0273-2289/93/3940—0701$03.60

Biodegradation of Chlorinated Aliphatic Hydrocarbon Mixtures in a Single-Pass Packed-Bed Reactor

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ABSTRACT

Aliphatic chlorinated compounds, such as trichloroethylene (TCE) and tetrachloroethylene (PCE), are major contaminants of ground water. A single-pass packed-bed bioreactor was utilized to study the biodegradation of organic waste mixtures consisting of PCE, TCE, and other short-chain chlorinated organics. The bioreactor consisted of two 1960-mL glass columns joined in a series. One column was packed with sand containing a microbial consortia enriched from a contaminated site. The other column provided a reservoir for oxygen and a carbon source of methane/propane that was recirculated through the reactor. Sampling was accomplished by both direct headspace and liquid effluent concentration analyses. The reactor was operated in a single-pass mode. Greater than 99% degradation of trichloroethylene, approaching drinking water standards, was observed when the bioreactor residence time ranged from 1.9 to 3.2 d. Typically, when the reactor was pulse-fed with methane, propane, and air, 1 mol of TCE was degraded/110 mol of substrate utilized. Perturbation studies were performed to characterize reactor behavior. The system's degradation behavior was affected by providing different carbon sources, a pulse feeding regime, supplementing microbial biomass, and by altering flow rates.

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

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INTRODUCTION

Chlorinated short-chain aliphatic compounds, such as trichloroethylene (TCE), tetrachloroethylene (PCE), and dichloroethylene (DCE), are some of the most prevalent contaminants of ground water (*1*). The National Priority List of the US Environmental Protection Agency lists TCE as the most frequently reported contaminant at hazardous waste sites (*2*). Recently, the EPA has determined these halogenated organics to be carcinogenic, and regulation on their existence in ground water is close to analytically detectable limits.

Typically, these compounds cannot be removed from ground water using conventional water-treatment techniques, such as coagulation, sedimentation, or filtration (*3*). Instead, chlorinated aliphatics are generally removed by air-stripping and adsorption (*4*). Unfortunately, these methods of removal have not destroyed the dangerous compound, but have just moved it from one phase to another. An alternative method for removing these alphatic compounds from water is biodegradation. Biodegradation has shown the potential to transform toxic compounds to nontoxic compounds, such as carbon dioxide, water, bases, salts, and inorganic acids.

The ability to degrade TCE has been observed in both pure cultures and mixed microbial consortia (*1,2,5–13*). Methanotrophs show the ability to degrade TCE and other chlorinated compounds (*2,5*). By utilizing methane monooxygenase, the enzyme that normally oxidized methane to methanol, the contaminant is oxidized, and water-soluble TCE breakdown products are utilized. Fortunately, most of the breakdown products can be metabolized by heterotrophic bacteria in mixed methane-enriched cultures. Other oxygenases, such as those formed during the oxidation of propane (*6,14*), toluene and other aromatics (*12,15*), and ammonia (*16*), have been found that are capable of contributing to TCE degradation.

TCE degradation has also been observed in cultures of *Pseudomonas* that are typically associated with aromatic degradative pathways that utilize *meta* fission (*12,17,18*). This aromatic pathway suggests that the toluene dioxygenase enzyme is responsible for degradation. Toulene or phenol was required to induce TCE degradation (*15*). Anaerobic studies have clearly shown biotransformation of PCE and TCE. In all cases, the transformation is the result of a reductive dehalogenation (*19–22*). Under anaerobic conditions, the rate of dechlorination decreases as chlorine molecules are removed (*19*).

Although degradation of the chlorinated organics is possible, there is a minimum concentration to which a single organic material can be decomposed under steady-state conditions. Biodegradation of such trace compounds can occur in general only if they are used as secondary substrates. The kinetics of degradation of low concentrations of mixed wastes is relatively unknown. Little knowledge has been assessed concerning

the interaction of the primary and secondary substrates, or of the impact sorption has on the ability to degrade trace organics.

The purpose of this study is to explore and understand further the cometabolic degradation abilities of microorganisms biodegrading mixed wastes in a packed-bed reactor. The reactor was seeded with a microbial community that exhibited the ability to degrade TCE when fed methane and/or propane under various conditions.

MATERIALS AND METHODS

Gases and Chemicals

National Welders (Charlotte, NC) supplied all gases. Methane was >98% pure, and oxygen and propane were >99.5% pure. All chemicals and reagents were obtained from Sigma Chemical Co. (St. Louis, MO) or from Mallinckrodt, Inc. (Paris, KY). Glass-distilled solvents and reagents were purchased from J. T. Baker Chemical Co. (Phillipsburg, NJ).

Bacterial Culture

The consortium used in seeding the bioreactor was obtained from several sites. A TCE degrading consortium capable of degrading concentrations >100 mg/L was isolated from the Savannah River Plant, Aiken, SC (*6*). From Ada, OK, methane- and propane-utilizing consortium was added to the reactor (23) along with a methanotroph isolate from a waste-disposal site near Oak Ridge, TN.

Bioreactor Configuration

The single-pass packed-bed bioreactor system, illustrated in Fig. 1, consisted of two borosilicate-glass columns (Pharmacia, Piscataway, NJ). The two columns (ID=5 cm, length=100 cm, total vol=1960 mL) were sealed using stainless-steel end caps equipped with Teflon™ O-rings. These columns provided a closed system. The lower column was inoculated with the consortia mentioned above and 50- to 70-mesh white quartz sand (Sigma Chemical Co., St. Louis, MO). The upper column provided oxygen, methane, and propane to the packed-bed on a recirculating basis (Fig. 1). The gases were pumped using an FMI lab pump model RP-G400 (Fluid Metering, Inc., Oyster Bay, NY) that distributed the gases to three equally spaced regions on the bottom of the packed bed. A 3% hydrogen peroxide solution was pumped by a Gilson Miniplus 2 peristaltic pump at the rate of 1–2 mL/h as an additional electron acceptor source. The flow was evenly distributed to three regions in the bottom of the reactor bisecting the gas flow inlets. The top of the packed-bed column was equipped with a gas outlet stream that recirculated into the upper column. All column connections were made of Swagelok® fittings.

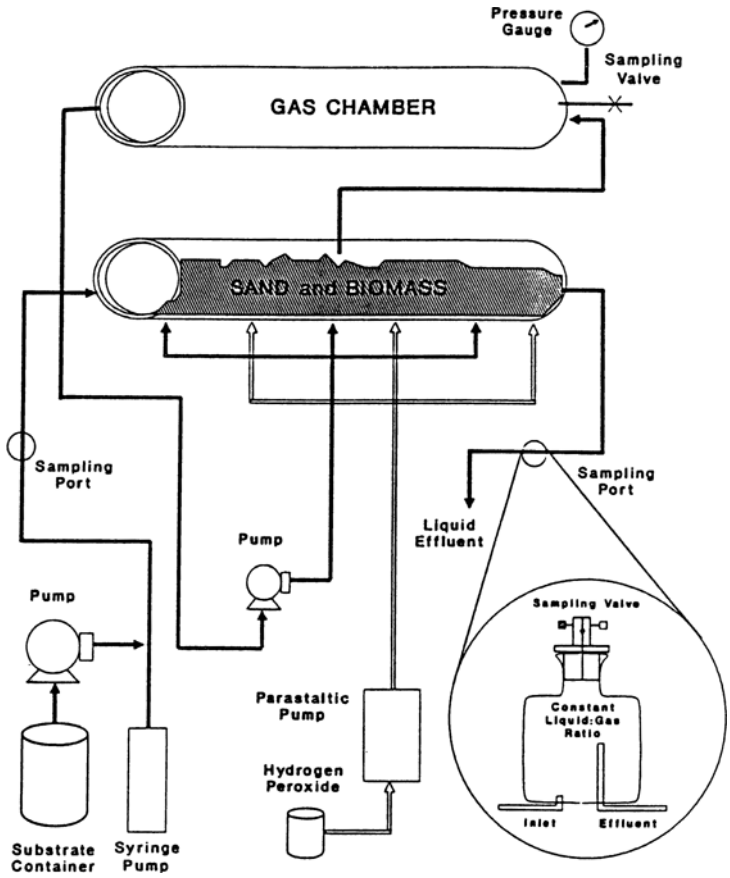

Fig. 1. Single-pass packed-bed bioreactor system with sampling port inset.

Chlorinated hydrocarbons were introduced into the reactor by an ISCO, model 314, high-pressure syringe metering pump equipped with a pressure monitor model 1590 (ISCO Instrument Specialties Co., Lincoln, NE). The syringe pump, having no headspace, allowed a constant concentration of the contaminants to enter the reactor. Medium was added to the reactor by the use of a Beckman model 11A pump. Medium and the organic contaminants were mixed in a tee, and the mixture initially entered a glass sampling port. The inlet and effluent sampling ports, detailed in Fig. 1, allowed for a constant liquid-to-gas gas ratio, from which GC gas headspace analyses were performed. A Teflon™ Mininert valve (Scientific Products, McGaw Park, IL) was attached to the glass sampling port providing a leak-tight sampling point. Liquid samples were taken from the effluent sampling port for oxygen concentration analysis. An MI-730 Micro-Oxygen Electrode equipped with an OM-4 oxygen meter (Microelectrodes, Inc., Londonderry, NH) was used to determine the concentration of oxygen in the effluent. All tubing connections in the

reactor system were of stainless steel. Connections to the inlet and outlet sampling ports utilized organic resistant Viton tubing (Cole-Parmer, Chicago, IL). The columns were covered with black sheeting to reduce growth of photosynthetic microorganisms and to prevent photolysis of the chlorinated compounds.

Prior to each degradation experiment, the inlet and outlet of the bioreactor were sealed, and the reactor was pressurized to 3 psi. The system was able to hold pressure for the 24-h test period allotted at the beginning of the run.

Medium

The medium entering the reactor system contained the following: $MgSO_4$, 0.055 g; $CaSO_4 \cdot 2H_2O$, 0.054 g; $NaNO_3$, 1.0 g; trace minerals II (*24*), 20 mL; 10× vitamin solution (*24*), 1 mL; 200-m*M* phosphate/bicarbonate buffer solution, 30 mL; nanopure-filtered water, 1000 mL. The pH of the medium was kept at 7.0.

Analytical Procedures

A Hewlett-Packard 5890A capillary gas chromatograph with a split-splitless injector was used to analyze the chlorinated hydrocarbons. The GC was equipped with an electron-capture detector (Hewlett-Packard) and a crosslinked methyl silicone capillary column (id of 0.2 mm, 0.33-μm film thickness). The oven temperature was operated isothermally at 60°C. Other operating parameters include the injection temperature of 150°C and the detector temperature of 200°C. The split ratio was set at 10:1. Helium was used as the carrier gas, and nitrogen as the makeup gas.

Methane and carbon dioxide percentages were analyzed using a Schimadzu GC-8A gas chromatograph. The GC was equipped with a 2.74-m, 3.2-mm diameter Carbosieve 8000 packed column and a thermal-conductivity detector. Both the injector and detector temperatures were 140°C, whereas the oven was operated isothermally at 130°C. Propane was analyzed using a Schimadzu GC-9A gas chromatograph equipped with a 2.44-m, 3.2-mm diameter Poropak N packed column and a flame-ionization detector. The isothermal temperature of the oven was 80°C, and the detector and injector temperature were 220°C.

The concentrations of methane, propane, and carbon dioxide were determined by comparing peak heights of prepared-gas standard-calibration curves to samples taken from the reactor. For halogenated compounds, this was done using a Nelson 2600 analytical chromatography system with version 4.0 (Nelson Systems, Cupertino, CA). Standard curves were obtained using the same liquid-to-gas ratio found in the reactor system. By using a constant ratio, this allowed for the direct calculation of volatile compound concentrations in the liquid phase by implementing Henry's Law.

A Waters model 431 conductivity detector (Waters, Milford, MA) was used for bromide ion detection. The eluent used was lithium borate/gluconate in conjunction with a Waters IC-PAK anion column. A Waters model 510 HPLC pump transported the eluent through the system at a rate of 1.2 mL/min. A Waters model WISP 710B intelligent sample processor was used as an automatic injection module for this high-performance liquid chromatography (HPLC) system. The system held a maximum of 48 samples and could inject 1- to 2000 μL samples.

Reactor Operation

The reactor was operated under two different regimes: continuous feeding for the duration of the experiment or feeding for a period of 5 d alternated with starvation for a period of 5 d. During the periods of continuous feeding, a carbon source was always present in the reactor. During the starvation period, no energy source was added to the reactor.

RESULTS AND DISCUSSION

Abiotic Results

Residence Time Distribution (RTD)

The nonideality of the bioreactor's flow behavior was defined by pulse injection perturbations. Potassium bromide served as the inert tracer in the RTD study. The reactor portrayed a typical Gaussian-shaped distribution curve (Fig. 2). Pulse perturbation experiments were conducted at different flow rates. These abiotic experiments provided information on the sand column's residence time distribution, Peclet number, and the void volume (porosity) within the reactor bed. Figure 2 represents the reactor's residence time distribution. The calculated average reactor dispersion coefficient was 3.9×10^{-3} cm^2/s, and the void volume was determined as $\epsilon = 0.42$.

Biotic Results

The bioreactor exhibited the ability to degrade $< 99\%$ of the TCE introduced into the reactor. Although this phenomenon did not occur during each experiment, the effluent from the bioreactor approached drinking water quality when the residence time was ≥ 1.9 d. Table 1 summarizes the typical results for TCE biodegradation. It should be noted that $> 99\%$ degradation was observed in both the starvation and feeding regimes. Because drinking water quality was not always attainable within the system, different perturbations were made on the system in order to optimize the degradation process. Data from these experiments are presented.

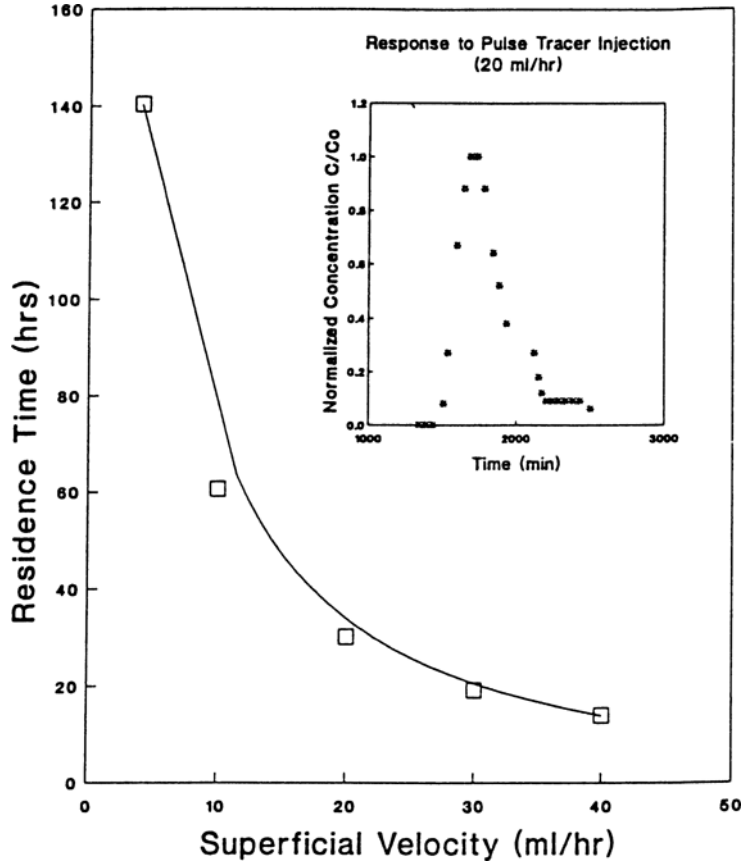

Fig. 2. Bioreactor residence time distribution with pulse tracer injection response.

Table 1
TCE Degradation Approaching Drinking Water Quality in Single-Pass Sand Column

Flow rate, mL/h	TCE Concentration		Propane consumed, mmol	Methane consumed, mmol	Rate of TCE degradation, mg L^{-1} d^{-1}	Substrate/ TCE loss, μmol/μmol
	Influent, mg/L	Effluent, mg/L				
18	0.75	0.08	0.9	0.06	0.36	292
16	0.45	0.04	0.01	0.08	0.19	28[a]
10	1.13	0.01	0.4	0.15	0.35	62[a]
18	1.73	0.017	0.4	0.27	0.91	46[a]

[a]Loss of substrate during TCE degradation-starvation phase. Reactor was fed methane plus propane for 1 d prior to experiment. If the substrate utilization during the feeding stage were included, the substrate/TCE loss would be >150. TCE: trichloroethylene.

Table 2
Comparison of Mixed-Waste Degradation in Bioreactor under Conditions of Pulsed Feeding and Continuous Feeding[a]

	$CHCl_3$	111-TCA	TCE[b]	112-TCA[c]	PCE[d]
Influent concentration (mg/L)	2.0	2.0	2.0	2.0	2.0
Effluent concentration for continuous feeding (mg/L)	0.41	0.35	1.1	0.32	1.18
Effluent concentration for starvation (mg/L)	0.40	0.37	0.75	0.32	0.88
Rate of organic degradation (mg/L d) continuous feeding	0.60	0.62	0.34	0.64	0.31
Rate of organic degradation (mg/L d) starvation	0.61	0.62	0.48	0.64	0.43

[a]The flow rate through the bioreactor was 13 mL/h. Steady state was reached after 5 d of operation. Oxygen concentration in the reactor was 3.14 mg/L after 8 d of operation in feeding mode. In starvatiaon phase, after 10 d of operation, oxygen concentration was 4 mg/L.
[b]TCE: trichloroethylene.
[c]TCA: trichloroethane.
[d]PCE: tetrachloroethylene.

Pulsed Feeding

Table 2 compares degradation of waste mixtures, including trichloroethylene (TCE), chloroform, 111-trichloroethane (111-TCA), 112-trichloroethane (112-TCA), and tetrachloroethylene (PCE). During the period of continuous feeding, methane and propane were always present in the reactor. During the starvation period, no energy source was added to the reactor. During such intervals, only the phosphate-bicarbonate buffer medium and the chlorinated hydrocarbons were being added to the reactor. Table 2 shows that there was comparable degradation ability during both the feeding and starvation periods. It is also noted that the μmole of substrate required per μmole of contaminant removed during the starvation phase is essentially halved. During the feeding period, 209 μmol of methane plus propane were required/μmol of chlorinated organic degraded vs 110 mol of primary substrate required during starvation feeding periods. This result indicates that the supply of a primary carbon source can be essentially halved with system degradation potential showing no adverse affects. It was observed that after 5 d of starvation, degradation activity in the reactor decreased. Phelps et al. (25) observed similar results in an expanded-bed bioreactor. This loss in degradation potential implies a depletion of the enzyme responsible for the epoxidation of the chlorinated compounds. This observation is expected in the absence of a primary substrate.

Table 3
Comparison of Degradation in Bioreactor
when Substrate is Limited to Only Methane or Only Propane

	Percent degradation, %				
Substrate	$CHCl_3$	111-TCA	TCE	112-TCA	PCE
Propane only	98	96	84	92	84
Methane only	58	53	39	49	35

Similar experiments were conducted that utilized the mixed-waste system seen in Table 2 with the addition of carbon tetrachloride. Preliminary results indicate 99% degradation of carbon tetrachloride is achievable when initial concentrations of the toxicant is ≤ 50 mg/L (data not shown here). Previous results indicated carbon tetrachloride degradation only under anaerobic conditions (*7,20,26*).

Propane vs Methane

Although methane facilitates TCE and other waste degradation (*2*), it is shown in Table 3 that the rates or percents of degradation when methane was the only electron donor were retarded when compared with those with propane as the carbon source. Similar results were observed in expanded-bed reactors (*27*). When propane was the only available primary substrate, results similar to that seen in the system containing both methane and propane were observed.

Mixed-Waste Degradation

A series of experiments was performed to test the ability of the bioreactor to degrade a mixture of aliphatic chlorinated compounds and aromatic compounds. The experiments were run for periods of 2–14 d with various flow rates. The aromatics in this mixed-waste system displayed the potential to be degraded consistently to below detectable limits when the residence time in the reactor ranged from 2.5–5 d. Table 4 indicates the bioreactor was resilient to such a mixture of compounds, and that degradation abilities are comparable to those observed in single-component or small-mixture waste systems. It should be noted that tetrachloroethylene was the only compound showing a drastic decrease in degradation potential. This result implies that more highly chlorinated wastes, such as PCE, are degraded less efficiently than the less chlorinated hydrocarbons in highly mixed-waste systems. Although TCE is considered a highly chlorinated compound, results indicate that TCE does not retard the metabolic efficiency within the mixed-waste system. Phelps et al. (*25*) suggested that many components in a mixed-waste system do not require additional nutrients or degradation time. This result is supported here.

Table 4
Degradation of Mixed Organic Wastes in Bioreactor[a]

Compound	Concentration of toxicant	
	In, mg/L	Out, mg/L
Trichloroethylene	5.5	0.57
cis-1,2-Dichloroethylene	15.8	≤0.005
trans-1,2-Dichloroethylene	2.7	≤0.005
1,1,2-Trichloroethane	2.1	0.22
1,1,1-Trichloroethane	7.2	0.68
Chloroform	10.9	1.7
Tetrachloroethylene	0.91	0.80
Methylene chloride	27.5	2.3
Benzene	3.57	0.40
Toulene	9.8	0.22
Xylene	1.8	≤0.005
Ethylbenzene	3.5	≤0.005

[a]The superficial velocity through the bioreactor was 7 mL/h

Table 5
First-Order Rate Constants
of Chlorinated Alphatics
in a Mixed-Waste System

Compound	k (h^{-1})
TCE	0.023
112-TCA	0.025
111-TCA	0.027
Chloroform	0.032
PCE	0.019

Modeling

Utilizing abiotic and biotic experiments performed at various flow rates, a model was developed to describe degradation within the packed-bed bioreactor. A first-order reaction rate was assumed ($-r_A = kC_A$), and the rate constant found for TCE degradation was $k = 0.023\ h^{-1}$. The rate constants for other compounds in a mixed-waste system were found and summarized in Table 5. These results suggest that the degradation rate of chlorinated aliphatics is controlled by extent of chlorination and length of carbon chain. The degradation abilities of the five compounds listed in Table 5 were compared with degradation observed for the large-mixture waste system composed of the compounds listed in Table 4, which include aromatic and aliphatic wastes. There were no data indicating that

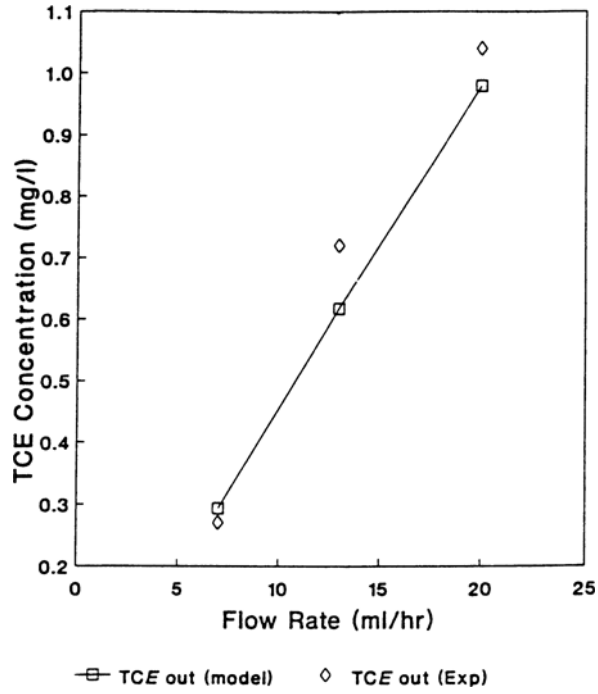

Fig. 3. Comparison of model predictions with experimental results.

the highly mixed-waste system inhibited any compound's ability to be degraded except that of PCE. Similar results have been observed by Strandberg et al. (*10*) in a fixed-film packed-bed bioreactor.

Utilizing a dispersed plug-flow reactor model and first-order reaction kinetics, a material balance across the reactor can be solved analytically (*28*). The solution is as follows:

$$(C_A / C_{Ao}) = \{4a \exp [(1\,uL / 2\,D)] / (1 + a)^2 \exp [(a\,uL / 2D)] - (1 - a)^2 \exp [- (a\,uL / 2\,D)]\} \quad (1)$$

where

$$a = [1 + 4\,k_T(D/uL)]^{1/2} \quad (2)$$

where, C_A is the effluent concentration of the toxicant (mg/L), C_{AO} is the inlet toxicant concentration (mg/L), u is the superficial velocity in the reactor (mL/h), L is the reactor bed length (m), D is the reactor dispersion coefficient (cm^2/s), k is the first-order reaction rate (h), and τ represents the reactor residence time (h). Figure 3 compares model predictions with experimental results. Experimental points plotted represent an average of the data obtained from 5–10 runs at each flow rate. Although chlorinated aliphatics are degraded cometabolically, this datum suggests first-order rate equations can be used to model such mixed-waste systems effectively.

SUMMARY

The single-pass packed-bed reactor system was utilized in this investigation to facilitate the understanding of chlorinated aliphatic hydrocarbon biodegradation. The reactor system represents a low-technology apparatus (system has been maintenance free for 2 yr) that exhibits the potential to degrade chlorinated aliphatics consistently to standards approaching drinking water quality. Possible field application could be as simple as a culvert filled with sand, with residence time, and therefore degradation potential, being controlled by the physical dimensions of the culvert.

The reactor was subjected to perturbations with the intent of degradation optimization. The bioreactor system showed resiliency when subjected to mixed wastes, including both aromatics and aliphatics. The aromatics in a mixed-waste system displayed the potential to be degraded consistently to below detectable limits when the residence time in the reactor was ≥ 2.5 d. The degradation potential for PCE in a highly mixed-waste system was the only compound exhibiting a depressed metabolism rate. Although previous results indicated carbon tetrachloride degradation only under anaerobic conditions, preliminary results of this study indicate that 99% aerobic degradation of carbon tetrachloride is achievable when initial concentrations of the toxicant is ≤ 50 mg/L. In conclusion, although the system contained many process variables, the ability to predict degradation potentials accurately was attainable by a simple analytical solution, the dispersion model, for packed-bed reactors assuming first-order reaction rate characteristics.

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