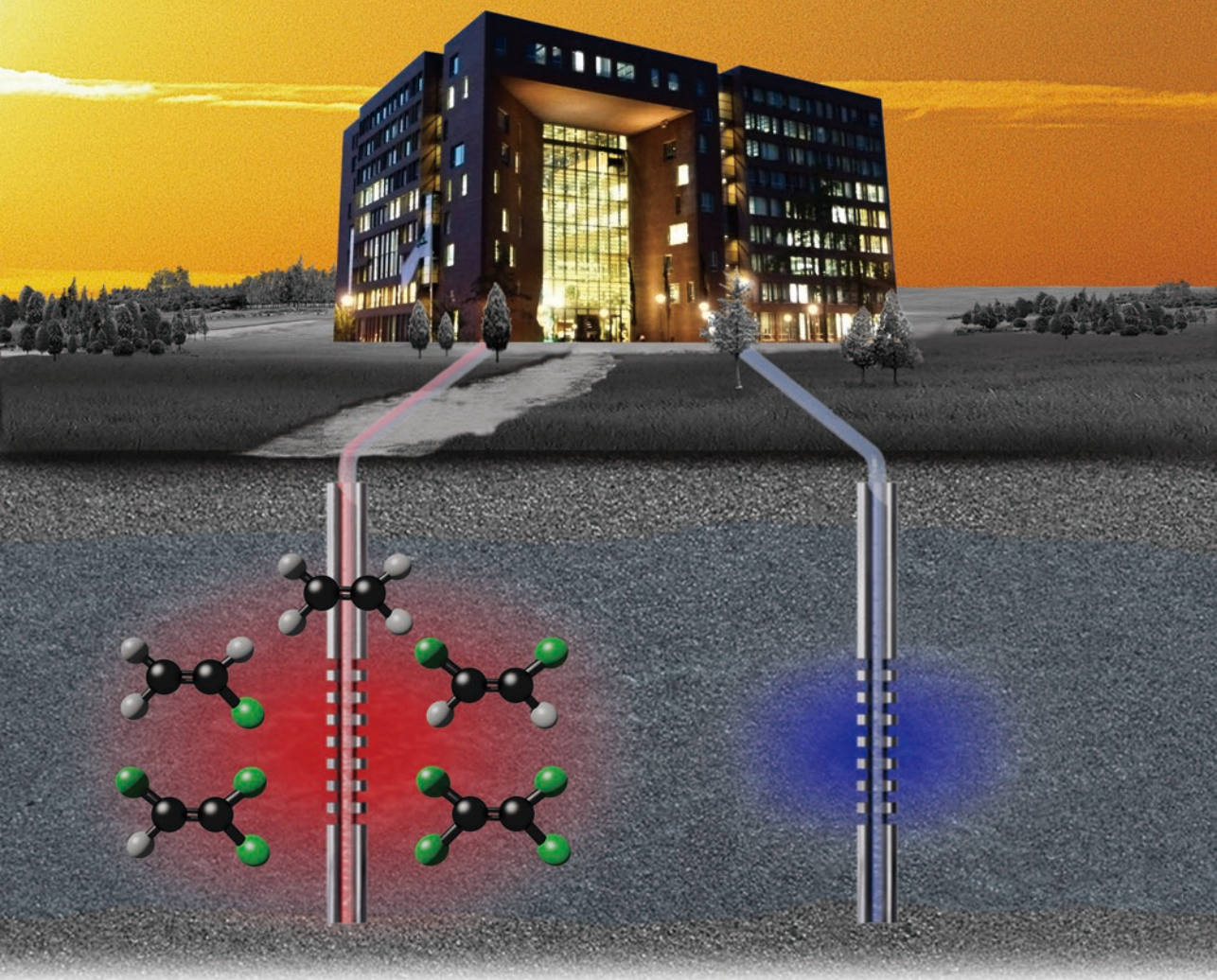# Bioremediation of Chlorinated Ethenes in Aquifer Thermal Energy Storage

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Bioremediation of Chlorinated Ethenes

in

Aquifer Thermal Energy Storage

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谨以此书献给我的父母

To my parents

Contents

Chapter I

General Introduction:

Challenges of ATES-ENA Concept in

Sustainable Urban Development

1.1 Background

Since the 1930s, chlorinated solvents, known as chlorinated volatile organic compounds (CVOCs), have been widely used in a variety of industrial sectors such as metal degreasing, textile dry-cleaning, chemical manufacturing, and the production of pharmaceuticals [1-3]. Due to leakage, improper disposal and accidents, CVOCs entered the environment in excess [4]. These compounds are by far the most prevalent organic contaminants in subsurface and groundwater all over the world because of the high amount of production and use during the past few decades [5]. For example, approximately 400,000 sites in the United States were confronted with CVOCs [2, 6]; more than 10,000 sites contaminated with CVOCs were found in the Netherlands especially in the urban areas [7]; about 260,000 tons of CVOCs were used in Europe [7]. Germany, France, United Kingdom, Sweden, Italy, Czech and Slovak Republic, and many other European and Asian countries, such as China and Japan also encounter contamination of CVOCs [5, 8-14]. The main representatives of chlorinated solvents are chlorinated ethenes, chlorinated methanes and chlorinated ethanes [3, 15, 16]. Common CVOCs that are often found in the subsurface are listed in Table 1.1.

Because of the high volatility, high specific density and low viscosity, and high solubility (up to 1000 mg/L or more), CVOCs are considered as highly mobile groundwater contaminants [4, 17]. They can spread in the subsurface both vertically and horizontally with groundwater movements. When existing as dense non aqueous-phase liquid (DNAPL), the characterization and remediation of CVOCs become even more challenging, due to the fact that DNAPL as contamination source continuously release dissolved compounds to the subsurface water systems [3]. The length of a DNAPL plume can range from 300 to 1500 m, which is generally 2 to 5 times longer than plumes of BTEX or polycyclic aromatic hydrocarbons (PAH) [1, 18, 19]. Besides their predominance in groundwater contamination, toxicity of CVOCs is another important issue. Some of them are known or suspected carcinogens, resulting in their presence in the groundwater environment being of even greater concern [12, 20-22]. The public health problem will be the most severe for countries where groundwater serves as a major drinking water resource. In the European Union (EU), 60% of the drinking water comes from groundwater. In the Netherlands, almost two third of the total

amount of drinking water comes from fresh groundwater ever since the 20th century [23]. In some countries, such as Denmark, groundwater is even the only source of drinking water [24].

Table 1.1 List of common chlorinated solvents.

Compounds	Abbreviation	Formula
Chlorinated Ethenes		
Perchloroethene or Tetrachloroethene	PCE	C_2Cl_4
Trichloroethene	TCE	C_2HCl_3
cis-1,2-Dichloroethene	cis-DCE	CH_2Cl_2
trans-1,2-Dichloroethene	trans-DCE	CH_2Cl_2
1,1-Dichloroethene	1,1,-DCE	CH_2Cl_2
Vinyl chloride	VC	$CHCl_3$
Chlorinated Methanes		
Carbon tetrachloride	CT	CCl_4
Chloroform	CF	$CHCl_3$
Methylene chloride dichloromethane	DCM	CH_2Cl_2
Chloromethane	CM	CH_3Cl
Chlorinated Ethanes		
1,1,1,2-Tetrachloroethane	PCA	C_2HCl_4
1,1,1-Trichloroethane	1,1,1-TCA	$C_2H_3Cl_3$
1,1,2-Trichloroethane	1,1,2-TCA	$C_2H_3Cl_3$
1,1-Dichloroethane	1,1-DCA	$C_2H_4Cl_2$
1,2-Dichloroethane	1,2-DCA	$C_2H_4Cl_2$
Chloroethane	CA	C_2H_5Cl

Physical technologies, like pump-and-treat, soil vapor extraction, soil excavation and dynamic underground stripping, were the first technologies that have been developed for CVOCs remediation since the 1970s, the time that CVOCs were recognized as highly accumulating pollutants in the subsurface [25]. However, these conventional technologies were neither very successful nor cost-effective. Subsequently, new remediation technologies based on biological and chemical approaches were developed, such as enhanced bioremediation which includes biostimulation and bioaugmentation, in situ chemical oxidation (ISCO), combination of ISCO and in situ bioremediation, permeable reactive barriers and walls [1-4, 10, 26, 27]. Among these technologies, bioremediation became more and more popular, especially after both co-metabolic and metabolic biodegradation pathways of CVOCs were elucidated [28]. In situ bioremediation has since then been discussed as a cost-effective and promising technique and used as alternative or additional remediation

approach for treating CVOCs contamination [29]. This technology proved often successful in practical remediation of CVOCs in the subsurface [1, 30-34]. However, in situ bioremediation is generally rather slow, sometimes with time frames estimated up to several decades, depending on the size and geochemistry of the site [3, 35]. This drawback has led and is leading to negative effects on urban and industrial spatial planning and thus often hampers the redevelopment of the contaminated sites. Integration of remediation into site redevelopment and combination of different technologies is becoming the direction and trend for dealing with chlorinated solvent groundwater pollution in many countries [36-38].

Nowadays, the demands for sustainable energy technology are increasing due to climate change and state and city plans to reduce CO_2 emissions from fossil energy resources. Aquifer thermal energy storage (ATES) is a growing major type of geothermal energy exploited in countries with suitable geology and aquifer systems. The ATES system stores cold or heat when available and retrieves it for use when needed, and is conceived and applied both as energy saving system and sustainable energy technology in urban areas [39-42]. These urban areas meanwhile often encounter groundwater contaminants, especially chlorinated solvents. Due to the unclear effects of ATES on the behavior and distribution of contaminants [43], as well as the concern on the quality of groundwater, ATES is currently not allowed to be installed in contaminated areas. However, considering the rapid development of ATES [44-46] and long duration for contaminated groundwater remediation, pressures on the renewed use of sites and underlying subsurface are also rising. Therefore, a combination concept has been put on the agenda to combine ATES and enhanced natural attenuation (ATES-ENA) based bioremediation [47-49]. High groundwater pumping volumes and rates, combined with elevated subsurface temperature around the warm well of ATES may create a good match to complete the remediation by natural attenuation in the lifetime of ATES systems, generally 30 years. This positive perspective was therefore chosen as the theme of research for this thesis, with a focus on chlorinated ethenes. Below we sketch the state of the art in natural attenuation and enhanced in situ bioremediation of chlorinated ethenes and ATES technology, identify potentials and knowledge gaps for applying this combined approach, and thus define the research topics to be addressed in this study.

1.1.1 Chlorinated ethenes

Chlorinated ethenes, as the most prevalent chlorinated solvents globally, are hydrocarbons with two carbon atoms and with chlorine substituted for some, or all of the hydrogen atoms. Their major chemical and physical properties are given in Table 1.2. Chlorinated ethenes that are most often detected in groundwater and subsurface comprise PCE, TCE, cis-DCE and VC. The parent chlorinated ethenes, PCE and TCE, were commonly used for dry cleaning operations and degreasing applications since the 1930s because of their non-flammable and stable chemical properties [4]. After entering the environment, PCE and TCE can be naturally biodegraded to less chlorinated by-product, such as cis-DCE and VC, under anaerobic condition. Similar to other chlorinated solvents, chlorinated ethenes can be present as dissolved phase in groundwater or soil moisture, or as pure phase (DNAPL) if they were released from industrial tanks and accumulated somewhere in the subsurface [3], or as sorbed phase in soil organic matter.

Table 1.2 Chemical and physical properties of chlorinated ethenes (based on [4, 16, 50, 51]).

Chemical	Molar mass (g/mol)	Solubility (mg/L)	K_{oc}	Log(K_{ow})	Intervention value (μg/L, NL)	Target value (μg/L, NL)
PCE	165.83	150	210-238	3.40	40	0.01
TCE	131.39	1,100	100	2.29	500	24
cis-DCE	96.95	3,500	36-49	1.86	20	0.01
VC	62.50	2,700	56	0.6	5	0.01

The parent compounds have higher sorption affinity compared to the two daughter products, as the organic carbon partition coefficient (K_{oc}) of chlorinated ethenes becomes lower and solubility becomes higher when the number of chlorine atoms becomes less (Table 1.2). In general, the solubility of chlorinated ethenes is considered relatively moderate to high in water, and the maximum soluble dissolved phase concentrations are far above the groundwater standards [4]. The concentration levels of chlorinated ethenes detected in groundwater are often much higher than the intervention values, which are 40, 500, 20 and 5 μg/L for PCE, TCE, cis-DCE and VC respectively in the Netherlands [51, 52]. The target values in groundwater for chlorinated ethenes are set even much lower (Table 1.2), due to their toxicity to human beings. All chlorinated ethenes are toxic, but VC in particular, and

this compound is classified in group 1 human carcinogen by the International Agency for Research on Cancer (IARC) [12]. In the past, the use of PCE and TCE for dry cleaning usually required high purity of these products. Therefore, a single spill of DNAPL generally did lead to a quality reduction of large volumes of groundwater, due to the strict intervention and target values. As a result of increasing evidence on toxicity, more strict environmental regulation, and improvement of the dry-cleaning process, decline in production and application of PCE and TCE began in the 1970s [15, 53]. In Australia, manufacturing of PCE was stopped in 1991 [54]. However, large amount of PCE, TCE and their daughter product are still present as resistant contaminants in the groundwater and subsurface in virtually all countries. As a consequence, different remediation technologies of chlorinated ethenes were required and have been developed. Among these remediation technologies, bioremediation became most popular due to its cost-effectiveness and has been explored in the past three decades [55].

1.1.2   History of chlorinated ethenes remediation

Starting in the 1970s, a wide variety of technologies have been developed for remediating contamination with chlorinated ethenes. These technologies refer to both in situ and ex situ treatments. Pump and treat was the first-developed ex situ and popular approach for physical remediation. It was widely applied in approximately 90% of the remediation projects until the late 1980s in the USA [56]. However, from the 1990s onward in situ remediation technologies for chlorinated ethenes became more common, because of the evident benefits of being relatively low-cost, highly compatible with other technologies and capable of addressing deep contamination [56, 57]. Although air sparging as physical treatment was most frequently selected in most of the early cases of in situ remediation, bioremediation was also chosen in many chlorinated ethene contaminated sites, becoming a mature technology from 2002 onwards [56, 58]. As for in situ bioremediation, both co-metabolic and metabolic biodegradation pathways were investigated in depth and the technology became the standard for especially dissolved chlorinated ethene groundwater plume [4, 59]. Compared to in situ physical technologies, attractive advantages of most in situ bioremediation technologies include low cost, possibility for complete destruction of contaminants, large scale applicability and highly compatible with other technologies, such as chemical treatment [56]. Enhanced reductive dechlorination (ERD) is currently by far the most common form of in

situ bioremediation. It involves and takes advantages of specific bacteria to dechlorinate CVOCs under anaerobic conditions by using natural or added electron donors. Under suitable conditions, the reductive dechlorination process allows complete conversion from PCE to non-toxic compound ethene via the pathway shown in Figure 1.1.

Reductive dechlorination was first studied for PCE and TCE biodegradation under methanogenic conditions [60] and has become the most dominant degradation mechanism for the CVOCs bio-removal [61]. Dechlorinating bacteria can use organic acids, such as lactate and acetate, and H_2 as electron donor to reduce PCE (that functions as an electron acceptor) to ethene. Although several dechlorinating species, such as *Desulfitobacterium hafniense*, *Dehalobacter restrictus*, *Clostridium bifermentans*, are able to degrade PCE into less chlorinated ethenes like TCE and cis-DCE, only the group of *Dehalococcoides mccartyi* can further reduce cis-DCE or VC to ethene [62-68]. Although *Dehalococcoides mccartyi* was discovered to be widely present in contaminated subsurface, strict requirements on the subsurface redox condition often lead to low biomass concentrations and slow growth and subsequent incomplete biodegradation of CVOCs and accumulation of cis-DCE and VC in the subsurface [69-71]. Therefore, bioaugmentation by supplying active biomass and biostimulation with addition of electron donor are commonly needed when implementing ERD. This means that characterization and monitoring of the biogeochemical conditions, such as redox condition, biomass concentration and electron donor substrate concentration is of high importance, as all of these conditions can possibly be a limiting factor of reductive dechlorination. Determination of these potential limiting factors prior to implementing bioremediation is essential to design a cost-effective remediation approach. This is also the case when in situ bioremediation of aquifers is considered in combination with other technologies, such as aquifer thermal energy storage which is introduced in the next section. Therefore, the determination of limiting factors is a specific topic of research addressed in this thesis in Chapter 2.
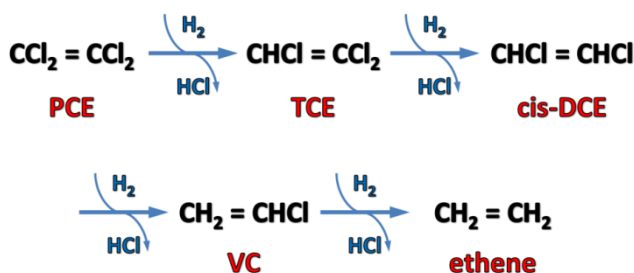
Figure 1.1 Reductive dechlorination pathway from PCE to ethene (modified from [4, 60]).

Despite the attractive benefits of in situ bioremediation, its drawbacks must not be overlooked. Besides the mentioned possible accumulation of toxic by-products, another essential commonly recognized limitation is the long duration of in situ bioremediation. Although, generally faster than pump and treat, ERD is still rather slow and meeting remediation targets of extensive contaminant plumes can take several decades. To overcome or reduce the negative impact of such limitation, an alternative could be to integrate the bioremediation by enhanced natural attenuation or engineered natural attenuation (ENA) project into a broader long term masterplan, such as redevelopment of the contaminated site, and make use of the time that is needed for remediation. In such situations, a combination with groundwater thermal energy storage becomes interesting, and the aspect of a decades' long application of bioremediation in such a combined approach is presented in Chapter 6.

1.1.3 Aquifer thermal energy storage (ATES)

To prevent dangerous anthropogenic influences in climate system and human induced climate change, the EU has signed up to reduce greenhouse gas (GHG) emissions by 20% in 2020 compared to the levels of 1990 under the Kyoto Protocol [72]. Further in the Copenhagen Conference of Climate Change 2009, the EU again confirmed to achieve this key target by 2020, and proposed to advance the target of 40% and 80%-95% reduction by 2030 and 2050 respectively [73]. In support of the reduction of GHG emissions, more use of sustainable energy and improving energy efficiency are two other main targets. For the Dutch government, the sustainability goals for 2020, compared to 1990, include 30% less GHG emissions, 20% energy savings, and 20% energy should be produced by sustainable technologies. For this matter, ATES, as a relatively new and sustainable technology, can

make a significant contribution to the goals for sustainable energy production. First applications of ATES occurred in 1990 in the Netherlands with five systems only. The number of ATES systems has increased to 2,740 in 2012 with an avoided fossil energy use of about 1103 MW [74]. In the Dutch policy to meet the EU targets, the number of ATES systems is planned to rise to about 20,000 in 2020 [44], possibly resulting in a reduction of CO_2 emission of approximately 11% in the Netherlands [75]. Besides the Netherlands, other countries, including Belgium, Germany, Turkey, Sweden, and the USA are also showing progressive development in application and increasing numbers of ATES [46, 76-80].

When applied as an open system, ATES mainly consists of a warm well and a cold well for groundwater extraction or injection, used for storage of heat and cold energy, respectively. The often applied doublet ATES systems operate in a seasonal mode, meaning the direction of groundwater flow in the system will reverse according to the season (Figure 1.2). During the summer, the groundwater is extracted from the cold well in the aquifer layer, receives heat from the building, is then injected in the warm well and stored there at a relatively high temperature. This process reverses and the groundwater flow direction is from warm well to cold well during the winter. Then warm groundwater and its heat energy is extracted and transferred to the building [39, 42]. In most times, the energy (either cold or heat) transfer form water to building or vice versa involves a heat pump for effective energy extraction, and depends on the temperatures of groundwater and building. The ATES system is typically operated with groundwater flow rate of hundreds cubic meter per hour within the system, and with temperatures of 6-7 ˚C and 20-25 ˚C for cold well and warm well, respectively [81-83]. Commonly, a distance of 50-200 m between cold and warm well is applied in most ATES systems, while these wells are generally 20-200 m deep in the subsurface [43, 84], although some high temperature systems can be several hundred meter deep [85].
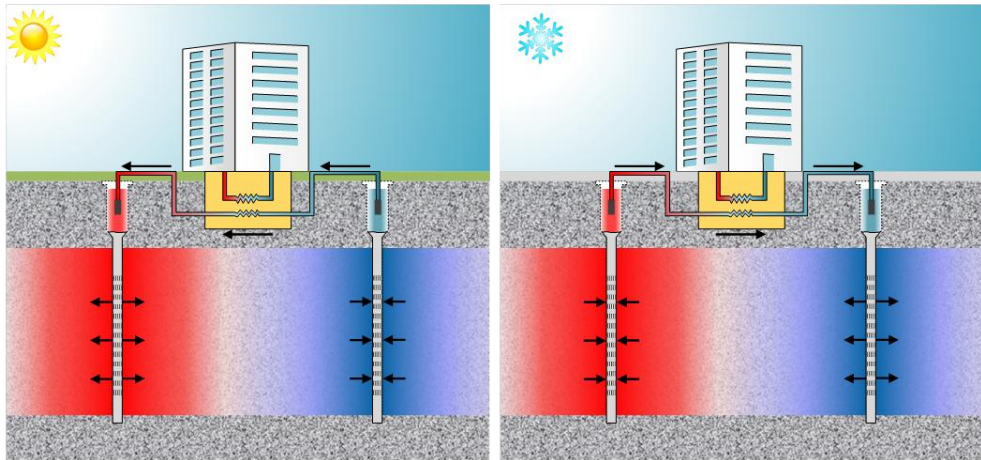
Figure 1.2 The principle of the operation of an ATES system.

1.2 Combination of enhanced bioremediation of CVOCs and ATES

1.2.1   Interference between CVOCs contamination and ATES

Due to the rapid development of ATES in the Netherlands and other urbanized areas in the world, the subsurface becomes increasingly intensively used. The CVOCs contaminations and suitable ATES aquifers are often found at a similar depth in the subsurface, especially in urbanized areas. Although regulations have been made to prevent application of ATES close to contaminated sites and their impacted subsurface, the risk of interference between groundwater contamination and ATES is rising, because of the increase in sustainable energy demand and subsequent growing numbers of ATES and slow progress of the in situ bioremediation projects. Moreover, the operation of ATES involves very large volumes of groundwater replacement. For example, in total 261 million m^3 groundwater was transported by the ATES systems in the Netherlands in 2013 [86]. Therefore, a major concern is the risk of groundwater quality deterioration through spreading of contaminants by ATES systems extracting water from CVOCs contaminated areas [43]. This encounter can probably enhance the dissolution of DNAPL in the subsurface by the increased mass transfer through high groundwater flow, and reallocate these dissolved contaminants in the subsurface in case no adequate measures are taken [87]. Such an occurrence of contaminant spreading will be more likely when background natural groundwater velocity is high, because part of the introduced

CVOCs plume might escape from the capture zone of ATES wells and thus not contained [43].

1.2.2 The ATES-ENA concept

In contrast to the concerns of spreading CVOCs contaminant, the challenge of an ATES-ENA concept has risen due to the need for sustainability of both energy and groundwater management. In 2010, this concept was firstly introduced in the Netherlands through the project "More with Subsurface Energy" (Meer met Bodemenergie, MMB) [48, 49], that also initiated this thesis project. Main practical and scientific rationales behind the combined concept include:

- bioremediation may be enhanced due to the higher temperature around the warm ATES well;

- environmental condition in the ATES subsurface can be more homogenized by seasonal extraction and injection of large volumes of groundwater;

- within its lifetime of 30 years, ATES can provide sustainable energy in contaminated urban area meanwhile in situ bioremediation is proceeding and potentially completed;

- ATES might be adapted to also function as a biostimulation tool, for example, for substrates injection and bioaugmentation.

These rationales make the ATES-ENA concept attractive and research studies and practical demonstration and valorization projects have been developed to further explore the possibilities [47-49, 83, 88, 89]. The relevant important processes and possible bottle necks in the ATES-ENA system that are explored in this thesis are illustrated in Figure 1.3. They include: temperature changes and CVOCs biodegradation in warm and cold well; potential biological and chemical clogging due to massive bacterial growth and iron precipitates, movement and redistribution of microorganisms and CVOCs, and biostimulation and bioaugmentation based on ATES.
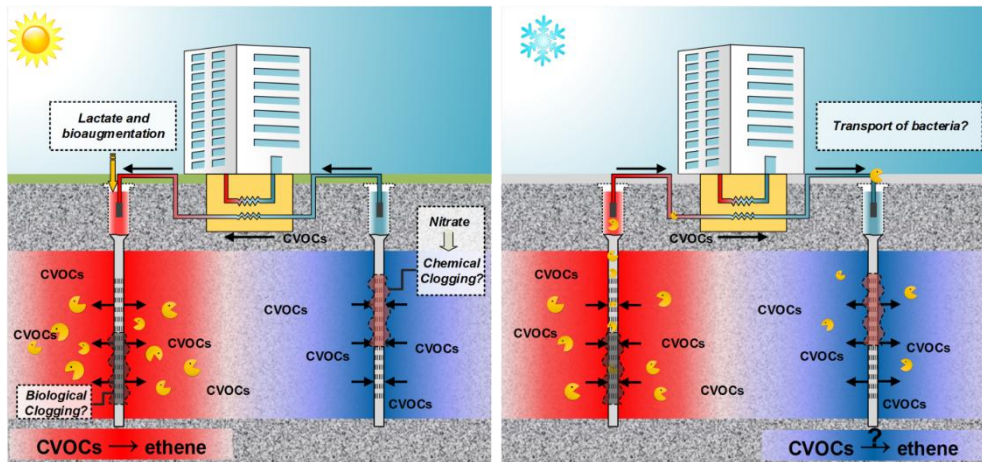
Figure 1.3 Illustration of relevant processes in the ATES-ENA system.

Groundwater temperature close to the ATES warm well can be more than 10 to 15 ˚C higher than the natural groundwater temperature in moderate climate regimes as in the Netherlands, which is about 10 ˚C. For biological conversions, the biodegradation rates increase by a factor of 1.5 to 2.5 (if the rate is limited by enzyme activity), when temperature is increased by 10 ˚C [90]. In this way, progress of CVOCs bioremediation may be enhanced by more than two times in the warm zone, during the half year operation of ATES. In the scientific literature, no reports are available on the temperature dependency of such biological dechlorination processes under in situ ATES conditions. To test these assumptions, well defined systems studies are needed and the results of such a study are presented in Chapter 3 of this thesis. Despite the expected inhibition of biodegradation around the cold well, dissolved CVOCs plumes can be transported to the warm zone where biodegradation may proceed prosperously. Furthermore, the elevation of temperature in groundwater may increase the amount of available organic carbon which can serve as electron donor for CVOCs biodegradation [91]. Besides temperature, the homogenizing consequence of ATES may be beneficial for remediation, by the continuous transfer of huge amounts of groundwater. This may result in mixing, normally prevented by subsurface heterogeneity, and leading to a better delivery of electron donor when added. Additionally, the redox potential in the subsurface is an important condition to be tuned to the level suitable for reductive dechlorination ([92], Chapter 2). For this, an adequate site subsurface

characterization on geochemistry is needed. A larger active bioremediation area might also be achieved due to the re-distribution of microorganisms in the subsurface mediated by the extensive groundwater movements.

In addition to the beneficial influence of ATES on biodegradation of CVOCs, research into the possible negative effects of the biological processes on the functioning of the ATES systems is also of importance. Biological and biogeochemical clogging by biomass and/or colloidal iron-oxides is the most important issue related to the possible limitation of ATES-ENA, as clogging is frequently observed in conventional groundwater extraction wells [93-98]. Therefore, prior to practical application of ATES-ENA, potential biological and biogeochemical clogging is one question that must be considered, before designing and implementing systems in practice. Also gaining a better understanding of the processes as a basis to develop remediation measures when such clogging occurs during operation is needed. For this reason microbial behavior and mobility, and chemical and biological clogging are research items addressed in this thesis in Chapters 4 and 5.

1.3 Research objectives and questions

The ATES-ENA concept appears attractive as such integration between groundwater treatment technology and sustainable subsurface energy technology provides a promising solution for redevelopment of urban areas in terms of improving the local environmental quality as well as achieving sustainable energy supply. Moreover, it will also reduce the negative interferences between groundwater contaminations and ATES systems. However, the concept at this moment is in an early stage and still relatively far from mature applications, also suffering from a lack of understanding of basic biogeochemical processes in aquifer systems under conditions of combining ATES and bioremediation. Therefore, efforts are required for the investigation of these processes as a base for further assessing the feasibility of the combination in practice. The research regarding to this matter should then focus on finding the essential process factors involved, revealing possible drawbacks, and providing a better understanding to design alternative options on better operation of the combined system. This thesis study aims to generate a more fundamental understanding of these processes and made use of laboratory experiments, modeling and conducted a small technological bench

scale pilot test. This PhD research dedicates to give better insight on the ATES-ENA combination with focus on the mutual effects between bioremediation and ATES by addressing the following research questions (RQ):

1) Which factor is limiting the CVOCs reductive dechlorination? (Chapter 2)

2) What are the effects of temperature variation and groundwater transport due to seasonal operation of ATES on CVOCs biodegradation? (Chapter 3)

3) What are the microbial responses of CVOCs reductive dechlorination upon changes of redox condition in ATES system? (Chapter 4)

4) What are potential clogging issues in the combination of ATES and enhanced bioremediation? (Chapter 5)

5) What are the anticipated outcomes when applying ATES as engineering tool of biostimulation in a CVOCs contaminated aquifer? (Chapter 6)

In addition, the feasibility of the combined approach is evaluated and possible recommendations as well as future perspectives on the application are given (Chapter 7).

1.4 Outline of this thesis

This thesis consists of seven chapters addressing important issues in ATES-ENA system, based on Figure 1.3 and research questions. An overview of the structure of this thesis and the flow of research focus in different chapters to meet the objectives and answer research questions is provided in Figure 1.4.
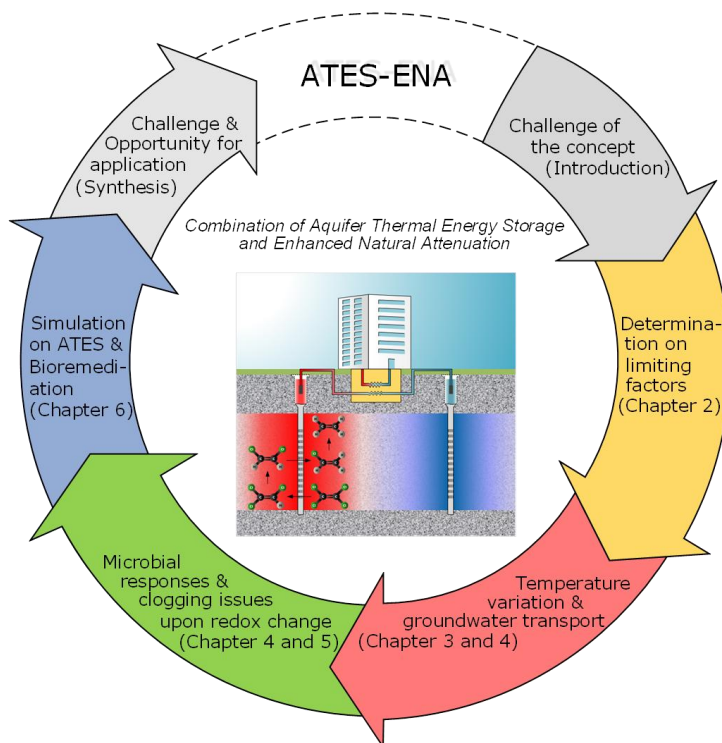
Figure 1.4 Structure of this thesis and research focus.

Chapter 2 presents the results of a step-wise batch study which consists of two separated experiments: redox conditioning by ascorbic acid and lactate, followed by microbial reductive dechlorination of PCE. Different scenarios are simulated and compared, namely: natural condition, with addition of electron donor, by applying bioaugmentation, and both electron donor and bioaugmentation application. The direct relationship between redox potential and PCE reductive dechlorination is assessed by the redox condition experiment. With another stepwise reductive dechlorination experiment, the need of site condition investigation and proper selection of appropriate biostimulation approaches, according to the goals of bioremediation projects is supported. In this way, determining the limiting factors for CVOCs bioremediation under ATES conditions (RQ 1) is addressed in this chapter.

Chapter 3 evaluates the effects of periodical changes on temperature and groundwater transport by ATES operation on biodegradation of cis-DCE, using straight forward batch

experiments (RQ 2). Another type of geothermal energy, borehole thermal energy storage (BTES) is also studied, a system where no groundwater is transported but only the temperature changes. Temperature levels of 5 ˚C and 25 ˚C are simulated as typical of the warm and cold wells in both ATES and BTES systems. The performances on cis-DCE biodegradation are discussed and compared among conditions of ATES, BTES, and a natural situation (at 10 ˚C) without temperature change and water transport.

Chapter 4 further investigates the impacts of ATES on the microbial responses of *Dehalococcoides* to evaluate the cis-DCE reductive dechlorination progress under condition when groundwater with high redox state is introduced by ATES operation (RQ 3). The microbial resilience of *Dehalococcoides* is tested by changing the environmental condition twice, i.e. by changing from redox condition suitable for reductive dechlorination to nitrate reducing conditions which are unfavorable for reductive dechlorination. These steps are simulated by adding lactate or nitrate to alter the redox potential in a recirculating column system with a flow rate of 10 mL/min (representing a distance of 1.3 m from ATES well filters). In this model ATES system, the activity, behavior and distribution of *Dehalococcoides* upon redox changes are addressed.

Chapter 5 studies the potential clogging issues in the combination of ATES and enhanced bioremediation (RQ 4). The same recirculating column setup as that in Chapter 4 was operated at two flow rates of 10 and 50 mL/min (representing in situ flow rates at 1.3 and 0.26 m distance to the ATES well filters). The experiments comprise a first phase with enhanced biological growth by lactate addition (to simulate a situation when using lactate to enhance bioremediation with ATES) and a second phase with enhanced iron precipitation by nitrate addition (to simulate a situation when ATES encounters groundwater with relatively high redox state). In a third phase lactate was added again with the aim to reverse the chemical clogging (to simulate potential seasonal recovery with ATES).

Chapter 6 develops and performs a reactive transport model to simulate the use of ATES as a continuous biostimulation tool for ERD with lactate addition on a hypothetical TCE contaminated aquifer that is assumed to be homogeneous. The relation between groundwater transport and biogeochemical processes in the capture zone of an ATES system is explored (RQ 5). In total 15 scenarios are considered in the model, dealing with different parameters

at various levels that include variations in electron donor dosage (3 concentration levels: 3.8, 1.9 and 0.38 mmol/L), temperature (3 pairs: 5/15 ˚C, 10/10 ˚C, 5/25 ˚C), biomass mobility (purely mobile or immobile), and pH limitation on Fe(III) reduction (absence and presence of such an effect). Besides interpretation of the predicted outcomes of the model, limitations of such reactive transport model are as well discussed.

Finally by integrating all findings, **Chapter 7** provides a general discussion and outlook on the feasibility, future perspectives, practical application, and limitations of the combination between enhanced bioremediation and ATES.

Chapter II

Effectiveness of Stimulating

PCE Reductive Dechlorination:

A Step-wise Approach

Effectiveness of stimulating PCE reductive dechlorination: A step-wise approach

Abstract

Reductive dechlorination of tetrachloroethene (PCE) and its daughter products in aquifers is often hampered by Fe(III) reducing conditions. Rigorous treatment to adjust the redox potential and stimulate dechlorination may be costly and potentially have negative effects on other aquifer functions. A step-wise experimental strategy was applied to investigate the effectiveness of various adjustment scenarios. Batch experiments with ascorbic acid (AA) and sodium lactate (SL) showed that 75 µmol electron equivalents per gram dry mass of aquifer material was required to reach a sufficiently low redox potential for the onset of PCE dechlorination. Similar effects of either AA or SL on the measured redox potential suggest electron donors are not specific. However, the relative rates of Fe(III) and sulphate reduction appeared to be specific to the electron donor applied. While redox potential stabilized around -450 mV after titration and sulphate was reduced to zero in both treatments, in the AA treatment a faster production of Fe^{2+} was observed with a final concentration of 0.46 mmol/L compared to only 0.07 mmol/L in the SL treatment. In subsequent batch experiments with aquifer material that was pre-treated with AA or SL, PCE reductive dechlorination occurred within 30 days. Further stimulation tests with extra electron donor or inoculum revealed that adding electron donor can accelerate the initiation of PCE biodegradation. However, bioaugmentation with dechlorinating bacteria is required to achieve complete reductive dechlorination to ethene. The findings from step-wise approaches are relevant for improving the cost-effectiveness of the design and operation of in situ bioremediation at initially unfavorable environmental conditions.

Keywords

2.1 Introduction

Tetrachloroethene (PCE) was introduced as a dry cleaning solvent in the 1930's [4, 99]. PCE together with its daughter products, trichloroethene (TCE), cis-dichloroethene (cis-DCE) and vinyl chloride (VC), which are commonly called chlorinated volatile organic compounds (CVOCs), are widespread groundwater contaminants throughout the world [2, 3, 22, 100]. Currently over 10,000 sites are contaminated with PCE in the Netherlands [7]. Meanwhile it was estimated that still more than 80% of the commercial dry cleaners use PCE in the United States in 2004 [101].

PCE has higher density than water, hence not only can it spread through mobilization of groundwater, but also sink down until trapped above finer grained layers. This can result in a large area of pure product contamination that is difficult to remediate with conventional techniques such as pump and treat [4, 102, 103]. PCE is potentially carcinogenic [104, 105] and difficult to characterize and to remediate. Therefore many studies have been directed at microbiological ways to diminish the threat [10, 106, 107]. Laboratory experiments [60, 108-111] focused on elaborating the dechlorination pathway and bacteria involved in PCE biodegradation at optimal conditions, while pilot or field tests [2, 32, 112-114] focused on stimulating bioremediation in groundwater or aquifer system by injecting sufficient substrate and bacteria.

PCE can be completely reduced to ethene via reductive dechlorination under sulphate reducing and methanogenic conditions [68, 115] when other conditions like temperature, availability of electron donor and nutrients, presence of specific microorganisms are suitable [71, 116-122]. Among these conditions, the redox condition proved to greatly affect the presence and activity of dechlorinating bacteria [4, 123-127], especially of "*Dehalococcoides*", which is the only group of bacteria capable to fully degrade CVOCs to ethene. Reductive dechlorination of CVOCs in aquifers is often hampered by unsuitable Fe(III) reducing conditions. Especially Fe(III) and SO_4^{2-} have been reported as main competitive electron acceptor to CVOCs [68, 128, 129]. Therefore, determining the redox condition is important in evaluating the reductive dechlorination potential of CVOCs in the field. Redox potential as measured electrochemically can be used as an indicator for aquifer redox conditions. Limited research was done so far on the role of redox potential in reductive

dechlorination processes of some chlorinated hydrocarbons, such as hexachloro-1,3-butadiene (HCBD) [130], halogenated methanes [131] and Pentachlorophenol (PCP) [132]. These studies showed that dechlorination can be monitored via the redox potential which can be used as an indicator of dechlorinating performance. However, research focused on assessing the direct relationship between redox potential and PCE reductive dechlorination is to our knowledge absent.

The aim of this study was to closely combine redox potential and PCE reductive dechlorination. Using redox potential as a criterion and indicator for PCE reductive dechlorination, we lowered the redox condition of aquifer material from Utrecht, Netherlands, by pre-treating the material with two different electron donors, ascorbic acid (AA) and sodium lactate (SL) in order to overcome the existing barrier for PCE biodegradation without adding excess electron donor, because excess electron donor may cause bio-chemical clogging in for example groundwater extraction wells or transport pipelines. Considering these possible negative interferences with other aquifer utilizations, and the ultimate view of application in large volumes of contaminated aquifer, we applied a step-wise approach, to estimate the minimum amount of electron donor needed for improving the redox potential of aquifer to initiate PCE reductive dechlorination.

2.2 Materials and methods

In summary, experiments consisted of two parts: 1) redox titrations with electron donor to estimate the minimum amount of electron donor needed to achieve redox potential values theoretically suitable for reductive dechlorination of PCE to occur; 2) PCE reductive dechlorination experiments in different combinations of pre-treatment and subsequent stimulation scenarios (Figure 2.1). More detailed information and codes on the experiment part 2 are given in Table 2.1.
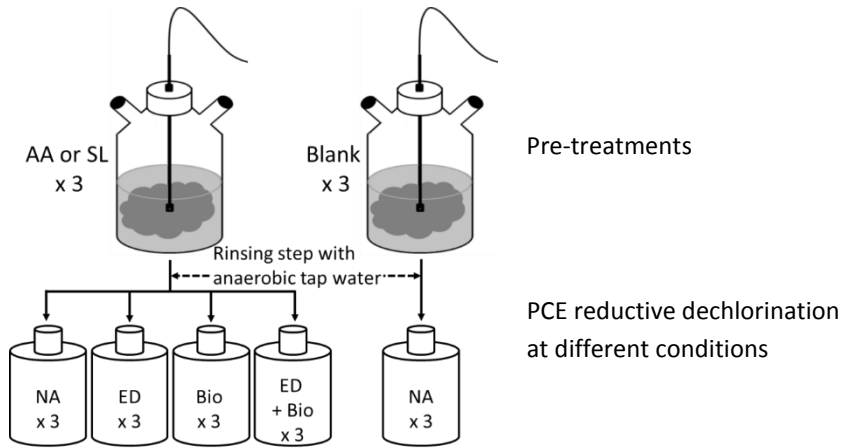
Figure 2.1 Schematic representation of experimental set-up for the reductive dechlorination batch tests after pre-treatment with AA or SL. NA: natural attenuation; ED: stimulation with electron donor; Bio: stimulation with inoculum.

Table 2.1 Overview of experimental set-up for the reductive dechlorination batch tests after pre-treatment. All dechlorination batches had 20 g wet aquifer material and a total volume of added liquid of 50 mL.

Pre-treatments[a]			Reductive dechlorination[a]					
Code	Electron donor	# of replicates	Code	PCE (105 mg/L)	SL (225 g/L)	Inoculum	Medium	# of replicates
AA	Ascorbic acid	3	AA1	2.5 mL	-	-	47.5 mL	2
			AA2	2.5 mL	0.5 mL	-	47 mL	3
			AA3	2.5 mL	-	5 mL	42.5 mL	3
			AA4	2.5 mL + 5 mL[b]	0.5 mL	5 mL	42 mL	3
SL	Sodium lactate	3	SL1	2.5 mL	-	-	47.5 mL	3
			SL2	2.5 mL	0.5 mL	-	47 mL	3
			SL3	2.5 mL	-	5 mL	42.5 mL	3
			SL4	2.5 mL + 5 mL[b]	0.5 mL	5 mL	42 mL	3
B	Blank	3	B1	2.5 mL	-	-	47.5 mL	3

[a] Abiotic controls with 0.1 g/L $HgCl_2$ were performed in both parts of experiment

[b] Second PCE spike on day 8

2.2.1   Basic materials

AA and SL were selected as electron donor, as AA has been widely used as a reactive chemical reductant for Fe(III) in the subsurface [133-135] and lactate as a well-known biological reductant, has also been used in many studies [136-139]. The half reactions of complete oxidation from AA or lactic acid to CO_2, as well as reduction of Fe(III) to Fe(II) and reduction of SO_4^{2-} to HS^- are given below:

$$C_6H_8O_6 + 6H_2O \rightarrow 6CO_2 + 20H^+ + 20e^- \tag{2.1}$$

$$C_3H_6O_3 + 3H_2O \rightarrow 3CO_2 + 12H^+ + 12e^- \tag{2.2}$$

$$Fe(III) + e^- \longrightarrow Fe(II) \tag{2.3}$$

$$SO_4^{2-} + 9H^+ + 8e^- \longrightarrow HS^- + 4H_2O \tag{2.4}$$
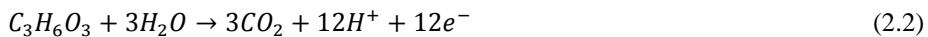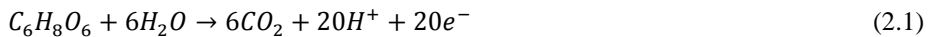
Aquifer material was collected at a site near Utrecht Central Station, the Netherlands. The aquifer mainly consists of fine sands. The CVOCs, especially VC and cis-DCE were found in this aquifer that is currently under Fe(III) reducing conditions. Details on the geochemical properties of the aquifer can be found in the report of [140]. Samples of this aquifer were selected since extensive re-construction of the area is being performed and a large number of groundwater extraction or injection wells are being installed or planned, for groundwater monitoring activities and aquifer thermal energy storage. Besides, this area was a case study area involved in the projects Meer Met Bodemenergie[1] and CityChlor[2]. The aquifer samples used in the experiments reported here came from a depth of 35 to 38 m below surface, where mostly VC and cis-DCE were present and with redox potential ranging from -112 to -151 mV.

All chemical solutions were made with anaerobic deionized water, which was first boiled and then purged with pure N_2 during cooling down to ambient temperature (22 ˚C). Ascorbic acid powder (≥99% purity, BDH Prolabo®) and sodium lactate powder (≥99% purity, Aldrich®) were used to prepare stock solutions of 18.9 g/L AA and 28.1 g/L SL respectively, for the redox titration experiment. These concentrations were aimed to be near-equivalent in electrons from AA and SL, based on complete oxidation to CO_2 (equations 1 and 2).

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[1] http://www.meermetbodemenergie.nl
[2] http://www.citychlor.eu/

Additional AA and SL stocks (200 g/L) were prepared for pre-treatments. PCE (99% anhydrous, Aldrich®) was used to prepare a stock solution of 105 mg/L. Anaerobic tap water was used as the bulk liquid in the titration and conditioning experiments, following the same procedure as with the deionised water. All biological reductive dechlorination batches received anaerobic medium containing per liter: 1.09 g Na_2HPO_4; 0.53 g KH_2PO_4; 1 g NH_4Cl; 48 mg $CaCl_2$ $2H_2O$; 54 mg $MgSO_4$ $7H_2O$; 1.2 mg $FeCl_2$ $4H_2O$; 1.2 mg $CoCl_2$ $6H_2O$; 0.3 mg $MnCl_2$ $4H_2O$; 0.018 mg $CuCl_2$ $2H_2O$; 0.03 mg $ZnCl_2$; 0.03 mg HBO_3; 0.054 mg $(NH_4)_6Mo_7O_{24}$ $4H_2O$; 0.06 mg Na_2SeO_3 $5H_2O$; 0.03 mg $NiCl_2.6H_2O$; 0.6 mg EDTA (tripex II); 0.216 mL 36% HCl; 0.3 mg resazurin (oxygen and pH indicator).

A mixed dechlorinating culture, provided by Bioclear BV (Groningen, NL), was fed with 20 mmol/L lactate and PCE. After 20 days of cultivation, when PCE was completely degraded to ethene, this mixed culture was used as inoculum.

2.2.2 Analytical methods

Dry weight of the aquifer material was determined by the difference of weight before and after 24 hours in a 105 ˚C oven.

Redox potential of the experimental systems was monitored by a Consort multi-channel (C3060) meter and data logger with ProSense (QIS) standard Pt redox electrode with Ag/AgCl as reference electrode (-199 mV vs. standard hydrogen electrode (SHE)) in saturated KCl solution [141]. During redox monitoring, a relatively high noise level in redox readings was observed, especially in the blanks, in both the redox titration experiment and the pre-treatments. Besides, it was observed that gas or liquid sampling momentarily interfered with the redox readings. In this paper, the measured redox potentials are therefore presented as smoothed values from the raw data, using the "smooth (Y, 500, 'loess')" function in MATLAB [3], which uses weighted linear least squares and a 2nd degree polynomial model[4] to fit and smooth the raw data. An example of raw data and smoothed data is provided in the supplementary material (see Figure S2.1 and S2.2).

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[3] http://www.mathworks.nl/
[4] http://www.mathworks.nl/help/curvefit/smooth.html

Dissolved Fe^{2+} concentration was determined with Hach Lange cuvette tests (LCK-320 0.2-6.0 mg/L Fe^{2+}/Fe^{3+}) on a Xion 500 spectrophotometer; SO_4^{2-} concentration was determined on a Dionex ICS 2100 with IonPac AS19 column and a conductivity detector. Analyses of AA and lactate were performed on HPLC with organic acids column (Ion 300) and Refractive Index (RI) detector. Volatile fatty acids (VFAs) were determined on a HewlettPackard (HP) 5890 series GC with packed column (10% Flurad on Supel-coport) and Flame Ionization Detector (FID). The pH was determined with Dosatest pH indicator strips from VWR Prolabo with a range of pH 6.0 to 8.0.

Prior to quantification of PCE, TCE and cis-DCE, the target components were extracted 2 min from the headspace using a 100 μm polydimethylsiloxane (PDMS) coated fiber. Quantification was performed on a Fisions 8000 series GC equipped with CP-Sil8 column (25 m $\times$ 0.53 mm × 5.0 μm) with helium as carrier gas and a FID detector. The temperature program started at 50 ˚C, ramped at 20 ˚C /min to 140 ˚C and held at 140 ˚C for 1.5 minutes. Injection was splitless (250 ˚C). VC and ethene were quantified by direct injection of 100 μL (using a glass syringe) on a HP6890 series GC equipped with a CP PoraBond Q column (25 m × 0.53 mm × 10 μm). Temperature was isothermal at 60 ˚C and detection was done by FID. CO_2 was quantified on a Shimadzu 2010 GC with Thermal Conductivity Detector (TCD) and with helium as carrier gas. Loop injection of 2 mL headspace sample was performed at 120˚C.

2.2.3   Experimental set-up

Redox titrations: 100 g of wet aquifer material and 250 mL anaerobic tap water were added into 500 mL double-side arm bottles (Figure 2.1) inside an anaerobic hood filled with 95% N_2 and 5% H_2. Bottles were closed by Teflon caps with gas tight connected redox electrodes. After removal from the hood, the headspace of the bottles was exchanged in 10 cycles of vacuuming and refilling with 98% N_2 and 2% CO_2 gas by automated headspace exchanger. Bottles were shaken at 150 rpm in a 25 ˚C cabinet. After 3 days of stabilization of the redox potential reading, 1 mL from 18.9 g/L AA stock or 28.1 g/L SL stock solution was added. After day 5, 1.65 mL AA stock or 1 mL SL stock (each time) was also added when the redox potential was stable for at least 2 days (see Figures S2.2 and S2.3). No addition of AA or SL was performed for the blanks. In total 6 additions of AA or SL were carried out in a period

of 19 days. The experiment was stopped on day 21, when no further decrease of redox potential was observed after the last addition. Redox titrations with AA, SL and blank were performed in duplicate.

PCE reductive dechlorination after pre-treatments: 200 g of wet aquifer material and 500 mL anaerobic tap water in 1 L double-side arm bottles were used in the pre-treatment step. The preparation procedures were the same as in the redox titration tests. All bottles were prepared at the same time, but the SL treatment was performed after the AA treatment for reasons of availability of the redox potential electrodes. Blanks were monitored throughout the whole duration of the experiment.

While the redox titration tests were aimed at determining the minimum amount of electron donor needed, an initial-surplus was used in the pre-treatment step to ensure suitable redox conditions at the start of microbial reductive dechlorination. Therefore at the end of pre-treatment, in total 1.5 mL from 200 g/L AA stock and 2.5 mL from 200 g/L SL stock were added respectively. After pre-treatment, bottles were transferred to the anaerobic hood and material from each pre-treatment and blank was mixed in 2 L beakers and rinsed with a total of around 3 L anaerobic tap water to remove remaining electron donors. The three steps of the rinsing procedure: 1) settling the slurry for 30 minutes; 2) pouring out the liquid until water level was close to sediment level; and 3) adding 1 L anaerobic tap water were repeated three times. After rinsing, no AA, lactate and VFA were detected in liquid sample.

The reductive dechlorination experiments were performed in 125 mL serum bottles with 20 g pre-conditioned wet aquifer material and 50 mL liquid. Liquid composition differed according to Table 2.1. Batches were prepared in the anaerobic hood and closed by Viton stoppers. Thereafter, the same headspace exchange procedure as mentioned before was performed for each batch bottle. Afterwards bottles were incubated on a shaker at 150 rpm in a 25 °C incubator.

All batches were spiked with 2.5 mL from 105 mg/L PCE stock leading to approximately 1.6 μmol PCE/batch on day 0. Second spiking with 5 mL from the same PCE stock was performed only in AA4 and SL4 batches. Tests of AA1, AA2, SL1 and SL2 lasted around 87 days, whereas AA3, AA4, SL3 and SL4 lasted around 30 days. Because of memory effects,

variance in effective biodegradation cannot simply be summarized using average values. Therefore, results of individual biological PCE reductive dechlorination test instead of average values are given in this paper. Examples of the different tests will be shown below, the rest of results are provided in the supplementary material.

2.3 Results

2.3.1   Redox titration

The effect of the stepwise addition of AA or SL on redox potential is shown in the bottom of Figure 2.2, where ΔRedox potential was calculated as the difference between the average of the blanks and the average of treatment replicates, using the smoothed redox potential value at the moment just before reductant was added (Figure S2.2, S2.3 and S2.4). At day 8, the redox potential in the AA and SL treatment was respectively 189 and 173 mV lower than that in the blank, after addition of approximately 1.07 mmol/L AA or 1.89 mmol/L SL in total after the second addition. These amounts of electron donor are comparable to approximately 6 mmol electron equivalent (eq) from both AA and SL per batch (80 g dry mass of aquifer material), based on equation 2.1 and 2.2.

Adding more electron donor did not lower the redox potential much further. Although small fluctuations were observed, ΔRedox potential in both treatments gradually stabilized between -150 and -200 mV, with in total approximately 3.82 mmol/L AA and 5.93 mmol/L SL added by the end of titration (Figure 2.2). The average values for the last redox potential reading of AA treatments, SL treatments and blanks were -423, -469 and -270 mV respectively (Figure S2.2, S2.3 and S2.4).
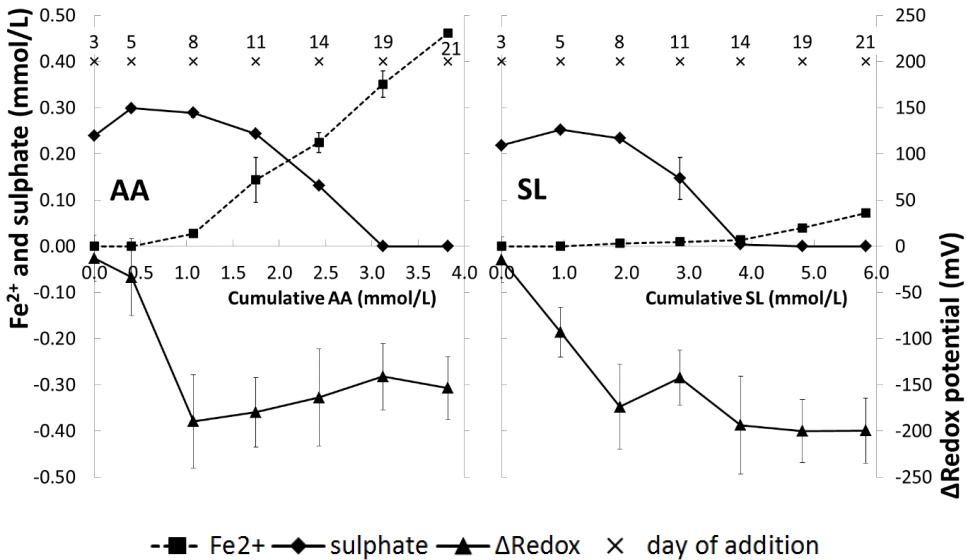
Figure 2.2 Fe^{2+} and sulphate evolution (top) and Redox change (bottom) in experimental system with titration of AA (left) and SL (right) respectively, compared to blank. Error bars represent standard errors or duplicates. In some points, errors bars cannot be seen as they are smaller than symbol size. Numbers with crosses indicate the time of measurement and subsequent electron donor additions.

Besides the redox potential, also Fe^{2+} and SO_4^{2-} were monitored as redox indicators, starting with the first addition of electron donor. In the AA treatment, Fe^{2+} increased from the second addition onwards, at an average rate of 0.03 mmol/L/day, ending up at 0.46 mmol/L (top left of Figure 2.2). The increase of Fe^{2+} was much slower in the SL treatment, starting only when SO_4^{2-} had decreased to zero, reaching a concentration of only 0.07 mmol/L by the end of observations (top right of Figure 2.2). From a starting concentration of 0.23 mmol/L, SO_4^{2-} was reduced to zero in both treatments (top of Figure 2.2). However, the rate of SO_4^{2-} decrease was faster in the SL than in the AA treatment. No Fe^{2+} was detected in the blanks throughout the experiment, but SO_4^{2-} was found to be slowly increasing to an average of 0.33 mmol/L at the end on day 21 (Figure S2.5), most probably due to analytical drift.

At the end of experiment no AA or lactate was detected. However, acetate and propionate were present and increased linearly with addition of both AA and SL (Figure S2.6 and S2.7). The final concentrations were 6.49 mmol/L acetate and 0.25 mmol/L propionate in the AA treatment, and 2.37 mmol/L acetate and 3.13 mmol/L propionate in the SL treatment. In

addition, CO_2 concentration in the headspace increased in both treatments as well, with the highest rate in the AA treatment (Figure S2.8). The developing trends of CO_2 are similar to that of Fe^{2+}. The final amounts of CO_2 in the headspace were 1.19 and 0.26 mmol in the AA and SL treatment respectively, while CO_2 in blanks always remained close to 0.10 mmol (Figure S2.8). At the end of redox titration experiment, the carbon mass balance was approximately 77% in the AA treatment and 81% in the SL treatment. In all batches, the pH was neutral throughout the experiment.

These results showed that the aquifer material can be changed from a redox potential at -250 mV, which is regarded at Fe(III) reducing conditions, to a redox potential at -400 mV or lower without reducing all Fe(III). It was shown that both iron and sulphate reduction was initiated. Sulphate reduction led to the conversion of sulphate till below the level of detection. As reductive dechlorination is reported to occur at sulphate reducing conditions it can be expected that at the set redox potential reductive dechlorination may occur.

2.3.2 PCE reductive dechlorination tests after pre-treatment

Before PCE reductive dechlorination was tested at different conditions, 3.19 mmol/L AA and 8.41 mmol/L SL were added respectively. As explained in the methods section, the purpose was to condition the aquifer material and set the redox potential to below -400 mV and to reach a comparable condition to redox titration.

During pre-treatment, the observed changes in redox potential, Fe^{2+} and sulphate in the AA and SL treatments were similar to those in the redox titration described above. After the redox potentials stabilized at -450 mV for more than 5 days, the pre-treated aquifer materials were rinsed as described in the methods section, and PCE reductive dechlorination tests started with different conditions summarized in Table 2.1.

For the aquifer material that was previously conditioned with AA or SL, in the natural attenuation (NA) tests without stimulation by either additional electron donor or inoculum, a lag phase of at least 30 days was observed before PCE started to degrade. The degradation was incomplete during the experiment and stopped at cis-DCE in AA1 and TCE in SL1 (Figure 2.3-A, 2.-3B, S2.9-A and S2.9-B). PCE degradation did not occur in B1 where aquifer material was not conditioned (Figure 2.3-C and S2.9-C).
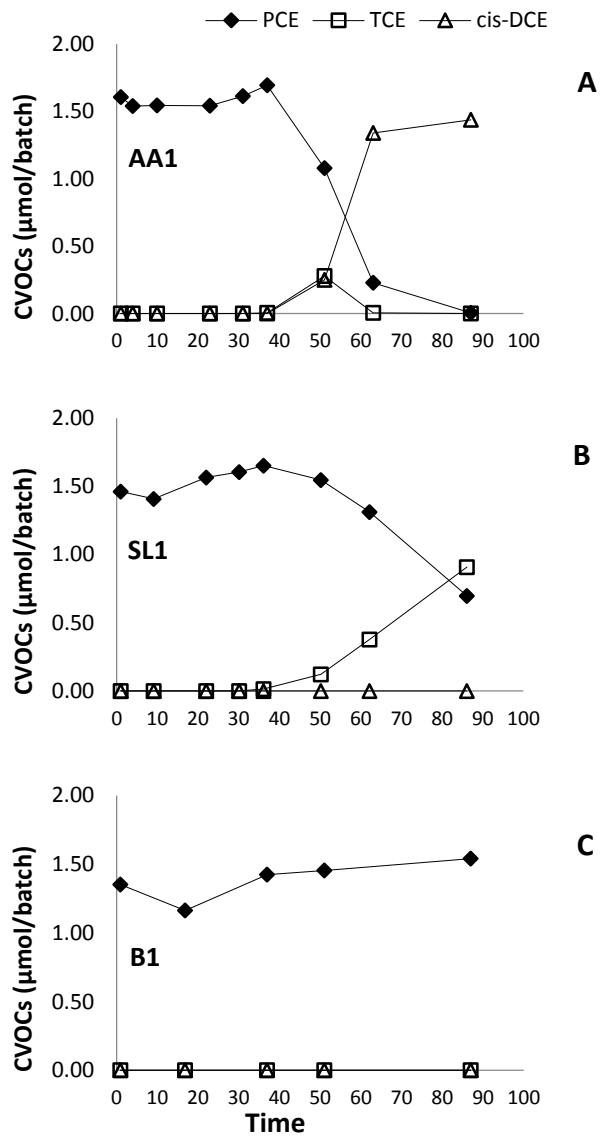
Figure 2.3 PCE reductive dechlorination for different experimental conditions (see Table 2.1) in natural attenuation tests.
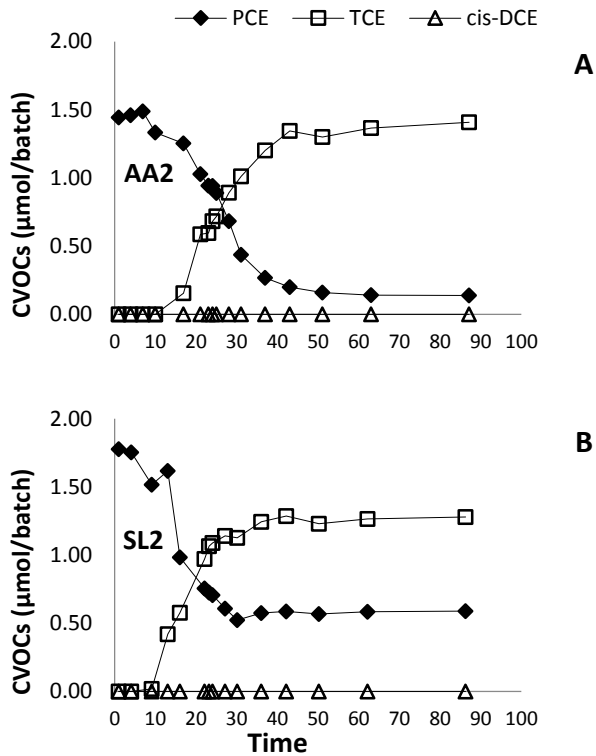
Figure 2.4 PCE reductive dechlorination for different experimental conditions (see Table 2.1) in electron donor stimulation tests.

In the electron donor (ED) stimulation tests, AA2 and SL2 behaved similarly (Figure 2.4). In general PCE was degraded with smaller lag phase compared to the NA tests, however, in one of the three AA2 batches no PCE degradation occurred throughout the experiment (Figure S2.10-A). In AA2, the first appearance of TCE was on day 16, while in SL2, it was observed on day 9. Additionally, cis-DCE was detected in one SL2 batch at the last sampling time (Figure S2.10-B).

Figure 2.5 PCE reductive dechlorination for different experimental conditions (see Table 2.1) in bioaugmentation tests.

In the bioaugmentation tests, complete PCE reductive dechlorination to ethene, with appearance of all intermediate CVOCs, was achieved within 30 days (Figure 2.5). The first decrease in PCE was already measured after 4 days. In AA3, VC seemed present as the only CVOC at day 10 (Figure 2.5-A and S2.11-A). However, ethene was detected after 20 days and the only remaining final product in all batches (Figure S2.11).
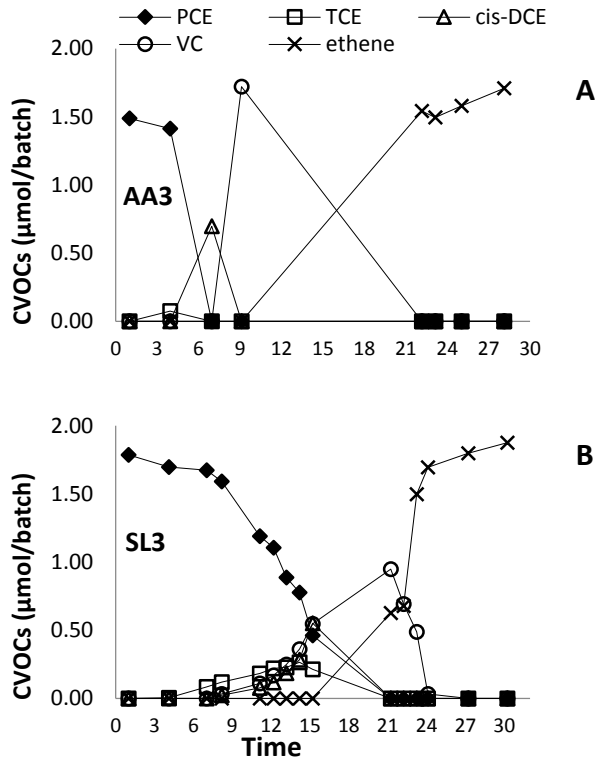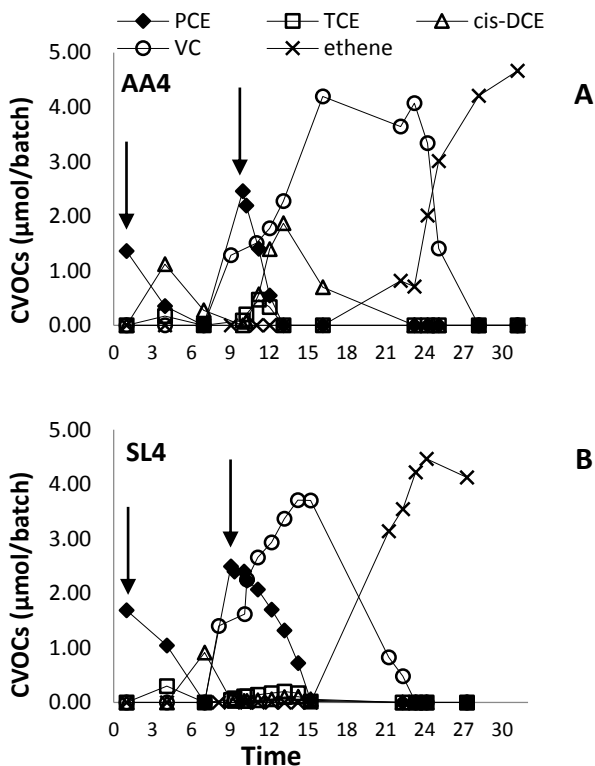
Figure 2.6 PCE reductive dechlorination for different experimental conditions (see Table 2.1) in electron donor + bioaugmentation tests. Arrows indicate two times of PCE spiking.

In the ED + bioaugmentation tests, PCE was degraded within 7 days without a lag phase (Figure 2.6). A second PCE spiking was also removed within 5 days and complete conversion to ethene was found in all batches at day 28. In contrast to bioaugmentation tests without additional electron donor, higher accumulation of VC was observed between day 10 and 20 in SL4 than in AA4 (Figure S2.12).

The summary of the performance of PCE reductive dechlorination in different scenarios mentioned above is given in Table 2.2. The CVOCs mass balance for the experiments is shown in Table S2.1. In all abiotic controls, biodegradation of PCE did not occur (Figure S2.13).

Table 2.2 Comparison of the performance on PCE reductive dechlorination in different scenarios.

Feature	No pre-treatment	With pre-treatment (AA or SL as indicated)							
		Natural attenuation		Electron donor (ED)		Bioaugmentation (Bio)		ED + Bio	
	B1	AA 1	SL1	AA2	SL2	AA3	SL3	AA4	SL 4
Reductive dechlorination	-	+		+		+		+	
Lag phase (day)	n.a.	37	30	16	9	< 4		0	
End products	PCE	PCE, TCE, cis-DCE		PCE, TCE, cis-DCE		ethene		ethene	
PCE degradation rate	n.a	Very slow		Slow		Fast		Very fast	

[a] Whether reductive dechlorination occurred or not is indicated by "+" or "-". Not applicable is indicated as n.a.

2.4 Discussion

2.4.1 Redox titration

Results from the redox titration revealed that, for the selected PCE contaminated aquifer that was initially under Fe(III) reducing conditions, a minimum addition of 75 µmol electron eq/g dry mass of aquifer material was needed to obtain suitable redox conditions. Additionally, electron donor that provides this amount of electron equivalents seems not specific, as AA and SL showed a similar final redox potential of -450 mV. Interestingly changes in Fe^{2+} and SO_4^{2-} could only be observed after the redox potential had been lowered. A possible explanation for this observation is provided by Christensen et al. who stated the reduction of ferric aqua ions to ferrous aqua ions is rapid while the reduction of structural Fe(III) to form ferrous aqua ions is a much slower reaction [142]. The observed, initially rapid decrease of redox potential in our study might thus be related to the reduction of a limited amount of aqueous electron acceptors. The slow increase of Fe^{2+} which was observed after the second addition of AA or SL then related to the reduction of structural Fe(III).

Based on equations 2.1-2.4, it should be noted that the amount of electrons accepted by Fe(III) and SO_4^{2-} is limited compared to the total amount of electron donor added. As normally the

contribution of Mn(IV) will be minor compared to Fe(III), and NO_3^- was not present in the experiments, the explanation could be that large part of the electron donor added was consumed in other processes, such as fermentation and growth of bacteria. Lactate can be fermented to acetate and propionate by some bacteria via the following reaction [143]:

$$3CH_3CH(OH)COO^- \rightarrow CH_3COO^- + 2CH_3CH_2COO^- + HCO_3^- + H^+ \qquad (2.5)$$
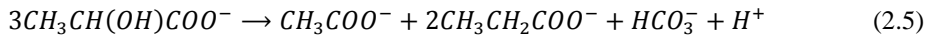
Based on the amount of acetate and propionate measured in the experiment, the fermentation of lactate could be the major process of lactate consumption. Meanwhile, Fe(III) and SO_4^{2-} were reduced by lactate, acetate and propionate. Such fermentation process of AA might be also possible. However, no specific evidence from literature could be found to support this hypothesis. After all, fermentation processes were reported to be important for PCE dechlorination, especially for acetate, in a PCE biodegradation by chitin fermentation [144].

In the experiment, the AA treatment showed a faster rate in Fe^{2+} production, while the SL treatment showed a faster rate in SO_4^{2-} reduction. Probably in the AA treatment most Fe^{2+} chelated with ascorbic acid as ferrous-ascorbate complex [145, 146]. This complexation process kept Fe^{2+} in a dissolved phase to be easily detected. In SL treatment, complexation between Fe^{2+} and lactate was probably much less, driving the transformation from SO_4^{2-} to sulfide, then to iron sulfide precipitate faster.

2.4.2 PCE reductive dechlorination tests after pre-treatment

No reductive dechlorination occurred under fully NA condition where no preconditioning with AA or SL was performed. This was due to the presence of competitive electron acceptors, such as Fe(III), Mn(IV) and sulphate. PCE reductive dechlorination is reported to be possible at redox potential below -200 mV referenced to SHE [1, 4], which was close to the final redox potential, approximately -450 mV referenced to Ag/AgCl electrode, of the pre-treated aquifer material in this study. Therefore in batches that received preconditioning with AA or SL, PCE reductive dechlorination could be stimulated as available Fe(III) and sulphate have been reduced. Besides, acetate as the fermentation product during the pre-treatment, could facilitate the growth of "*Dehalococcoides*" strains [147]. Hence, the growth of dechlorinating bacteria in batches received AA or SL before, could play an important role, causing the different PCE biodegradation performance between pre-treated and non-pre-treated

conditions. Further, stimulations with extra ED after preconditioning showed much earlier and faster reductive dechlorination compared to NA, but dechlorination process was not complete. Reductive dechlorination stopped at TCE probably because of the lack of microorganisms that can perform full reductive dechlorination [148].

After bioaugmentation, reductive dechlorination was complete from PCE to ethene, in spite of a few days' lag phase which was probably due to low concentration of electron donor. With extra ED and bioaugmentation, PCE reductive dechlorination occurred directly with complete reductive dechlorination in the end. Evolutions of CVOCs were in line with literature [149, 150]. In addition, the PCE removal rates in all inoculated treatments were comparable to earlier studies [151, 152]. Further, in most cases, ethene as a harmless end product from PCE reductive dechlorination, appeared around 20 days, which was similar to what Kao et al. showed in their experiments [153].

Apparently, performance of PCE reductive dechlorination is enhanced in the direction from natural conditions to more stimulated conditions. However, in any scenarios, costs such as time and additional chemicals or bacteria are inevitable for achieving specific purposes.

2.5 Conclusions and Implications

1. PCE reductive dechlorination was stimulated after improving the redox condition of the selected aquifer material by pre-treating with AA or SL.

2. 75 μmol electron equivalents per gram dry mass of aquifer material was the threshold to obtain a redox potential of -450 mV, which is theoretically suitable for PCE reductive dechlorination.

3. The impact of AA and SL on redox potential was not specific, while Fe(III) reduction by AA and SL behaved differently. The results showed faster iron mobilization by AA. This might induce a risk of iron oxide precipitation, when the mobilized iron, with groundwater extraction or transportation, is exposed to higher redox conditions.

4. Dechlorinating bacteria were needed to achieve complete reductive dechlorination from PCE to ethene, for the selected aquifer material.

The findings of this paper are relevant for improving the cost-effectiveness of the design and operation of in situ bioremediation. The redox potential of an aquifer can be used as a general indicator to evaluate the potential of PCE reductive dechlorination. The result from our step-wise experiments can be meaningful for dealing with in situ bioremediation and for applications in large volumes of contaminated aquifer. For achieving specific goals of in situ bioremediation projects on different CVOCs contaminated sites with various environmental conditions, the balance between cost and profit, and potential risks (e.g. bio-chemical well clogging due to bacteria growth and precipitation of metals) should be estimated before the design and operation. When addition of electron donors for improving redox conditions is necessary, the selection of electron donor is also of importance from a cost-effectiveness as well as optimization point of view.

Acknowledgement

Supporting material

Figure S2.1 Raw redox potential reading of AA treatment during redox titration experiment.
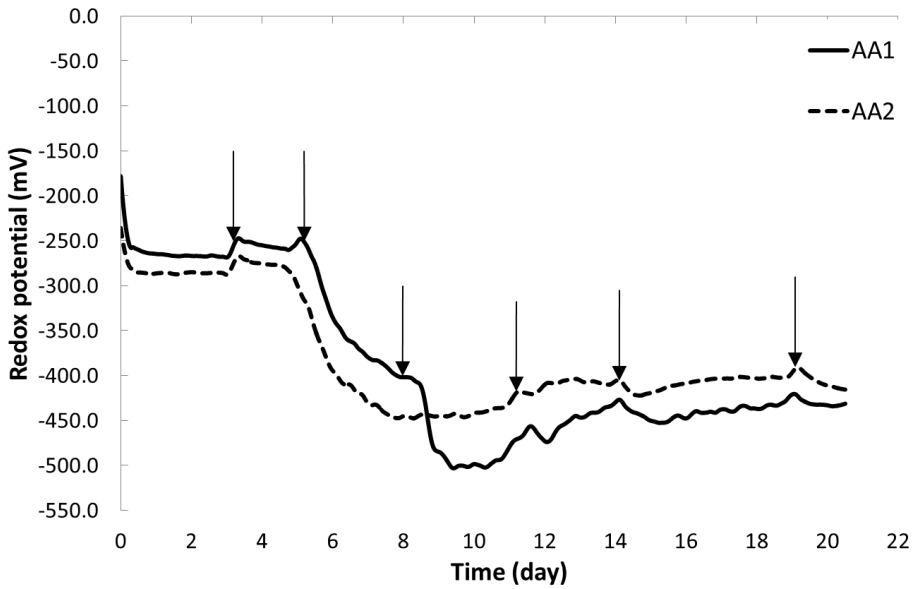
Figure S2.2 Smoothed redox potential evolution of AA treatment during redox titration experiment. Arrow indicates the time of addition.
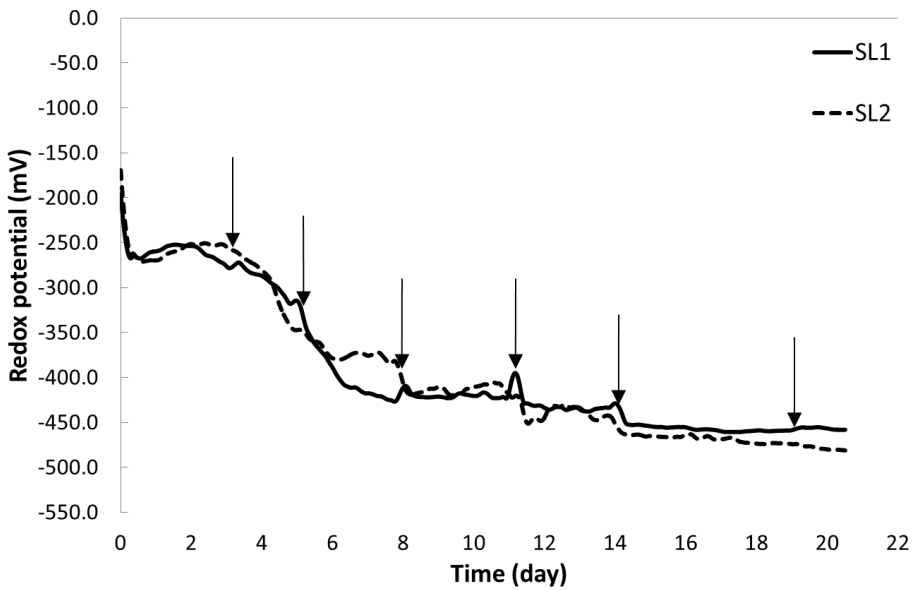
Figure S2.3 Smoothed redox potential evolution of SL treatment during redox titration experiment. Arrow indicates the time of addition.
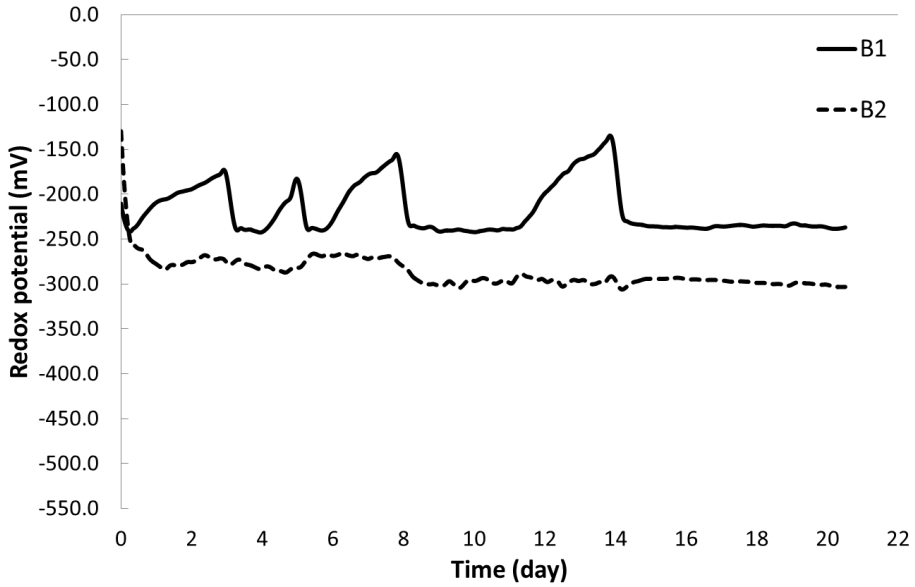
Figure S2.4 Smoothed redox potential evolution of blank during redox titration experiment.
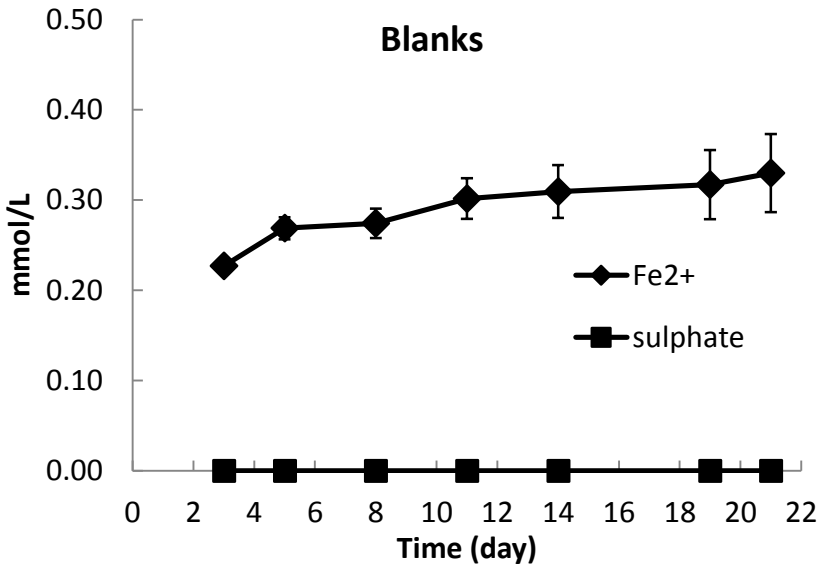
Figure S2.5 Fe^{2+} and sulphate in blanks during redox titration experiment.
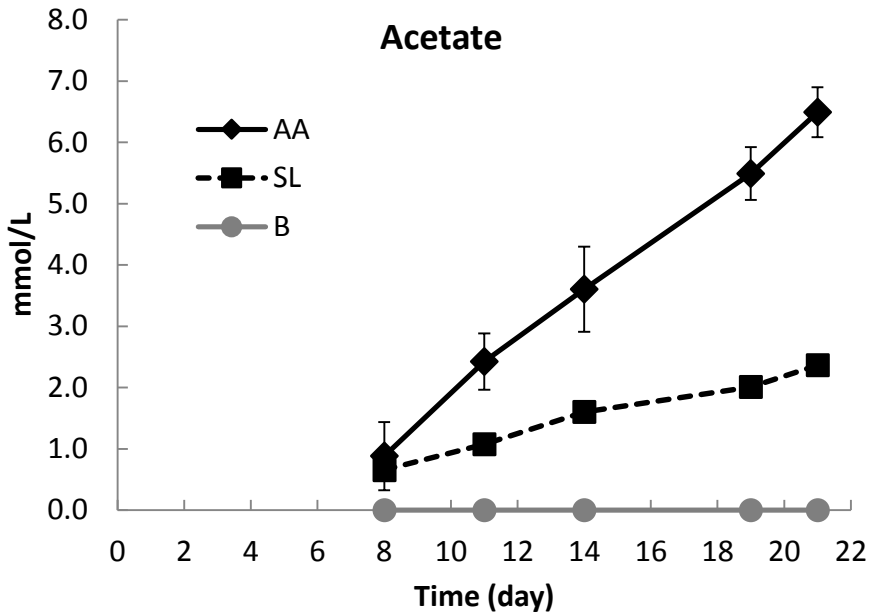
Figure S2.6 Acetate in AA, SL and blank during redox titration experiment.
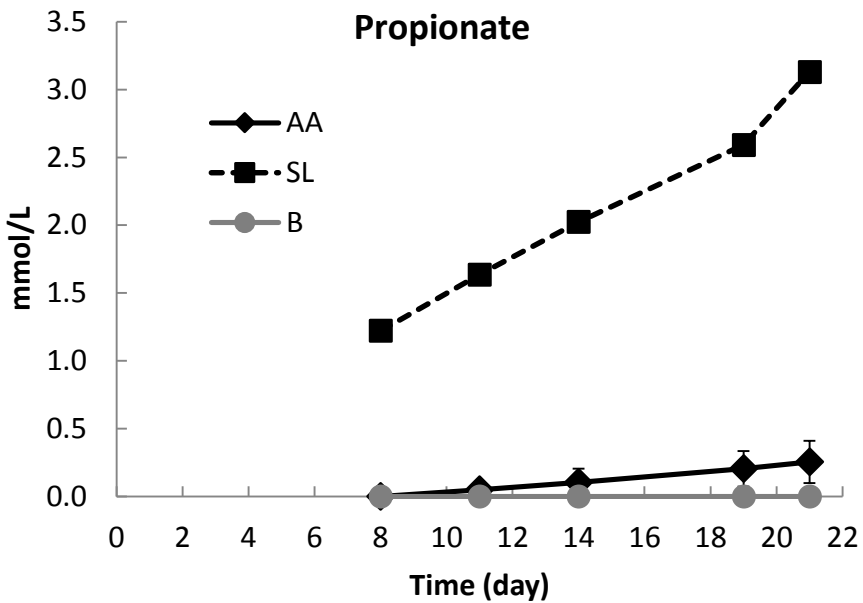
Figure S2.7 Propionate in AA, SL and blank during redox titration experiment.
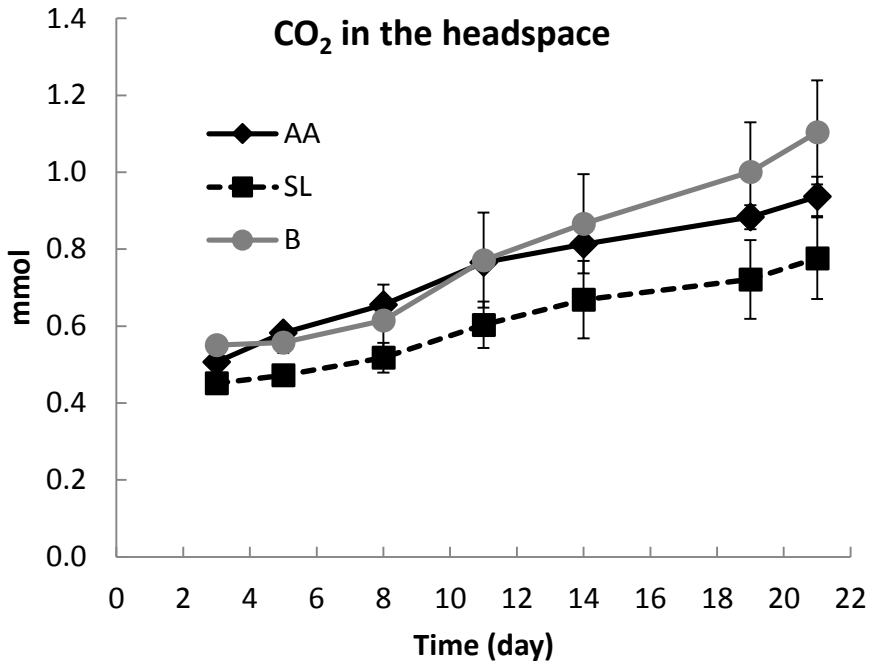
Figure S2.8 CO_2 in the headspace of AA, SL and blank batches during redox titration experiment.
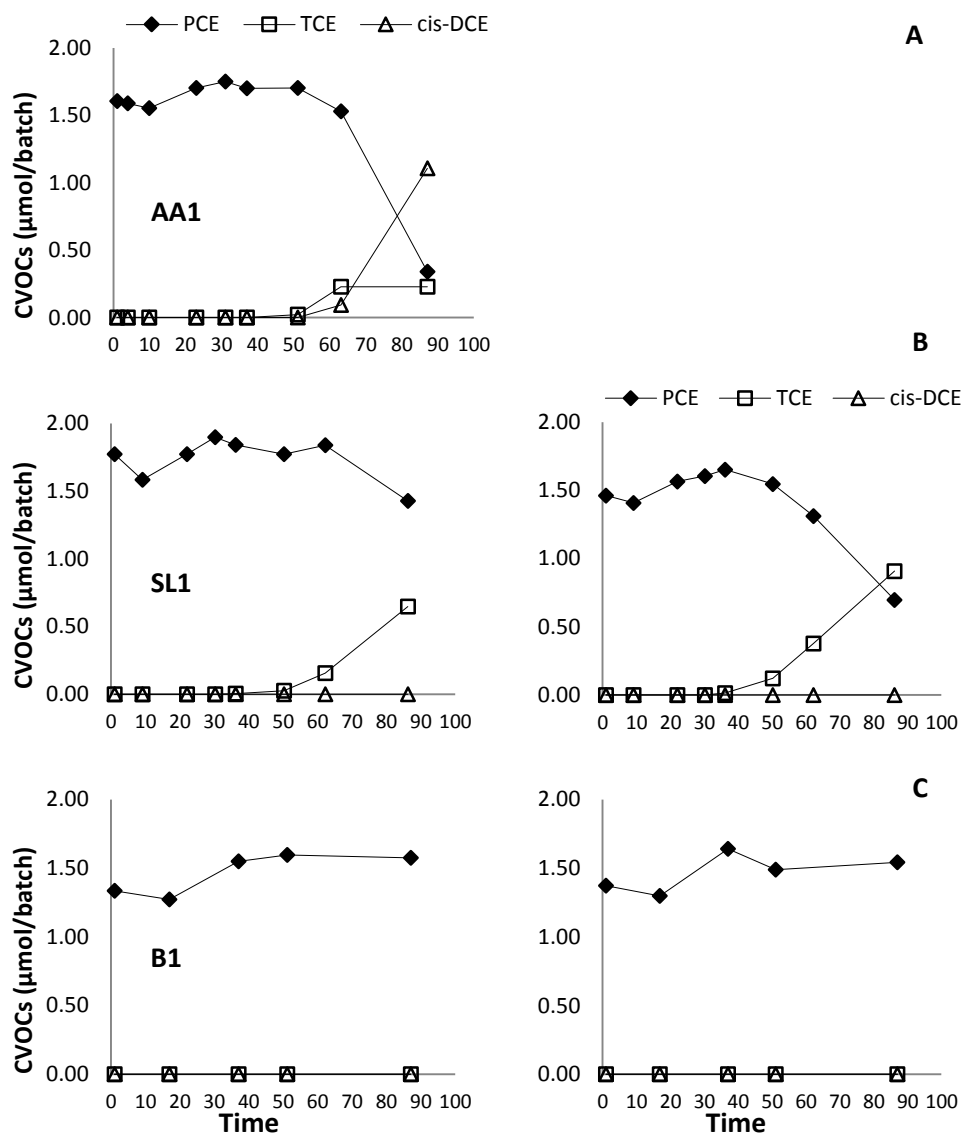
Figure S2.9 PCE reductive dechlorination in NA condition. AA conditioned (A), SL conditioned (B) and without redox conditioning (C).
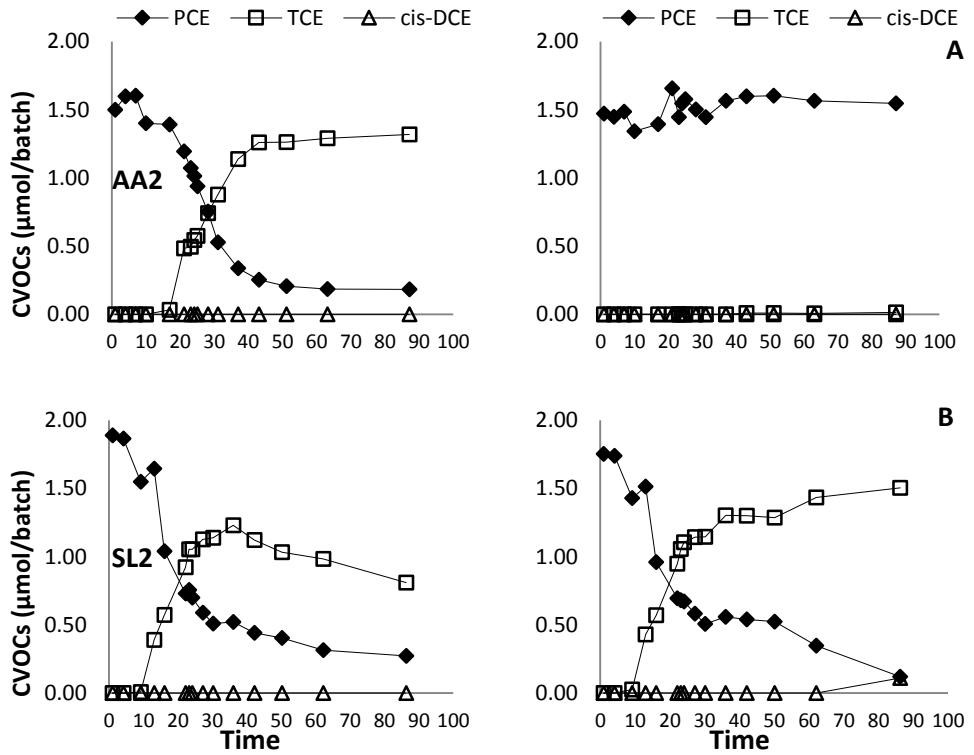
Figure S2.10 PCE reductive dechlorination in ED condition. AA conditioned (A) and SL conditioned (B).
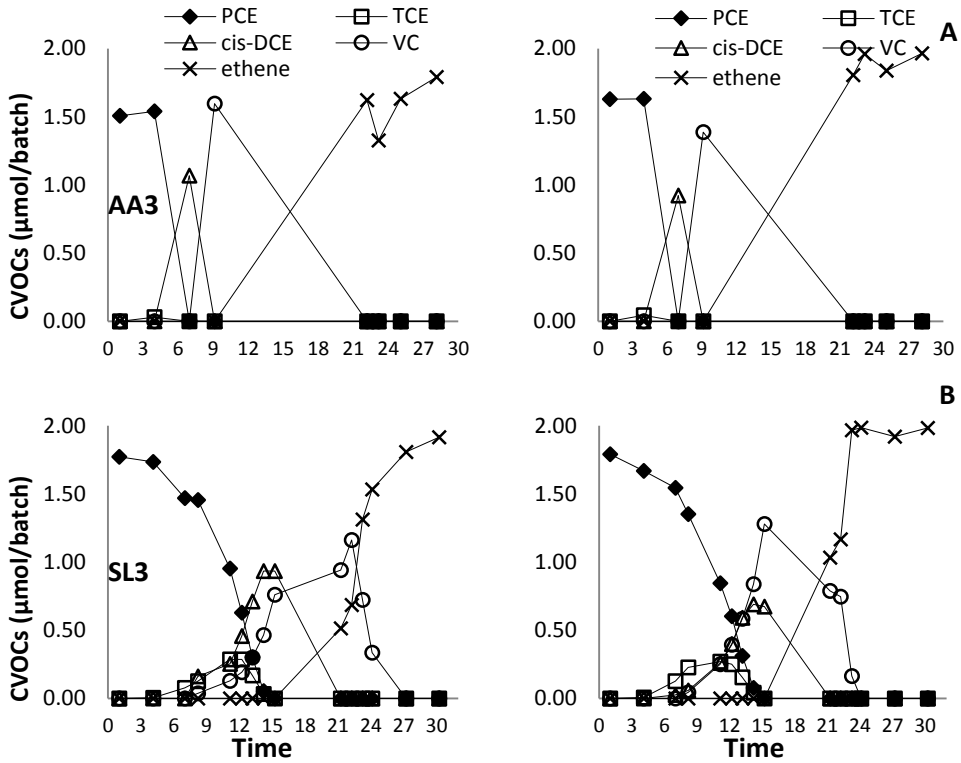
Figure S2.11 PCE reductive dechlorination in bioaugmentation condition. AA conditioned (A) and SL conditioned (B).
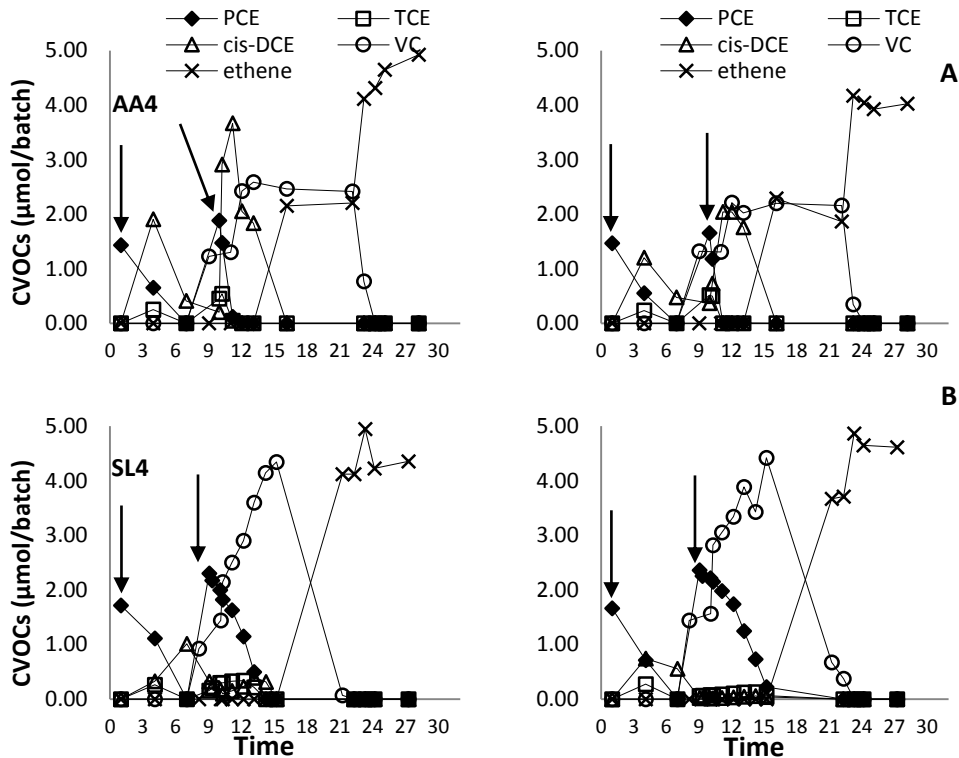
Figure S2.12 PCE reductive dechlorination in ED + bioaugmentation condition. AA conditioned (A) and SL conditioned (B). Arrows indicate two times of PCE spiking.

Table S2.1 CVOCs mass balance for reductive dechlorination experiments.

Tests	CVOCs mass balance
AA1	95%
AA2	95%
AA3	>99%
AA4	94%
SL1	>99%
SL2	97%
SL3	>99%
SL4	91%
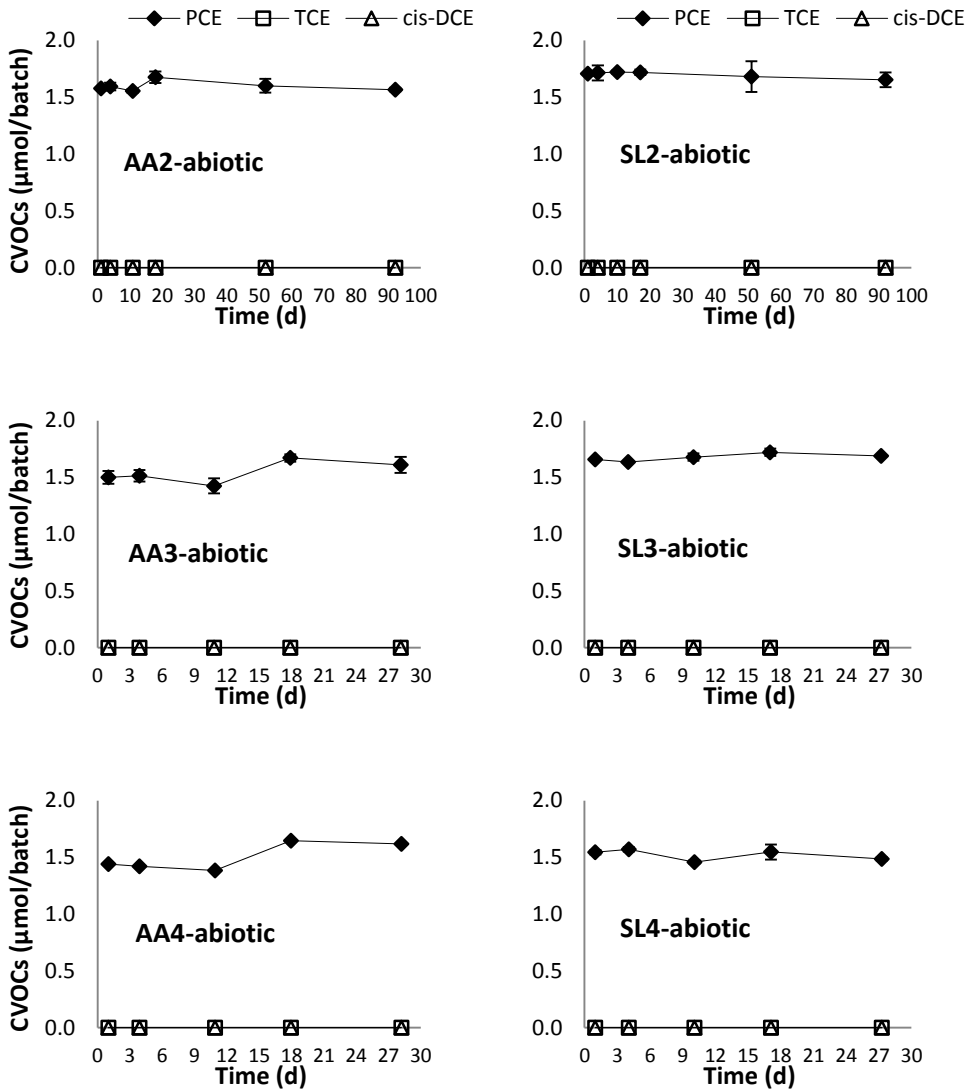
Figure S2.13 Averaged values of PCE, TCE, cis-DCE in abiotic controls for PCE reductive dechlorination tests on AA pre-treated (left) and SL pre-treated aquifer material (right). Error bar is standard error.

Chapter III

Biodegradation of cis-DCE in Simulated Underground Thermal Energy Storage Systems

Abstract

Underground thermal energy storage systems (UTES) showed a sharp rise in numbers in the last decades. Aquifer thermal energy storage (ATES) and borehole thermal energy storage (BTES) are the most widely used. The challenge for a new integrated concept, combining ATES and enhanced bioremediation, is the result of the need for both sustainable energy and sustainable groundwater management. To explore possible synergies of such combination, we performed batch experiments to study the effects of ATES by temperature and liquid exchange, and BTES by only temperature exchange, on cis-DCE reductive dechlorination. Compared to the natural situation (NS) at a constant temperature of 10 ˚C, we observed that under ATES conditions, the overall removal rate was 13 times higher, despite the lack of degradation during cold periods. Under BTES conditions, the overall removal rate was 8.5 times higher than under NS. Inoculation with *Dehalococcoides* revealed that their initial presence is a determining factor for the dechlorination process. Temperature became the dominant factor when *Dehalococcoides* concentration was sufficient. Furthermore, *Dehalococcoides* is preferentially attached to the soil matrix. The experiments with dynamic temperature regimes enhance the understanding of combination between UTES and bioremediation. Bioremediation showed to be best stimulated in the ATES warm well.

Keywords

3.1 Introduction

Since the 1970s, the use of underground thermal energy storage (UTES) for energy conservation has developed and proved to be a sustainable energy technique which is beneficial to the reduction of Greenhouse Gas (GHG) emissions [41, 154, 155]. Among different UTES systems, aquifer thermal energy storage (ATES) and borehole thermal energy storage (BTES) are considered the most common and cost-effective UTES systems [154, 156], which have been widely applied throughout Europe and North America [155]. To fulfill the EU's ambitions on GHG reduction by 2020 [157] and more demands on sustainable energy, the subsurface has been increasingly used for the purpose of developing more UTES systems. For instance in the Netherlands, the number of ATES systems dramatically increased from five in 1990 to over 1,300 in 2010 [82, 158, 159] and the potential number is estimated to be 20,000 in 2020 [44]; the number of BTES systems is even larger and has strongly grown as well (from 24 in 1996 to approximately 18,000 in 2006) [160, 161]. The principle of ATES is to store heat or cold energy when it is available and retrieve it when needed. During the summer, from pumped cool groundwater, the cold energy is extracted for cold demand in buildings and facilities. The heated groundwater is then injected into the warm well and stored in aquifer as heat source for winter [83, 154]. During the winter, the groundwater flow is reversed and so is the process. In BTES, groundwater is recirculated within closed loop made of high heat-conductive material. Depending on the temperature above ground, water inside BTES acts as heat sink (summer) or heat source (winter) [80]. Many studies have paid attention to the design, operation, application and efficiency of both ATES [41, 82, 84, 154, 156, 162] and BTES [41, 46, 154, 163] systems, including case study [46, 156, 164], laboratory experiment and field measurement [82, 165], and modeling [81, 82, 84]. The main impacts of ATES and BTES on subsurface conditions can be summarized by two aspects: groundwater displacement and temperature change. The open loop system ATES involves large volumes of displaced groundwater [84, 154] and wells with either high and low temperatures, typically ranging from 20-25 ˚C [83, 166, 167] for warm wells to 5-7 ˚C [43, 82, 156] for cold wells. In practice the temperature of warm well is often around 15 ˚C [162]; closed BTES systems have a fluctuating temperature impact on the subsurface due to seasonal [80, 154]. The operation and performance of UTES systems have been well investigated. Few of these have focused on the potential environmental effect or risk [43, 161,

164, 168], but none have closely investigated the impacts of periodic variation of groundwater conditions and fluctuation of temperature. For example the interaction between UTES systems and bio-chemical processes, mineral dissolution/precipitation, (in)organic compounds redistribution, and microorganism behavior have as yet been insufficiently studied. Utilization of the subsurface for UTES may coincide or interfere with other activities, hence research focusing on such aspects is required in support of better and integrated management of subsurface activities and resources. Especially the combination with groundwater remediation and the impact on the subsurface potential for natural attenuation of chlorinated volatile organic compounds (CVOCs) deserve attention, in view of increasing demands for installation of UTES systems in urban aquifers.

Aquifer contamination with CVOCs mainly involves tetrachloroethene (PCE), trichloroethene (TCE), cis-1,2-dichloroethene (cis-DCE) and vinyl chloride (VC). PCE and TCE are chlorinated solvents often used in dry cleaning and the metal degreasing industry. Together with their degradation products cis-DCE and VC they constitute the most prevalent organic contaminants in the subsurface of urban areas [2, 3, 10, 22, 43, 68, 92]. Due to their physical and chemical properties, which allow them travel with groundwater and sink to deeper aquifer layer, they are among the most difficult and costly contaminants to be cleaned up and characterized, especially when they exist as dense non aqueous phase liquid (DNAPL) [4, 68, 69]. Conventional techniques such as soil excavation, pump-and-treat or soil vapor extraction are mostly inefficient or too costly to properly remediate CVOC contaminants [102, 103]. Bio-based techniques become more and more attractive, such as monitored natural attenuation (MNA) and enhanced bioremediation [4, 31, 107], particularly when sufficient time is available, can in principle remediate up to set target-levels. Remediation based on biological transformation and biodegradation with proper stimulation approaches can be an effective way to reduce organic contaminants [169]. Groundwater in urban areas is often contaminated with chlorinated ethenes, whereas there is a high demand for sustainable energy in these areas [49, 170]. Hence, combining the natural attenuation potential of the subsurface with (bio)stimulation using existing engineered systems, in this case UTES systems, could be a promising integrated approach, not from a primary focus on CVOC remediation, but from the perspective of sustainable utilization of the subsurface [49, 88, 89].

Here, the effect is studied of only fluctuating temperature as simulation of BTES and the alternating displacement of warm groundwater to a cold well and vice versa as simulation of ATES, on the biodegradation of cis-DCE in laboratory batch experiments. The choice of cis-DCE as the target compound is motivated by the fact that under natural conditions, complete dechlorination of CVOCs is often limited by lack of electron donor or microorganisms, *Dehalococcoides* in particular, or unsuitable redox conditions [62, 69-71, 116], resulting in accumulation of cis-DCE and VC. Thus, the effectiveness of an enhanced bioremediation approach is demonstrated by its potential for complete degradation of cis-DCE via VC to ethene. Furthermore, *Dehalococcoides* is the only microorganism that metabolically converts cis-DCE and VC to ethene [68]. The dechlorination activity is of importance for combining UTES and enhanced natural attenuation, especially in the cold zone of ATES system where the temperature is even lower than natural groundwater temperature. Literature shows that low temperature could severely hamper the microbial dechlorination processes, particularly for cis-DCE [171-173]. However, considering ideal temperature condition, provided in the ATES warm zone, limited dechlorination activity in the cold zone might be compensated by possible transferring *Dehalococcoides* from the warm zone. In fact a preliminary batch experiment was performed prior to and as a basis for this study, and the results showed at 5 ˚C temperature, dechlorination of cis-DCE to ethene was achieved providing that *Dehalococcoides* concentration was high enough (twice as much as used in this study). Therefore, in this study the mobility of *Dehalococcoides* is also studied and discussed since the bioremediation in combination with ATES may be affected by such mobility.

3.2 Materials and methods

Batch experiments with different actions were performed in this study to simulate the seasonal functioning to ATES and BTES. ATES warm or cold well was simulated using batch bottle placing at 25 or 5 ˚C environment. Furthermore periodically exchanging liquid between the two temperatures while having aquifer material remained inside the bottle was performed to mimic seasonal change of groundwater flow in ATES. For BTES, change of season was mimicked by switching the entire batch bottle between 25 or 5 ˚C. Detailed procedures are given below. In real UTES systems, the duration of a seasonal operation is around 180 days. Here we shortened this duration to a period in which the cis-DCE is depleted

at 25 ˚C. The strategy then was to perform simulated change of season when cis-DCE was degraded in the 25 ˚C bottle. Consequently, both ATES and BTES bottles received an extra cis-DCE spiking after changing the season. In this setup UTES seasons were simulated in a relatively short period of time. Codes used in this studied are defined in Table 3.1 which gives the overview of experimental batches. The background color of red, blue and grey for figures in this study represent temperature level of 25, 5 and 10 ˚C respectively.

Table 3.1 Overview of experimental batches.

Code*	Mimicking:	*Dehalococcoides* inoculum	Temperature ˚C	# of replicates
AWb	ATES warm well	yes	25	3
AWc	ATES warm well	no	25	2
ACb	ATES cold well	yes	5	3
ACc	ATES cold well	no	5	2
BWb	BTES, starting at warm condition	yes	25	3
BWc	BTES, starting at warm condition	no	25	2
BCb	BTES, starting at cold condition	yes	5	3
BCc	BTES, starting at cold condition	no	5	2
NSb	Reference natural situation	yes	10	3
NSc	Reference natural situation	no	10	2

* Symbol b stands for biotic bottle, symbol c stands for control bottle without inoculum

3.2.1 Basic Material

Aquifer material, anaerobic waters, anaerobic mineral medium used in this experiment were the same as those in our previous study on limiting factors for PCE dechlorination [92]. 400 mg/L cis-DCE stock was prepared by dissolving 72 μL pure cis-DCE (≥97%, Aldrich®) into 230 mL anaerobic deionized water. Sodium lactate powder (≥99% purity, Aldrich®) was used to prepare stock solution 225 g/L as electron donor. A mixed culture of active dechlorinating bacteria, which contained *Dehalococcoides* ($>10^8$ cells/mL liquid), was obtained from Bioclear BV (Groningen, NL) as inoculum in the experiment.

3.2.2 Experimental Setup

All bottles were prepared in an aerobic hood filled with 95% N_2 and 5% H_2, using the same procedures as in our previous batch (125 mL serum bottle) experiment [92] on anaerobic reductive dechlorination of PCE. All biotic bottles initially had received 2.5 mL cis-DCE stock (equals to approximately 10.5 μmol cis-DCE), and contained 20 mmol/L lactate and

10% inoculum in the final liquid volume. Control bottles had the same recipe except inoculum (Table 3.1). At the end of preparation each bottle had in total approximately 20 g wet aquifer material and 50 mL liquid phase, and headspace was exchanged with 98% N_2 and 2% CO_2. All bottles were shaken at a speed of 150 rpm.

Batches of the ATES warm group (AW) and ATES cold group (AC) were incubated at 25 and 5 ˚C respectively. To simulate the periodic groundwater displacement by ATES, 40 mL liquid (80% of total liquid) was exchanged between the AW and equivalent AC bottle, once the concentration of cis-DCE in the AW bottle was below the level of detection. The liquid exchange procedure was as follows: 1) each bottle's stopper was penetrated by a needle with valve connected to a 50 mL syringe; 2) bottles together with syringe were put up side down to settle for about 2 hour to achieve a clear liquid layer; 3) while the bottles were still upside down, 40 mL liquid was extracted from each AW and AC bottle by syringes; 4) valves were close, and syringes were exchanged between AW and AC bottle; 5) 40 mL liquid from AW bottles was then injected into AC bottles and vice versa. The needle with valve would be left in the stopper after the first time liquid exchange event to perform the same procedures more conveniently and to avoid leakage from stoppers that might be over penetrated. With each liquid exchange event, an additional 2.5 mL cis-DCE stock was spiked into both AW and AC bottles.

Similarly, batches of the BTES warm group (BW) and BTES cold group (BC) started at temperature of 25 and 5 ˚C respectively. To simulate the fluctuating temperature of BTES, BW and BC bottles would be relocated from 25 to 5 ˚C and vice versa, once the cis-DCE was fully degraded in the bottle that was kept at 25 ˚C. Thus, both BW and BC batches had alternating temperature conditions over the duration of the experiment, the coding only indicates the starting temperature.

Batches simulating NS were always incubated at 10 ˚C and only received one cis-DCE spiking at the start.

3.2.3　Preliminary experiment

Four groups of bottles coded with A, B, C and D were tested prior the simulation experiments on UTES. Conditions differed from UTES experiment for these four groups were as followed:

group A and B were at 5 ˚C with 1% and 20% inoculum respectively; group C and D were at 10 ˚C with 1% and 20% inoculum, respectively. All bottles received one time of cis-DCE spiking. The preparation procedures were the same as stated in previous sub-section and each group was done in triplicate.

3.2.4   Analytical methods

A headspace sample was collected using a 100 μL glass syringe (Hamilton®) and needle. The quantification of CVOCs and ethene was performed by direct splitless injection on an HP6890 series GC equipped with a CP PoraBond Q column (25 m × 0.53 mm × 10 μm) and Flame Ionization Detector (FID). The temperature program started at 50 ˚C, ramped at 17.78 ˚C/min to 140 ˚C and was held at 140 ˚C for 1.5 minutes.

3.3 Results and discussion

3.3.1   Reductive dechlorination in preliminary experiment

Except in group A, ethene productions were observed in all other groups (Figure 3.1), even in group B with low temperature of 5 ˚C but with a 20 times higher *Dehalococcoides* concentration. This indicates reductive dechlorination process was initially limited by biomass concentration at low temperature. At the end of the experiment, ethene occupied approximately 19% of the amount of cis-DCE that was biodegraded ($\frac{C_{ethene}}{C_{VC}+C_{ethene}}$) in group B, while less than 5% in group C. Whereas group C has 2 times higher temperature and 20 times less biomass concentration compared to B. Remarkably, this percentage increased to 89% in group D whose difference compared to B was only 2 times higher temperature. From that we can conclude when biomass was not limiting the dechlorination process anymore, temperature would play a more important role than biomass concentration.
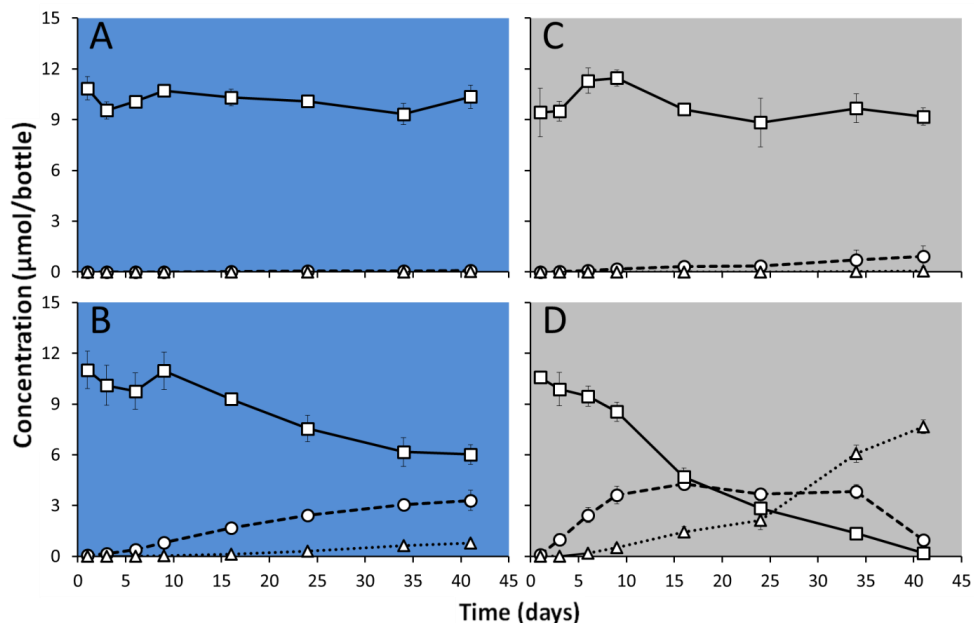
Figure 3.1 Concentration of cis-DCE (squares with straight line), VC (circles with dashed line) and ethene (triangles with dotted line) as a function of time in group A, B, C and D. Error bars indicate the standard deviation of the triplicates. When error bars are not visible, they are smaller than symbol size.

3.3.2   Reductive dechlorination in simulated ATES and BTES conditions

The behavior of CVOCs including production of ethene was similar in all those groups that were either at 25 or 5 °C, in the first 10 days. Immediate reductive dechlorination of cis-DCE was observed in AWb and BWb bottles, with similar degradation rate (Figure 3.2). In AWb bottles, with subsequent liquid exchanges and more cis-DCE spiking, the speed of cis-DCE depletion gradually increased, as the dechlorinating bacteria kept growing at a temperature that was always suitable [174]. Notably, complete dechlorination to ethene was achieved in almost every spiking period, except for the first period (Figure 3.2-AWb).
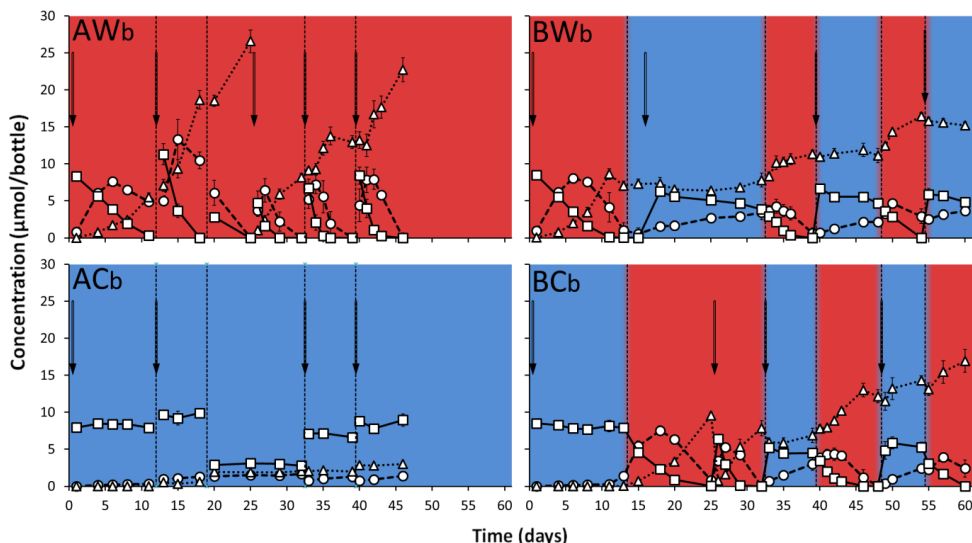
Figure 3.2 Concentration of cis-DCE (squares with straight line), VC (circles with dashed line) and ethene (triangles with dotted line) as a function of time in ATES groups (left) and BTES groups (right). Area of red and blue color represents the duration of bottles that stayed at 25 and 5 ˚C respectively. Black arrows indicate spiking of cis-DCE. Error bars indicate the standard deviation of the triplicates. When error bars are not visible, they are smaller than symbol size.

After the second time liquid exchange on day 19, no extra cis-DCE spiking was performed due to the relatively high remained amount of cis-DCE in ACb bottles from day 13 to day 18. So the cis-DCE concentration on day 20 after the second time of liquid exchange was smaller than other periods. On day 25, because of analytical limitations in ethene quantification, an extra headspace exchange was performed to remove all gas compounds, followed by a new start of the experiment. This was also done for the BCb bottles to prevent encountering same limitation later.

At the end of the experiment, day 46, all CVOCs were completely converted into ethene in AWb bottles (Figure 3.2-AWb). However, no evidence of reductive dechlorination was observed in ACb bottles during the entire experimental period (Figure 3.2-ACb). The remaining of cis-DCE shown in this case followed the similar pattern of the accumulative result of cis-DCE in previous studies [171, 175]. Although after each liquid exchange a small increase of VC and ethene concentration was measured in the ACb bottles, their

concentration remained at the same level within each spiking period. This was due to the entering of the exchange liquid from AWb bottles where VC and ethene were still present. At last CVOCs concentration in ACb remained the same level as that after last spiking. Even though about 80% of liquid was removed in AWb, the dechlorination activity increased when new cis-DCE was added. This would not be expected if the dechlorinating bacteria would preferentially be present in the liquid phase. Therefore, the fact that after each liquid exchange cis-DCE dechlorination was enhanced in AWb while dechlorination remained inactive in ACb (Figure 3.2-ATES) is attributed to the immobility of dechlorinating bacteria. Compared to other microorganism, such as *Geobacter*, *Dehalococcoides* is much less planktonic and largely prefers to attach to sediment or soil [176, 177], especially under poor or minimal-growth conditions [178]. Another study, upon dechlorination of carbon tetrachloride, showed that dechlorinating bacteria performed 2 to 5 times better when they are attached to the porous medium [179]. Therefore, liquid exchange barely transferred active *Dehalococcoides* bacteria from AWb bottles to ACb bottles and actually only changed some chemical conditions, like CVOCs concentrations. The growth of *Dehalococcoides* in AWb bottles could thus not compensate the hampered dechlorination due to the low temperature in ACb bottles in this experiment.

In contrast, both liquid and solid material in BTES bottles remained where it was, with only temperature changes. The *Dehalococcoides* were always active during all periods at 25 ˚C and apparently largely remained available when transferred to 5 ˚C. And the concentration of this remained available biomass was enough to allowed slow degradation from cis-DCE to VC in the periods of 5 ˚C. When the temperature was switched to 25 ˚C again in BWb/BCb bottles, decline of cis-DCE and increase of ethene were observed immediately (Figure 3.2-BTES), indicating that *Dehalococcoides* were capable of surviving at temperatures as low as 5 ˚C and became active again once the temperature was suitable [171, 175]. Unlike AWb bottles, BWb/BCb bottles stayed at 25 ˚C for a period followed by a period at 5 ˚C, the growth of *Dehalococcoides* in BWb/BCb bottles was then less optimal, leading to lower dechlorination performance compared to AWb bottles. In summary (Figure 3.2), reductive dechlorination during periods at 25 ˚C occurred at higher rates in AWb than in BWb/BCb bottles; reductive dechlorination during periods at 5 ˚C still occurred at low rates in BWb/BCb bottles, but not in ACb bottles.

In the control batches without inoculum, VC production was detected from day 25 onwards in AWc bottles and, probably because of the longer duration of the high temperature period, only in the BCc bottles and not in the BWc bottles (Figure 3.3). However, further dechlorination to ethene did not occur. The lack of *Dehalococcoides* in the aquifer material was responsible for the incomplete dechlorination process [92, 171, 180]. The appearance of VC in ACc bottles on day 34 onward was again due to liquid exchange from AWc bottles, where cis-DCE was degraded to VC in the period between day 25 and 32 (Figure 3.3-ATES).

3.3.3 Reductive dechlorination in NS condition

At the end of the experiment, nearly all cis-DCE was degraded in NSb bottles, with approximately 1.50 μmol residual VC remained as well (Figure 3.4). In the presence of *Dehalococcoides* and sufficient electron donor, the reductive dechlorination was not yet complete after 60 days experiment, but cis-DCE degradation and ethene production were evident at a normal groundwater temperature of 10 ˚C. Such finding is in line with a lactate and *Dehalococcoides* culture study at 10 ˚C [171]. However, in NSc the cis-DCE concentration remained at the same level throughout the experiment and no VC or ethene was detected. Hence, *Dehalococcoides* could be concluded as the main limiting factor for the occurrence of reductive dechlorination in the aquifer material tested here.

3.3.4 Performance on cis-DCE removal

Table 3.2 summarizes the performance on cis-DCE dechlorination in all biotic groups, including those in the preliminary experiment. The AWb batches achieved complete reductive dechlorination, not only in dechlorinating process from cis-DCE to ethene but also in the amount percentage (100%, all cis-DCE and VC were biodegraded to ethene at the end), with a removal rate of 1.14 μmol/day. However, this removal rate in AWb is underestimated, whereas it is strongly overestimated in ACb (0.69 μmol/day) in Table 3.2. This was because as discussed above, temperature of 5 ˚C was too low for biomass with concentration of 10% liquid phase to dechlorinate cis-DCE within the experimental time. The "removal" of cis-DCE in ACb was attributed to the liquid exchange which allowed cis-DCE being transferred to AWb and degraded there. Therefore, this amount of removed cis-DCE should be accounted for AWb, resulting in an actual removal of 1.83 μmol/day in AWb, while 0 in ACb. Two groups in BTES case had similar removal rates which were 0.56 and

0.65 µmol/day respectively (Table 3.2). The small difference was because of that the average temperature at which BCb stayed during the experiment was bit higher than BWb. In the NSb group, the removal rate was 0.14 µmol/day.
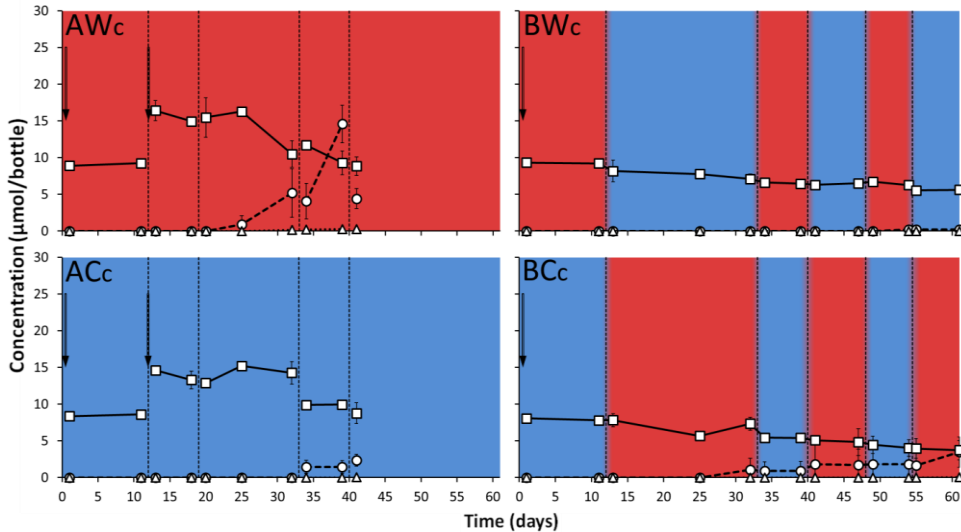
Figure 3.3 Concentration of cis-DCE (squares with straight line), VC (circles with dashed line) and ethene (triangles with dotted line) as a function of time in ATES controls (left) and BTES controls (right). Area of red and blue color represents the duration of bottles that stayed at 25 and 5 ˚C respectively. Black arrows indicate spiking of cis-DCE. Error bars indicate the standard deviation of the triplicates. When error bars are not visible, they are smaller than symbol size.
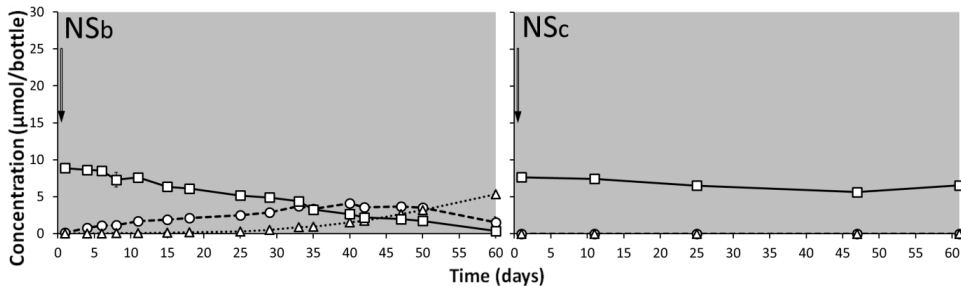
Figure 3.4 Concentration of cis-DCE (squares with straight line), VC (circles with dashed line) and ethene (triangles with dotted line) as a function of time in NSb (left) and NSc (right). Black arrows indicate spiking of cis-DCE. Error bars indicate the standard deviation of the triplicates (NSb) and duplicate (NSc). When error bars are not visible, they are smaller than symbol size.

Table 3.2 Performance and related condition of each experimental group.

Group	Total added cis-DCE (μmol)*	Residual CVOCs (μmol)**	Day	Removal Rate (μmol/day)***	Temperature (°C)	Biomass Concentration (%)
AWb	52.5	0	46	1.14	25	10
ACb	42	10.3	46	0.69	5	10
BWb	42	8.5	60	0.56	13.3[a]	10
BCb	42	3.2	60	0.65	16.7[a]	10
NSb	10.5	1.9	60	0.14	10	10
A	10.5	10.4	41	0.00	5	1
B	10.5	9.3	41	0.03	5	20
C	10.5	10.1	41	0.01	10	1
D	10.5	1.2	41	0.23	10	20

* Theoretical value, calculated based on the spiking times and stock concentration

** Measured concentration of cis-DCE and VC together at the end of experiment

*** Overall average removal rate, calculated as the difference between * and ** over experimental days

[a] Average temperature of BWb and BCb group during the experiment

Based on the performance of ACb and NSb together with the four pre-tested groups, interesting comparisons could be made. Group ACb and A both had no removal while group B, at the same temperature (5 °C) but with 2 times and 20 times higher biomass concentration compared to the former, had a removal rate of 0.03 μmol/day (Table 3.2). Similar comparison was between group NSb and C. Sufficient biomass concentration was required to initiate reductive dechlorination. Moreover, when biomass concentration was no longer limiting (in group NSb, B and D), the effect of temperature became more significant [171]. The removal rate was about 8 times higher in D than in B (in these two groups, biomass concentrations were the same, temperatures were 2 times different), while was less than 2 times difference between D and NSb (in these two group, temperatures were the same, biomass concentrations were 2 times different).

In summary, regarding to biotic groups, the overall performance in ATES was approximately 1.5 times and 13 times better than that in BTES and NS respectively. As in the ATES and BTES bottles, the environment was approximately half the time at 25 °C and half at 5 °C, the average system temperature could be estimated as 15 °C [181]. Therefore, the net increase in rate with ATES fluctuating conditions (with $\Delta T = 20$ °C and compared to a reference groundwater temperature of 11 °C) by a factor 1.5 as stated by Hartog could be an

underestimate. Instead, this study revealed a significant improvement by either ATES or BTES [181]. The explanation could be that bacterial growth is not included in the model presented by Hartog [181]. Once again the *Dehalococcoides* biomass concentration is shown to be paramount to either initiate or to achieve more complete dechlorination, not only in our study and other laboratory experiments [92, 171, 180], but also in pilot tests and in situ bioremediation projects [124, 174, 182]. Furthermore the role of temperature on reductive dechlorination becomes dominant as the growth of dechlorinating bacteria and corresponding dechlorination activity depended highly on temperature [120, 149, 171, 172].

3.4 Technological implications

In this research, we present the first laboratory simulation of the effects of UTES conditions on enhanced cis-DCE reductive dechlorination, mainly focusing on the aspects of fluctuating temperature and groundwater exchange between warm and cold well. From the groundwater contamination point of view, it appears that bioremediation of CVOCs can be promoted in a cost-effective manner using the UTES system, especially using ATES as vehicle for the bio-stimulation. Considering the long duration generally needed for enhanced natural attenuation of CVOCs contamination in groundwater, and the increasing demand for UTES systems, these results are highly relevant in view of integrated resource management for water and energy, thereby combining a sustainable energy use of the subsurface, with a faster clean-up of groundwater, a precious resource of drinking and industrial water.

Our results indicate that by applying ATES in combination with bioremediation, the overall cis-DCE removal performance can be simply intensified approximately 13 times compared to natural situation; and such improvement can be about 8.5 times better in the case of BTES. Although microorganisms would suffer of reduced activity at low temperature in the cold zone of an ATES system and dechlorination would be there stalled, the remaining CVOCs will be later transported to the warm zone where biodegradation is strongly enhanced. Moreover, this drawback can also be overcome by implementing more bioaugmentation. Within the typical lifespan of an ATES system of about 25 years, complete removal of CVOCs may now become a realistic and promising remediation goal. Eventually from a practical application point of view, selecting ATES or BTES to combine with enhanced

bioremediation requires further investigation and should be based on, the profile of the type of contamination (focus on dissolved CVOC plumes, i.e. extensive DNAPL source zones need to be addressed by a separate approach), the geochemical conditions of the contaminated aquifer and energy demand of the local area. As the dechlorinating microorganisms appear to preferably attach to the sediments rather than being transported with the groundwater, bioaugmentation would be recommended to be performed in the ATES warm wells. Though CVOCs would be transported due to seasonal groundwater movement in ATES, it is a better decision to place warm wells more close to the contaminated zones in the aquifer. This well placement issue is not that important for BTES. But unlike ATES which allows for injection of electron donor or dechlorinating microorganisms via existing wells, extra injection wells are needed to combine BTES with bioremediation and thus will be more costly. The combination of the sustainable energy production by UTES with biological groundwater treatment shows, based on laboratory experiments, good perspective especially for the ATES system.

Acknowledgement

Chapter IV

Combination of

Aquifer Thermal Energy Storage

and Enhanced Bioremediation:

Resilience of Reductive Dechlorination

to Redox Changes

Abstract

To meet the demand for sustainable energy, aquifer thermal energy storage (ATES) is widely used in the subsurface in urban areas. However, contamination of groundwater, especially with chlorinated volatile organic compounds (CVOCs), is often being encountered. This is commonly seen as an impediment to ATES implementation, although more recently combining ATES and enhanced bioremediation of CVOCs has been proposed. Issues to be addressed are the high water flow velocities and potential periodic redox fluctuation that accompany ATES. A column study was performed, at a high water flow velocity of 2 m/h, simulating possible changes in subsurface redox conditions due to ATES operation by serial additions of lactate and nitrate. The impacts of redox changes on reductive dechlorination as well as the microbial response of *Dehalococcoides* (DHC) were evaluated. The results showed that upon lactate addition reductive dechlorination proceeded well and complete dechlorination from cis-DCE to ethene was achieved. Upon subsequent nitrate addition reductive dechlorination immediately ceased. Disruption of microorganisms' retention was also immediate, and possibly detached DHC which preferred attaching to the soil matrix under biostimulation conditions. The detached DHC might be transferred to the liquid phase and eventually died off in nitrate reducing condition. Initially, recovery of dechlorination was possible, but required bioaugmentation and nutrient amendment in addition to lactate dosing. Repeated interruption of dechlorination and DHC activity by nitrate dosing appeared to be less easily reversible requiring more efforts for regenerating dechlorination. Overall, our results indicate that the microbial resilience of DHC in biosimulated ATES conditions is sensitive to redox fluctuations. Hence, combining ATES with bioremediation requires dedicated operation and monitoring on the aquifer geochemical conditions.

Keywords

4.1 Introduction

Aquifer thermal energy storage (ATES) has been developed since the 1970s and is considered as an energy saving and sustainable energy technology [41, 42, 155]. The principle of ATES is to store thermal energy when available and retrieve it for use when needed. In summer, groundwater is extracted from ATES cold well. At the heat exchanger the excessive heat from the building is transported to this water which is then re-injected into the aquifer at the ATES warm well. In the aquifer, a large fraction of heat remains available to be used in winter when the groundwater flow and energy extraction process will be reversed [42, 46]. Due to an increased demand for sustainable energy, existing ambitions to reduce greenhouse gas emission and its cost-effectiveness, ATES is increasingly being developed throughout the world, especially in the urban areas [39, 40, 157, 183]. For ATES development, Belgium, Germany, the Netherlands, Turkey, Sweden and the USA are currently leading [46, 76-80]. For example the number of ATES systems was 2740 in the Netherlands [159] and is expected to rise to 20,000 in 2020 [44]. Meanwhile, many urban areas are confronted with groundwater contaminations in the aquifer layer, especially with chlorinated solvents [8, 184-186]. Chlorinated solvents, in particular tetrachloroethene (PCE) and trichloroethene (TCE) have been applied widely as degreasers in industrial factories and for dry-cleaning since the 1930s [4, 99, 101]. Due to improper disposal, leakage and accidents, PCE and TCE together with their daughter products cis-dichloroethene (cis-DCE) and vinyl chloride (VC) have become the most prevalent groundwater contaminants in aquifers [2, 187]. These chlorinated volatile organic compounds (CVOCs) are often found at a depth in the subsurface similar to that at which ATES is usually applied [43, 188]. ATES involves the transportation of large volumes of groundwater, for instance 261 million m^3 groundwater was displaced by ATES in the Netherlands in 2013[170]. Hence, interference between CVOCs and ATES application at the same site can be a threat not only to the quality of groundwater and drinking water, but also to human health due to the vapour intrusion especially of the carcinogenic intermediate VC [189]. Faced with the long duration commonly needed for natural attenuation of CVOCs in the subsurface [27, 190] and the increasing demand for ATES, the combination of ATES and stimulated bioremediation might be prosperous to reduce the increasing pressure on the use of the subsurface. The increased temperature around the ATES warm well (20-25 ˚C) [43, 168] and using ATES as biostimulation tool -for example for addition of electron donor- are

the two main positive perspectives recognized for such combination (Chapter 6). Currently, two field scale tests are being performed in the Netherlands where ATES is applied at CVOCs contaminated sites [49, 168], which so far have not encountered practical problems. Furthermore, our previous laboratory study under simulated ATES conditions, focusing on cis-DCE biodegradation, showed 13 times higher degradation rates at increased temperature compared to biodegradation by enhanced natural attenuation at groundwater temperature of 10 ˚C (Chapter 3). These findings are promising, however, the impact on the microbial population is unclear, especially regarding the *Dehalococcoides* (DHC) species as the only group of bacteria strains capable of complete dechlorinating CVOCs to the harmless product ethene [62, 68, 70, 71, 116, 124].

The displacement of large volumes of groundwater by the ATES system will also have an impact on the local geochemical state of the aquifer. Redox conditions (of groundwater) in natural (undisturbed) aquifers can show a heterogeneous pattern over different depths and lateral positions [142, 191]. The mixing of large volumes of groundwater, together with temperature changes, may then result in an overall change of redox condition on the re-injected groundwater and thus the aquifer zone around the injection well [83, 192, 193]. Because of the seasonal operation of ATES, such change of redox condition can occur in both warm and cold zones which in the worst case can lead to well clogging by precipitation of iron (hydr)oxides when for example dissolved iron encounters nitrates or dissolved oxygen, [97, 194-197]. Also for microbiological processes in the combination of ATES and bioremediation of CVOCs the redox potential is a crucial factor, as DHC, which is primarily involved in the reductive dechlorination process, is sensitive to the redox condition [62, 124, 148, 198] and especially vulnerable to oxidized redox condition such as nitrate reducing and oxygenated condition [33]. Exposure to air or oxygen was found to cause irreversible inhibition of reductive dechlorination [198]. Several studies have revealed that DHC is natively found in many contaminated sites, though often at a very low number [174, 199]. Moreover, DHC is more abundant at contaminated sites with lower redox potential, where reductive dechlorination is well proceeding [127, 176, 180]. Therefore, changing redox condition of groundwater by ATES operation is expected to influence the CVOCs reductive dechlorination by affecting the activity of DHC. Previous studies showed the inhibition effect of nitrate on the dechlorination process of chloroaliphatic [173, 200-205] and chloroaromatic

compounds [206-212], while only very few studies focused on the responsible microorganism DHC upon its reaction to oxygen [198, 213, 214]. However these studies have not cover the situation of possible variation of redox conditions due to seasonal operation of ATES. This study aims to improve the understanding of the resilience of the dechlorinating process by bacteria that are active in contaminated aquifers upon disturbances by redox fluctuation that can be generated by the functioning of an ATES system. We focus on the warm well as we expect that most of the conversion of CVOCs is located at that area (Chapter 3). Dynamic redox conditions that might occur in an ATES system were simulated at lab scale by consequent nitrate and lactate additions, and were used to determine chemical changes as well as effects on the microbial community and its characteristics.

4.2 Materials and methods

4.2.1   Materials

Aquifer material from the city of Utrecht, similar as in our previous study [92] on PCE biodegradation in a Fe(III) reducing aquifer was used. All chemical stock solutions were prepared with anaerobic deionised water, which was purged with pure N_2 for at least 3 hours. Sodium lactate (SL) stock solution (225 g/L), cis-DCE stock solution (395 mg/L) and $NaNO_3$ stock solution (85 g/L) were prepared respectively with SL powder (≥99% purity, Aldrich®), $NaNO_3$ powder (≥99% purity, Aldrich®) and cis-DCE (99% anhydrous, Aldrich®). Anaerobic tap water, prepared similarly as anaerobic deionised water, from the lab was used as liquid medium in the column experiment.

A mixed culture containing 1.3-4.4 $\times 10^8$ DHC cells/mL liquid (or DNA copies/mL liquid, based on the assumption that 1 DNA-copy is equal to 1 cell) was provided by Bioclear BV (Groningen, NL). This culture was occasionally fed with cis-DCE and lactate prior to the experiment. Inoculation was performed when cis-DCE and VC were found absent in the headspace of the inoculum bottle.

4.2.2   Analytical methods

Redox potential and pH of the experimental systems was monitored by a Consort multi-channel (C3060) meter and data logger with ProSense (QIS) standard Pt redox

electrode with Ag/AgCl as reference electrode (-199 mV vs. standard hydrogen electrode (SHE)) in saturated KCl solution [141] and standard pH electrode (QIS) respectively. Temperature was also monitored and logged by C3060 meter. Logging interval of redox potential, pH and temperature was 1 minute.

Dissolved Fe^{2+}, SO_4^{2-}, lactate and volatile fatty acids (VFAs) were determined using the same analytical methods as in our previous study with redox titration method on PCE biodegradation in Fe(III) reducing aquifer [92]. Biogas was analysed and quantified for CO_2, CH_4 and O_2 in the headspace on a Shimadzu 2010 GC with Thermal Conductivity Detector (TCD) and with helium as carrier gas. Loop injection of 2 mL headspace sample was performed at 120 ˚C. Chlorinated ethene (cis-DCE and VC) and ethene were quantified by direct injection of 100 µL (with glass syringe) on a HP6890 series GC equipped with a CP PoraBond Q column (25 m × 0.53 mm × 10 µm) and Flame Ionization Detector (FID). Nitrogen gas was used as carrier gas at a flow rate of 25 mL/min. The temperature program started at 60 ˚C, ramped at 17.78 ˚C /min to 140 ˚C and held at 140 ˚C for 1.5 minutes.

Microbial analysis (total bacteria and DHC cell*) was done by BioClear BV. Before analyses, 5 mL effluent from the column was collected and stored at 4 ˚C, in a special storage kit from Bioclear BV.

4.2.3   Recirculating column set-up

Overview and specification
The schematic overview and specification of the column set-up are given in Figure 4.1, Table S4.1 and Figure S4.1.

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* http://www.microbialanalysis.com/
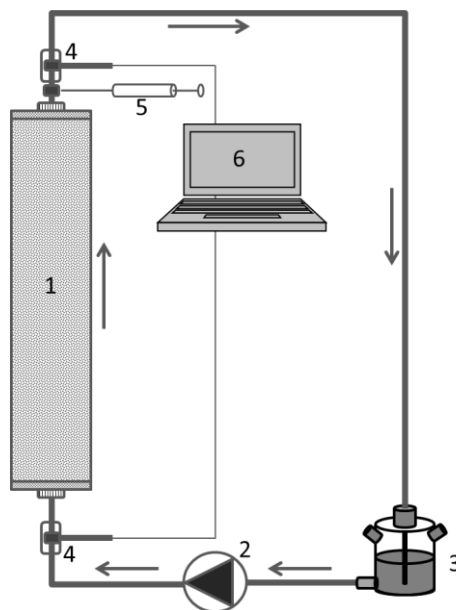
Figure 4.1 Schematic diagram of column set-up. 1: aquifer material with glass filters with pore size of 160 μm on the top and bottom of the column; 2: membrane pump; 3: buffering bottle; 4: redox and pH electrodes (bottom is for influent, top is for effluent); 5: liquid sampling port; 6: meter connected with computer. Arrow indicates the direction of water flow.

The various parts of the column set-up were connected by Teflon tubings (Ø4mm) with quick connectors with valves. The aqueous phase was recirculated by a SIMDOS® diaphragm metering pump (flow rate range: 1-100 mL/min). Glass filters (pore size with P2 specification: 100-160 μm) were placed on the top and bottom of the column to prevent particles from being flushed out. A 150 mL double-side armed bottle with approximately 100 mL headspace volume was placed in the middle of the system for headspace sampling and to prevent under-pressure during liquid and headspace sampling.

Preparation and maintenance of column

All individual parts of the column setup were filled inside an anaerobic hood (95% N_2 and 5% H_2) with either aquifer material or anaerobic tap water. The buffering bottle was additionally conditioned by exchanging the headspace 10 times with 99% N_2 and 1% CO_2 gas by an automatic headspace exchanger. The individual parts of the set-up were then attached by the quick connectors and, after opening the valves, the aqueous phase was

allowed to circulate at a flow rate of 10 mL/min. This flow rate is comparable to a groundwater velocity of 2 m/h, which is a realistic flow velocity in aquifers 1.3m away from an ATES well. This ATES system is assumed to operate with a flow rate of 100 m^3/h and with 20 m filter length (see calculation Figure S4.2).

During the first few days, the liquid level in the buffering bottles was lowered due to degassing of the aquifer column and tubing. Extra anaerobic tap water was injected in the bottle to maintain the liquid volume at approximately 50 mL. This procedure to maintain the liquid volume was also carried out later during the experiment, in case more gas bubbles were produced by degassing from the column. Moreover, after every liquid sampling, the same amount of anaerobic tap water as liquid sample was injected.

During the whole experimental period, small but continuous diffusion of oxygen was occurring, resulting in a volume concentration of about 1% in the headspace of the buffering bottle (see Figure 4.3). By lactate additions (Table 4.1) the redox condition in the columns was always kept anaerobic as can be observed from the redox measurements at the inflow and out flow of the sediment column (see Figure 4.2). Besides, some continuous removal of cis-DCE, VC and ethene from the column was also observed, most probably due to diffusion through the Teflon tubing [215, 216]. Parker et al. [216] showed a more than 10% loss of chlorinated organic in the stored sampling with Teflon material after eight hours. Therefore, a continuous dissipation of CVOCs was observed (see Figure 4.4), however, the formation and development trend of these compounds could still well be observed to determine biodegradation of cis-DCE and reflect the DHC resilience. The used tubing was the best to our knowledge that could be used in this experiment.

Experimental procedures

Dynamic redox conditions were simulated by adding lactate as electron donor to achieve lower redox potential or by adding nitrate as electron acceptor to raise the redox potential. In total, two cycles of low and elevated redox potential were performed. During the experiment, liquid was sampled at position 5 in Figure 4.1, while headspace sampling was done at the side arms of the buffering bottle. Addition of chemicals was also done via these side arms. The experiment lasted for 140 days with in total of 28 actions which are listed in Table 4.1.

Table 4.1 Overview of actions and related additions of different chemicals.

Action	Day	Lactate (225 g/L)	Inoculum (5 mL)	cis-DCE (5 mL; 395 mg/L)	NaNO$_3$ (5 mL; 85 mg/L)	other
1	20	1.5 mL				
2	30		1.3×10^8 cells/mL			
3	41		3.2×10^8 cells/mL	X		
4	45	0.5 mL				
5	48			X		
6	51			X		
7	55	0.5 mL				
8	56			X		
9	57				X	
10	62			X		
11	66	1 mL				
12	71	0.5 mL				
13	76			X		
14	80		2.5×10^8 cells/mL			
15	84	0.5 mL				
16	91			X		
17	92	0.5 mL				
18	93		2.9×10^8 cells/mL			
19	94					nutrient (2.5mL; Na$_2$HPO$_4$ 50 g/L, KH$_2$PO$_4$ 50 g/L, NH$_4$Cl 10 g/L)
20	98	0.5 mL		X		
21	101	0.5 mL				
22	104	0.5 mL		X		
23	105				X	
24	114	1 mL				
25	118					NH$_4$Cl (2.5mL; 10g/L)
26	125		3.6×10^8 cells/mL			
27	132		4.4×10^8 cells/mL			
28	134	0.5 mL				
29	135					vitamin B$_{12}$ medium (5 mL; 2 mg/L)

Important procedures included: 1) Monitoring initial behaviour of selected aquifer material in the column system in the adaptation period of 20 days; 2) Redox conditioning by adding lactate as electron donor to achieve an environmental condition with redox potential around -450 mV to stimulate reductive dechlorination [92]. For this purpose, three lactate additions on day 20, 66 and 114 were performed with 1.5, 1 and 1 mL lactate stock respectively (Table 4.1). Additional lactate additions of 0.5 mL lactate stock on day 45, 55, 71, 84, 92, 98, 101, 104 and 134 were to maintain the low redox potential that is suitable for DHC. The addition of electron donor was found to be necessary as the redox potential slowly increased, due to the continuous diffusion of O_2 through the Teflon tubing; 3) Inoculating the column system with DHC in the period without any chlorinated ethenes present to study the distribution of DHC in the model ATES system (day 30), the subsequent DHC inoculations on day 41, 80, 93, 125 and 132 (Table 4.1) were performed to start-up or to recover the reductive dechlorination; 4) Spiking of cis-DCE stock. In total 9 times of cis-DCE spiking with 5 mL cis-DCE stock for each; approximately 20 μmol cis-DCE/spiking (Table 4.1) were conducted on day 41, 48, 51, 56, 62, 76, 91, 98 and 104; 5) Two times nitrate stock additions (5 mL for each) as redox shock to bring up the redox potential on day 57 and 105; 6) Addition of 2.5 mL of nutrient solution containing 50 g/L Na_2HPO_4, 50 g/L KH_2PO_4 and 10 g/L NH_4Cl), 2.5 mL of 10 g/L NH_4Cl, and 5 mL vitamin B_{12} medium on day 94, 118 and 135 for promoting the recovery of reductive dechlorination.

4.3 Results

4.3.1   Redox potential and other redox indicators

During the first 20 days, the redox potential in the column was monitored without any redox conditioning steps. After an initial stabilization at -100 mV, the redox potential rapidly dropped to -350 mV on day 4, and further to -450 mV on day 6 (Figure 4.2). Thereafter, starting at day 12, the redox potential increased up to about 150 mV on day 20, ascribed to oxygen diffusion through the Teflon tubing.
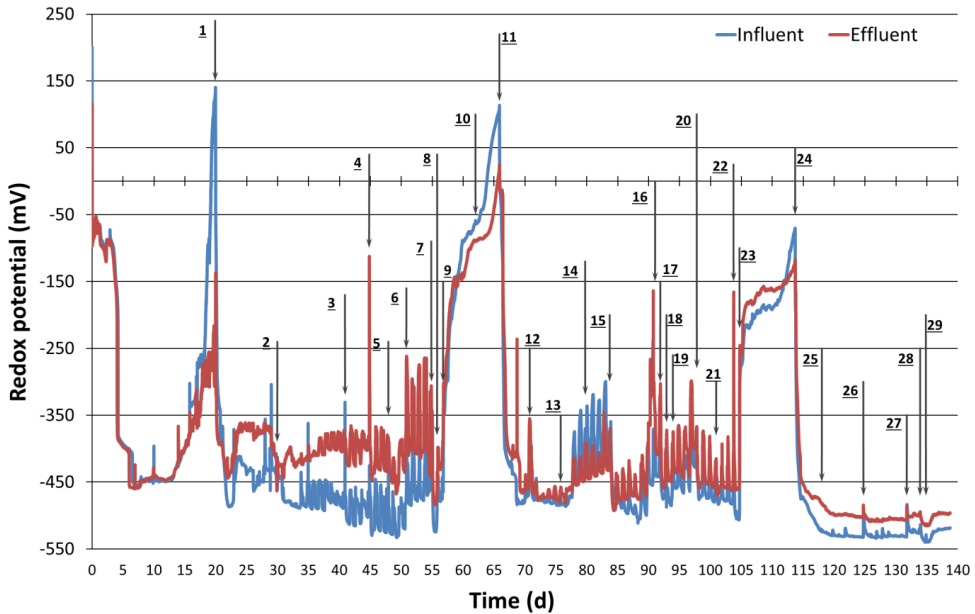
Figure 4.2 Redox potential of the influent (blue line) and the effluent (red line). Black arrows and with numbers indicate different actions listed in Table 4.1.

After addition of lactate (action, day 20), the redox potential in both influent and effluent decreased to -450 mV and remained stable at this low value. This low redox potential is reported to be suitable for reductive dechlorination and methanogenesis [4, 92, 142]. The nitrate addition on (action 9, day 57) resulted in an increase of redox potentials up to -150 mV within two days and continued to increase to 107 mV in the influent and 25 mV in the effluent during the following 8 days (Figure 4.2). The subsequent lowering of the redox potential was initiated by the addition of lactate on day 66 (action 11). Similar to the first lactate addition, the redox potential dropped sharply to below -400 mV within one day, and remained stable until day 105 when the second nitrate addition was performed (action 23). After this second nitrate addition, the redox potential increased up to -70 mV, which was around 170 mV below the value reached upon the first nitrate addition. Low redox potentials (from -400 to -500 mV) were again obtained after the 3rd addition of lactate on day 114 (action 24). Interestingly, the redox potential showed less noise after this lactate addition compared to earlier additions of lactate (Figure 4.2 after action 24).

Prior to any addition of lactate, acetate was detected with a maximum concentration of 0.27 mmol/L on day 7 and was consumed before the first lactate addition on day 20. Propionate was not detected at this stage. After the first addition of lactate, the concentration of acetate increased rapidly to 8.55 mmol/L, while the concentration of propionate increased to 2.43 mmol/L (Figure 4.3-A). The concentration of these two volatile fatty acids (VFAs) followed a pattern of rapid increase and gradual decrease with every lactate addition. After nitrate additions (action 9, day 57 and action 23, day 105), the decrease of VFA concentration was fast, however. Lactate was not detected along the experiment indicating that the production of acetate and propionate resulted from a fast process such as lactate fermentation [92, 143].

The initial concentration of Fe^{2+} in the aqueous phase was close to 148 μmol/L and decreased to below 4 μmol/L during the three weeks prior to the first addition of lactate (Figure 4.3-B). The first addition of lactate lead to a large increase of Fe^{2+} concentration up to 286 μmol/L on day 23 whereafter the concentration returned to low values (0-20 μmol/L). Subsequent additions of lactate did not result in elevated Fe^{2+} concentrations (Figure 4.3-B). Sulphate concentration, (1.8 mmol/L at the start), decreased steadily to about 1.0 mmol/L during the first 20 days. Thereafter the sulphate concentration declined quickly to negligible zero with first lactate addition (action 1, day 20). Only after nitrate addition (action 9, day 57; action 23, day105) elevated sulphate concentrations were observed (Figure 4.3-B).

Biogas analysis showed that the concentration of CO_2 increased already from the beginning of the experiment and the percentage reached a highest value of around 6% on day 23 (Figure 4.3-C). In the two periods when nitrate was added (action 9, day 57; action 23, day105), the CO_2 percentage rapidly decreased to about 2% and 1% respectively. After subsequent lactate addition (action 11, day 66; 24, day 114) the CO_2 gradually returned to values around 4%, with some fluctuations in the rest of periods (Figure 4.3-C). CH_4 was detected after the first lactate addition (action1, day 20). The highest CH_4 concentration was observed right before nitrate addition on day 57 (around 2%). After the second nitrate addition, the concentration slowly declined to less than 1% and further below 0.5%. The O_2 percentage in the headspace of the buffering bottle was stable at about 1% during the experiment (Figure 4.3-C).
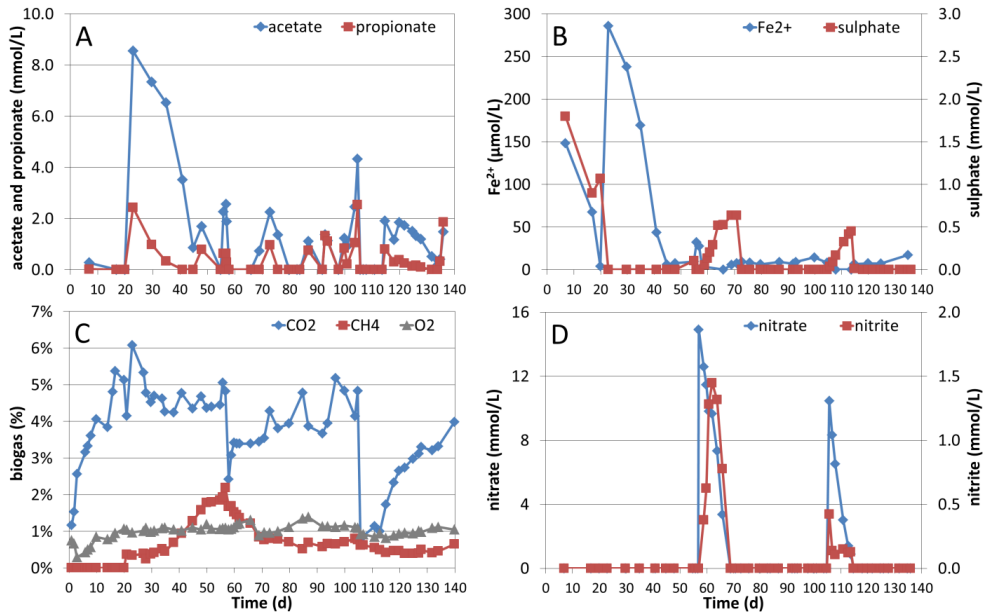
Figure 4.3 Concentrations of different compounds as a function of experimental time. A (top left) represents acetate and propionate concentration with unit of mmol/L in liquid sampling port; B (top right) represent Fe^{2+} (primary y-axis) and sulphate (secondary y-axis) concentration with unit of μmol/L and mmol/L respectively in liquid sampling port; C (bottom left) represents O_2, CH_4 and CO_2 gas composition in the headspace of the buffering bottle in percentage; D (bottom right) represent nitrate (primary y-axis) and nitrite (secondary y-axis) concentration with unit of mmol/L in liquid sampling port.

Neither nitrate nor nitrate was detected before nitrate addition on day 57. Nitrate concentration peaked at 15.0 mmol/L on day 58, after which the production of nitrite occurred with a maximum concentration of 1.45 mmol/L (Figure 4.3-D). After lactate addition (action 11, day 66), nitrate and nitrate concentrations rapidly decreased to below detection limits on day 69. A similar pattern was observed after the second nitrate addition although the peak concentrations for both nitrate (10.5 mmol/L) and nitrite (0.4 mmol/L) were lower than those in the first time. The nitrite peak was smaller and occurred simultaneously with nitrate, indicating the presence of adapted nitrate and nitrite reducing microbial community and thus faster denitrification process.

4.3.2   Reductive dechlorination and microbial population

First cis-DCE was spiked on day 41 (action 3) when the column remained at stable conditions regarding the redox indicators. Readily after spiking with cis-DCE the presence of VC and ethene was observed, indicating that the complete pathway of reductive dechlorination is occurring (Figure 4.4). When the redox went up to -250 mV unexpectedly, the production of VC and ethene ceased (day 52). However, after the lactate addition on day 55 (action 7), the production of these compounds resumed and reached 24.6 and 11.8 μmol for VC and ethene respectively.

After the first nitrate addition (action 9, day 57) the production of VC and ethene stopped, although all three compounds disappeared with comparable rates from the reactor (Figure 4.4), most probably due to diffusion via the via the Teflon tubing. Restoring the redox potential to its previous low values by the addition of lactate (action 11, day 66 and action 12, day 71) did not result in any noticeable recovery of reductive dechlorination. Even after adding fresh DHC (action14, day 80), two additions of lactate (action 15, day 84, action17, day 92), followed by DHC inoculum (action18, day 93 (DHC), dechlorination did not resume. Only after adding nutrients (action 19, day 94) the production of VC and ethene observably increased, which was further enhanced upon an extra addition of cis-DCE and lactate (action 20, day 98).

The second addition of nitrate (action 23, day 105) aimed at a repetition to test the resilience of reductive dechlorination after a redox shock. This time, reductive dechlorination as evidenced by production of VC and ethene, did not resume after addition of lactate (action 24, day 114), nutrients (action 25, day 118), and two DHC inoculum (action 26, day 125, action 27, day 132), again lactate addition (action 28, day 134) and finally addition of vitamin B_{12} (action 29, day 135).

The presence of DHC in the liquid phase followed with time is presented in Figure 4.5, as well as the concentration of the total bacteria in the liquid phase. During the first 30 days, DHC concentration was below detection limit. Readily after inoculation with 5 mL DHC (1.3×10^8 cells/mL), the DHC could be detected in liquid but below the quantification limit (red diamond on day 40 in Figure 4.5).
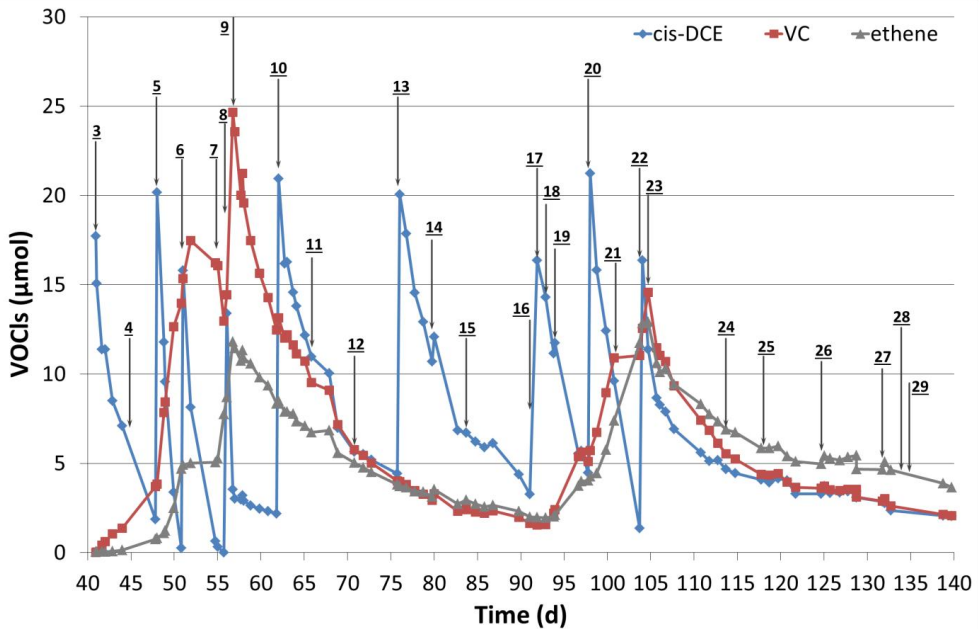
Figure 4.4 Concentration of cis-DCE (blue diamond), VC (red square) and ethene (grey triangle) as a function of experimental time. Black arrows and with numbers indicate different actions listed in Table 4.1.

After cis-DCE was spiked and reductive dechlorination occurred (day 40-56) the DHC concentration increased to 5.6×10^4 cells/mL, which was about 2% of the total bacteria concentration in the liquid phase. Interestingly, an increased concentration of DHC (3.1×10^5 cells/mL) was detected in the liquid only 2 days after nitrate addition, while reductive dechlorination had ceased. Shortly after this increase the concentration of DHC diminished to below detection limit in the next analysis on day 66 (Figure 4.5) and remained at this low concentration even after a new inoculation (action 14, day 80).

After a second inoculation (action 18, day 93) and the addition of nutrients (action 19, day 94), reductive dechlorination resumed and DHC concentration increased to 6.9×10^4 cells/mL, which was quantifiable though low compared to the amount of total bacteria (1.7×10^8 cells/mL).

The second nitrate addition showed that the DHC concentration gradually declined to 5×10^3 cells/mL after 10 days (Figure 4.5) (action 23, day 105). Aiming to resume the reductive dechlorination DHC was inoculated twice (action 26, day 125, action 27, day 132) resulting in a DHC concentration of 3.5×10^4 cells/mL in the liquid, at the end of experiment. However reductive dechlorination, as evidenced by an increase in ethene, did not resume.

The total bacteria concentration in the liquid phase with initial concentration of 2.8×10^5 cells/mL significantly expanded during the experiment. The maximum expansion reached 4 orders of magnitude on day 107 with concentration of 3.2×10^9 cells/mL, despite the final concentration reduced to 2.5×10^7 cells/mL. A variety of microbiological processes might be stimulated by this bacterial community, as was reflected by the responses upon redox chemistry and bioprocess indicators presented in Figure 4.3.
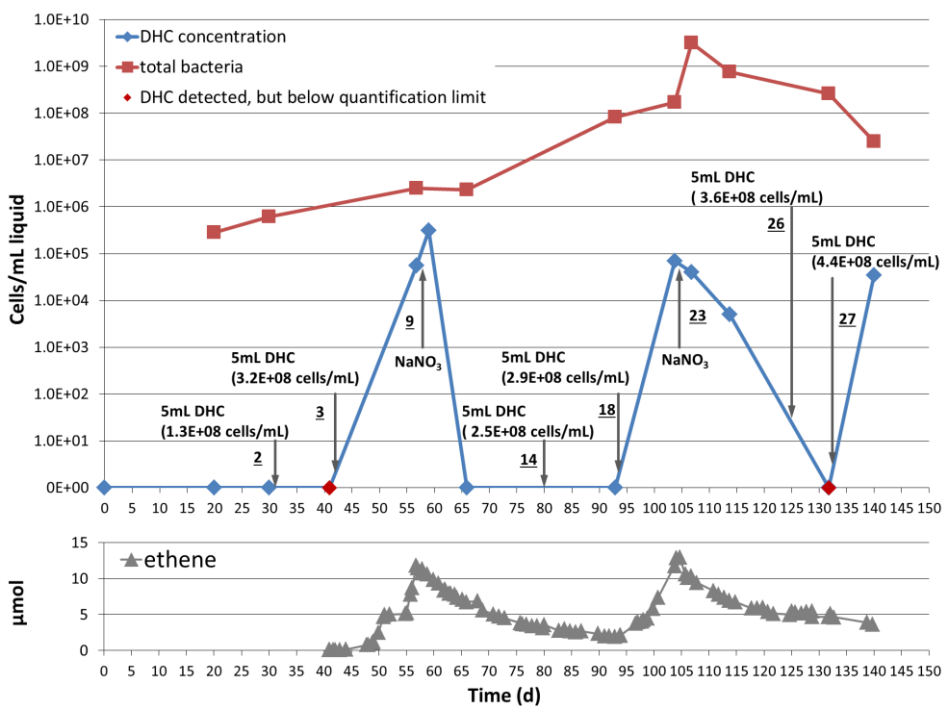
Figure 4.5 Change of DHC (blue or red diamond) and total bacteria (red square) concentrations in the liquid phase along time and with comparison to the change of ethene concentration (grey triangle) during the experiment. Black arrows indicate either addition of active DHC inoculum (action 2, 3, 14, 18, 26 and 27) or nitrate (action 9 and 23).

4.4 Discussion

4.4.1   Redox parameters

After the start of the experiment, the redox potential in both inflow and outflow showed a rapid lowering down to -380 mV at day 4, followed by a period of gradual decrease down to -400 mV at day 6 and again a rapid decrease down to -450 mV at day 7. Most probably organic matter is conversed in this phase which released by packing of the column and by organic particles that are disrupted by shear forces at the high liquid flow velocity [217]. The released organic matter can function as electron donor for biodegradation as we observed in a batch experiments with the same aquifer material at intensive shaking condition (Figure S4.3). The period of gradual decreasing redox potential between day 4 and 6 probably gave onset to the reduction of readily available Fe^{3+} species to dissolved Fe^{2+}, as evidenced by the remaining Fe^{2+} concentration measured at day 7.

The net removal of Fe^{2+} from the liquid phase together with sulphate points towards continued reduction of both iron and sulphate and subsequent precipitation as iron sulphides between day 7 and day 17. Then, however, the gradual increase in redox potential due to the oxygen diffusion inverted these processes as, between day 18 and 20, sulphate partly re dissolved. The on-going removal of Fe^{2+} from the liquid phase implies that during this period also iron re-oxidised, now precipitating again as iron (hydr)oxides. With the first lactate addition at day 20, the remaining sulphate was rapidly reduced to sulphide (Figure 4.3-B). The rapid increase in Fe^{2+} after this first lactate addition, when no DCE had yet been added to the system, seems to be related to the concurrent high acetate concentration (Figure 4.3-A), which suggests increased iron solubility due to Fe-acetate complexation [218]. Thereafter the Fe^{2+} concentration was always below 30 µmol/L, which can be caused by precipitation with sulphide again or carbonate, leading to formation of FeS and $FeCO_3$ with negligible solubility [219, 220].

After 12 days the readily available organic matter seemed to be depleted and could not donate electrons to sufficiently compensate the inflow of oxygen that was presumed to diffuse through the Teflon tubing into the system [221, 222]. This is supported by the observation that the redox potential of the inflow is continuously higher than the redox potential of the

outflow. By addition of lactate the effect of oxygen diffusion on the redox potential was negligible and a stable low redox potential was obtained.

After the nitrate additions (action 9, day 57 and action 23, day105) the redox potential elevated to 100 mV and -70 mV respectively (Figure 4.2). This increase resulted in a temporary increase of sulphate, as sulphide oxidation was occurring coupled with nitrate as terminal electron acceptor [223, 224]. In these nitrate reducing periods, Fe^{2+} as electron donor was also oxidized by nitrate leading to no detectable concentration in the dissolved phase [225]. The nitrate is then reduced to nitrite by lactate or VFAs (Figure 4.3-D) which is consistent with other studies [226, 227].

The build-up of both CO_2 and CH_4 in the gas phase, especially in the first period of biostimulation, is evidence of methanogenic redox conditions being achieved. The sudden decrease in CO_2 concentration in the headspace upon nitrate addition was attributed to the fast consumption of protons and electrons in the system for nitrate reduction [228, 229]. The consumption of protons led to a clear increase in pH level up to 7.6 and 8.0 (Fig. S4, influent), resulting in of the uptake of CO_2 (g) into HCO_3^- (aq) [230]. The decrease of methane concentration upon nitrate addition is attributed to the inhibition of methanogenesis by nitrate and diffusion of methane through the Teflon tubing [231, 232].

4.4.2   Resilience of Dehalococcoides and robustness of the reductive dechlorination process upon redox changes

At the start of the experiment, DHC was not detected which was expected as CVOCs were absent in the original samples and the redox conditions were unfavourable for reductive dechlorination [68, 92, 124, 129]. The absence of DHC in the original sample is furthermore supported by Cupples et al. who stated that the decay rate of DHC increases if the concentration of cis-DCE and VC as electron acceptor is less than 0.7 μmol [233]. Only after inoculation with 5 mL inoculum of around 108 cells/mL, DHC could be detected, although at a low level, whereas if all DHC was suspended about 107 cells/mL should be present. In fact during the whole experiment, the observed DHC concentration by sampling from the liquid phase was virtually negligible compared to the DHC that was inoculated. Even the quantifiable measurements observed in periods of active dechlorination showed that DHC concentrations were still less than 0.1% of the previously added amount, indicating that large

part of DHC is preferentially attached to the soil matrix. This finding is consistent with other studies which show DHC are less planktonic and mostly favour to attach to soil [176, 177]. Besides, Doong et al. showed the performance of dechlorinating bacteria is 2 to 5 times better with attachment to porous medium [179]. Furthermore, growth of DHC could be expected in the two active periods of reductive dechlorination [234-236], which could be the reason that quantifiable DHC were detected in the liquid phase on day 56 and 104. The small (one order of magnitude) increase in DHC concentration between day 57 and 59, with the subsequent measurement below detection, could be due to interruption by nitrate of DHC activity, leading to its detachment and transfer to the liquid phase with DHC dying off eventually. The balance between the rate of DHC detachment and DHC decay after the second nitrate dose seems slightly different. Interestingly, the final analysis showed a quantifiable DHC concentration in the liquid but without observable dechlorination proceeding. An explanation may be cis-DCE concentration, which was below 5 μmol after day 115 (Figure 4.4), was too low for DHC to metabolize or even survive, leading to some persisting inoculated cells to be observed in the liquid.

The inhibition of nitrate on reductive dechlorination found in this study upon nitrate addition was in line with previous research [204, 205, 237]. After the first nitrate shock, the reductive dechlorination process could be restarted, but only by adding new DHC and nutrients. After the second nitrate shock, no restart of the dechlorination process at any significant rate could be observed. Irreversible inhibition of dechlorination by elevated redox potential has been reported by several studies [198, 205, 238]. Yet, recovery of reductive dechlorination after applying chemical oxidation by permanganate and persulphate followed by bioaugmentation in laboratory experiments has also been described [238-240]. In a field test, recovery was even reported to occur without bioaugmentation, but ascribed to inflow of groundwater and recolonization by microorganisms from unaffected zones [26]. Although these studies are confronted with lag periods of up to hundreds of days, recovery of reductive dechlorination is proven with amendments which were mainly attributed to bioaugmentation. In contrast, our column was much less robust, even after several amendments including sufficient electron donor, nutrient, bioaugmentation and vitamin B_{12} which are beneficial to either DHC growth or reductive dechlorination [4, 241-244]. One reason could be the much higher flow rates in our experiment compared to other column systems [239, 240]. Another explanation

could be that the diffusion rate of cis-DCE, VC and ethene overcame the dechlorination rate, concealing any ongoing dechlorination process from being observed. The last cis-DCE spiking was performed on day 104 and its concentration had dropped to below 5 μmol after 10 days (Figure 4.4). Such low cis-DCE concentration might be insufficient to show measurable conversion into VC or ethene by DHC, while diffusion was always proceeding.

4.4.3 Implications for combining CVOCs bioremediation and ATES

Our results show that reductive dechlorination is sensitive to increased redox potentials. Therefore proper operation of ATES is recommended avoiding the mixing of oxidized groundwater into more reduced aquifers. Such approach is already in practice for iron rich groundwater, as iron precipitates and therefore clogging will occur with oxygenated water. Further it was shown that at the mimicked ATES conditions, active dehalogenating DHC cells appear to be preferentially attached to the soil matrix, instead of being mobile and transported with the water phase. Hence for the combination ATES and bioremediation of CVOCs, bioaugmentation by injecting mobile DHC will profit from this behaviour, as it can be expected that DHC augmentation will lead to attachment to the soil matrix after injection. From our experiment it is expected that inoculation with DHC in the warm well creates optimal conditions in the warm ATES well for reductive dechlorination at high biomass concentrations and at elevated temperature. These results show that practical application of the combination of ATES and in situ dehalogenation technologies can be promising by proper engineering, initial characterisation, continued monitoring of the groundwater systems, and well-designed bioaugmentation procedures.

Acknowledgement

Supporting material

Figure S4.1 Photo of the column set-up. 1: sediment column with glass filters on the top and bottom; 2: membrane pump; 3: buffering bottle; 4: redox and pH electrodes 5: liquid sampling port; 6: electrode wires connected to computer (not in this picture).

Table S4.1 Specification of column set-up.

Parameters	Value	Unit
Length	30.0	[cm]
Diameter	3.6	[cm]
Wet weight of aquifer material	625	[g]
Porosity	0.3	[-]
Pore volume	92	[mL]
Hydraulic Retention Time (HRT)	9.2	[min]
Volume of tubes and other connection parts	100	[mL]
Total liquid volume	240	[mL]
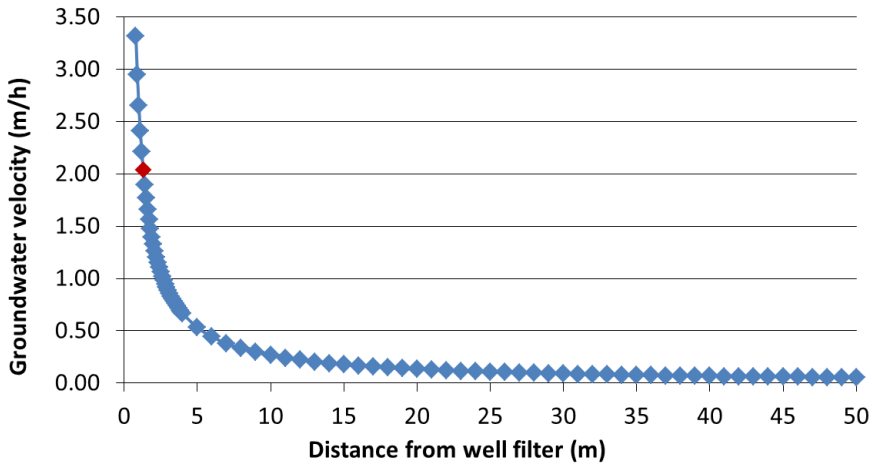
Figure S4.2 Groundwater velocity as a function of distance from well filter in ATES subsurface. The red diamond represents the mimicked water velocity in our column study.

This ATES operates with a flow rate of 100 m^3/h (Q) and with 20 m filter length (L). Using an aquifer porosity of 0.3 (ε), the relation between groundwater velocity (v) and distance from well filter (x) can be calculated via the following equation: $\mathrm{v} = \dfrac{Q}{2\pi x L \varepsilon}$

Therefore, the groundwater velocity is 2 m/h when it is 1.3 m from the well filter. A flow rate of 10 mL/min was applied in our column experiment, with the parameter given in Table S4.1, the water velocity in the column is calculated to be 2 m/h (flow rate over cross-section area and porosity).
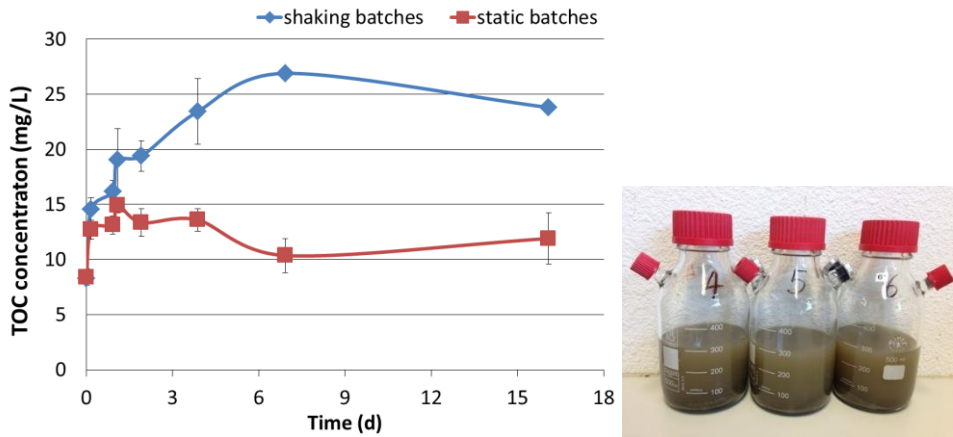
Figure S4.3 TOC (total organic carbon) concentration as a function of experimental time (left) in shaking (blue diamond) and static (red square) conditions in preliminary test upon effect of shaking on release of organic carbon at 25 ˚C and photo of shaking batches (right). Error bars represent standard deviation of the triplicates. When they are invisible, they are smaller than the symbols and hidden behind.

The preliminary experiment was performed using 500 mL double-side armed which contained 100 g wet aquifer material and 250 mL anaerobic tap water, with anaerobic headspace of 99% N_2 and 1% CO_2. One group of batches were shaken orbitally at a speed of 175 rpm, while other group of batches were always static during the experiment. The experiment was done in triplicate and at 25 ˚C. Liquid was periodically sampled for TOC measurements. TOC concentration was determined was measured by Hach Lange cuvette tests with Dr Lange LCK-385 (3-30 mg/L) on a Xion 500 spectrophotometer.
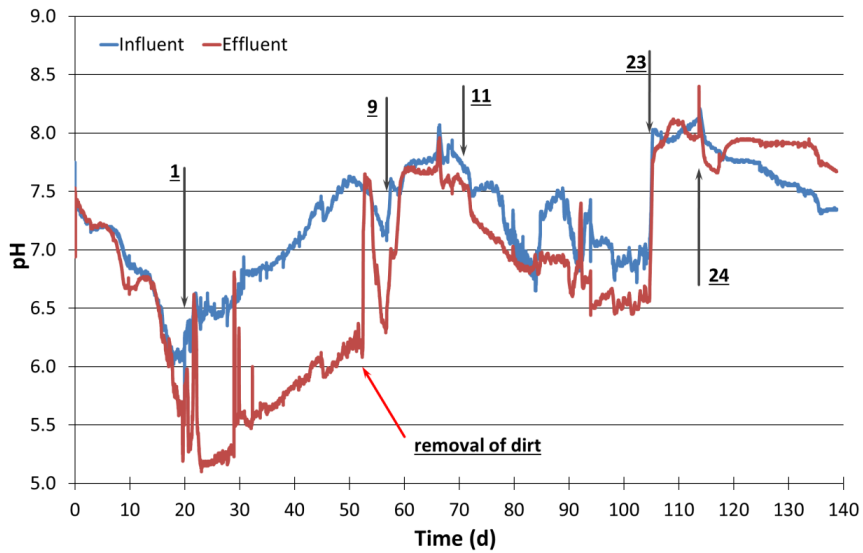
Figure S4.4 The pH of the influent (blue line) and the effluent (red line). Black arrows and with numbers indicate different actions listed in Table 4.1. Red arrow stands for the removal of dirt around the effluent pH electrode.

A relatively large deviation between pH readings of the influent and effluent was recorded from around day 23. This deviation lasted until day 52, when some dirt around the effluent pH electrode was observed. With removal of the dirt, pH readings of the influent and effluent were consistent thereafter.

Chapter V

Combination of

Aquifer Thermal Energy Storage

and Enhanced Bioremediation:

Biological and Chemical Clogging

Abstract

Interest in the combination concept of ATES and enhanced bioremediation has recently risen due to the demand for both renewable energy technology and sustainable groundwater management in urban areas. However, the impact of enhanced bioremediation on ATES is not yet clear. Of main concern is the potential for biological clogging, that might be enhanced and hamper the proper functioning of ATES. On the other hand, more reduced conditions in the subsurface by enhanced bioremediation might lower the chance of chemical clogging, which is normally caused by Fe-oxide precipitate. To investigate the possible effects of enhanced bioremediation on clogging with ATES, we conducted two recirculating column experiments with differing flow rates (10 and 50 mL/min), where enhanced biological activity and chemically promoted Fe-oxide precipitation were studied by addition of lactate and nitrate respectively. The pressure drop between the influent and effluent side of the column was used as a measure of the (change in) hydraulic conductivity, as indication of clogging in these model ATES systems. The results showed no increase in upstream pressure during the period of enhanced biological activity (after lactate addition) under both flow rates, while the addition of nitrate lead to significant buildup of the pressure drop. However, at the flow rate of 10 mL/min, high pressure buildup caused by nitrate addition could be alleviated by lactate addition. This indicates that the risk of biological clogging is relatively small with lactate as substrate for enhanced bioremediation and furthermore that lactate may counter chemical clogging. Because the pressure drop over the column is positively related to flow rate, at the flow rate of 50 mL/min the much higher buildup of pressure drop from chemical clogging could not be released in time through lactate addition, and an unforeseen blow-up of the column occurred. This implies that proper functioning of ATES systems at higher flow rate requires more careful operation. In summary, the effects of enhanced bioremediation on potential clogging with ATES are deemed positive, and a combined approach is indeed promising, especially in view of redevelopment opportunities for urban areas.

Keywords

5.1 Introduction

The demands for sustainable, low-carbon energy technology are increasing due to global climate change and the required reduction of greenhouse gas emissions from consumption of fossil fuels. For contributing to the non-fossil energy transition, aquifer thermal energy storage (ATES) has been developed and thereafter widely used as both sustainable energy and energy saving technology throughout the world during the past few decades [46]. Basically, an ATES system consists of a warm well and a cold well to store heat and cold energy. In summer groundwater is extracted from the cold well (at about 10 ˚C), heat is transferred from the building and the heated water is injected and stored underground in the warm well [42]. When heat is requested in autumn or winter the pumping direction and heat transfer process will be reversed. By now, the Netherlands and Sweden are the two countries where the development and application of ATES are most mature [46, 80]. While no official reports estimate the overall numbers of ATES system in the world, the increase of the number of ATES in for example the Netherlands is clear and obvious. There, the number of ATES systems increased from 33 in 1995 to 571 in 2005, and further to over 3000 at this moment [86]. The Dutch government wants to promote the implementation of ATES to around 20,000 systems in 2020 [44], to meet the targets for energy saving and reduction of CO_2 emission.

Due to the rapid development of ATES in urban areas, where often groundwater contaminations are found, the concern of negative interference with these groundwater contaminants has become an issue that needs attention to prevent further degradation of groundwater quality. Because operation of ATES involves the displacement of large volumes of groundwater, it could possibly lead to further spreading of contaminant plumes in the subsurface [43]. Therefore, currently strict permit conditions apply when implementing ATES in or close to contaminated sites. As a consequence, redevelopment of the urban area, in which sustainable energy is of importance, will be hampered. Meanwhile, the presence of aquifer contamination in itself is an issue that hinders urban redevelopment. Rigorous chemical and physical treatments are often too costly [3], especially when contamination has reached deeper in the aquifer such as chlorinated volatile organic compounds (CVOCs) and their pure products, dense non aqueous-phase liquids (DNAPLs) [4, 245]. Natural attenuation

of groundwater contaminants often takes a very long time (several decades) or is even stalled by unfavorable environmental conditions. Therefore, enhanced natural attenuation (ENA) using substrate addition or bioaugmentation has been developed [27, 31, 190]. Although the long duration of bioremediation can be improved, overall remediation still can hardly be achieved within the timeframe of urban redevelopment. Integration of remediation into site redevelopment and combination of different technologies is becoming the direction and trend for sustainable development.

To promote a master plan with both renewable energy technology and sustainable groundwater management for the redevelopment of the urban areas, an integration concept of applying ATES in contaminated sites has been recently proposed [47, 48, 89]. This concept tries to combine ATES and enhanced bioremediation of CVOCs, which are among the most common groundwater contaminants and often present at similar depths as ATES [1, 4]. Enhanced bioremediation has been popularly used to remediate chlorinated ethenes in the subsurface, due to its advantages of the potential for complete removal, high compatibility with other techniques, and low cost [56, 58, 246]. Besides, when combing bioremediation with ATES, the drawback of long duration is lessened, as the time required compares well to the life time of an ATES system. This offers a solution for stimulating the urban redevelopment, because it enables wider implementation of ATES, also in contaminated areas, with simultaneous enhanced removal of contamination. For example, the increased temperature around the warm well can improve the biodegradation of CVOCs [171], which has been demonstrated in Chapter 3. Besides, the seasonal operation of ATES guarantees that contaminants in the cold well, where microbiological activity will be hampered, can be transferred to the warm well and being degraded there (Chapter 3). The increased flow rate due to ATES was also shown not to hamper the reductive dechlorination (Chapter 4). In addition ATES can be used as a biostimulation tool from the engineering point of view, for example to inject substrate or microorganism to the subsurface (Chapter 6).

Accepting that the benefits anticipated for the combination concept of ATES and enhanced bioremediation outweigh the risk of contaminant spreading, a remaining issue of concern is potential biological clogging of ATES wells due to the enhanced growth of microorganisms [247]. Being a common phenomenon in many groundwater systems [248], clogging is also the most concerning problem during the operation of ATES [249, 250] as it will cause an

increase in pressure and resistance in the injection well [96]. Under normal conditions, without biostimulation, chemical clogging is a well know phenomenon for ATES, especially the precipitation of Fe and Mn oxide or hydroxide. This is caused by a change in groundwater chemistry [98, 249] and is mainly attributed to intrusion of oxygen if the ATES system is not very gas tight. Other cause of chemical clogging is because of ATES encountering water with nitrate due to vertical expanse of nitrate diffusion or regional groundwater flow. For an iron rich aquifer, for example in many sites in the Netherlands, this risk of chemical precipitation will be greater. Considering the more reduced redox condition in the aquifer when enhanced bioremediation is applied in combination with ATES, for example by lactate addition, the potential for chemical precipitation might actually be limited. By continuously maintaining this lower redox condition, chemical clogging might be prevented, avoiding costs for conventional de-clogging. However, the absence of biological clogging through promoted growth of bacteria due to biostimulation is not yet ensured. Therefore, the potential of biological clogging should be investigated in the combined system before its practical implementation. Moreover, the role of enhanced bioremediation on chemical clogging issues in ATES is of interest, as a chance of cost-effectively solving chemical clogging in ATES might lie in the combined system. Consequently, the aim of this study is to get insights into biological clogging caused by the combination of ATES and enhance bioremediation as well as into the possible effect of enhanced bioremediation on chemical clogging. Aiming at these two aspects, in preliminary column tests we first study the possible response of the ATES system to enhanced biological activity, and secondly investigate the effect of such bioenhancement on chemical clogging through Fe precipitates deliberately triggered by nitrate additions.

5.2 Materials and methods

A similar recirculating column system as described in Chapter 4 was also applied in this study. The experimental procedure consisted of two main actions: 1) enhancing biological activity by lactate addition; 2) promoting chemical precipitation by nitrate addition. After the second action, lactate would be added again to study whether chemical clogging can be reversed by biological reduction of the chemical precipitates. An overview of the actions of enhanced biological activity and promoted chemical precipitate that were performed in the

columns is shown in Table 5.1. The data for column 1 with flow rate of 10 mL/min is identical to in those in Chapter 4 (Table 4.1).

5.2.1   Materials

Material from an Fe(III) reducing sandy aquifer beneath the city of Utrecht (NL) was used for the column experiment [92]. Sodium lactate (SL, 225 g/L) and sodium nitrate (NaNO$_3$, 85 g/L) stock solution were prepared with anaerobic deionized water, which was purged with pure N$_2$ for over 3 hours, using SL and NaNO$_3$ powder (both ≥99% purity, from Aldrich®). Anaerobic tap water from the lab, prepared in the same way as anaerobic deionized water, was used as liquid medium for the column experiment.

5.2.2   Analysis

Monitoring of redox potential for the influent and effluent of the column system was conducted by a Consort multi-channel (C3060) meter and data logger with ProSense (QIS) standard Pt redox electrode with Ag/AgCl as reference electrode (-199 mV vs. standard hydrogen electrode (SHE)) in saturated KCl solution [141]. The interval of data records was set to 1 minute. Resistance between influent and effluent (pressure drop ΔP) of the column was indicated by pressure drop manometer (GDH200-13, from GREISINGER electronic GmbH) with measuring range of 0 to 2000 mbar. Analysis of Fe^{2+} was performed with Hach Lange cuvette tests (LCK-320 0.2-6.0 mg/L Fe^{2+}/Fe^{3+}) on a Xion 500 spectrophotometer.

5.2.3   Column experimental set-up

The recirculating column consisted of a glass column with length of 30 cm and inner diameter of 3.6 cm, the flow systems were operated by SIMDOS® diaphragm metering pumps (flow rate range: 1-100 mL/min), and. To avoid flush out of sand particles from the column, two glass filters with P2 pore size (100-160 μm) were placed on top and bottom inside the glass column. The same buffering bottle used in Chapter 4 was also connected within the column system here. Different compartments of the column set-up were connected with Teflon tubes (Ø4mm) with a quick connector with valve. An overview of the scheme of the column set-up is provided in Figure 5.1. The preparation and maintenance procedures are identical and presented in Chapter 4.
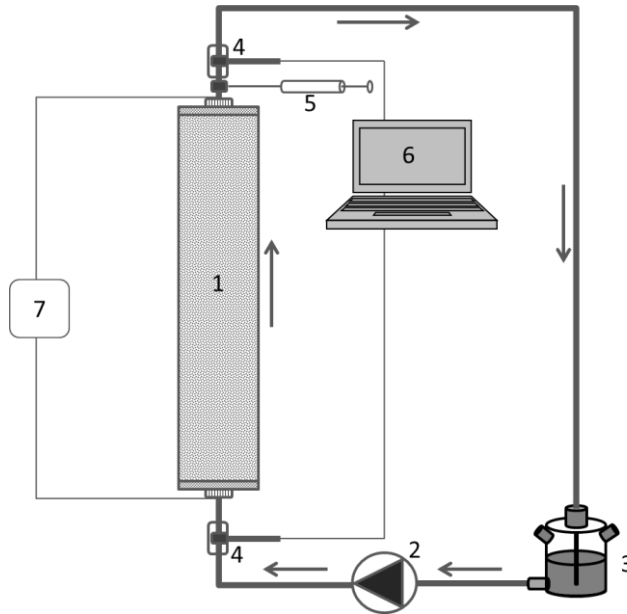
Figure 5.1 Schematic diagram of column set-up. 1: aquifer material with glass filters with pore size of 160 μm on the top and bottom of the column; 2: membrane pump; 3: buffering bottle; 4: redox and pH electrodes (bottom is for influent, top is for effluent); 5: liquid sampling port; 6: meter connected with computer; 7: Pressure drop (ΔP between influent and effluent). Arrow indicates the direction of water flow.

The two flow rates, 10 and 50 mL/min, were tested in two separate column systems with the same specifications. By applying these two flow rates, the water velocities within the column are comparable to the enhanced groundwater speeds at distances respectively 1.3 m and 0.26 m from the well filter of an ATES with 20 m filter length in the subsurface which is operated at a typical flow rate of 100 m^3/h (see Figure S4.2 in Chapter 4).

Column 1 (10 mL/min) lasted for 140 days, while column 2 (50 mL/min) lasted for 55 days. During operation of column 2, the pump was stopped from day 7 to 24 to cease the shear force caused by the high flow rate, aiming to better facilitate bacterial growth. The pump had to be stopped again at day 41 when, due to the high resistance in the column, water bypassed through the pressure drop meter instead, thereby damaging the meter.

Table 5.1 Overview two main actions during the column experiment.

Addition[a]	Column 1		Column 2	
	Total amount (mL)	Day	Total amount (mL)	Day
Lactate	2.5	20-56	6	3-37
Nitrate	5	57-65	20	38-54
Lactate	4	66-104	5	55
Nitrate	5	105-113	-	-
Lactate	1.5	114-140	-	-

[a] For either enhanced biological activity or chemically promoted Fe-oxide precipitation; - indicates no actions performed

5.3 Results and discussion

5.3.1 Column 1 (10 mL/min)

Pressure drop (ΔP), as the indicator of clogging of the column system during the experiment is given in Figure 5.2. The starting level was around 200 mbar with flow rate of 10 mL/min, and it slowly declined to about 150 mbar in the first 20 days. A sudden and sharp increase of ΔP to almost 700 mbar was observed on day 20 (Figure 5.2), with appearance of a faint red color in some of the tubes (Figure S5.1). We suspected that Fe precipitates started to be formed in the column and clog the system, due to accidental oxygen intrusion. Thereafter, lactate additions were performed in the period between day 20 and 56, to a total addition of 2.5 mL lactate stock. The pressure drop then first significantly dropped to below 100 mbar and gradually recovered and stabilized at a level of 150 mbar in this period (Figure 5.2). Along with lactate additions and stabilization of ΔP, the red color in the tubes disappeared.

Figure 5.2 The evolution of pressure drop (blue diamond) in column 1. Area with light blue or light red color stands for period of either enhanced biological activity or chemically promoted Fe-oxide precipitation.

With the intended nitrate addition (5 mL) for promoting chemical precipitation starting from day 57, pressure drop increased strongly, similar to the first peak observed on day 20. This steeply linear climb then reached a peak of about 600 mbar within 10 days (Figure 5.2). Meanwhile, we once more perceived red color appearing in some tubes of the column. Thereafter, the buildup of pressure drop was again eliminated by another period of lactate additions starting from day 66. This time, a lower pressure drop (below 50 mbar) compared to the first elimination was reached, and it slowly recovered to around 100 mbar. Surprisingly, during the second nitrate addition period (day 105-113), only a considerably smaller rise in pressure drop, up to 130 mbar, was observed, despite the same amount of nitrate was added (5 mL). Possible explanation could be that some reductive reagent was still present, as the redox potential remained negative (Figure 5.3). Eventually, entering the last lactate addition period, pressure drop was further lowered and maintained at a level of 50 mbar (Figure 5.2). In summary, pressure drop only elevated when oxidant was present in the column, and the presence of lactate as reductive reagent seemed to be able to reduce the pressure drop or prevent increase of pressure drop.
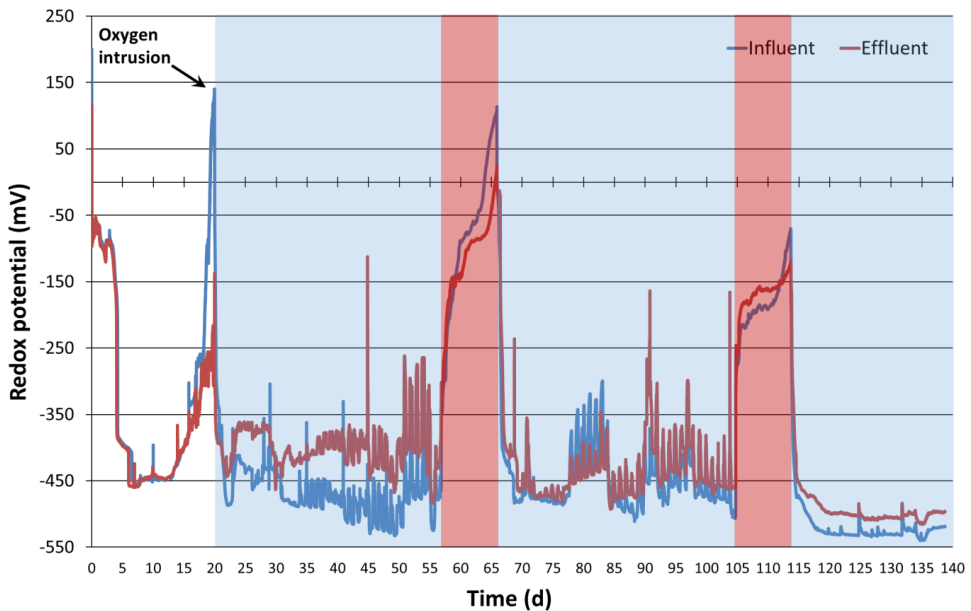
Figure 5.3 Redox potential of the influent (blue line) and effluent (red line) in column 1 (based on Figure 4.2 in Chapter 4). Area with light blue or light red color stands for period of either enhanced biological activity or chemically promoted Fe-oxide precipitation.

In general, the evolution of redox potential in column 1 (Figure 5.3) followed the same pattern as pressure drop. Three peaks of redox potential, in the period of oxygen intrusion and the two times of nitrate addition, were also observed, with maximum levels of about 150 mV, 100 mV and -70 mV respectively. In particular, the second nitrate-induced peak in redox potential was lower than the first one, and redox potential was still negative. Except for these periods with oxidants present in the column (oxygen or nitrate), redox potential generally remained close to -450 mV or even lower in periods of enhanced biological activity (blue areas in Figure 5.3).

The responses of pressure drop and redox potential upon the introduction of an oxidant, such as oxygen and nitrate, were obvious and fast. Besides, the changes in pressure drop and redox potential show a similar pattern. Increase of pressure drop was corresponding to increase of redox potential. The appearance of a red color indicated that most probably Fe precipitate was formed [251], which was proved by Fe analysis performed later in the experiment. To

this end, a small part of the red tubes was disconnected from the column (replaced by a fresh tube), the red material attached to the inner wall of this small tube was rinsed off and dissolved in HNO_3 acid. The acidified solution became rather red when pipetted into the Hach Lange kit (LCK-320) and showed to have a concentration of Fe^{3+} higher than detection limit. From this qualitative Fe^{3+} measurement, the red material was confirmed to contain Fe(III) precipitate. However, during the periods of enhanced biological activity with lactate additions, the red color disappeared and, more importantly, the pressure drop reduced sharply and remained relatively low. This implies that in our experiment, instead of causing biological clogging, lactate additions could lower the pressure drop and avoid buildup of resistance. This could not be due to that added substrate was insufficient, as research showed that biofilm formation can already occur at lower concentrations of substrate than used in our experiment [252, 253]. Therefore, a plausible explanation could be that the water velocity used in this case is higher than that in other studies [254], resulting in high shear force which is shown to be capable of detaching bacteria [255, 256]. As a result, extensive biofouling or biofilm could not be formed to increase pressure drop within the experimental time of this study.

5.3.2 Column 2 (50 mL/min)

The pressure drop in column 2 with a flow rate of 50 mL/min is given in Figure 5.4. Generally, during the enhanced biological activity period with a total addition of 6 mL of lactate stock, the pressure drop stayed around 700 mbar, which is about 5 times higher than that in column 1 in the first period of enhanced biological activity. The pressure drop reading was zero in periods between day 7 and 24, and between day 41 and 54, both because pumping was stopped. The first pumping stop was to operate the system in a batch mode to enlarge the chance of further growth and attachment of bacteria on the column filter or in the column. However, no increase of pressure drop was observed when pumping was on after over 13 days' batch mode (Figure 5.4).
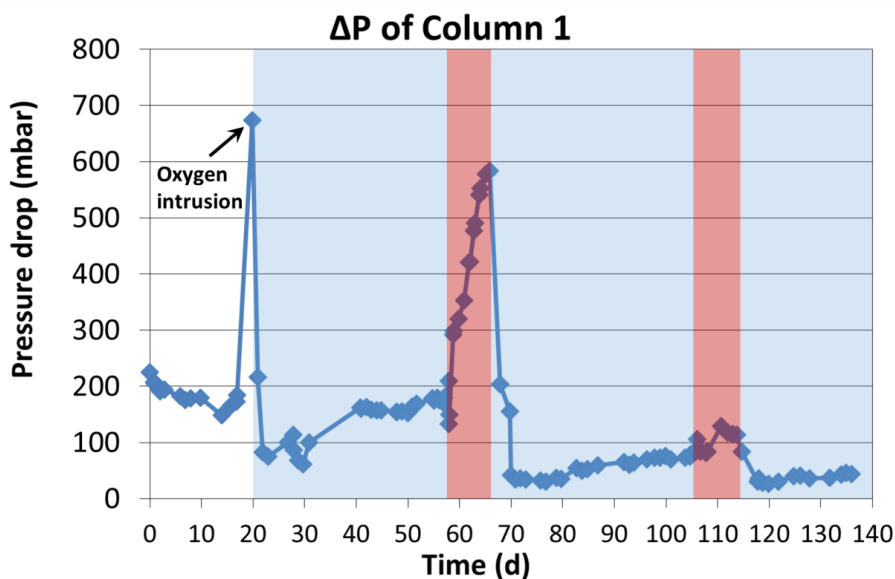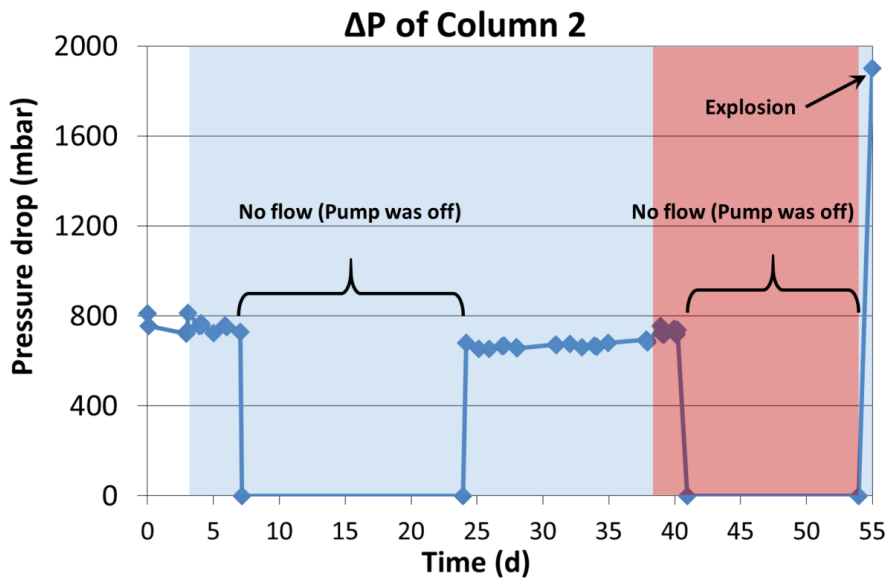
Figure 5.4 The evolution of pressure drop (blue diamond) in column 2. Area with light blue or light red color stands for period of either enhanced biological activity or chemically promoted Fe-oxide precipitation.

In contrast to the first intended stop, the second pumping stop was actually attributed to nitrate addition. After a slight increase in the pressure drop reading on the afternoon of day 38, the pressure drop meter was found to be filling with water on the next day. Without monitoring by the pressure meter, the pump had to be stopped. The fact that water preferred to go through the pressure drop meter instead of the column indicated a very high pressure drop in the column, although monitoring of pressure drop was not available at that period. This means that the resistance of the column was even higher than the resistance to pass through the meter whose measuring range is up to 2000 mbar. The proof was that a reading of over 1900 mbar was observed when a new pressure meter was connected and pumping was resumed (Figure 5.4), quickly followed with readings exceeding the upper measuring limit, and further followed with the sudden blow-up of column 2 (Figure S5.2). Although lactate (5 mL) was added right before starting pumping again (day 55), the blow-up of the column system at flow rate of 50 mL/min indicates that reduction of Fe precipitate could not proceed in time to lower the resistance of the column.

The redox potential of both influent and effluent dropped quickly to below -350 mV after lactate additions from day 3 (Figure 5.5). When the pump was stopped for the first time and thereafter, redox potentials remained at levels of about -350 mV and -450mV, respectively for influent and effluent. A pulse jump of redox potential was observed along with the startup of pumping on day 24, but soon the redox potential of both influent and effluent declined to -500 mV. After nitrate additions from day 38, redox potential increased to -250 mV within 3 days and afterward, under condition without flow of water, gradually rose to -150 mV as the end level of the experiment (Figure 5.5).

The initial pressure drop in column 2 was close to five times higher than that in column 2, due to the fact that pressure drop is proportionally related to velocity [257]. As the flow rate in column 2 was five times larger, no occurrence of clogging in the period of enhanced biological activity was expected due to an even higher shear force compared to column 1, despite more lactate was added. However, temporarily lowering the flow rate to zero also did not lead to biological clogging. This was probably due to the fact that organic acid fermenter and *Geobacter*, which are the majority of biomass in the system, were generally mobile (Chapter 4 and 6) [177]. The subsequent high dosage of nitrate in this column resulted in a much faster (and -because of the higher flow rate- also higher) buildup of pressure drop than in column 1 and led to complete clogging by Fe precipitate. In contrast to column 1, where chemical clogging could be removed by lactate addition, the higher water flow rate did not allow enough time for lactate induced biochemical processes to reduce and dissolve the Fe precipitate in the column. Eventually, continuing pumping at the high flow rate and the given resistance due to clogging, resulted in a sudden explosion, as the pressure buildup inside the system exceeded the strength of the column.
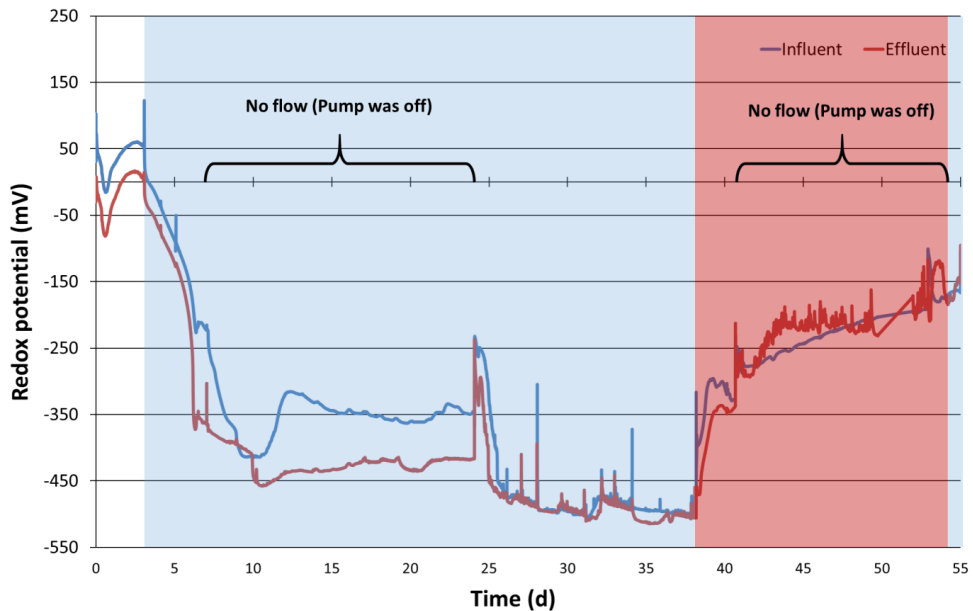
Figure 5.5 Redox potential of the influent (blue line) and effluent (red line) in column 2. Area with light blue or light red color stands for period of either enhanced biological activity or chemically promoted Fe-oxide precipitation.

5.4 Conclusions

Here we performed preliminary column tests to assess the possible impacts of enhanced bioremediation or ENA on the functioning of ATES, which was simulated by a recirculating column system, under two different flow rates (10 and 50 mL/min). We investigated the response of such model ATES system in periods of enhanced biological activity and chemically promoted Fe-oxide precipitation by adding lactate and nitrate respectively. The pressure drop as an indicator for clogging of the system was continuously monitored during the experiments. Within the period of enhanced biological activity, no increase of pressure drop was found. Instead, using lactate as the substrate for the biological enhancement could release the build-up of pressure drop, which was caused by nitrate addition. Such finding looks promising for the combination of ATES and enhanced bioremediation, as the concerned biological clogging seems not likely to occur, probably due to the high groundwater flow changed by the ATES system. Furthermore, the common problem for

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ATES operation, chemical clogging due to Fe precipitate formation by incoming nitrate or oxygen, can probably be prevented, because enhanced bioremediation normally requires low redox conditions. Under anaerobic and low redox conditions, the chemical precipitation process, mainly Fe(III) precipitations, in fact can be limited. However, regarding ATES systems with relatively high flow rates, cautious operation is still needed, as the impact of chemical clogging once it occurs, is direct and can be severe. In general, based on our preliminary column tests, we suggest that the combination of ATES and enhanced bioremediation could be indeed a promising integration between energy technology and groundwater management and be meaningful for the redevelopment of contaminated urban area.

Acknowledgement

Supporting material

Figure S5.1 Red color being observed in periods of chemically promoted Fe-oxide precipitation in column 1 and column 2.
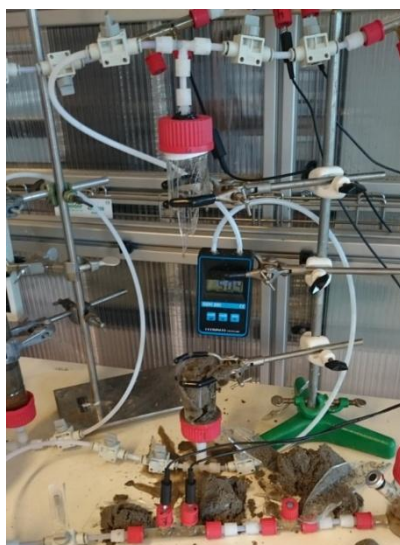
Figure S5.2 Picture of the blow-up of column 2 when restarting the pump, at the end of the period of chemically promoted Fe-oxide precipitation.

Chapter VI

Reactive Transport Modeling of

TCE Bioremediation Combined with

Aquifer Thermal Energy Storage

Abstract

Aquifer thermal energy storage (ATES) is increasingly being used to provide heating and cooling for buildings. Because many urbanized centres deal with contaminated soil and groundwater, an increasing number of ATES ambitions is confronted with the presence of contaminants. Hence, a well-designed combination of ATES with biostimulation could be a promising integrated technique, both for remediation of contaminants as for development of ATES. In this study, a reactive transport model was developed to simulate the use of ATES as a continuous biostimulation tool for enhanced reductive dechlorination (ERD) of a hypothetical trichloroethene (TCE) contaminated aquifer. Model results show that biostimulation by lactate addition reduces iron and sulphate in the capture zone of the ATES wells, after which dechlorination from TCE to ethene is possible. The progress of dechlorination is dictated by lactate dose and amounts of electron acceptors. Although microbial processes are known to be temperature dependent, temperature changes induced by thermal storage do not significantly influence the overall dechlorination process. Simulations also reveal that further study is required on: (1) reduction of iron oxide, related to increasing pH of the infiltrated groundwater, and (2) growth and mobility of bacteria related to well clogging, which is a main concern for biostimulation using ATES.

Keywords

6.1 Introduction

The subsurface is increasingly being used to provide heating and cooling for buildings and industrial processes through aquifer thermal energy storage (ATES) [40, 42]. In summer, ATES systems extract groundwater which is used for cooling by passing it through a heat exchanger. The heated groundwater is injected back into the aquifer, typically at a few hundred meters distance. This creates a volume of relatively warm groundwater. In winter, this warm groundwater is extracted and used for heating. This cools down the groundwater, which is again re-injected into the aquifer, such that it can be used for cooling in the next summer [39, 258]. Currently, ATES is used worldwide in many applications such as for air-conditioning of a supermarket in Turkey [76] and for heating and cooling of a hospital in Belgium [79], a college in the USA [77] and a governmental building in Germany [259]. The Netherlands and Sweden are considered to dominate the market in terms of implementation [46, 80]. For Sweden, Andersson [80] estimated that, in 2012, there were approximately 104 ATES systems with a total capacity of 110 MW. Based on yearly reports of the Dutch Central Bureau of Statistics (CBS), the number of ATES systems in the Netherlands in the same year was 2740, with a total estimated capacity of 1103 MW [74]. The majority of ATES systems is applied for offices and utility buildings in urban areas [42, 183]. Because many urbanized centres deal with contaminated soil and groundwater [170, 260], an increasing number of ATES ambitions is confronted with the presence of contaminants. Previous research shows that groundwater movement due to application of ATES in a contaminated aquifer can result in a larger contaminant flux to the aqueous phase due to increased dissolution of pure product and a larger volume of contaminated groundwater [43]. Also, temperature changes induced by ATES can impact redox processes, microbial communities [83] and geochemistry [261], and therefore the behaviour of contaminants. Regulations that prohibit extraction, injection or otherwise handling of contaminated groundwater narrow the opportunity window for ATES as a sustainable energy technology [47, 166, 167].

At the same time, however, there is a growing interest in combining ATES with bioremediation [83, 262]. In 2012, two pilot locations were studied, where, for the first time, ATES is combined with monitored natural attenuation [47, 140]. In both pilots no active biostimulation or bioaugmentation was applied, although this has been suggested as an

adequate method to be applied when natural biodegradation appears to be insufficient [48]. Both pilot locations are contaminated with chlorinated volatile organic compounds (CVOCs). Groundwater contaminated with CVOCs, in particular tetrachloroethene (PCE), trichloroethene (TCE), dichloroethene (DCE) and vinyl chloride (VC) [1, 7, 101, 263], is frequently encountered in urban areas [4, 148, 187, 264, 265]. Commonly applied as degreasers at dry cleaners and in chemical and metal processing factories, PCE and TCE, with DCE and VC as degradation products, have entered the groundwater after leakage or improper disposal [2, 25]. Since CVOCs are potentially carcinogenic, especially VC [25], their presence in groundwater is a threat to subsurface drinking water resources and public health through penetration into water infrastructure and vapour intrusion into indoor air [266-268]. When present as dense non aqueous-phase liquid (DNAPL), pure product CVOC can travel vertically through the subsurface and reach similar depth as where ATES is applied (20-200 m below ground level) [25, 43, 188]. Due to its low solubility, pure product may act as a source of dissolved contaminant and give rise to contaminant plumes with typical lengths that range from 300 m [1, 18] to 1500 m [19]. This makes physical remediation techniques such as pump-and-treat, soil vapour extraction and soil excavation either too costly or inefficient to properly remediate CVOC contaminated subsurface systems [102, 103]. Since biodegradation of CVOCs was recognized in the late 1970s [269], there is much attention on in situ reductive dechlorination as an effective way to remove organic contaminants [4, 31, 66, 169, 188, 270, 271]. Hence, the well-designed combination of ATES with natural attenuation or biostimulation could be a very promising integrated technique, both for remediation of CVOCs [48, 272] as for broadening the ATES window of opportunity.

Under natural conditions, reductive dechlorination is usually limited by, for example, unsuitable redox conditions or lack of electron donor or microorganisms, resulting in absent or incomplete biodegradation of CVOCs [70, 71, 116-118]. In these cases, addition of auxiliary electron donor combined with bio-augmentation is required to achieve reductive dechlorination from parent compounds to ethene [69, 92, 235, 273, 274]. Previous in situ bioremediation studies show that under field conditions 75-99% reductions are realized [245, 275]. Although in situ bioremediation is mainly targeted on more permeable zones in aquifers, due to diffusion of electron donor over time, also low-permeability zones can be treated [245]. Using ATES to deliver electron donor in a biostimulation approach, however, is different

from conventional in situ biostimulation for two reasons. First, as temperature is known to be a significant factor for the activity of microorganisms [43, 276], temperature changes induced by storage of cold and warm water may be expected to influence microbial growth and dechlorination. Secondly, ATES involves seasonal displacement of a large volume of groundwater (30,000-150,000 m^3) between the cold and warm storage. Therefore, potentially a large aquifer volume can be impacted by enhanced reductive dechlorination (ERD) activities. Thirdly, flow rates in typical ATES systems (20-100 m^3/hour) are much higher than those applied in normal ERD practices (around 2 L/min) [235, 273]. As a consequence, crucial for the influence of ATES on biodegradation is whether the microorganisms are transported by the large volumes of groundwater that are displaced by the ATES system, or remain attached to the aquifer matrix, and secondly, how planktonic or attached state affects the activity of the bacteria.

Therefore, optimization and an adequately engineered design of combined ATES and biostimulation as an enhanced bioremediation technique requires comprehensive study of both the biogeochemical aspects as well as characterization of subsurface conditions. Sophisticated modeling is a crucial step to explore the feasibility of the combined technique and direct future laboratory and field experiments. As shown by Chambon et al. [271] and Kouznetsova et al. [129] an increasing number of processes and interactions can be incorporated in numerical models. Although parameterization, especially for field applications, remains a challenge [271], these models can be used to study the relevance and sensitivity of interacting processes. In this study, a reactive transport model was developed to simulate the use of ATES as a continuous biostimulation tool for enhanced bioremediation of a hypothetical TCE contaminated aquifer. With this model we aim to explore the relation between transport and biogeochemical processes in the capture zone of an ATES system. In several scenarios, the influence of design conditions, i.e. storage temperatures and electron donor dose, were studied for their effect on bioremediation. Furthermore the effects of spreading of biomass upon assumptions regarding biomass mobility were simulated.

6.2 Method

6.2.1   Modeling approach

In our hypothetic case, ERD was achieved through lactate addition in both wells of an ATES system in a homogeneous confined aquifer. The reactive transport model was based on Malaguerra et al. [277], who successfully modelled competition between terminal electron acceptors and reactions kinetics in an ERD laboratory batch experiments presented in Scheutz et al. [235]. The model was implemented in the chemical reaction and transport code PHREEQC [278]. PHREEQC is a computer program that incorporates a wide range of biogeochemical reactions, such as kinetic and equilibrium reactions, surface complexation, chemical speciation and 1D transport processes. The constructed model included fermentation of lactate and propionate, iron reduction, sulphate reduction, methanogenesis and sequential reductive dechlorination of TCE, DCE and VC, as well as precipitation of iron minerals and calcite dissolution. Biomass growth and biochemical reactions were fully described by modified Michaelis-Menten kinetics. Competition between terminal electron acceptor processes was incorporated through inhibition factors. The main processes, inhibition and biomass species are presented in the Supporting materials (Table S6.1). The reaction kinetics have been described in detail in Malaguerra et al. [277]. First the batch model presented by Malaguerra et al. [277] was reproduced. Model results (Supporting materials, Figure S6.1) were consistent with the results presented in Malaguerra et al. [277], demonstrating correct reproduction of the original model. This batch model was modified to incorporate flow and transport for representing ERD using a typical doublet-well ATES system.

6.2.2   Double axi-symmetric flow tube model

Radial flow around the two wells of the ATES system was simulated using a double axi-symmetric flow tube model (DAFT) [261]. Using the DAFT model, we assume: a) sufficient distance between the two wells to exclude interference, b) radial symmetry of flow around the wells, and c) direct infiltration of the volume of water extracted from one well into the other well (Figure 6.1). The equality between extracted and injected water volume is completely in line with how ATES systems function. However, as in this model approach there is no above surface system, we thereby implicitly also assume that d) no significant

kinetic reactions occur in the above surface system. In real ATES systems, extracted water is directed through pipelines to a heat exchanger, where heat is exchanged either from or to the groundwater depending on the need for cooling or heating, after which the groundwater is directed through a different pipeline towards the injection well. Due to the high pumping rate and small volume of the pipeline network, residence time in the surface equipment is short (minutes to hours) compared to the residence time in the aquifer (approximately half a year), making the latter assumption reasonable.

6.2.3   Initial conditions, boundary conditions and discretization

Flux type boundary conditions were applied to the in- and outflow boundaries of the flow tube. To mimic seasonal storage of thermal energy, each year, flow was defined from left to right in Figure 6.1 for 180 days during summer, followed by 180 days in which the flux is defined in the opposite direction. This simulates extraction from the cold storage well and injection in the warm storage well in summer, and extraction from the warm storage well and injection into the cold storage well in winter. In the middle of the flow tube, a small cell (10 cm) was defined in which no kinetic reactions take place, but where addition of sodium lactate was defined at a constant rate (grey cell in Figure 6.1). Temperature in this cell was prescribed equal to the injection temperature of the ATES system. Initial conditions were chosen according to the initial conditions reported in Malaguerra et al. [277] and are summarized in the Supporting materials (Table S6.2). Dissolved TCE is assumed to be present throughout the entire model domain, as well as microorganisms. In some countries, such as in the Netherlands it is not allowed to re-inject contaminated groundwater into an aquifer [47, 166, 167]. Note that while operating an ATES system in a contaminated aquifer, all dissolved components including contaminants will be extracted from one well and re-injected into the other well. In this case an exemption should be acquired from such legislation. No DNAPL sources are considered. The domain was discretized by 81 grid cells that range in size from 4.74 m near the well to 0.38 m at the model boundary as calculated from equations (1) and (2) in Bonte et al. [261] to simulate radial flow around the wells of the ATES system. Each 180 day season was divided into 20 time steps of 9 days. Therefore, during 1 storage cycle, the injected water travels over 20 grid cells, which represents a total distance of 15 m from the injection well. A reference scenario (S1) was defined in which storage temperatures were set at typical values of 5 ˚C (cold storage) and 15 ˚C (warm storage)

[261] with an initial aquifer temperature of 10 ˚C. This model was run for a timeframe of 5 consecutive years.
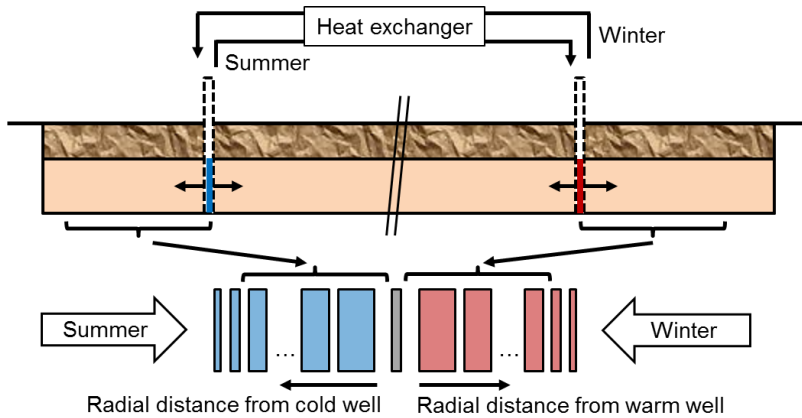
Figure 6.1 Schematic of ATES system (upper part) and how this is represented by the gridding of the double axi-symmetric flow tube (DAFT) model (lower part), lactate is added through the grey cell in the middle of the model domain, representing both the cold and warm well, depending on the season. The blue grid cells represent the aquifer around the cold storage well and the red cells the aquifer around the warm storage well.

6.2.4   Transport

In the original batch model [277], two types of iron oxide were incorporated, one accounting for the high bio-available fraction (e.g. ferrihydrite and lepidocrocite) and the other accounting for the low bio-available fraction (e.g. goethite). Both iron oxides were defined as aqueous species, such that the intermediate process of iron oxide dissolution is incorporated in the reduction kinetics. To prevent mobility of iron oxides in our transport model both types of iron oxide were defined as mineral phases with low solubility (log k = -10) that are directly used in the reaction network. This ensures that, before reaction, they are considered as primarily associated with the sediment phase. The initial amounts of high and low bio-available iron in the batch experiment were respectively 10.4 and 1.03 mmol/L [235, 277]. The batch experiment consisted of 100 g wet sediment and 200 mL groundwater. Under aquifer conditions, the sediment to groundwater ratio is approximately 1 kg of dry sediment on every 200 mL of groundwater (considering a quartz aquifer with a porosity of 35% and a quartz density of 2660 kg/m^3). Converting laboratory conditions to

aquifer conditions results in 104 and 10.3 mmol per L of pore volume of high and low bio-available iron oxide, according to the higher sediment to groundwater ratio. In the model as presented by Malaguerra et al. [277], siderite, pyrite and iron sulphide were allowed to precipitate. Preliminary model runs showed that more than 97% of the precipitate is siderite. Therefore, in our model runs pyrite and iron sulphide were not included which considerably reduced calculation times. Retardation factors for TCE, DCE and VC were set to 1.4, 1.2 and 1.1 respectively, similar to what was used in earlier modelling studies [279, 280]. These are typical values for sand and gravel aquifers, with low organic matter content, that are generally targeted for ATES. Thermal retardation was set to 2, representing a sand aquifer with a porosity of 35% [261].

6.2.5   Temperature dependence

The metabolic activity of microorganisms and their tolerance to geochemical changes are highly influenced by temperature. Optimum biological conversion rates are reported for psychrophilic (-20 to +10 ˚C) and mesophilic (20 to 45 ˚C) microbial systems [281-283]. For temperatures above and below the optimum temperature, microbial activity is slower or stops completely. Specifically for lactate-amended reductive dechlorination of TCE, Friis et al. [171] performed laboratory experiments at different temperatures and showed that TCE degradation rates increased approximately by a factor 10 when temperature was increased from 10 to 30 ˚C. For higher temperature, degradation rates decreased again with a factor 5 at 40 ˚C. Temperature dependence of the reaction kinetics was incorporated by Malaguerra et al. [277] using an Arrhenius type equation that was fitted to the experimental results of Friis et al. [171]. According to this relation, growth rates in a typical cold storage (5 ˚C) are approximately 1.7 times smaller than under undisturbed aquifer temperature (10 ˚C). In a typical warm storage (15 ˚C) the rates are approximately 1.7 times higher than under undisturbed aquifer conditions, while under maximum storage temperature that is allowed (25 ˚C) [167, 261], the growth rates are approximately 4.4 times higher. This selected range of temperature reflects common temperature used in Europe, e.g. in Austria, Denmark and the Netherlands [167].

6.2.6   Biomass mobility

In literature, different views exist on microbial transport and activity. Schaefer et al. [180] report that *Dehalococcoides* (DHC) concentrations associated with the solid phase are negligible compared to aqueous phase concentrations in a dechlorination column experiment. Amos et al. [177] show different behaviour for *Geobacter* and DHC in a bio-augmented PCE degradation column experiment. *Geobacter* bacteria were observed to grow and remain attached in the NAPL source zone but to be largely present in planktonic form in the plume. In contrast, DHC cells were primarily attached to the solid phase throughout the studied column. That bacterial growth and transport is also influenced by pore water flow velocities has been shown by Mendoza-Sanchez et al. [284], who studied the growth and transport of dechlorinating bacteria in a column experiment under different flow conditions. For low and medium flow rates (0.0036 and 0.080 m/d), attached biomass was only observed near the bioaugmentation injection points. In case of a high flow rate (0.51 m/d), a biofilm was observed throughout the whole column. However, whether planktonic or attached bacteria are more relevant in terms of dechlorination activity is yet unclear from the studies mentioned above. Also for other bacterial species, different views on growth and mobility are reported [285, 286]. Activity, attachment and detachment of bacteria depend on the physical/chemical properties of the sediment as well as the specific bacterial species [287]. To explore the influence of biomass mobility, two extreme cases were considered: (1) completely mobile biomass and (2) completely immobile biomass, while in both cases biomass is initially present throughout the aquifer model domain.

6.2.7   Model scenarios

Several scenarios were defined to study the influence of (1) lactate dose, (2) storage temperature and (3) biomass mobility. In the reference scenario (S1), lactate dose was set at 3.8 mmol/L, equal to the amount that was used in the batch experiments [235]. Storage temperatures were set at 5 ˚C (cold storage) and 15 ˚C (warm storage) to represent a typical ATES system. In the batch experiments, complete dechlorination was observed within 250 days. As ATES systems are typically designed to operate for 20 to 30 years, it may be considered to apply lactate at a lower dose. This has been studied by running additional simulations using a lactate dose of 1.9 and 0.38 mmol/L. The influence of storage temperatures was explored by setting the storage temperatures for the cold/warm well to

10/10 ˚C (i.e. no thermal storage, only pumping and addition of electron donor) and 5/25 ˚C (the maximum allowed storage temperature) [83]. An overview of the scenarios is presented in Table 6.1.

Preliminary model runs showed, remarkably, that pH in the infiltrated groundwater increases from 6.6 to 13.2. Apparently, the amount of buffer capacity available in the model is insufficient to cope with the large amount of protons that is used mainly for iron reduction, and to a lesser extent also for sulphate reduction and methanogenesis. Previous research indicates that iron reduction is hampered at pH > 7 due to lower solubility [288, 289] or blockage of sites available for microbial reduction [290]. Feedback between iron reduction and pH may prevent development of high pH values. Such hypothesis was tested with additional scenarios by incorporating an inhibition factor that limits iron reduction for pH values > 7. This was achieved by multiplying the iron reduction rate for pH > 7 with $10^{3\,(7\text{-pH})}$ based on a 3rd order dependence of Fe(III) dissolution on OH^- concentration [289, 291]. Support for and implications of this scenario are further discussed in the results and discussion section.

Table 6.1 Model scenarios.

Scenario	Lactate dose (mmol/L)	Injection temperature (˚C) cold/warm storage	Biomass mobility	pH limit on Fe-reduction
S1	3.8	5/15	Mobile	No
S2	1.9	5/15	Mobile	No
S3	0.38	5/15	Mobile	No
S4	3.8	10/10	Mobile	No
S5	3.8	5/25	Mobile	No
S6	3.8	5/15	Mobile	Yes
S7	1.9	5/15	Mobile	Yes
S8	0.38	5/15	Mobile	Yes
S9	3.8	10/10	Mobile	Yes
S10	3.8	5/25	Mobile	Yes
S11	3.8	5/15	Immobile	No
S12	1.9	5/15	Immobile	No
S13	0.38	5/15	Immobile	No
S14	3.8	10/10	Immobile	No
S15	3.8	5/25	Immobile	No

6.2.8 Presentation of results

Results are discussed on the amount of dechlorination, geochemical conditions and growth and distribution of biomass. The overall progress of dechlorination was expressed by the normalized chlorine number (N_{Cl}) [129] (equation 6.1). Here C_i refers to the concentration of TCE, DCE, VC and ethene. At the start of the simulation all contaminant is present as TCE and the normalized chlorine number is equal to 1. When TCE, DCE and VC are completely degraded to ethane, N_{Cl} becomes 0.

$$N_{Cl} = \frac{3 \cdot C_{TCE} + 2 \cdot C_{DCE} + C_{VC}}{3 \cdot (C_{TCE} + C_{DCE} + C_{VC} + C_{ETH})} \tag{6.1}$$

6.3 Results and discussion

First, results for the reference scenario (S1) and its equivalent with pH limited Fe-reduction (S6) are presented, followed by a discussion of the influence of the various parameters that were considered in the scenario analysis.

6.3.1 Reference scenario

Model results were post processed to represent a cross-section through the doublet well system according to Figure 6.1. Evolution of physical and geochemical conditions in space and time are shown in Figure 6.2. Here, the x-axis represents the horizontal distance from the well for the cold storage (left) and warm storage (right). The y-axis shows the time (days) since the start of the combined ERD-ATES system. Development of thermal plumes due to injection and withdrawal in the cold and warm storage is demonstrated in Figure 6.2-a. Concurrent with lactate addition, TCE is degraded to DCE (Figure 6.2-c) shortly followed by reduction of high bio-available iron oxides (Figure 6.2-g). As degradation of DCE to VC and ethene is inhibited by the presence of iron oxides and sulphate, this only occurs at a later time when methanogenic conditions have been established. Complete reduction of high and low bio-available iron oxides is reached within 600 days (2 storage cycles) in the zone that is affected by the injected electron donor. An expansion of this zone is observed for subsequent storage cycles. Within two storage cycles also the majority of the sulphate in the infiltrated water is reduced to sulphide (Figure 6.2-i).
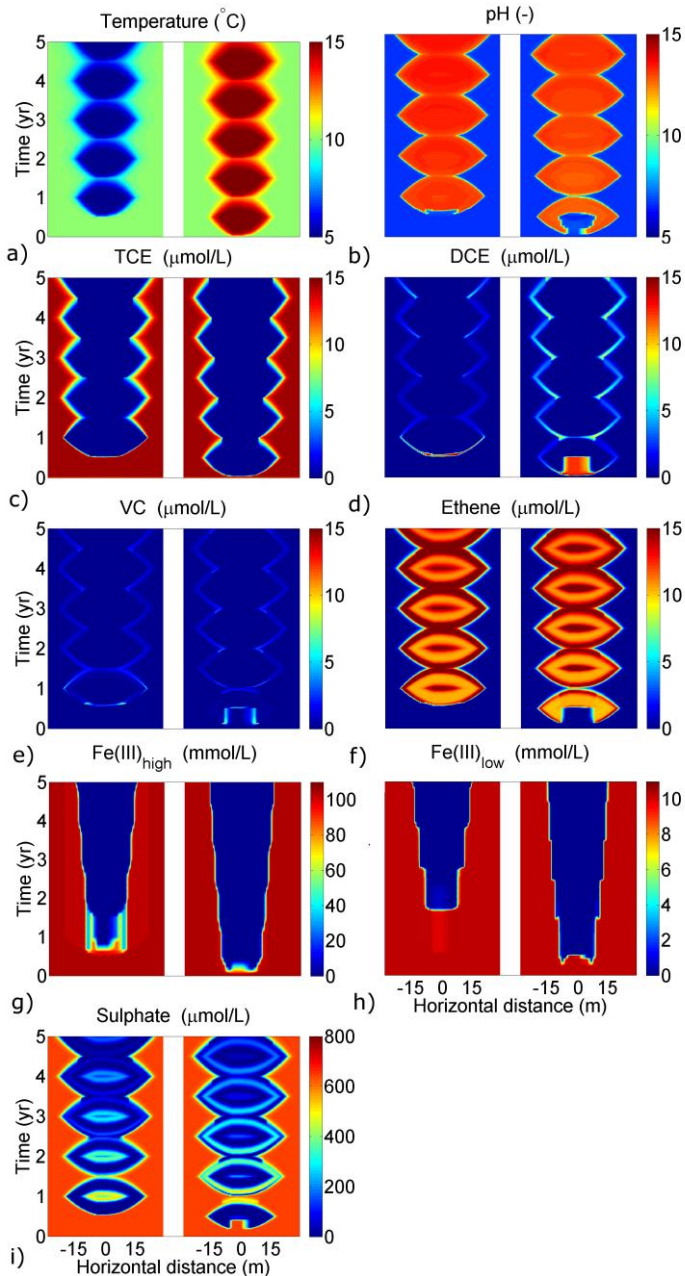
Figure 6.2 Development of aqueous species and minerals for the reference scenario S1. The x-axis depicts horizontal distance from the cold storage well (left) and heat storage well (right).

The domain average CVOCs and ethene concentrations (Figure 6.3) show that the majority of the TCE is fully degraded to ethene, with only a minor amount present as DCE and VC in the injection front (Figure 6.2). The constant total CVOCs concentration equal to the initial amount of TCE (14.5 μmol/L) in Figure 6.3 demonstrates a correct mole balance of contaminant and daughter products in the model.
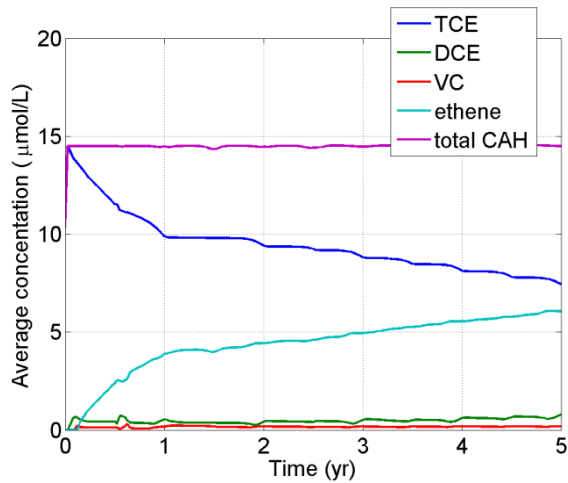
Figure 6.3 Domain average CVOCs concentrations for scenario S1.

6.3.2   Reference scenario with pH limited Fe-reduction

As shown in Figure 6.2-b, pH of the infiltrated groundwater in the model increases from 6.6 to 13.2 for scenario S1. This is surprising because dechlorination of CVOCs releases protons, and HCl formation after dechlorination can actually lower pH [69, 273, 292]. In fact, in some cases a pH buffer is added in ERD to prevent acidification, because reductive dechlorination is less effective at low pH [293]. Considering the relatively high amounts of both high and low bio-available iron, and the reaction order (Supporting materials, Table S6.1), the increase in pH in our model study can largely be attributed to reduction of iron oxides, and to a lesser extent also to sulphate reduction and methanogenesis. In the original batch model [277] pH stabilized at a level of 7.4 (Supporting materials, Figure S6.1). However, it must be noted that in the laboratory experiment [235] and batch model [277], the relative amount of bio-available iron was 10 times lower than under aquifer conditions because of the different groundwater to sediment ratio. Increasing pH due to iron reduction has been identified [294],

but no report of such considerable pH increase in laboratory or field studies was found. Two hypotheses to explain this discrepancy are (1) that, under field conditions, more buffer capacity is present, for example in the form of ion-exchanging clay minerals [295], or (2) reductive dissolution of Fe(III) is slowed down for increasing pH. The second explanation may be plausible as solubility of iron oxides decreases rapidly for pH levels above 7 [288]. Also, Wu et al. [290] show that microbial reduction of hematite reduces by a factor 10 when pH increases from 7 to 8.7 due to blockage of active surface sites by accumulation of biogenic Fe(II) and silicate on Fe(III) oxide and Fe(III)-reducing bacterial cell surfaces. As the rates for all the kinetically defined biochemical reactions in our model are independent of pH values, absence or presence of model buffer capacity and consequent model pH have no influence on the simulated dechlorination process. It may, however, be hypothesised that when iron reduction is inhibited, more electron donor becomes available for sulphate reduction and dechlorination, thereby increasing the overall dechlorination rate. This was explored by considering additional scenarios in which iron reduction was inhibited for pH > 7 (scenarios S6-S10). Results of the additional scenario S6 (Supporting materials, Figure S6.2), which, apart from the pH inhibition of reductive iron dissolution, is identical to the reference scenario, show that pH in the first storage cycle increases up to 8.7, and in later storage cycles stabilizes around 8. Indeed, dechlorination occurs slightly faster in this case as less electron donor is used by iron reduction (Figure 6.4). Also less electron donor is needed to reach similar dechlorination than in the non pH limiting scenarios. Our simulations indicate that the relation between laboratory and field processes, especially concerning the behaviour and reactivity of iron oxides in bioremediation efforts, and their pH dependency, is an important issue that requires further study. Such kinetic studies should involve laboratory batch or column experiments revealing pH dependencies and detailed pilot field studies related to competition for electron donor and effects of mass transport limitations [271].

6.3.3 Influence of electron donor dose

As addition of electron donor (lactate) and its fermentation products is the key factor in consecutive lowering of the redox conditions and reductive dechlorination, it comes as no surprise that lactate dose influences the dechlorination rate. In the reference scenario (S1) lactate dose was set at 3.8 mmol/L to achieve similar concentrations as in the batch experiments by Scheutz et al. [235]. Adding lactate at a lower dose slows down the reaction

(Figure 6.4-a). However, because ATES systems are typically designed to operate for 20 to 30 years, even with slow biodegradation a significant aquifer volume can be remediated. To compare dechlorination per unit of lactate added, scenarios with a lower dose have been run for a longer simulation time: 10 years (S2) and 50 years (S3). Results (Figure 6.4-b) show that, although dechlorination is slower at a lower dose, it also increases dechlorination per unit of lactate added. Similar influence of lactate dose is found for the scenarios with pH limitation on iron reduction and scenarios with immobile biomass (Figure 6.4). To cope with competition for electron donor between micro-organisms, a typical ERD approach is to supply an excess electron donor, effectively reducing all sulphate [296]. A similar approach could be suggested for a combined ATES-ERD concept. In addition, upon reaching sufficiently reduced conditions in the capture zone of the ATES system, the lactate dose can be lowered drastically. Given that typical groundwater volumes that are pumped by ATES systems are between 30,000 and 150,000 m^3/year per well [156], a continuously added dose of 3.8 mmol/L amounts to respectively 10 and 50 ton/year of sodium lactate used for the ERD treatment. In a pilot test reported by Lendvay et al. [199], dechlorination of 355 m^3 of contaminated aquifer was achieved within 99 days by biostimulation with approximately 23 kg of lactate. For an aquifer volume equivalent to 30,000 and 150,000 m^3 of groundwater, the amounts of lactate needed would be 5.6 and 28 ton respectively. However, based on a laboratory experiment performed by Ni et al. [92], the amount of lactate that would be needed to treat an equivalent volume of contaminated aquifer is much larger, respectively 82.5 and 412.5 ton. At a dose of 3.8 mmol/L, the latter would imply that at least 8 years of combined ATES-ERD are required for complete remediation of the volume of water that was influenced by lactate addition.

6.3.4   Influence of temperature

Although temperature changes do influence the maximum bacterial growth rates [171], temperature changes applied in our model do not have any impact on the overall progress of dechlorination regardless of the assumption on biomass mobility or pH limitation (Figure 6.4). As also indicated in [297], this is partly because increased growth rates in the warm storage are balanced by reduced growth rates in the cold storage. However, even in the scenario with a high storage temperature, virtually no effect is observed. Apparently other factors, such as total available electron donor, have a larger impact on the reaction kinetics.

It may be expected that the influence of temperature changes on (bio)geochemical reactions are more pronounced at high storage temperatures (e.g. 60 ˚C as considered in Bonte et al. [261]). Specifically for reductive dechlorination, the laboratory experiments of Friis et al. [171] show that the reaction rate in enriched lactate-amended cultures actually reduces for temperatures above 30 ˚C. Therefore, storage temperatures > 30 ˚C may not be desirable in a combined ATES-ERD concept.

6.3.5 Biomass mobility

As apparent from Figure 6.4, overall dechlorination is faster when biomass is assumed to be mobile compared to the immobile case. Also the total amount of dechlorination at the end of the simulation period is slightly higher in case of mobile biomass. In both simulations, the largest biomass growth was observed for iron reducers and secondly for lactate fermenters (Figure 6.5). Growth of DCE/VC degraders shows that degradation of DCE only occurs at a later time in case of immobile biomass (Figure 6.5-a and 6.5-b) which explains the slower dechlorination progress in this case (Figure 6.4). The average distribution of the different species of biomass during the last year of the simulation period shows that in the immobile case biomass is more concentrated close to the well, than in the mobile case. This is especially the case for lactate fermenters which, in the immobile scenarios, grow close to the well (Figure 6.5-d). Large amounts of attached biomass near the wells may lead to well clogging and thereby reduce the performance of an ATES system. Since the largest microbial growth is observed for lactate fermenters, it could be considered to use other electron donors [116, 298, 299]. As shown by Aulenta et al. [296] a mixture of hydrogen and acetate resulted in lower biodiversity and more effective dechlorination compared to lactate amended microcosms. An alternative approach to biostimulation by adding electron donor is bioaugmentation [273, 300]. As shown by Lendvay et al. bioaugmentation with DHC can speed up the dechlorination process compared to biostimulation without bioaugmentation [199].
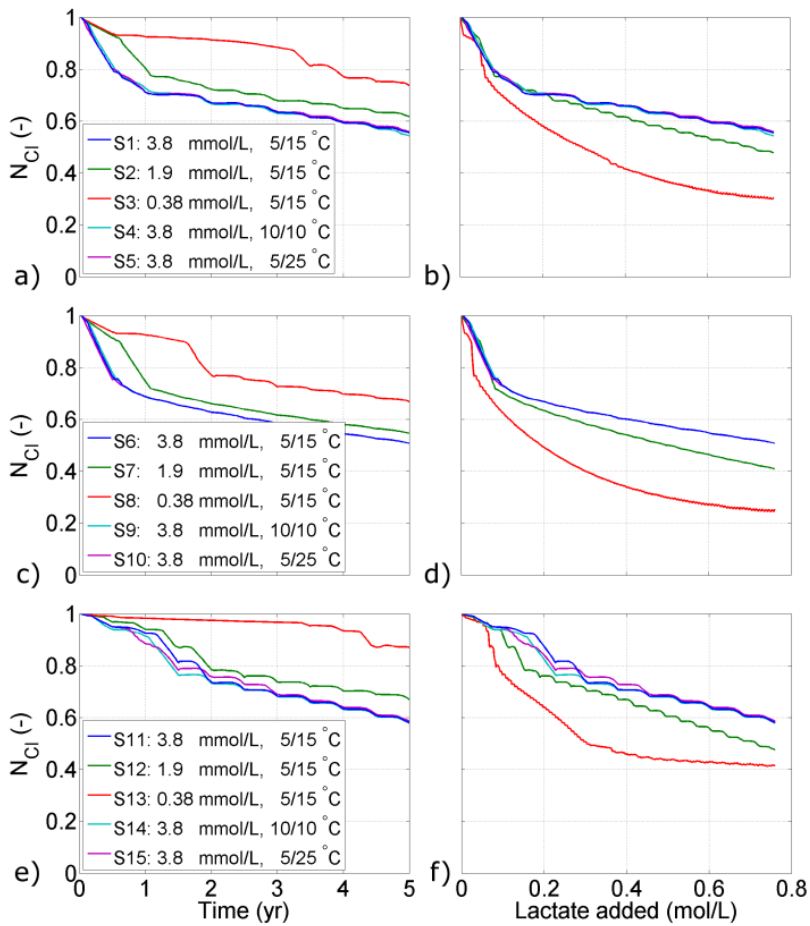
Figure 6.4 Spatially averaged normalized chlorine number in time (left panels) and against the amount of lactate added (right panels) for: (a)-(b) mobile biomass scenarios (S1-S5), (c)-(d) mobile biomass scenarios with pH limitation on iron reduction (S6-S10) and (e)-(f) immobile biomass scenarios (S11-S15).
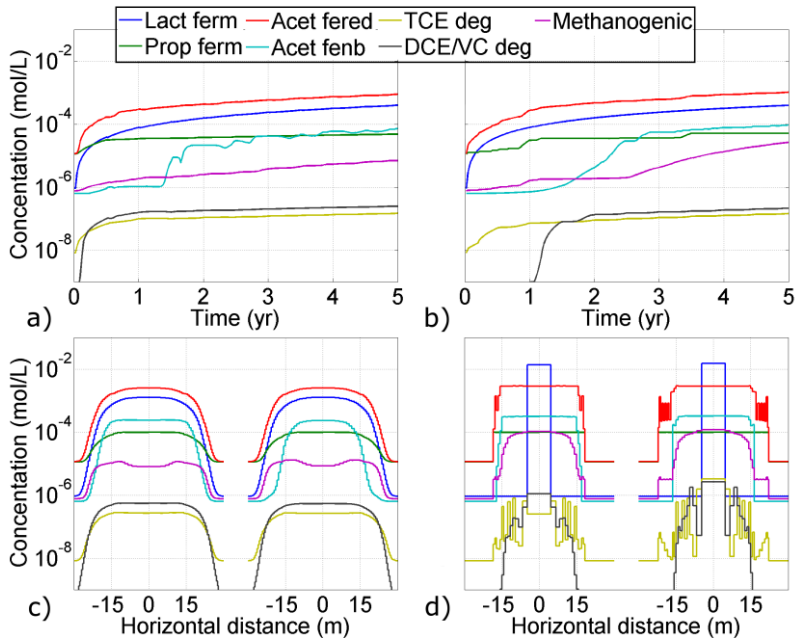
Figure 6.5 Spatially averaged biomass concentration for the mobile case S1 (a) and immobile case S11 (b) and biomass distribution averaged over the last year for the mobile case S1 (c) and immobile case S11 (d).

6.3.6   Limitations of the modeling approach

Note that in the 1D modelling approach adopted in this study, the aquifer was assumed to be homogeneous and transport of components was radially symmetric around the well of the ATES system. Therefore, no regional groundwater flow was incorporated. However, under field conditions, regional groundwater flow may transport part of the treated water including contaminant daughter products and fermentation products downstream outside of the capture zone of the ATES system. Similarly, new dissolved contaminant may enter the capture zone of the ATES system from upstream sources. In such case, the lactate dose should be chosen such that new contaminant that enters the capture zone of the well is degraded before it is transported downstream by regional groundwater flow, to prevent development of a contaminant plume. In this study, DNAPL was not incorporated in the model. In case DNAPL would be present, groundwater flow due to operation of the ATES system can enhance DNAPL dissolution, as shown by Zuurbier et al. [43]. This may lead to an increase in contaminant concentrations depending on the progress of ERD. For instance, if the

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treatment by ERD cannot compensate for the contaminant flux to the aqueous phase by dissolution of DNAPL, spreading of the contaminant plume can be expected.

6.4 Conclusions

Reductive dechlorination is a complex process, especially when competition with different terminal electron acceptors occurs [301], and taking into account transport and growth of microbial populations, mineral dissolution and precipitation and fermentation processes. The numerical model developed in this study provides a comprehensive tool to assess the development of biochemical processes in a combined ATES-ERD concept, which can be used to identify knowledge gaps and guide further research. Our model results suggest that dechlorination of TCE to ethene in the capture zone of an ATES well is possible when applying biostimulation by addition of electron donor. This is achieved by creating a zone around the wells where iron oxide and sulphate reductions do not occur anymore. After these electron acceptors have been depleted, a larger portion of the electron donor becomes available for dechlorination. The progress of dechlorination is dictated by lactate dose and amounts of electron acceptors. Although microbial processes are known to be temperature dependent, temperature changes induced by thermal storage did not significantly influence the overall dechlorination process in our simulations.

Simulations reveal several issues that require further study. Firstly, reduction of iron oxides in our simulation leads to increasing pH values that are not reported for laboratory or field studies. While fermentation of electron donor is widely studied, there is a limited number of reports on iron reduction in reductive dechlorination studies [271]. Also, our study shows that reductive dechlorination is faster when biomass is assumed to be mobile compared to the immobile case. Besides, in the immobile case, biomass, especially lactate fermenters, is more concentrated around the wells. Since well clogging due to microbial growth is a main concern for biostimulation using ATES, growth and mobility are important issues for further study. Another process that should be considered regarding clogging is the formation of methane bubbles due to methanogenesis [302]. Finally, study of field pilots is expected to improve the setting of boundary conditions for modelling and therefore model prediction which is needed to advance understanding of the combined ATES-ERD concept.

Acknowledgement

Supporting material

Table S6.1 presents the main kinetic processed that are incorporated in the model. Iron in the form of FeOOH is represented by Iron(III)$_{high}$ for the high bio-available component and Iron(III)$_{low}$ for the low bio-available component.

Table S6.1 Biochemical processes and inhibition (after [277]).

Process	Reaction equation	Inhibition by	Biomass species
Lactate fermentation	$3CH_3CH(OH)COO^- \rightarrow CH_3COO^- + 2CH_3CH_2COO^- + HCO_3^- + H^+$	-	Lact ferm
Propionate fermentation	$2CH_3CH_2COO^- + 3H_2O \rightarrow CH_3COO^- + HCO_3^- + H^+ + 3H_2$	Max biomass, Acetate	Prop ferm
Reduction of high bio-available iron with acetate	$CH_3COO^- + 8Iron(III)_{high} + 15H^+ \rightarrow 2HCO_3^- + 8Fe^{2+} + 12H_2O$		Acet fered
Reduction of low bio-available iron with acetate	$CH_3COO^- + 8Iron(III)_{low} + 15H^+ \rightarrow 2HCO_3^- + 8Fe^{2+} + 12H_2O$	Sulphate	Acet fenb
Reduction of high bio-available iron with hydrogen	$H_2 + 8Iron(III)_{high} + 4H^+ \rightarrow 2Fe^{2+} + 4H_2O$		-
Reduction of low bio-available iron with hydrogen	$H_2 + 2Iron(III)_{low} + 4H^+ \rightarrow 2Fe^{2+} + 4H_2O$	Sulphate	-
Hydrogen sulphate reduction	$4H_2 + SO_4^{2-} + H^+ \rightarrow HS^- + 4H_2O$	Iron(III)$_{high}$	-
Hydrogenotropic methanogenesis	$4H_2 + HCO_3^- + H^+ \rightarrow CH_4 + 3H_2O$	-	Methanogenic
TCE acetate dechlorination	$4C_2HCl_3 + CH_3COO^- + 4H_2O \rightarrow 4C_2H_2Cl_2 + 2HCO_3^- + 4HCl + H^+$	DCE, VC	TCE deg
DCE dechlorination	$C_2H_2Cl_2 + H_2 \rightarrow C_2H_3Cl + HCl$	TCE, VC	DCE/VC deg
VC dechlorination	$C_2H_3Cl + H_2 \rightarrow C_2H_4 + HCl$	TCE, DCE	DCE/VC deg

Table S6.2 Initial conditions for aqueous components, biomass species and minerals.

Initial groundwater conditions in the aquifer:	
pH	6.6
Temperature	10 ˚C
Acetate	0 mol/L
Propionate	10e-6 mol/L
Fe(+2)	9e-10 mol/L
TCE	14.5e-6 mol/L
Methane	6e-7 mol/L
Sulphate	640e-6 mol/L
Hydrogen	1e-10 mol/L
Cl(-1)	1.94e-3 mol/L
C(+4)	6.7e-3 mol/L
Initial biomass available (calculated per L pore volume)	
Lactate fermenters	9.14e-7 mol/L
Propionate fermenters	1.13e-5 mol/L
Iron reducers (high bio-available)	1.15e-5 mol/L
Iron reducers (low bio-available)	6.34e-7 mol/L
Methanogens	7.65e-7 mol/L
TCE degraders	8.22e-9 mol/L
DCE and VC degraders	5.02e-10 mol/L
Initial mineral species available (calculated per L pore volume)	
Calcite	17.93093 mol/L
Iron oxide (high bio-available)	1.04e-1 mol/L
Iron oxide (low bio-available)	1.03e-2 mol/L
CO_2(g)	0.00107 mol/L
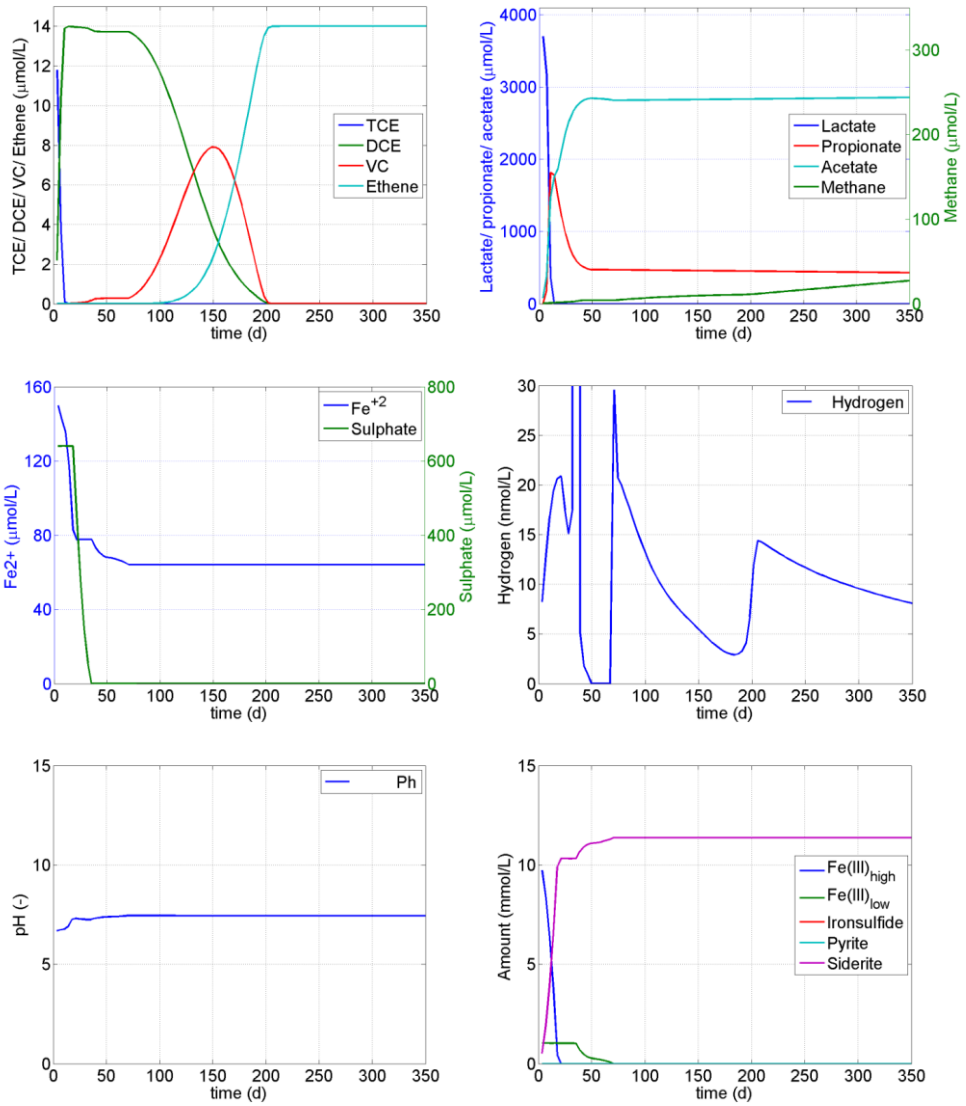
Figure S6.1 Results of the batch model.

In model scenario S6 iron reduction is inhibited for pH > 7. In this case, pH increases in the first storage cycle up to 8.7, and in later storage cycles stabilizes around 8.
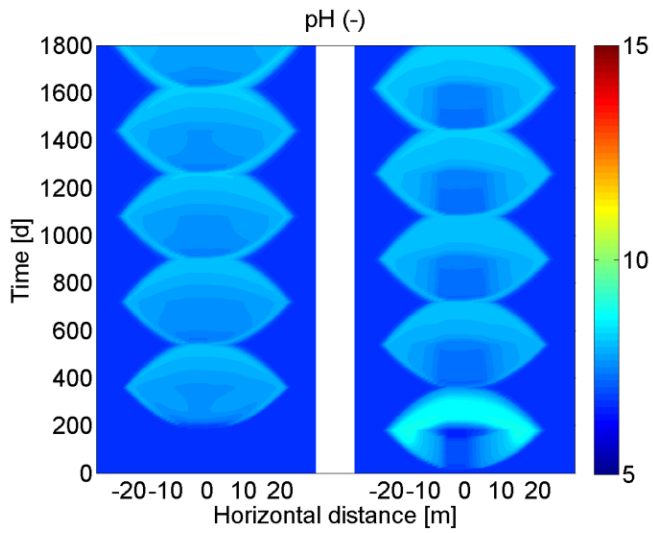
Figure S6.2 Development of pH levels in model scenario S6.

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Chapter VII

Opportunities of ATES-ENA toward

Sustainable Urban Development:

Future Perspectives in

the Netherlands and China

7.1 Introduction

7.1.1   What this thesis is about and why

With the rise of sustainability as guiding concept for societal progress and development, new technological concepts increasingly focus on integration of mature technologies from different fields (like climate mitigation and urban greening, combined energy and water efficiency), rather than invention of innovative yet immature ones. Integrations of techniques that are compatible, offer advantages and offset disadvantages in a cost-effective way, also have become the direction and trend for (re)development of urban areas. Within this context, this thesis investigated the feasibility of the integration of two subsurface technologies that individually are commonly applied in urbanized areas: enhanced natural attenuation (ENA) of chlorinated volatile organic compounds (CVOCs), for in situ bioremediation of contaminated groundwater and aquifer thermal energy storage (ATES) for increased energy efficiency. Both technologies are well matured, but the generally long duration of in situ bioremediation together with an increased demand for sustainable energy may lead to spatial as well as operational interferences. Whereas such interference at present is debated as unknown and unwanted, it can also be treated as an opportunity that enables implementation of ATES for energy supply with simultaneous removal of contamination in a redeveloping urban area (ATES-ENA). Therefore, this thesis aimed at gaining insights into the important processes and factors behind the combination of CVOCs bioremediation and ATES. By structuring the research into specific research questions and conducting laboratory experiments, including batch and column tests, as well as simulation modeling, conclusions could be drawn on the mutual impacts between ENA and operation and functioning of ATES. Below, we first briefly summarize the answers obtained to our research questions. In the later sections of this chapter, the main findings are then discussed from an integrated perspective, on which recommendations and future perspectives of the combined ATES-ENA approach, as presented in the final section, are based.

7.1.2   Concise answers to the research questions as presented in Chapter 1

In summary, the key findings of this thesis, in the form of straightforward answers to the research questions of section 1.3, are as following:

- Redox condition plays a dominant role for both ENA of CVOCs, and functioning of ATES. Suitable redox condition which requires proper regulation and monitoring is the guarantee for the combined system (RQ 1, Chapter 2, 4 and 5).

- Constantly elevated temperature in the warm well of ATES highly enhances the ENA of CVOCs. Groundwater transport by ATES can further improve the enhancement effect by increased temperature in the warm well. The synergism of these aspects generate a "1 + 1 > 2" effect (RQ 2, Chapter 3 and 6).

- Undesired change of redox condition caused by intrusion of oxygen, or nitrate due to vertical expanse of diffusion or regional groundwater flow can be harmful to both ENA of CVOCs and proper function of ATES (RQ 3, Chapter 4).

- Although concluding that the risk of biological clogging attributed to enhanced biological activity is relatively low may not yet solid, chemical clogging as common problem of ATES can be limited by biostimulation (RQ 4, Chapter 5).

- Coupling ATES and ENA is promising, and applying ATES as engineering tool for biostimulation, such as substrate injection and bioaugmentation, can be a cost-effective approach to support the combined system (RQ 5, Chapter 6). Hence, in this case enhanced natural attenuation can also be seen as engineered natural attenuation (ENA).

7.2 Relevant parameters in ATES-ENA system

A variety of important biological, chemical and physical parameters which are relevant to the ATES-ENA combined system were addressed through the previous five chapters of this thesis (Table 7.1). By investigating these parameters, different chapters are closely integrated, from which a comprehensive evaluation on the feasibility of ATES- ENA can be provided. As it is well known that redox conditions are essential for both ATES and ENA, this factor

was extensively studied. For understanding the mutual effects of both aspects, many efforts have been made to investigate the variation of temperature and groundwater transport due to ATES, and the development of biomass related to biostimulation. Last but not lease, necessity of bioaugmentation and choice of substrate concentration were also addressed from a more cost-effective point of view.

Table 7.1 Important parameters that were investigated in different chapters in this thesis.

Parameter	Chapter				
	2	3	4	5	6
Redox potential/condition	x		x	x	x
Temperature		x			x
Flow rate/water transport			x	x	x
Biomass concentration and mobility		x	x		x
Bioaugmentation	x	x	x		
Biological and chemical clogging			x	x	
Substrate concentration	x				x

7.3 The role of redox conditions

Except the transport of groundwater or bacteria, other relevant processes illustrated in Figure 1.3 are closely related to redox condition in the subsurface. Figure 7.1 shows the most ubiquitous processes in natural subsurface and relevant processes in the ATES-ENA combined system, with representative redox half reactions. As shown in Chapter 4, either promoting or suppressing these processes can be done by manipulating the redox conditions with electron donor or acceptor addition [142, 303], in which changes can be symbolized by redox potential (Chapter 2).

$E_H(V)^a$:

| Scale | -0.5 | 0 | +0.5 | +1.0 | Redox half reaction |

Process	Redox half reaction
O_2 red.	$O_2 + 4H^+ + 4e^- \rightarrow H_2O$
Denitrification	$2NO_3^- + 12H^+ + 10e^- \rightarrow N_2 + 6H_2O$
Mn(IV) red.	$MnO_2 + 4H^+ + 2e^- \rightarrow Mn^{2+} + 2H_2O$
Fe(III) red.	$FeOOH + 3H^+ + e^- \rightarrow Fe^{2+} + 2H_2O$
SO_4^{2-} red.	$SO_4^{2-} + 9H^+ + 8e^- \rightarrow HS^- + 4H_2O$
CO_2 red.	$CO_2 + 8H^+ + 8e^- \rightarrow CH_4 + 2H_2O$
Org.C ferm.	$CH_2O + H_2O \rightarrow HCOO^- + 3H^+ + 2e^-$
Org.C Ox.	$CH_2O + H_2O \rightarrow CO_2 + 4H^+ + 4e^-$
NO_3^- red.	$2NO_3^- + 4H^+ + 4e^- \rightarrow 2NO_2^- + 2H_2O$
NO_2^- red.	$2NO_2^- + 8H^+ + 6e^- \rightarrow 2N_2 + 4H_2O$
Fe_2O_3 red.	$Fe_2O_3 + 6H^+ + 2e^- \rightarrow 2Fe^{2+} + 3H_2O$
SO_4^{2-} red.	$SO_4^{2-} + 9H^+ + 8e^- \rightarrow HS^- + 4H_2O$
CVOCs red.	$C_2Cl_4 + 8H^+ + 8e^- \rightarrow C_2H_4 + 4HCl$
CO_2 red.	$HCO_3^- + 7H^+ + 8e^- \rightarrow CH_4 + 2H_2O$
AA Ox.	$C_6H_8O_6 + 8H_2O \rightarrow C_2H_3O_2^- + 4HCO_3^- + 17H^+ + 12e^-$
Lactate ferm.	$3C_3H_5O_3^- \rightarrow C_2H_3O_2^- + 2C_3H_5O_2^- + HCO_3^- + H^+$
Lactate Ox.	$C_3H_5O_3^- + 2H_2O \rightarrow C_2H_3O_2^- + HCO_3^- + 5H^+ + 4e^-$
Acetate Ox.	$C_2H_3O_2^- + 4H_2O \rightarrow 2HCO_3^- + 9H^+ + 8e^-$
Propionate Ox.	$C_3H_5O_3^- + 6H_2O \rightarrow 3HCO_3^- + 14H^+ + 12e^-$
Sulphide Ox.	$HS^- + 4H_2O \rightarrow SO_4^{2-} + 9H^+ + 8e^-$
Fe(II) Ox.	$2Fe^{2+} + 3H_2O \rightarrow Fe_2O_3 + 6H^+ + 2e^-$

Figure 7.1 Most relevant reduction-oxidation processes in the subsurface under different redox conditions (based on [303]). Upper part refers to representative half reactions in nature. Lower part refers to half reactions considered in this thesis. $E_H(V)$ is the standard reduction-oxidation potential. $C_6H_8O_6$, $C_3H_5O_3^-$, $C_3H_5O_2^-$ and $C_2H_3O_2^-$ standard for ascorbic acid (AA), lactate, propionate and acetate respectively.

7.3.1   Redox conditions and ENA

Characterisation of redox conditions in contaminated subsurface is considered as an important prerequisite for better understanding the behaviour and fate of groundwater contaminants [4, 142, 301, 303, 304]. Redox potential can be generally used to indicate the redox condition in the subsurface, and the intrinsic redox potential of a specific contaminated site also represents the potential or the progress of natural attenuation of groundwater contaminants [92, 305, 306]. Besides, in the case of combing enhanced bioremediation of CVOCs and ATES, redox condition is as well an essential factor, especially for reductive dechlorination of CVOCs. Such reductive dechlorination requires strict anaerobic condition to allow the transfer of electrons from electron donor to CVOCs as dominant electron acceptor [60, 68, 153]. However, many other reactants in the subsurface can compete with CVOCs for receiving electron, such as Fe(III), sulphate and CO_2. In particular, Fe(III) has been commonly revealed as the strongest electron competitor to CVOCs present in the subsurface, leading to inhibition of reductive dechlorination [68, 92, 128, 129, 307]. Successful reductive dechlorination mainly occurs after overcoming the Fe(III) reducing condition. Regarding to other common redox conditions, reductive dechlorination can proceed under sulphate or methanogenic conditions, despite some mild competition. Therefore, removal or reduction of Fe(III) in the subsurface is needed prior to reductive dechlorination process (Chapter 2 and 6). However, we found that complete removal of all Fe(III) is in fact not necessary and impossible considering the total amount of Fe(III) content present in the subsurface. Instead, a threshold of Fe(III) content exists for specific Fe(III) reducing aquifer which is actually limiting the reductive dechlorination. This threshold was considered and investigated as bio-available Fe(III) that needed to be reduced to onset reductive dechlorination in Chapter 2 using a redox titration method. To remove bio-available Fe(III) or to improve and achieve a suitable redox condition for reductive dechlorination, electron donor is generally required in enhanced bioremediation. Natural electron donor refers to soil organic matter (SOM), which is normally found to be present at limited level. It is the type and concentration of SOM that influence the redox conditions in the subsurface. With the fermentation process of SOM, redox condition is being changed. In many laboratory and field studies, organic acid such as lactate is widely used to improve the redox conditions [70, 137, 139, 174, 308]. As lactate can be directly utilized and its fermentation products can also further be converted into H_2 as electron donor, it was adopted as substrate to improve or

maintain favorable redox condition in most experiments performed in this thesis. In Chapter 4, we illuminated that presence of lactate was of necessity to prevent inhibiting compounds such as oxygen or nitrate and maintain the redox potential for reductive dechlorination. The low redox potential preserved by lactate was the key to maintain the activity of *Dehalococcoides* (DHC) that is responsible for complete reductive dechlorination (Chapter 4).

7.3.2   Redox conditions and ATES

Redox condition of the subsurface also plays an important role in proper operation of the common ATES system. As the most prevalent problem regarding to the functioning of ATES, chemical clogging is caused by high redox condition. Precipitation of Fe(III) minerals is the predominant phenomenon in chemical clogging in ATES, due to the increase of redox potential when encountering groundwater with high redox state like from nitrate reducing zone, or even by oxygen if the system is not strictly gas tight [98, 248-250]. As a consequence, characterization is essential by survey and monitoring of the redox condition and geochemistry in the aquifer in which the ATES system is installed. In contrast to normal ATES system, redox condition in the combined system of enhanced bioremediation and ATES might not be so critical for the aspect of ATES operation. The impact of redox condition on the functioning of ATES is limited by implementation of enhanced bioremediation, which demands low redox condition. By constantly sustaining low redox potential with substrate (lactate), chemical clogging by Fe precipitate could be prevented (Chapter 5).

7.4 Impacts of ATES operation on ENA

7.4.1   Temperature

The ATES system has impact on the temperature in the subsurface, as it creates in doublet systems a warm and a cold zone which are separated from each other [81-83]. This altered temperature leads to direct impacts on the ENA of CVOCs as biological activity is temperature dependent. The reductive dechlorination has been reported to be greatly affected by temperature, through the response of relevant microorganisms [171, 172, 175, 282, 309]. Generally for biological conversions, if the rate is limited by enzyme activity, an increase of 10 ˚C in temperature will improve the biodegradation rates by a factor of 1.5 to 2.5 [90].

Considering the temperature around the warm well of ATES which can be maintained at 20-25 ˚C, the overall remediation duration can be likely shortened half leastwise, as background temperature of groundwater in the Netherlands is about 10 ˚C.

In this thesis, the effect of temperature by ATES was studied with a fundamental batch experiment and by modeling in Chapter 3 and 6, respectively. Remarkably in Chapter 3, we found out the improvement related to cis-DCE removal was more than 13 times faster when comparing enhanced reductive dechlorination with ATES at 25 ˚C and without ATES at 10 ˚C. Although the dynamic nature of water transport (see section 7.4.2) in ATES was later proved to also assist the progress, another experiment mimicking borehole thermal energy storage (BTES), in which solely the subsurface temperature fluctuates, demonstrated an increase of overall cis-DCE removal rate of 8.5 times at 25 ˚C compared to ENA at 10 ˚C. The most essential reason was that DHC microorganisms could magnificently grow in the mimicked warm well with favorable temperature and organic substrate, resulting in enhancement of biomass concentration. In fact, the DHC concentration was detected at the highest level with complete and fastest reductive dechlorination process under temperature range of 20-30 ˚C [171, 310], which is close to temperature in the ATES warm well. However, in the modelling study (Chapter 6) the effect of higher temperatures was rather insignificant on enhancing the biodegradation rate. That is because only when the biomass concentration reaches an adequate level can temperature play a dominant role in reductive dechlorination. In contrast to Chapter 3, bioaugmentation of active DHC was not incorporated in the reactive transport model in Chapter 6. Although biomass growth was already included in the model, the difference of initial DHC concentration in the batch and model reflected different evolution of reductive dechlorination. Moreover, when biomass growth can perform at optimal conditions, as is occurring in an ATES well at 25 ˚C experiment, the bioconversion is accelerated by the increasing biomass. In fact at such warm well in ATES an autocatalytic process leads to much higher conversion rates than just could be realized by only temperature increase.

The mesophilic nature of DHC [310, 311], together with the elevated temperature which can be constantly remained in the warm well, and optimal conditions for biomass growth leads to higher bioremediation rates in ATES-ENA combined system compared to just ENA at normal subsurface temperature. Unfortunately, many non-high temperature ATES systems

are practically operated at temperatures below 20 ˚C in the warm well [77, 156, 312, 313], despite a maximum allowed range of 20-25 ˚C in the subsurface is generally accepted in several countries [166, 167]. The current operation state of ATES is not yet optimal for the CVOCs reductive dechlorination (Figure 7.2) due to either the requirement of energy balance or low efficiency of the ATES system. Further improvement on the system efficiency or a more flexible operation model will lead to a more optimal combined system targeting in contaminant remediation.
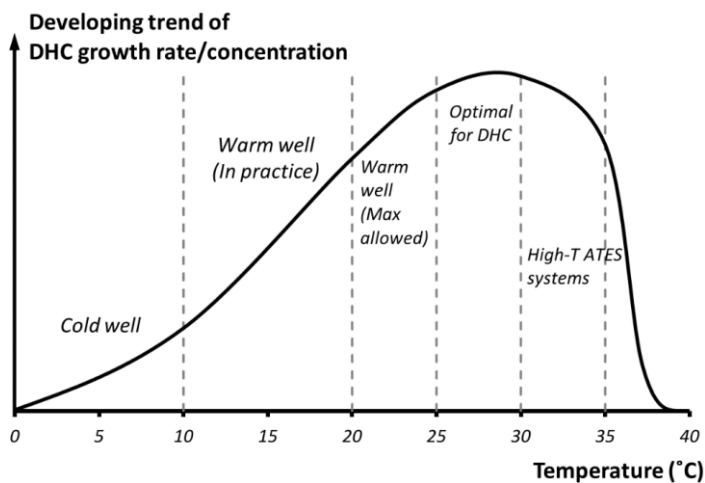
Figure 7.2 Estimated relationship between developing trend of DHC growth rate/concentration and temperature in ATES (based on [171, 172, 175, 310, 311]).

7.4.2   Groundwater transport

Comparing the common large length of CVOCs plumes and the relatively small influencing area of ATES, installing a warm well that captures most contaminants will not be feasible. This results in the presence of CVOCs also in the cold well of ATES of which the low temperature will suppress bioremediation [171, 172, 175, 309]. The inhibition effect in the cold well has been considered to counter the enhancement in the warm well [181]. However, such negative impact of cold well can be overcome by the groundwater transport due to the seasonal operation of ATES. Although in the cold well, low temperature (6-7 ˚C) remains and so does the inhibition, the stop of reductive dechlorination of a specific amount of CVOCs is actually temporary. During the summer, the CVOCs from the cold zone will be

transported together with the groundwater towards the warm well where reductive dechlorination always well proceeds, as the biomass in the warm well is active and growing in the attached mode (Chapter 3 and 4).

The batch experiment with periodical liquid exchanges for simulating seasonal operation of ATES in Chapter 3, and the reactive transport model in Chapter 6 have revealed the water transport process make meaningful contribution to the overall dechlorination progress. Besides the movement of CVOCs, the distribution of biomass is as well of importance. Hence, the mobility of biomass was investigated in Chapter 3, 4 and 6. From Chapter 3 in became clear that the DHC bacteria prefer to attach to the soil matrix, leading to the constant upgrading of dechlorination in warm well while no biomass developed in the cold well after several liquid exchanges. The immobility was confirmed in Chapter 4 with DNA microbial analysis. Comparing the DHC concentrations in the added inoculum and in the liquid phase of the column system, with dilution effect being taken into account, we noticed that over 99% of DHC indeed stayed within the aquifer column (Figure 4.5). This finding is consistent with other studies that report DHC is much less mobile compared to other microorganisms like *Geobacter* [176-178]. Consequently, the abundance of DHC and its immobility in the warm well contributed to the prodigious dechlorination progress, which therefore overcomes the obstacle of the low temperature in the cold well. On the other hand, both mobile and immobile biomass scenarios were simulated in Chapter 6 which predicted faster dechlorination progress in the mobile case. In particular, iron reducers and lactate fermenters were observed with largest suspended growth among all biomass species (Figure 6.5), indicating that ATES homogenizes and enlarges the area with more biological activity especially for regulating redox conditions. This assists in overcoming Fe(III) reducing and advancing redox condition favorable for reductive dechlorination. However, in the immobile scenarios, biomass growth was observed to occur mainly close to the well, indicating that higher risk of biological clogging could be realized from the less planktonic microorganisms in reality. In summary, we concluded that the seasonal groundwater transport by ATES greatly enhanced the dynamics in the subsurface system. Based on the results of this thesis, such increase in dynamics could positively affect the ENA of CVOCs mainly in two ways, which are 1) ensuring sufficient retention time of CVOCs contaminants within the capture zone of

ATES, and 2) improving the overall redox condition in the subsurface by better delivering electron donor.

7.5 Impacts of ENA on the functioning of ATES

As biofouling or biofilm formation leading to biological clogging has been observed in common groundwater activity and in ATES as well [98, 247, 248, 252-254, 257], a limitation for combining ATES and ENA thus exists in the potential biological clogging caused by biomass growth. Besides, in the case of solo ATES, chemical clogging has also been observed which is mainly attributed to intrusion of oxygen if the ATES system is not very gas tight or encountering water with nitrate due to vertical expanse of diffuse nitrate contamination or regional groundwater flow. Precipitation of Fe and Mn oxide or hydroxide is the most usual chemical clogging problem in ATES. In this thesis, the biological and chemical clogging issues were addressed in Chapter 4 and 5 with two recirculating columns with flow rates of 10 and 50 mL/min respectively. Pressure drop (ΔP) between the influent and effluent side of the column was monitored as the indicator of clogging of the column system. We studied the response of ΔP to two main actions: 1) enhancing biological activity by lactate addition; 2) promoting chemical precipitation by nitrate addition. The ΔP in these column tests did not remarkable increase in any periods of enhanced biological activity with several times of active DHC bioaugmentation, while significant increase of ΔP was observed after nitrate addition (Figure 5.2 and 5.4). Meanwhile, an expansion with four orders of magnitude in total bacteria was detected (Figure 4.5). These figures illustrated that biological clogging did not occur in our column systems within the timeframe of the experiment. In addition, instead of causing clogging, we surprisingly found that biostimulation could reduce buildup of pressure drop incurred by chemical clogging of Fe oxide. These results look promising for combining ATES and ENA, as they indicate the risk of biological clogging related to biostimulation is relatively small. Biostimulation itself that usually maintains a low redox condition may furthermore prevent chemical clogging in ATES.

Our preliminary column tests generated positive outcomes, yet deeper and more comprehensive work remains to be done, as these column tests may not be sufficiently representative of real ATES. Seeing that biological clogging also exists in the solo ATES

system, the risk in this matter regarding combination with enhanced bioremediation should not be underestimated. Therefore a concrete conclusion is not yet able to be presented and we recommend further exploration on such clogging issues, adopting our column tests as a fundamental framework, because potential clogging may greatly influence the feasibility of the combined system.

7.6 Future perspectives

7.6.1 Opportunities and challenges in the Netherlands

With the rise in the development of ATES in the Netherlands, ATES has gained much research interests regarding its impacts on groundwater and subsurface [43, 164, 165, 181, 192, 247, 261], where many complex biogeochemical processes occur. However, due to the difficulty to fully understand processes in heterogeneous subsurface and the concern of spreading groundwater contaminations and thus the deterioration of groundwater quality, as yet the ATES systems are limitedly applied in non-contaminated aquifers. Therefore, achieving the sustainability target especially in utilization of energy may be slowed down by this limitation, as the overlap between contaminated sites and ATES under the current situation is obvious (Figure 7.3) which leads to conceivable interference between each other.

Soil contaminated cites (2013)   Installed ATES systems (2010)
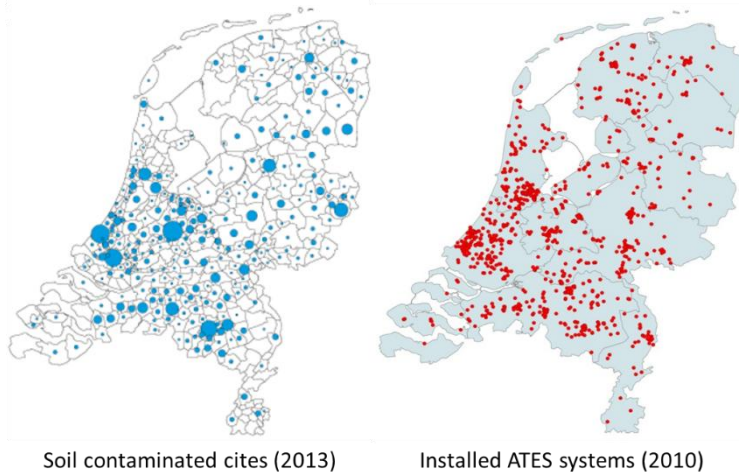
Figure 7.3 Maps of the Netherlands indicating the overlap between soil contamination and ATES systems (left [314], right [315]).

In this thesis, the perceived advantages of using ATES to engineer the natural attenuation have been confirmed by fundamental investigation, while its disadvantages like contaminant spreading or clogging were shown to be counteracted or manageable. A further step then lies in well-monitored large scale pilot tests in the field. At this moment two pilots are proceeding in Eindhoven and Utrecht [49, 168], which so far have not encountered practical problems. In the Netherlands, there are hundreds of areas with subsurface condition similar to these two sites, especially in urban areas. This is encouraging for much more potential cases or projects to be generated, for instance in North and South Holland provinces where ATES and subsurface contamination are most dense. In addition, from the long term development point of view, the case-based approach needs to advance to an integrated approach [168], because the overlapping situation reduces the cost-effectiveness of site-by-site approach due to the high chance of interference among cases. Finally, considering the relatively high Fe content in aquifers in the Netherlands, if integrating biostimulation can indeed counter the chemical clogging caused by Fe precipitate in ATES, the development perspective of the combination of ATES and enhanced would be magnificent.

7.6.2 Opportunities and challenges in China

In China, similar to the Netherlands, CVOCs contamination and other organic pollutants such as polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) are often detected in the subsurface of urbanized areas, especially in the eastern part [316-320]. In situ bioremediation approaches are also popularly used for these organic contaminants and many studies have focused on further developing and improving bioremediation technologies [321-325]. This provides a good base for integrating bioremediation with other systems. On the other hand, although it is reported that the first ATES was actually applied in Shanghai (China) in the 1960s [326, 327], the later development and implementation of standard ATES were less notable compared to those in the Netherlands. In fact until the 1980s, many cities in China had promoted the use of ATES, such as Beijing, Hangzhou, Nanchang and Tianjin as well as in other central-eastern cities. However, the overexploitation of groundwater due to imbalanced operation of the systems lead to land subsidence and lowering of groundwater level, and the development of ATES was hampered [328, 329]. With the problem being solved, and the driving force of the need for CO_2 reduction, ATES has been put back on the agenda and investigations have been carried out [327-330]. Currently still over 77% of energy

consumption in China is produced from coal while only 8% is from renewable sources [331]. Promotion of ATES application can make a great contribution towards reaching the goal of 15% renewable energy of China in 2020. Therefore, the opportunity actually lies in the development of ATES aiming at sustainable energy, with a side benefit on remediation of subsurface contaminants. Compared to the Netherlands, the advantage for using the combined system in China is that many more demonstration pilot tests are allowed for comprehensive research prior to real applications. The less intense pressure on subsurface use by ATES prevents interference among systems and can make them more flexible and compatible to enhanced bioremediation.

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Bibliography

Bibliography

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Summary

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The combination of enhanced natural attenuation (ENA) of chlorinated volatile organic compounds (CVOCs) and aquifer thermal energy storage (ATES) appears attractive because such integration provides a promising solution for redevelopment of urban areas in terms of improving the local environmental quality as well as achieving sustainable energy supply. It will reduce the current negative interference between groundwater contaminants and ATES systems that arises from the rapid increase of ATES system numbers and generally long duration of contaminated groundwater treatments. However, currently the implementation of the combined system is at an initial stage, and still requires comprehensive study before advancing to mature application. Studies should specifically focus on understanding of the basic biogeochemical processes in aquifer systems under conditions of ATES and enhanced bioremediation and their mutual impacts when combined in ATES-ENA. To this end, the research as reported in this thesis employed laboratory experiments and modeling approaches focused on finding the essential process factors involved in the combined system, revealing possible drawbacks, and providing a better understanding to design alternative options on better operation of the combined system.

Chapter 2 assessed the limiting factor for reductive dechlorination of PCE in an Fe(III) reducing aquifer, being the typical type of subsurface in the Netherlands. A step-wise batch study was performed which consisted of redox conditioning by lactate and ascorbic acid, followed by reductive dechlorination in different scenarios. For the sediment material sampled from the Fe(III) reducing aquifer, conditioning of the redox potential could stimulate PCE dechlorination. It was concluded that 75 μmol electron equivalents per gram dry mass of aquifer material was the threshold to obtain a redox potential of -450 mV, which is theoretically suitable for PCE reductive dechlorination. However, dechlorinating bacteria required for fully reductive dechlorination are generally lacking in Fe(III) reducing aquifers. Without bioaugmentation of dechlorinating bacteria, PCE could only be reduced to TCE or cis-DCE. The step-wise approach and findings obtained from different scenarios tested in this study are relevant for improving the cost-effectiveness of the design and operation of in situ bioremediation. The redox potential of an aquifer can be used as a general indicator to evaluate the potential for CVOCs reductive dechlorination. For achieving specific goals of

in situ bioremediation projects at different CVOCs contaminated sites with various environmental conditions, the balance between cost, benefit, and potential risks (e.g. bio-chemical well clogging due to bacteria growth and precipitation of metal-oxides) should be estimated before the design and operation of the ATES-ENA systems. This chapter provides insights into the essential factors that determine the feasibility of ATES-ENA.

In **Chapter 3**, the two most important impacts of ATES on enhanced bioremediation of CVOCs were investigated using batch experiments. Besides, another type of underground thermal energy storage system, the borehole thermal energy storage (BTES) was also studied as a comparison to ATES. Here cis-DCE was targeted as it is commonly found to accumulate in the subsurface due to incomplete dechlorination. Compared to a natural situation (NS) with sufficient electron donor and bioaugmentation at a constant temperature of 10 °C, we assessed the effect of ATES by exchanging liquid between bottles kept at 25 and 5 °C, and the effect of BTES by alternating temperature between 25 and 5 °C periodically. Under ATES warm condition, cis-DCE was dechlorinated to ethene and at an increasing rate with each liquid exchange, despite no biodegradation being observed under ATES cold condition. The overall removal rate under alternating ATES conditions reached 1.83 µmol cis-DCE/day, which was over 1.5 and 13 times faster than those in BTES and NS conditions. Most probably growth of biomass occurred under ATES warm condition, leading to an autocatalytic increase in conversion rates due to higher biomass concentration. Comparison between batches with or without *Dehalococcoides* inoculum revealed that their initial presence is a determining factor for the dechlorination process. Temperature then became the dominant factor when *Dehalococcoides* concentration was sufficient. The results also indicated that *Dehalococcoides* was preferentially attached to the soil matrix. This chapter highlights the importance of the dynamic temperature regimes in ATES on the bioremediation of CVOCs and recommends to implement biostimulation actions in the ATES warm well.

Further impacts of ATES related to change in redox condition on bioremediation of CVOCs, with focus on microbial responses of *Dehalococcoides*, were explored in **Chapter 4**. In this chapter, we adopted a recirculating column experiment with a flow rate of 10 mL/min (representing the flow velocity at a distance of 1.3 m from the center of a typical ATES well) to simulate the ATES system. To mimic potential periodic redox fluctuations that accompany ATES, serial additions of lactate and nitrate were performed. Firstly, also at the relatively

high liquid velocity (compared to normal bioremediation conditions) complete reductive dechlorination from cis-DCE to ethene was achieved in the column system. However, dechlorination was immediately terminated by subsequent nitrate addition due to direct interruption of *Dehalococcoides* retention to the soil matrix. In our column system, which was much more homogeneous than subsurface in reality, repeated interruption of dechlorination via *Dehalococcoides* was extremely severe. Such repeated interruption by nitrate dosing eventually led to less easily reversible while requiring more efforts for recovering dechlorination. In addition, the hypothesis of the immobility of *Dehalococcoides* was further confirmed by the microbial analysis of microorganism in the liquid phase where only less than 0.1% of the *Dehalococcoides* inoculum could be found back. Although some field studies demonstrated easier regeneration of *Dehalococcoides* in the subsurface after suffering oxidant, results from this chapter emphasized the sensitive resilience of *Dehalococcoides* which needs careful consideration in biostimulated ATES condition, and a functional combined system requires dedicated ATES operation and monitoring on the aquifer geochemical conditions.

The major concern on possible negative impact of enhanced bioremediation on ATES is biological clogging attributed to biomass growth. As chemical clogging due to Fe(III) precipitates is a common problem in the functioning of ATES, the clogging issues (both biological and chemical) should be addressed before practical application. The potential clogging issues in the combined system were then researched in **Chapter 5** using the same recirculating column system as in the previous chapter. For this purpose, two flow rates, 10 and 50 mL/min, were implemented. In the two columns, enhanced biological activity and chemically promoted Fe-oxide precipitation were studied by addition of lactate and nitrate respectively. Pressure drop (ΔP) between the influent and effluent of the columns was monitored to indicate clogging of the system. The results showed no increase in ΔP during the period of enhanced biological activity, with large amount of lactate and active inoculum being added, even when the concentration of total bacteria in the liquid phase increased by four orders of magnitude. Nitrate addition, however, caused significant increase of ΔP. Remarkably, in the column with higher flow rate (50 mL/min), an unforeseen blow-up occurred at the end of experiment, as the buildup of pressure in the system was higher than the strength of the glass column. However, in the column with flow rate of 10 mL/min, high

pressure buildup caused by nitrate addition could be alleviated by lactate addition. Such finding indicates that the risk of biological clogging related to biostimulation is relatively small, because by maintaining a low redox condition biostimulation itself may counter chemical clogging in ATES. Nevertheless, acknowledging that a column system cannot fully mimic real ATES conditions, additional tests are necessary to further investigate the clogging issues in the combined system.

In **Chapter 6**, we performed a simulation of ATES-ENA with a reactive transport model, using ATES as the engineering tool for lactate injection in a hypothetical TCE contaminated aquifer which is assumed to be homogeneous. Many relevant processes in the combined system were simulated, such as TCE, cis-DCE and VC dechlorination, sulphate and Fe(III) reduction, organic acid fermentation and oxidation and growth of different biomass. In total 15 scenarios are considered in the model, including variations in lactate dosage (three concentration levels: 3.8, 1.9 and 0.38 mmol/L), temperature (three pairs for the ATES cold/warm well: 5/15 ˚C, 10/10 ˚C, 5/25 ˚C), biomass mobility (purely mobile or immobile), and pH limitation on Fe(III) reduction (absence and presence of such an effect). In the five years' simulation by the model, complete dechlorination to ethene was achieved within 1 year, in the influence zone of the ATES wells, for the reference scenario with 3.8 mmol/L lactate, 5/15 ˚C ATES well temperatures and mobile biomass. Scenarios with lower dosage of lactate gave results with less dechlorination progress. Growth of biomass, especially iron reducer and lactate fermenter, was significant also in the first year (for both mobile and immobile biomass scenarios). Biomass also spread throughout the influence volume of ATES for both warm and cold wells. However, scenarios with different well-temperature pairs did not noteworthy differ in dechlorination progress. This could probably be due to biomass concentration being the limiting factor in this model setup, while temperature was not. Such situation was quite different than that in Chapter 3, of which experiment with bioaugmentation in the beginning. Besides, the model here could not include the important autocatalytic process (**Chapter 3**) which generated much faster dechlorination than just could be realized by only temperature increase in this chapter. In general, the modeling in this chapter suggests that applying ATES as engineering tool for biostimulation (substrate injection and bioaugmentation) can be a cost-effective approach to support the combined system.

Eventually in **Chapter 7**, overall discussions upon results gained from previous chapters were integrated and the research questions as presented in the introduction are reiterated. In addition, recommendation upon future study, and wider implications with future perspective for practical application are also discussed. We concluded that redox condition is the most essential factor in the ATES-ENA system. The mutual impacts of ATES and ENA were revealed to be quite positive. Elevated temperature in the ATES warm well synergizing with groundwater transport can provide "1 + 1 > 2" effect. Besides, ENA can probably reduce risk of chemical clogging in ATES, instead of causing biological clogging. The further investigation was recommended to perform with larger scale pilot tests. Finally, a brief review of possible applications was given for two countries, the Netherlands and China, which both have dense groundwater and subsurface contaminations around urban areas. The ATES technology is much more mature in the Netherlands, whereas in China, the advantage is the more flexible usage of subsurface. For both countries, ATES-ENA can provide cost-effective outcomes on both energy production and groundwater management.

Samenvatting

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Het samengaan van versnelde natuurlijke afbraak van vluchtige gechloreerde koolwaterstoffen (VOCls) in grondwater als saneringsmaatregel met de opslag van warmte en koude in watervoerende pakketten lijkt aantrekkelijk te zijn omdat een dergelijke koppeling gunstig kan zijn voor zowel de lokale milieukwaliteit als de verduurzaming van de energievoorziening. De verwachting is dat de negatieve interferentie die ontstaat wanneer Koude- en WarmteOpslagsystemen (KWO) worden geïnstalleerd in of nabij verontreinigd grondwater worden beperkt of zelfs worden tegengegaan. Dit is vooral van belang naarmate het gebruik van de ondergrond voor de opslag van energie toeneemt en de tijd die nodig is voor het voldoende verbeteren van de grondwaterkwaliteit lang is. De toepassing van deze combinatie staat echter nog in de kinderschoenen omdat nog onvoldoende duidelijk is hoe biochemische processen in de ondergrond worden beïnvloed door een combinatie van KWO en (gestimuleerde) biologische sanering en omgekeerd, hoe KWO en biologische sanering worden beïnvloed door bio-geochemische processen. Het experimentele en modelmatige onderzoek dat in dit proefschrift is beschreven richtte zich daarom op de identificatie van kritische processtappen van de combinatie KWO/saneren. Met de verkregen kennis van deze processtappen kunnen technologische risico's beter worden geschat en kunnen ontwerpen worden ontwikkeld die leiden tot een verdere verbetering van de lokale milieukwaliteit en energievoorziening.

In Hoofdstuk 2 wordt bekeken wat de beperkende factor is bij de reductieve dechlorering van tetrachlooretheen (PCE) in een watervoerend pakket onder ijzer reducerende omstandigheden. Deze omstandigheden komen veel voor in Nederlandse watervoerende pakketten. In een batch experiment werd daartoe eerst de redox potentiaal van een representatief monster verlaagd door lactaat of vitamine C toe te voegen. Het bleek dat per gram (droog) monstermateriaal 75 umol electron equivalenten nodig waren om een redox potentiaal (-450 mV) te verkrijgen wat volgens voorgaande onderzoeken geschikt is voor reductieve dechlorering. Na deze redox conditionering werd onder verschillende condities de afbraak van PCE gevolgd. De complete afbraak van PCE naar etheen werd alleen gezien wanneer speciale dechlorerende bacteriën aan het monster werden toegevoegd. Zonder deze toevoeging stopte de afbraak bij trichlooretheen (TCE) of cis-dichlooretheen (DCE). Dit lijkt

logisch aangezien dechlorerende bacteriën bij een lagere redox potentiaal actief zijn dan aanwezig was in het monster (ijzer reducerend). De gebruikte stapsgewijze aanpak en de gevonden resultaten die verkregen zijn in dit experiment zijn nuttig voor het verhogen van de kosten effectiviteit van ontwerp en uitvoering van in-situ bioremediatie. Om een eerste indruk te krijgen van de potentie van een watervoerend pakket voor microbiologische afbraak van VOCls kan de redox potentiaal worden gebruikt. Om meer specifieke doelen te behalen van in-situ bioremediatie met verschillende VOCls op uiteenlopende locaties met bijbehorende milieucondities, moeten kosten, baten en mogelijke risico's (zoals bijvoorbeeld het verstopt raken van de grondwaterputten door snelle groei van micro-organismen of door neerslagvormende chemische reacties) worden geschat voordat het ontwerp en aanleg van het KWO/sanerings systeem plaatsvind. Dit hoofdstuk geeft inzichten in de kritische factoren die de haalbaarheid van de combinatie WKO/saneren bepaald.

In hoofdstuk 3 worden experimenten beschreven die ingaan op twee belangrijke effecten van KWO op de gestimuleerde biologische afbraak van VOCls, namelijk temperatuurwisselingen en transport van grondwater. Hiertoe werd de vloeistoffase tussen verschillende batchflesjes, met temperaturen van 25 en 5 graden Celsius uitgewisseld. Ter vergelijking zijn ook een benadering van bodemwarmtewisselaars (BWW) onderzocht waarbij alleen de periodieke temperatuurwisselingen (25 of 5 graden C.) werd toegepast, en een natuurlijke situatie met voldoende electron donor en actieve bacteriën bij een constante temperatuur van 10 graden C. Bij alle condities werd cis-DCE gebruikt als VOCl omdat deze verontreiniging zich ophoopt wanneer de reductieve dechlorering stokt. Volledige afbraak tot etheen werd bereikt bij de KWO simulatie bij 25 graden C., terwijl bij 5 graden C. geen afbraak werd waargenomen. Na elke vloeistof uitwisseling nam de afbraaksnelheid toe tot een maximaal waargenomen afbraaksnelheid van 1,83 umol cis-DCE per dag. Deze snelheid was anderhalf keer sneller dan gezien werd bij BWW en 13 keer sneller dan de natuurlijke situatie. Zeer waarschijnlijk vond exponentiële groei van bacteriën plaats tijdens de warme periode. Een vergelijking tussen batches waarbij wel of juist geen actieve bacteriën (Dehalococcoides) werden toegevoegd laat zien dat de de initiële aanwezigheid van deze bacteriën van doorslaggevende betekenis is voor het proces van dechlorering. Indien de concentratie van Dehalococcoides bacteriën voldoende hoog is, wordt de temperatuur de bepalende parameter. Resultaten lijken aan te tonen dat Dehalococcoides bacterien bij voorkeur hechten aan de bodem matrix. Dit

hoofdstuk benadrukt de gevolgen van temperatuurveranderingen door KWO systemen op de biologische afbraak van VOCls. De resultaten laten zien dat stimuleren van biologische activiteit het best kan plaatsvinden in of nabij de warme grondwaterbron.

Een verkenning van de relatie tussen de beïnvloeding van de redoxtoestand door KWO en de biologische afbraak van VOCls, met name de respons van Dehalococcoides bacteriën is beschreven in hoofdstuk 4. Voor deze verkenning is gebruik gemaakt van een kolom experiment waarbij de waterfase continu gerecirculeerd werd met een debiet van 10 ml per minuut, hetgeen overeenkomt met de grondwatersnelheid die aanwezig is op een afstand van 1,3 meter van de grondwaterbron bij KWO. Om periodieke veranderingen van de redoxpotentiaal na te bootsen, welke mogelijk kunnen optreden in het veld, zijn opeenvolgend lactaat en nitraat aan de kolom toegevoegd. Bij deze hoge grondwatersnelheid (ten opzichte van reguliere biologische saneringscondities) werd in de recirculatiekolom cis-DCE volledig afgebroken tot etheen. Direct na toevoeging van nitraat stopte de dechlorering abrupt en spoelden de Dehalococcoides bacteri ën uit de kolom (bodem matrix). Herhaaldelijke onderbreking van de dechloreringsactiviteit door nitraat toevoeging had in ons kolomsysteem zeer ernstige gevolgen. De kolom met monstermateriaal, welke homogener was dan normaal gesproken in de praktijk wordt gevonden, veranderde qua redox eigenschappen vrijwel onomkeerbaar. Het koste steeds meer moeite de dechlorering weer op gang te brengen. De hypothese uit hoofdstuk 3 met betrekking tot de voorkeur van Dehalococcoides bacterien om te hechten aan de bodem matrix werd bevestigd door microbiologische analyse van de waterfase. Minder dan 0,1% van de toegevoegde bacteri ën werd in de waterfase teruggevonden. Hoewel in enkele veldexperimenten werd aangetoond dat Dehalococcoides betrekkelijk eenvoudig kon regenereren in de ondergrond na toevoeging van oxidanten, geven de resultaten in dit hoofdstuk aan dat de veerkracht van deze bacterie beperkt is. Bij de toepassing van de combinatie KWO/saneren zal daarom specifiek de geochemische toestand van het watervoerende pakket gemonitoord moeten worden en zal deze toestand betrokken moeten worden bij de aansturing van het KWO systeem.

De grootste zorg bij de combinatie KWO/saneren, met name als de biologische afbraak wordt gestimuleerd, betreft het verstopt raken van de grondwaterbronnen door de snelle groei van bacteri ën. Verstopping van grondwaterbronnen chemische reacties, zoals bijvoorbeeld de vorming van ijzer(III) neerslagen is eveneens een bedreiging maar de kans op dergelijke

verstoppingen kan redelijk goed worden voorspeld. In hoofdstuk 5 wordt het onderzoek beschreven naar de risico's van putverstopping door de combinatie KWO/saneren. Voor het onderzoek is gebruik gemaakt van dezelfde experimentele opstelling als beschreven is in het voorgaande hoofdstuk. Experimenten zijn ditmaal uitgevoerd in twee kolommen met een debiet van respectievelijk 10 en 50 ml per minuut. In beide kolommen werd de bacteriologische activiteit gestimuleerd door het toevoegen van lactaat en actieve bacteriën en werd de vorming van ijzer neerslagen versneld door het toevoegen van nitraat. De mate van verstopping in de kolom werd gevolgd door de drukval tussen ingaande en uitgaande waterstroom te meten. Tijdens de periode van gestimuleerde bacteriologische activiteit werd geen verhoging van de drukval waargenomen, zelfs niet toen de bacterieconcentratie in de waterfase met 4 ordegroottes was toegenomen. In tegenstelling hiermee resulteerde de toevoeging van nitraat in een duidelijke verhoging van de drukval. In de kolom met het hoge debiet zorgde de toename van de drukval zelfs voor het kapotgaan van de glazen kolom; de drukopbouw was groter dan de sterkte van de kolom. Bij de kolom met het lagere debiet kon de drukopbouw na toevoeging van nitraat verminderd worden door opnieuw lactaat toe te voegen. Deze bevindingen wijzen erop dat het risico van putverstopping bij stimuleren van de biologische activiteit beperkt is en dat een dergelijke stimulering de kans op putverstopping door neerslagvorming zelfs verkleint. Niettemin erkennen wij dat een kolom experiment een (zo goed mogelijke) benadering is van de realiteit. De risico's van putverstopping bij de combinatie KWO/saneren zouden bij voorkeur met aanvullende experimenten moeten worden onderzocht.

In hoofdstuk 6 is de combinatie KWO/saneren gesimuleerd met een reactief grondwatertransportmodel waarbij ter plaatse van de KWO grondwaterbronnen lactaat werd geïnjecteerd in een homogeen watervoerend pakket welke verontreinigd is met TCE. In het model zijn een aantal relevante processen opgenomen, namelijk de dechlorering van TCE, cis-DCE en VC, de reductie van sulfaat en ijzer(III), de fermentatie en oxidatie van organische verbindingen en de groei van verschillende typen bacteriën. In totaal zijn 15 scenario's bekeken, ondermeer bij verschillende concentraties van lactaat (3,8; 1,9 en 0,38 mmol/L), temperatuur van warme en koude bronparen (5/15; 10/10 en 5/25 graad C.), mobiliteit van de bacteriën (volledig mobiel en volledig immobiel) en pH limitatie bij ijzer(III) reductie (wel/geen limitatie). In de gesimuleerde periode van totaal 5 jaar vond bij het

referentiescenario (3,8 mmol/L lactaat, 5/15 graad C., mobiele bacteriën) binnen 1 jaar volledige afbraak van TCE tot etheen plaats binnen het invloedsgebied van de grondwaterbronnen. Verlaging van de lactaat concentratie resulteerde in een verminderde mate van afbraak. De groei van bacteriën, met name ijzer reducerende en lactaat fermenterende bacteriën, was eveneens een bepalende factor gedurende het eerste gesimuleerde jaar bij beide typen mobiliteit. De bacteriën verspreiden zich over het gehele invloedsgebied van het KWO system, zowel bij koude als warme zones. De invloed van temperatuur op de voortgang van afbraak leek beperkt te zijn, waarschijnlijk omdat de bacterie concentratie de beperkende factor was in de gebruikte modelopbouw. Deze observatie wijkt af van de bevindingen in hoofdstuk 3 waar gesteld is dat de initiële aanwezigheid van bacteriën van groot belang is. Daarnaast kon het model de zelfversterkende groei van bacteriën (exponentieel) niet meenemen, welk belang groter is dan de toename van temperatuur in het model. Over het algemeen suggereren de modelberekeningen in dit hoofdstuk dat het benutten van KWO grondwaterbronnen als mogelijkheid voor de toediening van substraat en bacteriën een kosteneffectieve benadering is van de combinatie KWO/saneren.

In hoofdstuk 7 worden de discussies van resultaten uit de voorgaande hoofdstukken geïntegreerd en wordt teruggekeken op de onderzoeksvragen die gesteld zijn het introducerende hoofdstuk. Daarnaast worden aanbevelingen gedaan voor vervolgonderzoek en wordt het perspectief voor praktische toepassing van de combinatie KWO/saneren besproken. De redoxtoestand blijkt overall de bepalende factor te zijn voor de kansrijke toepassing. De onderlinge beïnvloeding van KWO en (gestimuleerde) biologische sanering van VOCls is vrij gunstig, ondermeer omdat stimulering van (anaerobe) biologische activiteit het risico van putverstopping lijkt te verkleinen inplaats van te vergroten. Wij raden aan om hier aanvullend onderzoek naar te doen, met name met pilot testen op grotere schaal. Daarnaast kunnen met de lokale verhoging van temperatuur en het transport van grondwater synergetische voordelen worden behaald in combinatie met de activiteit van dechlorering. Tenslotte wordt een kort overzicht gegeven van mogelijke toepassingen van de combinatie KWO/saneren in China en Nederland. Hoewel de KWO technologie in Nederland beduidend verder is in haar ontwikkeling, heeft China het voordeel dat flexibeler gebruik kan worden gemaakt van de ondergrond. In beide landen biedt de combinatie KWO/saneren een kosten

effectieve oplossing voor zowel de productie van herwinbare energie en het beheersen van de grondwaterkwaliteit.

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About the Author

Zhuobiao Ni was born on June 11th, 1984 in Jieyang City, China. He finished his secondary schools in the Guangzhou City, where he also became a BSc student. Between 2003 and 2007, he was studying Environmental Science in South China Agricultural University. During this period, he obtained the 'Excellent Student' award in a consecutive two years. His BSc thesis entitled "Effect of Co-planting of *Sedum alfredii* and *Zea mays* on Absorbing of $Zn_3(PO_4)_2$-Zn in Solution" belonged to a PhD project focusing on phytoremediation of heavy metal. His tasks were performing aquaculture of *Sedum alfredii* and *Zea mays*, sampling and measuring Zn in water and plants, and analysing data. After his BSc graduation in 2007, he travelled abroad and started an Environmental Science MSc program, specialising in Environmental Technology, at Wageningen University in the Netherlands. During his MSc study in Wageningen, Zhuobiao was awarded the "Anne van den Ban" scholarship in 2008. Later he spent six months at Wetsus in Leeuwarden for his MSc thesis entitled "Ozonation of Methyl tertiary butyl ether adsorbed on zeolite or activated carbon, and regeneration of adsorbents". The MSc thesis was aiming to compare the performance of zeolite and activated carbon regarding adsorption, regeneration and evaluate these performances at different pH and temperature level. During his master internship at Voltea in Leiden in 2009, he contributed to develop a new method for capacitive salt water deionization. In 2010, Zhuobiao started his PhD at the department of Environmental Technology at Wageningen University. His PhD research dealt with the novel integration between sustainable energy technology and groundwater remediation technology. He finished his dissertation in September 2015. He hopes to continue using his PhD knowledge and to start a new challenge in his career.

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Ni, Z.; Smit, M.; Grotenhuis, T.; Hartog, N.; Rijnaarts, H., *Insights into factors limiting intrinsic biodegradation of chlorinated ethenes at Aquifer Thermal Energy Storage (ATES) conditions*. In Proceedings of Aquaconsoil, Barcalona, April 16-19, 2013.

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Ni, Z.; Smit, M.; van Gaans, P.; Rijnaarts, H.; Grotenhuis, T., *Combination of aquifer thermal energy storage and enhanced bioremediation: resilience of reductive dechlorination to redox changes*. Submitted.

Ni, Z.; van Gaans, P.; Rijnaarts, H.; Grotenhuis, T., *Combination of aquifer thermal energy storage and enhanced bioremediation: biological and chemical clogging*. Submitted.

Sommer, W.; Ni, Z.; Valstar, J.; van Gaans, P.; Grotenhuis, T.; Rijnaarts, H., *Reactive transport modeling of TCE bioremediation combined with aquifer thermal energy storage*. Submitted.

D I P L O M A

For specialised PhD training

The Netherlands Research School for the
Socio-Economic and Natural Sciences of the Environment
(SENSE) declares that

Zhuobiao Ni

born on 11 June 1984 in GuangDong, China

has successfully fulfilled all requirements of the
Educational Programme of SENSE.

Wageningen, 8 December 2015

the Chairman of the SENSE board

Prof. dr. Huub Rijnaarts

the SENSE Director of Education

Dr. Ad van Dommelen

The SENSE Research School has been accredited by the Royal Netherlands Academy of Arts and Sciences (KNAW)

KONINKLIJKE NEDERLANDSE
AKADEMIE VAN WETENSCHAPPEN

The SENSE Research School declares that **Mr Zhuobiao Ni** has successfully fulfilled all requirements of the Educational PhD Programme of SENSE with a work load of 32.8 EC, including the following activities:

SENSE PhD Courses

o Environmental Research in Context (2011)
o Basic statistics (2013)
o Research in Context Activity: 'Taking the initiative and creating informative Wikipedia pages in English and in Chinese on the subject of: Aquifer Thermal Energy Storage' (2015)

Other PhD and Advanced MSc Courses

o Phreeqc Hydrogeochemical Transport Modelling, Freie Universität Berlin (2011)
o OLI Stream Analyser, Wageningen University (2012)
o Scientific writing, Wageningen University (2012)
o Voice matters – Voice and Presentation Skills Training, Wageningen University (2013)

Management and Didactic Skills Training

o Supervising four MSc students with thesis entitled 'Effect of ascorbic acid and lactate addition on the redox conditions and biodegradation in an iron reducing and PCE contaminated soil' (2012), 'Effect Of Shaking On Soil Organic Matter And Redox Potential Of Fe(III) Reducing Aquifer' (2013), 'Effects of temperature and liquid transport on cis-DCE reductive dechlorination in Underground Thermal Energy Store (UTES)' (2014), and 'Identification of potential clogging problems in combination system of ATES and bioremediation' (2014)
o Supervising soil practical of the BSc course 'Introduction to Environmental Technology' (2011-2014)

Oral Presentations

o *Combination of Aquifer Thermal Energy Storage (ATES) & Bioremediation of Chlorinated Solvents*. 1st National Congress Soil Energy, 13-14 October 2011, Utrecht, The Netherlands
o Effects of Aquifer Thermal Energy Storage (ATES) on Bioremediation of Chlorinated Ethenes in Subsurface. 17th Bodem Breed Symposium, 29-30 November, Lunteren, The Netherlands
o *Insights into Factors Limiting Intrinsic Biodegradation of Chlorinated Ethenes (VOCls) at Aquifer Thermal Energy Storage (ATES) Conditions*. AquaConSoil 2013 Conference, 16-19 April 2013, Barcelona, Spain
o *Bioremediation of Chlorinated Ethenes in Underground Thermal Energy Storage*. AquaConSoil 2015 Conference, 9-12 June 2015, Copenhagen, Denmark

SENSE Coordinator PhD Education

Dr. ing. Monique Gulickx

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