ELSEVIER

International Biodeterioration & Biodegradation 33 (1994) 41–59

0964-8305/94/$7.00

Feasibility Testing for the On-site Bioremediation of Organic Wastes by Native Microbial Consortia

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ABSTRACT

Microbial consortia capable of degrading chlorinated hydrocarbons and organic solvents may exist in many contaminated sediments. These native microbial communities with the capability to bioremediate toxicants on-site may prove to be a resource during remediation efforts. For this study, microbial consortia capable of degrading trichloroethylene (TCE) and mixtures of chlorinated and aromatic toxicants were enriched from contaminated sediments and the feasibility of their participation in on-site bioremediation was examined. Batch studies were used to monitor changes in the microbial community structure by monitoring signature fatty acid biomarker trends during TCE degradation. Experimental bioreactors, which utilized sand as a support matrix for the microbial community, were constructed to study TCE and organic mixed waste degradation. In continuously recycled expanded-bed bioreactors and a single-pass packed-bed reactor, mixtures of organic wastes were degraded including: benzene, xylene, toluene, tetrachloroethylene, trichloroethylene, dichloroethylenes and vinyl chloride. Degradation proceeded to >99% depletion for many contaminants. Bioreactors were stable over an 18-month period of operation while using propane or methane plus propane as energy sources and oxygen as the electron acceptor. Biodegradation was most efficient when the bioreactors were pulsed-fed, maintaining the consortia in suboptimal conditions. For the single-pass packed-bed reactor, metabolic efficiencies of 20–70 μmol substrate per μmol TCE degraded were observed for pulse-feeding regimes while continuous substrate availability experiments

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required >180 μmol substrate per μmol TCE. Continuous feeding experiments utilizing mixtures of organic wastes showed metabolic efficiencies of 83–240 μmol substrate utilization per μmol total organic wastes degraded. These results demonstrated efficient and simultaneous degradation of organic solvents and chlorinated hydrocarbons by environmentally derived microorganisms in laboratory reactors.

INTRODUCTION

Chlorinated hydrocarbons and organic solvents are widespread contaminants of groundwater. The combination of high usage and disposal practices has resulted in the contamination of the subsurface beneath some sites (Fliermans *et al.*, 1988; Phelps *et al.*, 1989). Remediating contaminated aquifers will be a formidable task. Processes capable of mineralizing contaminants on-site may be more desirable than transferring toxicants to another site or to the atmosphere.

Biological transformation of trichloroethylene (TCE) has been demonstrated aerobically with pure cultures of methanotrophs (Little *et al.*, 1988; Wackett & Gibson, 1988), heterotrophs (Nelson *et al.*, 1987; Wackett & Gibson, 1988; Vandenbergh & Kunka, 1988; Wackett *et al.*, 1989) and mixed microbial consortia (Bouwer & McCarty, 1983; Fogel *et al.*, 1986; Fliermans *et al.*, 1988; Phelps *et al.*, 1990). Anaerobic biodegradation of chlorinated aliphatics occurs by reductive dechlorination which can result in the formation and accumulation of vinyl chloride (Bouwer & McCarty, 1983; Kleopfer *et al.*, 1985; Vogel & McCarty, 1985; Barrio-Lage *et al.*, 1986). Natural gas stimulates aerobic TCE degradation in unsaturated soil columns (Wilson & Wilson, 1985; Henson *et al.*, 1988), and various types of bioreactors (Strandberg *et al.*, 1989; Phelps *et al.*, 1990). Within these soil columns and reactors, TCE was degraded and the methanotrophic biomass that participated in the TCE mineralization process increased over time (Henson *et al.*, 1988; Wilson & Wilson, 1985). Heterotrophic consortia obtained from severely contaminated subsurface sediments (Fliermans *et al.*, 1988; Phelps *et al.*, 1989) degraded TCE at concentrations exceeding 100 mg/liter with propane, methane or yeast extract as energy sources. Combining native heterotrophic consortia with methane utilizing microorganisms facilitated the aerobic degradation of TCE (Phelps *et al.*, 1991).

The potential role of native microbial consortia for the on-site bioremediation of subsurface contaminants is of considerable interest. While the enrichment of consortia capable of detoxifying TCE demonstrates degradation potential, it does not assess the feasibility of on-site bior-

emediation of a contaminant plume. Feasibility testing should include assessment of co-contaminant degradation, degradation of expected intermediates, degrading efficiency, stability, and parameters necessary for upscaling the bioremediation process.

Recirculating bioreactors were utilized for the initial examination of degradation potential and efficiency over several 11-day periods. Degradation efficiency, kinetics, and long-term stability were further examined using single-pass sand column experiments over an 18-month period. In addition to substrate toxicant degradation, the ester-linked phospholipid fatty acid (PLFA) profile of the consortium was analyzed in growth experiments to ascertain physiological changes within the microbial consortia during toxicant degradation. Feasibility testing for the bioremediation of organic waste mixtures by microbial consortia was accomplished using laboratory bioreactors which examined degradation capabilities, reactor and consortia stability, and biological reaction kinetics. Batch experiments verified degradation potentials and analyzed the toxicant-degrading microbial community structure. By providing experimentally measured parameters, these studies are useful in assessing the bioremediation potential of native microorganisms at contaminated sites.

MATERIALS AND METHOD

Selection of microbial consortia

The consortium used for both test tube and bioreactor studies contained mixtures of propane and methane-oxidizing bacteria obtained from the vicinity of Ada, OK (Wilson & Wilson, 1985), a TCE-degrading consortium isolated from the Savannah River Plant, Aiken, SC (Fliermans *et al.*, 1988; Phelps *et al.*, 1989) plus a methanotroph isolated from a waste disposal site near Oak Ridge, TN. The consortium was maintained on a phosphate and bicarbonate buffered mineral salts medium (Fliermans *et al.*, 1988) supplemented with 5% methane and 3% propane (v/v, headspace).

Laboratory Techniques

All manipulations utilized syringe techniques for transferring solutions and inocula. Batch experiments utilized crimp-top tubes or serum vials (Bellco Glass, Inc. Vineland, NJ) sealed with Teflon-lined septa (Altech, Deerfield, IL). All incubations were at ambient temperature (23°C). Construction, maintenance, and operation of the total-recycle expanded-

bed bioreactors (Niedzielski *et al.*, 1989) and the single-pass packed-bed reactor (Lackey *et al.*, 1993) used in this study are described elsewhere. The expanded-bed reactor had a constant liquid volume of 230 ml, while the total headspace volume remained at 490 ml. In all experiments the headspace to liquid volume ratio was greater than 2·0. The TCE concentration varied between 2·0 and 150 mg/liter. Control reactors contained 0·2% (v/v) sodium azide and 0·5% (v/v) formalin to deter microbial contamination.

Gases, chemicals and reagents

Gases were supplied by MG Industries (Chattanooga, TN) and were greater than 99·5% pure. All chemicals were of reagent grade and were obtained from Mallinckrodt, Inc. (Paris, KY) or Sigma Chemical Co. (St Louis, MO). Glass distilled solvents and reagents were obtained from Burdick & Jackson Division (Mugkegon, MI). Vinylidene chloride and tetrachloroethylene were obtained from Aldrich (Milwaukee, WI) and vinyl chloride was purchased from Supelco (Bellefonte, PA).

Analytical procedures

Analytical procedures are described in detail elsewhere (Fliermans *et al.*, 1988; Phelps *et al.*, 1989, 1990; Niedzielski *et al.*, 1989). TCE and many chlorinated hydrocarbons were analyzed using a Hewlett-Packard 5890 gas chromatograph equipped with a 50-m Ultra Performance (Ultra 1, Hewlett-Packard) cross-linked methyl silicone capillary column (0·2 mm, 0·33 μm film internal diameter) and an electron capture detector (Hewlett Packard) as previously described (Fliermans *et al.*, 1988). Vinyl chloride, *cis*- and *trans*-1,2-dichloroethylene (DCE) were analyzed using a Schimadzu GC-9A gas chromatograph equipped with a 2·24 m, 3·2 mm diameter Poropak T packed column and a photoionization detector (HNU Systems, Newton, MA). Methane and CO_2 were assayed using a Schimadzu GC-8A gas chromatograph with a 2·74-m, 3·2-mm diameter Carbosieve 8000 packed column thermal conductivity detector. Propane was analyzed using a Schimadzu GC-9A gas chromatograph equipped with a ·244 m, 3·2 mm diameter Poropak N packed column and a flame ionization detector.

Batch vial culture conditions and analytics

The microbial consortium was grown as previously described by Phelps *et al.* (1990). The pH of the medium was kept at 7·0. Culture tubes were

inoculated and propane was added (10%, v/v) as the sole carbon source for triplicate samples at each of six sampling time points. TCE was added for a final concentration of 20 ppm to half of the tubes, while the remainder were left as controls. At each time point, three controls, three treated samples, and one uninoculated blank were sacrificed for analysis of PLFA synthesis, TCE and propane loss.

Lipid extraction and fractionation

For both bioreactor and test tube cultures a sample volume (20 ml) was lyophilized then extracted in a Bligh and Dyer (1955) single-phase solvent system modified to include phosphate buffer (White *et al.*, 1979) as described in Guckert *et al.*, (1991). General lipid classes were separated from the total lipid extract by silicic acid column chromatography as detailed in Guckert *et al.* (1985). The PHA-containing acetone fraction was retained for analysis of microbial physiological status. PHA was prepared for gas chromatography (GC) analysis by forming ethyl esters of the constituent PHA monomers by acid ethanolysis as described by Findlay and White (1983). The phospholipid-containing methanol fraction was used in the evaluation of bacterial membrane lipid profiles. The ester-linked PLFA were methylated, and the resultant fatty acid methyl esters (FAME) separated, quantified, and identified as described by Guckert *et al.* (1991).

Fatty acid nomenclature

Fatty acids are reported as $A{:}B\omega C$ where A designates the total number of carbon atoms, **B** the number of double bonds, and C the distance of the closest unsaturation from the aliphatic (ω) end of the molecule. The suffixes *c* for *cis* and *t* for *trans* refer to geometric isomers. The prefixes *i* and *a* refer to iso and anteiso methyl-branching, respectively (Kates, 1986). Cyclopropyl fatty acids are designated as *cy* with the ring position in parentheses relative to the aliphatic end.

RESULTS

Expanded-bed bioreactor experiments

Total-recycle expanded-bed bioreactors were well suited for evaluating the degradation potential for microorganisms (Niedzielski *et al.*, 1989; Phelps *et al.*, 1990, 1991). The bioreactors were used to monitor the response of

the microbial community during perturbation experiments as well as to provide information concerning short-term (days–weeks) stability on a microbial community attached to the substratum. In these studies toxicants were recirculated for 5–21 days during which time substrate, toxicants and products could be monitored. Table 1 shows results from methane- and propane-fed bioreactors which contained the microbial consortium enriched from a contaminated site. After 11 days of recirculation, 93% of the 20 mg/liter TCE was removed from the recirculating expanded-bed bioreactors, propane and methane were consumed, and carbon dioxide produced. In the absence of added microbial cultures, >95% of the TCE added to the systems was still present after 11 days. An average of 4·9 mmol propane plus 1·2 mmol methane were oxidized as substrates for a substrate/TCE loss ratio of 43 (μmol/μmol).

When mixtures of organic toxicants, including tetrachloroethylene (PCE), TCE, vinylidene chloride, vinyl chloride, benzene, toluene, and xylene were recirculated through the bioreactor, an average of 6·63 mmol propane plus 2·21 mmol methane were oxidized for a substrate/toxicant loss ratio of 40 (μmol/μmol). Benzene and xylene were degraded to detectable limits on the 8th day and toluene was depleted on the 10th day. Vinyl chloride was not detected within the reactor on the final day of the experiment. The system degraded PCE, TCE, and vinylidene chloride to 58, 81, and 78%, respectively. When the single ring aromatics as well as both high- and low-chlorinated compounds were added to the system, the aromatics and the lesser chlorinated compounds were rapidly degraded at little or no change in metabolic efficiency as represented in Table 1 by the substrate utilized/toxicant loss ratio. However, the more highly chlorinated compounds such as TCE were degraded to a lesser extent during the 11-day experiments.

Sand column experiments.

Several bench-scale studies using fluidized beds, expanded beds and trickling filter bioreactors for TCE degradation experienced difficulty in achieving effluent drinking water standards (Strandberg *et al.*, 1989; Phelps *et al.*, 1990). Table 2 shows results from a single-pass sand column capable of lowering 1–2 mg/liter influent TCE concentrations to near drinking water quality. The reactor was inoculated with the TCE degrading consortium described earlier and was operated for an 18-month period. Substrate and nutrients were provided to enhance microbial activity and attachment to the substratum for a month prior to initial experimentation. In these experiments a control reactor was not utilized, rather a perfluorocarbon (perfluoromethylcyclohexane) was added as a

TABLE 1
Comparison of TCE and Mixed Organic Waste Degradation in the Expanded-Bed[a] Bioreactor

Experiment	*Initial TCE conc. (mg/liter)*	*Average degradation in reactor (%)*	*Substrate consumed (μmol)*	*Substrate/toxicant (μmol/μmol)*	*Toxicant consumed (μmol/day/g dry wt biomass)*
TCE control	20	< 5	n.a.[b]	n.a.	n.a.
TCE	20	93	6100	43	107
Mixed organics	30	85	8840	40	167

[a]The expanded-bed bioreactor was continuously operated for an 11-day period. Propane and methane were maintained at 3% and 5% (v/v), respectively.
[b]n.a., not applicable.

TABLE 2
Comparison of TCE and Mixed Organic Waste Degradation in the Single-Pass Packed-Bed Bioreactor

Experiment[a]	*Initial TCE conc. (mg/liter)*	*Average TCE degradation in reactor (%)*	*Substrate consumed (μmol)*	*Substrate/ toxicant loss (μmol/μmol)*
TCE with CH_4 and C_3H_8 at day = 0 only (7 ml/h)	1·53	99	670	58[b]
TCE with CH_4 and C_3H_8 at day = 0 only (10 ml/h)	1·13	99	550	62[b]
TCE (18 ml/h)	0·75	89	1500	282
Mixed organics (7 ml/h)	10	84 (79)[c]	4140	83
Mixed organics (13 ml/h)	10	45 (33)[c]	3960	79

The single-pass packed-bed reactor was operated in both a continuous and pulse feeding regime. The numbers in the Table are representative of data observed during a single experiment. During each experiment, 3 data points were collected daily over a5-day period and then all 15 were averaged. Ranges of results from parallel experiments are presented in the text.

[a]Flow rate (ml/h) through the reactor is indicated in parentheses. Mixed organics included TCE, PCE, chloroform, 1,1,1-trichloroethane, and 1,1,2-trichloroethane. The initial concentration of all toxicants was 2 mg/liter.

[b]The packed-bed reactor was fed methane plus propane for at least 1 day prior to experiment. If the substrate utilization during the feeding stage were included, the substrate/organic waste loss would be >150.

[c]Percentage of mixture degraded is shown in parentheses.

conservative tracer. Effluent perfluorocarbons averaged 90 ± 1% of influent concentrations demonstrating retention of volatile chlorinated hydrocarbons. Flow rates of 18 ml/h resulted in >90% removal of 0·75–1·7 mg/liter TCE in a single pass, in turn resulting in approximately 1 mg of TCE degraded each day per liter of effluent volume with a substrate/TCE loss ratio of 127–292 (μmol/μmol). Experiments within the single-pass sand-column typically used lower inlet concentrations (1–2 mg/liter) than those used in the expanded-bed bioreactor (≈20 mg/liter). By comparison of the data in Table 1 and 2, it was observed that degradation of the lower versus higher concentrations of TCE was more energy intensive, exhibiting a substrate consumed/TCE degraded ratio 6·5 times larger than that observed when initial concentrations were higher, as in the expanded-bed bioreactor. At lower flow rates utilizing pulsed feeding regimes, 90–99% of the TCE was degraded resulting in molar degradation efficiencies of 28–62 (μmol/μmol). To obtain lower effluent concentrations of toxicants and maintain degradative efficiencies it was necessary to feed the reactor for a day, provide a starvation period several hours long, during which time propane and methane were utilized, and then add TCE

without additional propane or methane. TCE-degradative activity remained for 2 or more days before replenishing with substrates was necessary. If the substrate concentrations consumed during the non-TCE-degrading periods were included then the substrate/TCE ratio would probably be >150. Results from a mixed organic waste experiment showed a metabolic efficiency of 83 μmol substrate/μmol organic waste and a decrease in the overall degradation percentage within the reactor when compared with a single toxicant system (Table 2). When the flow rate within the reactor was both 7 and 10 ml/h, and TCE was the only degradable toxicant, the average TCE degradation was 99%. In contrast, during mixed organic waste experiments, the average TCE degradation at 7 and 13 ml/h were 84 and 45%, respectively. During the mixed organic waste experiments, the aromatics and the lower chlorinated compounds were degraded to 90–99% (results not shown). Thus, the retarded degradation potentials were a result of a pronounced decrease in degradation of the highly chlorinated organics in the waste mixture.

Comparison of mixed organic wastes degradation in test tube experiments, recirculating and single-pass bioreactors

Batch studies initially demonstrated toxicant removal by the native microbial consortia. Reactors inoculated with the consortia portrayed similar degradation trends, but differences between batch and reactor studies were observed. Degradation of mixed organic wastes by the hydrocarbon fed consortium in bioreactors and test tube batch cultures are shown in Table 3. The bioreactors were fed both methane and propane while batch cultures were fed only propane. A variety of chlorinated compounds were degraded, many to detection limits in both reactor and test tube experiments. Typical of the total-recycle bioreactors, TCE degradation ceased at 0·3 mg/liter, resulting in a loss of only 90% of the initial TCE concentration while batch cultures indicated a 99% degradation potential of TCE. Both reactors showed less extensive and less rapid TCE degradation than was expected from batch experiments. Degradation of PCE in batch studies by these consortia failed. In contrast, within the recirculating bioreactor and the single-pass packed-bed reactor 60% and 12%, respectively, of the PCE was lost despite the fact that degradation had not occurred in resting cell suspension or growing batch cultures. Current studies are re-examining PCE degradation by these consortia. Dichloroethylenes and vinyl chloride, suspected intermediates of TCE degradation, were degraded to detectable limits. Benzene, toluene and xylene, common groundwater contaminants associated with petroleum solvents, were also degraded to detectable limits. The single-pass sand

TABLE 3

Degradation of Mixed Organic Wastes by Propane-Fed Microbial Consortia in Test Tube, Expanded-Bed Bioreactor and Packed-Bed Reactor Experiments

Organic waste	*Conc. range in expanded-bed bioreactor Day 0 (mg/liter)*	*Average loss in reactor after 21 days (%)*	*Inlet sand column concentration (mg/liter)*	*Average loss in sand column 7 ml/h*[a]	*Day 0 test tube exp. conc. (mg/liter)*	*Average loss in test tube experiment after 30 days (%)*
Vinyl chloride	4–20	>99			5	>99
Vinylidene chloride	20	99				
1,1-Dichloroethane	1·1	>99				
1,1-Dichloroethylene	0·7	>99	15·8	>99	1	95
1,2-Dichloroethylene	3·0	>99	2·7	>99	1	95
1,1,1-Trichloroethane	1·2	>99	7·2	91	1	>99
Tetrachloroethylene	2·1–20	60–80	0·91	12		
Trichloroethylene	2·6–20	81–90	5·5	89	50	>99
Benzene	0·1–1	>99	3·57	89	1	>99
Xylene	0·1–5	>99	1·8	>99	1	>99
Toluene	0·05–5	>99	9·8	98	1	>99

The residence time in the single-pass sand column was 5 days.

[a]At 7 ml/h, the degradation rate coefficient when TCE was the only toxicant was 0·20 per day. Upon addition of organic waste mixtures the TCE degradation rate coefficient decreased to 0·16 per day.

column degraded 1,1-dichloroethylene, 1,2-dichlorethylene, and xylene to detectable limits.

Biological degradation rate constants associated with complex mixed-organic waste experiments in the recirculating expanded-bed and the single-pass packed-bed bioreactors are compiled in Table 4. Rate

TABLE 4

Biological Rate Constants of Organic Contaminants Associated With the Recirculating Expanded-Bed and Single-Pass Packed-Bed Bioreactors.

Compound	*Rate constants for recirculating expanded-bed bioreactor (per day)*	*Rate constants for single-pass packed-bed bioreactor (per day)*
Benzene	1·67 ± 0·25	0·59 ± 0·11
Xylene	1·67 ± 0·08	0·67 ± 0·13
Toluene	1·0 ± 0·11	0·42 ± 0·09
Vinyl chloride	0·91 ± 0·08	–
1,2-Dichloroethylene	–	0·37 ± 0·06
Trichloroethylene	0·79 ± 0·02	0·20 ± 0·06
Tetrachloroethylene	0·59 ± 0·05	0·16 ± 0·05

Results from both bioreactors indicated the following trends associated with the degradation rate constants of mixtures of organic wastes:

$$\left.\begin{matrix}\text{Benzene}\\ \text{Toluene}\\ \text{Xylene}\end{matrix}\right\} \gg \left\{\begin{matrix}\text{VC}\\ \text{DCE}\\ \text{VDC}\end{matrix}\right\} \gg \left\{\begin{matrix}\text{PCE}\\ \text{TCE}\end{matrix}\right.$$

constants in both bioreactors showed common trends. Higher first order rate constants were calculated for the aromatics than for the chlorinated organics in the mixtures. It was also noted that the rate constants for the lesser chlorinated compounds were larger than those of the more highly chlorinated toxicants. For example, in the expanded-bed bioreactor, the rate constants for benzene, vinyl chloride, and, trichloroethylene were 1·67, 0·91, and 0·79 per day, respectively.

Rate constants derived from the single-pass sand column appear to be similar. Representing degradation potentials in this manner can be deceiving if the longevity of the residence times within the reactor system are not noted. For example, compare the rate constants for TCE, 0·20 per day, and PCE, 0·16 per day. After a 10-day residence time, this correlates to a 87% degradation of TCE and a 79% of PCE; a significant difference.

Community structure of toxicant degrading microorganisms and consortia

Table 5 shows ester-linked phospholipid fatty acid profiles (PLFAME) of methanotrophic TCE degraders and bioreactor contents. The methano-

TABLE 5
Phospholipid Fatty Acid Profiles of Mixed Cultures and Bioreactors Contents

PLFAME[a]	*Methanotrophic TCE-degrading consortia*	*Native methylotrophic TCE-degrading consortia (bioreactor contents*	*Microbial contributions*[b]
i15:0		2·0	Act
15:0		2·2	Act
i16:0		0·7	
16:1ω7*c*	30	2·9	Ps
16:1ω7*t*	12·3	1·5	Ps & I
16:1ω5*c*	0·1	0·4	Ps & I
16:0	34	7·0	
10me16:0		1·1	
i17:1		0·4	
i17:0		1·4	Act
a17:0		1·8	
cy17:0	5·1	3·2	Ps
17:0		1·5	
18:2ω6		0·3	B & P
18:1ω9*c*		3·8	Ps & P
18:1ω8*c*/*t*		15·1	Ps & II
18:1ω7*c*	16·9	13·1	Ps
18:1ω7*t*	0·6	0·6	Ps & II
18:0	0·3	8·6	B & P
10me18:0		4·6	A
br19:1		0·8	
19:1ω12*c*		0·7	
cy19:0	0·3	23·4	Act & Ps
20:3ω6			P
20:5ω3		0·3	P
20:4ω6		0·7	P
20:0		1·2	P

[a]Analyzed as phospholipid fatty acid methyl esters (PLFAME) identified by gas chromatography.
[b]I = common PLFAME associated with Type I methanotrophs: II = Type II methanotrophs; P = PLFAME typically associated with protozoa; A = PLFAME typically associated with *Arthrobacter*; Act = PLFAME typically associated with *Actinomycetes*; Ps = PLFAME typically associated with *Pseudomonas*.

trophic TCE degrading consortium exhibited 16:1ω7t and 16:1ω5c PLFAME found in Type I methanotrophs (Nichols *et al.*, 1985) plus a small contribution of 18:1ω7c/t synthesized by some Type II methanotrophs. When contaminated groundwaters were treated in bioreactors, PLFAME characteristic of protozoa appeared. Upon microscopic examination, protozoan cysts were observed in the bioreactor contents. These results suggested successful colonization of methanotrophic consortia by

protozoan species. Preliminary results (data not shown) suggest that protozoan grazing upon bacteria can result in decreased efficiency of bioremediation. Quantifying PLFAME in bioreactors or during bioremediation may enable monitoring and modeling of the abundance and impact of protozoan species upon toxicant-degrading consortia.

Signature fatty acid biomarkers

Dissection of the signature PLFA biomarkers provided evidence that the presence of TCE affected community structure. Individual fatty acids were extracted from the entire fatty acid profile and summed for each microbial classification (Fig. 1(a–d)).

Gram-positive *Arthrobacter*-type signature biomarkers (terminally branched saturates) exhibited a statistically significant increase over the other fatty acid profile constituents as TCE was degraded in the stationary phase (Fig. 1(a)). Gram-positive *Actinomycete*-type biomarkers (10Me17:0, 10Me18:0) exhibited an increase in the control culture (Fig. 1(b)). Gram-negative *Pseudomonas*-type biomarkers (monoenoic and cyclopropyl PLFA) peaked during the first week and declined to a constant level in both the treated and control consortium (Fig. 1(c)). There was little correlation between TCE degradation and changes in Gram-negative biomarkers. A propane-utilizing bacterial biomarker (16:1ω6) increased as TCE was degraded and propane consumed (Fig. 1(d)). This biomarker increased in the control consortia during the log phase, but this trend was reversed during the stationary phase when the TCE was degraded.

These results indicate that reactor studies as well as test tube experiments are useful in feasibility testing, scale-up, and modeling of on-site bioremediation. Results indicate that reactors should be designed for the compound with the smallest rate constant. Batch studies provided microbial community structure and toxicant degradation potentials while reactor experiments provided a system response to perturbations, stability information, and degradation kinetics required for process scale-up.

DISCUSSION

Results demonstrated that bioreactors containing mixed consortia enriched from contaminated subsurface environments were capable of degrading TCE and mixed organic wastes while utilizing propane or methane plus propane as energy sources. Phospholipid fatty acid profiles

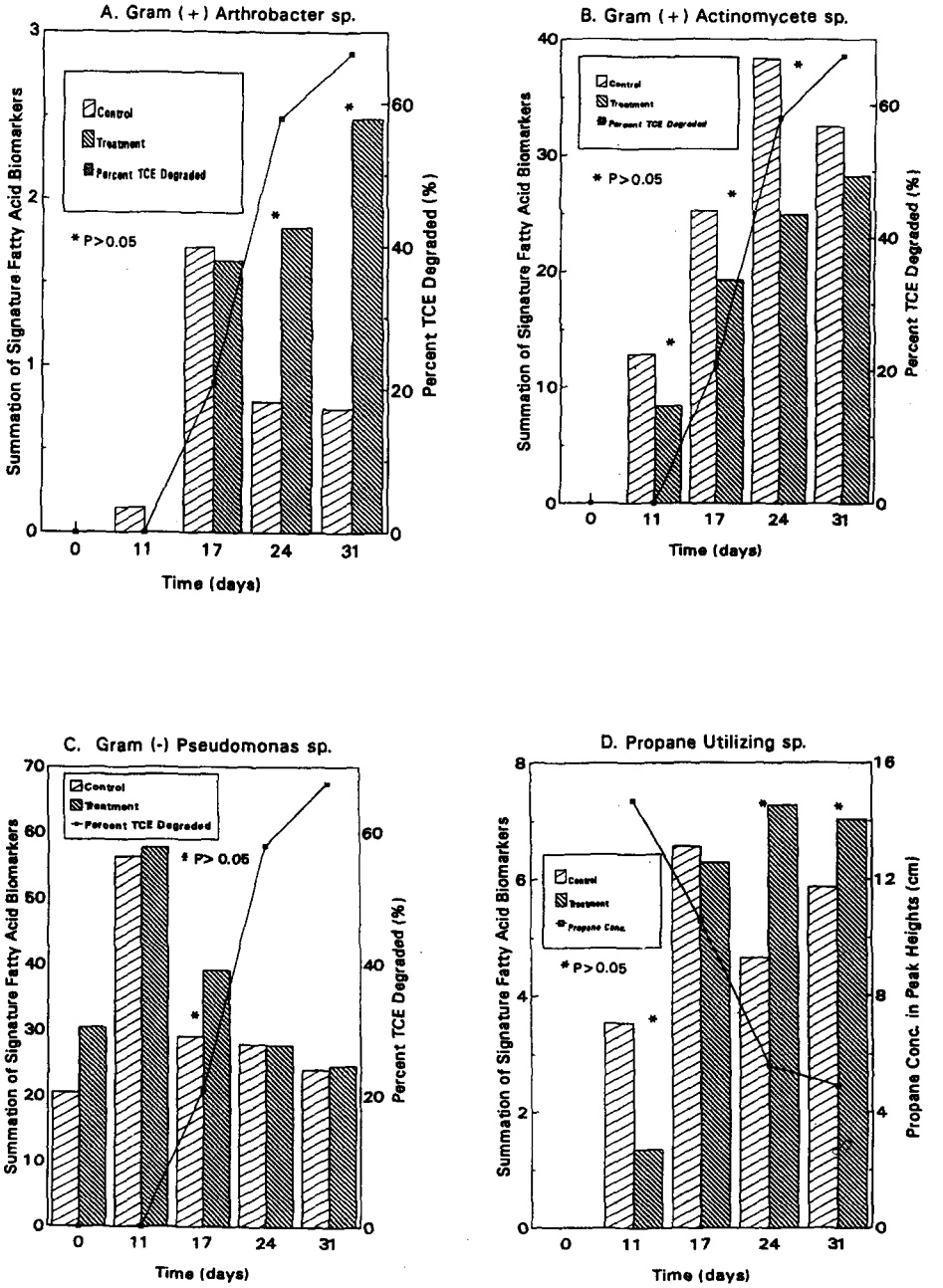

Fig. 1. Changes in microbial community structure during TCE degradation. (a) Summation of Gram-positive *Arthrobacter* species (Σi14:0, i15:0, i16:0, i17:0). (b) Summation of Gram-positive *Actinomycete* species (Σ 10Me17:0, 10Me18:0). (c) Summation of Gram-negative *Pseudomonas* species (Σ 16,18:1ωF.A., cy17,19:0, i17:1ω7, 17:1ω6). (d) Summation of propane-utilizing species (16:1ω6).

characterized the microbial community structure of the TCE-degrading microbial consortium and provided evidence of protozoan species colonizing with the consortia.

Reactor studies

In terms of TCE or mixed organic waste degradation efficiency (substrate utilized/toxicant degraded), pulsed feeding of microbial consortia resulted in the highest degradation rates and efficiency in both recycled bioreactors and single-pass sand columns. When the bioreactors were starved for several days (no substrate added), TCE degradation decreased significantly. This finding is in accord with the results presented by other researchers (Fliermans *et al.*, 1988; Little *et al.*, 1988; Strandberg *et al.*, 1989; Phelps *et al.*, 1990). This study, as well as previous work (Niedzielski *et al.*, 1989; Phelps *et al.*, 1990, 1991) shows the potential usefulness of recirculating expanded-bed bioreactors for examining degradation potentials and resiliency of the microbial community under various different operating conditions such as pH, medium, primary substrate availability, and organic waste mixtures.

Biological rate constants and degradation efficiencies were compared between reactor types. Higher first-order rate constants were observed in the recirculating expanded-bed bioreactor when rates were compared to those found in the single-pass sand column. Feasible explanations for this rate difference might include the different reactor configurations or that the lower inlet concentrations studied in the single-pass column were more difficult to degrade. Although rate constants between reactor types differed, trends associated with toxicant groups (benzene, toluene, and xylene; high and low chlorinated organics) were similar. Degradation was more efficient (μmol substrate/μmol toxicant) in the expanded-bed reactor but lower effluent TCE concentrations were achievable in the single-pass sand column. The single-pass packed-bed bioreactor provided a low-technological method for determining microbial stability as well as biodegradation rates associated with single toxicant and mixed-organic waste environments.

Bioreactors currently appear to be inefficient at lowering concentrations of highly chlorinated hydrocarbons to drinking water standards (Strandberg *et al.*, 1989; Phelps *et al.*, 1990). Incorporation of pulse-fed sand columns as an additional stage to bioreactors appears complimentary for complete biodegradation of residual chlorocarbons. Surprisingly, the sand columns lowered TCE concentrations to low part per billion levels at substrate/TCE ratios less than 300 (μmol/μmol). It could be hypothesized that sand columns may be used to model in-situ degradation efficiencies.

Accordingly, it may be possible to bioremediate chlorinated hydrocarbons within confined subsurface aquifers at substrate to TCE ratios less than 500.

Lipid biomarkers indicate protozoan and toxicant degraders

Lipid analysis provided a method to measure protozoan colonization, the stressed status of the microbial community, as well as providing signature lipid biomarkers yielding qualitative TCE degradation potentials. Monitoring the PLFAME provides a quantitative tool for assessing the abundance of protozoa within bioreactors as well as changes in the microbial community structure. It is possible to monitor the ratios of methanotrophic lipid markers (Nichols *et al.*, 1985) to those lipids typical of methylotrophs, arthrobacters, actinomycetes, or protozoa. In this study, signature lipid biomarkers provided information concerning the activity of the TCE-degrading consortia. This provides the potential to qualitatively identify degradation activity during an on-site or in-situ remediation program.

Idealized on-site bioremediation

Figure 2 is an artistic representation of on-site bioremediation of mixed organic wastes. Solid black circles represent concentrated wastes, including chlorinated hydrocarbons, with cumulative concentrations exceeding 1

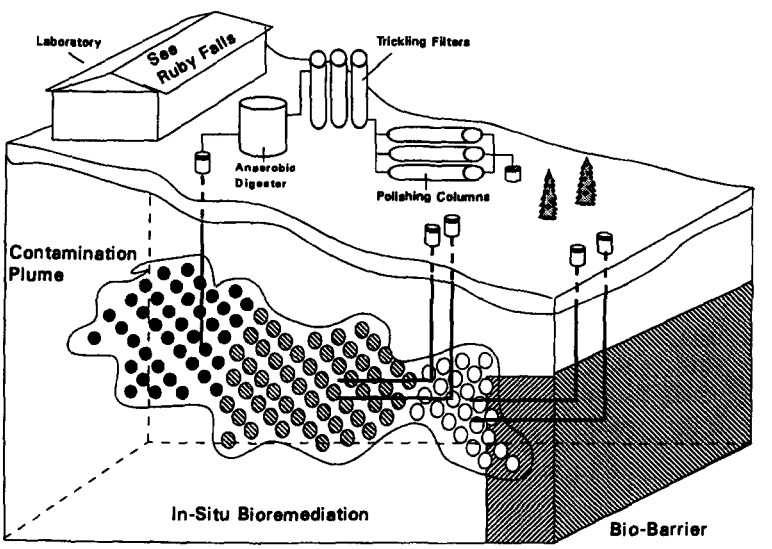

Fig. 2. Artistic representation of on-site and in-situ bioremediation.

g/liter. Excessive oxygen demand and nutrient costs may dictate pump-and-treat or evaporative extraction techniques coupled with surface bioreactors for remediation of concentrated wastes. Upon arrival at the surface, concentrated wastes could enter an anaerobic digester where readily digestible organics could be fermented without the cost of aeration. Reductive dechlorinations could also be accomplished anaerobically to facilitate more efficient aerobic degradation. Aerobic biological treatment in either up- or downward flow could result in >95% reduction of the toxicants, many being reduced to drinking water standards. Residual toxicants such as the final 0·2 mg/liter TCE could be removed in the polishing columns. This third stage could utilize biological treatment or chemical–physical means. Multiple polishing columns are depicted because biological treatment may require pulsed feeding with nutrients followed by a brief starvation and then toxicant degradation. Alternating between three polishing columns would enable simultaneous feeding, starving and toxicant degradation for continuous operations. Water exiting the polishing columns may be recycled through the system or utilized for in-situ bioremediation. Coupling of bioreactor designs enables complete detoxification of many contaminant mixtures.

The striped circles in Fig. 2 represent lesser contaminated subsurface aquifers suitable for in-situ bioremediation. Optimization of three-dimensional hydrological control and nutrient delivery may be accomplished by application of shallow horizontal wells. Horizontal wells may be suitable for upward or downward flow of nutrients during in-situ bioremediation and may additionally be suitable for vacuum extraction regimes. The open circles represent residual toxicant concentrations which could be degraded or immobilized by a down-gradient biological barrier. In addition to degrading organics, a biological curtain or wall at the plume boundary may be useful in binding or immobilizing heavy metals in exopolymers.

Previous studies and work presented here indicate the potential for bioremediation of environments contaminated with chlorinated ethenes and mixed organic wastes on-site and *in-situ*. This study suggests the use of both an expanded-bed and a single-pass packed-bed bioreactor to assess the feasibility of bioremediating a contaminated plume. Exploration of the degradative potential and the resiliency of the native microbial community could be examined in a recirculating expanded-bed bioreactor. The biodegradation rates associated with single toxicant and mixed organic waste degradation can be examined in single-pass packed-bed reactors. Lipid analysis provides useful information concerning protozoan grazing on toxicant degrading organisms as well as insight on the native population's ability to degrade TCE. Through complementing methodologies integrated bioremediation technologies could be realized which

remediate mixed organic wastes on-site rather than transferring the toxicants from one environment to another.

ACKNOWLEDGEMENTS

Research was supported by the Oak Ridge National Laboratory, US Department of Energy and the Savannah River Site. We thank Susan Pfiffner and Ken Malachowsky for technical assistance.

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