ANAEROBIC BIOTRANSFORMATION OF TCE BY HETEROTROPHIC ENRICHMENT CULTURES

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Abstract

The anaerobic dechlorination of trichloroethylene (TCE) was investigated in a treatability study using two different sewage sludges and TCE-contaminated soil used in the bioreactor. Methanol and hydrogen were selected to serve as electron donors for the *ex-situ* bioremediation processes. The TCE biodegradation tests were evaluated at two temperatures: 20°C and 30°C. The results obtained in the sewage experiments demonstrated faster TCE dechlorination rate in the first stage (TCE DCE) but the second stages (DCE VC ETH) of the dechlorination process were much slower. The addition of electron donors was found to significantly enhance the TCE biotransformation. However, non-examined parameters showed clear influence on TCE dechlorination in the tested samples. TCE dechlorination coursed in the way independent of electron donors and temperatures used. In the case of the bioreactor TCE contaminated soil completely TCE biodegradation in short time was observed. During the first 2 weeks of the incubation, high decrease of TCE (< 98%) was detected. The TCE decrease were correlated with significant increase of intermediates, e.g., *cis*-1,2-DCE, trans-1,2-DCE, 1,1-DCE, VC, and ethene. Results provide information, which may serve as a basis for determining the physical-chemical and microbiological parameters for the bioremediation processes at TCE contaminated sites.

Introduction

One of the most common environmental problems in most of industrialized countries is soil and groundwater contamination with volatile chemical solvents classified as Volatile Organic Compounds (VOCs) (1). Chlorinated solvents such as tetrachloroethene (PCE), trichloroethene (TCE), trichloroethane (TCA) and carbon tetrachloride (CT) are commonly used as degreasing agents at manufacturing, maintenance and service facilities all over the world and casually released into the environment, particularly to soils and groundwaters. Among the 129 priority pollutants promulgated by US EPA, 31 are halogenated compounds (2). Due to their suspected carcinoogenicity and ubiquitous contamination, an urgent need for understanding the fate, transport and transformation of such chemicals has arisen in the recent years. Several mechanisms for microbial biodegradation of chlorinated ethenes have been demonstrated in both laboratory and field studies. Anaerobic dechlorination has been proven to be an efficient method for complete PCE and TCE biotransformation to ethene under optimal conditions (1, 3).

This project tested the accelerated anerobic dechlroination of TCE by different innoculums under various environmental conditions.

Methods

Selection and characterization of biological materials

Sewage sludges from two sites and TCE contaminated soil were selected for the experiment (4). The materials were characterized for physico-chemical and microbiological parameters according to ISO standards and methods described by Alef et al. (5).

Microcosms study

Experiment with sewage sludge samples

The methods of Lee et al. (6) and Tokunaga et al. (7) were combined to perform the experiment. Two temperatures – 20°C and 30°C, and methanol and H_2 as electron donors were evaluated. The following combinations were prepared: **I**: S1+S2 + methanol + TCE – 20°C; S1+S2 + methanol + TCE - 30°C; **II**: S1+S2 + hydrogen + TCE – 20°C; S1+S2 + hydrogen + TCE - 30°C; **C I** (control I - abiotic): S1+S2 (autoclaved) + TCE - without methanol and hydrogen – 20°C, S1+S2 (autoclaved) + TCE - without methanol and hydrogen - 30°C; **C II** (control II): S1+S2 – without TCE, methanol and hydrogen – 20ºC; S1+S2 – without TCE, methanol and hydrogen - 30°C. Each set of the experiment was prepared in triplicate. Measurements were performed at the beginning of the experiment and then at day 7-49 days, until total removal of TCE was reached. Changes in TCE concentrations and daughter products were evaluated during 207 days of the experiment period.

Experiment with TCE contaminated soil

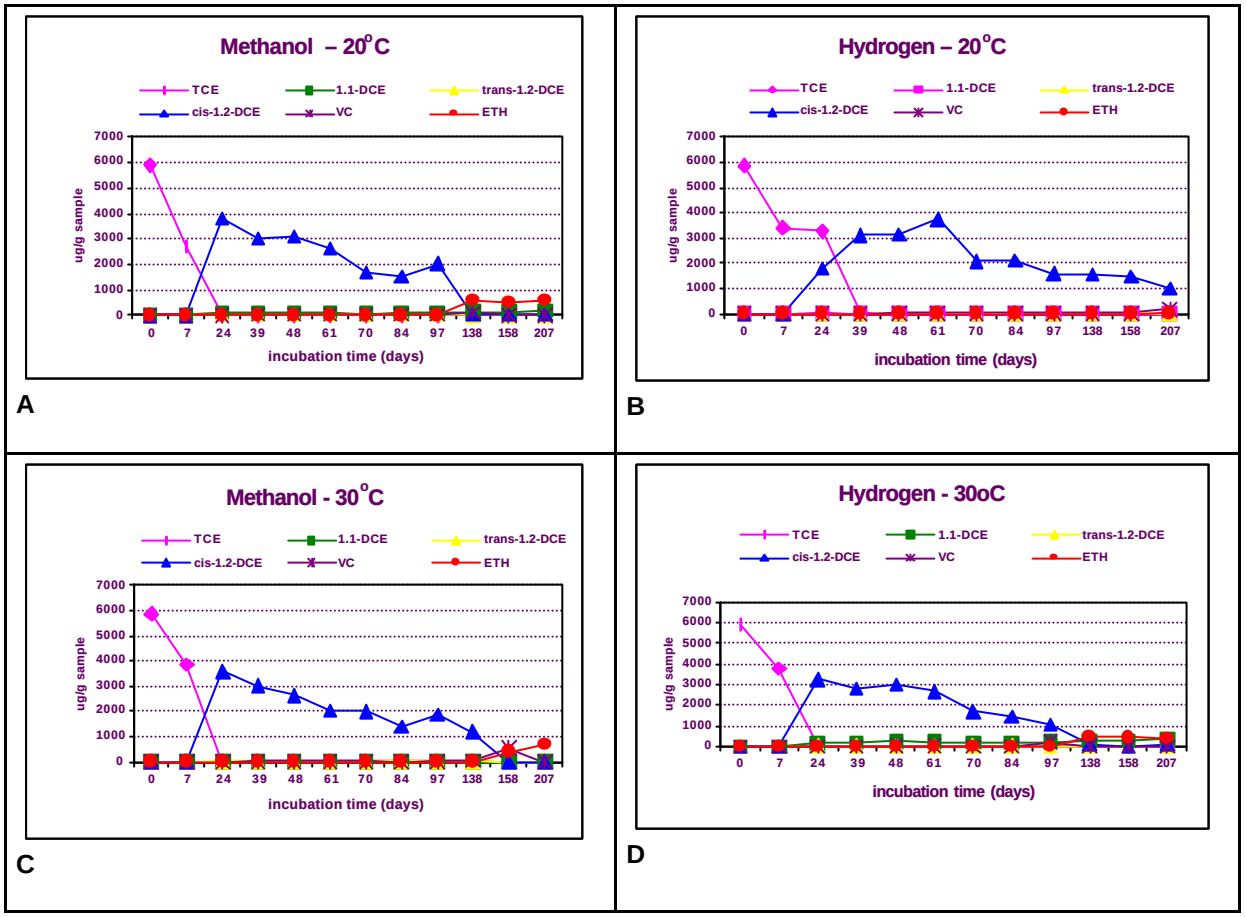
Seven grams of TCE-contaminated soil from the bioreactor (4) were used as inoculum for TCE dechlorination experiment that was carried out in the similar way as described by Lee et al. (6) and Doong and Wu (8). Methanol was used as electron donor. The experiment was performed in 20ºC. All tests were performed in triplicate.

Results

Experiment with sewage sludge samples

The results of the experiment with sewage sludge samples are presented in Figure 1 demonstrating was anaerobic TCE dechlorinat during the 207 day test. The formation of byproducts was observed only in the presence of living sludges, not autoclaved controls. Nearly complete TCE dechlorination was observed within 39 days of all microcosms. TCE removal was correlated with a simultaneous increase of DCE isomers, especially 1,2-*cis*-DCE and VC. Trace concentrations of subsequent DCE isomers, e.g. 1, 1-DCE and trans-1, 2-DCE were observed in all living cultures. However, as the incubation continued, lower chlorinated ethenes (the DCE isomers and VC) started to accumulate, albeit in very small amounts. Further measurements showed very slow course of the DCE to VC dechlorination process. At the same time only minute quantities of ETH were observed. A measurement made in 138th day of the experiment showed TCE dechlorination progress. A significant raise of VC and ETH concentrations was observed. Subsequent measurements showed the faster dechlorination rates in the DCE VC step. However, in samples with methanol as electron donor dechlorination rate were faster in DCE-VC and VC-ETH steps. The significant decrease of *cis*-1,2-DCE and ETH production were observed (Fig. 1C). During the experiment, the total amount of measured TCE byproducts did not balance the amount of TCE removed. TCE adsorbed onto the biomass as determined with the autoclaved (control) sludges. TCE dechlorination process proceeded independent of electron donors and temperatures tested (Fig.1). No significant influences of two electron donors (methanol and hydrogen) and temperatures (20ºC and 30°C) applied were observed on TCE biodegradation rates.

Figure 1: TCE, DCE, VC and ETH concentrations vs. time with methanol (A and C) and hydrogen (B and D) as electron donor in two temperatures: 20°C (A and B) and 30°C (C and D)



Experiment with TCE contaminated soil

The results of the experiment are presented in Table 1. During the first 2 weeks of incubation high decrease of TCE (< 98%) was observed, and intermediates byproducts including *cis*-1,2-DCE, trans-1,2-DCE, 1,1-DCE and VC were produced. TCE biodegradation rate was 378 µg/day in the first days of the incubation period. Traces of ethene were also observed. *Cis*-1, 2-DCE concentration was much higher than other byproducts and its significant decrease was observed during the next weeks of the incubation time (Tab. 1). Both TCE and *cis*-1, 2-DCE concentrations were depleted during the next sampling events at days 60 and 80. These decreases were correlated with significant increase of intermediates, e.g., 1,1-DCE and VC, and ethene. The VC production after 60 days was the highest (Tab. 1). Trans-1,2-DCE and 1,1-DCE were also transformed but at a much lower rate. A measurement made in 100th day of the experiment showed the progress in TCE dechlorination. After this time, TCE concentration was almost zero, small amounts of *cis*-1,2-DCE trans-1,2-DCE, VC were observed but the ETH concentration was much higher, and still increased in the next measurements (Tab.1).

Table 1: Changes of TCE and byproducts concentrations during the incubation period in the experiment with TCE contaminated soil.

Days	**TCE** ìM	***cis*-1,2-DCE** ìM	**trans-1,2-DCE** ìM	**1,1-DCE** ìM	**VC** ìM	**ETH** ìM
14	1,6	322	0,27	2,05	0,3	0,03
40	1,23	243,5	0,48	4,2	0,66	0,02
60	0,8	63	0,46	3,64	248,5	11,07
80	0,11	2,6	0,34	3	156,8	123,6
100	0,034	3,23	0,018	1,06	4,09	337,5
120	0,00	0,37	0,00	0,17	0,07	375

Discussion

This research showed that anaerobic TCE dechlorination was evident in combined sewage sludge samples and TCE contaminated soil. TCE was dechlorinated by sequential dehalogenation, first c-DCE and VC as intermediates and ethene produced as an end product (9). Ethane was not observed. In the sewage sludge samples the dechlorination process was slowly probably due to adaptation of microorganisms to TCE. Nearly complete TCE dechlorination to cDCE was observed after 50 days of the incubation period. Dechlorination rate was higher in the first stage of the process, e.g., TCE DCE. The next stages, DCE VC ETH were slower. The chlorinated ethenes were not dechlorinated in the presence of autoclaved sludge, indicating that the reactions were only catalyzed by living organisms. However, it was interesting that in the experiments described here small amounts of 1,1-DCE were formed. The formation of 1,1-DCE may have been caused by an abiotic reaction mediated by sulfides present in the medium (or sludge) (10, 11). No significant influences of two electron donors, i.e., methanol and hydrogen, and temperatures were observed in the batch experiment with sewage sludges beyond some acceleration of initial TCE dechlorination (Fig. 1). The lack of influences by electron donors and temperatures on the TCE biodegradation process were most likely due to the fact that sewage sludges used were rich in complex inorganic and organic compounds. However, the results found in this research indicated that methanol was a much better electron donor so it was used in TCE bioreactor. Why methanol is often the most effective source of reducing power remains unclear. DiStefano et al. (12) demonstrated that hydrogen was the direct electron donor used for dechlorination, thereby suggesting that methanol merely functioned as precursors for formation of an intermediate hydrogen pool.

In the case of TCE contaminated soil the degradation capacity was higher and metabolically active as the microbial community was adapted to the TCE dechlorination process. Isolated cultures from contaminated soil revealed the highest and stable TCE dechlorinating activity and are affiliated with different phylogenetic groups and have diverse physiological properties (13). The adaptation period, enhanced the capability of the consortium to dechlorinate TCE. The mixed culture was then acclimated to TCE dechlorination and production of the enzymes involved in this process. Reductive dehalogenation appears to be the predominant dechlorination pathway of chloroethenes found in the tested samples. The results obtained are compared to those described in the literature (13-15). Sewage sludges and TCE contaminated soil contain microorganisms which could be applied in remediation of TCE contaminated sites. However, microorganisms adapted to TCE have higher biodegradation potential. Bioremediation processes based on the activities of indigenous anaerobes are important in clean-up processes of TCE contaminated sites (natural attenuation).

This research is particularly important to control over dehalogenation *ex situ* or *off site* for the treatment of halogen-contaminated soils and waters. Further elucidation of the biochemical mechanisms of dehalorespiration is necessary in order to completely understand the process carried through bioremediation

Conclusions

1. The TCE biodegradation test with contaminated soil and sewage sludges revealed that indigenous TCE dechlorinating bacteria were in the examined samples.
2. In the sludge experiment, nearly complete TCE dechlorination was observed after 50 days of incubation. TCE removal was correlated with a simultaneous increase of DCE isomers, especially 1,2-cis-DCE and VC. The results showed faster dechlorination rate at the TCE DCE stage as compared with the DCE VC ETH stages of the process.
3. No influences of two electron donors, i.e., methanol and hydrogen, and tested temperatures were observed in the batch experiment with sewage sludges. TCE dechlorination proceeded independent of electron donors and temperatures tested. No significant influences of electron donors and temperatures on the TCE biodegradation process were due to that sewage sludges used were rich in complex inorganic and organic substances.
4. Complete TCE dechlorination under anaerobic conditions in a very short time was observed in batch experiments with TCE contaminated soil. The TCE and *cis*-1,2-DCE decreases were correlated with significant increase of the intermediates, e.g., 1,1-DCE, VC and ETH.
5. Microbial community found in the TCE contaminated soil should be characterized to determine the bacteria responsible for TCE dechlorination.
6. Results provide significant information on TCE biodegradation rates that may serve as a basis for determining physico-chemical and microbiological parameters for the bioremediation at other contaminated sites.

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