Biodegradation capacity in Utrecht

Determining biodegradation processes for chlorinated contaminants using innovative next level monitoring techniques

Investing in Opportunities
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Summary

The municipality of Utrecht has commissioned Bioclear to determine the biodegradation capacity for soil contaminants (especially chlorinated ethenes) in the subsurface of Utrecht as part of the CityChlor project. The main aim of this project is to determine if natural attenuation (NA) of the VOC (volatile chlorinated hydrocarbons) contamination occurs in the groundwater in the city centre of Utrecht and, if so, which biological processes play a role. The contamination mainly consist of the chlorinated hydrocarbons PCE and TCE, and the (intermediate) degradation products cis-DCE and VC which are found in the subsurface aquifers of the city centre. The source of the various plumes are not always known and the plumes often overlap therefore an area focused approach is preferred in Utrecht.

Within the city centre of Utrecht, several ATES systems are being employed and these systems are expected to have an effect on the degradation of the contaminants. The contribution of ATES systems to an area wide contaminated groundwater management approach is studied and its potential functioning as a "Bio-washing Machine" is being explored. This current research is focused on how the (bio)monitoring can be optimized.

Up until recently it was assumed that complete anaerobic degradation of PCE, TCE, cis-DCE and VC was only possible via reductive dechlorination. This process only occurs under methanogenic conditions. Under moderately reduced (iron to sulphate reducing) conditions, PCE and TCE can be degraded to cis-DCE and VC. The degradation of cis-DCE and VC to harmless compounds is hampered, resulting in accumulation of these degradation products. However groundwater plumes seem to stabilize and concentrations decrease, assuming there is another degradation process involved.

Biological micro-aerophilic degradation of cis-DCE is not widespread. To date, only one bacterium is known to use cis-DCE as a (single) carbon source, the bacterium Polaromonas sp. JS666. Bacteria that can degrade VC aerobically are not that unique and are found in samples from both contaminated and non contaminated sites. Epoxyalkane–coenzyme M transferase enzyme (*etnE*) and alkene monooxygenase (*etnC*) are two genes involved in VC degradation under micro-aerophilic degradation. These genes are a suitable biomarker for VC oxidation.

In this study the biodegradation capacity was determined by conducting molecular analysis on groundwater, compound specific stable isotope analysis, lab microcosms, analysis on BACTRAPS and performing molecular analysis on MicroTraps.

Molecular analyses on 19 groundwater samples, scattered over the city centre area of Utrecht were conducted in 2010 to determine whether *Dehalococcoides* (DHC) or *Polaromonas* bacteria and the genes (*etnE* and *etnC*) were present. In 2001 and 2008 similar analyses were performed on 11 groundwater samples. In 2001 and 2008 in six of the eleven wells the DHC bacteria were detected and in five of the wells the genes *etnE* and/or *etnC* were detected. Samples were obtained using the traditional groundwater sampling method. The occurrence of the genes positively correlate with the high concentration of VC in these monitoring wells.

Only in a selection of the sampled monitoring wells in 2010 (2/19) these genes were detected, which implies that the potential for micro-aerophilic degradation is limited to certain locations and spots. In a few wells conditions were favourable for reductive dechlorination, however not in all wells DHC was detected.

In order to assess the biodegradation capacity isotope monitoring of groundwater samples were conducted at a contaminated field site in Utrecht (Amsterdamsestraatweg). Using the isotopic analyses method it is possible to distinguish between anaerobic an aerobic degradation. The method is based on the principle that ^{12}C substrates are preferentially degraded in biological processes. The results indicated that biodegradation of VC was caused either by reductive dechlorination in conjunction with a substantial concentration decrease due to non-destructive processes (like dilution) or by aerobic VC degradation in conjunction with a low to moderate concentration decrease due to non-destructive processes. Aerobic VC degradation can (most probably) be excluded to a large extend at this site. The results of the isotope analyses strongly indicate that reductive dechlorination is the predominant pathway for VC biodegradation in the source area, in the plume at the Amsterdamsestraatweg other processes may play a role.

In order to assess whether in situ biodegradation of cis-DCE and VC occurs in Utrecht and to identify the micro organisms involved, BACTRAP studies were conducted. The BACTRAPS were deployed at two different sites, one with high PCE and TCE concentrations and one with high cis-DCE and VC concentrations. A BACTRAP® is an in situ microcosm containing ^{13}C-labelled substrate in order to trap bacteria. With regard to VC degradation strong indications for micro-aerophilic degradation were obtained in all sampled wells based on the molecular analyses on the BACTRAPs. The genes involved in micro-aerophilic degradation of VC (etnE and etnC) were detected. The isotope analyses were however focussed on gaining insight in the degradation process of cis-DCE. The isotope data show that CO_2 production occurred, this CO_2 could be derived from the 'naturally' occurring VC or from organic material.

A microcosm degradation study was conducted to investigate whether cis-DCE is converted to VC (reductive dechlorination) or to CO_2 (micro-aerophilic oxidation). Soil and groundwater samples from different locations in Utrecht were used to conduct a number of degradation batch tests. To make sure that any decrease in DCE concentration is the result of biodegradation a biotic and a sterile abiotic reference test were performed. The results showed that:

- In the abiotic reference test no decrease in concentration was observed, indication that any decrease in de biotic tests is a result of biological degradation;
- Biological VC degradation did occur after 6 weeks of incubation;
- The isotope analyses indicated that (micro-aerophilic) oxidation of VC has occurred (formation of labelled CO_2);
- The oxygen concentrations in the labtests were low (in the headspace lower than 1%);
- The degradation rate for VC was approximately 0,1 day^{-1};
- The relevant genes involved in micro-aerophilic degradation of VC were detected in the soil phase of the labtests;
- The bacteria involved in micro-aerophilic degradation are more prone to adhere to soil particles;
- The occurrence of biological degradation for cis-DCE could not be proven.

The MicroTraps are in-situ mesocosms which are used to validate and determine on site biodegradation (rate) of both reductive dechlorination and micro-aerophilic degradation. It is known from previous molecular analyses that the bacteria carrying the genes responsible for microaerophilic degradation are more likely to adhere to soil surface. Permeable tubes (HDPE) or MicroTraps (MT) filled with soil from the location were installed at the filter depth in a monitoring well over a period of 6 months. The monitoring wells selected show difference in VOC concentrations and/or redox conditions, implying different biological degradation processes are prevalent at these sites. Every 2 months soil from a MT was sampled, preserved and analyzed.

From the MicroTraps research the following can be concluded:

- In monitoring well at the Amsterdamsestraatweg the dechlorinating bacteria *Dehalococcoides* was detected, indicating that reductive dechlorination is the dominant active process on site.
- In monitoring well at the Nachtegaalstraat the dominant process is VC oxidation, the genes *etnE* and *etnC* were detected.
- In monitoring well 53 (near the ATES 'proeftuin' area) VC was the dominant contaminant present. The genes *etnE* and *etnC* were detected in the soil of the MicroTraps, it is therefore likely that micro-aerophilic degradation is the dominant process.

The total bacterial count was detected in higher numbers in the MicroTraps compared to groundwater samples, indicating that the MicroTrap sampling method is a representative method of detection.

The results as presented in this research confirm a degradation capacity for both reductive dechlorination and micro-aerophilic degradation in the subsurface of Utrecht. Previous results at the specific sites are confirmed by several lines of evidence using advanced monitoring tools. The predominant process is reductive dechlorination in monitoring wells with high concentrations of VOC. However in monitoring wells with low VC and cis-DCE concentrations and iron to sulphate reducing conditions it is more likely that micro-aerophilic degradation processes are dominant. The extrapolation of the detected degradation processes to the whole area is possible since the current research results provide several lines of evidence that micro-aerophilic degradation and reductive dechlorination processes are present at different locations within the area. However the results are based on a selection of monitoring wells distributed over the whole city centre of Utrecht. A strong correlation for occurrence of either of the processes remains to be investigated.

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Appendices

List of acronyms

ATES	Aquifer Thermal Energy Storage
Cis-DCE	Cis dichloroethylene
CSIA	Compound-specific stable isotope analysis
DHC	*Dehalococcoides*
DIC	Dissolved Inorganic Carbon
etnC	Alkene monooxygenase
etnE	Epoxyalkane–coenzyme M transferase enzyme
m-gl	Meters below ground level
MicroTrap	In situ mesocosm
MT	MicroTrap permeable tube used in the in situ mesocosm
NA	Natural attenuation: sum of processes that occur without active measures resulting in a decrease in contaminant mass
NPOC	Non purgeable organic carbon
PCE	Tertrachloroethylene
TCE	Trichloroethylene
VC	Vinylchloride
vcrA	Vinylchloride reductase
VOC	volatile chlorinated hydrocarbons

1 Introduction

The municipality of Utrecht has commissioned Bioclear to determine the biodegradation capacity for soil contaminants (especially chlorinated ethenes) in the subsurface of Utrecht as part of the CityChlor project. The experiments and activities have been performed as described in the quotations 20103770/6869 dated July 9th 2010; 20103770/7406 dated April 18th 2011; 20103770/7717 dated October 14th 2011 and 20103770/8240 dated July 17th 2012.

The main aim of this project is to determine if natural attenuation (NA) of the VOC (volatile chlorinated hydrocarbons) contamination occurs in the groundwater in the city centre of Utrecht and, if so, which biological processes play a role. Does the subsurface contain a biodegradation capacity? If this biodegradation capacity is present, is it possible to quantitatively determine the biodegradation capacity and are these processes occurring within the whole city centre area or only within specific sites or zones? When the biodegradation capacity is known and quantified and the contaminant flux from source zones to the groundwater is known, it will be possible to determine realistic boundaries for an area-oriented groundwater management approach.

1.1 Area based approach

Large-scale contamination of the deeper subsurface aquifers is present within the city centre of Utrecht. Contaminants mainly consist of the chlorinated hydrocarbons PCE and TCE, and the (intermediary) degradation products cis-DCE and VC. The contamination found in the deeper subsurface aquifers cannot always be retraced to the original source and/or the responsible party. Additionally, it has emerged that 'plumes' from different sources run into each other at depth. As a case-focused approach is thus illogical and unmanageable, an area-focused approach is the preferable option.

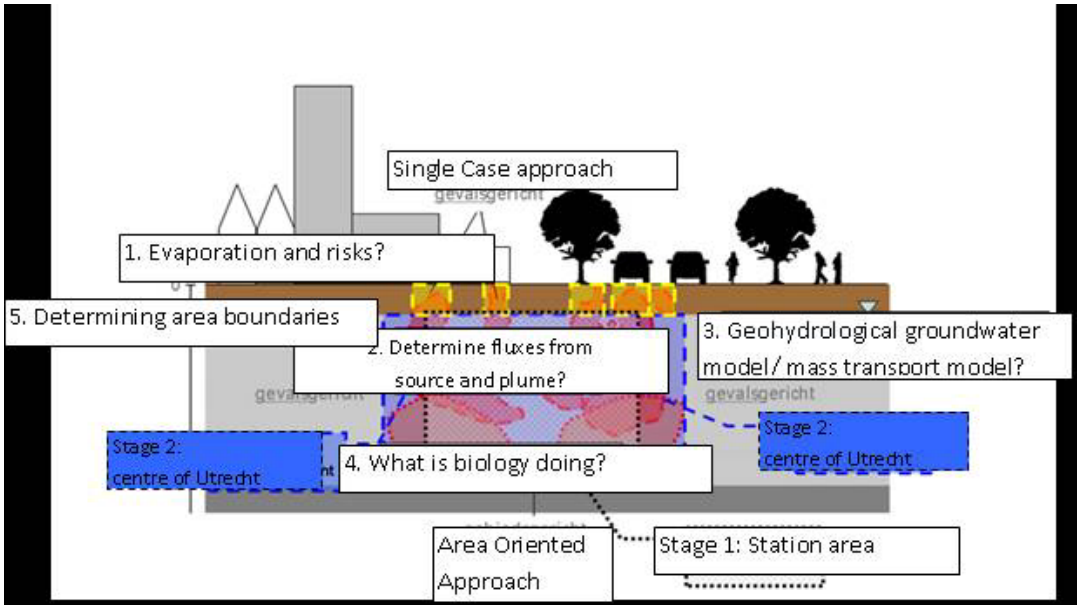

Figure 1 Schematic representation of the aspects addressed in the Utrecht CityChlor project

A number of aspects must be considered when determining the area and identifying controllable (= sustainable) aspects to manage risk, see Figure 1. Contamination will migrate into the (deeper) groundwater from the different source areas that are still present and have not yet been remediated. This means that these groundwater plumes will be continuously fed and continue to expand.

On the other hand, processes such as biological degradation, dilution, evaporation and large scale Aquifer Thermal Energy Storage (ATES) systems impact the concentration and physical distribution of groundwater components. Degradation of the contamination reduces the total mass and could prevent further migration. Therefore, contaminant degradation must be examined fully in a manner that allows proper estimation of plume behaviour, rather than only relying on numerical modeling with average degradation rates from literature. Such measurements contribute to the estimation of both the risks as a result of the contamination (do contaminants reach critical objects due to migration via groundwater flow?) and to determine the system boundaries of an area oriented approach.

Within the city centre of Utrecht, several ATES are being employed and these systems are expected to have an effect on the degradation of these contaminants. The contribution of ATES systems to an area wide contaminated groundwater management approach is studied and its potential functioning as a "Bio-washing Machine" is being explored. This research is focused on how the (bio)monitoring can be optimized.

The results from this biodegradation study will support the view of how to monitor area-focused groundwater management effectively and efficiently.

1.2 Readers guide

This research comprises of multiple experiments and activities in order to determine the biological degradation capacity in the subsurface of Utrecht. At first (Chapter 2), the processes responsible for the VOC biodegradation are described and how these can be affected in the subsurface. Secondly in chapter 3 the set-up of the research activities are described, which are divided in an quantitative and qualitative approach. In chapter 4 the sampling methods used for determining the biodegradation capacity are stated. In the following chapters 5, 6, 7 and 8 the results of the experiments are described and assessed. The conclusions are given in the final chapter number 10.

2 Degradation processes in the city centre area of Utrecht

Prior to the experiments and fieldwork elaborate knowledge on the reductive and oxidative degradation processes was gained by conducting a literature review, see Appendix 1. The results are summarized in this chapter.

Up until recently it was assumed that complete anaerobic degradation of PCE, TCE, cis-DCE and VC was only possible via reductive dechlorination. This process only occurs under highly reduced conditions in combination with dechlorinating biomass and sufficient organic carbon.

Under moderately reduced (iron to sulphate reducing) conditions, PCE and TCE can be degraded to cis-DCE and VC. These moderately reduced conditions are unfavourable for further reductive anaerobic degradation of cis-DCE and VC to the harmless end products ethane and/or ethane, resulting in an accumulation of these intermediate degradation products. However, at several sites groundwater plumes with cis-DCE and VC seem to stabilize and concentrations decrease, assuming there is another degradation process than reductive dechlorination involved. It was assumed that anaerobic oxidative degradation or micro-aerophilic degradation processes could be an explanation for this observation.

2.1 Reductive dechlorination

In the process of reductive dechlorination, PCE and TCE are degraded via cis-DCE and VC to ethene and/or ethane. This process is most efficient under highly reduced (methanogenic) conditions.

The bacteria involved in this process are *Dehalococcoides* spp. The specific gene involved in the critical step of degradation of VC to ethene, under strict anaerobic conditions, is *vcrA* (vinylchloride reductase).

The process of reductive dechlorination is up until now most intensively investigated. The VOC compound is used as electron acceptor, available organic carbon is used as electron donor. PCE and TCE can be degraded under iron reducing to methanogenic conditions to cis-DCE and VC. The intermediates cis-DCE and VC can be degraded to ethene or ethane but complete degradation to the harmless end products only occurs under the following conditions:

- Presence of strictly reduced (methanogenic) conditions.
- Sufficient organic carbon (TOC) available.
- Presence of dechlorinating biomass, *Dehalococcoides* sp.

If these criteria are not met the compounds cis-DCE and VC are likely to accumulate, unless other processes are involved.

2.2 Micro-aerophilic degradation

Until recently, it was assumed that under slightly less anaerobic conditions, like iron reducing conditions, degradation of cis-DCE and VC could also occur through a process known as anaerobic oxidation. This process was thought to take place under mildly anaerobic conditions. Oxidation could take place with nitrate, iron (III) sulphate or CO_2 as electron acceptor instead of oxygen. One reason for this proposed metabolic pathway was the observation that plumes with cis-DCE and VC in moderately reduced soils were stable even though complete reductive dechlorination to ethene or ethane did not occur. Recent research (Chuang, 2010) has shown that in these cases actually micro-aerophilic aerobic oxidation occur. During micro-aerophilic degradation cis-DCE or VC is metabolized and transformed into CO_2 with oxygen (in very low concentrations) as terminal electron acceptor.

Extensive research is still being conducted on this micro-aerophilic process. However, it is clear that other processes than reductive dechlorination are involved in the degradation of cis-DCE and VC, and should be taken into account when studying plume behaviour and the influence of NA processes on this behaviour.

It should be noted that it is generally accepted that PCE is not aerobically biodegradable, and that TCE can only be co-metabolized under aerobic conditions. The requirements for aerobic co-metabolic degradation of TCE are usually not met in soil and groundwater systems.

2.2.1 Cis DCE degradation

Biological micro-aerophilic degradation of cis-DCE is not widespread. To date, only one bacterium is known to use cis-DCE as a (single) carbon source i.e. electron donor (Jennings, 2009). This bacterium is called *Polaromonas* sp JS666. It is possible that more bacteria are capable of performing this degradation process, these bacteria have however not (yet) been identified. This bacterium is possibly also capable of using other substances as electron donor (and thus performing other reactions besides degradation of cis-DCE).

2.2.2 VC degradation

Bacteria that can degrade VC aerobically are not unique and are found in samples from both contaminated and non contaminated sites (Mattes, 2010). There are three groups of bacteria capable of VC degradation under aerobic conditions (Chuang, 2010).

- Methanotrophes
- Ethenotrophes
- VC assimilating bacteria

It is hard to differentiate aerobic ethene-assimilating bacteria which can degrade VC and non-VC degrading bacteria (Gossett, 2010). However in another research where bacteria were periodically exposed to VC, the micro organisms were capable to use VC as substrate (Yang, 2008). For an elaborate description on aerobic or micro-aerophilic degradation of VC and cis-DCE a literature review was conducted and is attached in Appendix 1. Epoxyalkane–coenzyme M transferase enzyme *(etnE)* and alkene monooxygenase *(etnC)* are two genes involved in VC degradation under micro-aerophilic degradation. These genes are a suitable biomarker for VC oxidation.

2.3 Abiotic influences on VOC degradation processes

It is known that VOC degradation can be hampered by environmental influences. Some of these conditions are stated below.

2.3.1 Hampered cis-DCE degradation

From literature it is known that in the presence of other chloroethenes, the degradation of cis-1.2-DCE is affected. When 1.1-DCE or TCE is present, cis-DCE can not be degraded by a cis-DCE aerobic metabolic degrading bacterial culture (in a batchtest setup). However in the presence of PCE, cis-DCE was degraded faster (Zhoa, 2009). With increasing VC concentrations the degradation rate of cis-DCE decreased.

2.3.2 Dissolved oxygen convection

Monitoring wells are a tool to assess the groundwater conditions in the subsurface. It is generally assumed that the protocols for groundwater sampling assure a sample that represents the actual conditions in the aquifer from which the sample is taken. However, within the monitoring well processes occur which can affect the local redox conditions around the monitoring well. Literature describes the phenomena of convection, which is convective transport from the top of the monitoring well to the deeper part of the well. With this oxygen is transported. It is possible that bacterial processes could be affected by dissolved oxygen convection in monitoring wells. It is reported that dissolved oxygen convection is influenced by seasonal changes. Wells with a small air pocket (isolation) above the waterline were more susceptible to thermally induced convection (Vroblesky, 2007).

2.4 Abiotic influences on plume behaviour

As described previously the total mass and/or concentrations of the contamination can also be influenced by abiotic processes. The plume behaviour can be affected by many processes some examples are; biological degradation, dilution, evaporation, adsorption and large scale Aquifer Thermal Energy Storage (ATES) systems. As can be expected many processes impact the plume and physical distribution of groundwater components. These processes do not necessarily result in a decrease in contaminant mass, which biodegradation processes do. Therefore it is important to apply tools and methods using several lines of evidence to be sure that an observed decrease in contamination concentrations are a result of biological degradation processes.

3 Assessment of the biodegradation capacity

In this research Bioclear investigated whether biodegradation of VOC occurs in Utrecht and, if so, through which degradation processes. Currently, most research into the degradation of VOC has focused on degradation through reductive dechlorination. For anaerobic degradation of *cis*-dichloroethene (cis-DCE) and vinylchloride (VC), strongly reducing methanogenic conditions are necessary and the presence of specific *Dehalococcoides* bacterial strains is required. In addition to reductive dechlorination, oxidative degradation processes, for example micro-aerophilic degradation may play a role in the degradation of VOC under less reducing conditions, under very low oxygen pressures (based on Dalton's law of partial pressures). However the degradation rate will be low.

For the area-oriented groundwater management approach it is therefore important to determine which degradation processes currently play a role in the subsoil of Utrecht and to assess all possible (known) degradation processes within the research project.

3.1 Research questions

The main research questions that are dealt with in this investigation are:

- Does the subsurface in Utrecht have a degradation capacity for VOC components (qualitative approach)?
- Which degradation process(es) are predominant in Utrecht?
- What is the extent of this biodegradation capacity (quantitative approach)?
- Is this degradation capacity expected to be present uniformly within the area for which an area-oriented approach is foreseen in the city centre of Utrecht?

The investigation is divided into two different approaches.

1. Qualitative approach: determine which degradation processes occur in the subsurface of Utrecht.
2. Quantitative approach: What is the (range of) degradation rates of the relevant degradation processes occurring in the subsurface of Utrecht.

3.2 Qualitative approach

First of all it is important to determine whether biodegradation in the subsurface of Utrecht is occurring; whether it may be via reductive dechlorination or via micro-aerophilic oxidation. If it is proven that degradation occurs that in itself is an added value for the Citychlor project because if degradation occurs (however minor it may be) the pollutant concentrations and mass will decrease in time. In this study the biodegradation capacity was determined by conducting molecular analysis on groundwater samples, BACTRAPS and MicroTraps,

performing compound specific stable isotope analysis and lab microcosms. The performed activities are described in more detail below.

3.2.1 Molecular analyses on groundwater samples

Molecular analyses on groundwater samples were conducted to determine whether *Dehalococcoides* bacteria were present. *Dehalococcoides* is, to date, the only species known to be able to completely degrade chlorinated ethenes, by means of reductive dechlorination, under strongly anaerobic conditions (methanogenic conditions). The bacteria are able to degrade cis-DCE and VC to the harmless end products ethene and/or ethane. The gene vinylchloride reductase (vcrA) is involved in the critical step of degradation of VC to ethene.

The compounds cis-DCE and VC are on the other hand also degradable under micro-aerophilic conditions. The genes epoxyalkane–coenzyme M transferase enzyme (*etnE),* alkene monooxygenase (*etnC)* and bacteria *Polaromonas* are involved in aerobic degradation of VC / cis-DCE. *Polaromonas* is, to date, the only bacteria known to be able to degrade cis-DCE under micro-aerophilic conditions. A molecular analysis for detection of this organism (*Polaromonas*) and these genes (*etnE* and *etnC*) was developed within this study.

The groundwater sampling protocol is described in chapter 4. The results of the groundwater samples analysis are described in chapter 5 *Groundwater sampling & molecular analyses*.

3.2.2 Compound specific stable isotope analyses (CSIA)

In order to assess biodegradation at a contaminated field site in Utrecht (Amsterdamsestraatweg) isotope monitoring of groundwater samples were conducted. With this analysis it is possible to determine whether biodegradation has occurred at the site and it is possible to distinguish between anaerobic and aerobic degradation processes. It is possible to determine the predominant degradation pathway for the lower chlorinated ethenes cis-DCE and VC, independent of the decrease in contaminant concentration. This detection method is based on the principle that ^{12}C substrates are preferentially degraded in biological processes.

Furthermore an isotope balance (calculated using the cumulative isotope ratio of all chlorinated ethenes) also gives insight into whether complete biodegradation occurs. Sampling was conducted by Bioclear and is described in chapter 4, the isotope analyses were conducted by Isodetect GmbH. The results are described in chapter 6.

3.2.3 BACTRAP analyses

In order to assess whether in situ biodegradation of cis-DCE and VC occurs in Utrecht and to identify the microorganisms involved, BACTRAP studies were conducted. In Figure 2 a picture of the BACTRAP is depicted. A BACTRAP® (provided by Isodetect GmbH) is an in situ microcosm containing ^{13}C-labelled substrate in order to trap bacteria. An elaborate description is given in paragraph 4.2.4. Isotope and molecular analyses were conducted on BACTRAPs which were placed in monitoring wells in Utrecht for a period of 3 months. In chapter 4 this sampling method is described in more detail. In chapter 8 the outcome of the experiments are described.

Figure 2 Picture of a BACTRAP

3.2.4 Lab microcosm degradation studies

A laboratory microcosm degradation study was conducted to investigate whether cis-DCE is converted to VC (reductive dechlorination) or to CO_2 (micro-aerophilic oxidation). Soil and groundwater samples from different locations in Utrecht were used to conduct a number of degradation tests. The sampling method is described in more detail in chapter 4, the results are given in chapter 7.

3.2.5 Molecular analyses on MicroTraps

The MicroTraps are in-situ microcosms which are used to validate and determine on site biodegradation (rate) of both reductive dechlorination and micro-aerophilic degradation. It is known from previous molecular analyses that the bacteria carrying the genes responsible for micro-aerophilic degradation are more likely to adhere to soil surfaces as they are present in the water phase. Permeable tubes (HDPE) or MicroTraps (MT) filled with soil from the location were installed at the filter depth in a monitoring tube over a period of 6 months. Every 2 months soil from a MT was sampled, preserved and analyzed. For an elaborated description of this sampling method see chapter 4, the results are lined out in chapter 9.

3.3 Quantitative approach

To determine system boundaries and boundary conditions for source zone treatment it is important to know the degradation rate and extent of degradation occurring in the subsurface of Utrecht.

To determine whether degradation of cis-DCE and/or VC can actually take place in the subsurface of Utrecht degradation tests were conducted. If degradation occurs in these test systems it is possible to determine a degradation rate. The following tests were conducted:

A. Degradation test with contaminated soil and groundwater sample and addition of
    a. cis-DCE
    b. VC (this test was included based on the pollutant data at Utrecht)
B. Degradation test with uncontaminated soil and groundwater sample and addition of contaminant (cis-DCE).

The reason degradation tests B were conducted is that degradation tests with uncontaminated soil samples give insight into whether degradation capacity is present in uncontaminated parts of Utrecht and whether there is an adaptation phase (lag phase).

4 Sampling methods

4.1 Fieldwork

In order to determine the biodegradation capacity field samples were taken. Soil and groundwater were anaerobically sampled and transported to Bioclear. Soil samples appropriate for the degradation tests were selected based on, among others, the concentrations of contaminants and macro-parameters. Groundwater samples selected for a specific purpose, eg. dialyser method or isotope analyses were sampled according to BRL SIKB 2000 'Veldwerk bij milieuhygiënisch bodemonderzoek' in combination with VKB protocol 2002 'Het nemen van grondwatermonsters'.

4.1.1 Analyses

- GC analyses on VOC, ethene, ethane and methane were performed by ACMAA laboratories located in Hengelo.
- Molecular analyses were performed by Bioclear.
- Isotopic analysis were conducted by Isodetect GmbH located in Leipzig Germany.

The sampling dates are presented in the tables and text of the topic of concern in this chapter. In the following paragraphs the different sampling methods are described.

4.2 Sampling methods

4.2.1 Traditional groundwater sampling

To determine the biodegradation capacity in the subsurface of Utrecht molecular analyses (Q-PCR) were conducted on DNA samples extracted from groundwater from monitoring wells located in the city centre of Utrecht. The groundwater samples were taken using standard DNA sampling protocol 100 mL sample with fixative, see Figure 3.

Figure 3 Groundwater sampling with standard sampling protocol

Based on the preliminary results it was expected that a larger volume of groundwater was necessary in order to detect the micro-organisms present in low concentrations, for this a new sampling method was applied, the dialyser method.

4.2.2 Dialyser method

The dialyser method was developed by Bioclear (within the research project Meer met Bodemenergie) to determine small quantities of micro-organisms in environmental groundwater samples. In this method a large volume of groundwater is sampled and concentrated into a smaller volume, thus increasing the chance of detecting specific genes/bacteria that may be present in low amounts. In Figure 4 the setup is depicted.

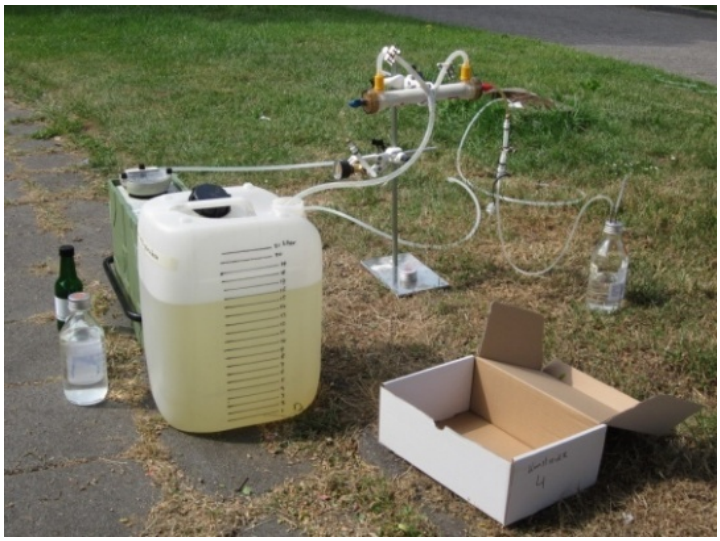

Figure 4 Sampling using the dialyser method

After consultation with the municipality of Utrecht it was decided to sample several monitoring wells with this new method to determine whether this sampling technique is needed in order to detect the genes/bacteria involved in micro-aerophilic degradation.

4.2.3 MicroTraps

In order to validate the determined biodegradation in the lab microcosmtests and investigate the on site biodegradation rate of both reductive dechlorination and micro-aerophilic degradation processes, in situ mesocosms or MicroTraps were installed, see Figure 5.

It is known from previous molecular analyses that the bacteria carrying the genes responsible for micro-aerophilic degradation are more likely to adhere to soil surface than they are present in the water phase, therefore MicroTraps were installed. Permeable tubes or MTs carrying soil from the location were installed at the filter depth in a monitoring tube over a period of 6 months time. The soil is permanently in contact with the surrounding groundwater and thus with the contamination and the bacteria. The bacterial community on the soil will adapt to the groundwater circumstances, and the soil in the MT can be analyzed for the genes responsible for contaminant degradation. Periodically, every 2 months soil from a permeable tube of the MicroTrap was sampled and preserved.

Groundwater samples for chemical analyses were taken before tubes were removed from the monitoring well. This data was used to correlate a decrease in contamination to the presence of specific genes and bacteria.

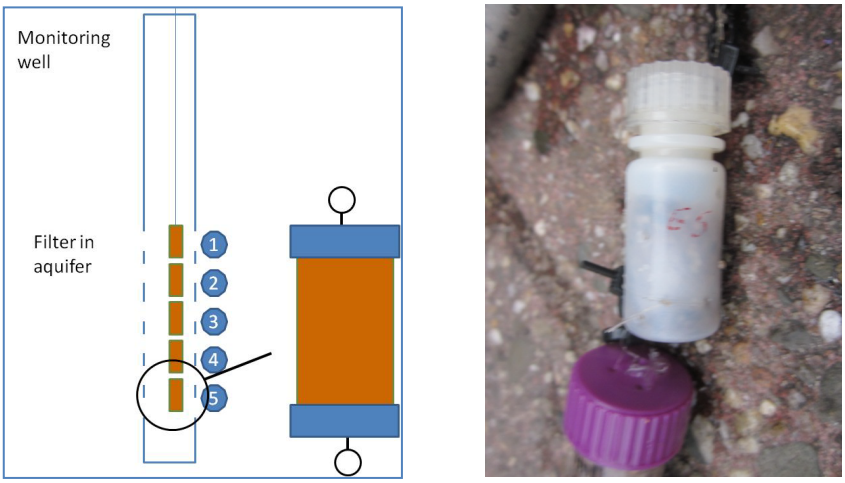

Figure 5 MicroTraps, consisting of five MTs containing soil from the site

4.2.4 BACTRAPS ©

In order to assess whether in situ biodegradation of cis-DCE and VC occurs in Utrecht and to identify the involved micro-organisms, BACTRAP studies were conducted. In Figure 2 a picture of the BACTRAP is shown.

Isotopes of an element have the same number of protons but differ in the number of neutrons, and therefore, they have different masses. Organic substances mainly consist of carbon which exhibits two stable isotopes. Carbon with the mass 13 (^{13}C, 6 protons and 7 neutrons) is the rare abundant stable isotope (1.1 %) compared to carbon with the mass 12 (^{12}C, 6 protons and 6 neutrons) which has an abundance of 98.9 %. ^{13}C-labelled compounds can be synthesized which have a significantly higher ^{13}C-abundance as their naturally present counterparts. These ^{13}C-labelled compounds are suitable as reactive tracers for studying biodegradation of pollutants.

These ^{13}C-labelled cis-DCE BACTRAPs are a powerful tool for providing evidence of in situ pollutant biodegradation. BACTRAPs consist of granular materials providing an appropriate surface for colonization of microorganisms and a reliable adsorption capacity for substrates like organic contaminants like cis-DCE. During reductive dechlorination cis-DCE is utilized as an electron acceptor. In this case, the ^{13}C label will be transformed into ^{13}C-labelled vinylchloride (as a metabolite of this process). Microbial oxidation of cis-DCE (and VC) leads to the formation of CO_2. The ^{13}C-label from cis-DCE will be incorporated in CO_2.

5 Groundwater samples & molecular analyses

5.1 Approach

The samples were taken as described previously in chapter 4. The molecular analyses (qPCR) were conducted on DNA samples extracted from groundwater from 19 monitoring wells distributed in the city centre of Utrecht. Appendix 3 gives an overview of the sampling points. In 2001 and 2008 samples were taken (within the project Meer met Bodemenergie) and analyzed for VOC and molecular occurrence. The monitoring wells are situated in the vicinity of the monitoring wells sampled in 2010 and 2012/2013. The analyses certificates are added in Appendix 6.

5.2 Results

The results from the samples acquired in the period from October – December 2010 show that *EtnC* was only detected in the samples from monitoring wells 67 (26 m-gl) and 53 (38 m-gl), 2.0 E+01 and 7.0 E+03 cells/mL respectively. In sample 53 (38 m-gl) *etnE* (1.0 E+03) was also detected. In none of the other samples *etnC*, *etnE* nor *Poloramonas* was detected.

In 2001 and 2008 concentrations of up to 1,300 µg/L VC were detected in monitoring well 106 (35 m-gl), the bacteria DHC was detected here in this well 2.1E+05 cells/mL. In six of the eleven wells the DHC bacteria were detected and in five of the wells the genes *etnE* and/or *etnC* were detected. The results of all the VOC and molecular analyses from 2001, 2008 and 2010 are shown in Appendix 3.

In a subsequent investigation groundwater samples were taken in the period from August 2012 to February 2013 for the comparison of the number of micro-organisms in the groundwater samples and the MicroTraps. The results are shown in Table 1 and Table 2.

In monitoring well AF2 groundwater was sampled and analyzed in February 2013, DHC was detected up to 6,50E+05 cells/mL. The high abundance of bacteria correspond with the high concentration of VOC.

The presence of the bacteria and genes correspond slightly with the VOC contamination. In the samples taken in 2010 high cis-DCE (75 µg/L) and VC (2,100 µg/L) concentrations were detected in well 53 (38 m-gl). In 2012/2013 the concentrations VC, in this monitoring well are comparable, the genes etnE and entC are detected once again. In 67 (26 m-gl) concentrations of up to 1 µg/L VOCl were detected. In a few wells conditions were favourable for reductive dechlorination, however the bacteria *Dehalococcoides* was not detected in any monitoring well in 2010.

Table 1 Genes and bacteria detected in groundwater of the monitoring wells NF 4.2 (9-11) and 53 (38 -39) in 2012/2013.

Monitoring well (depth m-gl)		**NF4.2 (9-11)**			**53 (38 - 39)**		
Analyses	Date	16-8-2012	12-12-2012	14-02-2013	16-8-2012	12-12-2012	14-02-2013
	Time point	start	t=4	t=6	start	t=4	t=6
contaminant	Unit						
Total Bacteria	cells/mL	6.00E+05	2.10E+04	9.40E+03	2.30E+03	2.40E+04	1.80E+04
Dehalococcoides sp.	cells/mL	<6.9E+1	<1.1E+2	< 5.5E+1	<7.5E+1	<7.1E+1	<4.5E+1
vcrA	cells/mL	<6.9E+1	<1.1E+2	< 5.5E+1	<7.5E+1	<7.1E+1	<4.5E+1
Polaromonas JS666	cells/mL	<6.9E+1	<1.1E+2	< 5.5E+1	<7.5E+1	<7.1E+1	<4.5E+1
etnC	cells/mL	<6.9E+1	<1.1E+2	< 5.5E+1	<7.5E+1	<7.1E+1	6.70E+01
etnE	cells/mL	<6.9E+1	<1.1E+2	< 5.5E+1	<7.5E+1	<7.1E+1	2.90E+02

Table 2 VOC concentrations in monitoring wells NF 4.2 (9-11) and 53 (38 -39).

Monitoring well (depth m-gl)		**NF4.2 (9-11)**				**53 (38 -39)**			
Analyses	Date	9-8-2012	-	12-12-2012	14-02-2013	10-8-2012	17-10-2012	12-12-2012	14-02-2013
	Time point	t=0	t=2	t=4	t=6	t=0	t=2	t=4	t=6
contaminant	Unit								
PCE	µg/L	2	-	<0.1	<0.1	<0.1	<0.1	1.4	<0.1
TCE	µg/L	1.9	-	<0.1	<0.1	<0.1	<0.1	0.57	<0.1
c-DCE	µg/L	48	-	26	20	5.5	11*	7.6	4.5
t-DCE	µg/L	0.6	-	<0.1	<0.1	0.49	0.82	1.3	0.66
VC	µg/L	4.1	-	690	560	1,100	750*	2,400	2,700
Ethene *	µg/L	-	-	29	24	-	7	10	12
Ethane *	µg/L	-	-	<1	<1	-	<1	<1	<1
Redox									
Nitrate	mg/L	2.2	-	0.71	<0.5	<0.5	<1	<0.5	<0.5
Sulphate	mg/L	66	-	76	69	80	7.0	54	64
Methane	µg/L	39	-	42	57	110	83	100	120
NPOC	mg TOC/L	5.3	-	2.5	2.2	4.6	-	5.0	4.3

- not measured, * The values at t=2 for ethene and ethane, c-DCE and VC in well 53 are indicative values. No samples for ethane and ethene were taken from monitoring well NF4.2 at t=2. There was a car on top of the monitoring well NF4.2 so it was not possible to take a sample.

The concentrations VC in 2010 and 2012/2013 in subsequent monitoring wells are comparable (cis-DCE 75, VC 2,100 µg/L), the genes *etnE* and *etnC* however were detected in both years.

Only in the last monitoring time point in 2012/2013 the genes were measured, this might be due to the average low bacterial number in the monitoring well 53. It should be mentioned that these results are derived from groundwater samples which are sampled in a traditional manner. In monitoring well NF 4.2 there is a decrease in PCE and TCE and an increase in the degradation products DCE and VC which would indicate reductive dechlorination, nevertheless no DHC were detected in the groundwater samples.

6 Isotope analysis CSIA

6.1 Approach

Compound-specific stable isotope analysis (CSIA) is an appropriate method for assessing (qualitatively) biodegradation in contaminated aquifers. According to the US-EPA the following criteria apply for evaluating CSIA data to determine whether pollutant biodegradation has occurred:

- Isotope ratio of pollutant (in the plume) is significantly more positive than its primary isotope signature (source zone).
- Changes in isotope ratios of > 2‰ along a groundwater flow path between two sampling points provide evidence of pollutant biodegradation (if the influence of a secondary contaminant source can be excluded as is the case at Amsterdamsestraatweg).

The site at the Amsterdamsestraatweg has a well defined plume and the groundwater flow direction is known, which makes it suitable for CSIA. The report written by Isodetect GmbH is presented in Appendix 2.

6.2 Results

At the site of the Amsterdamsestraatweg the following monitoring wells were sampled: 2015 (6-8 m-gl), 2015 (10-12 m-gl), 2015 (17-19 m-gl), 3006 (25 m-gl), 3021 (25 m-gl) and 3022 (25 m-gl), see Appendix 3 for the map. Monitoring wells 2015 and 3006 are located in the source zone. The other two wells are located downstream from the source zone.

The isotope analyses are usually expressed in delta (δ) per mil [‰] relative to the international standard as described in Appendix 2. The carbon isotope fractionation during reductive dechlorination of VC is significantly more pronounced (εC = -22 to -31 ‰) than the carbon isotope fractionation during aerobic degradation (εC = -3 to -8 ‰). The isotope fractionation during biodegradation of pollutants can be described by the Rayleigh equation.

Using the known isotope enrichment factors (εC) for VC biodegradation, specific ranges for VC degradation pathways can be determined from the correlation of isotope fractionation and concentration decrease according to the Rayleigh equation (Figure 6). The predominant pathway for in situ biodegradation of VC can be discovered by plotting the isotope and concentration data of VC determined at the site into the diagram. If the field data fall into the range of aerobic VC degradation, two interpretations are possible: (1) the predominant pathway is aerobic VC degradation and the influence of non-destructive processes is of minor extent or (2) the predominant pathway is reductive dechlorination of VC and the influence of non-destructive processes is of substantial extent. If field data are above a range of a specific pathway it can be excluded that this pathway is predominant for the in situ biodegradation. The results are shown in Figure 6.

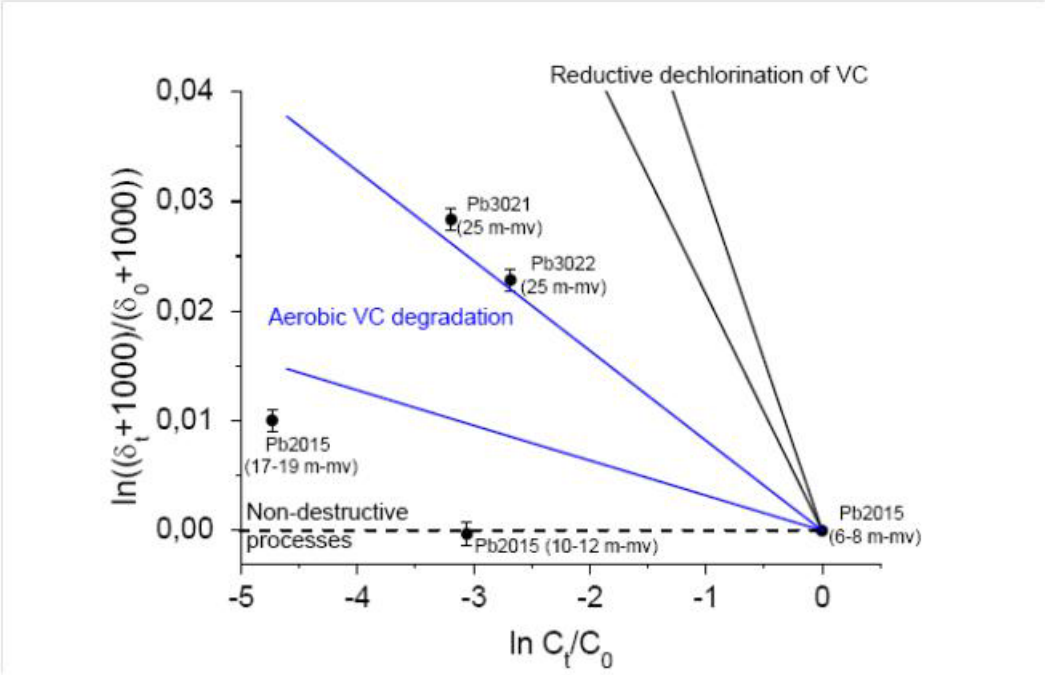

Figure 6. Rayleigh equation plot with specific ranges for aerobic VC degradation (blue) and for reductive dechlorination of VC (black). The dashed line represents the evolution for isotope and concentration data which are specific for non-destructive processes. The black points give the isotope and concentration data according to the Rayleigh equation for the different sampling points. The error bars were obtained from the expected error of isotope analysis (± 0.5‰).

The sampling point with the highest VC concentration (5,100 µg/L, Pb2015 (6-8 m-gl) was chosen as reference point (i.e. δ0 and C0 for the Rayleigh equation) for the evaluation of predominant VC degradation pathway at the field site using the Rayleigh equation approach.

Based on Pb2015 (6-8 m-gl) as reference point, the field data at Pb2015 (17-19 m-gl) indicated that biodegradation of VC was caused either by reductive dechlorination in conjunction with a substantial concentration decrease due to non-destructive processes (dilution) or by aerobic VC degradation in conjunction with a low to moderate concentration decrease due to non-destructive processes. The field data of the sampling points located downstream from the source zone Pb3021 (25 m-gl) and Pb3022 (25 m-gl) lie slightly above the range of aerobic VC degradation. Therefore, aerobic VC degradation can (most probably) be excluded to a large extend at these sampling points which gives strong indications that reductive dechlorination is the predominant pathway for VC biodegradation.

6.3 Conclusion prevalent process at the Amsterdamsestraatweg

The results indicated that biodegradation of VC was caused either by reductive dechlorination in conjunction with a substantial concentration decrease due to non-destructive processes (like dilution) or by aerobic VC degradation in conjunction with a low to moderate concentration decrease due to non-destructive processes. Aerobic VC degradation can (most probably) be excluded to a large extend in the source zone of this site. The results strongly indicate that reductive dechlorination is the predominant pathway for VC biodegradation. However to confirm these findings complementary research was required and degradation laboratory tests were conducted which are described in the following chapter.

7 Microcosms degradation test

7.1 Approach

The results of the groundwater molecular analyses (chapter 5) were used to determine the set-up of the microcosm degradation lab tests. These biodegradation tests were performed according to a protocol developed and validated within the framework of the Dutch Research Program for Biotechnical In-situ remediation (NOBIS project 97-4-04). This protocol, that was specially developed for the determination of anaerobic biodegradation of chlorinated ethenes, can also be used for the study of other degradation processes.

Soil and groundwater samples from different locations in Utrecht were used to conduct a number of degradation tests. The homogenized soil samples were mixed with groundwater from the same monitoring well. Separate degradation tests for cis-DCE and VC were incubated. The flasks were incubated in closed glass bottles during a period of approximately 6 months at room temperature (20°C). A picture of the flasks is given in Figure 7. Periodically GC analysis of the headspace were conducted in order to determine the concentrations cis-DCE, VC, ethene, ethane and methane. The samples for molecular analysis were taken from separate flasks (sacrificial experiment).

Figure 7 Picture of flasks used in the degradation experiments

7.1.1 Cis-DCE lab degradation tests

To determine whether degradation of cis-DCE occurs in the subsurface of Utrecht, laboratory degradation experiments were conducted. To make sure that any decrease in DCE concentration is the result of biodegradation a biotic and a sterile abiotic reference test was conducted. In this reference test the biodegradation is stopped by addition of a biocide. In this case a mixture of $HgCl_2$ and NaN_3 was used.

As depicted in Table 3, three tests were performed: one abiotic reference test , and two biotic tests. In one of these biotic tests ^{13}C labelled cis-DCE was added to the flasks. This was an uncontaminated sample (no contamination was present). This test was intended to determine whether degradation of cis-DCE could take place in clean soils and whether an adaptation period was required.

Table 3 Overview of performed tests to determine cis-DCE degradation

Batch test	Sample	Abiotic	*Cis*-DCE (1,000 µg/L)	13C labeled Cis-DCE
Cis-DCE degradation tests				
Batch A (abiotic)	53 (38 m-gl)	X	X	
Batch C	67 (26 m-gl)		X	
Batch D	67 (14 m-gl)			X

The preliminary results of labtest C showed a slight decrease in cis-DCE, this indicates biodegradation by either reductive dechlorination or oxidation. In these flasks bacteria for both processes were present (data not shown). The isotope analyses did not confirm this degradation. Therefore it was decided, after consultation with the municipality of Utrecht to continue the labtests for another 8 weeks.

The flasks from labtest D were spiked with ^{13}C labeled cis-DCE. The labeled contaminant is used to track the degradation process, using isotope analyses. In the first weeks GC analyses on cis-DCE, VC, ethene and ethane and methane were performed weekly. As the concentrations seemed stable, analysis were reduced. The isotope analyses were also performed on an abiotic flask at the start of the experiment, as a reference. During the incubation period isotope analysis were performed on a biotic flask if a decrease in contaminant concentration was observed in the biotic flasks.

Molecular analyses were conducted on both DNA and RNA samples. Results from DNA analyses indicate the presence of a degradation potential and the results from RNA analyses indicate whether degradation processes are actually occurring (activity of specific genes).

7.1.2 Results cis-DCE labtest

In Figure 8 the results of labtest D (cis-DCE) are shown. In labtest D soil and groundwater from monitoring well 67 (14 m-gl) was used.

The flasks were spiked with ^{13}C labeled cis-DCE. In labtest D fresh groundwater was added in order to assess the potential for micro-aerophilic degradation. No change was observed within the incubation period of 8 weeks. This is in line with the results seen in the preliminary batch experiment of this project, where no biological degradation of cis-DCE occurred.

As no degradation was observed in labtest D it was decided to try to induce the micro-aerophilic degradation of cis-DCE by the addition of groundwater that contained *Polaromonas* JS666 bacterium. These activities were conducted within the research project Ecolinc.

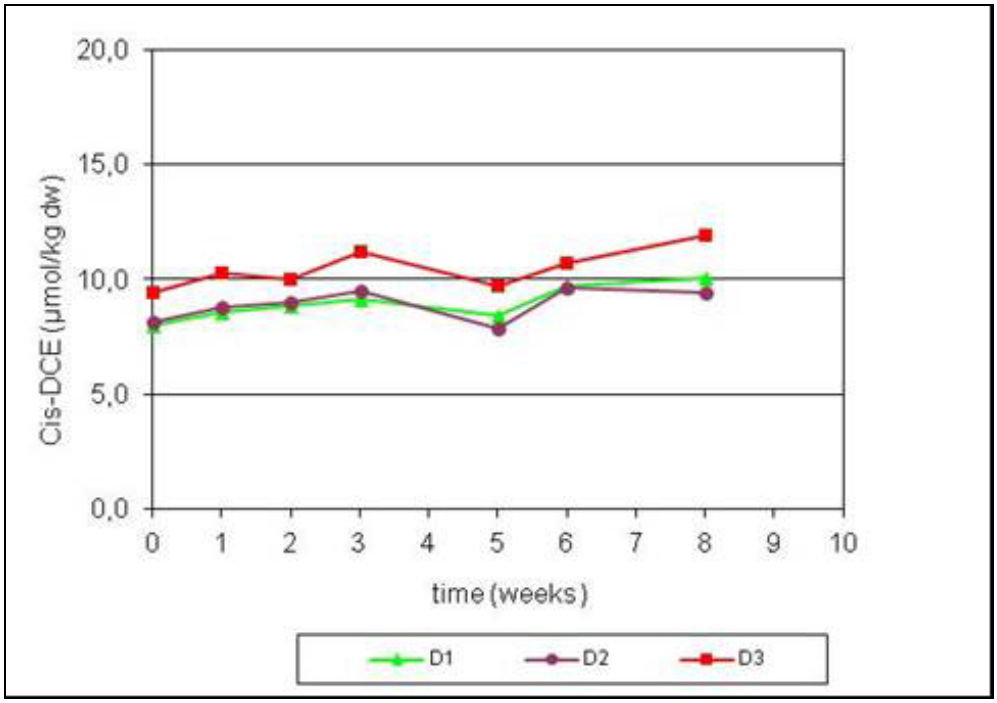

Figure 8 Concentration cis-DCE in flasks test D (soil and groundwater sampling well 67-14 m-gl).

The strain *Polaromonas* JS666 is described in literature to aerobically degrade cis-DCE. This bacterium was not detected in the groundwater from monitoring well 67 (14 m-gl) which was originally used for degradation test D. However, this bacterium was detected in groundwater of monitoring well 67 (26 m-gl), see appendix 2. Therefore remaining flasks of test D were spiked with concentrated groundwater from monitoring well 67 (26 m-gl) and incubated for an additional 6 weeks. During this additional incubation period no decrease in cis-DCE was observed (data not shown). There was no decrease observed, this might be due to low oxygen concentrations, however this was not investigated.

7.1.3 VC degradation tests

Laboratory degradation experiments were conducted in order to determine whether degradation of VC is possible in the subsurface of Utrecht. As depicted in Table 4 three tests were performed: one abiotic reference test and two biotic tests. In all tests VC was added to the flasks. Based on the preliminary tests additional tests were performed, these are described in table 5.

Table 4 Overview of performed tests to determine VC degradation

Batch test	Sample	Abiotic	VC (1,000 µg/L)
VC degradation tests			
Batch F (abiotic)	67 (14 m-gl)	X	X
Batch B	53 (38 m-gl)		X
Batch E	67 (14 m-gl)		X

Table 5 Overview of performed tests to determine VC degradation in extended tests

Spike	50 ml groundwater added	Abiotic	Labeled VC (2,500 µg/L)
VC degradation tests			
Batch A (abiotic)	Yes, well 53 (38 m-gl)	X	X
Batch B (VC)	Yes, well 53 (38 m-gl)		X
Batch E (VC)	Yes, well 67 (14 m-gl)		X
Batch F (abiotic)	Yes, well 67 (14 m-gl)	X	X

7.1.4 Results VC degradation tests

The results of the abiotic VC degradation tests showed no changes in VC concentrations during the incubation period of 6 weeks, see Appendix 4. This indicates that any decrease in the biotic flasks (test B and E) is caused by biological processes.

The preliminary results in the E-test and in the B-test suggest that degradation of VC might have occurred during the first few weeks of the test period see Figure 9. In Figure 9 the average VC concentration (of three flasks) is shown. The concentration of the contaminant VC decreased during incubation, see Figure 10 but no ethene was formed (the individual results are shown in Appendix 4). In some of the flasks micro-aerophilic degradation of VC seemed to occur (as not ethane was detected). To confirm these findings it was decided to extend the degradation test with another 8 weeks after consultation with the municipality of Utrecht. The flasks of tests A, B, E and F were spiked with ^{13}C labelled VC (see table 5). To minimize the chances for oxygen limitation in the flasks fresh groundwater was supplemented to each flask (it was assumed that the groundwater contained low oxygen concentrations; natural oxygen content).

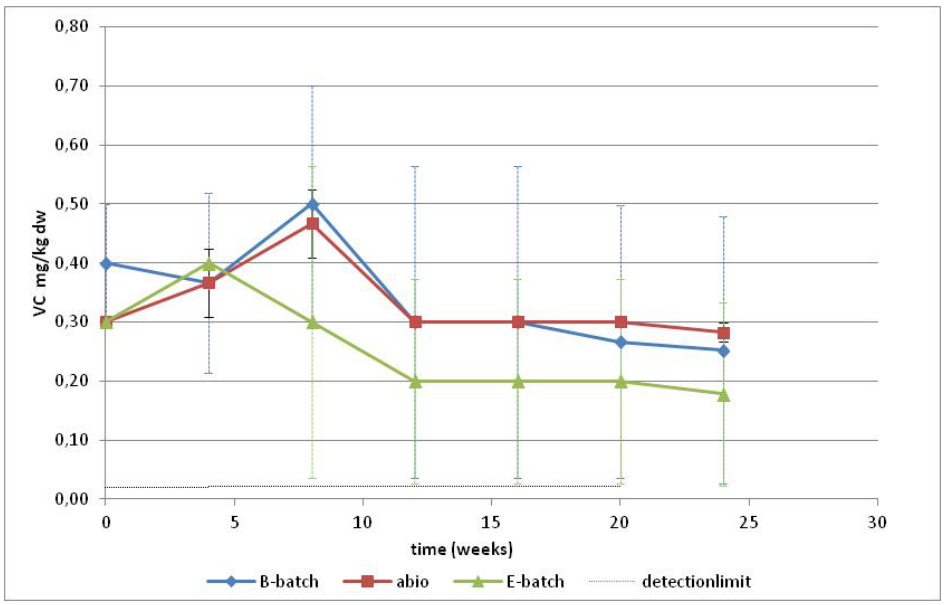

Figure 9 VC results for the abiotic test and the two biotic tests (B and E), errorbars for biotic test not shown

The results show that besides VC no other degradation product like ethene is formed and no other components like methane are present. Therefore only the results of VC are represented in the graphs, see figure Figure 10 and Figure 11.

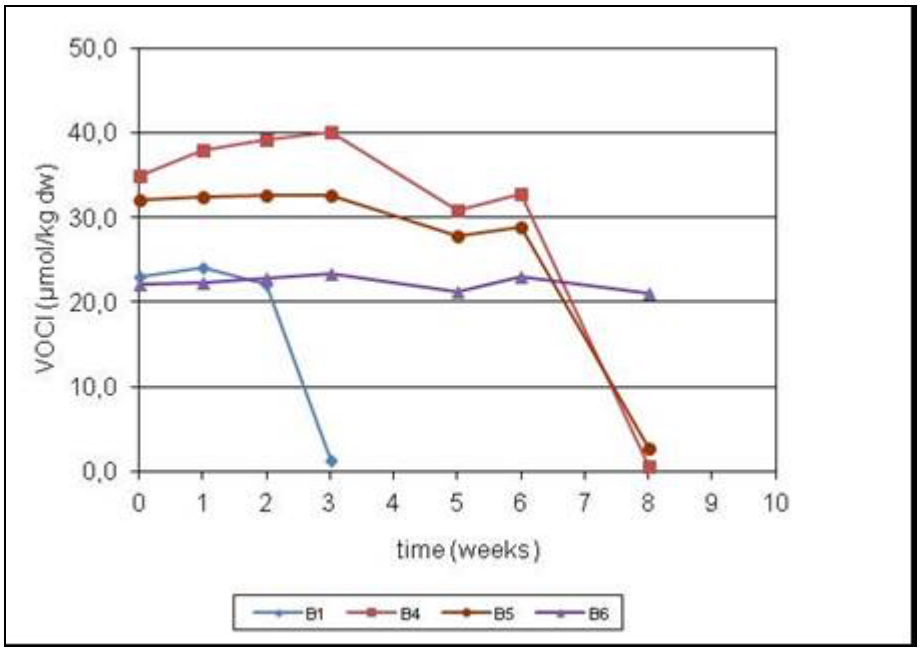

Figure 10 VC concentration in individual flasks test B (soil and groundwater from monitoring well 53 (38 m-gl)

In conclusion, the results show a decrease in VC concentration in labtest B, see Figure 10. Isotope analysis was performed on flask B4 as in this flask a decrease in VC was observed, the results are depicted in Appendix 4. If micro-aerophilic degradation occurs then VC is degraded to CO_2 (inorganic carbon), which will result in higher DIC (dissolved inorganic carbon) concentrations. DIC is a measure for CO_2. The isotope results show the formation of ^{13}C labeled CO_2 (increase in DIC), this indicates that degradation of VC by micro-aerophilic oxidation has occurred. Molecular analyses were performed on samples in which a decrease of VC was observed, this analyses provides an extra line of evidence for occurrence of micro-aerophilic degradation, the results are depicted in Table 6. As expected the number of copies (*etnC*) are higher in the soil phase as compared to the water phase. The occurrence of etnC in the soil phase of flask B4 confirms the biological degradation potential of the sample. Due to the high detection limit of the RNA analysis (10^6 cells/gr) no RNA was detected. The presence of RNA is an indication of the actual activity.

Table 6 Results molecular analyses on etnE, etnC and total bacteria on flask from VC test B

Molecular analysis	etnC		etnE		Total bacteria
DN/RNA	DNA	RNA	DNA	RNA	DNA
Flask B5					
Water phase (cells/mL)	3 x10^3	-	< 6 x10^2	-	
Soil phase (cells/gr)	3 x10^4	< 3 x10^6	< 5 x10^3	< 3 x10^6	2 x10^6

- not determined < : below detection limit

From the results a degradation rate is derived. The average degradation rate is 0.30 umol/day, however it should be mentioned that this is a rough estimation from the performed labtest, under laboratory conditions, at room temperature.

In Figure 11 the results of test E are represented. For this test soil and groundwater from monitoring well 67 (14 m-gl) was used.

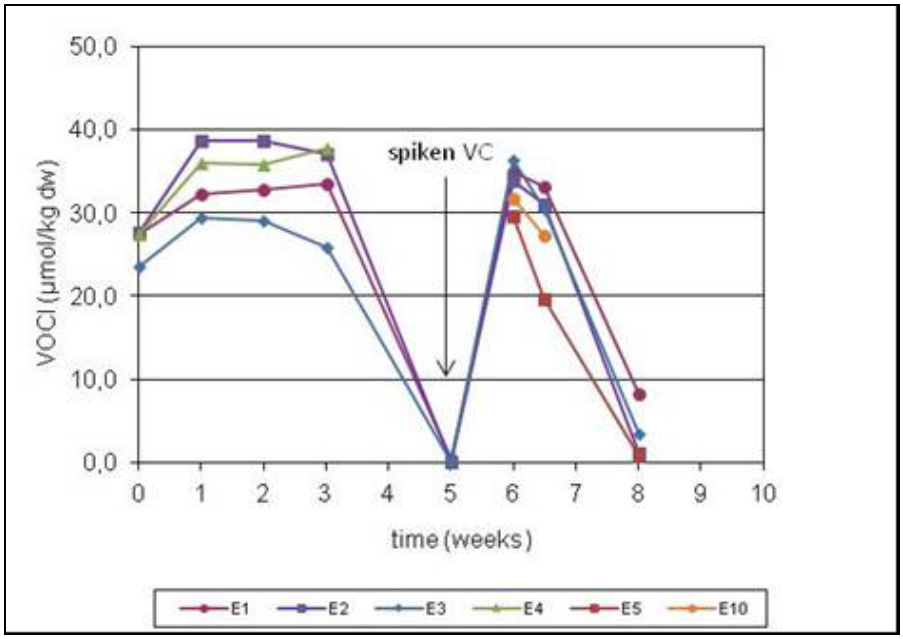

Figure 11 VC concentration in individual flasks labtest E (soil and groundwater monitoring well 67 (14 m-gl)

Results show a significant decrease in VC between week 3 and 5 of the incubation period. However during this period the flasks were not stored upside down (with the lid faced down), as described in the protocol. There is a minor possibility that VC leaked from these flasks or oxygen entered these flasks during this period. Therefore (Non-labelled) VC was spiked in week 5 in order to determine whether VC degradation could take place in these flasks. From the subsequent measurements a significant decrease in VC was observed, indicating a removal of the contaminant from the flask by biological degradation processes, and not as a result of leakage.

Table 7 shows the results of the molecular analyses on flask E1 (in which a decrease of VC was observed, Figure 11). Based on the molecular results this decrease in VC can be attributed to biological degradation (micro-aerophilic degradation). As was observed in test B in this test E the number of *etnC* in the soil phase was much higher than the number of copies in the water phase.

Table 7 Results molecular analyses on etnE, etnC and total bacteria on flask from VC test E

Molecular analysis	*etn*C		*etn*E		Total bacteria
	DNA	RNA	DNA	RNA	DNA
Flask E1					
Water phase (cells/mL)	< 6 x10^2	-	< 6,3 x10^2	-	
Soil phase (cells/gr)	3 x10^5	<1,3 x10^6	<1,3 x10^4	<1,3 x10^6	7,6 x10^6

- not determined < : below detection limit

7.1.5 Oxygen concentration biotic VC degradation tests

In addition to analyses mentioned in the above paragraphs, in a number of biotic flasks oxygen concentrations were measured to verify whether micro-aerophilic conditions (low oxygen concentrations) were present during the experiment. These analyses were conducted by the laboratory of Microbiology in Wageningen as part of the Ecolinc project. In Table 8 the results are shown.

Table 8 Oxygen concentration in headspace of flasks from test B and E

Flask	Headspace volume oxygen (%)	Concentration oxygen water phase (mg/L)
B6	0,1	0,05 mg/L
E2	0,6	0,25 mg/L

As can be seen in Table 8 the oxygen levels in the headspace of the flasks is low. Furthermore the diffusion constant of oxygen to the water phase is low, which means that based on these headspace results less than 0.5 mg/L oxygen is present in the water phase of the experiment. These conditions can be described as micro-aerophilic.

7.2 Conclusions degradation tests

To assess whether reductive dechlorination or micro-aerophilic oxidation occurs in the deeper subsurface of Utrecht anaerobic degradation microcosm tests were conducted. In these tests soil and groundwater samples from different locations were used to determine the biological degradation capacity. Soil and groundwater was used from three sites to which (^{13}C labeled) cis-DCE or VC was spiked. The degradation of both contaminants was followed through headspace measurements. Results showed degradation of VC under intrinsic (anaerobic/micro-aerophilic) conditions. However for cis-DCE degradation was less pronounced. In one of the flasks degradation occurred under anaerobic conditions after a long lagphase. In previous groundwater degradation capacity assessment the genes etnE and etnC, involved in micro-aerophilic degradation of VC, were detected in samples from monitoring wells 53 (38 m-bs) and 67 (14 m-bs). The results from the microcosm tests confirm the outcome derived with the other techniques used to determine degradation capacity, namely that there is a degradation potential for VC.

8 BACTRAPS

8.1 Approach

In order to assess whether in situ biodegradation of cis-DCE and VC occurs in Utrecht and to identify the micro-organisms involved BACTRAP studies were conducted. In monitoring wells 53 (38 m-gl) and 86 (14 m-gl) two BACTRAPs were placed (see Table 9); one loaded with ^{13}C-labelled cis-DCE and one loaded with ^{12}C cis-DCE, see Figure 12. The ^{13}C-labelled BACTRAP was analysed by Isodetect (isotope analyses) and the ^{12}C cis-DCE BACTRAP was analysed by Bioclear (molecular analyses). Furthermore one clean (unloaded) BACTRAP was placed in monitoring well 61 (26 m-gl). This BACTRAP was intended to detect bacteria able to biodegrade VC.

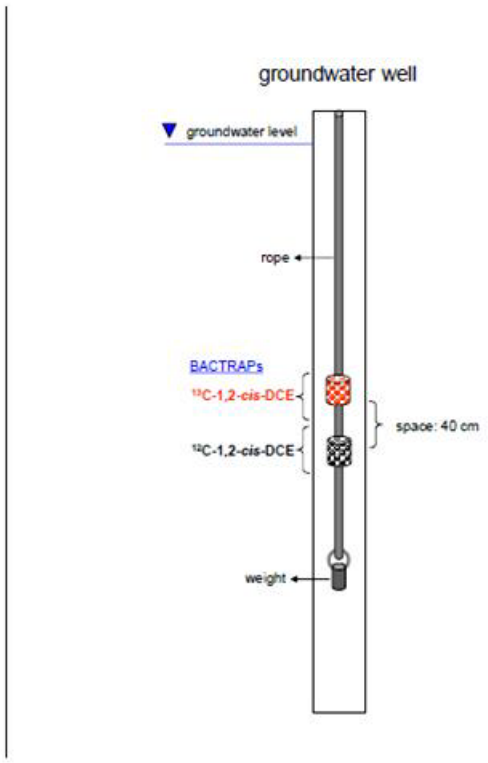

Figure 12 Schematization of BACTRAP installation

Isotope analyses were conducted before and after installation of the BACTRAPs. Analyses were conducted on VOCs (chlorinated volatile organic carbons, like cis-DCE and VC) and on DIC (dissolved inorganic carbon). DIC is a measure for CO_2. The isotope analyses were conducted by Isodetect. The molecular analyses were conducted by Bioclear and the analyses on VOC were conducted by Alcontrol laboratories.

Table 9 Overview of monitoring wells where BACTRAPS were placed

Monitoring well	Depth (m-gl)	Bactrap			Isotope *	VOC	Molecular analyses
		^{13}C *cis*-DCE + unloaded	^{12}C *cis*-DCE + unloaded	Unloaded			
53	38	X	X		2X	X	X
86	14	X	X		2X	X	X
61	26			X		X	X
Totaal		2	2	1	4 *	3	3

* before deployment of the BACTRAPs and after removing the BACTRAPs

8.2 Results

The results of the molecular analyses (qPCR) on the groundwater before deployment of the BACTRAPS and of the BACTRAPS after 3 months of incubation in the monitoring well are shown in Table 10. In the BACTRAP samples both DNA and RNA analyses were conducted. In general, the DNA analyses indicate the presence of genes and bacteria therefore indicating a potential degradation capacity. The RNA-analysis is a measure of the actual activity of the genes (degradation capacity is actually being used).

In all three monitoring wells VC is present before the BACTRAPs were installed. In two monitoring wells (pb 53 and pb 86) labelled cis-DCE was loaded on the BACTRAPs. The DNA results of all three BACTRAPs show a major increase in the genes involved in micro-aerophilic degradation of VC (*EtnE* and *EtnC*). The RNA analyses show that these genes are also active in the samples.

Table 10 QPCR results of groundwater and BACTRAPS

Monitoring well		Pb53 (38 m-gl)		Pb86 (14 m-gl)		Pb61 (26 m-gl)	
Sampling date		12-1-2011	27-4-2011	12-1-2011	27-4-2011	13-1-2011	27-4-2011
		groundwater	BACTRAP	groundwater	BACTRAP	groundwater	BACTRAP
Molecular analysis *							
etnC (DNA	Cells/mL	7E+03	1E+06	< 4	1E+05	<	8E+04
etnC (RNA)	Cells/mL		2E+08		2E+06		4E+06
etnE (DNA)	Cells/mL	1E+03	5E+05	< 4	3E+04	<	1E+04
etnE (RNA)	Cells/mL		3E+07		3E+06		< 4E+05
Polaromonas (DNA)	Cells/mL	< 19	< 2E+03	< 4	< 2E+03	<[1)]	< 3E+03
Dehalococcoides (DNA)	Cells/mL	< 13	< 2E+03	< 4	< 2E+03	<	< 3E+03
vcrA (DNA)	Cells/mL	< 13	< 2E+03		< 2E+03		< 3E+03
vcrA (RNA)	Cells/mL		< 3E+05		< 4E+05		< 4E+05
Contamination							
PCE	µg/L	< 0.1		3.6		< 0.1	
TCE	µg/L	0.1		4.4		< 0.1	
cis-1,2-DCE	µg/L	28		67		1.2	
trans-1,2-DCE	µg/L	2.1		5.9		< 0.1	
VC	µg/L	970		61		240	
Redox and other parameters							
pH	-	6.98		6.69		7.22	
temperature	°C	12.0		13.7		13.3	
Oxygen	mg/L	0.43		0.41		0.04	
conductivity	µS/cm	1169		910		1131	
Redox	mV H_2	109		149		89	

1) *Polaromonas* was detected in low numbers when the dialyser sampling method was applied

The detection of *etnE* and *etnC* RNA provides evidence that degradation of VC (or ethene) occurs in these monitoring wells. The bacteria involved in micro-aerophilic degradation of cis-DCE (BACTRAPS in monitoring wells pb53 and pb86 were loaded with cis-DCE), *Polaromonas*, was not detected in numbers above the detection limit. Thus these results do not provide an insight in the occurrence of micro-aerophilic degradation of cis-DCE in Utrecht. The bacteria *Dehalococcoides*, were not detected in the BACTRAPS. This suggests that (at these monitoring wells) no reductive dechlorination occurs.

8.2.1 Isotope analysis

The results of the isotope analysis on the BACTRAPS represent carbon isotope signatures of the VOC and DIC in the monitoring wells Pb53 (38 m-gl) and Pb86 (14 m-gl) before and after BACTRAP installation. An elaborate description of the results is given in Appendix 5.

In both monitoring wells, the carbon isotope signatures for DIC showed significantly more negative values after than before the BACTRAP installation. This demonstrates that the ^{13}C-cis-DCE was not biodegraded via microbial oxidation which would have led to more positive isotope signatures of DIC. The more negative carbon isotope signatures measured might be explained by the mineralisation of organic compounds in the groundwater. 'Naturally' occurring VC present in the groundwater also forms a part of this 'organic compound' fraction. The degradation of organic compounds (including VC) leads to the formation of CO_2 exhibiting negative carbon isotope signatures. Thus these negative DIC values could be caused by the oxidation of VC to CO_2. This suggests that micro-aerophilic degradation of VC does occur and that micro-aerophilic degradation of cis-DCE does not occur.

In pb53 (38 m-gl), carbon isotope ratios of cis-DCE could not be determined neither before nor after the BACTRAP installation because the concentration of cis-DCE was too low for reliable isotope analysis. The isotope ratio of VC was significantly more positive after the BACTRAP installation than before BACTRAP installation, indicating that the ^{13}C-cis-DCE was possibly degraded to ^{13}C-labelled VC. However, the isotope ratio of VC was within the range of expected values arising from isotope fractionation of natural occurring VC during biodegradation. Thus, besides VC derived from ^{13}C-cis-DCE from the BACTRAP, VC which was already present in pb53 (38 m-gl) might be biodegraded leading to changes in carbon isotope ratios from -20.9 ‰ (before BACTRAP installation) to +12.9 ‰ (after BACTRAP installation). No indications of a predominant degradation pathway can be derived based on the changes in VC isotope ratios alone. The results from the molecular analysis however indicate that micro-aerophilic degradation could play an important role in this monitoring well.

In pb86 (14 m-gl), carbon isotope ratio of cis-DCE and VC could only be determined after BACTRAP installation and were highly positive. Hence, the positive carbon isotope signature for VC might be explained by the microbial reduction of the ^{13}C-cis-DCE to ^{13}C-VC providing strong indication for reductive dechlorination of cis-DCE at pb86 (14 m-gl). This is contrary to what was indicated by the molecular analysis, no *Dehalococcoides* was detected. The sampling for molecular analyses was conducted using the traditional sampling protocol. The results from DNA-analysis on the samples obtained using the dialyser sampling method, show a number of DHC < 4 cell/mL, which confirm previous findings.

8.3 Conclusions BACTRAP biodegradation assessment

Based on the isotope analysis there are indications that reductive dechlorination of cis-DCE occurs in monitoring well 86 (14m-gl). These results are not confirmed by the molecular data. The bacteria involved in reductive dechlorination (*Dehalococcoides*) were not detected in this monitoring well (below detection limit). The molecular analysis do not provide insight into the possible degradation process of cis-DCE, the bacterium *Polaromonas* was not detected. The isotope analysis confirmed this observation (no indications for microbial oxidation of cis-DCE were observed).

With regard to VC degradation, strong indications for micro-aerophilic degradation were obtained based on the molecular analyses. The genes involved in micro-aerophilic degradation of VC (*etnE* and *etnC*) were detected. The isotope analyses were however focussed on gaining insight in the degradation process of cis-DCE. But based on the DIC values it is possible to determine whether microbial oxidation processes are occurring (transformation of VC into CO_2). The isotope data show that CO_2 production occurred, this CO_2 could be derived from the 'naturally' occurring VC or from organic material.

9 In situ mesocosms (MicroTraps)

9.1 Approach

From previous results (chapters 6, 7 and 8) it can be concluded that bacteria capable of micro-aerophilic degradation are detected in (a selection of) monitoring wells in the city centre of Utrecht. In order to validate the determined biodegradation and investigate the in- situ biodegradation rate of both reductive and micro-aerophilic degradation MicroTraps were deployed in the monitoring wells.

As described in paragraph 4.2.3 the MicroTraps (MT) consist of permeable tubes containing soil from the site. These tubes were installed at a specific filter depth in the monitoring well over a period of 6 months. In Table 11 an overview is given. Every 2 months, soil from an MT was sampled and preserved in the lab for molecular analyses (DNA). The soil was analysed for the presence of the bacteria/genes responsible for contaminant degradation (*Dehalococcoides, vcrA, etnC, etnE, Polaromonas*).

Table 11 Overview of monitoring wells with MicroTraps

Monitoring well	Depth filter (m-gl)	depth MT (m-gl)	Date placed	Comments	Site	MW upstream (m-gl)	MW downstream (m-gl)
67	14	13.5	15 aug 2012		City centre	404 (12-13)	107bis (8.7)
AF2.1	5.6 – 9.6	9.5	10 aug 2012	First tube removed 15 aug.	Amsterdamse-straatweg	2016 (4-6)	AF1 (6-10)
NF4.2	9-11	10.5	20 aug 2012	Not in NF4.1 (5-8), conc. to low *	Nachtegaalstraat	NF3.1 (5-8)	2002 (7-8)
53	38	37	15 aug 2012		Near ATES system	308 (39-40)	502 (29-30)

*The results of NF4.1 are added in appendix 1

Periodically groundwater from the MicroTrap monitoring wells was sampled and analyzed for VOC, redox parameters (sulphate, nitrate, methane), NPOC and the degradation products ethane and ethene. In addition at the start and end (t=start and t=6 months) of the deployment monitoring wells up- and downstream from these wells were monitored, Table 11, see location map in Appendix 3. Groundwater samples from the wells 53 and NF4.2 were also analyzed on the previously mentioned genes and bacteria for comparison with previous results (see chapter 5).

The analysis certificates are added in Appendix 6.

9.2 Results monitoring wells start and end

In Table 12, Table 13 and Table 14 the results of the chemical analyses are presented.

Table 12 Results groundwater analyses in MicroTrap monitoring wells AF2.1 and 67 and the up and downstream wells start.

Monitoring well depth (m-gl)		2016 (4-6)	AF 2.1 (6-10)*	AF 1.1 (6-10)	404 (12-13)	67 (14-15)	107bis (8.7- 9.7)
t=0		16-8-2012	9-8-2012	9-8-2012	16-8-2012	10-8-2012	16-8-2012
Analyses	Unit	Up	MT	Down	Up	MT	Down
contaminant							
PCE	µg/L	<0.1	16,000	<0.1	29	0.17	<0.1
TCE	µg/L	<0.1	5,100	<0.1	17	0.79	<0.1
c-DCE	µg/L	24	29,000	5.5	7.8	1.2	<0.1
t-DCE	µg/L	<0.1	110	<0.1	0.22	<0.1	<0.1
VC	µg/L	54	1,700	7	0.13	<0.1	<0.1
Redox							
Oxygen	mg/L	0.18	-	0.22	0.58	-	0.13
Nitrate	mg/L	-	<0.5	-	-	5.9	-
Sulphate	mg/L	-	<8	-	-	55	-
Methane	µg/L	27,000	15,000	8,000	<8	<8	78
NPOC	mg TOC/l	-	9.4	-	-	3.1	-
Redox condition		methano	methano	methano		Nitr/iron	

- Not analyzed * Redox samples from 16-8-2012 Methano: methanogenic Nitr/iron: nitrate to iron reducing conditions

In all the monitoring wells that contain a MicroTrap VOC was detected. The concentrations in MicroTrap well 67 are too low to be able to monitor a decrease in contaminant concentration. Therefore in this monitoring well no further groundwater monitoring was performed.

9.2.1 Redox conditions

In all four wells containing the MicroTraps the redox conditions were assessed, both at the time of deployment and after 6 months. At the Amsterdamsestraatweg, AF2 methanogenic conditions prevail, in the MT as well as the up and downstream wells high methane concentrations were detected. The redox conditions in this aquifer are highly reduced and favourable for reductive dechlorination. In monitoring well 67 nitrate to iron reducing conditions are prevalent. In well NF4.2, at the Nachtegaalstraat nitrate to iron reducing conditions occur, the conditions at the time of deployment and after 6 months is comparable. In monitoring well 53 no nitrate was detected, the redox conditions are therefore probably iron to sulphate reducing. The conditions at the start and after 6 months are similar. The available carbon, NPOC, was in all wells fairly low, 4.6 mg TOC/L in well 53 up to 9.6 mg TOC/L in well AF2.1.

Table 13 Results groundwater analyses in MicroTraps monitoring wells AF4.2 and 53 and the up and downstream wells, start.

Monitoring well depth (m-gl)		NF3.1 (5-8)	NF4.2 (9-11)*#	2002 (7-8)	308 (39-40)	53 (38-39)	502 (29-30)
t=0		9-8-2012	9-8-2012	16-8-2012	16-8-2012	10-8-2012	16-8-2012
Analyses	Unit	Up	MT	Down	Up	MT	Down
contaminant							
PCE	µg/L	20	2	<0.1	<0.1	<0.1	<0.1
TCE	µg/L	4.1	1.9	<0.1	<0.1	<0.1	<0.1
c-DCE	µg/L	0.54	48	0.32	2	5.5	2.2
t-DCE	µg/L	<0.1	0.6	<0.1	0.57	0.49	<0.1
VC	µg/L	15	4.1	11	280	1,100	22
Redox							
Oxygen	mg/L	0.01	-	0.07	0.22	-	0.18
Nitrate	mg/L	-	2.2	-	-	<0.5	-
Sulphate	mg/L	-	66	-	-	80	-
Methane	µg/L	190	39	130	230	110	25
NPOC	mg TOC/l	-	5.3	-	-	4.6	-
Redox condition			Nitr/iron			Iron	

- Not analyzed nitr/iron: nitrate to iron reducing conditions Iron: iron reducing conditions

Table 14 Results groundwater analyses in MicroTrap monitoring wells AF2.1, NF 4.2, 53 and the up and downstream wells after 6 months.

Monitoring well depth (m-gl)		2016 (4-6)	AF 2.1 (6-10)*	AF 1.1 (6-10)	NF3.1 (5-8)	NF4.2 (9-11)	2002 (7-8)	308 (39-40)	53 (38-39)	502 (29-30)
t=6		14-2-2013	14-2-2013	14-2-2013	19-2-2013	14-2-2013	14-2-2013	14-2-2013	14-2-2013	14-2-2013
Analyses	Unit	Up	MT	Down	Up	MT	Down	Up	MT	Down
contaminant										
PCE	µg/L	<0.1	150,000	0.15	0.16	<0.1	<0.1	<0.1	<0.1	<0.1
TCE	µg/L	<0.1	36,000	<0.1	0.72	<0.1	<0.1	<0.1	<0.1	<0.1
c-DCE	µg/L	30	30,000	0.12	46	20	0.32	1.7	4.5	2.4
t-DCE	µg/L	0.12	2,600	<0.1	0.31	<0.1	<0.1	0.54	0.66	<0.1
VC	µg/L	76	5,200	0.12	46	560	50	530	2,700	25
Redox										
Nitrate	mg/L	-	<0.5	-	-	<0.5	-	-	<0.5	-
Sulphate	mg/L	-	<8	-	-	69	-	-	64	-

Methane	µg/L	23,000	15,000	9,000	120	57	65	200	120	19
NPOC	mg TOC/L	-	8.0	-	-	2.2	-	-	4.3	-

9.3 Results MicroTraps wells

9.3.1 Monitoring VOC concentrations

In well AF 2.1 the highest VOC concentrations were measured, there are however some fluctuations in the concentrations in time. The concentrations measured at t=2 are higher compared to previous measurements. It is expected that the fluctuations are a result of the flux from the source. This however makes the well unsuitable for assessing the degradation rate. At t =2 months monitoring well NF 4.2 was not available for sampling, a car was parked at the site. The results are presented in Table 16.

Table 15 VOC concentrations in monitoring wells AF2.1 and MW 67 in 2012/2013.

Monitoring well (depth m-gl)		**AF 2.1 (6-10)**				**67 (14-15)**			
Analyses	Date	9-8-2012	17-10-2012	12-12-2012	14-2-2013	10-8-2012	17-10-2012		
	Time point	t=0	t=2	t=4	t=6	t=0	t=2	t=4	t=6
contaminant	Unit								
PCE	µg/L	16,000	100,000	88,000	150,000	0.17	0.3	-	-
TCE	µg/L	5,100	23,000	21,000	36,000	0.79	1.6	-	-
c-DCE	µg/L	29,000	37,000	33,000	30,000	1.2	1.7	-	-
t-DCE	µg/L	110	190	240	2,600	<0.1	<0.1	-	-
VC	µg/L	1,700	2,700	1,900	5,200	<0.1	0.13	-	-
Ethene	µg/L	229	61*	110	58	-	<1	-	-
Ethane	µg/L	223	49*	170	120	-	<1	-	-
Redox									
Nitrate	mg/L	<0.5	-	<0.5	<0.5	5.9	-	-	-
Sulphate	mg/L	<8	-	<25	<8	55	-	-	-
Methane	µg/L	15,000	-	14,000	15,000	<8	-	-	-
NPOC	mg TOC/L	9.4	-	10	8	3.1	-	-	-
Redox condition	-	Methano		Methano		Nitrate			
Grade of dechlorination	%	-	22	23	23	-	-	-	-

* The values for ethene and ethane at t=2 are indicative values.

The grade of dechlorination is a measure used for natural attenuation of PCE, it is the ratio of mother- and its daughter compounds. If PCE and TCE are the dominant compounds present at a site then the grade of dechlorination is low, implying that limited dechlorination occurred.

However when more degradation products like cis-DCE, VC and ethene and ethane are present the grade of degradation is higher. An increasing grade of degradation in time implies that natural attenuation is prevalent at the site, see equation below (in concentration mol/L).

$$([TCE] + 2[DCEs] + 3[VC] + 4[ethene] + 4[ethane])/(4 * ([PCE] + [TCE] + [DCEs] + [VC] + [ethene] + [ethane]) * 100\%$$

Table 16 VOC concentrations in monitoring wells

Monitoring well (depth m-gl)		NF4.2 (9-11)				53 (38 -39)			
Analyses	Date	9-8-2012	-	12-12-2012	14-2-2013	10-8-2012	17-10-2012	12-12-2012	14-2-2013
	Time point	t=0	t=2	t=4	t=6	t=0	t=2	t=4	t=6
Contaminant	Unit								
PCE	µg/L	2	-	<0.1	<0.1	<0.1	<0.1	1.4	<0.1
TCE	µg/L	1.9	-	<0.1	<0.1	<0.1	<0.1	0.57	<0.1
c-DCE	µg/L	48	-	26	20	5.5	11*	7.6	4.5
t-DCE	µg/L	0.6	-	<0.1	<0.1	0.49	0.82	1.3	0.66
VC	µg/L	4.1	-	690	560	1,100	750*	2,400	2,700
Ethene *	µg/L	-	-	29	24	-	7	10	12
Ethane *	µg/L	-	-	<1	<1	-	<1	<1	<1
Redox									
Nitrate	mg/L	2.2	-	0.71	<0.5	<0.5	<1	<0.5	<0.5
Sulphate	mg/L	66	-	76	69	80	7.0	54	64
Methane	µg/L	39	-	42	57	110	83	100	120
NPOC	mg TOC/l	5.3	-	2.5	2.2	4.6	-	5.0	4.3
Redox conditions		Nitr/ iron				iron			
Grade of dechlorination	%	-	-	77	77		75	75	75

- not measured; Nitr/iron: nitrate to iron reducing conditions; Iron: iron reducing conditions; * The values at t=2 for ethane and ethane, c-DCE and VC in well 53 are indicative values. No samples for ethane and ethene were taken. There was a car on top of the monitoring well NF4.2 so it was not possible to take a sample.

9.4 Molecular analyses

The results of the molecular analyses on groundwater, soil and MicroTraps are presented below.

Table 17 Genes and bacteria present in soil and MicroTrap of the monitoring well AF2.

Monitoring well (depth m-gl)		AF 2 (6-10)				
Analyses	Date	14-8-2012	15-8-2012	5-10-2012	12-12-2012	14-2-2013
	Time point	start (soil)	t=0	t=2	t=4	t=6
Contaminant	Unit					
Total Bacteria	cells/gr	1,20E+09	6,80E+09	2,10E+09	2,20E+08	8,00E+08
Dehalococcoides sp.	cells/gr	3,00E+05	1,50E+05	1,50E+05	3,10E+04	9,10E+04
vcrA	cells/gr	4,90E+04	2,50E+04	2,10E+04	8,40E+03	3,80E+04
Polaromonas JS666	cells/gr	2,10E+04	1,30E+05	< 8,5e+003	<3,3E+3	< 8,2E+3
etnC	cells/gr	< 9,6e+003	< 1,4e+004	< 8,5e+003	<3,3E+3	2,2E+4
etnE	cells/gr	< 9,6e+003	< 1,4e+004	< 8,5e+003	<3,3E+3	<8,2E+3

Table 18 Genes and bacteria present in soil and MicroTrap of the monitoring well NF 4.2.

Monitoring well (depth m-gl)		NF4.2 (9-11)		
Analyses	Date	14-8-2012	12-12-2012	14-2-2013
	Time point	start (soil)	t=4	t=6
Contaminant	Unit			
Total Bacteria	cells/gr	4,90E+07	7,70E+07	6,80E+07
Dehalococcoides sp.	cells/gr	<2,8E+3	<1,4E+3	<6,1E+3
vcrA	cells/gr	<2,8E+3	<1,4E+3	<6,1E+3
Polaromonas JS666	cells/gr	<2,8E+3	<1,4E+3	<6,1E+3
etnC	cells/gr	<2,8E+3	6,70E+03	1,20E+05
etnE	cells/gr	<2,8E+3	<1,4E+3	1,20E+04

Table 19 Genes and bacteria present in soil and MicroTrap of the monitoring well MW53.

Monitoring well (depth m-gl)		53 (38 -39)			
Analyses	Date	14-8-2012	05-10-12	12-12-2012	14-2-2013
	Time point	start (soil)	t=2	t=4	t=6
Contaminant	Unit				
Total Bacteria	cells/gr	1,10E+08	1,50E+08	1,70E+08	1,50E+08
Dehalococcoides sp.	cells/gr	<1,9E+3	<1,5+E3	<2,4E+3	<6,3E+3
vcrA	cells/gr	<1,9E+3	<1,5+E3	<2,4E+3	<6,3E+3
Polaromonas JS666	cells/gr	<1,9E+3	<1,5+E3	<2,4E+3	<6,3E+3
etnC	cells/gr	3,20E+03	8,20E+04	9,40E+04	4,20E+04
etnE	cells/gr	<1,9E+3	3,80E+04	1,90E+05	7,30E+04

The data in Table 1 showed the molecular results on the groundwater (traditional sampling method) samples. The number of total bacteria measured in NF4.2 was up to 6 x10^5 cells/mL and in well 53 up to 2.4 x 10^4 cells/mL, in the latter well also etnE and etnC were detected after 6 months, no other genes/ bacteria were detected in these groundwater samples in 2012/2013. The data of the soil and MT results on the other hand show up to 6.8 x 10^9 cells/gr, see Table 17. The total bacterial count, DHC and *vcrA* is most abundant in AF 2 with equivalent high concentrations of VOC. So with high concentrations of chlorinated compounds a high bacterial count is expected. After 6 months also the gene etnC was detected. In the other wells NF4.2 and MW53 the genes *etnE* and *entC* were detected above the detection limit. It was not possible to sample NF4.2 in October (t=2), therefore no results are available.

9.4.1 Degradation processes in monitoring well Amsterdamsestraatweg AF2

As shown in previous tables, prior deployment, the *Dehalococcoides* spp., and *vcrA* are present in relatively high quantities in AF2 in the MTs. The dominant process here is reductive dechlorination. On average an amount of 1 x 10^3 cells DHC/mL is often sufficient to degrade high levels of VOC. The redox conditions are highly reduced and it is likely for reductive dechlorination to occur here. In the measurements, in time no prevalent VOC concentration decrease is detected, however the presence of the degradation products ethene and ethane (see Table 15) is another line of evidence for reductive dechlorination. Degradation rates for cis-DCE at the location are derived from the results obtained based on the number of *Dehalococcoides* present in soil, MT and groundwater and vary in the range from 0.007 to 0.07 µmol/l day.

On the other hand the strain *Polaromonas* was also detected. The *Polaromonas* strain JS666 is involved in the metabolic degradation of cis-DCE, this requires oxygen. This strain is measured only in the first two time points. It is possible that during deployment oxygen might have leached in, this was however consumed rapidly since no *Polaromonas* was detected after two months of incubation. There was *etnC* detected after 6 months which would indicate there are some micro-aerophilic conditions prevalent, however in the other time points there were no signs for the micro-aerophilic degradation of VC. It is known that VC degraders are widely spread, these bacteria are most abundant downstream of the pollution source (Coleman, 2003).

Based on the abundance of *Dehalococcoides* present, the process of reductive dechlorination is the dominant process.

In the upstream well (2016) a slight increase in degradation products (DCE and VC) is seen in time, in the downstream well a decrease in cis-DCE and VC is detected compared to the previous measurement. It is assumed that reductive degradation is the dominant process. The strongly reduced conditions (high methane concentrations) are an evidence.

9.4.2 Processes in the vicinity of the Nachtegaalstraat NF4

At this site considerable lower concentrations of chlorinated solvents were measured compared to the Amsterdamsestraatweg, mainly cis-DCE and VC. The redox conditions are nitrate to iron reducing, which is not favourable for reductive dechlorination by DHC. In the MicroTraps no reductive dechlorinating related bacteria nor genes were measured. After four months (t=4) the functional gene *etnC* was detected, involved in the first degradation step of VC. The *EtnC* possessing bacteria were identified in a VC-contaminated water samples.

The redox conditions are favourable so it is likely that VC oxidation will occur. Both the genes *etnC* and *etnE* were detected in the monitoring well after 6 months of incubation. The level of VC has increased compared to the measurement at the time of deployment, however it is likely that VC is formed as a result of reductive dechlorination more upstream. In the upstream well (NF3.1) low concentrations of PCE and TCE are detected. In addition the levels of nitrate and NPOC showed a decrease, as a result of microbial activity.

In the groundwater few bacteria were detected. A total bacterial count of 6,0 x 10^5 cells/mL was measured which is relatively low for an environmental groundwater sample. In the soil from the MicroTraps on the other hand numbers of 4,9 x 10^7 cells/gr were measured, this proves the adherence preference of bacteria.

9.4.3 Processes in monitoring well 53 near ATES system

In monitoring well 53 no nitrate was detected, the redox conditions are therefore iron to sulphate reducing. The conditions are favourable for micro-aerophilic degradation. The groundwater results show that there are few bacteria present. In de groundwater no functional genes nor DHC were detected. In the MicroTrap and soil samples however the bacteria are abundant, up to 1.7 x 10^8 cells/gr (
Table 18). The genes responsible for micro-aerophilic degradation *etnC* and *etnE* were detected at all timepoints up to 9.4 x 10^4 and 1.9 x 10^5 cells/gr, which indicates that micro-aerophilic degradation does occur on site. It should be mentioned that the wells sampled after two and four months (t=2 and t=4), were not located at 38 m-gl but at 8 m-gl. Therefore the MicroTraps were more susceptible to convection of oxygen as described before. The results after 6 months however confirm the previous results, micro-aerophilic degradation is occurring. There is a slight increase in VC, possibly from upstream degradation of DCE.

No variations in the contaminant concentrations and redox conditions are observed over time. Therefore it can be concluded that VC oxidation is prevalent on site however degradation is slow, no degradations rates can be extracted from the findings.

9.5 Conclusions MicroTrap degradation assessment

From the results the following can be concluded:

- In monitoring well AF2 concentrations of 230,000 µg total VOC/L (t=6) were detected. TCE was measured in the highest concentration of 150,000 µg/L. The redox conditions are methanogenic and in the MicroTraps the dechlorinating bacteria *Dehalococcoides* was detected, indicating that reductive dechlorination is the dominant active process at the source zone.
- In monitoring well NF4 concentrations of 700 µg total VOC/L at t=4 were detected. VC was detected at the highest concentrations of 690 µg/L. The redox conditions are nitrate to iron reducing. The dominant process is VC oxidation, the genes *etnE* and *etnC* were detected in relative high numbers.
- In monitoring well 53 VC was the dominant contaminant present in concentrations up to 2,700 µg/L at 6 months. The redox conditions are iron to sulphate reducing. The genes *etnE* and *etnC* were detected in the MicroTraps, it is therefore likely that micro-aerophilic degradation is the dominant process.

The total bacterial count was detected in higher numbers in the MicroTraps compared to groundwater samples, indicating that the MT sampling method is a representative method of bacterial and molecular detection.

In several monitoring wells (NF4 and 53) additional MTs are still present for additional molecular analysis (if required).

10 Conclusions

10.1 Background and aim

In this study the biodegradation capacity was determined by conducting molecular analysis on groundwater, compound specific stable isotope analysis, lab microcosms, analysis on BACTRAPS and molecular analysis on MicroTraps.

10.2 Groundwater

Molecular analyses on 19 groundwater samples, scattered over the city centre area of Utrecht were conducted to determine whether *Dehalococcoides* (DHC) or *Polaromonas* bacteria and the genes *(etnE* and *etnC*) were present. Samples were obtained using the traditional groundwater sampling method. In monitoring well 67 potential for micro aerophilic degradation appeared to be present, the gene etnC was detected in low numbers. In well 53 both etnE and etnC were detected indicating micro-aerophilic degradation. The occurrence of these genes positively correlates with the high concentration of VC in these monitoring wells. Only in a selection of the sampled monitoring wells (2/19) these genes were detected, this is probably biased by the sampling method (traditional groundwater sampling see §10.5) but could also imply that the potential for micro-aerophilic degradation is limited to certain locations and spots. In an additional (traditional) groundwater sampling (in 2001 and 2008) in wells with high VC concentrations significant high numbers of DHC were detected. In six of the eleven wells the DHC bacteria were detected and in five of the wells the genes *etnE* and/or *etnC* were detected.

A few monitoring wells had favourable conditions for reductive dechlorination, however in a limited number of the wells bacteria for reductive dechlorination were detected.

10.3 Isotope analyses

The results from monitoring wells located at the contaminated site Amsterdamsestraatweg indicated that biodegradation of VC in the plume was caused either by reductive dechlorination in conjunction with a substantial concentration decrease due to non-destructive processes (like dilution) or by aerobic VC degradation in conjunction with a low to moderate concentration decrease due to non-destructive processes. Aerobic VC degradation can (most probably) be excluded to a large extend at the source zone of this site. The results of the isotope analyses strongly indicate that reductive dechlorination is the predominant pathway for VC biodegradation in the source zone. In the plume aerobic processes are likely to occur.

10.4 Degradation lab tests

The results from the microcosm labtests showed that:

- In the abiotic test no decrease of DCE nor VC was observed, indicating that the decrease in the biotic flasks is a result of the biological degradation.
- Biological VC degradation did occur after 6 weeks of incubation with fresh groundwater (which could contain minor concentrations of oxygen)
- The isotope analyses in the microcosm flasks indicates that (micro-aerophilic) oxidation of VC has occurred (formation of ^{13}C labelled CO_2).
- The oxygen concentrations in the labtests were low (in the headspace lower than 1%).
- The degradation rate for VC was approximately 0,1 day^{-1}.
- The relevant genes involved in micro-aerophilic degradation of VC were detected in the soil phase of the labtests.
- The bacteria involved in micro-aerophilic degradation are more prone to adhere to soil particles.
- The occurrence of biological degradation for cis-DCE could not be proven.

Based on the results so far it seems that micro-aerophilic degradation of VC can occur in the subsurface of Utrecht, this is most probably the predominant pathway in the plume.

10.5 BACTRAPS and MicroTraps

BACTRAPS were placed in a number of monitoring wells, in these wells isotope analyses were conducted to determine the prevalent degradation process. Based on the isotope analysis there are indications that reductive dechlorination of cis-DCE occurs in monitoring well 86 (14m-gl). These results are not confirmed by the molecular data. The bacteria involved in reductive dechlorination (*Dehalococcoides*) were not detected in this monitoring well (below detection limit). The molecular analysis don't give insight into the possible degradation process of cis-DCE. With regard to VC degradation strong indications for micro-aerophilic degradation were obtained based on the molecular analyses on the BACTRAPs. The genes involved in micro-aerophilic degradation of VC (etnE and etnC) were detected. The isotope analyses were however focussed on gaining insight in the degradation process of cis-DCE. The isotope data show that CO_2 production occurred, this CO_2 could be derived from the 'naturally' occurring VC or from organic material.

From the MicroTrap research the following can be concluded:
- In the monitoring well located at the Amsterdamsestraatweg, with high concentrations of PCE and TCE and methanogenic redox conditions, the dechlorinating bacteria *Dehalococcoides* was detected in high numbers. Indicating that reductive dechlorination is the dominant active process on site.
- In the monitoring well located at the Nachtegaalstraat, with high concentrations of cis-DCE and VC the dominant process is VC oxidation, the genes *etnE* and *etnC* were detected.
- In monitoring well 53, where micro-aerophilic degradation was expected VC was the dominant contaminant present. The genes *etnE* and *etnC* were detected in the MicroTraps, it is therefore expected that micro-aerophilic degradation is the dominant process.

The total bacterial count was higher in the MicroTraps compared to groundwater samples, indicating that the MicroTrap sampling method is a representative method for the detection of bacteria present in the subsurface.

10.6 Degradation rate

Based on the degradation tests the degradation rate for VC under aerobic conditions was determined at 0,1 day^{-1} . The degradation rate in-situ is expected to be lower. Furthermore this process requires oxygen, the speed of the process will be dependent on the oxygen present.

The 1st order degradation (rate) is dependent on the contaminant concentration, this means that the degradation rate decreases with a decrease in contaminant concentration. The redox conditions probably determine which degradation process occurs. To determine the degradation rate in-situ based on the number of *Dehalococcoides* spp. data from literature [Suarez and Rifai 1999)] was used. The number of *Dehalococcoides* spp. varied in the different samples, this implies that there is a broad range in degradation rate at the site. Based on the *Dehalococcoides* number and contaminant concentration we expect the degradation rates to vary. In table 20 the expected degradation rates are given. The degradation rates are higher in the source zone compared to the plume areas. Locally no reductive dechlorination capacity was detected at all. Thus in some areas no degradation will take place as both the capacity for reductive dechlorination as well as for micro-aerophilic degradation is absent.

Table 20. Degradation rates in Utrecht (day^{-1}) these are based on literature [Suarez and Rifai, 1999] and number of *Dehalococcoides* bacteria.

Contaminant	Reductive proces source	Reductive proces plume	Oxidative proces plume area
PCE	0,08	0,008-0,0008	
TCE	0,023	0,002-0,0002	
DCE	* 0,013	0,001-0,0001	
VC	0,007	0,001- 0,0001	0,1-0,02

* 90th percentile data used as the maximum degradation rate reported by Suarez and Rifai was regarded as unrealistic (partially based on the statistical data: the maximum reported degradation rate was 0.1 day^{-1} whereas the 90th percentile data point was 0,013 day^{-1})

10.7 Optimisation of the sampling method

In the soil samples from the MicroTraps a significant increase in detected total bacteria and degradation specific genes and bacteria was shown compared to groundwater samples. The BACTRAP and MicroTrap methods are comparable quantification tools. The MicroTrap sampling method is an improved technique to quantify bacteria and genes in the environment because MTs:

- Contains (local) sand instead of granulates. These granulates actively abstract contaminants and subsequently bacteria from the close vicinity. It is therefore likely that the numbers detected are an over prediction of the actual number of bacteria in the environment as a result of the activated carbon properties.
- Granulate in the BACTRAP is a foreign adhesion material, and therefore the expression of the detected cells/copies will be per BACTRAP instead of cells/gr soil. The results only indicate the presence of certain genes and bacteria but quantification of the results is difficult. Therefore

MicroTraps not only represent the actual number of bacteria and genes present in the environment, but the results can also be easily extrapolated to actual in situ numbers.

This technique is highly suitable for detecting bacteria which are prone to adhere to soil surface, the slow growing micro-organisms.

10.8 Summary

The main research questions that are dealt with in this investigation are summarized below.

Does the subsurface in Utrecht have a degradation capacity (qualitative approach)?
Yes, there is a capacity for biodegradation in the subsurface of Utrecht because in monitoring wells distributed in the whole city centre area bacteria and genes responsible for reductive dechlorination or micro-aerophilic degradation were identified.

Which degradation process(es) are predominant in Utrecht?
The predominant process is reductive dechlorination in monitoring wells with high concentrations of PCE and TCE. However in monitoring wells with high VC and cis-DCE concentrations and iron to sulphate reducing conditions it is more likely that micro-aerophilic degradation processes are dominant. These processes are heterogeneously distributed throughout the city center of Utrecht.

What is the extent of this biodegradation capacity (quantitative approach)?
In several monitoring wells scattered over the city centre area the potential for biodegradation was confirmed. The degradation rate is however dependent on the contaminant concentration at a site. At the Amsterdamsestraatweg a degradation rate was derived based on the number of DHC present, the rate varies in the range from 0.007 to 0.07 umol/l/dag for DCE. Furthermore based on the *Dehalococcoides* numbers it is also possible to determine that degradation rates are higher in de source zone than in the plume area. We expect degradation rates to vary between 0.05 and 0.005 day^{-1} for PCE, 0.02 and 0.002 day^{-1} for TCE, 0.1 and 0.001 day^{-1} for DCE and 0.007 and 0.001 day^{-1} VC. The degradation rates are higher in the source zone compared to the plume areas.

Is this degradation capacity expected to be present in the whole area within the city centre of Utrecht?
The extrapolation of the detected degradation processes to the whole area is possible since the current research results provide several lines of evidence that micro-aerophilic degradation and reductive dechlorination processes are present at different locations within the area. However the results are based on a selection of monitoring wells distributed over the whole city centre of Utrecht. A strong correlation for occurrence of either of the processes remains to be investigated. Furthermore within this project a new sampling method was developed, this method should be applied to more monitoring wells in Utrecht to be able to determine the extent of micro-aerophilic degradation capacity.

10.9 Future perspective

The current research provides evidence for the degradation capacity in the subsurface of Utrecht. The degradation process in the subsurface with high concentrations of PCE and TCE is predominantly reductive dechlorination, although this process has not been detected in many samples from Utrecht (in the current project).

It is known that temperature can effect degradation rates. The ATES system will evidently have an effect on the temperature in the subsurface. Within project MMB, Meer Met Bodemenergie the temperature effect biodegradation was investigated. The effect of change in temperature on micro-aerophilic degradation was investigated within the project of Meer Met Bodemenergie.

Furthermore the correlation between degradation processes and site (redox)conditions is still unknown. In order to extrapolate the degradation capacity data to the overall Utrecht city centre area it is important to investigate the correlation of redox conditions and VOC concentrations in relation to occurrence of micro-organisms and genes involved in the degradation processes. Therefore we propose to do a groundwater characterization on the 19 monitoring wells sampled previously. Furthermore we suggest to take samples using the new sampling method (MicroTrap).

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Appendices

Appendix 1 Literature review micro-aerophilic degradation

1.1 Achtergrondinformatie afbraak chloorethenen

De biologische afbraak van chloorethenen kan via zowel anaerobe (reductieve dechlorering) als aerobe (aerobe oxidatie) processen verlopen. Onder de juiste anaerobe omstandigheden zijn alle gechloreerde ethenen afbreekbaar. Aerobe afbraak treedt alleen op voor *cis*-DCE en VC.

Tot voor kort werd aangenomen dat in anaerobe bodempakketten ook afbraak van *cis*-DCE en VC mogelijk was via anaerobe oxidatie, waarbij nitraat, ijzer(III), sulfaat of CO_2 als elektronenacceptor wordt gebruikt in plaats van zuurstof. Een van de redenen voor deze voorgestelde afbraakroute was de waarneming dat pluimen met *cis*-DCE en VC in matig gereduceerde bodempakketten stabiel bleken te zijn zonder dat er volledige reductieve afbraak tot etheen of ethaan optrad. Recent onderzoek heeft aangetoond dat er in deze gevallen echter geen sprake is van anaerobe oxidatie maar van micro-aerofiele aerobe afbraak in overwegend anaerobe bodempakketten.

Hieronder worden de verschillende processen en bijhorende condities verder toegelicht.

1.1.1 Reductieve dechlorering

Bij reductieve dechlorering worden PER en TRI via *cis*-DCE en VC omgezet in etheen/ethaan. Reductieve dechlorering verloopt goed onder sterk gereduceerde (methanogene) omstandigheden.

Dit is het afbraakproces voor chloorethenen dat tot op heden het best bestudeerd is. In dit afbraakproces wordt VOCl als elektronacceptor gebruikt en wordt organisch koolstof als elektrondonor wordt gebruikt. PER en trichlooretheen (TRI) kunnen middels reductieve dechlorering onder ijzerreducerende tot methanogene condities worden afgebroken, hierbij wordt *cis*-dichlooretheen (cis-DCE) als afbraakproduct gevormd. Indien volledige afbraak plaatsvindt dan worden achtereenvolgens via cis-DCE de afbraakproducten vinylchloride (VC) en uiteindelijk het onschadelijke etheen en/of ethaan gevormd. Voor de volledige anaerobe afbraak van chloorethenen via reductieve dechlorering moet voldaan worden aan onderstaande randvoorwaarden:

- Aanwezigheid van gereduceerde omstandigheden.
- Aanwezigheid van voldoende organisch koolstof (TOC).
- Aanwezigheid van de dechlorerende bacteriën, met name *Dehalococcoides* sp.

In 2002 is ook aangetoond dat (an)aerobe oxidatie van etheen naar CO_2 onder sulfaatreducerende condities plaatsvindt. Dit betekent dat onder sulfaatreducerende condities reductieve dechlorering tot etheen plaats kan vinden maar dat vervolgens geen etheen of ethaan ophoping plaats hoeft te vinden.

1.1.2 Micro-aerofiele afbraak cis-DCE en VC

Bradley [1996, 1997, 1998a en 1998b] heeft in laboratorium experimenten onder verschillende redoxcondities het optreden van (anaerobe) oxidatie van VC en cis-DCE gerapporteerd. Uit meerdere onderzoeken van andere wetenschappers blijken de bevindingen van Bradley [2003] over anaerobe oxidatie niet te kloppen. Fang [2009] heeft in 350 afbraaktesten geen anaerobe oxidatie van cis-DCE en VC kunnen aantonen. De testen zijn uitgevoerd onder ijzer en mangaan reducerende condities. Schmidt

(2008) heeft eveneens in 40 afbraaktesten onder nitraat, mangaan, ijzer en sulfaat reducerende condities geen anaerobe oxidatie van cis-DCE en VC kunnen aantonen. In deze afbraaktesten vond in het begin van de test wel afname van VC plaats. De concentratie afname stopte echter op het moment dat de zuurstofconcentratie lager werd dan 0,3 mg/l en de afbraaktest echt anaeroob werd. Onder nitraat of ijzer reducerende condities kunnen toch lage zuurstofconcentraties aanwezig zijn [Gossett 2010]. Dat op enkele praktijklocaties oxidatieve afbraak plaats lijkt te vinden is volgens Fang [2009] en Gossett [2010] mogelijk het gevolg van zeer lage zuurstofconcentraties die naar de diepte doordringen. In 2008 heeft ook Bradley aangegeven dat anaerobe oxidatie van VOCl in feite waarschijnlijk micro-aerofiele oxidatie is geweest.

Zuurstof concentraties van 0,1 mg/l liggen vaak in de buurt van de detectiegrens van meetapparatuur, waardoor het aantonen van lage zurustofoncentraties en het verbruik daarvan voor afbraak in de praktijk niet goed mogelijk is.

Micro-aerofiele oxidatie van cis-DCE

Biologische aerobe afbraak van *cis*-DCE is geen wijdverspreide microbiologische eigenschap. Tot op heden is er slechts één bacterie waarvan bekend is dat deze *cis*-DCE als (enige) koolstofbron kan gebruiken, de zogeheten *Polaromonas* sp JS666. Bioclear heeft een moleculaire analyse ontwikkeld voor de detectie van dit organisme [Jennings 2007]. De afbraakroute van cis-DCE (metabolisch) is tot op heden niet opgehelderd.

Micro-aerofiele afbraak van VC

De aerobe afbraak van VC is wel wijd verspreid. Er zijn drie groepen bacteriën die VC onder aerobe omstandigheden kunnen afbreken [Chuang et al 2010]:

- Methanotrofen
- Ethenotrofen
- VC-assimilerende bacteriën

Bacteriën die VC kunnen afbreken komen zowel op VC verontreinigde als op niet verontreinigde locaties voor [Mattes et al 2010]. Het is lastig om onderscheid te maken tussen aerobe etheen-afbrekers die wel VC kunnen afbreken en zij die VC niet kunnen afbreken [Gossett 2010]. Onderzoek van Yang heeft echter aangetoond dat etheen-afbrekers die langdurig worden blootgesteld aan VC na verloop van tijd in staat zijn om VC als groeisubstraat te gebruiken [Yang 2008].

De meest veel voorkomende VC-assimilerende bacterien in het milieu zijn *Mycobacterium* stammen (bijv. JS60, JS61, JS616, JS617 [Coleman 2002b]). Verder zijn er ook stammen van *Pseudomonas*, *Nocardiodes* (bijv JS614), *Ochrobactrum* en *Ralstonia* die VC kunnen afbreken. De genoemde JS strains hebben allen een lage Ks waarden voor VC en zuurstof, met andere woorden bij zeer lage VC concentraties en/of zeer lage zuurstofconcentraties zijn ze in staat om VC af te breken [Mattes]. Afbraak van VC is waargenomen bij zeer lage zuurstofconcentraties van 0,1-0,3 mg/l [Smidt & Tiehm 2008]. Er is slechts 2 mol zuurstof nodig om 1 mol VC af te breken (circa 1:1 op massabasis) [Gossett 2010]. Gossett geeft aan dat het niet mogelijk is om op basis van zuurstofmetingen vast te stellen/uit te sluiten of

aerobe afbraak van VC op een locatie kan optreden, omdat VC oxidatie ook kan plaatsvinden bij zuurstofconcentraties van 0,02 mg/l [Gossett 2010]. Gossett verwacht dat de VC-oxideerders op het raakvlak tussen strikt anaerobe en aerobe zones voorkomen. Aerobe afbraak verloopt snel, dus VC zal niet lang aanwezig blijven in aerobe zones.

In uitgevoerde experimenten blijkt dat de *Mycobacterium* soorten geen lagfase hebben terwijl de *Nocardiodes* species wel een lange lagfase hebben maar een veel snellere afbraak vertonen [Mattes 2010].

Optimum temperatuur van Mycobacterium is 30 °C, de optimale zuurgraad (pH) bedraagt 6-6,5. Er is veel variatie in kinetische parameters tussen de bekende aerobe VC-afbrekers. De lagfase varieert tussen 20-110 dagen. VC-afbrekers komen het meeste voor stroomafwaarts van anaerobe kernzone. VC afbrekers komen wijd verspreid voor en de aanwezigheid wordt niet alleen bepaald door de aanwezigheid van VC [Coleman 2002].

Afbraaksnelheid: 60 µmol/l in 12 dagen als alleen cis aanwezig is. In de aanwezigheid van 10 µmol/ l VC nam de afbraaksnelheid af (20 dagen) en in de aanwezigheid van 110 µmol/l VC nog verder af (50 dagen). Als VC concentratie hoger is dan cis concentratie dan wordt eerst alle VC afgebroken en vervolgens pas de cis-DCE. Aanwezigheid van VC heeft dus nadelig effect op afbraak cis-DCE. [Zhou et al 2010].

Gossett beschrijft een proefopzet waarbij door middel van het inbrengen van LDPE (low density polyethylene) buisjes de micro-aerofiele afbraak van VC gevolgd kan worden. Afbraaksnelheden en proefopzet beschreven [Gossett 2010]

Alle ethenotrofen en VC assimilerende bacteriën gebruiken voor de eerste stap in de afbraakroute van VC het enzym alkene monooxygenase (AKMO groep gecodeerd door *etnABCD* gencluster) en voor de tweede stap epoxyalkane coenzym M transferase (EaCoMT groep waaronder *etnE*) [Chuan et al 2010]. AKMO valt onder de soluble di-iron monooxygenase enyzmen (SDiMo). VC wordt omgezet naar chlorooxirane (VC epoxide) en vervolgens met behulp van het *etnE* enzym verder afgebroken (zie onderstaande figuur). De eerste twee stappen zijn middels experimentele data reeds opgehelderd, de overige stappen zijn nog speculatief. *etnC* (en soortgelijke genen) zijn alleen aangetoond op etheen of VC verontreinigde locaties [Chuang et al 2010]. Abe heeft in 2009 uitgebreid onderzoek gedaan naar de chemische samenstelling van het grondwater en moleculaire analyses waarbij hij gekeken heeft naar de verdeling van anaerobe VC afbrekers (*Dehalococcoides* spp., DHC) en aerobe VC afbrekers. Hieruit bleek dat DHC vooral aanwezig is op locaties met een redoxpotentiaal rond sulfaatreducerend of lager en waar organisch rijk materiaal aanwezig was. Het potentiaal voor aerobe afbraak was over de gehele locatie aanwezig, ook in diepe anoxische monsters (DNA analyse uitgevoerd op *etnE,* geen RNA analyse uitgevoerd). Hierbij werd opgemerkt dat er vooralsnog geen onderscheid gemaakt kan worden in *etnE* genen voor etheen afbraak en *etnE* genen voor VC afbraak. Dus de aanwezigheid van *etnE* wil niet per definitie zeggen dat er VC-assimilerende bacteriën aanwezig zijn maar betekent dat er een potentieel aanwezig is voor metabole (growth-linked) VC afbraak [Abe et al 2009, Mattes 2010]. Op etheen

verontreinigde locaties zijn voornamelijk *etnC* en vergelijkbare genen aangetoond [Coleman 2006]. Op de twee VC verontreinigde locaties zijn alleen *etnC* genen aangetroffen [Coleman 2006].

Bij Bioclear is een moleculaire analyse ontwikkeld om het *etnC* gen, het *etnE* gen en *Polaromonas* aan te tonen.

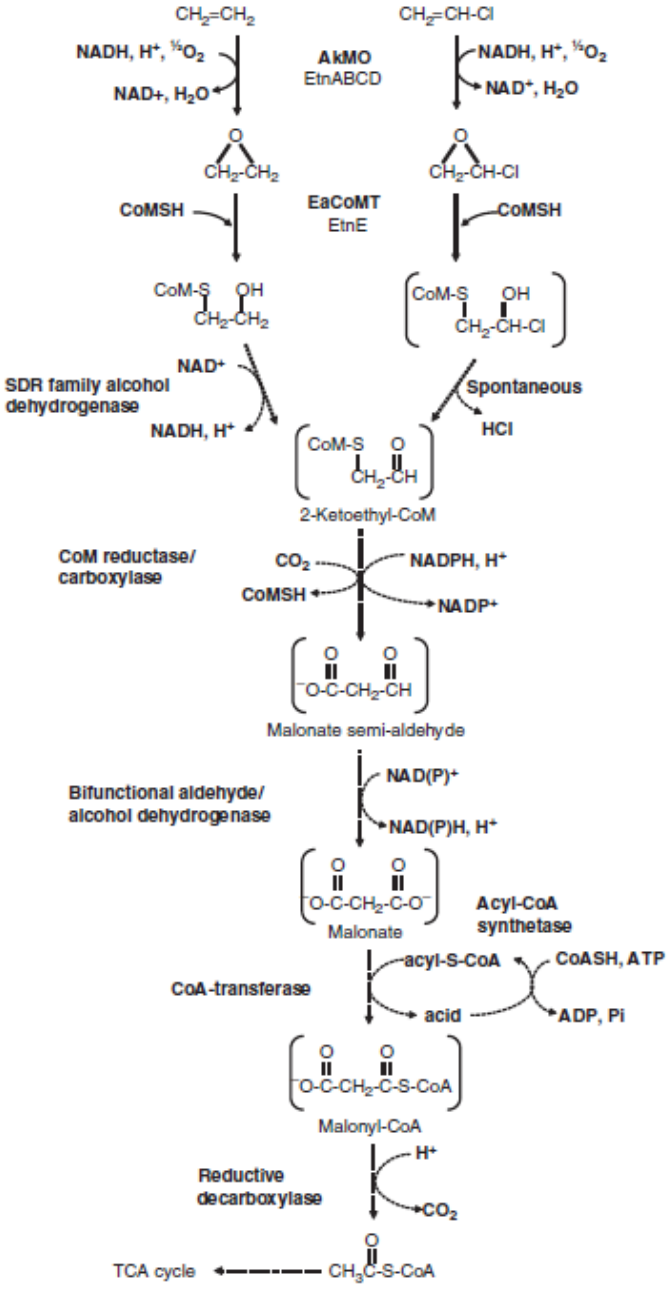

Fig. 4. Proposed VC and ethene biodegradation pathways in aerobic assimilators. Solid arrows depict the biochemical reactions that are supported by experimental evidence. Dotted arrows indicate the proposed biochemical reactions, and the proposed metabolic intermediates are enclosed in parentheses.

Fig 1. Afbraakroute zoals weergegeven in Mattes 2010.

Appendix 2 Results isotope analyses

Isodetect
Umweltmonitoring GmbH

24th of January 2011

Evaluation of Biodegradation at a CVOC-polluted Field Site using Carbon Isotope Analyses

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Field site:	Amsterdamsevaartweg, The Netherlands
Examination period:	October 2010
Persons in charge:	Dr. Anko Fischer / Dr. Heinrich Eisenmann
Report volume:	11 Pages 1 Figure 1 Table 3 Appendices

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Isodetect
Umweltmonitoring GmbH

Contents

1 Background

In order to identify contaminant sources and to assess biodegradation at a field site contaminated by chlorinated volatile organic compounds (CVOCs) Bioclear b.v. entrusted

Isodetect GmbH (Branch Leipzig)
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with an isotope monitoring of groundwater samples.

Most abundant pollutants at the field site are chlorinated ethenes. The higher chlorinated ethenes tetrachloroethene (PCE) and trichloroethene (TCE) occur in minor concentrations compared to the degradation products dichloroethene (DCE) and vinylchloride (VC) (see Fig. 1 in Appendix B) indicating biodegradation by reductive dechlorination at the field site. However, knowledge about the amount and sustainability of contaminant biodegradation as well as predominant degradation pathways especially for lower chlorinated ethenes is missing.

2 Purpose and scope

Compound-specific stable isotope analysis (CSIA) is an appropriate method for assessing pollutant biodegradation in contaminated aquifers *(1)*. Thus, a qualitative and quantitative estimation of the biodegradation of chlorinated ethenes at the field site should be performed by isotope monitoring.

The scope of this study included the following tasks:

- Measurement of carbon stable isotope ratios of chlorinated ethenes;
- Verification of pollutant biodegradation using changes in isotope ratios of chlorinated ethenes;
- Evaluation of predominant biodegradation pathways using changes in isotope ratios of chlorinated ethenes;
- Assessment of pollutant biodegradation using changes in isotope ratios of chlorinated ethenes.

Successive isotope monitoring campaigns should focus on the comprehensive evaluation of pollutant biodegradation at the field site including the identification of predominant degradation pathways and the assessment of the sustainability of pollutant biodegradation.

3 Summary

The isotope monitoring provided evidence of complete biodegradation of chlorinated ethenes at the site. Strong indications were revealed for reductive dechlorination as predominant pathway for VC biodegradation. Based on conservative calculation parameters for the source isotope signature, complete biodegradation of chlorinated ethenes yielded <5-25% within the source zone and 29-65% downstream from the source zone, respectively.

4 Methodology

4.1 Sampling

Samples were taken by Bioclear according to "normal good practices" in October 2010. For isotope analysis, 6 groundwater samples in total were taken from the wells Pb2015 (6-8 m-mv), Pb2015 (10-12 m-mv), Pb2015 (17-19 m-mv), Pb3006 (25 m-mv), Pb3021 (25 m-mv), and Pb3022 (25 m-mv). The concentrations of CVOC and hydrogeochemical parameter were measured by analytical laboratory entrusted by Bioclear. The results of the chemical analyses are given in Appendix A. For the isotope analysis, 1 L groundwater were filled in glass bottles and immediately preserved with NaOH-pellets. The bottles were shipped via courier service to Isodetect in Munich. The samples were stored at 4 °C in the dark until isotope analysis.

The monitoring wells Pb2015 (6-8 m-mv, 10-12 m-mv, 17-19 m-mv) and Pb3006 (25 m-mv) are located within the contaminant source zone. The sampling points Pb3021 (25 m-mv) and Pb3022 (25 m-mv) are located downstream from the source zone.

4.2 Carbon isotope analysis of CVOC

Gas chromatography combustion isotope ratio mass spectrometry (GC-C-IRMS) was applied to determine the stable carbon isotope composition of the CVOC in the groundwater samples. All samples were measured in at least two replicates. Isotope analysis of groundwater samples was done via (1) accumulation by a purge-and-trap unit; (2) transfer to a gas chromatograph split/splitless injector at -150°C; (3) injection at a split ratio of 1:3 to 1:100 to a capillary column (VOCOL 60 m x 0.25 mm ID); (4) separation of compounds in GC; (5) combustion of CVOC to CO_2-molecules; (6) transfer and further separation of $^{12}CO_2$ vs. $^{13}CO_2$ in an isotope ratio mass spectrometer (Thermo Finnegan MAT 253). According to different concentrations of CVOC, pre-dilution and variable injection split ratios were applied. Authentic laboratory standards were used for identification of chlorinated ethenes and to improve the accuracy of the isotope analyses by linear correction of isotope values.

Isotope ratios are conventionally expressed in the delta notation ($\delta^{13}C$) in per mil [‰] relative to an international standard according to:

$$\delta^{13}C_{sample}\ [‰] = \left(\frac{R_{sample} - R_{standard}}{R_{standard}}\right) \cdot 1000 \qquad (1)$$

where R_{sample} and $R_{standard}$ are the $^{13}C/^{12}C$-ratios of the sample and an international standard, respectively. Vienna Pee Dee Belemnite (VPDB, $^{13}C/^{12}C = (11237.2 \pm 2.9) \times 10^{-6}$) was used as standard for the analysis of carbon isotope signatures *(2)*.

The reproducibility of isotope analyses given by the standard deviation of the replicate measurements was ≤0.5 ‰. Reliable isotope ratios could be measured for concentrations ≥5 µg/L.

Due to peak overlaps, isotope ratios of *trans*-DCE could not be determined for all samples. The same problem appeared for VC of sample Pb3006 (25 m-mv).

4.3 Evaluation of carbon isotope data

The evaluation of carbon isotope data for providing evidence of pollutant biodegradation can be conducted based on the criteria given by the US-EPA *(1)*:

- The isotope ratio of a pollutant is significantly more positive than its production depended, primary isotope signature. Primary isotope signatures of contaminants are mostly more negative than -22 ‰. Hence, isotope ratios those are more positive than -20 ‰ can be explained due to biodegradation.
- Changes in isotope ratios of ≥2 ‰ along a groundwater flow path between two sampling points provide evidence of pollutant biodegradation if the influence of a secondary contaminant source with a more positive isotope signature can be excluded. Smaller changes in isotope ratios can be interpreted as strong (>1 ‰) and low (>0.5 ‰) indication for pollutant biodegradation.

The isotope ratios obtained at the field site were evaluated based on the criteria provided by the US-EPA.

To assess whether complete biodegradation of chlorinated ethenes takes place at a contaminated site, an isotope balance can be used calculating cumulative isotope ratio of all CVOC. When the cumulative isotope ratio is more positive than the source isotope signature then complete biodegradation of chlorinated ethenes is indicated. Since biodegradation leads to changes in the isotope ratio of primary CVOC, the cumulative isotope ratio can also be used for assessing the source isotope signature.

The cumulative isotope ratio of all CVOC ($\delta^{13}C_{CVOC}$) was calculated by multiplying the molar concentration of each chlorinated ethene (C_i) with its respective carbon isotope signature ($\delta\ ^{13}C_i$), adding all contributions and dividing it by the total molar concentration of all chlorinated ethenes (C_{CVOC}) (Eq. 2) *(3)*.

$$\delta^{13}C_{CVOC}[‰] = \frac{\sum(C_i * \delta^{13}C_i)}{C_{CVOC}} \quad (2)$$

A detailed description of basics for isotope data evaluation with respect to qualitative and quantitative assessment of pollutant biodegradation is given in Appendix B.

5 Results and discussion

5.1 Qualitative interpretation

The highest CVOC concentrations were detected at sampling point Pb2015 (6-8 m-mv), which is located in the contaminant source zone (Tab. 1). There, the primary contaminants PCE and TCE are already exhausted and biodegradation products DCE and VC are accumulated. The isotope ratio of *cis*-DCE (-16.2 ‰) is significantly more positive than the expected primary isotope signatures of contaminants (< -22 ‰). Therefore, biodegradation of *cis*-DCE at sampling point Pb2015 (6-8 m-mv) was evident. The isotope ratio of VC (-29.6 ‰) was considerably more negative than the isotope ratio of *cis*-DCE indicating an accumulation of VC. Since ethene was detected at the sampling point (Appendix A) it can be assumed that VC was biodegraded. Due to the negative carbon isotope ratio of VC, the formation of VC seems to be more pronounced than its biodegradation.

Substantially smaller CVOC concentrations were determined at Pb2015 (10-12 m-mv). Nevertheless, the isotope ratios of *cis*-DCE (-12.2 ‰) and VC (-29.9 ‰) were similar to these of Pb2015 (6-8 m-mv), therefore, the same conclusion can be drawn for Pb2015 (10-12 m-mv): *cis*-DCE is biodegraded and VC accumulates.

For Pb2015 (17-19 m-mv), isotope ratios of *cis*-DCE (-10.1 ‰) and VC (-19.8 ‰) were more positive than the expected primary isotope signatures of contaminants (< -22 ‰) providing evidence for the biodegradation of these two pollutants.

Primary pollutants PCE and TCE were detected in higher concentrations at Pb3006 (25 m-mv). The isotope ratio of PCE (-23.1 ‰) does not give an indication of biodegradation since this value was in the range of primary isotope signatures of pollutants (< -22 ‰). The isotope ratio of TCE (-19.3 ‰) was more positive than primary isotope signatures of pollutants (< -22 ‰), hence, biodegradation of TCE at Pb3006 (25 m-mv) was evident. Compared to the

isotope ratios of PCE and TCE, *cis*-DCE exhibits a more negative isotope signature (-26.0 ‰) leading to the assumption that *cis*-DCE accumulates at Pb3006 (25 m-mv). Nevertheless, the elevated VC concentration indicates the biodegradation of *cis*-DCE. The relative negative isotope signature of *cis*-DCE suggests that the formation of *cis*-DCE seems to be more pronounced as its biodegradation. The isotope ratio of VC could not be determined because interfering compounds led to an overlap of the VC peak. Hence, information on VC biodegradation for Pb3006 (25 m-mv) cannot be derived based on isotope data. The detection of ethene at this sampling point indicated that biodegradation of VC has occurred.

At Pb3021 (25 m-mv) and Pb3022 (25 m-mv) located downstream from the source zone, only VC could be detected in substantial concentrations. The isotope ratios of VC were at both sampling points more than 2 ‰ more positive than those within the source zone and those of primary pollutants (< -22 ‰) providing evidence for biodegradation of VC at Pb3021 (25 m-mv) and Pb3022 (25 m-mv), respectively.

The cumulative isotope ratios of CVOC ($\delta^{13}C_{CVOC}$) provide information on the primary isotope signature of CVOC, those isotope signature of the compound that emerge from the production process and has not be influenced by biodegradation processes, and the complete biodegradation of chlorinated ethenes. Since the primary pollutants PCE and TCE are exhausted to a large extent, the primary isotope signature of PCE and TCE could not be determined at the field site. The source isotope ratio remains within the reductive dechlorination cascade (Fig. 1) as long as no substantial consumption of the end member of the cascade (VC) occurs. The most negative cumulative isotope signature was determined at Pb2015 (10-12 m-mv) (-24.4 ‰). This $\delta^{13}C_{CVOC}$-value can be regarded as an indication for the source isotope signature of the primary pollutants PCE and TCE. Since the detection of ethene indicated a complete reductive dechlorination, an enrichment of ^{13}C can be expected within the cascade leading to a more positive $\delta^{13}C_{CVOC}$-value compared to the source isotope signature of the primary pollutants PCE and TCE. Therefore, the source isotope signature of primary pollutants should be more negative than the $\delta^{13}C_{CVOC}$-value for Pb2015 (10-12 m-mv) (-24.4 ‰). Based on the assumption that the $\delta^{13}C_{CVOC}$-value at Pb2015 (10-12 m-mv) is representative for the source isotope signature, a conservative interpretation of the biodegradation of chlorinated ethenes was conducted. This avoids an overestimation of the biodegradation of chlorinated ethenes.

Compared to the $\delta^{13}C_{CVOC}$-value for Pb2015 (10-12 m-mv) (-24.4 ‰), the cumulative isotope signature of Pb3006 (25 m-mv) was nearly similar (-23.8 ‰) suggesting that only a limited complete biodegradation of chlorinated ethenes took place at this sampling point. Moderately higher differences for the $\delta^{13}C_{CVOC}$-values of Pb2015 (6-8 m-mv) and Pb2015 (17-19 m-mv) indicated the complete biodegradation of chlorinated ethenes at these sampling points. Substantial differences of cumulative isotope signatures were obtained at Pb3021 (25 m-mv)

and Pb3022 (25 m-mv) providing evidence for the complete biodegradation of chlorinated ethenes.

Tab. 1: Concentrations, individual and cumulative isotope ratios of CVOC.

Sample/ Location	CVOC	$\delta^{13}C$ [‰]	Conc. [µg/L]	Conc. [µmol/L]	Σ Conc. $_{CVOC}$ [µmol/L]	$\delta^{13}C_{CVOC}$ [‰]
Pb2015 (6-8 m-mv) source zone	PCE	n.d.	0.43	0,003	178.6	-22.3
	TCE	n.d.	0.96	0,007		
	cis-DCE	-16.2	9400	97		
	VC	-29.6	5100	82		
Pb2015 (10-12 m-mv) source zone	PCE	n.d.	0,1	0,001	5.6	-24.4
	TCE	n.d.	0,1	0,001		
	cis-DCE	-12.2	170	1,8		
	VC	-29.9	240	3,8		
Pb2015 (17-19 m-mv) source zone	PCE	n.d.	0,1	0,001	0.8	-18.3
	TCE	n.d.	0,1	0,001		
	cis-DCE	-10.1	13	0,1		
	VC	-19.8	45	0,7		
Pb3006 (25 m-mv) source zone	PCE	-23.1	390	2,4	10.8	-23.8
	TCE	-19.3	280	2,1		
	cis-DCE	-26.0	510	5,3		
	VC	n.d.	61	1,0		
Pb3021 (25 m-mv) downstream from the source zone	PCE	n.d.	0,1	0,001	3.4	-1.7
	TCE	n.d.	0,1	0,001		
	cis-DCE	n.d.	0,1	0,001		
	VC	-1.7	210	3,4		
Pb3022 (25 m-mv) downstream from the source zone	PCE	n.d.	0,1	0,001	5.6	-7.2
	TCE	n.d.	0,1	0,001		
	cis-DCE	n.d.	0,39	0,004		
	VC	-7.2	350	5,6		

n.d. - not detectable.

5.2 Evaluation of degradation pathways

Chlorinated ethenes can be biodegraded via reductive dechlorination (see Fig. 1 in Appendix B) or via metabolic or cometabolic aerobic breakdown. In order to figure out which of these pathways are predominant for the VC biodegradation at the site, changes in isotope ratios of VC in correlation to the concentration decrease of VC were evaluated.

The carbon isotope fractionation during reductive dechlorination of VC is significantly more pronounced (ε_C = -21.5 to -31.1 ‰ *(4-8)*) than carbon isotope fractionation during aerobic degradation (ε_C = -3.2 to -8.2 ‰ *(8-11)*). The isotope fractionation during biodegradation of pollutants can be described by the Rayleigh equation (see Eq. 1 in Appendix B). Using the known isotope enrichment factors (ε_C) for VC biodegradation, specific ranges for VC

degradation pathways can be determined from the correlation of isotope fractionation and concentration decrease according to the Rayleigh equation (Fig. 1). The predominant pathway for *in situ* biodegradation of VC can be discovered by plotting the isotope and concentration data of VC determined at the site into the diagram. For the interpretation of the field data, it has to be taken into account that the VC concentration decrease within an aquifer may result from non-destructive processes like dispersion, dilution, volatilization and sorption, respectively, for which isotope effects can be neglected in nearly all circumstances. The influence of non-destructive processes may lead to a shifting of field data below the range of the actual pathway and, therefore, to inconclusive interpretations. For example, if field data fall into the range of aerobic VC degradation, two interpretations are possible: (1) the predominant pathway is aerobic VC degradation and the influence of non-destructive processes is of minor extent or (2) the predominant pathway is reductive dechlorination of VC and the influence of non-destructive processes is of substantial extent. If field data are above a range of a specific pathway it can be excluded that this pathway is predominant for the *in situ* biodegradation.

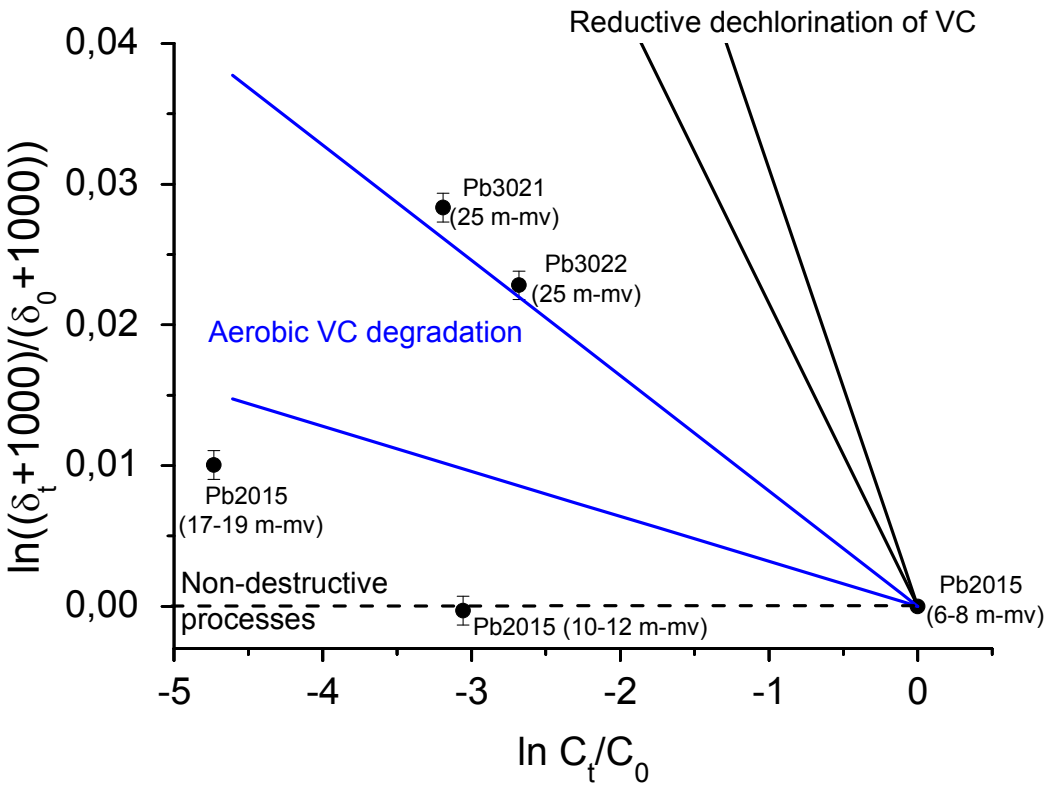

Fig. 1: *Rayleigh equation plot with specific ranges for aerobic VC degradation (blue) and for reductive dechlorination of VC (black). The dashed line represents the evolution for isotope and concentration data which are specific for non-destructive processes. The black points give the isotope and concentration data according the Rayleigh equation for the sampling points. The error bars of the black points were obtained from the expected error of isotope analysis (±0.5 ‰).*

The sampling point with the highest VC concentration (5100 µg/l, Pb2015 (6-8 m-mv)) was chosen as reference point (i.e. δ_0 and C_0 for the Rayleigh equation, see Eq. 1 in Appendix B) for the evaluation of predominant VC degradation pathway at the field site using the Rayleigh equation approach. Since the field data of Pb2015 (10-12 m-mv) lie on the line which is specific for non-destructive processes (Fig. 1) the concentration decrease of VC seemed to be mainly caused by non-destructive processes at this sampling point.

Based on Pb2015 (6-8 m-mv) as reference point, the field data at Pb2015 (17-19 m-mv) (i.e. δ_t and C_t for the Rayleigh equation, see Eq. 1 in Appendix B) indicated that biodegradation of VC was caused either by reductive dechlorination in conjunction with a substantial concentration decrease due to non-destructive processes or by aerobic VC degradation in conjunction with a low to moderate concentration decrease due to non-destructive processes.

The field data of the sampling points located downstream from the source zone Pb3021 (25 m-mv) and Pb3022 (25 m-mv) lie slightly above the range of aerobic VC degradation (Fig. 1). Therefore, aerobic VC degradation can be excluded to a large extend at these sampling points which gives strong indications that reductive dechlorination is the predominant pathway for VC biodegradation. To confirm the reductive dechlorination as predominant pathway for VC biodegradation at the field site, chlorine isotope analysis additionally to carbon isotope analysis should be carried out. The ratio for chlorine and carbon isotope fractionation is substantially different for reductive dechlorination and for aerobic degradation of VC suggesting that the reaction mechanisms can be differentiated at the field scale using a dual isotope approach *(8)*.

5.3 Quantitative interpretation

Using the Rayleigh equation, pollutant biodegradation within groundwater systems can be estimated by the changes in isotope ratios. Appropriate enrichment factors are necessary for proper calculations. A summary of enrichment factors for contaminant biodegradation is available in the literature *(1)* and internet, respectively *(12)*. Due to the detection of breakdown products and the Rayleigh equation approach, it could be figured out that the reductive dechlorination seemed to be the dominant process for the microbial removal of CVOC. The overall enrichment factor for complete reductive dechlorination can be assumed between -22 and -51 ‰ *(3)*. The more negative enrichment factor leads to a conservative estimation of complete reductive dechlorination and the more positive enrichment factor to a progressive estimation. The $\delta^{13}C_{CVOC}$-value for Pb2015 (10-12 m-mv) was defined as source isotope signature ($\delta^{13}C_0 \triangleq$ -24.4 ‰).

Within the source zone, complete biodegradation of chlorinated ethenes was of minor extent (<5%) at Pb3006 (25 m-mv). Relative low complete biodegradation of chlorinated ethenes was obtained at Pb2015 (6-8 m-mv) (4-9%) and at Pb2015 (17-19 m-mv) (12-25%), respectively.

Downstream from the source zone, moderate to substantial complete biodegradation of chlorinated ethenes was estimated for Pb3021 (25 m-mv) (36-65 %) and for Pb3022 (25 m-mv) (29-55 %), respectively.

6 Conclusions and outlook

The isotope data provided evidence for the complete degradation of chlorinated ethenes at the Amsterdamsevaartweg site. Based on a Rayleigh equation approach, strong indications were obtained that reductive dechlorination is the predominant biodegradation pathway for VC downstream from the contaminant source. Isotope data were used for estimating complete biodegradation of chlorinated ethenes. The results revealed moderate to substantial biodegradation downstream from the contaminant source.

Successive isotope monitoring campaigns should focus on the assessment of predominant degradation pathways of DCE and VC for zones of the contaminant plume that have not investigated so far. This would provide comprehensive knowledge on processes which are relevant for *Natural Attenuation* of pollutants at the site. Next to the carbon isotope analysis, chlorine isotope analysis should be carried out to obtain a more distinct differentiation of predominant degradation pathways.

Moreover, the sustainability of pollutant biodegradation should be evaluated during successive isotope monitoring campaigns. In order to assess the future development of pollutant biodegradation at the field site, the carbon isotope data of the current isotope monitoring will be compared with carbon isotope data which will regularly be obtained for the investigated aquifer transect.

7 References

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Appendix

A Concentrations of pollutants and hydrogeochemical parameter in groundwater samples taken from the Amsterdamsevaartweg site on 6th of October 2010

Peilbuis	Pb2015 (6-8 m-mv)	Pb2015 (10-12 m-mv)	Pb2015 (17-19 m-mv)	Pb3006 (25 m-mv)	Pb3021 (25 m-mv)	Pb3022 (25 m-mv)
Datum	6-10-2010	6-10-2010	6-10-2010	6-10-2010	6-10-2010	6-10-2010
diepte pb	7,74	11,62	18,45	25,45	25,02	25,01
diepte gw	1,05	1,03	1,03	1,1	0,64	1,78
temperatuur	14,5	14,4	14,2	14,9	14,1	14,4
geleidbaarheid	1091	963	952	724	965	989
pH	6,5	6,9	7,0	7,0	7,1	7,2
redox	67	44	88	62	82	83
zuurstof	0,15	0,22	0,17	0,15	0,13	0,22
nitraat	<0,23	<0,23	<0,23	<0,23	<0,23	<0,23
ijzer	16.000	11.000	6.700	5.500	7.700	7.800
sulfaat	<8	<8	41,8	58	30	52
methaan	16.000	7.200	750	19.000	1.100	2.300
PER	0,43	0,1	0,1	390	0,1	0,1
TRI	0,96	0,1	0,1	280	0,1	0,1
cis-DCE	9.400	170	13	510	0,1	0,39
trans-DCE	1.500	45	3,7	130	0,1	0,17
som DCE	11.000	210	16	640	0,2	0,56
VC	5.100	240	45	61	210	350
etheen	2.200	76	7	17	57	37
ethaan	1.400	700	36	800	12	35
dechloreringsgraad	76,0	93,6	88,6	81,0	85,5	82,7

B Basics of isotope data interpretation

Isotopes of an element have the same amount of protons, but differ in the number of neutrons, and therefore, they have different masses. Carbon exhibits two stable isotopes; one with the mass 12 (^{12}C - 6 protons and 6 neutrons) and one with the mass 13 (^{13}C - 6 protons and 7 neutrons). The quotient between the amounts of the heavy and the light isotopes is called isotope ratio or isotope signature (e.g. $^{13}C/^{12}C$).

Since biodegradation of molecules with heavy isotopes is less pronounced than that of molecules with light isotopes, the isotope signature of primary contaminants (e.g. PCE) becomes more positive during the microbial decomposition. This process is called isotope fractionation. Pollutant biodegradation within contaminated aquifers can be assessed because of the substance-specific proportionality of isotope fractionation and contaminant decrease *(1)*. The proportionality is expressed by the isotope enrichment factor ε.

Under anoxic conditions, primary CVOCs like PCE and TCE can be used as electron acceptors *(2,3)*. Generally, they are degraded by reductive dechlorination (Fig. 1). Thereby, chlorine is split off successively and the metabolites DCE and VC are formed. The complete reductive dechlorination leads to the formation of the non-toxic ethene.

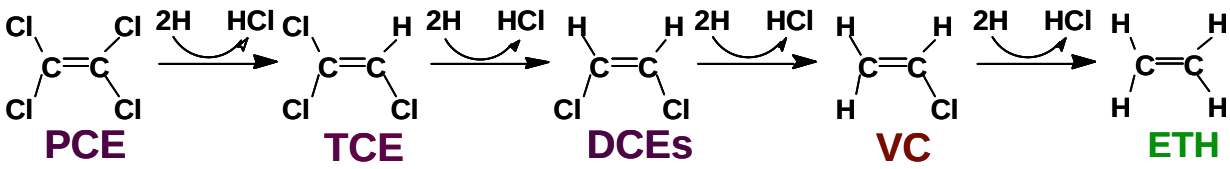

Fig. 1: Scheme of reductive dechlorination for chlorinated ethenes: PCE - Tetrachloroethene, TCE - Trichloroethene, DCE - Dichlorothene, VC - Vinylchloride, ETH - Ethene.

The isotope ratio of primary CVOCs (PCE or TCE) becomes more and more positive during the reductive dechlorination. In the beginning of biodegradation, the breakdown products DCE and VC are depleted in ^{13}C, i.e. they have a more negative isotope ratio than the PCE or TCE (Fig. 2). In the further course of reductive dechlorination, the breakdown products are enriched in ^{13}C. For example, if TCE as primary CVOC is completely converted to DCE, the accumulated DCE will exhibit the same isotope signature that TCE had before biodegradation. The further biodegradation of DCE leads to more positive isotope ratios compared to primary isotope ratio of TCE. Assuming a complete reductive dechlorination and no further biodegradation of ethene, the accumulated ethene should have the same isotope ratio than the primary CVOC before biodegradation.

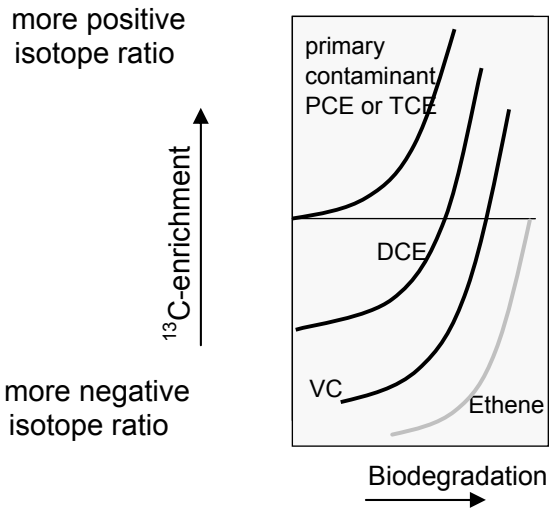

Fig. 2: *Evolution of isotope signatures during reductive dechlorination of primary CVOC PCE or TCE.*

The commonly used mathematical description of microbial isotope fractionation processes is the Rayleigh equation (Eq. 1), where δ_t is the isotope signature of the substrate at a certain time t of biodegradation, δ_0 is the initial isotope signature of the substrate, C_{Bt}/C_0 is the fraction of substrate remaining during biodegradation at a certain time t, and ε is the isotope enrichment factor given in ‰ *(4)*:

$$\frac{(\delta_t + 1000)}{(\delta_0 + 1000)} = \left(\frac{C_{Bt}}{C_0}\right)^{\frac{\varepsilon}{1000}} \tag{1}$$

The extent of contaminant biodegradation is commonly expressed as percentage, B [%], of the initial contaminant concentration removed due to biodegradation:

$$B[\%] = \left(1 - \frac{C_{Bt}}{C_0}\right) \cdot 100 \tag{2}$$

Combining this expression with the Rayleigh equation allows quantifying contaminant biodegradation within a time interval or along a flow path of the contaminant in a given environment using measured isotope signatures *(1)*. Required data are the initial isotope ratio of the contaminant at a starting point in time or in space (generally the contaminant source) and the isotope ratio of the remaining contaminant at a temporal or spatial observation point (generally a downstream well). The amount of contaminants degraded between the initial and observation point (x) is then given by

$$B[\%] = \left(1 - \frac{C_{Bx}}{C_0}\right) \cdot 100 = \left[1 - \left(\frac{\delta_x + 1000}{\delta_0 + 1000}\right)^{\left(\frac{1000}{\varepsilon}\right)}\right] \cdot 100 \qquad (3)$$

using the Rayleigh equation (Eq. 1).

Moreover, *in situ* zero- and first-order biodegradation rate constants (k, λ) can be estimated by changes in isotope ratios for expected groundwater flow pathway between the initial and observation point using a Rayleigh equation based approach *(1)*. Biodegradation rate constants related to the distance of the groundwater flow pathway (s) can be obtained from:

zero-order rate constant (k_s):

$$k_s = \frac{C_0 - \left(C_0 \times \frac{(100 - B[\%])}{100}\right)}{s} \qquad (4)$$

first-order rate constant (λ_s):

$$\lambda_s = \frac{-1000}{\varepsilon \cdot s} \ln\left(\frac{\delta_x + 1000}{\delta_0 + 1000}\right). \qquad (5)$$

The time-dependent *in situ* zero- and first-order biodegradation rate constants (k_s, λ_t) can be determined by taking into account the travel time of the pollutants.

zero-order rate constant (k_t):

$$k_t = \frac{C_0 - \left(C_0 \times \frac{(100 - B[\%])}{100}\right)}{t} \qquad (6)$$

first-order rate constant (λ_t):

$$\lambda_t = \frac{-1000}{\varepsilon \cdot t} \ln\left(\frac{\delta_x + 1000}{\delta_0 + 1000}\right) \qquad (7)$$

The travel time (t) can be assessed by the groundwater flow velocity (v) and the distance between the initial and observation point (s):

$$t = \frac{s}{v}. \qquad (8)$$

The first-order biodegradation rate constants can be used to calculate biological half life distances ($s_{1/2}$) or times ($t_{1/2}$), which gives the distance or time needed for the biodegradation to halve the initial pollutant concentration *(5)*.

$$s_{1/2} = \frac{\ln 2}{\lambda} \tag{9}$$

$$t_{1/2} = \frac{\ln 2}{\lambda} \tag{10}$$

C References of appendix

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Appendix 3 Map and results sampling groundwater locations

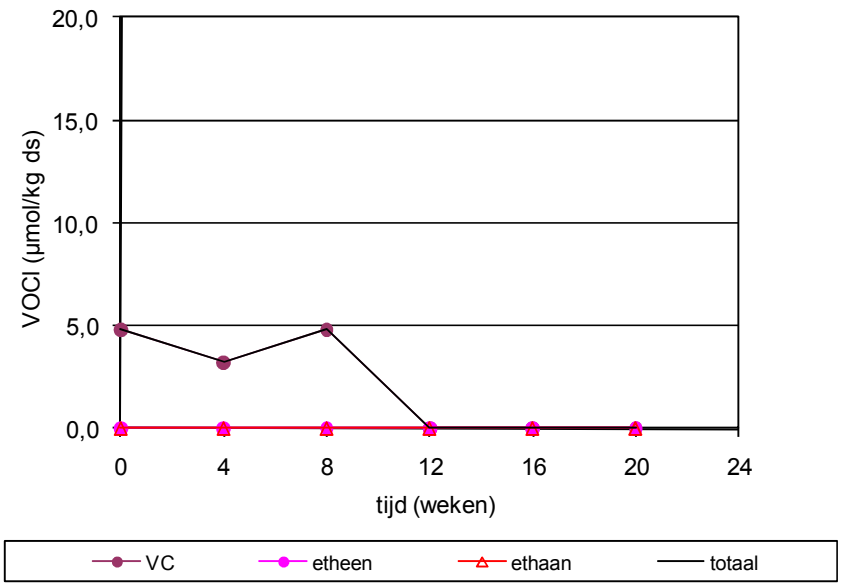
Q-PCR results based on groundwater sampling with traditional sampling method, sampled in 2010

Monitoring well (depth m-gl))	unit	Pb67 (14)	Pb67 (26)	Pb52 (26)	Pb53 (38)	Pb61 (26)	Pb85 (26)	Pb85 (38)	Pb86 (14)	Pb86 (24)	CU14 (26)	CU25 (38)	CU28 (14)	CU28 (26)	CU28 (45)	CU30 (26)	gra306 (6)	ROGHK11 (26)	ROGHK11 (35)	Rog 105
Molecular analyses																				
etnC	cells/mL	<14	20	< 13	7E+03	<	< 3	< 3	< 4	< 14	< 3	< 13	< 31	< 18	< 25	< 13	< 3	< 13	< 13	< 13
etnE	cells/mL	<14	< 14	< 13	1E+03	<	< 3	< 3	< 4	< 14	< 3	< 13	< 31	< 18	< 25	< 13	< 3	< 13	< 13	< 13
Polaromonas	cells/mL	<14	< 14	< 13	< 19	<	< 3	< 3	< 4	< 14	< 3	< 13	< 31	< 18	< 25	< 13	< 3	< 13	< 13	< 13
DHC	cells/mL	<14	< 14	< 13	< 13	<	< 3	< 3	< 4	< 14	< 3	< 13	< 31	< 18	< 25	< 13	< 3	< 13	< 13	< 13
bvcA	cells/mL			< 13	< 13							< 13	< 31	< 18	< 25	< 13		< 13	< 13	< 13
vcrA	cells/mL			< 13	< 13							< 13	< 31	< 18	< 25	< 13		< 13	< 13	< 13
Contaminant																				
PER	µg/L	<	<	<0.1	<	< 0.1			89	8	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	2.4	<0.1	<0.1	
TRI	µg/L	<	0.16	6	0.17	< 0.1			14	0.12	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	6	<0.2	<0.2	
cis-DCE	µg/L	<	0.98	11	75	1.2			87	7.8	0.22	<0.1	0.87	0.73	<0.1	0.43	32	0.79	0.11	
VC	µg/L	<	0.97	60	2100	240			64	2	0.65	0.22	5.3	48	30	37	16	0.56	<0.2	
Redox and other parameters																				
depth gw	m- bk pb	3.9	3.9	2.5	2.8	2.6	3.5	3.5	2.5	2.4	1.7	5.4	3.1	3.1	3.1	2.1	4.3	2.1	2.1	2.5
pH	-	7.4	7.4	7.4	7.2	7.2	7.0	7.0	6.7	7.2	7.0	7.2	7.1	7.2	7.0	7.1	6.6	8.1	7.5	7.5
Temperature	°C	14.5	14.4	15.2	14	13.3	13.5	13.5	13.7	13.4	12.5	16.8	15.2	15.2	14.8	15.3	12.8	14.5	14.4	17.3
Oxygen	mg/L	0.13	0.08	0.11	0.15	0.04	0.04	0.04	0.41	0.11	0.21	0.24	0.4	0.04	0.3	0.09	0.49	0.33	0.23	0.3
Conductivity	µS/cm	1098	1009	839	857	1131	1186	1133	910	986	1136	980	798	1228	641	799	1023	627	1118	750
redox	mV H_2	122	38	127	135	89	78	88	149	78	83	76	82	78	113	91	287	62	61	318

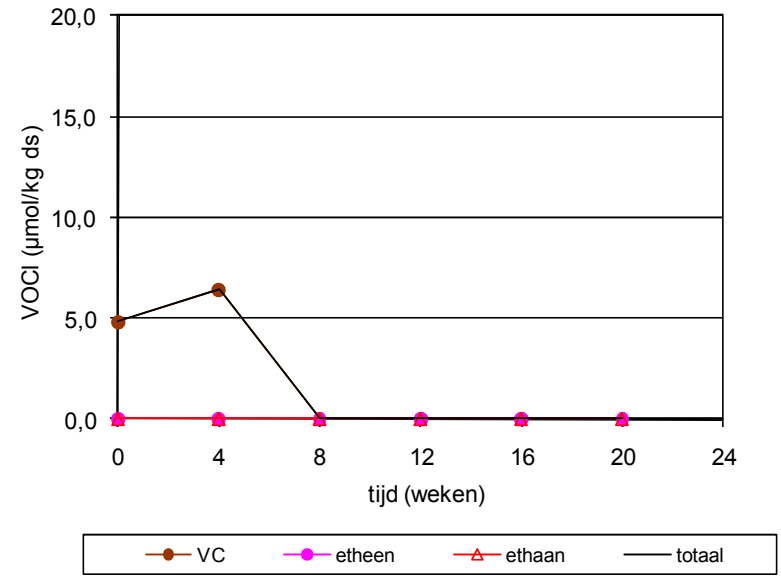
Results Q-PCR on DNA samples available at Bioclear, sampling with the traditional sampling method from 2001 and 2008

Monitoring well (depth m-gl))	unit	pb 106 (20)	pb B (23)	pb 203	pb 205	pb 511	pb 607	pb1003 (38)	pb1K1 (18)	pb 106 (35)	pb B (41)	pb 1W2 (39)
Sampling date		25-2-2008	25-2-2008	24-9-2001	24-9-2001	24-9-2001	24-9-2001	3-11-2008	4-11-2008	25-2-2008	25-2-2008	28-10-2008
Molecular analyses												
etnC	cells/mL	< 125	3E+04	2E+04	9E+02	< 250	< 250	< 125	3E+04	8E+02	3E+02	< 250
etnE	cells/mL	< 125	< 125	3E+03	< 125	< 250	< 250	< 125	< 125	1E+03	< 125	< 250
Polaromonas	cells/mL	< 125	3E+02	< 125	< 125	< 250	< 250	< 125	3E+02	< 125	< 125	< 250
DHC	cells/mL	3,9E+04	1,7E+03	<130	<130	7,0E+05	7,2E+03	<250	<250	2,1E+05	1,3E+03	<250
bvcA	cells/mL											
vcrA	cells/mL											
Contaminant												
PER	µg/L	<0,1	<0,1					<	<	<0,1	<0,1	<
TRI	µg/L	<0,1	<0,1					<	<	<0,1	<0,1	<
cis-DCE	µg/L	2,20	1,50					<	<	0,69	<0,1	<
VC	µg/L	110	110					32	46	1300	280	45
kinetics												
Degradation rate (based on DHC)	mV H_2	0,008	0,0009	-	-	0,07	0,002	-	-	0,03	0,001	-

Appendix 4 VC degradation lab test results



GC results flask B1



GC results flask E3

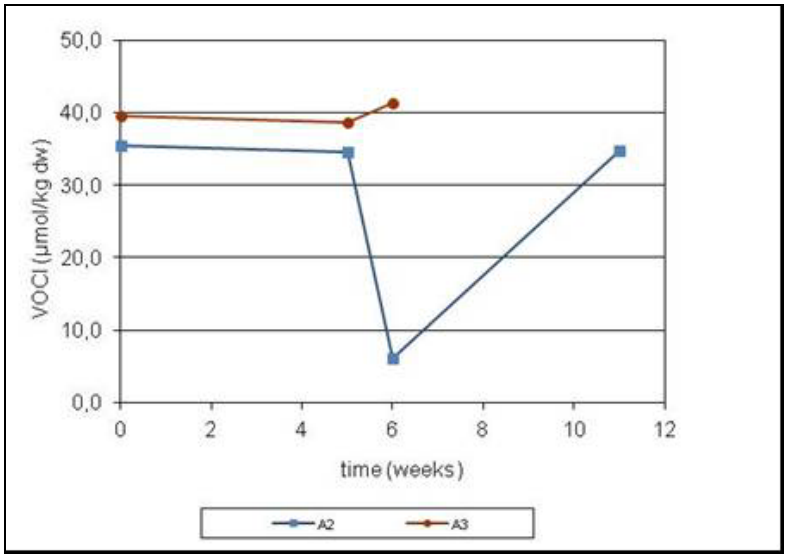


Figure 13 VC concentration in abiotic VC test, test A

Appendix 5 Report isotope analysis BACTRAP samples

Isodetect
Umweltmonitoring GmbH

25th of July 2011

Assessment of *in situ* biodegradation of *cis*-1,2-dichloroethylene at a CVOC-polluted field site in Utrecht using BACTRAPs

Ordering party:	Bioclear b.v. Rozenburglaan 13 9727 DL Groningen The Netherlands
Contact person:	Shakti Lieten
Contractor:	Isodetect Umweltmonitoring GmbH Ingolstädter Landstr. 1 · D-85764 Neuherberg
Field site:	Utrecht, The Netherlands
Examination period:	January 2011 - April 2011
Persons in charge:	Dr. Petra Bombach, Dr. Anko Fischer
Report volume:	12 Pages 2 Tables 4 Figures 2 Appendices

.....
Dr. Petra Bombach

……..
Dr. Anko Fischer

Contents

1 Background

In order to assess *in situ* biodegradation of *cis*-1,2-dichloroethene (*cis*-DCE) at a field site in Utrecht contaminated with chlorinated volatile organic compounds (CVOCs) Bioclear b.v. entrusted

Isodetect GmbH
Permoserstr. 15
04318 Leipzig

with BACTRAP studies.

Most abundant pollutants at the field site are chlorinated ethenes. The higher chlorinated ethenes tetrachloroethene (PCE) and trichloroethene (TCE) occur in minor concentrations compared to the degradation products dichloroethene (DCE) and vinyl chloride (VC) indicating biodegradation by reductive dechlorination at the field site.

2 Purpose and scope

In situ microcosms loaded with a ^{13}C-labelled compound (BACTRAP®$_s$) are an appropriate method for assessing qualitatively the biodegradation of a specific compound in the environment /1,2,3/. The scope of this study included the following tasks:

- Assessment of *in situ* biodegradation of *cis*-1,2-dichloroethylene;
- Characterisation of *in situ* biodegradation with respect to reductive dechlorination and oxidative biodegradation, respectively.

3 Summary

The BACTRAP study provided strong indication for reductive dechlorination of *cis*-DCE at the site. No indications for microbial oxidation of *cis*-DCE were revealed as a dominant pathway for *cis*-DCE biodegradation.

4 Basics

Isotopes of an element have the same number of protons but differ in the number of neutrons, and therefore, they have different masses. Organic substances mainly consist of

carbon which exhibits two stable isotopes. Carbon with the mass 13 (^{13}C, 6 protons and 7 neutrons) is the rare abundant stable isotope (1.1 %) compared to carbon with the mass 12 (^{12}C, 6 protons and 6 neutrons) which has an abundance of 98.9 %. ^{13}C-labelled compounds can be synthesized which have a significantly higher ^{13}C-abundance as their counterparts naturally found. These ^{13}C-labelled compounds are suitable as reactive tracers, e.g., for studying biodegradation of pollutants.

In situ microcosms so called BACTRAP®s (bac - bacteria, trap - trap) loaded with ^{13}C-labelled substrates provide a powerful tool for providing evidence of *in situ* pollutant biodegradation (Figure 1) /1,2,3/. BACTRAPs consist of materials providing an appropriate surface for colonisation of microorganisms and a reliable adsorption capacity for substrates like organic contaminants.

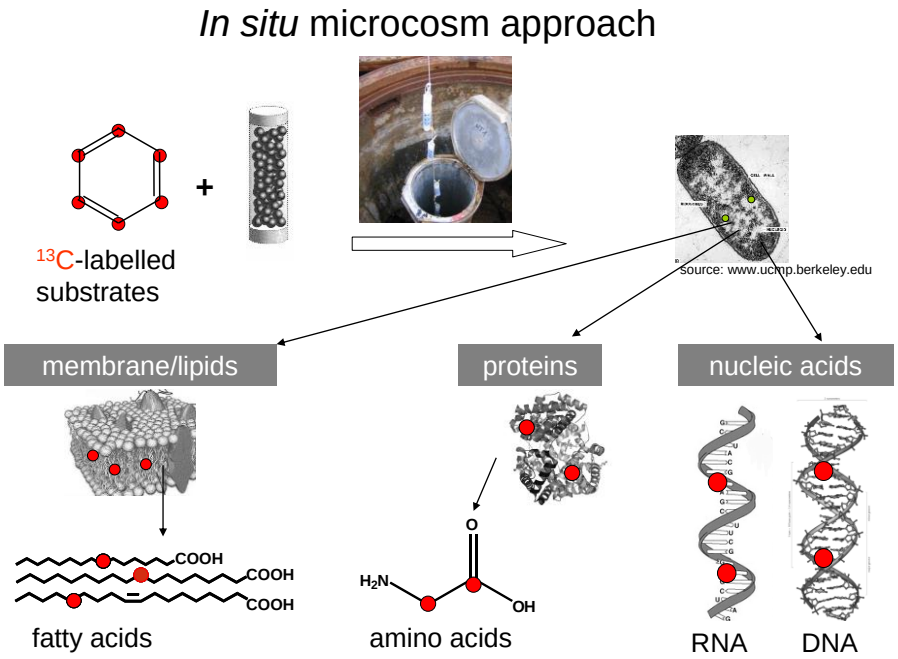


Figure 1: Concept of the *in situ* microcosm approach: The microcosms are loaded with a ^{13}C-labelled substrate and deployed in a well for several weeks. During the incubation, microbial communities from the groundwater colonise the *in situ* microcosms. Microorganisms that are able to use the ^{13}C-labelled substrate incorporate the ^{13}C in their biomass such as fatty acids, amino acids and nucleic acids.

The BACTRAPs are loaded with a ^{13}C-labelled substrate and deployed in an aquifer for several weeks. During the deployment, microbial communities from the groundwater colonise the growth and storage material. Microorganisms that are able to use the ^{13}C-labelled substrate incorporate the ^{13}C-carbon in their biomass so that the evidence for biodegradation

can be provided by the detection of a significant ^{13}C-enrichment in microbial biomass components such as fatty acids (Figure 1). For this reason, fatty acids are extracted from the BACTRAPs after placement in the groundwater and the ^{13}C-incorporation in the fatty acids is analysed.

5 Methodology

5.1 BACTRAP study

BACTRAPs were composed by filling 1 g heated BioCoal® into perforated teflon tubes. Subsequently, the BACTRAPs were loaded each with 50 mg of ^{12}C-*cis*-DCE or ^{13}C-*cis*-DCE via gas phase under reduced pressure. All microcosms were stored in anoxic sterile water until their installation in wells at the field site. One BACTRAP loaded with ^{13}C-*cis*-DCE and one BACTRAP loaded with ^{12}C-*cis*-DCE were each fixed to a rope and were placed in the wells Pb86 and Pb53 at a depth of 14 m and 38 m below ground level, respectively. An overview about the BACTRAP installation is given in Figure 2. BACTRAP studies took place from 11th of January 2011 until 27th of April 2011. After these 106 days of incubation, all BACTRAPs were taken out of the wells. BACTRAPs loaded with ^{13}C-*cis*-DCE were cooled transported to Isodetect GmbH to prevent microbial activities. After the BACTRAPs arrived they were stored at -80°C until analysis. BACTRAPs loaded with ^{12}C-*cis*-DCE were used for molecular analysis by Bioclear b.v..

Fatty acids were extracted from the BioCoal® beads according to a modified Bligh&Dyer method /4/ described by White and Ringelberg (1998) /5/. To analyse the fatty acids with gas chromatography-mass spectrometry (GC-MS), the lipids were transesterified to fatty acid methyl ester (FAME) by a mild alkaline methanolysis /6/. The dried FAME fraction was dissolved in *n*-hexane containing 21:0 FAME as internal standard. For the identification and quantification of the FAMEs, an Agilent 7890A gas chromatograph coupled to an Agilent 5975C mass spectrometer (Agilent Technologies, Palo Alto, U.S.) was used. The FAMEs were separated on a HP5-ms column (30 m x 0.32 mm x 0.25 µm) by injecting 2 µl of FAME extracts with a split ratio of 1:1 using a specific temperature program. The FAMEs were identified by co-injection of an authentic standard mix (bacterial acid methyl ester mix, Sigma-Aldrich, Germany). The fatty acids are designated in the form of A:BωC where A is the number of carbon atoms, B is the number of double bonds and C is the distance of the closest double bond from the aliphatic end of the molecule (unsaturation, ω-nomenclature). Absolute and relative amounts of fatty acids were determined according to the concentration of the added internal FAME standard.

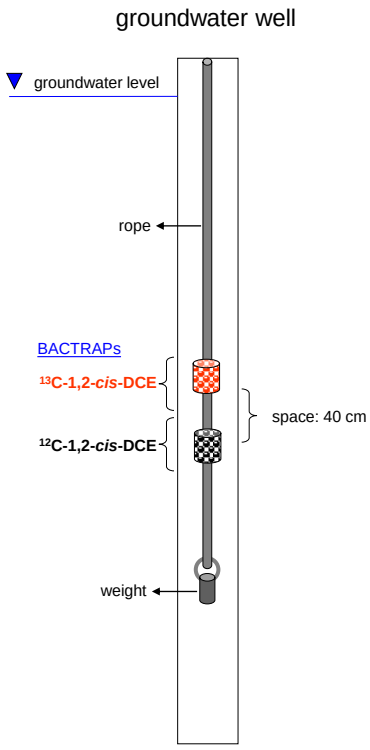


Figure 2: Outline of BACTRAP installation.

The **carbon isotope composition** of the FAMEs was analysed using gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS). The system consisted of a gas chromatograph (6890 series, Agilent Technology, Palo Alto, CA) coupled via a Conflow III interface (Thermo Electron, Germany) to a MAT 252 mass spectrometer (Thermo Electron, Germany). The carbon isotope ratio $^{13}C/^{12}C$ of fatty acids has been analysed and given in δ-notation relative to the Vienna Pee Dee Belemnite standard (VPBD, International Atomic Agency).

$$\delta^{13}C\,[‰] = \left(\frac{(^{13}C/^{12}C)_{Probe}}{(^{13}C/^{12}C)_{Standard}} - 1 \right) \times 1000 \qquad (1)$$

The reproducibility of isotope analyses given by the standard deviation of the replicate measurements was ≤0.5 ‰.

5.2 Carbon stable isotope analysis of DIC and CVOC

To determine the **stable carbon isotope composition of dissolved inorganic carbon (DIC) and of chlorinated ethenes** in the monitoring wells before and after BACTRAP installation, GC-C-IRMS was applied /7/. After sampling, an oversaturated salt solution was produced by adding sodium chloride to the groundwater to fix the samples and to increase the headspace DIC concentration. Before measurement, all samples were acidified to pH 2 to put the CO_2/carbonic acid/bicarbonate/carbonate equilibrium towards CO_2. Aliquots (50 - 100 µl) of headspace samples were injected into a gas chromatograph (Agilent 6890; Palo Alto, USA) in split mode (split ratio was set at 1:20 to 1:50) using a split/splitless injector at 250 °C. The CO_2 were separated on a CP-PoraPLOT Q-capillary column (27.5 m × 0.32mm ID × 10 µm FD; Chrompack, Niederlande) using an isothermally program at 40°C. All samples were measured in at least two replicates. The reproducibility of isotope analyses given by the standard deviation of the replicate measurements was with one exception (methane in Pb53) ≤0.7 ‰. Carbon isotope composition of CVOC was determined in the same manner using a stepwise heating temperature program.

6 Results and interpretation

The basics for the microbial degradation of CVOC are given in the appendix A.

6.1 BACTRAP study

Total amount of total lipid fatty acids (TLFA) were quite similar in ^{13}C-*cis*-DCE amended BACTRAPs installed in the monitoring wells Pb53 (38 m-mv) and Pb86 (14 m-mv), demonstrating a comparable colonisation by groundwater microorganism (see Figure 3 and Figure 4). Fatty acid profiles gained from both BACTRAPs were dominated by the saturated fatty acids 14:0 and 16:0 and the unsaturated fatty acids 16:1ω7 and 18:1ω7. The unsaturated fatty acids 16:1ω7 and 18:1ω7 are characteristic fatty acids for bacteria, especially for gram-negative bacteria /8/. The saturated fatty acids 12:0 and 18:0 which are widespread in prokaryotes and eukaryotes /8/ were detected only in smaller amounts.

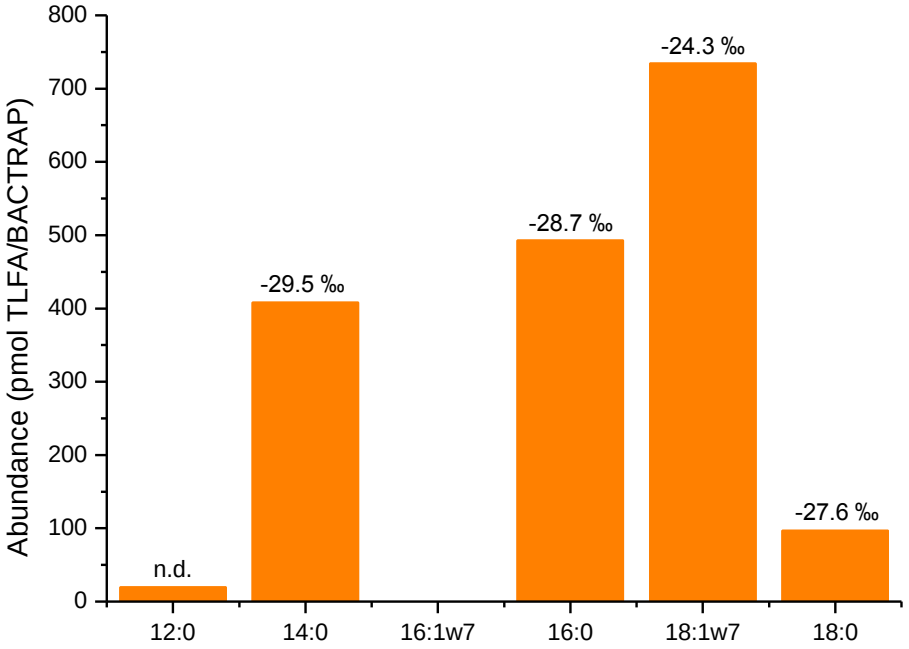


Figure 3: Abundance and carbon isotope signatures (values above columns) of total lipid fatty acids (TLFA) extracted from ^{13}C-*cis*-DCE loaded BACTRAPs after exposure in monitoring well Pb53 (38 m-mv). n.d. = not detectable

Natural abundances of carbon isotope signature (δ^{13}C) in fatty acids range from -20‰ to -36‰ /9,10/. Therefore, carbon isotope signatures of fatty acids which are significantly more positive clearly indicate the incorporation of ^{13}C-carbon from the provided ^{13}C-substrate in the bacterial biomass. The isotope signature of the fatty acids extracted from

the BACTRAPs installed in Pb53 (38 m-mv) ranged from -24.3 ‰ to -29.5 ‰, indicating that the bacterial growth was not based on the metabolisation of ^{13}C-*cis*-DCE but utilisation of a non-labelled carbon source from the groundwater (see Figure 3). Carbon isotope signatures of fatty acids extracted from the BACTRAP in Pb86 (14 m-mv) showed similar values in the range of -17.0 ‰ up to -28.1 ‰ (Figure 4), thus also not providing evidence for the microbial oxidation of *cis*-DCE as anabolism substrate. For some fatty acids, no reliable isotope signature could be determined due to the low abundance of this fatty acid or an insufficient separation of fatty acids from other organic compounds which were also concentrated during the fatty acid extraction process. Despite this fact, no ^{13}C-enrichment could be observed for any fatty acid, indicating that *cis*-DCE was degraded via microbial oxidation. In this case, *cis*-DCE acts as carbon source and the ^{13}C-label would be incorporated into the microbial biomass.

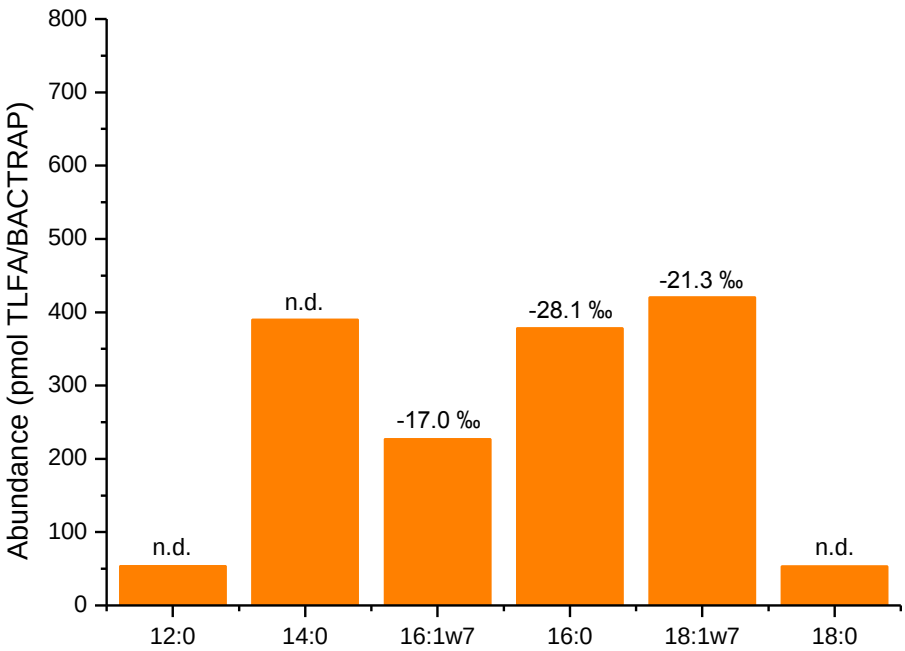


Figure 4: Abundance and carbon isotope signatures (values above columns) of total lipid fatty acids (TLFA) extracted from ^{13}C-*cis*-DCE loaded BACTRAPs after exposure in monitoring well Pb86 (14 m-mv). n.d. = not detectable

Another method for assessing microbial oxidation of *cis*-DCE is the analysis of ^{13}C-incorporation into CO_2. The microbial oxidation of *cis*-DCE leads to the formation of CO_2 thus the ^{13}C-label will be incorporated in the formed CO_2. Therefore, beside analysis of ^{13}C-enrichment into microbial biomass, the ^{13}C-incorporation into CO_2 was analysed (see chapter 6.2).

6.2 Carbon isotope analysis of DIC

At both monitoring wells, the carbon isotope signatures for DIC showed significantly more negative values after than before the BACTRAP installation (Table 1). This demonstrates that the ^{13}C-*cis*-DCE was not biodegraded via microbial oxidation which would have led to more positive isotope signatures of DIC. Carbon isotope signatures of DIC detected before the BACTRAP installation were with -17.1 (Pb86 (14 m-mv) and -13.2 ‰ Pb53 (38 m-mv) in the range described for carbon isotope signatures of DIC in groundwater (-20 ‰ to 0 ‰) /11/. The tendency to more negative carbon isotope signatures might be explained by the mineralisation of organic compounds in the groundwater. The degradation of organic compounds leads to the formation of CO_2 exhibiting the same or a more negative carbon isotope signature than the organic compounds. Primary isotope signatures of organic compounds are mostly more negative than -22 ‰ /12/. Hence, carbon isotope ratios of DIC more negative than -22 ‰ can be explained by biodegradation of organic compounds.

Methane could only be detected in sufficient abundance for carbon isotope analysis after the BACTRAP installation. At Pb53 (38 m-mv) the carbon isotope signature was +10.0 ‰ suggesting that either methane underwent oxidation at this monitoring well leading to carbon isotope fractionation /13/ or the methanogenesis of ^{13}C-enriched degradation products derived from the biotransformation of ^{13}C-*cis*-DCE (e.g. ethene) caused the positive isotope ratio of methane. Carbon isotope signature of methane at Pb86 (14 m-mv) was with -63.5 ‰ much more negative. Such strongly negative isotope signature can be attributed to methanogenic processes leading to the formation of methane with carbon isotope signatures in the range from -110 ‰ to -50 ‰ /13/.

Table 1: Carbon isotope ratios of DIC and methane before and after BACTRAP installation.
n.d. = not detectable; DIC = dissolved inorganic carbon

monitoring well	analyte	before BACTRAP installation δ^{13}C (‰)	after BACTRAP installation δ^{13}C (‰)
Pb53 38 m-mv	DIC	-13.2	-21.2
	methane	n.d.	+10.0
Pb86 14 m-mv	DIC	-17.1	-25.4
	methane	n.d.	-63.5

6.3 Carbon isotope analysis of chlorinated ethenes

Another potential process for the microbial degradation of *cis*-DCE is reductive dehalogenation in which the *cis*-DCE is utilised as electron acceptor. In this case, the ^{13}C label will not be transformed into the biomass but ^{13}C-labelled vinyl chloride is formed as a metabolite during the reductive dechlorination. In order to assess whether the *cis*-DCE on the BACTRAPs was biodegraded via reductive dechlorination, carbon isotope signatures of the CVOC in the monitoring wells Pb53 (38 m-mv) and Pb86 (14 m-mv) before and after BACTRAP installation was determined.

For Pb53 (38 m-mv), carbon isotope ratios of *cis*-DCE could not be determined neither before nor after the BACTRAP installation because the concentration of *cis*-DCE was too small for reliable isotope analysis. The isotope ratio of VC were significantly more positive after the BACTRAP installation (+12.9 ‰) than before BACTRAP installation (-20.9 ‰), indicating that the offered ^{13}C-*cis*-DCE was possibly reduced leading to the formation of ^{13}C-labelled vinyl chloride (Table 2). However, the isotope ratio of VC was within the range of expected values arising from isotope fractionation of natural abundance VC during biodegradation. Thus, besides VC derived from ^{13}C-*cis*-DCE from BACTRAP, VC which was already present in Pb53 (38 m-mv) might be biodegraded leading to changes in carbon isotope ratios from -20.9 ‰ (before BACTRAP installation) to +12.9 ‰ (after BACTRAP installation). Assuming this scenario, an anaerobic VC biodegradation via reductive dechlorination of at least 66 % and aerobic VC of at least 98 % might be expected for the duration of BACTRAP exposure. No indications of a predominant degradation pathway can be derived based on the changes in VC isotope ratios alone. Further studies are needed to provide information whether oxidation or reductive dechlorination leads to VC biodegradation.

For Pb86 (14 m-mv), carbon isotope ratio of *cis*-DCE and VC could only be determined after BACTRAP installation and were with +2414.1 ‰ and +237.5 ‰ highly positive. Hence, the positive carbon isotope signatures for VC might be explained by the microbial reduction of the ^{13}C-*cis*-DCE to ^{13}C-VC providing strong indication for reductive dechlorination of *cis*-DCE at Pb86 (14 m-mv). The highly positive isotope value for *cis*-DCE determined at Pb86 (14 m-mv) after the BACTRAP installation stem from the ^{13}C-*cis*-DCE on the BACTRAPs which desorb in tiny amounts from the BACTRAPs to the surrounding groundwater.

Table 2: Carbon isotope ratios of *cis*-DCE and vinyl chloride before and after BACTRAP installation. n.d. = not detectable

monitoring well	CVOC	before BACTRAP installation $\delta^{13}C$ (‰)	after BACTRAP installation $\delta^{13}C$ (‰)
Pb53 38 m-mv	*cis*-DCE	n.d.	n.d.
	VC	-20.9	+12.9
Pb86 14 m-mv	*cis*-DCE	n.d.	+2414.1
	VC	n.d.	+237.5

7 Conclusion

The BACTRAP study including the carbon isotope analysis of DIC and CVOC in groundwater before and after BACTRAP installation suggested that reductive dechlorination is the predominant biodegradation pathway for *cis*-DCE at the site. Neither microbial biomass formed on the BACTRAPs during groundwater incubation nor the DIC produced during mineralisation showed significant ^{13}C-incorporation providing no evidence for microbial oxidation of *cis*-DCE. In contrast, significantly ^{13}C-labelled vinyl chloride was detected Pb86 (14 m-mv) after BACTRAP removal providing indication for reductive dechlorination of *cis*-DCE.

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Appendix A: Basics of microbial degradation of CVOC

Under anoxic conditions, *cis*-DCE can be degraded by reductive dechlorination in which the *cis*-DCE is utilised as electron acceptor. The reductive dechlorination is a stepwise process in which the chlorine of the primary CVOC like PCE and TCE is split off successively and the metabolites DCE and VC are formed (Figure A1). The complete reductive dechlorination leads to the formation of the non-toxic ethene /A1, A2/.

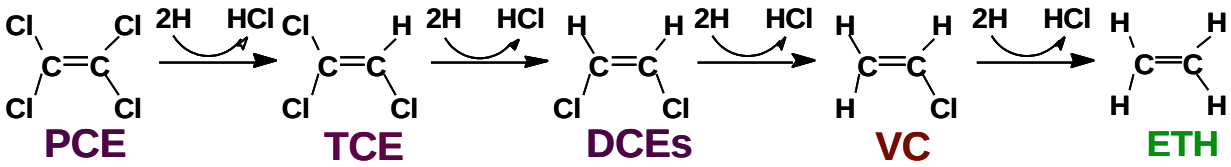

Figure A1: *Scheme of reductive dechlorination for chlorinated ethenes: PCE - Tetrachloroethene, TCE - Trichloroethene, DCE - Dichlorothene, VC - Vinylchloride, ETH - Ethene.*

Microbial oxidation of *cis*-DCE is another potential process for biodegradation of those leading to the formation of CO_2. Contrary to the reductive dechlorination, *cis*-DCE acts as electron donor during this degradation process. Microbial oxidation of *cis*-DCE has been shown under oxic, hypoxic and anoxic conditions /A1, A3-A5/.

Appendix B: References

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/A3/ Bradley PM, Chapelle FH (1998) Microbial mineralization of VC and DCE under different terminal electron accepting conditions. Anaerobe 4:81-87

/A4/ Bradley PM, Chapelle FH (2011) Microbial mineralization of dichloroethene and vinyl chloride under hypoxic conditions. Ground Water Monit. R.: in press

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Appendix 6 Analyses certificates

ACMAA B.V. ANALYTISCH CHEMISCH MILIEU ADVIESBUREAU ALMELO

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Opdrachtgever : Bioclear B.V.
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Opdrachtgegevens:

Opdrachtcode	: 20103770	Labcomcode:	: 1208020BCL
Rapportnummer	: P120800179 (v1)	Datum opdracht	: 09-08-2012
Opdracht omschr.	: biocapacitietsbepaling	Startdatum	: 09-08-2012
Bemonsterd door	: Opdrachtgever	Datum rapportage	: 16-08-2012

Monstergegevens:

Nr.	Labnr.	Monsteromschrijving	Monstersoort	Datum bemonstering
1	M120800388	: 3770 083~AF 2 (6-10 m-mv)	Grondwater	09-08-2012

Resultaten:

	Parameter	Intern ref. nr.	Eenheid	1
	Mvb. SIKB AS3000	MVB-VBH-AS3000-W01		+
	Sulfaat		mg/l	<8,0
S	Nitraat (als NO3)	DIV-NO3-01	mg/l	<0,50
	Methaan		µg/l	15000
	Tot. org. koolstof (als NPOC)		mg TOC/l	9,4 (1)

S = door RvA geaccrediteerd conform SIKB AS3000.

Opmerkingen:

1 = Deze bepaling is uitbesteed aan derden. Dit laboratorium is voor deze bepaling geaccrediteerd.

Hoofd lab. Ing. H. Punte

Handtekening:

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Onderzoeksrapport

Opdrachtgever:

Opdrachtgever : Bioclear B.V.
Aanvrager : Dhr. A. Nipshagen
Adres : Postbus 2262
Postcode en plaats : 9704 CG Groningen

Opdrachtgegevens:

Opdrachtcode	: 20114081	Labcomcode:	: 1208016BCL
Rapportnummer	: P120800200 (v1)	Datum opdracht	: 09-08-2012
Opdracht omschr.	: Fluxmeting A'damsestraatweg en Nachtegaalstr Utrecht	Startdatum	: 09-08-2012
Bemonsterd door	: Opdrachtgever	Datum rapportage	: 15-08-2012

Monstergegevens:

Nr.	Labnr.		Monsteromschrijving	Monstersoort	Datum bemonstering
1	M120800448	:	4081 020~AF1 (boorgat 1.1) (6-10 m-mv)	Grondwater	09-08-2012
2	M120800449	:	4081_021~(boorgat 1.2) (14-16 m-mv)	Grondwater	09-08-2012
3	M120800450	:	4081_022~(boorgat 1.2) (18-20 m-mv)4081_022~(boorgat 1.2)	Grondwater	09-08-2012
4	M120800451	:	4081_023~AF2 (boorgat 2.1) (6-10 m-mv)	Grondwater	09-08-2012

Resultaten:

	Parameter	Intern ref. nr.	Eenheid	1	2	3	4
	Mvb. SIKB AS3000	MVB-VBH-AS3000-W01		+	+	+	+
	Vluchtige organische halogeen verbindingen						
S	Dichloormethaan	GC-VLUCHTIG-01	µg/l	<0,20	<0,20	<0,20	<20
S	1,1-Dichloorethaan	GC-VLUCHTIG-01	µg/l	<0,50	<0,50	<0,50	<50
S	1,2-Dichloorethaan	GC-VLUCHTIG-01	µg/l	<0,10	<0,10	<0,10	<10
S	Trans-1,2-Dichlooretheen	GC-VLUCHTIG-01	µg/l	<0,10	<0,10	<0,10	110
S	Cis-1,2-Dichlooretheen	GC-VLUCHTIG-01	µg/l	5,5	6,7	0,50	29000
S	Trichloormethaan (Chloroform)	GC-VLUCHTIG-01	µg/l	<0,10	<0,10	<0,10	<10
S	Tetrachloormethaan (Tetra)	GC-VLUCHTIG-01	µg/l	<0,10	<0,10	<0,10	<10
S	1,1,1-Trichloorethaan	GC-VLUCHTIG-01	µg/l	<0,10	<0,10	<0,10	<10
S	1,1,2-Trichloorethaan	GC-VLUCHTIG-01	µg/l	<0,10	<0,10	<0,10	<10
S	Trichlooretheen (Tri)	GC-VLUCHTIG-01	µg/l	<0,10	<0,10	<0,10	5100
S	Tetrachlooretheen (Per)	GC-VLUCHTIG-01	µg/l	<0,10	<0,10	<0,10	16000
S	Dichl.ethenen (som cis+trans)	GC-VLUCHTIG-01	µg/l	5,6 (1,2)	6,7 (1,2)	0,57 (1,2)	29000 (1)
S	Vlucht.chl.koolw.stoffen (som)	GC-VLUCHTIG-01	µg/l	6,6 (2)	7,7 (2)	1,6 (2)	51000 (3,2)
S	Vinylchloride	GC-VLUCHTIG-01	µg/l	7,0	21	27	1700

S = door RvA geaccrediteerd conform SIKB AS3000.

Opmerkingen:

1 = Methode vluchtige aromatische en gehalogeneerde koolwaterstoffen : GC-MS
2 = Bij de som zijn de waarden "< rapportagegrens" vermenigvuldigd met factor 0,7 zoals beschreven in 'AS3000, bijlage 3'.
3 = De rapportagegrens is verhoogd, omdat bij de analyse een verdunningsstap noodzakelijk was. Dit als gevolg van het in verhoogde concentratie voorkomen van één of meerdere componenten.
4 = In het chromatogram is MTBE en/of ETBE boven de rapportagegrens waargenomen.

Verpakking bij monster: M120800450 (4081_022~(boorgat 1.2) (18-20 m-mv)4081_022~(boorgat 1.2) (18-20 m-mv))
AM110000652

Hoofd lab. Ing. H. Punte

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Opdrachtgever:

Opdrachtgever	:	Bioclear B.V.
Aanvrager	:	Dhr. A. Nipshagen
Adres	:	Postbus 2262
Postcode en plaats	:	9704 CG Groningen

Opdrachtgegevens:

Opdrachtcode	:	20114081	Labcomcode:	:	1208016BCL
Rapportnummer	:	P120800200 (v1)	Datum opdracht	:	09-08-2012
Opdracht omschr.	:	Fluxmeting A'damsestraatweg en Nachtegaalstr Utrecht	Startdatum	:	09-08-2012
Bemonsterd door	:	Opdrachtgever	Datum rapportage	:	15-08-2012

Monstergegevens:

Nr.	Labnr.		Monsteromschrijving	Monstersoort	Datum bemonstering
5	M120800452	:	4081 024~(boorgat 2.2) (14-16 m-mv)	Grondwater	09-08-2012
6	M120800453	:	4081_025~(boorgat 2.2) (18-20 m-mv)	Grondwater	09-08-2012
7	M120800454	:	4081_026~AF3 (boorgat 3.1) (6-10 m-mv)	Grondwater	09-08-2012
8	M120800455	:	4081_027~NF1 (boorgat 1.1) (5-8 m-mv)	Grondwater	09-08-2012

Resultaten:

	Parameter	Intern ref. nr.	Eenheid	5	6	7	8
	Mvb. SIKB AS3000	MVB-VBH-AS3000-W01		+	+	+	+
	Vluchtige organische halogeen verbindingen						
S	Dichloormethaan	GC-VLUCHTIG-01	µg/l	<0,20	<0,20	<0,20	<0,20
S	1,1-Dichloorethaan	GC-VLUCHTIG-01	µg/l	<0,50	<0,50	<0,50	<0,50
S	1,2-Dichloorethaan	GC-VLUCHTIG-01	µg/l	<0,10	<0,10	<0,10	<0,10
S	Trans-1,2-Dichlooretheen	GC-VLUCHTIG-01	µg/l	<0,10	<0,10	<0,10	2,5
S	Cis-1,2-Dichlooretheen	GC-VLUCHTIG-01	µg/l	19	2,9	<0,10	280
S	Trichloormethaan (Chloroform)	GC-VLUCHTIG-01	µg/l	<0,10	<0,10	<0,10	<0,10
S	Tetrachloormethaan (Tetra)	GC-VLUCHTIG-01	µg/l	<0,10	<0,10	<0,10	<0,10
S	1,1,1-Trichloorethaan	GC-VLUCHTIG-01	µg/l	<0,10	<0,10	<0,10	<0,10
S	1,1,2-Trichloorethaan	GC-VLUCHTIG-01	µg/l	<0,10	<0,10	<0,10	<0,10
S	Trichlooretheen (Tri)	GC-VLUCHTIG-01	µg/l	11	1,4	<0,10	110
S	Tetrachlooretheen (Per)	GC-VLUCHTIG-01	µg/l	31	16	<0,10	440
S	Dichl.ethenen (som cis+trans)	GC-VLUCHTIG-01	µg/l	19 (1,2)	3,0 (1,2)	0,14 (1,2)	280 (1)
S	Vlucht.chl.koolw.stoffen (som)	GC-VLUCHTIG-01	µg/l	61 (2)	21 (2)	1,1 (2)	840 (2)
S	Vinylchloride	GC-VLUCHTIG-01	µg/l	6,7	17	<0,10	11

S = door RvA geaccrediteerd conform SIKB AS3000.

Opmerkingen:

1 = Methode vluchtige aromatische en gehalogeneerde koolwaterstoffen : GC-MS

2 = Bij de som zijn de waarden "< rapportagegrens" vermenigvuldigd met factor 0,7 zoals beschreven in 'AS3000, bijlage 3'.

3 = De rapportagegrens is verhoogd, omdat bij de analyse een verdunningsstap noodzakelijk was. Dit als gevolg van het in verhoogde concentratie voorkomen van één of meerdere componenten.

4 = In het chromatogram is MTBE en/of ETBE boven de rapportagegrens waargenomen.

Verpakking bij monster: M120800452 (4081_024~(boorgat 2.2) (14-16 m-mv))

G83670434

Verpakking bij monster: M120800454 (4081_026~AF3 (boorgat 3.1) (6-10 m-mv))

AM11000071%

Verpakking bij monster: M120800455 (4081_027~NF1 (boorgat 1.1) (5-8 m-mv))

AM110009617

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Opdrachtgegevens:

Opdrachtcode	:	20114081	Labcomcode:	:	1208016BCL
Rapportnummer	:	P120800200 (v1)	Datum opdracht	:	09-08-2012
Opdracht omschr.	:	Fluxmeting A'damsestraatweg en Nachtegaalstr Utrecht	Startdatum	:	09-08-2012
Bemonsterd door	:	Opdrachtgever	Datum rapportage	:	15-08-2012

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Bemonsterd door	: Opdrachtgever	Datum rapportage	: 15-08-2012

Monstergegevens:

Nr.	Labnr.	Monsteromschrijving	Monstersoort	Datum bemonstering
9	M120800456	: 4081 028~(boorgat 1.2) (9-11 m-mv)	Grondwater	09-08-2012
10	M120800457	: 4081_029~(boorgat 1.2) (13-15 m-mv)	Grondwater	09-08-2012
11	M120800458	: 4081_030~NF2 (boorgat 2.1) (5-8 m-mv)	Grondwater	09-08-2012
12	M120800459	: 4081_031~(boorgat 2.2) (9-11 m-mv)	Grondwater	09-08-2012

Resultaten:

	Parameter	Intern ref. nr.	Eenheid	9	10	11	12
	Mvb. SIKB AS3000	MVB-VBH-AS3000-W01		+	+	+	+
	Vluchtige organische halogeen verbindingen						
S	Dichloormethaan	GC-VLUCHTIG-01	µg/l	<0,20	<0,20	<0,20	<0,20
S	1,1-Dichloorethaan	GC-VLUCHTIG-01	µg/l	<0,50	<0,50	<0,50	<0,50
S	1,2-Dichloorethaan	GC-VLUCHTIG-01	µg/l	0,17	0,12	<0,10	0,12
S	Trans-1,2-Dichlooretheen	GC-VLUCHTIG-01	µg/l	0,19	<0,10	0,47	0,24
S	Cis-1,2-Dichlooretheen	GC-VLUCHTIG-01	µg/l	47	4,5	110	17
S	Trichloormethaan (Chloroform)	GC-VLUCHTIG-01	µg/l	<0,10	<0,10	<0,10	<0,10
S	Tetrachloormethaan (Tetra)	GC-VLUCHTIG-01	µg/l	<0,10	<0,10	<0,10	<0,10
S	1,1,1-Trichloorethaan	GC-VLUCHTIG-01	µg/l	<0,10	<0,10	<0,10	<0,10
S	1,1,2-Trichloorethaan	GC-VLUCHTIG-01	µg/l	<0,10	<0,10	<0,10	<0,10
S	Trichlooretheen (Tri)	GC-VLUCHTIG-01	µg/l	0,36	0,13	16	11
S	Tetrachlooretheen (Per)	GC-VLUCHTIG-01	µg/l	1,4	0,13	46	74
S	Dichl.ethenen (som cis+trans)	GC-VLUCHTIG-01	µg/l	47 (1)	4,6 (1,2)	110 (1)	17 (1)
S	Vlucht.chl.koolw.stoffen (som)	GC-VLUCHTIG-01	µg/l	49 (2)	5,8 (2)	180 (2)	100 (2)
S	Vinylchloride	GC-VLUCHTIG-01	µg/l	83	8,0	54	33

S = door RvA geaccrediteerd conform SIKB AS3000.

Opmerkingen:

1 = Methode vluchtige aromatische en gehalogeneerde koolwaterstoffen : GC-MS
2 = Bij de som zijn de waarden "< rapportagegrens" vermenigvuldigd met factor 0,7 zoals beschreven in 'AS3000, bijlage 3'.
3 = De rapportagegrens is verhoogd, omdat bij de analyse een verdunningsstap noodzakelijk was. Dit als gevolg van het in verhoogde concentratie voorkomen van één of meerdere componenten.
4 = In het chromatogram is MTBE en/of ETBE boven de rapportagegrens waargenomen.

Verpakking bij monster: M120800456 (4081_028~(boorgat 1.2) (9-11 m-mv))
AM110009538

Verpakking bij monster: M120800457 (4081_029~(boorgat 1.2) (13-15 m-mv))
AM11000946A

Verpakking bij monster: M120800458 (4081_030~NF2 (boorgat 2.1) (5-8 m-mv))
AM11000302$

Verpakking bij monster: M120800459 (4081_031~(boorgat 2.2) (9-11 m-mv))
AM110002610

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Opdrachtgegevens:

Opdrachtcode	:	20114081	Labcomcode:	:	1208016BCL
Rapportnummer	:	P120800200 (v1)	Datum opdracht	:	09-08-2012
Opdracht omschr.	:	Fluxmeting A'damsestraatweg en Nachtegaalstr Utrecht	Startdatum	:	09-08-2012
Bemonsterd door	:	Opdrachtgever	Datum rapportage	:	15-08-2012

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Bemonsterd door	:	Opdrachtgever	Datum rapportage	:	15-08-2012

Monstergegevens:

Nr.	Labnr.		Monsteromschrijving	Monstersoort	Datum bemonstering
13	M120800460	:	4081 032~(boorgat 2.3) (13-15 m-mv)	Grondwater	09-08-2012
14	M120800461	:	4081_033~NF3 (boorgat 3.1) (5-8 m-mv)	Grondwater	09-08-2012
15	M120800462	:	4081_034~(boorgat 3.2) (9-11 m-mv)	Grondwater	09-08-2012
16	M120800463	:	4081_035~(boorgat 3.2) (13-15 m-mv)	Grondwater	09-08-2012

Resultaten:

	Parameter	Intern ref. nr.	Eenheid	13	14	15	16
	Mvb. SIKB AS3000	MVB-VBH-AS3000-W01		+	+	+	+
	Vluchtige organische halogeen verbindingen						
S	Dichloormethaan	GC-VLUCHTIG-01	µg/l	<0,20	<0,20	<2,0	<0,20
S	1,1-Dichloorethaan	GC-VLUCHTIG-01	µg/l	<0,50	<0,50	<5,0	<0,50
S	1,2-Dichloorethaan	GC-VLUCHTIG-01	µg/l	<0,10	<0,10	<1,0	0,38
S	Trans-1,2-Dichlooretheen	GC-VLUCHTIG-01	µg/l	<0,10	0,54	1,1	<0,10
S	Cis-1,2-Dichlooretheen	GC-VLUCHTIG-01	µg/l	0,54	150	230	4,6
S	Trichloormethaan (Chloroform)	GC-VLUCHTIG-01	µg/l	<0,10	<0,10	<1,0	<0,10
S	Tetrachloormethaan (Tetra)	GC-VLUCHTIG-01	µg/l	<0,10	<0,10	<1,0	<0,10
S	1,1,1-Trichloorethaan	GC-VLUCHTIG-01	µg/l	<0,10	<0,10	<1,0	<0,10
S	1,1,2-Trichloorethaan	GC-VLUCHTIG-01	µg/l	<0,10	<0,10	<1,0	<0,10
S	Trichlooretheen (Tri)	GC-VLUCHTIG-01	µg/l	<0,10	4,1	1,2	0,14
S	Tetrachlooretheen (Per)	GC-VLUCHTIG-01	µg/l	0,50	20	2,2	0,42
S	Dichl.ethenen (som cis+trans)	GC-VLUCHTIG-01	µg/l	0,61 (1,2)	150 (1)	230 (1)	4,7 (1,2)
S	Vlucht.chl.koolw.stoffen (som)	GC-VLUCHTIG-01	µg/l	2,0 (2)	170 (4,2)	250 (3,2)	6,4 (2)
S	Vinylchloride	GC-VLUCHTIG-01	µg/l	15	51	380	96

S = door RvA geaccrediteerd conform SIKB AS3000.

Opmerkingen:

1 = Methode vluchtige aromatische en gehalogeneerde koolwaterstoffen : GC-MS

2 = Bij de som zijn de waarden "< rapportagegrens" vermenigvuldigd met factor 0,7 zoals beschreven in 'AS3000, bijlage 3'.

3 = De rapportagegrens is verhoogd, omdat bij de analyse een verdunningsstap noodzakelijk was. Dit als gevolg van het in verhoogde concentratie voorkomen van één of meerdere componenten.

4 = In het chromatogram is MTBE en/of ETBE boven de rapportagegrens waargenomen.

Verpakking bij monster: M120800460 (4081_032~(boorgat 2.3) (13-15 m-mv))

AM110002676

Verpakking bij monster: M120800461 (4081_033~NF3 (boorgat 3.1) (5-8 m-mv))

AM110002766

Verpakking bij monster: M120800462 (4081_034~(boorgat 3.2) (9-11 m-mv))

AM110002878

Verpakking bij monster: M120800463 (4081_035~(boorgat 3.2) (13-15 m-mv))

AM110002823

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Opdrachtcode	:	20114081	Labcomcode:	:	1208016BCL
Rapportnummer	:	P120800200 (v1)	Datum opdracht	:	09-08-2012
Opdracht omschr.	:	Fluxmeting A'damsestraatweg en Nachtegaalstr Utrecht	Startdatum	:	09-08-2012
Bemonsterd door	:	Opdrachtgever	Datum rapportage	:	15-08-2012

Hoofd lab. Ing. H. Punte

Handtekening:

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Onderzoeksrapport

Opdrachtgever:

Opdrachtgever : Bioclear B.V.
Aanvrager : Dhr. A. Nipshagen
Adres : Postbus 2262
Postcode en plaats : 9704 CG Groningen

Opdrachtgegevens:

Opdrachtcode	: 20114081	Labcomcode:	: 1208016BCL
Rapportnummer	: P120800200 (v1)	Datum opdracht	: 09-08-2012
Opdracht omschr.	: Fluxmeting A'damsestraatweg en Nachtegaalstr Utrecht	Startdatum	: 09-08-2012
Bemonsterd door	: Opdrachtgever	Datum rapportage	: 15-08-2012

Monstergegevens:

Nr.	Labnr.	Monsteromschrijving	Monstersoort	Datum bemonstering
17	M120800464	: 4081 036~NF4 (boorgat 4.1) (5-8 m-mv)	Grondwater	09-08-2012
18	M120800465	: 4081_037~(boorgat 4.2) (9-11 m-mv)	Grondwater	09-08-2012
19	M120800466	: 4081_038~(boorgat 4.2) (13-15 m-mv)	Grondwater	09-08-2012

Resultaten:

	Parameter	Intern ref. nr.	Eenheid	17	18	19
	Mvb. SIKB AS3000	MVB-VBH-AS3000-W01		+	+	+
	Vluchtige organische halogeen verbindingen					
S	Dichloormethaan	GC-VLUCHTIG-01	µg/l	<0,20	<0,20	<0,20
S	1,1-Dichloorethaan	GC-VLUCHTIG-01	µg/l	<0,50	<0,50	<0,50
S	1,2-Dichloorethaan	GC-VLUCHTIG-01	µg/l	<0,10	0,38	<0,10
S	Trans-1,2-Dichlooretheen	GC-VLUCHTIG-01	µg/l	0,60	<0,10	<0,10
S	Cis-1,2-Dichlooretheen	GC-VLUCHTIG-01	µg/l	48	21	0,17
S	Trichloormethaan (Chloroform)	GC-VLUCHTIG-01	µg/l	<0,10	<0,10	<0,10
S	Tetrachloormethaan (Tetra)	GC-VLUCHTIG-01	µg/l	<0,10	<0,10	<0,10
S	1,1,1-Trichloorethaan	GC-VLUCHTIG-01	µg/l	<0,10	<0,10	<0,10
S	1,1,2-Trichloorethaan	GC-VLUCHTIG-01	µg/l	<0,10	<0,10	<0,10
S	Trichlooretheen (Tri)	GC-VLUCHTIG-01	µg/l	1,9	<0,10	<0,10
S	Tetrachlooretheen (Per)	GC-VLUCHTIG-01	µg/l	2,0	<0,10	<0,10
S	Dichl.ethenen (som cis+trans)	GC-VLUCHTIG-01	µg/l	48 (1)	21 (1,2)	0,24 (1,2)
S	Vlucht.chl.koolw.stoffen (som)	GC-VLUCHTIG-01	µg/l	53 (4,2)	22 (2)	1,2 (2)
S	Vinylchloride	GC-VLUCHTIG-01	µg/l	4,1	330	19

S = door RvA geaccrediteerd conform SIKB AS3000.

Opmerkingen:

1 = Methode vluchtige aromatische en gehalogeneerde koolwaterstoffen : GC-MS
2 = Bij de som zijn de waarden "< rapportagegrens" vermenigvuldigd met factor 0,7 zoals beschreven in 'AS3000, bijlage 3'.
3 = De rapportagegrens is verhoogd, omdat bij de analyse een verdunningsstap noodzakelijk was. Dit als gevolg van het in verhoogde concentratie voorkomen van één of meerdere componenten.
4 = In het chromatogram is MTBE en/of ETBE boven de rapportagegrens waargenomen.

Verpakking bij monster: M120800464 (4081_036~NF4 (boorgat 4.1) (5-8 m-mv))
AM11000300.

Verpakking bij monster: M120800465 (4081_037~(boorgat 4.2) (9-11 m-mv))
AM11000958D

Verpakking bij monster: M120800466 (4081_038~(boorgat 4.2) (13-15 m-mv))
AM11000956B

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Aanvrager	:	Dhr. A. Nipshagen
Adres	:	Postbus 2262
Postcode en plaats	:	9704 CG Groningen

Opdrachtgegevens:

Opdrachtcode	:	20114081	Labcomcode:	:	1208016BCL
Rapportnummer	:	P120800200 (v1)	Datum opdracht	:	09-08-2012
Opdracht omschr.	:	Fluxmeting A'damsestraatweg en Nachtegaalstr Utrecht	Startdatum	:	09-08-2012
Bemonsterd door	:	Opdrachtgever	Datum rapportage	:	15-08-2012

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Onderzoeksrapport

Opdrachtgever:

Opdrachtgever : Bioclear B.V.
Aanvrager : Mevr. J. Wittebol
Adres : Postbus 2262
Postcode en plaats : 9704 CG Groningen

Opdrachtgegevens:

Opdrachtcode	: 20103770	Labcomcode:	: 1208024BCL
Rapportnummer	: P120800236 (v1)	Datum opdracht	: 10-08-2012
Opdracht omschr.	: biocapacitietsbepaling	Startdatum	: 10-08-2012
Bemonsterd door	: Opdrachtgever	Datum rapportage	: 16-08-2012

Monstergegevens:

Nr.	Labnr.	Monsteromschrijving	Monstersoort	Datum bemonstering
1	M120800580	: 3770 082~67 (14 m-mv)	Grondwater	10-08-2012
2	M120800581	: 3770_085~53 (38 m-mv)	Grondwater	10-08-2012

Resultaten:

	Parameter	Intern ref. nr.	Eenheid	1	2
	Mvb. SIKB AS3000	MVB-VBH-AS3000-W01		+	+
	Vluchtige organische halogeen verbindingen				
S	Dichloormethaan	GC-VLUCHTIG-01	µg/l	<0,20	<0,20
S	1,1-Dichloorethaan	GC-VLUCHTIG-01	µg/l	<0,50	<0,50
S	1,2-Dichloorethaan	GC-VLUCHTIG-01	µg/l	<0,10	<0,10
S	Trans-1,2-Dichlooretheen	GC-VLUCHTIG-01	µg/l	<0,10	0,49
S	Cis-1,2-Dichlooretheen	GC-VLUCHTIG-01	µg/l	1,2	5,5
S	Trichloormethaan (Chloroform)	GC-VLUCHTIG-01	µg/l	<0,10	<0,10
S	Tetrachloormethaan (Tetra)	GC-VLUCHTIG-01	µg/l	<0,10	<0,10
S	1,1,1-Trichloorethaan	GC-VLUCHTIG-01	µg/l	<0,10	<0,10
S	1,1,2-Trichloorethaan	GC-VLUCHTIG-01	µg/l	<0,10	<0,10
S	Trichlooretheen (Tri)	GC-VLUCHTIG-01	µg/l	0,79	<0,10
S	Tetrachlooretheen (Per)	GC-VLUCHTIG-01	µg/l	0,17	<0,10
S	Dichl.ethenen (som cis+trans)	GC-VLUCHTIG-01	µg/l	1,3 (1,2)	5,9 (1)
S	Vlucht.chl.koolw.stoffen (som)	GC-VLUCHTIG-01	µg/l	3,1 (2)	6,9 (2)
S	Vinylchloride	GC-VLUCHTIG-01	µg/l	<0,10	1100
S	Sulfaat	DIV-SO4-W02	mg/l	55	80
S	Nitraat (als NO3)	DIV-NO3-01	mg/l	5,9	<0,50
	Methaan		µg/l	<8	110
	Tot. org. koolstof (als NPOC)		mg TOC/l	3,1 (3)	4,6 (3)

S = door RvA geaccrediteerd conform SIKB AS3000.

Opmerkingen:

1 = Methode vluchtige aromatische en gehalogeneerde koolwaterstoffen : GC-MS
2 = Bij de som zijn de waarden "< rapportagegrens" vermenigvuldigd met factor 0,7 zoals beschreven in 'AS3000, bijlage 3'.
3 = Deze bepaling is uitbesteed aan derden. Dit laboratorium is voor deze bepaling geaccrediteerd.

Verpakking bij monster: M120800580 (3770_082~67 (14 m-mv))

AM110000674
AM110000720

Verpakking bij monster: M120800581 (3770_085~53 (38 m-mv))

AM110003060
F55902351
AM110009527

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Aanvrager	:	Mevr. J. Wittebol
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Postcode en plaats	:	9704 CG Groningen

Opdrachtgegevens:

Opdrachtcode	:	20103770	Labcomcode:	:	1208024BCL
Rapportnummer	:	P120800236 (v1)	Datum opdracht	:	10-08-2012
Opdracht omschr.	:	biocapacitietsbepaling	Startdatum	:	10-08-2012
Bemonsterd door	:	Opdrachtgever	Datum rapportage	:	16-08-2012

Hoofd lab. Ing. H. Punte

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Postcode en plaats : 9704 CG Groningen

Opdrachtgegevens:

Opdrachtcode : 20103770
Rapportnummer : P120800369 (v1)
Opdracht omschr. : biocapacitietsbepaling
Bemonsterd door : Opdrachtgever

Labcomcode: : 1208034BCL
Datum opdracht : 16-08-2012
Startdatum : 16-08-2012
Datum rapportage : 23-08-2012

Monstergegevens:

Nr.	Labnr.		Monsteromschrijving	Monstersoort	Datum bemonstering
1	M120800930	:	3770_086~StroomO pb67_ 404 (13)	Grondwater	16-08-2012
2	M120800931	:	3770_087~StroomA pb67_107bis (8,7)	Grondwater	16-08-2012
3	M120800932	:	3770_088~StroomO pbAF2_2016(4-6)	Grondwater	16-08-2012
4	M120800933	:	3770_089~StroomA pbAF2_AF1(6-8)	Grondwater	16-08-2012

Resultaten:

	Parameter	Intern ref. nr.	Eenheid	1	2	3	4
	Mvb. SIKB AS3000	MVB-VBH-AS3000-W01		+	+	+	+
	Vluchtige organische halogeen verbindingen						
S	Dichloormethaan	GC-VLUCHTIG-01	µg/l	<0,20	<0,20	<0,20	
S	1,1-Dichloorethaan	GC-VLUCHTIG-01	µg/l	<0,50	<0,50	<0,50	
S	1,2-Dichloorethaan	GC-VLUCHTIG-01	µg/l	<0,10	<0,10	<0,10	
S	Trans-1,2-Dichlooretheen	GC-VLUCHTIG-01	µg/l	0,22	<0,10	<0,10	
S	Cis-1,2-Dichlooretheen	GC-VLUCHTIG-01	µg/l	7,8	<0,10	24	
S	Trichloormethaan (Chloroform)	GC-VLUCHTIG-01	µg/l	<0,10	<0,10	<0,10	
S	Tetrachloormethaan (Tetra)	GC-VLUCHTIG-01	µg/l	<0,10	<0,10	<0,10	
S	1,1,1-Trichloorethaan	GC-VLUCHTIG-01	µg/l	0,37	<0,10	<0,10	
S	1,1,2-Trichloorethaan	GC-VLUCHTIG-01	µg/l	<0,10	<0,10	<0,10	
S	Trichlooretheen (Tri)	GC-VLUCHTIG-01	µg/l	17	<0,10	<0,10	
S	Tetrachlooretheen (Per)	GC-VLUCHTIG-01	µg/l	29	<0,10	<0,10	
S	Dichl.ethenen (som cis+trans)	GC-VLUCHTIG-01	µg/l	8,0 (1)	0,14 (1,2)	24 (1,2)	
S	Vlucht.chl.koolw.stoffen (som)	GC-VLUCHTIG-01	µg/l	55 (2)	1,1 (2)	25 (2)	
S	Vinylchloride	GC-VLUCHTIG-01	µg/l	0,13	<0,10	54	
	Methaan		µg/l	<8	78	27000	8000

S = door RvA geaccrediteerd conform SIKB AS3000.

Opmerkingen:

1 = Methode vluchtige aromatische en gehalogeneerde koolwaterstoffen : GC-MS

2 = Bij de som zijn de waarden "< rapportagegrens" vermenigvuldigd met factor 0,7 zoals beschreven in 'AS3000, bijlage 3'.

Verpakking bij monster: M120800930 (3770_086~StroomO pb67_ 404 (13))

AM110000865

Verpakking bij monster: M120800931 (3770_087~StroomA pb67_107bis (8,7))

AM110000966

Verpakking bij monster: M120800932 (3770_088~StroomO pbAF2_2016(4-6))

AM110000854

Verpakking bij monster: M120800933 (3770_089~StroomA pbAF2_AF1(6-8))

M120800933

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Postcode en plaats	:	9704 CG Groningen

Opdrachtgegevens:

Opdrachtcode	:	20103770	Labcomcode:	:	1208034BCL
Rapportnummer	:	P120800369 (v1)	Datum opdracht	:	16-08-2012
Opdracht omschr.	:	biocapacitietsbepaling	Startdatum	:	16-08-2012
Bemonsterd door	:	Opdrachtgever	Datum rapportage	:	23-08-2012

Hoofd lab. Ing. H. Punte

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Opdrachtcode : 20103770
Rapportnummer : P120800369 (v1)
Opdracht omschr. : biocapacitietsbepaling
Bemonsterd door : Opdrachtgever

Labcomcode: : 1208034BCL
Datum opdracht : 16-08-2012
Startdatum : 16-08-2012
Datum rapportage : 23-08-2012

Monstergegevens:

Nr.	Labnr.		Monsteromschrijving	Monstersoort	Datum bemonstering
5	M120800934	:	3770 090~StroomO pbNF4 NF3 (5-8)	Grondwater	16-08-2012
6	M120800935	:	3770_091~StroomA pbNF4_2002 (7-8)	Grondwater	16-08-2012
7	M120800936	:	3770_092~StroomO pb53_308 (39-40)	Grondwater	16-08-2012
8	M120800937	:	3770_093~StroomA pb53_502 (29-30)	Grondwater	16-08-2012

Resultaten:

	Parameter	Intern ref. nr.	Eenheid	5	6	7	8
	Mvb. SIKB AS3000	MVB-VBH-AS3000-W01		+	+	+	+
	Vluchtige organische halogeen verbindingen						
S	Dichloormethaan	GC-VLUCHTIG-01	µg/l		<0,20	<0,20	<0,20
S	1,1-Dichloorethaan	GC-VLUCHTIG-01	µg/l		<0,50	<0,50	<0,50
S	1,2-Dichloorethaan	GC-VLUCHTIG-01	µg/l		<0,10	<0,10	<0,10
S	Trans-1,2-Dichlooretheen	GC-VLUCHTIG-01	µg/l		<0,10	0,57	<0,10
S	Cis-1,2-Dichlooretheen	GC-VLUCHTIG-01	µg/l		0,32	2,0	2,2
S	Trichloormethaan (Chloroform)	GC-VLUCHTIG-01	µg/l		<0,10	<0,10	<0,10
S	Tetrachloormethaan (Tetra)	GC-VLUCHTIG-01	µg/l		<0,10	<0,10	<0,10
S	1,1,1-Trichloorethaan	GC-VLUCHTIG-01	µg/l		<0,10	<0,10	<0,10
S	1,1,2-Trichloorethaan	GC-VLUCHTIG-01	µg/l		<0,10	<0,10	<0,10
S	Trichlooretheen (Tri)	GC-VLUCHTIG-01	µg/l		<0,10	<0,10	<0,10
S	Tetrachlooretheen (Per)	GC-VLUCHTIG-01	µg/l		<0,10	<0,10	<0,10
S	Dichl.ethenen (som cis+trans)	GC-VLUCHTIG-01	µg/l		0,39 (1,2)	2,6 (1)	2,3 (1,2)
S	Vlucht.chl.koolw.stoffen (som)	GC-VLUCHTIG-01	µg/l		1,4 (2)	3,6 (2)	3,2 (2)
S	Vinylchloride	GC-VLUCHTIG-01	µg/l		11	280	22
	Methaan		µg/l	190	130	230	25

S = door RvA geaccrediteerd conform SIKB AS3000.

Opmerkingen:

1 = Methode vluchtige aromatische en gehalogeneerde koolwaterstoffen : GC-MS

2 = Bij de som zijn de waarden "< rapportagegrens" vermenigvuldigd met factor 0,7 zoals beschreven in 'AS3000, bijlage 3'.

Verpakking bij monster: M120800934 (3770_090~StroomO pbNF4_NF3 (5-8))

M120800934

Verpakking bij monster: M120800935 (3770_091~StroomA pbNF4_2002 (7-8))

AM110000922

Verpakking bij monster: M120800936 (3770_092~StroomO pb53_308 (39-40))

AM110000797

Verpakking bij monster: M120800937 (3770_093~StroomA pb53_502 (29-30))

AM110000911

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Postcode en plaats	:	9704 CG Groningen

Opdrachtgegevens:

Opdrachtcode	:	20103770	Labcomcode:	:	1208034BCL
Rapportnummer	:	P120800369 (v1)	Datum opdracht	:	16-08-2012
Opdracht omschr.	:	biocapacitietsbepaling	Startdatum	:	16-08-2012
Bemonsterd door	:	Opdrachtgever	Datum rapportage	:	23-08-2012

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Opdrachtgegevens:

Opdrachtcode	: 20103770	Labcomcode:	: 1208044BCL
Rapportnummer	: P120800516 (v1)	Datum opdracht	: 22-8-2012
Opdracht omschr.	: biocapaciteitsbepaling	Startdatum	: 22-8-2012
Bemonsterd door	: Opdrachtgever	Datum rapportage	: 29-8-2012

Monstergegevens:

Nr.	Labnr.	Monsteromschrijving	Monstersoort	Datum bemonstering
1	M120801336	: 3770 084~NF 4 (5-8 m-mv)	Grondwater	22-8-2012

Resultaten:

	Parameter	Intern ref. nr.	Eenheid	1
	Mvb. SIKB AS3000	MVB-VBH-AS3000-W01		+
S	Sulfaat	DIV-SO4-W02	mg/l	66
S	Nitraat (als NO3)	DIV-NO3-01	mg/l	2,2
	Methaan		µg/l	39
	Tot. org. koolstof (als NPOC)		mg TOC/l	5,3 (1)

S = door RvA geaccrediteerd conform SIKB AS3000.

Opmerkingen:

1 = Deze bepaling is uitbesteed aan derden. Dit laboratorium is voor deze bepaling geaccrediteerd.

Hoofd lab. Ing. H. Punte

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Opdrachtgever:

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Aanvrager : Mevr. S. Lieten
Adres : Postbus 2262
Postcode en plaats : 9704 CG Groningen

Opdrachtgegevens:

Opdrachtcode	: 20103770	Labcomcode:	: 1210010BCL
Rapportnummer	: P121000789 (v1)	Datum opdracht	: 17-10-2012
Opdracht omschr.	: Citychlor	Startdatum	: 17-10-2012
Bemonsterd door	: Opdrachtgever	Datum rapportage	: 24-10-2012

Monstergegevens:

Nr.	Labnr.	Monsteromschrijving	Monstersoort	Datum bemonstering
1	M121003127	: 3770 097~67 (14 m-mv)	Grondwater	17-10-2012
2	M121003128	: 3770_098~AF 2 (6-10 m-mv)	Grondwater	17-10-2012
3	M121003129	: 3770_100~53 (38 m-mv)	Grondwater	17-10-2012

Resultaten:

	Parameter	Intern ref. nr.	Eenheid	1	2	3
	Vluchtige organische halogeen verbindingen					
Q	Dichloormethaan	GCMS-VLUCHTIG-01	µg/l	<0,20	<0,20	<0,20
Q	1,1-Dichloorethaan	GCMS-VLUCHTIG-01	µg/l	<0,50	<0,50	<0,50
Q	1,2-Dichloorethaan	GCMS-VLUCHTIG-01	µg/l	<0,10	<0,10	<0,10
Q	Trans-1,2-Dichlooretheen	GCMS-VLUCHTIG-01	µg/l	<0,10	190	0,82
Q	Cis-1,2-Dichlooretheen	GCMS-VLUCHTIG-01	µg/l	1,7	37000	11 (4)
Q	Trichloormethaan (Chloroform)	GCMS-VLUCHTIG-01	µg/l	<0,10	<0,10	<0,10
Q	Tetrachloormethaan (Tetra)	GCMS-VLUCHTIG-01	µg/l	<0,10	<0,10	<0,10
Q	1,1,1-Trichloorethaan	GCMS-VLUCHTIG-01	µg/l	<0,10	<0,10	<0,10
Q	1,1,2-Trichloorethaan	GCMS-VLUCHTIG-01	µg/l	<0,10	<0,10	<0,10
Q	Trichlooretheen (Tri)	GCMS-VLUCHTIG-01	µg/l	1,6	23000	2,9 (4)
Q	Tetrachlooretheen (Per)	GCMS-VLUCHTIG-01	µg/l	0,30	100000	13 (4)
Q	Dichl.ethenen (som cis+trans)	GCMS-VLUCHTIG-01	µg/l	1,8 (1,2)	37000 (1)	12 (1)
Q	Vlucht.chl.koolw.stoffen (som)	GCMS-VLUCHTIG-01	µg/l	4,6 (2)	160000 (2)	28 (2)
Q	Vinylchloride	GCMS-VLUCHTIG-01	µg/l	0,13	2700	3500 (5)
	Ethaan		µg/l	<1 (3)	49 (3)	<1 (3)
	Etheen		µg/l	<1 (3)	61 (3)	7 (3)

Q = door RvA geaccrediteerd.

Opmerkingen:

1 = Methode vluchtige aromatische en gehalogeneerde koolwaterstoffen : GC-MS
2 = Bij de som zijn de waarden "< rapportagegrens" vermenigvuldigd met factor 0,7 zoals beschreven in 'AS3000, bijlage 3'.
3 = Het monster is onjuist aangeleverd, het resultaat is een indicatieve waarde.
4 = Het gehalte bevat carry-over. Het gehalte dient daarom als indicatief te worden beschouwd.
5 = Het gehalte is boven de detector lineariteit. Het gehalte dient daarom als indicatief te worden beschouwd.

Verpakking bij monster: M121003127 (3770_097~67 (14 m-mv))
AM11000298A

Verpakking bij monster: M121003128 (3770_098~AF 2 (6-10 m-mv))
AM110002913

Verpakking bij monster: M121003129 (3770_100~53 (38 m-mv))
AM110002867

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Aanvrager	:	Mevr. S. Lieten
Adres	:	Postbus 2262
Postcode en plaats	:	9704 CG Groningen

Opdrachtgegevens:

Opdrachtcode	:	20103770	Labcomcode:	:	1210010BCL
Rapportnummer	:	P121000789 (v1)	Datum opdracht	:	17-10-2012
Opdracht omschr.	:	Citychlor	Startdatum	:	17-10-2012
Bemonsterd door	:	Opdrachtgever	Datum rapportage	:	24-10-2012

Hoofd lab. Ing. H. Punte

Handtekening:

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Onderzoeksrapport

Opdrachtgever:

Opdrachtgever : Bioclear B.V.
Aanvrager : Mevr. J. Wittebol
Adres : Postbus 2262
Postcode en plaats : 9704 CG Groningen

Opdrachtgegevens:

Opdrachtcode : 20103770
Rapportnummer : P121200520 (v1)
Opdracht omschr. : Citychlor Utrecht
Bemonsterd door : Opdrachtgever

Labcomcode: : 1212041BCL
Datum opdracht : 12-12-2012
Startdatum : 12-12-2012
Datum rapportage : 18-12-2012

Monstergegevens:

Nr.	Labnr.		Monsteromschrijving	Monstersoort	Datum bemonstering
1	M121201718	:	3770 111~53 (38 m-mv) t=4	Grondwater	12-12-2012
2	M121201719	:	3770_109~AF 2 (6-10 m-mv) t=4	Grondwater	12-12-2012

Resultaten:

	Parameter	Intern ref. nr.	Eenheid	1	2
	Mvb. SIKB AS3000	MVB-WATER-01		+	+
	Vluchtige organische halogeen verbindingen				
S	Dichloormethaan	GCMS-VLUCHTIG-01	µg/l	<0,20	<0,20
S	1,1-Dichloorethaan	GCMS-VLUCHTIG-01	µg/l	<0,50	<0,50
S	1,2-Dichloorethaan	GCMS-VLUCHTIG-01	µg/l	<0,10	<0,10
S	Trans-1,2-Dichlooretheen	GCMS-VLUCHTIG-01	µg/l	1,3	240
S	Cis-1,2-Dichlooretheen	GCMS-VLUCHTIG-01	µg/l	7,6	33000
S	Trichloormethaan (Chloroform)	GCMS-VLUCHTIG-01	µg/l	<0,10	<0,10
S	Tetrachloormethaan (Tetra)	GCMS-VLUCHTIG-01	µg/l	<0,10	<0,10
S	1,1,1-Trichloorethaan	GCMS-VLUCHTIG-01	µg/l	<0,10	<0,10
S	1,1,2-Trichloorethaan	GCMS-VLUCHTIG-01	µg/l	<0,10	<0,10
S	Trichlooretheen (Tri)	GCMS-VLUCHTIG-01	µg/l	0,57	21000
S	Tetrachlooretheen (Per)	GCMS-VLUCHTIG-01	µg/l	1,4	88000
S	Dichl.ethenen (som cis+trans)	GCMS-VLUCHTIG-01	µg/l	8,9 (1)	33000 (4,1)
S	Vlucht.chl.koolw.stoffen (som)	GCMS-VLUCHTIG-01	µg/l	12 (2)	140000 (2)
S	Vinylchloride	GCMS-VLUCHTIG-01	µg/l	2400	1900
S	Nitraat (als NO3)	DIV-AA2-01	mg/l	<0,50	<0,50
S	Sulfaat	DIV-AA2-01	mg/l	54	<25
	Ethaan		µg/l	<1	170
	Etheen		µg/l	10	110
	Methaan		µg/l	100	14000
	Tot. org. koolstof (als NPOC)		mg TOC/l	5,0 (3)	10 (3)

S = door RvA geaccrediteerd conform SIKB AS3000.

Opmerkingen:

1 = Methode vluchtige aromatische en gehalogeneerde koolwaterstoffen : GC-MS
2 = Bij de som zijn de waarden "< rapportagegrens" vermenigvuldigd met factor 0,7 zoals beschreven in 'AS3000, bijlage 3'.
3 = Deze bepaling is uitbesteed aan derden. Dit laboratorium is voor deze bepaling geaccrediteerd.
4 = In het chromatogram zijn één of meerdere aromatische componenten boven de rapportagegrens waargenomen.

Verpakking bij monster: M121201718 (3770_111~53 (38 m-mv) t=4)

F55685258
M121201718
AM11000949D
AM11000250+

Verpakking bij monster: M121201719 (3770_109~AF 2 (6-10 m-mv) t=4)

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Opdrachtgegevens:

Opdrachtcode	:	20103770	Labcomcode:	:	1212041BCL
Rapportnummer	:	P121200520 (v1)	Datum opdracht	:	12-12-2012
Opdracht omschr.	:	Citychlor Utrecht	Startdatum	:	12-12-2012
Bemonsterd door	:	Opdrachtgever	Datum rapportage	:	18-12-2012

F55685438
AM11000311$
AM110009415
M121201719

Hoofd lab. Ing. H. Punte

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Opdrachtgegevens:

Opdrachtcode	: 20103770	Labcomcode:	: 1212054BCL
Rapportnummer	: P121200624 (v1)	Datum opdracht	: 14-12-2012
Opdracht omschr.	: Citychlor Utrecht 2	Startdatum	: 14-12-2012
Bemonsterd door	: Opdrachtgever	Datum rapportage	: 20-12-2012

Monstergegevens:

Nr.	Labnr.	Monsteromschrijving	Monstersoort	Datum bemonstering
1	M121202119	: 3770 110~NF4.2 (9-11 m-mv) t=4	Grondwater	14-12-2012

Resultaten:

	Parameter	Intern ref. nr.	Eenheid	1	
	Mvb. SIKB AS3000	MVB-WATER-01		+	
	Vluchtige organische halogeen verbindingen				
S	Dichloormethaan	GCMS-VLUCHTIG-01	µg/l	<0,20	
S	1,1-Dichloorethaan	GCMS-VLUCHTIG-01	µg/l	<0,50	
S	1,2-Dichloorethaan	GCMS-VLUCHTIG-01	µg/l	0,80	
S	Trans-1,2-Dichlooretheen	GCMS-VLUCHTIG-01	µg/l	<0,10	
S	Cis-1,2-Dichlooretheen	GCMS-VLUCHTIG-01	µg/l	26	
S	Trichloormethaan (Chloroform)	GCMS-VLUCHTIG-01	µg/l	<0,10	
S	Tetrachloormethaan (Tetra)	GCMS-VLUCHTIG-01	µg/l	<0,10	
S	1,1,1-Trichloorethaan	GCMS-VLUCHTIG-01	µg/l	<0,10	
S	1,1,2-Trichloorethaan	GCMS-VLUCHTIG-01	µg/l	<0,10	
S	Trichlooretheen (Tri)	GCMS-VLUCHTIG-01	µg/l	<0,10	
S	Tetrachlooretheen (Per)	GCMS-VLUCHTIG-01	µg/l	<0,10	
S	Dichl.ethenen (som cis+trans)	GCMS-VLUCHTIG-01	µg/l	26	(1,2)
S	Vlucht.chl.koolw.stoffen (som)	GCMS-VLUCHTIG-01	µg/l	28	(2)
S	Vinylchloride	GCMS-VLUCHTIG-01	µg/l	690	
S	Nitraat (als NO3)	DIV-AA2-01	mg/l	0,71	
S	Sulfaat	DIV-AA2-01	mg/l	76	
	Ethaan		µg/l	<1	
	Etheen		µg/l	29	
	Methaan		µg/l	42	
	Tot. org. koolstof (als NPOC)		mg TOC/l	2,5	(3)

S = door RvA geaccrediteerd conform SIKB AS3000.

Opmerkingen:

1 = Methode vluchtige aromatische en gehalogeneerde koolwaterstoffen : GC-MS
2 = Bij de som zijn de waarden "< rapportagegrens" vermenigvuldigd met factor 0,7 zoals beschreven in 'AS3000, bijlage 3'.
3 = Deze bepaling is uitbesteed aan derden. Dit laboratorium is voor deze bepaling geaccrediteerd.

Verpakking bij monster: M121202119 (3770_110~NF4.2 (9-11 m-mv) t=4)

AM110003295
M121202119
F55685405
AM110003734

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Opdrachtgegevens:

Opdrachtcode	:	20103770	Labcomcode:	:	1212054BCL
Rapportnummer	:	P121200624 (v1)	Datum opdracht	:	14-12-2012
Opdracht omschr.	:	Citychlor Utrecht 2	Startdatum	:	14-12-2012
Bemonsterd door	:	Opdrachtgever	Datum rapportage	:	20-12-2012

Hoofd lab. Ing. H. Punte

Handtekening:

Analysecertificaat

Opdrachtgever:

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Postcode en plaats	:	9704 CG Groningen

Opdrachtgegevens:

Opdrachtcode	:	20124388	Labcomcode:	:	1302055BCL
Rapportnummer	:	P130200375 (v1)	Datum opdracht	:	08-02-2013
Opdracht omschr.	:	Mon. Centrum Utrecht Citychlor3	Startdatum	:	08-02-2013
Bemonsterd door	:	Opdrachtgever	Datum rapportage	:	14-02-2013

Monstergegevens:

Nr.	Labnr.		Monsteromschrijving	Monstersoort	Datum bemonstering
1	M130200981	:	4388 047~308 (19-20)	Grondwater	08-02-2013
2	M130200982	:	4388_048~308 (29-30)	Grondwater	08-02-2013
3	M130200983	:	4388_049~308 (39-40)	Grondwater	08-02-2013
4	M130200984	:	4388_050~WKO-DH (24-25)	Grondwater	08-02-2013

Resultaten:

	Parameter	Intern ref. nr.	Eenheid	1	2	3	4
	Mvb. SIKB AS3000	MVB-WATER-01		+	+	+	+
	Metalen						
	Calcium	ICP-MET-01	µg/l	140000	94000	130000	130000
	IJzer (II)		mg/l	1,2 (1)	1,1 (1)	0,12 (1)	0,11 (1)
Q	Mangaan	ICP-MET-01	µg/l	1600	810	380	770
	Natrium	ICP-MET-01	µg/l	52000	76000	100000	55000
	Vluchtige organische halogeen verbindingen						
S	Dichloormethaan	GCMS-VLUCHTIG-01	µg/l	<0,20	<0,20	<0,20	<0,20
S	1,1-Dichloorethaan	GCMS-VLUCHTIG-01	µg/l	<0,50	<0,50	<0,50	<0,50
S	1,2-Dichloorethaan	GCMS-VLUCHTIG-01	µg/l	<0,10	<0,10	<0,10	<0,10
S	Trans-1,2-Dichlooretheen	GCMS-VLUCHTIG-01	µg/l	0,46	2,4	0,54	0,69
S	Cis-1,2-Dichlooretheen	GCMS-VLUCHTIG-01	µg/l	16	5,1	1,7	18
S	Trichloormethaan (Chloroform)	GCMS-VLUCHTIG-01	µg/l	<0,10	<0,10	<0,10	<0,10
S	Tetrachloormethaan (Tetra)	GCMS-VLUCHTIG-01	µg/l	<0,10	<0,10	<0,10	<0,10
S	1,1,1-Trichloorethaan	GCMS-VLUCHTIG-01	µg/l	<0,10	<0,10	<0,10	<0,10
S	1,1,2-Trichloorethaan	GCMS-VLUCHTIG-01	µg/l	<0,10	<0,10	<0,10	<0,10
S	Trichlooretheen (Tri)	GCMS-VLUCHTIG-01	µg/l	1,7	<0,10	<0,10	4,8
S	Tetrachlooretheen (Per)	GCMS-VLUCHTIG-01	µg/l	<0,10	<0,10	<0,10	<0,10
S	Dichl.ethenen (som cis+trans)	GCMS-VLUCHTIG-01	µg/l	17 (2)	7,4 (2)	2,3 (2)	19 (2)
S	Vlucht.chl.koolw.stoffen (som)	GCMS-VLUCHTIG-01	µg/l	19 (3)	8,4 (3)	3,3 (3)	25 (3)
S	Vinylchloride	GCMS-VLUCHTIG-01	µg/l	5,9	3400	530	2,5
	Bromide		mg/l	0,15 (4)	0,18 (4)	0,16 (4)	0,13 (4)
	Sulfide		mg/l	<0,1 (1)	<0,1 (1)	<0,1 (1)	<0,1 (1)
S	Chloride	DIV-AA2-01	mg/l	66	120	110	75
S	Nitraat (als NO3)	DIV-AA2-01	mg/l	<0,50	<0,50	<0,50	<0,50
S	Sulfaat	DIV-AA2-01	mg/l	140	57	130	76
	Ethaan		µg/l	<1	<1	<1	<1
	Etheen		µg/l	<1	86	2	<1
	Methaan		µg/l	30	360	200	52
	Carbonaat		mg/l	390 (1)	380 (1)	520 (1)	440 (1)

Zie volgende pagina

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Opdrachtgegevens:

Opdrachtcode	:	20124388	Labcomcode:	:	1302055BCL
Rapportnummer	:	P130200375 (v1)	Datum opdracht	:	08-02-2013
Opdracht omschr.	:	Mon. Centrum Utrecht Citychlor3	Startdatum	:	08-02-2013
Bemonsterd door	:	Opdrachtgever	Datum rapportage	:	14-02-2013

Monstergegevens:

Nr.	Labnr.		Monsteromschrijving	Monstersoort	Datum bemonstering
1	M130200981	:	4388 047~308 (19-20)	Grondwater	08-02-2013
2	M130200982	:	4388_048~308 (29-30)	Grondwater	08-02-2013
3	M130200983	:	4388_049~308 (39-40)	Grondwater	08-02-2013
4	M130200984	:	4388_050~WKO-DH (24-25)	Grondwater	08-02-2013

Resultaten:

	Parameter	Intern ref. nr.	Eenheid	1	2	3	4
	Bicarbonaat		mg/l	390 (1)	380 (1)	520 (1)	440 (1)
	Tot. org. koolstof (als NPOC)		mg TOC/l	4,4 (4)	4,5 (4)	5,6 (4)	4,7 (4)
Q	Ammonium (als N)	DIV-KJEL-01	mg/l	4,5 (5)	1,7 (5)	3,4 (5)	<1,0 (5)
	Opgelost organisch koolstof		mg DOC/l	3,5 (4)	3,1 (4)	4,5 (4)	4,2 (4)

Q = door RvA geaccrediteerd.

S = door RvA geaccrediteerd conform SIKB AS3000.

Opmerkingen:

1 = Deze bepaling is uitbesteed aan derden.

2 = Methode vluchtige aromatische en gehalogeneerde koolwaterstoffen : GC-MS

3 = Bij de som zijn de waarden " rapportagegrens" vermenigvuldigd met factor 0,7 zoals beschreven in AS3000, bijlage 3 .

4 = Deze bepaling is uitbesteed aan derden. Dit laboratorium is voor deze bepaling geaccrediteerd.

5 = Ureum en vluchtige amines interfereren op deze analyse. Indien aanwezig kunnen ze voor een verhoogd analyseresultaat zorgen.

Hoofd lab. Ing. H. Punte

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Postcode en plaats	:	9704 CG Groningen

Opdrachtgegevens:

Opdrachtcode	:	20124388	Labcomcode:	:	1302055BCL
Rapportnummer	:	P130200375 (v1)	Datum opdracht	:	08-02-2013
Opdracht omschr.	:	Mon. Centrum Utrecht Citychlor3	Startdatum	:	08-02-2013
Bemonsterd door	:	Opdrachtgever	Datum rapportage	:	14-02-2013

Monstergegevens:

Nr.	Labnr.		Monsteromschrijving	Monstersoort	Datum bemonstering
5	M130200985	:	4388 053~WKO-LN (24-25)	Grondwater	08-02-2013
6	M130200986	:	4388_054~WKO-LN (29-30)	Grondwater	08-02-2013
7	M130200987	:	4388_055~WKO-LN (34-35)	Grondwater	08-02-2013

Resultaten:

	Parameter	Intern ref. nr.	Eenheid	5	6	7
	Mvb. SIKB AS3000	MVB-WATER-01		+	+	+
	Metalen					
	Calcium	ICP-MET-01	µg/l	150000	150000	150000
	IJzer (II)		mg/l	0,31 (1)	3,8 (1)	0,13 (1)
Q	Mangaan	ICP-MET-01	µg/l	2700	1500	1100
	Natrium	ICP-MET-01	µg/l	54000	54000	65000
	Vluchtige organische halogeen verbindingen					
S	Dichloormethaan	GCMS-VLUCHTIG-01	µg/l	<0,20	<0,20	<0,20
S	1,1-Dichloorethaan	GCMS-VLUCHTIG-01	µg/l	5,1	2,4	<0,50
S	1,2-Dichloorethaan	GCMS-VLUCHTIG-01	µg/l	<0,10	<0,10	<0,10
S	Trans-1,2-Dichlooretheen	GCMS-VLUCHTIG-01	µg/l	0,29	0,13	<0,10
S	Cis-1,2-Dichlooretheen	GCMS-VLUCHTIG-01	µg/l	6,8	3,3	0,17
S	Trichloormethaan (Chloroform)	GCMS-VLUCHTIG-01	µg/l	<0,10	<0,10	<0,10
S	Tetrachloormethaan (Tetra)	GCMS-VLUCHTIG-01	µg/l	<0,10	<0,10	<0,10
S	1,1,1-Trichloorethaan	GCMS-VLUCHTIG-01	µg/l	<0,10	<0,10	<0,10
S	1,1,2-Trichloorethaan	GCMS-VLUCHTIG-01	µg/l	<0,10	<0,10	<0,10
S	Trichlooretheen (Tri)	GCMS-VLUCHTIG-01	µg/l	0,77	0,23	<0,10
S	Tetrachlooretheen (Per)	GCMS-VLUCHTIG-01	µg/l	<0,10	<0,10	<0,10
S	Dichl.ethenen (som cis+trans)	GCMS-VLUCHTIG-01	µg/l	7,1 (2)	3,5 (2)	0,24 (2,3)
S	Vlucht.chl.koolw.stoffen (som)	GCMS-VLUCHTIG-01	µg/l	14 (3)	6,7 (3)	1,2 (3)
S	Vinylchloride	GCMS-VLUCHTIG-01	µg/l	0,31	17	0,69
	Bromide		mg/l	0,19 (4)	0,14 (4)	0,13 (4)
	Sulfide		mg/l	<0,1 (1)	<0,1 (1)	<0,1 (1)
S	Chloride	DIV-AA2-01	mg/l	62	68	92
S	Nitraat (als NO3)	DIV-AA2-01	mg/l	<0,50	<0,50	<0,50
S	Sulfaat	DIV-AA2-01	mg/l	140	150	150
	Ethaan		µg/l	<1	1	<1
	Etheen		µg/l	<1	<1	<1
	Methaan		µg/l	97	77	37
	Carbonaat		mg/l	430 (1)	420 (1)	420 (1)

Zie volgende pagina

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Opdrachtgegevens:

Opdrachtcode	:	20124388	Labcomcode:	:	1302055BCL
Rapportnummer	:	P130200375 (v1)	Datum opdracht	:	08-02-2013
Opdracht omschr.	:	Mon. Centrum Utrecht Citychlor3	Startdatum	:	08-02-2013
Bemonsterd door	:	Opdrachtgever	Datum rapportage	:	14-02-2013

Monstergegevens:

Nr.	Labnr.		Monsteromschrijving	Monstersoort	Datum bemonstering
5	M130200985	:	4388 053~WKO-LN (24-25)	Grondwater	08-02-2013
6	M130200986	:	4388_054~WKO-LN (29-30)	Grondwater	08-02-2013
7	M130200987	:	4388_055~WKO-LN (34-35)	Grondwater	08-02-2013

Resultaten:

	Parameter	Intern ref. nr.	Eenheid	5	6	7
	Bicarbonaat		mg/l	430 (1)	420 (1)	420 (1)
	Tot. org. koolstof (als NPOC)		mg TOC/l	5,2 (4)	4,7 (4)	3,6 (4)
Q	Ammonium (als N)	DIV-KJEL-01	mg/l	4,5 (5)	3,2 (5)	<1,0 (5)
	Opgelost organisch koolstof		mg DOC/l	4,2 (4)	3,9 (4)	3,0 (4)

Q = door RvA geaccrediteerd.

S = door RvA geaccrediteerd conform SIKB AS3000.

Opmerkingen:

1 = Deze bepaling is uitbesteed aan derden.

2 = Methode vluchtige aromatische en gehalogeneerde koolwaterstoffen : GC-MS

3 = Bij de som zijn de waarden " rapportagegrens" vermenigvuldigd met factor 0,7 zoals beschreven in AS3000, bijlage 3 .

4 = Deze bepaling is uitbesteed aan derden. Dit laboratorium is voor deze bepaling geaccrediteerd.

5 = Ureum en vluchtige amines interfereren op deze analyse. Indien aanwezig kunnen ze voor een verhoogd analyseresultaat zorgen.

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Opdrachtgegevens:

Opdrachtcode	:	20103770	Labcomcode:	:	1302076BCL
Rapportnummer	:	P130200564 (v1)	Datum opdracht	:	14-02-2013
Opdracht omschr.	:	Citychlor Utrecht 3	Startdatum	:	14-02-2013
Bemonsterd door	:	Opdrachtgever	Datum rapportage	:	22-02-2013

Monstergegevens:

Nr.	Labnr.		Monsteromschrijving	Monstersoort	Datum bemonstering
1	M130201554	:	3770 118~AF 2 (6-10 m-mv) gw t=6	Grondwater	14-02-2013
2	M130201555	:	3770_120~StroomO pbAF2_2016(4-6) gw t=6	Grondwater	14-02-2013
3	M130201556	:	3770_121~StroomA pbAF2_AF1(6-8) gw t=6	Grondwater	14-02-2013
4	M130201557	:	3770_122~NF4.2 (9-11 m-mv) gw t=6	Grondwater	14-02-2013

Resultaten:

	Parameter	Intern ref. nr.	Eenheid	1	2	3	4
	Mvb. SIKB AS3000	MVB-WATER-01		+	+	+	+
	Vluchtige organische halogeen verbindingen						
S	Dichloormethaan	GCMS-VLUCHTIG-01	µg/l	2500	<0,20	<0,20	<0,20
S	1,1-Dichloorethaan	GCMS-VLUCHTIG-01	µg/l	<5000 (1)	<0,50	<0,50	<0,50
S	1,2-Dichloorethaan	GCMS-VLUCHTIG-01	µg/l	<1000 (1)	<0,10	<0,10	0,60
S	Trans-1,2-Dichlooretheen	GCMS-VLUCHTIG-01	µg/l	2600	0,12	<0,10	<0,10
S	Cis-1,2-Dichlooretheen	GCMS-VLUCHTIG-01	µg/l	30000	30	0,12	20
S	Trichloormethaan (Chloroform)	GCMS-VLUCHTIG-01	µg/l	2200	<0,10	<0,10	<0,10
S	Tetrachloormethaan (Tetra)	GCMS-VLUCHTIG-01	µg/l	1700	<0,10	<0,10	<0,10
S	1,1,1-Trichloorethaan	GCMS-VLUCHTIG-01	µg/l	2100	<0,10	<0,10	<0,10
S	1,1,2-Trichloorethaan	GCMS-VLUCHTIG-01	µg/l	<1000 (1)	<0,10	<0,10	<0,10
S	Trichlooretheen (Tri)	GCMS-VLUCHTIG-01	µg/l	36000	<0,10	<0,10	<0,10
S	Tetrachlooretheen (Per)	GCMS-VLUCHTIG-01	µg/l	150000	<0,10	0,15	<0,10
S	Dichl.ethenen (som cis+trans)	GCMS-VLUCHTIG-01	µg/l	33000	30	0,19 (2)	20 (2)
S	Vlucht.chl.koolw.stoffen (som)	GCMS-VLUCHTIG-01	µg/l	230000 (2)	31 (4,2)	1,2 (4,2)	22 (2)
S	Vinylchloride	GCMS-VLUCHTIG-01	µg/l	5200	76 (4)	0,12 (4)	560
	Sulfaat		mg/l	<8,0			
S	Nitraat (als NO3)	DIV-AA2-01	mg/l	<0,50			<0,50
S	Sulfaat	DIV-AA2-01	mg/l				69
	Ethaan		µg/l	120	6200	120	<1
	Etheen		µg/l	58	150	<1	24
	Methaan		µg/l	15000	23000	9000	57
	Tot. org. koolstof (als NPOC)		mg TOC/l	8,0 (3)			2,2 (3)

S = door RvA geaccrediteerd conform SIKB AS3000.

Opmerkingen:

1 = De rapportagegrens is verhoogd, omdat bij de analyse een verdunningsstap noodzakelijk was. Dit als gevolg van het in verhoogde concentratie voorkomen van n of meerdere componenten.

2 = Bij de som zijn de waarden " rapportagegrens" vermenigvuldigd met factor 0,7 zoals beschreven in AS3000, bijlage 3 .

3 = Deze bepaling is uitbesteed aan derden. Dit laboratorium is voor deze bepaling geaccrediteerd.

4 = In het chromatogram is MTBE en/of ETBE boven de rapportagegrens waargenomen.

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Opdrachtcode	:	20103770	Labcomcode:	:	1302076BCL
Rapportnummer	:	P130200564 (v1)	Datum opdracht	:	14-02-2013
Opdracht omschr.	:	Citychlor Utrecht 3	Startdatum	:	14-02-2013
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Opdracht omschr.	:	Citychlor Utrecht 3	Startdatum	:	14-02-2013
Bemonsterd door	:	Opdrachtgever	Datum rapportage	:	22-02-2013

Monstergegevens:

Nr.	Labnr.		Monsteromschrijving	Monstersoort	Datum bemonstering
5	M130201558	:	3770 126~StroomA pbNF4 2002 (7-8) gw t=6	Grondwater	14-02-2013
6	M130201559	:	3770_127~53 (38 m-mv) gw t=6	Grondwater	14-02-2013
7	M130201560	:	3770_129~StroomA pb53_502 (29-30) gw t=6	Grondwater	14-02-2013

Resultaten:

	Parameter	Intern ref. nr.	Eenheid	5	6	7
	Mvb. SIKB AS3000	MVB-WATER-01		+	+	+
	Vluchtige organische halogeen verbindingen					
S	Dichloormethaan	GCMS-VLUCHTIG-01	µg/l	<0,20	<0,20	<0,20
S	1,1-Dichloorethaan	GCMS-VLUCHTIG-01	µg/l	<0,50	<0,50	<0,50
S	1,2-Dichloorethaan	GCMS-VLUCHTIG-01	µg/l	<0,10	<0,10	<0,10
S	Trans-1,2-Dichlooretheen	GCMS-VLUCHTIG-01	µg/l	<0,10	0,66	<0,10
S	Cis-1,2-Dichlooretheen	GCMS-VLUCHTIG-01	µg/l	0,32	4,5	2,4
S	Trichloormethaan (Chloroform)	GCMS-VLUCHTIG-01	µg/l	<0,10	<0,10	<0,10
S	Tetrachloormethaan (Tetra)	GCMS-VLUCHTIG-01	µg/l	<0,10	<0,10	<0,10
S	1,1,1-Trichloorethaan	GCMS-VLUCHTIG-01	µg/l	<0,10	<0,10	<0,10
S	1,1,2-Trichloorethaan	GCMS-VLUCHTIG-01	µg/l	<0,10	<0,10	<0,10
S	Trichlooretheen (Tri)	GCMS-VLUCHTIG-01	µg/l	<0,10	<0,10	<0,10
S	Tetrachlooretheen (Per)	GCMS-VLUCHTIG-01	µg/l	<0,10	<0,10	<0,10
S	Dichl.ethenen (som cis+trans)	GCMS-VLUCHTIG-01	µg/l	0,39 (2)	5,1	2,4 (2)
S	Vlucht.chl.koolw.stoffen (som)	GCMS-VLUCHTIG-01	µg/l	1,4 (2)	6,1 (2)	3,4 (2)
S	Vinylchloride	GCMS-VLUCHTIG-01	µg/l	50	2700	25
S	Nitraat (als NO3)	DIV-AA2-01	mg/l		<0,50	
S	Sulfaat	DIV-AA2-01	mg/l		64	
	Ethaan		µg/l	<1	<1	<1
	Etheen		µg/l	2	12	4
	Methaan		µg/l	65	120	19
	Tot. org. koolstof (als NPOC)		mg TOC/l		4,3 (3)	

S = door RvA geaccrediteerd conform SIKB AS3000.

Opmerkingen:

1 = De rapportagegrens is verhoogd, omdat bij de analyse een verdunningsstap noodzakelijk was. Dit als gevolg van het in verhoogde concentratie voorkomen van n of meerdere componenten.

2 = Bij de som zijn de waarden " rapportagegrens" vermenigvuldigd met factor 0,7 zoals beschreven in AS3000, bijlage 3 .

3 = Deze bepaling is uitbesteed aan derden. Dit laboratorium is voor deze bepaling geaccrediteerd.

4 = In het chromatogram is MTBE en/of ETBE boven de rapportagegrens waargenomen.

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Opdrachtcode	:	20103770	Labcomcode:	:	1302076BCL
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Opdracht omschr.	:	Citychlor Utrecht 3	Startdatum	:	14-02-2013
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Opdrachtgegevens:

Opdrachtcode	:	20103770	Labcomcode:	:	1302089BCL
Rapportnummer	:	P130200720 (v1)	Datum opdracht	:	19-2-2013
Opdracht omschr.	:	Citychlor Utrecht 4	Startdatum	:	19-2-2013
Bemonsterd door	:	Opdrachtgever	Datum rapportage	:	21-2-2013

Monstergegevens:

Nr.	Labnr.		Monsteromschrijving	Monstersoort	Datum bemonstering
1	M130201983	:	3770 125~StroomO pbNF4 NF3 (5-8) gw t=6	Grondwater	19-2-2013

Resultaten:

	Parameter	Intern ref. nr.	Eenheid	1	
	Mvb. SIKB AS3000	MVB-WATER-01		+	
	Vluchtige organische halogeen verbindingen				
S	Dichloormethaan	GCMS-VLUCHTIG-01	µg/l	<0,20	
S	1,1-Dichloorethaan	GCMS-VLUCHTIG-01	µg/l	<0,20	
S	1,2-Dichloorethaan	GCMS-VLUCHTIG-01	µg/l	<0,10	
S	Trans-1,2-Dichlooretheen	GCMS-VLUCHTIG-01	µg/l	0,31	
S	Cis-1,2-Dichlooretheen	GCMS-VLUCHTIG-01	µg/l	46	
S	Trichloormethaan (Chloroform)	GCMS-VLUCHTIG-01	µg/l	<0,10	
S	Tetrachloormethaan (Tetra)	GCMS-VLUCHTIG-01	µg/l	<0,10	
S	1,1,1-Trichloorethaan	GCMS-VLUCHTIG-01	µg/l	<0,10	
S	1,1,2-Trichloorethaan	GCMS-VLUCHTIG-01	µg/l	<0,10	
S	Trichlooretheen (Tri)	GCMS-VLUCHTIG-01	µg/l	0,72	
S	Tetrachlooretheen (Per)	GCMS-VLUCHTIG-01	µg/l	0,16	
S	Dichl.ethenen (som cis+trans)	GCMS-VLUCHTIG-01	µg/l	46	
S	Vlucht.chl.koolw.stoffen (som)	GCMS-VLUCHTIG-01	µg/l	47	(1,2)
S	Vinylchloride	GCMS-VLUCHTIG-01	µg/l	46	(1)
	Ethaan		µg/l	1	
	Etheen		µg/l	3	
	Methaan		µg/l	120	

S = door RvA geaccrediteerd conform SIKB AS3000.

Opmerkingen:

1 = In het chromatogram is MTBE en/of ETBE boven de rapportagegrens waargenomen.

2 = Bij de som zijn de waarden " rapportagegrens" vermenigvuldigd met factor 0,7 zoals beschreven in AS3000, bijlage 3 .

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Document description

Title: Biodegradation capacity in Utrecht - using innovative next level technologies

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Translations:

Summary: In this study the biodegradation capacity was determined by conducting molecular analysis on groundwater, compound specific stable isotope analysis, lab microcosms, analysis on BACTRAPS and performing molecular analysis on MicroTraps.

Investing in Opportunities

This project has received European Regional Development Funding through INTERREG IV B.

INTERREG IVB