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Biodegradation of Chlorinated Aliphatics and Aromatic Compounds in Total-Recycle Expanded-Bed Biofilm Reactors

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ABSTRACT

Ground-water contamination by chlorinated aliphatic compounds is a major cause for concern because of their toxicity. This study examined the biodegradation of trichloroethylene and aromatic compounds by microbial consortia enriched from contaminated subsurface sediments. The consortia were capable of utilizing methane and propane as sources of carbon and energy. Two continuously recycled expanded-bed bioreactors were inoculated with (1) the subsurface consortium, and (2) *P. fluorescence, P. putida* (strains pRB1401 and pWWO), and *M. trichosporium* OB3b. An uninoculated reactor containing 0.2% sodium azide and 0.5% formalin served as the control. Methane (5% v/v) and propane (3% v/v) were maintained by batch feeding through the course of the experiment. Greater than 97% degradation of trichloroethylene was observed over a period of 12 d. More than 99% of benzene, toluene, and xylene were degraded within the first 7 d. Dissolved oxygen levels were measured and found to be in the range 4.9–6.5 mg/L throughout the experiments.

Index Entries: Mixed-waste biodegradation; bioreactors; chlorinated aliphatics; aromatics; bioremediation.

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INTRODUCTION

The contamination of soils, sediments and ground water by chlorinated aliphatic and aromatic compounds is a major cause for concern. Chlorinated aliphatic compounds are very toxic, and some are carcinogenic. Ground-water contamination has resulted from the widespread use of various chlorinated aliphatic and aromatic compounds, like trichloroethylene, tetrachloroethylene, benzene, toluene, and xylenes. Their improper disposal has led to the occurrence of these volatile organic compounds in ground water at spill sites and at disposal sites. In the U.S. E.P.A. List of Priority Pollutants (*1*), chlorinated aliphatics form one of the nine groups. Many soils and subsurface aquifers that are subjects for remediation contain >1000 mg/L of chlorinated hydrocarbons (*2–4*).

Removal of organic compounds from water is currently accomplished by physical and chemical methods, such as air stripping, land filling, carbon adsorption, and incineration (*5*). Air stripping and incineration, although widely used, cause deterioration of air quality, which may result in a disturbance in the ozone layer. Land filling and other physical techniques move the problem from one location to another, since the contaminants are not degraded or tranformed. These techniques will not be suitable for remediation of chlorinated hydrocarbon contamination in deep vadose zones or aquifers.

Trichloroethylene, a chlorinated aliphatic compound, is degraded cometabolically by methanotrophs (*6,7*) and by heterotrophic microorganisms (*2,8,9*). Biodegradation of trichloroethylene has been shown to be stimulated by methane and propane (*7,10–14*). Bacterial cultures capable of degrading aromatics have been well documented (*14–22*). Biodegradation of mixed organic waste that contained trichloroethylene, benzene, toluene, and xylene in a total-recycle expanded-bed biofilm reactor is reported in this study.

MATERIALS AND METHODS

Gases and Chemicals

Methane and oxygen were obtained from Linde Specialty Gases, Union Carbide Corp., Danbury, CT. Propane was supplied by Holston Gases, Knoxville, TN. Propane and oxygen were more than 99.8% pure, and methane was more than 98% pure. Trichloroethylene was purchased from Mallinckrodt Chemical Co., Paris, KY. Benzene, toluene, and xylene were purchased from Aldrich Chemical Co., Inc., Milwaukee, WI. All chemicals were of reagent grade.

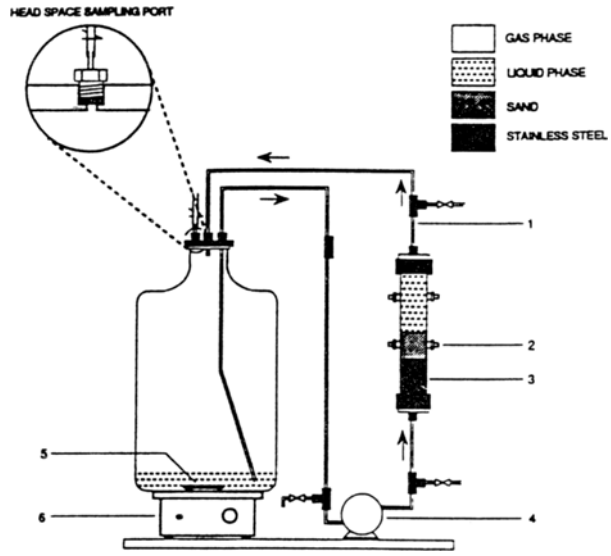

Fig. 1. Schematic of the continuous-recycle expanded-bed bioreactor. 1—1/8-in stainless-steel tubing. 2—Solids sampling port with Mininert valves. 3—Stainless-steel lower end cap. 4—Metering pump. 5—Stir bar. 6—Stir plate.

Bacterial Cultures

Two mixed culture consortia that degrade trichloroethylene have been maintained in this laboratory. The primary trichloroethylene degrading consortium was obtained from the Savannah River Plant, Aiken, SC (*2*), referred to as the SRP consortium. Methane and propane oxidizing bacteria isolated from a waste disposal site near Oak Ridge, TN, capable of degrading trichloroethylene (*6*) were added to the SRP consortium, referred to as the Reactor consortium. Pure cultures of *Pseudomonas putida* (strains pRB1401 and pWWO), *Pseudomonas fluorescence*, and *Methylosinus trichosporium* OB3b were obtained from G. S. Sayler.

The growth medium for the SRP consortium contained the following (per liter): $MgSO_4 \cdot 7H_2O$, 0.055 g; $CaSO_4 \cdot 2H_2O$, 0.054 g; NH_4NO_3, 1.48 g; trace mineral and vitamin solutions, 2.0 m*M* phosphate-bicarbonate buffer solution, pH 7.0 (*11*). Resazurin (2.0 mg/L) was added to monitor the redox of the liquid phase. Five percent methane and 3% propane (vol/vol, headspace) were added to the two cultures. The *Pseudomonas putida* and *P. fluorescens* were grown on Tryptic-Soy broth (Difco, Inc.). *Methylosinus trichosporium* OB3b was grown on a mineral salts medium (*23*).

Design of the Total-Recycle Expanded-Bed Bioreactor System

The total-recycle expanded-bed biofilm reactors were based on the bioreactors developed by Niedzielski et al. in our laboratory (*11*). Figure 1

depicts a schematic of the improved form of the reactor. The gas-recharge column in the earlier reactor was replaced with a 2.5-gal glass carboy outfitted with a 3/8-in stainless-steel plate fastened by a clamp. A Teflon™ washer was used to seal the stainless-steel plate to the carboy. High gas-liquid mass-transfer rates in the recharge vessel were obtained by using a Teflon™-coated stir bar spinning at a high speed in the liquid medium. The stainless-steel plate top-plate was equipped with four 1/8-in Swagelock® fittings. One fitting was connected to the inlet at the bottom of the glass column via a metering pump, one fitting connected to the top of the glass column, and another fitting served as a port for head-space sampling. The fourth fitting served as a port for the addition of gases into the carboy. The expanded-bed column consisted of a 30×5 cm borosilicate glass chromatography column (Spectrum Medical Industries, Inc., Los Angeles, CA). The Teflon™ end-caps on the expanded-bed column were replaced with stainless-steel end-caps. This minimized the loss of volatile organic compounds. The column contained 180 g of quartz sand (Sigma Chemical Co., St. Louis, MO). This sand served as the solid support for the biofilm. The walls of this column had been specially designed with ports fitted with Mininert® valves (Dynatech Precision Sampling, Baton Rouge, LA) for sampling of the biofilm in the sand bed. The stainless-steel end cap at the top of the column was provided with an 1/8-in Swagelock® fitting, and this fitting was connected to the glass carboy with 1/8-in stainless-steel tubing. The threaded fittings were sealed with Teflon™ tape. The total liquid-phase volume in the reactor was 850 mL, and the total gas-phase volume in the glass carboy was 9000 mL, resulting in a liquid:head-space volume ratio of 1:10.58.

The reactors were inoculated with bacterial cultures as described earlier. A continuous-recycle flow rate of 35 mL/min was maintained with a fluid metering pump (Fluid Metering, Inc., Oyster Bay, NY). A stainless-steel "Tee" was provided at the inlet and outlet of the column to allow sampling of the liquid media for disssolved oxygen (DO). Dissolved oxygen levels were monitored using an OM-4 Oxygen Meter (Microelectrodes, Inc., Londonderry, NH). The reactors were operated at room temperature (24–25°C). At the beginning of each experiment, the head space was replaced with oxygen, and appropriate amounts of methane and/or propane were added. The gas inlet port was then sealed, and the toxicants were added (trichloroethylene 20 mg/L, xylene 5 mg/L, and the others at 1 mg/L) through the head-space sampling port.

Analytical Procedures

Methane and carbon dioxide were determined using a Shimadzu GC-8A equipped with a 2.7-m long, 3.2-mm diameter Carbosieve 8000 packed column with a thermal conductivity detector. The following conditions were used: oven temperature, 110°C; detector and injector temperature,

140°C. The carrier gas was helium. Propane, benzene, toluene, and xylene were analyzed with a Shimadzu GC-9A equipped with a 2.4-m long, 3.2-mm diameter Poropak N packed column and a flame ionization detector. The following conditions were used: oven temperature, 210°C; and injector temperature, 220°C. Trichloroethylene was analyzed with a Shimadzu GC-9A equipped with a 2.4-m long, 3.2-mm diameter Poropak T column and a photo-ionization detector (HNU Systems, Newton, MA). The following conditions were used: oven temperature, 115°C; injector temperature, 160°C; and detector temperature, 230°C. The carrier gas was helium. Concentrations of methane, propane, and carbon dioxide were determined by comparing peak heights of samples to those of prepared gas standard calibration curves. The peak heights of the volatile organic compounds sampled from the reactors were compared to peak heights of the respective compounds. The standards prepared had the same gas phase:liquid phase ratio as that of the reactors.

RESULTS AND DISCUSSION

Biodegradation experiments were performed with semicontinuous feeding of methane and propane. The concentrations of methane and propane were maintained at 3% v/v and 5% v/v, respectively, in reactor 1. Reactor 2 had only methane, and the concentration was maintained at 5% v/v. Uninoculated control reactor inhibited with 0.2% sodium azide and 0.5% formalin retained more than 92% of initial amounts of the compounds added to the reactors. The liquid-phase pH remained at 7.2 ± 0.1 in the reactors throughout the time-course of the experiments. A wide range of rates and extent of degradation of different compounds in the two reactors was observed. The two reactors had different inocula. The initial concentrations of various compounds in the reactors were as follows: trichloroethylene—20 mg/L, benzene—1 mg/L, toluene—1 mg/L, xylene—5 mg/L.

More than 99% degradation of trichloroethylene (of 50 mg/L) by a mixed culture of heterotrophic microorganisms has been demonstrated in our laboratory (*2*). The degradation rate of trichloroethylene in reactor 1 (SRP consortium) stayed almost constant at 1.58 mg/L/d throughout the 11-day period (Fig. 2A). Propane was utilized at a rate faster than that for methane, indicative of a larger population of propane-utilizing bacteria. Earlier it has been shown in this laboratory that this consortium grown on methane alone showed very little trichloroethylene degradative activity (*12*). When grown on propane alone, the trichloroethylene degradation was similar to that observed when methane and propane were maintained at constant levels in the reactors. The trichloroethylene degradation in reactor 2 was much faster during the first 7–8 d (2.2 mg/L d) and then

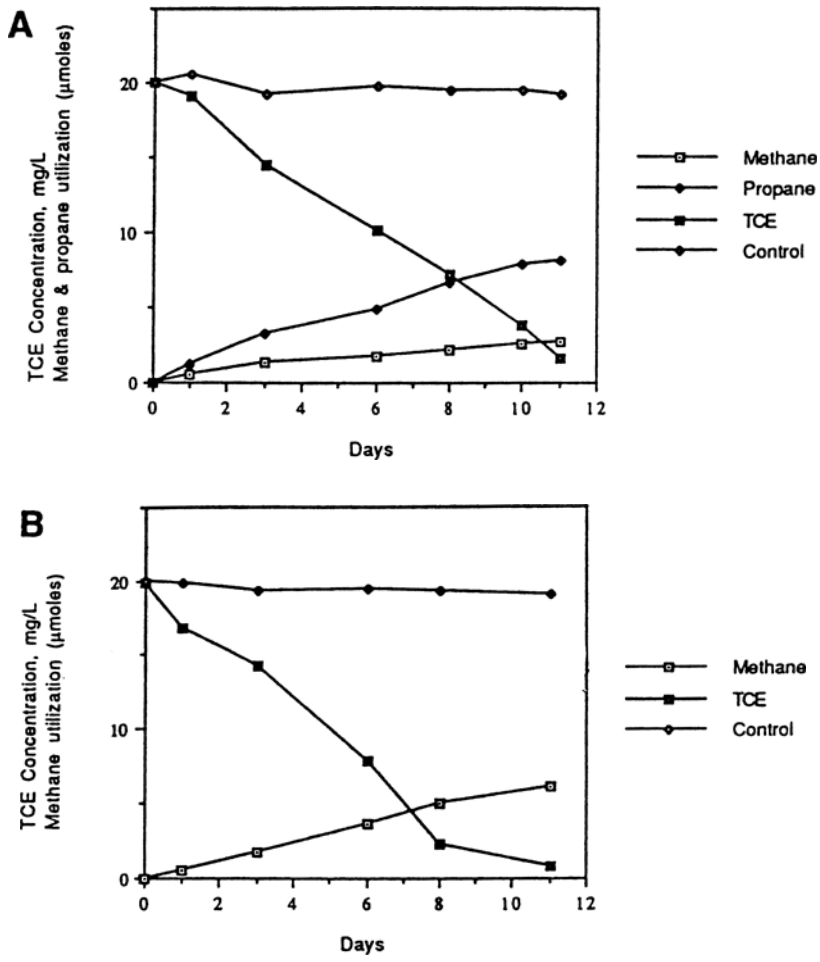

Fig. 2. Biodegradation of trichloroethylene and the utilization of methane and propane in the bioreactors. A: Reactor 1: SRP Consortium. B: Reactor 2: *P. fluorescence, P. putida* (strains pWWO and pRB1401), and *M. trichosporium* OB3b.

slowed to 0.5 mg/L/d (Fig. 2B). Similar results were observed in other studies (*12,13*), where degradation of trichloroethylene became less efficient at low trichloroethylene concentrations. No volatile intermediates of trichloroethylene degradation were detected in either of the reactors during the time-course of the experiment.

The trichloroethylene degradation rates (in the presence of mixed waste) observed in this study are almost an order of a magnitude higher than those reported earlier from this laboratory (*14*). Decreased rates of trichloroethylene observed in reactor 2 after 8 d agreed well with other studies (*12,13*). These studies have reported a decrease in trichloroethylene degradation rates as concentrations of trichloroethylene decreased to approx 500 µg/L. The degradation of aromatic compounds, like benzene, toluene, and xylene, was also found to be faster than found earlier in this

laboratory (*14*). The presence of methane and propane did not seem to affect degradation of aromatics, like benzene, toluene, and xylene. These results suggest that this consortium is capable of degrading mixtures of organic wastes (*6,8,9,12*).

Some of the heterotrophic cultures capable of utilizing toluene also degrade trichloroethylene when induced with toluene (*8*). Induction by toluene or other aromatic compounds was not necessary for the degradation of trichloroethylene by this consortium. Reactor 1 was inoculated with the SRP consortium alone. Reactor 2, which had been inoculated primarily with a trichloroethylene-degrading methanotroph (*M. trichosporium* OB3b), showed initial higher rates and then much slower rates of trichloroethylene degradation at lower concentrations of trichloroethylene.

Benzene, toluene, and xylene were degraded to below detectable limits by reactor 1 within 8–10 d (Fig. 3A). In this reactor, the degradation rate of xylene (833 μg/L/d) was faster than the degradation rates of benzene and toluene (167 and 100 μg/L/d respectively). Reactor 2, which was inoculated mainly with a methanotroph in addition to the *Pseudomonas*, dedegraded toluene and xylene to below detectable limits in 8 and 11 d, respectively (Fig. 3B). The rate of degradation of xylene was higher (455 μg/L/d) than toluene (125 μg/L/d). The degradation of benzene was much slower. Only about 60% of benzene (initial concentration 1 mg/L) was degraded in 11 d. The control reactor retained more than 87% of these aromatic compounds (Fig. 4).

Results from these experiments show that total-recycle expanded-bed bioreactors inoculated with aerobic cultures are capable of biodegrading over 90–92% of trichloroethylene at an initial concentration of 20 mg/L, while utilizing propane and or methane as the energy source. Differences within the microbial populations in the two reactors were evident from the differences in the utilization of the substrates, carbon dioxide produced, and trichloroethylene degraded. The culture in reactor 1 was represented by a more diverse microbial population than the cultures in reactor 2. The methanotrophic bacteria are known to cometabolize trichloroethylene, the soluble methane monooxygenase playing a major role in this step of cometabolism (*6*). Biodegradation of chlorinated alkenes has been reported by cultures stimulated with methane or natural gas (*6,7,10,13*). This study suggests that propane may also play an important role in promoting trichloroethylene degradation. Benzene, toluene, and xylene were also degraded by these cultures to below detectable limits, whereas the control reactors retained more than 87% of the initial amounts of these compounds added to the reactors.

The dissolved oxygen concentration in the reactors was monitored using a dissolved oxygen probe. At the end of 10 d, the dissolved oxygen concentration in the effluent from the expanded-bed column of reactor 1 was > 4.9 mg/L, indicating aerobic conditions. The dissolved oxygen concentrations in the effluent from reactor 2 were higher than that in reactor 1 (Table 1). Although the bulk liquid phase was aerobic, it is likely that

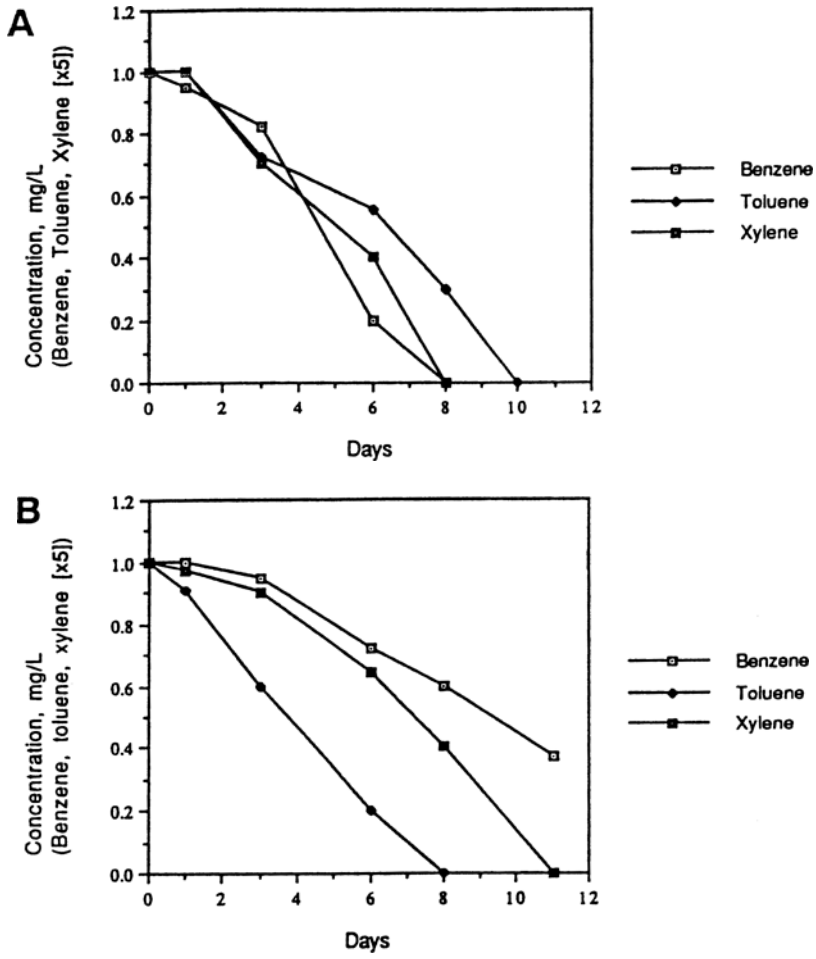

Fig. 3. Biodegradation of benzene, toluene, and xylene in the bioreactors. A: Reactor 1: SPR Consortium. B: Reactor 2: *P. fluorescence, P. putida* (strains pWWO and pRB1401), and *M. trichosporium* OB3b.

some anaerobic microniches existed within the biofilm. This could be attributed to the possible channeling effects of the liquid phase through the sand bed.

These bioreactor experiments demonstrated that the mixed cultures were capable of degrading more than 95% of 20 mg/L trichloroethylene and other aromatic wastes under aerobic conditions, utilizing propane and/or methane as the carbon and energy sources. The consortia used methane and propane or propane as energy sources. It has been shown that these consortia are stable and used a variety of energy sources for growth, but could not use methane as the sole energy source. The degradation rates dropped significantly if the consortium was starved of propane (*12*).

Table 1
Dissolved Oxygen Levels
in the Bioreactors at the End of 10 D at 25°C

	Dissolved oxygen, mg/L	
	Inlet	Outlet
Reactor 1[a]	5.62	4.89
Reactor 2[b]	6.69	6.53

[a]Reactor 1: SRP consortia.
[b]Reactor 2: *P. fluorescence*+*P. putida* (pWWO and pRB1401 strains) and *M. trichosporium* OB3b.

Table 2
Degradation Rates of Trichloroethylene,
Benzene, Toluene, and Xylene in the Bioreactors

	Degradation rates, μg/L/d	
Compound	Reactor 1	Reactor 2
TCE	1580. ± 25	2200. ± 32
Benzene	167. ± 15	55. ± 6
Toluene	100. ± 11	125. ± 12
Xylene	833. ± 42	455. ± 23

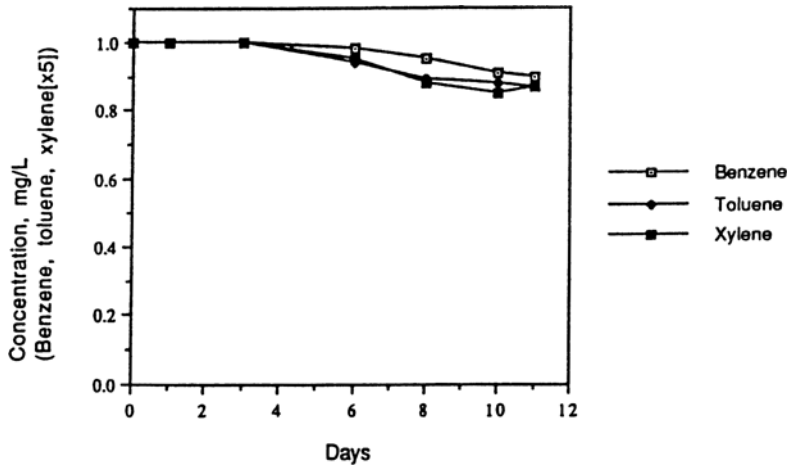

Fig. 4. Uninoculated control reactor (0.2% sodium azide and 0.5% formalin) Reactor 3: Uninoculated control (0.2% sodium azide and 0.5% formalin).

CONCLUSIONS

The bioreactor system developed in this work was suited for the biodegradation of chlorinated aliphatic and aromatic compounds, which represent major contaminants in ground waters. The design of the gas-recharge vessel allows for larger gas-to-liquid phase ratios, thus holding large volumes of gases and maintaining aerobic conditions for more than 12–14 d. Degradation of trichloroethylene, benzene, toluene, and xylene occurred under aerobic conditions. The results of this investigation provided insight into the degradation of mixed organic waste by various consortia in laboratory bioreactors. Biodegradation of trichloroethylene by a methanotroph in the presence of methane gas in the bulk phase was observed. The bioreactor system is also suited for studying the community structure in the biofilm with the help of phospholipid, as well as gene probe analyses.

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