# BIOREMEDIATION POTENTIAL OF TOXICS BY MANIPULATION OF DEEP TERRESTRIAL SUBSURFACE ECOSYSTEMS

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## ABSTRACT

Mixed physiological types of bacteria in consortia recovered from subsurface contaminated sediments degrade mixed organic wastes containing carbon-rich (benzene, xylene, toluene) and halogenated hydrocarbon substrates (chlorobenzene, trichloroethylene, dichloroethylenes, vinyl chloride) in column bioreactors when provided with oxygen and methane and/or propane substrates. In expanded bed bioreactors degradation proceeds to 99% completion for several organic and chlorocarbon contaminants (60% for tetrachloroethylene) to carbon dioxide on repeated cycles in 21 days with little loss of volatiles in the control bioreactor except for a 70% loss of vinyl chloride in the control. Biodegradation is most efficient when the microbial consortia is maintained in a suboptimal nutritional state which can be monitored by ratios of endogenous storage lipid (poly beta-hydroxy alkanoic acid, PHA) to total phospholipid ester-linked fatty acids (PLFA). Under the best conditions the efficiency of biodegradation was 50-65 moles substrate (propane or propane + methane)/mole of TEC degraded. The microbial communities showed a rich diversity of microbes based on PLFA biomarkers. The effects of adding methane and/or propane in inducing specific subsets of the microbial community can readily be detected in the PLFA biomarker. Despite the presence of carbon rich substrates (benzene, toluene, xylene) in the mixed wastes, no evidence of plugging of interstitial spaces by exopolysaccharide was detected.

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Approximately 90 thousand tons of trichloroethylene (TCE) were used in the United States in 1985 (28) and quite possibly greater amounts in previous decades. As a consequence of this high usage, many subsurface aquifers are contaminated with chlorinated ethenes and some contain chlorocarbons in excess of 1,000 mg per liter (6, 7, 14). Many chlorocarbons are carcinogens or suspected carcinogens which are recalcitrant in many natural environments (5). Present technologies for reclaiming groundwater contaminated with chlorocarbons include pumping waters to the surface and either air-stripping or adsorbing the pollutants onto sorbents (8, 14). Processes which result in the mineralization of chlorocarbons rather than transferring the potential carcinogens to other media would be more desirable.

Biological degradation of TCE has been shown to occur with pure cultures and mixed microbial consortia (6, 7, 13, 16, 17, 23-30). Chlorinated alkenes may be converted anaerobically by reductive dehalogenation to known carcinogens such as vinyl chloride (1, 4, 12). Natural gas has been shown to stimulate aerobic TCE degradation in unsaturated soil columns (10, 30), and trickling filter reactors (25). Methanotrophic biomass in soil columns and reactors increased over time, TCE was degraded, and it was hypothesized that methanotrophs could be an important constituent in the TCE mineralization process (19, 25, 30). Pure cultures of methanotrophs capable of degrading TCE at concentrations of 1 mg $L^{-1}$ have been obtained (13) and methane mono-oxygenase appeared to play a key role in TCE oxidation. Methanotrophs and propane oxidizers contain unique phospholipid ester-linked fatty acid (PLFA) biomarkers which enables their monitoring within bioreactors (18, 19, 20, 24). It was also discovered that TCE degradation in methanotrophs and propane oxidizers was related to poly beta-hydroxyalkanoate (PHA) accumulation (20, 24). Addition of methane or propane to aerated subsurface soils from several widely dispersed test sites have always resulted in the appearance of the signature PLFA biomarkers of the type II methanotrophs or the actinomycetes type propane degraders. Thus it may be possible to monitor nutritional status of TCE degrading microbial consortia by phospholipid (PLFA) biomarkers and storage polymers (PHA).

Heterotrophic microorganisms have been shown to degrade TCE aerobically when enriched with an aromatic compound, such as toluene or phenol (16, 17). Toluene dioxygenase has been shown to be involved in oxidation of ug quantities of TCE during whole cell studies with Pseudomonas putida F1 (29). Heterotrophic consortia obtained from severely

contaminated subsurface sediments (6, 22, 23) degrade TCE at concentrations exceeding 100 mg $L^{-1}$ with propane, methanol or yeast extract as energy sources. Combining heterotrophic consortia with methane utilizing microorganisms enabled propagation of aerobic TCE degradation in bioreactors supplemented with methane or propane (21). The purpose of this report is to examine the potential of bioremediation with successful monitoring of complex microbial consortia capable of degrading chlorocarbons and mixed-organic wastes using gaseous hydrocarbons as the energy source.

## MATERIALS AND METHODS

### Gases, Chemicals and Microbial Inocula.

All gases were supplied from MG Industries (Chattanooga, TN). Propane and oxygen were greater than 99.5% pure and methane was greater than 98% pure. All chemicals were of reagent grade and were obtained from Mallinkrodt, Inc. (Paris, KY) or Sigma Chemical Co. (St. Louis, MO). Glass-distilled solvents and reagents were purchased from J. T. Baker Chemical Co. (Phillipsburg, NJ). Subsurface sediments were recovered from contaminated sites at Savannah River Site by methods and procedures previously described (6, 22).

Mixtures of propane and methane-oxidizing bacteria were obtained from Ada, OK (19), TCE-degrading consortia, which were capable of degrading TCE at concentrations in excess of 50 mg/L (6), were isolated from the Savannah River Plant, Aiken, SC and methanotrophs, which were capable of degrading TCE at concentrations less than 1 mg/L (13), were isolated from a waste disposal site near Oak Ridge, TN were utilized in this study. [1,2 $^{14}C$] TCE (10 mCi/mmol) was purchased from New England Nuclear Corp. (Boston, MA).

### Laboratory Techniques.

Construction, maintenance and operation of the bioreactors used in this study are described elsewhere (21). The liquid phase in the reactors was the same medium that was employed for maintaining TCE-degrading test cultures. The medium contained, per liter: $MgSO_4$, 0.055 g; $CaSO_4$ $2H_2O$, 0.054 g; $NH_4NO_3$, 1.48 g; trace minerals II, 10 mL (15); 10X vitamin solution, 1 mL (15); 200 mM phosphate/bicarbonate buffer solution, 10 mL and deionized water, 1000 mL and organic substrates. The specified were added as sterile solutions to the basal medium. Resazurin was added (1 mL of a 0.2% solution/L) to visually monitor the redox of the liquid phase.

The total liquid phase volume for the bioreactor experiments was 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 nominal liquid phase TCE concentration varied between 2.0 and 150 mg $L^{-1}$. Controls contained 0.2% sodium azide and 0.5% formalin to deter microbial contamination.

Analytical Procedures.

TCE was analyzed using a Hewlett Packard 5890 gas chromatograph equipped with a 50m Ultra Performance (Ultra 1, Hewlett Packard) cross-linked methyl silicone capillary column (0.2 mm, 0.33 um film internal diameter) and an electron capture detector (Hewlett Packard) as previously described (21). Vinyl chloride, cis- and trans-1,2-dichloroethylene (DCE) were analyzed using a Schimadzu GC-9A gas chromatograph equipped with an 2.44 m, 3.2 mm diameter Poropak T packed column and a photoionization detector (HNU Systems, Newton, MA). While methane and $CO_2$ were assayed using a Schimadzu GC-8A gas chromatograph equipped with a 2.74 m, 3.2 mm diameter Carbosieve 8000 packed column and a TCD detector. Propane was analyzed using a Schimadzu GC-9A gas chromatograph equipped with an 2.44 m, 3.2 mm diameter Poropak N packed column and a flame ionization detector as described elsewhere (21). The limits of detection for sampled compounds were as follows: TCE, 0.1 ug/L; cis-1,2-DCE, 10 ug/L; trans-1,2-DCE, 10 ug/L; Vinyl Chloride, 10 ug/L; propane, $CH_4$, and $CO_2$, 0.05% (v/v). Detection limits for chlorinated compounds are nominal liquid phase concentrations. Reproducibility was obtained at a signal to noise ratio (S/N) greater than 3.0.

Total phospholipids were extracted from frozen and lyophilized bioreactor contents by a modification (3) of the single phase chloroform-methanol method of Bligh and Dyer (2). Phospholipids were separated on silicic acid columns, fractions collected and subjected to methanolysis (3). Methylesters of the phospholipid fatty acids were analyzed by capillary-gas liquid chromatography (GC) as previously described (3, 9). The fatty acid methyl esters (FAME) were analyzed on a Hewlett Packard 5880 GC at 50°C in the splitless mode using a nonpolar cross-linked methyl silicone fused silica column (50 m x 0.2 mm internal diameter). Peak areas were quantified using a Nelson Analytical (Perkin Elmer) laboratory data system operated with an internal standard program as previously described (9). The structures of the PLFA and PHA were confirmed by gas chromatography/mass spectrometry using the derivatizations and methods described (9, 18, 19, 24). Fatty acids were designated by the total number of carbon atoms: number of double bonds followed by the position of the unsaturation from the aliphatic end. Suffixes c and t indicate cis and trans geometry while i represents iso-branching.

## RESULTS

### Enrichment of Subsurface Consortia.

The subsurface consortia capable of degrading TCE at concentrations of >100 mg $L^{-1}$ to less than 10 ug $L^{-1}$ and the mixed-organic wastes were obtained from a subsurface environment severely contaminated with TCE. As shown in Table 1, there was an area in the subsurface where biological activities could not be detected by radioisotope incorporation activity measures. The subsurface zone containing undetectable biological activity corresponded to high levels of TCE in the sediments. Groundwaters from nearby wells have exhibited TCE concentrations >200 mg $L^{-1}$. Above and beneath the biologically inhibited region were zones of intense biological activity. The TCE degrading consortia was enriched from sediments beneath the TCE plume in the 50-60 m depth sediments.

TCE degrading enrichments were successful under a variety of conditions (Table 2). Enrichments and transfer tubes typically contained 50 mg $L^{-1}$ TCE (assuming all TCE was in the liquid phase with a gas to liquid volume ratio of 2-3:1) and nutrient sources were added at approximately 10mM. TCE alone was not utilized as an energy/nutrient source for growth or degradation. Glucose stimulated TCE degradation aerobically and anaerobically in initial enrichments but TCE degrading activities were always lost upon subsequent transfers. Trypticase: yeast extract and propane were the most successful energy sources at providing stable TCE degradation with frequent transfers. As shown in Table 2, several consortia were capable of both 99% degradation at 50 mg $L^{-1}$ and significantly lowering the initial concentration 150 mg$L^{-1}$ TCE over a 6 week period.

Aerobic degradation of TCE proceeded to carbon dioxide as demonstrated by GC-GPC results (Figure 1). By chromatographically separating carbon dioxide from TCE we could successfully demonstrate radioactive carbon dioxide formation without the use of trapping agents. Greater than 60% of the TCE could be accounted for by $^{14}CO_2$ from radiolabeled $^{14}C$ TCE experiments.

### Bioreactor Experiments.

Figure 2 shows the design of continuous recycle expanded-bed bioreactors used for TCE degradation studies. Previous studies performed with the bioreactor have indicated that in the absence of added microbial cultures, over 98±2% of the TCE added to the systems at nominal liquid concentrations of 20 mg/L was still present after 5 days. Experimental reactors inoculated with test cultures did not show any indication of TCE loss until several weeks after inoculation.

Table 3 shows TCE degradation after 9 days in either the methane or propane stimulated aerobic reactors. After nine days of recirculation 95% of the TCE in the inhibited control reactor remained. In contrast, 50-75% of the TCE was removed from the test reactors, propane and methane were consumed and carbon dioxide produced.

In other experiments (data not shown) reactor 3 displayed a over 86% loss in TCE, while reactor 2 showed over 43% loss after 5 days in pulsed feeding experiments where reactors were fed on Day 0 only. Reactor 2 was fed only on day zero with 5% methane in the headspace and 200 mg $L^{-1}$ tryptone/yeast extract (YE/TRY) while reactor 3 initially contained 5% methane and 3% propane (v/v) in the headspace. Reactor 1 (control) showed no loss of analytes. The liquid phase pH remained at 7.2 in all reactors throughout the time course of the experiment. The results of continuous substrate experiments (data not shown) indicated 90% and 85% removal of the initial TCE added to reactors 2 and 3 respectively after five days. Carbon dioxide production increased as compared to the pulsed addition experiments. When substrate was not added to the reactors (starvation), the rates of TCE removal were less than 10% of those seen in either the "propane only" or mixed substrate propane/methane experiments (data not shown). When the medium pH was changed from 7.2 to 7.5, only 15% of the initial TCE concentration was degraded (data not shown). The amount of methane and propane utilized and as the amount of carbon dioxide produced also declined sharply.

Degradation of mixed organic wastes by the hydrocarbon fed consortium in the bioreactors is shown in Table 4. In this experiment the bioreactors were fed propane and the medium was replaced with waters directly recovered from a well in the outflow of a hazardous waste site. The water was contaminated with a variety of hydrocarbons, some of which were growth substrates and some of which were not. A variety of chlorinated compounds were degraded, most to the detection limits of the assays employed. 60% of the tetrachloroethylene in the mixture was degraded in the bioreactors despite the fact that cell suspension or growth cultures isolated from the bioreactors never degraded it. 91% of the TCE was degraded in the bioreactor. All other contaminants shown were degraded by more than 99%. Benzene, toluene and xylene were degraded and these results have been corroborated in cell suspension and growth cultures (data not shown). With the exception of vinyl chloride the control reactors showed little substrate loss.

No volatile TCE intermediates were detected in either of the test reactors during any of the experiments performed. The control reactor typically contained 0.5 mg $L^{-1}$ of trans-1,2-DCE and 20 ug $L^{-1}$ vinyl chloride after five days. This was possibly a result of chemical

decomposition of TCE in the presence of the microbial growth inhibitors in the control reactor liquid phase (sodium azide, formalin), or trace contaminants found in the reagent TCE used in this study.

PLFA profiles of the microorganisms residing within the reactors are shown in Table 5. The presence of Type II methanotrophs was demonstrated by 18:1w8c and 18:1w7t PLFA (18, 19). Actinomycete type propane degrading microorganisms were evidenced by high levels of 10 Me18:0 in the PLFA (24) and evidence of a small eukaryotic community was shown by a preponderance of 20 carbon fatty acids (3, 9). Both bioreactors exhibited diverse microflora corresponding to approximately 20 grams dry weight of cell mass or $2 \times 10^9$ cells per gram of substratum.

Efficiency of TCE degradation may be judged by the TCE degraded per mole of substrate or the TCE degraded per gram of biomass each day. Table 6 compares variations of efficiency in cell suspensions and expanded bed bioreactors with those of methanotrophic trickling filters. Induced cell suspensions required fewer moles of substrate per mole TCE degraded. Thick biomass mats of trickling filters probably contributed to the lower metabolic efficiencies (100-500 moles substrate per mole TCE degraded). Expanded bed bioreactors maintained their activity for months while requiring only 50-160 mole of substrate per mole TCE degraded. Expanded bed bioreactors containing the subsurface consortia degraded up to 80 mg TCE each day per gram dry weight of cells, approaching 10% of their dry weight each day. Cell suspensions were nearly as efficient . The thick biomass films of trickling filters resulted in lower biomass efficiencies.

## DISCUSSION

Results of these experiments demonstrate that bioreactors containing aerobic mixed culture consortia recovered from contaminated subsurface environments are capable of biologically mineralizing TCE and its products in the presence of other organic contaminants while utilizing propane or methane/propane energy sources. The waste water utilized in the experiments reported in Table 4 were taken directly from a well in the contamination outflow of a hazardous waste site. Control reactors retained 98±2% of the initial TCE added following 5 days of operation establishing that the losses were the result of biodegradation and not leakage of volatile contaminants. Vinyl chloride in the bioreactors leaked. In sealed suspension tests vinyl chloride was readily degraded and quantitatively retained in the controls.

In terms of TCE degradation efficiency (substrate

utilized/TCE degraded), propane when employed as the sole carbon source was the most efficient when considering the total amount of TCE removed from the system during any of the daily substrate replenishment experiments. When the bioreactors were starved (no substrate added), TCE degradation decreased significantly. This finding is in accord with the results presented by other researchers (6, 13).

Pulsed substrate additions (fed, starved conditions) showed that the bioreactors had a substrate/TCE degraded ratio of approximately 55 when degrading over 85% of the initial nominal liquid phase TCE concentration of 20 mg $L^{-1}$. In pulsed and daily substrate replenishment experiments, the threshold TCE concentration was approximately 0.5 mg/L. This limit of TCE degradation was observed if experiments were allowed to proceed for 8 to 10 days. At the present time the apparent "threshold" level of 0.5 mg $L^{-1}$ in the recycling bioreactor is not clearly understood as the same consortia when utilized as cell suspensions in sealed tubes reproducibly degrade 99.9% of the TCE present at liquid phase concentrations of 20 mg $L^{-1}$ (data not shown).

In previous studies involving methane or natural gas stimulation of degradation, TCE was converted to $CO_2$, cell-bound material or water-soluble products (13, 16, 25, 30). The methanotrophic biomass increased in methane-enriched soil columns which led workers to believe that methanotrophs played a major role in decreasing the TCE concentration (13, 20, 24, 30). It is believed that the monooxygenase of methanotrophs can oxidize and dechlorinate TCE (13). Several researchers have observed chlorinated alkene biodegradation in methane or natural gas-enriched mixed cultures obtained from various environmental sources (7, 10, 13, 24, 25, 30). Our results suggest that propane or propane amended methane may be a suitable substrate for promoting TCE degradation and mixed microbial consortia may offer stability and promise for pilot scale studies. Propane-oxidizing bacteria can epoxidate ethylene (11) and investigators have hypothesized that the enzymes which epoxidate ethylenes may transform TCE (30).

The most exciting studies show that the consortium recovered from the subsurface was able to degrade the mixed organic wastes with both the carbon-rich substrates and the chlorinated hydrocarbons that require suboptimal microbial growth conditions. The substrates in solution used for the studies reported in Table 4 were recovered directly from the well-head from a hazardous waste site and applied to the column.

Technologies that define the nutritional status of the microbiota which optimizes the biodegradation of volatile chlorinated hydrocarbons can be modified from analyses like

those illustrated in Table 5. Recovery of biofilms on coupons incubated in test wells in areas of in situ bioremediation could be examined for the presence of specific PLFA. The ratios of incorporation of isotopes into PLFA and PHA can, in some cases, be correlated to ratios of protein to PHA detected non-destructively by using diffuse reflectance infrared (Fourier Transforming) (DRIFT) microscopy. Coupons lyophilized in the field could be rapidly examined by the DRIFT in the field and correlated with remediation effectiveness and provided a potential monitor for control of the co-metabolic break down of certain products. Shifts in the microbial community structure could be correlated to shifts in frequency and activity of specific genes measured by nucleic acid probes once the microbial ecology of these complex consortia is understood.

Previous studies and the work presented here indicate the potential for bioremediation of subsurface environments contaminated with both chlorinated ethenes and carbon rich organic substrates. Figure 3 shows a diagram of how a bioremediation plant could operate at a site containing mixed organic wastes. The highly concentrated wastes could be pumped to the surface where they are treated in a series of bioreactors culminating in high quality water. An initial anaerobic reactor would foster reductive dechlorination and oxidation of readily degradable organics. These reactions would occur without the cost of extensive aeration. After primary treatment, water could enter trickling filters where pollutants could be lowered to the 500 ug $L^{-1}$ range as seen in the bioreactor utilized in this study. Tertiary treatment could include polishing bioreactors where trace levels of contaminants would be removed. Redesigned air-stripping apparatus could be utilized as bioreactors for removing organic materials from effluents gases. In less contaminated portions of the subsurface in-situ treatments could be used in conjunction with horizontal wells for nutrient delivery and vacuum extraction. At the plume fringe a biological curtain or wall could be generated by stimulating in situ exopolymer formation to redirect ground water circulation or to bind metals in exopolymers. Through complementing methodologies integrated bioremediation technologies could be realized to mineralize toxic wastes rather than transferring toxicants from one environment to another.

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Table 1. Activities of microorganisms from sediments contaminated with trichloroethylene.

| Depth (m) | [$^{14}$C]Acetate Incorporation into lipids (dpm)[a] | [$^{3}$H]Thymidine Incorporation into DNA (dpm)[b] | Trichloroethylene concentration (ug per kg soil)[c] |
|---|---|---|---|
| 3 | 147 | 1700 | - |
| | 820 | 0 | - |
| 10 | 7600 | 0 | - |
| | 180 | 1900 | - |
| | 660 | 12,000 | 670 |
| 20 | 680 | 4200 | 320 |
| | 2,000 | 11,000 | 190 |
| | 13,000 | 0 | 250 |
| 30 | 130,000 | 0 | 16,000 |
| | 0 | 0 | 310,000 |
| | 0 | 0 | 42,000 |
| | 0 | 0 | 59,000 |
| 40 | 0 | 0 | 361,000 |
| | 0 | 0 | 373,000 |
| | 290 | 0 | 38,000 |
| 50 | 82 | 0 | - |
| | 170,000 | 74,000 | - |
| | 30,000 | 1100 | - |
| 60 | 210,000 | 36,000 | - |
| | 2200 | 3800 | - |
| | 130 | 0 | - |
| | 53,000 | 0 | - |
| 70 | 2300 | 1600 | - |
| | 250 | 2100 | - |
| 75 | 0 | 2000 | - |

a. Incorporation of [$^{14}$C]acetate into lipids expressed as dpm/day determined from duplicate time course experiments.
b. Incorporation of [$^{3}$H]thymidine into macromolecules expressed as above.
c. TCE results from an independent source. Nearby monitoring wells screened at 50-65 m depth contained TCE at concentrations exceeding 20 mg $L^{-1}$.

Data from Phelps et. al., 1989, Geomicrobiology J. 6: 157-170.

Table 2. Aerobic and anaerobic degradation of TCE by microbial consortia utilizing a variety of energy sources.

| Aerobic Energy source | 50 mg $L^{-1}$ TCE (percent degraded) | 150 mg $L^{-1}$ (percent degraded) |
|---|---|---|
| Methane | 21-91 | 23 |
| Acetate | 21-99 | 18 |
| Trypticase:yeast ext. | 75-99 | 37 |
| Glucose | 0-93[a] | 18 |
| Methanol | 34-99 | 0 |
| Propane | 24-99 | 8 |
| Propane plus methane | 24-95 | - |
| TCE | 0 | 0 |
| **Anaerobic Energy source** | | |
| Glucose | 98[a] | - |
| Methanol | 95 | - |

a. Upon subsequent transfers glucose fed cultures lost TCE degrading capabilities.

Cultures were incubated at ambient temperature 4-6 weeks prior to analysis.

Data summarized from Fliermans et al., 1988, Appl. Environ. Microbiol., 54: 1709-1714 and Phelps et. al., 1989, Geomicrobiol. J. 6: 157-170.

Table 3. Degradation of TCE in bioreactors fed methane and propane.

| Sample | TCE (mg $L^{-1}$) | Methane (mmol $L^{-1}$) | Propane (mmol $L^{-1}$) | $CO_2$ (mmol $L^{-1}$) |
|---|---|---|---|---|
| Day 0 | | | | |
| Control 1 | 20 | 2.25 | 0.93 | ND |
| Reactor 2 | 20 | 2.25 | 0.91 | ND |
| Reactor 3 | 20 | 2.25 | ND | ND |
| Day 9 | | | | |
| Control 1 | 19 | 2.25 | 0.93 | ND |
| Reactor 2 | 5 | 0.66 | ND | 0.91 |
| Reactor 3 | 9 | 0.23 | ND | 0.89 |

ND = not detectable.
Reactor 1 was an uninoculated control inhibited with 0.5% formalin and 0.2% sodium azide. Reactors 2 and 3 contained TCE degrading consortia. Data from Niedzielski et. al., 1989, J. Microbiol. Methods (in press).

Table 4. Degradation of mixed organic wastes by propane fed microbial consortia.

| Organic Wastes | Concentration Day 0 (mg $L^{-1}$) | % Loss in Control, Day 21 (%) | Average loss in bioreactors (%) |
|---|---|---|---|
| Vinyl Chloride | 4.0 | 70 | >99 |
| 1,1-dichloroethane | 1.1 | 25 | >99 |
| 1,1-dichloroethylene | 0.7 | 2 | >99 |
| 1,2-dichloroethylene | 3.0 | | |
| 1,1,1-trichloroethane | 1.2 | 22 | >99 |
| trichloroethylene | 2.6 | 0 | 91 |
| tetrachloroethylene | 2.1 | 0 | 60 |
| Benzene, xylene, tol. | 1.4 | 30 | 99 |

Analyses performed in accordance with EPA procedure 624.

Table 5. Significant phospholipid fatty acids from bioreactor contents.

| PLFAME[1] | Mole % Reactor PM | Reactor SM |
|---|---|---|
| i15:0 | 2.0 | 1.4 |
| 15:0 | 2.2 | 2.2 |
| 16:1w7c | 2.9 | 4.3 |
| 16:1w7t | 1.5 | 1.4 |
| 16:0 | 7.0 | 8.4 |
| cy17:0 | 3.2 | 3.1 |
| 17:0 | 1.5 | 2.3 |
| 18:0[2] | 7.1 | 8.8 |
| 18:1w9c | 3.8 | 4.1 |
| 18:1w8c/t | 15.1 | 16.2 |
| 18:1w7c | 13.1 | 11.3 |
| 18:1w7t | 0.6 | 0.8 |
| 10me18:0 | 4.6 | 4.5 |
| cy19:0 | 23.4 | 17.9 |
| 20:4w6 | 0.7 | 0.5 |
| 20:0 | 1.2 | 1.3 |
| Cell mass mgdw/reactor.[2] | 25 | 16 |

1. Analyzed as phospholipid-fatty-acid methyl-esters (PLFAME).
2. Assuming 100 umoles of PLFAME per g dry weight of cells with 70 g substratum per reactor.

Table 6. Variations in efficiency of trichloroethylene degradation by microorganisms.

| Microbial system | Metabolic efficiency (mol substrate per mol TCE) | Daily biomass efficiency (mg TCE per gram dry wgt. cells) |
|---|---|---|
| Mixed consortia cell suspension | 20-30[a] | 10-20 (60) |
| Mixed consortia expanded bed | 50-160 | 15-40 (80) |
| Methanotrophic[b] trickling filter | 100-500 | 0.5-3 |

a. Typical range of values with the maximum vale in parenthesis.
b. Data from Strandberg and Donaldson, 1989 E.S.T. (in press).

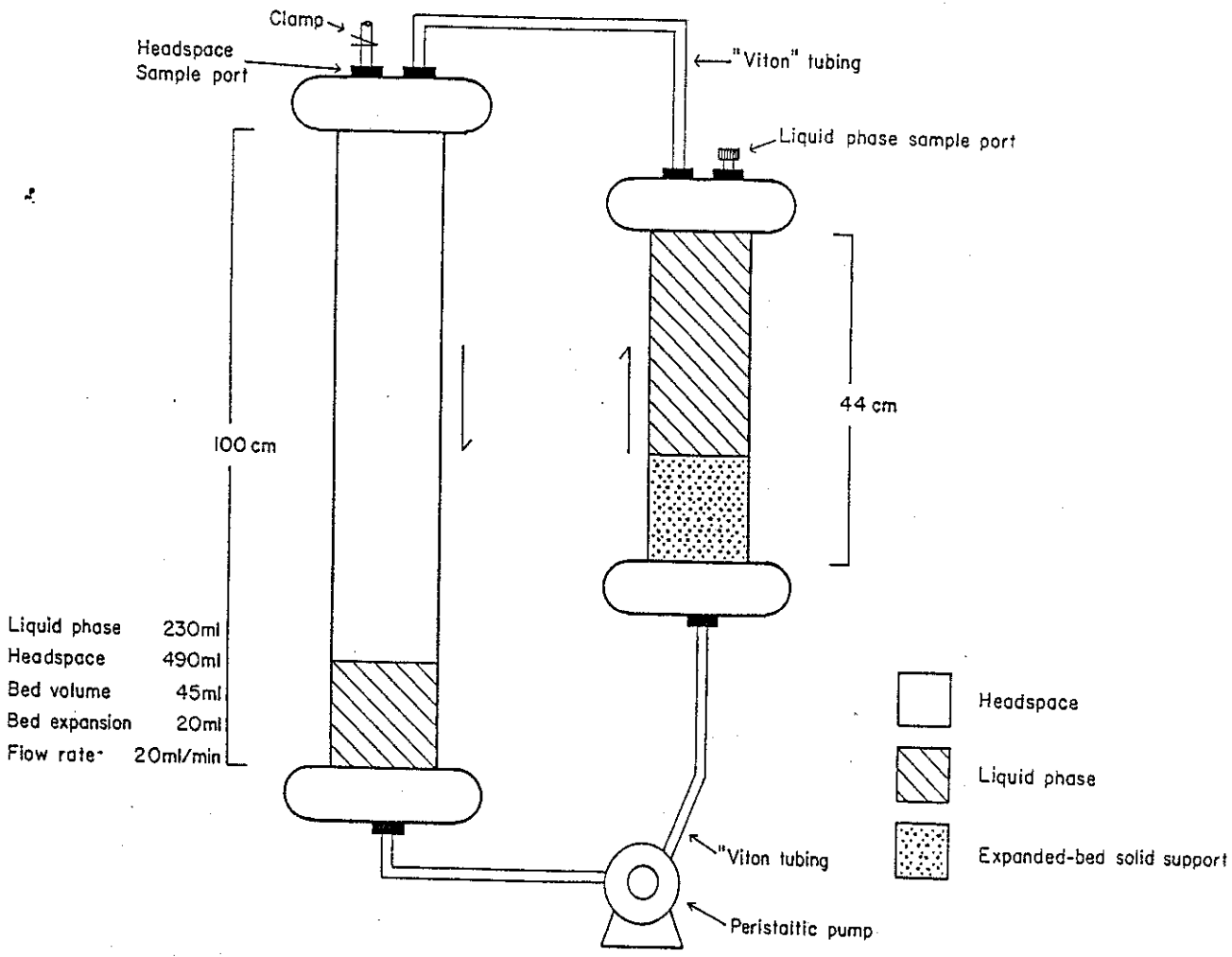


Figure 2. Diagram of the expanded bed reactors used for the degradation of TCE and mixed organic wastes. From Niedzielski et al., J. Microbiol. Methods, 1989 ( in press).

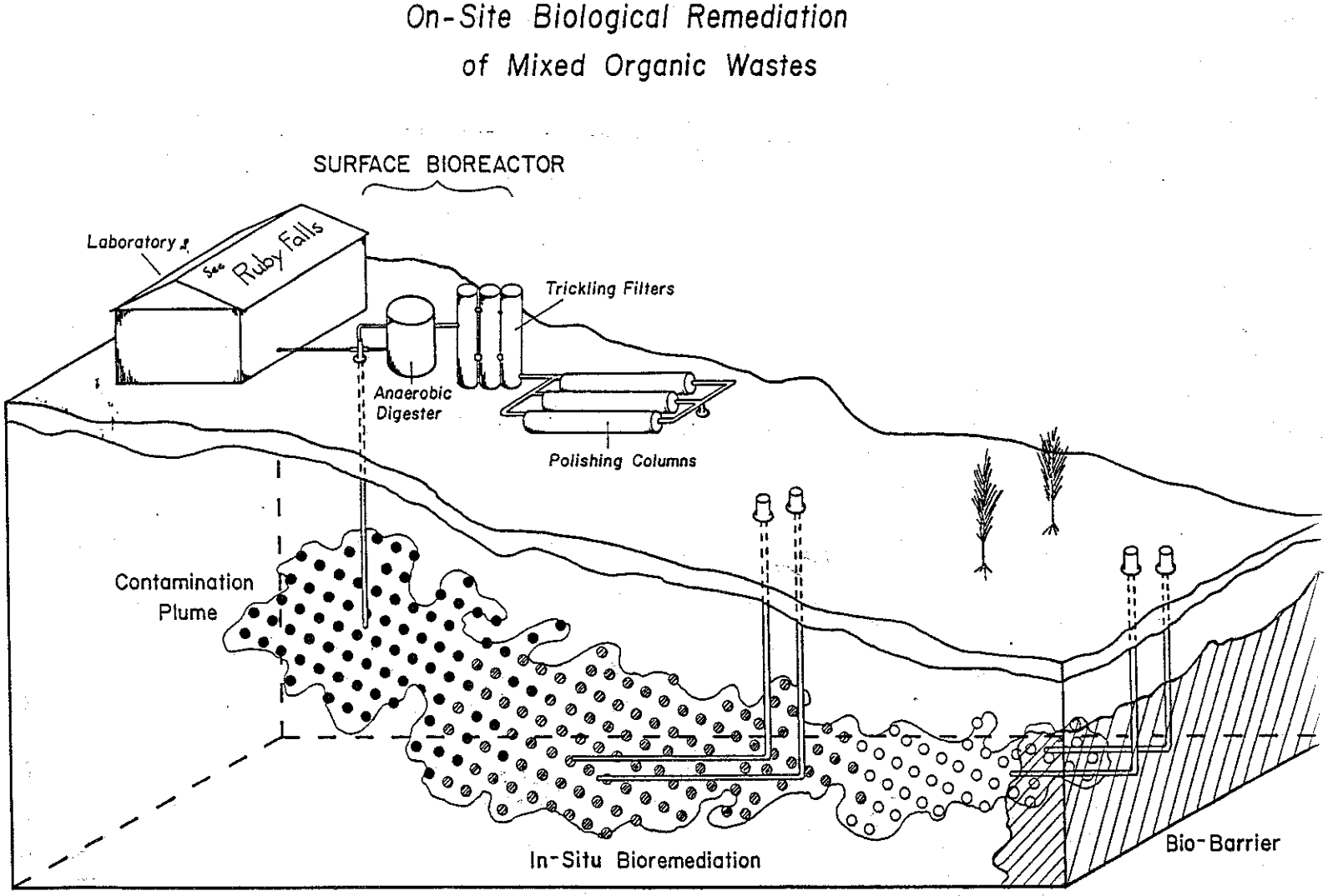


Figure 3. Schematic of bioremediation at a contaminate site. Surface bioramediation treatments would be combined horizontal well grids for in-situ treatments and a biological barrier preventing spread of plume.