SUBSTRATE INTERACTIONS DURING THE DEGRADATION OF BENZENE, TOLUENE, AND *p*-XYLENE AND THEIR EFFECTS ON SORPTION IN A FIXED BED BIOLOGICAL ACTIVATED CARBON REACTOR

By

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ABSTRACT

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Two *Pseudomonas* species (designated strains B1 and X1) were isolated from an aerobic pilot-scale fluidized-bed reactor treating groundwater containing benzene, toluene, and *p*-xylene (BTX). Strain B1 grew with benzene and toluene as the sole sources of carbon and energy, and it cometabolized *p*-xylene in the presence of toluene. Strain X1 grew on toluene and *p*-xylene, but not benzene. Competitive inhibition and cometabolic transformation were observed during batch rate experiments using paired substrates (BT, TX, or BX) and the two strains. These were mathematically modeled and rate parameters were determined. Coupling was achieved by defining two transformation capacity terms for the cometabolizing culture. Cometabolism increased decay rates, and the observed yield for strain B1 decreased in the presence of *p*-xylene.

The biodegradation kinetic study showed that cometabolism resulted in the formation of *p*-xylene dihydrodiol. In the absence of growth substrate, *p*-xylene dihydrodiol was further degraded to 3,6-dimethyl pyrocatechol. These intermediate and byproduct compounds were identified and quantified. The effect of these byproduct compounds on activated carbon sorption revealed that they compete with the target compounds.

Fixed-bed biological activated carbon (BAC) columns and biofilm columns were seeded with strain B1 and operated until steady-state conditions were reached. *p*-xylene dihydrodiol was initially adsorbed by the activated carbon, but eventually saturated the adsorption capacity of the column and appeared in the column effluent. Perturbation studies showed the extended removal capacity of target compounds in BAC systems. The response to the perturbation was buffered in the BAC systems as a result of the adsorption capacity of the activated carbon.

The operation of columns treating toluene and *p*-xylene showed different patterns of degradation depending on the bacterial species present. Shock loading to the reactors by increasing the favorably degraded compound resulted in a significant increase in the effluent concentrations of both compounds, while the increase of effluent concentrations resulting from the increase of the less favorably degraded compound was smaller. The formation of byproducts was reduced in both cases.

Solio Deo Gloria

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The fear of the LORD is the beginning of wisdom,
and knowledge of the Holy One is understanding. (Proverbs 9:10)

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NOMENCLATURE

Fundamental quantities

L	length
M	mass, in general
M_c	mass of non-growth substrate
M_g	mass of growth substrate
M_b	mass of bacteria

English symbols

b	first order endogenous decay constant (T^{-1})
C_e	concentration of solute at adsorption equilibrium (ML^{-3})
H_C	Henry's constant
I_L	concentration of inhibitor in liquid phase (M_gL^{-3})
k_c	maximum specific rate of utilization of the non-growth substrate in the absence of growth substrate ($M_cM_b^{-1}T^{-1}$)
k_g	maximum specific rate of utilization of growth substrate ($M_gM_b^{-1}T^{-1}$)
K_F	constant representing the sorptive capacity
K_I	inhibition coefficient (M_gL^{-3})
$K_{s,c}$	half saturation coefficient of the non-growth substrate (M_cL^{-3})
$K_{s,g}$	half saturation coefficient of the growth substrate (M_gL^{-3})

n	constant representing the sorption affinity
q_e	mass of solute adsorbed per mass of adsorbent (MM^{-1})
S_c	concentration of non-growth substrate (M_cL^{-3})
S_g	concentration of growth substrate (M_gL^{-3})
S_L	concentration of substrate in liquid phase (M_gL^{-3})
t	time (T)
T_c^{b*}	true biomass transformation capacity in the absence of endogenous decay ($M_cM_b^{-1}$)
T_c^g	growth substrate transformation capacity ($M_cM_g^{-1}$)
V_L	volume of liquid phase (L^3)
V_G	volume of gas phase (L^3)
X	active organism concentration (M_bL^{-3})
X_o	initial concentration of active organisms (M_bL^{-3})
Y_m	maximum yield or true growth yield ($M_bM_g^{-1}$)
Y	observed yield ($M_bM_g^{-1}$)

Greek symbols

μ	specific growth rate (T^{-1})

CHAPTER 1

INTRODUCTION AND RESEARCH OBJECTIVES

1.1 Introduction, Background, and Problem Statement

1.1.1 Introduction

Discovery of pollutants in aquifers and water supply wells has led to the realization that groundwater supplies can no longer be assumed to be pristine sources of drinking water requiring little or no treatment. Groundwater quality has historically been largely dependent upon nature. However, there are now numerous identifiable threats of serious, widespread contamination. These threats are generally classified by their causes : 1) waste disposal activities, 2) industrial and commercial release due to accidents, 3) agricultural operations, and 4) artificial recharge of ground water with contaminated water. Among the many contaminants responsible for deteriorating groundwater quality, organic contaminants are the most common health-threatening chemicals detected. The greatest difficulties in groundwater remediation have been encountered at organic contamination sites. Much of the groundwater contamination of this type is caused by leakage, spilling, or disposal of organic liquids originally immiscible with water (nonaqueous-phase liquids-NAPLs) into the ground. Subsequent dissolution of the NAPL and transport of the dissolved constituents by groundwater is thought to generate many plumes. Dissolved organic contaminants that are less soluble in water and that have a tendency to bind to aquifer media, and thus retarded and move at rates slower than the rate at which groundwater flows.

1.1.2 Engineering Background

Once its quality has deteriorated, groundwater is either unusable or has to undergo treatment before use, depending on the extent of the problem. However, in most instances it is not practical to restore a contaminated aquifer to its former condition. Many remediation techniques are available such as pump-and-treat, venting , and biorestoration. It is difficult to return aquifers to drinking-water quality, but restoration methods are often able to lower the contaminant concentration significantly. Almost all remediation of contaminated groundwater is based on groundwater extraction from wells or drains.

Among the many remediation techniques available, carbon adsorption is currently the focus of renewed investigation because of its potential for removing many of the contaminants being regulated under the 1986 amendments to the SDWA (Safe Drinking Water Act). The U.S. Environmental Protection Agency (EPA) designated granular activated carbon (GAC) treatment as a "Best Available Technology (BAT)" for treating a variety of regulated synthetic organic chemicals. "BAT" implies that while the treatment technique is not perfect, it is one of the best approaches currently available.

1.1.3 Biological Activated Carbon Process

In many cases, biological growth is observed during the operation of fixed-bed granular activated carbon columns. This growth has previously been deemed undesirable, and steps have been taken to combat it. Efforts to prevent the growth of microorganisms in activated carbon adsorbers are often unsuccessful (Bishop *et al.* 1967). On the other hand, many researchers now view biological activity on activated carbon as beneficial.

The possibility of combining biological and physicochemical treatment was first proposed in the early 1970's by Weber *et al.* (1970; 1971; 1972) and has since been an active research area. The European practice of BAC involves the deliberate growth of microorganisms in the adsorber for the purpose of treating drinking water. This has lead to the common use of the terms "Biofilm Activated Carbon (BFAC)" or "Biological Activated Carbon (BAC)" which describe these combined processes (Rice and Robson 1982). Since BAC is not normally preceded by filtration, it can function as both filter and adsorber. When BAC does follow filtration, its primary function is adsorption. In any case, the carbon particles serve as a support medium for the biomass, and biological activity is the major means of contaminant removal.

One of the major advantages claimed for BAC is that it can treat a broader range of dissolved organics than granular activated carbon alone, including highly biodegradable but non-adsorbable or slightly adsorbable organic compounds (Benedek 1980). A removal technique that is right for one set of circumstances is not necessarily right for another. It is imperative that time be taken to choose the right tool for the job and then to refine and optimize the selected technology for the particular conditions before implementation. BAC is advantageous because it contains both physicochemical and biological treatment elements.

Another proposed advantage is that there is an *in-situ* regenerating capability of the carbon by the biological activity (bioregeneration of carbon). This can extend the operating period, which is a most critical factor in deciding the cost of operation. BAC can also effectively cope with shock situations by adsorbing high concentrations, then releasing sorbed contaminants back into solution where they become available for microbial degradation. One important application is the treatment of contaminated groundwater.

1.1.4 Problem Statement

Benzene, toluene and xylene (BTX) are among the many contaminants regulated under the 1986 SDWA amendments. These three organic compounds are commonly detected at sites contaminated by petroleum products. Of the 567 sites of environmental contamination listed on the proposed priority list in the State of Michigan in 1989, 234 sites (41.3%) were contaminated by benzene, toluene, or xylene or by some combination of the three (DNR 1989). The number of sites contaminated by BTX has increased annually as more contaminated sites are identified (Table 1-1). BTX are less strongly adsorbed to the soil matrix than the aliphatic components in fuel. BTX are known to biodegrade aerobically (Kincannon *et al.* 1983a; 1983b; Richards and Shieh 1986; Tabak *et al.* 1981) and to be sorbed by activated carbon. For these reasons, BAC treatment can be an excellent approach to take advantage of integrated physicochemical and biological methods.

Table 1-1. Sites of environmental contamination listed on the priority lists in the States of Michigan - ACT 307 (DNR 1989)

year	sites contaminated by BTX	total contaminated sites listed	% BTX/total
1989	234	567	41.3
1991	1198	2848	42.1
1992	1663	3396	49.0

1.2 Research Objectives and Overview

The overall objective of this study is to investigate the role of multi-substrate degradation in the operation of packed-bed biological activated carbon systems under

various combinations of substrates and with different populations of organisms. Specific objectives include characterizing the different biodegradation patterns for each organism used and mathematically quantifying substrate interactions. Another objective is studying the effect of a metabolic byproduct on sorption. A further objective is the qualitative description of the integrated result of substrate interaction and sorption. This research is divided into three phases. Each phase constitutes one chapter in the dissertation. A brief description of each chapter, with its particular objectives, follows.

The first phase, presented in chapter 3, covers the isolation of microorganisms and degradation kinetics. The objectives of this phase of the study are: 1) to isolate BTX-degrading organisms from a biological activated carbon system, 2) to determine kinetic parameters for single substrate degradation, 3) to categorize the pattern of multiple substrate utilization, and 4) to quantify substrate interactions in the biodegradation of paired substrates (BT, TX, and BX). The information obtained from this phase was used in the interpretation of later experimental data .

The second phase, presented in chapter 4, focuses on cometabolism of *p*-xylene and the byproducts produced. The effect of these byproducts on the removal of target compounds is studied. For this purpose, the identification of byproducts is essential. This identification is accomplished by GC-MS analysis and by comparative studies using other organisms producing known byproducts. The effect of the formation of byproducts on the sorption of the major target compound is investigated using a bottle-point adsorption isotherm method with and without byproducts. The formation of byproducts and their effect in the reactor is studied in a packed-bed column. The role of adsorption in the cometabolism-base BAC operation is investigated by a comparison of the dynamic loading of growth substrate with that of non-growth substrate.

The third phase, presented in chapter 5, emphasizes the investigation of the effects of different patterns of degradation on general BAC operation. Also, the population effect is studied to show the importance of specific populations of microorganisms in treating particular compounds. How the treatment mechanism reacts with the dynamic loadings in the system under different conditions of substrate degradation is also investigated.

The engineering significance of the findings put forth in this dissertation is discussed in the last chapter, along with recommendations for future studies.

1.3 Production, Use, and Properties of BTX

Benzene, toluene, and *p*-xylene are listed among the top 50 chemicals produced in the United States during the last several years. Annual production of these three compounds during 1987 through 1990 are shown in Table 1-2.

Table 1-2. Annual production of benzene, toluene, and *p*-xylene in the United States during 1987-1990 (Chemical and Engineering News 1991).

unit : billions of lb.

Compound	1987	1988	1989	1990
benzene	11.57	11.81	11.94	11.86
toluene	7.00	6.45	5.83	6.10
p-xylene	5.16	5.60	5.34	5.20

Benzene is present not only in all crude oils but also in refined oils. Benzene was discovered by Faraday in 1825 and named "bicarburet of hydrogen". It was produced

commercially primarily by the coal carbonization industry until the late 1950's, when the production of petroleum-derived benzene became its leading source (Ayers and Nuder 1964). At the present time, more than 90% of benzene is produced from this latter source (Howard and Durkin 1974). Benzene is derived from petroleum through two processes: catalytic reforming followed by solvent extraction and fractional distillation to remove benzene and other aromatic hydrocarbons; and dealkylation of toluene or xylene (Ayers and Nuder 1964). Benzene is an intermediate compound in the production of other chemicals, including phenol, chlorobenzene, ethyl benzene, and cyclohexane. Benzene and chemically transformed benzene products are used to make plastics, synthetic rubber, and numerous polystyrene compounds. Benzene is also used as a solvent in paints and coatings, and in the preparation of artificial and natural leathers. The major sources of benzene contamination occur in (1) the production of chemicals and processings (2) coating operations and (3) storage and transportation. Contamination can be caused by spills, leaks, and effluents from industries (USEPA 1976). Benzene can be found in virtually any waste from industries producing or utilizing benzene .

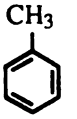
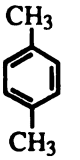
Toluene is a natural component of coal and petroleum (Kirk and Othmer 1963). The primary producers of toluene in the US are the coke and petroleum industries. The primary direct uses of toluene in industry are as a gasoline component and as a solvent. An important indirect use of toluene is its conversion to benzene (Kirk and Othmer 1963).

Xylene is produced primarily from petroleum by the reforming process (Kirk and Othmer 1963). Xylene is used mainly as a gasoline additive and as a solvent. It is not used as extensively as benzene or toluene. There are three isomers of xylene: *para-*, *ortho-*, and *meta*-xylene.

Benzene, toluene, and xylene act as neurotoxins. All three compounds affect cell permeability with possible concomitant shifts in blood ions. Possible carcinogenic, teratogenic, and mutagenic effects have been observed. Details of BTX toxicity can be found elsewhere in toxicological studies (Dean 1985).

The physical and chemical properties of these three compounds are summarized on Table 1-3.

Table 1-3. Physical and chemical properties of benzene, toluene, and *p*-xylene (Verschueren 1983)

Compound	benzene	toluene	*p*-xylene
Structural Formula			
Synonyms	benzol cyclohexatriene phene phenyl hydride	methacide methyl benzene phenylmethane toluol	*p*-xylol 1,4-dimethyl benzene
Molecular Weight	78.11	92.10	106.17
Specific Gravity	0.8786	0.867	0.86
Solubility (20°C) in water (mg/l)	1780	515	198
log P_{oct} at 20°C	2.13	2.69	3.15
Henry's Constant (mg/l / mg/l at 20°C)	.2345	.2601	.2967
Melting Point (°C)	5.5	-95.1	13.26
Boiling Point (°C)	80.1	110.8	138.4

CHAPTER 2

BACKGROUND

This chapter provides a general summary of important findings regarding biological activated carbon (BAC), the adsorption and biodegradation of organic compounds, and the characteristics of benzene, toluene, and *p*-xylene relevant to their removal from contaminated groundwater. The concepts underlying BAC and its research history are described in the first section, followed by two sections on removal mechanisms: adsorption and degradation. The section on adsorption is not intended to be comprehensive; it is focused on multi-component adsorption in granular activated carbon systems. On the other hand, the section on degradation is divided into three sub-sections : 1) kinetics of microbial degradation, 2) multi-substrate degradation, and 3) degradation of BTX and its pathway. The last section briefly reviews current research most directly related to this study.

2.1 Biofilm Activated Carbon

2.1.1 Development of Biofilm on Granular Activated Carbon

The development of a biofilm can be observed easily on any surface submerged in an aqueous environment. A biofilm life cycle involves 1) transport and adsorption of organic molecules and microbial cells to the surface, 2) attachment of microorganisms, 3) microbial growth and metabolism, and 4) the detachment of the biofilm (Trulear and Characklis 1982; Characklis 1984). The surface structure of activated carbon is suitable for

the first two steps in the biofilm life cycle because of its morphological and adsorptive characteristics (Pirbazari *et al.* 1990). Since activated carbon in water and wastewater applications invariably comes in contact with microorganisms, the development of microbial activity on activated carbon occurs naturally. The growth of biofilm depends on a variety of biological, chemical, and physical factors. Transport and interfacial transfer processes probably control the ultimate thickness of a biofilm in high hydrodynamic shear -stress environments. Microbial activity within the biofilm can influence the microbial population distribution and product formation. Detachment mechanisms can be divided into erosion (continuous removal of small particles from the biofilm) and sloughing (detachment of large fragments of biofilm). Erosion is the result of hydrodynamic shear stress at the biofilm-water interface. On the other hand, the sloughing is triggered by transitions in the environment. Sloughing can be observed at high substrate loadings or low shear stresses (Characklis *et al.* 1990).

2.1.2 History and Definition

Weber *et al.* (1970) demonstrated that physicochemical treatment could meet the more stringent demands of tertiary treatment. In their study, they suggested the direct application of physicochemical processes, specifically granular activated carbon (GAC), for the treatment of wastes containing dissolved organic matter. With high organic loading, biological activity occurred in the GAC systems. Their results demonstrated that biological activity did not hinder adsorption, and it apparently enhanced the overall capacity for removal of organics. Further work by Weber *et al.* (1972) revealed the possibility of a biologically extended-bed physicochemical treatment. They operated an aerobic expanded adsorption system for nine months without regeneration. They attributed the adsorption bed longevity to "bioregeneration" - the utilization of adsorbed organic materials by bacteria.

Organics adsorbed on adsorption sites are used by organisms, and the sorption sites recover their adsorption capacity. They compared anthracite and activated carbon as packing materials for support of biomass growth and concluded that activated carbon was superior because of its shock-absorbing capability and its extended residence time for acclimation. The early findings of Weber and co-workers have been the subject of considerable debate by researchers in the subsequent years. Over the time, the term biological activated carbon or BAC has come to be defined as the simultaneous combination of GAC adsorption of dissolved organic materials coupled with aerobic biological oxidation of organic materials (Rice and Robson 1982).

2.1.3 Research on Biofilm Activated Carbon System

Several publications have reviewed the technology of BAC (AWWA Committee 1981; Miller and Rice 1978; DiGiano 1981; Rice and Robson 1982). Research on this subject started in late 1960's and continues today. Among the many directly and indirectly related studies, the most important works have been chronologically reviewed below.

Studies regarding the microbial activity on the surface of activated carbon can be found prior to 1970 but these are not generally considered to be part of the BAC literature. Rather, they report difficulties in creating a bacteria-free activated carbon column and suggest approaches for dealing with the biofilm. Bishop *et al.* (1967) reported unexpected increases in output turbidity and TOC from carbon beds. They explained that biological activity, caused by the heavy load of BOD, was responsible. To prevent column failure, predisinfection using chlorination or ozonation were recommended. However, Weber *et al.* (1970) incidentally observed improvement of the treatment by the microbial growth on activated carbon surfaces. The overall capacity for organic removal was enhanced and the

effective operation period was extended. This observation indicated the possibility for the co-existence of microbiological and sorptive activities in the adsorption column.

After Weber *et al.* reported the positive effect of microbial activity in the adsorber, other researchers started to examine these processes and demonstrated the practical application of combined processes to various kinds of waste removal. Perrotti and Rodman (1973; 1974) showed enhanced TOC removal from textile dye wastewater in a reactor containing both mixed activated carbon and activated sludge as compared to either system alone. They proposed that extracellular enzymes promoted high rate degradation of the organic matter adsorbed on the carbon. Other applications include the treatment of refinery and petrochemical wastewaters by Ford and Buerklin (1972). They predicted that 'biological-carbon systems will probably be the most prevalent application of carbon for refinery wastewater treatment in the immediate future.

During mid-1970s', BAC became the focus of increased research attention for industrial waste applications. Techniques to improve the performance were developed by including preoxidation steps before activated carbon treatment. Preozonation oxidizes a fraction of the total organic carbon (TOC) there by reducing the organic loading and rendering the load more readily degradable. In addition, the ozonation saturates the solution with oxygen and subsequently satisfies the condition for aerobic biodegradation (Miller and Rice 1978).

About the same time, many European water treatment practices applied ozone coupled with activated carbon for reducing organics concentrations in drinking water (Van Der Kooij 1976a; Van Der Kooij 1976b; Sontheimer 1978; Sontheimer *et al.* 1979). The application of BAC to treat drinking water raised a question concerning the contamination

by bacteria. However, with post-chlorination, sufficient disinfecting residuals to protect distribution system was achieved.

The addition of powdered activated carbon (PAC) to activated sludge was developed during this period and commercialized under the name "PACT" (DeWalle and Chian 1977; Rodman, Shunney and Perrotti 1978; Nayar and Sylvester 1979). PAC is used to remove soluble organics and to aid clarification. The addition of PAC to primary clarifiers reduced the load of BOD and solids to downstream process and the addition to activated sludge system controlled organic loading. The PACT process differes somewhat from other BAC approaches, however, the use of two mechanisms in one reactor may involve similar mechanism.

Studies during the 1980's focused on specific mechanisms and different working conditions. A number of mathematical models were developed (Andrews 1979; Andrews and Tien 1976; Andrews and Tien 1977; Andrews and Tien 1981; Chang and Rittmann 1987; Tien and Wang 1982; Ying and Weber Jr. 1979) and operating experiences were reviewed (AWWA Committee 1981; DiGiano 1981). Table 2-1 compares models developed by different researchers. These models differ in their description of; 1) biofilm kinetic (growth and decay), 2) substrate transport, and 3) adsorption equilibrium. Each model, however, yields similar patterns of target compound concentrations in the effluent from BAC systems.

Among the studies on mechanisms, Speitel used a unique approach to study bioregeneration in the fixed-bed adsorption column (Speitel 1985; Speitel and DiGiano 1987; Speitel *et al.* 1987; Speitel and DiGiano 1988; Speitel *et al.* 1988; Speitel Jr. *et al.* 1989). Radio-labeled chemicals were partially adsorbed on certain depths of activated

Table 2-1. Comparisons of models by different researchers

Model by	No. of limiting substrate	Biofilm		Mass transfer				Adsorption equation		Reactor
		thickness	kinetics	axial dispersion	external film	diffusion biofilm	internal diffusion	ads. model	competitive adsorption	configuration
Ying & Weber	1	Variable	Monod & Decay	no	yes	no	surface diffusion	Freundlich	no	fixed bed
Speital	2	Variable	Monod, Decay & Sloughing	no	yes	yes	surface diffusion	Freundlich	yes	fixed bed
Kim & Pirbazari	1	Variable	Monod & Decay	yes	yes	yes	surface diffusion	Freundlich	no	fluidized bed with recycle
Wisecarver & Fan	2 (Phenol & O_2)	Fixed	Haldane & Monod	yes	yes	yes	no	no	no	three phase fluidized bed
Benedek	1	Fixed	Zero-order	yes	assume steady state	yes	linear driving force	linear	no	fixed bed
Schulhof	1	Variable	1st order decay, Cell diffusion	no	no	no	linear driving force	linear	no	fixed bed
Chang & Rittmann	1	Variable	Monod, Decay & Sloughing	no	yes	yes	surface diffusion	Freundlich	no	completely mixed biofilm
Andrew & Tien	1	Variable	1st order	no	no	yes	linear driving force	linear	no	fluidized with recycle

carbon and regeneration was studied by measuring labeled carbon contents of effluent substrate, CO_2, and cells. Consumption of pre-adsorbed labeled carbon was observed as the biofilm developed.

Some researchers questioned the enhanced performance in BAC and sometimes did not observe enhanced removal (Lowry and Burkhead 1980; Maqsood 1977; Olmstead 1989; Peel and Benedek 1983).

2.1.4 Adsorption, Desorption and Biological Activity

Sorption and biological degradation are the basic removal mechanisms in the BAC process. The beneficial effect of BAC results from a combination of adsorption and biodegradation in a single reactor, processes which are complementary and synergistic. This can be understood by considering the different types of organic compounds : 1)adsorbable and biodegradable, 2) adsorbable but non-biodegradable, 3) biodegradable but non-adsorbable, and 4) non-adsorbable and non-biodegradable (Benedek 1980). Most organic contaminants are in categories 1), 2), and 3) suitable for treatment in a BAC system. BAC, which has both adsorption and biodegradation in one system, can be claimed to remove broader spectrum of organic compounds than the alternative systems.

Adsorbing potentially toxic compounds onto activated carbon may also protect the microbial system (Gardner, Suidan and Kobayashi 1988) As a general rule, many toxic compounds are adsorbed easily while the compounds which are not adsorbable tend to be readily biodegradable. The biofilm may thus function as a filtering device to remove easily biodegradable compounds before adsorption. Speitel *et al.* (1989) conducted experiments with mixtures of biodegradable and nonbiodegradable compounds in columns containing pre-equilibrated GAC. Utilization of biodegradable compounds by microbial activity

reduced competition between biodegradable and nonbiodegradable compounds for adsorption sites. They concluded that removal of biodegradable compounds from the mixture through microbial activity may extend the service life of the GAC.

The adsorption capacity of activated carbon can smooth out periods of high influent concentration, even in the later stages of operation when little adsorption capacity remains. The effectiveness of BAC systems in buffering the fluctuation of influent concentration or shock loading has been proven experimentally (Chudyk and Snoeynik 1984). Buffering occurs because of the adjustment in adsorption equilibrium brought about by an increase in the feed concentration. During such a loading, biodegradable organics may pass through the biofilm and become adsorbed by activated carbon. When baseline conditions are again established, the accumulated organics desorb and degrade as they pass through the biofilm. Thus, some adsorption sites can be regenerated.

Bioregeneration has been a controversial topic in BAC studies and its definition may be different among different researchers. Some researchers hypothesized and noted that there was no bioregeneration (Lowry and Burkhead 1980). Maqsood *et al.* (1977) for example, compared the isotherms of the spent carbon before and after bioregeneration. No difference in isotherm data was observed. However, more researchers have confirmed bioregeneration. Mechanisms which are most likely to make bioregeneration possible appear to be desorption and the subsequent diffusion to an external biofilm.

Speitel (1985) examined the rate and extent of bioregeneration of ^{14}C-labeled phenol and *p*-nitrophenol that had been preadsorbed in a granular activated carbon column. A recovery of radioactivity in the effluent from the GAC column indicated that preadsorbed compounds were desorbed and subsequently biodegraded. Bioregeneration was observed to range from 8 to 15 percent for phenol and 5 to 22 percent for *p*-nitrophenol over a 10

day period. The potential for bioregeneration was observed to have increased as sorption capacity and surface diffusivity increased. The author suggested two causes of substrate desorption: (1) a reduction in biofilm/GAC interfacial concentration resulting from substrate uptake within the biofilm, and (2) a reduction in the surrounding bulk liquid concentration caused by biological activity in upstream portions of the GAC column.

Li and DiGiano (1983) observed higher biodegradation rates and specific growth rates on GAC than on sand or coal. Their evidence for enhanced biodegradation and higher specific growth rates pointed to utilization of adsorbed substrate rather than better attachment on GAC. The enhanced specific growth rate also increased as particle size was decreased. Dobrevski and Zvezdova (1989) investigated the effect of activated carbon pore structure on the process of carbon regeneration. Based on their results, it seems that the process of bioregeneration depends on the volume of pores with radii of 5-50 nm. This study implied that the internal diffusion was an important factor for resupplying sorbed substrate to the biofilm. Other authors such as Kim *et al.* (1986), Andrews and Tien (1976), Suidan *et al.* (1978) and Schultz and Keinath (1984), observed a similar bioregeneration.

Understanding the combined mechanisms of biodegradation and adsorption occurring in the same reactor is still limited. Whether and to what extent there is a synergistic effect needs further study.

2.2 Multi-Component Adsorption

If each component in solution is adsorbed independently of the others, a multicomponent adsorption system can be described by simply using single-solute theory.

In actuality, the solutes generally interfere with each other. This requires an understanding of equilibrium equation processes including interference effects (Cooney and Lightfoot 1966). Multicomponent adsorption was first studied for gas-phase adsorption (Gariepy and Zwiebel 1971; Kidnay and Myers 1966; Thomas and Lombardi 1971). Gariepy and Zwiebel (1971) studied the binary adsorption of a gas mixture onto fixed beds. Even though they showed some agreement between the experimental and predicted data, their mathematical model did not include the effect of diffusional resistance within the adsorbent particles which has been shown experimentally to be rate controlling for activated carbon.

One of the special characteristics in multicomponent adsorption is that there can be an effluent jump of the less adsorbable component, as shown on Figure 2-1. This jump is caused by the displacement of the previously adsorbed component by a compound that is more strongly sorbed. Gariepy *et al.* (1971) termed this less adsorbable compound the "non-key component" as opposed to the more strongly sorbed compound "key

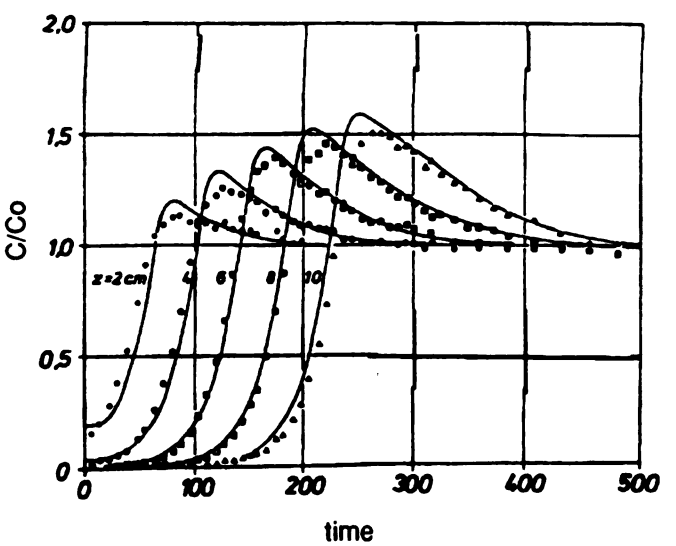


Figure 2-1. Example of effluent jump from *p*-chlorophenol replaced by *p*-nitrophenol (after Merk *et al.* 1980)

component". The height of jump is affected by the inlet compositions of the two components, and it increases with increasing bed length. Thomas and Lombardi (1971) studied the binary adsorption of a benzene and toluene mixture onto a fixed bed of activated carbon. They used a simple semi-empirical model with the assumption that surface diffusion is a rate-controlling mechanism within the particle and is governed by linear driving force kinetics. They correlated the adsorption of both components using a separation factor to describe the relative affinity of the adsorbent for toluene and benzene. Cooney and Strusi (1972) tried to solve the two solute problem by considering two single solute problems (which they called a pseudo-binary approach) and by solving them independently. They used the linear driving force approximation to describe the diffusion within the adsorbent.

Many researchers have used a Langmuir Isotherm model modified to describe multi-component adsorption. However, Jain and Snoeyink (1973) proposed a model of bisolute systems with the hypothesis that a certain amount of adsorption occurs without competition. The basic assumption in the Langmuir equation is that the surface of the adsorbent is homogeneous with respect to energy and that all sites are equally likely to adsorb either solute. Adsorption onto activated carbon does not comply with the assumption of constant site energies. Jain and Snoeyink's modified version provided a reasonably good description of the data. Fritz *et al.* and Merk *et al.* (Fritz *et al.* 1980; Fritz and Schlunder 1980; Merk *et al.* 1980) examined different models using experimental results they obtained. They used a Freundlich isotherm. This equation has some disadvantages such as the restriction of applicability to a limited concentration range, but it is also suitable for highly heterogeneous surfaces. They used both single component batch experiments and fixed bed experiments, and compared the results of these experiments to model predictions based on the assumption that diffusion is rate-controlling.

The prediction of multicomponent adsorption performance was made (Crittenden *et al.* 1985) using the simplified form of the ideal adsorption theory (IAT) which was originally developed by Myers and Prausnitz (1965). The simplified form allowed the rapid prediction of bi-solute adsorption. The deviation between the prediction and the experimental result was significant using only single solute data if there existed significant internal diffusion resistance and marked displacement. Predictions for more weakly adsorbed solutes in bi-solute systems were less accurate than predictions for more strongly adsorbed compounds. The possible prediction of bi-solute performance using single solute data was evaluated. The deviations were reduced using data for preloaded carbon, which gives the information about the displacement effect, along with single solute data.

2.3 Microbiological Degradation of Organic Compounds

2.3.1 Kinetics of Microbiological Degradation

Many factors influence the biodegradability of individual organic pollutants. One way of comparing biodegradability is by comparing kinetic parameters. The most commonly employed rate equation is that of Monod (1949), which can be expressed as:

$$-\frac{dS}{dt} = \frac{\mu_{max} \cdot S \cdot X}{Y \cdot (K_S + S)} \qquad (2\text{-}1)$$

where, μ_{max}/Y (which is often represented by k) is the maximum specific rate of substrate utilization (mg substrate per mg biomass-min) at time t. X and S are active biomass concentration (mg/L) and substrate concentration (mg/L). μ_{max} and K_S can be obtained by integrating the equation (2-1) with known S and X values over a certain time period. Batch

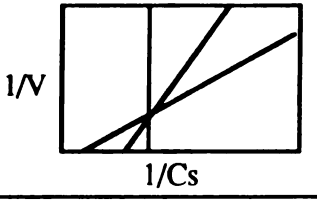
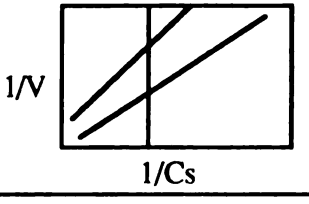
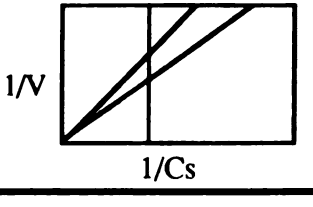
experiments are generally easy to perform by measuring the biomass increase and substrate utilization (Sundstrom and Klei 1979). In a batch experiment, the reactors containing the pollutant of interest at different concentrations as the sole organic constituent are inoculated with small quantities of biomass capable of performing the desired biodegradation. The increase in biomass concentration in each reactor can be measured as a function of time and analyzed to obtain μ_{max}.

2.3.2 Multisubstrate Degradation

In the metabolism of multiple substrates, frequently reported degradation patterns include diauxie (Monod 1942), simultaneous utilization (Deshpande, Chakrabartl, and Subrahmanyam 1987), and competitive inhibition (Law and Button 1977; Strand, Bjelland, and Stensel 1990). Diauxie or sequential utilization results from catabolite repression or catabolite inhibition (Yoon, Klinzing, and Blanch 1977). The substrate consumption rate of multiple-substrates is often affected by the interactions between enzymatic processes. An enzymatic inhibitor will reduce the rate of an enzymatically catalyzed reaction by binding with either free enzyme or with the enzyme-substrate complex (Grady and Lim 1980). Three different types of inhibition are presented on Table 2-2. The mechanism, the mathematical expression, and Lineweaver-Burk plots are compared.

Simultaneous utilization of substrates may or may not result in growth. Simultaneous utilization of substrates that do not support growth is termed cometabolism (Law and Button 1977). As defined by Dalton (1982), cometabolism is the transformation of a non-growth substrate in the "obligate presence of a growth substrate or another transformable compound". More generally, cometabolism also includes transformations by resting cells if no growth results. The term "cometabolism" is often used with

Table 2-2. Comparisons of enzyme inhibition (Grady and Lim 1980)

Type of inhibition	Reaction†	Mathematical expression††	Lineweaver-burk plots
Competitive inhibition	E + S = ES = E + P E + I = EI	$r_p = \frac{V_m C_s}{K_m \cdot (1 + \frac{C_I}{K_I}) + C_s}$	
Uncompetitive inhibition	E + S = ES = E + P ES + I = ESI	$r_p = \frac{V_m C_s}{K_m + C_s \cdot (1 + \frac{C_I}{K_I})}$	
Noncompetitive inhibition	E + S = ES = E + P E + I = EI ES + I = ESI	$r_p = \frac{V_m C_s}{(K_m + C_s) \cdot (1 + \frac{C_I}{K_I})}$	

† In reaction equations, E : free enzyme, S : substrate, ES : enzyme-substrate complex, P : product, I : inhibitor, EI : enzyme-inhibitor complex, ESI : enzyme-substrate-inhibitor complex

†† In mathematical expression, r_p : reaction rate, V_m : maximum specific velocity, C_s : substrate concentration, K_m : Michaelis-Menten constant, K_I : inhibition constant, C_I : inhibitor concentration

"cooxidation". Cooxidation is defined as the process in which microorganisms oxidize a substance without utilizing the energy derived from this oxidation to support growth (Leadbetter and Foster 1959). The proof of cometabolism can be easily observed by the disappearance of substrate and the accumulation of end product without an increase in bacterial numbers (Horvath 1972). The inability to grow on some substrates is not the result of an organism's inability to attack the substrate but often results from its inability to assimilate the products of oxidation (Foster 1962). Oxidation of the substrate may induce the formation of enzymes capable of oxidizing other substrates.

Recently, several mathematical models have been developed for cometabolism (Alvarez-Cohen and McCarty 1991; Criddle *et al.* 1990; Saez and Rittmann 1991; Schmidt *et al.* 1985). Most of these expressions describe resting cell transformation. The important concept in these models is introduction of transformation capacity (Alvarez-Cohen and McCarty 1991; Saez and Rittmann 1991). Transformation of non-growth substrate gets its reducing power from cells grown previously on growth substrate. Transformation of non-growth substrate in the presence of growth substrate is not well understood. In the presence of growth substrate, reducing power can be obtained from the growth substrate itself (Saez and Rittmann 1991). Recently Criddle (1993) proposed a single unstructured model to describe non-growth substrate transformation in the presence of growth substrate by coupling the transformation of non-growth substrate to consumption of growth substrate and biomass.

2.3.3 Degradation of BTX and Its Pathway

Degradation as a Single Component

Benzene and toluene have been known as moderately to easily biodegradable compounds (Kincannon *et al.* 1983a; Kincannon *et al.* 1983b; Richards and Shieh 1986; Tabak *et al.* 1981). The ability of bacteria to utilize aromatic hydrocarbons for growth was first demonstrated in 1908 by Stormer who isolated bacteria which could grow on toluene and xylene. In 1913, Sohngen reported the utilization of benzene by microorganisms. In 1968, Gibson *et al.* isolated a strain of *Pseudomonas putida* that would grow with ethylbenzene as the sole source of carbon and energy. This organism would also grow with toluene and toluene-grown cells rapidly oxidized benzene to catechol. Claus and Walker (1964) isolated a *Pseudomonas* species and an *Achromobacter* species that would grow with toluene as the sole source of carbon and energy. *p*-xylene is also known to be oxidized by *Nocardia corallina* (Jamison, Raymond, and Hudson 1969).

Degradation Pathway for BTX

Many researchers have studied biodegradation of BTX compounds (Dagley *et al.* 1964; Dagley, Evans and Ribbons 1960; Davey and Gibson 1974; Davies and Evans 1964; Davis, Hossler and Stone 1968; Evans 1963; Franklin *et al.* 1983; Gibson 1968; Gibson, Mahadevan and Davey 1974; Hayaishi and Nozaki 1969; Hou ; Kitagawa 1956; Nozaka and Kusunose 1968; Nozaka and Kusunose 1969; Omori, Horiguchi and Yamada 1967; Omori and Yamada 1969; Omori and Yamada 1970a; Omori and Yamada 1970b; Ribbons and Eaton 1982). The pathways for BTX are summarized on Table 2-3 along with the microorganisms involved and references. The degradative pathway of benzene to catechol and *cis,cis* -muconic acid was first demonstrated by Marr and Stone (1961). In 1968,

Table 2-3. Degradation pathway of BTX compounds

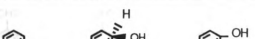
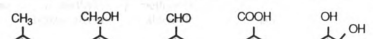
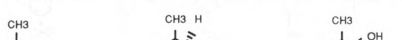
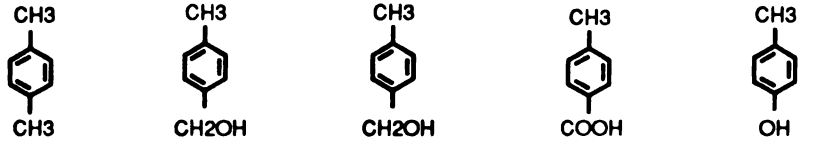
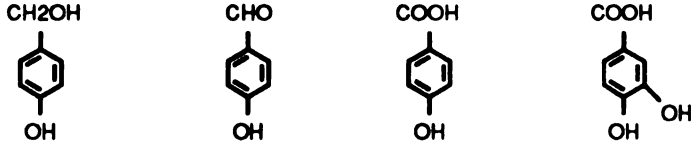
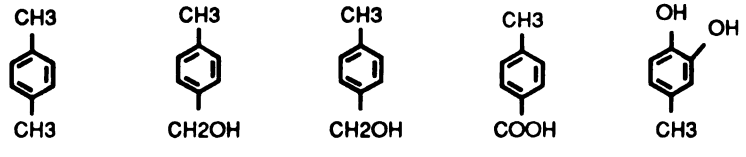
Oxidation reaction	Microbial species	Reference
 benzene - *cis*-benzene glycol - catechol	*Pseudomonas aeruginosa*	Marr and Stone 1961
 toluene - benzyl alcohol - benz aldehyde - benzoic acid - catechol	*Pseudomonas aeruginosa*	Kitagawa 1956 Nozaka and Kusunose 1968
 toluene - *cis*-2,3-dihydroxy-2,3-dihydrotoluene - 3-methyl catechol	*Pseudomonas mildenberger* *Pseudomonas putida*	Claus and Walker 1964 Nozaka and Kusunose 1968 Gibson et al. 1970
 *p* -xylene - *cis* -3,6-dimethyl- 3,5-cyclohexadiene-1,2-diol (*p* -xylene dihydrodiol) - 3,6-dimethyl pyrocatechol * *p*-xylene was cometabolized with the presence of succinate	*Pseudomonas putida 39/D*	Gibson, Mahadevan, and Davey 1974

Table 2-3. (cont'd)

Oxidation reaction	Microbial species	Reference
 *p* -xylene - *p* -methyl benzyl alcohol - *p* -methylbenz- aldehyde - *p* -toluic acid - *p* -cresol  - *p* -hydroxy benzylalcohol - *p* -hydroxy- benzaldehyde - *p* -hydroxy benzoicacid - protocatechuic acid	*Pseudomonas aeruginosa*	Nozaka and Kusunose 1968 Omori, Horiguchi, and Yamada 1967 Omori abd Yanada 1970
 *p* -xylene - *p* -methyl benzylalcohol - *p* -tolue- aldehyde - *p* -toluic acid - 4-methyl catechol - 2-hydroxy-5-methylmuconic semialdehyde - Ring Fission	*Pseudomonas Pxy*	Davey and Gibson 1974

Gibson found that *cis* - benzene glycol was an intermediate in the conversion of benzene to catechol.

Microbial degradation of substituted benzene including toluene and xylene is initiated by oxidation of the methyl group or the aromatic ring, depending on the organism. Oxidation of the aromatic ring is similar to the degradation of benzene. Toluene is oxidized to 3-methyl catechol. This pathway is reported by Claus and Walker (1964) and supported by others (Gibson *et al.* 1970; Nozaka and Kusunose 1969).

The pathway of *p* -xylene degradation is also well documented. Two pathways for bacterial oxidation of *p* -xylene have been reported. Gibson *et al.* (1974) showed the initial oxidation products formed from *p*- and *m*- xylene by *Pseudomonas putida* 39/D by aromatic ring oxidation. Gibson's proposed pathway of *p*-xylene produces diol and catechol. Similar observation was made from *Nocardia corallina* (Jamison, Raymond, and Hudson 1969). However, this strain further transformed 3,6-dimethyl pyrocatechol to α,α'- dimethyl-*cis,cis* muconic acid by ring cleavage. Oxidation of the methyl substituent is the second case of degradation. This pathway is investigated more thoroughly than the aromatic ring oxidation. Even though proposed pathways are described slightly differently by different researchers, all researchers commonly found that *p*-xylene produces *p*-toluic acid as a byproduct.

2.4 The Effect of Byproducts on Sorption

The presence of background organic matter can significantly affect the behavior of target organic compounds in GAC columns (Summers *et al.* 1989). Metabolic byproducts, which are formed as a result of biodegradation, can also affect the sorption of target

compounds. Schultz and Keinath (1984) studied the adsorption of metabolic end product (MEP) by powdered activated carbon during the degradation of phenol substrate. They found 62 % of the MEP could be adsorbed by PAC and also observed this adsorbed MEP was not desorbed by dilution or displaced by the target compound, phenol. Chang's study (Chang 1985) with ^{14}C labeled compound also showed competition between microbial products and the target compound, acetate. Olmstead (1989) showed the effect of microbial products on adsorption using bovine serum albumin (BSA) as the representative protein. His results did not indicate a significant difference between isotherms in the absence or presence of BSA. On the other hand, the adsorption with GAC preloaded with soluble microbial product (SMP) did show the different sorption patterns.

2.5 Multicomponent BAC System

Little is known regarding the performance of solute mixtures in BAC systems. Multi-component systems involve both competitive adsorption and interference in biodegradation. Speitel, Jr. *et al.* (1989) have studied the performance of bisolute mixture in a BAC column using *p*-nitrophenol and trichloroethylene (TCE), under the assumption that TCE is not aerobically biodegradable. This study showed the displacement effect of one chemical by the others, but did not show biological interactions in the removal of these two compounds. The assumption that TCE is not aerobically degradable may not be valid, however. In 1985, Wilson and Wilson showed that aerobic metabolism of TCE occured when soil microflora were exposed to natural gas in air. Recent studies by Nelson *et al.* (Nelson, Montgomery, and Pritchard 1988; Nelson *et al.* 1987) showed that TCE can be cometabolized in the presence of phenolic compounds. To study substrate interactions in the BAC systems, kinetic characterization of each species should precede the BAC experiment.

CHAPTER 3

KINETICS OF COMPETITIVE INHIBITION AND COMETABOLISM IN THE BIODEGRADATION OF BENZENE, TOLUENE, AND *p*-XYLENE BY TWO *PSEUDOMONAS* ISOLATES

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3.1 Introduction

Benzene, toluene, xylene (BTX) are commonly found in soils and groundwater at sites contaminated by gasoline and other petroleum products. Laboratory investigations of biodegradation have often focused on single substrates alone, neglecting the fact that environmental contamination by single substrate is uncommon. The vast majority of field situations involve degradation of multiple substrates. Complex degradation patterns are frequently observed in such cases. Techniques are needed to quantify these kinetic interactions to permit more rational design and operation of engineered systems.

In the metabolism of multiple substrates, frequently reported degradation patterns include diauxie (Monod 1942), simultaneous utilization (Deshpande, Chakrabartl and Subrahmanyam 1987), and competitive inhibition (Law and Button 1977; Strand, Bjelland and Stensel 1990). Diauxie or sequential utilization results from catabolite repression or catabolite inhibition (Voice *et al.* 1992). Simultaneous utilization of substrates may or may not result in growth. Simultaneous utilization of substrates that do not support growth is

termed cometabolism (Grady 1985). As defined by Dalton and Stirling (1982), co-metabolism is the transformation of a non-growth substrate in the "obligate presence of a growth substrate or another transformable compound". More generally, cometabolism also includes transformations by resting cells if no growth results.

A number of substrate interactions have been observed during hydrocarbon degradation. Bauer and Capone (1988) reported a stimulatory effect of benzene on the degradation of anthracene. Meyer, Marcus and Bergman (1984) observed diauxic degradation of benzene in the presence of phenol. Growth on toluene induced non-specific dioxygenase activity, permitting cometabolism of trichloroethylene (TCE) (Nelson *et al.* 1987). Goldsmith Jr. and Balderson (1988) evaluated BTX degradation kinetics in an enrichment culture. They obtained first-order kinetic parameters for individual compounds and demonstrated differences in the rates of degradation among substrates. Interactions between benzene and other aromatic carbons were evaluated by Arvin *et al.* (1989). These researchers concluded that benzene degradation was enhanced in the presence of either toluene or *o*-xylene. The effect of different substrate combinations were evaluated statistically using factorial experiments. Recent studies by Alvarez and Vogel (1991) revealed interactions during BTX degradation by organisms from aquifer enrichments and by two pure-culture organisms. Factorial experiments were designed and interactions evaluated by comparing changes in lag period and in pseudo zero-order biodegradation rates. Induction, inhibition, and cometabolism were observed. These researchers observed enhanced degradation of benzene and *p*-xylene in the presence of toluene by a *Pseudomonas* strain. In none of these studies was a detailed quantitative interpretation of substrate interactions attempted. Quantification is needed to facilitate comparison of cultures from different sources, and to advance the design and operation of engineered systems (Grady 1990).

The objectives of this study were: 1) to isolate BTX-degrading organisms with different degradation characteristics from a biological activated carbon fluidized bed treatment system, 2) to determine kinetic parameters for single substrate degradation, 3) to categorize the pattern of multiple substrate utilization, and 4) to quantify substrate interactions in the biodegradation of paired substrates (BT, TX, and BX).

3.2 Materials and Methods

3.2.1 Growth Medium and Chemicals

HCMM2 mineral salts medium was prepared as described elsewhere (Ridgway *et al.* 1990). This medium has been previously used for the isolation of gasoline degrading bacteria. Solid medium was prepared by addition of 15 g/L Bacto agar (Difco laboratories, Detroit, Mich.) to the mineral medium. Reagent grade benzene, toluene, and *p*-xylene were obtained from Aldrich Chemical Co., Milwaukee, Wis.

3.2.2 Isolates

BTX-degrading isolates were obtained from an aerobic pilot-scale activated carbon fluidized bed reactor located at Michigan State University. Details of reactor operation and design are described elsewhere (Voice *et al.* 1992). After a three month period of continuous operation treating BTX-contaminated water, activated carbon particles with well developed biofilms were removed from the fluidized-bed reactor. Approximately five particles were placed in a 25-mL glass vial containing 10 mL of phosphate buffer solution and ten 4 mm diameter glass beads. The vial was vigorously shaken and allowed to stand for 15 minutes until all large floc had settled. A dilution series was then prepared from the

supernatant. Spread plates for each dilution were exposed to vapors of benzene, toluene, or *p*-xylene in sealed chambers at room temperature (~24°C). After 48 hours, isolates were picked, streaked, and re-incubated. This procedure was repeated 5 to 8 times. Colonies isolated by growth upon vapors of a single pure compound, such as benzene, were then tested for growth in chambers containing other substrates, such as toluene or *p*-xylene. Growth of isolates was also evaluated in separate BTX vapor chambers in which the substrates were paired (BT, TX, BX) or combined (BTX).

Two isolates, designated strains B1 and X1, were selected for further investigation based on their distinctive degradation patterns. Strain B1 did not degrade *p*-xylene, and strain X1 did not degrade benzene. Each strain was characterized and identified on a GN MicroPlate® test panel using Biolog's MicroLog® software. Strain B1 was identified as *Pseudomonas fragi*, with a high confidence rating in a MicroLog® search; strain X1 was identified as *Pseudomonas fluorescens*, but with a low confidence rating. Fatty acid analysis performed by Microbial ID, Inc., Newark, Del., confirmed the *Pseudomonas* classification. Strain X1 had a high correlation with the fatty acid profile for a library strain of *Pseudomonas fluorescens*.

3.2.3 Inoculum Preparation

Picked colonies were transferred to sealed 250-mL glass bottles with 100 mL HCMM2 liquid medium containing in the liquid phase: 10 mg/L of either benzene, toluene, or *p*-xylene, or 5 mg/L of paired combinations of these substrates. Immediately after these substrates had completely degraded (as indicated by GC analysis), the resulting cell suspension (~15 mg/L) was used as an inoculum for batch experiments.

3.2.4 Kinetic Experiments

In all kinetic experiments, benzene, toluene, and *p*-xylene were assayed using a headspace sampling method (Gossett 1985). Ninety-eight milliliters of HCMM2 mineral media were added to a 250-mL glass bottle (VWR catalog no. 16151-300) and sealed with a pressure-tested Teflon Mininert valve (Alltech catalog no. 95326). The bottle was covered with aluminum foil and autoclaved for 20 minutes at 121°C . After cooling to room temperature, BTX substrates were added to the bottles from stock solutions. The bottles were then sealed and shaken for 12 hours. This procedure ensured complete dissolution of the hydrocarbons and equilibrated the headspace with the solution. The liquid-phase concentration at the initiation of an experiment was approximately 10 mg/L for each compound. For the paired substrate experiments, the total carbon source was doubled. Batch kinetic experiments were conducted using the inoculum directly (~15 μg/mL) or immediately after degradation of a second spike of growth substrate (~30 μg/mL).

In experiments to assess substrate interactions and effects on growth, two milliliters of the 30 μg/mL inoculum was transferred to the bottle leaving 150 mL of headspace. The bottles were shaken on a rotary shaker at 100 rpm. Rates of mass transfer of BTX between the gas and aqueous phases under these conditions (0.2 min^{-1}, 0.16 min^{-1}, and 0.12 min^{-1} for B,T, and X, respectively) were higher than the maximum biodegradation rates ensuring rapid equilibration with the liquid phase and justifying use of gas-phase measurements for biodegradation experiments. After inoculation, headspace gas samples (0.5 mL) were withdrawn from the bottle using a 1.0- mL Precision gas-tight syringe (Alltech catalog no. 050033) equipped with a side-port needle (Alltech catalog no. 943052), and assayed by injection onto a Hewlett Packard 5890 gas chromatograph equipped with a DB-624 capillary column (30m x .549 mm, J & W Scientific) and a flame ionization detector. The

carrier was helium (15 mL/min). Oven temperature began at 40°C and increased at a rate of 30°C/min to 200°C, where it remained constant for 2 minutes.

Gas sampling continued until growth substrate concentrations dropped below the detection limit (~0.01 ppm). A sterile control was also assayed to assess abiotic losses of the target compounds. Growth was monitored with optical density measurement at 560 nm using a Shimadzu UV spectrophotometer (path length = 1 cm). Biomass concentration was estimated from a correlation of optical density (O. D.) to dry weight. For strain B1, biomass concentration (mg/L) = (O.D. - .0046) / .0026 (98% correlation); for strain X1, biomass concentration (mg/L) = (O.D. - .0060) / .0025 (99% correlation).

In experiments to assess competitive inhibition, the 15 μg/mL inoculum was used directly in the same bottle in which it had grown. After depletion of the original growth substrate, paired combinations of substrates were added. Two substrate combinations, benzene-toluene and toluene-xylene, were tested with strain B1. In both cases, the amount of benzene or xylene was held constant while the amount of toluene was varied (700, 1400, 2800 μg corresponding to ~5, 10, 20 mg/l in the liquid phase respectively). Three levels of inhibitor were used (700, 1400, 2800 μg). The inhibitory effects of benzene and xylene on toluene were evaluated by comparing the initial degradation rate of toluene. Changes in the concentration of biomass and inhibitor over the initial period of degradation (60 minutes) were insignificant (<5%).

3.2.5 Identification of Byproducts

UV spectroscopy at 279 nm revealed the presence of one or more byproducts arising from transformation of *p* -xylene by *Pseudomonas* strain B1. Three UV-absorbing products were subsequently separated on a Gilson high pressure liquid chromatograph

equipped with a fraction collector and a UV detector. These fractions were identified by comparing their HPLC run time and elution patterns on thin layer chromatography with known standards. *Pseudomonas putida* strain 39/D and *Pseudomonas putida* strain F1, two strains known to transform *p*-xylene, were used to provide standards matching two of the three peaks. *Pseudomonas putida* strain 39/D converts *p*-xylene to *p*-xylene dihydrodiol and *Pseudomonas putida* strain F1 converts *p*-xylene to 3,6-dimethyl pyrocatechol (Gibson 1968; Gibson *et al.* 1970; Gibson *et al.* 1968; Gibson, Mahadevan and Davey 1974). The third peak was identified as 2,5-dimethyl phenol using an authentic standard (Aldrich Chemical Co., Milwaukee, Wis.). This identification was confirmed by ether extraction and gas chromatography/mass spectroscopy as per Gibson *et al.* (1974).

3.2.6 Estimation of Decay Rates and Maximum Yields

Decay rates were measured in the absence of non-growth substrate (*p*-xylene) and in its presence, at three different aqueous- phase concentrations (6, 9 and 12 mg/l) added immediately after the growth substrate (toluene) had disappeared. Optical density and *p*-xylene concentrations were monitored throughout the decay period (120 minutes). The decay rate was obtained from the slope of a ln(X) vs. time plot. The maximum yield was obtained by incorporating the single-substrate toluene degradation data into the expression $\mu = Y_m(-dS/Xdt)-b$. This expression was solved by varying Y_m to determine the best fit to the growth curve.

3.2.7 Parameter Estimation for Single Substrates

A Monod expression was used to estimate kinetic parameters for single-substrate biodegradation in batch experiments:

$$\frac{-dM}{dt} = \frac{kS_L X V_L}{K_s + S_L} \qquad (3\text{-}1)$$

Equation (3-1) was modified using Henry's constants to correct for the continuous supply of target compound from the headspace:

$$M = S_L \cdot (V_L + H_C \cdot V_G) \qquad (3\text{-}2)$$

Substituting equation (3-2) into equation (3-1) yields:

$$-\frac{dM}{dt} = \frac{k \cdot M \cdot X \cdot V_L}{K_S \cdot (V_L + H_C \cdot V_G) + M} \qquad (3\text{-}3)$$

The half saturation coefficient, K_S, and the initial biomass, X_o, were estimated by non-linear parameter estimation using an integrated form of equation (3-3), where $X=X_o+Y\cdot(M_o-M)/V_L$. Approximate values for X_o were obtained by measuring the optical density of the inoculum. However, because model predictions were sensitive to the choice of X_o (Figure 3-1), X_o was selected as a fitting parameter, but was constrained to the 95% confidence interval for the inoculum as determined by O.D. measurements. Statistical analysis was performed by the statistical package SYSTAT 5.0 (SYSTAT, Inc.). K_S values obtained by non-linear parameter estimation using the analytical solution to equation (3-3) were the same as values obtained by a least-squares minimization procedure using a numerical solution for equation (3-3). Figure 3-2 illustrates the sensitivity of model predictions to K_S values for a fixed X_o.

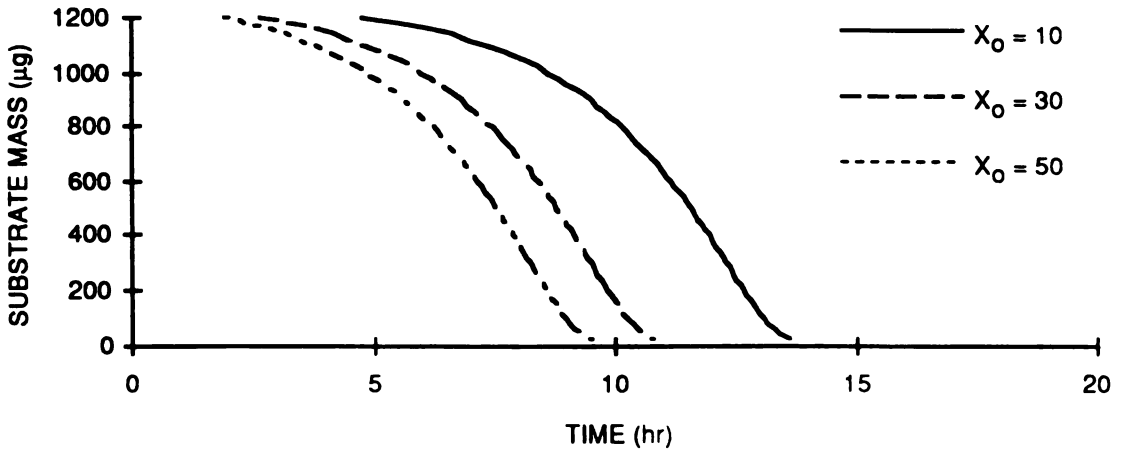


Figure 3-1. Sensitivity test for different initial biomass X_0 (µg) with the same half saturation constant K_S of 1 mg/l.

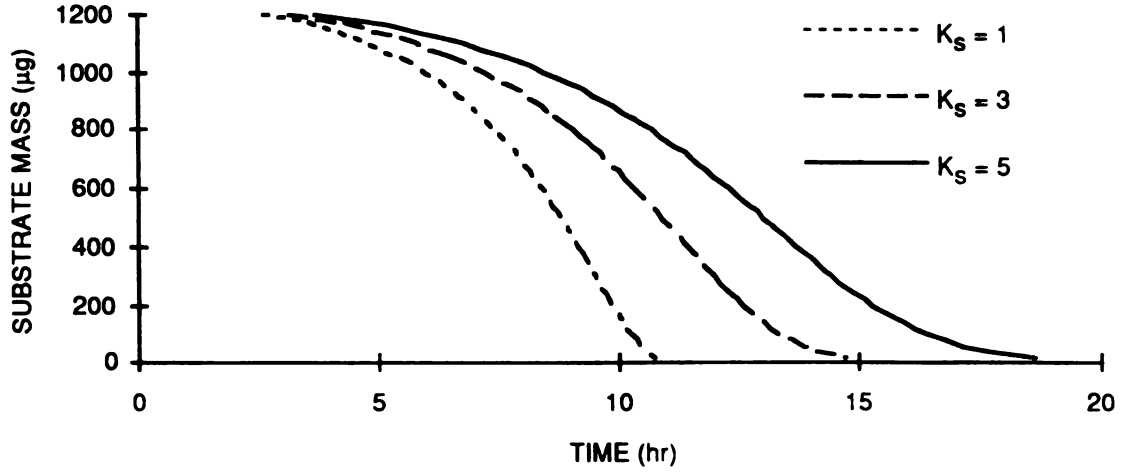


Figure 3-2. Sensitivity test for different half saturation constant K_S (mg/l) with the same initial biomass of 30 µg.

3.2.8 Quantification of Competitive Inhibition

Competitive inhibition was modeled with the following expressions (Machado and Grady 1989) :

$$-\frac{dM_1}{dt} = \left(\frac{\mu_{max,1}}{Y_1}\right) \cdot \frac{S_{L,1} \cdot X \cdot V_L}{K_{S,1} \cdot \left(1 + \frac{I_{L,1}}{K_{I,1}}\right) + S_{L,1}} \qquad (3\text{-}4)$$

$$-\frac{dM_2}{dt} = \left(\frac{\mu_{max,2}}{Y_2}\right) \cdot \frac{S_{L,2} \cdot X \cdot V_L}{K_{S,2} \cdot \left(1 + \frac{I_{L,2}}{K_{I,2}}\right) + S_{L,2}} \qquad (3\text{-}5)$$

To account for partitioning of substrate from the gas phase into the liquid phase, equation (3-2) was substituted into equations (3-4) and (3-5). K_S, μ_{max}, and Y were previously obtained from single-substrate experiments. $K_{I,benzene}$ was measured in a batch experiment; $K_{I,toluene}$ was estimated using the best fit to the degradation curve.

For competitive inhibition between benzene and toluene, growth was described using equation (3-6). A lag term was introduced numerically into this expression to account for the observed lag between substrate removal and biomass production (see Discussion).

$$X = X_o + \frac{Y_{benzene} \cdot (M_{o,benzene} - M_{benzene})}{V_L} + \frac{Y_{toluene} \cdot (M_{o,toluene} - M_{toluene})}{V_L} \qquad (3\text{-}6)$$

3.2.9 Quantification of Cometabolism

The following expressions were used to quantify cometabolism. Details of this model are provided elsewhere (Criddle 1993).

$$-\frac{dM_g}{dt} = k_g \cdot \left(\frac{S_g}{K_{S,g} + S_g}\right) \cdot X \cdot V_L \tag{3-7}$$

$$-\frac{dM_c}{dt} = \left(T_c^g \cdot \left(\frac{-dM_g}{X \cdot V_L \cdot dt}\right) + k_c\right) \cdot \left(\frac{S_c}{K_{S,c} + S_c}\right) \cdot X \cdot V_L \tag{3-8}$$

$$\frac{dX}{dt} = Y_m \cdot \left(\frac{-dM_g}{V_L \cdot dt}\right) - b - \frac{1}{T_c^{b^*}} \cdot \left(\frac{-dM_c}{V_L \cdot dt}\right) \tag{3-9}$$

Equations (3-7) - (3-9) were modified using equation (3-2) to account for partitioning from the gas phase and solved using a second order Runge-Kutta numerical solution. T_c^g was estimated using non-linear curve fitting procedures. $T_c^{b^*}$ was obtained by monitoring biomass decay and loss of the non-growth substrate in the decay period.

3.3 Results

The parameter estimation results for single-substrate degradation experiments are summarized in Table 3-1. Multi-substrate degradation studies revealed three different substrate utilization patterns: no interaction, competitive inhibition, and cometabolism. These patterns are given in Table 3-2 for each organism and for each substrate pair.

Table 3-1. Single compound biodegradation kinetic parameters for strains B1 and X1.

Strains	Substrates	μ_{max} (day $^{-1}$)	Yield (mg/mg)	K_S (mg/l)
B1	Benzene(3)	8.05±3.10	1.04±.09	3.17±0.82
	Toluene(3)	13.03±1.83	1.22±.01	1.96±0.91
	Xylene	-	-	-
X1	Benzene	-	-	-
	Toluene(6)	10.84±2.77	0.99±.25	1.88±1.26
	Xylene(3)	12.85±1.73	0.52±.03	4.55± 0.37

* Numbers in parenthesis are the number of experiments run. Parameters estimated are shown ± one standard deviation.

Table 3-2. Degradation patterns for each combination by each strain.

	Substrate Combination*		
	B/T	T/X	B/X
Strain B1	Competitive inhibition of B by T	Cometabolic degradation of X	Cometabolic degradation of X
Strain X1	T was not affected and B was not degraded	Competitive inhibition of X by T	X was not affected and B was not degraded

* B=benzene, T=toluene, and X=*p*-xylene

As shown in Figure 3-3, strain B1 removed benzene and toluene to concentrations below the detection limit (.01 ppm) in a 12-hour period. Strain B1 grew faster on toluene than on benzene, as evidenced by the larger μ_{max} values in Table 3-1. Consequently, the degradation rate of toluene was faster than that of benzene as shown on Figure 3-3. The rate of degradation of either benzene or toluene in the presence of the other was slower than the degradation rate of either substrate alone. To evaluate the nature of inhibitive interactions, a double-reciprocal plot (1/M vs. -1/(dM/dt)) was prepared for toluene degradation in the presence of varied concentrations of benzene. As shown in Figure 3-4, benzene and toluene are competitive inhibitors for strain B1.

Substituting equation 3-2 into equation 3-4 and inverting the resulting expression yields:

$$-\frac{1}{\frac{dM_1}{dt}} = \frac{1}{M_1} \cdot \frac{K_{S,1} \cdot \left(1 + \frac{I_{L,1}}{K_{I,1}}\right) \cdot (V_L + H_C \cdot V_G)}{k_1 \cdot X \cdot V_L} + \frac{1}{k_1 \cdot X \cdot V_L} \qquad (3\text{-}10)$$

The competitive inhibition parameter $K_{I,benzene}$ was obtained graphically from the Lineweaver-Burke plot described mathematically by equation (3-10) and illustrated in Figure 3-4. A value of 3.10±0.12 mg/L was obtained. This value compares well with the $K_{S,benzene}$ value obtained in single substrate experiments (3.17±0.82, Table 3-1). $K_{I,toluene}$ was estimated by curve fitting, using values provided in Table 3-1. A value of 1.71 mg/L was obtained. This value compares well with the value for $K_{S,toluene}$ (1.96±.91 mg/L) obtained in single substrate experiments. The $K_{S,toluene}$ value obtained from competitive inhibition experiments (Figure 3-4) was 1.23 mg/L.

Unlike strain B1, strain X1 did not degrade benzene, whether it was added alone or together with toluene or *p*-xylene (data not shown). In addition, the presence of benzene

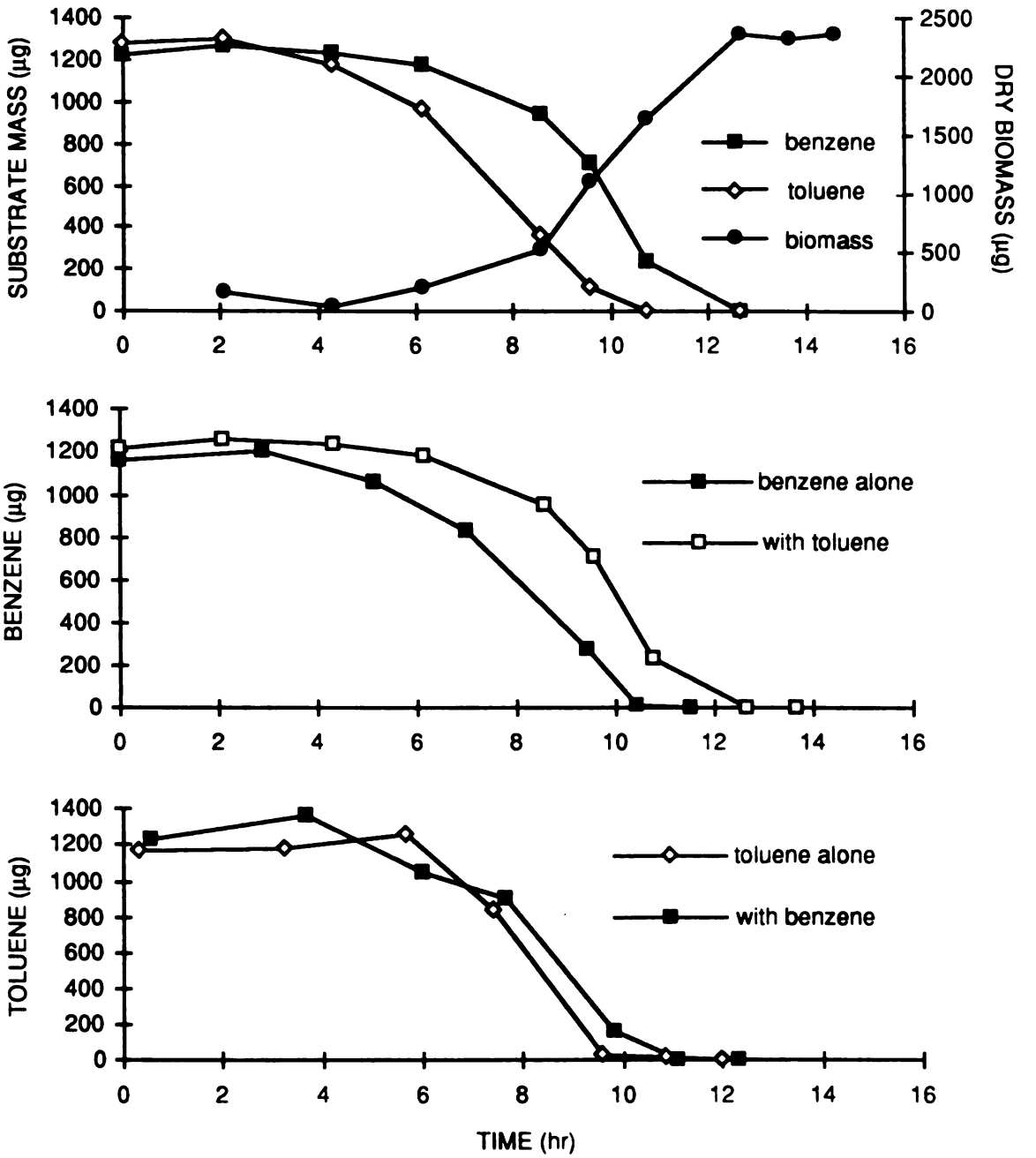


Figure 3-3. Typical curves illustrating competitive inhibition in the degradation of benzene and toluene by strain B1.

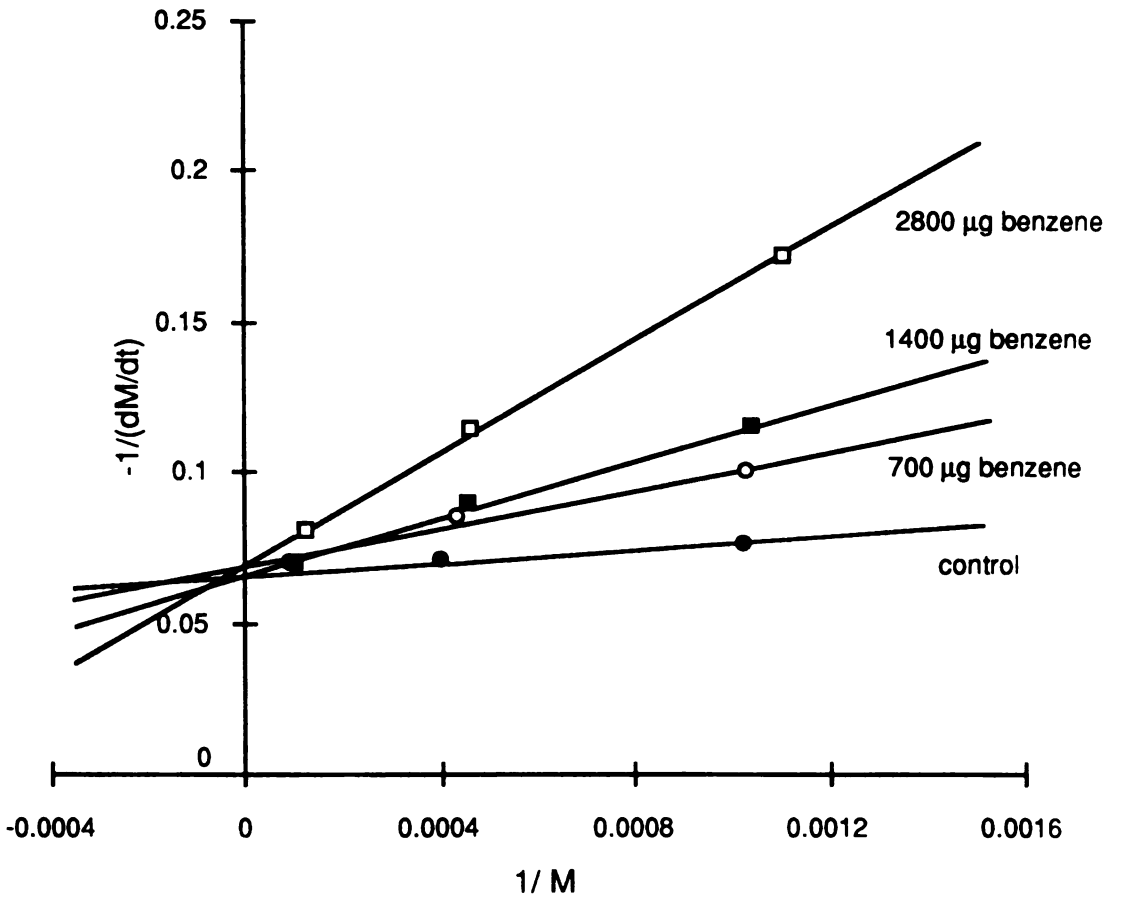


Figure 3-4. Lineweaver-Burke plot for the degradation of toluene alone and with benzene by strain B1.

did not affect the degradation rate of toluene or *p*-xylene. Mutual inhibition was observed in the degradation of toluene and xylene by strain X1. Strain X1 degraded *p*-xylene faster than toluene. However, toluene had a greater effect on the degradation of *p*-xylene than *p*-xylene had on toluene degradation, as evidenced by the smaller value of K_S for toluene.

Figure 3-5 illustrates cometabolic degradation of *p*-xylene in the presence of toluene by strain B1. In single substrate experiments, strain B1 was incapable of transforming *p*-xylene. However, *p*-xylene was transformed when combined with a growth substrate, such as benzene or toluene. Transformation of *p*-xylene slowed significantly when the growth substrate disappeared, but increased when a growth substrate (either benzene or toluene) was again added. Competitive inhibition experiments with toluene and *p*-xylene provided no evidence for competitive inhibition between these compounds in the concentration range studied. Products of the cometabolic transformation were tentatively identified as *p*-xylene dihydrodiol , 3,6-dimethyl pyrocatechol, and 2,5-dimethyl phenol.

Table 3-3 summarizes decay measurements for different concentrations of *p*-xylene in the absence of growth substrate. The decay rate increased with increasing concentrations of *p*-xylene, but appeared to reach a maximum value. Table 3-4 summarizes measured or estimated parameters for modeling degradation of toluene and *p*-xylene by strain B1. Similar observations were achieved for the degradation of benzene and *p*-xylene by strain B1.

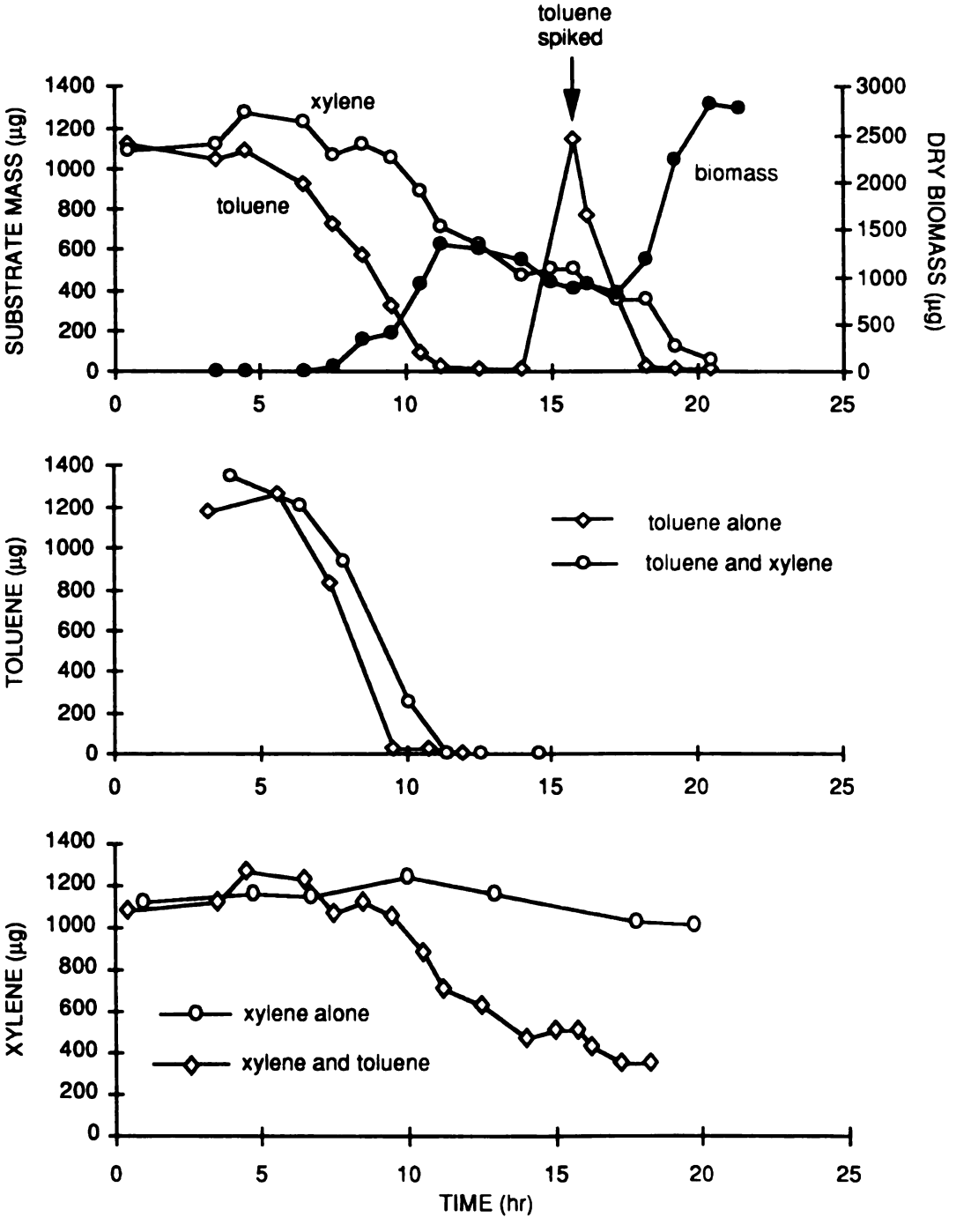


Figure 3-5. Typical curves illustrating cometabolic degradation of xylene in the presence of toluene by strain X1

Table 3-3. Decay rate of strain B1 with different initial *p*-xylene concentrations.

Initial *p*-xylene concentration (mg/l)	biomass decay rate (day^{-1})
0	0.41*
6	0.68
9	1.21
12	1.19

* Endogeneous decay rate

Table 3-4. Measured and estimated parameters for modeling degradation of toluene and cometabolism of *p*-xylene by strain B1.

Parameters	Value
Measured parameters	
μ_{max} for toluene	13.03 day^{-1}
K_S for toluene	1.96 mg/l
Endogeneus decay coefficient, b	0.41 day^{-1}
k for *p*-xylene [a]	0.71 mg xylene/mg biomass day^{-1}
Estimated parameters	
Y_{max} for toluene [b]	1.27 mg biomass/mg toluene
T_C^G - toluene transformation capacity [c]	0.45 mg xylene/mg toluene
$T_C^{b^*}$ - biomass transformation capacity [d]	2.4 mg xylene/mg biomass
K_S for xylene [e]	-

a. measured graphically from the initial disappearance of non-growth substrate (xylene).
b. estimated from equation 3-10.
c. estimated by curve fitting model of the degradation data for non-growth substrate (xylene) disappearance while growth substrate (toluene) was present.
d. estimated by curve fitting of degradation data for the non-growth substrate (xylene) after growth substrate (toluene) had completely degraded.
e. K_S was not determined, but was assumed to be much less than the xylene concentration.

3.4 Discussion

The two *Pseudomonas* isolates demonstrated very different substrate preferences for BTX. This observation calls into question the extent to which sweeping generalizations regarding the ease of degradability of BTX can or should be made for mixed cultures, especially in the absence of an intimate understanding of the processes and populations present (Arvin *et al.* 1989). In this study, three different patterns of degradation were observed in dual substrate experiments (no interaction, competitive inhibition, and cometabolism), and these patterns required different modeling approaches for their quantification.

In conventional Monod modeling approaches, substrate utilization is tightly (instantaneously) coupled to biomass production, as indicated by equation (3-4). However, in the single-substrate batch BTX experiments, the disappearance of substrate actually *preceded* biomass production. The same phenomenon was observed in some of the dual-substrate experiments. Figure 3-6 shows how experimental data and model predictions compare for the degradation of benzene and toluene by strain B1. Using single-substrate yield coefficients and assuming a tight coupling of substrate disappearance to biomass formation, the model predicted biomass appearance at an earlier point in time than observed (solid line in Figure 3-6), suggesting that a time lag occurs between the uptake of substrate and the production of biomass. The possibility that this lag was due to slow air/water mass transfer was eliminated based on the high measured mass-transfer rates (data not shown), and the fact that hindered air/water mass transfer would result in an apparent lag in *substrate* consumption, not biomass production. An alternate explanation for the time lag is that biomass formation results from the slow degradation of a BTX metabolite. This hypothesis was supported by optical density measurements showing continued increase in biomass after all of the substrate had disappeared from the system. By

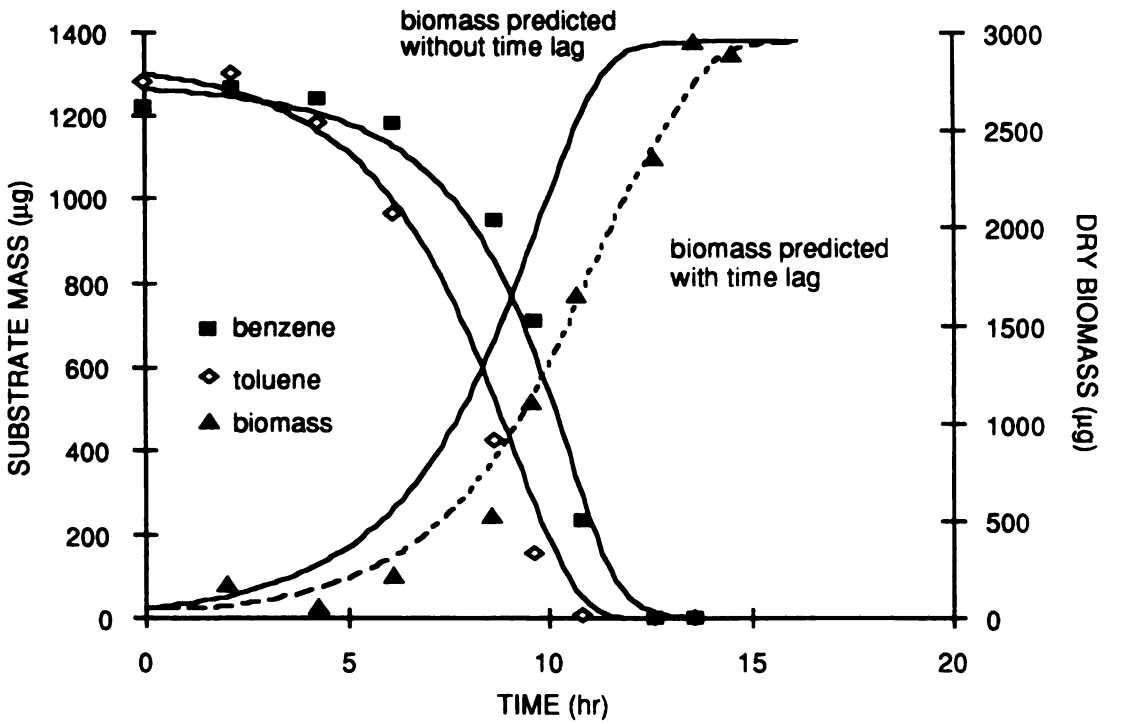


Figure 3-6 . Comparisons between experimental data and model (estimated by Runge-Kutta numerical solution) for competitive inhibition of benzene and toluene by strain B1 ($K_{S,benzene}$ = 3.17 mg/l, $K_{S,toluene}$ = 1.96 mg/l, X_O = 50µg, $K_{I,benzene}$ = 3.10 mg/l, $K_{I,toluene}$ = 1.71 mg/l, and time lag = 70 min)

numerically incorporating a time lag into the model (dotted line of Figure 3-6), good fits to the biomass data were obtained using equations (3-4) - (3-5). Sharma and Ahlert (1977) noted that while the Monod equation predicts an instantaneous coupling of substrate degradation with growth, such a relationship is not observed in non-steady state systems. Storer and Gaudy (1969) reported growth rate hysteresis in their system, and showed that the Monod equation does not fully describe transient behavior.

In this work, K_S values obtained in single substrate experiments agreed remarkably well with K_I values obtained in competitive inhibition experiments. This observation suggests that the inhibition coefficient of a competitive inhibitor may be approximated by its single substrate half saturation coefficient as suggested by other researchers (Machado and Grady 1989; Voice *et al.* 1992).

An important aspect of this work was the successful quantification of cometabolic transformation rates in the presence of growth substrate. Many studies have addressed cometabolic transformation by resting cells in the absence of growth substrate (Alvarez-Cohen and McCarty 1991a; Alvarez-Cohen and McCarty 1991b; Saez and Rittmann 1991). This situation rarely occurs in practice, however. More often, the growth substrate is added to enhance the degradation of non-growth substrate by supplying electrons and energy for more rapid and extensive transformation. An important observation was the reduction in yield for cells grown in the presence of *p*-xylene. This effect can also be expected for cometabolizing populations (Bauer and Capone 1988), and agrees with predictions for the integrated model used to quantify cometabolism (Criddle 1993).

The principal UV-absorbing byproducts were identified as *p*-xylene dihydrodiol and 3,6-dimethyl pyrocatechol. The proposed pathway agrees with that proposed by Gibson, *et al.* (1974) and is illustrated in Figure 3-7. After a long period of incubation or

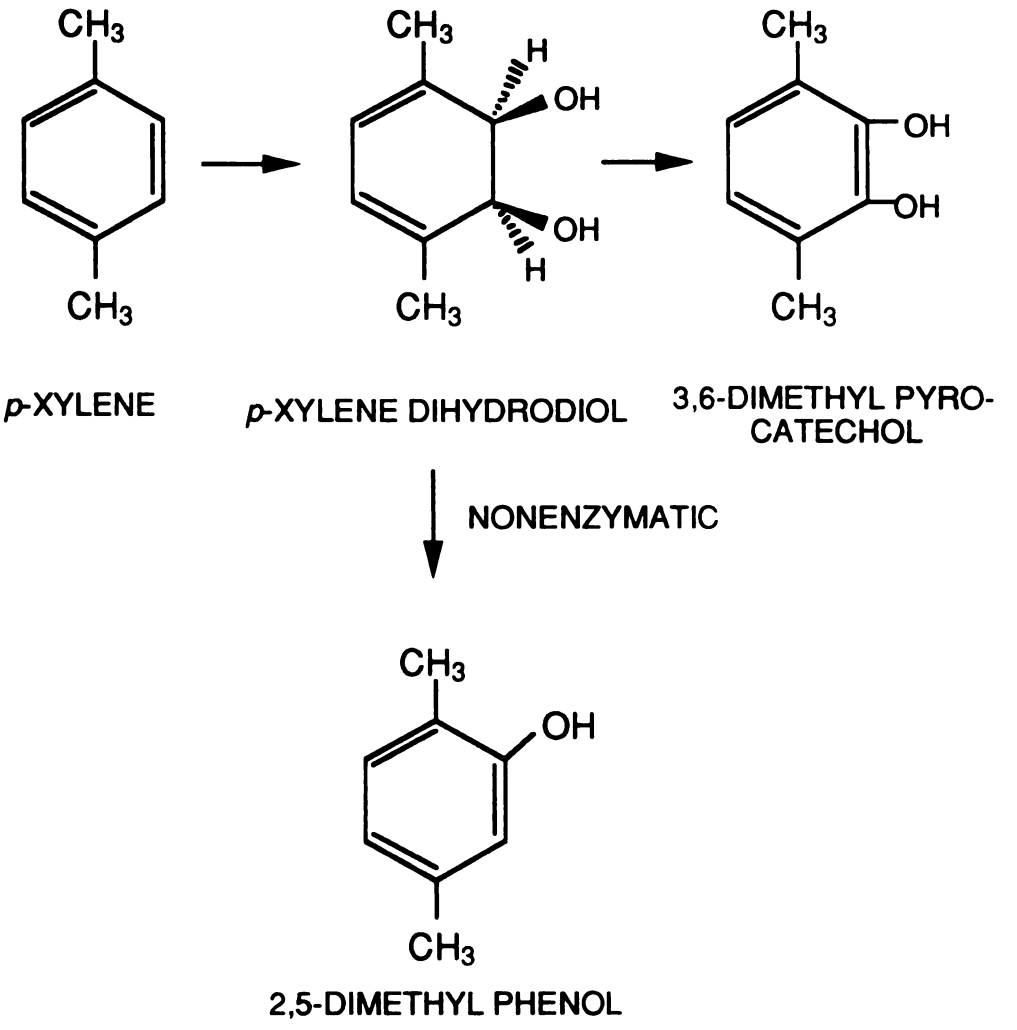


Figure 3-7. Pathway proposed for the oxidation of *p*-xylene (Gibson *et al.* 1974).

upon lowering the pH, 2,5-dimethyl phenol was produced. The formation of 2,5-dimethyl phenol continued even after cells were removed from the solution by centrifugation (5,000 rpm) and filtration through a 0.22 μm filter, and appears to be abiotic as suggested by Gibson *et al.* (1974).

The integrated model for cometabolism used here predicts that the rates of decay are dependent upon the concentration of non-growth substrate. Similar observations were made by Janke and Ihn (1989) with aniline (non-growth substrate) on cells grown with sodium acetate as a growth substrate. Sáez and Rittmann (1991) observed that biomass decay could be correlated with transformation of non-growth substrate during the decay period. The same observation was noted in this work, that is, when the growth substrate (toluene or benzene) was no longer present, further disappearance of non-growth substrate (*p*-xylene) correlated with biomass disappearance (Figure 3-8). Production of UV-absorbing byproducts also correlated with the loss in biomass (Figure 3-8 and Figure 3-9). The non-growth substrate did not contribute to biomass formation, only to its loss. A loss in biomass upon transformation of the non-growth substrate seems reasonable given the dependency of dioxygenase systems upon cell reducing power. In initial attempts to describe the transformation of *p*-xylene, a single transformation capacity term was used for both the growth and decay periods. However, this approach did not describe the accelerated transformation of *p*-xylene observed during the growth period. By adding a transformation capacity term from the growth substrate (equation 3-8), a reasonable fit to the data for the whole degradation curve was obtained (Figure 3-8).

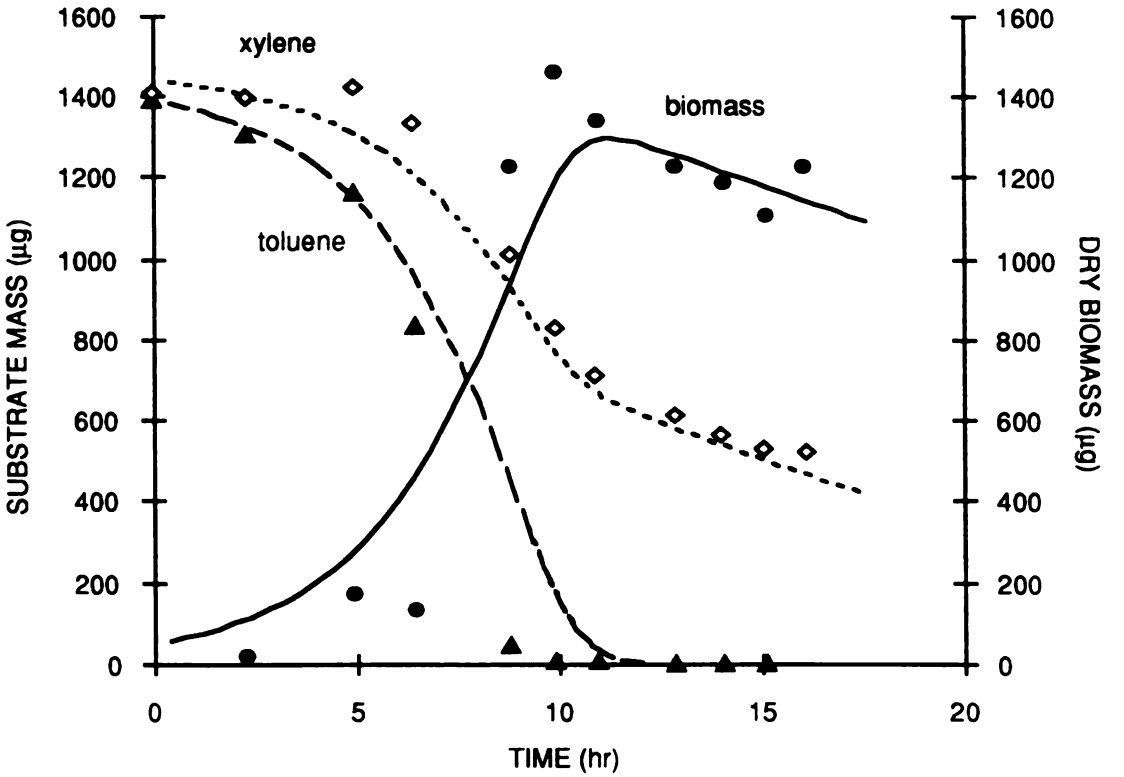


Figure 3-8. Modeling of cometabolism - degradation of toluene and xylene by strain B1 with parameters on Table 3-4. Initial concentration of each substrate in the liquid phase is approximately 10 mg/l.

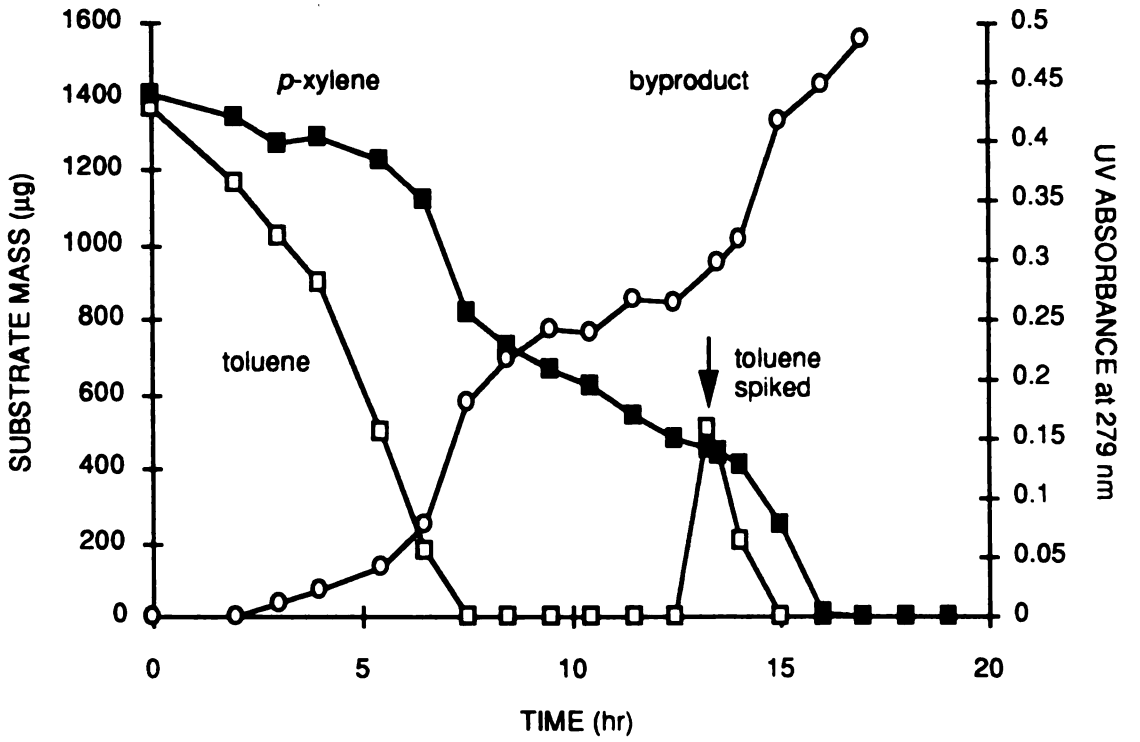


Figure 3-9. Formation of byproducts as monitored by absorbance at 279 nm for transformation of *p*-xylene with toluene as growth substrate by strain B1

3.5 Conclusions

Two *Pseudomonas* species with distinctive patterns of BTX degradation were isolated. Kinetic expressions, incorporating competitive inhibition, a lag time, and coupled cometabolic degradation were developed, and these expressions were used to quantify complex substrate interactions. The results suggest that paired competitive substrate interactions can be quantified using single-substrate kinetic parameters and batch degradation experiments. Cometabolic transformation during the growth phase can be modeled using two transformation capacity terms: one for the growth substrate, and a second for the biomass. The results also suggest that modeling and interpretation of BTX degradation in mixed-culture systems should be done with care, as differences in transformation may be due to differences in the microorganism present and to the complex enzymatic interactions possible within a single organism.

CHAPTER 4

KINETICS OF COMETABOLIC DEGRADATION OF *p*-XYLENE BY *PSEUDOMONAS SP.* STRAIN B1 AND THE INTERACTION OF DEGRADATION AND ADSORPTION IN FIXED-BED BIOLOGICAL ACTIVATED CARBON SYSTTEMS

4.1 Introduction

Benzene and methyl substituted benzene compounds are commonly detected in soils and groundwater at sites contaminated by gasoline and other petroleum products. Among the many remediation techniques available, carbon adsorption is widely used because of its potential to remove many of the contaminants regulated under the 1986 amendments to the Safe Drinking Water Act (SDWA). The US Environmental Protection Agency designated granular activated carbon (GAC) treatment as the '"best available technology" for treating a variety of regulated synthetic organic chemicals. In operation, it is commonly found that microorganisms readily colonize the carbon surface, a factor which must be considered in maintaining such systems. Biological activated carbon (BAC), exploits this phenomenon by encouraging organism growth and the resultant degradation of organic compounds delivered to the column. By combining adsorption and biodegradation in a single system, BAC has been shown to have the effluent protection capability of GAC and the efficiency of biological systems.

Most BAC research has focused on single substrates, despite the fact that multiple substrate contamination is common in field systems. It is known that complex interactions occur between substrates in both adsorption and degradation processes. Speitel *et al.* (1989) have investigated the performance of a BAC column receiving a mixture *p*-nitrophenol and trichloroethylene (TCE). This study showed the sorption displacement effect of one chemical by the other, however, complex interactions in the degradation processes were not considered.

Adsorption interactions can be viewed in two ways. First, a variety of approaches have been developed to describe adsorption dynamics of multiple substrates or solutes (Fritz *et al.* 1980c; Digiano *et al.* 1980; Frick *et al.* 1980). These typically require an understanding of both the mass-transfer processes and adsorption energies of all species and a description of the interactions involved as solutes compete for adsorption sites. An alternative approach is to focus only on one compound of interest and study its behavior in a characterized background solution. For example, water known to contain humic materials measured only as total organic carbon, has been shown to reduce the capacity of activated carbon for certain target compounds (Summers *et al.* 1989).

Simultaneous biological utilization of substrates in multi-substrate systems may or may not result in growth. When growth is not supported, the process is termed cometabolism (Grady 1985). As defined by Dalton and Stirling (1982), cometabolism is the transformation of a non-growth substrate in the "obligate presence of a growth substrate or another transformable compound". More generally, cometabolism also includes transformations by resting cells if no growth results. The inability to grow on some substrates is not the result of an organism's inability to attack the substrate but often results from its inability to assimilate the products of oxidation (Foster 1962). Oxidation

of the substrate may induces the formation of enzymes capable of oxidizing other substrates.

Cometabolic transformation typically results in the production of byproducts that are not further utilized by the cometabolizing population. These compounds have the potential to interfere with adsorption in BAC systems, but no evidence was found in the literature that this has been studied. In a previous study in this laboratory, the formation of byproducts from the cometabolic degradation of *p*-xylene with toluene as a growth substrate was shown (Chang *et al.* 1993). These by-products were not identified and details of transformation kinetics were not investigated. The current study was designed to investigate these unresolved issue relating to the cometabolic degradation of *p*-xylene in BAC systems.

The aerobic degradation pathways for *p*-xylene have been well established by others and can be classified into two groups depending on which part of the structure is oxidized first. The first class involves the oxidation of the aromatic ring (Gibson *et al.* 1974; Jamison *et al.* 1969) while the second involves oxidation of a methyl substituent (Davey and Gibson 1974; Davis *et al.* 1968; Evans *et al.* 1991; Nozaka and Kusunose 1968; Omori *et al.* 1967; Omori and Yamada 1969; Omori and Yamada 1970a; Omori and Yamada 1970b). Gibson *et al.* (1974) showed that the initial oxidation products formed from *p*-xylene by *Pseudomonas putida* 39/D were produced by aromatic ring oxidation. This transformation was achieved by cooxidation in the presence of succinate as a growth substrate. The proposed pathway is shown in Figure 3-7. Presently, more is known about oxidation of methyl substituents than about aromatic ring oxidation. Even though the pathways proposed by different researchers differ slightly, they all hypothesized the formation of *p*-toluic acid and further degradation by ring fission.

The objectives of this study were to (1) identify and quantify byproducts from the cometabolic degradation of *p*-xylene by *Pseudomonas sp.* strain B1 with toluene as a growth substrate, (2) study the kinetics of byproduct formation, (3) study the effect of byproducts on adsorption in batch and flow-through systems, and (4) investigate the role of cometabolic degradation in the performance of a fixed-bed BAC system.

4. 2 Materials and Methods

4.2.1 Isolates

Strain B1 was isolated from an aerobic pilot-scale actiated carbon fluidized-bed reactor, as described in the previous paper reporting the degradation kinetics of two different BTX degrading isolates (Chang *et al.* 1993). This organism used benzene and toluene as growth substrates, and it cometabolized *p*-xylene, in the presence of toluene. The strain was characterized and identified as a *Pseudomonas* based on a GN MicroPlate® test panel. This identification was confirmed by fatty acid analysis (Microbial ID, Newark, Delaware).

4.2.2 Inoculum Preparation

Picked colonies were transferred to sealed 250-mL glass bottles with 100 mL HCMM2 liquid medium (Ridgway *et al.* 1990) containing 10 mg/L of toluene. After the toluene had completely degraded, a second addition was provided. Immediately following the disappearance of the second addition, an inoculum was withdrawn for kinetic experiments. Two milliliters of grown cells (~ 0.3 mg/mL) served as the inoculum

in the batch degradation experiments. Five milliliters of concentrated pure culture of strain B1 (~ 90 mg/mL) were used for column inoculation.

4.2.3 Batch Kinetic Experiments

Pseudomonas sp. strain B1 was transferred through a Mininert® valve into a sealed 250-mL glass bottles with buffered HCMM2 mineral salt medium containing both toluene and *p*-xylene. pH of the mineral medium was 6.9~7.1 before substrates were added. The bottle was shaken on a rotary shaker at 100 rpm. Headspace gas samples (0.5 mL) were periodically withdrawn from the bottle using a 1.0 mL gas-tight syringe equipped with a side-port needle, and assayed by injection onto a gas chromatograph equipped with a DB-624 column (30 m x .549 mm, J & W Scientific) and a flame ionization detector using 15 mL/min of helium as a carrier gas. The oven temperature began at 40°C and was increased at a rate of 30°C/min to 200°C, where it remained constant for 2 minutes. Liquid samples (200 µL) were also withdrawn and measured by a high pressure liquid chromatograph equipped with a C_{18} reverse-phase column and a UV detector at 280 nm. The mobile phase consisted of 40 % water and 60 % methanol with 0.1 % acetic acid and was delivered at a rate of 1.2 mL/min.

4.2.4 Identification and Quantification of Byproducts

The byproducts of cometabolism were collected from the batch kinetic experiments by centrifugation at of a liquid aliquot at 5000 rpm for 20 min followed by filtration through a 0.22 µm Millipore filter to remove cells. The solution was then concentrated by one of two methods. The first involved the extraction of the byproduct solution with ethyl acetate followed by drying in a rotary vacuum dryer as described by Gibson *et al.* (1974). The concentrated sample was then redissolved in 1 mL of

methanol. In the second method the sample was frozen using dry ice and placed in a vacuum freeze dryer until the water portion was completely removed. The dried sample was redissolved in 1 mL of deionized water. Concentrated samples were injected into a GC-MS for further analysis. Thin layer chromatography (TLC) was used to compare the byproducts from strain B1 to those produced by *Pseudomonas putida* 39/D and *Pseudomonas putida* F1 (provided by David T. Gibson, University of Iowa). The former organism has been shown to accumulate *p*-xylene dihydrodiol (*p*-XDHD), while the latter accumulates 3,6-dimethyl pyrocatechol (3,6-DMPC) (Gibson *et al.* 1974). Details of the TLC method are provided elsewhere (Gibson *et al.* 1974; Zylstra *et al.* 1990).

Because of difficulties in obtaining commercially available *p*-XDHD and 3,6-DMPC quantification was made by producing byproducts from *Pseudomonas putida* 39/D and *Pseudomonas putida* F1. A known amount of *p*-xylene was added to batch systems and the resulting concentration of the accumulated byproduct was stoichiometrically calculated. This allowed for preparation of a standard curve for HPLC analysis.

4.2.5 Pure Compound Adsorption Isotherm Experiments

Activated carbon (Filtrasorb-400, Calgon Corporation) was prepared by sieving to obtain a 30x40 mesh fraction (average particle diameter of 0.5 mm). After sieving, the GAC was rinsed with distilled water to remove oil and fines and then dried overnight at 100°C in an oven. The GAC was then stored in a sealed container until use.

Pure byproduct isotherms were performed by spiking a series of bottles containing either *Pseudomonas putida* 39/D or *P. putida* F1 with different concentrations of *p*-xylene. When complete degradation of *p*-xylene was observed, the solution in each bottle

was filtered to remove cells, and the concentration of byproduct quantified. These solutions were then used in bottle-point isotherms by adding a fixed amount of activated carbon (~5 mg), equilibrating by shaking at 100 rpm for 72 hours, and measuring the final byproduct concentrations. Isotherms for toluene and *p*-xylene were also performed using stock solutions of these compounds.

4.2.6 Effect of Strain B1 Byproducts on Sorption of Target Compounds

Two liters of solution containing byproducts were prepared by degrading approximately 28 mg of *p*-xylene by *Pseudomonas sp.* strain B1 with toluene as a growth substrate. Approximately 50 mg of toluene was added incrementally until *p*-xylene completely disappeared from solution. A control solution was prepared by adding the same amount of toluene to a 2-liter bottle without *p*-xylene seeded with strain B1. Bottle point isotherm experiments with activated carbon were performed by adding a series of concentrations of toluene and *p*-xylene to a filtered solution containing the byproduct and a control (which did not contain any byproduct compound).

4.2.7 Fixed-Bed Column Experiments

Adsorption column experiments were conducted in 1.5 cm diameter columns to study the effect of byproduct formation on the removal of target compounds in a packed bed system. A schematic of the experimental set-up is shown in Figure 4-1. To isolate the effects of adsorption from biodegradation, parallel systems containing activated carbon and baker product or non-activated carbon (provided by Calgon Corporation) were employed. Baker product has been shown to provide a similar environment for the growth of microorganisms, but has little adsorption capacity (Voice *et al.* 1992). This medium was prepared in the same manner as the GAC.

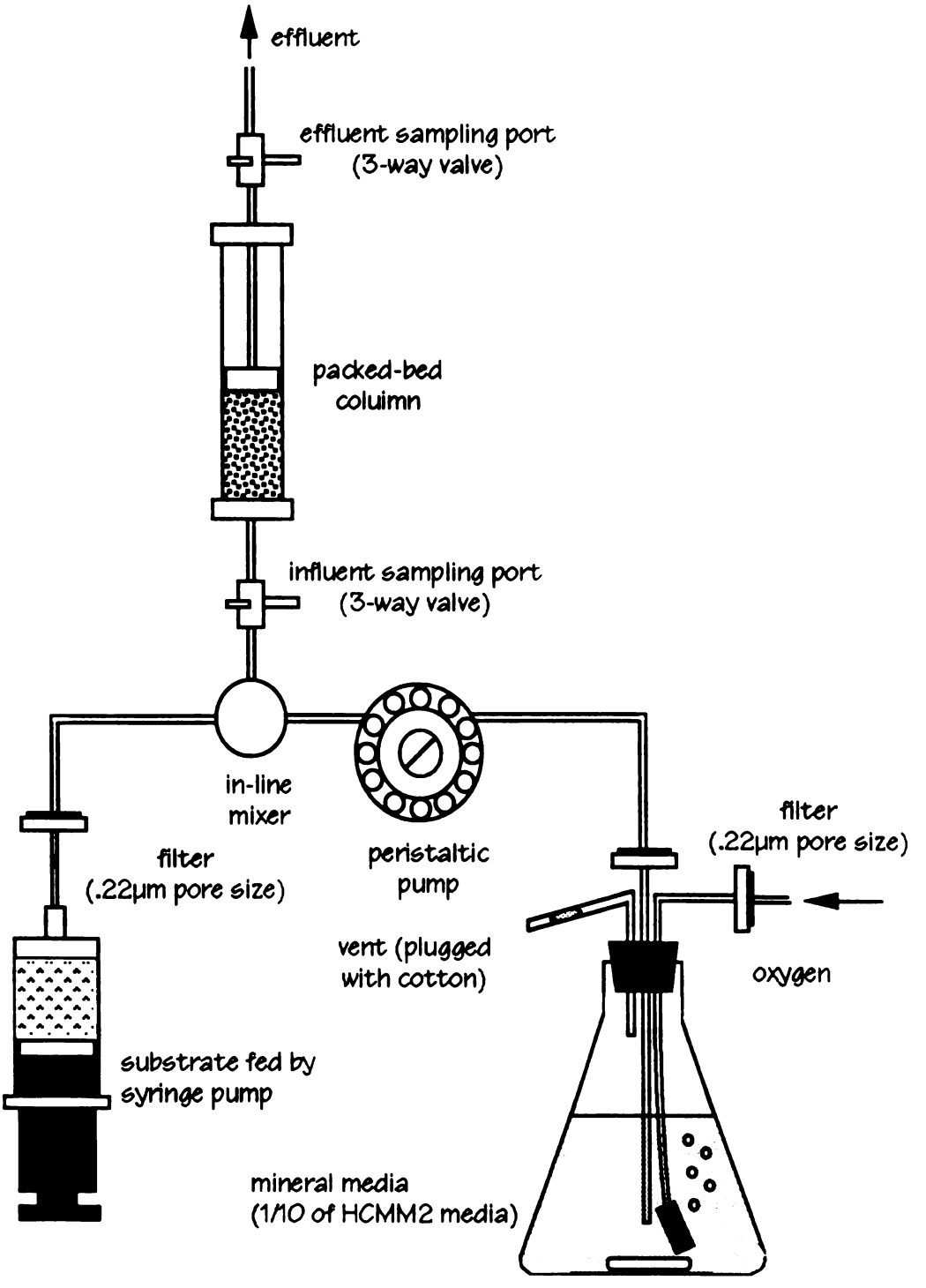


Figure 4-1. Schematic diagram of the column apparatus.

A bed depth of 2 cm and a flow rate of 1 mL/min were used to maintain a hydraulic residence time of approximately 2 min. The whole system was autoclaved before inoculation and kept sterile using filters. Prepared inoculum was injected into the column and left overnight without flow for attachment. Column operation was initiated by supplying both substrates (toluene and *p*-xylene) and mineral medium (1/5 concentration of original HCMM2 medium). Unattached microorganisms were flushed out as soon as the column operation started. The columns were operated until steady-state effluent concentrations were reached. Additional column experiments involved various influent perturbations such as the removing the supply of one of the target compounds or oxygen.

4. 3 Results

Figure 4-2 shows a HPLC chromatogram of the solution containing byproducts from the cometabolic degradation of *p*-xylene and the end product solution obtained from the degradation of toluene with strain B1. As the chromatograms show, the byproducts were formed only when xylene was degraded. These peaks were initially designated compounds A, B, and C corresponding to their order of appearance in the chromatogram. Each of the three peaks were collected from the HPLC effluent in separate aliquots and compared to previously identified *p*-xylene degradation products using thin layer chromatography after samples were concentrated. It was found that compound A corresponded to the byproduct of *p*-xylene degradation by *P. putida* F1, both having an R_f factor of 0.53. Compound B matched the byproduct produced by *P. putida* 39/D with an R_f factor of 0.33. Compound C, which was obtained by decreasing the pH of a solution containing compound B, produced the formation of a new spot at $R_f = 0.84$,

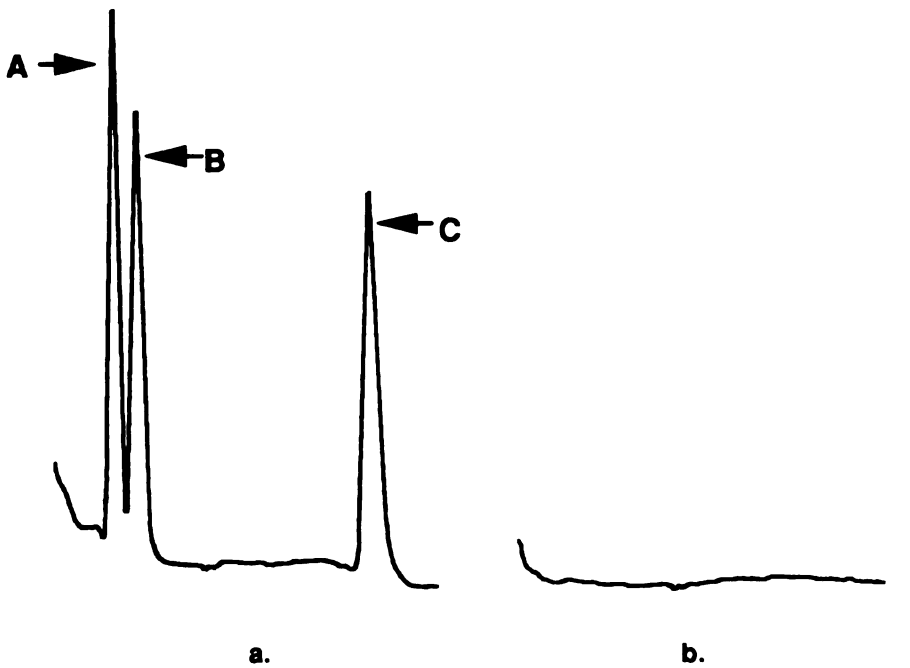


Figure 4-2. HPLC chromatograms of two solutions: a) solution containing byproducts from *p*-xylene degradation with toluene as growth substrate and b) final solution after degradation of toluene by *Pseudomonas sp.* strain B1. Peaks A, B, and C are byproducts of *p*-xylene cometabolism.

which corresponded to that produced by 2,5-dimethyl phenol obtained commercially. This identification was confirmed by comparison of GC-MS spectra.

Based on these observations, it is proposed that compound A is 3,6-dimethyl pyrocatechol (3,6-DMPC), compound B is *cis*-3,6-dimethyl-3,5-cyclohexadiene-1,2-diol (*p*-xylene dihydrodiol, *p*-XDHD), and compound C is 2,5-dimethylphenol (2,5-DMP).

Figure 4-3 shows substrate and byproduct concentrations over the initial 60 hours of a rate experiment. It can be seen that when the growth substrate (toluene) is completely removed from solution, the rates of *p*-xylene degradation and *p*-XDHD formation decrease. The formation of 3,6-DMPC began after the disappearance of toluene and lagged behind the formation of *p*-XDHD. The same experiment is plotted over the course of 300 hours in Figure 4-4, where it is compared to a similar study in which the cells were removed from solution after 30 hours. With the cells present, the concentration of *p*-XDHD slowly decreased from the maximum value reached during the first 60 hours, and the 3,6-DMPC continued to increase. In the system where cells were removed, the 3,6-DMPC remained constant following removal, and the *p*-XDHD decreased only slightly. After 30 days of extended observation, the *p*-XDHD was completely removed from the system containing cells and the 3,6-DMPC and 2,5-DMP increased significantly. HPLC analysis of a sample in which the pH was lowered by adding one drop of 4N sulfuric acid revealed that the peak of *p*-XDHD disappeared, and a peak of 2,5-DMP appeared. The rate of formation of 2,5-DMP appears to correspond to the rate of *p*-XDHD degradation.

A mass balance on *p*-xylene and byproducts during the kinetic experiment is shown in Figure 4-5. Analysis of five replicates of this experiment indicated that the measured concentrations of these four compounds represented 105 ± 10% of the *p*-xylene

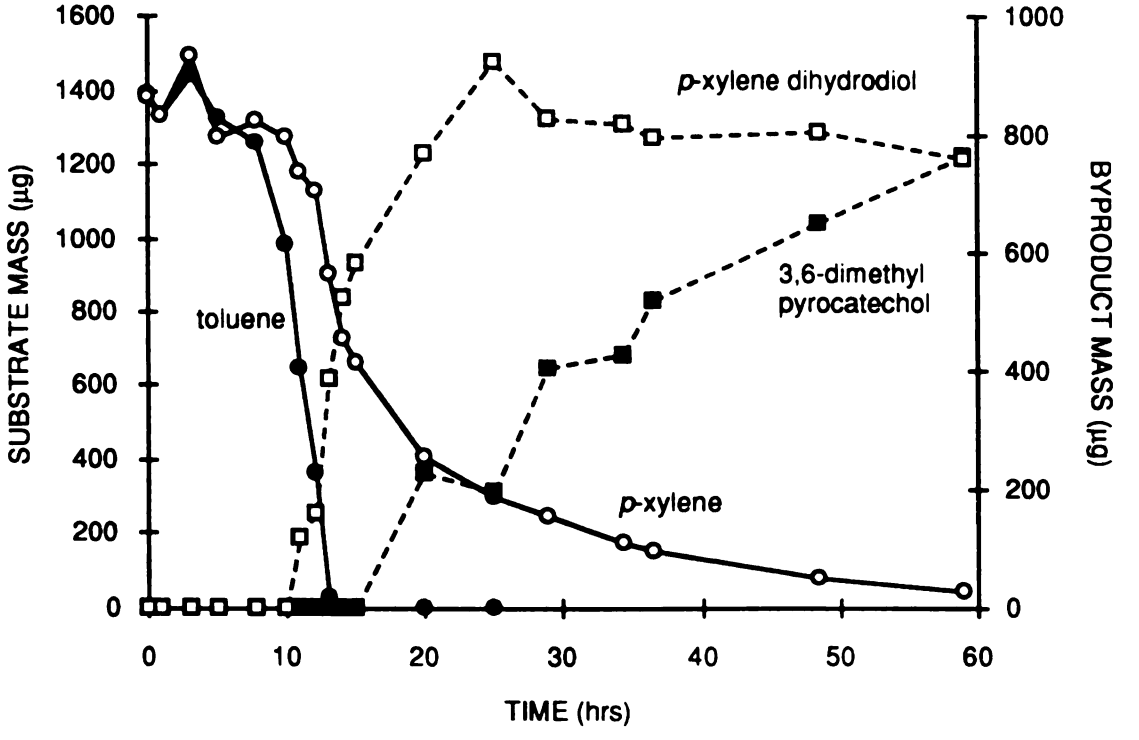


Figure 4-3. Byproduct formation kinetics (initial 60 hrs) for degradation of toluene and *p*-xylene.

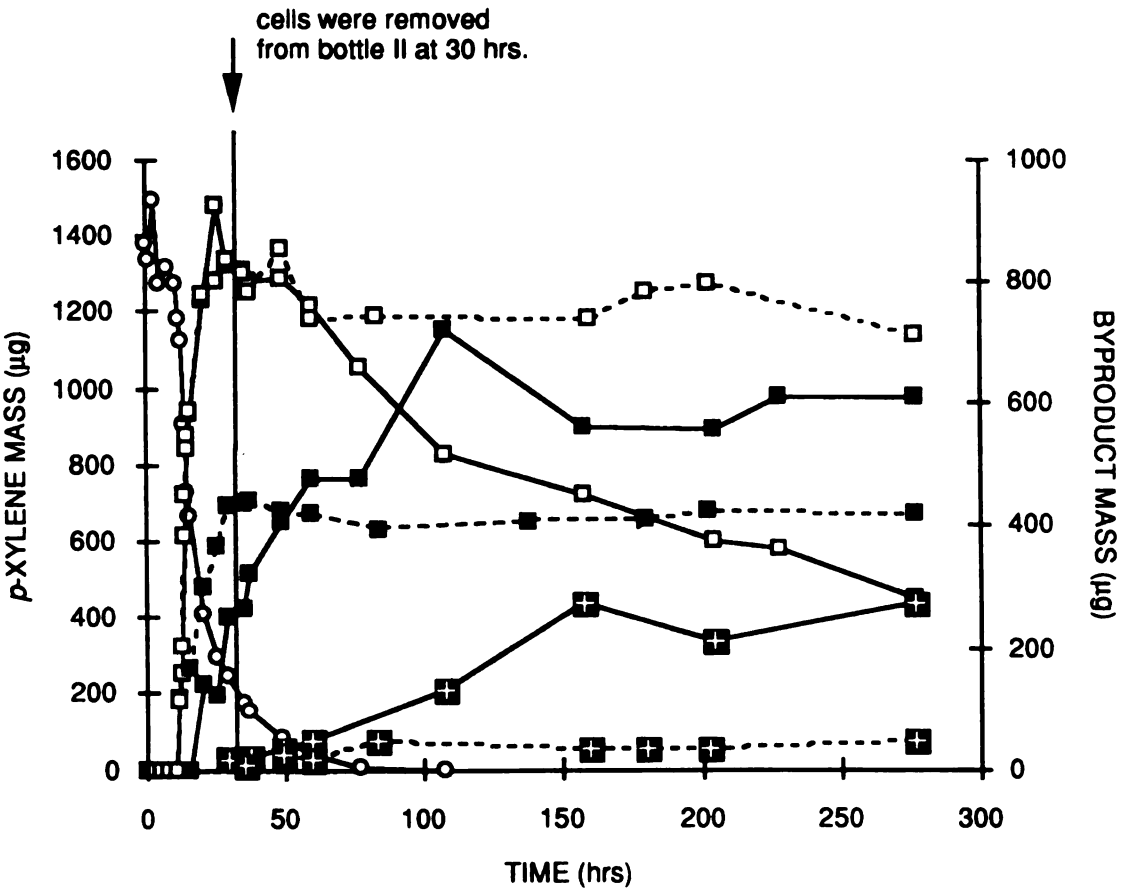


Figure 4-4. Byproduct formation kinetics (up to 300 hrs) for the transformation of *p*-xylene (extended result of Figure 4-3). bottles were compared. Bottle No. I (———) contained biomass until the end of the experiment. Cells were removed from bottle No. II (------) after 30 hours (o ; *p*-xylene, □ ; *p*-xylene dihydrodiol, ■ ; 3,6-dimethyl pyrocatechol, ⊞ ; 2,5-dimethyl phenol).

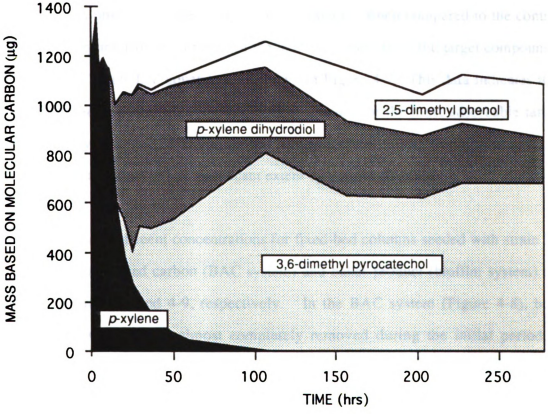


Figure 4-5. Mass balance between *p*-xylene and byproducts based on the mass of molecular carbon.

added on a molecular carbon basis. This confirms the contention that further degradation did not occur in these systems.

The effect of the byproducts on the adsorption of toluene and *p*-xylene by activated carbon is shown in Figure 4-6. A slight decrease in the sorption of both compounds was observed in the presence of byproducts, when compared to the control solution. Adsorption isotherms for all of the individual byproduct and target compounds, and the best-fit Freundlich equations are shown in Figure 4-7. This data indicates that 2,5-DMP is readily adsorbed, comparable to that found for the most adsorbable target compound, *p*-xylene. *p*-XDHD was sorbed somewhat less, falling below toluene. 3,6-DMPC was not adsorbed to any significant extent by activated carbon.

Influent and effluent concentrations for fixed-bed columns seeded with strain B1 and containing activated carbon (BAC system) and baker product (biofilm system) are shown in Figures 4-8 and 4-9, respectively. In the BAC system (Figure 4-8), both toluene and *p*-xylene were almost completely removed during the initial period of operation. As expected on the basis of adsorption alone, toluene breakthrough occurred first, followed by *p*-xylene. *p*-Xylene concentrations did not fall after breakthrough but appear to increase at a slower rate and did not reach saturation (i.e. influent) levels as would be expected from adsorption alone. In contrast, toluene and *p*-xylene were found in the effluent of the biofilm system (Figure 4-9) from the onset of operation.

The behavior of the byproducts in the two systems was similar, in that both 3,6-DMPC and *p*-XDHD were initially not found in the BAC system effluent. In the biofilm system, *p*-XDHD appeared at the beginning of column operation, followed by 3,6-DMPC, as would be expected on the basis of the proposed mechanism (Figure 3-7). It is interesting to note that 3,6-DMPC appears before *p*-XDHD in the BAC system.

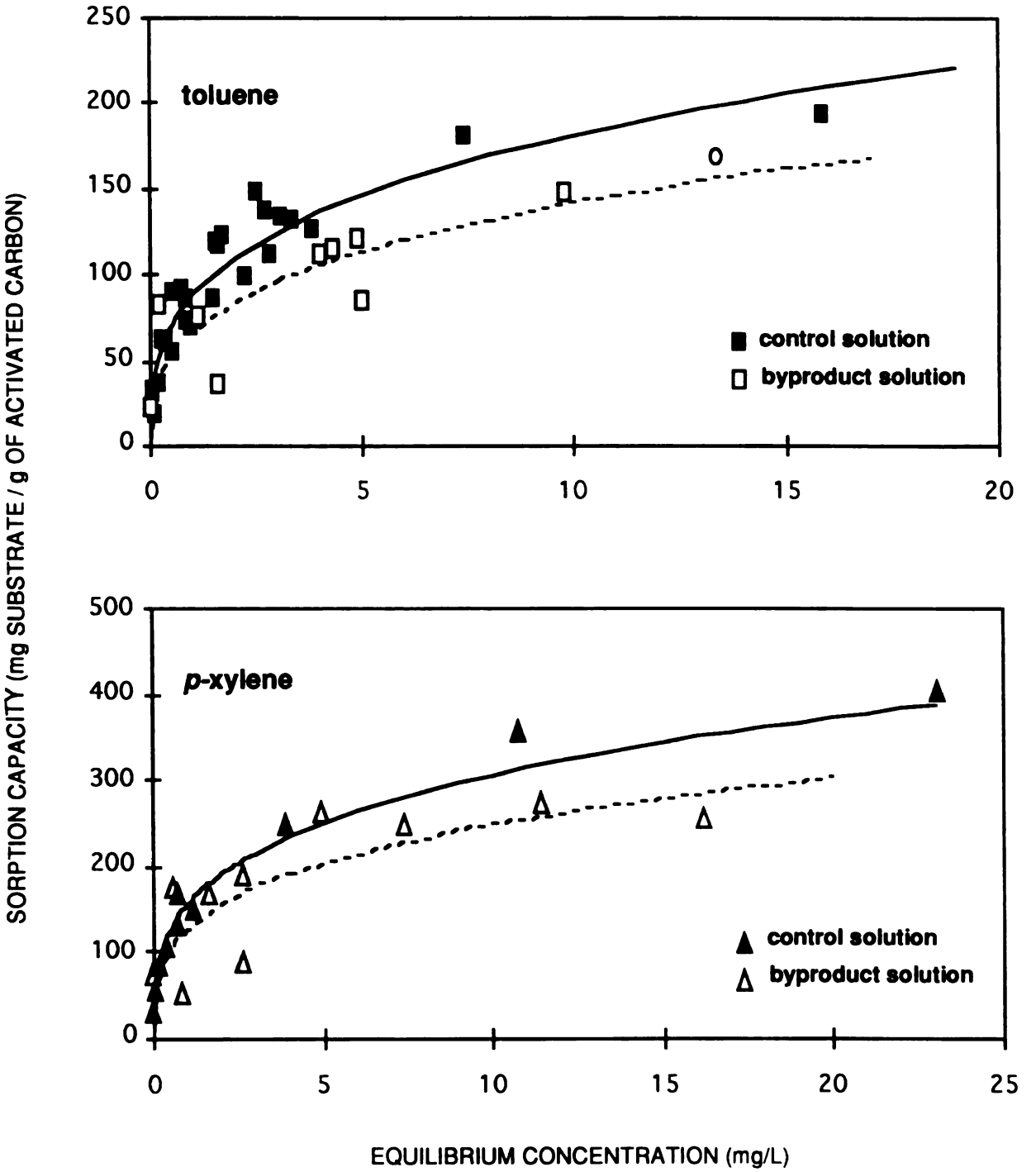


Figure 4-6. Sorption isotherms of toluene and *p*-xylene prepared in control solution and in solution containing byproducts.

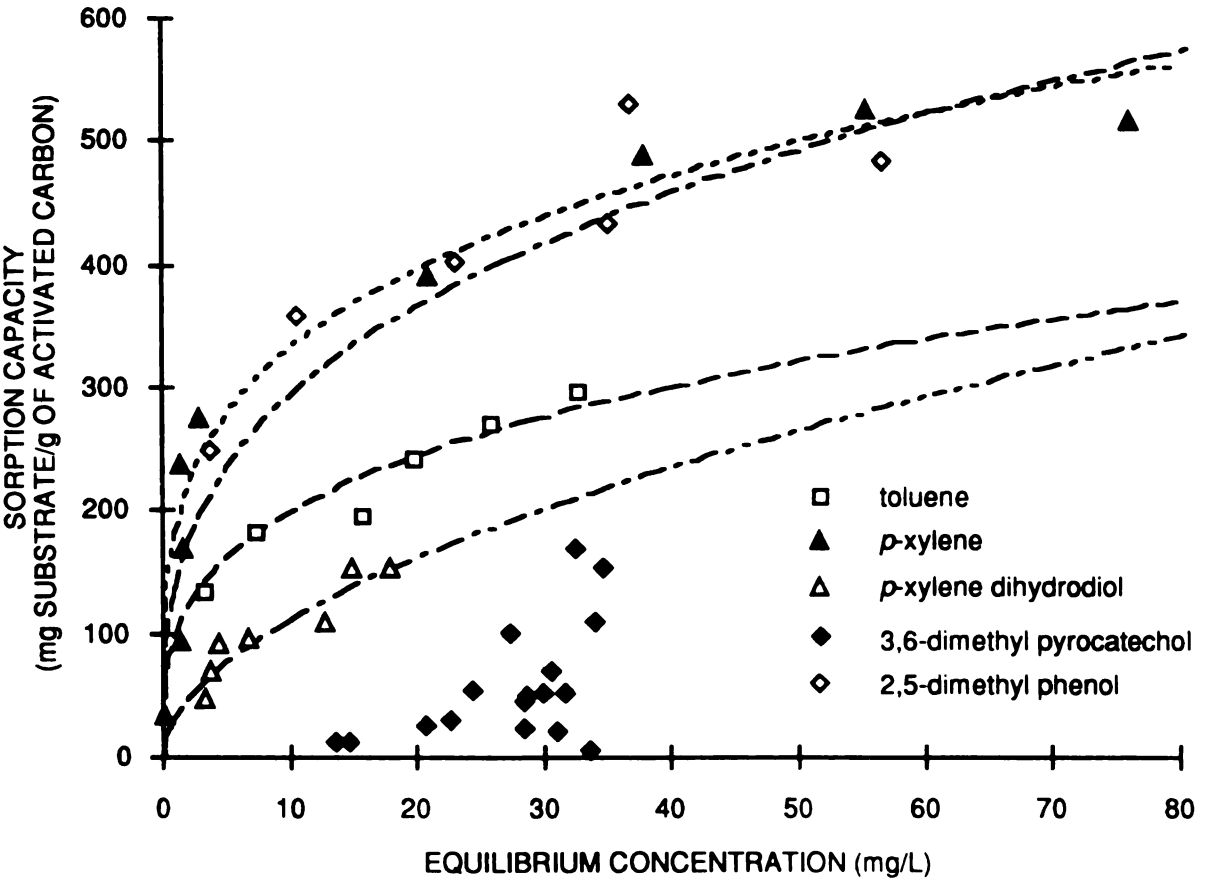


Figure 4-7. Comparison of isotherms for toluene, *p*-xylene and byproducts ; *p*-xylene dihydrodiol, 3,6-dimethyl pyrocatechol, and 2,5-dimethyl phenol.

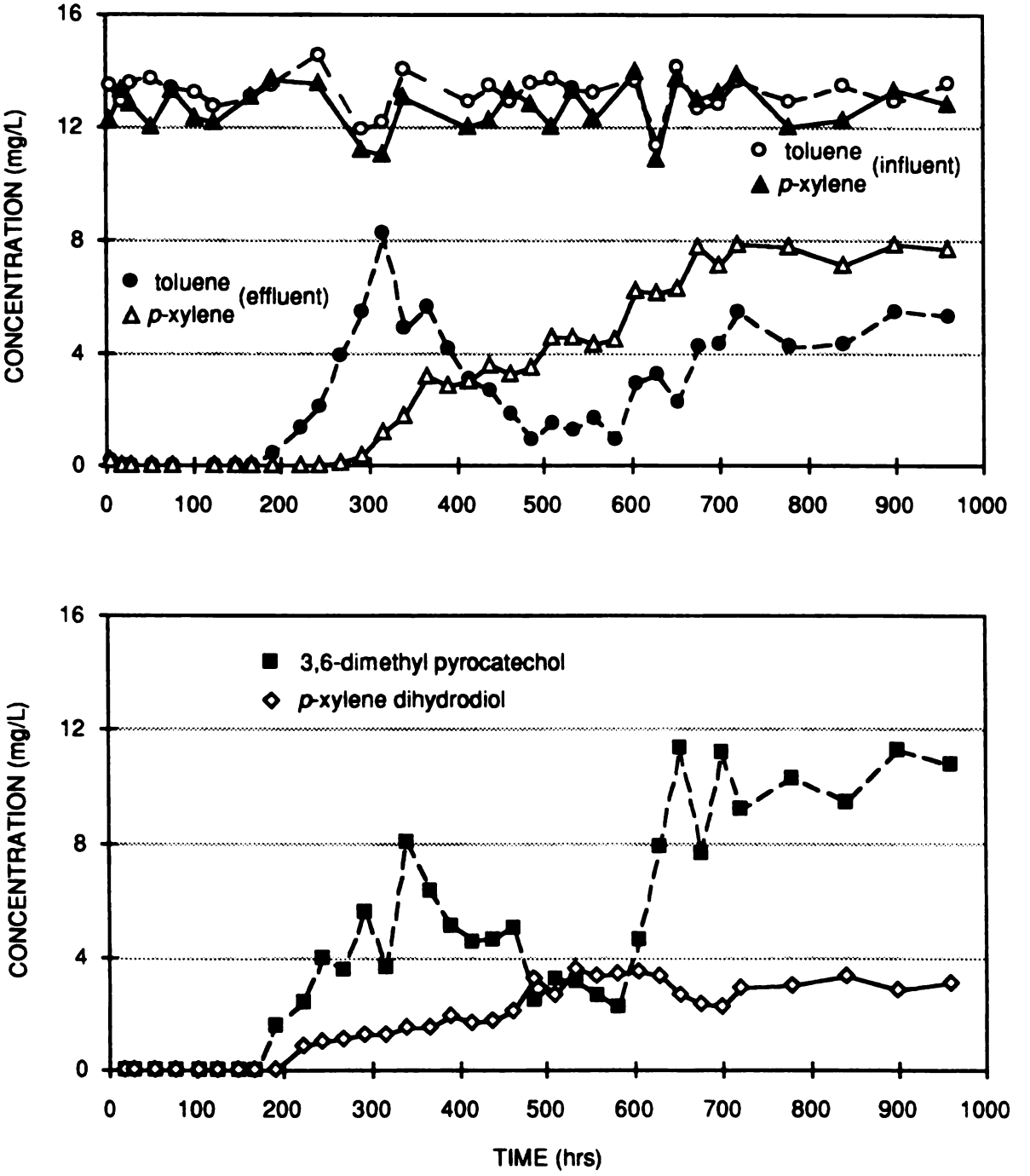


Figure 4-8. Effluent profiles of target compounds (top) and byproducts (bottom) from BAC column seeded with strain B1.

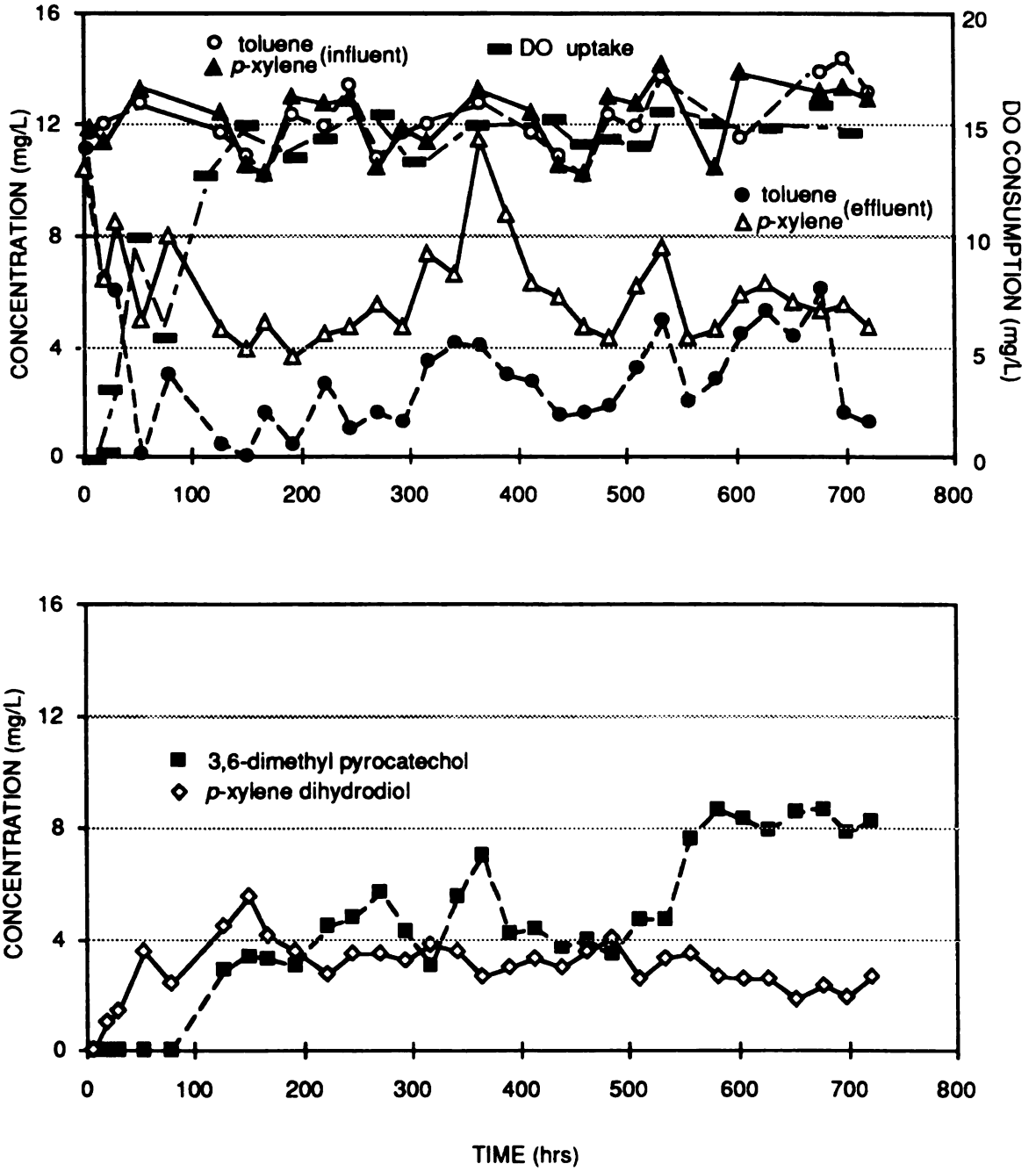


Figure 4-9. Effluent profiles of target compounds and oxygen consumption (top) and byproducts (bottom) from biofilm column grown on Baker product seeded with strain B1.

To further investigate the interactions between biodegradation and adsorption in fixed-bed columns, a series of experiments involving temporary changes in the influent concentrations of columns that had reached steady-state operating conditions were conducted. The effect of removing the supply of toluene for 24 hours is shown in Figure 4-10. A substantial increase in *p*-xylene concentrations occurred during this period in the biofilm system, whereas only a very minor increase was found for BAC. Effluent 3,6-DMPC concentrations fell during this interval but significant levels were still observed in the effluents of both BAC and biofilm systems. The decrease is somewhat more rapid with the biofilm system. Upon resumption of the toluene feed, a sharp increase in effluent toluene was observed in the biofilm system, whereas this change and that of other compounds monitored changed only slowly in the BAC system.

Removing the supply of *p*-xylene primarily affected the concentrations of byproducts, as shown in Figure 4-11. Both 3,6-DMPC and *p*-XDHD levels dropped off rapidly in the biofilm system and somewhat more slowly in the BAC system. The concentrations of both byproducts also returned to their previous levels more rapidly in the biofilm systems upon restoration of the *p*-xylene feed. It can be also be noted that the concentration of *p*-xylene in the BAC system effluent decreased only slightly during the period of no *p*-xylene supply and then increased only slightly upon resumption. This is in contrast to the rapid drop followed by a sharp rise in the biofilm system.

When the supply of oxygen to the BAC and biofilm systems was interrupted, byproduct concentrations fell in both systems, as shown in Figure 4-12. The decrease was slightly slower in the BAC system. Both target compounds rose rapidly to influent levels in the biofilm system but increased only slightly in the BAC system.

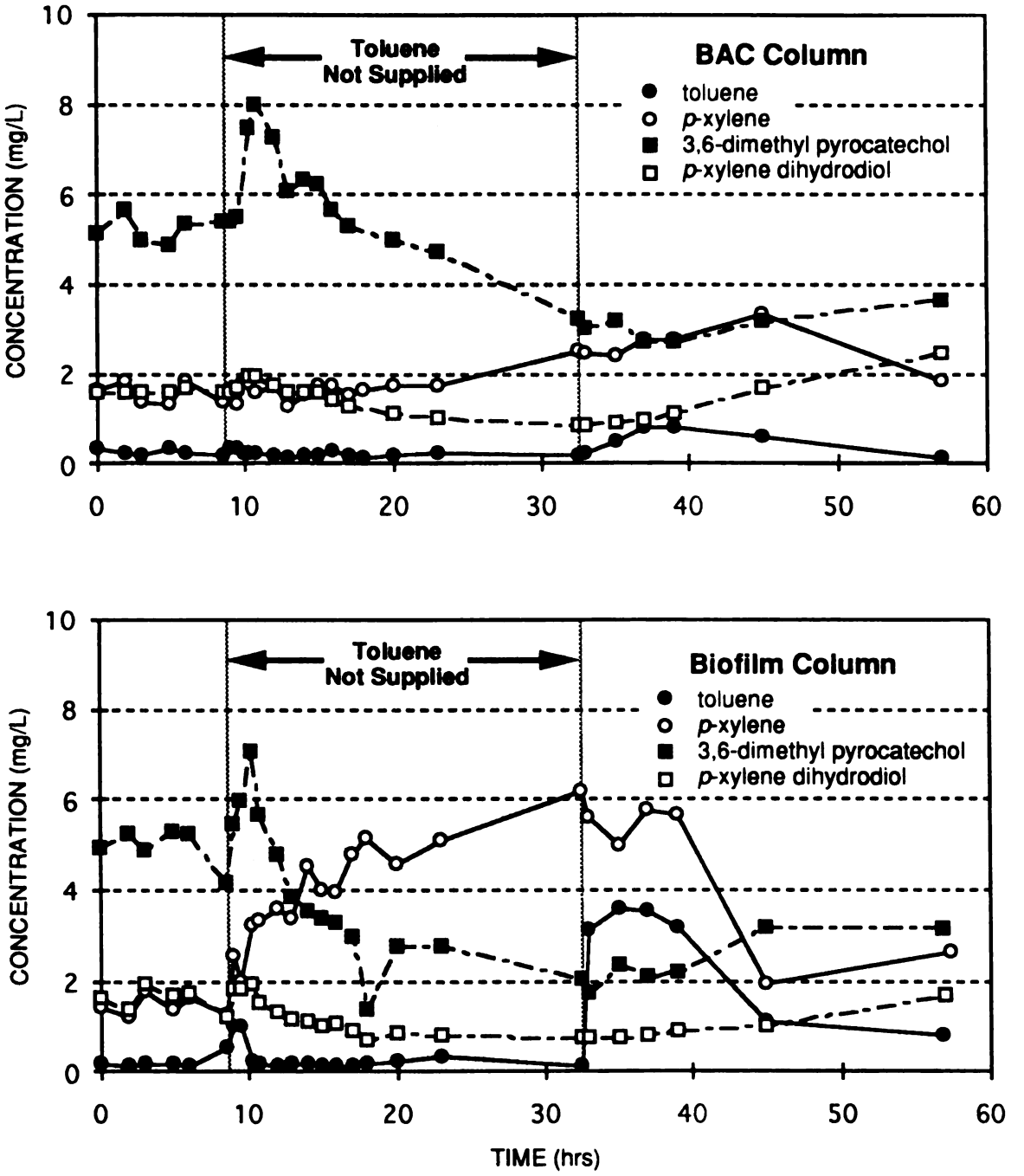


Figure 4-10. Effluent profiles of target compounds and byproducts from BAC column (top) and biofilm column (bottom) seeded with strain B1 after disconnecting the supply of toluene (only *p*-xylene was provided during 24 hrs period - shaded area).

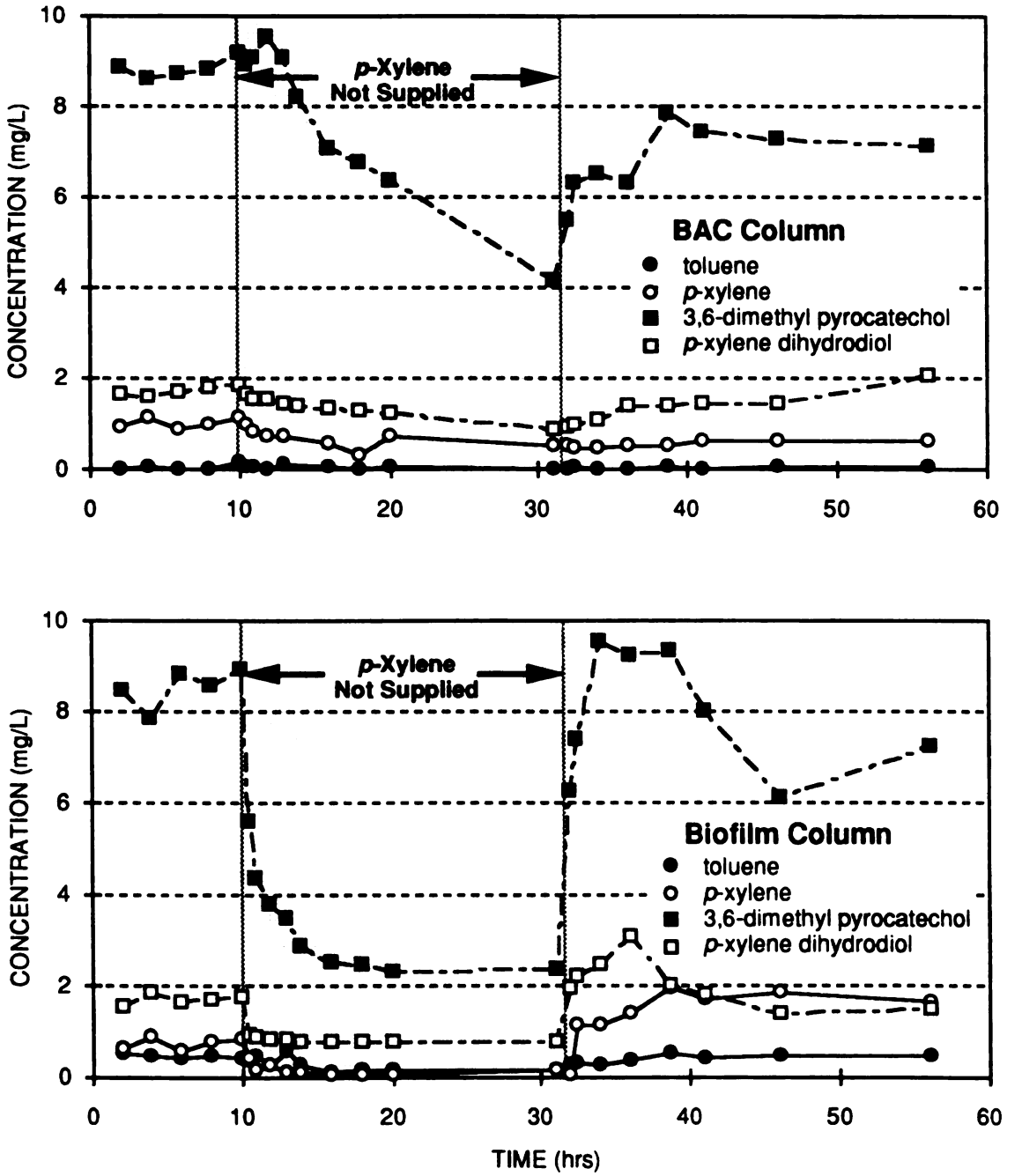


Figure 4-11. Effluent profiles of target compounds and byproducts from BAC column (top) and biofilm column (bottom) seeded with strain B1 after disconnecting the supply of *p*-xylene (only toluene was provided during 22.5 hrs period - shaded area).

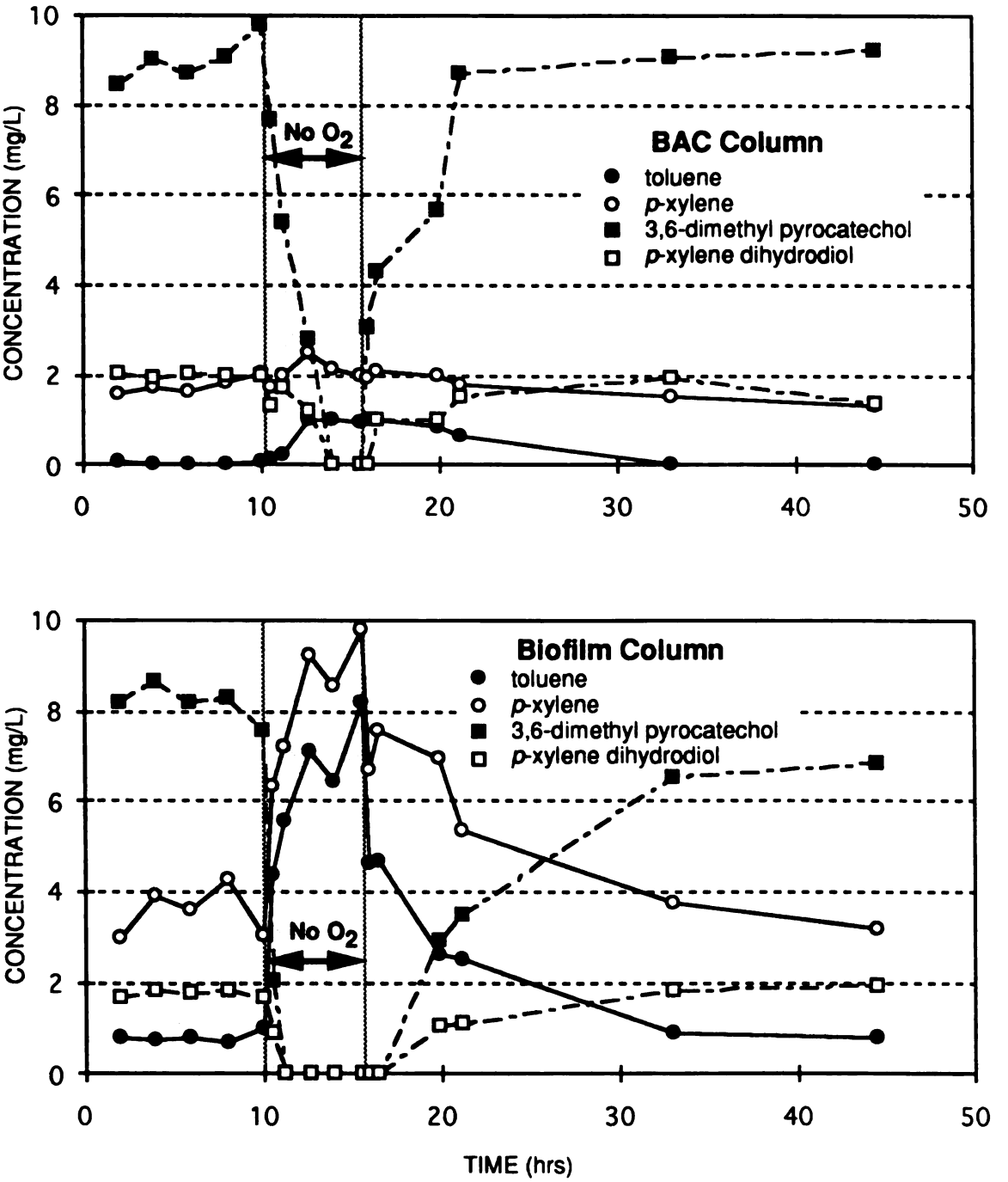


Figure 4-12. Effluent profiles of target compounds and byproducts from BAC column (top) and biofilm column (bottom) seeded with strain B1 after disconnecting the supply of oxygen for 5 hrs (shaded area).

4.4 Discussion

When cells acquire proper enzyme and transformation capacity in the presence of growth substrate, then they can utilize other non-growth substrate by cometabolism. Recently, Criddle (1993) developed a model which considers cometabolic degradation kinetics in both the presence and absence of the growth substrate. This conceptual framework, in conjunction with the *p*-xylene degradation mechanism proposed by Gibson (1974), provides the basis for interpreting the biodegradation rate data.

The degradation of toluene, the growth substrate, proceeds rapidly after an initial acclimation period, as expected (Figure 4-3). The degradation of *p*-xylene to 3,6-DMPC is a sequential two-step process (Figure 3-7). *p*-xylene degradation lags that of toluene only slightly, and proceeds at a similar rate, until the supply of growth substrate is depleted. This is matched by the production of *p*-XDHD, the first product in the degradation sequence. Formation of the second degradation product, 3,6-DMPC, does not begin until the growth substrate is depleted (Figure 4-4). In other experiments, it was observed that with the continuous addition of toluene to solution, only *p*-XDHD accumulated in the system and the addition of *p*- xylene to resting cells produced 3,6-DMPC without accumulating *p*-XDHD even though the reaction was slow. It thus appears that the second step occurs only in the presence of resting cells.

Two potential explanations can be offered for this behavior. 1) Enzymes obtained in two situations are different. In the presence of growth substrate the organism can only convert *p*-xylene to *p*-XDHD. In the absence of growth substrate, however, the organism can convert either *p*-xylene or *p*-XDHD to 3,6-DMPC. 2) Enzymes are same in both cases. However, the transformation of *p*-XDHD to 3,6-DMPC is competing with the

degradation of toluene. This competition diminishes when toluene is completely degraded, so the enzyme can devote itself to the conversion of *p*-XDHD to 3,6-DMPC.

The formation of both *p*-XDHD and 3,6-DMPC is clearly the result of microbial activity. Gibson *et al.* (1974) proposed that the formation of 2,5-DMP from *p*-XDHD proceeds via a non-enzymatic pathway. It was found that this reaction was accelerated by the addition of acid. 2,5-DMP was not observed at significant levels until after the concentration of *p*-XDHD reached its maximum value. In systems containing cells for the entire period, only 3,6-DMPC and 2,5-DMP were found in solution after 30 days. 2,5-DMP also appeared in the bottle without cells, however, the rate of the formation was not as fast as that in the bottle with cells. It is not clear that the formation of 2,5-DMP from *p*-XDHD is strictly an abiotic process as claimed by Gibson *et al.* (1974).

The slight reduction in sorption of both target compounds in the presence of byproducts, suggests that the effects of degradation and adsorption in BAC systems will be primarily additive rather than synergistic over short periods of time such as that used to produce the byproduct solution for the adsorption experiments. It should be noted that 2,5-DMP is equally adsorbable as *p*-xylene, and thus may compete with this target compound if it is formed in significant quantities. Since the rate of formation of 2,5-DMP is somewhat slower than that of the other byproducts, we might expect accelerated loss of adsorption capacity over longer periods of time.

In column studies, the additive effects of degradation and sorption can be inferred by comparing the BAC and biofilm systems (Figures 4-8 and 4-9). Adsorption serves to remove both target compounds in the BAC system prior to development of the biofilm. The spike of toluene results from adsorption breakthrough, followed by development of the degradation capacity. This spike does not occur with *p*-xylene, presumably because

the higher adsorption capacity of the carbon for this compound allows it to be retained until degradation capacity is fully developed. It is interesting to note that the biofilm system appears to plateau at slightly better removal rates. This may result from the better biofilm development throughout the depth of the column due to the longer period of exposure to the target compounds. Alternatively, the elevated toluene levels in the BAC system may result from release of previously adsorbed material desorbing due to displacement by the more adsorbable xylene.

Adsorption of the target compounds also appears to prevent the formation of byproducts during the start-up period. In the biofilm systems, we observe the formation of *p*-XDHD shortly after start-up with the first evidence of *p*-xylene degradation. As would be expected from the mechanism, this is followed by the formation of 3,6-DMPC. It is interesting to note that in the BAC system, 3,6-DMPC is observed in the column effluent prior to *p*-XDHD, even though the former compound is the degradation product of the later. This can be explained by considering that the carbon exhibits some adsorption capacity for *p*-XDHD but not 3,6-DMPC, and thus the *p*-XDHD is retarded in its release from the column.

The interactions between cometabolic degradation and adsorption under pseudo-steady-state conditions (well developed biofilm) can be seen more clearly in the studies in which one of the target compounds or oxygen is removed from the influents of the two columns (Figures 4-10 - 4-12). Because *p*-xylene degradation requires a growth substrate, this reaction could not proceed during the period of no toluene feed in the biofilm system, as evidenced by the rapid increase of *p*-xylene in the effluent (Figures 4-10). Resumption of the toluene feed restored xylene degradation, although a significant lag was observed in the biofilm column before either *p*-xylene or toluene degradation returned to previous levels. During this period *p*-xylene degradation continued at nearly

the previous rate in the BAC system, presumably because the previously adsorbed toluene desorbed and provided the means for the cometabolic process to continue. This result must be inferred, however, because desorbed toluene was degraded and thus similar low levels of toluene were found in the two systems. Other concentration changes were also dampened by adsorption in the BAC system, and only a slight increase in toluene concentration was observed upon resumption of the feed.

This mechanistic interpretation is substantiated by the study in which the *p*-xylene feed was temporarily interrupted. In the biofilm system the levels of both *p*-XDHD and 3,6-DMPC decreased substantially, but stabilized at non-zero levels. This may be caused by the utilization of *p*-xylene trapped in the system either by the slight adsorption capability of Baker products or by cells as biosorption. The concentrations of *p*-xylene and both byproducts decreased only slowly in the BAC system. This confirms that the activated carbon serves as a reservoir, in this case of *p*-xylene, which allows the degradation reaction to proceed. Resumption of the *p*-xylene feed produced concentration spikes in the biofilm but not the BAC system, similar to those observed for the toluene interruption.

The conclusion that activated carbon dampens concentration changes is further supported by the study in which biodegradation is stopped completely for an interval by not supplying dissolved oxygen to the columns (Figure 4-12). In the biofilm system, both target compounds increased to influent levels and byproducts decrease to zero, very rapidly after the oxygen supply is eliminated. In contrast, only a slight increase in toluene and *p*-xylene concentrations was observed in the BAC system because in the absence of biodegradation, adsorption becomes operative and dampens the system response. It was noted that the byproduct concentrations fell somewhat more slowly in

the BAC system, perhaps because the *p*-XDHD desorbed from the carbon and degradation to 3,6-DMPC proceeded in the absence of oxygen.

4.5 Conclusion

Pseudomonas sp. strain B1 cometabolically degraded *p*-xylene in the presence of toluene as a growth substrate. Three different byproduct compounds were produced. *p*-XDHD was formed by growing cells and a further degraded compound, 3,6-dimethyl pyrocatechol was formed by resting cells. 2,5-dimethyl phenol was also formed. The formation was faster in the bottle with cells than the one in the bottle without cells.

The byproducts of cometabolic *p*-xylene degradation reduced the sorption of the target compounds only slightly. It was found that the performance of BAC column systems could be explained by considering the effects of degradation and adsorption to be additive but not synergistic. Activated carbon serves as a reservoir for those compounds and byproducts that adsorb, and thus induced concentration changes are dampened. This was exhibited in 1) removal of target compounds and byproducts prior to full development of a biofilm, 2) continued cometabolic degradation in a period when the growth substrate was not supplied to the column, 3) dampening of concentration spikes following periods where the supply of target compounds was interrupted and 4) reduced effluent levels of target compounds when biodegradation was eliminated altogether due to lack of oxygen.

CHAPTER 5

EFFECT OF MICROBIAL PROCESS ON THE OPERATION OF BIOLOGICAL ACTIVATED CARBON SYSTEM DURING SHOCK LOADINGS

5.1 Introduction

Biological activated carbon (BAC) systems have been shown to provide superior performance to conventional activated carbon adsorption systems. The capability to biologically degrade substrates can reduce the sorption load and extend the service time of the reactor. The adsorption capability, in return, can add the capacity to minimize the effects of shock loading on microorganisms during a perturbation event.

The definition by Rice and Robson (Rice and Robson 1982) describes BAC as a simultaneous combination of granular activated carbon adsorption of dissolved organic materials coupled with aerobic biological oxidation of organic materials. In most BAC studies, a strictly aerobic environment is maintained by providing excessive amounts of oxygen throughout the system (Chang and Rittmann 1987; Olmstead 1989; Speitel *et al.* 1988; Speitel *et al.* 1989; Voice *et al.* 1992). As a result, the adsorptive characteristics of the BAC system are often neglected by the active biological degradation. During the steady-state operation of a BAC fluidized-bed system with excess oxygen, Voice *et al.* (1992) observed that the major removal mechanism in the system is biodegradation. They also found that sorption became important during changes in influent concentration. This is

clearly one of the advantages of the BAC system. However, how different types of substrate degradation processes can affect the operation of BAC systems treating more than a single substrate has not previously been shown.

Different types of microorganisms have different capabilities for degrading substrates. These capabilities can be quantitatively expressed by kinetic parameters which show how fast they utilize substrate, how efficiently they grow by utilizing substrate, or how fast they grow. *Pseudomonas sp.* strain B1 and strain X1 were previously isolated from BAC reactors treating benzene, toluene, and *p*-xylene (Chang *et al.* 1993). Strain B1 degrades benzene and toluene as growth substrates and also cometabolically degrades *p*-xylene in the presence of either growth substrate. Resting cells of strain B1, which were previously grown on benzene or toluene, can also degrade *p*-xylene by cometabolism. Strain X1 degrades toluene and *p*-xylene; however, it can not degrade benzene.

In this research, the effect of different degradation patterns on the operation of BAC systems was studied. Strain B1 and strain X1 were inoculated in two reactors and two substrates were fed to show how the BAC system seeded with different species of microorganisms would respond to the same combination of multiple substrates. A control was run by inhibiting any microbiological activities (designated GAC). The appropriateness of the application of batch degradation data to column operation was compared. Experiments to investigate the role of adsorption during perturbation were performed by applying different shock loadings and observing the performance of the reactor. The shock loading was applied by increasing one of the substrate concentrations two-fold. The role of adsorption during the failure of microbial activity in the system was studied by removing the supply of oxygen. Cross-sectional matrices of the column after 40 days of operation were examined by the scanning electron microscope (SEM) to investigate the population distribution of microorganisms at different locations in the column.

5.2 Materials and Methods

5.2.1 Isolates

Two *Pseudomonas* isolates, designated strains B1 and X1, were previously isolated based on their distinctive degradation patterns (Chang *et al.* 1993). Each strain was characterized and identified on a GN MicroPlate® test panel using Biolog's MicroLog® software. Strain B1 was identified as *Pseudomonas fragi*, with a high confidence rating in a MicroLog® search; strain X1 was identified as *Pseudomonas fluorescens*, but with a low confidence rating. Fatty acid analysis performed by Microbial ID, Inc. (Newark, Del.), confirmed the *Pseudomonas* classification. Strain X1 had a high correlation with the fatty acid profile for a library strain of *Pseudomonas fluorescens*.

5.2.2 Inoculum Preparation

Pure colonies of strain B1 and strain X1 were picked from the previously grown nutrient agar plate and transferred to sealed 250-mL glass bottles with 100 mL HCMM2 liquid medium (Ridgway *et al.* 1990) containing toluene and *p*-xylene, respectively. After each substrate had completely degraded, a second addition was provided. Additions of each substrate were continued until concentrated pure cultures of strain B1 and strain X1 (approximately 90 μg/mL) were obtained. Five milliliters of culture were seeded in the column.

5.2.3 Batch Kinetic Experiment for Degradation

A detailed method for the kinetic experiment is described elsewhere (Chang *et al.* 1993). In this research, degradation of toluene and xylene by strain B1 and X1 were compared. Each substrate was assayed as single substrate or combination.

5.2.4 Packing Media

Two different media, GAC and a nonadsorbent cabon material (Baker product), were used in this study. The GAC used was Filtrasorb 400. Baker product is the same material as GAC, but has not been activated and has therefore little adsorption capacity. Both media were provided by the Calgon corporation (Pittsburgh, PA). Both media were prepared by sieving to obtain a 30x40 mesh fraction. The average particle diameter was 0.5 mm. After seiving, the GAC was rinsed with distilled water to remove oil and fines and then dried overnight at 100°C. The GAC was then stored in sealed container until use.

5.2.5 Batch Isotherms for Toluene and *p*-Xylene

Isotherm assays for toluene and *p*-xylene were conducted using serum bottles (160 mL) sealed with teflon-coated septa. Twenty milligrams of sterilized activated carbon were used with the desired initial concentration of toluene and *p*-xylene dissolved in HCMM2 mineral medium. The serum bottles were shaken (6 rpm) at 24°C for 5 days to reach equilibrium. Controls were run without activated carbon to ensure that there was no biological degradation. The liquid-phase concentration was then analyzed to determine the residual concentration of toluene and *p*-xylene. The solid-phase concentration was calculated using a mass balance. Isotherms for the Baker product were not prepared.

However, previous studies (Voice *et al.* 1992) showed that sorption capacity by the Baker product was negligible.

5.2.6 Fixed Bed Reactor

A flow-through experiment was conducted using 1.5 cm diameter columns (OmniFit USA, Atlantic Beach, NY). A complete description of the set-up is provided in Chapter 4 and shown in Figure 4-1. Two different packing media, activated carbon and Baker product, were used to compare biofilm on both activated carbon and inert media. The bed depth was 2 cm and the flow rate was 1 ml /min to maintain a hydraulic residence time of approximately 2 min. The whole system was autoclaved before seeding and kept sterile using filters and autoclaving. Prepared inoculum was injected through the column, left overnignt for attachment, and flushed with mineral medium to remove residual inoculum solution. Pumping was started by supplying mineral medium solution (1/5 concentration of original HCMM2 midium) in which toluene and xylene had been dissolved using a syringe pump (Harvard Apparatus, Model 22) and inline mixer. The commencement of pumping was defined as time zero. Five columns were operated simultaneously for parallel comparisons. One GAC column was operated abiotically to evaluate adsorption. Two sets of two columns each were packed with activated carbon and Baker product. One set of two columns was seeded with strain B1 and other two were seeded with strain X1. The steady-state columns were operated until the GAC column reached complete exhaustion and the biological columns reached steady-state (both BAC and biofilm). During this experiment, the effect of different microbial populations on the operation of BAC and biofilm column was studied by monitoring the effluent concentrations of the target compounds. Also, concentrations of byproduct from columns seeded with strain B1 were measured to monitor cometabolism.

5.2.7 Perturbation Experiment

Several perturbation experiments were performed to obtain information regarding the role of each substrate, the role of each mechanism (adsorption and biodegradation), and the role of different degradation patterns (cometabolism and competitive inhibition) in the system. The following shocks were applied to each column :

1. The effect of increased influent concentration of toluene ; which showed a different degradation rate and a different effect compared to *p*-xylene during the degradation by each strain ; was studied in this experiment. Toluene is a growth substrate for both strains. Strain B1 utilizes toluene faster than it does *p*-xylene. However, toluene is less preferably utilized to *p*-xylene, by strain X1.

2. A similar experiment to the above shock loading with toluene was performed by doubling the influent concentration of *p*-xylene, which is sorbed more strongly than toluene.

3. Nitrogen was used to supply the mineral media instead of oxygen to eliminate obligate aerobic activity.

5.2.8 Analytical Method

BTX liquid samples (1 ml) from column effluents were collected in 22 ml head-space vials and assayed by automatic headspace analysis. The analysis system included a Hewlett Packard HP 19395A automatic headspace sampler and a Perkin-Elmer Autosystem gas chromatograph, equipped with a DB-624 capillary column (30 m x .549 mm, J & W

Scientific) and a flame ionization detector. The carrier gas was helium (15 ml/min). Oven temperature began at 60°C and increased at a rate of 10°C/min to 160°C, then increased at a rate of 25°C/min to 200°C, where it remained constant for 1 minute. Liquid samples (4 ml) were also collected from columns seeded with strain B1 and filtered using sterile 0.22 μm filters. The concentrations of two byproducts - 3,6-dimethyl pyrocatechol and *p*-xylene dihydrodiol (Chang *et al.* 1993) - were then measured by a Gilson Gradient System high pressure liquid chromatograph (HPLC) equipped with a C_{18} reverse-phase column and a UV detector at 280 nm. The mobile phase was composed of 40 % water and 60 % methanol with 0.1 % acetic acid. The flow rate was 1.2 ml/min.

5.2.9 Scanning Electron Microscopy (SEM)

Samples of both virgin and biofilm coated activated carbon and baker product (from the fluidized bed reactor) were fixed at 4°C for 1-2 hours in 4% glutaraldehyde buffered with 0.1 m sodium pnosphate (pH 7.4). Following a brief rinse in the buffer, samples were dehydrated in a ethanol series (25%, 50%, 75%, 95%) for 10-15 min at each gradation followed by three 10 min shifts in 100% ethanol. A Balzers critical point dryer, using liquid carbon dioxide as the transitional fluid, was used to critical point dry the samples. Samples were sputter coated with gold (28 nm thickness) in an Emscope Sputter Coater model SC 500 purged with argon gas, and examined in a JEOL JSM-35CF Scanning Electron Microscope (Japan Electron Optics Ltd.).

5.3 Results

Typical degradation curves for toluene and xylene by the two strains are shown in Figure 5-1 and Figure 5-2. Figure 5-1 illustrates cometabolic degradation of *p*-xylene by

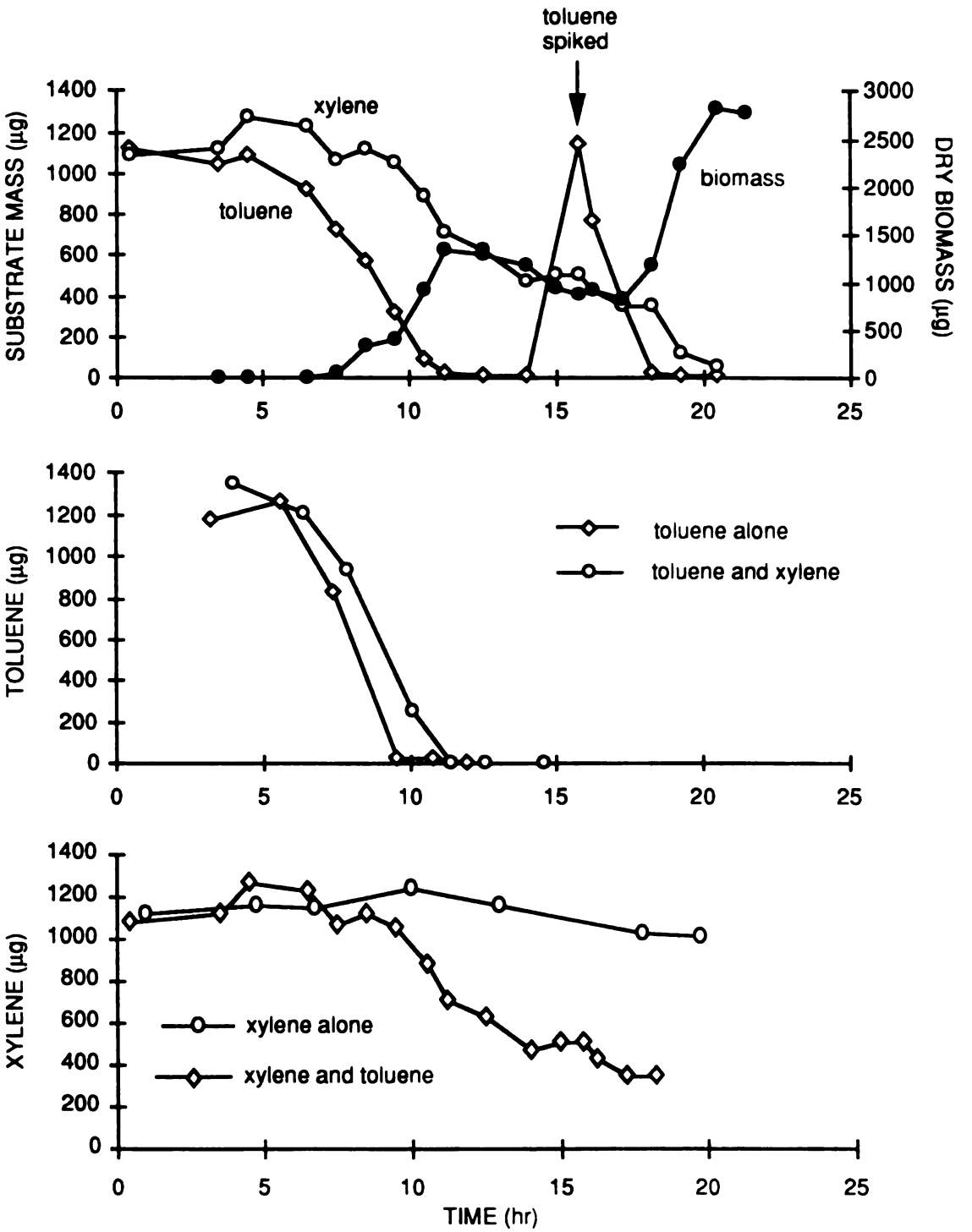


Figure 5-1. Degradation curves illustrating the degradation of toluene and *p*-xylene by strain B1.

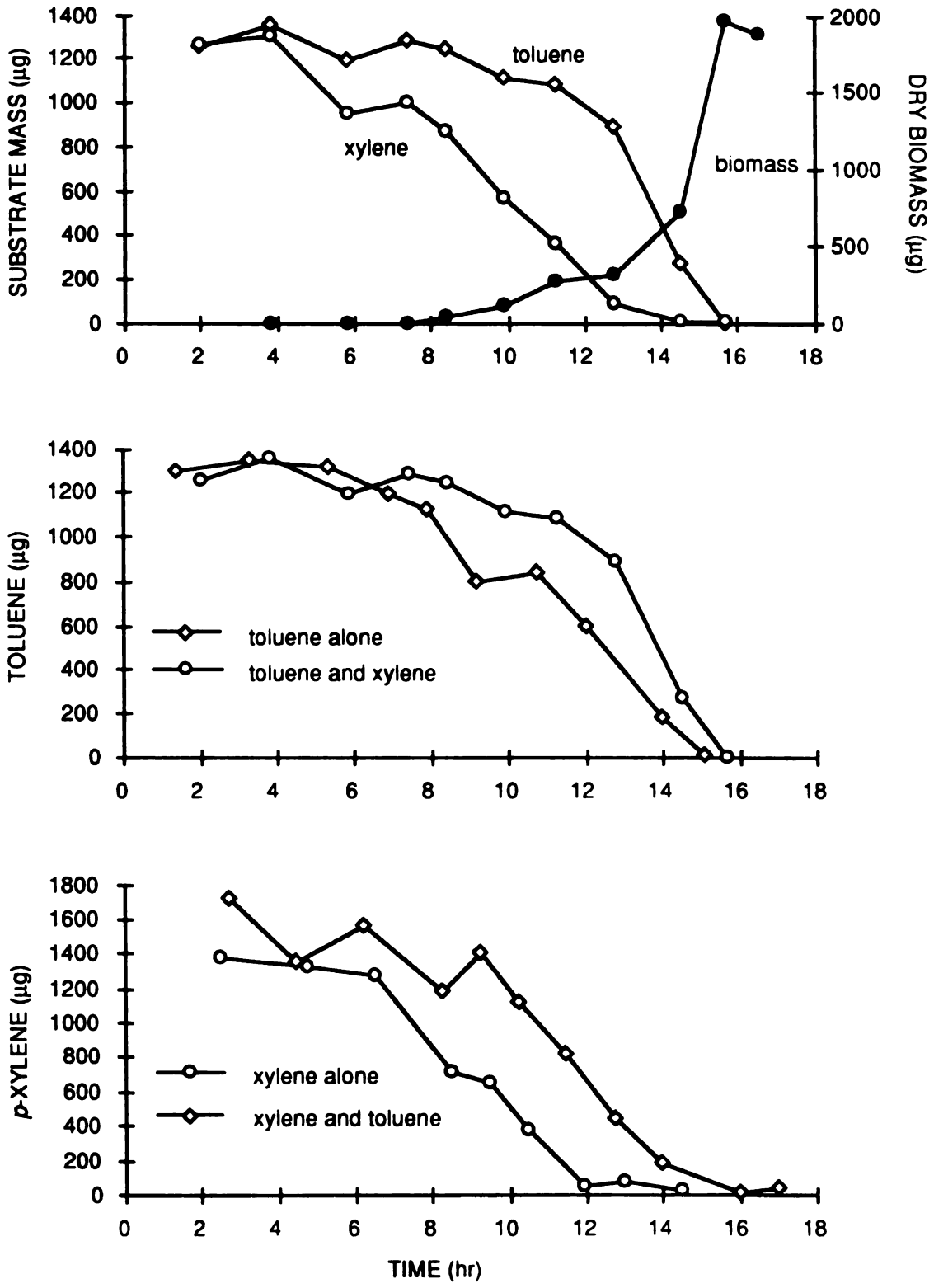


Figure 5-2. Degradation curves illustrating the degradation of toluene and *p*-xylene by strain X1

strain B1. In single substrate experiments, strain B1 was incapable of transforming *p*-xylene. However, *p*-xylene was transformed when combined with a growth substrate, such as toluene. Transformation of *p*-xylene slowed significantly when the growth substrate disappeared, but increased when toluene was again added. Products of the cometabolic transformation were previously identified as *p*-xylene dihydrodiol, 3,6-dimethyl pyrocatechol, and 2,5-dimethyl phenol. Strain X1 utilizes both compounds as growth substrates. The rate of degradation of *p*-xylene was faster than that of toluene. The presence of either compound with the other affects the rate of degradation of each substrate as shown in Figure 5-2.

The single-solute adsorption isotherms for toluene and *p*-xylene were described using the Freundlich isotherm model;

$$q_e = K_F \times (C_e)^n \qquad (5\text{-}1)$$

where, q_e = mass of solute adsorbed per mass of adsorbent, C_e = concentration of solute at equilibrium, and K_F and n are constants representing the sorptive capacity and affinity (intensity), respectively. The values of the constants were determined by a non-linear curve fitting procedure and tabulated in Figure 5-3 with the two isotherms. The larger K_F value and the shape of the isotherm indicates *p*-xylene is more readily adsorbed than toluene.

Figure 5-4 compares effluent profiles of toluene and *p*-xylene from three different activated carbon columns ; 1) BAC seeded with strain B1, 2) BAC seeded with strain X1, and 3) GAC as a control. BAC columns show slower appearance of target compounds in the effluent than GAC. In BAC columns, effluent profiles show distinctively different patterns for each different microorganism with which they were seeded. In the strain B1 seeded BAC column, toluene was observed in the effluent earlier than *p*-xylene, however,

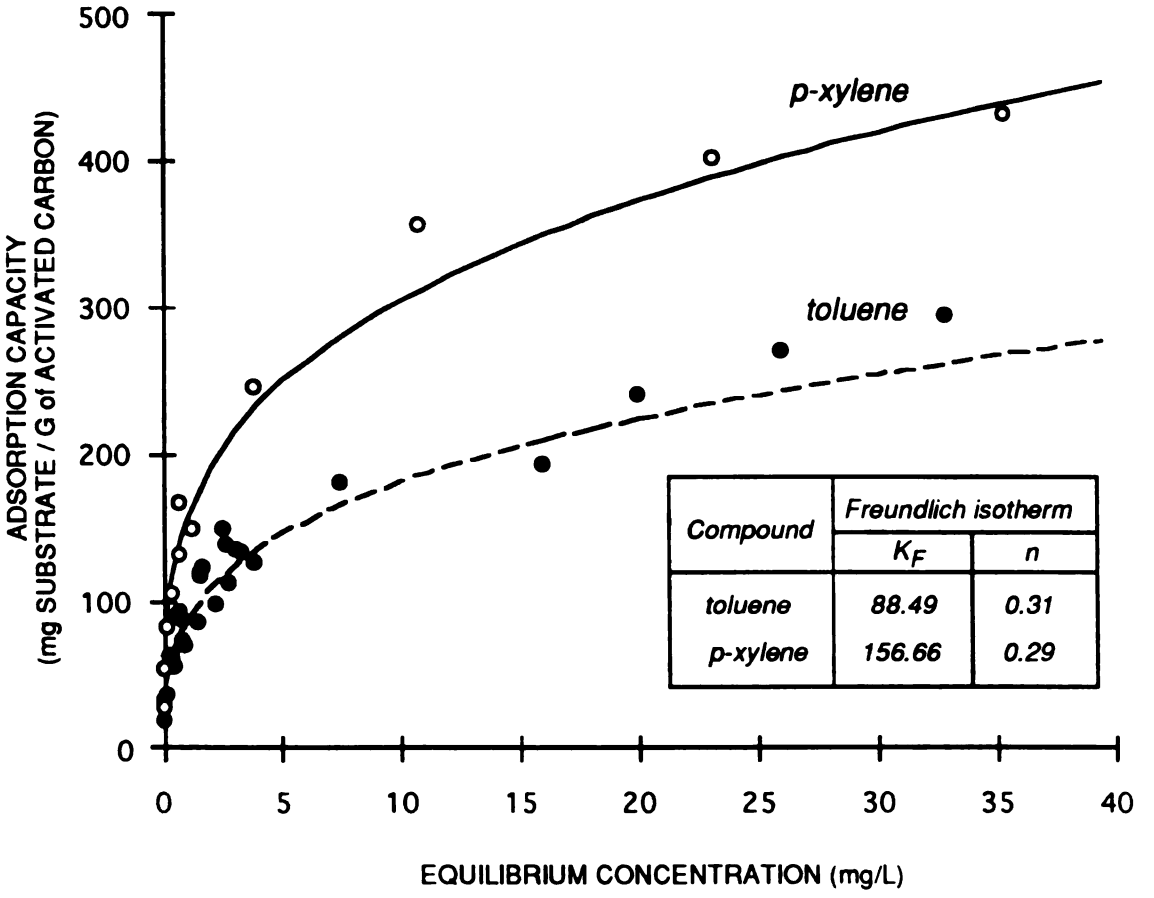


Figure 5-3. Isotherms for toluene and *p*-xylene with Freundlich parameter estimation.

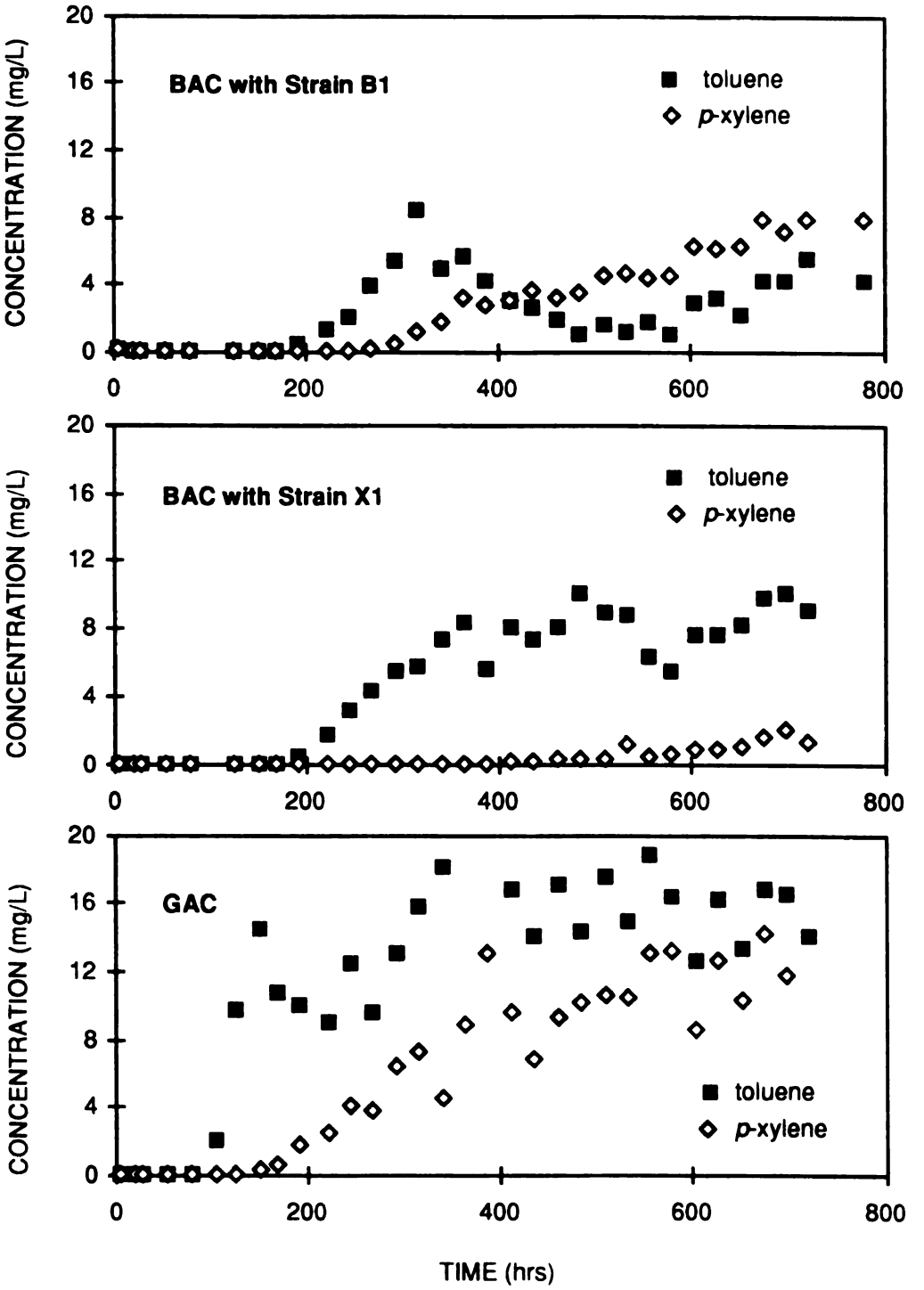


Figure 5-4. Effluent profiles of toluene (■) and *p*-xylene (◇) for 1) BAC seeded with strain B1, 2) BAC seeded with strain X1, and 3) GAC.

it eventually decreased. *p*-xylene in the effluent shows a typical breakthrough pattern. The BAC column seeded with strain X1 shows low effluent concentration of *p*-xylene, however, toluene shows a breakthrough pattern. The GAC column shows typical breakthrough curves found for bi-solute systems.

Figure 5-5 shows the byproducts in the effluent from the cometabolic degradation of *p*-xylene in columns seeded with strain B1. In the biofilm column, *p*-xylene dihydrodiol was formed immediately after starting the operation of the column. 3,6-Dimethyl pyrocatechol was not formed until 100 hrs of bed operation. Then, eventually, the formation of 3,6-dimethyl pyrocatechol increased and after approximately 200 hrs of bed operation, it bypassed the formation of *p*-xylene dihydrodiol. However, in the BAC column, byproducts were not observed until 200 hrs of operation. Figure 5-6 compares the effluent profile of *p*-xylene from the GAC system and the accumulated area graph of *p*-xylene, *p*-xylene dihydrodiol, and 3,6-dimethyl phenol, in the BAC system all on a molecular carbon basis. There was a good agreement between the two lines.

The results of a shock load resulting from increased toluene concentrations are provided in Figure 5-7 and Figure 5-8. Figure 5-7 shows effluent profiles of target compounds and byproducts during the shock in columns seeded with strain B1. In these columns, doubling the influent concentration of toluene not only increased the effluent concentration of toluene but also that of *p*-xylene. However, the formation of byproducts was retarded during this shock. Figure 5-8 compares effluent profiles of target compounds from BAC and biofilm columns seeded with strain X1. Doubling the toluene significantly affected the effluent toluene concentration. However, *p*-xylene concentration was only slightly affected.

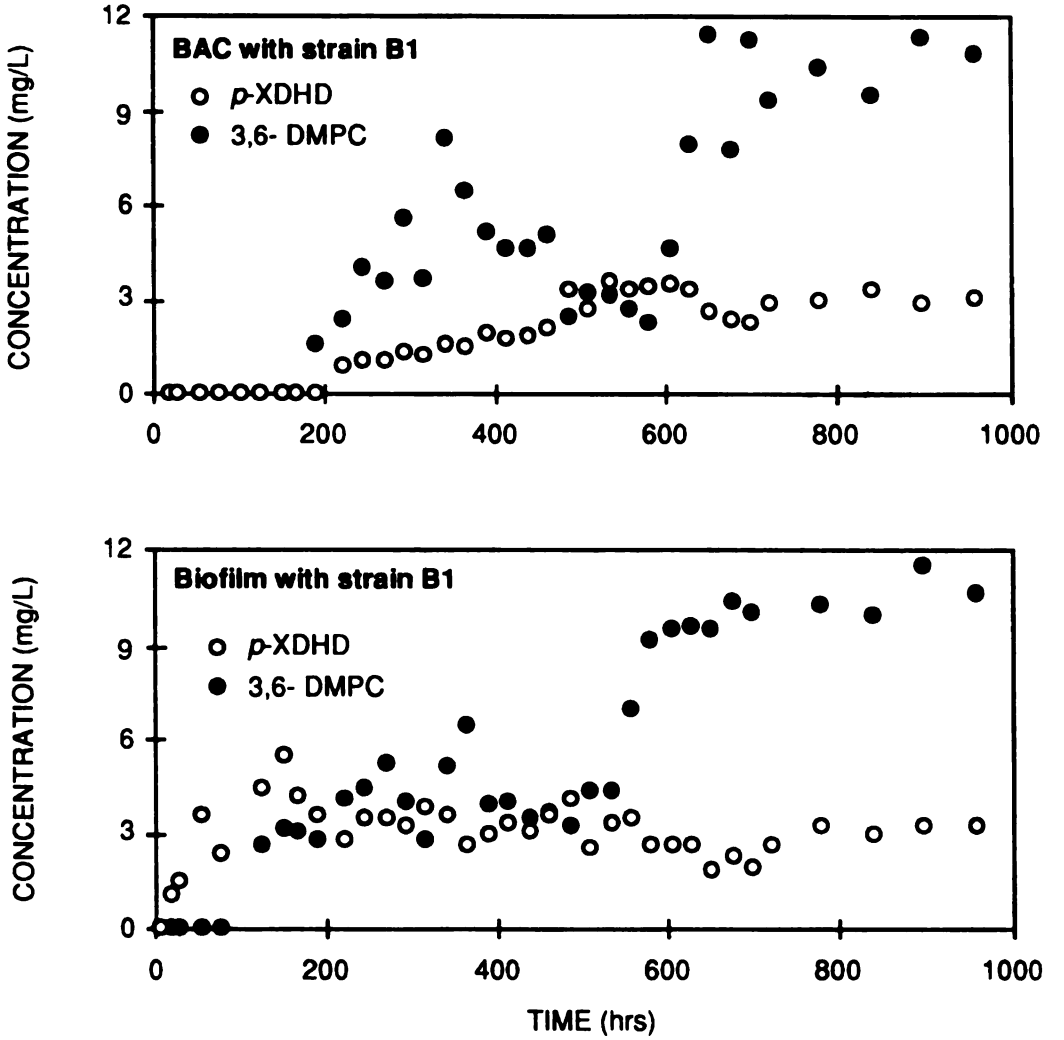


Figure 5-5. Effluent profiles of byproducts, *p*-xylene dihydrodiol (o) and 3,6-dimethyl pyrocatechol (●) from 1) BAC and 2) Biofilm from columns seeded with strain B1.

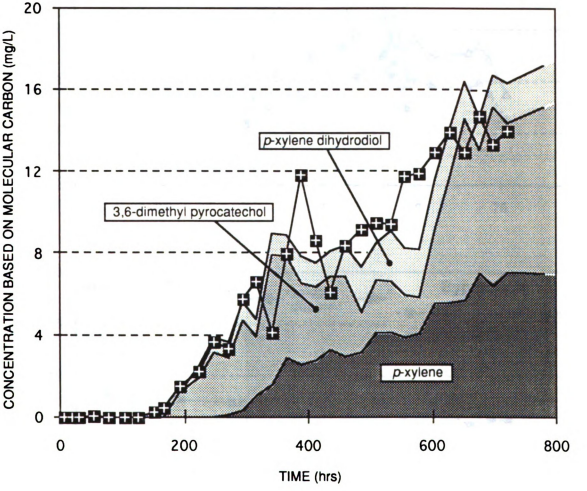


Figure 5-6. Effluent profile of *p*-xylene from GAC column (-⊞-) and accumulated area graph of *p*-xylene, *p*-xylene dihydrodiol, and 3,6-dimethyl pyrocatechol from BAC based on the concentration of molecular carbon.

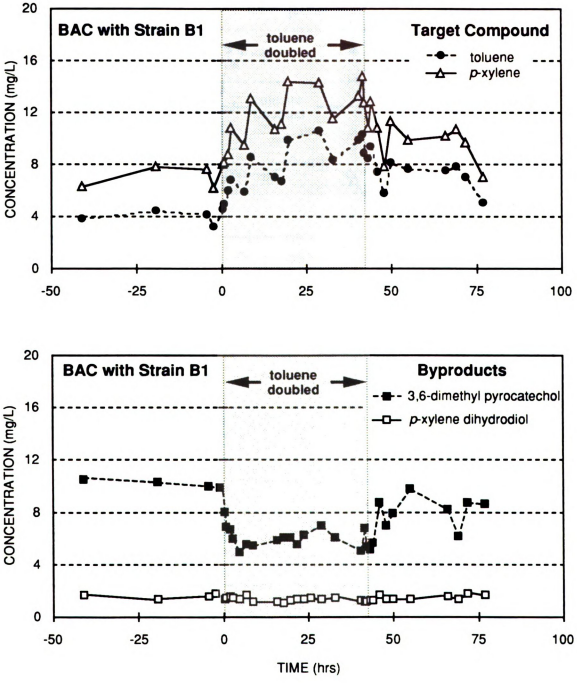


Figure 5-7. Effluent profiles of target compounds and byproducts during shock loadings by doubling toluene concentration in BAC column seeded with strain B1.

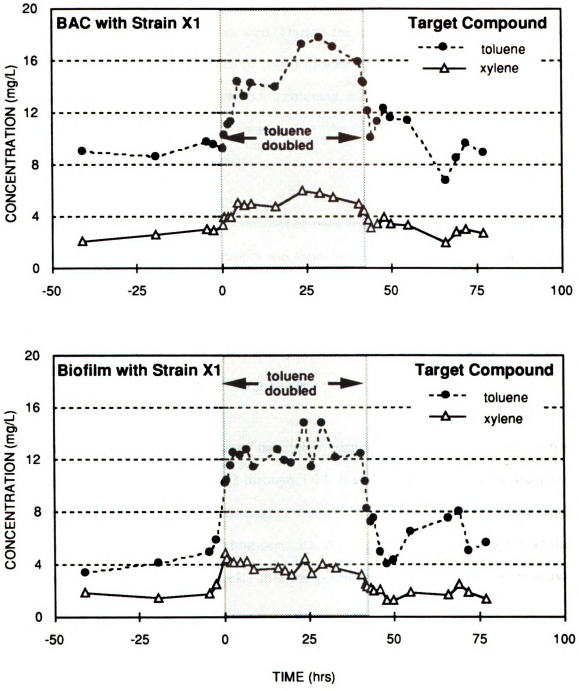


Figure 5-8. Effluent profiles of target compounds during shock loadings by doubling toluene concentration in BAC and biofilm columns seeded with strain X1.

Figure 5-9 shows *p*-xylene shock-loading result for columns seeded with strain B1. These columns showed increased effluent concentrations of *p*-xylene. There was a slight increase in toluene concentrations as well. During the shock period, the formation of byproducts was diminished. Figure 5-10 shows effluent profiles of target compounds in columns seeded with strain X1. In the BAC columns, effluent concentrations were only slightly affected by the shock. However in the biofilm columns, concentrations of both compounds were increased considerably.

Figure 5-11 compares effluent profiles of both BAC columns (seeded with strain B1 and strain X1) after the oxygen supply was terminated. Concentrations of toluene and *p*-xylene from the column seeded with strain B1 increased and ultimately reached the same concentration as the influent. The concentration of toluene from the column seeded with strain X1 reached influent levels faster than *p*-xylene, which also increased. However, there were more sorption capacity left for *p*-xylene.

Scanning electron microscopy of the carbon particles from different depths of the column are compared in Figure 5-12 through 5-14. It can be seen that carbon particles obtained near the influent are more throughly covered by biomass. However, those from the effluent side still show bare carbon surfaces. A Column section collected near the influent at low magnification suggests that the pores are kept open even with active biological growth.

5.4 Discussion

The two *Pseudomonas* isolates demonstrated very different substrate preferences for toluene and *p*-xylene. Especially during multi-substrate degradation, their degradation

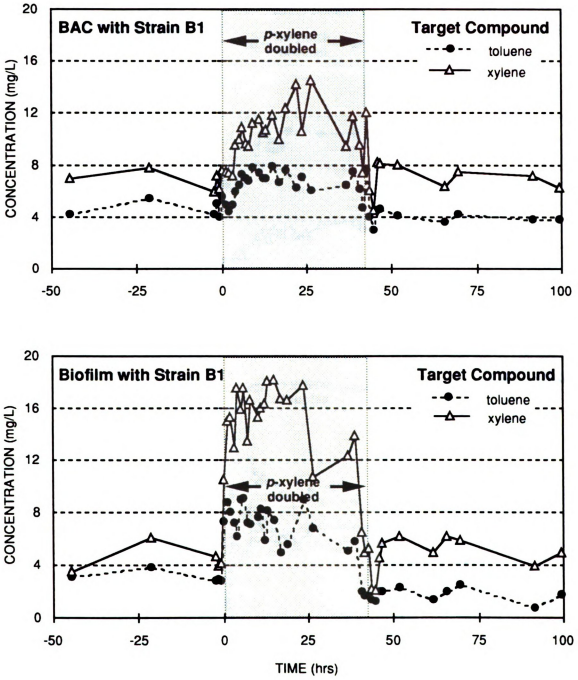


Figure 5-9. Effluent profiles of target compounds during shock loadings by doubling *p*-xylene concentration in BAC and biofilm columns seeded with strain B1.

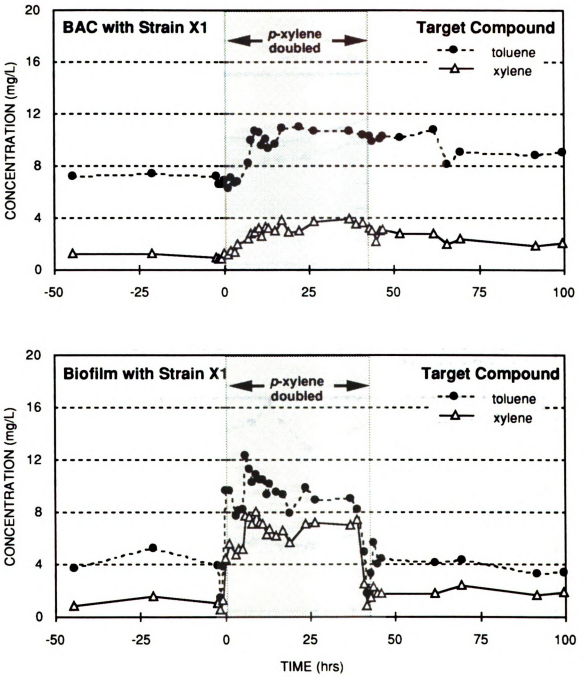


Figure 5-10. Effluent profiles of target compounds during shock loadings by doubling *p*-xylene concentration in BAC and biofilm columns seeded with strain X1.

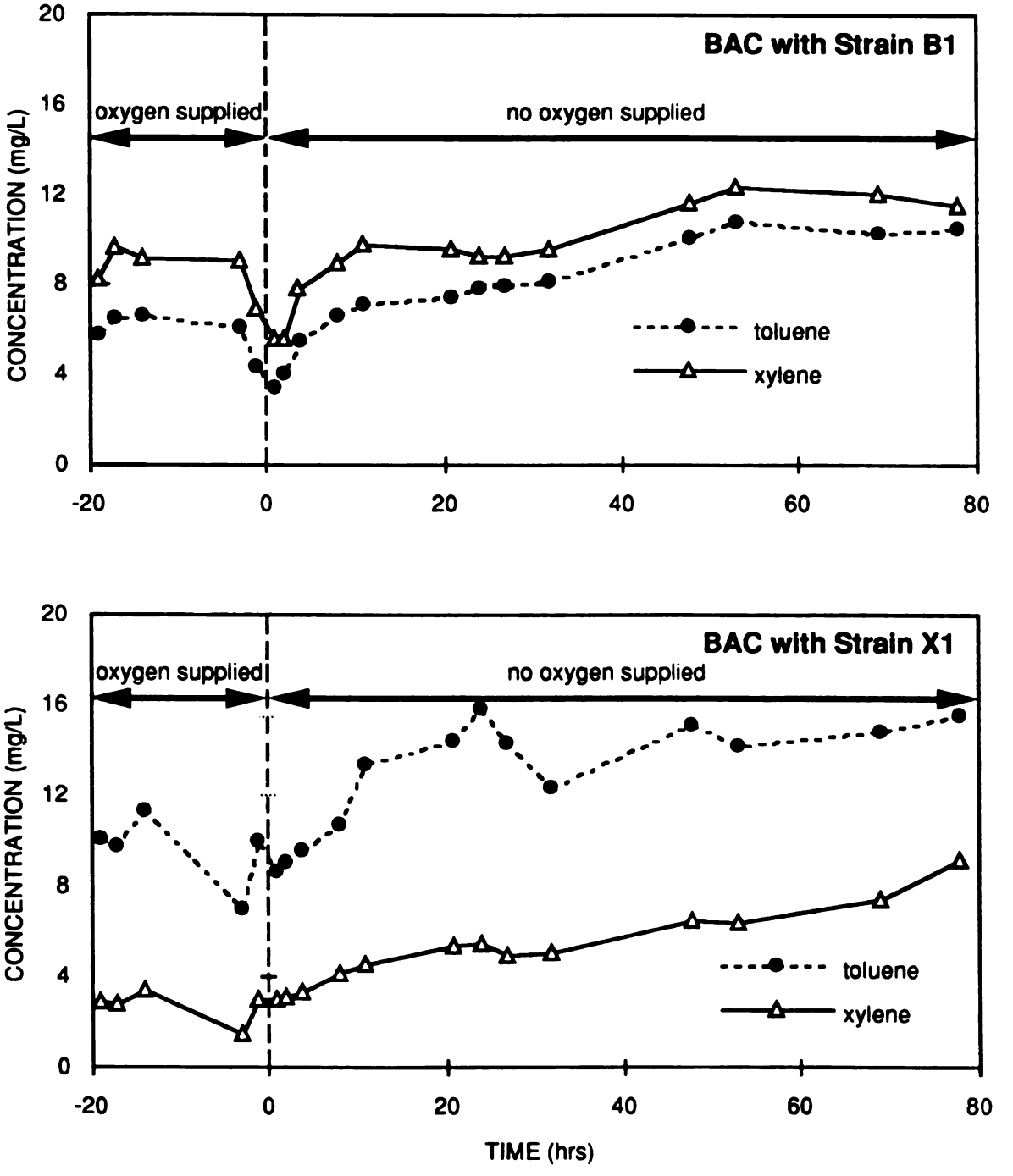


Figure 5-11. Effluent profiles of target compounds in BAC columns after the microbiological activity was eliminated.

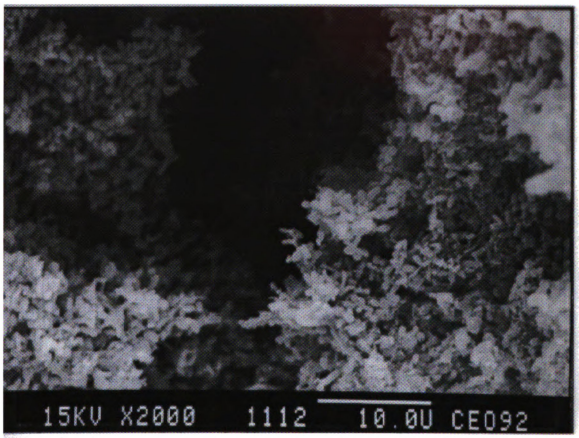


Figure 5-12. Scanning electron micrograph of carbon particles obtained near influent side of the reactor.

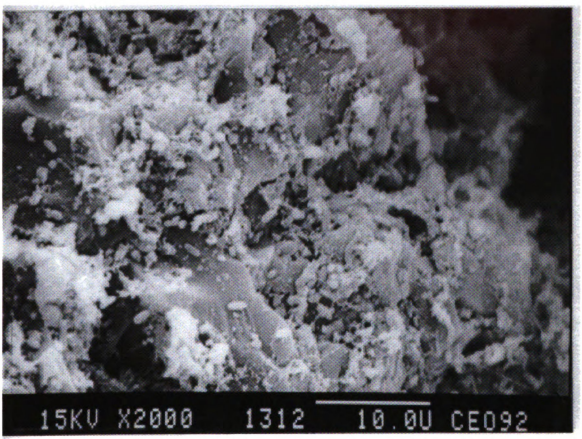


Figure 5-13. Scanning electron micrograph of carbon particles obtained near effluent side of the reactor.

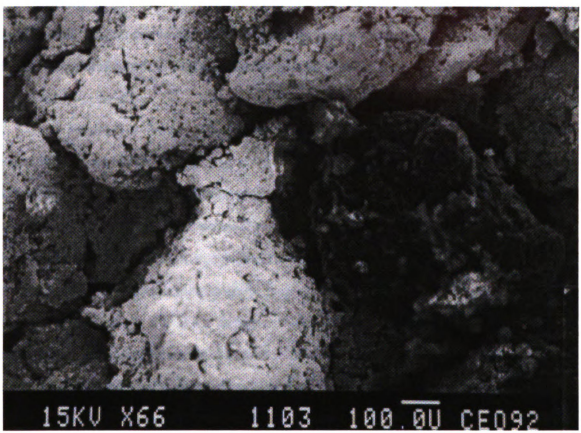


Figure 5-14. Scanning electron micrograph of matrix of carbon particles near influent side of the reactor.

pattern exhibited not only opposite preferences for one compound over the other, but also the effect of one compound on the degradation of others. From the adsorption study, the single compound isotherms show that *p*-xylene is more adsorbable than toluene. These kinds of complexities make the prediction of the operation of BAC difficult.

In this study, the initial seeding of different species of microorganisms showed considerable differences in degradation patterns and in consequence, the importance of a dominant population of microorganisms in the treatment of BAC was observed.

As Figure 5-4 shows, the effluent profiles of *p*-xylene were greatly influenced depending on which microorganisms were present in the columns. During the start-up period, the major removal mechanism for BAC is adsorption. The measurement of oxygen consumption (data not shown) showed that microbiological removal in the BAC system developed more slowly than in the biofilm columns. Toluene started to appear in the effluents of both BAC columns at about the same time, although it was decreased as the biomass increased in the BAC column seeded with strain B1. *p*-xylene is a more adsorbable compound; however, because it is a less favorably degraded compound than toluene by strain B1, it ultimately reached higher effluent concentrations than toluene.

The BAC system seeded with strain X1 demonstrated different performance with respect to *p*-xylene. *p*-xylene is more favorably degraded by strain X1 than toluene (Figure 5-2) and it is also more favorably adsorbed. Consequently, both adsorption and biodegradation effectively removed *p*-xylene and it was not observed in the effluent until 600 hrs of operation.

Based on the previous batch studies, *p*-xylene dihydrodiol is formed when there is a enough growth substrate, and 3,6-dimethyl pyrocatechol is formed from *p*-xylene

dihydrodiol under the limiting growth substrate condition. During the start-up period, only *p*-xylene dihydrodiol was formed. This is evidenced from the result of the biofilm column (Figure 5-5). As the biomass increased, it depleted toluene quickly and caused a growth substrate limitation. This resulted in the formation of 3,6-dimethyl pyrocatechol. In the BAC system, the slightly adsorbable *p*-xylene dihydrodiol reached breakthrough at about 200 hrs of bed operation. It is not clear why the profile of 3,6-dimethyl pyrocatechol changed (increased, decreased, then re-increased) until it reached steady-state. It is noteworthy that the profile matched the toluene effluent profile which also increased, then decreased (Figure 5-5).

The concentrations of byproducts and *p*-xylene were converted to a molecular carbon basis to graphically show a mass balance on these compounds. Good matches between these accumulated concentrations with the *p*-xylene profile from GAC (Figure 5-6) sugget that the difference between profiles of *p*-xylene in the two columns can be interpreted as the result of microbiological degradation.

The addition of toluene to the bottle containing *p*-xylene with strain B1 caused enhanced degradation of *p*-xylene in the batch experiment (Figure 5-1). It was hypothesized that the increased toluene concentration in the influent would cause enhanced degradation of *p*-xylene with increased amount of byproducts formation. However, the column experiment revealed a different result. The increased influent concentration of toluene caused an increase in toluene concentration as well as *p*-xylene in the effluent (Figure 5-7). This can be explained as the result of competition. The increase of the more favorable substrate (toluene) caused the microorganisms to utilize toluene instead of the increasing cometabolic degradation of *p*-xylene. Byproduct formation also decreased as less *p*-xylene was degraded. The biofilm system (data not shown) showed an immediate increase of both target compounds as well as an immediate decrease of byproducts. The

BAC system showed sorptive buffering, which is suggested to be an advantage of the BAC systems (Voice *et al.* 1992).

The shock loading of the less favorably degraded compound had a smaller impact on the more favorably degraded compound. The shock loading of toluene in columns seeded with strain X1 showed *p*-xylene was only slightly disturbed by the presence of increased toluene (Figure 5-8). The response of shock loading was more rapid for the biofilm. The increased influent concentration of toluene instantly raised the effluent concentration as well.

For strain B1, *p*-xylene is the less desirable substrate. The increased influent *p*-xylene concentrations caused increased effluent concentrations of both compounds. However, the amount of increase in toluene concentration was less significant than that of *p*-xylene. Figure 5-7 and Figure 5-9 shows that the effect on the more degradable compound by the increase of the less degradable compound is not as significant as the reverse situation.

In the BAC system with strain X1, more adsorption sites were available, because the microbiological activity removes the more adsorbable compound (*p*-xylene) from the system. This was advantageous for later shock loadings. The *p*-xylene shock for the biofilm column seeded with strain X1 showed that concentrations of both compounds increased significantly. This result was consistent for the shock loading of toluene in columns seeded with strain B1. In other words, shock loading of the favorably degraded compound affects the effluent concentrations of both compounds significantly. However, the BAC system with strain X1 shows a much diminished shock effect in the effluent. The shock loading was buffered by adsorption.

Without microbiological activity, the only removal mechanism in BAC is adsorption. As observed during the shock-loading studies, the BAC system with strain X1 had more adsorption capacity than the BAC system with strain B1. During the oxygen depravation degradation study, both BAC systems showed a slow increase of target compounds in the effluent . Even though others reached equilibrium with influent after 60 hrs, *p*-xylene was continuously removed by adsorption in the BAC system seeded with strain X1 .

The observations by SEM differentiate carbon particles obtained near both the influent and effluent sides of the reactor. Activated carbon particles collected near the influent, where substrate is abundant, show complete coverage of biomass. However, the effluent side gets less substrate and this causes less biomass to grow. If the reactor is deep enough, this phenomena should be more distinct. Biomass will develop up to the height providing sufficient growth substrate and dissolved oxygen. Beyond this level, the uncolonized activated carbon will remain and can be reserved as backup for an unusual case of influent loading. As the column section shows, the pores between the carbon particles remain open. The shear force of the flow is believed to take some biomass away from the surface of the biofilm surrounding the activated carbon particles.

5.5 Conclusion

BAC and biofilm columns seeded with two *Pseudomonas* species - strain B1 and X1 - were compared with GAC in treating two substrates; toluene and *p*-xylene. The effluent profiles of the target compounds were greatly influenced by the microorganisms in the systems. Shock loading was applied to the reactors by increasing one of the target compounds. Shock loading of the favorably degraded compound resulted in a significant

increase in the effluent concentrations of both compounds. The effect of shock loading by the less favorably degraded compound was smaller. Both cases of shock loadings affected the degradation of *p*-xylene and resulted in the reduced formation of byproducts. The BAC systems were less affected by perturbations. It is recommended that BAC columns be designed with enough longitudinal length to provide residual adsorption capability to handle variations in unusual influent loading.

CHAPTER 6

DISSERTATION SUMMARY AND RECOMMENDATIONS

6.1 Summary

The purpose of this research was to develop a better understanding of BAC systems for the treatment of BTX as multiple substrates. For this purpose, microbiological kinetic studies were performed. Two *Pseudomonas* species (designated strains B1 and X1) were isolated from an aerobic pilot-scale fluidized-bed reactor treating groundwater containing benzene, toluene, and *p*-xylene (BTX). Strain B1 grew with benzene and toluene as the sole sources of carbon and energy, and it cometabolized *p*-xylene in the presence of toluene. Strain X1 grew on toluene and *p*-xylene, but not benzene. In single substrate experiments, the appearance of biomass lagged the consumption of growth substrates, suggesting that substrate uptake may not be growth rate-limiting for these substrates. Batch tests using paired substrates (BT, TX, or BX) revealed competitive inhibition and cometabolic degradation patterns. Competitive inhibition was modeled by adding a competitive inhibition term to the Monod expression. Cometabolic transformation of non-growth substrate (*p*-xylene) by strain B1 was quantified by coupling *p*-xylene transformation to consumption of growth substrate (toluene) during growth and to loss of biomass during the decay phase. Coupling was achieved by defining two transformation capacity terms for the cometabolizing culture: one that relates consumption of growth substrate to the consumption of non-growth substrate, and a second that relates consumption of biomass to the consumption of non-growth substrate. Cometabolism

increased decay rates, and the observed yield for strain B1 decreased in the presence of *p*-xylene.

The formation of byproduct observed during the cometabolic degradation of *p*-xylene was extensively investigated. Three byproducts - *p*-xylene dihydrodiol, 3,6-dimethyl pyrocatechol, and 2,5-dimethylphenol - were identified. The byproduct formed during cometabolic degradation was found to depend the conditions under which the cells obtained transformation capacity. During the presence of the growth substrate, the primary byproduct formed was *p*-xylene dihydrodiol. However, after the complete degradation of toluene (growth substrate), the major byproduct was 3,6-dimethyl pyrocatechol.

p-xylene dihydrodiol was found to be very adsorbable to activated carbon, while 3,6-dimethyl pyrocatechol was not. Batch isotherm experiments showed that the presence of byproducts in solution interfered with the sorption of the target compounds. Benzene was the most significantly affected of the three compounds. The formation of byproducts during the operation of fixed-bed biological activated carbon (BAC) system was studied by comparing BAC reactors to biofilm reactors during the absence of either the growth substrate or the non-growth substrate. In the BAC reactor, cometabolic transformation continued in the absence of both substrates, while the transformation stopped immediately in the biofilm column.

BAC system clearly showed extended service time when compared to GAC systems. However, two BAC columns seeded with different species showed significant differences in effluent profiles of the target compounds. During the start-up period, the major removal mechanism was adsorption. As the biomass increased, biodegradation became more important than adsorption. BAC also showed a buffering effect during the influent perturbations. The effect of a shock-load by the growth substrate on the non-

growth substrate was more significant than that by the non-growth substrate on the growth substrate. Designing the BAC reactor with enough depth to retain adsorption capacity is recommended to cope with variations in influent loading.

6.2 Recommendations for Future Studies

Continuation of this work should consider the following issues that require further investigation.

- Degradation and competition of other substrates with BTX by the two species (strain B1 and X1) should be studied.

- Perform kinetic studies on a mixed culture. The two (strain B1 and X1) species could be combined and the degradation of target compounds and byproduct formation be monitored.

- Investigate the toxicity of the byproduct and the long-term effects of shock loads on the formation of byproducts.

- Develop model to describe multi-substrate biofilm and adsorption dynamics to describe different patterns of degradation such as competitive inhibition or cometabolic degradation.

APPENDICES

APPENDIX A

MEDIA PREPARATION

Composition of HCMM2 mineral-salts medium

Add the following and take volume to 1 L with deionized water :

Major ions

KH_2PO_4	1.36 g
Na_2HPO_4	1.42 g
KNO_3	0.50 g
$(NH_4)_2SO_4$	2.38 g
$MgSO_4 \cdot 7H_2O$	0.05 g
$CaCl_2$	0.01 g

Trace elements

H3BO4	2.862 mg
MnSO4•H2O	1.538 mg
Fe(NH4)2(SO4)2•6H2O	3.529 mg
CuSO4•5H2O	0.0392 mg
ZnCl2	0.0209 mg
CoCl2•6H2O	0.0405 mg
$Na_2MoO4 \cdot 2H2O$	0.0252 mg

After Ridgway *et al.* (1990)

Check pH (6.9-7.1). Adjust if needed.
Solidified medium is prepared by adding 18 g Bacto agar.
Medium for column expeirment is diluted from above HCMM2 medium by 1/10.

Composition of phosphate and magnesium buffer

Add the following and take volume to 1 L with deionized water :

KH_2PO_4	8.5 mg
K_2HPO_4	21.75 mg
Na_2HPO_4	17.70 mg
$MgSO_4$	11.0 mg

After Chang (1987)

Phosphate and magnesium buffer was used for the dilution of cell containing solution.

APPENDIX B

ISOLATION PROCEDURES

Pure culture microorganisms were isolated from the laboratory-scale activated carbon fluidized reactor located at Michigan State University. Details of reactor operation and design are described elsewhere (Voice *et al.*, 1992). After a three month period of continuous operation treating BTX-contaminated water, activated carbon particles with well developed biofilms were removed from the fluidized-bed reactor. Approximately five particles were placed in a 25-ml glass vial containing 10 ml of phosphate buffer solution and ten glass beads 4 mm in diameter. The vial was vigorously shaken and allowed to stand for 15 minuites until all large floc had settled.

Test tubes with 9 ml phosphate buffer solution were prepared after autoclaving. Then 1 ml of solution from the above 25-ml glass vial was transfered to make a solution of dilution 10^{-1}. This step was repeated to succeeding tubes until a solution of dilution 10^{-7} was made. From each tube of this dilution series, 0.2 ml of solution were spreaded on plates of HCMM2 solid media (Appendix A). Three plates were prepared from each dilution.

The plates were exposed to benzene, toluene, or *p*-xylene in completely sealed chambers . After 48 hours, isolates were picked, streaked, and re-incubated. This procedure was repeated 5 to 8 times. One to three distinctive colonies were identified from each carbon source and were named appropriately by using the abbreviation of each substrate followed by a sequence number. Colonies isolated by growth upon vapors of a single pure compound, such as benzene, were then tested for growth in chambers containing other substrates, such as toluene or *p*-xylene. The extent of growth was

recorded with regard to the formation of colonies. The results from the observations are provided in Table B-1. Details of the isolation procedure are described on Figure B-1.

Table B-1. Growth of isolated colonies in each carbon source

Strain	benzene	toluene	*p*-xylene
B1	++	++	-
T1	+	++	+
T2	+	++	+
X1	-	++	++
X2	+	++	++

++ : Active growth + : Moderate growth - : No growth

Among these strains, strain B1 and strain X1 showed distinct differences in degradation characteristics. Strain B1 grew on either benzene or toluene; however, it did not grow on p-xylene. On the contrary, Strain X1 grew on xylene, but it did not grow on benzene. Because of their different degradation patterns, these two strains were selected for further investigation.

The results of scanning electron microscopy (SEM) of these two strains are shown in Figure B-2. Materials and methods for SEM are provided in Appendix C.

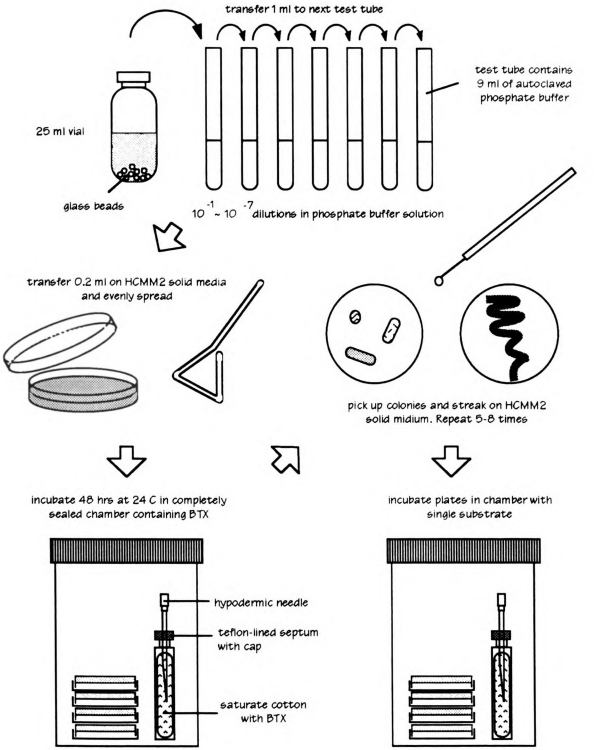
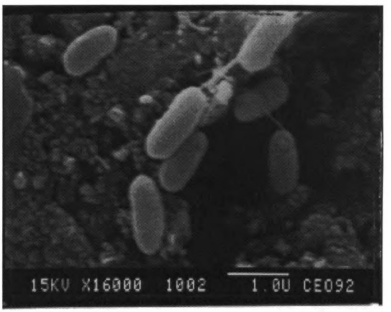
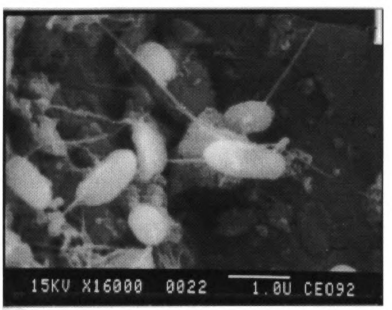


Figure B-1. Schematic details of isolation procedures



Pseudomonas sp. strain B1



Pseudomonas sp. strain X1

Figure B-2. SEM of *Pseudomonas sp.* stain B1 and strain X1

APPENDIX C

SCANNING ELECTRON MICROSCOPY SAMPLE PREPARATION PROCEDURES

1. Fix samples at 4 C for 1 hour in 4% glutaraldehyde buffered with 0.1 M sodium phosphate at pH 7.4. Following a brief rinse in the buffer, dehydrate samples in a ethanol series (25%, 50%, 75%, 95%) for 10-15 min. at each gradation followed by three 10 min. changes in 100% ethanol.

2. Critical point dry samples in a Balzers critical point dryer using liquid carbon dioxide as the transitional fluid.

3. Coat samples with gold (28 nm thickness) in an Emscope Sputter Coater model SC 500 purged with argon gas.

4. Examine samples in a JEOL JSM-35CF Scanning Electron Microscope (Japan Electron Optics Ltd.)

APPENDIX D

CODE FOR COMETABOLIC DEGRADATION MODELING

```
REM
REM         MODELING COMETABOLISM
REM
REM         Developed using 2nd order Runge-Kutta
REM         numerical method by Myung K. Chang
REM
REM         March 22, 1992
REM
REM

REM         Input parameters for growth substrate
CLS
DIM         SG(5000), SN(5000), X(5000), GS(5), NS(5), XC(5)
PRINT       "Modeling of Cometabolism":PRINT
GOSUB       1000
INPUT       "Do you want to input data from :  1. Key-board   or   2. Input data ";YN

IF YN = 1 THEN GOTO 50

REM         Read data from File.
READ        MMX, Y, KSG, TCG, k, KSN, FCO, B, TCB, SG(1), SN(1), X(1), dt
GOTO 100
50 CLS
PRINT       "Parameters for growth substrate"
PRINT
INPUT       "  1. Maximum specific growth rate : Umax (1/day) = ";MMX
INPUT       "  2. Yield coefficient : Y (mass biomass/mass growth substrate) = ";Y
INPUT       "  3. Half saturation coefficient : Ks (mg/l) = ";KSG

REM         Input parameters for non-growth substrate
PRINT       "Parameters for-non-growth substrate"
PRINT
INPUT       "  4. Transformation capacity : Tcg (mass of NGS/mass of GS) = "; TCG
INPUT       "  5. Maximum specific growth rate of utilization : k (mass of NGS/mass
              biomass-day) = "; k
INPUT       "  6. Half saturation coefficient : Ks (mg/l) = ";KSN

REM         Input other parameters
PRINT       "Other parameters"

INPUT       "  7. Fractional coefficient : fco = ";FCO
INPUT       "  8. Decay coefficient : b (1/day) = ";B
INPUT       "  9. Biomass transformation capacity : Tcb (mass of NGS/mass of
              biomass) = "; TCB
PRINT
```

```
REM         Information on initial conditions
PRINT       "Information on initial conditions":PRINT
INPUT       "  10. Initial growth substrate mass : Sg (ug) = ";SG(1)
INPUT       "  11. Initial non-growth substrate mass Sng (ug) = "; SN(1)
INPUT       "  12. Initial biomass amount : Xo (ug) = "; X(1)
INPUT       "  13. Time interval for analysis : delta T (min) = "; dt

GOTO 100

REM         Print all information on the screen for check

100 CLS

PRINT       "Summary of input parameters":PRINT
PRINT       "  1. Maximum specific growth rate : Umax = ";MMX; "(1/day)"
PRINT       "  2. Yield coefficient : Y = ";Y; "(mass biomass/mass growth substrate)"
PRINT       "  3. Half saturation coefficient : Ks = ";KSG; "(mg/l)"
PRINT       "  4. Transformation capacity : Tcg = "; TCG; "(mass of NGS/mass of
              GS)"
PRINT       "  5. Maximum specific growth rate of utilization : k = "; k;  "(mass of
              NGS/mass biomass-day)"
PRINT       "  6. Half saturation coefficient : Ks = ";KSN; "(mg/l)"
PRINT       "  7. Fractional coefficient : fco = ";FCO
PRINT       "  8. Decay coefficient : b = ";B; "(1/day)"
PRINT       "  9. Biomass transformation capacity : Tcb = "; TCB; "(mass of
              NGS/mass of biomass)"
PRINT       "  10. Initial growth substrate mass : Sg = ";SG(1); "(ug)"
PRINT       "  11. Initial non-growth substrate mass Sng = "; SN(1);  "(ug)"
PRINT       "  12. Initial biomass amount : Xo = "; X(1);  "(ug)"
PRINT       "  13. Time interval for analysis : delta T = "; dt; "(min)"

90 GOSUB 1000

INPUT       "Do you want to change any parameter"; YN$

IF YN$ = "n" OR YN$ = "N" THEN GOTO 300
IF YN$ <> "y" AND YN$ <> "Y" THEN GOTO 90

80 GOSUB 1000

INPUT       "Print number to change"; num
PRINT

IF num = 1 GOTO 110
IF num = 2 GOTO 120
IF num = 3 GOTO 130
IF num = 4 GOTO 140
IF num = 5 GOTO 150
IF num = 6 GOTO 160
IF num = 7 GOTO 170
IF num = 8 GOTO 180
IF num = 9 GOTO 190
IF num = 10 GOTO 200
IF num = 11 GOTO 210
```

```
IF num = 12 GOTO 220
IF num = 13 GOTO 230

GOTO 80
110 INPUT "  1. Maximum specific growth rate : Umax (1/day) = ";MMX
GOTO 100
120 INPUT "  2. Yield coefficient : Y (mass biomass/mass growth substrate) = ";Y
GOTO 100
130 INPUT "  3. Half saturation coefficient : Ks (mg/l) = ";KSG
GOTO 100
140 INPUT "  4. Transformation capacity : Tcg (mass of NGS/mass of GS) = "; TCG
GOTO 100
150 INPUT "  5. Maximum specific growth rate of utilization : k (mass of NGS/mass
       biomass-day) = "; k
GOTO 100
160 INPUT "  6. Half saturation coefficient : Ks (mg/l) = ";KSN
GOTO 100
170 INPUT "  7. Fractional coefficient : fco = ";FCO
GOTO 100
180 INPUT "  8. Decay coefficient : b (1/day) = ";B
GOTO 100
190 INPUT "  9. Biomass transformation capacity : Tcb (mass of NGS/mass of biomass)
       = "; TCB
GOTO 100
200 INPUT "  10. Initial growth substrate mass : Sg (ug) = ";SG(1)
GOTO 100
210 INPUT "  11. Initial non-growth substrate mass Sng (ug) = "; SN(1)
GOTO 100
220 INPUT "  12. Initial biomass amount : Xo (ug) = "; X(1)
GOTO 100
230 INPUT "  13. Time interval for analysis : delta T (min) = "; dt
GOTO 100

REM          Start calculations

300 MMX = MMX / (24*60)
k = k / (24*60)
B = B / (24*60)

REM          Decide the number of loop.

NL = INT(1200/dt)
CLS
FOR i = 2 TO NL
GS (1) = dt*(-MMX/Y*SG(i-1)*X(i-1)/(KSG*139.015+SG(i-1)))
NS (1) = dt*(TCG*GS(1)-k*X(i-1))*SN(i-1)/(KSN*144.505+SN(i-1))
XC (1) = dt*(Y*(-GS(1))*(1-FCO)-B*X(i-1)+NS(1)/TCB)
GS (2) = dt*(-MMX/Y*(SG(i-1)+GS(1)/2)*(X(i-1)+XC(1)/2)/(KSG*139.015+(SG(i-
              1)+GS(1)/2)))
NS (2) = dt*(TCG*GS(2)-k*(X(i-1)+XC(1)/2))*(SN(i-
              1)+NS(1)/2)/(KSN*144.505+(SN(i-1)+NS(1)/2))
XC (2) = dt*(Y*(-GS(2))*(1-FCO)-B*(X(i-1)+XC(1)/2)+NS(2)/TCB)
GS (3) = dt*(-MMX/Y*(SG(i-1)+GS(2)/2)*(X(i-1)+XC(2)/2)/(KSG*139.015+(SG(i-
              1)+GS(2)/2)))
```

```
NS (3) = dt*(TCG*GS(3)-k*(X(i-1)+XC(2)/2))*(SN(i-
            1)+NS(2)/2)/(KSN*144.505+(SN(i-1)+NS(2)/2))
XC (3) = dt*(Y*(-GS(3))*(1-FCO)-B*(X(i-1)+XC(2)/2)+NS(3)/TCB)
GS (4) = dt*(-MMX/Y*(SG(i-1)+GS(3))*(X(i-1)+XC(3))/(KSG*139.015+(SG(i-
            1)+GS(3))))
NS (4) = dt*(TCG*GS(4)-k*(X(i-1)+XC(3)))*(SN(i-1)+NS(3))/(KSN*144.505+(SN(i-
            1)+NS(3)))
XC (4) = dt*(Y*(-GS(4))*(1-FCO)-B*(X(i-1)+XC(3))+NS(4)/TCB)

REM Calculation of New Step

SG(i) = SG(i-1)+(GS(1)+2*GS(2)+2*GS(3)+GS(4))/6
SN(i) = SN(i-1)+(NS(1)+2*NS(2)+2*NS(3)+NS(4))/6
X(i) = X(i-1)+(XC(1)+2*XC(2)+2*XC(3)+XC(4))/6
NEXT i

REM Open output file.

PRINT :PRINT :PRINT
GOSUB 1000
INPUT       "What file name you want for output data";file$
OPEN file$ FOR OUTPUT AS #1
WRITE #1, "Time(hr)", "GS", "NGS", "Biomass"

FOR j=1 TO 50
KM=j*NL/50
kl=KM*dt
t=kl/60
WRITE #1, t, SG(KM), SN(KM), X(KM)
NEXT j

CLOSE

INPUT       "Do you want to repeat calculation with different parameters"; YN$
IF YN$ <> "Y" AND YN$ <> "y" THEN GOTO 500
MMX = MMX * (24*60)
k = k * (24*60)
B = B * (24*60)
GOTO 100

DATA        15.00,1.28, 3.17, .48, .5, .8, .026, .41, 2.4, 1390.708, 1437.74, 50, 1

500 STOP

END

1000 FOR II = 1 TO 3
        SOUND 500,1:SOUND 400,1
     NEXT II

RETURN
```

LIST OF REFERENCES

AWWA Committee, 1981. An assessment of microbial activity on GAC. Journal AWWA, 73:81-83.

Alvarez, P. J. J. and T. M. Vogel, 1991. Substrate interactions of benzene, toluene, and *para*-xylene during microbial degradation by pure cultures and mixed culture aquifer slurries. Appl. and Env. Microbiol., 57(10) :2981-2985.

Alvarez-Cohen, L. and P. L. McCarty, 1991a. A cometabolic biotransformation model for halogenated aliphatic compounds exhbiting product toxicity. ES & T, 25(8) :1381-1387.

Alvarez-Cohen, L. and P. L. McCarty, 1991b. Product toxicity and cometabolic competitive inhibition modeling of chloroform and trichloroethylene transformation by methanotrophic resting cells. Appl. and Env. Microbiol., 57(4) :1031-1037.

Andrews, G. F. and C. Tien, 1976. The interreaction of bacterial growth adsorption and filtration in carbon columns treating liquid waste. AIChE Symposium Series, 71:164-175.

Andrews, G. F. and C. Tien, 1977. New approach to bacterial kinetics in wastewater. Journal of the Environmental Engineering, 103(6) :1057-1074.

Andrews, G. F., 1979. The expansion of a fluidized bed containing biomass. AIChE Journal, 25:720-723.

Andrews, G. F. and C. Tien, 1981. Bacterial film growth in adsorbent surfaces. AIChE Journal, 27(3) :396-403.

Arvin, E., B. K. Jensen and A. T. Gundersen, 1989. Substrate interactions during aerobic biodegradation of benzene. Appl. and Env. Microbiol., 55(12) :3221-3225.

Ayers, G. W. and R. E. Nuder, 1964. Benzene. New York, NY: John Wiley and Sons.

Bauer, J. E. and D. G. Capone, 1988. Effects of co-occurring aromatic hydrocarbons on degradation of individual polycyclic aromatic hydrocarbons in marine sediment slurries. Appl. and Env. Microbiol., 54(7) :1649-1155.

Benedek, A., 1980. Simultaneous biodegradation and activated carbon adsorption-a mechanistic look. In M. J. McGuire (Eds.), Activated carbon adsorption of organic from the aqueous phase (pp. 273-302). Ann Arbor, MI: Ann Arbor Science Inc.

Bishop, D. F., L. S. Marshall, T. P. O'Farrell, R. B. Dean, B. O'Connor and R. V. Villiers, 1967. Studies on activated carbon treatment. Journal WPCF, 39(2) :188-194.

Chang, H. T., 1985. Mathematical modeling and scanning electron microscopic study of biofilms on adsorptive media . University of Illinois, Urbana-Champaign, IL

Chang, H. T. and B. E. Rittmann, 1987. Mathematical modeling of biofilm on activated carbon. , 21:273-288.

Chang, M.-K., T. C. Voice and C. S. Criddle, 1993. Kinetics of competitive inhibition and cometabolism in the biodegradation of benzene, toluene, and *p*-xylene by two *pseudomonas* isolates. Biotechnology and Bioengineering, 41(11):1057-1065

Characklis, W. G., 1984. Biofilm development: a process analysis. Microbial Adhesion and Aggregation:137-157.

Characklis, W. G., G. A. McFeters, and K. C. Marshall, 1990. Physiological ecology in biofilm systems. In W. G. Characklis and K. C. Marshall (Eds.), Biofilms (pp. 341-394). Wiley Interscience.

Chemical and Engineering News, 1991. Facts and figures. 69(25) :28-33.

Chudyk, W. A. and V. L. Snoeynik, 1984. Bioregeneration of activated carbon saturated with phenol. ES & T, 18(1) :1-5.

Claus, D. and N. J. Walker, 1964. The decomposition of toluene by soil bacteria. Journal Gen. Microbiol., 36:107-122.

Cooney, D. and E. Lightfoot, 1966. Multicomponent fixed-bed sorption of interfering solutes. , 5(1) :25-32.

Cooney, D. and F. Strusi, 1972. Analytical description of fixed-bed sorption of two langmuir solutes under nonequilibrium conditions. Ind. Eng. Chem. Fundam, 11(1) :123-126.

Criddle, C. S., 1993. The kinetics of cometabolism. Biotechnology and Bioengineering, 41(11):1048-1056

Criddle, C. S., J. T. DeWitt and P. L. McCarty, 1990. Reductive dehalogenation of carbon tetrachloride by *escherichia coli*.. Appl. and Env. Microbiol., 56(11) :3247-3254.

Crittenden, J., P. Luft, D. Hand, and L. Orvitz, 1985. Prediction of Multicomponent Absorption Equilibria Using Ideal Absorbed Solution Theory, ES & T, 19(11) : 1037-1043

Dagley, S., P. J. Chapman, D. T. Gibson and J. M. Wood, 1964. Degradation of the benzene nucleus by bacteria. Nature, 202(4934) :775-778.

Dagley, S., W. C. Evans and D. W. Ribbons, 1960. New pathways in the oxidative metabolism of aromatic compounds by micro-organisms. Nature, 188(4750) :560-566.

Dalton, H. and D. I. Stirling, 1982. Co-metabolism. Philos. Trans. R. Soc. London Ser. B, 297:481-496.

Davey, J. F. and D. T. Gibson, 1974. Bacterial metabolism of *para*- and *meta*-xylene: oxidation of a methyl substituent. Journal of Bacteriology, 119(3) :923-929.

Davies, J. I. and W. C. Evans, 1964. Oxidative metabolism of naphthalene by soil pseudomonads. Biochemical Journal, 91:251-261.

Davis, R. S., F. E. Hossler and . W. Stone, 1968. Metabolism of *p*- and *m*-Xylene by species of *pseudomonas*. Canadian Journal of Microbiology, 14:1005-1009.

Dean, B. J., 1985. Recent findings on the genetic toxicology of benzene, toluene, xylenes and phenols. Mutation Research, 154:153-181.

DNR, 1989. Michigan sites of environmental contamination ACT 307. The State of Michigan.

Deshpande, S. D., T. Chakrabartl and P. V. R. Subrahmanyam, 1987. Mixed-substrate utilization by acclimated activated sludge in batch and continuous-flow stirred tank reactors. ES & T, 21(10) :1003-1008.

DeWalle, F. B. and E. S. K. Chian, 1977. Biological regeneration of powdered activated carbon added to activated sludge units. Water Research, 11:439-446.

Digiano, F. A., 1981. Influence of biological activity on GAC performance. In W. W. Eckenfelder (Eds.), Proceedings: Conference on applications of adsorption in wastewater treatment (pp. 285-305). Nashville, TN: Enviro Press

Dobrevski, I. and L. Zvezdova, 1989. Biological regeneration of activated carbon. Water Science Technology, 21(1) :141-143.

Evans, W. C., 1963. The microbiological degradation of aromatic compounds. Journal Gen. Mirobiol., 32:177-184.

Evans, P. J., D. T. Mang and L. Y. Young, 1991. Degradation of toluene and *m*-xylene and transformation of *o*-xylene by denitrifying enrichment cultures. Appl. and Env. Microbiol., 57(2) :450-454.

Ford, D. L. and M. A. Buercklin, 1972. The interrelationship of biological and carbon adsorption systems for the treatment of refinery and petrochemical wastewaters. In S. H. Jenkins (Ed.), Advances in Water pollution Research, Pergamon Press.

Foster, J. W., 1962. Hydrocarbons as substrates for microorganisms. Journal Microbiol. Serol, 28:241-274.

Franklin, F. C. H., P. R. Lehbrach, R. Lurz, B. Rueckert, M. Bagdasarian and K. N. Timmis, 1983. localization and functional analysis of transposon mutations in regulatory genes of the tol catabolic pathway. Journal of Bacteriology, 154(2) :676-685.

Fritz, W. and E. Schlunder, 1980. Competitive adsorption of two dissolved organics onto activated carbon - I. Chemical Engineering Science, 36:721-730.

Fritz, W., W. Merk and E. Schlunder, 1980. Competitive adsorption of two dissolved organics onto activated carbon - II. Chemical Engineering Science, 36:731-741.

Gardner, D. A., M. T. Suidan and H. A. Kobayashi, 1988. Role of GAC activity and particle size during the fluidized-bed anaerobic treatment of refinery sour water stripper bottoms. Journal WPCF, 60(4) :505-513.

Gariepy, R. L. and I. Zwiebel, 1971. Adsorption of binary mixtures in fixed beds. AIChE Symp. Series, 67:17-24.

Gibson, D. T., J. R. Koch and R. E. Kallio, 1968. Oxidative degredation of aromatic hydrocarbons by microorganisms.I.Enzymatic formation of catechol from benzene. Biochemistry, 7(7) :2653-2662.

Gibson, D. T., J. R. Kock, C. L. Schuld and R. E. Kallio, 1968. Oxidative degradation of aromatic hydrocarbons by microorganisms. II. Metabolism of halogenated aromatic hydrocarbon. Biochemistry, 7(11) :3795-3802.

Gibson, D. T., 1968. Microbial degradation of aromatic compounds. Science, 161(3846) :1093-1097.

Gibson, D. T., M. Hensley, H. Yoshioka and T. J. Mabry, 1970. Formation of (+)-cis-2,3-dihydroxy-1-methylcyclohexa-4,6-diene from toluene by *pseudomonas putida*. Biochemistry, 9(7) :1626-1630.

Gibson, D. T., V. Mahadevan and J. F. Davey, 1974. Bacterial metabolism of *para*- and *meta*-xylene: Oxidation of the aromatic ring. Journal of Bacteriology, 119(3) :930-936.

Goldsmith Jr., C. D. and R. K. Balderson, 1988. Biodegradation and growth kinetics of enrichment isolates on benzene, toluene and xylene. Water Science Technology, 20(11) :505-507.

Gossett, J. M., 1985. Anaerobic degradation of C1 and C2 chlorinated hydrocarbons. Tyndall Air Force base: Air Force Engineering and Service Center.

Grady, C. P. L., Jr. and H. C. Lim, 1980. Biological wastewater treatment-theory and application. New York: Marcel Dekker, Inc.

Grady, C. P. L., Jr., 1985. Biodegradation : Its measurement and microbiological basis. Biotechnology and Bioengineering, 27:660-674.

Grady, C. P. L., Jr., 1990. Biodegradation of toxic organism: Status and potential. Journal of Environmental Engineering, 116(5) :805-828.

Hayaishi, O. and M. Nozaki, 1969. Nature and mechanism of oxygenases. Science, 164(3878) :389-396.

Horvath, R. S., 1972. Microbial co-metabolism and the degradation of organic compounds in nature. Bacteriological review, 36(2) :146-155.

Hou, C. T. Microbial transformations of important industrial hydrocarbons. In J. P. Rosazza (Eds.), Microbial transformation of bioactive compounds (pp. 82-107). Boca Raton, Florida: CRC press.

Howard, P. H. and P. R. Durkin, 1974. Sources of contamination, ambient levels, and fate of benzene in the environment. USEPA. EPA 560/5-75-005.

Jain, K. S. and V. L. Snoeyink, 1973. Adsorption from bisolute systems on activated carbon. Journal WPCF, 45(12) :2463-2479.

Jamison, W. V., R. L. Raymond and J. O. Hudson, 1969. Microbial hydrocarbon co-oxidation - III. Isolation and characterization of an α, α' - dimethyl - *cis, cis* -muconic acid producing strain of *Nocardia corallina*. Appl. Microbiol., 17:853-856.

Janke, D. and W. Ihn, 1989. Cometabolic turnover of aniline, phenol and some of their monochlorinated derivatives by the rhodococcus mutant strain AM 144. Archies of Microbiology, 152:347-352.

Kidnay, A. J. and A. L. Myers, 1966. A simplified method for the prediction of multicomponent adsorption equilibria from single gas isotherms. AIChE Journal, 12(5) :981-986.

Kim, R., E. S. K. Chian, W. H. Cross and S. S. Cheng, 1986. Adsorption, desorption, and bioregeneration in an anaerobic, granular activated carbon reactor for the removal of phenol. Journal WPCF, 58(1) :35-40.

Kincannon, D. F., E. L. Stover, V. Nichols and D. Medley, 1983a. Removal mechanisms for toxic priority pollutants. Journal WPCF, 55(2) :157-163.

Kincannon, D. F., A. Weinert, R. Padorr and E. L. Stover, 1983b. Prediction treatability of multiple organic priority pollutant wasterwaters from single-pollutant treatability studies. :641-650.

Kirk, R. E. and D. Othmer, 1963. Kirk-Othmer encyclopedia of chemical technology (2nd ed.). New York: John Wiley and Sons.

Kitagawa, M., 1956. Studies on the oxidation mechanism of methyl group. Journal Biochemistry, 43(4) :553-563.

Law, A. T. and D. K. Button, 1977. Multiple-carbon-source-limited growth kinetics of a marine coryneform bacterium. Journal of Bacteriology, 129(1) :115-123.

Leadbetter, E. R. and J. W. Foster, 1959. Oxidation products formed from gaseous alkanes by the bacterium *pseudomonas methanica*. Arch. Biochem. Biophys., 82:491-492.

Li, Y. L. and F. A. Digiano, 1983. Availability of sorbed substrate for microbial degradation on granular activated carbon. Journal WPCF, 55:392-399.

Lowry, J. D. and C. E. Burkhead, 1980. The role of adsorption in biologically extended activated carbon columns. Journal WPCF, 52:389-398.

Machado, R. J. and C. P. Leslie Grady Jr., 1989. Dual substrate removal by an axenic bacterial culture. Biotechnology and Bioengineering, 33:327-337.

Maqsood, R., 1977. Low-temperature organic removal and denitrification in activated carbon columns. Journal WPCF, 49:2107-2117.

Marr, E. K. and R. W. Stone, 1961. The bacterial oxidation of benzene. Journal Bacteriol., 81:425.

Merk, W., W. Fritz and E. U. Schlunder, 1980. Competitive adsorption of two dissolved organics onto activated carbon - III. Chemical Engineering Science, 36:743-757.

Meyer, J. S., M. D. Marcus and H. L. Bergman, 1984. Inhibitory interactions of aromatic organics during microbial degradation. Environmental Toxicology and Chemistry, 3:583-587.

Miller, G. W. and R. G. Rice, 1978. European water treatment practices - the promise of Biological Activated Carbon. Civil Engineering, 48(2) :81-83.

Monod, J., 1942. Recherches sur la croissance des cultures bacteriennes. Paris: Hermann and Cie, Paris.

Monod, J., 1949. The growth of bacterial cultures. Annual Review of Microbiology, 3:371-394.

Nayar, S. C. and N. D. Sylvester, 1979. Control of phenol in biological reactors by addition of powdered activated carbon. Water Research, 13:201-205.

Nelson, M. J. K., S. O. Montogomery, W. R. Mahaffey and P. H. Pritchard, 1987. Biodegradation of trichloroethylene and involvement of an aromatic biodegradative pathway. Appl. and Env. Microbiol., 53(5) :949-954.

Nelson, M. J. K., S. O. Montgomery and P. H. Pritchard, 1988. Trichloroethylene metabolism by microorganisms that degrade aromatic compounds. Appl. and Env. Microbiol., 54(2) :604-606.

Nozaka, J. and M. Kusunose, 1968. Metabolism of hydrocarbons in microorganisms part I. Oxidation of *p*-xylene and toluene by cell-free enzyme preparations of *pseudomonas aeruginosa*. Agr. Biol. Chem., 32(8) :1033-1039.

Nozaka, J. and M. Kusunose, 1969. Metabolism of hydrocarbons in microorganisms Part II. Degradation of toluene by cell-free extracts of *Pseudomonas mildenbergii*. Agr. Biol. Chem., 33(6) :962-964.

Olmstead, K. P., 1989. Microbial interference with the adsorption of target organic contaminants by granular activated carbon. University of Michigan, Ann Arbor, MI.

Omori, T., S. . Horiguchi and K. Yamada, 1967. Studies on the utilization of hydrocarbons by Microorganisms Part X. Screening of Aromatic Hydrocarbon-Assimilating Microorganisms and *p*-Toluic Acid Formation from *p*-Xylene. Agr. Biol. Chem., 31(11) :1337-1342.

Omori, T. and K. Yamada, 1969. Studies on the utilization of hydrocarbons by microorganisms Part XIII. Oxidation of *m*-xylene and Pseudocumene by *Pseudomonas aeruginosa*. Agr. Biol. Chem., 33(7) :979-985.

Omori, T. and K. Yamada, 1970a. Studies on the utilization of hydrocarbons by microorganisms Part XVI. Detection of metabolic intermediates of xylene and *pseudomonas*. Agri. Biol. Chem., 34(5) :659-663.

Omori, T. and K. Yamada, 1970b. Studies on the Utilization of hydrocarbons by microorganisms Part XVII. Metabolism of *p*-xylene and related compounds. Agr. Biol. Chem., 34(5) :664-669.

Peel, R. G. and A. Benedek, 1983. Biodegradation and adsorption within activated carbon adsorbers. Journal WPCF, 55:1168-1173.

Perrotti, A. E. and C. A. Rodman, 1973. Enhancement of biological waste treatment by activated carbon. Chemical Engineering Progress, 69:63-64.

Perrotti, A. E. and C. A. Rodman, 1974. Factors involved with biological regeneration of activated carbon. AIChE Symp. Series, 70(144) :317-325.

Pirbazari, M., T. C. Voice and W. J. Weber, Jr., 1990. Evaluation of biofilm development on various natural and synthetic media. Hazardous Waste & Hazardous Materials, 7(3) :239-250.

Ribbons, D. W. and R. W. Eaton, 1982. Chemical transformations of aromatic hydrocarbons that support the growth of microorganisms. In A. M. Chakrabarty (Eds.), Biodegradation and detoxification of environmental pollutants (pp. 59-84). Boca Raton, Florida: CRC Press, Inc.

Richards, D. J. and W. K. Shieh, 1986. Biological fate of organic priority pollutants in the aquatic environment. Water Research, 20(9) :1077-1090.

Rice, R. G. and C. M. Robson., 1982. Biological Activated Carbon - enhanced aerobic biological activity in GAC systems. Ann Arbor: Ann Arbor Sciene Publisher

Ridgway, H. F., J. Safarik, D. Phipps and D. Clark, 1990. Identification and catabolic activity of well-derived gasoline-degrading bacteria from a contaminated aquifer. Appl. and Env. Microbiol., 56(11) :3565-3575.

Rodman, C. A., E. L. Shunney and A. E. Perrotti, 1978. Biological regeneration of activated carbon. In P. N. Cheremisinoff and F. Ellerbusch (Eds.), Carbon adsorption (pp. 449-483). Ann Arbor, MI: Ann Arbor Sciene Publisher

Saez, P. B. and B. E. Rittmann, 1991. Biodegradation kinetics of 4-chlorophenol, an inhibitory co-metabolite. Journal WPCF, 63(6) :838-847.

Schmidt, S. K., S. Simkins and M. Alexander, 1985. Models for the kinetics of biodegradation of organic compounds not supporting growth. Appl. and Env. Microbiol., 50(2) :323-331.

Schultz, J. R. and T. M. Keinath, 1984. Organic removal mechanisms in biophysical treatment systems. Water Science Technology, 17:1043-1054.

Sharma, B. and R. C. Ahlert, 1977. Nitrification and nitrogen removal. Water Research, 11:897-925.

Sohngen, N. L., 1913. Centr. Bakteriol. Parasitenk. Abt. II, 37:595.

Sontheimer, H., E. Heilker, M. R. Jekel, H. Nolte and F. H. Vollmer, 1978. The mulheim process. Journal AWWA, 79:393-396.

Sontheimer, H., 1979. Applying oxidation and adsorption techniques: A summary of progress. Journal AWWA, 79(11) :612-617.

Speitel, G. E., Jr., 1985. Bioregeneration of granular activated carbon : quantification at low substrate concentrations by radiochemical techniques. University of North Carolina, Chaple Hill, NC

Speitel, G. E., Jr. and F. A. DiGiano, 1987. The Bioregeneration of GAC Used to Treat Micropollutants. Journal AWWA, 87:64-73.

Speitel, G. E., Jr., K. Dovantzis and F. A. Digiano, 1987. Mathematical modeling of bioregeneration in GAC Columns. Journal of Environmental Engineering, 113(32):48-56.

Speitel, G. E., Jr., C. J. Lu and M. H. Turakhia, 1988. Biodegradation of synthetic organic chemicals in GAC beds. Wat. Sci. Tech., 20(11/12) :463-465.

Speitel, G. E., Jr., and F. A. DiGiano, 1988. Determination of microbial kinetic coefficients through measurement of initial rates by radiochemical techniques. Water Research, 22(4) :829-835.

Speitel, G. E., Jr., C. J. Lu, M. Turakhia and X.-J. Zhu, 1989. Biodegradation of trace concentration of substituted phenol in granular activated carbon columns. Environ. Sci. & Tech., 23(1) :68-74.

Speitel, G. E., Jr., C. J. Lu, X. J. Zhu and M. H. Turakhia, 1989. Biodegradation and adsorption of a bisolute mixture in GAC Columns. Journal WPCF, 61(2) :221-229.

Storer, F. F. and J. Gaudy A. F., 1969. Computational analysis of transient response to quantitative shock loadings of heterogeneous populations in continuous culture. Environ. Sci. & Tech., 3(2) :143-149.

Stormer, K., 1908. Zentralbl. Bakteriol. Parasitenk. Infek., 20:282.

Strand, S. E., M. D. Bjelland and H. D. Stensel, 1990. Kinetics of chlorinated hydrocarbon degradation by suspended cultures of methane-oxidizing bacteria. Journal WPCF, 62(2) :124-129.

Suidan, M. T., P. Fox and J. T. Pfeffer, 1978. Water Science and Technology, 19:229-236.

Summers, R. S., B. Haist, J. Koekler, J. Ritz, G. Zimmer and H. Sontheimer, 1989. The influence of background organic matter on GAC adsorption. Journal AWWA, 89(5) :66-74.

Sundstrom, D. W. and H. E. Klei, 1979. Wastewater Treatment. New Jersey: Prentice-Hall.

Tabak, H. H., S. A. Quave, C. I. Mashni and E. F. Barth, 1981. Biodegradability studies with organic priority pollutant compounds. Journal WPCF, 53(10) :1503-1518.

Thomas, W. J. and J. L. Lombardi, 1971. Binary adsorption of benzene-toluene mixtures. Trans. Instn Chem. Engrs., 49:240-250.

Tien, C. and S. C. Wang, 1982. Dynamics of adsorption columns with bacterial growth outside adsorbents. The Canadian Journal of Chemical Engineering, 60:363-376.

Trulear, M. G. and W. G. Characklis, 1982. Dynamics of biofilm processes. Journal WPCF, 54(9) :1288.

USEPA, 1976. Health effects of benzene: a review, part VI: Occupational exposure to benzene. Federal register, 42(103) :27452-27459 EPA 560/5-76-003.

Van Der Kooij, D., 1976a. Process during biological oxidation in filters. In Sentheiner (Ed.), Translation of report on special problem of water technology, 9 (pp. 689-699). EPA 600/9 - 76 - 030.

Van Der Kooij, D., 1976b. Some investigations into the presence and behaviour of bacteria in activated carbon filters. In Sentheiner (Ed.), Translations of Reports on Special Problems of Water Technology, 9 (pp. 348-354).

Verschueren, K., 1983. Handbook of environmental data on organic chemicals. New York: Van Nostrand Reinhold Company.

Voice, T. C., D. Pak, X. Zhao, J. Shi and R. Hickey, 1992. Biological activated carbon in fluidized bed reactor for the treatment of groundwater contaminated with volatile aromatic hydrocarbons. Water Research, 26(10) :1389-1401.

Weber, W. J., Jr., C. B. Hopkins and R. Bloom Jr., 1970. Physico-chemical treatment of wastewater. Journal WPCF, 42:83-99.

Weber, W. J., Jr., C. B. Hopkins and R. Bloom Jr., 1971. Expanded-bed adsorption for treatment of sewage effluents. Water-1970:541-553.

Weber, W. J., Jr., L. D. Friedman and R. Bloom Jr., 1972. Biologically-extended physicochemical treatment. :.

Wilson, J. T. and B. H. Wilson, 1985. Biotransformation of trichloroethylene in soil. Appl. Env. Microbiol., 49(1) :242-243.

Ying, W. C. and W. J. Weber Jr., 1979. Bio-physicochemical adsorption systems model. Journal WPCF, 51(11) :2661-2677.

Yoon, H., G. . Klinzing and H. W. Blanch, 1977. Competition for mixed substrates by microbial populations. Biotechnology and Bioengineering, 19:1193-1210.

Zylstra, G. J., S. Chauhan and D. T. Gibson Degradation of chlorinated biphenyls by *escherichia coli* containing cloned genes of the *pseudomonas putida* f1 toluene catabolic pathway. In Proceeding of the15th annual research symposium on Land Disposal, Remedial Action, Incineration, and Treatment of Hazardous Wastes. USEPA.