This is to certify that the

dissertation entitled

Biological Activated Carbon In Fluidized Bed Reactors
For The Treatment Of Groundwater Contaminated
With Volatile Aromatic Hydrocarbons

presented by

Xianda Zhao

has been accepted towards fulfillment
of the requirements for

Ph.D. degree in Environmental Engineering

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Major professor

Date 11-17-94

BIOLOGICAL ACTIVATED CARBON IN FLUIDIZED BED REACTORS FOR THE TREATMENT OF GROUNDWATER CONTAMINATED WITH VOLATILE AROMATIC HYDROCARBONS

By

Xianda Zhao

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Civil and Environmental Engineering

1994

ABSTRACT

BIOLOGICAL ACTIVATED CARBON IN FLUIDIZED BED REACTORS FOR THE TREATMENT OF GROUNDWATER CONTAMINATED WITH VOLATILE AROMATIC HYDROCARBONS

By

Xianda Zhao

Laboratory and pilot-scale fluidized bed systems employing granular activated carbon (GAC) as a biomass carrier were used for the remediation of groundwater contaminated with the gasoline constituents, benzene, toluene and xylene (BTX). These systems (designated GAC-FBR) were evaluated in start-up, pseudo-steady-state and step-load rate increase modes of operation. The role of adsorption and the change in adsorption capacity of GAC in the systems were investigated. The formation of partial oxidation products was also investigated under steady-state and extremely high organic loading conditions.

The role of adsorption was investigated by comparing identical systems with adsorptive (GAC) and non-adsorptive (non-activated carbon) biofilm carriers. The GAC-FBR system, which had both adsorption and biodegradation capacities, showed higher organic removal and more stable performance over the non-adsorptive bioreactor (FBR) during transient conditions such as start-up and step-load increases. However, under steady-state organic loading conditions (3 and 6 kg COD/m^3-day) BTX removals were comparable for the GAC-FBR and biological only (FBR) systems and dominated by biodegradation. More than 90 percent of BTX was removed in both systems.

At steady-state, no intermediate decomposition products were detected in the effluents of either system. These materials were formed, however, during single substrate concentration step increases (twenty, twelve and seven-fold step increases in the organic loading rates of benzene, toluene and *p*-xylene, respectively). The concentration of byproducts was found to be lower in the GAC-FBR effluent than in the FBR system.

The adsorption capacity of biocoated GAC was maintained at more than 70% of its initial level during the first two months of system operation. After six months of operation, the remaining capacity was approximately 50% of the initial value. It was determined that this was not due to adsorption of the primary substrate, but may result from the adsorption of other materials produced by the biofilm. No direct relationship was found between the amount of biomass on the carbon and the remaining adsorption capacity.

ACKNOWLEDGMENTS

I would like to give my greatest appreciation to my advisor Dr. Thomas Voice of the Department of Civil and Environmental Engineering for his encouragement, understanding, and assistance in my research.

A special thank you is given to Dr. Robert Hickey of the Michigan Biotechnology Institute for introducing me to fluidized bed technology and teaching me how to write a research paper (let the data speak for itself and never exaggerate). Thank you also to Mr. Dan Wagon at the Michigan Biotechnology Institute for teaching me the tricks of the laboratory and making my job much easier.

I would also like to thank Dr. Craig Criddle of the Department of Civil and Environmental Engineering for his fresh ideas which opened my eyes to new view points and helped me think beyond my research. I would like to thank Dr. Stephen Boyd of the Department of Crop and Soil Sciences for his understanding and help to insure my study program ran smoothly, and for being the first person to introduce the soil environment into my study. I would also like to thank Dr. William Saul, chairmen of the Department of Civil and Environmental Engineering, for serving on my committee and rescuing me from the last minute regulation change.

I have been at MSU for seven years and have so many fellow students and friends who gave me considerable assistance and made my Spartan life more enjoyable. I can't write every name in this very small space but my heart will always be with them. I would like to mention a few names to remember their specific contributions. Dr. Daewon Pak

introduced me to biological reactors and helped me to establish my experimental protocol. Dr. Myung-Keun Chang, as my fellow graduate student, worked very closely with me and discussed many important ideas. Mr. Yanlyang Pan, who was once my fellow graduate student, helped me to understand and operate my first piece of analytical equipment (the IC) in my research and continuously helped me on many different analytical methods since then. Mr. Kwunhwan Doh conducted a series of experiments with me and kindly allowed me to present the results in chapter five of this dissertation. I would like to thank Mr. Chien-Chun Shih and Mr. I-yuang Hsu for their valuable comments on many aspects of the environmental engineering field. I would also thank Ms. Mara Hollinbeck for her help correcting my not so good English.

I give a very special thank you to my grandfather for his encouragement and effort which made it possible for me to study in the US. I would also like to thank my granduncles, grandaunts, aunts and uncles for the encouragement and help in making us life more enjoyable.

I have a very special thank you for my parents. They taught me how to work hard and value life.

My greatest thank you goes to my lovely wife Jing and our daughter Aisling. They make my life as happy as it can be. Jing, who was my classmate in college and my fellow graduate student at MSU, not only makes my home life very comfortable and enjoyable, but also helped me to conduct many experiments in my research. She also is the co-author of chapter six in this dissertation. Aisling, my lovely daughter, you came into my life just over a year ago, but you make my life so rich and bring happiness to my heart. My greatest love to Jing and Aisling.

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NOMENCLATURE

English Symbols

b	decay coefficient (1/day)
C_e	equilibrium concentration of toluene in water (mg/L)
dt_S	empty bed hydraulic retention time of the section.
f_b	fraction of electrons diverted for byproduct.
f_e	fraction of electrons diverted for energy.
f_S	fraction of electrons diverted for synthesis.
f_d^B	fraction of electron donor originating from benzene
f_d^T	fraction of electron donor originating from toluene
f_d^X	fraction of electron donor originating from xylene
g	electron equivalent mass of the electron donor (g)
h	electron equivalent mass of biomass
k	maximum specific utilization rate of substrate (mg substrate/mg VSS-day)
K_f	a constant for adsorption isotherm
K_s	half-saturation coefficient (mg/L)X concentration of biomass (mg VSS/L)
M_b	amount of toluene removed biologically
M_{COD}	difference between the influent and effluent COD
M_{DO}	mass of oxygen consumed in the FBR
$1/n$	constant for adsorption isotherm
Q	oxygen utilization quotient
Q_{SS}	removal index at pseudo steady-state

q_e	additional amount of toluene adsorbed (mg/g)
q_o	initial amount of adsorbed toluene (mg/g)
R	average degradation rate in the section (mg/L-min)
R_a	half reaction for the reduction of an electron acceptor for energy
R_b	half reaction for the reduction of an electron acceptor for byproduct
R_d	half reaction for the oxidation of an electron donor
R_e	half reaction for the reduction of an electron acceptor for synthesis
S	concentration of substrate (mg/L)
S_H	concentration of substrate at higher point of the section (mg/L)
S_L	concentration of substrate at lower point of the section (mg/L)
S_0	initial concentration of substrate (mg/L)
t	time (day)
X	concentration of biomass (mg VSS/L)
X_0	initial concentration of biomass (mg VSS/L)
Y	net yield coefficient of biomass (mg VSS/mg substrate)
M_b	production of byproduct (mg, or mg/L)
M_o	consumption of oxygen (mg, or mg/L)
M_s^B	consumption of benzene (mg, or mg/L)
M_s^T	consumption of toluene (mg, or mg/L)
M_s^X	consumption of *p*-xylene (mg, or mg/L)

Greek Symbols

μ	specific growth rate constant (1/hr)
μ_m	maximum specific growth rate constant (1/hr)

CHAPTER 1

INTRODUCTION AND RESEARCH OBJECTIVES

1.1 Introduction

The contamination of groundwater by volatile organic compounds (VOCs) is being reported with increasing frequency. More than 40% of U.S. population uses groundwater as a drinking water supply, often without any treatment other than disinfection. Groundwater contamination is thus a serious public health concern. Among the most common types of groundwater contamination are those resulting from petroleum products. In 1989, the department of natural resources in the State of Michigan reported that 41.3 % of their registered sites contained benzene, toluene or xylene (BTX) related contamination (DNR 1989). The presence of these materials frequently results in elevated levels of BTX in groundwater due to the fact that these compounds are relatively water soluble and hence are poorly retarded by the soil matrix.

The conventional approaches to remediation of BTX contaminated groundwater involve either liquid-phase adsorption by granular activated carbon (GAC) or air stripping. In these processes, the VOCs are simply transferred from one phase to another (McCarty 1983; Voice 1989). Further treatment or disposal of the receiving phase is often required. Biological treatment of contaminated groundwater would appear to be a desirable alternative to such techniques. This approach has the potential to destroy the pollutant and is often less expensive than physical-chemical treatment. Biological processes are frequently perceived

as being less stable than physical-chemical approaches, however, and thus are not always considered for groundwater treatment.

The beneficial aspects of integrated biodegradation/adsorption systems were reported in early work on the use of GAC for secondary and tertiary treatment of municipal wastewater (Weber *et al.* 1970). In systems designed as adsorbers, it has been shown that biodegradation increases the period between GAC regeneration cycles (Bouwer and McCarty 1982; Chudyk and Snoeyink 1984; De Laat *et al.* 1985; Kim *et al.* 1986; Gardner *et al.* 1988; Speitel *et al.* 1989a; 1989b). In biofilm systems, the use of GAC as a biomass carrier has been shown to provide for removal of compounds resistant to biodegradation (Suidan *et al.* 1983; 1987). In a treatment process for groundwater contaminated with relatively low levels of volatile organic compounds, a biological granular activated carbon fluidized-bed reactor (GAC-FBR) system has been demonstrated to provide both the efficiency of biological removal and the positive effluent protection capability of activated carbon adsorption (Hickey *et al.* 1990b; 1991a).

1.2 Research Objectives

This project was designed to investigate and exploit the advantages of the biological activated carbon fluidized bed system (GAC-FBR) for the treatment of gasoline contaminated groundwater. The primary objective was to elucidate the roles of the two removal mechanisms, adsorption and biodegradation, under a variety of operating conditions. Secondary objectives included using this information to better define the conditions under which GAC-FBR systems might be used and to explore the implications to system design and operation.

1.3 Overview of the Chapters

This project was designed in six phases. Each phase constitutes one chapter in the dissertation. The investigation and discovery of the advantages of GAC-FBR systems were the major focus in the first phase. In the second phase, the interaction of BTX and the role of adsorption during a single-substrate step-load increase were investigated. Formation of byproducts in FBRs during transient conditions was explored in the third phase. In the fourth phase, adsorption in a GAC-FBR was monitored by measurement of adsorbed toluene on the GAC carriers. Because of the importance of biodegradation in the GAC-FBR, the biodegradation kinetics of BTX were determined quantitatively in the fifth phase. In the sixth phase, the change in adsorption capacity of the GAC-FBR was measured in terms of adsorption isotherms. The dissertation concludes with a summary of the most important results from this project along with several recommendations for the design and operation of GAC-FBR systems.

CHAPTER 2

BACKGROUND

2.1 Definition and development of biological activated carbon

It has been understood that activated carbon can removal undesirable contaminants from water since some time in the nineteenth century (Kornegay 1978). The production of synthetic organic chemicals has grown exponentially since 1930 (Schwarzenbach *et al.* 1993). Many of these compounds have entered the aqueous environment and contaminated drinking water sources. The use of activated carbon has therefore grown widely in recent years as a means of removing such pollutants. In addition to adsorbing aqueous contaminants, activated carbon has also been shown to provide a very favorable environment for the growth of microorganisms (Pirbazari *et al*. 1990). Development of a microbial community on the carbon surface has generally been regarded as an undesirable, but unavoidable aspect of activated carbon usage.

In the early 1970's, scientists and engineers discovered that bacterial activity appeared to extend the life of carbon adsorbers used in drinking water treatment processes in Western Europe. They investigated the effect of pretreatment and the requirement of post-treatment for the process. A report written by AWWA Research and Technical Practice Committee on Organic Contaminants summarized and discussed these studies and suggested further research in this area (Committee Report 1981). Rice and Robson have also presented a very comprehensive review of this topic (Rice and Robson 1982).

During this same period, researchers and engineers working on physical-chemical treatment (PCT) and tertiary treatment of municipal wastewater had also identified the beneficial effect of biological activity in the GAC adsorption process. In these processes, the activated carbon was designed to remove recalcitrant pollutants through adsorption. It is not surprising that microorganisms grew in these systems. In what can be viewed as one of the first integrated biological activated carbon systems, Weber *et al.*. demonstrated the benefits of biological activity in expanded-bed adsorbers for treatment of municipal wastewater (Weber *et al.* 1970; 1972a).

Following these initial discoveries, Rice *et al.* defined the biological activated carbon (BAC) process as a system for water or wastewater treatment "in which aerobic microbial activity is deliberately promoted in granular activated carbon (GAC) adsorber systems" (Rice *et al.* 1980). In 1981 DiGiano summarized the results of previous studies and identified the following key features of biological activated carbon systems (DiGiano 1981):

a). The adsorption capability of a BAC system serves "to remove those difficult to biodegrade, but adsorbable, compounds."

b). The adsorbed compounds may create a high concentration region in the macropore to hasten the acclimation of bacteria and the resulting biodegradation.

c). The GAC could prevent escape of compounds during the adoption and acclimation period of microbial growth.

d). The adsorption capability is able to smooth out periods of high influent concentration, even when little adsorptive capacity remains.

e). The adsorption capability will also protect the microbial system from potentially toxic compounds.

2.2 Activated carbon

Activated carbon is manufactured from many carbon-based materials including bituminous coal, coconut shells, lignite, wood and pulp mill residues (Rice and Robson 1982). As a result of the activation process, activated carbon exhibits a high degree of porosity and an extensive associated surface area (Weber and Vliet 1980). The carbon particles can be thought of as stacks of graphite planes. The channels and interstices which pass through or between the planes are called macropores and have diameters larger than 1000 Angstroms. The micropores are within and parallel to the planes with diameters between 10 and 1000 Angstroms (Weber 1972b). Commonly used activated carbon has a specific surface area of 800 to 1200 m^2/g (Weber and Vliet 1980). About 99% of the available surface for adsorption consists of micropores while only 1% of the surface sites are due to the macropores (Rice and Robson 1982).

2.3 Adsorption, Desorption and Regeneration

2.3.1 Adsorption

Adsorption is a phenomenon of accumulation and concentration of substances at a surface or interface (Weber 1972b). Adsorption phenomena are commonly sub-divided into three categories: exchange adsorption, physical adsorption and chemical adsorption. Exchange adsorption results from the electrostatic attraction of ions to the charged sites at the surface. Exchange adsorption can be a reversible process by changing the direction of electrostatic attraction. Physical adsorption, which is also generally considered reversible, involves weak van der Waals forces. In this process, the adsorbed molecule is not affixed to a specific site but remains free to undergo transitional movement within the interface

(Weber 1972b). Chemical adsorption results when there is a specific reaction between the adsorbed substances and the surface sites. Chemical adsorption is usually considered an irreversible process. The primary adsorption processes for GAC in water treatment are physical adsorption and chemical adsorption (Rice and Robson 1982).

Activated carbon can adsorb a variety of compounds. Weber and Gould investigated the adsorption of several organic pesticides from aqueous solution and concluded that adsorption on activated carbon is an effective way to remove those pollutants from water. Giusti *et al.* presented results demonstrating the adsorption ability of 93 petrochemicals on GAC and generalized that the higher molecular weight compounds are favored for adsorption by activated carbon (Giusti *et al.* 1974). Youssefi and Faust reported that GAC is capable of removing low molecular-weight halogenated hydrocarbons from water under conditions simulating treatment plant operation (Youssefi and Faust 1980). The adsorption of polycyclic aromatic hydrocarbons (PAHs) on activated carbon was studied by Borneff (1980). He concluded that 99 - 99.9% of soluble PAHs could be eliminated from water by adsorption on activated carbon . The U.S. Environmental Protection Agency conducted adsorption studies on 140 toxic organic and concluded that pesticides, PAHs, phthalates, phenolics, and substituted benzenes are readily adsorbed by activated carbon (Dobbs and Cohen 1980). Certain low molecular weight compounds with high polarity, which include low molecular weight amines, nitrosamines, glycols, and certain ethers, were found not to be amendable to treatment using activated carbon adsorption.

The adsorption capacity of GAC can be evaluated by an adsorption isotherm measurement. One of the accepted procedures used to generate isotherms is termed the bottle point method (Randtke and Snoeyink 1983). In this technique, a series of bottles, each containing activated carbon, are equilibrated with an aqueous solution of the target

compound. After equilibrium is achieved, the concentration of the compound in the liquid phase is measured and the amount of solute in the solid phase is estimated by mass balance.

There are several models that are commonly used to describe adsorption equilibria. The Langmuir model describes a single-layer adsorption model. The Brunauer, Emmett, Teller (BET) model represents multilayer adsorption. Both models assume uniform energies of adsorption on the surface. Another widely used equation for adsorption by activated carbon is the Freundlich equation which is essentially empirical, but can be shown to be a special case of Langmuir model which assumes heterogeneous surface energies vary as a function of surface coverage (Weber 1972b).

2.3.2 Multi-Component Adsorption

In most practical applications, more than one adsorbable compound is present in the water to be treated. Interactive and competitive effects can occur in such multi-component systems. Jain and Snoeyink (1973)reported the results obtained from a study of several bisolute systems which included neutral and anionic *p*-nitrophenol, benzenesulfonate, and *p*-bromophenol. They concluded that competitive adsorption could occur when the substances tend to occupy the same adsorption, sites while only minor competitive effects occurred when the two species were adsorbed on different kinds of sites. Fritz *et al.* studied competitive adsorption in bisolute water systems (Fritz *et al.* 1980). They investigated the adsorption equilibrium and kinetics in a batch reactor and a fixed-bed adsorber. A *p*-nitrophenol and phenol bisolute system showed a higher reduction in adsorptive capacity compared to a single-solute system. The displacement of more weakly adsorbed compounds by stronger adsorbing compounds was found in both the batch reactor and the fixed-bed adsorber.

An ideal adsorbed solution theory (IAST) has been used to describe the adsorption equilibrium of multi-components (Crittenden *et al.* 1985; Annesini *et al.* 1987). This theory is based upon the assumption that the single-solute surface loading and the mixture loading cause the same spreading pressure. Crittenden *et al.* (1985) presented a prediction of multicomponent adsorption of seven mixtures that contained various combinations of two, three and six solutes, which include chloroform, bromoform, trichloroethene, tetrachloroethene, 1,2-dibromform, and chlorodibromomethane, using the IAST and Freundlich isotherm equations. The results show an average percent error of 29% and 16% for the liquid phase and solid phase concentrations, respectively. Annesini *et al.* (1987) reported satisfactory agreement between the IAST coupled with the Langmuir isotherm equation and experimental results for four bisolute mixtures included methylisobutylketone, acetone, propionaldehyde, and sucrose.

2.3.3 Desorption

Desorption can occur when there are changes in the conditions under which equilibrium was established. If the concentration of the substance at the liquid-solid interface decreases, the adsorbed substance will have a tendency to return to the liquid phase. Depending upon the type of adsorption, some or all adsorbed substances may not be released. Desorption can also occurs when a mixture of substances is fed into a fixed-bed absorber (Fritz *et al.* 1980). As the mixture enters the absorber, the different substances will be adsorbed at different depths in the unit. The less favored compounds for GAC adsorption will be adsorbed at greater depths in the absorber while the more strongly favored compounds will be adsorbed closer to the inlet. Since the substances enter the column continuously, the more strongly favored compounds will compete and replace the less favored compounds in the deeper region of the column. This desorptive release of the less strongly adsorbed compounds, is known as the chromatographic effect.

2.3.4 Regeneration

After the adsorption capacity of activated carbon is exhausted, the carbon must either be disposed of regenerated to recover its capacity. Three potential reactivating techniques are chemical, steam, and thermal (Clark and Benjamin W. Lykins 1989). Chemical reactivating involves using inorganic or organic regenerates to recover the capacity. The recovery efficiency is not very high, especially for low molecular weight compounds (Martin and Ng 1985). Steam reactivation is used primarily when adsorbate recovery is desired as it often causes the carbon to lose its ability to remove low concentrations of organic (Clark and Benjamin W. Lykins 1989). Thermal regeneration systems are the most widely used. Detailed design considerations have been reviewed by others (Zanitsch and Lynch 1978; Clark and Benjamin W. Lykins 1989). According to a study of the cost for GAC filters and contactors in the Cincinnati Water Works from 1979 to 1980, more then 70% of the operating and maintenance costs were due to regeneration processes (Clark and Benjamin W. Lykins 1989). Neukrug *et al.* (1984) reported similar results.

2.4 Biofilm Development on Activated Carbon

Microorganisms can survive and grow in an environment which provides sufficient water, substrate, and nutrients at suitable pH and temperature conditions. These requirements are not difficult to accomplish in an engineered reactor. The growth of a biofilm involves several physical, chemical and biological processes (Characklis 1984):

a). Transfer of organic molecules and microbial cells to the wetted surface.

b). Adsorption of organic molecules to the wetted surface creating a "conditioned" surface.

c). Attachment of microbial cells to the "conditioned" surface.

d). Attached microbial cell metabolism resulting in more attached cells and associated material.

e). Detachment of portions of the biofilm.

The studies of microbial growth on GAC mainly rely upon direct microscopic observation and viable cell counts. Klotz *et al.* (1976) found that the number of microorganism colonies reached 10^6 to 10^7 per gram of wet GAC for a filter treating river water. Van der Kooij reported that cell counts were 10 time higher on a GAC sample than a sand sample when the columns were fed with non-chlorinated tap water . Latoszek and Benedek (1979) studied the microorganisms on GAC for the treatment of domestic wastewater and found 10^9 bacteria per gram of wet carbon at 25 °C and $2x10^8$ at 5 °C. Brewer and Carmichael (1979) reported that the GAC filter could retain and accumulate bacteria and fungi based on the difference between influent and effluent cell counts in a drinking water system. Bancroft *et al.* (1983) studied the overall growth rate of bacteria on GAC contactors treating river water by measuring the cell count in the influent and effluent and on the GAC. They found an exponential decrease in specific growth rate of the population for the first 40 days after which time it remained constant.

The scanning electron microscope (SEM) is able to magnify the details of a solid surface and has therefore been used to investigate the colonization of microorganisms on GAC. Weber *et al.* (1978a) examined GAC exposed to coagulated-settled sewage and a 5 mg/L solution of humic acid using this approach. After seven days of exposure in a completely mixed batch reactor, a diverse community of microorganisms was found on the surface of the GAC. An expanded-bed charged with clean GAC was fed a 5 mg/L solution of humic acid for 10 days and the GAC particles were also examined by SEM. It was reported that the biofilm was non-homogeneous and full surface coverage was not observed. However, this may result from the relatively short growth period use in this study.

An investigation of microorganism coverage on GAC in a two-stage process was conducted by Petrovic *et al.* (1989) The two-stage process was structured with an activated sludge system and a fixed bed GAC adsorber system for the purification of mixed oil refinery and municipal wastewater. An average of 10^7 cells per gram was counted on the GAC. Examination of scanning electron microscopy results showed heterogeneity of the microflora on the GAC. During the colonization period, the cells appeared as individuals or in small groups. After one year of operation, a rich extracellular matrix on the GAC was observed. A homogeneous covering was found on some of the GAC while a non-homogeneous cobweb-like covering was found on the remainder.

Five different biofilm support media including GAC, carbonaceous adsorbent resin, anion-exchange resin, silica sand and glass beads were used to compare initial cell attachment and cultivation on the media surface (Pirbazari *et al.* 1990). Each of the media was added in each of several individual completely-mixed-batch reactors and seeded with activated-sludge from the secondary stage of a municipal wastewater treatment plant. The reactor was filled with the effluent from the primary stage of the municipal wastewater treatment plant. After 24 hours of stirring at 100 rpm without aeration, air was provide to each reactor for 40 hours. The particles were then removed and processed for examination by SEM. The results showed activated carbon provided a more favorable surface for the attachment of microorganisms compared to the other media. The sand contained less growth than the GAC. The authors concluded that adsorption on GAC partially helped the growth of microorganisms.

Based upon this research, it has been concluded that GAC provides a surface that promotes colonization and growth by microorganisms, making it a desirable biofilm support material.

2.5 Microbial Degradation of Benzene, Toluene and *p*-Xylene (BTX)

Since the early part of this century, it has been known that microorganisms are able to metabolize benzene, toluene and *p*-xylene (BTX) (Stormer 1908; Sohngen 1913). Numerous investigations have been conducted on the degradation pathways, type of organisms, and the degradation rates of BTX. There are several articles which review the efforts in these areas (Evans 1963; Gibson 1968a; Ribbons and Eaton 1982; Gibson and Subramanian 1984).

The accepted degradative pathways (Figure 2-1) for benzene involve transformation to *cis*-benzene glycol by a dioxygenase and subsequent oxidation of *cis*-benzene glycol to catechol (Marr and Stone 1961; Gibson *et al.* 1968b; Hou 1982). The catechol is degraded by a ring cleavage reaction involving either the β-ketoadipate pathway (*ortho* cleavage) or the *meta* fission pathway (*meta* cleavage) (Gibson and Subramanian 1984). In *ortho* cleavage, the dihydroxylated aromatic ring is opened to produce *cis*, *cis*-muconic acid, which is further metabolized to β-ketoadipic acid and then oxidized to succinic acid and acetyl-CoA by the tricarboxylic cycle. In *meta* cleavage, the dihydroxylated aromatic ring is opened to produce 2-hydroxy-*cis*, *cis*-muconic semialdehyde. Formic acid, pyruvic acid, and acetaldehyde are further metabolic products. All of the products from both cleavage mechanisms are used further for either biosynthesis or energy generation through the citric acid cycle, which produces CO_2 and ATP by electron transport and oxidative phosphorylation (Lehninger 1982).

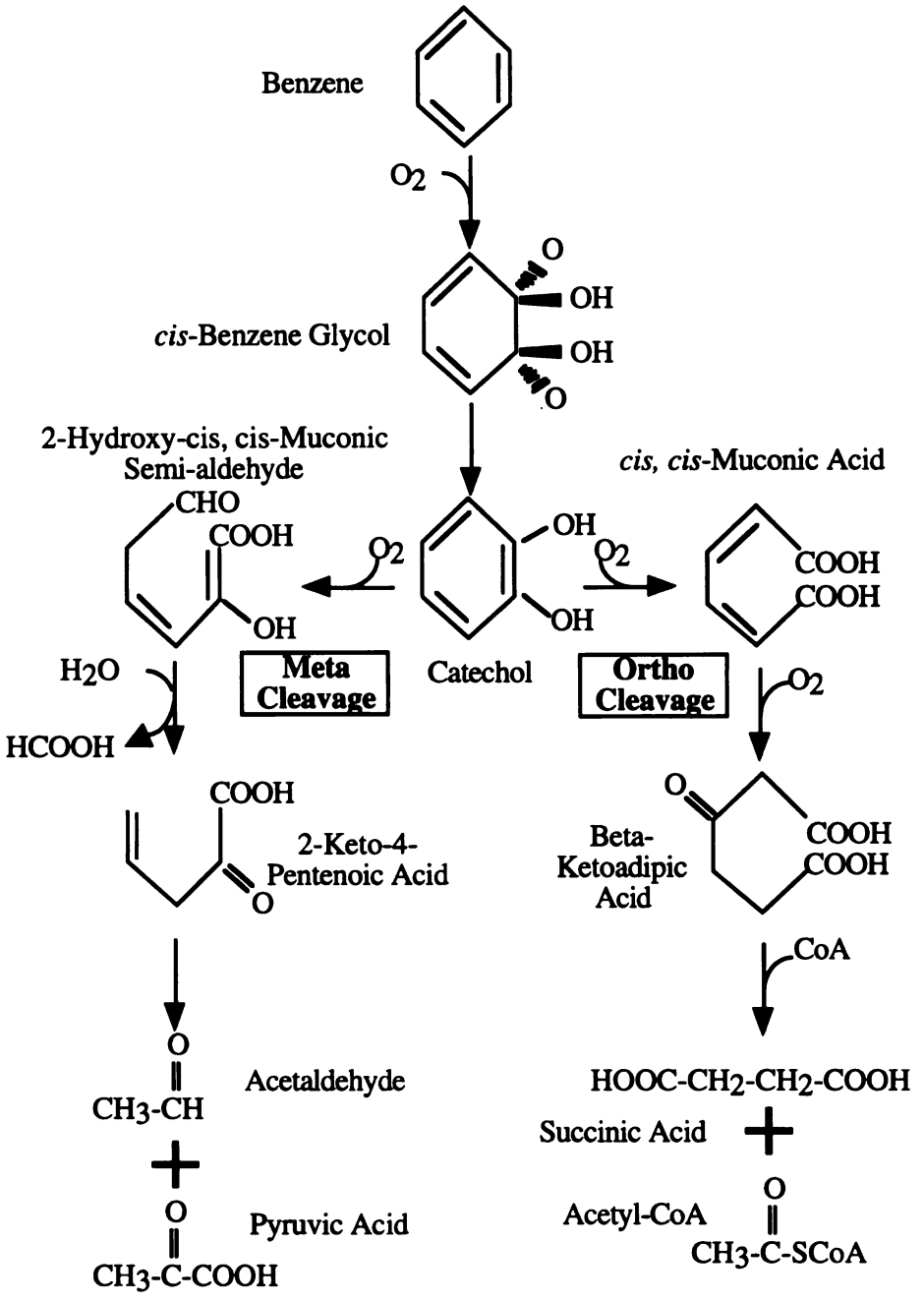

Figure 2-1. Pathways for benzene biotransformation (modified from Atlas and Bartha 1998).

As a result of the methyl group on the aromatic ring, the initial steps in the biodegradation of toluene can occur either by oxidation of the methyl group to form benzoic acid or hydroxylation of the ring (Figure 2-2) (Zylstra and Gibson 1991). Depending upon the hydroxylated position on the ring, the products are *p*-cresol, *o*-cresol, *m*-cresol, or *cis*-toluene dihydrodiol. A catechol will be produced as a result of the transformation of the benzoic acid which can be further degraded through *meta* or *ortho* cleavage of the aromatic ring. *p*-cresol can be hydroxylated again to form protocatechuate and this can be broken down by *meta* ring cleavage. A further product of *o*-cresol, *m*-cresol, or *cis*-toluene degradation is dihydrodiol 3-methylcatechol which is a substrate for *meta* ring cleavage.

The biotransformation of *p*-xylene can also occur through one of two pathways (Figure 2-3): aromatic ring oxidation or oxidation of the methyl substituent. Gibson *et al.* (1974) studied ring oxidation of *p*-xylene by *Pseudomonas putida* 39/D. The *p*-xylene was oxidized first to *cis*-*p*-xylene dihydrodiol and further to 3,6-dimethylprocatechol, which can be degraded further by the *ortho* ring fission pathway. The *cis*-*p*-xylene dihydrodiol can also be transformed to 2,5-dimethyl phenol by a non-enzymatic reaction (Gibson *et al.* 1974). Methyl substituent oxidation of *p*-xylene has been reported by several researchers (Davis *et al.* 1968; Omori and Yamada 1970a; 1970b; Davey and Gibson 1974). The proposed pathways involve two variations. In the first, one of the methyl groups is oxidized to form a carboxylic acid, which is then transformed to 4-methylcatechol. The aromatic ring of 4-methylcatechol can be broken by *meta* ring fission. The second pathway involves oxidation of both methyl groups before ring fission.

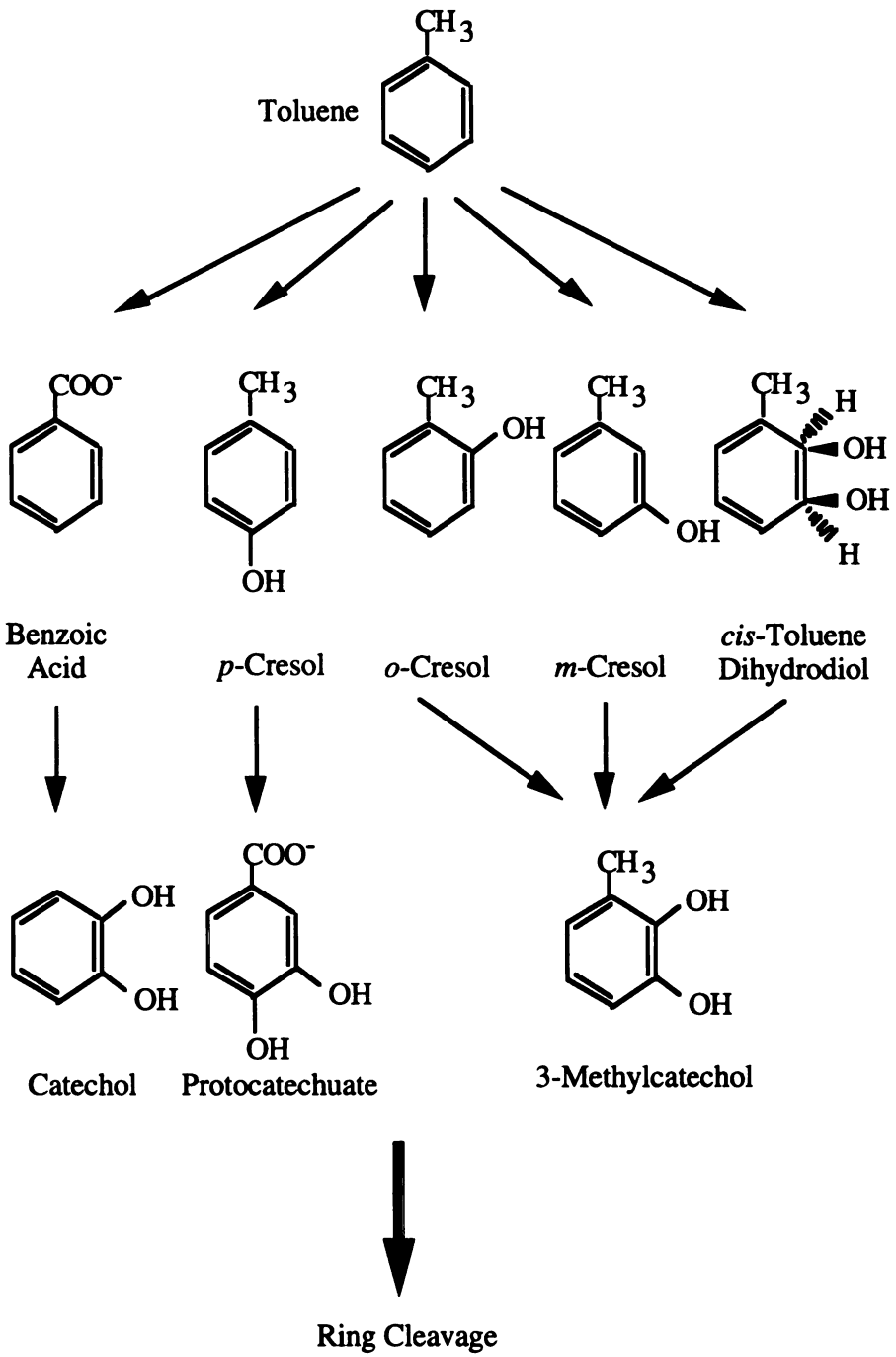

Figure 2-2. Pathways for toluene biotransformation (modified from Zylstra and Gibson 1991).

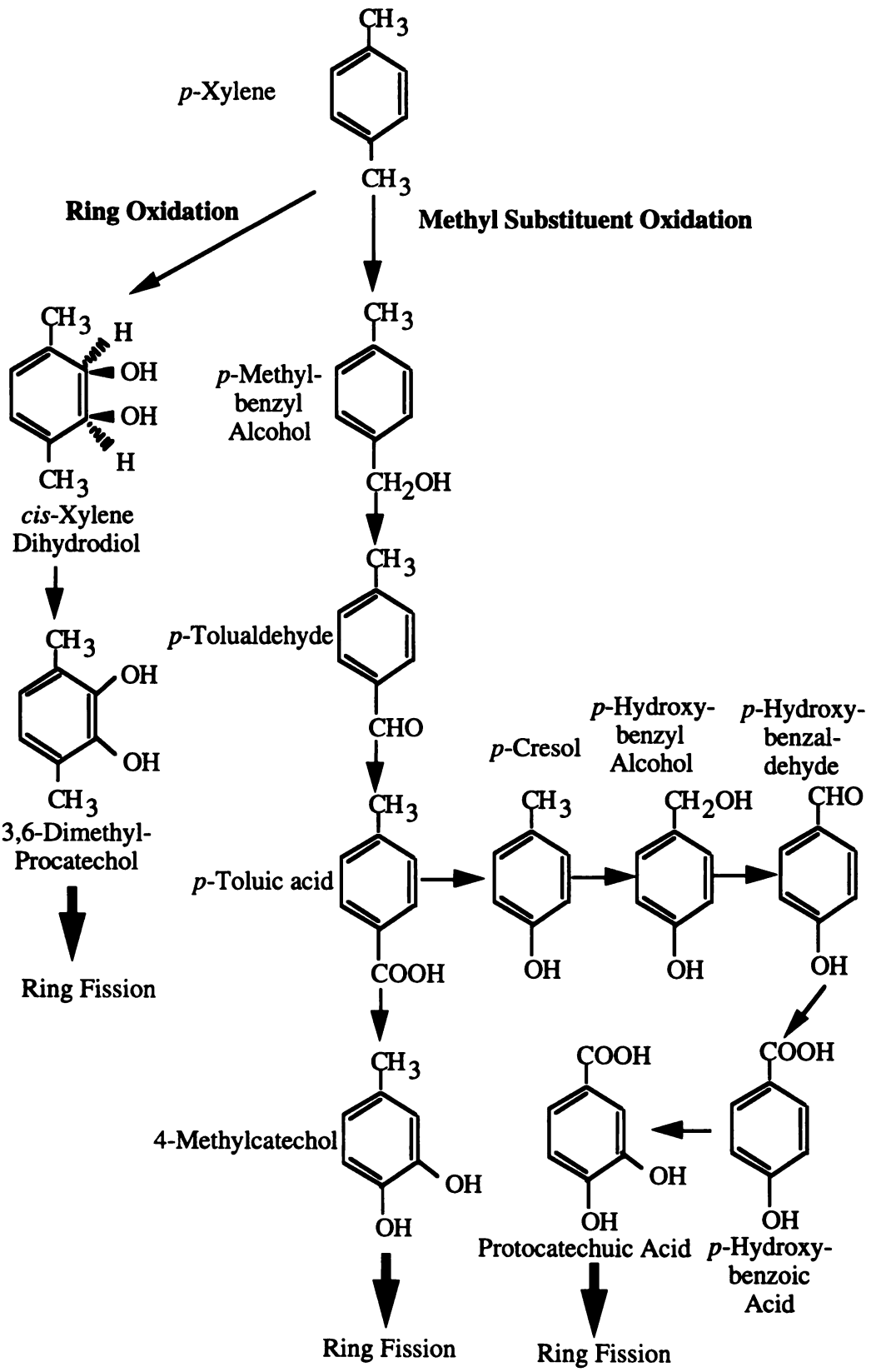

Figure 2-3. Pathways for *p*-xylene biotransformation.

In a study of cometabolic degradation of *p*-xylene by *Pseudomonas sp.* strain B1 (pure culture system) in a fixed-bed biological activated carbon system, Chang (1994) noted the formation of several partial oxidation products. These products were identified as 3,6-dimethyl procatechol and *p*-xylene dihydrodiol from the degradation of *p*-xylene when toluene was utilized as both carbon and energy sources. They were found in the effluent of a biological activated carbon fixed-bed and a biological fixed-bed (without adsorption) during the entire experimental period (about 30 days).

It is clear that various intermediate products (byproducts) will be generated as part of the degradation reactions. It is also clear that the BTX can be utilized as sole source of energy and carbon by various microorganisms. With a fully developed mixed-culture community, the byproducts will not normally accumulate because of the enzyme regulation systems in the microorganisms. In a continuous flow bioreactor, a community of microorganisms will develop to utilize the substrate resource to obtain the maximum benefit. The community will not produce more byproducts than are needed because such production costs, rather than yields, energy. Under transient conditions, however, byproducts may be generated at a rate higher than they are consumed, causing these compounds to accumulate in the reactor or be washed out in the effluent.

2.6 Microbial Degradation Kinetics of Benzene, Toluene and *p*-Xylene (BTX)

One of the most widely accepted rate equations is the Monod equation (Monod 1949) shown here.

$$\mu = \frac{\mu_m S}{K_s + S} \qquad (2\text{-}1)$$

S is the concentration of substrate (mg/L), μ is the specific growth rate constant (1/hr), μ_m is the maximum specific growth rate constant (1/hr), and K_s is the half-saturation coefficient (mg/L). The Monod equation is strictly an empirical equation (Grady and Lim 1980). The kinetic parameters can be evaluated by conducting a batch experiment. In this approach, the compound (as a sole organic constituent) is introduced into a reactor containing the required nutrients, electron acceptor, and growth factors. The reactor is then inoculated with a known amount of microorganisms. Subsequent samples are taken to monitor degradation of the compound and growth of the microorganisms. The Monod coefficients can then be estimated from Eqn. 2-1.

Several studies have been conducted to estimate the Monod coefficients for the biodegradation of BTX (Table 2-1). There are tremendous differences in the values reported, however. This is not unexpected given that different organisms and widely varied environments were used in these studies.

Interactions among BTX during biodegradation has been evaluated by several researchers. Goldsmith (1988) showed a slightly higher maximum utilization rate for toluene in a BTX mixture by an enriched mixed culture. Arvin *et al.* (1989) studied substrate interactions during aerobic biodegradation of benzene. They found that the degradation rates of benzene were higher when toluene or xylene was also present in the environment. Alvarez and Vogel (1991) investigated the interactions of BTX during biodegradation by two pure cultures and a mixed culture from an aquifer. The enhanced degradation patterns of benzene and *p*-xylene in the presence of toluene was revealed. The degradation of benzene was inhibited by the presence of *p*-xylene for a toluene degrader. In their studies, toluene could not be degraded by a benzene degrader unless benzene was also presence. Recent studies by Chang *et al.* (1993) indicated competitive inhibition and cometabolism during biodegradation of BTX by two *Pseudomonas* isolates. Strain B1, which grew on benzene and toluene as sole sources of carbon and energy, has the ability to

transform *p*-xylene by consumption of the growth substrates (benzene and toluene) or consumption of biomass during cometabolic degradation. The degradation of benzene, however, was inhibited by toluene even though the degradation rate of toluene was not affected. For strain X1, which grew on toluene and *p*-xylene but not benzene, the degradation of *p*-xylene was inhibited by the presence of toluene, but the degradation rate of toluene was not affected. Quantification of competitive inhibition and cometabolism was performed in their studies. For a system treating a mixture of BTX, complete degradation patterns should be expected. Such patterns may depend upon environmental conditions, composition of the waste, and the maturity of the microbial population

Table 2-1. Biodegradation kinetic coefficients for aerobic biodegradation of BTX (modified from Alvarez *et al.* 1991)

Substrate	k	Ks	Y	k/Ks	Culture	References
Benzene	4.7	10.8	0.39	0.44	mixed	(Grady *et al.* 1989)
	8.3	12.2		0.68	aquifer	(Alvarez *et al.* 1991)
	7.7	3.17	1.04	2.43	*Pseudomonas* sp. B1	(Chang *et al.* 1993)
Toluene	0.004	0.33	0.01	0.012	*Pseudomonas* sp. T2	(Button 1985)
	11	0.43	0.28	25.5	*Pseudomonas* sp. T2	
	0.013	0.034	0.1	0.38	*Pseudomonas* sp. T2	(Robertson and Button 1987)
	0.33	0.044	0.1	7.7	*Pseudomonas* sp. T2	
	0.49	0.65	0.43	0.75	aquifer	(MacQuarrie *et al.* 1990)
	4.32	0.15		28.8	denitrifying sewage sludge	(Jorgensen *et al.* 1990)
	10.7	1.96	1.22	5.45	*Pseudomonas* sp. B1	(Chang *et al.* 1993)
	10.9	1.88	0.99	5.82	*Pseudomonas* sp. X1	
p-Xylene	51.4	4.55	0.25	11.3	*Pseudomonas* sp. X1	

Where: k is the maximum specific utilization rate of substrate (mg substrate/mg VSS-day).

K_s is the half-saturation coefficient (mg/L).

Y is the yield coefficient for the biomass (mg VSS/mg substrate).

2.7 Bioregeneration of BAC

Following the discovery of the benefits of bioactivity on operating adsorption systems, some researchers attempted to use biological methods to regenerate exhausted GAC (Rodman and Skunney 1971). Rodman *et al.* (1978) summarized their studies in a 1978 article. A textile dye wastewater was adsorbed by a GAC column for 10 hours followed by back flushing with the liquor from a completely mixed activated sludge reactor for 13 hours. The process accomplished a 99% removal efficiency of color for a period of 4 months. The removed COD represented eight times the single adsorption capacity of the GAC. Sigurson and Robinson (1978) developed a mathematical model to estimate the time required for bioregeneration of the GAC. In their study, a phenol solution was treated using a GAC column. After the carbon equilibrated with the liquid-phase phenol, the carbon was regenerated with a mixed liquid from a fermentor in a back flush mode. They found that the bioregenerated carbon had recovered only 47 percent of the fresh carbon phenol adsorption capacity. They concluded that the low recovery may be due to occupation of certain adsorption sites by excreted products or products of the intermediary metabolism. Wallis and Bolton (1982) studied a similar regeneration system loaded with phenol. They demonstrated that the regeneration efficiency increased with an increase in regeneration contact time to a certain point (about 29 hours), then the efficiency would decrease. A maximum of 87% of the original adsorptive capacity was recovered after a 29 hour contact time. In 1987 Goeddertz *et al.* (1988) named this type of bioregeneration process "Off-line Bioregeneration". They used a similar system which contained a GAC expanded bed and an aeration tank. After the GAC was saturated with phenol, a desired amount of biomass was added in the aeration tank and the mixed liquid was recirculated in the GAC column and the aeration tank. After the regeneration period, the GAC was saturated again with phenol to measure the regeneration efficiency. The experiments were conducted with different initial biomass levels and duration of regeneration. The results

showed that 64% to 75% regeneration efficiencies can be achieved for the maximum regeneration cycle of 4 days. The higher the concentration of initial biomass, the faster the system approached the maximum GAC regeneration efficiency for the GAC.

In situ bioregeneration phenomenon, which is described as the adsorption, desorption, and biodegradation which occurs in an integrated reactor, was also studied by several researchers. Chudyk and Snoeyink (1984) studied columns filled with phenol-saturated GAC. The columns were inoculated and fed with phenol at two different concentrations of dissolved oxygen (4 mg/L and 9 mg/L). The results from a mass balance calculation (using influent and effluent phenol concentrations and the DO consumption) showed that bioregeneration of GAC occurred at a DO concentration of 9 mg/L while no bioregeneration was found when the influent DO was 4 mg/L. They also demonstrated the effect of bioregeneration on GAC columns in response to transient loading. There is a more detailed discussion of this transient loading effect in a later section. Kim *et al.* (1986) also provided evidence for *in situ* bioregeneration in an anaerobic granular activated carbon reactor for the removal of phenol. They found that the total carbon in biogas production was higher than the total carbon removed from the influent with a relatively low concentration of phenol in the influent. Therefore, bioregeneration of the GAC occurred.

Speital and DiGiano (1987) studied the bioregeneration of GAC used to treat very low concentrations of pollutants (less than 100 μg/L). They selected two model organic contaminants, phenol and paranitrophenol (PNP). Both chemicals have similar biodegradation characteristics, but PNP has a higher adsorptive capacity for GAC. Using a radiochemical method, they demonstrated that 8% to 15% of pre-sorbed phenol and 5% to 22% of PNP were bioregenerated within 10 days. Mathematical modeling suggested that the bioregeneration rate was controlled by diffusion of sorbed substrate to the outer surface of the GAC particle. Suidan and his colleagues (Khan *et al.* 1981; Suidan *et al.* 1981; 1983; Wang *et al.* 1984; 1986; Suidan *et al.* 1987) extensively studied anaerobic treatment of coal

gasification wastewater using BAC expanded-beds. They found that the BAC processes were able to remove pollutants from the influent during a long initial start-up phase (about 100 days) and consequently part of adsorbed pollutants were removed by bioregeneration.

The effect of chemical characteristics of an organic compound on bioregeneration has also been studied. Speitel *et al.* (Speitel *et al.* 1988; 1989a; 1989c) conducted a series of studies to further understand this effect. Four chemicals (phenol, dichlorophenol (DCP), pentachlorophenol (PCP), and paranitrophenol (PNP)) were used in their studies. The GAC in the fixed-bed reactor was saturated with radiolabled substrates, then a stream of the same non-radiolabled substrates was fed to the reactor at equilibrium concentrations. The portion of substrate removed by bioregeneration could be monitored by the production of radiolabled CO_2. The effect of bioregeneration is highly dependent on the adsorption and desorption characteristics of the substrate. The moderately adsorbable chemicals such as phenol and PNP could be removed from the surface of carbon significantly by bioregeneration. The absence of bioregeneration of highly adsorbable chemicals such as DCP and PCP was linked to irreversible adsorption.

2.8 Interaction of Adsorption and Biodegradation in the BAC Process

Bouwer and McCarty (1982) conducted a study on the removal of trace chlorinated organic compounds by BAC systems. A mixture of chlorobenzene (CB), 1,2-dichlorobenzene (1,2-DCB), 1,3-dichlorobenzene (1,3-DCB), 1,4-dichlorobenzene (1,4-DCB), 1,2,4-trichlorobenzene (1,2,4-TCB), chloroform (CLF), 1,1,1-trichloroethane (1,1,1-TCLE), and tetrachloroethylene (TECE) with sodium acetate was continuously applied to two upflow columns for 2 years. One of the columns (BAC) was filled with a homogeneous mixture of GAC and glass beads and another (BC) contained only glass beads. The empty-bed detention time was 60 min and the hydraulic loading was 6.0 m/day

for both columns. The concentration of each chlorinated compound was between 10 and 30 µg/L and the dissolved oxygen concentration was maintained above 5 mg/L in the effluent to ensure aerobic conditions existed throughout the columns. Removal efficiencies of 95%-98% of the chlorinated organic compounds were achieved in the BAC column initially. Complete breakthrough of CLF, 1,1,1-TCLE, and TECE, however, occurred after 4, 6, and 18 months, respectively. The high removal efficiency of chlorinated benzenes continued during the two years of operation. The chlorinated benzenes were also removed in the BC column after a period of bio-acclimation. The removal of CLF, 1,1,1-TCLE, and TECE, however, was not observed in the BC column. In order to investigate the role of adsorption in the columns, carbon-14 labeled CB and 1,4-DCB were fed to the columns as tracer compounds and the column was operated in a "shut off" mode by maintaining anoxic conditions in the feed. Nearly complete removal of both compounds continued to occur in the BAC column, but removal of CB almost completely stopped and only 39% of 1,4-DCB was removed in the BC column. Adsorption became the dominant removal mechanism in the BAC column during the "shut off". After restoring aerobic conditions and changing to nonlabeled substrates, a measurable amount of ^{14}C activity was found in both columns in the first 6 hours. Then, the $^{14}CO_2$ production in the BC column dropped rapidly to background levels. The $^{14}CO_2$ production in the BC column, however, was found for more than two weeks leading to the conclusion that bioregeneration occurred in the BAC column.

The BAC systems are expected to remove more non-biodegradable but adsorptive compounds, in a mixture of non-biodegradable and biodegradable chemicals than a strictly adsorptive system. This effect is expected because biodegradation of some compounds can reduce competition on the GAC active sites for adsorption. De Laat *et al.* (1985) studied the treatment of two mixtures of salicylic acid with 4-chlorobenzoic acid and 4-nitrophenol with 2-methyl 4,6-dinitrophenol in GAC adsorbers and BAC systems. Salicylic acid and 4-nitrophenol were presented as the biodegradable compounds, while 4-chlorobenzoic acid

and 2-methyl 4,6-dinitrophenol were used as non-biodegradable compounds. The two chemicals from each of the mixtures showed competitive adsorption in the sterile GAC adsorbers. When the mixture of salicylic acid and 4-chlorobenzoic acid was introduced into a BAC filter, salicylic acid did not breakthrough and breakthrough of 4-chlorobenzoic acid was significantly delayed compared with the breakthrough curve in a GAC adsorber. The amount of adsorbed 4-chlorobenzoic acid was close to the adsorptive value in a single solute system. In the case of treatment of a mixture of 4-nitrophenol and 2-methyl 4,6-dinitrophenol in the BAC reactor, the system performed as an adsorber initially for almost 45 days. When significant biodegradation of 4-nitrophenol occurred, the effluent concentration of 4-nitrophenol decreased dramatically to almost zero and the adsorption of 2-methyl 4,6-dinitrophenol increased. As a result, the concentration of 2-methyl 4,6-dinitrophenol decreased in the effluent. The concentration of 2-methyl 4,6-dinitrophenol in the effluent, however, rebounded to the higher level after 20 days. This can be explained due to exhaustion of the adsorption capacity. The total amount of adsorbed 2-methyl 4,6-dinitrophenol was significantly higher than the value in the GAC adsorber with the mixture and equated to the adsorbed amount in a single solute GAC adsorber.

DeWaters and DiGiano (1990) studied the influence of ozonated and non-ozonated natural organic matter (NOM) on the removal of phenol in a BAC bed. The ozonated NOM was less adsorptive and more biodegradable than non-ozonated NOM. The radio-labeled phenol was fed to the reactors under three conditions. First, the phenol and ozonated NOM were fed from start-up of the reactors (t = 0 hour). Second, the phenol and non-ozonated NOM were fed after biofilm development (t = 210 hours). Third, the phenol without NOM was fed after start-up of the reactors (t = 0 hour). High $^{14}CO_2$ production was found in the presence of NOM because the NOM was believed to compete with phenol for the adsorption sites on the activated carbon and more phenol was available for biodegradation. There was less $^{14}CO_2$ production when no NOM was introduced because the adsorbed

phenol was not directly available to the microorganisms. When the phenol was introduced into the reactor with a developed biofilm, $^{14}CO_2$ production was observed immediately.

Li and DiGiano (1983) determined the availability of sorbed substrate for biodegradation on GAC using an infinite batch recycle reactor. The four organic substrates, o-cresol, acetophenone, phenol, and benzoic acid, were selected in their studies. Ottawa silica sand, anthracite coal, and three different sizes of GAC (Filtrasorb 400) were used to compare the effect of biofilm carrier. The GAC particles were loaded with substrate and inoculated with a tertiary culture before use in the reactor. Constant substrates concentrations were maintained in the reactor by adding the appropriate amount of substrates with time. They found the surface characteristics of the particle had little effect on bioactivity, but higher biodegradation rates and specific growth rates were observed on GAC compared to sand or coal. Therefore, the utilization of internally sorbed substrate rather than better attachment on GAC may contribute to this enhancement. They also found that the enhanced specific growth rate was increased further with an increase in sorbed substrate concentration and decreased with an increase in particle size.

Several researchers found that there is, however, no evidence to prove that bioenhancement occurs under steady-state conditions or when the adsorption capacity is exhausted. Lowry and Burkhead (1980) compared fixed-bed reactors charged with sand, coal, and GAC for the treatment of hexanol and sodium acetate. There was no significant difference in the performance of the reactors under steady-state conditions. Peel and Benedek (1983) reported the results from a comparison of effluent data from adsorption systems and data from a model prediction. They suggest that activated carbon does not have an inherent bioenhancement capability. Zhang *et al.* (1991) reported that there were no difference in terms of removal efficiency of phenol between BAC and biological aerated filters after the start-up period.

Even though the bioenhancement claim seems unlikely for BAC systems, activated carbon adsorption provides a very important mechanism for removal of non-biodegradable or inhibited compounds. Wang *et al.* (1984) reported that adsorption was an essential mechanism for removal of methylquinoline from a polycyclic N-aromatic compounds mixture in an anaerobic activated carbon expanded-bed reactor. Suidan *et al. (1987)* studied the treatment of synthetically prepared coal gasification wastewater in anaerobic activated carbon expanded-bed systems. The phenols, primarily phenol and the C_2-phenols, account for 60 to 80 percent of the COD in coal gasification wastewater (Singer and Yen 1980). The *o*- and *m*-cresol were not degraded anaerobically and the high concentrations of those compounds inhibited the biodegradation of the other aromatic compounds (Suidan *et al.* 1987). Methane production in the reactors decreased when breakthrough of *o*- and *m*-cresol occurred. GAC replacement became necessary and an increase in methane production followed immediately after replacement. Further studies to determine the relationship between carbon replacement rate and reactor performance were conducted by Nakhla *et al.* (1988) . They found that the soluble COD in the effluent decreased linearly with an increase in GAC replacement rate. After a replacement rate was selected, the minimum required volumetric COD loading rate was determined.

When loading transients occur in a BAC system, adsorption may serve to dampen concentration changes. Chudyk and Snoeyink (1984) demonstrated that a BAC system was able to smooth out the 150-fold phenol pulses if bioregeneration had removed enough adsorbed phenol. Lowry and Burkhead (1980), however, reported that the GAC carrier was not able to provide better COD removal than other biological filters charged with sand and coal when the systems were subjected to a two-fold sodium acetate (less favorable for adsorption) step increase.

The adsorption capacity of GAC can decrease with an increase in usage of the BAC system. This reduction could be due to adsorption of some compounds from the influent

but may also be caused by microorganism growth on the surface of the carbon. Suidan *et al.* (1983) reported that the adsorption capacity of GAC decreased after the carbon was exposed to a phenol mixture, which contained some compounds resistant to biodegradation, in an anaerobic expanded-bed reactor for a period of time. The capacity was, however, slightly regained after a period of bioregeneration. Zhang *et al.* (1991) found that a similar phenomena occurred in an aerobic BAC filter to treat phenol. They did not, however, observe the effect bioregeneration had on the reactor.

Weber and Ying (1978b) assert that breakthrough of toluene sulfonate in an expanded-bed GAC adsorber with sucrose pre-saturated-biocoated activated carbon was earlier than an adsorber with sucrose pre-saturated activated carbon without the biocoat. When Schultz and Keinath (1984) conducted a study of powdered activated carbon treatment (PACT) process mechanisms in an activated sludge system, they found that the biomass in the PACT culture impedes the rate of mass transfer of phenol to the PAC surface.

Olmstead (1989) studied the effect of microbial interference on GAC adsorption . In his experiment, a column containing GAC particles was fed glucose for 1 -2 weeks. After the biofilm developed, the carbon was removed from the column in sections. Because of the plug-flow pattern in the column, more bioactivity and biomass were expected on the influent end of the column. Biocoated GAC with different amount of biomass could be obtained. For trichloroethylene (TCE), he found that the multiplier term in the Freundlich isotherm equation, K_f, decreased with increasing biomass on the carbon while the slope term of the equation, n, remained constant. The adsorption of *para*-toluene sulfonate (PTS), which has a much lower adsorption capacity than TCE, was not decreased significantly. The author was not able to isolate the effect of a sample protein (BAS) on the adsorption of PTS, but, discovered that preloading soluble microbial products (SMP) on the GAC reduced the amount of PTS adsorbed. Results from column experiments

investigating adsorption of dodecylbenzene sulfonate (DBS) confirmed that a smaller amount of DBS was adsorbed by the biocoated GAC.

2.9 Application of BAC processes

2.9.1 Drinking Water Treatment

The early stages of understanding BAC processes started in the drinking water treatment field. Researchers in Western Europe discovered and studied the beneficial use of the bacterial activity to extend the service life of activated carbon processes (Rice and Robson 1982). The BAC process was combined with pretreatment (including clarification, filtration, and oxidation by ozonation) and post-treatment (mainly disinfection) to achieve high quality drinking water.

Ozonation can oxidize organic matter to what is termed assembled organic carbon (AOC). The adsorption capacity of GAC for AOC is less than the capacity for the natural organic carbon (Glaze *et al.* 1986), but the biodegradability of AOC is higher (Somiya *et al.* 1986). Janssens *et al.* (1985) examined a GAC filtration system in a water production center. They compared four different processes using different ozone dosages (0, 2, 4, 8 mg/L). They found that ozonation could increase filter service time by about 60% to 65% due to the significant increase in AOC. Larger increases in AOC correspond to higher dosages of ozone. They also observed that a dosage higher than 2 mg/L provided no additional effect on total organic carbon removal.

The BAC process is often followed by a disinfection procedure to remove microorganisms. Tobin *et al.* (1981) found two secondary pathogenic organisms (*Pseudomonas aeruginosa* and *Flavobacterium*) in the effluent of a point-of-use GAC filter. Camper *et al.* (1985; 1986; 1987) studied the release of colonized granular activated

carbon particles from the GAC bed to the treated drinking water. They found that three enteric pathogens *Yersinia enterocolitica O:8, Salmonella trphimurium,* and *enterotoxigenic Escherichia coli* were able to colonize sterile GAC. The heterotrophic plate count bacteria were found in 41.4% of 201 GAC-treated drinking water samples. They also found that the GAC supported greater numbers of the coliform *Kebsiella oxytoca* than sand or anthracite. Suffet (1980) suggested the use of backwashing to control the detachment of microorganisms from the BAC process. LeChevallier *et al.* (1984), however, reported that the microorganisms attached on GAC, which can wash out with the treated water, resisted disinfection by chlorination. Treatment plant operators need to be aware that the microorganisms could penetrate disinfection barriers and enter drinking water supplies.

2.9.2 Industry Wastewater Treatment

The complexity of the components in industrial wastewater is well known. Some of the constituents may be biologically degradable, but others may be non-biodegradable and may even be toxic to the microbial process. BAC systems which can provide adsorption and biodegradation in an integrated reactor are appropriate candidates to treat such complicated waste. BAC systems have demonstrated high removal efficiencies and stable performance for the treatment of many wastes such as phenolic wastewater, slaughterhouse wastewater, musty odor compounds, geosmin, textile wastewater, and metal-cutting-fluids wastewater. A summary of the remarks related to those systems is presented in Table 2-2.

Table 2-2. BAC processes for treatment of industrial wastewaters

Wastewater	Major Components	Reactors	Loading Rate (kg COD/m^3-d)	Removal Efficiency
Phenols	Catechol 200-1000 mg/L	two anaerobic expanded-bed reactors in series (35 °C)	1 - 5 (in the first reactor)	Catechol > 99% COD 93%, 94%, 96%
	Remark: Adsorption removed majority of COD in the first 140 days with a bioregeneration period thereafter (Suidan *et al.* 1981).			
	o-Cresol 256 mg/L	two anaerobic expanded-bed reactors in series (35 °C)	1.9 - 2.2 (in the first reactor)	Breakthrough
	Remark: 50 % of *o*-Cresol breakthrough after 200 days in the first reactor and 340 days in the second reactor. Reactor performance was similar as adsorbers (Suidan *et al.* 1981).			
	Phenol 360 - 3000 mg/L	an anaerobic expanded-bed reactor (35 °C)	0.9 -7.2	phenol 99.9%, COD 98%
	Remark: Bioregeneration was evident (Wang *et al.* 1986).			
	Phenol 200-1000 mg/L	three anaerobic expanded-bed reactors in series (35 °C)	1 - 5 (in the first reactor)	phenol > 98%
	Remark: Adsorption removed the most of COD in the first 135 days and a bioregeneration period was occurred thereafter (Khan *et al.* 1981).			
Coal Gasification	Phenol 200-1000 mg/L; Cresols 170-850 mg/L	berl-saddle packed anaerobic filter and an anaerobic GAC expanded-bed reactor in series (35 °C)	0.8 - 1.7 (in the first reactor)	COD > 90%
	Remark: No significant removal occurred in the berl-saddle reactor and GAC replacement was necessary to prevent microbial toxic levels of some components (Suidan *et al.* 1983; Suidan *et al.* 1987).			
Polycylic N-aromatic compounds	Indole 50-300 mg/L; Quinoline 50-300 mg/L Methylquinoline 50-300 mg/L	anaerobic expanded-bed reactor (35 °C)	0.3 - 2.2	COD > 95% No target compounds were detected in the effluent.
	Remark: Bioregeneration was evident. Methylquinoline was removed strictly by adsorption (Wang *et al.* 1984).			
Musty odor	2-methyl-isoborneol (MIB) 1.6 mg/L geosmin 1.5 mg/L	batch filter	2.7	99 %
	Remark: More than 50% of substrates were biodegraded in the seeded GAC filters, but there was no significant removal in the seed sand filter after 43-54 bed volumes. The seed time was one day (Yagi *et al.* 1988)			

CHAPTER 3

COMPARISON OF BIOFILM CARRIERS IN FLUIDIZED BED REACTOR SYSTEMS

3.1 Introduction

The ever increasing presence of volatile organic compounds (VOCs) in water supply wells has led to the realization that groundwater can no longer be considered as an inherently pristine source of drinking water. Contamination by VOCs can be caused by spills of hazardous materials and leaks from underground storage tanks and pipelines. Because many people depend on groundwater as the sole source of their potable water supply, the degradation of these water reserves has serious public health ramifications.

One of the most widespread contamination problems is the release of gasoline and other petroleum fuels into the subsurface. The constituents of principle concern are the aromatic components, benzene, toluene, and xylenes (BTX), which are less strongly adsorbed to the soil matrix than the aliphatic components in fuel. The BTX components are, therefore, more mobile in the soil and therefore more likely to contaminant water supplies.

The most widely used remediation techniques, liquid-phase adsorption using granular activated carbon (GAC) and air stripping, simply transfer contaminants from one phase to another (McCarty 1983; Voice 1989). Further treatment or disposal of the receiving phase is required. Biological treatment would appear to be a desirable alternative to such concentration techniques because it has the potential to completely destroy the contaminant compounds and it is generally less expensive than physical-chemical treatment

processes. Biological treatment has not been widely accepted for groundwater treatment, however, due to the widespread impression that biological systems are not sufficiently stable to consistently meet the stringent discharge limitations that are often required. In cases where biological treatment has been employed, it is typically followed by GAC adsorption for effluent polishing and to provide back-up treatment in the event of failure of the biological system.

A promising new approach, termed biological activated carbon (BAC), integrates biological removal and granular activated carbon adsorption into a single unit process. The beneficial aspects of integrated biodegradation/adsorption systems were reported in the early work on the use of GAC for secondary and tertiary treatment of municipal wastewater (Weber *et al.* 1970). Researchers observed that adsorption columns continued to effectively remove organic material far beyond the point at which adsorption capacity would normally be exhausted (Bouwer and McCarty 1982; Kim *et al.* 1986; Rittmann 1987; Gardner *et al.* 1988; Speitel *et al.* 1989a; 1989b; 1989c). This removal has been shown to be the result of growth of microorganisms on the surface of the GAC particles, and the subsequent biodegradation of the waste constituents by these communities. In some systems this phenomenon has been regarded as a fortuitous benefit because less frequent carbon regeneration is required. In others, however, it has proven to be problematic because the biomass can grow to the point where it interferes with the hydraulic operation of the system and it may impede the adsorption of non-degradable compounds. Little attention has been given in the literature to understanding how adsorption and biodegradation work together in biological activated carbon systems to affect both effluent quality and system stability. In addition, BAC has only recently been considered as a potential treatment process for groundwater contaminated with relatively low levels of toxic materials, such as petroleum hydrocarbons or chlorinated solvents (Hickey *et al.* 1990b).

In this research an integrated BAC system consisting of a biological fluidized bed reactor employing granular activated carbon as a biofilm support (BAC) was evaluated and compared to identical systems with adsorptive removal only (GAC) and biological removal only (FBR) for the remediation of groundwater contaminated with the gasoline constituents, benzene, toluene, and xylene. It has been hypothesized that BAC systems provide for faster start-up times and better protection against shock-loads than similar systems with only biological removal, while preserving the benefits of low operating costs and pollutant destruction (Hickey 1990a). These hypotheses were evaluated by comparing the breakthrough profiles, steady-state removals, and system responses to step increases in applied organic loading rates for the three systems.

3.2 Materials and Methods

3.2.1 Media and Chemicals

Two different support media, GAC and a non-adsorbent carbon material (non-activated carbon) were used in this study. The GAC used was Calgon Filtrasorb 400 (Calgon Co., Pittsburgh, PA). Non-activated carbon, termed "baker product" by the manufacturer, is the same material as the GAC that it has not undergone the activation step in the manufacturing process and has therefore little adsorption capacity. Both media were sieved to obtain a 20 x 30 mesh fraction, resulting in an average particle diameter of 0.75 mm. After sieving, the GAC and baker product were rinsed with distilled water to remove oil and fines and dried overnight at 100 ^{o}C. The media were then stored in sealed containers until needed.

Benzene, toluene, and p-xylene (BTX) were obtained from except Chemical Co., Milwaukee, WI. Ammonium chloride and potassium phosphate dibasic were obtained from J. T. Baker Chemical Co., Phillipsburg, NJ. All chemicals were reagent grade. The water

supplied to Michigan State University was used directly as a source of groundwater. This relatively hard water (450 mg/L as $CaCO_3$) is pumped from a deep aquifer underlying the campus and distributed without further treatment.

3.2.2 Determination of Adsorptive Capacity of BTX

Isotherms for each BTX compound were performed using serum bottles (160 ml) sealed with Teflon-coated septa. Activated carbon (10 mg, 20 mg, and 30 mg for benzene, toluene, and xylene, respectively) was added to oven dried serum bottles, which were then capped and sterilized. The serum bottles were subsequently filled, without leaving a head space, with water (pH = 7.0) containing the desired concentration of BTX. Ten bottles, each with different initial concentrations between 1 and 20 mg/L, were prepared for each compound. The bottles were tumbled at 6 rpm and 15 ^{o}C for 7 days to ensure that equilibrium was obtained. The liquid-phase concentration was then analyzed to determine the residual amount of BTX. The solid-phase concentration was calculated by difference. An isotherm was also performed for xylene with non-activated carbon using the same procedure.

3.2.3 Fluidized Bed Reactors

The laboratory-scale fluidized bed reactors used in this study were glass columns with a 2.5 cm diameter and 92 cm height (Figure 3-1). All reactors were operated as one-pass systems with no recycle. The GAC-FBR and FBR (biological only) systems were charged with activated carbon and non-activated carbon, respectively, and seeded with a mixed culture. This culture was taken from a pilot-scale fluidized bed system that was originally seeded with activated sludge and was subsequently supplied with BTX as the sole carbon source. The reactors were provided with sufficient dissolved oxygen and

essential nutrients to encourage biofilm formation and permit complete biological oxidation of the BTX. The feed water was oxygenated to a concentration sufficient to maintain an effluent DO of more than 2.0 mg/L using pure oxygen and was supplemented with nitrogen and phosphorous at a stoichiometric ratio of 100/5/1:COD/N/P. A mixture of benzene, toluene, and xylene, in 1:1:1 volumetric ratio, was injected into this stream via a syringe pump and dissolved using an mixer. A recycle loop around the mixer was employed to create turbulent flow inside the mixer and help ensure complete BTX dissolution. This BTX contaminated water was then pumped to the bottom of the reactors using peristaltic pumps with variable speed drives. To limit microbial growth, the GAC system (adsorption only; no biological activity) was operated under anaerobic conditions and received no additional nitrogen or phosphate. During the course of this experiment the in-line water temperature averaged 15 °C ± 1.0 °C.

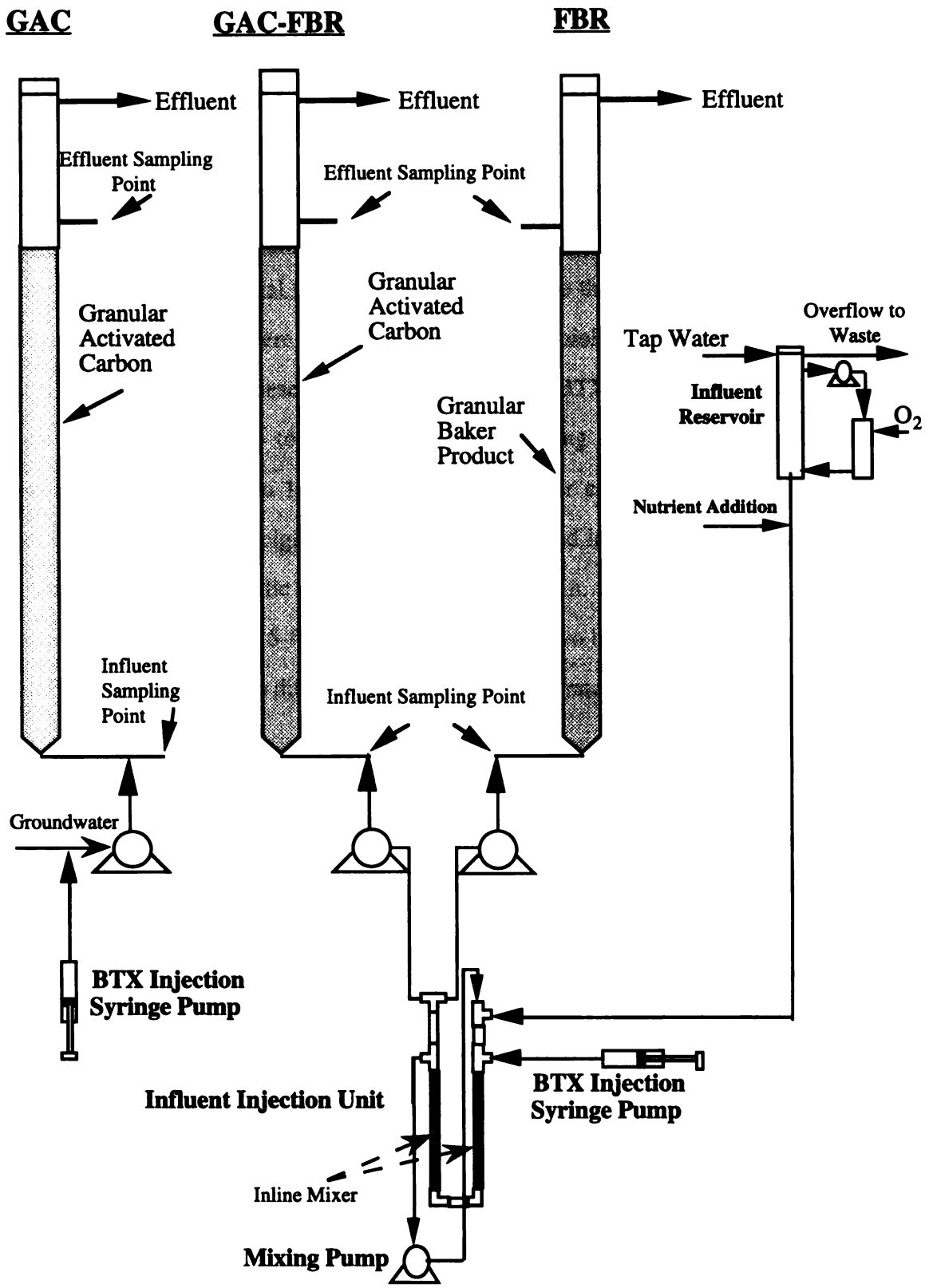

Figure 3-1. Schematic of fluidized bed reactor systems.

Breakthrough profiles were obtained for the three reactor types (GAC, GAC-FBR , and FBR) using a 500 ml working volume (92 cm working height) and a 180 ml initial charge of media, which produced a settled bed height of approximately 33 cm. BTX contaminated water was fed at a flow rate of 140 ml/min to each reactor during this period, resulting in a hydraulic flux rate of 0.29 m^3/min-m^2 and an empty bed hydraulic retention time of 3.6 min. The initial fluidized bed height for the three reactors was 43 cm. After steady-state conditions were reached in the units with biological activity, higher loading rates were applied to these reactors by increasing BTX concentration 1.5-fold and increasing the flow rate to 300 ml/min. The working volume of each reactor was concurrently increased to 1000 ml by adding another column unit and subsequently increasing the working heights to 184 cm. This resulted in a hydraulic flux rate of 0.61 m^3/min-m^2 and hydraulic retention time of 3.3 min. Step load increases in BTX concentration (ca. 3- and 5-fold) were applied to the two biological reactors (GAC-FBR and FBR) operating at two different steady-state loading rates.

3.2.4 Analytical Methods

Aqueous BTX concentrations were determined using an automated headspace sampler (Perkin-Elmer, model HS-101) coupled to a gas chromatograph (Perkin-Elmer model 8700) equipped with a flame ionization detector (FID) using helium as a carrier gas. Samples collected from the fluidized bed reactors (5 ml or 10 ml) were transferred to 20 ml glass vials with Teflon-coated septa and aluminum seals. The samples were equilibrated for 1 hour at 80 °C and an aliquot of the headspace gas was injected onto a 30 m x 0.53 mm DB-624 column (J & W Scientific). The detection limit of this method was 1 µg/l for each BTX component. The accuracy for measurements of a sample containing a concentration of 1.5 ug/l was ± 0.1 µg/l.

Influent and effluent dissolved oxygen concentrations were analyzed using an polarographic electrode coupled to a digital pH/millivolt meter. If the DO concentration exceeded the limit of the instrument (15 mg/L), the sample was diluted to the measurable range using water of a known (low) DO concentration. DO consumption was calculated as the difference between inlet and effluent DO.

3.2.5 Scanning Electron Microscopy

Samples of both virgin and biofilm coated activated carbon and non-activated carbon were fixed at 4 ^{o}C for 1-2 hours in 4% glutaraldehyde buffered with 0.1 m sodium phosphate (pH 7.4). Following a brief rinse in the buffer, samples were dehydrated in a ethanol series (25%, 50%, 75%, 95%) for 10-15 min at each gradation followed by three 10 min changes in 100% ethanol. A Balzers critical point dryer, using liquid carbon dioxide as the transitional fluid, was used to dry the samples. Samples were sputter coated with gold to a thickness of 20 nm (Emscope Sputter Coater model SC 500), purged with argon gas and examined via scanning electron microscopy (Japan Electron Optics Ltd., model JSM-35CF).

3.3 Results

3.3.1 Adsorption Characteristics Of BTX

The single solute adsorption isotherm of each BTX compound can be related through the Freundlich isotherm.

$$q_e = K_f C_e^{\frac{1}{n}} \qquad (3\text{-}1)$$

where q_e = mass solute adsorbed/mass adsorbent

C_e = concentration of solute in solution, mass/volume

K_f, $\frac{1}{n}$ = empirical parameters representing the sorption capacity and non-linearity, respectively.

The experimental data was fit to the Freundlich equation using non-linear regression analysis, resulting in the parameter estimates shown in Table 3-1. The K_F values of BTX were observed to follow the expected order: xylene > toluene > benzene. This means that the amount of xylene adsorbed per unit mass of activated carbon is greater than that of toluene which in turn is greater than that of benzene for a given aqueous concentration. The K_F value for xylene with the non-activated carbon at 15 °C was 2.7% of that found for GAC, thereby substantiating the assumption that this material had negligible adsorption capacity. The adsorption capacity of baker product for benzene and toluene was not measured, but it can be assumed that it is also low relative to GAC.

How to experiment ?

Table 3-1. Adsorption characteristics of BTX

Media	Compounds	Temperature °C	Freundlich Isotherm		Correlation Coefficient
			K_f*	1/N	
Activated	Benzene	15	30	0.45	0.99
Carbon	Toluene	15	83	0.44	0.99
	Xylene	15	147	0.28	0.97
Non-active	Xylene	15	4	0.56	0.98
Carbon					

* $(mg/g)(L/mg)^{1/n}$

Note: BTX: Benzene, Toluene and *p*-Xylene

3.3.2 Breakthrough Profiles

Start-up and breakthrough profiles for the three systems are shown in Figures 3-2 and 3-3. The initial fluidized bed height for the all reactors was 43 cm. The concentration of

each BTX component in the inlet was maintained at approximately 1 mg/L during this period.

In the GAC system, where adsorption was the only removal mechanism, substantial breakthrough of benzene and toluene occurred after 160 and 460 hours, respectively. Xylene concentration in the effluent increased slowly up to 0.3 mg/L by 800 hours. These breakthrough profiles indicate that the GAC unit can be treated as upflow fixed bed reactor with essentially plug-flow liquid transport conditions. This is because liquid-solid, two-phase fluidized beds become stratified according to particle size and particle motion in the GAC unit can be assumed to be confined to reasonably defined patterns of localized movement. To predict the time required for substantial breakthrough of BTX, an equilibrium column model (ECM), which neglects mass transfer resistance and uses ideal adsorbed solution theory to include the competitive effects in multicomponent mixtures, was used. The ECM has been shown to be able to calculate the elution order of the adsorbates in multicomponent mixture using single solute isotherms (Crittenden *et al.* 1987). The predicted and actual breakthrough times for BTX are presented in Table 3-2. Differences between measured and predicted breakthrough times are likely due to competitive interactions among adsorbates and non-ideal adsorption conditions.

Table 3-2. Substantial breakthrough time for each BTX compound in the GAC system

Compounds	Breakthrough Time		
	GAC system (hrs)	Predicted using ECM (hrs)	Predicted based on single solute isotherm (hrs)
Benzene	160	218	286
Toluene	460	585	790
Xylene	>800	1300	1400

Note: GAC: Granular Activated Carbon;
ECM: Equilibrium Column Model

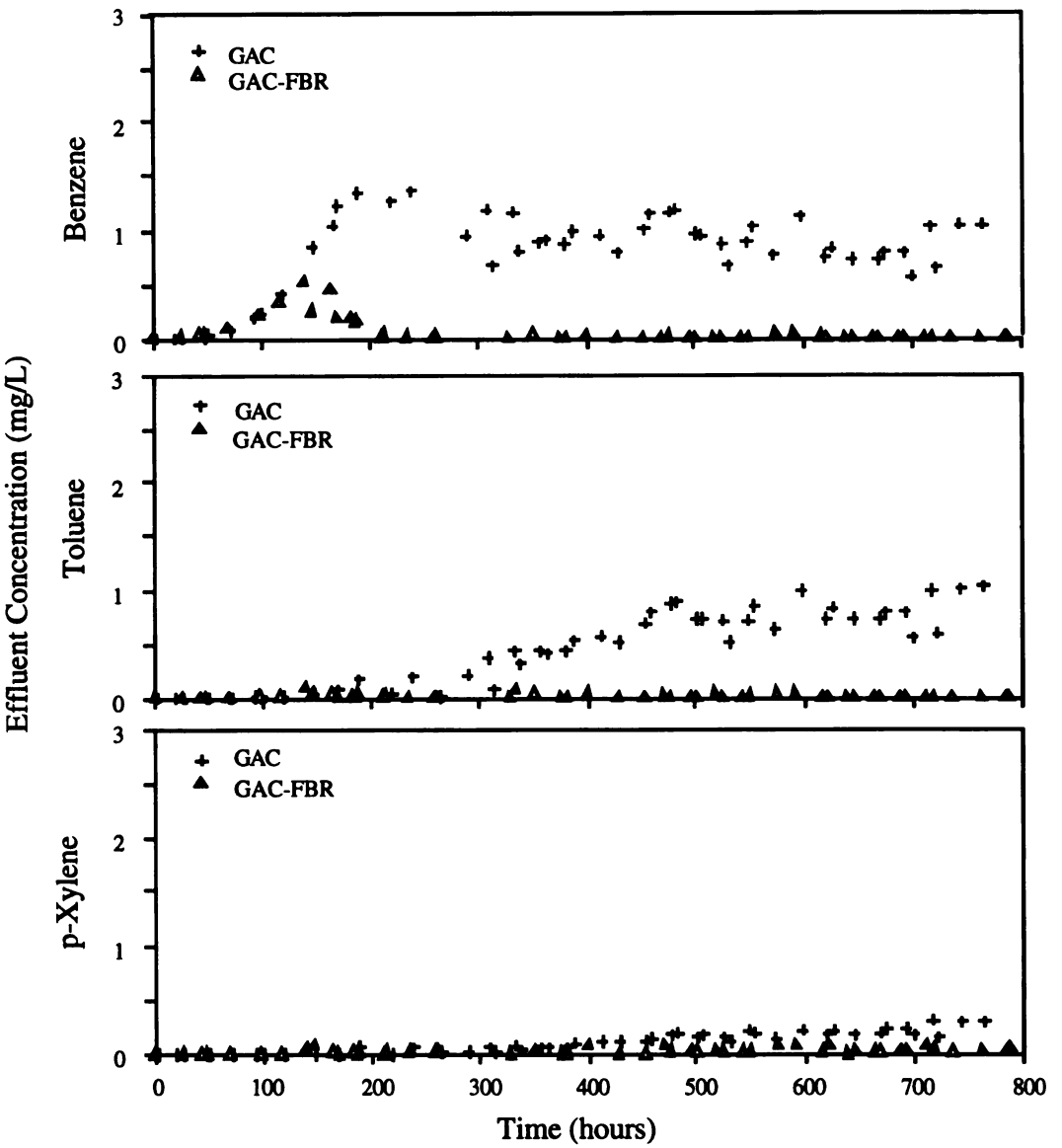

Figure 3-2. Comparison of breakthrough profiles for GAC and GAC-FBR systems. (hydraulic flux rate: 0.29 m^3 /min-m^2 ; empty bed hydraulic retention time: 3.6 min; influent concentration of each BTX component in the influent was maintained at ca. 1 mg/l.)

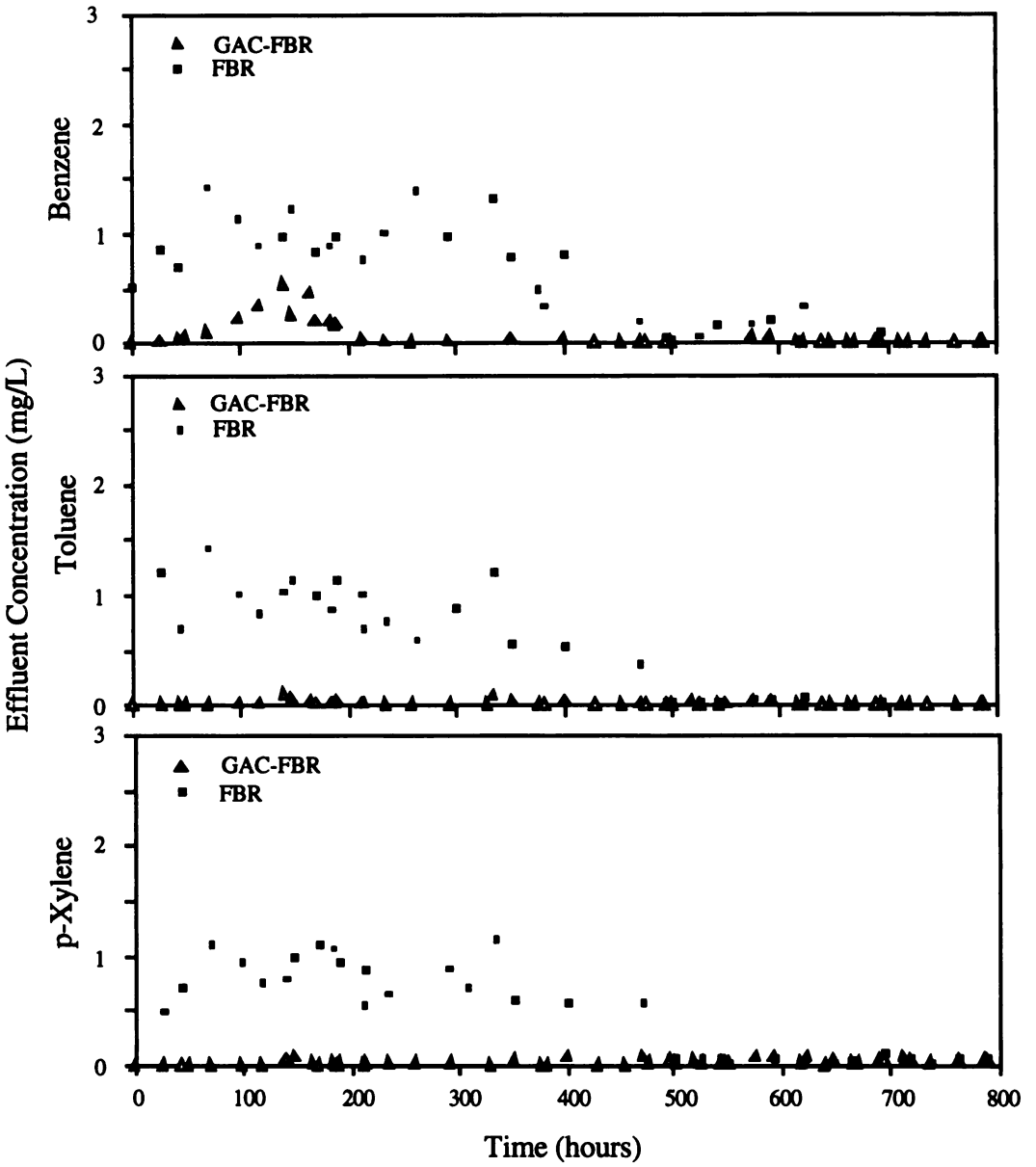

Figure 3-3. Comparison of breakthrough profiles for GAC-FBR and FBR systems. (hydraulic flux rate: 0.29 m^3 /min-m^2 ; empty bed hydraulic retention time: 3.6 min; influent concentration of each BTX component in the influent was maintained at ca. 1 mg/l.)

In the FBR system, where biodegradation was the only significant mechanism, breakthrough of BTX occurred soon after introduction of the influent (Figure 3-3). After approximately 400 hours, sufficient biomass had developed on the surface of the non-activated carbon so that biodegradation became significant and the concentrations of BTX in the effluent began to decrease. At the same time, the bed height and DO consumption began to increase.

The GAC-FBR system, where both adsorption and biodegradation are possible, initially performed much like the GAC system. Effluent benzene concentrations increased up to 50% of the in-line levels by 140 hours, in the same manner as the GAC system. After this initial period, however, the GAC-FBR system performed quite differently: the benzene concentration began to decrease, while the DO consumption and bed height started to increase. Low concentrations of toluene and xylene were also detected in the effluent during this start-up period, but these also decreased with the onset of biological activity. These compounds were detected in the effluent slightly earlier than in the GAC system. This may be a result of particle motion changing from well-defined patterns of localized movement at the beginning of operation to more random patterns as surface growth developed and the seeded particles became more buoyant and started migrating in the reactor (Shieh and Keenan 1986). This type of movement would serve to increase the amount of mixing in the reactor which would produce faster breakthrough. An alternative explanation is that the developing biofilm interferes with adsorption, again resulting in earlier breakthrough.

Even though the same amount of inoculum was added to both the GAC-FBR and FBR systems, the time required until noticeable biodegradation commenced was significantly less in the GAC-FBR reactor. Previous reports have attributed this to the fact that the activated carbon surface has a large number of crevasses and ridges that provide a sheltered environment for colonization. It can be seen from Figure 3-4, however, that the

non-activated carbon used in this study has a very similar surface texture. This suggests that some other property of the activated carbon, such as the adsorption capacity, is responsible for faster colonization rates.

A comparison of the biological systems during start-up, that is until a constant biomass develops and steady-state operating conditions are reached, reveals that the GAC-FBR system produced a higher quality effluent and allowed considerably less BTX to leave the reactor untreated than did the FBR system. The mass of each BTX compound which left the system untreated was determined by calculating the area under the effluent concentration curves using the trapezoidal rule (Burden *et al.* 1981). These results, which are presented in Table 3-3, show that the FBR system allowed approximately 15 times more BTX to leave the system untreated than was observed for the GAC-FBR system.

Table 3-3. Summary of performance of FBRs during the start-up period

Reactor	Compounds	Accumulated Mass of BTX		Average Conc. in effluent (µg/l)
		added (mg)	in effluent (mg)	
GAC -FBR	Benzene	3789	349	83
	Toluene	3652	61	15
	Xylene	3474	75	18
FBR	Benzene	4351	2925	696
	Toluene	3969	2388	569
	Xylene	3568	2149	512

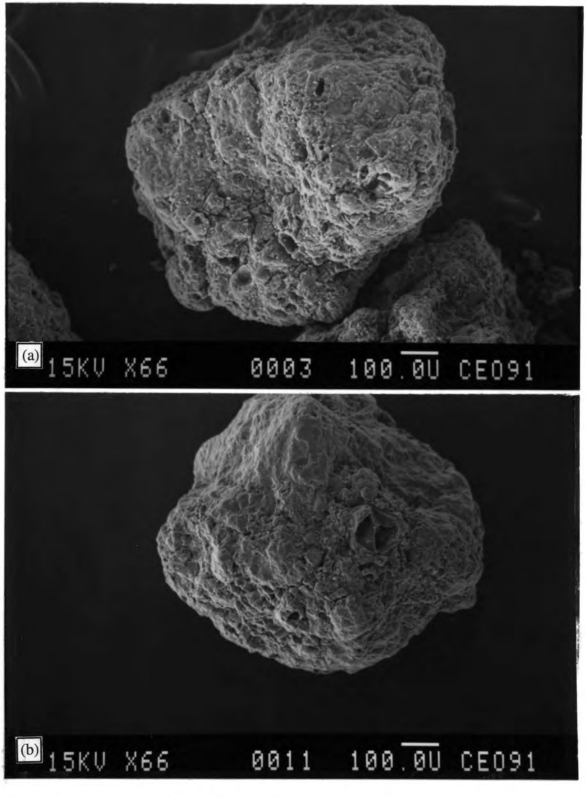

Figure 3-4. SEM of a particle without biological growth. (a) activated carbon, x 66 and (b) non-activated carbon (baker product), x 66.

3.3.3 Comparison of Steady State BTX Removal Between GAC-FBR and FBR Systems

The reactors were assumed to be operating under steady- state conditions when constant DO consumption, consistent with that expected to be required for degradation of the added BTX, was found in the effluent. In this initial test, the average total COD in the in-line to FBR was calculated to be 7.25 mg/L, or an applied organic loading rate of 2.9 kg COD/m^3-day. More than 90 percent of the BTX (94% for benzene and toluene and 90% for xylene) was removed (Table 3-4). The average DO consumed was 4.6 mg/L. The average total COD in the inlet to GAC-FBR system was somewhat higher, 9.6 mg COD/l or 3.8 kg COD/m^3-day. Despite the higher loading rate to the GAC-FBR system, greater substrate removal was observed: 99% for benzene and toluene and 92% for xylene. The average DO consumed was 6.1 mg/L.

Performance of the two systems at a higher loading rate was also examined. With a total COD loading to both reactors of 6.0 kg COD/m^3-day, the two systems performed comparably. As shown in Table 3-5, removal rates averaged 94%, 90% and 81% for benzene, toluene, and xylene. The GAC-FBR system was found to consume slightly more dissolved oxygen.

Table 3-4. Comparison of steady state BTX removals in GAC-FBR and FBR systems

Reactor	Compound	Influent (µg/L)	Effluent (µg/L)	Removal (%)	DO uptake / COD removal	Organic Loading Rate kg COD /m^3-day
GAC -FBR	Benzene	1066 (±303)	3 (±2)	99.7	0.66	3.8
	Toluene	1023 (±340)	5 (±5)	99.6		
	Xylene	996 (±340)	81 (±53)	92.0		
FBR	Benzene	834 (±151)	45 (±40)	94.6	0.68	2.9
	Toluene	758 (±380)	40 (±40)	94.7		
	Xylene	732 (±340)	68 (±42)	90.7		

3.3.4 Response of FBR Reactors to Step Load Increases in BTX Concentration

The GAC-FBR and FBR systems were subjected to several step-load increases to determine whether the use of an adsorptive biomass carrier contributed to system stability. A 96-hour, three-fold step-load increase from a base organic loading rate of approximately 3.0 kg COD/m^3-day was introduced to the two FBRs by increasing the BTX concentration in the in-line, while keeping the flow constant. BTX concentrations in the in-line and effluent are presented in Figures 3-5 and 3-6 for the FBR and GAC-FBR systems, respectively. The FBR system showed an immediate response to the step load increase (Figure 3-5). Benzene and toluene concentrations increased from 40 to 200 µg/l and xylene increased from 70 to 1000 µg/l. DO consumed increased from 4.6 to 13 mg/L within one hour. After initiating a similar increase to the GAC-FBR system, effluent concentrations of the BTX remained at nearly identical levels to those observed prior to the increase. DO consumption increased from 6 to 13 mg/L within one hour. After the loading rate was returned to the base loading level, DO consumption remained at a level higher than that observed during the period prior to the increase. Consumption of DO thereafter decreased slowly to the previous steady-state value.

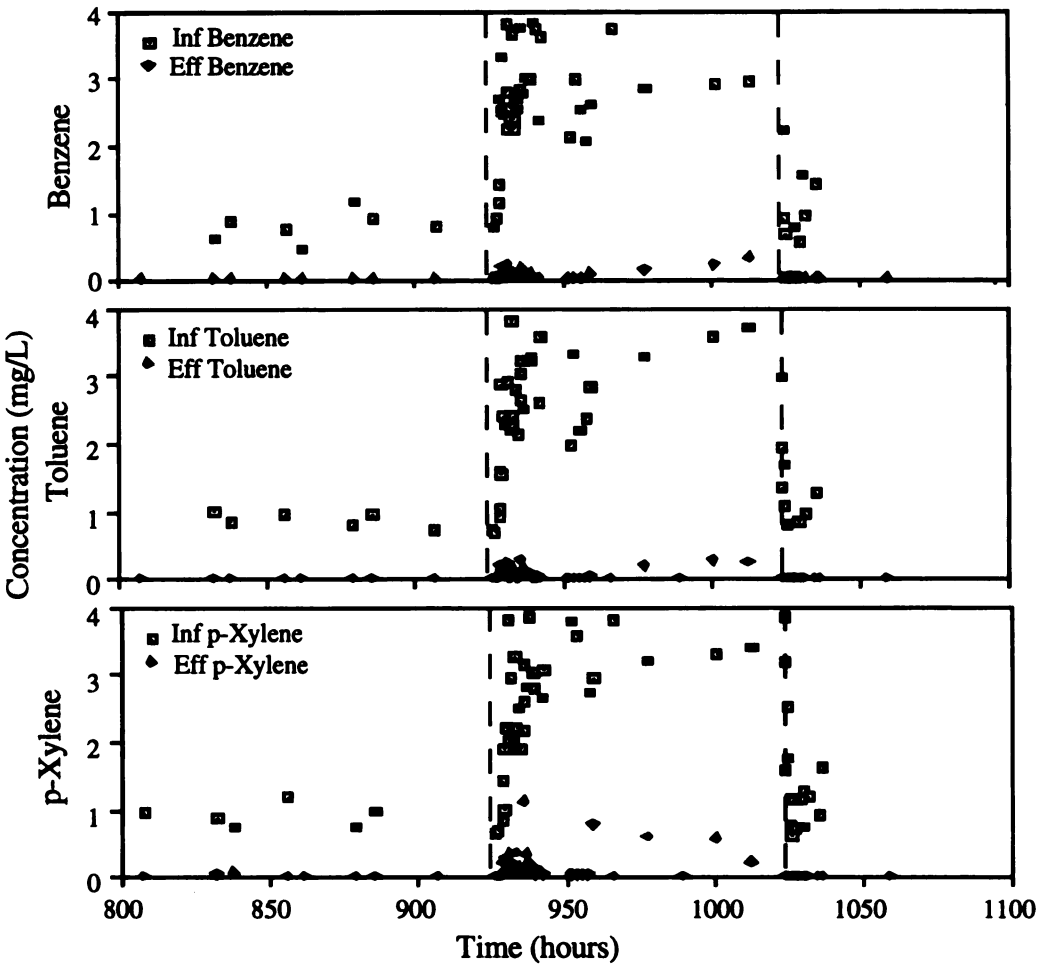

Figure 3-5. The response of FBR system to a 96 hour, 3-fold step load increase from steady state loading level of 2.9 kg COD/m^3 -day. The load increase started at hour number 929 and ended at hour number 1025. (hydraulic flux rate: 0.29 m^3/min-m^2; hydraulic retention time: 3.6 min)

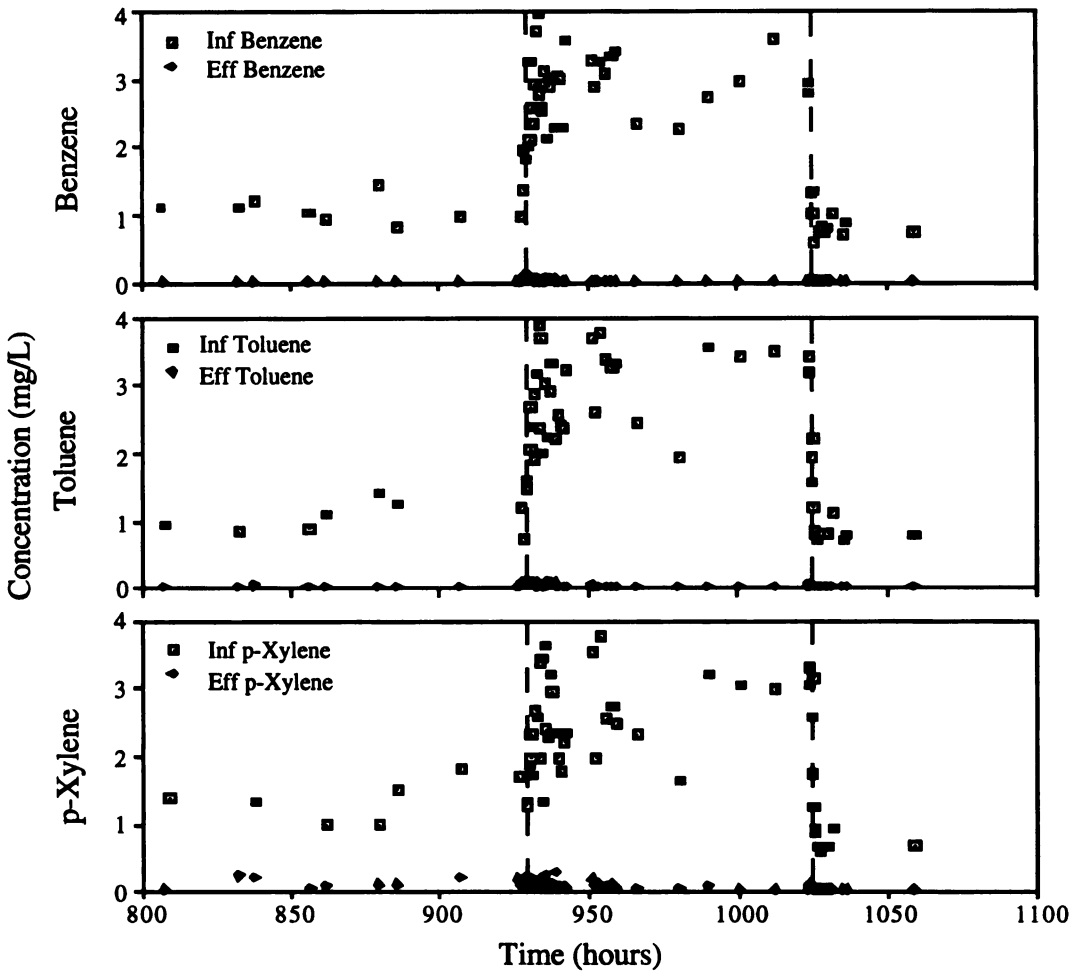

Figure 3- 6. The response of GAC-FBR system to a 96 hour, 3-fold step load increase from steady state loading level of 3.8 kg COD/m^3-day. The load increase started at hour number 929 and ended at hour number 1025. (hydraulic flux rate: 0.29 m^3/min-m^2; hydraulic retention time: 3.6 min)

Because the performance of neither biological system was seriously perturbed by the 3-fold step-load increase, the reactors were subsequently subjected to a five-fold step load increase from a higher base loading level (6.0 kg COD/m^3-day). Step-load increases of this magnitude were conducted for two different durations, 4 and 8 hours. These results, shown in Table 3-5, show that during the increases, BTX removal and DO consumption in the GAC-FBR system were higher than in the FBR system. The higher DO consumption indicates enhanced bioactivity, which may result from a greater amount of biomass or more active cells in the GAC-FBR system. While more dissolved oxygen was consumed, the ratio of DO consumed to COD removed for the GAC-FBR system was lower than for the FBR system (Table 3-5). This indicates that some of COD (BTX) was being removed concurrently via adsorption.

Table 3-5. Summary of FBR responses to step-load increases from base loading level of 6.0 kg COD/m^3-day

State	Reactor	Compound	Influent (µg/L)	Effluent (µg/L)	Subst. removal (µg/L)	DO consumed (mg/L)	DO consumed / COD removed (mg/mg)
Steady State	GAC -FBR	Benzene	1571 (±303)	86 (±40)	1485	8.6	0.68
		Toluene	1531 (±330)	152 (±60)	1379		
		Xylene	1382 (±302)	254 (±100)	1128		
	FBR	Benzene	1628 (±142)	92 (±52)	1536	8.3	0.68
		Toluene	1479 (±170)	148 (±85)	1331		
		Xylene	1267 (±152)	237 (±123)	1030		
4 hrs load increase	GAC -FBR	Benzene	6628 (±820)	1214 (±452)	5414	21	0.47
		Toluene	6334 (±830)	1479 (±430)	4855		
		Xylene	5666 (±802)	1660 (±560)	4006		
	FBR	Benzene	6569 (±853)	2509 (±550)	4060	19	0.60
		Toluene	5996 (±863)	2464 (±535)	3532		
		Xylene	5244 (±873)	2465 (±538)	2779		
8 hrs load increase	GAC -FBR	Benzene	7863 (±550)	1403 (±280)	6457	23	0.45
		Toluene	7503 (±525)	1850 (±336)	5653		
		Xylene	6313 (±568)	2197 (±340)	4116		
	FBR	Benzene	6918 (±600)	2487 (±390)	4431	19	0.60
		Toluene	6222 (±610)	2821 (±410)	3401		
		Xylene	4911 (±450)	2625 (±400)	2286		

3.3.5 Extent of Surface Coverage of the Media by the Biofilms

Microbial attachment to the external surface of GAC has been shown using scanning electron microscopy (Weber *et al.* 1978a; Pirbazari *et al.* 1990). These studies employed completely mixed batch reactors to contact carbon with chemically coagulated and settled sewage for up to 17 days. Under these conditions, a contiguous, uniform biofilm over the entire surface area was not formed. Localized concentrations of bacteria were observed to occur in crevices and areas which are sheltered from fluid shear forces. During this current study, scanning electron microscopy (SEM) was used to examine the extent of biofilm coverage on the GAC and baker product. Samples of GAC that had been taken from the fluidized bed reactors while they were operated under steady state conditions are shown in Figures 3-7. Contiguous biofilms were observed on the support media in both biological systems. These films were quite thick (100 to 200 um) on both the activated and non-activated carbons. The organisms observed on the surface of both carriers had similar morphologies.

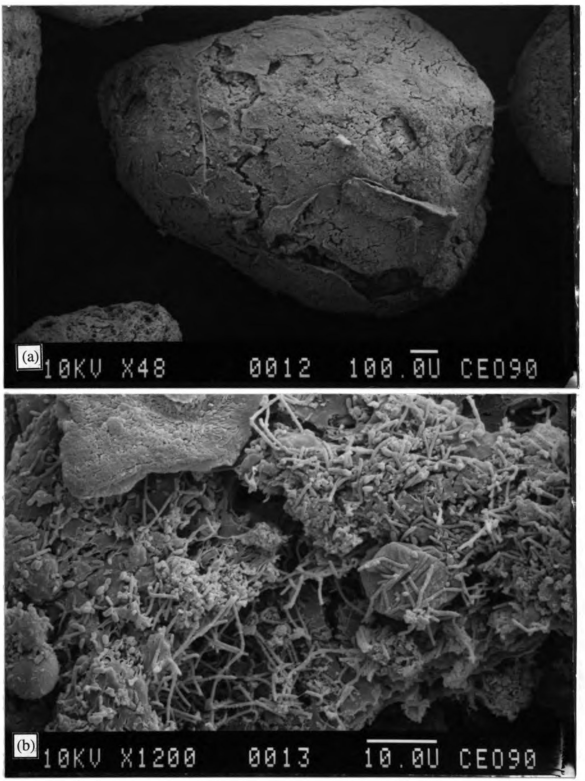

Figure 3-7. SEM of a GAC with biological growth. (a) x 48 and (b) x 1200.

3.4 Discussion

A comparison of the performance of the three reactor systems during start-up reveals one of the most distinctive characteristics of GAC-FBR systems. The adsorption capacity of the biomass support serves to protect effluent quality until the biodegradation capability of the system is established. Only benzene was released at significant concentrations, and this proved to be short-lived. Since activated carbon has a relatively low capacity for benzene, the amount of carbon in these systems was not sufficient to adsorb the total benzene loading during the time required to develop a biofilm. GAC has a higher adsorption capacity for toluene and xylene, so the system had the ability to remove these compounds during the start-up period. The total mass of toluene and xylene released during start-up was 30-40 times greater in the FBR system than in the GAC-FBR system. It thus appears that effluent protection during start-up is a straightforward design consideration involving the amount of carbon in the reactor, the loading rate, the time required to develop a biofilm, and the adsorption capacity of the carbon for the compounds of interest. A second start-up characteristic of GAC-FBR is that these systems developed a biofilm and began degrading BTX faster than the biological system employing non-adsorbing support media. This is supported by visual observation of the biofilm, and data collected on oxygen consumption and BTX in the effluent. This is consistent with previous findings showing that GAC particles support richer microbial communities than other materials (Pirbazari *et al.* 1990). It has been hypothesized that this results from the ability of the carbon to concentrate chemicals necessary for microbial growth while providing an environment that is well protected from fluid shear forces for cell attachment. Since the activated and non-activated carbons were observed to have similar surface textures, it is unlikely that the differences can be explained by shear-force protection alone.

Under steady-state conditions at an organic loading rate of 3.8 kg COD/m^3-day and a hydraulic retention time of 3.6 minutes, the GAC-FBR system produced effluent benzene

and toluene concentrations of 3 and 5 µg/l, respectively. The FBR system, operating at a somewhat lower loading rate of 2.9 kg COD/m^3-day had effluent benzene and toluene levels of 45 and 40 µg/l. Karlson et al. (Karlson and Frankenberger 1989) conducted a batch assay using a mixed bacterial culture capable of utilizing gasoline as a sole carbon source. They reported that 22-35 µg/l of benzene did not provide enough carbon source to sustain an active bacterial population. Higher BTX concentrations were not degraded to levels below the 20-40 µg/l concentration range. One possible explanation for the low effluent concentrations in the GAC-FBR system is that the adsorption capability of the activated carbon may serve to enrich substrate and oxygen levels, thereby allowing the bacterial population to utilize BTX despite the low concentrations in the bulk solution. Alternatively, the low effluent levels reached in the GAC-FBR systems may result from continued effluent polishing via adsorption. At the higher loading rate of 6.0 of kg COD/m^3-day performance of the GAC-FBR and FBR systems were comparable. Effluent concentrations in both systems were above the critical minimum range of 20-40 µg/l discussed above. At this loading rate it appears that adsorptivity of the biomass support has little effect on steady-state performance and the systems are dominated by biofilm processes.

The abilities of the GAC-FBR and FBR systems to respond to in-line shock loads, as simulated by the step concentration increase, demonstrates another distinctive feature of systems with adsorptive biomass supports. Effluent quality during the step increase was better for the GAC-FBR system in all cases. During the highest loading rate applied in the step increase experiments, the GAC-FBR system was found to remove 82, 74 and 64 percent of the benzene, toluene and xylene applied, compared to 65, 56, and 46 percent in the FBR system.

It was previously suggested that the superior performance of the GAC-FBR system during the step increase could be attributed to adsorption. This conclusion is

supported by the lower ratios of DO consumed to COD removed in the GAC and the implied assumption that the observed differences could not be explained entirely by changes in metabolic efficiency. If we assume that efficiency remains constant at the value found under steady-state conditions (where it is assumed that only biodegradation is active), we can calculate the amounts of COD removed by biodegradation and by adsorption during the step increase. This analysis is presented in Table 3-6. From this data it appears that biodegradation is essentially the same in both systems and the lower effluent levels in the GAC-FBR system are attributable to additional removal by adsorption.

Table 3-6. Summary of FBR performance during step load increases

State	Reactor	Compound	BTX Mass (mg)		DO consumed (mg)	Removed COD (mg)	
			Influent	Effluent		Biodegra.	Adsorp.
4 hrs load increase	GAC -FBR	Benzene	479	96	1513	2225*	1105†
		Toluene	514	110			
		Xylene	413	133			
	FBR	Benzene	477	190	1355	2261‡	N/A
		Toluene	440	189			
		Xylene	383	196			
8 hrs load increase	GAC -FBR	Benzene	1128	208	3443	5063*	2086†
		Toluene	1075	276			
		Xylene	899	327			
	FBR	Benzene	987	347	2842	4541‡	N/A
		Toluene	886	392			
		Xylene	698	375			

* Amount of COD removed by biodegradation equals the DO consumed divided by 0.68.
† Amount of COD removed by adsorption equals the total COD removal minus the amount of COD removed by biodegradation.
‡ Amount of COD removed by biodegradation equals the total COD removal.

Elevated levels of DO consumption following a step increase and subsequent return to original loading levels were observed in the GAC-FBR but not in the FBR systems. This is strong circumstantial evidence for bioregeneration of adsorption sites on the GAC. The BTX adsorbed during the load increase were apparently desorbed from the carbon to

the biofilm when bulk aqueous phase concentration decreased. Such a conclusion is also consistent with the observation that there was slightly more DO consumption under the initial steady-state conditions, since this may have resulted from desorption and degradation of the BTX adsorbed during start-up.

3.5 Conclusions

This study demonstrates that the use of activated carbon as a biomass carrier in fluidized bed reactors produces a system in which both adsorption and biodegradation affect substrate removal. To a large extent, the performance of such biological activated carbon systems is controlled by the additive contributions of these two removal mechanisms. During the start-up period, before a fully functional biomass has developed, the substrate is removed primarily by adsorption. After the biomass is established and steady-state conditions are reached, system performance is dominated by biodegradation. The system retains adsorption capacity under steady state conditions, however, as evidenced by the response of the system to in-line concentration shocks. Lower effluent concentration levels were found in GAC-FBR systems during a step concentration increase than in similar systems using a non-adsorbing biomass carrier. The data suggest that this results from adsorption of a portion of the increase, and that this material can be subsequently desorbed and biodegraded when the inlet concentration returns to pre-shock levels.

The combination of adsorption and biodegradation in a single reactor system also produced two synergistic effects. First, biomass developed more quickly on the surface of activated carbon than it did on non-activated carbon with a similar surface texture. Second, higher steady-state substrate removal was observed in the GAC-FBR system at lower loading levels (3.8 kg COD/m^3-day). A potential explanation for both of these observations is that the adsorptivity of the activated carbon surface serves to concentrate substances

including the substrates, nutrients, and oxygen on the carrier surface. This concentration effect may promote more rapid colonization and may allow degradation to occur when the substrate concentrations in the bulk solution are too low to support growth.

CHAPTER 4

SINGLE SUBSTRATE STEP-LOADING RATE INCREASES

4.1 Introduction

The beneficial aspects of integrated biodegradation/adsorption systems were reported in the early work on the use of GAC for secondary and tertiary treatment of municipal wastewater (Weber *et al.* 1970). In systems designed as adsorbers, it has been shown that biodegradation increases the period between GAC regeneration cycles (Bouwer and McCarty 1982; Chudyk and Snoeyink 1984; De Laat *et al.* 1985; Kim *et al.* 1986; Gardner *et al.* 1988; Speitel *et al.* 1989a; 1989b; 1989c). In biofilm systems, the use of GAC as a biomass carrier has been shown to provide for removal of compounds resistant to biodegradation (Suidan *et al.* 1983; Suidan *et al.* 1987). In a treatment process for groundwater contaminated with relatively low levels of volatile organic compounds, a biological activated carbon fluidized-bed reactor (GAC-FBR) system has been demonstrated to provide both the efficiency of biological removal and the positive effluent protection capability of activated carbon adsorption (Hickey *et al.* 1990b; 1991a; Voice *et al.* 1992).

In the previous chapter, the performance of adsorptive and non-adsorptive biofilm carriers in biological fluidized-bed reactors (FBR) was compared for the treatment of groundwater contaminated with benzene, toluene and *p*-xylene (Voice *et al.* 1992). The results demonstrated that the GAC-FBR system functioned primarily as a biological reactors under steady-state conditions, but that the GAC carrier contributed significantly

to overall removal during transient conditions. This study extends the previous work by characterizing the interactions (such as inhibition) among benzene, toluene and *p*-xylene before and during a step-load increase in the influent concentration of one of these substrates while the other two were held constant.

4.2 Materials and Methods

4.2.1 Media and Chemicals

GAC (Calgon Filtrasorb 400, Calgon Co., Pittsburgh, PA) and non-activated carbon, termed "baker product" by the manufacturer, were used as carrier media in two fluidized-bed reactor systems. The baker product is the same material as GAC except that it has not undergone the activation step in the manufacturing process and has little adsorptive capacity (Voice *et al.* 1992). Both media were sieved to obtain a 20 x 30 mesh fraction (average particle diameter 0.75 mm). Prior to addition to the reactors, the GAC and baker product were rinsed with distilled water to remove fines and dried at 100 °C for 24 hours.

Benzene, toluene, *p*-xylene (BTX), potassium phosphate dibasic and ammonia chloride were obtained from J.T. Baker Chemical Co., Phillipsburg, NJ. All chemicals were reagent grade. The water supplied to Michigan State University was used directly as a source of groundwater. This relatively hard water (450 mg/L as $CaCO_3$) is pumped from a deep aquifer underlying the campus and distributed without further treatment.

4.2.2 Biodegradation Kinetic Assays

After a mature biofilm developed on the baker product, a small amount of biomass coated material was removed from the FBR. The biomass was stripped from the carrier particles, purged with oxygen to remove residual BTX, and homogenized by shaking the biomass using glass beads in a 300 mL glass bottle. Kinetic assays were performed using a 50 mL glass syringe equipped with a Lure-lock valve. A stirring bar (6 mm in diameter and 15 mm long) was placed in the syringe. The syringe was placed on a magnetic stirrer to provide mixing. The growth media was groundwater amended with nitrogen and phosphorous at a weight ratio of 100/5/1:COD/N/P. Prior to addition of the BTX this water was oxygenated with pure oxygen to obtain an initial dissolved oxygen concentration of greater than 30 mg/L. Stock substrates of benzene, toluene and *p*-xylene were dissolved in tap water and then measured amounts of these solutions were transferred to the syringes. The initial concentration of BTX was varied from 1200 to 3900 µg/L for each substrate. A constant ratio of B:T:X of 1:1:1 was used for all assays. The initial concentration of biomass was varied from 20 to 80 mg VSS/L. After biomass was introduced into the syringe, a one-mL liquid sample was taken. Subsequent one-mL samples were taken at regular intervals to track degradation. Assays were conducted in triplicate.

The net yield coefficient (Y) was measured using a similar procedure as used in the kinetic assay except only one substrate was added to the syringes at a time. Liquid samples were taken at the beginning and end of experiment to measure the concentration of substrate and biomass. Biomass concentrations (VSS) were measured according to Standard Methods (Greenberg *et al.* 1985). All assays were conducted at 20 °C.

4.2.3 Fluidized-Bed Reactors

The laboratory-scale fluidized-bed reactors were constructed using a 2.5 cm diameter by 184 cm height glass column (Figure 4-1) to produce a system with a 1000-mL working volume. Two reactors were used in this study to allow comparison of adsorptive and non-adsorptive biofilm carriers. The GAC-FBR (combined adsorption and biodegradation) and FBR (biodegradation only) were charged with activated carbon and baker product, respectively. The reactors were operated as single pass systems with no recycle. Both were inoculated with a mixed culture obtained from a pilot-scale FBR that was originally seeded with activated sludge and was subsequently supplied with BTX as the sole carbon source for more than two years (Hickey *et al.* 1990b). Dissolved oxygen (DO) was supplied to the reactors by oxygenating the reactor feed water with pure oxygen to a concentration sufficient to maintain an effluent DO of more than 4.0 mg/L. Nitrogen and phosphorus were supplemented at a weight ratio of 100/5/1:COD/N/P. A mixture of benzene, toluene and *p*-xylene, at a 1:1:1: volumetric ratio, was injected into feed water using a syringe pump (Harvard Apparatus 22) and dissolved using an in-line mixer. A recycle loop around the mixer was employed to create turbulent flow inside the mixer and help ensure complete BTX dissolution. This BTX contaminated groundwater was then pumped to the bottom of the reactors using peristaltic pumps (Watson-Marrow 502E with 501R head). The bed height was controlled by mixing the bed twice a week with a long thin wire brush that sheared some biomass from the carrier medium. The reactors were charged with 180 mL media initially, which produced a settled bed height of 33 cm. BTX contaminated water was fed at flow rate of 200 mL/min to each reactor, resulting in a hydraulic flux rate of 0.41 m/min, an empty bed hydraulic retention time of 5 min and organic loading rate about 3 kg COD/m^3-day. The temperature of the feed water was 15 ± 1 °C.

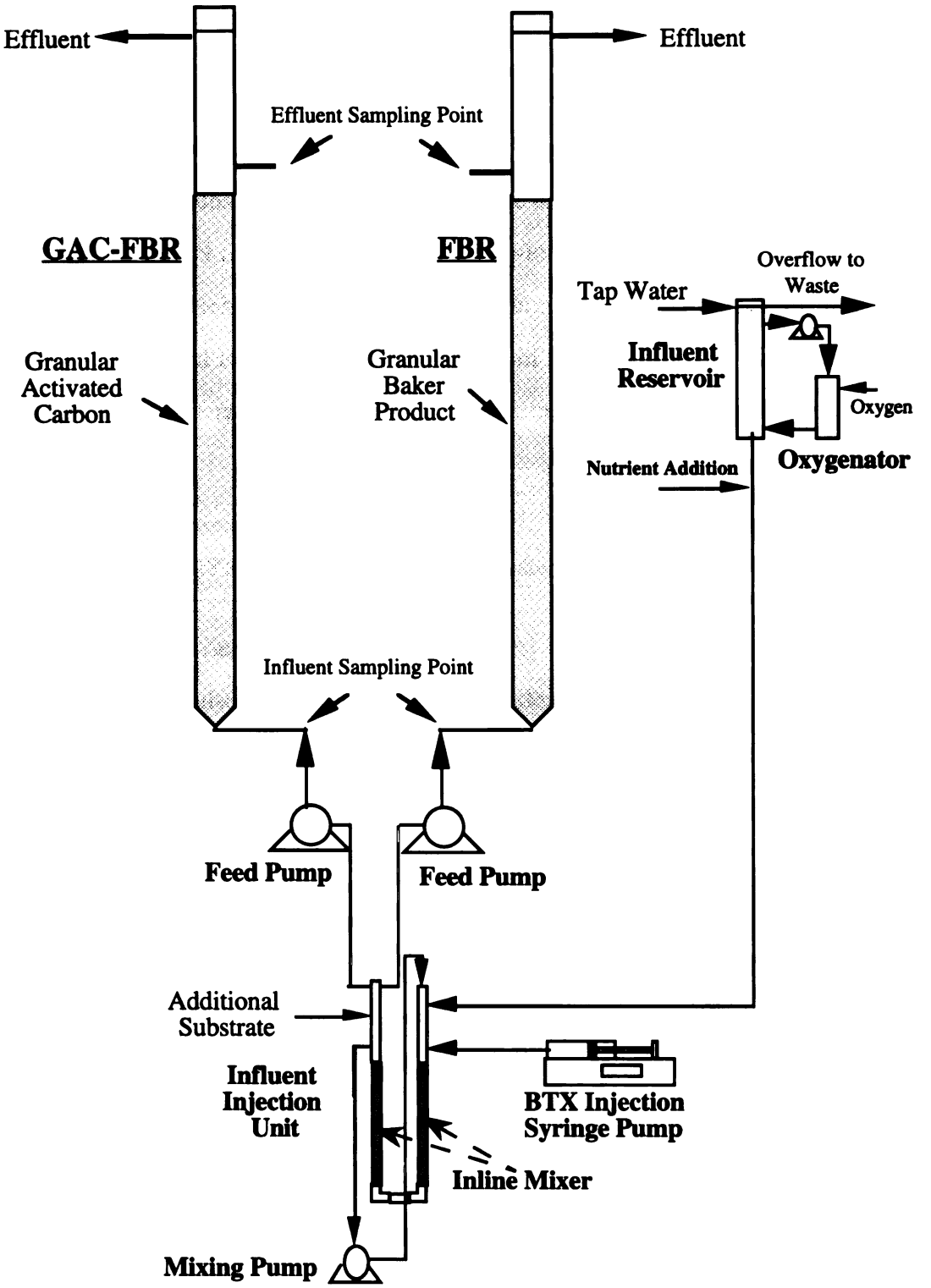

Figure 4-1. Schematic of fluidized-bed reactor systems

4.2.4 Step-Loading Rate Increase Experiments

After steady-state conditions reached, as determined by biomass growth and BTX removal, the organic loading rates were increased seven-fold by raising the concentration of one of the BTX substrates in the feed water. The organic loading rates during the step-load increases were 9 to 11 kg COD/m^3-day. Step-loading increases were maintained for four hours, after which the concentration was returned to the steady-state loading level. During the increased loading period, the amount of DO and essential nutrients supplies were concurrently increased to ensure that these factors did not limit performance.

4.2.5 Extraction of BTX from Seeded GAC and Baker Product

Biomass carrier (GAC and baker-product) samples were collected from each reactor, dried with adsorbent tissues and then transferred to 17 mL glass vials containing 15 mL of methanol. The vials were capped with Teflon®-coated septa and aluminum seals and tumbled at 6 rpm for three days. Analysis involved transferring 100 µL of the extract to 20 mL headspace vials containing 10 mL NaCl saturated water and 120 µL 6 M HCl. The vials were sealed with Teflon®-coated septa and aluminum crimps seal and analyzed by headspace chromatography. After samples were withdrawn for analysis, the carbon was dried at 90 oC for 24 hours and weighed on an analytical balance. The extraction efficiencies for a clean GAC with a known amount of BTX were 93%, 79% and 66% for benzene, toluene and *p*-xylene, respectively.

4.2.6 Analytical Procedures

BTX concentrations were determined by using a gas chromatograph equipped with a flame ionization detector (FID) using helium as a carrier gas (Perkin-Elmer, model

8700) coupled with an automated headspace sampler (Perkin-Elmer, model HS-101). Separation was accomplished using a 30 m x 0.53 mm DB-624 column (J & W Scientific). Water samples, collected from the fluidized-bed reactors (5 mL or 10 mL), were transferred to 20 mL glass vials with Teflon®-coated septa and aluminum crimps seals. The samples were equilibrated for 1 hour at 80 °C and an aliquot of the headspace gas was injected into the gas chromatograph. The detection limit of this procedure was 1 µg/L for each BTX component. The accuracy for measurements of split samples containing a concentration of 1.5 µg/L was ±0.1 µg/L

DO concentrations in the influent and effluent were measured using a polarographic electrode (Orion 97-08-00) coupled to a digital pH meter (Orion 611). If the DO concentration exceeded the limit of the instrument (15 mg/L), the sample was diluted 1:1 or 1:2 with tap water of a known low DO concentration.

4.3 Results

4.3.1 Steady-State Conditions

During steady-state operation, both fluidized-bed systems performed similarly; the removal rates were approximate 99 percent for all substrates. Influent and effluent substrate concentrations and DO consumption are shown in Table 4-1. Effluent concentrations were typically less than 4 µg/L, 8 µg/L and 15 µg/L for benzene, toluene and *p*-xylene, respectively. Total DO consumption was 9.36 mg/L for GAC-FBR and 7.71 mg/L for the FBR. The slightly higher DO consumption in the GAC-FBR was attributed to slightly higher loading rate for GAC-FBR (3.21 kg COD/m^3-day vs. 3.07 kg COD/m^3-day).

Table 4-1. Summary of fluidized-bed system performance at steady-state and during a step-load increase

State	Reactor	Compound	Influent (µg/L)	Effluent (µg/L)	DO Consumed (mg/L)
Steady-State	GAC-FBR	Benzene	1234 ±141	4 ±3	9.36 ±0.22
		Toluene	1211 ±146	8 ±7	
		p-Xylene	1125 ±109	15 ±8	
	FBR	Benzene	1188 ±130	1 ±0	7.71 ±0.65
		Toluene	1157 ±125	3 ±2	
		p-Xylene	1070 ±65	8 ±4	
Benzene loading increase	GAC-FBR	Benzene	9756 ±384	3 ±4	19.90 ±0.72
		Toluene	1367 ±57	2 ±4	
		p-Xylene	1291 ±64	5 ±3	
	FBR	Benzene	9312 ±871	5 ±6	20.35 ±1.03
		Toluene	1298 ±127	10 ±6	
		p-Xylene	1224 ±127	36 ±8	
Toluene loading increase	GAC-FBR	Benzene	1456 ±187	4 ±6	17.53 ±2.19
		Toluene	9734 ±1153	16 ±18	
		p-Xylene	1198 ±143	32 ±13	
	FBR	Benzene	1535 ±76	2 ±3	18.28 ±1.88
		Toluene	10566 ±482	18 ±16	
		p-Xylene	1253 ±57	32 ±33	
p-Xylene loading increase	GAC-FBR	Benzene	1246 ±243	2 ±4	17.05 ±0.57
		Toluene	1185 ±234	2 ±3	
		p-Xylene	8421 ±1704	19 ±8	
	FBR	Benzene	1214 ±204	4 ±4	15.96 ±1.59
		Toluene	1151 ±197	5 ±3	
		p-Xylene	8357 ±1488	680 ±234	

4.3.2 Biodegradation Kinetics

The degradation kinetics for benzene, toluene and *p*-xylene by a mixed culture obtained from the FBR were studied in batch experiments. Degradation rate parameters were estimated using a kinetic equation and a mass balance on biomass growth:

$$\frac{dS}{dt} = -\frac{kSX}{K_s + S} \tag{4-1}$$

$$X = X_0 + Y(S_0 - S) \tag{4-2}$$

Where: S is the concentration of substrate (mg/L).

S_0 = initial concentration of substrate (mg/L).

X = concentration of biomass (mg VSS/L).

X_0 = initial concentration of biomass (mg VSS/L).

k = maximum specific utilization rate of substrate (mg substrate/mg VSS-day).

K_s = half-saturation coefficient (mg/L).

Y = net yield coefficient of biomass (mg VSS/mg substrate).

t = time (day).

Combining Eqn. 4-1 and Eqn. 4-2, integrating and rearranging results in:

$$t = \frac{1}{k}\left[\frac{K_s}{(X_0 + YS_0)}\ln\left(\frac{S_0}{S}\right) - \left(\frac{K_s}{(X_0 + YS_0)} + \frac{1}{Y}\right)\ln\left(\frac{X_0}{X_0 + Y(S_0 - S)}\right)\right] \tag{4-3}$$

The net yield coefficient (Y) was calculated using Eqn. 4-2. The values of k and K_s were determined using a nonlinear regression analysis (Systate 5.1, SYSTAT, Inc.) of Eqn. 4-3. These results are presented in Table 4-2. The biodegradation pattern for these compounds is shown in Figure 4-2. When the concentration of each of the BTX constituents was greater than 530 μg/L, the degradation rate for toluene was highest and followed by benzene degradation. If the concentrations were lower than 530 μg/L,

however, the rate of benzene degradation was fastest. The degradation rate for *p*-xylene was the lowest for the concentration range tested.

Table 4-2. Biodegradation rate parameters for BTX by a mixed culture

Substrate	k (mg/mg/day)	Ks (mg/L)
Benzene	0.99 ±0.12	0.19 ±0.16
Toluene	1.48 ±0.21	0.47 ±0.23
p-Xylene	0.73 ±0.16	0.58 ±0.58

To understand the effect of biodegradation rate on performance of the biological fluidized-bed at steady-state, a concentration profile for the bottom half of FBR is shown in Figure 4-3. The average degradation rates were calculated using Eqn. 4-4 for each compound in first three sections of a FBR.

$$R = \frac{S_L - S_H}{dt_s} \qquad (4\text{-}4)$$

Where: R = average degradation rate in the section (mg/L-min).

S_L = concentration of substrate at lower point of the section (mg/L).

S_H = concentration of substrate at higher point of the section (mg/L).

dt_S = empty bed hydraulic retention time of the section.

The degradation rate for toluene was highest in first section. After the first section, the degradation rate for benzene became highest because the concentrations of BTX were lower than 530 μg/L.

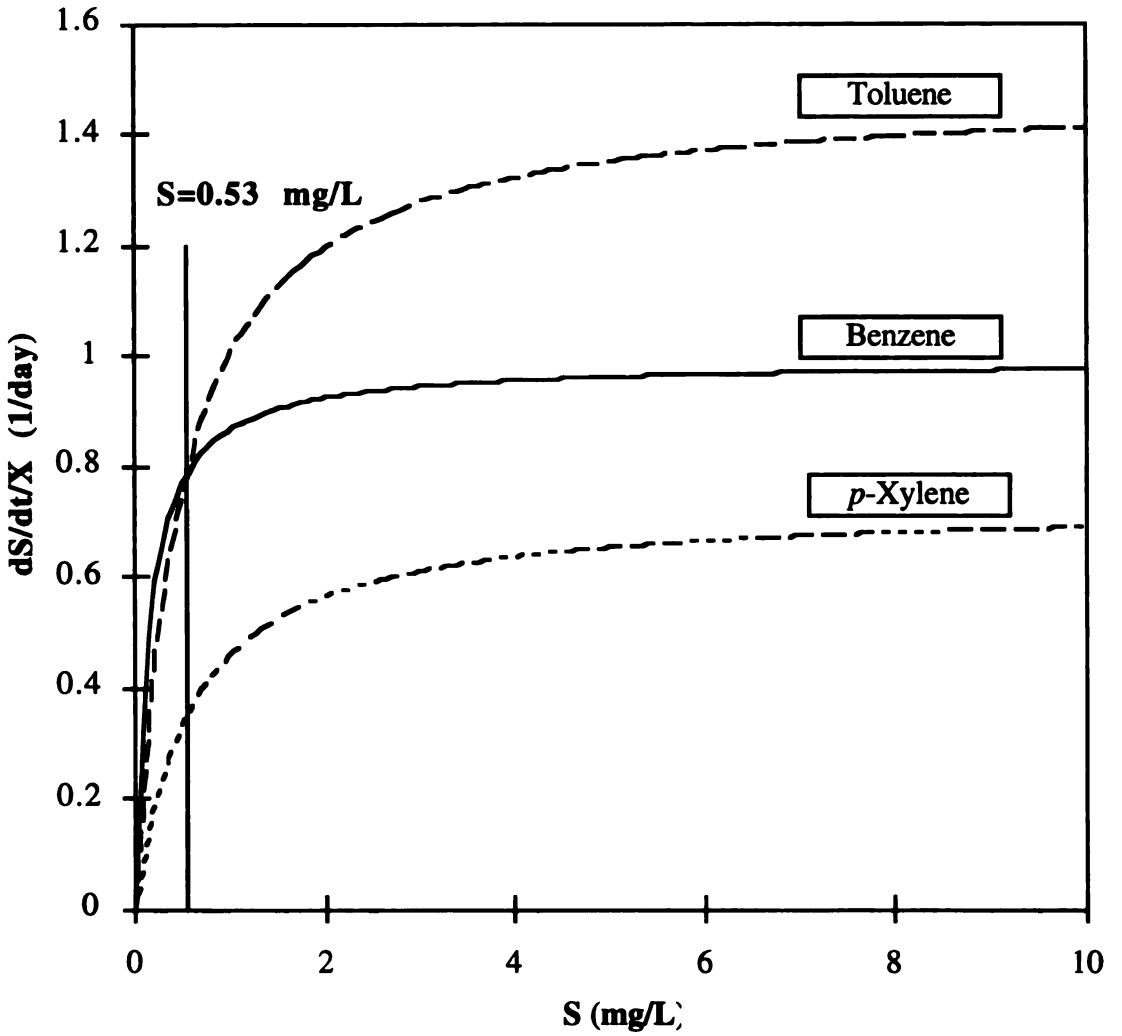

Figure 4-2. The biodegradation rate patterns for BTX in a mixed culture

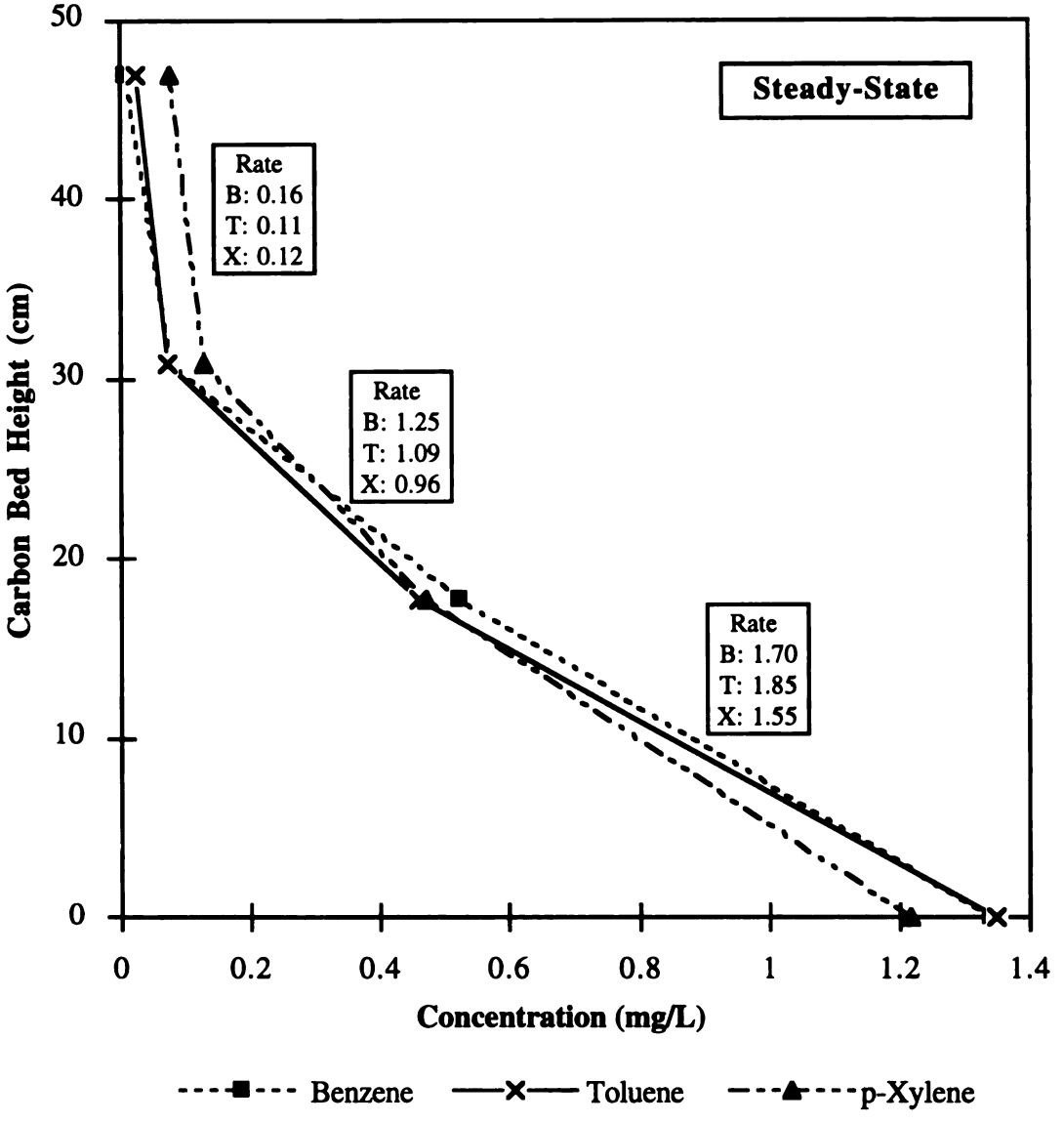

Figure 4-3. Profile of BTX concentrations and degradation rates in a FBR under steady-state conditions

4.3.3 Step-Load Increase Experiments

After steady-state conditions were achieved, the concentration of benzene in the influent was increased seven-fold while the concentrations of toluene and *p*-xylene were keep constant. The concentrations of nutrients and DO were also increased corresponding to the loading increase. The step-load increase was maintained for four hours; the loading rate was subsequently decreased to the initial steady-state level. The step-load increase experiments were repeated for both toluene and *p*-xylene.

The substrate concentration and the DO consumption during the benzene step-load increase are shown in Table 4-1. The concentration of BTX in effluent of the fluidized-bed systems is shown in Figure 4-4. The DO consumption and effluent quality of the two systems were similar. The removal of BTX was greater than 97 percent. *p*-Xylene concentrations in the effluent of the FBR were slightly higher than in GAC-FBR and higher than the value at steady-state. The average concentration of benzene and toluene in the effluents were similar to the steady-state levels.

During the benzene step-load increase, the increase in the concentration of benzene could effect the degradation rate of toluene and *p*-xylene (Figure 4-5). The degradation rate for benzene increased from the steady-state value of 1.70 mg/L-min to 9.35 mg/L-min in the bottom section, from 1.25 mg/L-min to 4.95 mg/L-min in second section and from 0.16 mg/L-min to 4.87 mg/L-min in third section. The degradation rates for toluene and *p*-xylene did not change in the bottom section compared to steady-state value. The degradation rates of toluene and *p*-xylene did, however, decrease in the second section despite the fact that the average concentration for toluene was higher than the value at steady-state (410 vs. 260 μg/L). Increases in the toluene and *p*-xylene degradation rates were observed in third section.

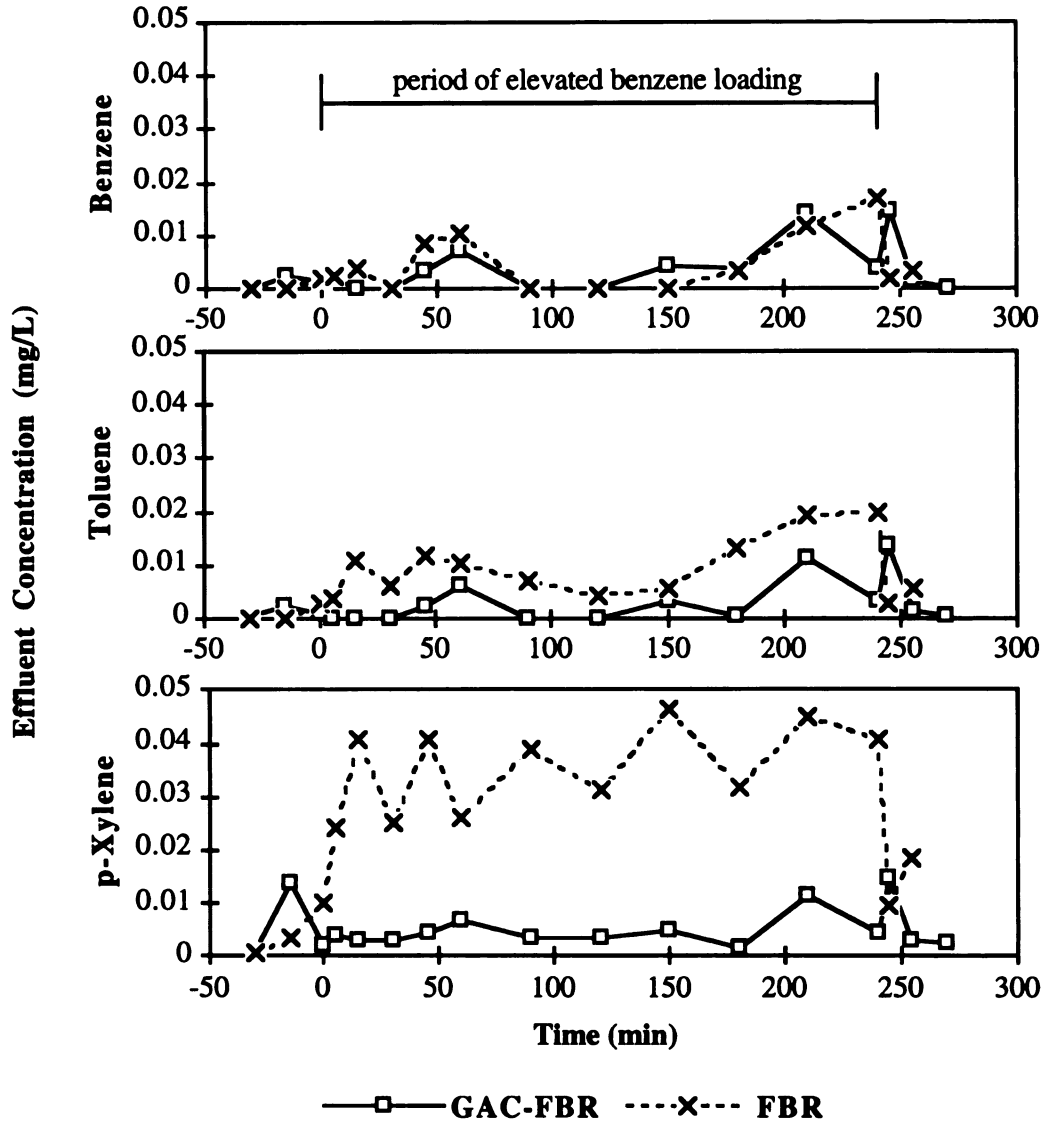

Figure 4-4. Substrate concentrations in the effluent of fluidized-bed systems during a benzene step-load increase experiment

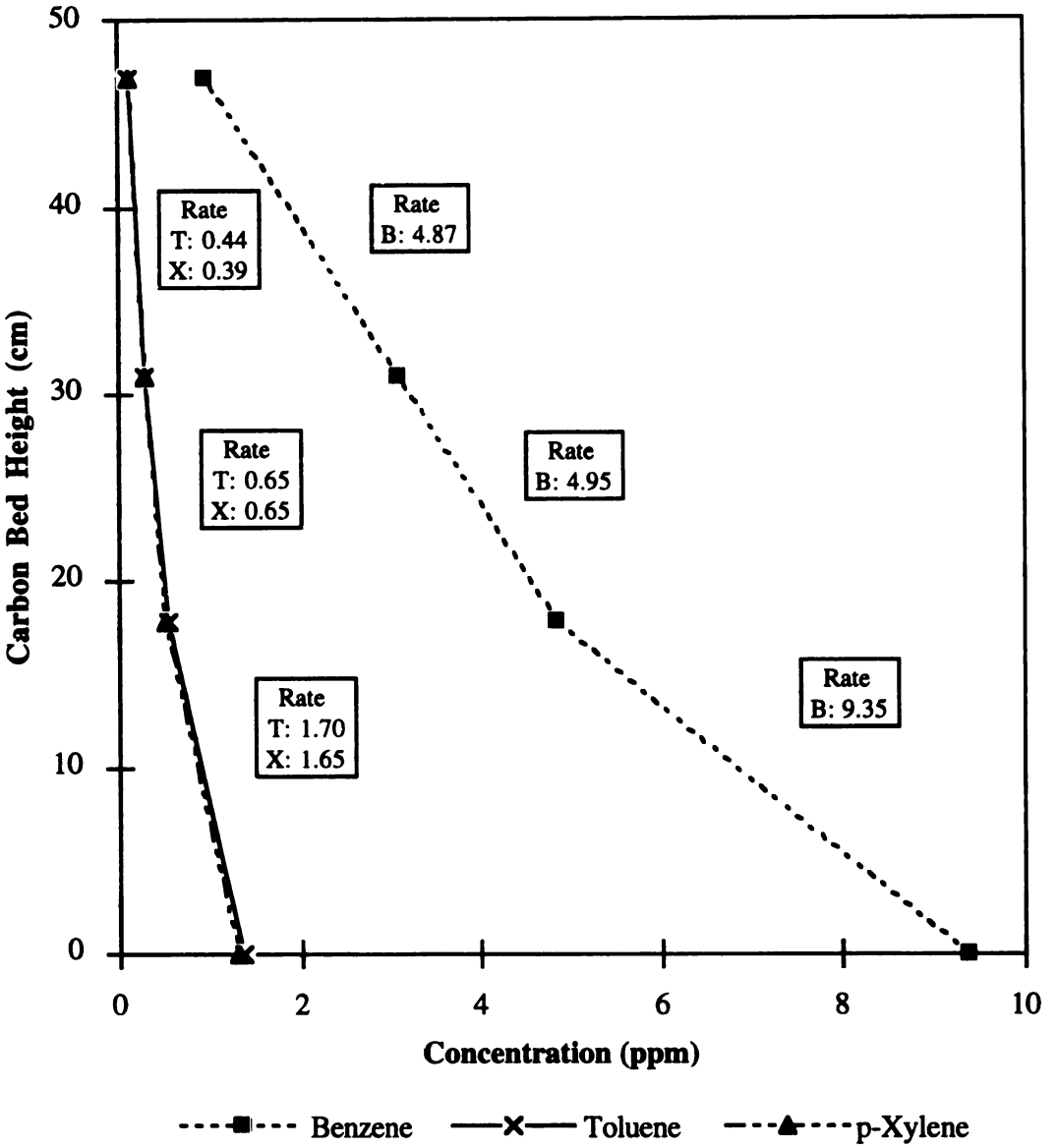

Figure 4-5. Profile of BTX concentrations and degradation rates in a FBR at end of benzene step-load increase

The two fluidized-bed systems performed almost identically during the toluene step-load increase (Table 4-1, Figure 4-6). The concentration of toluene and *p*-xylene in the effluent streams were slightly higher than the concentrations observed at steady-state (from 3 - 8 μg/L compared to 16 - 18 μg/L for toluene and 8 - 15 μg/L compared to 32 μg/L for *p*-xylene). The concentration of benzene in the effluent did not change when compared to the steady-state levels.

During the *p*-xylene step increase, the effluent concentration of benzene and toluene were the same for the GAC-FBR and FBR. These values were essentially the same as the values observed at steady state (about 3 μg/L and 4 μg/L for benzene and toluene, respectively). The concentration of *p*-xylene in effluent of GAC-FBR was, however, significantly lower than in the FBR (Table 4-1, and Figure 4-7) The total mass of *p*-xylene in the effluent of the non-adsorption system (FBR) was 34 times higher (19 μg/L compared to 680 μg/L) than in the GAC-FBR (Figure 4-8).

The concentration profile of substrate was measured in both systems at the end of each step increase. The profiles of BTX at the end of the benzene step increase are plotted in Figure 4-9. There were no significant differences in terms of the concentrations of BTX and oxygen consumption in the two fluidized-bed systems. This indicates the same biodegradation rate in the two fluidized-bed systems. Likewise, similar concentration profile patterns were observed for both reactors during the toluene step-load increase. During the *p*-xylene step-load increase experiment, the concentration profiles of benzene and toluene were similar for both the adsorptive and non-adsorptive systems (Figure 4-10). The nearly identical DO profiles indicates that similar level of biological activity occurred in two fluidized-bed systems. The *p*-xylene concentration profiles were, however, very different (Figure 4-10). Lower concentrations of *p*-xylene were observed at all heights in the GAC-FBR compared to the FBR.

A small amount of carrier media were removed from the GAC-FBR and subjected to solvent extraction prior to and at the conclusion of each step-load increase to determine changes in the amount of BTX adsorbed. During the benzene step-load increase, no change in the concentrations of adsorbed BTX were observed. However, there was a slight but significant change in adsorbed BTX during the toluene step-load increase based on 90% confidence levels. Likewise a small mass of *p*-xylene and toluene were adsorbed during the *p*-xylene step-load increase (Figure 4-11).

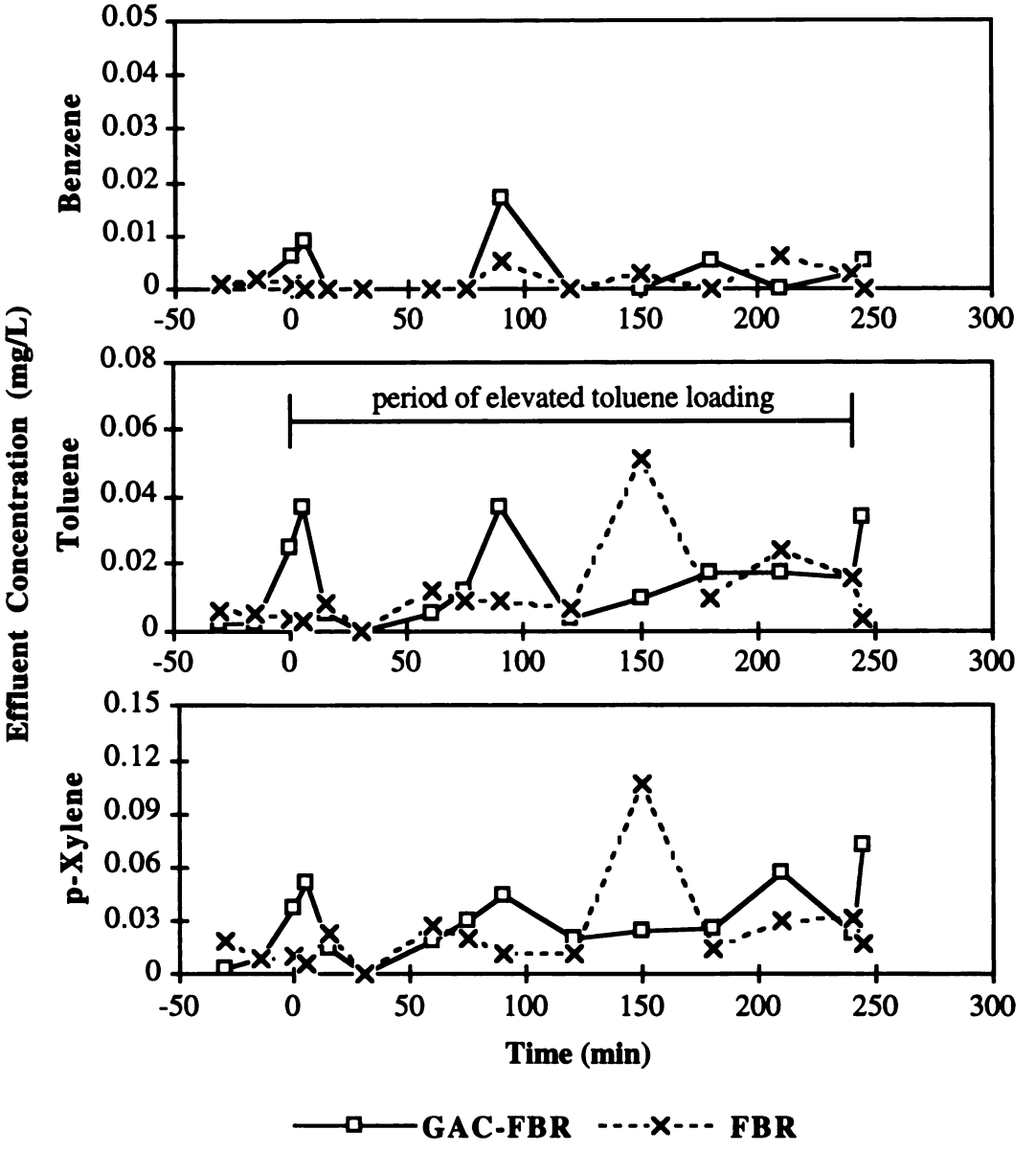

Figure 4-6. Substrate concentrations in the effluent of fluidized-bed systems during a toluene step-load increase experiment

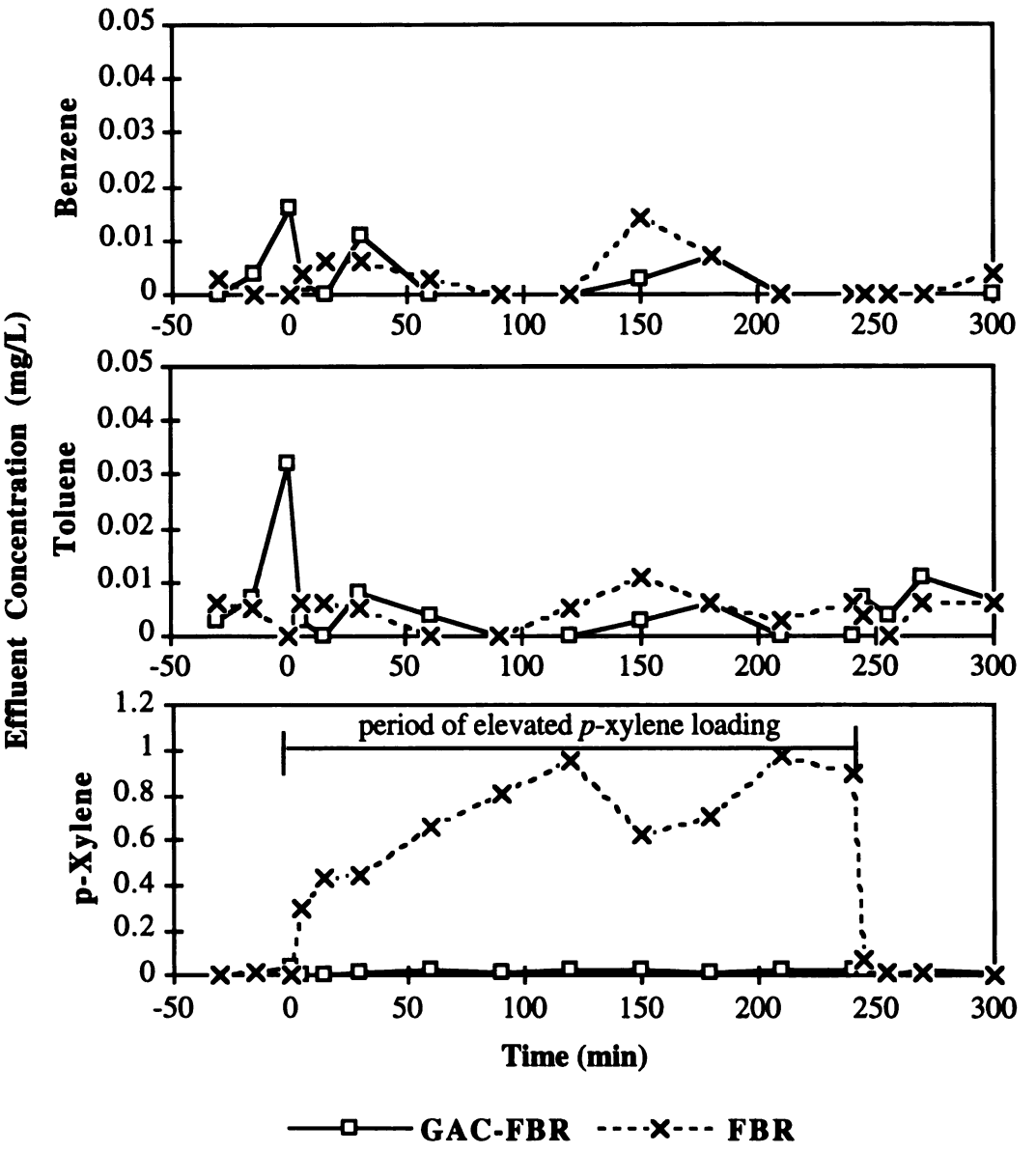

Figure 4-7. Substrate concentrations in the effluent of fluidized-bed systems during a *p*-xylene step-load increase experiment

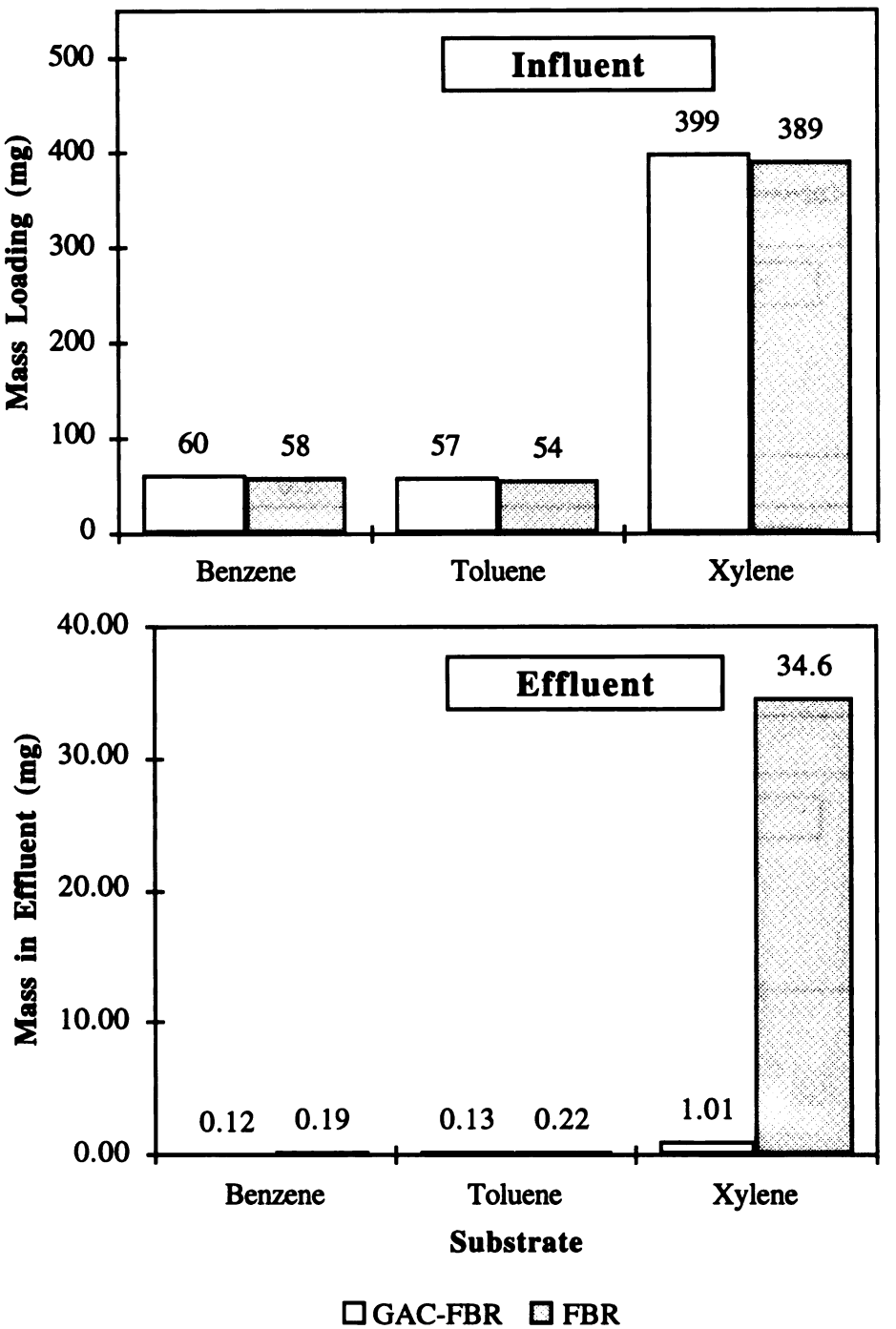

Figure 4-8. The responses of fluidized-bed systems to a *p*-xylene step-load increase

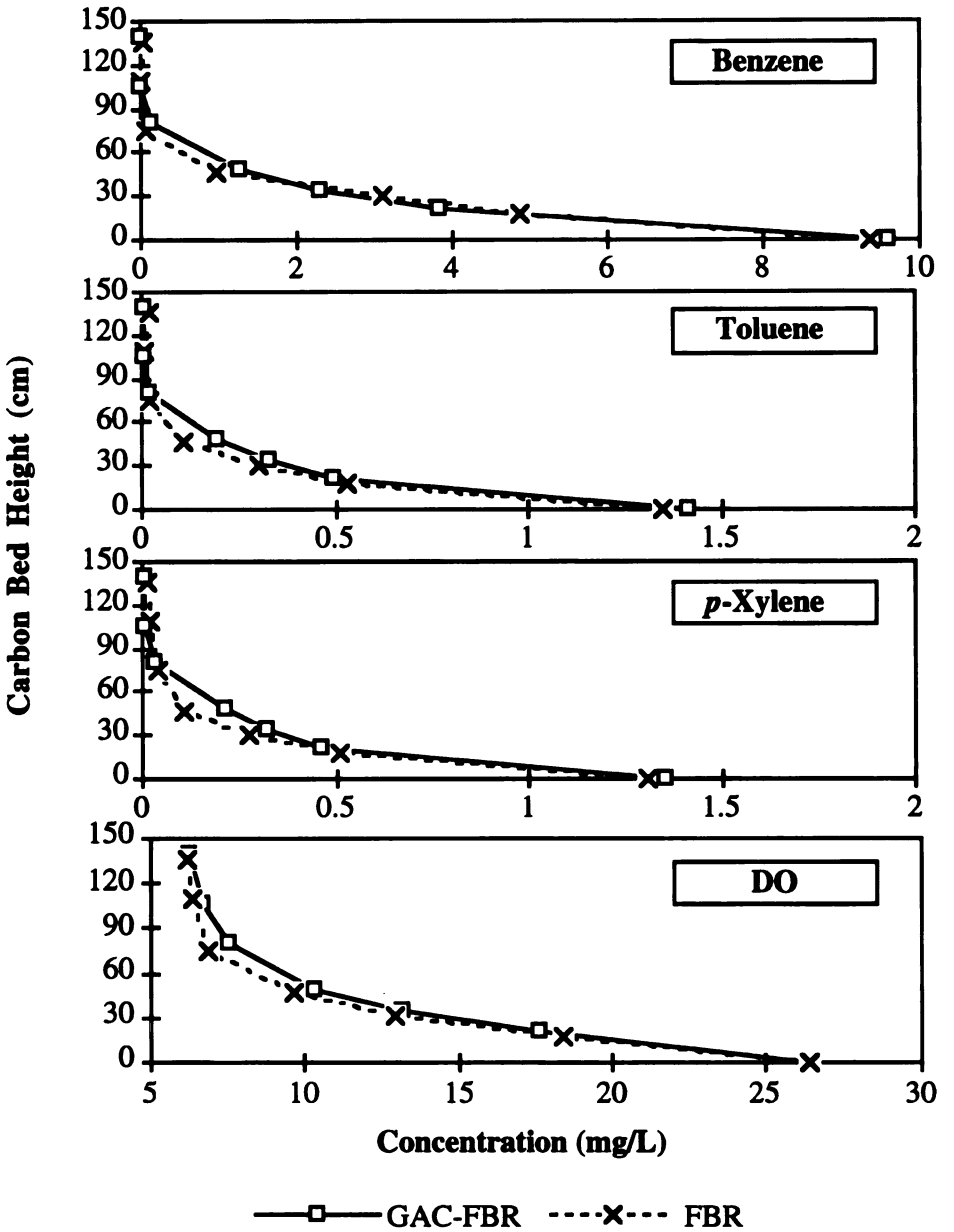

Figure 4-9. Concentration profiles of substrates and DO in fluidized-bed systems at the end of a benzene step-load increase experiment

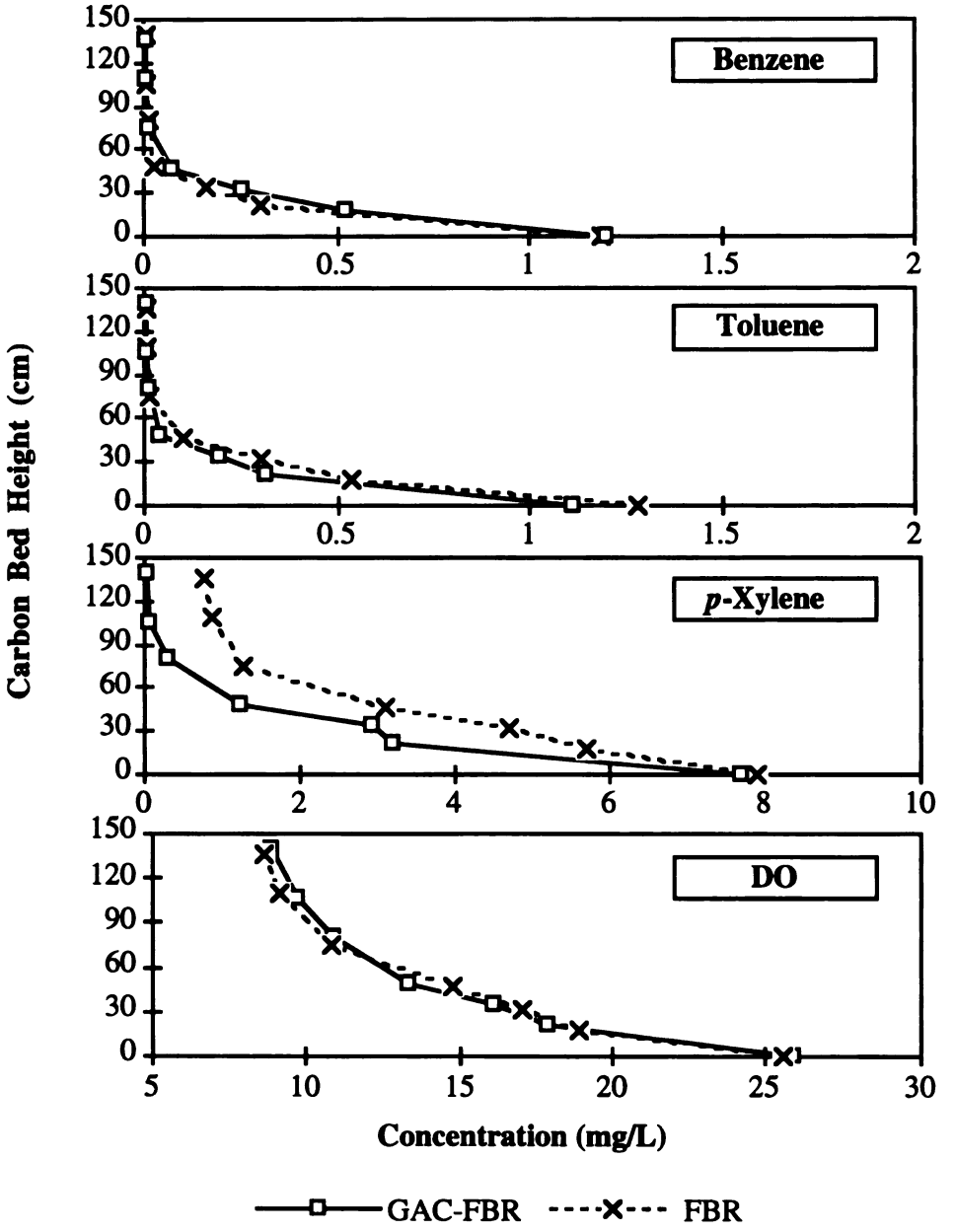

Figure 4-10. Concentration profiles of substrates and DO in fluidized-bed systems at the end of a *p*-xylene step-load increase experiment

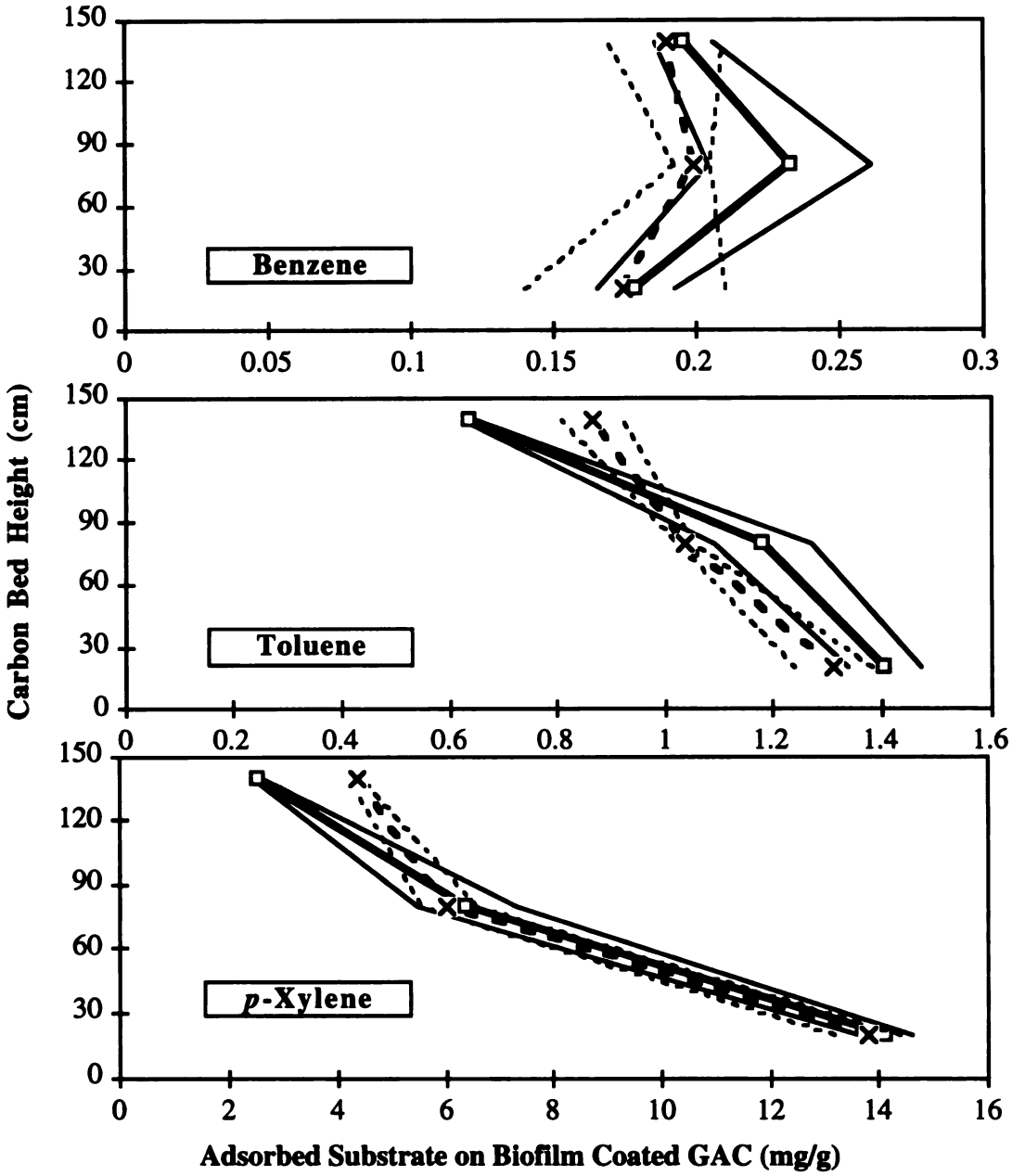

Figure 4-11. Profiles of adsorbed substrates on GAC-FBR before and *p*-xylene step-load increase; Squares represents the average value before the step load increase; Crosses represents the average value at end of the step load increase; Bands represent the 90 % confidence intervals.

4.4 Discussion

There are two mechanisms for removal of organic contaminants in GAC-FBR systems: biodegradation and adsorption. Under dynamic loading conditions, the removal of an organic chemical depends upon the rate of biodegradation and the adsorption capacity of activated carbon for the compound in addition to other factors such as biofilm thickness. Three substrates, benzene, toluene and *p*-xylene, were used in this study. The ranking of adsorption capacities for these compounds from high to low is *p*-xylene, toluene and benzene (Dobbs and Cohen 1980). The maximum rate of biodegradation for this biofilm community, however, is fastest for toluene followed by benzene and then *p*-xylene. The maximum rate of biodegradation of *p*-xylene is approximately half of the value of toluene (Table 4-2). As indicated by the low half-saturation coefficient (K_S) value, the rate of biodegradation of benzene at low concentrations (less than 530 µg/L) is the greatest. The ability of the fluidized-bed systems to remove BTX is highly related to these characteristics.

Under steady-state conditions at an organic loading rate of 3 kg COD/m^3/day and an empty bed retention time of 5 min, the GAC-FBR performed as a biological reactor. The high concentration of biomass that fully coated the GAC was able to remove 99 percent of the influent BTX. The concentrations of BTX in effluents from two systems were essentially the same as was observed in a previous study (Voice *et al.* 1992).

The low concentrations of BTX in the effluent are due to the mixed culture which has the relatively low K_S values and the high biomass concentrations attainable in the fluidized-bed systems. Higher K_S values were reported for pure and mixed cultures isolated from aquifer material (Goldsmith and Balderson 1988; Alvarez *et al.* 1991). The continuous feed of low concentrations of BTX in fluidized-bed systems apparently enhanced the growth of microorganisms with low K_S values.

During the step-load increase of benzene, the two systems performed similarly and the effluent concentrations of all three compounds remained at essentially the steady-state level (Table 4-1). Because of the high k value for benzene and the high biomass levels in fluidized-bed systems, the reactors are able to maintain high quality effluents due to biodegradation alone. The increase in *p*-xylene concentration over that found at steady state indicates that competitive inhibition due to the high concentration of benzene may have occurred.

The toluene step increase did not effect benzene removal in either fluidized-bed system. Even though the a high k value was found for toluene, the high value of K_S resulted in low degradation rates at low concentrations (Table 4-1). The concentrations of the toluene in the effluent were higher than the values at steady state. Although the mass of toluene on the GAC carrier increased slightly during toluene step increase, the two systems performed essentially as biological reactors. The concentrations of *p*-xylene increased by a factor of two from the steady-state value. There was no significant change in the concentration of *p*-xylene in the influent and no evidence of desorption during the toluene step increase. The increase in *p*-xylene concentration in the effluents is probably due to substrate competition between toluene and *p*-xylene.

Our previous results demonstrated that adsorption is an important removal mechanism for BTX during a step-load increase experiment where the loading rate increased from 6 kg COD/m^3-day to 16 kg COD/m^3-day. The amount of BTX removed by biological oxidation during this step-load increase was about 9 kg COD/m^3-day (Voice *et al.* 1992). In the present study, the organic loading rates were increase from 3 kg COD/m^3-day to 11 kg COD/m^3-day during the benzene and toluene step-load increases. Apparently, biodegradation was sufficient in the current study to remove all of the benzene and toluene added during the step-load increase. It thus appears that

adsorption serves to remove the portion of a shock-load that can not be degraded by the biofilm.

The *p*-xylene step-load increase provided the most convincing evidence of the role of adsorption in GAC-FBR systems. Due to the relatively low biodegradation rate of *p*-xylene (compared to toluene and benzene), high concentrations were found in the effluent of the FBR system but not in GAC-FBR system (Table 4-1 and Figure 4-10). The similar DO profiles in two systems indicated similar biodegradation rates (Figure 4-10). Apparently the adsorption capacity present in the GAC-FBR prevented the escape of the *p*-xylene in the effluent (Figures 8 and 9) Extraction the GAC carrier verified that the adsorption was responsible for maintaining in high treatment efficiencies during the step increase (Figure 4-11).

4.5 Conclusions

The results from this study demonstrate the roles of the biodegradation and adsorption in the fluidized-bed systems treating BTX. Under steady-state conditions with an organic loading of 3 kg-COD/m^3-day, both systems removed more than 99 percent of the applied BTX and the GAC-FBR functioned primarily as a biological reactor. When the two systems were subjected to the seven-fold step-load increases in benzene and toluene, biodegradation in the fluidized-bed system was sufficient to maintain a high effluent quality. For a substrate that is biodegraded more slowly, such as *p*-xylene, adsorption onto the GAC carrier contributed significantly to removal and helped to provide more robust performance in the GAC-FBR. Because of the complicated substrate interactions, the concentration of some substrates (e.g. benzene and toluene) can effect the degradation rate of other substrates in a fluidized-bed systems and other biological treatment systems.

CHAPTER 5

FORMATION OF PARTIAL OXIDIZED PRODUCTS IN THE FBR SYSTEMS AT SINGLE STEP LOADING INCREASES

5.1 Introduction

The contamination of soil and groundwater by petroleum products is a common occurrence in many parts of the country. Of the numerous gasoline components, benzene, toluene and xylenes (BTX) are the most mobile, and thus the most likely to contaminate water supplies. One of most common approaches to remediate the contaminated groundwater involves a "pump and treat" procedure, in which groundwater is removed from the subsurface, treated in an above-ground system and discharged to either a publicly owned treatment works or a surface water, or is returned to the aquifer. Increasing attention is being given to biological treatment because these systems can destroy the pollutant and are often less expensive than physical-chemical treatment. Recent work has shown that biological activated carbon in fluidized bed reactor (GAC-FBR) systems provide a high level of removal efficiency and stability in the treatment of BTX contaminated water (Hickey *et al.* 1990b; 1991a; Voice *et al.* 1992; Zhao *et al.* 1993).

Since the early part of this century, it has been known that microorganisms are able to metabolize hydrocarbons, including BTX (Stormer 1908; Sohngen 1913). Numerous investigations have been conducted on the degradation pathways, type of organisms, and the degradation rates of BTX. The degradative pathway for benzene

involves the transformation to *cis*-benzene glycol by a dioxygenase and subsequent oxidation of *cis*-benzene glycol to catechol (Marr and Stone 1961; Gibson *et al.* 1968b; Hou 1982). The catechol is degraded by a ring cleavage reaction involving either the β-ketoadipate pathway (*ortho* cleavage) or the *meta* fission pathway (*meta* cleavage) (Gibson and Subramanian 1984). In *ortho* cleavage, the dihydroxylated aromatic ring is opened to produce *cis*, *cis*-muconic acid, which is further metabolized to β-ketoadipic acid and then oxidized to succinic acid and acetyl-CoA by the tricarboxylic cycle. In *meta* cleavage, the dihydroxylated aromatic ring is opened to produce 2-hydroxy-*cis*, *cis*-muconic semialdehyde. Formic acid, pyruvic acid, and acetaldehyde are further metabolic products. All of the products from both cleavage mechanisms are used further for either biosynthesis or energy generation through the citric acid cycle, which produces CO_2 and ATP by electron transport and oxidative phosphorylation (Lehninger 1982).

As a result of the methyl group on the aromatic ring, the initial steps in the biodegradation of toluene can occur either by the oxidation of the methyl group to form benzoic acid or hydroxylation of the ring (Zylstra and Gibson 1991). Depending upon the hydroxylated position on the ring, the products are *p*-cresol, *o*-cresol, *m*-cresol, or *cis*-toluene dihydrodiol. The catechol will be produced as a result of the transformation of the benzoic acid and this can be further degraded through *meta* or *ortho* cleavage of the aromatic ring. *p*-Cresol can be hydroxylated again to form protocatechuate and this can be broken by *meta* ring cleavage. A further product of *o*-cresol, *m*-cresol, or *cis*-toluene degradation is dihydrodiol 3-methylcatechol which is a substrate for *meta* ring cleavage.

The biotransformation of *p*-xylene can also be characterized in two categories: aromatic ring oxidation and oxidation of the methyl substituent. Gibson *et al.* (Gibson *et al.* 1974) studied ring oxidation of *p*-xylene by *Pseudomonas putida* 39/D. The *p*-xylene was oxidized first to *cis*-*p*-xylene dihydrodiol and further to 3,6-dimethylprocatechol,

which can be degraded further by the *ortho* ring fission pathway. The *cis-p*-xylene dihydrodiol can also be transformed to 2,5-dimethyl phenol by a non-enzymatic reaction (Gibson *et al.* 1974). Methyl substituent oxidation of *p*-xylene has been reported by several researchers (Davis *et al.* 1968; Omori and Yamada 1970a; 1970b; Davey and Gibson 1974). The proposed pathways involve two variations. In the first, one of the methyl groups is oxidized to from a carboxylic acid, which is then transformed to 4-methylcatechol. The aromatic ring of 4-methylcatechol can be broken by *meta* ring fission. The second pathway involves the oxidation of both methyl group before ring fission.

In a study of cometabolic degradation of *p*-xylene by *Pseudomonas sp.* strain B1 in fixed-bed biological activated carbon systems, Chang (1994) demonstrated the formation of partial oxidized products in this pure culture system when toluene was utilized as carbon and energy source. The partial oxidized products were identified as 3,6-dimethyl pyrocatechol and *p*-xylene dihydrodiol. These partial oxidized products were found in the effluents of biological activated carbon fix-bed and a biological fix-bed (without adsorption) for the duration of the experiment (about 30 days).

It is clear that various partial oxidized products will be generated as a part of BTX degradation reactions. It is also clear that the BTX can be utilized as a sole energy and carbon source by various microorganisms. With a fully developed mixed culture community, the partial oxidized products will not normally accumulate because of the enzyme regulation systems in the microorganisms. In a continuous flow bioreactor, a community of microorganisms will develop to utilize the substrate resource for maximum benefit. The community will not produce more partial oxidized products than are needed because such production costs, rather than yields, energy. Under transient conditions, however, partial oxidized products may be generated at a rate higher than

they are consumed, and these compounds may accumulate in the reactor or be washed out in the effluent.

BTX are listed as priority pollutants by the U.S. Environmental Protection Agency (EPA). Benzene is an acute and chronic toxicant, and is listed as a suspected human carcinogen (Patnaik 1992). Toluene and xylene have similar acute toxicity to that of benzene, but less severe chronic effects. A limited amount of information is available about most of the partial oxidized products of BTX degradation. Pyrocatechol, for example, has higher acute oral and percutaneous toxicities than that of phenol, which is an EPA priority pollutant (Patnaik 1992). Cresols exhibit similar toxicity to those of phenol, and *p*-cresol has been found to be hepatotoxic and nephrotoxic (Patnaik 1992). Even on the basis of this limited information, it is clear that the potential public health impacts of BTX degradation products should be considered. The disappearance of BTX in a treatment process does not ensure that the environmental impacts of the contamination are eliminated.

The performance of adsorptive and non-adsorptive biofilm carriers in biological fluidized-bed reactors for the treatment of groundwater contaminated with BTX has been evaluated in this laboratory (Voice *et al.* 1992; Zhao *et al.* 1993). The GAC-FBR system demonstrated the ability to remove more BTX from the influent than a traditional FBR (non-adsorptive biofilm carrier) during step-load increases. The present work focuses on the effect of adsorption on the extent of mineralization of BTX during transient step loading in the FBRs. The formation of partial oxidized products in both GAC-FBR and FBR systems was compared during a step-load increase in influent concentration of one substrate while the other two were held constant.

5.2 Materials and Methods

5.2.1 Media and Chemicals

Granular activated carbon (GAC Calgon Filtrasorb 400, Calgon Co., Pittsburgh, PA) and non-activated carbon, termed "baker product" by the manufacturer, were used as carrier media in two FBR systems. Baker product is the same material as GAC except that it has not undergone the activation step in the manufacturing process and has little adsorptive capacity (Voice *et al.* 1992). Both media were sieved to obtain a 20 x 30 mesh fraction (average particle diameter 0.75 mm). Prior to addition to the reactors, the GAC and baker product were rinsed with distilled water to remove fines and dried at 100 °C for 24 hours.

Benzene, toluene, *p*-xylene (BTX), potassium phosphate dibasic and ammonia chloride were obtained from J.T. Baker Chemical Co., Phillipsburg, NJ. All chemicals were reagent grade. Michigan State University tap water was used directly as a source of groundwater. This relatively hard water (450 mg/L as $CaCO_3$) is pumped from a deep aquifer underlying the campus and distributed without further treatment.

5.2.2 Fluidized Bed Reactors

Laboratory-scale fluidized bed reactors were constructed using a 2.5 cm diameter by 184 cm height glass column to produce a system with a 1000-mL working volume (Figure 5-1). Two reactors were used in this study to allow comparison of adsorptive and non-adsorptive biofilm carriers. The GAC-FBR (combined adsorption and biodegradation) and FBR (biodegradation only) were charged with activated carbon and baker product, respectively. The reactors were operated as single-pass systems with no recycle. Both were inoculated with a mixed culture obtained from a pilot-scale FBR that

was originally seeded with activated sludge and was subsequently supplied with BTX as the sole carbon source for more than two years (Hickey *et al.* 1990b). Dissolved oxygen (DO) was supplied to the reactors by oxygenating the reactor feed water with pure oxygen to a concentration sufficient to maintain an effluent DO of more than 4.0 mg/L. Nitrogen and phosphorus were supplemented at a weight ratio of 100/5/1:COD/N/P. A mixture of benzene, toluene and *p*-xylene, at a 1:1:1: volumetric ratio, was injected into feed water using a syringe pump (Harvard Apparatus 22) and dissolved using a mixing tank (2 liters) and an in-line mixer. A recycle loop around the mixing tank was employed to create complete mixing conditions inside the tank and to help complete BTX dissolution. This BTX contaminated groundwater was then pumped to the bottom of the reactors using peristaltic pumps (Watson-Marrow 502E with a 501R head). The bed height was controlled by mixing the bed bi-weekly with a long thin wire brush to shear some biomass from the carrier medium. The reactors were charged with 180 mL media initially, which produced a settled bed height of 33 cm. BTX contaminated water was fed at flow rate of 200 mL/min to each reactor, resulting in a hydraulic flux rate of 0.41 m/min, an empty bed hydraulic retention time of 5 min and organic loading rate about 2.2 kg COD/m^3-day. The temperature of the feed water was 15±1 °C.

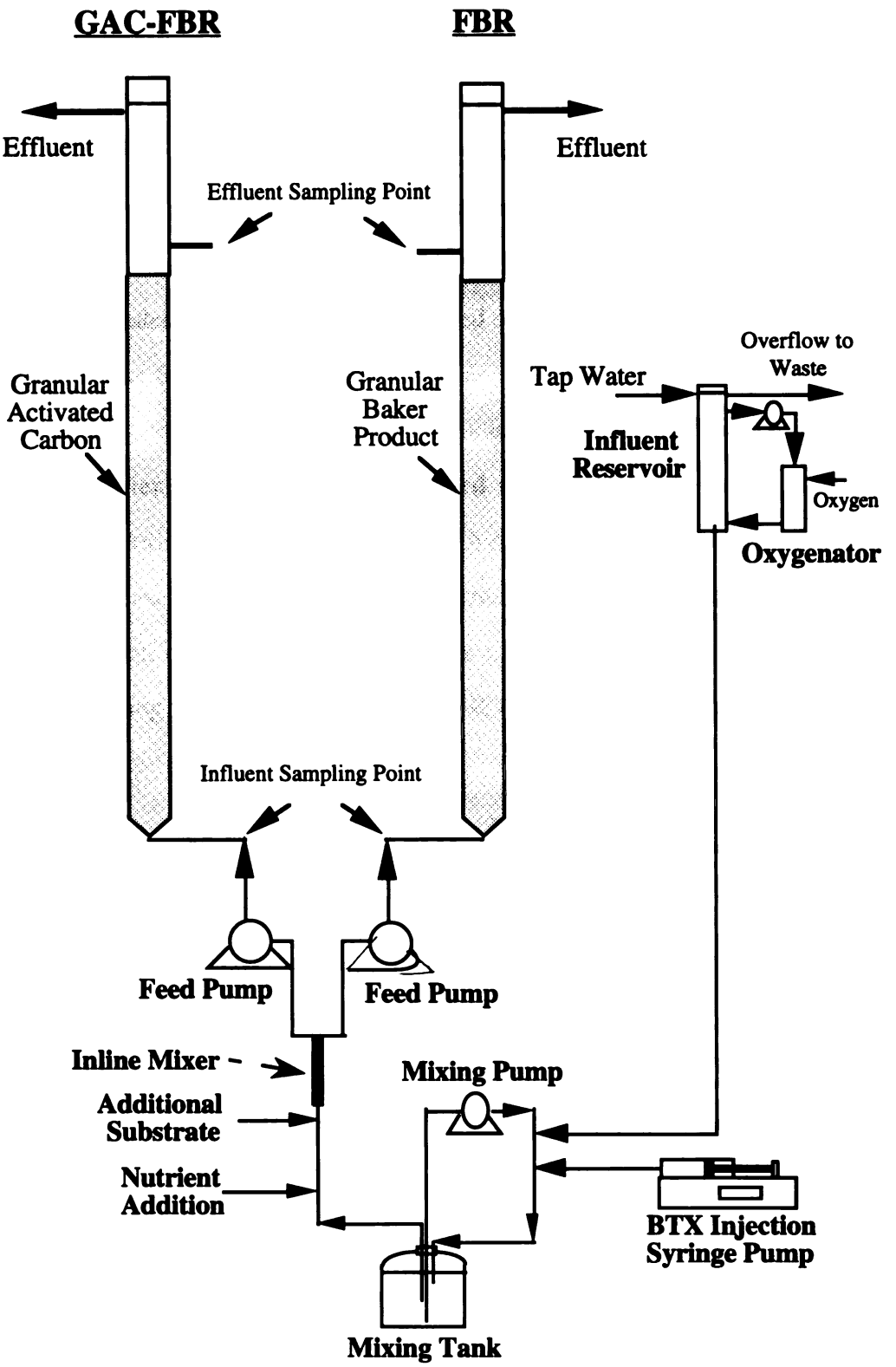

Figure 5-1. Schematic of fluidized-bed reactor systems

5.2.3 Step-Load Increase Experiments

In the first phase of the experiment, the presence of partial oxidized products in the reactor effluents was investigated during a step-load increase of *p*-xylene under different concentrations of dissolved oxygen and nutrients in the influents. After steady-state conditions were reached, as determined by biomass growth and BTX removal, the organic loading rates (Figure 5-2) were increased seven-fold by raising the concentration of *p*-xylene in the feed water. The increase was maintained for five hours. In the first hour of increase (referred to as HLL: high organic, low oxygen, and low nutrients), the oxygen and nutrients supplies were maintained at steady-state level. During the next hour of loading period (HHL: high organic, high oxygen, and low nutrients), the amount of DO was increased 2.5-fold. Essential nutrients supplies were then increased (HHH: high organic, high oxygen, and high nutrients). Finally, the loading sequences were reversed: beginning with HLH, proceeding to HLL and returning to the initial condition (LLL).

In phase two of the experiment, after steady-state conditions were established again, the organic loading rates were increased twenty, twelve and seven-fold for benzene, toluene, and *p*-xylene, respectively. The increases were achieved by raising the concentration of one of the BTX substrates in the feed water. Step-load increases were maintained for four hours. During the increased loading period, the feed concentrations of DO and essential nutrients were increased to ensure that these factors did not limit degradation.

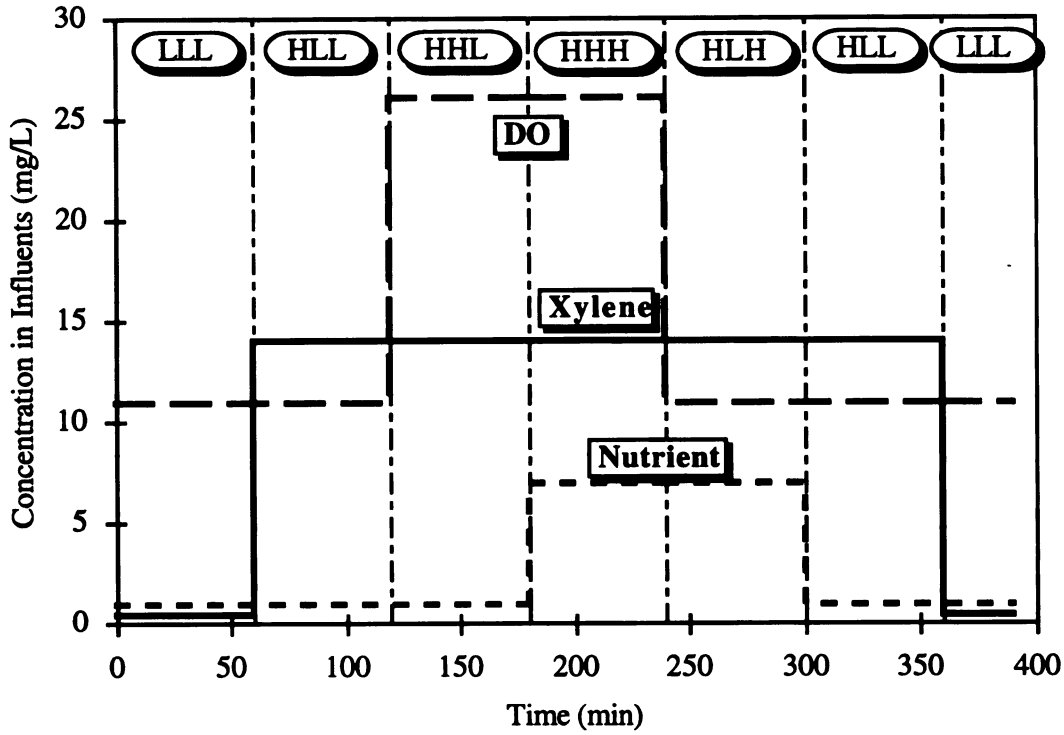

Figure 5-2. Experimental conditions for a xylene step load increase

5.2.4 Analytical Procedure

BTX concentrations were determined using a gas chromatograph equipped with a flame ionization detector (FID) using helium as a carrier gas (Perkin-Elmer, model 8700), coupled with an automatic headspace sampler (Perkin-Elmer, model HS-101). Separation was accomplished using a 30 m x 0.53 mm DB-624 column (J & W Scientific). Water samples collected from the fluidized bed reactors (5 mL or 10 mL) were transferred to 20 mL glass vials with Teflon®-coated septa and aluminum crimp-seals. The samples were equilibrated for 1 hour at 80 °C and an aliquot of the headspace gas was injected into the gas chromatograph. The detection limit of this procedure was 1

μg/L for each BTX component. The accuracy for measurements of split samples containing a concentration of 1.5 μg/L was ± 0.1 μg/L

DO concentrations in the influent and effluent were measured using a polarographic electrode (Orion 97-08-00) coupled to a digital pH meter (Orion 611). If the DO concentration exceeded the limit of the instrument (15 mg/L), the sample was diluted 1:1 or 1:2 with tap water of a known low DO concentration.

The amount of partial oxidized products in the effluent of FBRs can be measured by several methods. Two analytical procedures, non-volatile organic carbon (non-volatile TOC) measurement and the measurement of adsorption on the ultraviolet spectrophotometer, are easily conducted in a general analytical laboratory. Oxidation on the organic molecule, results in partial oxidized products that are less volatile than the parent compounds. A simple gas stripping process can separate the partial oxidized products from the parent compounds. The carbon in the partial oxidized products can be measured by a total organic carbon analyzer. Before ring cleavage, the partial oxidized products contain the conjugated aromatic ring structure which will show ultraviolet adsorption in the wavelength of 200 nm to 300 nm (McMurry 1988; Willard et al. 1988). Formation of partial oxidized products was monitored by measurement of adsorption on the ultraviolet spectrophotometer. Chang (1994) reported that the 3,6-dimethyl pyrocatechol, 2,5-dimethylphenol, and *p*-xylene dihydrodiol, which are the partial oxidized products of degradation of p-xylene, can be measured by a high pressure liquid chromatograph equipped with a C_{18} reverse-phase column and a UV detector at wavelength of 280 nm.

The concentration of non-volatile total organic carbon (non-volatile TOC) was measured on a total organic carbon analyzer (Simazdu, Model TOC-500) equipped with an auto-sample injector (Simazdu, ASI-502). Before the measurement of non-volatile TOC, the water sample was acidified with hydrochloric acid and purged with oxygen for

4 minutes to remove inorganic carbon and volatile organic carbon. A water sample spiked with BTX and catechol (one of the expected intermediates) was tested to ensure the efficacy of the procedure. The results showed that the BTX and inorganic carbon were removed completely and there was no significant removal of the non-volatile organic carbon (catechol). The TOC detection limit was 0.6 mg/L and the accuracy for measurements of standard solution of 1 mg/L was ± 0.2 mg/L.

The absorbance of ultraviolet and visible light by water samples was measured by a spectrophotometer (Perkin-Elmer, model Lambda 6 UV/VIS). Before measurement, samples were passed through a 0.22 μm filter to remove suspended solids and purged with oxygen for two minutes. A test showed that the purging procedure was able to remove all BTX from the sample. The samples were scanned from a wavelength of 600 nm to a wavelength of 200 nm with a slit of 4 and scan speed of 200 nm per min. The absorbance was also determined at a wavelength of 265 nm. Deionized water was used as reference sample.

5.2.5 Stoichiometry of Biological Reactions

The removal of substrates in a GAC-FBR system can result from either adsorption or biodegradation. Biodegradation can further be divided into that which is used 1) as an electron donor, 2) for the growth for biomass, and 3) to form incomplete oxidation products. The overall reaction (R) can be written as:

$$R = R_d + f_e R_a + f_s R_c + f_b R_b \qquad (5\text{-}1)$$

where: R_d is the half reaction for the oxidation of an electron donor.

R_a is the half reaction for the reduction of an electron acceptor for energy.

R_e is the half reaction for the reduction of an electron acceptor for synthesis.

R_b is the half reaction for the reduction of an electron acceptor for byproduct.

f_e is the fraction of electrons diverted for energy.

f_s is the fraction of electrons diverted for synthesis.

f_b is the fraction of electrons diverted for byproduct.

The half reactions for R_d, R_a, R_c, and R_b can be written as:

Electron donor half reaction (R_d):

Benzene: $0.0333C_6H_6 + 0.4H_2O = 0.2CO_2 + H^+ + e^-$

Toluene: $0.0278C_7H_8 + 0.389H_2O = 0.194CO_2 + H^+ + e^-$

p-Xylene: $0.0238C_8H_{10} + 0.381H_2O = 0.19CO_2 + H^+ + e^-$

Electron acceptor half reaction for energy (R_a):

$$0.25O_2 + H^+ + e^- = 0.5H_2O$$

Electron acceptor half reaction for cell synthesis (R_c):

$$0.25CO_2 + 0.05NH_3 + H^+ + e^- = 0.05C_5H_7O_2N + 0.4H_2O$$

Electron acceptor half reaction for byproduct (R_b):

$$\text{Organic} + 0.5O_2 + H^+ + e^- = \text{Byproduct} + H_2O$$

In this system there are three electron donors in the influent. The terms f_d^B, f_d^T, and f_d^X denote the fraction of electron donor originating from benzene, toluene, and xylene, respectively. The half reaction for the production of byproduct is presented in a general form since specific partial oxidized products were not identified in this study.

The overall reactions (R)are: $R = f_d^B R_d^B + f_d^T R_d^T + f_d^X R_d^X + f_e R_a + f_s R_c + f_b R_b$

$$f_d^B + f_d^T + f_d^X = 1$$

$$f_e + f_s + f_b = 1$$

An expression for the overall reactions for BTX biodegradation under steady-state conditions (without the production of byproduct, f_b=0) is:

$$\begin{aligned}&0.0333f_d^B C_6H_6 + 0.0278f_d^T C_7H_8 + 0.0238f_d^X C_8H_{10} + 0.25f_e O_2 + 0.05f_s NH_3\\ &= 0.05f_s C_5H_7O_2N + \left(0.2f_d^B + 0.194f_d^T + 0.19f_d^X - 0.25f_s\right)CO_2\\ &+\left(0.5f_e^B + 0.4f_s^B - 0.4f_d^B - 0.389f_d^T - 0.381f_d^X\right)H_2O\end{aligned}$$

Because the partial oxidized products were not explicitly identified, the overall reactions under the step-load increase conditions can not be written.

If yield (Y) data is available, the values of f_S, can be estimated by the relationship (Criddle et al. 1991):

$$f_s = Y\left(\frac{g}{h}\right) \qquad (5\text{-}2)$$

where: g is the electron equivalent mass of the electron donor, g.

h is the electron equivalent mass of biomass (5.65 g for ammonia as the nitrogen source).

The values of f_e and f_S may be calculated from the consumption of substrates and oxygen under steady-sate conditions as the following:

The calculation of the fraction of electrons at steady-state:

$$\frac{M_s^B}{(0.0333)(78)f_d^B} = \frac{M_s^T}{(0.0278)(92)f_d^T}$$

$$\frac{M_s^T}{(0.0278)(92)f_d^T} = \frac{M_s^X}{(0.0238)(106)f_d^X}$$

$$\frac{M_s^T}{(0.0278)(92)f_d^T} = \frac{M_o}{(0.25)(32)f_e}$$

Where: M_o = consumption of oxygen (mg, or mg/L).

M_s^B = consumption of benzene (mg, or mg/L).

M_s^T = consumption of toluene (mg, or mg/L).

M_s^X = consumption of *p*-xylene (mg, or mg/L).

M_b = production of byproduct (mg, or mg/L).

Because there is no production of partial oxidized products at steady-state condition,

$$f_b = 0$$

and

$$f_s = 1 - (f_e + f_b)$$

An estimate of the fraction of BTX removed by the two mechanisms, biodegradation and adsorption, was performed. Two assumptions were made in order to perform this calculation. The first was that the removal of BTX in the FBR results only from biodegradation. The second was that the microbial communities in both reactors were similar, allowing the use of the same values for f_e, f_S, and f_b. The amount of biologically removed BTX could be estimated using dissolved oxygen consumption data:

$$(M_s^B)_{BAC-FBR} = \left(\frac{M_s^B}{M_o}\right)_{FBR} (M_o)_{BAC-FBR}$$

$$(M_s^T)_{BAC-FBR} = \left(\frac{M_s^T}{M_o}\right)_{FBR} (M_o)_{BAC-FBR}$$

$$(M_s^X)_{BAC-FBR} = \left(\frac{M_s^X}{M_o}\right)_{FBR} (M_o)_{BAC-FBR}$$

$$(M_b)_{BAC-FBR} = \left(\frac{M_b}{M_o}\right)_{FBR} (M_o)_{BAC-FBR}$$

The fraction removed by adsorption was calculated from the difference of the total removed BTX and the biologically removed BTX. The adsorbed byproduct in the GAC-FBR was estimated from the difference between the calculated amount of byproduct from biodegradation and the amount in the effluent.

5.3 Results

5.3.1 Steady-State Conditions

Under steady-state conditions, BTX removal rates were greater than 98% in both reactors. The consumption rate of DO in both reactors were similar. It is believed that the primary removal mechanism is biodegradation in both systems. The same conclusion was presented in our previous studies (Voice *et al.* 1992; Zhao *et al.* 1993). No byproduct was found in the effluents under these conditions. The values of f_e and f_s, were calculated from this and previous studies (Voice *et al.* 1992) and the results are summarized in the Table 5-1. The average value of f_e and f_s, were 0.70 and 0.30, respectively. A yield coefficient was determined in a batch experiment which was described in our previous study (Zhao *et al.* 1993). Assuming that f_b is approximately zero, the yield coefficient can be used to calculate f_e and f_s values of 0.72 and 0.28, respectively. The observation that similar values were found in the batch and both types of FBR systems.

Table 5-1. Electron balances at steady-state

Loading Rate (kg COD/m^3-day)	Reactor	f_e	f_s	Ref.
2.2	GAC-FBR	0.84	0.16	this study
2.2	FBR	0.73	0.27	
2.2	GAC-FBR	0.68	0.32	
2.2	FBR	0.65	0.35	
3.8	GAC-FBR	0.65	0.35	Voice *et al.* 1992
2.9	FBR	0.68	0.32	
6.0	GAC-FBR	0.69	0.31	
6.0	FBR	0.70	0.30	
	Average	0.70	0.30	
	SD	0.06	0.06	
Calculated from yield data		0.72	0.28	Y=0.2 g VSS/g COD

5.3.2 Step-Load Increases

In the first phase of the experiment, the organic loading rates were increased from 2.2 to 14.9 kg COD/m^3-day by raising the concentration of *p*-xylene in the feed water from 0.5 to 14 mg/L. Step-loading increases were maintained for five hours. During the increased loading period, the supply of DO was increased from 11 to 27 mg/L in the second and third hours of the increase, and the amount of essential nutrients supplies were increased in the third and fourth hours.

The spectra of absorbance for the effluent of FBR before, during (HHH), and after a xylene step load increase is shown in Figure 5-3. The absorbance ($\lambda < 300$ nm) increased significantly during the step-load increase. A wavelength of 265 nm was selected as an indicator of the presence of partial oxidized products. Removal percentages for benzene, toluene, and *p*-xylene, DO consumption, and effluent absorbance at 265 nm are shown in Figure 5-4. Benzene and toluene removal were

similar in both reactors with greater than 90% of the two compounds being removed under most loading conditions (Figure 5-4a, b). Removal dropped from steady-state values of greater than 99% to 96% in the initial hour of shock loading. Removal increased to 99% in the second hour with an increase in DO concentration. Effluent DO levels increased from 2 mg/L to 8 mg/L in this interval and DO consumption increased from 10 mg/L to 17 mg/L (Figure 4d). Increasing the nutrient levels in the third hour did not appear to effect performance. Similar results were found in the fourth and fifth hours as first the DO and then nutrient levels were decreased.

The removal of *p*-xylene (Figure 5-4c) in the GAC-FBR was consistently higher than in the FBR (75% vs. 55%). The average absorbance at 265 nm in the effluent of the GAC-FBR was also lower than that from the effluent of the FBR (0.064 vs. 0.072 , Figure 5-4e.). The higher removal of xylene was observed under high DO conditions in both reactors (80% vs. 72% for GAC-FBR and 62% vs. 50% for FBR) and the absorbances were also higher (0.074 vs. 0.058 for GAC-FBR and 0.082 vs. 0.067 for FBR).

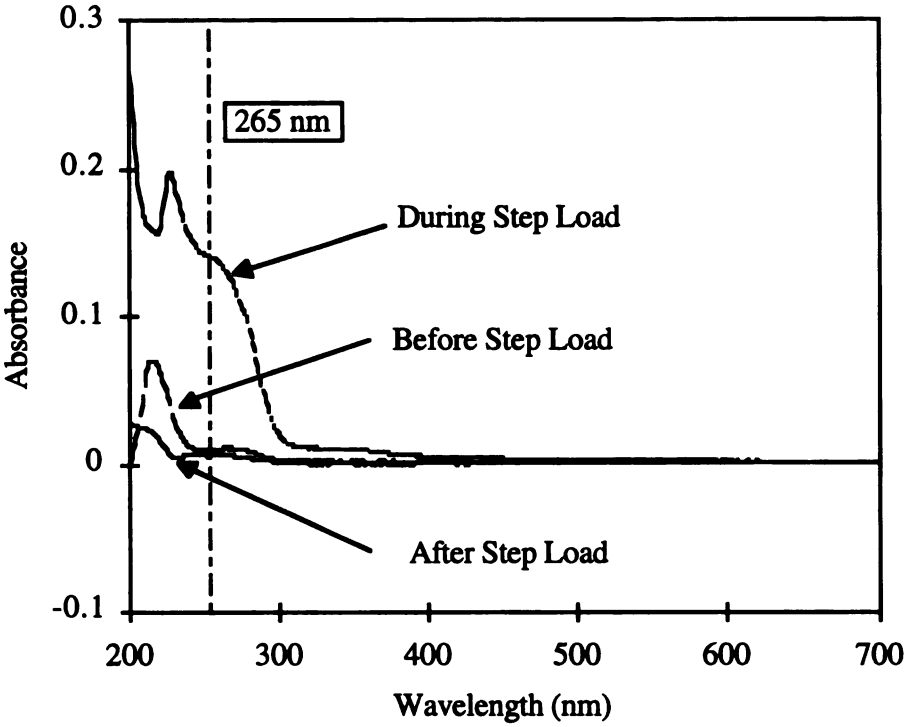

Figure 5-3. Spectra for the effluent of FBR before, during (HHH), and after a xylene step load increase

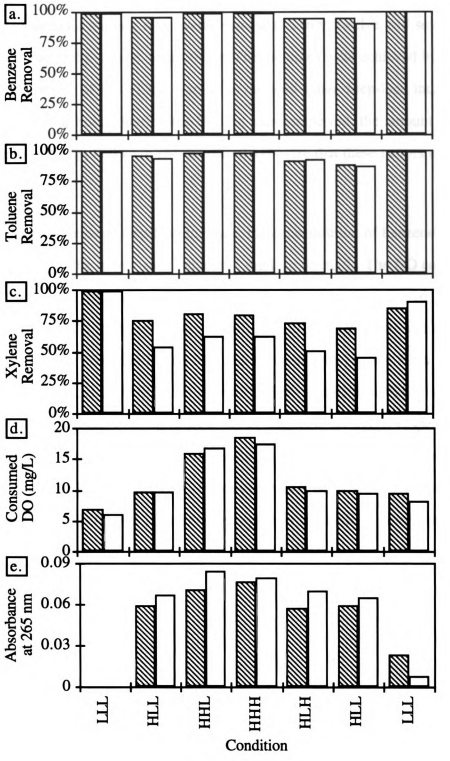

Figure 5-4. Removal of BTX, consumption of DO, and production of byproducts in the GAC-FBR and FBR during a xylene step load increase with different oxygen and nutrient supply

In phase two of the experiment, three step-load increases were applied to both reactors. The organic loading rates were increased twenty, twelve and seven-fold for benzene, toluene, and *p*-xylene, respectively. The increases were achieved by raising the concentration of one of the BTX substrates in the feed water. Step-load increases were maintained for four hours. During the loading increase period, the amount of DO and essential nutrients supplied were also increased to ensure that these factors did not limit performance.

During the benzene step-load increase, the concentration of benzene in the feed was increased from the steady-state value of 1 mg/L to 45 mg/L. The DO concentration in the effluents of both reactors were maintained at greater than 10 mg/L. The concentrations of BTX in the influents and effluents, DO consumption and non-volatile TOC production, are shown in Figure 5-5. The removal rates (Figure 5-5 a, b, c; Table 5-2) for applied BTX in the GAC-FBR were 63%, 85%, and 93% for BTX, respectively. The removal rates in the FBR were 24%, 55%, and 89% for BTX, respectively. DO consumption was similar in the two systems during the step-load increase, but it did not drop to pre-increase levels in the GAC-FBR system as it did in the FBR system (Figure 5-5d). Non-volatile TOC was considerably higher in the FBR system throughout the step-load increase compared to the GAC-FBR (Figure 5-5e).

Table 5-2. BTX removal during step-load increases

Load Increase	Reactor	Compound	Total Mass (mg)			Removal (%)
			Influent	Effluent	Removed	
Benzene	GAC-FBR	Benzene	2175	793	1383	64
		Toluene	41	7	34	85
		p-Xylene	29	2	27	93
	FBR	Benzene	2154	1617	536	25
		Toluene	40	18	22	55
		p-Xylene	28	3	25	89
Toluene	GAC-FBR	Benzene	95	19	76	80
		Toluene	1340	203	1137	85
		p-Xylene	116	31	85	73
	FBR	Benzene	95	66	29	30
		Toluene	1333	883	450	34
		p-Xylene	115	101	14	12
p-Xylene	GAC-FBR	Benzene	51	0.2	50.8	99
		Toluene	59	1	58	98
		p-Xylene	647	56	591	91
	FBR	Benzene	50	2	48	96
		Toluene	57	4	53	93
		p-Xylene	638	270	368	58

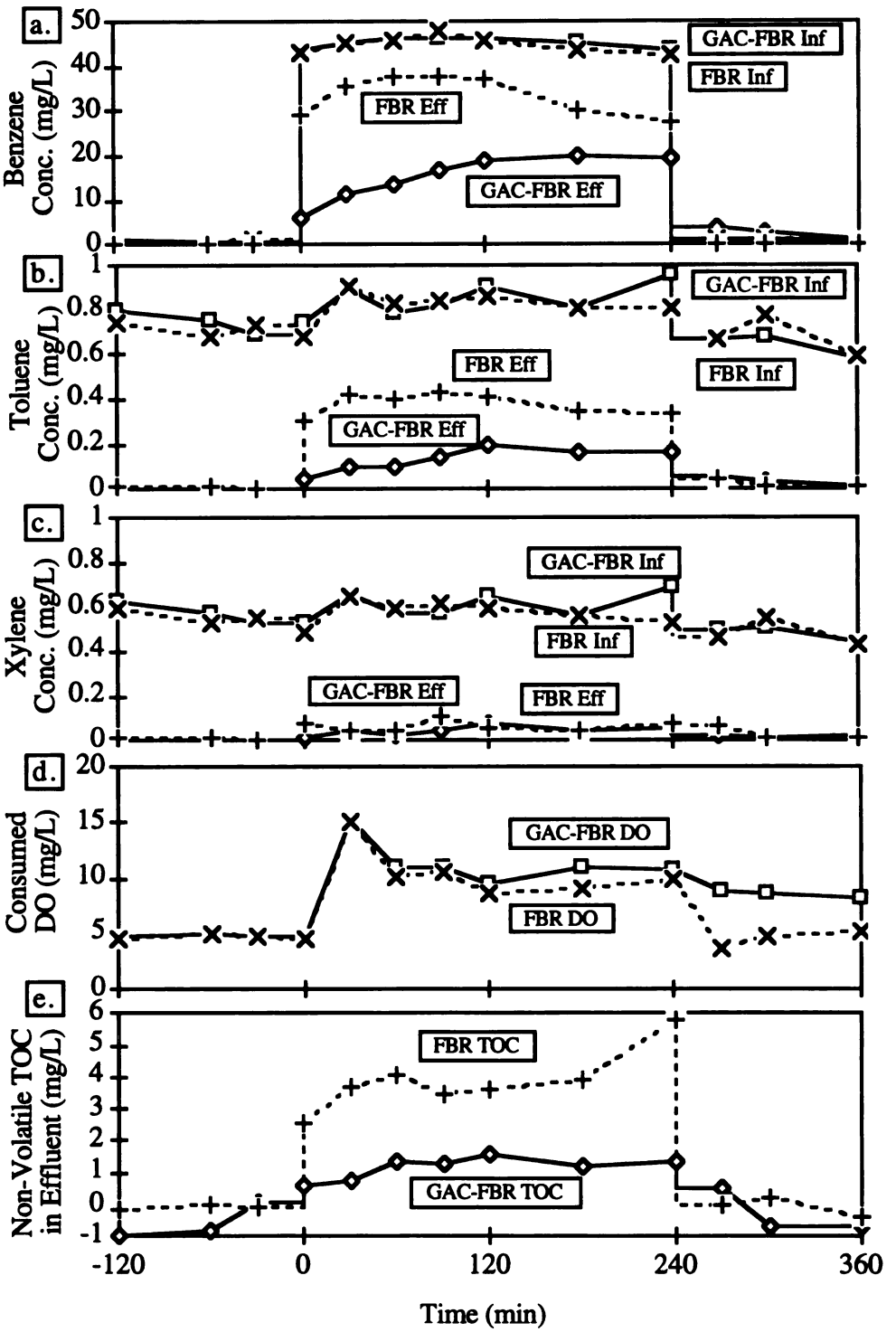

Figure 5-5. Performance of the GAC-FBR and FBR during a benzene step load increase

Of the total BTX fed in the GAC-FBR, 64% was removed. From the mass balance calculation of GAC-FBR, 35% was estimated to result from adsorption and 28% due to biodegradation (Table 5-3). The production of byproduct accounted for 10% of the BTX that was removed in the GAC-FBR (Table 5-3). However, only 3% of the removed BTX was detected in the effluent of GAC-FBR as the partial oxidized products In the FBR removal of 26% of the applied BTX was biological, with 9% of the total showing up in the effluent as partial oxidized products (Table 5-3). Three times as much non-volatile TOC (partial oxidized products) was found in the effluent of FBR than in the GAC-FBR.

Table 5-3. Carbon balances during the step load increases (unit: % of influent carbon)

Load Increase	Reactor	Total Removal of BTX	Removal by				
			Adsorp-tion	Biodegradation			
				Bio-mass and CO_2	Byproduct		Total
					in Eff.	Adsorbed	
Benzene	GAC-FBR	64.2%	35.6%	18.6%	2.9%	7.1%	28.6%
	FBR	26.1%	N/A	17.0%	9.2%	N/A	26.1%
Toluene	GAC-FBR	83.9%	44.8%	20.9%	3.4%	14.8%	39.1%
	FBR	32.1%	N/A	17.2%	14.9%	N/A	32.1%
Xylene	GAC-FBR	93.0%	32.3%	43.2%	11.4%	6.1%	60.7%
	FBR	63.4%	N/A	45.2%	18.2%	N/A	63.4%

After the reactors were returned to steady-state conditions again, the concentration of toluene in the influents was increased from 0.9 mg/L to 27 mg/L for four hours. DO concentrations in the effluents were maintained at greater than 10 mg/L. The concentrations of BTX in the influents and effluents of both reactors, DO consumption and non-volatile TOC production are shown in Figure 5-6. Due to a failure of the substrate feeding system, high concentrations of all three compounds were introduced into both reactor systems for the first 30 minutes. BTX removal rates, after recovery from this situation, were 80%, 85%, and 73% respectively in the GAC-FBR.

Removals in the FBR were 31%, 34%, and 12% for BTX, respectively. DO consumption was higher and non-BTX production lower, in the GAC-FBR system during the increase period. As in the case of benzene, DO consumption continued at elevated levels in the GAC-FBR system after the OLR increase period for more than 48 hours.

A carbon balance for the toluene increase experiment is presented in Table 5-3. Approximately 84% of the total BTX fed to the GAC-FBR was removed. As with the benzene step increase, 45% was estimated to result from adsorption and 39% due to biodegradation in GAC-FBR (Table 5-3). About 18% of removed BTX was transformed to the partial oxidized products in GAC-FBR (Table 5-3). However, only 3% of removed BTX was found in the effluent of the GAC-FBR as partial oxidized products. In the FBR 32% of the applied BTX removed biologically, with 15% of the total showing up in the effluent as partial oxidized products (Table 5-3). More than four times as much non-volatile TOC (partial oxidized products) were found in the effluent of FBR compared to the GAC-FBR.

The last step load increase experiment was a xylene increase (Figure 5-7). The concentrations of xylene in the influents were increased from 0.6 mg/L to 13 mg/L and, as with previous increases, the concentrations of DO in the effluents were maintained greater than 10 mg/L. The concentrations of BTX in the influents and effluents, DO consumption and non-BTX TOC production, for both reactors are shown in Figure 5-7. The removal rates (Table 5-2) of applied BTX in the GAC-FBR were 99%, 98%, and 91% for BTX, respectively compared to 96%, 93%, and 57% in the FBR, respectively.

The carbon balance for both reactors is shown in Table 5-3. Because the xylene step load increase was less than the benzene and toluene increases (the organic loading rates were increased twenty, twelve and seven-fold for benzene, toluene, and xylene, respectively.), The removal of BTX in the GAC-FBR remained at 93%, but removal

dropped to 63% in the FBR. About 32% of loaded BTX was removed by the activated carbon in the GAC-FBR and the 60% of the removed BTX was degraded biologically. In the FBR, 18% of the BTX was removed as products, all of which were present in the effluent. Similar to the other two step increases, the carbon in the GAC-FBR was able to reduce the amount of partial oxidized products in the effluent from 17% of applied BTX to 11%.

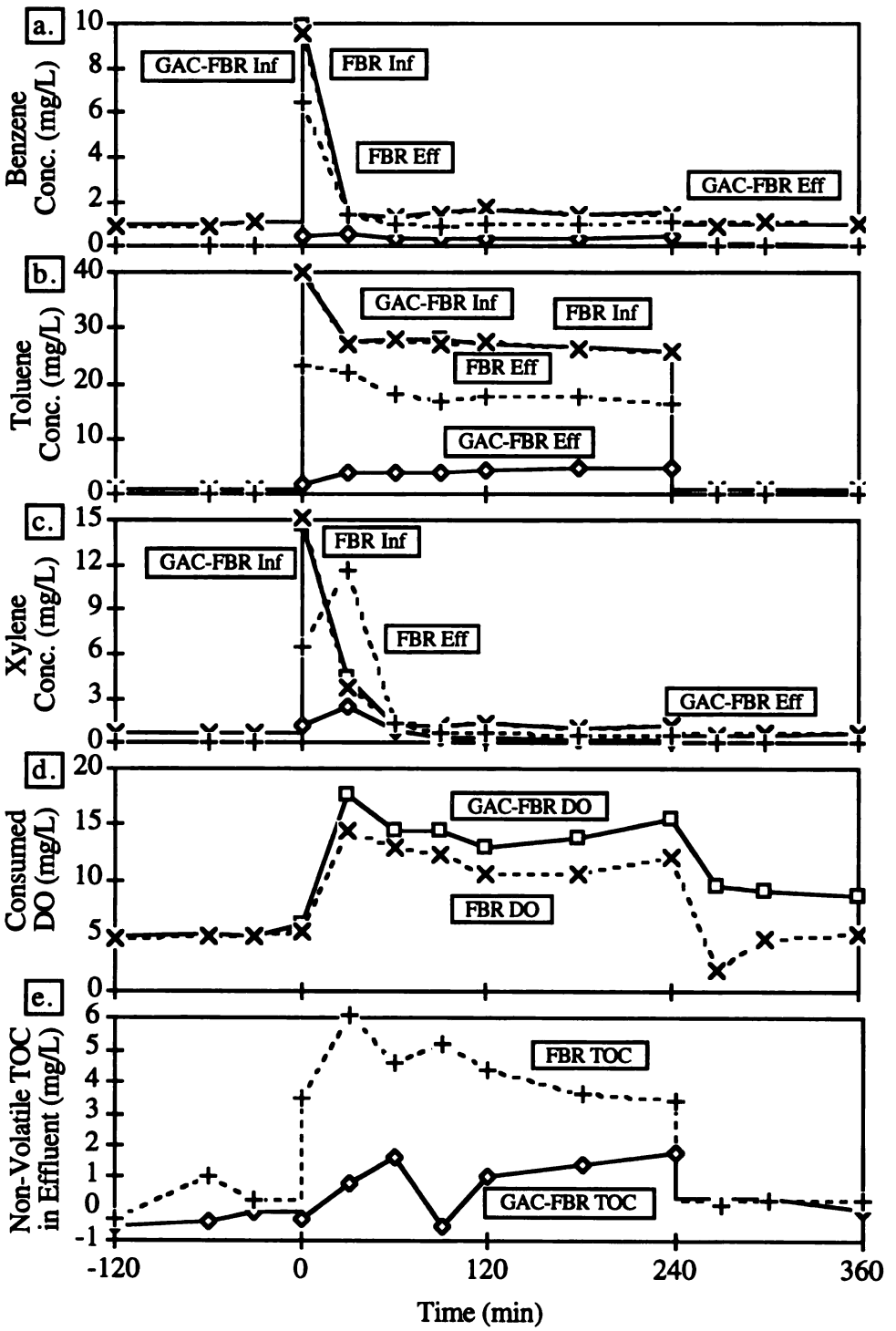

Figure 5-6. Performance of the GAC-FBR and FBR during a toluene step load increase

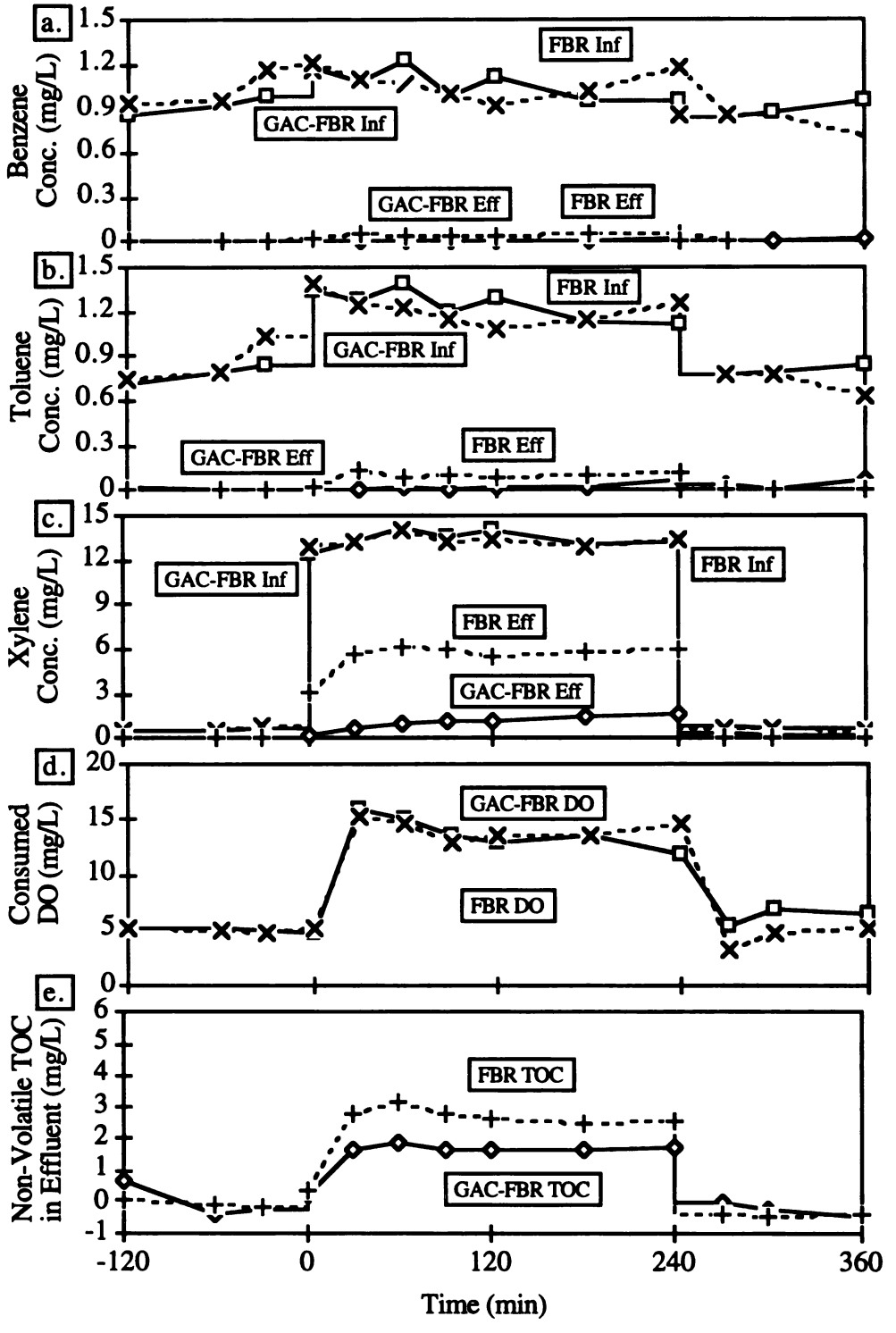

Figure 5-7. Performance of the GAC-FBR and FBR during a xylene step load increase

5.4 Discussion

The GAC-FBR performs as a biological reactor under steady-state conditions (Voice *et al.* 1992; Zhao *et al.* 1993). The calculated yields of biomass from mass balances at different loading conditions produce similar values to that obtained in batch experiments. This suggests that the production of partial oxidized products is not significant under steady-state conditions. This is consistent with the hypothesis that microorganisms obtain maximum energy from complete mineralization and will naturally form a community capable of mineralization under constant feed conditions.

Under transient conditions, such as those that occur during a step-load increase, the community is presented with a feed condition different from that around which it was organized. Changes in community structure are slow, and can not occur to any significant level during short-term perturbations. As a result, the community will not operate at full efficiency, and complete mineralization may not occur. This was demonstrated in this study. In the all of the step load increase experiments, partial oxidized products were found in the effluents of both reactor types.

The formation of partial oxidized products is a result of incomplete oxidation and consumes oxygen as a reactant. When the amount of available DO was varied under step-loading conditions, higher DO consumption corresponded to higher byproduct concentrations in the effluent (Figure 5-4). Total BTX removal also increased at higher DO levels, however.

The levels of essential nutrients (N and P) in the influent feeds were not a significant factor in BTX removal and the production of partial oxidized products over the short time intervals studied. A related study of nutrient pulse feeding conducted on a GAC-FBR system showed that a nutrient supply cycle of 30 minutes on and 30 minutes off did not effect BTX removal (Xing and Hickey 1994) . When nutrients were withheld

for two days, BTX removal decreased approximately 10% from the steady-state level. The present study confirms and extends this observation by demonstrating that short term variations in nutrient supply do not effect the removal of BTX or the formation of partial oxidized products.

The mass balance calculations in this study were based upon the assumption that, in the FBR, BTX is removed only by biodegradation, and that both reactors have similar levels of bio-activity. The first assumption may slightly overestimate biodegradation because the non-activated carbon has a small amount of adsorption capacity (Voice *et al.* 1992). The second assumption is only supported by similarity of performance of the two systems under steady-state conditions. As a result, these calculations must be viewed as estimates. Nevertheless, the results do indicate the relative magnitudes of adsorption and biodegradation in these systems.

During the benzene and toluene step-load increases, about half of the removed BTX was adsorbed by the GAC in the GAC-FBR. Consequently, the total removals of BTX were much higher in the GAC-FBR than that in the FBR (64% vs. 26% for benzene step load increase and 84% vs. 32% for toluene step load increase). There were also lower concentrations of partial oxidized products in the effluent of the GAC-FBR. This system also had slightly higher levels of DO consumption. In the first experiment it was found that increased DO consumption corresponded to increased BTX removal and increased byproduct formation. We would thus expect the slightly higher DO consumption in the GAC-FBR to indicate slightly higher byproduct formation, and thus the lower byproduct levels in the effluent must result from adsorption of the byproduct compounds by the activated carbon.

During the xylene step-load increase, 35% of the removed BTX was adsorbed by the carbon in the GAC-FBR and 35% of the partial oxidized products were removed by adsorption. Because the amount of the xylene increase was lower than the benzene and

toluene increases, biodegradation could remove larger portion of influent BTX and adsorption constituted a smaller portion of the total removal.

During the step-load increases, a significant amount of the biologically removed BTX resulted in the production of partial oxidized products (approximately 35%, 46%, and 29% for benzene, toluene, and xylene increases, respectively). Since the disappearance of BTX is normally used as the only indicator of treatment system performance, the relatively high levels of byproduct compounds in the effluent corresponding to these percentages would not be observed. This may pose a potential health problem. The activated carbon biofilm carrier in the GAC-FBR prevents these partial oxidized products from leaving the reactor. In concept, such systems could be designed to insure protection against certain types of shock loads. As shown in previous studies, this effluent protection capability applies equally well to BTX. During the toluene step load increase experiment, high concentrations of BTX inadvertently entered both reactors during the first 30 minutes due to an operational error. The GAC-FBR demonstrated the ability to maintain low levels of BTX in the effluent. The FBR system, however, was not able to handle the increase (Figure 5-6).

5.5 Conclusions

The production of partial oxidized products was not observed under steady-state conditions, at a organic loading rate of 2.2 kg COD/m^3-day. Under application of a step-load increase, however, significant concentrations of partial oxidized products were produced. The production of partial oxidized products was not effected by the nutrient levels but increased with an increase in DO in the influent. A significant amount of the biologically removed BTX resulted in the production of partial oxidized products (35%, 46%, and 29% for benzene, toluene, and xylene increases, respectively). use of activated

carbon as a biofilm carrier in the GAC-FBR dramatically increased the total removal of BTX and reduced the amount of partial oxidized products in the effluent.

CHAPTER 6

ROLE OF ADSORPTION IN GRANULAR ACTIVATED CARBON FLUIDIZED BED REACTORS

6.1 Introduction

Groundwater contamination by volatile aromatic hydrocarbons is widespread as a result of leaks and spills from petroleum storage tanks, pipelines, and production facilities. Benzene, toluene, and xylenes (BTX) are frequently observed far from the contaminant source because of the relatively high water solubility and low soil/water partition coefficients of the these petroleum constituents. Remediation of groundwater contaminated with BTX is often accomplished using either air stripping or activated carbon adsorption. Recently, a technique referred to as biological activated carbon (BAC) has been used treat BTX contaminated groundwater. In the Granular Activated Carbon-Fluidized Bed Reactor (GAC-FBR) system, BAC has been shown to provide both the efficiency of a biological removal system and the positive effluent protection capability of activated carbon adsorption (Hickey *et al.* 1991a; Voice *et al.* 1992).

The precise roles of the two removal mechanisms in GAC-FBR systems -- biodegradation and adsorption -- have not been well defined. However, several hypotheses have been formulated based on the overall performance of operating systems. It has been observed that microorganisms colonize granular activated carbon (GAC) surfaces faster than surfaces that are either smoother or less adsorptive (Pirbazari *et al.* 1990). In

addition, BAC systems are observed to perform well at low substrate concentrations. It is thought that granular activated carbon provides a favorable environment for microbial growth by locally enriching the concentrations of substrates, oxygen and nutrients, and by providing a surface with sufficient texture to protect a sparse biofilm from fluid shear forces (Kim and Pirbazari 1989; Speitel *et al.* 1989a). In addition to promoting biofilm development, activated carbon provides a removal mechanism during initial the reactor start-up period (DiGiano 1981).

In previous BAC studies, considerable attention has been given to the question of whether substrate which adsorbs can later be biodegraded, a phenomenon referred to as bioregeneration (Khan *et al.* 1981; Suidan *et al.* 1981; 1983; Wang *et al.* 1984; Kim *et al.* 1986; Wang *et al.* 1986; Speitel and DiGiano 1987; Suidan *et al.* 1987). Speitel and co-workers (Speitel and DiGiano 1987; Speitel *et al.* 1988; 1989) conducted a series experiments designed to help determine how bioregeneration varied among four chemicals: phenol, dichlorophenol (DCP), pentachlorophenol (PCP), and paranitrophenol (PNP). The bioregeneration rate, as measured by the production of radio-labeled CO_2, which was produced by the biodegradation of pre-adsorbed radio-labeled substrates, was found to be highly dependent on the adsorption and desorption characteristics of these compounds. Moderately absorbable chemicals such as phenol and PNP could be significantly removed from carbon by bioregeneration. In contrast, compounds with a great affinity for activated carbon, such as DCP and PCP, were observed to exhibit a high degree of irreversibility of adsorption. These authors also observed that the diffusive transport resistance in GAC may control the rate of bioregeneration.

Under the types of operating conditions that can be expected in BAC treatment systems, it is hypothesized that activated carbon will protect the microorganisms by removing materials that may be toxic or inhibitory (Suidan *et al.* 1987). Adsorption will

serve to hold these compounds in the reactor for longer than the hydraulic retention time, thereby allowing time for the organisms to acclimate, and in some cases to degrade, these potentially toxic compounds (Andrews and Tien 1982). The ability of GAC to adsorb and desorb compounds has led researchers to suggest that BAC systems may be able to better respond to changes in influent concentrations than systems employing non-adsorbing carrier particles. During an influent concentration spike, excess substrate could be adsorbed and then, following the spike, the substrate would be desorbed and biodegraded. It is thought that the biofilm can thereby serve to bioregenerate the activated carbon (Kim *et al.* 1986).

Previous work in this laboratory investigated the performance of GAC-FBR systems designed to remove BTX from groundwater under both constant influent and spike-loading conditions. By comparing the GAC-FBR to an identical system using a non-adsorbing biofilm carrier, it was observed that the GAC-FBR functioned primarily as a biological reactor under steady-state conditions, but that the GAC carrier contributed significantly to overall system removal capacity during transient conditions. It was hypothesized and demonstrated numerically that this resulted from adsorption/desorption of the substrate by the GAC. However this conclusion was not experimentally verified (Voice *et al.* 1992). The purpose of the present study was to verify this hypothesis by monitoring the amount of adsorbed substrate before, during and after a step-increase in influent toluene concentration to a laboratory scale GAC-FBR reactor.

6.2 Materials and Methods

6.2.1 Fluidized-Bed Reactor

The experimental set-up used for the GAC-FBR studies is illustrated in Figure 6-1. The reactor was constructed using two sections of 5 cm i.d. x 92 cm long glass pipe,

containing side sampling ports. The working volume was 4 liters. The reactor was operated as a one-pass system without recycle, at a flow rate of approximately 900 mL/min. This provided an empty bed contact time of approximately 4 minutes. A peristaltic pump (Watson-Marlow 503S with a 501R head) was used to introduce groundwater into the column after it had been pre-oxygenated using pure oxygen. Greater than 25 mg/L of dissolved oxygen could be achieved in the influent. Toluene was introduced into the influent line using a syringe pump (Harvard Apparatus 22). An in-line mixer combined with a recirculation pump (Watson-Marrow 502E with a 501R head) was employed to increase the efficiency of mixing and dissolution of the toluene. A nutrient solution was added to the influent just prior to the feed stream entering the column to minimize any bacterial growth in the tubing.

In full-scale GAC-FBRs the bed height is maintained at a set control level using one of several proprietary systems (Hickey *et al.* 1991b). In this laboratory pilot-scale system, control of the bed was maintained by mixing the bed once daily with a long thin wire that sheared some of the biomass from the GAC carrier.

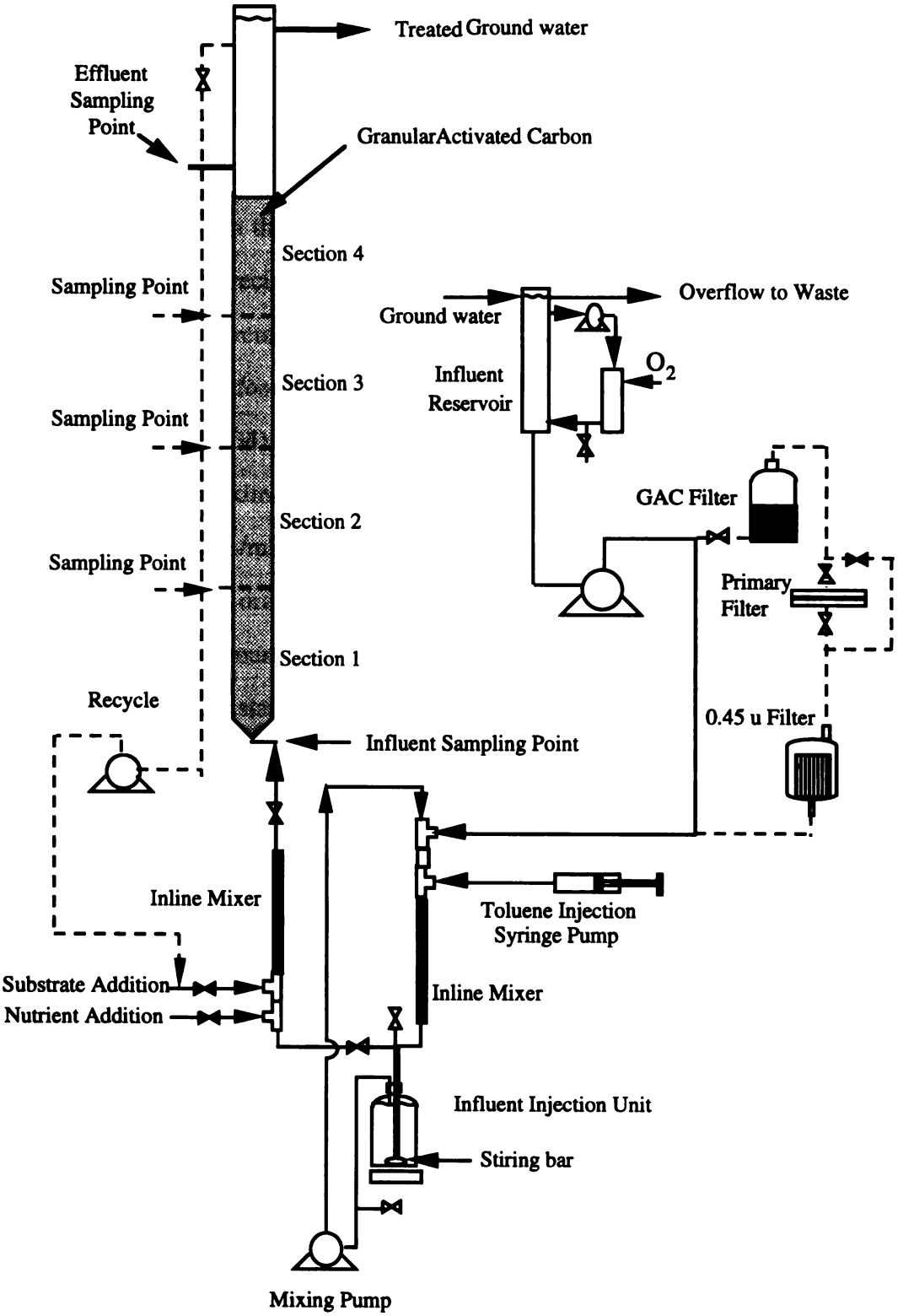

Figure 6-1. Schematic of the experimental GAC-FBR system

In order to concurrently investigate the ability of inoculated bacterial strains to compete with an indigenous microfilm (results reported elsewhere) the system was initially heat sterilized and the influent feed water was subsequently sterilized by 0.22 mm filter . Prior to start-up, the glass column was filled with a 20% chlorine bleach solution which was recirculated through the system for two days. This was followed by a 0.2 M HCl solution for which was recirculated for one day. The reactor was subsequently flushed with deionized water to remove the acid. Initially, the reactor was inoculated with three bacterial cultures: *Pseudomonas pickettii* (PKU1), *Psueodmonas putida* (PaW1) and *Pseudomonas cepacia* (G4) obtained from the Center for Microbial Ecology at Michigan State University. Approximately 450 mL of cell suspension containing 3.37×10^7, 1.58×10^8 and 9.50×10^7 cells/mL respectively were added to the reactor (direct counts were obtaining using acridine orange stained samples). The reactor was operated in a recycle mode for the first two hours to ensure sufficient contact between the inoculum and the GAC carrier. During the start-up period (initial seventeen days of operation) a filter system was placed in the influent water supply line to prevent organisms in the raw water feed from entering the reactor. This allowed the inoculated organisms the opportunity to preemptively colonize the GAC. The filter system contained activated carbon, a glass-fiber filter and a 0.45 mm filter. After day 17, when the biofilm was well established on the carrier particles, the filter system was removed.

6.2.2 Materials

Activated carbon (Calgon Filtrasorb 400) was sieved to select only particles passing a No. 20 sieve (0.85 mm) and retained on a No. 30 sieve (0.65 mm). The geometric mean diameter of the media was 0.70 mm. The carbon was washed several times with deionized water to remove carbon fines, dried in an oven at 105 °C, and sterilized in an autoclave. Five hundred grams of carbon were added to the reactor. Toluene was used as the sole

source of carbon for the biofilm. The nutrient solution was formulated to maintain a ratio of 100/5/1: COD/N/P using NH_4Cl and KH_2PO_4 and boiled deionized water.

6.2.3 Step-OLR Increase Procedure

After a stable biofilm had developed and the reactor reached pseudo-steady-state conditions at a COD loading rate of 5.4 kg COD/m^3-day, the OLR was increased by a factor of 4 without changing the hydraulic loading rate. An additional oxygen diffuser was added to the influent line to insure that dissolved oxygen availability did not limit biodegradation. The nutrient concentration was increased proportionally to the increase in OLR. The step-OLR increase was maintained for 77 hours, after which time the influent was reduced to the previous loading rate. The influent dissolved oxygen and nutrient concentrations were not decreased for an additional 50 hours, in order to ensure that these factors did not limit biological removal of the adsorbed toluene during this period. When oxygen consumption decreased to the pre-step-OLR increase level, the nutrient and oxygen feeds were reduced to the initial conditions.

6.2.4 Extraction Procedure

To measure the amount of toluene adsorbed onto the GAC, carbon samples (0.03 to 0.1 g) were collected from reactor sampling points located at 0%, 25%, 50%, 75%, and 100% of the carbon bed height, placed in aluminum dishes, separated from the liquid using adsorbent tissue and transferred to 15 mL glass vials. The vials were capped with Teflon-faced septa and aluminum seals. Fifteen mL of methanol was added to each vial as the extraction solvent, and the vials were tumbled at 6 rpm for 3 to 4 days. This methanol extract was stored for no more than 3 days at 4 °C prior to analysis. Analysis involved transferring 0.1 mL of the extract to a 20 mL headspace vial containing 10 mL NaCl saturated water and 0.12 mL 6 M HCl, sealing the vials with Teflon-faced septa, and

analyzing this solution by headspace gas chromatography. The mass of carbon extracted was determined by drying at 90 °C overnight, desiccating, and weighing on an analytical balance.

In order to determine the effect of adsorption time on extraction efficiency, a carbon sample was collected from a GAC-FBR which was operated in a manner similar to the system used in this study. Two adsorption isotherm assays were conducted by the bottle-point method, (Voice *et al.* 1992) using different equilibrium contact times (7 days and 35 days). Adsorbed toluene was determined using five successive extractions with methanol. No differences in recovery were found between the two isotherms using either the concentration in the first extract or the total mass recovered in all five. The total mass recovered was greater than 90% of that lost from solution in all cases.

In this study, only one extraction was performed for each carbon sample and thus, the extraction efficiency is dependent upon the ratio of the volume of extraction solvent to mass of carbon used in the extraction procedure. A standard multiple extraction calculation was used to estimate the total amount of toluene adsorbed using the amount recovered in the first extraction and the results of five successive extraction tests conducted at different solvent to carbon ratios (Schwarzenbach *et al.* 1993).

6.2.5 Analytical Measurements

Ten-mL influent and effluent samples were transferred directly to 20 mL headspace vials for analysis and sterilized with 0.12 mL 6 M HCl. Both these samples and the methanol extracts described above were analyzed using an automatic headspace sampler (Hewlett-Packard 19395A) coupled to a gas chromatograph (Perkin-Elmer Autosystem) equipped with a flame ionization detector using helium as a carrier gas. Samples were

equilibrated at 90 °C for 1 hour and an aliquot of the headspace gas was injected onto a 30 m x 0.53 mm DB-624 column (J & W Scientific). An overall relative standard deviation of 4% was found for toluene sample collection and analysis. The method detection limit was determined to be 0.2 μg/L.

Influent and effluent dissolved oxygen (DO) concentrations were determined using a polarographic electrode (Orion 97-08-00) and pH meter (Orion 611). Samples were diluted 1:1 or 1:2 with tap water of a known low DO concentration.

6.3 Results

The operation and performance of the GAC-FBR reactor system can be conveniently divided into four phases: 1) *start-up*, the period from inoculation to development of a mature biofilm; 2) *pseudo-steady-state*, the period after development of a mature biofilm, as evidenced by stable influent, effluent toluene concentrations and DO consumption levels, 3) *step-OLR increase*, the period of elevated influent concentration, and 4) *recovery*, the period following the step-OLR increase during which the influent concentration was restored to the original level.

6.3.1 Phase 1: Start-up

Immediately following inoculation, the fluidized bed height was 96.5 cm. Scanning electron microscopy of carbon particles removed from the middle of the bed revealed that organisms were attached to the carbon surfaces. There was no appreciable DO consumption during the first 4 to 5 days (Figure 6-2); toluene concentrations averaged 2700 μg/L in the influent and 18 μg/L in the effluent. This indicates that adsorption was the primary removal mechanism during this period. This is confirmed by measurements of

adsorbed toluene, which is shown both as a function of time and bed height in Figure 6-3. Toluene concentrations increased sharply at all bed positions during the first 10 days of operation, with higher toluene levels found at lower bed positions.

DO consumption increased sharply between days 4 and 8 and continued to increase until day 10 as the toluene degradation capability of the biofilm developed. After day 10, DO consumption decreased slowly from the maximum value of 8 mg/L to a pseudo-steady-state average value of 5.6 mg/L. During this period, the levels of adsorbed toluene decreased and the distribution of adsorbed toluene became more uniform throughout the bed. This suggests that as the degradation capability of the biofilm developed, not only was the toluene in solution degraded but the toluene adsorbed on the GAC was desorbed and degraded as well. This is graphically illustrated in Figure 6-4 which shows that the total amount of adsorbed toluene decreased significantly after reaching a peak value observed at day 10.

Although the bed height of the reactor did not increase significantly during the first 20 days (Figure 6-5), the level of DO consumption confirmed that biodegradation was occurring. After day 20, an observable amount of biomass began to accumulate on the surface of the GAC particles, and the bed height began to increase rapidly.

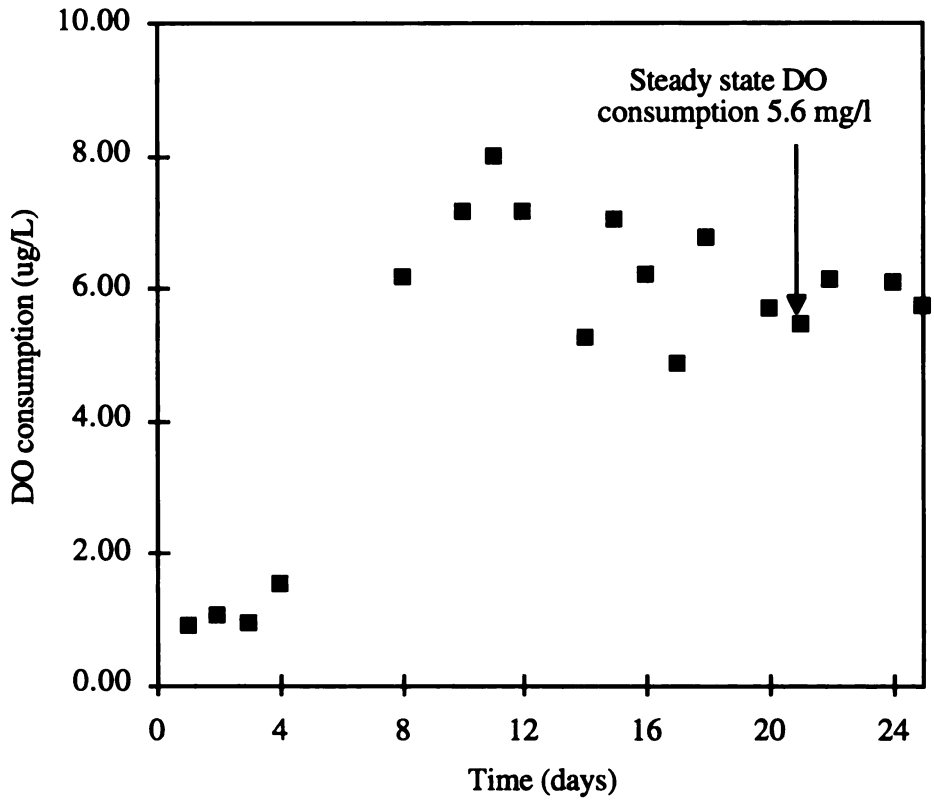

Figure 6-2. DO consumption during the startup phase (hydraulic flux rate: 0.44 m/min ; empty bed hydraulic retention time: 4.4 min; influent concentration of toluene was maintained at 2.75 mg/L)

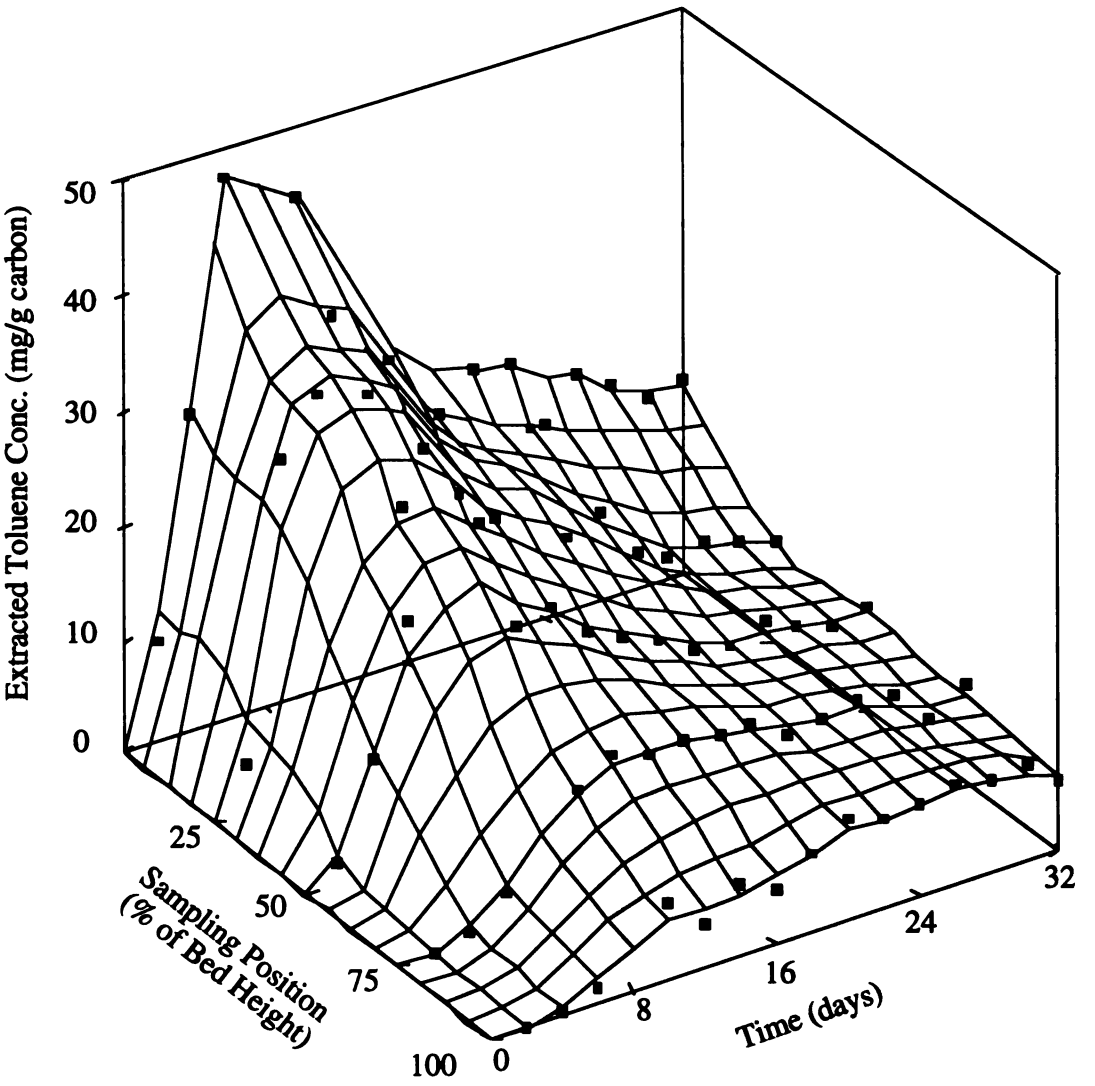

Figure 6-3. The profile of adsorbed of toluene during the startup phase (hydraulic flux rate = 0.44 m/min; empty bed hydraulic retention time = 4.4 min; influent toluene concentration =2.75 mg/l.)

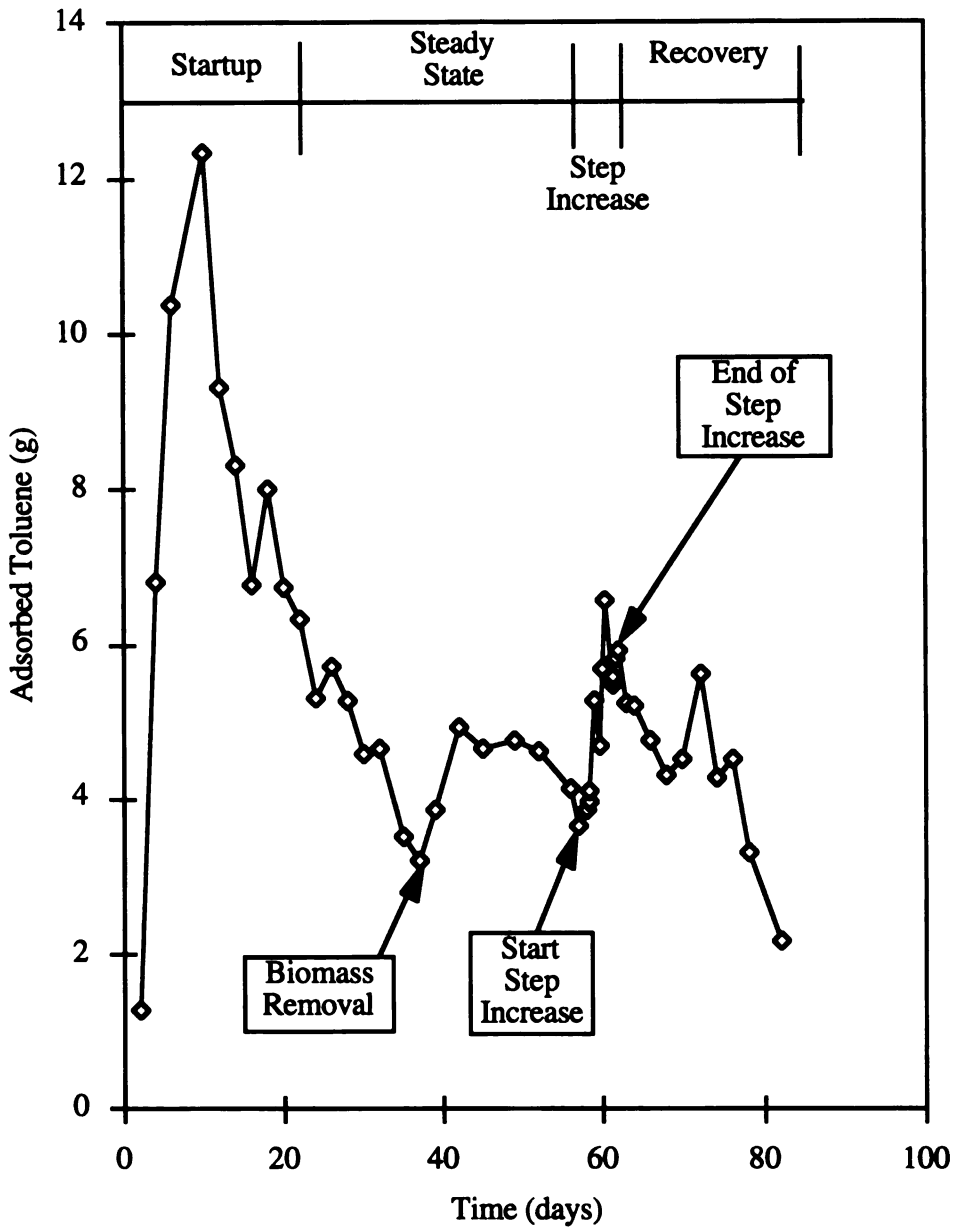

Figure 6-4. Total mass of adsorbed toluene in the GAC-FBR system

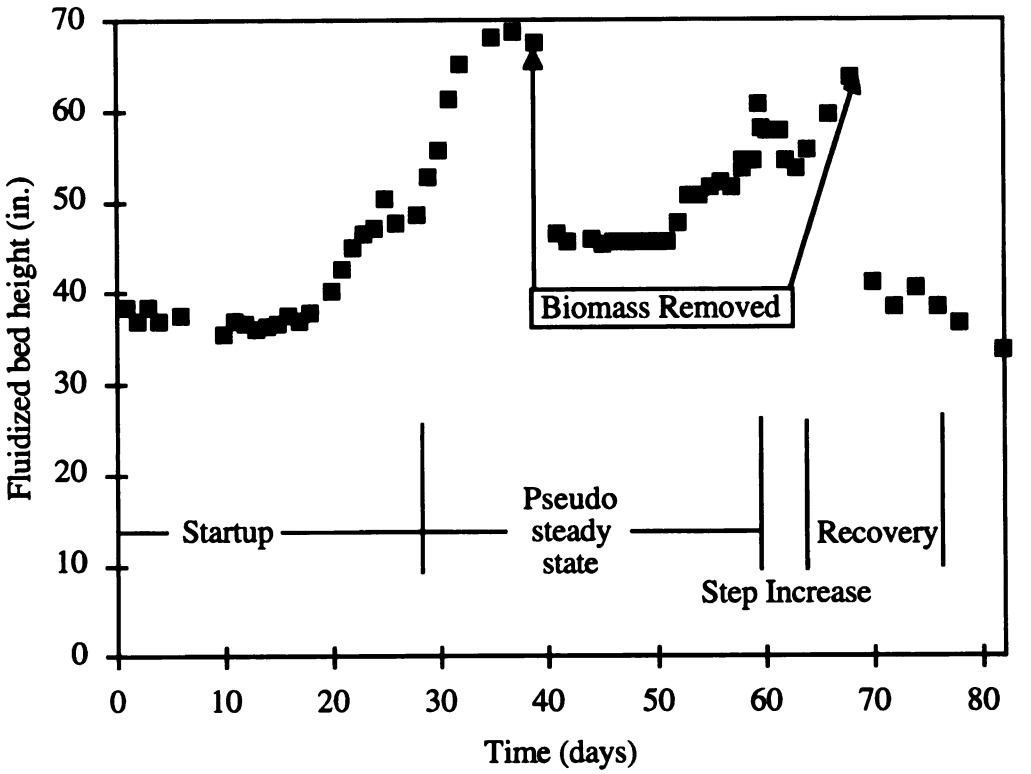

Figure 6-5. Height of the carbon bed in the GAC-FBR system

6.3.2 Phase 2: Pseudo Steady-State

The reactor was assumed to be operating under pseudo-steady-state conditions when constant DO consumption, commensurate with that required for degradation of the added toluene, was observed. The system performance stabilized with average effluent toluene concentrations and DO consumption of 14 μg/L and 5.6 mg/L, respectively. This corresponded to an overall toluene removal rate of 99.4%.

In an attempt to assess the influence of the biofilm thickness on adsorption and biological removal within the profile of the bed, all but a thin film of biomass was removed

from the GAC particles in the bed on day 39 by vigorous mixing. Prior to biomass removal, 81% of the DO consumption in the system occurred in the first section of the reactor (15 cm). After the removal of a significant amount of the total biomass, the amount of biodegradation in the first section of the bed decreased and a greater bed height was required to obtain the same level of toluene removal. This resulted in an increase in the aqueous phase toluene concentration throughout the profile of the bed and a concomitant increase in adsorbed toluene level (Figure 6-6). With the thinner biofilm, adsorption became, temporarily, an important removal mechanism for toluene in the lower portion of the bed. This demonstrates that the GAC had additional adsorptive capacity available. As is illustrated in Figure 6-4, the mass of adsorbed toluene subsequently leveled-off and then decreased over the next several weeks as the biofilm regrew.

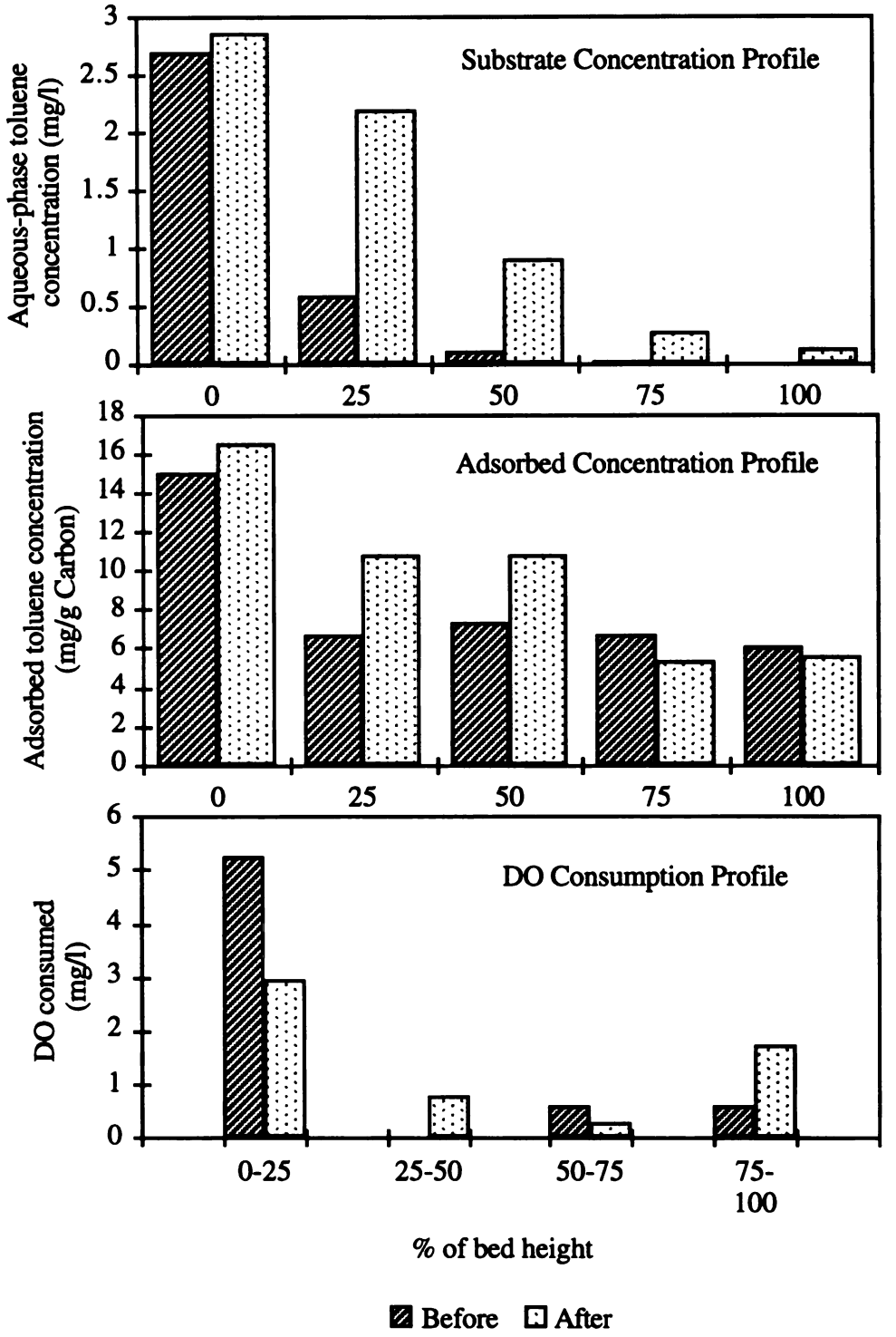

Figure 6-6. Concentration profile aqueous-phase toluene, extracted toluene and DO consumption before and after biomass removal

6.3.3 Phase 3: Step-OLR Increase

The reactor system was subjected to a step-OLR increase starting on day 58. The OLR was increased by approximately 400% by increasing the influent concentration to 11,000 μg/L for a 77 hour period. The DO consumption increased substantially and rapidly, with most of the increase occurring in the first section of the bed (Figures 6-7 and 6-8). In response to the increased aqueous-phase concentration, the concentration of adsorbed toluene also increased rapidly (Figure 6-4). The increase in bioactivity and adsorption did not completely mitigate the effects of the increase in influent toluene at this elevated OLR; the effluent concentration increased to an average value of 520 μg/L during this period in which the system was challenged with an applied organic loading of 22.6 kg COD/m^3-d (removal was 95.1 % or 21.5 kg COD/m^3-d).

A comparison of aqueous-phase toluene concentrations, adsorbed toluene concentrations, and DO consumption before and during the step-OLR increase is shown in Figure 6-9. It is clear that the increased influent toluene concentration served to increase aqueous toluene concentrations throughout the profile of the bed. These higher levels drove an increase in adsorption, which served to dampen the changes in the aqueous phase. As expected, the higher concentrations also resulted in increasing biodegradation and a corresponding increase in oxygen consumption.

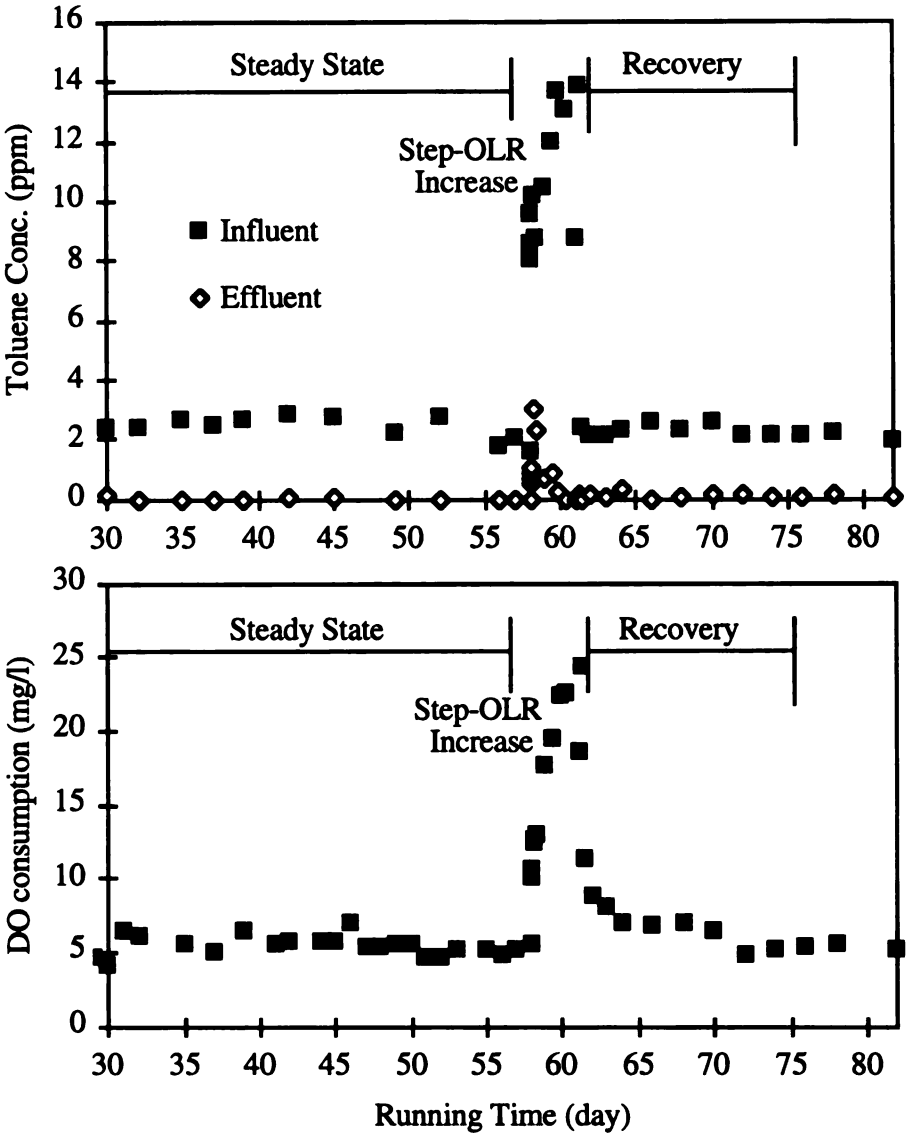

Figure 6-7. Toluene concentration in the influent and effluent and DO consumption during the pseudo-steady-state, step-OLR increase and recovery phases

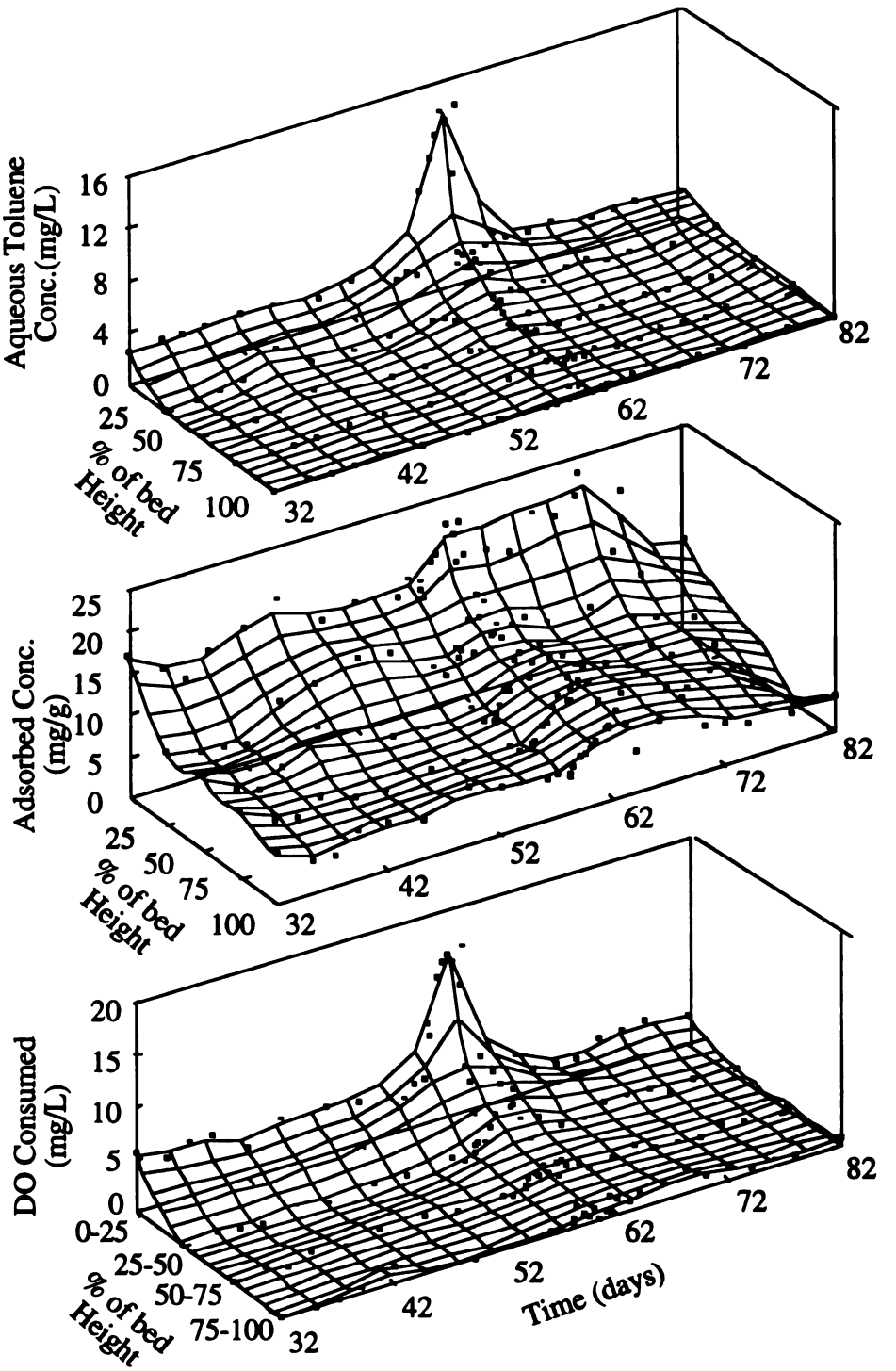

Figure 6-8. Concentration profiles of aqueous-phase toluene, adsorbed toluene and DO consumption

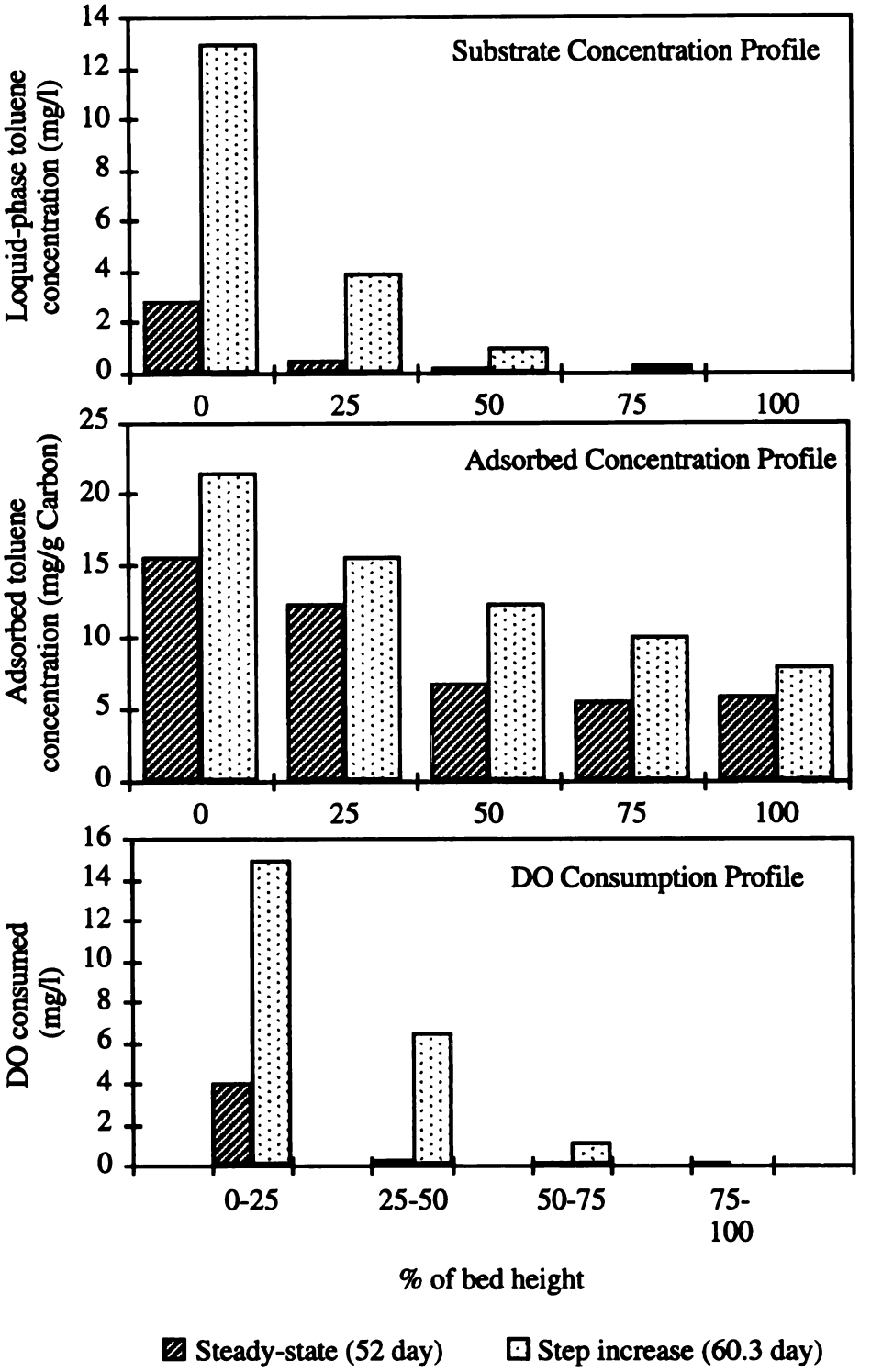

Figure 6-9. The profiles of aqueous-phase toluene, adsorbed toluene and DO consumption during the pseudo-steady-state and step increase periods.

6.3.4 Phase 4: Recovery

After 77 hours of the step-OLR increase, the organic loading rate was returned to the original level. DO consumption decreased from 24.3 to 11.3 mg/L within 4 hours, but did not return to the pre-step OLR increase level of 5.6 mg/L for another 50 hours (Figure 6-7). The total amount of adsorbed toluene, which had increased significantly during the step OLR increase, began to slowly decrease, but did not decline to the pre-OLR step increase level until 17 days after the OLR was reduced.

6.4 Discussion

Average performance of the system during each of the four phases is shown in Table 6-1. For the purpose of comparing reactor performance during different phases of operation, an oxygen utilization quotient (Q) can be defined as the ratio of the mass of DO consumed to COD removed,

$$Q = \frac{M_{DO}}{M_{COD}} \qquad (6\text{-}1)$$

where Q= the oxygen utilization quotient (when all COD is removed biologically, the Q will equals to fe which is the fraction of electrons diverted for energy.), M_{DO} = the mass of oxygen consumed in the FBR, and M_{COD} = the difference between the influent and effluent COD. In the absence of adsorption, Q indicates the fraction of COD consumed that was used for energy by the biomass, while the remainder was used for cell synthesis (i.e. biomass production). During the pseudo-steady-state phase, the average Q was observed to be 0.74, which is consistent with previous reports for toluene degradation in FBR systems (Hickey *et al.* 1991a; Voice *et al.* 1992). Since it has been demonstrated that

biodegradation is the primary removal mechanism and the production of incomplete oxidation products (byproducts) was negligible during steady-state, this value can be considered a property of the biological (biofilm) removal mechanism. Assuming that the fraction of energy diverted to cell synthesis was not significantly different during the other phases of operation, changes in the value Q from the pseudo-steady-state value will reflect the effect of adsorption and desorption on substrate removal and the production and degradation of byproducts in the system.

Table 6-1. Summary of overall performance of the reactor

Phase	Time (day)	Influent Conc. (μg/l)	Effluent Conc. (μg/l)	COD Loading Rate kg/m^3-Day	Removal %	Q $\left(\frac{DO}{COD}\right)$
Startup	1-25	2700 (±250)	18 (±12)	5.8	99.3	0.11-0.74
Steady state	25-58	2500 (±200)	14(±5)	5.3	99.4	0.74
Step-OLR increase	58-61	11000 (± 1400)	520(±270)	22.6	95.1	0.58
Recovery	61-82	2300 (±150)	34(±25)	4.9	98.5	1.51-0.77

As shown in Figure 6-10, Q increased from essentially zero to the pseudo-state-state value of 0.74 over the initial part of the start-up phase. This indicates that more COD was removed initially than can be explained by biodegradation (as indicated by oxygen consumption), and thus adsorption must be responsible. This was confirmed by the concentration of toluene extracted from the GAC (a steady increase in the amount of adsorbed toluene, Figure 6-4). After establishment of a biofilm capable of degrading toluene, a continual decrease in the amount of adsorbed toluene was observed (Figure 6-4). The desorbed toluene was subsequently degraded by microorganisms in the biofilm. During the pseudo-steady-state period the value of Q remained essentially constant as would be anticipated if biodegradation was the predominate removal mechanism.

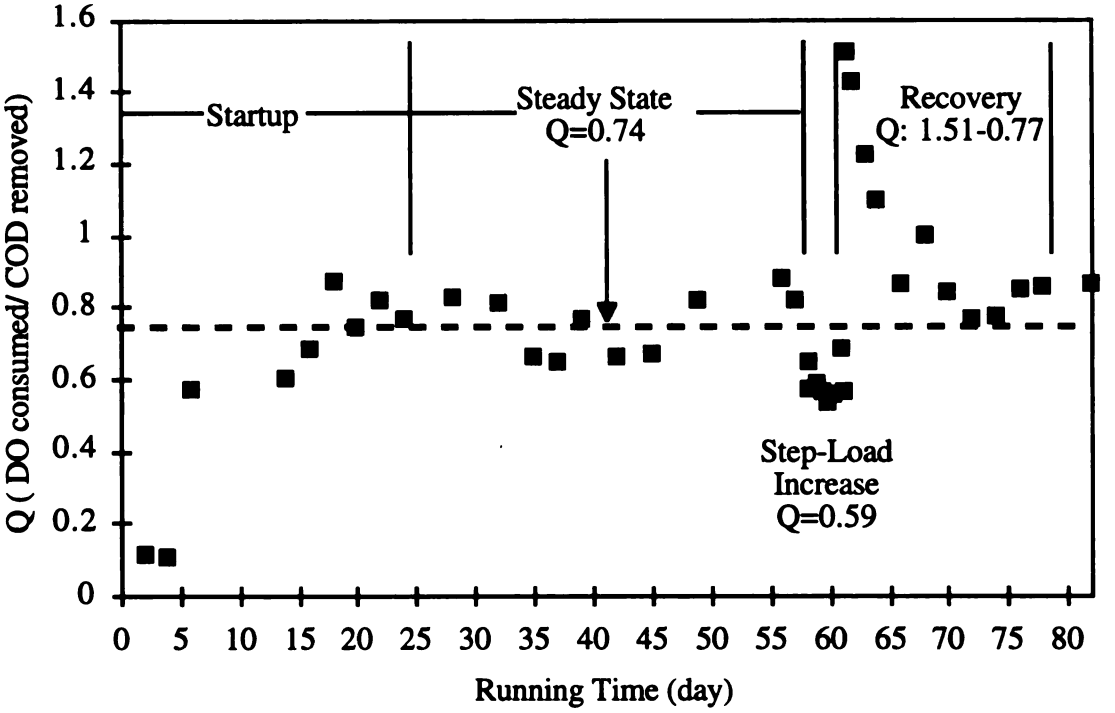

Figure 6-10. The oxygen utilization quotient (Q) for the overall experiment

During the step OLR increase, the value of Q decreased to an average value of 0.59. As was found during the start-up period, more COD was removed than could be explained by biodegradation; this was accompanied by a measured increase in the amount of adsorbed toluene (Figure 6-4). This appears to confirm the hypothesis of previous work (Hickey *et al.* 1991b; Voice *et al.* 1992) that adsorption serves to reduce the effect of concentration increases.

During the recovery phase, the value of Q increased to a maximum value of 1.51 immediately after the loading was reduced. Approximately 20 days was required before a return to the pseudo-steady-state value of 0.74 was observed. This indicates that during this period more dissolved oxygen was consumed than necessary for removal of the influent toluene. Based on Figure 6-4 it can also be seen that amount of adsorbed toluene

decreased, rapidly at first and then somewhat more slowly, during the recovery period. Since the effluent concentration did not increase, it must be concluded from these two observations that the excess toluene adsorbed during the step increase was both desorbed and biodegraded after the OLR was returned to 5 kg COD/m^3-day. This indicates that adsorption serves not only to protect the system against concentration increases but that, at least for toluene, adsorption is reversible and dampens the systems against increases or decreases in concentration. Furthermore, the elevated DO consumption levels observed after the OLR was returned to the steady-state level confirms that the biofilm can regenerate the adsorptive capacity of the carbon. This process of bioregeneration should provide a means by which GAC-FBR systems can handle repeated transient loading increases.

Speitel and DiGiano (1987) demonstrated that diffusive transport resistance is the rate-limiting step in bioregeneration of GAC using a homogeneous surface diffusion model. While this study was not designed to quantitatively evaluate the desorption rate, the results showed that the bioregeneration was a slow process. It can be inferred from previous work that this probably results from such a limitation. More information on the kinetics of desorption, degradation, and time varying concentration gradients would be needed to fully understand this issue.

The large increase in Q during the recovery period can't be explained by toluene desorption alone. In a separate study conducted in this laboratory, it was found that during an extremely high shock-OLR period not all of the toluene was completely oxidized; some intermediate products were detected (Zhao *et al.* 1994). These intermediate oxidation products may have also adsorbed during the step-OLR period and subsequently desorbed and were degraded during the recovery period. This would be consistent with the observation that Q was larger than could be accounted for by desorption and degradation of

inlet toluene only. Additional experimentation would be necessary to substantiate this explanation.

The oxygen utilization quotient index can be used to estimate the amount of toluene removed biologically,

$$M_b = \frac{M_{DO}}{(3.13)Q_{ss}} \qquad (6\text{-}2)$$

where: M_b = the amount of toluene removed biologically, M_{DO} = the mass of DO consumed through the profile of the reactor, Q_{SS} = the removal index at pseudo steady-state (0.74), and (3.13) = conversion factor for mass of COD to mass of toluene. The total amount of toluene removed from the liquid phase, as determined by influent and effluent concentrations, and the amount removed biologically, as determined by equation 6-2, are shown in Figure 6-11. It can be seen that during the step-OLR increase, total removal exceeded the amount that could be accounted for by complete oxidation. The difference between these two curves is the amount of toluene adsorbed from the start of the step-OLR increase. After the OLR was returned to normal, the biological removal rate exceeded the total removal rate, as evidenced by the greater slope of the lower line in Figure 6-11, and the amount toluene adsorbed onto the GAC is reduced. At approximately day 69, 11 days after the end of the step-OLR increase, the two curves meet, indicating that the mass of toluene adsorbed during the increase has been removed by bioregeneration. However, because of the potential production of the byproducts, the results from Eqn. 2 could under-estimate the biological removal of toluene during the step-OLR increase and over-estimate biological removal during recovery. As such, this analysis must be treated as a simplification of a presumably more complex set of processes.

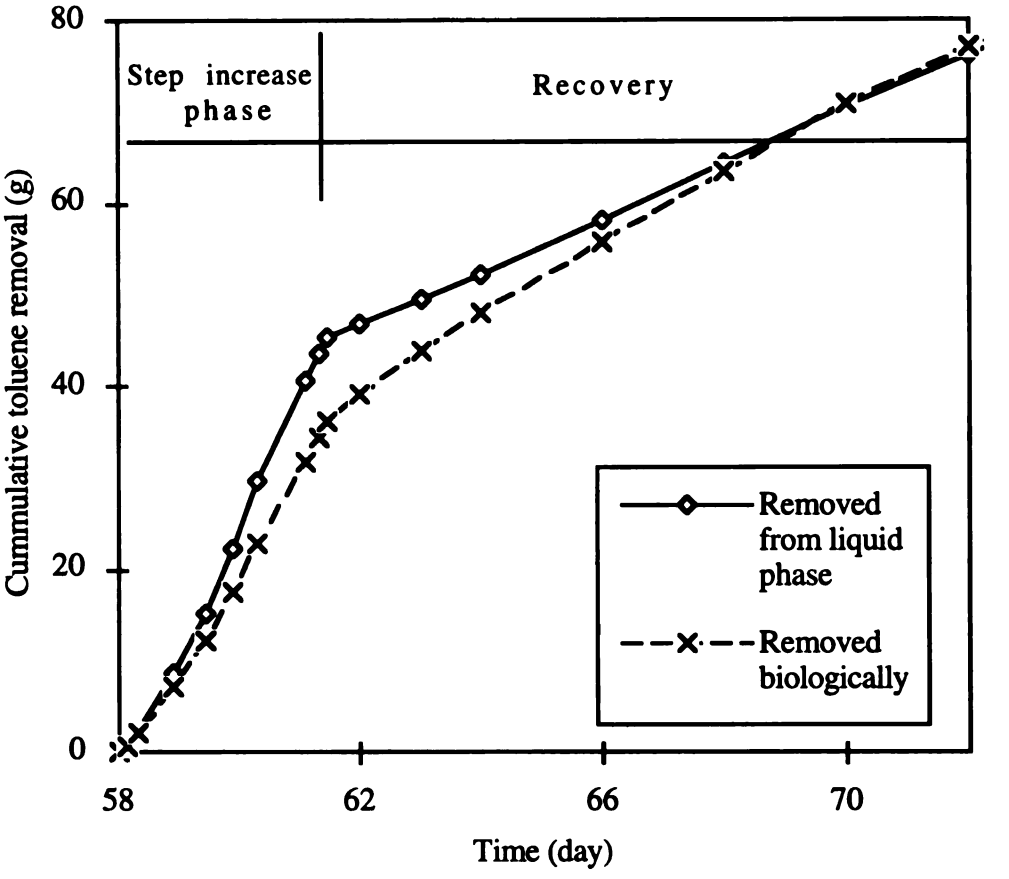

Figure 6-11. Cumulative toluene removed from the aqueous phase and that removed biologically during the step-OLR increase and recovery phases

6.5 Conclusions

This study provides conclusive evidence that a major role of adsorption in integrated biological activated carbon (BAC) systems is to dampen concentration changes during loading transients. During the start-up period in a GAC-FBR before a biofilm is established, the substrate (toluene) was adsorbed rather being released, as would occur in a biological-only system. Upon establishment of a biofilm capable of degrading the substrate, influent toluene was biologically oxidized and a portion of the previously adsorbed toluene was desorbed and degraded. Under continued constant organic loading rate conditions, the system stabilized and functioned as a biological reactor. During a 77 hour, 400% step-OLR increase, adsorption again became important; excess substrate was observed to accumulate on the GAC carrier particles. This material was desorbed and degraded over an 11 day period following resumption the initial organic loading rate of 5.3 kg COD /m^3-d. Thus it was established that the dampening effect of activated carbon is not exhausted, but is recovered via bioregeneration.

It is clear from these results that GAC-FBR systems are advantageous for treating BTX-contaminated waters when variations in loading rates are expected or when positive removal is required during the start-up period. Furthermore, this concept provides a foundation upon which future studies can be conducted in order to develop a rational design procedure which incorporates criteria for transient loading performance.

CHAPTER 7

ESTIMATION OF KINETIC PARAMETERS FOR BIODEGRADATION OF BENZENE, TOLUENE AND XYLENE

7.1 Introduction

Biological fluidized bed reactors (FBR) have demonstrated the ability to treat groundwater contaminated with petroleum hydrocarbons (Hickey *et al.* 1990b; 1991a; Voice *et al.* 1992). Even when granular activated carbon particles are used as the biofilm carrier, biodegradation remains the most important removal mechanism in the system, especially under steady-state conditions (Voice *et al.* 1992). Biodegradation kinetics, therefore, are very important to describe this system and assist engineers during process design.

A kinetic experiment can be used to evaluate the rate of biodegradation. One of the most widely accepted rate equations is the Monod equation (Monod 1949) shown here.

$$\mu = \frac{\mu_m S}{K_s + S} \qquad (7\text{-}1)$$

S is the concentration of substrate (mg/L), μ is the specific growth rate constant (1/hr), μ_m is the maximum specific growth rate constant (1/hr), and K_s is the half-saturation coefficient (mg/L). It should be noted that the Monod equation is strictly an empirical equation (Grady and Lim 1980). Lawrence and McCarty (1970) modified the equation to:

$$\frac{dS}{dt} = -\frac{kSX}{K_s + S} \qquad (7\text{-}2)$$

Where: $\mu = \frac{YdS}{Xdt}$ and $\mu_m = kY$

X is the concentration of biomass (mg VSS/L), k is the maximum specific utilization rate of substrate (mg substrate/mg VSS-day), K_s is the half-saturation coefficient (mg/L), and t is time (day). Y is the yield coefficient of biomass (mg VSS/mg substrate).

The kinetic parameters can be evaluated by a batch experiment. In this approach, a compound, as the sole organic constituent, is introduced into a reactor containing the required nutrients, electron acceptor, and growth factors. The reactor is then inoculated with a known amount of microorganisms. Samples are collected periodically to monitor degradation of the compound and growth of the microorganisms. The kinetic coefficients can then be estimated from Eqn. 7-2.

Several studies have been conducted to estimate the kinetic coefficients for the biodegradation of BTX (Table 2-1). There is a tremendous difference in the reported values, however, and different values for different environments should be expected. For the mixed culture used during this work, the parameters had to be evaluated.

Interactions between BTX during biodegradation have been evaluated by several researchers. Goldsmith (1988) showed a slightly higher maximum utilization rate for toluene in a BTX mixture by an enriched mixed culture. Arvin *et al.* (1989) studied substrate interaction during aerobic biodegradation of benzene. They found that the degradation rates of benzene were higher when toluene or xylene was also present in the environment. Alverez and Vogel (1991) investigated the interactions of BTX during biodegradation by two pure cultures and a mixed culture from an aquifer. The enhanced degradation patterns of benzene and *p*-xylene in the presence of toluene was revealed. The degradation of benzene was inhibited by the presence of *p*-xylene for a toluene

degrader. In their studies, toluene could not be degraded by a benzene degrader unless benzene was also present. Recent studies by Chang *et al.* (1993) revealed competitive inhibition and cometabolism during biodegradation of BTX by two *Pseudomonas* isolates. Strain B1, which grew on benzene and toluene as sole sources of carbon and energy, has the ability to transform *p*-xylene by consumption of the growth substrates (benzene and toluene) or consumption of biomass during cometabolic degradation. The degradation of benzene, however, was inhibited by the toluene even though the degradation rate of toluene was not effected. For strain X1, which grew on toluene and *p*-xylene but not benzene, the degradation of *p*-xylene was inhibited by the presence of toluene, but the degradation rate of toluene was not effected. Competitive inhibition and cometabolism effects were quantified in their studies. For a system treating a mixture of BTX, complete degradation patterns should be expected. Such patterns may depend upon the environmental conditions, composition of the waste, and maturity of the microbial population

In this chapter, the kinetic parameters for the degradation of BTX by a mixed culture (from a biological fluidized bed reactor treating BTX contaminated groundwater) were estimated.

7.2 Materials and Methods

7.2.1 Biodegradation Kinetic Assays

Biomass was collected from a biological fluidized bed (FBR) reactor treating groundwater contaminated with benzene, toluene, and *p*-xylene (Voice *et al.* 1992). Non-activated carbon (without adsorption capacity) was used as the biofilm carrier in the FBR. After a mature biofilm developed, a small amount of biomass coated material was removed from the FBR. The biomass was stripped from the carrier particles, purged with

oxygen to remove residual BTX, and homogenized by shaking the biomass using glass beads in a 300 mL glass bottle. Kinetic assays were performed using a 50 mL glass syringe equipped with a Luer-lock valve. A stirring bar (6 mm in diameter and 15 mm long) was placed in the syringe and the syringe was placed on a magnetic stirrer to provide mixing. The growth media consisted of groundwater amended with nitrogen and phosphorous at a weight ratio of 100/5/1:COD/N/P. Prior to addition of the BTX this water was oxygenated with pure oxygen to obtain an initial dissolved oxygen concentration of greater than 30 mg/L. Stock substrates of benzene, toluene and *p*-xylene were dissolved in tap water and then measured amounts of these solutions were transferred to the syringes. The initial concentration of BTX varied from 1.2 to 15 mg/L for each substrate. Three different substrate compositions were used in the assays. The first composition contained only one substrate (benzene, toluene or *p*-xylene) as a single substrate and seven experiments were conducted. The second composition contained two substrates (benzene and toluene, benzene and *p*-xylene or toluene and *p*-xylene) as a dual substrate system with a 1:1 substrate ratio and six experiments were conducted. The third composition contained all three substrates (benzene, toluene and *p*-xylene) in a constant ratio of B:T:X of 1:1:1 and five experiments were conducted. The initial concentration of biomass varied from 13 to 120 mg VSS/L for all of the assays. After biomass was introduced into the syringe, a one-mL liquid sample was taken. Subsequent one-mL samples were taken at regular intervals to track degradation. Biomass concentrations (VSS) were measured according to Standard Methods (Greenberg *et al.* 1985). All assays were conducted at 20 °C.

7.2.2 Yield Coefficient Assay

The net yield coefficient (Y) was measured using a procedure similar to that used for the kinetic assay. One substrate was added to the syringes at a time for the single

substrate measurement. Benzene and toluene were used to estimate the yield coefficients for the dual substrate condition. All three substrates were added to the system to estimate the coefficient under the three substrate condition. Liquid samples were taken at the beginning and end of each experiment (about 6 to 8 hours) to measure the substrate and biomass concentrations.

7.2.3 Decay Coefficient Assay

One hundred mL of biomass in growth media (100 mg VSS/L) was transferred to a 250 mL glass bottle with cap. The biomass was collected from the FBR and treated using a similar procedure as used in the kinetic assay. Subsequent one-mL samples were taken at regular intervals to track the disappearance of the biomass. The concentration of biomass was determined by an optical density method using a spectrophotometer (PE Lambda 6 UV/VIS) at 500 nm. The optical density was converted to VSS using an external calibration curve with the same mixed culture.

600 nm ?

7.2.4 Theory

The modified Monod equation (Eqn. 7-2) was used to evaluate the degradation capability of the microorganisms in the FBR. The growth of biomass can be described as:

$$X = X_0 + Y(S_0 - S) \qquad (7\text{-}3)$$

where: S = concentration of substrate (mg/L).

S_0 = initial concentration of substrate (mg/L).

X = concentration of biomass (mg VSS/L).

X_0 = initial concentration of biomass (mg VSS/L).

Y = net yield coefficient of biomass (mg VSS/mg substrate).

Combining Eqn. 7-2 and 7- 3, integrating and rearranging results in:

$$t = \frac{1}{k}\left[\frac{K_s}{(X_0 + YS_0)}\ln\left(\frac{S_0}{S}\right) - \left(\frac{K_s}{(X_0 + YS_0)} + \frac{1}{Y}\right)\ln\left(\frac{X_0}{X_0 + Y(S_0 - S)}\right)\right] \quad (7\text{-}4)$$

The decay of biomass without substrate can be described as follows:

$$\frac{dX}{dt} = -bX \quad (7\text{-}5)$$

where: b = decay coefficient (1/day)

The integrated form of Eqn. 7-5 is:

$$\ln\left(\frac{X}{X_o}\right) = -bt \quad (7\text{-}6)$$

$$X = X_o e^{-bt} \quad (7\text{-}7)$$

The net yield coefficient (Y)can be expressed as follows:

$$Y = \frac{X - X_0}{S_0 - S} \quad (7\text{-}8)$$

7.2.5 Estimation of Parameters

A decay coefficient was estimated using Eqn. 7-7 and a non-linear regression computer software program (Solver, Excel 4.0). The net yield coefficients were calculated using Eqn. 7-8. The kinetic parameters, maximum specific utilization rate of substrate (k) and half-saturation coefficient (K_s), were estimated using Eqn. 7-4 and non-linear regression. The following criteria were applied during selection of experimental data and parameter estimation.

1). discard data sets with fewer than 6 usable data points;

2). discard data sets with an initial substrate concentration higher than 45 mg COD/L;

3). discard data set with end substrate concentrations higher than the GC detection limit (about 15 μg/L for each of BTX);

4). if a lag-phase was observed, discard the data points during the lag-phase, as defined by a rate of substrate disappearance significantly (50%) lower than the rate at later time period, and use a calculated initial substrate concentration estimated from the intercept of an extension-line of the first three points after the lag-phase on a graph of remaining substrate concentration vs. time;

5). estimate k and K_s simultaneously.

7.2.6 Chemicals and Analytical Procedure

Benzene, toluene, *p*-xylene (BTX), potassium phosphate dibasic and ammonia chloride were obtained from J.T. Baker Chemical Co., Phillipsburg, NJ. Michigan State University tap water was used directly as a source of groundwater. This relatively hard water (450 mg/L as $CaCO_3$) is pumped from a deep aquifer underlying the campus and distributed without further treatment.

BTX concentrations were determined using a gas chromatograph equipped with a flame ionization detector (FID) using helium as the carrier gas (Perkin-Elmer, model 8700), coupled with an automatic headspace sampler (Perkin-Elmer, model HS-101). Separation was accomplished using a 30 m x 0.53 mm DB-624 column (J & W Scientific). Water samples collected from the kinetic assays (1 mL) were transferred to 20 mL glass vials with Teflon®-coated septa and aluminum crimp-seals. The samples were equilibrated for 1 hour at 80 °C and an aliquot of the headspace gas was injected into the gas chromatograph. The detection limit of this procedure was 10 μg/L for each BTX

10 ppb discrepancy with p 39

component. The accuracy for measurements of split samples containing a concentration of 15 μg/L was ± 1 μg/L.

7.3 Results

7.3.1 Estimation Method Verification

In order to test the variability of the results from the estimation procedure, a 15 point degradation curve was generated based upon Eqn. 7-4 using the parameters in Table 7-1. The substrate concentration (S) was then randomly varied between -10 % and +10 % of the theoretical value and ten groups of generated data sets were generated (Table 7-2 and Figure 7-1). The parameters were estimated from the data sets using Eqn. 7-4 with a non-linear parameter estimation procedure. The results are shown in Table 7-1. The relative standard deviation of the each created point was approximately 6%. The results showed that the estimation can be significantly affected by the variance in data which resulted in a large error being introduced to the estimation of Ks (the relative standard deviation is 36%).

Table 7-1. Procedure verification for parameter estimation

Parameters	Theoretical Value	Summary Results for 10 Tests	% SD
Xo	114.2	114.2	
So	9.1	8.92	6.91%
Y	1.116	1.116	
Ks	0.299	0.285	35.91%
k	2.248	2.194	11.61%
r^2	1.000	>0.985	

Table 7-2 Evaluated data sets

Time (min)	Substrate Concentration (mg/L)					
	Theoretical Value	Evaluated Values				
		High	Low	Average	SD	% SD
0.00	9.100	9.818	8.214	8.918	0.616	6.9%
6.35	8.000	8.651	4.340	7.987	0.960	6.2%
12.09	7.000	7.561	6.488	6.938	0.317	4.6%
17.81	6.000	6.558	5.578	5.954	0.411	6.9%
23.53	5.000	5.361	4.589	4.948	0.296	6.0%
29.25	4.000	4.386	3.638	4.030	0.257	6.4%
35.03	3.000	3.255	2.760	2.935	0.194	6.6%
40.93	2.000	2.163	1.806	2.017	0.112	5.6%
47.23	1.000	1.096	0.915	1.050	0.058	5.6%
48.62	0.800	0.868	0.747	0.805	0.048	6.0%
51.76	0.400	0.431	0.366	0.400	0.022	5.4%
55.45	0.100	0.109	0.093	0.102	0.005	5.0%
56.78	0.050	0.054	0.047	0.052	0.0027	5.1%
59.46	0.010	0.011	0.009	0.010	0.0007	7.1%
63.05	0.001	0.0011	0.0009	0.001	0.0001	5.4%

7.3.2 Decay Coefficient

Two decay curves are shown in Figure 7-2. The biomass concentration decreased until day 9 without significantly changing thereafter. The decay coefficients were calculated based upon Eqn. 7-7 using a non-linear regression procedure and presented in Table 7-3. The data from the first 9 and 8 days was used. The average decay coefficient value was found to be 0.049 1/day.

Table 7-3. Decay coefficient determination

Experiment Dates	Usable Days	n	r^2	b (1/day)
9/15/92-9/24/93	9.06	7	0.988	0.053
9/3/93-9/17/92	7.88	7	0.994	0.046
			Average	0.049

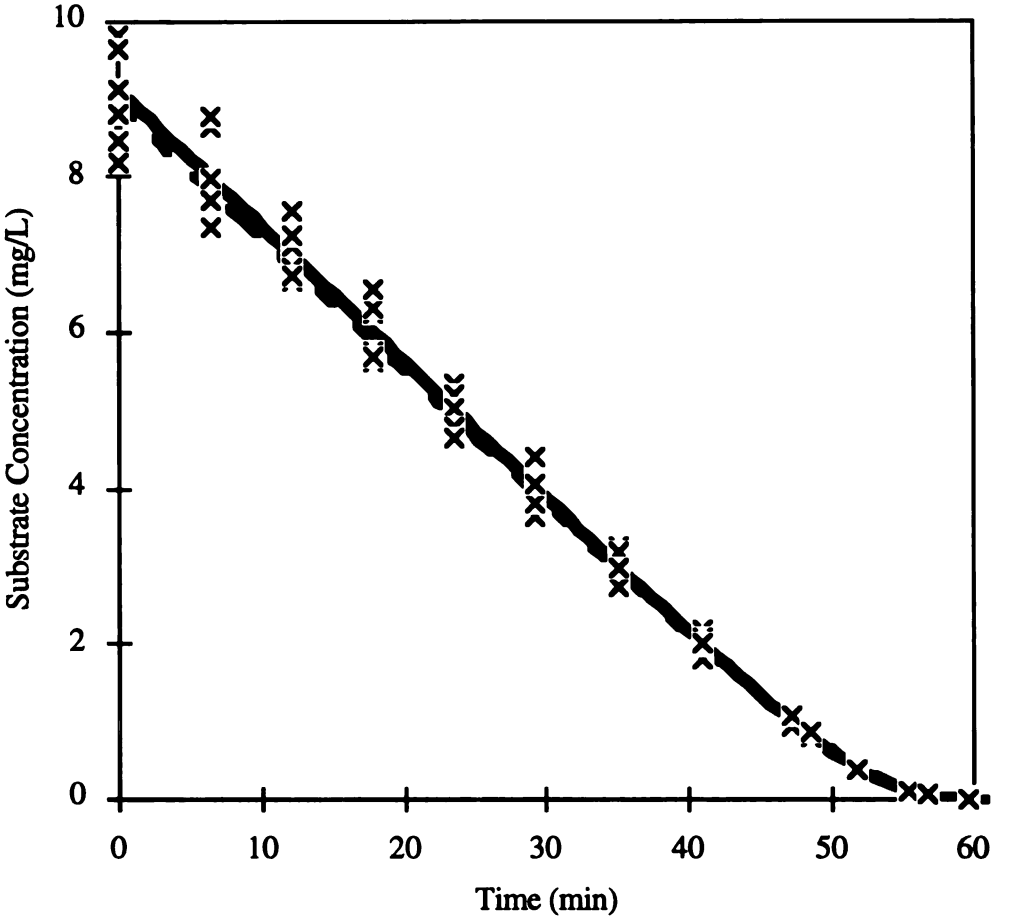

Figure 7-1. Verification of parameter estimation protocol

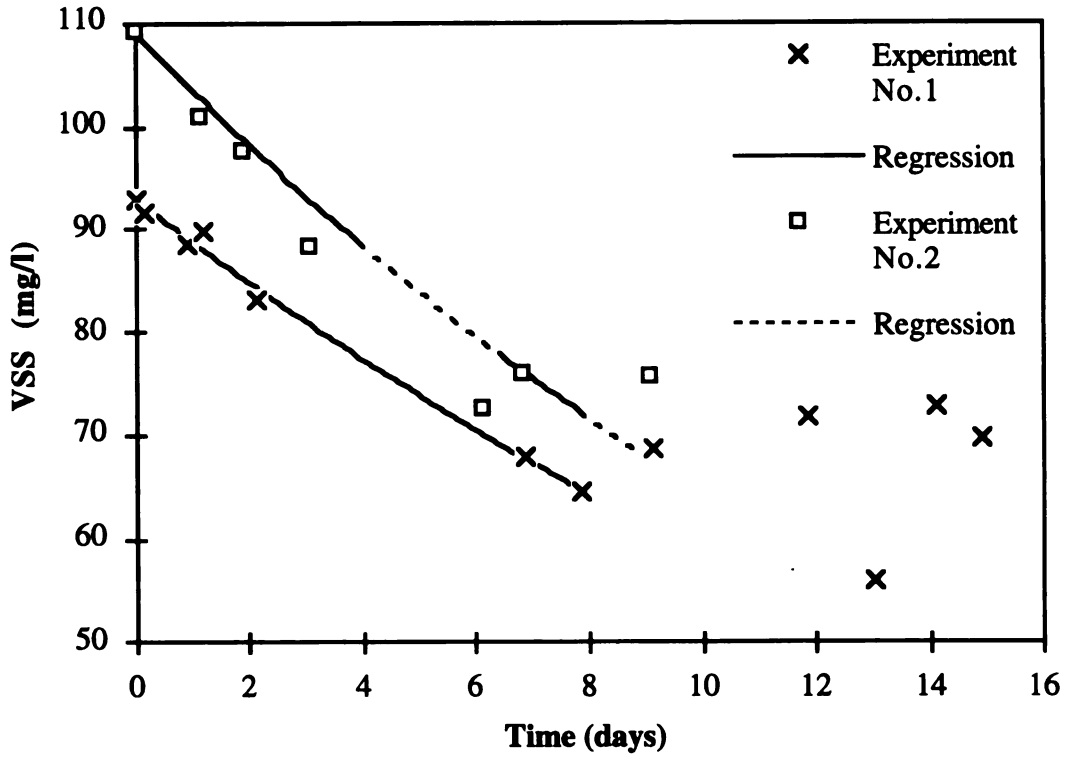

Figure 7-2. Decay of biomass harvested from the FBR

7.3.3 Yield Coefficient

The yield coefficients were determined under different combinations of the substrates, single substrate(the initial concentration of benzene, toluene, and xylene were 18 mg/L, 10 mg/L, and 17 mg/L, respectively), two substrates (the initial concentration of benzene and toluene were 4.8 mg/L and 5.1 mg/L, respectively), and three substrates (the initial concentration of benzene, toluene, and xylene were 4.8 mg/L, 5.1 mg/L, and 3.9 mg/L, respectively). The net yield coefficient was calculated from Eqn. 7-8, which includes the effect of biomass decay. The coefficients are reported in Tables 7-4 and 7-5. The net yield coefficient in a biological fluidized bed reactor treating groundwater contaminated with BTX was also measured by tracking the waste biomass and the COD

consumption during a two week period. The net yield coefficient was 0.23 mg VSS/mg COD at a solids retention time of 20 days.

Table 7-4. Net yield coefficients for single substrates

Substrate	n	Net Yield Coefficient	
		Y (mg VSS/mg substrate)	SD (mg VSS/mg substrate)
Benzene	3	1.12	0.15
Toluene	3	1.13	0.13
p-Xylene	3	0.92	0.01

Table 7-5. Net yield coefficients for multiple substrates

Substrate	n	Net Yield Coefficient	
		Y (mg VSS/mg COD)	SD (mg VSS/mg COD)
Benzene and Toluene	1	0.22	-
Benzene Toluene and *p*-Xylene	2	0.17	0.04

7.3.4 Estimation of Biodegradation Kinetic Parameters

The kinetic parameters, maximum specific utilization rate of substrate (k) and half-saturation coefficient (K_s), were estimated under different substrate combinations: one substrate (B, T, and X), two substrates (BT, BX, and TX), and three substrates (BTX). The disappearance of each substrate was measured allowing the parameters to be estimated using Eqn. 7-4 and non-linear regression.

In order to avoid large variation during kinetic parameter estimation, multiple kinetic assays were conducted. Seven assays were conducted for each substrate in the

single substrate condition. Six assays were conducted for each combination of two substrates. Five assays were conducted for the three substrate case. The initial biomass and substrate concentrations were varied to determine the effect on parameter estimation. Compared to the large variation in the parameters, however, the initial concentration effect was not significant. Therefore, the results reported in this chapter are a combinations all estimations without considering the initial concentration effect.

7.3.4.1 One Substrate

In this phase of the study, one substrate was added to the syringe with the results indicating the ability of the mixed culture to degrade one substrate without interaction effects caused by other substrate. Six sets of data satisfied the criteria for kinetic estimation of the degradation of benzene. The initial concentrations of biomass and benzene were 13.8 to 114.2 mg VSS/L and 1.5 to 9.6 mg/L, respectively. Five sets of data satisfied the criteria for kinetic estimation of the degradation of toluene. The initial concentrations of biomass and toluene were 49.2 to 114.2 mg VSS/L and 8.5 to 11.1 mg/L, respectively. Six sets of data satisfied the criteria for kinetic estimation of the degradation of xylene. The initial concentrations of biomass and xylene were 27.6 to 228.4 mg VSS/L and 1.1 to 12.1 mg/L, respectively. Typical biodegradation curves for each single substrate system are shown in Figures 7-3, 7-4 and 7-5. The kinetic parameters are reported in Tables 7-6, 7-7, and 7-8. The average maximum specific utilization rates of substrate (k) are 2.09, 3.71, and 1.59 1/day for benzene, toluene, and xylene, respectively. The average half-saturation coefficient values (K_s) are 0.25, 0.84, and 0.63 mg/L for benzene, toluene, and xylene, respectively.

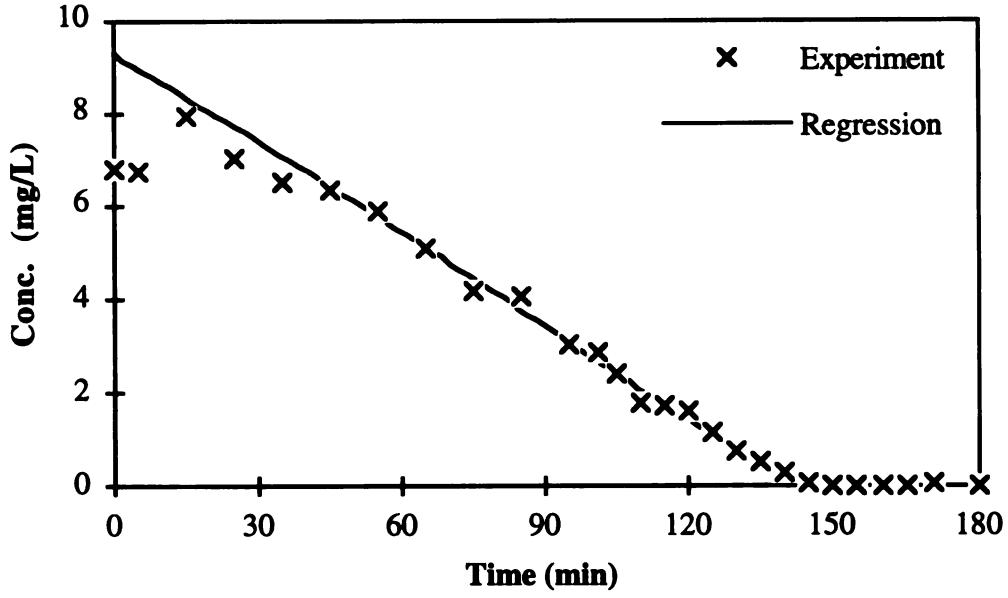

Figure 7-3. Typical biodegradation trend of benzene as a single substrate in a mixed culture; (estimation results: k=1.87 1/day; K_s=0.16 mg/L; R^2=0.989; n=19).

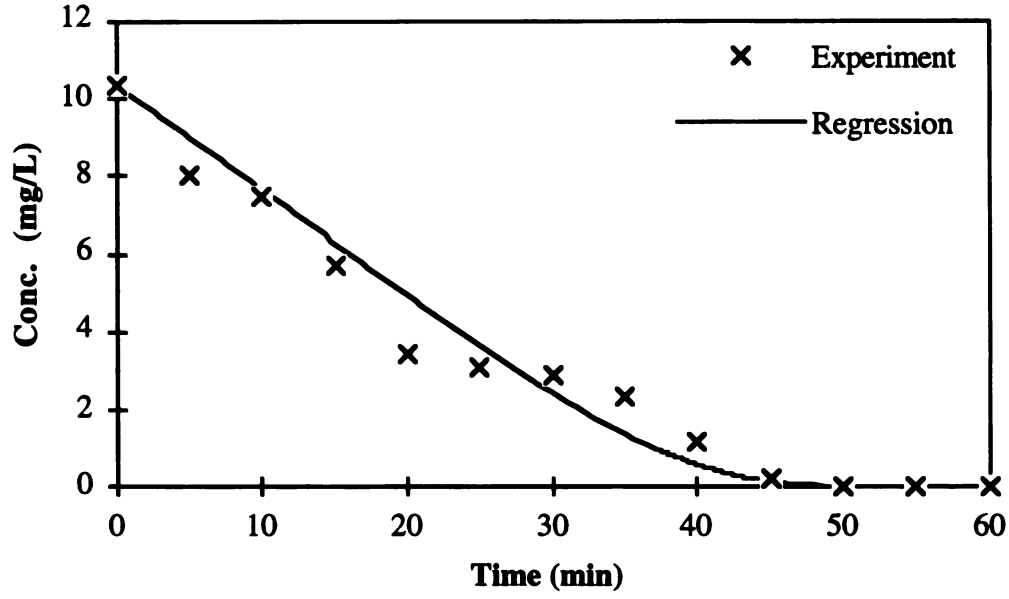

Figure 7-4. Typical biodegradation trend of toluene as a single substrate in a mixed culture; (estimation results: k=3.70 1/day; K_s=0.91 mg/L; R^2=0.970; n=13).

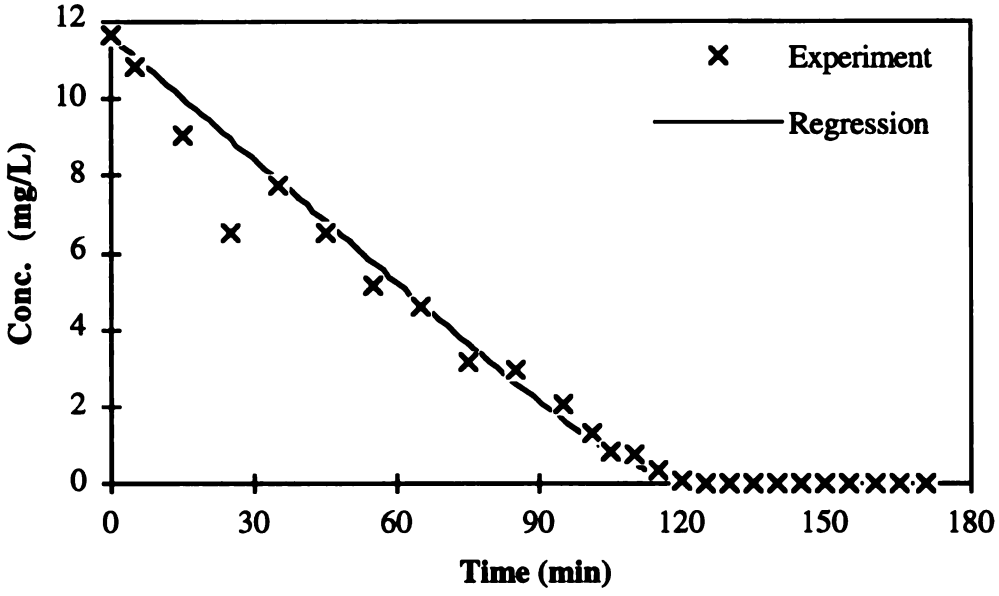

Figure 7-5. Typical biodegradation trend of *p*-xylene as a single substrate in a mixed culture; (estimation results: k=1.83 1/day; K_s=0.56 mg/L; R^2=0.991; n=19).

Table 7-6. Kinetic parameters for biodegradation of benzene by a mixed culture with and without other substrates present

Substrate	Other Substrate	Parameter	Average	n	90 % Confidence Interval	
					Upper	Lower
Benzene	-	k (1/day)	2.09	6	2.31	1.87
		Ks (mg/L)	0.25		0.34	0.16
	Toluene	k (1/day)	1.32	4	1.49	1.14
		Ks (mg/L)	0.10		0.16	0.04
	p-Xylene	k (1/day)	2.39	6	3.13	1.66
		Ks (mg/L)	0.43		0.64	0.22
	Toluene & *p*-Xylene	k (1/day)	1.08	5	1.77	0.39
		Ks (mg/L)	0.18		0.32	0.04

Table 7-7. Kinetic parameters for biodegradation of toluene by a mixed culture with and without other substrates present

Substrate	Other Substrate	Parameter	Average	n	90 % Confidence Interval	
					Upper	Lower
Toluene	-	k (1/day)	3.71	5	4.83	2.58
		Ks (mg/L)	0.84		0.95	0.73
	Benzene	k (1/day)	3.15	4	4.30	2.00
		Ks (mg/L)	0.90		1.24	0.56
	p-Xylene	k (1/day)	3.12	2	-	-
		Ks (mg/L)	0.53		-	-
	Benzene & *p*-Xylene	k (1/day)	2.12	5	3.71	0.54
		Ks (mg/L)	0.71		1.13	0.30

Table 7-8. Kinetic parameters for biodegradation of xylene by a mixed culture with and without other substrates present

Substrate	Other Substrate	Parameter	Average	n	90 % Confidence Interval	
					Upper	Lower
p-Xylene	-	k (1/day)	1.59	6	1.99	1.19
		Ks (mg/L)	0.63		0.97	0.28
	Benzene	k (1/day)	1.33	6	1.61	1.04
		Ks (mg/L)	0.17		0.27	0.07
	Toluene	k (1/day)	1.16	4	1.38	0.95
		Ks (mg/L)	0.15		0.21	0.09
	Benzene & Toluene	k (1/day)	0.79	5	1.05	0.53
		Ks (mg/L)	0.13		0.27	-0.01

7.3.4.2 Two Substrates

Two substrates were added to the syringe simultaneously in the second phase of this study to produce results indicating the ability of the mixed culture to degrade two interacting substrates. A constant ratio of two substrates (B:T, B:X, and T:X) of 1:1 was used in most of the assays.

Four sets of data satisfied the criteria for kinetic estimation of the degradation of benzene and toluene. The initial concentrations of biomass, benzene, and toluene were 48.3 to 64.6 mg VSS/L, 3.5 to 6.5 mg/L, and 4.9 to 6.4 mg/L, respectively. Typical biodegradation curves are shown in Figure 7-6. The kinetic parameters are reported in Tables 7-6 and 7-7. The average values of maximum specific utilization rate of substrate (k) are 1.32 and 3.15 1/day for benzene and toluene, respectively. The average values of half-saturation coefficient values (K_s) are 0.10 and 0.90 mg/L for benzene and toluene, respectively.

There were six sets of data which satisfied the criteria for kinetic estimation of the degradation of benzene and xylene. The initial concentrations of biomass, benzene, and xylene were 24.0 to 96.0 mg VSS/L, 1.5 to 7.0 mg/L, and 1.3 to 6.8 mg/L, respectively. Typical biodegradation curves are shown in Figure 7-7. The parameters are reported in Tables 7-6 and 7-8. The average maximum specific utilization rates of substrate (k) are 2.39 and 1.33 1/day for benzene and xylene , respectively. The average value of half-saturation coefficients (K_s) are 0.43 and 0.17 mg/L for benzene and xylene, respectively.

Only two sets of data satisfied the criteria for kinetic estimation of toluene and xylene degradation. The initial concentrations of biomass, toluene , and xylene were 48.3 and 64.6 mg VSS/L, 3.9 and 4.3 mg/L, and 2.4 and 2.6 mg/L, respectively. The initial concentrations of toluene were higher than the concentrations of xylene. Typical biodegradation curves are shown in Figure 7-8. The parameters are reported in Tables 7-7 and 7-8. The average value of maximum specific utilization rate of substrate (k) are 3.12 and 1.18 1/day for toluene and xylene , respectively. The average value of half-saturation coefficients (K_s) are 0.53 and 0.13 mg/L for toluene and xylene , respectively.

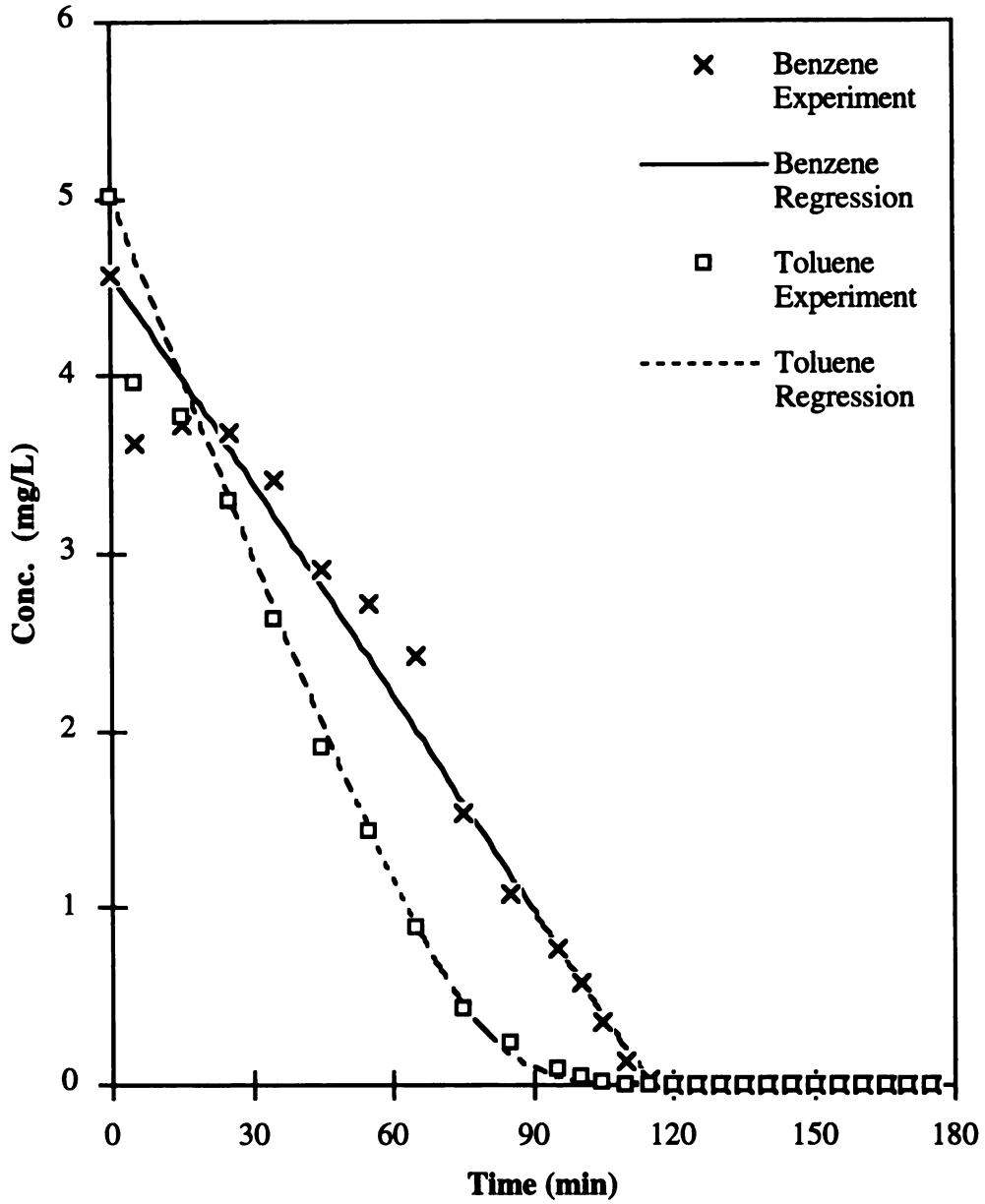

Figure 7-6. Typical biodegradation patterns of benzene and toluene as dual substrates in a mixed culture; (estimation results for benzene: k=1.12 1/day; K_s=0.03 mg/L; R^2=0.973; n=16; estimation results for toluene: k=2.17 1/day; K_s=0.58 mg/L; R^2=0.973; n=16).

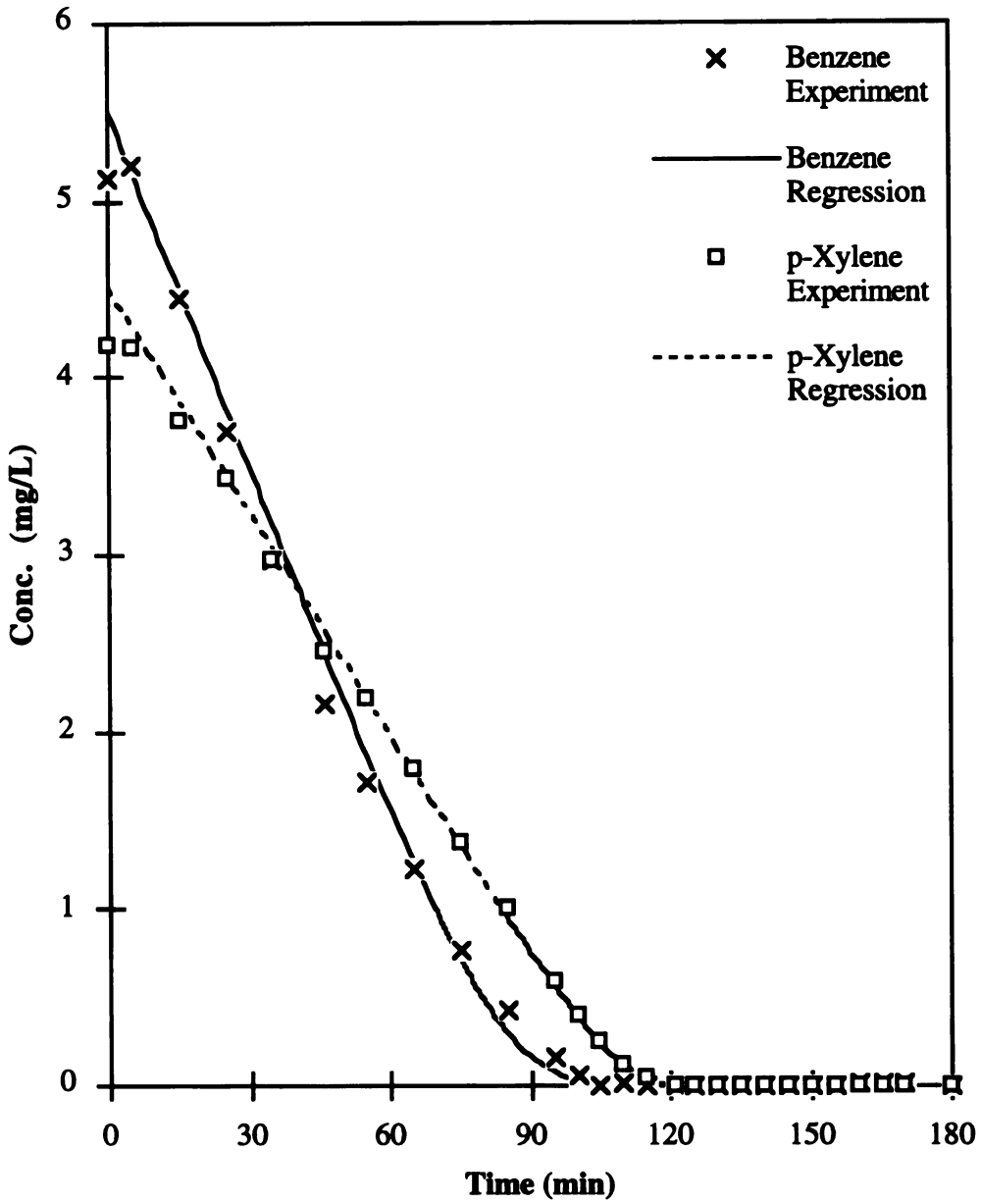

Figure 7-7. Typical biodegradation patterns of benzene and *p*-xylene as dual substrates in a mixed culture; (estimation results for benzene: k=2.15 1/day; K_s=0.45 mg/L; R^2=0.991; n=12; estimation results for *p*-xylene: k=1.29 1/day; K_s=0.16 mg/L; R^2=0.998; n=15).

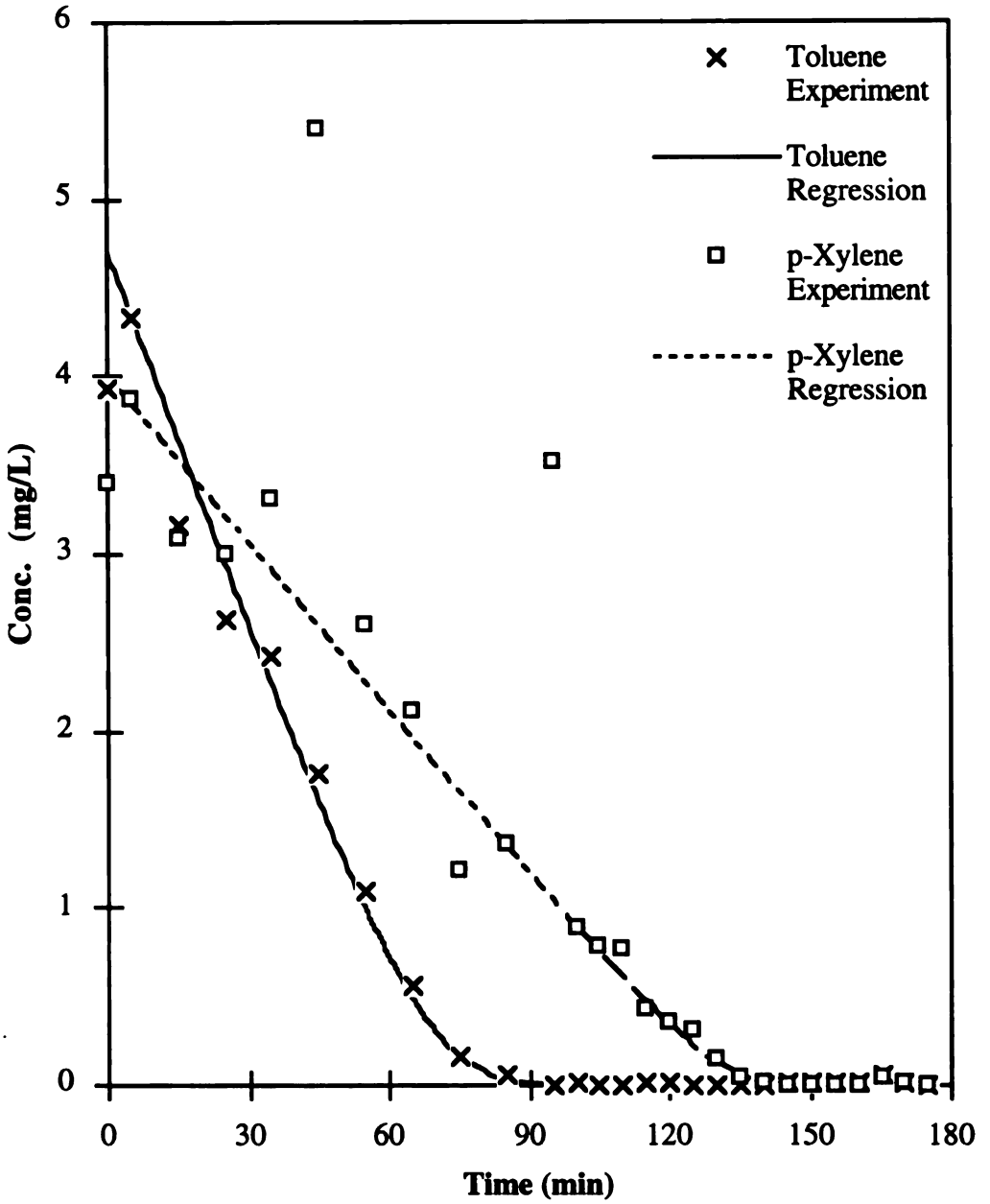

Figure 7-8. Typical biodegradation patterns of toluene and *p*-xylene as dual substrates in a mixed culture; (estimation results for toluene: k=2.32 1/day; K_s=0.49 mg/L; R^2=0.988; n=10; estimation results for *p*-xylene: k=0.96 1/day; K_s=0.14 mg/L; R^2=0.979; n=18).

7.3.4.3 Three Substrates

All five sets of data satisfied the criteria for kinetic estimation under the three substrate condition. The initial concentrations of biomass, benzene, toluene , and xylene were between 22.8 and 63.7 mg VSS/L, 1.3 and 3.4 mg/L, 1.2 and 3.5 mg/L, and 1.1 and 3.1 mg/L, respectively. Typical biodegradation curves are shown in Figure 7-9. The parameters are reported in Tables 7-6, 7-7, and 7-8. The average value of the maximum specific utilization rate of substrate (k) are 1.08, 2.12, and 0.79 1/day for benzene, toluene, and xylene , respectively. The average value of half-saturation coefficients (K_s) are 0.18, 0.71, and 0.13 mg/L for benzene, toluene, and xylene, respectively.

To investigate the substrate effect interaction has on the degradation rate, three experiments were conducted for the BTX system. In the experiments, the initial concentration of one of the three substrates was three times higher than the other two compounds (Table 7-9). The initial biomass concentrations were 63.7 mg VSS/L. The parameters were estimated and are reported in Table 7-9.

Table 7-9. Kinetic parameters for biodegradation of BTX by a mixed culture with varying initial concentrations.

X_0 (mg/L)	Benzene			Toluene			*p*-Xylene		
	S_0 (mg/L)	k (1/day)	K_s (mg/L)	S_0 (mg/L)	k (1/day)	K_s (mg/L)	S_0 (mg/L)	k (1/day)	K_s (mg/L)
63.7	5.74	1.67	0.37	1.34	1.08	0.72	1.34	0.36	0.05
63.7	1.76	0.47	0.02	6.47	2.57	0.69	1.58	0.36	0.018
63.7	1.72	0.73	0.31	1.59	2.27	1.84	5.81	1.00	0.35

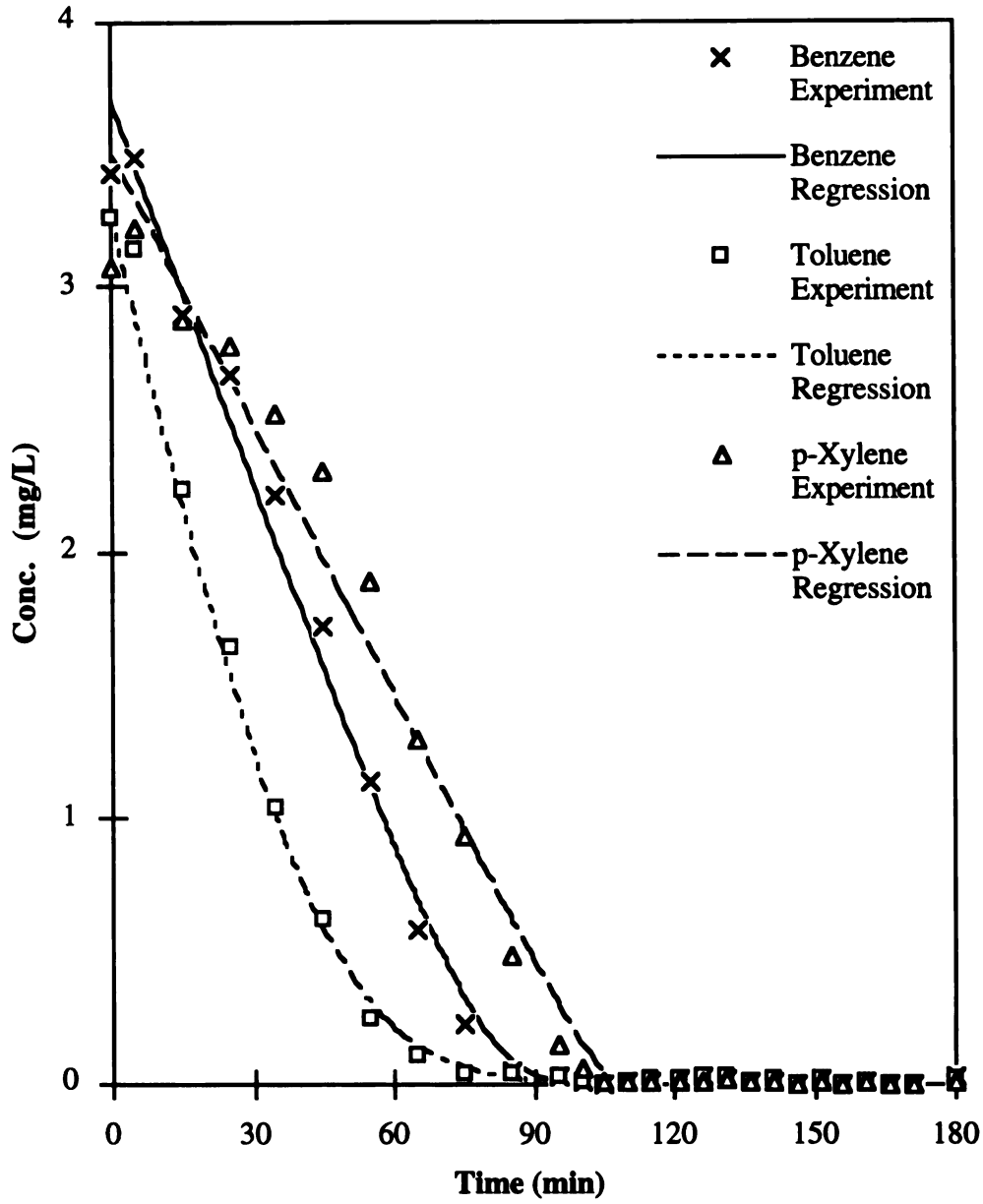

Figure 7-9. Typical biodegradation patterns of benzene, toluene and *p*-xylene as multiple substrates in a mixed culture; (estimation results for benzene: k=1.15 1/day; K_s=0.24 mg/L; R^2=0.992; n=11; estimation results for toluene: k=2.43 1/day; K_s=1.33 mg/L; R^2=0.987; n=12; estimation results for *p*-xylene: k=0.92 1/day; K_s=0.05 mg/L; R^2=0.982; n=14).

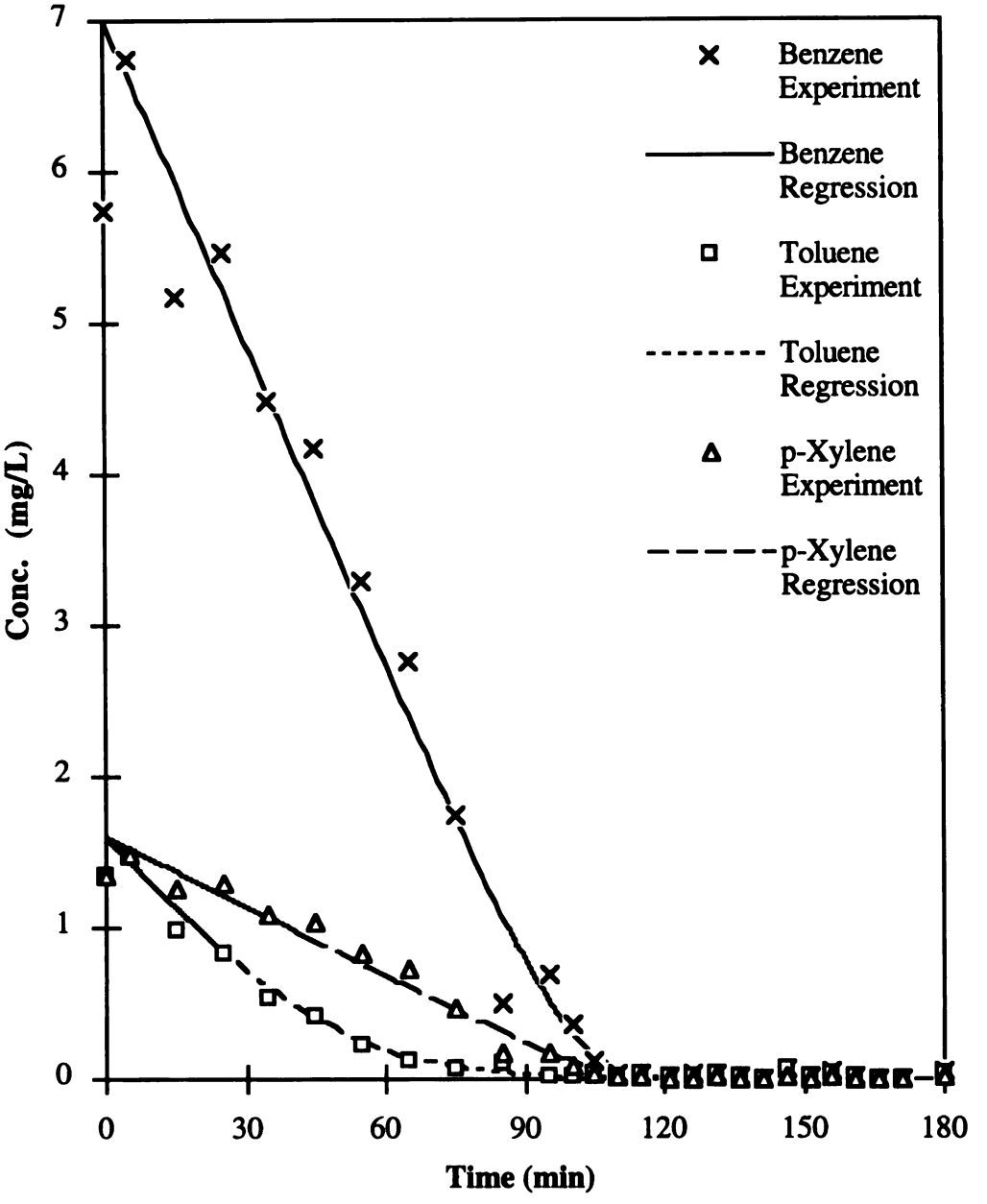

Figure 7-10. Biodegradation patterns of benzene, toluene and *p*-xylene as multiple substrates with a high initial benzene concentration in a mixed culture; (estimation results for benzene: k=1.67 1/day; K_s=0.37 mg/L; R^2=0.986; n=16; estimation results for toluene: k=1.08 1/day; K_s=0.72 mg/L; R^2=0.978; n=11; estimation results for *p*-xylene: k=0.36 1/day; K_s=0.05 mg/L; R^2=0.985; n=15).

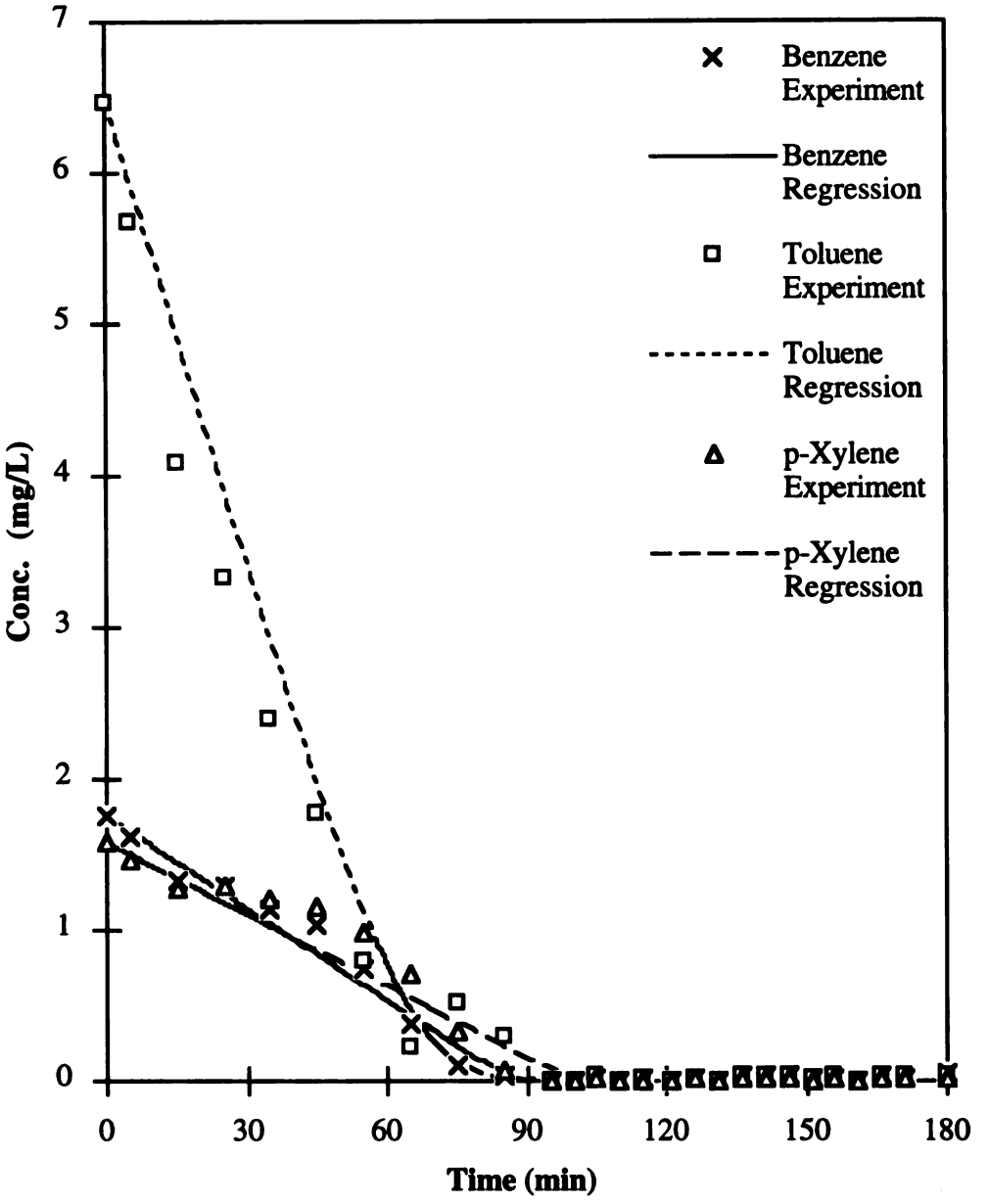

Figure 7-11. Biodegradation patterns of benzene, toluene and *p*-xylene as multiple substrates with a high initial toluene concentration in a mixed culture; (estimation results for benzene: k=0.47 1/day; K_s=0.02 mg/L; R^2=0.976; n=11; estimation results for toluene: k=2.57 1/day; K_s=0.69 mg/L; R^2=0.949; n=12; estimation results for *p*-xylene: k=0.36 1/day; K_s=0.01 mg/L; R^2=0.971; n=12).

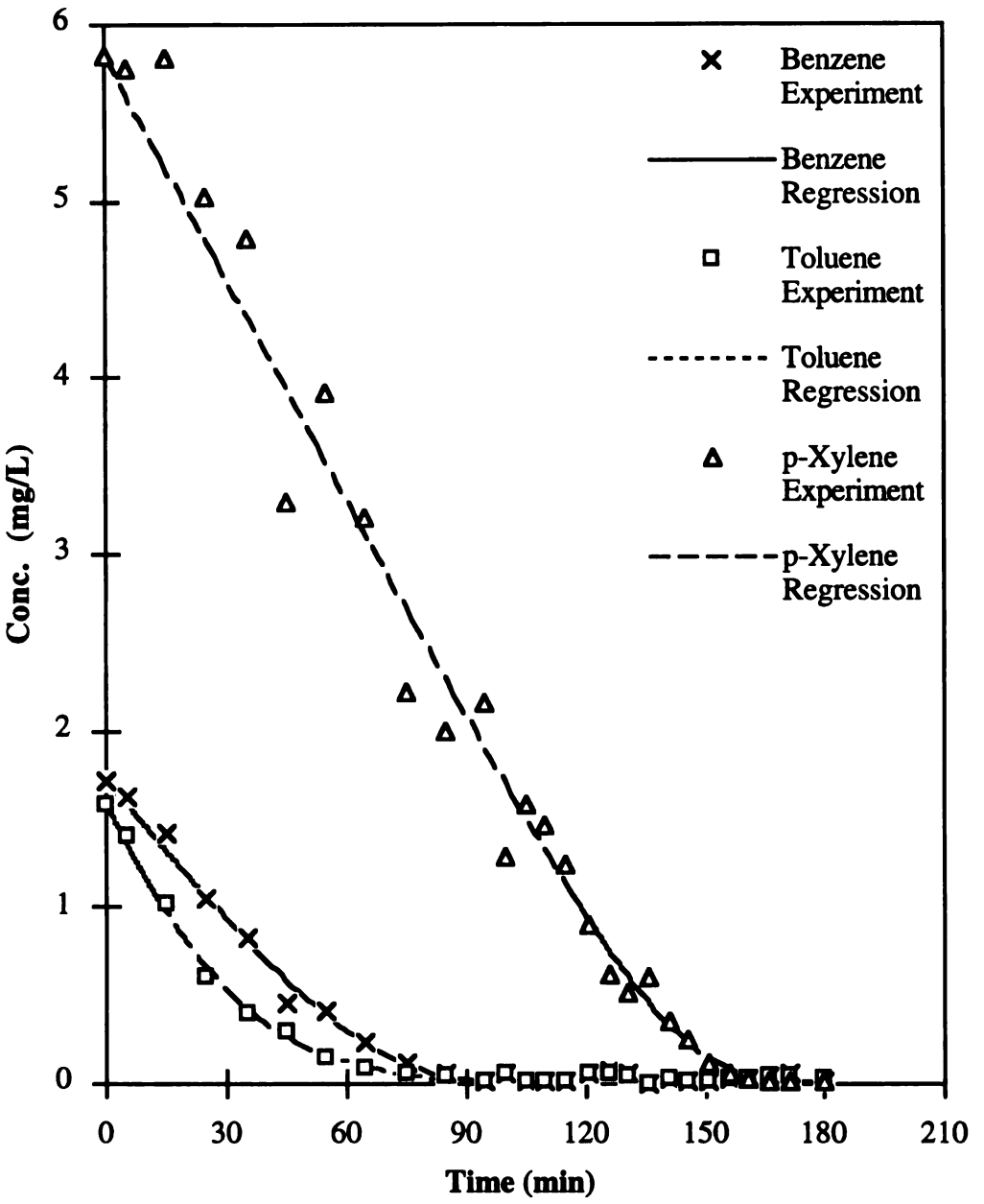

Figure 7-12. Biodegradation patterns of benzene, toluene and *p*-xylene as multiple substrates with a high initial *p*-xylene concentration in a mixed culture; (estimation results for benzene: k=0.73 1/day; K_s=0.31 mg/L; R^2=0.994; n=11; estimation results for toluene: k=2.27 1/day; K_s=1.84 mg/L; R^2=0.995; n=11; estimation results for *p*-xylene: k=1.00 1/day; K_s=0.35 mg/L; R^2=0.986; n=19).

7.4 Discussion

The net yield coefficient for each BTX compound is about 0.36 mg VSS/mg COD, which is a typical value for aerobic microorganisms. Each of the BTX contaminants could be degraded by the organisms as a sole substrate. When both benzene and toluene were present in the system, the net yield coefficient decreased to 0.22 mg VSS/mg COD. When all three substrates were available to the microorganisms, the coefficient decreased again to 0.17 mg VSS/mg COD. Chang (1993) reported cometabolism of BTX by two *Pseudomonas* isolates isolated from the same FBR system used in this study. He documented a similar yield coefficient value but a much larger value of decay coefficient for toluene by a *Pseudomonas* strain B1 which could grow on benzene or toluene. He also found an increase in decay rate with an increase in xylene concentration, while the xylene was cometabolized by strain B1. Therefore, a decrease in the net yield coefficient was expected in the multiple substrate system.

Estimation of kinetic parameters is delicate work due to the significant variation which can be associated with the procedure. The relative standard deviation in k for the generated data sets was 11.6% based on the verification test. The degradation ability of the mixed culture for BTX was high as indicated by the high k and relatively low Ks. In the verification test, the relative standard deviation of Ks was 35.9% for the sets of generated data which had a relative standard deviation of 6%. Therefore, the comparison of the k values at different conditions was possible, but it was very difficult to draw any conclusions regarding a trend in the Ks under those conditions.

There are several reasons for the variation in estimation results. Because of the low value of Ks, there are a limited number of points which occur during the transient period of the degradation curve. This transient phase is located between the end of the zero-order degradation period and the point at zero substrate concentration. Therefore, an accurate estimate of Ks was very difficult. A second reason for variation is due to

collection of biomass from the FBR. Because the biofilm thickness was approximately 0.1 mm, it is possible that different activity levels and even different populations of microorganisms occur at different depths within the biofilm. At the time of measurement, because the FBR was operated without recycle which allowed a significant substrate concentration gradient to develop along the height of the reactor, the activity was found to decrease with an increase in reactor height. When each of the kinetic assays was conducted, the biofilm coated particles were collected from the same position in the reactor and the biomass was stripped from the carrier particle. However, this did not guarantee that the biomass obtained had the same activity.

The mixed culture in the fluidized bed reactor can degrade BTX. The rates, however, were different for the individual substrates and affected by the presence of the other substrates. Using a single substrate kinetic test allowed the potential ability of the community to degrade each substrate to be found. This community had the highest maximum specific utilization rate for toluene, followed by benzene, and finally by xylene, which was the lowest.

When multiple substrates were present in the system, inhibited degradation of some substrates occurred. The xylene degradation rates were decreased by the presence of benzene and toluene. However, the benzene degradation rates were decreased by toluene while remaining virtually unaffected by the presence of xylene. The presence of benzene and xylene did not effect the toluene degradation rates. The maximum specific utilization rates were also effected by the initial concentrations of substrate. When the initial concentrations of benzene were three times higher than the other substrate concentrations (Table 7-9), the value of k for benzene was higher and the values of k for toluene and xylene were lower than the values for equal concentration cases. A similar phenomena occurred for high concentrations of toluene. When the concentration of xylene was higher, the k for xylene increased and the k for benzene decreased, while the

k value for toluene remained unchanged. Even though the maximum specific utilization rates were changing based on initial concentrations, all of the substrates were utilized simultaneously. Therefore, sequential utilization was not observed.

Kinetic parameters for the degradation of BTX have been estimated by several researchers. There are tremendous differences in the values reported for BTX degradation. Comparison of those results is difficult and not very useful, because many different microorganisms are able to degrade BTX and the rates are affected by environmental conditions. The community structure in the FBR is very complicated. Chang (1993) isolated five different strains with different degradation characteristics from the same FBR. Those strains were not necessarily the dominant groups in the community. Inhibition of the substrates was observed, however, due to the complex nature of the community and the large variance in the estimation of Ks, the type of inhibition was not determined.

Large variation in kinetic parameters was found in this study. This fact should not limit use of the results. Because of the continuous change in activity and population of the microorganisms in the bioreactor, the variation in substrate utilization rates are expected. Engineers will need to consider such uncertainty in their designs (such as increase the height of reactor).

7.5 Conclusions

The yield and decay coefficients for a mixed culture treating BTX were evaluated and the kinetic parameters for degradation of BTX were determined. The degradation rate for toluene was the highest, while the rate for xylene was the lowest. There was inhibition between all three substrates but the type of the inhibition was not determined. The effect of inhibition on toluene degradation was limited. A large variance during estimation of

Ks was found and may be due to its low value for this mixed culture. The parameters, however, are useful for bioreactor design with a consideration of uncertainty.

CHAPTER 8

ADSORPTION CAPACITY OF BIOFILM COATED ACTIVATED CARBON IN A BIOLOGICAL FLUIDIZED BED REACTOR SYSTEM

8.1 Introduction

Biological granular activated carbon fluidized bed reactors (GAC-FBR) have been shown to have the ability to remove the gasoline contamination from groundwater and provide both the efficiency of biological removal and the positive effluent protection capability of activated carbon adsorption (Hickey *et al.* 1990b; 1991a). In the previous chapters, the role of the adsorption in a GAC-FBR was discussed. The adsorption capacity was found to be a very important factor during transient conditions. However, the change in adsorptive capacity during operation of the GAC-FBR has not been fully explored.

The adsorptive capacity of GAC can decrease with increased usage in BAC systems. This reduction could be due to adsorption of some non-degradable compounds from the influent but may also be caused by microorganism growth on the surface of the carbon. Suidan *et al.* (1983) reported that the adsorption capacity of GAC decreased after the carbon was exposed to a phenol mixture, which contained some compounds resistant to biodegradation, in an anaerobic expanded-bed reactor. The capacity was, however, slightly regained after a period of bioregeneration. Zhang *et al.* (1991) found that a similar phenomena occurred in an aerobic BAC filter used to treat phenol. They did not, however, investigate the effect bioregeneration had on the reactor. Weber and Ying (1978b) asserted that breakthrough of toluene sulfate in an expanded-bed GAC adsorber with a biofilm

grown on sucrose was earlier than an adsorber without the biocoat. When Schultz and Keinath (1984) conducted a study of powdered activated carbon treatment (PACT) process mechanisms in an activated sludge system, they found that the biomass in the PACT culture impedes the rate of mass transfer of phenol to the PAC surface. Olmstead (1989) studied the effect of microbial interference on GAC adsorption. In his experiment, a column containing GAC particles was fed glucose for 1 -2 weeks. After the biofilm developed, the carbon was removed from the column in sections to obtain the biocoated GAC with different amount of biomass. Because of the plug-flow pattern in the column, more bioactivity and biomass were expected at the influent end of the column. He found that the multiplier term in the Freundlich isotherm equation, K_f, for trichloroethylene (TCE) decreased with increasing biomass on the carbon while the slope term of the equation, n, remained constant. The adsorption of *para*-toluene sulfate (PTS), which has a much lower K_{ow} than TCE, did not decrease significantly. The author failed to determined the effect of a sample protein (BAS) had on the adsorption of PTS, but, discovered that preloading soluble microbial products (SMP) on the GAC reduced the amount of PTS adsorbed.

In this chapter, the adsorption capacity of biofilm coated activated carbon from a biological fluidized bed reactor which treated toluene contaminated water was determined periodically over an extended period of operation.

8.2 Materials and Methods

8.2.1 Media and Chemicals

Granular activated carbon (GAC Calgon Filtrasorb 400, Calgon Co., Pittsburgh, PA) was used as the carrier media in the GAC-FBR. The media were sieved to obtain a 18 x 20 mesh fraction (average particle diameter 0.90 mm). Prior to addition to the reactors, the GAC was rinsed with distilled water to remove fines and dried at 100 ^{o}C for 24 hours.

All chemicals were obtained from J.T. Baker Chemical Co., Phillipsburg, NJ and were reagent grade. The water used to feed the reactor was from an industrial grade soft water system which produces water with a hardness of 103 mg $CaCO_3$/L.

8.2.2 Fluidized Bed Reactors

Pilot-scale fluidized bed reactor was constructed using a 7.6 cm diameter by 322 cm high polyvinyl acetate (PVC) column to produce a system with a 14.7-L working volume (Figure 8-1). The reactor was charged with activated carbon and operated as single-pass systems with no recycle. The reactor were inoculated with a mixed culture obtained from a laboratory-scale FBR that was supplied with toluene as the sole carbon source. Dissolved oxygen (DO) was supplied to the reactors by oxygenating the reactor feed water with pure oxygen to a concentration sufficient to maintain an effluent DO exceeding 4.0 mg/L. Nitrogen and phosphorus were supplemented at a weight ratio of 100/5/1:COD/N/P. Toluene was injected into the feed water using a syringe pump (Harvard Apparatus 22). A mixing tank (15 liters) and a recycle loop around the mixing tank were installed in the second run of this study to improve dissolution of the pure toluene in the influent. This toluene contaminated groundwater was then pumped to the bottom of the reactor using peristaltic pumps (Watson-Marrow 601S). The bed height was controlled by a peristaltic pump installed on the top of the carbon bed to shear some biomass from the carrier medium. The reactor was charged with 2850 g of media initially to produce a settled bed height of 156 cm. Toluene contaminated water was fed at a flow rate of 2.2 L/min resulting in a hydraulic flux rate of 0.48 m/min, an empty bed hydraulic retention time of 6.7 min, and an organic loading rate of about 1.4 kg COD/m^3-day. The temperature of the feed water was 20±1 °C and the pH was about 8.

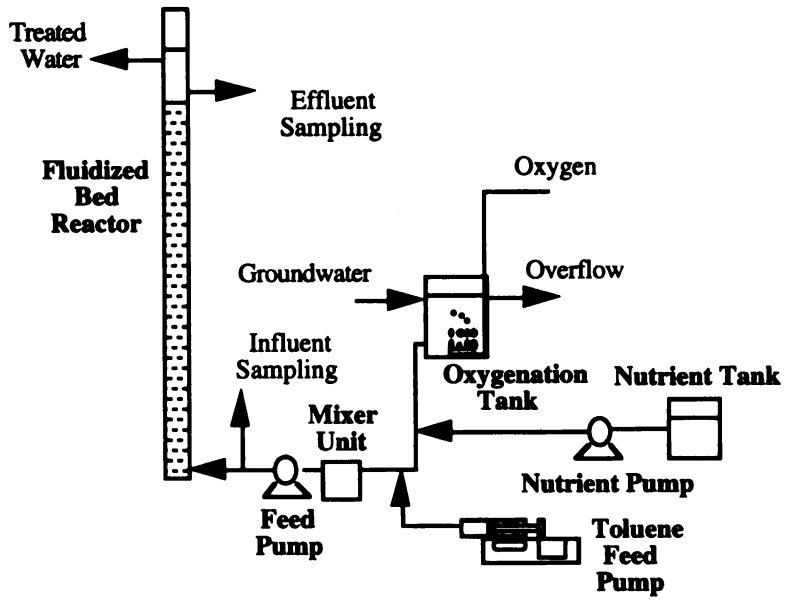

Figure 8-1. Schematic of the experimental GAC-FBR system.

8.2.3 Collection and Sterilization of Biofilm Coated Activated Carbon

Carbon samples were collected from two points in the reactor (50 cm and 150 cm from the bottom of the reactor) using a sampling bottle (50 mL). The bottle was filled with water, closed with a rubber stopper and lowered into the reactor to the desired height. The stopper was then removed and the carbon allowed to fill in the bottle. After one minute fill, the bottle was removed from the reactor. The carbon was transferred to 50 mL serum bottles filled with the effluent water (headspace free) and sealed with Teflon-coated septa and aluminum seals.

The carbon and water filled bottles were sterilized by gamma radiation in a cobalt-60 irradiator located at the Phoenix Memorial Laboratory (Ann Arbor, Michigan). The samples were sterilized for one hour at a dose rate of 2 Mrad/hr. The effect of the

sterilization procedure was tested by plating the sterilized carbon samples on nutrient agar. There was no colony growth after two weeks incubation at 25 °C.

There are two reasons to consider that the sterilization procedure may change the adsorption characteristics of the GAC. The first possibility is that gamma radiation may change the carbon molecular structure and adsorption characteristics. The second possibility is that the radiation may result in biomass alteration, creating adsorbable substances which may reduce the adsorption capacity. Therefore, two tests were conducted to investigate the effect of sterilization on the adsorption capacity of the GAC. In the first test, two clean GAC samples, one with and one without sterilization, were used for the isotherm studies. The results indicate that sterilization does not effect the adsorption capacity of the GAC. In the second test, a clean GAC sample (1 g) was mixed with 20 mL of 500 mg VSS/L fresh biomass to create a similar concentration of biomass on the biofilm coated carbon. The mixture was sterilized by gamma radiation. After a 48 hour contact time, this mixture was used to perform an isotherm study along with a clean GAC sample for comparison purpose. The results are reported in Table 8-1 and show that the biomass addition does not significantly effect the adsorption capacity of the clear GAC.

8.2.4 Measurement of Biofilm Thickness

Carbon particles were photographed after the sterilization using a stereo microscope (Olympus SZH) equipped with a camera system (Olympus AD Exposure Control Unit). Because the biofilm color (light yellow) was significantly different from the color of the carbon (black), the biofilm thickness could be directly measured from the photograph. The thickness of biofilm on the carbon was often not uniform, therefore, three particles were selected randomly and five measurements were taken from each particle at equally distributed locations. An average value was then calculated.

Table 8-1. Effect of biomass and base washing on isotherm parameters

Day	Carbon Description	Data Point	Isotherm Parameters			Biofilm Thickness (μm)
			q_o (mg/g)	K_f (mg/g)	1/n	
Effect of Biomass						
-	Clean GAC	10	-	71.9±3.4‡	0.39±0.02	-
-	Clean GAC with Biomass	10	-	78.8±5.4	0.35±0.04	-
64	Non-disturbed BAC	10	6.38	66.7±2.8	0.37±0.02	160
64	Biofilm Removed	10	5.90	62.5±0.9	0.36±0.01	76
122	Non-disturbed BAC	29	3.71	34.4±1.2	0.54±0.02	76
122	Biofilm Removed	10	3.36	37.5±0.7	0.54±0.02	9
Base Wash (0.5 M NaOH)						
374	Non-disturbed BAC	10	0.88	27.9±3.1	0.57±0.05	-
374	Washed with NaOH	10	0.85	32.2±1.7	0.47±0.03	-

‡: 95 % confident interval

8.2.5 Extraction Procedure

To measure the amount of adsorbed toluene, the carbon samples (0.03 to 0.1 g) were placed in aluminum dishes, separated from the liquid using adsorbent tissue and transferred to 15 mL glass vials. Fifteen mL of methanol was added to each vial as the extraction solvent, then, the vials were capped with Teflon-faced septa and aluminum seals and tumbled at 6 rpm for 3 to 4 days. Analysis involved transferring 0.1 mL of the extract to a 20 mL headspace vial containing 5 mL D.I. water and 0.12 mL 6 M HCl, sealing with Teflon-faced septa, and analyzing by headspace gas chromatography. Three successive extractions were performed. The mass of carbon was determined by drying at 90 °C overnight, desiccating, and weighing on an analytical balance. The total mass of adsorbed toluene was calculated based on the summary of three extractions. The efficiency of the extraction procedure is higher than 90% (Shi *et al.* 1994).

8.2.6 Adsorption Isotherm Assay

The isotherm solution was prepared by adding calcium carbonate (5 mg as Ca/L), magnesium carbonate (0.6 mg as Mg/L), and sodium bicarbonate (15 mg/L) to D.I. water and adjusting to pH 8.0 by adding sodium hydroxide. The toluene solution was prepared by dissolving toluene in the isotherm solution followed by filtration (0.22 μm filter, Whatman PolyCap 75 AS) for sterilization. 160 mL serum bottles were then filled, capped and stored at 4 °C until use.

Isotherms for toluene were performed using serum bottles (160 mL) sealed with Teflon-coated septa. The serum bottles contained the desired volume of isotherm solution and biofilm coated activated carbon (60 mg) and were then filled, without leaving a head space, with toluene solution to achieve a desired concentration of toluene. Ten to fifteen

bottles, each with different initial concentrations between 10 and 70 mg/L, were prepared for each assay. The bottles were tumbled at 6 rpm and 20 °C for 7 days to ensure that equilibrium was obtained. The mass of carbon was determined by drying at 90 °C overnight, desiccating, and weighing on an analytical balance. After equilibration, five milliliter liquid samples were transferred to headspace vials, sealed with Teflon-coated septa, and analyzed by headspace gas chromatography to determine the residual amount of toluene. The solid-phase concentration was calculated by difference. An isotherm assay was also performed with new activated carbon. All of the serum bottles and septa were sterilized by autoclave.

To evaluate the equilibration time, four isotherm studies were performed using different equilibration times (4, 14, 21, and 28 days, respectively). Each isotherm curve contained five points and the equilibrium concentrations in the water ranged from 0.5 mg/L to 10 mg/L. There were no significant differences in the adsorption results, therefore, seven days was chosen as a convenient time to allow equilibrium to be occurred.

In order to study the effect the amount of biomass on the carbon adsorption, the biofilm on a carbon sample collected on day 122 was removed prior to sterilization by shaking in the presence of a plastic tube to knock the biofilm free. The carbon was then used in an isotherm study. The same procedure was performed on a carbon sample collected on day 64, and the results were compared to those found previously without biofilm removal.

8.2.7 Sodium Hydroxide Wash

The protein and other biological products can be digested and dissolved by a base wash. To investigate the possibility of the base wash for the recovery of the adsorption capacity, a carbon sample collected on day 374 was digested with 0.5 M sodium hydroxide

(NaOH) at 20 °C for 24 hours and washed with 0.5 M NaOH three times. Then, the carbon rinsed with isotherm solution until the pH of the leachate reached 8. An isotherm experiment was performed on this carbon and the adsorption parameters were estimated.

8.2.8 Estimation of Adsorption Parameters

The extended Freudlich isotherm equation was used to estimate the adsorption characteristics of the carbon. This relationship follows.

$$q_e + q_o = K_f C_e^{\frac{1}{n}} \qquad (8\text{-}1)$$

where: q_e is the additional amount of toluene adsorbed (mg/g).

q_o is the initial amount of adsorbed toluene (mg/g).

K_f is a constant.

C_e is the equilibrium concentration of toluene in water (mg/L).

1/n is a constant.

The initial amount of adsorbed toluene was measured by the extraction procedure described previously. The isotherm data was fit to the equation using a non-linear regression computer software program (Solver, Excel 4.0, Microsoft Inc.) and both parameters were estimated simultaneously. The 95% confidence intervals for each parameter were also determined (Beck and Arnold 1977).

8.3 Results

8.3.1 Fluidized Bed Performance and Collection of Carbon Samples

The fluidized bed reactor was operated in two phases. The first phase lasted for 406 days. The long term effect of operation time on the adsorption capacity of biocoated GAC

was monitored and the recovery of capacity after stop of toluene feed was investigated. The second phase lasted for 77 days and the short term changes of the adsorption capacity were determined.

The influent and effluent toluene concentrations and the DO consumption in the first phase are shown in Figure 8-2. The average influent and effluent concentrations of toluene were 1.71 mg/L and 0.10 mg/L, respectively. The average toluene removal rate was 94%. The first carbon sample was collected at day 94 and collected every 30 days thereafter until day 202 while toluene feed was stopped. In order to understand the capacity recovery potential, toluene feed was stopped and the nutrient supplies were reduced by half at day 202. To investigate the effect of supplied nutrient levels on the recovery of the adsorption capacity, nutrients stopped being supplied at day 259 and resumed on day 304. Carbon samples were collected on days 255, 304, and 374. On day 375, the toluene feed was resumed to allow new biomass growth for the start of the second run. All of the carbon samples were collected from mid-depth of the carbon bed (150 cm).

The FBR was operated for 77 days for the second phase. A modification of the influent mixing unit produced a more stable and higher influent toluene concentration than that in the first phase. The influent and effluent toluene concentration and the DO consumption are shown in Figure 8-3. The average influent and effluent toluene concentrations were 2.73 mg/L and 0.08 mg/L, respectively. The average toluene removal rate was 97%. Significant DO consumption started on day 5 and the carbon bed height reached a stable level at day 50. The carbon samples were collected from the start of the second run. In the first week, the carbon samples were collected twice a week, then collection was reduced to once a week for the next four weeks. For the final six weeks, carbon was sampled every two weeks. The carbon samples were collected from the bottom portion of FBR at 50 cm and the middle portion of the FBR at 150 cm.

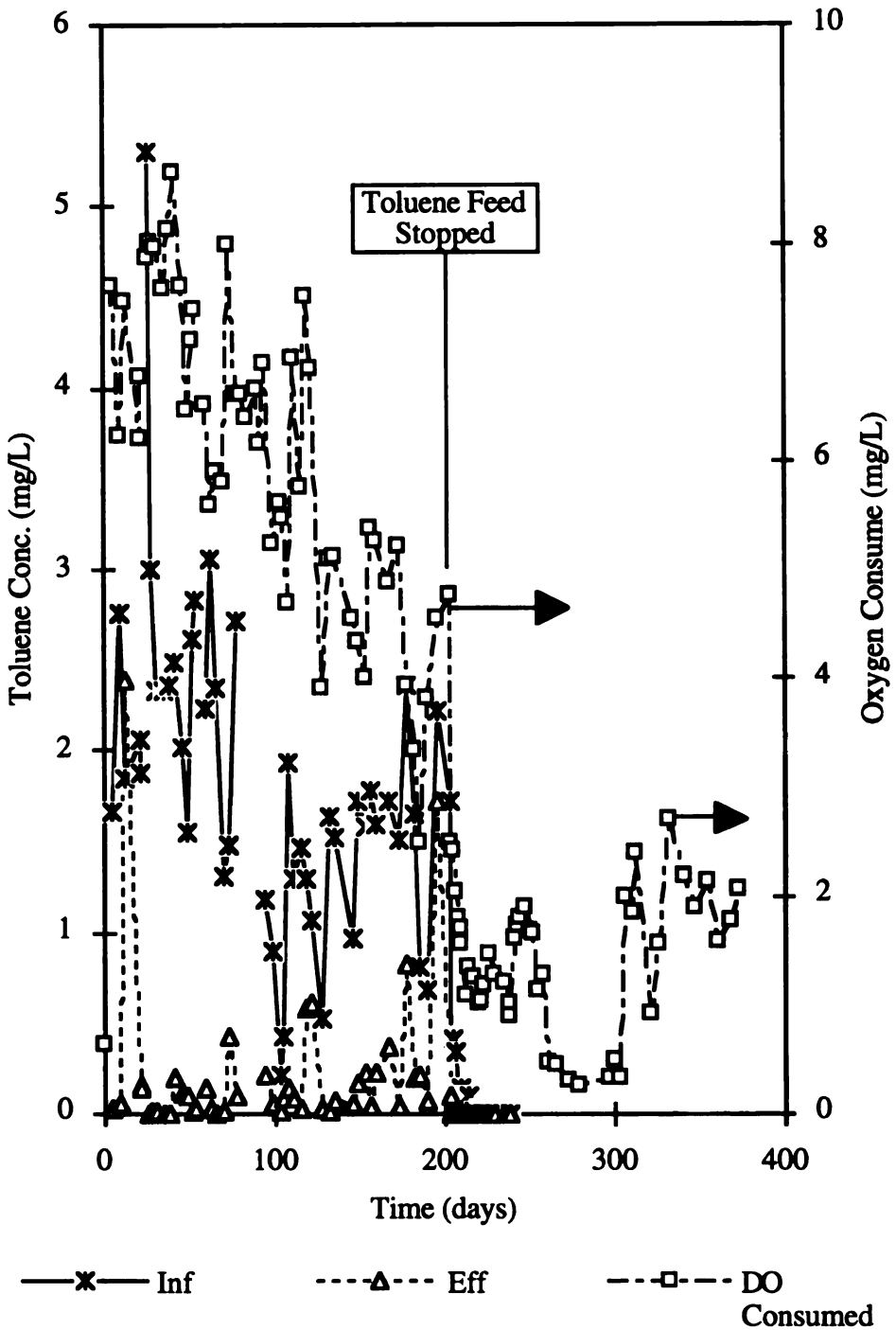

Figure 8-2. Influent and effluent toluene concentrations and DO consumption in a GAC-FBR during the first phase.

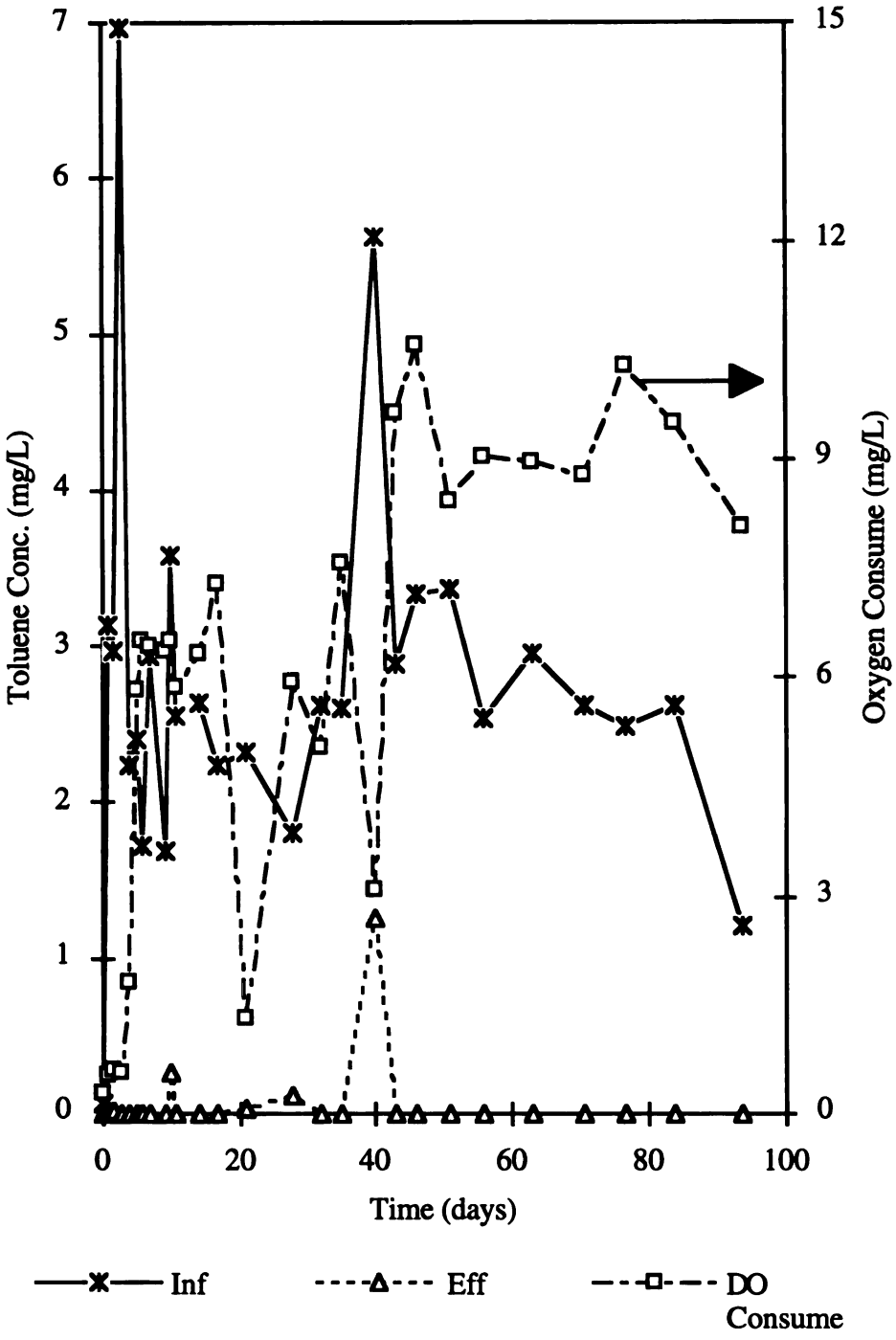

Figure 8-3. Influent and effluent toluene concentrations and DO consumption in a GAC-FBR during the second phase.

8.3.2 Extraction of Adsorbed Toluene on Carbon

The adsorbed toluene concentration reached its highest value at day 4 in the bottom of the reactor followed by a sharp decrease after day 7 (Figure 8-4). Significant DO consumption started on day 5 which indicated that biodegradation became significant. On day 27, the adsorbed toluene concentration at the bottom of the FBR reached a level similar to that on carbon in the middle of the reactor. The adsorbed toluene concentration reached its highest value on day 21 in the middle of the reactor and decreased gradually thereafter (Table 8-2 and Figure 8-4). Discontinuation of toluene feed did not increase the desorption rate.

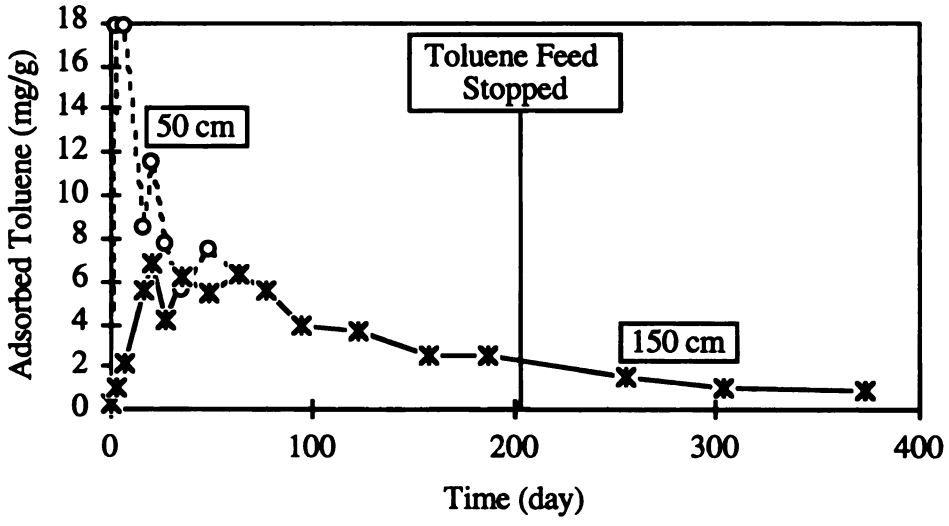

Figure 8-4. Adsorbed toluene on biocoated GAC in the middle (150 cm) and bottom portion of the GAC-FBR

Table 8-2. Adsorption isotherm parameters for GAC collected from mid-height in a GAC-FBR (150 cm)

Day Sampled	Data Points	Isotherm Parameters			Biofilm Thickness (μm)
		q_o (mg/g)	K_f (mg/g)	1/n	
Clean GAC	70	-	79.3±3.3‡	0.37±0.03	-
The Second Phase					
0	10	0.30	79.3±5.9	0.43±0.06	ND
4	10	1.11	71.5±1.4	0.38±0.02	4
7	10	2.13	69.6±1.4	0.37±0.02	5
17	20	5.61	62.6±1.7	0.44±0.01	54
21	20	6.87	61.0±1.1	0.42±0.03	52
28	10	4.16	67.5±1.7	0.44±0.01	25
35	20	6.22	64.5±3.0	0.35±0.04	21
49	10	5.48	56.7±3.9	0.43±0.04	148
64	10	6.38	66.7±2.8	0.37±0.02	160
77	10	5.58	46.3±1.2	0.42±0.02	76
The First Phase					
94	42	4.01	40.9±2.5	0.48±0.02	31
122	29	3.71	34.4±1.2	0.54±0.02	76
158	44	2.56	35.7±0.9	0.51±0.01	42
187	20	2.54	37.7±1.2	0.51±0.02	23
203	Stop Toluene Feed				
255	15	1.52	34.4±1.5	0.46±0.03	12
304	16	1.02	32.5±1.1	0.49±0.02	ND
374	10	0.88	27.9±3.1	0.57±0.05	ND

‡: 95 % confident interval

ND: non-detectable

8.3.3 Adsorption Capacity

The isotherm parameters are presented in Table 8-2 and Figure 8-5. Adsorption capacities throughout the experiment were calculated by comparing to the capacity at day 0. The K_f value decreased slowly in the first 60 days and quickly in the following 30 days to a low level. The K_f value continued to decrease after the toluene feed stopped. Values of 1/n increased slightly throughout the experimental period. Because of the non-linear relationship between the equilibrium concentration and the adsorption capacity, the capacities were also evaluated at three hypothetical concentrations of toluene (0.1, 3, and 10 mg/L to represent the concentrations at effluent, influent, and a high influent shock conditions, respectively) and the results are plotted in Figure 8-6. The adsorption capacities remained between 70% to 100% of the initial value for all three concentrations in the first 60 days and dropped to 40%, 50%, and 55% for 0.1, 3, and 10 mg/L, respectively, at later times. The adsorption capacities decreased slightly and stopping the toluene feed did not increase the capacities. The isotherm parameters for the carbon collected from the bottom of the reactor were also estimated (Table 8-3) and found to be similar to those corresponding to carbon in the middle portion of the reactor.

Table 8-3. Adsorption isotherm parameters for GAC collected from the bottom portion of a GAC-FBR (50 cm)

Day Sampled	Data Points	Isotherm Parameters			Biofilm Thickness (μm)
		q_o (mg/g)	K_f (mg/g)	1/n	
0	10	0.20	77.8±2.9‡	0.39±0.03	ND
4	10	17.87	77.0±1.6	0.36±0.02	4
7	10	17.84	76.3±1.5	0.30±0.02	12
17	20	8.45	65.1±1.7	0.39±0.01	6
21	20	11.54	63.9±0.5	0.37±0.01	16
49	10	7.45	47.6±3.6	0.52±0.04	119

‡: 95 % confident interval

ND: non-detectable

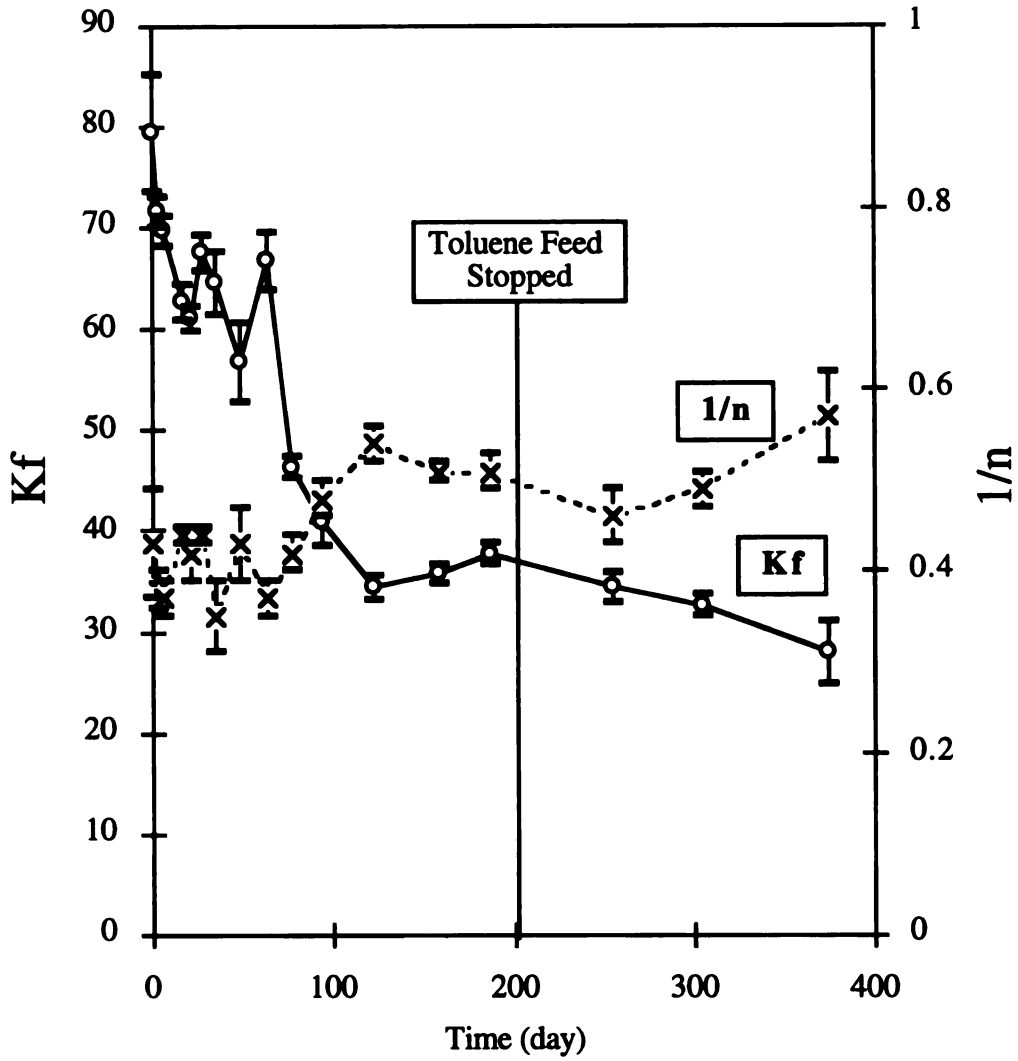

Figure 8-5. Isotherm parameters for biocoated GAC.

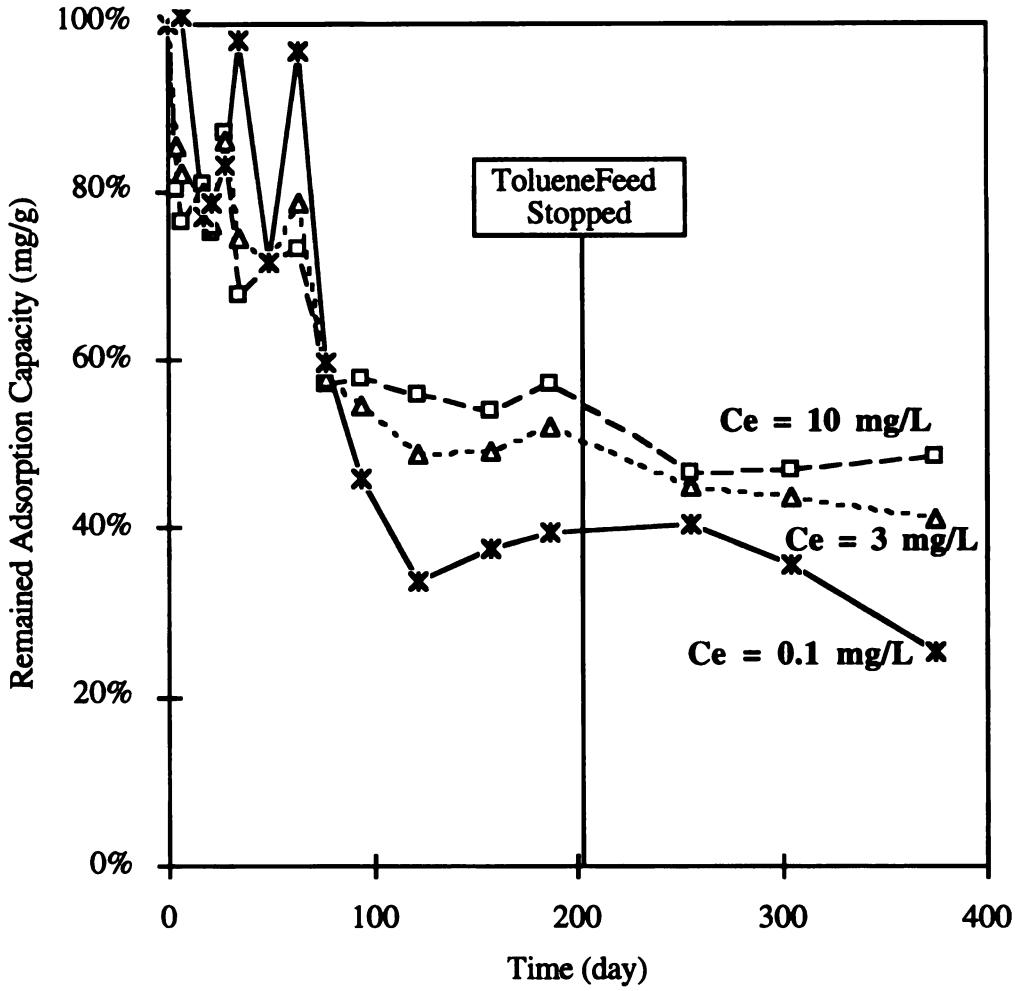

Figure 8-6. Remaining capacity at three hypothetical equilibrium concentrations (0.1, 3, and 10 mg/L representing the concentrations at effluent, influent, and a high influent shock load, respectively.).

8.3.4 Effect of Biomass on Adsorption

The biofilm thicknesses on the carbon varied during this experiment. No consistent relationship was found between adsorption capacity and biofilm thickness. After toluene feed stopped, the reactor operated in a decay mode , therefore, the biofilm was decomposed and was reduced to non-detectable levels. The adsorption capacity, however, did not increase.

To investigate the effect of biofilm thickness on adsorption, the biofilm on two carbon samples (day 64 and day 122) were removed before sterilization and the carbon was used in the adsorption isotherm experiment. The average biofilm thicknesses were reduced from 160 to 76 μm and 76 to 9 μm for the day 64 and day 122 samples, respectively. The isotherm parameters are similar to those for the non-disturbed carbon (Table 8-1 and Figure 8-7).

Clean GAC was mixed with biomass before sterilization and contacted with the biomass for 48 hours. An adsorption isotherm was conducted using this carbon. The biomass addition did not effect the isotherm results (Table 8-1 and Figure 8-8).

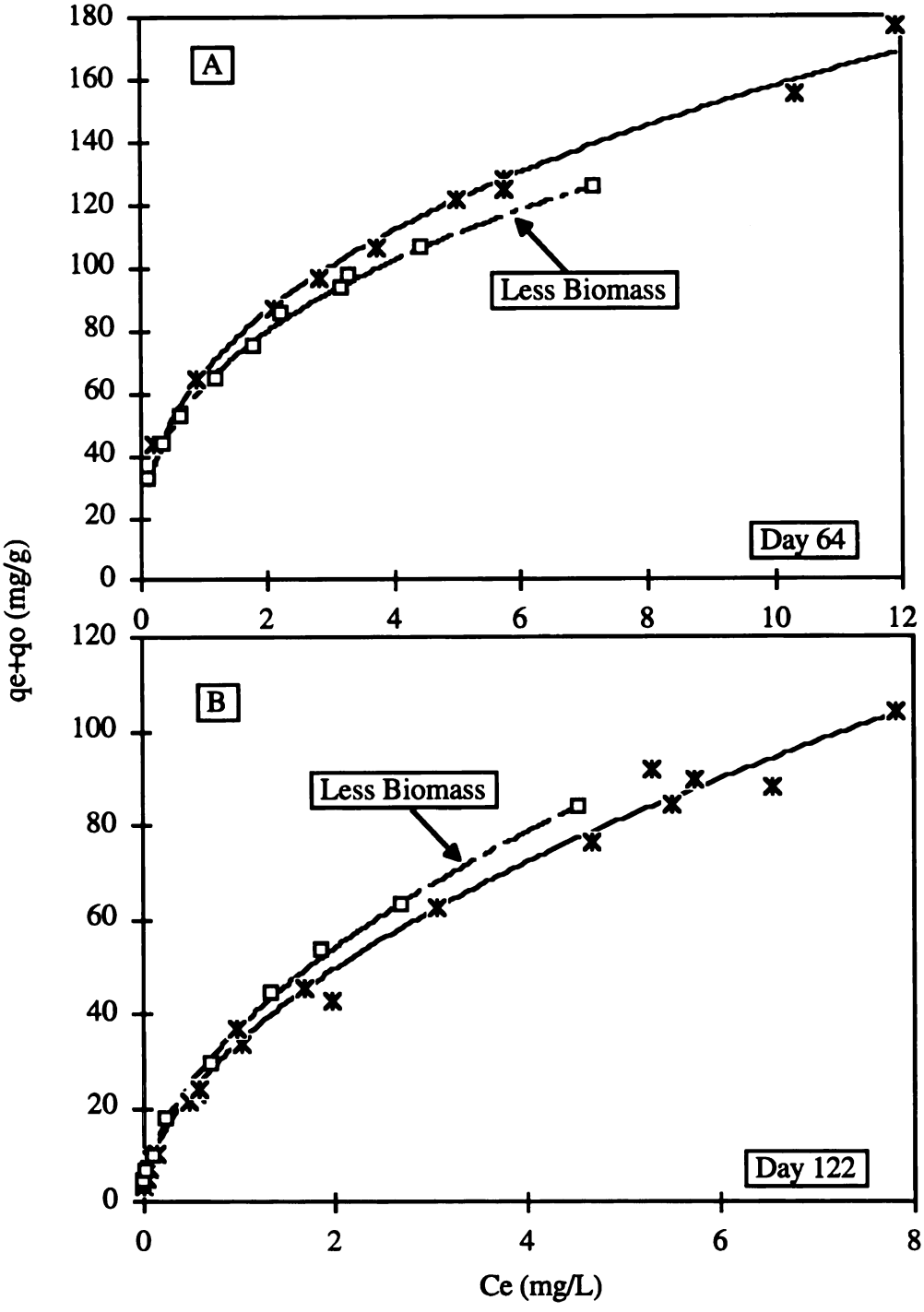

Figure 8-7. Adsorption isotherms for carbon with and without biomass removal.
(A: carbon collected at day 64, biofilm thicknesses were 160 and 76 mm for non-disturbed carbon and biomass removed carbon, respectively;
B: carbon collected at day 122, biofilm thicknesses were 76 and 9 mm for non-disturbed carbon and biomass removed carbon, respectively.)

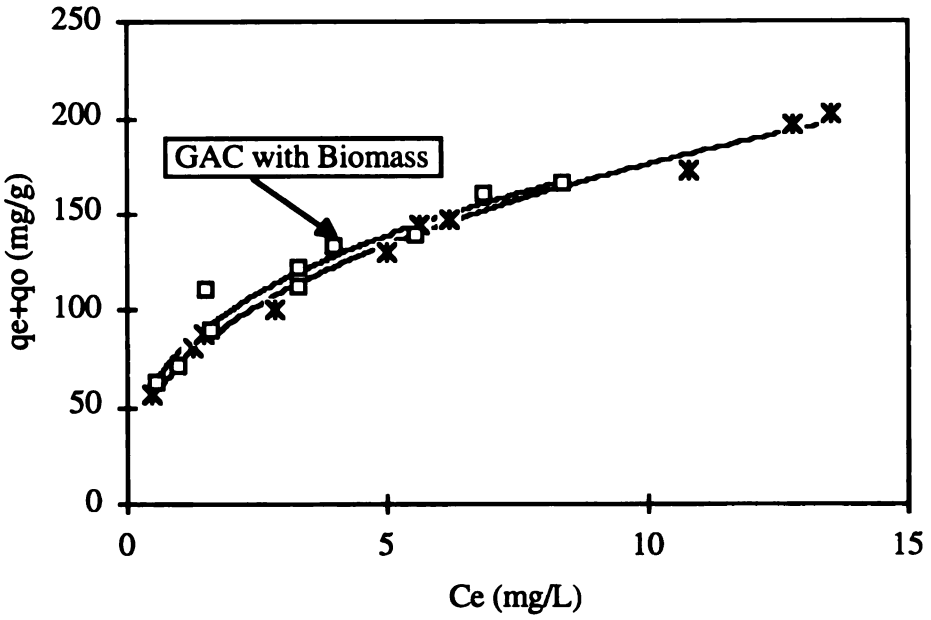

Figure 8-8. Adsorption isotherm for GAC with and without contact with biomass.

8.3.5 Sodium Hydroxide Wash

A carbon sample collected at day 374 was digested and washed with 0.5 M sodium hydroxide (NaOH). Some unknown brown substance was observed in the solution. The adsorption pattern did not change significantly although the K_f increased slightly (Table 8-1 and Figure 8-9).

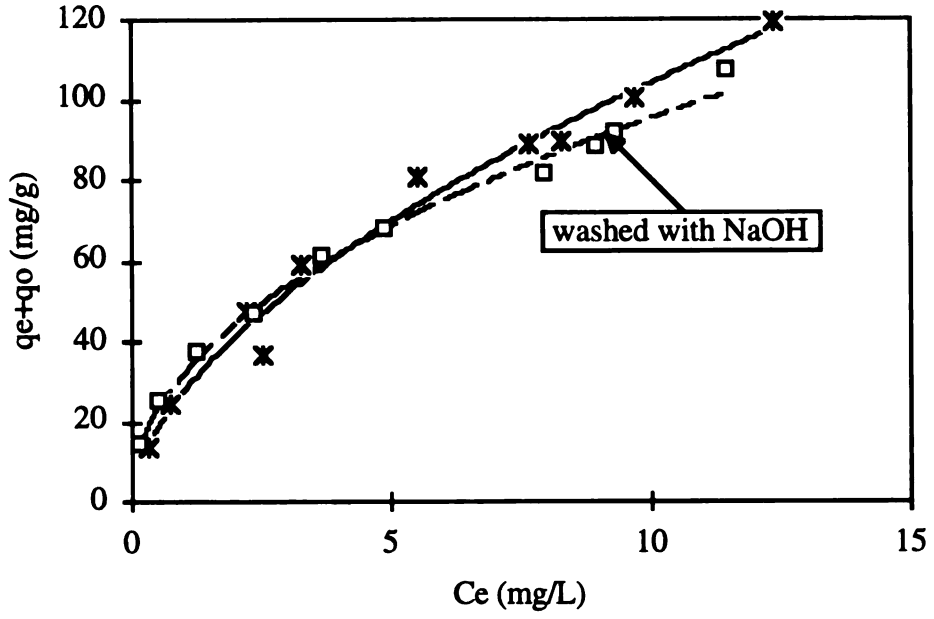

Figure 8-9. The effect of NaOH wash on the adsorption isotherm

8.4 Discussion

Adsorption is an important removal mechanism in biological activated carbon fluidized bed reactors. In the previous chapters, we have demonstrated that adsorption reduced the concentrations of target compounds and byproducts in the effluent during the transient loading condition. Adsorption capacity, however, decreased with an increase in usage of the GAC-FBR.

GAC-FBRs were operated at steady-state conditions without intentionally imposing any perturbations. The adsorbed toluene on the carbon decreased after one week of operation while DO consumption reached steady-state levels (ratio between consumed DO and COD was 0.7 to 0.8). The adsorption capacity of the biocoated carbon decreased slightly during the first two months and was maintained at greater than 70% of the initial level. The samples, which were collected from two portions of the reactor, indicate a

uniform distribution of the adsorption capacity in the GAC-FBR. After the biofilm covered the carbon particles, migration of the particles was expected in the reactor because of the change in particle density and operation of the biomass control device. This migration could increase particle mixing in the reactor, therefore, sampling carbon from any portion of the reactor could represent the remaining capacity in the reactor.

After the biomass developed fully in GAC-FBR, which took about three weeks, the adsorption capacity decreased gradually. The rate of decrease, however, was accelerated after two months of operation to reach a new low level followed by a continued gradual decrease. After six months of use, the remaining capacity was approximately 40%, 52%, and 57% of it's initial value for the equilibrium toluene concentrations of 0.1, 3, and 10 mg/L, respectively. The remaining capacity may still provide a positive response when the organic loading rate was increased.

Bioregeneration is a term used to describe the use of biodegradation to recover adsorption capacity. In this study, the adsorbed toluene on biocoated GAC decreased, however, the adsorption capacity did not recover. Therefore, bioregeneration did not occur. After toluene feed stopped, the adsorption capacity decreased continuously even though the adsorbed toluene level decreased as well. This indicates that the decrease in capacity is not due to the adsorbed toluene but rather due to biomass growth. After toluene feed stopped, a small amount of organic carbon was available in the influent and the DO consumption was continuous. The DO consumption decreased after the nutrient supply was stopped and increased immediately after the nutrient supply was resumed. The bioactivity never stopped in this reactor. It is not clear what chemicals or factors cause the decrease in adsorption capacity. However, it is known that microorganisms can produce soluble microbial products (SMP) and adsorption of SMP can reduce the adsorption capacity (Olmstead 1989). When clean GAC was mixed with biomass for 48 hours, the adsorption capacity of the GAC did not change. This indicates that if the SMP causes the reduction in capacity, the

carbon has to contact the SMP for a long period to allow a large amount of SMP to be adsorbed by the carbon. The sodium hydroxide wash did not improve the recovery. If the carbon is temporarily exhausted by toluene due to a transient loading condition, bioregeneration can occur and the capacity can be recovered to the pre-transient level.

Olmstead (1989) reported that adsorption of TCE decreased with an increase in biomass on the carbon. In this study, there is direct relationship between the amount of biomass on the carbon and the remaining adsorption capacity. For example, at days 21 and 158, the carbon had a similar biocoat, but the capacity was much higher on day 21 than on day 158 (Table 8-2). When some biomass was removed from the carbon, the adsorption isotherm did not change significantly (Figure 8-7). In Olmstead's study, the GAC particles were fed with glucose for one to two weeks and coverage of the biofilm on the GAC was not reported. During this current study, a contiguous biofilm was found using stereo microscope examination. In a previous study, the contiguous biofilm was also observed on the carbon by scanning electron microscopy (SEM) examination of sample from a similar GAC-FBR system (Voice *et al.* 1992). It is unlikely that biofilm thickness could influence the adsorption capacity.

The adsorption capacity found using an isotherm study indicates the potential of biocoated GAC to adsorb the target chemical. The rate of adsorption, however, is another very important factor which effects reactor performance during transient conditions. This issue has not been addressed in this study therefore further experimentation is required.

8.5 Conclusions

This study provides conclusive evidence of the effect of biofilm growth on the adsorption capacity of GAC. The adsorption capacity of biocoated carbon was maintained at more than 70% of initial levels during the first two months. After six months of

operation, the remaining capacity was approximately 40%, 52%, and 57% of the initial value for the equilibrium toluene concentrations of 0.1, 3, and 10 mg/L, respectively. If the adsorption removal mechanism is used to provide a buffer during the transient conditions, this remaining capacity may be sufficient without carbon replacement. The adsorbed toluene on biocoated GAC decreased continuously after the biofilm developed, however, the adsorption capacity did not recover and the bioregeneration did not occur. There is no direct relation between the amount of biomass on the carbon and the remaining adsorption capacity.

CHAPTER 9

DISSERTATION SUMMARY AND ENGINEERING DESIGN IMPLICATIONS

9.1 Summary

The objectives of this project were to investigate and exploit the advantages and limitations of biological activated carbon fluidized bed systems for treatment of gasoline contaminated groundwater. The systems were tested under different conditions such as start-up, steady-state with different organic loading rates, step loading rate increases, and recovery. The research focused on several aspects including adsorption removal mechanisms, biodegradation removal mechanisms, interaction between multiple substrates, and adsorption capacity maintenance.

9.1.1 Importance of Using GAC as a Carrier Medium in FBR Systems

A comparison of fluidized bed reactor systems with (1) adsorption removal capacity only using granular activated carbon (GAC) without microbial growth, (2) combined biological and adsorptive removal mechanisms using GAC with microbial growth and (3) biological removal only using non-activated carbon with microbial growth was performed. These three systems were fed groundwater contaminated with benzene, toluene and xylene (BTX). The breakthrough profiles, steady-state removal of BTX, and system responses to applied organic step loading rate increases were investigated. The use of activated carbon as a biomass carrier in fluidized bed reactors produces a system in which both adsorption and

biodegradation affect substrate removal. During start-up, even though the same amount of inoculum was added to the two biological systems, the time (ca. 200 hrs) required until effective biodegradation commenced in the system employing GAC was less than that (ca. 500 hrs) observed for the system employing the non-adsorptive biomass carrier (non-activated carbon). Complete breakthrough of BTX did not occur in the system with combined removal mechanisms and the development of a contiguous biofilm was more rapid. To a large extent, the performance of such biological activated carbon systems is controlled by the additive contributions of these two removal mechanisms. During the start-up period, before a fully functional biomass has developed, the substrate is removed primarily by adsorption. The system retains adsorption capacity under steady state conditions, however, as evidenced by the response of the system to in-line concentration shocks. Lower effluent concentration levels were found in BAC systems during a step concentration increase than in similar systems using a non-adsorbing biomass carrier. The data suggest that this results from adsorption of a portion of the increase, and that this material can be subsequently desorbed and biodegraded when the in-line concentration returns to pre-shock levels.

The biocoated GAC were collected from the granular activated carbon fluidized bed reactor (GAC-FBR) fed with toluene contaminated groundwater. The amount of adsorbed toluene on the GAC was measured at various points in time by performing a solvent extraction of a sample of the GAC during start-up, pseudo-steady-state, step-load-increase and recovery phases of operation. This study provides conclusive evidence that a major role of adsorption in integrated biological activated carbon (BAC) systems is to dampen concentration changes during loading transients. During the start-up period in the GAC-FBR before a biofilm was established, the substrate (toluene) was adsorbed rather being released, as would occur in a biological-only system. Upon establishment of a biofilm capable of degrading the substrate, influent toluene was biologically oxidized and a portion of the previously adsorbed toluene was desorbed and degraded. Under continued constant

organic loading rate conditions, the system stabilized and functioned as a biological reactor. During a 77 hour, 400% step-OLR increase, adsorption again became important; excess substrate was observed to accumulate on the GAC carrier particles. This material was desorbed and degraded over an 11 day period following resumption of the initial organic loading rate of 5.3 kg COD /m^3-d. Thus it was established that the dampening ability of activated carbon is not exhausted, but is recovered via bioregeneration.

It is clear from these results that GAC-FBR systems are advantageous for treating BTX-contaminated waters when variations in loading rates are expected or when positive removal is required during the start-up period. Furthermore, this concept provides a foundation upon which future studies can be conducted in order to develop a rational design procedure which incorporates criteria for transient loading performance.

9.1.2 Biodegradation in FBR Systems

After the biomass is established and steady-state conditions are reached, system performance is dominated by biodegradation. Scanning electron microscopy was used to examine the extent of surface coverage of the GAC and non-activated carbon by the biofilm. Particles from both systems were observed to be completely covered by a contiguous, thick biofilm. Under constant, steady-state, organic loading conditions (3 and 6 kg COD/m^3-day) BTX removals were comparable for the GAC-FBR and biological only (FBR) systems. More than 90 percent of BTX was removed in both systems.

The yield and decay coefficients for a mixed culture treating BTX were evaluated and the kinetic parameters for degradation of BTX were determined. The degradation rate for toluene was the highest, while the rate for xylene was the lowest. There was inhibition between all three substrates but the type of the inhibition was not determined. The effect of inhibition on toluene degradation was limited. A large variance during estimation of Ks was

found and may be due to its low value for this mixed culture. The parameters, however, are useful for bioreactor design with a consideration of uncertainty.

9.1.3 Interaction of Multiple Substrates

Parallel fluidized bed systems were operated to treat groundwater containing one milligram per liter each of benzene, toluene and p-xylene (BTX). Granular activated carbon (GAC) was used as the carrier media for microbial growth in one system and granular non-activated carbon (with very low adsorption capacity) was used in another. The performance of the systems during the single-substrate step-loading increases were investigated. When the two systems were subjected to seven-fold step-load increases in benzene and toluene, biodegradation in the fluidized-bed system was sufficient to maintain a high effluent quality. For a substrate that is biodegraded more slowly, such as *p*-xylene, adsorption onto the GAC carrier contributed significantly to removal and helped to provide more robust performance in the BAC-FBR. Because of the complicated substrate interactions, the concentration of some substrates (e.g. benzene and toluene) can effect the degradation rate of other substrates in a fluidized-bed systems and other biological treatment system. Extraction of the GAC carrier and oxygen consumption in the GAC-FBR verified that both adsorption and biodegradation were responsible for maintaining high treatment efficiencies during the step increases.

9.1.4 Formation of Byproducts

Groundwater containing benzene, toluene and p-xylene (BTX) was treated in a fluidized bed reactor (FBR) using non-activated carbon as the biofilm carrier and a biological activated carbon fluidized bed reactor (GAC-FBR) using activated carbon as the biofilm carrier. Formation of metabolic intermediate products at steady-state and during

single-substrate step-loading increases was determined by measuring non-volatile organic carbon (non-volatile TOC) and UV spectra. At steady-state (organic loading rate of 2.2 kg-COD/m^3-day), no intermediate decomposition products were detected in the effluent. Both reactors were subjected to a seven-fold *p*-xylene step increase under different concentrations of dissolved oxygen and nutrients in the influent. The production of byproducts was not effected by the nutrient levels but increased with an increase in influent DO. Both systems were subsequently subjected to twenty, twelve and seven-fold step increases in the organic loading rates of benzene, toluene and *p*-xylene, respectively. A significant amount of the biologically removed BTX resulted in the production of byproducts (35%, 46%, and 29% for benzene, toluene, and xylene increases, respectively). Effluent TOC corresponded to the appearance of new peaks in the UV spectra. The concentration of byproducts was less in the BAC-FBR effluent than in the FBR system. The combination of biological and adsorptive removal mechanisms resulted in enhanced removal of BTX and byproducts and more stable operation of the BAC-FBR system.

9.1.5 Adsorption Capacity in a GAC-FBR

Biocoated GAC was collected from a GAC-FBR treating groundwater contaminated with toluene at different times. The adsorption capacity, as described by the adsorption isotherm, was measured. The results provide conclusive evidence of the effect biofilm growth has on the adsorption capacity of GAC. The adsorption capacity of biocoated carbon was maintained at more than 70% of its initial levels during the first two months. After six months of operation, the remaining capacity was approximately 40%, 52%, and 57% of its initial value for the equilibrium toluene concentrations of 0.1, 3, and 10 mg/L, respectively. If the adsorption removal mechanism is used to provide a barrier for handling the transient condition, this remaining capacity may be sufficient to avoid carbon

replacement. The amount of adsorbed toluene on biocoated GAC decreased continuously after the biofilm developed, however, the adsorption capacity did not recover. No direct relationship was found between the amount of biomass on the carbon and the remaining adsorption capacity.

9.2 Engineering Design Implications

- Biological activated carbon fluidized bed systems have very high treatment efficiencies when fed groundwater contaminated with volatile aromatic hydrocarbons and provide stable performance.

- Using GAC as the biofilm carrier allowed for more rapid biomass development during the start-up period. The adsorption capacity of the GAC prevented breakthrough of some strongly adsorptive compounds, thereby, avoiding use of additional treatment components such as an adsorption column may not be necessary.

- At a constant, steady-state organic loading rate of 3 kg-COD/m^3-day, the GAC-FBR is able to remove 98% of the applied BTX. At a constant, steady-state organic loading rate of 6 kg-COD/m^3-day, the GAC-FBR is able to remove 90% of the applied BTX (B:T:X of 1:1:1 volume to volume).

- During steady-state conditions, the performance of the GAC-FBR is dominated by biodegradation. Engineers, therefore, do not need to consider the adsorption capacity in design of such conditions.

- The biokinetic parameters reported in this dissertation can be used to design the GAC-FBR systems with a help of modeling tool. Because of the continuous change in activity and population of the microorganisms in the bioreactor,

however, variation in substrate utilization rates are expected. Engineers will need to consider such uncertainty in their designs.

- During a transient condition such as an organic loading rate increase, the combination of biological and adsorption removal capacity results in enhanced BTX removal and more stable operation.

- 70% of the initial adsorption capacity of biocoated carbon can be maintained after the first two months. After six months of operation, the remaining capacity was approximately 40%, 52%, and 57% of its initial value for the equilibrium concentrations of 0.1, 3, and 10 mg/L, respectively. If the adsorption removal mechanism is used to provide a barrier for handling the transient condition, this remaining capacity may be enough to make new carbon replacement unnecessary.

- Formation of byproducts was not found during steady-state condition. At extremely high organic loading rate increases (twenty, twelve and seven-fold step increases in the organic loading rates of benzene, toluene and *p*-xylene, respectively), a significant amount of the biologically removed BTX, however, resulted in the production of byproducts (35%, 46%, and 29% for benzene, toluene, and xylene increases, respectively). The GAC-FBR can minimize the concentration of byproducts in the effluent, however, longer hydraulic retention time and higher carbon bed height are required to compensate for this effects

- A two-step procedure should be considered for design of a GAC-FBR treating biodegradable substrates. First, the reactor volume and height are calculated from a steady-state organic loading rate without considering adsorption effects. Second, the parameters are checked with a possible high organic loading rate to verify that effluent quality is maintained. The combined effect of the adsorption and biodegradation removal mechanisms should be considered for the removal of

substrates. The production of byproducts should also be considered with adsorption being the only removal mechanism for the byproducts. The reactor volume and height can be adjusted from the steady-state value to satisfy the effluent requirements.

9.3 Recommendations for Future Studies

- Investigate the effects of usage time of biocoated GAC on adsorption kinetics of toluene.
- Investigate the relationship between increased organic loading rates and production of byproducts. Develop a method to identify the byproducts and investigate their adsorption characteristics.
- Develop a single substrate dynamics model to describe adsorption and biodegradation including production and adsorption of byproducts. Use this model to develop a rational design procedure for GAC-FBR incorporating steady-state and shock loading conditions.
- Understand the long-term effects of high organic loading rate on reactor performance and the production of byproducts.
- Identify the cause of reduced adsorption capacity in GAC-FBR systems.
- Study multiple substrate adsorption isotherms and kinetics for biocoated GAC.
- Develop a dynamic model to describe multiple substrates in GAC-FBR system including adsorption and biodegradation of the substrates and production and adsorption of byproducts under high organic loading rate conditions.

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