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INSTITUTE OF ENVIRONMENT & RESOURCES
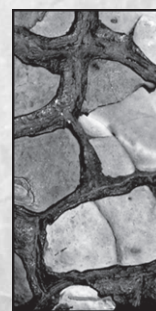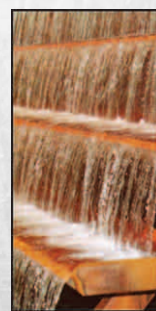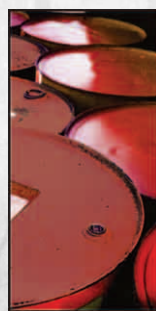
The potential for reductive dechlorination after thermal treatment of TCE-contaminated aquifers

Anne Kirketerp Friis

Ph.D. Thesis

January 2006

Institute of Environment & Resources

Technical University of Denmark

The potential for reductive dechlorination after thermal treatment of TCE-contaminated aquifers

Project period: 2003-2005

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PREFACE

This thesis is based on research for a PhD project undertaken from 2003 to 2005. The main supervisor was Professor Poul L. Bjerg (E&R, DTU), and co-supervisors were Associate Professor Hans-Jørgen Albrechtsen (E&R, DTU) and Professor Kent S. Udell (University of California, Berkeley, USA). Furthermore, Dr Gorm Heron (TerraTherm, California, USA), Professor Elizabeth Edwards (University of Toronto, Canada), Evan Cox and Dr Melanie Duhamel (GeoSynthec Consultants, Canada) contributed scientific input to the project. This thesis comprises a summary and is accompanied by seven journal papers and a technical note. Please note that the papers are not included in this www-version but can be obtained from the library at Institute of Environment & Resources, Bygningstorvet, Building 115, Technical University of Denmark, DK-2800 Lyngby (library@er.dtu.dk).

I	**Friis A.K.,** Albrechtsen H.-J., Heron G., and Bjerg P.L. Redox processes and release of organic matter after thermal treatment of a TCE-contaminated aquifer. *Environmental Science and Technology.* 39 (15), 5787-5795, 2005.
II	**Friis A.K.,** Edwards E., Albrechtsen H.-J., Udell K., Duhamel M., and Bjerg P.L. Dechlorination after thermal treatment of a TCE-contaminated aquifer: Laboratory experiments. Submitted.
III	**Friis A.K.,** Albrechtsen H.-J., Cox E., and Bjerg P.L. The potential for bioaugmentation after thermal treatment of TCE-contaminated groundwater: Laboratory experiments. Submitted.
IV	**Friis A.K.,** Cox E., and Albrechtsen H.-J. Effects of thermal treatment on microbial diversity and community structure in a contaminated aquifer. Submitted.
V	**Friis A.K.,** Heimann A.C., Jakobsen R., Albrechtsen H.-J., Cox E., and Bjerg P.L. Temperature dependence of anaerobic TCE-dechlorination in a highly enriched *Dehalococcoides*-containing culture. Submitted.
VI	**Friis A.K.,** Kofoed J.L.L., Heron G., Albrechtsen H.-J., and Bjerg P.L. Optimizing bioaugmentation after field-scale thermal treatment of a TCE-contaminated aquifer. Submitted.
VII	**Friis A.K.,** Heron G., Albrechtsen H.-J., Udell K., and Bjerg P.L. Anaerobic dechlorination and redox activities after full-scale Electrical Resistance Heating (ERH) of a TCE-contaminated aquifer. Submitted.
VIII	**Friis A.K.** Technical note: Phase distribution of chlorinated ethenes at elevated temperatures.

Publications co-authored and related to the topic of this thesis but *not* comprised in this thesis include two journal papers and one extended conference paper:

>Heron G., Baker R., LaChance J., and **Friis A.K.** Using heat to accelerate remediation. Eighth International In Situ and On-Site Bioremediation Symposium, Baltimore, Maryland, June 6–9, 2005.

Heimann A.C., **Friis A.K.**, Scheutz C., and Jakobsen R. Dynamics of reductive TCE dechlorination at various temperatures and two distinct H_2 supply scenarios. Accepted for publication in Biodegradation in 2006.

Heimann A., **Friis A.K.**, and Jakobsen, R. Effects of sulfate on anaerobic dechlorination of chloroethenes under transient and steady-state conditions. *Water Research*. 39 (15), 3579-3586, 2005.

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ABSTRACT

This thesis presents the results of an investigation of 'the potential for reductive dechlorination after thermal treatment of TCE-contaminated aquifers' with focus on reductive dechlorination of trichloroethene (TCE) after electrical resistance heating (ERH) and includes seven journal papers and a technical note describing the work undertaken for this PhD project.

Chlorinated ethenes as perchloroethene (PCE) and TCE were useful to industry because of their physico-chemical properties. PCE is most commonly known for its use in the dry-cleaning industry, whereas TCE was previously used as a degreasing solvent. Due to their widespread use, chlorinated ethenes are commonly detected groundwater contaminants. They are among the most commonly found groundwater pollutants and are detected at approximately 80% of all superfund sites in the USA. Remediation of these compounds from polluted soil and groundwater is a challenging task because of various factors including the pollutants complex spreading pattern in the subsurface, their low aqueous solubility that exceeds the drinking water limits, diffusion from free-phase product, and their higher density than water. Due to this, various innovative remediation technologies have been developed and applied to reach regulatory goals.

Thermally enhanced remediation technologies overcome many factors affecting the removal of DNAPL mass. However, these technologies are often expensive, and a coupling of more than one technology may reduce the overall cost and time.

Chlorinated ethenes can also be biologically degraded, which makes them interesting from a remediation perspective. In nature, higher chlorinated ethenes as PCE/TCE can be degraded anaerobically by reductive dechlorination, where the chlorinated ethenes act as electron acceptors. Each step in the process occurs under specific redox conditions and is carried out by different microorganisms. The first steps from PCE/TCE to *cis*-dichloroethene (*c*DCE) can be carried out by a variety of microorganisms, whereas dechlorination of *c*DCE to ethene has been documented only in the presence of *Dehalococcoides*. Biological remediation can be applied by biostimulation (injection of electron donors) or by bioaugmentation (injection of dechlorinating microorganisms and electron donors), the latter being successful at several sites. The use of bioaugmentation has been suggested as a follow-up technology after thermal treatments.

One advantage of combining biological and thermal remediation technologies is that elevated subsurface temperatures after heating can stimulate metabolic activity. Although conditions during thermal treatment are similar to those reached in an autoclave, some microorganisms survive and are capable of degrading various hydrocarbons. Microbial investigations carried out as part of this work demonstrated that, although the genetic

diversity decreased after heating in closed microcosms, functional and catabolic diversity reoccurred within 400 days after heating. However, little is known about the post-thermal redox conditions and the survival of dechlorinating microorganisms, which control the potential for reductive dechlorination.

The work in this thesis demonstrated that reduced conditions could be obtained in the field after full-scale ERH and that microorganisms present after heating were capable of dechlorinating TCE to *c*DCE. This activity was not observed in closed microcosms heated to 100°C for 10 days. It suggested that microorganisms, capable of dechlorinating TCE to *c*DCE, survived the harsh treatment in the field or were transported into the treated area with groundwater during cooling and prior to sampling of aquifer materials. Also, a release of dissolved organic carbon (DOC) was observed in the aqueous phase of microcosms that were closed and heated, but not in field samples collected after ERH. This suggested that DOC was either not released at the field scale or transported downstream of the heated area with groundwater. Released DOC can stimulate reductive dechlorination.

Despite an inflow and/or survival of microorganisms after ERH, dechlorination was weakened in half of the treatments, while it remained the same in the remaining microcosms as compared with dechlorination in unheated microcosms. The environmental conditions demonstrated lower redox activity, similar/lower DOC concentrations and similar pH and alkalinities after ERH.

TCE was not dechlorinated or dechlorination stalled at *c*DCE in heated microcosms. Microcosms were therefore bioaugmented using a mixed culture (KB-1^{TM}) containing *Dehalococcoides*, sulfate reducers, methanogens and fermentors. Upon bioaugmentation, dechlorination of TCE to ethene was observed in the majority of microcosms, including laboratory-heated and *in-situ* heated aquifer materials. This demonstrated a potential and need for bioaugmentation after ERH.

The combination of thermal treatment and enhanced bioremediation can be applied either concomitantly or sequentially. The application of the two technologies concomitantly would involve thermal treatment of a hot spot that is surrounded by biological active zone created by the addition of electron donors alone or in conjuction with bioaugmentation in the perimeter zone. Temperatures in the perimeter zone can be elevated due to conductive heat transfer from the thermal treatment. The elevated temperatures are shown to increase metabolic dechlorinating activity. Bioremediation can, at these sites, be applied at temperatures of approximately 30°C. Sequential thermal treatment and bioremediation can also be applied. In this process, bioremediation is initiated after thermal treatment when temperatures have decreased to approximately 30°C. Finally, the solutions can be combined, where biological treatment is used both in the perimeter zone and inside the thermally treated area during cooling.

When thermal treatment is applied in combination with bioaugmentation, an optimum temperature of approximately 30°C for rapid dechlorination should be the aim. Electron donors are also commonly added during bioaugmentation to obtain reduced subsurface conditions and reductive dechlorination. Electron donors can further stimulate dechlorination of TCE to *c*DCE, which can be carried out by a variety of subsurface microorganisms. Electron donors can therefore be added at higher temperatures (at the beginning of the cooling period) to obtain anaerobic conditions and dechlorination of TCE to *c*DCE.

The focus of this thesis was the combination of thermal treatment and bioremediation for reductive dechlorination. However, the combination of these two technologies can also be applied in respect of aerobically degraded contaminants such as oils and BTEX. Furthermore, this combination has the advantage that microorganisms capable of degrading various hydrocarbons have been observed to survive thermal treatment.

DANSK SAMMENFATNING

I denne afhandling undersøges 'potentialet for reduktiv deklorering efter en termisk oprensning af TCE-forurenede akvifærer' med fokus på reduktiv deklorering af triklorethen (TCE) efter elektrisk opvarmning (ERH). Arbejdet, der er udført under dette ph.d.-projekt, er ud over afhandlingen beskrevet i syv artikler samt en teknisk note.

Klorerede ethener som fx perklorethen (PCE) og TCE har vist sig nyttige for industrien grundet deres fysiske-kemiske egenskaber. PCE er primært kendt for sit omfattende brug ved rensning af tøj, hvorimod TCE primært er blevet benyttet som affedtningsmiddel. På grund af den omfattende brug er klorerede ethener ofte fundet i forurenet grundvand. Stofferne er relateret til alvorlige grundvandsforureninger og er fundet i ca. 80% af alle 'superfund sites' i USA. Remediering af disse stoffer i forurenet jord og grundvand vanskeliggøres af forskellige faktorer der inkluderer forureningens komplekse spredningsmønster i undergrunden, lav vandopløselighed der er højere end drikkevands krav, diffusion fra en fri fase forurening og højere densitet end vand. Forskellige innovative remedieringsteknologier er derfor udviklet og anvendt for at nå regulatoriske mål.

Termiske teknologier har overvundet mange faktorer der påvirker fjernelse af DNAPLs. Men disse teknologier er ofte dyre og en kombination af mere end en teknologi kan måske reducere total omkostningerne og varigheden af en oprensning.

Biologiske remedieringsteknikker er interessante, da klorerede ethener også kan nedbrydes biologisk. I grundvandsmiljøer kan klorerede ethener, som fx PCE/TCE, nedbrydes anaerobt ved reduktiv deklorering, hvor de klorerede ethener virker som elektronacceptorer. Hvert trin i denne proces foregår under specifikke redox-forhold og er udført af forskellige mikroorganismer. Det første trin fra PCE/TCE til *cis*-diklorethen (*c*DCE) kan udføres af forskellige mikroorganismer, hvorimod deklorering af *c*DCE til ethen kun er dokumenteret under tilstedeværelse af *Dehalococcoides*. Biologisk deklorering kan benyttes som remedieringsteknik enten ved biostimulering (injektion af elektrondonorer) eller ved bioaugmentering (injektion af deklorerende mikroorganismer og elektrondonorer). Deklorering stopper ofte ved *c*DCE i biostimulerede akvifærer og fortsætter til ethen efter bioaugmentation med blandingskulturer, der indeholder *Dehalococcoides*. Bioaugmentation er således blevet foreslået som en opfølgende teknologi efter termisk oprensning.

En fordel ved at kombinere biologisk og termisk remedieringsteknologier er, at de forhøjede temperaturer i undergrunden efter en termisk oprensning kan stimulere metabolsk aktivitet. Selvom forholdene under en termisk oprensning minder om dem, der er opnået i en autoklave (dvs. 120° C og 2 atm. tryk 10 m under grundvandsspejlet), overlever nogle mikroorganismer og er i stand til at nedbryde forskellige hydrokarboner. I denne afhandling

påvises at, funktionel og katabolsk diversitet i uopvarmede mikrokosmer var identisk med diversiteten i laboratorieopvarmede mikrokosmer efter 400 dages inkubation og felt-opvarmede mikrokosmer inden for 300 dage. Dette var tilfældet, selv om genetisk diversitet reduceredes efter opvarmning i laboratorieopvarmede mikrokosmer. Til trods for at viden om posttermiske redox-forhold og overlevelse af deklorerende bakterier er begrænset, er denne viden nødvendig for at forudsige potentialet for posttermisk reduktiv deklorering.

Resultaterne fra denne afhandling har vist, at der var reducerede forhold efter ERH, og at mikroorganismer, der overlevede opvarmningen, kunne deklorere TCE til *c*DCE. Denne mikrobielle aktivitet blev ikke observeret i lukkede mikrokosmer, der havde været opvarmet til 100° C i 10 dage. Dette indikerer, at mikroorganismer, der kunne deklorere TCE til *c*DCE, overlevede den hårde behandling i felten eller var transporteret ind i det opvarmede område med grundvand under køling og efter prøveudtagning. Frigivelse af opløst organisk kulstof (DOC) observeredes også i lukkede mikrokosmer, men ikke efter ERH. Dette viser, at DOC enten ikke var frigivet efter ERH i fuld skala eller var transporteret nedstrøms det opvarmede område med grundvandet. Frigivet DOC kan stimulere reduktiv deklorering.

Aktiviteten af dekloreringen blev svækket i halvdelen af mikrokosmerne, mens aktiviteten forblev den samme i den anden halvdel som i ikke-opvarmede mikrokosmer på trods af transport og/eller overlevelse af mikroorganismer. Sammenligning af forholdene i feltområdet før og efter opvarmningen viste, at der var lavere redox-aktivitet og identiske/lavere DOC koncentrationer efter opvarmningen, mens pH og alkalinitet forblev uændret.

I opvarmede mikrokosmer stoppede deklorering ved *c*DCE, eller TCE forblev ikke-nedbrudt. Mikrokosmerne blev derfor bioaugmenteret med en blandingskultur (KB-1™), der bl.a. indeholder: *Dehalococcoider,* sulfatreducerende, methanogene og fermenterende mikroorganismer. Efter bioaugmentation blev TCE dekloreret til ethen i størstedelen af mikrokosmerne både med laboratorieopvarmede samt *in-situ*-opvarmede sedimenter. Dette faktum har påvist et potentiale og behov for bioaugmentation efter ERH.

Kombinationen af termisk oprensning og bioremediering kan udføres enten samtidigt eller sekventielt. Anvendelse af de to teknologier samtidigt involverer brug af termisk oprensning i den inderste zone af kildeområdet der er omkranset af en biologisk aktiv zone dannet ved tilførsel af elektron donor eller med bioaugmentation i den omkringliggende zone. Temperaturer i den omkringliggende zone kan øges på grund af konduktiv varmeoverførsel fra den termiske oprensning. De forhøjede temperaturer øger dekloreringsaktiviteten. Sekventiel termisk oprensning og bioremediering kan også benyttes. På disse lokaliteter kan bioremediering anvendes umiddelbart efter den termiske oprensning, når temperaturerne er faldet til ca. 30° C. Derudover kan disse løsninger

kombineres, hvorved biologisk oprensning kan anvendes både i den omkringliggende zone og i det termisk behandlede område efter behandlingen.

Når termisk oprensning og bioaugmentation kombineres er den optimate temperatur for hurtig deklorering omkring 30° C. Elektrondonorer er også ofte tilført under bioaugmentation for at opnå reducerede forhold og reduktiv deklorering i undergrunden. Tilførsel af elektrondonorer kan derfor også stimulere deklorering af TCE til *c*DCE, hvilket kan udføres af forskellige mikroorganismer. Elektrondonorer kan derfor med fordel tilføres ved højere temperaturer for at opnå reducerede forhold og deklorere TCE til *c*DCE.

Denne afhandling fokuserer på kombinationen af termisk oprensning og bioremediering for reduktiv deklorering. Denne kombination kan dog også anvendes til aerob nedbrydning af forureninger med olie og BTEX. Den aerobe kombination har ydermere den fordel, at mikroorganismer, der er i stand til at nedbryde forskellige hydrokarboner, har vist sig at kunne overleve termiske oprensninger.

1. INTRODUCTION

Groundwater contamination with chlorinated ethenes represents one of the greatest threats to our drinking water supply and to the ecosystem due to their neurotoxic and potential carcinogenic effects (U.S.EPA, 2005a; WHO, 2004). In subsurface environments, chlorinated ethenes can exist as dense non-aqueous phase liquids (DNAPLs). Various factors make it difficult to locate the pollution and challenges remediation technologies including the complex spreading pattern of DNAPLs, low aqueous solubility, exchange with free-phase product and higher density than water. For these reasons, various innovative technologies have been developed and tested (NRC, 1994; NRC, 1997; NRC, 1999). Although some of these technologies have proven capable of removing substantial mass (Heron et al., 2005b), some contaminants may remain within the porous medium even when treatment is most effective (Fountain et al., 1995; Sale and McWhorter, 2001). As a result, down-gradient contamination can occur and may require further remediation.

Enhanced biodegradation may attenuate contaminant mass flux to levels that achieve regulatory compliance at down gradient wells (Christ et al., 2005). Use of aggressive source-zone treatment followed by bioremediation may therefore be an attractive remediation alternative (de Blanc et al., 1997; Rao et al., 2002; Zoller, 1998; Zoller and Rubin, 2001).

In general, aggressive source zone treatment is not beneficial to the microbial activity. However, certain technologies are suggested to be more promising for stimulation of microbial activity as a polishing step after source-zone treatment, as for example thermal treatment (**Figure 1**). During thermally enhanced remediation, high temperatures similar to those in an autoclave can be reached in the subsurface (120°C and 2 atm pressure 10 m below groundwater table (Heron et al., 1998a)); however, long-term monitoring provides evidence that microbial activity rebounds after field-scale steam treatment (Richardson et al., 2002; Smith et al., 1998; Smith et al., 2000). These surviving microorganisms even demonstrate a capability of degrading various hydrocarbons (Balshaw-Biddle et al., 2000; Huesemann et al., 2002; Krauter et al., 1995; Richardson et al., 2002). Until now, knowledge about the physical, chemical and biological effects of thermal treatment on subsurface environment remains sparse and research is needed to determine the potential for post-thermal bioremediation.

This thesis presents the results of experiments to evaluate the effect of thermal treatment on the subsequent application of biological reductive dechlorination of TCE. Experiments were designed to determine the effects of thermal treatment on (1) environmental conditions; (2) survival of dechlorinating microorganisms; (3) functional and catabolic diversities of surviving microorganisms; (4) the potential for bioaugmentation to enhance

reductive dechlorination; and (5) the applicability of the technologies, including optimal temperature conditions for bioaugmentation after thermal treatment.

A full-scale application of electrical resistance heating (ERH) was conducted at the U.S. Army's Ft. Lewis, East Gate Disposal Yard (EGDY), WA, USA, a former disposal site for liquid and solid waste. This disposal site was heavily contaminated, primarily with TCE. This field site was selected because sediment and groundwater samples could be obtained before and after full-scale ERH. Redox conditions and dechlorination were evaluated after heating in closed microcosms in the laboratory (appendix **I, II, III**) and in the field (appendix **VI, VII**). The effect on biological diversity from both heating techniques was also determined (appendix **IV**). All these results were compared with a parallel setup containing unheated microcosms. The temperature optima for complete dechlorination were determined to estimate the optimum bioaugmentation temperature (appendix **V**). Finally, phase distribution of chlorinated ethenes at elevated temperatures was determined (appendix **VIII**).
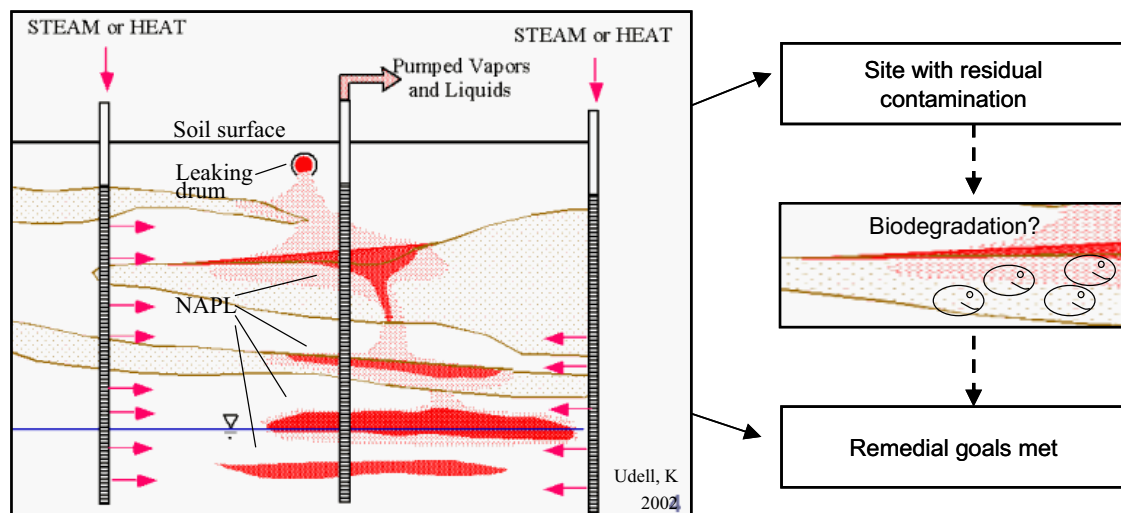
Figure 1. Conceptual figure of biodegradation after thermal treatment. The question is whether biodegradation is an option for remediation of residual contaminants after thermal treatment to reach remedial goals.

2. CHLORINATED ETHENES IN THE SUBSURFACE

Chlorinated ethenes consist of hydrocarbons where one or more hydrogen atoms are replaced by a chloride atom, i.e. tetrachloroethene (PCE), TCE, *cis*-dichloroethene (*c*DCE) and vinyl chloride (VC). The most commonly used chlorinated ethenes are PCE and TCE because they are ideal solvents for numerous applications. Due to their widespread use and subsequent disposal, they are contaminants commonly found at hazardous waste disposal sites, especially in urban industrial areas. They are persistent in nature and produce toxic and carcinogenic intermediates. VC is the most hazardous of all the chlorinated ethenes. In groundwater systems, VC often originates from anaerobic biodegradation of higher chlorinated ethenes. In Denmark, no maximum contaminant level (MCL) is set for each compound in groundwater, but since they are unwanted in groundwater, a MCL of 1 µg/l (Miljøstyrelsen, 1996) is set for all organic carbohydrates; although VC has an MCL of 0.2 µg/l (Miljøstyrelsen, 2003). In the USA, PCE and TCE have an MCL of 5 µg/l, whereas the MCLs of *c*DCE and VC are 70 µg/l and 2 µg/l, respectively (U.S.EPA, 2004d).

2.1. History of production and use of PCE and TCE

PCE and TCE were useful to industry because of their rapid evaporation rates, low flammability and reactivity and ability to quickly and efficiently dissolve a wide range of organic substances (Doherty, 2000b). In general, the largest historical end uses were within the areas of vapor degreasing, metal cleaning and dry-cleaning, though they were also used for processing adhesives, pharmaceuticals and textiles, as extraction solvents, paint solvents and coating solvents as well as feedstocks for production of other chemicals (Doherty, 2000b). PCE is most commonly known for its use in the dry-cleaning industry, whereas TCE was previously used as a degreasing solvent (Ullmann, 2005). This section focuses on the use of TCE because it is the main contaminant of interest of my research.

TCE was first prepared in 1864 by E. Fischer (Ullmann, 2005) although little attention was given to TCE as a commercial chemical before production began in Germany in 1910. In 1912, TCE was produced for laundries and for textiles and varnishes and as an extraction agent for fats. However, this stopped after animal feed with soybean defatted with TCE was identified as the source of cattle poisoning, which led to extensive losses of cattle in Europe between 1923 and 1925 (Doherty, 2000a). TCE production in the United States began in the early 1920s (**Figure 2**). TCE was used as a replacement for petroleum distillates in the dry-cleaning industry and became the solvent of choice for vapor degreasing in the 1930s. The use of TCE as a degreaser decreased in the 1960s due to more stringent environmental policies and increasing popularity of TCA (Doherty, 2000a) although production rates steadily increased with a peak in the 1970s (Ullmann, 2005).
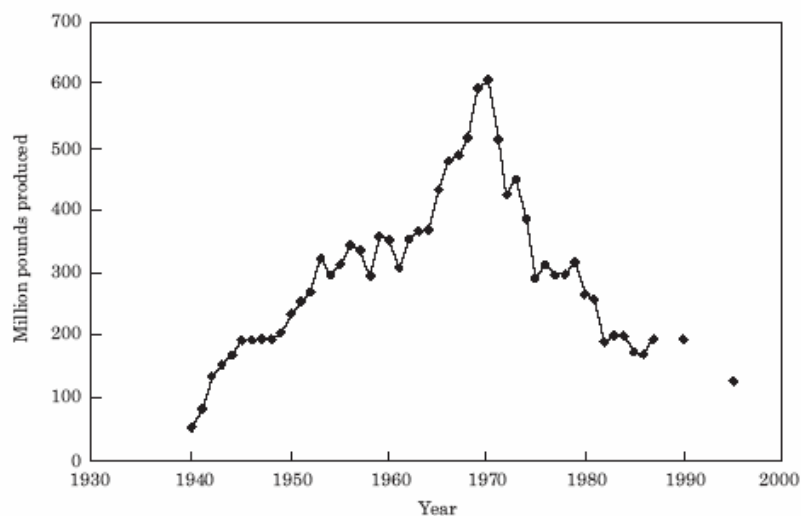
Figure 2. US production of TCE. Adapted from (Doherty, 2000a).

In 1965, Los Angeles Country Air Pollution Control District (APCD) proposed Rule 66, a regulation to limit solvent emissions from industrial facilities; however, after a storm of controversy, all chlorinated solvents were exempted, except for TCE (Doherty, 2000a). After 1970, the use of TCE began to decline due to a combination of several regulatory and economic factors. Also, the Clean Air Act (CAA) controlled TCE as a volatile organic carbon (VOC) due to its suspected contribution to ozone and smog formation, and the National Cancer Institute (NCI) found that TCE caused cancer in mice. In 1976, TCE was included on the EPA's Hazardous Substance List; and in 1977, the Food and Drug Administration (FDA) banned direct and indirect use of TCE in food (Doherty, 2000a).

2.2. Physico-chemical properties of chlorinated ethenes

The chlorinated ethenes TCE, *c*DCE, and VC are volatile compounds (Henry's constant > 0.04 at average groundwater temperatures of 10°C) with viscosities lower than water (Table 1). VC, which is the most carcinogenic of the chlorinated ethenes, is distinguished by a lower density than water and by higher volatility and lower sorption than the higher chlorinated ethenes. This leads to a higher risk of indoor air problems above sites contaminated with VC compared to TCE-contaminated sites.

Table 1. Physico-chemical properties of PCE, TCE, *c*DCE, and VC at standard temperature and pressure (25°C and 101.325 kPa) unless otherwise mentioned according to Ullmann (2005). Values were consistent with those from Chemfinder (2005) and AVJ (2001). Chemical structures are demonstrated in **Figure 6**.

Property	PCE	TCE	*c*DCE	VC
Density (g/cm^3)	1.623	1.478	1.282	0.910
Vapor pressure, 20 °C (kPa)	1.90	5.78	24.0	333
Solubility in water (wt %)	0.015	0.107	0.550	0.110
Viscosity (Pa s)	0.88×10^{-3}	0.58×10^{-3}	0.47×10^{-3}	0.19×10^{-3}

The physico-chemical properties of chlorinated ethenes are highly dependent on temperature. At elevated temperatures, volatilization increases and sorption decreases, resulting in a higher fraction of the chlorinated ethenes in headspace (**Figure 3**). This effect is used for remediation purposes such as thermal treatment, where temperatures are increased to the boiling point to extract contaminants in the vapor phase from the subsurface (chapter 4).
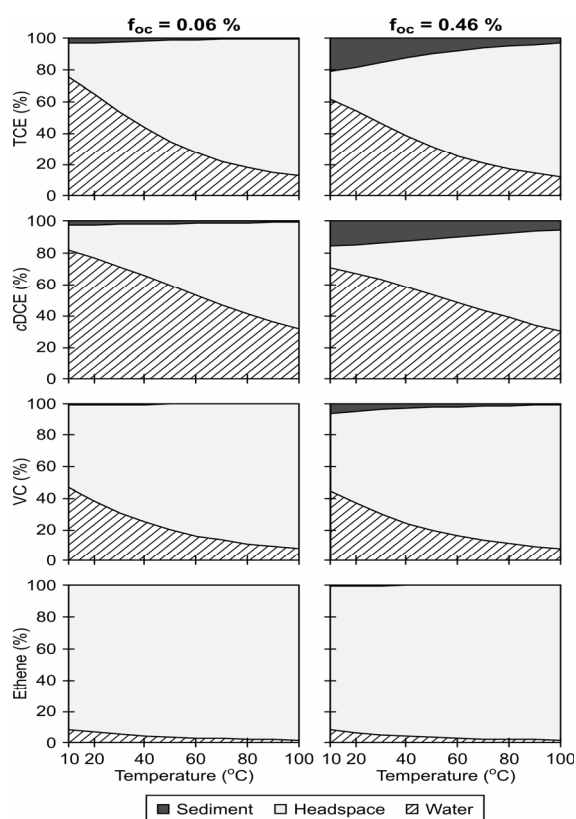
Figure 3. Theoretical distribution of chlorinated ethenes and ethene as a function of temperature. Modified from Friis (**VIII**, 2005). The distribution was estimated in microcosms containing 100 g sediment, 200 mL water and 250 mL air (i.e. headspace) with varying fraction of organic carbon (f_{oc}) on the sediment. Sorption was estimated as $K_d = f_{oc} \times C_{oc} / C_w$ where C is the concentration of a given compound subscribed by w, water or oc, octanol. Temperature dependencies on Henry's law constant for distribution between headspace and water were obtained from the literature for TCE (Staudinger and Roberts, 2001), *c*DCE (U.S.EPA, 2004a), VC (Shimotori and Arnold, 2003), and ethene (Lane, 1991) as described in Friis (**VIII**, 2005).

2.3. Subsurface pollution with chlorinated ethenes

A number of physico-chemical properties of chlorinated ethenes become important in the light of their distribution in groundwater environment. The larger density than water and the hydrophobicity of TCE and *c*DCE (Table 1) makes it possible for these contaminants to form dense non-aqueous phase liquids (DNAPLs). When released to the environment, DNAPLs will spread deep into the soil and migrate downwards until they reach a layer which they cannot penetrate (Stumm and Morgan, 1996), as shown in **Figure 4**. Horizontal spreading of the dissolved phase is observed in the unsaturated zone through diffusion and convection and in the saturated zone depending on contaminant water solubility and groundwater flow (Bedient et al., 1999).

VC, on the other hand, has a lower density than water. In some cases, TCE has also been observed in light non-aqueous phase liquid (LNAPL), which can occur in mixtures of contaminants. This complex spreading pattern of NAPLs makes it difficult to determine where the pollution is located in the subsurface and therefore challenges the remediation technologies.
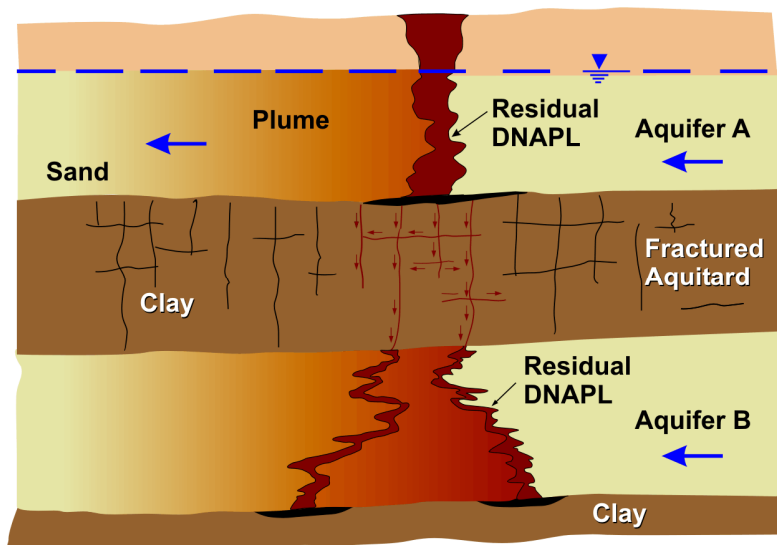
Figure 4. Spreading of DNAPL in the subsurface. Modified from Miljøstyrelsen (1996).

2.4. Biodegradation of chlorinated ethenes

In the subsurface, chlorinated ethenes are only degraded to a limited extent. Biological degradation of chlorinated aliphatics is observed in both aerobic and anaerobic environments (**Figure 5**) (Bjerg et al., 1999; Bradley, 2000; Davis et al., 2002; Witt et al., 2002). This biological transformation can occur either as energy-coupled or non-energetic transformation. In energy-coupled transformation, microorganisms grow in a process termed reductive dechlorination (also called dehalorespiration and halorespiration). Non-energetic transformation occurs by cometabolic degradation, where chlorinated ethenes are degraded by an enzyme synthesized by the cell for metabolism of another compound (Bradley, 2000).

2.4.1. Degradation pathways for chlorinated ethenes

Aerobic degradation can occur both cometabolically and by oxidation, where the chlorinated ethenes act as electron donors. Aerobic oxidation has been observed for *c*DCE and VC, whereas aerobic cometabolic dechlorination of TCE, *c*DCE, and VC (Bradley and Chapelle, 2000; Davis et al., 1990) has been observed when various microorganisms are used (Alvarez-Cohen and Speitel, 2001; Wilson and Wilson, 1985). Although aerobic cometabolic and oxidative degradation of chlorinated ethenes is relatively fast, compared to reductive dechlorination (Wiedemeier et al., 1999), these processes are rarely documented in nature. They can, however, be important at mixed layers such as on the edge of a contaminant plume, at the border between groundwater and surface waters and in top covers of landfills (Scheutz et al., 2004).

Anaerobic degradation can occur either cometabolically, by oxidation or by reductive dechlorination (Bradley, 2000; Hopkins and McCarty, 1995; McCarty et al., 1998; Smith et al., 1997), as demonstrated in **Figure 5**. In nature, reductive dechlorination is the most common degradation path (Fennell et al., 2001), whereas anaerobic cometabolic degradation and oxidation rarely is documented. Anaerobic oxidation of *c*DCE and VC to CO_2 can occur in groundwater environments. In this process, *c*DCE oxidation is usually the rate- limiting step and generally requires Mn-reducing conditions. VC oxidation generally requires Fe-reduced conditions and mineralization increases after addition of Fe(III) (Bradley, 2000). The advantage of anaerobic oxidation is that *c*DCE can be degraded without production of the carcinogenic VC. Toxic degradation products can be accumulated during reductive dechlorination of PCE, TCE, and *c*DCE (Bradley, 2000).

Aerobic Conditions | Anaerobic Conditions

Tetrachloroethene
(PCE)

↓ reductive dechlorination

CO_2 ← cometabolism ← Trichloroethene
(TCE)

(Wilson & Wilson, 1995) (methanotrophs)
(Nelson et al., 1999) (toluene oxidizers)
(Wackelt et al., 1999) (propane oxidizers)
(Vanell et al., 1990) (ammonia oxidizers)
(Hopkins et al., 1993) (phenol oxidizers)
(Ensign et al. 1992) (ethene oxidizers)

↓ reductive dechlorination

CO_2 ← cometabolism (as for TCE) ← Dichloroethene (cis-1,2-DEC) → anaerobic oxidation (manganese reducers) → CO_2 (Bradley et al., 1998)

(Klecka et al., 1997)
(Coleman et al. 2002 b)
oxidation

↓ reductive dechlorination

CO_2 ← cometabolism (as for TCE) ← Vinyl Chloride (VC) → anaerobic oxidation (iron reducers) → CO_2 (Bradley & Chapelle, 1998)

(Hartmans et al., 1995)
(Davis & Carpenter, 1990)
(Cox et al., 1995)
(Coleman et al. 2002 a)
oxidation

↓ reductive dechlorination

CO_2 ← cometabolism ← Ethene → Ethane (deBruin et al., 1992)

(Freedman & Gossett, 1999)
(Holliger et al., 1993)
(Wild et al., 1995)
(Maymo-Gatell et al., 1997)
(He et al., 2003)

Figure 5. Pathways for the degradation of chlorinated ethenes (GeoSyntec Consultants, 2004)**.**

2.4.2. Reductive dechlorination of chlorinated ethenes

Reductive dechlorination is currently believed to be the most common degradation pathway for PCE and TCE in nature. It was first recognized in 1983 for PCE and TCE to VC (Bouwer and McCarty, 1983), and later, in 1989, it was demonstrated that VC could be further reduced to ethene (Freedman and Gossett, 1989). In this process, the chlorinated ethenes, PCE or TCE, are sequentially dechlorinated to *c*DCE, VC and finally to ethene (**Figure 6**). The chlorinated ethenes act as electron acceptors (Holliger et al., 1993) and the majority of degraders use H_2 as their primary and direct electron donor for dechlorination (Fennell et al., 1997b). H_2 is also consumed by other organisms as sulfate reducers and methanogens.

In general, it is believed that excess H_2 levels can favor other microorganisms as methanogens and outcompete dechlorination, whereas too low H_2 levels undermines the energy gain for dechlorination (Fennell et al., 1997b; Fennell and Gossett, 1998; Heimann

et al., 2005a). Each dechlorinating step occurs under a specific range of redox conditions and with a community shift in dominant degraders (He et al., 2003a). In general, more reduced conditions are needed for each sequential step in reductive dechlorination (Chapelle, 1996), and ethene production commonly occurs under methanogenic conditions (Ballapragada et al., 1997; Maymo-Gatell et al., 1999; Vogel and McCarty, 1985). However, ethene can be produced with no or little methane production and few methanogens (Duhamel et al., 2004).
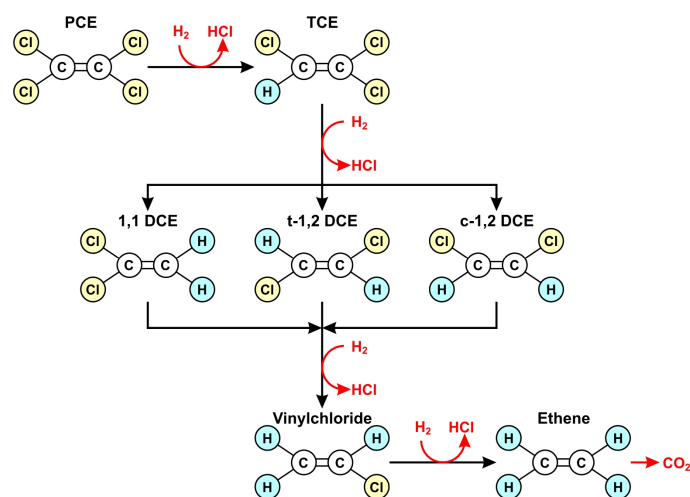
Figure 6. Anaerobic reductive dechlorination.

The first identified organism capable of securing complete dechlorination to ethene was *Dehalococcoides (Dhc) ethenogens* 195 (Maymo-Gatell et al., 1999). This organism dechlorinated PCE to VC through reductive dechlorination and VC to ethene cometabolically. Recent research suggests that diversification of its reductive dechlorination functions is mediated by recent genetic exchange and amplification and that the ancestor is a nitrogen-fixing autotroph (Seshadri et al., 2005). Only organisms restricted to the *Dhc* genus are known to be capable of securing complete dechlorination to ethene (Table 2). It is, however, worth noting that not all *Dhc* can utilize chlorinated ethenes as electron acceptors, but use substrates such as polychlorinated dibenzodioxins and chlorobenzenes (Bunge et al., 2003).

In the subsurface, natural reductive dechlorination is rarely complete to ethene due to lack or low presence of electron donors and/or specific dechlorinating microorganisms (e.g. Fennell and Gossett, 1998; Hendrickson et al., 2002; Yang and McCarty, 1998). This can be overcome or occur at higher rates in subsurface environments upon addition of electron donors and/or specific dechlorinating microorganisms are injected into the subsurface (section 5.2).

Table 2. Degradation of chlorinated solvents carried out by selected identified microbes. Red. decl. is reductive dechlorination where microorganisms grow using chlorinated ethenes.

Dechlorination	Process	Organism(s)	References
PCE → TCE	Red. dechl.	*Dehalococcoides ethenogenes* 195	(MaymoGatell et al., 1997)
		Dehalobacter restrictus strain PER-K23	(Holliger et al., 1993)
		Dehalobacter restrictus strain TEA	(Wild et al., 1996)
		*Dehalospirillum multivorans**	(Scholz-Muramatsu et al., 1995)
		Sulforospirillum halorespirans strain PCE-M2	(Luijten et al., 2003)
		Desulfomonile tiedjei	(Fathepure and Tiedje, 1994)
		Desulfuromonas michiganensis	(Sung et al., 2003)
		Desulfitobacterium strain PCE1	(Gerritse et al., 1996)
		Desulfitobacterium strain PCE-S	(Miller et al., 1998)
		Desulfitobacterium strain TCE1	(Gerritse et al., 1999)
		Desulfitobacterium strain Y51	(Suyama et al., 2001)
		Desulfitobacterium metallireducens	(Finneran et al., 2002)
		Desulfuromonas chloroethenica	(Krumholz, 1997)
		Clostridium bifermentans strain DPH-1	(Chang et al., 2000)
		Enterobacteriaceae strain MS-1	(Sharma and McCarty, 1996)
	Cometabolic	*Dehalococcoides* sp. strain BAV1	(He et al., 2003b)
TCE → cDCE	Red. dechl.	*Dehalococcoides ethenogenes* 195	(MaymoGatell et al., 1997)
		Dehalococcoides sp. strain FL2	(Loffler et al., 2000)
		Dehalobacter restrictus strain PER-K23	(Holliger et al., 1993)
		Dehalobacter restrictus strain TEA	(Scholz-Muramatsu et al., 1995)
		*Dehalospirillum multivorans**	PCE-M2 (Luijten et al., 2003)
		Sulforospirillum halorespirans strain	(Sung et al., 2003)
		Desulfuromonas michiganensis	(Miller et al., 1998)
		Desulfitobacterium strain PCE-S	(Gerritse et al., 1999)
		Desulfitobacterium strain TCE1	(Suyama et al., 2001)
		Desulfitobacterium strain Y51	(Finneran et al., 2002)
		Desulfitobacterium metallireducens	(Krumholz, 1997)
		Desulfuromonas chloroethenica	(Chang et al., 2000)
		Clostridium bifermentans strain DPH-1	(Sharma and McCarty, 1996)
		Enterobacteriaceae strain MS-1	
	Cometabolic	*Dehalococcoides* sp. strain BAV1	(He et al., 2003b)
cDCE → VC	Red. dechl.	*Dehalococcoides ethenogenes* 195	(MaymoGatell et al., 1997)
		Dehalococcoides strain FL2	(Loffler et al., 2000)
		Dehalococcoides strain VS	(Cupples et al., 2003)
		Dehalococcoides sp. strain BAV1	(He et al., 2003b)
VC → ethene	Red. dechl.	*Dehalococcoides* strain VS	(Cupples et al., 2003)
		Dehalococcoides sp. strain BAV1	(He et al., 2003b)
	Cometabolic	*Dehalococcoides ethenoges* strain 195	(Maymo-Gatell et al., 1999)
		Dehalococcoides sp. strain FL2	(Loffler et al., 2000)

* Renamed to *Sulfurospirillum multivorans*.

3. *IN-SITU* REMEDIATION TECHNOLOGIES USED FOR CHLORINATED ETHENES

Various innovative technologies have been developed and tested to enhance contaminant removal of chlorinated ethenes from polluted sites (NRC, 1994; NRC, 1997; NRC, 1999). Early efforts to remediate the subsurface for chlorinated solvents involved use of 'pump-and-treat' systems. In this technology, groundwater is extracted, treated and either discharged to a sewer or surface water or injected downstream the source zone. However, even though this technique was initially aimed at removing the source of contamination, the time frames for sufficient clean-up were unreasonable long because NAPLs continued dissolving contaminants in the groundwater (Bedient et al., 1999). Subsequently, innovative technologies for remediating sites contaminated with chlorinated solvents and DNAPLs have evolved on the basis of *in-situ* physical, chemical and biological processes (Table 3). Although some observations in this section may be generally applicable to any DNAPL and dissolved phase plume, as well as remediation of volatile organic carbon (VOC) the focus is on remediation technologies used at sites contaminated with DNAPL of chlorinated ethenes.

Table 3 Overview of *in-situ* remediation technologies used for remediation of chlorinated ethenes. Contaminant phases include vapor, sorbed, dissolved, and DNAPL.

Remediation technology		Subsurface zone	Contaminant phase	Potential*
Physical	Pump-and-treat	Saturated	Primarily dissolved	+
	Soil-vapor extraction (SVE)	Unsaturated/ saturated	All	+++
	Multiphase extraction (MPE)	Unsaturated/ saturated	All	+++
	Air sparging	Saturated	Dissolved and vapor	+
	Thermal treatments	Unsaturated/ saturated	Primarily DNAPLs	+++
	In-situ flushing	Saturated	All but vapor	+++
	Containment (e.g. PRB)	Mainly saturated	Varies w/ application	vary
	Biophysical (phytoremediation)	Shallow saturated	Site-specific	+
Chemical	Chemical oxidation	Unsaturated/ saturated	DNAPL and dissolved	+
	Chemical reduction	Saturated	DNAPL and dissolved	n.g.
	Metal-enhanced (e.g. Fe^0, ZVI)	Saturated	DNAPL and dissolved	n.g.
Biological	Aerobic degradation	Unsaturated/ saturated	E.g. edge of plume	n.g.
	Anaerobic degradation	Saturated	All (DNAPL is developed)	+++

*The potential of the technologies for VOC-contaminated soils is obtained from the US EPA's suggestions (U.S.EPA, 2005b), where '+++' indicates better technology and '+' average technology; 'n.g.' indicates not given in the reference; and 'vary' indicates variation from + for deep well injection to +++ for permeable reactive barriers (PRB). ZVI indicate zero valent iron.

3.1. Physical remediation technologies

Physical properties of chlorinated ethenes that can be manipulated for remediation purposes are primarily volatility, solubility, viscosity and sorption. A widely used *in-situ* technology for removal in the unsaturated and saturated zone is soil vapor extraction (SVE). In this

technology, soil vapor is extracted under vacuum, whereby a flow of air through the soil causes chlorinated ethenes to volatilize and partition into the vapor phase. The US EPA designates this technology as the presumptive remedy for soils contaminated with VOCs, defined as technologies including multiphase extraction (MPE), *in-situ* air sparging and thermally enhanced SVE.

In MPE, a high vacuum (typically 0.6 to 0.8 atm) is applied to the subsurface to simultaneously extract soil vapor, groundwater and a separate phase liquid (U.S.Army Corps of Engineers, 1999; U.S.EPA, 1997). This technology is best suited at sites with finer-grained soils because these are more difficult to dewater. However, studies indicate that the degree of dewatering may be less than previously anticipated (Baker et al., 1999). *In-situ* air sparging can be used to treat dissolved-phase chlorinated ethenes. In this technology, air is injected beneath the water table to volatilize the contaminants to the vadose zone, where they are removed by SVE. In some cases, this can also stimulate aerobic degradation. A challenge of this technology is that DNAPLs may mobilize vertically into a deeper zone in the aquifer due to the increased mobility at elevated temperatures, making remediation even more difficult (Henry et al., 2003).

The removal rate of contaminants can be drastically increased at elevated temperatures (Heron et al., 1998c). Thermal treatment is efficient and can remediate heavily contaminated sites with free phase DNAPL (Heron et al., 2005b). Limitations of these technologies include the high costs associated with heating the subsurface and equipment as well as the risk of downward mobilization, particularly in the case of steam injection, where a condensate front develops. However, different methods have been suggested to minimize this risk including injection of air mixed with steam or by heating the layer underlying the contaminated zone prior to the thermal treatment (Schmidt et al., 2002). Thermal treatment will be further discussed in chapter 4.

Another physical remedy is *in-situ* flushing, where various flushing agents can be injected to the subsurface to increase the solubility and/or mobility of the contaminant. DNAPL is then displaced by the continuous flooding of the subsurface (ITRCWG, 2000). Furthermore, water flooding can be applied aswell.

Also, containment strategies can be applied. These include physical structures or hydraulic barriers to control movement of dissolved-phase plume (Pankow et al., 1996) as well as complete encapsulation. Permeable reactive barriers (PRB) have been used to remediate dissolved contaminants. PRB can contain various chemical agents that may reduce, oxidize and/or degrade contaminants (Wickramanayake et al., 2000).

Finally, biophysical removal has been tested using wetlands or other vegetation for remediation of shallow groundwater systems. Phytoremediation can for example be applied

although it is subject to certain limitations. These include the depth at which plants' root systems can uptake water and contaminants as well as the potential toxicity to the root system of high concentrations of chlorinated solvents (Nietch et al., 1999).

All the physical methods suffer from general limitations:

1. Subsurface heterogeneity will make it difficult to recover contaminants having migrated or diffused into low-permeability layers such as silts and clays. The treatment occurs preferentially in the permeable zones, leading to long tailing in removal (resulting in treatment times of decades to centuries or ineffective treatment).
2. The mass-transfer limitations are primarily caused by:
   a. Low solubility and dissolution rates of the chlorinated ethenes in water
   b. Ineffective contact of DNAPL with flowing fluids
   c. Low volatility at ambient temperatures
   d. Adsorption onto solid surfaces (organic matter, clay minerals)

This means that the use of these technologies has transitioned from remediation towards containment, where some mass is removed and an inward gradient is established to prevent contaminants from escaping – especially since it has been realized that site restoration in most cases will not be feasible due to the mass-transfer limitations. The thermal technologies discussed in chapter 4 aim to overcome these limitations.

3.2. Chemical remediation technologies

Chemical processes involve destruction of chlorinated solvents using reductive or oxidative chemical reactions as well as indirect destruction of contaminants through manipulation of subsurface geochemical conditions (Siegrist et al., 2001). Metal-enhanced dechlorination can also be applied using low valence colloidal or zero valent iron either in nanoscale or granular form (Dries et al., 2005; Lien and Zhang, 2001; Rosenthal et al., 2004). Direct chemical oxidation involves the use of an oxidant. The most common oxidants are ozone, permanganate, and Fenton's reagent (H_2O_2 catalyzed with Fe^{2+}). The application of chemical processes can be carried out with a variety of physical methods. In all cases, successful delivery relies on the subsurface geology and hydrogeology. For example, fine grained sediments are less amenable to chemical oxidation because of difficulties in delivering the reactant into the affected aquifer. Moreover, limitations include competitive reactions in the subsurface.

3.3. Biological remediation technologies

Biological remediation processes involve aerobic and anaerobic destruction of chlorinated solvents, where anaerobic reductive dechlorination is most widely applied (section 2.4. and

discussed in chapter 5). The challenges facing anaerobic dechlorination as a remediation technology include limited transport of electron donor and microorganisms in the porous media, maintenance of a dechlorinating population as well as regulatory objection to injecting microorganisms into the subsurface. There is also a risk of producing intermediates that are even more toxic as VC.

The effectiveness and applicability of biodegradation for DNAPL source zones is evolving. Groundwater bacteria can only grow in the aqueous phase and not in DNAPL, and the dechlorination rate is therefore controlled by the dissolution rate. However, dechlorinating microorganisms have been shown to flourish at the DNAPL/dissolved interface and to cause an increased dissolution rate of chlorinated ethenes into the aqueous phase (Cope and Hughes, 2001; Yang and McCarty, 2000). Nevertheless, bioaugmentation of PCE/TCE may be applicable to remediate dissolved-phase plumes (Adamson et al., 2003) and used in conjunction with other remedies as polishing technology.

4. THERMAL TREATMENT

Thermally enhanced remediation technologies are promising for removal of contaminants at heavily contaminated sites (Heron et al., 2005b; U.S.EPA, 2004c). These technologies are based on a temperature increment in the soil and groundwater environment resulting in enhanced recovery of contaminants. Heat can be applied in various ways to the subsurface. The methods include injection of hot air, hot water, or steam; thermal conduction using heat blankets or thermal wells; low-frequency electrical heating; radio frequency heating; microwave heating; and/or combinations of such methods (e.g. Davis, 1997; Heron et al., 1998c; Smith and Hinchee, 1993). By 2004, the most commonly applied thermal technologies in the USA were steam injection, electrical heating and a combination of these two (U.S.EPA, 2004c). Thermal conduction heating (TCH), also called *in-situ* thermal desorption, has since 2000 been applied at 15 field sites (Heron, 2005).

4.1. Mechanisms during thermal treatment

The main reason for the wide application of thermal treatment is that the mobility of the contaminant increases at elevated temperatures (Davis, 1997; Davis, 1998). The single most important removal mechanism is increased vapor pressure. Vapor pressure is a measure of volatility of a compound when it is present as a free phase liquid. The high temperature dependence of vapor pressure leads to an increased fraction of the contaminants in the gas phase (**Figure 3**), which increases the ease of extracting contaminants in soil vapor (Heron et al., 1998c). The larger fraction of contaminant mass in the gaseous phase (**Figure 3**) results in lower influence of increased solubility and decreased interfacial tension with increasing temperature on removal of contaminants (Heron et al., 1998b). The effects of heating on physical/chemical properties are shown in Table 4.

Table 4. The effects of heating on physical/chemical properties of TCE.

Effect of heating	Property	Effect of heating on TCE	Reference
Increase	Vapor pressure	Factor of 18 for 10-100°C	Heron et al., 1998b
	Henry's constant	Factor of 22 for 10 to 95°C	Heron et al., 1998a
	Aqueous solubility	40% for 10 to 90°C	Heron et al., 1998b
	Diffusion, water	31% for 10 to 100°C	Heron et al., 1998b
	Diffusion, gas	51% for 10 to 100°C	Heron et al., 1998b
Decrease	Interfacial tension	< 20% for 0 to 100°C	Sleep & Ma, 1997
	Viscosity	50% for 0 to 100°C	Sleep & Ma, 1997; Imhoff et al., 1997
	Sorption	Unknown decrease	Heron et al., 1998b
	Density	10-15% for 0 to 100°C	Heron et al., 1998b

The vapor pressure is also influenced by the presence of two immiscible fluids (i.e. water and an organic compound) at the same time. Immiscible fluids boil when the sum of their vapor pressures exceeds the pressure of the surroundings. Since both contribute to the total vapor pressure, the boiling point of the mixture is lower than that of each fluid (Stewart and Udell, 1988). This effect results in boiling at lower temperatures, which can increase extraction of contaminants. For TCE, which has a pure liquid boiling point of 87°C, the boiling temperature in water is 73°C. For PCE, which has a pure liquid boiling point of 121°C, the boiling temperature in water is 88°C.

Removal of contaminants from the heated zone is mainly based on SVE and/or MPE (Davis, 1997). During this extraction, steam stripping occurs when the vapor phase is removed continuously, thus preventing an equilibrium and increasing the mass of extracted contaminant (Davis, 1997). Another removal mechanism during thermal treatment is in-situ wet oxidation or hydrous pyrolysis oxidation (HPO). However, the existence and extent of this mechanism are still debated. The mechanism of HPO is, that in soil with high water content and presence of oxygen, a chemical oxidation of organic compounds may take place at temperatures up to 100ºC releasing CO_2 (Heron and Heron, 2003; Knauss et al., 1999; Ridley and Martinelli, 1994).

4.2. Steam injection

Steam injection, also termed steam enhanced extraction (**Figure 7**), was initially developed by the petroleum industry for enhancing oil recovery, and has more recently been adapted to remediate soil and aquifers (Davis, 1997; Udell and Stewart, 1989). Steam injection has been applied at some sites in the USA (U.S.EPA, 2004c) and also at five locations in Denmark (e.g. Heron and Heron, 2003; Larsen et al., 2004). It has been applied in unsaturated as well as saturated zones (Smith and Hinchee, 1993) and is generally more efficient in porous media, such as sand (Heron et al., 1998c), than in low permeable soils (Balshaw-Biddle et al., 2000).
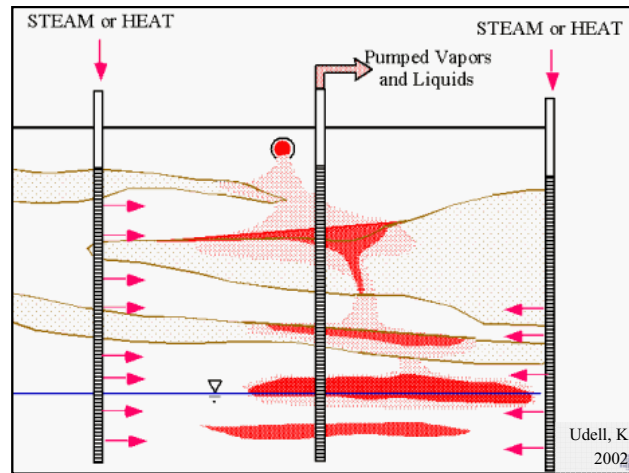
Figure 7. Steam injection in a cross-section of a contaminated aquifer.

When applied at a field site, steam is injected into the subsurface via wells surrounding the contaminant, and the subsurface is heated to approximately 100-120°C (Heron et al., 1998c). The elevated temperatures volatilize high and semi-volatile contaminants. The removal of contaminants is based on migration of contaminant vapor and flow of contaminant liquid. As the steam front advances through the contaminated area, more contaminant accumulates and the concentration may exceed residual saturation. When this occurs, the contaminant becomes mobile and can migrate vertically due to gravitational forces (Katalusky and Udell, 2002; Sleep and Ma, 1997) resulting in one of the most severe drawbacks of this technology. As previously described, it has been proposed that downward migration can be avoided by heating the area below the contaminant, although this may be difficult or by using mixtures of steam and air instead of pure steam to increase control on heating and maintain specific temperatures (Schmidt et al., 2002).

4.3. Electrical resistance heating (ERH)

Soil and groundwater can be heated by applying an electrical current to the subsurface. ERH uses the natural electrical resistance within the subsurface, where energy is dissipated through resistive losses (U.S.EPA, 2004c). In this technique, electrodes are inserted into the ground in triangular or hexagonal arrays and three-phase or six-phase electricity is applied to the electrodes (**Figure 8**). The installation of electrodes generally depends on space constraints either at the surface or in the subsurface. When ERH is applied in unsaturated zones or when full steaming conditions are achieved in the subsurface, water is typically injected to maintain good electrical contact and prevent excessive drying or voltage breakdown at the electrodes (U.S.EPA, 2004b).

Figure 8. Electrical resistance heating (ERH) at Ft. Lewis EGDY, 2005. Electrodes inserted at a distance of approximately 5 m.

The current is carried away from the electrodes by water in the pore space. When an area is heated and dries out, the resistance increases and the current will find alternate directions. The whole area will therefore theoretically be heated with time. The natural resistance to electrical current in soils will cause a loss of energy as heat. The current will preferentially travel through clay and silt layers as they have higher water content. In addition, presence of ions in clay will increase the current flow and thereby heat deposition in such layers (Davis, 1997). This method is therefore suitable for soils with low-permeable layers that steam vapors cannot penetrate rapidly (Beyke, 1998).

4.4. Thermal conductive heating (TCH)

Another way of applying heat to the subsurface is by direct heating with heating elements or heat blankets (TCH or in-situ thermal desorption (ISTD)). The heating elements can be heated to temperatures up to 1,000ºC, but lower temperatures are used for chlorinated solvent sites as the soil and water are heated to the boiling point, where in-situ steam generation leads to effective treatment. This technique is based on thermal conduction, and therefore heating boreholes are placed relatively close (less than 6 meters apart). In addition, soil close to the elements will dry out as soon as the temperature is high enough for the water to boil, creating a zone with increased permeability to allow the generated steam to migrate for recovery. The advantages of this technique are that it is easy to control and reach a specific target temperature, and that even higher temperatures can be reached, thus making it possible to remediate polluted soil for less volatile compounds (Heron, 2005).

4.5. Other thermal technologies

ERH can be combined with steam injection in aquifers interbedded with low permeable lenses or in situations where a lower aquitard has been impregnated with DNAPLs (U.S.EPA, 2004c). This technology is termed dynamic underground stripping (DUS) and is efficient at removing volatile and semi-volatile organics (Heron et al., 1998c; Newmark and Aines, 1995).

4.6. Elevated temperatures after thermal treatment

Temperatures after thermal treatment are important when it comes to predicting the potential for biological remediation because elevated temperatures can increase the metabolic activity, but too high temperatures can destroy the microorganisms (**V**, Friis et al., 2005e).

The duration of the temperature decrease after thermal treatment can be affected by groundwater flow, properties of the porous media (especially water content), size of the treated area, depth below the surface and the area above or below the groundwater table. Krauter et al. (1995) reported that the groundwater remained between 45 and 75°C two years after steam injection was completed at Lawrence Livermore National Laboratory Site, California, USA. In contrast, at a Danish site where cooling was applied by extracting the groundwater after steam injection, temperatures between 30 and 50°C were measured only 1.5 months after completion (Heron, 2003). Areas surrounding the heated zone can also hold elevated temperatures and cool similarly as the heated zone. A temperature example before, during and after a full-scale ERH is illustrated in **Figure 9**. In general, exponential temperature decreases are observed after thermal treatment.
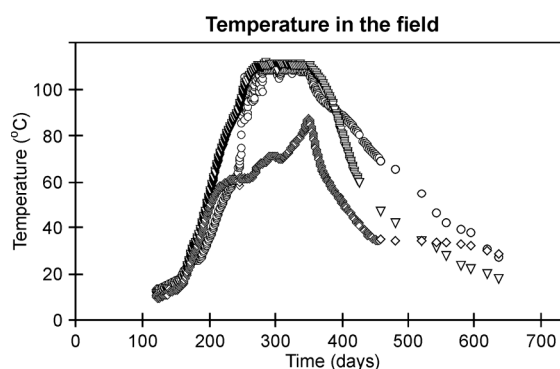

Figure 9. Temperature development before, during and after ERH at a field site. Three samples within hot-spot are illustrated from the Ft. Lewis field site, WA, USA.

4.7. Residual contamination after thermal treatment

After thermal treatment application, the presence of residual contaminant will depend on the properties of the soil and contaminants as well as the duration of the thermal treatment process and maximum temperatures reached (Davis, 1998). Theoretically, a residual water saturation of contaminants will always be left behind (Stewart and Udell, 1988). In general, thermal treatment will preferentially remediate volatile compounds, and lower removal rates may be observed for sorbing contaminants as PAHs (Richardson et al., 2002) and contaminants with a significant water solubility (Stewart and Udell, 1988). Slow removal of contaminants may also occur in low permeable soils such as in clay lenses (e.g. Balshaw-Biddle et al., 2000) and in heterogeneous soils (e.g. Sleep and McClure, 2001). Previous studies demonstrate cleanup to concentrations ranging from below detection limit (Heron et al., 2005b) to 20 µg TCE/kg soil, 295 µg TCE/L groundwater (Udell et al., 2000) or approximately 180 µg PCE/L in groundwater (Jørgensen et al., 2003).

Apart from residual contamination dissolved in water, a potentially more important fraction may stem from "hidden" residual contamination (Heron, 2003). The problem occurs from contaminants present in areas not having reached the high temperatures during thermal treatment and so not having been remediated. These areas may include low permeable zones (Balshaw-Biddle et al., 2000), overlying unsaturated zones where contaminant condenses due to lower temperatures (Huesemann et al., 2002), and underlying colder regions. Immediately after thermal treatment, contaminants appear to be removed since low concentrations are observed in high permeable zones, although a rebound effect may occur, from unheated areas where contaminants can dissolve slowly into the water phase. Unfortunately, this is rarely monitored and reported, and the extent of this phenomenon is therefore not quantified.

Residual or leftover contamination occurs sometimes even if thermal treatment is 100% effective, simply because trace amounts of contaminants may exist outside the area being treated. The target treatment zone is defined on the basis of discrete soil and water samples and is therefore never a perfect delineation of the contaminated zone. As thermal treatment progresses and vapors and liquids are extracted in order to maintain pneumatic and hydraulic control, contaminants from the surrounding zones will enter and prevent complete remediation. This may leave small amounts of contaminants in and around the treated zones (Heron, 2005). Since many sites require cleaning to concentrations near non-detect or maximum contaminant levels (MCLs), a polishing method well suited for removing the residual contaminants would be a necessity, especially for the less effective thermal methods.

5. BIOREMEDIATION OF CHLORINATED ETHENES

Bioremediation can be applied to subsurface environments by injecting electron donor(s) into the subsurface, a process termed biostimulation, and/or by injecting specific dechlorinating microorganisms, a remediation process termed bioaugmentation. This is performed to overcome the requirements for complete dechlorination of PCE/TCE to ethene which includes: (1) presence of electron donors for dechlorination, commonly H_2 (Fennell et al., 1997b; Loffler et al., 1999), (2) reducing conditions, (3) dechlorinating microorganism, and (4) presence of chlorinated electron acceptors at concentrations high enough to sustain growth of dechlorinating microorganisms. However, the last requirement is commonly fulfilled at PCE/TCE contaminated sites.

5.1. Biostimulation

Biostimulation is applied to obtain reduced subsurface conditions and to supply the reductive dechlorination process with electron donor. Numerous laboratory and field studies have been conducted to examine the potential for stimulating reductive dechlorination of chlorinated ethenes using a wide variety of electron donors, including lactate, methanol, ethanol, acetate, proprionate, butyrate, benzoate (Fennell et al., 1997a; He et al., 2002) and more complex molecules as molasses, oil and HRC^{TM} (hydrogen releasing compound), (Koeningsberg and Farone, 2005). The most commonly applied donors include H_2, lactate, propionate, methanol, butyrate and ethanol (Ballapragada et al., 1997; Carr and Hughes, 1998; Fennell et al., 1997a; Freeborn et al., 2005; Yang and McCarty, 1998). When organic acids and alcohols are provided as electron donors, secondary-fermenting or syntrophic bacteria are responsible for production of acetate, CO_2 and H_2 (Freeborn et al., 2005).

H_2 can be consumed by dechlorinating and other microorganisms, and it is important to deliver low H_2 partial pressures to provide a selective advantage to dechlorinators over methanogens (Ballapragada et al., 1997; Carr and Hughes, 1998; Fennell and Gossett, 1998; Loffler et al., 1999; Yang and McCarty, 1998). Electron donors that are slowly fermented, such as propionate and butyrate, typically provide a steady source of electrons with corresponding low H_2 partial pressures, whereas rapidly fermented substrates such as lactate typically provide a quicker release of electrons with corresponding high H_2 partial pressures (Heimann et al., 2005a).

Table 5. Examples of field studies with biostimulation of chlorinated ethenes.

Chlorinated ethene		Location of field study	Donor	Reference
Pre	Post*	All in USA		
TCE	*c*DCE	Dover Air Force Base	Lactate	Ellis et al., 2000
PCE/*c*DCE	*c*DCE	Kelly Air Force Base	Methanol, acetate	Major et al., 2002
TCE/*c*DCE	Ethene	Naval Air Station	Lactate	Leigh et al., 2000
PCE	*c*DCE	Oregon dry cleaning	HRC	Anderson et al., 2000
PCE/TCE/*c*DCE/VC	*c*DCE	Illinois Industry	HRC	Schuhmacher et al., 2000
PCE/TCE/*c*DCE/VC	*c*DCE	Florida dry cleaning	HRC	Lodato et al., 2000
PCE/TCE	Ethene	Bachman Road site	Lactate	Lendway et al., 2003
TCE	VC/Ethene	Twin Cities Ammunition	H_2	Edstrom et al., 2005

* indicates the main constituent after treatment.

Biostimulation in PCE/TCE-contaminated aquifers resulted in dechlorination to *c*DCE at five sites and ethene production at three sites (Table 5). This demonstrates that dechlorination often stalls at *c*DCE upon biostimulation. The reason for the stalled dechlorination is still debated. On the one hand it is believed that microorganisms, capable of securing dechlorination to ethene, are present at all sites, but the number of such organisms may be small (Nyer et al., 2002). However, the majority of researchers find that *Dehalococcoides* are required for complete dechlorination to ethene (Hendrickson et al., 2002; Major et al., 2003) and that these dechlorinating microorganisms can be added to subsurface environments when absent or when the numbers are too low to achieve dechlorination within a reasonable timeframe. Another challenge faced during biostimulation is sufficient distribution of electron donors in the subsurface to the area of interest where the contamination is located.

5.2. Bioaugmentation

Bioaugmentation is a remediation technology where microorganisms and electron donors are injected into the subsurface to degrade contaminants and to establish favorable redox conditions. The fact that different microorganisms carry out different dechlorinating steps (Table 2) leads to the use of mixed consortia for enhanced bioremediation. These include KB-1[TM] (Major et al., 2002), the Pinellas culture (Ellis et al., 2000), the Bachman road culture also called BC2 or bio-dechlor (He et al., 2003b; Lendvay et al., 2003; Loffler et al., 2000), and multiple mixed cultures from Bioremediation Consulting Inc. (GeoSyntec Consultants, 2004). Complete dechlorination of PCE/TCE to ethene has been obtained at sites bioaugmented with these cultures (Table 6). Other dechlorinating cultures not tested at field sites include the "Cornell" enrichment culture (Maymo-Gatell et al., 2001; MaymoGatell et al., 1997), Victoria enrichment (Cupples et al., 2003), Toronto main enrichment (Dennis et al., 2003), ANAS enrichment (Richardson et al., 2002), LEC1 enrichment (Adamson and Parkin, 2000), and Cape Canaveral enrichment (Fennell et al., 2001).

Table 6. Examples of field studies with bioaugmentation of chlorinated ethenes. * indicates the main constituent after treatment.

Chlorinated ethene Pre	Post*	Location	Geology	Culture	Reference
PCE, TCE, chloroform	cDCE, ongoing	Caldwell superfund site, NJ, USA	Fractured bedrock	KB-1[TM]	Finn et al., 2003
PCE, TCE	Ethene	Evenblij site, Hooeveen, Holland	Sand	On-site anaerobic bioreactors.	Henssen et al., 2001
TCE	Ethene	Cape Canaveral, AFS, FL	Sand, silt and with shells	KB-1[TM]	McMaster et al., 2002
TCE	Ethene	Dover AFB, DE	Fine sand and silt	Pinellas	Ellis et al., 2000
TCE	Ethene	Aerojet superfund site, Sacramento, CA	Fluvial deposits with sand and gravel	KB-1[TM]	Cox et al., 2000
PCE	Ethene	Bachman Road residential wells	Fine to medium grained sand	Bachman Road (Bio-Dechlor)	Lendvay et al., 2003
PCE	Ethene	Kelly AFB, TX	Unconsolidated alluvial deposits	KB-1[TM]	Major et al., 2002
TCE	Ethene	Industrial facility, Boston, MA	Unconsolidated fluvial deposits.	KB-1[TM]	Chang et al., 2002; Chang et al., 2003
TCE	VC/ Ethene	Industrial facility	Fractured bedrock	KB-1[TM]	Chartrand et al., 2005

Several factors are known to affect culture performance in dechlorination during bioaugmentation, including exposure to oxygen (MaymoGatell et al., 1997), competition for electron donor with other electron acceptors (e.g. sulfate, Heimann et al., 2005), type and concentration of electron donor (Fennell et al., 1997b; Gerritse et al., 1999) (section 3.2.3), temperature (V, Friis et al., 2005e), pH, concentration of chlorinated solvents (Harkness et al., 1999) and presence of co-contaminants as chloroform and TCA. As with most microbial processes, groundwater pH can limit dechlorinating activity at pH levels below 6 and above 9 (Fennell and Gosset, 2003; Middeldorp et al., 1999). Temperatures below 4°C and above 40°C can hinder dechlorination (V, Friis et al., 2005e).

The drawbacks of bioaugmentation include the risk of uneven distribution of microorganisms, especially in tight subsurface soils, and excess methane production as a result of electron donor amendments, which may be caused by competitive substrate utilization by methanogens over dechlorinating microorganisms (Fennell and Gossett, 1998; Yang and McCarty, 1998), and the potential for clogging by methanogens (Baveye et al., 1998; Yang and McCarty, 2002). Furthermore, the challenges include maintenance of the dechlorinating population, capable of securing complete dechlorination to ethene and suitable redox conditions, as well as regulatory objection against injecting microorganisms into the subsurface. The use of bioaugmentation in DNAPL source zones is still discussed, as previously described. Nevertheless, the successful field-scale demonstrations suggest that the technology is viable despite the challenges (Ellis et al., 2000; Lendvay et al., 2003; Major et al., 2002).

5.3. The culture used for bioaugmentation: KB-1™

The culture used for the research presented in this thesis, was KB-1™, which was enriched from soil and groundwater microcosms from a TCE-contaminated site in Ontario, Canada (Edwards et al., 2001). The community structure has been thoroughly characterized (Duhamel, 2005). It consists of at least two *Dehalococcoides*, capable of dechlorinating TCE to ethene, and geobacter, which can dechlorinate TCE to *c*DCE alongside *Dehalococcoides*. Furthermore, the presence of *Spirochete*, *Sporomusa*, *Methanomicrobiales*, *Methanomethylovorans* and *Methanosarcina* was also frequently detected in KB-1™ (**Figure 10**), though their function is largely redundant for reductive dechlorination, apart from *Sporomusa*, which can increase VC dechlorination rates in co-cultures with *Dehalococcoides* (Duhamel, 2005).
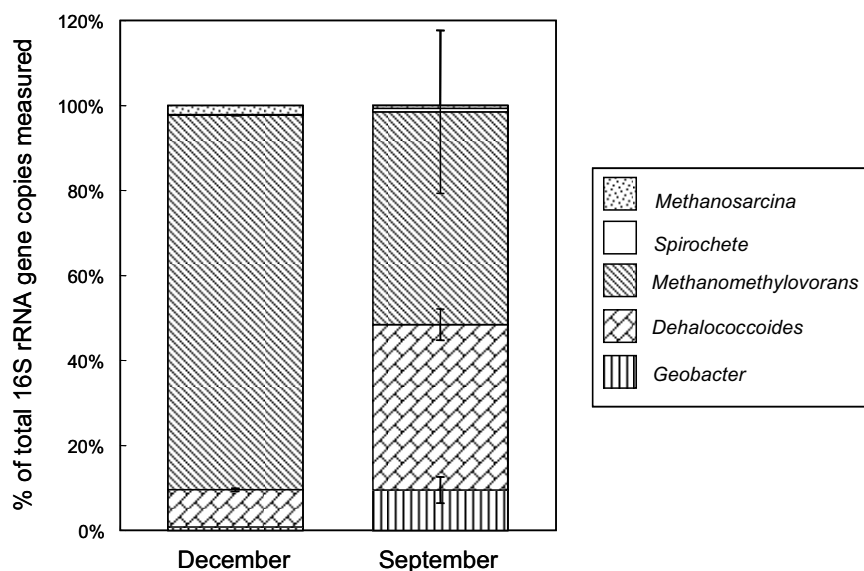
Figure 10. Community structure in KB-1™ in September and December 2004 as % age of total 16S rDNA copies. (Unpublished results, with permission from Duhamel, M.).

The first successful application of KB-1™ demonstrated with a control plot to verify the effect of bioaugmentation was at Kelly Air Force Base, Texas, USA (**Figure 11**, Major et al., 2002). Prior to addition of KB-1™, the site was stimulated with methanol and acetate, resulting in conversion of 1 mg PCE/L to *c*DCE. Upon addition of KB-1™ in a recirculation system, dechlorination was nearly complete to ethene within 200 days. Since then, KB-1™ has been used for bioaugmentation at more than 50 sites in the USA and Europe.

Figure 11. Distribution of observed and modeled chlorinated ethenes and ethene concentration after bioaugmentation in a field study using KB-1^{TM} at Kelly Air Force Base, Texas, USA. A, B, C, D, demonstratd 72, 93, 115, and 142 days after bioaugmentation, respectively. The predicted concentrations were determined using the sequential first-order decay and production equations. Permission granted, Major et al. (2002).
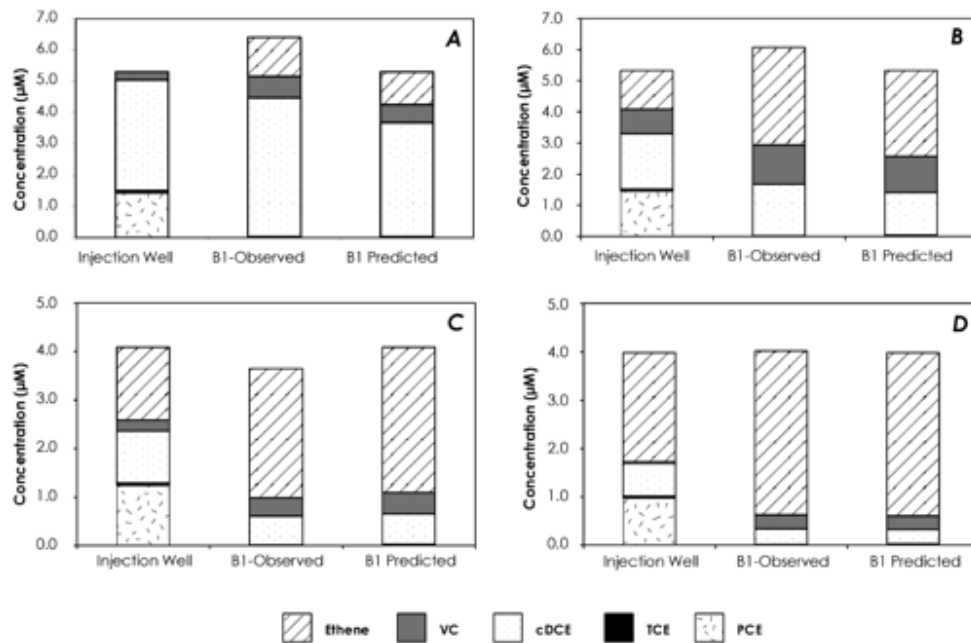
6. CHALLENGES OF COUPLING THERMAL TREATMENT WITH REDUCTIVE DECHLORINATION

Certain aggressive source-zone technologies are suggested to be more promising for stimulation of reductive dechlorination as a polishing step after source-zone treatment. Although steam injection or hot water injection is likely to generate aerobic environments immediately after treatment (Kaslusky and Udell, 2002), ERH is expected to create anaerobic conditions. Thermal treatment is also expected to influence the microbial community, but long-term monitoring provides evidence that microbial activity may rebound after field-scale steam treatment (**IV**, Friis et al., 2005c; Richardson et al., 2002; Smith et al., 2000). Potential advantages of coupling thermal treatment with bioremediation include:

- o Elevated temperatures after thermal treatment can stimulate metabolic activity (V, Friis et al., 2005e)
- o The DNAPL is removed by thermal treatment, reducing the total mass. This eases access for dechlorinating bacteria to contaminant after thermal treatment
- o Possibility of re-cycling equipment used for thermal treatment

However, before the technology is applied, certain challenges must be overcome, such as distribution of electron donors and dechlorinating microorganisms, especially in low permeable layers, which can be remediated using ERH or TCH. Until now, research in this area has been sparse, and knowledge about the physical, chemical and biological effects of thermal treatment on the subsurface environment is needed to determine the potential for post-thermal bioremediation.

6.1. Effect of heat on microorganisms

The effect of heat on microorganisms was investigated to predict and understand the implications of applying thermal treatment to subsurface microbial communities. As early as the 18th century, Pasteur started heated wine to 55°C for several minutes to kill the microorganisms that destroyed the wine. This initiated extensive research to determine how heat could destroy or immobilize microorganisms harmful to food without destroying the food itself. Today, heat is one of the safest and most reliable methods of food preservation (Pelczar et al., 1993). The technique is based on knowledge about heat resistance of various species indicating which conditions are needed to destroy harmful microorganisms. Especially spore-forming microorganisms can withstand elevated temperatures and pressures and are therefore likely to cause food spoilage. Extensive research has therefore focused on thermal death times of these microorganisms (Pelczar et al., 1993).

6.1.1. Biomolecular stability at elevated temperatures

The effect of heat on microorganisms can be regarded as the effect on intracellular molecules and on the major classes of macromolecules. Surprisingly, certain small molecules are unstable in vitro at the same temperature conditions as those prevailing when they are found in growing hyperthermophiles (Daniel and Cowan, 2000). This appears to be caused by a range of mechanisms, including rapid turnover, metabolic channeling and local stabilization inside the cells (i.e. in vivo, Daniel and Cowan, 2000).

Proteins have been demonstrated to be stable at temperatures in vivo which are substantially higher than those held in vitro. Protein structural analysis has indicated that conformational stability may not be the limiting factor for growth because proteins possess a high level of thermostability exceeding 130°C (Adams, 1993). Stability of DNA can be achieved at elevated salt concentrations, polyamined, cationic proteins and supercoiling rather than manipulation of C-G ratios (Daniel and Cowan, 2000). The stability and presence of RNA are widely debated (Cowan, 2004). On the one hand, it is suggested that thermophiles represent the origin of life (Di Giulio, 2003), despite the fact that organisms capable of growing at 110°C do not contain functional RNA (Daniel and Cowan, 2000), and that only minor modification would have been necessary to stabilize RNA in vivo (Kowalak et al., 1994). On the other hand, the taxonomic tree constructed from RNA is based on the idea that the original ancestors contained RNA.

Lipids, constituting the major fraction of monolayer membranes, are chemically very stable as diether-linked and seem potentially capable of maintaining membrane integrity at elevated temperatures (Daniel and Cowan, 2000). The stability at elevated temperatures may not apply to other cell constituents, such as coenzymes (e.g. NAD/P(H)) and low molecular weight metabolites (e.g. AMP, ADP, ATP, acetyl CoA, acetyl phosphate and carbamoyl phosphate) (Cowan, 2004). It has therefore been suggested that small molecules will dictate the upper temperature limit for life.

It is not clear what the upper temperature for life is (**Figure 12**), or which factors will set this limit. However, the search for finding the upper temperature for life has evolved within the last 60 years and the maximum temperature has increased with research in this area.
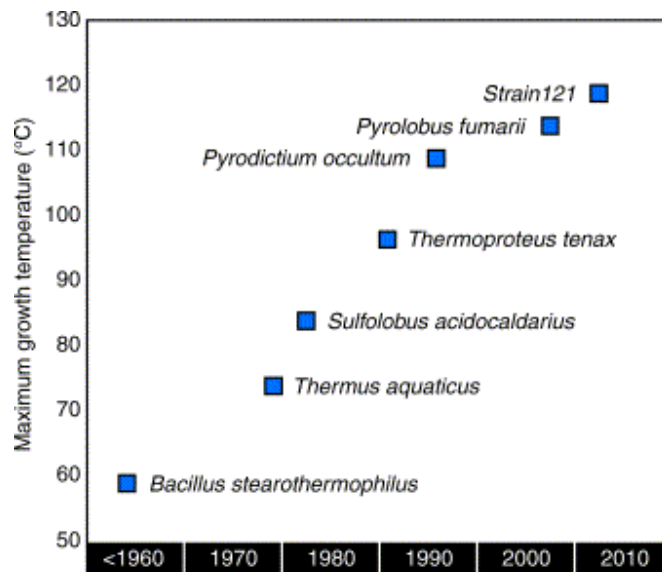
Figure 12. The rise of the upper temperature limit for life. Modified from Cowan (2004). Data points indicate when new organisms were reported that significantly extended the upper limit.

6.1.2. Microbial enhanced oil recovery (MEOR)

The petroleum industry uses microorganisms to degrade subsurface contaminants at elevated temperatures by microbial enhanced oil recovery (MEOR) (Donaldson et al., 1989; Donaldson, 1991). This technology combines microbial degradation with steam injection to enhance oil recovery from subsurface wells. Bacteria are injected into the subsurface to produce CO_2 and/or CH_4 by fermentation and neutralization of acid products, thus lowering pH and decreasing the viscosity of residual oil (Andersen, 2003; Donaldson et al., 1989). Especially the decreased viscosity can increase oil recovery. Despite the decreased viscosity, concerns remain that injected microorganisms may not substantially increase the recovery compared to the risk of microbial degradation in the oil reservoir (Donaldson et al., 1989).

Nevertheless, Donaldson et al. (1989) revealed interesting results from microorganisms surviving conditions at a depth of 2000 m, temperatures around 60°C and fluid pressures of 20 MPa. Microorganisms changed from rod-shaped to spherical and adapted to high salinity, pressure, temperature and absence of essential nutrients (Donaldson et al., 1989). Correspondingly, rod and cocci-shaped anaerobic bacteria were observed in oil field brine. These bacteria were both Gram-positive and Gram-negative (Bhupathiraju et al., 1991). From these studies, survival of bacteria in extreme environments could again be confirmed (Margesin and Schinner, 2001). In these environments, rod/cocci-shaped bacteria that were both Gram-positive and Gram-negative were detected.

6.1.3. Increased metabolic dechlorination rate at elevated temperatures

The temperature effect on reductive dechlorination was investigated in cultures with KB-1[TM] from 4°C to 60°C (**Figure 13**, **V**, Friis et al., 2005e). This demonstrated that the temperature optima for TCE reduction to *c*DCE was around 30°C, whereas dechlorination rates of *c*DCE and VC to ethene were optimal between 15 and 30°C. The increased metabolic rate at temperatures of 15°C to 30°C can be an advantage for bioaugmentation at these temperatures.
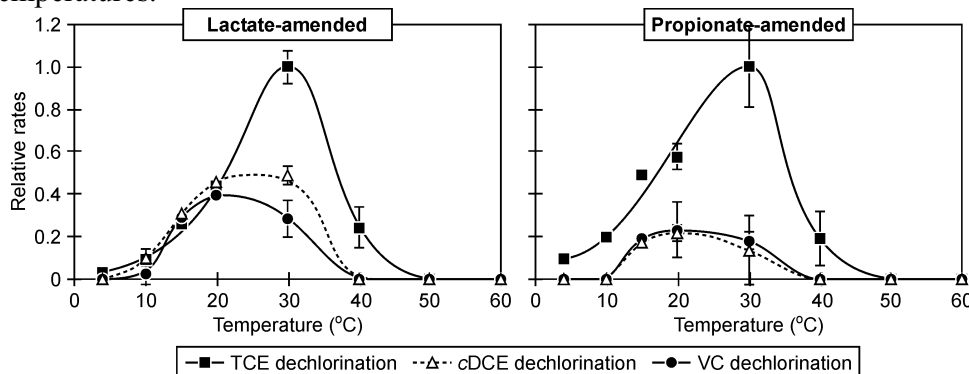

Figure 13. Relative dechlorination rates estimated using zero order degradation as a function of temperature Modified from Friis et al. (**V**, 2005e). All rates were normalized with respect to the highest rate (i.e., TCE dechlorination at 30°C in lactate and propionate-amended microcosms). Bars indicate $\pm$ one standard deviation of rates from triplicate batches at 10, 20, 30, and 40°C.

6.2. Microbial survival and rebound after thermal treatment

In soils, a large diversity and quantity of microorganisms can be detected. According to estimates, 4×10^6 to 5×10^8 bacteria, 6×10^3 to 1×10^6 fungi, 1×10^3 to 1×10^5 algae, and 1×10^3 to 5×10^5 protozoa can be found in only 1 g of soil (Pelczar et al., 1993). Studies estimating the effect of heating on specific microorganisms can therefore not be directly converted to the effects likely to be seen in soils. Moreover, this is complicated by the effect of sediment particles, which may protect microorganisms from various physical effects. This section discusses studies that investigated the effect of heating on microbial activity in sediment samples. Unfortunately, the data are sparse in most studies and cannot be obtained from peer-reviewed publications.

As demonstrated in a study (Krauter et al., 1995), several bacterial strains survived two years of dynamic underground stripping at the Gasoline Pad Site, USA. The survivors were of the Archaea and Bacteria domain (Richardson et al., 2002). They were found to be mainly thermophilic spore-forming gram-positive bacteria and yeast, where the bacteria possessed a higher degree of saturated fatty acids in their lipid membranes compared to the indigenous population (Krauter et al., 1995). In a demonstration site at the Savannah River Site, with radio frequency heating, the survivors were of the bacteria domain and no fungi

were present (Eddy-Dilek et al., 1993). This survival of microbes indicates that a potential for microbial degradation remained after thermally enhanced remediation.

A decrease in both microbial activity (Balshaw-Biddle et al., 2000) and the total number of microbes after steam injection was observed in the top soil (Bender et al., 2004) and in the subsurface (Dablow et al., 1995; Krauter et al., 1995; Richardson et al., 2002). Overall, the effect of heating demonstrated that the activity and/or number of microorganisms decreased in four studies and increased in three studies, and that the effect varied with incubation time, contaminant and depth of soil samples (Table 6). These results were inconsistent and could neither be correlated with specific analytical methods, nor with experimental setup as to whether the results were obtained from laboratory or field studies. However, other factors as rapid cooling may also have influenced these results as described in Richardson et al. (2002). This demonstrates that no general conclusions can be made from microbial activity after thermal treatment.

Table 7. The effect of heating sediment on microbial activity and number of microorganisms. Colony forming units (CFU) are heterotrophic plate counts unless otherwise stated. Most probable number (MPN) and CFU detect the culturable and viable part of a microbial community, whereas direct epiflourescent microscopy (DEM) and microcopic counts using acridine orange counts (AODC) detect the total number of microorganisms. SI indicates pilot or full-scale steam injection, whereas ERH indicates electrical resistance heating.

Activity after heating	Analytical method	Comments	Reference
Decreased	CFU and MPN after autoclaving (121°C, 15 psi, 45min)		Hendrix et al., 1997
Decreased	CFU and AODC 4 weeks after SI	Higher decrease in activity close to injection wells	Krauter et al., 1995
Decreased	CFU and CO_2 production after SI		Balshaw-Biddle et al., 2000
Decreased	CFU and CO_2 production after SI	Large pre and post-SI populations after one week	Dablow et al., 1995
Increased	DEM 6 weeks after SI		Richardson et al., 2002
Increased	AODC immediately and 1 month after ERH	AODC may not relate to activity	Eddy-Dilek et al., 1993
Increased	CFU for petrophilic microorganisms 30 and 90 days after SPH		Dettmer, 2002.
Decreased/ unchanged	DEM after steaming soil columns	Activity reached pre-steamed level within 30 days	Richardson et al., 2002
Decreased / increased	CFU after heating sand in open buckets to 25-110°C	Decrease with diesel and increase with PAHs	Huesemann et al., 2002
Decreased / increased	CFU and MPN 6-8 months after SI and SPH	Decrease below 15 mbs and increase above 10 mbs	Yoon, 2003
Unchanged	CFU app. 8 to 17 months after SI	Top soils collected app. 0.4 mbs., max reached 53°C	Bender et al., 2004

The effect of heating on microbial diversity is rarely documented, as described in a study by Friis et al. (**IV**, 2005c). Bender et al. (2004) demonstrated that the microbial community was changed in top soils overlying a steam injection heated to a maximum of 53°C. In contrast, Keeley et al. (2004) demonstrated that the microbial community (estimated using PLFA analyses) was significantly similar before and after steam injection and ERH. These two studies corresponded with the results obtained in a Friis et al. (**IV**, 2005c) study, where microcosms 300 days after full-scale ERH and after 400 days of incubation in laboratory-heated microcosms were similar to unheated microcosms.

The microorganisms that survived treatment were capable of degrading various hydrocarbons (Balshaw-Biddle et al., 2000; Bender et al., 2004). Post-thermal communities were demonstrated to degrade contaminants such as gasoline (Krauter et al., 1995), phenanthrene (Richardson et al., 2002), heavy PAHs and diesel heavier than C-21 (Huesemann et al., 2002). The capability of naturally degrading chlorinated solvents was explored in a study by Friis et al., (**II**, 2005d), where a post-ERH community dechlorinated TCE to cDCE. Recent investigations demonstrate that dechlorination is only partial to cDCE when bioaugmented with KB-1^{TM} at 40°C, even after cooling to 10°C. This indicates that microorganisms capable of securing complete dechlorination to ethene are unable to survive at elevated temperatures around 40°C and suggests that they are unable to survive at the higher temperatures reached during thermal treatment.

Table 8. Contaminant degradation by survivors after thermal treatment. PAH stands for polyaromatic hydrocarbons. The PAHs in Huesemann et al., (2002) were naphthalene, phenantrene, anthracene, pyrene, chrysene and perylene.

Degradation by survivors	Analytical method	Comments	Reference
Phenanthrene	Enrichment cultures after steaming soil in columns	Lag phase of more than 15 days	Richardson et al., 2002.
Phenanthrene	Enrichment cultures 6 months after SI	Lag phase of 6 months	Richardson et al., 2002.
Heavier PAHs and diesel heavier than C-21	Heating sand in open buckets to 25-110°C	Degradation rate increased at 50 and 70°C	Huesemann et al., 2002
Gasoline	Enrichment culture 4 weeks after pilot scale SI		Krauter et al., 1995
TCE to cDCE	Microcosm studies 6 months after ERH		**I**, Friis et al., 2005b

For remediation purposes, an increased microbial activity was observed upon addition of various nutrients, (Dablow et al., 1995). At another application where nutrients were added to the subsurface in Skokie, IL, USA, approximately 10,400 kg of TCE and TCA (representing 36% of the total mass removed) were estimated to have been biodegraded by reductive dechlorination after electrical heat application (Dettmer, 2002). This stimulating process was patented by (Aiken et al., 2005), where naturally occurring hydrocarbon-

degrading microbes are enhanced using nutrients after steam injection. In another patented *in-situ* thermal enhanced remediation strategy (Taylor et al., 1998) aerobic and anaerobic thermophiles are used after a dynamic underground stripping for biodegradation as a polishing technique.

In conclusion, heating can drastically change the diversity of subsurface populations. The effect of thermal treatment on microbial activity varies and may be related to various lengths of cooling as well as repopulation at field sites. However, the survival of some microorganisms indicates that the potential for microbial degradation of contaminants is not eliminated by thermal treatment, and furthermore that degradability can be enhanced by adding nutrients and/or specific degraders.

6.3. Biogeochemical conditions after thermal treatment

Characterization of post-thermal redox conditions is essential when it comes to evaluating the potential for reductive dechlorination because each step in this process occurs under specific redox conditions (section 3.3.). Knowledge about the effect of heating on the abundance and character of electron acceptors (Fe, Mn, SO_4^{2-}) and electron donors (organic matter) in the subsurface remains limited. The redox conditions will in turn also depend on the applied thermal technology. After steam injection or hot water injection, it is likely that aerobic conditions will be created immediately after treatment, given that air is injected during treatment for DNAPL mobility control (Kaslusky and Udell, 2002). On the other hand, conditions after ERH are expected to create anaerobic conditions (Smith et al., 2000) stimulating reductive dechlorination. This is also supported by an expected release of H_2 from the electrodes (McNab et al., 2000) and stripping of gasses from the subsurface (Eddy-Dilek et al., 1993). Any thermal treatment can potentially release dissolved organic carbon (DOC) during heating (**I**, Friis et al., 2005b).

Redox conditions after thermal treatment not only depend on the thermal technique, but also on the survival of the microbial communities as well as the presence of electron donors and electron acceptors. At a Danish site, the redox conditions were aerobic before and during steam injection and became more reduced after treatment (Larsen et al., 2004). Correspondingly, non-volatile organic carbon (NVOC) concentrations increased from 6 mg/L to 38 mg/L during heating (Larsen, 2003). This may stem from consumption of released electron donors.

Investigating the effect of heating on biogeochemical conditions also formed part of this thesis (**I**, Friis et al., 2005b; **II**, Friis et al., 2005f). In this research, redox conditions, dissolved organic carbon, pH and alkalinity were compared in microcosms exposed to three treatments. Microcosms held sediment that was unheated, laboratory-heated (heated in closed microcosms in incubators to 100°C for 10 days resembling thermal treatment) or

field-heated (sediment samples were obtained after full-scale heating when the site had cooled to approximately 40°C). This research generally demonstrated that there was little effect on redox activity in unamended microcosms, and that the majority of microcosms remained Fe/Mn-reducing (**Figure 14**).
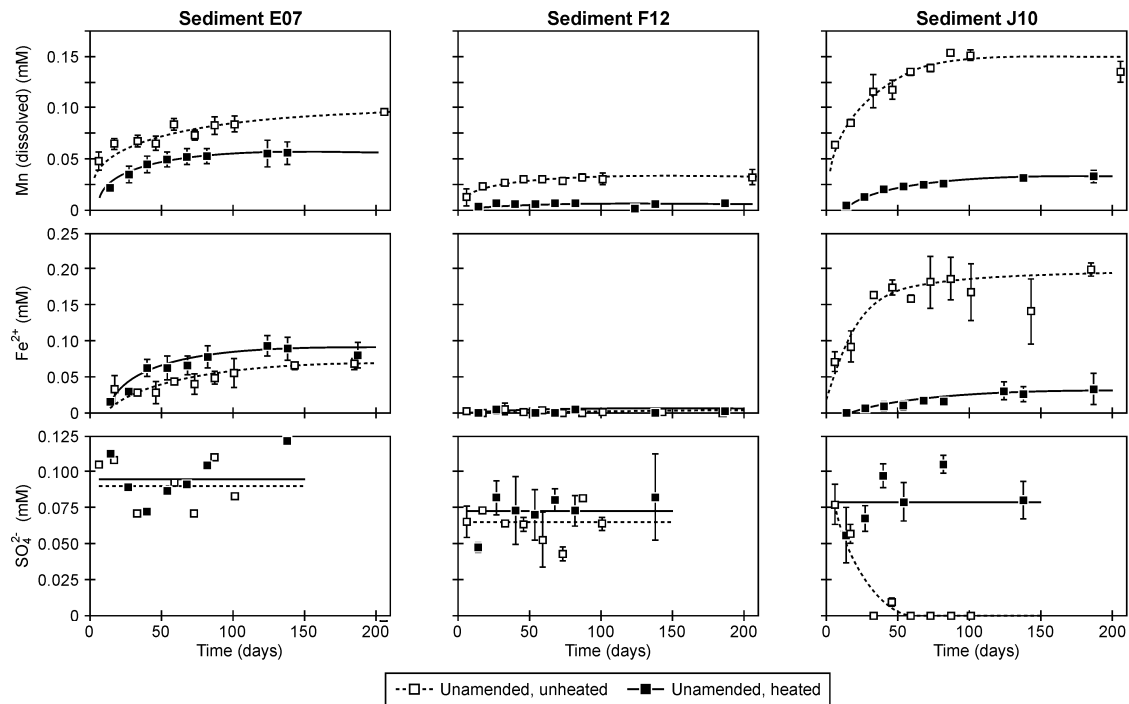

Figure 14. Aqueous concentration of redox-sensitive parameters in unamended unheated, or heated microcosms. All of the data points represent average values from triplicate bioassays by including standard deviations. If no error bars are shown, then the standard deviations were too small to be illustrated, except for SO_4^{2-}, where only one assay is shown. Modified from Friis et al. (**I**, 2005b).

However, in lactate-amended microcosms, a clear effect was observed on the redox activity in the following order: laboratory-heated microcosms < unheated microcosms < microcosms with field-heated sediment (**VII**, Friis et al., 2005f). The concentrations of dissolved organic carbon (DOC) were in the opposite order: microcosms with field-heated sediments < unheated microcosms < laboratory-heated microcosms. This suggests that microorganisms were present in field-heated sediments or not destroyed by ERH. Microorganisms could have been transported into the site with groundwater. DOC, on the other hand, was either not released in field-heated sediments or transported downstream prior to sampling. These effects are conceptualized in **Figure 15**.
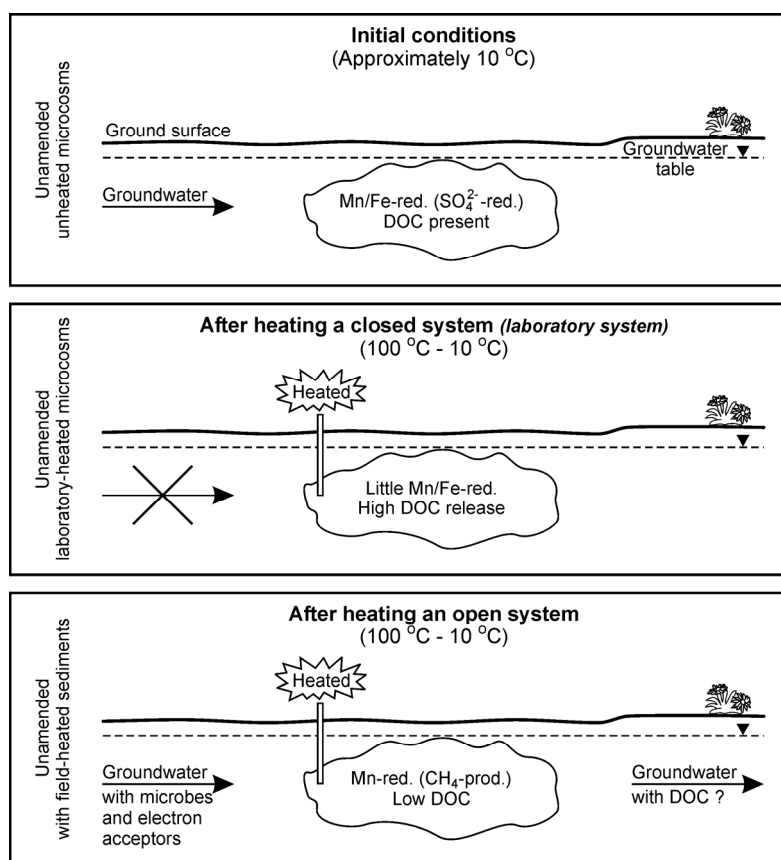
Figure 15. Conceptual model of the effect of thermal treatment on redox activity. Modified from Friis et al. (**VII**, 2005f). Redox activity was present prior to heating, but was limited by heating in closed microcosms. However, in an open system, groundwater can carry microorganisms capable of reducing Mn/Fe and SO_4^2 and producing CH_4. Processes indicate reactions in unamended microcosms, while those in brackets indicate reactions in lactate-amended microcosms, all after 200 days of incubation.

6.4. Inhibited or stalled dechlorination after thermal treatment

Previous studies demonstrated that some microorganisms are able to survive thermal treatment and capable of degrading various contaminants (Dablow et al., 1995; Krauter et al., 1995; Richardson et al., 2002). However, the potential for chlorinated ethenes has not yet been investigated (Dettmer, 2002). Correspondingly, little is known about the effect of remediation technologies on indigenous dechlorinators. Nonetheless, it is known that *Chlostridium bifermentas* (Chang et al., 2000) and *Desulfitobacterium metallireducens* (Finneran et al., 2002), dechlorinating PCE and TCE to *c*DCE, can form spores and thus withstand high temperatures and pressures.

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Investigating the effect of thermal treatment on dechlorination also formed part of this PhD thesis (**II**, Friis et al., 2005f; **VII**, Friis et al., 2005d). Unheated microcosms with groundwater and aquifer materials demonstrated that dechlorination could proceed to *c*DCE in unamended sediments and to ethene in lactate-amended sediments (**II**, Friis et al., 2005d). 16S DNA detection by nested PCR confirmed the presence of dechlorinators including *Dehalococcoides.* In comparison, no dechlorination of TCE was observed in unamended and lactate-amended microcosms heated in closed microcosms to 100°C for 10 days after 200 days of incubation. The decrease in the presence of dechlorinators upon heating was confirmed by nested PCR (**II**, Friis et al., 2005d). However, within 200 to 400 days of incubation, TCE was dechlorinated to *c*DCE in two out of six replicas. These microcosm studies suggest that dechlorinating activity is inhibited or postponed and that the presence of dechlorinators decreases after heating in closed microcosms.
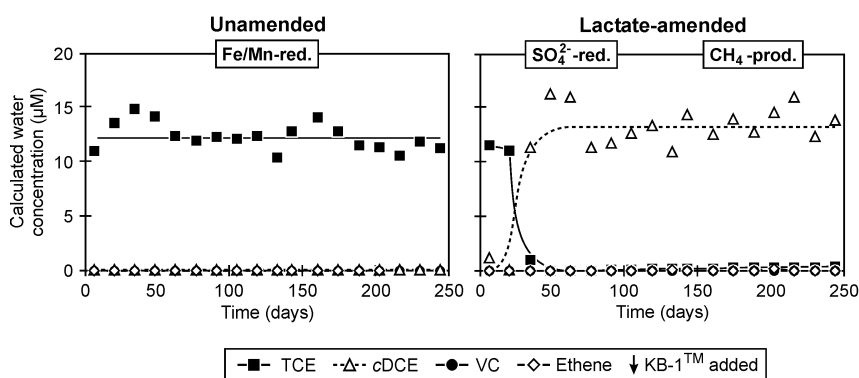

Figure 16. Fate of chlorinated organics in microcosms with field-heated sediments. Modified from Friis et al. (**VII**, 2005f).

These conditions were compared to dechlorination in sediments collected after full-scale thermal treatment (**VII**, Friis et al., 2005f). In these microcosms, TCE was dechlorinated to *c*DCE within 200 days of incubation upon lactate-amendment (**Figure 17**). This suggested that microorganisms capable of dechlorinating TCE to *c*DCE were transported into the heated site during cooling (**Figure 17**). The dechlorination stalled at *c*DCE, corresponding to the larger diversity of microorganisms capable of completing this dechlorination step compared with the steps for complete dechlorination to ethene.
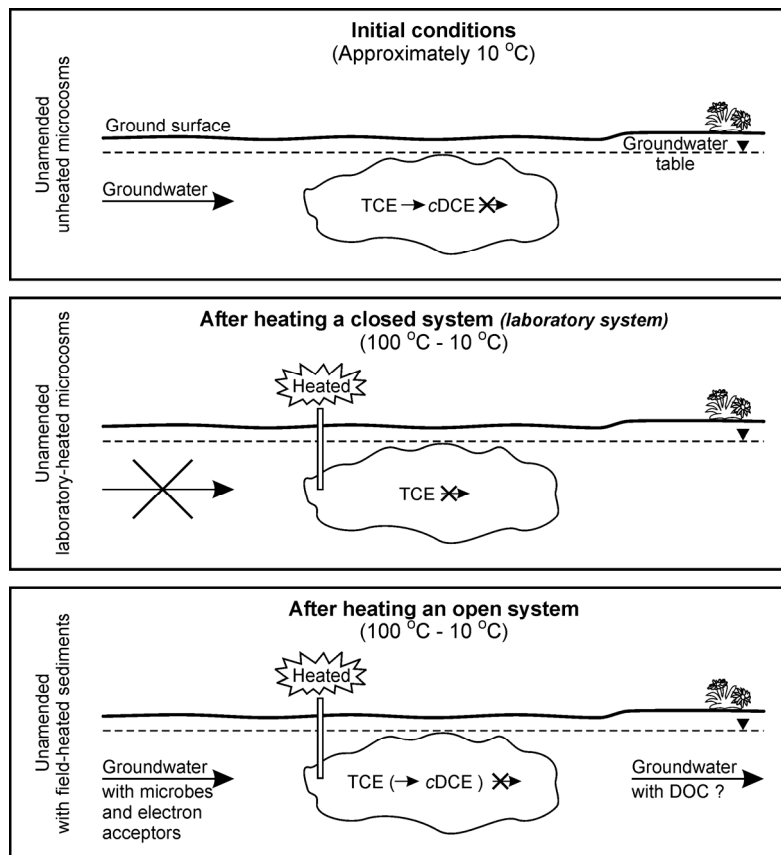
Figure 17. Conceptual model of the effect of thermal treatment on dechlorination. Modified from Friis et al. (**VII**, 2005f). TCE was dechlorinated past *c*DCE in unheated microcosms and at the field site. Although TCE was not dechlorinated after laboratory-heating, TCE was dechlorinated to *c*DCE in sediments collected after ERH. This suggested that microorganisms with bacteria capable of dechlorinating TCE to *c*DCE were transported into the site during cooling. Processes indicate reactions in unamended microcosms, while those in brackets indicate reactions in lactate-amended microcosms when these behaved differently, all after 200 days of incubation.

6.5. Coupling bioaugmentation with thermal treatments

Concerns are that microbial activity and potential for complete dechlorination to ethene can decrease or be postponed after thermal treatment (**II**, Friis et al., 2005f; **VII**, Friis et al., 2005d). However, after thermal treatment, the redox conditions, pH and alkalinity were demonstrated to remain within normal aquifer levels (I, Friis et al., 2005b; VII, Friis et al., 2005f), and bioaugmentation may therefore be feasible to obtain complete dechlorination to ethene (**Figure 198)**. The potential for this technology was demonstrated by successful dechlorination to ethene in microcosms with field-heated sediments (**Figure 19**, **VII**, Friis et al., 2005f) and laboratory-heated microcosms with sediment from two out of three locations (**III**, Friis et al., 2005a).
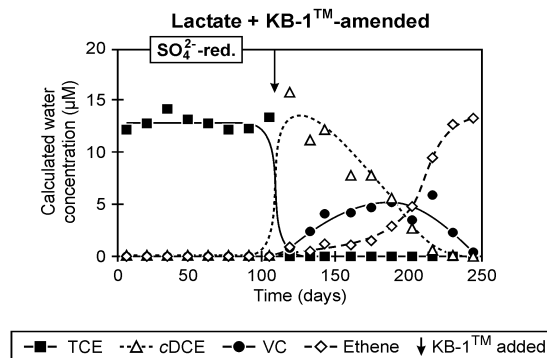
Figure 18. Dechlorination upon bioaugmentation in field-heated sediments. Modified from Friis et al. (**VII**, 2005f).
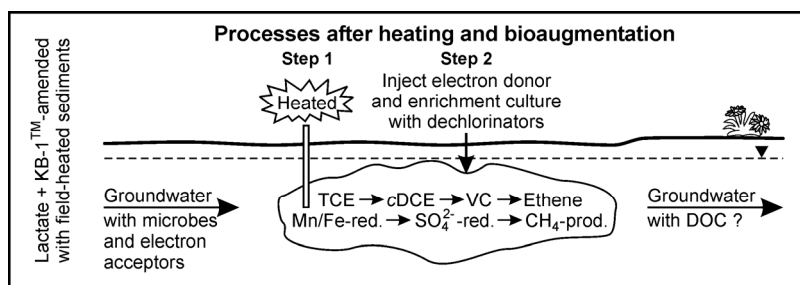

Figure 19. Coupling bioaugmentation with thermal treatments. Modified from Friis et al. (**VII**, 2005f).

Synergies between bioaugmentation and thermal treatment technologies include:
- o Increased temperatures after heating can increase metabolic activity of reductive dechlorination and stimulate biological activity (V, Friis et al., 2005e; VI, Friis et al., 2005g).
- o Redox conditions after thermal treatment were similar to those observed prior to heating, and organic matter was released during heating in closed microcosms (I, Friis et al., 2005b; VII, Friis et al., 2005f). The organic matter released may, however, be transported downstream the treated zone.
- o The concentration of contaminant will decrease, thus limiting the potential for exceeding high toxic concentrations (Krauter et al., 1995).

On the other hand, efforts must be made to overcome the challenges related to the distribution of electron donors and microorganisms, maintenance of dechlorinating microorganisms, and potential regulatory objections against injecting microorganisms into the subsurface. Furthermore, additional research will be beneficial before the technology is applied.

7. APPLICATION OF THERMAL TREATMENT AND BIOAUGMENTATION

The combination of thermal treatment technologies and bioaugmentation provides an opportunity to capitalize on synergies between treatments using one or more of the following approaches (described in **VI**, Friis et al., 2005g):

- o Thermal treatment of hot spots surrounded by a bioremediation zone and plume treatment. Temperatures in the perimeter zone can be elevated by thermal treatment and thereby increase metabolic activity. Minimization of the thermally treated zone reduces overall cost while ensuring compliance as shown in **Figure 20, A**.
- o Sequential thermal and biological treatment for sites with stringent goals and recalcitrant contaminants (**Figure 20, B + C**). This can also be used at sites where thermal remediation is unsuccessful, e.g. due to inhomogeneous heating.
- o Combined solutions where biological treatment is used both in a perimeter zone and inside hot spots during cooling as shown in **Figure 20, A + C**. Some the thermal treatment wells may be used for electron donor circulation and bioaugmentation.
- o Plume removal by bioaugmentation downstream a thermal treatment (not shown). The conditions downstream a thermally treated area can hold elevated temperatures and elevated concentrations of organic carbon (as previously discussed). These conditions may increase dechlorination rates and minimize added electron donor addition after bioaugmentation.

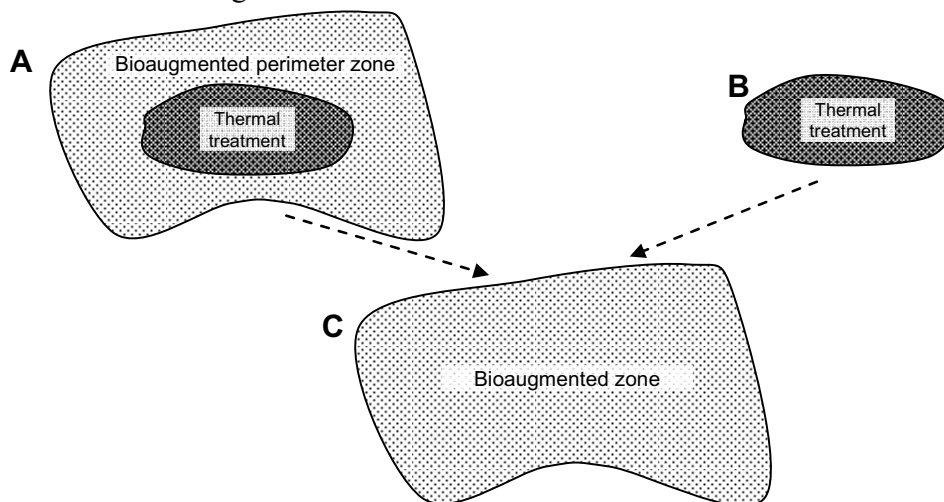**Figure 20. Conceptual model of combinations of bioaugmentation with thermal treatment demonstrated as plan view**. A: Thermal treatment of hot spot surrounded by bioremediation zone and plume treatment (i.e. concomitant bioaugmentation and thermal treatment). B + C: Sequential thermal and biological remediation; or A + C; combined solution where bioaugmentation is applied in the perimeter zone and inside hot spot during cooling.

Friis et al. (**VI**, 2005g) observed the effects of bioaugmentation on TCE dechlorination in sediments collected after full-scale thermal treatment. Bioaugmentation was performed at 40°C, 30°C, 20°C, and 10°C during the cooling process. This research demonstrated that dechlorination of TCE to *c*DCE occurred in lactate-amended microcosms at temperatures around 40°C. Lactate-amendment may therefore be performed at elevated temperatures, allowing time for mixing with native groundwater, establishment of anaerobic conditions, and dechlorination of TCE to *c*DCE prior to bioaugmentation (**Figure 21**). Bioaugmentation is most favorably started in areas of the site that are cooled to approximately 30°C (**Figure 21**).
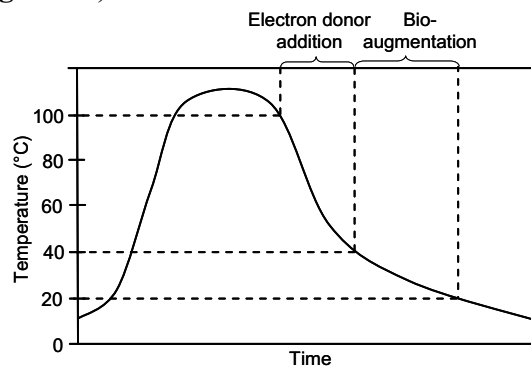
Figure 21. Conceptual figure of optimal post-thermal bioaugmentation. Electron donor (lactate) amendment can be performed at temperatures above 40°C, allowing time for mixing with native groundwater, establishment of anaerobic conditions and e.g. dechlorination of TCE to *c*DCE. Bioaugmentation is most favorably begun in areas where the site cooled to below 40°C at approximately 30°C to gain advantage of the increased metabolic rate at elevated temperatures and to prevent microorganisms from being disabled as a result of too high temperatures.

The results in Friis et al. (**VI**, 2005g) indicate that dechlorination of TCE can proceed to ethene when these approaches are successfully applied and the challenges of distributing and maintaining dechlorinating microorganisms are overcome.

8. CONCLUSION

Chlorinated ethenes like PCE and TCE were useful to industry because of their physico-chemical properties, including rapid evaporation rates, low flammability and reactivity and their ability to quickly and efficiently dissolve organic substrates. PCE is most commonly known for its use in the dry-cleaning industry, whereas TCE was previously used as a degreasing solvent. Due to their widespread use and physico-chemical properties, chlorinated ethenes are commonly found groundwater contaminants present as a free-phase, i.e. DNAPLs.

DNAPLs are difficult to remediate due to their complex and unpredictable spreading pattern in the subsurface. The large extent of groundwater contamination with these compounds has led to use of various innovative remediation technologies. Especially, thermally enhanced extraction has proven effective in remediating heavily contaminated sites although this is not always enough to reach regulatory goals.

Another commonly used remediation technology is biodegradation, where chlorinated ethenes are degraded anaerobically through a process termed reductive dechlorination. Each step in the process occurs under specific redox conditions and is carried out by different microorganisms. The first steps from PCE/TCE to *c*DCE can be carried out by a variety of organisms, whereas *c*DCE to ethene has been documented only in the presence of *Dehalococcoides.*

Bioremediation has proven successful when electron donors and a mixed consortia with dechlorinating microorganisms are injected into the subsurface, i.e., by bioaugmentation. Bioaugmentation has been suggested as a polishing technology after thermal treatment to capitalize on synergies between the individual treatment processes. Elevated temperatures after thermal treatment can increase the metabolic activity of reductive dechlorination. However, little is known about post-thermal environmental conditions such as redox sensitive parameters, pH, alkalinities, presence of organic matter etc.

This thesis focused on the potential for bioaugmentation of TCE after full-scale ERH. This was evaluated by determining the effects of thermal treatment on (1) environmental conditions, (2) survival of dechlorinating microorganisms, and (3) the potential for bioaugmentation to enhance reductive dechlorination.

The specific findings from the thesis include:
  o The environmental conditions in field-heated sediments suggested lower redox activity, similar/lower DOC concentrations and similar pH and alkalinities after full-scale ERH compared to the situation before treatment. Microorganisms from

field-heated sediments were capable of reducing Fe/Mn and SO_4^{2-} and producing CH_4 although this activity was demonstrated only upon electron donor addition.

o The degree of dechlorination remained the same (three treatments out of six) or decreased (three treatments out of six) after full-scale ERH in unamended and lactate-amended microcosms compared to dechlorination in sediments obtained before ERH. This demonstrated that microorganisms capable of dechlorinating TCE to *c*DCE were present in field-heated sediments although the diversity of dechlorinating microorganisms decreased after heating in closed systems. In general, the genetic diversity decreased after heating in closed systems, but catabolic and functional diversity reoccurred within 400 days of incubation in closed microcosms and within 300 days after full-scale thermal treatment.

o Bioaugmentation resulted in complete dechlorination to ethene after full-scale ERH demonstrated by addition of lactate and the anaerobic dechlorinating culture, KB-1™, to field-heated sediments. Bioaugmentation rates can be optimized during cooling at temperatures around 30°C.

From these observations, different processes associated with thermal remediation were suggested. First of all, dissolved organic matter (DOC) was either not released during full-scale ERH or released and transported downstream prior to sampling. Secondly, microorganisms capable of dechlorinating TCE to *c*DCE, reducing Fe/Mn and producing SO_4^{2-} as well as methanogenesis were either not destroyed by heating *in situ* or transported into the site with groundwater.

These findings suggest that there is a potential for combining bioaugmentation with ERH to obtain complete reduction of TCE to ethene. The combination can either be applied by using the technologies concomitantly, where the source zone is thermally treated and the perimeter is bioaugmented, or sequentially, where thermal treatment first is used to remove the bulk of the contaminants and bioaugmentation then is applied as a polishing technology. However, in order for applications to be successful, efforts must be made to overcome challenges related to the distribution of electron donors as well as the distribution and maintenance of dechlorination microorganisms.

9. PERSPECTIVES

Aggressive thermal treatment can be combined with bioaugmentation either to decrease costs and resources used for remediation at a specific site, or to increase confidence in meeting strict regulatory goals. For example, the costs of thermal treatment decrease by concomitant thermal treatment of the source zone and bioaugmentation in the perimeter zone. The large costs of a remediation project can be broken down into design, construction, operation, demobilization and reporting (Heron et al., 2005a). In Denmark, costs for operation will be higher because of the higher energy prices (Friis and Jensen, 2004). If the thermal source zone is reduced by bioaugmentation in the perimeter zone, the costs of construction and operation can be limited, whereas the costs of design and reporting remain unchanged (**Figure 22**). However, the applicability of this technology and the associated costs will depend on the specific site as well as the contaminant.
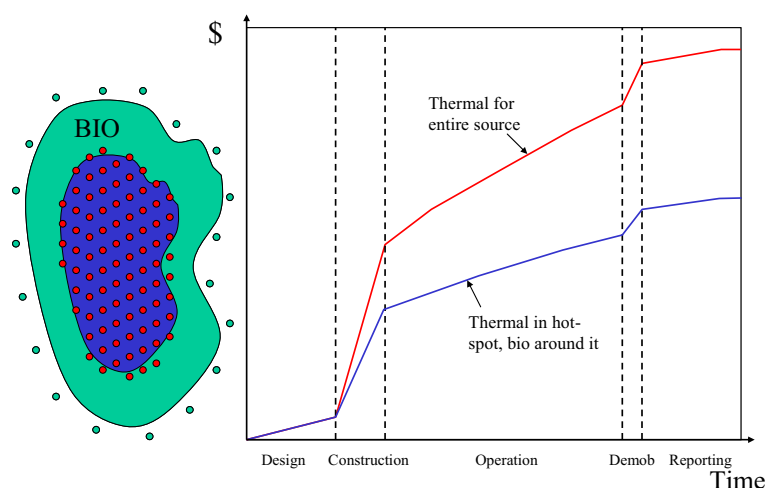
Figure 22 Conceptual decrease in costs from combining bioaugmentation with thermal treatment of source zone in USA. Modified from Heron et al. (2005a).

This thesis focused on the use of ERH followed by bioaugmentation of TCE-polluted sites, although other combinations of thermal technologies as well as contaminants can also be applied. In general, it is believed that reduced conditions are obtained after ERH, whereas other thermal techniques as steam injection or hot water injection can result in aerobic conditions (**Figure 23**). During TCH, conditions can be manipulated to be more oxidizing (by the injection of air) or more reducing (by allowing boiling to remove oxygen and the formation of acetone – a readily available electron donor used to react with electron acceptors).

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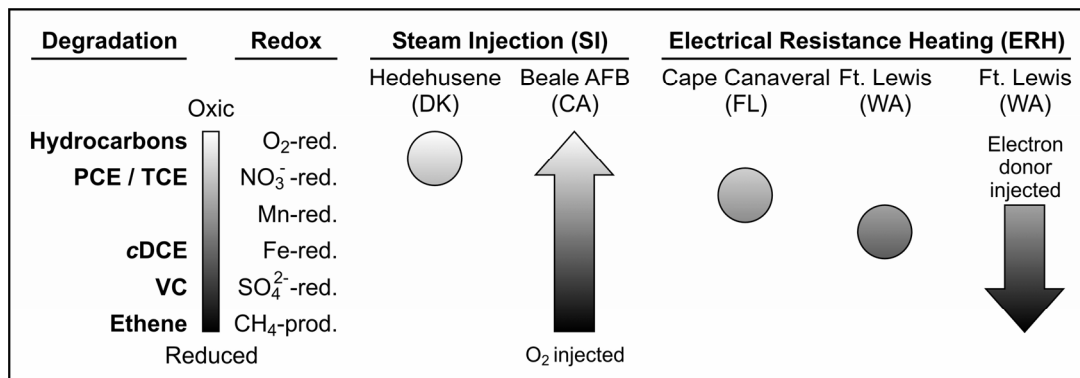**Figure 23. Redox conditions after thermal treatment at field sites.** The redox conditions after steam injection were oxic prior to heating and reduced NO_3^-/Mn after treatment (Larsen, 2003). A study demonstrated that oxic conditions were obtained when O_2 was injected (Heron, 2005). These are favorable for degradation of hydrocarbons. Conditions after ERH remained slightly anaerobic. However, when electron donors were injected, conditions became methanogenic and favorable for complete dechlorination of PCE/TCE to ethene. Contaminant degradation demonstrates general redox zonation during dechlorination, as previously discussed (section 2.4).

In the event of aerobic conditions after thermal treatment, aerobic biodegradation processes of oil and BTEX, for example, could be stimulated. A field study demonstrated that redox conditions could be manipulated and changed from possibly methanogenic to aerobic upon injection of air (Carroll et al., 2004). In addition, existing evidence suggests that microorganisms which survived thermal treatment were capable of degrading contaminants as gasoline (Krauter et al., 1995), phenanthrene (Richardson et al., 2002), heavy PAHs and diesel heavier than C-21 (Huesemann et al., 2002). The results from the current thesis suggest that microorganisms and electron acceptors may be transported into the heated site with groundwater, thus possibly increasing the potential for presence of ubiquitous contaminant-degrading microorganisms after thermal treatment. However, further research will be needed in this area, including pilot and field-scale demonstrations, to establish that other challenges such as transport of bacteria will also be overcome.
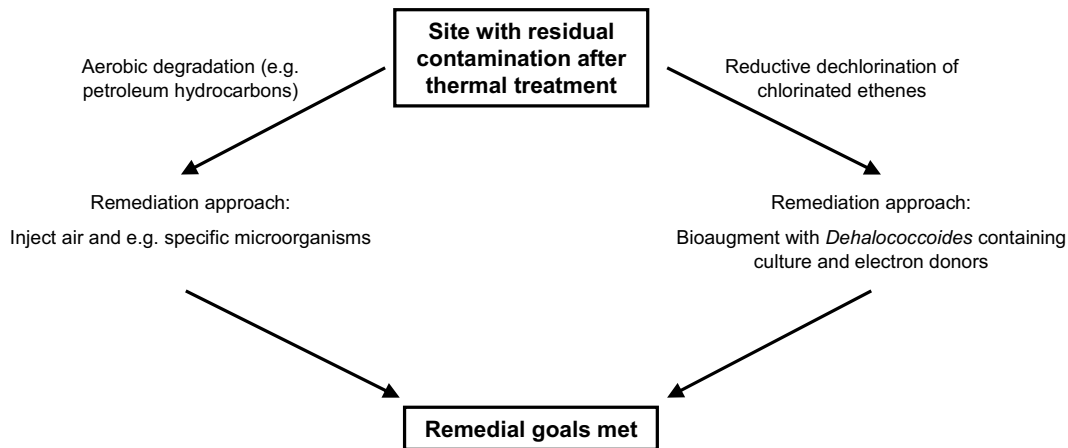
Figure 24. Flow chart of possible remediation technologies after thermal treatment.

Resent research has focused on alternative treatment trains. For example, use of thermally enhanced chemical oxidation followed by biological degradation has been suggested for TCE dechlorination (Hrapovic et al., 2005). In these various combinations of technologies, it is advantageous when the final step of the first technology is beneficial to the sequential remediation technology. In future, an increased number of various treatment trains are expected to be investigated.

Finally, environmental standards have changed as analytical methods have developed, leading to more stringent cleanup standards with time as we have become able to detect contaminants at lower concentrations. If this trend continues, one can expect that future remediation efforts will be targeted to even more stringent standards than today. This may generate an even larger need to use inexpensive polishing techniques such as biostimulation and augmentation after aggressive methods such at thermal ones are applied to remove most of the mass.

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APPENDICES

The papers are not included in this www-version but can be obtained from the library at Institute of Environment & Resources, Bygningstorvet, Building 115, Technical University of Denmark, DK-2800 Lyngby (library@er.dtu.dk).

I   **Friis A.K.,** Albrechtsen H.-J., Heron, G., and Bjerg P.L. Redox processes and release of organic matter after thermal treatment of a TCE-contaminated aquifer. *Environmental Science and Technology*. 39 (15), 5787-5795, 2005.

II   **Friis A.K.,** Edwards E., Albrechtsen H.-J., Udell K., Duhamel M., and Bjerg P.L. Dechlorination after thermal treatment of a TCE-contaminated aquifer: Laboratory experiments. Submitted.

III   **Friis A.K.,** Albrechtsen H.-J., Cox E., and Bjerg P.L. The potential for bioaugmentation after thermal treatment of TCE-contaminated groundwater: Laboratory experiments. Submitted.

IV   **Friis A.K.,** Cox E., and Albrechtsen H.-J. Effects of thermal treatment on microbial diversity and community structure in a contaminated aquifer. Submitted.

V   **Friis A.K.,** Heimann A.C., Jakobsen R., Albrechtsen H.-J., Cox E., and Bjerg P.L. Temperature dependence of anaerobic TCE-dechlorination in a highly enriched *Dehalococcoides*-containing culture. Submitted.

VI   **Friis A.K.,** Kofoed J.L.L., Heron G., Albrechtsen H.-J., and Bjerg P.L. Optimizing bioaugmentation after field-scale thermal treatment of a TCE-contaminated aquifer. Submitted.

VII   **Friis A.K.,** Heron G., Albrechtsen H.-J., Udell K., and Bjerg P.L. Anaerobic dechlorination and redox activities after full-scale Electrical Resistance Heating (ERH) of a TCE-contaminated aquifer. Submitted.

VIII   **Friis A.K.** Technical note: Phase distribution of chlorinated ethenes at elevated temperatures.