Constraints in Anaerobic Microbial Dechlorination, Fermentation, and Sulfate-Reduction Induced by High Concentrations of Tetrachloroethylene

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Abstract Anaerobic bioremediation of tetrachloroethylene (PCE) under high concentration conditions is difficult. Anaerobic dechlorination of PCE occurs with synergetic reactions, fermentation, and sulfate-reduction; however, the way in which high concentrations of PCE affects these reactions is still poorly understood. This study aims to elucidate how high concentrations of PCE affect fermentation and sulfate-reduction, as well as PCE dechlorination. Laboratory dechlorination tests were performed using a wide concentration range of PCE between 2 and 125 mg/L added to a microbial consortium that had been continuously cultivated in the laboratory and completely dechlorinated PCE for over 4 years. Fermentation of lactate, reduction of sulfate, and dechlorination of PCE were monitored in addition to microbial activities based on RNA. All three reactions, fermentation, sulfate-reduction, and PCE dechlorination were observed to be inhibited. The inhibition for fermentation, sulfate-reduction, and dechlorination occurred when PCE concentrations were higher than 125, 75, and 30 mg/L, respectively. The fermenter, *Anaerotignum*, and the sulfate-reducer, *Desulfosporosinus*, were active when the dechlorination was inhibited with 30 mg/L of PCE. These

findings suggest that there is interference of PCE dechlorination, despite the occurrence of fermentation and sulfate reduction. Bioaugmentation with a PCE dechlorinator that is tolerant to high PCE concentrations can be a possible solution for bioremediation of PCE when its concentrations are greater than 30 mg/L.

Keywords Biodegradation · High concentration · Chloroethylenes · Microbial community · RNA

1 Introduction

Soil and groundwater in many industrial sites are contaminated with high concentrations of tetrachloroethylene (PCE) (US EPA 2003). PCE has been used as an effective solvent in the dry cleaning and manufacturing industries (US EPA 2002; Zhang and Yoshikawa 2016). Once PCE is released into subsurface due to illegal and/or careless disposal from these industries, the high concentration of contaminant slowly partitions into groundwater; then, it can migrate long distances dependent on geological characteristics of the site and pollute large area (EPA 1998; US EPA 2003). Thus, besides the removal of pollutant source, remediation of the area near the pollutant source with high concentrations is a significant issue. Bioremediation, the remediation of soil or water using microorganisms, is considered difficult in instances where there is a high concentration of PCE (US EPA 2003; Juwarkar et al. 2010;

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Zhang and Yoshikawa 2016). In such cases, remediation conventionally consists of other engineering technologies such as in situ hydraulic containment, off-site disposal, and institution controls. Bioremediation has been largely selected for use in groundwater with a low concentration of pollutants (US EPA 2017). While the difficulty of bioremediation of PCE at high concentrations is known in practice, the reason for this difficulty at the microbial community scale has not been fully understood.

Anaerobic dechlorination of PCE occurs due to microbial degradation alongside other microbial activities. One essential role played by other microorganisms is fermentation (Parsons 2004). To enable the reductive dechlorination of chloroethylenes, such as PCE and its toxic intermediate products; tetrachloroethylene (TCE), *cis*-dichloroethylene (*cis*-DCE), and vinyl chloride (VC), fermenting substrates to organic acids and producing molecular hydrogen are indispensable. The organic acids are used as carbon sources and/or electron donors for the dechlorinators of chloroethylenes, and molecular hydrogen is also used as their electron donor (e.g., Scholz-Muramatsu et al. 1995; Krumholz 1997; Sung et al. 2006; Löffler et al. 2013). Microbes also play an essential role in lowering the oxidation-reduction potential (ORP). Sulfate-reducing bacteria support the reductive dechlorination due to decreasing the ORP (Parsons 2004). With a declining ORP, the rate of PCE dechlorination increases (Hirl and Irvine 1997). A previous laboratory study demonstrated the inhibition of anaerobic dechlorination of PCE when its concentration is higher than 90 mg/L with PCE dechlorinating isolates (Amos et al. 2007). However, the mechanisms at play that impact the synergetic reactions and PCE dechlorination in the microbial community are still poorly understood.

In this study, we aimed to reveal the effects of high PCE concentrations on the microbial community. Laboratory degradation tests with a wide PCE concentration range between 2 and 125 mg/L were carried out with a microbial consortium that had been continuously cultivated in the laboratory and completely dechlorinated PCE for over 4 years. We analyzed the changes in microbial structure at the RNA level as well as the fermentation, sulfate reduction, and PCE dechlorination processes, to elucidate whether and how high concentrations of PCE influences these processes in the microbial consortium.

2 Materials and Methods

2.1 Biodegradation Tests

2.1.1 Enrichment Culture as Microbial Source

A soil sample was collected from a site contaminated with toxic volatile organic compounds including PCE and other contaminants (Yoshikawa et al. 2017). The soil was mixed with a mineral solution and anaerobically incubated with PCE at a concentration of 30 mg/L as per Yoshikawa et al. (2016, 2017). Sodium acetate, sodium lactate, yeast extract, and methanol were added as carbon sources and/or electron donors. Following dechlorination of chloroethylenes, a portion of culture was transferred to the fresh mineral solution at a ratio of 1:5 (v/v) to enrich the microorganisms degrading chloroethylenes. The enrichment culture was allowed to stably dechlorinate chloroethylenes and produce ethylene for over 4 years. VC is more toxic compared with its parent compound, and excessive accumulation of VC or the degradation ends by VC should be avoided. Previous studies (Yoshikawa et al. 2017) showed that when the concentration of PCE was less than 30 mg/L and with the existence of *Dehalococcoides*, PCE could be completely degraded to ethylene which is a harmless substance.

2.1.2 Biodegradation Test with Different Concentrations of PCE

An aliquot of 32 mL of the enrichment culture and 160 mL of the fresh mineral solution were mixed in each of 220 mL test bottles. Each bottle was sealed with an aluminum cap and a Teflon-coated butyl rubber septum, followed by replacement of the headspace gas with N₂. Then, sodium lactate, sodium acetate, yeast extract, and methanol were added. PCE was added for concentrations of 2, 6, 30, 75, and 125 mg/L, which equated to 2.4, 7.1, 35.5, 88.7, and 147.9 µmol/bottle, respectively. We named the conditions PCE-2, PCE-6, PCE-30, PCE-75, and PCE-125 to correspond to the 2, 6, 30, 75, and 125 mg/L PCE concentrations, respectively. The test bottles were placed in a dark shaking incubator (150 rpm, 30 °C). We performed the biodegradation test in triplicate. We also prepared bottles with no PCE added to analyze initial concentrations of organic acids, SO₄²⁻, and the microbial community. This

dataset represented day 0 in this study. We also prepared bottles with 30 mg/L of PCE as sterile controls.

2.2 Changes in Chemical Properties

2.2.1 Fermentation in Culture Solution

To evaluate the fermentation of organic matter, organic acids including lactate, acetate, and propionate were analyzed during the experimental timeframe. We collected 1 mL of culture solution periodically from each of the test bottles to a plastic tube. This was then centrifuged (14,000 rpm, 4 °C, 4 min) to divide the supernatant and pellet. The supernatant was filtered with a 0.22- μ m polytetrafluoroethylene (PTFE) syringe filter (Membrane Solutions, Plano, TX, USA) for the analysis of organic acids. A high-performance liquid chromatograph (HPLC) system was used (Shimadzu) with two equivalent columns installed (Shim-Pack SCR-102H, Shimadzu) to separate the organic acids accurately. The organic acids were detected with a COD-10A detector (Shimadzu) using 0.005 mmol/L detection limits. Pyruvate and succinate were also analyzed, but the concentrations of them were below the detection limits and not shown in this paper. The pellet left in the plastic tube was used for subsequent RNA extraction.

2.2.2 Sulfate Reduction in Culture Solution

We monitored SO_4^{2-} concentrations over time to investigate sulfate reduction. The filtered supernatant, as described in Section 2.2.1, was applied to analyze SO_4^{2-} with an ion chromatography (IC-2010, Tosoh, Tokyo, Japan) installed with a column (TSKgel SuperIC-Anion HS, Tosoh). The detection limit was 0.001 mmol/L.

2.2.3 Dechlorination of PCE

We monitored PCE concentrations, its intermediate products, and the harmless non-chlorinated product, ethylene, in the headspace gas of the test bottle. We analyzed PCE and its intermediate products with a gas chromatography (GC-2014, Shimadzu, Kyoto, Japan) and a gas chromatography–mass spectrometry (GC-MS-QP2010 Ultra, Shimadzu), and ethylene with a gas chromatography (GC-2014). The selection of GC or GC-MS was based on the concentrations of PCE and its intermediate products to be analyzed. GC and GC-MS were used to analyze PCE and its intermediate

products when their concentrations were greater than 1 mg/L, and less than 1 mg/L, respectively. Capillary columns of GS-Q (0.53 mm diameter, 30-m length; Agilent Technologies, Santa Clara, CA, USA) and RTX-624 (0.25 mm diameter, 60-m length, Restek, Bellefonte, PA, USA) were used for the separation in GC and GC-MS, respectively. For the GC analysis, the column temperature was maintained at 80 °C for 1.0 min, then increased to 220 °C at a speed of 35 °C per min, and maintained at 220 °C for 2.0 min. A 25–200 μ L aliquot of headspace gas was withdrawn from each test bottle and injected to the GC manually. For the GC-MS analysis, the column temperature was maintained at 100 °C for 3.5 min, increased to 170 °C at a speed of 20 °C per min. A 25–100 μ L aliquot of headspace gas was withdrawn from each test bottle and injected to the GC-MS manually. We calculated concentrations in the culture solution using the Henry's law constant (Mackay and Shiu 1981). The Henry's law constants for the PCE, TCE, *cis*-DCE, VC, and ethylene at 25 °C were 0.93, 0.48, 0.31, 0.95, and 8.76, respectively. Because the constants are sensitive to the temperature, the room temperature was continuously controlled at 25 °C during the analyses. The detection limit for the chloroethylenes with GC-MS were 0.001 mg/L. This was equivalent to 0.001, 0.001, 0.002, and 0.003 μ mol/bottle for PCE, TCE, *cis*-DCE, and VC, respectively, whereas that of ethylene analyzed with GC was 0.028 mg/L, equivalent to 0.20 μ mol/bottle. To ensure the precision of gas analyses, the linearities of calibration curves were confirmed to be $R^2 > 0.97$ and $R^2 > 0.99$ for the GC and GC-MS analyses, respectively.

2.3 Changes in Microbiological Properties

2.3.1 Extraction of RNA and Synthesis of cDNA

We periodically extracted RNA to assess the microbial communities as active microbial assemblies. The pellet collected with the centrifugation described in Section 2.2.1 was soaked in RNAlater™ Stabilization Solution (Thermo Fisher Scientific, Waltham, MA, USA) and stored at –80 °C until RNA extraction. Extraction was carried out using NucleoSpin® RNA (Macherey-Nagel, Düren, Germany), followed by additional DNA digestion with DNA-free™ Kit (Thermo Fisher Scientific). cDNA was synthesized from the RNA extract with PrimeScript™ 1st strand cDNA

Synthesis Kit (Takara Bio, Kusatsu, Japan) containing random 6-mer primers.

2.3.2 Next-Generation Sequencing-Based Amplicon Sequencing

Microbial communities were determined with next-generation sequencing (NGS)-based amplicon sequencing of V4 region of 16S rRNA transcripts. The region of the cDNA was amplified with universal primers containing an adapter sequence for Illumina sequencer, 515F and 806R (Caporaso et al. 2012) and PrimeSTAR® GXL DNA Polymerase (Takara Bio, Kusatsu, Japan). Following the purification of the first PCR products with magnetic beads, specifically AMPure XP beads (Beckman Coulter, Brea, CA, USA), products were applied to a second PCR to add index sequences. Purified second PCR products were sequenced 300-bp paired end with Illumina Miseq (Illumina, San Diego, CA, USA). We performed data processing of the sequence data using QIIME 1 (Caporaso et al. 2010) and mothur (v.1.42.3) (Schloss et al. 2009). Non-chimeric sequences obtained from the QIIME 1 procession were grouped into operational taxonomic units (OTUs, 97% similarity) with the mothur program. As reference taxonomy to affiliate taxonomy of the sequences, EzBioCloud 16S database (Yoon et al. 2017) was applied to the sequences.

3 Results

3.1 Fermentation of Organic Compounds

The 10.2 mmol/L of lactate in the culture solutions in the PCE-2, PCE-6, PCE-30, and PCE-75 tests immediately reduced, and the concentration was under the detection limit within 3 days (Fig. 1a). PCE-125 had a slower reduction, taking 6 days to achieve a concentration under the detection limit (Fig. 1a). Alongside lactate reduction, increments in acetate and propionate concentrations occurred (Fig. 1 b and c). We observed an increment of acetate from 10.4 to 13.7, 13.9, 13.7, and 13.4 mmol/L in PCE-2, PCE-6, PCE-30, and PCE-75, respectively, during days 0–3 (Fig. 1b). The concentration of acetate in PCE-125 at day 3 was 12.0 mmol/L, lower than that under other test conditions, and then increased to 13.9 mmol/L at day 6. The increment of propionate had a similar tendency to that of acetate (Fig.

1c). The concentrations of propionate in PCE-2, PCE-6, PCE-30, and PCE-75 had increments from 2.4 to 8.2, 8.2, 8.4, and 8.2 mmol/L, respectively, during days 0–3. Concentrations of propionate in PCE-125 were only 4.7 mmol/L at day 3, which then increased to 8.3 mmol/L during days 3–6.

3.2 Sulfate Reduction

The concentrations of SO_4^{2-} in culture solutions reduced from 0.8 to 0.03 mmol/L during days 0–6 in PCE-2, PCE-6, and PCE-30 tests (Fig. 2). In PCE-75, SO_4^{2-} reduction was observed later, during days 3–9. The culture solution of PCE-125 kept the concentration of SO_4^{2-} around 0.8 mmol/L until day 24, then the test showed the reduction of SO_4^{2-} within the following 10 days.

3.3 Anaerobic Microbial Dechlorination of PCE

PCE dechlorination occurred in the tests of PCE-2, PCE-6, and PCE-30 (Fig. 3a–c). PCE was sequentially dechlorinated; TCE, *cis*-DCE, and VC were produced. Ethylene production was then confirmed in the PCE-2 and PCE-6 tests over the 34 days of the experiment. PCE dechlorination to TCE was observed within the initial 6 days in both PCE-2 and PCE-6 tests, while slower PCE dechlorination continued in PCE-30 test until day 24. The dechlorination rates of PCE were 0.33, 0.60, and 0.19 $\mu\text{mol/bottle/day}$ in PCE-2, PCE-6, and PCE-30 tests, respectively. When tests were performed with 75 and 125 mg/L of PCE in PCE-75 and PCE-125, PCE dechlorination did not occur within the experimental time frame (Fig. 3 d and e). The dechlorination of PCE did not occur in the sterile control bottles too (data not shown). The mass balance between VC and ethylene was not obvious (Fig. 3). One possible decomposition product, specifically ethane, of VC, was also analyzed but not detected during our experiments. This means that there would be unknown decomposition pathways and byproducts that were involved in the degradation reactions, and future research should further develop and confirm this phenomenon.

3.4 Microbial Diversity Based on Transcripts of 16S rRNA

Microbial communities based on cDNA at genus level with 29,853 sequences in each community are shown in Fig. 4. *Anaerotignum* accounted for a large percentage

Fig. 1 Changes in concentrations of organic acids over the test period, **a** lactate, **b** acetate, and **c** propionate, with different initial concentrations of PCE. The error bars show standard errors

Fig. 2 Sulfate reduction under the conditions with different initial concentrations of PCE. The error bars show standard errors

of the microbial communities in the PCE-2, PCE-6, PCE-30, and PCE-75 culture solutions during days 1–3. The genus was also predominant in microbial communities in PCE-125 culture solution during days 3–9. The proportion of *Desulfosporosinus* increased up to 19.0, 16.4, and 28.0%, in PCE-2, PCE-6, and PCE-30 tests on day 3, respectively. As for PCE-75 and PCE-125 tests, the genus increased to 27.4% in PCE-75 culture solution on day 6, and 24.5% in PCE-125 culture solution on day 24. The genera of *Sulfurospirillum*, *Desulfitomonas*, and *Geobacter* were not observed in any of the communities. The genus *Desulfitobacterium* was observed in relatively low ratios from 0.01–0.04, 0.01–0.03, 0.02–0.05, 0.03–0.12, and 0.07–0.34%, in PCE-2, PCE-6, PCE-30, PCE-75, and PCE-125 culture solutions during days 1–24, respectively. More specifically, the ratios of genus *Desulfitobacterium* were 0.02, 0.02, 0.04, 0.07, and 0.08% in day 9, and 0.02, 0.01,

Fig. 3 Anaerobic dechlorination of PCE under different initial concentration conditions. The initial concentrations were **a** 2 mg/L (2.4 μmol/bottle), **b** 6 mg/L (7.1 μmol/bottle), **c** 30 mg/L

(35.5 μmol/bottle), **d** 75 mg/L (88.7 μmol/bottle), and **e** 125 mg/L (147.9 μmol/bottle), respectively. The error bars show standard errors

0.03, 0.10, and 0.16% in day 24, in PCE-2, PCE-6, PCE-30, PCE-75, and PCE-125 culture solutions, respectively. The abundance of genus *Desulfitobacterium*

in the PCE-125 culture solution was higher than that in any other culture solution with a lower PCE concentration.

Fig. 4 The relative abundance of different microbial genus over-time based on cDNA amplicon sequencing. Initial PCE concentrations of PCE-2, 2 mg/L (2.4 $\mu\text{mol/bottle}$); PCE-6, 6 mg/L (7.1 $\mu\text{mol/bottle}$); PCE-30, 30 mg/L (35.5 $\mu\text{mol/bottle}$); PCE-75,

75 mg/L (88.7 $\mu\text{mol/bottle}$); and PCE-125, 125 mg/L (147.9 $\mu\text{mol/bottle}$). The less abundant genus (<1%) were integrated into others

The structures of *Anaerotignum*, *Desulfosporosinus*, and *Desulfobacterium* are shown in Fig. 5. The genus *Anaerotignum* included two major OTUs; OTU0001 and OTU0008 (Fig. 5a). The OTU0001 representative over 69.6% of relative abundance of *Anaerotignum* in the cDNA communities of PCE-2, PCE-6, PCE-30, and PCE-75, while the microbial community of PCE-125 on day 6 contained 45.2% of OTU0001 and 54.3% of OTU0008. For *Desulfosporosinus* and *Desulfobacterium*, OTU0004 and OTU0036, respectively, accounted for more than 90.5% of the relative abundance of the genus (Fig. 5 b and c).

4 Discussion

4.1 Effects of High PCE Concentrations on Lactate Fermentation

Lactate reduction and the increment of cDNA abundance of *Anaerotignum* were induced simultaneously (Figs. 1 and 4). The representative sequence of *Anaerotignum* OTU0001 (Fig. 5) in this study was similar to *Anaerotignum propionicum* JCM1430 (Collins et al. 1994; Ueki et al. 2017) and *Anaerotignum neopropionicum* DSM3847 (Ueki et al. 2017) with 100.0% identity. The strains JCM1430 and DSM3847 are gram-positive and spore-forming bacteria, which ferment lactate to acetate and propionate (Tholozan et al. 1992; Ueki et al. 2017). It is reasonable

that *Anaerotignum* was one of the fermenters in this study. Molecular hydrogen is produced during the fermentation process of organic acids including lactate, assisting with the anaerobic dechlorination of chlorinated ethylenes (Parsons 2004). In terms of hydrogen production, adequate conditions for production may have occurred when there was a PCE concentration of 75 mg/L or lower. Lactate fermentation and increments in the relative abundance of *Anaerotignum* was delayed for 3 days under a PCE concentration of 125 mg/L compared to other concentrations (Figs. 1 and 4). Furthermore, with a PCE concentration of 125 mg/L, *Anaerotignum* OTU0008 accounted for a higher percentage on day 6, compared to other concentrations (Fig. 5). One possible reason for the continued fermentation under a PCE concentration of 125 mg/L may be due to fermentation by a more PCE-tolerant strain, *Anaerotignum* OTU0008, compared to *Anaerotignum* OTU0001.

4.2 Impact of High PCE Concentrations on Sulfate Reduction

The decreasing SO_4^{2-} and increasing proportion of the genus *Desulfosporosinus* occurred concurrently when PCE concentrations were between 2 and 75 mg/L (Figs. 2 and 4). The obtained sequence of *Desulfosporosinus* OTU0004 was close to *Desulfosporosinus meridiei* S5 (Robertson et al. 2001) with 99.6% of identity. The strain is gram-negative/

Fig. 5 The relative abundances of the top three OTUs overtime classified into **a** *Anaerotignum*, **b** *Desulfosporosinus*, and **c** *Desulfitobacterium*. PCE-2, 2 mg/L (2.4 $\mu\text{mol/bottle}$) of initial concentration of PCE; PCE-6, 6 mg/L (7.1 $\mu\text{mol/bottle}$) of initial concentration of PCE; PCE-30, 30 mg/L (35.5 $\mu\text{mol/bottle}$) of

initial concentration of PCE; PCE-75, 75 mg/L (88.7 $\mu\text{mol/bottle}$) of initial concentration of PCE; and PCE-125, PCE-125 with 125 mg/L (147.9 $\mu\text{mol/bottle}$) of initial concentration of PCE. The less abundant genus (< 1%) were integrated into others

gram variable and sulfate-reducing bacteria, originated from groundwater contaminated with organic solvents including benzene, toluene, ethylbenzene, and xylene (BTEX). This suggests that the *Desulfosporosinus* observed in this study was a sulfate reducer, under the existing PCE concentrations. The *Desulfosporosinus* activity and sulfate reduction were interfered at PCE concentrations over 75 mg/L. As the concentration increased, a longer lag time for starting sulfate reduction was needed.

As opposed to *Anaerotignum*, the fermenter, the same *Desulfosporosinus*, OTU0004 was activated despite the PCE concentration (Fig. 5b). The results indicate that the same species of *Desulfosporosinus* may adapt to high PCE concentrations after a lag time. As reported with gram-negative bacteria, there may be some mechanisms that can be considered for adaptation. For example, modifying the cell membrane to decrease the permeability of the solvent, activating solvent efflux pumps to reduce accumulation of

the solvent in a cell, and inactivating the solvent with an enzymatic reaction (Sardessai and Bhosle 2002; Torres et al. 2011).

4.3 Constraints in Anaerobic Microbial Dechlorination of PCE at High PCE Concentrations

Anaerobic PCE dechlorination was inhibited when PCE concentrations were above 30 mg/L and completely interfered when PCE concentrations were above 75 mg/L (Fig. 3). The solubility of PCE at 20 °C is about 150 mg/L. This means that bioremediation is basically not applicable to remediate the source zone and its surrounding areas where concentrations of PCE are higher than 30 mg/L. However, the bioremediation can be effectively used to remediate the groundwater contaminated plume where the concentrations of PCE are less than 30 mg/L. Other technologies should be adopted to clean up the source zone and its surrounding areas, but discussion on this topic is beyond the scope of this paper.

This study was carried out with a microbial consortium, though the results were similar to those tested with PCE dechlorinating isolates (Amos et al. 2007). Amos et al. (2007) found that the dechlorination of PCE for PCE concentrations above 90 mg/L had ceased when they tested with *Sulfurospirillum multivorans*, *Desulfuromonas michiganensis* BB1, *Geobacter lovleyi* SZ, and *Desulfitobacterium* sp. Viet1. The representative sequence of *Desulfitobacterium* OTU0036 (Fig. 5c) was similar to known dechlorinating strains, *Desulfitobacterium* sp. Viet1 (100.0%, Löffler et al. 1997), *Desulfitobacterium hafniense* PCE-S (99.2%, Miller et al. 1997), and *Desulfitobacterium hafniense* Y51 (99.2%, Suyama et al. 2001). The cDNA-based communities in this study did not contain similar species to known PCE degrading strains of *S. multivorans* (Neumann et al. 1994; Scholz-Muramatsu et al. 1995), *Desulfuromonas chloroethenica* (Krumholz 1997), *D. michiganensis* (Sung et al. 2003), and *G. lovleyi* (Sung et al. 2006). Considering previous studies, one possible dechlorinating bacterium in this study could be *Desulfitobacterium*. The genus of *Desulfitobacterium* can also utilize the sulfate-reducing product, sulfite, as an electron acceptor (Christiansen and Ahring 1996). Our results suggest that anaerobic dechlorination is constrained with high PCE concentrations even when dechlorinating microorganisms were alive with other electron acceptors. Future studies need to be performed

to investigate the expression of the functional genes encoding PCE dehalogenase and sulfite reductase. The outcomes of such studies will facilitate better understanding on the mechanisms of inhibition of dechlorination associated with high PCE concentrations.

5 Conclusions

We found that the inhibition of PCE dechlorination with high PCE concentrations occurs even when synergetic anaerobic reactions of fermentation and sulfate-reduction proceed. Among fermenters and sulfate reducers, strategies to overcome the toxicity of PCE were observed; different species of fermenters, *Anaerostignum*, were activated, while the same species of sulfate-reducer, *Desulfosporosinus*, adapted to the high concentrations after a lag time. However, we did not observe such strategies on dechlorinating bacteria during the experiment. To perform successful bioremediation of PCE under concentrations over 30 mg/L, the bioaugmentation of dechlorinating microorganisms tolerant to high concentrations of PCE is considered as one possible solution.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that there is no conflict of interest.

Code Availability Not applicable.

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