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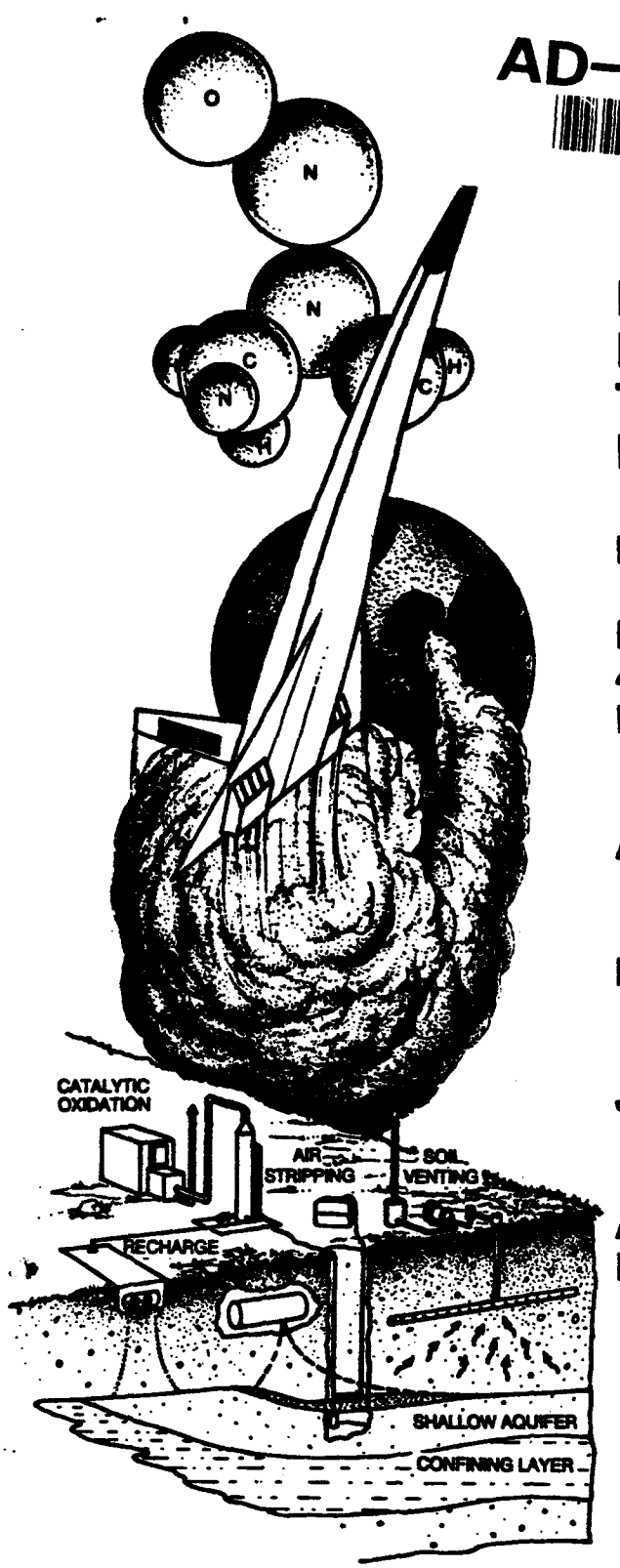

LIQUID-PHASE BIOREACTOR FOR DEGRADATION OF TRICHLOROETHYLENE AND BENZENE

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13. ABSTRACT *(Maximum 200 words)*

The objective of this project was to design and operate a bench-scale biological treatment system capable of degrading trichlorethylene (TCE) and benzene, each present at concentrations up to 50 ppm, to concentrations of 4 ppb or less per contaminant. A two-stage reactor system was designed and fabricated to allow for the sequential degradation of benzene followed by TCE since TCE demonstrated no inhibitory effects on benzene degradation. A first-stage fixed-film reactor system demonstrated greater than 96% biodegradation of benzene from an artifically contaminated groundwater stream over a two month period of continuous operation. The liquid exiting the first-stage reactor was stripped to transfer the TCE and residual benzene into the air phase which was treated into a vapor phase bioreactor. The second-stage unit biodegraded greater than 90% of the TCE and greater than 90% of the residual benzene load. The two stage treatment system demonstrated the feasibility of biological treatment for a mixture of nonchlorinated and chlorinated organic contaminants in groundwater.

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EXECUTIVE SUMMARY

A. OBJECTIVE

The objective of this project was to test a bench-scale biological system designed to mineralize dilute concentrations of benzene (a model fuel component) and trichloroethylene (TCE) from contaminated groundwater. Benzene (petroleum-based fuels) and TCE represent two classes of chemicals (nonchlorinated and chlorinated organic chemicals) commonly found in contaminated groundwater. The operation of a bench-scale reactor would establish the feasibility of biologically based treatment and provide operational parameters critical for development of a field demonstration system.

B. BACKGROUND

TCE has been extensively used as a degreasing agent and represents one of the most prevalent organic contaminants found in both soils and groundwater. Benzene is a major component of petroleum-based fuels. Fuels have found their way into the environment through leaking storage tanks and spills often associated with transfer operations. Environmental release of these chemicals is regulated and both chemicals are either known or suspected carcinogens. Available physical/chemical treatment technologies for removal of organic chemicals from contaminated groundwater include activated carbon adsorption, air stripping, vapor extraction and catalytic oxidation. These treatment methods can be costly and operationally complex and sometimes merely act to transfer the contaminants to another phase which requires subsequent treatment. Biological systems offer the possibility of a cost-effective destruction technology for many classes of contaminants, including nonchlorinated and chlorinated organic chemicals.

Benzene and other hydrocarbon fuel components have been known to be aerobically biodegradable for many years. Biological treatment systems for fuel hydrocarbons have been demonstrated for both contaminated water

and soils. However, these systems have not been capable of effectively treating TCE or other chlorinated organic chemicals that cocontaminate many sites. TCE biodegradation has only been demonstrated more recently. Although bench-scale systems have demonstrated initial success in TCE degradation, there has been limited success using pilot-scale demonstration units. Effective treatment of both nonchlorinated and chlorinated organic chemicals in a unified system poses potential problems that this project was designed to identify and address.

Under contract with the DOE's Office of Technology Development (OTD), Envirogen has developed a bench-scale bioreactor that gives better than 90 percent destruction of TCE from a contaminated groundwater or air stream. This reactor has been demonstrated to perform equally well with either of two biocatalysts that have been tested in the system. The economic analysis generated as part of the OTD project indicate typical savings of 70 to 80 percent using the biological treatment system as compared to carbon adsorption. A 4000-Liter bioreactor has been constructed by Envirogen and will soon undergo pilot-scale field testing.

C. SCOPE

The first objective of the work under this contract was to establish the microbial requirements for the cotreatment of both TCE and benzene. A static assay was used to assess benzene and TCE mineralization, inhibitory interactions and toxic effects under a variety of conditions. This bottle assay involved the addition of active biomass to a serum bottle followed by the addition of benzene and/or TCE. The bottle was immediately sealed with a chemically unreactive septum and the concentrations of benzene and/or TCE were determined by injecting a fixed volume of headspace gas onto a gas chromatograph (GC). Effective biological treatment often depends on the selection of the best microorganism. The more difficult of the two chemicals to biodegrade was TCE; therefore, organisms capable of this activity were isolated and characterized. Benzene degradative microorganisms were isolated from sewage sludge to augment overall biocatalytic capability.

Once basic growth, induction, inhibition and toxicity parameters were characterized, a two-stage treatment system was designed, fabricated and tested. This two-stage treatment system offered the simplest design with the greatest degree of operational flexibility against the broadest range of contaminants. Several types of bioreactors were available for bench-scale testing including fixed film, suspended-growth, fluidized-bed, trickling-filter and vapor-phase reactor designs. A fixed-film reactor was chosen for treatment of benzene initially due to its simplicity of design and operation. A suspended-growth reactor was also tested as part of this investigation. The effectiveness of treatment was assessed using GC methods established to quantify benzene and TCE concentrations in air and water samples entering and leaving the test reactors. GC methods allowed for direct analysis of the chemicals of interest and the potential identification of intermediate chemicals accumulating in either the air or water phases. A complete mass balance for benzene and TCE was deemed essential for accurately assessing system performance. These bench-scale units provided operational information for fully functional scaled down bioreactors.

D. RESULTS

TCE-degradative microorganisms were not capable of degrading benzene unless they had been induced following growth with toluene and/or phenol. TCE did not inhibit benzene degradation over the range of concentrations tested. Benzene-degradative organisms did not degrade TCE. These observations suggested that more than one microorganism would be required for the mineralization of both benzene and TCE in a cotreatment system. Sequential degradation of benzene followed by TCE would be feasible since the TCE demonstrated no significant inhibitory effects on benzene degradation.

The ultimate goal of a biological system is to effectively treat a wide range of nonchlorinated and chlorinated organic cocontaminants including, but not limited to benzene and TCE. With this broad goal and the findings that a single organisms would have limited capabilities, a staged reactor system incorporating more than one type of microorganism offers the

simplest comprehensive design. A two-stage treatment system was fabricated and tested for its ability to mineralize 50 ppm benzene and 50 ppm TCE. A first-stage fixed-film unit was designed for benzene degradation. Operational performance typically demonstrated greater than 96 percent biodegradation of benzene from an artificially contaminated groundwater stream over a two month period of continuous operation . The undegraded benzene exited the reactor in either the air or liquid effluent streams. Oxygen was supplied as either pure O_2 or as air with similar effects on benzene degradation performance. TCE in the contaminated groundwater elicited no significant inhibitory or toxic effects on benzene degradation. As expected with this first-stage unit, there was minimal activity against TCE with less than a 28 percent decrease between influent and effluent TCE loads. An alternative first-stage reactor design, a suspended-growth reactor, was also tested with greater than 96 percent of the benzene degraded and essentially no degradation of the TCE. Both first-stage treatment systems performed as designed resulting in the destruction of benzene from an average of 45 ppm to below 1 ppm in the liquid stream.

The second-stage unit was designed to degrade an influent load of 50 ppm TCE with <5ppm residual benzene. The liquid exiting the first-stage unit is stripped to transfer the TCE and "residual" benzene into the air phase. The combined air phases from the stripper and the offgas from the first-stage unit are combined and passed through the second-stage vapor-phase reactor. Following first-stage treatment and subsequent stripping, the levels of both benzene and TCE should be below the discharge limits of 5 ppb in the treated groundwater effluent. To model the second-stage system, a synthetic mixture of TCE and benzene were passed through an air stripper then treated in a vapor-phase reactor. Benzene loads to the second-stage reactor were lowered in relationship to TCE loads to simulate the effluent from a moderately successful first-stage unit operation. The second-stage unit biodegraded >90 percent of the TCE and >90 percent of the residual benzene loads over the 6 days of testing. There were no apparent inhibitory effects of benzene on TCE degradative performance.

E. CONCLUSIONS

The two-stage treatment system demonstrated the feasibility of biological treatment of a mixture of nonchlorinated and chlorinated organic contaminants in groundwater. The system demonstrated robust performance and provided operational parameters which allow for designing a pilot-scale treatment system for field evaluation. This staged system design allows for the treatment of a range of nonchlorinated and chlorinated organic contaminants including, but not limited to benzene and TCE. By augmenting the microbial population within the reactors, additional chemical contaminants would be treatable. Treatment of the more easily degradable contaminants in a first-stage unit lowers the complexity of the wastes entering the second-stage unit which inherently requires a greater level of operational control due to the nature of the contaminants being treated, the chlorinated organic chemicals.

F. RECOMMENDATIONS

The scope of work should now be expanded to include a more complex waste typically found at contaminated sites. Contaminated groundwater is likely to include more components than just benzene and TCE. Some modifications in design and operation may be required when using a more realistic synthetic waste or authentically contaminated groundwater. Optimally, authentic site water from the chosen field site should be used in bench-scale units to generate information to aid in the design of a pilot-scale unit.

Further work could also assess the cause of TCE reduction in the fixed-film reactor. One likely possibility for this observed activity is anaerobic dechlorination. An anaerobic dechlorination process could be developed into an effective biological treatment process which would possess a wider range of activity towards the chlorinated organic chemicals often found as groundwater contaminants. The next logical stage of work would perform a field evaluation of this system to assess effectiveness and costs of biological treatment compared to other technologies.

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PREFACE

This report was prepared by Envirogen, Inc., 4100 Quakerbridge Road, Lawrenceville NJ 08648, under Contract Number FO8635-91-C-0198 for the Air Force Civil Engineering Support Agency (AFCESA/RAVW), Tyndall AFB FL 32403-5319.

This report summarizes work done between 1 June 1991 and 30 November 1991. The HQ AFCESA/RAVW project officer was Capt Catherine M. Vogel.

This report has been reviewed by the Public Affairs Office and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nationals.

This report has been reviewed and is approved for publication.

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LIST OF ABBREVIATIONS

BSM .....basal salts medium

CL.....clarifier

CSTR.....continuously stirred tank reactors

DO.....dissolved oxygen

DOD.....Department of Defense

DOE.....Department of Energy

GC.....gas chromatograph

OTD.....Office of Technology Development

TCE.....trichloroethylene

VOC.....volatile organic chemicals

SECTION I:

INTRODUCTION

A. OBJECTIVE

The objective of this research was to develop a biological treatment system to mineralize dilute concentrations of a mixture of chlorinated and nonchlorinated organic chemicals found in groundwater pumped from contaminated aquifers. This Phase I research project focused on the design, fabrication and testing of a bench-scale system to establish proof-of-concept for biologically treating a mixture of benzene and TCE. The bench-scale system was also used to establish operational parameters and design criteria for a Phase II field demonstration project.

B. BACKGROUND

The most commonly used technologies for removal of organic chemicals from contaminated groundwater are activated carbon adsorption, air stripping, vapor extraction and catalytic oxidation. The first three methods merely transfer the contaminants from the groundwater to another phase which requires subsequent treatment. The last technology tends to be costly and operationally complex especially for the chlorinated organic chemicals which damage the catalyst support.

TCE, one of the components in this study, is a volatile chlorinated organic compound that has been used extensively as a solvent and degreasing agent. Its heavy use in cleaning engines has led to extensive groundwater contamination at DOD sites. It is believed that TCE is the most prevalent organic contaminant of both soils and groundwater (19). Studies indicate that TCE may be carcinogenic (6). The Safe Drinking Water Act reflects a concern over TCE contamination by limiting its concentration in drinking water to 5 ppb or less. Potentially, a single 55-gallon drum of TCE could contaminate over 10 billion gallons of water to levels above the

drinking water standard. TCE is a prevalent contaminant within both the DOE and DOD and at Superfund Sites.

In general, biotransformation of chlorinated organic chemicals falls into two major categories, anaerobic dehalogenation and aerobic oxidation. Anaerobic biotransformation of TCE can lead to the formation of vinyl chloride, a potent carcinogen (16). Because many subsurface regions are anoxic, a significant potential exists for the accumulation of biologically formed vinyl chloride from TCE. Within the last few years, a broad variety of microorganisms have been shown to aerobically degrade TCE and related chlorinated organic chemicals under selected conditions. Aerobic biotransformation generally requires induction of monooxygenase or dioxygenase enzymes which fortuitously oxidize TCE. Inducing substrates for these oxygenases include toluene, ethyl benzene, phenol, methane, propane and ammonia (1, 2, 9, 10, 12, 15, 18, 20, 21, 22).

The feasibility of using TCE-degrading microorganisms in bioreactors has previously been demonstrated. Four different types of reactors have been used to investigate the degradation of TCE and other VOCs: a continuous recycle, expanded-bed reactor (11), a fixed-film, packed-bed reactor (14), a submerged aerobic bioflim reactor (13) and a recirculating suspended-growth reactor (3). Based on such work, Envirogen has been developing bench-scale bioreactors for degradation of TCE for over 2 years.

A bench-scale TCE bioreactor has been developed and tested. When fed a cosubstrate, *Pseudomonas cepacia* G4, ENV110 or *Pseudomonas mendocina* rapidly oxidize >90 percent of the TCE within a suspended-growth bubble-column reactor. The reactor incorporates a ceramic type of air sparger which generates small bubbles. This simple reactor design has demonstrated reliable performance with straightforward operation with a biocatalyst lifetime up to least 30 days.

At some sites, TCE is the sole contaminant whereas at numerous other sites it is only one of many chemicals found. These cocontaminants can be both chlorinated and nonchlorinated. TCE, historically used as a solvent, is

often found with fuels and nonchlorinated solvents at contaminated sites. Fuel contamination originated from leaking storage tanks and accidental spills during transfer operations Benzene is probably the most significant health concern within the mixture of chemicals found in fuels and represents the second component of the mixture used in this study. Benzene and many of the other nonchlorinated hydrocarbons found in fuels tend to be readily degradable under aerobic conditions. Although both benzene and TCE have been shown to be degradable separately at the bench scale, treatment of mixtures of chlorinated and nonchlorinated contaminants has not been clearly demonstrated. This project attempts to bring all of these components together into a unified treatment system which can be scaled up to field systems.

C. SCOPE

The Phase I technical objectives were broken down into 5 basic tasks:

1. Establish whether TCE degradative organisms were capable of also degrading benzene.
2. If a single class of organisms was not found to be capable of degrading both TCE and benzene, then organisms capable of benzene metabolism were to be added to augment the test program.
3. Characterize conditions for enhancing microbial degradation of both TCE and benzene.
4. Characterize any inhibitory or toxic interactions of TCE and benzene on the degradative microorganisms.
5. Design and operate a bench-scale treatment system for the biodegradation of both TCE and benzene.

The last task of this series was most important and would provide the essential information for the design and operation of a field-scale test system.

SECTION II:

METHODOLOGY

A. BACTERIAL STRAINS, CULTURE CONDITIONS AND CHEMICALS.

Three strains of TCE-degradative microorganisms were used in this study. *P. cepacia* G4 was obtained from the U.S. Environmental Protection Agency Environmental Research Laboratory, Gulf Breeze, FL where it had been previously isolated and characterized (3, 4, 8, 9). Two unique TCE-degradative organisms, ENV110 and ENV113, have been isolated at Envirogen from sewage sludge prior to initiation of work on this project. These two strains had been grown with a combination of phenol and toluene and were selected for their ability to degrade TCE. All organisms were grown with either 2 mM phenol and 20 mM lactate or toluene (in a hydrocarbon feeder) in a defined basal salts medium (BSM), pH 7.5 (5).

An undefined mixed population of benzene degradative microorganisms was used in this study. Enrichments for these organisms were performed using sewage sludge as an inoculum and benzene as the sole carbon source for growth. Cultures were grown in a 250 mL shake flask with benzene supplied from a glass reservoir suspended above the culture within the headspace of the flask. BSM was used to supply required trace metals for growth. This culture was transferred weekly over the course of this project and was used as the initial inoculum for both fixed-film and suspended-growth bioreactors.

B. METHODS FOR QUANITFYING TCE AND BENZENE

Aqueous TCE and benzene concentrations were quantified using a Varian 3400 gas chromatograph (GC) equipped with two detectors, photo ionization and electron capture. Water samples contaminated with TCE and/or benzene were extracted for 1 hour with n-pentane with recoveries greater than 99 percent. The organic layer was then transferred to a 2 mL autosampler vial for injection onto the GC. Separation was accomplished by

injecting 1 μL of the pentane extract onto a VOCOL (Supelco, Inc., Bellefonte, PA) capillary column run isothermally at 50°C with 5 mL/min helium carrier gas flow. Standards were prepared by adding known amounts of TCE and benzene in methanol, measuring detector response, then calculating concentrations from a standard curve. The detection limit for benzene was about 100 ppb and for TCE was about 25 ppb for aqueous extractions using these methods.

TCE and benzene concentrations in air were quantified using the same GC, detectors, and VOCOL column operated isothermally at 90°C. Air samples were directly injected onto the GC using a 10 μL gastight syringe. Standards were prepared by injecting a standard mixture of benzene and TCE in methanol into an evacuated serum bottle (61 mL) sealed with a Teflon®-lined septum. Once the liquid evaporated, the pressure was equalized by venting air into the bottle using a syringe needle. A 10 μL gastight syringe was then used to inject a gas sample from the bottle onto the GC. Concentrations were calculated from a standard curve. The detection limit was about 2 μg/L for benzene and about 1 μg/L for TCE using direct air phase injections.

A second Varian 3400 gas chromatograph equipped with an electron capture detector and a 16-port stream selection valve was used for continuous gas-phase monitoring of TCE entering and exiting the vapor-phase TCE bioreactor. A 50 μL sample loop was flushed with vapor entering or exiting the vapor-phase TCE reactor. The contents of this sample loop were then injected onto a VOCOL column held isothermally at 100°C. Samples were collected continuously at defined intervals and concentrations calculated from a standard curve. Only TCE was monitored continuously using this instrument.

C. BOTTLE ASSAY PROTOCOL

TCE and benzene degradation kinetics and inhibitory interactions were determined using a bottle assay. In this standard assay, a 10 mL liquid microbial suspension was placed into a 50 mL serum bottle (actual volume of

61 mL). A known amount of TCE and/or benzene was added to a bottle which was immediately sealed with a Teflon®-lined septum and placed on a shaker at 200 rpm. Periodically, 10 µL of headspace gas was withdrawn through the septum using a gastight syringe and injected onto a GC for quantification. For volatile organic chemicals such as TCE and benzene, which equilibrate rapidly between air and water, the gas-phase analysis provides a clear representation of the total amount of chemical in the sealed bottle. Chemical concentrations in live experimental and killed controls are calculated by comparison to a standard curve. Degradation rates are calculated for the disappearance of total chemical from the bottle normalized to the microorganism content expressed as total protein. Microbial protein concentrations were determined using the BCA method (Pierce Chemical Co., Rockford, Ill.), using bovine serum albumin as a protein standard.

D. FIXED-FILM BIOREACTOR DESIGN AND OPERATION

The process diagram of the fixed-film bioreactor for the degradation of benzene is depicted in Figure 1. The main reactor vessel was constructed from a 10 cm diameter by 60 cm glass chromatography column with threaded Teflon® plugs in each end (Kontes Glass, Vineland, NJ) with an empty bed volume of 4.7 Liters. The column was packed with 1-inch Jaeger Tri-Pack (Jaeger Products, Inc., Spring, TX). A 1-Liter glass vessel functioned as an equalization vessel and gas/liquid separator. All tubing used to connect the reactor components was composed of Teflon®. A vane pump was used to circulate liquid through the reactor at a flow rate of about 300 mL per minute which gave an upflow velocity past the packing of about 4 mL per minute per cm^2 (1 gallon per minute per square foot). The influent water originated directly from a groundwater well which was adjusted to a pH between 7 and 7.4 using sodium hydroxide. TCE, benzene and nutrients were continuously added to this water just prior to entry into the reactor using metered pumps and a mixing vessel to minimize pulsing in the influent stream. Typical flow rates for the influent contaminated groundwater ranged between 14 and 20 mL per minute, giving a hydraulic

residence time of about 6 hours. Oxygen was supplied to the reactor either as pure O_2 or as compressed air with flow rates controlled and measured by a calibrated rotameter. An in-line static mixer was used to maximize oxygen mass transfer into the aqueous phase. Dissolved oxygen levels were monitored using a dissolved oxygen probe placed into the liquid of the equalization vessel. The reactor was inoculated with a mixed culture of benzene-degradative organisms grown at room temperature in shake flasks with benzene as the sole carbon source. TCE and benzene concentrations were monitored by extracting triplicate samples of the influent and effluent water stream (① and ②). The offgas from the surge tank was also monitored for TCE and benzene by direct gas injection (❸).

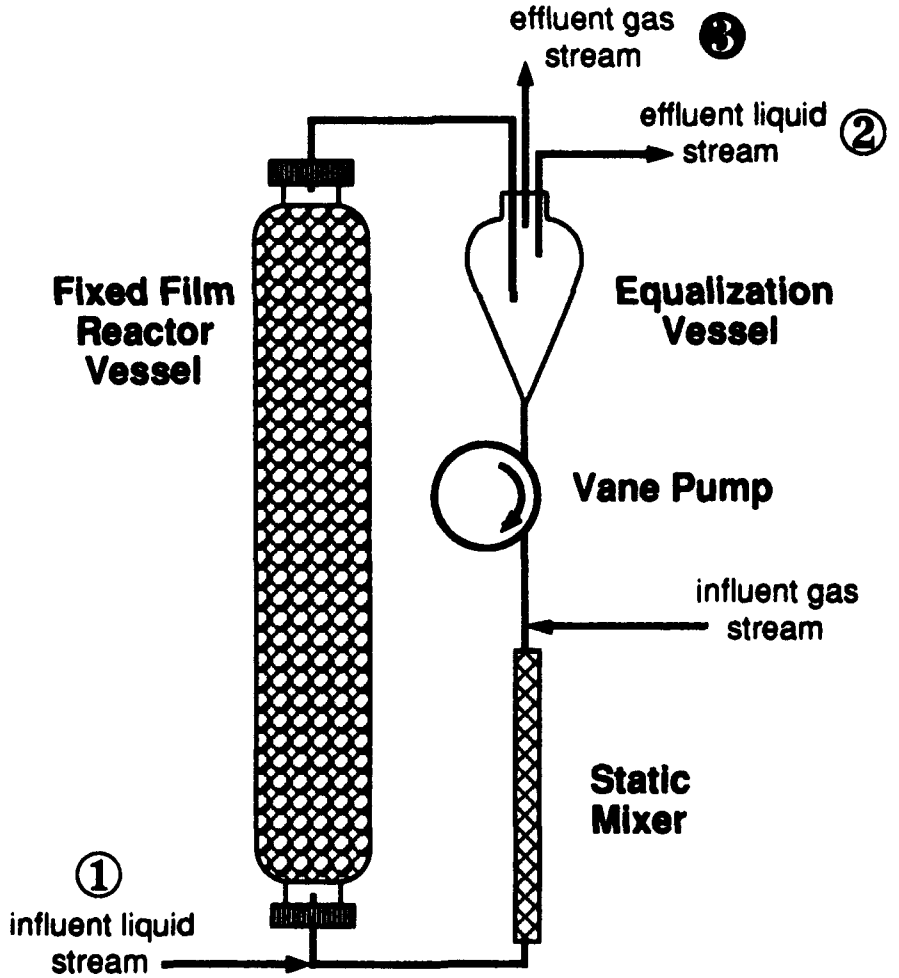

Figure 1. Diagram of the Fixed-Film Bioreactor. Sample locations are marked ① and ② for liquid samples and ❸ for gas samples.

E. SUSPENDED-GROWTH BIOREACTOR DESIGN AND OPERATION

The process diagram of the suspended-growth bioreactor for the degradation of benzene is depicted in Figure 2. The reactor was composed of 3 vessels, 2 continuously stirred tank reactors (CSTR 1 and CSTR 2) and a clarifier (CL). The volume of each CSTR was about 2 Liters and a volume of about 1.5 Liters for the clarifier. Settled solids were pumped back to the first CSTR through a Teflon line using a peristaltic pump at a flow rate of 15 mL per minute. Overhead mixers provided agitation to each CSTR. A rake rotated at 1 rpm promoted the movement of solids to the bottom of the clarifier for recycle. All three vessels were covered to minimize fugitive losses of benzene and TCE from the air above the liquid. Contaminated groundwater was prepared as described above and introduced at a rate of about 15 mL per minute.The hydraulic residence time for this system was about 6 hours. Oxygen was supplied to CSTR 1 as pure O_2 and compressed air was supplied to CSTR 2 with flow rates controlled and measured by calibrated rotameters. Dissolved oxygen levels were monitored in each chamber using a calibrated dissolved oxygen probe. The reactor was inoculated with a mixed culture of benzene-degradative organisms grown in shake flasks with benzene as the sole carbon source. Diatomaceous earth, R-685 (Manville Corp., Denver, CO) and flocculating polymers (Betz Laboratories Inc., Trevose, PA) were added to promote flocculating and settling of the microbial biomass. TCE and benzene concentrations were monitored by extracting triplicate liquid samples of the influent stream, effluent stream and the contents of each reaction vessel (①, ③, ⑤ and ⑥). TCE and benzene in the offgas from each CSTR were also monitored by direct gas injection (❷ and ❹).

F. VAPOR-PHASE REACTOR DESIGN AND OPERATION

The process diagram of the TCE vapor-phase bioreactor for the degradation of TCE is depicted in Figure 3. The main reactor vessel was constructed from a 10 cm diameter by 60 cm glass chromatography column with threaded Teflon® plugs in each end (Kontes Glass, Vineland, NJ) with an empty bed volume of 4.7 Liters. The reactor was filled with 3 L of a

suspension of *P. cepacia* G4 which had been grown with phenol and lactate as described previously. A mixture of TCE and benzene in water was pumped into an air-stripping vessel with TCE/benzene contaminated air then passing into the vapor-phase reactor at a flow rate of 100 mL per minute. The air-stripping vessel was packed with glass wool. The TCE concentration in the influent and effluent gas was continuously and automatically monitored by GC (❶ and ❷). Separate analyses for both TCE and benzene were performed periodically by manually injecting 10 µL of both influent and effluent gas onto a GC from these two same sample locations. The reactor was fed 85 percent (by wt) phenol in water at a feed rate of 0.2 grams phenol per liquid Liter volume per day.

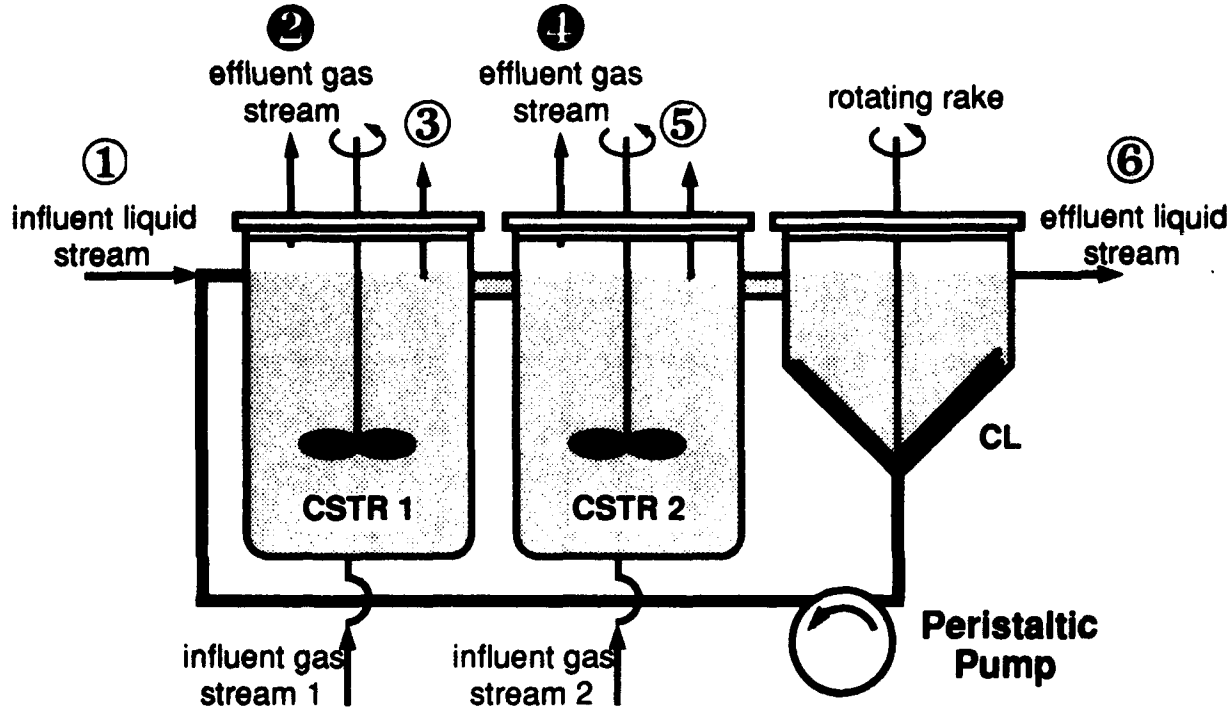

Figure 2. Diagram of the Suspended-Growth Bioreactor. Sample locations are marked ①, ③, ⑤ and ⑥ for liquid samples and ❷ and ❶ for gas samples.

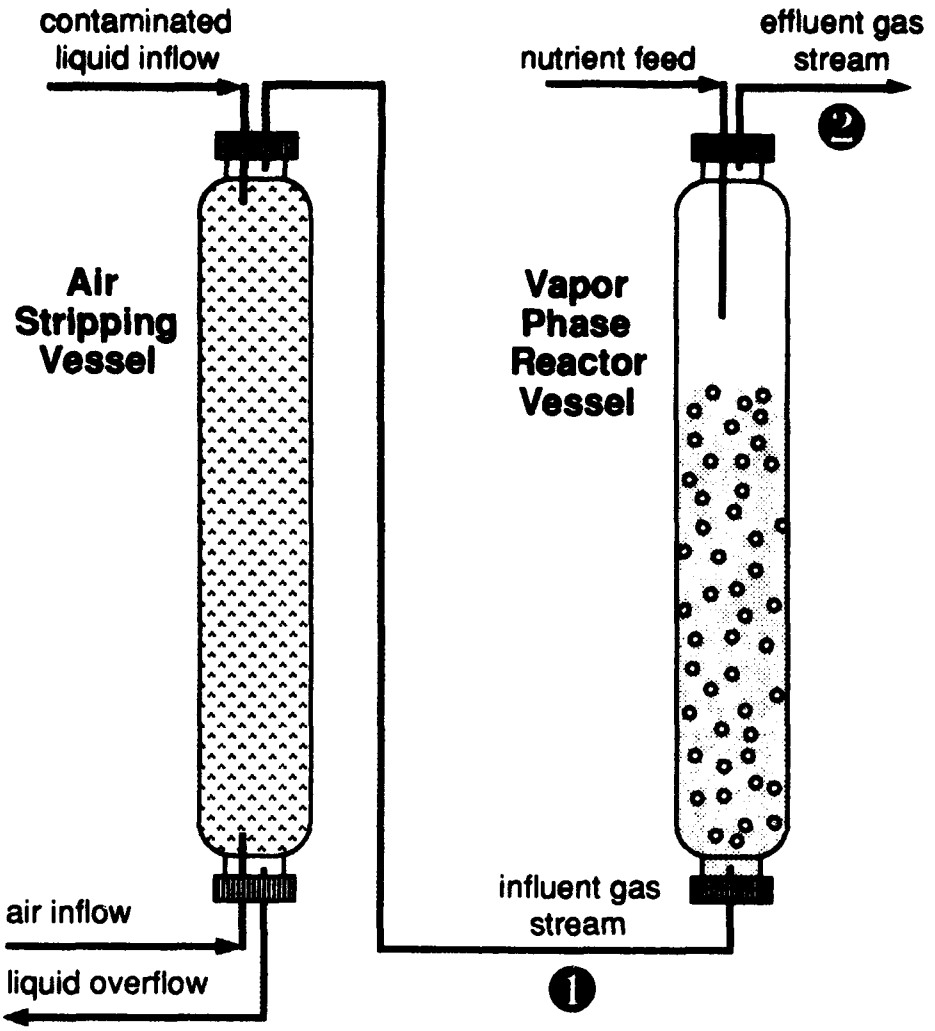

Figure 3. Diagram of the Vapor-Phase Bioreactor. Sample locations are marked ❶ and ❷ for gas samples.

SECTION III:

TEST RESULTS

A. SELECTION AND CHARACTERIZATION OF MICROORGANISMS.

The simplest reactor configuration would be a single vessel containing microorganisms capable of degrading all contaminants in the influent water. The simplest microbial culture would be a single organism capable of degrading all contaminants. TCE degradation is a cometabolic process requiring a unique population of microorganisms. This population of organisms needs to be added and/or maintained to assure TCE degradation activity within a treatment system. Several TCE-degradative organisms were compared for differences in their ability to degrade TCE (Figure 4). Using a bottle assay, there was no significant difference in the specific rate of TCE degradation between the three strains tested following growth with phenol. All three organisms exhibited a specific activity for TCE degradation of 1.0 ± 0.2 μmole per min per g protein under these conditions.

TCE-degradative organisms were next assessed for their ability to degrade benzene to determine whether a single-stage treatment system was feasible. All three TCE-degradative strains failed to grow with benzene as the sole source of carbon. However, following growth with toluene, these three strains were capable of degrading 50 ppm benzene to below detection limits following a 24-hour incubation using the bottle assay. These results demonstrate that all three strains tested were comparable in their ability to degrade TCE and any one should be adequate for further study. These results also demonstrate that, although benzene does not appear to act as an inducer, it is a degradable substrate. Furthermore, these results suggest that a single-stage system for treatment of benzene and TCE would require either a different microbial strain, a mixed population of compatible microorganisms or significantly more complex operating conditions to support activity of a single strain. A flexible and simple approach would incorporate a staged design.

Enrichments for microorganisms capable of growth with benzene as the sole carbon source were performed. Once an active population was obtained, it was assayed for benzene degradative activity using the bottle assay (Figure 5). Initial studies yielded a specific benzene degradation rate of 1.9 μmole per minute per gram protein with an apparent decrease in degradation rate at low benzene concentrations.The amount of benzene added corresponded to 50 ppm if all of the benzene was dissolved in the aqueous phase. These same cultures failed to degrade 50 ppm TCE in a 24-hour period.

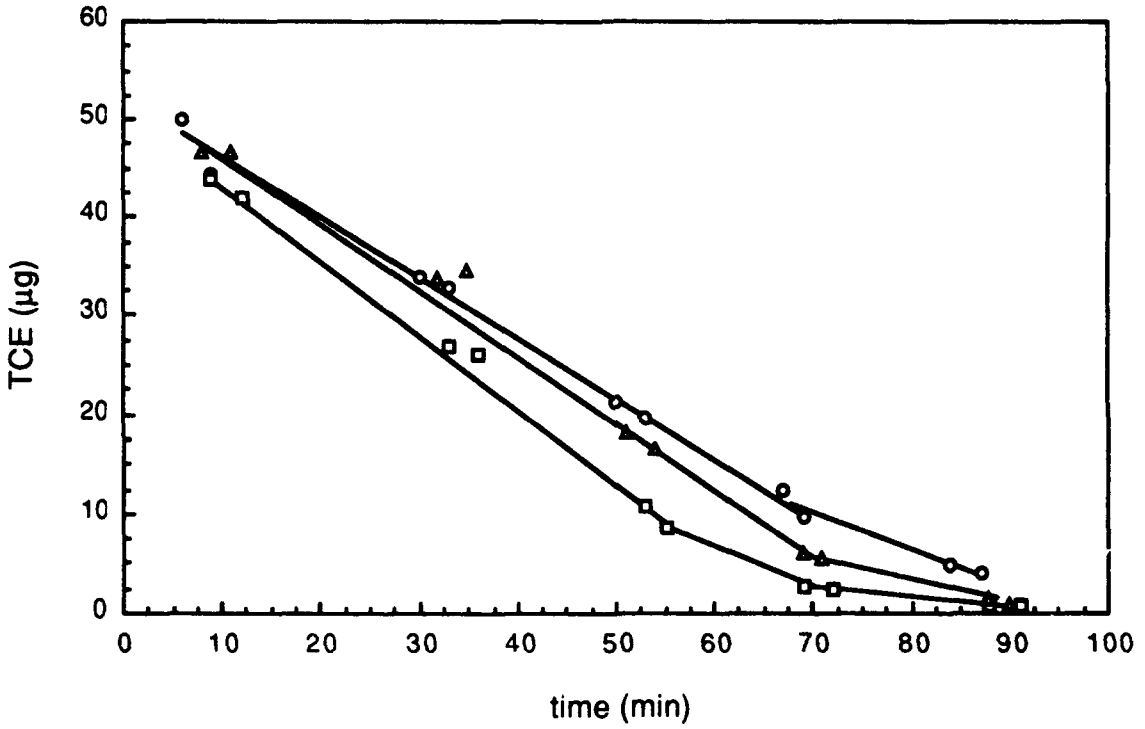

Figure 4. TCE degradation by Three Unique Microbial Strains. The standard bottle assay was used to assess TCE degradation using a 10 mL suspension of cells grown with toluene as the sole carbon source at 0.52, 0.47 and 0.54 mg/mL of cell protein for G4 (O), ENV110 (□) and ENV113 (Δ), respectively. The total amount of TCE in the bottle was plotted against the time of incubation

Inhibition of benzene degradation by TCE was then assessed. Approximately 900 μg each of benzene and TCE were added to 10 mL of a mixed benzene enrichment in a bottle assay and both benzene and TCE degradation were monitored (Figure 6). If all of the benzene and TCE were

dissolved in the aqueous phase their concentrations would be about 100 ppm each. Benzene degradation rates were higher in this experiment, 11.7 µmole per minute per gram protein, with no measurable inhibition of benzene degradation by TCE being observed. Again, no degradation of TCE was observed during the initial time course or following an additional incubation of 24 hours. Although TCE was not degraded by this enrichment of benzene-degradative organisms, TCE exhibited neither inhibitory effects nor any apparent toxic effects on benzene degradation.

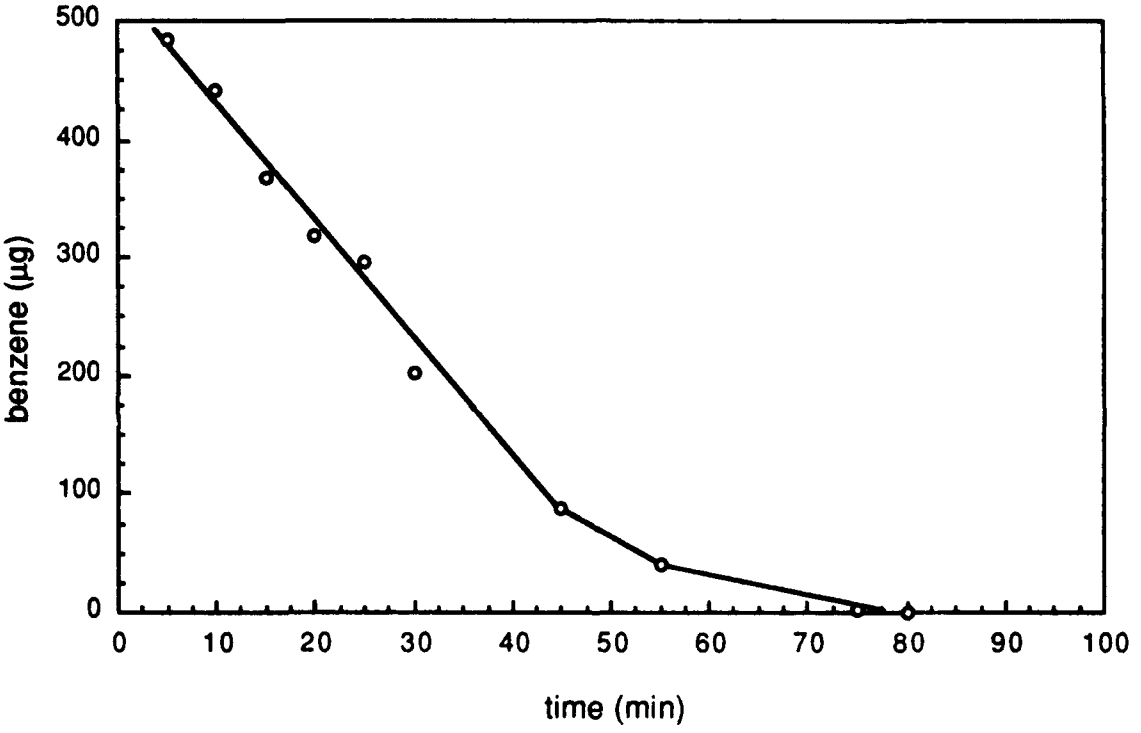

Figure 5. Benzene Degradation by a Mixed Microbial Culture. The standard bottle assay was used to assess benzene degradation using a 10 mL suspension of cells grown with benzene as the sole carbon source at 0.68 mg cell protein/mL. The total amount of benzene in the bottle was plotted against the time of incubation

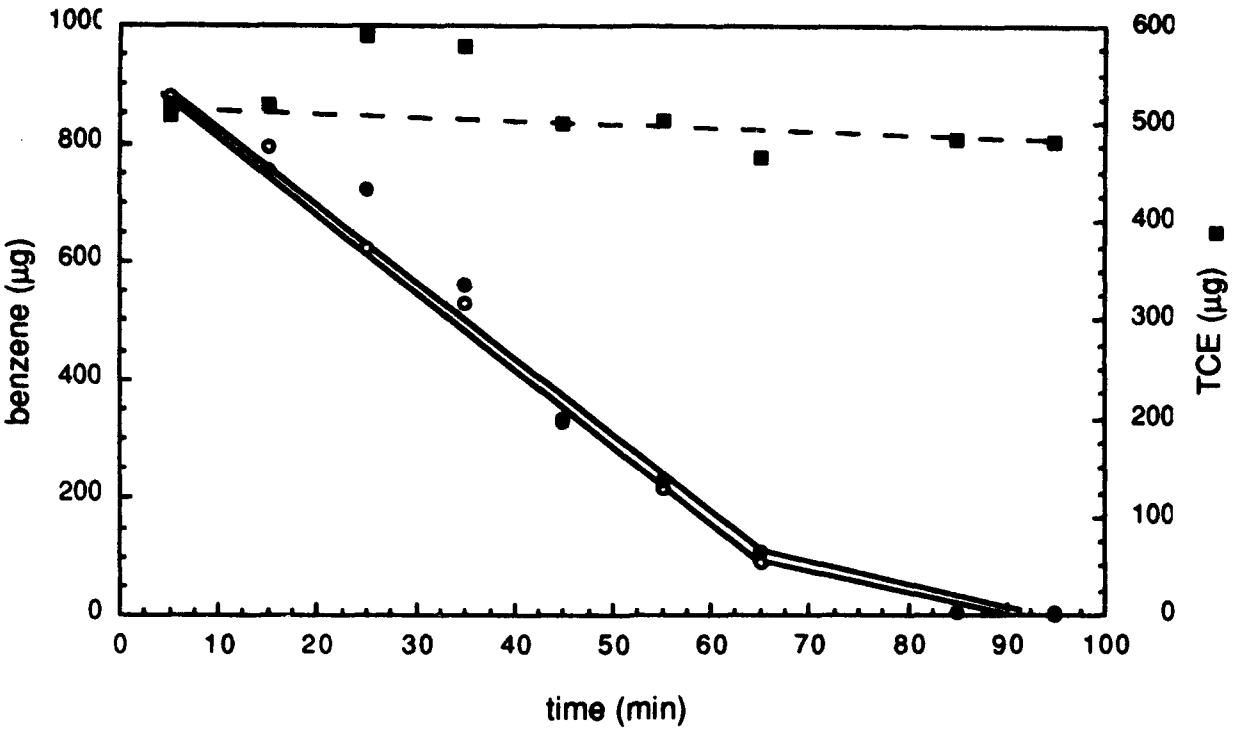

Figure 6. The Effect of TCE on Benzene Degradation. The standard bottle assay was used to assess any inhibitory effects of TCE on benzene degradation using a 10 mL suspension of cells grown with benzene as the sole carbon source at 0.15 mg cell protein/mL. Each of two bottles had 900 µg benzene added and one had 500 µg TCE added. The total amount of benzene in the bottle was plotted against the time of incubation with (●) and without (○) TCE. For the bottle with both benzene and TCE additions, the total amount of TCE (■) was also plotted.

B. FIRST-STAGE REACTOR PERFORMANCE EVALUATION

A fixed-film bioreactor was designed and constructed for the degradation of the benzene component in a mixture of benzene and TCE. This was the first treatment step of a two-stage treatment system with a second-stage unit designed for TCE degradation. A control fixed-film reactor system was constructed and operated to determine abiotic losses of benzene and TCE. Benzene and TCE were added to the reactor and allowed to equilibrate for 48 hours. The reactor contained no packing and had no inflow of either water or air. The reactor was operated with the normal levels of liquid recirculation within the reactor. Following equilibration,

initial aqueous concentrations of benzene and TCE were 68 and 102 ppm, respectively. After a 194-hour incubation, benzene and TCE concentrations dropped to 31 and 33 ppm, respectively. Maximal abiotic losses through joints, fittings, vessel walls and tubing walls for benzene and TCE were 0.19 and 0.36 ppm/hour or about 0.3 percent/hour. The actual mass losses would be proportional to the aqueous concentration and would therefore be less for rapidly degraded components such as benzene in an actively degrading system.

An active fixed-film reactor was constructed using a mixed culture of benzene-degradative organisms as an inoculum. This fixed-film reactor was initially operated for 3 days in a fed-batch mode with benzene to promote acclimation and growth of biomass. The flow of contaminated groundwater was then increased to 15 mL per minute resulting in a hydraulic residence time of 6 hours. Initial operation of this system was with 45 ppm benzene as the sole contaminant with no TCE in the influent stream. Oxygen demand was supplied by flowing air into the reactor at a flow rate of 42 mL per minute. Liquid within the reactor was recycled at 300 mL per minute to provide liquid shear past the biomass entrapped in the packing. This allowed for a more even biomass distribution throughout the reactor and a lower oxygen demand with a single pass through the system. The hydraulic residence time for a single pass through the fixed-film reactor was about 30 minutes whereas the overall hydraulic residence time was about 6 hours. Therefore, on a single pass through the reactor only about 1/12 of the oxygen needed to be supplied on a liquid volume basis. Under these conditions, 91.1 percent of the benzene was biodegraded with 8.4 percent exiting in the air stream and 0.5 percent exiting in the effluent liquid stream. While work on other aspects of the project was ongoing, overall reactor operation was monitored for 8 weeks. During this time, there was evidence of heterogeneity in the biomass within the reactor with apparent anaerobic zones forming from the buildup of solids. Reactor performance remained steady at 90 to 95 percent benzene degradation.

More systematic performance characteristics were developed following this initial phase of operation. The fixed-film reactor was disassembled, cleaned and restarted with biomass from the previous run used as an inoculum. The reactor was initially operated for 11 days under the same conditions as listed above, then the conditions were varied and performance parameters were monitored as indicated (Table 1). The benzene load was increased to determine volumetric performance. As the load increased the percent degradation decreased, although the total amount of benzene degraded increased dramatically. When the source of oxygen was switched from air to pure O_2 the rate and extent of benzene stripping decreased. Over this 4-week period, the fixed-film reactor continued to perform well as a treatment system for benzene.

The next stage of operation included both benzene and TCE in the influent water. Operation was continued with the addition of TCE at average influent groundwater concentrations for benzene and TCE of 36 ppm and 24 ppm respectively. Oxygen was supplied as pure O_2 at 6 mL per minute. Over a 5-week period, reactor performance parameters were monitored (Table 1). On average, >96 percent of the benzene was biodegraded when TCE was present. At the end of 3 weeks of operation on this mixed waste stream, the oxygen supply was switched back to air with only minimal effects on the total degradation of benzene. Differences between TCE influx and efflux varied somewhat over this time period. Following the switch from O_2 to air, greater amounts of TCE were stripped into the air stream although the difference between influx and total efflux was about the same. On average, there was a 28 percent decrease between TCE influx and efflux the system. Though the initial inoculum did not demonstrate TCE degradative activity, either aerobic or anaerobic TCE-degradative organisms may have entered the unit with the groundwater.

At one point during this period of fixed-film reactor operation, performance stability was monitored. Samples of influent water, effluent treated water and effluent gas were collected in triplicate at several times over an 8-hour interval and analyzed for benzene and TCE (Figures 7 and 8).

Table 1: FIRST-STAGE FIXED-FILM BIOREACTOR PERFORMANCE.

day	liquid flow (ml/min)	gas flow (ml/min)	% DO	chemical	liquid influent (mg/L)	liquid effluent (mg/L)	liquid influent) (mg/hr)	liquid effluent (mg/hr)	gas effluent (mg/hr)	decrease (mg/hr)	% decrease
8	17	42 (air)	ND	benzene	44.8 ± 2.2	0.25 ± 0.01	46.4	0.24	3.90	42.3	91.1
11	17	42 (air)	ND	benzene	45.2 ± 4.4	0.30 ± 0.03	46.7	0.30	1.44	45.0	96.3
22	20	47 (air)	21	benzene	72.0 ± 1.4	13.20 ± 1.21	84.3	15.46	7.98	60.9	72.2
23	20	32 (O_2)	20	benzene	152.6 ± 5.6	28.96 ± 1.03	187.7	35.5	12.86	139.3	74.2
29	7.5	7 (O_2)	20	benzene	80.2 ± 0.9	<0.1	36.1	<0.1	0.01	34.0	99.7
30	15	3 (O_2)	2	benzene	15.9 ± 0.2	<0.1	14.3	<0.1	0.01	14.2	99.2
(1)				TCE	8.8 ± 0.1	4.3 ± 0.2	8.0	1.8	0.06	6.1	76.7
34	15	5 (O_2)	18	benzene	39.6 ± 1.4	<0.1	35.6	<0.1	0.02	35.5	99.7
(5)				TCE	23.6 ± 1.5	16.1 ± 0.1	21.2	14.5	0.48	6.2	29.3
40	13	4 (O_2)	18	benzene	34.9 ± 0.8	<0.1	28.1	<0.1	0.01	27.0	99.6
(11)				TCE	18.2 ± 0.7	13.5 ± 0.3	14.6	10.9	0.66	3.0	20.8
42	15	6 (O_2)	24	benzene	62.5 ± 11.1	6.9 ± 1.0	56.2	6.2	0.03	50.0	88.9
(13)				TCE	32.8 ± 7.6	15.9 ± 0.4	29.5	17.6	0.72	11.2	37.9
44	15	7 (O_2)	33	benzene	37.4 ± 4.1	2.4 ± 0.4	34.6	2.1	0.04	32.5	93.8
(15)				TCE	33.2 ± 4.7	25.5 ± 3.1	29.7	22.8	1.02	5.9	19.8
51	15	6 (O_2)	36	benzene	33.6 ± 0.4	1.2 ± 0.1	29.6	1.0	0.04	28.6	96.5
(22)				TCE	24.1 ± 0.5	16.7 ± 0.8	20.7	14.6	1.08	5.0	24.3
55	15	65 (air)	19	benzene	32.7 ± 0.6	0.4 ± 0.1	29.8	0.4	0.46	28.9	97.1
(26)				TCE	23.0 ± 0.5	7.2 ± 0.2	21.0	6.4	20.70	<0	<0
63	15	22 (air)	7	benzene	34.1 ± 1.2	0.4 ± 0.1	30.9	0.4	0.06	30.4	98.5
(32)				TCE	27.6 ± 1.0	11.9 ± 1.8	25.1	10.9	5.46	8.7	34.8

TCE was added to the influent liquid on Day 29 following the reactor performance monitoring. The number in parentheses indicates the time from start of TCE additions. ND indicates information not determined. <0 indicates more mass exiting the reactor than entering giving a negative difference. <0.1 indicates integrated peak area was below detection limits. Standard deviation for 3 replicate samples are indicated by ± values.

There was a dramatic difference between influent and effluent benzene concentrations in the fixed-film reactor. Minimal amounts of benzene exited the reactor in liquid and gas streams. There was an order of magnitude difference between influent and effluent benzene concentrations thereby necessitating the use of a logarithmic Y-axis in Figure 7. In contrast, most of the influent TCE exited the reactor in the effluent water and air with some apparent decreases either by sorption to reactor internal components or through biodegradation (possibly aerobic and/or anaerobic). Although there was some variation in the influent benzene and TCE loads over this 8-hour time interval, overall reactor operation was stable.

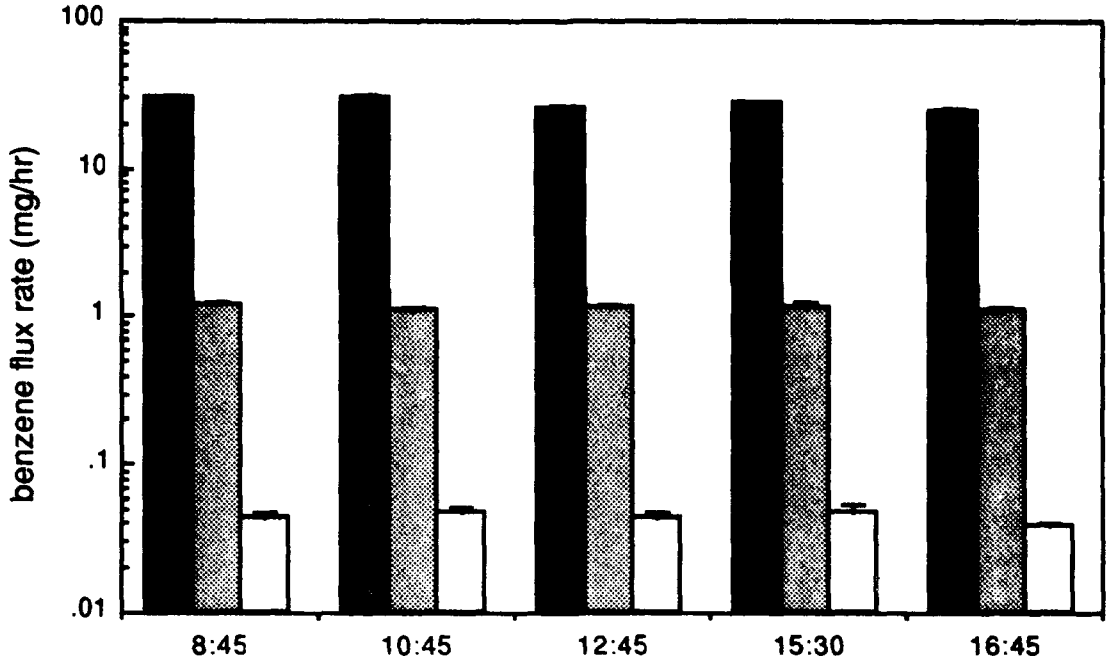

Figure 7. Benzene Fluxes in the Fixed-Film Bioreactor. Triplicate samples of influent water (■), effluent water (▨) and effluent gas (□) were collected and analyzed for benzene levels at intervals over 8 hours. The benzene flux rate expressed in mg/hr were plotted as bars on a logarithmic scale for each stream monitored at the times indicated. Error bars were included for the standard deviation for the replicates.

To verify that decreases in benzene and TCE were a consequence of biological activity, a sample of biomass was removed from the top of the

fixed-film reactor and analyzed for aerobic degradative activity using a bottle assay. Within 4 hours, under aerobic conditions, 50 ppm benzene had been biodegraded below detection limits, whereas TCE levels had not measurably decreased from 50 ppm initial levels, even following a 28-hour incubation. Although TCE does not appear to be biodegraded under aerobic conditions, decreases may still be attributed to uncharacterized anaerobic biological activity. In contrast, decreases in benzene clearly resulted from biodegradative activity. On average, >96 percent of the influent benzene was biodegraded with minimal amounts exiting the reactor in effluent water and air streams.

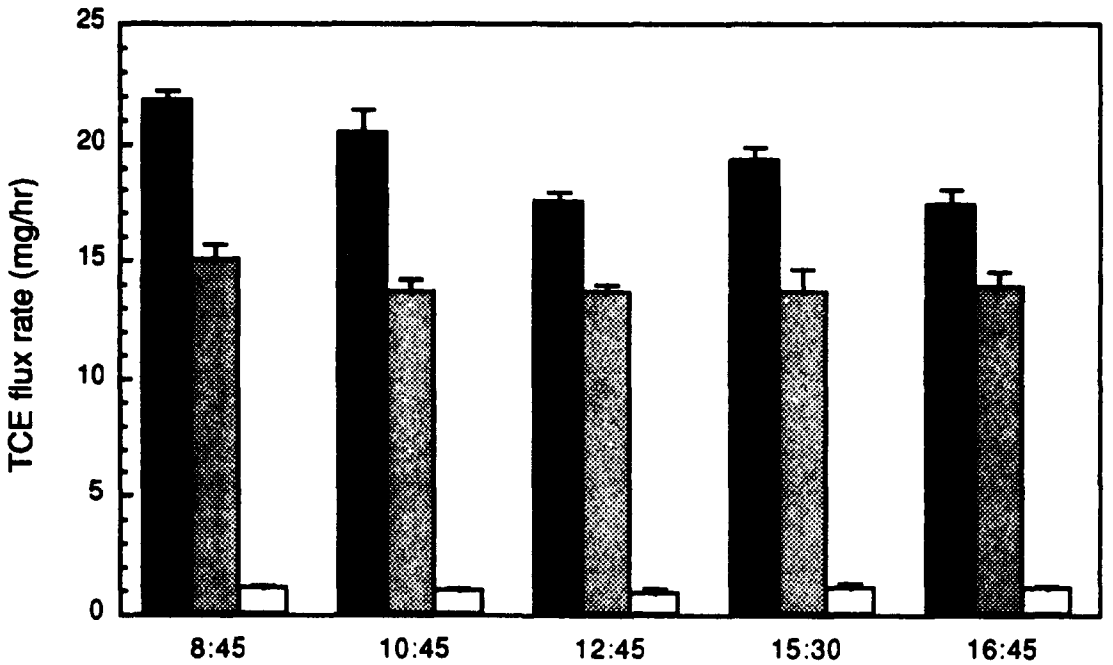

Figure 8. TCE Fluxes the Fixed-Film Bioreactor. The same samples described in Figure 7 were also analyzed for TCE. TCE flux rates expressed in mg/hr were plotted at the times indicated for influent water (■), effluent water (▨) and effluent gas (□) (note this plot does not use a logarithmic scale). Error bars are included for the standard deviation for the replicates.

Buildup of solids within a fixed-film reactor may pose an operational problem at higher organic loadings. Other reactor configurations offer greater levels of process control than does the fixed-film reactor and may

offer advantages in the treatment of high-strength contaminated waters. One alternative first-stage reactor configuration, a suspended-growth reactor, was constructed and operated in parallel to the fixed-film system. Over a 3-week period, various reactor performance parameters were monitored (Table 2). The average influent concentrations of benzene and TCE were 39 ppm and 26 ppm, respectively. Benzene degradation ranged from 86 to 99 percent of the influent benzene with minimal amounts exiting in water and air effluent streams. Most of the influent TCE exited the reactor in the effluent water and air. Abiotic losses of benzene were assumed to be less than those for TCE because the steady-state concentration was lower. TCE acted as a conservative tracer with nearly a complete accounting of the influent TCE appearing either in the effluent liquid or gas. Some variability was observed in the influent concentrations which accounts for some variations observed in the concentrations at various points within the reactor. This was due to the time required for a defined volume of liquid to flow through the reactor operated at a HRT of 6 hours with a HRT of about 2 hours for each chamber. These variations were accentuated in this design in comparison to the fixed-film reactor which had a greater degree of internal mixing due to recirculation. The overall performance of the suspended-growth reactor was comparable to that of the fixed-film system averaging better than 96 percent benzene degradation.

C. SECOND-STAGE REACTOR PERFORMANCE EVALUATION

The second-stage vapor-phase bioreactor was designed for the biodegradation of TCE. *P. cepacia* G4 was used as the active biomass in the vapor-phase TCE reactor. Abiotic controls and unreactive chemicals tracers were used to determine physical losses from the system. These experiments demonstrated that equilibrium was reached within 10 hours of operation with 90 to 95 percent recovery of the influent chemicals. A mixture of TCE and benzene was prepared at a ratio of 10 to 1 which

Table 2: FIRST-STAGE SUSPENDED-GROWTH BIOREACTOR PERFORMANCE.

day	chemical	liquid influent (mg/L)	C1 (mg/L)	C2 (mg/L)	liquid effluent (mg/L)	liquid influent (mg/hr)	liquid effluent (mg/hr)	gas[1] effluent (mg/hr)	%[2] decrease C1	% decrease total
1	benzene	34.2 ± 0.8	4.9 ± 0.6	4.6 ± 0.1	4.7 ± 0.7	36.9	4.2	0.72	75.0	86.7
	TCE	21.2 ± 0.5	13.3 ± 0.7	11.1 ± 0.1	10.1 ± 0.5	22.9	9.1	10.58	9.0	14.1
2	benzene	42.4 ± 0.8	1.0 ± 0.3	0.1 ± 0.1	<0.1[3]	45.3	<0.1	0.09	95.4	99.6
	TCE	24.3 ± 0.4	17.8 ± 0.2	15.7 ± 0.5	14.0 ± 0.1	26.0	15.1	6.08	1.4	18.5
4	benzene	39.8 ± 8.7	1.2 ± 0.2	1.0 ± 0.1	0.8 ± 0.1	33.4	0.7	0.03	93.9	97.8
	TCE	30.4 ± 4.6	25.4 ± 3.6	24.8 ± 3.5	26.8 ± 4.5	25.6	22.5	6.25	4.8	<0[4]
11	benzene	39.4 ± 1.7	3.2 ± 0.2	0.8 ± 0.1	0.6 ± 0.1	29.8	0.6	0.21	83.9	97.3
	TCE	27.6 ± 1.2	19.6 ± 1.6	17.3 ± 0.9	16.7 ± 1.2	21.0	16.0	10.43	0	<0[4]

The dissolved oxygen level was greater than 20 percent throughout the reactor on all days monitored. Liquid flow rates varied between 14 and 18 ml/min. Concentrations are average of triplicate extractions. Standard deviation for 3 replicate samples are indicated by ± values.

1 - total gas effluent from CSTR1 and CSTR2.

2 - Calculated for mass fluxes through CSTR1 only.

3 - below detectable limits.

4 - more TCE exiting system than entering as discussed in the conclusions.

reflected a worst-case situation for benzene degradation in the first-stage system. Some benzene carry over into the second-stage unit was to be expected, so it was added to determine effects on the performance of the second-stage unit. TCE fluxes entering and exiting the reactor in the air stream were monitored continuously over a 5 day period (Figure 9). Over this time period, greater than 90 percent of the TCE was continuously degraded. On Days 1, 4 and 6, separate analyses were performed to quantify TCE and benzene concentrations in the influent and effluent gas (Table 3). Both the TCE within this system and the "residual" benzene were degraded. This confirms earlier experiments in which induced TCE-degrading organisms were capable of benzene metabolism. The overall performance of the vapor-phase TCE bioreactor averaged >90 percent degradation of both TCE and benzene.

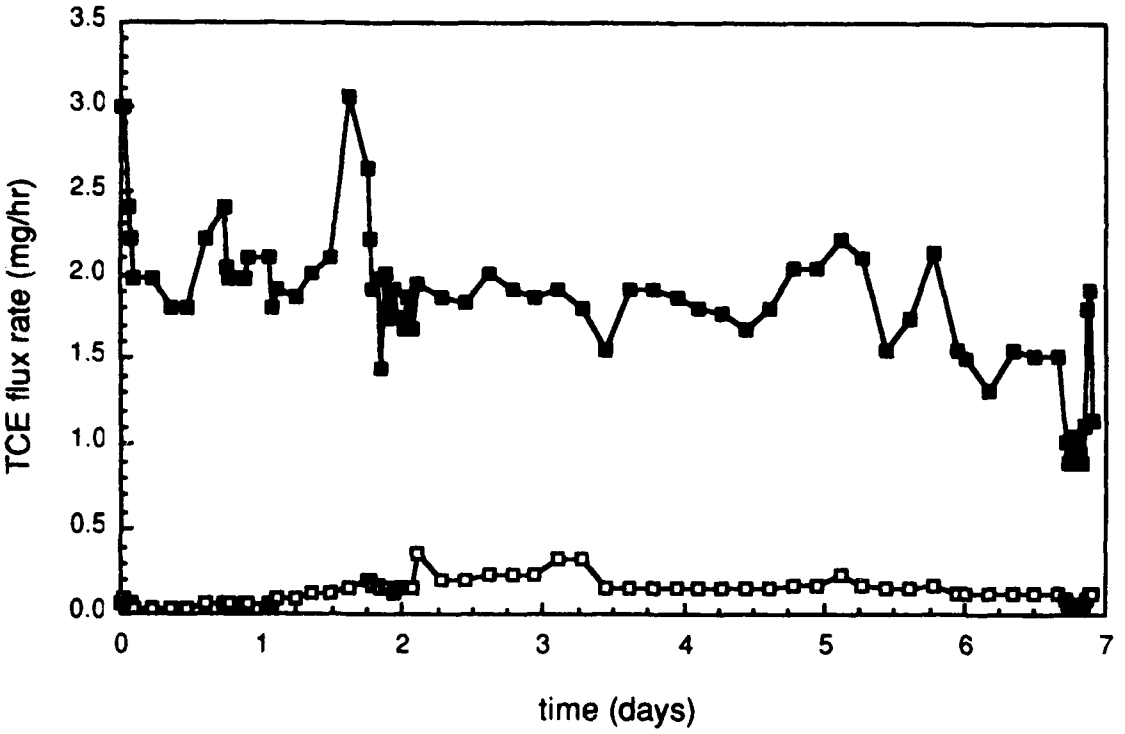

Figure 9. TCE Fluxes in the Vapor-Phase Reactor. TCE concentrations were monitored continuously by direct sampling and analysis using a GC. TCE influx (■) and efflux (□) rates were calculated as mg/hr and plotted against time of operation.

Table 3 SECOND-STAGE VAPOR-PHASE BIOREACTOR PERFORMANCE.

day	influent TCE (mg/hr)	effluent TCE (mg/hr)	% decrease	influent benzene (mg/hr)	effluent benzene (mg/hr)	% decrease
1	1.80 ± 0.26	0.35 ± 0.04	81	0.19 ± 0.03	0.033 ± 0.013	83
4	2.12 ± 0.02	0.26 ± 0.02	88	0.29 ± 0.03	0.002 ± 0.001	99
6	2.17 ± 0.06	0.21 ± 0.01	90	0.27 ± 0.01	0.003 ± 0.001	99

SECTION IV:

CONCLUSIONS

The objective of this project was to develop a novel biological treatment system to mineralize a mixture of nonchlorinated (benzene) and chlorinated (TCE) organic chemicals in groundwater pumped from contaminated aquifers. Mineralization of many classes of nonchlorinated organic chemicals by microorganisms is well established, with biological destruction offering a convenient, low-cost, reliable method for effective treatment. Systems are currently available for biological treatment of common nonchlorinated organic chemicals such as those found in gasoline or jet fuel. The presence of TCE in the contaminated stream will impact both the design and performance of systems designed exclusively for nonchlorinated organic mineralization. The novelty and difficulty of this project centers on the mineralization of chlorinated organic chemicals, such as TCE, while maintaining biological activity against the more easily degradable nonchlorinated organic chemicals.

The overall project goal was to design and operate a bench-scale treatment system for the mineralization of both benzene and TCE. The initial investigation focused on interactions between benzene and TCE identifying possible effects on growth of organisms and degradation of the target chemicals. Three strains of TCE-degradative organisms failed to grow with benzene as the sole carbon source although benzene was degraded by these microorganisms if the requisite enzymes had been induced following growth with toluene. These results are consistent with the observations that a constitutive mutant of *P. cepacia* G4 can grow on benzene (M. Shields, personal communication). Although a mixed population of benzene-degradative organisms failed to degrade TCE, TCE did not inhibit benzene degradation. These observations suggested that at least two types of microorganisms were required for simultaneous benzene and TCE degradation.

Given the overall objective of this project, biotreatment of chlorinated and nonchlorinated organic chemicals, a two-stage treatment system was designed, constructed and tested. The first-stage treats the readily metabolizable, nonchlorinated organic chemicals, such as benzene. The second-stage targets the more recalcitrant, chlorinated organic chemicals such as TCE. TCE degradation requires a higher level of process control and the maintenance of specialized degradative strains. These requirements, in turn, dictate that the second unit will be more complex to build and operate. This modular system (Figure 10) offers several advantages over a single-stage system. The first-stage unit can be one of several classical bioreactor designs available, selected to match the characteristics of the contaminated aquifer to be treated. If no chlorinated organic chemicals are present, this unit can function separately. Several criteria useful in selection of the specific design to be implemented include organic load, hydraulic load, chemical composition of target organic chemicals and other water characteristics such as iron or suspended solids which can impact operation. The second-stage, can also function independently if nonchlorinated organic chemicals are absent.

Two basic reactor configurations were evaluated for the first-stage benzene treatment unit. Three key issues were addressed with the bench-scale reactors: (1) extent of benzene biodegradation, (2) extent of TCE degradation, with complete mass balances for both contaminants, and (3) impact of TCE on first-stage unit operation and impact of benzene on second-stage unit operation. Two units were used to assess first-stage operations. A fixed-film reactor was used as the primary design tested and a suspended-growth reactor provided additional process information. The effect of gas flow on stripping was evaluated in conjunction with use of either air or O_2 as the supply of oxygen to the reactor. A vapor-phase unit was used to assess second-stage operations.

Benzene was effectively degraded within a fixed-film reactor with no apparent negative effects when TCE was present in the influent water. At influent benzene concentrations between 30 and 50 ppm, greater than 96

percent of the benzene was biodegraded on average over a 63-day period. At higher loadings, 50 to 150 ppm, performance dropped to a low of 72 percent degradation but the total amount of benzene degraded increased significantly from an average of 34 mg/hour to 139 mg/hour. Decreased efficiency with increased volumetric performance suggests that benzene degradation is concentration-dependent. A multistage system may be required to reduce the effluent benzene concentrations, although the second-stage reactor can function to polish the benzene.

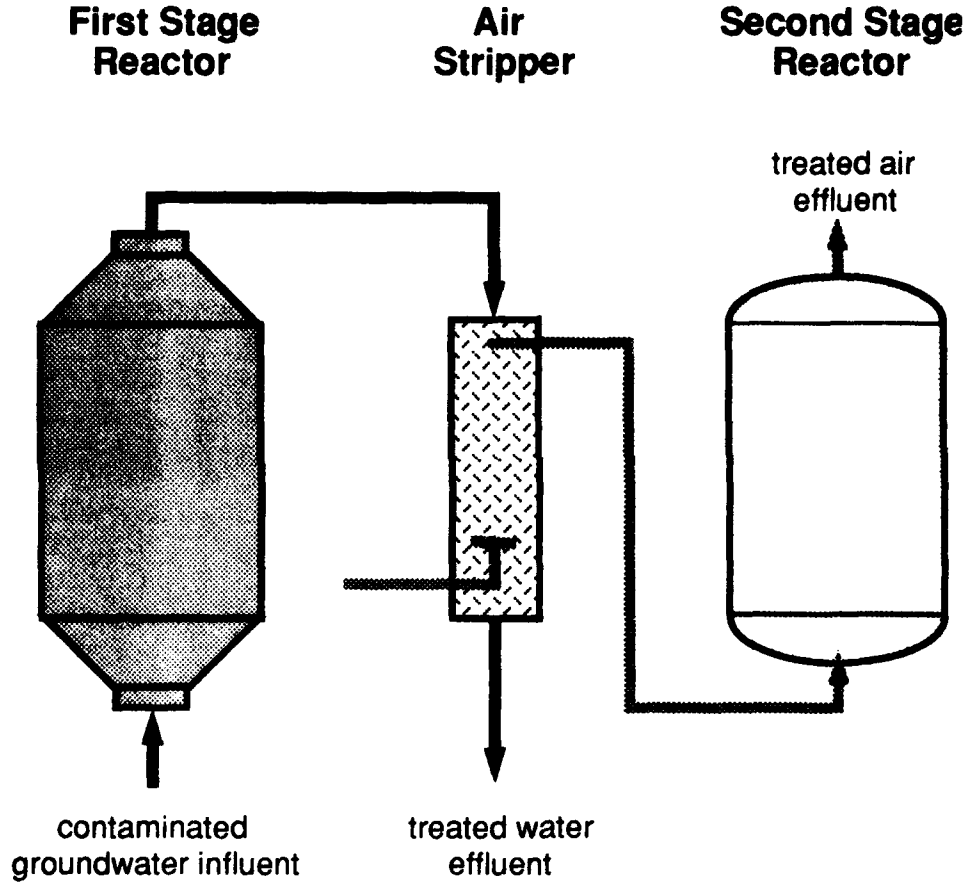

Figure 10. Diagram of the Two-Stage Biotreatment System.

Another aspect of performance evaluation concerned volatile organic stripping rates. Although stripping of volatiles did increase with increased gas flow rates, the effects on benzene degradation efficiency were minimal. Greater effects were observed for the more recalcitrant component, TCE. Although the apparent decrease in TCE flowing through the reactor

remained essentially unchanged at about 6 mg/hour, more of the TCE exited the reactor through the offgas when higher gas flow rates were used. Minimal amounts of gas should be supplied to provide oxygen for activity to minimize stripping, with air affording adequate performance and simple operation.

There was a relatively constant decrease in TCE between influent and effluent flows with the fixed-film reactor. Abiotic loss experiments clearly established that maximal losses of TCE under these conditions from this reactor should not exceed 1 mg/hour. Over the first day of operation, there was a 77 percent decrease which was likely due to initial sorption to biomass and plastic within the reactor. Following the switch from O_2 to air on Day 25, there was a greater amount of TCE exiting the reactor than entering. This was likely due to desorption of TCE from biomass and plastic following a shift in equilibrium conditions. Typically, there was a decrease in TCE of about 6 mg/hour. Bottle assays performed under strict aerobic conditions failed to demonstrate any significant TCE degradation by biomass harvested from the reactor. This was consistent with initial studies using benzene-degradative organisms. Another explanation is that there are anaerobic zones within the biofilm layers of the reactor which could possess dechlorination activity. Identification of this type of activity could be the focus of further investigation.

A suspended-growth reactor was also operated as a first-stage unit for benzene degradation. This reactor models an activated sludge process. Performance of this unit was comparable to that of the fixed-film reactor with greater than 96 percent benzene degradation. This unit demonstrated insignificant decreases in TCE which would be consistent with the hypothesis that decreases observed with the fixed-film unit may originate with anaerobic dechlorination. A CSTR would be well aerated with a lower likelihood than the fixed-film reactor of having anaerobic biofilm zones. The benefits of staging reactors could be seen from this study whereby decreases in first-chamber performance was compensated by the second-stage operation leading to a high overall performance.

There were some anomalous results with this system due to the flow-through configuration and multistage operation. Samples were collected from each of the three vessels (CSTR1, CSTR2 and CL) at the same time. The residence time in each chamber was about 1 hour so a pulse of higher concentration would show up in the second chamber after about 1 hour later. If there had been any decrease in influent concentrations of TCE during the 2 to 3 hours preceding sampling, there would be a greater concentration in CSTR 2 and/or CL than in CSTR1. This was observed on Day 4 of the suspended-growth reactor operation. This behavior would only be observed for recalcitrant chemicals and not for degradable components.

The second-stage unit was designed for TCE degradation. This vapor-phase reactor has been under development with significant evaluation of operation with TCE as the sole contaminant already completed. This study augmented this previous work by treating a mixture of benzene and TCE. A mixture of benzene and TCE (1:10 mass ratio) was fed to the vapor-phase unit to evaluate inhibitory effects and operational performance. This mixture was used to model the effluent from a poorly performing first-stage unit. Under these conditions, about 90 percent of the TCE and up to 99 percent of the benzene loads were degraded continuously over a 6-day operating period. There was no significant inhibition of TCE degradation by benzene observed under these conditions. Other volatile aromatic contaminants, such as toluene, ethylbenzene and xylene(s), would likely be degraded in this vapor-phase TCE reactor since they all are degradable by induced or constitutive *P. cepacia* G4 (M. Shields, personal communication). By this point in the treatment process, essentially all of the degradable and volatile components have been reduced in the liquid effluent stream. Minimal amounts of unreacted volatile chemicals exiting the second-stage unit could be passed over activated carbon as a final polishing step to prevent fugitive emissions for the system.

SECTION V:

RECOMMENDATIONS

Selection of an individual reactor configuration depends on the characteristics of the contaminated groundwater. If the actual contaminated groundwater has an organic loading below about 20 ppm, then a fixed-film reactor may be optimal due to simplicity of construction and operation. At higher organic loadings, alternative designs such as suspended-growth or fluidized-bed reactors with higher levels of process control may be better suited designs with comparable overall efficiencies. A suspended-growth system may also offer higher volumetric performance capabilities if the amount of suspended solids can be kept high. Other influent characteristics such as suspended solids and dissolved metals would impact selection of a specific first-stage treatment unit. Treatment of a more complex mixture of nonchlorinated organic chemicals may also suggest the use of one reactor type over another. Further studies could evaluate the performance of bench-scale units treating authentically contaminated groundwater or using gasoline or jet fuel instead of benzene to approximate more typical groundwater contamination. This type of study with more complex mixtures of chemicals would help in assessment of the optimal design for a pilot-scale treatment system.

Further work could focus on several technical issues. (1) Are the observed decreases of TCE in the fixed-film reactor due to anaerobic dehalogenation and can this process be increased? An anaerobic dechlorination system may expand the capability of this remediation process. (2) Expand the range of chemicals, both nonchlorinated and chlorinated, in the mixture entering the bench-scale units to more closely model actual contaminated sites. (3). Use contaminated groundwater from a site to be remediated to evaluate performance and system design modifications based on actual conditions. 4) Design and operate a pilot-scale treatment system for remediation of a contaminated aquifer.

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