Chemosphere xxx (xxxx) xxx

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Examining aerobic degradation of chloroethenes mixture in consortium composed of *Comamonas testosteroni* RF2 and *Mycobacterium aurum* L1

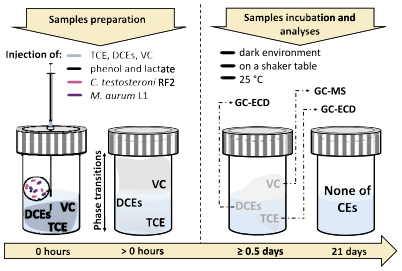
Michal Zalesak [a], Jan Ruzicka [a, *], Robert Vicha [b], Marie Dvorackova [a]

[a] Department of Environment Protection Engineering, Tomas Bata University in Zlin, Faculty of Technology, Vavreckova 275, 760 01, Zlin, Czech Republic
[b] Department of Chemistry, Tomas Bata University in Zlin, Faculty of Technology, Vavreckova 275, 760 01, Zlin, Czech Republic

HIGHLIGHTS

- The consortium was able to degrade TCE, all three DCE isomers and VC.
- Removals of chloroethenes were accomplished by cometabolic and metabolic degradation.
- Degradation of VC slightly influenced degradation of other chloroethenes.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 28 April 2020
Received in revised form
7 September 2020
Accepted 23 October 2020
Available online xxx

Handling Editor: Chang-Ping Yu

Keywords:
Comamonas
Cometabolism
Consortium
Chloroethenes
Mycobacterium

ABSTRACT

An environmental isolate *Comamonas testosteroni* RF2 has been previously described to cometabolize trichloroethene (TCE), 1,2-cis-dichloroethene (*c*DCE), 1,2-trans-dichloroethene (*t*DCE), and 1,1-dichloroethene (1,1DCE) when grown on phenol and lactate sodium. In this study, three vinyl chloride (VC) degrading strains, *Mycobacterium aurum* L1, *Pseudomonas putida* PS, and *Rhodococcus ruber* Sm-1 were used to form consortia with the strain RF2 in terms to achieve the removal of VC along with above-mentioned chloroethenes. Degradation assays were performed for a binary mixture of *c*DCE and VC as well as for a mixture of TCE, all DCEs and VC. The consortium composed of *C. testosteroni* RF2 and *M. aurum* L1 showed to be the most efficient towards the removal of *c*DCE (6.01 mg L^{-1}) in the binary mixture with VC (10 mg L^{-1}) and was capable of efficiently removing chloroethenes in the mixture sample at the initial concentrations of 116 μg L^{-1} for TCE, 662 μg L^{-1} for *c*DCE, 42 μg L^{-1} for *t*DCE, 16 μg L^{-1} for 1,1DCE, and 7 mg L^{-1} for VC with a removal efficiency of nearly 100% for all of the compounds. Although complete removal of VC took a significantly longer time than the removal of other chloroethenes, the consortium composed of C. *testosteroni* RF2 and M. *aurum* L1 displayed strong bioremediation potential for aquifers with downstream contamination characterized by the presence of less chlorinated ethenes.

1. Introduction

Due to a range of different industrial activities, chlorinated ethenes are among the most common groundwater contaminants worldwide (Nishino et al., 2013). In general, despite the toxic nature

* Corresponding author.
E-mail addresses: michal.zalesak@iaeste.cz (M. Zalesak), ruzickaj@utb.cz (J. Ruzicka), rvicha@utb.cz (R. Vicha), dvorackova@utb.cz (M. Dvorackova).

https://doi.org/10.1016/j.chemosphere.2020.128770
0045-6535/

and persistence of chloroethenes, these xenobiotics are subject to various microbial degradations under certain conditions. Tetrachloroethene (PCE) and trichloroethene (TCE) can be readily reduced to less chlorinated ethenes under anaerobic conditions by bacteria in the genera *Dehalobacter* (Holliger et al., 1998), *Dehalospirillum* (Neumann et al., 1996) and *Dehalococcoides* (Fletcher et al., 2008). Although some *Dehalococcoides* (Maymo-Gatell et al., 1999; Ismaeil et al., 2017) and *Propionibacterium* (Chang et al., 2011) species were reported to be able to reduce PCE up to ethene, the tendency of chloroethenes to undergo reductive dechlorination decreases with a decreasing number of chlorine substituents (Tiehm and Schmidt, 2011). Hence, significant accumulation of 1,2-cis-dichloroethene (*c*DCE) and vinyl chloride (VC) downstream of reductive dechlorination is a major issue at many sites contaminated by PCE and TCE (Cupples et al., 2004). Among others, to a lesser extent, the process can lead to the formation of 1,2-trans-dichlorothene (*t*DCE) and 1,1DCE that along with *c*DCE and VC form persistent contamination plumes (Zhao et al., 2010; Tiehm and Schmidt, 2011).

Conversely to PCE, less chlorinated ethenes are more susceptible to microbial degradation under aerobic conditions (Mattes et al., 2010). In fact, plenty of bacteria can degrade such chloroethenes utilizing cometabolic or metabolic pathways under aerobic conditions; many of which were thoroughly reviewed by Dolinova et al. (2017). Cometabolic processes are applicable for degradation of a whole range of chloroethenes except for PCE that is broadly considered as non-biodegradable under aerobic conditions although its degradation by *Pseudomonas stutzeri* OX1 was described by Ryoo et al. (2000). In comparison, metabolic degradation, also known as direct oxidation, is restricted to VC (Gossett, 2010; Jin et al., 2010), *c*DCE (Jennings et al., 2009; Cloelle et al., 2010; Schmidt et al., 2010), and rarely *t*DCE (Olaniran et al., 2008). Furthermore, metabolic degradation of DCE*s* might be affected by other chloroethenes, as found in the study of Zhao et al. (2010), where *c*DCE degradation by an enrichment culture was significantly slowed down in samples containing a mixture of chloroethenes. On the other hand, in the case of VC, a metabolic pathway is believed to be a more efficient process for removing the compound compared to aerobic cometabolism (Frascari et al., 2015). Hence, the combination of bacterial direct oxidation of VC and cometabolism of TCE along with all DCEs offers an interesting clean-up strategy for certain aquifers characterized by a downstream accumulation of less chlorinated ethenes.

While field application of aerobic cometabolism at sites contaminated by chlorinated ethenes has been thoroughly investigated using either biostimulation or bioaugmentation as a bioremediation approach (Steffan et al., 1999; Frascari et al., 2015), direct bacterial oxidation of the same has been investigated only under laboratory conditions (Hartmans et al., 1985; Verce et al., 2000; Tiehm et al., 2008). Most of the field studies have focused on stimulating the aerobic cometabolism in autochthonous microbial populations applying different growth substrates along with various sources of oxygen. Out of these studies, the most significant results were obtained when applying methane (Semprini et al., 1990; Kim et al., 2008), phenol (Hopkins et al., 1993), toluene (Hopkins and McCarty, 1995), and propane (Kim et al., 2008) as primary substrates. In particular, using phenol as a biostimulative agent has shown to be an effective strategy to remove the contaminants. Hopkins et al. (1993) accomplished removals of TCE (45 μg L^{-1}, >90%) and *c*DCE (45 μg L^{-1}, >90%) by stimulating phenol utilizing microorganism in a shallow confined aquifer with phenol and oxygen. Furthermore, the subsequent study at the same site showed a high degradation capacity of phenol utilizing microorganisms towards a mixture of chloroethenes, intentionally introduced into the aquifer (Hopkins and McCarty, 1995). The study described rapid removals of TCE (250 μg L^{-1}, >90%); *c*DCE (125 μg L^{-1}, >90%); *t*DCE (125 μg L^{-1}, 74%); 1,1DCE (65 μg L^{-1}, 50%); and VC (60 μg L^{-1}, 98%). Hence, the site application of phenol as well-soluble, non-volatile, biostimulative agent showed to be highly efficient for the removal of a wide range of chloroethenes.

However, a treated site may lack autochthonous strains capable of such process, which can be overcome by pregrown microbial inoculum, preferably strain(s) possessing degrading capacity towards several chloroethenes. Nevertheless, until now, no study has described bioaugmentation of aquifers contaminated by chlorinated ethenes using pregrown phenol utilizing inoculum, presumably due to a lack of data available from laboratory experiments.

A prime candidate for a field application may be phenol utilizing strain *Comamonas testosteroni* RF2, which was described to efficiently degrade TCE, *c*DCE, *t*DCE, and 1,1DCE in mixed samples under laboratory conditions (Zalesak et al., 2017). Although the study showed that strain RF2 was fully incapable of VC degradation, the compound did not influence cometabolic degradation of *c*DCE by the strain. Thus, based on these promising findings, we decided to design a bacterial consortium degrading TCE, *c*DCE, *t*DCE, 1,1DCE, and VC under laboratory conditions, and we investigated three different consortia, each composed of phenol utilizing strain RF2 along with one of the VC degrading strains, specifically *Mycobacterium aurum* L1, *Pseudomonas putida* PS and *Rhodococcus ruber* Sm-1, which were purchased from German Collection of Microorganisms and Cell Cultures (DSMZ); these strains represent VC degraders well available for the whole scientific community. Whereas *M. aurum* L1 was described to degrade VC metabolically (Hartmans et al., 1985), other two strains, *R. ruber* Sm-1 (Phelps et al., 1991) and *P. putida* PS (Castro et al., 1992), were described to degrade VC cometabolically. The diversity of these strains allows us to assess the most suitable strain for the purposes of this study.

2. Materials and methods

2.1. Chemicals and nutrient agars

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

The composition of a mineral salt medium (MSM, pH 7.4) was identical to our previous study (Zalesak et al., 2017).

Tryptone yeast extract agar (TYA), Reasoner's 2A agar (R2A, both Himedia), and Middlebrook agar were used as solid growth media. Middlebrook agar was prepared according to a guideline published by DSMZ that is available online.

2.2. Description of strains

Comamonas testosteroni RF2 was isolated earlier from activated sludge fed by phenol. It is deposited in the Czech Collection of Microorganisms under the catalogue number CCM 7350.

Mycobacterium aurum DSM-6695, *Pseudomonas putida* DSM-7189, and *Rhodococcus ruber* DSM-7511 were purchased from DSMZ.

M. aurum DSM-6695 was isolated from contaminated soil in Arnhem, Netherlands, and designated as *M. aurum* L1 (Hartmans et al., 1985). It is an aerobic strain with an optimum growth temperature of 28 °C; it utilizes ethene and vinyl chloride as the only carbon and energy source.

P. putida DSM-7189 was isolated from a sample of soil collected in California, USA, and designated as *P. putida* PS (Castro and Belser, 1990). It is an aerobic strain with an optimum growth temperature

of 28 °C, which utilizes 3-chloropropan-1-ol and chloroallylalcohols as its carbon sources. The resting cells of the strain previously grown on the above-listed sources are capable of vinyl chloride mineralization (Castro et al., 1992).

R. ruber DSM-7511 was isolated from contaminated subsurface sediments at the Savannah River Site in the USA and designated as *R. ruber* Sm-1 (Phelps et al., 1991). It is an aerobic strain with an optimum growth temperature of 25 °C, which utilizes a variety of alkanes, benzene, toluene, phenol, benzoate, naphthalene or biphenyl as its sole carbon source. Further, it degrades cometabolically VC, TCE, and other chlorinated aliphatic compounds (Malachowsky et al., 1994).

Original designations of all strains are used throughout the study.

2.3. Growth conditions

The strain RF2 was routinely grown on TYA plates enriched with phenol (200 mg L^{-1}) for three days. *M. aurum* L1 was cultivated either onto the Middlebrook or R2A agar for two weeks; *P. putida* PS and *R. ruber* Sm-1 were cultivated on R2A agar for three days. All the strains were incubated at 25 °C. Grown bacterial cells were harvested and dispersed in sterile saline to obtain the cell density 10^8 CFU mL^{-1}, and finally utilized in degradation assays.

2.4. Growth of bacterial strains on carbon sources

The tests were performed in 40 mL sterile glass vials sealed with sterile (UV irradiated) gas-tight septum caps (Wheaton). Each vial contained 10 mL of MSM, a cell suspension (10 μL), and an appropriate carbon source: sodium benzoate, phenol, or 3-chloropropan-1-ol (200 mg L^{-1}). Prepared samples were incubated either for 3 days (strains RF2, *P. putida* PS, and *R. ruber* Sm-1) or 14 days (*M. aurum* L1) as described for degradation assays, and bacterial growth was monitored by the optical density at 600 nm (OD_{600}) measurements: the portions of 200 μL were pipetted into three wells of a microplate and measured using TECAN SUNRISE spectrophotometer. Blank tests (MSM with carbon source and MSM + inoculum) were performed in parallel.

2.5. Degradation assays

All degradation assays were performed as previously described by Zalesak et al. (2017). Shortly, experiments were conducted in 40 mL glass vials in quadruplicates for each sampling time, in addition to which abiotic blanks were always carried out. Each vial contained 10 mL of MSM, cell suspension of selected strains (each 10 μL), 200 mg L^{-1} of appropriate carbon source (described in detail in Table 2), and different concentrations of chloroethenes, the latter being added as methanolic solutions. Samples were not further enriched in the course of incubation. The concentrations of TCE and all DCEs listed are intended as actual concentrations in the liquid phase, whilst concentrations of VC are intended as "all in the liquid phase".

Degradation assays were performed in three series. (1) In the first series, three different consortia were tested for their ability to degrade *c*DCE (6.09 mg L^{-1}) in the presence of VC (10 mg L^{-1}). (2) The second series investigated a degradation course 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}) in mixed samples by a consortium composed of strain RF2 and *M. aurum* L1. (3) The third series examined the course of degradation of TCE (117.2 μg L^{-1}), *c*DCE (671 μg L^{-1}), *t*DCE (44.09 μg L^{-1}), and 1,1DCE (16.13 μg L^{-1}) by the strain RF2 alone.

2.6. Analytical methods

Each sample was taken by gas-tight syringe through a septum and each vial was used for one measurement only. TCE and DCEs were extracted by the Purge and Trap method (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 in length, 0.53 mm (inner diameter) and 3 μm in film thickness, supplemented with an electron-capture detector (ECD), as previously described (Zalesak et al., 2017). The oven temperature was set at 30 °C min^{-1}, up to a maximum of 150 °C, and maintained until all the compounds had eluted.

The loss of VC during the tests was monitored in a gas phase using GC-MS Shimadzu QP-2010 equipped with the Equity-1 (30 m, 0.32 mm, 1 μm) column, and He as carrier gas at constant linear velocity (58.8 cm s^{-1}) was used; GC method: 40 °C/10 min, 20 °C min^{-1} 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). Propane-2-ol (IPA) was used as an internal standard and typical ion *m*/*z* 62 and 45 were selected as characteristic ions for VC and IPA, respectively. This approach enabled unambiguous observation of two peaks with no interference with other compounds, which were present in the sample (other chloroethenes and degradation intermediates). The SIM mode was stopped at 4.99 min and the full-scan mode was applied for the residual time of the analysis (*m*/*z* 40–450).

3. Results

3.1. Utilization of carbon sources by VC degrading strains and strain RF2

VC degrading strains along with strain RF2 were examined for their ability to utilize benzoate sodium, 3-chloropropan-1-ol, and phenol as carbon sources in terms to find out a potential competition between strain RF2 and each of the VC degrading strains for these carbon sources; the results are shown in Table 1.

The results in Table 1 confirmed the suitability of benzoate sodium and 3-chloropropan-1-ol for the growth of *R. ruber* Sm-1 and *P. putida* PS, respectively. Also, strain RF2 could utilize both compounds, particularly benzoate sodium was found as a suitable growth substrate. Further tests with phenol showed that while the *R. ruber* Sm-1 efficiently utilized the compound, *P. putida* PS could utilize phenol only slightly. Finally, *M. aurum* L1 showed no signs of growth on phenol.

3.2. cDCE degradation by three consortia in the presence of VC

Being the predominant chloroethene at many sites affected by chlorinated solvents, *c*DCE, the compound previously found to be efficiently degraded by the strain RF2, was chosen for the first series

Table 1
Utilization of carbon sources by the strains.

Strain	Carbon source (200 mg L^{-1})		
	Benzoate sodium	3-chloropropan-1-ol	Phenol
C. testosteroni RF2	++	+	++
R. ruber Sm-1	++	−	++
P. putida PS	N	++	+
M. aurum L1	N	N	−
MSM + carbon source	−	−	−

N: not tested; (−) no growth, OD_{600} < 0.02; (+) slight growth, OD_{600} 0.07–0.09. (++) significant growth, OD_{600} 0.13–0.15.
Initial and final levels of inoculated blanks (MSM + each inoculum): OD_{600} < 0.02.

of assays examining *c*DCE degradation in a binary mixture with VC. The assay aimed to investigate the influence of each VC degrading strain and VC itself (10 mg L^{-1}) on degradation course of *c*DCE (6.09 mg L^{-1}) by strain RF2. The assays were performed with three bacterial consortia, each composed of the strain RF2 and one of the VC degrading strains. The carbon sources used for the growth of strains were selected on the base of the preliminary growth tests as well as the findings of the previous work (Zalesak et al., 2017) examining cometabolic degradation of TCE and DCEs by the strain RF2 (Table 2). The results of the degradation courses of *c*DCE in the three consortia over a course of 8 days are given in Fig. 1.

Although the results showed that *c*DCE in a binary mixture with VC was efficiently degraded in all three consortia, the degradation courses differed in each consortium as seen in Fig. 1. Whereas a complete removal of *c*DCE in consortium containing *M. aurum* L1 occurred within 4 days of the assay, the removal of the compound in consortia comprising *R. ruber* Sm-1 or *P. putida* PS took 6 days. Owing to the fastest removal of *c*DCE in a consortium containing *M. aurum* L1, the consortium was chosen for main degradation assays aiming to examine its degradation potential towards a mixture of chloroethenes.

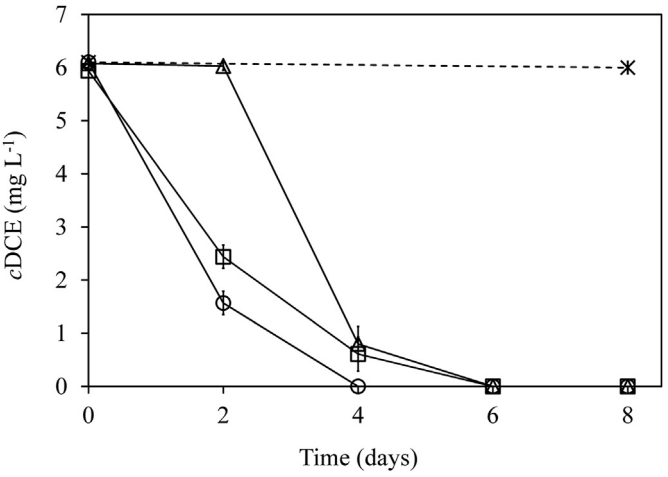


Fig. 1. Degradation curves of *c*DCE in the presence of VC by three consortia; (○) *C. testosteroni* RF2 + *M. aurum* L1; (□) *C. testosteroni* RF2 + *P. putida* PS; (Δ) C. *testosteroni* RF2 + *R. ruber* Sm-1; (×) abiotic blank.

3.3. Degradation of TCE, DCEs and VC in a mixed sample

Degradations of TCE, all DCEs, and VC in mixed samples by a consortium composed of the strain RF2 and *M. aurum* L1 were examined. The composition of the mixture and the chloroethene concentrations were chosen to simulate a groundwater contamination plume with *c*DCE as a predominant intermediate of TCE anaerobic dehalogenation. Additionally, the oddly high concentration of VC, in the context of the other chloroethenes within the mixture, was intentionally applied in terms to investigate its influence on degradation efficiency of strain RF2 towards TCE and DCEs as well as to examine degradation capacity of *M. aurum* L1 towards VC in the mixture. The assay lasted 21 days and its course over time is shown in Fig. 2 A,B.

In parallel, degradation of TCE and all DCEs in another mixture was studied in the strain RF2 alone, i.e. without applying VC and *M. aurum* L1. The comparison of the two tests aimed to examine the influence of VC and its degradation products on the degradation of TCE and DCEs in the strain RF2. The parallel assay lasted 6 days and the results are given in Fig. 2C.

While Fig. 2 A shows the percentage removal of highly volatile VC (7 mg L^{-1}), Fig. 2 B tracks the decrease in concentrations of other chloroethenes within the same mixture. In general, all chloroethenes were completely degraded at the end of the assay. Whereas *c*DCE (662 μg L^{-1}) and 1,1DCE (16.02 μg L^{-1}) were fully removed within the first 2 days of the assay, the removal of TCE (115.7 μg L^{-1}) took 3 days. Furthermore, *t*DCE (42.01 μg L^{-1}) was significantly degraded after two-days lasting lag phase and its complete degradation was accomplished during the last three days of the assay. In contrast, despite the rapid percentage removal of VC in the first two days of the assay, its complete degradation required a significantly longer time and was achieved after 21 days. The substantial decrease in removal rate of VC, observed between days 2 and 7, when only 4.32% of the compound was removed, was the significant phenomenon of the process. Here, the production of a toxic intermediate chlorooxirane might be the possible cause of the temporary slowdown in the degradation rate of the compound that is discussed further in the paper.

Interestingly, the parallel assay showed that RF2 alone completely removed TCE and all DCEs one day faster than the consortium composed of the strain RF2 and *M. aurum* L1 in presence of VC (Fig. 2C). In detail to the parallel test, despite the significantly higher concentration of *c*DCE (671 μg L^{-1}) compared to other chloroethenes in the mixture, the removal of the compound along with 1,1DCE (16.13 μg L^{-1}) lasted just 1 day, whilst degradation of TCE (117.2 μg L^{-1}) took 2 days. *t*DCE (44.09 μg L^{-1}) was nearly fully degraded in 3 days; after that, it showed only trace amounts in GC-ECD chromatograms.

The efficient removals of TCE and DCEs by both the consortium (Fig. 2 B) and RF2 alone (Fig. 2C) was fostered by abiotic blanks in which no or negligible reductions in TCE and DCEs concentrations were observed (data not shown for simplicity).

4. Discussion

The results of this study demonstrated high degradation efficiency of the consortium composed of *C. testosteroni* RF2 and *M. aurum* L1 towards a mixture of chloroethenes containing TCE, all DCEs, and VC. Further, the study showed that *M. aurum* L1 did not utilize phenol for the growth (Table 1) and thus it could not compete with strain RF2 for the carbon source fundamentally. On the contrary, *P. putida* PS or *R. ruber* Sm-1, growing on 3-chloropropan-1-ol or benzoate sodium, respectively, could potentially compete for these carbon sources with strain RF2 that was proven to utilize both compounds (Table 1).

Possible interactions between each VC degrading strain and strain RF2 as well as the effects of VC on degradation efficiency of cDCE in strain RF2 were firstly evaluated in samples containing a binary mixture of *c*DCE and VC, inoculated with three designed consortia listed in Table 2. Results showed that *c*DCE (6.09 mg L^{-1})

Table 2
Designed consortia and applied carbon sources used for degradation of *c*DCE in presence of VC.

Consortium	RF2 + *R. ruber* Sm-1	RF2 + *P. putida* PS	RF2 + *M. aurum* L1
Carbon source(s)	phenol (200 mg L^{-1})	phenol (150 mg L^{-1}) 3-chloropropan-1-ol (50 mg L^{-1})	phenol (100 mg L^{-1}) lactate (100 mg L^{-1})

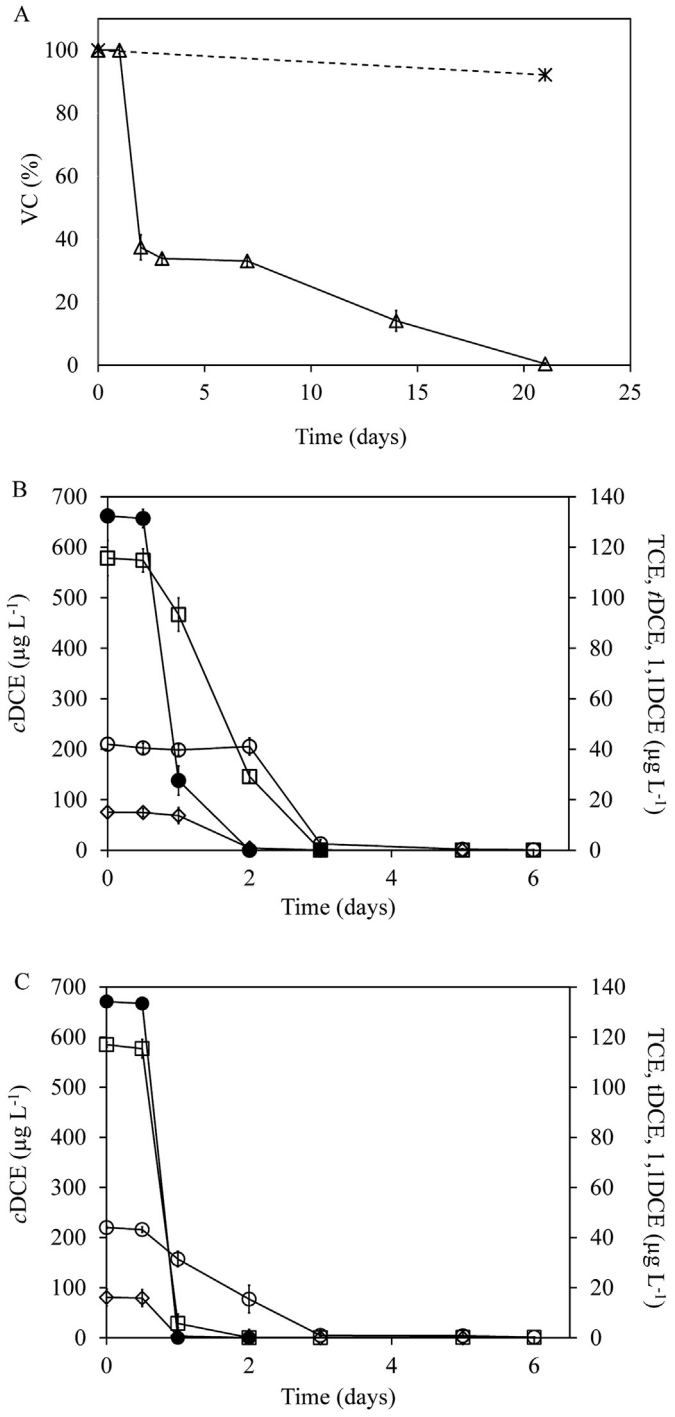


Fig. 2. A,B: Degradation curves of TCE, all DCEs, and VC in a mixed sample by a consortium composed of *C. testosteroni* RF2 and *M. aurum* L1; Fig. 2. C: Degradation curves of TCE and all DCEs in a mixed sample by *C. testosteroni* RF2 alone; (□) TCE; (●) *c*DCE; (○) *t*DCE; (◊) 1,1DCE; (Δ) VC; (×) abiotic blank.

was completely removed in all three consortia, however, its degradation courses differed in each consortium (Fig. 1). The fastest removal of *c*DCE lasted four days and was seen in a consortium containing *M. aurum* L1. This result was accompanied with the rapid production of 2,2-dichloracetaldehyde, described as the first intermediate of *c*DCE transformation in strain RF2 (Zalesak et al., 2017), which was clearly recorded on all chromatograms between the second and eighth day of the assay (data not shown). In comparison, complete removal of the same amount of *c*DCE in consortia containing either *P. putida* PS or *R. ruber* Sm-1 took two days longer and the initial production of 2,2-dichloracetaldehyde was less rapid. This phenomenon was particularly distinct in the consortium encompassing *R. ruber* Sm-1, where 2,2-dichloracetaldehyde was recorded no earlier than the fourth day of the assay. Such a result may be due to initial inhibition of *c*DCE degradation by higher phenol concentration (200 mg L^{-1}) applied for supplying both members of the consortium. Thus, findings of the first degradation series indicated that the consortium composed of strain RF2 and *M. aurum* L1 forms the most suitable tool for removal of chloroethenes in mixed samples.

Indeed, the consortium could efficiently remove TCE, all DCEs, and VC in the mixture sample (Fig. 2A and B). However, despite the initial rapid degradation of VC, complete removal of the compound lasted 21 days. On the contrary, the consortium was able to completely remove other chloroethenes within 2–6 days. Longer degradation time of VC resulted from a temporary slowdown in its degradation course that occurred between the third and seventh day of the assay. This could be explained by the temporary production of chlorooxirane during the initial rapid degradation phase of VC, described by Hartmans and de Bont (1992). Although chlorooxirane may be further transformed by epoxyalkane coenzyme M transferase (Coleman and Spain, 2003), it may also be partially released from the cells of *M. aurum* L1 in the course of VC degradation (Hartmans and de Bont, 1992) and eventually affect other microbial processes. Hence, in the parallel test, cometabolic degradation of mixed samples containing TCE and all DCEs was examined in strain RF2 alone. Here it was found that the bacterium fully removed all the compounds in the mixture one day faster than the consortium in the previous test (compare Fig. 2B and C). The findings from both degradation tests suggest that chlorooxirane not only affected VC degradation but also influenced the removal rate of other chloroethenes in the strain RF2. Besides, further reasons for the observed temporary slowdown of the VC degradation might exist, such as the influence of TCE-epoxide and DCE-epoxides, which could be formed during the cometabolic degradation of chloroethenes by the strain RF2. Especially 1,1DCE-epoxide, a transformation intermediate of 1,1DCE, was found to be strongly reactive and often causing the death of microbial cells (Forkert, 1999). However, some bacteria can break down a certain proportion of this compound without adversely affecting their cells (Verce et al., 2001). Thus, it might be hypothesized that dissipation of TCE-epoxide, DCE-epoxides, and possibly other intermediates, in particular 2,2-dichloroacetaldehyde that showed its peak in the sixth day of the assay (data not shown), could lead in the restoring of VC degradation observed after the seventh day of the assay (Fig. 2 A).

Among others, although resting cells of *M. aurum* L1 were earlier described to partially degrade all three DCEs (Hartmans and de Bont, 1992), the results of this study did not show any contribution of the strain towards the removal of DCEs under growing condition, as the complete removal of the compounds in strain RF2 alone was notably faster than in the consortium (Fig. 2B and C).

Finally, despite the degradation of chloroethenes in the mixture by the consortium composed of *C. testosteroni* RF2 and *M. aurum* L1 involves a complex of processes potentially influencing degradation rate of the compounds, the two strains proved substantial suitability for bioremediation applications at sites characterized by the occurrence of less chlorinated ethenes.

5. Conclusions

The results of this study showed that consortium composed of *C. testosteroni* RF2 and *M. aurum* L1 can degrade TCE, all DCEs, and

VC in a mixture sample. Each strain in the consortium played a crucial role in the removal of certain chloroethenes within the mixture. While TCE and DCEs were primarily degraded by the strain RF2, the degradation of VC was exclusively ensured by *M. aurum* L1. Removal of chloroethenes was accomplished by a combination of aerobic cometabolism of TCE and DCEs along with metabolic degradation of VC, which makes it an interesting option for in-situ bioaugmentation downgradient of reductive dechlorination process commonly characterized by the accumulation of *c*DCE, VC, and residual TCE.

Credit author statement

Michal Zalesak: Investigation – growth and degradation assays, GC-ECD analyses, Methodology, Writing - Original draft preparation, Reviewing and Editing; **Jan Ruzicka**: Conceptualization, Writing - Original draft preparation, Reviewing and Editing; **Robert Vicha**: Investigation – vinyl chloride measurement by GC-MS; **Marie Dvorackova**: Investigation – GC-ECD analyses.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was supported by the Internal Grant Agency of Tomas Bata University in Zlin (projects numbers IGA/FT/2017/003 and IGA/FT/2018/009).

References

Castro, C.E., Belser, N.O., 1990. Biodehalogenation: oxidative and reductive metabolism of 1,1,2-trichloroethane by *Pseudomonas putida* – biogeneration of vinyl chloride. Environ. Toxicol. Chem. 9, 707–714 https://doi.org/10.1002/etc.5620090603.

Castro, C.E., Wade, R.S., Riebth, D.M., Bartnicki, E.W., Belser, O.N., 1992. Biodehalogenation: rapid metabolism of vinyl chloride by a soil *Pseudomonas* sp. Direct hydrolysis of vinyl C-Cl bond. Environ. Toxicol. Chem. 11, 757–764 https://doi.org/10.1002/etc.5620110605.

Chang, Y.C., Ikeutsu, K., Toyama, T., Choi, D., Kikuchi, S., 2011. Isolation and characterization of tetrachloroethylene and *cis*-1,2-dichloroethylene dechlorinating *propionibacteria*. J. Ind. Microbiol. Biotechnol. 10, 1667–1677 https://doi.org/10.1007/s10295-011-0956-1.

Cloelle, G.S.G., Fang, L., James, M.G., 2010. Microcosm assessment of *Polaromonas* sp. JS666 as a bioaugmentation agent for degradation of *cis*-1,2-dichloroethene in aerobic, subsurface environments. Groundwater Monit. Res. 10, 106–113 https://doi.org/10.1111/j.1745-6592.2010.01283.x.

Coleman, N.V., Spain, J.C., 2003. Epoxyalkane: coenzyme M transferase in the ethene and vinyl chloride biodegradation pathways of *Mycobacterium* strain JS60. J. Bacteriol. 185, 5536–5545 https://doi.org/10.1128/JB.185.18.5536-5545.2003.

Cupples, A.M., Spormann, A.M., McCarty, P.L., 2004. Vinyl chloride and *cis*-dichloroethene dechlorination kinetics and microorganism growth under substrate limiting conditions. Environ. Sci. Technol. 38, 1102–1107 https://doi.org/10.1021/es0348647.

Dolinova, I., Strojsova, M., Cernik, M., Nemecek, J., Machackova, J., Sevcu, A., 2017. Microbial degradation of chloroethenes: a review. Environ. Sci. Pollut. Res. Int. 24, 13262-13262. https://doi.org/10.1007/s11356-017-8867-y.

Fletcher, K.E., Ritalahti, K.M., Pennell, K.D., Takamizawa, K., Loffler, F.E., 2008. Resolution of culture *Clostridium bifermentans* DPH-1 into two populations, a *Clostridium* sp. and tetrachloroethene-dechlorinating *Desulfitobacterium hafniense* strain JH1. Appl. Environ. Microbiol. 74, 6141–6143 https://doi.org/10.1128/AEM.00994-08.

Forkert, P.G., 1999. 1,1-Dichloroethylene induced Clara cell damage is associated with in situ formation of the reactive epoxide. Am. J. Respir. Cell Mol. Biol. 20, 1310–1318 https://doi.org/10.1165/ajrcmb.20.6.3525.

Frascari, D., Zanaroli, G., Danko, A.S., 2015. In situ aerobic cometabolism of chlorinated solvents: a review. J. Hazard Mater. 283, 382–399 https://doi.org/10.1016/j.jhazmat.2014.09.041.

Gossett, J.M., 2010. Sustained aerobic oxidation of vinyl chloride at low oxygen concentrations. Environ. Sci. Technol. 44, 1405–1411 https://doi.org/10.1021/es9033974.

Hartmans, S., de Bont, J.A.M., 1992. Aerobic vinyl chloride metabolism in *Mycobacterium aurum* L1. Appl. Environ. Microbiol. 58, 1220–1226 https://doi.org/10.1128/AEM.58.4.1220-1226.1992.

Hartmans, S., de Bont, J.A.M., Tramper, J., Luyben, K.C.A.M., 1985. Bacterial degradation of vinyl chloride. Biotechnol. Lett. 7, 383–388 https://doi.org/10.1007/BF01166208.

Holliger, C., Hahn, D., Harmsen, H., Ludwig, W., Schumacher, W., Tindall, B., Vazquez, F., Weiss, N., Zehnder, A.J.B., 1998. Dehalobacter restrictus gen. nov. and sp. nov., a strictly anaerobic bacterium that reductively dechlorinates tetra- and trichloroethene in an anaerobic respiration. Arch. Microbiol. 169, 313–321 https://doi.org/10.1007/s002030050577.

Hopkins, G.D., McCarty, P.L., 1995. Field evaluation of in-situ aerobic cometabolism of trichloroethylene and three dichloroethylene isomers using phenol and toluene as the primary substrates. Environ. Sci. Technol. 29, 1628–1637 https://doi.org/10.1021/es00006a029.

Hopkins, G.D., Semprini, L., McCarty, P.L., 1993. Microcosm and in situ field studies of enhanced biotransformation of trichloroethylene by a phenol utilizing microorganism. Appl. Environ. Microbiol. 59, 2277–2285 https://doi.org/10.1128/AEM.59.7.2277-2285.1993.

Ismaeil, M., Yoshida, N., Katayama, A., 2017. Identification of multiple dehalogenase genes involved in tetrachloroethene-to-ethene dechlorination in a *Dehalococcoides*-dominated enrichment culture. BioMed Res. Int. 2017, 1–12 https://doi.org/10.1155/2017/9191086.

Jennings, L.K., Chartrand, M.M.G., Lacrampe-Couloume, G., Lollar, B.S., Spain, J.C., Gossett, J.M., 2009. Proteomic and transcriptomic analyses reveal genes upregulated by *cis*-dichloroethene in *Polaromonas* sp. strain JS666. Appl. Environ. Microbiol. 75, 3733–3744 https://doi.org/10.1128/AEM.00031-09.

Jin, Y.O., Cheung, S., Coleman, N.V., Mattes, T.E., 2010. Association of missense mutations in epoxyalkane coenzyme M transferase with adaptation of *Mycobacterium* sp. strain JS623 to growth on vinyl chloride. Appl. Environ. Microbiol. 76, 3413–3419 https://doi.org/10.1128/AEM.01320-09.

Kim, Y., Istok, J.D., Semprini, L., 2008. Single-well, gas-sparging tests for evaluating the in situ aerobic cometabolism of *cis*-1,2-dichloroethene and trichloroethene. Chemosphere 71, 1654–1664 https://doi.org/10.1016/j.chemosphere.2008.01.021.

Malachowsky, K.J., Phelps, T.J., Teboli, A.B., Minnikin, D.E., White, D.C., 1994. Aerobic mineralization of trichloroethylene, vinyl chloride, and aromatic compounds by *Rhodococcus* species. Appl. Environ. Microbiol. 60, 542–548 https://doi.org/10.1128/AEM.60.2.542-548.1994.

Mattes, T.E., Alexander, A.K., Coleman, N.V., 2010. Aerobic biodegradation of the chloroethenes: pathways, enzymes, ecology, and evolution. FEMS Microbiol. Rev. 34, 445–475 https://doi.org/10.1111/j.1574-6976.2010.00210.x.

Maymo-Gatell, X., Anguish, T., Zinder, S.H., 1999. Reductive dechlorination of chlorinated ethenes and 1,2-dichloroethane by *Dehalococcoides ethenogenes* 195. Appl. Environ. Microbiol. 65, 3108–3311.

Neumann, A., Wohlfarth, G., Diekert, G., 1996. Purification and characterization of tetrachloroethene reductive dehalogenase from *Dehalospirillum multivorans*. J. Biol. Chem. 28, 16515–16519 https://doi.org/10.1074/jbc.271.28.16515.

Nishino, S.F., Shin, K.A., Gossett, J.M., Spain, J.C., 2013. Cytochrome P450 initiates degradation of *cis*-dichloroethene by *Polaromonas sp.* strain JS666. Appl. Environ. Microbiol. 79, 2263–2272 https://doi.org/10.1128/AEM.03445-12.

Olaniran, A.O., Pillay, D., Pillay, B., 2008. Aerobic biodegradation of dichloroethenes by indigenous bacteria isolated from contaminated sites in Africa. Chemosphere 73, 24–29 https://doi.org/10.1016/j.chemosphere.2008.06.003.

Phelps, T.J., Malachowsky, K.J., Schram, R.M., White, D.C., 1991. Aerobic mineralization of vinyl chloride by a bacterium of the order *Actinomycetales*. Appl. Environ. Microbiol. 57, 1252–1254 https://doi.org/10.1128/AEM.57.4.1252-1254.1991.

Ryoo, D., Shim, H., Canada, K., Barbieri, P., Wood, T.K., 2000. Aerobic degradation of tetrachloroethylene by toluene-o-xylene monooxygenase of *Pseudomonas stutzeri* OX1. Nat. Biotechnol. 18, 775–778.

Schmidt, K.R., Augenstein, T., Heidinger, M., Ertl, S., Tiehm, A., 2010. Aerobic biodegradation of *cis*-1,2-dichloroethene as sole carbon source: stable carbon isotope fractionation and growth characteristics. Chemosphere 78, 527–532 https://doi.org/10.1016/j.chemosphere.2009.11.033.

Semprini, L., Roberts, P.V., Hopkins, G.D., McCarty, P.L., 1990. A field evaluation of in-situ biodegradation of chlorinated ethenes. Part 2, Results of biostimulation and biotransformation experiments. Groundwater 28, 715–727 https://doi.org/10.1111/j.1745-6584.1990.tb01987.x.

Steffan, R.J., Sperry, K.L., Walsh, M.T., Vainberg, S., Condee, C.W., 1999. Field-scale evaluation of in situ bioaugmentation for remediation of chlorinated solvents in groundwater. Environ. Sci. Technol. 33, 2771–2781 https://doi.org/10.1021/es9812204.

Tiehm, A., Schmidt, K.R., Pfeifer, B., Heidinger, M., Ertl, S., 2008. Growth kinetics and stable carbon isotope fractionation during aerobic degradation of *cis*-1,2-dichloroethene and vinyl chloride. Water Res. 42, 2431–2438 https://doi.org/10.1016/j.watres.2008.01.029.

Tiehm, A., Schmidt, R.S., 2011. Sequential anaerobic/aerobic biodegradation of chloroethenes-aspects of field application. Curr. Opin. Biotechnol. 22, 415–421 https://doi.org/10.1016/j.copbio.2011.02.003.

Verce, M.F., Ulrich, R.L., Freedman, D.L., 2000. Characterization of an isolate that uses vinyl chloride as a growth substrate under aerobic conditions. Appl. Environ. Microbiol. 66, 3535–3542 https://doi.org/10.1128/AEM.66.8.3535-3542.2000.

Verce, M.F., Ulrich, R.L., Freedman, D.L., 2001. Transition from cometabolic to growth-linked biodegradation of vinyl chloride by a *Pseudomonas* sp. isolated on ethene. Environ. Sci. Technol. 35, 4242–4251 https://doi.org/10.1021/es002064f.

Zalesak, M., Ruzicka, J., Vicha, R., Dvorackova, M., 2017. Cometabolic degradation of dichloroethenes by *Comamonas testosteroni* RF2. Chemosphere 186, 919–927 https://doi.org/10.1016/j.chemosphere.2017.07.156.

Zhao, H.-P.Z., Schmidt, K.R., Tiehm, A., 2010. Inhibition of aerobic metabolic *cis*-1,2-dichloroethene biodegradation by other chloroethenes. Water Res. 44, 2276–2282 https://doi.org/10.1016/j.watres.2009.12.023.