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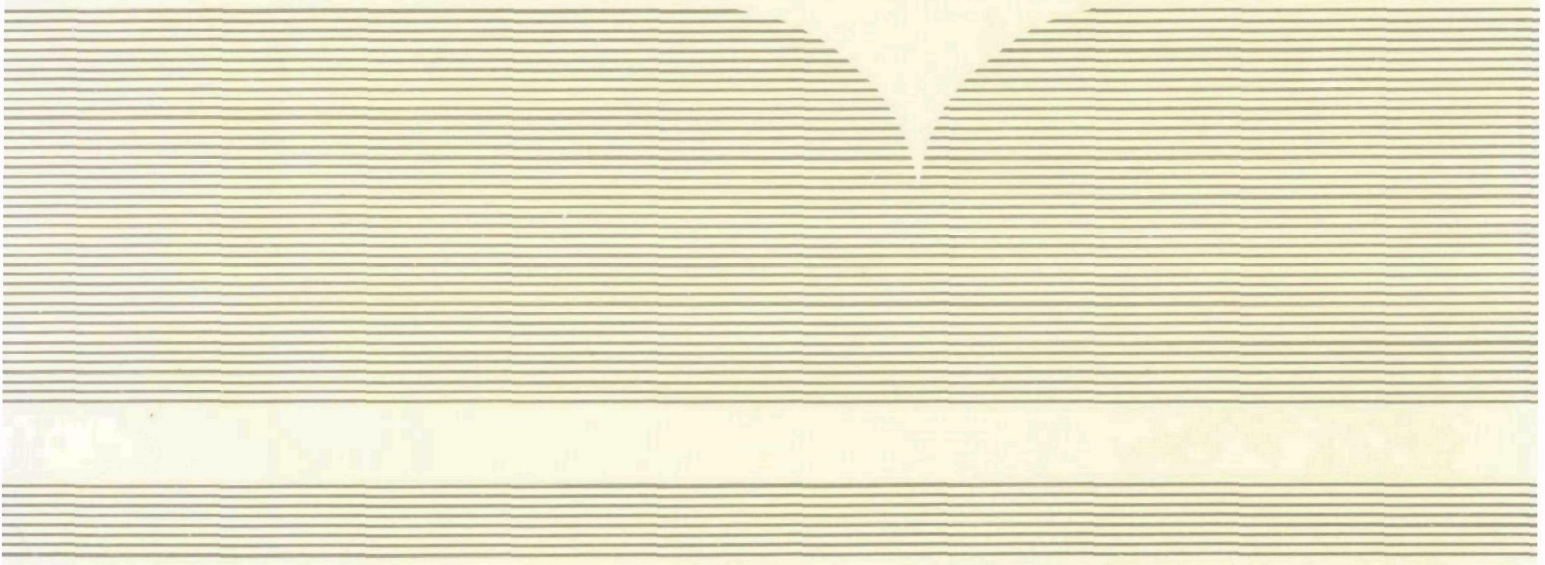

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INNOVATIVE PROCESSES FOR RECLAMATION OF CONTAMINATED SUBSURFACE ENVIRONMENTS

by

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16. ABSTRACT

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 concentration 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 by primary substrates consisting of gaseous aliphatic hydrocarbons; the removal of alkylbenzenes from air streams by biofilms utilizing these compounds as primary substrate 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 employment of fixed-film bioreactors in systems for above ground treatment of contaminated ground water and vadose zone gases.

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FOREWORD

EPA is charged by Congress to protect the Nation's land, air, and water systems. Under a mandate of national environmental laws focused on air and water quality, solid waste management and the control of toxic substances, pesticides, noise and radiation, the Agency strives to formulate and implement actions which lead to a compatible balance between human activities and the ability of natural systems to support and nurture life.

The Robert S. Kerr Environmental Research Laboratory is the Agency's center of expertise for investigation of the soil and subsurface environment. Personnel at the Laboratory are responsible for management of research programs to: (a) determine the fate, transport and transformation rates of pollutants in the soil, the unsaturated and the saturated zones of the subsurface environment; (b) define the processes to be used in characterizing the soil and subsurface environment as a receptor of pollutants; (c) develop techniques for predicting the effect of pollutants on ground water, soil, and indigenous organisms; and (d) define and demonstrate the applicability and limitations of using natural processes indigenous to the soil and subsurface environment for the protection of this resource.

This report describes research conducted to evaluate the feasibility of innovative biological treatment of chlorinated hydrocarbons and alkylbenzenes in engineered systems. Cometabolism of trichloroethylene and 1,1,1-trichloroethane by hydrocarbon-utilizing organisms is demonstrated. Also shown is the ability of soil-bed reactors to treat benzene, toluene, ethylbenzene, and *o*-xylene in the vapor phase.

Clinton W. Hall

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Director
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ABSTRACT

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 concentration 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 steams by biofilms sustained by primary substrates consisting of gaseous aliphatic hydrocarbons; the removal of alkylbenzenes from air streams by biofilms utilizing these compounds as primary substrate 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 employment of fixed-film bioreactors in systems for above ground treatment of contaminated ground water and vadose zone gases.

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SECTION 1

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 groundwater sources (Tchobanoglous and Schroeder, 1985). 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.

In 1985, the total industrial production of the four most used chlorinated solvents -- trichloroethylene (TCE), tetrachloroethylene (PCE), 1,1,1-trichloroethane (TCA), and methylene chloride -- was 1.64 billion pounds (Storck, 1987). These large productions result in extensive release to the environment through spilling, industrial wastewater discharge, landfilling, and volatilization. A review of American and European drinking-water sources conducted by Folkard (1986) revealed that the compounds most likely to be identified in contaminated ground water are halogenated hydrocarbons of low molecular weight. TCE, PCE, and TCA were the most prevalent because of their extensive use. Westrick *et al.* (1984) reported similar results based on a U.S. Environmental Protection Agency survey in the United States. In this study, the compounds found most commonly in finished ground water supplies were TCE, PCE, *cis*-1,2-dichloroethylene (*cis*-DCE), *trans*-1,2-dichloroethylene (*trans*-DCE) and 1,2-dichloroethane with benzene and toluene also frequently found. The most common sources of these contaminants were accidental spills, failure of underground storage tanks and associated plumbing, and inappropriate disposal to landfills, evaporation pits, and sludge disposal lagoons.

CONVENTIONAL TREATMENT

Currently air stripping is the most widely used method of removing TCE and other low-molecular-weight chlorinated compounds from contaminated ground water because of its significantly lower capital and operating costs compared with other methods. Frequent use in the treatment of waters containing the soluble constituents of petroleum products is also seen. Air stripping achieves approximately 99 percent removal of TCE and offers a high degree of flexibility in response to prevailing operating conditions (Folkard, 1986). The countercurrent packed towers appears to be the most appropriate system in that it provides the most liquid interfacial area and allows for high air-to-water ratios (Canter and Knox, 1985).

Carbon adsorption is less widely used than air stripping because of the high cost associated with the disposal or thermal regeneration of the spent carbon. Activated carbon adsorption can be up to four times as expensive as air stripping due to costs associated with the landfilling at a hazardous waste disposal site or thermal regeneration of the used carbon (Knox and Canter, 1988). However, adsorption is a well-established technology for reducing the concentrations of TCE and related compounds to less than 5 µg/l (Love and Eilers, 1982). Carbon adsorption is efficient but costly; air stripping is both efficient and inexpensive. Both

treatments have the disadvantage of transferral to another medium without ultimate destruction of the compounds. Biological treatment has the potential to be a final treatment by metabolizing the contaminants of interest to carbon dioxide, water, and cellular constituents.

INNOVATIVE BIOLOGICAL TREATMENT

Although biological treatment of easily metabolizable organic compounds has been used for decades to treat municipal and industrial wastes, only in the past ten years has biological treatment of recalcitrant compounds or innovative treatment of easily degraded compounds been attempted. This report will focus on innovative treatment of water or air streams contaminated with TCE and/or TCA using engineered systems and soil bed reactors to treat waste air streams containing hydrocarbons. The biological treatment of the chlorinated compounds will rely on the cometabolism of the contaminant using methane or butane as the primary substrate. The hydrocarbon waste streams will be remediated using a soil bed containing bacteria capable of metabolizing the compounds to water, carbon dioxide, and trace inorganic salts.

The contaminated water stream may come from pumping polluted ground water to control plume migration or water from product recovery wells. Contaminated air streams are produced during soil remediation procedures, air stripping of contaminated ground water, or vapor collection from manufacturing or treatment processes.

In addition to being an ultimate treatment for contaminated air or water, biological treatment may also be more economical than many alternative processes. Knox and Canter (1988) have presented an economic evaluation of the cost of conventional treatment for the low molecular-weight halogenated organics. In an initial economic analysis, the biological treatment of TCE using propane as a primary substrate was found to be more cost effective than carbon adsorption but less so than air stripping (Wilson and White, 1986). Treatment of waste hydrocarbon vapor streams using biofilters has shown to be the most economical technology available when compared to conventional treatments such as thermal degradation or activated carbon (Kosky and Neff, 1988).

ENVIRONMENTAL FATE

In oxygenated subsurface materials, TCE and TCA are not ordinarily biodegradable and tend to persist in those environments (Ghiorse and Wilson, 1988; Wilson *et al.*, 1981). TCE and TCA are biodegraded only in subsurface materials where oxygen is not available by undergoing a reductive dechlorination. The daughter products of TCE are the dichloroethylenes and vinyl chloride (VC) and those of TCA are 1,1-dichloroethane and chloroethane (Barrio-Lage *et al.*, 1986; Barrio-Lage *et al.*, 1987; Vogel and McCarty, 1985). TCA may also be nonbiologically transformed to 1,1-dichloroethylene (1,1-DCE) by elimination and to acetic acid by hydrolysis (Haag *et al.*, 1986) with the potential of further biodegradation of 1,1-DCE to VC. These products of transformation of TCE and TCA are more mobile in ground waters than the parent compounds, and, in the case of VC, more carcinogenic. TCE is resistant to hydrolysis, with an estimated half-life between 0.9 to 2.5 years (Vogel *et al.*, 1987). Most abiotic transformations are very slow in contrast with biotic transformation (Bitton *et al.*, 1986).

Because they are soluble in water, benzene, toluene, ethylbenzene, and the xylenes are the indicators of groundwater contamination from petroleum products. These aromatic compounds are readily biotransformed in oxygenated environments, with recent work also indicating a biological fate in the absence of molecular oxygen (Grbić-Galić and Vogel, 1987; Vogel and Grbić-Galić, 1986; Wilson *et al.*, 1986).

AEROBIC BIOTRANSFORMATION OF TRICHLOROETHYLENE

Although aerobic biodegradation of TCE and related compounds does not occur under ordinary aerobic conditions (Bouwer and McCarty, 1982; Wilson *et al.*, 1981;) recent work has shown that bacteria that oxidize gaseous hydrocarbons such as methane or propane are also able to cometabolically oxidize TCE and other low molecular weight halogenated compounds (Wilson and Wilson, 1985; Wilson and White, 1986; Fogel *et al*, 1986; Henson *et al.*, 1988, Wackett *et al.*, 1989).

The biochemistry of methanotrophs to oxidize methane and cometabolize TCE has been described by Horvath, 1972; Atlas, 1981; Fogel *et al.*, 1987; Wilson *et al.*, 1987; and Little *et al.*, 1988. Methane-utilizing bacteria oxidize methane first to methanol and then to formaldehyde, which can be converted to biomass or to CO_2 as follows:

$$CH_4 \text{---> } CH_3OH \text{ ---> } CH_2O \begin{matrix} \text{---> HCOOH ---> } CO_2 \\ \text{---> biomass} \end{matrix}$$

The first step in methane oxidation is performed by the enzyme methane monooxygenase (MMO), which obtains oxygen directly from molecular oxygen. MMO is a highly non-specific enzyme able to insert an oxygen into a wide variety of nongrowth compounds. MMO may exist as either a soluble or particulate form; the soluble MMO has been isolated from both type I and type II methanotrophs and is associated with a broader substrate specificity than the particulate form (Burrows *et al.*, 1984). Oxygenations by the enzyme include hydroxylation on n-alkanes such as ethane, the expoxidation of alkenes, and dechlorination of aliphatic and aromatic substances.

The first step in degradation of TCE by methanotrophs is expoxidation to form TCE epoxide, which is unstable and hydrolyzes rapidly in water at neutral pH with half-lives on the order of seconds. The products of hydroxylation are dichloroacetic acid, glyoxilic acid, and formic acid (Fogel *et al.*, 1987), all of which can be further degraded by methanotrophs and other heterotrophic organisms. These reactions are shown in Figures 1.1 and 1.2 (Wilson *et al.*, 1987; and Little *et al.*, 1988).

The use of alkanes such as methane, propane, or butane as primary substrates for cooxidation of chlorinated compounds has advantages. First, the alkane serves as the primary source of carbon and energy needed to sustain a stable microbial community when the target pollutant is present in trace amounts, as in the case of TCE in ground waters. An advantage of butane over methane is its increased solubility in water (60 mg/l versus 24 mg/l) and thus a potentially increased concentration in a biofilm. Next, these alkanes are commonly used industrial chemicals that are easily found and inexpensive. They are nontoxic to humans, and therefore not considered pollutants, and easily biodegraded.

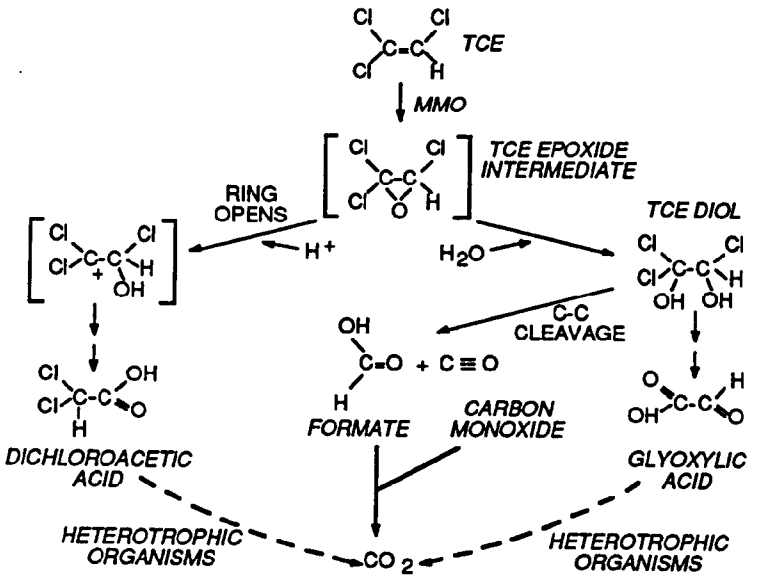


Figure 1.1. Proposed Mechanism of TCE Breakdown by Methanotrophic Bacteria (Little, et al., 1988).

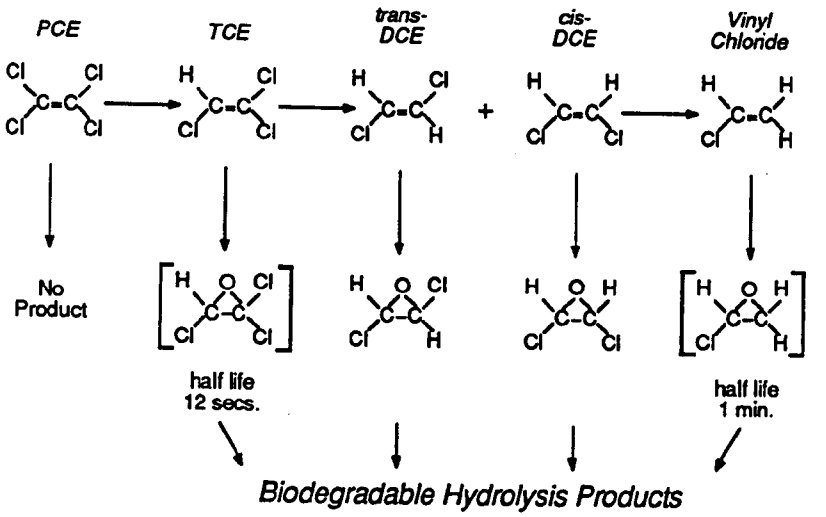


Figure 1.2. Biotransformation of Chlorinated Ethylenes and Their Breakdown Products (Wilson, et al., 1987).

PREVIOUS STUDIES ON BIOTRANSFORMATION OF TRICHLOROETHYLENE

The earliest study to evaluate the feasibility of TCE oxidation by methane-utilizing bacteria was conducted with unsaturated soil at the U.S. Environmental Protection Agency's R.S. Kerr Environmental Research Laboratory in Ada, Oklahoma (Wilson and Wilson, 1985; Wilson and Wilson, 1987). Trichloroethylene was shown to degrade aerobically to carbon dioxide in an unsaturated soil column exposed to a mixture of natural gas in air (0.6 percent). A removal of one order of magnitude was observed during the 2-day residence time of water in the column.

In a second study (Wilson and White, 1986), a glass column 152 cm tall by 5 cm in diameter was packed with 127 cm of coarse sand followed by 10 cm of a mixture on Lincoln plus coarse sand at the top. A mixture of propane in air was supplied to the top of the column, and a solution of 800 µg/l of TCE and 760 µg/l of TCA was continuously pumped to the head of the column concurrently with the air. After 13 days of acclimation, removals of TCE from 80 percent to 95 percent were observed.

Recent work with a fixed-film, packed-bed bioreactor with a residence time of approximately 50 minutes and using methane as the primary substrate treated synthetic ground water containing TCE and DCE (Strandberg *et al.*, 1989). The initial concentrations of 1 mg/l each were reduced >50 percent for TCE and >90 percent for DCE.

A field demonstration project recently completed by Battelle Columbus at Tinker Air Force Base in Oklahoma has shown reductions of 260 µg/l TCE in influent ground water to approximately 110 µg/l in effluent from fixed-film bioreactors using natural gas as the primary substrate (U.S. Department of the Air Force, Progress Report). Mannville's Celite biocatalyst carrier was used as the solid support. This is the first known field demonstration of remediation of TCE-contaminated ground water in a surface bioreactor using the cooxidation process. The substantial removal of TCE during one retention time in the bioreactor indicates the potential for this remediation technique.

Stanford University conducted a pilot study of *in-situ* biodegradation of TCE by stimulating the native bacterial population capable of degrading the contaminant within the aquifer under saturated conditions (Roberts *et al.*, 1989). The study was conducted on a shallow confined sand and gravel aquifer at Moffett Naval Air Station. An extraction well and injection wells were installed six meters apart, with three intermediate monitoring wells. Bromide was used as a conservative tracer, with TCE continuously injected at average concentrations of 100 µg/l during the initial stage and 60 µg/l during the later stages.

Complete methane utilization was observed within a few weeks, confirming the presence of indigenous methanotrophic bacteria. Mass balances indicated that under the influence of active biostimulation with methane and oxygen, approximately 20 to 30 percent of the TCE was degraded within 2 meters of travel in the test zone. The limited degree of TCE transformation was attributed to the high degree of chlorination of the TCE molecule, resulting in a slow rate of oxidation, limited methane-oxidizing population, and possible competitive inhibition of TCE degradation by methane. No intermediate products were found, suggesting completely aerobic conditions and treatment. Another interesting finding was the relatively high sorption of TCE on the aquifer material (Roberts *et al.*, 1989).

As part of the Moffett Station *in-situ* biodegradation project, batch studies with mixed cultures from the site were conducted to determine the effects of changing the methane, oxygen, TCE, and biomass concentrations (Henry and Grbić-Galić, 1987). Three mixed cultures were enriched from the Moffett Field aquifer or from effluent from a saturated column. In general, TCE transformation was rapid. All three cultures transformed TCE in the absence of methane. One mixed culture transformed TCE significantly faster when no methane was present; however, the rate of transformation for the other two mixed cultures when no methane was present was significantly slower than when methane was provided. The removal of TCE was found to be highly dependent on the types of methanotrophs used and the relative effects of the main variables on the metabolic capacity of the mixed cultures. The degradation of TCE when no methane was present was an interesting finding, as was the incorporation of ^{14}C into the cell.

Recent work by Tsien *et al.*, (1989), has observed degradation of TCE by the *Methylosinus trichosporium 0B3b*, a type II methanotroph, in both continuous and batch cultures following the appearance of soluble MMO. In cultures with suppressed MMO, the oxidization of TCE increased with increasing soluble MMO concentrations.

In a study by Little *et al.* (1988) two pure strains of TCE-degrading type I methanotrophic bacteria were isolated from mixed ground water cultures enriched from ground and surface water at a site contaminated by chlorinated alkenes. Pure cultures converted 1 to 2 percent of radiolabeled TCE to cell biomass, 10 to 16 percent to CO_2, and 15 to 22 percent to water-soluble breakdown products. This accounts for approximately 26 to 40 percent of the TCE added, from which one-third was converted to CO_2. This study confirmed the incorporation of some ^{14}C carbon into cell biomass even for pure cultures, and showed that pure cultures accumulated more water-soluble breakdown products, suggesting the need for mixed cultures for complete mineralization.

Fliermans *et al.* (1988) searched for heterotrophic enrichment cultures able to degrade TCE aerobically at high concentrations (i.e., 50-300 mg/l). More than 400 enrichments and incubation mixtures obtained from subsurface sediments which had been heavily contaminated with short-chain chlorinated solvents were examined for TCE utilization after a one-month incubation period. Concentrations from 50-300 mg/l TCE were used, and the studies included radioisotope activity measurements and isolation enrichments. There was a significant removal of TCE by the enriched cultures using several substrates including methane, propane, methanol, acetate, and trypticose plus yeast extract. The enrichment cultures were stable and used a variety of energy sources for growth but could not use methane as a sole source of carbon and energy. Moreover, they did not have the phospholipid biomarker typical of methanotrophs (Ringelberg *et al.*, 1989).

The toxicity of TCE to methanotrophs has been studied by Janssen *et al.* (1987). The authors reported that when methane is used as a sole carbon source, complete inhibition occurs at concentration levels of 30-35 µm for most chlorinated aliphatic hydrocarbons and 45 µm specifically for TCE, which is equivalent to 6 mg/l. The addition of methanol caused a relief from growth inhibition of some specific steps in the assimilation of methane, rather than a general inhibitory effect. Lee *et al.* (1988) stated that inhibition of TCE in certain mixed cultures

starts at a concentration of about 1 mg/l, and pointed out the importance of defining this toxicity threshold limit.

Strand *et al.* (1988) studied the kinetics of TCE degradation by suspended cultures on methane-oxidizing bacteria using a closed system reactor. It was found that methane oxidation followed Michaelis-Menton kinetics, with K_s = 0.67 mg methane/l and r_m = 47.2 µg methane/mg VSS-hr. TCE removal followed first order kinetics with a rate constant of 3.7×10^{-4} l/mg VSS-hr for concentrations less than 3000 µg/l. TCE biodegradation was not inhibited by the presence of dissolved methane concentrations in excess of 0.25 mg/l. In the absence of methane, the culture continued to degrade TCE, but the degradation rate gradually decreased until it ceased after 104 hours.

A more recent discovery is the cometabolism of TCE by bacteria that degrade aromatic compounds, specifically phenol, toluene, and cresol (Wackett and Gibson, 1988). Nelson *et al.* (1986) isolated a bacteria designated G4 from a number of soil and water samples that were screened for the biological capacity to metabolize TCE. Further investigation by Nelson *et al.* (1987) revealed that strain G4 degrades TCE only when preinduced with phenol, toluene, *o*-cresol, or n-cresol. Recent studies have shown TCE degradation by *Escherichia coli* containing the toluene dioxygenase genes cloned from *Pseudomonas putida* F1 (Zylstra *et al.*, 1989).

VAPOR PHASE TREATMENT OF ALKYLBENZENES

Use of soil-bed bioreactors has been shown to be effective for a number of different applications, and has the advantage of completely destroying the contaminant. Soil bed systems have been shown to be effective for controlling such air waste streams as rendering plant emissions (Prokop and Bohn, 1985) and wood and coal flue gases (Duncan *et al.*, 1982). Natural gas has been shown to be readily degradable by soil microbes (Hoeks, 1972; Bohn, 1977). Propane, isobutane, and n-butane have been effectively removed using a soil bioreactor system (Kampbell *et al.*, 1987). Ethylene and acetylene have also been shown to be removed by soils (Bohn, 1977). Additionally, soil-bed bioreactors allow a substantial savings in investment and maintenance cost. Soil beds are estimated to cost about $8 per cubic foot per minute (cfm) compared to $30 - $100/cfm for activated carbon systems (Bohn and Bohn, 1986).

Work has been done which indicates that some hydrocarbon streams may be effectively controlled with a soil-bed bioreactor (Kampbell *et al.*, 1987). A volume of soil is excavated and a piping network is placed within a layer of gravel. The purpose of the gravel layer is to help distribute the injected gases. The excavated soil is then treated (if necessary) and back filled over the gravel layer and distribution pipe. Peat or compost could also be substituted for the soil, although soil is reported to have the greatest removal efficiency (Bohn and Bohn, 1986).

A minimum residence time of one minute for gases within a soil filter has been proposed (Bohn, 1975), but actual contact times probably need to be much longer depending on the desired amount of removal. Residence time can be controlled by varying the inlet volumetric flow rate or by planning for a larger bioreactor with either greater surface area or greater depth.

Factors such as soil type, moisture content, temperature, and the particular compound of interest greatly influence the biodegradation potential. In general, the type of soil which would accommodate the largest microbial population under a given set of environmental conditions will probably produce the greatest biodegradation activity (Schoen and Winterlin, 1987; Abou-Assaf and Coats, 1987). The type of soil (sandy, loamy, or clayey) will determine the internal pore structure which in turn will determine the adsorptive capacity of the soil and the magnitude of the microbial population (Prokop and Bohn, 1985). Typically, population increases of several orders of magnitude have been observed for hydrocarbon-utilizing microorganisms after hydrocarbon spills (Atlas, 1981). Increases in the total biomass after hydrocarbon exposure are sometimes not observed because exposure to hydrocarbons leads to an increase in the number of hydrocarbon degrading microbes but not the overall population present in the sediment may not increase (Heitkamp *et al*, 1987). As the waste gas passes through the soil bed, hydrocarbon compounds are adsorbed and may be retained for an extended length of time before degradation by microbial action (Prokop and Bohn, 1985). Soil beds may be designed on the basis of upflow rates of waste gases of 0.2 to 1.0 m/min with backpressures of approximately 10 cm of water (Pomeroy, 1982).

An environment which favors microbial growth and activity should also favor hydrocarbon degradation. Soil moisture content has a large influence on microbial activity. The optimum moisture content of a soil is difficult to specify. It is generally thought that maximum hydrocarbon oxidation occurs in the same moisture range which satisfies higher plants (Bohn, 1977). More specific studies have shown that increasing the moisture content from 4 to 11 to 20 percent increases CO_2 removal in a soil column from 74 to 85 to 95 percent, respectively (Duncan, Bohn, and Burr, 1982). Other studies specify that soil moisture contents of 9 to 20 percent (wet mass basis) are adequate for removal of components of waste gases containing organic sulfides, aldehydes, amines and organic acids (Prokop and Bohn, 1985). Significant increases in degradation of carbofuran were observed as soil moistures were increased from 11 to 27 percent (Abou-Assaf and Coats, 1987).

Higher moisture contents do not always ensure faster hydrocarbon removals. Optimum removal rates have been reported to occur at moderate moistures, 22 percent, when compared to higher, 30 percent, and lower, 15 percent, moisture contents (Abou-Assaf and Coats, 1987). Soils which are at or near the water holding capacity are reported to result in near anaerobic conditions (oxygen transport limiting) which would inhibit degradation by aerobic organisms (Abou-Assaf and Coats, 1987; Schoen and Winterlin, 1987). Excess water is also thought to fill up the soil pore spaces and prevent adsorption of the hydrocarbon compounds (Prokop and Bohn, 1985). In one example of restricted mobility, propane was reported to be removed more rapidly by soils having 10 percent moisture that the same soils at 20 percent moisture (Edinger *et al.*, 1987). It has been reported that the greatest aerobic degradation of simple or complex organic material can be expected to occur at 50 to 70 percent of a soil's water holding capacity (Dibble and Bartha, 1979).

Temperature also has a direct influence on microbial activity. Microbial activity can be expected to double with each 10° rise up to around 35°C, at which point microbial activity falls off rapidly (Prokop and Bohn, 1985). In some cases little or no increase of microbial

activity is seen above 20°C (Dibble and Bartha, 1979). Alternatively, even greater increases are sometimes encountered. Hoeks (1972) reported 4- to 5-fold increases in oxygen consumption from natural gas degradation accompanying a temperature increase of 13° to 20°C. Propane removal rates have also been shown to increase 10 fold over the temperature range of 2° to 25°C (Edinger *et al.*, 1987). A minimum temperature limit of 10°C for microbial activity has been suggested (Prokop and Bohn, 1985). Actual soil-bed biofilters should be warmed enough by the influent gas temperature and microbial respiration to perform satisfactorily in cold weather (Bohn, 1975). Soil-bed biofilters located in Quebec City, Canada, Germany, and Racine, Wisconsin, have reportedly operated successfully throughout the winter months even when shut down during the weekends (Prokop and Bohn, 1985).

Rates of biodegradation vary widely between compounds and reaction systems. N-alkanes seem to be the most readily degradable hydrocarbons with longer chained n-alkanes degrading more readily than shorter chained compounds (Atlas, 1981). Research completed by Bohn (1977) and Kampbell *et al.* (1987) support this generalization. Bohn reported butane degradation rates (100 mg/kg soil/hr) an order of magnitude greater than methane degradation (5 mg/kg soil/hr). Kampbell reported slightly higher degradation rate constants for n-butane (0.1 - 0.55 hr^{-1}). Branched alkanes also display greater resistance to biodegradation than n-alkanes (Atlas, 1981). Kampbell's work illustrates this greater resistance to degradation of substituted alkanes with reported rate constants for isobutane (0.04 - 0.31 hr^{-1}) which were half as large as the rate constants for n-butane (0.1 - 0.55 hr^{-1}). Substituted cycloalkanes degrade more rapidly than unsubstituted cycloalkanes, while methyl substitution seems to inhibit aromatic hydrocarbon degradation (Atlas, 1981). Halogenated hydrocarbons seem to be the most resistant to microbial degradation and so tend to be environmentally persistent. The amount of halogen substitution and location of the halogen substitution influence the resistance to biodegradation (Boyer *et al.*, 1987). Swindoll *et al.*, 1988, showed the much greater resistance associated with halogen substituted compounds with reported removal rate constants for chlorobenzene (0.46 (10^{-5} hr^{-1}) which were 4 orders of magnitude less than the removal rate constants of toluene (0.050 hr^{-1}) and phenol (0.058 hr^{-1}).

Successful degradation of a compound by microorganisms depends in a large part on the availability of the compound to the microorganism. Two factors which can greatly influence availability are water solubility and adsorption to the soil matrix. Compounds which have limited solubility in water will not be available to microorganisms because soil microorganisms develop primarily in the water films around soil particles and water filled capillaries between the soil particles (Boyer *et al.*, 1987). Compounds which sorb strongly onto soils will also not be available to microorganisms for biodegradation. Compounds which are irreversibly sorbed onto soil organic matter may be isolated and protected from extracellular degradation (Mihelcic and Luthy, 1988). The degree of adsorption is influenced by the size of the compound (which influences volatility) and by the amount of humic material or organic carbon present in the soil. The type of soil also strongly influences adsorptive capacity. Surface area increases as the grain size decreases, hence soils composed of clay sized particles would have greater surface area for adsorption than soils composed of sand sized particles.

RESEARCH OBJECTIVES

The results of the research presented in this report addressed the feasibility of above-ground aerobic biological treatment of TCE and/or TCA in contaminated air or water streams in laboratory-scale fixed-film bioreactors. A variety of media including coarse sands, berl saddles, and Celite biocatalysts were used as the solid support for the biofilm. Natural gas, pure methane, and pure butane were used as the primary substrates to support the cooxidation process required for aerobic degradation of TCE. Also presented are the results of the treatment of hydrocarbon gas streams in soil-bed reactors. The results can then be applied to the design of above ground treatment units to biologically treat low molecular weight chlorinated compounds or hydrocarbons in air or water streams.

The cooperative research effort was conducted both at the University of Oklahoma, Norman, Oklahoma, and at the R. S. Kerr Environmental Research Laboratory, Ada, Oklahoma. The objectives of the research at the R. S. Kerr Laboratory were to test the feasibility of treating an air stream containing TCE and TCA from a soil venting or air stripping operation using butane as the primary substrate and two Celite biocatalyst carriers as the solid support. Also, the use of soil-bed reactors to treat waste streams containing vapor phase benzene, toluene, ethylbenzene, and *o*-xylene was examined. The research conducted at the University of Oklahoma focused on liquid phase treatment of TCE and examined the optimization of the organic and hydraulic loadings in bioreactors by controlling a series of variables including air flow, water flow, primary substrate concentration, TCE concentration, and packing media. Quantification of removal efficiencies under varying conditions such as air-to-liquid ratios, methane-TCE concentrations, and flow rates was studied.

SECTION 2

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

1. Bioreactors consisting of short sand columns supporting fixed biofilms utilizing methane as the primary substrate appear capable of removing as much as 55 to 60 percent of TCE from influent water containing approximately 500 µg/l of this pollutant, provided operating parameters are optimized.

2. If concentrations of TCE approach 1000 µg/l, removal of contaminant from influent waters will probably be inhibited, although utilization of primary substrate by the biofilm continues. When influent TCE concentrations are near 1500 µg/l, methanotrophic organisms constituting the biofilm will likely be completely inhibited.

3. The percentage removal of TCE from aqueous streams by fixed-film bioreactors appears to increase with increasing TCE concentrations until the inhibitory concentration is approached.

4. Operating conditions such as water flow, flow rates for air and methane, and required nutrient concentrations to attain maximum TCE removal from polluted water by fixed-film bioreactors are dependent on the supporting media and geochemistry of the treated water. Hence, determination of optimized operating parameters for specific situations will probably be required, at least until a more comprehensive data base on the effects of operating variables is developed.

5. Multiport injection of primary substrate (methane) at various depths within the bed of bioreactors containing sand as supporting medium appears to result in increased removal of TCE from influent water, probably because of better distribution and increased mass of the biofilm. Increased TCE removal can also be achieved by operating two bioreactors in series.

6. Limited observations indicate that biofilms developed and sustained on natural gas as primary substrate, are at least as effective in removing TCE from influent water as biofilms developed and sustained on pure methane. The probable ability of the more complex substrate to support a greater diversity of microorganisms may be advantageous in terms of biofilm sensitivity to concentration inhibition by TCE.

7. Fixed-film bioreactors supplied with primary substrate on an intermittent basis to encourage endogenous respiration exhibit decreased removal of TCE from influent water in comparison to similar bioreactors provided with a continuous supply of primary substrate.

8. The potential utility of fixed-film bioreactors for on site, above ground treatment of ground water contaminated with relatively low levels of TCE (probably 500 µg/l or less) appears to be promising and worthy of further evaluation and development. Such systems should not be considered for treatment of waters containing TCE in the neighborhood of 1000 µg/l or higher.

9. Bioreactors consisting of fixed biofilms sustained by butane as primary substrate and supported on two sizes of diatomaceous earth packing materials are capable of removing more than 90 percent of TCE and TCA from influent air streams under optimum

conditions. Removals were stable in a bioreactor with the smaller size solid support. However, rates of removal of the chlorinated hydrocarbons in another bioreactor with the larger support were observed to decline severely after several days of reactor operation, possibly as the result of build up of inhibitory products in recirculated nutrient solution or predation by protozoa.

10. The potential utility of fixed-film bioreactors for removal of volatile chlorinated hydrocarbons from contaminated air streams generated by air stripping of polluted ground water or soil venting applications appears high, provided problems associated with operation of bioreactor systems over extended periods of time can be overcome.

11. Soil bioreactors appear to be 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.

12. Biological removal of alkylbenzenes from air during passage through soil is sensitive to the flux of alkylbenzenes. At a give flow rate, the biomass will adjust to remove the same fraction of alkylbenzenes regardless of initial concentration (over the range of 1 to 35 mg/l air). At a given concentration, the biomass will adjust to remove the same fraction as flow rates are decreased (0.9 to 0.2 ml air/cm^2 cross section/m).

13. In these studies, the extent of removal of the various volatile aromatic hydrocarbons from contaminated vapor streams by soil bioreactors varied significantly with the soil sample used to prepare the bioreactor. This probably reflects qualitative and quantitative differences in the microbial ecosystems of the different soils.

14. Soil moisture content appears to be very important in determining the effectiveness of soil bioreactors in removing volatile aromatic hydrocarbons from contaminated air streams.

RECOMMENDATIONS

1. Bioreactors comprised of fixed biofilms utilizing light aliphatic hydrocarbons primary substrate should be further developed and evaluated in laboratory and pilot-scale studies for treatment of ground water containing low levels (500 µg/l or less) of TCE. Particular attention should be given to the following:

a. The effect of TCE removal of employing propane, butane, or mixtures of light hydrocarbons, including natural gas, as primary substrate for biofilm organisms should be investigated. Comparative susceptibility to TCE concentration inhibition effects of biofilms utilizing various primary substrates should be evaluated.

b. Materials other than sandy soils, such as ceramic materials and diatomaceous earth products, should be examined as support media for fixed biofilms capable of cometabolizing TCE in aqueous streams.

c. Variations in bioreactor system configurations to achieve increased contaminant removal from contaminated water streams should be rigorously examined. This should include further studies of the use of columns in series and the use of multiport injection of primary substrate, better distribution and increase in biomass throughout the depth of the bioreactor.

2. Bioreactors utilizing light aliphatic hydrocarbons as primary substrates to treat contaminated air streams should be subjected to further laboratory and pilot-scale studies. Particular attention should be directed to the following:

a. The effect of recirculation of the effluent fluids on long-term stability of TCE and TCA removal should be addressed.

b. The ability of different mixed cultures from various soils on their abilities to oxidize TCE and TCA should be better defined using propane or aviation gasoline as a primary substrate.

c. The potential for inhibition of TCE oxidization by TCA should be described.

d. Determine any potential effects by protozoal predation of the biomass on the efficiency of the removal of TCE and TCA.

3. Soil based bioreactors to treat air streams contaminated with petroleum hydrocarbons are ready for pilot-scale evaluation. Successful demonstration will require clean understanding of the following:

a. The effect of soil moisture on mass transport limitations and microbial biodegradation rates will have to be determined for the particular soil used in the demonstration.

b. The effect of other hydrocarbons in the spill on removal of the aromatics must be determined.

c. The influence of physical properties of the soil such as grain size distribution, organic carbon content, and soil moisture release characteristics should be defined to identify the most appropriate soils for use in bioreactors.

SECTION 3

LIQUID PHASE TREATMENT OF TRICHLOROETHYLENE

METHODS AND MATERIALS

Laboratory experiments were conducted to determine the effects of changing environmental conditions in unsaturated fixed-film bioreactors using methane as the primary substrate, the goal being to remove TCE as a secondary substrate by the cometabolism process.

Lincoln fine sand was used as the inoculum in completely mixed reactors in which air and natural gas were bubbled to develop enrichment cultures. The enrichment cultures were then used to promote growth in laboratory-scale packed columns using methane as the sole source of carbon. Air and water were injected, mineral nutrients were added, and the percent removal of methane monitored. Finally, TCE was injected as a solution in the influent water, and the percent removal monitored. After the completion of the enrichment and acclimation process, the columns were operated over a range of hydraulic and organic loadings during which the system performance was assessed by monitoring the influent and effluent concentrations of methane and TCE, as well as the flows of water, air, and methane.

Two silica sands were used in the study as packing media. The sands were obtained from water filter supplies and prescreened and washed to remove any organic matter. The saturated hydraulic permeability (Darcy's constant) was determined by a constant head permeameter according to ASTM method C127. The sieve analysis was performed according to ASTM D 422-63 using standard U.S. Sieve Series.

A schematic of the packed column bioreactor design is depicted in Figure 3.1. The columns were designed so that all surfaces in contact with the feed water solution containing TCE are either glass or teflon. The reactors were constructed of borosilicate glass columns 61 cm long by 6 cm in diameter, filled with 6 cm of glass beads at the bottom, 48 cm of coarse sand as the main packing media, and 6 cm of glass beads at the top. The entire media was supported by a porous aluminum plate. Two headspace air sections in both ends of the columns were left for even distribution of both air and liquid flows as well as to separate both phases for sampling purposes.

The TCE solution was fed into one of the three top ports of the column by means of a peristaltic pump with variable speed drives (Masterflex Pumps), using pump head number 14 (Masterflex Heads). The same water flow was withdrawn from one of the three bottom ports of the columns using the same head size so that a continuous water flow will be established.

A similar peristaltic pump was used to pull a slight vacuum from one of the three ports to establish an effluent air flow equal to the influent mixture of methane and air flow. To achieve desired concentrations of methane in the influent, an air cylinder was connected by tygon tubing to one of the top ports, with the pressure set to atmospheric by a PVC cylinder with an open orifice that allowed excess air to bleed off. Methane was then injected in the influent tubing. Sufficient tubing length was provided for proper mixing prior to the sampling port. The control column was constructed identically to the other columns, but without the primary substrate added.

A concentrated aqueous solution (250 mg/l) of TCE was prepared in a 50 gal. HDPE container with a floating lid and a sealed cover. A pump was installed for mixing, and several ports with ball valves were placed in different locations of the container for adequate recirculation. Tap water from a ground water supply, showing TCE concentrations below detectable levels, was aerated for at least 244 hours before the solution was prepared. The concentrated aqueous solution of TCE in the required volume was injected with a glass syringe through a septum port installed in the discharge line of the pump. The solution was recirculated for 30 minutes for proper mixing and then connected to the system.

A concentrated aqueous solution of ammonium sulfate and potassium phosphate monobasic was prepared to produce a N/P ratio of 5/1. The concentrated solution contained 51 g/l of ammonium sulfate equivalent to 10.6 g/l of nitrogen, and 9.0 g/l of potassium phosphate monobasic equivalent to 2.0 g/l of phosphorus. The amount of oxygen required for 100 percent degradation of methane was estimated. The concentrated aqueous solution was diluted to provide a ratio of 100/5/1 for oxygen, nitrogen, and phosphorus.

The flow of methane was regulated by a cartridge pump (ISMATEC) with speed control. To achieve the desired mixture of air and methane, a peristaltic pump was used to withdraw a constant flow of air from the bottom of the columns. The flows of air, methane, and water was changed by controlling the speed of the peristaltic pumps. Experimentation was made with an air flow of 4 to 12 ml/min, a water flow of 1.8 to 8.5 ml/min, and a range of methane flow of 0.017- 0.25 ml/min. Influent methane concentrations from 1 mg/l to 16 mg/l were injected, and TCE concentrations from 80 µg/l to 1400 µg/l were utilized.

The development of an enrichment culture was accomplished by bubbling air and natural gas in three 1-liter, completely mixed reactors seeded with Lincoln sand. After two weeks of operation, the reactor which showed the most extensive growth was selected as the inoculum for the rest of the experiment. Next, an enriched biofilm was established in a packed column bioreactor fed exclusively with methane as the sole source of carbon and energy, with air and water for aerobic conditions and a suitable environment for microbial growth.

As part of this experiment, two identical columns were assembled in series as depicted in Figure 3.2. A known concentration of TCE was injected at one of the upper ports of column 1, and the effluent water was pumped through Teflon tubing to column 2 by a peristaltic pump. Air and methane were injected in a second port located at the top of column 1; additional methane was injected in the effluent air line that interconnects both columns to insure an equal influent methane concentration in both bioreactors. At the bottom of column 2, the air and water effluents were withdrawn by peristaltic pumps. Sampling and flow measurements were conducted at the locations shown in Figure 3.2. Prior to assembling the columns in series, both bioreactors were operated separately for at least 10 days to allow acclimation.

To observe how the distribution of microorganisms and contact times affect the removal of TCE, a column was assembled as a replicate of another one in regard to influent TCE concentration, water flow, air flow and total influent mass of methane; however, the methane was injected at the top, one-third and two-thirds points of the multiport column by inserting 1/16 in. stainless steel tubing through ports located in the top and bottom caps of the column, as shown in Figure 3.3. The total methane flow was monitored in the three lines by bubble

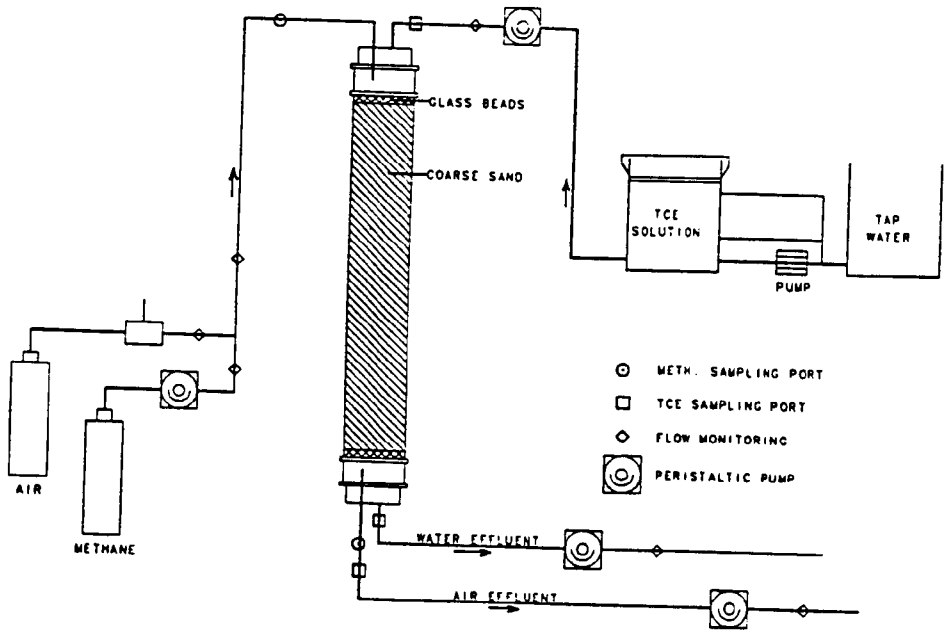


Figure 3.1. Schematic of Fixed-film Bioreactor.

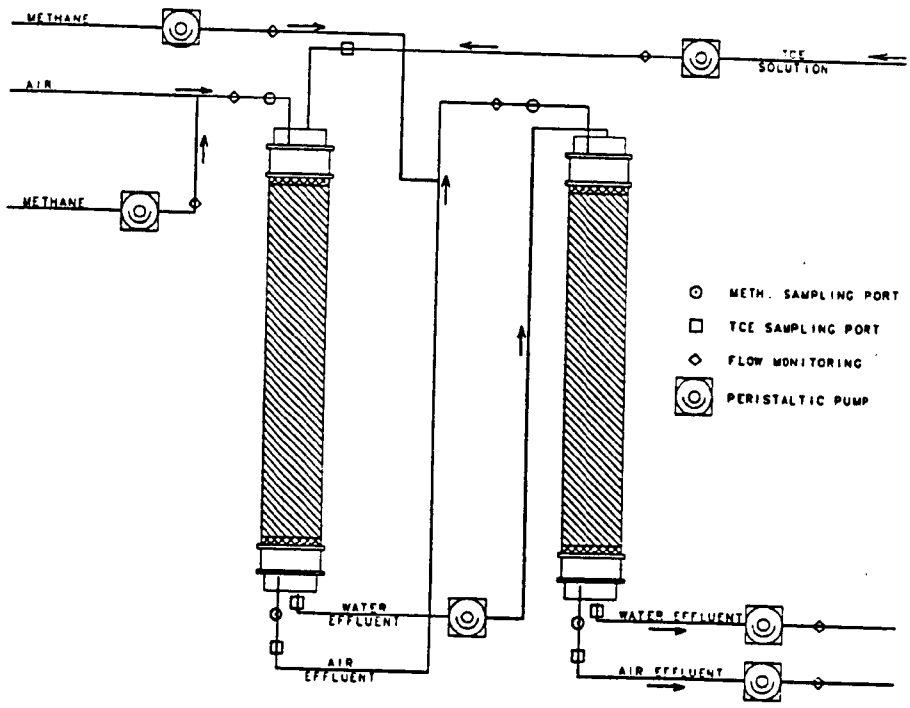


Figure 3.2. Schematic of Bioreactors in Series.

flowmeters to insure that the flow, and therefore the mass of methane, was the same as a parallel control column with all the methane injected at the top.

Removal of methane under unsteady- and steady-state conditions was studied by utilizing a column with four sampling ports located at 0, 8, 15, and 61 cm, and monitoring the remaining methane concentration at each port for several influent concentrations, keeping the other variables constant; for example, the water flow, air flow, and TCE concentration. Figure 3.4 depicts this arrangement. Sampling points 1 and 4 were taken from the influent and effluent air lines respectively; sampling points 2 and 3 were taken from two 1/16 in. stainless steel tubes inserted in the column through two ports located at the top and bottom caps of the column. Prior to sampling the columns a small amount of air was gently withdrawn with a gas tight syringe to remove the stagnant air, then the samples were taken as described earlier. Methane and TCE were monitored.

The unsteady-state study was performed by installing a new column that was be seeded with microorganisms taken from an existing column. The removal of methane over time was monitored hourly for the first day and twice a day the remaining period until steady state conditions were reached at all the sampling points. The steady-state study was conducted by changing the influent methane concentration and monitoring the methane concentration until no further changes were observed at all sampling points. The information collected from these studies, in conjunction with the estimation of the dry cell mass per unit volume, was used to explore the development of a model for methane removal.

The pressure drop through the packed column bioreactors was estimated at steady state conditions using a sensitive differential manometer. Gage pressures were measured at the influent and effluent lines and differential pressures were determined between both lines as a double check.

To estimate the average retention time of a column for a given set of conditions, a known concentration of sodium chloride was injected at the top of the column without disturbing the normal operation of the flows. The change in electrical conductivity in the water effluent was monitored with an electrical conductivity meter every 5 minutes until the concentration of sodium chloride was constant for three consecutive readings. A normalized response curve provided information not only about residence times, but also about the time span between the first appearance of the tracer and the time when the effluent concentration equaled the influent concentration.

At the end of the experiment, the columns were disassembled to estimate the dry mass of cells per unit volume of media. Ten cubic centimeters of sand was collected at five different depths (0 cm, 8 cm, 15 cm, 30 cm, and 61 cm) and weighted after drying at 103°C. The residual sand was dried at 550°C for two hours, then cooled in a desiccator and washed with a solution of chromic and sulfuric acid. The samples were then dried at 550°C., cooled and weighed again. The same procedure was performed for the control column. The dry mass of cells per unit volume was calculated as follows:

$$\frac{\text{Dry mass of cells}}{\text{Volume of media}} = \frac{(A - B) - (C - D)}{V}$$

Where,

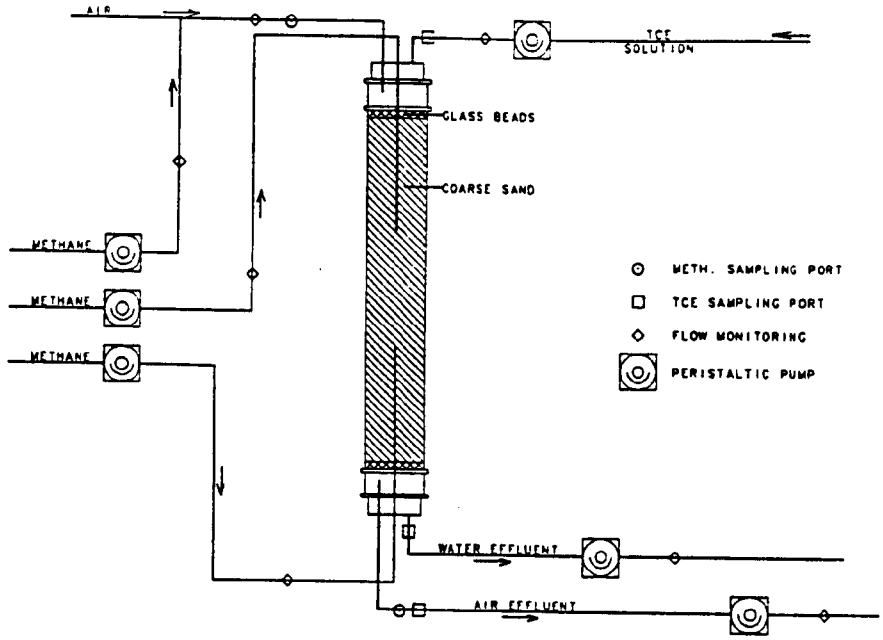


Figure 3.3. Schematic of Bioreactor with Multiport Methane Injection.

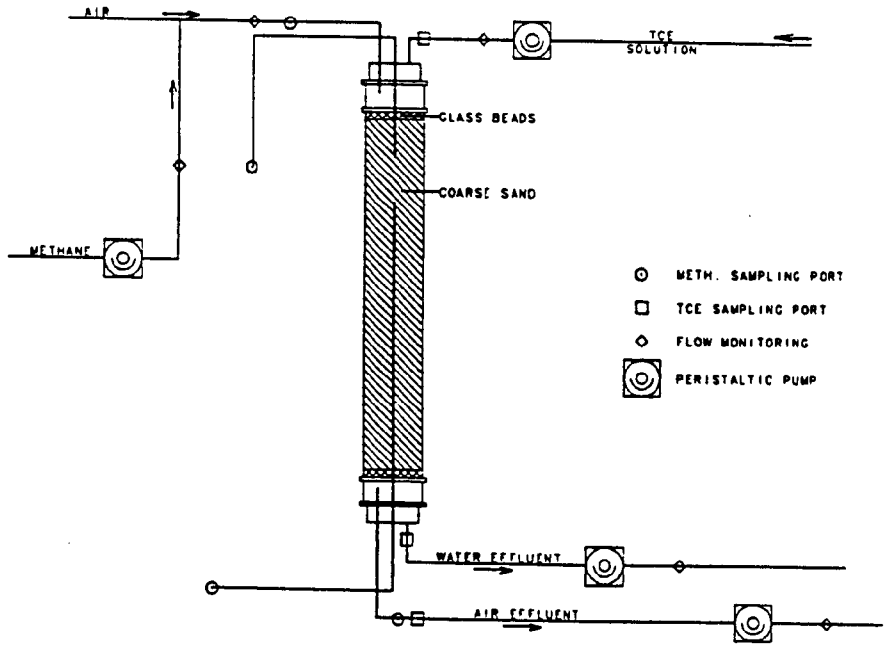


Figure 3.4. Schematic of Bioreactor with Intermediate Methane Sampling Ports.

A = weight of sample in column fed with methane after drying at 103 °C
B = weight of sample in column fed with methane after drying at 550 °C
C = weight of sample from control column after drying at 103 °C
D = weight of sample from control column after drying at 550 °C
V = initial sample volume

Quick connectors were installed in the influent lines of the columns for sampling purposes. Teflon plug valves were connected directly at the bottom caps of the columns to temporarily divert the flow for sampling, allowing for rapid connection of the sample bottles without disturbing the normal flow. Liquid samples were collected in 10 ml-bottles with screw-cap lids and Teflon-faced septa. The bottles were left in place long enough for 10 to 20 flushings before the samples were taken without headspace.

TCE gas samples were collected by using a gas-tight syringe to withdraw a given volume of air from a sampling port provided with a septum. The sample was then injected into a packed resin trap (Tenax GC) using nitrogen as the carrier gas.

Duplicate gaseous samples for methane were collected in the influent and effluent lines by using a gas-tight syringe with push-button valves to withdraw a volume of air from the sampling port, then immediately injected into an FID gas chromatograph for analysis.

Trichloroethylene was analyzed by a Hewlett Packard 5880 gas chromatograph equipped with a Hewlett Packard 7675A purge and trap analyzer. Modification of purge-and-trap Method 624, modified for GC/ECD, found in "Methods for Organic Chemical Analysis of Municipal and Industrial Wastewater," EPA-600/4-82-957, July, 1982, was used for the analyses. After the purging was completed, the sorbent column was desorbed onto the gas chromatograph with temperature-programming and electron capture detection (ECD). The limit of detection was 1 µg/l. Methane was analyzed on a Tracor 560 Gas Chromatograph using isothermal programming and Flame Ionization Detection (FID).

RESULTS AND DISCUSSION

The research program involved an acclimation period and several steady-state phases. The program was designed to explore the following issues:

(a) Hydraulic performance of the sands used to construct bioreactors.
(b) Effect of biofilm growth on the pneumatic and hydraulic performance of the bioreactors.
(c) Effect of air to liquid ratio on TCE removal.
(d) Effect of methane concentration on TCE removal.
(e) Effect of TCE concentration on TCE removal.
(f) Effect of delivery schedule of methane on TCE removal.
(g) Comparison of TCE removals with natural gas and methane.

To explore these issues, the fixed-film bioreactors were operated over a range of TCE concentrations, methane concentrations, and air and water flows for two coarse sands used as packing media. Different arrangements of the columns were made for one of the coarse sands during the last phases of the program, with the aim being to improve the biodegradation of TCE.

System performance was assessed by monitoring the influent and effluent concentrations of methane and TCE along with the hydraulic characteristics of the columns.

Two coarse silica sands (sands 2 and 3) were used as packing material. Both sands were prescreened and washed to remove organic matter. The characteristics of the sands used during the experimentation are shown in Table 3.1. Adsorption tests for TCE were performed to determine the Freundlich isotherm constants; however, no adsorption was detected even after three days of contact for TCE concentrations of 465 μg/l. These results confirm the absence of significant organic matter, clay or silt.

Table 3.1. Characteristics of Coarse Sands

Coarse Sand	Porosity n_1 (%)	Density (g/cm^3)	Saturated Hydraulic Permeability (cm/day)	Coefficient of Uniformity (Cu, D60/D10)	Effective Size (mm)	Mean Size (mm)	Unified Soil Classification
2	38.0	2.67	5.97×10^3	1.66	0.75	0.72	Poorly Graded Sand
3	39.9	1.61	1.50×10^4	1.74	1.60	1.40	Poorly Graded Sand

Tap water from a local ground water source was used to prepare the TCE solution. The quality characteristics of the ground water are shown in Table 3.2. A summary of the hydraulic characteristics of the system for the two sands is shown in Table 3.3. The selected water flows were well below the saturated hydraulic conductivity (Table 3.3).

The unsaturated hydraulic retention time was estimated by using sodium chloride as a tracer. The electrical conductivity in the effluent was measured over time and the data were normalized to get the response curves. The results are summarized in Table 3.3. As expected, the unsaturated hydraulic retention times were greater for sand 2 than for sand 3 due to its smaller mean particle size.

Although a narrow range of grain sizes were selected to provide a uniform flow, the elapsed time from the first appearance of the tracer to the time when the effluent concentration equalled the influent concentration was remarkably longer than expected. Unsaturated packed columns are not truly plug-flow reactors but a combination of plug-flow and mixed reactors due mainly to internal short circuiting, wall effects, the tortuosity of the channels and the mixing effect produced by the air stream. Therefore, when a liquid is applied to the column, various portions of the liquid follow different flow patterns. With lateral and longitudinal mixing, different residence times result for different particles of fluid even though they were part of the same mass of fluid when applied to the column. Additionally, part of the liquid is held on the bed, part of the liquid adheres to the grains by molecular attraction, other portions of the liquid are held at the points of contact between the grains by capillary forces, and finally, some liquid flows over the grains by gravity and percolates downward.

Table 3.2. Analysis of Major Constituents of Ground Water

Constituent	Concentration mg/l
Calcium	10.4
Magnesium	10.2
Sodium	153
Carbonate ion	5.4
Bicarbonate ion	310
Chloride ion	41
Hardness	68 as $CaCO_3$
Alkalinity	340 as $CaCO_3$
pH	8-8.25 units
TCE	< 1 µg/l

The effect of microbial growth on the unsaturated hydraulic retention time was studied by feeding three columns with methane at concentrations of 3.2, 14, and 24 mg/l in air, keeping all the other conditions constant.

Table 3.3. Hydraulic Characteristics of Systems with Sand Media 2 and 3

Water Flow (ml/min)	Air Flow (mg/min)	Media	Porosity	Saturated Hydraulic Retention Time (min)	Average Unsaturated Hydraulic Retention Time (min)	Hydraulic Loading ($ft^3/d/ft^2$)
1.85	7.5	Sand 2	0.38	253.8	125.0	4.3
4.2	7.5	Sand 2	0.38	111.8	75.0	9.8
6.0	7.5	Sand 2	0.38	78.2	57.5	14.0
4.2	7.5	Sand 3	0.40	117.4	45.0	9.8
4.2	11.0	Sand 3	0.40	117.4	47.5	9.8
6.0	6.4	Sand 3	0.40	82.2	37.5	14.0
8.4	11.0	Sand 3	0.40	58.7	27.5	19.6

The dry density of the microorganisms per unit volume of sand was determined with influent methane concentrations of 24, 14, 8.9, and 3.5 mg/l, respectively (Figure 3.5). Most of the biomass developed in the upper 15 cm of the columns, with biomass decreasing sharply by 30 cm. Thereafter, the biomass remained low and fairly constant.

The observed methane removal was in excess of 98 percent at all four methane concentrations. Although the observed microbial growth was significantly greater in the column receiving the higher concentration of methane (Figure 3.5), little difference was observed in the residence times of water (Figure 3.6). Because most of the biofilm developed in the top 8 to 15 cm of the columns, microbial growth did not significantly affect the residence time in the column.

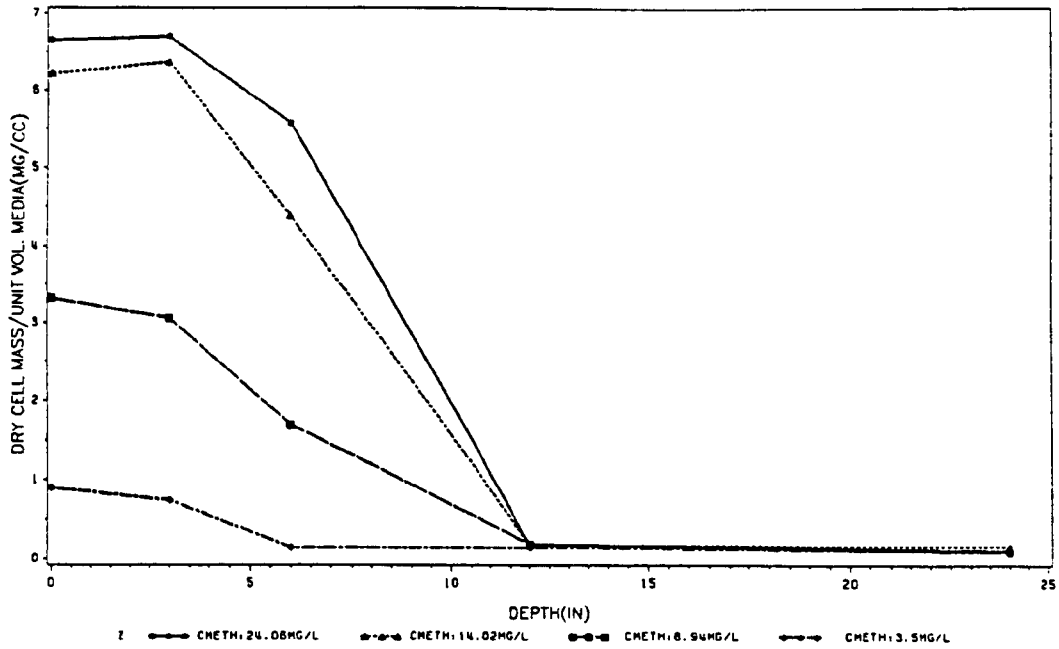


Figure 3.5. Variation of Dry Cell Mass Per Unit Volume of Sand as a Function of the Influent Methane Concentration. (Water Flow: 6.3 ml/min; Air Flow: 6.0 ml/min; Influent TCE Concentration: 270 µg/l; Media: Sand 3).

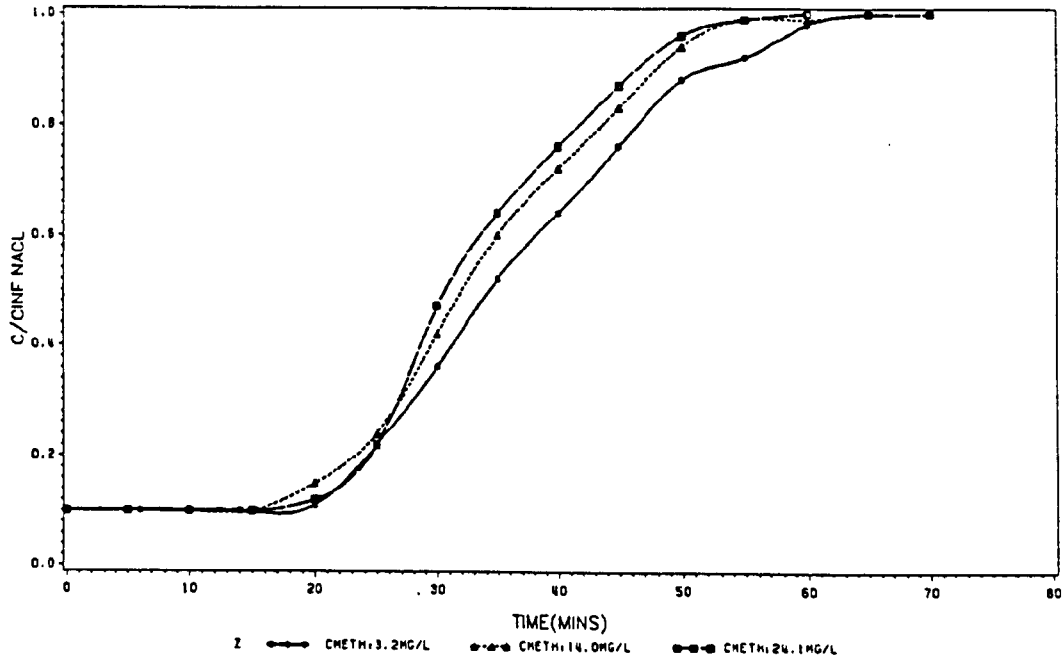


Figure 3.6. Normalized Breakthrough Response of Sodium Chloride For Several Influent Methane Concentrations. (Water Flow: 6.0 ml/min; Air Flow: 6.4 ml/min; Influent TCE Concentration: 270 µg/l; Media: Sand 3; Average Residence Time: 37.5 minutes).

Pressure drops were measured for some of the air-water combinations by means of a precise differential water manometer (Table 3.4). The losses through the columns increased as a result of either increasing the water or air flows; however, an increase in the water flow rate had a greater impact on the system. The effect of microbial growth on pressure drop was assessed by measuring the pressure drop in the five columns under steady state operations with regard to methane; other measured conditions were nearly constant (see Table 3.4). Although the growth of microorganisms did not affect the unsaturated hydraulic retention time, it affected the pressure drop significantly, which increased from 0.19 in. of water/ft in a clean column to more than 0.44 in. of water/ft in the column acclimated to 25 mg/l methane (Figure 3.7, Table 3.4).

Since air stripping is to be expected in any packed tower fed with a volatile chemical, the air-to-water ratio is a fundamental parameter in designing an aerobic bioreactor for treating TCE. Air is provided as the source of oxygen; however, if the TCE is not completely removed by the methanotrophs, some TCE will leave the system in the air stream.

An estimation of the effect of the air-to-water ratio on the stripping of TCE was developed by plotting the TCE mass ratio in the air and water effluent versus the volumetric air to water ratio. The results are shown in Figures 3.8 and 3.9. The following relations were obtained by linear regression of the collected data:

$$\frac{\text{TCE in effluent air } (\mu g)}{\text{TCE in effluent water } (\mu g)} = 0.728 \frac{\text{Air}}{\text{Water}} - 0.092 \quad \text{(sand 2)}$$

correlation coefficient = 0.95

$$\frac{\text{TCE in effluent air } (\mu g)}{\text{TCE in effluent water } (\mu g)} = 1.068 \frac{\text{Air}}{\text{Water}} - 0.644 \quad \text{(sand 3)}$$

correlation coefficient = 0.94

These relations hold independent of the influent TCE concentration, the percent removal of TCE, or the influent methane concentration, excepting for the special condition when the TCE concentration was greater than 1400 µg/l for an air-to-liquid ratio greater than 2.6. These results may be due to the microbial growth developing in the upper 8 to 15 cm of the columns. If TCE is biodegraded in the upper section, the remaining longer segment of the column will allow a mass transfer equilibrium to develop between air and water. Based on the information above, a system with a low air-to water ratio is recommended.

Pure methane was used as the primary substrate because it has been proven by others to support cometabolism for the removal of TCE and related compounds. The removal of methane without the addition of nutrients ranged from 30 to 70 percent (data not shown). After the addition of nitrate and phosphate, in the ratio of 100/20/5 for COD/N/P, the removal of methane was two orders of magnitude higher and independent of the influent methane concentration (Table 3.5).

The removal of methane with depth in the column bioreactors was studied by taking air samples at 8, 15, 30, 41, and 61 cm (Table 3.5). This was accomplished by installing a new column seeded with microorganisms from one existing column, and increasing the methane

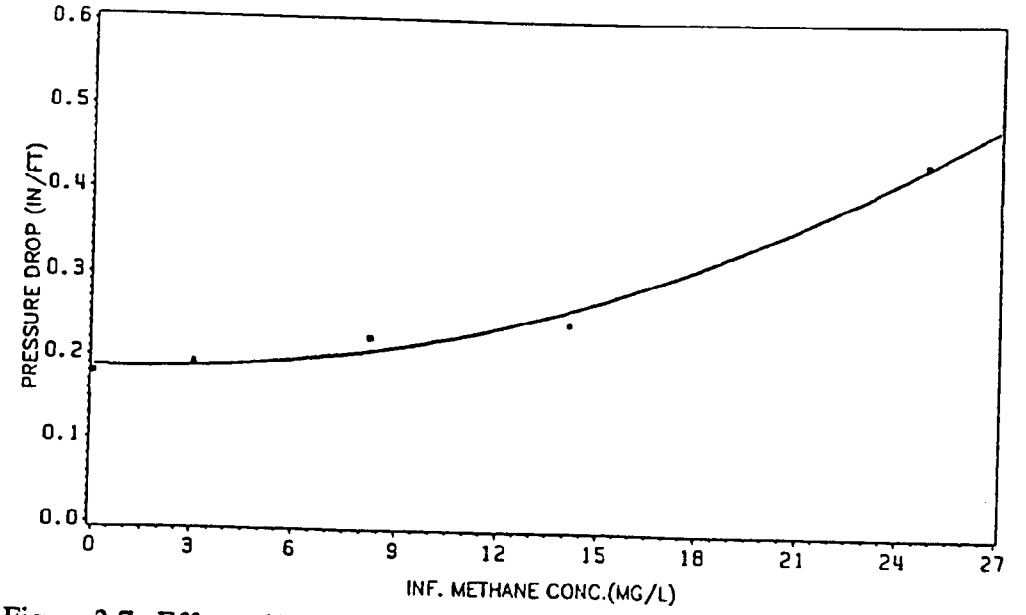


Figure 3.7. Effect of Microbial Growth on Pressure Drop at Several Steady State Conditions: (Water Flow: 4 ml/min; Air Flow: 7 ml/min; % Methane Removal: 99+; Media: Sand 3).

Table 3.4: Pressure Drop for Unsaturated Packed Columns

Media	Water Flow (ml/min)	Air Flow (ml/min)	Methane Concentration in Air (mg/l)	Pressure Drop (in water/ft of column)
Sand 2	4.2	7.5	0	1.40
Sand 2	6.1	7.7	0	1.67
Sand 3	4.2	7.5	0	0.11
Sand 3	6.0	7.5	0	0.18
Sand 3	4.2	11.0	0	0.19
Sand 3	8.5	11.0	0	0.41
Sand 3	5.9	6.7	0	0.18
Sand 3	5.9	6.6	3.0	0.19
Sand 3	5.9	6.6	8.3	0.23
Sand 3	5.9	6.6	14.1	0.24
Sand 3	5.9	6.6	24.9	0.44

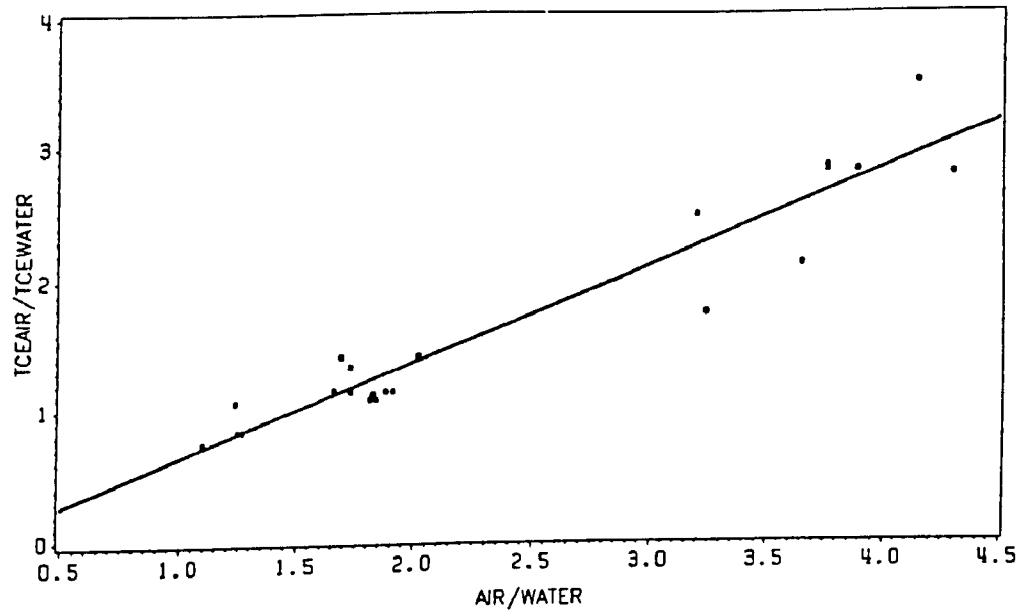


Figure 3.8. Relation Between Basic Column Air-to-Water Ratio and Ratio of TCE Mass in the Air and Water Phases of the Effluent for Sand 2.

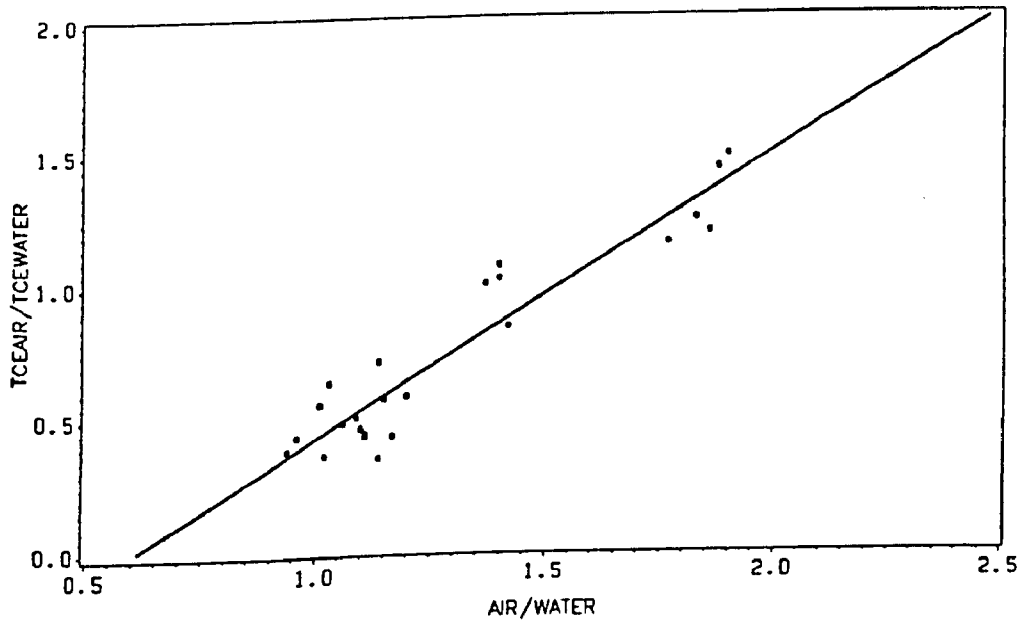


Figure 3.9. Relation Between Basic Column Air-to-Water Ratio and Ratio of TCE Mass in the Air and Water Phases of the Effluent for Sand 3.

concentration stepwise from 3.7 to 24 mg/l while keeping the water and air flow nearly constant. After each methane increment, the methane concentration in the intermediate ports and effluent were measured until steady state conditions were reached. Additionally, samples at 30 cm and 41 cm were taken in two columns with an influent methane concentration of 3.8 and 14 mg/l, respectively. The influent TCE concentration for the study of methane removal with depth was 440 µg/l.

Table 3.5. Removal of Methane at Different Depths for Several Influent Methane Concentrations

	Methane Concentrations and Removal									
Influent	8 cm		15 cm		30 cm		41 cm		61 cm	
Conc. (mg/l)	Conc. (mg/l)	% Removal	Conc. (mg/l)	% Removal	Conc. (mg/l)	% Removal	Conc. (mg/l)	% Removal	Conc (mg/l)	% Removal
3.8	0.1	97.8	0.04	99.1	----	----	0.01	99.7	0.01	99.7
9.7	0.3	97.0	0.06	99.4	----	----	----	----	0.03	99.7
13.9	2.4	82.6	0.33	97.6	0.05	99.6	----	----	0.03	99.8
24.1	7.1	70.3	1.8	95.5	----	----	----	----	0.03	99.9

More than 95 percent of the methane was removed in the top 8 cm when the influent methane concentration was less than 10 mg/l. The same removal was achieved in the top 15 cm when the influent methane concentration was between 13 mg/l and 25 mg/l. In all the cases more than 99 percent of the methane was removed throughout the length of the column. The columns responded to an increase in the influent methane concentration within a few days (Table 3.6).

The effects of influent methane concentrations were studied by keeping all the variables constant while varying the influent methane concentration. At a water flow of 6.1 ml/min, an air flow of 6.2 ml/min and an influent TCE concentration of 430 µg/l, the percent removal of TCE increased as the influent methane concentration increased (Figure 3.10). Even though the percent removal increased with an increment in the influent methane concentration, the specific mass of TCE removal per unit mass of methane consumed decreased (Tables 3.7, 3.8); this means that a large increment in the concentration of the primary substrate produced only a relatively small increment in TCE removal.

Table 3.6. Elapsed Time for Complete Acclimation After Increasing the Influent Methane Concentration

Initial Methane Concentration (mg/l)	Methane Concentration After Increment* (mg/l)	Elapsed Time for Complete Acclimation (days)
0	4	7
4	10	5
10	14	4
14	24	4

*Steady state was reached in the order of days for all the sampling ports.

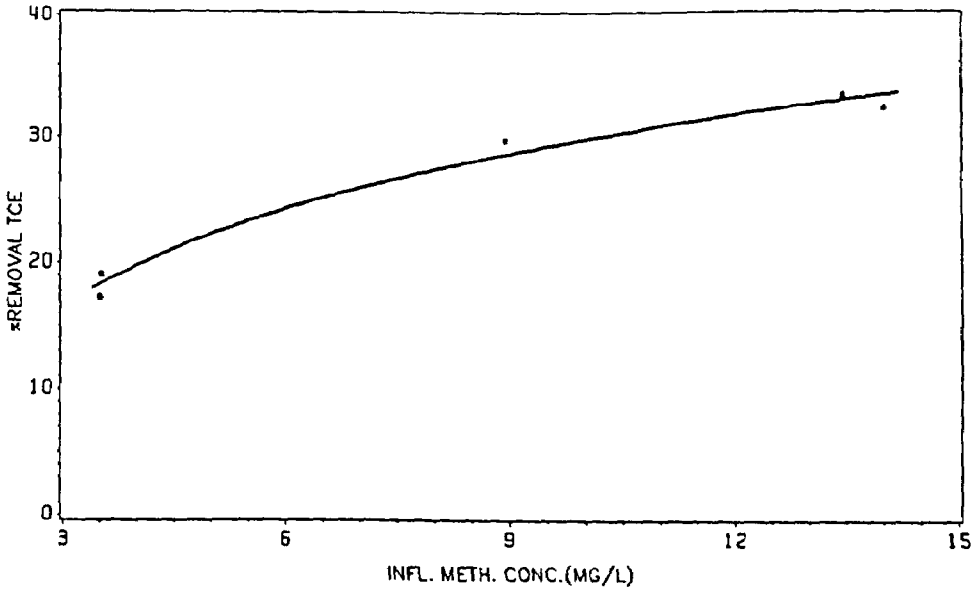


Figure 3.10. Effect of Influent Methane Concentration on TCE Biodegradation. (Water Flow: 6.1 ml/min; Air Flow: 6.2 ml/min; Influent TCE Concentration: 430 µg/l; Media: Sand 3).

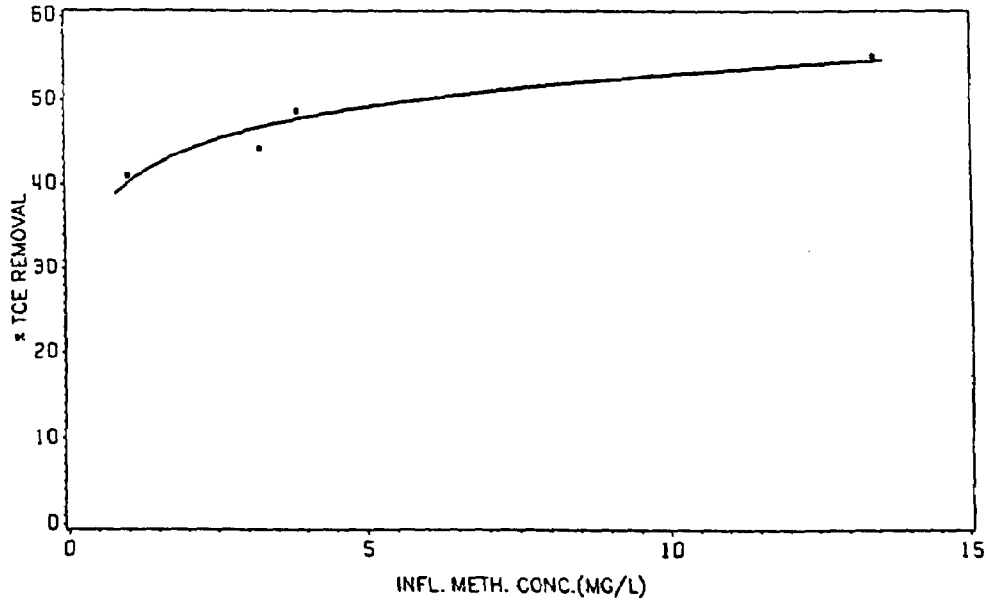


Figure 3.11. Effect of Influent Methane Concentration on TCE Biodegradation. (Water Flow: 4.1 ml/min; Air Flow: 7.6 ml/min; Influent TCE Concentration: 430 µg/l; Media: Sand 3).

In a second experiment, the conditions were kept similar in regard to air flow and influent TCE concentration (air flow: 7.6 ml/min, influent TCE concentration: 430 µg/l), but the water flow was reduced to 4 ml/min (Figure 3.11). The data obtained was in agreement with the first experiment, but the percent removals were higher, and could be attributed to the increased retention time.

The amount of TCE removed per unit of methane consumed ranged from 3.4 to 30 µg of TCE per gram of methane (Table 3.8), except for one isolated case when the methane concentration was 1 mg/l. As was noticed before, although an increase in the influent methane concentration resulted in a greater removal of TCE, the specific TCE utilization decreased significantly. For example, when the influent methane concentration was reduced from 13 to 3 mg/l the specific utilization increased from 2 to 10 µg of TCE removed per mg of methane consumed. Conversely, an increase in the influent TCE concentration increased the specific TCE utilization.

Several models were tried to fit the data collected from the study of methane consumption versus depth for sand number 3 (Table 3.5). The model that best fit the data followed Monod kinetics. For the development of the model it was assumed that the inhibitory effect of TCE was negligible. The model is somewhat limited because a constant air flow rate was used during the study of methane removal. The model is as follows:

$$\ln(S/So) = K_1(1/So - 1/S) - K_2H^n$$

where

So = influent methane concentration (mg/l)

S = methane concentration at depth H (mg/l)

H = depth

K_1,K_2= constants

As the model is not linear with regard to H, regression analysis was performed with an iterative process to evaluate n, the results are shown below:

$K_1 = 0.022$

$K_2 = 2.018$

$n = 0.31$

$r^2 = 0.967$

The effect of the influent TCE concentration was assessed by establishing a set of conditions that were kept constant (i.e., water flow, air flow, methane inflow) while the TCE concentration was changed. As the influent TCE concentration increases, the percent removal increased (Figure 3.12 - Figure 3.17). This behavior has been reported by Grady and Lim (1980) when they stated "...when the applied flow rate is low, an increase in flow rate will cause a relatively large reduction in the resistance to mass transfer, consequently, the rate of removal can increase. As further increases are made in the flow rate, however, additional reduction in the mass transfer resistance becomes negligible so that no benefits accrue to offset the deleterious effect of the decreased time of contact."

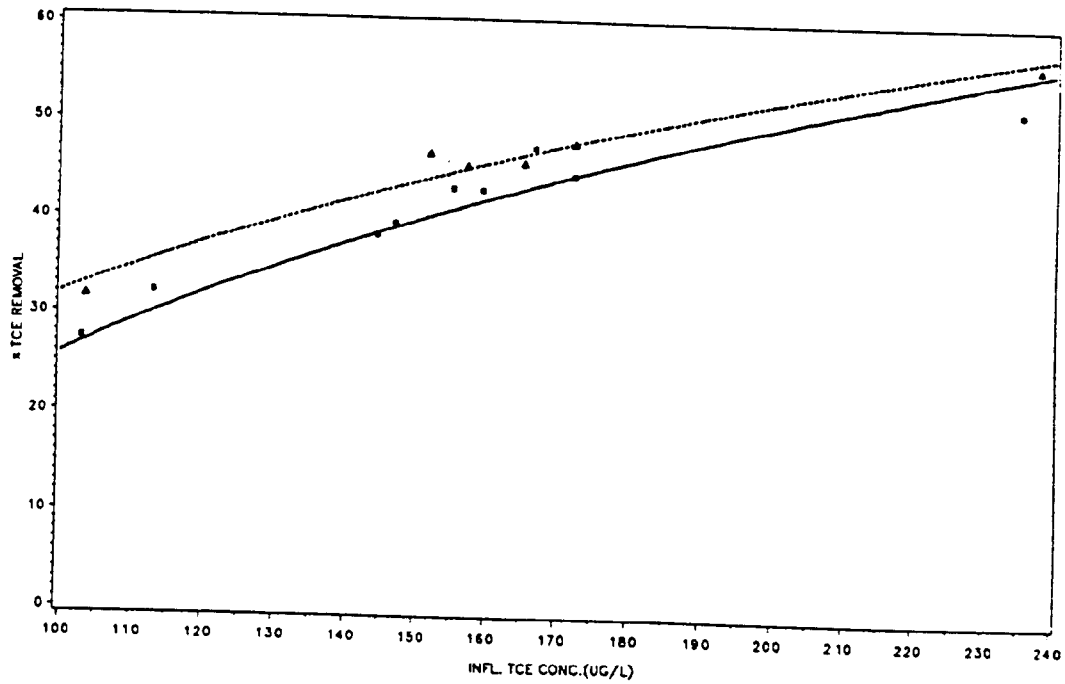


Figure 3.12. Effect of Influent TCE Concentration on TCE Biodegradation. (Water Flow: 6.1 ml/min (Upper Curve, 4.1 ml/min (Lower Curve); Air Flow: 7.4 ml/min; Influent Methane Concentration: 15 mg/l; Media: Sand 2).

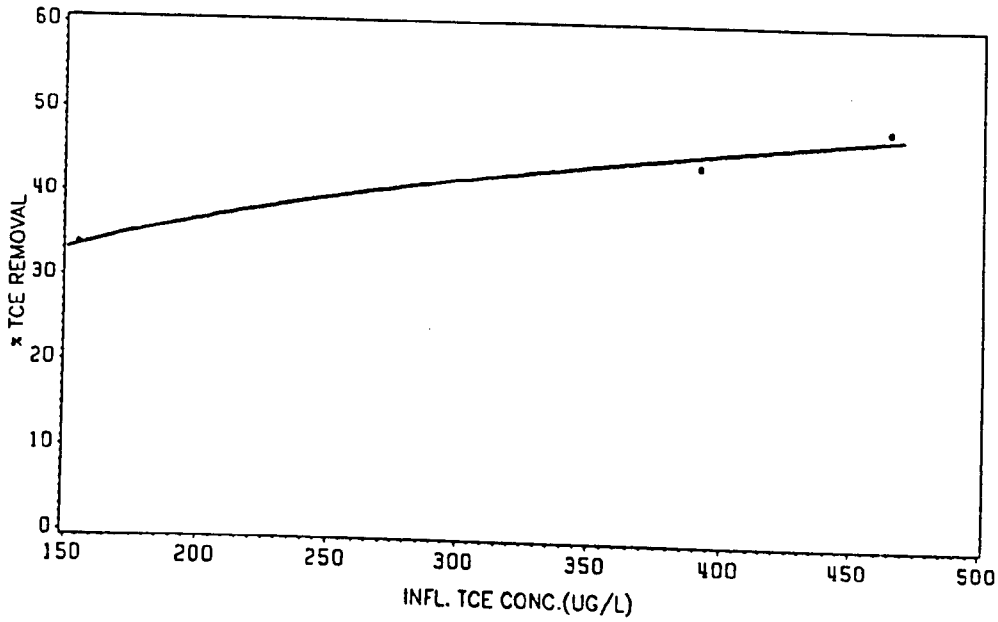


Figure 3.13. Effect of Influent TCE Concentration on TCE Biodegradation. (Water Flow: 6.1 ml/min; Air Flow: 7.6 ml/min; Influent Methane Concentration: 15 mg/l; Media: Sand 2).

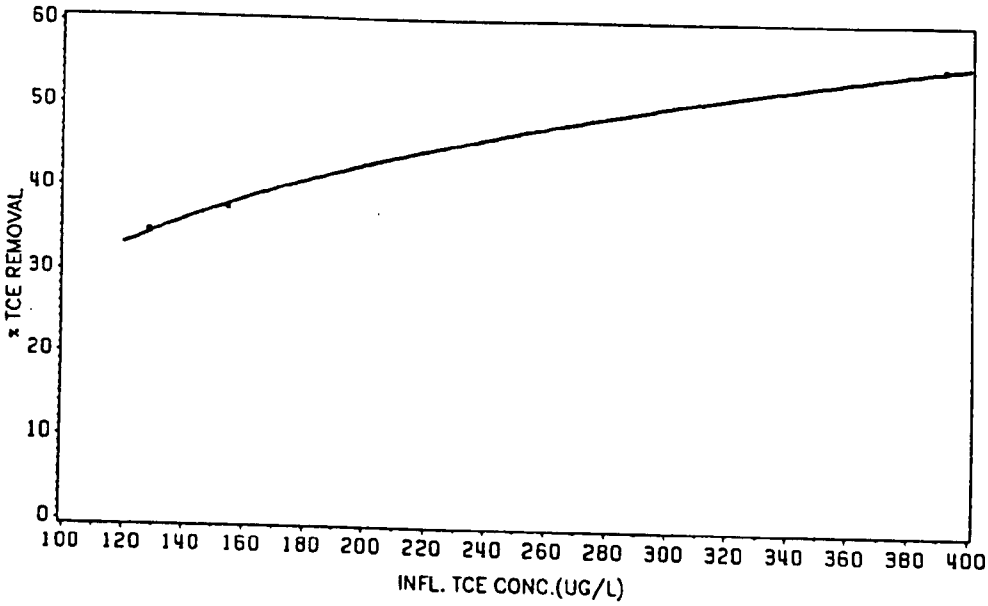


Figure 3.14. Effect of Influent TCE Concentration on TCE Biodegradation. (Water Flow: 4.2 ml/min; Air Flow: 7.3 ml/min; Influent Methane Concentration: 14 mg/l; Media: Sand 2).

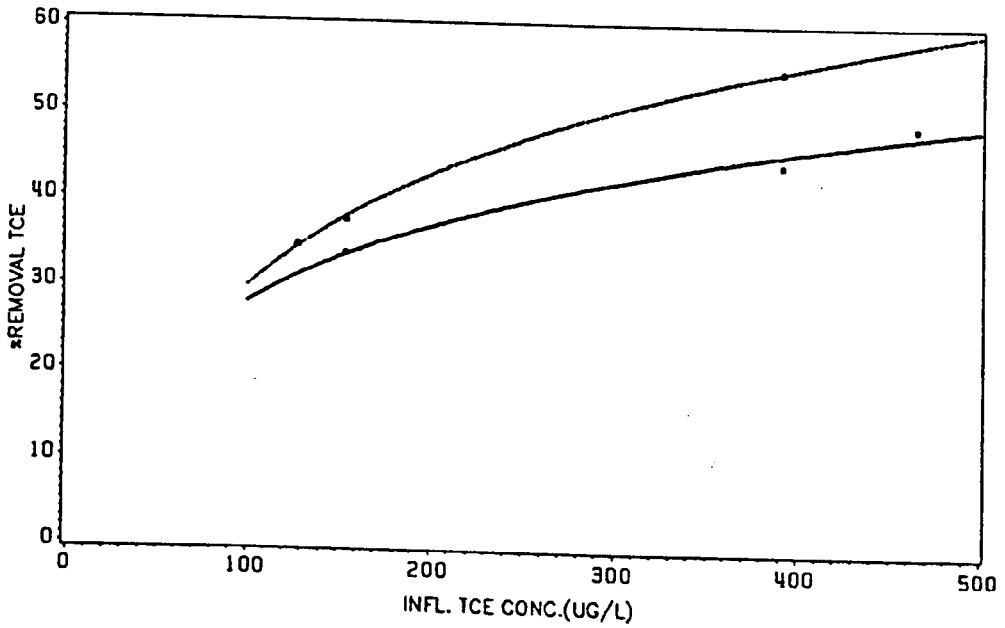


Figure 3.15. Effect of Influent TCE Concentration on TCE Biodegradation. (Water Flow: 4.1 ml/min; Air Flow: 7.5 ml/min; Influent Methane Concentration: 13 mg/l (upper curve), 3.4 mg/l (lower curve); Media: Sand 3).

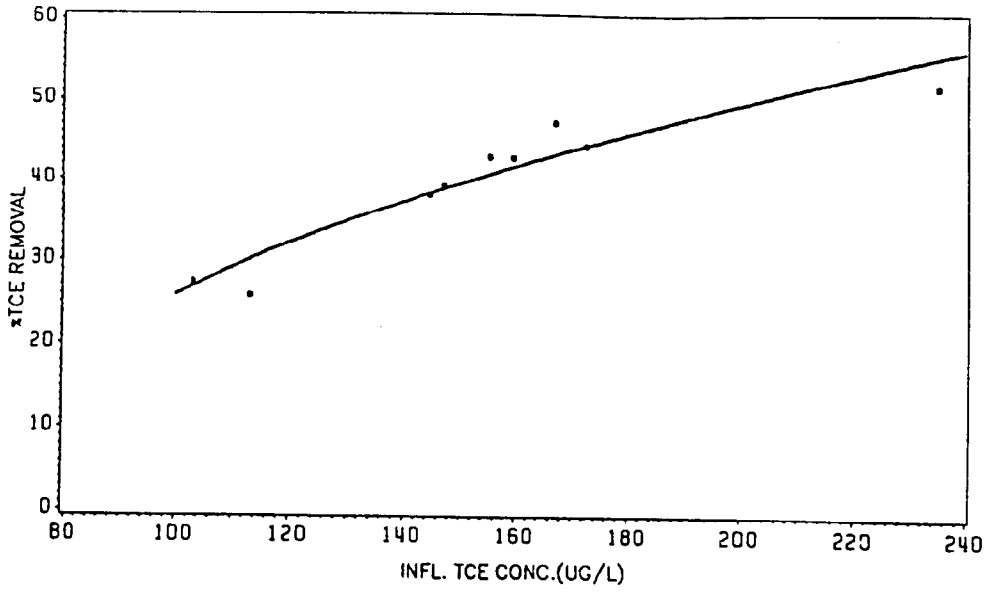


Figure 3.16. Effect of Influent TCE Concentration on TCE Biodegradation. (Water Flow: 4.1 ml/min; Air Flow: 7.6 ml/min; Influent Methane Concentration: 13.4 mg/l; Media: Sand 3).

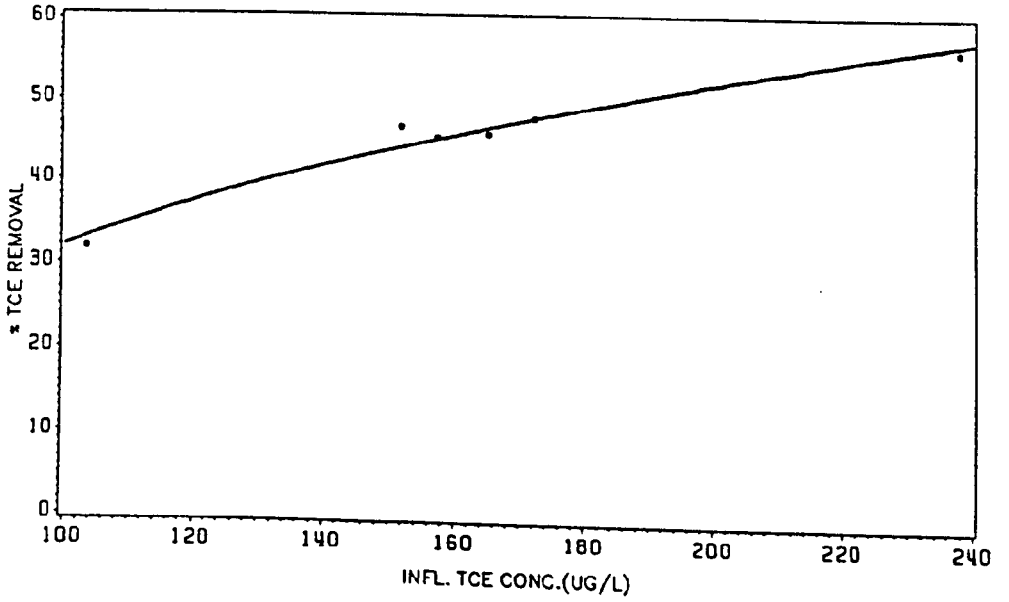


Figure 3.17. Effect of Influent TCE Concentration on TCE Biodegradation. (Water Flow: 4.1 ml/min; Air Flow: 7.6 ml/min; Influent Methane Concentration: 13.4 mg/l; Media: Sand 3).

Table 3.7. Decrease of TCE Removal Resulting from Inhibition of Methanotrophs

Influent Methane Concentration (mg/l)	Influent TCE Concentration (µg/l)	% Removal Methane	TCE Removal % ± SD*
3.8	470	98.8	48.6 ± 3.8
1.0	460	95.1	40.9 ± 3.0
3.0	1070	96.5	48 to 0
0.70	1070	86.5	41 to 0
2.7	1500	91.2	1.4 ± 2.3
0.7	1500	69.7	0.1 ± 0.8
6.7	1400	98.3	9.5 ± 4.4
1.4	1400	94.1	1.2 ± 1.7

* $n \geq 10$

At TCE concentrations as high as 1000 µg/l or greater, a significant impact was observed for the columns with an inflow methane concentration of 0.7 mg/l, in this case there was a 13 percent reduction. With regard to the removal of TCE, the system was far more sensitive to inhibition as can be seen in Tables 3.7 and 3.8. For an influent TCE of 1070, µg/l the percent removal of TCE gradually decreased to zero over a period ranging from 12 to 24 days, even though a relatively high percent removal of methane was being achieved. An increase of TCE to 1460 µg/l further reduced the percent removal of methane and a total inhibition of the microbiota removing TCE occurred; however, as the influent methane concentration was increased to 6.7 mg/l for one column, the percent TCE removal increased to 9.5 which was still low.

Previous studies have shown that the inhibition of methanotrophs occurred at lower concentrations when pure methane was used as the sole carbon source (Janssen *et al.*, 1987). The authors reported that the addition of another substrate caused a relief from growth inhibition and suggested that the toxicity is a result of the inhibition of some specific steps in the assimilation of methane, rather than a general inhibitory effect. It is anticipated that a combination of substrates will enhance the microbial capacity to resist inhibition at such low concentrations.

Inhibition of methane oxidation by higher concentrations of TCE was observed to be a function of both microbial density and TCE concentration. Figure 3.18 shows the effect of the influent TCE concentration on methane removal for a low microbial density (i.e., influent methane concentration = 0.8 mg/l). As the TCE concentration was increased the percent removal decreased in a parabolic trend ($r^2 = 0.998$). At TCE concentration greater than 1000 µg/l the impact on the microbiota was significant, and TCE removals less than 80 percent of the maximum observed were noted. A similar but less pronounced effect was noted when the influent methane concentration was 3.2 mg/l (Figure 3.19).

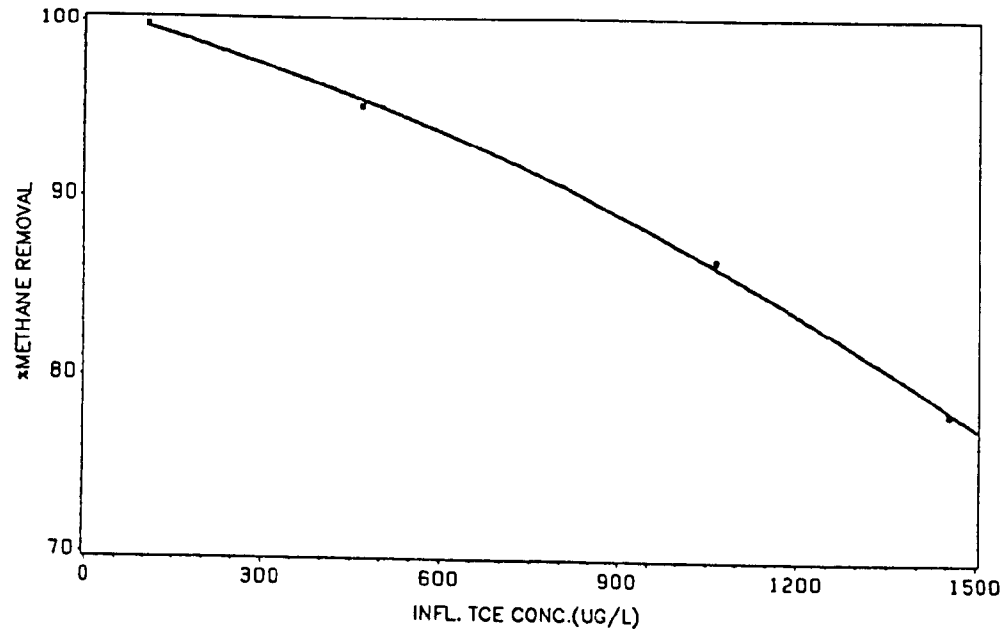


Figure 3.18. Inhibition of Methanotrophs by TCE for an Influent Methane Concentration of 0.8 mg/l.

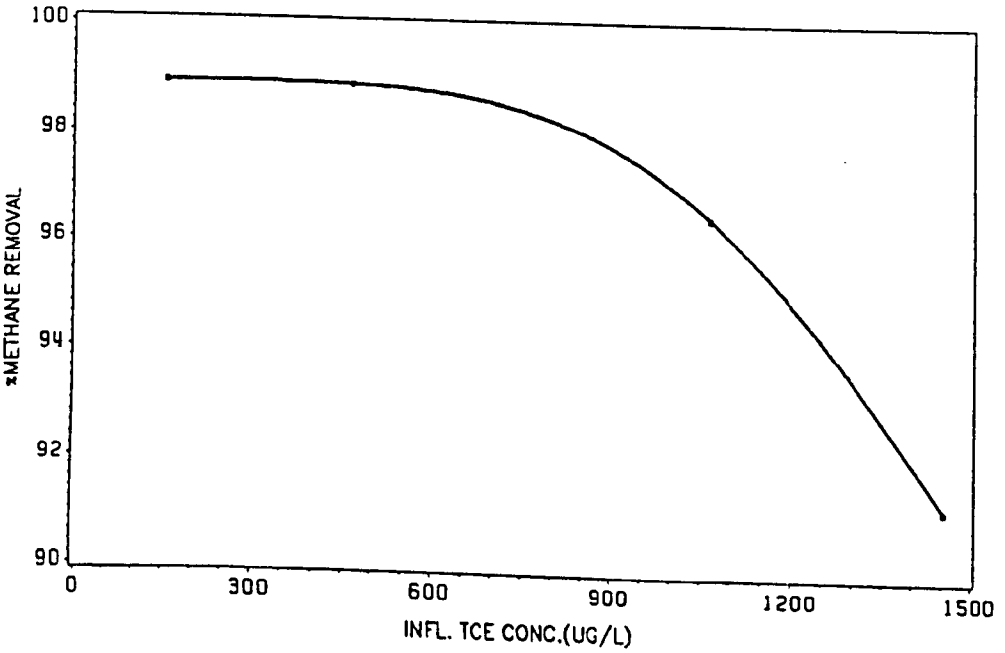


Figure 3.19. Inhibition of Methanotrophs by TCE for an Influent Methane Concentration of 3.2 mg/l.

Table 3.8: Specific TCE Utilization Rate Per Unit Mass of Methane Consumed for Sand 3

Water Flow (ml/min)	Air Flow (ml/min)	Influent Methane Concentration (mg/l)	Influent TCE Concentration (µg/l)	% TCE Removal	µg TCE Consumed per mg Methane Consumed (µg TCE/mg Methane)
4.1	7.6	13	150	38	2.3
4.0	7.7	13	390	44	8.5
5.9	6.8	13	270	27	4.6
6.0	6.6	14	41	33	8.5
6.4	6.4	13	430	34	9.6
4.2	7.3	3	150	34	10
4.1	7.5	3	390	49	30
4.0	7.7	4	460	49	30
6.2	6.0	3	430	20	25
6.1	5.7	4	440	17	24
5.9	6.5	24	270	35	3.5
6.1	6.2	9	420	30	14
4.1	7.6	1	460	41	102

The inhibitory effect was somewhat offset with the higher initial methane concentration, but the inhibition effect was noticeable. When the inhibitory effect of TCE was incorporated into the model (mentioned previously), the following equation resulted:

$$\ln(Se/So) = K_1(1/So - 1/Se) - K_4 H^n / (1 + CTCE/K_I)$$

where

CTCE = Influent concentration of TCE (µg/l)

K_1 = TCE inhibition constant (µg/l)

K_1 K_4 = constants.

Regression analysis was performed following the same process as described above with the following results:

$K_1 = 0.0284$

$K_4 = 2.961$

$K_I = 763.44$

$\eta = 0.31$

$r^2 = 0.9568$

An effort was made to optimize the schedule of the application of methane to enhance TCE removal. Four experimental conditions were considered: (1) Column operated as before; (2) Two columns in series with more methane injected into the second column; (3) Multiport injection of methane into one column; (4) Intermittent injection of methane (endogenous respiration).

Five columns were operated simultaneously, one of them as a single regular column, two of them in series, another one with multiport injection, and the last one as a control with no methane injection. In a latter time period, four of the five columns were subjected to endogenous

respiration by intermittently injecting methane (every other day) and monitoring the removal of TCE and methane in the endogenous period.

For the columns in series, the effluent air and water flows from the first column were injected to the top of the second column; however, methane was injected in both columns since the effluent methane concentration from the first column approached zero. The total mass of methane injected was twice as much as in one single regular column. In the columns with multiport injection, methane was injected at three different ports spaced at the third points in the columns. The total mass of methane was the same as the mass injected in one single column. A constant influent TCE concentration of 420 μg/l was injected until steady state was reached (Table 3.9). Greater than 99 percent removal of methane was achieved for all the columns. The TCE removal in the first column in series (34 percent) was similar to the removal observed in the single regular columns (33 percent); this means that the former behaved as a single column. The first of the columns in series was used for comparison purposes.

The multiport injection column showed a 10.5 percent increase in TCE removal efficiency in comparison with a single column. Although the same mass of methane was introduced to the multiport column as the single column, it was concluded that a better distribution of the microorganisms significantly improved the removal of TCE.

The total TCE removal of the columns in series was 54 percent which is 20 percent greater than the removal observed in the first column. From this result it can be concluded that columns in series produced a beneficial improvement in TCE removal.

Next, the TCE concentration was reduced to 270 μg/l (Table 3.9) until steady state operations were reached. Greater than 99 percent removal of methane was achieved for all the columns. The removal of TCE followed a pattern similar to the one described at 430 μg/l, but the percent removed decreased in agreement with the general findings that as the influent TCE decreases the percent removal decreases.

The multiport injection column removed more TCE than the single column, confirming the beneficial use of an even distribution of methanotrophs for the same mass of methane. A 17 percent increase in TCE removal was observed in the columns in series with regard to the single column. Removal was significantly greater in the columns in series. The total removal was 44 percent.

Finally, the systems were subjected to endogenous respiration by feeding with methane for 24 hours and then shutting off the methane source for 24 hours. Methane samples were taken 1 hour after the methane source was reconnected to the columns. The TCE concentration was kept constant at 270 μg/l. The results, presented in Table 3.9, were taken one hour after the methane source was reconnected to the columns.

The percent methane removal during the feeding phase was greater than 99 percent for all the columns, this shows that the methane removals were relatively unaffected even with alternate periods of methane addition. The TCE removal followed a pattern similar to the case with continuous methane addition, but the percent removals were lower and more variable, indicating that intermittent methane addition did produce a detrimental effect on TCE removal.

Microorganisms growing on natural gas as a primary substrate would probably be more diverse in structure than microorganisms living on pure methane. Three experimental

Table 3.9. Removal of TCE and Methane

	Influent TCE Conc.	Influent Water Flow (ml/m)	Air Flow (ml/m)	Methane Conc. (mg/l)	Methane Removal %	TCE Removal % ± SD*
Single column	410	6.0	6.6	14	99.8	33 ± 1.9
First column in Series	430	6.1	6.4	13	99.8	34 ± 2.9
Second column in series (total removal)	--	6.1	6.5	13	99.8	54 ± 4.3
Multiport Injection	420	6.0	6.6	14	99.6	44 ± 5.3
Control Column	410	6.0	6.1	0.0	--	1.6 ± 2.4
First Column in Series	270	6.0	6.8	13	99.8	27 ± 4.3
Second Column in Series (total removal)	--	6.0	7.0	14	99.8	44 ± 4.6
Multiport Injection	270	5.9	6.7	14	99.7	34 ± 4.1
Intermittent Methane Addition						
First Column in Series	270	5.8	6.7	13	99.8	16 ± 9.1
Second Column in Series (total removal)	270	5.8	7.0	14	99.8	31 ± 5.0
Multiport Injection	270	5.7	6.6	14	99.6	22 ± 3.8

* $n \geq 10$

Table 3.10: Summary of Results of Bioreactors Fed with Natural Gas

Media	Water Flow (ml/min)	Air Flow (ml/min)	Gas Flow (ml/min)	Influent TCE Concentration (µg/l)	TCE Removal (%)
Sand 3	3.8	6.7	0.3	77	38
Sand 3	3.6	6.7	0.2	107	51
Sand 3	3.7	6.4	0.1	190	22
Sand 3	3.9	7.5	0.1	910	55
Berl Saddle	5.4	6.9	0.2	150	57
Berl Saddle	5.4	6.6	0.1	210	54
Berl Saddle	5.4	7.0	0.2	910	38

reactors were designed to assess the response of the methanotrophs exposed to natural gas to degrade TCE. A summary of the results is shown in Table 3.10.

Removal of TCE with sand 3 ranged from 22 to 55 percent, with the highest efficiency obtained for a maximum TCE concentration of 910 µg/l. No inhibitory effects were noticed at this high concentration. The percent removal of TCE in the column packed with berl saddles ranged from 38 to 57 percent. It is assumed that some inhibition occurred at an influent TCE concentration of 910 µg/l as the percent removal decreased in contrast with the general observed pattern that the percent TCE removal increased as a function of the influent TCE concentrations. The high percent removal achieved in the berl saddle reactor might have occurred because the methanotrophs were more evenly distributed than in the coarse sand reactor, allowing a longer contact between the biofilm and the TCE-laden water. Flow in the berl saddle reactor was quiescent compared with that of the coarse sand packed reactor, resulting in less TCE stripped.

It appears that similar percent TCE removals were attained by the bioreactor packed with sand 3 and fed with natural gas than the bioreactor fed with pure methane. The relatively high percent removal obtained for the low influent methane and TCE concentrations of the former was never achieved for the bioreactors fed with pure methane. The inhibitory effect of high concentrations of TCE was lower in the natural gas-bioreactors; concentration in the order of 910 µg/l did not appear to repress the microbial activity as high removals of both methane and TCE were attained. Greater diversity of microorganisms would probably be promoted using natural gas as a primary substrate compared to pure methane. (Wilkinson *et al.*, 1974).

SECTION 4

VAPOR PHASE TREATMENT OF TRICHLOROETHYLENE AND 1,1,1-TRICHLOROETHANE

METHODS AND MATERIALS

The treatment of air streams contaminated with TCE and/or TCA is needed because of soil venting for remediation of unsaturated subsurface materials and air stripping of ground water pumped to the surface. Diverse geochemistries of ground waters may present problems to biological-based systems for treating water; treating air would circumvent this complication. Another potential advantage would be the dilution of high concentrations of TCE in water by the 10-to-1 or 20-to-1 air-to-water ratios of stripping towers.

Two bioreactors were constructed using borosilicate glass columns 60 cm long by 5 cm in diameter. A schematic of the bioreactors is shown in Figure 4.1. Celite biocatalyst carrier R-635 by Mannville was used as the solid support for microbial growth in bioreactor A; bioreactor B was filled with Celite biocatalyst 630 by Mannville. These diatomaceous-silica supports vary in diameter, distributions of porosity, and mechanical integrity. The porous nature of the supports allows for great surface areas per unit of volume and increases the chances of biomass survival after periods of shock loadings. The R630 is a sphere with a mesh size of (3/5) and a surface area of 1.3 m^2/g. No information on the R635 was provided by Mannville, however, the R630 was a larger sphere.

The headspaces of the columns were plumbed to receive vapors of TCE and TCA, a mixture of butane (technical grade, 95.0 percent minimum purity in the liquid phase; Linde Specialty Gases) and air, and a nutrient media. The nutrient media solution contained 100 mg/l NH_4Cl, 100 mg/l K_2PO_4, 20 mg/l $MgSo_4$, 1 mg/l $FeSO_4$, and 1 mg/l $CaCl_2$.

A slurry of Rollin muck soil previously adapted to n-butane was the inoculum for the biofilm (Kampbell *et al*, 1987). Ten grams of the soil was added to 250 ml of the nutrient media and circulated throughout the columns until substantial removals of butane were observed in the effluent gas port.

Four pairs of butane sampling ports and air sampling ports are located along each column (Figure 4.1). The butane-enriched air was pulled throughout the column by a vacuum line placed in the last air sampling port. An overflow line returns the effluent media to the nutrient solution reservoir to provide recirculation of that solution to the columns.

Influent and effluent butane samples were collected by gas-tight syringe at the appropriate ports (H,J,L,N). Air was pumped into a neat solution of TCE and TCA (D) by a peristaltic pump (C) to saturate the air going to the column with TCE and TCA vapors. The peristaltic pump also pulled a slight vacuum which mixed the air and butane as well as metering the flow of butane from a cylinder (A). Influent concentrations of TCE and TCA were determined by inserting a Tenax TA trap in the first air port (I) located in the headspace of the column and placing the vacuum line (O) onto the end of the trap with an air flow of 13 ml/min.

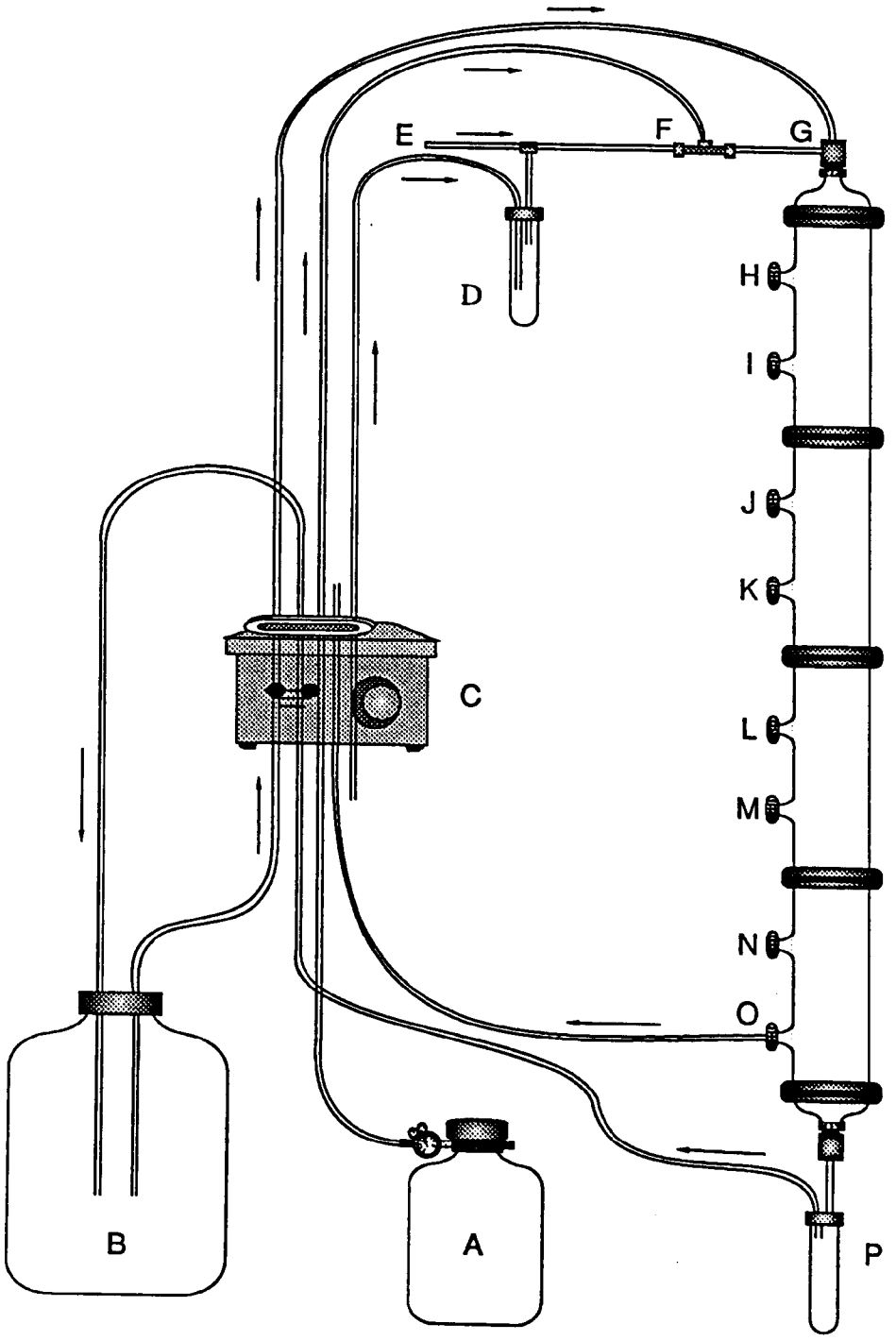


Figure 4.1. Schematic of Bioreactors Treating Air Streams. A. Butane (primary substrate); B. Nutrient media reservoir; C. Peristaltic pump; D. Chemical reservoir; E. Influent air; F. Influent butane; G. Influent nutrient media; H.J.L.N. Sample port by syringe; I.K.M.O. Sample port by trap; P. Effluent water sampling port.

The air retention time of the column containing the R630 carrier was 18 minutes while that of the column containing the R635 carrier was 20 minutes. Additional air samples could be taken at the remaining ports located along the column (K,M,O). The initial concentration were approximately 90 µg TCE/l air, 200 µg TCA/l air, and 8 mg butane/l air. The chemicals used in the bioreactor study were high purity TCE and TCA obtained from Aldrich Chemical Co., Milwaukee, WI. All purities were at least 97 percent.

The effluent nutrient solution was transferred to a serum bottle then purged onto a Tenax TA trap. The traps from both air and solution samples were analyzed by EPA Method 624 (1982), modified for GC/FID. Each trap was desorbed at 225°C for 5 minutes onto a Megabore DB 624 column. The column was held at 30°C for 5 minutes then programmed at 8°C/minute to 175°C and held for 15 minutes; detection was by flame ionization. Influent and effluent butane samples were analyzed by the method of Mindrup (1978).

Biomass estimates were determined by acridine-orange direct counts. Approximately 300 milliliters of the effluent waters were examined for protozoa, with both ciliates and amoeba observed . The amoeba were identified as *Arcella vulgaris*; the ciliates were identified as the genus *Tetrahymena*.

RESULTS AND DISCUSSION

Reactors A and B were exposed to butane approximately 2.5 weeks before the addition of TCE (90 µg/l air) and TCA (200 µg/l air) on day 18. Removals of TCE, TCA, and butane were 82 percent, 93 percent, and 65 percent, respectively, on day 19 in bioreactor A (Tables 4.1 and 4.2). TCE removals did not remain constant over the next seven days, with a maximum removal of 94 percent observed on day 25. Removals of TCA gradually decreased to 73 percent during this same time interval.

In bioreactor B, respective removals of TCE, TCA, and butane were 74 percent, 76 percent, and 59 percent on day 19 (Tables 4.3 and 4.4). Decreases in removal efficiencies of TCE were seen after day 20, with no removal of TCE observed on day 28; the removals of TCA gradually decreased to 28 percent on day 28.

To test for the ability of the organisms to metabolize greater quantities of chemical, the concentrations were increased to approximately 770 µg TCE/l air and 990 µg TCA/l air on day 28. The ability of bioreactor A to remove TCE and TCA was dramatically reduced with maximum removals on day 32 of 24 percent for TCE and on day 31 of 16 percent for TCA. Interestingly, the removals of TCE and TCA in bioreactor B remained similar to those at the lower concentrations, with maximum removals of TCE and TCA of 25 percent and 21 percent, respectively, on day 30.

Removals of butane were seen throughout the length of the columns (Tables 4.5 and 4.6), indicating that the Celite biocatalyst carriers allowed microbial growth throughout the entire length of the bioreactor. The larger biomass should result in increased removals of TCE and TCA.

Table 4.1. Removal of TCE from Bioreactor Packed with R635

Time (Days)	Butane	TCE			
	Quantity Applied (mg/L air)	Fraction Remaining			
		Air	Air	Water	Total
		% Influent			
1	7.6	98.4			
9	8.3	61.9			
15	7.6	34.2			
19	8.5	35.1	10.9	7.5	18.4
21	6.7	60.2	9.2	11.7	20.9
22	7.2	55.9	36.2	15.3	51.5
25	6.2	67.9	4.8	1.3	6.1
29	10.5	59.7	56.7	32.6	89.3
31	7.6	84.7	68.4	14.3	82.7
32	7.5	75.8	60.5	15.8	76.3

Table 4.2. Removal of TCA from Bioreactor Packed with R635

Time (Days)	Butane	TCA			
	Quantity Applied (mg/L air)	Fraction Remaining			
		Air	Air	Water	Total
		% Influent			
1	7.6	98.4			
9	8.3	61.9			
15	7.6	34.2			
19	8.5	35.1	1.0	5.8	6.8
21	6.7	60.2	10.6	7.6	18.3
22	7.2	55.9	23.7	9.4	33.1
25	6.2	67.9	23.9	2.9	26.8
29	10.5	59.7	87.3	11.2	98.4
31	7.6	84.7	79.4	6.3	85.7
32	7.5	75.8	87.5	6.2	93.7

Table 4.3. Removal of TCE from Bioreactor Packed with R630

Time (Days)	Butane: Quantity Applied (mg/L air)	TCE: Fraction Remaining: Air	TCE: Fraction Remaining: Air	TCE: Fraction Remaining: Water	TCE: Fraction Remaining: Total
		% Influent			
1	9.3	98.9			
3	9.7	77.9			
5	8.9	62.2			
15	8.7	49.3			
19	10.7	41.2	14.4	11.5	25.9
22	8.8	60.5	40.2	8.1	48.3
25	7.0	73.3	66.3	11.8	78.1
26	7.6	66.0	40.1	11.9	52.0
28	8.6	87.5	90.3	21.9	112.2
30	7.7	83.4	59.1	16.2	75.3

Table 4.4. Removal of TCA from Bioreactor Packed with R630

Time (Days)	Butane: Quantity Applied (mg/L air)	TCA: Fraction Remaining: Air	TCA: Fraction Remaining: Air	TCA: Fraction Remaining: Water	TCA: Fraction Remaining: Total
		% Influent			
1	9.3	98.9			
3	9.7	77.9			
5	8.9	62.2			
15	8.7	49.3			
19	10.7	41.2	18.8	5.4	24.2
22	8.8	60.5	44.4	7.2	51.6
25	7.0	73.3	70.0	5.8	75.8
26	7.6	66.0	61.4	6.3	67.7
28	8.6	87.5	65.3	6.4	71.7
30	7.7	83.4	73.1	6.2	79.3

Table 4.5. Gas Removals In Bioreactor A With R635

Day	Port 1 (H)* mg/l	Port 2 (J)* mg/l	Port 3 (L)* mg/l	Port 4 (N)* mg/l
1	7.6	nd**	nd	7.4
3	8.8	nd	nd	7.9
4	7.9	nd	nd	7.2
8	8.3	6.6	6.0	5.2
12	8.2	5.1	2.0	1.0
14	7.6	5.2	3.6	3.7
16	9.2	4.1	3.9	2.9
18	8.5	6.5	4.5	3.0
19	7.1	6.0	2.8	1.6
25	7.3	6.4	5.6	4.6
27	10.5	9.3	8.7	6.4
29	7.6	7.3	6.8	6.4
30	7.0	6.2	5.9	5.4

*see Figure 4.1
**not determined

Table 4.6. Gas Removals In Bioreactor B With R630.

Day	Port 1 (H)* mg/l	Port 2 (J)* mg/l	Port 3 (L)* mg/l	Port 4 (N)* mg/l
1	9.3	nd**	nd	9.2
3	9.7	nd	nd	7.5
4	8.9	nd	nd	5.6
8	9.7	8.3	7.6	6.3
12	9.1	6.9	4.5	2.2
14	8.7	7.4	5.9	4.3
16	6.2	5.6	4.8	3.8
18	10.7	8.4	6.3	4.4
19	8.6	7.4	6.1	4.1
25	7.6	6.9	5.9	5.0
27	8.6	7.7	7.6	7.3
29	7.8	7.3	7.0	6.5
30	12.0	12.0	10.4	9.2

*see Figure 4.1
**not determined

No products of biotransformation were determined in this study. However, CO_2 and cellular constituents have been observed as the final products of biotransformation in studies using ^{14}C-labeled TCE (Wilson and Wilson, 1985; Fogel *et al.*, 1986) or CCL_3 (Strand and Shippert, 1986) with methane as the primary substrate. The probable intermediate products of the oxidation of low molecular weight alkanes such as methane, propane, or butane are alcohols and ketones, which are easily metabolized in oxygenated systems (Haber *et al.*, 1983; Atlas, 1981). Epoxides are probably formed during the oxidation of chlorinated ethylenes with rapid conversion to biodegradable hydrolysis products. Recent work has shown that oxidation of TCA yields 2,2,2-trichloroethanol as a chlorinated intermediate (Oldenhuis *et al.*, 1989).

In this study, the pH of the effluent media solution changed from 6.5 to 5.0 during the study interval. It is possible that sufficient quantities of butyric acid might have been formed from the oxidation of butane (van Ginkel *et al.*, 1987) with the resultant change of pH. This change in pH would probably decrease the efficiency of the removal process. The reactive nature of the epoxide produced by the oxidation of TCE would also decrease the efficiency of removal by potentially destroying biomass. The effect of these intermediate compounds on biofilms in engineered systems and the effluent waters to be disposed should not be ignored.

The biotransformation of TCA seen in this study has also been seen in saturated soil columns containing poorly sorted silty sand and gravel from a semi-confined alluvial aquifer with propane as the primary substrate (Wilson *et al.*, 1987). However, other studies have not observed the biological removal of TCA when using an unsaturated soil column containing Lincoln fine sand with natural gas as the primary substrate (Henson *et al.*, 1988) or in fixed-film bioreactors using propane as the primary substrate (Wilson and White, 1986). The three soils differ greatly in texture, organic content, and clay content, but the effect of these differences on the distribution of organisms capable of cometabolically degrading TCA is not known.

A bacterial count of 1.8×10^{10} showed approximately 6400 protozoans in an acridine-orange direct count smear on a microscopic slide. Since ciliates and amoebas are free-living protozoans, they can be found in every type of fresh and salt water, soils, or other moist environment, including fixed-film bioreactors. Bioreactors can hold many factors influencing the proliferation and distribution of ciliates and amoebas. These factors are the temperature, light, chemical composition, acidity, kinds of food and the amount, and the adaptability of the individual protozoan to various changes of the habitats and environment. The protozoa were probably enriched along with bacteria from the Rollin muck soil used as an inoculum for the bioreactor. The proliferation of a butane-utilizing bacterial consortia would provide an ample food supply for the protozoa to eat. It was not determined if the presence of protozoa was detrimental to the performance of the bioreactor. The reduction of biomass associated with protozoan predation would provide no benefit if TCE oxidation were associated with the resting cell phase of growth. Only if an actively growing biomass were required for cometabolism of TCE would protozoan predation seem beneficial.

SECTION 5

VAPOR PHASE TREATMENT OF ALKYLBENZENES

METHODS AND PROCEDURES

The laboratory methods and procedures developed and used for the soil bioreactor and microcosm studies are presented. A description of the experimental apparatus and experimental set-up are explained along with a review of procedures for sample gathering and analysis.

The chemicals used in the vapor phase treatment project were Baker spectraphotometric grade benzene, Baker reagent grade toluene, Eastman industrial grade ethylbenzene, and Aldrich 97 percent purity *o*-xylene. Chemical purity was confirmed with analysis by gas chromatography.

Soil columns were constructed of 7.6 cm internal diameter beaded process pipe. The column length varied from 86.4 cm to 96.5 cm. The columns were filled with soil by lightly tamping the soil into the column as well water was pumped up through the bottom of the column. The well water was obtained from a local source near Ada, Oklahoma. This technique allowed settling of the soil without physically packing and destroying the natural soil aggregates. Once filled, the water was pumped out of the column; and an air stream was introduced into the bottom of the column to establish a flow up through the column.

A hydrocarbon vapor stream was generated by purging air through vials containing the desired hydrocarbon and then gathering the individual vapors into one stream to be directed into the bottom of the soil column. Figure 5.1 shows a schematic of the soil column apparatus. Air was pumped through the vials with a Technicon Autoanalyzer pump and the rate was controlled by using different sized pump tubing. Tubing was replaced once a week. All lines were made of Technicon pump tubing or teflon tubing.

A solvent vapor generator was constructed to produce a vapor stream which could be injected into crimp-top serum bottles. The solvent vapor generator consisted of a glass manifold which distributed an air supply through several flowmeters. The flowmeters then adjusted the individual air streams which were bubbled through vials containing the individual aromatic hydrocarbons. The streams were gathered into a single stream which could be injected into the soil microcosm. Figure 5.2 illustrates the solvent vapor generator which was located in an exhaust hood.

Sample analysis was performed using a Varian 3700 gas chromatograph. The Varian 3700 was equipped with a flame ionization detector and a 0.32 cm by 182.9 cm stainless steel packed column containing 5 percent SP - 1200 / 1.75 percent Bentone 34 on 100/120 Supelcoport. Nitrogen (N_2) was used as the carrier gas at 40 ml/min and the operating temperatures for the injector and detector were 170°C and 180°C, respectively. The column temperature was programmed for an initial temperature of 50°C for one minute, ramp up 8°C/min to 130°C, and hold for one minute. A Tekmar LSC-2 was used to add an internal standard to each sample prior to desorbing onto the Varian 3700 for analysis.

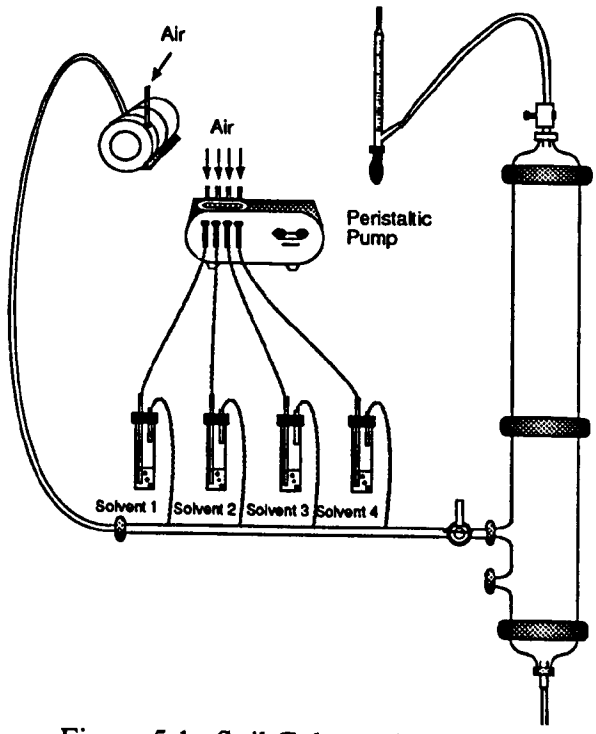


Figure 5.1. Soil Column Apparatus.

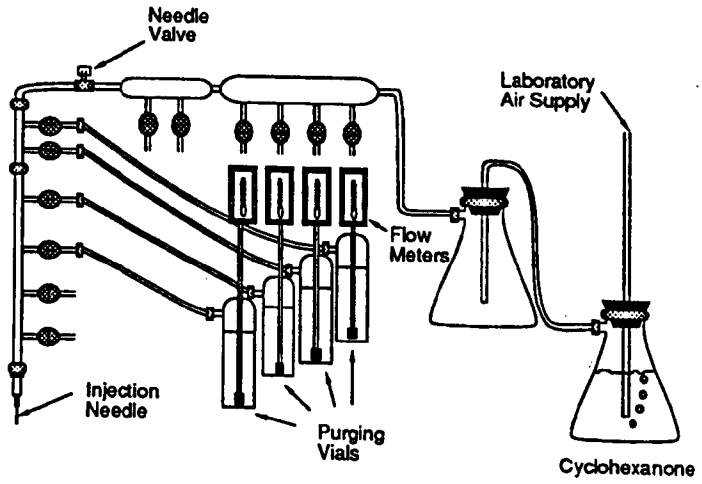


Figure 5.2. Solvent Vapor Generator Apparatus.

The hydrocarbon vapor stream was sampled at the inlet and the outlet of the soil column. Samples were collected onto Tenax traps. Each trap consisted of a 0.64 cm by 10 cm stainless steel tube which contained five cm of 60/80 mesh Tenax held in place with glass wool plugs. Hydrocarbon removal was determined by comparison of the inlet and outlet concentrations. Flow rates were measured prior to each sampling period. Upon completion of the soil column study, the columns were taken apart and sections of soil were stored in separate sealed containers.

Moisture determinations were conducted on the acclimated soil from the soil columns. Soil microcosms were then prepared to determine the hydrocarbon removal rate. A predetermined amount of acclimated soil was added to crimp top serum bottles; 50 and 160 ml serum bottles were used for the microcosm studies. 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 using the solvent vapor generator. The injection was accomplished by holding a silicon-Teflon septa over the mouth of the serum bottle and injecting vapors through the septa. An outlet line connected to a soap film bubble meter allowed monitoring of the injection volume. After injection of a predetermined volume of vapors, an aluminum faced Teflon disk was inserted between the bottle top and the punctured septa. Both septa and disk were then crimped onto the serum bottle. This sealed the serum bottle with an unbroken aluminum seal. Neglecting to insert the additional disk between the bottle top and the punctured septa would result in loss of the hydrocarbons within 24 hours.

Sampling of a microcosm was accomplished by connecting a Tenax trap to the microcosm with a short section of teflon tubing. Ten ml of tap water spiked with an internal standard were then injected into the bottle. The slurry was purged for 9 minutes (for 50 ml bottles) or 15 minutes (for 160 ml bottles) with nitrogen at a flow rate of 40 ml/min. The bottle was shaken on a bottle shaker during the purging period. The sample was desorbed onto a Varian 3700 gas chromatograph with a Tekmar LSC-2 for analysis. The microcosm studies were conducted in triplicate with samples of acclimated soil and controls of unacclimated soil. Soil column and microcosms were conducted in a constant temperature box maintained at 12°C.

RESULTS AND DISCUSSION

The experimental data and results from this research is presented in two sections: soil column data and microcosm data. The soil column experiments examined the variables of column preparation, inlet concentration, inlet flow rate, and soil type. Microcosm experiments examined the effect of different soil moistures on hydrocarbon removal rates.

The rate of biodegradation should be dependent on one of two factors: (1) transport of the carbon source to the soil microbe; or (2) utilization of the carbon source by the microbe. Soil microbes exist within water films and pore space waters of the soil matrix. The carbon source must move from the vapor (gas) phase across the gas/liquid interface and through the liquid to the microbe. Diffusion is the primary driving force and diffusion is dependent upon the concentration gradient. Transport, or availability, will probably be the limiting step under conditions of lower concentrations because the concentration gradient (driving force) will be low.

Moving the carbon source to the microbe would take longer than its utilization by the microbe. High vapor phase concentrations will produce a large driving force for transport, thus utilization by soil microbes will probably be the limiting step.

Soil columns need to be consistently packed to minimize variations in loading which may influence interpretation of the removal efficiency of the soil column. Duplicate columns of Rubicon sand were used to examine packing variability. Table 5.1 presents a summary of the removals obtained for the individual compounds in each column. Comparison of the two columns reveals a good correlation at both hydrocarbon loading rates. Duplication between the columns was adequate for experimentation purposes for each of the compounds. Removal differences were due to the slight difference in the initial inlet concentrations. It can be concluded that the method of column packing is reproducible and minimizes packing variation.

Table 5.2 contains a summary of the removals of each compound for the three soils. In general, when the inlet concentrations were increased, the relative percent of removal decreased only very slightly or remained the same which indicates the overall removal process follows first-order kinetics. When the inlet concentrations were decreased from a higher level, the relative percent removal increased due to the initially inflated biomass to hydrocarbon ratio. The greatest change was for benzene. The high level of removal was only temporary, however. As the biomass to hydrocarbon ratio adjusted back to the initial value, the percent removals were approaching their initial values. Percent removals which are independent of loading rate are characteristic of first-order removal kinetics.

Residence time within a soil column is determined by the flow rate of the inlet stream. Reducing the flow rate in half will theoretically double the residence time but, at the same time, will cut the loading rate in half. The biomass within the soil column will in turn adjust to the new loading rate and, as shown in the previous section, would produce the same percent removal if the residence time were equal and first-order removal kinetics were controlling the removal process. Since the residence time increases with reductions in the flow rate, increases in percent removal may be expected from the soil columns. The increases in removal will follow first-order removal kinetics until removal becomes transport limited. Removals then become a combination of the rate of the removal reaction and the rate of transport. During periods of low loading rates the concentration gradient between the bulk fluid and the reaction site (which drives the transport process) will be small and transport would be reduced. As the retention times are increased within a given column, the outlet concentration would asymptotically approach a minimum concentration which is limited by the transport of the reactant to the reaction site. As the outlet concentration approaches the minimum concentration allowed by the transport limitations, increases in retention time would produce diminishing increases in percent removals.

Table 5.3 provides a summary of the compound removals for the varying flow rate experiment. In general, decreased flow rates resulted in increased percent removals. In the Durant loam soil column, decreasing the flow rate from 40 ml/min to 20 ml/min increased removals which indicated the removal process was controlled by first-order removal kinetics. Further reduction in the flow rate in the Durant loam soil column resulted in diminished increases in the removals which indicated that transport was becoming limiting in the removal process.

Table 5.1. Hydrocarbon Removal In Duplicate Soil Columns
Rubicon Sand Soil Columns

Soil Type	Compound	Avg Inlet ug/ml	Percent Remaining*
Column F	Benzene	3.1	66.4 ± 4.9
	Toluene	1.1	64.8 ± 3.0
	Ethylbenzene	0.9	53.2 ± 4.4
	O-Xylene	1.0	64.8 ± 5.6
Column G	Benzene	3.1	67.5 ± 5.1
	Toluene	1.5	57.6 ± 4.8
	Ethylbenzene	1.1	58.1 ± 2.4
	O-Xylene	1.1	62.4 ± 3.4
Column F	Benzene	20.3	70.2 ± 4.2
	Toluene	4.5	68.7 ± 3.3
	Ethylbenzene	1.3	61.2 ± 5.0
	O-Xylene	0.9	68.2 ± 4.0
Column G	Benzene	20.5	68.8 ± 3.9
	Toluene	4.7	66.9 ± 4.3
	Ethylbenzene	1.2	62.2 ± 5.1
	O-Xylene	0.9	66.2 ± 6.0

* mean ± sample SD, n = 19
40 ml/min average inlet flow rate

Flow rate reductions in the Dougherty sand soil columns also produced increases in the percent removals. Observed percent removal was possibly limited by transport effects. A reduction in the inlet flow rate may not produce an increase in the percent removal which follows purely first-order removal kinetics with no concentration minimum. An alternative to decreasing the inlet flow rate (and reducing the biomass) would be to keep the flow rate constant and simply double the length of the column. This would allow greater percent removals up to the point that transport becomes the limiting step in the removal process. Further experiments need to be conducted to determine the minimum concentrations caused by transport limitations. Further work is needed to examine grain size distribution, organic carbon content, and other factors which may reduce the minimum concentration.

Table 5.4 presents a summary of the removals of each compound obtained by the three soils examined under similar operating conditions. Table 5.5 lists the final moisture contents of the soil columns. The columns were constructed by settling the soil with water to minimize destruction of the natural soil aggregates. Initial moisture contents were probably higher than the moisture contents determined at the end of the column experiments. The amount of drying which took place in the columns is unknown but each column still had adequate soil

Table 5.2. Hydrocarbon Removal At Varying Inlet Concentrations
Average flow rates and concentrations

Soil Type	Flow Rate ml/min	Compound	Inlet Conc ug/ml	Percent Remaining*
Rubicon Sand Column F	40	Benzene	3.1	66.4 ± 4.9
			20.3	70.2 ± 4.2
		Toluene	1.1	64.8 ± 3.0
			4.5	68.7 ± 3.3
		Ethylbenzene	0.9	53.2 ± 4.4
			1.3	61.2 ± 5.0
		O-Xylene	1.0	64.8 ± 5.6
			0.9	68.2 ± 4.0
Rubicon Sand Column G	40	Benzene	3.1	67.5 ± 5.1
			20.5	68.8 ± 3.9
		Toluene	1.5	57.6 ± 4.8
			4.7	66.9 ± 4.3
		Ethylbenzene	1.1	58.1 ± 2.4
			1.2	62.2 ± 5.1
		O-Xylene	1.1	62.4 ± 3.4
			0.9	66.2 ± 6.0
Dougherty Sand	10	Benzene	35.2	69.1 ± 2.4
			4.1	4.2 ± 1.0
			3.4	16.0 ± 8.5
		Toluene	9.5	76.7 ± 2.2
			2.9	73.3 ± 4.7
			0.9	69.1 ± 2.9
		Ethylbenzene	3.9	72.2 ± 3.1
			4.0	72.8 ± 10.2
			1.8	61.7 ± 8.3
		O-Xylene	2.6	82.2 ± 3.4
			4.9	64.5 ± 23.2
			2.4	desorption
Durant Loam	10	Benzene	35.6	69.9 ± 1.8
			3.9	8.5 ± 5.9
			3.9	26.0 ± 5.8
		Toluene	10.3	76.4 ± 1.9
			3.8	65.0 ± 9.4
			1.6	69.5 ± 3.3
		Ethylbenzene	3.6	73.1 ± 3.2
			3.7	73.5 ± 8.0
			1.6	74.8 ± 4.9
		O-Xylene	2.6	77.2 ± 3.6
			4.5	74.8 ± 11.4
			2.2	84.6 ± 4.1

* mean ± SD, n ≥ 6

Table 5.3. Hydrocarbon Removal At Varying Flow Rates
Average flow rates and concentrations

Soil Type	Flow Rate ml/min	Compound	Inlet Conc ug/ml	Percent Remaining
Durant Loam	40	Benzene	31.2	85.4 ± 3.2
		Toluene	8.7	86.2 ± 1.9
		Ethylbenzene	3.4	85.3 ± 1.2
		O-Xylene	2.7	86.4 ± 1.8
	20	Benzene	33.0	71.3 ± 2.4
		Toluene	8.9	79.4 ± 2.1
		Ethylbenzene	3.3	74.3 ± 2.7
		O-Xylene	2.5	72.7 ± 2.6
	10	Benzene	35.6	69.9 ± 1.8
		Toluene	10.3	76.4 ± 1.9
		Ethylbenzene	3.6	73.1 ± 3.2
		O-Xylene	2.6	77.2 ± 3.6
Dougherty Sand	40	Benzene	33.1	76.2 ± 3.8
		Toluene	9.6	90.8 ± 0.9
		Ethylbenzene	3.5	82.0 ± 5.1
		O-Xylene	2.4	92.0 ± 3.0
	20	Benzene	31.9	75.4 ± 2.7
		Toluene	8.7	79.4 ± 2.5
		Ethylbenzene	3.6	72.0 ± 2.6
		O-Xylene	2.5	79.7 ± 2.2
	10	Benzene	35.2	69.1 ± 2.4
		Toluene	9.5	76.7 ± 2.2
		Ethylbenzene	3.9	72.2 ± 3.1
		O-Xylene	2.6	82.2 ± 3.4

* mean ± SD, n ≥ 6

moisture after three to four months of operation. Inhibition due to excessive moisture contents was not directly addressed and was not observed in the experiments performed in this study. Table 5.6 lists the individual characteristics of each soil examined in this study. The main physical differences between the soils are the grain size distributions and the permeabilities.

Additional differences between the soil columns were the length of the columns (50 to 75 cm) and the types of microbial populations present in each soil. The Rubicon sand provided the greatest removal for each component tested of the three soils examined. The Rubicon sand had the greatest moisture content throughout the soil column. The columns of Durant loam and Dougherty sand each had upper sections of lower moisture contents. Later microcosm experiments showed the upper sections had reduced microbial activities. The residence times of the components within the active microbial zones in the Durant loam and Dougherty sand soil columns were less than the residence time within the Rubicon sand column. Direct comparison of the removals between the columns is not possible due to the differences but relative removals of the hydrocarbons can be addressed.

Table 5.4. Hydrocarbon Removal By Different Soils
Averaged inlet concentrations

Soil Type		Compound	Inlet Concentration µg	Percent Remaining*
Rubicon Sand	(F)	Benzene	20.3	70.2 ± 4.2
	(G)		20.5	68.8 ± 3.9
Dougherty Sand			33.1	76.2 ± 3.8
Durant Loam			31.2	85.4 ± 3.2
Rubicon Sand	(F)	Toluene	4.5	68.7 ± 3.3
	(G)		4.7	66.9 ± 4.3
Dougherty Sand			9.6	90.8 ± 0.9
Durant Loam			8.7	86.2 ± 1.9
Rubicon Sand	(F)	Ethylbenzene	1.8	61.2 ± 5.0
	(G)		1.2	62.2 ± 5.1
Dougherty Sand			3.5	82.0 ± 5.1
Durant Loam			3.4	85.3 ± 1.2
Rubicon Sand	(F)	*O*-Xylene	0.9	68.2 ± 4.0
	(G)		0.9	66.2 ± 6.0
Dougherty Sand			2.4	92.0 ± 3.0
Durant Loam			2.7	86.4 ± 1.8

* mean ± SD, n ≥ 6
40 ml/min flow rate

Rubicon sand soil columns removed nearly equal percentages of each hydrocarbon. Equal utilization of the hydrocarbons possibly reveal a diverse microbial community within the Rubicon sand. The columns removed 25.6-35.1 percent of the input benzene, 28.0-37.4 percent of the input toluene, 32.9-43.8 percent of the input ethylbenzene, and 27.8-39.8 percent of the input *o*-xylene. Evidence of transport limitations due to different water solubilities was not present in this portion of the soil column experiment.

Table 5.5. Soil Column Moisture Contents

Soil Type		Percent Moisture Content (Wet Basis)	Percent Of Saturation
Rubicon Sand			
Column F	lower 15 cm	17.7	saturated
	middle 25 cm	11.2	63
	upper 25 cm	9.4	53
Column G	lower 15 cm	13.4	76
	middle 25 cm	9.5	54
	upper 25 cm	10.0	56
Durant Loam	lower 28 cm	19.6	88
	upper 25 cm	11.4	51
Dougherty Sand	lower 48 cm	10.4	46
	upper 25 cm	6.8	30

Dougherty sand showed a distinct preference for benzene (20-27 percent removal) and virtually ignored *o*-xylene (5-11 percent removal). The Dougherty sand soil column had the lowest percent water saturation values of the soil columns studied. In this case the low water content would eliminate the possibility of transport limitations causing the low *o*-xylene utilization. Possibly the types of microorganisms present in the Dougherty sand soil simply could not utilize the doubly methylated *o*-xylene.

Table 5.6. Soil Characteristics

Soil Type	Moist Bulk Density (gm/cm^3)	Permeability (in/hr)	Soil Reaction pH	% Organic Matter	%Passing Sieve no.4 4.7 mm	no.10 2.0 mm	no.40 0.42 mm	no.200 0.074 mm
Dougherty Sand*		0.63 - 2	5.6 - 6.5	0.79	100	100	68 - 80	13 - 30
Durant Loam*		< 0.6	5.6 - 6.5	0.75	100	100	95 - 100	55 - 85
Rubicon Sand**	1.35 - 1.55	6 - 20	4.5 - 6.0	.5-1.0	95-100	75-100	35-70	0 - 15

* (USDA/SCS, 1973)
** (USDA/SCS, 1986)

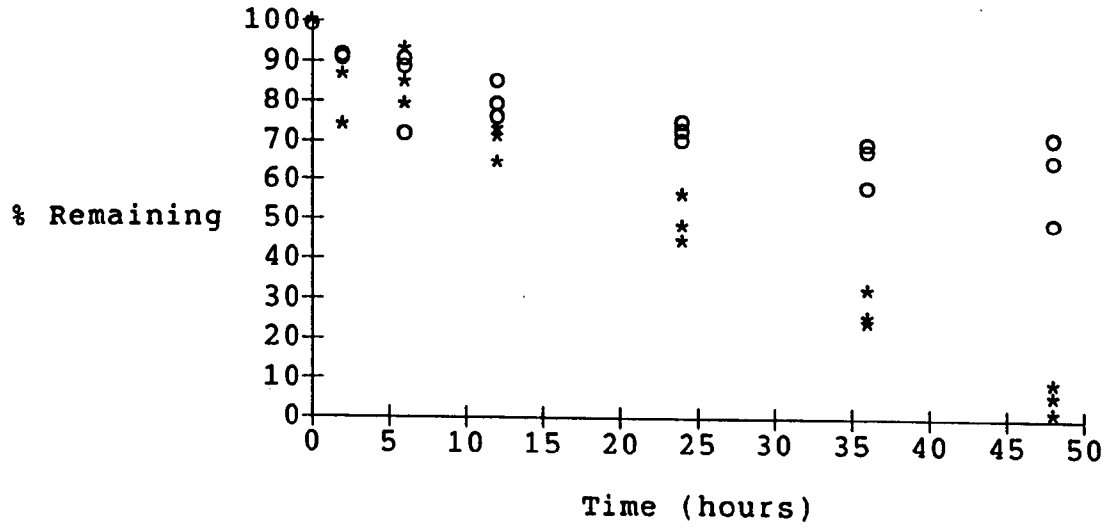
Similarly, the Dougherty sand soil column does not effectively remove toluene (8.3-10.1 percent removal). Again, possibly the presence of the methyl group inhibits the microorganisms from utilizing the hydrocarbon. Alternatively, the Dougherty sand soil column did show a potential for ethylbenzene utilization (12.9-23.1 percent removal). The types of microbes present in the Dougherty sand seem to make a distinction between the ethyl substituted ethylbenzene and the methyl substituted toluene and *o*-xylene. Alternatively, the low relative moisture content of the Dougherty sand soil column may cause the inhibition of the microbes from utilizing the methyl substituted hydrocarbons. Additional studies of Dougherty sand soil at other moisture contents would have to be performed to determine the possible cause of the lack of methyl substituted hydrocarbon utilization.

Durant loam soil also showed a balanced removal of the four hydrocarbons. The Durant loam soil column removed 11.4-17.8 percent of the benzene, 11.9-15.7 percent of the toluene, 13.6-15.9 percent of the ethylbenzene, and 11.8-15.4 percent of the *o*-xylene introduced into the column. The relative moisture content of the column was 51-88 percent of the saturation value. This moisture content was close to the optimum range of 50-70 percent of saturation capacity suggested by Dibble and Bartha (1979). Moistures within this optimum range possibly allow microbial development which utilize the available hydrocarbon sources equally.

The rate of change of hydrocarbon concentration was monitored with soil microcosms. Figures 5.3 and 5.4 are examples of experimental data expressed as percent remaining versus time. Rate constants were determined for each contaminant of interest for each of the soils. The rate of removal was assumed to be pseudo first-order or zero-order. First-order rate constants were determined using the integral method for determining the reaction rate (Hill, 1977). First-order reaction rates should produce straight line graphs of ln(concentration) versus time. The slope of the resulting straight line is the rate constant of the removal reaction. Zero-order reactions are straight line relationships when data are plotted as reaction time versus concentration. The slope of the line is then the reaction rate constant.

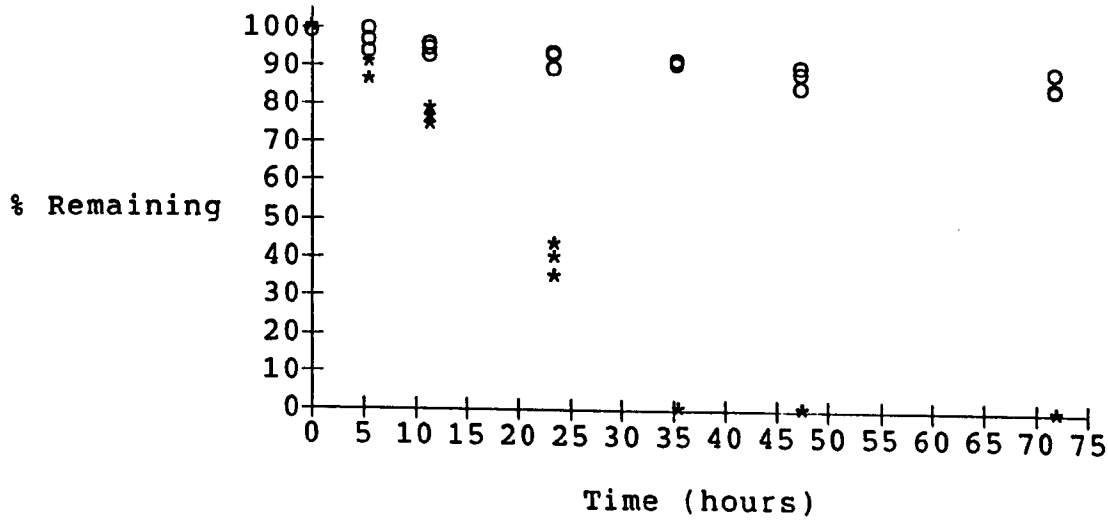
A least squares line was fit to the removal data for zero-order and first-order relations and the accompanying r-squared values were calculated. R-squared is the statistic that implies how well a model fits a set of data. R-squared equal to one implies a perfect fit. The r-squared values were used to determine whether the microcosm data displayed kinetics of zero- or first-order. Table 5.7 lists the rate constants determined for the removal of each component. Least squared fits which had nearly equal r-squared values for both zero-order and first-order list the rate constants for both. Rate constants are listed as negative values to clarify that the reactions are removal reactions.

Most of the removal reactions did not display a clear distinction between first-order removals or zero-order removals. The r-squared values for the zero-order and first-order relations were nearly equal in most cases. Table 5.8 lists the microbial densities determined for each acclimated soil at the various moisture contents. In all but one case, greater microbial densities accompanied higher moisture contents. In two out of three cases, greater moisture contents were associated with faster removal rates. Figures 5.5 and 5.6 graphically illustrate the effect of moisture content on removal rates. The Durant loam produced the largest benzene



Moisture Content 13.4%
o - Control
* - Sample

Figure 5.3. Toluene Removal in Rubicon Sand Microcosm.



Moisture Content 19.6%
o - Control
* - Sample

Figure 5.4. Benzene Removal in Durant Loam Microcosm.

Table 5.7. Removal Rate Constants For Hydrocarbons In Soil Microcosms

Soil Type	Moisture Content (wet basis)	gms Soil per Microcosm	Compound	Initial Conc. (ug/ml)	Reaction Constants Zero (ug/min)	First (min^{-1})
Rubicon Sand						
	13.4 %	27.3	Benzene	3.46	-2.966	-0.039
			Toluene	3.32	-3.039	
			Ethylbenzene	3.59		-0.121
			O-Xylene	3.95		-0.032
	9.5 %	12.5	Benzene	3.61	-0.860	-0.005
			Toluene	3.46	-1.187	-0.007
			Ethylbenzene	3.53	-3.075	-0.032
			O-Xylene	3.87		-0.013
	10.0 %	13.3	Benzene	3.36	-0.869	-0.005
			Toluene	3.43	-1.177	-0.007
			Ethylbenzene	3.52	-2.803	-0.026
			0-Xylene	3.81	-1.866	-0.013
Dougherty Sand						
	10.4 %	6.2	Benzene	2.48	-2.849	-0.102
			Toluene	2.65	-1.362	-0.014
			Ethylbenzene	1.74		-0.025
			O-Xylene	3.13	-0.794	-0.006
	6.8 %	5.9	Benzene	2.64	-1.474	
			Toluene	2.55	-0.750	-0.006
			Ethylbenzene	1.71	-1.135	
			O-Xylene	2.62	-0.444	-0.003
Durant Loam						
	19.6 %	21.7	Benzene	3.33	-4.626	
			Toluene	3.12	-2.062	-0.045
			Ethylbenzene	1.94	-0.660	-0.012
			O-Xylene	3.02	-0.705	-0.007
	11.4 %	6.9	Benzene	2.53	-3.076	
			Toluene	2.48	-1.616	
			Ethylbenzene	1.50	-0.655	-0.012
			O-Xylene	2.31	-0.714	-0.007

removal rate constant. Rubicon sand had the largest ethylbenzene and *o*-xylene removal rate constants.

Rubicon sand had the largest toluene removal rate constant at the higher moisture content. At lower moistures, Durant loam had the largest removal rate constant for toluene. Ethylbenzene and *o*-xylene removal seemed to be the most first-order related of the contaminants tested. Shorter sampling times may be required to produce better first-order correlations for the other components.

Table 5.8. Microbial Densities
Acridine Orange Fluorescent Direct Bacteria Counts

Soil Type	Percent Moisture (wet basis)	Microbial Density* (Cells per g soil)
Rubicon Sand (G)	13.4	$18.0(10)^8$
	9.5	$22.2(10)^8$
	10.0	$26.6(10)^8$
Durant Loam	19.6	$9.1(10)^8$
	11.4	$6.2(10)^8$
Unacclimated soil		$0.4(10)^8$
Dougherty Sand	10.4	$32.8(10)^8$
	6.8	$17.4(10)^8$
Unacclimated soil		$18.6(10)^8$

* Acridine-orange Direct Counts follow the Poisson Distribution. The standard deviation of the microbial density is equal to the square root.

In general, the Rubicon sand exhibited a preference for ethylbenzene followed by toluene, benzene, and *o*-xylene when tested at the higher moisture content. The microbes present in the Rubicon sand evidently contained microbial strains which preferentially utilized the ethyl and methyl substituted benzenes over benzene itself. Higher moisture contents possibly restrict the transport of *o*-xylene causing the removal rate constant for *o*-xylene to be slightly less than for benzene. At the lower moisture content, a larger surface area would be available for adsorption. And in the case of lower moisture contents, the resulting removal rate constant for *o*-xylene is greater than either of the removal rate constants for benzene or toluene. At the lower moisture content the order of preference was ethylbenzene, *o*-xylene, toluene, and benzene. The order of preference corresponds well with the results obtained from the soil column studies. Ethylbenzene was removed the most in the soil column, followed by nearly equal removals of toluene and *o*-xylene, with benzene being removed the least.

Dougherty sand removed benzene the fastest of the four components tested. Ethylbenzene removal was second to benzene and preceded toluene and *o*-xylene removal. The lesser removals of *o*-xylene probably reflect increased resistance to biodegradation due to additional methyl substitution of *o*-xylene. A considerable reduction in microbial activity was

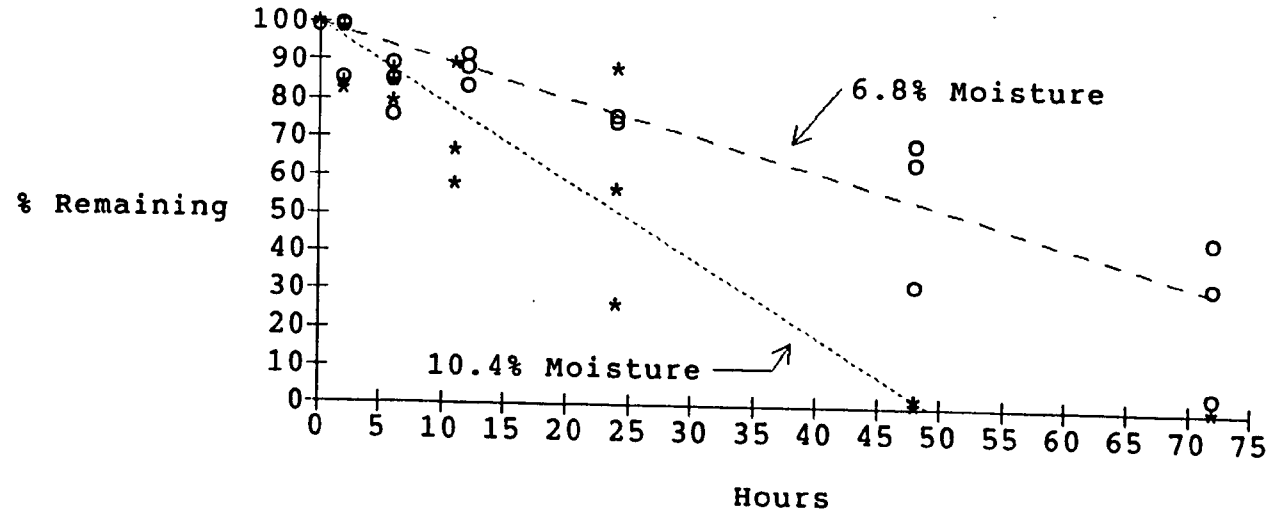


Figure 5.5. Moisture Effect on Benzene Removal, Dougherty Sand Microcosm.

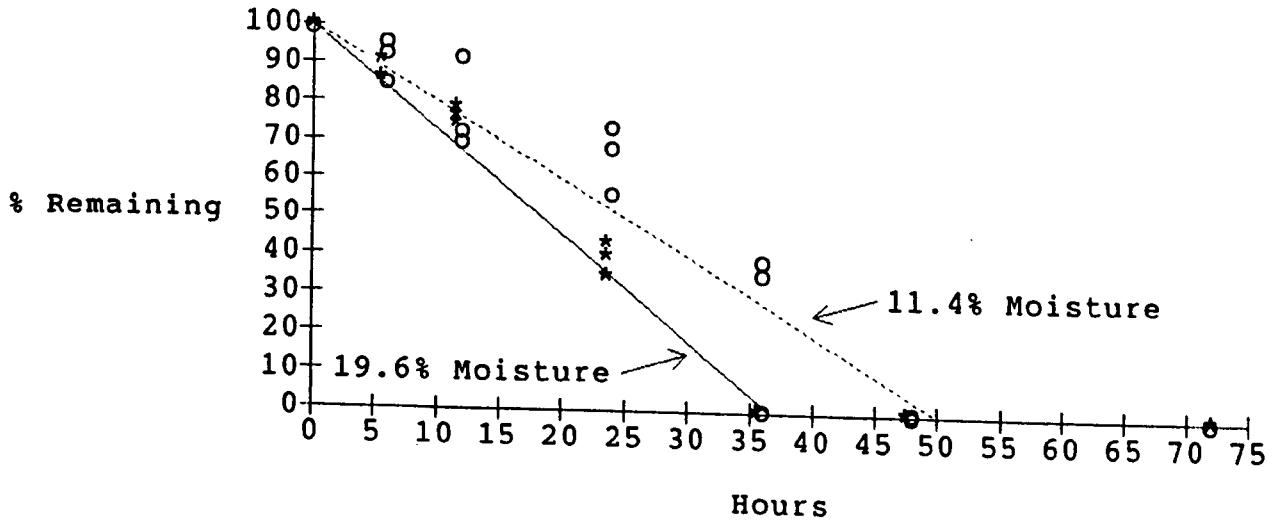


Figure 5.6. Moisture Effect on Benzene Removal, Durant Loam Microcosm.

observed between the 10.4 percent (46 percent of saturation capacity)and 6.8 percent (30 percent of saturation capacity) moisture contents as illustrated in Figure 5.5. The 10.4 percent moisture content is close to the optimum moisture range of 50-70 percent of saturation which has been reported by Dibble and Bartha (1979).

Removal rate constants developed from Durant loam were greatest for benzene followed by toluene, *o*-xylene, and ethylbenzene. The removal constants of ethylbenzene and *o*-xylene were almost equal. Nearly equal solubilities which influenced the transport of these components along with the presence of suitable utilizing microorganisms probably contributed to the nearly equal removal rates. Moisture content was an important parameter of hydrocarbon removal as illustrated in Figure 5.6. Removal inhibition from excess moisture was not observed for the limited number of moisture contents evaluated. Additional studies need to be performed to determine optimum soil moistures.

Direct comparison of the experimental reaction rates with previously reported values is not possible because of the different types of experimental setups, but some generalizations may be drawn. Thomas (1987) reported benzene removals for soil-water systems approximately 1000 times less than the benzene removals obtained in this study (Thomas:0.0004-0.0024 /min versus 0.9-4.0 /min). Slower transport through water than air and limited availability of oxygen in aqueous systems compared to air systems may contribute to this difference. Swindoll (1988) has reported removal rates for toluene removal in aqueous systems which are within an order of magnitude to the values obtained in this study (Swindoll:0.00083 min^{-1} compared to 0.006-0.045 min^{-1}). The values obtained in the aqueous system are less than those obtained in the air system.

The rates of aromatic hydrocarbon removal in the bioreactors were equivalent to rates of biological removal of aliphatic hydrocarbons published for other soils. From Table 5.7 the zero-order rate for benzene degradation in the Rubicon sand at 13.4% moisture is equivalent to 0.109 µg/g soil/min. The rate of removal of propane and butanes in a pilot-scale bioreactor at Racine, Wisconsin, was 0.113 µg/g soil/min (Kampbell *et al.*, 1987). Hoeks (1972) showed that soils from The Netherlands could degrade methane at a zero-order rate of 0.083 mg/g soil/min after a suitable period of acclimation.

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