Treatment of Groundwaters Contaminated with Aromatic Hydrocarbons in a Fluidized Bed Reactor

by

Arturo Massol-Deyá
Biology Department
University of Puerto Rico
Mayagüez, PR 00681

and

Ning-Hsi Tang
Civil Engineering Department
University of Puerto Rico
Mayagüez, PR 00681

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Abstract

The contamination of groundwater by aromatic hydrocarbons and chlorinated solvents has become a serious threat to human health. Bioremediation, nonetheless, has emerged as the leading technology for the detoxification of contaminated groundwater.

In this investigation, fluidized bed reactors (FBR) using granular activated carbon as a supporting medium were used to study biodegradation of benzene, toluene and p-xylene (BTX) under aerobic conditions by a microbial consortium. Experiments were conducted to determine the removal efficiency of BTX compounds fed individually as well as in mixture and these systems' response to step-increase in organic loading. In addition, kinetic constants of these compounds (benzene, toluene, and p-xylene) were determined by following the Monod model.

The first bioreactor was fed with a BTX mixture as substrate for 51 days after which the feeding was changed to benzene as the sole carbon source for 28 days. The second bioreactor was also fed firstly with toluene as the sole substrate for 51 days after which the feeding was changed to p-xylene as the sole carbon source for 14 days, and finally this reactor was fed again with toluene for also 14 days. For an average influent concentration of less than 4.0 mg/l for either toluene or benzene, higher than 95% removal was obtained, thus, achieving complete mineralization. Individual feeding of p-xylene to the bioreactor originally fed with toluene showed less than 30% removal for an average influent concentration of 1.5 mg/l. However, in the presence of benzene and toluene (BTX mixture), p-xylene was cometabolically transformed with higher than 80% removal. In the presence of p-xylene, the observed removal efficiency of toluene was slightly reduced.

In the step-increase study of influent concentration from 3.6 mg/l to 8.0 mg/l, the benzene reactor showed marked decline in removal efficiency to less than 60%. The toluene reactor showed no change in removal efficiency after the change in influent concentration from 3.5 mg/l to 7.0 mg/l. However, the removal efficiency was reduced to around 75% when the influent toluene concentration was changed from 7.0 mg/l to 17 mg/l. Likewise, the BTX reactor showed no change in removal efficiency after the change in total BTX concentration from 2.5 mg/l to 5.0 mg/l. However, the removal efficiency was considerably reduced to 75%, 60%, and 40% for benzene, toluene, and p-xylene respectively when the influent total BTX concentration changed from 5.0 mg/l to 10 mg/l. These results indicate that the FBR responds well to even a slight increase in the influent concentration. However, this reactor needs time to reach the steady state if the change in influent concentration is drastic. More study is needed to confirm this observation.

Treament of contaminated groundwater can be achieved efficiently by the biological GAC-FBR process. Better understanding of the biological FBR process will lead to better design and cost-benefit ratio.

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CHAPTER 1

INTRODUCTION

Many people depend on groundwater for their principal source of potable water. The contamination of groundwater by aromatic hydrocarbons and chlorinated solvents has become a serious threat to human health. Puerto Rico is ranked among the top states, commonwealths or territories in the U.S. in total toxic releases into the environment (US EPA, 1993a). According to the United States Environmental Protection Agency's (US EPA) 1991 annual Toxic Release Inventory, over 300 toxic chemicals were directly released into the air, water or land, or injected to the groundwater (US EPA, 1993a).

The increasing presence of volatile organic compounds (VOCs) in water supply wells has led to the understanding that groundwater can no longer be considered as an inherently pristine source of drinking water. Contamination by VOCs may be caused by spills of hazardous materials and leaks from underground storage tanks and/or distribution systems.

1.1 Justification

One widespread contamination problem is caused by the release of gasoline and other petroleum fuels into the subsurface (Karlson and Frankenberger, 1989). The major constituents of concern are aromatic hydrocarbons such as benzene, toluene, and xylenes (BTX) which are less adsorbed by the soil matrix than aliphatic hydrocarbons.

The BTX compounds are more mobile in the soil matrix and, therefore, more likely to contaminate groundwater supplies (Voice et al., 1992).

The cost of traditional remedial programs can range from several thousands to millions of dollars per site. Bioremediation, nonetheless, has emerged as the leading technology for the detoxification of many contaminated sites. Bioremediation is a managed or spontaneous process in which biological, especially microbiological, catalysis acts on pollutant compounds, thereby eliminating environmental contamination. The EPA Bioremediation Resource Guide states that the EPA is committed to identifying the most effective and efficient means of addressing the thousands of hazardous waste sites in the United States, including Puerto Rico. Therefore, the Office of Solid Waste and Emergency Response's (OSWER's), Technology Innovation Office (TIO) at the EPA is working in conjunction with the EPA regional research centers and industries to identify and further the implementation of innovative treatment technologies (US EPA, 1993b).

The success of these technologies is dependent on a variety of environmental factors including temperature, hydrogeology, pH, nutrients, electron acceptors, and substrate composition and bioavailability (Choi et al., 1992; US EPA, 1993c). The impact of these factors on the growth, survival and performance of microorganisms is important to the bioremediation process. The most widely used bioremediation techniques have been developed in mild temperate regions, while the dominant processes of pollutant degradation in tropical environments remain poorly understood. Information is needed for the development or implementation of bioremediation technologies in areas with tropical climate such as that of Puerto Rico. This understanding will lead to the development of better designs, safer and more cost-effective strategies for bioremediation or treatment systems.

In situ biodegradation focuses on activating microbial processes for the destruction of environmental pollutants in the contaminated sites. These processes are gaining attention because the microorganisms may play a role in alleviating environmental pollution problems. Bioremediation strategies for pollution control merely bring the broad biodegradative capabilities of microorganisms into focus on a cluster of organic compounds considered as undesiderable environmental pollutants. Recent use of the terms *in situ* bioremediation and environmental biotechnology in both scientific and popular literature implies that knowledge of biodegradation has produced several reliable technologies that are fully operable and capable of eliminating pollutants from contaminated sites (Chiang et al., 1989; Forster and Wase, 1987; Kim et al., 1995; Madsen, 1991; McCarty, 1988; Wang et al., 1990). Both spontaneous and commercially managed *in situ* biodegradation processes promise to play a major role in alleviating some of society's environmental problems, such as contamination of drinking water supply and soils (Madsen, 1991).

Moving the pollutants away from the spill site for physical, chemical or biological treatment in a reaction vessel (i.e., bioreactor) is an alternative commonly known as *ex situ* remediation. One of the most widely used remediation techniques is liquid-phase adsorption using granular activated carbon (GAC) and air stripping. This is simply a transfer of contaminants from one phase to another (McCarty, 1983; Voice, 1989). Further treatment or disposal of the receiving phase is required. Biological treatment appears to be a desirable alternative to such techniques because it has the potential to completely destroy the contaminant compounds. In addition, this strategy is generally less expensive than physical-chemical treatment. Bioremediation has not been widely accepted for groundwater treatment because of a widespread assumption that biological systems are not sufficiently stable to consistently meet the stringent concentration level limitations that are often required. In cases where biological

treatment has been employed, it is typically followed by GAC adsorption for effluent polishing and providing of back-up treatment in the event of partial or total failure of the biological system.

A promising approach known as biological activated carbon (BAC) integrates biological removal with granular activated carbon adsorption into a single unit process (Karlson and Frankenberger, 1989; Voice et al., 1992). Both biological treatment and carbon adsorption have inherent advantages for remediation of groundwater contaminated with BTX compounds. Biological treatment will destroy the contaminants in a cost-effective manner. On the other hand, carbon adsorption is a positive removal mechanism that ensures high quality water in the effluent. Furthermore, GAC in a fluidized bed reactor (FBR) represents a great surface area matrix for attached microbial growth. Coupling these two substrate removal processes into a single system could be a way of capitalizing on the inherent advantages of both approaches (Mueller et al., 1990; Sutton and Mishra, 1994). One additional benefit of combining these removal mechanisms in a FBR system is the reduced loss of BTX from volatilization since predissolution of oxygen is used in place of conventional aeration (Hickey et al., 1991).

This study evaluated the use of integrated GAC-B/FBR system to remediate contaminated groundwaters with BTX compounds. In addition, it also evaluated the reactor's performance in relation to operational perturbations such as step-increase of organic loading and changes in primary substrate composition.

1.2 Objectives

Experiments were conducted to discern differences in structure and function of degradative communities in response to operational perturbations such as changes in the type of substrate, sudden changes in substrate concentrations, step-increase in organic loading and impact of introduced populations on reactor's performance and their fate.

The specific objectives of this research are:

- to determine the efficiecy of degradation of toluene, benzene and p-xylene feeding separately and in a BTX mixture, using aerobic bacteria in a GAC-B/FBR system.
- to evaluate the system responses to a step increase in organic loading application under different operational conditions with respect to the type of substrate (toluene, benzene and p-xylene) feeding separately and in a mixture.
- to determine kinetic parameters of toluene, benzene and p-xylene biodegradation such as maximum specific cell growth rate (μ_m) and half-velocity coefficient for substrate utilization (K_s) in a GAC-B/FBR system.

CHAPTER 2

LITERATURE REVIEW

2.1 Pollutant types

Many synthetic chemicals are discharged into the environment in the form of herbicides, pesticides and industrial effluents. These chemicals could accumulate and cause detrimental changes to natural ecosystems. One particular concern is the contamination of drinking water sources by the toxic, water-soluble and mobile petroleum components such as benzene, toluene, and xylenes (BTX). Microbial degradation of these compounds in aquatic environments or in soils occurs naturally, therefore, it can serve as a significant attenuation mechanism (Baker et al., 1987; Chiang et al., 1989; Gibson and Subramanian, 1984; Karlson and Frankenberger, 1989; Kim et al., 1995; Madsen, 1991; McCarty, 1988).

2.2 Bioremediation technologies

Bioremediation technologies focus on the use of microorganisms that possess the catabolic potential for the degradation of pollutant compounds. Biological reactors based on fixed bacterial films have been used for wastewater treatments for many years. (Bouwer and McCarty, 1982; Oppelt and Smith, 1981; Sutton and Mishra, 1994; Weber et al., 1970). Many groundwater bioremediation programs at contaminated sites are based on groundwater extraction by wells or drains, usually accompanied by treatment of the extracted water in fixed biofilm reactors prior to disposal or reinjection to the aquifer (groundwater pump-and-treat remediation) (Chiang et al., 1989; Karlson and Frankenberger, 1989)

2.3 Microbial populations

Microbial populations capable of utilizing BTX compounds as carbon and energy source either aerobically or anaerobically are widely distributed in nature (Gibson and Subramanian, 1984; Lee et al., 1994). In most cases these populations have been developed and utilized under mild temperate conditions, obtaining good results; but we have little knowledge of the behavior and efficiency of these and other native microbial colony of biodegradation of BTX compounds under tropical climate conditions.

2.4 Biodegradation and adsorption in coupled systems

The beneficial aspects of integrated biodegradation/adsorption systems were reported in a earlier 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; Chudyk and Snoeyink, 1984; Gardner et al., 1988; Kim et al., 1986; Rittmann, 1987; Speitel et al., 1989a,b,c). The removal process has been shown to result from growth of microorganisms on the surface of the GAC particles and the subsequent biodegradation of the waste constituents. The characteristically long sludge retention time (SRT) attainable in the biological fluidized bed reactor minimizes the changes of inhibition due to microbially toxic or inhibitory feed inputs. Tolerance to such conditions is further achieved by promotion of physical-chemical adsorption through the use of GAC as the fluidizing media. The use of GAC provides additional benefits including a faster removal rate upon the start-up phase, enough buffer for toxic shock loads and a greater removal of slowly degradable or recalcitrant compounds. During reactor start-up, the activated carbon adsorption serves as the main removal mechanism of wastewater

constituents by concentrating them on the carbon surface. Microbial growth and biofilm formation becomes an important removal mechanism in addition to extending the life of the carbon through bioregeneration (Sutton and Mishra, 1994).

For other systems this phenomenon has been regarded as a fortuitous benefit because less frequent carbon regeneration was required. In other cases, however, biofilm formation 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 exclude the adsorption of non-biodegradable compounds. Little attention has been given to understand how adsorption and biodegradation processes interact in GAC systems and its effects in both effluent quality and system stability. In addition, there is insufficient information on how the GAC process configuration affects systems efficiency and operational control.

2.5 Granular activated carbon in a fluidized bed reactor system (GAC-FBR)

Recently, the GAC-FBR system has been used for the treatment of groundwater contaminated with relatively low levels of toxic materials such as petroleum hydrocarbons or chlorinated solvents (Hickey et al., 1990; Voice et al., 1992). In a GAC-FBR system, the contaminated water is forced upward at a velocity sufficient to expand or fluidize the bed beyond the point at which the frictional drag on GAC particles is equal to the downward force by gravity. Once at or beyond the point of minimum fluidization, the GAC media particles provide a vast surface area for biological growth, in part leading to the development of a biomass concentration aproximately five to ten times greater than that normally achievable in more conventional bioreactors (Mueller et al., 1990; Sutton and Mishra, 1994).

2.6 Adsorption characteristics of BTX

The single solute adsorption isotherm relationship for each compound can be described using the Freundlich isotherm:

$$\frac{x}{m} = k_f * C_e^{\frac{1}{n}}$$, where

x/m = mass solute adsorbed/mass adsorbent

C_e = concentration of solute in solution, mass/volume

k_f, n = empirical parameters representing the sorption capacity and non-linearity, respectively.

Experimental data were fit to the Freundlich equation using non-linear regression analysis resulting k_f values of BTX to follow the expected order: p-xylene > toluene > benzene (Voice et al., 1992). This means that the amount of p-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 equilibrium aqueous concentration.

2.7 Biodegradation of volatile aromatic hydrocarbons

A biological fluidized bed reactor (B-FBR) and a biological activated carbon fluidized bed reactor (A/B-FBR) were evaluated operating under steady-state conditions and with BTX as substrate (Voice et al., 1992). The average total COD in the influent to the B-FBR was 7.25 mg/l, or an applied organic loading rate of 2.9 kg COD/m^3-day. More than 90% of the BTX (94% for benzene and toluene and 90% for p-xylene) was removed, the average dissolved oxygen DO consumed was 4.6 mg/l. The average total COD in the influent to the A/B-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 A/B-FBR system, greater substrate removal was observed: 99% for benzene and toluene and 92% for p-xylene. The average DO consumed was 6.1 mg/l. With a total COD loading to both reactors of

6.0 kg COD/m^3-day the two systems performed comparably, removal rates averaged 94, 90 and 81% for benzene, toluene and p-xylene, respectively.

Data from a laboratory, pilot-scale biological FBR, using granular activated carbon (GAC) as the support media (GAC-FBR), operated at various benzene, toluene, ethylbenzene, and xylene (BTEX) concentrations and organic loading rates, showed that greater than 99% of degradation of total BTEX was achieved at an organic loading rate of 3.0 kg COD/m^3-day or less and an empty bed hydraulic retention time of 5.0 minutes (Hickey et al., 1991). System performance was extremely robust, easily handling a tenfold step increase in loading due to the combined adsorptive capability of the biofilm-coated GAC and ability to subsequently bioregenerate the GAC.

A microbial consortium and Pseudomonas strain (PPO1) were used in studying biodegradation of benzene, toluene and p-xylene under aerobic conditions (Alvarez and Vogel, 1991; Sook et al., 1994). Studies involved removal of each compound individually as well as in mixtures with the others. Both the pure culture and the consortium exhibited a qualitatively similar behavior toward each one of the three compounds. Neither culture was able to biodegrade p-xylene. Both cultures degraded benzene following Monod kinetics, and toluene following Andrews (Haldane) inhibitory kinetics. Benzene and toluene mixtures were removed under cross-inhibitory (competitive inhibition) kinetics. In the presence of benzene and/or toluene, p-xylene was cometabolically utilized by both cultures, but was not completely mineralized. Benzene and toluene were completely mineralized (Chang et al., 1993; Gibson and Subramanian, 1984; Sook et al., 1994). Cometabolic removal of p-xylene reduced the yields on both benzene and toluene.

CHAPTER 3

THEORETICAL CONSIDERATIONS

3.1 Adsorption

Adsorption, in general, is a process of collecting soluble substances that are in solution or in suitable interface. The interface can be between the liquid and a gas, a solid or another liquid. From the point of view of a liquid-solid interface, adsorption refers to the ability of certain solids to concentrate on their surface, substances from the surrounding medium.

For many years, the adsorption process has not been used extensively in wastewater treatment, but demands for a better quality of treated wastewater effluent have led to an intensive examination and the use of the process of adsorption on activated carbon (Tchobanoglous and Burton, 1991). The activated carbon in this case is used to remove a portion of the remaining dissolved organic matter.

3.1.1 Activated carbon production

Activated carbon is prepared by first making a char from materials such as almond, coconut, and walnut hulls, other woods, and coal. The char is produced by heating the material to a red heat in a retort to drive off hydrocarbons but with an insufficient supply of air to sustain combustion. The char particles are then activated by

exposure to an oxidizing gas at a high temperature. This gas develops a porous structure in the char and thus creates a large internal surface area.

3.1.2 Treatment with granular activated carbon (GAC)

A fixed-bed column is often used as a means of contacting wastewater with GAC. The water is applied to the top of the column and withdrawn from the bottom. The carbon is held in place with a underdrain system at the bottom of the column.

Expanded-bed and moving-bed carbon contactors have also been developed to overcome the problems associated with headloss buildup. In the expanded-bed system the influent is introduced at the bottom of the column and is allowed to expand, much as a filter bed expands during the backwash. In the moving-bed system, spent carbon is replaced continuously with fresh carbon (Tchobanoglous and Burton, 1991).

3.1.3 Analysis of the adsorption process

The adsorption process takes place in three steps: macrotransport, microtransport and sorption. Macrotransport involves the movement of the organic material through the water to the liquid-solid interface by advection and diffusion. Microtransport involves the diffusion of the organic material through the macropore system of the GAC to the adsorption sites in the micropores and submicropores of the GAC granule. Adsorption occurs on the surface of the granule and in the macropores and mesopores, but the surface area of these parts of the GAC granule is so small compared with the surface

area of the micropores and submicropores that the amount of material adsorbed there is usually considered negligible (Tchobanoglous and Burton, 1991). Sorption is the term used to describe the attachment of the organic material to the GAC. The term sorption is used because it is difficult to differentiate between chemical and physical adsorption. When the rate of sorption equals the rate of desorption, equilibrium has been achieved and the capacity of the carbon has been reached.

The quantity of adsorbate that can be taken up by an adsorbent is a function of both the characteristics of concentration of adsorbate and the temperature. Generally, the amount of material adsorbed is a function of the concentration at a constant temperature; the resulting function is called an adsorption isotherm. Equations that are often used to describe the experimental isotherm data were developed by Freundlich, by Langmuir, and by Brunauer, Emmet and Teller (BET isotherm) (Tchobanoglous and Burton, 1991). Of the three, the Freundlich isotherm is used most commonly to model the adsorption characteristics of the activated carbon used in water and wastewater treatment.

3.1.4 Freundlich isotherm

The empirically derived Freundlich isotherm is shown as follows

$$\frac{x}{m} = k_f * C_e^{\frac{1}{n}}$$

where,

x/m = amount adsorbate adsorbed per unit weight of absorbent (carbon).

C_e = equilibrium concentration of adsorbate in solution after adsorption.

k_f, n = empirical constants.

the constants in the Freundlich isotherm can be determined from experimental data by plotting (x/m) versus C_e, rewriting the equation above as

$$\log(\frac{x}{m}) = \log k_f + \frac{1}{n}\log C_e$$

3.1.5 Adsorption of mixtures

In the application of adsorption to wastewater treatment, mixtures of organic compounds are always encountered. Typically, there is a depression of the adsorptive capacity of any individual compound in a solution of many compounds, but the total adsorptive capacity of the adsorbent may be larger than the adsorptive capacity with a single compound. The amount of inhibition due to competing adsorbates is related to the size of the molecule being adsorbed, their adsorptive affinities, and their relative concentrations (Tchobanoglous and Burton, 1991).

3.1.6 Effects of sorption on microbial degradation

The effect of the sorption of compounds onto solid surfaces on biodegradation depends strongly upon the type of compound which is sorbed and the availability of the sorbed substrate for microbial transformation (Criddle et al., 1991). If it is assumed that

sorbed compounds are completely protected from microbial action, sorption would result in a decreased microbial exposure to the compound. This would decrease bioactivity if the sorbate was a useful substrate, or it would increase bioactivity if the sorbate was inhibitory or toxic.

Conversely, if it is assumed that sorbed compounds are readily available for transformation, sorption would increase microbial exposure to the sorbate at the surface while decreasing exposure to suspended organisms. Hence, the effects on suspended organisms would parallel those described above while the organisms in contact with the surface would be oppositely affected. For attached organisms, sorption of beneficial compounds would increase bioactivity while sorption the toxicants and inhibitory compounds could cause bioactivity decrease.

3.2 Fluidized bed reactor (FBR)

In a FBR system, the contaminated water flows upward through a bed of fine-grained material such as activated carbon, sand and others, at sufficient velocity to suspend, to expand or to fluidize the media beyond the point at which the frictional drag on particles is equal to the downward force by gravity. Fluidization significantly increases the specific surface area and allows high biomass concentrations in the reactor, approximately five to ten times greater than that normally achievable in more conventional reactors. The reactor requires a relatively small space and is relatively simple to operate (Mueller et al., 1991; Sutton and Mishra, 1994).

3.3 Bioremediation

3.3.1 Definitions

Bioremediation is a managed or spontaneous process in which biological, especially microbiological, catalysis act on pollutants compounds, thereby remedying or eliminating environmental contamination.

Biodegradation is a subset of biotransformation which causes simplification of an organic compound's structure by breaking intramolecular bonds by microbiological, usually enzymatic, catalysis. Alterations include those that increase (e.g., condensation reactions) and decrease (e.g., mineralizations reactions) the number or complexity of intramolecular bonds (Madsen, 1991).

Mineralization is the conversion of an organic molecule into its inorganic constituents (e.g., CO_2, NO_3^-, SO_4^{-2}, PO_4^{-3}). Mineralization occurs when an organic compound is altered by central catabolic and anabolic cellular mechanisms. The responsible organism(s) typically benefit from mineralization reactions thus, microbial growth is expected, and a portion of the carbon in the original organic molecule is usually incorporated into biomass (Madsen, 1991).

Cometabolism is the fortuitous modification of one molecule by an enzyme which routinely acts on another primary substrate molecule. The primary substrate supports growth of microorganisms that produce one or more enzymes of low specificity that also act on the cometabolized substrate. The cometabolized substrate is

usually altered only slightly and does not enter catabolic and anabolic pathways of the microbial cell. Therefore, the responsible organism does not benefit from cometabolic reactions. Microbial growth does not result and cometabolic reactions are not expected to accelerate. However, other organisms may be able to mineralize products of cometabolism (Chang et al., 1993; Madsen, 1991).

3.3.2 Microbial process in porous media

Microorganisms consist of a broad group of life forms, including virus, bacteria, protozoa, algae and fungi. The bacteria are the most diverse organisms within this group with respect to their ability to carry out a wide range of transformations of both organic and inorganic compounds (Criddle et al., 1991). A single bacterium is on the order of one micrometer in size, and as such, will not settle, but will remain suspended by Brownian motion. Bacteria can, however, become attached to surfaces presented by porous media, or may form large agglomerates with other bacteria of sufficient size so that they settle, or become lodged within the pore spaces of porous media. Thus, either through attachment, or agglomerations, bacteria may remain in place within porous media, and not move with the fluid itself. The major transformations that occur within porous media are generally brought about by attached bacteria or those contained in agglomerates. The transformation of a chemical contained in the fluid necessitates that it be transported to the microorganisms and into the agglomerates or surfaces film of bacteria for transformation. The rates of biotransformation are here generally governed

by physical processes of mass transport and diffusion as well as by the biological processes itself (Criddle et al., 1991).

3.3.3 Transformations of contaminants

Bacteria mediate the transformation of organic and/or inorganic compounds in order to obtain energy for growth and to synthesize cellular material. The energy yielding reactions are generally oxidation-reduction reactions that involve the transfer of electrons from a donor to an acceptor (Criddle et al., 1991). Reduced compounds such as organic chemicals, ammonia, sulfides, Fe(II), and H_2, can serve as electron donors for energy, and are generally considered as the food for bacteria. The electrons released during oxidation are transferred to electron acceptors, the most common ones being oxygen, nitrate, sulfate and carbon dioxide. When oxygen is present, it is the acceptor used, and the process is termed aerobic. In the absence of oxygen (anoxic or anaerobic conditions), the electron acceptors tend to be used by different bacterial species in order of the energy provided, with nitrate being used first (with reduction to nitrite and nitrogen gas), sulfates next (with reduction to sulfide), and carbon dioxide last (with reduction to methane).

A great variety of reduced carbon and inorganic chemicals can serve as the electron donor for biological reactions. It is increasingly recognized that a great variety of organic and inorganic chemicals may also be used as electron acceptors. Thus, the potencial number of reactions that can be mediated by bacteria is very large. While most

transformations brought about by bacteria are directly beneficial for energy or synthesis, some are brought about fortuitously by enzymes produced by bacteria for other purposes. There is considerable interest today in exploiting such fortuitous reactions in order to broaden the range of xenobiotic chemicals that can be degraded by biological processes (Criddle et al., 1991).

3.4 Microbial attachment to surfaces

3.4.1 Introduction

The vast majority of exposed solid/liquid interfaces found in nature are colonized by microorganisms. The solid component of such interfaces are composed of a broad array of materials: animate objects such as teeth, roots and digestive tract walls, and inanimate solids such as ship hulls, soil grains, glass slides, soils and reactors walls. The concept that solid/liquid interfaces are capable of affecting microbial activity has long been acknowledged (Criddle et al., 1991). Delineating precisely the nature of these effects and the conditions under which they occur is still the subject of much research. For the purposes of this discussion, it is beneficial to explore the mechanisms and advantages of attachment.

3.4.2 Mechanisms of attachment

There are several steps involved in the attachment of microorganisms to solid surfaces. The preliminary step is sorption from solution of an organic macromolecular

film to the surface. This occurs at a rate which is extremely rapid compared to the rate of microbial transport, and hence can be considered instantaneous. These films consist primarily of glycoproteins, proteoglycans and end products humic residues which are biologically produced. Due to the net negative charge of most natural surfaces, as well as the predominance of negatively charged bacterial surfaces, the altered properties imparted by the conditioning film play a key role in the initial microbial attraction. The film enables the organisms to overcome the inherent electrostatic repulsion in order to adhere to the surface. This organic layer may also affect the physical properties, wettability and perceived charge of the surface (Criddle et al., 1991).

The second step in microbial adhesion involves the transport of microorganisms to the surface. Microbial transport may occur by one or more of several processes. Under quiescent conditions, the two major transport processes are diffusion to the surface by Brownian motion and active transport. Active transport applies only to motile organisms and is often a response to a perceived concentration gradient of some biologically relevant compound. This chemotaxic response may be positive, as in the case of available substrates, or negative, as in the case of inhibitory substances or toxins. Under conditions of flowing solute or turbulence, advective transport becomes the predominant process and is potentially having several orders of magnitude faster than diffusion or active transport.

Initial bacterial adhesion is generally reversible, the result of physicochemical processes. Surface characteristics such as charge, hydrophobicity and conditioning film

accumulation of both the solid interface and microbial membrane, affect the degree of reversible sorption (Criddle et al., 1991). Highly hydrophobic organisms will be drawn to hydrophobic surfaces, and oppositely charged organisms and surfaces will naturally attract. For surfaces and microorganisms of like charge, Van der Waal's forces of attraction and electrostatic forces the repulsion interact such that adhesion occurs at the secondary minimum of the overlapping double layers described by theory of colloidal stability. At this stage, the microorganisms may be detached by moderate shear forces and are still capable of exhibiting Brownian motion and flagellar motility.

Irreversible attachment occurs after some critical residence time, and is characterized by the production of extracellular polymers, fibrils and adhesives. The adhesion produced by these polymers result in a strong anchoring, which is resistant to substantial shear forces and devoid of Brownian motion or motility (Criddle et al., 1991).

Colonization of surfaces occurs as irreversible sorbed bacteria reproduce and the daughter cells remain adhered. With the growth of microcolonies, extracellular polymers are produced which form a connective framework in which it is possible for the organisms to maintain a microenvironment with conditions which are substantially different from those in the surrounding system. As the attached colonies merge, an extended layer of inmobilized cells interconnected by a matrix of polymers and fibrils is formed. This layer is referred to as a biofilm, and can consist of a thin homogeneous populations of cells or can grow to thicknesses in excess of centimeters. In these

biofilms, many different microenvironments may exist which are capable of supporting a mixed microbial consortia consisting of both procaryotic and eucaryotic microorganisms (Criddle et al., 1991). It is only in systems with high substrate and nutrients concentration that deep heterogeneous films developed. Typically, over time, consumption of substrate in a deep biofilm leads to substrate deprivation at the surface, promoting decay of the attached microorganisms. This decay undercuts the integrity of the adhering exopolymer framework and can cause the biofilm to detach and slough off the surface. This leaves a surface which is littered with exopolymers and ripe for recolonization, allowing the attachment cycle to begin anew.

3.4.3 Advantages of attachment

The predominance of attached microorganisms within the broad array of naturally occurring environmental conditions suggests that there must be advantages of attachment (Criddle et al., 1991). The perceived advantages are condition specific and include the following:

- Preservation of position: Many microorganisms are known to preferentially attach in the presence of substrate, preserving their position near a food source and eluding washout.
- Optimization of substrate and waste transport: The transport of substrate to cells and the transport of waste products away from cells occur by advective as well as diffusive transport for attached cells. Suspended microorganisms tend to be

transported within their locus of surrounding liquid and therefore benefit mainly from diffusive transport alone.

- Protection from predators: Attached organisms are protected from virus and bdellovidrio attack as well as grazing protozoa due to the sequestration of the cell and subsequent diminishment of area exposed for attack.
- Maintenance of microenvironment: Conditions which are advantageous for growth can be maintained within a community of attached organisms which are not achievable for single suspended cells. Examples include maintenance of anaerobic conditions and optimal pH as well as avoidance of toxic shock loadings, temperature shocks and desiccation.
- Proximity to substrate: Accumulation of substrates at solid surfaces and their subsequent availability for microbial uptake.

3.5 Kinetics of transformation

3.5.1 Organism growth rate

Organic compounds are generally utilized by microorganisms for energy and growth, and thus the rate of substrate utilization is a function of the growth rate of microorganisms (Criddle et al., 1991; Tchobanoglous and Burton, 1991). Microbial growth rate is generally based upon the original formulation by Monod (1942), which was subsequently modified by Van Uden (1967) to consider organisms decay as well:

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

$$\mu_m = k_m Y_m \qquad (2)$$

also, $$\mu = \frac{dX / dt}{X} \qquad (3)$$

where, b = decay coefficient, day^{-1}

k_m = maximum specific rate of substrate utilization, day^{-1}

K_s = affinity or half-velocity coefficient, mg/l

μ = specific growth rate, day^{-1}

μ_m = maximum specific growth rate, day^{-1}

S = rate-limiting subtrate concentration, mg/l

t = time, days

X = concentration of microorganisms, mg/l

Y_m = maximum organism yield, mg organism / mg substrate

According to equation (1) microorganisms increase in concentration if the substrate concentration exceeds the rate of decay. When S is zero, microorganisms decay, and biomass concentration decrease.

3.5.2 Substrate utilization rate

If the concentration of all but one of the substances needed for bacterial growth is in excess of growth needs, then the limiting substance is termed the growth-limiting

substrate, or simply, the substrate. Substrate utilization generally follows a similar kinetic model as that for bacterial growth:

$$\frac{dS}{dt} = -\frac{k_m XS}{S + K_s} \qquad (4)$$

Although equation (4) has the same form as the well known Michaelis-Menten expression for enzymatic degradation, it is an empirical relationship based on observed patterns of substrate consumption by whole cells, and its coefficients may or may not be related to the activity of a specific enzyme (Criddle et al., 1991; Tchobanoglous and Burton, 1991).

When substrate concentration is low, the rate of utilization is directly proportional to the substrate and organism concentrations:

$$-\frac{dS}{dt} = \frac{k_m}{K_s} XS \qquad \text{where } S << K_s \qquad (5)$$

The ratio k_m/K_s represents a second order rate constant (k') with units of mg/l-day. Equation (5) is often used for low substrate concentration in soils and groundwater.

At high substrate concentration, substrate utilization becomes independent of substrate concentration, and first order with respect to organisms concentration:

$$-\frac{dS}{dt} = k_m X \qquad \text{where } S >> K_s \qquad (6)$$

The value Y_m represents the maximum yield of organisms that can result from substrate utilization. Because organism decay occurs during substrate utilization, the net yield of organisms is always less than the maximum yield, and is defined by:

$$Y_n = \frac{dX/dt}{-dS/dt} \qquad (7)$$

The relationship between net and maximum yields and growth rate is found by combining equations (1) through (4) and (7):

$$Y_n = Y_m \frac{\mu}{\mu + b} \qquad (8)$$

In order to obtain the various coefficients for microbial growth and substrate utilization, experimental data will be obtained, and from a linear relationship between growth rate and substrate utilization some of these coefficients can be determined:

$$\mu = Y_m \left(\frac{-dS/dt}{X}\right) - b \qquad (9)$$

In a graph of μ versus (-dS/dt)/X the slope of the straight line represents Y_m and the y-axis intercept is -b.

CHAPTER 4

EXPERIMENTAL PROCEDURES

4.1 Medium and chemicals

The GAC used was Calgon filtrasorb F-200 (Calgon Co., Pittsburgh, PA) which was rinsed with distilled water to remove oil and fine powder and sterilized in an autoclave at 121 ^{0}C. The medium was then stored in sealed containers until needed.

Benzene, toluene and p-xylene (BTX), ammonium chloride (NH_4Cl) and potassium phosphate dibasic (K_2HPO_4) were obtained from Fisher scientific chemicals (Fair Lawn, N.J.). All chemicals were of reagent grade.

The laboratory tap water was used as the feed. This water contained chlorine residual (around 1.5 mg/l). A GAC prefilter was used for the removal of the chlorine residual (effluent Cl_2 concentration < 0.02 mg/l).

4.2 Inoculum

Five different types of bacteria with recognized capacity of degrading BTX compounds were isolated from tropical soils in Puerto Rico previously contaminated with aromatic hydrocarbons. The isolation was carried out in the Microbial Ecology Laboratory in the Biology Department at the University of Puerto Rico, Mayagüez Campus (UPRM).This microbial consortium was used initially to seed the two reactors.

4.3 Fluidized bed reactors set-up

Two laboratory-scale GAC-B/FBR reactors were constructed in the Environmental Engineering Laboratory of Civil Engineering Department at the University of Puerto Rico, Mayagüez Campus. Each reactor consisted of a glass column packed with approximately 200 ml of sterilized granular activated carbon. The first

reactor was 2.5 cm in diameter and 110 cm in height, and the second was 3.2 cm in diameter and 100 cm in height. Other components in the system were: a 1.5 m^3 water storage tank, a prefilter with GAC for reducing chlorine residual concentration in tap water, the aerated tap water feed, a syringe pump model 200 (Kd Scientific, Inc., Boston, MA) for BTX feed, a feeder controlled by a clock for nutrient addition, a mixer and a mixing flask, peristaltic pumps and a sedimentation tank. The reactors were operated as an upflow system without recycle. A schematic diagram of the set-up is shown in Figure 4.1.

The feed water was oxygenated to a concentration sufficient to maintain an effluent DO concentration higher than 2.0 mg/l. This was accomplished by the combination of the air flow rate and the type of aerator. Nitrogen and phosphorus (NH_4Cl-K_2HPO_4) were added to the aerated water at a COD:N:P ratio of 100:5:1. Finally, the tap water was amended with the BTX mixture or individual compounds at an influent concentration of approximately 3.5 to 7.0 mg/l. These concentrations are commonly found in many contaminated sites. The feeding point of the aromatic compounds was different from that of the nitrogen and phosphorus nutrients. The feeding point of the nutrients was very close to the entrance of the reactor in order to avoid bacterial growth inside the feeding line and the mixing flask. The mixing flask was needed to dissolve the aromatic compounds in the tap water. The amount of the feed for BTX mixture and individual compounds was controlled by a syringe pump (Kd scientific, model 200) with a variable flow rate drive. The amount of the feed for nutrients was gravitationally controlled by a clock. The aerated tap water, together with the aromatic compounds and the nutrients, was pumped into the bottom of the reactors, by using a peristaltic pump with variable speed drive.

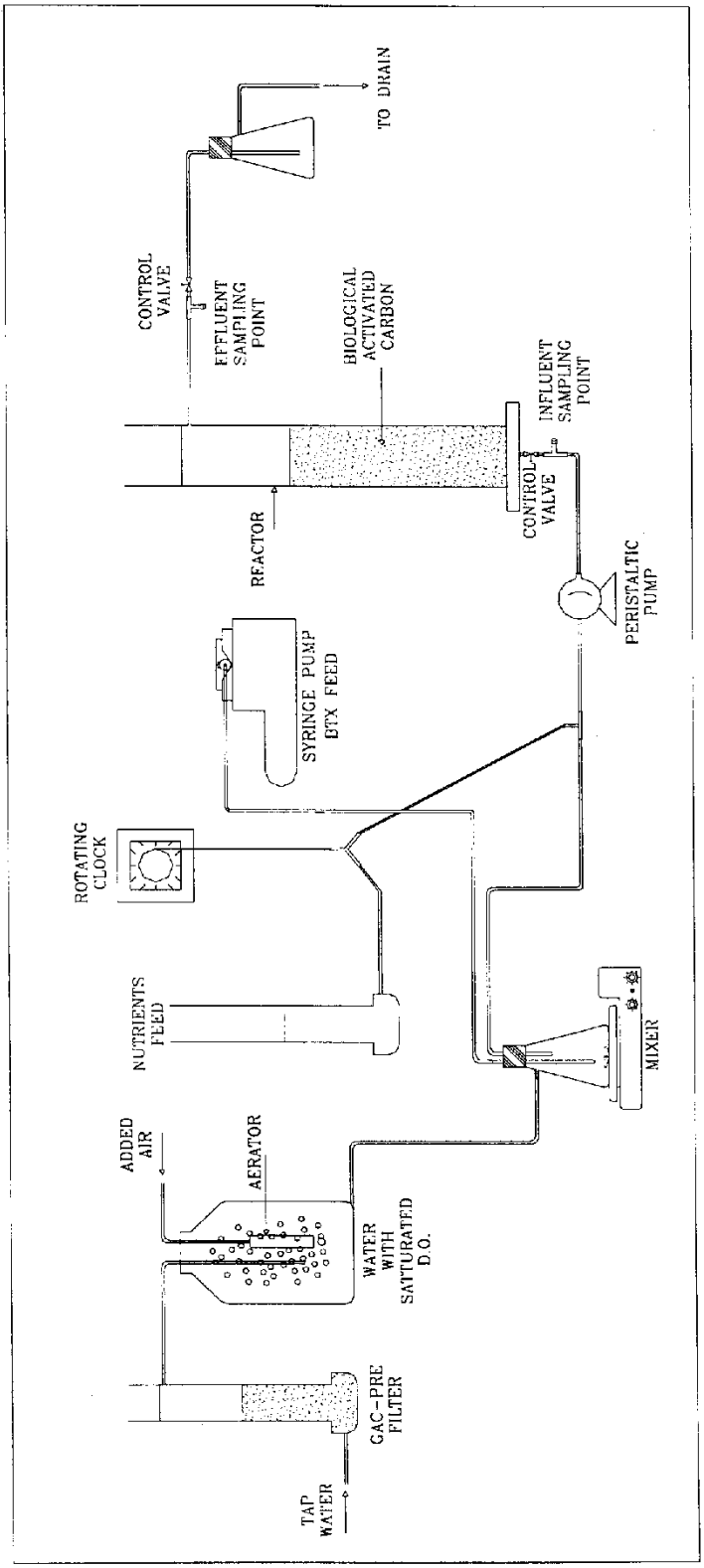

Fig. 4.1 Schematic diagram of biological activated carbon fluidized bed reactor (GAC-B/FBR) system

The reactors were initially seeded with a consortium of BTX degrading bacterial population. Approximately 1.0 L of inoculum containing higher than 10^9 cells/ml was recirculated in the bioreactor for over 12 hours to allow bacteria to attach to the GAC particles. At this point, the amended water was pumped at a flow rate of 110 ml/min into each reactor, resulting in a hydraulic flux rate of 0.22 m^3 /min-m^2 and 0.14 m^3 /min-m^2 and empty bed hydraulic retention times of 4.9 min and 7.3 min for reactors 1 and 2, respectively. The initial fluidized bed height was 45 cm for reactor 1 and 26 cm for reactor 2. As soon as the system started operating normally, the BTX concentrations at the influent and effluent sampling ports of the reactors were monitored periodically. Oxygen consumption, bed height, pH and temperature were also measured continuously until removal efficiency reached steady-state.

4.4 Analytical methods

4.4.1 BTX concentration

Aqueous BTX concentration was determined by analyzing headspace samples in a gas chromatograph, Perkin-Elmer model AS9000 (Perkin-Elmer Corp., Norwalk, CT.), equipped with a Flame Ionization Detector (FID) using helium as a carrier gas and attached to a PC computer with Turbochrom software version 4.0 <4J28> (PE Nelson, a division of Perkin-Elmer Corp., San Jose, CA). Samples collected from the biological fluidized bed reactors (10 ml), were fixed with 2 drops of 5N HCl solution and transferred to 20-ml glass vials with teflon-coated septa and aluminum seal. The samples were equilibrated for 1.0 hr at 80 ^{0}C and an aliquot (1.0 ml) of the headspace gas was injected manually by using a gastight syringe of 1.0 ml (Hamilton, Reno, NV), onto a 30 m in length and 0.25 mm in diameter fused silica capillary column (Perkin-Elmer Corp.). The detection limit of this method was 10 μg/l for each BTX component.

4.4.2 Dissolved oxygen

Influent and effluent dissolved oxygen concentrations were analyzed by using a YSI (Yellow Springs Instrument Co., Inc., Yellow Springs, Ohio.) polarographic electrode. The probe was air-calibrated fifteen minutes before being used. If DO concentration exceeded the limit of the instrument (20 mg/l), the sample was diluted to the measurable range by using water of a known (low) DO concentration. DO uptake was calculated as the difference between influent and effluent DO of the reactor.

4.4.3 pH measurement

pH measurements of the influent and effluent samples were analyzed through the use of an electrode (VWR Scientific, San Francisco, CA). attached to a digital pH meter Orion model 720A (Orion Research Inc., Boston, MA).

4.4.4 Temperature

Temperature of influent and effluent samples was measured with a mercury thermometer placed into each sample.

4.4.5 Protein determination

Protein by Folin Reaction (Lowry Assay) colorimetric method for the estimation of proteins was used. The absorbance, $A_{750,}$ was measured in a spectrophotometer Spectronic 601 (Milton Roy, Co., Rochester, N.Y.). Bovine serum albumin served as the standard in the calibration curve.

4.5 Determination of biodegradation kinetics

The substrate utilization rate and organism growth rate of the BTX mixture and its individual components by the consortium of bacteria were measured. The procedure used is described in the following sections.

4.5.1 Inoculum

A sufficient sample of GAC with its suitable biofilm was extracted from the corresponding fluidized bed reactor fed previously with the component under study. This reactor had already reached steady-state conditions. The GAC was resuspended in a solution of cell extraction buffer (CEB) and placed in a rotary shaker (medium velocity) to remove the biofilm. The CEB with cells and without GAC was centrifugated and the cell pellet was resuspended in fresh mineral medium (BSM) (20 to 30 ml). This inoculum was stored in a tightly closed container until the moment of the experiment.

4.5.2 Experiment set-up

Tests with benzene alone, toluene alone and BTX mixture as substrate were performed. For the removal of a single component, six different concentrations (5, 10, 15, 20, 25 and 30 mg/l approximately) of each compound were added to the serum bottles containing 100 ml of fresh mineral medium (BSM) previously oxygenated over 20 mg/l DO. For the BTX mixture, various concentrations of each compound were added to the serum bottles. Fifty ml of mineral medium with the suitable concentration was mixed with 4 to 8 ml of inoculum, chosen to give appropriate sampling intervals at high or low substrate levels. This mixture was transferred into a glass syringe (without headspace) of 50 ml capacity (Perfektum micro-mate, Popper & Sons, Inc.) and into which a small magnetic bar (stirrer) was placed. The syringe had a valve to keep it

tightly closed, thus, avoiding volatilization. These reactors were placed on a mixer for complete mixing. Substrate utilization rate of each component individully and in the mixture was monitored by taking 2.0 ml liquid samples at various time intervals. The samples were placed into 20-ml glass vials containing 13 ml of saline solution (30% NaCl) acidified with HCl (pH < 2.0). These vials were sealed with teflon-coated septa and aluminium crimp caps. These samples were stored in the refrigerator for subsequent analysis according to item 4.4.1. Increase in microbial biomass was determined by withdrawing samples of 2.0 ml mixed liquor from the 50 ml syringe reactor and storing them in a probe tube. The microbial cells were digested by the addition of 1N NaOH prior to protein determination according to item 4.4.5.

CHAPTER 5

EXPERIMENTAL RESULTS AND DISCUSSION

5.1 Removal efficiencies

5.1.1 Toluene reactor

The influent-effluent toluene concentration, % removal of toluene, dissolved oxygen uptake, and bed height, are shown in Fig. 5.1. The first reactor was used as the toluene reactor. During the 51 days of study, this reactor was oxygenated with air to maintain in the influent an average dissolved oxygen concentration of around 8.0 mg/l, except on days 25 to 37 and 40 to 46 when pure oxygen was added. The initial fluidized bed height was 45 cm. The average pH in the influent was 7.5 (±0.3) and the average temperature was 24.0 (±1.7) °C. The average concentration of toluene in the influent was somewhat higher than 3.50 mg/l which corresponds to the theoretical organic loading rate of 0.92 kg COD/m^3-day.

In this type of system where both adsorption and biodegradation were possible, initial removal of toluene was mainly attributed to the adsorption by the granular activated carbon. After the initial period of 10 days, however, the toluene concentration in the effluent began to decrease while the bed height started to increase drastically because biomass had developed on the surface of the GAC on account of the rapid biodegradation of toluene. The addition of pure oxygen increased DO uptake, however, the additional cost could not be justified because the percentage of toluene removal did

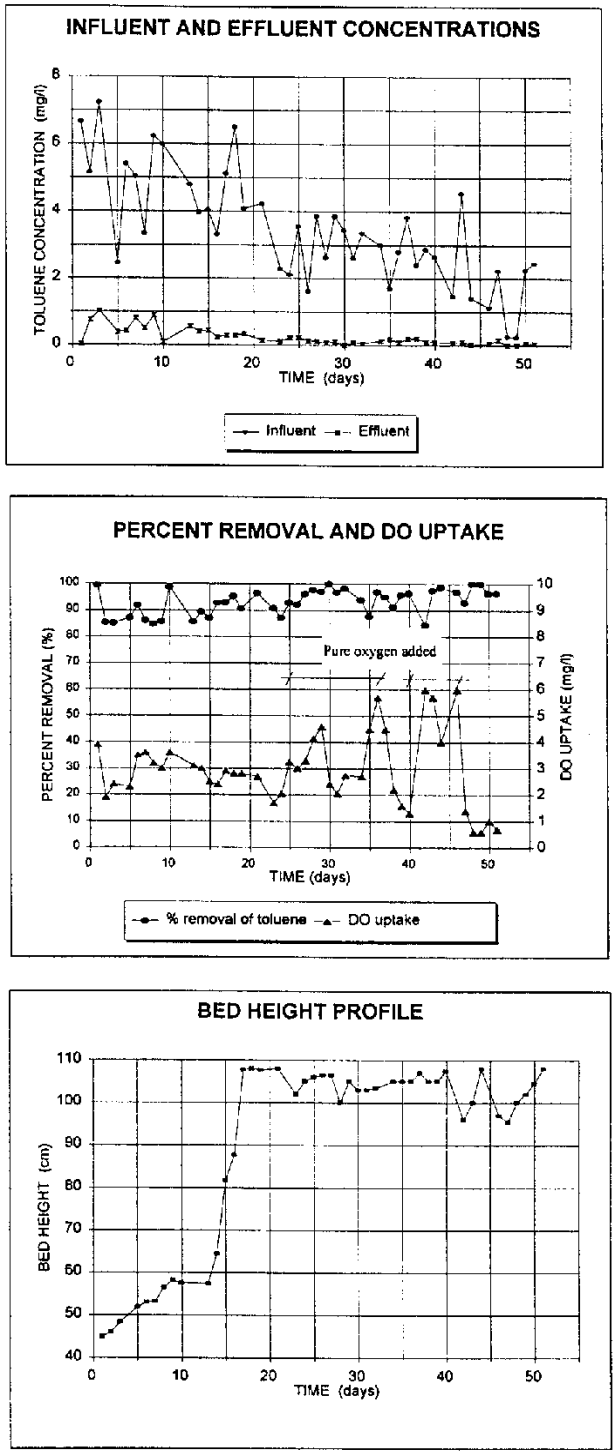

Fig. 5.1 Performance of a GAC-B/FBR system fed with toluene as sole added carbon source. Hydraulic flux rate: 0.22 m^3/min-m^2, empty bed hydraulic retention time: 4.9 min.

not increase significantly. The average pH of 7.2 (±0.2) in the effluent was less than that in the influent because of the production of CO_2 during mineralization or biodegradation process. After day 13, the bed height began to increase rapidly. This could have been a result of particle motion changing from well-defined patterns of localized movement at the beginning of the operation to more random patterns as surface biological growth developed and the particles became more buoyant and capable of migrating upward in the reactor. This type of particle movement would increase the amount of mixing and fluidization in the reactor, but could result in the loss of particles in the effluent. The average value of the ratio grVSS/grGAC of 3.75, 1.33 and 0.32 for the top, middle and bottom of the reactor, respectively, shows that the increase of the buoyancy of the particles was proportional to the amount of biological growth on the particles.

The reactor was assumed to be operating under steady-state condition when a toluene effluent concentration under 0.2 mg/l was observed. In this condition, more than 95% of the toluene was removed. The average DO consumed was 3.0 (±1.4) mg/l.

5.1.2 BTX reactor

The influent-effluent benzene, toluene and p-xylene concentration, % removal of benzene, toluene and p-xylene BTX mixture, DO uptake, and bed height, are shown in Figs. 5.2. and 5.3. The second reactor was used as the BTX reactor. The initial fluidized bed height was 26 cm. The average dissolved oxygen concentration in the influent and the average temperature of this reactor were the same of those of the toluene reactor

since both reactors were operated at same time and shared the same source of flow. The average pH of 7.6 (±0.3) in the influent was slightly higher than that of the toluene reactor. The average pH of 7.3 (±0.2) in the effluent was less than that of the influent. This lower pH was caused by the CO_2 production from the biodegradation of the organic compounds in the wastewater flow.

The cell growth in the initial stage of this reactor appeared to be much slower than that in the toluene fed reactor. This phenomenon was demonstrated by the contrast observed between no increase in bed height during the first 10 days of the experiment and a significant increase in bed height (14 cm) during the same period of time in the reactor fed with toluene alone. Apparently, the initial high percentage removal of each contaminant was due to the carbon adsorption. The bed height began to increase after the first 10 days of feeding. Although the DO uptake remained relatively constant, the increase of the bed height showed the accumulation of the biocell on the surface of the carbon particles. After this point, the removal of the contaminants was increasingly due to the biological oxidation rather than the carbon adsorption. The DO uptake profile indicates no significant increase in DO uptake even after the bed height increase which occurred after day 10. The DO uptake remained at around 2.8 (±1.2) mg/l from the initial day to day 25. This indicates some form of biological activity on the surface of the carbon particles beginning from the first day.

Biomass was added to the toluene fed reactor in both days 17 and 30 because the cells grew very slowly and the reactor seemed to be unstable, especially for toluene

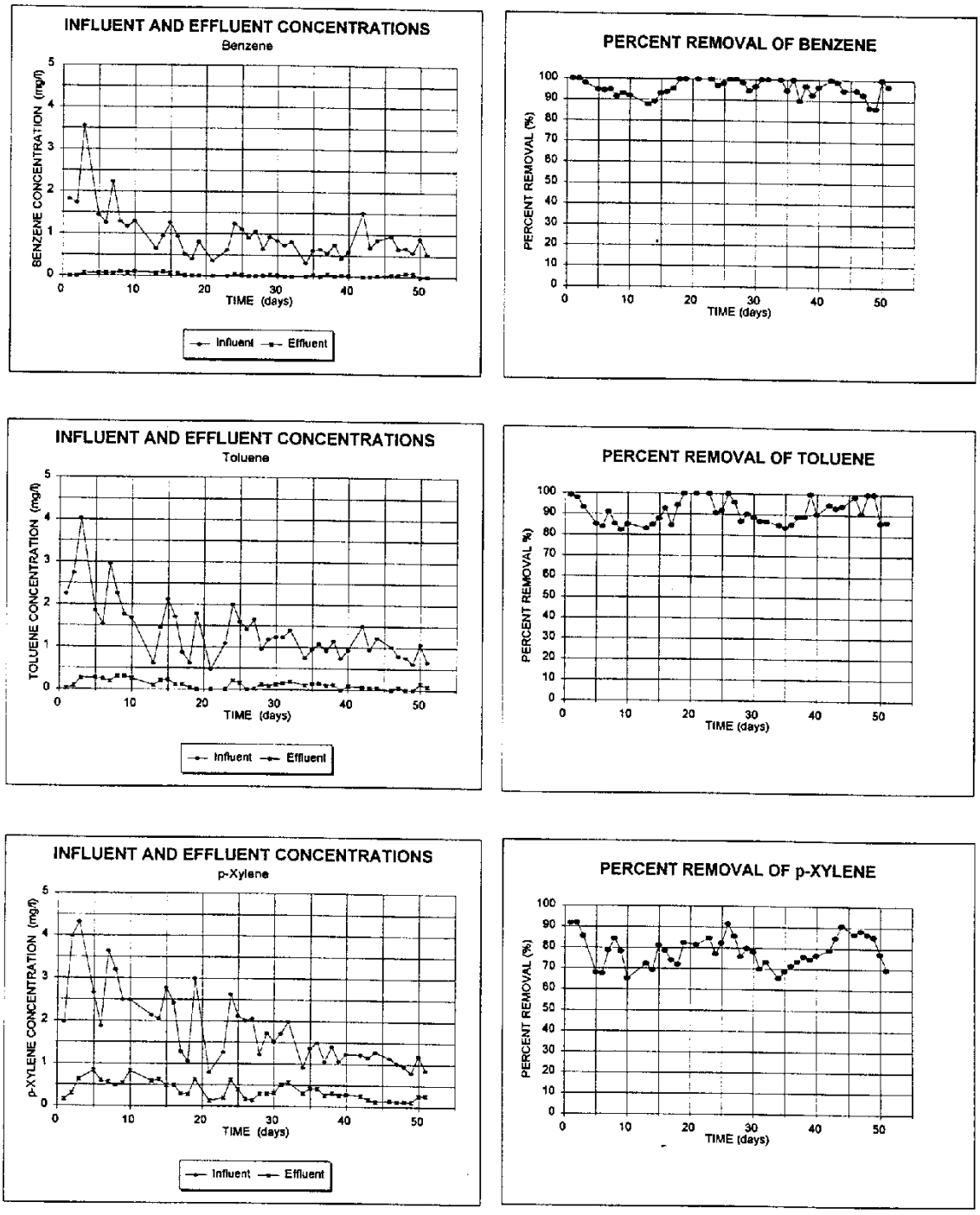

Fig. 5.2 Experimental results of a GAC-B/FBR system fed with a BTX mixture in ratio 1:1:1 as carbon source. Hydraulic flux rate: 0.14 m^3/min-m^2, empty bed hydraulic retention time: 7.3 min.

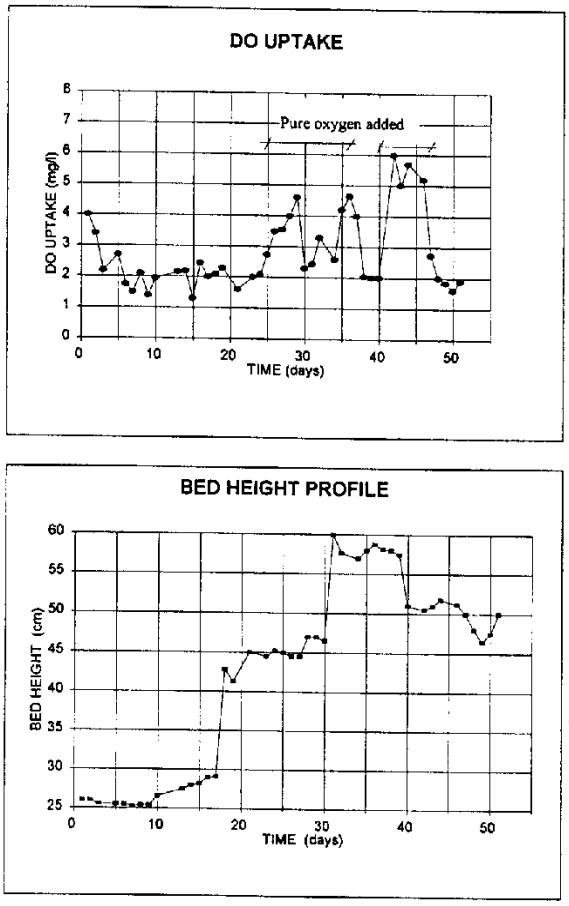

Fig. 5.3 Operational conditions in a GAC-B/FBR system fed with BTX mixture in ratio 1:1:1 as substrate.

and p-xylene removal. The average influent concentration were 1.0, 1.4 and 1.8 mg/l corresponding to the theoretical organic loading rates of 0.18, 0.25 and 0.32 kg COD/m^3-day for benzene, toluene and p-xylene, respectively. These was a likelihood that the rising of DO uptake from day 25 to day 46 was caused by the addition of pure oxygen. However, the percentage of removal did not show significant change during that period.

When the reactor reached steady-state condition, the average percent removals obtained were 96%, 91% and 79% for benzene, toluene and p-xylene, respectively. The average DO uptake was 2.8 (±1.2) mg/l.

5.1.3 Benzene reactor

The reactor which had been fed with BTX mixture was used for studying the performance of reactor in response to the change of the primary carbon source. This reactor operated under the same condition as the others. The influent-effluent benzene concentration, % removal of benzene, DO uptake, and bed height, are shown in Fig. 5.4. The dissolved oxygen concentration in the influent was maintained at an average value of 6.7 (±0.6) mg/l with the addition of air only. The average temperature in the reactor was 20.2 (±2.0) ^{o}C and the influent and effluent pH 7.4 (±0.2) and 7.1 (±0.2) exhibited a similar trend as that of the toluene reactor. The initial fluidized bed height was 27 cm, because a portion of the GAC with cell growth was removed from the BTX reactor for a better fluidization condition. The reactor showed a slow cell growth during the first 12 days. After this initial acclimation period, the bed height began to increase rapidly, thus, resulting in good fluidization and, therefore, more cell growth and biological activity. The effluent concentration was less than 0.15 mg/l with a benzene removal efficiency reaching 95%. The average DO uptake was at 2.9 (±0.8) mg/l. This performance was maintained during the entire period of 28 days after a change in substrate feeding.

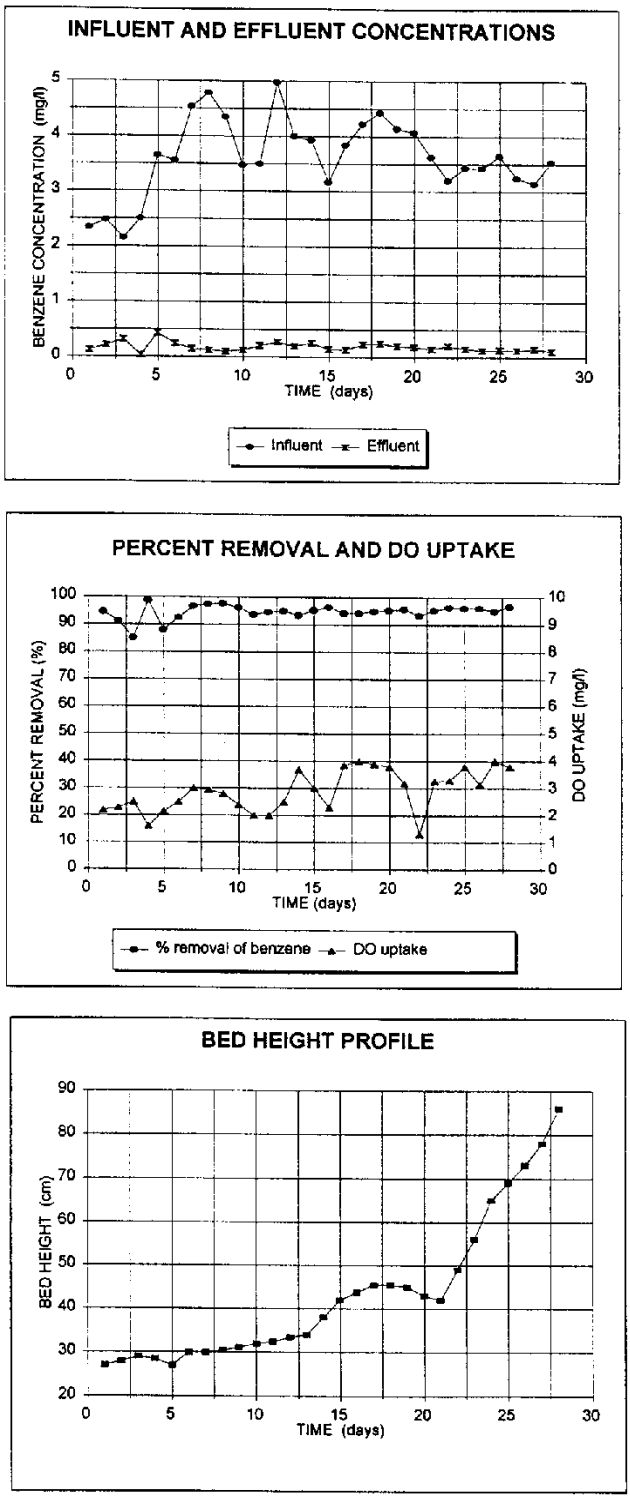

Fig. 5.4 Performance of a GAC-B/FBR system raised with BTX mixture and fed with benzene as substrate for a 28-day period at an average concentration of 3.6 mg/l.

In this reactor, the growth of biomass appeared to be less than that in the toluene reactor and no excess of biomass was produced. The grVSS/grGAC ratios were 0.22, 0.13, and 0.44 grVSS/grGAC for the top, middle and bottom of the column, respectively. These results suggest that this reactor was younger than the toluene reactor, with ratios of 3.75, 1.33, and 0.32 grVSS/grGAC in terms of cell growth and fluidization condition where the patterns of movement, the buoyancy, and the migration of GAC particles with biofilm were not totally defined.

5.1.4 p-Xylene reactor

A reactor acclimated to toluene alone as the carbon source and with excellent cell growth and removal efficiency was used for studying its performance in response to the change of the primary carbon source from toluene to p-xylene. The influent-effluent p-xylene concentration, % removal of p-xylene DO uptake, and bed height are shown in Fig. 5.5. This reactor was operated under conditions similar to those of other reactors described previously. The dissolved oxygen concentration in the influent was maintained at an average value of 8.9 (±0.2) mg/l with the addition of air only. The average temperature in the reactor was 19.6 (±1.0) °C and the initial fluidized bed height was 97 cm. The average DO uptake was 0.9 (±0.5) mg/l and the influent pH was 7.9 (±0.1). The change in pH between influent and effluent was negligible. Both the DO uptake and the stable pH value showed little biological activity within the reactor. The bed height profile also showed a continuous downward trend.

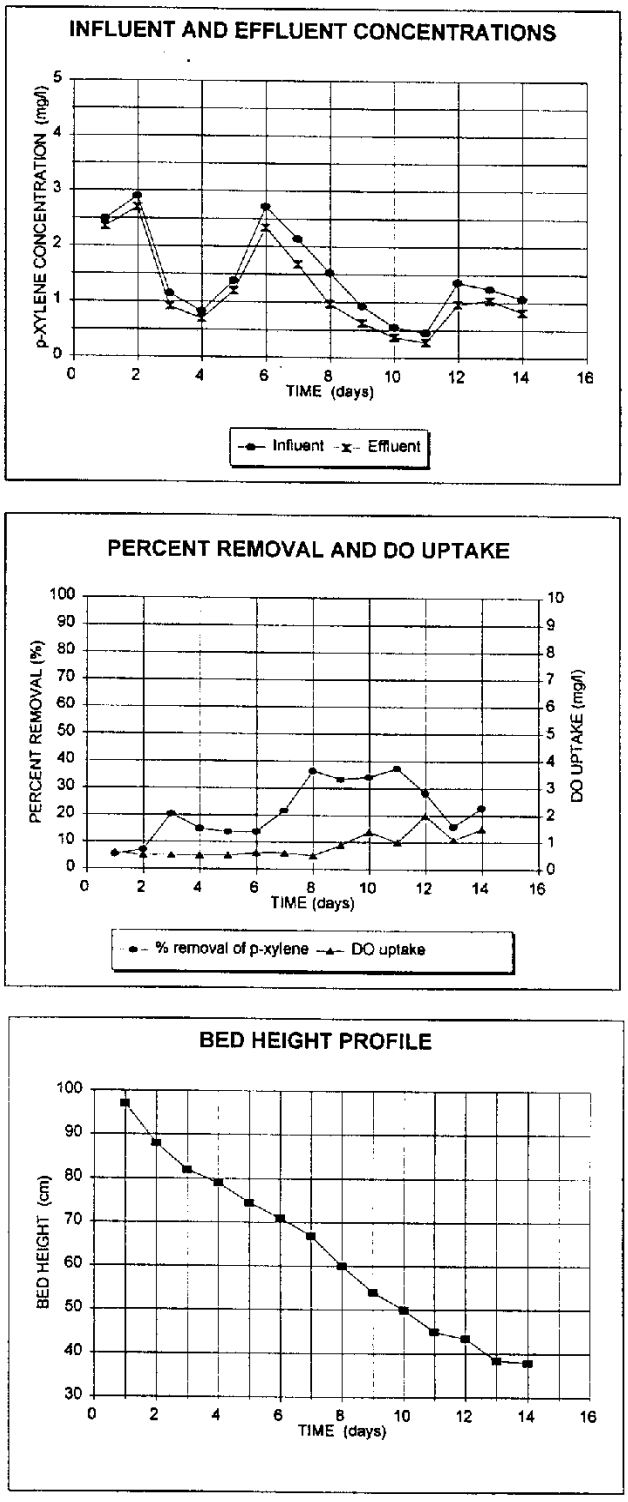

Fig. 5.5 Performance of a GAC-B/FBR system raised with toluene as carbon source and fed with p-xylene as substrate for a 14-day period.

The microbial consortium was obviously unable to biodegrade p-xylene and the microorganisms could not use it as the sole carbon source. This confirms the observation that p-xylene was only cometabolically utilized in the presence of benzene and toluene. The percent removal in this reactor was unstable and only 32% was observed with an average influent p-xylene concentration of 1.5 mg/l corresponding to a theoretical organic loading rate of 0.40 kg COD/m^3-day.

5.1.5 Recovery time study - toluene reactor

After a 14-day period of feeding GAC-B/FBR with p-xylene alone the condition of the reactor deteriorated because it was difficult for the microbial consortium to biodegrade p-xylene as the sole carbon source. This reactor was subjected to a 14-day recovery period test which involved feeding it with toluene as the sole carbon source. The results of the test are presented in Fig. 5.6. The initial bed height was 24 cm, but GAC particles had sufficient biomass on their surface. The average temperature in the reactor was 20.9 (±2.9) °C and the pH values in the influent and effluent were 7.3 (±0.2) and 7.1 (±0.2), respectively. The influent toluene concentration was unstable; it changed from 4.0 to 11.0 mg/l and later dropped to 5.0 mg/l, but the average influent concentration was 6.60 mg/l corresponding to a theoretical organic loading rate of 1.94 kg COD/m^3-day.

The recovery of the reactor was immediate, the toluene effluent concentration showed a continuous downward trend from 3.0 to 1.0 mg/l, while dissolved oxygen

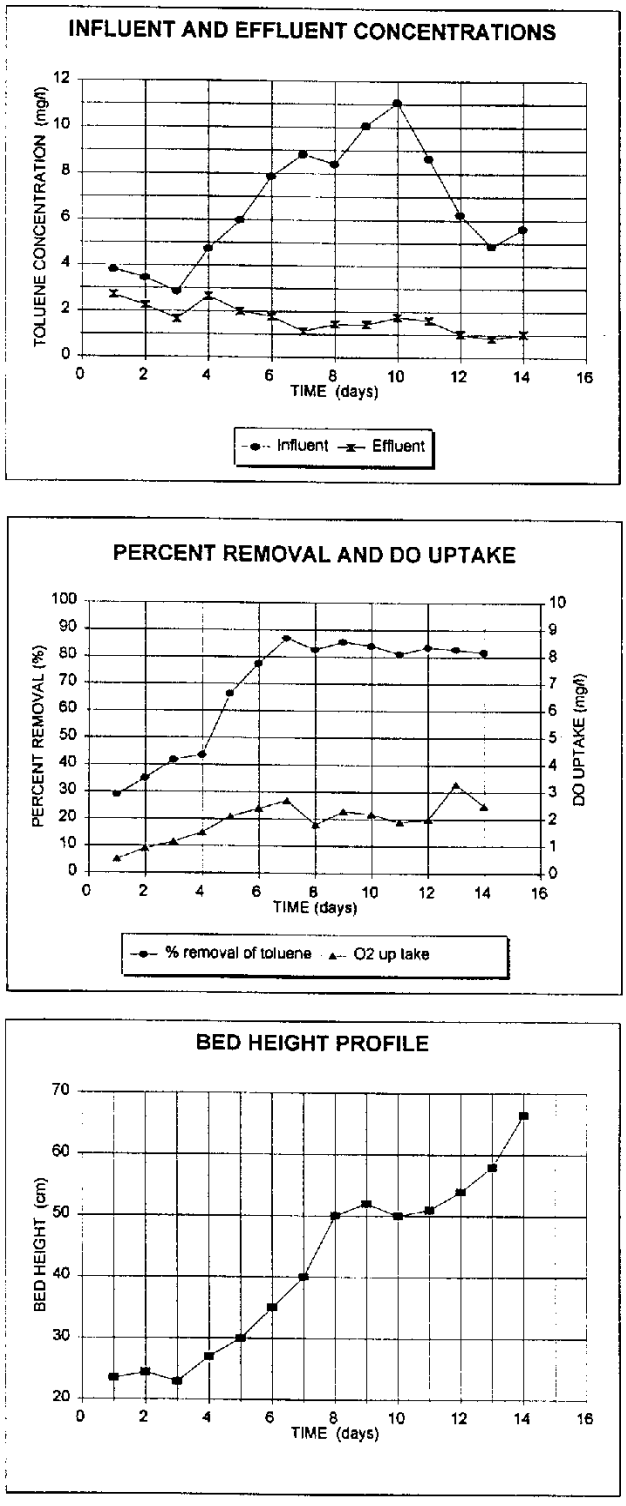

Fig. 5.6 Performance of a GAC-B/FBR system fed with average influent toluene concentration of 6.6 mg/l during recovery-time study following 14-day period of feeding with p-xylene alone as the substrate

uptake, bed height profile and percent removal showed a continuous upward trend from 0.5 mg/l, 24 cm and 30% to 3.0 mg/l, 67 cm and 85%, respectively. After day 3, the DO uptake, bed height and percent removal increased rapidly. This provided evidence of the expanding biological activity. Another aspect that showed biological activity was the downward trend of the influent dissolved oxygen concentration which went from 9.0 to 7.0 mg/l because cells grew in the mixing flask, pipelines and peristaltic pump of the system. The lower percent removal (85%) compared to that of the original toluene reactor could have been caused by the increase of influent concentration from 3.5 to 6.6 mg/l.

Table 5.1 shows a summary of the obtained results followed by a discussion.

Table 5.1 Summary of the experimental results

Reactor	Feeding Compound	Average concentration Influent (mg/l)	Effluent (mg/l)	Average removal (%)	Average DO consumed (mg/l)	Average organic loading ($kgCOD/m^3$-day)
Toluene	Toluene	3.5 ±1.8	0.20 ±0.20	93 ±5	3.0 ±1.4	0.92
BTX	Benzene	1.0 ±0.6	0.04 ±0.05	96 ±4		0.17
	Toluene	1.4 ±0.8	0.12 ±0.10	91 ±6	2.8 ±1.2	0.25
	p-Xylene	1.8 ±1.0	0.38 ±0.24	79 ±8		0.33
Benzene	Benzene	3.6 ±0.8	0.19 ±0.09	95 ±3	2.9 ±0.8	0.65
p-Xylene	p-Xylene	1.5 ±0.8	1.21 ±0.77	22 ±11	0.9 ±0.5	0.40
Toluene recovery	Toluene	6.6 ±2.6	1.67 ±0.61	69 ±21	2.0 ±0.7	1.74

5.2 Organic loading step increase

The GAC-B/FBR systems were subjected to several step-load increases to determine whether the use of a well developed biomass contributed to system stability.

A 48-hr, 2-fold step-load increase was introduced to each of the two reactors, one fed with toluene alone and the other with a BTX mixture. This was carried out by increasing the substrate concentration in the influent while keeping the flow constant. The first step-load increase for the toluene reactor was from 3.5 to 7.0 mg/l corresponding to a theoretical organic loading rate of 0.92 to 1.85 kg COD/m^3-day and the second step-load increase was from 7.0 to 17 mg/l or from 1.85 to 5.0 kg COD/m^3-day. Toluene concentration in the influent and effluent, percent removal and DO uptake are presented in Fig. 5.7. In the first step-load increase, the effluent toluene concentration remained nearly the same as that prior to the increase of organic loading and showed only a slight disturbance during the first 5 hours. After that, the reactor responded to the increase and the percent removal reached more than 90% and

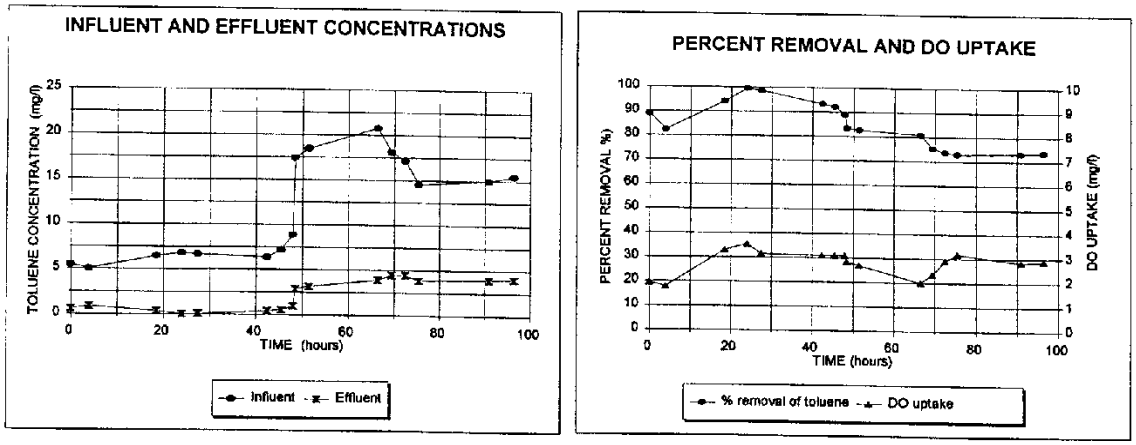

Fig. 5.7 Performance of GAC-B/FBR system during a 48-hr 2-fold step-load increase of influent toluene concentration from 3.5 to 7.0 and to 17.0 mg/l.

oxygen consumption was at an average of 3.0 mg/l. The toluene reactor showed an immediate response to the second step-load increase and the effluent toluene concentration increased to 3.5 mg/l; the percent removal went down to less than 75% and the DO uptake remained at an average value of 3.0 mg/l.

The first step-load increase experiment for the BTX reactor was from 2.5 to 5.0 mg/l of total BTX corresponding to a theoretical organic loading rate of approximately 0.45 to 0.90 kg COD/m^3-day and the second from 5.0 to 10 mg/l or 0.90 to 1.80 kg COD/m^3-day.

Benzene, toluene and p-xylene concentrations in the influent and effluent, percent removal, and oxygen uptake are presented in Fig. 5.8. In the first step-load increase, the effluent BTX concentration remained at levels nearly identical to those observed prior to the increase of organic loading. Dissolved oxygen consumption rose to 3.0 mg/l and percent removals were over 95% for both toluene and benzene compounds and over 85% for p-xylene. In the second step-load increase, the BTX reactor showed an immediate response; the effluent concentrations increased gradually to 1.0, 2.0 and 2.5 mg/l for benzene, toluene and p-xylene, respectively. The dissolved oxygen uptake dropped to 2.0 mg/l and the percent removal showed a significant downward trend with 75%, 60% and 40% for benzene, toluene, and p-xylene, respectively. The stability of the system followed this order: benzene> toluene>p-xylene.

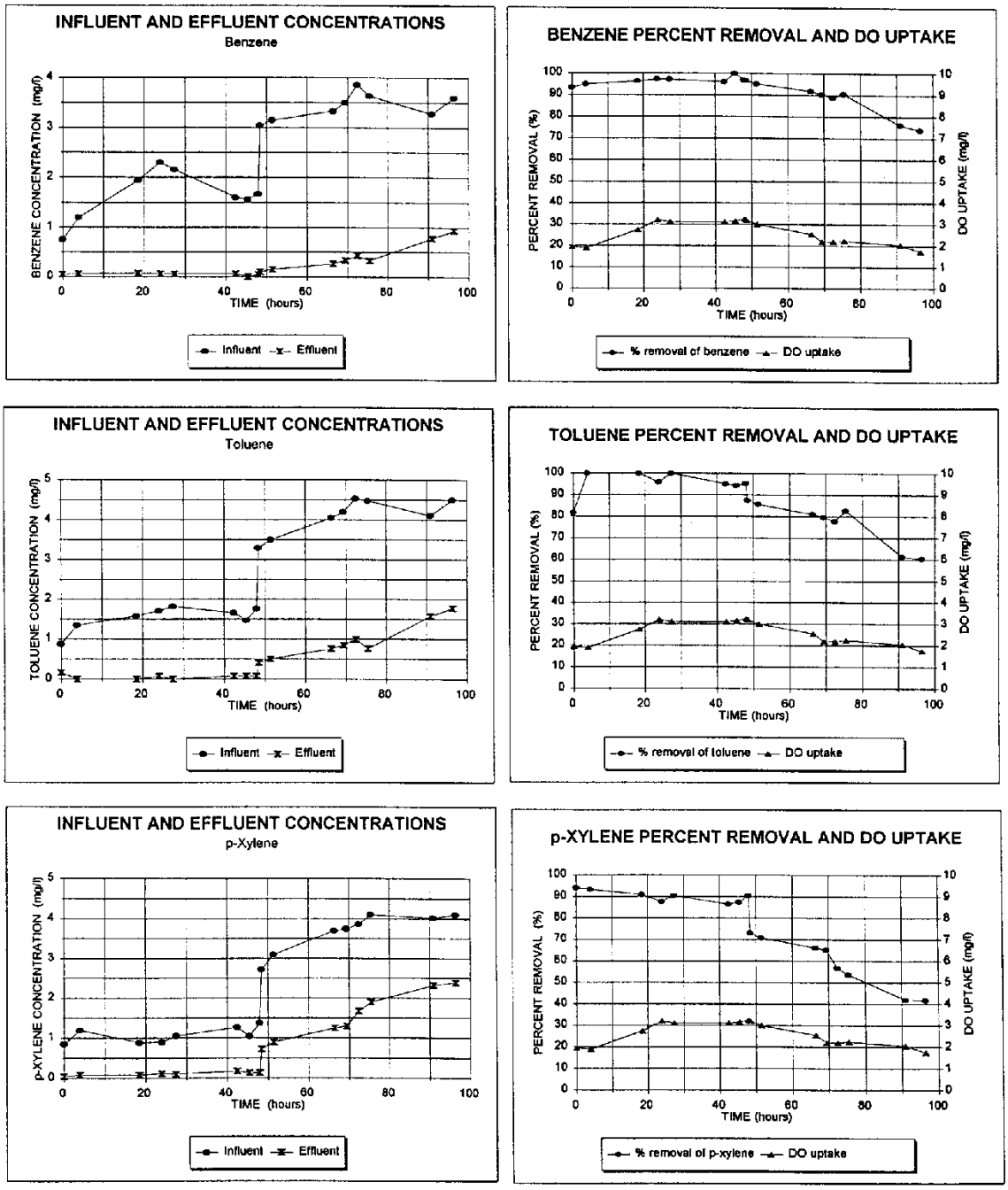

Fig. 5.8 Behavior of GAC-B/FBR system during a 48-hr 2-fold step-load increase of influent BTX mixture concentration from 2.5 to 5.0 and to 10 mg/l.

A similar test was performed for GAC-B/FBR using benzene alone as the carbon source, but only one step-load increase of 64 hr duration was applied. The influent benzene concentration was increased from 3.6 to 8.0 mg/l corresponding to a theoretical organic loading rate of approximately 0.65 to 1.44 kg COD/m^3-day. The benzene reactor showed an immediate response to the step-load increase. Benzene concentrations in the influent and effluent, as well as percent removal and dissolved oxygen uptake are shown in Fig.5.9. Benzene effluent concentration increased from 0.5 to 3.0 mg/l on the average. The percent removal dropped to under 60% and dissolved oxygen uptake went downward from 4.0 to 2.5 mg/l.

The findings discussed above show the sensitivity and instability of all systems operating under high influent concentrations or great applied organic loading rates.

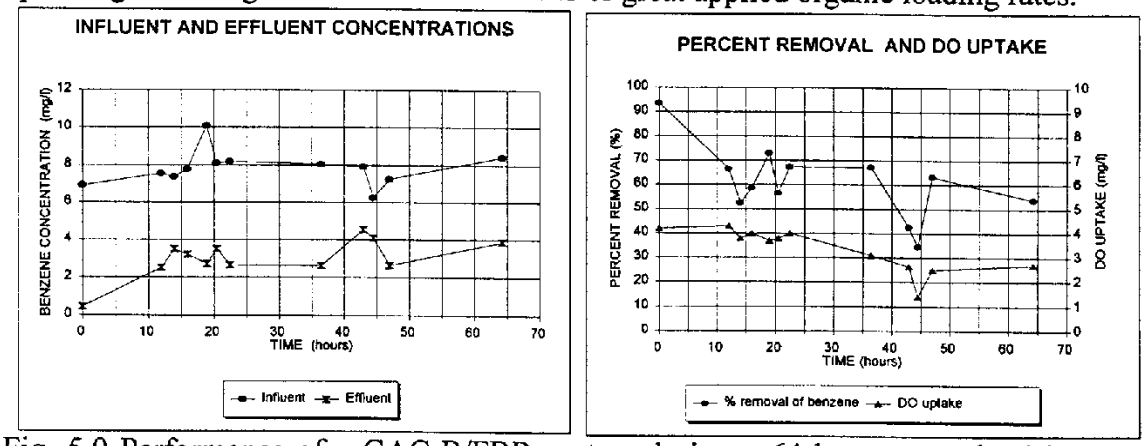

Fig. 5.9 Performance of a GAC-B/FBR system during a 64 hr one step-load increase benzene influent concentration from 4.0 to 8.0 mg/l.

5.3 Optimal bed height

The GAC-B/FBR systems using either toluene or benzene alone were subjected to one additional test for determining the optimal bed height in the reactor. Dissolved

oxygen consumption and substrate utilization profiles in the biological fluidized bed reactor were made. The results of the test are shown in Fig. 5.10. The profiles suggest that the optimal bed height was 50 cm for both toluene and benzene reactors. There was a significant substrate reduction and dissolved oxygen uptake occurred inside the mixing flask, tubing, and the peristaltic pump.

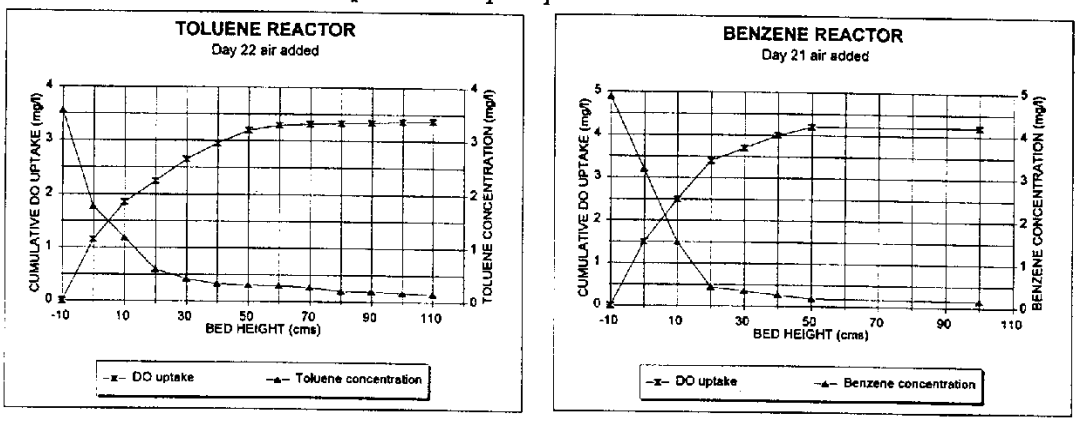

Fig. 5.10 Dissolved oxygen uptake and substrate utilization profiles of the GAC-B/FBR fed with toluene or benzene as carbon source.

5.4 Determination of biodegradation kinetics

The microbial consortium exhibited a similar behavior toward each of the three compounds. Experiments for kinetic constants determination were carried out only for toluene alone, benzene alone, and the BTX mixture since the microbial consortium was not able to biodegrade p-xylene alone (as previously discussed, p-xylene was only cometabolically utilized). The initial dissolved oxygen concentration was raised to more than 25 mg/l (pure oxygen added) and the final DO concentration was lowered to less than 2.0 mg/l. The average pH was 6.80 and the average temperature was 24 °C. These conditions were the same for all tests.

Experimental data for substrate utilization rate were obtained for each of the six different initial concentrations for feeding a compound alone or in a mixture. Data appeared in a range with a rapid downward trend were adjusted to the best fit of an exponential equation. Other data were not included because of certain limiting factors, especially the low DO concentration which affected the biodegradation process. The cell growth was measured by protein concentration and was determined at time 0 min and 60 min. With this information, the average yield coefficient was calculated. Afterwards, the cell growth curve was calculated by combining the substrate utilization curve and the average yield coefficient for each compound alone or in a mixture. The final step was the determination of the specific growth rate for each initial substrate concentration in the feed. The specific growth rate was determined by using the cell growth curve in fitting the equation $\frac{dX}{dt} = \mu X$.

Finally, kinetic constants for each compound were determined by using the Monod kinetic model. Kaleidagraph computer software was used in estimating μ_m and K_s of the kinetic equation $\mu = \frac{\mu_m S}{K_s + S}$ by fitting the μ and S data points. Figs. 5.11 to 5.13 show the raw experimental data and the best fit substrate utilization curves as well as the calculated cell growth curves. Fig. 5.14 shows the specific growth rate versus substrate relationship. Table 5.2 gives the estimated kinetic constants for each feeding pattern.

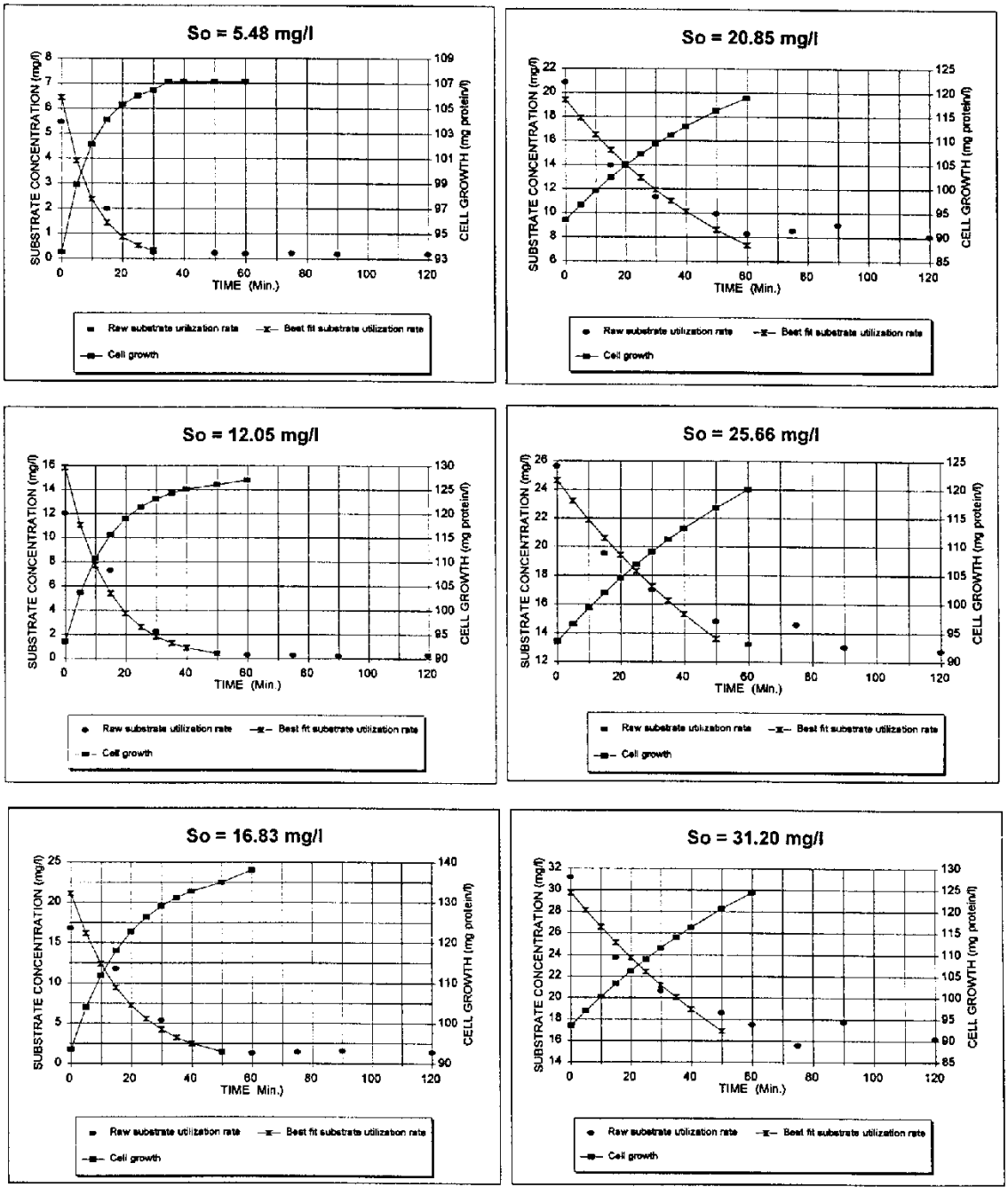

Fig. 5.11 Kinetic constant determination experiment: Substrate utilization raw data, best fit curve and the calculated cell growth curve for a reactor using toluene as sole carbon source.

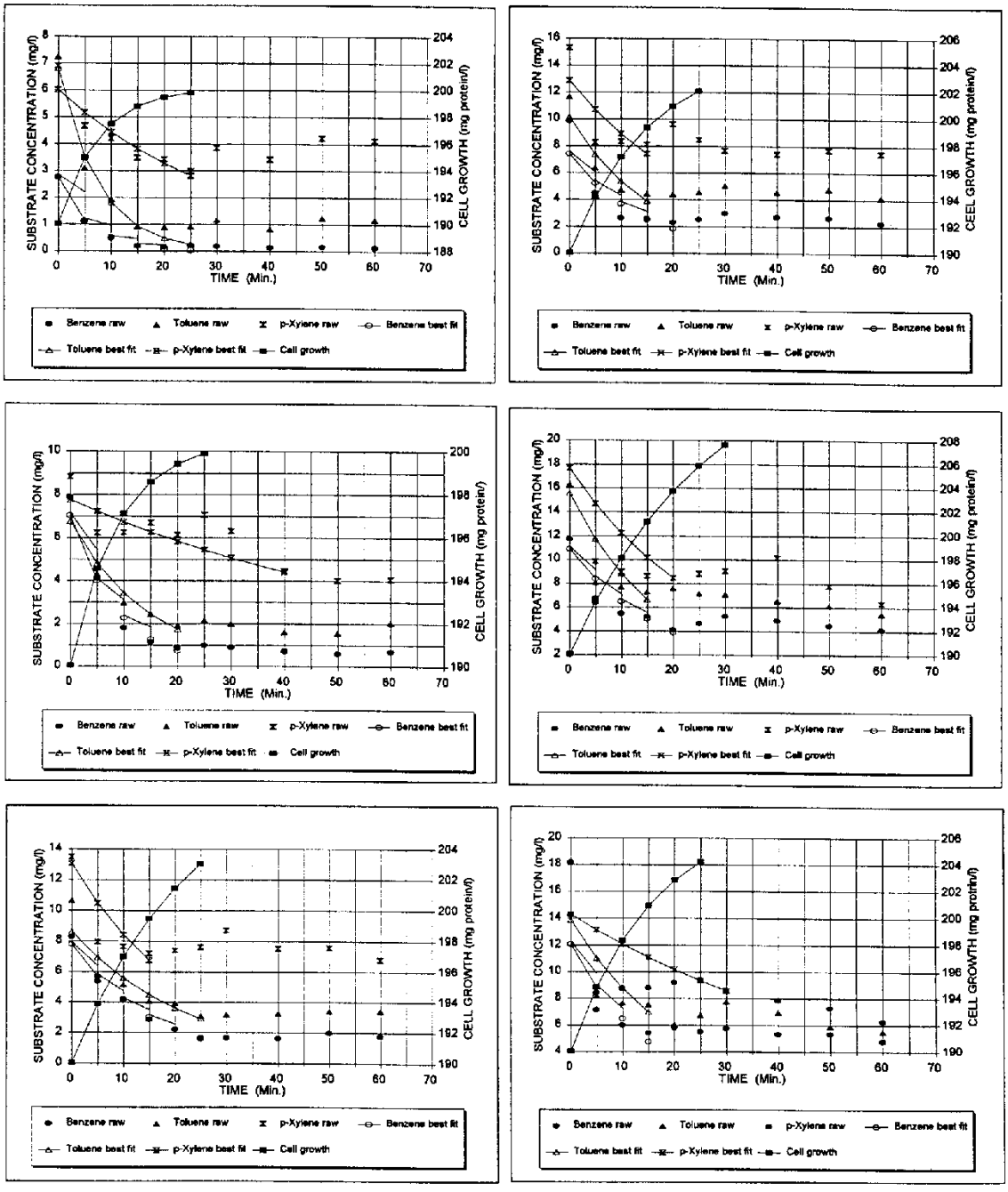

Fig. 5.12 Kinetic constant determination experiment: Substrate utilization raw data, best fit curve and the calculated cell growth curve for a reactor using BTX mixture as the carbon source.

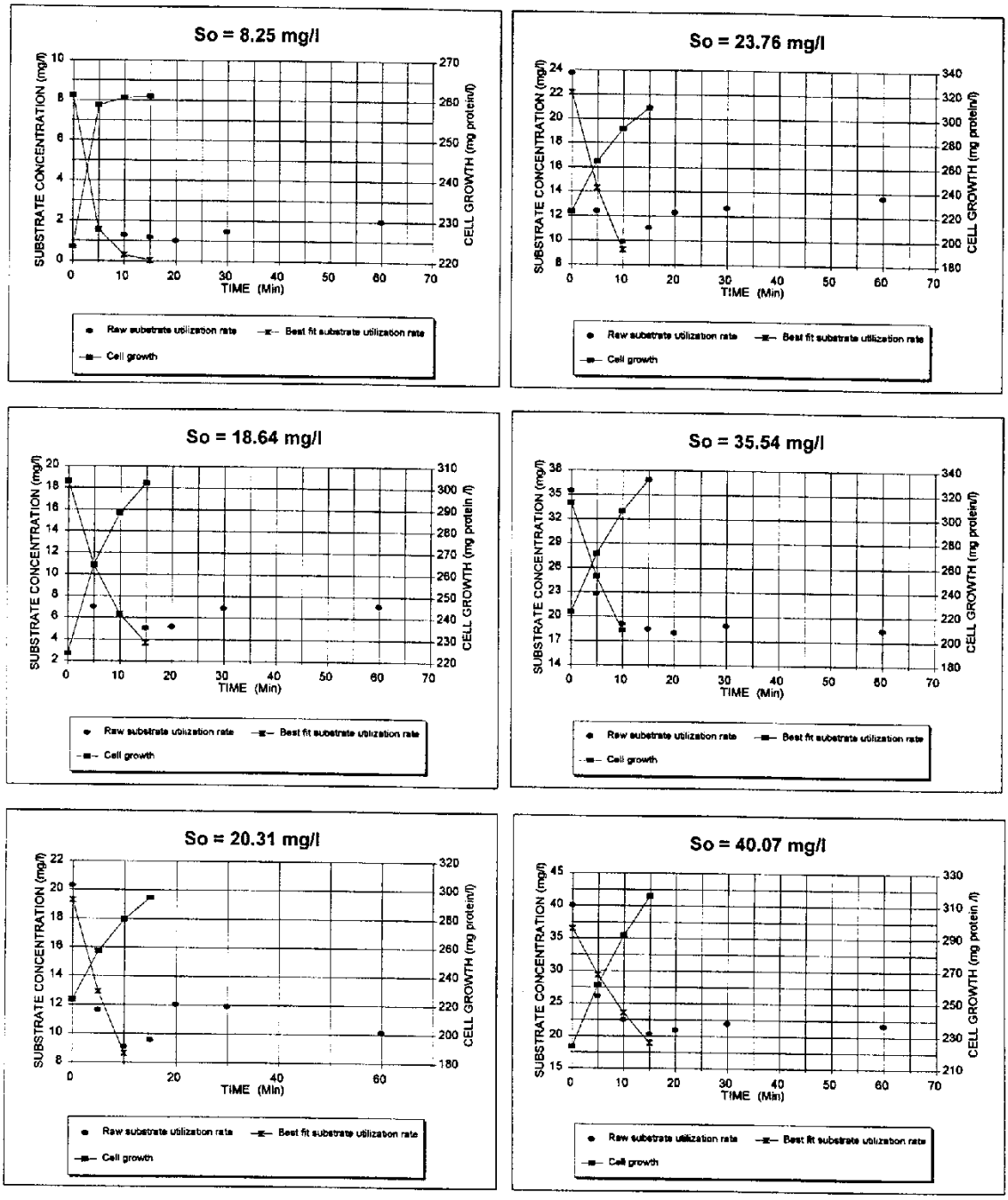

Fig. 5.13 Kinetic constant determination experiment: Substrate utilization raw data, best fit curve and the calculated cell growth curve for a reactor using benzene as sole carbon source.

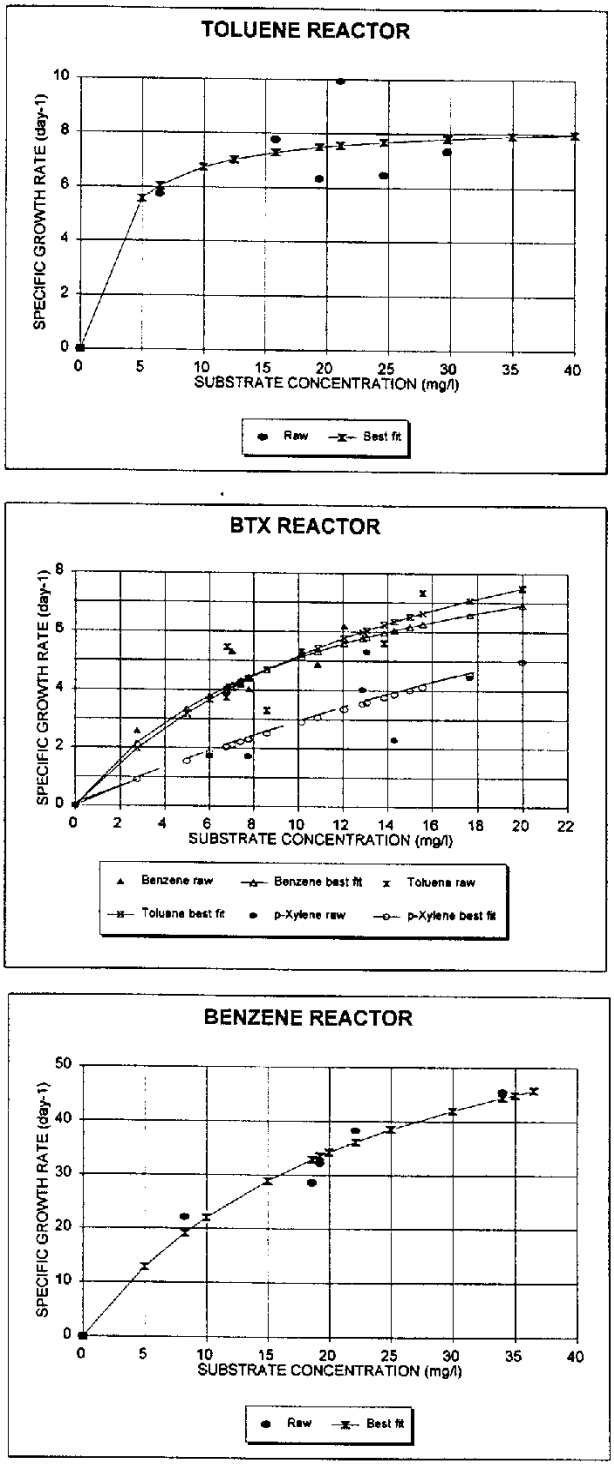

Fig. 5.14 The specific growth rate versus substrate concentration results.

Table 5.2 Biodegradation kinetics constants for a microbial consortium fed with toluene alone, benzene alone, and a BTX mixture.

CONSTANTS	COMPOUNDS				
			BTX MIXTURE		
	Toluene	Benzene	Benzene	Toluene	p-Xylene
Maximum specific growth rate μ_m (day^{-1})	8.50	76.75	10.66	13.68	19.01
Half-velocity coefficient K_s (mg/l)	2.62	24.80	10.90	16.50	56.20
r square	0.92	0.99	0.98	0.92	0.84

The maximum specific cell growth rate (μ_m) values of each of the three compounds in a BTX mixture present themselves in the following pattern: p-xylene > toluene > benzene. The half-velocity coefficient (K_s) follows the same pattern, but with more difference between compounds especially for p-xylene. For individual feeding of toluene and benzene, the maximum specific cell growth rate (μ_m) was very different; it was approximately 10 times greater for benzene than for toluene. The half-velocity coefficient (K_s) followed the same pattern. In the previous discussion, it was mentioned that toluene and cometabolic p-xylene biodegradation in a BTX mixture inhibited benzene biodegradation but strengthened the toluene and p-xylene biodegradation. Benzene as the sole carbon source was more easily and rapidly biodegraded by the

microbial consortium than toluene as the sole added carbon source. The kinetic biodegradation constants for p-xylene feeding in a BTX mixture responded only to cometabolic utilization.

CHAPTER 6

CONCLUSIONS

1. The use of granular activated carbon as a biomass carrier in a fluidized bed reactor produced a system in which both adsorption and biodegradation affected the substrate removal. During the start-up phase and before a fully functional biofilm developed, the contaminants were removed primarily by adsorption processes. However, after the biofilm was established and under steady-state conditions, substrate removal was dominated by biodegradation as determined by biomass accumulation and oxygen uptake.

2. The high percentage of substrate removals obtained in the GAC-B/FBR with more than 95% for toluene and benzene in feeding alone, 96%, 91% and 79% in a BTX mixture for benzene, toluene and p-xylene respectively showed that the microbial consortium have good capabilities for biodegrading these aromatic hydrocarbons.

3. The microbial community preacclimated for toluene degradation was not fully able to biodegrade p-xylene when added individually with less than 35% removal efficiency.

4. When p-Xylene was cometabolically utilized by the microbial consortium in the presence of benzene and toluene, approximately 80% removal was obtained.

5. Cometabolic removal of p-xylene affects the efficiency of removal for both benzene and toluene biodegradation.

6. According to the step-load increase tests, the bioreactor showed good stability and percent removal when the influent concentration was less than 7.0 mg/l; above this concentration the bioreactor became unstable and lower percent removal was observed.

7. After feeding the microbial consortium with p-xylene for 2 weeks in which period the microorganisms experienced low attainable carbon source the GAC-B/FBR preacclimated with toluene recovered toluene degradation capabilities after 3 days.

8. The maximum specific cell growth rate (μ_m) had a similar behavior for toluene feeding alone and in a BTX mixture, being 8.50 and 13.68 day^{-1}, respectively. Nevertheless, it was significantly different for benzene feeding individually and in a BTX mixture; this turned out to be 76.75 and 10.66, day^{-1}, respectively.

9. The half-velocity coefficient (K_s) had a different behavior for toluene (2.62 mg/l) and benzene (24.80 mg/l) as the sole added carbon source; it was nearly 10 times

greater for benzene. The maximum specific cell growth rate (μ_m) followed the same pattern, 8.50 and 76.75 day^{-1}, respectively.

10. In the BTX fed bioreactor, benzene biodegradation rate was reduced by the presence of toluene and p-xylene, while the biodegradation rate of both toluene and p-xylene was enhanced.

11. Benzene as the sole added carbon source was more easily and rapidly biodegraded by the microbial consortium than toluene as the sole added carbon source.

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APPENDIX A

EXPERIMENTAL DATA FOR GAC-B/FBRs SYSTEMS - REMOVAL EFFICIENCIES

Table A.1 Removal efficiencies in a GAC-B/FBR system fed with toluene as substrate.

TOLUENE REACTOR - REMOVAL PERCENT									
DAY (days)	OXYGEN UP TAKE (mg/l)	BED HEIGHT (cm)	INFLUENT 1 (mg/l)	EFFLUENT 1 (mg/l)	REMOVAL PERCENT 1 (%)	INFLUENT 2 (mg/l)	EFFLUENT 2 (mg/l)	REMOVAL PERCENT 2 (%)	REMOVAL PERCENT AVERAGE (%)
1	3.90	45.00	6.91	0.02	99.71	6.42	0.04	99.38	99.54
2	1.90	46.00	5.14	0.75	85.41	5.19	0.76	85.36	85.38
3	2.40	48.30	6.34	1.26	80.13	8.13	0.78	90.41	85.27
5	2.30	51.90	3.04	0.75	75.33	1.90	0.02	98.95	87.14
6	3.50	53.00	4.66	0.39	91.63	6.16	0.47	92.37	92.00
7	3.60	53.20	6.50	1.34	79.38	3.61	0.25	93.07	86.23
8	3.20	56.50	3.35	0.82	75.52	3.35	0.20	94.03	84.78
9	3.00	58.20	5.48	0.79	85.58	6.99	0.99	85.84	85.71
10	3.60	57.50	7.50	0.18	97.60	4.49	0.00	100.00	98.80
13	3.10	57.40	3.24	0.71	78.09	6.36	0.41	93.55	85.82
14	3.00	64.40	4.66	0.36	92.27	3.29	0.44	86.63	89.45
15	2.50	81.70	2.99	0.59	80.27	5.09	0.30	94.11	87.19
16	2.40	87.70	3.66	0.23	93.72	2.99	0.24	91.97	92.84
17	2.90	107.70	3.75	0.43	88.53	6.51	0.15	97.70	93.11
18	2.80	108.00	7.22	0.31	95.71	5.80	0.29	95.00	95.35
19	2.80	107.70	3.41	0.52	84.75	4.77	0.15	96.86	90.80
21	2.70	108.00	4.80	0.14	97.08	3.67	0.15	95.91	96.50
23	1.70	102.00	0.90	0.14	84.44	3.69	0.09	97.56	91.00
24	2.05	105.00	1.66	0.37	77.71	2.58	0.08	96.90	87.31
25	3.25	106.00	4.61	0.26	94.36	2.53	0.21	91.70	93.03
26	3.00	106.50	1.59	0.05	96.86	1.67	0.20	88.02	92.44
27	3.30	106.50	3.02	0.18	94.04	4.74	0.07	98.52	96.28
28	4.15	100.00	2.97	0.14	95.29	2.31	0.00	100.00	97.64
29	4.60	105.00	3.61	0.12	96.68	4.12	0.10	97.57	97.12
30	2.40	103.00	4.68	0.00	100.00	2.25	0.00	100.00	100.00
31	2.05	103.00	2.17	0.09	95.85	3.07	0.07	97.72	96.79
32	2.75	103.50	3.58	0.10	97.21	3.15	0.01	99.68	98.44
34	2.70	105.00	2.17	0.26	88.02	3.84	0.00	100.00	94.01
35	4.50	105.00	1.39	0.25	82.01	2.04	0.13	93.63	87.82
36	5.70	105.00	2.50	0.07	97.20	3.12	0.10	96.79	97.00
37	4.50	107.00	4.50	0.33	92.67	3.18	0.07	97.80	95.23
38	2.20	105.00	1.87	0.15	91.98	2.98	0.27	90.94	91.46
39	1.60	105.00	1.99	0.11	94.47	3.78	0.11	97.09	95.78
40	1.30	107.50	2.92	0.12	95.89	2.41	0.07	97.10	96.49
42	6.00	96.00	0.22	0.06	72.73	2.73	0.10	96.34	84.53
43	5.70	100.00	4.63	0.12	97.41	4.49	0.11	97.55	97.48
44	4.00	108.00	0.54	0.00	100.00	2.30	0.06	97.39	98.70
46	6.00	97.00	0.31	0.00	100.00	1.97	0.12	93.91	96.95
47	1.40	95.50	2.29	0.05	97.82	2.19	0.26	88.13	92.97
48	0.60	100.00	0.03	0.00	100.00	0.50	0.00	100.00	100.00
49	0 60	102.00	0.06	0.00	100.00	0.44	0.00	100.00	100.00
50	1.00	104.50	1.36	0.07	94.85	3.17	0.06	98.11	96.48
51	0.70	108.00	1.74	0.12	93.10	3.20	0.00	100.00	96.55
Average	2.96		3.25	0.30	90.96	3.66	0.18	95.43	93.20
Std-Dev	1.38		1.97	0.33	8.04	1.70	0.22	4.18	4.95

Table A.2 Benzene removal efficiencies in a GAC-B/FBR system fed with BTX mixture as substrate.

BTX REACTOR - REMOVAL PERCENT BENZENE									
DAY (days)	OXYGEN UP TAKE (mg/l)	BED HEIGHT (cm)	INFLUENT 1 (mg/l)	EFFLUENT 1 (mg/l)	REMOVAL PERCENT 1 (%)	INFLUENT 2 (mg/l)	EFFLUENT 2 (mg/l)	REMOVAL PERCENT 2 (%)	REMOVAL PERCENT AVERAGE (%)
1	4.00	26.00	1.80	0.00	100.00	1.85	0.00	100.00	100.00
2	3.40	26.00	2.22	0.00	100.00	1.25	0.00	100.00	100.00
3	2.20	25.60	3.04	0.08	97.37	4.08	0.06	98.53	97.95
5	2.70	25.51	1.44	0.00	100.00	1.47	0.15	89.80	94.90
6	1.75	25.51	1.15	0.00	100.00	1.38	0.15	89.13	94.57
7	1.50	25.30	3.31	0.00	100.00	1.17	0.12	89.74	94.87
8	2.10	25.40	0.99	0.11	88.89	1.59	0.09	94.34	91.61
9	1.40	25.40	1.12	0.08	92.86	1.23	0.08	93.50	93.18
10	1.95	26.60	1.60	0.11	93.13	1.01	0.09	91.09	92.11
13	2.15	27.50	0.80	0.10	87.50	0.51	0.06	88.24	87.87
14	2.20	28.00	0.84	0.11	86.90	1.07	0.09	91.59	89.25
15	1.30	28.20	0.87	0.09	89.66	1.68	0.05	97.02	93.34
16	2.45	29.00	0.88	0.00	100.00	1.00	0.12	88.00	94.00
17	2.00	29.10	0.45	0.04	91.11	0.60	0.00	100.00	95.56
18	2.10	42.80	0.38	0.00	100.00	0.44	0.00	100.00	100.00
19	2.30	41.30	1.06	0.00	100.00	0.57	0.00	100.00	100.00
21	1.60	45.00	0.26	0.00	100.00	0.47	0.00	100.00	100.00
23	2.00	44.50	0.65	0.00	100.00	0.60	0.00	100.00	100.00
24	2.10	45.30	0.96	0.00	100.00	1.55	0.09	94.19	97.10
25	2.75	45.00	0.86	0.00	100.00	1.38	0.05	96.38	98.19
26	3.50	44.50	0.89	0.00	100.00	0.92	0.00	100.00	100.00
27	3.55	44.50	0.58	0.00	100.00	1.56	0.00	100.00	100.00
28	4.00	47.00	0.67	0.02	97.01	0.61	0.00	100.00	98.51
29	4.60	47.00	0.75	0.08	89.33	1.12	0.00	100.00	94.67
30	2.30	46.50	0.61	0.04	93.44	1.09	0.00	100.00	96.72
31	2.45	60.00	0.76	0.00	100.00	0.71	0.00	100.00	100.00
32	3.30	57.70	0.62	0.00	100.00	1.02	0.00	100.00	100.00
34	2.60	57.00	0.37	0.00	100.00	0.26	0.00	100.00	100.00
35	4.20	58.00	0.60	0.06	90.00	0.65	0.00	100.00	95.00
36	4.65	58.80	0.65	0.00	100.00	0.65	0.00	100.00	100.00
37	4.00	58.20	0.60	0.04	93.33	0.53	0.07	86.79	90.06
38	2.05	58.00	0.99	0.00	100.00	0.52	0.03	94.23	97.12
39	2.00	57.50	0.36	0.03	91.67	0.50	0.03	94.00	92.83
40	2.00	51.00	0.73	0.05	93.15	0.47	0.00	100.00	96.58
42	6.00	50.50	1.50	0.00	100.00	1.55	0.00	100.00	100.00
43	5.00	51.00	0.87	0.00	100.00	0.52	0.01	98.08	99.04
44	5.70	51.80	1.13	0.00	100.00	0.61	0.06	90.16	95.08
46	5.20	51.20	1.00	0.00	100.00	0.93	0.09	90.32	95.16
47	2.75	50.00	0.92	0.08	91.30	0.41	0.02	95.12	93.21
48	2.00	48.00	0.70	0.09	87.14	0.68	0.09	86.76	86.95
49	1.85	46.50	0.60	0.11	81.67	0.56	0.05	91.07	86.37
50	1.60	47.50	0.96	0.00	100.00	0.87	0.00	100.00	100.00
51	1.90	50.00	0.59	0.00	100.00	0.49	0.03	93.88	96.94
Average	2.82		0.98	0.03	96.17	0.98	0.04	95.86	96.02
Std- Dev	1.23		0.63	0.04	5.16	0.64	0.05	4.65	3.95

Table A.3 Toluene removal efficiencies in a GAC-B/FBR system fed with BTX mixture as substrate.

BTX REACTOR - REMOVAL PERCENT TOLUENE									
DAY (days)	OXYGEN UP TAKE (mg/l)	BED HEIGHT (cm)	INFLUENT 1 (mg/l)	EFFLUENT 1 (mg/l)	REMOVAL PERCENT 1 (%)	INFLUENT 2 (mg/l)	EFFLUENT 2 (mg/l)	REMOVAL PERCENT 2 (%)	REMOVAL PERCENT AVERAGE (%)
1	4.00	26.00	2.21	0.02	99.10	2.30	0.02	99.13	99.11
2	3.40	26.00	4.01	0.16	96.01	1.48	0.00	100.00	98.00
3	2.20	25.60	3.95	0.39	90.13	4.12	0.15	96.36	93.24
5	2.70	25.51	1.94	0.30	84.54	1.80	0.25	86.11	85.32
6	1.75	25.51	1.26	0.20	84.13	1.83	0.29	84.15	84.14
7	1.50	25.30	4.48	0.17	96.21	1.45	0.20	86.21	91.21
8	2.10	25.40	1.96	0.38	80.61	2.59	0.25	90.35	85.48
9	1.40	25.40	1.86	0.37	80.11	1.69	0.26	84.62	82.36
10	1.95	26.60	2.08	0.37	82.21	1.29	0.15	88.37	85.29
13	2.15	27.50	0.52	0.10	80.77	0.70	0.10	85.71	83.24
14	2.20	28.00	1.43	0.25	82.52	1.48	0.18	87.84	85.18
15	1.30	28.20	1.71	0.29	83.04	2.55	0.18	92.94	87.99
16	2.45	29.00	1.78	0.12	93.26	1.65	0.12	92.73	92.99
17	2.00	29.10	0.49	0.09	81.63	1.27	0.15	88.19	84.91
18	2.10	42.80	0.52	0.00	100.00	0.73	0.08	89.04	94.52
19	2.30	41.30	2.57	0.00	100.00	1.02	0.00	100.00	100.00
21	1.60	45.00	0.31	0.00	100.00	0.65	0.00	100.00	100.00
23	2.00	44.50	0.98	0.00	100.00	1.20	0.00	100.00	100.00
24	2.10	45.30	1.53	0.07	95.42	2.48	0.34	86.29	90.86
25	2.75	45.00	1.27	0.00	100.00	1.94	0.31	84.02	92.01
26	3.50	44.50	1.21	0.00	100.00	1.63	0.00	100.00	100.00
27	3.55	44.50	0.76	0.06	92.11	2.55	0.00	100.00	96.05
28	4.00	47.00	1.00	0.12	88.00	0.92	0.13	85.87	86.93
29	4.60	47.00	0.69	0.10	85.51	1.68	0.08	95.24	90.37
30	2.30	46.50	0.86	0.13	84.88	1.62	0.12	92.59	88.74
31	2.45	60.00	1.45	0.15	89.66	1.02	0.16	84.31	86.98
32	3.30	57.70	1.08	0.11	89.81	1.73	0.28	83.82	86.81
34	2.60	57.00	0.66	0.11	83.33	0.84	0.11	86.90	85.12
35	4.20	58.00	0.85	0.15	82.35	1.04	0.15	85.58	83.96
36	4.65	58.80	1.01	0.19	81.19	1.16	0.12	89.66	85.42
37	4.00	58.20	1.13	0.14	87.61	0.72	0.07	90.28	88.94
38	2.05	58.00	1.43	0.16	88.81	0.86	0.09	89.53	89.17
39	2.00	57.50	0.64	0.00	100.00	0.84	0.00	100.00	100.00
40	2.00	51.00	1.04	0.14	86.54	0.85	0.05	94.12	90.33
42	6.00	50.50	1.50	0.03	98.00	1.53	0.12	92.16	95.08
43	5.00	51.00	1.23	0.09	92.68	0.67	0.04	94.03	93.36
44	5.70	51.80	1.68	0.02	98.81	0.78	0.08	89.74	94.28
46	5.20	51.20	0.95	0.02	97.89	1.10	0.00	100.00	98.95
47	2.75	50.00	0.73	0.07	90.41	0.89	0.08	91.01	90.71
48	2.00	48.00	0.75	0.00	100.00	0.80	0.00	100.00	100.00
49	1.85	46.50	0.64	0.00	100.00	0.60	0.00	100.00	100.00
50	1.60	47.50	1.16	0.18	84.48	1.01	0.12	88.12	86.30
51	1.90	50.00	0.90	0.13	85.56	0.42	0.05	88.10	86.83
Average	2.82		1.40	0.13	90.64	1.38	0.11	91.70	91.17
Std-Dev.	1.23		0.92	0.11	7.18	0.72	0.10	5.74	5.77

Table A.4 p-Xylene removal efficiencies in a GAC-B/FBR system fed with BTX mixture as substrate.

BTX REACTOR - REMOVAL PERCENT p-XYLENE									
DAY (days)	OXYGEN UP TAKE (mg/l)	BED HEIGHT (cm)	INFLUENT 1 (mg/l)	EFFLUENT 1 (mg/l)	REMOVAL PERCENT 1 (%)	INFLUENT 2 (mg/l)	EFFLUENT 2 (mg/l)	REMOVAL PERCENT 2 (%)	REMOVAL PERCENT AVERAGE (%)
1	4.00	26.00	1.95	0.14	92.82	2.00	0.18	91.00	91.91
2	3.40	26.00	5.67	0.39	93.12	2.32	0.21	90.95	92.03
3	2.20	25.60	4.78	0.85	82.22	3.87	0.42	89.15	85.68
5	2.70	25.51	2.78	0.55	80.22	2.54	1.12	55.91	68.06
6	1.75	25.51	1.67	0.54	67.66	2.08	0.67	67.79	67.73
7	1.50	25.30	5.24	0.42	91.98	2.05	0.71	65.37	78.68
8	2.10	25.40	3.03	0.54	82.18	3.37	0.46	86.35	84.26
9	1.40	25.40	2.66	0.65	75.56	2.34	0.44	81.20	78.38
10	1.95	26.60	2.96	0.80	72.97	2.02	0.86	57.43	65.20
13	2.15	27.50	3.17	0.89	71.92	1.10	0.30	72.73	72.33
14	2.20	28.00	2.04	0.56	72.55	2.05	0.70	65.85	69.20
15	1.30	28.20	2.39	0.62	74.06	3.18	0.37	88.36	81.21
16	2.45	29.00	2.58	0.35	86.43	2.28	0.66	71.05	78.74
17	2.00	29.10	0.68	0.21	69.12	1.91	0.40	79.06	74.09
18	2.10	42.80	0.82	0.29	64.63	1.30	0.27	79.23	71.93
19	2.30	41.30	4.11	1.12	72.75	1.90	0.15	92.11	82.43
21	1.60	45.00	0.60	0.16	73.33	0.99	0.10	89.90	81.62
23	2.00	44.50	1.20	0.10	91.67	1.35	0.30	77.78	84.72
24	2.10	45.30	1.98	0.38	80.81	3.28	0.87	73.48	77.14
25	2.75	45.00	1.78	0.16	91.01	2.46	0.65	73.58	82.29
26	3.50	44.50	1.67	0.10	94.01	2.37	0.25	89.45	91.73
27	3.55	44.50	0.87	0.23	73.56	3.26	0.07	97.85	85.71
28	4.00	47.00	1.23	0.24	80.49	1.22	0.35	71.31	75.90
29	4.60	47.00	0.98	0.25	74.49	2.48	0.36	85.48	79.99
30	2.30	46.50	1.26	0.32	74.60	1.79	0.32	82.12	78.36
31	2.45	60.00	1.93	0.57	70.47	1.51	0.46	69.54	70.00
32	3.30	57.70	1.53	0.29	81.05	2.46	0.85	65.45	73.25
34	2.60	57.00	1.06	0.37	65.09	0.77	0.26	66.23	65.66
35	4.20	58.00	1.23	0.30	75.61	1.51	0.57	62.25	68.93
36	4.65	58.80	1.66	0.43	74.10	1.35	0.42	68.89	71.49
37	4.00	58.20	1.34	0.28	79.10	0.78	0.25	67.95	73.53
38	2.05	58.00	1.80	0.35	80.56	1.02	0.29	71.57	76.06
39	2.00	57.50	0.99	0.27	72.73	1.14	0.27	76.32	74.52
40	2.00	51.00	1.33	0.31	76.69	1.16	0.27	76.72	76.71
42	6.00	50.50	1.25	0.28	77.60	1.20	0.23	80.83	79.22
43	5.00	51.00	1.39	0.19	86.33	0.92	0.15	83.70	85.01
44	5.70	51.80	1.59	0.16	89.94	0.99	0.08	91.92	90.93
46	5.20	51.20	1.15	0.20	82.61	1.15	0.10	91.30	86.96
47	2.75	50.00	0.95	0.15	84.21	1.09	0.08	92.66	88.44
48	2.00	48.00	0.90	0.12	86.67	0.98	0.13	86.73	86.70
49	1.85	46.50	0.85	0.10	88.24	0.76	0.13	82.89	85.57
50	1.60	47.50	1.35	0.22	83.70	1.04	0.3	71.15	77.43
51	1.90	50.00	1.04	0.35	66.35	0.67	0.18	73.13	69.74
Average	2.82		1.89	0.37	79.19	1.77	0.38	77.99	78.59
Std-Dev.	1.23		1.20	0.23	8.17	0.82	0.25	10.60	7.52

Table A.5 Removal efficiencies in a GAC-B/FBR system fed with benzene as substrate.

BENZENE REACTOR - REMOVAL PERCENT									
DAY (days)	OXYGEN UP TAKE (mg/l)	BED HEIGHT (cm)	INFLUENT 1 (mg/l)	EFFLUENT 1 (mg/l)	REMOVAL PERCENT 1 (%)	INFLUENT 2 (mg/l)	EFFLUENT 2 (mg/l)	REMOVAL PERCENT 2 (%)	REMOVAL PERCENT AVERAGE (%)
1	2.20	27.00	2.26	0.15	93.36	2.43	0.10	95.88	94.62
2	2.28	28.00	2.30	0.20	91.30	2.65	0.23	91.32	91.31
3	2.50	29.00	2.19	0.35	84.02	2.12	0.29	86.32	85.17
4	1.60	28.50	2.65	0.00	100.00	2.36	0.06	97.46	98.73
5	2.15	27.00	3.50	0.30	91.43	3.80	0.57	85.00	88.21
6	2.50	30.00	4.17	0.18	95.68	2.95	0.31	89.49	92.59
7	3.00	30.00	5.01	0.18	96.41	4.06	0.12	97.04	96.73
8	2.95	30.50	4.98	0.15	96.99	4.59	0.10	97.82	97.40
9	2.80	31.20	4.44	0.12	97.30	4.26	0.08	98.12	97.71
10	2.40	32.00	3.23	0.15	95.36	3.72	0.11	97.04	96.20
11	2.00	32.50	3.13	0.25	92.01	3.88	0.17	95.62	93.82
12	2.00	33.50	6.94	0.40	94.24	3.02	0.15	95.03	94.63
13	2.50	34.00	4.06	0.22	94.58	3.94	0.18	95.43	95.01
14	3.70	38.00	3.82	0.16	95.81	4.05	0.36	91.11	93.46
15	3.00	42.00	3.02	0.14	95.36	3.32	0.16	95.18	95.27
16	2.30	43.80	3.68	0.13	96.47	4.01	0.15	96.26	96.36
17	3.85	45.50	4.00	0.23	94.25	4.45	0.25	94.38	94.32
18	4.00	45.50	4.57	0.31	93.22	4.29	0.19	95.57	94.39
19	3.90	45.00	4.21	0.25	94.06	4.07	0.17	95.82	94.94
20	3.80	43.00	3.96	0.20	94.95	4.19	0.18	95.70	95.33
21	3.20	42.00	3.41	0.16	95.31	3.85	0.15	96.10	95.71
22	1.30	49.00	3.08	0.22	92.86	3.33	0.20	93.99	93.43
23	3.28	56.00	3.25	0.15	95.38	3.61	0.17	95.29	95.34
24	3.30	65.00	3.30	0.11	96.67	3.55	0.14	96.06	96.36
25	3.80	69.00	3.54	0.13	96.33	3.76	0.15	96.01	96.17
26	3.15	73.00	3.02	0.12	96.03	3.48	0.13	96.26	96.15
27	4.00	78.00	2.98	0.15	94.97	3.32	0.16	95.18	95.07
28	3.80	86.00	3.67	0.13	96.46	3.41	0.10	97.07	96.76
Average	2.90		3.66	0.19	94.67	3.59	0.18	94.70	94.69
Std-Dev.	0.78		0.98	0.08	2.81	0.64	0.10	3.22	2.78

Table A.6 Removal efficiencies in a GAC-B/FBR system fed with p-xylene as substrate.

p-XYLENE REACTOR - REMOVAL PERCENT									
DAY (days)	OXYGEN UP TAKE (mg/l)	BED HEIGHT (cm)	INFLUENT 1 (mg/l)	EFFLUENT 1 (mg/l)	REMOVAL PERCENT 1 (%)	INFLUENT 2 (mg/l)	EFFLUENT 2 (mg/l)	REMOVAL PERCENT 2 (%)	REMOVAL PERCENT AVERAGE (%)
1	0.60	97.00	2.54	2.41	5.12	2.44	2.30	5.74	5.43
2	0.50	88.00	2.96	2.80	5.41	2.85	2.60	8.77	7.09
3	0.50	82.00	1.07	0.83	22.43	1.23	1.00	18.70	20.56
4	0.50	79.00	0.88	0.66	25.00	0.78	0.74	5.13	15.06
5	0.50	74.50	1.52	1.44	5.26	1.25	0.97	22.40	13.83
6	0.60	71.00	2.76	2.40	13.04	2.68	2.28	14.93	13.98
7	0.60	67.00	2.21	1.96	11.31	2.07	1.40	32.37	21.84
8	0.50	60.00	1.47	0.92	37.41	1.60	1.03	35.63	36.52
9	0.90	54.00	0.88	0.55	37.50	0.99	0.70	29.29	33.40
10	1.40	50.00	0.49	0.28	42.86	0.63	0.47	25.40	34.13
11	1.00	45.00	0.43	0.27	37.21	0.48	0.30	37.50	37.35
12	2.00	43.50	1.53	0.98	35.95	1.20	0.95	20.83	28.39
13	1.10	38.50	1.16	1.04	10.34	1.33	1.04	21.80	16.07
14	1.50	38.00	1.10	0.86	21.82	1.04	0.79	24.04	22.93
Average	0.87		1.50	1.24	22.19	1.47	1.18	21.61	21.90
Std-Dev.	0.47		0.82	0.83	13.92	0.76	0.71	10.33	10.66

Table A.7 Removal efficiencies in a GAC-B/FBR system fed with toluene as substrate during recovery-time study following p-xylene feeding.

RECOVERY TIME STUDY TOLUENE REACTOR - REMOVAL PERCENT									
DAY (days)	OXYGEN UP TAKE (mg/l)	BED HEIGHT (cm)	INFLUENT 1 (mg/l)	EFFLUENT 1 (mg/l)	REMOVAL PERCENT 1 (%)	INFLUENT 2 (mg/l)	EFFLUENT 2 (mg/l)	REMOVAL PERCENT 2 (%)	REMOVAL PERCENT AVERAGE (%)
1	0.50	23.50	3.75	2.63	29.87	3.89	2.80	28.02	28.94
2	0.90	24.50	3.50	2.28	34.86	3.42	2.21	35.38	35.12
3	1.15	23.00	2.87	1.81	36.93	2.84	1.51	46.83	41.88
4	1.50	27.00	4.43	2.44	44.92	5.03	2.90	42.35	43.63
5	2.10	30.00	6.10	2.14	64.92	5.84	1.87	67.98	66.45
6	2.40	35.00	7.99	2.00	74.97	7.76	1.55	80.03	77.50
7	2.70	40.00	8.76	1.11	87.33	8.90	1.20	86.52	86.92
8	1.80	50.00	8.54	1.81	78.81	8.31	1.10	86.76	82.78
9	2.30	52.00	10.25	1.54	84.98	9.88	1.35	86.34	85.66
10	2.20	50.00	11.78	1.96	83.36	10.40	1.56	85.00	84.18
11	1.90	51.00	8.60	1.55	81.98	8.73	1.70	80.53	81.25
12	2.00	54.00	6.57	1.16	82.34	5.90	0.89	84.92	83.63
13	3.30	58.00	4.70	0.80	82.98	5.03	0.86	82.90	82.94
14	2.50	66.50	5.83	1.13	80.62	5.44	0.92	83.09	81.85
Average	1.95		6.69	1.74	67.78	6.53	1.60	69.76	68.77
Std-Dev.	0.74		2.71	0.55	21.32	2.45	0.66	21.63	21.40

APPENDIX B

OPERATIONAL CONDITIONS IN GAC-B/FBRs SYSTEMS

Table B.1 Operational conditions in a GAC-B/FBR system fed with toluene

TOLUENE REACTOR					
DAY	TEMPERATURE	pH		DISSOLVED OXYGEN	
		INFLUENT	EFFLUENT	INFLUENT	EFFLUENT
(days)	(oC)				
1	22.00	7.84	7.27	8.10	4.20
2	22.00	7.54	7.14	8.60	6.70
3	22.00	7.50	6.90	8.60	6.20
5	26.00	7.81	7.38	8.40	6.10
6	23.00	7.78	7.32	8.70	5.20
7	23.00	7.60	7.09	8.70	5.10
8	22.00	7.83	7.36	8.00	4.80
9	22.00	7.53	7.05	7.90	4.90
10	22.00	7.68	7.20	7.70	4.10
13	23.50	7.55	7.24	7.60	4.50
14	23.00	7.65	7.40	7.50	4.50
15	23.00	7.69	7.36	6.80	4.30
16	22.00	7.50	7.11	8.00	5.60
17	22.00	7.60	7.40	7.40	4.50
18	25.50	7.40	7.05	6.60	3.80
19	27.00	7.38	7.07	6.25	3.45
21	23.50	7.70	7.39	7.00	4.30
23	23.00	7.40	7.14	7.10	5.40
24	22.50	7.62	7.29	6.75	4.70
25	27.00	7.05	7.05	11.80	8.55
26	27.00	6.90	6.86	9.80	6.80
27	25.00	7.11	6.98	11.60	8.30
28	23.50	6.90	6.84	13.15	9.00
29	23.00	7.15	7.03	16.35	11.75
30	24.00	6.94	6.85	9.00	6.60
31	23.00	6.90	6.80	11.25	9.20
32	26.00	7.31	7.20	11.05	8.30
34	24.00	7.46	7.29	11.05	8.35
35	24.00	7.55	7.38	18.30	13.80
36	24.00	7.40	7.18	19.50	13.80
37	23.50	7.45	7.30	15.70	11.20
38	23.00	7.54	7.10	7.05	4.85
39	26.50	7.58	7.29	6.25	4.65
40	26.00	7.50	7.30	5.90	4.60
42	25.00	7.41	7.31	18.20	12.20
43	24.00	7.34	7.24	18.20	12.50
44	24.00	7.32	7.20	15.00	11.00
46	28.00	7.69	7.53	19.70	13.70
47	27.00	7.40	7.20	6.40	5.00
48	24.50	7.90	7.77	8.40	7.80
49	24.50	7.43	7.20	8.30	7.70
50	24.00	7.59	7.48	6.10	5.10
51	23.00	7.46	7.38	6.00	5.30
Averag	24.01	7.46	7.21	9.99	7.03
Std-Dev	1.68	0.26	0.20	4.11	3.07

Table B.2 Operational conditions in a GAC-B/FBR system fed with BTX mixture

BTX REACTOR					
DAY	TEMPERATURE	pH		DISSOLVED OXYGEN	
		INFLUENT	EFFLUENT	INFLUENT	EFFLUENT
(days)	(oC)				
1	22.00	8.03	7.56	9.50	5.50
2	22.00	7.63	7.15	9.70	6.30
3	22.00	7.40	6.97	8.00	5.80
5	26.00	7.84	7.41	8.20	5.50
6	23.00	7.83	7.57	8.65	6.90
7	23.00	7.64	7.29	8.30	6.80
8	22.00	7.93	7.59	8.20	6.10
9	22.00	7.72	7.42	7.90	6.50
10	22.00	7.82	7.50	7.85	5.90
13	23.50	7.81	7.53	8.35	6.20
14	23.00	7.93	7.60	8.00	5.80
15	23.00	7.94	7.68	7.60	6.30
16	22.00	7.79	7.43	8.55	6.10
17	22.00	7.85	7.50	8.10	6.10
18	25.50	7.53	7.22	6.60	4.50
19	27.00	7.66	7.28	6.80	4.50
21	23.50	7.91	7.65	7.80	6.20
23	23.00	7.83	7.60	8.80	6.80
24	22.50	7.80	7.54	7.55	5.45
25	27.00	7.12	7.08	13.25	10.50
26	27.00	7.09	6.91	10.80	7.30
27	25.00	7.30	7.15	12.40	8.85
28	23.50	7.10	6.95	15.60	11.60
29	23.00	7.24	7.08	17.35	12.75
30	24.00	7.14	6.94	10.20	7.90
31	23.00	7.02	6.90	11.90	9.45
32	26.00	7.49	7.29	12.35	9.05
34	23.50	7.57	7.32	11.30	8.70
35	24.00	7.65	7.40	17.80	13.60
36	24.00	7.67	7.20	19.00	14.35
37	23.50	7.50	7.31	15.85	11.85
38	23.00	7.59	7.21	7.10	5.05
39	26.50	7.63	7.30	6.30	4.30
40	26.00	7.45	7.20	6.20	4.20
42	25.00	7.40	7.23	17.90	11.90
43	24.00	7.44	7.20	17.60	12.60
44	24.00	7.32	7.02	14.10	8.40
46	28.00	7.63	7.42	12.80	7.60
47	27.00	7.50	7.11	6.70	3.95
48	24.50	8.00	7.65	8.60	7.35
49	24.50	7.61	7.33	8.50	7.40
50	24.00	7.87	7.56	7.40	5.80
51	23.00	7.90	7.46	7.20	5.30
Averag	24.00	7.61	7.32	10.29	7.51
Std-Dev	1.69	0.27	0.22	3.70	2.73

Table B.3 Operational conditions in a GAC-B/FBR system fed with benzene

BENZENE REACTOR					
DAY	TEMPERATURE	pH		DISSOLVED OXYGEN	
		INFLUENT	EFFLUENT	INFLUENT	EFFLUENT
(days)	(oC)				
1	20.50	7.77	7.52	7.50	5.30
2	20.50	7.70	7.45	7.45	5.10
3	20.00	7.64	7.35	7.40	4.90
4	21.00	7.65	7.45	6.80	5.20
5	20.50	7.46	7.27	5.85	3.70
6	19.50	7.60	7.31	6.60	4.10
7	18.00	7.61	7.32	7.10	3.80
8	18.00	7.58	7.29	6.80	3.70
9	19.00	7.55	7.25	6.50	3.50
10	19.50	7.52	7.32	6.20	3.80
11	19.50	7.42	7.30	5.70	3.70
12	20.50	7.32	7.20	6.30	4.30
13	18.00	7.37	7.18	6.50	4.00
14	19.00	7.30	7.05	7.30	3.60
15	18.50	7.16	6.90	6.60	3.60
16	17.50	7.20	6.92	6.80	3.70
17	17.00	7.23	6.97	7.00	3.15
18	18.00	7.20	6.90	6.50	3.80
19	19.00	7.15	6.93	6.80	4.00
20	22.00	7.46	7.17	6.60	3.70
21	21.50	7.52	7.15	6.40	3.20
22	25.00	7.39	7.22	5.00	3.70
23	23.00	7.20	7.02	6.50	3.90
24	21.00	7.07	6.61	6.90	3.60
25	23.00	7.30	7.05	6.80	3.75
26	22.00	7.49	7.10	6.90	3.55
27	24.00	7.64	7.21	6.90	2.90
28	21.00	7.15	6.62	7.30	3.50
Averag	20.21	7.42	7.14	6.68	3.88
Std-Dev	2.01	0.20	0.23	0.55	0.59

Table B.4 Operational conditions in a GAC-B/FBR system fed with p-xylene

p-XYLENE REACTOR					
DAY	TEMPERATURE	pH		DISSOLVED OXYGEN	
		INFLUENT	EFFLUENT	INFLUENT	EFFLUENT
(days)	(oC)				
1	21.00	7.78	7.81	8.60	8.00
2	20.50	7.85	7.87	8.80	8.30
3	20.00	7.91	7.96	9.10	8.60
4	21.00	7.99	7.96	8.70	8.20
5	20.50	7.96	7.98	8.70	8.20
6	19.50	7.95	7.98	8.90	8.30
7	18.00	7.98	7.94	9.40	8.80
8	19.00	7.96	7.90	9.20	8.70
9	18.50	7.99	7.93	9.10	8.20
10	19.50	7.95	7.76	9.00	7.60
11	19.50	8.00	7.82	8.90	7.90
12	20.50	7.71	7.46	9.10	7.10
13	18.00	7.71	7.54	8.80	7.70
14	19.00	7.54	7.39	8.60	7.10
Averag	19.61	7.88	7.81	8.92	8.05
Std-Dev	1.02	0.14	0.20	0.24	0.53

Table B.5 Operational conditions in a GAC-B/FBR system fed with toluene during recovery-time study.

RECOVERY TIME STUDY TOLUENE REACTOR					
DAY	TEMPERATURE	pH		DISSOLVED OXYGEN	
		INFLUENT	EFFLUENT	INFLUENT	EFFLUENT
(days)	(oC)				
1	18.50	7.44	7.33	8.80	8.30
2	18.00	7.47	7.32	9.00	8.10
3	17.00	7.51	7.33	9.15	8.00
4	18.00	7.40	7.25	8.80	7.30
5	18.50	7.42	7.30	8.30	6.20
6	19.00	7.30	7.15	7.70	5.30
7	20.50	7.24	7.11	7.00	4.30
8	25.00	7.43	7.21	5.70	3.90
9	26.00	7.20	7.00	7.20	4.90
10	20.50	7.00	6.80	6.90	4.70
11	22.00	7.15	6.92	6.80	4.90
12	23.00	7.25	7.04	6.75	4.75
13	24.00	7.49	7.23	6.70	3.40
14	22.00	6.81	6.58	6.50	4.00
Averag	20.86	7.29	7.11	7.52	5.58
Std-Dev	2.87	0.20	0.22	1.10	1.69

APPENDIX C

ORGANIC STEP-LOADING INCREASE IN GAC-B/FBRs SYSTEMS

Table C.1 Responses for 48-hr. 2-fold step-load increase in a GAC-B/FB system fed with toluene as carbon source.

ORGANIC LOADING STEPS TOLUENE REACTOR				
TIME	OXYGEN	SUBSTRATE CONCENTRATION		
	UP TAKE	INFLUENT	EFFLUENT	REMOVAL PERCENT
(hours)	(mg/l)	(mg/l)	(mg/l)	(%)
0.00	1.95	5.48	0.60	89.05
4.00	1.80	5.06	0.89	82.41
18.50	3.30	6.49	0.37	94.30
24.00	3.55	6.85	0.04	99.42
27.50	3.15	6.70	0.09	98.66
42.50	3.10	6.43	0.43	93.31
45.50	3.10	7.21	0.55	92.37
48.00	3.10	8.91	0.98	89.00
48.50	2.85	17.43	2.88	83.48
51.50	2.70	18.50	3.18	82.81
66.50	2.00	20.79	3.95	81.00
69.50	2.35	18.12	4.46	75.39
72.50	2.90	17.20	4.50	73.84
75.50	3.20	14.57	3.93	73.03
91.00	2.85	15.00	4.00	73.33
96.50	2.90	15.50	4.07	73.74

Table C.2 Benzene responses for 48-hr. 2-fold step-load increase in a GAC-B/FBR system, fed with a BTX mixture as carbon source.

ORGANIC LOADING STEPS BTX REACTOR BENZENE				
TIME	OXYGEN	SUBSTRATE CONCENTRATION		
	UP TAKE	INFLUENT	EFFLUENT	REMOVAL PERCENT
(hours)	(mg/l)	(mg/l)	(mg/l)	(%)
0.00	1.95	0.76	0.05	93.42
4.00	1.90	1.20	0.06	95.00
18.50	2.75	1.94	0.07	96.39
24.00	3.20	2.30	0.06	97.39
27.50	3.10	2.16	0.06	97.22
42.50	3.10	1.60	0.06	96.25
45.50	3.15	1.56	0.00	100.00
48.00	3.25	1.67	0.05	97.01
48.50	3.20	3.04	0.10	96.71
51.50	3.00	3.15	0.15	95.24
66.50	2.55	3.33	0.27	91.89
69.50	2.20	3.50	0.34	90.29
72.50	2.20	3.86	0.43	88.86
75.50	2.25	3.64	0.34	90.66
91.00	2.05	3.28	0.78	76.22
96.50	1.75	3.60	0.94	73.89

Table C.3 Toluene responses for 48-hr. 2-fold step-load increase in a GAC-B/FBR system, fed with a BTX mixture as carbon source.

ORGANIC LOADING STEPS BTX REACTOR TOLUENE				
TIME	OXYGEN	SUBSTRATE CONCENTRATION		
	UP TAKE	INFLUENT	EFFLUENT	REMOVAL PERCENT
(hours)	(mg/l)	(mg/l)	(mg/l)	(%)
0.00	1.95	0.88	0.16	81.82
4.00	1.90	1.35	0.00	100.00
18.50	2.75	1.59	0.00	100.00
24.00	3.20	1.71	0.07	95.91
27.50	3.10	1.82	0.00	100.00
42.50	3.10	1.66	0.08	95.18
45.50	3.15	1.47	0.08	94.56
48.00	3.25	1.76	0.08	95.45
48.50	3.20	3.29	0.41	87.54
51.50	3.00	3.50	0.50	85.71
66.50	2.55	4.05	0.76	81.23
69.50	2.20	4.20	0.85	79.76
72.50	2.20	4.54	1.00	77.97
75.50	2.25	4.48	0.77	82.81
91.00	2.05	4.11	1.58	61.56
96.50	1.75	4.50	1.78	60.44

Table C.4 p-Xylene responses for 48-hr. 2-fold step-load increase in a GAC-B/FBR system, fed with a BTX mixture as carbon source.

ORGANIC LOADING STEPS BTX REACTOR p-XYLENE				
TIME	OXYGEN	SUBSTRATE CONCENTRATION		
	UP TAKE	INFLUENT	EFFLUENT	REMOVAL PERCENT
(hours)	(mg/l)	(mg/l)	(mg/l)	(%)
0.00	1.95	0.85	0.05	94.12
4.00	1.90	1.20	0.08	93.33
18.50	2.75	0.89	0.08	91.01
24.00	3.20	0.90	0.11	87.78
27.50	3.10	1.06	0.10	90.57
42.50	3.10	1.27	0.17	86.61
45.50	3.15	1.05	0.13	87.62
48.00	3.25	1.38	0.13	90.58
48.50	3.20	2.72	0.73	73.16
51.50	3.00	3.10	0.90	70.97
66.50	2.55	3.70	1.25	66.22
69.50	2.20	3.75	1.30	65.33
72.50	2.20	3.87	1.67	56.85
75.50	2.25	4.10	1.90	53.66
91.00	2.05	4.02	2.33	42.04
96.50	1.75	4.10	2.39	41.71

Table C.5 Responses for 64-hr. one step-load increase in a GAC-B/FBR system fed with benzene as carbon source.

ORGANIC LOADING STEPS BENZENE REACTOR				
TIME (hours)	OXYGEN UP TAKE (mg/l)	SUBSTRATE CONCENTRATION		
		INFLUENT (mg/l)	EFFLUENT (mg/l)	REMOVAL PERCENT (%)
0.00	4.20	6.92	0.44	93.64
12.00	4.30	7.54	2.52	66.58
14.00	3.80	7.38	3.50	52.57
16.00	4.00	7.78	3.22	58.61
19.00	3.70	10.11	2.71	73.19
20.50	3.80	8.11	3.52	56.60
22.50	4.00	8.20	2.66	67.56
36.50	3.10	8.07	2.64	67.29
43.00	2.65	7.95	4.57	42.52
44.50	1.40	6.27	4.10	34.61
47.00	2.50	7.25	2.66	63.31
64.30	2.70	8.44	3.89	53.91

APPENDIX D

OPTIMAL BED HEIGHT IN GAC-B/FBRs SYSTEMS

Table D.1 GAC-B/FBR system fed with toluene as substrate

OXYGEN UP TAKE AND TOLUENE CONCENTRATION PROFILE			
HEIGHT (cms)	DISSOLVED OXIGEN (mg/l)	ACCUMULATIVE OXYGEN UP TAKE (mg/l)	TOLUENE CONCENTRATION (mg/l)
-10	6.70	0.00	3.56
0	5.55	1.15	1.77
10	4.85	1.85	1.19
20	4.45	2.25	0.60
30	4.05	2.65	0.42
40	3.75	2.95	0.33
50	3.50	3.20	0.31
60	3.40	3.30	0.30
70	3.38	3.32	0.27
80	3.36	3.34	0.20
90	3.35	3.35	0.20
100	3.33	3.37	0.17
110	3.32	3.38	0.15

Table D.2 GAC-B/FBR system fed with benzene as substrate

OXYGEN UP TAKE AND BENZENE CONCENTRATION PROFILE			
HEIGHT (cms)	DISSOLVED OXIGEN (mg/l)	ACCUMULATIVE OXYGEN UP TAKE (mg/l)	BENZENE CONCENTRATION (mg/l)
-10	6.70	0.00	4.90
0	5.20	1.50	3.20
10	4.20	2.50	1.50
20	3.30	3.40	0.45
30	3.00	3.70	0.36
40	2.70	4.00	0.27
50	2.50	4.20	0.19
100	2.50	4.20	0.15

APPENDIX E

BIODEGRADATION KINETICS CONSTANTS DETERMINATION

Table E.1 Protein measurement for all compounds studied in biodegradation kinetics constants tests.

PROTEIN MEASUREMENT											
COMPOUND	INITIAL CONCENTRATION (So in mg/l)	ABSORBANCE						PROTEIN CONCENTRATION			YIELD (Y) (mg/mg)
		TIME 0 min.			TIME 60 min.			TIME 0 min. (mg/l)	TIME 60 min. (mg/l)	DELTA (mg/l)	
		Sample 1	Sample 2	Average	Sample 1	Sample 2	Average				
BENZENE	8.25	0.08	0.12	0.10	0.14	0.15	0.14	163.95	234.91	70.96	11.39
	18.64	0.15	0.16	0.15	0.20	0.21	0.20	251.22	331.16	79.93	6.95
	20.31	0.15	0.17	0.16	0.20	0.20	0.20	262.64	321.37	58.73	5.80
	23.76	0.11	0.12	0.12	0.14	0.15	0.15	187.60	236.54	48.94	4.81
	35.54	0.14	0.14	0.14	0.16	0.15	0.16	228.38	252.85	24.47	1.43
	40.07	0.14	0.16	0.15	0.15	0.18	0.17	247.15	270.80	23.65	1.29
							Average	223.49	274.61		5.28
TOLUENE	5.48	0.01	0.03	0.02	0.02	0.04	0.03	32.63	52.20	19.58	3.72
	12.05	0.04	0.06	0.05	0.05	0.06	0.06	79.93	93.80	13.87	1.18
	16.83	0.05	0.05	0.05	0.07	0.06	0.07	80.75	106.85	26.10	1.68
	20.85	0.08	0.09	0.08	0.10	0.10	0.10	137.03	157.42	20.39	1.62
	25.66	0.06	0.06	0.06	0.07	0.06	0.06	98.69	105.22	6.53	0.52
	31.20	0.08	0.08	0.08	0.11	0.12	0.11	132.14	185.97	53.83	3.93
							Average	93.53	116.91		2.11
BTX	16.93	0.11	0.12	0.12	0.12	0.13	0.13	190.05	204.73	14.68	1.28
	24.62	0.06	0.10	0.08	0.10	0.13	0.12	130.51	187.60	57.10	3.21
	32.44	0.11	0.11	0.11	0.11	0.13	0.12	181.89	193.31	11.42	0.56
	36.87	0.07	0.11	0.09	0.11	0.14	0.12	146.00	200.65	54.65	2.38
	45.72	0.12	0.16	0.14	0.15	0.18	0.16	232.46	267.54	35.07	1.18
	50.71	0.14	0.18	0.16	0.15	0.18	0.17	259.38	269.98	10.60	0.31
							Average	190.05	220.64		1.49

Table E.2 Substrate utilization rate and cell growth rate adjusted by best fit equation for a reactor fed with toluene

KINETICS OF BIODEGRADATION - TOLUENE REACTOR																		
TIME (min.)	S (mg/l)	X (mg/l)	ln(X)	S (mg/l)	X (mg/l)	ln(X)	S (mg/l)	X (mg/l)	ln(X)	S (mg/l)	X (mg/l)	ln(X)	S (mg/l)	X (mg/l)	ln(X)	S (mg/l)	X (mg/l)	ln(X)
0	6.46	93.53	4.54	15.87	93.53	4.54	21.13	93.53	4.54	19.43	93.53	4.54	24.65	93.53	4.54	29.78	93.53	4.54
5	3.91	98.90	4.59	11.08	103.64	4.64	16.18	103.98	4.64	17.91	96.72	4.57	23.23	96.53	4.57	28.15	96.98	4.57
10	2.37	102.16	4.63	7.73	110.70	4.71	12.39	111.97	4.72	16.52	99.66	4.60	21.89	99.37	4.60	26.60	100.24	4.61
15	1.43	104.13	4.65	5.40	115.62	4.75	9.48	118.10	4.77	15.24	102.37	4.63	20.62	102.03	4.63	25.14	103.33	4.64
20	0.87	105.32	4.66	3.77	119.06	4.78	7.26	122.79	4.81	14.05	104.87	4.65	19.43	104.55	4.65	23.76	106.24	4.67
25	0.53	106.04	4.66	2.63	121.46	4.80	5.56	126.38	4.84	12.96	107.18	4.67	18.31	106.92	4.67	22.45	109.00	4.69
30	0.32	106.48	4.67	1.84	123.14	4.81	4.26	129.13	4.86	11.95	109.31	4.69	17.25	109.15	4.69	21.22	111.60	4.71
35				1.28	124.31	4.82	3.26	131.23	4.88	11.02	111.27	4.71	16.25	111.25	4.71	20.05	114.06	4.74
40				0.89	125.12	4.83	2.50	132.84	4.89	10.16	113.08	4.73	15.32	113.23	4.73	18.95	116.38	4.76
50				0.44	126.09	4.84	1.46	135.02	4.91	8.64	116.28	4.76	13.60	116.86	4.76	16.93	120.65	4.79
60										7.35	119.01	4.78	12.07	120.08	4.79	15.12	124.47	4.82

Table E.3 Benzene utilization rate and cell growth rate adjusted by best fit equation for a reactor fed with a BTX mixture.

KINETICS OF BIODEGRADATION - BTX REACTOR BENZENE																		
TIME (min.)	S (mg/l)	X (mg/l)	ln(X)	S (mg/l)	X (mg/l)	ln(X)	S (mg/l)	X (mg/l)	ln(X)	S (mg/l)	X (mg/l)	ln(X)	S (mg/l)	X (mg/l)	ln(X)	S (mg/l)	X (mg/l)	ln(X)
0	2.78	190.05	5.25	7.04	190.05	5.25	7.80	190.05	5.25	7.43	190.05	5.25	10.89	190.05	5.25	12.07	190.05	5.25
5	1.15	192.47	5.26	4.01	194.57	5.27	5.69	193.19	5.26	5.24	193.31	5.26	8.42	193.73	5.27	8.85	194.84	5.27
10	0.48	193.48	5.27	2.28	197.14	5.28	4.16	195.48	5.28	3.70	195.61	5.28	6.51	196.57	5.28	6.49	198.36	5.29
15	0.20	193.89	5.27	1.30	198.60	5.29	3.04	197.15	5.28	2.61	197.23	5.28	5.03	198.77	5.29	4.76	200.94	5.30
20				0.74	199.44	5.30	2.22	198.37	5.29				3.89	200.47	5.30	3.49	202.83	5.31
25							1.62	199.26	5.29									

Table E.4 Toluene utilization rate and cell growth rate adjusted by best fit equation for a reactor fed with a BTX mixture.

KINETICS OF BIODEGRADATION - BTX REACTOR TOLUENE																		
TIME (min.)	S (mg/l)	X (mg/l)	ln(X)	S (mg/l)	X (mg/l)	ln(X)	S (mg/l)	X (mg/l)	ln(X)	S (mg/l)	X (mg/l)	ln(X)	S (mg/l)	X (mg/l)	ln(X)	S (mg/l)	X (mg/l)	ln(X)
0	6.81	190.05	5.25	6.77	190.05	5.25	8.61	190.05	5.25	10.16	190.05	5.25	15.58	190.05	5.25	13.86	190.05	5.25
5	3.51	194.97	5.27	4.82	192.95	5.26	6.93	192.55	5.26	7.38	194.18	5.27	11.74	195.77	5.28	11.03	194.27	5.27
10	1.81	197.50	5.29	3.44	195.02	5.27	5.58	194.56	5.27	5.37	197.19	5.28	8.84	200.08	5.30	8.78	197.63	5.29
15	0.93	198.81	5.29	2.45	196.49	5.28	4.49	196.18	5.28	3.90	199.37	5.30	6.66	203.33	5.31	6.98	200.30	5.30
20				1.74	197.54	5.29	3.62	197.48	5.29				5.02	205.78	5.33	5.56	202.42	5.31
25							2.92	198.53	5.29									

Table E.5 p-Xylene utilization rate and cell growth rate adjusted by best fit equation for a reactor fed with a BTX mixture.

KINETICS OF BIODEGRADATION - BTX REACTOR p-XYLENE																		
TIME (min.)	S (mg/l)	X (mg/l)	ln(X)	S (mg/l)	X (mg/l)	ln(X)	S (mg/l)	X (mg/l)	ln(X)	S (mg/l)	X (mg/l)	ln(X)	S (mg/l)	X (mg/l)	ln(X)	S (mg/l)	X (mg/l)	ln(X)
0	6.02	190.05	5.25	7.77	190.05	5.25	13.08	190.05	5.25	12.89	190.05	5.25	17.68	190.05	5.25	14.32	190.05	5.25
5	5.18	191.31	5.25	7.24	190.84	5.25	10.49	193.90	5.27	10.73	193.27	5.26	14.72	194.47	5.27	13.15	191.79	5.26
10	4.45	192.40	5.26	6.74	191.58	5.26	8.42	196.99	5.28	8.93	195.95	5.28	12.25	198.14	5.29	12.08	193.39	5.26
15	3.82	193.33	5.26	6.28	192.27	5.26	6.76	199.46	5.30	7.43	198.18	5.29	10.20	201.20	5.30	11.10	194.85	5.27
20	3.29	194.13	5.27	5.85	192.91	5.26				6.19	200.04	5.30	8.49	203.75	5.32	10.19	196.20	5.28
25				5.45	193.51	5.27				5.15	201.58	5.31	7.06	205.87	5.33	9.36	197.44	5.29
30				5.08	194.07	5.27				4.29	202.87	5.31	5.88	207.63	5.34	8.60	198.57	5.29
40				4.40	195.07	5.27				2.97	204.83	5.32				7.25	200.58	5.30
50																6.12	202.27	5.31
60																5.16	203.69	5.32
90																3.10	206.77	5.33

Table E.6 Substrate utilization rate and cell growth rate adjusted by best fit equation for a reactor fed with benzene.

KINETICS OF BIODEGRADATION - BENZENE REACTOR																		
TIME (min.)	S (mg/l)	X (mg/l)	ln(X)	S (mg/l)	X (mg/l)	ln(X)	S (mg/l)	X (mg/l)	ln(X)	S (mg/l)	X (mg/l)	ln(X)	S (mg/l)	X (mg/l)	ln(X)	S (mg/l)	X (mg/l)	ln(X)
0	8.25	223.49	5.41	18.64	223.49	5.41	19.28	223.49	5.41	22.15	223.49	5.41	34.02	223.49	5.41	36.50	223.49	5.41
5	1.57	258.76	5.56	10.87	264.50	5.58	12.92	257.09	5.55	14.30	264.93	5.58	24.94	271.44	5.60	29.35	261.24	5.57
10	0.30	260.65	5.56	6.34	288.42	5.66	8.66	279.60	5.63	9.23	291.68	5.68	18.28	306.59	5.73	23.60	291.60	5.68
15				3.70	302.37	5.71										18.98	316.01	5.76