolites reaching the soil surface. A better determination of the conditions of this degradation is necessary to enable effective control of mitigating vapor flow.

8 Acknowledgments

This study was supported by the Interreg IV B, the fund of the Picardy region and ADEME.
It was conducted with the collaboration of the Center for Hydrogeology and Geothermics (CHYN, Neuchâtel).

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Document description

Title: Attenuation of the vinyl chloride in the vadose zone - Study of VC degradation in the unsaturated zone at different scales
INERIS reference: DRC-13-114341-03490A
Number of Pages: 37

Date of publication: May 17 2013
Contact: Olivier Bour (olivier.bour@ineris.fr) & Remy Albrecht
Key words: VC, attenuation, vadose, microcosms, aerobic oxidation, biodegradation

Investing in Opportunities
This project has received European Regional Development Funding through INTERREG IV B.
INTERREG IVB