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Master Thesis

Conducting microcosm experiments to select suitable pollutant-degrading microbial consortia for perchloroethene remediation.

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Affidavit

I hereby declare that I have authored this master thesis independently, and that I have not used any assistance other than that which is permitted. The work contained herein is my own except where explicitly stated otherwise. All ideas taken in wording or in basic content from unpublished sources or from published literature are duly identified and cited, and the precise references included.

I further declare that this master thesis has not been submitted, in whole or in part, in the same or a similar form, to any other educational institution as part of the requirements for an academic degree.

I hereby confirm that I am familiar with the standards of Scientific Integrity and with the guidelines of Good Scientific Practice, and that this work fully complies with these standards and guidelines.

Vienna, 16th August Celia FERNÁNDEZ BALADO *(manu propria)*

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Abstract

Perchloroethene (PCE) is one of the most frequent contaminants found in groundwater. It is widely used as a solvent in the industry and its chemical properties makes it a persistent and difficult to degrade pollutant. Reductive dichlorination is a biological remediation process in which organohalide respiring bacteria (OHRB) transform PCE to other toxic metabolites until the harmless ethene. This study investigated the dichlorination performance of two commercially available mixed microbial communities (KB1 and Bioclear b.v.) for further use in physical-biological remediation filters. Microcosm (MC) experiments and compound specific isotope analysis (CSIA) were used to monitor PCE degradation, and a phospholipid fatty acid (PLFA) extraction was carried out to investigate the microbial community's structure. Results showed that the type of electron source for the bacteria and the presence of Dehalococcoides sp. are key factors for the complete degradation of PCE. In addition, no significant PCE isotope fractionation could be observed, meaning that applying CSIA might not always be possible to quantify degradation. The KB1 culture, when incubated with lactate, is suitable for complete PCE dichlorination and represents a potential option for its use in combination with biochar for physical-biological remediation.

Kurzfassung

Perchlorethen (PCE) ist einer der häufigsten Schadstoffe, die im Grundwasser gefunden werden. Es wird in großem Umfang als Lösungsmittel in der Industrie verwendet und ist aufgrund seiner chemischen Eigenschaften ein persistenter und schwer abbaubarer Schadstoff. Die reduktive Dichlorierung ist ein biologischer Sanierungsprozess, bei dem Organohalogenid-atmende Bakterien (OHRB) PCE in andere toxische Metaboliten bis hin zum harmlosen Ethen umwandeln. In dieser Studie wurde die Dichlorierungsleistung von zwei kommerziell erhältlichen gemischten mikrobiellen Gemeinschaften (KB1 und Bioclear b.v.) für die weitere Verwendung in physikalisch-biologischen Sanierungsfiltern untersucht. Zur Überwachung des PCE-Abbaus wurden Mikrokosmusexperimente (MC) und eine verbindungsspezifische Isotopenanalyse (CSIA) verwendet, und zur Untersuchung der Struktur der mikrobiellen Gemeinschaft wurde eine Phospholipidfettsäureextraktion (PLFA) durchgeführt. Die Ergebnisse zeigten, dass die Art der Elektronenquelle für die Bakterien und das Vorhandensein von Dehalococcoides sp. entscheidende Faktoren für den vollständigen Abbau von PCE sind. Darüber hinaus konnte keine signifikante PCE-Isotopenfraktionierung beobachtet werden, was bedeutet, dass die Anwendung von CSIA möglicherweise nicht immer möglich ist, um den Abbau zu quantifizieren. Die KB1-Kultur ist, wenn sie mit Laktat bebrütet wird, für eine vollständige PCE-Dichlorierung geeignet und stellt eine potenzielle Option für die Verwendung in Kombination mit Biokohle zur physikalisch-biologischen Sanierung dar.

1. Introduction

1.1 Perchloroethene and groundwater contamination.

Perchloroethene (PCE) is a chlorinated hydrocarbon (CHC) with molecular formula C_2Cl_4. It is considered one of the most common groundwater and soil contaminants due to its wide use in industrial production (Huang et al., 2014). It is a colourless, volatile, dense non-aqueous phase liquid (DNAPL) with an ethereal odour, and it is also known as tetrachloroethylene, perchloroethylene, tetrachloroethene and perchlor. Its high density (1.62 g/cm^3) and low solubility in water (150 mg/L at 25 °C) makes it a potential persistent source of pollution once it reaches the subsurface (Dong et al., 2017). Its chemical structure is shown in Figure 1

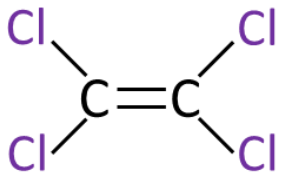

Figure 1. Chemical structure of perchloroethene (PCE)

PCE is used as a solvent in the dry cleaning of textiles, as a vapour and liquid degreasing agent in metal-cleaning operations, as a heat transfer medium, as a starting material in the manufacture of fluorohydrocarbons and in some consumer products such as fragrances, spot removers or water repellents. It is mainly released directly to the atmosphere during use (especially during dry-cleaning) where it has a half-life of about 3-4 months. When it is present in industrial liquid wastes, it can escape from the waste site and end in the surface water and soil. As PCE does not easily volatilize from soil and it is reasonably mobile, it can leach and contaminate groundwater (ATSDR, 2019). In addition, it is only biodegraded in water under anaerobic conditions and at a low rate by the process of reductive dechlorination, therefore it may persist for several months in groundwater (Nijhuis et al., 2010).

According to the European Chemicals Agency (ECHA) 2021, PCE is toxic to aquatic life with long-lasting effects, and it is suspected of causing cancer. Perchloroethene exposure may lead to kidney, nervous system, liver, and reproductive system damage (ATDSR, 2019). In Austria, 69,628 contaminated sites and old deposits of waste are registered in which CHC are the most frequent pollutants found in significant quantities with a 90% prevalence (Granzin and Valtl, 2021) (Figure 2).

Undoubtedly, groundwater has a huge environmental value, and it plays an essential role providing drinking water worldwide. It is the largest reservoir of fresh water after glaciers and ice caps in our planet and it is an important source for irrigation in agriculture, for domestic and industrial uses (Margat & Gun, 2013). It is of urgent need to reduce the use of CHC and to continue developing and applying economic and effective remediation methods to diminish the threat and ensure both quality and protection of groundwater and the environment.

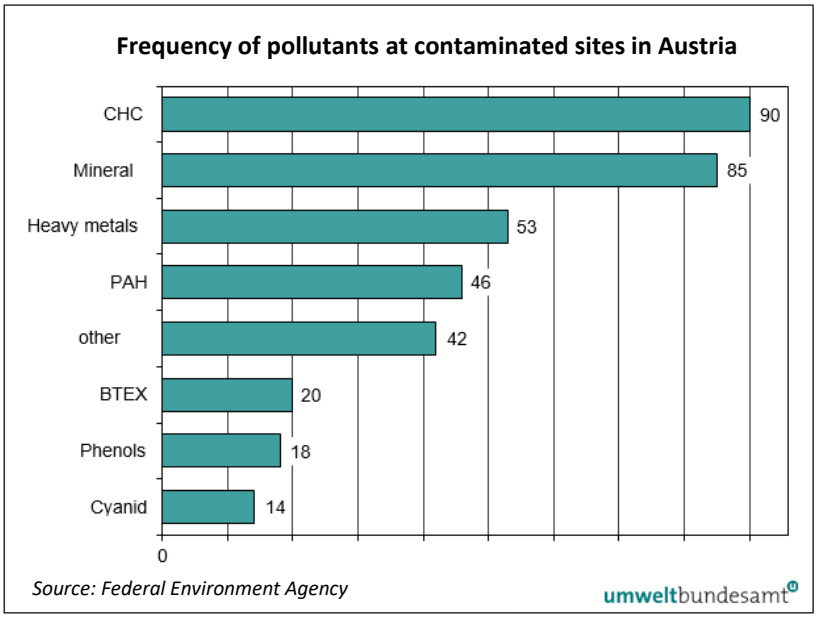

Figure 2. Frequency of pollutants found in significant quantities at contaminated sites in Austria (Granzin and Valtl, 2021, translated)

1.2 PCE remediation techniques

DNAPLs, such as PCE, are challenging to degrade due to their chemical properties (low solubility in water, low viscosity, and higher density than water). Depending on the location of treatment, remediation methods can be divided into *ex situ* and *in situ*. In general, *ex situ* processes involve pump (water) or extract (gas) and treat technologies on-site or off-site. On the other hand, *in situ* technologies such as permeable reactive barriers (PRBs) require a deep knowledge about the subsurface region where the technique is applied, and they are a more efficient, less disruptive, and sustainable remediation option since they keep the underground structures intact and they are applicable in urban areas (ÖVA, 2012). In addition, there is another *in situ* remediation option known as natural attenuation. Natural attenuation is a passive remediation method that reduces contaminant concentrations by physical (binding contaminants to soil particles), chemical (dilution and dispersion) and biological (biodegradation) natural processes (Khan et al., 2004). A proper way to identify and monitor natural attenuation processes is compound-specific isotope analysis (CSIA) which will be explained more in detail in the following sections.

Depending on the mode of action applied, CHC decontamination processes can be differentiated into chemical, adsorptive and biological (Figure 3).

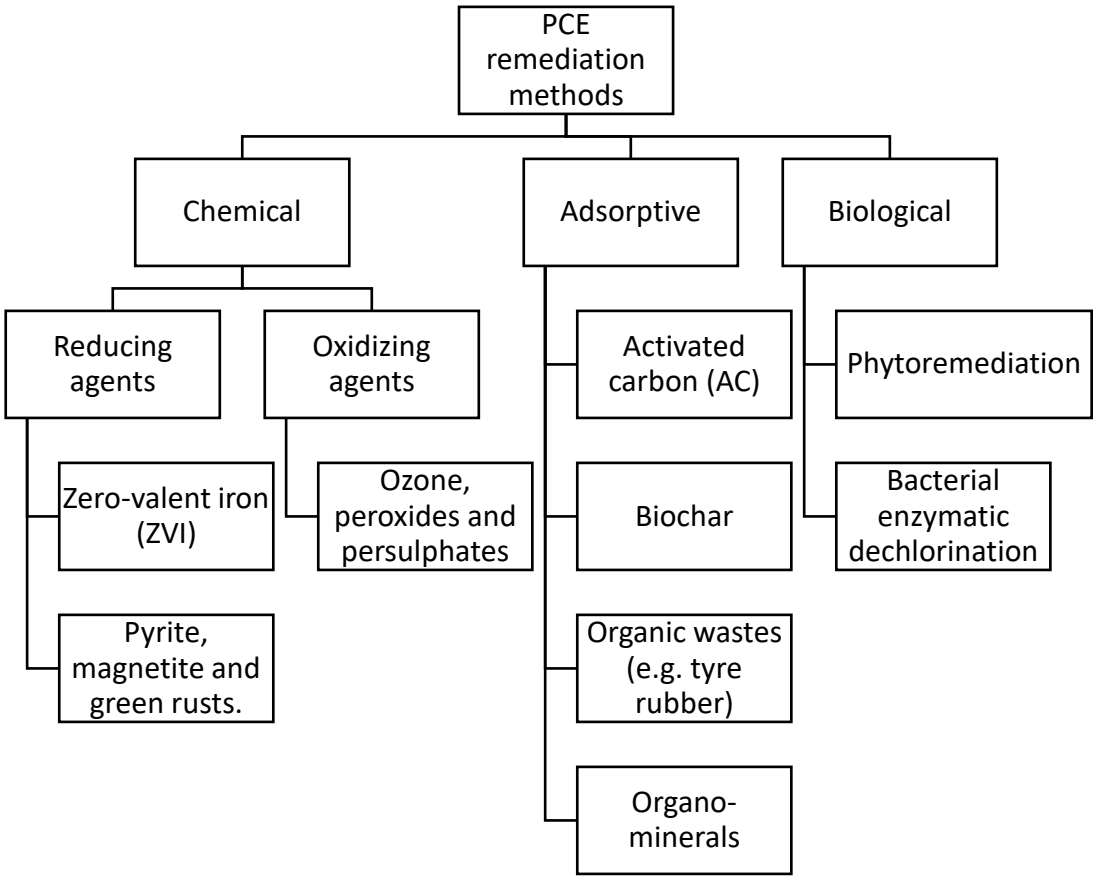

Figure 3. Classification of PCE remediation methods.

Zero-valent iron (ZVI) is considered as an efficient PCE remediation method through chemical reduction to ethene (Mueller et al., 2012). Other Fe-based chemical reduction methods involving reactive minerals such as pyrite, magnetite and green rusts have also been shown to contribute to PCE dechlorination (Liang et al., 2009). In situ chemical oxidation (ISCO) conforms an additional chemical decontamination process in which permanganates, ozone, peroxides, and persulphates are used for direct oxidation of CHCs or by the formation of free radicals (ÖVA, 2012).

Among adsorptive materials, activated carbon (AC) is, since the last three decades, a well-stablished and the most used material to remove CHCs from groundwater (Samuel & Osman, 1987). Activated carbon is a carbonaceous sorbent treated with oxygen to increase surface area and microporosity. However, biochar has gained more attention for contaminant sorption in the last years because it has a similar C structure to activated carbon with high surface area (Beesley et al., 2011). Moreover, it is a more cost-effective and climate-neutral material as it derives from biomass and less energy is required on its production (Thompson et al., 2016). Other sorbents used for PCE comprise organic wastes such as tyre rubber (Obiri-Nyarko et al., 2014) and minerals with organic anions substitutions such as organo-hydrotalcites (Alonso-de-Linaje et al., 2019).

Lastly, biological methods for CHCs remediation include phytoremediation and bacterial enzymatic dechlorination. Some plants and algae present specific dehalogenases for oxidative degradation of trichloroethene and they can also enhance bacterial degradation by root interactions with microorganisms in the rhizosphere. Phytoremediation can however lead to uncomplete degradation and accumulation of the contaminant in plant tissue (Perelo, 2010). Bacterial remediation methods consist in the use of certain aerobic or anaerobic bacteria which, by enzymatic dechlorination, cleavage the carbon-chlorine bond. Aerobic bacteria can use trichloroethene, dichloroethane and vinylchloride as electron or carbon sources and replace the chlorine atom with an hydroxyl group derived from water in the hydrolytic or derived from O_2 in the oxygenolytic dechlorination (Arora & Bae, 2014; Kastner, 1991). In the other hand, anaerobic bacteria can use the chlorinated ethenes as electron acceptors in a process called reductive dechlorination or dehalorespiration. In this anaerobic biodegradation process, chlorine atoms from PCE are replaced with H_2 forming less chlorinated but still toxic intermediate metabolites namely trichloroethene (TCE), cis/trans-dichloroethene (cDCE, tDCE) and vinylchloride (VC) until the harmless ethene (Löffler et al., 2013) (Figure 4). The potential for aerobic dechlorination increases with decreasing chlorination degree whereas anaerobic degradation of chlorinated ethenes with higher chlorine number tends to increase with lower reduction potential (Imfeld, 2008).

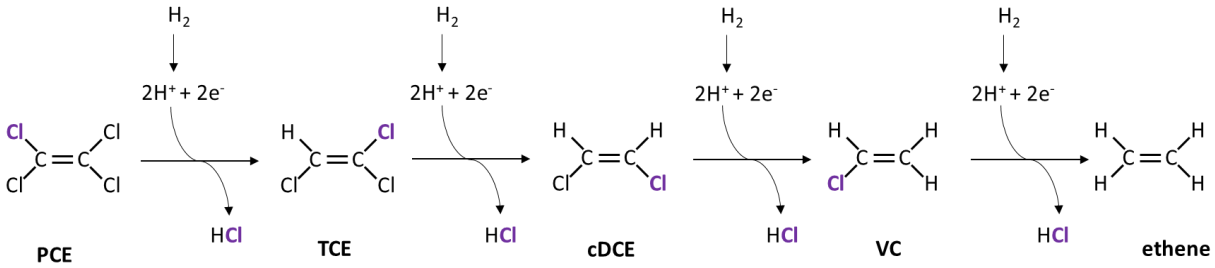

Figure 4. Reductive dechlorination of PCE.

1.3 Bacterial reductive dechlorination of PCE.

The potential of certain microorganism to perform reductive dechlorination provides a major advantage of biological remediation methods compared to chemical and adsorptive remediation techniques.

Different genera of bacteria are known to use chlorinated ethenes as terminal electron acceptors such as *Geobacter*, *Desulfitobacterium*, *Desulfuromonas*, *Sulfurospirillum*, *Dehalobacter*, *Dehalogenimonas* and *Dehalococcoides.* They can be further classified as non-obligate orhano-halide respiring bacteria (OHRB) in the case of the phyla Firmicutes (e.g., *Desulfitobacterium*) and Proteobacteria (e.g., *Desulfuromonas* and *Geobacter*), or as obligate OHRB in the case of the phyla *Chloroflexi* to which *Dehalogenimonas* and *Dehalococcoides* belong. Non-obligate OHRB have versatile metabolisms and relatively large genomes whereas obligate OHRB have a very restricted metabolism and smaller genomes (Maphosa et al., 2012).

Many microorganisms from these OHRB genera have been isolated and are known to use PCE, TCE and cDCE as terminal electron acceptors, however only the obligate organohalide-respirers Dehalococcoides spp. and Dehalogenimonas spp. have been shown as capable of degrading VC to ethene (Maymó-Gatell et al., 1997; Leitner et al., 2017; Yang et al., 2017).

The electron transport chain of OHRB presents dehydrogenases responsible of deriving electrons from electron donors, and a key enzyme called reductive dehalogenase (RDase) that catalyze the halogen removal from the chlorinated compounds. Obligate OHRB possess a higher number of RDase homologous genes compared to non-obligate and, interestingly, a specific vinylchloride RDase has been purified from Dehalococcoides sp. whose genes can be used as biomarkers in bioremediation (Maphosa et al., 2012).

Apart from DNA, RNA and protein analysis, the detection of dehalogenating bacteria can also be carried out by analysis of phospholipid fatty acids (PLFA) which can be used as signature biomarkers. Fatty acids present different chain structures which are typical of distinct microbial groups and can be classified into ester-linked and ether-linked fatty acids. Phospholipid ether-linked fatty acids (PLEL) are widespread in the cell membranes of Archaea (e.g., Methanogens) whereas phospholipids ether linked (PLFA) are prevalent in Gram-positive (e.g. Firmicutes), Gram-negative (e.g. Chloroflexi, Proteobacteria) and ubiquitous bacteria (Frostegård et al., 1991). Furthermore, they are a reliable biomarker for viable microbial communities because they are rapidly degraded after cell death (Lanekoff & Karlsson, 2010). PLFAs are a useful tool to study microbial diversity and biomass and community structure at contaminated sites or in commercial microbial consortia.

During reductive dechlorination, the replacement of the chlorine atoms occurs with the addition of a proton and two electrons which can come directly from H_2 or from organic sources. For example, the fermentation of pyruvate, ethanol, lactate, propionate, and butyrate directly provide H_2 and can promote dechlorination (Wang et al., 2018). These organic substrates can be fermented by other microorganisms in the contaminated site or in the microbial consortium used in the bioremediation process and become available to the OHRB. In such situations the organohalide-respirers might have competition with other microorganisms for the H_2 (Fennell et al., 1997) (Figure 5).

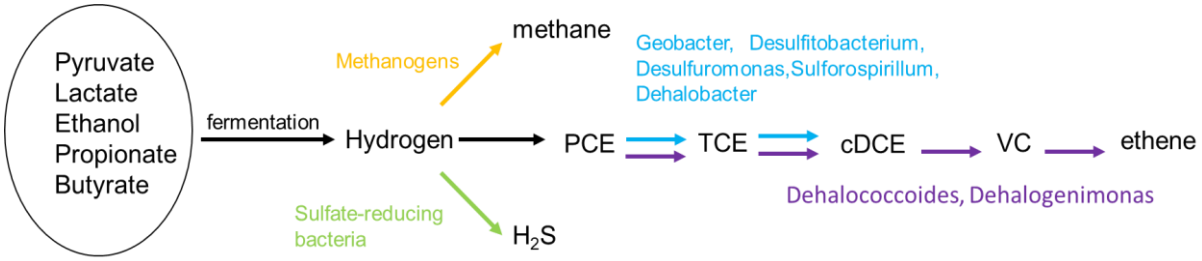

Figure 5. Simplification of organic substrate utilization by OHRB and possible competitor. (Adapted from Waller,2010).

1.4 Compound specific isotope fractionation, microcosms experiments and CHARBAK project.

During reductive dichlorination an isotopic effect may be produced due to the presence of heavy and light isotopes in the chlorinated ethenes. As lighter isotopes are preferred in enzymatic reactions, the rate of biodegradation is reduced, and the heavier isotope accumulates in the residual molecule. This is known as compound specific isotope fractionation and can be used to evaluate, quantify, and qualify PCE degradation (Meckenstock et al., 2004).

Microcosms (MCs) experiments are an appropriate method to assess the reductive dechlorination performance of different microbial communities and enable to conduct compound specific isotope analysis (CSIA). By using MCs, a suitable habitat for microorganisms can be created. They allow to determine the formation of metabolites, their shifts in isotopic ratios and whether certain electron donors stimulate dichlorination. CHARBAK is a project developed by the Institute of Soil Research and the Institute of Soil Physics and Rural Water Management from the University of Natural Resources and Life Sciences of Vienna, with the partnership of the Austrian Institute of Technology. The project is concerned with the development of physical-biological filters for groundwater remediation. The goal is to create a cost-effective and climate neutral filter in which the biochar adsorbs the PCE and serves as habitat for the microbial biofilm. The lifespan of the filter is expected to be higher than conventional physical filters because the contaminant degrading microbial communities would break down the adsorbed PCE preventing filter clogging. For this purpose, biochar from different biomasses was produced, and characterized and its sorption capacity was tested. To combine biochar and microbial communities it is needed to select the appropriate consortia capable of efficiently dechlorinate PCE. This thesis is embedded in this section and its focus was to monitor and interpret PCE degradation under controlled laboratory conditions in MCs experiments with the help of CSIA (Figure 6).

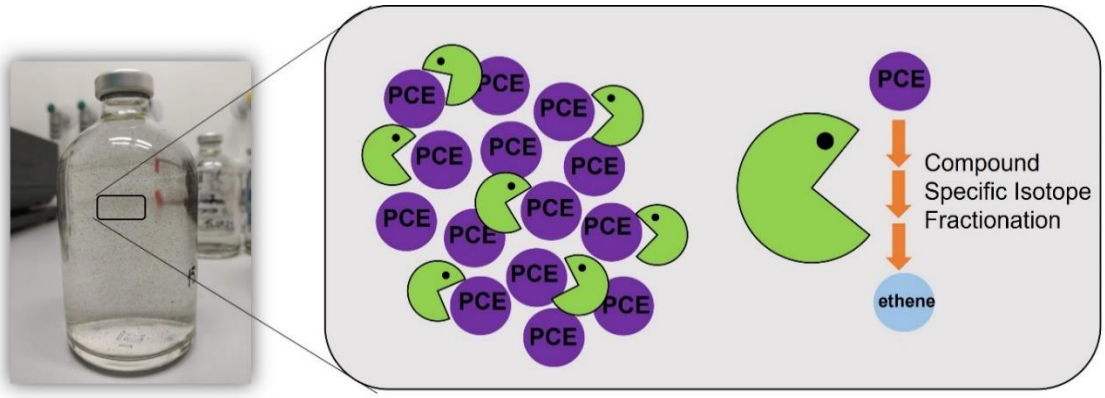

Figure 6. Eschmatic overview of the dehalorespiration studied in MCs experiments.

1.5 Aim and hypothesis

A deep comprehension of the reductive dichlorination is essential to apply organohalide respiring bacteria in remediation of PCE. In addition, the identification of suitable cultures able to completely degrade PCE to ethene for their use in the physico-chemical filter is needed. The main goal of this thesis was to understand and investigate the degradation performance of two different commercially available bacteria consortia containing organohalide-respirers (KB1® and Bioclear earth b.v.) and to elucidate which one was more suitable for its subsequent use in combination with biochar in the filter. The research included:

i) Incubation of bacteria in MCs experiments and compound specific isotopic analysis to assess reductive dechlorination and the formation of metabolites.

ii) Then, a closer examination of the microorganisms in the communities was undertaken to gain a more complete picture of the community abundance and diversity and to relate it with their dichlorination performance. For this, a phospholipid fatty acids (PLFA) extraction was

carried out which provided information about biomass content and community structure in the different MCs.

Hypothesis 1:

The degradation performance of PCE is dependent on the type of bacteria consortia and the source of electrons used in the incubation media.

Hypothesis 2:

The presence of certain species of bacteria such as Dehalococcoides sp. strongly influence the complete transformation of PCE to ethene.

2. Material and Methods

2.1 Bacterial consortia.

Two different commercially available anaerobic mixed microbial communities were used: KB-1® and Bioclear earth b.v.. KB-1® is a consortium derived from naturally occurring bacteria in soil and groundwater, produced by SiREM laboratory, Canada, and formed in 98% by the genera *Dehalococcoides*, *Geobacter* and *Methanomethylovorans* (SiREM, 2013). Bioclear earth b.v. is a consortium containing dechlorinating bacteria, produced by the company with the same name (Bioclear earth b.v., Netherlands), and from which a detailed microbial characterization is not available in literature. Both consortia were stored at 4 °C until their use and they were always handled in a glovebox flushed with Ar (purity of 5.0, Messer Austria GmbH) with an O_2 concentration below 80 ppm.

2.2 Preparation of anaerobic medium for OHRB consortia.

The medium used to incubate the bacterial consortia was prepared according to the principles of the Hungate technique (Hungate, 1969; Miller & Wolin, 1974). For 1 L medium the following amounts were added in a 1L volumetric flask: 10 mL of salt stock solution (NaCl, 100 g; MgCl2 x 6H2O, 50 g; KH2PO4, 20 g; NH4Cl, 30 g; KCl, 30 g; CaCl2 x 2H2O, 1.5 g), 1 mL of trace metal solution (HCl (25% solution, w/w), 10 mL; FeCl2 x 4H2O, 1.5 g; CoCl2 x 6H2O, 0.19 g; MnCl2 x 4H2O, 0.1 g; ZnCl2, 70 mg; H3BO3, 6 mg; Na2MoO4 x 2H2O, 36 mg; NiCl2 x 6H2O, 24 mg; CuCl2 x 2H2O, 2 mg), resazurin (0.9 µM) as a redox indicator, sodium acetate (6mM) and/or lactate (4mM) as electron donors (Fennell et al., 1997). Subsequently, MilliQ water was added to a final volume of 1L. The medium was then transferred to a 1L Shott® flask and flushed with N2 gas with a bubble diffuser while stirring during 10 min to exchange the dissolved O2. Finally, it was autoclaved and stored in the dark at 4 °C until use.

2.3 Microcosm (MC) experiments.

Two MC experiments were conducted to test the degradation capacity of KB-1® and Bioclear earth b.v..Seven replicates per culture were prepared in an anoxic glove tent (SylaTech GmbH) flushed with Ar 5.0 (Messer Austria GmbH).

The previously prepared anaerobic medium was amended with Na2S x 9H20 (3mM) to achieve an oxidation-reduction potential lower than -120mV (referred to standard hydrogen electrode Eh) suitable for bacterial dehalogenation (Löffler, 2013); 1mL HCl (17% v/v) to adjust the pH value to 7.0; and cyanocobalamin (36 nM) to optimize the growth of *Dehalococcoides* (He et

al., 2007). After the medium turned from pink to colourless showed by the Resazurin, indicating suitable reducing conditions, it was transferred to 120 mL glass. Then, 10mL bacteria inoculum was added into the respective microcosms. The microcosms bottles were weighed before and after the addition of the media and bacteria to control the amount of media added.

The MCs bottles were sealed with PTFE/butyl rubber septa and aluminium crimp caps to ensure an airtight seal. After a first measurement to confirm no detectable chlorinated ethene contamination, PCE (162 µM) in pure phase was added to each microcosm. They were then incubated on a shaker (70 ppm) in the dark at room temperature (22 °C) and liquid samples of 10 µL volume were taken regularly with a gas-tight syringe (SGE, Trajan Scientific Australia Pty Ltd) to measure concentration and isotope ratio of all detectable chlorinated ethenes (Figure 7). Details on the dates of PCE additions and mL of media per microcosm can be found in the Supplement Material (SM) (Table SM2,Table SM3,Table SM4).

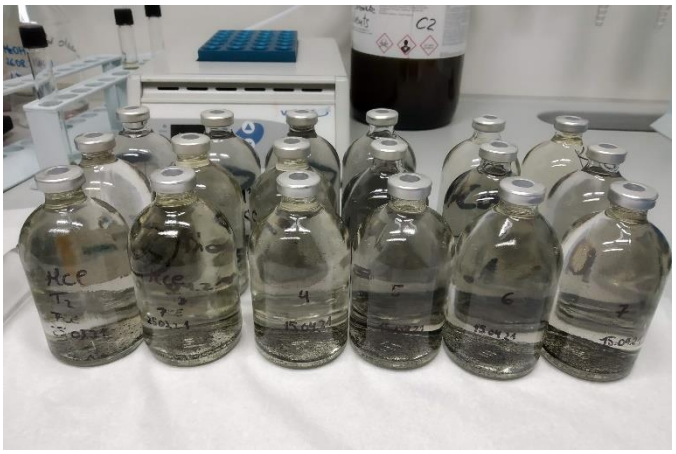

Figure 7. Glass bottles (120 mL) used for the microcosm experiment with PTFE/butyl rubber septa and crimp caps to ensure anaerobic conditions.

2.4 Compound specific isotope analysis.

Carbon stable isotope ratios and concentration of the CEs were determined using purge and trap autosampling-Gas Chromatography (GC)-Mass Spectrometry (MS)-Isotopic Ratio Mass Spectrometry (IRMS). Carbon isotope ratios ($\delta^{13}C$) were expressed in per mill (‰) and reported in δ-notation according to Eq.1:

$$\delta^{13}\mathrm{C} = \left(\frac{R_{sample}}{R_{standard}} - 1\right) \times 1000 \qquad (1)$$

where R is the heavy-to-light isotope ratio of the element ($R = {}^{13}C/{}^{12}C$). By convention, samples are measured relative to the international standard Vienna Peedee Belemnite Standard (VPDB standard). The 10 µL fluid samples from the MCs were transferred to 20mL vials containing 10 mL of Milli-Q water. In the purge and trap auto sampler (VSP4000, IMT GmbH, Vohenstrauss, Germany), the fluid samples were purged for 20 min at 40 °C with He (purity of 4.0, Linde Tulln, Austria) at a pressure of 1200 hPa. The purged CEs were carried with the He stream to a

Cryocooled Tenax trap cooled to -90 °C with liquid nitrogen (LN_2) (Messer Austria, Gumpoldskirchen, Austria) where they were frozen and adsorbed. Thereupon, trapped samples were desorbed at 200 °C and transferred to the GC column (GS-Gas-Pro column, 30 m, 0.32 mm ID, Agilent, Vienna, Austria). The initial temperature program was 35 °C and it was held for 3 min, then ramped to 140 °C at 40 °C/min and finally to 240 °C at 20 °C/min and held for 2 min. Analytes were separated due to their different boiling points and their interaction with the GC-column. The gas flow at the GC-column was split. One part (1/10) was analyzed by mass spectrometry (ISQ, ThermoFisher, Vienna, Austria) and the second part (9/10) was converted to CO2 in the combustion furnace (GC-Isolink,ThermoFisher, Vienna, Austria) at 1000 °C which was analyzed by isotope ratio mass spectrometry (IRMS) (Delta V, ThermoFisher, Vienna, Austria) to measure the intensities of the mass-to-charge (m/z) ratios of the masses 44, 45 and 46 (Leitner et al., 2017; Leitner et al., 2018).

In order to quantify the CEs concentrations and for the calibration of δ values, a calibration standard containing PCE (21.8 mg/L), TCE (25 mg/L), cDCE (16.2 mg/L), tDCE (15 mg/L) and VC (8.2 mg/L), and a calibration standard containing ethene (10 mg/L), were used.

2.5 Calculation of degradation rates and carbon isotope enrichment factor.

The rate of degradation of chlorinated ethenes strongly depends on the concentration of the substrate. First-order kinetics are adequate for describing reductive dichlorination (Schaerlaekens et al., 1999). The degradation rate constant (*k*) of PCE was first calculated according to the following equation:

$$k\ in\ day^{-1} = \ln\frac{\left(\frac{C_t}{C_0}\right)}{t} \tag{2}$$

where C_t and C_0 is the concentration of PCE at time t_0 and time t_t. Then, *k* was calculated by a linear regression with individual intercepts of C_t and t. Each data point used in the linear regression hade to meet the following requirements according to Leitner et al., 2017: (a) C_0 represented the most recent maximum concentration of PCE and (b) C_t had to show a shift in concentration of at least − 0.5 mg/L to C_0.

The carbon isotope enrichment factor (ε) was calculated using the Rayleigh equation:

$$\varepsilon\ in\ ‰ = \frac{\ln\frac{R_t}{R_0}}{\ln\frac{C_t}{C_0}} \times 1000 \tag{3}$$

Where R is the heavy-to-light isotope ratio of the element ($R = {}^{13}C/{}^{12}C$) at a given time (R_t) and at time zero (R_0) with the corresponding concentrations in the liquid phase (C_t, C_0) (Rayleigh, 2009). For the linear regression, apart from the requirements mentioned for the concentration in the calculation of the degradation rate, δ values needed to meet the same conditions: (a) R_0

represented the δ value corresponding to the most recent maximum concentration of PCE and (b) R_t had to show a shift in δ of at least − 0.5‰ to R_0).

2.6 Calculation of quantification limit for IRMS

Purge and trap (P&T) is one of the injection methods with the higher sensitivity for CSIA with GC-IRMS which allows low limits of quantification (in the order of low µg/L) (Smallwood et al., 2001). Knowing the limit of quantification is important to ensure the reliability of the GC-IRMS analysis. The limit of quantification for the different analytes at IRMS was calculated doing a calibration with five different concentrations (three replicates each) of the standards (2, 5, 10, 15 and 20 µL) according to Jochmann et al., 2006. The mean $\delta^{13}C$ value of the three highest concentrations levels was determined and a ±0.5‰ interval was set around it. This procedure was repeated consecutively using always in addition the $\delta^{13}C$ value of the next lower concentration level. The limit of quantification is then defined as the last point which is inside the mentioned interval and/or has a standard deviation of the triplicates higher than ±0.5‰.

Minimum quantification limits for $\delta^{13}C$ expressed as µmol injected in the GC were 0.044 µmol for PCE, 0.050 µmol for TCE, 0.032 µmol for cDCE, 0.164 µmol for VC and 0.050 µmol for ethene.

The limit of quantification for the CEs concentrations was calculated by a linear regression with the mean concentration of µmol injected in GC. First, the three highest concentrations were used for the regression and then the next lower concentration level was consecutively added to it. The limit of quantification for the CEs was defined as the last point added in which the linear regression had an $R^2 \geq 0.95$. Minimum quantification limits for the CEs concentrations were the same as for $\delta^{13}C$ except for VC which was 0.016 µmol and for ethene which was 0.020 µmol.

2.7 PLFA analysis.

A PLFA analysis was performed to describe the size and structure of the microbial community based on the phospholipids present in the cell membranes. The samples from the MCs containing Bioclear and KB1 consortia were transferred to 500 mL Schott bottles, frozen and then freeze-dried. Only glassware which was previously rinsed with chloroform and Pasteur pipets were used during the procedure and pipets were always rinsed with the organic solvents before use. PLFAs were extracted according to Watzinger & Hood-Nowotny, 2019 with a slight difference in the Bligh and Dyer extraction step.

Firstly, in the extraction step, 8 mL of a 1:2:0.8 ratio (v/v/v) one phase solution of chloroform, methanol containing 19:0 PLFA first standard (15 nmol) and citrate buffer were directly added to the Schott bottles containing the freeze-dried sample. The pH was measured to ensure that the citrate buffer was able to set a value of 4.0 and the bottles were stored overnight in the dark. Then, the Schott bottles were rinsed with 4 mL of Bligh and Dyer solution and the whole volume was transferred to centrifuge tubes. From here, every step was done following the protocol of Watzinger & Hood-Nowotny, 2019. Secondly, PLFAs were separated with methanol using solid phase extraction (SPE) silica columns (500 mg, ISOLUTE, Biotage) and the 13:0 FAME second standard (36nmol) diluted in methanol-toluene was added. Finally, PLFAs were hydrolyzed and converted to fatty acid methyl esters (FAME) using KOH methanol under a stream of N2 at 40 °C. The dried residues were dissolved in 100 uL of isooctane and 2.5 uL were measured by GC-C-IRMS (gas chromatography – combustion – isotopic ratio mass spectrometry). The sample was injected via a programmable temperature vaporizer (PTV) with

the TriPlus RSH autosampler to the GC (Trace 1310) which was connected to the IRMS (Delta V Plus) via a combustion furnace (Isolink). The temperature programme was conducted at an initial temperature of 70 °C which was held for 2 min, ramped to 160 °C at 15 °C/min, then ramped to 240 °C at 2.5 °C/min and finally ramped to 280 °C at 15 °C/min and hold for 3 min. FAMEs were identified by chromatographic retention times and comparison with the internal standards 13:0 and 19:0 and a bacterial FAME mix. Some photos from the procedure are shown in Figure 8.

The nomenclature used to call the fatty acids was as follows: the total number of carbons, followed by the number of double bonds with the position of the double bond closest to the methyl end (w) of the molecule. Suffixes “c” and “t” indicate the configuration cis and trans of the double bonds and the prefixes “a” and “i” refer to iso and anteiso in the branched fatty acids. Finally, methyl groups are indicated by “Me” preceded by a number indicating the position of the group and “cy” refers to cyclopropane fatty acids.

The total microbial PLFAs concentration from Bioclear and from KB1 were compared by using a non-parametric Welch t-test because the variances were unequal. The PLFA abundances were then converted to relative data to have the PLFA fingerprint. This data was subjected to Principal Component Analysis to study the differences in the microbial community composition between the two consortia. All statistical analyses were carried out using the software R version 4.0.4.

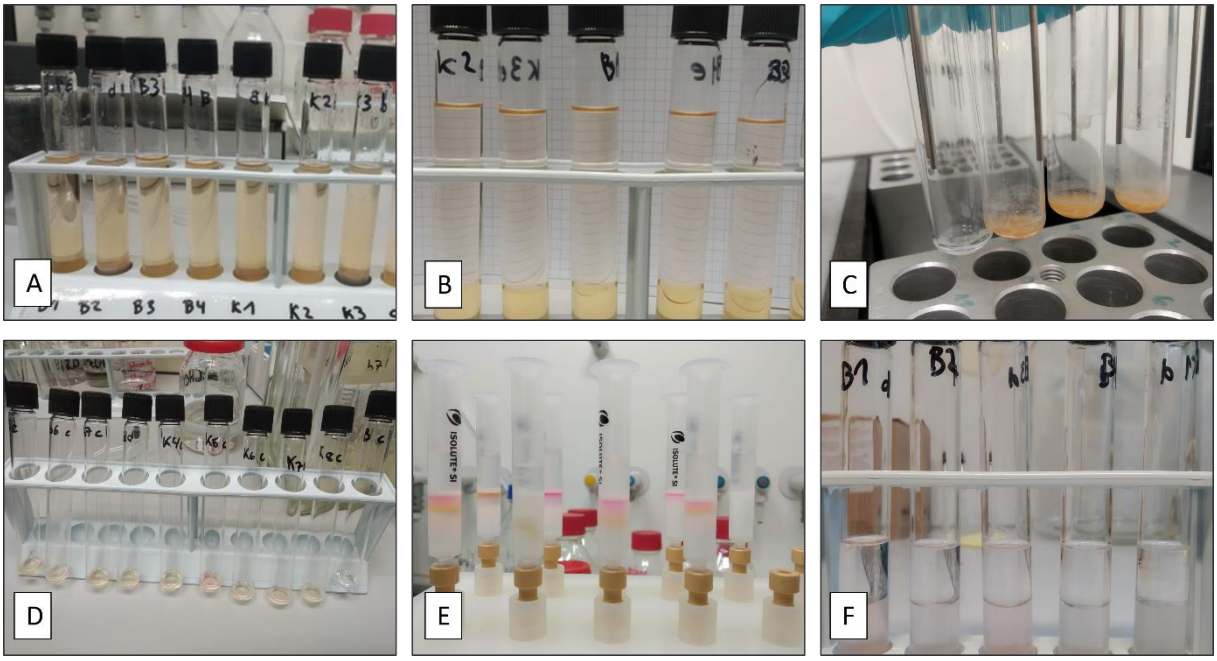

Figure 8. PLFA extraction procedure. A) Blight and Dyer extractant transferred from the Schott bottles to the centrifuge tubes. B) The two phases after chloroform and water addition can be observed (upper part water and chloroform, lower part chloroform with the total lipids extracted from the sample). C) Sample with chloroform dried at 40°C in the heating block concentrator with a flow of N_2 gas. D) Samples after dried were resuspended in 0.5 mL of chloroform for the lipid separation step. E) Fractionation of lipid extract in SPE silica columns. Neutral lipids are extracted with chloroform, glycolipids with acetone and phospholipids with methanol. F) Partition of aqueous and organic phases after methylation of phospholipids (upper phase hexane with the FAMEs).

3. Results and discussion

3.1 Bioclear consortia

***Reductive dechlorination of PCE and formation of metabolites**.*

The degradation behaviour and the δ ^{13}C values of the 7 MCs containing Bioclear consortia are shown in Figure 9 and Figure 10. Continuation of Figure 9.Figure 10 (exact values per MC can be found in Table SM2). The media in all microcosms was amended with acetate as electron donor. Results for single MCs can be interpreted in two sections divided by a line in the graphs which represents the moment when the incubation media was exchanged. Data for MCs 2 and 3 is shown from day 20 due to inconsistent results of the standards and the impossibility of calculating concentrations at the beginning of the experiment.

Before the media exchange, two additions of PCE (162.8 µmol/L each) were done at days 17 and 27 in MCs 2 and 3. In the rest of the bottles the additions were done three days before day zero (first sampling time) and at day 7. These PCE additions were done to evaluate the recovery of the contaminant and they explain the steady increase on cDCE formation. In MCs 4 to 8 the measured cDCE (317.6 to 328.4 8 µmol/L) before media exchange corresponds indeed to the amount of PCE added. In MCs 2 and 3 the measured cDCE reaches almost 500 µmol/L due to another PCE amendment done at the beginning of the experiment (data not shown).

All the PCE was transformed to cDCE with no further dichlorination demonstrating uncomplete degradation in the Bioclear consortia. Partial dechlorination and accumulation of large amounts of cDCE is consistent with findings from other investigations. Summer et al., 2020, reported accumulation of cDCE in Bioclear and only further degradation to ethene when combined with micro zero-valent iron (mZVI). They also reported the presence of Dehalococcoides spp in the culture. OHRB need H^2 as electron donor which is produced by anaerobic corrosion of ZVI stimulating complete bacterial dichlorination. Furthermore, PCE dichlorination is thermodynamically and kinetically more favourable than less chlorinated ethenes leading to cDCE accumulation specially when persistent PCE additions are done (Adamson et al., 2004). In addition to cDCE, small portions of TCE were formed. This could be explained by a cometabolic dichlorination process in which the PCE is transformed by organisms in the culture serving neither as electron nor carbon source such as by acetogenic and methanogenic bacteria (Middeldorp et al., 1999).

Due to the accumulation of cDCE the media was exchanged and a new PCE amendment was done at day 50 in MCs 2 and 3 and at day 30 in the rest of the bottles. Dechlorination after media exchange show the same pattern as already described except in microcosms 2, 3 and 4 in which almost no formation of metabolites could be detected. In these microcosms, cDCE levels before media exchange reached 474 µmol/L. It has been reported that at high concentrations, organohalide substrates itself can be toxic or inhibitory to dehalorespiring bacteria (Adrian & Löffler, 2016). Duhamel et al., 2002 demonstrated reduced activity of OHRB cultures at cDCE concentrations of 1000 µmol/L. Although concentrations observed in this study where lower, microcosms with the highest cDCE levels were the ones showing no degradation after the media was exchanged. The toxicity of high cDCE concentrations could have led to the decease of the bacteria.

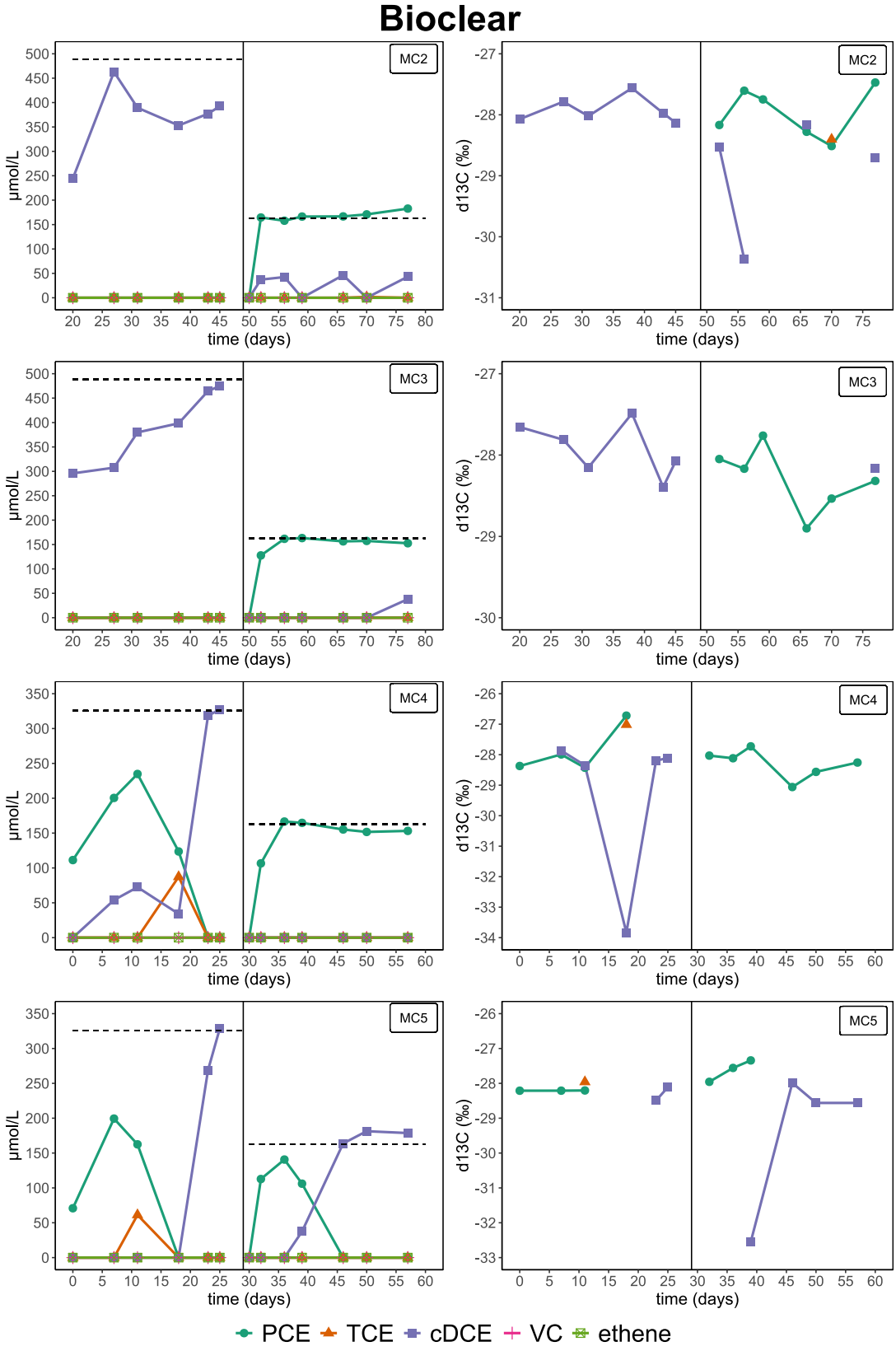

Figure 9 Temporal changes in concentration (µmol/L) (left column) and in δ ^{13}C (‰) (right column) of PCE, TCE, cDCE, VC and ethene in microcosms with Bioclear. The microcosms IDs are given in the upper right part of each graph. Vertical lines indicate the moment in which the incubation media was exchanged. Horizontal lines indicate the concentration value of the srthinitially added PCE. Time 20 (MC 2 and 3) and time 0 (rest of MCs) represent the first measurement after PCE addition.

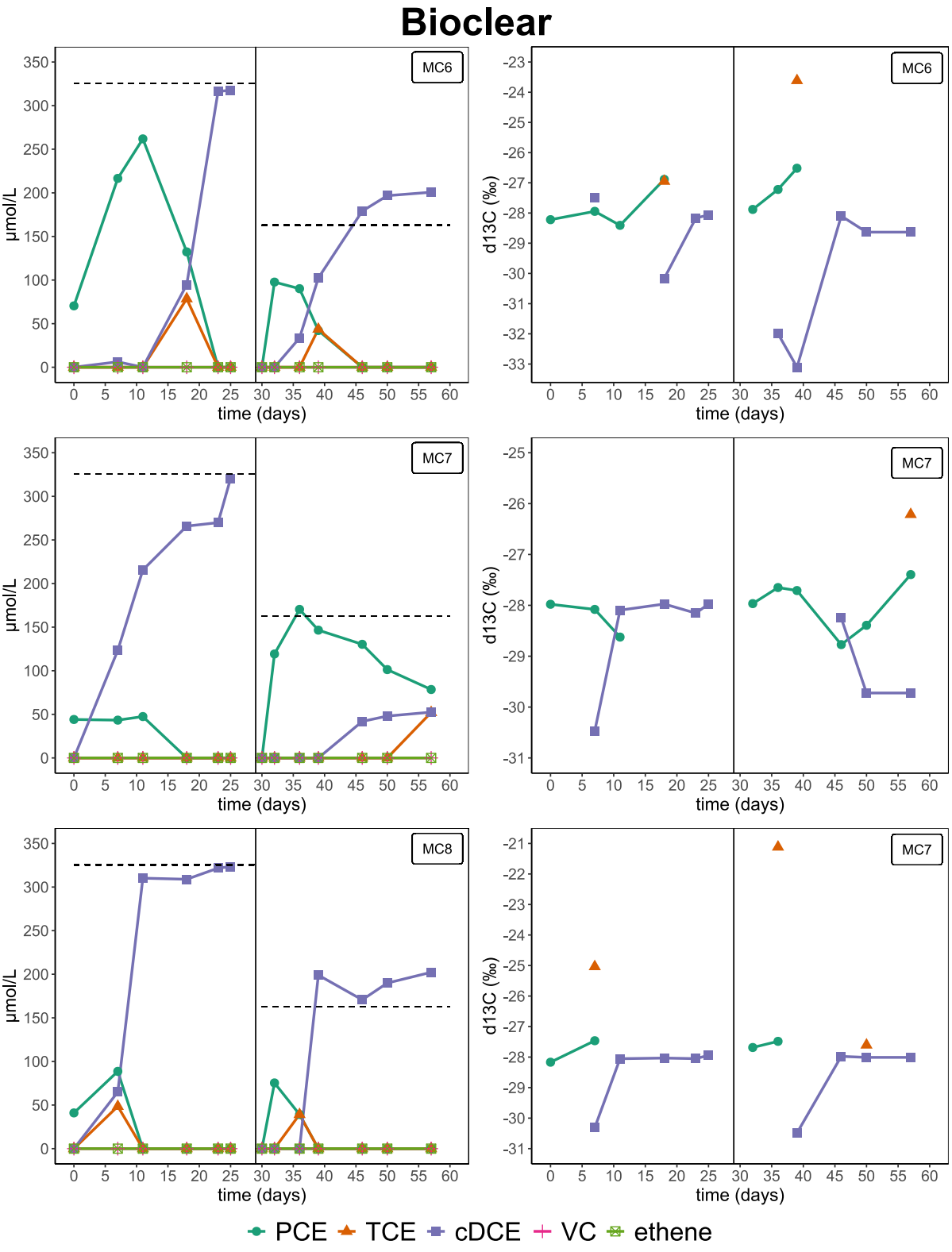

Figure 10. Continuation of Figure 9.

In the microcosms showing degradation after media exchange, the complete conversion of PCE to cDCE occurred in 10-15 days (MC5, MC6 and MC8). Due to the fast degradation, the fraction of remaining PCE was below the limit of quantification (0.05 µmol injected in GC) within consecutive samplings. Hence, degradation rates according to the requirements stablished in the section 2.9 ("Calculation of quantification limit for IRMS") could not be calculated.

However, the consortia in MC7 exhibited a slower degradation being possible to still detect PCE 29 days after the addition (the experiment finished without observing complete transformation to cDCE in this bottle). The number of data points allowed to calculate a degradation rate constant of -0.036 day^{-1} (R^2=0.99, n=5). Despite of being the slowest of the microcosms showing formation of DCE after media exchange, its degradation rate is higher than the reported in aquifer samples (-0.002-0.007 day^{-1}) (Schaerlaekens et al.,1999)

Isotopic effects and enrichment during reductive dichlorination

As indicated in the previous section, the high activity of PCE dechlorination did not allow to calculate degradation rates in MCs 5,6 and 8 meaning no isotopic enrichment value could be assigned either. δ values for PCE showed no trend among microcosms with a maximum shift of 1.4‰ (from -27.9 to -26.5 ‰) (Figure 9 and Figure 10). Different studies reported a minimal carbon isotope fractionation of PCE in comparison to other chlorinated ethenes (Cichocka et al., 2007; Leitner et al., 2017). The lower PCE dichlorination activity in MC 7, however, allowed to calculate an enrichment value of -3.1 ‰ (R^2=0.98, n=3). In contrast to PCE carbon isotope fractionation, cDCE δ values showed a maximum negative value of -33.1‰ getting rapidly enriched again towards the initial δ values of PCE.

The isotope fractionation of PCE occurring during the cleavage of chemical bonds might be affected by the rate limitation of reaction steps preceding the cleavage (Northrop, 2003). For example, the cell membrane resistance to the PCE can influence its uptake and affect the reaction step before the discrimination reducing the isotope fractionation (Imfeld, 2008). At the same time, fractionation is a degradation bottleneck, meaning that if lower PCE fractionation is observed, there is no rate-limitation for degradation. This could be an explanation for the fast dechlorination in MC 5,6 and 8 and the impossibility to calculate an enrichment factor.

3.2 KB1 consortia

Reductive dechlorination of PCE and formation of metabolites.

The degradation behaviour and the δ ^{13}C values of the 7 MCs containing KB1 consortia are shown in Figure 11 and Figure 12 (exact values per MC can be found in Table SM3). The media in all microcosms was amended with acetate as electron donor. PCE (162.8 µmol/L) was added two days before day zero (first sampling time). The formation of metabolites was very similar to that in Bioclear consortia but occurred faster. TCE was produced in small portions and PCE was completely transformed to cDCE in 7 days. No further formation of metabolites after cDCE could be seen leading to its accumulation within consecutive sampling times. However, KB1 contains Dehalococcoides spp. and has been reported to fully dechlorinate PCE to ethene (Major et al., 2002(Major et al., 2002); Duhamel et al., 2002; Dorothea et al., 2020). The reason of incomplete dechlorination in both KB1 and Bioclear cultures in this study has more to do with the lack of appropriate conditions in the incubation media than with the lack of appropriate organisms that completely degrade PCE to ethene as it will be discussed in sections 3.3 and 3.4

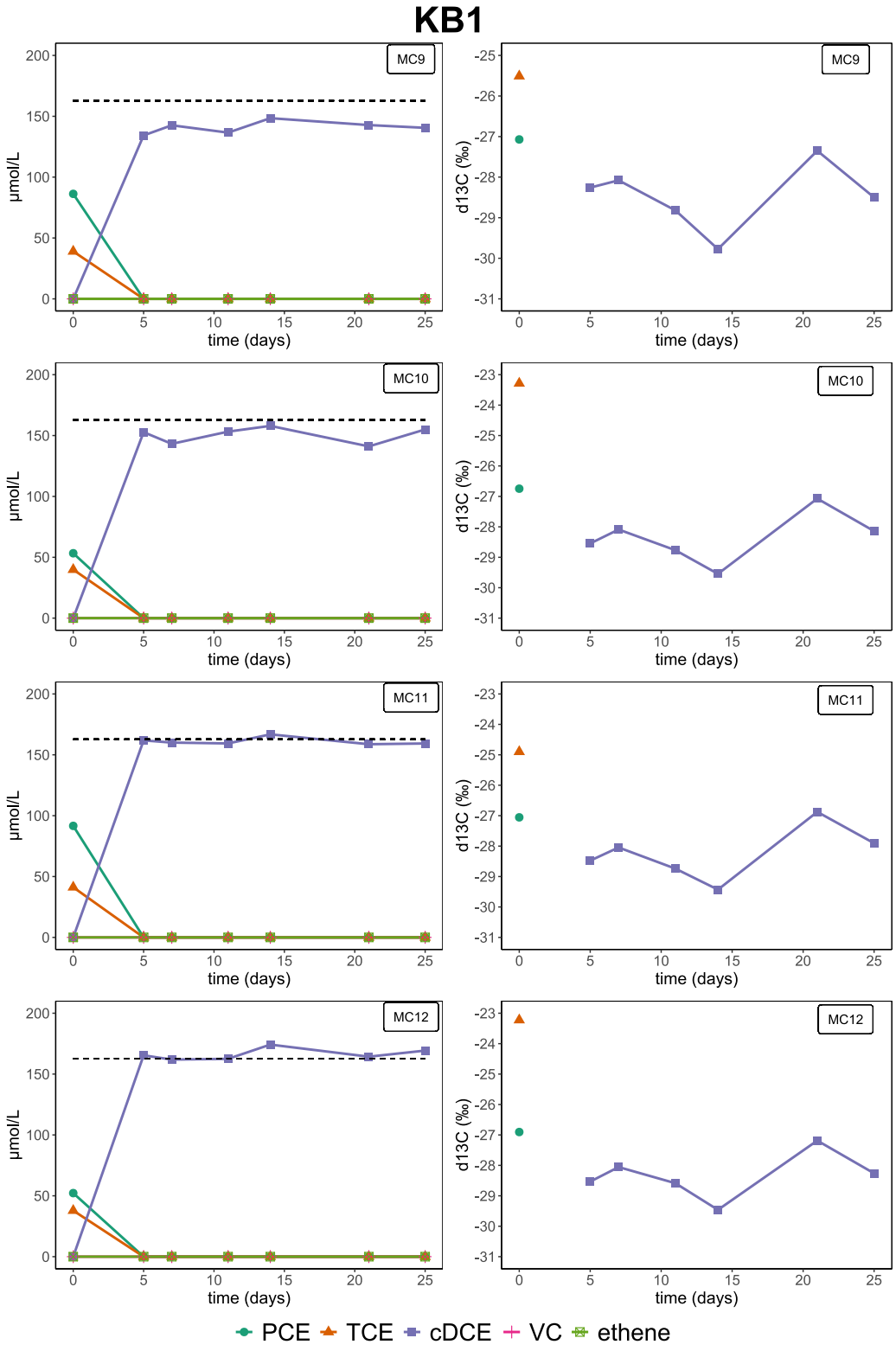

Figure 11. Temporal changes in concentration (µmol/L) (left column) and in δ ^{13}C (‰) (right column) of PCE, TCE, cDCE, VC and ethene in microcosms with KB1. The microcosms IDs are given in the upper right part of each graph. Horizontal dashed lines indicate the concentration value of the initially added PCE. Time 0 (rest of MCs) represent the first measurement after PCE addition.

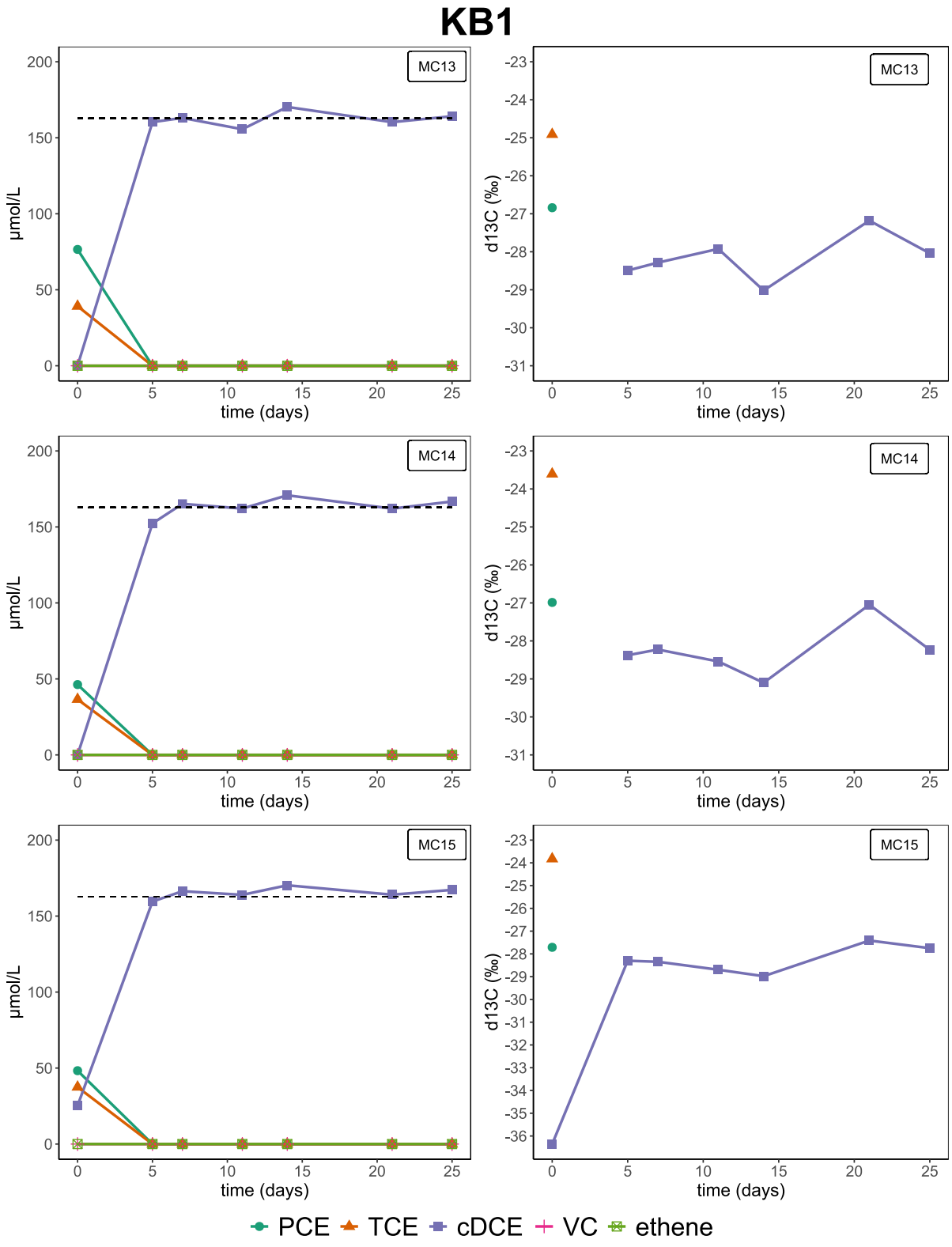

Figure 12. Continuation Figure 11.

In line with results for Bioclear, degradation rates could not be calculated due to the fast transformation of PCE to cDCE and absence of fraction levels over the quantification limit. It is possible that decreasing the sampling time interval to a daily basis may give enough data to study degradation rates.

Isotopic effects and enrichment during reductive dichlorination

δ values for PCE and TCE could only be detected in the first sampling time (Figure 11 and Figure 12). All MCs showed a value for PCE in the range of -27.7 to -26-74 ‰. cDCE becomes less and less enriched until it reaches its maximum concentration and then shows again an enrichment going up to PCE delta values. The less enriched cDCE is found in MC15 in which cDCE formation is detected when PCE dissolution to the liquid phase was still ongoing. For MC15 δ of DCE ranged from -36.4 to -27.4 ‰ and for the remaining microcosms, from -28.5 to -26.8 ‰.

As discussed in section 3.1, the rapid transformation of PCE and its small fractionation seems to indicate no rate-limiting step for degradation.

Finally, the replicates in both consortia, especially in KB1, showed high qualitative and quantitave reproducibility and mass balances were maintained throughout the experiment

3.3 Influence of electron donors on reductive dichlorination

H_2 is known to be the electron source for reductive dichlorination (Fennell and Gosset., 1997). Hence, it is very important to understand the fate and determine the substrates provided as electron donors to the incubation media. Different organic substrates such as lactate, pyruvate, formate, ethanol, glycerol, propionate and methanol have been described as a source of electrons when subjected to fermentation (Wang et al., 2008). Production of H_2 by fermentation using a combination of acetate and lactate has also been reported (Matsumoto & Nishimura, 2007). However, studies using KB1 announce methanol, ethanol, lactate, propionate and molasses but not acetate, as viable sources of H2 for complete dichlorination (Duhamel et al., 2002; Summel et al., 2020).

In order to reveal the influence of electron sources, lactate (4mM) was added to the KB1 culture. In Figure 13, the results for MC16 which was kept under measurement longer than its KB1 counterparts, are presented (exact values per MC can be found in Table SM4). Organisms in this microcosm showed transformation of PCE mainly to cDCE with fractions of VC from day 25. When lactate and an amendment of PCE were added at day 41 inside the glovebox, a huge increase in ethene formation was observed.

PCE was rapidly dissolved to the liquid phase and the almost complete conversion to ethene took place in 14 days. This indicated that lactate could serve as good substrate for sustaining complete dichlorination of PCE in KB1 culture.

In the case Bioclear, an addition of lactate was also done but no degradation could be observed due to errors in the used experimental conditions. Further investigation with Bioclear should be done to elucidate the extend of PCE dechlorination when adding lactate to the culture.

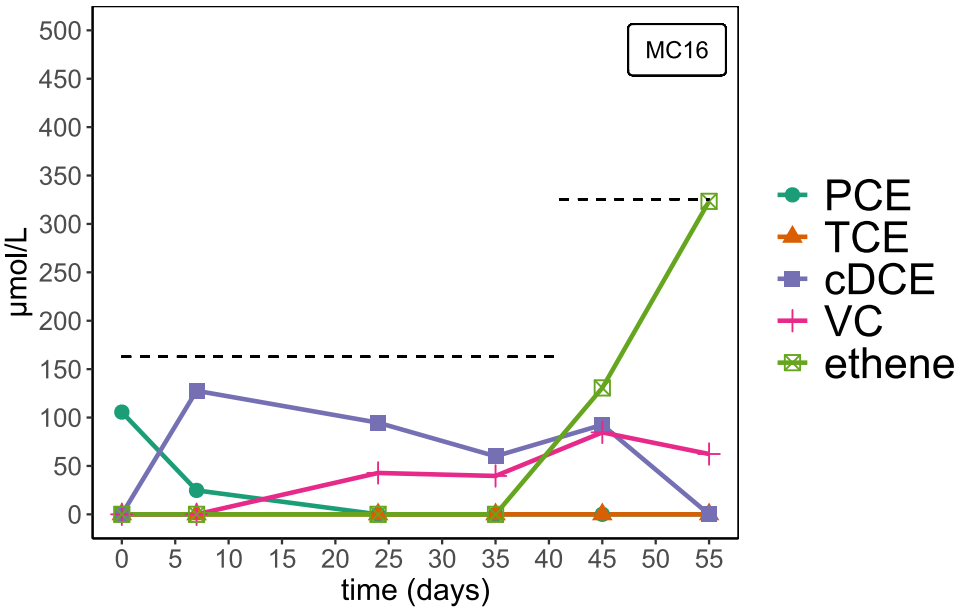

Figure 13. Temporal changes in concentration (µmol/L) of microcosm 16. Lactate addition to the media was done in day 41. Horizontal lines indicate the concentration value of the added PCE.

3.4 PLFA analysis

Total microbial biomass and community composition.

The PLFA extraction was done to discover the possible differences in community structure between Bioclear and KB1 and explain the degradation behaviours observed. The analysis of the samples in the GC-C-IRMS allowed to identify 28 PLFAs and the two cultures show a slightly different composition pattern (Figure 14). The fatty acids 16:0 (Bioclear: 69.2 nmol/l, KB1: 28.4 nmol/L),16:1w7c (Bioclear: 60.4 nmol/l, KB1: 25.1 nmol/L),16:1w6c (Bioclear: 16.8 nmol/l, KB1: 6.8 nmol/L),14:0 (Bioclear: 23.3 nmol/l, KB1: 27.9 nmol/L), and i15:0 (Bioclear: 28.1 nmol/l, KB1: 13.7 nmol/L), predominated in both cultures. The exact mean concentrations for each PLFA can be found in Table SM1. The Welch t-test revealed that the mean microbial PLFAs concentration was significantly higher in Bioclear (283.4± nmol/L) than in KB1 consortia (162.9 ± nmol/L) (p=0.014) (Figure 15).

It is known that the predominant organisms conforming the KB1 culture are Dehalococcoides, Geobacter and Methanomehylovorans (SiREM, 2013). Geobacter sp., Dehalobacter sp. and some sulphate reducers are able to degrade PCE to cDCE whereas Dehalogenimonas sp. and Dehalococcoides are reported to perform complete degradation of PCE to ethene (Maymó-Gatell et al., 1997). Geobacter species contain mainly the fatty acid 16:1w7c together with i15:0 and 16:0 (Lovley et al., 1993). These fatty acids where dominant in both cultures with a higher abundance in Bioclear indicating presence of bacteria carrying out incomplete PCE dechlorination. The fatty acids 10Me16:0, 16:0, 14:0 and 18:0 are reported to be dominant in pure cultures of Dehalococcoides sp. (Löffler 2013). These fatty acids were detected in both cultures with a higher abundance in KB1 demonstrating the presence of OHRB which perform complete dehalorespiration.

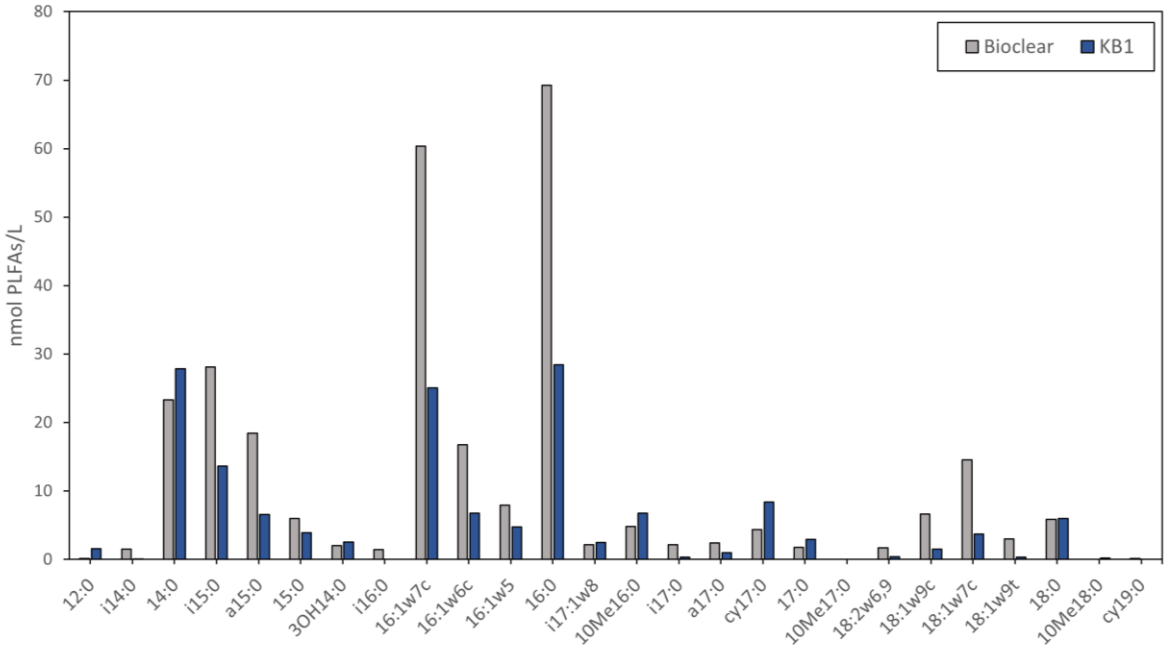

Figure 14. Fatty acids detected from PLFA extraction in Bioclear and KB1 microcosms. Values are the mean PLFA concentrations (nmol/L) of the cultures' replicates

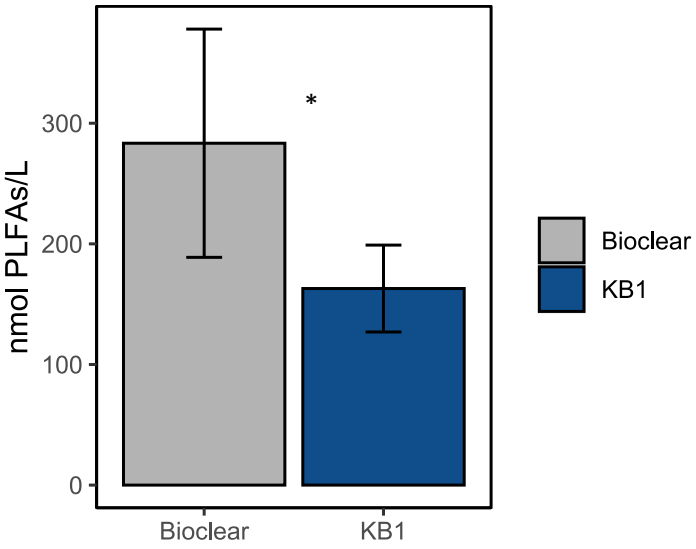

*Figure 15. Concentration of PLFAs (mean ± SD) in Bioclear and KB1. *: significant differeces (p<0.05).*

To gain a better understanding on the microbial community composition of both cultures, a PCA was performed (Figure 16). This enabled to identify the most important PLFAs in defining each consortium. Principal component 1 explained 47.5% of the total variance differentiating KB1 and Bioclear communities. In line with the results mentioned above, PLFAs belonging to Dehalococcoides sp. (10Me16:0,14:0, 18:0) defined KB1 culture. Fatty acids i15:0, a15:0, 16:0 and i17:0 are representative of sulphate reducers bacteria (Edlund et al., 1985), which do not perform complete dichlorination and defined Bioclear culture.

These results support the production of ethene observed in KB1 cultures. Despite the lower abundance of total PLFAs in KB1, the presence of Dehalococcoides together with the addition of lactate as the electron donor led to full transformation of PCE to ethene. In the case of Bioclear, PLFA results show that it is a consortium with a lower prevalence of complete degraders which suggests the culture would perform worst on fully dechlorinating PCE. As mentioned in the previous section, the study of lactate addition in Bioclear needs to be investigated to relate degradation behaviour with PLFA composition.

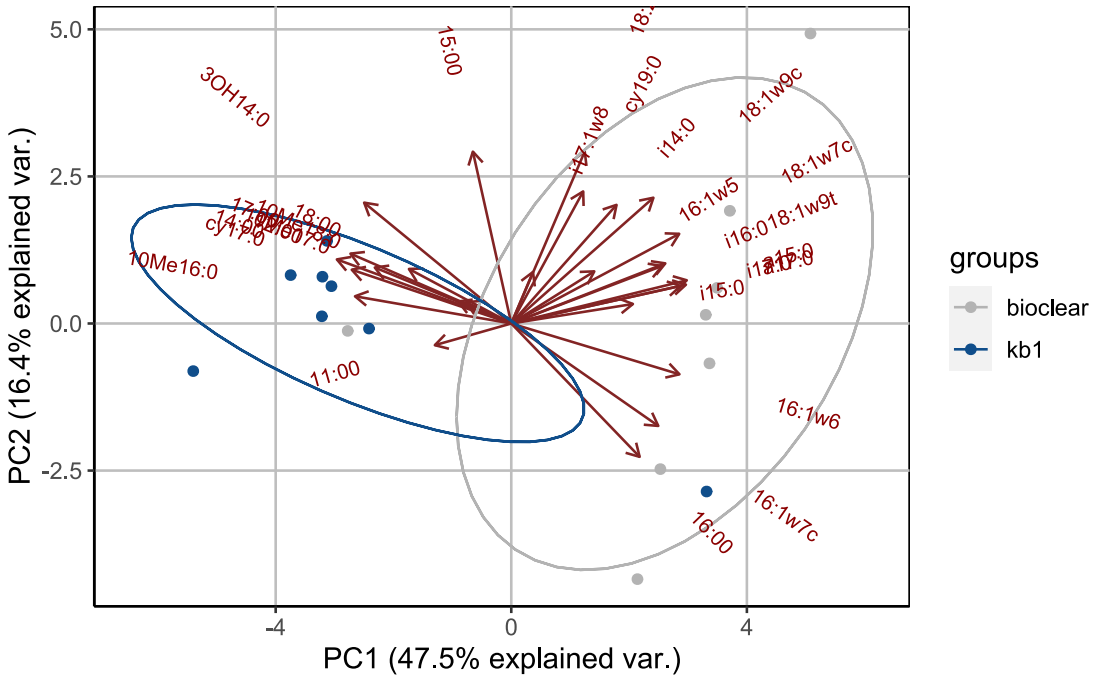

Figure 16. Principal component analysis of the PLFAs showing the distribution of the samples along the first two principal components.

4. Conclusion and Outlook

In order to select suitable consortia for PCE dechlorination, an exhaustive investigation on their degradation requirements (e.g., carbon and electron source), on the formation of metabolites and on their community structure needs to be done.

This study found that the use of compound specific isotope analysis to quantify degradation might not always be possible. The results of the degradation experiments showed a fast microbial dichlorination and no observable PCE isotope fractionation in most of the cases. Transport processes across membranes or to active sites can lower the isotope effects and huge variations in bacterial enrichment behaviour have been reported before(Cichocka et al., 2007; Cichocka et al., 2008**;** Aeppli et al., 2009). The isotope fractionation resulted to not be a rate-limiting step for degradation.

The complete transformation of PCE to ethene was only achieved when lactate instead of acetate was used as the source of electrons in the KB1 culture. The fate of the electron donor and its fermentation products are key in reductive dichlorination studies. Lactate seems to be effectively fermented to acetate and H_2 by members of the consortia and the H_2 can then be used by OHRB able to completely degrade PCE to ethene.

From the results of the PLFA extraction in the KB1 culture, it can be said that a higher abundance of bacteria from the genus Dehaloccocoides than from Geobacter or sulphate reducers is related with complete transformation of PCE to ethene. Despite of not having enough information to make conclusions for the Bioclear culture, it seems clear that KB1 with lactate as electron donor is a promising option for PCE remediation and a suitable choice for its use in combination with biochar.

Biochar has advantages over other physical and chemical remediation methods such as activated carbon (higher production costs and no additional environmental benefits), ZVI (necessity of high amounts when electrons are consumed by the anaerobic corrosion) or chemical oxidation (an excess of oxidative agents is needed when high amounts of natural oxidable substances are present) (ÖVA, 2012; Schöftner et al., 2015). Biochar filters are reported to have high removal efficiency of PCE (e.g. spruce biochar shows 93% removal from 20mg/L initial PCE concentration) however, they can become saturated, and their lifespan gets reduced (Siggins et al., 2020). The idea of the physical-biological filters is to use the biochar as a surface for the stablisment of a dehalorespiring bacteria biofilm capable of degrading the contaminant and prolong the lifespan of the system (Figure 17)

The KB1 culture can potentially be combined with biochar to study its biodegradation when PCE is being adsorbed. The use of CSIA to investigate the adaptation of the community to the conditions of the microcosms with biochar and the remediation performance of the physical-biological filter must be constantly revised due to the lack of fractionation observed in this study.

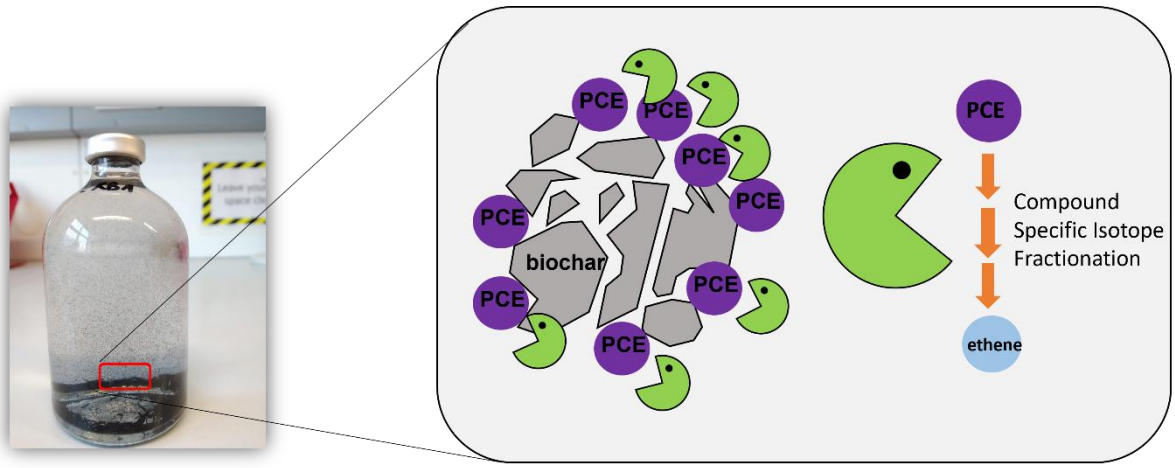

Figure 17. Eschmatic overview of the dehalorespiration and adsorption in MCs experiments

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6. Supplementary material

Table SM1. Mean PLFA concentrations values (nmol/L) of the KB1 and Bioclear cultures replicates.

PLFAs	Bioclear	KB1	PLFAs	Bioclear	KB1
12:0	0.1	1.6	10Me16:0	4.8	6.8
i14:0	1.5	0.1	i17:0	2.2	0.3
14:0	23.3	27.9	a17:0	2.4	1.0
unknown	0.0	7.5	cy17:0	4.4	8.4
i15:0	28.1	13.7	17:0	1.7	2.9
a15:0	18.5	6.6	10Me17:0	0.0	0.0
15:0	6.0	3.9	18:2w6,9	1.7	0.4
3OH14:0	2.1	2.6	18:1w9c	6.6	1.5
i16:0	1.4	0.0	18:1w7c	14.6	3.7
16:1w7c	60.4	25.1	18:1w9t	3.0	0.4
16:1w6c	16.8	6.8	18:0	5.9	6.0
16:1w5	7.9	4.8	10Me18:0	0.0	0.2
16:0	69.2	28.4	cy19:0	0.1	0.0
i17:1w8	2.2	2.5			

Table SM2. Sampling days, PCE addition (µmol/L), concentration of metabolites (µmol/L) and mL of media in Bioclear microcosms.

Sample ID	Consortium	sampling day	PCE addition before measurement (µmol/L)	PCE (µmol/L)	TCE (µmol/L)	cDCE(µmol/L)	VC (µmol/L)	Ethene (µmol/L)	Media (mL)
MC2	Bioclear	20	162.8	0.0	0.0	244.5	0.0	0.0	117.8
MC3		20	162.8	0.0	0.0	295.8	0.0	0.0	118.7
MC4		0	162.8	111.3	0.0	0.0	0.0	0.0	118.0
MC5		0	162.8	70.8	0.0	0.0	0.0	0.0	117.8
MC6		0	162.8	70.4	0.0	0.0	0.0	0.0	117.8
MC7		0	162.8	44.1	0.0	0.0	0.0	0.0	118.3
MC8		0	162.8	41.0	0.0	0.0	0.0	0.0	118.0
control1		0	162.8	137.5	0.0	0.0	0.0	0.0	118.4
MC2		27	162.8	0.0	0.0	462.8	0.0	0.0	117.8
MC3		27	162.8	0.0	0.0	307.5	0.0	0.0	118.7
MC4		7	162.8	200.5	0.0	54.0	0.0	0.0	118.0
MC5		7	162.8	199.4	0.0	0.0	0.0	0.0	117.8
MC6		7	162.8	216.6	0.0	6.2	0.0	0.0	117.8
MC7		7	162.8	43.4	0.0	123.4	0.0	0.0	118.3
MC8		7	162.8	88.6	48.5	65.0	0.0	0.0	118.0
control1		7	162.8	221.0	0.0	0.0	0.0	0.0	118.4
MC2		31		0.0	0.0	389.4	0.0	0.0	117.8
MC3		31		0.0	0.0	379.8	0.0	0.0	118.7
MC4		11		234.8	0.0	72.4	0.0	0.0	118.0
MC5		11		162.5	61.2	0.0	0.0	0.0	117.7
MC6		11		261.9	0.0	0.0	0.0	0.0	117.8
MC7		11		47.5	0.0	215.6	0.0	0.0	118.3
MC8		11		0.0	0.0	310.2	0.0	0.0	118.0
control1		11		210.0	0.0	0.0	0.0	0.0	118.4

MC2		38		0.0	0.0	353.1	0.0	0.0	117.8
MC3		38		0.0	0.0	398.4	0.0	0.0	118.7
MC4		18		123.7	87.3	34.1	0.0	0.0	118.0
MC5		18		0.0	0.0	0.0	0.0	0.0	117.7
MC6		18		132.3	78.5	94.7	0.0	0.0	117.8
MC7		18		0.0	0.0	265.8	0.0	0.0	118.3
MC8		18		0.0	0.0	308.9	0.0	0.0	118.0
control1		18		263.2	0.0	0.0	0.0	0.0	118.4
control 2		18		27.1	0.0	0.0	0.0	0.0	118.4
MC2		43		0.0	0.0	377.1	0.0	0.0	117.8
MC3		43		0.0	0.0	464.6	0.0	0.0	118.6
MC4		23		0.0	0.0	318.3	0.0	0.0	118.0
MC5		23		0.0	0.0	268.9	0.0	0.0	117.7
MC6		23		0.0	0.0	316.2	0.0	0.0	117.8
MC7		23		0.0	0.0	269.9	0.0	0.0	118.3
MC8		23		0.0	0.0	321.7	0.0	0.0	118.0
control1		23		256.3	0.0	0.0	0.0	0.0	118.4
control 2		23		132.5	0.0	0.0	0.0	0.0	118.4
MC2		45		0.0	0.0	392.4	0.0	0.0	117.8
MC3		45		0.0	0.0	474.8	0.0	0.0	118.6
MC4		25		0.0	0.0	327.5	0.0	0.0	118.0
MC5		25		0.0	0.0	328.5	0.0	0.0	117.7
MC6		25		0.0	0.0	317.6	0.0	0.0	117.8
MC7		25		0.0	0.0	320.6	0.0	0.0	118.3
MC8		25		0.0	0.0	323.4	0.0	0.0	118.0
control1		25		253.0	0.0	0.0	0.0	0.0	118.4
control 2		25		140.0	0.0	0.0	0.0	0.0	118.4
		media exchanged							
MC2		50	162.8	0.0	0.0	0.0	0.0	0.0	117.8

MC3		50	162.8	0.0	0.0	0.0	0.0	0.0	118.5
MC4		30	162.8	0.0	0.0	0.0	0.0	0.0	118.1
MC5		30	162.8	0.0	0.0	0.0	0.0	0.0	117.8
MC6		30	162.8	0.0	0.0	0.0	0.0	0.0	117.7
MC7		30	162.8	0.0	0.0	0.0	0.0	0.0	118.3
MC8		30	162.8	0.0	0.0	0.0	0.0	0.0	118.0
control1		30	162.8	0.0	0.0	0.0	0.0	0.0	118.5
control 2		30	162.8	0.0	0.0	0.0	0.0	0.0	118.4
MC2		52		164.5	0.0	37.1	0.0	0.0	117.8
MC3		52		127.7	0.0	0.0	0.0	0.0	118.5
MC4		32		106.6	0.0	0.0	0.0	0.0	118.1
MC5		32		112.7	0.0	0.0	0.0	0.0	117.8
MC6		32		97.6	0.0	0.0	0.0	0.0	117.7
MC7		32		119.2	0.0	0.0	0.0	0.0	118.3
MC8		32		75.4	0.0	0.0	0.0	0.0	118.0
control1		32		257.9	0.0	0.0	0.0	0.0	118.5
control 2		32		166.5	0.0	0.0	0.0	0.0	118.4
MC2		56		157.9	0.0	42.4	0.0	0.0	117.8
MC3		56		161.7	0.0	0.0	0.0	0.0	118.5
MC4		36		166.6	0.0	0.0	0.0	0.0	118.1
MC5		36		140.7	0.0	0.0	0.0	0.0	117.8
MC6		36		90.1	0.0	33.6	0.0	0.0	117.7
MC7		36		170.3	0.0	0.0	0.0	0.0	118.2
MC8		36		39.2	39.2	86.1	0.0	0.0	118.0
control1		36		277.5	0.0	0.0	0.0	0.0	118.5
control 2		36		138.9	0.0	0.0	0.0	0.0	118.4
MC2		59		166.4	0.0	44.7	0.0	0.0	117.8
MC3		59		163.2	0.0	0.0	0.0	0.0	118.5
MC4		39		164.7	0.0	0.0	0.0	0.0	118.1

MC5		39		106.1	0.0	37.5	0.0	0.0	117.8
MC6		39		42.1	43.7	102.4	0.0	0.0	117.7
MC7		39		146.6	0.0	0.0	0.0	0.0	118.2
MC8		39		0.0	0.0	199.0	0.0	0.0	118.0
control1		39		261.6	0.0	0.0	0.0	0.0	118.5
control 2		39		165.2	0.0	0.0	0.0	0.0	118.4
MC2		66		166.7	0.0	0.0	0.0	0.0	117.7
MC3		66		156.5	0.0	0.0	0.0	0.0	118.5
MC4		46		155.2	0.0	0.0	0.0	0.0	118.1
MC5		46		0.0	0.0	163.8	0.0	0.0	117.8
MC6		46		0.0	0.0	179.1	0.0	0.0	117.7
MC7		46		130.3	0.0	41.9	0.0	0.0	118.2
MC8		46		0.0	0.0	170.7	0.0	0.0	118.0
control1		46		249.4	0.0	0.0	0.0	0.0	118.5
control 2		46		168.1	0.0	0.0	0.0	0.0	118.4
MC2		70		170.8	1.7	0.0	0.0	0.0	117.7
MC3		70		157.3	0.0	0.0	0.0	0.0	118.5
MC4		50		151.7	0.0	0.0	0.0	0.0	118.1
MC5		50		0.0	0.0	181.3	0.0	0.0	117.8
MC6		50		0.0	0.0	196.8	0.0	0.0	117.7
MC7		50		101.3	0.0	48.0	0.0	0.0	118.2
MC8		50		0.0	0.0	189.9	0.0	0.0	118.0
control1		50		250.4	0.0	0.0	0.0	0.0	118.5
control 2		50		164.2	0.0	0.0	0.0	0.0	118.3
MC2		77		182.7	0.0	43.2	0.0	0.0	117.7
MC3		77		152.7	0.0	38.1	0.0	0.0	118.5
MC4		57		153.2	0.0	0.0	0.0	0.0	118.1
MC5		57		0.0	0.0	178.7	0.0	0.0	117.8
MC6		57		0.0	0.0	200.7	0.0	0.0	117.7

MC7		57		68.4	52.1	52.5	0.0	0.0	118.2
MC8		57		0.0	0.0	202.3	0.0	0.0	118.0
control1		57		257.5	0.0	0.0	0.0	0.0	118.5
control 2		57		183.5	0.0	0.0	0.0	0.0	118.3

Table SM3 Sampling days, PCE addition (µmol/L), concentration of metabolites (µmol/L) and mL of media in KB1 microcosms

Sample ID	Consortium	sampling day	PCE addition before measurement (µmol/L)	PCE (µmol/L)	TCE (µmol/L)	cDCE (µmol/L)	VC (µmol/L)	Ethene (µmol/L)	mL media
9	KB1	0	162.8	86.3	39.0	0.0	0.0	0.0	116.4
10		0	162.8	53.3	39.9	0.0	0.0	0.0	117.0
11		0	162.8	91.6	41.2	0.0	0.0	0.0	117.4
12		0	162.8	52.2	37.9	0.0	0.0	0.0	116.8
13		0	162.8	76.5	39.2	0.0	0.0	0.0	117.4
14		0	162.8	46.3	36.5	0.0	0.0	0.0	118.7
15		0	162.8	48.3	37.5	25.3	0.0	0.0	116.5
control		0	162.8	82.5	0.0	0.0	0.0	0.0	117.3
9		5		0.0	0.0	134.3	0.0	0.0	116.4
10		5		0.0	0.0	152.8	0.0	0.0	117.0
11		5		0.0	0.0	161.9	0.0	0.0	117.4
12		5		0.0	0.0	165.4	0.0	0.0	116.7
13		5		0.0	0.0	160.3	0.0	0.0	117.4
14		5		0.0	0.0	152.3	0.0	0.0	118.7
15		5		0.0	0.0	159.5	0.0	0.0	116.5
control		5		69.0	0.0	0.0	0.0	0.0	117.3
9		7		0.0	0.0	142.6	0.0	0.0	116.4
10		7		0.0	0.0	143.2	0.0	0.0	117.0
11		7		0.0	0.0	160.1	0.0	0.0	117.4

12		7		0.0	0.0	161.9	0.0	0.0	116.7
13		7		0.0	0.0	163.1	0.0	0.0	117.4
14		7		0.0	0.0	165.2	0.0	0.0	118.7
15		7		0.0	0.0	166.4	0.0	0.0	116.5
control 1		7		142.4	0.0	0.0	0.0	0.0	117.3
control 2		7		26.3	0.0	0.0	0.0	0.0	119.1
9		11		0.0	0.0	136.6	0.0	0.0	116.4
10		11		0.0	0.0	153.2	0.0	0.0	117.0
11		11		0.0	0.0	159.3	0.0	0.0	117.4
12		11		0.0	0.0	162.5	0.0	0.0	116.7
13		11		0.0	0.0	155.6	0.0	0.0	117.4
14		11		0.0	0.0	162.1	0.0	0.0	118.7
15		11		0.0	0.0	164.0	0.0	0.0	116.5
control 1		11		141.7	0.0	0.0	0.0	0.0	117.3
control 2		11		87.4	0.0	0.0	0.0	0.0	119.1
9		14		0.0	0.0	148.5	0.0	0.0	116.4
10		14		0.0	0.0	157.9	0.0	0.0	117.0
11		14		0.0	0.0	166.8	0.0	0.0	117.3
12		14		0.0	0.0	174.2	0.0	0.0	116.7
13		14		0.0	0.0	170.3	0.0	0.0	117.4
14		14		0.0	0.0	170.8	0.0	0.0	118.7
15		14		0.0	0.0	170.2	0.0	0.0	116.5
control 1		14		137.0	0.0	0.0	0.0	0.0	117.3
control 2		14		111.4	0.0	0.0	0.0	0.0	119.1
9		21		0.0	0.0	142.8	0.0	0.0	116.3
10		21		0.0	0.0	141.1	0.0	0.0	117.0
11		21		0.0	0.0	158.7	0.0	0.0	117.3
12		21		0.0	0.0	164.3	0.0	0.0	116.7
13		21		0.0	0.0	160.3	0.0	0.0	117.4

14		21		0.0	0.0	162.0	0.0	0.0	118.7
15		21		0.0	0.0	164.1	0.0	0.0	116.5
control 1		21		135.2	0.0	0.0	0.0	0.0	117.3
control 2		21		154.4	0.0	0.0	0.0	0.0	119.1
9		25		0.0	0.0	140.5	0.0	0.0	116.3
10		25		0.0	0.0	155.0	0.0	0.0	117.0
11		25		0.0	0.0	159.3	0.0	0.0	117.3
12		25		0.0	0.0	169.3	0.0	0.0	116.7
13		25		0.0	0.0	164.1	0.0	0.0	117.4
14		25		0.0	0.0	166.7	0.0	0.0	118.7
15		25		0.0	0.0	167.2	0.0	0.0	116.5
control 1		25		128.5	0.0	0.0	0.0	0.0	117.2
control 2		25		147.1	0.0	0.0	0.0	0.0	119.1

Table SM4 Sampling days, PCE addition (µmol/L), concentration of metabolites (µmol/L) and mL of media in microcosm 16

Sample ID	Consortium	sampling day	PCE addition before measurement (µmol/L)	PCE (µmol/L)	TCE (µmol/L)	cDCE (µmol/L)	VC (µmol/L)	Ethene (µmol/L)	mL media
MC16	KB1	0	162.8	105.6	0.0	0.0	0.0	0.0	119.0
MC16		7	162.8	24.7	0.0	127.6	0.0	0.0	119.0
MC16		24	162.8	0.0	0.0	94.7	42.7	0.0	118.9
MC16		35	162.8	0.0	0.0	60.0	39.7	0.0	118.9
MC16		45	162.8	0.0	0.0	92.5	84.7	130.6	118.9
MC16		55	162.8	0.0	0.0	0.0	62.3	323.3	118.9