COMPLETE DEGRADATION OF TETRACHLOROETHYLENE WITH AN ANAEROBIC GROUNDWATER CULTURE - EXPERIMENTAL AND NUMERICAL INVESTIGATION.

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Abstract

Dissolution and biodegradation are the two key processes in the remediation of a contaminated site with dense non-aqueous phase liquids (DNAPLs). Here, we present preliminary experimental and numerical modelling results in order to provide us with the knowledge for the design of a 2 dimensional tank experiment. This tank experiment focusses on the flow and biodegradation near a pure DNAPL. We determined kinetic parameters of an anaerobic dechlorinating culture, which is able to degrade high concentrations of PCE (approximately 0.5 mM). These kinetic parameters will be used for numerical modelling.

1. INTRODUCTION

Widespread use of chlorinated solvents in dry cleaning industry and in metal degreasing operations has caused major soil and groundwater pollution. The chlorinated solvents, for example tetrachloroethylene (PCE), belong to the group of dense non-aqueous phase liquids (DNAPLs). A spill of DNAPL causes serious groundwater pollution problems because it is immiscible with water and has a higher density. Due to these properties, a DNAPL can migrate vertically and penetrate the groundwater table when released on the land surface. Over the whole trajectory of the migrating DNAPL, a residual zone of immobile blobs of DNAPL can be retained. It can continue downward migration until it reaches a low-permeable layer, which may result in DNAPL pooling.

Due to the sensitivity of DNAPL to the small-scale heterogeneities of the soil, it is very hard to predict the location of DNAPL blobs and pools in deeper soil layers. This makes DNAPL contaminations difficult to remove. In addition, the large contact area between DNAPL and water, especially in residual configurations, might result in a continuous delivery of DNAPL to the groundwater for decades [*Schwille*, 1998]. There are several techniques, such as excavation, pump and treat, chemical oxidation, thermal methods, and co-solvent and surfactant flushing, which have been employed, with varying success, for DNAPL cleanup. But they all suffer from technical and/or economical disadvantages. For some time, biodegradation is seen as a cost-effective method to remediate dissolved plumes and, recently, as a post-treatment method after a chemical or physical remediation technology [*Christ et al.*, 2005]. This is the result of a growing interest in the possibility of biodegradation near DNAPL source zones [*Adamson et al.*, 2003]. This is believed to

cause an enhancement of dissolution from DNAPL, which will, consequently, lead to a faster remediation of sites contaminated with DNAPL.

The potential of biodegradation near DNAPL source zones has been investigated in two ways. First, various researchers [*Nielsen and Keasling*, 1999, *Dennis et al.*, 2003, *Yu and Semprini*, 2004, *Isalou et al.*, 1998] reported a degradation of tetrachloroethylene (PCE) at high concentrations (>0.3 mM) in laboratory experiments. However, the main focus of biodegradation studies was on the possibilities of enhancing DNAPL biodegradation, for example, by the use of a specific electron donor [*Carr and Hughes*, 1998, *Fennell et al.*, 1997, *Yu and Semprini*, 2002]. Secondly, a few studies investigated the interplay between flow and biodegradation near DNAPLs mainly through numerical experiments on enhanced dissolution. *Seagren et al.* [1993] started to model flushing and biodegradation in the presence of pure DNAPL to determine in which situations the biodegradation had a positive effect on the dissolution rates. Also experiments were performed to determine the dissolution enhancement [*Yang and McCarty*, 2000, 2002, *Cope and Hughes*, 2001], usually in column experiments. Questions remain on how the flow rate can affect the dissolution rate. The aim of our study is to investigate this issue through numerical and laboratory experiments.

Here, we present results from batch experiments with PCE as the basis for our numerical and 2D experimental investigations. In particular, values for kinetic parameters of the dechlorinating culture are obtained. These parameters are used for our numerical model, for the design of a large sandbox experiment, and to determine how to run the experiments within suitable time frames. Sandbox experiments will be conducted in an two-dimensional tank of dimensions 1 (h) by 2 (l) by 0.05 m (d) and filled with natural sand. The aim of the tank experiment is to provide more insight into the (2D) flow and degradation of a DNAPL in the presence of pure product. Groundwater will flow horizontally through the medium after a spill of DNAPL will be introduced at the top of the sandbox by means of a volume of PCE that will be infiltrated. This PCE will flow downwards into the sandbox and provide a small pool of free product located at the bottom of the tank. The sandbox will then be inoculated with the same dechlorinating culture as in the batch experiments and the nutrients will be provided via the inflow solution. Before the start of the tank experiment, numerical simulations will be performed accounting for DNAPL dissolution and biodegradation. We will report here on the preliminary stages of our preparation for the design and simulation of our tank experiments.

2. MATERIAL AND METHODS

2.1. **Chemicals.** Commercially available PCE, TCE, and cDCE were used in the kinetic experiments. PCE, TCE, cDCE (99.9%) were obtained from Merck and were used in the preparation of analytical standards and PCE for the addition to the batch experiments.

2.2. **Culture medium and growth conditions.** Commonly, PCE is sequentially degraded to trichloroethylene (TCE), cis-dichloroethylene (cDCE), vinyl chloride (VC), ethene (ETH) and methane [*McCarty*, 1997]. This degradation process is called dehalogenation, or dechlorination, in which a chlorine atom is biologically replaced by a hydrogen atom during each step. The only known micro-organisms that can completely degrade PCE are *Dehalococcoides ethenogenes* [*Maymó-Gatell et al.*, 1995].

We used a dechlorinating culture provide by Bioclear B.V. from a PCE-contaminated site, Evenblij in Hoogeveen (The Netherlands). It was delivered as groundwater samples that were collected anaerobically in green-glass bottles. In a glove bag, 10 ml of this groundwater and 90 ml of an anaerobic medium were transferred to 120 ml glass vials closed with a viton stop. The medium was autoclaved and contained: 2 mM Na-acetate, 5 mM K-L-lactate, 1.6 mM NH_4Cl, 0.37 mM KH_2PO_4, and 16.4 mM $NaHCO_3$. Resazurin (1 mg/l) (Sigma-Aldrich) was added as a redox indicator. To completely reduce the medium, a few grains of sodium-dithionite ($Na_2O_4S_2$, Aldrich) were added. After the addition of the groundwater, PCE dissolved in methanol (50 mM PCE) was added to obtain concentrations in the range of 0 to 0.5 mM PCE in the vials (for comparison: the solubility of PCE in water is approximately 0.9 mM aqueous concentration). During cultivation at 20 ℃ on a shaker, the 120 ml vials were covered with aluminium foil to prevent growth of photosynthetic micro-organisms.

2.3. **Analytical methods.** The total concentration of each chlorinated ethene was determined by a gas chromatograph (GC) equipped with two detectors: an electron capture detector (ECD) and an flame ionization detector (FID). At various times after cultivation, 1 ml water samples were transferred to 2 ml vials containing 30 μl of H_3PO_4 to prevent further degradation of the chlorinated ethenes in the measurement vials. After allowing time for equilibration between water sample and the headspace of 2 ml vials, a Solid Phase Micro Extraction (SPME) (Supelco) device was put for 5 minutes in the headspace. The SPME fiber, has a polydimethylsiloxane (PDMS) coating and a thickness of 100 μm. Chlorinated ethenes in the headspace would adsorb to the PDMS coating. After adsorption of the volatile compounds, the SPME fiber was inserted into the inlet of a gas chromatograph for 3 minutes for desorption at 240 ℃. Chromatographic separation was achieved using a 30 m x 0.31 μm GS-GasPro column with helium as the carrier gas. The GC oven was initially set at 30 ℃ for 3 minutes, heated at 30 ℃/min to 180 ℃ and at 25 ℃/min to 230 ℃, and kept at 230 ℃ for 10 minutes. To prevent overshoot in the GC column, samples with a concentration higher than 0.1 mM PCE were diluted with distilled water.

3. MODEL DEVELOPMENT

In the tank experiment, two phases will be present, water and DNAPL. Governing equations for mass transport of mobile components within each phase are written. The PCE phase is present in a pooled configuration and as immobile blobs along the flow path of the DNAPL. We will assume that the DNAPL in all configurations are immobile and, thus, only single-phase flow will be considered. The mass balance equation for the components in the water phase can be written as:

$$\frac{\partial n S^w C_i^w}{\partial t} + \underline{\nabla} \cdot (\underline{q}^w C_i^w - \underline{\nabla} \cdot (n S^w \underline{\underline{D}} \cdot \underline{\nabla} C_i^w) = r_i^{\text{diss}} - r_i^{\text{ads}} - \sum_{j=1}^{N^x} r_{ij}^{\text{deg}} + \sum_{j=1}^{N^x} r_{ij}^{\text{prod}} \qquad (1)$$

where n [-] is porosity, S^w [-] is water saturation, C_i^w [M/L^3] is water phase concentration of component i, $\underline{q}^w$ [L/T] is water phase Darcy velocity vector, $\underline{\underline{D}}$ [M/L^{2}T] is the diffusion-dispersion tensor, r_i^{diss} [M/L^{3}T] is the dissolution rate of DNAPL component i

into the water, r_i^{ads} [M/L^{3}T] is the adsorption rate of component i, r_{ij}^{deg} [M/L^{3}T] is the degradation of component i by bacterial consortium j, and r_{ij}^{prod} [M/L^{3}T] is the production of component i due to the degradation of the mother product by bacterial consortium j. The mass balance equation for the components in the DNAPL phase can be written as:

$$\frac{\partial n S^n C_i^n}{\partial t} = -r_i^{\text{diss}} \tag{2}$$

where S^n [-] is DNAPL saturation, and C_i^n [M/L^3] is DNAPL phase concentration of component i. The degradation of the components is performed by bacteria, whose growth and decay are modelled according to the following mass balance equation:

$$\frac{\partial X_j}{\partial t} = -r_j^{\text{decay}} + \sum_{i=1}^{N^w} (Y_{ij} r_{ij}^{\text{deg}}) \tag{3}$$

where X_j [M/L^3] is the biomass concentration, r_j^{decay} [1/T] is the decay constant, and Y_{ij} [-] a yield coefficient. This equation can be written for various bacterial consortia. To complete the set of governing equations, appropriate relationships for r_i^{diss}, r_i^{ads}, r_{ij}^{deg}, r_{ij}^{prod}, and r_j^{decay} are needed. In this paper, we are only interested in the biodegradation rate, r_{ij}^{deg}. In particular, we have performed batch experiments to obtain information on the coefficients in the r_{ij}^{deg} term. We assume that the complete degradation of PCE to ethene is achieved by one bacterial consortium (so, the subscript j may be dropped). A number of models have been developed to describe anaerobic dechlorination. The most common formula is the Michaelis-Menten relationship that prescribes a linear increase in the growth rate followed by a stationary phase with higher substrate concentrations:

$$r_i^{\text{deg}} = \mu_{\max,i} X \frac{C_i^w}{K_{m,i} + C_i^w} \tag{4}$$

where $\mu_{\max,i}$ [1/T] is the maximum specific growth rate, and $K_{m,i}$ [M/L^3] is the half-saturation constant. The index i stands for the components PCE (i=1), TCE (i=2), and cDCE (i=3). Here we assume that the concentration of electron donor is high enough as not to pose any limitations. The mass balance equation 1 in our batch systems, where no soil and no pure phase DNAPL are present, reduces to:

$$\frac{dC_i^w}{dt} = -\mu_{\max,i} X \frac{C_i^w}{K_{m,i} + C_i^w} + \mu_{\max,i} X \frac{C_{i-1}^w}{K_{m,i-1} + C_{i-1}^w} \tag{5}$$

where the second term on the right hand side accounts for production of TCE by PCE, or cDCE by TCE. Here we have assumed that the concentration of all the components are given in mol/l, so we do not need to take into account the molecular weight of the components. Equation 3, reduces to:

$$\frac{dX}{dt} = -k^{\text{decay}} X + Y \sum r_i^{\text{deg}} \tag{6}$$

where k^{decay} [1/T] is the decay coefficient. We assume that the microbial consortia can grow from the degradation of all chlorinated ethenes. Therefore, we make a summation of the degradation rates of all chlorinated ethenes. Furthermore, the bacterial growth is not

dependent on the DNAPL component that is degraded, therefore, the yield coefficient, Y, is a constant.

4. RESULTS AND DISCUSSION

As mentioned earlier, number of solutions containing dissolved PCE with initial concentrations in the range of 0 to 0.5 mM were cultured and then concentrations of PCE, TCE, and cDCE were plotted as a function of time. The plots of measured PCE concentrations as a function of time for various initial PCE concentrations are given in Figure 1. It is obvious that biodegradation of PCE occurs even at a concentration of 0.5 mM. It is also clear that the higher the initial concentration, the longer the time to full degradation. However, a lag phase, during which the bacterial culture should grow or adapt to the conditions, seems to be independent of the initial PCE concentration. In these experiments, the lag phase is difficult to see because of the absence of a measurement point at day 0. However, the first measurement points shown in the graph correspond to the intended initial PCE concentration in the vials. A possible explanation for the slow start in biodegradation is, that, first, fermentative bacteria had to convert lactate into hydrogen, which is the major source of electron donor in reductive dechlorination [*DiStefano et al.*, 1992]. The maximum rate at which PCE is degraded is approximately 0.025 mM/day.

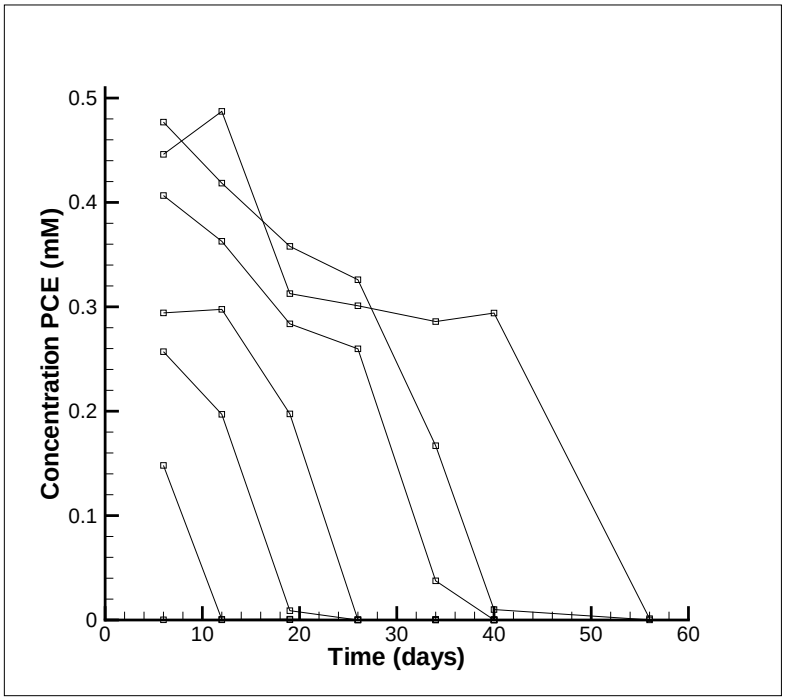

FIGURE 1. Degradation of PCE with time, with different initial PCE concentrations.

PCE degrades into TCE, which rapidly transfers to cDCE in all batches (an example is shown in Figure 2). Sometimes TCE concentrations were not detected as the degradation of TCE occurs fast. An incomplete mass balance for the chlorinated ethenes occurs after day 12. However, this corresponds to the appearance of an unidentified peak on

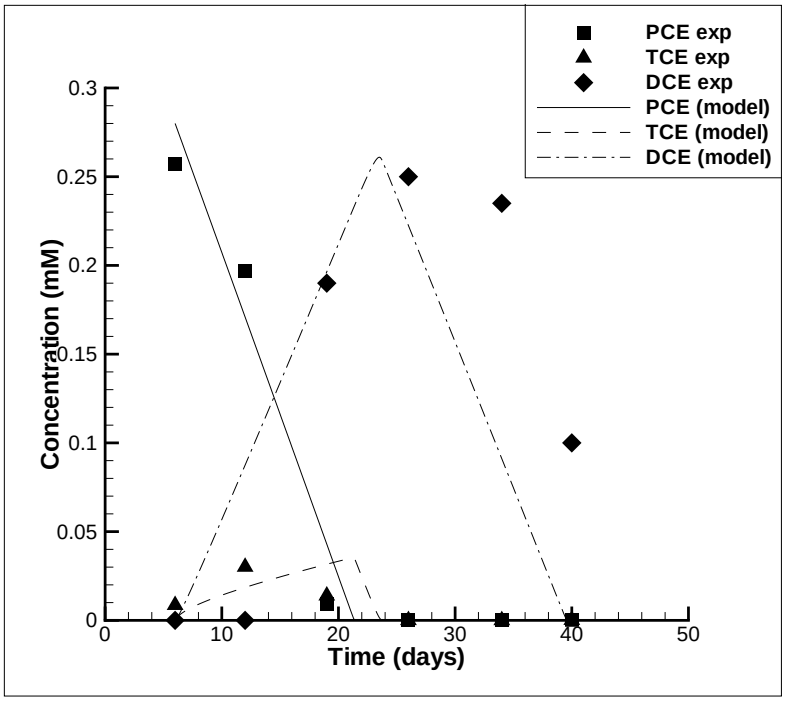

FIGURE 2. Degradation of PCE, TCE, and cDCE in batch experiment and model simulation.

the gas chromatogram, which probably is VC, but was not calibrated. After day 34, another peak appears, which could be ethene. As there is no cDCE measured at day 12 in the experiments, this could have been degraded into VC. However, at day 19 a high cDCE concentration is measured. This suggests that high TCE concentrations affect the cDCE degradation. *Yu et al.* [2005] showed that cDCE dechlorination was inhibited by TCE but it was unaffected or very weakly inhibited by PCE. This type of inhibition is called competitive inhibition and is modelled with a modification to the Michaelis-Menten equation on the half-saturation constant. The equivalent of equation 5 for cDCE reads:

$$\frac{dC^w_{DCE}}{dt} = -\mu_{\max,\mathrm{DCE}} X \frac{C^w_{DCE}}{K_{m,DCE}(1+\frac{C^w_{TCE}}{K_{I,TCE}}) + C^w_{DCE}} + \mu_{\max,\mathrm{TCE}} X \frac{C^w_{TCE}}{K_{m,TCE} + C^w_{TCE}} \quad (7)$$

A kinetic model was developed according to equation 5 and 6. Furthermore, an inhibition of TCE was set for cDCE degradation, following equation 7. The model was initiated on day 6, to account for the time in which the fermentative bacteria produced the required electron donor. It is beyond the scope of our studies to include fermentative bacteria as well, but it can be an option to include later on, in a similar way as *Fennell and Gossett* [1998] did. The kinetic parameters used in the model simulation are presented in Table 1.

The obtained model results (see Figure 2) are in good agreement with the observed data points. However, the appearance and degradation of cDCE in the numerical model is too fast. The production and the onset of the degradation of cDCE occurs in the model simultaneously with PCE and TCE, which is not according to the observations. A determination of the initial biomass concentration of the dechlorinating bacteria used should be

made, as well as an extension of the experiments to higher initial PCE concentrations to investigate the capabilities of the culture. In particular, the initial biomass concentration has a significant effect on the value of the kinetic parameters and model results. We have determined this by varying the initial biomass concentration.

TABLE 1. Kinetic parameters used in the model simulation.

	PCE	*TCE*	*cDCE*
$\mu_{\max}$ [a] (1/s)	$1.5{\cdot}10^{-7}$	$1.4{\cdot}10^{-7}$	$1.33{\cdot}10^{-7}$
K_m [a] (M)	$1{\cdot}10^{-7}$	$1{\cdot}10^{-6}$	$1{\cdot}10^{-7}$
$K_{I,\mathrm{TCE}}$ [a]	-	-	$1{\cdot}10^{-9}$
Biomass, X			
Initial concentration [b] (M)		$1.4{\cdot}10^{-3}$	
Y [c]		0.26	
K_d [d] (1/s)		$6.65{\cdot}10^{-9}$	

[a] Obtained from the experimental data fitting.
[b] Converted from *Yu and Semprini* [2004].
[c] Comparable to *Yu and Semprini* [2004], *Chu et al.* [2004].
[d] $0.05{\cdot}\mu_{\max,\mathrm{PCE}}$, following *Ghoshal and Luthy* [1998].

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