Available online at www.sciencedirect.com
ScienceDirect
Journal of Hazardous Materials 149 (2007) 582–589
Journal of Hazardous Materials
www.elsevier.com/locate/jhazmat

Dechlorinating ability of TCE-fed microcosms with different electron donors

Iraklis Panagiotakis [a,*], Daniel Mamais [a], Marina Pantazidou [a], Matina Marneri [a], Maria Parapouli [b], Efstathios Hatziloukas [b], Valter Tandoi [c]

[a] *School of Civil Engineering, National Technical University of Athens, Zografou 15780, Athens, Greece*
[b] *Department of Biological Applications & Technologies, University of Ioannina, University Campus, Ioannina 45110, Greece*
[c] *Water Research Institute, National Research Council (IRSA-CNR), Via Reno 1, Rome 00198, Italy*

Available online 5 July 2007

Abstract

The main objective of the work presented herein is to assess the effect of different electron donors (butyric acid and methanol) on the dechlorinating activity of two microbial cultures where active methanogenic populations are present, in an effort to evaluate the importance of the electron donor selection process. The ability of each anaerobic culture to dechlorinate TCE, when enriched with either butyric acid or methanol, was verified based on the results of gas chromatography. In addition, the fluorescent in situ hybridization (FISH) and the polymerase chain reaction (PCR) methods gave positive results for the presence of *Dehalococcoides* spp. According to results of the batch tests conducted in this study, it appears that the selection of the electron donor for stimulating TCE dechlorination depends on microbial culture composition; therefore, the decision on the appropriate electron donor should be based on site-specific microcosm studies.

Keywords: TCE remediation; Reductive dechlorination; Electron donor; Methanol; Butyric acid

1. Introduction

After more than 20 years of research, laboratory and field evidence indicates that trichloroethene (TCE) can be degraded efficiently to innocuous constituents by passing through a series of intermediate products including *cis*-dichloroethene (cDCE), the less common 1,1DCE and tDCE, vinyl chloride (VC) and ethene [1].

As these dechlorination processes are made possible by bacteria, the presence of suitable microorganisms that can achieve biologically mediated dechlorination is a requisite condition. Anaerobic dechlorination of TCE to cDCE is carried out by various microorganisms, which are commonly found in the subsurface environment [2]. However, the next steps from cDCE to VC and finally to ethene appear to be limited only to one genus of dechlorinating bacteria, namely *Dehalococcoides* [3], which may not be ubiquitous in the groundwater environment [4]. Each of the successive dechlorinating steps requires two electrons (for each chlorine that is replaced by hydrogen), and therefore adequate quantity of electron donor [1]. Under these favorable conditions, bacteria gain energy and grow, as one or more chlorine atoms of chlorinated ethenes are replaced with hydrogen in an anaerobic environment. In this reaction, chlorinated compounds serve as electron acceptors while hydrogen (although there is evidence that acetate could also drive the dechlorination of cDCE and VC to ethene [5]), supplied by fermentation of organic substrates, typically serves as the direct electron donor [3,6,7]. Dechlorinating bacteria often have to compete with other microbial groups that consume electron donors in processes such as methanogenesis, acetogenesis and sulfate reduction. Studies conducted in the last decade indicate that appropriate selection of electron donor might impact the efficiency and the sustainability of dechlorinating activity in situ, because in some cases it creates a competitive advantage for dechlorinating bacteria [6–8]. Laboratory-scale experiments may be very useful tools to study the suitability of different electron donors for different in situ conditions when the appropriate dechlorinating microorganisms are present. During the past 15 years, several research studies addressed the relative efficiency of various electron donors, such as acetate [5,6], formate [6],

* Corresponding author. Tel.: +30 210 772 1592; fax: +30 210 772 2377.
E-mail address: ipanag@hydro.civil.ntua.gr (I. Panagiotakis).

0304-3894/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.jhazmat.2007.06.113

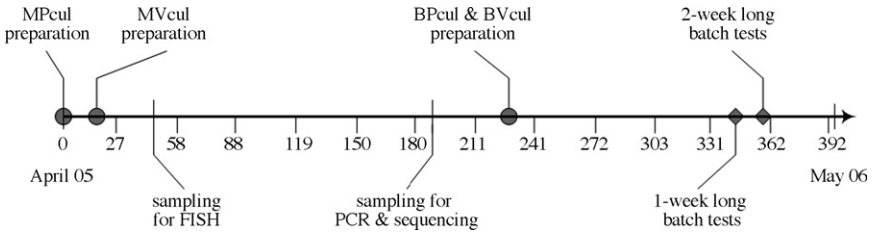

Fig. 1. Time-line of enrichment process, molecular characterization tests and batch tests.

lactate [6,7], propionate [6,7], butyrate [6,9], ethanol [7] and methanol [9,10]. The results of most of these studies, which were conducted using tetrachloroethene (PCE) rather than TCE, are consistent in showing that dechlorinators are able to outcompete other hydrogenotrophic bacteria (e.g., methanogens) in mixed cultures when fed with substrates that are able to ensure low but steady H_2 concentrations [7,8]. Substrates that are fermented slowly and tend to sustain low H_2 generation rates include volatile fatty acids, such as propionic and butyric acid [7].

The main objective of the work presented herein was to assess the effect of electron donor selection on TCE-dechlorinating ability of two different anaerobic microbial cultures that exhibited to a satisfactory degree both the ability to dechlorinate TCE and the ability to produce methane. Two electron donors were selected, methanol and butyric acid. Hydrogen production, due to both methanol and butyric acid fermentation, is dependent upon hydrogenotrophs (e.g., methanogens, sulfate-reducing bacteria, dechlorinating bacteria) to maintain partial H_2 pressure low enough to render the reactions of H_2 production thermodynamically favorable [11,12]. However, in the case of methanol, a very complex food chain could be supported by its fermentation and several reactions could take place, including the reaction which is responsible for H_2/CO_2 production [11]. In contrast, butyric acid fermentation is known to yield acetate and H_2 following a single reaction [12]. That is why it is possible to estimate hydrogen production by butyric acid fermentation, unlike methanol, where the reaction of H_2/CO_2 production may or may not take place.

2. Materials and methods

In order to evaluate the effect of electron donor (butyric acid and methanol) selection on TCE dechlorination, four dechlorinating cultures were developed, derived from two anaerobically digested sludges. All dechlorinating cultures were maintained in a semi-batch fashion with a mean hydraulic residence time of 48 days and weekly culture amendments of 0.5 mM TCE and 1.0 mM methanol or 0.3 mM of butyric acid. The ability of each culture to achieve a stable dechlorinating activity was verified by gas chromatography, as well as by the fluorescent in situ hybridization (FISH) and polymerase chain reaction (PCR) methods. Dechlorinating cultures enriched with methanol and butyric acid were used as source cultures to conduct batch experiments in order to acquire a detailed picture of comparative culture performance. During the batch experiments, the formation of dechlorination products and methane was intensively monitored. The next section describes in detail the materials used and the experimental methods followed during culture enrichment and batch studies.

2.1. Dechlorinating culture enrichment and growth

Four mixed cultures were enriched and used in this study. Each culture was inoculated with anaerobically digested sludge from either the wastewater treatment plant (WWTP) of Psyttalia (Pcul), Athens, or from the WWTP of Volos (Vcul). Both plants receive significant contribution of industrial discharges, estimated to be on the order of 20%.

The cultures from Psyttalia and Volos were maintained for approximately 11 months and were fed on a weekly basis with gradually increasing concentration of TCE, reaching a value of 0.5 mM (nominal concentration, i.e., total amount added to the bottle divided by liquid volume) [13]. As shown in Fig. 1, from the start of the experiments (April 2005), and for a period of approximately 6 months, both cultures were fed with methanol. The ability of each culture to dechlorinate TCE to cDCE and vinyl chloride was verified through weekly measurements of the above compounds by gas chromatography (in later weeks ethene was also measured), as well as by FISH and PCR. Following evidence that both methanol-fed cultures developed the ability to dechlorinate TCE to cDCE, vinyl chloride and ethene, each was split into two cultures: one that continued to be fed with TCE and methanol (MPcul & MVcul), while the other was fed with TCE and butyric acid (BPcul & BVcul) (Table 1). Enrichment of the four cultures continued for another 5 months (from November 2005 till March 2006) in order to reach steady-state conditions. Thereafter biomass samples were obtained from the four parent cultures to conduct batch experiments with methanol and butyric acid.

The microbial consortia were cultured in flasks of 2.2 l with openings modified to accept previously autoclaved grey-butyl Teflon-lined septa 20 mm (Wheaton, Millville, NJ) and aluminum crimp caps. The cultures were incubated without mixing, at room temperature, approximately 20 °C throughout the

Table 1
Nominal concentration of TCE and electron donors in dechlorinating cultures

Electron donor and acceptor	BPcul	MPcul	BVcul	MVcul
TCE (mM)	0.5	0.5	0.5	0.5
Butyric acid (mM)	0.3	–	0.3	–
Methanol (mM)	–	1.0	–	1.0

Table 2
Probes and materials used in FISH

Probe	Target organisms	Sequence (5′–3′)	Fluorophore label	Formamide concentration (%)
EUB338I	Bacteria	GCTGCCTCCCGTAGGAGT	Texas Red	30
DHE1259t	*Dehalococcoides* spp.	AGCTCCAGTTCACACTGTTG	Alexa 488	30

year, and in the dark. The cultures operated in a semi-batch mode. Every 7 days, a volume of 200 ml was replaced by fresh Reduced Anaerobic Mineral Medium (RAMM) with vitamins and yeast extract. This is the aqueous medium consisting of all the compounds that are necessary for a healthy and active dechlorinating culture. The composition of this medium has been described elsewhere [14]. The medium was prepared every week and the following ingredients were added per 100 ml of medium: 1 ml of vitamin solution [15], 0.1 ml yeast extract 10%, w/v (weight/volume), 0.5 ml $Na_2S{\cdot}9H_2O$ 5%, w/v, and 2 ml $NaHCO_3$ 10%, w/v.

Before each re-feeding, reactors were purged with a CO_2/N_2 (30:70) gas mixture to remove volatile compounds, including all chlorinated ethenes as well as methane and ethene. Methanol (1.0 mM) and butyric acid (0.3 mM) were added to the cultures in order to provide two times in excess the required reducing equivalents for complete dechlorination of TCE to ethene, i.e., 6 meq/l, assuming that both methanol and butyric acid were oxidized completely.

2.2. Analytical procedures

2.2.1. Gas chromatography

Chlorinated ethenes, ethene and methane were measured with a Perkin-Elmer Autosystem XL gas chromatograph equipped with a PLOT column GS-GasPro (30 m, 0.32 mm diameter, J&W, Folsom, CA) and flame ionization detector (FID) by injecting 50 μl of reactor headspace with a gas-tight 100 μl syringe, while injector and detector were held at 220 °C and 250 °C, respectively. Chlorinated ethenes were quantified using a temperature program (start at 50 °C, increase to 180 °C at a rate of 15 °C/min, hold for 2 min at 180 °C, increase to 250 °C at a rate of 25 °C/min), while methane (MTH) and ethene (ETH) by an isothermal method (50 °C). Standards of ethene, methane and chlorinated ethenes were prepared by adding a known amount of each compound to serum bottles of 160 ml that had the same, 6:10, headspace/liquid ratio as the reactor.

2.2.2. Molecular methods for Dehalococcoides spp. detection in cultures

Both FISH and PCR molecular assays were employed to detect the presence of *Dehalococcoides* spp. in the enriched parent cultures.

2.2.3. FISH assay

Dehalococcoides spp. detection was performed using probe DHE1259t developed by Yang and Zeyer, labeled with fluorophore Alexa 488, viewable under epifluorescence microscope equipped with specific filter set for Texas Red/FITC [16]. It has to be noted that this probe is only one of the two oligomers developed by Yang and Zeyer in order to cover the *Dehalococcoides* genus. Probe DHE1259t was selected based on the facts that (a) it is tested and (b) it covers 75% of the known *Dehalococcoides* spp., including the strains most commonly detected, in comparison to the second oligomer, DHE1259c, which covers only the remaining 25%. In addition, for the visualization of all bacteria, eubacterial probe EUB338I was employed labeled with fluorophore Texas Red, viewable under epifluorescence microscope equipped with specific filter set for Texas Red/FITC.

The paraformaldehyde-fixed samples were hybridized and washed according to Yang and Zeyer [16]. Sequences and target sites of the probes used in this study are presented in Table 2. Slides were mounted with Citifluor solution (Citifluor Ltd, United Kingdom) and examined at X1000 magnification with a Nikon Eclipse 50i epifluorescence microscope equipped with specific filter set for the Texas Red/FITC fluorescence dye.

2.2.4. PCR assay

2.2.4.1. DNA extraction procedure. Total community bacterial DNA from culture samples was extracted using standard procedures [17].

2.2.4.2. PCR procedure. Amplification of putative *Dehalococcoides*-specific 16S ribosomal DNA sequences (16S rDNA) was performed using primers DHC1–DHC1377, which are reported to be specific for *Dehalococcoides* strains [4] and yield

Table 3
Concentrations of TCE and electron donors in batch experiments

Parent culture	Initial TCE concentration (mM)	Concentration of electron donor added	
		First week of experiments	Second week of experiments
BPcul	0.5	0.3 mM butyric acid	3.0 mM H_2
MPcul	0.5	1.0 mM methanol	3.0 mM H_2
BVcul	0.5	0.3 mM butyric acid	3.0 mM H_2
MVcul	0.5	1.0 mM methanol	3.0 mM H_2

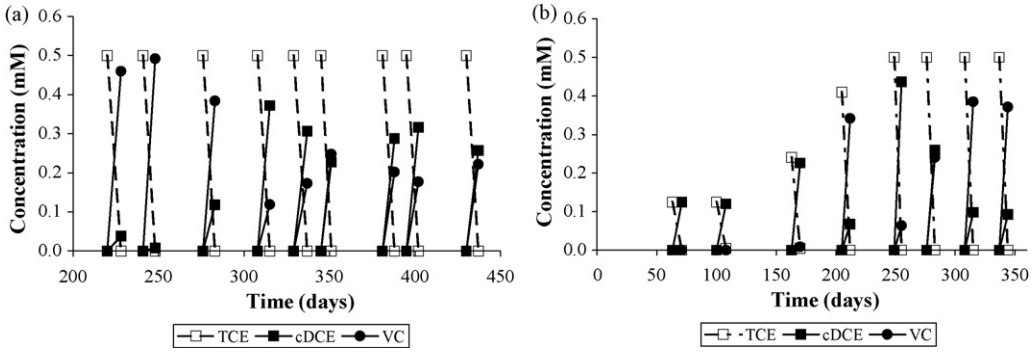

Fig. 2. Dechlorination patterns in (a) BPcul and (b) MPcul during the enrichment period.

an amplicon approximately 1377 base pair (bp) long. Amplification conditions were as described elsewhere [4]. Amplification products were visualized by gel electrophoresis using standard procedures [17] and cloned using the system Zero Blunt PCR Cloning Kit (Invitrogen, Inc., San Diego, CA). Gel electrophoretic analyses were performed using bacteriophage λ DNA digested with restriction enzyme *Hind*III, which served as a molecular mass marker. Putative recombinant plasmids were initially analyzed using restriction digestion with enzyme *Eco*RI. Further analysis involved sequencing performed by Macrogen (Korea). Sequences were further aligned to the ones deposited in Data Banks using the program Blast (National Center for Biotechnology Information, NCBI).

2.3. Batch dechlorination tests

The four enriched cultures (BPcul, MPcul, BVcul and MVcul) were used as source cultures to conduct batch experiments to investigate and compare TCE dechlorination. The batch tests presented herein were part of an experimental program designed to investigate a series of issues of interest for dechlorination, such as the role of electron donor, the influence of the feeding rate of the electron donor, possible degradation pathways of electron donors, etc. The batch dechlorination tests described herein lasted 14 days and were conducted using various types of electron donors, as shown in Table 3. From each culture, 100 ml were withdrawn and transferred to continuously mixed serum bottles of 160 ml, under anaerobic conditions, where TCE (0.5 mM) and butyric acid (0.3 mM) or methanol (1.0 mM) as well as yeast extract were initially added. The amount of electron donor added corresponded to twice the stoichiometric amount required for complete dechlorination of TCE to ethene, i.e., 6 meq/l, assuming that both methanol and butyric acid were oxidized completely. After 7 days of operation, H_2, twice in excess (3.0 mM) of the stoichiometrically required quantity to completely dechlorinate TCE to ethene (i.e., 6 meq/l), was added, in order to assess the presence of an active methanogenic population and to investigate if H_2 was the ultimate electron donor of the dechlorination process.

2.4. Calculations

The dechlorination rates of the cultures were compared on the basis of the total amount of chloride, n_{chloride}, produced from dechlorination over time per mol of TCE, as shown in the following expression:

$$n_{\text{chloride}}(t) = \frac{C_{\text{cDCE}}(t)}{C_{\text{TCEo}}} + \frac{2C_{\text{VC}}(t)}{C_{\text{TCEo}}} + \frac{3C_{\text{ETH}}(t)}{C_{\text{TCEo}}} \quad (1)$$

where $n_{\text{chloride}}(t)$ is the total amount of chloride produced from dechlorination per mol of TCE, at measurement time, t; $C_{\text{cDCE}}(t)$ is the cDCE nominal concentration (mM) at measurement time, t; $C_{\text{VC}}(t)$ is the VC nominal concentration (mM) at measurement time, t; $C_{\text{ETH}}(t)$ is the ETH nominal concentration (mM) at measurement time, t; C_{TCEo} is the TCE nominal initial concentration (mM).

According to Eq. (1), the possible values of n_{chloride} vary from 0, for no dechlorination, to 3, for complete dechlorination.

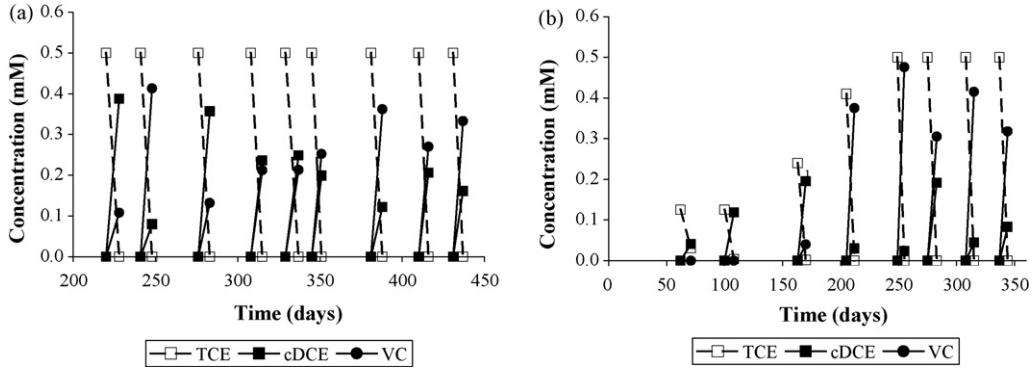

Fig. 3. Dechlorination patterns in (a) BVcul and (b) MVcul during the enrichment period.

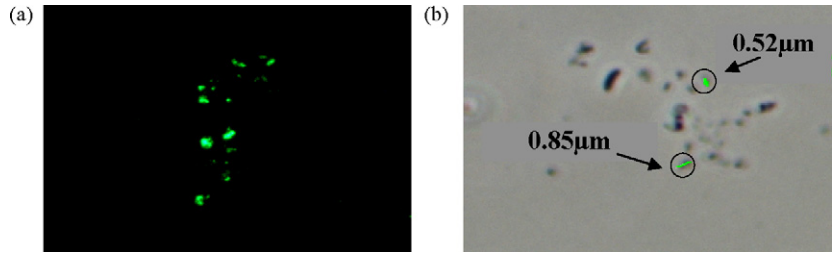

Fig. 4. (a) *Dehalococcoides* spp. cells in MVcul bound to probe DHE1259t labeled with fluorophore Alexa 488 and visualized under filter for Texas Red/FITC. (b) Pale, coccoid *Dehalococcoides* spp. cells observed with phase contrast at ×1000 magnification are indicated with arrows [different microscopic fields are shown in (a) and (b)].

3. Results and discussion

3.1. Enriched TCE dechlorinating cultures

Following 11 months of acclimation, four TCE-acclimated cultures were developed from two anaerobically digested activated sludges. The ability of each culture to achieve a stable dechlorinating activity was verified by gas chromatography as well as FISH and PCR methods.

At the end of this acclimation period (approximately day 350 in Fig. 1) all cultures had reached a stable TCE dechlorinating ability as indicated by the concentration values of chlorinated ethenes measured in the parent cultures, which are shown in Figs. 2 and 3. Dechlorination results for the methanol-enriched cultures are shown for their entire maintenance period, which lasted about 1 year (MPcul and MVcul in Figs. 2b and 3b, respectively). For the butyric acid-fed cultures, which were maintained longer, results are shown until a 100 days later (BPcul and BVcul in Figs. 2a and 3a, respectively). Figs. 2 and 3 show that all parent cultures dechlorinated TCE, although at different rates. Ethene, measured only after day 300, was found to range between 0.01 and 0.1 mM at the end of the week-long cycle.

The ability of the four enriched cultures to dechlorinate TCE was also verified using both FISH and PCR assays conducted during the initial acclimation period when parent cultures were operated on methanol only. *Dehalococcoides* spp. was successfully detected in both parent cultures using the probe DHE1259t developed by Yang and Zeyer [16]. The in situ hybridization results for MVcul are displayed in Fig. 4a. As shown in Fig. 4a, *Dehalococcoides* spp. hybridized with DHE1259t probe appeared as irregular cocci. Additionally, phase-contrast microscopic observations showed that hybridized cells were pale irregular cocci with diameters mostly in the 0.4–0.80 μm range (Fig. 4b). As noted in Section 2.2.2, probe DHE1259t covers 75% of the known *Dehalococcoides* spp., while the second oligomer, DHE1259c, covers the remaining 25%. Therefore, using only probe DHE1259t was deemed adequate for the detection of *Dehalococcoides* spp. However, for quantification purposes, probe DHE1259c has also to be added to the hybridization mixture.

Sequencing of cloned PCR products confirmed the presence of *Dehalococcoides* spp. in both cultures fed with methanol, since PCR products showed a 99% similarity to *Dehalococcoides* spp. 16S rDNA, when compared to the ones deposited in Data Banks. As mentioned, primers DHC1–DHC1377, reported to be specific for *Dehalococcoides* strains [4], yield an amplicon approximately 1377 base pair (bp) long. DNA extracted from samples from both parent cultures yielded the expected amplification products when subjected to PCR. These products are presented in Fig. 5a and marked by arrows. Successful cloning of the above amplification products was initially confirmed by restriction analysis of the putative recombinant plasmids using restriction enzyme *Eco*RI. A representative picture of this analysis is shown in Fig. 5b.

Several different recombinant plasmid clones were constructed using amplification products of DNA templates derived from bacterial cultures originating from Volos and Psyttalia cultures. Several representative clones were sequenced and proven to be identical regarding the rDNA sequences they contained. The sequence of clone pDehV1 was deposited in the GenBank database under accession number EF630361.

According to the above information, the working assumption prior to the conduct of the batch experiments was that all enriched cultures had developed a bacteria population capable of dechlorinating TCE to cDCE, vinyl chloride and, ultimately, ethene.

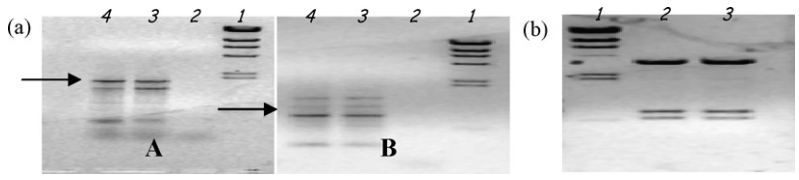

Fig. 5. (a) Detection of *Dehalococcoides* group 16S rDNA sequences in the dechlorinating cultures. (A) MVcul—lane 1: λDNA/*Hind*III; lane 2: no DNA control; lanes 3–4: PCR products with primers DHC1–DHC1377. (B) MPcul—lane 1: λDNA/*Hind*III; lane 2: no DNA control; lanes 3–4: PCR products with primers DHC1–DHC1377. (b) Digestion of putative recombinant plasmids from MVcul and MPcul. Lane 1: λDNA/*Hind*III; lane 2: putative recombinant plasmid from MVcul; lane 3: putative recombinant plasmid from MPcul.

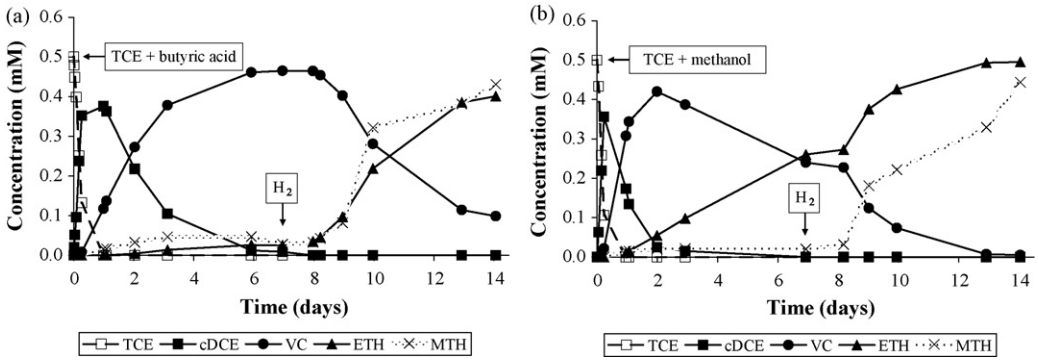

Fig. 6. Dechlorination patterns in (a) BPcul and (b) MPcul.

3.2. Comparative dechlorination behavior in BPcul and MPcul

As shown in Fig. 6, both BPcul and MPcul exhibited the ability to exhaust TCE (0.5 mM) completely, during the first day of operation and cDCE during the first feeding period. Differences emerged in the production rate of the byproducts. At the end of the first period (day 7), both cultures had significant VC concentrations, 0.24 and 0.47 mM, with MPcul having almost 49% lower VC concentration than BPcul. The opposite was observed, as expected, for ethene: at the end of the first feeding cycle, the concentration in MPcul (0.26 mM) was almost 90% higher compared to that of BPcul (0.03 mM). This difference in dechlorination efficiencies is better illustrated when dechlorination is expressed in terms of the total amount of chloride produced (Eq. (1)), as shown in Fig. 7a. On the basis of $n_{chloride}$, MPcul demonstrated 20% higher dechlorinating efficiency than BPcul at the end of the first feeding period. As soon as H_2 was added, VC concentrations declined markedly in both cultures, while ethene concentrations rose. This accelerated dechlorination process is reflected by $n_{chloride}$, which reached a value of 3 (the maximum value) in MPcul and 2.8 in BPcul, at the end of the second feeding period (day 14). The repeatability of these experiments was evaluated with 1-week long batch tests conducted earlier (see Fig. 1). As shown in Fig. 7b, the same trend was observed, providing evidence that the repeatability of the batch experiments is very satisfactory.

3.3. Comparative dechlorination behavior in BVcul and MVcul

Quick TCE degradation was observed in these cultures as well: TCE (0.5 mM) was completely exhausted during the first day of operation (Fig. 8). At the end of first feeding period (day 7), cDCE is still present, VC is close to 0.3 mM and ethene is increasing. The two cultures demonstrated more or less the same dechlorinating activity, based on both the composition of dechlorination products, as well as the overall dechlorination behavior expressed by $n_{chloride}$, which at day 7 reached values of 2.0 and 2.2 for MVcul and BVcul, respectively (Fig. 7a). Similarly to Pcul, the addition of H_2 accelerated the dechlorination process. The concentration of VC approached zero and $n_{chloride}$ reached values close to 3 (the possible maximum value). Similarly to Pcul, the repeatability of the observed behavior was evaluated with comparisons to the results of 1-week long batch tests. As shown in Fig. 7b, the same trend was observed providing evidence that the repeatability of the batch experiments is very satisfactory for Vcul as well.

3.4. Methanogenic activity

During the first week of the batch experiments, methanogenic activity remained very low, with methane concentrations remaining lower than 0.05 mM in all experiments. However, this finding is not attributed to a lack of methanogenic populations. As soon as H_2 was added, methane concentrations increased in

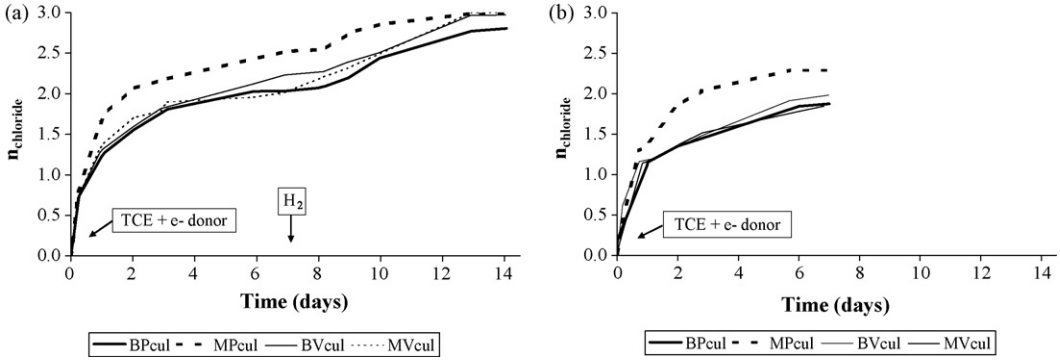

Fig. 7. Dechlorination progress on the basis of Cl^- removed per mol TCE in (a) 2-week long batch tests and (b) 1-week long batch tests.

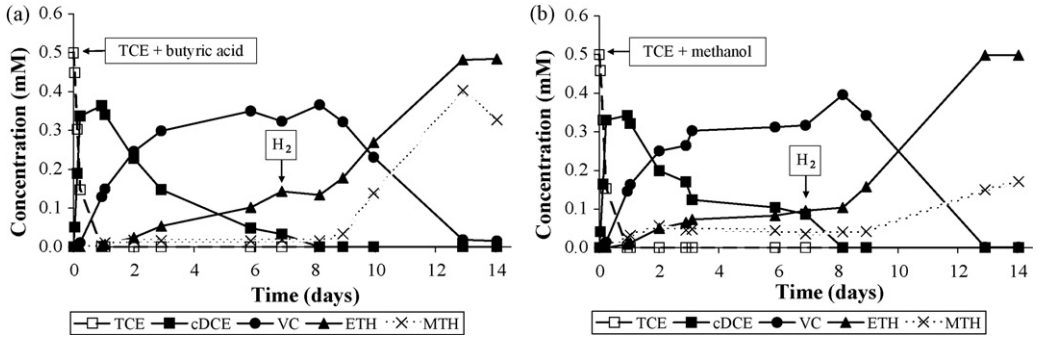

Fig. 8. Dechlorination patterns in (a) BVcul and (b) MVcul.

all cultures, reaching at the end of the second feeding period values equal to 0.43 mM (BPcul), 0.44 mM (in MPcul, which had a value of 0.22 mM close to the end of dechlorination corresponding to a value of $n_{chloride} = 2.9$), 0.33 mM (BVcul) and 0.17 mM (MVcul). It is relevant to report here briefly results from subsequent batch experiments using butyric acid, not described herein. In those later experiments, appreciable methanogenesis was observed already from the first feeding cycle while significant dechlorination took place. These later experiments also suggested that TCE at the maximum concentration used in this study (0.5 mM) is not toxic to methanogens.

4. Conclusions

The results of the batch experiments conducted using two anaerobic cultures showed that both low molecular weight substrates employed, methanol and butyric acid, resulted in the development of mixed bacteria cultures of dechlorinating and methanogenic bacteria. Evidence of the presence of active TCE dechlorinating and methane producing bacteria in each culture was drawn by the significant ethene and methane production obtained when hydrogen gas was added in excess of the stoichiometrically required amount to completely dechlorinate TCE. In addition, *Dehalococcoides* spp. was identified in all microbial cultures developed, with the fluorescent in situ hybridization (FISH) and polymerase chain reaction (PCR) methods.

Both methanol and butyric acid appeared to create suitable conditions for dechlorination, albeit at different rates. For Pcul, methanol appeared to be a more efficient electron donor than butyric acid, while butyric acid and methanol had more or less the same dechlorinating efficiency for Vcul. Butyric acid appeared to have a more consistent dechlorinating efficiency, with a difference of only 10% between the two cultures, while this difference for methanol reached 20%. In the presence of excess hydrogen, it was observed that dechlorination of TCE was completed to ethene, indicating that H_2 was probably the ultimate electron donor for both dechlorinating cultures.

Overall, the experimental results presented herein are consistent with the inconclusive studies published so far, mostly for PCE dechlorination, regarding the importance of the electron donor. It appears that the effect of the electron donor depends on the make up of the entire microbial community and the prevalent site-specific environmental conditions. In other words, it is possible that batch tests conducted for the selection of electron donor at a TCE-affected site may not yield a conclusive outcome, like the tests performed in this study with the Volos culture (Vcul). In cases where it is not possible to distinguish among electron donors on the basis of dechlorination rates alone, then alternative selection criteria can be used, such as, whether an electron donor has a simpler degradation pathway compared to the others, whether its role in the metabolic activity is well known or whether it produced consistent dechlorination results. According to this rationale, butyric acid would be a better candidate for further evaluation at a TCE-affected site.

Acknowledgments

This project is co-funded by the European Social Fund (75%) and National Resources (25%) – (EPEAEK II) – PYTHAGORAS.

References

[1] D.L. Freedman, J.M. Gossett, Biological reductive dechlorination of tetrachloroethylene and trichloroethylene to ethylene under methanogenic conditions, Appl. Environ. Microbiol. 55 (1989) 2144–2151.
[2] F.H. Chapelle, Ground-water biology and biochemistry, John Wiley & Sons Inc., New York, 1992.
[3] X. Maymó-Gatell, Y.-T. Chien, J.M. Gossett, S.H. Zinder, Isolation of a bacterium that reductively dechlorinates tetrachloroethene to ethene, Science 276 (1997) 1568–1571.
[4] E.R. Hendrickson, J.A. Payne, R.M. Young, M.G. Starr, M.P. Perry, S. Fahnestock, D.E. Ellis, R.C. Ebersole, Molecular analysis of *Dehalococcoides* 16S ribosomal DNA from chloroethene-contaminated sites throughout North America and Europe, Appl. Environ. Microbiol. 68 (2002) 485–495.
[5] J. He, Y. Sung, M.E. Dollhopf, B.Z. Fathepure, J.M. Tiedje, F.E. Löffler, Acetate versus hydrogen as direct electron donors to stimulate the microbial reductive dechlorination process at chloroethene-contaminated sites, Environ. Sci. Technol. 36 (2002) 3945–3952.
[6] B.S. Ballapragada, H.D. Stensel, J.A. Puhakka, J.F. Ferguson, Effect of hydrogen on reductive dechlorination of chlorinated ethenes, Environ. Sci. Technol. 31 (1997) 1728–1734.
[7] D.E. Fennell, J.M. Gossett, S.H. Zinder, Comparison of butyric acid, ethanol, lactic acid, and propionic acid as hydrogen donors for the reductive dechlorination of tetrachloroethene, Environ. Sci. Technol. 31 (1997) 918–926.
[8] C.R. Smatlak, J.M. Gossett, S.H. Zinder, Comparison kinetics of hydrogen utilization for reductive dechlorination of tetrachloroethene and methanogenesis in an anaerobic enrichment culture, Environ. Sci. Technol. 30 (1996) 2850–2858.

[9] F. Aulenta, J.M. Gossett, M.P. Papini, S. Rossetti, M. Majone, Comparative study of methanol, butyrate, and hydrogen as electron donor for long-term dechlorination of tetrachloroethene in mixed anaerobic cultures, Biotechnol. Bioeng. 91 (2005) 743–753.
[10] T.D. DiStefano, J.M. Gossett, S.H. Zinder, Reductive dechlorination of high concentrations of tetrachloroethene to ethene by an anaerobic enrichment culture in the absence of methanogenesis, Appl. Environ. Microbiol. 57 (1991) 2287–2292.
[11] R. Cord-Ruwisch, B. Ollivier, Interspecific hydrogen transfer during methanol degradation by *Sporomusa acidovorans* and hydrogenophilic anaerobes, Arch. Microbiol. 144 (1986) 163–165.
[12] B. Schink, Energetics of syntrophic cooperation in methanogenic degradation, Microbiol. Mol. Biol. Rev. 61 (1997) 262–280.
[13] I. Panagiotakis, D. Mamais, M. Pantazidou, M. Marneri, M. Parapouli, E. Hatziloukas, Development of dechlorinating cultures for TCE bioremediation, in: Proceedings of the third European Bioremediation Conference, Chania, Crete, July 4–7, 2005, P036.
[14] V. Tandoi, T.D. DiStefano, P.A. Bowser, J.M. Gossett, S.H. Zinder, Reductive dehalogenation of chlorinated ethenes and halogenated ethanes by a high-rate anaerobic enrichment culture, Environ. Sci. Technol. 28 (1994) 973–979.
[15] W.E. Balch, R.S. Wolfe, New approach to the cultivation of methanogenic bacteria: 2-mercaptoethanesulfonic acid (HS-CoM)-dependent growth of *Methanobacterium ruminantium* in a pressurized atmosphere, Appl. Environ. Microbiol. 32 (1976) 781–791.
[16] Y. Yang, J. Zeyer, Specific detection of *Dehalococcoides* species by fluorescence in situ hybridization with 16S rRNA-targeted oligonucleotide probes, Appl. Environ. Microbiol. 69 (2003) 2879–2883.
[17] J. Sambrook, D.W. Russell, Molecular cloning. A laboratory manual, 3rd ed., Cold Spring Harbor Laboratory Press, New York, 2001.