Microbial degradation of chlorinated ethenes and its potential application for in-situ bioremediation

Ing. Michal Záležák, Ph.D.

Doctoral Thesis Summary

Tomas Bata University in Zlín

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Faculty of Technology

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Mikrobiální degradace chlorovaných ethylenů a její potenciální využití pro in-situ bioremediace

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ABSTRACT

The doctoral thesis summary deals with microbial degradation of chlorinated ethenes and its potential application for in-situ bioremediation. The theoretical part of the work shortly explains the main reasons for soil and groundwater contamination by chloroethenes along with their transport and a fate in a subsurface environment. The work then outlines common procedures that must be undertaken prior to selecting a suitable remediation method at contaminated sites. Further, the work summarizes various microbial processes leading to a transformation and degradation of all chlorinated ethenes and outlines several options for the use of these processes within in-situ bioremediation of affected sites. Special attention is paid to aerobic cometabolic degradation of all three dichloroethenes by pure bacterial strains in a mineral salt medium. The theoretical part of the work ends with an overview of the possible field applications for in-situ bioremediation of sites polluted by chloroethenes.

A following experimental part of the work deals with degradation of selected chlorinated ethenes by *Comamonas testosteroni* strain RF2 and by several consortia in the mineral salt medium. At first, strain RF2 was tested to investigate its capacity for degrading 1,2-*cis*-dichloroethene (*c*DCE), 1,2-*trans*-dichloroethene (*t*DCE), and 1,1-dichloroethene (1,1DCE). Degradation assays were performed for single DCEs, as well as for a mixture of DCEs with TCE, which resembled contaminated plume in groundwater. Strain RF2 was capable of efficiently removing all three dichloroethenes (DCEs) at the initial aqueous concentrations of 6.01 mg L^{-1} for *c*DCE, 3.80 mg L^{-1} for *t*DCE and 0.65 mg L^{-1} for 1,1DCE, with a removal efficiency of 100 % for *c*DCE, 65.8 % for *t*DCE, and 46.8 % for 1,1DCE. Furthermore, complete removal of TCE, *c*DCE and 1,1DCE (122.5 μg L^{-1}, 84.3 μg L^{-1} and 51.4 μg L^{-1}, respectively) were observed in a mixture sample that also contained 72.33 μg L^{-1} of *t*DCE, which was removed to the amount of 72.3%. Moreover, degradation of *c*DCE (6.01 mg L^{-1}) led to a 92.2 % release of inorganic chloride, and 2,2-dichloroacetaldehyde was determined as the first intermediate of *c*DCE transformation. Further, a consortium composed of the strain RF2 and vinyl chloride (VC) utilizing *Mycobacterium aurum* DSM-6695 was tested to investigate its capacity for degrading TCE (115.7 μg L^{-1}), *c*DCE (662 μg L^{-1}), *t*DCE (42.01 μg L^{-1}), 1,1DCE (16 μg L^{-1}), and VC (7 mg L^{-1}; "all in a liquid phase") in mixed samples. The consortium was able to nearly completely remove all the compounds in the mixed sample within 21 days of the assay.

The findings of this thesis suggest that the consortium composed of the strain RF2 and *M. aurum* DSM-6695 exhibits the potential to remediate groundwater contaminated with chlorinated ethenes.

Keywords: Chlorinated ethenes, microbial degradation and transformation, in-situ bioremediation, *Comamonas testosteroni* RF2

ABSTRAKT

Teze dizertační práce krátce shrnuje problematiku mikrobiální degradace chlorovaných ethylenů a jejím potenciální využití pro in-situ bioremediace. V teoretické části práce jsou stručně popsány hlavní důvody kontaminace půd a podzemních vod chlorovanými ethyleny i jejich následný transport v podzemním prostředí. Práce dále stručně shrnuje běžné postupy při monitorování znečištěných lokalit, jež vedou k zvolení vhodné sanační metody. Následně jsou popsány různé mikrobiální procesy vedoucí k transformaci a degradaci chlorovaných ethylenů, načež jsou nastíněny známé způsoby využití těchto procesů pro in-situ bioremediace znečištěných lokalit. Zvláštní pozornost je věnována aerobní kometabolické degradaci všech tří dichloroethenů čistými bakteriálními kmeny v prostředí minerálního média. Teoretická část práce je zakončena kapitolou, která shrnuje možnosti technického řešení in-situ bioremediací, cílených na odstranění chlorovaných ethylenů z půd a podzemních vod.

Navazující experimentální část práce se zabývá degradací vybraných chlorovaných ethylenů kulturou *Comamonas testosteroni* RF2 a několika bakteriálními konsorcii v prostředí minerálního média. Kmen RF2 byl zvolen pro pokusy kometabolické degradace 1,2-cis-dichlorethylenu (*c*DCE), 1,2-trans-dichlorothylenu (*t*DCE), 1,1-dichlorethylenu (1,1DCE) a vinyl chloridu (VC). Degradační testy byly provedeny jednak pro jednotlivé dichlorethyleny (DCEs) a rovněž pro směs DCEs s TCE, simulující podzemní vodu znečištěnou těmito látkami. Kmen RF2 byl schopen odstraňovat všechny DCEs (dávkovány samostatně) při počátečních koncentracích v kapalné fázi: 6,01 mg L^{-1} *c*DCE, 3,80 mg L^{-1} *t*DCE a 0,65 mg L^{-1} 1,1DCE, s účinností odstranění 100% pro *c*DCE, 65,8 % pro *t*DCE a 46,8 % pro 1,1DCE. Úplné odstranění *c*DCE vedlo k uvolnění 92,2% anorganických chloridů. Dále bylo zjištěno úplné odstranění TCE, *c*DCE a 1,1DCE (122,5 µg L^{-1}, 84,3 µg L^{-1} a 51,4 µg L^{-1}) ve vzorcích obsahujících modelovou podzemní vodu. Ve stejných vzorcích rovněž došlo k odstranění 72,3 % *t*DCE o koncentraci 72,33 µg L^{-1}. Sledování kinetiky degradace *c*DCE ukázalo na existenci dvou metabolitů rozkladu, přičemž jako první meziprodukt transformace *c*DCE byl zjištěn 2,2-dichloracetaldehyd. V neposlední řadě bylo studováno konsorcium kmene RF2 s bakterií *Mycobacterium aurum* DSM-6695, jež metabolicky rozkládá VC. Toto konsorcium bylo podrobeno experimentu současné degradace TCE (115,7 µg L^{-1}), *c*DCE (662 µg L^{-1}), *t*DCE (42,01 µg L^{-1}), 1,1DCE (16 µg L^{-1}) a VC (7 mg L^{-1}, „vše v kapalné fázi“) a ukázalo schopnost téměř úplně odstranit všechny sloučeniny ve směsném vzorku do 21 dnů, čímž prokázalo značný potenciál pro jeho případné využití v rámci čištění podzemních vod znečištěných chlorovanými ethyleny.

Klíčová slova: Chlorované etheny, mikrobiální degradace a transformace, in-situ bioremediace, *Comamonas testosteroni* RF2

CONTENTS

1. CURRENT STATE OF THE ISSUES DEALT WITH

1.1 Structure and toxicity of CEs

Chlorinated ethenes (CEs) are represented by tetrachloroethene, commonly referred as perchloroethene (PCE); trichloroethene (TCE); *cis*-1,2-dichloroethene (*c*DCE); *trans*-1,2-dichloroethene (*t*DCE); 1,1-dichloroethene (1,1-DCE); and vinyl chloride (VC). CEs possess two carbon centres joined by a carbon-carbon double bond known as a π-bond system, which does not allow covalently bonded chlorine substituents to rotate freely in the plane perpendicular to the direction of the π –bond (Cwiertny and Scherer, 2010). Also, due to the double-bonded carbon centres of CEs, they can accommodate at most two chlorine substituents. Thus, PCE with its four chlorine substituents is the most chlorinated ethene; chemical structures of all CEs are depicted in Fig. 1.1.

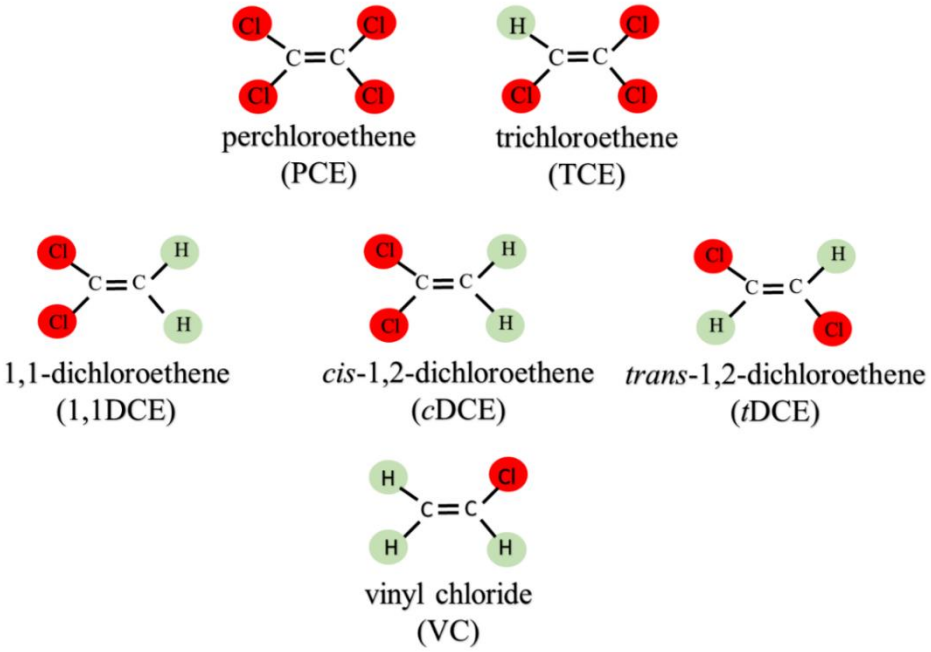

Fig 1.1 Chemical structure of chlorinated ethenes

CEs have several undesirable properties that include high toxicity, environmental resistance, and ability to accumulate in living and non-living components of an environment, including humans. PCE and TCE are the suspected human carcinogens, DCEs are highly toxic compounds, and VC is well known as the human carcinogen (Binbin et al., 2014)

1.2 Industrial uses of CEs and their occurrence in the environment

Generally, CEs are volatile organic compounds that belong to a class of chlorinated organic solvents, which are used for a variety of commercial and industrial purposes, especially as degreasers, cleaning solutions, and paint thinners (Cwiertny and Scherer, 2010; Cloelle et al., 2010; Nishino et al., 2013). Because of their chlorine-containing chemical structure, CEs can efficiently dissolve organic materials like fats and greases and to serve as raw materials or intermediates in the production of other chemicals (NSW, 2011)

The extensive use of perchloroethene (PCE) and trichloroethene (TCE) as dry cleaning and degreasing solvents for industrial purposes has caused world-wide contamination of soils and groundwater. In particular, TCE has become a major groundwater contaminant on a global scale. Moreover, natural attenuation of PCE and TCE often leads to the formation of less-chlorinated *cis*-1,2-dichloroethene (*c*DCE) and vinyl chloride (VC), which may persist in soils and particularly groundwater plumes. Also, to a lesser extent, *trans*-1,2-dichloroethene (*t*DCE) and 1,1-dichloroethene (1,1DCE) may be formed as well. Therefore, contaminated groundwater with prevailing anaerobic conditions may contain a mixture of residual TCE, DCEs, and VC even after a period of several years or even decades.

1.3 Fate and transport of CEs in a subsurface environment

The fate and transport of CEs in a subsurface environment along with their degradability by both biotic and abiotic processes are all closely related to their physicochemical parameters as well as to the geochemical and hydrological conditions of contaminated sites, such as organic matter content of the subsurface, soil porosity, hydraulic conductivity, and hydraulic gradient (Huling and Weaver, 1991; Pant and Pant, 2010; Kret et al., 2015). Appendix 1.1 summarizes some of the most important physicochemical parameters of CEs, which influence their distribution in an unsaturated zone (vadose zone) and groundwater, i.e. their partitioning between soil, water, air, and non-aqueous phase liquids (NAPLs), occurring as dense (DNAPLs) and light (LNAPLs) (Cwiertny and Scherer, 2010). Typically, the resulting distribution of CEs in the contaminated subsurface is a highly complex process that results in their non-uniform occurrence in soils and groundwater, which is caused by limited water solubility, high volatility, moderate hydrophobicity, and relative density of these compounds (Fig 1.2).

Given the severity and extent of the environmental pollution caused by chlorinated ethenes, innovative remediation technologies ensuring efficient, low-cost, and environmentally friendly clean-up methods for their removal are essential. In-situ bioremediation of CEs-polluted sites, which includes techniques universally based on the use of microorganisms capable of degrading the targeted

compounds seems like a promising clean-up method due to its relatively low cost and environmentally-friendly character.

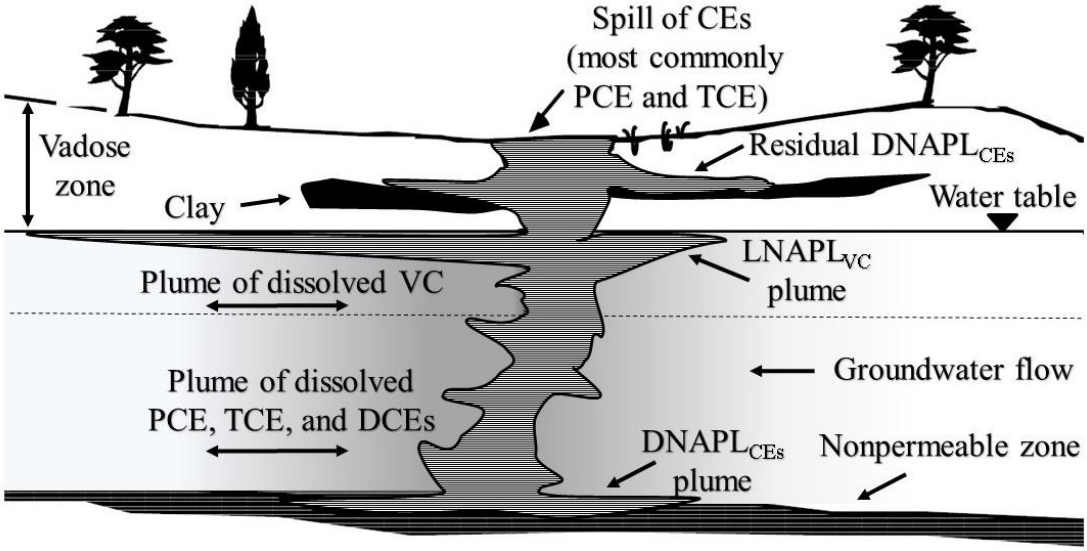

Fig. 1.2 Likely transport of CEs in the subsurface[①]

1.4 Site investigation and monitoring

A site investigation is an integral part of a whole in-situ bioremediation process and plays an irreplaceable role in the selection of an appropriate clean-up method. The principal objectives of the site investigation should be as follows: (1) gather information about the extent of soil and groundwater contamination including horizontal and vertical subsurface distribution of CEs in all phases, i.e. groundwater, soil gas, DNAPL, LNAPL and portions adsorbed on organic matter; (2) examine prevailing environmental conditions and likely degradative processes of CEs; (3) a determination of a cost-effective clean-up strategy that ensures sufficient removal of contaminants within an acceptable time frame (EPA Victoria, 2006; WDNR, 2014; Kret et al., 2015).

1.5 Bacterial strategies for degradation and transformation of CEs

Bacteria have evolved several strategies for enzyme-catalysed dechlorination and degradation of CEs; specifically, anaerobic reductive dechlorination (organohalide respiration), aerobic metabolic degradation, and aerobic cometabolic degradation are the best known processes, which may be applied for in-situ bioremediation of polluted sites.

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① Inspired by US EPA, 1991*; $DNAPL_{CEs}$: chlorinated ethenes as dense non-aqueous phase liquids; $LNAPL_{VC}$: vinyl chloride as light non-aqueous phase liquids*

1.5.1 Anaerobic reductive dechlorination (organohalide respiration)

Organohalide respiration, also known under the term metabolic reductive dehalogenation, is a process in which CEs serve as the only electron acceptors that accept electrons from other compounds, e.g. propionate, butyrate, lactate, methanol, or ethanol, which are fermented in a subsurface to H2 or acetate and serve as the actual electron donors utilized by dechlorination bacteria (Aulenta et al., 2006, 2007; Frascari et al., 2013). In other words, organohalide respiration of CEs is a replacement of the chlorine substituent in the chloroethene molecule with a hydrogen atom; each dechlorination step consumes two electrons and two protons and releases H^+ and Cl^- (Loffler et al., 2013) Further, reductive dehalogenase enzymes (RDases) are the key enzymes responsible for catalysing a cleavage of the carbon-chlorine bond in CEs during organohalide respiration (Futamata et al., 2009).

In general, organohalide respiration of CEs often leads to the accumulation of less chlorinated ethenes, particularly *c*DCE and VC, that may accumulate at sites where PCE and TCE are degraded through this process; however, anaerobic reduction of an entire range of CEs to ethene has been observed (Bourg et al., 1992; Fennell et al., 2001; Aeppli et al., 2010; Imfeld et al., 2011; Frascari et al., 2015). Typically, a number of bacterial strains of the *Dehalococcoides* genus are involved in the complete dechlorination of CEs (He et al., 2005; Lee et al., 2008). Nevertheless, reduction of DCEs and VC has been found to be very limited not only because of the lower potential of these compounds for accepting electron(s) but also due to the fact that *Dehalococcoides* are very sensitive to oxygen, and generally less robust towards changes in environmental conditions than other organohalide-respiring bacteria (Smidt and de Vos, 2004; Tiehm and Schmidt, 2011).

1.5.2 Aerobic metabolic degradation (direct aerobic oxidation)

Aerobic metabolic degradation, also known as direct aerobic oxidation, is a process in which bacteria utilize CEs as their growth substrate, i.e. CEs serve as the sole sources of organic carbon and energy. Oxidative dechlorination is a result of mono and/or dioxygenases that catalyse the incorporation of one or two oxygen atom(s) into the molecule of chloroethene (Frascari et al., 2015).

Generally, several bacteria have been identified as capable of aerobic metabolic degradation, mostly of VC (Dolinova et al., 2017). Indeed, VC has been found to be directly oxidized by a variety of bacterial strains, such as *Mycobacterium aurum* strain L1 (Hartmans et al., 1985), *Mycobacterium* sp. strains JS60, JS61, JS616, and JS617 (Coleman et al., 2002) or *Pseudomonas putida* strain AJ (Danko et al., 2004). On the contrary, direct aerobic TCE and DCEs oxidation occur rarely, but some studies on their direct oxidation exist (Schmidt and Tiehm 2008; Dey and Roy 2009; Kim et al., 2010).

1.5.3 Aerobic cometabolic degradation

Aerobic cometabolism occurs when the enzymes originally produced for the degradation of bacterial growth substrates (auxiliary/primary substrates) fortuitously catalyses the oxidation of a non-growth substrate (CEs) (Semprini, 1997). Key enzymes involved in the cometabolic oxidation of CEs are different mono and/or dioxygenases; their production is initiated by variety of suitable growth substrates, such as isoprene (van Hylckama Vlieg et al., 1998), methane (Kim et al., 2008), o-xylene (Li et al., 2014), propene (Ensign et al., 1992; Kim et al., 2008), toluene (Azizian et al., 2007), phenol (Hopkins and McCarty, 1995) and many others (Findlay et al., 2016). Among the commonly produced cometabolic enzymes belongs methane monooxygenase (Fox et al., 1990), ammonia monooxygenase (Arciero et al., 1989), phenol monooxygenase (Fries et al., 1997), toluene monooxygenase, toluene-2,3-dioxygenase (Byrne et al., 1995; Johnson and Olsen, 1995), and alkene monooxygenase (Ensign et al., 1992), all of which have relatively broad substrate specificity and are produced by a variety of bacteria.

Many bacteria are capable of cometabolic oxidation of TCE, *c*DCE and VC, though fewer strains are capable of the same process in the case of *t*DCE and 1,1DCE, and only *Pseudomonas stutzeri* OX1 has been reported to be able to degrade PCE cometabolically (Ryoo et al., 2000). Up to date list of bacteria capable of degrading TCE, *c*DCE and VC via cometabolic oxidation can be found in a study published by Dolinova et al. (2017). In most cases, *t*DCE and 1,1DCE are not the major contaminants in a subsurface compare to PCE, TCE, *c*DCE and VC; which might be a reason why less research has been devoted to the degradation of 1,1DCE, *t*DCE, or mixtures of all DCEs and their potential mutual interactions. Indeed, the presence of *t*DCE and 1,1DCE in a subsurface might influence a rate of the degradation of other CEs, especially *c*DCE and VC (Verce et al., 2002).

In general, the cometabolic oxidation of CEs is slower (and usually time-limited) than their direct oxidation, however, it has a potential to degrade a broader spectrum of CEs, such as TCE, and particularly all DCEs. Hence, its potential for removal of these chloroethenes by bacterial strains utilizing different primary substrates should be studied.

1.6 Bacterial cometabolic degradation of DCEs in mineral salt medium (MSM)

Several studies have described cometabolic oxidation of all DCEs in MSM by pure cultures and obtained various results (Ewers et al., 1990; Ensign et al., 1992; Hartmans and De-Bont, 1992; Chang and Alvarez-Cohen, 1996; Vardar and Wood, 2005). Therein, it was found that two isoprene (2-methyl-1,3-butadiene) utilizing bacteria, *Alcaligenes denitrificans* ssp. *xylosoxidans* JE 75 and

Rhodococcus erythropolis JE 77, could oxidize all the given DCEs. Primarily, a high initial specific rate of conversion was discerned for 1,1DCE. However, degradation of the compound caused an 80% reduction in cell activity within the first 20 min of the process (Ewers et al., 1990). As distinct from it, *Xanthobacter* cells (strain Py2) utilizing propene as a growth substrate were able to degrade *c*DCE and *t*DCE, although degradation of 1,1DCE proved less efficient (Ensign et al., 1992). Similarly, *Mycobacterium aurum* L1, growing on VC, removed 1,1DCE less efficiently; this showed initial oxidation rates for *c*DCE > *t*DCE > 1,1DCE (30, 25 and 10 nmol min^{-1} (mg of dry weight)$^{-1}$, respectively) (Hartmans and De-Bont, 1992). Also, methane-oxidizing *Methylosinus trichosporium* OB3b expressing the particulate methane monooxygenase showed a transformation capacity (Tc) for all DCEs; the highest Tc was observed for *t*DCE, while the levels of Tc for 1,1DCE and *c*DCE were significantly lower (Chang and Alvarez-Cohen, 1996). Finally, *Pseudomonas stutzeri* OX1, possessing toluene-o-xylene monooxygenase, degraded all DCEs individually as well as in different mixtures when it was grown either on toluene or o-xylene (Vardar and Wood, 2005).

1.7 Main approaches for in-situ clean-up of CEs-polluted sites

Monitored natural attenuation (MNA), biostimulation, and bioaugmentation belong among the three main clean-up approaches based on the ability of microorganisms to degrade CEs at polluted sites. While MNA relies on monitoring of spontaneous natural processes that reduce concentrations of CEs, biostimulation and bioaugmentation, both bioremediation techniques, are based on enhanced degradation process(es) using microorganisms (indigenous or introduced) along with other manipulations to remove CEs within a delimited and controlled subsurface environment. Therefore, for the purpose of this work, MNA relying on natural processes is not considered as a bioremediation technique for sites clean-up; although MNA is commonly referred as a passive bioremediation approach in a scientific community (US EPA, 2000). Hence, in view of the above-mentioned, MNA is seen as a stand-alone clean-up strategy that is based on monitoring of a contamination plume and its surrounding area in terms to determine whether the natural attenuation of CEs is occurring at sufficient rates to attain site-specific treatment goals (US EPA, 1998).

At sites, where MNA is not sufficient enough to meet treatment goals, biostimulation and/or bioaugmentation must be applied (Lacinova et al., 2013; Dolinova et al., 2016). Firstly, biostimulation includes modification of existing conditions in a subsurface environment in a manner favouring specific degradation process controlled by indigenous microbial populations (Mihopoulos et al., 2002). For instance, to enhance organohalide respiration of PCE and TCE, injection of suitable electron donor(s) is crucial. On the other hand, to support/initiate aerobic degradation processes of less chlorinated ethenes, a supply of electron acceptors (predominantly O_2) is essential and may be followed

by the addition of carbon source(s) and nutrients (N, P) if necessary. Whereas enhancing the direct oxidation of CEs requires a supply of only oxygen and nutrients, aerobic cometabolism must include additional injection of the primary substrate(s) that induce production of a catabolic enzyme(s). Secondly, for in-situ bioremediation of sites that do not harbour suitable indigenous microbial population(s) capable to degrade CEs, bioaugmentation is the only option. This approach involves all the aspects of the biostimulation along with an addition of a pre-cultured exogenous bacterial strain or consortium with proven degradation activity towards CEs (Steffan et al., 1999; Ellis et al., 2000).

1.8 Field application of the main in-situ clean-up approaches

1.8.1 In-situ MNA of CEs-polluted sites

MNA generally employs a network of monitoring wells that are installed at a site in a manner to cover the largest possible plume area with respect to the likely migration paths of the plume in the future. Typically, at least one monitoring well is placed directly in a source zone and several other wells are placed downstream and upstream of the zone with respect to regional hydrological conditions as shown in Fig. 1.3

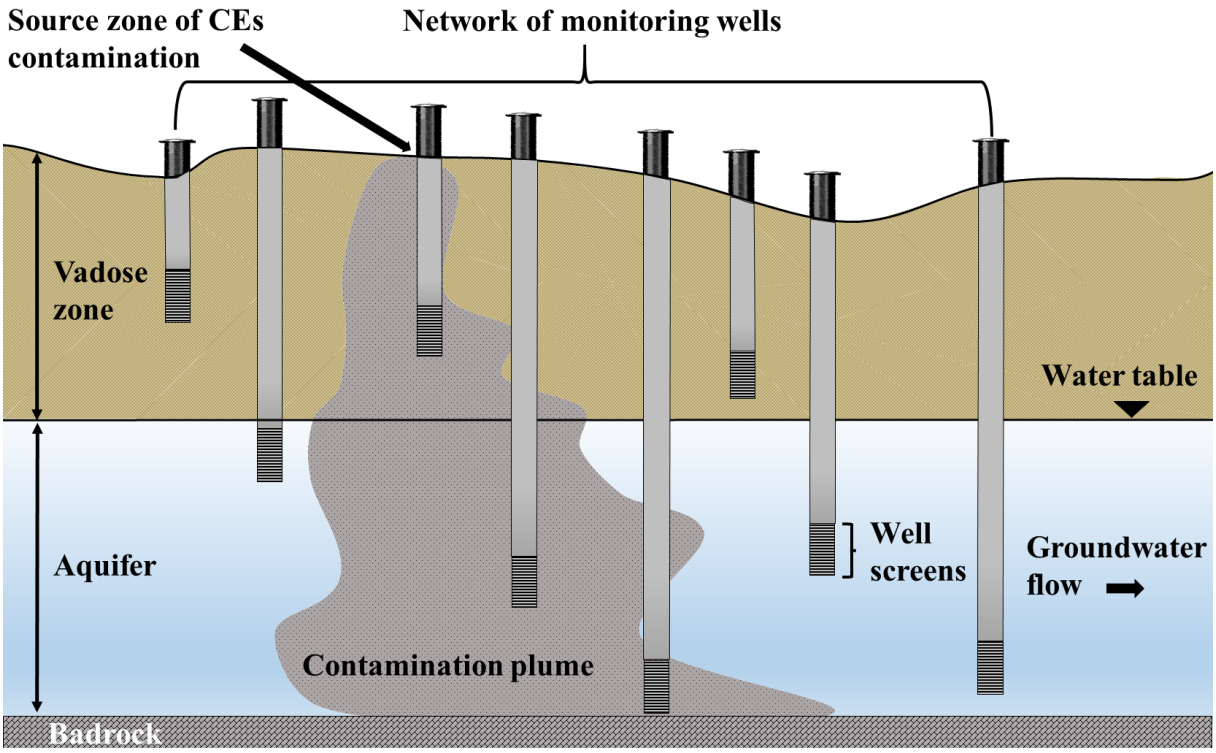

Fig. 1.3 Schematic of a site monitoring using a network of monitoring wells

Such network of monitoring wells ensures collection of field data, which are ideally used to document the loss of CEs and formation of their daughter compounds, presence and distribution of biogeochemical indicators of natural attenuation, and direct microbiological evidence (Witt et al., 2002). Only the

collection of these heterogeneous data ensures sufficient evidence about ongoing natural attenuation of CEs at the site. The loss of CEs and formation of their daughter compounds are predominantly monitored by analysing collected samples of soil, soil gases, groundwater, and alternatively from samples of tree cores; however, three core sampling is restricted to only shallow subsurface layers containing tree roots (Larsen et al., 2008). While the loss of CEs and formation of their daughter compounds is a primary line of evidence about ongoing natural attenuation at the site, monitoring of biogeochemical indicators, such as redox potential (Eh), redox state, specific conductance, pH, dissolved oxygen (DO), total organic carbon (TOC), presence of hydrogen, methane, and dissolved chloride can be used to determine under what conditions natural attenuation of CEs is occurring (Holmes et al., 1998; Witt et al., 2002). Further, identification of microbial species and/or their functional genes involved in CEs degradation serves as a third line of evidence about ongoing natural attenuation, which allows assessing the biodegradation potential (capacity) of indigenous microbial populations. Besides these three crucial lines of evidence, an approximate time needed to achieve attenuation goals at sites may be estimated by using Natural Attenuation Software that models variety of attenuation processes, e.g. contaminant concentration changes, redox processes, advection, dispersion, sorption, and diffusion and dissolution of $NAPL_{CEs}$ (Mendez et al., 2004).

MNA generally requires much longer time frames to achieve clean-up objectives in comparison with bioremediation approaches, biostimulation and bioaugmentation. Moreover, MNA is not appropriate where imminent site risks are present, i.e. humans or environmental receptors exposure (US EPA, 2012b). Also, if possible, groundwater clean-up should ensure drinking water standards within a reasonable timeframe, which is not applicable in the case of MNA (US EPA, 2012b).

1.8.2 Biostimulation of CEs-polluted sites

Biostimulation allows considerable flexibility in technical design of clean-up systems, which depends on a part of subsurface that is treated, i.e. vadose zone or aquifer as well as on the method selected to establish a bioreactive zone, the part of a subsurface environment in which microbial degradation of CEs is stimulated (Das and Dash, 2014). The remedy systems for biostimulation commonly contain a variety of injection wells used to deliver biostimulative agents (electron acceptors, electron donors, primary substrates or nutrients) into a subsurface environment along with a network of monitoring wells. In general, the systems should be designed in a manner ensuring the formation of an extensive bioreactive zone in the subsurface and sufficient retention of CEs within the zone (Semprini, 1997, Frascari et al., 2015). In the last few decades, many studies have examined a variety of systems for biostimulation of either anaerobic reductive dechlorination or cometabolic aerobic degradation of CEs in a vadose zone and groundwater. (Hopkins et al., 1993; Bennett et al., 2007; Kim et al., 2008; Dugat-

Bony et al., 2012). This effort has led to the development of three main strategies for establishment of the bioreactive zone; (1) direct injection of biostimulative agents into the treatment zone; (2) groundwater extraction amendment and reinjection in a closed loop system; (3) recirculation of amended groundwater using recirculation wells (Semprini, 1997; Frascari et al., 2015).

Systems for a direct injection of biostimulative agents

This strategy uses injection wells for direct introduction of biostimulative agents into a delimited zone of the contaminated subsurface and thus allowing to establish a bioreactive zone through which contamination plume gradually migrates. Simplified wells that are commonly used to stimulate the microbial degradation of CEs in a subsurface environment are shown in Fig. 1.4.

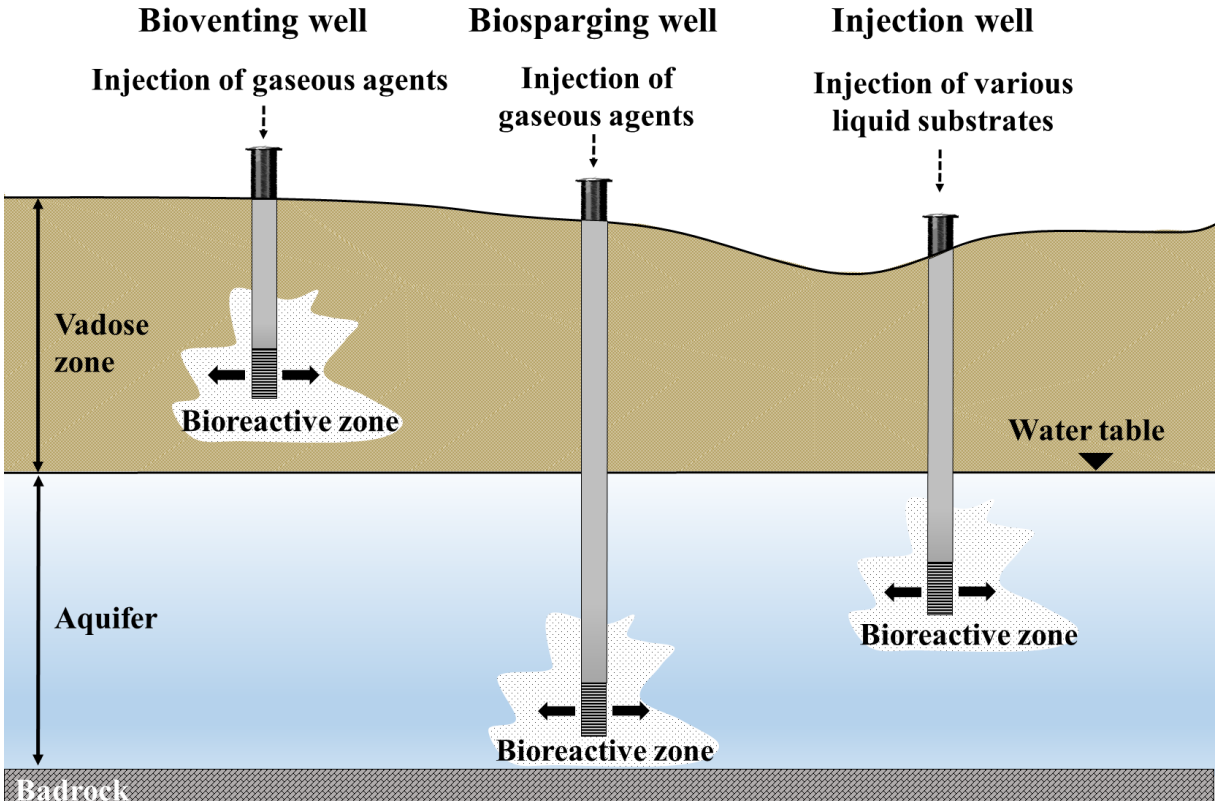

Fig. 1.4 Wells for biostimulation of microbial degradation of CEs in a subsurface environment[②]

This strategy appears as the only option to stimulate microbial degradation of CEs in a vadose zone by using bioventing wells for direct injection of gaseous substrates and oxygen into the bioreactive zone (Frascari et al., 2015). Also, the systems for direct injection of biostimulative agents (both liquid and gaseous) can be used for bioremediation of CEs-polluted aquifers. Whilst it is

[②] The position of the monitoring boreholes is not shown for simplicity

a common practice that a variety of injection wells are used to introduce liquid agents into aquifers, gaseous agents are delivered through biosparging wells.

The main disadvantage of the systems for direct injection of biostimulative agents is the formation of a narrow bioreactive zone that is typically formed in the immediate vicinity of each injection well. Hence, further migration of contaminants beyond the treatment zone is highly likely (Hoelen et al., 2006).

Closed loop systems

These systems are utilized for bioremediation of aquifers and generally consist of a pair of injection and extraction wells connected to a surface gas-tight tank for amendment of groundwater extracted from an aquifer prior to its re-injection into the aquifer. A simplified scheme of a typical closed loop system is shown in Fig. 1.5.

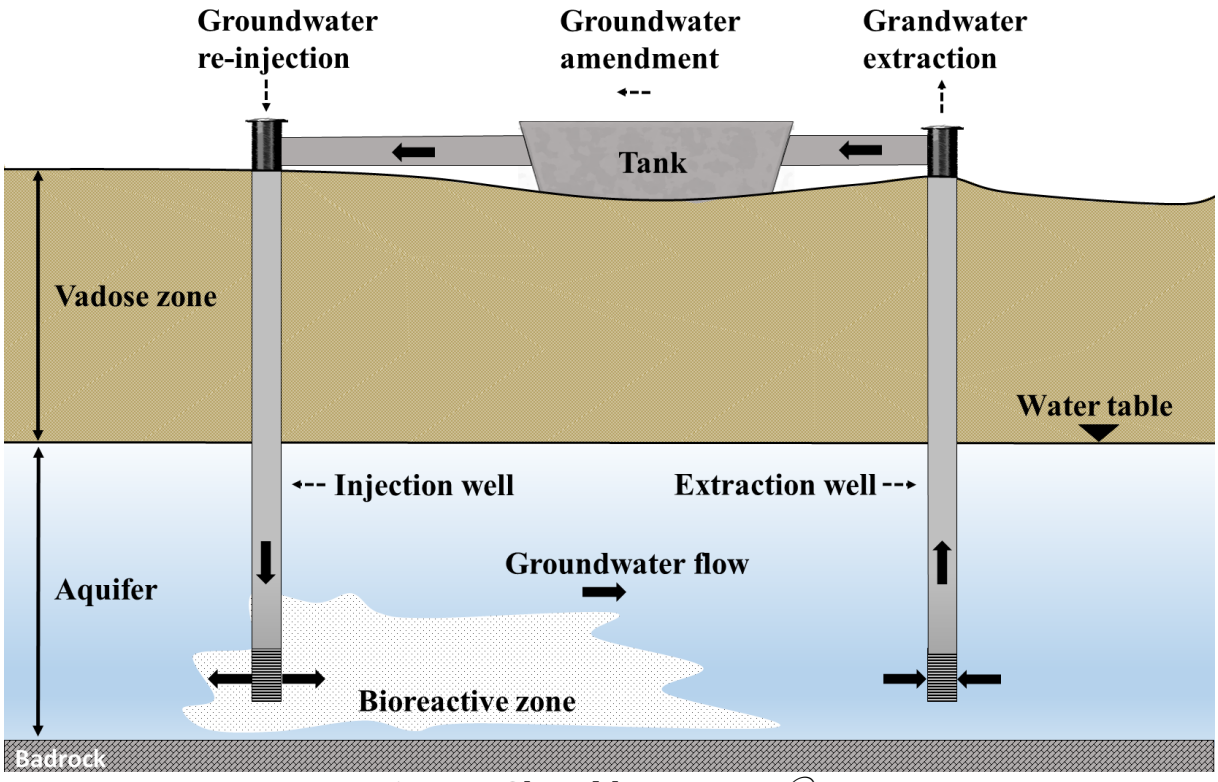

*Fig. 1.5 Closed loop system*③

Closed loop systems promote recirculation of polluted groundwater through an established bioreactive zone within a delineated part of an aquifer that usually extends several meters between the injection and extraction wells.

A full-scale application of these systems as the only remedy strategy is unlikely due to two main reasons. First of all, only a limited bioreactive zone can be

③ The position of the monitoring boreholes is not shown for simplicity

established within these systems (1 to 2 meters from the injection well) and second, pumping of groundwater to the surface is costly.

Systems employing recirculation wells

Another strategy for in-situ bioremediation of aquifers is an application of systems employing subsurface recirculation wells equipped with a submersible pump installed between two screens, one of which serves for extraction of groundwater and second for its discharge (Semprini, 1997). A simplified scheme of a system employing two recirculation wells for treatment of two underlying aquifers is illustrated in Fig. 1.6.

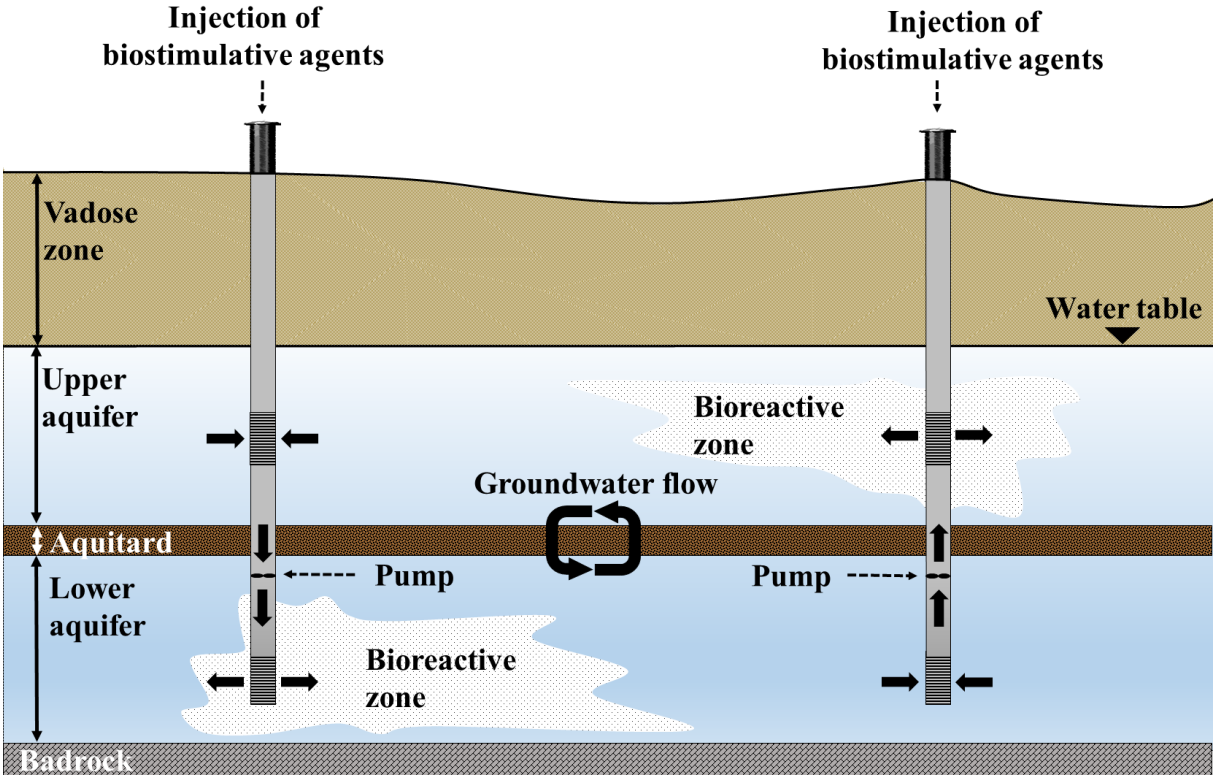

*Fig. 1.6 System employing two recirculation wells*④

Biostimulative agents are introduced into the wells through feed lines and mixed with CEs-contaminated groundwater using mixers placed inside the wells; each well ensures the formation of a bioreactive zone around the discharge screen as well as recirculation of groundwater across the zone allowing more time for biological reactions to occur (McCarty et al., 1998). In general, these systems ensure a treatment of aquifers without a need to pump groundwater on the surface, hence the complete process occurs in-situ, which is less expensive compared to the previously reviewed closed loop systems (Frascari et al., 2015). Also, the

④ Inspired by the work of Hoelent et al. (2006), and Bennett et al. (2007); the position of the monitoring boreholes is not shown for simplicity

bioreactive zones formed within the systems employing recirculation wells are broader and thus offering more efficient removal of targeted CEs.

1.8.3 Bioaugmentation of CEs-polluted sites

Two different bioaugmentation approaches have been developed for in-situ bioremediation of CEs-polluted sites. While in the first approach, large amounts of microbial suspensions are injected to the subsurface with the aim to achieve rapid removal of contaminants before the injected cells are inactivated or perished (Duba et al., 1996), the second approach aims to achieve prolonged survival and growth of the added microorganisms along with long-term degradation of targeted contaminants within an established bioreactive zone in the subsurface (Semprini et al., 2007). Whereas the rapid clean-up approach can only be applied for treatment of aquifers, the second approach, relying on the establishment of the bioreactive zone, allows treating not only aquifers but also low permeable matrices, such as clay layers.

For the purpose of this work, the two bioaugmentation approaches are further referred to as a rapid bioaugmentation approach and a long-term bioaugmentation approach.

At first, rapid bioaugmentation of aquifers is an efficient clean-up strategy ensuring significant removal of CEs within a few days of operation (Duba et al., 1996). In comparison, biostimulation may require several months of operation to achieve the same removal efficiency. However, the high efficiency of the fast bioaugmentation approach is time-limited and its application seems to be restricted to only highly contaminated parts of aquifers underlying the source of contamination. Secondly, the long-term bioremediation approach has shown to be a promising clean-up strategy for CEs-polluted aquifers and low permeable matrices (Scheutz et al., 2010; Verce et al., 2015). Especially bioaugmentation of clay deposits showed a great promise for reducing contamination within the matrix and so diminishing the risk of secondary contamination of aquifers. On the other hand, the efficiency of the site bioaugmentation containing 1,1DCE showed to be only temporal and so not suitable as the long-term clean-up strategy (Semprini et al., 2007).

2. OBJECTIVES OF THE THESIS

A theoretical part of the doctoral thesis aimed to write a comprehensive summary dealing with an influence of the physicochemical properties of CEs on their transport and a fate in a subsurface environment, and to outline common procedures of a site investigation as well as to review different bacterial strategies for degradation and transformation of CEs, and the possible use of these strategies for in-situ bioremediation of contaminated soils and groundwater. Within this aim was also to summarize field studies (not included in the Doctoral Thesis Summary) that have achieved significant removal of chloroethenes at polluted sites. Such a review should be later published in an appropriate scientific journal with an impact factor.

In an experimental part of the thesis, the main aim was to investigate the microbial degradation of all three dichloroethene isomers (DCEs), trichloroethene (TCE), and vinyl chloride (VC) in a mineral salt medium (MSM) by *Comamonas testosteroni* RF2. Within that objective were performed studies focusing on the degradation of single compounds as well as on degradation of the compounds in mixed samples. Degradation studies investigating the ability of *C. testosteroni* RF2 to degrade various mixtures of the chloroethenes were performed in terms to imitate model conditions in contaminated groundwater. In addition, kinetics of single DCEs in *C. testosteroni* RF2 along with tracking transformation intermediates of the compounds were studied too. Also, in order to find a suitable consortium capable of degrading VC and other chloroethenes in mixed samples, several bacterial consortia containing *C. testosteroni* RF2 and one of the following strains capable of degrading VC, *Mycobacterium aurum* DSM-6695, *Pseudomonas putida* DSM-7189, and *Rhodococcus ruber* DSM-7511, were studied for their degradation potential.

3. METHODOLOGY OUTLINE

3.1 Important chemicals

TCE (99%), *c*DCE (99.1%), *t*DCE (99.7%), 1,1DCE (99.9%), VC (99.5%), chloroacetyl chloride (min. 99.0%), Mercury(II) thiocyanate, and sodium lactate were obtained from Sigma-Aldrich. Ordinary chemicals were purchased from local suppliers.

3.2 Bacterial strains

Comamonas testosteroni RF2 isolated from activated sludge fed by phenol (Ruzicka et al., 2002).

Mycobacterium aurum DSM-6695 isolated from contaminated soil; purchased from German Collection of Microorganisms and Cell Cultures (DSMZ).

Pseudomonas putida DSM-7189 isolated from a sample of soil; purchased from DSMZ.

Rhodococcus ruber DSM-7511 isolated from contaminated subsurface sediments; purchased from DSMZ.

3.3 Degradation assays

The assays were performed in 40 mL sterile glass vials sealed with sterile (UV irradiated) gas-tight septum caps (WHEATON). Experiments were always conducted at least in two duplicates, in addition to which abiotic blanks were always carried out. Each vial contained 10 mL of mineral salt medium (MSM), a cell suspension (10 μL), phenol (100 mg L^{-1}), lactate sodium (100 mg L^{-1}) and the methanolic solutions of chlorinated ethenes. All the chloroethene concentrations listed are intended as actual concentrations in a liquid phase unless otherwise stated. Test vials were incubated in darkness on a shaker (150 rpm) set to semi-continuous mode at 25 ºC for 7- 23 days, according to the objective of the research.

3.4 Analysis of samples containing chloroethenes

The chloroethenes were first extracted by the Purge and Trap method in the concentrator Teckmar LSC 2000 and monitored on a Hewlett Packard 5890 Series II GC device equipped with a Quadrex capillary column (Methyl phenyl cyanopropyl silicone) at 29.87 m length, 0.53 mm (inner diameter) and 3 μm in film thickness, supplemented with an electron-capture detector (ECD), as previously described (Ruzicka et al., 2011). Initial concentrations of CEs in prepared samples, as well as the determination of the achieved removals of the compounds, were performed by using previously assembled calibration curves.

3.5 Analysis of samples containing vinyl chloride

Samples containing VC were prepared and cultivated identically as described in section 3.3. The analysis of samples was carried out at the Institute of Chemistry at the Tomas Bata University in Zlín using an internal standard method with propane-2-ol as the internal standard. Samples analysis was performed by using GC-MS Shimadzu QP-2010 equipped with the Equity-1 (30 m, 0.32 mm, 1μm) column, and He as carrier gas at constant linear velocity (58.8 $cm \cdot s^{-1}$) was used; GC method: 40 °C/10 min, 20 °C/min to 250 °C, hold for 14.5 min, IS 200 °C/70 eV; MS method: acquisition was started at 0.41 min and two mass-selected ions were detected (SIM mode).

3.6 Cell survival after dichloroethene degradation

The preparation of samples was identical to the degradation assays described above. Samples were prepared in duplicates for each dichloroethene tested. Colony-forming units (CFUs) grown on Tryptone Yeast Agar were directly counted and compared with the number of CFUs determined in parallel samples without the given dichloroethene(s).

3.7 Determination of intermediates of *c*DCE and *t*DCE degradation

Degradation samples were prepared as described in section 3.3; however, the determination of the intermediates was performed in cooperation with two institutes, namely: Institute for testing and certification (ITC) in Zlín, Czech Republic; and Regional Hygiene Station of the Olomouc Region based in Olomouc, Czech Republic.

Solid Phase Microextraction (SPME) and Purge and Trap extraction were used along with GC-MS to determine the intermediates produced during cometabolic degradation of *c*DCE and *t*DCE in *C. testosteroni* RF2

3.8 Determination of chloride release

Conditions during dichloroethene degradation resembled those in the degradation assays described above; with the exception of MSM that was replaced by the chloride-free mineral medium. After a certain period of degradation, the liquid phases of the samples were analysed for the presence of inorganic chlorides. Prior the determination of chloride concentration in the samples according to the Iwasaki method (Iwasaki et al., 1952), bacterial cells were removed by filtration through a 0.22 μm pore-sized syringe filter (Millex). Also, blank tests without the appropriate dichloroethene as well as tests without cells and with the dichloroethene were performed in parallel; obtained results were subtracted.

4. RESULTS AND DISCUSSIONS

4.1 Cometabolic aerobic degradation of single DCEs

C. testosteroni RF2 was subjected to degradation assays of *c*DCE, *t*DCE and 1,1DCE separately. Phenol and lactate were applied throughout all the assays with RF2 because the application of these substrates ensured sufficient multiplication of the bacterial cells as well as induction of the desired enzyme in previous assays with *c*DCE (data not shown). Each degradation test lasted for seven days, and at least two different concentrations of each isomer were applied. The data obtained on removing the DCEs and subtracted numbers of CFUs at the end of the chosen tests are given in Table 4.1.

Table 4.1 Cometabolic degradation of single DCEs by *Comamonas testosteroni* RF2 (means ± SD), including abiotic blanks⑤

CE	Initial concentration "all in liquid phase" (mg L^{-1})	Actual initial concentra-tion (mg L^{-1})	Compound removal (%)	Cell number (10^7 CFU mL^{-1})	
				DCE degradation	Blank test without dichloro-ethene
*c*DCE	1.93	1.72	100	11 ± 1.5	11 ± 2.5
*c*DCE	7.06	6.01	100	N	N
*c*DCE[a]	7.06	6.01	<0.5	N	N
*t*DCE	1.35	0.97	72.2 ± 3.0	8 ± 0.5	5 ± 0.5
*t*DCE	6.92	3.80	65.8 ± 1.0	N	N
*t*DCE[a]	6.92	3.80	<0.5	N	N
1,1DCE	0.91	0.25	100	N	N
1,1DCE	1.33	0.37	65.5 ± 7.9	3 ± 1.0	28 ± 1.0
1,1DCE	6.91	1.77	1.6 ± 0.9	0.001	28 ± 1.0
1,1DCE[a]	6.91	1.77	1.6 ± 0.9	N	N

The results listed in Table 4.1 showed that the strain is able to degrade all three DCE isomers with prominent activity towards *c*DCE, as the entire 6.01 mg L^{-1} of this compound was removed at the end of the assay. In comparison to *c*DCE

⑤ [a] *Abiotic blank (without cells); N: not tested;* * *Initial concentration "all in a liquid phase": calculated and injected dosage of chloroethenes into test vials; Actual initial concentration: measured concentrations of chloroethenes in a liquid phase after equilibrium partitioning using GC-ECD system*

degradation, efficiencies for removing 1,1DCE and *tDCE* were significantly lower, yet still interesting for potential strain utilization in bioremediation processes. Despite *t*DCE did not influence RF2 growth, this compound was not fully degraded in any of the assays. In contrast, 1,1DCE appeared to be the only dichloroethene with a toxic effect against RF2 cells under higher tested concentrations. While *c*DCE and *t*DCE degradation led to the same or slightly reduced cell counts as the blank tests without *c*DCE and *t*DCE enrichments, samples with higher 1,1DCE concentrations showed significantly reduced numbers of living cells. This reduction was especially dramatic if 1.77 mg L^{-1} of 1,1DCE was applied; under such a condition, bacterial growth was completely inhibited. Above all, 1,1DCE (1.77 mg L^{-1}) completely inhibited the degradation of *c*DCE (6.01 mg L^{-1}) in mixed samples (data not shown). The results of 1,1DCE degradation (1.77 mg L^{-1}) highlighted a key importance of RF2 growing cells for the degradation and this fact was fostered by abiotic blanks in which no or negligible reductions in DCEs concentrations were observed.

4.2 Cometabolic degradation of a quaternary mixture containing TCE and all three DCEs

The RF2 strain was examined for its ability to degrade all DCEs along with TCE in a prepared mixture that included 1,1DCE, *c*DCE, *t*DCE and TCE (51.4 µg L^{-1}; 144.3 µg L^{-1}; 91.6 µg L^{-1}; 122.5 µg L^{-1}, respectively). The composition of the mixture and the chloroethene concentrations were chosen to resemble groundwater pollution at a site affected by chlorinated ethenes. The results for removing the chloroethenes after seven days of the assay are given in Table 4.2

*Table 4.2 Cometabolic degradation of a quaternary mixture of chloroethenes by Comamonas testosteroni RF2 (means ± SD)*⑥

Compound	Initial concentration "all in a liquid phase" (µg L^{-1}) *	Actual initial concentration (µg L^{-1}) *	Compound removal (%)
TCE	154.2	122.5 ±1.70	100
*c*DCE	170.7	144.3 ± 3.46	100
*t*DCE	151.4	91.6 ± 5.86	79.0 ± 3.7
1,1DCE	158.6	51.4 ± 4.21	100

When all the chloroethenes were used at concentrations corresponding to approx. 150 µg L^{-1} "all in liquid phase", *C. testosteroni* RF2 was able to

⑥ * *Initial concentration "all in a liquid phase": calculated and injected dosage chloroethenes into test vials; Actual initial concentration: measured concentrations of chloroethenes in a liquid phase after equilibrium partitioning using GC-ECD system*

completely remove 1,1DCE, *c*DCE and TCE and a significant portion of *t*DCE (79.0 ± 3.7 %). The results obtained can be considered valuable proof that RF2 bacterium is able to degrade not only single DCEs but also all three isomers along with TCE in the given mixture of CEs. Furthermore, no abiotic reductions in TCE and DCEs concentrations were observed during the assay (data not shown).

4.3 Chloride production during *c*DCE degradation

Chloride release after degradation of chloroethenes is an important indicator of DCE dechlorination. *c*DCE was chosen for this assay as it is one of the most significant pollutants occurring in groundwater. Moreover, this compound was readily degraded by RF2 in previous experiments. In order to investigate the dechlorination of potentially formed chlorinated intermediates, the assay lasted 22 days and the course of the assay over time is shown in Fig. 4.1.

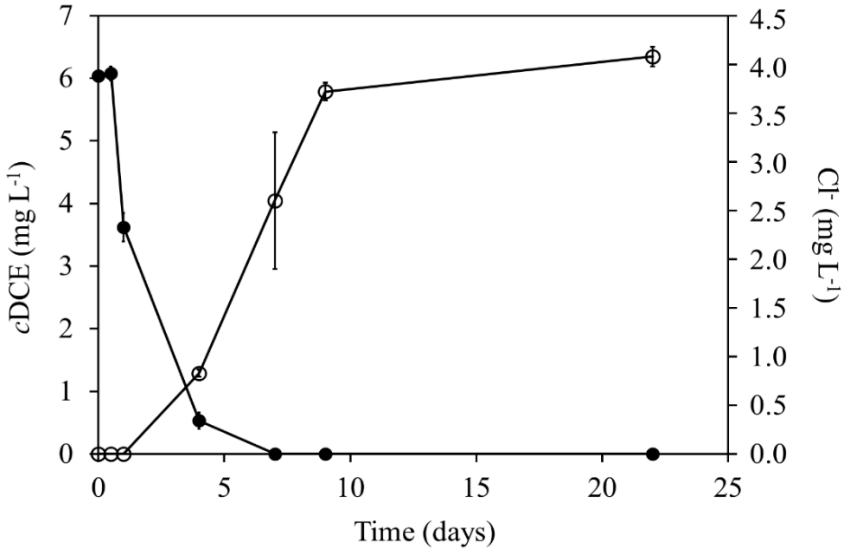

Fig. 4.1 Release of inorganic chlorides during cDCE degradation[7]

Generally, a decrease in *c*DCE concentration was clearly accompanied by the production of chlorides. However, although *c*DCE was completely removed in 7 days, a further increase in chloride concentration occurred in the next few days. Based on these results, the formation of the two unstable chlorinated intermediates that appeared on the chromatogram during the assay is the most probable explanation for the chloride release observed in the later stage of the experiment. Finally, over 92 % mineralization of the chlorine originally bound in *c*DCE was discerned after 22 days of the assay (Fig. 4.1).

[7] *(●) cDCE; (o) inorganic chlorides (abiotic blanks subtracted); cDCE concentrations are actual concentrations of the compound in a liquid phase at given times*

4.4 DCEs degradation kinetics and metabolites monitoring

So as to monitor the formation of *c*DCE and *t*DCE intermediates, further assays on the degradation kinetics of these DCEs were performed. Monitoring the *c*DCE and *t*DCE intermediates lasted until no intermediate was detected by GC-ECD. The resultant unknown intermediates were named I1 and I2 respectively; their formation and dissipation along with *c*DCE and *t*DCE degradation are given in Fig. 4.2.

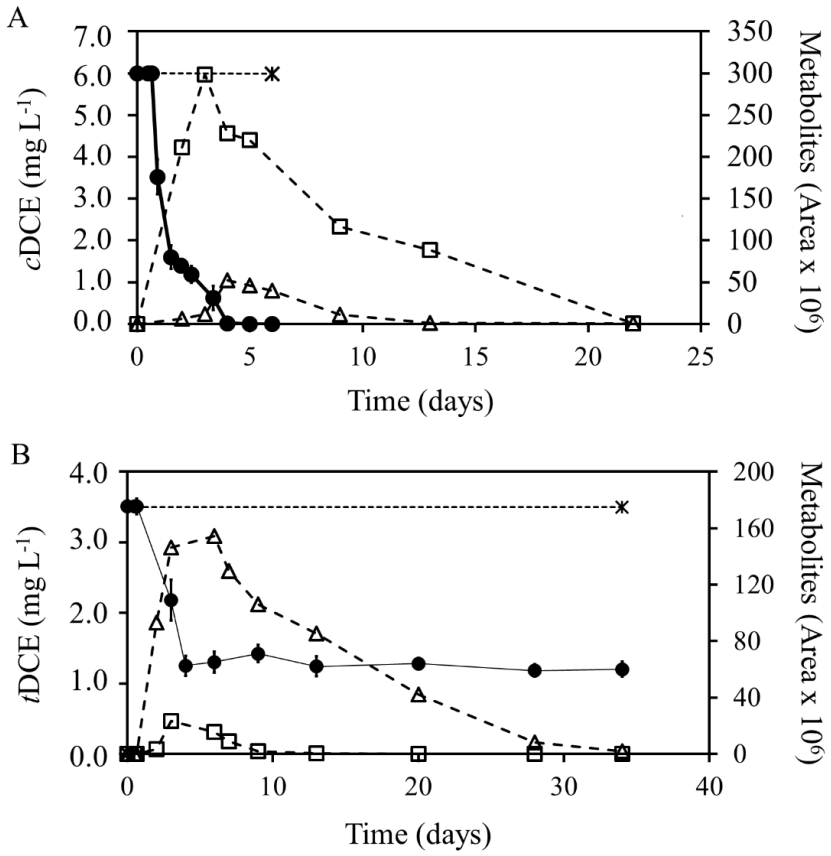

Fig. 4.2 cDCE (A) and tDCE (B) degradation kinetics and formation of intermediates[8]

Fig. 4.2 shows that during individual degradation of both *c*DCE and *t*DCE, certain amounts of the two unknown intermediates (I1 and I2) transiently accumulated during the first few days of the assays but subsequently disappeared later. In the course of *c*DCE degradation, I1 had a major response on GC-ECD,

[8] *(●) cDCE and tDCE; (□) intermediate I1; (Δ) intermediate I2; both intermediates are expressed as peak areas; (x) abiotic tests; cDCE and tDCE concentrations are actual concentrations of the compounds in a liquid phase at given times*

followed by a considerably reduced response of I2, whereas degradation of *t*DCE resulted in exactly the reverse formation of peaks, with the main response for I2 and a significantly lesser response for I1.

Moreover, interestingly, whereas degradation of *c*DCE and *t*DCE resulted in the formation of the two intermediates, degradation of 1,1DCE (under the identical conditions) did not result in any of the two intermediates produced during degradation of *c*DCE and *t*DCE even though 1,1DCE was completely degraded as shown Fig. 4.3.

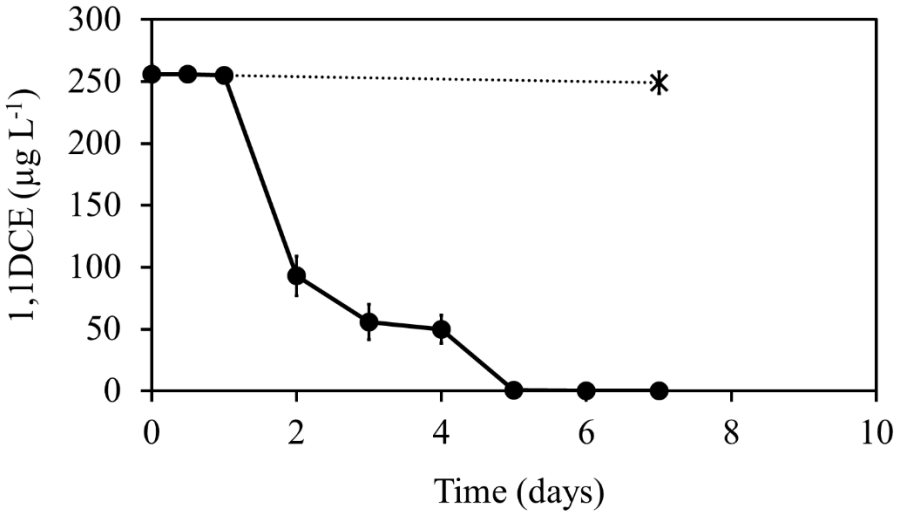

Fig. 4.3 Degradation kinetics of 1,1DCE[9]

4.5 Determination of *c*DCE and *t*DCE intermediates in the RF2 strain

A great effort was made to identify the I1 and I2 intermediates. To this end, I1, which showed a major peak in GC-ECD chromatograms during *c*DCE degradation, was detected by both SPME-GC-MS and Purge and Trap-GC-MS as 2,2-dichloroacetaldehyde; however, I2 was not detected by these methods.

Considering the possible reactions for the initial steps of *c*DCE degradation controlled by monooxygenase would mean that various amounts of an aldehyde, such as 2,2-Dichloroacetaldehyde and *c*DCE epoxide, could be produced (Nishino et al., 2013); the proposed pathways for *c*DCE transformations in RF2 strain are described in Fig. 4.4.

[9] *(●) 1,1DCE; (x) abiotic tests; 1,1DCE concentrations are actual concentrations of the compound in a liquid phase at given times*

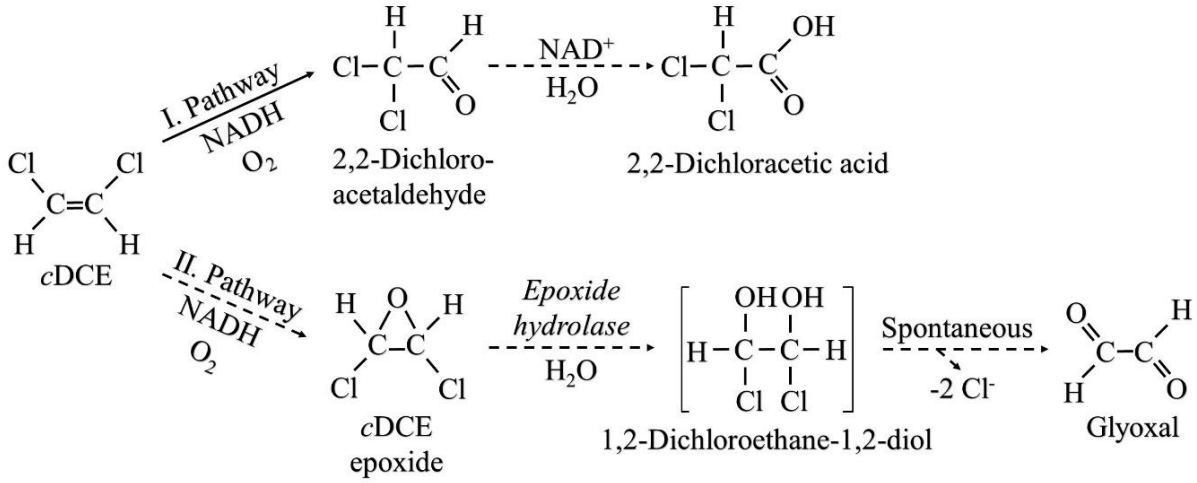

Fig. 4.4 Potential degradation pathways for cDCE degradation in RF2[10]

Consequently, it can be hypothesized that I2 might be 1,2-dichloroethane-1,2-diol, which was described as a product of hydrolysis of the *c*DCE epoxide (Nishino et al., 2013). However, this could not be clearly proven due to the fact that the compound is not commercially available, hence was not tested in this study. On the basis of the results obtained, it is not possible to state which of the two pathways, as described in Fig. 4.4, prevails during *c*DCE degradation. This is because the responses of both intermediates in the ECD chromatograms are significantly influenced by their molecular structure. Nevertheless, the previously reported 92.31 ± 2.26 % release of chlorides clearly demonstrates the high level of desired mineralization of organically bound chlorine.

Further, it may be supposed that ***t***DCE degradation pathways proceed similarly as in the case of *c*DCE, except the *t*DCE epoxide formation instead of the *c*DCE epoxide in the II. pathway. However, even though the removal of *t*DCE (6.92 mg L^{-1}; 65.8 ± 1.0 %) was lower than the removal of *c*DCE (7.06 mg L^{-1}; 100 %) under same conditions, its transformation resulted in the more significant production of the unknown intermediate (I2) than in the case of *c*DCE transformation. Hence, it might be speculated that other degradation pathway, such as the proposed II. pathway in Fig. 4.4, is more preferred than the I. pathway with 2,2-dichloracetaldehyde as the main transformation intermediate. Therefore, it can be stated that a position of chlorine atoms in the DCE molecules not only plays a crucial role in the removal efficiency of the compounds, but it may also be a key factor in determining a distribution of degradation pathways. Nevertheless, to the best knowledge of the author of the thesis, the exact determination of *t*DCE degradation pathways in aerobic bacterial strains has yet not been done by any study.

[10] *Solid lines: degradation steps based on the results of the present study; Dashed lines: degradation steps proposed by Nishino et al. (2013)*

4.6 Cometabolic degradation of binary mixtures containing VC and *c*DCE

VC and *c*DCE are commonly formed at the edges of contamination plumes in groundwater originally polluted by PCE and TCE. Thus, bacterial strains capable of degrading both compounds are of interest for bioremediation purposes.

Several assays were performed in terms to evaluate the ability of RF2 to cometabolically degrade VC and *c*DCE in binary mixtures. While the applied concentration of *c*DCE (1.18 mg L^{-1}) was the same throughout all the assays, the concentration of VC (0.67 mg L^{-1}; 0.93 mg L^{-1}), as well as the duration of the assays, differed. Despite the variety of the assays, none of the applied concentrations of VC in binary mixtures with *c*DCE (1.18 mg L^{-1}) resulted in neither an inhibitory nor a toxic effect against bacterial cells. Most importantly, it exerted no influence on *c*DCE degradation, which was completely removed (data not shown).

4.7 *c*DCE and VC degradation kinetics by different bacterial consortia

Degradation kinetics of *c*DCE (6.09 mg L^{-1}) and VC (10 mg L^{-1}; "all in a liquid phase") in binary mixtures were examined by three consortia, each of which contained the strain RF2 and one of the DSMZ strains. Growth substrates for the consortia were selected based on the results of the previous tests (not shown) and are listed in Table 4.3 along with consortia designations.

Table 4.3 Bacterial consortia used for degradation of *c*DCE and VC in a binary mixture

Consortium designation	Composition of a bacterial consortium	Used organic substrates
RF2-Rr	RF2 + R. ruber DSM-7511	phenol (200 mg L^{-1})
RF2-Pp	RF2 + P. putida DSM-7189	phenol (150 mg L^{-1}) + 3-chloropropan-1-ol (50 mg L^{-1})
RF2-Ma	RF2 + M. aurum DSM-6695	phenol (100 mg L^{-1}) + lactate (100 mg L^{-1})

Unfortunately, due to the technical issue with GC-ECD, monitoring the loss of VC using the system appeared to be tricky and no VC removal could be confirmed as no peak in a retention time for VC appeared on chromatograms, including the initial concentrations of the compound (10 mg L^{-1}; "all in a liquid phase"). In contrast, degradation kinetics of *c*DCE (6.09 mg L^{-1}) was well monitored and the results obtained for each consortium are shown in Fig. 4.5.

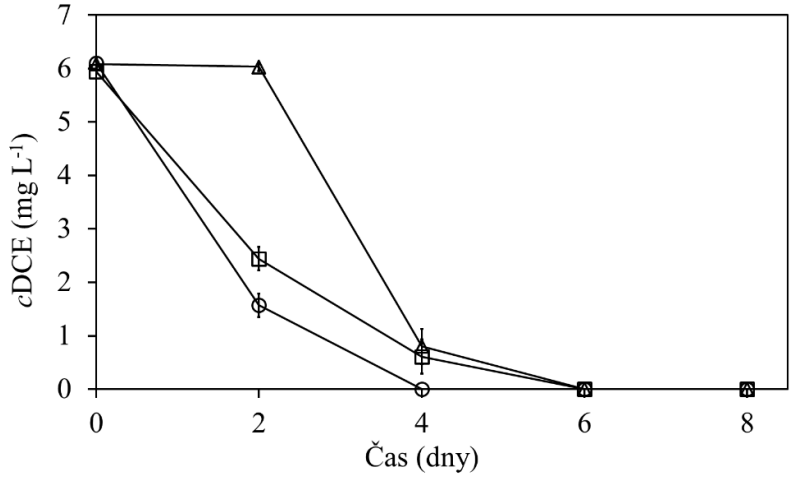

Fig. 4.5 Degradation kinetics of cDCE in a binary mixture with VC by different bacterial consortia[ⅱ]

Although the results showed that *c*DCE in a binary mixture with VC was efficiently degraded by the strain RF2 in all three consortia, the degradation kinetics of the compound by each consortium differed as seen in Fig. 4.5. Whereas *c*DCE was completely removed within the first four days of the assay by a consortium composed of RF2 and *M. aurum* DSM-6695, the removal of the compound by other two consortia, composed of RF2 and *R. ruber* DSM-7511 or *P. putida* DSM-7189, took more time. The faster removal of *c*DCE may be attributed to the partial degradation of the compound by the *M. aurum* DSM-6695. Indeed, the strain was described to be able to partially degrade all three dichloroethenes when grown on VC (Hartmans and De-Bont, 1992). Also, there is no substrate competition between the strain RF2 and *M. aurum* DSM-6695, which cannot utilize phenol. In comparison, *P. putida* DSM-7189 and especially *R. ruber DSM*-7511 can compete with RF2 for phenol as well as RF2 can compete with *P. putida* DSM-7189 for 3-chloropropan-1-ol.

4.8 Degradation kinetics of TCE, all DCEs and VC in mixed samples

Degradation kinetics of TCE (115.7 μg L^{-1}), all DCEs (662 μg L^{-1} of *c*DCE; 42.01 μg L^{-1} of *t*DCE; 16.02 μg L^{-1} of 1,1DCE), and VC (7 mg L^{-1}; "all in a liquid phase") in mixed samples by a consortium composed of the strain RF2 and *M. aurum* DSM-6695 was examined with the aim to ideally achieve a complete removal of all chloroethenes. The consortium was selected based on the previous

[ⅱ] *(○) C. testosteroni RF2 and M. aurum DSM-6695; (□) C. testosteroni RF2 and P. putida DSM-7189; (Δ) C. testosteroni RF2 and R. ruber DSM-7511; cDCE concentrations are actual concentrations of the compound in a liquid phase at given times*

results obtained from the degradation kinetics of *c*DCE in a binary mixture with VC. While removals of TCE and DCEs were monitored by GC-ECD, the system that has already been proven to be a reliable analytical method for monitoring of these compounds, VC removals were monitored by GC-MS as described in methods. Results of all measurements are given in Fig. 4.6.

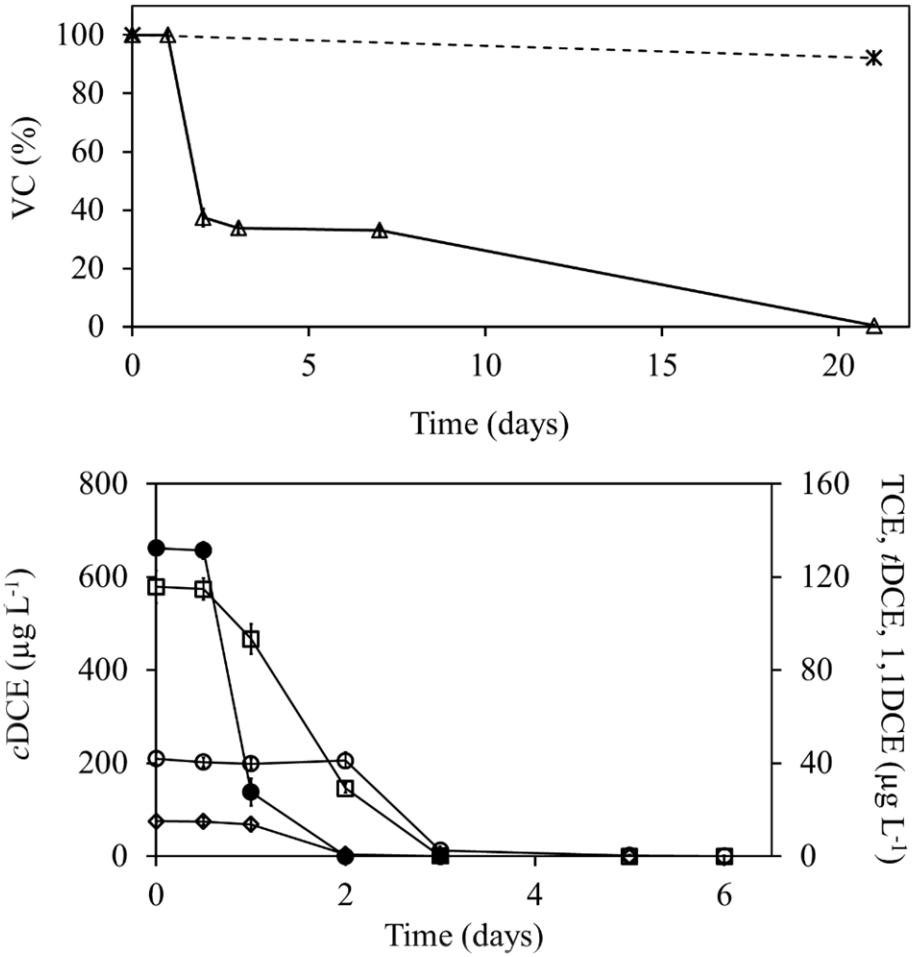

Fig. 4.6 Degradation kinetics of TCE, all DCEs, and VC in mixed samples by a bacterial consortium composed of C. testosteroni RF2 and M. aurum DSM-6695[12]

Fig. 4.6 shows that all chloroethenes were practically fully removed after 21 days of the assay. Whereas TCE and all DCEs were removed during first 6 days, complete removal of VC lasted longer. However, a real degradation time required for the complete removal of VC could have been shorter than 21 days as

[12] *(□) TCE; (●) cDCE; (○) tDCE; (◊) 1,1DCE; (Δ) VC; (x) abiotic tests; CEs concentrations are actual concentrations of the compounds in a liquid phase at given times*

no sample analyses were made between days 7 and 21. The decision to not analyse the samples during the mentioned period was made intentionally as only negligible removal of VC was observed between days 2 and 7 (4.32 %). Among others, 99.6 % of *t*DCE (42 μg L^{-1}), the compound that was not fully degraded in previous assays, was removed which could be explained either by its lower concentration applied in the assay or due to its partial cometabolic degradation by *M. aurum* DSM-6695. Furthermore, Fig. 4.11 shows that degradation of *t*DCE (42.01 μg L^{-1}) was initiated only after the complete removal of *c*DCE (662 μg L^{-1}) and 1,1DCE (16.02 μg L^{-1}) and significant removal of TCE (115.7 μg L^{-1}; 74.8 ± %) and VC (7 mg L^{-1}; 62.8 ± 2.98). Thus, the results suggest that cells of the strain RF2 and, perhaps to a lesser extent, *M. aurum* DSM-6695 have a lower affinity towards *t*DCE than *c*DCE, 1,1DCE, and TCE.

Further, with regard to investigate a possible influence of VC degradation by *M. aurum* DSM-6695 on degradation efficiency of other chloroethenes by RF2, the previous assay was repeated but no VC nor *M. aurum* DSM-6695 was added into degradation samples containing TCE (117.2 μg L^{-1}), *c*DCE (671 μg L^{-1}), *t*DCE (44.09 μg L^{-1}), 1,1DCE (16.13 μg L^{-1}), and cells of RF2; results are shown in Fig. 4.7.

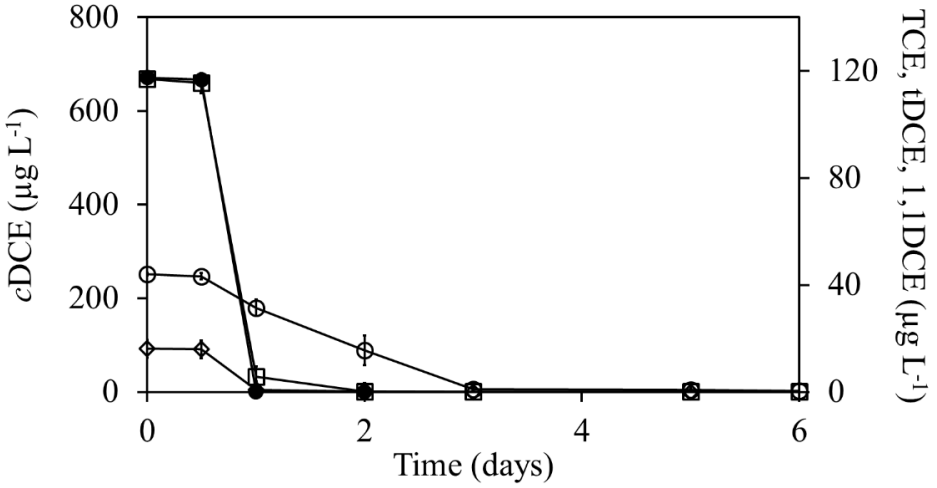

Fig. 4.7 Degradation kinetics of TCE and all DCEs by RF2

Degradation kinetics of chloroethenes in Fig. 4.7 shows that RF2 could alone remove all the compounds. Furthermore, all chloroethenes were removed in significantly shorter times if compared with results, where VC and *M. aurum* DSM-6695 were applied. Especially efficient degradation was observed for *c*DCE (671 μg L^{-1}) and 1,1DCE (16.13 μg L^{-1}), which were both completely removed during the first day. Also, TCE (117.2 μg L^{-1}) was nearly fully removed after 2 days of the assay, which is 1 day less than its degradation in the previous case. Finally, *t*DCE (44.09 μg L^{-1}) degradation was initiated during the first day when 28.7 ± 4.69 % of the compound was removed.

Based on these findings, it could be suggested, though unexpectedly, that RF2 itself can completely degrade low concentrations of *t*DCE. Moreover, it seems that rather than *M. aurum* DSM-6695 itself, but transformation intermediates of VC might have a temporary adverse effect on degradation kinetics of chloroethenes by RF2, i.e. reduction of degradation rates of the compounds. The most probable transformation intermediate with such an impact on RF2 cells could be chlorooxirane, which was described to be formed during the initial step in VC metabolism in *M. aurum* L1 that is catalysed by alkene (Hartmans and De-Bont, 1992).

Nevertheless, even though the reactive epoxide chlorooxirane could be formed during degradation of VC in *M. aurum* DSM-6695, and thus might also adversely affect degradation rates of chloroethenes by RF2, its effect was not significant enough to prevent the complete degradation of chloroethenes in this thesis. Therefore, the consortium composed of RF2 and *M. aurum* DSM-6695 displays potential for in-situ bioremediation of CEs-polluted subsurface.

5. CONCLUSION

The results of this thesis proved that phenol growing cells of the strain RF2 could degrade all DCEs along with TCE either as single compounds or in a mixture of these chloroethenes. The strain showed prominent activity towards *c*DCE, as the entire 6.01 mg L^{-1} of this compound was removed at the end of the assay. Further, despite *t*DCE (3.80 mg L^{-1}) did not influence RF2 growth, this compound was not fully degraded, which was most probably due to deficient molecular "lock and key" conformation between the molecules of phenol-2-monooxygenase of the strain RF2 and *t*DCE. Nevertheless, very low concentrations of *t*DCE (44.09 ± 2.52) in samples with other chloroethenes were almost completely degraded. Next, 1,1DCE turned out to be the most troublesome isomer, as only the low concentration of 0.25 mg L^{-1} was degraded completely and just partial removal of 0.37 mg L^{-1}) was observed. Furthermore, higher concentrations of 1,1DCE (1.77 mg L^{-1}) proven to be toxic towards RF2 cells and completely inhibited the degradation of *c*DCE (6.01 mg L^{-1}) in mixed samples with 1,1DCE.

Among others, the strain RF2 could not degrade any of the applied VC concentrations. Nevertheless, the ability of strain RF2 to degrade other chlorinated ethenes remained unaffected in presence of VC, which enabled construction of a consortium composed of strains RF2 and *M. aurum* DSM-6695. The consortium efficiently removed TCE, all DCEs and VC in a subsequent assay; although degradation of VC in *M. aurum* DSM-6695 temporarily affected degradation rates of other chloroethenes in strain RF2, nearly complete removal of the applied compounds was observed within three weeks of the assay.

The importance of these results can be supported by the fact that the application of phenol as the primary substrate for a field remediation has already been successfully tested (Hopkins and McCarty, 1995). Furthermore, it should be noted that all the assays performed herein resembled natural conditions and that *c*DCE, which is the most common intermediate produced during PCE and TCE anaerobic dehalogenation in groundwater, was efficiently removed both by strain RF2 and its consortium with *M. aurum* DSM-6695. In addition, the ability of the strain RF2 to degrade only low 1,1DCE concentrations should not pose a serious issue, as the production of this compound during natural attenuation of higher chlorinated ethenes was rarely observed (Schmidt and Tiehm, 2008; Tiehm and Schmidt, 2011). Also, *M. aurum* DSM-6695 could degrade VC in consortium with strain RF2 in a mixed sample containing TCE and all DCEs including toxic 1,1DCE. Therefore, the degradation of dichloroethenes and TCE in strain RF2 and the degradation of their mixture with VC in a consortium of the above-mentioned strains appeared to be a practical process for sufficient remediation of contaminated groundwater.

6. CONTRIBUTION OF THE THESIS TO SCIENCE AND PRACTICE

This thesis focused on the bacterial degradation of chlorinated ethenes (CEs) in mineral salt medium (MSM); the compounds, which are a significant source of soil and groundwater pollution worldwide. Although many studies have investigated the bacterial degradation of the compounds in MSM, the number of strains and consortia capable to degrade a broader range of chloroethenes is very limited. Hence, it is obvious that the research aiming to isolate new strains or consortia and further examine their degradation ability towards chloroethenes is desirable, and it might potentially lead to the application in practice for in-situ bioremediation of polluted sites by chloroethenes.

The contribution of the thesis to science and practice is as follow:

- It is the very first study describing the cometabolic degradation of all three dichloroethenes by a pure bacterial strain, *Comamonas testosteroni* RF2, utilizing phenol.
- Degradation of *c*DCE, the most common isomer found in polluted groundwater by perchloroethene and trichloroethene, in the RF2 lead to the high production of inorganic chlorides (92.2 %).
- 2,2-dichloroacetaldehyde was determined as one of the main transformation intermediates in RF2, and one of the degradation steps in *c*DCE degradation catalysed by monooxygenase was shown.
- Phenol-growing cells of the strain RF2 could degrade all three dichloroethenes along with trichloroethene, which is not usual for a pure bacterial strain, and it indicates its potential suitability for in-situ bioremediation applications.
- Most importantly, the consortium composed of the strain RF2 and *M. aurum* DSM-6695 could remove trichloroethene, all three dichloroethenes and vinyl chloride in mixed samples.
- The result shown in the thesis could be used for a further study focusing on improving the degradation efficiency of vinyl chloride by the consortium used in the present study.
- The degradation ability of the consortium towards chloroethenes could potentially be examined in a pilot-scale study.

7. LIST OF PUBLICATIONS

Articles published in journals with impact factor:

ZALESAK, M., J. RUZICKA, R. VICHA and M. DVORACKOVA, 2017. Cometabolic degradation of dichloroethenes by *Comamonas testosteroni* RF2. *Chemosphere*. November 2017, vol. 186, p. 919-927. ISSN 0045-6535. Available from: doi: 10.1016/j.chemosphere.2017.07.156

Journal Impact factor: 4.427 (2017); 4.551 (5 years)
Quartile in Category: Q1

WITCZAK, A., H. ABDEL-GAWAD, **M. ZALESAK** and A. POHORYLO, 2017. Tracking residual organochlorine pesticides (OCPs) in green, herbal, and black tea leaves and infusions of commercially available tea products marketed in Poland. *Food Additives and Contaminants Part A – Chemistry Analysis Control Exposure and Risk Assessment*. 2018, vol. 35, iss. 3, p. 479-486. ISSN 1944-0049. Available from: doi: 10.1080/19440049.2017.1411614

Journal Impact factor: 2.129 (2017); 2.23 (5 years)
Quartile in Category: Q2/Q3

MERKOVA, M., **M. ZALESAK**, E. RINGLOVA, M. JULINOVA and J. RUZICKA, 2018. Degradation of the surfactant Cocamidopropyl betaine by two bacterial strains isolated from activated sludge. *International Bioremediation and Biodegradation*. February 2018, vol. 127, p. 236-240. ISSN 0964-8305. Available from: doi: 10.1016/j.ibiod.2017.12.006

Journal Impact factor: 3.562 (2017); 3.631 (5 years)
Quartile in Category: Q1/Q2

Articles that are being prepared:

ZALESAK, M. and J. RUZICKA, 2019. Evaluation of the potential techniques for in situ bioremediation of chlorinated ethenes: A review.

Attended conferences:

ZALESAK, M., 2016. Human Activities as a Cause of Introducing of Chlorinated Ethenes into the Environment: Their Fate, Potential Risks and Biological Methods of Removal. II. International Conference Human Ecology, Szczecin, Poland. ISBN 978-83-7663-214-8

ZALESAK, M. and J. Ruzicka, 2018. Chlorinated ethenes in groundwater: Significant factors affecting their transport in a subsurface environment and the use of microorganisms for their controlled removal. I. Conference “Mlada Voda Brehy Mele 2018”, Brno, Czech Republic. ISBN 978-80-270-3802-2

Attended workshops:

CYBERWARER 2018 - NATO ARW Workshop - Physical and Cyber Safety in Water Critical Infrastructure. October 2018, Oslo, Norway – Kiel, Germany.

8. REFERENCES

AEPPLI, C., T.B. HOFSTETTER, H.I.F. AMARAL, R. KIPFER, P.R. SCHWARZENBACH and M. BERG, 2010. Quantifying in-situ transformation rates of chlorinated ethenes by combining compound-specific stable isotope analysis, groundwater dating, and carbon isotope mass balances. *Environmental Science and Technology* [online]. May 2010, vol. 44, iss. 10, p. 3705-3711 [cit. 2018-02-21]. ISSN 0013-936X. Available from: doi: 10.1021/es903895b

ARCIERO, D., T. VANNELLI, M. LOGAN and A.B. HOOPER, 1989. Degradation of trichloroethylene by the ammonia-oxidizing bacterium *Nitrosomonas europa*. *Biochemical and Biophysical Research Communications* [online]. March 1989, vol. 159, iss. 2, p. 640-643 [cit. 2018-03-09]. ISSN 0006-291X. Available from: doi: 10.1016/0006-291X(89)90042-9

AULENTA, F., A. PERA, S. ROSSETTI, M.P. PAPINI and M. MAJONE, 2007. Relevance of side reactions in anaerobic reductive dechlorination microcosms amended with different electron donors. *Water Research* [online]. January 2007, vol. 41, iss. 1, p. 27-38 [cit. 2018-03-09]. ISSN 0043-1354. Available from: doi: 10.1016/j.watres.2006.09.019

AULENTA, F., M. POTALIVO, M. MAJONE, M.P. PAPINI and V. TANDOI, 2006. *Biodegradation* [online]. June 2006, vol. 17, iss. 3, p. 193-206 [cit. 2018-04-06]. ISSN 0923-9820. Available from: doi: 0.1007/s10532-005-4218-7

AZIZIAN, M.F., J.D. ISTOK and L. SEMPRINI, 2007. Evaluation of the in-situ aerobic cometabolism of chlorinated ethenes by toluene-utilizing microorganisms using push-pull tests. *Journal of Contaminant Hydrology* [online]. February 2007, vol. 90, iss. 1-2, p.105-124 [cit. 2018-03-11]. ISSN 0169-7722. Available from: doi: 10.1016/j.jconhyd.2006.09.015

BINBIN, H., L. CHAO, W. CHAOHAI and Z. GUANGMING, 2014. Chlorinated volatile organic compounds (Cl-VOCs) in environment - sources, potential human health impacts and current remediation technologies. *Environment International* [online]. October 2014, vol. 71, p. 118-138. [cit. 2017-06-11]. ISSN: 0160-4120. Available from: doi.org/10.1016/j.envint.2014.06.013

BOURG, A.C.M., C. MOUVET and D.N. LERNER, 1992. *Quarterly Journal of Engineering Geology* [online]. 1992, vol. 25, iss. 4, p. 359-370 [cit. 2018-03-23]. ISSN 0481-2085. Available from: doi: 10.1144/GSL.QJEG.1992.025.04.10

BYRNE, A.M., J.J. KUKOR and R.H. OLSEN, 1995. Sequence-analysis of the gene-cluster encoding toluene-3-monooxygenase from *Pseudomonas pickettii* PKO1. *Gene* [online]. 1995, vol. 154, iss. 1, p. 65-75 [cit. 2018-03-26]. ISSN 0378-1119. Available from: doi: 10.1016/0378-1119(94)00844-I

CHANG, H.L. and L. ALVAREZ-COHEN, 1996. Biodegradation of individual and multiple chlorinated aliphatic hydrocarbons by methane-oxidizing cultures. *Applied and Environmental Microbiology* [online]. September 1996, vol.

62, iss. 9, p. 3371-3377. [cit. 2018-02-24]. ISSN: 0099-2240. Available from: PMC168134

CLOELLE, G.S.G., L. FANG and M.G. JAMES, 2010. Microcosm assessment of *Polaromonas* sp. JS666 as a bioaugmentation agent for degradation of cis-1,2-dichloroethene in aerobic, subsurface environments. *Ground Water Monitoring and Remediation* [online]. May 2010, vol. 30, iss. 2, p.106-113 [cit. 2017-06-11]. ISSN 1069-3629. Available from: doi:10.1111/j1745-6592.2010.001283.x

COLEMAN, N.V., T.E. MATTES, J.M. GOSSETT and J.C. SPAIN, 2002. Phylogenetic and kinetic diversity of aerobic vinyl chloride-assimilating bacteria from contaminated sites. *Applied Environmental Microbiology* [online]. December 2002. vol. 68, iss. 12, p. 6162-6171 [cit. 2018-02-14]. ISSN 0099-2240. Available from: doi: 10.1128/AEM.68.12.6162-6171.2002

CWIERTNY, D.M. and M.M. SCHERER, 2010. In-situ Remediation of Chlorinated Solvents. Chapter 2 – Chlorinated solvents chemistry: structures, nomenclature and properties [online]. New York: Springer, May 2010, p. 29-36. [cit. 2018-03-27] ISBN 978-1-4419-1400-2. Available from: doi:10.1007/978-1-4419-1401-9

DANKO, A.S., M.Z. LUO, C.E. BAGWELL, R.L. BRIGMON and D.L. FREEDMAN, 2004. Involvement of linear plasmids in aerobic biodegradation of vinyl chloride. *Applied and Environmental Microbiology* [online]. October 2004, vol. 70, iss. 10, p. 6092-6097 [cit. 2018-04-06]. ISSN 0099-2240. Available from: doi: 10.1128/AEM.70.10.6092-6097.2004

DAS, S. and H.R. DASH, 2014. Microbial bioremediation: A potential tool for restoration of contaminated areas. *Microbial Biodegradation and Bioremediation* [online]. New York, US, Elsevier, 2014, p. 1-21 [cit. 2018-09-19]. ISBN 978-0-12-800482-1; 978-0-12-800021-2. Available from: doi: 10.1016/B978-0-12-800021-2.00001-7

DEY, K. and P. ROY, 2009. Degradation of trichloroethylene by Bacillus sp.: Isolation strategy, strain characteristics, and cell immobilization. *Current Microbiology* [online]. September 2009, vol. 59, iss. 3 p. 256-260 [cit. 2018-01-26]. ISSN 0343-8651. Available from: doi: 10.1007/s00284-009-9427-6

DOLINOVA, I., M. CZINNEROVA and L. DVORAK, 2016. Dynamics of organohalide-respiring bacteria and their genes following in-situ chemical oxidation of chlorinated ethenes and biostimulation. *Chemosphere* [online]. August 2016, vol. 157, p. 276-285 [cit. 2018-04-07]. ISSN: 0045-6535. Available from: 10.1016/j.chemosphere.2016.05.030

DOLINOVA, I., M. STROJSOVA, M. CERNIK, J. NEMECEK, J. MACHACKOVA and A. SEVCU, 2017. Microbial degradation of chloroethenes: a review. *Environmental Science and Pollution Research* [online]. May 2017, vol. 24, iss. 15, p. 118-138. [cit. 2018-01-11]. ISSN 0944-1344. Available from: doi:10.1007/s11356-017-8867-y

DUBA, A.G., K.J. JACKSON, M.C. JOVANOVICH, R.B. KNAPP and R.T. TAYLOR, 1996. TCE remediation using in-situ resting-state bioaugmentation.

Environmental Science and Technology [online]. June 1996, vol. 30, iss. 6, p. 1982-1989 [cit. 2018-09-20]. ISSN 0013-936X. Available from: doi: 10.1021/es950730k

DUGAT-BONY, E., C. BIDERRE-PETIT, F. JAZIRI, M.M. DAVID, J. DENONFOUX, D.Y. LYON, J.Y. RICHARD, C. CURVERS, D. BOUCHER, T.M. VOGEL, E. PEYRETAILLADE and P. PEYRET, 2012. In-situ TCE degradation mediated by complex dehalorespiring communities during biostimulation processes. *Microbial Technology* [online]. February 2012, vol. 5, iss. 5, p. 642-653 [cit. 2018-09-19]. ISSN 1751-7907. Available from: doi: 10.1111/j.1751-7915.2012.00339.x

ELLIS, D.E., E.J. LUTZ, J.M. ODOM, R.J. BUCHANAN, C.L. BARTLETT, M.D. LEE, M.R. HARKNESS and K.A. DEWEERD, 2000. *Bioaugmentation for accelerated in-situ anaerobic bioremediation* [online]. January 2000, vol. 34, iss. 11, p. 2254-2260 [cit. 2018-04-08]. ISSN: 0013-936X. Available from: doi: 10.1021/es990638e

ENSIGN, S.A., M.R. HYMAN and D.J. ARP, 1992. Cometabolic degradation of chlorinated alkenes by alkene monooxygenase in a propylene-grown *Xanthobacter* strain. *Applied Environmental Microbiology* [online]. September 1992, vol. 58, iss. 9, p. 3038-3046 [cit. 2018-03-09]. ISSN 0099-2240. Available from: doi: PMC183045

EPA Victoria, 2006. Hydrogeological assessment (groundwater quality) guidelines [online]. September 2006, Victoria [cit. 2018-03-29]. ISBN 0-7306-76587. Available from: https://www.epa.gov/sites/production/files/2014-09/documents/pvicvi.pdf

EWERS, J., D. FREIERSCHRODER and H.J. KNACKMUSS, 1990. Selection of trichloroethene (TCE) degrading bacteria that resist inactivation by TCE. *Archives of Microbiology* [online]. September 1990, vol. 154, iss. 4, p.410-413 [cit. 2018-03-19]. ISSN 0302-8933. Available from: doi: 10.1007/BF00276540

FENNELL, D.E., A.B. CARROLL, J.M. GOSSETT and S.H. ZINDER, 2001. Assessment of indigenous reductive dechlorinating potential at a TCE-contaminated site using microcosm, polymerase chain reaction analysis, and site data. *Environmental Science and Technology* [online]. May 2001, vol. 35, iss. 9, p. 1830-1839 [cit. 2018-03-23]. ISSN 0013-936X. Available from: doi: 10.1021/es0016203

FINDLAY, M., D.F. SMOLER, S. FOGEL and T.E. MATTES, 2016. Aerobic vinyl chloride metabolism in groundwater microcosms by methanotrophic and ethenetrophic bacteria. *Environmental Science and Technology* [online]. April 2016, vol. 50, iss. 7, p. 3617 - 3625 [cit. 2018-04-06]. ISSN 1520-5851. Available from: doi: 10.1021/acs.est.5b05798

FOX, B.G., J.G. BORNEMAN, P.L. WACKETT and J.D. LIPSCOMB, 1990. Haloalkene oxidation by the soluble methane monooxygenase from *Methylosinus trichosporuim* OB3B – mechanistic and environmental implications.

Biochemistry [online]. July 1990, vol. 29, iss. 27, p. 6419-6427 [cit. 2018-03-23]. ISSN: 0006-2960. Available from: doi: 10.1021/bi00479a013

FRASCARI, D. G. ZANAROLI and A.S. DANKO, 2015. In-situ aerobic cometabolism of chlorinated solvents: A review. *Journal of Hazardous Materials*, [online]. February 2015 vol. 283, p. 382-399. [cit. 2018-01-11]. ISSN 0304-3894. Available from: doi: 10.1016/j.jhazmat.2014.09.041

FRASCARI, D., S. FRARACCIO, M. NOCENTINI and D. PINELLI, 2013. Aerobic/anaerobic/aerobic sequenced biodegradation of a mixture of chlorinated ethenes, ethanes and methanes in batch bioreactors. *Bioresource Technology* [online]. January 2013, vol. 128, p. 479-486 [cit. 2018-03-08]. ISSN 0960-8524. Available from: doi: 10.1016/j.biortech.2012.10.026

FRIES, M.R., L.J. FORNEY and J.M. TIEDJE, 1997. Phenol and toluene degrading microbial populations from aquifer in which successful trichloroethene cometabolism occurred. *Applied Environmental Microbiology* [online]. April 1997, vol. 63, iss. 4, p. 1523-1530 [cit. 2018-04-04]. ISSN 0099-2240. Available from: PMC1389555

FUTAMATA, H., S. KAIYA, M. SUGAWARA and A. HIRAISHI, 2009. Phylogenetic and Transcriptional Analyses of a Tetrachloroethene-Dechlorinating Dehalococcoides Enrichment Culture TUT2264 and Its Reductive-Dehalogenase Genes. *Microbes and Environments* [online]. February 2009, vol. 24, iss. 4, p. 330-337 [cit. 2018-03-23]. ISSN 1342-6311. Available from: doi: 10.1264/jsme2.ME09133

HARTMANS, S. and J. DEBONT, 1992. Aerobic vinyl-chloride metabolism in Mycobacterium-aurum L1. *Applied and Environmental Microbiology* [online]. April 1992, vol. 58, iss. 4, p. 1220-1226 [cit. 2018-03-20]. ISSN 0099-2240. Available from: PMC195578

HARTMANS, S., J. DEBONT, J. TRAMPER and K.C.A.M. LUYBEN, 1985. Bacterial-degradation of vinyl-chloride. *Biotechnology Letters* [online]. 1985, vol. 7, iss. 6, p. 383-388. [cit. 2018-02-24]. ISSN 1664-302X. Available from: doi: 10.1007/BF01166208

HE, J., Y. SUNG, R. KRAJMALNIK-BROWN, K.M. RITALAHTI and F.E. LOFFLER, 2005. Isolation and characterization of Dehalococcoides sp strain FL2, a trichloroethene (TCE) and 1,2-dichloroethene respiring anaerobe. *Environmental Microbiology* [online]. September 2005, iss. 9, p. 1442-1450. [cit. 2018-04-08]. ISSN 1462-2912. Available from: doi: 10.1111/j.1462-2920.2005.00830.x

HOELEN, T.P., J.A. CUNNINGHAM, G.D. HOPKINS, C.A. LEBRON and M. REINHARD, 2006. Bioremediation of cis-DCE at a sulfidogenic site by amendment with propionate. *Groundwater Monitoring and Remediation* [online]. 2006, vol. 26, iss. 3, p. 82-91 [cit. 2018-09-19]. ISSN 1069-3629. Available from: doi: 10.1111/j.1745-6592.2006.00088.x

HOLMES, MW., P. MORGAN, G.M. KLECKA, N.J. KLIER, R.J. WEST, J.W. DAVIES, D.E. ELLIS, E.J. LUTZ, J.M. ODAM, T.A. EI, F.H. CHAPELLE,

D.W. MAJOR, J.J. SALVO and M.J. BELL, 1998. The natural attenuation of chlorinated ethenes at Dover Air Force Base, Delaware, USA. 6th International FZK/TNO Conference on Contaminated Soil (ConSoil 98). Edinburg, Scotland, May 1998. *Contaminated Soil'98* [online]. vol. 1-2, p. 143-152 [cit. 2018-02-14]. ISSN 0-7277-2675-7

HOPKINS, G.D. and P.L. MCCARTY, 1995. Field evaluation of in-situ aerobic cometabolism of trichloroethylene and three dichloroethylene isomers using phenol and toluene as the primary substrates. *Environmental Science and Technology* [online]. Jun1995, vol. 29, iss. 6, p. 1628-1637 [cit. 2017-06-11]. ISSN 0013-936X. Available from: doi:10.1021/es00006a029

HOPKINS, G.D., L. SEMPRINI and P.L. MCCARTY, 1993. Microcosm and in-situ field studies of enhanced biotransformation of trichloroethylene by a phenol-utilizing microorganism. *Applied and Environmental Microbiology* [online]. July 1993, vol. 59, iss. 7, p. 2277-2285 [cit. 2018-09-11]. ISSN 0099-2240. Available from: PMID: 8357259

HULING, S.G. and J.W. Weaver, 1991. Ground Water Issue: Dense non-aqueous phase liquids [online]. Washington: US EPA/540/4-91-002 [Cit. 2018-03-27]. Available from: https://www.epa.gov/sites/production/files/2015-06/documents/dnapl_issue_paper.pdf

IMFELD, G., H. PIEPER, N. SHANI, P. ROSSI, M. NIKOLAUSZ, I. NIJENHUIS, H. PASCHKE, H. WEISS and H.H. RICHNOW, 2011. Characterization of groundwater microbial communities, dechlorinating bacteria, and in-situ biodegradation of chloroethenes along a vertical gradient. *Water Air and Soil Pollution* [online]. Jun 2011, vol. 221, iss. 1-4, p. 107-122 [cit. 2018-04-01]. ISSN 0049-6979. Available from: doi: 10.1007/s11270-011-0774-0

IWASAKI, I., S UTSUMI and T. OZAWA, 1952. New colorimetric determination of chloride using mercuric thiocyanate and ferric ion. *Bulletin of the Chemical Society of Japan* [online]. 1952, vol. 25, iss. 3, p. 226-226 [cit. 2018-04-10]. ISSN 1860-6768. Available from: doi: 10.1246/bcsj.25.226

JOHNSON, G.R. and R.H. OLSEN, 1995. Nucleotide-sequence analysis of genes encoding a toluene benzene-2-monooxygenase from Pseudomonas sp strain JS150. *Applied and Environmental Microbiology* [online]. September 1995, vol. 61, iss. 9, p. 3336-3346 [cit. 2018-04-06]. ISSN 0099-2240. Available from: PMC167614

KIM, S., W. BEA, J. HWANG and J. PARK, 2010. Aerobic TCE degradation by encapsulated toluene-oxidizing bacteria, Pseudomonas putida and Bacillus spp. *Water Science and Technology* [online]. 2010, vol. 62, iss. 9, p. 1991-1997. [cit. 2018-02-01]. ISSN 0273-1223. Available from: doi: 10.2166/wst.2010.471

KIM, Y., J.D. STOK and L. SEMPRINI, 2008. Single-well, gas-sparging tests for evaluating the in-situ aerobic cometabolism of cis-1,2-dichloroethene and trichloroethene. *Chemosphere* [online]. April 2008. vol. 71, iss. 9, p. 1654-1664 [cit. 2018-03-21]. ISSN 0045-6535. Available from: doi: 10.1016/j.chemosphere.2008.01.021

KRET, E., A. KIECAK, G. MALINA, I. NIJENHUIS and A. POSTAWA, 2015. Identification of TCE and PCE sorption and biodegradation parameters in a sandy aquifer for the fate and transport modelling: batch and column studies. *Environmental Science and Pollution Research* [online]. July 2015, vol. 22, iss. 13, p. 9877-9888. [cit. 2018-01-11]. ISSN 0944-1344. Available from: doi: 10.1007/s11356-015-4156-9

LACINOVA, L., M. CERNIKOVA, J. HRABAL and M. CERNIK, 2013. In-situ combination of bio and abio remediation of chlorinated ethenes. *Ecological Chemistry and Engineering S-Chemia i Inzenieria Ekologiczna S* [online]. 2013, vol. 20, iss. 3, p. 463-473. [cit. 2018-04-09]. ISSN 1898-6196. Available from: doi: 10.2478/eces-2013-0034

LARSEN, M., J. BURKEN, J. MACHACKOVA, U.G. KARLSON and S. TRAPP, 2008. Using tree core samples to monitor natural attenuation and plume distribution after a PCE spill. *Environmental Science and Technology* [online]. March 2008, vol. 42, iss. 5, p. 1711-1717 [cit. 2018-09-19]. ISSN 0013-936X. Available from: doi: 10.1021/es0717055

LEE, P.H.K., T.W., MACBETH, K.S. SORENSON, R.A. DEEB and L. ALVAREZ-COHEN, 2008. Quantifying genes and transcripts to assess the in-situ physiology of Dehalococcoides spp. In a trichloroethene-contaminated groundwater site. *Applied and Environmental Microbiology* [online]. May 2008, vol. 74, iss. 9, p. 2728-2739 [cit. 2018-04-11]. ISSN 0099-2240. Available from: doi: 10.1128/AEM.02199-07

LI, J.H., R.A. DE TOLEDO, J. CHUNG and H. SHIM, 2014. Removal of a mixture of 1,2-cis dichloroethylene/trichloroethylene/benzene, toluene, ethylbenzene, and xylenes from contaminated soil by *Pseudomonas plecoglossicida*. *Journal of Chemical Technology and Biotechnology* [online]. December 2014, vol. 89, iss. 12 p. 1934-1940 [cit. 2018-04-01]. ISSN 0268-2575. Available from: doi: 10.1002/jctb.4279

LOFFLER, F.E., J. YAN, K.M. RITALAHTI, L. ADRIAN, E.A. EDWARDS, K.T. KONSTANTINIDIS, J.A. MULLER, H. FULLERTON, S.H. ZINDER and A.M. SPORMANN, 2013. Dehalococcoides mccartyi gen. nov., sp nov., obligately organohalide-respiring anaerobic bacteria relevant to halogen cycling and bioremediation, belong to a novel bacterial class, *Dehalococcoidia* classis nov., order *Degalococcoidale*s ord. nov and family *Dehalococcoidaceae* fam. Nov., within the phylum Chloroflexi. *International Journal of Systematic and Evolutionary Microbiology* [online]. February 2013, vol. 63, p. 625-635 [cit. 2018-04-01]. ISSN 1466-5026. Available from: doi: 10.1099/ijs.0.034926-0

MCCARTY, P.L., M.N. GOLTZ, G.D. HOPKINS, M.E. DOLAN, J.P. ALLAN, B.T. KAWAKAMI and T.J. CARROTHERS, 1998. Full scale evaluation of in-situ cometabolic degradation of trichloroethylene in groundwater through toluene injection. *Environmental Science and Technology* [online]. January 1998, vol. 32, iss. 1, p. 88-100 [cit. 2018-09-03]. ISSN 0013-936X. Available from: doi: 10.1021/es970322b

MENDEZ, E., M. WIDDOWSON, S. BRAUNER, F. CHAPELLE and C. CASEY, 2004. Natural Attenuation Software (NAS): a computer program for estimating remediation times of contaminated groundwater. 10th International Conference on Development and Application of Computer Techniques to Environmental Studies. Ancona, Italy, Jun 2004. *Development and Application of Computer Techniques to Environmental Studies X* [online]. vol. 11, p. 185-194 [cit. 2018-09-19]. ISSN 1462-6098

MIHOPOULOS, P.G., M.T. SUIDAN, G.D. SAYLES and S. KASKASSIAN, 2002. Numerical modelling of oxygen exclusion experiments of anaerobic bioventing. *Journal of Contaminant Hydrology* [online]. October 2002, vol. 58, iss. 3-4, p. 209-220 [cit. 2018-03-10]. ISSN 0169-7722. Available from: doi:10.1016/S0169-7722(02)00037-2

NISHINO, S.F., K.A. SHIN, J.M, GOSSETT and J.C SPAIN, 2013. Cytochrome P450 initiates degradation of *cis*-dichloroethene by *Polaromonas* sp. strain JS666. *Applied and Environmental Microbiology* [online]. April 2013, vol. 79, iss. 7, p. 2263-2272 [cit. 2017-06-11]. ISSN 0099-2240. Available from: doi:10.1128/AEM.03445-12

NSW, Office of Environment and Heritage, 2011. Management of chlorinated solvents – Strategic environmental compliance and performance review [online]. Sydney, September 2011, [cit. 2018-03-27]. ISBN 987-1-74293-302-3. Available from: https://www.epa.nsw.gov.au

PANT, P. and S. PANT, 2010. A review: Advances in microbial remediation of trichloroethylene (TCE). *Journal of Environmental Sciences* [online]. January 2010, vol. 22, iss. 1, p. 116-126 [cit. 2018-02-16]. ISSN 1001-0742. Available from: doi: 10.1016/S1001-0742(09)60082-6

RUZICKA, J., J. MULLER, D. VIT, V. HUTECKA, J. HOFFMANN, H. DATKOVA and M. NEMEC, 2002. Biotransformation of trichloroethene by pure bacterial cultures. *Folia Microbiologica* [online]. 2002, vol. 47, iss. 5, p. 467-472 [cit. 2018-09-19]. ISSN 0015-5632. Available from: https://doi.org/10.1007/BF02818782

RUZICKA, J., M. DVORACKOVA and M. KOUTNY, 2011. Trichloroethylene degradation by two phenol utilizing strains of Comamonas testosteroni. *Asian Journal of Chemistry* [online]. October 2011, vol. 23, iss. 10, p. 4255-4259 [cit. 2018-03-19]. ISSN 0970-7077. Available from: http://www.asianjournalofchemistry.co.in/User/ViewFreeArticle.aspx?ArticleID=23_10_3

RYOO, D., H. SHIM, K. CANADA, P. BARBIERI and T.K. WOOD, 2000. Aerobic degradation of tetrachloroethylene by toluene-o-xylene monooxygenase of *Pseudomonas stutzeri* OX1. *Nature Biotechnology* [online]. July 2000, vol. 18, iss. 7, p. 775-778 [cit. 2018-04-09]. ISSN 1087-0156. Available from: doi: 10.1038/77344

SAYLES, G.D., P. MIHOPOULOS and M.T. SUIDAN, 1997. Anaerobic bioventing of PCE. 4th International In-Situ and On-Site Bioremediation

Symposium. New Orleans, LA, April 28-May 01, 1997. *Battelle Press* [online]. vol. 1-2, p. 143-152 [cit. 2018-02-14]. ISBN:1-57477-031-4

SCHEUTZ, C., M.M. BROHOLM, N.D. DURANT, E.B. WEETH, T.H. JORGENSEN, P. DENNIS, C.S. JACOBSON, E.E. COX, J.C. CHAMBON and P.L. BJERG, 2010. Field evaluation of biological enhanced reductive dechlorination of chloroethenes in clayey till. *Environmental Science and Technology* [online]. July 2010, vol. 44, iss. 13, p. 5134-5141 [cit. 2018-09-11]. ISSN 0013-936X. Available from: doi: 10.1021/es1003044

SCHMIDT, KR. and A. TIEHM, 2008. Natural attenuation of chloroethenes: identification of sequential reductive/oxidative biodegradation by microcosm studies. *Water Science and Technology* [online]. 2008, vol. 8, iss. 1, p. 104-111 [cit. 2018-03-22]. ISSN 0273-1223. Available from: doi: 10.2166/wst.2008.729

SCHWARZENBACH, R.P., P.M. GSCHWEND and D.M. IMBODEN, 2003. Environmental Organic Chemistry. John Wiley & Sons, Inc., Hoboken, NY, USA. p. 1313

SEMPRINI, L., 1997. Strategies for the aerobic co-metabolism of chlorinated solvents. *Current Opinion in Biotechnology* [online]. June 1997, vol. 8, iss. 3, p. 296-308. [cit. 2018-03-24]. ISSN 0958-1669. Available from: doi: 10.1016/S0958-1669(97)80007-9

SEMPRINI, L., M.E. DOLAN, M.A.B. MATHIAS, G.D. HOPKINS and P.L. MCCARTY, 2007. Laboratory field and modelling studies of bioaugmentation of butane-utilizing microorganisms for the in-situ cometabolic treatment of 1,1-dichloroethylene, 1,1-dichloroethane, and 1,1,1-trichloroethane. *Advances in Water Resources* [online]. June-July 2007, vol. 30, iss. 6-7, p. 1528-1546. [cit. 2018-09-11]. IDS Number: 173WD. ISSN 0309-1708. Available from: doi: 10.1016/j.advwatres.2006.05.017

SMIDT, H. and W.M. de VOS, 2004. Anaerobic microbial dehalogenation. *Annual Review of Microbiology* [online]. 2004, vol. 58, p. 43-73 [cit. 2018 -04-11]. ISSN: 0066-4227, PMID: 15487929. Available from: doi: 10.1146/annurev.micro.58.030603.123600

STEFFAN, R.J., K.L. SPERRY, M.T. WALSH, S. VAINBERG and C.W. CONDEE, 1999. Field-scale evaluation of in-situ bioaugmentation for remediation of chlorinated solvents in groundwater. *Environmental Science and Technology* [online]. August 1999, vol. 33, iss. 16, p. 2771-2781 [cit. 2018-04-06]. ISSN 0013-936X. Available from: doi: 10.1021/es9812204

TIEHM, A. and R.S. SCHMIDT, 2011. Sequential anaerobic/aerobic biodegradation of chloroethenes-aspects of field application. *Current Opinion in Biotechnology* [online]. Jun 2011, vol. 22, iss. 3, p. 415-421 [cit. 2017-06-11]. ISSN 0958-1669. Available from: doi:10.1016/j.copbio.2011.02.003

US EPA, 1998. Technical protocol for evaluation natural attenuation of chlorinated solvents in groundwater [online]. September 1998, Washington: EPA/600/R-98/128, [cit. 2018-03-29]. Available from: https://clu-in.org/download/remed/protocol.pdf

US EPA, 2000. Engineered approaches to in-situ bioremediation of chlorinated solvents [online]. July 2000, Washington: EPA 542-R-00-008, [cit. 2018-09-09]. Available from: https://clu-in.org/download/remed/engappinsitbio.pdf

US EPA, 2012b. A citizen's guide to monitored natural attenuation [online]. September 2012, EPA 542-F-12-014 [cit. 2018-08-28]. Available from: https://cluin.org/download/citizens/a_citizens_guide_to_monitored_natural_attenuation.pdf

VARDAR, G. and T.K. WOOD, 2005. Protein engineering of toluene-o-xylene monooxygenase from *Pseudomonas stutzeri* OX1 from enhanced chlorinated ethene degradation and o-xylene oxidation. *Applied Microbiology and Biotechnology* [online]. September 2005, vol. 68, iss. 4, p. 510-517 [cit. 2018-02-26]. ISSN 0175-7598. Available from: doi: 10.1007/s00253-005-1923-4

VERCE, M.F., C.K. GUNSCH, A.S. DANKO and D.L. DREEDMAN, 2002. Cometabolism of cis-1,2-dichloroethene by aerobic cultures grown on vinyl chloride as the primary substrate. *Environmental Science and Technology* [online]. May 2002, vol. 36, iss. 10, p. 2171-2177 [cit. 2018-04-06]. ISSN 0013-936X. Available from: doi: 10.1021/es011220v

VERCE, M.F., V.M. MADRID, S.D. GREGORY, Z. DEMIR, M.J. SINGLETON, E.P. SALAZAR, P.J. JACKSON, R.U. HALDEN and A. VERCE, 2015. Along-term field study of in-situ bioremediation in a fractured conglomerate trichloroethylene source zone. *Bioremediation Journal* [online]. January 2015, vol. 19, iss. 1, p. 18-31 [cit. 2018-09-20]. ISSN 1088-9868. Available from: doi: 10.1080 / 10889868.2014.978836

VLIEG, J.E.T.V., J. KINGMA, A.J. VAN DEN WIJNGAARD and D.B. JANSSEN, 1998. A glutathione S-transferase with activity towards cis-1,2-dichloroepoxyethane is involved in isoprene utilization by Rhodococcus sp. strain AD45. *Applied and Environmental Microbiology* [online]. August 1998, vol. 64, iss. 8, p. 2800-2805 [cit. 2018-03-20]. ISSN 0099-2240. Available from: PMC106775

WDNR, 2014. Understanding chlorinated hydrocarbon behaviour in groundwater: Guidance on the investigation, assessment and limitations of monitored natural attenuation [online]. *Wisconsin Department of Natural Resources.* Madison: October 2014, RR-699, [cit. 2018-03-29]. Available from: http://dnr.wi.gov/files/pdf/pubs/rr/rr699.pdf

WITT, M.E., G.M. KLECKA, E.J. LUTZ, T.A. EI, N.R. GROSSO and F.H. CHAPELLE, 2002. Natural attenuation of chlorinated solvents at area 6 air force base: groundwater biogeochemistry. *Journal of Contaminant Hydrology* [online]. July 2002, vol. 57, iss. 1-2, p. 61-80 [cit. 2018-09-06]. ISSN 0169-7722. Available from: doi: 10.1016/S0169-7722(01)00218-2

9. LIST OF SYMBOLS AND ABBREVIATIONS

DNAPLs	dense non-aqueous phase liquids
EaCoMT	epoxyalkane coenzyme M transferase
LNAPLs	light non-aqueous phase liquids
NAPLs	aqueous phase liquids
TCE-RDase	trichloroethene reductive dehalogenase enzyme
1,1DCE	1,1-dichloroethene
*c*DCE	*cis*-1,2-dichloroethene
CEs	chlorinated ethenes
CFUs	colony forming units
DCEs	dichloroethenes
$DNAPL_{CEs}$	chlorinated ethenes as dense non-aqueous phase liquids
ECD	electron capture detector
GC	gas chromatography
I1	intermediate 1
I2	intermediate 2
K_H	Henry's law constant
Koc	carbon-water partitioning coefficient
Kow	octanol-water partitioning coefficient
$LNAPL_{VC}$	vinyl chloride as light non-aqueous phase liquids
MNA	monitored natural attenuation
MS	mass spectroscopy
MSM	mineral salt medium
PCE	perchloroethene
PCE-RDase	perchloroethene reductive dehalogenase enzyme
P^o	vapour pressure
RDase	reductive dehalogenase enzyme
RF2-Ma	Consortium composed of *C. testosteroni* RF2 + *M. aurum*
RF2-Pp	Consortium composed of *C. testosteroni* RF2 + *P. putida*
RF2-Rr	Consortium composed of *C. testosteroni* RF2 + *R. ruber*
VC-RDase	vinyl chloride reductive dehalogenase enzyme
S	water solubility
Tc	transformation capacity
TCE	trichloroethene
*t*DCE	*trans*-1,2-dichloroethene
TOC	total organic carbon
VC	vinyl chloride

10. LIST OF TABLES, FIGURES, AND APPENDIXES

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11. CURRICULUM VITAE

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March 2016 – June 2016

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August 2015 – September 2015

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Observation and biometric measurement of agricultural plants at West Pomeranian University of Technology, Szczecin, Poland
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IGA/FT/2016/012
IGA/FT/2017/003
IGA/FT/2018/009
The Water Harmony Erasmus +

- Funded by EU Erasmus + Programme - Key Action 2 - Capacity building in the field of higher education
- Official website: https://www.waterh.eu/en/water-harmony-erasmus/
- Facebook: https://www.facebook.com/WHEplus/

12. APPENDIXES

Appendix 1.1 Summary of some important physicochemical properties of CEs at 25 ºC

Compound	Molar weight (g mol^{-1})	Carbon oxidation state	Density (g cm^{-3})	Solubility in water (g L^{-1})	Log (Kow)	Log (Koc)	Vapour pressure (kPa)	Henry's Law Constant $K_{(H)}$ (x 10^{-3} atm.m^3 mol^{-1})	Relative vapour density	Boiling point (°C)
PCE	165.8	+ II	1.63	0.15	2.9	2.42	2.41	26.3	1.12	121.1
TCE	131.4	+ I	1.46	1.10	2.42	1.81	9.87	11.7	1.35	87.2
*c*DCE	96.9	0	1.28	3.50	2.0	1.77	27	7.40	1.63	60.2
*t*DCE	96.9	0	1.26	6.26	2.09	-	44.3	9.38	1.97	48.5
1,1DCE	96.9	0	1.22	3.34	1.32	-	80.3	23.0	2.86	32.0
VC	62.5	- I	0.91	2.76	1.38	0.39	353.8	79.2	2.2	-13.4

Ing. Michal Zálešák, Ph.D.

Microbial degradation of chlorinated ethenes and its potential application for in-situ bioremediation

Mikrobiální degradace chlorovaných ethylenů a její potenciální využití pro in-situ bioremediace

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