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Robert S. Kerr
Environmental Research Laboratory
Ada, OK 74820

Research and Development

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Project Summary

Innovative Processes for Reclamation of Contaminated Subsurface Environments

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Research to better assess the capabilities and limitations of fixed-film bioreactors for removing selected organic contaminants from ground water or from contaminated vapor streams produced by air stripping of polluted ground water and by soil venting operations is described. Work was focused on volatile chlorinated aliphatic hydrocarbons and light aromatic constituents of distilled petroleum products, two groups of compounds which have been identified in polluted ground water more frequently and usually in higher concentrations than other organic pollutants. Laboratory scale fixed-film bioreactors containing soil or diatomaceous earth materials were employed to study the cometabolic removal of trichloroethylene and related compounds from aqueous and vapor streams by biofilms sustained on primary substrates consisting of gaseous aliphatic hydrocarbons. Additionally, the removal of alkylbenzenes from air streams by biofilms using these compounds as primary substrates was examined in laboratory scale soil bioreactors. The biodegradation processes involved and the effects of bioreactor operating parameters and systems configurations on contaminant removal were evaluated. Results obtained indicate a significant potential for utilization of fixed-film bioreactors in systems for above ground treatment of contaminated ground water and vadose zone gases.

This Project Summary was developed by EPA's Robert S. Kerr Environmental Research Laboratory, Ada, OK, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

Introduction

Ground water is the primary source of drinking water in the United States, with estimates of approximately 80 percent of all drinking water supplies obtained from ground water sources. Historically, ground water has been used for drinking water without major treatment other than removal of minerals and final disinfection. During the past few years, however, hundreds of synthetic chemicals have been detected in a significant number of drinking water supplies, thus raising concerns about the potability and purity of such waters.

Of the many organic chemicals that have been detected in polluted ground water, two groups of compounds have been identified more frequently and usually in higher concentration than any others. These are volatile chlorinated aliphatic hydrocarbons, such as trichloroethylene (TCE) and related substances, and light aromatic constituents of distilled petroleum products. Effective methodologies for removing these pollutants from contaminated subsurface environments and restoring the quality of polluted ground waters are urgently needed.

This report describes research conducted to better define the feasibility of treatment methodologies employing fixed-film bioreactors for removal of volatile chlorinated and light aromatic hydrocarbons from aqueous and vapor streams contaminated with these substances. Such methodologies would have significant utility for above ground treatment of polluted ground water and of gaseous streams produced by air stripping of ground water or by soil venting operations. The work consisted of three segments, conducted concurrently. In one segment, treatment of trichloroethylene (TCE) in solution in water by biofilms sustained on a primary substrate of methane or natural gas was studied, using laboratory scale bioreactors containing sand or beryl saddles as supporting medium for the biofilm. Another segment of the research effort examined vapor phase treatment of TCE and 1,1,1-trichloroethane (TCA) in laboratory scale bioreactors containing biofilms sustained by butane and supported on diatomaceous earth materials. The third segment consisted of studies of the vapor phase treatment of selected alkylbenzenes in soil bioreactors by biofilms utilizing these compounds as primary substrates.

Liquid Phase Treatment of Trichloroethylene

The removal of TCE from contaminated aqueous streams was studied in laboratory packed column bioreactors such as that depicted schematically in Figure 1. Bioreactors were designed so all surfaces in contact with feed water solutions containing TCE were either glass or Teflon. Columns (6 x 61 cm) were packed with rewashed coarse sand (specific surface area of 1599 or 673 ft^2/ft^3) for most experiments, although beryl saddles were used as the support for the biofilm in one case. The systems were operated in the unsaturated mode, with liquid flow rates ranging between 1.9 and 8.4 ml/min (equivalent to hydraulic loadings between 4.3 and 19.6 $ft^3/day/ft^2$). Biofilms of methanotrophic microorganisms capable of cometabolizing TCE were established in the reactors, utilizing enrichment cultures prepared from Lincoln fine sand. Influent water was amended with nitrogen and phosphorus to enhance methane utilization and biofilm development. After appropriate acclimation, the systems were operated over a range of hydraulic and organic loadings, during which bioreactor performance was assessed by monitoring influent and effluent concentrations of methane and TCE, as well as flows of water and air.

TCE removals in the bioreactor columns packed with coarse sand generally ranged from 20 to 60 percent for most of the experimental conditions examined in this study. The specific percentage of removal appeared to be a function of several interrelated parameters, including particularly the influent TCE and methane concentrations, the packing media for the columns, and water flow.

The percentage of TCE removed in the bioreactors was found to increase significantly with increasing influent concentrations up to at least 500 $\mu g/L$ of TCE, as shown by Figure 2. However, when TCE influent concentrations approached 1000 $\mu g/L$, removal was observed to be drastically reduced in bioreactors constructed from either of the coarse sand supporting media. Evaluation of methane utilization in bioreactors receiving various input concentrations of TCE revealed that significant inhibition of methanotrophic microorganisms comprising the biofilm was likely to occur when TCE levels were in the vicinity of 1000 $\mu g/L$ or higher, as indicated by Figure 3. Inhibition appeared to be related to influent methane concentration, with biofilms sustained on low concentrations of methane appearing to be most susceptible. Although complete inhibition of methane utilization did not occur until influent TCE concentrations exceeded 1500 $\mu g/L$, the ability of the microbes to cometabolize TCE was apparently lost completely when their capability for metabolizing the primary substrate was only partially compromised.

Influent methane concentrations ranging from less than 1 mg/L to more than 15 mg/L in air delivered to the bioreactors were observed to sustain biofilms capable of removing TCE in the bioreactors. The percentage of TCE removed increased with increasing influent methane concentration for influent TCE concentrations below inhibitory levels, as shown in Figure 4. However, the rates of increase were relatively small, so the specific mass of TCE removed per unit mass of methane consumed was less at higher methane concentrations. Nevertheless, significantly higher removals of TCE were attained in bioreactors receiving higher influent levels of methane, provided other operating parameters were the same. This is illustrated in Figure 2, which presents TCE removals at varying influent TCE concentrations for systems operating under identical conditions except for influent methane concentrations, which were either 3.4 or 13.4 mg/L in air.

In one set of experiments, natural gas was substituted for pure methane as primary substrate for the microbes com-

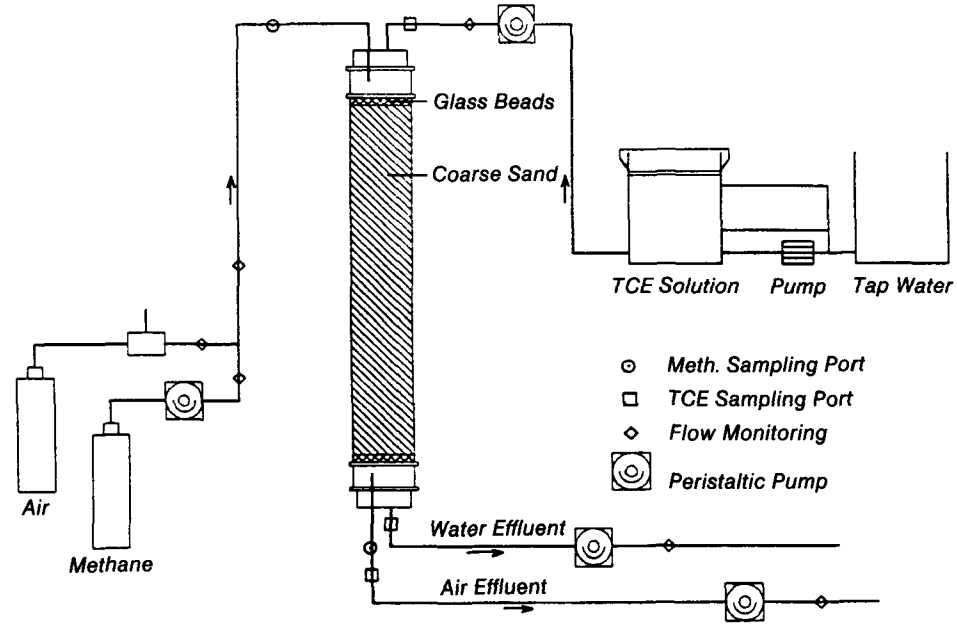


Figure 1. *Schematic of fixed-film bioreactor.*

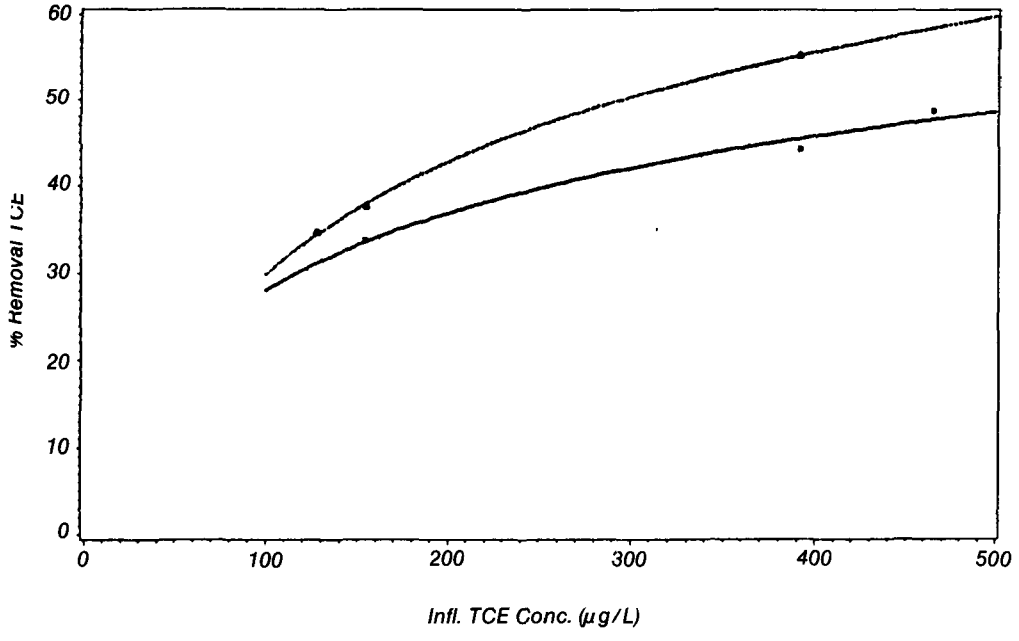


Figure 2. *Effect of influent TCE concentration on TCE biodegradation. (Water flow: 4.1 mL/min; Air flow: 7.5 mL/min; Influent methane concentration: 3.4 mg/L; Media: Sand 3).*

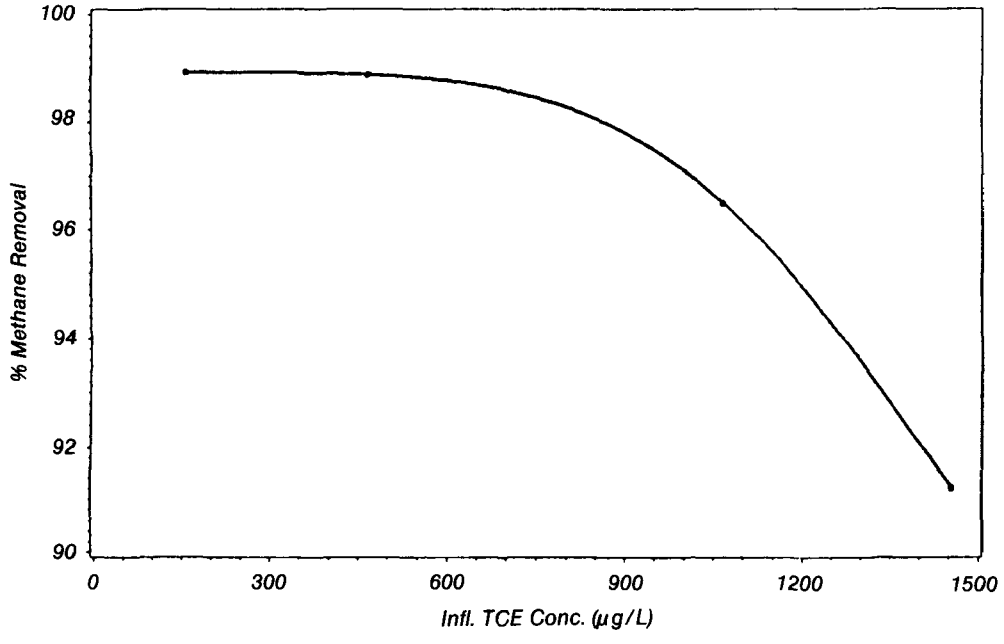


Figure 3. *Inhibition of methanotrophs by TCE for an influent methane concentration of 3.2 mg/L.*

prising the biofilm. Although the data obtained were not sufficient for definitive conclusions, removals of TCE in bioreactors operating on natural gas appeared to be at least as good as removals attained in those operating on pure methane. Especially at influent TCE concentrations in excess of 900 µg/L, removals in the natural gas bioreactors seemed noticeably higher. This may reflect less susceptibility of biofilms sustained on natural gas to inhibition by high TCE concentrations. Such biofilms would be expected to be composed of more diverse microbial population than biofilms developed on pure methane.

Changes in TCE removal by the bioreactors were also noted when water flow rates were varied. For bioreactors packed with the sand of higher specific surface area and, hence, having longer unsaturated hydraulic retention times, percentages of TCE removed increased steadily as water flow was increased in the range of 3.8 to 6.2 mL/min (8.9 to 14.5 $ft^3/day/ft^2$). This was believed to be the result of reduced resistance to mass transfer at the higher flow rates. Highest rates of TCE removal in bioreactors packed with the sand of lower specific surface area seemed to be achieved when water flow was in the neighborhood of 4 mL/min (9.3 $ft^3/day/ft^2$). This probably reflected the negation of beneficial effects of improved mass transfer at higher flow rates by deleterious effects of decreased contact time resulting from shorter unsaturated hydraulic retention times in these columns.

Both of the coarse sands utilized as packing media in these studies served relatively effectively as supports for biofilms capable of cometabilizing TCE, although the operating parameters to achieve optimum TCE removal were different for each, as illustrated by the observations concerning water flow rates noted above. Bioreactors packed with the sand of greater surface area were found to be somewhat more susceptible to clogging by excessive biomass when influent concentrations of methane were very high. In limited studies with bioreactors packed with beryl saddles as biofilm support media, much higher percentages of low influent concentrations of TCE were removed than in comparable systems packed with coarse sand. This may have resulted from better distribution of the microorganisms throughout the length of the beryl saddles column, with concomitant longer contact of the biofilm with the TCE-laden water. However, at high TCE concentration (910 µg/L), removal was significantly less in the beryl saddle system, possibly indicating increased susceptibility of the biofilm to inhibition by TCE.

Changes in the configuration of the bioreactor systems were also investigated in order to improve the effectiveness of the systems for removing TCE from influent water streams. This included operating two columns in series and operating single columns with multiport injection of the primary substrate, methane. Standard fixed-film bioreactor columns (Figure 1), packed with coarse sand and modified as appropriate, were used.

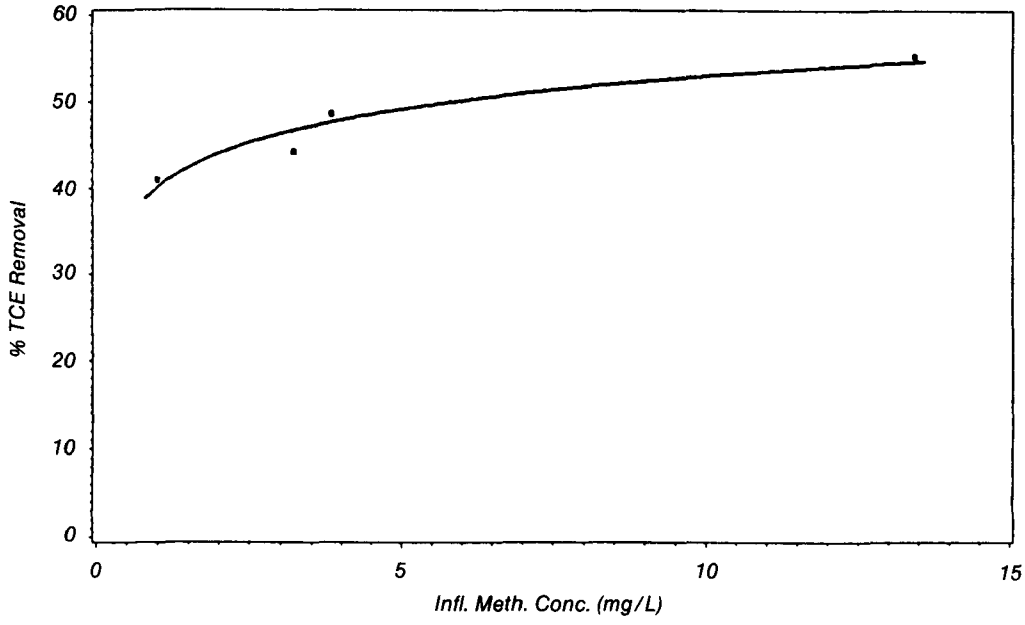


Figure 4. *Effect of influent methane concentration on TCE biodegradation. (Water flow: 4.1 mL/min; Air flow: 7.6 mL/min; Media: Sand 3).*

In the systems constructed with two bioreactor columns in series, effluent liquid and vapor streams from the first column were introduced directly onto the top of the second column, together with sufficient methane to achieve an influent methane concentration in the second column equal to that in the first column. Since the effluent from the first column usually contained essentially no methane, the total mass of this compound introduced to the system was double that introduced in a single column system.

Multiport injection systems consisted of single bioreactor columns modified to achieve introduction of methane simultaneously at the top and at points one-third and two-thirds down the length of the column, as shown in Figure 5. The objective was to obtain a more uniform distribution of the biofilm throughout the supporting media. An examination of columns receiving methane only at the influent end revealed most of the biofilm development occurred within the first 8 to 15 cm of packing. The total mass of methane introduced to a multiport injection column was the same as that introduced to a single column receiving methane only at the influent end.

As Table 1 shows, removals of TCE achieved both in bioreactor systems constructed with two columns in series and with single columns with multiport injection were significantly higher than removals achieved by single column systems in which methane was introduced only at the influent end. The data presented, representing two sets of experiments using different influent concentrations of TCE, indicate that columns in series can provide 60 to 65 percent increases in TCE removal over that attainable in single columns with single point methane injection, while single columns with multiport injection of methane can be expected to remove 27 to 30 percent more TCE than single column, single point injection systems. Systems constructed with single bioreactor columns with multiport methane injection may be especially promising because they appear to provide significant increases in TCE removal essentially without any requirement for additional equipment or supplies.

Vapor Phase Treatment of Trichloroethylene and 1,1,1-Trichloroethane

The cometabolic removal of TCE and TCA from contaminated air streams was studied in bioreactors using two sizes of Manville's Celite diatomaceous earth products (R630 and R635) as the solid support for microbial growth and butane as the primary substrate. The bioreactors were constructed using borosilicate glass columns 60 cm long by 5 cm in diameter. The headspaces of the columns were plumbed to receive vapors of TCE and TCA, a mixture of butane in air, and a nutrient media solution. To inoculate the columns, a slurry of Rollin muck soil previously adapted to *n*-butane was circulated until substantial removals of butane were observed at approximately 2.5 weeks.

Addition of chemical began on day 18 with influent concentrations of 90 μg TCE/L air, 200 μg TCA/L air, and 8 mg butane/L air. Influent and effluent concentrations of butane, TCE, and TCA were monitored daily. Respective removals of TCE, TCA, and butane in the bioreactor with the R635 solid support were 82 percent, 93 percent, and 65 percent on day 19. Removals on day 19 for the bioreactor utilizing the R630 support were 74 percent for TCE, 76 percent for TCA, and 59 percent for butane. On day 28, the influent concentrations were increased to 770 μg TCE/L air and 990 μg TCA/L air. At these influent concentrations, maximum removals for TCE and TCA ranged from 16 to 25 percent with both solid supports.

Density estimates of bacteria and protozoa in the recirculated fluids were determined by acridine-orange direct counts. The recirculated water contained a bacterial count of 1.8×10^{10} /mL. The water also contained approximately 6400 protozoa/mL.

No products of biotransformation were determined in this study. The probable intermediates of oxidation of low molecular weight alkanes such as methane, propane, or butane are readily degradable alcohols and ketones. Recent work has shown 2,2,2-trichloroethanol is the chlorinated intermediate of TCA oxidation. The oxidation of chlorinated ethylenes most likely yields an epoxide with rapid conversion to biodegradable hydrolysis products.

Vapor Phase Treatment of Alkylbenzenes

The objective of this study was to examine various soils for their ability to remove aromatic hydrocarbon vapors from waste air streams. Three soils of differing textures were tested for their ability to remove vapors of benzene, toluene, ethylbenzene, and *o*-xylene. The soils examined were Rubicon sand from Traverse City, Michigan; Durant loam from

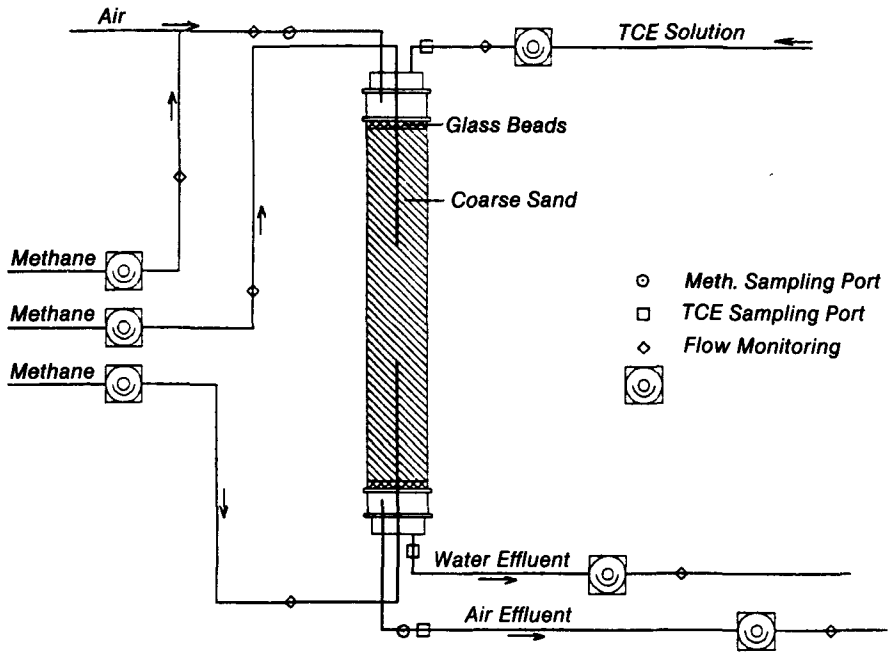


Figure 5. *Schematic of bioreactor with intermediate methane sampling ports.*

Table 1. *Removal of TCE by Bioreactors Incorporating Single Column, Two Columns in Series, and Single Column with Multiport Injection*

Bioreactor Configuration	Influent TCE Conc. mg/L	Influent Water Flow mL/min	Air Flow mL/min	Methane Conc. mg/L	Methane Removal %	TCE Removal %
Single Column	420	6.0	6.4	13.5	99.8	33.6
Two Columns in Series	420	6.0	6.5	13.3	99.8	54.0
Single Column with Multiport Injection	420	5.9	6.6	13.8	99.6	44.1
Control Column	420	6.0	6.1	—	—	0.0
Single Column	267	5.9	6.8	13.5	99.8	26.8
Two Columns in Series	267	5.9	7.0	13.6	99.8	44.2
Single Column with Multiport Injection	267	5.9	6.7	14.2	99.7	34.1
Control Column	267	6.0	6.2	—	—	0.0

Ada, Oklahoma; and Dougherty sand from Stratford, Oklahoma. The variables of hydrocarbon loading rate, soil type, and soil moisture were examined to determine their influence on removal efficiencies. The soils were originally acclimated in soil columns and then used for batch microcosm studies. Reaction constants were developed from the batch experiments.

A 7.6 cm internal diameter beaded process pipe was used to construct the soil columns. The column length varied from 86.4 cm to 96.5 cm. The columns were filled with soil, then an air stream was introduced into the bottom of the column to establish flow up through the bioreactor. Hydrocarbon removal was determined by comparison of the inlet and outlet concentration. Flow rates were measured prior to each sampling period. Upon completion of the studies, the columns were taken apart and sections of soil were stored in separate sealed containers for later use in batch experiments.

Soil microcosms were prepared from the acclimated soil using 50 and 160 mL serum bottles. The amount of soil added to a bottle was dependent upon the moisture content and estimated porosity of the soil. A headspace to open pore space ratio of 60:1 was used so that hours in a microcosm would correspond to minutes in a soil bed. Each microcosm was injected with hydrocarbon vapors and sampled at predetermined time intervals.

The hydrocarbon loading rate was varied by changing the inlet hydrocarbon concentration and by changing the flow rate of the inlet vapor stream. Inlet concentration did not significantly affect the percentage of removal when the concentration was adjusted from a lower to higher concentration, 6 μg/mL to 26 μg/mL of total hydrocarbons. Changing the inlet concentration from a higher to lower concentration, 50 μg/mL to 12 μg/mL total hydrocarbons, resulted in greatly increased removals of benzene (31 percent removal increased to 96 percent removal), and to a lesser extent, increased removals of toluene (removals increased from 23 percent to 31 percent). The increased removal activity was short-lived and diminished as the biomass to hydrocarbon ratio readjusted to the reduced loading rate. Reducing the inlet flow rate produced greater removals until the removal process became transport limited. At this point, further reduction of the inlet flow rate resulted in little or no increase in hydrocarbon removal.

Although all soils examined displayed an ability to remove a portion of each vapor stream component, soil types had a large influence on removals. Rubicon sand produced the most efficient removals for the four contaminants tested. Dougherty sand removed some benzene but substantially smaller amounts of the other components. The Durant loam soil column removed almost equal percentages of each of the four components and displayed the most consistent first-order

removal characteristics. Benzene removals in microcosms constructed from Durant loam soil are shown in Figure 6.

Soil moisture content had a great influence on the hydrocarbon removal rate in the microcosm experiments, with the highest removal rate constants found on the higher moisture content (Figure 7). Closer examination of soil moisture levels is needed to determine the points of low moisture and high moisture inhibition. In addition, aromatic hydrocarbon removal in the presence of typical gasoline alkanes should be tested. Factors which may affect transport limitations, including grain size distribution, organic carbon content, and moisture content, need to be examined.

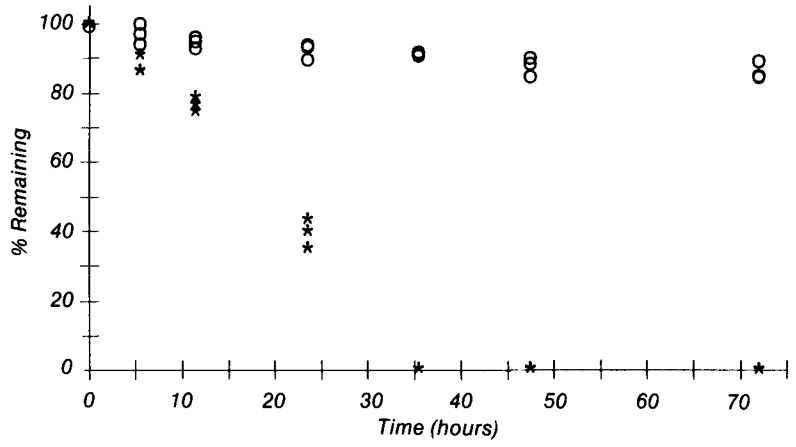
Conclusions and Recommendations

The results obtained in this research indicate that fixed-film bioreactors have significant potential utility for removal of volatile chlorinated and light aromatic hydrocarbons from polluted ground water and from gaseous streams produced by air stripping of ground water or by soil venting.

Fixed-film bioreactors utilizing light aliphatic hydrocarbons as primary substrates are indicated by this work to be capable of removing up to at least 60 percent of TCE from influent water containing less than approximately 1000 μg/L of this pollutant. Several interrelated parameters, including influent concentrations of TCE and primary substrate, geochemistry and flow rate of water, flow rate of air, nature of the supporting media for the biofilm, and system configuration appear to govern the extent of TCE removal by these systems. Additional laboratory and pilot-scale studies related to the effects of these parameters are needed to further develop and evaluate optimized fixed-film bioreactor methodologies for treatment of ground water contaminated by TCE. In particular, propane, butane, and mixtures of light hydrocarbons, including natural gas, should be evaluated as primary substrate for biofilm organisms, and additional porous media, especially ceramic materials and diatomaceous earth products, should be examined as support media for fixed biofilms capable of cometabolizing TCE in aqueous streams. Also, variations in bioreactor configurations, such as multiport injection of primary substrate and columns in series, should be more rigorously examined.

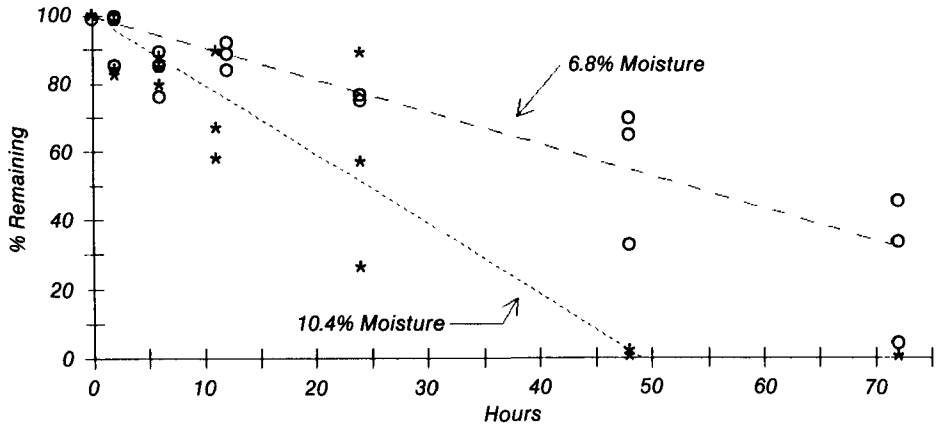
Bioreactors consisting of fixed biofilms sustained by butane as primary substrate and supported on diatomaceous earth media are shown by this research to be capable of removing more than 90 percent of TCE and TCA from vapor streams. These systems have high potential utility for treatment of contaminated vapor streams produced by air stripping of polluted ground water and soil venting operations. However, major questions remain unanswered regarding the effect of type of primary substrate and biofilm supporting media, nutrient solution recirculation, and protozoal predation on the extent of pollutant removal and long-term stability of bioreactor operation. Additional laboratory and pilot-scale studies should be undertaken to address these questions.

Soil bioreactors were found capable of removing at least 35 to 40 percent of volatile aromatic hydrocarbons such as benzene, toluene, ethylbenzene, and o-xylene from air streams containing mg/L concentrations of these contaminants. The extent of removal of the



Moisture Content 19.6%
o —*Control*
* —*Sample*

Figure 6. *Benzene removal in Durant loam microcosm.*



Lines Generated Using Removal Rate Constants
o —*6.8% Moisture*
* —*10.4% Moisture*

Figure 7. *Moisture effect on benzene removal, Dougherty sand microcosm.*

individual compounds appears to be dependent on the type of soil used in preparation of the bioreactor, probably reflecting differences both in physical properties and microbial ecosystems of different soils. The development of optimized methodologies employing soil bioreactors for treating vapor streams laden with aromatic hydrocarbons will require further studies to better define characteristics of soils most appropriate for bioreactor construction.

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***William J. Dunlap** is the EPA Project Officer (see below).*
The complete report, entitled "Innovative Processes for Reclamation of Contaminated Subsurface Environments," (Order No. PB 90-199 514/AS; Cost: $17.00, subject to change) will be available only from:
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