Chapter 1

General Introduction
Pollution by chlorinated compounds

Organohalide compounds constitute one of the largest groups of chemicals (Häggblom and Bossert, 2003). Due to the wide variety in applications of these compounds, such as their use as solvents, degreasers, biocides, and pharmaceuticals, they have been produced and used in large quantities in the last 80 years (Häggblom and Bossert, 2003). Combined with a lack of environmental awareness and legislation, this has caused a large influx of these products into the environment and hence environmental pollution, of groundwater in particular.

Organohalides are organic compounds containing one or more substituted halogen atoms (see Table 1 for examples). The majority of the organohalide pollutants are chlorinated, although brominated (Br), fluorinated (F) or iodinated (I) forms also have industrial applications and are of environmental concern, in particular fluorinated surfactants are (Häggblom and Bossert, 2003; Löffler et al., 2003). Chlorinated ethenes (CEs; Table 1) are among the most frequently reported groundwater contaminants due to spillage and leakage (Häggblom and Bossert, 2003). The major CE is perchloroethylene (also known as perchlorethene, tetrachloroethylene, tetrachloroethene or PCE), which has been widely used as primary solvent in the dry-cleaning industry and as metal degreasing agent. This compound and its associated degradation products, trichloroethene (TCE), dichloroethene (DCE) isomers and vinyl chloride (VC), are the smallest compounds in the alkene group (compounds that have a single carbon-carbon double bond), and can also be classified as chlorinated aliphatic hydrocarbons (chains of C atoms that do not contain a benzene ring) (Ramamoorthy and Ramamoorthy, 1997) (Table 1). All CEs have been placed in the group of priority pollutants by the U.S. Environmental Protection Agency (U.S. EPA) with demonstrated (vinyl chloride)(EPA, 2000) or suspected (cis-DCE, TCE and PCE) (EPA, 2009, 2011, 2012) carcinogenicity to humans.

Besides their vast industrial production, natural sources of CEs exist. PCE and TCE can originate from marine micro- and macro-algae (Abrahamsson et al., 1995) and volcanic emissions (Jordan et al., 2000), while VC is formed during the oxidative degradation of organic matter in terrestrial environments (Keppler et al., 2002). More importantly, VC and dichlorinated ethenes (DCEs) occur in groundwater primarily as the result of in situ microbial degradation of higher CEs (PCE and TCE) resulting in the formation of these thereby secondary pollutants (Bradley, 2000). VC is of particular concern, as it is the most carcinogenic among the CEs. Thus, VC has the lowest regulatory limit for its presence in drinking water (2 µg/L compared to 5 µg/L for PCE and TCE (Table 1).
Table 1. Physico-chemical properties of chlorinated ethenes and ethene, the end product of dechlorination $^a$

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Molecular Formula</th>
<th>Chemical structure</th>
<th>Molecular Weight (g/mol)</th>
<th>Carbon Oxidation State</th>
<th>Density (g/mL) (approx. 20 to 25°C)</th>
<th>Solubility (mg/L) (approx. 20 to 25°C)</th>
<th>Vapor Pressure (mm Hg, 20°C)</th>
<th>Henry’s Law Constant (atm·m$^3$/mol)</th>
<th>Octanol/Water Partition Coefficient (log Kow)$^c$</th>
<th>Soil Organic Carbon/Water Partition Coefficient (log Koc)$^d$</th>
<th>USEPA MCL (mg/L)$^e$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetrachloroethene (PCE)</td>
<td>C$_2$Cl$_4$</td>
<td>![C2Cl4_structure]</td>
<td>165.8</td>
<td>+II</td>
<td>1.63</td>
<td>150</td>
<td>14.01</td>
<td>0.0132</td>
<td>2.53</td>
<td>2.42</td>
<td>0.005</td>
</tr>
<tr>
<td>Trichloroethene (TCE)</td>
<td>C$_3$HCl$_3$</td>
<td>![C3HCl3_structure]</td>
<td>131.4</td>
<td>+I</td>
<td>1.46</td>
<td>1,100</td>
<td>60.0</td>
<td>0.0072</td>
<td>2.42</td>
<td>2.03</td>
<td>0.005</td>
</tr>
<tr>
<td>Cis-1,2-dichloroethene (cis-DCE)</td>
<td>C$_3$H$_2$Cl$_2$</td>
<td>![C3H2Cl2_structure]</td>
<td>96.94</td>
<td>0</td>
<td>1.28</td>
<td>3,500</td>
<td>200</td>
<td>0.0030</td>
<td>0.70</td>
<td>1.65</td>
<td>0.070</td>
</tr>
<tr>
<td>Trans-1,2-dichloroethene (trans-DCE)</td>
<td>C$_3$H$_2$Cl$_2$</td>
<td>![C3H2Cl2_structure]</td>
<td>96.94</td>
<td>0</td>
<td>1.26</td>
<td>6,300</td>
<td>340</td>
<td>0.0073</td>
<td>2.06</td>
<td>1.77</td>
<td>0.100</td>
</tr>
<tr>
<td>1,1-dichloroethene (1,1-DCE)</td>
<td>C$_3$H$_2$Cl$_2$</td>
<td>![C3H2Cl2_structure]</td>
<td>96.94</td>
<td>0</td>
<td>1.22</td>
<td>2,250</td>
<td>500</td>
<td>0.021</td>
<td>2.13</td>
<td>1.81</td>
<td>0.007</td>
</tr>
<tr>
<td>Vinyl Chloride (VC)</td>
<td>C$_2$H$_3$Cl</td>
<td>![C2H3Cl_structure]</td>
<td>62.51</td>
<td>-I</td>
<td>0.91$^b$</td>
<td>1,100</td>
<td>2.660</td>
<td>0.218</td>
<td>0.60</td>
<td>1.23</td>
<td>0.002</td>
</tr>
<tr>
<td>Ethene</td>
<td>C$_2$H$_4$</td>
<td>![C2H4_structure]</td>
<td>28.05</td>
<td>-II</td>
<td>Gas</td>
<td>131</td>
<td>30.800</td>
<td>8.60</td>
<td>1.13</td>
<td>2.48</td>
<td>---</td>
</tr>
</tbody>
</table>

$^a$ Modified from (Parsons, 2004)

$^b$ Source (Cwiertny and Scherer, 2010).

$^c$ log of octanol/water partition coefficient (dissolution coefficient)

$^d$ log of soil organic carbon/water partition coefficient (soil sorption coefficient)

$^e$ USEPA Maximum Contaminant Level; mg/L = milligrams per liter
The natural production and consumption of CEs are generally balanced at low steady-state concentrations of these compounds. However, the industrial production of CEs and associated spills have often disrupted the natural balance between production and consumption and dramatically increased their concentrations (Löffler et al., 2003). The number of chlorine atoms in CEs directly affects their physical and chemical properties. As the number of chlorine atoms increases, density generally increases, while vapor pressure and aqueous solubility generally decreases. Also hydrophobicity (i.e. a high octanol/water partition coefficient) and susceptibility to soil sorption (a high organic carbon/water partition coefficient) increases with the number of chlorine atoms in CEs (Table 1). CEs (with the exception of VC) are denser than water, hence once released into the subsurface they tend to migrate as dense non-aqueous phase liquids (DNAPLs) below the groundwater table through the water-saturated zone and sink to the bottom of aquifers, serving as reservoirs (of mainly PCE and TCE) for long-term solubilization (Christ et al., 2004; Dugat-Bony et al., 2012). In general, the properties of the more highly chlorinated compounds (Table 1) result in slow rates of subsurface transport, compared to less chlorinated compounds (USEPA, 2000).

Although many countries have implemented stringent guidelines and laws regarding the use and disposal of chlorinated solvents, past practices and accidental spills have resulted in extensive contamination of vast areas. The recognition of the toxicity and persistence of PCE and its degradation products has led to extensive efforts to decipher the mechanisms of its degradation and to develop suitable clean-up techniques for DNAPL sources and contaminated groundwater (McCarty, 2010).

(Bio)degradation of chloroethenes

Microorganisms have evolved a variety of biochemical pathways to degrade chloroorganic compounds. Depending on the oxidation state of the carbon atoms in CEs and on the prevailing environmental conditions (aerobic or anaerobic), degradation can occur via reductive dechlorination (RD) or oxidative degradation, where CEs act as electron acceptors or donors, respectively (Bradley, 2003). In general, PCE or TCE which have a high number of chlorine substituents (corresponding to a high oxidation state of their carbon atoms; Table 1) are more susceptible to reduction while DCE and VC more easily undergo oxidative degradation (Vogel et al., 1987). CE oxidation and reduction are generally Gibbs energy yielding except in case of co-metabolism. In the latter case, CEs are converted by an enzyme or co-factor that is central to the metabolism of another growth substrate, e.g. the ammonia mono-oxygenases of nitrifiers can also act on TCE (Sayavedra-Soto et al., 2010). Co-
metabolism does not yield any Gibbs energy or obvious growth benefit for the microbe mediating the reaction (Bossert et al., 2003).

Moreover, transformation of CEs can also occur by abiotic mechanisms. Abiotic agents such as zero-valency metals (Lee et al., 2001), sulfide minerals or green rusts, and permanganate have been shown to enhance the reductive degradation and oxidation of CEs, respectively (Tobiszewski and Namieśnik, 2012). However, the majority of environmental CE degradation is biology mediated (Holliger et al., 2003).

**Anaerobic biodegradation of chloroethenes**

Under anaerobic conditions, microorganisms are able to grow using CEs as respiratory terminal electron acceptors (TEA), coupled to ATP synthesis via RD. In this process, chlorine atoms are sequentially replaced with hydrogen atoms to yield a step-wise transformation, accepting 2 electrons per step, of PCE via TCE, cis-1,2-DCE and VC to ultimately the non-toxic end product ethene (Fig. 1; Table 1) (Holliger et al., 1999; Smidt and de Vos, 2004). While cis-1,2-DCE has been reported as main dechlorination intermediate, other dichloroethenes (Table 1), i.e. trans-1,2-dichloroethene (trans-DCE) and 1,1-dichloroethene (1,1-DCE), are also sometimes produced (Zhang et al., 2006; Cheng et al., 2010). PCE and TCE can be reduced to cis-DCE as end product by bacteria belonging to several genera (some examples in Fig. 1). However, only members of the genus *Dehalococcoides*, now called *D. mccartyi*, are capable of performing the complete reduction of PCE or TCE to ethene (Fig. 1).

In addition, the lesser CEs (DCE and VC) may also be degraded by anaerobic microbial oxidation to CO₂ (Bradley, 2003; Mattes et al., 2010) (Fig. 1). Anaerobic oxidation seems to occur at many sites and with various TEAs (other than CEs), although the potential for anaerobic oxidation is higher for VC than for DCE and the rate of oxidation increases when redox conditions in groundwater become energetically more favorable (e.g. nitrate-reducing conditions are more favorable than sulfate-reducing conditions) (Bradley, 2003). However, knowledge of the mechanisms and enzymes of anaerobic chloroethene oxidation still remains scarce due to the lack of pure cultures or defined mixed cultures capable of mineralization of DCE or VC (Bradley and Chapelle, 2010; Mattes et al., 2010).

**Aerobic biodegradation of chloroethenes**

In the presence of oxygen, the lower CEs can be oxidized co-metabolically or degraded metabolically (Fig. 1). During aerobic co-metabolic oxidation, non-specific oxygenases fortuitously oxidize CEs, during the conversion of primary growth substrates, such as methane, ethene, ammonium or aromatic hydrocarbons (Fogel et al., 1986; Chauhan et al., 1998; Koziollek et al., 1999; Sayavedra-Soto et al., 2010). However, co-metabolic
degradation may yield chlorinated epoxides as intermediates, which if they are not metabolized further may lead to secondary toxicity (Mattes et al., 2010). Aerobic co-metabolism of TCE, DCE and VC is quite common (Frascari et al., 2008; Zhang and Tay, 2012). So far Pseudomonas stutzeri OX1 is the only species known to be capable of aerobically co-metabolizing PCE (Ryoo et al., 2000).

Aerobic oxidation of VC as sole carbon and Gibbs energy source has been shown to occur frequently, while aerobic DCE oxidation is rare (Mattes et al., 2010), perhaps because the midpoint potential of the oxidation of the latter differs less from that of the reduction of
oxygen. Indeed, at present, there is no evidence that PCE or TCE (with an even higher midpoint potential than DCE; Fig.2) are susceptible to aerobic metabolic oxidation. Hence, although the lower chlorinated compounds may be transformed under aerobic conditions, the higher chlorinated compounds are recalcitrant to aerobic degradation. In addition, groundwater quickly becomes anaerobic upon pollution as the result of rapid oxygen consumption coupled to the metabolism of this pollution (Bradley, 2000). Also, many aquifers are by nature anaerobic, e.g. due to high natural organic matter content (van Breukelen et al., 2003). Therefore, anaerobic reductive dechlorination (RD) appears to be the predominant transformation pathway for PCE degradation in the subsurface, and will be discussed in detail in subsequent sections.

**Dechlorinating bacteria – phylogeny and physiology**

In the latest decades, a large number of CE-respiring microorganisms belonging to a variety of genera, i.e. Desulfitobacterium, Dehalobacter, Sulfurospirillum, Desulfomonile, Desulfuromonas, Geobacter and Dehalococcoides, have been isolated and characterized in terms of their physiology, biochemistry, phylogeny and genetics (Löffler et al., 2003; Smidt and de Vos, 2004; Maphosa et al., 2010b; Hug et al., 2013). However, most of these microbes cannot completely detoxify PCE and produce cis-DCE as end product. Degradation to ethene has only been observed for *D. mccartyi* strains (Fig. 1) (Maymó-Gatell, 1997; Löffler et al., 2013). Therefore, *D. mccartyi* strains have received over recent years major attention in relation to their application in the bioremediation of CEs (Taş et al., 2010). However, *D. mccartyi* strains differ in their dechlorination capabilities (Fig. 1). Some strains can just perform a few RD steps and/or perform some steps through slow co-metabolic transformation (Fig. 1). Therefore, establishing the presence of this genus on the basis of the detection of its 16S rRNA gene in a CE polluted site does not guarantee successful in situ dechlorination (Stroo et al., 2010; Taş et al., 2010). Besides, *Dehalococcoides* spp. are strictly organohalide-respiring bacteria (OHRB); only organohalide compounds serve as respiratory electron acceptors. They have specific nutritional needs, using only hydrogen as electron donor, requiring acetate as carbon source for biomass assimilation and needing an exogenous source of vitamins for growth. This restricts their growth and efficiency of dechlorination (Löffler et al., 2013).

Compared to *D. mccartyi*, OHRB belonging to the phylum *Proteobacteria* exhibit a more versatile metabolism and can use a great variety of electron donors and acceptors for growth. For example, dechlorinating *Desulfuromonas* isolates can use several organic
compounds as electron donors (including acetate, but not hydrogen) and acceptors (fumarate, ferric iron, sulfur) besides PCE and TCE (Sung et al., 2003). *Geobacter lovleyi* strain SZ uses both acetate and hydrogen as well as pyruvate as electron donor for RD of PCE to *cis*-DCE, and, like other members of this genus, reduce iron (Sung et al., 2006). *Sulfurospirillum multivorans* is also a well studied PCE-dechlorinating bacterium, where fumarate or nitrate can act as alternative TEA (John et al., 2009).

The phylum *Firmicutes* contains both organohalide-respiring generalists (which not only use organohalides as TEA) as well as obligate OHRB. Members belonging to *Desulfitobacterium* genus are characterized by their metabolic versatility (Villemur et al., 2006). The spectrum of halogenated organic compounds used differs substantially from one strain to another, and even between strains belonging to the same species. Similarly, the variety of electron acceptors (e.g. fumarate, nitrate, sulfite, metals, humic acids) and donors (e.g. hydrogen, formate, lactate, pyruvate) used is large and differs among species. Most *Desulfitobacterium* strains can degrade PCE, TCE and other halogenated organic compounds by organohalide respiration, with the exception of some strains that can perform RD of PCE into TCE (e.g. KBC1, DCB-2, PCP1, TCP-A, PCE1 show only weak activity concerning conversion of TCE into DCE) and *Desulfitobacterium hafniense* strain DP7, isolated from an organohalide-“free” environment and incapable of dechlorinating PCE or chlorophenols (Villemur et al., 2006). In contrast, members of *Dehalobacter* genus grow exclusively via organohalide respiration (Rupakula et al., 2013). Some isolates belonging to this genus have been reported to be able to dechlorinate PCE and TCE: TCP1, 12DCB1, 13DCB1, PER-K23 and TEA (Maillard and Holliger, 2016), using hydrogen as sole electron donor, and acetate as source of carbon for biomass assimilation.

Biogeographic studies have revealed the global distribution of *D. mccartyi* and *Desulfitobacterium* spp., and their occurrence in both polluted and pristine environments (Lanthier et al., 2001; Hendrickson et al., 2002). This has lead Krzamarzick et al. to suggest that OHRB are not only important for bioremediation purposes but also for the natural biogeochemical chlorine cycle (Krzmarzick et al., 2012). *Dehalococcoides mccartyi* strains are often detected when ethene formation occurs (Hendrickson et al., 2002). Members of the genus *Desulfitobacterium* have been isolated from very diverse locations (Villemur et al., 2006), and have for instance also been detected in many different types of soil sampled in Canada (Lanthier et al., 2001). *Dehalococcoides* spp. and *Desulfitobacterium* spp. frequently coexist in CE polluted sites (e.g. (Maphosa et al., 2010a)). Besides, (Rouzeau-Szynalski et al., 2011) revealed the concomitant presence of both genera in the majority of environmental
samples and enrichments they analyzed. Although *Dehalobacter* was detected as well, the number of samples in which this genus was observed was considerably lower (Rouzeau-Szynalski et al., 2011). Hence, *Desulfitobacterium* spp. and *Dehalococcoides* spp. appear to be the most wide-spread genera involved in CE-respiration. Numerous isolates of these genera (Villemur et al., 2006; Hug et al., 2013; Löffler et al., 2013) have been extensively studied in the recent years and used as model organisms to investigate the mechanism of RD (Nonaka et al., 2006). The genomes of several isolates have been sequenced, including those of some *Dehalococcoides mccartyi* strains (Kube et al., 2005; Seshadri et al., 2005; McMurdie et al., 2009), and of *Desulfitobacterium hafniense* strain Y51 (Nonaka et al., 2006) and strain DCB2 (Kim et al., 2012). Metabolic inferences made on the basis of *D. mccartyi* genome information (Kube et al., 2005; Seshadri et al., 2005; McMurdie et al., 2009) support the results obtained from physiological studies on this genus (Löffler et al., 2013). They confirmed their narrow metabolic repertoire, and the inability of the majority of sequenced strains to synthesize corrinoid, requiring external corrinoid vitamin B12 for RD. Hence, they depend on other microorganisms for growth. Comparative genome studies have highlighted the much broader range of metabolism of *D. hafniense* Y51 strain as compared to *D. mccartyi* 195 (Nonaka et al., 2006), which confers to *Desulfitobacteria* a potential importance for bioremediation purposes.

**Desulfitobacterium hafniense Y51 as model microorganism in PCE respiration**

*D. hafniense* strain Y51 is a strictly anaerobic Gram-positive bacterium which belongs to the family *Peptococcaceae*, order *Clostridiales*, class *Clostridia*, in the phylum *Firmicutes*. It was isolated from a PCE polluted soil in Japan (Suyama et al., 2001). *D. hafniense* Y51 features a high metabolic versatility (Nonaka et al., 2006). It can use several electron donors, such as lactate, formate, pyruvate and vanillate, and in addition to its ability to respire CEs it can utilize sulfate, sulfite, nitrate, and fumarate as electron acceptors (Lee et al., 2001; Villemur et al., 2006; Peng et al., 2012). In addition to CEs (PCE and TCE), chloroethanes such as hexachloroethane, pentachloroethane, tetrachloroethanes, and 1,1,1,2,2,3,3-heptachloropropane are dechlorinated by *D. hafniense* Y51, but complete dechlorination is not achieved (Furukawa et al., 2005). Halo-aromatic compounds are not respired by *D. hafniense* Y51 (Furukawa et al., 2005). *D. hafniense* Y51 dechlorinates PCE to cis-DCE via TCE by the reductive dehalogenase PceA, even at concentrations as high as 1 mM (i.e. at water saturation) and as low as 0.6 µM (Suyama et al., 2001, 2002). Maximum dechlorination rates ($V_{\text{max}}$ 70 nmol min$^{-1}$ mg cell protein$^{-1}$) by cell extracts were observed between 0.4 and
0.6 mM of PCE (Furukawa et al., 2005).

Unlike *D. mccartyi* strain 195, *D. hafniense* Y51 displays motility (Suyama et al., 2001) and is self-sufficient for nucleotides, amino acids, and the corrinoid cofactor vitamin B$_{12}$ (Nonaka et al., 2006), with the latter being essential for dechlorination (Smidt and de Vos, 2004). In addition, as its genome contains genes encoding methyl-accepting chemotaxis proteins (Nonaka et al., 2006), likely chlorinated compounds might act as chemo-attractants for this strain as suggested for the closely related *Desulfitobacterium hafniense* DCB-2 (Gábor et al., 2008). All these features of *D. hafniense* Y51 have been investigated in monoculture and under “non-stress conditions”. Therefore, there is still a lack of knowledge about the effects on dechlorination of its interactions with other community members, as well as its behavior under environmentally relevant conditions, such as nutrient limitation and pollutant stress.

**Environmental conditions affecting reductive dechlorination of chloroethenes**

Redox conditions are one of the major factors affecting the efficiency of RD of CEs (Bradley, 2000). Microorganisms tend to use the electron acceptors that enable the largest free energy harvest preferentially. Hence, in the presence of oxygen the reduction of CEs is an unfavorable process, also because of toxic effects of oxygen, and oxygen respiring will prevail over CE-respiring microorganisms if only because of the higher growth yields (Bradley, 2000).

Redox potentials for half reactions of CE reduction range from +0.57 V for PCE to TCE down to +0.39 V for *cis*-DCE to VC in aqueous solution at pH 7 and a temperature of 25°C (Vogel et al., 1987) (Fig. 2). This range suggests that anaerobic RD is less favorable than denitrification and more favorable than partial reduction to nitrite (Fig. 2). However, under field conditions, often more reducing conditions (i.e. than iron reduction) are required to enable dechlorination of the least oxidized *cis*-DCE and VC compounds, with complete dechlorination to ethene occurring under sulfate reducing or methanogenic conditions (Bradley, 2000) (Fig. 2). The higher CEs PCE and TCE are more susceptible to reduction because they are the most oxidized (i.e. they have a higher redox potential), and their RD can occur under nitrate- and ferric-iron reducing conditions (Bradley, 2000). Their reduction also yields more Gibbs energy per step during RD to ethene (Dolfing, 2003) and the organisms using that Gibbs energy for growth can grow at a higher growth yield, leading to more biomass capable of this RD. Conversely, *cis*-DCE and VC may degrade at lower reaction rates because they are the least-oxidized/most-reduced among the CEs, and may be more
prone to undergo aerobic or anaerobic oxidation than RD (Bradley, 2000). As a result, RD is often incomplete and frequently leads to the accumulation of *cis*-DCE and VC (Bradley, 2000; Mattes et al., 2010).

**Fig. 2.** Standard oxidation-reduction potentials for relevant environmental TEA (terminal electron accepting) processes (Modified from Madigan et al., 2012) and redox couples of chlorinated ethenes (PCE, TCE, *cis*-DCE, VC) and ethene (Vogel et al., 1987). As indicated on the left, the amount of Gibbs energy released during TEA reduction (e.g. by hydrogen as the electron donor) decreases with decreasing (i.e. less positive) redox potential of the TEA redox couple. Conversely, lower *in situ* hydrogen concentrations are associated to TEA processes with increasing redox potential, as the hydrogen is more reactive. This is illustrated by the triangle on the right-hand side. Environmental *in situ* redox conditions required for RD are also indicated. Optimal range for complete RD refers to the range of *in situ* reducing conditions where degradation of the smallest CEs is normally observed: *cis*-DCE to VC (under sulfate-reducing) and VC to ethene (under methanogenic conditions) according to Bradley and Chapelle, 2010).

*In situ* hydrogen concentrations play an important role in determining whether *cis*-DCE and VC accumulate or not, as well as the observed differences in redox conditions under which PCE and its degradation intermediates are reduced (Luijten et al., 2004). Although OHRB oxidize several types of organic compounds, hydrogen has been identified as the most important electron donor for complete RD in the environment (Smatlak et al., 1996; Löffler et al., 1999; Mazur and Jones, 2001). Hydrogen threshold concentrations (the minimal hydrogen concentration that can be consumed under specific reducing conditions by a microorganism)
for PCE and TCE reduction are relatively low (0.05-0.9 nM), while conversion of cis-DCE and VC to ethene can only proceed at hydrogen levels of 0.1 to 2.5 nM and 2 to 24 nM, respectively (Fig. 3). The highest hydrogen concentrations are generally observed under the least energetic redox conditions, i.e. where potential electron acceptors have the lowest redox potential; and decrease with increasing redox potential (i.e. conditions enabling methanogenesis > conditions enabling sulfate reduction > conditions enabling iron reduction > conditions enabling denitrification) (Lovley, 1988; Luijten et al., 2004; Röling et al., 2007; Fig. 2). The in situ hydrogen concentration has therefore been suggested as a suitable indicator to delineate redox zones and the expected extent of RD (Lovley, 1988; Luijten et al., 2004).

OHRB can out-compete methanogenic archaea, acetogenic bacteria, and sulfate-reducing bacteria at low hydrogen concentrations (Fig. 3). However, they may have to compete fiercely for available hydrogen with other anaerobic organisms which can also afford a high affinity for hydrogen because of the favorable energetics, such as nitrate- and iron-reducing bacteria (Luijten et al., 2004) (Fig. 3). Therefore, the limiting availability of hydrogen due to the presence of competitors, inadequate redox conditions, low growth yields because of limited energetics and a potential absence of cis-DCE- and VC-respiring microorganisms (e.g. appropriate D. mccartyi strain), might be the main causes of incomplete anaerobic RD and of accumulation of the least chlorinated compounds (Alleman et al., 2010).

OHRB are only able to oxidize simple fermentation products (e.g. lactate, formate, hydrogen, acetate). They do not consume complex organic molecules such as polymers (e.g. polysaccharides) or the corresponding monomers (e.g. sugars). Primary fermenting microorganisms can convert these complex organics into hydrogen, small organic acids and alcohols. Therefore, in the natural environment the growth and activity of OHRB depend on the activity of non-dechlorinating bacterial guilds (Fig. 3). More importantly and counter-intuitively, OHRB themselves do not control the rate of CE reduction in anaerobic microbial networks. Control resides almost primarily with the fermenting microorganisms (Röling et al., 2007).

Furthermore, research on D. mccartyi in particular suggests that OHRB are easier to grow and that their physiology is more robust within mixed consortia containing fermenting microorganisms, which apart from electron donors also provide cofactors to the OHRB (Maymó-Gatell, 1997; Duhamel et al., 2004; Holmes et al., 2006; Löfler et al., 2013). Since these OHRB lack fundamental and canonical metabolic pathways, D. mccartyi have been
Fig. 3. Anaerobic organic-matter degrading, dechlorinating microbial network, indicating interactions of organohalide respiring bacteria (OHRB) with other major microbial guilds, and the flux of carbon and energy through the microbial guilds; modified from (Schink, 1997). Hydrogen threshold concentrations (Luijten et al., 2004) for different redox processes are shown. T.E.A indicates terminal electron accepting.
found to increase growth and dehalogenation rates when grown with excess of vitamin B$_{12}$ or in consortia that produce cobalamin (He et al., 2007; Löffler et al., 2013). Similarly, metabolically restricted *Dehalobacter* spp. can only dechlorinate β-hexachlorocyclohexane in the presence of the fermenting *Sedimentibacter* spp., which is postulated to provide cobalamin to the former (van Doesburg et al., 2005; Maphosa et al., 2012b).

Reduction of CEs under field conditions occurs in a highly complex microbial context, where multiple functional groups interact (Macbeth et al., 2004; Miller et al., 2007). In these complex systems the OHRB members may represent only a small percentage of the total microbial community (Maphosa et al., 2012a). Nevertheless, most studies on OHRB in environmental settings tend to focus on the dechlorinating populations while little attention is paid to the fermenting guild and their impact on organohalide respiration activity (Daprato et al., 2007). Thus, it is crucial to obtain more understanding on the extent to which the community composition, in particular the contribution of the non-OHRB component, may affect PCE degradation, and organohalide respiration in general. Such knowledge may contribute to more rational design and optimization of bioremediation strategies.

**Bioremediation strategies for chloroethene pollution**

Many CE-remediation technologies just involve the excavation of polluted soil and extraction of groundwater with treatment at the surface (pump-and-treat) or physicochemical methods such as *in situ* thermal (electrical resistive heating, thermal conductive heating) and chemical processes (oxidative and reductive) (Mccarty, 2010). Besides being relatively expensive, these methods are invasive to the environment, which make them unsustainable solutions in the long term (Wenning et al., 2006). Bioremediation is an alternative that provides the possibility to degrade toxic pollutants present in soils, water, sediments or air, into less toxic forms, using natural biological activity, primarily in microorganisms. The contaminants may be transformed, reduced or eliminated by living organisms through reactions that occur as part of their metabolism (Vidali, 2001). Strategies for the bioremediation of organohalide pollutants by RD comprise monitored natural attenuation, biostimulation and bioaugmentation (Skipper, 1999).

**Monitored natural attenuation**

When OHRB are present and active in a contaminated environment, RD of CEs can naturally occur without a need for human intervention. This process is referred to as natural attenuation, or as passive or intrinsic bioremediation (Röling and van Verseveld, 2002). Natural attenuation does not occur at all CE contaminated sites due to for example
deficiencies in nutrients or microbial communities, or it may not be an adequate remedy to reduce pollution to regulatory levels within an acceptable timeframe (Henry, 2010). Hence, site-specific characterization to determine the potential for intrinsic bioremediation, verifying that degradation is occurring and monitoring its capacity for contaminant removal in the long term, is required in order to rely on natural attenuation (Röling and van Verseveld, 2002).

When monitoring reveals that natural attenuation is not occurring or not fast enough, *in-situ* engineering, i.e. active bioremediation strategies such as biostimulation and bioaugmentation, need to be considered. In practice, CE polluted areas are usually subjected to active bioremediation, rather than to the passive approach of monitored natural attenuation, particularly when DNAPL is present (Moretti, 2005).

**Biostimulation**

Biostimulation comprises the enhancing of anaerobic CE reduction by the addition of fermentable organic substrates (e.g. molasses, organic acids like butyrate) to generate hydrogen indirectly, or by injecting the electron donor (e.g. hydrogen or acetate) directly into the subsurface to stimulate the growth and activity of indigenous OHRB. The degradation of the added fermentable substrates depletes dissolved oxygen and other TEAs present in the aquifer (e.g., nitrate, iron, sulfate), creating an anaerobic environment with a low (highly negative) ambient redox potential, and depleted in TEAs that may potentially compete with CE for electrons, thereby stimulating complete RD of CEs (Alleman et al., 2010).

There are many fermentable organic substrates that may be injected into the subsurface to enhance the activity of OHRB (Alleman et al., 2010). Substrates mainly differ in the rate at which the material is degraded to a form that is suitable for utilization by ORHB, in the complexity of their composition, and in their cost. Soluble molasses, a waste product from the sugar industry, are widely applied to enhance CE degradation in the aqueous phase, due to their great potential for uniform distribution throughout the aquifer and their relatively low cost (Henry, 2010). However, an engineering challenge in applying biostimulation in the field is ensuring adequate substrate loading rates (Henry, 2010) so as to optimize and sustain bioremediation over time.

Other amendments may be applied towards secondary biostimulation, such as the injection of inorganic nutrients (nitrogen, phosphorous, potassium) and yeast extract to promote microbial growth and development, and of compounds contributing to pH buffering (such as sodium bicarbonate) in systems with insufficient natural buffering capacity. Amendments with sulfate and ferric iron can indirectly stimulate abiotic degradation, as after their biological reduction iron sulfide (FeS) is formed, and this compound can directly
Biostimulation has the potential of complete detoxification of CE compounds in situ, with little impact on the environment and at relatively low cost, when native microbial communities capable of the RD are present, as demonstrated in several case studies (Parsons, 2004; Nelson et al., 2005). Failed cases of biostimulation are rarely described in literature. However in 2004, Parsons (Parsons, 2004) estimated that biostimulation applications to that date had only been successful in achieving “site closure” or “no-further-action” in less than 10 percent of cases. The lack of success related to failures to adequately characterize and understand the site hydrogeology, failure to deliver effectively the substrate throughout the targeted treatment zone, failure to induce sufficiently reducing conditions, or a lack of appropriate microbial communities (Parsons, 2004).

**Bioaugmentation**

The abundances and types of indigenous OHRB may limit natural attenuation or biostimulation of CE degradation. Therefore, a frequently applied strategy to attempt to enhance biodegradation is adding microorganisms (bioaugmentation), by injecting the contaminated area with exogenous OHRB, along with any required nutrients (biostimulation). The aim is to complement or replace the native community if this one is incapable of biodegrading the target contaminants (Stroo et al., 2010). For sites containing competent OHRB but at low abundance or with poor distribution throughout the contaminated area, an alternative is to inoculate the polluted area with native microorganisms, which are enriched in the laboratory from populations obtained from the site (Gentry et al., 2004). Bioaugmentation can reduce the lag time before RD occurs and decrease the duration of bioremediation (Stroo et al., 2010).

However, before considering bioaugmentation, the contaminated site should be evaluated to ensure that the proper geochemical conditions have been achieved (generally through biostimulation to achieve anaerobic groundwater with low redox potential), and to determine that native OHRB are not capable to complete the dechlorination of CEs within a certain time period upon biostimulation (Stroo et al., 2010).

Bioaugmentation, in general, has been viewed with skepticism in the past, since in general the added microorganisms did not survive due to predation, parasitism, failure to compete for substrates or unfavorable environmental conditions (Goldstein et al., 1985). However, in case of CE pollution, bioaugmentation appears to be a suitable approach for cleaning groundwater (Bradley and Chapelle, 2010; Stroo et al., 2010). Modification of environmental conditions through biostimulation (favoring RD) provides OHRB with a
selective advantage, allowing these organisms to compete with other subsurface microbes and to colonize the subsurface upon bioaugmentation (Stroo et al., 2010). For example, for sites where *D. mccartyi* strains were not detected and dechlorination was arrested at cis-DCE, the addition of mixed cultures containing *D. mccartyi* led to complete dechlorination to ethene and the establishment of these OHRB in the bioaugmented area (Ellis et al., 2000; Major et al., 2002).

Nowadays, several cultures for use in bioaugmentation procedures are commercially available and more are being developed (Stroo et al., 2010). All commercially developed bioaugmentation cultures are mixed cultures that contain one or more *D. mccartyi* strains with different dechlorination capabilities, as well as fermenting microorganisms. The advantage of the use of mixed cultures over pure cultures, is that the fermenting microorganisms in the mixed cultures supply *D. mccartyi* with electron donors and cofactors, enabling faster growth of the mixed cultures compared to pure cultures (Stroo et al., 2010).

**The relevance of Desulfitobacterium for in situ bioremediation**

Relatively little attention has been paid so far to the presence and importance of other OHRB that co-occur with *D. mccartyi* in bioaugmented or non-engineered CE polluted environments, possibly because solely *D. mccartyi* can completely dechlorinate CEs. However, in practice degradation to ethene may often result from cooperation between several OHRB with specificities for different RD reactions. Molecular monitoring has revealed the coexistence of distinct OHRB at a location in The Netherlands at which ethene production from CEs was observed (Maphosa et al., 2010a). Complete TCE degradation was mediated by complex OHRB associations (*Sulfurospirillum*, *Dehalobacter*, *Desulfitobacterium*, *Geobacter* and several *D. mccartyi* strains) in four biostimulated, chlorinated solvent-contaminated sites in France, as revealed by employing the so-called Dechloarray, a microarray targeting genes encoding enzymes involved in chlorinated solvent biodegradation (Dugat-Bony et al., 2011), and by pyrosequencing (Dugat-Bony et al., 2012). OHRB associations in enrichments derived from CE-contaminated sites have frequently been described in the scientific literature, of which the most frequently reported are *Desulfitobacterium* and *D. mccartyi* (Yang et al., 2005; Bunge et al., 2007; Rouzeau-Szynalski et al., 2011), *Geobacter* and *D. mccartyi* (Duhamel and Edwards, 2007) and *Dehalobacter* and *D. mccartyi* (Daprato et al., 2007).

Kinetic modeling suggested that biostimulation may sustain different types of OHRB, and could cause them to function as PCE-to-cis-DCE (e.g. *Desulfitobacterium*) and DCE-to-
ethene (D. mccartyi) specialists (Becker, 2006). Maximum specific substrate utilization rates ($q_{\text{max}}$) and half-saturation constants ($K_S$) for PCE and TCE reported for D. mccartyi strains are lower than those of other OHRB-like Desulfitobacterium spp. (Becker, 2006; Huang and Becker, 2009), perhaps because of limitation by total enzyme capacity or reductant availability. Thus, the higher PCE and TCE dechlorination capacity of Desulfitobacterium could provide it with an advantage in the competition with D. mccartyi for PCE and TCE present at high concentrations. The products of RD of PCE and TCE would feed the D. mccartyi however. As and where PCE and TCE become depleted, the D. mccartyi would then take a greater share of TCE and PCE reduction. In this way different niches for the OHRB could emerge and the overall process should be speed up as compared to a situation with D. mccartyi as sole OHRB present (Becker, 2006). Enabling the coexistence of these OHRB might therefore be an attractive and effective strategy for bioremediation of PCE contaminated sites.

D. hafniense Y51 offers great potential for application in bioaugmentation of PCE pollution, if only due to its high metabolic versatility and self-sufficiency for vitamin B$_{12}$ (see section “Desulfitobacterium hafniense Y51 as model microorganism in PCE respiration” for more details). Possibly D. hafniense Y51 is capable of chemotaxis towards chlorinated compounds, as suggested for D. hafniense DCB-2 (Gábor et al., 2008). Motility and ability for chemotaxis towards pollutants have been proposed as key factors in selecting strains for bioaugmentation purposes (Parales and Haddock, 2004).

Next to bioaugmentation with D. hafniense Y51 and this together with D. mccartyi in order to achieve complete RD, also other bioaugmentation strategies may lead to completely biology-mediated detoxification of CEs. The accumulated end product cis-DCE of D. hafniense Y51 constitutes a carbon and energy source for aerobic oxidizing bacteria (Mattes et al., 2010). Indeed, sequential anaerobic-aerobic degradation has allowed for efficient and complete mineralization of PCE, both in natural and in engineered settings (Gerritse et al., 1995, 1997; Tiehm and Schmidt, 2011).

Bioaugmentation solely with D. hafniense Y51 might also be a promising and sustainable remediation technique when combined with an abiotic Fe$^0$ treatment (Lee et al., 2001). The relatively inexpensive and non-toxic Fe$^0$ has been shown to efficiently reduce the cis-DCE produced by D. hafniense Y51 into non-chlorinated end products. Additionally, Fe$^0$ might enhance RD of PCE by maintaining pH and low redox potential, and by producing hydrogen (through the corrosion of Fe$^0$ in anaerobic water), which in turn can be used as electron donor for OHRB (Lee et al., 2001). Hence, bioaugmentation with the highly efficient
OHRB *D. hafniense* Y51, in combination with other aerobic or anaerobic biotic processes, or in combination with abiotic treatments, may provide for potent bioremediation of higher CEs in aquifers.

As stated previously, surprisingly little attention has been paid to the non-dechlorinating fermenting guild to date, despite its apparent importance since CE dechlorination in the environment does not only depend on metabolic capacities of individual OHRB, but also on their interactions with other community members such as fermenting organisms. Insight in environmental factors affecting the identity and behavior of this crucial fermenting guild in relation to growth, survival and functioning of *D. hafniense* Y51 should prove beneficial to optimizing *in situ* bioremediation. As a speculative example: possibly the rate of molasses-stimulated dechlorination can be enhanced by additional bioaugmentation with co-cultures of *D. hafniense* Y51 and specific fermenting strains. Furthermore, also insight on the robustness to stresses that will prevail under natural conditions is limited for *D. hafniense* Y51 considered for bioaugmentation. Investigation of both these issues also requires consideration of the experimental approaches to use, which will be the topic of the next two sections.

**Microbial physiology in the context of environmental conditions**

Natural environments are often characterized by low concentrations of essential nutrients (e.g. carbon, nitrogen) for microbial growth, as the result of the low bioavailability of these resources and the metabolic activities of indigenous microbial populations. Consequently, growth of bacteria is slow in most environments (Harder and Dijkhuizen, 1983; Langwaldt et al., 2005; Egli, 2010), as they are subject to nutrient starvation and other environmental stresses. In the laboratory, microorganisms are generally studied by batch cultivation under non-limiting conditions (e.g. excess of carbon) and at growth rates much higher than observed in their natural environment (Harder and Dijkhuizen, 1983). While cell environment, cell composition and physiological state change during batch culturing, continuous culturing in chemostats allows the maintenance of stable growth conditions, and hence the same physiological state. More importantly, chemostats allow cultivating at low growth rates and high cell densities, which facilitates the acquisition of multiple reliable biological samples for further analysis (Hoskisson and Hobbs, 2005). Chemostat cultivation enables reproducible experiments employing growth conditions that more closely mimic environmental conditions (Stouthamer and Bettenhausen, 1975; Kovárová-Kovar and Egli, 1998; Hoskisson and Hobbs, 2005). On the other hand of course, chemostat conditions may
not really mimic the natural situation of the various limiting nutrients (i.e. chemostat by
definition operates with a single limiting nutrient and in nature there might certainly be more
than one limiting nutrient); simultaneous presence of perhaps competitive microorganisms at
different specific growth rates and scenarios of feast and famine. In addition chemostats are
not particularly suited for the study of strong stresses as wash out may ensue.

Continuous culturing enables one to study some of the diverse strategies that
microorganisms have evolved to adapt to restrictive and dynamic conditions. Some
heterotrophic bacteria possess high metabolic flexibility vis-à-vis nutrient limitation, as for
example the anaerobic toluene-degrader and iron-reducing bacterium *Geobacter metallireducens* (Marozava et al., 2014). During carbon limitation, *G. metallireducens*
derepressed some metabolic pathways involved in the degradation of substrates typically
found in anoxic environments, although these carbon sources were not present in their growth
environment. This phenomenon of the “relief of carbon catabolite repression” is a well-
documented strategy found in other bacteria under starvation. It enables fast responses when
new carbon sources become available in the environment of these cells (Egli, 2010;
Marozava et al., 2014), but comes at the cost of increased protein synthesis. At low growth
rates *G. metallireducens* also expressed proteins related to the utilization of alternative
electron acceptors, as well as to signal transduction and motility. All this suggests that this
microorganism has a great potential for survival under environmental stress conditions
(Marozava et al., 2014).

Detailed studies on the physiology of *Desulfitobacterium* under environmentally
relevant conditions are needed to improve our understanding of this genus and to predict their
effectiveness in dechlorination and their competitiveness under in situ bioremediation
conditions. Several chemostat-based studies on *Desulfitobacterium* spp. have already been
performed. For example, continuous culturing of *Desulfitobacterium hafniense* TCE1
(formerly *D. frappieri* TCE1) (Gerritse et al., 1999) under lactate-limiting conditions and
with excess of mixed TEAs (nitrate, fumarate, sulfite and PCE) showed that PCE
dechlorination by this strain was completely suppressed under these conditions. This suggests
that a low ratio of electron donor to electron acceptor may compromise successful
dechlorination by *Desulfitobacterium* in the presence of other TEAs (Gerritse et al., 1999).
Drzyzga and colleagues (Drzyzga et al., 2001; Drzyzga and Gottschal, 2002) showed that
potential syntrophic associations between *D. hafniense* TCE1 and sulfate-reducing
*Desulfovibrio* species can occur in the (near) absence of sulfate (depletion of TEAs is a
common condition upon biostimulation). The sulfate-reducing microorganisms convert to
fermentation under these conditions, while *D. hafniense* TCE1 kept the hydrogen partial pressure very low by oxidizing it with PCE as electron acceptor, hence forcing the sulfate-reducers into further fermentation. However, in the presence of sulfate, sulfate reducers outnumbered *D. hafniense* TCE1 and sulfate reduction was the dominating process, which indicates the importance of applying carbon source in excess, in order to deplete native TEA such as sulfate. Such syntrophic associations between sulfate-reducing bacteria and *Desulfitobacterium* spp. may be of great significance in anaerobic CE polluted environments, since it is well known that RD is especially favored by highly reducing conditions (Drzyzga and Gottschal, 2002).

**Advanced molecular tools for monitoring anaerobic dechlorination**

What neither chemostat nor batch cultivation do particularly well is the robust maintenance of a diverse system of microbes with different specific growth rates growing under substrate limited conditions. It is good therefore that a wide range of culture-independent technologies has emerged in recent years that hold promise for evaluating the presence and activities of appropriate microbial species and communities *in situ*, allowing to monitor natural attenuation or to assess contaminated sites as candidates for engineered bioremediation (Maphosa et al., 2010b). These approaches are also suitable for fundamental studies on the interaction of *D. hafniense* Y51 with its abiotic and biotic environments, although here coculture studies could be more powerful with respect to discovering mechanisms.

The ecogenomics toolbox comprises both new “omics” techniques (e.g., (meta)genomics, (meta)transcriptomics, (meta)proteomics) and the traditional physiological analysis, DGGE and qPCR measurements. Together they provide insights into microbial community structure, dynamics, and functioning at CEs polluted sites (Maphosa et al., 2010b). Molecular screening techniques such as (i) PCR assays for the detection of *Dehalobacter*, *Desulfitobacterium* and *D. mccartyi* (Smits et al., 2004) or reductive dehalogenases- (RDase) encoding genes (*pceA, tceA, vcrA*, and *bvcA*) involved in sequential dechlorination of PCE to ethene by *D. mccartyi* (Ritalahti et al., 2006; Behrens et al., 2008), or (ii) pan genome microarrays containing thousands of probes that encompass both genus-specific as well as strain-specific genes for *D. mccartyi* (Hug et al., 2011) and *Desulfitobacterium* (Tian et al., manuscript in preparation) or (iii) large-scale sequencing “meta-omics” approaches that allow to represent the whole community of organisms (Segata et al., 2013), are all examples of molecular tools that are available to date and that can be applied at CEs-contaminated field sites undergoing bioremediation.
Furthermore, the advances in culture-independent approaches have stimulated the development of systems microbiology, a discipline that allows to build a comprehensive picture of how a microbial network is functioning and might be tweaked (Röling et al., 2010; Röling and van Bodegom, 2014; Röling, 2015). Relatively simple systems biology approaches such as ecological control analysis (ECA) provide a first step towards this complex and ambitious goal, by elucidating which functional group has the control of a specific flux within a microbial network (Röling et al., 2007). These developments may contribute to improved control over CE degradation through rational engineering of microbial community performance.
Main objectives of this thesis
The highly chlorinated ethene PCE is a prevalent groundwater contaminant. Utilizing the activity of anaerobic organohalide respiring bacteria (OHRB) might enable an efficient, cost-effective treatment for PCE degradation. Desulfitobacterium hafniense strain Y51 is a PCE-degrading organism with high metabolic versatility, which confers great potential for bioaugmentation. More knowledge on the (eco)physiology of this OHRB is required if it is to be considered for use in bioremediation of PCE contaminated sites. To this end, the objective of the research described in this thesis was to advance the understanding of the system of environmental factors and interactions (abiotic and biotic) that controls the activity of the OHRB Desulfitobacterium hafniense Y51, design a new strategy for PCE-bioremediation using this OHRB in a new microbial network, test it experimentally, and validate our design.

In order to serve this objective, conventional culture-dependent methods (batch and chemostat cultivation) and culture-independent molecular methods (16S rRNA gene-based techniques), including “omics” tools (transcriptomics and proteomics) were applied to generate insight into the physiology of D. hafniense Y51 under relevant environmental conditions and to characterize both phylogenetically and physiologically its interactions with other members in microbial networks.

Thesis Outline
Chapter 2 provides insight into the physiology of Desulfitobacterium hafniense Y51 under relevant environmental conditions, with the aim of predicting its behavior in potential bioremediation scenarios. Low growth rates and various well-defined limitations (carbon and TEA) were achieved by cultivation in chemostat. Physiological characterization in combination with microarrays and proteomic analysis was carried out and the results were compared to physiology at maximum growth rates under non-limiting conditions in batch cultures.

The work described in Chapter 2 employed mono-cultures of D. hafniense growing on lactate. In some natural environments, fermenting microorganisms supply D. hafniense with fermentation products such as lactate. Since D. hafniense Y51 depends on organic compounds supplied by fermenting microorganism, Chapter 3 focuses on the potential effect of redox conditions that are relevant for D. hafniense (i.e. nitrate-, ferric iron-, sulfate- and CO₂-reduction) on the identity and performance of its feeder of reducing equivalents, i.e. the fermenting guild. The time-courses of glucose fermentation and electron-acceptor reduction
obtained in enrichment cultures under the four redox conditions were correlated with phylogenetic information derived from 16S rRNA gene-based pyrosequencing analysis. This chapter reveals clear differences in terms of identities and physiology of the glucose-fermenting guild in response to variation of the dominant redox process.

These findings led to the research presented in **Chapter 4**, which investigates in batch cultures whether differences in fermenting guilds affect the dechlorination of PCE by *D. hafniense* Y51. The consortia described in **Chapter 3** were co-cultured with *D. hafniense* with PCE as sole electron acceptor. Glucose, as analogue of the molasses that are frequently used for *in situ* biostimulation, provided for the electrons required for dechlorination through fermentation. Combining metabolite measurements and Illumina sequencing, this chapter elucidates that the redox history of the local community plays a critical role on the RD of PCE by *D. hafniense* Y51.

In order to simulate a bioaugmentation situation after a PCE spill, the consortia established during the experiments described in **Chapter 4**, were inoculated in **Chapter 5** into native and sterilized sediments, artificially polluted with PCE and amended with glucose. In sterile sediments the type of dechlorinating consortia inoculated, exerted a clear effect on dechlorination rates while in natural sediment, with the original microbial community present, bioaugmentation with consortia had no positive influence on PCE degradation. The chapter further describes the effects of the native microbial communities on complete dechlorination of PCE and the influence of the type of consortia on *D. hafniense* Y51 performance based on the phylogenetical information obtained from culture-independent molecular methods (16S rRNA gene-based techniques). Additionally, physiologically responses of *D. hafniense* Y51 in the presence of PCE, in a simulated field scenario, were analyzed by the use of a pangenome microarray.

**Chapter 6** discusses the main findings of the research described in this thesis and evaluates it with respect to environmental significance and implications for *in situ* bioremediation of PCE by *D. hafniense* Y51. It concludes with a substantial number of notes, each of which constitutes advice for when and how biostimulation and bioaugmentation should be put in place.