Degradation of TCE Using Sequential Anaerobic Biofilm and Aerobic Immobilized Bed Reactor

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ABSTRACT

Bacteria capable of degrading Trichloroethylene (TCE) were isolated from contaminated wastewaters and soil sites. The aerobic cultures were identified as *Pseudomonas aeruginosa* (four species) and *Pseudomonas fluorescens*. The optimal conditions for the growth of aerobic cultures were determined. The minimal inhibitory concentration values of TCE for *Pseudomonas* sps. were also determined. The aerobic cells were immobilized in calcium alginate in the form of beads. Degradation of TCE by the anaerobic and dichloroethylene (DCE) by aerobic cultures was studied using dual reactors - anaerobic biofilm and aerobic immobilized bed reactor. The minimal mineral salt medium saturated with TCE was pumped at the rate of 1 ml per hour into the anaerobic reactor. The MMS medium saturated with DCE and supplemented with xyelens and toluene (3 ppm each) was pumped at the rate of 1 ml per hour into the fluidized air-uplift-type reactor containing the immobilized aerobic cells. The concentrations of TCE and DCE and the metabolites formed during their degradation by the anaerobic and aerobic cultures were monitored by GC. The preliminary study suggests that the anaerobic and aerobic cultures of our isolates can degrade TCE and DCE.

INTRODUCTION

Trichloroethylene (TCE) is a volatile chlorinated organic compound which is used extensively as a solvent and degreasing agent (Storck, 1987). TCE and other chlorinated wastes have been shown to migrate through soils from disposal sites and threaten groundwater aquifers across the nation (Folsom *et al.*, 1990). The U.S. Environmental Protection Agency has classified TCE as a priority pollutant on the basis of its ubiquity, suspected carcinogenicity and propensity to be anaerobically degraded to vinyl chloride in groundwater (U.S. Environmental Protection Agency, 1980). Due to its widespread contamination of soil and water and its potential health threat, TCE has received much attention recently.

The application of bioremediation to solve environmental problems has increased tremendously in the last 15 to 20 years (Wackett and Gibson, 1988; Tsien *et al.*, 1989; Zylstra *et al.*, 1989; Harker and Kim, 1990; Folsom *et al.*, 1990; Folsom and Chapman, 1991; Malachowsky *et al.*, 1994). Several pure and mix cultures which possess the ability to act on TCE have been isolated and characterized from different ecosystems contaminated with these compounds (Fliermans *et al.*, 1988; Baek, N.H., and P.R. Jaffe, 1989; Folsom *et al.*, 1990; Folsom and Chapman, 1991; Alvarez-Cohen *et al.*, 1992; Malchowsky *et al.*, 1994). Bouwer and McCarty (1983) reported that the chlorinated alkenes may be converted anaerobically by reductive dehalogenation to known carcinogens such as vinyl chloride. Similarly tropic enrichment cultures can degrade TCE aerobically in the presence of certain aromatic compounds such as toluene or phenol (Nelson *et al.*, 1987). Keeping the above ongoing research in mind, a preliminary study was carried out to study the degradation of TCE by anaerobic and aerobic cultures in continuous bioreactors.

MATERIALS AND METHODS

Chemicals

Trichloroethylene, Dichloroethylene of 99% purity were purchased from Aldrich Chemical Company. Toluene and xylenes were purchased from Curtin Matheson Scientific Company, Kennesaw, GA. The gases were puchased locally. All other chemicals were obtained from Sigma Chemical Company Co., St. Louis, MO.

Media and Culture Conditions

The sterile minimal minaral salt (MMS) medium (pH 7.0) used for the isolation of aerobic microorganisms capable of degrading TCE in the presence of, xylenes and toluene contained the following (g/L): K_2HPO_4, 4.3; KH_2PO_4, 3.4: $MgCl_2.H_2O$, 0.3, NH_4Cl, 1.0, the medium was amended with 0.5 ml of the trace element solution containing the following (mg/L): $MnCl_2.4H_2O$, 1.0; $FeSO_4.7H_2O$, 0.6; $CaCl_2.H_2O$, 2.6; $NaMoO_4.\ 2H_2O$, 6.0.

MMS plates were prepared by adding 15 g of agar (Difco Laboratories, Detroit, MI) to 1 liter of the medium. Unless otherwise stated, the microorganisms were grown in the medium containing xylenes and toluene as the sole source of carbon and energy with trace amount of TCE.

Isolation and Identification of Cultures

The bacteria capable of degrading trichloroethylene in the presence of xylene and toluene were isolated from contaminated soil and water samples collected from industrial sites. A 1:10 dilution of each sample was made with sterile MMS medium and the suspension was incubated at room temperature for 1 h. One milliliter of the suspension was inoculated into sterile Pyrex test tubes containing 9 ml of the medium supplemented with different concentrations of TCE (ranging from 25 ppm to saturation concentration) and xylenes and toluene (3 ppm each). The tubes were then incubated at 25°C for 7 days and examined for turbidity. After 5 to 7 transfers, the turbid samples were streaked onto plates containing TCE and xylenes and toluene as sources of carbon and energy. Colonies that grew on agar plates containing TCE and xylenes and toluenes but not on control plates were then selected for identification.

Identification of the Bacteria

Identification of bacteria was based on the classification scheme described in *Bergey's Manual of Systematic Bacteriology* (Palleroni, 1984). Tests were performed as described in the manual (Palleroni, 1984) or as described by Smibert and Krieg(1981). Subsequently, the isolates were biochemically characterized by using commercially available diagnostic kits from the API 20E test kit (Analytab Products, Plainview, N.Y.). The identification was reconfirmed using Biolog MicroID system in Biology Department, University of Alabama, Birmingham, AL.

Growth Medium for Anaerobic Cultures

The basic growth medium for anaerobic cultures contained the following (mg/L): $(NH_4)_2HPO_4$, 80; NH_4Cl, 1000; K_2HPO_4, 200; NaCl, 10; $CaCl_2$, 10; $MgCl_2$, 50; $CoCl_2.6H_2O$, 1.5; $CuCl_2.2H_2O$ 0.2; $Na_2MoO_4.H_2O$, 0.23; $ZnCl_2$, 0.19; $NiCoO_4.6H_2O$, 0.2; $FeSO_4.7H_2O$, 1.0; $AlCl_3.6H_2O$,0.4; H_3BO_3, 0.38; Biotin, 0.02; Folic acid, 0.02; Pyridoxine-HCl, 0.1 Riboflavin, 0.05; Thiamine, 0.05; Nicotinic acid, 0.05; Pathothenic acid, 0.05; Vitamin B_{12}, 0.0001; p-aminobenzoic acid, 0.2 and Resazurin, 1.0; supplimented with 0.5% carbon source (Glucose) and saturated with TCE.

Optimal Conditions

The optimal temperature for the growth of the bacteria was determined by inoculating 50 ml of the MMS medium containing TCE and 3 ppm of xylenes and toluene into 250 ml Pyrex flasks, which were then incubated at 5, 15, 25, 35, 45, and 55°C. Growth was monitored after 72 h of incubation. The optimal pH was determined by inoculating the medium adjusted to pH 3.0 to 9.0. The inoculated cultures were then incubated at 25°C. The growth was measured after 72 h of incubation.

Minimal Inhibitory Concentrations (MIC)

Since TCE is highly toxic and can not be used as a carbon source, it is necessary to determine the levels at which the experiments could be carried out without greatly affecting the organisms. The minimal inhibitory concentration values of TCE for the isolates of *Pseudomonas* sp.s were determined by conducting the experiments in 100 ml Qorpak bottles filled with MMS medium supplemented with different concentrations of TCE (10 - 2500 ppm) and 3 ppm of xylenes and toluene as a carbon source and also inducers of mono- and dioxyganase enzymes. The medium was inoculated with 1 ml of cell suspension (A_{660} 1.0) of xylenes- and toluene-utilizing cultures. The experiment was run at 25°C for 120 h and the MIC values of TCE for the isolates were determined by monitoring the bacterial growth. The MIC is defined as the lowest concentration of the inhibitor above which no growth is observed.

Immobilization of Aerobic Cultures in Alginate

Aerobic mix culture consortium was harvested from the MMS medium supplemented with constant saturation of toluene and xylenes at exponential phase by continuous centrifugation at 15,000 x g and the pellet was washed three times with 25 mM phosphate buffer. The cell paste (300-400 mg/100 ml) suspended in 100 ml of 0.85% normal saline was mixed with 100 ml of sterile 4% sodium alginate. The alginate-cell mixture was extruded dropwise through a 25-gauge needle from a height of about 20 cm into cold 0.2 M $CaCl_2$. Each drop was hardened into a bead containing entrapped cells. The beads were further hardened by stirring them in $CaCl_2$ for 30 min and stored at 5°C for 24 h.

Optimal Conditions

Maximum growth was obtained after 120 h of incubation at 25-30°C. No growth was observed at 55°C. The isolate was found to grow well between pH 6.0 and 7.0 . However, maximum cell growth was obtained at pH 6.5 and 7.0. No growth was observed below pH 5.0 or above pH 7.0.

Immobilization

The typical yield following immobilization of cells with alginate was about 0.6-0.8 g of beads/ml of cell-alginate suspension. Individual beads had a diameter of 1-2 mm and an average wet and dry wt of 13 and 0.6 mg, respectively. At the time of immobilization, each bead contained approximately 1.5-2.5 x 10^8 viable cells, as determined by pour plate method of disrupted beads (O'Reilly and Crawford, 1989).

Minimal Inhibitory Concentration Values of TCE

The minimal inhibitory concentration values of TCE for *Pseudomonas* sp.s are presented in the Table 1. The MIC values of *Pseudomonas aeruginosa* #II and *Pseuodmonas aeruginosa* #IV were found to be 1024 ppm followed by *Pseudomonas aeruginosa* # III. The MIC values for both *Pseudomonas aeruginosa* # I and *Pseudomonas* fluorescens were found to be 32 ppm. (Table 1).

Table 1. Minimal Inhibitory Concentration Values of TCE for *Pseudomonas* Sp.s

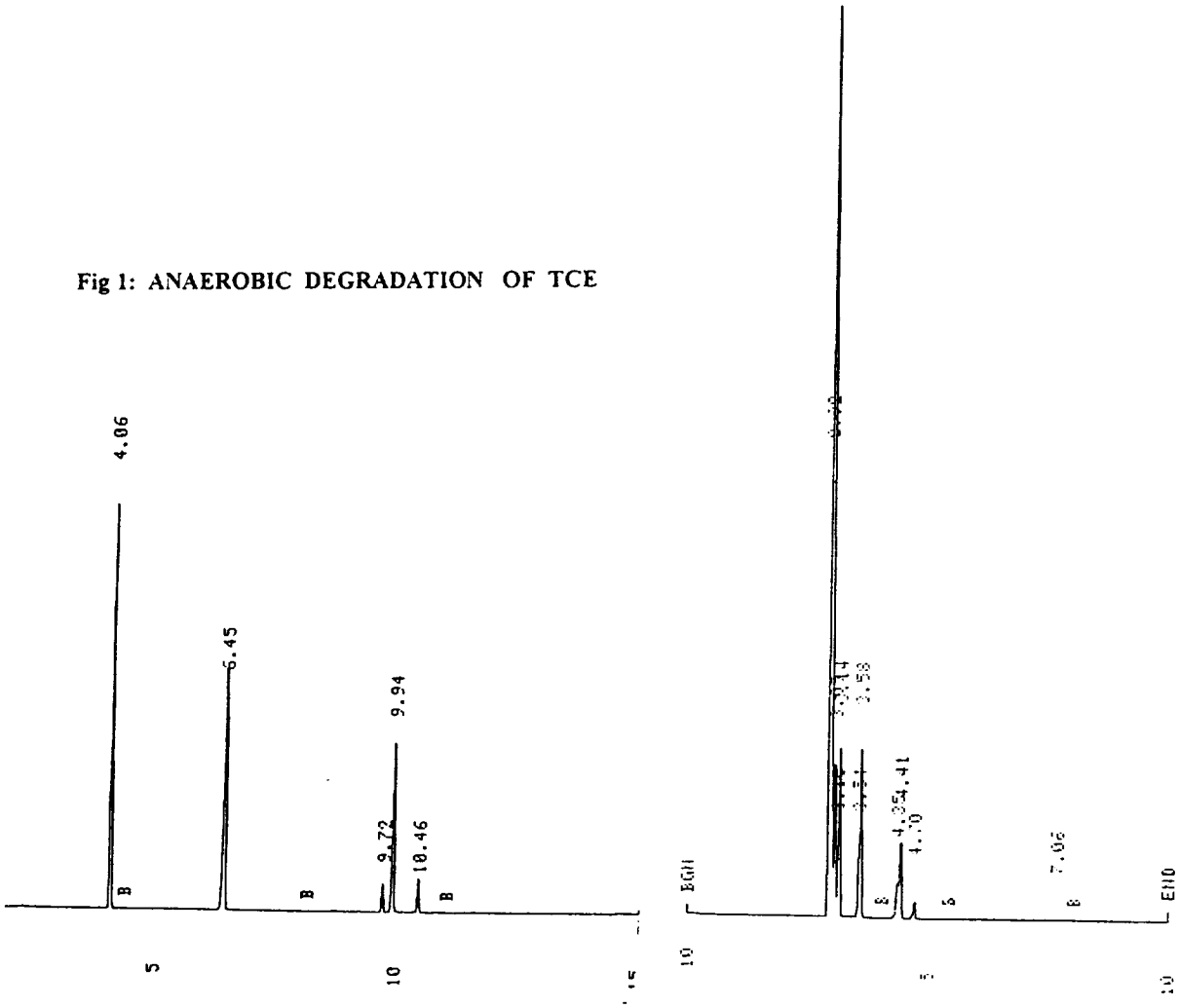
Culture #	Name of the Culture	MIC Value [ppm]
1	Pseudomonas aeruginosa	32
2	Pseudomonas aeruginosa	1024
3	Pseudomonas aeruginosa	256
4	Pseudomonas aeruginosa	1024
5	Pseudomonas fluorescens	32

Degradation of TCE and DCE

Figure 1 indicate the degradation of TCE by the anaerobic cultures. The anaerobic bacterial cells were able to degrade or dechlorinate TCE into different compounds (Fig.1) which were detected in GC. Figure 2 shows that the immobilized cells of aerobic mix cultures were able to degrade DCE. The products and concentrations of TCE and DCE have not been identified and quantified in the present investigation.

Fig 2: DEGRADATION OF DCE BY IMMOBILIZED CELLS OF *PSEUDOMONAS* Sps.

Fig 1: ANAEROBIC DEGRADATION OF TCE



DISCUSSION

Trichloroethylene (TCE), an Enviornmental Protection Agency Priority Pollutant, is widespread in the environment and relatively resistant to biodegradation in soil and the subsurface. Under aerobic conditions, TCE is usually cometabolized and thus requires the presence of a cosubstrate such as methane, ammonia, toluene, xylene. Nelson *et al.* (1988), Little *et al.*(1988), and Arciero *et al* (1989) have shown that toluene-, methane-, and ammonia-oxidizing bacteria, respectively, biodegrade TCE. It is genetically evident that toluene dioxygenase (Nelson *et al.*,1988; Wackett and Gibson, 1988) and toluene monooxygenase (Winter *et al.*, 1989) are biocatalysts active in TCE degradation (Nelson *et al.*, 1988). The results from the present study indicate the anaerobic dechlorination of TCE and degradation of DCE by immobilized cells of aerobic cultures.

The studies on the confirmation of TCE and DCE degradation and the identification and quantification of metabolites formed during their degradation are in progress. The optimization of degradation of TCE using various carbon soruces such as glucose, formate, succinate, corn syrups, etc., is also being carried out. The work on the degradation of DCE in the presence of various concentrations of xylenes and toluene which act as inducers of mono- and dioxygenases is also in progress.

ACKNOWLEDGEMENT

The present work was supported by NASA Grant No. NAGW -3969.

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