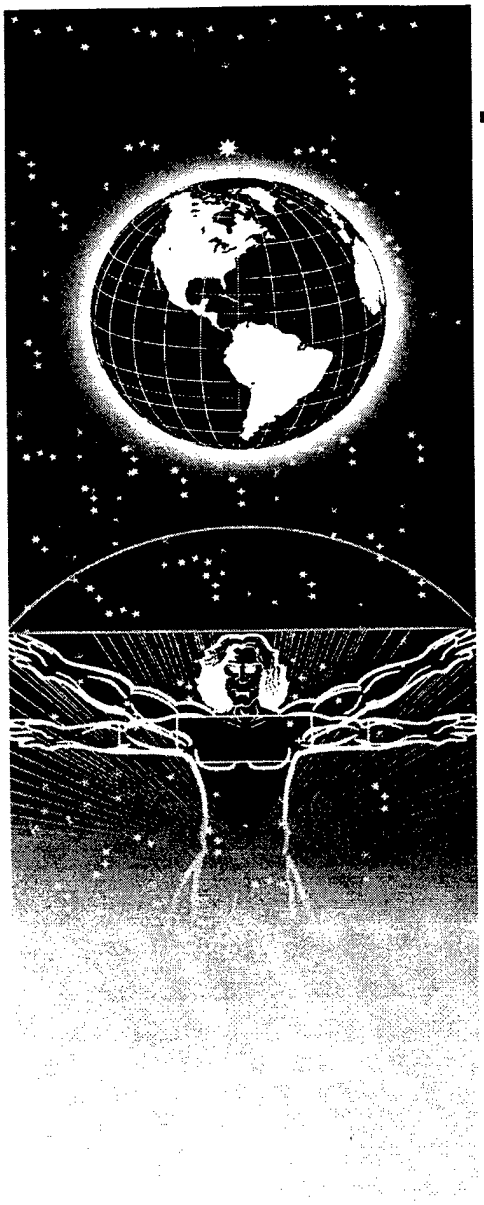
AL/EQ-TR-1993-0008



UNITED STATES AIR FORCE ARMSTRONG LABORATORY

Chlorobenzene Bioreactor Demonstration

Dennis Miller, Dr. Jim Spain, Dr. William Wallace, and Catherine Vogel

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
P. O. BOX 1198, Ada, Oklahoma 74820

ARMSTRONG LABORATORY ENVIRONICS DIRECTORATE (AL/EQ)
139 Barnes Drive, Suite 2
Tyndall Air Force Base, Florida 32403-5323

April 1997

Approved for public release; distribution is unlimited.

Environics Directorate
Environmental Risk Management Division
139 Barnes Drive
Tyndall Air Force Base FL
32403-5323

NOTICES

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency thereof, nor any employees, nor any of their contractors, subcontractors, nor their employees, make any warranty, expressed or implied, or assume any legal liability or responsibility for the accuracy, completeness, or usefulness or any privately owned rights. Reference herein to any specific commercial process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency, contractor, or subcontractor thereof. The views and opinions of the authors expressed herein do not necessarily state or reflect those of the United States Government or any agency, contractor, or subcontractor thereof.

When Government drawings, specifications, or other data are used for any purpose other than in connection with a definitely Government-related procurement, the United States Government incurs no responsibility or any obligations, whatsoever. The fact that the Government may have formulated or in any way supplied the said drawings, specifications, or other data, is not to be regarded by implication, or otherwise in any manner construed, as licensing the holder or any other person or corporation; or as conveying any rights or permission to manufacture, use, or sell any patented invention that may in any way be related thereto.

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

This report has been reviewed and is approved for publication.

CATHERINE M. VOGEL, P.E.
Biotechnology Technical Area Manager

ALLAN M. WEINER, LtCol, USAF
Chief, Risk Management

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	March 1997	Final Report Mar 92 - Nov 92

4. TITLE AND SUBTITLE
Chlorobenzene Bioreactor Demonstration

5. FUNDING NUMBERS
MIPR-N92-16

6. AUTHOR(S)
Dennis Miller, Dr. Jim Spain, Dr. William Wallace, Catherine Vogel

7. PERFORMING ORGANIZATION NAMES(S) AND ADDRESS(ES)
United States Environmental Protection Agency (USEPA), P. O. Box 1198, Ada, OK 74820
AL/EQM-OL, 139 Barnes Dr, Suite 2, Tyndall AFB, FL, 32403-5323

8. PERFORMING ORGANIZATION REPORT NUMBER
RW57935105-01-1

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
AL/EQM-OL
139 Barnes Drive, Suite 2
Tyndall AFB FL 32403-5323

10. SPONSORING/MONITORING AGENCY REPORT NUMBER
Final Report
AL/EQ 1993-0008

11. SUPPLEMENTARY NOTES
Availability of this report is specified on reverse of front cover. AL/EQM Project Officer: Catherine M Vogel (904) 283-6208, DSN 523-6208

12a. DISTRIBUTION/AVAILABILITY STATEMENT
This technical report has been reviewed by the Public Affairs Office (PA) and is releasable to the National Technical Information Service (NTIS) where it will be available to the general public, including foreign nationals.

12b. DISTRIBUTION CODE
B

13. ABSTRACT (Maximum 200 words)
The technical objective of this research was to test whether above-ground, fixed-film bioreactors can biodegrade complex mixtures of chlorinated aromatic compounds in groundwater. Specifically, the project was designed to test the metabolic capabilities of *Pseudomonas* Strain JS150 in the field. The approach involved a preliminary field study at Robins AFB GA using two above-ground, fixed-film bioreactors, one colonized with *Pseudomonas* JS150 and one colonized with indigenous groundwater bacteria, to measure degradation of chlorinated aromatic compounds. Independent variables measured included temperature, pH, dissolved oxygen, and fluctuations in microbial populations. Results from the bench-scale column study indicated that the JS150 isolate had the greatest number of desirable qualities and would be the best selection to utilize in a biofilm reactor. Results from the study indicate that both reactors provided substantial chlorobenzene removal (greater than 95%). Data indicate that bioreactor inoculation may be useful for reducing startup time. No degradation intermediates were detected from either the inoculated or uninoculated reactors.

14. SUBJECT TERMS
Bioremediation, fixed-film, bioreactor, chlorinated aromatic compounds, *Pseudomonas* Strain JS150, chlorobenzene, ex situ

15. NUMBER OF PAGES
52

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF REPORT	19. SECURITY CLASSIFICATION OF REPORT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	UL

(The reverse of this page is blank.)

PREFACE

This report was prepared by the USEPA, P.O. Box 1198, Ada, OK 74820, USAF MIPR No. MIPR-N92-16, for the Armstrong Laboratory Environics Directorate (AL/EQ), Suite 2, 139 Barnes Drive, Tyndall Air Force Base, Florida 32403-5319.

This technical objective of the research described in this final report was to test whether above-ground, fixed-film bioreactors can biodegrade complex mixtures of chlorinated aromatic compounds in groundwater. Specifically, the project was designed to test the metabolic capabilities of *Pseudomonas* Strain JS150 in the field. The approach involved a preliminary field study at Robins AFB GA using two above ground, fixed-film bioreactors, one colonized with *Pseudomonas* JS150 and one colonized with indigenous groundwater bacteria to measure degradation of chlorinated aromatic compounds. Independent variables measured included temperature, pH, dissolved oxygen, and fluctuations in microbial populations. Results from the bench-scale column study indicated that the JS150 isolate had the greatest number of desirable qualities and would be the best selection to utilize in a biofilm reactor. Results from the study indicate that both reactors provided substantial chlorobenzene removal (greater than 95%). Data indicate that bioreactor inoculation may be useful for reducing startup time. No degradation intermediates were detected from either the inoculated or uninoculated reactors.

The AL/EQM project officer was Catherine M. Vogel.

(The reverse of this page is blank.)

EXECUTIVE SUMMARY

A. OBJECTIVE

The purpose of this field study is to demonstrate the ability of a biological treatment system to remediate ground water contaminated with chlorobenzene.

B. BACKGROUND

Due to widespread industrial use, halogenated solvents are frequently released into the environment and become sources of ground water contamination. Often, an engineered remediation system will employ some form of pump-and-treat technology which may include air-stripping and carbon adsorption to treat the withdrawn ground water. Because many types of halogenated compounds are resistant to microbial transformation, biological treatment is often overlooked when developing treatment system options. This is unfortunate because some of the commonly used solvents, such as chlorobenzene, are amenable to bioremediation. Reduced cleanup costs may be realized with bioremediation because biological treatment has the potential to provide complete destruction of pollutants at a fraction of the cost of the other options.

C. SCOPE

This report discusses the three objectives of this project: inoculum selection, field performance evaluation of pilot-scale reactor, and biomass development. Section I provides an overview of the background material and description of the field site. Section II discusses the initial bench-scale packed-column experiments which were conducted to select the most suitable microbial strain for use in the pilot reactor. The pilot-scale reactor and field study evaluation is described in Section III. Section IV discusses the microbiology monitoring conducted throughout the field study evaluation. Section V lists study conclusions, and Section VI provides recommendations.

D. METHODOLOGY

Bench-scale packed-columns were utilized to evaluate four microbial strains of chlorobenzene degraders to compare their ability to colonize the packing material and overall chlorobenzene removal. One isolate was then selected for field trials as the inoculum in a pilot-scale reactor. The pilot study examined the ability of a biological system to aerobically treat chlorobenzene-contaminated site water under actual field conditions at Robins AFB, GA. Treatability of secondary contaminants and influence of pH and dissolved oxygen (DO) on the removal efficiency were evaluated. Biomass growth and displacement of the inoculated microbial strain were monitored throughout the field evaluation.

E. TEST DESCRIPTION

The strains employed for the bench-scale evaluation include *Pseudomonas* sp. strains JS150, 1604, 1627B, and 1640B. Chlorobenzene concentrations and solution pH were monitored to evaluate microbial colonization of the packing material. Flow rate, inlet concentration, and rate of air addition were varied to compare the removal efficiencies of four

microbial strains. Based on the results of the bench-scale evaluation, one microbial strain was selected as the inoculum in a pilot-scale reactor.

For the field study, two 120 liter, up-flow, packed-bed reactors were operated in parallel to allow comparison of the removal efficiencies of the indigenous population of ground water microbes and the selected inoculant. Flow rate, DO, pH, and contaminant concentrations were monitored throughout the course of the field trial. In addition to the primary contaminant, secondary contaminant concentrations of benzene and *m*-dichlorobenzene were monitored.

Monitoring of the microbial makeup of the reactors was accomplished by periodically shutting down the column operation to allow aseptic sampling of the packing material. A fixed amount of the packing material was plated on agar spread plates to determine microbial population, chlorobenzene utilizer population, and presence of *Pseudomonas* sp. strain JS150.

F. RESULTS

Bench-scale results showed a single inoculation of the *Pseudomonas* sp. strain JS150 isolate resulted in significant chlorobenzene removal within eight days. The three *Pseudomonas* sp. strains from Kelly, AFB (1604, 1627B, and 1640B) required multiple inoculations and did not develop significant chlorobenzene removal ability for at least 12 days. All microbial strains provided >95% chlorobenzene removal when the columns were operated with empty bed residence times (EBRT) > 30 min. At higher flow rates, increasing the loading rate decreased the removal efficiency for each microbial strain except *Pseudomonas* sp. strain JS150 which remained at >80%. At the higher loading, the column inoculated with *Pseudomonas* sp. strain JS150 exhibited a utilization rate almost 50% greater than the rate observed for the other three columns. Based on the rate of colonization and utilization capacity, *Pseudomonas* sp. strain JS150 was selected as the reactor inoculum for the pilot-scale field trial.

Results from the field study showed the inoculated reactor exhibited immediate chlorobenzene removal capacity of >95%. The uninoculated reactor required eight days of operation before equaling the removal capacity of the inoculated reactor. At a 45-min EBRT flow rate, the inoculated and uninoculated reactors displayed steady-state removal efficiencies of 86% and 83%, respectively, when inlet pH was maintained between 6.8 and 7.2. The outlet DO was > 4.0 mg O_2/L. During the specified operating conditions, the corresponding outlet concentrations averaged 1.31 ppm chlorobenzene in the inoculated reactor and 1.59 ppm chlorobenzene in the uninoculated reactor.

Microbial monitoring demonstrated the top port of the uninoculated reactor originally contained no chlorobenzene degraders at Time 0. Throughout the first 16 days of operation an increase in chlorobenzene degraders was observed at both top and bottom ports. By Day 16, the numbers of chlorobenzene degraders had risen to 100% of bacteria present. In the inoculated reactor, the top port contained 1.0×10^6 and the bottom port contained 4.47×10^5 chlorobenzene degraders per gram of pellets at Time 0. The number of chlorobenzene degraders increased throughout the reactor during the first 16 days of operation. By Day 16, the indigenous chlorobenzene degraders had increased to make up 37% and 47% of the population in the top and bottom ports, respectively. *Pseudomonas* sp. strain JS150 was not detected after Day 16, but the overall chlorobenzene-degrading population increased for the top port and declined at the bottom port by Day 36.

G. CONCLUSIONS

Results of the laboratory- and pilot-scale studies indicate chlorobenzene can be successfully remediated with biological treatment. The *Pseudomonas* sp. strain JS150 was an effective inoculant for biological reactor systems for the treatment of chlorobenzene-contaminated ground water. The *Pseudomonas* sp. strain JS150 readily populates the Manville Celite support material, and, once established, the *Pseudomonas* sp. strain JS150 displays tolerance and rapid recovery to temporal pH shifts. The DO content is directly related to the chlorobenzene utilization rate and becomes limiting when effluent concentrations fall below the 4.0 to 4.5 mg O_2/L range. Use of the caustic buffer solution to adjust solution pH limited the maximum removal efficiency which could be attained in the field study. Inoculating a reactor with a selected strain provides the benefits of immediate and rapid contaminant removal ability. This suggests that inoculation may be useful for reducing start-up time for reactors in the field.

H. RECOMMENDATIONS

The presence of high iron concentrations within the ground water at the Robins AFB site possibly precludes the use of any *in-situ* aerobic treatment option and may limit treatment schemes to above-ground systems. Biological treatment can effectively reduce chlorobenzene concentrations and should be considered as a possible treatment option. Inoculation of the reactor prior to use with an isolate like the *Pseudomonas* sp. strain JS150 is encouraged in order to provide immediate chlorobenzene treatment capacity and minimize startup time at the site. A less caustic buffering solution should be employed to encourage more biomass growth and enhance the removal efficiency. Additionally, utilization of the recycle stream during the flow-through operation would provide greater resistance to upsets. Complete removal of the secondary contaminants could not be achieved with this particular packed bed reactor configuration and further treatment will be required (especially for benzene). A second treatment process, such as activated carbon, is needed to reduce the benzene concentrations to acceptable levels. However, use of a bioreactor as a pretreatment could result in at least an order of magnitude reduction in the contaminant loading onto the carbon and greatly reduce the carbon usage rate. Alternatively, use of a fluidized-bed or expanded-bed reactor may provide higher treatment capacities and also be resistant to system upsets. Further study would be required to determine whether a different reactor configuration would eliminate the need for a secondary treatment system, such as an activated carbon unit. An overall economic analysis would be required to properly size a minimum cost treatment system.

(The reverse of this page is blank)

TABLE OF CONTENTS

TABLE OF CONTENTS
(continued)

LIST OF FIGURES

LIST OF TABLES

SECTION I

INTRODUCTION

A. OBJECTIVE

The objective of this research project was to evaluate the potential of biological treatment in the remediation of chlorobenzene-contaminated ground water. Bench-scale column experiments were conducted to select a microbial strain most suitable to biofilm formation and removal of chlorobenzene. Once microbe selection was completed, a pilot-scale reactor was prepared and transported to a selected field location for a series of field evaluations. The removal efficiency of the inoculated reactor was compared to the removal efficiency obtained from a reactor populated with an indigenous population. Biomass growth and displacement of the inoculated microbial strain were monitored throughout the field evaluation.

B. BACKGROUND INFORMATION

Chlorobenzene is a commonly used industrial solvent and is used in the production of other chemicals. Primary uses of chlorobenzene include: solvent for pesticide formulations, manufacture of diisocyanate, diphenyl oxide, and phenylphenol; intermediate in the synthesis of other halogenated organics; and degreasing automobile parts (1). Due to its common usage, there will inevitably be soil and ground water contamination in areas where these compounds are manufactured, stored, used, and disposed. Chlorobenzene has been identified as one of the most commonly occurring contaminants at National Priorities List hazardous waste sites (1).

Contacting contaminated ground water with activated carbon is a common remediation technique for organic contaminants. Unfortunately, activated carbon systems are typically expensive and merely shift the contaminant burden onto the carbon. The spent carbon must be managed, either by incineration or storage in a waste disposal facility. Biological removal is one treatment alternative which can destroy the contaminant rather than transferring the compound to another medium. Low concentrations of substituted benzenes are effectively treated by wastewater treatment facilities (2,3), but high loading rates can result in low removal efficiencies for the haloaromatic compounds (4,5). Various studies have reported the ability of isolated microorganisms to utilize aromatic and haloaromatic compounds as sole carbon sources (6 - 14). Efficient biological removal of haloaromatic contaminants at high loading rates may be possible if the microbe population includes selected bacteria capable of mineralizing haloaromatic compounds. Field studies have displayed successful removal of other halocarbon compounds with both selected isolates and indigenous mixed populations (15-18). Targeting the population to the contaminant is reported to be more efficient at contaminant removal than the traditional activated sludge systems of industrial wastewater treatment (19). Targeting a biological treatment process to specifically remove chlorobenzene may result in an efficient and economical treatment system.

C. SITE DESCRIPTION

Robins AFB, Warner Robins, GA, was chosen as a suitable location for the field study. An area of ground water contaminated with solvents and heavy metals was identified on a 40 acre tract within the base boundary. The identified area was once the site of a Fire Protection Training Area, a landfill, and a laboratory chemical disposal area. The training activities and waste disposal practices were eventually discontinued, and the area was reclaimed for recreational activities. Soils within this area are characterized as relatively heterogeneous with discontinuous regions of interlayer sand, silt, clayey sand, and clay. The surface soil in the area of contamination is composed of a sand to silty sand layer which varies in thickness from 3 to 6 meters. The aquifer lies within this surface soil unit, and the water table varies from 1.5 to 3 meters below the surface. A large clay unit underlies this area and defines the lower boundary of the aquifer. A Resource Conservation and Recovery Act (RCRA) Facility Investigation report (20) revealed the presence of a plume of chlorobenzene within the water table aquifer. The plume was separate from the heavy metal contamination identified in the area, and secondary organic contaminants were present in negligible amounts as compared to the chlorobenzene concentrations. A well was installed within the area of highest contaminant concentration to provide the source water for this field study. Figure 1 shows the location of the extraction well with respect to the area monitoring wells (20). Analysis of this well water confirmed chlorobenzene was the major contaminant, (approximately 10 ppm), with trace amounts of benzene, o-xylene, toluene, and dichlorobenzenes. Table 1 summarizes the characteristic parameters of the site waters.

TABLE 1 INLET WATER PARAMETERS

Parameter	Value	EPA Drinking Water Standard Maximum Contaminant Level
Dissolved Oxygen	1.59 mg O_2/liter	
pH	6.04	
Temperature	22.5°C	
Iron	10.0 mg/liter	
Calcium	8.0 mg/liter	
Chlorobenzene	9.670 mg/liter	0.1 mg/liter[1]
Benzene	0.11 mg/liter	0.005 mg/liter[21]
m-Dichlorobenzene	0.088 mg/liter	
p-Dichlorobenzene	Not Detected	0.075 mg/liter[21]
o-Dichlorobenzene	<0.01 mg/liter	0.6 mg/liter (tentative)[21]
Toluene	<0.01 - 0.86 mg/liter	2.0 mg/liter (tentative)[21]
o-Xylene	<0.01 - 0.094 mg/liter	0.44 mg/liter[22]

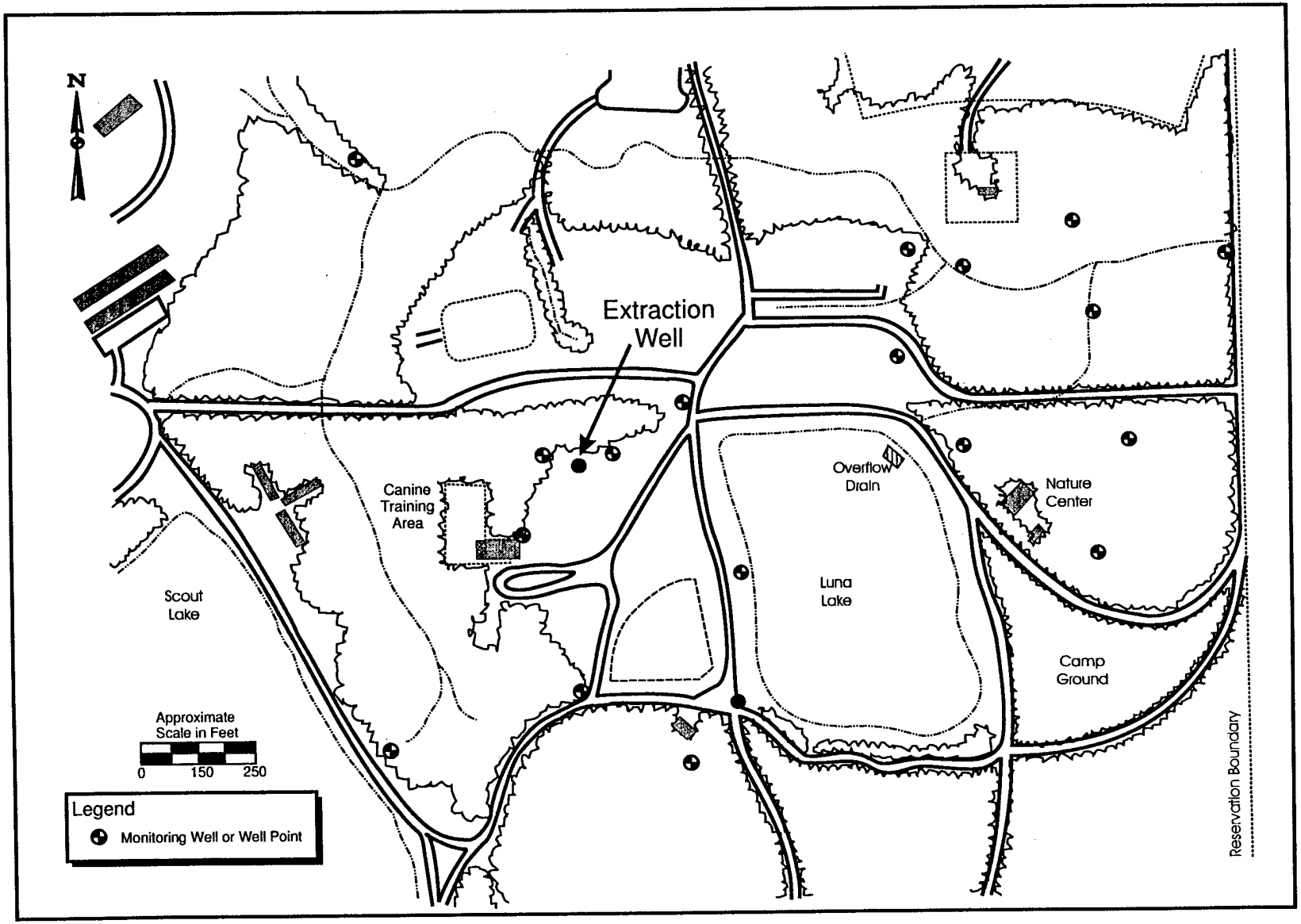


Figure 1 Location of Pilot Study Extraction Well

D. SCOPE OF STUDY

This project consisted of a combination of three objectives:

1. Selection of a microbial strain to populate the bioreactor.
2. Conduct a field study to compare removal efficiency of the selected microbe with the indigenous ground water microbes.
3. Monitor the development of biomass within reactors and examine for possible displacement of selected microbes by indigenous populations by monitoring cell numbers throughout the duration of the experiment.

SECTION II

SELECTION OF MICROBIAL STRAIN

A. OBJECTIVE

Chlorobenzene is identified as one of the most commonly occurring contaminants at National Priorities List hazardous waste sites (1). The purpose of this study was to develop information to select the most efficient microbial strain for use in a future field test to evaluate the biological treatment of chlorobenzene in a fixed-film reactor. Biofilms of four microbial strains of known chlorobenzene utilizers were evaluated, under varied loading conditions, for their ability to remove chlorobenzene from ground water.

B. INTRODUCTION

Biofilms are composed of microbial communities that have colonized onto a solid support. Biofilms play an important role in removing pollutants from natural aquatic environments (23) and also provide the cleansing mechanism for trickling filters, rotating biological contactors, biological activated carbon, biofilters, etc., in water and wastewater treatment (24). Formation of a biofilm is controlled by the transport of organic matter and microbes to the support surface, microbial attachment to the support, bacterial growth and cell maintenance, and the partial detachment and wasting of the biofilm (25). The amount of biomass which can develop depends on the concentration of the limiting substrate and rate of biofilm wasting. For steady-state biofilms, growth of the biomass equals the amount lost from wasting, and substrate utilization is equal to the rate of substrate diffusion into the biofilm. The rate of wasting reportedly varies for different species of microbes (26) and increases for increasing thickness of the biofilm (27). Substrate uptake in a biofilm is commonly limited by mass transfer (28-31). The mass transfer is a combination of diffusional resistance through the liquid phase and diffusional resistance into the biofilm. The amount of liquid-phase diffusional resistance is partially dependent upon the flow rate of the contaminant stream through the reactor. Alternatively, biofilm resistance to substrate diffusion is species-dependent, and microbes with high specific growth rates may exhibit diminished growth rates within a biofilm if diffusional resistance is great (32).

Engineered biofilms with single or selected species populations are commonly utilized in industrial fermentations and water treatment systems to target a specific product or recalcitrant contaminant. Typical examples include glucose to ethanol fermentation (33), glucose to acetone-butanol-ethanol fermentations (34), sulfide removal (35), 2-chlorophenol removal (36), methanotroph removal of halogenated and aromatic solvents (37), removal of chlorinated aliphatics (15), and organophosphate/organochlorine removal (16). Often several microbial strains are available that are capable of utilizing the same substrate but display varying utilization efficiencies. Selection of microbial strains based on comparison of kinetic parameters such as the specific utilization rate (k), half saturation concentration (K_s), etc., may not result in the most efficient process. Kinetic parameters are typically determined from suspended growth in batch reactors or flow-through chemostats. The type of reactor system used for the kinetic determinations affects the observed values of the kinetic parameters (38, 39). Additionally, these systems do not address the potential species variations in the rate of fixed-film wasting or

diffusional resistance of substrate into the biofilm. Both the rate of wasting and the diffusional resistance influence the eventual thickness and amount of biomass which can form in a reactor. The overall rate of substrate removal by a biofilm depends on the total biomass in a system (40). Selection of a microbial strain based on the ability to form a biofilm and accumulate biomass may be a better criteria than comparison of individual kinetic parameters. Biofilm formation can, in turn, be indirectly monitored by observing the overall substrate removal within a reactor.

C. METHODS AND MATERIALS

1. Equipment

Samples were analyzed for chlorobenzene with a Varian 3400 gas chromatograph (GC) equipped with a DB624, 75-meter (m), 3.0-micrometer (μm) megabore column and an flame ionization detector (FID). Gas samples were directly injected onto the column and liquid samples were purged with a Varian LSC-2000 purge-and-trap concentrator. High-purity helium was utilized as the carrier gas at a flow rate of 24 milliliters (ml)/min. The injector and detector were maintained at 190°C. A temperature program for chlorobenzene analysis was the following: 70°C initial oven temperature, hold 2 min, 15°C/min ramp to a 170°C final temperature and hold for 2 min. Samples were quantifiable down to 85 micrograms (μg) chlorobenzene/liter (L) air for gas samples and 24 μg chlorobenzene/L water for aqueous samples.

The reactor columns were constructed of borosilicate beaded process pipe with an inner diameter of 3.81 centimeters (cm) and an overall length of 30.48 cm. Distance between inlet and outlet ports was 26.7 cm which provided an empty bed volume of 304 ml. Figure 2 provides a detail of the column assembly and location of sampling ports. Each column was filled with approximately 175 grams (g) of a diatomaceous biocatalyst support material (Manville Celite Biocatalyst carrier, R635). Each pellet is cylindrical in shape, 6 millimeters (mm) in diameter and approximately 13 mm in length. The carrier has a mean pore diameter of 20 μm and a surface area of 0.27 m^2/g. Height of the packing in each column was 25.4 cm, and the gravity drained pore volume varied between 92 and 98 ml. The sample tubes were constructed of stainless steel capillary tubing. Figure 3 presents the entire system of pumps and columns. The laboratory air supply was filtered and passed through an activated carbon tube to provide a clean air source to the columns. GC analysis of the air stream confirmed no detectable contaminants were present. All transfer lines and connector materials were either Teflon® or stainless steel.

2. Sampling Method

Gas samples (250 microliter (μl) volumes) were gathered from the desired stream locations with a gas-tight syringe and directly injected onto the GC for determination of chlorobenzene concentration. Aqueous samples were gathered in 4.8 ml Teflon®-capped vials and stored at 4° C until time of analysis. The samples were then allowed to warm to room temperature before determination of chlorobenzene content by purge-and-trap analysis following EPA method 604.

Samples gathered for pH determination were collected in 24-ml volatile organic

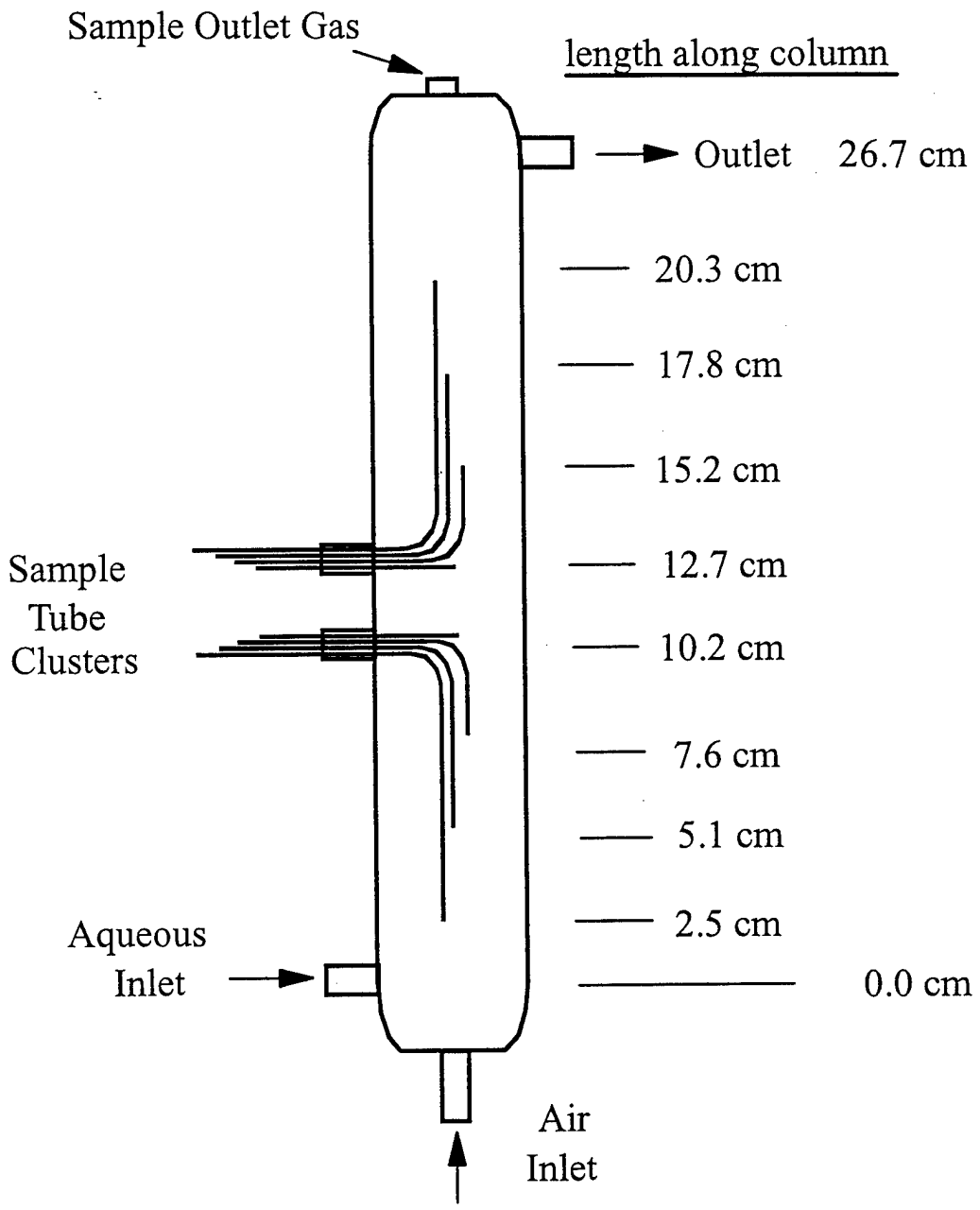


Figure 2 Column Apparatus and Sampling Locations

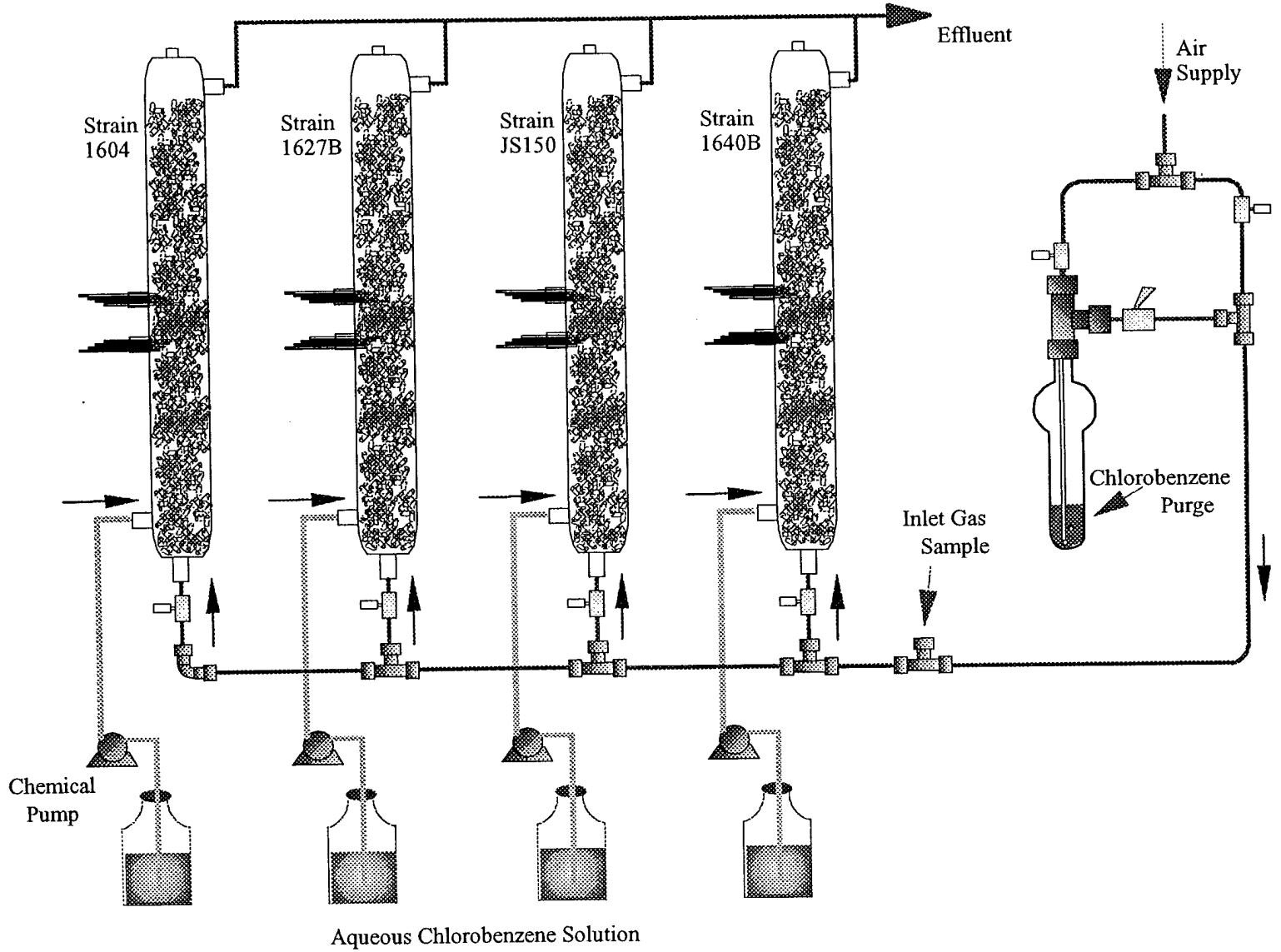


Figure 3 Bench-Scale Apparatus

analysis (VOA) vials and measured immediately. During batch operation, the 24 ml volumes drained from the columns for pH determination were replaced with fresh minimal salts medium, MSB (41).

3. Solutions

MSB was employed as the aqueous solution for the flow-through trials. The solution was autoclaved prior to usage to minimize addition of outside microbial sources to the columns during a trial. Batches of chlorobenzene-contaminated solution were prepared by dilution of a concentrated aqueous stock solution of chlorobenzene with MSB to the desired concentration. The concentrated stock solution was prepared in an aluminum foiled screw-top Erlenmeyer flask by dissolving 300 µl of chlorobenzene (Kodak chemicals, GC grade) in one liter of MSB. The concentration of the resulting stock solution was approximately 300 mg chlorobenzene per liter. Additional flow-through trials were conducted with uncontaminated ground water from the field study site. The site water was pretreated by filtering through a 0.2-µm filter to remove particulates and iron present in the water. Desired concentrations of chlorobenzene in site water were prepared following the same procedure as used for the previous trials.

4. Microbial Strains

The four strains of chlorobenzene utilizers examined in this study were obtained from Tyndall AFB, FL. The strains include: *Pseudomonas* sp. strain JS150, a modified strain developed and characterized at Tyndall AFB (42) and three strains isolated from a chlorobenzene spill site at Kelly AFB, TX (*Pseudomonas* sp. strains 1604, 1627B, and 1640B).

5. Column Operation

a. Startup - A column inoculum was prepared for each microbial strain by transferring an agar plate colony growing on chlorobenzene vapor to a dilution tube of 4.5 ml trypticase soy broth solution. The inoculum was incubated at 30°C for several days until the solution appeared cloudy. Each designated column was inoculated by adding the inoculum to the top of the column packing and then filling the remaining column volume with MSB solution. The columns were operated as a batch system by introducing chlorobenzene vapor and air into the bottom of the column at a rate of 20 ml/min. Chlorobenzene concentrations in the gas stream and pH of the solution were monitored daily. The initial inlet chlorobenzene concentration was maintained at 2300 - 2500 µg/L air. The inlet concentration was incrementally increased to 10,000 µg/L air in response to the increasing microbial utilization so the exiting gas stream concentration was close to zero. The initial pH of the column solution was 7.2, and the solution was replaced when the pH was observed to decrease below a range of 6.4 to 6.6.

b. Trials - The columns were operated in a once-through, up-flow basis for both the inlet aqueous stream and the inlet air stream. Aqueous stream flow rates were varied to provide different contaminant loadings and residence times within the columns. Air flow rates were maintained between 20 to 25 ml/min. Flow-through experiments were conducted

throughout the day, and the columns were switched to a batch operation overnight. Chlorobenzene was added to the columns via the air stream during overnight operations to maintain the biofilm activity. The contaminant solution was regulated through the columns by chemical metering pumps, and all lines and connections were assembled from stainless steel or Teflon®.

D. RESULTS

1. Development of Biofilms

Typical results from monitoring pH and chlorobenzene utilization during biomass development are illustrated in Figure 4 for the *Pseudomonas* sp. strain 1604. The biomass was assumed to be a biofilm; however, the thickness was not measured. Difficulty was encountered in maintaining a steady chlorobenzene inlet concentration and air flow rate. Each of these factors greatly influenced the utilization rate and are the cause of the rapid variations in the utilization rates observed between Days 4 to 6 and 13 to 14. Chlorobenzene removal activity was slow in developing for *Pseudomonas* sp. strain 1604, so the column was reinoculated on Day 7 and again on Day 10. The removal activity was observed to increase, and the pH of the buffer solution began to decline between Day 11 and Day 12. Chlorobenzene utilization rapidly increased over the next few days as the microbe population expanded and a rapid drop in pH was observed on Day 14 when the pH was recorded at 5.7. The entire volume of buffer was replaced, and the pH again fell to 5.8 within 24 hours. On Day 15, the buffer solution was again replaced and the pH was observed to immediately begin decreasing. At this time, column operation was switched from batch to continuous flow to maintain the pH between 6.8 and 7.2. The two other Kelly *Pseudomonas* sp. strains, 1627B and 1640B, displayed very similar startup histories. Multiple inoculations were required before an increasing chlorobenzene removal activity was observed on Day 12. Both columns displayed the rapid decline in pH over the next few days until column operations were switched to flow-through operation.

In contrast, the column inoculated with the *Pseudomonas* sp. strain JS150 isolate required only one inoculation and displayed much faster biofilm formation. Figure 5 reveals a steadily declining pH, beginning the day following inoculation of the column. The chlorobenzene utilization rate rapidly increased within eight days of inoculation compared to the 12 to 13 days required for the other strains. Again, the decreased utilization recorded between Days 8 and 9 is a result of variation in the inlet concentration and flow rate. After Day 8, the column containing *Pseudomonas* sp. strain JS150 displayed similar rapid decreases in buffer pH as previously described for the other strains. By Day 10, the biofilm activity was sufficiently established to allow the column operation to be switched from batch to continuous flow.

Monitoring the column startup revealed the *Pseudomonas* sp. strain JS150 to be the most efficient isolate at forming a biofilm. The more rapid biofilm development by *Pseudomonas* sp. strain JS150 indicates a greater growth rate and matrix attachment affinity than the strains 1604, 1627B, or 1640B. Inoculation of a field-scale unit with the *Pseudomonas* sp. strain JS150 would minimize the startup time required to populate the reactor. Greater rate of growth and attachment ability also suggest that the *Pseudomonas* sp. strain JS150 may be better able to withstand possible displacement by indigenous microbes under actual ground water treatment conditions.

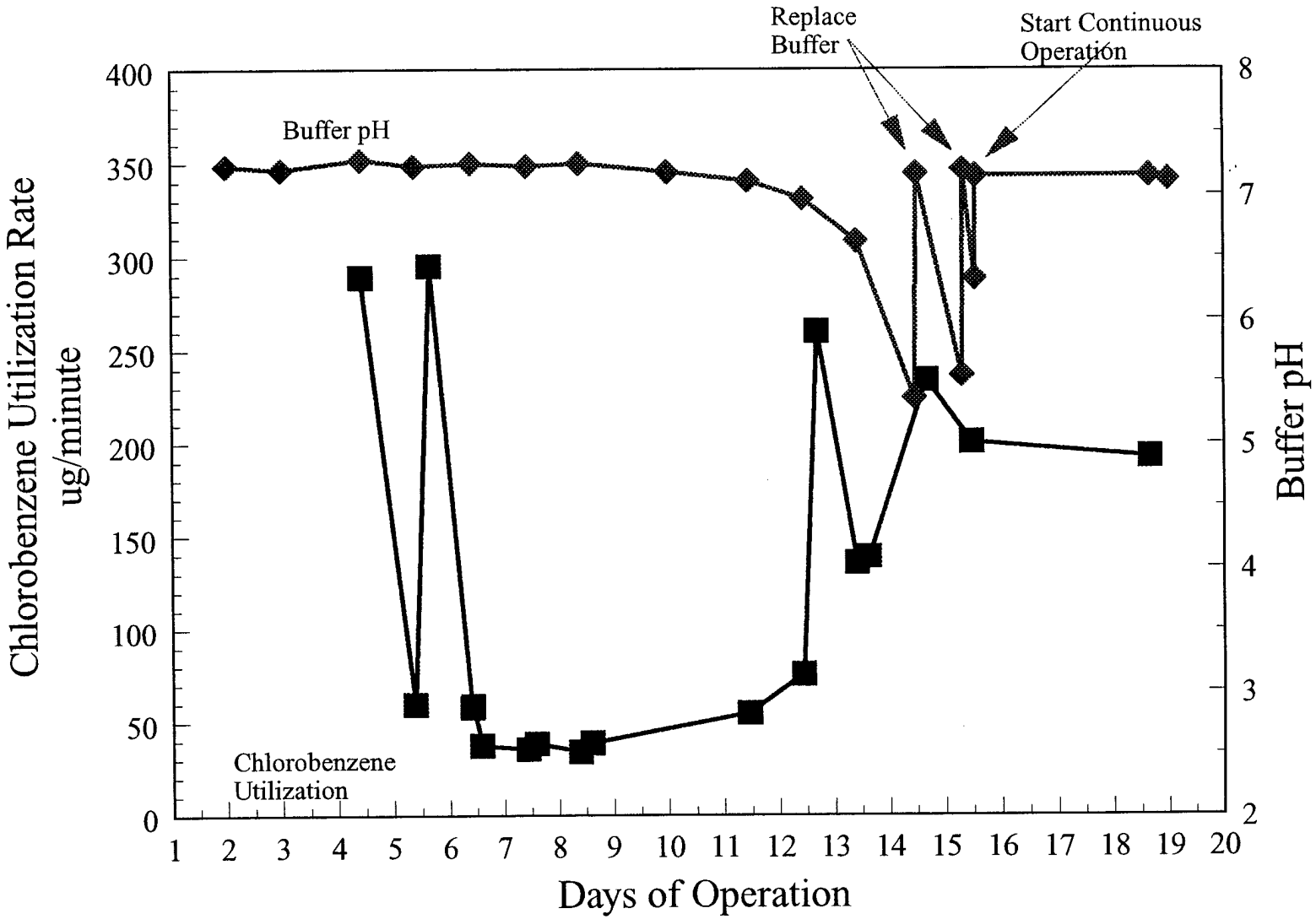


Figure 4 *Pseudomonas sp.* strain 1604 Biofilm Development

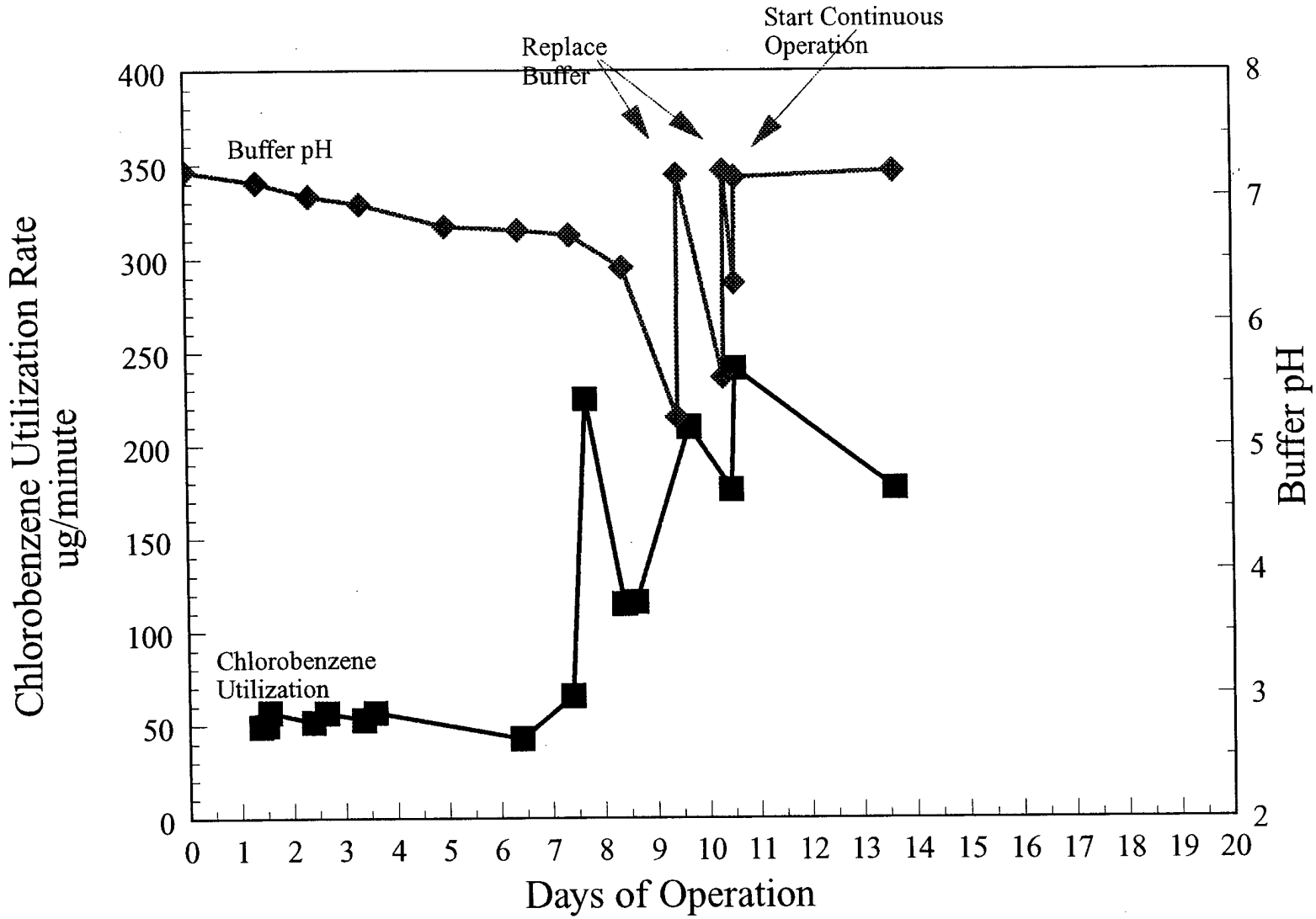


Figure 5 *Pseudomonas sp.* strain JS150 Biofilm Development

The four strains were also maintained as individual streak plate cultures on MSB agar plates and a carbon source. The cultures were exposed to individual carbon sources, and the relative rates of growth were subjectively observed for each strain. Averages of the growth rate observations are presented in Table 2 for the various carbon sources.

TABLE 2 RELATIVE GROWTH OF STRAINS FOR VARIOUS CARBON SUBSTRATES

	1604	1627B	1640B	JS150
Benzene	++	+	++	+++
Chlorobenzene	++	+	++	++++
Toluene	+++	+	+++	++++
Naphthalene	++	+	++	+++
o-Dichlorobenzene	++	+	+++	++
o-Xylene	+	++	-	+++

+ growth observed
- no growth observed
Control plates MSB agar plates without carbon sources.

Similar to the results observed during the column startup, the *Pseudomonas* sp. strain JS150 exhibited the most vigorous agar plate growth rate of the four strains. Streak plate growth also showed that the *Pseudomonas* sp. strain JS150 could utilize a wide range of substrates, as has been previously reported (42). The ability to utilize both chloro- and methyl-substituted aromatics is generally considered uncommon (12). Two of the three *Pseudomonas* sp. strains from Kelly AFB, 1604 and 1640B, displayed the ability to also grow on a wide variety of substrates, although not as vigorously as the *Pseudomonas* sp. strain JS150. Further growth experiments would be required to confirm substrate utilization ability.

2. Effect of Flow Rate on Utilization and Removal Efficiency

After development of batch mode biofilms, MSB solution was continuously circulated through the columns while vaporous chlorobenzene was introduced through the air stream. The columns were operated at 10 ml MSB solution/min and 20 to 25 ml air/min containing 9 to 10 mg chlorobenzene/L for five days. Similar operating conditions were maintained for each column to allow comparison of biomass production. Day-long, flow-through trials were then conducted to examine the dynamic removal of chlorobenzene from the aqueous stream. During the trials, the input of chlorobenzene was switched from an air stream vapor to chlorobenzene dissolved in the aqueous phase. The characteristic chlorobenzene removal capacity of each column was measured to provide an indirect indication of microbial substrate affinity and the amount of biomass present. Inlet chlorobenzene concentration and aqueous flow rate were varied to examine the removal efficiency of each column. Results of the individual removal trials are presented in Table 3. The flow trials found all the biofilms were capable of removing 98% or more of the inlet chlorobenzene for EBRTs greater than 15 min.

TABLE 3 FIXED-FILM UTILIZATION OF CHLOROBENZENE

EBRT[a] minutes	Inlet Concentration µg/L	Loading Rate µg/min	Outlet Concentration µg/L	Utilization Rate µg/min	% Removal
1604					
43.4	7312	51	24	51	99.7
43.4	11369	80	39	79	99.7
38.0	17153	137	153	136	99.1
33.8	5127	46	< 24	46	> 99.5
6.1	8242	412	1560	334	81.1
5.8	11475	597	3887	395	66.1
1627B					
46.9	6465	42	<24	42	>99.6
38.0	6031	48	< 24	48	> 99.6
6.3	6941	333	1730	250	75.1
6.1	5961	298	4507	73	24.4
6.0	13292	678	5458	400	58.9
1640B					
42.2	20004	144	717	139	96.4
40.5	6215	47	39	46	99.4
32.0	10330	98	43	98	99.6
14.5	10664	224	809	207	92.4
10.9	16744	469	3560	369	78.7
6.3	8106	389	1788	303	77.9
6.3	8960	430	1121	376	87.5
5.8	11777	612	5501	326	53.3
5.3	9864	562	1474	478	85.1
JS150					
50.7	2819	17	< 24	17	> 99.1
43.4	8900	62	46	62	99.5
35.8	7846	67	124	66	98.4
10.5	12589	365	2374	296	81.1
6.6	13153	605	2441	493	81.4
6.1	8904	445	1761	357	80.2
6.1	8952	448	3901	253	56.4
5.6	13812	746	2272	623	83.6
5.5	12549	690	2493	553	80.1

[a] Empty bed residence time

The observed removal efficiency was independent of the inlet concentrations employed and higher inlet concentrations produced greater utilization rates. This indicates the chlorobenzene

addition was less than the removal capacity of each biofilm. In later trials, the flow rates were increased to minimize the liquid transport resistance and ensure a reaction-controlled removal. Additionally, the shorter EBRTs magnified the differences in the chlorobenzene removal ability between each biofilm. As shown in Figure 6, the amount of chlorobenzene utilized by each column was similar at the longer EBRTs, but the differences in column utilization became much more pronounced for shorter EBRTs. Higher flow rates increase the convective transport and decrease the diffusive resistance. The shorter EBRT data presented in Figure 6 show a much wider range of chlorobenzene utilization than observed for flow rates with longer EBRTs. The chlorobenzene loading approached the saturation capacity of each biofilm and magnified the variations in the utilization capacities of each microbial strain. Decreased removal efficiencies are also a result of shorter EBRTs due to decreased contact times within the column. Figure 7 displays the removal efficiencies observed at EBRTs greater than and less than 15 min. In some cases, the removal efficiencies were also observed to be affected by the inlet concentration at the shorter EBRTs. This further suggests that the amount of chlorobenzene utilization was approaching the saturation capacity of the biofilm.

3. Effect of Inlet Concentration on Utilization and Removal Efficiencies

The effect of the inlet concentration on the removal efficiency was most pronounced during the high flow rate trials (6-min EBRT). Figure 8 provides a comparison of the removal efficiencies and the amount of utilization observed for each biofilm at two inlet concentrations. The figure reveals the biofilms had similar removal ability at the lower inlet concentration range of 6900 to 8900 μg chlorobenzene/L. Removal efficiencies ranged from 75% to 88% of the inlet chlorobenzene during the lower concentration trials in which the biofilm of strain 1627B produced the smallest reduction of chlorobenzene and the biofilm of strain 1604 produced the largest. The high inlet concentration range of 11,500 to 13,300 μg chlorobenzene/L produced large reductions in the removal efficiencies for all biofilms except the biofilm of *Pseudomonas* sp. strain JS150. As shown in Figure 8, the removal efficiency of the *Pseudomonas* sp. strain JS150 biofilm remained constant at 80% removal, while the removal efficiencies of the other three columns dropped from 15 to 35 percentage points. Examination of the utilization rates in Figure 8 reveals the rate of chlorobenzene utilization was greatest in the column containing the *Pseudomonas* sp. strain JS150 for both concentration trials. Under the conditions examined, the *Pseudomonas* sp. strain JS150 was either able to develop a greater biomass or possessed faster removal kinetics than the biofilms of the other strains. In either case, the saturation capacity was greater for the column with *Pseudomonas* sp. strain JS150 than the other three columns evaluated.

4. Concentration Profiles

Further continuous-flow trials were conducted with the column containing the *Pseudomonas* sp. strain JS150 to resolve the longitudinal concentration profile within the column. The change in the chlorobenzene concentration was determined from samples gathered along the column via the sample tube clusters illustrated in Figure 2. The column activity was maintained by operating the column as a batch system between profile trials. Switching the modes of operation maintained a constant biofilm activity with a similar amount of biomass and biofilm distribution for all trials. Results of the profile delineation for three separate flow trials

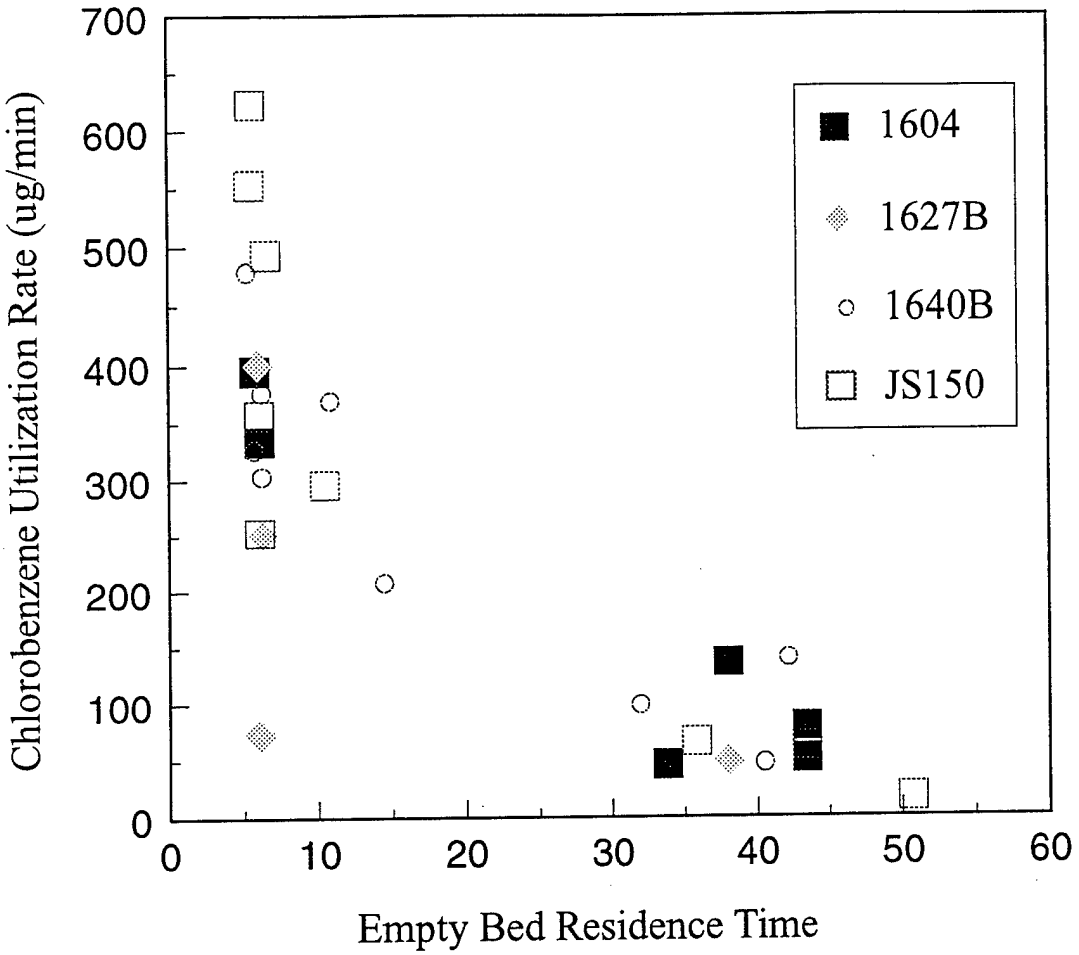


Figure 6 Chlorobenzene Utilization

Flow-Through Packed Column

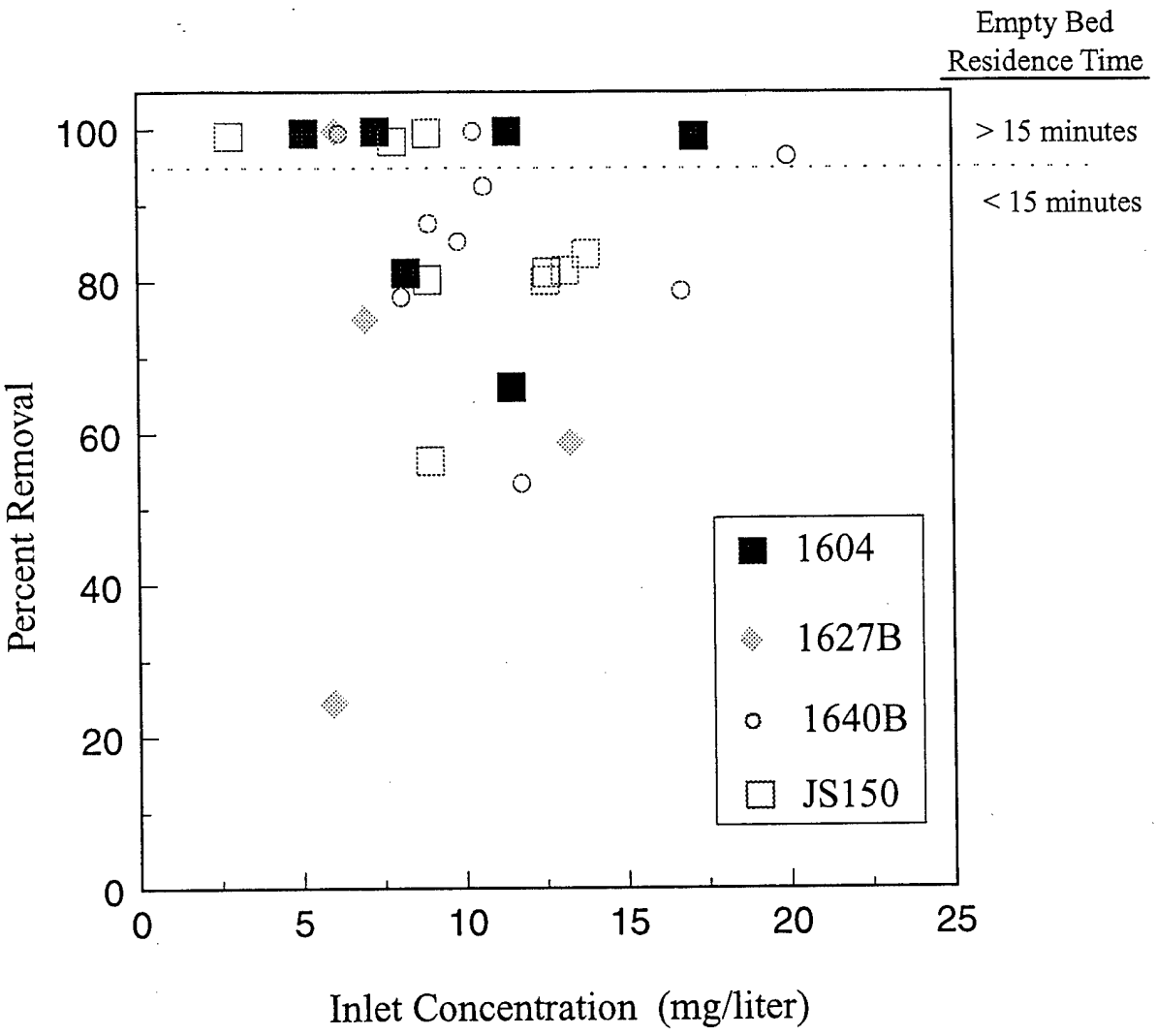


Figure 7 Chlorobenzene Removal Efficiency

Flow-Through Packed Column

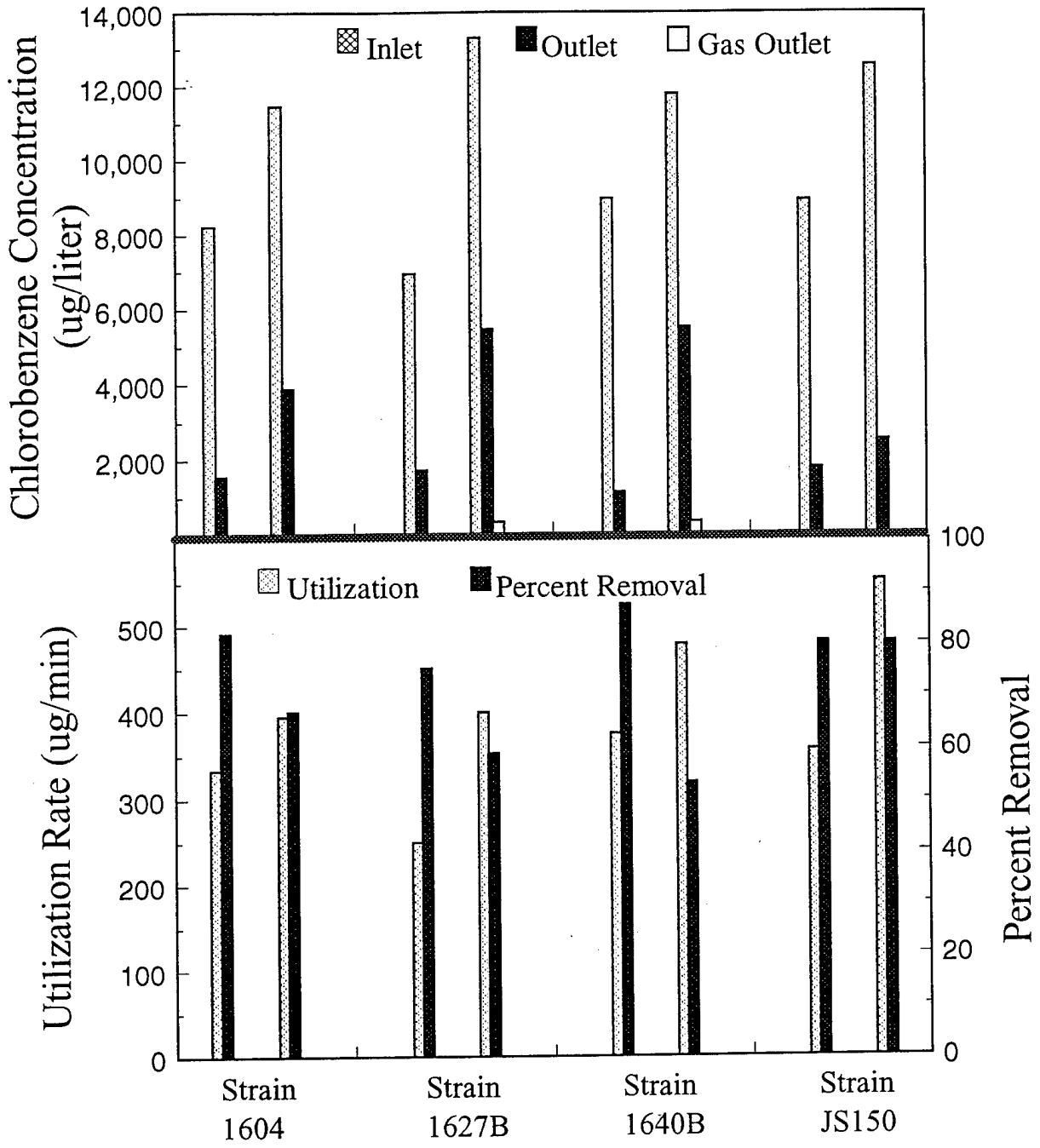


Figure 8 Comparison of Biofilm Activity

5.8 - 6.3 EBRT Range

are presented in Figure 9. The first profile trial examined the saturated removal capacity of the biofilm by operating at a high inlet concentration and a short EBRT. Under saturated conditions, the concentration profile provided an indirect representation of the biomass distribution. As shown in Figure 9, a similar rate of removal was exhibited from the Inlet position to Port 3 during the 5.6 minute EBRT trial. The discordant value observed at Port 1 was interpreted as caused by preferential flow between the inlet and the sample port. The shape of the profile suggests the greatest amount of biomass to be within the initial 7 to 10 cm of the column. This is typical of a packed column in which the density of a steady state biomass decreases from the inlet to the outlet in response to the decreasing substrate concentration. For the second trial, the flow rate was reduced to produce a long EBRT. Allowing greater contact times should produce a steeper short-term concentration profile if the removal mechanism is still reaction-controlled. The profile developed for the 40-min EBRT trial shows the rate of decline of the concentration is less than the rate observed for the previous trial. In this instance, the removal mechanism had switched from a controlled reaction to a transport-limited system. The third profile trial examined a midrange flow rate at a high inlet concentration and a stoichiometrically adjusted air flow rate. As shown in Figure 9, the change in the concentration profile for the 10.5-min EBRT between the inlet and Port 2 produces a utilization rate nearly equal to the rate observed for the 5.6-min EBRT profile. The removal was again controlled by the rate of reaction. Observing the same amount of removal for two separate flow trials suggests the removal capacity of the biofilm was saturated, and the observed removal behaves as a zero order system. Removal observed between Port 2 and the outlet during the 10.5-min EBRT trial was much less than expected. Reduction of the air flow rate to produce a more stoichiometric amount of oxygen possibly induced an oxygen limitation which reduced the substrate removal. The air flow was greater than the estimated requirement for complete removal of the chlorobenzene. However, it was less than the total oxygen demand which would also include oxygen for endogenous respiration and undissolved oxygen which was unavailable.

5. Site Water Treatability

Results of the site water treatability trials are listed in Table 4. Air flows were optimized to 2.1 to 3.8 ml/min during the site water trials in order to provide a more stoichiometric amount of oxygen for chlorobenzene degradation. Comparing the removal results of the site water trials to the previous trials which utilized MSB solution (Table 3) reveal the site water may have contributed to a reduction in the biofilm activity in two of the six trials. Similar rates of utilization were observed for trials with similar loading rates for the biofilms of the *Pseudomonas* sp. strains 1604, 1627B, and 1640B. The *Pseudomonas* sp. strain 1604 biofilm utilized 136 and 170 μg chlorobenzene/min at loading rates of 137 and 201 μg chlorobenzene/min during trials with MSB solution and site water. The removal efficiency was slightly less during the site water trial (84.6% verses 99.1%), due to the higher loading rate and shorter contact time employed. Utilizations of 250 and 229 μg chlorobenzene/min were observed for the biofilm of *Pseudomonas* sp. strain 1627B during loadings of 333 and 316 μg chlorobenzene/min during MSB solution and site water trials, respectively. In this case the removal efficiencies are also very similar for both solutions. The site water trials of the *Pseudomonas* sp. strain 1640B biofilm produced utilization rates of 143 and 187 μg chlorobenzene/minute from loadings of 145 and 246 μg chlorobenzene/min. The removal

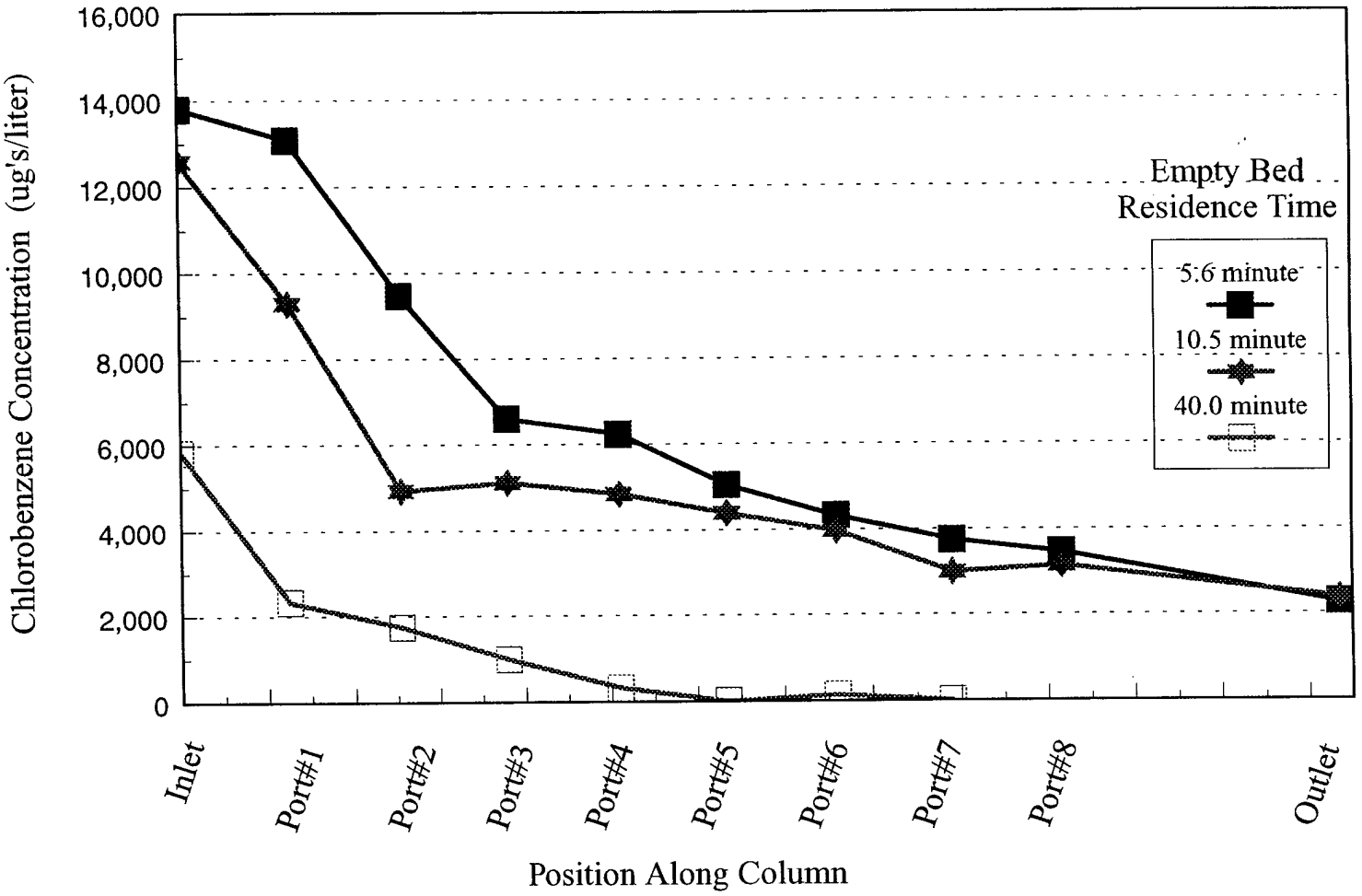


Figure 9 Concentration Profile Along Column Inoculated with *Pseudomonas sp.* strain JS150

obtained during the low loading rate trial is similar to the utilizations and efficiencies previously observed for similar loadings during the MSB solution trials. (Utilizations of 139 and 207 μg chlorobenzene/min were observed for loading rates of 144 and 244 μg chlorobenzene/minute during trials with MSB solution). The chlorobenzene utilization obtained from the site water trial conducted at the greater loading rate of 246 μg chlorobenzene/min was approximately 10% less the utilization observed at the corresponding trial with MSB solution. This reduction in removal ability is possibly an indication of inlet concentration toxicity, inadequate oxygen input into the system, or that the site water provided a less optimum medium for growth than the MSB solution. Utilization of chlorobenzene in the column populated with the *Pseudomonas* sp. strain JS150 during the site water trial was almost 50% less than the utilization which was observed during MSB solution trials. A loading of 277 μg chlorobenzene/min during the site water trial produced a utilization rate of 158 μg chlorobenzene/min and a removal efficiency of 57.1%. During trials with MSB solution, a utilization rate of 296 μg chlorobenzene/min and removal efficiency of 81.1% was obtained at a loading of 365 μg chlorobenzene/min. As suggested in Section 4, the removal observed in this MSB trial may have been oxygen-limited. Air flow rates into the column were approximately the same for both the MSB and site water trials (2.9 and 2.6 ml/min, respectively) so the removal observed during the site water trial may have been oxygen-limited also. Additional factors contributing to the reduced removal activity may include toxicity of the inlet chlorobenzene concentration and less than optimum growth media provided by the site water.

TABLE 4 SITE WATER TREATABILITY TRIALS

EBRT	Inlet Concentration	Loading Rate	Outlet Concentration	Utilization Rate	% Removal
minute	μg/liter	μg/min	μg/liter	μg/min	
1604					
27.6	18230	201	2815	170	84.6
1627B					
20.3	3847	58	< 24	58	> 99.4
16.4	17093	316	4718	229	72.4
1640B					
25.3	12072	145	195	143	98.4
23.4	18947	246	4565	187	75.9
JS150					
22.5	20501	277	8800	158	57.1

E. CONCLUSIONS

Experimental results indicate the *Pseudomonas* sp. strain JS150 possesses a greater growth rate and is more efficient at populating the support matrix than the other three strains of chlorobenzene utilizers. All strains of biofilms can remove >98% of the chlorobenzene introduced into the columns if sufficient contact time is allowed. The column populated with the

Pseudomonas sp. strain JS150 displayed the greatest chlorobenzene utilization rate and had the highest saturation capacity. In some cases, the biofilm oxygen demand was greater than the amount needed for chlorobenzene degradation alone and minimizing the air flow can potentially limit chlorobenzene removal. All strains of biofilms were capable of treating chlorobenzene-contaminated site water.

To be suitable for use as an inoculum for a pilot-scale fixed-film bioreactor, a microbial strain should possess the following beneficial characteristics:

1. Provide an efficient inoculation onto the column support material.
2. Display a rapid growth rate which may allow greater resistance to possible displacement.
3. Develop a stable biofilm population within the shortest amount of time.
4. Be able to form a large amount of biomass and express a rapid chlorobenzene utilization rate.
5. Be tolerant of changes in operating parameters such as pH, flow rate, Do, etc. which are more difficult to control in the field.

Results of the experimental studies indicate the *Pseudomonas* sp. strain JS150 most effectively meets the beneficial characteristics listed above and is considered the best selection to utilize as an inoculum in a pilot-scale reactor.

SECTION III

FIELD STUDY EVALUATION

A. OBJECTIVE

The objective of the field study was to evaluate the effectiveness of an above-ground biological treatment system to degrade chlorobenzene in ground water. Two fixed-bed up-flow reactors were employed in this field effort. The evaluation focused on the following objectives:

1. Evaluate treatability of chlorobenzene with a biological reactor.
2. Compare efficiencies of indigenous microbes with an isolated strain inoculum.
3. Determine treatability of secondary contaminants.
4. Evaluate operating parameters, i.e. pH and DO.

B. INTRODUCTION

Identical packed columns were configured to operate in parallel to allow comparison of the removal efficiencies of each. The packing material was cleaned prior to transporting the treatment system to the field site. One reactor was inoculated with the selected microbial culture several days before deployment to the field. The second reactor was allowed to populate with indigenous microbes.

C. METHODS AND MATERIALS

1. Equipment

Volatile contaminant concentrations were determined by GC analysis. An HP 5890 GC equipped with a 183-cm, 0.318-cm diameter stainless steel packed column and a photo ionization detector, PID, was utilized for the analysis. The column support was 5.0% SP 1200 and 1.75% Bentone 34. A 30 ml/min flow of high-purity helium was utilized as the carrier gas. Injector and detector temperatures were maintained at 220°C. A Varian LSC-2 purge-and-trap concentrator was utilized for analysis of the aqueous samples following EPA Method 602 and gaseous samples were analyzed by direct injection onto the GC. The estimated concentrations required to produce a response at the preset 10,000 unit area rejection limit are listed in Table 5.

TABLE 5 10,000 AREA UNIT RESPONSE CONCENTRATIONS

Contaminant	ppm
Benzene	0.004
Toluene	0.002
Chlorobenzene	0.002
o-Xylene	0.002
m-Dichlorobenzene	0.003

Fatty acid analysis was conducted by HPLC. Compound separation was achieved on an Interaction Ion-300 column, and 0.3 millimoles (mM) 1-octanesulfonic acid was utilized as the mobile phase. A Waters 431 conductivity detector and a 490 optical detector operated at 210 ηm were utilized for compound detection.

DO was measured with a YSI Model 57 DO meter. An Orion Model 611 pH meter was utilized to measure pH.

Each bioreactor consisted of a 30.5 cm diameter stainless steel column 183 cm long. Sample port locations are illustrated in Figure 10. Auxiliary fittings and transfer lines were also stainless steel. Each bioreactor was equipped with a recycle line which connected the effluent line to the inlet line pump (not shown in figure). Recycle of the effluent waters was utilized only during packing washing and reactor inoculation. The column was packed with Manville Celite biocatalyst carrier R-635 (described previously) to a height of 165 cm. The corresponding empty bed volume was120 L, and the drained void volume prior to field operation was measured as 65 and 59 L for the indigenous and inoculated reactors, respectively. The bioreactors were operated as once-through, up-flow reactors.

The entire bioreactor treatment system is shown in Figure 11. High iron content of the well water (73 mg/l as reported by an independent water analysis) necessitated the use of a water softener to pre-treat the inlet stream prior to the biological treatment. The bioreactors, water softener, and analytical equipment were housed within a trailer provided by Tyndall AFB, FL. A sand filter was later installed in the pretreatment line to remove clay-like particulates from the inlet stream. Liquid flow rates to each bioreactor were monitored with in-line flow-meters. The inlet water characteristics (after sand filter and water softener pretreatment) were previously listed in Table 1 along with the Drinking Water Standards for the organic contaminants of interest.

A phosphate buffer solution [2.58 molar (M) K_2HPO_4, 0.37 M KH_2PO_4, and 0.35 M $NaNO_3$] was initially added to control the pH of the inlet stream. The buffer solution was later modified (1.87 M Na_2CO_3, 0.04 M KH_2PO_4, and 0.08 M $NaNO_3$) after the first week of operation to minimize release of phosphate into the environment. All reactor effluent was collected for disposal to the base wastewater treatment facility.

The DO was controlled by addition of technical grade oxygen to the contaminant stream.

2. Packing Preparation

The reactor packing was washed prior to the field study by recirculating an alkaline solution of pH > 9 through the reactors for 24 hours. This was followed by rinsing with tap water and then circulating an acidic solution of pH< 2 for 24 hours; next, the reactor packing was rinsed with tap water. One reactor was inoculated with a chlorobenzene utilizing isolate, *Pseudomonas* sp. strain JS150, seven days prior to transporting the treatment system to the field. The inoculum of *Pseudomonas* sp. strain JS150 isolate was prepared in a 20 L batch fermenter utilizing chlorobenzene as the growth substrate. The designated reactor was inoculated by adding 27 L suspended cells, with a cell density absorbance of 1.2 absorbance units at 600 ηm, and 10 L minimal medium (41) to the top of the column packing. The remaining volume was filled with tap water. The solution was continuously circulated throughout the reactor and

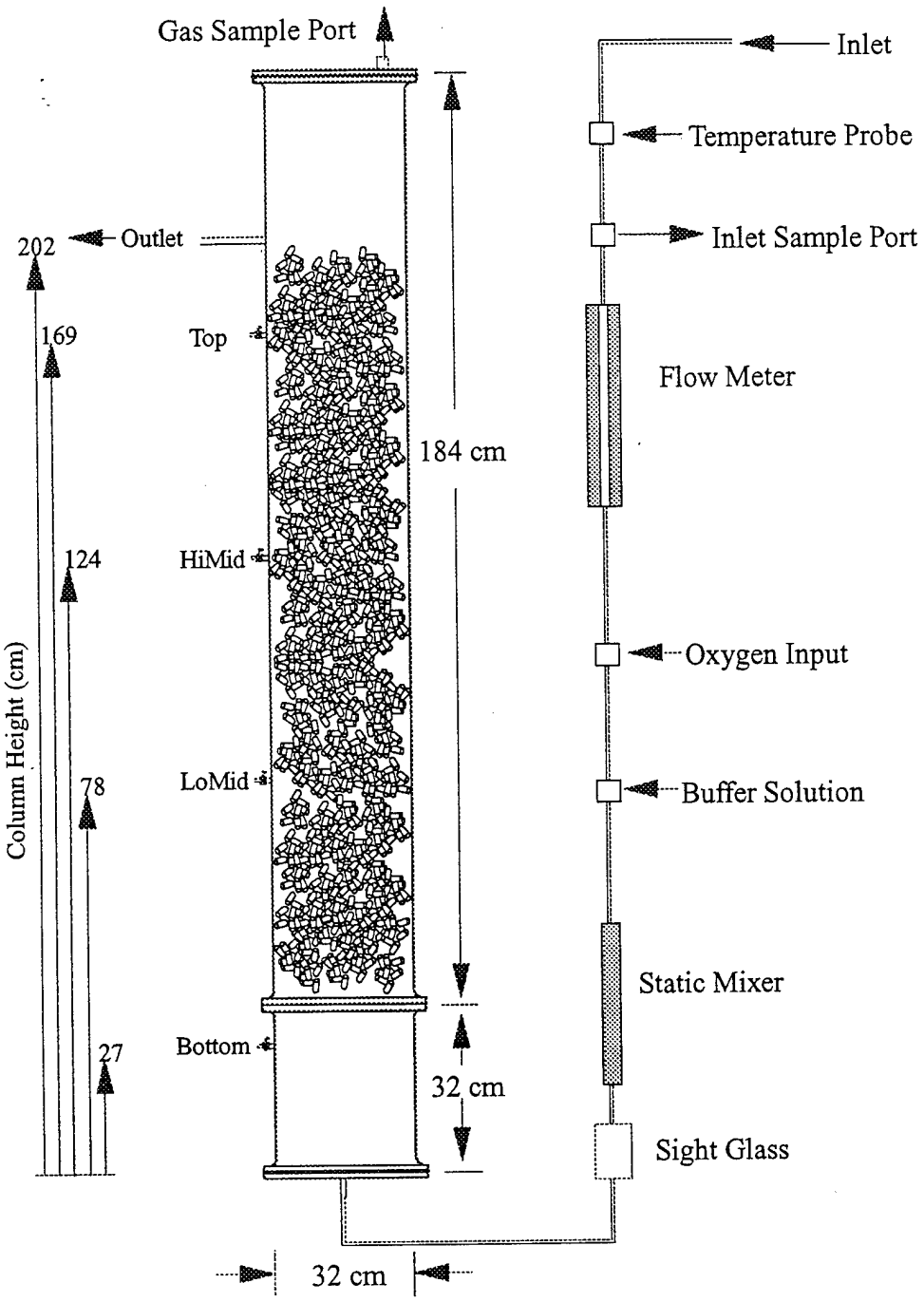


Figure 10 Detail of Sample Port Location

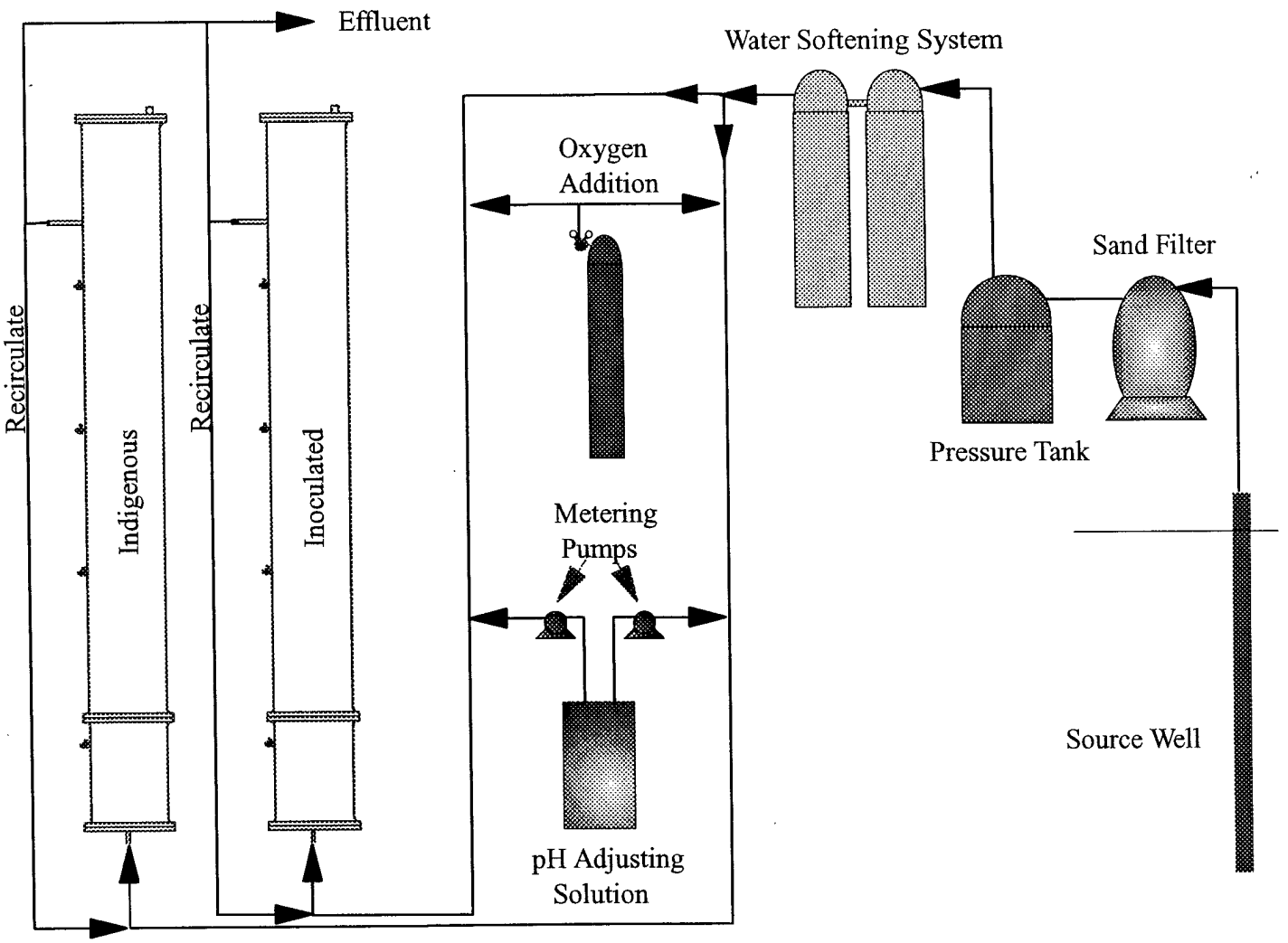


Figure 11 Schematic of Treatment System (not to scale).

chlorobenzene vapor was supplied via the inlet air stream. The solution pH was monitored and maintained between 6.6 and 7.2 by addition of 10N sodium hydroxide and minimal medium as needed.

3. Contaminant Sampling

Organic contaminant concentrations were monitored in both the aqueous and gaseous streams. Aqueous samples were collected with a 5-ml Teflon® luer lock, TLL, gas-tight syringe. A detail of the column side-wall sampling ports is illustrated in Figure 12. The sampling port was designed to sample solution passing through the packing and minimize concentration variations caused by wall effects. To sample a desired port location, an initial 5 ml was drawn from the sample port and discarded. A second 5-ml aqueous sample was then obtained for analysis of the purgeable organics. Aqueous samples for DO and pH were gathered with a 50-ml TLL gas-tight syringe through the same sampling port locations. Gaseous samples were gathered with a 250-μL gas sampling syringe. The outlet gas sampling port was sealed with a septum which allowed sampling of the head-space above the drain level at the top of the reactor. The head-space sample was analyzed by direct injection onto a GC.

D. RESULTS

1. Biotreatability of Chlorobenzene

Studies have shown various types of bacteria can live and grow on chlorobenzene as a primary carbon source (6, 8, 10, 12-14, 42). Continuous biological removal of chlorobenzene was observed during this field trial for periods of 15 and 30 days for the uninoculated and inoculated reactors, respectively. Figure 13 illustrates the chlorobenzene concentrations observed throughout the course of this study at the inlet and outlet sampling ports of the reactor inoculated with *Pseudomonas* sp. strain JS150. Table 6 presents the removal efficiencies for each reactor at different EBRTs. In all cases, the chlorobenzene effluent concentrations were less in the inoculated reactor than in the indigenous reactor. Steady-state operation (15 days) examined flow rates which correspond to a 45-min EBRT. The indigenous and inoculated reactors displayed removal efficiencies of 83% to 86%, respectively, when inlet pH was maintained between 6.8 and 7.2 and the outlet DO was greater than 4.0 mg O_2/L. During the specified operating conditions the corresponding outlet concentrations averaged 1.31 to 1.59 ppm chlorobenzene. This concentration of chlorobenzene was greater than the 0.1 ppm Drinking Water Standards Maximum Contaminant Level (MCL) as listed in Table 1. The minimum sustainable outlet concentration of a biological reactor is limited by the concentration which is required to maintain the biofilm. To develop and maintain the biofilm, a substrate has to be available above a minimum concentration, S_{min} (40). Once a reactor decreases the contaminant concentration to S_{min}, increasing the reactor residence time will not produce sustainable effluent concentrations below S_{min} (43). During short term periods of operation, effluent concentrations may be reduced to a concentration below the S_{min} if a second substrate limitation does not become significant (44).

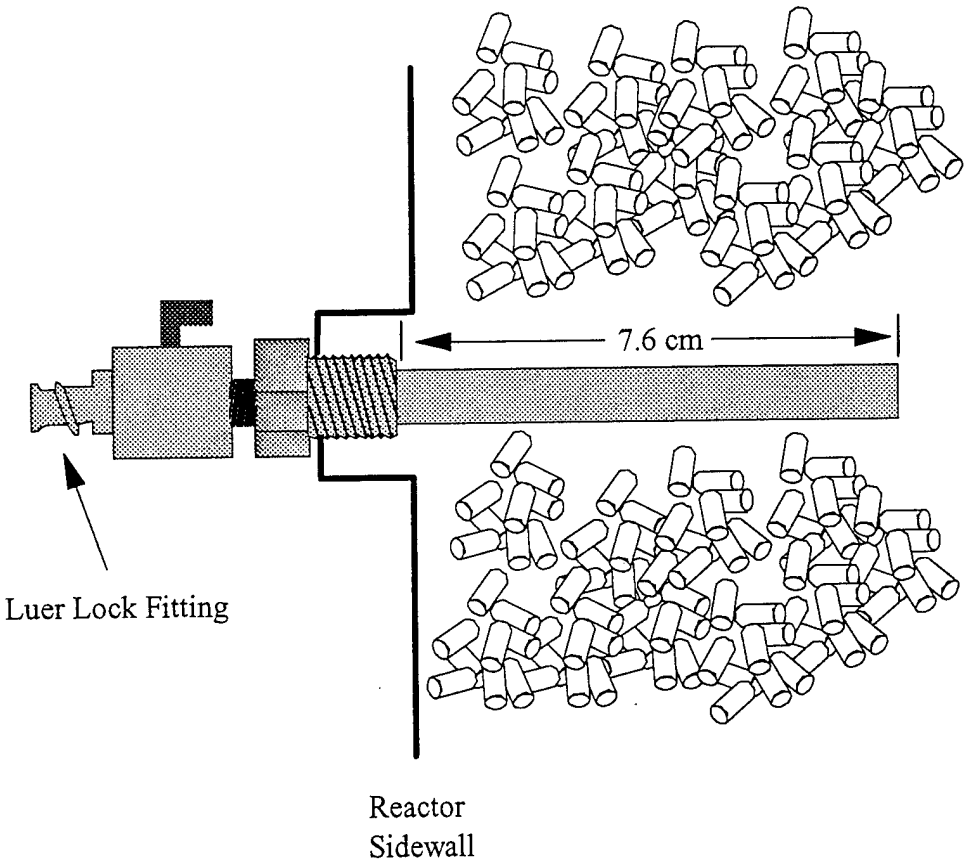


Figure 12 Cutaway View of Sampling Port

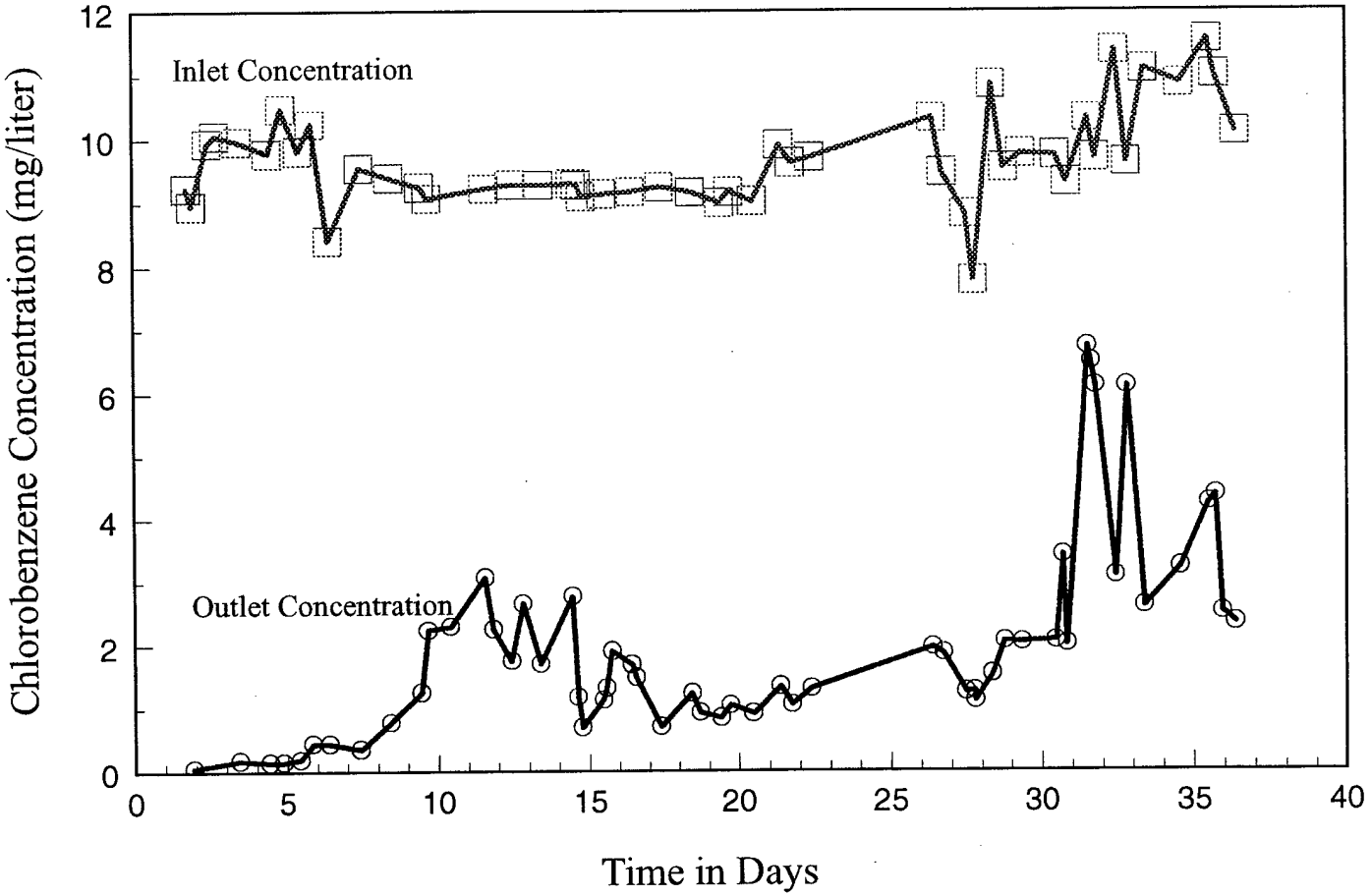


Figure 13 Inlet & Outlet Chlorobenzene Concentrations of Reactor Inoculated With *Pseudomonas sp.* strain JS150

TABLE 6 BIOREACTOR REMOVAL EFFICIENCIES

	Inlet mg/liter	Effluent mg/L	% Removal	Removal Rate mg/min
125 min EBRT				
Uninoculated - maximum removal after populating reactor				
Chlorobenzene	9.91	0.23	97.7	9.20
Benzene	0.20	0.03	85.3	0.17
m-Dichlorobenzene	0.17	0.03	83.8	0.14
Inoculated - length of operation = 2.5 days				
Chlorobenzene	9.39	0.06	99.3	8.67
Benzene	0.18	0.03	84.4	0.14
m-Dichlorobenzene	0.15	<0.01	93.5	0.13
70 minute EBRT				
Uninoculated - length of operation = 2 days				
Chlorobenzene	9.39	0.96	89.8	14.66
Benzene	0.08	0.03	66.1	0.09
m-Dichlorobenzene	0.10	0.04	58.1	0.10
Inoculated - length of operation = 2.5 days				
Chlorobenzene	10.03	0.19	98.1	17.11
Benzene	0.19	0.04	77.3	0.26
m-Dichlorobenzene	0.17	0.02	85.5	0.25
45 minute EBRT				
Inoculated - length of operation = 3 days				
Chlorobenzene	9.37	0.50	94.6	23.03
Benzene	0.15	0.03	75.6	0.30
m-Dichlorobenzene	0.14	0.04	70.0	0.26
45 minute EBRT (change buffer solution)				
Uninoculated - length of operation = 15 days				
Chlorobenzene	9.15	1.59	83.4	21.09
Benzene	0.10	0.04	60.8	0.18
m-Dichlorobenzene	0.10	0.05	47.7	0.13
Inoculated - length of operation = 15 days				
Chlorobenzene	9.43	1.31	86.1	21.82
Benzene	0.09	0.03	63.4	0.15
m-Dichlorobenzene	0.09	0.04	55.5	0.14

At concentrations below S_{min} the biomass will begin to diminish and will eventually disappear. High removal efficiencies (>95%) were observed in both reactors for short-term operation periods of three days or less during longer EBRT operation. Determination of microbial

densities at the lower and upper portion of both reactors indicate the biomass population was increasing during these periods of operation, and approximately equal numbers of total microbes were distributed throughout the reactor (SECTION IV). The growing population and equal distribution of microbes suggest additional chlorobenzene removal may be sustainable with longer reactor retention times. Results listed in Table 6 indicate this EBRT was between 70 and 125 min for the inoculated reactor.

Efforts to determine the concentration profile along the length of the reactor were unsuccessful. Contaminant concentrations at the Hi-Mid and Top sampling positions were consistently lower than the outlet concentrations. This discrepancy suggests that preferential flow was occurring in both reactors and further development work is needed in either the sampling procedures, the sample port configuration, or both.

2. Reactor Startup.

Initial contaminant removal by the inoculated reactor, as shown in Figure 14, reveals significant removal activity (>95%) for the first eight days of the study. From Day 8 to Day 14 the removal efficiency decreased to an average removal of 86%. Figure 13 illustrates this level of removal was sustainable through Day 31. Reduced oxygen addition (outlet concentration below 4.5 mg DO/L), switching the buffer solution from a phosphate buffer to caustic soda, and the appearance of clay-like particulates in the inlet stream all possibly contributed to the decrease in the removal efficiency observed over the operation period of Day 8 to Day 14. The reduced DO was corrected by flow adjustment, and the inlet particulates were eliminated by addition of a sand filter to the pretreatment process. Even after these problems were corrected, the chlorobenzene removal efficiency of the inoculated reactor never surpassed 90% removal for the remainder of the study. This suggests the change in the buffer solution during Day 9 diminished the reactor efficiency. Sampling for microbe density on Day 16 revealed a 20% reduction in the total colony numbers but an increase in the chlorobenzene utilizers within both reactors (see SECTION VI). Use of the more caustic buffer or possibly the reduction in the phosphate or nitrate concentration may have contributed to diminished microbial activity and lower overall microbial densities within the reactors.

The uninoculated reactor, also illustrated in Figure 14, initially showed no signs of biological activity for the first five days of operation. After Day 5, significant differences between the inlet and outlet concentrations were apparent. By Day 8, the removal activity of the indigenous reactor produced an outlet concentration equal to the outlet concentration observed for the inoculated reactor. The flow rate into the indigenous reactor was less than the flow rate into the inoculated reactor, so the corresponding rate of contaminant removal was also less. On Day 9, the indigenous reactor flow rate was increased to 2.75 L/min to match the 45-min EBRT of the inoculated reactor. The rate of buffer addition was also increased to compensate for the additional flow into the indigenous reactor. Measurement of pH on Day 10 revealed the buffer flow rate was too great, and the reactor was subjected to a pH spike of at least 9.15. Analysis of inlet and outlet samples for chlorobenzene content confirmed the biological activity within the reactor had ceased. Comparison of the chlorobenzene removal rate of each reactor was delayed several days to allow recovery of the indigenous population.

After recovery from the buffer pulse, which involved the indigenous reactor only, a steady removal of chlorobenzene was maintained from Day 15 to Day 30. Equal flow rates

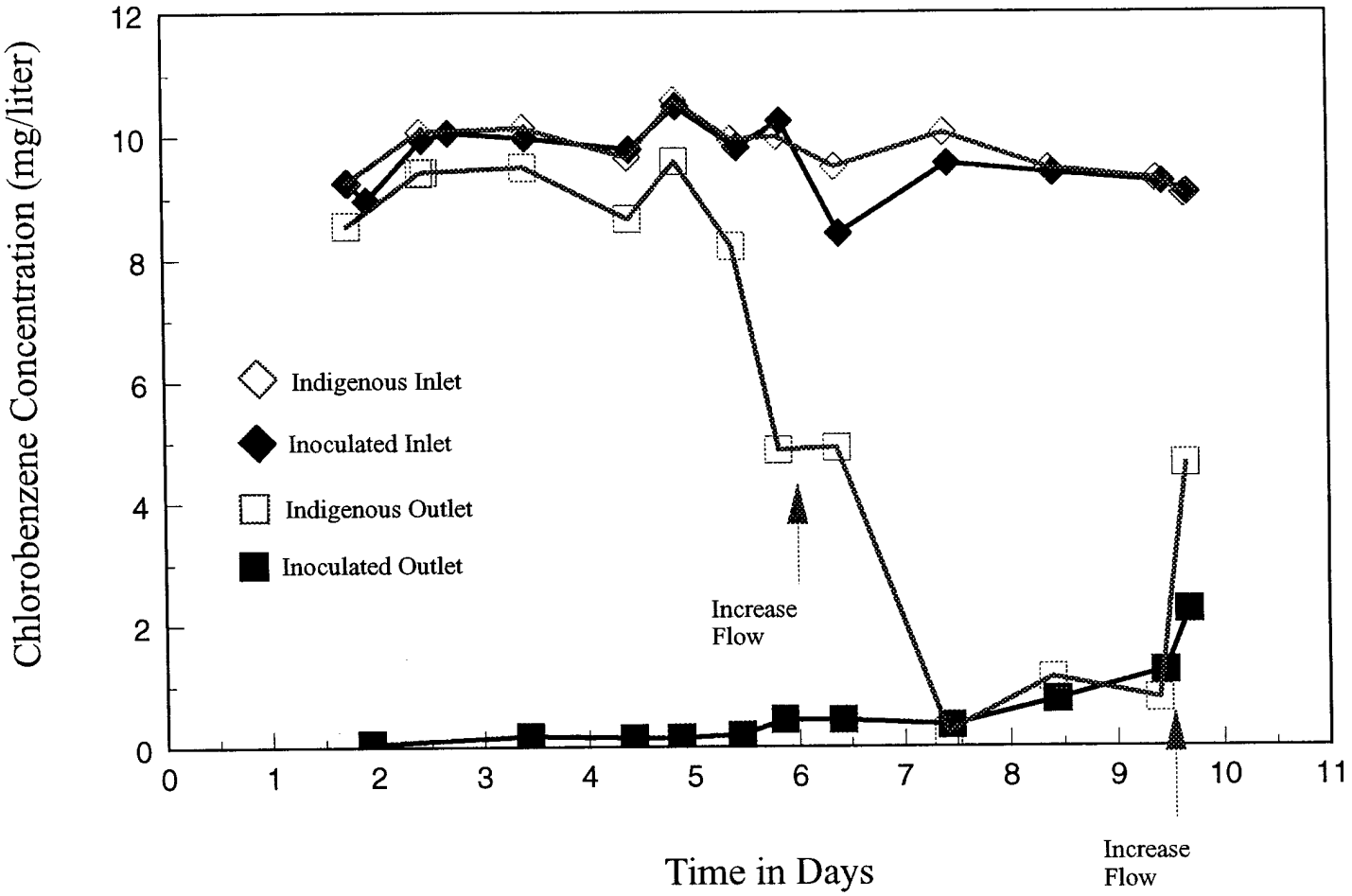


Figure 14 Inlet and Outlet Chlorobenzene Concentrations (Days 1 to 8)

were maintained for both columns during this time period to allow a direct comparison of removal efficiencies. Concentration data from the inlet and outlet ports is presented in Figure 15. Chlorobenzene removal efficiencies of 83% and 86% were observed throughout this time period for the indigenous and inoculated reactors, respectively. Three concentration peaks are apparent in the indigenous outlet. Each peak represents periods in which the effluent DO concentration fell below 4.5 mg O_2/L.

3. Treatability of Secondary Contaminants

The site water also contained trace amounts of numerous other contaminants. The secondary contaminants present in the greatest concentrations were benzene, toluene, *o*-xylene, and *m*-dichlorobenzene. Benzene was the only secondary contaminant present at concentrations above the MCL specified by Federal Drinking Water Standards. At this time, *m*-dichlorobenzene is not a regulated contaminant, but the inlet concentrations observed for *m*-dichlorobenzene fell between the MCL of the two other dichlorobenzene isomers. Bioreactor removal efficiencies for benzene and *m*-dichlorobenzene are included in the removal summary listed in Table 6. Benzene and *m*-dichlorobenzene inlet concentrations displayed greater variability throughout the length of the study than chlorobenzene concentrations. Examination of the pumping rate in the well reveals the inlet concentrations were inversely related to the total flow of the contaminant stream as illustrated in Figure 16.

Both reactors could remove a portion of the secondary contaminants, but neither reactor completely eliminated the secondary contaminants from the aqueous stream. Simultaneous degradation of methyl- and chloro-substituted benzenes by a single microorganism is considered uncommon (12). The mixed population of the indigenous reactor probably includes separate sub-populations which remove only the benzene or the chlorinated benzenes. The *Pseudomonas* sp. strain JS150 was able to utilize both benzene and m-dichlorobenzene (42) in mixed contaminant streams. Each reactor displayed approximately the same removal efficiency for benzene (approximately 85% removal for 125 min EBRTs), but neither reduced the effluent concentration below 0.028 mg/L.

The microbial population differences are more apparent for removal of m-dichlorobenzene. The inoculated reactor displayed greater removal efficiencies and consistently removed m-dichlorobenzene to a lower concentration than the indigenous reactor for all flow rates. At the longest residence time (125 min EBRT), the *Pseudomonas* sp. strain JS150 isolate was able to reduce the *m*-dichlorobenzene to concentrations which were detectable but below the quantification limit of 0.01 mg/L (one-third the lowest calibration standard). Removal of m-dichlorobenzene by the indigenous reactor was very similar to the benzene removal efficiency at all flow rates.

Utilization of secondary contaminants has been reported to increase as the inlet concentration is decreased (45,46). A decrease in the inlet concentration of secondary contaminants was observed during this study during the 20-min residence time trial. A decrease in the removal efficiencies for benzene and *m*-dichlorobenzene was observed rather than an increase. The decreased efficiency may also indicate that changing the buffer solution proved detrimental to the system.

Samples were gathered from each reactor for HPLC analysis of fatty acid content. Results indicate that the inlet stream contained 3.7 ppm acetic acid, (SD 0.9) and trace amounts

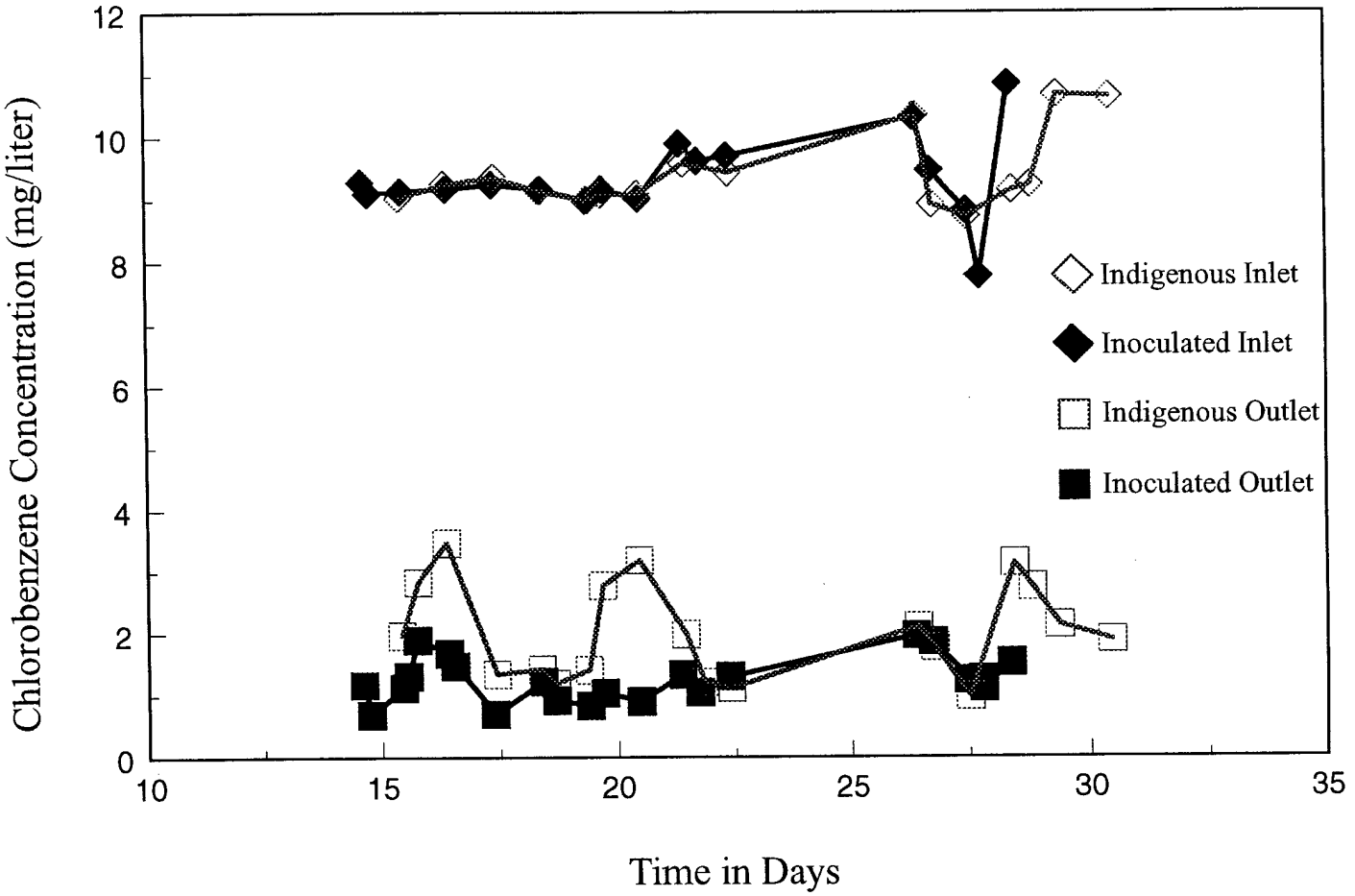


Figure 15 Inlet and Outlet Chlorobenzene Concentrations (Days 15 to 30)

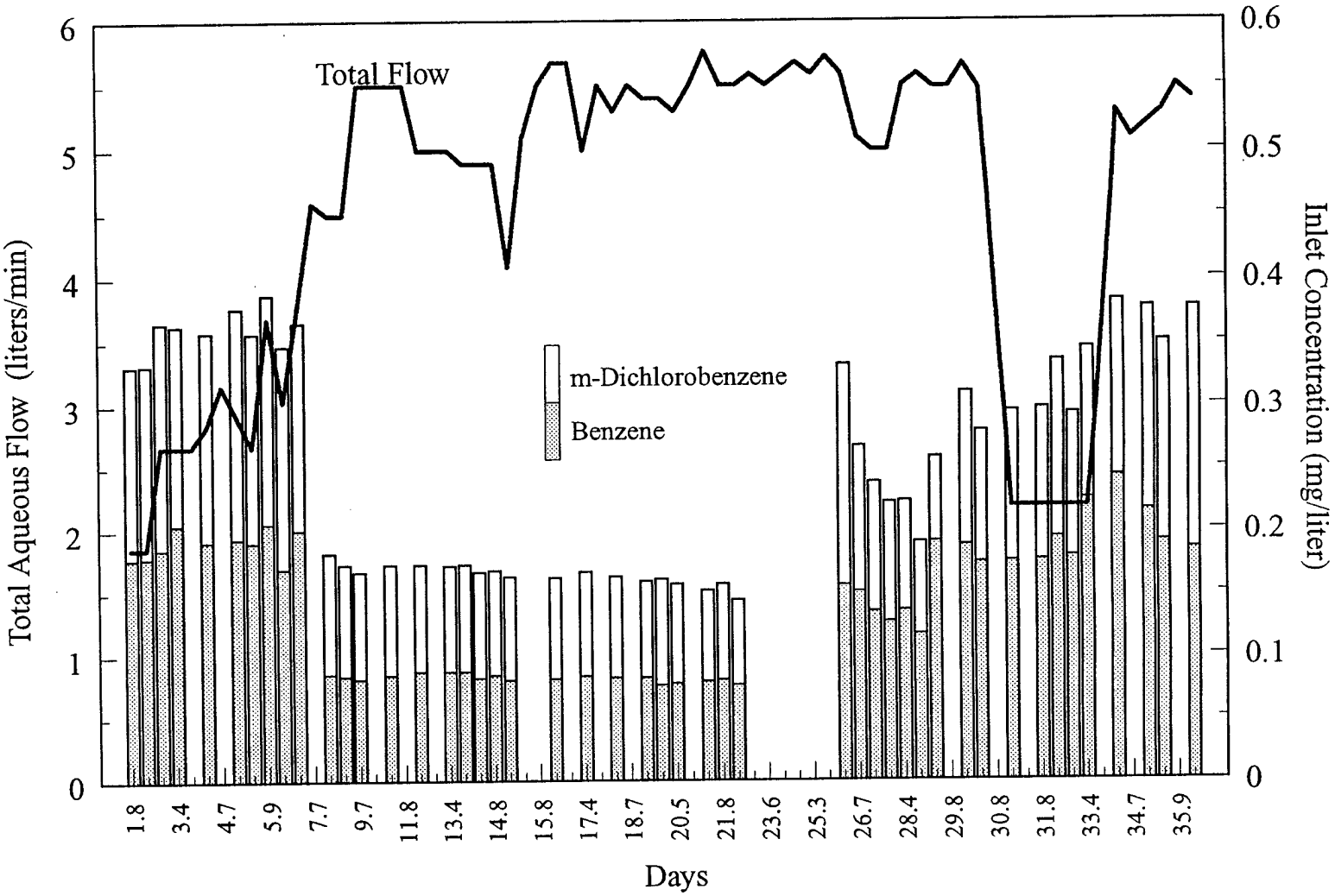


Figure 16 Effect of Flow Rate on Secondary Contaminant Concentration

of formic acid. The samples gathered at the bottom port indicate that the acetic acid was removed very rapidly within the bottom mixing chamber. Acetic acid was not detected above the bottom mixing chamber. These results demonstrate that short chain fatty acids were not competing with the ground water contaminants as a carbon and energy source for microbial growth. These results also indicate that the oxygen consumption in the reactors was due to the metabolism of the ground water contaminants and not the short chain fatty acids. Sample analysis results are listed in Appendix B.

4. Effect of pH

The low pH of the inlet stream required the addition of a buffer solution to increase the inlet pH to a range of 6.8 to 7.2. The monitoring results are presented in Appendix A. Addition of the buffer solution was not started until Day 5 and the inoculated reactor was subjected to a lower inlet pH, 6.1 to 6.4, for the first four days of operation. The bioreactor population of the *Pseudomonas* sp. strain JS150 was tolerant of this brief period of lower pH values and produced high chlorobenzene removal efficiencies. The pH of the inoculated reactor also fell to 6.1 during prefield preparation, inhibiting chlorobenzene removal. Similar brief periods of acidic tolerance were reported in previous bench-scale studies (discussed in SECTION II) in which the pH fell below 6.0. Two episodes of transient pH increases were encountered over the course of the field study in which the pH jumped above 9.0. The first buffer spike affected only the indigenous column and completely inactivated the chlorobenzene removal ability of the biofilm. The second episode occurred on Day 30 when the buffer addition rate was not reduced in response to a decrease in the ground water flow rate and resulted in greatly diminished contaminant removal capability in both reactors. The contaminant removal ability of the inoculated reactor, though greatly diminished, was not inhibited as greatly as the indigenous reactor. One explanation is that the indigenous microorganisms were naturally acclimated to a slightly acidic environment (inlet pH of 6.1) and may have been more sensitive to alkaline conditions. Alternatively, the flow patterns within the inoculated reactor may have been more channelized so the pH increase affected only a limited portion of the reactor. The possibility of channeling is also supported by the problems previously discussed concerning determination of the concentration profile. Additional trials would be required to determine if adding buffer to adjust the pH is actually required to maintain the biofilm activity within the reactors.

5. Effect of Dissolved Oxygen Content

The inlet stream had a low DO content of 1.59 mg O_2/L The low DO may be a result of oxygen scavenging by the iron present in the water or is possibly an indicator of up gradient aerobic biological activity. The low inlet stream DO required oxygen addition for aerobic degradation. Periods of operation which displayed diminished chlorobenzene removals correspond to time periods when a reduced amount of DO was detected in the outlet stream. Decreases in removal efficiencies may also be the result of increasing the flow rate if the contaminant removal was limited by degradation kinetics. To collectively examine flow rate and total amount of degradation obtained in a reactor, the values are combined into a rate of chlorobenzene removal. Figures 17 and 18 display the effect of DO on the amount of chlorobenzene removed per time within each reactor. In every case except one, the removal

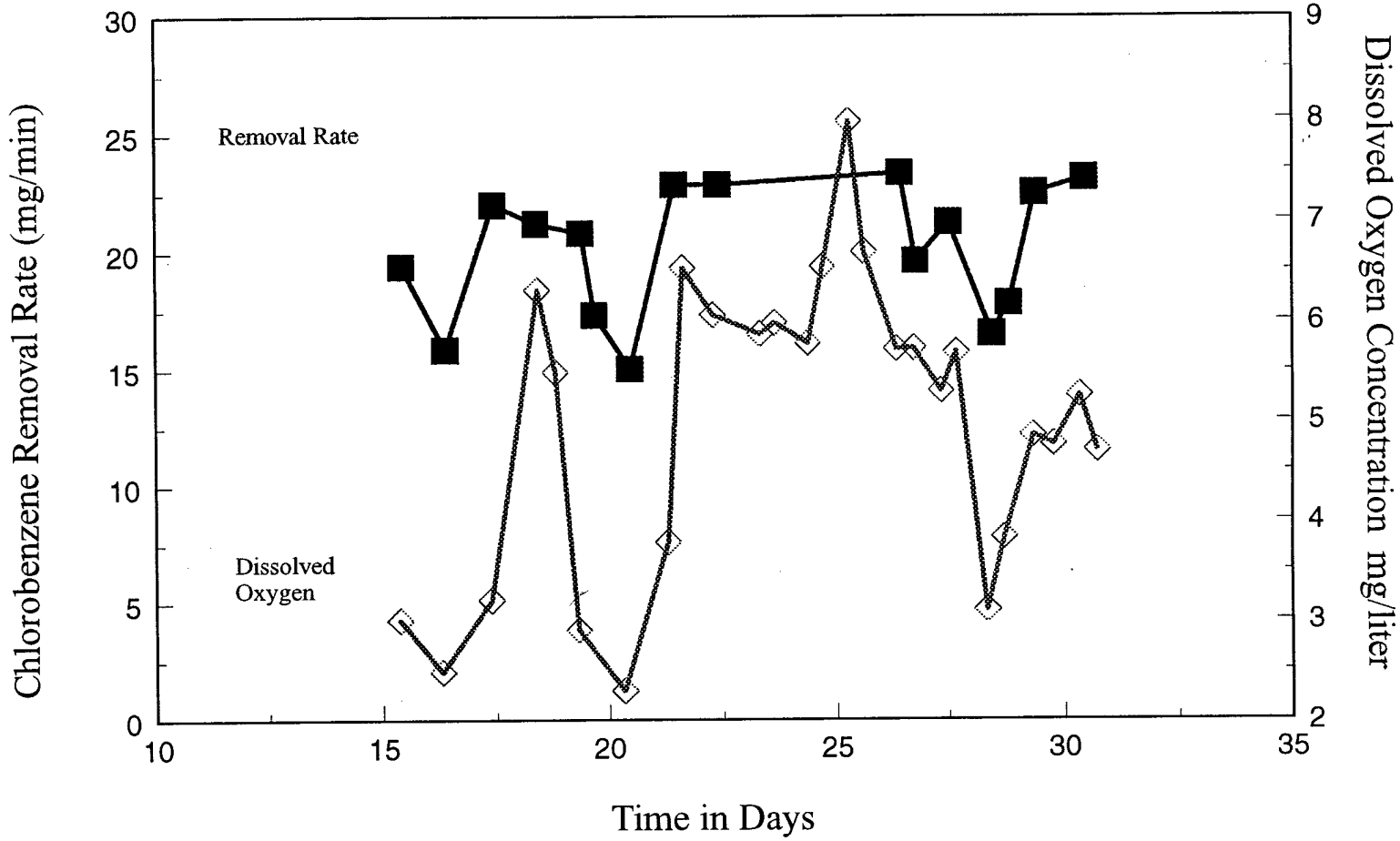


Figure 17 Uninoculated Reactor - Relationship of DO and Removal Rate

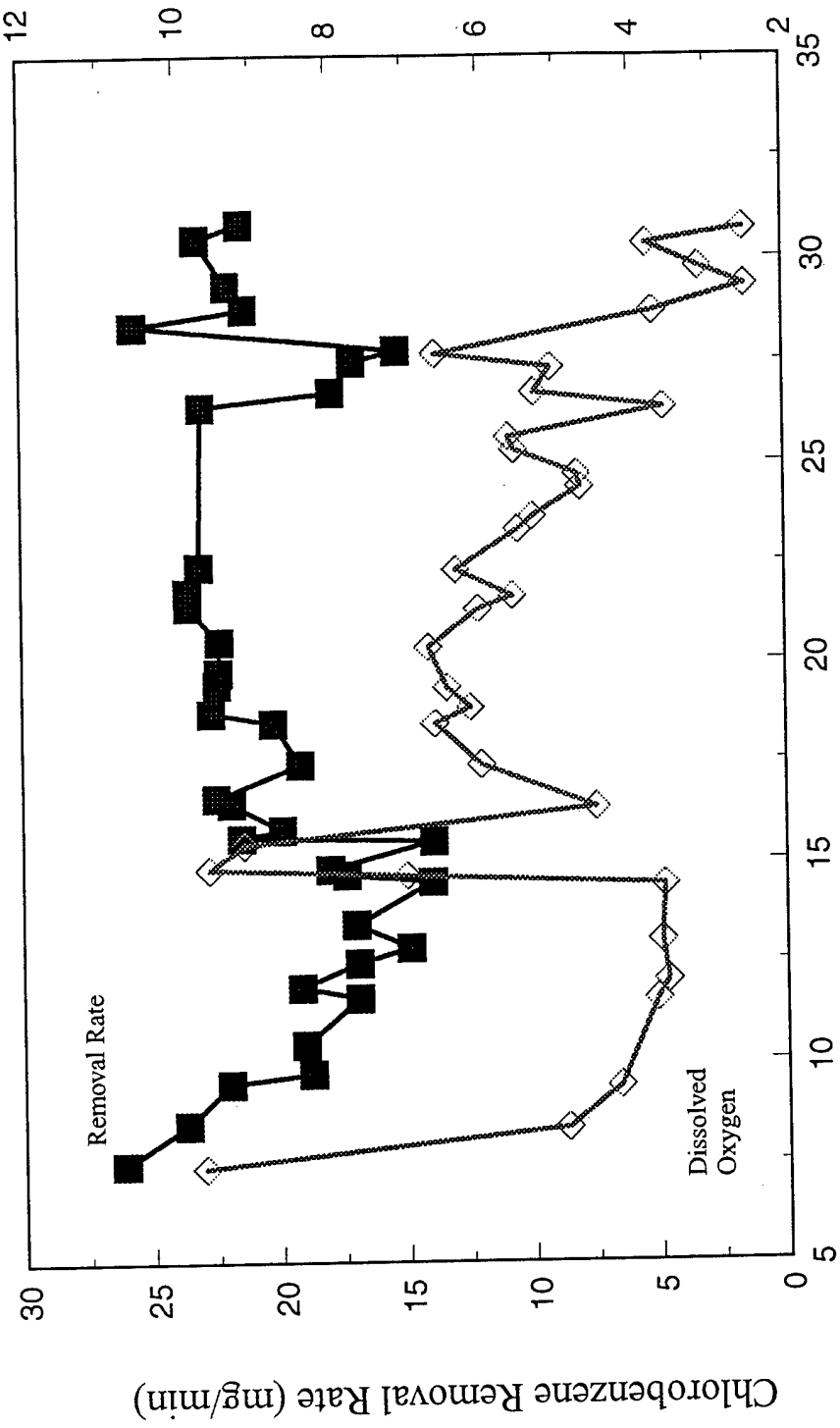


Figure 18 Inoculated Reactor - Relationship of DO and Removal Rate

rate of chlorobenzene mirrored the increase or decrease of DO in the outlet stream. The single exception occurred for the inoculated reactor at approximately Days 15 to 16. The exception of decreasing chlorobenzene removal rate during increased DO content was caused by a sudden reduction of the inlet flow rate into the reactor. Decreasing the flow rate lowers the rate chlorobenzene is introduced into the system and causes the DO content to increase at the same time. In general, as the DO level in the outlet stream fell below the range of 4.0 - 4.5 mg/L, the contaminant removal rate also decreased. The DO content of 4.0 mg/L appears to be a lower limit of oxygen availability for the microbial population. Contaminant removal in other fixed film systems has also been observed to be oxygen-limited when the DO was present at or below 4.0 mg/L (47). The fraction of the chlorobenzene removal which can be identified as losses due to stripping from the water is less than 0.5%.

E. CONCLUSIONS

Results of this field study indicated biological treatment of chlorobenzene was effective throughout the entire length of the field study. Both reactors provided substantial chlorobenzene removal, >95%. The reactor inoculated with the *Pseudomonas* sp. strain JS150 required an EBRT 70 to 125 minutes to produce an outlet chlorobenzene concentration equal to or less than the 0.1 mg/liter MCL specified by EPA Drinking Water Standards. Inoculation of a reactor before system startup provided immediate contaminant removal ability. Neither reactor was effective at reducing the benzene concentration to below the EPA Drinking Water Standard MCL, but the inoculated reactor proved more effective at removing *m*-dichlorobenzene. Both indigenous and inoculated biofilms were tolerant of transient lowering of the pH to 6.0 to 6.1 levels, but neither biofilm could survive pH increases above 9. DO becomes limiting when effluent concentrations fall below 4.0 to 4.5 mg O_2/L range.

SECTION IV

MICROBIOLOGY MONITORING

A. OBJECTIVE

The objective of monitoring the microbial population of the field reactors was to examine the following parameters:

1. Rate of colonization of packing material by indigenous bacteria.
2. Distribution of bacteria within reactors.
3. Possible displacement of inoculated strain by the indigenous bacteria.

B. INTRODUCTION

Technologies for treatment of contaminated ground water brought to the surface with pump-and-treat systems include air-stripping, carbon sorption, solar detoxification, and bioremediation. Bioremediation provides the potential for complete destruction of pollutants at a fraction of the cost of the other options. A key question in the use of bioremediation is whether inoculation with microorganisms provides an advantage over the use of indigenous strains. To help evaluate this question, separate biomass populations were utilized in the dual pilot-scale bioreactors (Section III). One reactor was pre-inoculated with *Pseudomonas* sp. strain JS150, a strain selected for its ability to colonize the reactor packing and degrade a wide variety of chlorinated compounds. The indigenous bacteria in the contaminated ground water were allowed to colonize the second reactor. The reactors were operated for 36 days at various flow rates, while degradation rates and microbial populations were monitored. Operation of the pilot-scale bioreactors was periodically interrupted to allow sampling of the packing material. Bacteria from the sampled packing material was then evaluated for total microbes, chlorobenzene utilizers in general, and the presence of *Pseudomonas* sp. strain JS150.

C. METHODS AND MATERIALS

1. Media

Nutrient agar and MSB were diluted to one-tenth strength and one-half strength, respectively (41). The buffer and nutrient solution contained 2.58 M K_2HPO_4, 0.37 M KH_2PO_4 and 0.35 M $NaNO_3$ at a pH of 8.5. The phosphate buffer was replaced with a sodium bicarbonate buffer (1.87 M Na_2CO_3, 0.04M KH_2PO_4, and $NaNO_3$) at a pH of 13 on Day 9. Dilutions blanks were made with full strength MSB. Colony forming units (CFU) were enumerated by spreading appropriate dilutions on plates containing one-half strength MSB agar at pH 7.0 with bromthymol blue (0.04 g/L) as a pH indicator.

2. Organisms

Use of the *Pseudomonas* sp. strain JS150 was described previously. *Pseudomonas* sp.

strain JS150 was originally isolated for its ability to grow on 1,4-dichlorobenzene (12). It also has been shown to grow on toluene, chlorobenzene, salicylate, naphthalene, phenol, benzene, ethylbenzene, p-hydroxybenzoate, benzoate, and several 1,4-dihalogenated benzenes (42).

3. Microbial Sampling

Both reactors were filled with water from the test well and operated in the recycle mode with oxygen added at a rate of 80 ml/min for 14 h after which the Time 0 sampling was performed. At the appropriate times, 1 to 3 pellets of column packing material were removed aseptically, weighed to approximately 1 g wet weight, and crushed by circular grinding for 5 min with a mortar and pestle in 4.5 ml one-half strength MSB. Samples were spread on MSB media and nutrient agar. Samples on MSB media were incubated in the presence of chlorobenzene vapor at 22°C as described previously (48). Control plates were treated as above but without chlorobenzene vapor.

4. Bacterial Identification

Pseudomonas sp. strain JS150 was differentiated from indigenous strains by catalase and oxidase tests and the ability to grow on naphthalene, toluene, chlorobenzene, and o-DCB (42).

5. Reactor Inoculation

a. Uninoculated Reactor - The uninoculated reactor was filled with water from the test well and operated in the recycle mode with oxygen added at a rate of 80 ml/min for 14 hours, after which the first sampling was performed and designated Time 0. The ground water flow rate was maintained at 1 L/min for the next seven days.

b. Inoculated Reactor -The inoculated reactor was inoculated at Tyndall AFB with 27 L of MSB medium containing a culture (1.2 absorption units at 600 ηm) of *Pseudomonas* sp. strain JS150 grown on chlorobenzene. The reactor was then operated in the recycle mode for the next six days while supplying oxygen at 135 ml/min which contained approximately 3.2 mg/L vaporous chlorobenzene. All flows to the reactor were then shut off, and the system was transported to the field. One day was required for transport, and one day was needed for reactor setup. Thus, air flow and water recycle were reestablished within 48 hours. Contaminated ground water flow was started the following day.

D. RESULTS

1. Uninoculated Reactor Microbial Colonization

The uninoculated reactor top port contained 8.6×10^5 CFU/g of pellet material at Time 0 with no chlorobenzene degraders present (Tables 7 and 8). After two days, the entire reactor was contaminated with *Pseudomonas* sp. strain JS150, after which there was a significant

increase in chlorobenzene removal as illustrated in Figure 13. The cross contamination of the reactors possibly originated from the common drain line during system shutdown to allow the packing material to be sampled. As a result of the change in pH on Day 9 (discussed in Section III), all biodegradation was inhibited until Day 12. The data was insufficient to determine whether the pH extreme reduced the microbial numbers, inhibited biodegradation, or both. Although the actual numbers vary from the top and bottom sampling ports in the indigenous reactor, a similar increase in chlorobenzene degraders occurred at both top and bottom ports. (Table 8). By Day 16, the numbers of chlorobenzene degraders had risen to 100% of bacteria present.

TABLE 7 ENUMERATION OF BACTERIA ON NUTRIENT AGAR

Time (days)	Uninoculated Reactor		Inoculated Reactor	
	Top Port	Bottom Port	Top Port	Bottom Port
0	0.86[a] (0.6)[b]	0.51 (0.09)	364 (29)	307 (13)
1	0.65 (0.11)	0.81 (0.7)	450 (7)	419 (3)
2	3.5 (0.6)	1.7 (0.5)	418 (20)	612 (5)
4	17 (17)	23 (6)	613 (41)	887 (18)
8	80 (45)	86 (5)	508 (16)	595 (38)
16	15 (38)	12.8 (4)	110 (35)	150 (34)
36	23 (17)	13 (2)	44 (10)	34 (5)

[a] Cell counts expressed as 10^6 cells per g of pellet.
[b] Standard deviation given in parenthesis.

TABLE 8 ENUMERATION OF BACTERIA ON CHLOROBENZENE MEDIA

Time (days)	Uninoculated Reactor		Inoculated Reactor	
	Top Port	Bottom Port	Top Port	Bottom Port
0	0.0[a]	0.0	1.0 (0.15)[b]	0.45 (0.07)
1	0.0067 (0.0003)	0.0064 (0.0004)	1.6 (1.1)	2.9 (1.2)
2	0.014 (0.005)	0.003 (0.001)	2.0 (1.3)	3.5 (2.5)
4	0.648 (0.5)	0.134 (0.04)	1.4 (0.8)	1.6 (0.7)
8	28.9 (15)	5.8 (1.6)	2.6 (0.9)	4.0 (2.2)
16	20.9 (53)	18.5 (12)	7.5 (5)	29.4 (13)
36	35.5 (5)	2.2 (22)	40.6 (11)	19.6 (12)

[a] Cell counts expressed as 10^6 cells per g of pellet.
[b] Standard deviation given in parenthesis.

2. Indigenous Colonization of Inoculated Reactor

The top port of the inoculated reactor contained 3.6×10^8 organisms/g of pellets and 1.0×10^6 chlorobenzene degraders at Time 0 (Tables 7 and 8). The number of culturable organisms remained fairly constant from Time 0 to Day 8 (Table 7); the same was true for the bottom port. Initially, the number of bacteria present at the bottom port of the inoculated reactor that degraded chlorobenzene was 4.47×10^5 bacteria/g of pellets. This number increased six-fold by the next 24 hour period This rapid increase was surprising and may be the result of a sampling error at Time 0. During Days 1 through 8, a stable bacterial population was maintained with cell numbers similar for the bottom and top ports. A decrease in total bacterial populations was observed from Day 8 to Day 36, reaching 4.3 and 3.3×10^7 cells/g of pellets for the top and bottom ports, respectively. The low number of total bacterial populations on Day 36 may have been a direct result of the previously discussed pH spike which occurred on Day 30 prior to the sampling. The number of chlorobenzene degraders increased throughout the reactor during the first 16 days of operation. By Day 16, the indigenous population had increased to make up 37% in the top port and 47% in the bottom port of the chlorobenzene degrading population (Table 9). *Pseudomonas* sp. strain JS150 was not detected after Day 16, but the overall chlorobenzene degrading population was observed to have increased for the top port and declined at the bottom port when sampled on Day 36.

The indigenous organisms were catalase positive, oxidase negative. The indigenous organisms were unable to grow on naphthalene or toluene but demonstrated growth on chlorobenzene and *o*-dichlorobenzene. Ten randomly selected indigenous isolates were examined with Biolog GN Microplates (Biolog, Inc., Hayward Calif.) but were only poorly identified as *Pseudomonas glathei* or Genus identification *Pseudomonas fluorescens* subgroup C.

TABLE 9 PERCENTAGE OF TOTAL BACTERIA THAT WERE PSEUDOMONAS SP. STRAIN JS150

Time (days)	Uninoculated Reactor				Inoculated Reactor			
	Top Port		Bottom Port		Top Port		Bottom Port	
0	0/10[a]	0%	0/10[a]	0%	30/30	100%	30/30	100%
1	0/10[a]	0%	0/10[a]	0%	30/30	100%	29/30	97%
2	22/30	73%	3/10	30%	30/30	100%	30/30	100%
4	27/30	90%	30/30	100%	27/30	90%	29/30	97%
8	30/30	100%	29/30	97%	28/30	93%	16/30	53%
16	1/30	3%	0/30	0%	19/30	63%	16/30	53%
36	0/30	0%	0/30	0%	0/30	0%	0/30	0%

[a] Isolates grew poorly on chlorobenzene without production of yellow color on pH indicator plates.

E. CONCLUSIONS

These data suggest that strain *Pseudomonas* sp. strain JS150 can degrade chlorobenzene under field conditions and that it can be replaced by indigenous chlorobenzene degraders. The initial colonization of the uninoculated reactor by strain *Pseudomonas* sp. strain JS150 would suggest that during the first days of bioreactor operation strain *Pseudomonas* sp. strain JS150 multiplied. One possible explanation for this is that the buffer used for the first eight days was potassium phosphate with ammonium sulfate as a nitrogen source. Perhaps this buffer encouraged the survival of strain *Pseudomonas* sp. strain JS150. This nutrient-buffer was shown in preliminary studies to be favorable for culturing *Pseudomonas* sp. strain JS150 in the laboratory. Although strain *Pseudomonas* sp. strain JS150 was replaced by the indigenous bacteria between Day 8 and Day 16, the inoculated column demonstrated immediate and rapid removal of contaminants from the beginning of the experiment. This suggests that inoculation may be useful for reducing start-up time for reactors in the field.

During the course of this study, the bioreactor was subjected to excessive pH shifts. The alkaline pH extremes inhibited 90% to 100% of the chlorobenzene degradation for two three-day periods. After three days, there was a rapid increase in biodegradation. After five days, the degradation rates had returned to the original levels. Rapid recovery of the bioreactor degradation rates indicated that all the bacteria were not killed and suggested that only the outer layers of the pellet material were affected by the pH extremes. Organisms deep within the pellets could provide an inoculum for recolonizing the bioreactor. Previous studies have indicated that bacteria penetrate the diatomaceous earth pellets which may afford the organisms some protection to environmental conditions (49).

Degradation intermediates were not detected from either the inoculated or uninoculated reactor. This was known for strain *Pseudomonas* sp. strain JS150 in the laboratory, but it was important to verify this under field conditions.

SECTION V

CONCLUSIONS

Results of the laboratory- and pilot-scale studies indicate chlorobenzene can be successfully remediated with biological treatment. The *Pseudomonas* sp. strain JS150 is an effective inoculant for biological reactor systems for the treatment of chlorobenzene-contaminated ground water. The *Pseudomonas* sp. strain JS150 readily populates the Manville Celite support material, and, once established, the *Pseudomonas* sp. strain JS150 displays tolerance and rapid recovery to temporal pH shifts. DO content is directly related to the chlorobenzene utilization rate and becomes limiting when effluent concentrations fall below 4.0 to 4.5 mg O_2/L range. Use of the caustic buffer solution to adjust solution pH limited the maximum removal efficiency which could be attained in the field study. Inoculating a reactor with a selected strain provides the benefits of immediate and rapid contaminant removal ability. This suggests that inoculation may be useful for reducing start-up time for reactors in the field.

SECTION VI

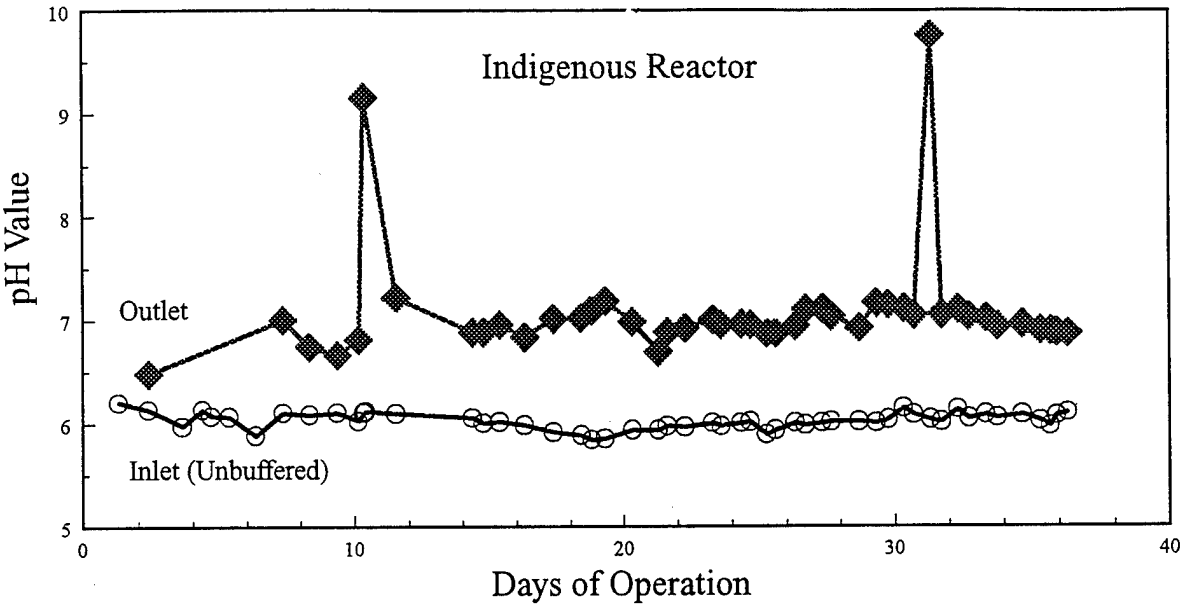
RECOMMENDATIONS

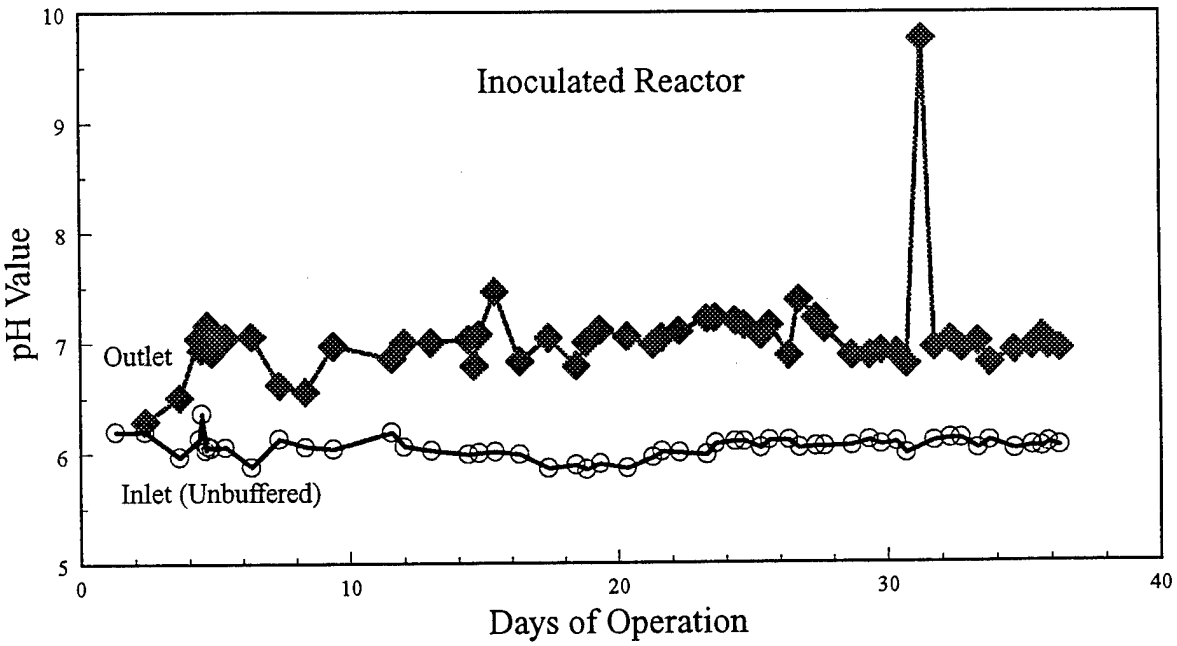
The presence of the high iron concentrations within the ground water at the Robins AFB, GA, site possibly precludes the use of any *in-situ* aerobic treatment option and may limit treatment schemes to above-ground systems. Biological treatment can effectively reduce chlorobenzene concentrations and should be considered as a possible treatment option. Inoculation of the reactor prior to use with an isolate like the *Pseudomonas* sp. strain JS150 strain is encouraged in order to provide immediate chlorobenzene treatment capacity and minimize startup time at the site. A less caustic buffering solution should be employed to encourage more biomass growth and enhance the removal efficiency. Additionally, utilization of the recycle stream during the flow-through operation would provide greater resistance to upsets. Complete removal of the secondary contaminants could not be achieved with this particular packed-bed reactor configuration, and further treatment will be required (especially for benzene). A second treatment process such as activated carbon is needed to reduce the benzene concentrations to acceptable levels. However, use of a bioreactor as a pretreatment could result in an order of magnitude, or greater, reduction in the contaminant loading onto the carbon and greatly reduce the carbon usage rate. Alternatively, use of a fluidized-bed or expanded-bed reactor may provide higher treatment capacities and also be resistant to system upsets. Further study would be required to determine whether a different reactor configuration would eliminate the need for a secondary treatment system such as an activated carbon unit. An overall economic analysis would be required to properly size a minimum cost treatment system.

Acknowledgments: The authors would like to thank Billy Haigler and Shirley Nishino for excellent technical advice and Erica Becvar for critical reading of the manuscript. This research was supported in part by an appointment to the Postgraduate Research Program at the Tyndall AFB administered by the Oak Ridge Institute for Science and Education through an interagency agreement between the U.S. Department of Energy and Tyndall AFB.

APPENDIX A

REACTOR pH MONITORING DATA





APPENDIX B

FATTY ACID ANALYSIS RESULTS

Operating Conditions:

Uninoculated Reactor
- Flow Rate - 2750 ml/min
- Inlet CB Conc - 9.90 mg/L
- Outlet CB Conc - 5.60 mg/liter
- Outlet pH - 6.87
- Outlet DO - 5.71 mg O_2/L

Inoculated Reactor
- Flow Rate - 2649 ml/min
- Inlet CB Conc - 10.11 mg/L
- Outlet CB Conc - 2.34 mg/liter
- Outlet pH - 6.94
- Outlet DO - 4.19 mg O_2/L

Fatty Acid Analysis Results
All values listed as mM x 10^4

Sample	ID #	UV-Formic[a]	CON-Formic[b]	UV-Acetic	CON-Acetic
Indigenous Inlet	1	0.0	0.0	719.4	648.2
Bottom	2	0.0	35.8	0.0	131.7
LoMid	3	0.0	0.5	0.0	0.0
HiMid	4	0.0	31.7	0.0	0.0
Top	5	0.0	24.0	0.0	0.0
Outlet	6	0.0	4.7	0.0	0.0
Inoculated Inlet	7	0.0	8.3	334.9	654.5
Bottom	8	0.0	35.9	0.0	0.0
LoMid	9	0.0	0.0	0.0	0.0
HiMid	10	0.0	70.8	0.0	0.0
Top	11	0.0	0.0	0.0	0.0
Outlet	12	0.0	0.0	0.0	0.0
Blank	13	0.0	0.0	0.0	0.0
Indigenous Inlet	14	0.0	24.8	568.8	758.0
Inoculated HiMid	15	0.0	0.0	0.0	0.0

[a]UV- optical detector
[b]CON- conductivity detector

REFERENCES

1. US Public Health Service, Toxicological Profile for Chlorobenzene, ATSDR/TP-09-06, 1990.

2. Anthony, R.M. and L.H. Breimhurst, "Determining Maximum Influent Concentrations of Priority Pollutants for Treatment Plants," Journal WPCF, vol 53(10), pp. 1457-1468, 1981.

3. Martin, E.J. and J.H. Johnson, Hazardous Waste Management Engineering, Chapter 3, Van Nostrand Reinhold Company Inc., pp. 81-226, 1987.

4. Schmidt, E., M. Hellwig, and H.J. Knackmuss, "Degradation of Chlorophenols by a Defined Mixed Microbial Community," Appl. Environ. Microbiol., vol 46(5), pp. 1038-1044, 1983.

5. Schmidt, E.,"Response of a Chlorophenols Degrading Mixed Culture to Changing Loads of Phenol, Chlorophenol and Cresols," Appl. Microbial. Biotechnol., vol 27, pp. 94-99, 1987.

6. Reineke, W. and H.J. Knackmuss, "Microbial Metabolism of Haloaromatics: Isolation and Properties of a Chlorobenzene-Degrading Bacterium," Appl. Environ. Microbiol., vol 47(2), pp. 395-402, 1984.

7. Schraa, G., M.L. Boone, N.S.M. Jetten, A.R.W. van Neerven, P.J. Colberg, and A.J.B. Zehnder, "Degradation of 1,4-Dichlorobenzene by *Alcaligenes* sp. Strain A175," Appl. Environ. Microbiol., vol 52(6), pp. 1374-1380, 1986.

8. Spain, J.C. and S.F. Nishino, "Degradation of 1,4-Dichlorobenzene by a *Pseudomonas* sp.," Appl. Environ .Microbiol., vol 53(5), pp. 1010-1019, 1987.

9. Spain, J.C. and D.T. Gibson, "Oxidation of Substituted Phenols by *Pseudomonas putida* F1 and *Pseudomonas* sp Strain JS6," Appl. Environ. Microbiol., vol 54(6), pp. 1399-1404, 1988.

10. Haigler, B.E., and J.C. Spain, "Degradation of p-Chlorotoluene by a Mutant of *Pseudomonas* sp. Strain JS6," Appl. Environ. Microbiol., vol 55(2), pp. 372-379, 1989.

11. van der Hoek, L.G.C.M. Urlings, and C.M. Grobben, "Biological Removal of Polycyclic Aromatic Hydrocarbons, Benzene, Toluene, Ethylbenzene, Xylene, and Phenolic Compounds from Heavily Contaminated Ground water and Soil," Environ. Tech. Lett, vol 10, pp. 184-194, 1989.

12. Pettigrew, C.A., B.E. Haigler, and J.C. Spain, "Simultaneous Biodegradation of Chlorobenzene and Toluene by a *Pseudomonas* Strain," Appl. Environ. Microbiol., vol 57, pp. 157-162, 1991.

13. Fritz, H. W. Reineke, E. Schmidt, "Toxicity of Chlorobenzene on *Pseudomonas* sp. Strain RHO1, a Chlorobenzene-Degrading Strain," Biodegradation, vol 2, pp. 165-170, 1992.

14. Nishino, S.F., J.C. Spain, L.A. Belcher, and C.D. Litchfield, "Chlorobenzene Degradation by Bacteria Isolated from Contaminated Groundwater," Appl. Environ. Microbiol., vol 58(5), pp. 1719-1726, 1992.

15. Miller, G.P., R.J. Portier, D.G. Hoover, D.D. Friday, J.L. Sicard, "Biodegradation of Chlorinated Hydrocarbons in an Immobilized Bed Reactor," Environ. Progress, vol 9(3), pp. 161-164, 1990.

16. Portier, R.J., J.A. Nelson, J.C. Christianson, J.M. Wilkerson, R.C. Bost, and B.P. Flynn, "Biotreatment of Dilute Contaminated Ground Water Using an Immobilized Microbe Packed Bed Reactor," Environ. Progress, vol 8(2), pp. 120-125, 1989.

17. Friday, D.D. and R.J. Portier, "Development of an Immobilized Microbe Bioreactor for VOC Application," Environ. Progress, vol 10(1), pp. 30-39, 1991.

18. Flathman, P.E., D.E. Jerger, and P.M. Woodhull, "Remediation of Dichloromethane (DCM)-Contaminated Ground Water," Environ. Progress, vol 11(3), pp. 202-209, 1992.

19. Portier, R.J., S.J. Palmer, S.E. Lantz, and K. Fugisaki, "Application of Immobilized Bacteria Technology for the Degradation of Chemicals in Aqueous Waste Streams," presented at the 197th ACS National Meeting, Dallas, TX, April 9-14, 1989, vol 29(1), pp. 79-81, 1988.

20. CH2M Hill, Inc. RI/FS Report Zone 3 for Robins AFB GA, Oct 1990.

21. Calabrese, E.J., C.E. Gilbert, and H. Pastides, Safe Drinking Water Act, amendments, regulations and standards, 1st ed., Lewis Publishers, Chelsea, Michigan, 1989.

22. US Public Health Service, Toxicological Profile for Xylenes, ATSDR/TP-90-30, 1990.

23. Applegate, D.H. and J.D. Bryers, "Effects of Carbon and Oxygen Limitations and Calcium Concentrations on Biofilm Removal Processes," Biotech. and Bioeng., vol 37, pp. 17-25, 1991.

24. Kinner, N.E. and T.T. Eighmy, "Biological Fixed-Film Systems," Journ WPCF, vol 59(6), pp. 395-401, 1987.

25. Characklis, W.G., "Fouling Biofilm Development: A Process Analysis," Biotech. and Bioeng., vol 23, pp. 1923-1960, 1981.

26. Banks, M.K. and J.D. Bryers, "Bacterial Species Dominance Within a Binary Culture

Biofilm," Appl. Environ. Microbiol., vol 57(7), pp. 1974-1979, 1991.

27. Trulear, M.G. and W.G. Characklis, "Dynamics of Biofilm Processes," Journ. WPCF, vol 54(9), pp. 1288-1301, 1982.

28. La Motta, E.J., "Kinetics of Growth and Substrate Uptake in a Biological Film System," Appl. Environ. Micorbiol., vol 31(2), pp. 286-293, 1976.

29. Siegrist, H. and W. Gujer, "Mass Transfer Mechanisms in a Heterotrophic Biofilm," Water Research, vol 19(11), pp. 1369-1378, 1985.

30. Logan, B.E. and J.W. Dettmer, "Increased Mass Transfer to Microorganism with Fluid Motion," Biotech. and Bioeng., vol 35, pp. 1135-1144, 1990.

31. Lewandowski, Z., G. Walser, and W.G. Characklis, "Reaction Kinetics in Biofilms," Biotech. and Bioeng., vol 38, pp 877-882, 1991.

32. Siebel, M.A. and W.G. Characklis, "Observations of Binary Population Biofilms," Biotech. and Bioeng., vol 37, pp. 778-789, 1991.

33. Steinmeyer, D.E. and M.L. Shuler, "Continuous Operation of a Pressure-Cycled Membrane Bioreactor," Biotechnol. Prog., vol 6, pp. 286-291, 1990.

34. Park, C.H., M.R. Okos, and P.C. Wankat, "Characterization of an Immobilized Cell, Trickle Bed Reactor During Long Term Butanol (ABE) Fermentation," Biotech. and Bioeng., vol 36, pp. 207-217, 1990.

35. Buisman, C.J., B. Wit, and G. Lettinga, "Biotechnological Sulphide Removal in Three Polyurethane Carrier Reactors: Stirred Reactor, Biorotor Reactor and Upflow Reactor," Water Research, vol 24(2), pp. 245-251, 1990.

36. Armenante, P.M., G. Lewandowski, and I.U. Haq, "Mineralization of 2-Chlorophenol by *P. Chrysosporium* Using Different Reactor Designs," Hazardous Waste & Hazardous Materials, vol 9(3), pp. 213-229, 1992.

37. Phelps, T.J., J.J. Niedzielski, K.J. Malachowsky, R.M. Schram, S.E. Herbes, and D.C. White, "Biodegradation of Mixed-Organic Wastes by Microbial Consortia in Continuous-Recycle Expanded-Bed Bioreactors," Environ. Sci. Tech., vol 25(8), pp. 1461-1465, 1991.

38. Hooijmans, C.M., M.L. Stoop, M. Boon and K.C.A.M. Luyben, "Comparison of Two Experimental Methods for the Determination of Michaelis-Menton Kinetics of an Immobiolized Enzyme," Biotech. and Bioengg., vol 40, pp. 16-24, 1992.

39. Cooney, M.J. and K.A. McDonald, "The Monod Constant for Growth for *Candida utilis* on Ammonium in Continuous and Batch Cultures," Biotechnol. Prog., vol 9(1), pp. 93-96,

1993.

40. Rittmann, B.E. and P.L. McCarty, "Model of Steady-State-Biofilm Kinetics," Biotech. and Bioeng., vol 22, pp. 2343-2357, 1980.

41. Stanier, R.Y., N.J. Palleroni, and M. Doudoroff, "The Aerobic Pseudomonad: a Taxonomic Study," J. Gen. Microbiol., vol 53, pp. 1010-1019, 1966.

42. Haigler, B.E., Pettigrew, C.A., and J.C. Spain, "Biodegradation of Mixtures of Substituted Benzenes by *Pseudomonas* sp. Strain JS150," Appl. Environ. Microbiol., vol 58(7), pp. 2237-2244, 1992.

43. Rittmann, B.E. and P.L. McCarty,"Evaluation of Steady-State-Biofilm Kinetics," Biotech. and Bioeng., vol 22, pp. 2359-2373, 1980.

44. Rittmann, B.E. and P.L. McCarty, "Substrate Flux Into Biofilms of Any Thickness," Journ of Environ Eng, vol 107(EE4), pp. 831-849, 1981.

45. Stratton, R.G., E. Namkung, and B.E. Rittmann, "Secondary Utilization of Trace Organics by Biofilms on Porous Media," Journ. AWWA, pp. 463-469, Sept. 1983.

46. Manem, J.A. and B.E. Rittmann, "Removing Trace-Level Organic Pollutants in a Biological Filter," Journ. AWWA, pp. 152-157, April 1992.

47. Jeris, J.S., R.W. Owens, R. Hickey, and F. Flood, "Biological Fluidized-bed Treatment for BOD and Nitrogen Removal," Journ. WPCF, pp. 816-831, May 1977.

48. Spain., J.C., C.A. Pettigrew and B. E. Haigler, "Biodegradation of Mixed Solvents by a Strain of *Pseudomonas,*" Environmental Biotechnology for Waste Treatment, G.S. Sayler, R. Fox and J.W. Blackburn (ed.), pp. 175-184, Plenum Press, New York, 1991.

49. Heitkamp., M.A., W.J. Adams and L.E. Hallas, "Glyphosate Degradation by Immobilized Bacteria: Laboratory Studies Showing Feasibility for Glyphosate Removal from Waste Water," Can. J. Microbiol. vol 38, pp. 921-926, 1992.