

# **A groundwater velocity and dissolved oxygen on bioremediation of gasoline-contaminated sandy aquifers**

**Niaz Mohammed**

Civil Engineering

May 1995

## **Abstract**

Benzene, Toluene and Xylene (BTX) compounds are the main constituents of gasoline and their presence in groundwater is common because of hydrocarbon spill and leakage of storage tanks. These compounds are relatively highly soluble and mobile in the subsurface and are toxic even at very low concentrations. Bioremediation is the most widely used technique among all the currently employed methods for treating BTX contaminated soil and groundwater. Various factors affecting bioremediation, such as dissolved oxygen, nutrient, temperature, pH, etc. have been well studied in the laboratory soil columns and microcosms. The effect of soil permeability (as groundwater velocity) has been investigated in this study using a pilot scale sand tank model. The effect of dissolved oxygen (DO) and contaminant concentration are also included in the study using a pilot scale sand tank model. Numerical models have been developed using finite difference and orthogonal collocation to simulate one dimensional transport with time-dependent pore water velocity. The modeling process includes sorption given by linear isotherm and biodegradation given by a variety of kinetics such as first-order, zero-order, Monod, Michaelis-Menten, Haldane and many other inhibitory and non-inhibitory kinetics. A variety of initial and boundary conditions such as Dirichlet's, Neuman's, mixed, decaying, etc. have been modeled. Three models (first-order and/or zero-order, non growth associated Monod, and Monod) have been inverted using a Gauss-Marquardt-Levenberg algorithm to assess the transport parameter, such as retardation constant ( $R$ ), first-order rate constant ( $\mu$ ) and zero-order rate constant ( $\gamma$ ). A  $3 (2^2)$  factorial experiment has been conducted to study three factors, groundwater velocity, BTX concentration and dissolved oxygen (DO). Observed concentration data collected from the sand tank model have been used for estimating the transport parameters. The data has been found to fit well to first-order/zero-order as well as to Monod model. Groundwater velocity has been found to be the most significant factor governing the rate of biodegradation (determined from the first-order rate constant) of BTX compounds. Dissolved oxygen and BTX concentration have also been found to be significant factors.

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# **Effect of Groundwater Velocity and Dissolved Oxygen on Bioremediation of Gasoline-Contaminated Sandy Aquifers**

BY

**NIAZ MOHAMMED**

A Dissertation Presented to the  
FACULTY OF THE COLLEGE OF GRADUATE STUDIES  
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In Partial Fulfillment of the  
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**DOCTOR OF PHILOSOPHY**  
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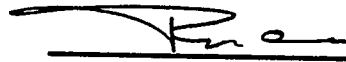
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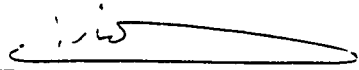
  
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*Dedicated*  
*to my beloved parents, wife and son*

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## NOTATIONS

<b>A, B</b>	Collocation matrices
<b>b</b>	Decay coefficient of the Monod kinetics
<b>C</b>	Volume averaged solute (BTX) concentration (mg/l)
<b>D</b>	Coefficient of mechanical dispersion
<b>D<sub>m</sub></b>	Coefficient of molecular dispersion (m)
<b>e</b>	Void ratio
<b>h</b>	Finite difference grid interval (m)
<b>L</b>	Length of the sand tank
<b>k</b>	Specific substrate utilization rate (mg-substrate/mg-cells/day)
<b>k<sub>m</sub></b>	Maximum specific substrate utilization rate (mg/l)
<b>K<sub>d</sub></b>	Coefficient of linear isotherm
<b>K<sub>s</sub></b>	Half saturation coefficient (mg-substrate/l)
<b>M</b>	Number of internal collocation matrix
<b>M<sub>2</sub></b>	Total number of collocation matrix including the boundaries ( $M_2 = M + 2$ )
<b>Q</b>	Darcy flow
<b>q</b>	Darcy flow per unit area
<b>R</b>	Retardation constant
<b>t</b>	Time (day)
<b>U</b>	Numerical solution of the concentration (C)
<b>v</b>	Linear pore water velocity
<b>X</b>	Microbial concentration (both attached and suspended, mg/l)
<b>x</b>	Cartesian coordinate
<b>Y</b>	Yield coefficient (mg cells produced/mg substrate consumed)
<b>Z</b>	Dimensionless x ( $Z = x/L$ ), also collocation matrix
<b>Φ</b>	Porosity
<b>α</b>	Coefficient of dispersivity
<b>γ</b>	Zero order rate constant
<b>γ<sub>w</sub></b>	Zero order rate constant in the liquid phase
<b>γ<sub>s</sub></b>	Zero order rate constant in the solid phase
<b>λ</b>	Solute source decay coefficient
<b>μ</b>	First order rate constant
<b>μ<sub>w</sub></b>	First order rate constant in the liquid phase
<b>μ</b>	First order rate constant in the solid phase
<b>ρ<sub>b</sub></b>	Bulk density of the porous medium

## **ABSTRACT**

**Full Name : Niaz Mohammed**

**Title of Study : EFFECT OF GROUNDWATER VELOCITY AND DISSOLVED OXYGEN ON  
BIOREMEDIATION OF GASOLINE-CONTAMINATED SANDY AQUIFERS**

**Major Field : Civil Engineering (Water Resources and Environmental)**

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Benzene, Toluene and Xylene (BTX) compounds are the main constituents of gasoline and their presence in groundwater is common because of hydrocarbon spill and leakage of storage tanks. These compounds are relatively highly soluble and mobile in the subsurface and are toxic even at very low concentrations. Bioremediation is the most widely used technique among all the currently employed methods for treating BTX contaminated soil and groundwater. Various factors affecting bioremediation, such as dissolved oxygen, nutrient, temperature, pH, etc. have been well studied in the laboratory soil columns and microcosms. The effect of soil permeability (as groundwater velocity) has been investigated in this study using a pilot scale sand tank model. The effect of dissolved oxygen (DO) and contaminant concentration are also included in the study. Numerical models have been developed using finite difference and orthogonal collocation to simulate one dimensional transport with time-dependent pore water velocity. The modeling process includes sorption given by linear isotherm and biodegradation given by a variety of kinetics such as first-order, zero-order, Monod, Michalis-Menten, Haldane and many other inhibitory and non-inhibitory kinetics. A variety of initial and boundary conditions such as Dirichlet's, Neuman's, mixed, decaying, etc. have been modeled. Three models (first-order and/or zero-order, non growth associated Monod, and Monod) have been inverted using a Gauss-Marquardt-Levenberg algorithm to assess the transport parameter, such as retardation constant (R), first-order rate constant ( $\mu$ ) and zero-order rate constant ( $\gamma$ ). A  $3(2^2)$  factorial experiment has been conducted to study three factors, groundwater velocity, BTX concentration and dissolved oxygen (DO). Observed concentration data collected from the sand tank model have been used for estimating the transport parameters. The data has been found to fit well to first-order/zero-order as well as to Monod model. Groundwater velocity has been found to be the most significant factor governing the rate of biodegradation (determined from the first-order rate constant) of BTX compounds. Dissolved oxygen and BTX concentration have also been found to be significant factors.

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## خلاصة الرسالة

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عنوان الدراسة : أثر سرعة المياه الجوفية والأكسجين الذائب على المعالجة الحيوية لمكامن المياه الرملية الملوثة بالجازولين  
التخصص : هندسة هندسة ( هندسة موارد المياه والبيئة )

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تعتبر مركبات البنزين والتولوين والزيلين المكون الرئيسي للجازولين كما أن وجود هذه المركبات في المياه الجوفية قد أصبح أمراً شائعاً وذلك بسبب تدفق وتسرب المركبات الهيدروكربونية من أهمها صهاريج التخزين ، وهذه المركبات قابلة للذوبان والتحرك بصورة عالية نسبياً في الطبقات التحت سطحية ، كما أنها سامة حتى إذا وجدت بتراكيز منخفضة جداً من بين التقنيات المستخدمة لعلاج التربة والمياه الجوفية الملوثة بمركبات البنزين والتولوين والزيلين ، فإن المعالجة الحيوية تعتبر أكثر التقنيات إستعمالاً لهذا الغرض . وهناك عدة عوامل تؤثر في عملية المعالجة الحيوية ومن جملة هذه العوامل تركيز الأكسجين الذائب ، المواد المغذية والحرارة والرقم الهيدروجيني . وقد درست هذه العوامل بدقة في المعمل بإستعمال (أعمدة من التربة) والميكروكوزمز . وقد قامت هذه الدراسة بدراسة أثر نفاذية التربة (كسرعة المياه الجوفية) وذلك بإستخدام نموذج تجريبي لصهريج من الرمل وقد تضمنت الدراسة أثر الأكسجين الذائب وتركيز الملوث . وكما قامت الدراسة بتطوير نموذج رياضي بإستخدام طرق الحل العددية وذلك لمحاكاة الإنتقال ذو البعد الواحد مع السرعة الزمانية للماء عبر الثقوب . وقد إشتملت عملية النمذجة على نموذج إمتصاص بإستعمال معادلة خطية لتساوي درجة الحرارة وكذلك على نموذج التحلل الحيوي بإستعمال عدة نماذج للتغيرات الحيوية مثل نماذج من الدرجة الأولى ومونود وميكاليس - منتن وهالدين وغيره من النماذج المانعة وغير المانعة . وقد تضمنت عملية النمذجة عدة من الحالات المبدئية والحدودية مثل دير يشلت ونيومان وغيرها . وقد قامت الدراسة بقلب ثلاث نماذج بإستعمال لوغاريتم جوس - ماركارد - ليفينبرنج وذلك لتقييم عوامل الإنتقال مثل ثابت التعريف (R) وثابت معدل الدرجة الأولى (M) وثابت معدل الدرجة الصغرى 8 ( ) . وقد قامت الدراسة بدراسة ثلاث عوامل هي سرعة المياه الجوفية وتراكيز مركبات البنزين والتولوين والزيلين وتركيز الأكسجين المذاب حيث إستعملت معلومات التراكيز الملاحظة والمجمعة من خزان الرمل لتقدير عوامل النقل . وقد وجد بأن تلك المعلومات المجمعة تتماشى بصورة جيدة مع نماذج الدرجة الأولى والدرجة الصغرى وكذلك نموذج مونود . وكما وجد بأن سرعة المياه الجوفية تعد العامل المهم الذي يتحكم في معدل التحلل الحيوي للمركبات المذكورة ، كما وجد بأن تركيز الأكسجين المذاب يلعب دوراً مهماً في ذلك .

درجة الدكتوراه

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## Chapter 1

# INTRODUCTION

# 1 INTRODUCTION

## 1.1 Problem Statement

### *1.1.1 Common Organic Pollutants*

Groundwater contamination by organic compounds represents a serious public health problem. Sixty five classes of such compounds are considered hazardous and more than 100 organic compounds have been designated by the U. S. Environmental Protection Agency (EPA) as priority pollutants [Robinson et al.; 1990; Pollution Engg., 1989]. At least 33 toxic organic chemicals have been found in drinking water wells from 40 states in United States [Robinson et al., 1990].

Organics treated with bioremediation can be classified into the following groups: (1) Gasoline and its constituents notably benzene, toluene, ethylbenzene and xylenes (BTEX) and other monoaromatic hydrocarbons; (2) Polynuclear aromatic hydrocarbons (PAHs) such as naphthalene, anthracene, etc.; (3) Phenols including chlorinated phenols, (CP, TCP, PCP) and other pesticides, (4) Chlorinated aliphatic hydrocarbons (CAHs) including trichloroethylene (TCE), trichloroethane (TCA), pentachloroethylene (PCE), dichloroethane (DCA), vinyl chloride (VC), chloroform, carbon tetrachloride and other synthetics, and (5) Other organics such as alcohols, aldehydes, dioxins, PCBs, DDT, nitrotoluenes, and so on.

Many of the monoaromatic hydrocarbons are soluble components of gasoline (BTEX compounds) and result because of hydrocarbon spill or leakage. Benzene, Toluene and Xylene (BTX) compounds are the main constituents of gasoline and their presence in groundwater is most common among other organic pollutants [Lee et al., 1988; Wilson et

al., 1986]. These compounds are relatively highly soluble and mobile in the subsurface and they are toxic at very low concentrations. Benzene has a very low standard in drinking water of 5 µg/L. The state of Virginia Water Control Board established a total BTEX concentration of less than 1 ppm as an allowable limit in the groundwater. The  $10^{-6}$  risk of cancer for benzene is 0.67 µg/L, and toluene is 1 µg/L.

Groundwater contamination by chlorinated aliphatic hydrocarbons (CAHs), such as PCE, TCE, etc. had resulted from their widespread use and disposal in the environment, and also from accidents and leaks at chemical disposal sites. Trichloroethylene (TCE) has been used widely for about 50 years as an industrial solvent, in metal processing, electronics, printing, pulp and paper, and textiles. Tetrachloroethylene (PCE), also known as perchloroethylene is used as a solvent for chlorinated rubber, in degreasing, as fumigant, in dry-cleaning, as insecticides, weed killer and in manufacturing of rubber, bleach, paints, varnish, dust remover, etc. TCE and PCE have been assigned a maximum contaminant level (MCL) of 5 µg/L each. Chlorinated aliphatic hydrocarbons are the most pervasive contaminants and their prevalence has been reported by many authors including Hopkins et al. [1993], Roberts et al. [1990], Speital and Alley [1991]. In the group of the chlorinated aliphatic solvent, vinyl chloride (VC) has been identified as a carcinogenic agent and PCE, TCE, VC have been identified as possible carcinogens [Yeh and Kastnberg, 1991].

Phenolic compounds and polynuclear aromatic hydrocarbons have contaminated several places in United States [Ehrlich et al., 1982; Mueller et al., 1991, US. EPA, 1990; Wilson and Jones, 1992]. Phenol and its chlorinated derivatives 2-CP, 2,4-DCP, 2,4,6-TCP, TCP are the common phenolic contaminants. TCP is used in wood and glue preservatives, in textile, as a defoliant and disinfectant. PCP is used in pesticides, herbicides, algacides, fungicide, and wood preservative. Although phenolic compounds

are not carcinogens, humans exposed to phenols in water at concentration as high as 1130 mg/L exhibited a significant increase in cases of diarrhea, mouth sores, dark urine, and burning of the mouth [US. EPA, 1980]. PAH are ubiquitous in the environment and are found at high concentrations in many industrial sites, particularly those associated with the petroleum, gas production and wood preservative industries [Wilson and Jones, 1992]. Some PAH compounds such as anthracene, benzo(a)pyrene, and phenanthrene are carcinogens and mutagens [World Health Organization, 1983].

### ***1.1.2 Sources of Organic Contamination***

There are myriads of organic, inorganic, biological and nuclear sources of groundwater contamination. These sources are given by Nielsen [1989]. Organic contaminants enter into groundwater mainly from the following sources:

- leaking underground tanks,
- accidental spill of petroleum products,
- tanker spills and leaks from petroleum refinery and bulk storage facilities.
- petroleum pipeline breaks,
- septic tanks and cesspools,
- oil and gas well drilling operations,
- subsurface waste injection wells,
- land application of sludge,
- application of herbicides and insecticides to agricultural land,
- solid waste (sanitary) landfill, and
- hazardous waste landfills and surface impoundments.

Leaking underground storage tanks (USTs) are a major source of groundwater contamination by petroleum hydrocarbons. Approximately 75,000 to 100,000 tanks out of total 1.4 million in U. S. A. are leaking [Hutchins et al., 1991]. There are approximately 90,000 confirmed releases only for the two years (1989-1990) [OUST, 1990]. The EPA estimates that 35% of the existing USTs in the United States are not liquid tight and are leaking [Frankenberger, 1991]. However this figure has been contested by the American Petroleum Institute, which reports that a more realistic number is 2%. Waste materials released from industry and agriculture are responsible for considerable contamination of soil and water. In the United States, there are approximately 14,000 industrial sites producing about 265 million tons of hazardous waste annually [Levin and Gealt, 1993]. The accidental oil spills from tanker and storage facilities have become a global problem. The *Amoco Cadiz* spill of 220,000 tons of oil happened in 1978 along the Brittany Coast [Swannel & Head, 1994]. The *Exxon Valdez* oil spill [Atlas, 1991; Bragg et al., 1994; Pritchard, 1991; Pritchard and Costa, 1991] of approximately 200,000 barrels of crude oil in the Prince William Sound, Alaska in March 1989 resulted in contamination of about 2,000 km of Rocky Intertidal Shorelines. Since January, 1991, the world's attention has focused on the water in the Persian Gulf. The deliberate dumping of oil order by Saddam Hussein in the Persian Gulf has far exceeded the horrible Exxon Valdez (USA) Spill in notoriety [Keeler, 1991]. Gulf oil spill has been estimated to be 40 times larger than the *Exxon Valdez* spill of Alaska [Koons and Johns, 1992]. More than 8,000,000 barrels of crude oil spilled over 640 km of Saudi coastline [Tawfiq and Olsen, 1993]. Remediation of the contaminated aquifers usually require costly treatment techniques. In some cases, the aquifer must be abandoned in favor of alternate water supplies.

## 1.2 Remediation Technologies

Traditional technologies for remediation of contaminated groundwater have relied heavily on pump-and-treat technologies. A review of Superfund Records of decision (RODs) indicates that pump-and-treat technologies were the treatment of choice in 68% of the sites for which a final remediation technology has been specified [Travis and Doty, 1990]. In the pump-and-treat systems, wells are installed at the contaminated site for removal of groundwater. Groundwater is pumped to the surface and the contaminants are removed using appropriate physical, chemical, or biological treatment system. The treated groundwater is then discharged either to surface waters, or to a publicly owned treatment works (POTW), or in some cases back into the aquifer [Haley et al., 1989].

Recently improved pump-and-treat so called "smart pump-and-treat" technology has also been suggested [Hoffman, 1993] because the conventional pump-and-treat groundwater remediation is criticized for being too expensive and time-consuming, especially when cleanup standard are very low levels.

A wide range of physical, chemical, and biological treatment technologies are available for application with hazardous materials and contaminated land. These technologies can be grouped on the basis of the scientific and engineering principles involved, or, on the basis of how and where the technology is implemented (i.e., containment on site, treatment in situ, treatment on-site; treatment or disposal off-site). A number of physical, thermal, chemical and biological treatment technologies applied on site are given in Table 1.1.

Table 1.1 Examples of contaminated land on-site remediation technologies [Ellis, 1992].

Technique	Prime Objectives	Common Problems
Synthetic liner	Containment	Short and long range resistance to contaminants
Modified Clay liner	Containment	Quality control during installation; durability
Jet grouting	Containment	Quality control during installation; and expense of plants
Slurry walls	Containment	Quality control during installation
Ground freezing	Containment	High cost
Product recovery	Containment removal	difficulty in full recovery
Stabilization	Reduction of contaminant mobility	Often ineffective with organics
Solidification	Reduction of contaminant mobility	Long term efficacy; interfaces in mixed wastes
Chemical treatment	Immobilization or destruction	Quality control; lack of targeting; leachate and air emissions
Vitrification	Immobilization or destruction	Containment of gaseous releases, expense; soil variability
Vacuum extraction	Containment removal	Limited to volatile organics in the vadose zone
Air stripping	Containment removal	Limited range of pollutants; in situ sparging can create groundwater mounding
Land spreading	Biological destruction/dispersion	Quality control; run off; can exacerbate pollutants problems
Land farming	Biological destruction	Accumulation of inorganics; control of application rates
Composting	Biological destruction	Limited range of contaminants and treatability rates vary
Treatment bed	Biological destruction	Limited to range of organic pollutants
In-situ (biological)	Destruction; Stabilization	Quality control is difficult and a high degree of monitoring is needed
Soil flushing	Physical removal in situ	Long period of treatment; difficulties in contaminant spreading
Soil washing	Physical removal ex situ	High cost; problem of residue
Pump and treat	Physical removal on site	Limited to groundwater; long periods are required; can exacerbate pollution
Thermal	High temperature oxidation	High cost; residues and off-gas require treatment
Material handling	Volume reduction; pretreatment	Noise; dust; vibration; odor
Contaminant delineation	Volume reduction	Accuracy depends on methodology

The definitions, objectives, problems, and the range of applications of treatment technologies are given by Ellis [1992]. Predicted effectiveness of few selected treatment technologies in decontaminating soil is reported by Bradshaw et al., [1992]. Several reports are available on the comparative efficacy of treatment technologies. Eight distinct

techniques have been evaluated and ranked by Haiges et al. [1989]. Table 1.2 depicts the ranking of these technologies based on the criteria of feasibility, cost, time, efficiency and adverse impacts. Bioremediation is the option preferred by Haiges, although it is almost the slowest technique and gives lower treatment levels than most competing methods. Actually, the main objective of most commercially available techniques is to reduce the toxic effect of organic pollutants. This is best achieved by encouraging microbial degradation of these compounds, since the end products of biodegradation are usually innocuous. This preference for bioremediation reflects US stringent standards on environmental emissions. Vacuum extraction, which has successfully been used on several UK sites [Texaco News, 1991] is distinctly cheaper, but entails air pollution risk unless complex air cleaners and filters can be included. Thermal destruction methods rated poorly because of their costs and the difficulties in ensuring acceptable emission standards. However, this judgment may be outdated, since the newer oxygen enhanced incinerators [Anonymous, 1989] are able to double treatment rates despite their reduced capital costs.

Table 1.2 Ranking of techniques for the treatment of soils contaminated with light oils

[Haiges et al. 1989]

Technique	Technical feasibility	Achievable treatment levels	Adverse impacts	costs	Time of treatment	Overall ranking
Bioremediation	3	5	1	4	7	1
Soil washing	6	2	4	5	2	2
Soil flushing	4	4	3	8	4	3
Land farming	5	3	2	3	5	4
Vacuum extraction	2	6	5	2	6	5
Passive venting	1	8	6	1	8	6
Thermal destruction	7	1	8	7	1	7
Stabilization	8	7	7	6	3	8

<sup>a</sup> 1 indicates best, 8 indicates worst.



Many authors including Brown et al., [1991], Bradshaw et al., [1992], Roberts et al. [1993], and Levin and Gealt [1993] have reported the relative cost of different technologies in USA, UK, Holland and other countries. Bioremediation has been used extensively for treating contaminated soil in Europe and the U.S.A. The state of usage is discussed in detail by Porta [1994], Devine [1994]. Over 50 Companies in Germany offer biological treatment and even more companies operate in the U.S.A., in Netherlands, and Denmark [Ellis, 1992]. Bioremediation currently is the most commonly available of these newer techniques in the United Kingdom and essentially mirrors the natural degradation of organic material to water and carbon dioxide [Bradshaw et al., 1992].

Bioremediation was identified as the main natural process by which volatile hydrocarbons were removed from the *Amoco Cadiz spill* [Swannel & Head, 1994]. The largest bioremediation project was undertaken for the *Exxon Valdez* oil spill [Bragg et al., 1993]. Bioremediation was proposed to mitigate the long term damage created by the Gulf oil spill. The effectiveness of bioremediation to the Gulf oil spill has been studied by Fayad et al. [1992]. According to a recent report [Journal of the Air and Waste Management Association, 1993] bioremediation is expected to become the potential growth market over next five years.

In order to optimize a remediation program, it may be necessary to combine treatment methods with a range of engineering techniques [Ellis, 1992]. For example, four techniques have been used together in order to clean-up contaminated sites. These are free phase recovery, venting, bioremediation and groundwater extraction. Venting the ground aids volatilization of pollutants in the vadose zone and enhances natural biodegradation by allowing increased oxygen supply into the contaminated area. This can be further enhanced with forced aeration below the groundwater table or in the vadose zone.

Biodegradation also aids removal of contaminants in the dissolved and adsorbed phases and this can be effective in the saturated zone, capillary fringe and vadose zone.

### **1.3 State of the Art**

Numerous studies have been conducted in the laboratory and in the field to determine the various aspects of bioremediation such as biodegradability of various toxic organics under different conditions, various factors affecting bioremediation, and modeling biodegradation in the laboratory and field. Biodegradability of different toxic organics and factors affecting biodegradation will be discussed in detail in the next chapter. A brief review of modeling is given below:

A large number of models has been summarized by Javandel et al. [1984], Khondaker et al. [1990] for groundwater flow and transport in saturated and unsaturated porous media. Groundwater flow may be one-dimensional, two-dimensional, three dimensional, transient or steady, the aquifer may be saturated, unsaturated, bounded, unbounded, and the solute transport process may have advection, dispersion, decay, ion-exchange, leaching, or dissolution. The capabilities of many computer models to simulate contaminant transport are, in most cases, limited to specific type of flow, for specific type of aquifer and for a specific number of transport processes. Moreover models coupling with chemical and biological reactions are very few. Bioremediation invariably involves biological reactions modeled by first-order, zero-order, Monod, and a variety of other kinetic models. The objective of this review is not to critically evaluate all the available models with respect to their supported features, but to make an informative summary of the models pertinent to bioremediation studies.

A recent review of models applied to biodegradation in groundwater has been given by Bedient and Refai [1992]. From the viewpoint of biodegradation kinetics, they divided the models in four categories:

- (1) First-Order Decay
- (2) Biofilm Models (including kinetic expressions)
- (3) Instantaneous Reaction Model
- (4) Dual-Substrate Monod Model

Three different conceptual models have been adopted in the past for the development of mathematical models of bacterial growth and biologically reactive solute transport in saturated porous media [Molz, 1987; Baveye and Valocchi, 1989; Widdowson, 1991]: *Strickly Macroscopic model*, *Microcolony model*, and *Biofilm model*. The Biofilm model is based on the assumption that the solid particles covering the aquifer material are uniformly covered by a *biofilm* in which consumption of the substrate and electron donor takes place. The microcolony concept assumes that bacteria grows not in fixed films, but in small discrete colonies or microcolonies. Strickly macroscopic model is the traditional model used over last few decades. It is characterized by the absence of any assumption concerning the microscopic configuration and distribution of the pores.

In a series of studies, Rittman and McCarty [1980], Bouwer and McCarty [1984], Kissel et al. [1984], Suidan and Wang [1985], Suidan et al. [1987] used the biofilm concept to simulate the removal of organics by attached microorganisms. The kinetic and mass transfer criteria by Rittman and McCarty [1980], Suidan et al. [1987] indicate the assumption of a fully penetrated biofilm without external or internal mass transfer limitations. This permits a greatly simplified model since diffusion into the biofilm need not be considered. The assumption that the biomass is essentially attached to aquifer material (i.e., not mobile) is supported by Harvey et al. [1984]. Modeling the biodegradation of

organics that are degraded in presence of oxygen generally requires that oxygen, substrate, and microbial mass be simulated. Borden et al. [1984] showed that in many field situations large variations in microbial population and growth kinetics have little effect on contaminant distribution. This is due to the very high rates of microbial growth relative to the groundwater flow. That is, the microbial growth reaches equilibrium rapidly relative to the groundwater flow. This is supported in part by biofilm studies [Bakke, 1986] that show the biofilm thickness reaching a maximum early in the experiment and remaining constant. Recent studies by Taylor and Jeffe [1990a, 1990b, 1990c, 1990d] however indicated that the biofilm growth may continue for a long time depending on type of substrate and substrate concentration. Using methanol as the growth substrate, they observed that the permeability reduction in laboratory sand column may continue as long as 365 days and by an amount of more than 99 percent. However with substrate like BTX, such growth can never be achieved, because such compounds are soluble only in very low concentration and growth of degrading microorganisms is inhibited still at lower concentration. For the purpose of present study, the temporal effect of biofilm growth on contaminant distribution and diffusion of contaminant into the biofilm will be neglected.

McCarty et al. [1981] assumed that substrate concentration within the biofilm changes only in the direction which is normal to the surface of the biofilm and that all the required nutrients are in excess except the rate-limiting substrate. The model employs three basic processes: mass transport from the bulk liquid, bio-decomposition within the biofilm, and biofilm growth and decay. The authors evaluated the applicability of the biofilm model to aerobic subsurface biodegradation using a laboratory column filled with glass beads. The experimental data and the model predictions were relatively consistent.

Kissel et al. [1984] developed differential equations describing mass balances on solutes and mass fractions in a mixed-culture biological film within a completely mixed

reactor. The model incorporates external mass transport effects, Monod kinetics with internal determination of limiting electron donor or acceptor, competitive and sequential reactions, and multiple active and inert biological fractions which vary spatially. Results of hypothetical simulations involving competition between heterotrophs deriving energy from an organic solute and autotrophs deriving energy from ammonia and nitrite were presented.

Molz et al. [1986] and Widdowson et al. [1987] presented one-dimensional and two-dimensional models for aerobic biodegradation of organic contaminants in ground water coupled with advective and dispersive transport. A microcolony approach was utilized in the modeling effort, microcolonies of bacteria are represented as disks of uniform radius and thickness attached to aquifer sediments. A boundary layer of a given thickness was associated with each colony across which substrate and oxygen are transported by diffusion the colonies. Their results indicated that biodegradation would be expected to have a major effect on contaminant transport when proper conditions for growth exist. Simulations of two-dimensional transport suggested that under aerobic conditions microbial degradation reduces the substrate concentration profile along longitudinal sections of the plume and retards the lateral spread of the plume. Anaerobic conditions developed in the plume center due to microbial consumption and limited oxygen diffusion into the plume interior.

Widdowson et al. [1988] also extended their previous work to simulate oxygen and/or nitrate based respiration. Basic assumptions incorporated into the model include a simulated particle-bound microbial population comprised of heterotrophic, facultative bacteria in which metabolism is controlled by lack of either an organic carbon-electron donor source (substrate), electron acceptor (oxygen and or nitrate), or mineral nutrient, or all three simultaneously.

Borden and Bedient [1986] developed the first version of the BIOPLUME model. They developed a system of equations to simulate the simultaneous growth, decay, and transport of micro-organisms combined with the transport and removal of hydrocarbons and oxygen. Rifai and Bedient [1987] developed the second version of the program called BIOPLUME II which allows for prediction of naturally occurring biodegradation as well as in situ bioremediation. The model is based on USGS 2-D solute transport code, MOC [Konikow and Bredehoeft, 1978] and used instantaneous reaction model for the aerobic and first-order for anaerobic biodegradation. USEPA decision support system, OASIS [Newell et al., 1990] mainly contains two solute transport models: (1) BIOPLUME II and (2) one dimensional analytical solute transport model (ODAST) developed by van Genuchten [1982].

Borden et al. [1986] applied the first version of the BIOPLUME model to simulate biodegradation at the Conroe Superfund site in Texas. Oxygen exchange with the unsaturated zone was simulated as a first-order decay in hydrocarbon concentration. The loss of hydrocarbon due to horizontal mixing with oxygenated ground water and resulting biodegradation was simulated by generating oxygen and hydrocarbon distributions independently and then combining by superposition. Simulated oxygen and hydrocarbon concentrations closely matched the observed values.

Srinivasan and Mercer [1988] presented a one-dimensional, finite difference model for simulating biodegradation and sorption processes in saturated porous media. The model formulation allows for accommodating a variety of boundary conditions and process theories. Aerobic biodegradation was modeled using a modified Monod function; anaerobic biodegradation is modeled using Michaelis-Menten kinetics. In addition, first-

order degradation was allowed for both substrates. Sorption was incorporated using linear, Freundlich, or Langmuir equilibrium isotherms for either substrate.

MacQuarrie et al. [1990] utilized a similar approach to Borden et al. [1986] and Rifai et al. [1987,1987a] to develop a biodegradation model. The advection-dispersion equation was coupled with a dual-Monod relationship. The system of equations was solved using an iterative principal direction finite element technique. Comparisons of numerical results with the results of a laboratory column experiment showed that the model equations adequately describe the behavior of toluene, dissolved oxygen, and the microbial population, without considering solute diffusion through stagnant fluid layers or biofilms. The authors concluded that in a two-dimensional shallow aquifer setting, an organic plume experiences mass loss, spreading controlled by the availability of dissolved oxygen, and skewing in the direction of ground water flow.

MacQuarrie and Sudicky [1990] utilized the model developed by MacQuarrie et al. [1990] to examine plume behavior in uniform and random flow fields. In uniform ground water flow, a plume originating from a high-concentration source will experience more spreading and slower normalized mass loss than a plume from a lower initial concentration source because dissolved oxygen is more quickly depleted. Large ground water velocities produced increases in the organic solute mass loss because of increased mechanical mixing of the organic plume with oxygenated ground water.

Recently, Odencrantz et al. [1990] presented a contaminant transport model which allows for different biodegradation kinetics. Monod kinetics and biofilm kinetics are compared in a two-dimensional transport model, where the differential equations are solved using a nonlinear operator splitting. Results indicated that the two models could differ for a large enough biofilm thickness.

Celia et al. [1989] presented two papers on development of a numerical biodegradation model designed to handle co-metabolism, multiple substrates, and aerobic and anaerobic metabolism. The model is currently one-dimensional and therefore has limited applications to field sites.

Semprint and McCarty [1991, 1992] developed a one dimensional nonsteady state model having features similar to models described by Molz et al. [1986] and Bordent and Bedient [1986]. The model supports 1-D transport with advection, dispersion, and sorption, Monod kinetics for electron donor and electron acceptor, cometabolic transformation. They verified the model with field bioremediation results for chlorinated aliphatic compound.

Chen et al. [1992] developed a one dimensional model for transport and biodegradation of Benzene and Toluene in the subsurface environment. Modeled processes include mass exchange between the constituent phase (solid, liquid, gas, and biomass), advective and dispersive transport and biotransformation as well as biomass production.

Parker and van Genuchten [1984] developed a one-dimensional analytical model that supports advection, dispersion, sorption and biodegradation with first-order and/or zero-order decay. The outstanding feature of the model is that it can be used for nonlinear least-square fit to analyze breakthrough curves to estimate transport parameters. The model has been successfully used in a number of studies [Mohammed, 1988; Chen et al. 1992]. The model will be described in detail in Section 8.



## 1.4 Research Needs

Although the process of bioremediation has been utilized for the decades in the field of wastewater engineering, its application to soil and groundwater at hazardous waste site is fairly new and still undergoing intensive development. Many authors [Alexander, 1991; J. Air Waste Manage. Assoc., 1991; Pritchard, 1991; Nicholas, 1992] have discussed the research needs in bioremediation. USEPA convened a meeting in February, 1990, to discuss biotechnological solutions to environmental problems. As a result of that meeting, EPA formed a Bioremediation Action Committee (BAC) and established six subcommittees of BAC to facilitate further development of the technologies. Four major areas of high priority research has been given by Alexander, [1991]: determining factors that govern the availability of pollutants for bioremediation and devising ways to increase their availability for microbial destruction; improving the design of processes for bioremediation; overcoming problems associated with scale-up from simple laboratory systems to field operations; and developing innovative bioremediation processes.

Many compounds that are normally quickly destroyed by microorganisms apparently are not easily degraded in polluted soils, subsoil, and aquifers because they are not readily available. The chemicals may be sorbed, dissolved in nonaqueous phase liquids, present in physically inaccessible state, or bound in some other way that prevent microorganisms with biodegradative enzymes from carrying out rapid transformations [Alexander, 1991]. Research designed to explain and overcome the problems of poor availability to microorganisms of chemicals that are otherwise easily destroyed should make bioremediation more useful. EPA workshop recommended several other different research needs for in situ bioremediation and above-ground bioreactors as well as for land treatment and composting. Scale-up from laboratory to field sometimes poses difficulties, and related issues were considered. In addition, because of difficulties in bioremediation of

complex wastes - including those that contain substances toxic to the biodegrading microorganisms-and of compounds that are only biologically transformed by cometabolism, an exploratory program is needed to seek innovative processes for complex wastes and pollutants transformed only by cometabolism. Staps [1989] recommended the following research areas:

- availability of contaminants to microorganisms
- insufficient knowledge about the behavior of oxygen and hydrogen peroxide in the soil, especially the increase in the stability of hydrogen peroxide.
- incomplete degradation of benzene, toluene and xylene; residual concentration of contaminants and metabolites.
- insufficient knowledge about the possibility of transport of microorganisms through the soil.
- behavior of oxygen and hydrogen peroxide in the soil
- feasibility for total mineralisation of contaminants and metabolites
- alternative oxygen sources or electron acceptors for aerobic degradation
- possibility for transport of microorganisms through soil and the effect of inoculation
- optimization of nutrient addition
- bio-availability.

## **1.5 Objectives**

An overabundance of lab studies on the affect of diverse factors on biodegradation has been reported. These will be reported in the next chapter. However most of these studies were conducted in laboratory microreactors such as microcosms, soil columns, etc. Although in many soil columns small flow of water has been maintained, kinetic

parameters have been determined using batch analysis. In many studies only the overall removal of pollutants have been reported from measured concentrations at the inlet and the outlet of the microreactor. However in actual field studies many other phenomena such as advection, dispersion, and biodegradation come into the picture. The effect of groundwater velocity or soil permeability plays an important role in the transport of nutrients, growth and decay of microorganisms. However studies reported on this aspect are still lacking. BTX compounds has been selected because, contamination of groundwater by gasoline or BTX compounds is the most severe problem around the world. Besides it was also outlined in the previous section that research is needed on transport and biodegradation of BTX compounds. Since scale-up from laboratory to field scale experimental results in the most important issue, present study will be performed in a big pilot scale model so that comparison between existing laboratory results and the present results can assist in a better scale-up.

A pilot scale sand tank model will be used to simulate the steady one dimensional flow and kinetic parameters will be estimated by fitting the observed data to the solution of one dimensional advection dispersion equation allowing sorption and biodegradation given by first-order and zero-order kinetics. Although, quite a few analytical and numerical models exists for one dimensional solute transport, none of them is suitable for actual bioremediation studies because of the following reasons:

- almost all the existing models are based on constant parameters and it is very difficult to have some of the parameters, such as groundwater velocity, as constants during the bioremediation process,
- recent studies in the laboratory columns have indicated substantial permeability changes due to microbial growth [Taylor and Jeff, 1991; Essa, 1993] and gas production [Morgan & Watkinson, 1992]. In such conditions the use of a mean value for the groundwater velocity may cause substantial error.

Solute transport modeling with time-dependent parameters is still in its infancy. Barry and Spostio [1989] described a procedure to compute the analytical solution of the advection dispersion equation with time dependent transport parameters in case of nonreactive solutes only.

The basic objective of the proposed research is to the study bioremediation of saturated sandy aquifer contaminated with gasoline residues under aerobic condition and at a scale that simulates field conditions and facilitates scale-up of laboratory findings. The specific objectives of this study are

- to develop a one dimensional model to simulate one dimensional transport of BTX compounds with sorption and biodegradation given by first-order and/or zero-order kinetics and a variety of other kinetics, such as Michaelis-Menten and Monod kinetics. The model will consider time-dependent velocity and a variety of initial and boundary condition to cover most possible cases of biodegradation studies in the laboratory and in the field,
- to develop a computer program for easy inversion of the above models to compute various transport parameters,
- to assess the fate of BTX compounds in groundwater by measuring the spatial and temporal concentration profile for various velocities, concentration, and dissolved oxygen ( $\text{H}_2\text{O}_2$ ) in a pilot scale laboratory sand tank model,
- to compute sorption and kinetic parameters of first-order and zero-order model by nonlinear least square fitting of the experimental data collected from the sand tank experiment,
- performing statistical analysis to determine the dependence of transport parameters on velocity, solute concentration, and dissolved oxygen (as hydrogen peroxide).

## Chapter 2

# **BIOREMEDIATION**

## 2 BIOREMEDIATION

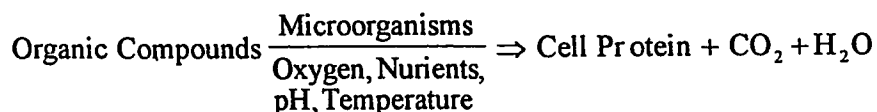
### 2.1 Introduction

Biological processes have been used for over 100 years for the treatment of organic-bearing municipal and industrial wastewaters that does not contain toxic chemicals. About 3 decades ago, it was first realized that wastewater containing toxic organics like phenol can also be treated because the degrading microorganisms maintain the concentration of the chemicals below the toxic threshold. Biological treatment of such wastewaters is now common. Many compounds that were once believed to be refractory to biological action are now recognized as being transformed naturally by the native microorganisms in the environment [McCarty, 1988]. However, the application of bioremediation for the treatment of organic-contaminated soil and groundwater is fairly new and still undergoing intensive development [Gabriel, 1991]. Hoff [1993] divided the history of bioremediation into three development periods: (1) courtship period, **Pre-1989**, this period is primarily a research period, (2) honeymoon period, **1989-1991**, in this period bioremediation as a technology received wide attention and interest, (3) maturing or establishing period, **1992 to the present**, when bioremediation has achieved a certain level of acceptance.

### 2.2 Definitions

Bioremediation is a term that encompasses biological methods for the clean-up of contaminated land and water [Bradshaw et al., 1992]. It can imply the complete restoration of a site so that its original multifunctional use is recovered, or, reclamation of a site for a particular intended use. A number of definitions of bioremediation has appeared in literature that have more or less the same meaning. *Bioremediation* has been defined as the acceleration of the *biodegradation* process through the addition of nutrients

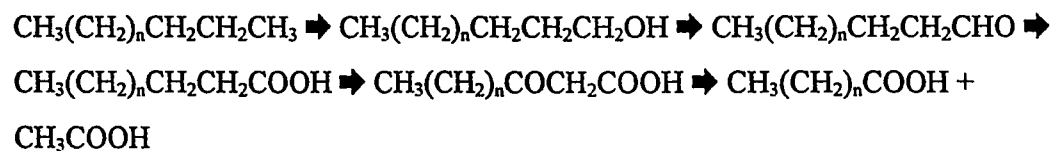
and other materials to contaminated media using techniques such as aeration, venting and temperature control [Hoff, 1993]. *Biodegradation* in context of organic pollutants is a natural process whereby bacteria and other microorganisms alter and break down organic molecule into substances, eventually producing carbon dioxide and water or methane.



Although the ultimate aim of bioremediation is to degrade organic compounds completely to harmless constituents such as CO<sub>2</sub> and water, many intermediate metabolites that are more toxic and more soluble than the parent compounds can be formed [Wilson and Jones, 1993]. A more restrictive definition of bioremediation is postulated by Baker and Herson [1991], who construe bioremediation as an in situ treatment technology that uses microorganisms to detoxify and degrade contaminating xenobiotic materials for the remediation of contaminated aquifers and subsurface soils. This definition has been supported by Baker et al. [1988], Lee et al. [1988] and Soczo and Visscher [1987].

Fundamentally there are two types of bioremediation: *enhanced biodegradation* or *biostimulation* and *bioaugmentation* [Baker and Herson, 1991]. Enhanced Biodegradation or biostimulation uses alterations of site's physical/chemical characteristics to increase biodegradation by indigenous microorganisms. In this method, biodegradation of contaminants by indigenous organisms is stimulated by the addition of supplemental inorganic nutrients (mainly nitrogen and phosphorous), electron acceptors or organic substrate to the subsurface environment. Bioaugmentation relies on the addition to the site of microorganisms selected for their ability to degrade the contaminants.

Pathways of aerobic and anaerobic biodegradation of BTX has been depicted by many authors including Lapinkas [1989], Kuhn et al. [1988], Lovely and Lonergan [1990], Grbic-Galic and Vogel [1987], Zeyer et al. [1990]. The actual biochemical pathways for bacterial degradation of hydrocarbon contamination depends on the substrate metabolized and the type of microorganisms involved. Both aliphatic and aromatic compounds are biologically mineralized in a step-wise fashion. Aliphatic terminal carbon oxidation (to alcohols) is the first stage conversion, followed by a dehydrogenation reaction to corresponding aldehydes as shown below:



Oxidation continues in the third stage conversion to the corresponding fatty acids, which then undergo bacterial oxidation to yield the fatty acid plus acetic acid. The acetic acid is then degraded further to yield carbon and energy for assimilatory purposes.

The first phase of aromatic metabolism is often the modification or removal of substituents on the benzene ring followed by a step wise conversion of catechol. Catechol is of primary importance as it represents the hydroxylated forms of benzene or phenol. Figure 2.1 illustrates the degradation of catechol, which completes the bioconversion process, generating fatty acids which fuel TCA cycle. This itself acts as an efficient



receptor for the input of biochemical intermediates from catabolic pathways and is a principal source of metabolic energy in the form of adenosine triphosphate (ATP).

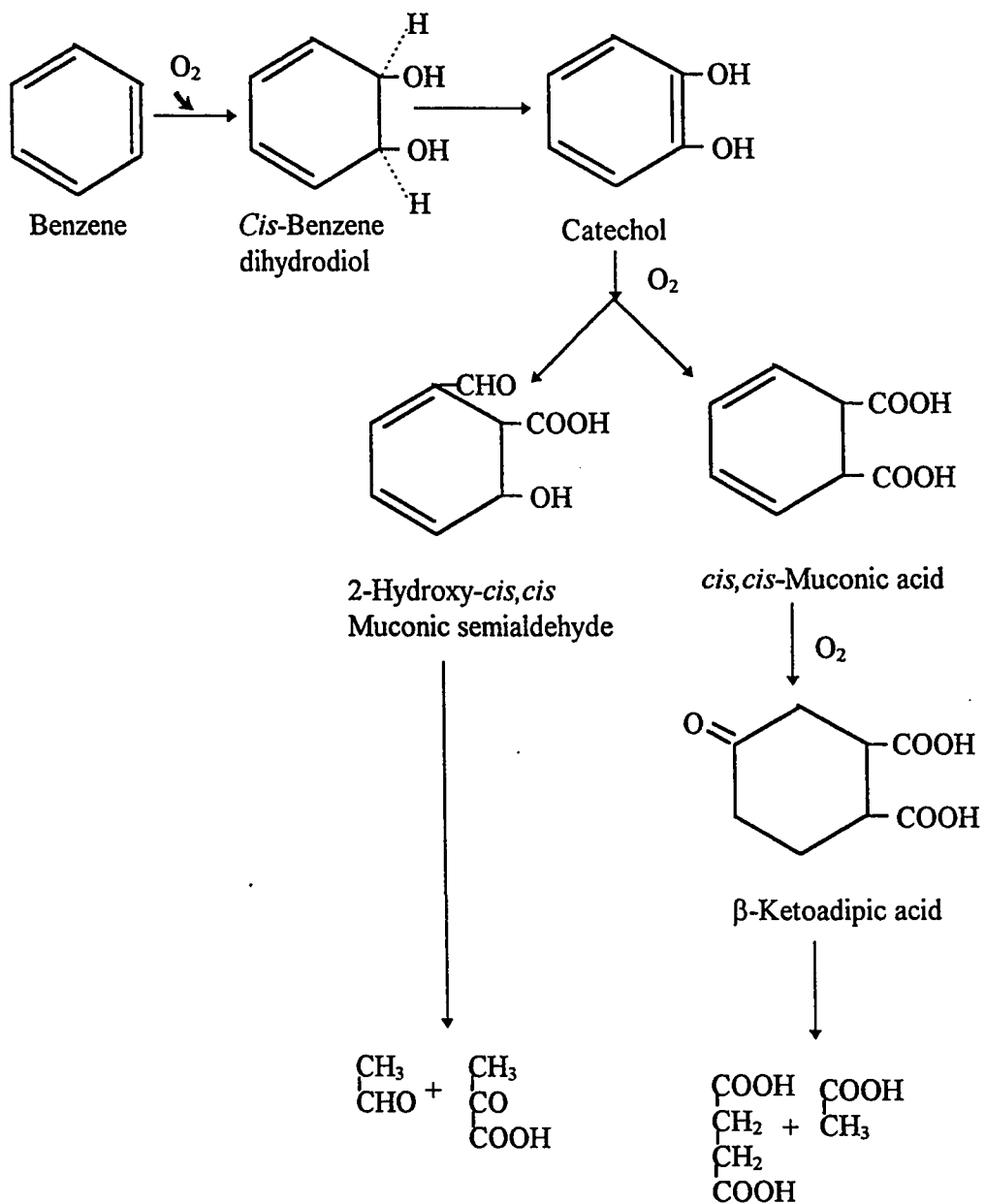


Figure 2.1 Pathways of aerobic biodegradation of benzene [Lapinkas, 1989]

## 2.3 Biodegradation of Toxic Organics

### 2.3.1 Microbially mediated reactions

A number of authors including Lee et al. [1988], McCarty [1988, 1991], Morris and Novak [1989], Torpy et al. [1989], and Zitomer and Speece [1993] have documented the various mechanisms for biodegradation of organic compounds.

Microorganisms participate in two classes of chemical reactions for their energy and growth: *gross reactions* and *synthetic reactions*. The gross reactions also called *redox reactions* are oxidation-reduction reactions in which an electron is transferred from one chemical to another. Redox reactions are of particular importance to hazardous waste and groundwater contamination problems. The microorganisms promote the second class of reactions utilizing smaller amounts of material. These synthetic reactions produce the highly specialized chemicals necessary for life, such as proteins, carbohydrates, and DNA. The microorganisms promote these reactions for chemical products rather than energy.

Electrons are not found in isolation in water solutions (which make up all living organisms). In redox reaction, the electron is donated by one species and accepted by another. Such reactions can take place in aerobic environments in which oxygen serves as a terminal electron acceptor, or in anaerobic process where nitrates, sulfates, carbon dioxide, or the organic compounds themselves serve as electron acceptors. The organic matter or the food is the electron donor.

The redox reactions produced by various combinations of reductions and oxidations can be classified as follows. Respiration, the "*oxidation by oxygen*" of organic food to carbon dioxide and water, is the basic metabolism of all multicelled organisms and most single-celled organisms. If oxygen is abundant but no organic matter is available, the reduction of

oxygen can be combined with the sulfide, iron, ammonia, or hydrogen oxidations in chemoautotrophy. If organic food is abundant, but oxygen is absent, *anaerobic respiration* is accomplished by microorganisms which combine the oxidation of organic matter with the reduction of inorganic species like nitrate, sulfate, and carbon dioxide.

There are also chemoautotrophic organisms which can oxidize methane to carbon dioxide. They are called methanotropic organisms and are found in abundance where methane is being evolved by other microbiological processes, or where it is seeping from natural gas deposits. They are abundant in soils surrounding gas leaks in natural gas delivery systems.. Some of the methanotropic organisms are important as they are capable of aerobic degradation of chlorinated solvents such as trichloroethylene and tetrachloroethylene.

If neither organic matter nor methane are available, organisms which can oxidize sulfide may become dominant. Sulfide is not common in natural aerobic environments, but it does occur. Sulfide-oxidizing bacteria are found in springs, for example, where sulfide generated in deep aquifers reaches the surface. They may also become important where a variety of pollutant processes produce a release of sulfide.

Springs which bring anaerobic groundwater into contact with the atmosphere may also support cultures of iron-oxidizing bacteria. Oxidation of ferrous iron ( $\text{Fe}^{++}$ ) is the next reaction on the oxidations list (assuming no organic matter is present, so there is no alcohol). It will occur readily when ferrous iron, oxygen, and the appropriate bacteria are present. It can occur in water delivery systems, where the metallic iron of the pipes is first oxidized to the ferrous form and then to the ferric ( $\text{Fe}^{3+}$ ) form.

If oxygen is abundant and there is no organic matter, methane, sulfide, or ferrous iron, the presence of ammonia ( $\text{NH}_3$ ) may still prompt biological activity. Many species are capable of converting ammonia to nitrite, and then to nitrate. This occurs in sewage treatment plants, where the process is the first step in "nitrification-denitrification," used to remove the nitrogen species which pollute lakes and rivers.

The most energetically favorable redox reaction is the reduction of nitrate to nitrogen gas. Denitrification occurs in soils saturated with water, to the dismay of rice farmers who value the nitrate as a plant nutrient. They must replace it with expensive artificial fertilizers. An anaerobic environment is intentionally created in some sewage treatment processes, with the objective of removing nitrate. This prevents nitrate accumulation, which can cause nuisance blooms of algae in waters where the sewage is being disposed. Converting the nitrate to nitrogen gas, which is released harmlessly to the atmosphere, solves the problem. For subsurface waters, nitrate reduction is another way in which organic material may be decomposed. In a few cases, nitrate has been added to aquifers to promote anaerobic degradation of contaminants. It is an effective oxidizing agent for this application because it is a negative ion, and so is not adsorbed strongly by the soil. It can move readily to the site of contamination. Unlike oxygen itself, it can be supplied in high concentrations because its solubility in water is high. Under some conditions, the nitrate will all be consumed by the conversion to nitrogen gas. At other times, however, substantial amounts of nitrogen will be converted to ammonia. In fermentative nitrate reduction, organisms use nitrate as an electron acceptor by producing ammonia. If the nitrate is all consumed, microbially-mediated redox reactions may continue, using ferric iron ( $\text{Fe}^{3+}$ ) to accept the electrons. The ferrous iron ions produced may be important in water quality.

The next reaction on the list, in order of energy, is the conversion of carbohydrate to alcohol. Coupling this reaction with the oxidation of organic matter produces a special kind of anaerobic respiration called fermentation.

In many cases, sulfate will be the next oxidizing agent utilized by the microorganisms. This is particularly so in seawater environments, where sulfate is present in high concentration. As it is used to oxidize organic matter to carbon dioxide, sulfide is generated. The sulfide has many secondary effects in the local environment. When the sulfate is exhausted, further anaerobic microbiological activity may occur using carbon dioxide as an electron acceptor.

**Cometabolism:** Microorganisms utilize gross reactions to generate the energy necessary for life. They carry out synthetic reactions in smaller amounts to produce the building materials of the cells. In some cases, however, microorganisms are unable to metabolize a substance as the sole source of carbon and energy but can transform these substances if provided an alternate growth substrate called cosubstrate. This phenomenon is known as *cometabolism*, *cooxidation*, or *cotransformation*. Co-metabolism is defined as the degradation of a compound only in the presence of other organic material which serves as the primary energy source [Brock et al., 1984].

Cometabolic reactions are decomposing reactions, that is, the reactions involve the breakdown of hydrocarbons into small molecules like carbon dioxide, in a way that releases energy. They differ from the gross reactions in that the cells are not capable of utilizing this energy for growth. They must have another organic present as the primary substrate on which they live.

The phenomenon has its importance for the cleanup of subsurface hazardous waste. Very often, the hazardous materials are those which the microorganisms cannot use as a primary substrate, but which are decomposable as a cosubstrate. Cleanup by biodegradation will therefore employ the addition of primary substrate. An easily biodegradable and innocuous substrate like acetate is added to the soil or water, and a vigorous culture of microorganisms develops. While they go about the business of consuming the acetate, the bacteria also cometabolizes the hazardous waste, and the soil is decontaminated.

**Sequential Reactions:** Often, the degradation of a complex organic molecule to carbon dioxide and water is done in many steps, involving several species. It may require a consortium of microorganisms, rather than a single species. Slater and Lovatt (1984) have emphasized the importance of microbial communities in biodegradation, and classified some possible relationships.

In one kind of community, the activities of the first species may provide the nutrients necessary for the second, allowing it to survive. Examples have been found in which each of the two species provides a nutrient for the other. Sometimes the reaction that is beneficial to a second organism is cometabolic for the first. In other communities, an organism may serve by degrading harmful products produced by another. This prevents self-inhibition and allows biodegradation to proceed.

Organisms may also cooperate in a combined metabolic attack on a difficult substrate. One organism may break crucial chemical bonds in the food molecule. The product molecule becomes the substrate for another organism, which breaks more bonds, and so on.

### ***2.3.2 Application of Different Processes of Biodegradation***

A number of authors including McCarty [1988, 1991], Morris and Novak [1989], Torpy et al. [1989], and Zitomer and Speece [1993] have documented the various mechanisms for biodegradation of organic compounds.

**Aerobic treatment methods:** Conventional treatment processes for contaminated soil and waters mostly rely on aerobic processes that cover a wide range of treatment including land treatment, land farming, and wastewater treatment. Land treatment encompasses solid phase land treatment, composting, liquid/solid treatment, liquid phase treatment (in activated sludge, sequencing batch reactors, fixed-film bioreactors), in situ treatment. Aerobic wastewater treatment methods includes activated sludge, trickling filters, etc.

**Anaerobic treatment methods:** Interest in anaerobic biotechnology for industrial wastewater treatment has greatly increased during the past decade. Today, anaerobic processes are recognized as feasible unit operations for treatment of high-strength industrial wastewater [Zitomer and Speece, 1993]. Benefits of anaerobic treatment often cited include lower electrical power requirements; production of methane which may be used for heating or power generation; and lower sludge production. In conventional anaerobic digestion, organic material is solubilized and converted to organic acids by a set of organisms (acetogens) and then to methane by another distinct set of organisms, called methanogens. The environment in which the methanogenic bacteria survive must be practically free from oxygen. The conversion of energy from the organic contaminants to cell mass is 20 times less than in aerobic digestion and therefore considerably less biological sludge is produced [Torpy et al. 1989].

Sequential biodegradation of toxic compounds, for example anaerobic treatment followed by aerobic treatment has proved to very effective in biodegrading a wide range of organic compounds such as trichlorophenols, tetrachlorophenols, pentachlorophenols, Chloroform, TCE, TCA, DCE, DCA, dichlorobenzene, trichlorobenzene, hexachlorobenzene, trichlorobiphenyls, tetrachlorobiphenyls, pentachlorobiphenyls, hexachlorobiphenyls, DDT, BTEX compounds, sucrose, glucose, etc [Zitomer and Speece, 1993].

### ***2.3.3 Biodegradability of Organic Compounds***

Table 2.1 shows biodegradability prospects of several important class of organic pollutants. It was first introduced by Wilson and McNabb [1983] and was based on their cautious extrapolation from the behaviour of these compounds in natural system and their admitted limited experience with their behaviour in the subsurface environment.



Table 2.1 Prospect of biotransformation of selected organic pollutants in water table aquifer [Wilson and McNabb, 1983].

Class of Compounds	Aerobic water, concentration		Anaerobic water
	> 100 µg/L	< 10 µg/L	
<i>Halogenated Aliphatic Hydrocarbons</i>			
Trichloroethylene	none	none	possible*
Tetrachloroethylene	none	none	possible*
1,1,1-Trichloroethene	none	none	possible*
Carbon Tetrachloride	none	none	possible*
Chloroform	none	none	possible*
Methylene Chloride	possible	improbable	possible
1,2-Dichloroethene	possible	improbable	possible
<i>Brominated methanes</i>	improbable	improbable	probable
<i>Chlorobenzenes</i>			
1,2-Dichlorobenzene	probable	possible	none
1,4-Dichlorobenzene	probable	possible	none
1,3-Dichlorobenzene	improbable	impossible	none
<i>Alkyl benzene</i>			
Benzene	probable	possible	none
Toluene	probable	possible	none
Xylenes	probable	possible	none
Styrene	probable	possible	none
<i>Phenol and Alkyl Phenols</i>	probable	probable	probable†
<i>Chlorophenols</i>	probable	possible	possible
<i>Aliphatic Hydrocarbons</i>	probable	possible	none
<i>Polynuclear Aromatic Hydrocarbons</i>			
Two or three rings	possible	possible	none
Four or more rings	improbable	improbable	none

\* Possible, probably incomplete

† Probable, at high concentration

In another report the feasibility of hydrocarbon biodegradation and their relative level of recalcitrance was given by Lapinkas [1989]. Lapinkas classified the organic substrate as simple, intermediate and recalcitrant according to their relative difficulty of being biodegraded as shown in Table 2.2.

Table 2.2. Feasibility of biodegradation: hydrocarbons and their relative recalcitrant  
[Lapinkas, 1989]

Substrate type	Group of organics	Example
<b>Simple</b>	Volatile aliphatics and aromatics	Alkenes, alkadienes and alkynes
	Heavy aliphatics and aromatics	Saturated alkanes and cyclic hydrocarbons
	Phenolic compounds	Phenol, cresols, xylenols, and naphthols
<b>Intermediate</b>	Volatile halogenated hydrocarbons	Chlorinated or bromated
	Heavy halogenated hydrocarbons	Chlorinated or bromated
	polyuclear aromatic hydrocarbons	Mono, di, and trinuclear aromatic compounds
<b>Recalcitrant</b>	Residuum	Asphalts, asphaltenes and resinous compounds
	Tars and waxes	Asphaltic compounds and paraffinic compounds
	Pesticides	Aniline, diuron, PCB, DDT

Lee et al. [1988] summarized the biodegradability of some organic compounds from actual experimental works conducted by a number of authors. As shown in Table 2.3 almost all of these studies were conducted in aerobic environment and with or without bioaugmentation. Bioaugmentation and acclimation is a very important factor deciding the biodegradability of many organic compounds. Many compounds that are found to be biodegraded in contaminated soil are reported to be nonbiodegradable in uncontaminated soil from the same site [Thomas and Ward, 1992].

Table 2.3 Organic compounds that have been shown to be biodegradable in the subsurface [Lee et al., 1988]

Compound	Soil from contaminated area	Aerobic
<i>Natural Compounds</i>		
Glucose	No	Yes
Glutamic acid		
Arginine		
<i>Solvents</i>		
Acetone	Yes	Yes
Ethanol		
Isopropanol		
Tert-butanol	Yes	Yes
Methanol		
Bromodichloromethane		
<i>Aromatics</i>		
Benzene	No	Yes
Toluene	No	Yes
Xylene	No	Yes
Methylated benzenes	Yes	Yes
Chlorinated benzenes		
Chlorinated phenols	Yes	Yes
Naphthalene		
Dibenzofuran		
Flurene		
Phenanthrene		
Chlorobezenc		

**Cometabolism:** Aerobic and anaerobic microorganisms are known to degrade certain organic pollutants when the organisms are grown in the presence of the pollutants with other organic compounds which becomes the primary energy source for growth. McCarty [1988] and Torpy et al. [1989] indicated that co-metabolism can biodegrade many recalcitrant organic compounds. Table 2.4 shows a list of primary and secondary substrates that can be transformed by bacteria.

Table 2.4 Biological process and environmental conditions under which different compounds may be transformed by bacteria [McCarty, 1988]

Substrate type	Environment	Example
Primary substrate	Aerobic & anaerobic	Glucose, acetone, isopropanol, acetone, benzoate, phenol
	Aerobic primarily	Alkanes, benzene, toluene, xylene, vinyl chloride, 1,2-dichloroethane, chlorobenzene
Secondary substrate (Co-metabolism)	Oxidations	Trichloroethylene, dichloroethylene, dichloroethane, vinyl chloride, chloroform
	Reduction	1,1,1-Trichloroethane, trichloroethylene, tetrachloroethylene, dichloroethylene, dichloroethane, carbon tetrachloride, chloroform, DDT, lindane, polychlorinated biphenyls

The aerobic biodegradability of some toxic organic compounds in activated sludge system is given in Table 2.5. The extent of biodegradation is related to the bacterial oxygen consumption by comparing the BOD to the theoretical oxygen demand (TOD) of the compounds. TOD is computed from reaction stoichiometry and is the theoretical amount of oxygen required to totally oxidize a compound to carbon dioxide and other inorganic products.

Table 2.5 Aerobic biodegradability of organic compounds (Eckenfelder & Grau, 1992]

Readily biodegradable <sup>a</sup>	Moderately biodegradable <sup>b</sup>	Slightly biodegradable under studied conditions <sup>c</sup>	Refractory under studied conditions <sup>d</sup>
Cyclohexane Octane Phenol Methanol 1-Propanol 1-Butanol 1-Pentanol 1-Hexanol 1-Octanol 2-Chloropropionic acid Diethanol amine Acetonitrile Acrylonitrile Benzene Toluene Xylene Benzyl alcohol  Nitrobenzene Naphthalene Pyridine Quinoline <i>m</i> -Cresol <i>p</i> -Cresol 4-Chlorophenol 4-Nitrophenol	1-Decanol 1-Dodecanol Acetone Ethylbenzene 2-Furaldehyde Benzonitrile 4-Bromophenol Hydroquinone	Decane 1,3-Dichloropropane Ethyl ether Phenanthrene	Dodecane Dichloromethane Chloroform 1-Chloropropane 1-Chlorobutane 1-Chloropentane 1-Chlorohexane 1-Chlorodecane 1,2-Dichloroethylene 3-Chloro-1,2-propane  Isopropylether Trichloroacetic acid Chlorobenzene 1,3-Dichlorobenzene 1,4-Dichlorobenzene 1,2,4-Trichlorobenzene 1,2,4,5-Tetrachlorobenzene Hexachlorobenzene Benzidine
<sup>a</sup> Biochemical oxygen demand (BOD)/theoretical biochemical oxygen demand (TOD), ≥50%. <sup>b</sup> BOD/TOD, 25-50%. <sup>c</sup> BOD/TOD, 10-25%. <sup>d</sup> BOD/TOD, < 10%.			

A list of organic compounds that have been reported to be biodegradable under aerobic, denitrifying, sulphate reducing, methanogenic conditions as primary or secondary substrates is given in Table 2.6

Table 2.6 List of common groundwater pollutant biodegraded under different conditions

Organic Compounds	Environment	References
<b>Monoaromatic hydrocarbons</b>		
BTEX (benzene, toluene, ethylbenzene and xylenes)	Acrobic environment	Anonymous [1989], Anid & Vogel [1990], Anid et al. [1993], Alvarez & Vogel [1991], Alvarez & Vogel [1991], Barker et al. [1987], Bayly and Barbour [1984], Borden [1994], Bouwer, [1989], Berwanger & Barker, [1988], Chen et al. [1992], Chiag et al. [1989], Gibson and Subramanian [1984], Graves et al. [1994], Hutchins et al. [1992], Lee et al. [1988], Lodaya et al. [1991], Major et al. [1988], Robinson et al. [1990], Warith et al. [1991]
	Anaerobic environment	Action and Barker [1992], Barker et al. [1987], Coresuil and Weber [1994], Karlson & Frankenberger [1989], Suflita [1993]
	Denitrifying	Anid et al. [1993], Arcangeli and Arvin, [1994], Jansen et al. [1989], Hutchins [1991, 1993], Hutchins & Wilsons, [1994], Hutchins et al. [1989, 1991, 1992], Kuhn et al. [1988], Major et al. [1988], Ramanand et al. [1994], Zeyer et al. [1986, 1990]
	Sulfate-reducing	Edwards et al. [1991], Ramanand et al. [1994],
	Methanogenic	Garbic-Galic and Vogel [1987], Ramanand et al. [1994], Vogel and Garbic-Galic [1986], Wilson et al. [1986, 1986b], Wilson et al., [1994]
	Dissimilatory iron reducing	Lovely and Lonergan [1990]
	Competitive inhibition and Cometabolism	Chang et al. [1993]
<b>Polyaromatic hydrocarbon (PAH)</b>		
only Naphthalene	Acrobic	Glaze et al. [1986], Erickson et al. [1993]
	Anaerobic (mainly denitrifying)	Ehrlich et al. [1982], Mihelic and Luthy [1988a, 1988b, 1991], Klecka et al. [1990],
PAH	Acrobic	Adenuga et al. [1992], Borden and Bedient [1987], Brubaker and Stroo [1992], Bewley et al. [1989], Castaldi, [1994], Cardinal and Stenstorm [1991], Durant et al. [1994], Field et al. [1994], Johnson and Leuschner [1991], Lewis [1993], McGinnis et al. [1991], Mueller et al. [1991, 1991a], Mueller et al. [1994], Sutherland [1992], Secch et al. [1994], Steiber et al. [1994], Symons et al. [1988], Van der Hock et al. [1989], Wang et al. [1990], Warith et al. [1991], Weissenfels et al. [1990]
	Anaerobic	Blum et al. [1986], Durant et al. [1994], Ellis [1991], Johnson and Leuschner [1991], Mihelic and Luthy [1988a, 1988b, 1991]
PAH (oil, cresote, etc.)	Acrobic	Aust, [1989], Hilderbrand and Wilson [1991], Morgan and Watkinson [1990], Prince and Sambasivam [1993], Scherrer and Mille [1990],

Table 2.6 List of common groundwater pollutant biodegraded under different conditions (contd.)

Organic Compounds	Environment	References
<b>Chlorinated Aliphatic Hydrocarbons (CAHs)</b>		
TCE, TCA, DCA, DCE, VC, etc	(Aerobic)	Cox et al. [1994, 1994a], Fennel [1993], Mahaffey et al. [1992], McClellan et al. [1989], Roberts et al. [1990], Wilson et al. [1986]
	(Anaerobic)	Barrio-Lage et al. [1986, 1990], Beeman et al., [1994], Cox et al. [1994a], Criddle et al. [1986], Phelps et al., [1994], Semprint et al. [1987], Singhal et al. [1990], Stucki et al. [1992], Vargas & Ahlert [1987], Wilson et al. [1986]
	Aerobic Co-metabolism (CH <sub>4</sub> )	Alvarez-Cohen and McCarty, [1991], Arvin [1991], Broholm et al., [1992], Broholm et al., [1992], Dolan & McCarty [1994], Dugan et al., [1990], Fogel et al., [1986], Henry & Grbic-Galic., [1990], Lanzarone and McCarty [1991], Legrand, [1994], Little et al., [1988], McFarland et al., [1991], McNab and Narasimhan [1994], More et al., [1989], Oldenhus et al., [1989], Semprint et al. [1990, 1992, 1994], Speitel & Alley, [1991], Strandberg et al. [1989], Tsien et al., [1989], Wackett & Householder., [1989], Wilson & Wilson [1985], Yagi et al. [1994]
	Aerobic Co-metabolism (propane)	Keenan et al., [1994], Wackett et al., [1989]
	Aerobic Co-metabolism (propene)	Ensign et al., [1992]
	Aerobic Co-metabolism (ethylene)	Hartmans & Debont, [1992]
	Aerobic Co-metabolism (toluene)	LaPat-Polasko et al., [1994], Nelson et al., [1986], Nelson et al., [1987], Nelson et al., [1988], Shields et al., [1994], Wackett & Gibson., [1988], Winter et al., [1989]
	Aerobic Co-metabolism (phenol)	Coyle, [1994], Folsom et al., [1990], Harker & Kim., [1990], Hopkins et al., [1993], Montgomery et al., [1989], Nelson et al., [1986], Semprint et al., [1994]
	Aerobic Co-metabolism (ammonia)	Arciero et al., [1989], Vannelli et al., [1990]
	Aerobic Co-metabolism (isoprene)	Ewers et al., [1990]
	Aerobic Co-metabolism (isopropyl benzene)	Dabrock et al. [1992]
	Aerobic Co-metabolism (2,4-D)	Harker & Kim, [1990]
	Aerobic Co-metabolism (JP-4 oil)	Kampbell & Wilson., [1994]
	Co-metabolism (Anaerobic)	Bake and Jaffe [1989], Back et al. [1990], Barrio-Lage et al. [1986], Bouwer & McCarty [1983], Dugan et al., [1990], Fiorenza et al., [1994], Kleopfer et al. [1985], Semprint et al. [1987], Singhal et al., [1990], Vogel and McCarty [1985, 1987]

Table 2.6 List of common groundwater pollutant biodegraded under different conditions  
(contd.)

Organic Compounds	Environment	References
PCE	Co-metabolism (Aerobic)	McNabbb & Narasimhan [1994], Rasmussen et al. [1994]
	Co-metabolism (Anaerobic)	Beeman et al., [1994], Carter and Jewell [1993], Chu and Jewell, [1994], Fiorenza et al., [1994], Smith and Ferguson, [1994]
	Sequential (Anaerobic-aerobic)	Fathepure & Vogel, [1991]
Carbon Tetrachloride	Aerobic	
	Anarobic	Bhattacharya and Ataman [1989], Truex et al., [1994], Wu and Doong [1993]
	Anoxic	Stensel & Dejong, [1994]
Chloroform	Co-metabolism (Aerobic)	Rahni et al. [1986], Strand and Schippert [1986]
	Sequential (Anaerobic-aerobic)	Fathepure & Vogel, [1991]
<b>Phenolic Compounds</b>		
Phenol, cresol, etc.	Aerobic	Arvin et al. [1991], Bettman et al. [1984], Brown et al., [1990], Ehrlich et al. [1982], Evangelista et al., [1990], Klecka et al., [1990], Kumaran & Parhad, [1984], Lewandowski, et al., [1986], Namkoong et al. [1989],
	Anaerobic	Blum et al. [1985, 1986], Fedorak & Rudey [1986], Kobayashi et al, [1989], O'Connor & young [1989], Pai & Wang [1990], Pitrowski, [1989], Sloan, [1987], Suidan et al., [1991], Wang et al. [1989], Young & Rivera, [1985]
Chlorophenols (CP, DCP, TCP, PCP, etc.)	Aerobic	Carberry and Benzing [1991], Chudoba et al., [1989], Dasappa & Loehr, [1991], Ettala et al., [1992], Frick and Crawford, [1986], Jacobson et al., [1991], Jarvinen et al., [1994], Klecka & Maier, [1988], Koch et al. [1991], McGinnis et al., [1991], Mikesell & Boyd, [1985], Puhakka et al., [1991], Puhakka et al., [1991], Ravikumar, [1990], Smith and Novak [1987], Yucel, [1989]
	Anaerobic	Hakulinen et al., [1985], Henderksen et al., [1991], Litchfield et al., [1994], Mikesell and Boyd [1985, 1988], Nevalainen et al., [1993], Puhakka et al., [1991], Smith and Novak [1987]



Table 2.6 List of common groundwater pollutant biodegraded under different conditions (contd.)

Organic Compounds	Environment	References
<b>Miscellaneous organics</b>		
Alcohols	Aerobic	Wilson and Novak [1986], Morris and Novak [1989]
Ethylene glycols	Aerobic	Costa [1985], Mcgahey, [1990]
Chlorinated organics	Aerobic	Closman and Speitel [1989], Matsumoto [1985]
	Anaerobic	Atlallah and Butz [1985], Kim and Maier [1986]
Esters	Aerobic & anaerobic	Shanker et al. [1985]
Nitrophenol	Aerobic	Yucel [1989]
Dinitrophenol	Aerobic	Silverstein et al. [1990]
Nitrotoluene	Aerobic	Struijs & Stoltenkamp [1986]
TNT	Aerobic	Selivanovskaya [1987]
PCB	Aerobic	Anonymous, [1985], Focht & Brunner, [1985], Shannon et al., [1991], Unterman et al., [1985]
	Anaerobic	Berthouex & Gan, [1991]
	Aerobic and Anaerobic	Abramowicz [1993], Brunner et al., [1985]
	Sequential	Bowlds [1992], Vogel, [1991]
DDT	Aerobic and Anaerobic	Sharma et al. [1987]
Pesticides (Isopfenfos)	Anaerobic	Chapman et al. [1986]
Dioxin (DCDD, TCDD)	Aerobic	Bumpus & Aust, [1986], Gold et al., [1994]

Table 2.6 has been compiled from about 220 lab and field studies. Seventy eight percent of these studies were conducted in the laboratory and remaining 22% in the field. 56.5% studies were carried out in aerobic environment, 36.1% in anaerobic environment and 7.5% in both aerobic and anaerobic conditions. Almost all the major pollutants such as BTEX, PAH, CAH were biodegraded under both aerobic and anaerobic conditions. CAHs were mainly biodegraded in cometabolic conditions with a variety of primary substrates shown in Table 2.6 Figure 2.2 shows the relative number of studies on different organic compounds. Figure 2.3 shows the actual number of studies in aerobic, anaerobic and both (aerobic and anaerobic) conditions.

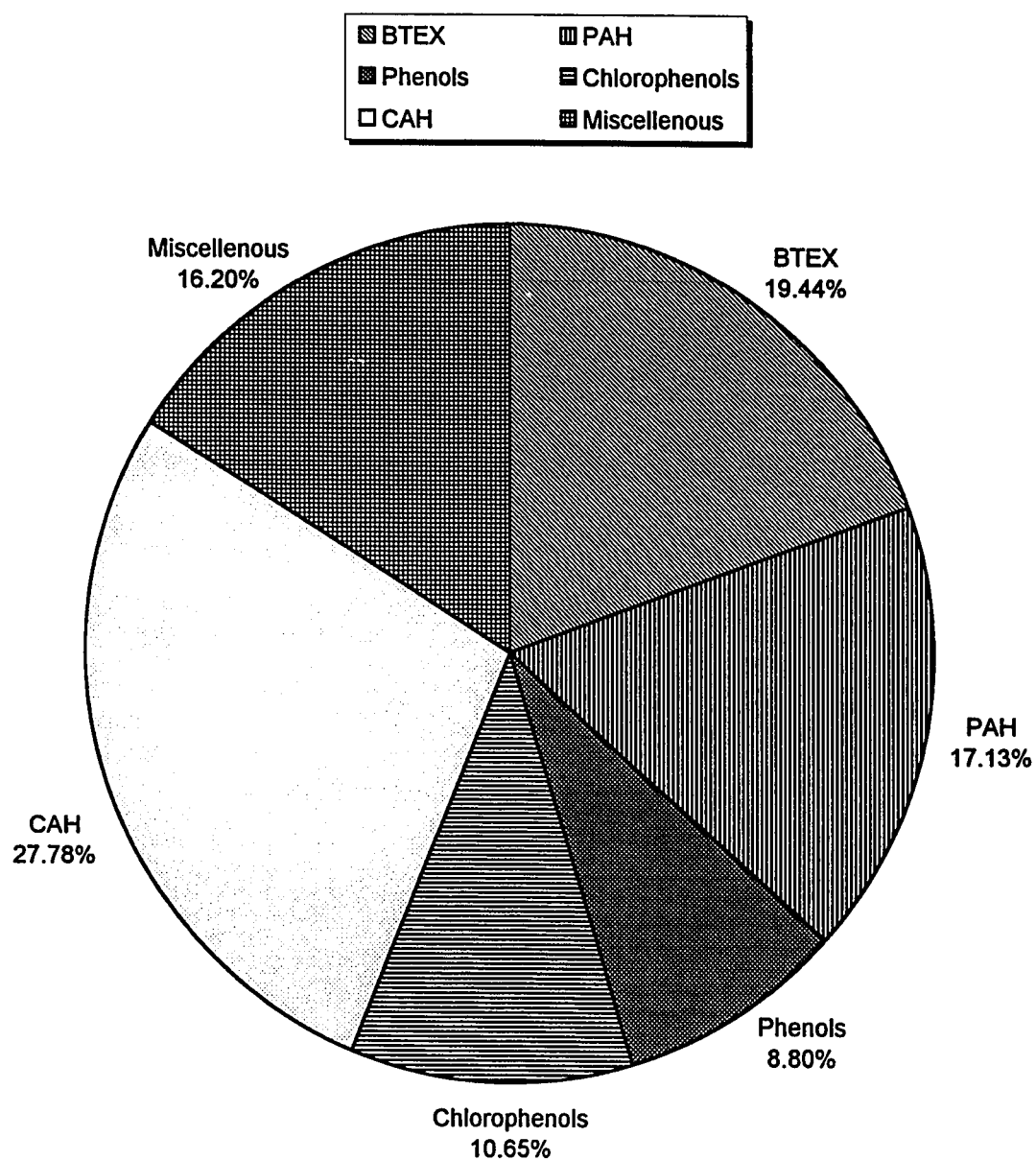


Figure 2.2 Relative number of studies on different organics

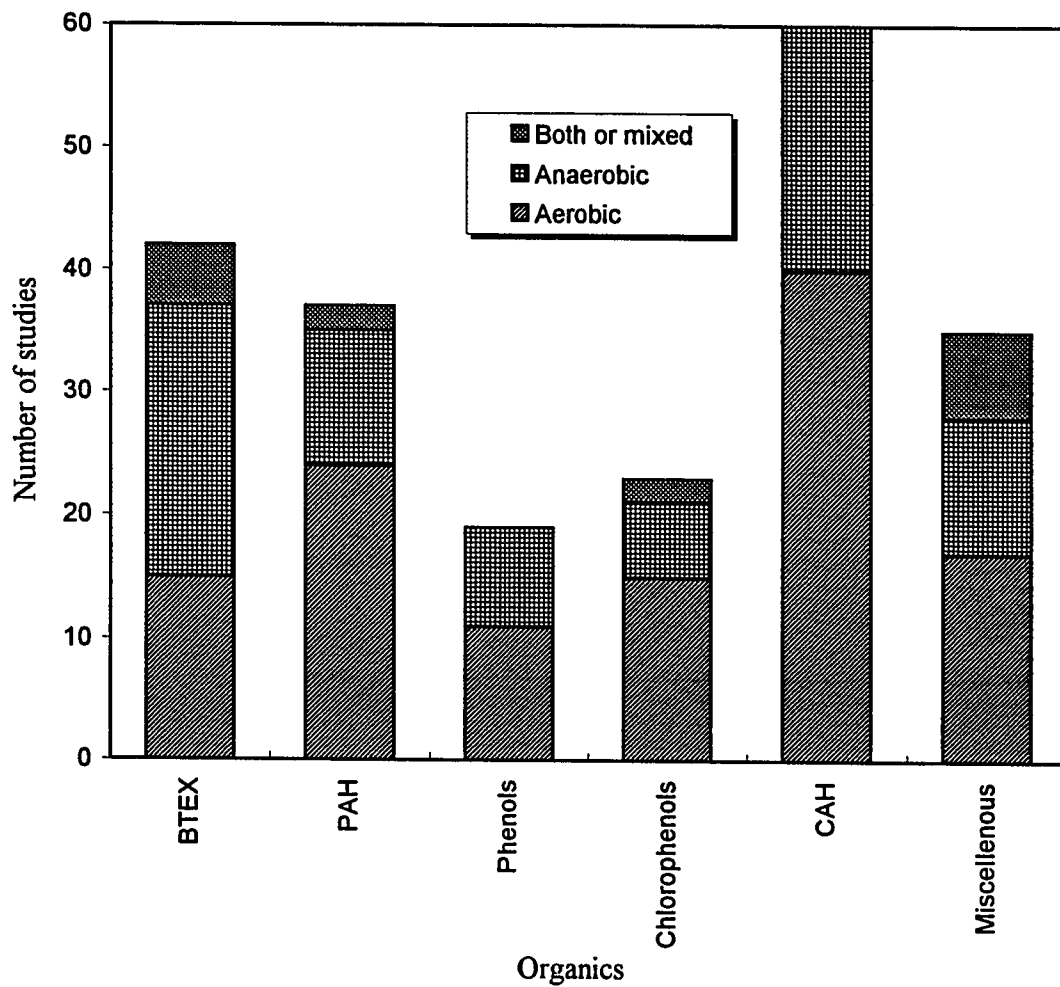


Figure 2.3 Number of studies in aerobic, anerobic and both condition

## 2.4 Rate-limiting Factors Affecting Bioremediation

Numerous researchers have directed their efforts to determine factors that affect biodegradation under the real-world conditions. Much of this work has been made possible by the use of microcosms that allow for the experimental manipulation of microbial communities while retaining some of complexities of the natural environment [Parkes, 1982; Pfarl et al., 1990; Prichard and Bourquin, 1984; Trevors, 1988; and Wilson and Noonan, 1984]. Rate-limiting factors affecting bioremediation have been discussed by many authors and reviewers including Atlas [1988], Autry & Ellis, [1992], Baker and Herson [1991], Fiorenza et al. [1991], Focht [1988], Lapinkas [1989], Lee et al. [1988], McCarty [1991] and Thomas and Ward [1992]. A review of the factors has been depicted by Frankenberger, [1991]. Rate limiting factors for field applications of bioremediation technologies can be classified according to two principal sources [Autry & Ellis, 1992]:

- Biochemical/Microbiological factors
- Environmental factors

### 2.4.1 *Biochemical/Microbiological Factors*

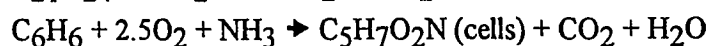
The principle biochemical rate limiting factor is the absence of bacterial population or species that is capable of degrading hydrocarbon compounds. Because hydrocarbon-degrading bacterial species is ubiquitous in nature, it is highly unlikely that any soil system would be governed by this factor for biodegradation to occur. Studies [Gaffney, 1990; Robinson et al., 1990] show that the addition of acclimated microorganisms (bioaugmentation) to the soil system greatly enhance the biodegradation rate. However, in one site, the addition of acclimated microorganisms to petroleum contaminated soil did not significantly change the biodegradation rate of these compounds [Compeau, et al., 1991]. This observation tends to imply that sufficient number of bacteria capable of

biodegrading hydrocarbons were already present in that site [Autry & Ellis, 1992]. These conflicting results of bioaugmentation are discussed at length by Atlas [1991].

#### **2.4.2 Environmental Factors**

Various environmental factors affecting bioremediation are soil permeability [Lapinkas, 1989], oxygen supply [Atlas, 1991; McCarty et al., 1984; Mueller et al., 1989a; Swindoll et al. 1988], nutrient availability [Barker et al., 1988; Mueller et al., 1989a; Swindoll et al., 1988], temperature [Atlas, 1981; Larson, Clinckemaille, and VanBelle, 1981], pH [Torpy et al., 1989], moisture [Frankenberger, 1991], contaminant concentration [Atlas, 1981; Simkins and Alexander, 1984], geochemistry and hydrogeology [Litchfield, 1993], addition of surfactants [Bewley, 1992; Hunt et al., 1994; Ducreux et al., 1994; Wilson and Jones, 1993], contaminant chemical structure, etc. Few important factors are discussed below:

**Oxygen Requirement:** Aerobic degradation is the most attractive of the microbial processes for degradation of gasoline component in groundwaters because it proceeds at a more rapid rate and does not produce the noxious by-products associated with anaerobic decomposition [Noonan and Curtis, 1990]. As an example, the biodegradation rate constant for carbofuran is 0.047/day aerobically and 0.026/day anaerobically [Lyman et al., 1982]. For aerobic degradation, significant quantities of oxygen must be available to the microbes. The ratio of oxygen mass to hydrocarbon mass required for complete aerobic degradation to CO<sub>2</sub> (mineralization) has been estimated to range from the 3:1 ratio used in BIOPLUME II model [Rifai et al., 1987] to 1.03-1.7 (1.03:1 for benzene, 1.4:1 for toluene, 1.7:1 for xylene when the simultaneous production of cell mass is considered [Chiang et al., 1989]. The above ratios are computed from the stoichiometric balanced equations:



where  $\text{C}_{21}\text{H}_{24}$  represents the formula of BTX compounds.

Barker et al. [1987] computed that 23.2 mg/L of oxygen is required for 1 mg/L of BTX in groundwater. This is a high ratio compared to the theoretical requirement. Wilson et al. [1986] noted that in well-oxygenated groundwater containing 4 mg/L of molecular oxygen, microbes can degrade only 2 mg/L of benzene. Lodaya et al. [1991] however indicated very high removal of BTX compounds (Concentration upto 250 mg/L) in an immobilized activated sludge reactor using  $\text{H}_2\text{O}_2$  at very low concentration (2 mg/L of oxygen).

As microbes consume oxygen during the biodegradation of hydrocarbons, an aerobic groundwater can quickly become anaerobic. This onset of anaerobic conditions is the most significant factor in limiting the rate of biodegradation in groundwater environment [Raymond, 1987]. Three means of increasing the dissolved oxygen content of groundwater are commonly used in in-situ bioremediation: injection of air, liquid oxygen, and hydrogen peroxide. According to Raymond [1987], the saturation concentration of oxygen in water from air injection is about 10 mg/L. Depending on temperature pure oxygen can provide about 40 mg/L of dissolved oxygen. Hydrogen peroxide injection can provide between 250-400 mg/L of dissolved oxygen. This very high amount of hydrogen peroxide makes it an excellent choice to maintain aerobic condition of a groundwater system. However, it must be asserted that the addition of oxygen to reduced subsurface environments containing iron and manganese can result in rapid clogging. Furthermore, peroxide concentration as low as 100 mg/L can be toxic to microorganisms [Fiorenza, 1991; Texas Research Institute, 1982]. To avoid toxicity peroxide is added in a stepwise

manner, from 50 to 1000 mg/L, to allow subsurface microflora to adapt to the oxidant [Thomas and Ward, 1992]. Other problems associated with peroxide include rapid decomposition and off-gassing of O<sub>2</sub> to the surface and plugging of the region undergoing treatment. At a field experiment at Eglin Air Force Base, FL, using H<sub>2</sub>O<sub>2</sub> at an initial concentration of 500 mg/L, problems with off-gassing and flow impedance were observed and attributed to microbial degradation of H<sub>2</sub>O<sub>2</sub> [Spain, 1989].

**Nutrient Requirement:** Macronutrients such as nitrogen and phosphorous are often limiting in the subsurface and must be supplied to ensure biological degradation of hydrocarbons [Noonan and Curtis, 1990, Fiorenza et al., 1991]. Mulkins-Phillips and Stewart [1974] found that phosphorous limited the rate and extent of growth of a *Nocardia* sp. on 1% v/v Bunket C fuel oil. Laboratory experiments prior to the beginning of a bioremediation project in Ambler, PA. [Raymond et al., 1976] indicated that the native microflora could be stimulated by the addition of inorganic nitrogen, phosphorous salts and air. Thornton-Manning et al. [1987] found that both rate and extent of degradation of phenol can be increased by the addition of nitrogen and phosphorous. Swindoll et al. [1988] found that addition of inorganic nitrogen, phosphorous to pristine Lula aquifer sediments had a mixed effect on biodegradation of different compounds. Degradation of p-nitrophenol was increased by nutrient supplement, while degradation of ethylene dibromide and toluene were inhibited by the same treatment. Wilson et al. [1983] reported that more than 97% of toluene was degraded in microcosms from pristine aquifer without additional oxygen or nutrient. Thomas et al. [1989] found that no enhancement of naphthalene and 2-methylnaphthalene mineralization from the addition of nutrients to samples from a creosote-contaminated site in Conroe, Texas.

The quantity of nutrients required for degradation is generally expressed as a ratio of the nutrients to the carbon source. The carbon : nitrogen : phosphorous ratio necessary to

enhance the bioremediation can vary from 100:10:1 to 100:1:0.5, depending on the type of treatment used (aerobic or anaerobic) and the location of the contaminant (liquid or solid phase) [Torpy et al., 1989]. According to McCarty [1991], for aerobic biodegradation the optimal concentration of nitrate nitrogen are in the range of 2 to 8 pounds per 100 pounds of organic material and the phosphorous requirement is about one-fifth of this. Bossert and Bartha, [1984] suggest a C-N-P ratio of 160:1:0.08 for petroleum products. Dibble and Bartha [1979] determined the optimal C:N and C:P ratios of 60:1 and 800:1 respectively for oil sludge biodegradation. Ellis et al. [1990] maintained a C:N:P ratio of 70:5:1 for a pilot scale in situ treatment system where oil hydrocarbons were reduced from 185 to 26 mg/kg within 15 weeks. C-N-P ratio as high as 2:1:1 has also been reported for acetate biodegradation [Prince and Sambasivam, 1993]. However high inorganic salt content is also toxic to microorganisms [Torpy et al., 1989]. Initial excessive levels of nitrogen may expose the microorganisms to nitrogen burns [Lapinkas, 1989]. Moreover, nitrate-N concentration in groundwater higher than 10 mg/L have deleterious health effects, particularly in children.

Micronutrients sulfur and trace nutrient K, Mg, Ca, Fe, Na, Co, Zn, Mo, Cu and Mn are typically needed for optimal growth, although in very small quantities. The micronutrients and trace nutrient would not therefore limit growth of microbes in aquifer systems as often as oxygen deficiency does.

**Moisture:** The aerobic degradation of organics in soils depends on soil moisture. The moisture content of the contaminated soils affects the biodegradation of oils due to dissolution of the residual compounds, dispersive action, and the need for microbial locomotion to sustain high activity. The moisture content of soil affects microbial locomotion, solute diffusion, substrate supply, and the removal of metabolic byproducts [Frankenberger, 1991]. Excessive moisture will limit the gaseous supply of oxygen for



enhanced decomposition of hydrocarbons. Several authors including Frankenberg, [1991], Loehr [1991], USEPA [1988], USEPA [1990] have cited range of moisture in which biodegradation is optimum. Most studies indicate that optimum moisture content is within 50% to 70% of the water holding capacity [Frankenberg, 1991]. Other optimum ranges cited are 30%-90% and 40%-80%. Both extremes, waterlogging and desiccation will affect the effectiveness of bioremediation projects.

**Soil permeability:** This is one of the most important factor in in-situ bioremediation. Since water is the carrier for all nutrients, microbial inoculum and dissolved oxygen required to contact the contaminating substrate, therefore it is essential that certain degree of permeability must exist within the soil for a successful bioremediation. In situ bioremediation is not recommended for soil with permeability less than  $10^{-4}$  cm/s. [Thomas and Ward, 1992, Lapinkas, 1989]. In USA most of the in situ bioremediation has been applied to soils ranging in conductivity from  $10^{-3}$  to 2.1 cm/s [Staps, 1989].

**Contaminant Concentration:** Alexander [1985] reported that the rates of mineralization of some organic compounds are directly proportional to their concentration, and there is a threshold level below which certain compounds usually subject to biodegradation are not converted to  $\text{CO}_2$  and  $\text{H}_2\text{O}$ . Smith and Novak [1987] also found straight line relationship of log-log plot of initial concentration of phenolic compounds and zero-order degradation rate. However, at the higher concentrations of hydrocarbons in groundwater, microbial toxicity may occur [Cooney, 1984]. As the concentration of contaminants decreases and microbial population become adapted to the compounds, the microbes may be able to overcome the effects of toxicity and degrade the compounds.

**Temperature:** All biological transformations are affected by temperature. Generally, as the temperature increases, biological activity also increase up to a temperature where

enzyme desaturation occurs. Temperature affects the biodegradation rate in two ways. Both the specific growth rate of degrading microorganism and the activity of the enzymes responsible for contaminant oxidation are temperature dependent. Hydrocarbon degrading microorganisms have been isolated at temperature as low as  $-1^{\circ}\text{C}$  to as high as  $70^{\circ}\text{C}$  [Bartha and Atlas, [1977]. The optimum temperature for biological degradation as reported in the review of Frankenberger [1991] varies from  $18^{\circ}\text{C}$  to  $30^{\circ}\text{C}$ . Song et al., [1990] suggested that the optimum temperature for bioremediation of petroleum products is  $27^{\circ}\text{C}$ . According to Lapinkas [1989], the optimum biodegradation of hydrocarbon occurs in temperature range of  $30-40^{\circ}\text{C}$ . However, Focht [1988] reported that: unlike enteric bacteria, many soil bacteria do not grow optimally at  $37^{\circ}\text{C}$ , some bacteria may not even survive at  $30^{\circ}\text{C}$ . The optimal temperature reported by him for the *Pseudomonas* bacteria is  $25-30^{\circ}\text{C}$ . Bhattacharya [1990] has reported a temperature between  $20-30^{\circ}\text{C}$  as the optimal temperature. However substantial rate of mineralization of arctic diesel spiked to an Alaska spill was achieved at low temperatures ( $5^{\circ}\text{C}$  to  $20^{\circ}\text{C}$ ) upon addition of nutrients [Frankenberger, 1991].

**pH:** The ideal pH range to promote biodegradation of oils in soil is within the neutral to slightly alkaline range [Frankenberger, 1991]. Most studies indicate that pH 7 to 8 is optimum for degradation of petroleum hydrocarbons. Dibble and Bartha [1979] found that biodegradation of n-alkanes in minimal in acidic soil (pH 3.7); liming with  $\text{CaCO}_3$  to pH 7.8 promoted the rate of  $\text{CO}_2$  evolution from soil receiving oil sludge. Lime was also added by Song et al. [1990] to adjust the pH to 7.5-7.6 for enhancing hydrocarbon degradation. Most bacteria grow best at neutral to slightly alkaline pH and grow very poorly or do not grow at all below pH 5 [Focht, 1988]. Other studies have indicated optimum pH range of (6-9), (7.4), (8.0), (6.5-9.5), (6-10), (5-8) [Frankenberger, 1990]. Laboratory studies has also shown that at or above pH values 9.5, hydrocarbon

degradation is inhibited [Frankenberger, 1991]. Bhattacharya [1990] and Verheul et al. [1988] have reported that the neutral pH is the optimal pH.

**Surfactant addition:** Hydrophobic organic compounds (HOCs) especially PAHs tend to partition into soil and thereby limiting the bioavailability and biodegradation of these compounds. The use of surfactants, synthetic or biogenic, has been considered as a way of enhancing bioremediation efficiency by increasing the accessibility of contaminants to microorganisms, nutrients, and even oxygen [Ducreux, 1994]. However high concentration of these chemicals required to extract HOCs may inhibit biodegradation. Furthermore, synthetic surfactants may adversely affect the permeability of the cell membrane, thus reducing or eliminating the biodegradative potential of indigenous microorganisms [Hunt, 1994]. For soil:water ratio of 1:7-1:2, more than 0.1% by volume of surfactant was required to initiate solubilization, and 1% by volume resulted in 70-90% solubilization [Wilson and Jones, 1993]. Degradability of surfactants used is also important to limit further contamination. Application concentrations in excess of the critical concentrations have usually been reported successful. However, contradictory results on the activation of *in situ* biodegradation of PAH in soil-water laboratory system have been published [Aronstein et al., 1991, Laha and Luthy, 1991].

## 2.5 Selected Lab Studies

In order to optimize conditions for biodegradation, it is important to obtain background information about a site, such as pollutant concentration, various chemical and physical analysis of the soil (e.g., pH, inorganic N and P, particle size analysis), population density of the degrading microorganisms, and biodegradation potential with respect to natural unamended biodegradation rates vs. accelerated rates upon the addition

of biostimulating agent. Laboratory feasibility studies are usually performed for assessing the optimal conditions with respect to the above factors as well as other environmental parameters including oxygen supply, and moisture content. Selected published lab studies on various organics are shown in Table 2.7.

Table 2.7 Selected laboratory studies on different organic compounds

Researchers	Reactor	Soil	Treatment	Plate Count	Conc.	Removal and Kinetics
<b>BTEX compounds</b>					(mg/L)	
Alvarez and Vogel, 1991	Aquifer sand bottle	sand	Nutrients, vitamins, bio-augmentation		50 + 50 + 0 + 50	> 99% in 8, 15, 43 days Pseudo 1st-order removal
Alvarez et al., 1991	Batch incubator	sand	Nutrients, vitamins, air & oxygen	in the order of $10^6$	upto 250+250 +0+0	Removed below 100 ppm each. Monod $K_s = 12.2$ (B), 17.4 (T), $k = 8.3$ (B), 9.9 (T)
Anid et al., 1993	Aquifer columns	Sand	$\text{NO}_3$ and $\text{H}_2\text{O}_2$		BTEX = 200	25%-95% in 42 days Benzene with $\text{H}_2\text{O}_2$ only
Arcangeli & Ervin, 1994	Biodrum system	Biofilm system	Nutrients + $\text{NO}_3$ , pH, Temperature		BTEX = 60, High conc.	1st order for $\text{Co} = 2-3$ mg/L, Monod for higher ( $K_s = 0.4-0.85$ ), zero order for $\text{Co} = 8-30$ mg/L, Only TEX removed
Hutchins et al., 1991	Microcosm	Sand, Gravel, Clay	Nutrients + $\text{NO}_3$	$9.8 \times 10^7 - 1.4 \times 10^8$	9 + 6 + 4 + 4	1st-order, 0.016-0.38/day for contaminated and 0.022-0.067/day for uncontaminated soil
Lodaya et al., 1991	PBR with recirculation	calcium aginate sand	Nutrients + $\text{H}_2\text{O}_2$		150+100 + 0 + 255	more than 90% in 81 hours modeled with Monod
Weber and Corseuil, 1994	Sand column	sand	Nutrients + $\text{NO}_3$	$5.0 \times 10^5 - 3.0 \times 10^6$	2 + 2 + 0 + 4	> 99% removal in 3-5 days. Monod kinetics
Weber and Corseuil, 1994a	BAC reactor, microcosm	AC and sand	No treatment	$3.0 \times 10^6 - 6.0 \times 10^6$	BTEX = 0.025 - 9	> 99% for B in 25 h, upto 50% for T, X, in 250 h
<b>PAHs compounds</b>					(mg/kg)	
April et al., 1990	Batch reactor, soil column	sandy loam	No treatment		490-6646 mg/kg	upto 70% in one year
Breedveld and Briseid, 1994	Soil column	Coarse sand	Nutrient, moisture, pH, aeration	$1.0 \times 10^5 - 1.0 \times 10^8$	2 mg/L	upto 67% in 170 days
Brubaker and Stroo, 1992	Reactors, and soil column	fine soil (high C)	Oxygen		19-11700 mg/kg	upto 90% in reactors in 12 weeks, upto 94% in columns in 22 weeks
Erickson et al., 1993	Microcosm	MGP site soil	Nutrient, pH, temperature	$4.5 \times 10^5 - 4.6 \times 10^8$	150 mg/kg	about 50% removal in 3 months (overall)
McGinnis, 1991	Steel box	muddy, fluvial deposit	Nutrient, moisture, pH, aeration		PAH = 14612 PCP = 236	PAH (75%-100%), and PCP (33%-96%) in 84 days (1st order kinetics)
Mihelcic and Luthy, 1991	Soil slurry	well graded fine soil	Nutrient, pH, nitrate (35-135) mg/L	$7.0 \times 10^6 - 9.0 \times 10^7$	$1.0 \times 10^{-7}$ mol/ml	55%-100% in 9 months, Monod kinetics
Morgan and Watkinson, 1990	Soil slurry	sandy	Nutrient, temperature	$1.0 \times 10^7 - 1.0 \times 10^8$	< 15 mg/kg	upto 99.3%
Muller et al., 1994	Shaking flask, respirometer	varied	Nutrient, pH, temperature	$\text{Log}(\text{CFU}) = 6-7.3$ (total)	500 mg/L	Microbial ecology studied
Wang et al., 1989	outdoor lysimeter	sandy loam	Nutrient, pH, DO		60 mg/g	67.5%-87.5% in 12 weeks, 100% in 20 weeks

Table 2.7 Selected laboratory studies on different organic compounds

Researchers	Reactor	Soil	Treatment	Plate Count	Conc.	Removal and Kinetics
<b>CAHs compounds</b>						(mg/L)
Barrio-Lage et al., 1987	Microcosm	muck, sand, rock	No treatment		TCE= 5 ppm	> 99% in 1600 hours
Broholm et al., 1993	117 ml glass bottle		Nutrient, methane, pH	$1.0 \times 10^7$ - $6.0 \times 10^7$	TCE = 0.5	Michalis-Menten Kinetics 28%-55% in 30 days
Chu and Jewell, 1994	AAFEb	diatomaceous bed	Nutrient, pH, sucrose, temperature		PCE=10-26,	43-99% removal in 10-15 days, Monod kinetics, $K_s = 22.9$
Coyle, 1994	CSTR, batch reactor		Phenol, temperature		TCE= 0.1-18	47-85% in 8 hour
Lanzarone and McCarty, 1991	Sand column	Sand	Nutrient, O <sub>2</sub> , methane (or propane)		TCE= 1.5 - 4.5	20%-50% removal in one year, no degradation at 4.5 ppm
LaPat-Polasko et al., 1994	soil column		Phenol or (salicylic acid, tyrosine, H <sub>2</sub> O <sub>2</sub> )		TCE= 0.25-1.25	60-85% in 8 hour
McCellen et al., 1989	Microcosm	salt media	Nutrient, pH, DO	$4.3 \times 10^6$ - $1.0 \times 10^7$	TCF= 0.56-6.7	47% to 33% in 18-80 days
Speitel & Alley, 1991	Recirculating batch reactor	sandy clay	Nutrient, O <sub>2</sub> , methane		TCE=7 $\mu\text{g/g soil}$ , DCA=60-613	TCE: 1st order (1.76 $\mu\text{g/g/day}$ ), zero-order (0.884/day) DCA: 1st order (0.768 $\mu\text{g/g/day}$ ), zero-order (1.59/day) 95% in 40 days
Wilson and Wilson, 1985	Soil Column	Sand	Air with 6% natural gas		TCE = 0.015	> 95% in two weeks
Yagi et al., 1994	Microcosm		Nutrient, H <sub>2</sub> O <sub>2</sub> , methane, pH, temperature	$1.0 \times 10^6$	TCE= 0.1-1.0	95% at 0.1 ppm in 3 days 80% in one day 15%-25% in 7 days at 1 ppm
<b>Phenolic compounds</b>						(mg/L)
Brown et al., 1990	Electrolytic respirometer		Nutrient, DO, temperature		100 mg/L of COD	Parameters of Monod and Andrews kinetics were estimated
Jarvinen and Puhakka, 1994	1-L fluidized bed reactor		Nutrient, DO, temperature, pH		Total = 45.3	TCP, TeCP (99%) PCP (82%) in 12 days
Namkoong et al., 1989	150 ml soil reactor	fine sandy loam	moisture, DO, temperature		700, 500, 90 mg/kg of phenol, cresol and DCP	upto 100 % in 6-11 days 1st order kinetics
Smith and Novak, 1987	Microcosm	Silty sand	No treatment, anaerobic after 2-3 days	$1.0 \times 10^5$ - $3.0 \times 10^7$	1000, 1000, 130, 55 ppm of phenol, CP, DCP, TCP	Phenol and CP: Upto 100% in 17-25 days DCP: 60-90% in 65 days TCP and PCP: upto 100% in 30-65 days

## 2.6 Engineering Systems

Bioremediation operations may be made either on-site or off-site, in situ or ex-situ. Irrespective of the type of operation, bioremediation involves the deployment of microorganisms to detoxify or mineralize hazardous chemicals. Such chemicals are utilized as sources of nutrients and/or energy by microorganisms or are degraded by means of cometabolic transformations. Depending on the mode of form of application, bioremediation is categorized in three forms [Gabriel 1991]: *In-situ, aboveground, and reactors*. In situ bioremediation (ISB) involves the in-place microbial degradation of subject contaminants in the soil/water matrix. No excavation of the contaminated soil takes place. However, groundwater pumping and/or vacuum aeration is typically required to circulate oxygen and nutrients through the aquifer. The aboveground form of bioremediation involves the excavation of contaminated soil and treatment in an above-grade systems. The complexity of above-grade systems may range from open window composting to construction of a lined containment area enclosed within a green-house structure. Reactors for bioremediation may come in the form of mobile or fixed tank units. Excavation soil is combined with water to form a slurry which is stirred in a batch or continuous cycle mode. After contaminant degradation has occurred the "clean" slurry is dewatered and disposed. Another variation of this classification is given by Thayer [1991]: *land treatment, bioreactors, and in-situ treatment*. Ryan et al. [1991] also classified bioremediation in three groups of engineering systems: solid-phase treatment using unlined land treatment systems or prepared bed reactors, - slurry phase treatment systems completed either in-place or within tanks or impoundments, and- in situ treatment systems.

Nicholas [1992] classified bioremediation into three types: fertilizers, seeding and open-water applications. Fertilizers and seeding are synonymous to biostimulation and bioaugmentation respectively. Open-water application is the use of seeding or fertilizers in the open water such as in the open sea having as oil spill. Fiorenza et al. [1991] divided

the bioremediation technology into following three methodologies: *in situ*, *bioreactors*, and *bioventing*. According to them *in situ* bioremediation is a variation of pump and treat technology, with the biological treatment occurring in the subsurface environment. Ideally contaminants dissolved in groundwater and present in the soil matrix are both degraded by the indigenous microorganisms; however it is most effective for the biodegradation. The bioreactor category consists of methods that use either the soil matrix, the groundwater, or a combination of the two as the substrate and include the following methodologies: conventional land treatment with or without excavation, composting of contaminated materials, liquid-solid contactors, and withdrawal of groundwater and treatment in specialized reactors. Bioventing is a variation of vapor or vacuum extraction and is also a *in situ* technology. In bioventing, the degradation of fuel hydrocarbons located in the vadose zone is stimulated by the injection of air. Sufficient retention time is allowed so that the volatile organic compounds (VOCs) are biodegraded rather than volatilized

Table 5 shows a number of biotreatment system applied for treatment of industrial and hazardous waste. Table 6 summarizes the advantages, disadvantages and application of four most common bioremediation technologies applied in soil and groundwater. In all of its physical modes, bioremediation is typically promoted or enhanced by the introduction of nutrients, oxygen and water. In the case of *in situ* systems, these limiting factors are provided through injection and extraction wells.



Table 2.8 Types of biotreatment processes [Levin and Gealt, 1993]

Type	Principle	Comments	Safety Issues
Land farming	Soil mixed with nutrients and tilled in situ.	Requires lining to contain microbes and material.	Lining and cap have leakage and aging problems; monitoring and treating can be difficult.
Soil slurry (tank or lagoon)	Soil and water agitated together in reactor.	No temperature control	Little control over degradation process; effluent can be monitored and treated.
Subsurface reclamation (in situ)	Water, nutrients, and oxygen (electron acceptor) pumped through soil	Enhanced growth of entire indigenous population. Primary applications: oil and gasoline spills.	Organic contamination of groundwater as a result of mobilization of compounds; no control over dispersal of microbes or degradation products.
Soil treatment system	Wash procedure to solubilize adsorbed contaminants.	Pretreatment necessary to maximize efficacy.	Effluent goes to SBR; washed soil can be monitored before replacing at site.
Sequencing batch reactor (SBR)	Microbial digestion in liquid suspension	Allows control of reaction conditions	Release of microbes to environment; can monitor for microbes and pollutants.
Aqueous treatment system	Immobilized microbes or enzymes in flow-through system	Requires soluble organic material.	No microbial release; effluent can be monitored and treated.
Fixed-film bioreactor	Microbes/enzymes on plastic media in column to maximize surface area and nutrient exchange.	Can treat low concentrations of organic material	No microbial release; recycling of pollutants permits enhanced degradation and monitoring.

Table 2.9 Comparison of biological remediation technologies [Roberts et al., 1993]

Technology	Advantages	Disadvantages	Application/contaminant
Land farming	Simple procedure Inexpensive Currently accepted method	Slow degradation rate Residual contamination often not removed High exposure risks May require long incubation period	Surface contamination Aerobic process Low to medium contamination levels Pentachlorophenol Oil and gasoline PAH
Composting	More rapid reaction rates Inexpensive Self-heating	Needs bulking agents Require aeration Nitrogen addition often necessary High exposure risks Residual contamination Incubation periods are months to year	Surface contamination Aerobic process Can treat high contamination levels Aerobic sewage sludges Oil and gasoline
In situ	Relatively inexpensive Low exposure risks Excavation not required	Low degradation rates Less control over environmental parameters Need good hydrogeological site characterization Incubation periods are months to years	Deep contamination Aerobic or nitrate reducing conditions Low to medium contamination levels Oil and gasoline Chlorinated aromatics Chlorinated hydrocarbons
Slurry bioreactor	Good control over parameters Good microbe/compound contact Enhances desorption of compound from soil Incubation periods are days to weeks	High capital outlay Limited by reactor size High exposure risks	Surface contamination Recalcitrant compounds Soils that bind compound tightly Aerobic or anaerobic process

There are a number of variations of the bioremediation process that will be described in the following section. In a typical system, the groundwater is pumped to the surface. Nutrients to optimize microbial activity and a source of oxygen (such as  $H_2O_2$ ) are added via a mixing tank. The groundwater is then returned to the soil and the process continues. A typical system, shown in Figure 2.4 consists of injection and production wells and equipment for addition and mixing of nutrient and a source of oxygen. This was the original setup used by Raymond et al. [1976] for treating gasoline below the water table. Several variations of this system as applied to above and below the water table has been depicted by many authors including Lee et al. [1988], Litchfield [1993], McDonald and Rittmann[1993]. Different types of aerobic and anaerobic bioreactors are depicted by Armenante [1993]. The process of bioventing as compared with conventional soil venting is discussed in detail by Reisinger et al. [1994] and Eyk [1994].

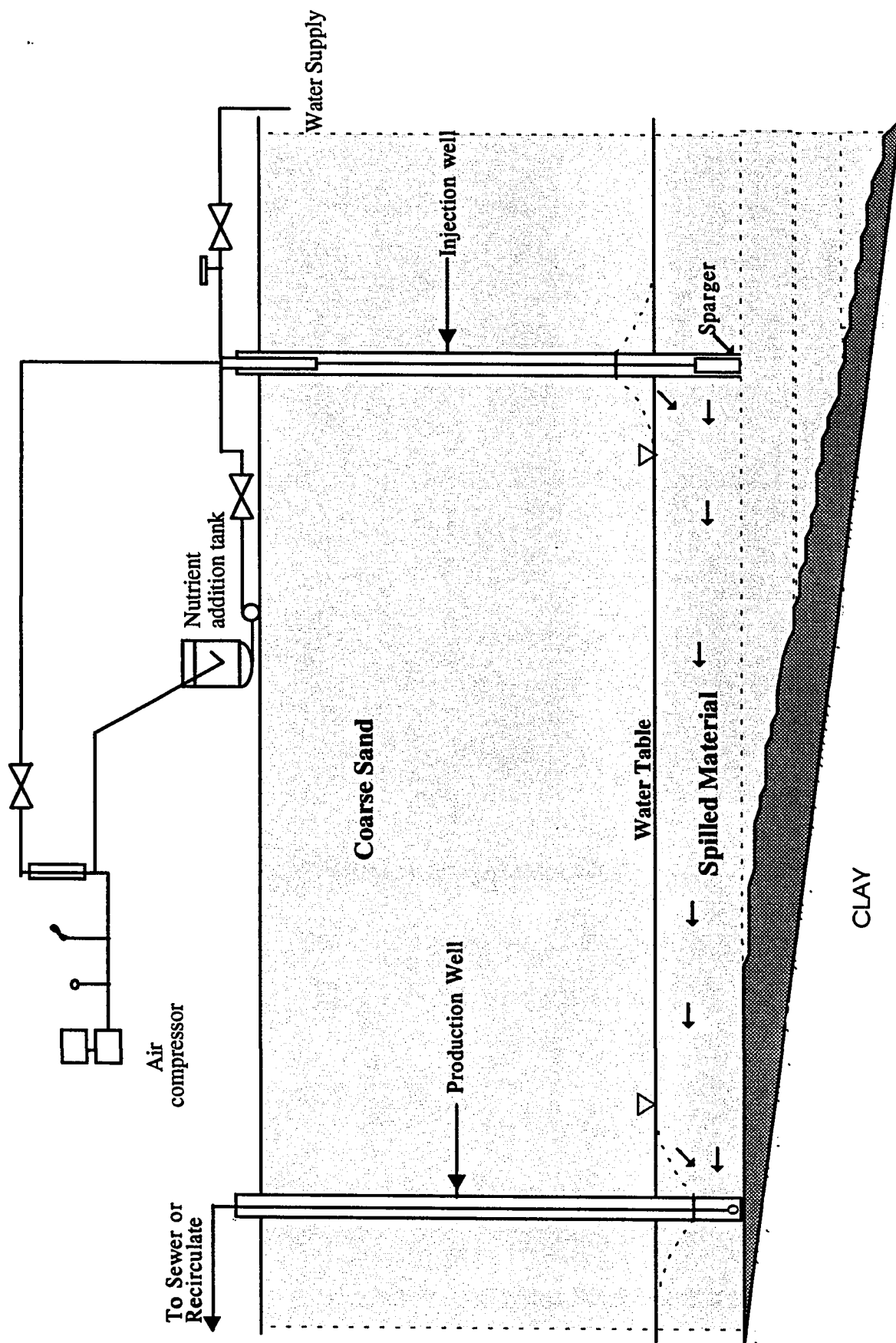


Figure 3 Typical in situ bioremediation scheme

## 2.7 Selected Full-Scale And Pilot Studies

Solid-phase bioremediation has been used for over 30 years for the remediation of petroleum contaminated soils in unlined land treatment systems [API, 1983]. The use of prepared bed reactors was introduced in the last decades with few of the first applications were done by Patnode [1987] and [Torpy et al., 1989]. In situ applications were pioneered in 1972 by Sun Refining to remediate a gasoline spill [Raymond et al., 1976]. Since then, a number of engineering advancements in nutrient and oxygen delivery systems has been made [Ryan et al., 1991]. It was estimated that more than 100 in situ projects had been implemented before 1991 [Ryan et al., 1991]. Most of the applications have been related to light petroleum derivatives associated with gasoline and diesel contamination. Slurry-phase systems are fairly recent innovation. Most applications involved treatment of sludges and contaminated soils resulting from the closure of impoundments containing petroleum refining wastes, petroleum production wastes and petrochemical waste [Ryan et al., 1988].

A summary of twenty different type of bioremediation applications on different organics from Superfund Record of Decisions (RODs) has been given by Ryan et al. [1991]. A summary of 132 case studies undertaken by US remediation companies is depicted by Devine [1994]. More than 80% (106) were at field or full-scale level and more than 62% (82) were on petroleum-related waste; 21 cases (16%) were on in-situ treatment of water and groundwater only and 53 cases (40%) were on land treatment and bioreactors. Selected published case studies of the in situ bioremediation of gasoline, diesel and oil are presented in Table 5. More published case studies on PAHs and wood preservatives are shown in Table 6.

Table 2.10 Selected published case studies of the in situ bioremediation of gasoline, diesel and oil (Litchfield, 1993)

Location	Type and Amount of Contaminant	Full field (F) or pilot (P) test	Geology* Area	Nutrient addition	Electron Acceptor	Duration of treatment	Level of treatment	Problems	Company®	Reference
Watsonville, California	Gasoline 1000 gal	F	CL,ML,SC NS, SW	NH <sub>4</sub> <sup>+</sup> , phosphates	H <sub>2</sub> O <sub>2</sub>	13 months	> 90%	NS	DERS	Litchfield et al., 1989
Long Island, New York	Gasoline 10000 gal	F	GL, CL lenses	NH <sub>4</sub> <sup>+</sup> , phosphates	H <sub>2</sub> O <sub>2</sub>	64 months	> 99%	NS	DERS	Lee and Raymond, 1991
Northern Indiana	Gasoline 80000 gal	P	GM	NH <sub>4</sub> <sup>+</sup> , phosphates	H <sub>2</sub> O <sub>2</sub>	6 months	63-80%	Ca, Mg, Fe	IT	Anonymous, 1987
Michigan refinery	petroleum hydrocarbons	P	NS	NS	aerated water in basin	106 days	78-90%	Rain and flooding	OHM	Schmitt and Caplan
Southern California	Gasoline NS	F	GL, CL, & SM lenses	RESTORE™ 375	H <sub>2</sub> O <sub>2</sub>	6 months	84 to >99%	Another spill	IT	Brubaker and Exner, 1988
Oakland, California	Gasoline, 5000 ppm in soil	F	GM, ML	NS	H <sub>2</sub> O <sub>2</sub>	9 months	80%	NS	HL	Mote et al., 1990
Canada	Gasoline NS	F	Fill GP, fractured bedrock	NH <sub>4</sub> <sup>+</sup> , phosphates	H <sub>2</sub> O <sub>2</sub>	6 months	A = 95% B=40-50 % C = 85 %	Sorbed material	GTI	Brown et al., 1989
Upper Rhine Valley, Germany	Oil spill, est. 17 tons	F	SW, ML	NH <sub>4</sub> <sup>+</sup> , phosphates	Nitrate 300 ppm	24 month	> 95%	Iron and methane	NS	Warner, 1985
Camp, Grayling Army Airbase, Grayling, Michigan	Diesel, 16000 - 25000 ppm in soil	F	SW, CL lenses	NS	Aboveground bioreactor aerated water recycled	11 months	> 95%	NS	Hunter	Lieberman et al., 1989

Table 2.10 Selected published case studies of the in situ bioremediation of gasoline, diesel and oil (continued)

Location	Type and Amount of Contaminant	Full field (F) or pilot (P) test	Geology*	Area	Nutrient addition	Electron Acceptor	Duration of treatment	Level of treatment	Problems	Company©	Reference
Eastern Missouri	Gasoline, 30,000 gal	F	Impermeable tile fractured bedrock	360 X 720 ft	NS	H <sub>2</sub> O <sub>2</sub>	32 months	> 99%	NS	J. Mathes	Bell and Hoffman, 1991
Southern California	Gasoline, 200 ppm total TPH	F	SC	Groundwater at 60 ft and approximately 40 X 50 ft on the surface	ACT™ (NH <sub>4</sub> <sup>+</sup> , phosphates)	H <sub>2</sub> O <sub>2</sub>	10 months	> 99%	Low permeability	CAA	Fogel et al., 1991
Amsterdam, The Netherlands	BTEX / mineral oil, 200 and 6000 mg/kg soil, respectively	F	NS	NS	NS	Oxygenated water	3 months	79% of the oil; 98% BTEX	NS	DRM	Steps, 1988
Arnhem, The Netherlands	Mineral oil, 10,000 mg/kg soil	F	NS	21 ft of unsaturated soil to groundwater	NS	KNO <sub>3</sub>	Ongoing	After 2 months, 5-56%	NS	DRM	Steps, 1988
Eastern Pennsylvania	Gasoline 900 gal	F	SP, MH	540 X 400 ft	NH <sub>4</sub> <sup>+</sup> , phosphates	H <sub>2</sub> O <sub>2</sub>	24 months	≈ 99 %	Drought water table	GTI	Litchfield et al., 1988

\*Abbreviations are based on the Unified Soil Classification System

©Company names: DERS = DuPont Environmental Remediation; OHM = OH Materias, Inc.; IT = Interantional Technology Corp., GTI = Groundwater Technology Inc.; Hunder = Hunder Bioscience, Inc.; J. Mathes = Jon Mathes & Associates; CAA = Cambridge AnalyticalAssociates Bioremediation Systems; DRM = De Ruiter Milieutechnologie B. V.

NS = not stated.

Table 2.11 Full scale bioremediation projects on PAH compounds

Researchers	Type of soil	Contamination	Type of project	Conditions	Time and Removal
Bewley et al., 1989	30500 m <sup>3</sup> fill and clayey soil	Disused refinery, crude and mineral oil 9763 mg/kg dry soil	Layered, homogenous, treatment bed	Moisture added, inoculated in layers with microbes from site, nutrients, surfactants, bed rotovation	12 months, about 50% in 8 wks, about 99% in 12 months
Brubaker and Stroh, 1992	MGP site soil (1%-50% organic) 3%-26% fines	Hypothetical PAH 1000, 250, 100 mg/kg	(1) Pump and treat vs (2) in situ treatment	(1) moisture, aeration, nutrients (2) nutrients, DO injected	0.8%-26% in 12 weeks 1st order k=0.024-0.054 for unsaturated and (0.19-0.57) for saturated soil
Ellis et al., 1990	Silty clay, Sandy clay, gravel	Oil from refinery site, 2000 m <sup>3</sup> 12980 mg/kg	(1) Treatment bed, 45m x 8m x .6m, HPDE liner (2) In situ, extraction and infiltration (10m x 20m x 8m)	(1) moisture (15%), temperature (25 °C), aeration, nutrients, surfactant, microbial inoculations (2) aeration, nutrients, surfactant, microbial inoculations	(1) 90% in 34 weeks (2) 86% in 15 weeks
Ellis et al., 1991	Sandy clay, clay-loam, bricks, stones	(1) Creosote 10-32000 mg/kg soil 0.9-4.5m depth (2) Creosote 3500 m <sup>3</sup> 10000-30000 mg/kg	(1) In situ treatment 15000 m <sup>3</sup> sheet pile contaminant (2) Treatment bed, concrete based, gravel underlayer, leachate collection, 80m x 60m x 0.75m	(1) MSM DO 8.5 mg/L + 35% H <sub>2</sub> O <sub>2</sub> , nutrients, microbial inoculations surfactants, temperature (2) Moisture (20% w/w), MSM, nutrients, microbial inoculations (10 <sup>6</sup> cell/g soil, surfactants (5%), bed rotovation (2 weekly)	(1) about 60% in 4 months (2) 42% in 35 days, 64% in 4 months
Jerger et al., 1994	From clay to gravel (40% sand)	Creosote and PAHs from wood preserving wastes 6,100 m <sup>3</sup> of sludge	Soil classification/washing and treatment of the concentrate in slurry reactors, HPDE liner, leachate collection system Company: OHM	Nutrients (8-16 mg/l), microbial inoculations (log(CFU) = 6.0-9.4), DO (1-6 ppm), temperature (33 °C), pH (7)	85-95% in 20-30 days (mainly in first 10 days)
Lewis, 1993	Coarse sand	Creosote	Slurry phase treatment with five EIMCO Biolift reactors, 64 litres each	Nutrients, microbial inoculations (9.3 x 10 <sup>7</sup> cell/g soil), surfactants, temperature, agitation, aeration	90%-96% in 12 weeks, 97.4% 2- and 3-ring PAHs, 90% 4- and 6 ring PAHs



Table 2.11 Full scale bioremediation projects on PAH compounds (continued)

Researchers	Type of soil	Contamination	Type of project	Conditions	Time and Removal
Johnson and Leuschner, 1991		2 sites (1) Coal tar from MGP (2) Creosote PAHs and PCP	CROW process followed by (1) in situ treatment of residuals in slurry reactor and (2) inocula development in flush water	Aerobic and anaerobic treatment with and without surfactant and (1) nutrients, microbial inoculations, mixing, air or nitrogen flow, pH, temperature, HRT=5 days, DO > 3 ppm (2) all of the above + NO <sub>3</sub> HRT=10 days, DO > 0.5 ppm (anaerobic) moisture (8-12%), temperature (23 °C), tiling, nutrient	The CROW process removed 60% from site 1 and 80% from site 2 (1) 76% (17 to 4), 80% with surfactant in 6 weeks both aerobically and anaerobically 96% (160 to 6) and 82% (160 to 30) without CROW (2) 99% in aerobic, 97% in anaerobic 20% to 95% in 90 days (depending on group of PAH and nutrient addition)
Muller et al., 1991b	Sandy subsurface and surface soil Clay to gravel	Creosote	Landfarming chamber	(1) tilling, nutrients, microbial inoculations (2) nutrients, microbial inoculations, DO, pH, temperature (3) nutrient, oxygen	(1) PAHs: 90% (300 to 30 mg/kg) in 140 days: PCP (100%) (2) 100% of both PAHs and PCP
Piotrowski, 1991		Creosote, PCP, diesel	(1) Land treatment unit (LTU), one-acre (2) Extraction and treatment with aboveground bioreactors (3) In situ treatment	(1) moisture (80%), temperature (11-28 °C in winter and 18-34 °C in summer), nutrients, microbial inoculations 7 x 10 <sup>5</sup> cell/g soil	PAHs: 1485 to 35 mg/kg in 207 days PCP: 680 to 6 mg/kg in 207 days
Seech et al., 1994	Fine sandy loam	Creosote (PCP and PAHs, and TPH)	on-site/ex situ treatment bed, 12 tonnes of soil, 0.5m deep, HPDE liner, enclosed with steel structure	(1) Nutrients, with or without bacteria (2) Nutrients, DO, pH, and bacteria (10 <sup>7</sup> to 10 <sup>8</sup> ) (3) Nutrients, oxygen, pH	(1) 50% , mostly by sorption (2) 95% , mostly by biodegradation (3) 54%-96% in 3-12 weeks
Tremaine et al., 1994		Creosote	Wastewater treatment by (1) Wetlands (2 m <sup>2</sup> ) (2) Fixed-film bioreactors and Soil treatment (3) Land treatment (3-12 wks)	Recirculation and treatment using (1) Lab: upflow aerated column (UAC) and RBC (2) Site: UAC and Trickling filter	(1) PAHs: 99-100% ; BTEX (94-100%), Phenols (40-97%) in 37-146 days, (2) PAHs: 67-100%; BTEX: 69-79%; Phenols: 9-86% in 38 days
Van der Hock et al., 1989		Former asphalt plant-PAHs, BTEX, phenols 1.5 hectare area upto a depth of 10m PAHs= 6.1, BTEX= 5.5; Phenols= 12 mg/L			

CROW®: Contained Recovery of Oily Wastes, HDPE = High-density polyethylene

## 2.8 Advantages and Disadvantages of Bioremediation

Many authors [Lee et al. 1988; Lapinkas, 1989; Gabriel, 1991; Nicholas, 1992; Noonan and Curtis, 1990] summarized the advantages and disadvantages, strength and weakness, potentials and pitfalls of bioremediation. A summary of the advantages of bioremediation is given below:

- can be used to treat hydrocarbons and certain organic compounds, especially water-soluble pollutants and low levels of other compounds that would be difficult to remove by other methods,
- environmentally sound because it does not usually generate waste products and typically results in complete degradation of contaminants,
- utilizes the indigenous microflora and does not introduce potentially harmful organisms,
- fast, safe, and generally economical,
- relatively simple technology compared with other on-site treatment technologies,
- little or no excavation required, minimal site disruption, and reduced potential for public exposure,
- treatment move with the groundwater, good for short-term treatment of organic contaminated groundwater,
- treatment process includes restoration of both soil and groundwater.

Litchfield [1993] mentioned four major factors which can limit the application of in situ bioremediation: time, metabolic by-products or recalcitrance, geochemistry and hydrogeology, and environmental factors. Other disadvantages of bioremediation discussed by Lee et al. [1988] are as summarized below:

- cannot be used where a quick startup is needed, acclimatization microorganism typically takes 4-6 weeks,
- it is not successful in a start/stop mode; that is it must be continued 24 hours per day, 7 days a week,
- can be inhibited by heavy metals and some organics,
- difficult to degrade chlorinated hydrocarbons, may create carcinogenic vinyl chloride,
- bacteria can plug the soil and reduce circulation,
- introduction of nutrients can adversely affect nearby surface waters,

- residues may cause taste and odor problems,
- labor and maintenance requirements may be high, especially for long-term treatment,
- long-term effects are unknown,
- may not work for aquifers with permeability that do not permit adequate circulation of nutrients.
- Other notable problems encountered by practitioners are [Gabriel, 1991]:
- Insufficient coordination/integration of the diverse staff or expertise required
- Regulatory barriers
- Unrealistic clean-up goals and/or expectations
- Scale up from bench/pilot level to the field
- Dispersed and/or unavailable data base, or lack of awareness
- Failure to consider full range of remediation options or configurations
- Liability for failure to achieve goals.

Attempts at bioremediation can be hampered or doomed from the outset if the project team does not include appropriate or diverse expertise. The typical team should have experience in microbiology, engineering, hydrogeology, soil science and chemistry.

# **Chapter 3**

# **EXPERIMENTAL SETUP**

### 3 EXPERIMENTAL SETUP

The experimental setup consists of a big sealed tank filled with sand and acclimated microorganisms capable of biodegrading BTX compounds. The purpose of the sand tank was to simulate steady one-dimensional flow coupled with BTX transport through saturated sandy soil. BTX compounds were pumped with syringe pumps and water containing nutrients and  $H_2O_2$  was pumped with a metering pump. A detailed description of the sand tank model and experimental procedure is given below.

#### 3.1 Physical Description

##### 3.1.1 General set-up

As shown in Figure 3.1, the overall dimension of the whole tank is 860 cm long  $\times$  30 cm wide  $\times$  30 cm high. The base, top as well as sides are made of 10-mm-thick plexy glass sheet. At both end of each tank are water tanks (30 cm  $\times$  30 cm  $\times$  30 cm) separated from the sand tank by screens. The screens are made up of perforated plexy glass-sheet facing the water tank and rubbing pad at the middle and a piece of cloth facing the sand. This screen confines the sand medium and provides inflow and exiting flow uniformly across the width of the tank. Sampling ports are located at every 100 cm along the center of the tank. Two piezometer ports are located at two ends of the enclosed sand to monitor the head and permeability changes. The sampling ports are located in center-line of the side walls and made of a stainless steel tube (1/8 in) penetrating 15 cm to the sand. At the outer end of the tube a small tygon tube (1/8 in)

is tightly fitted and clamped by a Hoffman screw. By opening the Hoffman screw samples are taken using a syringe. First few milliliters of samples were discarded to ensure procurement of a representative sample from the center of the tank. The piezometers are placed at the top in order to determine the hydraulic conductivity of the medium.

### ***3.1.2 Inflow & Outflow***

A metering pump (Chem-feed Injector, Cole Parmer, Model 50000-073) with a capacity of upto 120 ml/min (1.9 GPH) was used to pump the water mixed with nutrient through the sand tank. This pump is capable of pumping at any steady flow rate upto 120 ml/min at a pressure not exceeding 125 psi. BTX chemicals and  $H_2O_2$  were pumped with Syringe pump (Sage model 152 syringe pump by Orion, U.S.) Figures 3.2 and 3.3 presents two photographs showing the sand tank model with the pumps. Depending on the syringe size (5 to 100 ml), the chemical flow rate can be set to 102 different setting with the flow rate dial and syringe size setting. The minimum flow achievable flow rate is 0.016 ml/hr with a 5 ml syringe and the maximum flow rate is 99 ml/hr with a 100 ml syringe.

### ***3.1.3 Sand***

The raw sand was collected from SAFWA area (on the way from Dhahran to Ras Tanura) in the Eastern province of Saudi Arabia selected primarily due to its coarseness and uniformity. The sand was then sieved to discard the too coarses and too fines so that a porous media with relatively high permeability can be formed. In situ

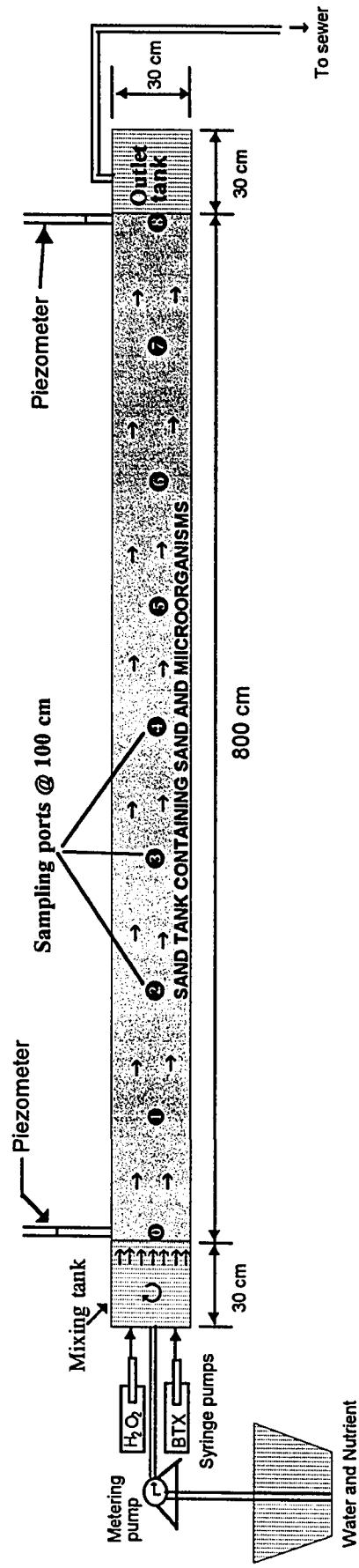
bioremediation is not recommended for soil with permeability less than  $10^{-4}$  cm/s. [Thomas and Ward, 1992]. In USA most of the in situ bioremediation has been applied to soils ranging in conductivity from  $10^{-3}$  to 2.1 cm/s [Staps, 1989]. The hydraulic conductivity of the medium under study is about 0.35 cm/s. The sieve analysis is roughly as below. The sand is brownish yellow-colored and fairly round-shaped.

U. S. Sieve No	Percentage Retained (by weight)	
	Individual	Cumulative
16	0.0	0.0
20	75.0	75.0
30	25.0	100.0

## 3.2 BTX Compounds

### 3.2.1 Benzene ( $C_6H_6$ )

Benzene is a clear, colorless to light yellow watery-liquid with an aromatic or gasoline-like odor. It has a specific density of 0.87366 at 25/4° C, solubility 1800 mg/L at 25° C, boiling point 80.1 ° C and vapor pressure 95.2 mm at 25° C [Montgomery and Welton, 1990]. Benzene is widely used in the manufacturing of ethylbenzene (preparation of styrene monomer), dodecylbenzene (for detergent), cyclohexane (for nylon), nitrobenzene, aniline, maleic anhydride, diphenyl, benzene hexachloride, benzene sulfonic acid, phenol, dichlorobenzene, insecticides, pesticides, fumigants, explosives, aviation fuel, flavors, perfume, medicine, dyes, and other organic chemicals; paints, coatings, plastics, raisins; food processing, photographic chemicals; nylon intermediates; paint removers; rubber cement; antiknock gasoline; solvent. It is highly carcinogenic and immediately dangerous to life or health (IDLH) at 2,000 ppm.



**Figure 3.1 Schematic of the sand tank model**



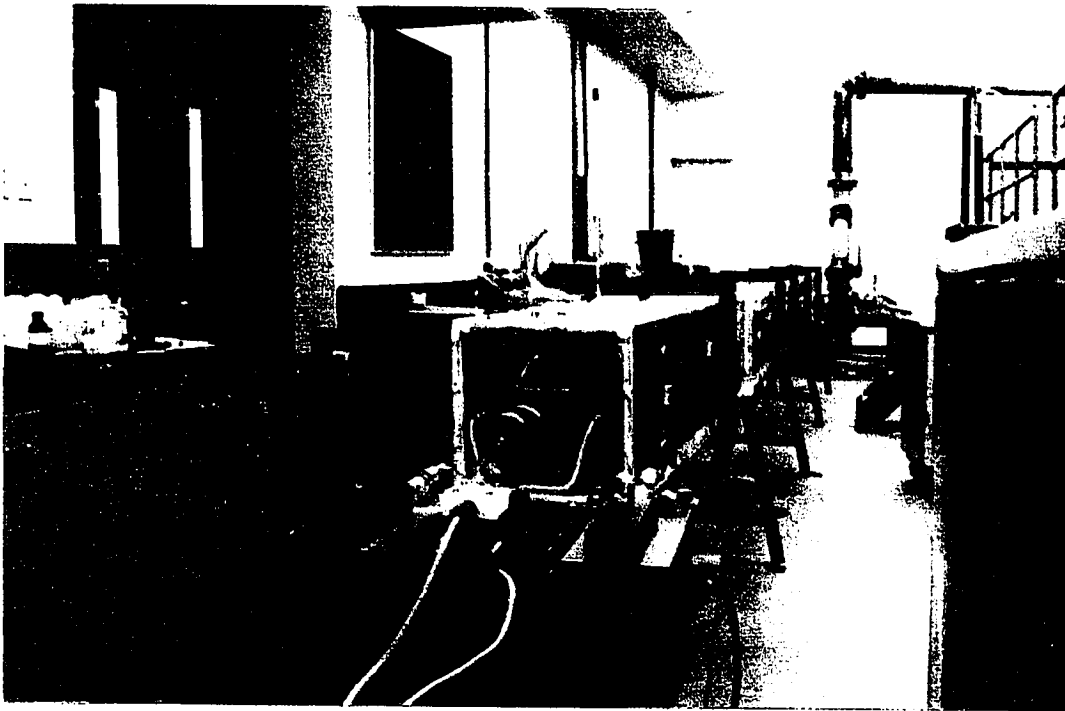


Figure 3.2 Photograph showing overall view of the sand tank model including the pumps

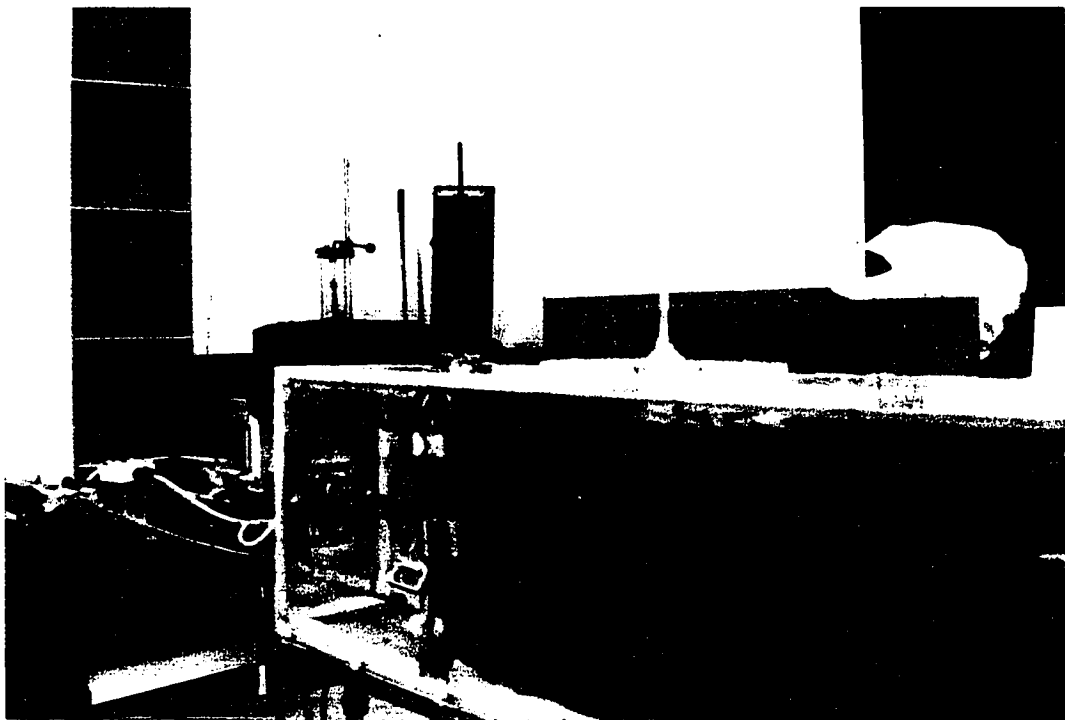


Figure 3.3 Photograph showing the exploded view of the a portion of the sand tank model including the mixing tank and the pumps

### 3.2.2 Toluene ( $C_7H_8$ )

Toluene is a colorless, water white liquid with a pleasant odor similar to benzene. It has a specific density of 0.86233 at 25/4° C, solubility 535 mg/L at 25° C, boiling point 110.6 ° C and vapor pressure 22 mm at 20° C [Montgomery and Welcom, 1990]. Toluene is widely used in the manufacturing of caprolactum, saccharin, medicines, dyes, perfumes, benzoic acid, trinitrotoluene (TNT), and other benzene derivatives; solvents for paints and coatings, gums, resins, rubber, oils, and vinyl compounds; adhesive solvent in plastic toys and model airplanes; diluent and thinner for nitrocellulose lacquers; detergent manufacturing; aviation gasoline and high-octane blending stock; preparation of toluene diisocyanate for polyurethane resins. It is highly carcinogenous and immediately dangerous to life or health (IDLH) at 2,000 ppm.

### 3.2.3 O-Xylene ( $C_8H_{10}$ )

O-xylene is a clear colorless, liquid with a specific density of 0.87596 at 25/4° C, solubility 204 mg/L at 25° C, boiling point 144.4° C and vapor pressure 4.34 mm at 25° C [Montgomery and Welcom, 1990]. o-Xylene is used in the preparation of phthalic anhydride, terephthalic acid, isophthalic acid; solvent for alkyl resins, lacquers, enamels, rubber cements; manufacturing of dyes, pharmaceuticals, and insecticides; motor fuels. It is highly carcinogenous and immediately dangerous to life or health (IDLH) at 1,000 ppm.

A summary of important properties of benzene, toluene, and o-xylene is presented in Table

3.1.

Table 3.1 Important properties of BTX compounds

Compounds (formula)	Specific density at (25/4° C)	Solubility, mg/L at (25/4° C)	Boiling point ° C	Vapor pressure mm
Benzene (C <sub>6</sub> H <sub>6</sub> )	0.87366	1800	80.1	95.2
Toluene (C <sub>7</sub> H <sub>8</sub> )	0.86233	535	110.6	22.0
o-Xylene (C <sub>8</sub> H <sub>10</sub> )	0.87596	204	144.4	4.34

The allowable limits of BTX compounds in soils and groundwater depend on the nature and concentration of polluted substances. A test framework used in Netherlands [NVP, 1990] is built up of three levels of pollutions designated as A, B, and C. Level A is a reference value below which there is no demonstrable pollution. Level B is an assessment value, pollutants above this level should be investigated more thoroughly. Level C is the assessment value above which pollutants must be treated.

Present in Compounds	Soil (mg/Kg soil)			Groundwater µg/L		
	A	B	C	A	B	C
Benzene	0.05	0.5	5	0.2	1	5
Toluene	0.05	3	30	0.2	15	50
o-Xylene	0.05	5	50	0.2	20	60

### 3.3 Sand Tank Preparation

#### 3.3.1 Background work

The top of the sand tanks has fifteen-cm-dia holes at every 50 cm. Sand was placed into the empty tanks through these holes in layers of approximately 3 cm. With placement of each layer, the sand was hand rodded and tamped to achieve a high degree of consolidation. The lids of these holes were screwed and sealed. Water was passed through the sand at high velocity by pumping. After few days of pumping, when the sand subsided,

pumping was stopped and the lids were open again. More sand was put and gently rodded and tapped to allow any entrained air to escape. This procedure was actually repeated until no further subsiding of sand was observed. High degree of compaction was needed to ensure a uniform porous media and to avoid any kind of short circuiting of flow.

Pumping was then resumed at various flow rates. The flow rate through the tanks was measured by collecting the effluent from the outlet tube for a period of time.

A permeability device was used to simulate the compaction corresponding to the mean permeability. The porosity of the sand medium was determined to be 0.36 by weighing the amount of sand needed to fill a known volume using a specific gravity of 2.65.

### ***3.3.2 Acclimation of the microorganisms***

Acclimation is defined as the amount of time between exposure of microorganisms to a substrate and detection of substrate biodegradation [Thomas and Ward, 1992]. Acclimatization may occur as a result of an increase in the number of contaminant-degrading organisms, genetic changes which confer degradation capabilities, enzyme induction, and depletion of a substrate which is preferably metabolized [Wiggins et al., 1987]. Detection of pollutant biodegradation within a relatively short incubation period (days to weeks) also has been reported for samples of uncontaminated subsurface material [Wilson et al., 1983, Swindoll et al., 1988, Aelion et al., 1989]. The 7-day screening test for microbial degradation of benzene and toluene revealed rapid adaptation at concentration of 5 and 10 mg/L [Tabak et. al., 1981]. Lodaya et al. [1991] also

acclimated microorganism to a mixture of BTX compounds using 10 ppm (each) solution for seven days.

In the present study, the microorganisms in raw sewage collected from North Aramco Watewater Treatment Plant, Dhahran were acclimated to a mixture of BTX compounds for two weeks. An increase in the plate count of the mixed culture confirmed the acclimatization. Chemical analysis of the raw sewage is listed in Table 3.2.

Table 3.2 Chemical properties of the raw sewage

Parameter	Average	Range
BOD	110	80-140
TSS (mg/l)	90	80-120
VSS (mg/l)	70	64-96
COD(mg/l)	200	180-250
Alkalinity(mg/l)	150	120-180
TKN (mg/l)	15	12-25
Total P (mg/l)	5	4-7
pH		6-9

Twenty liters of raw sewage were put into the sand tank model and BTX compounds at concentration of 5 ppm each and  $H_2O_2$  at concentration starting from 50 ppm (to 200 ppm at step of 50 ppm every other day) were slowly pumped (at velocity of 0.5 meters per day) into the sand tank for two weeks. Plate count of the mixed species indicated acclimatization of BTX degrading bacteria.

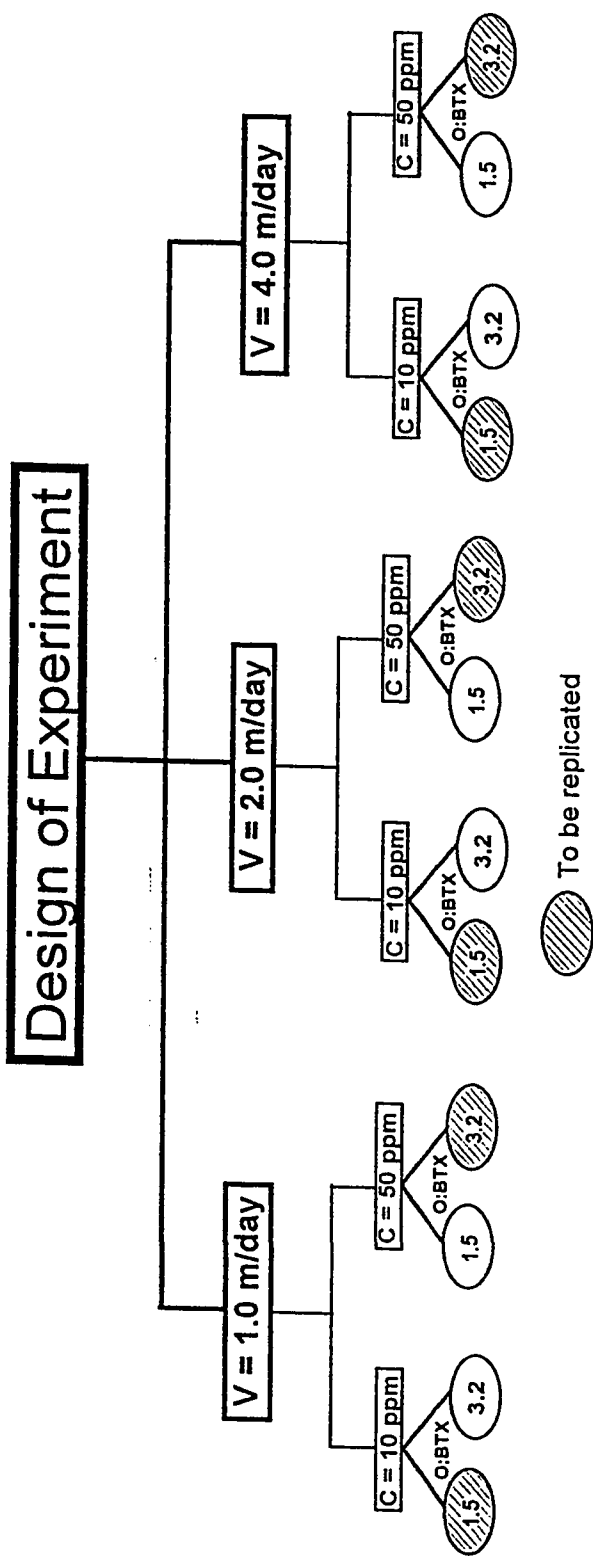
### 3.4 Experimental Procedure

#### 3.4.1 Design of Experiment

The experimental variables are shown in Figure 3.4. A  $3(2^2)$  factorial design has been used to study the three factors, groundwater velocity, BTX concentration and dissolved oxygen (DO) on biodegradation rate of BTX compounds. Experimental runs involving low concentration and low DO and those involving high concentration and high DO has been replicated. Thus a total of 18 experimental runs has been performed of which 6 runs are replicated. The observations will be modeled by the linear statistical model

$$y_{ijkl} = \mu + \tau_i + \beta_j + \gamma_k + (\tau\beta)_{ij} + (\tau\gamma)_{ik} + (\beta\gamma)_{jk} + (\tau\beta\gamma)_{ijk} + \varepsilon_{ijkl} \quad \left\{ \begin{array}{l} i = 1,2,3 \\ j = 1,2 \\ k = 1,2 \\ l = 1(\text{or } 2) \end{array} \right. \dots\dots\dots (3.1)$$

where  $y_{ijkl}$  represents the observation taken under the  $i$ th level of velocity,  $j$ th level of DO,  $k$ th level of concentration in the  $l$ th replicate (in few cases of levels we have only one replicate).  $\mu$  is the overall mean effect,  $\tau_i$  is the mean of the  $i$ th level of the velocity,  $\beta_j$  is the mean of the  $j$ th level of the  $j$ th level of the DO,  $\gamma_k$  is the mean of the  $i$ th level of the concentration,  $(\tau\beta)_{ij}$  is the mean of the interaction effect between  $i$ th level of the velocity and  $j$ th level of DO,  $(\tau\gamma)_{ik}$  is the mean of the interaction effect between  $i$ th level of velocity and  $k$ th level of concentration,  $(\beta\gamma)_{jk}$  is the mean of the interaction effect between  $j$ th level of DO and  $k$ th level of concentration,  $(\tau\beta\gamma)_{ijk}$  is the mean of the interaction effect between  $i$ th level of velocity,  $j$ th level of DO and  $k$ th level of concentration,  $\varepsilon_{ijkl}$  is a random error component. All the factors and their interaction are fixed for the present study.



Other Environmental Parameters:

Nutrients: C:N:P (100:10:1)

Temperature: 25° C

Figure 3.4 Design of experiment

### 3.4.2 Control Runs

As shown in Table 3.1, the BTX compounds are highly volatile, benzene being the highest of all. Their solubility are also very low. To account for the volatilization losses, three control runs has been performed at three velocities with BTX concentration in between high and low values.

### 3.4.3 Typical Experiment

Variables adjusted in a typical experiment was pore water velocity, BTX concentration and DO. Pore water velocity was adjusted by setting the flow through the metering pump according to the computed value of porosity. Concentration of the BTX compounds and DO was adjusted by setting the flow rates of the syringe pumps. Peizometer readings were taken at every cycle of the detention time (3 to 4 times for every runs) to compute the hydraulic conductivity, porosity and pore water velocity changes. Darcy's formula was applied to find the coefficient of hydraulic conductivity K for different discharges. Change of pore water velocity (v) and porosity ( $\Phi$ ) was computed using the following equations [Engineering Properties of Soil and their measurement by Joseph E Bowle, 99 p.]: Since the initial porosity is known, porosities at subsequent time step can be computed.

$$\frac{K_2}{K_1} = \left( \frac{e_2}{e_1} \right)^2 \dots\dots\dots (3.2)$$

where e is the void ratio defined as the ratio of void volume to the solid volume. Since  $\Phi$  is the ratio of void volume to the total volume, it can be shown that

$$e = \frac{\Phi}{1 - \Phi} \dots\dots\dots (3.3)$$

$$\Phi = \frac{e}{1 + e} \dots\dots\dots (3.4)$$



The pore water velocity,  $v$  was computed from the known darcy velocity as follows:

$$v = \frac{Q}{A\Phi}$$

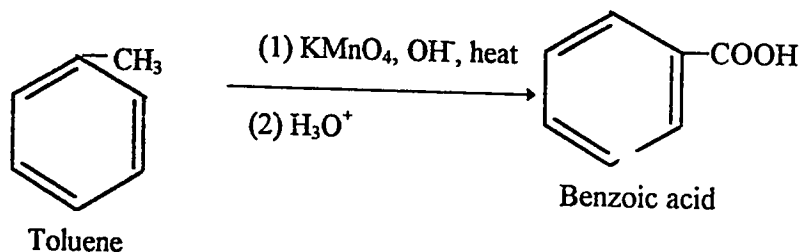
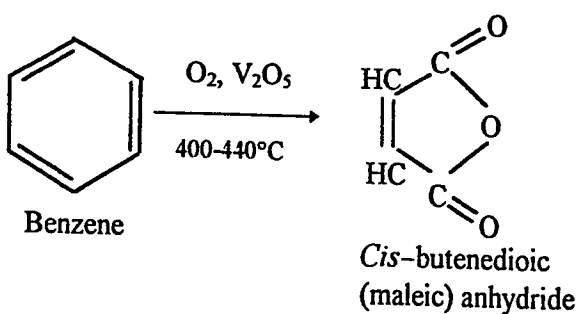
The compounds were determined by the flame ionization detector (FID) of the gas chromatograph (GC) [Corseuil, 1994; Frankenberger and Emerson, 1989; Lodaya et al., 1991; Nubbe et. al., 1990]. A number of GC procedure including direct injection of aqueous sample [Karlson & Frankenberger, 1989; Robinson et al., 1990], microsolvent extraction [Barker et al., 1987], purge-and-trapp [Chiang et al., 1989; Hutchins et al., 1991; Corseuil, 1994], headspace analysis [Anid et al., 1993; Corseuil, 1994] has appeared in literature for BTX analysis using GC. Direct injection of liquid samples in a Varian 6000 Gas Chromatograph equipped with FID and a 2-m 3% OV-1 on Chromosorp WHP (80/100 mesh) stainless steel packed column. A typical elution sequence in terms of retention time (min) of BTX consisted of benzene, 1.16; toluene 2.35; and o-xylene, 4.43. The operating condition consisted of the following: Sample size 1  $\mu$ L;  $N_2$ ; 13 ml/min,  $H_2$ ; 50 ml/min; and air 500 ml/min; column temperature, 50-325  $^{\circ}$ C, 15  $^{\circ}$ C/min; detector temperature, 340  $^{\circ}$ C.

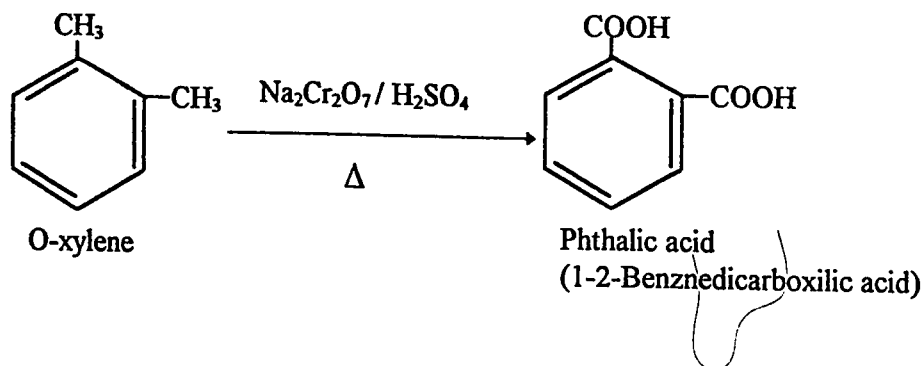
#### ***3.4.4 Correction for Abiotic Losses***

The various reasons of abiotic losses are sorption, volatilization, and chemical oxidation by  $H_2O_2$ . Corrective actions were taken to account for BTX removal due to these abiotic factors.

**Chemical oxidation** - Theoretical background of Chemical oxidation of benzene and alkyl benzenes has been reported in many standard texts [Organic Chemistry by Rowland Brown, 1975; Basic Principles of Organic Chemistry by Roberts and Caserio, 1977; Organic Chemistry by Solomons, 1984]. Benzene is very stable towards the action of ordinary oxidizing agent, such as  $\text{KMnO}_4$ ,  $\text{H}_2\text{O}_2$ ,  $\text{CrO}_3$ ,  $\text{HNO}_3$ . Under high temperature and pressure benzene can be oxidized to cis-butenedioic (maleic) anhydride by air with a vanadium pentoxide catalyst.

In the case of alkyl benzenes (toluene, xylenes, ethyl benzenes, etc.) the side chain or the alkyl group is similarly oxidized. For example, toluene is oxidized to benzoic acid by heating with dilute nitric acid or with potassium permanganate. O-xylene can be oxidized to Phthalic acid (1-2-Benzenedicarboxylic acid) by heating with sulphuric acid with hot sodium dichromate.





However, it is extremely unlikely that BTX compounds can be chemically oxidized by  $\text{H}_2\text{O}_2$  at the experimental condition (concentration, temperature). Lu [1994] performed a number of batch studies to conclude that BTX compounds are not oxidized by  $\text{H}_2\text{O}_2$  at  $\text{H}_2\text{O}_2$  concentration as high as 2,000 mg/L. Therefore chemical oxidation has been ignored in this study.

**Volatilization** - Volatilization losses in the inlet mixing tank was accounted by measuring the input concentration ( $C_0$ ) at a point which is just in the sand media at differential distance from the screen separating inlet tank and the sand tank. To account for volatilization losses in the sand media, BTX compounds were transported through the sand media sterilized with 2 gm/L mercuric chloride. The removal was estimated with first order kinetics. The gross first order removal by biodegradation and volatilization was corrected by subtracting from the first-order removal by volatilization.

**Sorption** - Removal by sorption is automatically counted when the retardation constant  $R$  is estimated. Sorption parameter ( $R$ ) was also computed from the control runs performed by sterilizing the sand tank with mercuric chloride. Besides batch studies with pure sand was performed to compute  $R$ .

As a cross check, a set of batch tests have also been conducted in the laboratory with a set of fourteen-ml-culture-tubes filled with 5 gm of pure sand and solution of BTX mixture of different concentrations. The guideline have been taken from a recent study [Zytner, 1994] conducted to assess the sorption of BTX compounds on different soil including sand. However no headspace was left in the samples and proper mixing was achieved using a rotary shaker. The mixing time was 7 days [Zytner, 1994]. However, duplicate samples were kept in the shaker for upto 2 weeks and no change of equilibrium concentration was observed. Although, all the tubes were sealed with silicon sealant, control tubes of the same concentration were kept in the rotary shaker to determine any loss due to stripping or volatilization. The concentrations selected for the volatilization blanks were identical to those used in the sorption study. One blank was prepared for every concentration. The results will be presented in Chapter 6.

#### ***3.4.5 Bacteria Plate Count***

Total bacteria plate count was conducted at the end of every run to monitor the growth and activity of the microorganisms in the sand tank. Soil samples were collected from five different locations along the length of sand tank. Total count of the mixed species ranged between  $10^6$  to  $10^9$ . Bacteria plate counts within the range of  $10^6$  to  $10^9$  colony-forming

units are considered to reflect an acceptable bacteria growth rate for successful biodegradation [Skiba et al., 1991]. Bacteria counts below  $10^6$  could indicate that bacteria were not receiving enough nutrients and food source. Counts above  $10^9$  could indicate that bacteria were too populated and could toxify themselves.

#### ***3.3.6 Volatile solids Measurement***

In this study the determination of biomass was accomplished by determining the volatile solids which refer to the portion of solids that evaporate during ignition of the soil sample at 550 C. The fixed solids after the evaporation of the volatile fraction constitute the biomass density. The sample sampling technique used for total counts is used.

#### ***3.3.7 Dissolved Oxygen Measurement***

Sand tank influent and effluent dissolved oxygen were measured to check possible oxygen limitations. The DO was measured every three days using the Winkler method of the azide modification. The DO concentrations at the effluent were always sufficient to prevent oxygen limitations.

## Chapter 4

# TRANSPORT SIMULATION

## 4 TRANSPORT SIMULATION

### 4.1 General

There are many natural processes that affect chemical transport from point to point in the subsurface. These natural processes can be arbitrarily divided into three categories: (i) Physical (advection, mechanical dispersion, molecular diffusion, density stratification, immiscible phase flow, fractured media flow ) (ii) Chemical (oxidation-reduction reactions, radionuclide decay, ion-exchange, complexation, co-solvation, immiscible phase partitioning, sorption), (iii) Biological (microbial population dynamics, substrate utilization, biotransformation, adaptation, co-metabolism). A satisfactory level of understanding of all these processes is not complete yet and consequently there are lack of theories which adequately describe or predict subsurface contaminant transport. Most attempts at quantifying contaminant transport have relied on a solution of some form of a well-known governing equation referred to as advection-dispersion equation. Advection refers to the transport of contaminants at the same velocity as the average linear velocity of groundwater given by Darcy's law. Dispersion carries solute mass from areas of high to low concentration.

Aerobic and anaerobic biodegradation of organics in the laboratory and the field has been modeled by first-order [Barrio Lage et al., 1987; Berthouex, 1991; Chiang et al., 1989; Hutchins et al., 1991; Lyman et al., 1982; Major et al., 1988; Schmidt et al., 1985; Smith and Novak, 1986; Strandberg et al., 1989; Vogel and McCarty, 1987], zero-order [Barker et al., 1987; Hutchins et al., 1991], and mixed-order [Mihelcic and Luthy, 1991, Speitel and Alley, 1991] kinetics.

van Genuchten [1980], van Genuchten and Alves [1981], and Parker and van Genuchten [1985] published a number of reports giving analytical solutions of one

dimensional advection dispersion equation supporting adsorption and first-order and/or zero-order production and decay for a number of initial and boundary conditions. The analytical solutions are based on constant pore water velocity and constant initial media concentration. In many experimental conditions, it is very difficult to maintain a constant velocity. Substantial permeability changes due to microbial growth [Taylor and Jeff, 1991; Essa, 1993] and gas production [Morgan & Watkinson, 1992] have been reported. Furthermore, it is also very difficult to have a constant initial concentration in few cases of laboratory experiments. The solution of advection dispersion equations in the present study therefore considers time dependent velocity and spatially variable background concentration.

## 4.2 Governing Equations

The advection-dispersion equation is derived by combining a mass-balance equation with an expression for the gradient of mass flux. One dimensional advection-dispersion equation allowing sorption and first and/or zero order biodegradation is given by [Parker and van Genuchten, 1984]:

$$\frac{\rho_b}{\Phi} \frac{\partial S}{\partial t} + \frac{\partial C}{\partial t} = D \frac{\partial^2 C}{\partial x^2} - v \frac{\partial C}{\partial x} + \mu_w C + \mu_s \frac{\rho_b}{\Phi} S + \gamma_w C + \gamma_s \frac{\rho_b}{\Phi} S \dots\dots\dots (4.2.1)$$

where C is the volume averaged resident concentration of the solute in the liquid phase; S is the adsorbed concentration per unit mass of the solid phase;  $\rho_b$  is the bulk density of the porous medium ;  $\Phi$  is the effective porosity; D is the dispersion coefficient, v is the seepage or average pore water velocity in the x direction;  $\mu_w$  and  $\mu_s$  are rate constants for first-order decay in the liquid and solid phase of the soil respectively; and  $\gamma_w$  and  $\gamma_s$  are the corresponding zero-order decay coefficients. The derivation of Equation (4.2.1) can be



found in Appendix A. Considering the case where adsorption is defined by a linear or linearized isotherm of the form

$$S = K_d C \dots\dots\dots (4.2.2)$$

where  $K_d$  is an empirical distribution constant. Substituting (2) into (1) we get

$$D \frac{\partial^2 C}{\partial x^2} - v \frac{\partial C}{\partial x} + \mu C + \gamma = R \frac{\partial C}{\partial t} \dots\dots\dots (4.2.3)$$

where the dimensionless retardation factor  $R$  is defined as

$$R = 1 + \rho_b K_d / \Phi \dots\dots\dots (4.2.4)$$

and the new rate constants  $\mu$  and  $\gamma$  are given by

$$\mu = \mu_w + \mu_s \rho_b K_d / \Phi \dots\dots\dots (4.2.5)$$

$$\gamma = \gamma_w + \gamma_s \rho_b K_d / \Phi \dots\dots\dots (4.2.6)$$

### 4.3 Boundary Conditions

Solutions of Equation (4.2.3) needs appropriate initial and boundary conditions. The initial condition is usually of the form

$$C(x,0) = C_i \dots\dots\dots (4.3.1)$$

$$C(x,0) = f(x) \dots\dots\dots (4.3.2)$$

where  $C_i$  is the constant initial concentration of the media and in the second case it is a function of  $x$  along the length of the media. Depending on experimental conditions, a variety of boundary conditions, such as Dirichlet's, Neuman's, mixed, and decaying can be applied at the inlet boundary [van Genuchten, 1982; Srinivasan and Mercer, 1988]. Depending on whether the measured concentration is flux-averaged or volume averaged [Parker and van Genuchten, 1985], the boundary conditions that can be applied at the inlet boundary ( $x=0$ ) are given by

$$C(0,t) = g(t) \dots\dots\dots (4.3.3)$$

$$C - \frac{D}{v} \frac{\partial C}{\partial x} \Big|_{x=0} = g(t) \dots\dots\dots (4.3.4)$$

Where  $g(t)$  is the concentration of the solute injected at the inlet boundary which is either a constant or a function of time. For the outlet boundary the following condition can be applied [Parker and van Genuchten, 1984; Kreft and Zuber, 1978].

$$\frac{\partial C}{\partial x}(\infty, t) = \text{finite} \dots\dots\dots (4.3.5)$$

For a finite system of length  $L$ , a frequently used boundary condition is

$$\frac{\partial C}{\partial x}(L, t) = 0 \dots\dots\dots (4.3.6)$$

#### 4.4 Analytical Solutions

Equation (4.2.1) can be solved analytically using Laplace transforms if the initial and boundary conditions are given by Equations (4.3.1), (4.3.3), (4.3.4), and (4.3.5). The solution is based on certain assumptions: (1) the flow is steady and uniform, (2) the medium is homogeneous and isotropic, (3) the fluid is incompressible, (4) only saturated flow is considered. However the solution is available for the limited cases of  $g(t)$  given below.

$$g(t) = C_0$$

$$g(t) = C_0 e^{-\lambda t}$$

$$g(t) = C_a + C_b e^{-\lambda t}$$

For  $g(t) = C_0$  and for initial and boundary conditions (4.3.1), (4.3.3), and (4.3.5), the solution is as follows [van Genuchten, 1981; van Genuchten and Alves 1982; Parker and van Genuchten 1985].

$$C(x,t) = C_i + (C_0 - C_i) A(x,t) + B(x,t)$$

where

$$A(x, t) = \frac{1}{2} \operatorname{erfc} \left[ \frac{Rx - vt}{2(DRt)^{1/2}} \right] + \frac{1}{2} \exp \left( \frac{vx}{D} \right) \operatorname{erfc} \left[ \frac{Rx + vt}{2(DRt)^{1/2}} \right]$$

$$B(x, t) = \frac{\gamma}{R} \left\{ t + \frac{Rx - vt}{2v} \operatorname{erfc} \left[ \frac{Rx - vt}{2(DRt)^{1/2}} \right] - \frac{Rx + vt}{2v} \exp \left( \frac{vx}{D} \right) \operatorname{erfc} \left[ \frac{Rx + vt}{2(DRt)^{1/2}} \right] \right\}$$

It was difficult to use this form of solution in the present study because of two main reasons:

1. The initial condition in the sand tank was in most cases a function of  $x$  (length of the sand tank)
2. The groundwater velocity ( $v$ ) which is assumed to be constant in the analytical solution was a function of time.

Numerical solution with the method of finite difference and orthogonal collocation has been used to solve the transport equations of the present study.

#### 4.5 Finite Difference Solution

In the advection dispersion equation given by (4.2.3), the hydrodynamic dispersion coefficient,  $D$  is given by [Bear, 1979, Freeze and Cherry, 1979]

$$D = \alpha v + D_m$$

where  $\alpha$  is the coefficient of dispersivity and  $D_m$  is the coefficient of molecular diffusion which is very small compared to the mechanical dispersion. Neglecting the molecular diffusion and taking  $v$  as a function of time, Equation (4.2.3) can be written as

$$\alpha v(t) \frac{\partial^2 U}{\partial x^2} - v(t) \frac{\partial U}{\partial x} + \mu U + \gamma = R \frac{\partial U}{\partial t} \dots \dots \dots (4.5.1)$$

where  $U$  represent the finite difference solution corresponding to the analytical solution

C. Equation (4.5.1) can be written in dimensionless form with respect to the length of the sand tank. Substituting  $x = ZL$  such that  $z$  varies from 0 to 1 for  $x = 0$  to  $L$ , we have

$$\frac{\alpha v(t)}{L^2} \frac{\partial^2 U}{\partial Z^2} - \frac{v(t)}{L} \frac{\partial U}{\partial Z} + \mu U + \gamma = R \frac{\partial U}{\partial t}$$

#### 4.5.1 Initial and Boundary Conditions

$$U(Z,0) = f(ZL) \quad \text{at } t = 0 \dots\dots\dots (4.3.2)$$

$$U(0,t) = g(t) \quad \text{at } Z = 0 \dots\dots\dots (4.3.3)$$

$$\frac{\partial U}{\partial Z}(1,t) = 0 \quad \text{at } Z = 1 (x = L) \dots\dots\dots (4.3.6)$$

#### 4.5.2 Finite Difference Formulation

The following finite difference scheme which is similar to the Crank-Nicholson's has been used to solve equation (4.5.1) subject to initial condition (4.3.2) and boundary conditions (4.3.3) and (4.3.6).

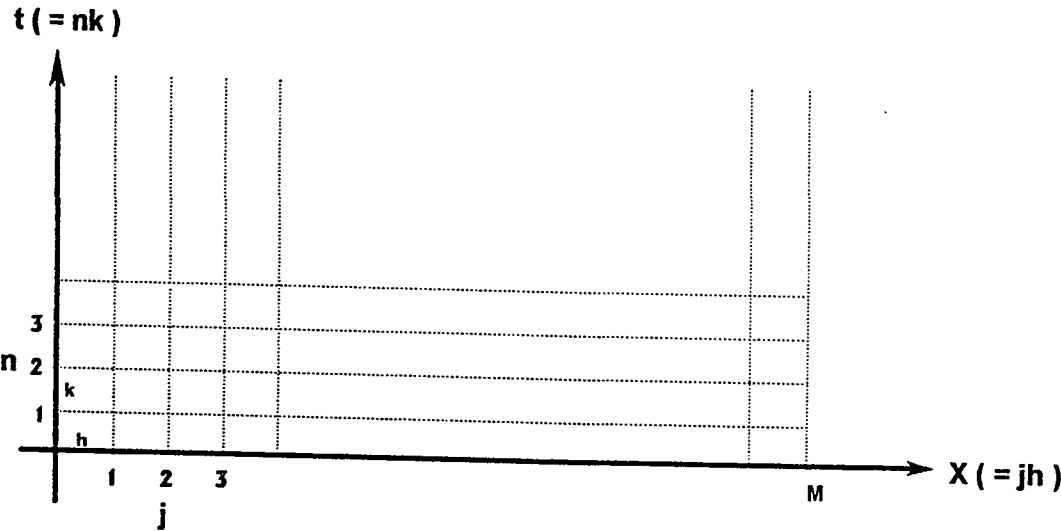


Figure 1 Finite difference grid in space and time

$$\begin{aligned} \frac{R}{4k} \left\{ (U_{j+1}^{n+1} - U_{j+1}^{n-1}) + (U_j^{n+1} - U_j^{n-1}) \right\} &= \frac{\alpha v(t)}{2h^2 L^2} \left\{ (U_{j+1}^{n+1} - 2U_j^{n+1} + U_{j-1}^{n+1}) + (U_{j+1}^n - 2U_j^n + U_{j-1}^n) \right\} \\ -\frac{v(t)}{4hL} \left\{ (U_{j+1}^{n+1} - U_{j-1}^{n+1}) + (U_{j+1}^n - U_{j-1}^n) \right\} &+ \frac{\mu}{2} \{U_j^n + U_j^{n+1}\} + \gamma \dots\dots\dots (4.5.2) \end{aligned}$$

which simplifies to

$$-aU_{j-1}^{n+1} + bU_j^{n+1} + cU_{j+1}^{n+1} = aU_{j-1}^n + dU_j^n + eU_{j+1}^n + f\{U_j^{n-1} + U_{j+1}^{n-1}\} + \gamma \dots\dots\dots (4.5.2)$$

where

$$\begin{aligned} a &= \frac{\alpha v(t)}{2h^2 L^2} + \frac{v(t)}{4hL} \\ b &= \frac{R}{4k} + \frac{2\alpha v(t)}{2h^2 L^2} - \frac{\mu}{2} \\ c &= \frac{R}{4k} + \frac{v(t)}{4hL} - \frac{2\alpha v(t)}{2h^2 L^2} \\ d &= \frac{\mu}{2} - \frac{2\alpha v(t)}{2h^2 L^2} \\ e &= \frac{\alpha v(t)}{2h^2 L^2} - \frac{v(t)}{4hL} \\ f &= \frac{R}{4k} \end{aligned}$$

Where a, b, c, d, e, f are time dependent (except f) constants.. Substituting j = 1 in (FDE-0) we have

$$-a U_0^{n+1} + b U_1^{n+1} + c U_2^{n+1} = a U_0^n + d U_1^n + e U_2^n + f\{U_1^{n-1} + U_2^{n-1}\} + \gamma \dots\dots\dots (FDE-1)$$

With the boundary condition (4.3.3), this can be put in the form

$$b U_1^{n+1} + c U_2^{n+1} = d U_1^n + e U_2^n + f\{U_1^{n-1} + U_2^{n-1}\} + \gamma + 2ag(nk) \dots\dots\dots (FDE-1)$$

Substituting j = 2, 3, .....M in (FDE-0) we have

$$-a U_1^{n+1} + b U_2^{n+1} + c U_3^{n+1} = a U_1^n + d U_2^n + e U_3^n + f\{U_2^{n-1} + U_3^{n-1}\} + \gamma \dots\dots\dots (FDE-2)$$

$$-a U_2^{n+1} + b U_3^{n+1} + c U_4^{n+1} = a U_2^n + d U_3^n + e U_4^n + f \{ U_3^{n-1} + U_4^{n-1} \} + \gamma \dots\dots\dots (FDE-3)$$

..  
..  
..

$$-a U_{M-1}^{n+1} + b U_M^{n+1} + c U_{M+1}^{n+1} = a U_{M-1}^n + d U_M^n + e U_{M+1}^n + f \{ U_M^{n-1} + U_{M+1}^{n-1} \} + \gamma \dots\dots\dots (FDE-M)$$

Applying the outlet boundary condition (4.3.6) ( $U_{M+1} = U_{M-1}$ ) in (FDE-M) we have

$$(c-a) U_{M-1}^{n+1} + b U_M^{n+1} = a U_{M-1}^n + d U_M^n + e U_{M+1}^n + f \{ U_M^{n-1} + U_{M+1}^{n-1} \} + \gamma \dots\dots\dots (FDE-M)$$

Writing in matrix form, the finite difference equations (FDE-1) to (FDE-M) can be written as

$$\begin{bmatrix} b & c & & & 0 & 0 \\ -a & b & c & & 0 & 0 \\ \dots & -a & b & c & & \\ \dots & \dots & \dots & \dots & \dots & \dots \\ \dots & \dots & \dots & \dots & \dots & \dots \\ 0 & 0 & & -a & b & c \\ 0 & 0 & & c-a & b & \end{bmatrix} \begin{bmatrix} U_1^{n+1} \\ U_2^{n+1} \\ U_3^{n+1} \\ \dots \\ \dots \\ U_{M-1}^{n+1} \\ U_{M+1}^{n+1} \end{bmatrix} = \begin{bmatrix} f_1 \\ f_2 \\ f_3 \\ \dots \\ \dots \\ f_{M-1} \\ f_M \end{bmatrix}$$

$$\begin{aligned} f_1 &= dU_1^n + eU_2^n + f(U_1^{n-1} + U_2^{n-1}) + \gamma + 2ag(nk) \\ f_2 &= aU_1^n + dU_2^n + eU_3^n + f(U_2^{n-1} + U_3^{n-1}) + \gamma \\ f_3 &= aU_2^n + dU_3^n + eU_4^n + f(U_3^{n-1} + U_4^{n-1}) + \gamma \\ &\dots \\ &\dots \\ f_M &= aU_{M-1}^n + dU_M^n + eU_{M+1}^n + f(U_M^{n-1} + U_{M+1}^{n-1}) + \gamma \end{aligned}$$

U at any time step can be easily computed by solving the above tridiagonal system of linear equations with Thomas algorithm. However the U values at two previous time steps

must be known. To compute  $U^1$  with the above scheme, we need both  $U^0$  (known from the initial condition) and  $U^{-1}$ . This can not be computed with the given boundary conditions. The above scheme is therefore valid for time steps  $U^2$  and onward. To calculate the  $U$  values at  $t=1$  another scheme is required. The following can be used.

$$\frac{R}{2k}(U_j^1 - U_j^0) = \frac{\alpha v(t)}{h^2}(U_{j+1}^0 - 2U_j^0 + U_{j-1}^0) - \frac{v(t)}{2h}(U_{j+1}^0 - U_{j-1}^0) + \mu U_j^0 + \gamma$$

Since all  $U_j^0$  are known from Eqn. (IC-1),  $U_j^1$  can be explicitly determined using the above scheme. Thus

$$U_j^0 = f(x)$$

$$U_j^1 = U_j^0 + \frac{2k}{R} \left\{ \frac{\alpha v(t)}{h^2}(U_{j+1}^0 - 2U_j^0 + U_{j-1}^0) - \frac{v(t)}{2h}(U_{j+1}^0 - U_{j-1}^0) + \mu U_j^0 + \gamma \right\}$$

$j = 2, 3, \dots, M-1$

for  $j = 1$

$$U_1^1 = U_1^0 + \frac{2k}{R} \left\{ \frac{\alpha v(t)}{h^2}(U_2^0 - 2U_1^0 + C_0) - \frac{v(t)}{2h}(U_2^0 - C_0) + \mu U_1^0 + \gamma \right\}$$

and for  $j = M$

$$U_M^1 = U_M^0 + \frac{2k}{R} \left\{ \frac{\alpha v(t)}{h^2}(2U_{M-1}^0 - 2U_M^0) + \mu U_M^0 + \gamma \right\}$$

The same scheme has been used to solve the PDE with the second type of inlet boundary condition given by (4.3.4). Because of different inlet boundary condition, the tridiagonal system was modified as follows:





parameter values ( $\alpha$ ,  $v$ ,  $R$ ,  $\mu$ ,  $\gamma$ ) are shown on the table as well as on the figures. According to sign convention used in CXTFIT, the sign of the first-order rate constant,  $\mu$  was taken positive for CXTFIT computation. From the table, it is obvious that at steady state the two solutions are almost identical. A slight discrepancy can be noted at the unsteady state. This is mainly due to the different boundary condition at the outlet boundary. The slope of the analytical solution should be zero at infinite  $x$ , whereas the slope of finite difference solution curve should be zero at  $x = L$  ( $Z = 1$ ). However at steady state, the two solutions coincides.

Table 4.1 Comparison of finite difference and analytical solution

Inlet boundary condition ►		$U(0,t) = 10$		$U - \frac{D}{v} \frac{\partial U}{\partial x} \Big _{x=0} = 10$	
T (day)	Distance (m)	Analytical	Finite difference	Analytical	Finite difference
2	0	10.0000	10.0000	9.8636	9.8636
2	1	8.1975	8.1976	8.0842	8.0842
2	2	6.6996	6.6960	6.6053	6.6005
2	3	5.4495	5.3934	5.3680	5.3035
2	4	4.2985	4.0610	4.1972	3.9579
2	5	2.7860	2.5792	2.6303	2.4740
2	6	1.1354	1.3130	1.0317	1.2429
2	7	.4171	.6198	.3971	.5912
2	8	.3184	.3832	.3173	.3756
4	0	10.0000	10.0000	9.8636	9.8636
4	1	8.1975	8.1976	8.0842	8.0843
4	2	6.6997	6.6998	6.6054	6.6056
4	3	5.4549	5.4551	5.3766	5.3768
4	4	4.4205	4.4207	4.3554	4.3556
4	5	3.5609	3.5608	3.5068	3.5066
4	6	2.8464	2.8440	2.8015	2.7986
4	7	2.2519	2.2368	2.2142	2.1974
4	8	1.7487	1.7158	1.7149	1.6790
6	0	10.0000	10.0000	9.8636	9.8636
6	1	8.1975	8.1976	8.0842	8.0843
6	2	6.6997	6.6998	6.6054	6.6056
6	3	5.4549	5.4551	5.3766	5.3768
6	4	4.4205	4.4207	4.3554	4.3556
6	5	3.5609	3.5611	3.5068	3.5070
6	6	2.8465	2.8467	2.8015	2.8018
6	7	2.2528	2.2530	2.2155	2.2157
6	8	1.7595	1.7748	1.7285	1.7435

Parameters:  $C_0 = 10$  mg/L,  $C_i = 2$  mg/L,  $v = 4$  m/day,  $R = 1.5$ ,  $\alpha = 7$  cm,  $\mu = -0.75$  /day,  $\gamma = -0.50$  mg/L/day

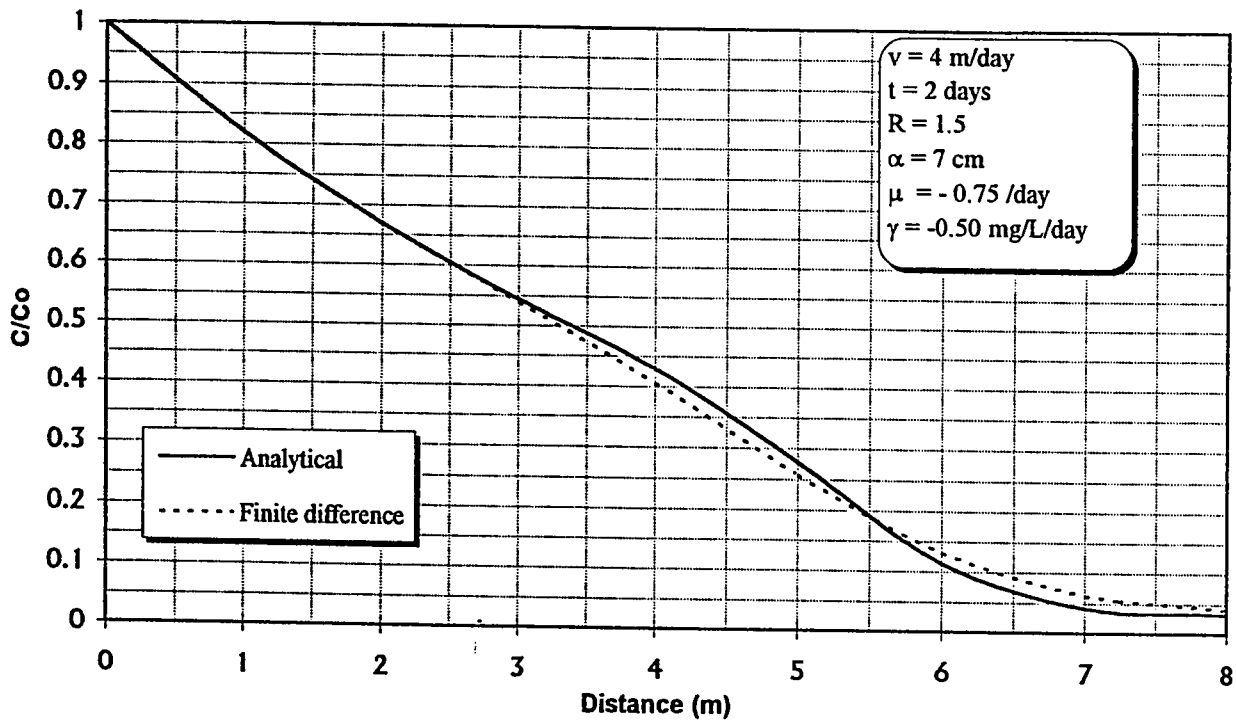


Figure 4.2 Comparison of numerical and analytical solution at  $t = 2$  day [ $C(0,t) = C_0$ ]

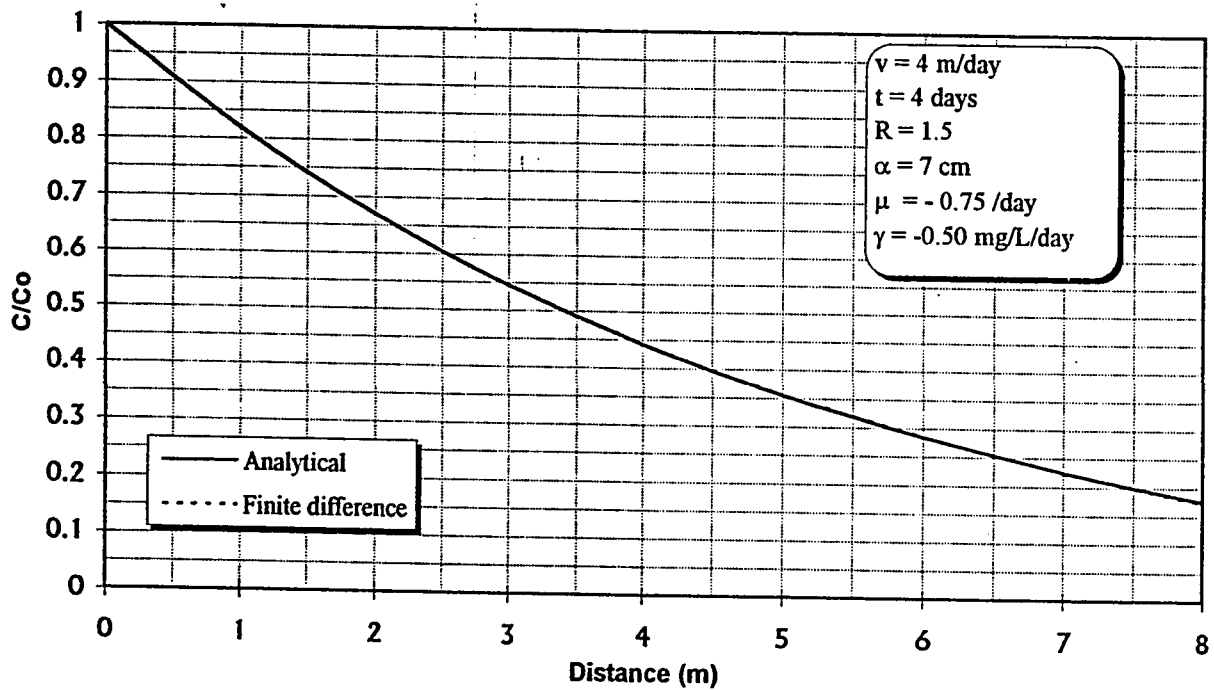


Figure 4.3 Comparison of numerical and analytical solution at  $t = 2$  day [ $C(0,t) = C_0$ ]

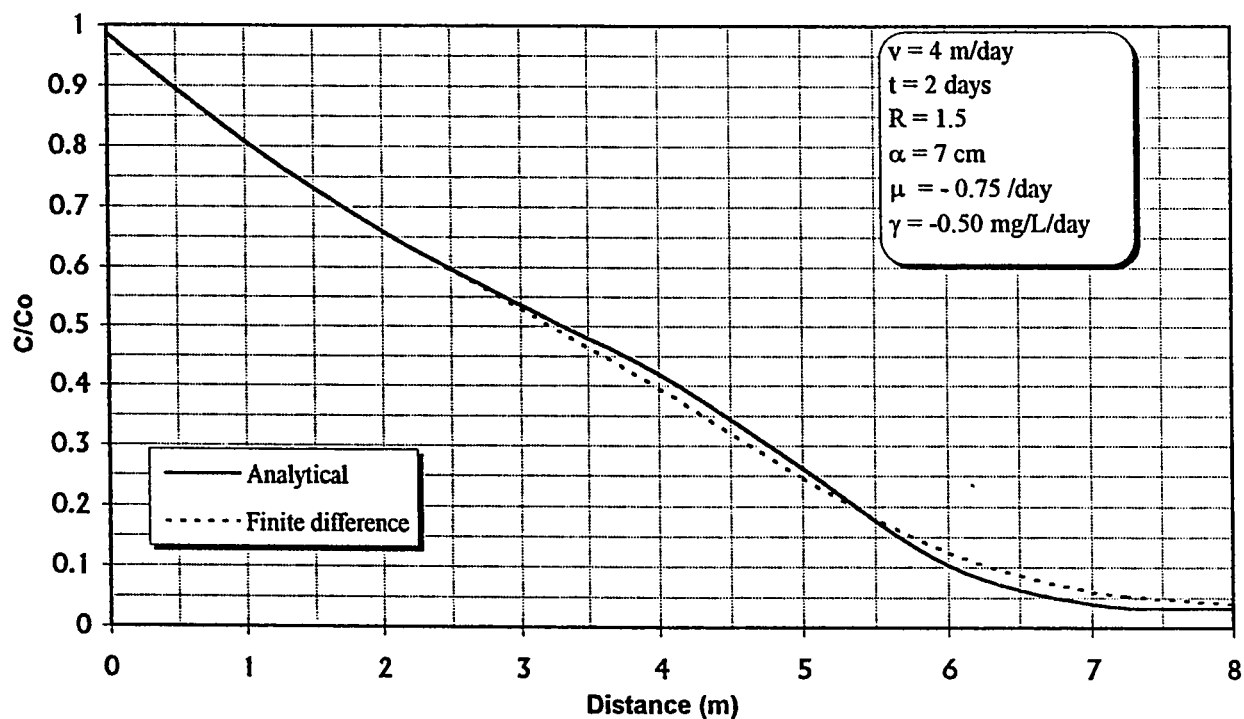


Figure 4.4 Comparison of numerical and analytical solution at  $t = 2$  day

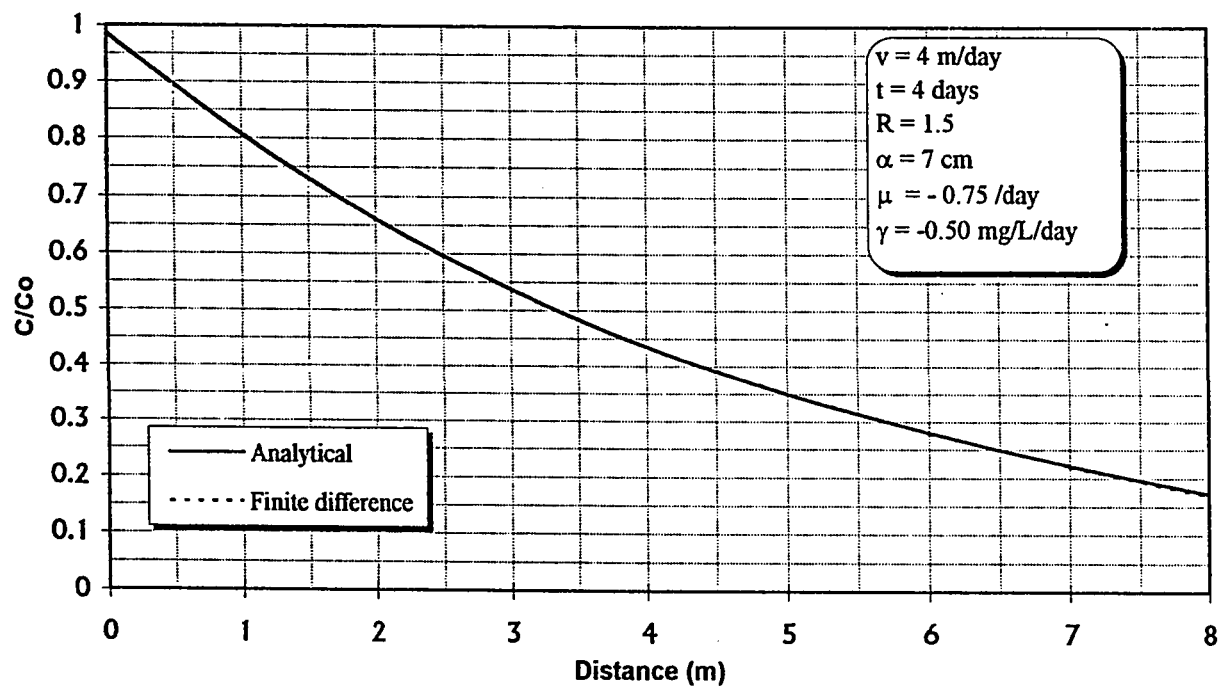


Figure 4.5 Comparison of numerical and analytical solution at  $t = 2$  day

#### ***4.5.4 Discussion of the Finite Difference Solution***

The grid interval used was very small and could have been little bigger. Since the system is tridiagonal, attempt was made to optimize the computational efficiency by taking smaller grid intervals and bigger time steps. Time steps from 0.01 to 0.1 days were found to have negligible effect on the steady state solution. However a small step size was found to improve slightly the solution at unsteady state. A variable time step starting from 0.02 day can be used for efficient computation.

### **4.6 Solution by Orthogonal Collocation**

#### ***4.6.1 Why Orthogonal Collocation ?***

Although biodegradation kinetics are mainly given by first-order and zero-order kinetics, use of Monod kinetics, including or ignoring bacterial growth and decay, has also been reported by Alvarez et al., [1991], Arcangeli & Ervin, [1994], Barrio Lage et al., [1987], Chu and Jewell, [1994], Coreseuil and Weber, [1994], Lodaya et al., [1991], and Mihelcic and Luthy, [1991]. Monod kinetics including bacterial growth have been criticized by few authors on the ground that conversion of substrate is necessarily associated by an increase in biomass as long as the substrate concentration is above a lower limit. However studies [Jones, 1970] have found that there may be a substantial conversion of substrate without a proportional increase in biomass. Other kinetics such as Haldane kinetics are used by Brown et al. [1990] and Zarooq et al. [1993]. Although this was beyond the main objective of the present study, models for Monod and Michaelis-Menten Kinetics were developed and investigated for BTX biodegradation in this study.

Although finite difference solution was very accurate compared to the analytical solution, it was very difficult to use the finite difference scheme for growth and non-growth biodegradation kinetics other than first-order and zero-order ones. This was very easy with the method of orthogonal collocation. The method was first used for solving the first-order/zero-order model that was solved with finite difference technique. The computer program was later very easily modified for other kinetic model. It will be obvious from the following analysis that the change in coding required to adapt the FORTRAN code, for other kinetic model, was very simple. In many cases change of only very few statements were required. A brief description of the orthogonal collocation method for solving the first-order and zero-order model is given below:

#### 4.6.2 First-order and Zero-order kinetics

Substituting  $x = ZL$  and dividing by  $R$

$$\frac{\partial U}{\partial t} = \frac{\alpha v(t)}{L^2 R} \frac{\partial^2 U}{\partial Z^2} - \frac{v(t)}{LR} \frac{\partial U}{\partial Z} + \frac{\mu}{R} U + \frac{\gamma}{R} \quad (4.6.1)$$

which can be written as

$$\frac{\partial U}{\partial t} = f_1(t) \frac{\partial^2 U}{\partial Z^2} - f_2(t) \frac{\partial U}{\partial Z} + \mu' U + \gamma' \quad (4.6.2)$$

The above PDE will be first solved for initial and boundary conditions shown below:

$$U(Z, 0) = f(Z) \quad (4.3.2)$$

$$U(0, t) = g(t) \quad (4.3.3)$$

$$\frac{\partial U}{\partial Z}(1, t) = 0 \quad (4.3.6)$$

Let  $U$  be approximated by non-symmetric polynomials of the type

$$U(Z, t) = (1-Z)U(0, t) + ZU(1, t) + Z(1-Z) \sum_{i=1}^M a_i(t) P_{i-1}(Z) \quad (4.6.3)$$

where  $a_i$  are arbitrary coefficients and  $P_i$  are the non-symmetric polynomials defined by the condition

$$\int_0^1 w(Z) P_n(Z) P_m(Z) dZ = 0 \dots\dots\dots (4.6.4)$$

$$n = 0, 1, 2, \dots, m-1$$

$W(Z) = 1$  in the present study

Let

$M$  = Number of internal collocation points.

$M2 = M + 2$  = Total number of points including boundaries where  $U$  is to be computed.

$M1 = M + 1$

Writing Equation (2) in collocation form ( using  $\frac{\partial U}{\partial Z} = \sum_{i=1}^{M2} A_{j,i} U_i$  and  $\frac{\partial^2 U}{\partial Z^2} = \sum_{i=1}^{M2} B_{j,i} U_i$  ) we have

$$\frac{dU_j}{dt} = f_1(t) \sum_{i=1}^{M2} B_{j,i} U_i - f_2(t) \sum_{i=1}^{M2} A_{j,i} U_i + \mu' U_j + \gamma' \dots\dots\dots (4.6.5)$$

Equation (4.6.5) represents  $M2$  coupled ordinary differential equations for solving  $M2$  values of  $U$  at every time step. Proper way of solving this equation is to solve  $M2$  values of  $U$  simultaneously using any numerical technique such as Gill's method. Many subroutines are available in IMSL, SSPSYS to solve this type of coupled ODEs. Gill's method is given in many Numerical books. These methods were partially investigated to solve the above equation. The method used in this study is relatively simple and faster. The idea is to substitute the known values of  $U_i$  from previous time step in the first two terms and obtain  $M2$  independent ODEs for solving  $M2$  values of  $U_j$  as follows:

$$\frac{dU_j}{dt} = \mu' U_j + F(t) \dots\dots\dots (4.6.6)$$

$U_j$  can now be computed at subsequent time steps by integrating the above equations numerically by employing numerical techniques such as Eulers method or Runge Kutta

method. The collocation matrices A and B can be generated by the procedures described by Finlayson [1972]. The solution of  $U_j$  is valid only at the internal collocation points. The collocation points between  $z = 0$  to 1 can also be found along with A and B matrices. The boundary solutions,  $U_1$  and  $U_{M2}$  can be computed from the boundary conditions as follows:

**Incorporating the boundary conditions:**

**Case 1:** When the inlet boundary condition is given by (4.3.3)

$$U_1 = g(t) \dots \dots \dots (4.6.7)$$

At the outlet we have

$$A_{M2,1}U_1 + \sum_{i=2}^{M1} A_{M2,i}U_i + A_{M2,M2}U_{M2} = 0$$

or

$$U_{M2} = \frac{1}{A_{M2,M2}} \left[ -\sum_{i=2}^{M1} A_{M2,i}U_i - A_{M2,1}g(t) \right] \dots \dots \dots (4.6.8)$$

Now using the values of  $U_1$  and  $U_{M2}$  from Equations (4.6.7) and (4.6.8) respectively in Equation (4.6.5) we have

$$\begin{aligned} \frac{dU_j}{dt} = & g(t)(f_1(t)B_{j,1} - f_2(t)A_{j,1}) \\ & + (f_1(t)B_{j,M2} - f_2(t)A_{j,M2}) \frac{1}{A_{M2,M2}} \left[ -\sum_{i=2}^{M1} A_{M2,i}U_i - A_{M2,1}g(t) \right] \\ & + f_1(t) \sum_{i=2}^{M1} B_{j,i}U_i - f_2(t) \sum_{i=2}^{M1} A_{j,i}U_i + \mu'U_j + \gamma' \dots \dots \dots (4.6.9) \end{aligned}$$

Now integrating Equation (4.6.9) we get the solution of U at internal collocation points. Substituting internal values in Equation (4.6.7) and (4.6.8) we get the solution at the boundaries.

Equation (4.6.9) can be simplified with  $V(t) = V_0 + V_1t + V_2t^2 + V_3t^3$



$$\begin{aligned}\frac{dU_j}{dt} &= f_1(t) \left[ g(t)B_{j,1} + B_{j,M2}S_1 + S_2 \right] + f_2(t) \left[ -g(t)A_{j,1} - A_{j,M2}S_1 - S_3 \right] + \mu'U_j + \gamma' \\ &= f_1(t)C_1(t) + f_2(t)C_2(t) + \mu'U_j + \gamma'\end{aligned}$$

where

$$S_1 = \frac{1}{A_{M2,M2}} \left[ -\sum_{i=2}^{M1} A_{M2,i} U_i - A_{M2,1} g(t) \right]$$

$$S_2 = \sum_{i=2}^{M1} B_{j,i} U_i$$

$$S_3 = \sum_{i=2}^{M1} A_{j,i} U_i$$

With the above substitutions, Equation (4.6.9) can be put in the form of

$$\frac{dU_j}{dt} = W_0 + W_1(t) * t + W_2(t) * t^2 + W_3(t) * t^3 + \mu'U_j + \gamma' \dots\dots\dots (4.6.10)$$

where

$$W_0 = C_1(t) \frac{\alpha V_0}{L^2 R} + C_2(t) \frac{V_0}{LR}$$

$$W_1 = C_1(t) \frac{\alpha V_1}{L^2 R} + C_2(t) \frac{V_1}{LR}$$

$$W_2 = C_1(t) \frac{\alpha V_2}{L^2 R} + C_2(t) \frac{V_2}{LR}$$

$$W_3 = C_1(t) \frac{\alpha V_3}{L^2 R} + C_2(t) \frac{V_3}{LR}$$

**Case 2:** When the inlet boundary condition is given by (4.3.4)

Let  $S = L/\alpha$ , from the inlet boundary conditions

$$\frac{\partial U}{\partial Z} = S[U_1 - g(t)] \dots\dots\dots (4.6.11)$$

$$\sum_{i=1}^{M2} A_{1,i} U_i = S[U_1 - g(t)]$$

or

$$A_{1,1}U_1 + \sum_{i=2}^{M1} A_{1,i}U_i + A_{M2,1}U_{M2} = S[U_1 - g(t)]$$

or

$$(A_{1,1} - S)U_1 + A_{1,M2}U_{M2} = -Sg(t) - \sum_{i=2}^{M1} A_{1,i}U_i \dots\dots\dots (4.6.12)$$

At the outlet we have

$$A_{M2,1}U_1 + \sum_{i=2}^{M1} A_{M2,i}U_i + A_{M2,M2}U_{M2} = 0$$

or

$$A_{M2,1}U_1 + A_{M2,M2}U_{M2} = -\sum_{i=2}^{M1} A_{M2,i}U_i \dots\dots\dots (4.6.13)$$

Applying Crammers rule in Equations (4.6.12) and (4.6.13) we have

$$U_1 = \frac{1}{S_1} \left\{ -A_{M2,M2} \left( Sg(t) + \sum_{i=2}^{M1} A_{1,i}U_i \right) + A_{1,M2} \sum_{i=2}^{M1} A_{M2,i}U_i \right\} \dots\dots\dots (4.6.14)$$

$$U_{M2} = \frac{1}{S_1} \left\{ A_{M2,1} \left( Sg(t) + \sum_{i=2}^{M1} A_{1,i}U_i \right) - (A_{1,1} - S) \sum_{i=2}^{M1} A_{M2,i}U_i \right\} \dots\dots\dots (4.6.15)$$

where

$$S_1 = (A_{1,1} - S)A_{M2,M2} - A_{1,M2}A_{M2,1} \dots\dots\dots (4.6.16)$$

Now using the values of  $U_1$  and  $U_{M2}$  from Equations (4.6.14) and (4.6.15) respectively in Equation (4.6.5) we have

$$\begin{aligned} \frac{dU_j}{dt} = & \frac{1}{S_1} \left( f_1(t)B_{j,1} - f_2(t)A_{j,1} \right) \left[ -A_{M2,M2} \left( Sg(t) - \sum_{i=2}^{M1} A_{1,i}U_i \right) + A_{1,M2} \sum_{i=2}^{M1} A_{M2,i}U_i \right] \\ & + \frac{1}{S_1} \left( f_1(t)B_{j,M2} - f_2(t)A_{j,M2} \right) \left[ A_{M2,1} \left( Sg(t) + \sum_{i=2}^{M1} A_{1,i}U_i \right) - (A_{1,1} - S) \sum_{i=2}^{M1} A_{M2,i}U_i \right] \end{aligned}$$

$$+ f_1(t) \sum_{i=2}^{M1} B_{j,i} U_i - f_2(t) \sum_{i=2}^{M1} A_{j,i} U_i + \mu' U_j + \gamma' \dots\dots\dots (4.6.16)$$

Equation (4.6.16) can also be simplified and put in the form of (4.6.10)

#### 4.6.3 Monod and Michaelis Menten Kinetics

The transport equation involving the Monod kinetics as the reaction term can be represented by the coupled system:

$$R \frac{\partial U}{\partial t} = \alpha v(t) \frac{\partial^2 U}{\partial x^2} - v(t) \frac{\partial U}{\partial x} + \frac{kXU}{K_s + U} \dots\dots\dots (PDE-1)$$

$$\frac{dX}{dt} = \frac{YkXU}{K_s + U} - bX \dots\dots\dots (PDE-2)$$

where X is the microbial concentration (total cell mass attached to the solid phase and suspended with liquid phase per unit volume of liquid phase, mg/l), k is the maximum specific substrate utilization rate (mg-substrate/mg-cells/day),  $K_s$  is the half saturation coefficient (mg-substrate/l), Y is the yield coefficient (mg cells produced/mg substrate consumed), b is the overall biomass loss due to shear and decay (/day).

A special case of the Monod equation that does not include microbial growth and decay is often referred to as the Michaelis Menten Kinetics. The transport equation involving the Michaelis Menten Kinetics can be written as:

$$R \frac{\partial U}{\partial t} = \alpha v(t) \frac{\partial^2 U}{\partial x^2} - v(t) \frac{\partial U}{\partial x} + \frac{k_m U}{K_s + U} \dots\dots\dots (PDE-3)$$

Where  $k_m = kX$ . The solution of the Monod system (PDE-1 and PDE-2) using the method of orthogonal collocation is described below:

Using the same analysis as in section (4.6.1) and with same sets of initial and boundary conditions the following equation can be obtained:

$$\frac{dU_j}{dt} = f_1(t) \sum_{i=1}^{M2} B_{ji} U_i - f_2(t) \sum_{i=1}^{M2} A_{ji} U_i + \frac{kX_j U_j}{R(K_s + U_j)} \dots\dots\dots (4.6.17)$$

$$\frac{dX_j}{dt} = \frac{YkX_j U_j}{K_s + U_j} - bX_j \dots\dots\dots (4.6.18)$$

The above two equations represents (2\*M2) coupled ordinary differential equations for solving M2 values of  $U_j$  and  $X_j$  at every time step. One way to solve this system is to solve all these unknowns simultaneously by any numerical method such as Gill's method. The method used in the present study is as follows:

Substituting the known values of  $U_j$  from previous time step,  $X_j$  can be easily obtained by integrating equation (4.6.18) analytically between two time steps as follows:

$$\int_{x_1}^{x_2} \frac{dX}{X} = \int_{t_1}^{t_2} \left\{ \frac{YkU_j}{K_s + U_j} - b \right\} dt$$

We can now substitute  $X$  in the third term in the right hand side of Equation (4.6.17) and the known values of  $U_j$  from previous time step in the first two terms and we obtain M2 independent ODEs for solving M2 values of  $U_j$  as follows.

$$\frac{dU_j}{dt} = W_0 + W_1(t) * t + W_2(t) * t^2 + W_3(t) * t^3 + \frac{kX_j U_j}{R(K_s + U_j)} \dots\dots\dots (4.6.19)$$

If we have a correction factor, for example, first order volatilization removal given by  $-K_v U$  this can be easily incorporated in the above equation as follows:

$$\frac{dU_j}{dt} = W_0 + W_1(t) * t + W_2(t) * t^2 + W_3(t) * t^3 + \frac{kX_j U_j}{R(K_s + U_j)} - K_v U_j \dots\dots\dots (4.6.20)$$

#### 4.6.4 Other Kinetics

Inspection of ODEs given by (4.6.10) and (4.6.20) reveals that whatever the kinetics of biodegradation is, we need to change only the kinetic term in the final ODE and therefore

the transport equation can be solved with virtually any kinetics with minimum change of the FORTRAN code. This makes the method very suitable for a wide range of kinetics such as Haldane kinetics and other inhibitory and non-inhibitory kinetic model. However if growth is involved in the kinetic model, for example in Monod kinetics, we need to put two or three statements to solve the value of  $X$  before solving the value of  $U$ .

#### ***4.6.5 Checking the Collocation Solution***

As mentioned earlier, a number of methods are available to solve a system of coupled ODEs derived in the orthogonal collocation method. Runge Kutta method and Gill's method are widely used. A number of routines are available in IMSL and SSP. The system of ODEs (4.6.5) was solved by IMSL routine DIVPAG, Gill's Runge Kutta method. These programs solve the system of equation simultaneously. In the method used in the present study, the coupled system given by (4.6.5) and (4.6.17) are decoupled by partial substitutions of  $U$  values described previously. As shown in Table 4.2 and Figures 4.6 and 4.7 the results are almost identical but the present method is at least twice as fast as the other ones. However for a stiff system, where  $U$  is very time-sensitive, a smaller time step will be required to obtain the same degree of accuracy. Depending on time sensitivity, an adjustable time step may be used for efficient and accurate computation.

Table 4.2 Comparison of analytical solution with the collocation solution

Distance X	Time T	Analytical Solution	Solution by collocation method and Error					
			Gill's	% Error	IMSL	% Error	Present	% Error
0.0000	2.00	9.8636	9.8593	-0.04	9.8586	0.05	9.8590	0.05
0.1588	2.00	9.5585	9.5517	-0.07	9.5499	0.09	9.5513	0.08
0.8133	2.00	8.3918	8.4044	0.15	8.3989	-0.08	8.4052	-0.16
1.8979	2.00	6.7442	6.7226	-0.32	6.7119	0.48	6.7212	0.34
3.2663	2.00	5.0648	5.1040	0.77	5.0860	-0.42	5.1054	-0.80
4.7337	2.00	3.0983	3.0968	-0.05	3.0159	2.66	3.0569	1.34
6.1021	2.00	0.9188	1.0203	11.04	0.9493	-3.32	0.9630	-4.81
7.1867	2.00	0.3631	0.3099	-14.65	0.3074	15.34	0.2990	17.66
7.8412	2.00	0.3198	0.3048	-4.68	0.3196	0.06	0.3164	1.08
8.0000	2.00	0.3173	0.3091	-2.59	0.3242	-2.17	0.3217	-1.37
0.0000	4.00	9.8636	9.8730	0.10	9.8728	-0.09	9.8733	-0.10
0.1588	4.00	9.5585	9.5729	0.15	9.5717	-0.14	9.5733	-0.15
0.8133	4.00	8.3918	8.3673	-0.29	8.3606	0.37	8.3666	0.30
1.8979	4.00	6.7442	6.7736	0.44	6.7646	-0.30	6.7745	-0.45
3.2663	4.00	5.0860	5.0562	-0.59	5.0411	0.88	5.0554	0.60
4.7337	4.00	3.7176	3.7453	0.75	3.7316	-0.38	3.7457	-0.76
6.1021	4.00	2.7365	2.7120	-0.90	2.6969	1.45	2.7123	0.89
7.1867	4.00	2.1155	2.1402	1.17	2.1249	-0.44	2.1395	-1.13
7.8412	4.00	1.7902	1.7804	-0.55	1.7639	1.47	1.7790	0.63
8.0000	4.00	1.7149	1.7481	1.94	1.7315	-0.97	1.7466	-1.85
0.0000	6.00	9.8636	9.8712	0.08	9.8706	-0.07	9.8712	-0.08
0.1588	6.00	9.5585	9.5697	0.12	8.5679	10.36	9.5697	-0.12
0.8133	6.00	8.3918	8.3726	-0.23	8.3670	0.30	8.3726	0.23
1.8979	6.00	6.7442	6.7684	0.36	6.7578	-0.20	6.7684	-0.36
3.2663	6.00	5.0860	5.0591	-0.53	5.0456	0.79	5.0591	0.53
4.7337	6.00	3.7176	3.7456	0.75	3.7306	-0.35	3.7456	-0.75
6.1021	6.00	2.7366	2.7097	-0.98	2.6952	1.51	2.7097	0.98
7.1867	6.00	2.1176	2.1406	1.09	2.1264	-0.42	2.1407	-1.09
7.8412	6.00	1.7999	1.7905	-0.52	1.7769	1.28	1.7905	0.52
8.0000	6.00	1.7285	1.7594	1.79	1.7459	-1.01	1.7594	-1.79

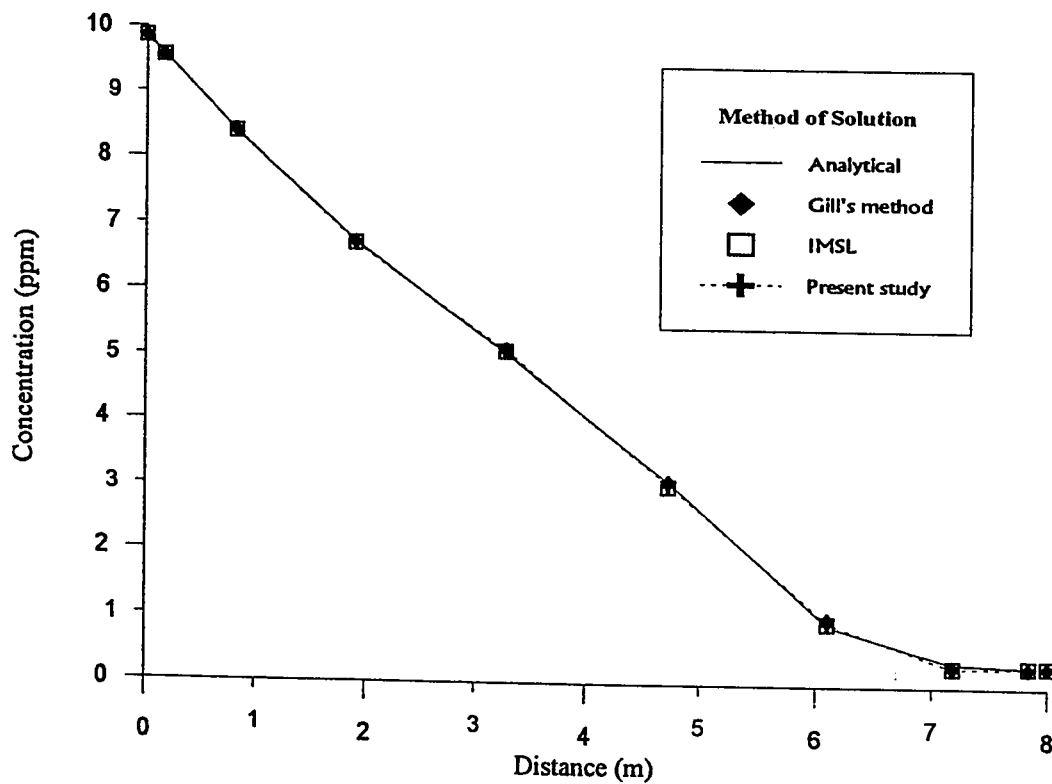


Figure 4.6 Comparison of solution with different method of solving the resulting ODEs  
( $v = 4$  m/day,  $t = 2$  days,  $C_o = 10$  ppm)

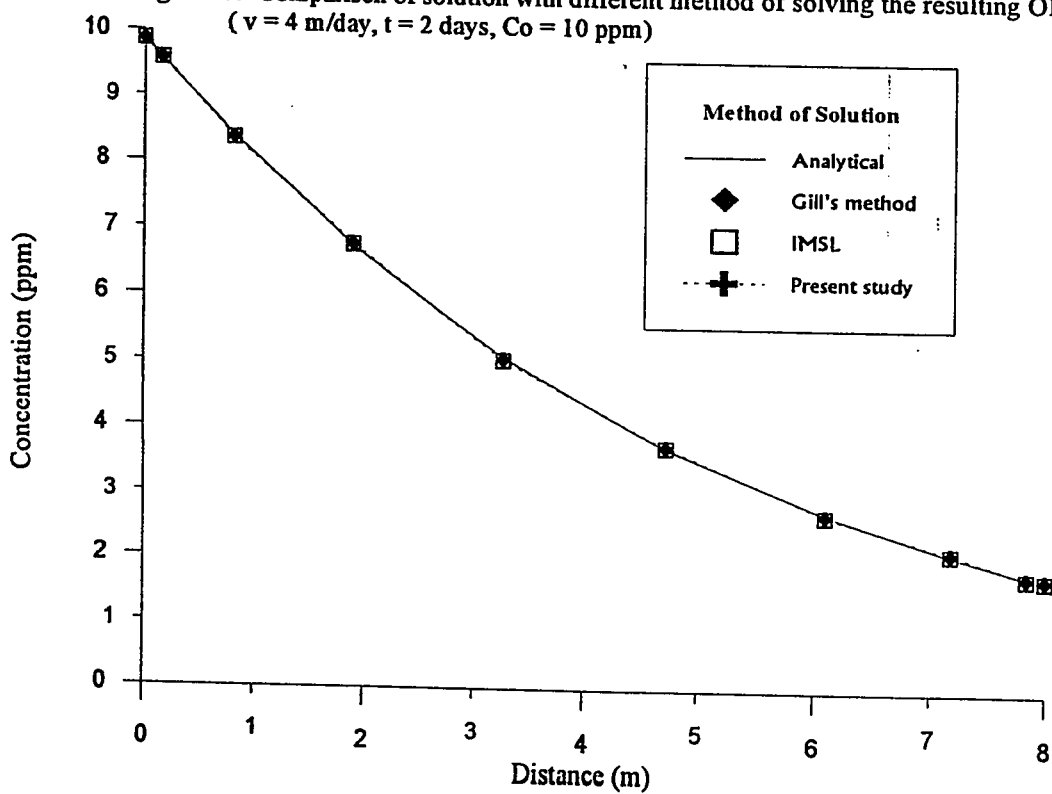


Figure 4.7 Comparison of different methods of solving the resulting ODEs  
( $v = 4$  m/day,  $t = 4$  days)

#### **4.6.6 Discussions on the Collocation Solution Method**

In the collocation methods, concentration is obtained at the collocation points that are irregularly spaced. Solution desired at any point other than the collocation points can be obtained by Spline or other interpolation method. The number of collocation points can be increased for higher accuracy. For the type of problems presented in this article, the optimum number of internal collocation points was found to be eight. More points did not improve the accuracy significantly but slowed down the solution. However slight fluctuation was observed at the unsteady state in one or two points near the outlet boundary. This can be easily removed by interpolation or smoothening after discarding the abnormal values. Higher values of collocation points was also found to improve the fluctuation. Value of time step was found to depend on the time sensitivity of the problem. For the solution given in Table 4.2, a time step of 0.02 day was found to be optimum. Smaller time step was found to have negligible effect on the steady state solution.

#### **4.7 Comparison of Finite Difference and Orthogonal Collocation Solution**

As mentioned earlier, the method of orthogonal collocation computes the solution only at the collocation points which are not necessarily the points where the solution with finite difference method is obtained. For the sake of comparison, cubic spline interpolation was used to compute the solution of orthogonal collocation at the desired points. Both solutions has been found to be very accurate compared with the analytical solution. The accuracy has been found to depend on time step and grid interval or number of collocation points. However for the same computational time, the method of orthogonal collocation has been found to be more accurate especially at the unsteady state. Table 4.3 and figures 8, 9, 10, and 11 shows numerical and analytical solution for both the boundary condition (4.3.3) and (4.3.4).



Table 4.3 Comparison of numerical solution with analytical solution for special cases

Boundary Condition ➔		$C(0,t) = 10.00$			$C - \alpha \frac{\partial C}{\partial x}(0,t) = 10.00$		
T (day)	Distance	Analytical	Finite diff.	Collocation	Analytical	Finite diff.	Collocation
2	0	10.0000	10.0000	10.0000	9.8636	9.8636	9.8593
2	1	8.1975	8.1976	8.2086	8.0842	8.0842	8.0956
2	2	6.6996	6.6960	6.6745	6.6053	6.6005	6.5858
2	3	5.4495	5.3934	5.4933	5.3680	5.3035	5.4122
2	4	4.2985	4.0610	4.2565	4.1972	3.9579	4.1561
2	5	2.7860	2.5792	2.7288	2.6303	2.4740	2.6145
2	6	1.1354	1.3130	1.1730	1.0317	1.2429	1.0881
2	7	0.4171	0.6198	0.3627	0.3971	0.5912	0.3428
2	8	0.3184	0.3832	0.3162	0.3173	0.3756	0.3221
4	0	10.0000	10.0000	10.0000	9.8636	9.8636	9.8731
4	1	8.1975	8.1976	8.1613	8.0842	8.0843	8.0583
4	2	6.6997	6.6998	6.7213	6.6054	6.6056	6.6371
4	3	5.4549	5.4551	5.4271	5.3766	5.3768	5.3546
4	4	4.4205	4.4207	4.4172	4.3554	4.3556	4.3540
4	5	3.5609	3.5608	3.5804	3.5068	3.5066	3.5265
4	6	2.8464	2.8440	2.8191	2.8015	2.7986	2.7741
4	7	2.2519	2.2368	2.2857	2.2142	2.1974	2.2482
4	8	1.7487	1.7158	1.7791	1.7149	1.6790	1.7462
6	0	10.0000	10.0000	10.0000	9.8636	9.8636	9.8712
6	1	8.1975	8.1976	8.1719	8.0842	8.0843	8.0651
6	2	6.6997	6.6998	6.7193	6.6054	6.6056	6.6301
6	3	5.4549	5.4551	5.4295	5.3766	5.3768	5.3558
6	4	4.4205	4.4207	4.4192	4.3554	4.3556	4.3580
6	5	3.5609	3.5611	3.5759	3.5068	3.5070	3.5252
6	6	2.8465	2.8467	2.8128	2.8015	2.8018	2.7719
6	7	2.2528	2.2530	2.2815	2.2155	2.2157	2.2473
6	8	1.7595	1.7748	1.7882	1.7285	1.7435	1.7594

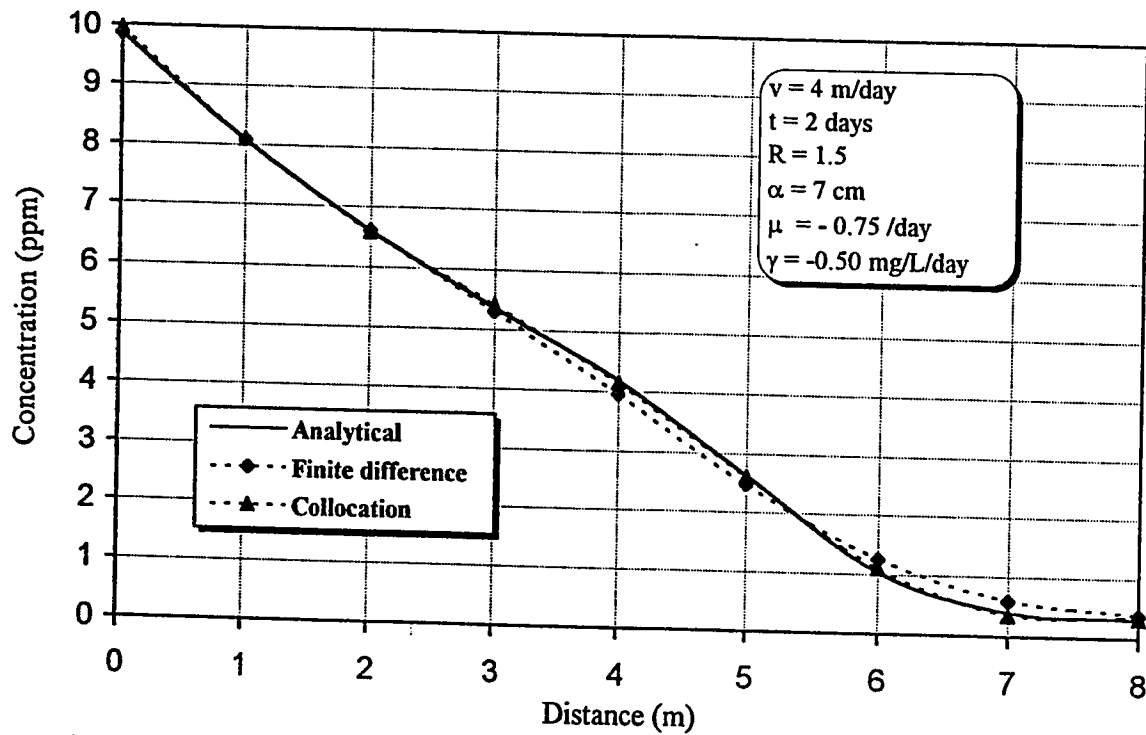


Figure 4.8 Comparison of finite difference and orthogonal collocation solution ( $t = 2 \text{ day}$ )

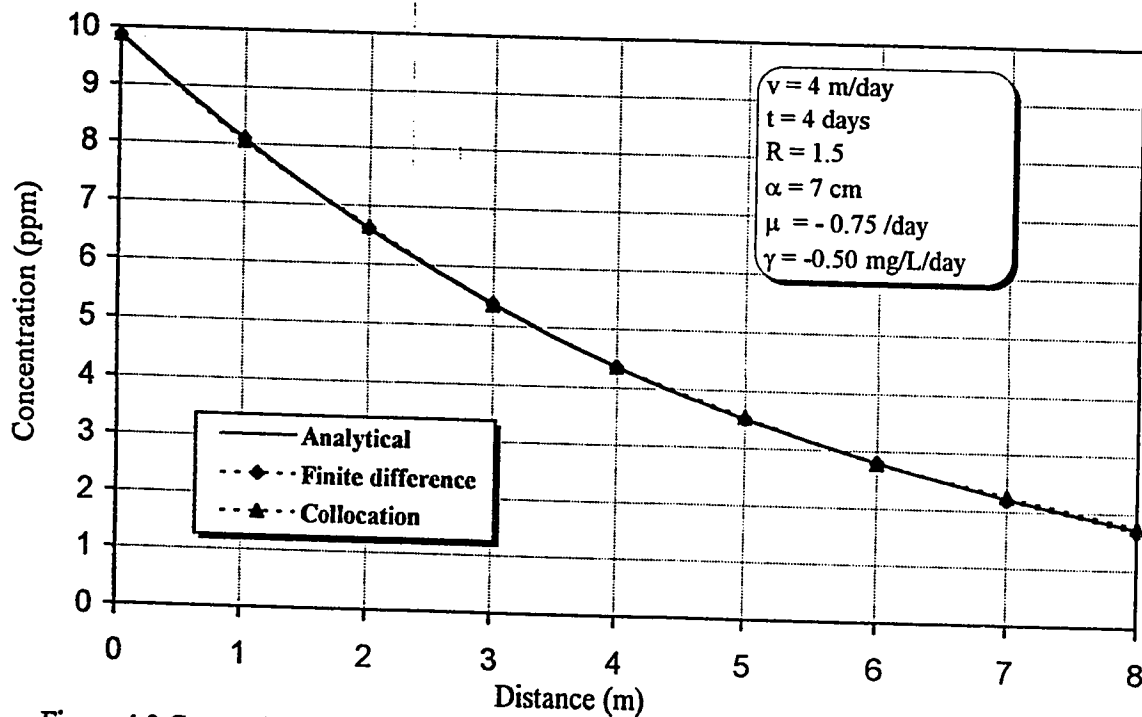


Figure 4.9 Comparison of finite difference and orthogonal collocation solution ( $t = 4 \text{ day}$ )

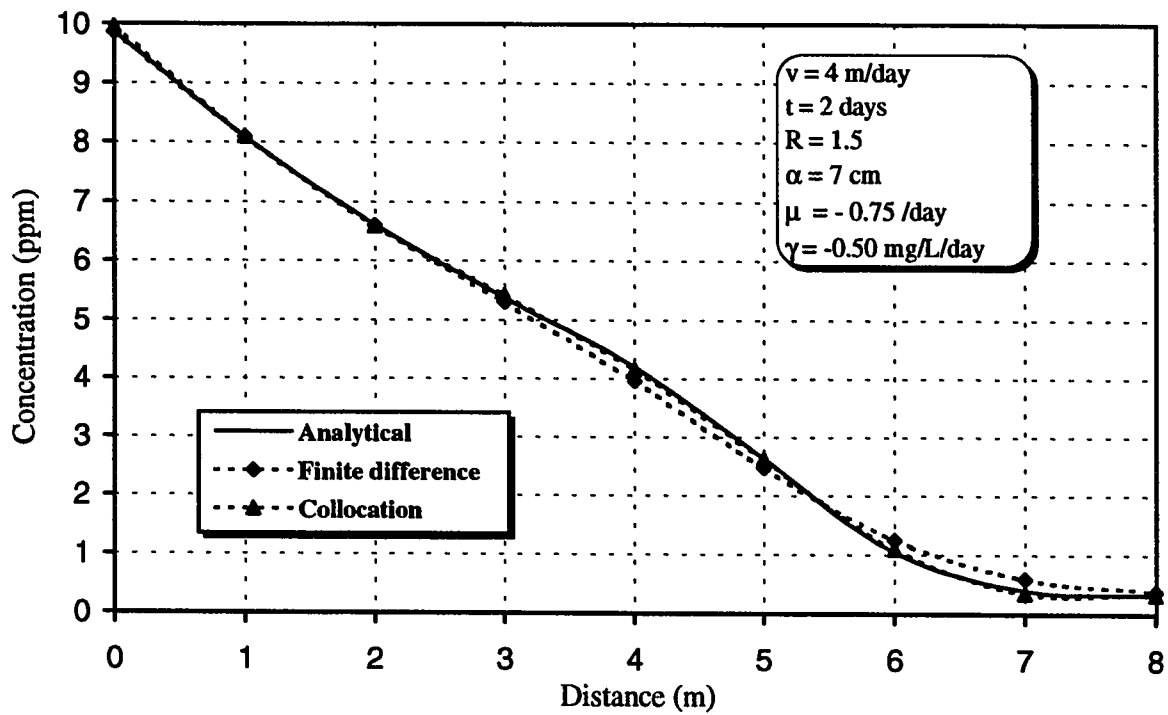


Figure 4.10 Comparison of finite difference and orthogonal collocation solution

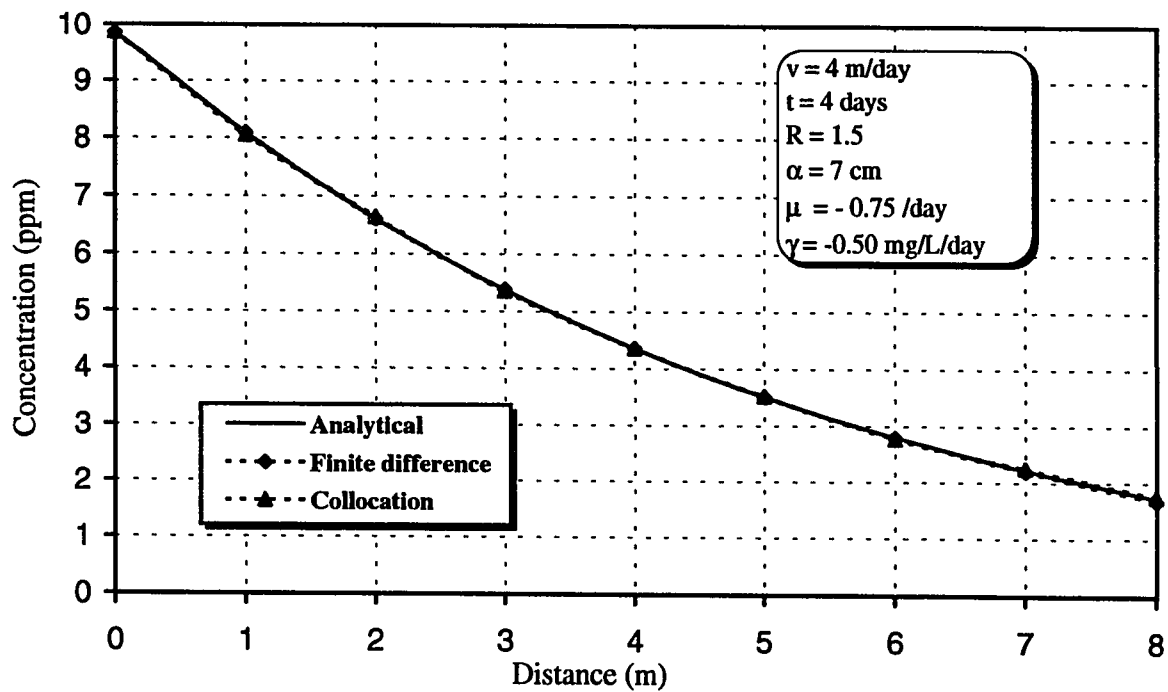


Figure 4.11 Comparison of finite difference and orthogonal collocation solution

## Chapter 5

# **PARAMETER ESTIMATION**

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## 5 PARAMETER ESTIMATION

### 5.1 General

The one dimensional equation advection dispersion equation with first-order and zero-order biodegradation has five transport parameters.

- (1) Coefficient of dispersivity ( $\alpha$ )
- (2) Pore water velocity ( $v$ )
- (3) Retardation constant ( $R$ )
- (4) First order rate constant ( $\mu$ )
- (5) Zero order rate constant ( $\gamma$ )

Transport equation involving the Monod kinetics has seven parameters:  $\alpha$ ,  $v$ ,  $R$ ,  $k$ ,  $K_s$ ,  $Y$ ,  $b$ . The Michaelis Menten model involves five parameters. Parameters need to be quantified before the pertinent model can be used for actual prediction purposes. From previous study [Mohammed, 1988], it was found that dispersivity ( $\alpha$ ) does not change significantly because of microbial growth. Therefore the mean  $\alpha$  value obtained from the control runs has been fixed for parameter estimation in the subsequent runs. The other reasons of fixing the dispersivity value is that estimating this along with other parameters ( $R$ ,  $\mu$ ,  $\gamma$  in case of the first-order/zero-order model) very often results in very high  $\alpha$  values due to possible experimental error that affect other parameters. The pore water velocity has been treated as a time dependent parameter and was measured. The other parameters were estimated.

### 5.2 Methods of Parameter Estimation

Several methods of analysis are available for determining the dispersion coefficient  $D$ . Rifai et al. [1956] proposed a method for calculating  $D$  from the slope of breakthrough

curve. Rose and Passiura [1971] and Passiura et al. [1970] discussed a procedure which allows D and R to be determined from a plot of  $\ln(t)$  versus C on probability paper. Agneessens et al. [1978] used the moments to obtain D from pulse-type effluent curves. Another method, based upon a non-linear least square analysis of the effluent data, was used by Elprice and Day [1977], Laudelout and Dufey [1977], Agneessens et al. [1978] and Le Renard [1979]. In particular, least-square inversion methods have proved to be accurate and reliable tools for assessing parameters [van Genuchten, 1985]. The method is based on fitting an appropriate form of analytical solution of the governing equation to the effluent data. This method has found wide application for parameter estimation. Few recent applications includes Allayla et al. [1991], Chen et al. [1992], and Anid et al. [1993]. In the present study, the parameters are estimated by the nonlinear least square fit described below.

### 5.3 Nonlinear Least Square Fit

Basically nonlinear least square fit is an unconstrained optimization problem where the objective function is minimized with the parameters. The objective function is defined as:

$$S = \sum (y_{\text{obs}} - f)^2$$

Where  $y_{\text{obs}}$  are the observed data and  $f(x, t, \text{parameters})$  are the corresponding values computed from the nonlinear equation. Several methods are available for parameter estimation by fitting observed data to nonlinear equations using the principle of least square or best fit. For example

- **Gauss Newton method**, uses the Taylor's series expansion of the function,
- **Steepest Descent method**, involves the sum of squares of residuals w.r.t. parameters,
- **Lavenberg-Marquardt method**, derived from the Gauss Newton method and steepest descent method
- **Secant method** - a methods that does not use derivatives like the first three methods and so on.

The theoretical background, advantages and disadvantages are given in many books including Draper and Smith [1982], Bates and Watts [1982], Dennis and Schnabel [1982], Davies [1954]. The algorithm of Lavenburg and Marquardt can be found in Lavenburg [1944] and Marquardt [1963]. A brief description of the applicability of the methods for the present study is given below:

### 5.3.1 Gauss Newton Method

Gauss-Newton method forms the basis of many important and successful practical methods for nonlinear least square. This is the most efficient method for many problems and, for few linear problems, the optimization can be obtained in one trial. The determination of transport parameters for the present study is based on this method. The theoretical background will therefore be described in this section.

Keeping  $v$  as a time dependent and known parameter, the solution of the advection-dispersion equation for the first-order/zero-order kinetics can be written as

$$U = f(x, t, \alpha, R, \mu, \gamma)$$

Expanding this in a first order Taylor's series about an initial guess  $\alpha$ ,  $R$ ,  $\mu$ , and  $\gamma$  we have

$$f(x, t, R+dR, \alpha+d\alpha, \mu+d\mu, \gamma+d\gamma) \approx f(x, t, \alpha, R, \mu, \gamma) + \frac{\partial f}{\partial R} dR + \frac{\partial f}{\partial \alpha} d\alpha + \frac{\partial f}{\partial \mu} d\mu + \frac{\partial f}{\partial \gamma} d\gamma \quad (18)$$

$$\text{The objective function } S \text{ is defined as: } S = [U_{\text{obs}} - f(x, t, \alpha, R, \mu, \gamma)]^2 \quad (19)$$

In the Gauss-Newtons method  $S$  is minimized by solving

$$[V^T V](\delta) = [V^T](U_{\text{obs}} - f) \quad (20)$$

where  $(\delta) = [dR, d\alpha, d\mu, d\gamma]$ .  $U_{\text{obs}}$  are the observed concentrations and  $f$  are the corresponding computed values.  $V$  is a  $(N \times 4)$  size matrix,  $N$  being the number of data

points, and 4 is the number of parameters. The linear systems of equations represented by matrix equation (20) are often referred to as "*normal equations*" and the matrix  $(V^T V)$  is often referred to as "*normal matrix*". This is in analogy with the least square parameters estimation of the linear equation

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4 + \dots$$

$$\text{by } (X^T X)\beta = X^T Y$$

The elements of V are as follows:

$$V_{11} = \frac{\partial f(x_1, t_1, R, \alpha, \mu, \gamma)}{\partial R}, \quad V_{12} = \frac{\partial f(x_1, t_1, R, \alpha, \mu, \gamma)}{\partial \alpha}, \quad V_{21} = \frac{\partial f(x_2, t_2, R, \alpha, \mu, \gamma)}{\partial R}$$

and so on.

Procedure used:

In the Taylor's expansion, all terms including second and higher order derivatives are neglected.

For a linear system this is not needed because the values of all higher order derivatives are zero. Thus for a linear system the least square fitting can be done in one step. For a nonlinear system an iterative procedure as described below can be used:

1. The initial guess of the parameters are supplied or input from the available information.
2. With this guess the advection dispersion equation is solved and sum of squares of the residuals is computed.
3. To compute the V matrix, a forward or central finite difference scheme is used, as shown below. Using forward difference:

$$V_{11} = [f(x_1, t_1, R+\Delta R, \alpha, \mu, \gamma) - f(x_1, t_1, R, \alpha, \mu, \gamma)]/\Delta R$$

$$V_{12} = [f(x_1, t_1, R, \alpha+\Delta \alpha, \mu, \gamma) - f(x_1, t_1, R, \alpha, \mu, \gamma)]/\Delta \alpha$$

$$V_{21} = [f(x_2, t_2, R+\Delta R, \alpha, \mu, \gamma) - f(x_2, t_2, R, \alpha, \mu, \gamma)]/\Delta R$$

and so on.



A central difference scheme as shown below was used to check the accuracy.

$$V_{11} = [f(x_1, t_1, R + \Delta R, \alpha, \mu, \gamma) - f(x_1, t_1, R - \Delta R, \alpha, \mu, \gamma)] / 2(\Delta R)$$

$$V_{12} = [f(x_1, t_1, R, \alpha + \Delta \alpha, \mu, \gamma) - f(x_1, t_1, R, \alpha - \Delta \alpha, \mu, \gamma)] / 2(\Delta \alpha)$$

$$V_{21} = [f(x_2, t_2, R + \Delta R, \alpha, \mu, \gamma) - f(x_2, t_2, R - \Delta R, \alpha, \mu, \gamma)] / 2(\Delta R)$$

and so on.

4. With the V matrix computed in step 3, the ( $\delta$ ) matrix is computed and the initial guess of parameters is corrected using

$$R = R + \delta_1$$

$$\mu = \mu + \delta_2$$

$$\gamma = \gamma + \delta_3$$

5. With the revised parameters, a second iteration is made repeating from step 2 and the sum of squares of residuals are computed. This iteration process is repeated until no substantial improvement in the reduction in the sum of squares of residuals is obtained.

### 5.3.2 Steepest Descent method

This is also an iterative process for finding the minimum of  $S(R, \mu, \gamma)$  by moving from the initial guess along the vector with components

$$\delta_g = - \left\{ \frac{\partial S}{\partial R}, \frac{\partial S}{\partial \alpha}, \frac{\partial S}{\partial \mu}, \frac{\partial S}{\partial \gamma} \right\}$$

Various modifications of this method have been employed. For the present problem the procedure can be applied as follows.

The transport equation can be solved with various levels of  $R, \mu, \gamma$  (usually selected in a factorial design) to compute the sum of squares of residual ( $S$ ) and to fit  $S$  with a first order polynomial

$$S = \beta_0 + \beta_1 R + \beta_2 \mu + \beta_3 \gamma$$

From this equation  $\beta$  can be solved and, differentiating it with respect to  $R$ ,  $\mu$ ,  $\gamma$ , a local minimum of  $S$  can be obtained. The procedure can be repeated for a global minima. The speed of convergence depends on the selection of factors in the factorial design. While theoretically, the steepest descent method will converge, it may do so with agonizing slowness after some rapid initial progress in convergence. Slow convergence is particularly likely when the contours of  $S(\text{parameters})$  are attenuated or banana shaped as they are often in practice. The steepest descent method is, on the whole, less favored than the Gauss Newton method because of reasons discussed by Draper and Smith, [1982]. The method of steepest descent was not used in the present study but the gradients matrix  $\delta_g$  was computed at every iteration of the Gauss Newton methods to cross check the minimization of  $S$ . At the minimization those gradients were close to zero. These values are also used to determine the values of  $\Delta R$ ,  $\Delta \mu$ ,  $\Delta \gamma$  that are used to compute the  $V$  matrix. This will be elaborated in the following sections.

### ***5.3.3 Lavenburg-Marquardt's Algorithm***

Lavenburg-Marquardt's method is the most widely used optimization technique used for a wide variety of practical problems. Optimization routines of SAS, IMSL and many other inverse groundwater problems have used this method. Model independent parameter independent program, PEST [1994] available in the market has also used Gauss-Levenburg-Marquardt method. The nonlinear least square fit of the one dimensional advection dispersion equation having analytical solution with constant parameter and constant initial concentration have also used Marquardt maximum neighborhood method [Parker and van Genuchten, 1985].

Basically the Lavenburg-Marquardt Compromise is an interpolation between the Gauss Newton method and the steepest descent method. The theory is described in detail by Marquardt [1963] and only a brief description is given below. Starting from a certain

point, if the Gauss Newtons method is applied a certain correction vector ( $\delta_g$ ) is obtained. An interesting feature of this vector is that this is often very large but the right directions. In other words the sign of the components are right but the magnitude is often very wrong. This results mainly because of the singularity of the derivative matrix  $V$  caused by the collinearity of the columns. Steepest descent method can be obtained to get a suitable step size using Marquardt's procedure. In any iteration of the Gauss Newton's method, when ( $\delta_g$ ) is computed, ( $\delta_g$ ) can also be easily computed as follows:

$$\begin{aligned}\partial S/\partial R &= [S(R+\Delta R, \mu, \gamma) - S(R, \mu, \gamma)]/\Delta R \\ \partial S/\partial \mu &= [S(R, \mu+\Delta \mu, \gamma) - S(R, \mu, \gamma)]/\Delta \mu \\ \partial S/\partial \gamma &= [S(R, \mu, \gamma+\Delta \gamma) - S(R, \mu, \gamma)]/\Delta \gamma\end{aligned}$$

To move from these current values of the parameters, one must move within  $90^\circ$  of ( $\delta_g$ ), otherwise the movement will take place in the positive direction of the above gradients and  $S$  will get larger locally. Marquardt found that for a large number of practical problems, the angle lies between  $80^\circ$  and  $90^\circ$ . In matrix notation, this angle can be incorporated in the Gauss Newton increment by

$$\delta(k) = (V^T V + \lambda I)^{-1} V^T (Y_{obs} - f)$$

as suggested by Lavenburg [1944]

$$\delta(k) = (V^T V + \lambda D)^{-1} V^T (Y_{obs} - f)$$

as suggested by Marquardt [1963], where  $\lambda$  is the conditioning factor and  $D$  is a diagonal matrix having elements equal to the diagonal element of  $V^T V$ . This is called the Lavenburg-Marquardt compromise because the direction of  $\delta(k)$  is intermediate between the Gauss Newton method ( $\lambda \rightarrow 0$ ) and the direction of steepest descent ( $\lambda \rightarrow \infty$ ). The direction of steepest descent is given by  $V^T (Y_{obs} - f) / |V^T (Y_{obs} - f)|$

Lavenburg's Marquardt's compromise was not necessary for estimating the parameters of first-order/zero-order model and the non-growth associated Michaelis Menten model because the number of parameters to be estimated were only three. However, inversion of the Monod model, where five parameters were involved, needed the Lavenburg-Marquardt technique.

#### 5.4 Optimization Technique Used in the Present Study

The nonlinear least square optimization of the present study is mainly based on the Gauss Newton method. The unmodified Gauss method was first attempted at every iteration of the optimization process. However, the unmodified Gauss method did not work in few cases of first-order/zero-order model inversion and in almost all cases of Monod inversion. The reason is obviously the existence of more parameters in the case of Monod model inversion. Marquardt suggested two methods to ameliorate the ill-conditioning of the normal equations: scaling and conditioning. The normal equation (20) can also be written as:

$$A\delta = g$$

This matrix can be scaled as follows to have the diagonal elements as 1.

$$A^* = (a_{ij}^*) = \left( \frac{a_{ij}}{\sqrt{a_{ii}} \sqrt{a_{jj}}} \right)$$

$$g^* = (g_i^*) = \left( \frac{g_i}{\sqrt{a_{ii}}} \right)$$

The solution of the original correction vector  $\delta$  can be obtained by solving  $A^*\delta^* = g^*$  and using

$$\delta_i = \left( \frac{\delta_i^*}{\sqrt{a_{ii}}} \right)$$

Although, the scaled system and the original systems of normal equations are mathematically equivalent, the scaled system is numerically far superior. Marquardt and Lavenberg also suggested changing the diagonal element of the  $(A^*)$  matrix by adding a factor  $\lambda$ , or multiplying by  $(1+\lambda D)$ , where  $D$  is the diagonal element of the normal matrix. Usually when  $\lambda$  is small, the convergence is much faster and the objective function is minimized fast. But very often the process does not converge without conditioning of the normal matrix. It can be proved that there always exist a  $\lambda$ , however large, for which the objective function is always minimized. Usually the higher the  $\lambda$ , the slower the convergence.

Marquardt's method worked in many cases of inversion of the present study. However, the convergence was very slow in many cases. Therefore, in addition to Marquardt's scaling and conditioning, few more techniques have been adopted for an efficient and accurate minimization. They are as follows:

(1) *Proper values finite difference intervals*, such as  $\Delta R$ ,  $\Delta \mu$ ,  $\Delta \gamma$  for computation of gradient matrix: Theoretically these values should be as small as possible for accuracy in the gradient computation. However, it has been found that very small values of these increments results in very-ill conditioned normal matrix and slower convergence with large  $\lambda$ . Again with high  $\Delta R$ ,  $\Delta \mu$ ,  $\Delta \gamma$  values the convergence processes oscillated and or the minimization was only partially achieved. It was found that these  $(\Delta R, \Delta \mu, \Delta \gamma)$  values should be kept changing depending on the sum of squares of residual and the magnitudes of the gradient vector  $(\delta_g)$ . By trial and error, the best  $\Delta R$ ,  $\Delta \mu$ ,  $\Delta \gamma$  values for the first iteration of the minimization process has been incorporated depending on the sum of square of residuals and the gradient vector in the previous trial. These values were changed in the subsequent iteration for a rapid and very accurate minimization.

(2) *Damping the correction vector*: It was observed that in few cases of optimization, specially for Monod model inversion, some of the correction vector computed are always amplified. Instead of choosing a high  $\lambda$ , the  $\delta$  values were damped with proper factors. This resulted in a more efficient convergence. This process is very often referred to as the *damped Gauss Newton method*.

(3) *Setting upper and lower bounds of the parameters*: Very often the values of the parameters corrected with the  $\delta$  values were too high or too low. For example, negative R or R less than 1 is meaningless. Therefore setting lower and upper values of the parameters also helps the convergence of the optimization process.

With all the above technique, the optimization process always converged regardless of very rough initial guesses of the parameters. The fitting procedure used by CXTFIT [Parker and van Genuchten, 1984] needs non-zero guesses of parameters with the correct sign, because the fitting procedure is unable to change the sign of the parameters. However, the present study has overcome this limitation. Even with very erroneous guesses of the parameters with respect to both sign and direction 100% accurate minimization was achieved in few trials.

Computation of the gradient matrix using central difference almost doubled the number of times the advection dispersion equation is solved in each trial. For example, in case of estimating 3 parameters, the transport equation is solved 4 (1+3) times in every trial in case of forward difference, whereas in case of central difference, the equation is solved 7 (1+2\*3) times. The central difference was tested and found not to be superior than the forward difference. Depending on the values of  $\Delta R$ ,  $\Delta \mu$ ,  $\Delta \gamma$  the central difference often resulted in very ill conditioned matrix. The central difference was found to be more sensitive to the values of  $\Delta R$ ,  $\Delta \mu$ ,  $\Delta \gamma$ . In most cases, the iteration process diverged unless

Lavenburg [1944] and Marquardt [1963] modification of the  $V^T V$  matrix was incorporated. Another observed problem is oscillation of the convergence process as reported in the literature. Multiplying the  $\delta$  values with a factor less than one damped the oscillation but slowed down the convergence. The initial guess was found to play very important role in the convergence process. In many instances, a very rough guess of the parameters resulted in the divergence of the iteration process. However this limitation has been overcome by choosing proper  $\Delta R$ ,  $\Delta \mu$ ,  $\Delta \gamma$  values at each iteration.

## 5.5 Examples of the Least Square Fit

A computer program has been developed to estimate the parameters using the above algorithm. It was very difficult to use the IMSL routine for the optimization because it needs a finite difference Jacobean similar to the  $V$  matrix as input and that cannot be easily computed. Moreover all the programs have been developed on the PC and the PC version of the IMSL is not available. To test the program performance and accuracy, a set of hypothetical values of concentration data ( $C(x,t)$ ) was input to the program. The data was computed with  $R = 2.0$ ,  $\mu = -0.80$  /day,  $\gamma = +0.40$  mg/L/day. The initial concentration was 10 mg/L. The initial media concentration was  $C(x) = 10 - x$ . The groundwater velocity  $v$  was given by  $v(t) = 4.0 + 0.1t$ . As shown in the Table 5.1 and Table 5.2, 100% accurate minimization was obtained in very few iterations with very rough initial guesses. Table 5.1 shows the optimization of all the four parameters starting from zero initial guesses of reaction constants. Since  $R$  and  $\alpha$  cannot be zero, they were assumed as 1. Table 5.2 shows estimation of three parameters (keeping  $\alpha$  as constant) with more erroneous initial guesses of the parameters.

Table 5.1 Part of computer program output showing convergence of the least square parameters

Iteration No	Dispersivity Constant	Retardation Constant	First-order Constant	Zero-order Constant	Sum of squares of Residuals
1	1.00000	1.00000	.00000	.00000	738.92499
2	1.49520	1.65730	-.38470	-.20000	62.24965
3	6.95654	1.83468	-.71806	.35075	3.07349
4	8.53524	1.98997	-.80016	.40650	.01986
5	7.21317	2.00340	-.79688	.39063	.00081
6	7.06231	2.00036	-.79943	.39792	.00003

Table 5.2 Part of computer program output showing the convergence of the least square parameters ( $\alpha$  kept constant)

Iteration No	Dispersivity Constant	Retardation Constant	First-order Constant	Zero-order Constant	Sum of squares of Residuals
1	7.00000	1.00000	.50000	-.50000	3898.28362
2	7.00000	1.02396	.97913	-15.58675	4645.73205
3	7.00000	1.05446	.44479	-9.56915	468.09890
4	7.00000	1.21024	-.15919	-3.99775	31.43939
5	7.00000	1.49420	-.73127	-.14147	3.18030
6	7.00000	1.89001	-.86389	.67999	.34921
7	7.00000	2.00665	-.80179	.40905	.00088
8	7.00000	1.99945	-.80019	.40049	.00000



# **Chapter 6**

## **RESULTS & DISCUSSIONS**

## 6 RESULTS & DISCUSSIONS

### 6.1 General

Besides computer simulation, the present study involves large amount of experimental work as discussed in Chapter 3. Important properties of the porous medium (hydraulic conductivity, porosity etc.) and sand (gradation, shape, etc.) were mentioned in that chapter. The theoretical background of transport simulation has been described in Chapter 4. The theoretical background of the nonlinear least square fit of the experimental data has been covered in Chapter 5. The summary of experimental results and least square fit is presented in table 6.1.

Table 6.1 Summary of experimental data analysis

Expt. No. ↓	Conc. (ppm)	Velocity (m/day)	O:BTX	Computed R			Computed -μ (/day)		
				B	T	X	B	T	X
CTRL - 1	10	4	1.5	1.04	1.07	1.03	0.052	0.038	0.029
CTRL - 2	30	2	3.2	1.05	1.05	1.04	0.054	0.039	0.028
CTRL - 3	50	1	3.2	1.05	1.06	1.04	0.052	0.037	0.027
BIO1.1.1(1)	10	4	1.5	1.08	1.15	1.05	0.30	0.25	0.21
BIO1.1.1(2)	10	4	1.5	1.03	1.08	1.02	0.32	0.24	0.22
BIO1.1.2	10	4	3.2	1.04	1.11	1.06	0.39	0.31	0.25
BIO1.2.1(1)	10	2	1.5	1.06	1.10	1.07	0.42	0.36	0.30
BIO1.2.1(2)	10	2	1.5	1.08	1.05	1.06	0.45	0.37	0.31
BIO1.2.2	10	2	3.2	1.05	1.09	1.02	0.60	0.47	0.35
BIO1.3.1(1)	10	1	1.5	1.08	1.08	1.04	0.65	0.55	0.45
BIO1.3.1(2)	10	1	1.5	1.10	1.12	1.08	0.67	0.56	0.47
BIO1.3.2	10	1	3.2	1.10	1.15	1.07	0.75	0.60	0.54
BIO2.1.1	50	4	1.5	1.07	1.10	1.05	0.32	0.29	0.22
BIO2.1.2(1)	50	4	3.2	1.04	1.07	1.06	0.39	0.33	0.29
BIO2.1.2(2)	50	4	3.2	1.08	1.10	1.03	0.40	0.32	0.28
BIO2.2.1	50	2	1.5	1.07	1.09	1.06	0.49	0.40	0.30
BIO2.2.2(1)	50	2	3.2	1.09	1.10	1.06	0.60	0.53	0.46
BIO2.2.2(2)	50	2	3.2	1.05	1.08	1.04	0.61	0.54	0.47
BIO2.3.1	50	1	1.5	1.05	1.09	1.04	0.70	0.64	0.53
BIO2.3.2(1)	50	1	3.2	1.11	1.10	1.07	0.80	0.70	0.60
BIO2.3.2(2)	50	1	3.2	1.07	1.13	1.08	0.81	0.72	0.63

A total of twenty one experiments including three control runs have been conducted with varying concentration, velocity and DO. As shown in Table 6.1, experimental runs involving low concentration and low DO and those involving high concentration and high DO have been replicated. The replicates are indicated within parenthesis in the experiment numbers. The concentration shown in the Table are concentration of each of BTX compounds. The velocity shown are the initial velocity that is subject to change during the experimental runs depending on change of effective porosity or hydraulic conductivity.

## 6.2 Control Runs

The objective of the control runs is to assess the removal of the BTX compounds in the sand tank due to abiotic sources, namely volatilization, chemical oxidation and sorption. As reported in literature [Lodaya et al., 1991], volatilization removal has been accounted by a first-order removal. For the total removal, Equation (4.5.1) can be written as

$$\alpha v(t) \frac{\partial^2 U}{\partial x^2} - v(t) \frac{\partial U}{\partial x} + (\mu_{\text{biodegradation}} + \mu_{\text{volatilization}}) U + \gamma = R \frac{\partial U}{\partial t}$$

The values of  $\mu$  computed in the main design points gives the sum of first-order coefficient attributed to biodegradation and volatilization. If  $U_c$  represents the concentration measured in the control runs, then

$$\alpha v(t) \frac{\partial^2 U_c}{\partial x^2} - v(t) \frac{\partial U_c}{\partial x} + (\mu_{\text{volatilization}}) U_c + \gamma = R \frac{\partial U_c}{\partial t}$$

Thus  $\mu$  computed from the control runs is  $\mu_{\text{volatilization}}$  in the above equation. As shown in Table 6.1, mean first order removal rate ranged from 0.028/day for xylene to 0.052/day for benzene. The first-order volatilization removal for each of BTX compounds has been found to statistically independent of velocity and input concentration. The biodegradation removal in the main experimental runs has been computed by subtracting the volatilization removal from the total first-order removal.

In case of Monod, the transport equation in Section 4.6.3 has been modified as follows:

$$R \frac{\partial U}{\partial t} = \alpha v(t) \frac{\partial^2 U}{\partial x^2} - v(t) \frac{\partial U}{\partial x} + \frac{kXU}{K+U} - \mu_{\text{volatilization}} U$$

The solution of this equation with known values of  $\mu_{\text{volatilization}}$  accounts for the volatilization removal.

As mentioned in Chapter 3, removal due to chemical oxidation has been neglected in this study. The results of sorption isotherm plotted from the batch studies outlined in Chapter 3 is shown in Figure 6.1. The high values of  $R^2$  indicates that sorption isotherm can be safely modeled with linear isotherm assumed in the transport equation. Although Zytner [1994] modeled the sorption of BTX compounds by Freundlich isotherm ( $S = K_d C_e^{1/n}$ ), the values of  $n$  is very close to unity suggesting that linear isotherm could also be used. Sorption data of the present study was also fitted by Freundlich isotherm as shown in Figure 6.2. It can be noted that the values of  $R^2$  was not improved from the linear isotherms. The values of  $n$  has also found to be close to 1. From the slope of the linear isotherms ( $K_d$ ), the retardation constants ( $R$ ) has been computed using the relation:

$$R = 1 + \rho_b K_d / \Phi$$

From Figure 6.1, the slope  $K_d$  has the unit of  $[VC/m)/C]$ , or  $[ml(mg/l)/gm]/mg/l$ ,  $\rho_b$  has the unit of  $gm/ml$ ,  $R$  for benzene can be computed as follows:

$$R = 1.0 + \frac{(1.78 \text{ gm/ml})(0.0075 \text{ ml/gm})}{0.355} = 1.04$$

The  $R$  values for pure sand for benzene, toluene, and o-xylene has been found to be 1.04, 1.05, and 1.03 respectively. These values has good agreement of  $R$  values computed by least square fit of the three control runs as shown in Table 6.1.

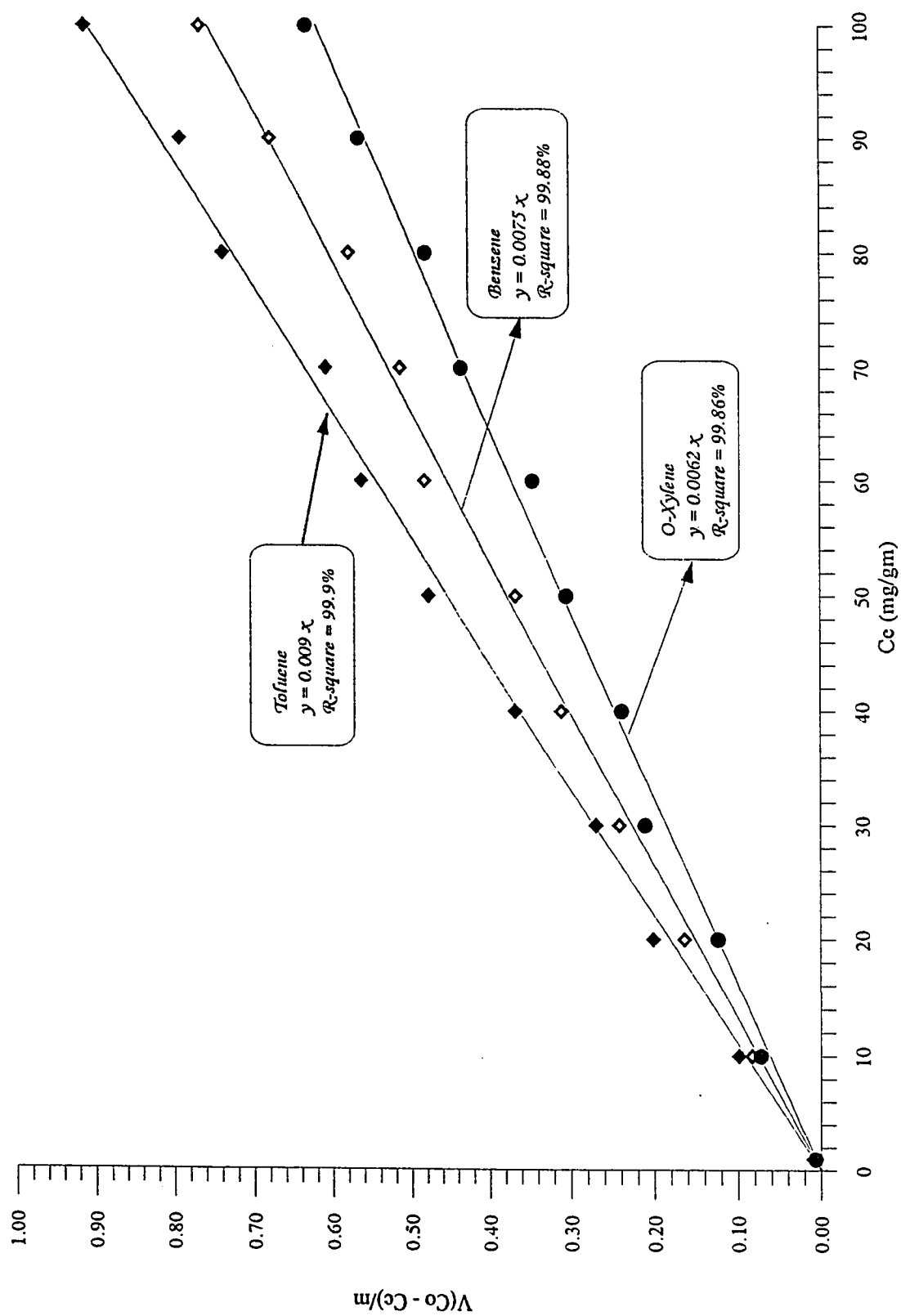


Figure 6.1 Sorption isotherm (linearized) of BTX compounds

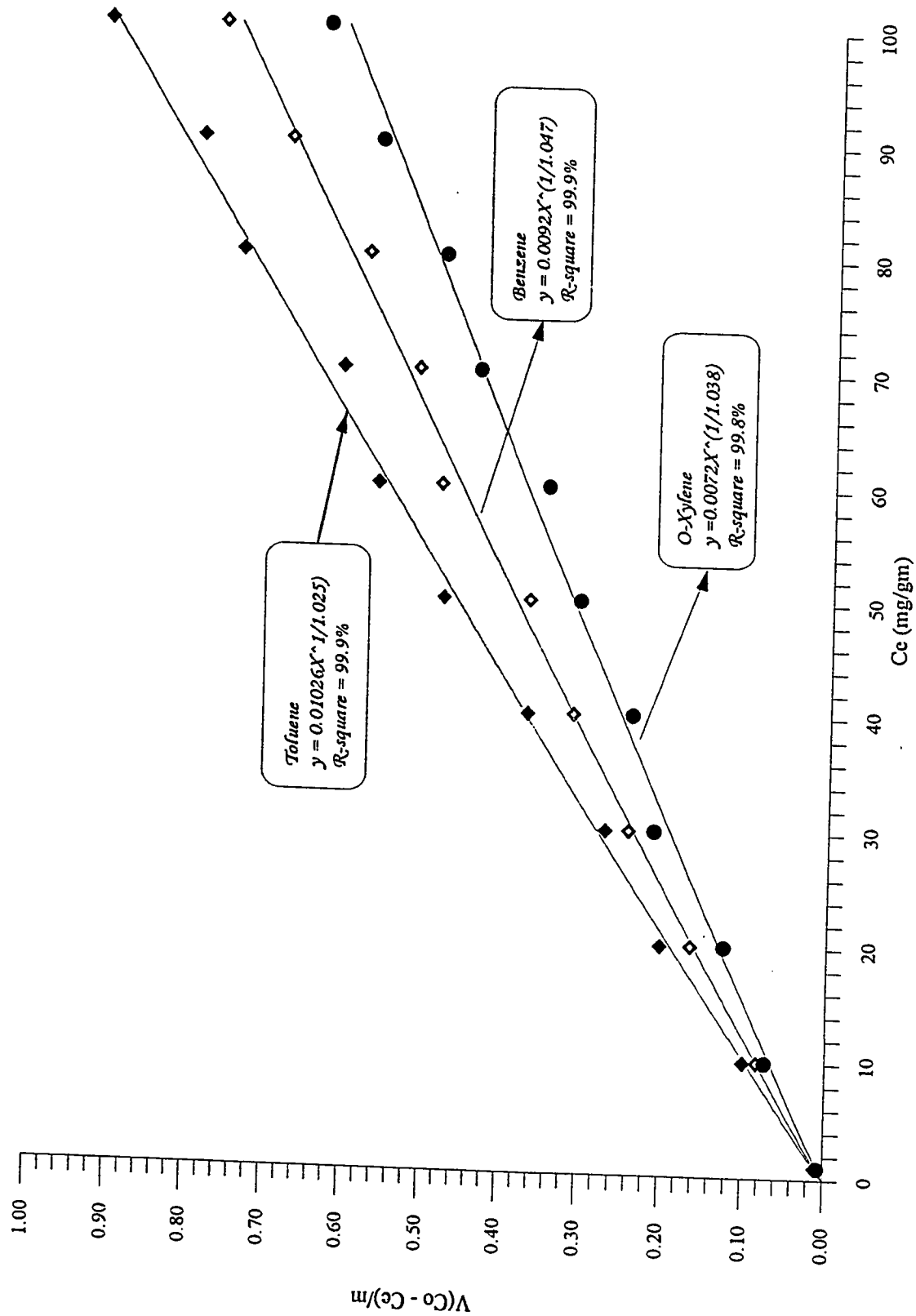


Figure 6.2 Sorption isotherm (Freundlich) of BTX compounds

## 6.3 Experimental Results

### 6.3.1 Order of Experiments

The order of the main experiments is shown in Table 6.2. Randomness was maintained within velocity and DO. Since the same sand tank model was used in repeated runs and since it was imperative that the initial BTX concentration in the sand tank be less than the input concentration, all experiments with low concentration were first performed. The data sheets used to record the experimental data are given in Appendix B. The data shows that benzene has the highest potential of being biodegraded and xylene has the least potential.

Table 6.2 Order of experiments

Serial #	Date	Experiment #	Replicate	Concentration (PPM)	Velocity (m/day)	O:BTX
1	01/08/94	BIO1.1.1 (1)	1	10	4	1.5
8	06/10/94	BIO1.1.1 (2)	2	10	4	1.5
2	07/08/94	BIO1.1.2		10	4	3.2
3	11/08/94	BIO1.2.1 (1)	1	10	2	1.5
7	28/09/94	BIO1.2.1 (2)	2	10	2	1.5
4	19/08/94	BIO1.2.2		10	2	3.2
5	27/08/94	BIO1.3.1 (1)	1	10	1	1.5
9	12/10/94	BIO1.3.2 (2)	2	10	1	1.5
6	12/09/94	BIO1.3.2		10	1	3.2
10	27/10/94	BIO2.1.1		50	4	1.5
14	12/12/94	BIO2.1.2 (1)	1	50	4	3.2
18	23/01/95	BIO2.1.2 (2)	2	50	4	3.2
12	18/11/94	BIO2.2.1		50	2	1.5
15	18/12/94	BIO2.2.2 (1)	1	50	2	3.2
17	11/01/95	BIO2.2.2 (2)	2	50	2	3.2
11	02/11/94	BIO2.3.1		50	1	1.5
13	26/11/94	BIO2.3.2 (1)	1	50	1	3.2
16	26/12/94	BIO2.3.2 (2)	2	50	1	3.2

### 6.3.2 Observed BTX concentration

Observed BTX concentration was found to depend on the velocity, DO, input concentration, as well as the initial (porous media) concentration. Since the experiments were conducted in a random order, the initial concentrations were in some case lower and in some cases higher compared to the pseudo steady state concentrations. As shown in Figures 6.3 - 6.6, the observed concentration reached a quasi steady state after 2 to 3 cycle of detention time ( $T_d$ ) defined as follows:

$$T_d = \frac{\text{Length of the sand tank (m)}}{\text{pore water velocity (m/ day)}}$$

It can be noted that the concentration at the first cycle is sometimes higher than the steady state concentration. The reason is obviously higher initial concentration from the previous run.

Observed BTX concentration at 2 cycles of time at three different velocities is shown in Figures 6.7 - 6.10. These figures illustrate that the higher the velocity, the lower the removal for benzene, for toluene, as well as for xylene. Typical effect of DO on observed benzene, toluene, and o-xylene concentrations at 2 cycles of time is illustrated in figures 6.11-6.16 respectively. These figures demonstrates that at higher DO higher removal of benzene, toluene and xylene is obtained at all velocity and concentration level. The effect of concentration on observed benzene, toluene, and o-xylene concentrations at 2 cycles of time is illustrated in Figures 6.17 and 6.18. These figures shows that at higher concentration higher removal of benzene, toluene and xylene is obtained. The prominent effect of groundwater velocity on the observed pseudo steady state removal of benzene, toluene, and o-xylene is further illustrated in Figures 6.19 and 20.



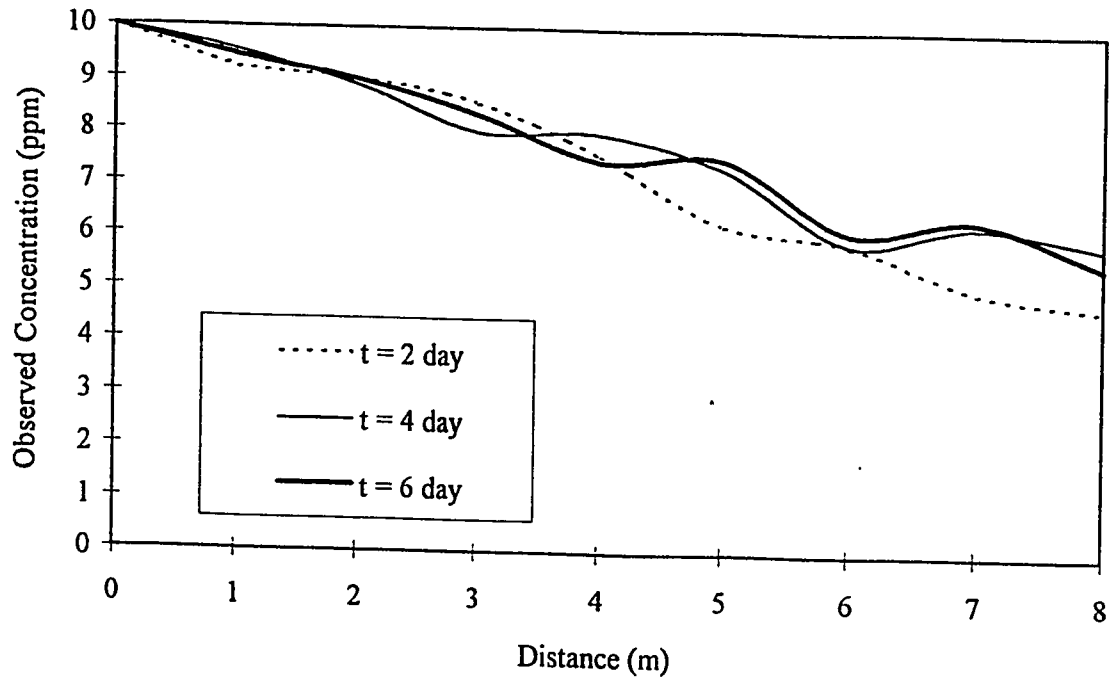


Figure 6.3 Observed benzene concentration at  $v = 4$  m/day [Expt. No. 1.1.1(2)]

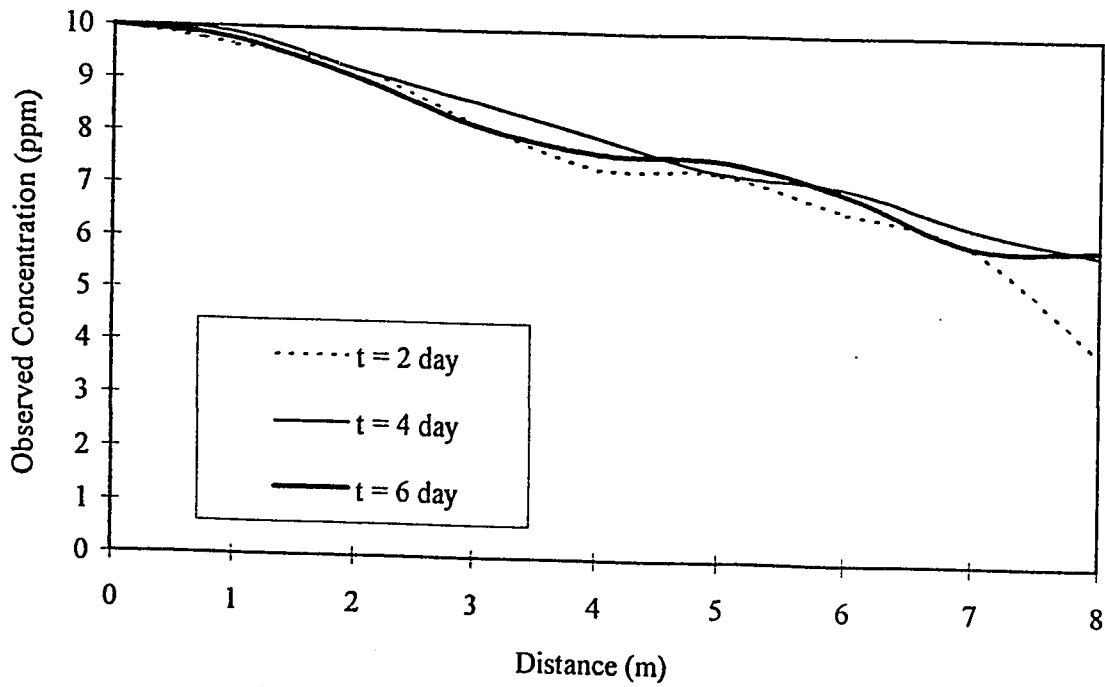


Figure 6.4 Observed toluene concentration at  $v = 4$  m/day [Expt. No. 1.1.1(2)]

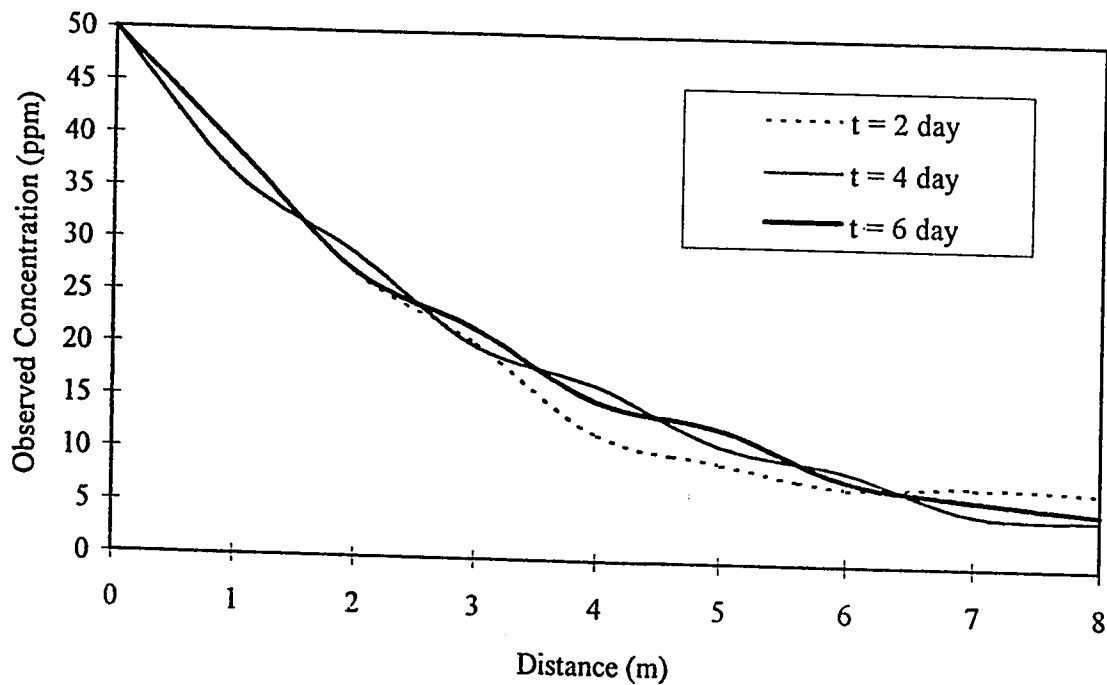


Figure 6.5 Observed benzene concentration at  $v = 2$  m/day [Expt. No. 2.2.2(1)]

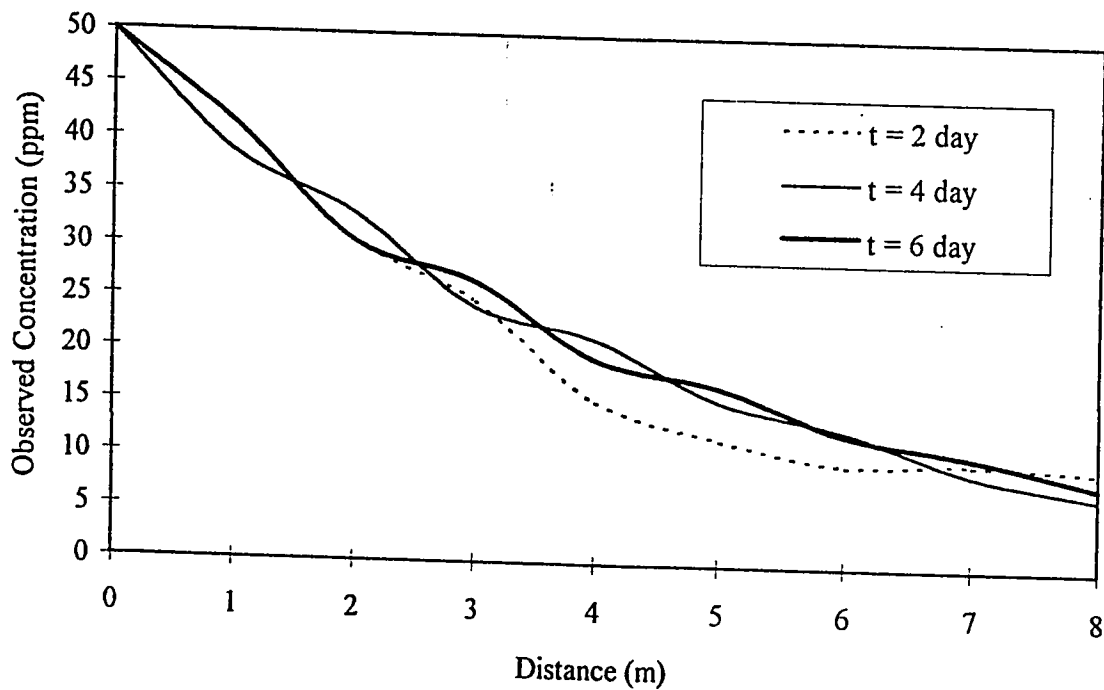


Figure 6.6 Observed o-xylene concentration at  $v = 2$  m/day [Expt. No. 2.2.2(1)]

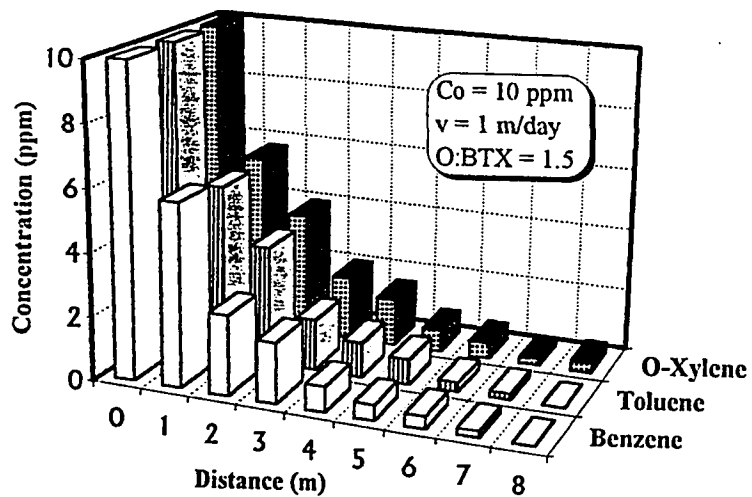
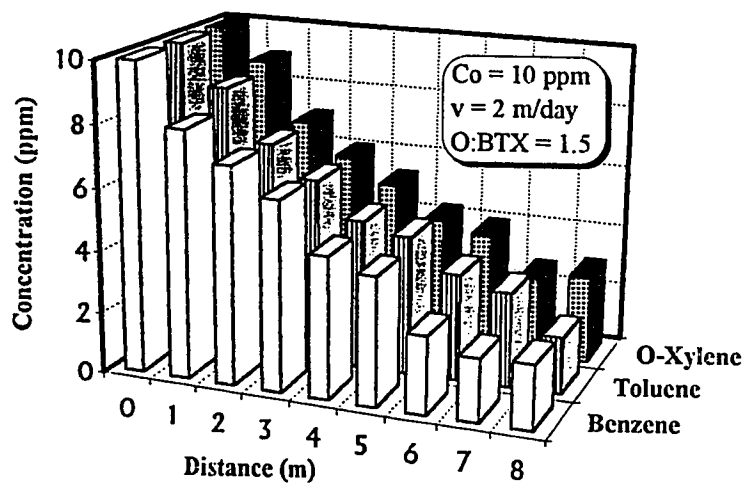
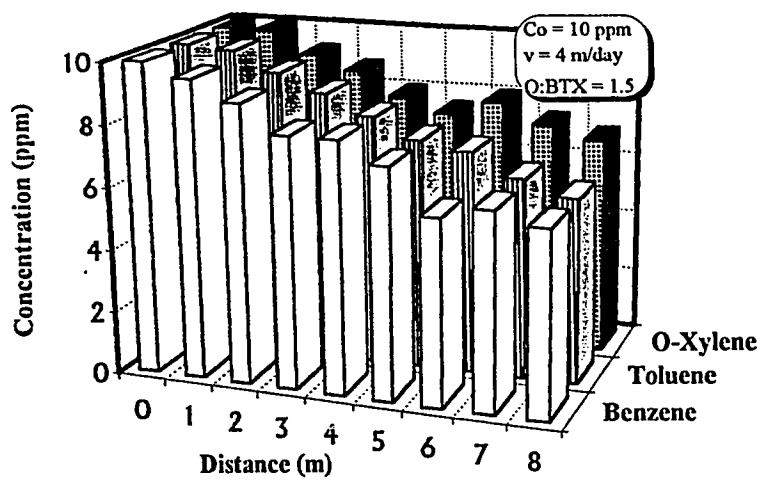


Figure 6.7 Observed BTX concentration at  $C=10$ ,  $O:BTX = 1.5$

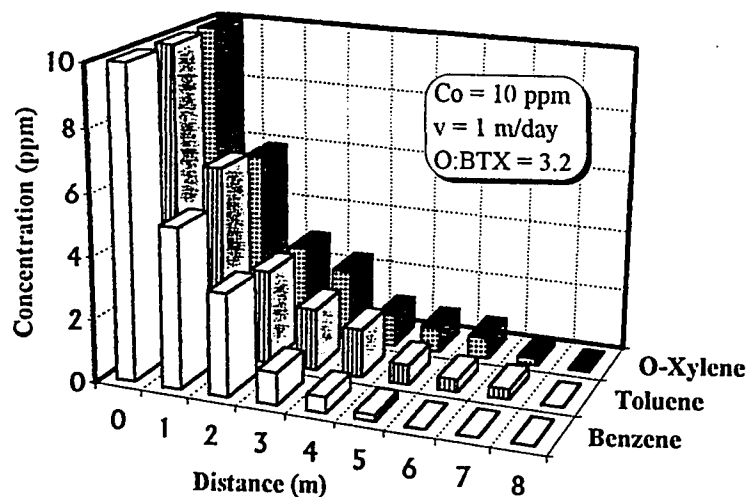
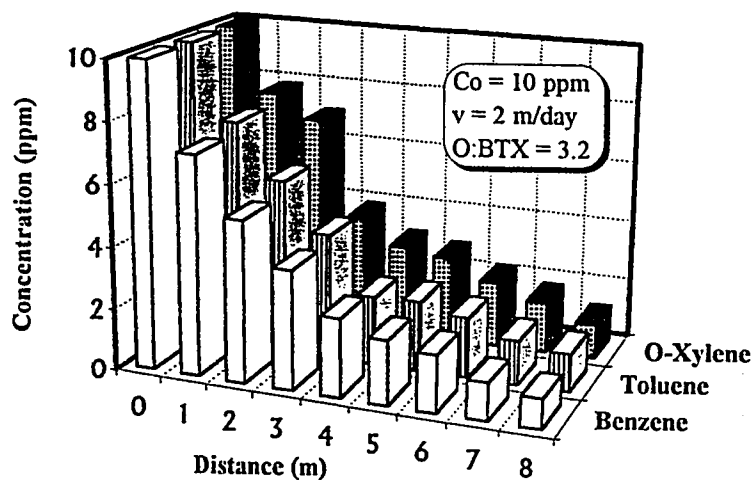
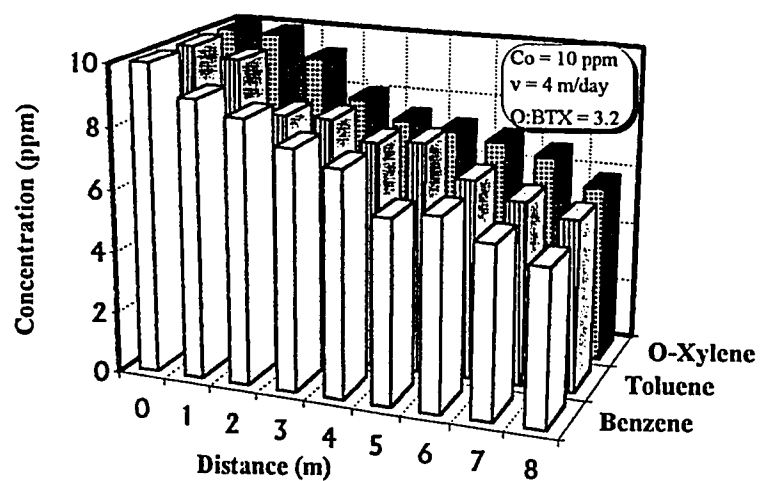


Figure 6.8 Observed BTX concentration at  $C = 10$ ,  $O:BTX = 3.2$

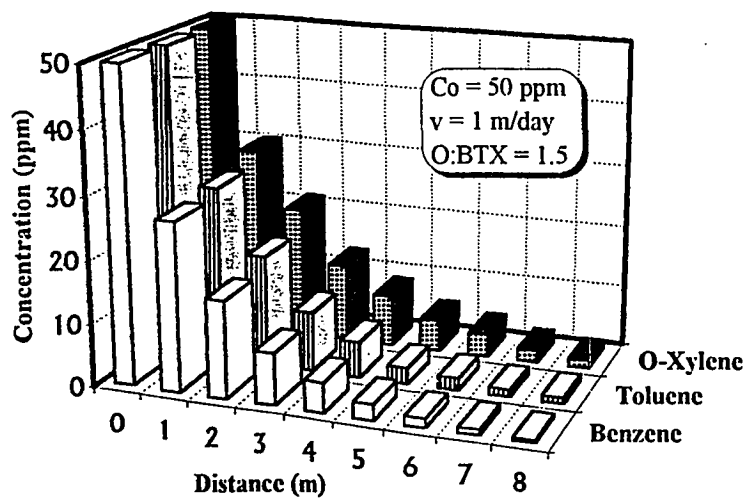
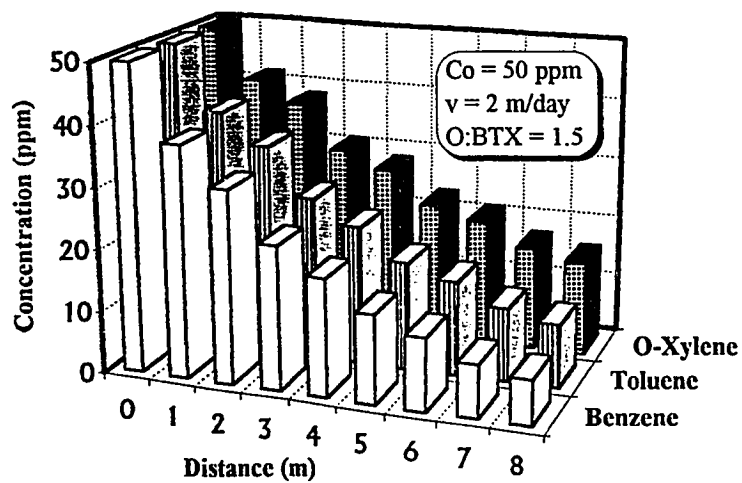
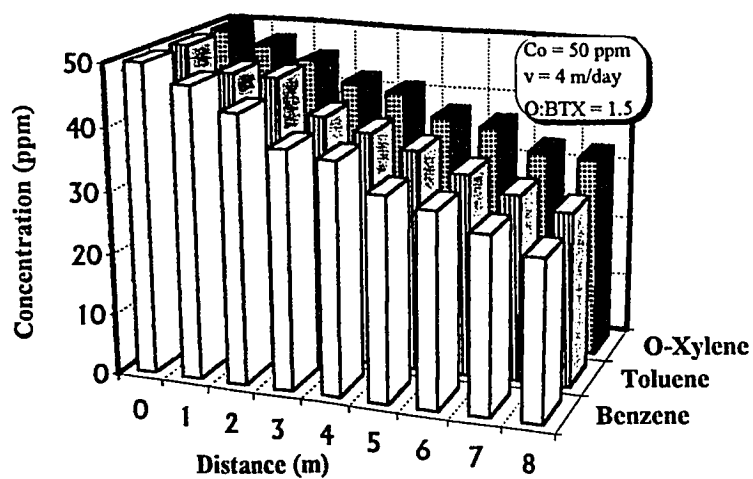


Figure 6.9 Observed BTX concentration at  $C = 50$ ,  $O:BTX = 1.5$

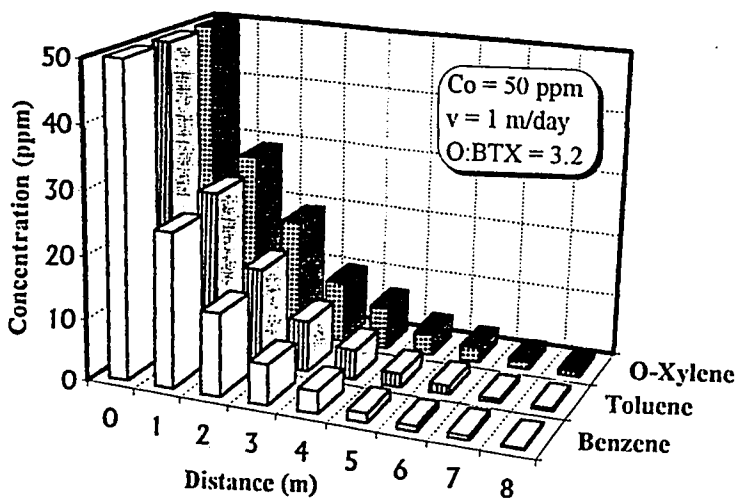
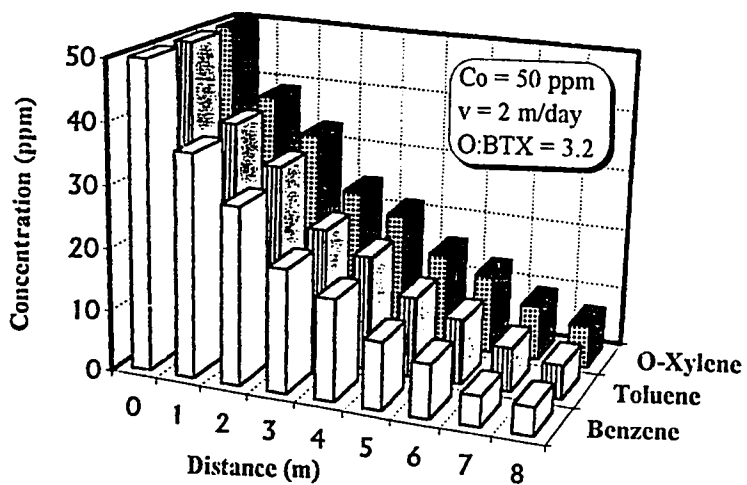
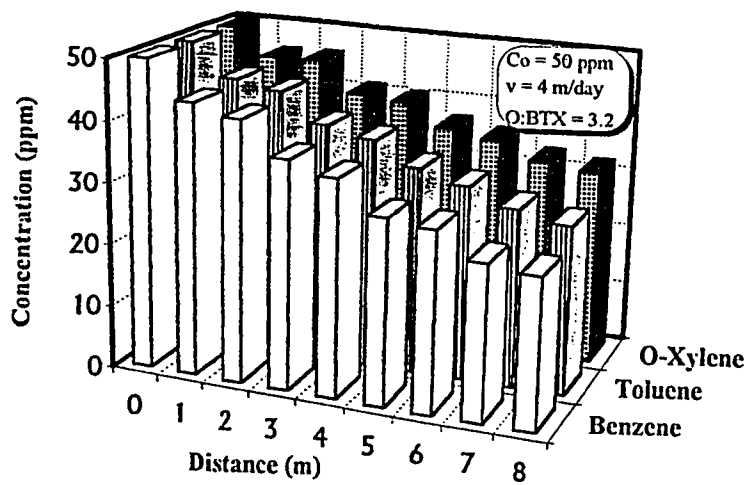


Figure 6.10 Observed BTX concentration at  $C = 50$ ,  $O:BTX = 3.2$

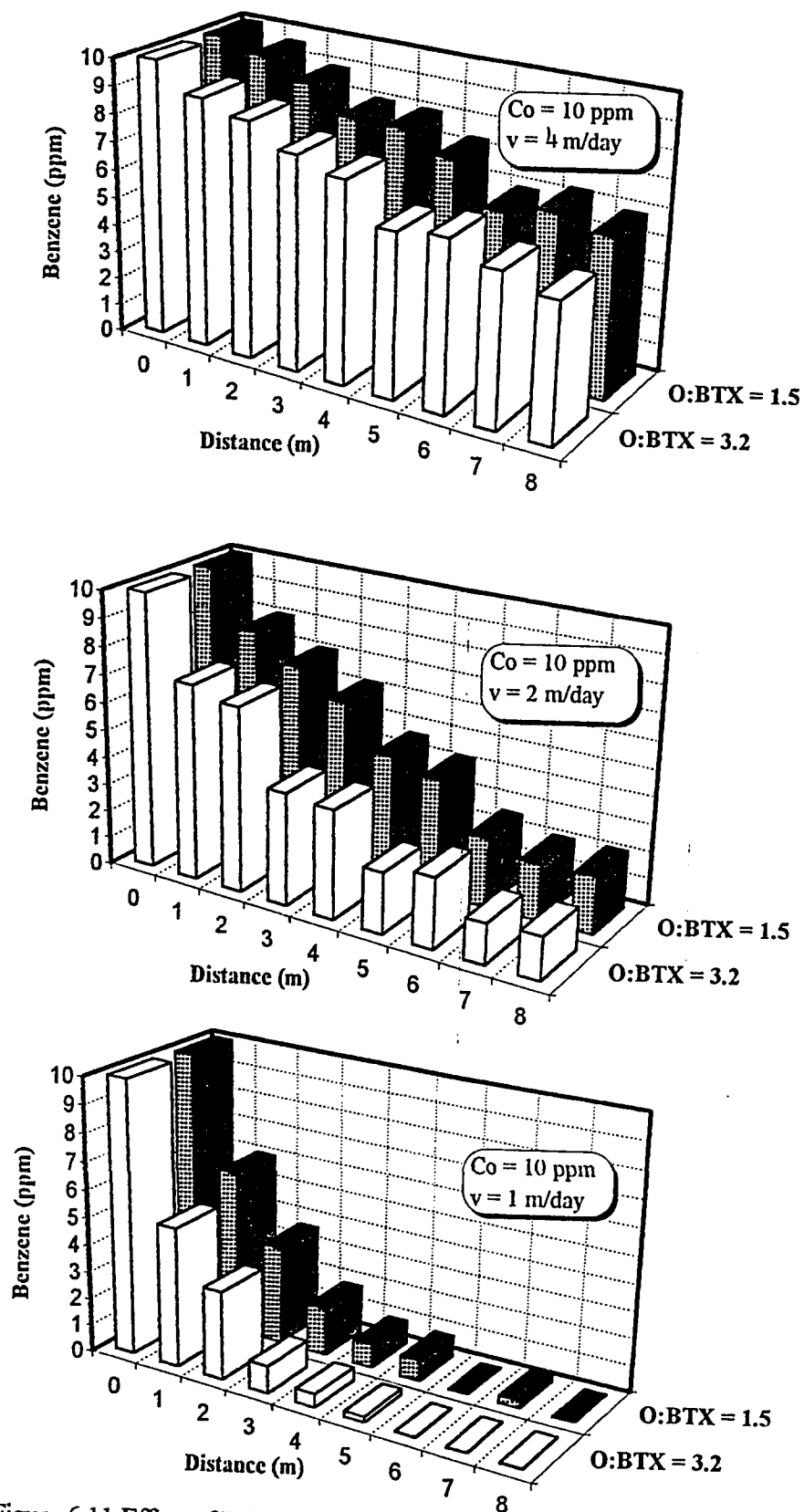


Figure 6.11 Effect of DO on pseudo steady state concentration of benzene

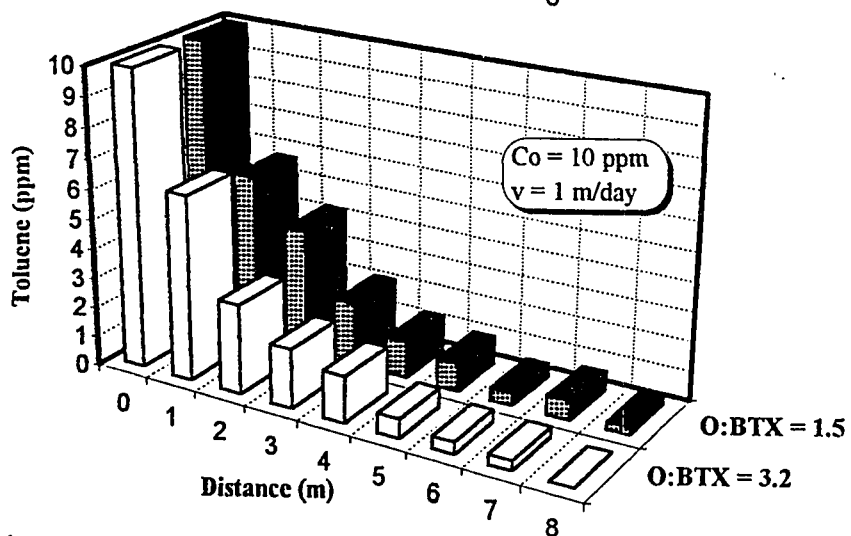
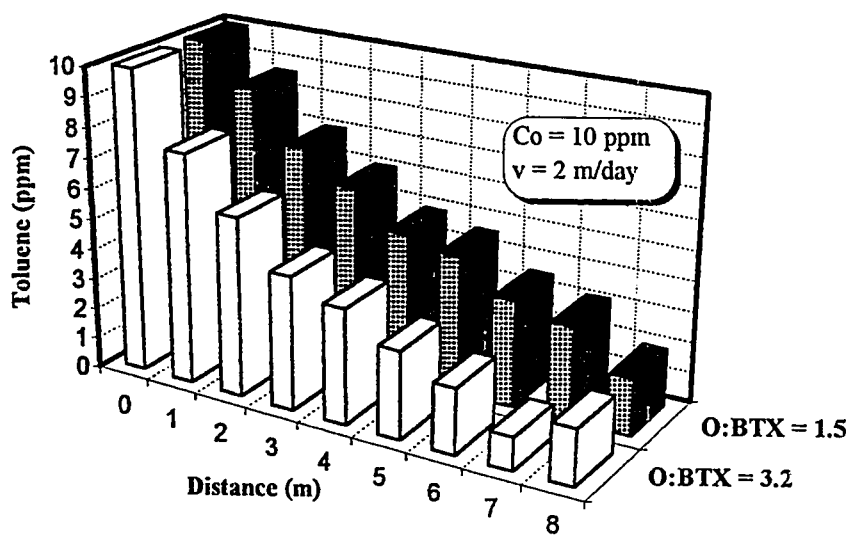
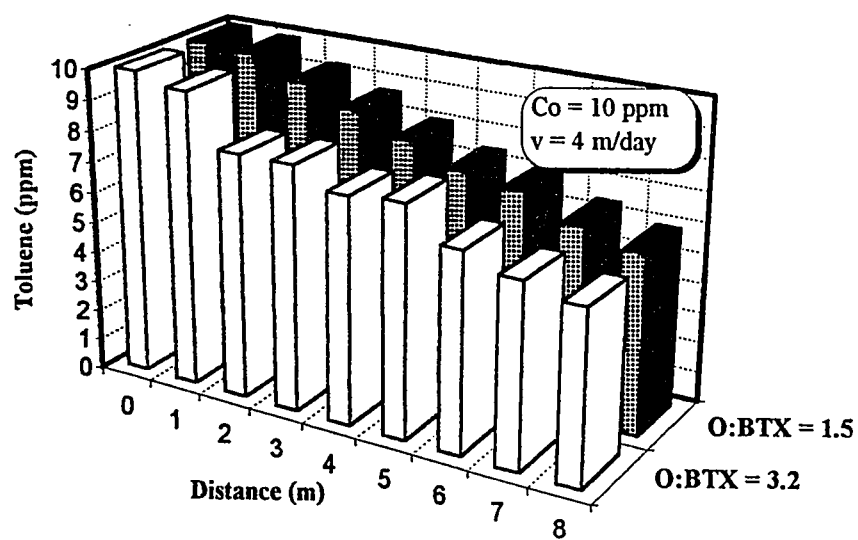


Figure 6.12 Effect of DO on pseudo steady state concentration of toluene



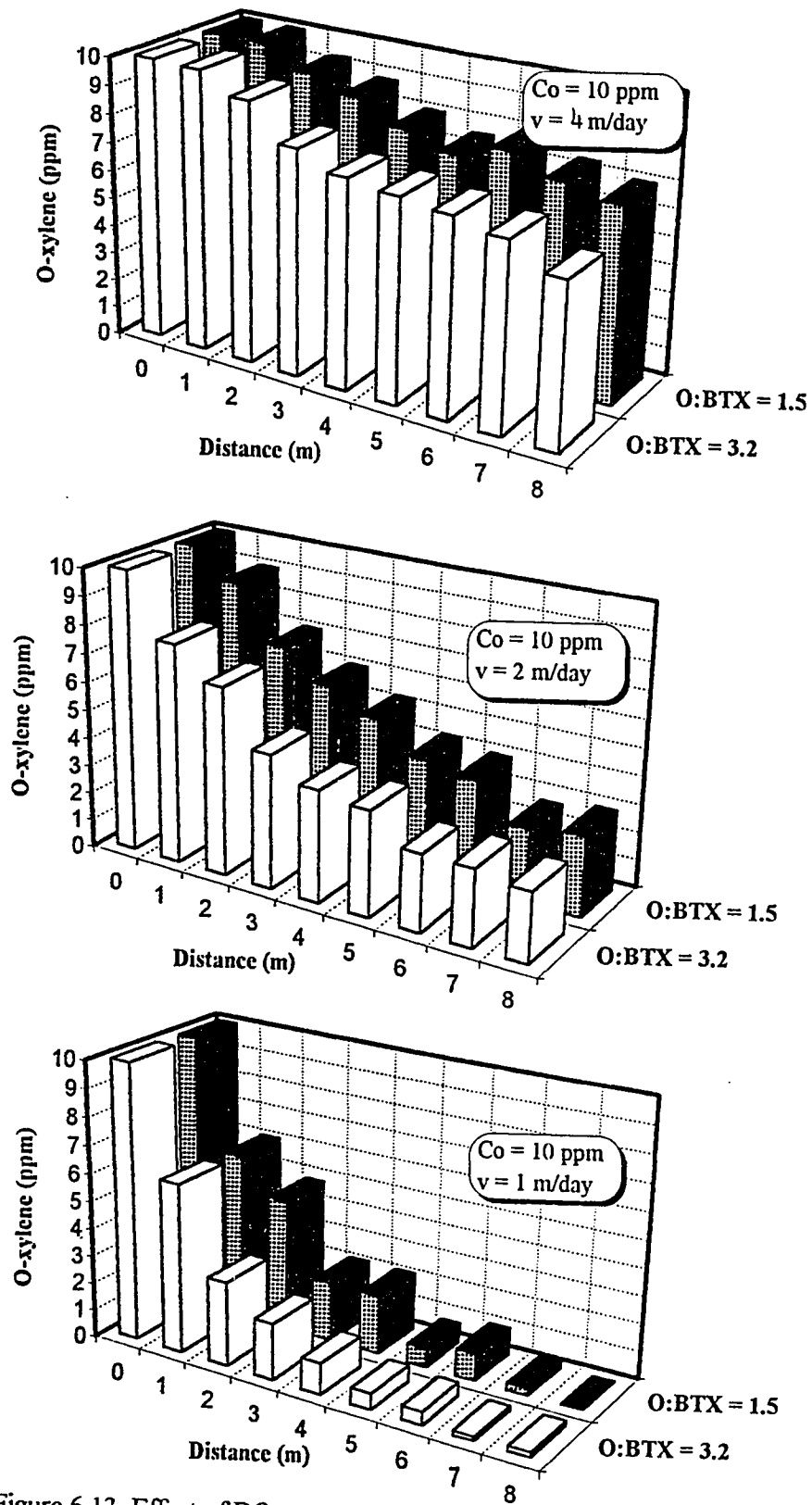


Figure 6.13 Effect of DO on pseudo steady state concentration of o-xylene

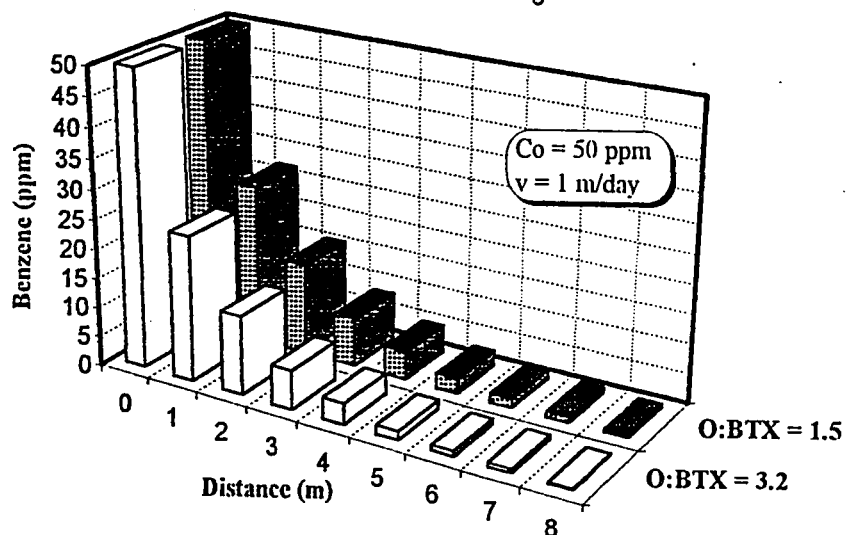
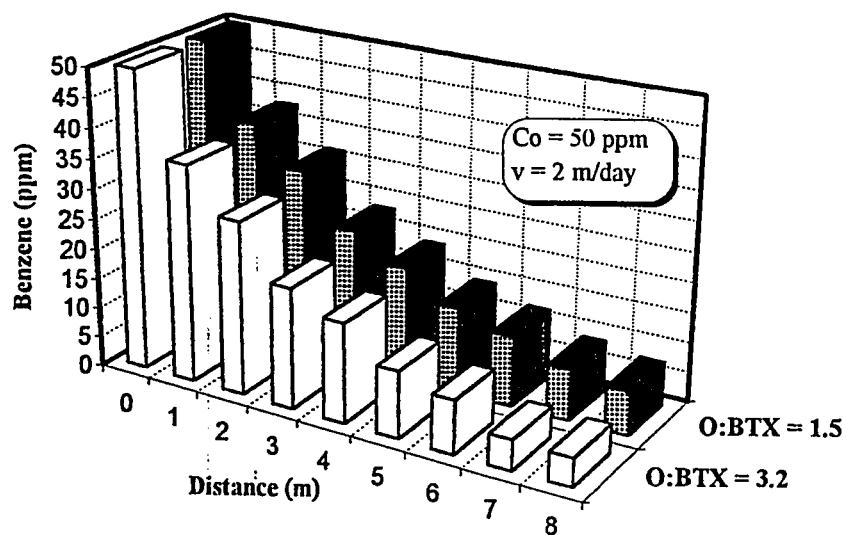
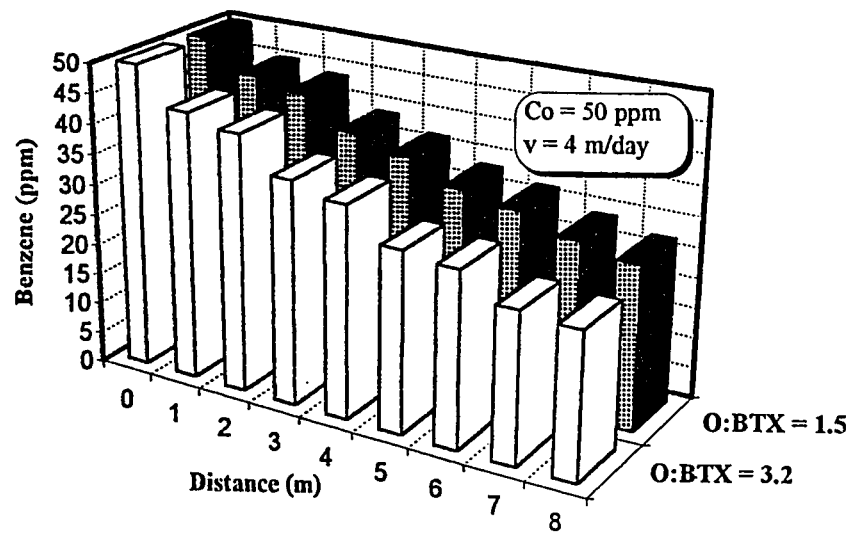


Figure 6.14 Effect of DO on pseudo steady state concentration of benzene ( $C=50$ )

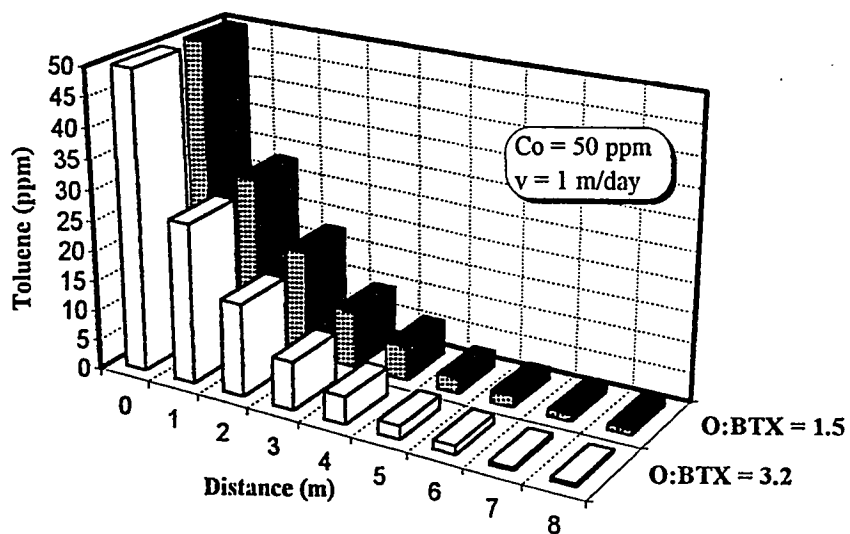
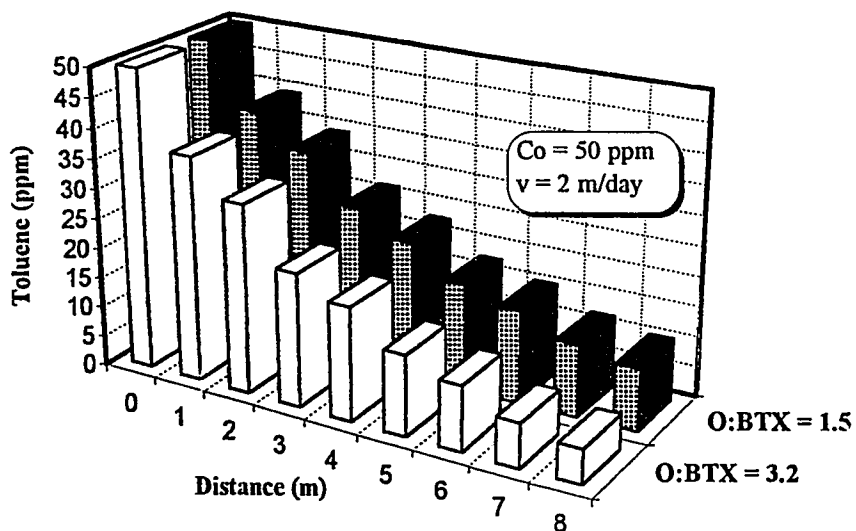
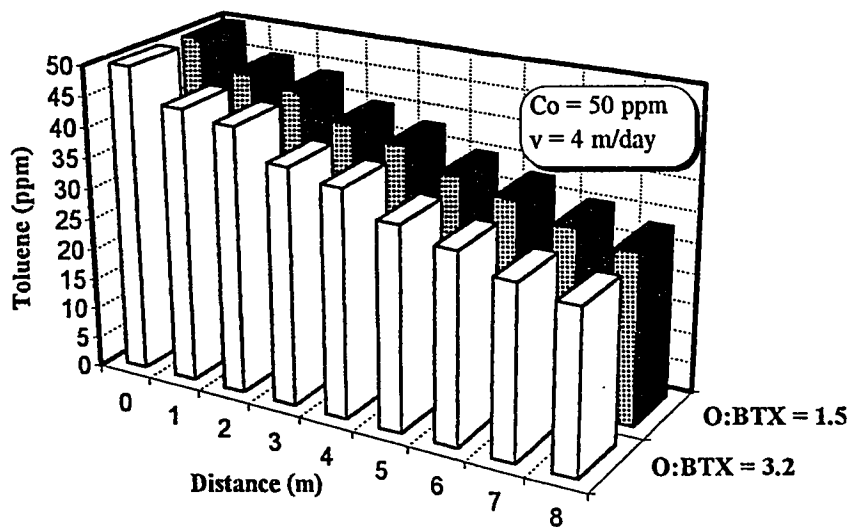


Figure 6.15 Effect of DO on pseudo steady state concentration of toluene ( $C=50$ )

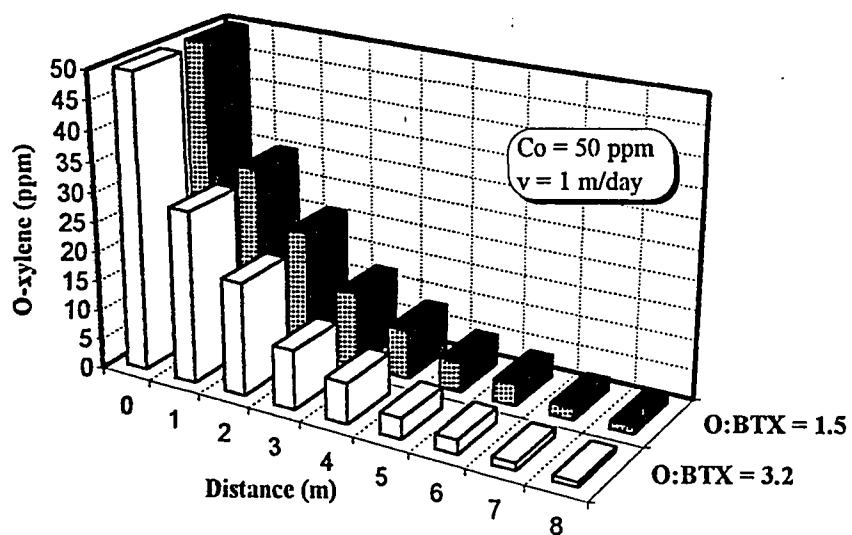
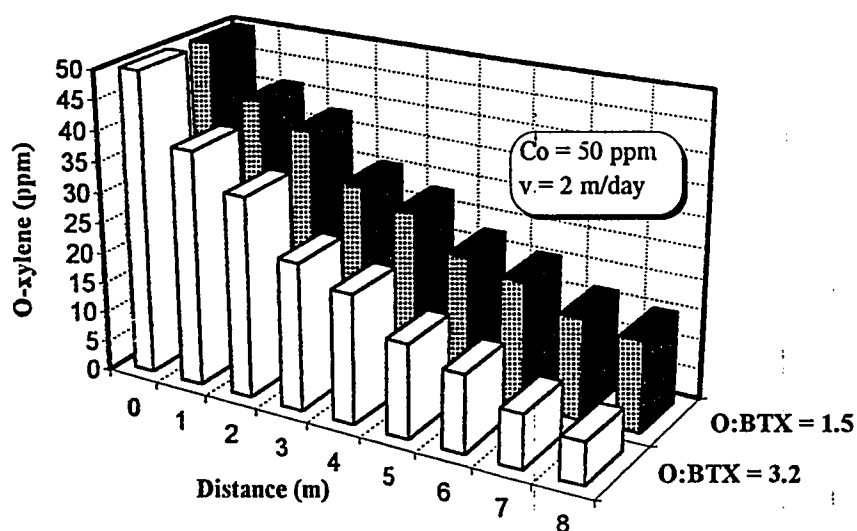
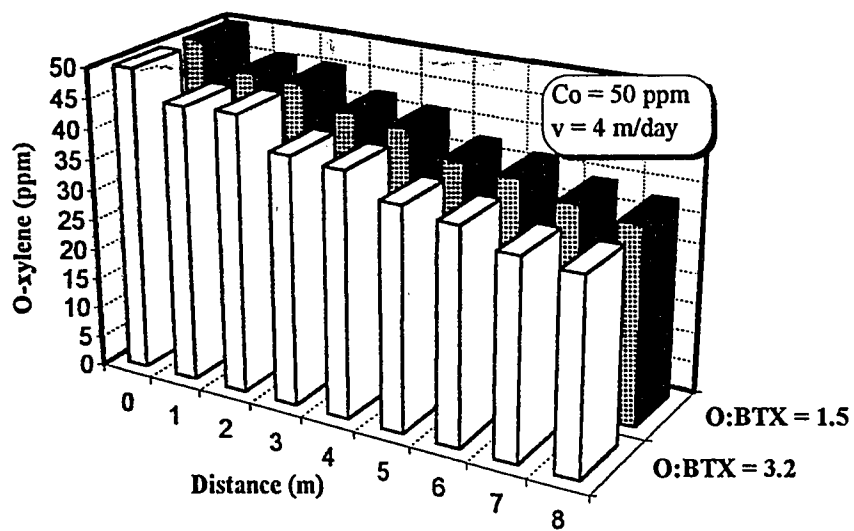


Figure 6.16 Effect of DO on pseudo steady state concentration of o-xylene ( $C=50$ )

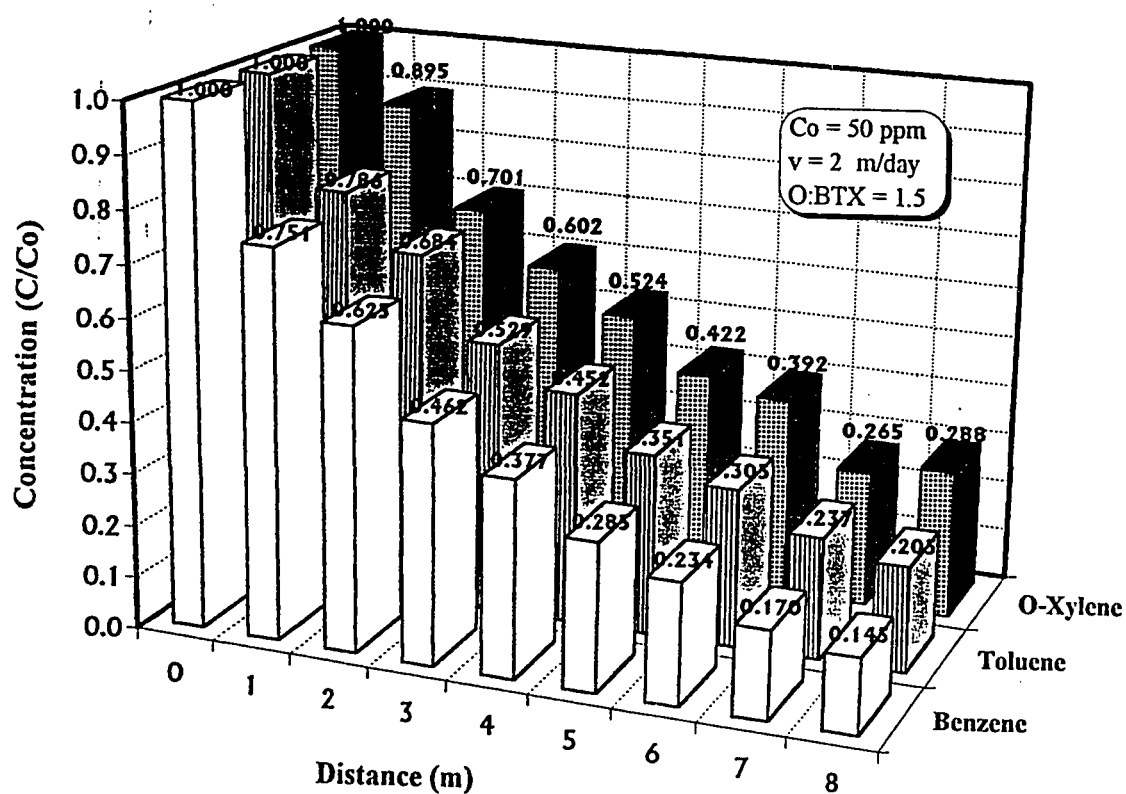
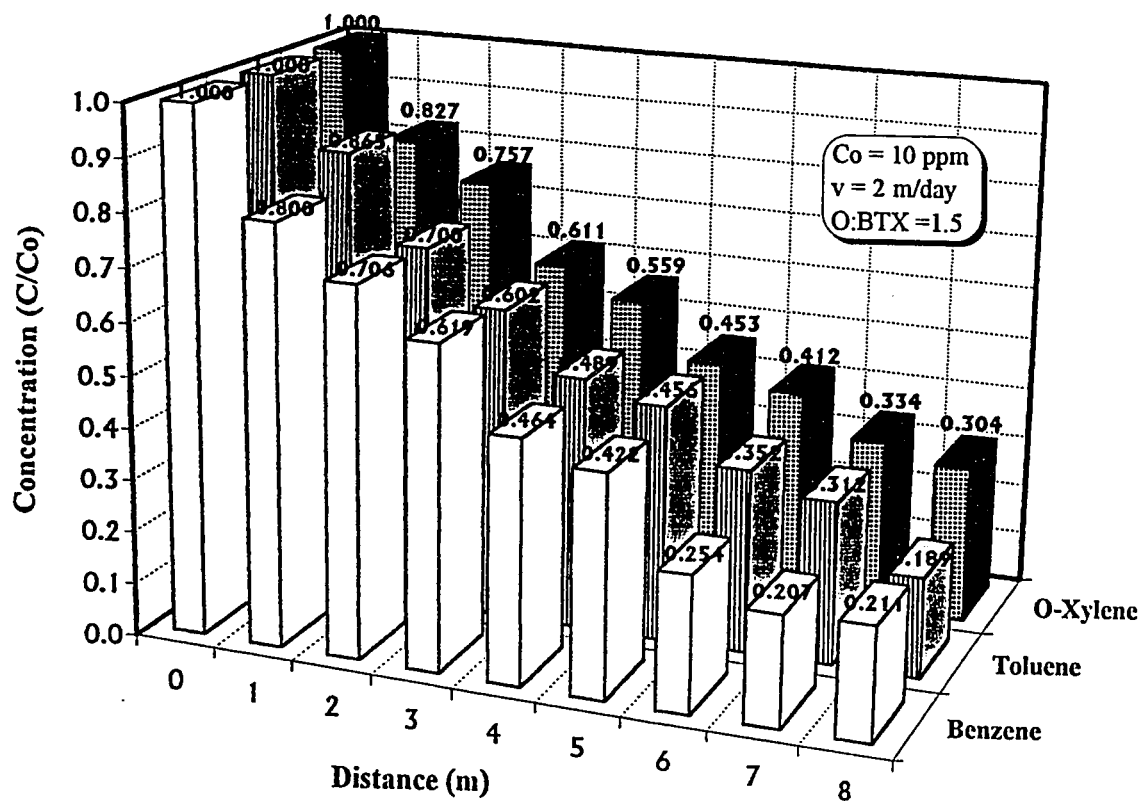


Figure 6.17 Effect of concentration on pseudo steady state concentration of BTX compounds

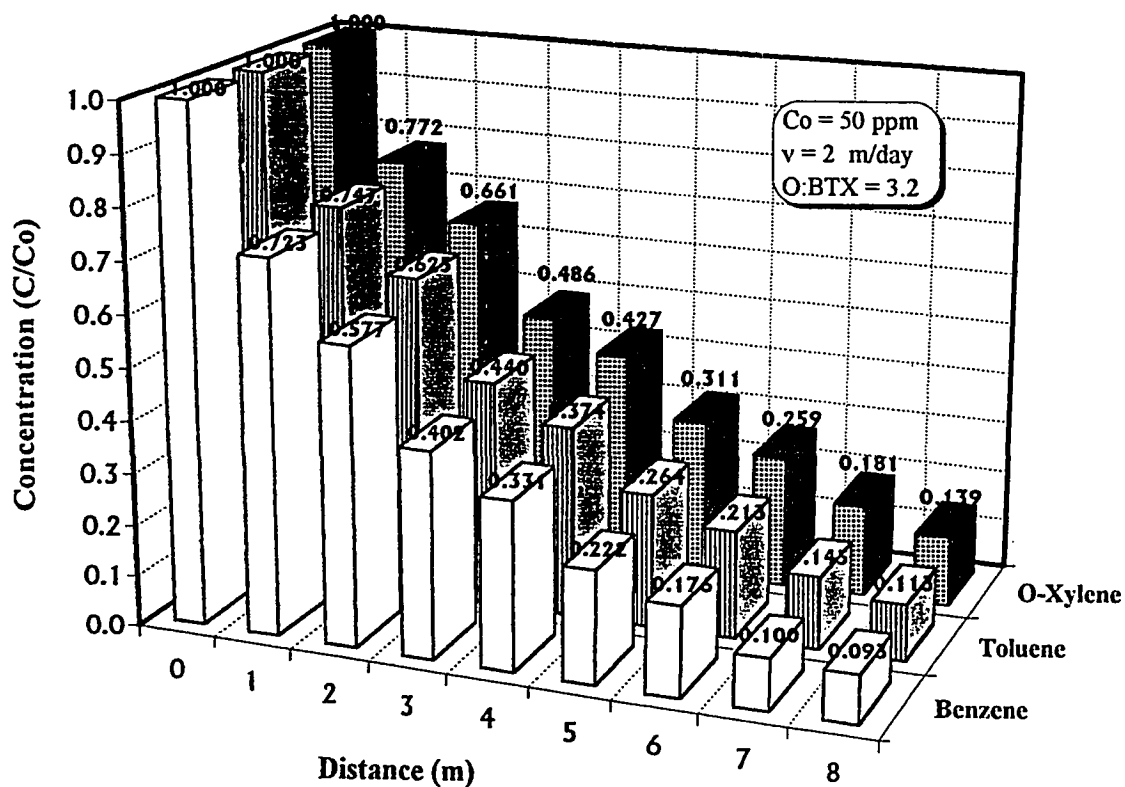
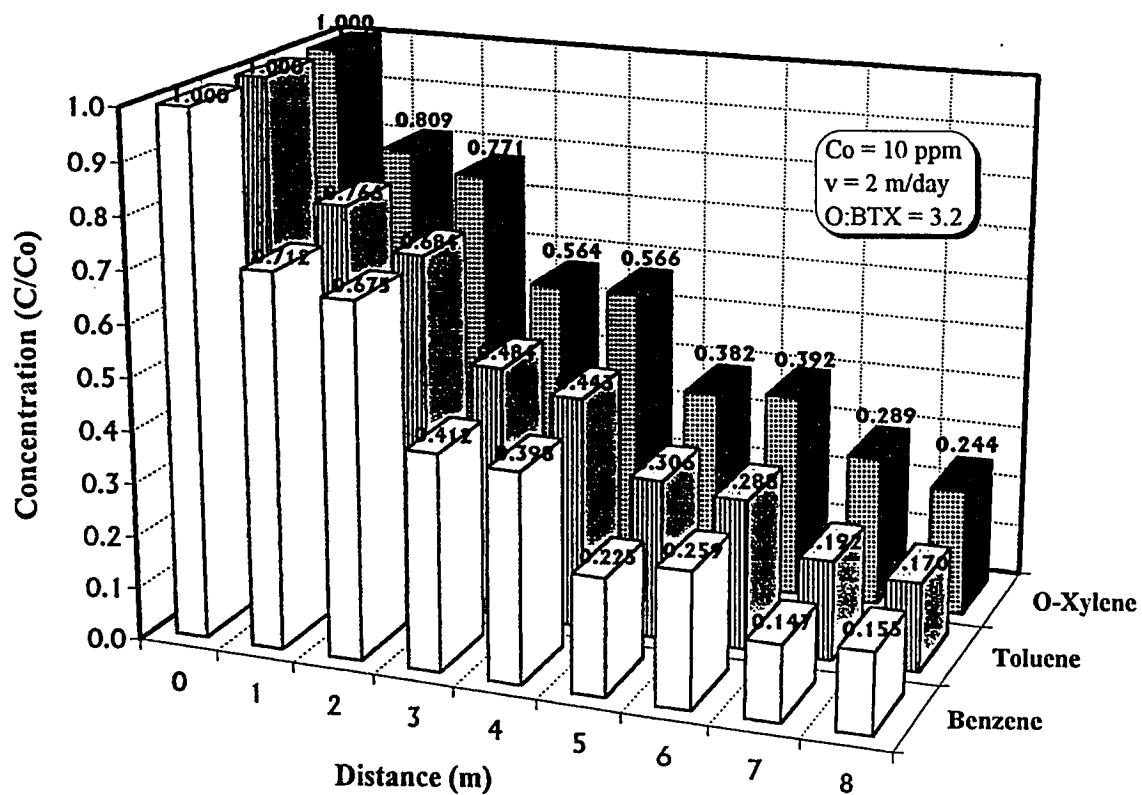


Figure 6.18 Effect of concentration on pseudo steady state concentration of BTX compounds

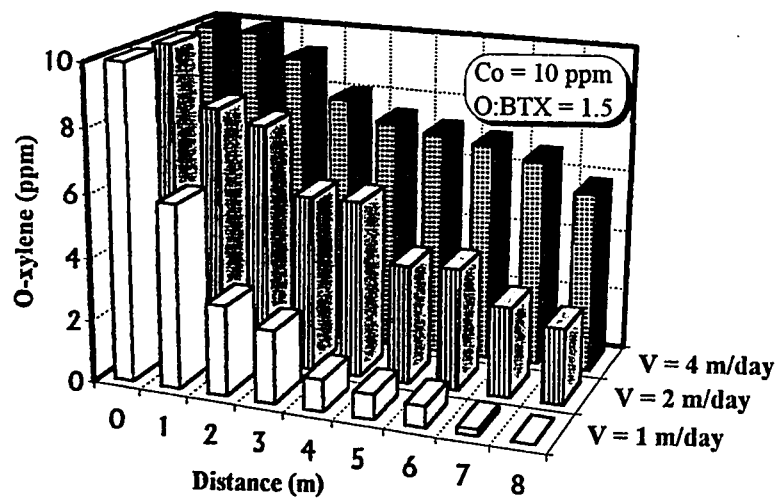
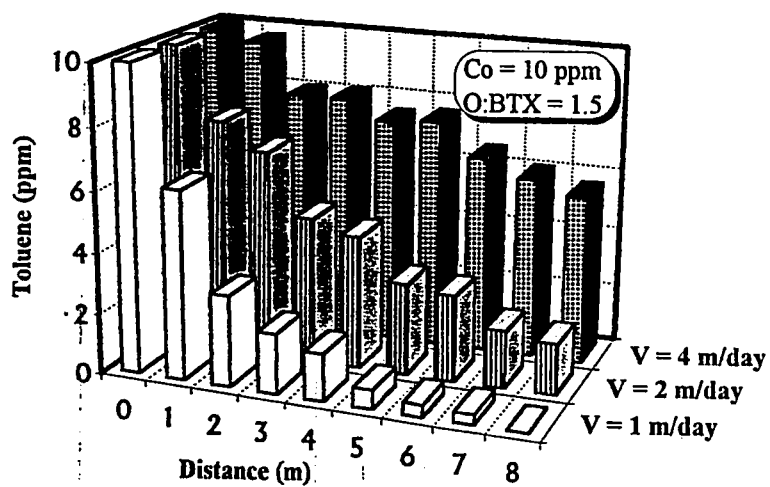
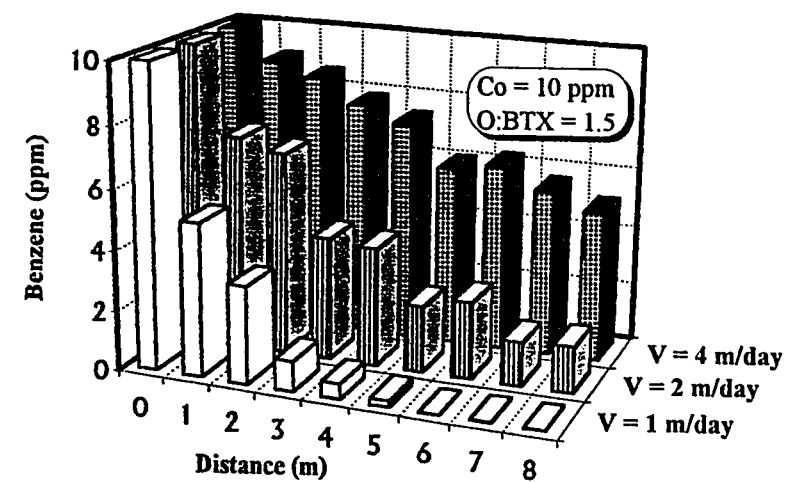


Figure 6.15 Effect of velocity on pseudo steady state concentration of BTX compounds

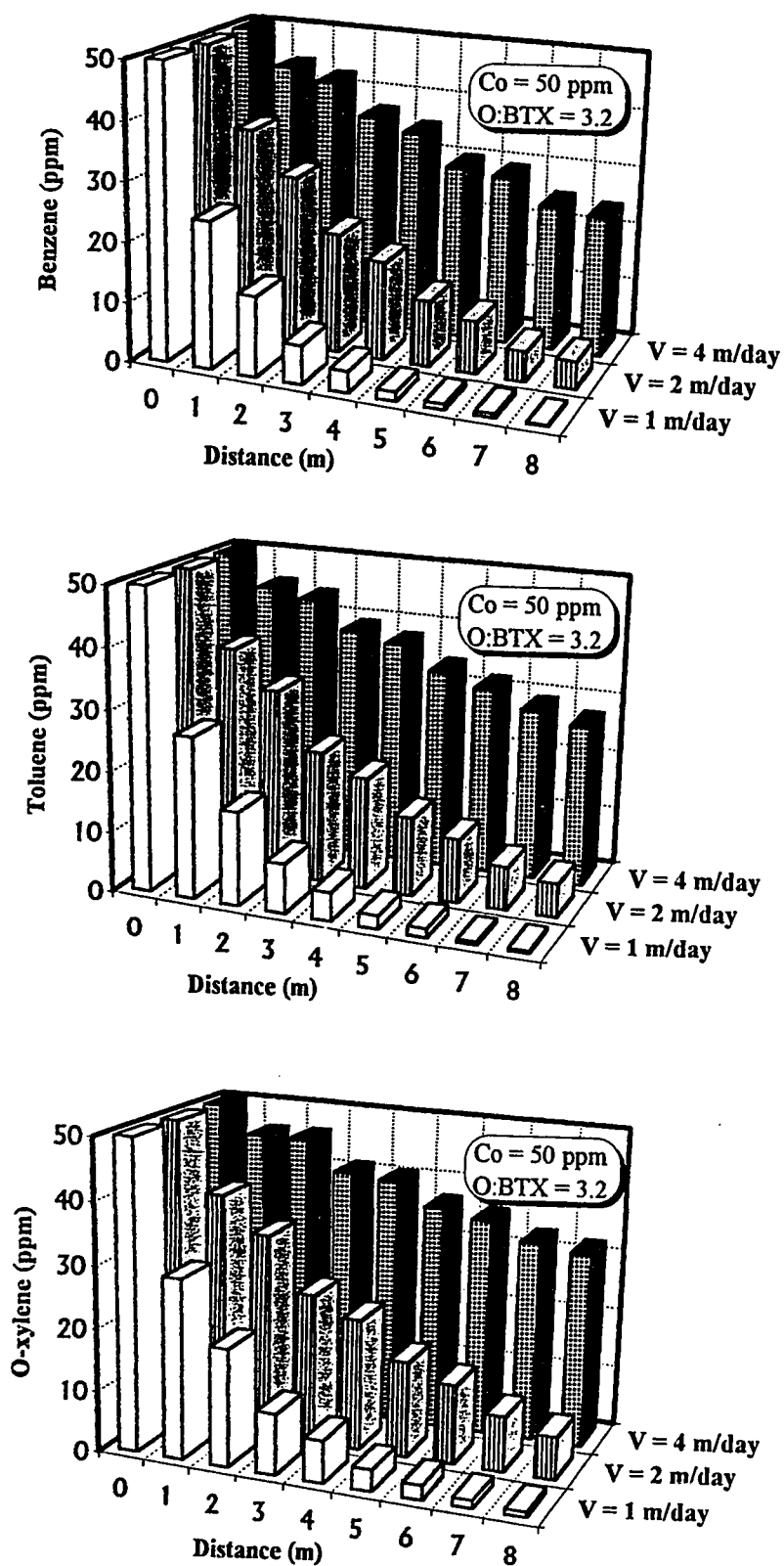


Figure 6.20 Effect of velocity on pseudo steady state concentration of BTX compounds



### ***6.3.3 Changes of Porosity and Permeability***

As mentioned in Chapter 3, only the change of hydraulic conductivity (K) was monitored during the course of experimental period. The effective porosity, defined as the amount of pores (as a ratio of the total volume) interconnected and available for flow and transport, has been computed using Equations (3.2), (3.3) and (3.4). Because of lack of a proper tracer (a tracer, insoluble in water having no toxic effect on the mixed culture) the porosity and pore water velocity were computed in an indirect way. The porosity and hydraulic conductivity profile during the experimental period are depicted by Figures 6.16 and 6.17. Initial hydraulic conductivity and porosity of the sand was 262 cm/s and 0.355 respectively and the minimum hydraulic conductivity and porosity of the medium was computed to be 48 cm/s and 0.165. The mean porosity was 0.2075 with a standard deviation of 0.0544. The mean hydraulic conductivity was 82.74 with a standard deviation of 49.87.

A continued decrease in hydraulic conductivity and porosity was observed when experiments were conducted from high to low velocity. On the other hand, in cases of experimental run where velocity was increased from the previous run, a relatively steady or sometimes increasing hydraulic conductivity and porosity was computed.

### ***6.3.4 Change of Linear Velocity***

In all experiment the flow thorough the sand tank was kept constant by the metering pump. Since the effective porosity of the medium was keeping on changing, the linear velocity given by Equation 3.4 ( $v = Q/A\Phi$ ) also kept on changing. Usually the linear velocity increased as shown in Figure 6.18. However, in few cases where the velocity in the previous design experiment had been lower, the velocity in the current experiment run was somewhat constant or even decreasing because of high shear.

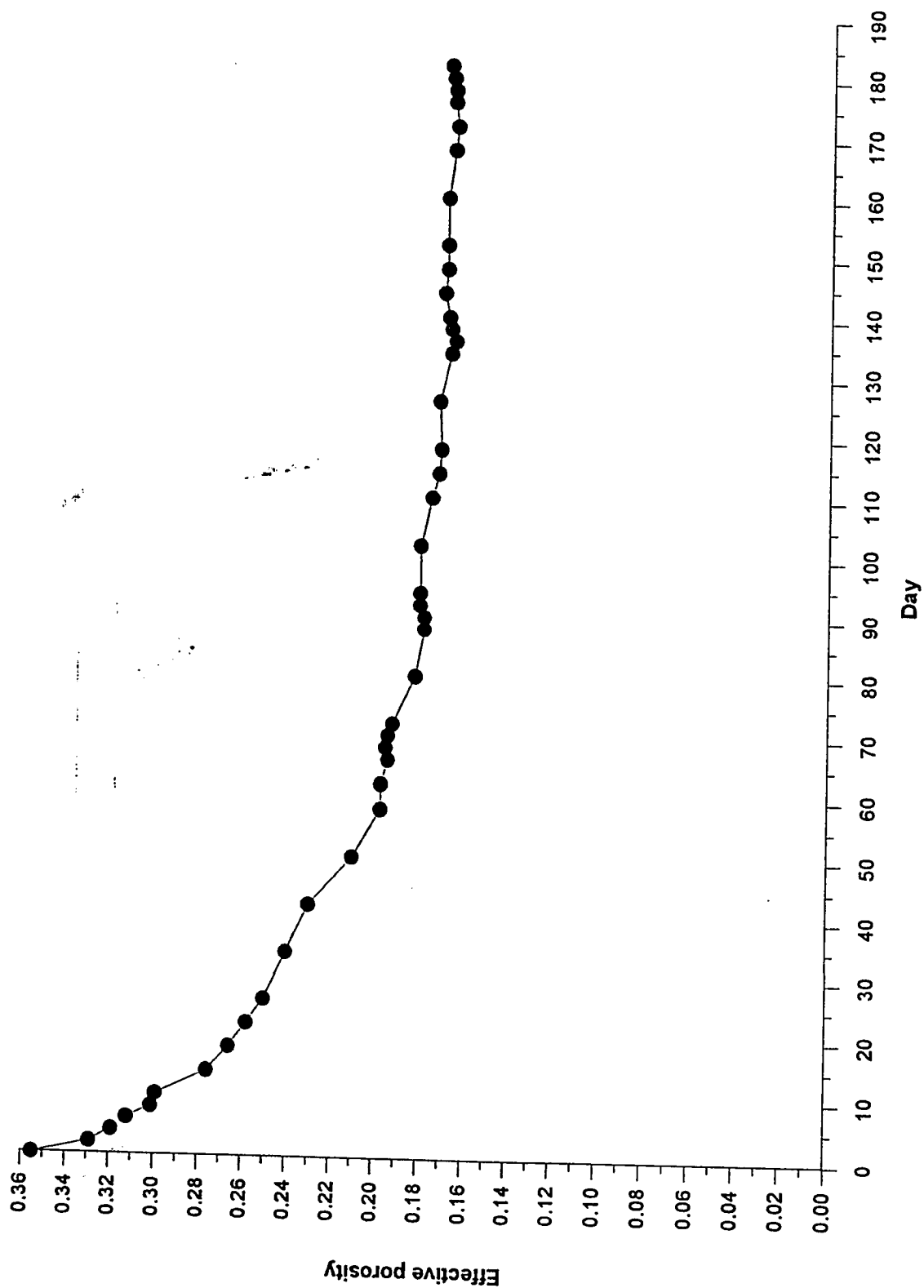


Figure 21 Change of effective porosity during the course of experiment

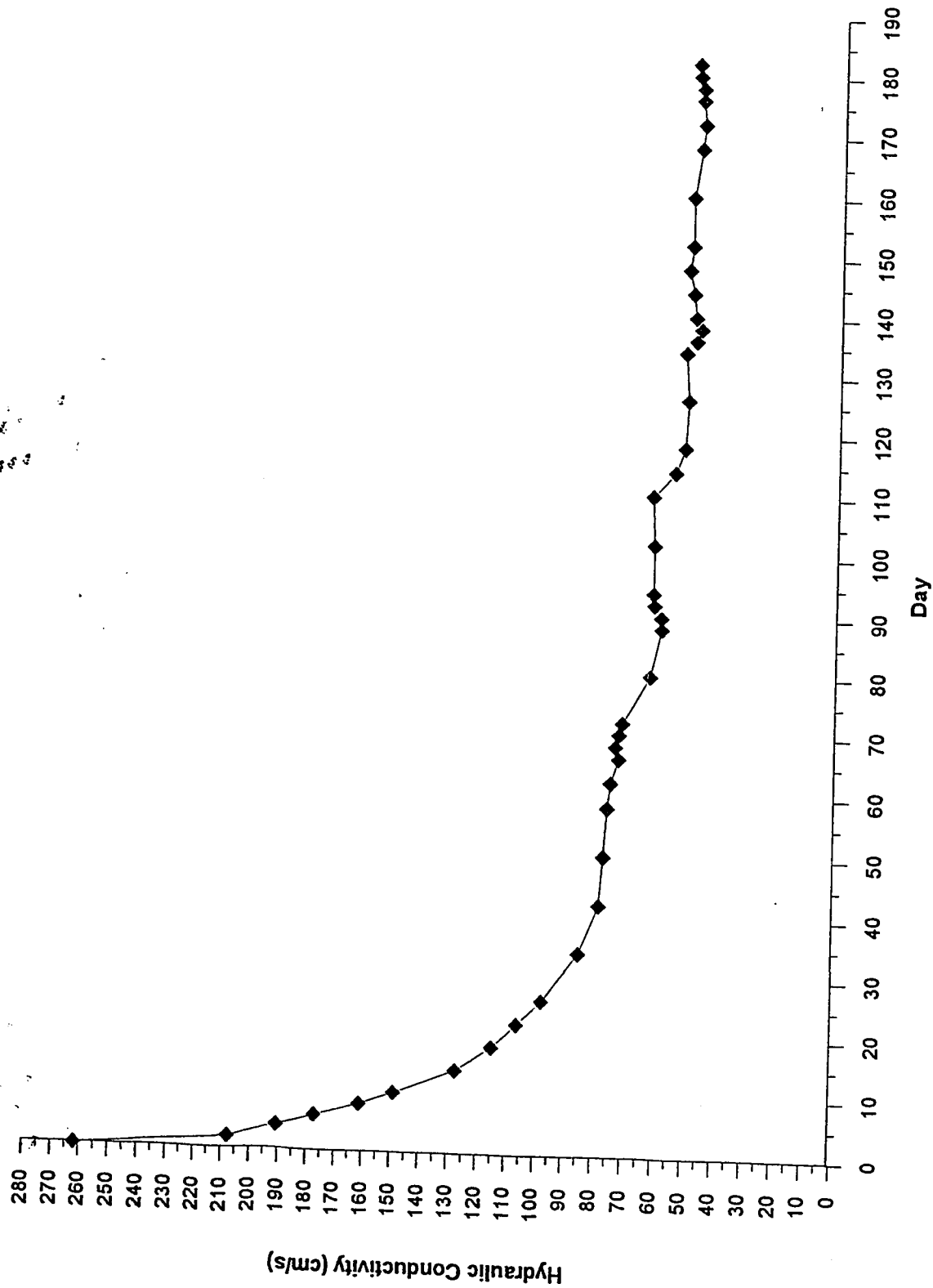


Figure 22 Change of hydraulic conductivity during the course of experiment

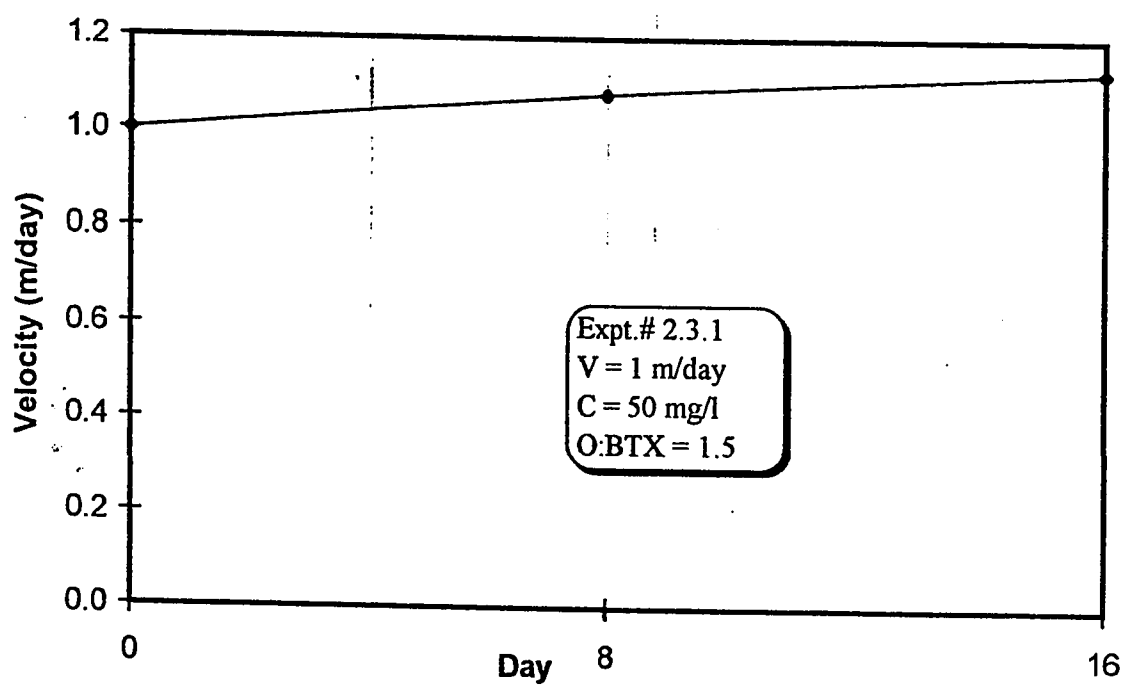
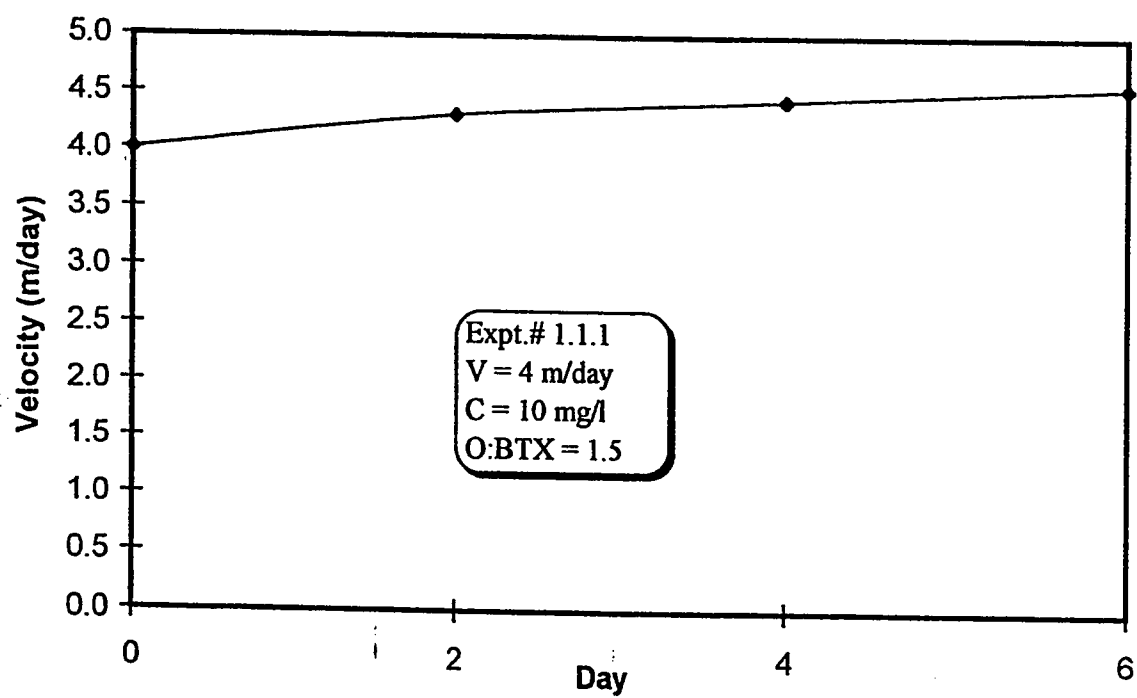


Figure 6.23 Change of linear velocity during two experimental run

### 6.3.5 Plate Count

Microbial count and volatile suspended solid (VSS) for soil samples taken from the center of the sand tank has been monitored at the end of every run. Samples were taken from five different locations along the length of the sand tank.

The plate count varied along the length of the column. The typical variation of the total cell count are shown in Figure 19. The results of mean plate count and VSS is shown in Table 6.3. Figure 6.20 shows the fluctuation of the mean plate count computed as the arithmetic mean of the five locations where samples were collected. There was a steady build-up of the microbial mass as long the velocity in the subsequent runs were reduced. However increasing the velocity resulted in a lowering of the plate count, obviously due to shear loss. The least square polynomial fit of the plate count data shown by the dotted line in Figure 6.20 illustrates the trend of the microbial mass to be build-up in the sand tank. The initial plate count was very low ( $3.9 \times 10^7$  cells/gm of soil), but high count (about 10 times the initial count) was also observed in during the experimental period. The highest count was  $4.45 \times 10^8$  cells/gm of soil.

Plate count shown in the shaded region was missing and has been approximated using the Monod transport model. The microorganism concentration,  $X$  (mg/l) has been computed from the total count as follows [Coreseuil and Weber, 1994]:

$$X = (\text{Nos. of cells/gm of soil}) \times (2 \times 10^{-10} \text{ mg/cell}) \times (1.6 \text{ gm of soil/ml}) \times (1000 \text{ ml/l}),$$

or,  $X = (\text{Nos. of cells} \times 3.2 \times 10^{-7}) \text{ mg/l}$

Table 6.3 Plate count and VSS during the course of experiment

Expt. No. ↓	C	V	O	Plate Count ( $\times 10^{-7}$ ) at distance						VSS ( $\mu\text{g/gm}$ )
				1.0	2.5	4.0	5.5	7.0	Mean	
BIO1.1.1(1)	10	4	1.5	4.5	4.1	3.8	3.7	3.4	3.9	31
BIO1.1.1(2)	10	4	1.5						7.8	
BIO1.1.2	10	4	3.2						5.8	
BIO1.2.1(1)	10	2	1.5						6.9	
BIO1.2.1(2)	10	2	1.5						10.4	
BIO1.2.2	10	2	3.2						9.3	
BIO1.3.1(1)	10	1	1.5						6.6	
BIO1.3.1(2)	10	1	1.5						10.7	
BIO1.3.2	10	1	3.2	19.7	15.2	8.9	7.5	4.7	11.2	65
BIO2.1.1	50	4	1.5	15.6	12.7	10.3	7.7	7.2	10.1	42
BIO2.1.2(1)	50	4	3.2	23.4	17.8	14.5	13.4	12.9	16.4	45
BIO2.1.2(2)	50	4	3.2	55.6	45.6	34.4	24.5	23.4	36.7	47
BIO2.2.1	50	2	1.5	24.2	16.7	11.4	7.8	5.9	13.2	53
BIO2.2.2(1)	50	2	3.2	60.5	38.9	29.4	13.5	11.2	30.7	56
BIO2.2.2(2)	50	2	3.2	86.3	59.3	36.7	18.8	12.9	42.8	59
BIO2.3.1	50	1	1.5	49.8	25.6	13.4	7.8	6.9	20.7	58
BIO2.3.2(1)	50	1	3.2	50.2	23.5	11.7	6.8	5.3	19.5	69
BIO2.3.2(2)	50	1	3.2	110.6	57.5	31.2	15.6	9.1	44.8	73

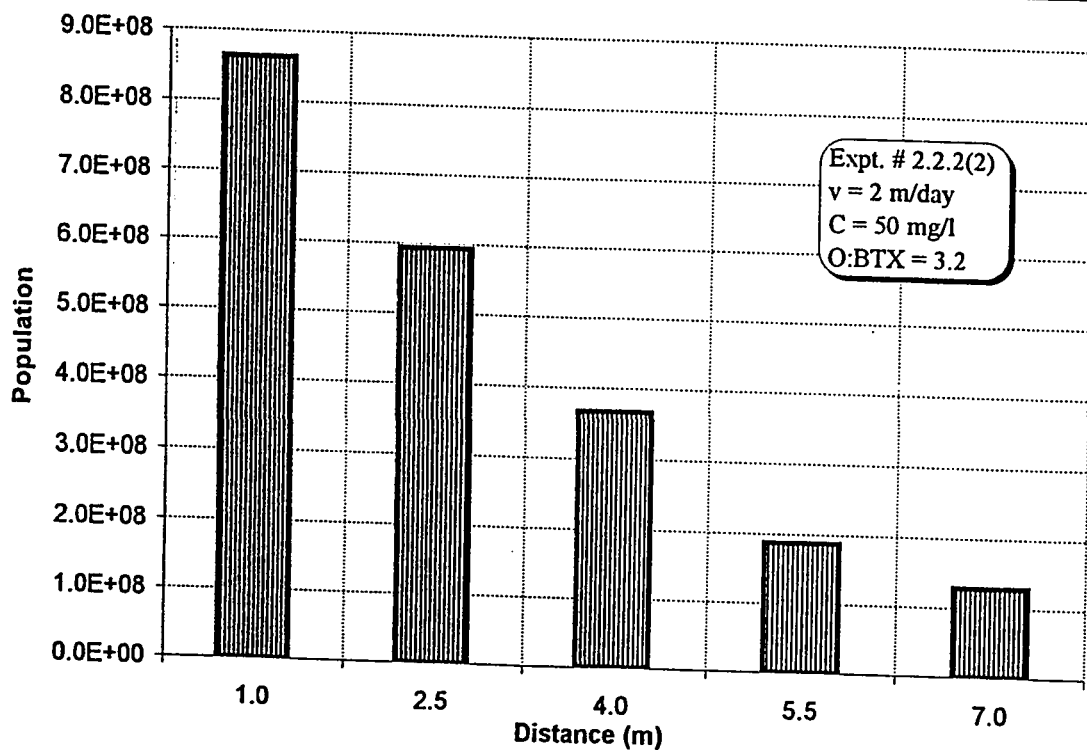
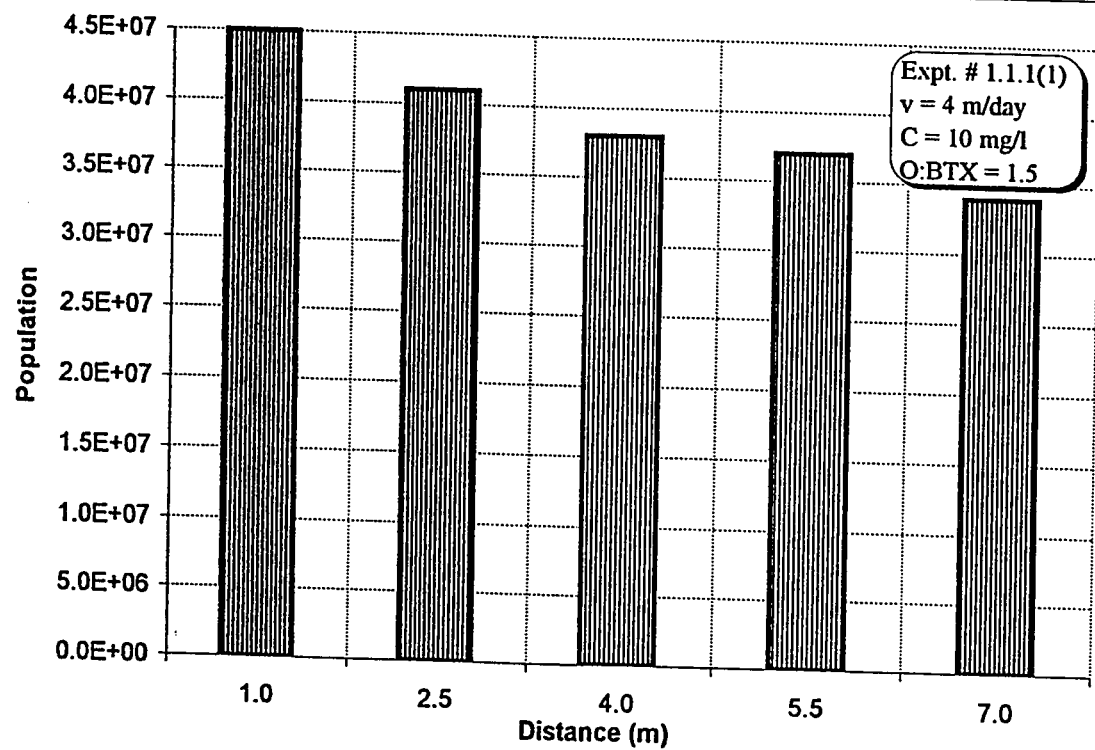


Figure 6.24 Microorganism profile at the end of two experimental run

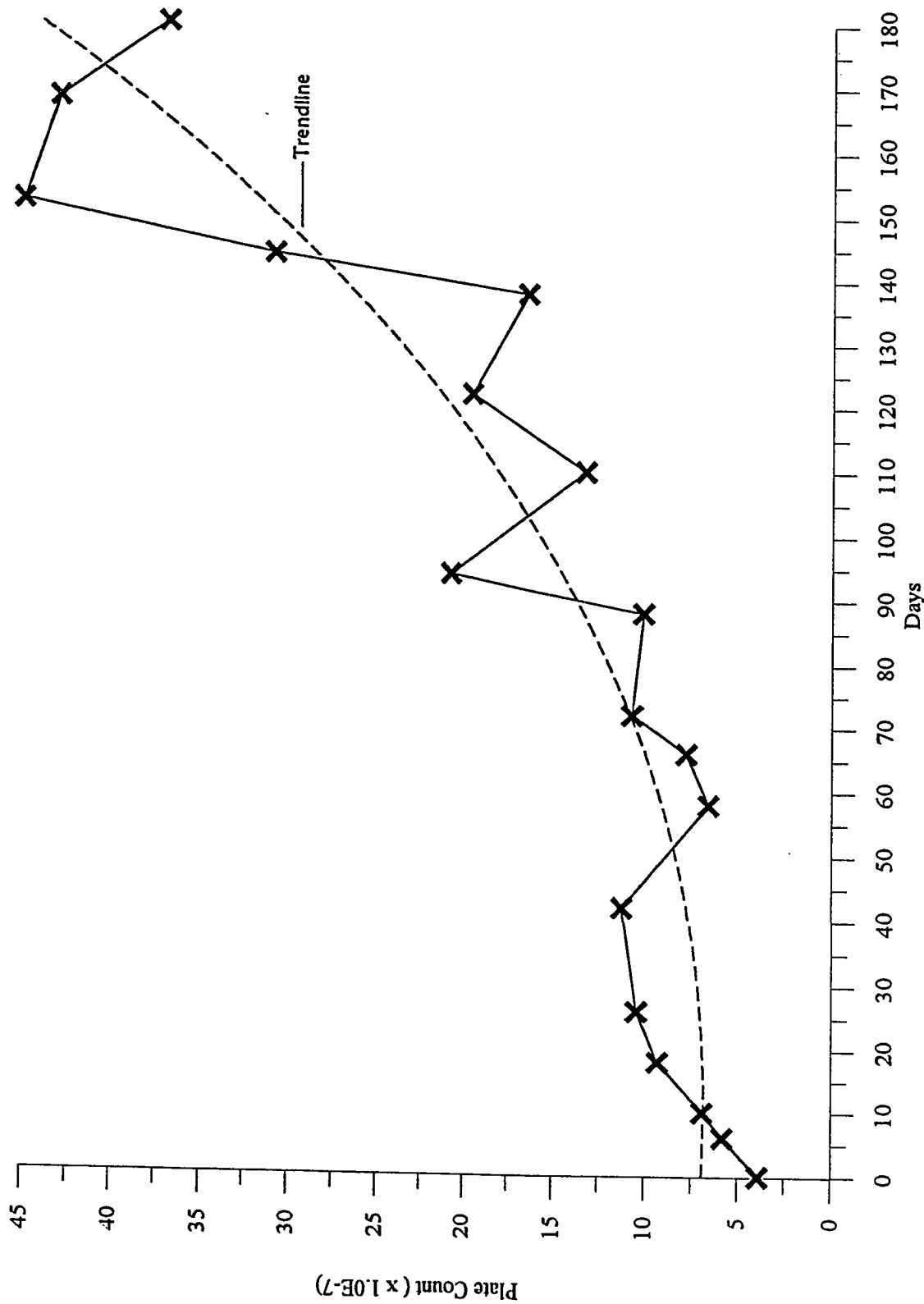


Figure 6.25 Fluctuation of plate count (X) during the experimental period



## 6.4 Modeling and Inversion Results

### 6.4.1 General

The simulation of the transport process has been described in detail in Chapter 4. As shown in Tables 4.1, 4.2, and 4.3 as well as in Figures 4.2 to 4.9, the solution of the transport equation by both finite difference and orthogonal collocation method was highly accurate as compared with existing analytical solutions. The sorption of the BTX compounds on the sand studied has been found to be negligible, but the assumption of linear equilibrium isotherm has been verified by batch test. The first-order and zero-order kinetics has been found to be satisfactory to describe the biodegradation process. The non-growth-associated Michaelis-Menten kinetics has found to be equally good to describe the biodegradation process. However, the Monod kinetics with a continuing growth of microorganism as long as the substrate concentration is above the lower limit has been found to very poor in describing the biodegradation process. Obviously the growth of the microorganism reaches steady state in the early stage of a typical experimental run. This has been verified by the plate count in few experimental run. Jones [1970] found that substrate utilization might not be necessarily associated with bacterial growth and considerable proportion of substrate might be consumed without an equivalent increase in the microbial cell. Alvarez et al. [1991] observed that microbial count did not increase significantly from the initial count prior to the addition of BTX compounds to the time of biodegradation. They used a constant value of microbial mass ( $X$ ) in the Monod model for prediction purpose. Lodaya et al. [1991] also used Monod kinetics ignoring bacterial growth and decay.

It has been found that the Monod kinetics with a constant value of  $X$  (cell mass, mg/l) computed from the plate count at the end of each experimental run can be used to fit the data of that run. However, the fit has not found to be superior to the first-order and zero-

order model in case of low concentration of BTX compounds. Results of fitting the experimental data to Monod kinetics allowing and ignoring microbial growth will be depicted in Section 6.4.3. The following section (Section 6.4.2) is devoted to the detailed data analysis with the first-order and zero-order model.

#### ***6.4.2 First-order/Zero-order Model***

Experimental data from all twenty one experimental runs including three control runs has been analyzed to compute the parameters of the first-order and zero-order model by the nonlinear least square fit discussed in Chapter 5. The computed parameters are given in Table 6.1. Typical observed and fitted concentration profiles for three different velocities at the first and second detention time cycle are shown in Figures 6.26-29. The values of  $R^2$  shown on the figures is not for the data shown on one figure but for the all data at two or three dimensionless time. It can be noted that the shape of the curves at high velocity ( $v = 4$  m/day) is not the same as those at lower velocities. This is because of low biodegradation rate at the high velocity. In fact, for a nonreactive solute transport the shape of the curve is always concave-downward. Figures 30 shows the predicted (fitted by the first-order/zero-order model) steady state removal of benzene, toluene, and o-xylene at three velocities.

The mean dispersivity computed from three control run was 7.02 cm for benzene, 6.47 cm for toluene and 6.11 cm for xylene. As indicated in Chapter 5, these values have been kept constant in the parameter estimation for the main experimental runs. The biodegradation in most cases has been found to be first-order, in few cases the fitting procedure computed small values of zero-order rate constant ( $\gamma$ ). For the sake of analysis of variance for drawing conclusions, the zero-order rate constant was set to zero for all cases.

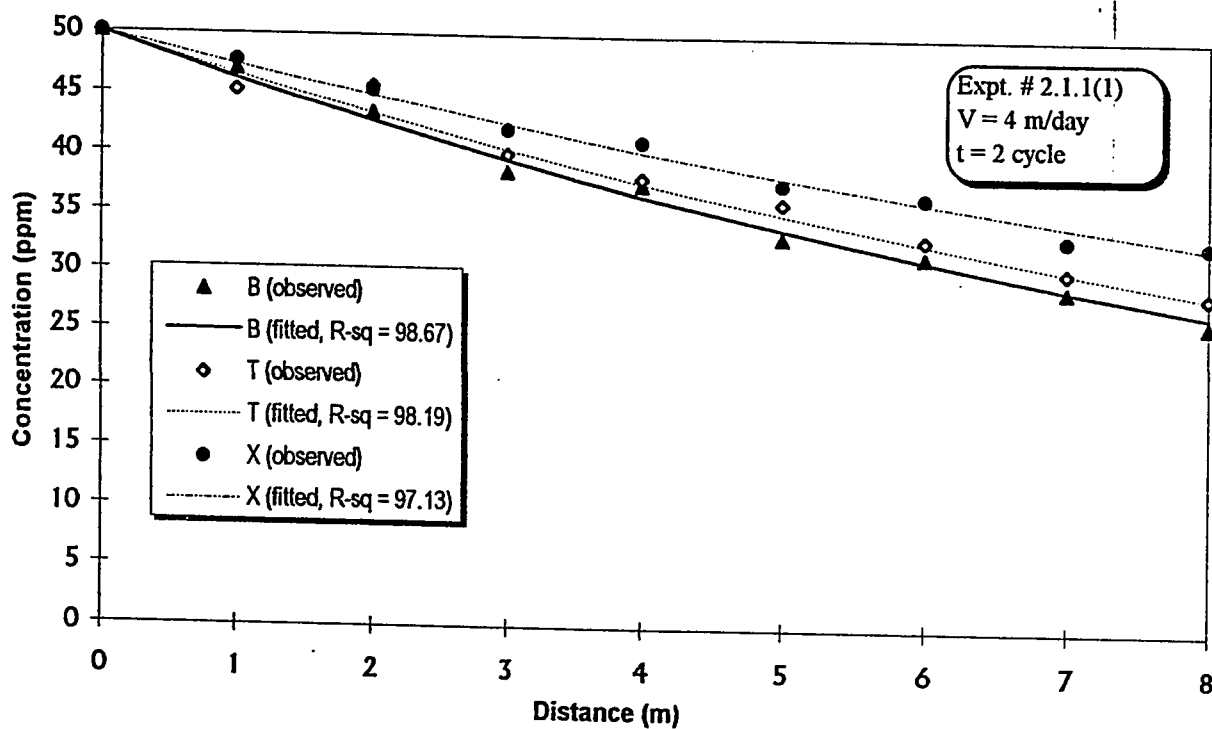
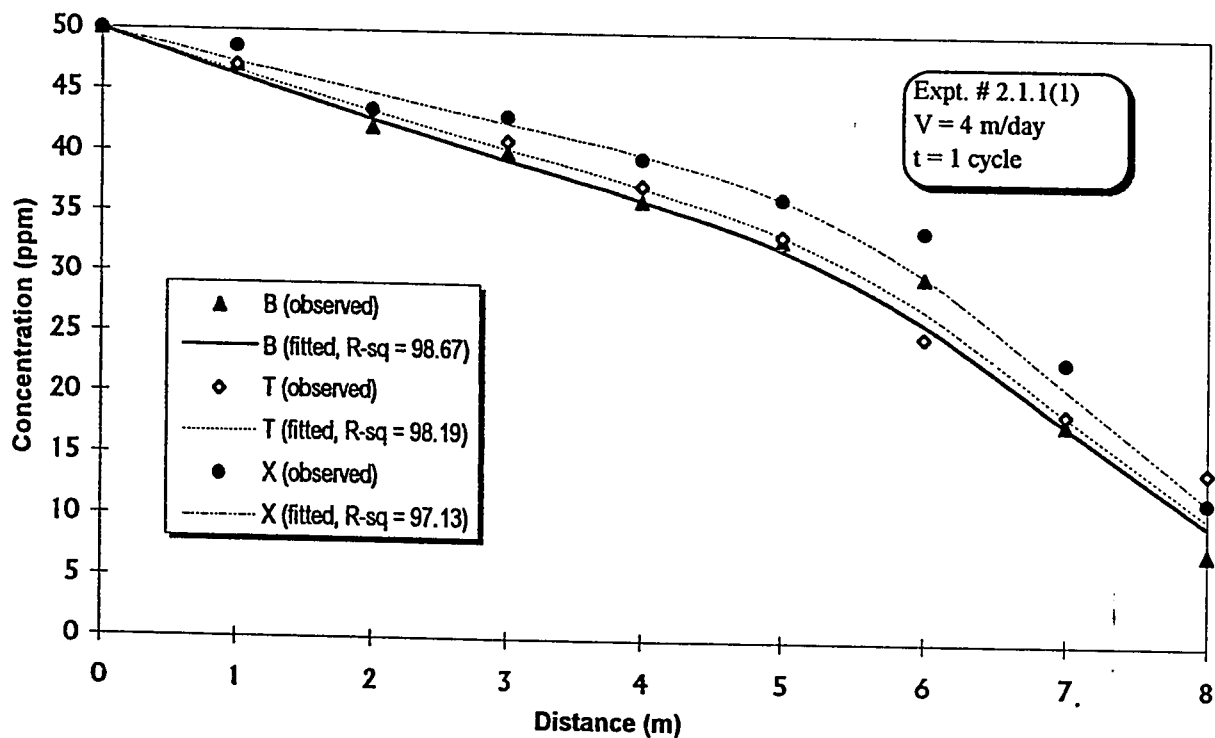


Figure 6.26 Typical observed and fitted BTX concentration at  $v = 4$  m/day

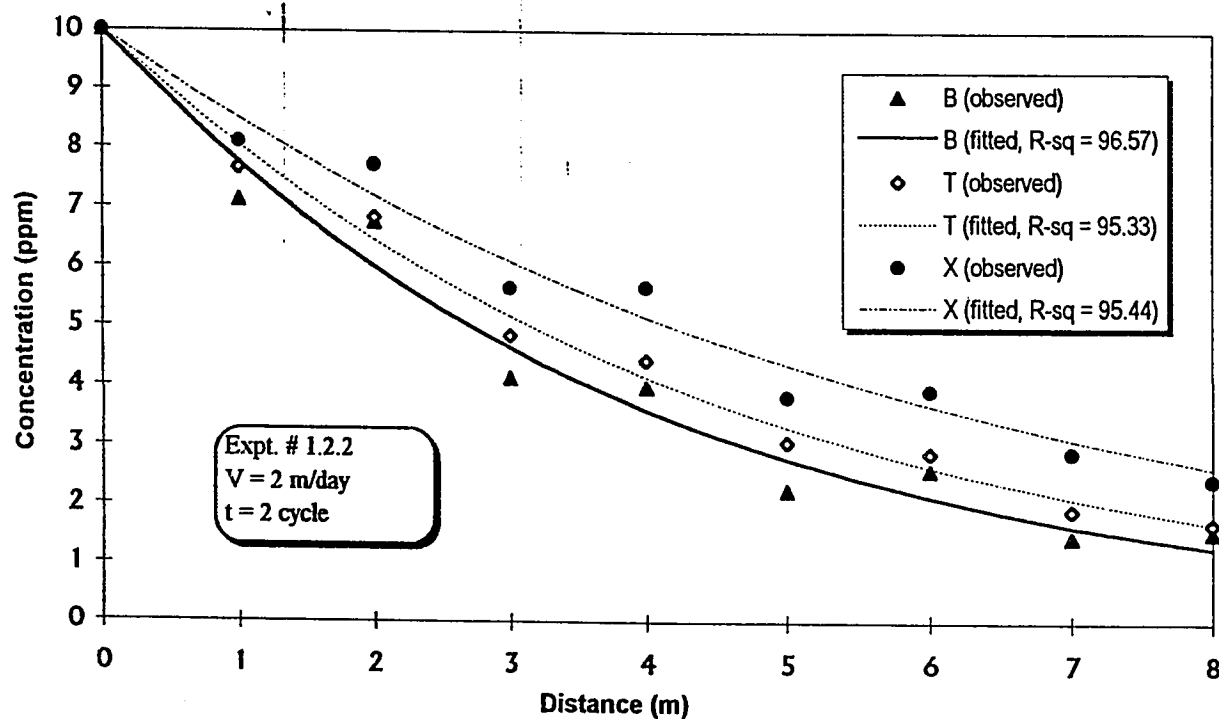
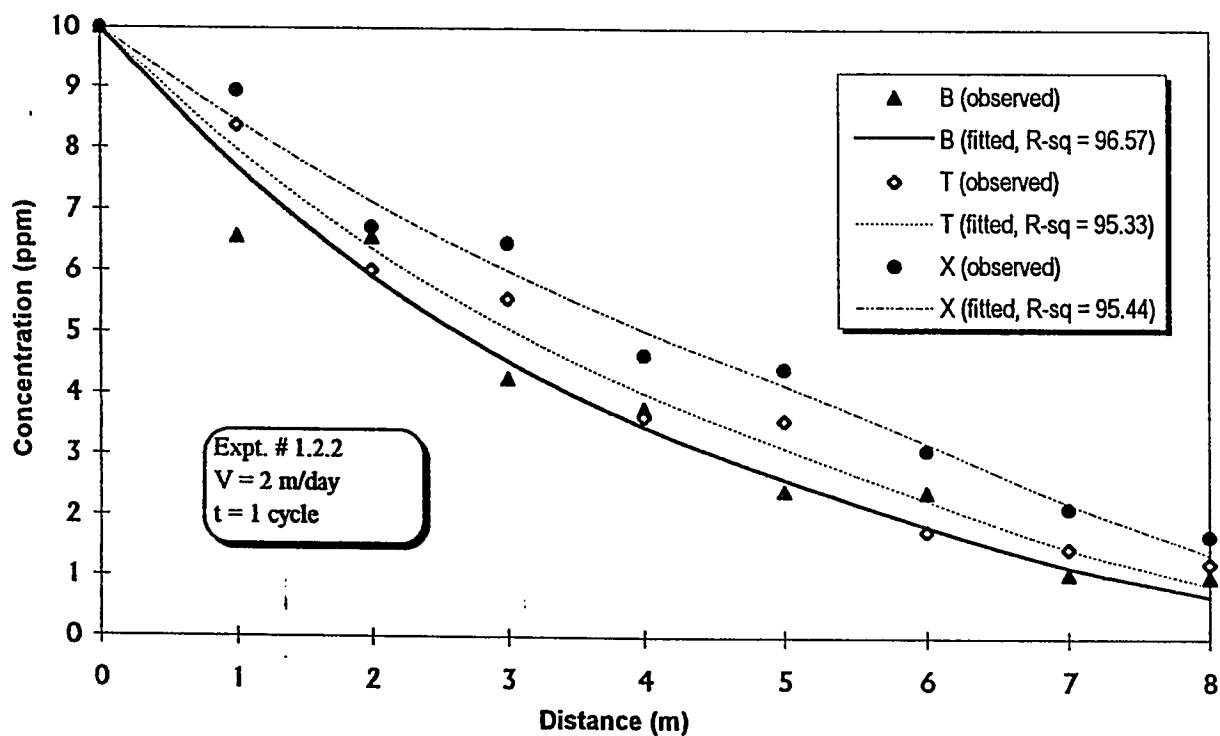


Figure 6.27 Typical observed and fitted BTX concentration at  $v = 2$  m/day

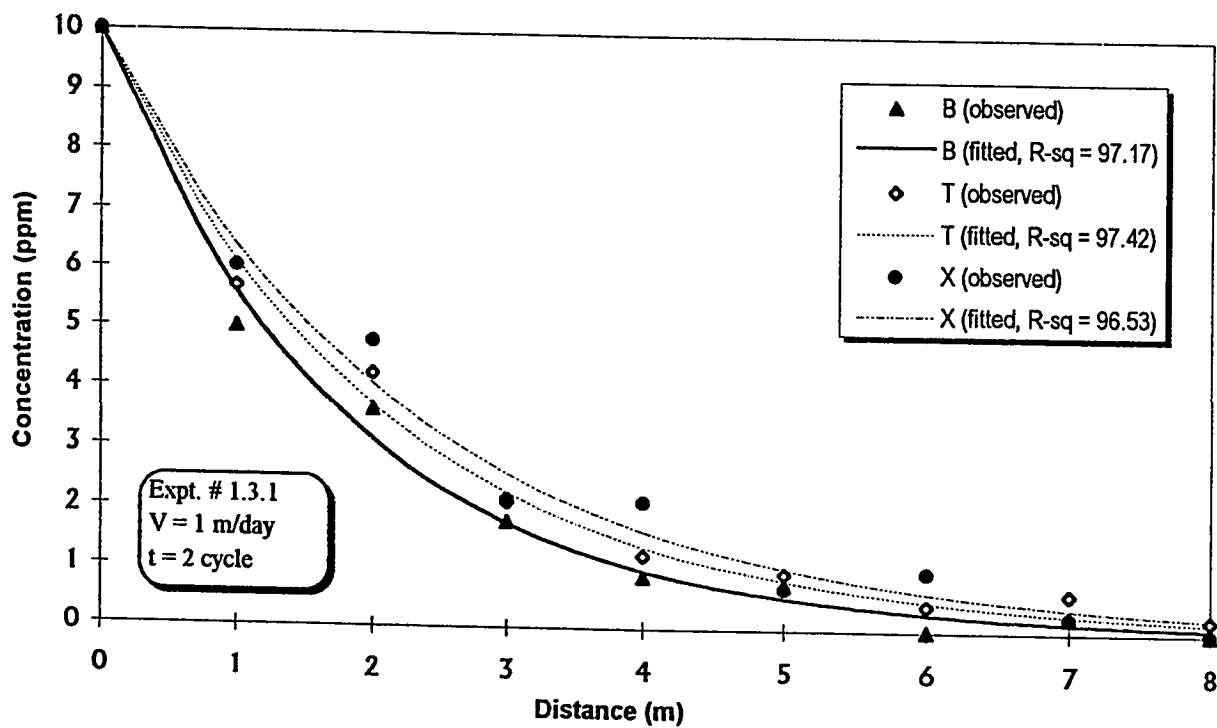
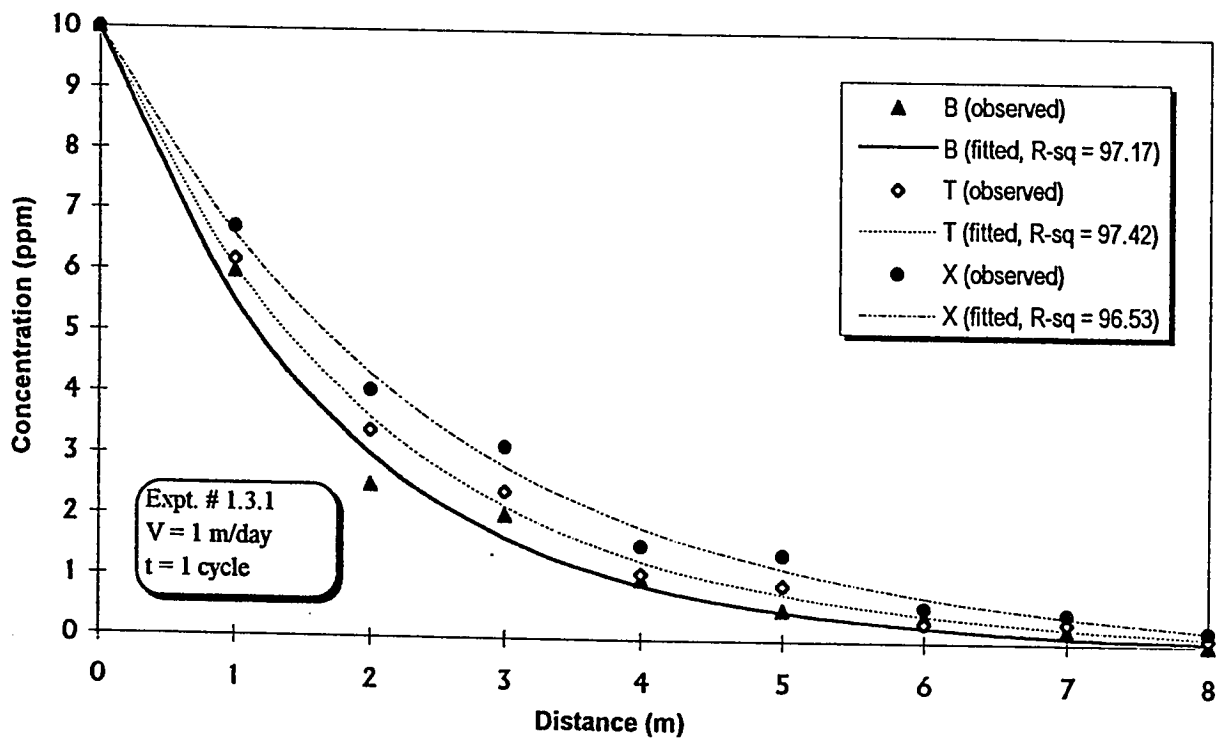


Figure 6.28 Typical observed and fitted BTX concentration at  $v = 1$  m/day

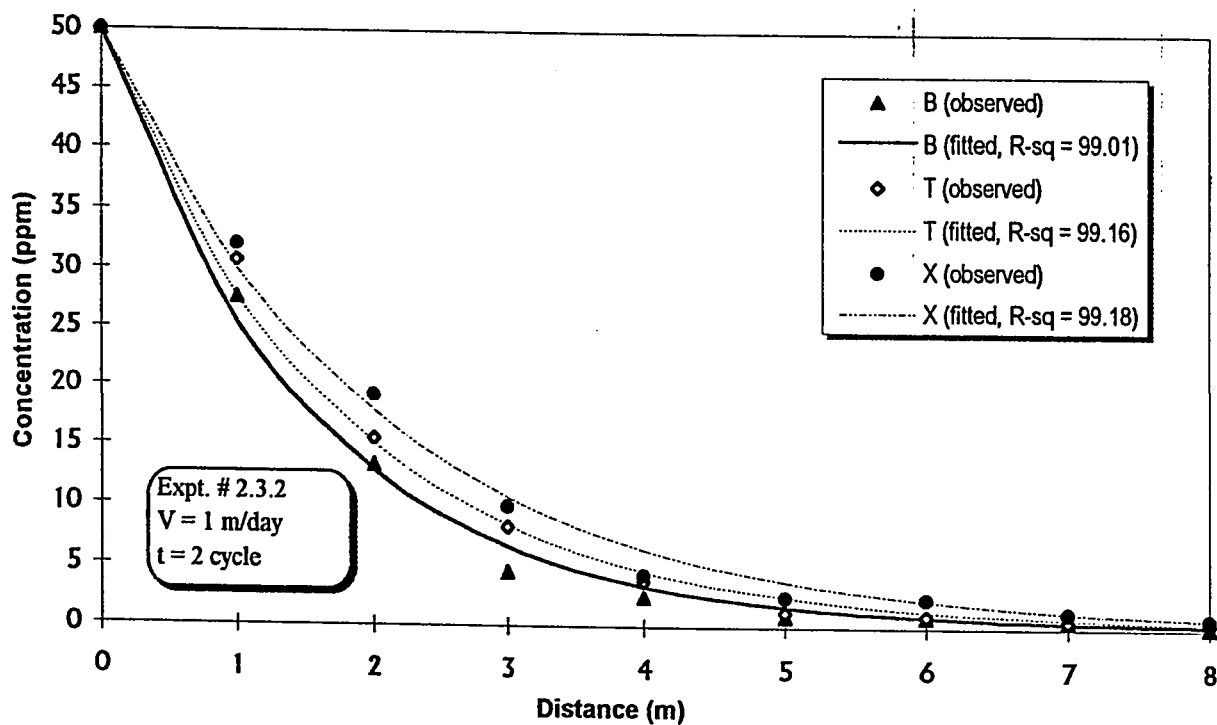
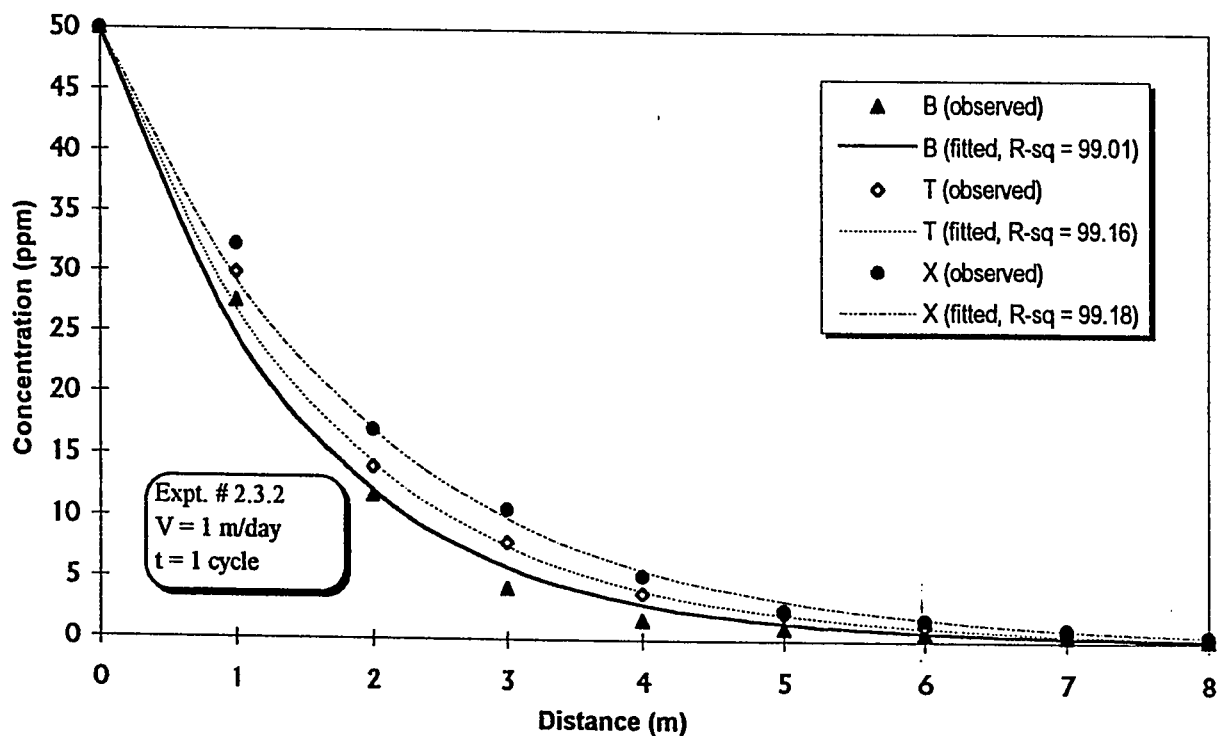


Figure 6.29 Typical observed and fitted BTX concentration at  $v = 1$  m/day,  $C = 50$  mg/l

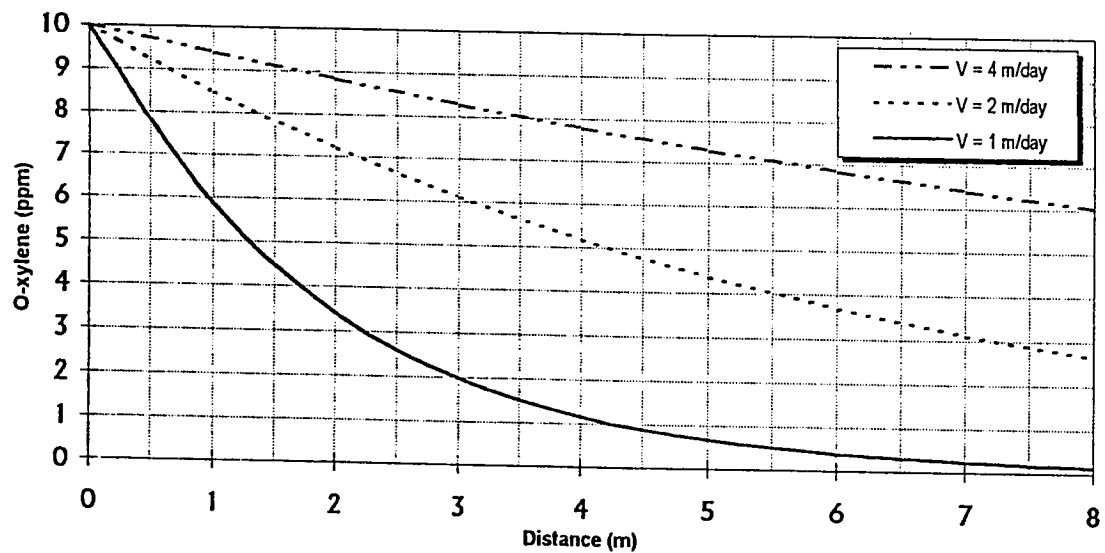
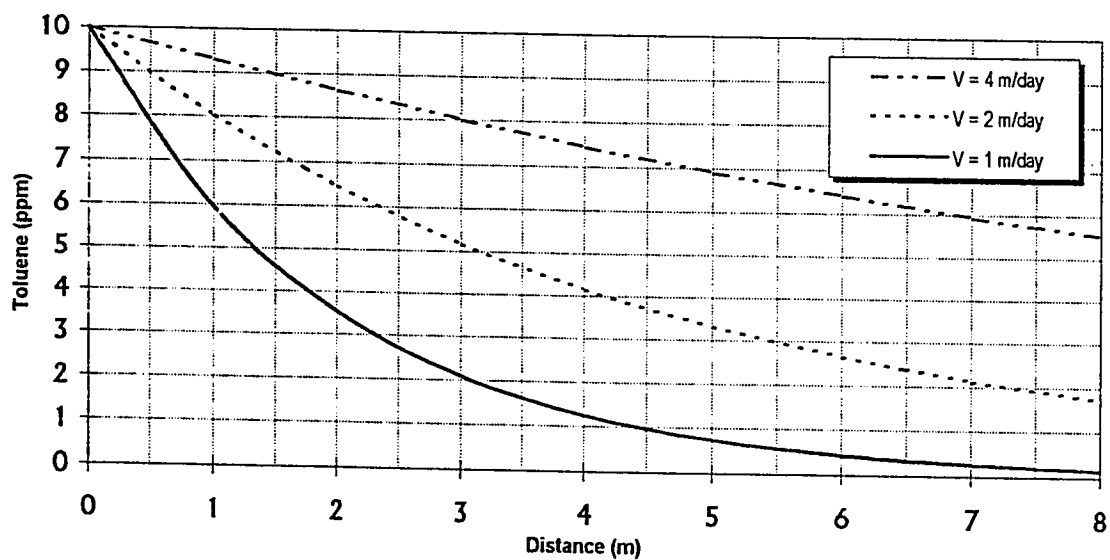
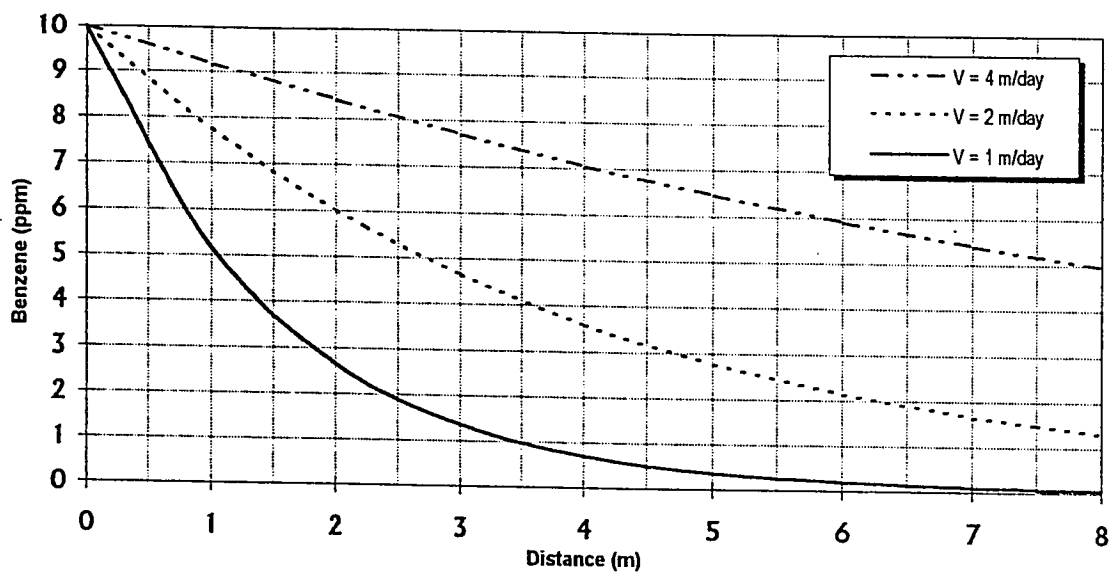


Figure 6.30 Predicted steady state BTX concentration ( $C_0 = 10$  ppm, O:BTX = 3.2)

#### *6.4.3 Monod and the Michaelis-Menten Model*

Although, this was not the main objective of the present study, the Monod and Michaelis Menten models have been also inverted and the observed data were analyzed to study the suitability of these models in predicting the BTX fate in saturated sandy soil. This also helped in evaluating the first-order model compared with these two models. It has been found that all the observed BTX concentration data can be fitted with the non-growth associated Monod kinetics or the Michaelis Menten Kinetics.

The values of  $k_m$ ,  $K_s$  and  $k$  for benzene, toluene, and xylene are shown in Table 6.5. In case of high concentration the values of  $K_s$  was increased to above 30 mg/l.  $R^2$  was slightly lowered (1-3%) in case of low concentration (10 mg/l of BTX each), but improved (1-3%) in case of high (50 mg/l). Thus the observed data in case of high concentration fitted better to the Michaelis Menten kinetics than to first-order/zero-order model. Again in almost 90% of the cases (16 out of 18), the value of  $R^2$  was higher than 95% for all fittings to first-order/zero-order or the Michaelis Menten model. Therefore the fits to first-order/zero-order model are good to the range of the concentration of BTX compounds studied.



Table 6.5 Least square Parameters of the Michaelis Menten and Monod model

Compound	$k_m$ (mg/l/d)	$k^\otimes$ (mg/l/d)	$K_s$ (mg/l)	Y	b
Benzene	4.11 - 10.48	0.28 - 0.34	5.54 - 10.16	0.50 - 1.02	0.091 - 0.12
Toluene	3.87 - 10.25	0.26 - 0.30	6.32 - 10.47	0.48 - 1.21	0.100 - 0.13
Xylene	3.61 - 9.98	0.24 - 0.27	6.79 - 10.81	0.52 - 1.26	0.095 - 0.14

$\otimes$  Computed from  $k_m$  using the total microbial concentration, X (mg/l)

Observed and fitted (to the Michaelis Menten model) BTX concentration profile for three different velocities at first and second detention time cycle are illustrated in Figures 6.31, 6.32, and 6.33 respectively. Comparing these figures with Figures 6.26, 6.27, and 6.28, it can be noted that  $R^2$  is slightly improved in case of high concentration data.

Although the Monod model allowing microbial growth and decay offers two more parameters (Y and b) for fitting, it has been found to be slightly inferior in predicting the BTX concentration as the values of  $R^2$  were lowered in almost all cases. The  $k$  and  $K_s$  values of the Monod kinetics were almost in the same ranges as the Michaelis Menten kinetics. Comparison of all three model in predicting the BTX concentration profile are presented in Figures 6.34 - 6.36.

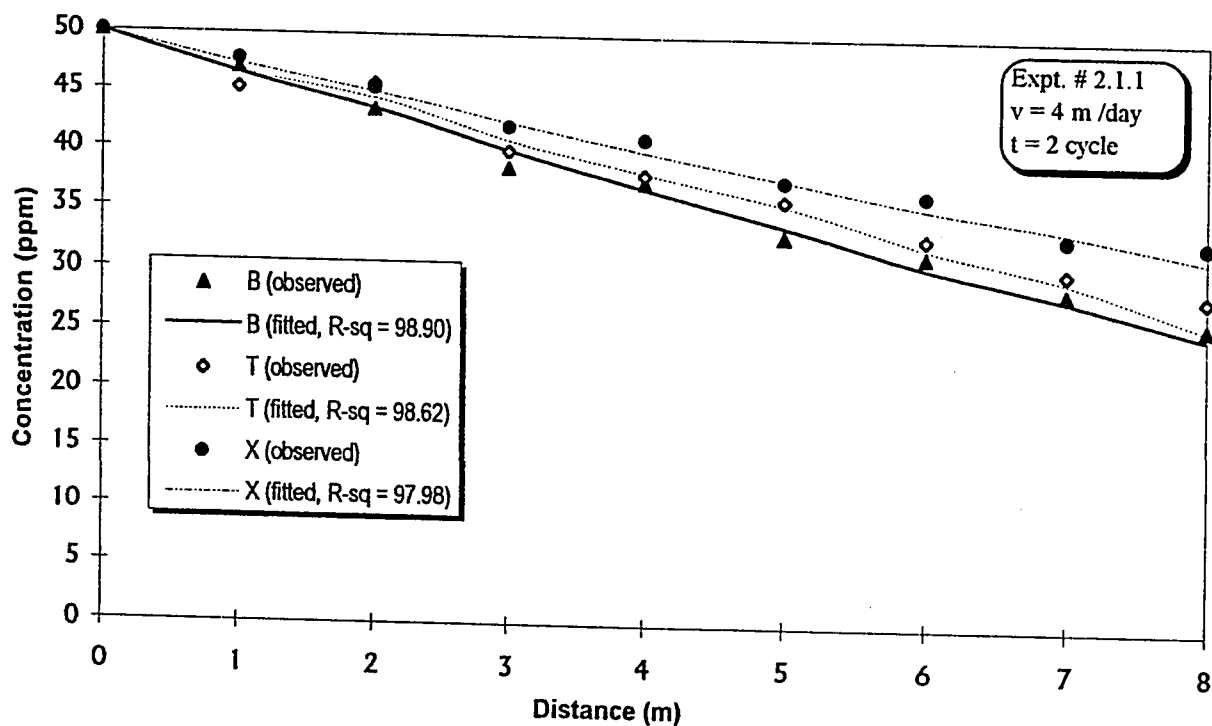
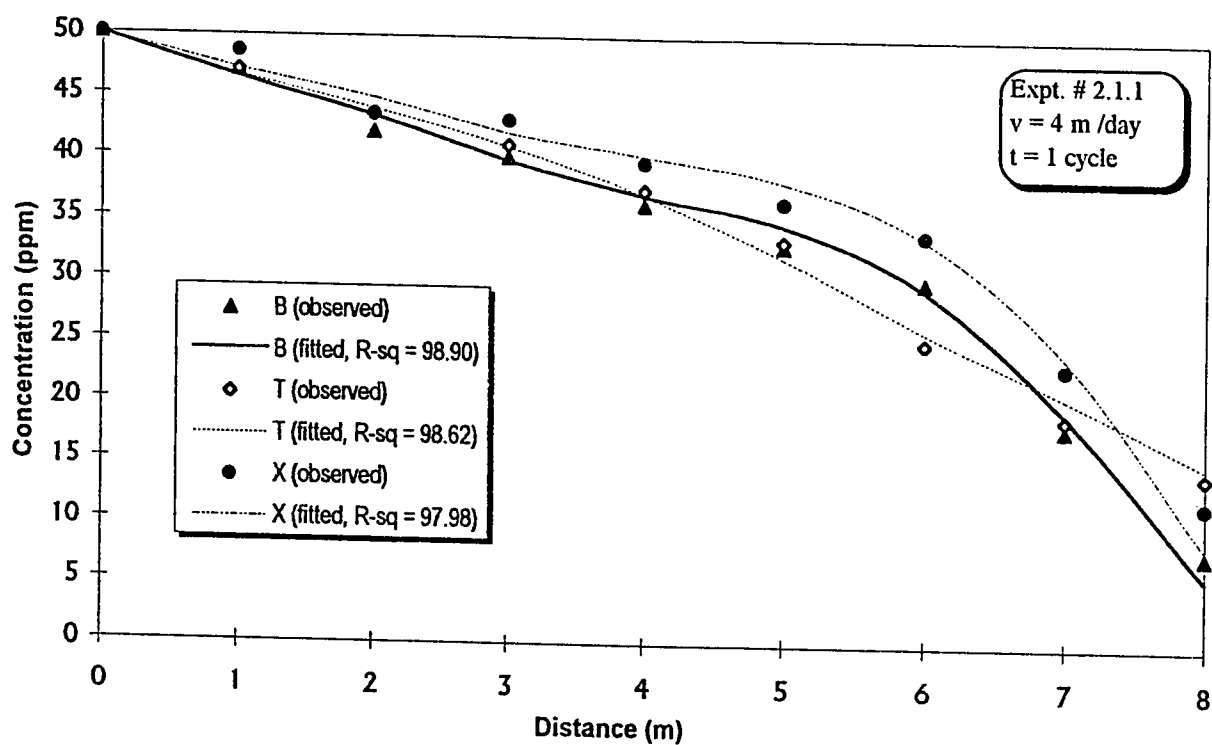


Figure 6.31 Typical observed and fitted (to Michalis Menten model) BTX concentration for  $v = 4 \text{ m/day}$

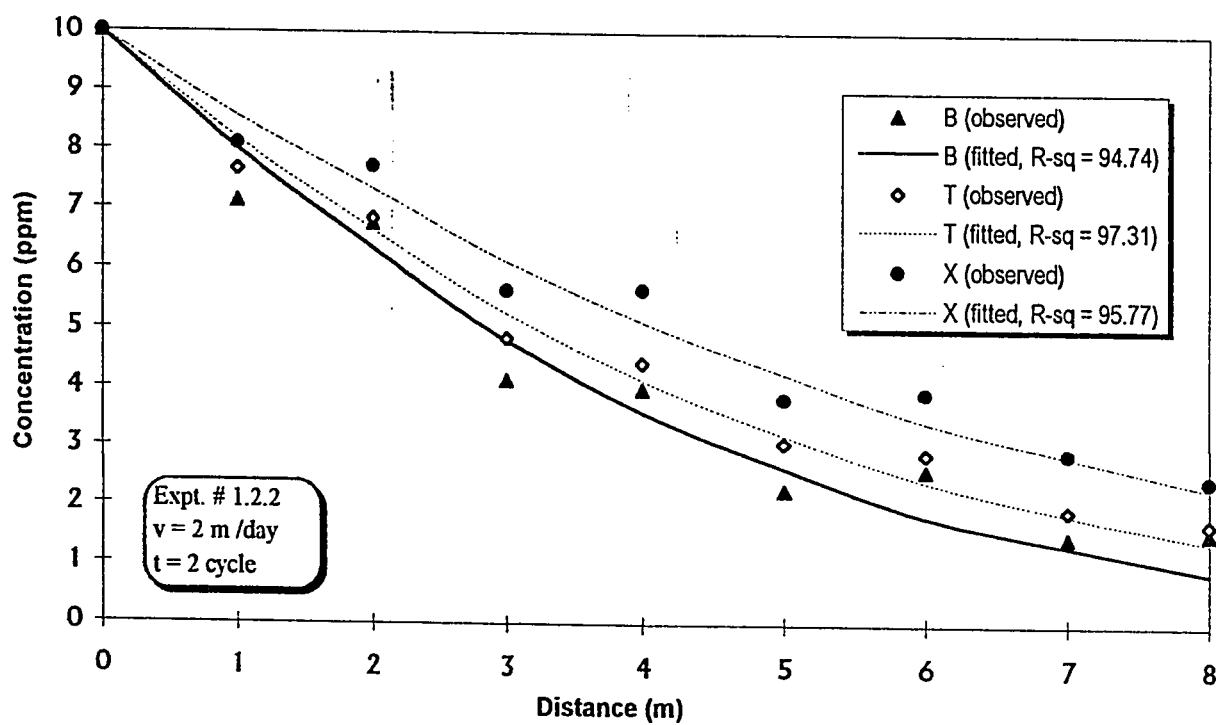
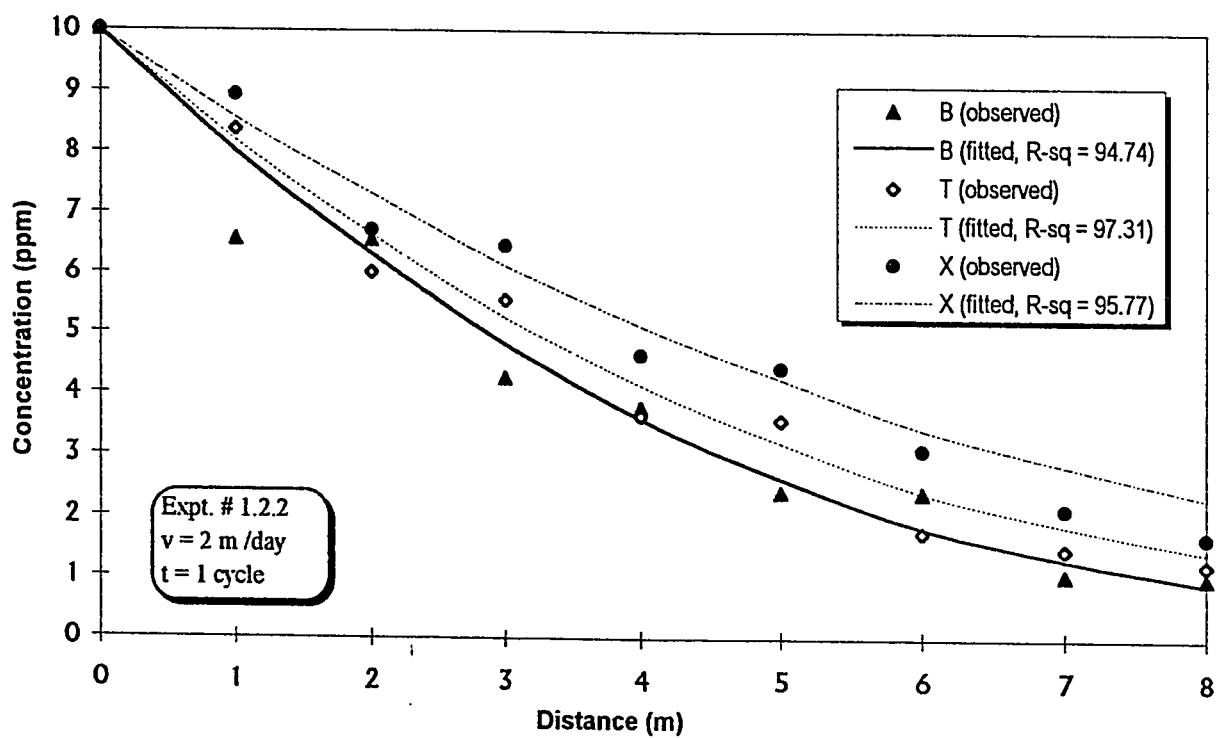


Figure 6.32 Typical observed and fitted (to Michalis Menten model) BTX concentration for  $v = 2 \text{ m/day}$

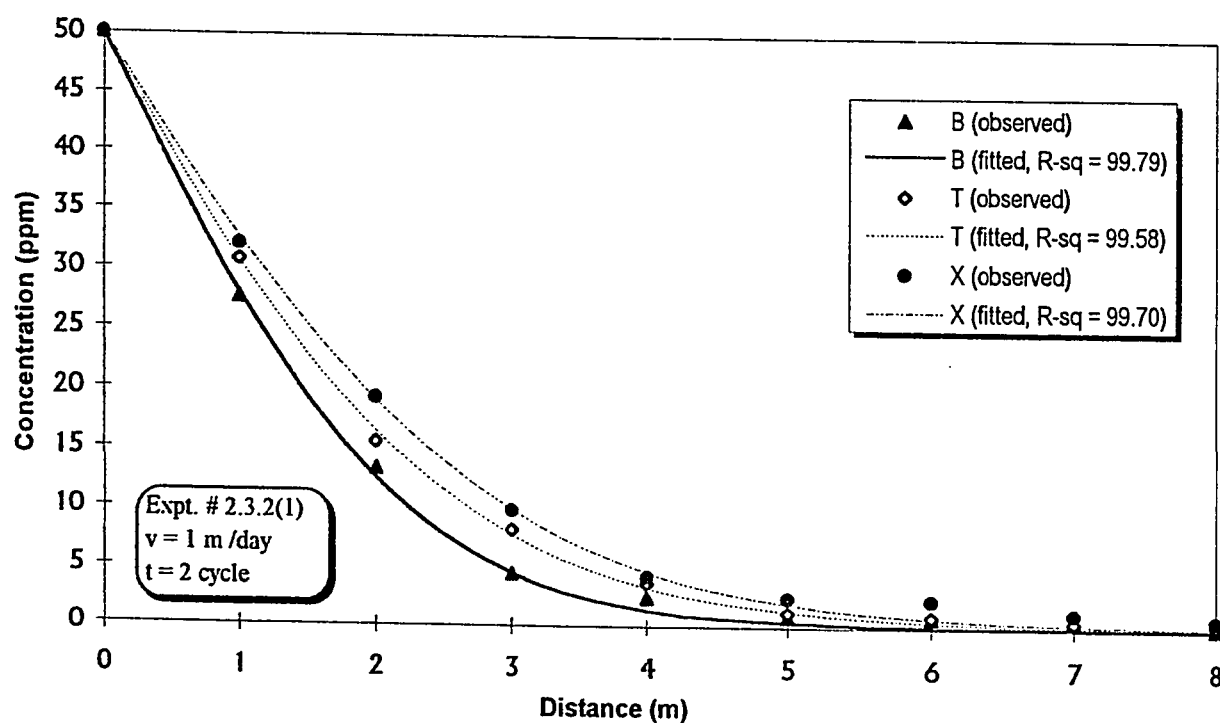
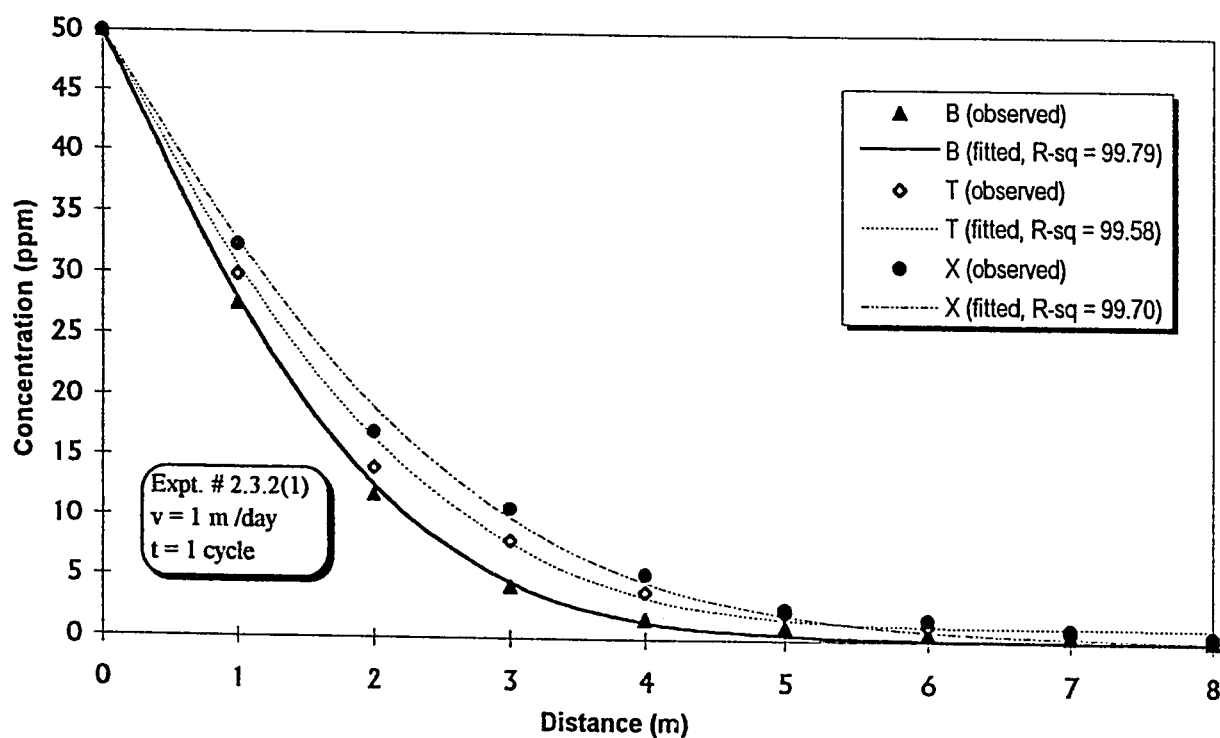


Figure 6.33 Typical observed and fitted (to Michalis Menten model) BTX concentration for  $v = 1 \text{ m/day}$

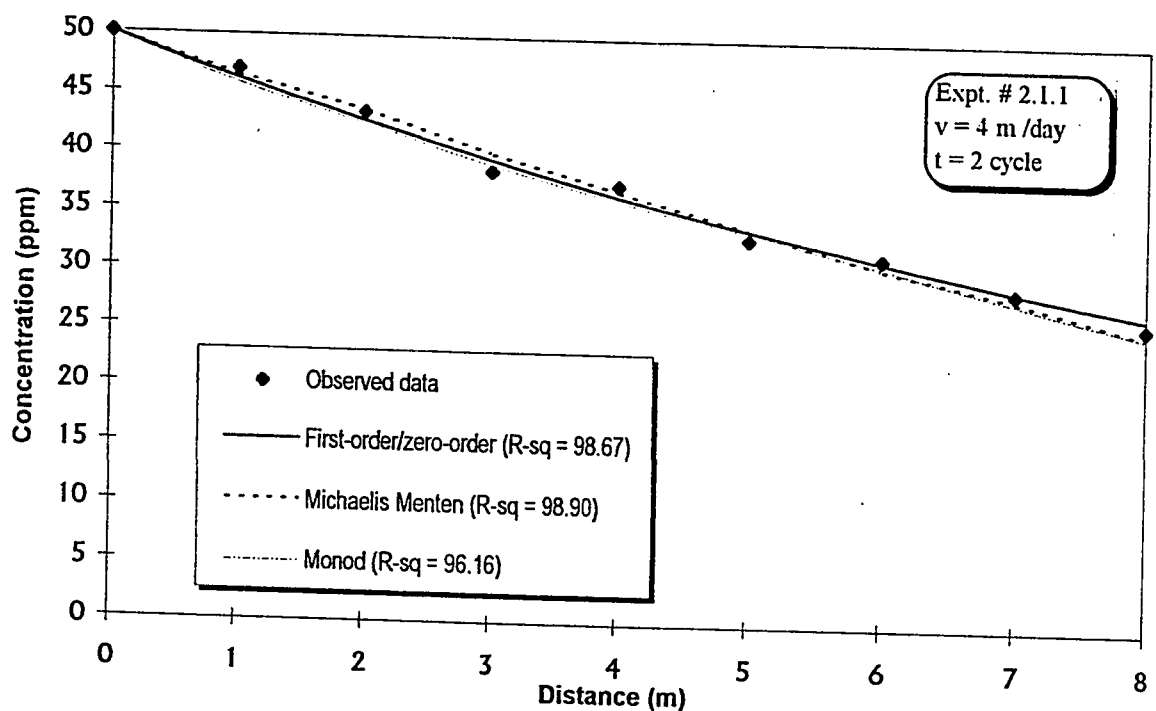
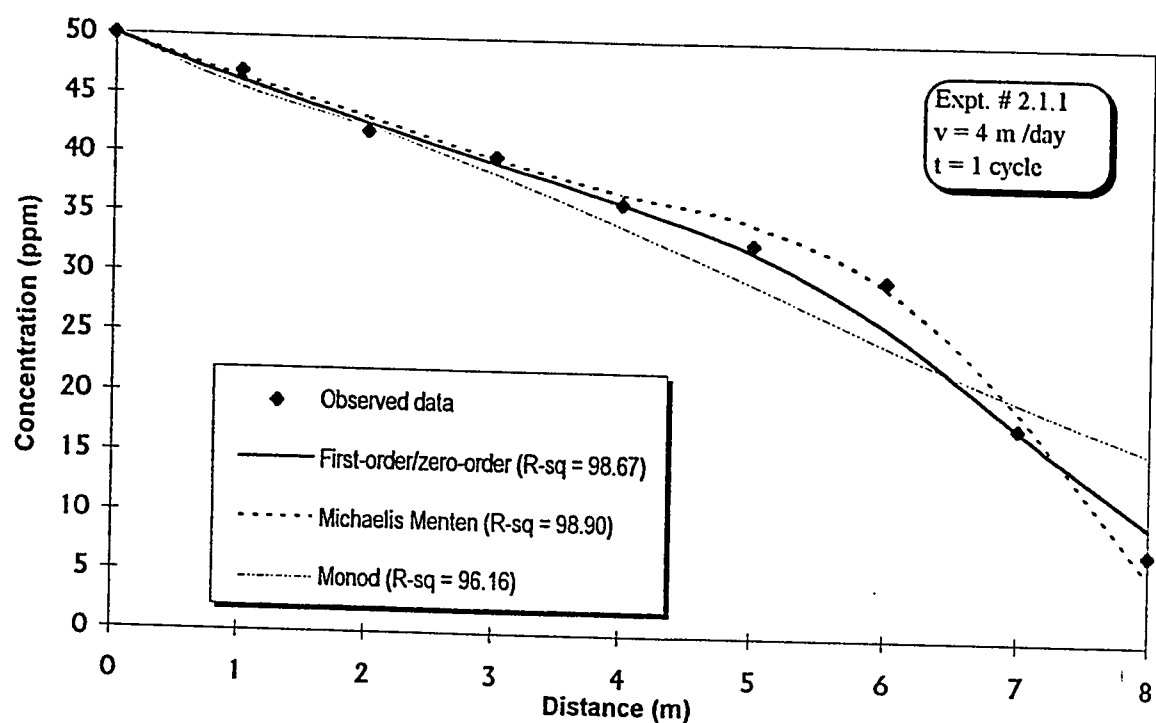


Figure 6.3.4 Comparison of different models in simulating the observed benzene concentration

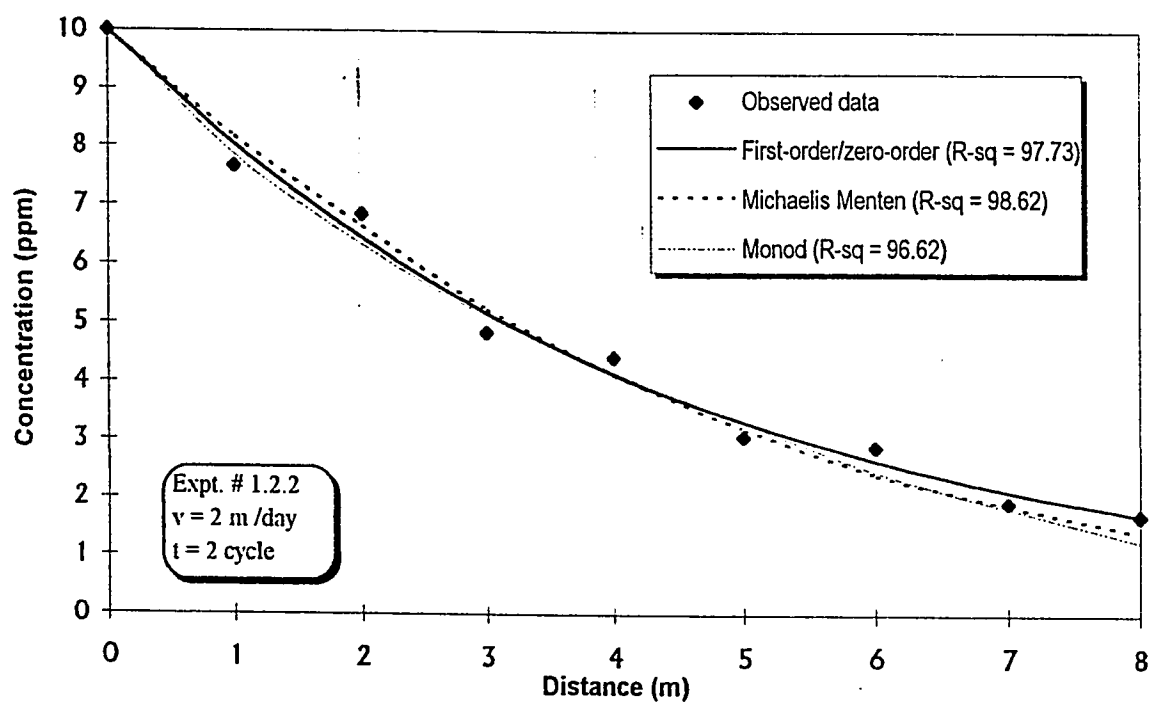
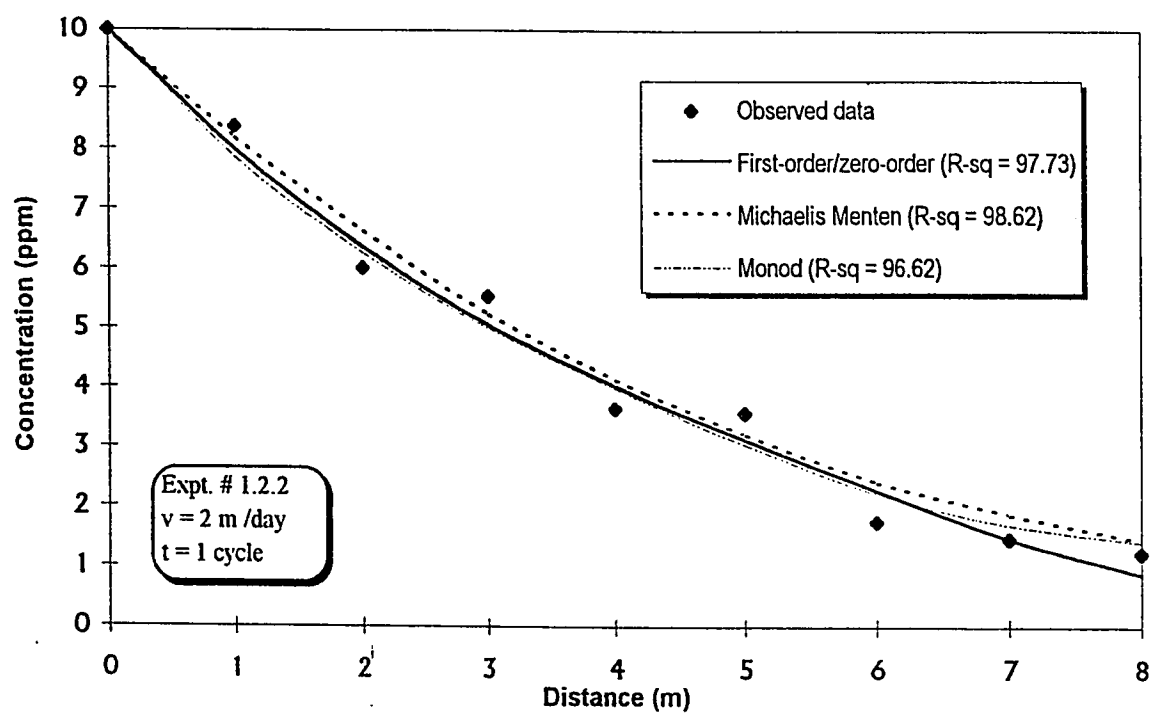


Figure 635 Comparison of different models in simulating the observed toluene concentration

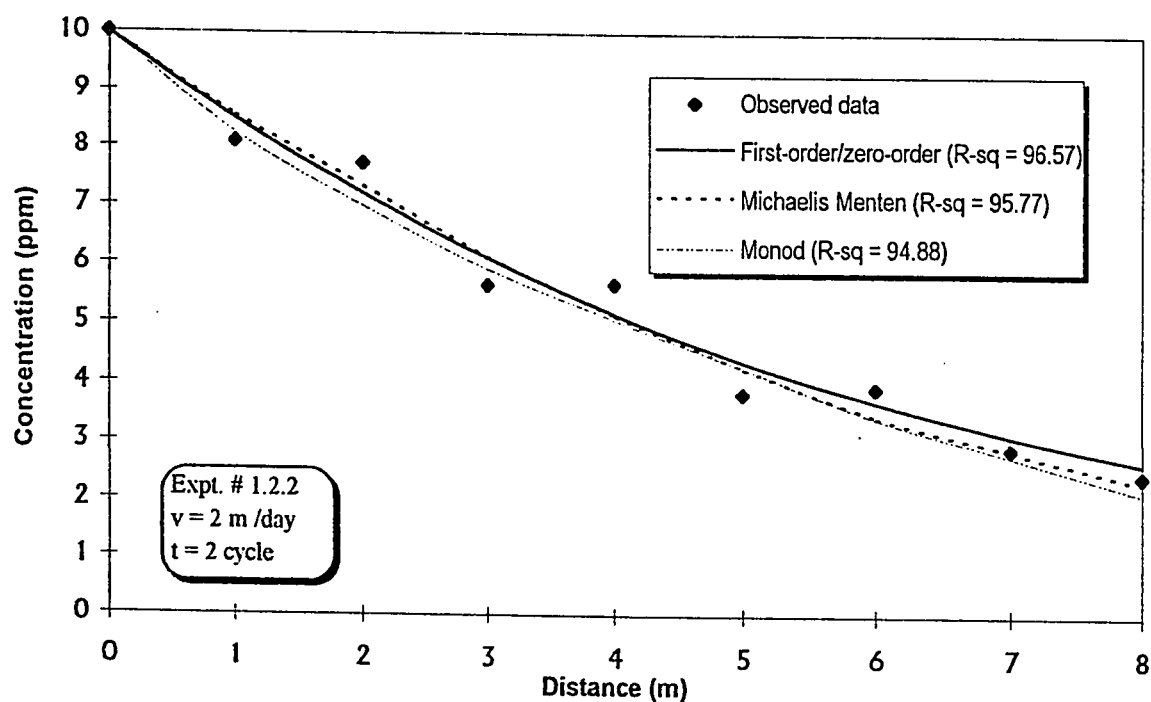
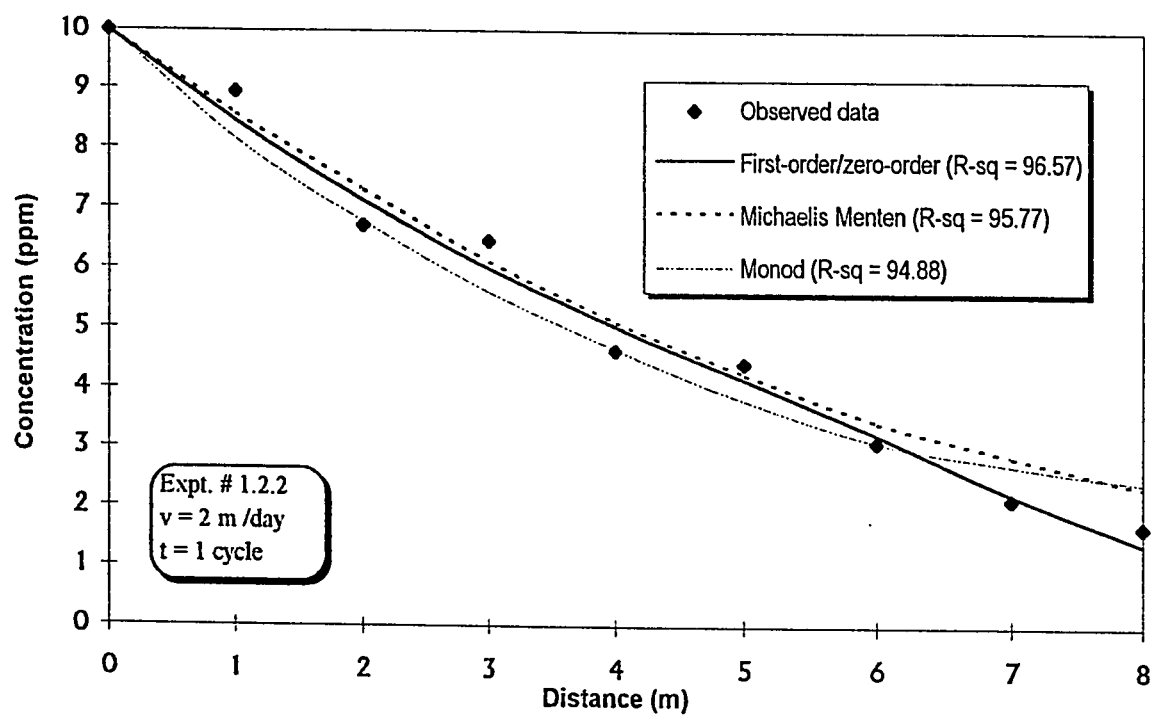


Figure 636 Comparison of different models in simulating the observed o-xylene concentration

## 6.5 Analysis of Variance

### 6.5.1 General

The analysis of variance of the first-order rate constant, retardation constant, cell count, and porosity has been performed for the three main factors of the study: velocity, DO, and concentration. Approximate methods are available [Montgomery, 1982] for the analysis of variance for the type unbalanced factorial design used in the present study. Exact method of analysis, which is based on fitting a regression line given by Equation (3.1), has been used in the present study. The method is available in SAS procedure, GLM (Generalized Linear Models). The detailed results from SAS including analysis of variance, regression parameters, means, etc. are given in Appendix C. The result showing the significance of the factors given by the F value and confidence is presented in this Chapter. The goodness of regression fitting given by the coefficient of determination ( $R^2$ ) and the coefficient of variation is also included. The coefficient of determination ( $R^2$ ) in fraction or percentage is defined as the fraction or percentage of observed data accounted by the fitted model. The standard deviation has been root mean square error (RMSE) as follows:

$$\text{Standard Deviation} \approx \sqrt{\frac{\text{Error Sum of Square}}{\text{Error Degree of Freedom}}}$$

The percentage coefficient of variation (CV %) has been computed as follows:

$$\text{Coefficient of variation} = \frac{100 \times \text{Standard Deviation}}{\text{Mean}}$$

High  $R^2$  (> 95%) and low CV (< 10%) usually imply a good model.

### 6.6.2 First-order Rate Constant ( $\mu$ )



The analysis of variance (ANOVA) of the first-order biodegradation rate constant ( $\mu$ ) for benzene, toluene, and xylene is shown in Tables 6.6, 6.7, and 6.8 respectively. The ANOVA table shows that all the main factors (V, C, O) are significant at more than 99% confidence level. The highest value of F imply that groundwater velocity is the factor of highest significance for the biodegradation rate of BTX compounds. It is also apparent that in each case of BTX compounds, the concentration is less significant than DO. Few of the second order interactions have been found to be significant, but no general conclusion can be made for all benzene, toluene, and o-xylene. The third order interaction has been found to be insignificant in all cases.

Table 6.9 shows the analysis of variance for the benzene biodegradation rate with the interaction CO and VCO pulled with the error. It can be noted that the  $R^2$  has changed from 99.79 to 99.61 which is about 0.1%. Which means that these interactions can be easily neglected. Table 6.10 shows the analysis of variance ignoring all the interactions. The value of  $R^2$  has dropped to 98.61% which is only about 1%. Analysis with the toluene and o-xylene biodegradation rate yielded the same result, however the value of  $R^2$  dropped slightly more than benzene (1% - 2%). Therefore the interactions can be neglected in modeling biodegradation rate ( $\mu$ ) of BTX compounds. As shown in Table 6.11, velocity alone can account for about 86% of the variability of the biodegradation rate of benzene. For toluene and o-xylene the value of  $R^2$  has been found to be 84% and 82% respectively. Which means that other factors are more significant in case of toluene and xylene. Figures 6.37, 6.38, and 6.39 illustrates the significance of interactions for the biodegradation rate of benzene, toluene and o-xylene. More or less parallel lines shows that interaction are most insignificant in case of benzene, whereas intersecting lines (Figure 6.38 and 6.39) shows that they have slight significance in case of toluene and o-xylene.

Table 6.6 Analysis of variance of first-order biodegradation rate ( $\mu$ ) of benzene  
[Factors: V (velocity), O (O:BTX ratio), C (concentration)]

	Factor	Degree of Freedom	Sum of Square	Mean sum of square	F value	Significance (%)
<i>Main factors</i>	V	2	0.3802	0.1901	1140.65	99.99
	O	1	0.0427	0.0427	256.27	99.99
	C	1	0.0036	0.0036	21.60	99.65
<i>Interactions</i>	VO	2	0.0031	0.0015	9.32	98.55
	VC	2	0.0008	0.0004	2.45	83.20
	OC	1	0.0001	0.0001	0.60	53.20
	VOC	2	0.0004	0.0002	2.45	83.20
<i>Error</i>		6	0.0010	0.0001667		
<i>Total</i>		17	0.4982			

Mean of $\mu$	Standard Deviation	Coefficient of variation	R Square (%)
0.48466	0.0129	2.6637	99.7993

Table 6.7 Analysis of variance of first-order biodegradation rate ( $\mu$ ) of toluene  
[Factors: V (velocity), O (O:BTX ratio), C (concentration)]

	Factor	Degree of Freedom	Sum of Square	Mean sum of square	F value	Significance (%)
<i>Main factors</i>	V	2	0.3002	0.1501	1501.03	99.99
	O	1	0.0235	0.0235	235.11	99.99
	C	1	0.0144	0.0144	144.11	99.99
<i>Interactions</i>	VO	2	0.00377	0.00188	18.86	99.74
	VC	2	0.0030	0.0015	15.08	99.54
	OC	1	0.00004	0.00004	2.45	83.20
	VOC	2	0.0006	0.0004	4.36	93.23
<i>Error</i>		6	0.0006	0.0001		
<i>Total</i>		17	0.4126			

Mean of $\mu$	Standard Deviation	Coefficient of variation	R Square (%)
0.41588	0.0100	2.4000	99.8546

Table 6.8 Analysis of variance of first-order biodegradation rate ( $\mu$ ) of o-xylene  
[Factors: V (velocity), O (O:BTX ratio), C (concentration)]

	Factor	Degree of Freedom	Sum of Square	Mean sum of square	F value	Significance (%)
<i>Main factors</i>	<b>V</b>	2	0.2318	0.01159	818.14	99.99
	<b>O</b>	1	0.0200	0.0200	141.67	99.99
	<b>C</b>	1	0.0132	0.0132	93.95	99.99
<i>Interactions</i>	<b>VO</b>	2	0.0008	0.0008	3.08	87.98
	<b>VC</b>	2	0.0028	0.0014	10.06	98.79
	<b>OC</b>	1	0.0012	0.0012	8.65	97.41
	<b>VOC</b>	2	0.0007	0.00035	2.53	84.03
<i>Error</i>		6	0.00085	0.000141		93.23
<i>Total</i>		17	0.31425			

Mean of $\mu$	Standard Deviation	Coefficient of variation	R Square (%)
<b>0.35700</b>	<b>0.0119</b>	<b>3.33400</b>	<b>99.7295</b>

Table 6.9 Analysis of variance of first-order biodegradation rate ( $\mu$ ) of benzene  
(some of the interactions is pulled with the error)  
[Factors: V (velocity), O (O:BTX ratio), C (concentration)]

	Factor	Degree of Freedom	Sum of Square	Mean sum of square	F value	Significance (%)
<i>Main factors</i>	<b>V</b>	2	0.4300	0.2150	1009.64	99.99
	<b>O</b>	1	0.0427	0.0427	200.56	99.99
	<b>C</b>	1	0.0036	0.0036	19.60	99.65
<i>Interactions</i>	<b>VO</b>	2	0.0031	0.0015	7.29	98.69
	<b>VC</b>	2	0.0008	0.0004	1.92	79.75
<i>Error</i>		9	0.0019	0.0002		
<i>Total</i>		17	0.4982			

Mean of $\mu$	Standard Deviation	Coefficient of variation	R Square (%)
<b>0.48466</b>	<b>0.0129</b>	<b>3.0109</b>	<b>99.6153</b>

Table 6.10 Analysis of variance of first-order biodegradation rate ( $\mu$ ) of benzene  
[Factors: V (velocity), O (O:BTX ratio), C (concentration)]

	Factor	Degree of Freedom	Sum of Square	Mean sum of square	F value	Significance (%)
<i>Main factors</i>	<b>V</b>	2	0.4300	0.2150	413.09	99.99
	<b>O</b>	1	0.0427	0.0427	82.06	99.99
	<b>C</b>	1	0.0036	0.0036	6.92	97.92
<i>Error</i>		13	0.0067	0.00052		
<i>Total</i>		17	0.4982			

Mean of $\mu$	Standard Deviation	Coefficient of variation	R Square (%)
<b>0.48466</b>	<b>0.0129</b>	<b>4.7073</b>	<b>98.6418</b>

Table 6.11 Analysis of variance of first-order biodegradation rate ( $\mu$ ) of benzene  
[Factors: V (velocity), O (O:BTX ratio), C (concentration)]

	Factor	Degree of Freedom	Sum of Square	Mean sum of square	F value	Significance (%)
<i>Main factors</i>	<b>V</b>	2	0.4300	0.2150	47.31	99.99
<i>Error</i>		15	0.0681	0.00454		
<i>Total</i>		17	0.4982			

Mean of $\mu$	Standard Deviation	Coefficient of variation	R Square (%)
	<b>0.0129</b>	<b>13.9090</b>	<b>86.3174</b>

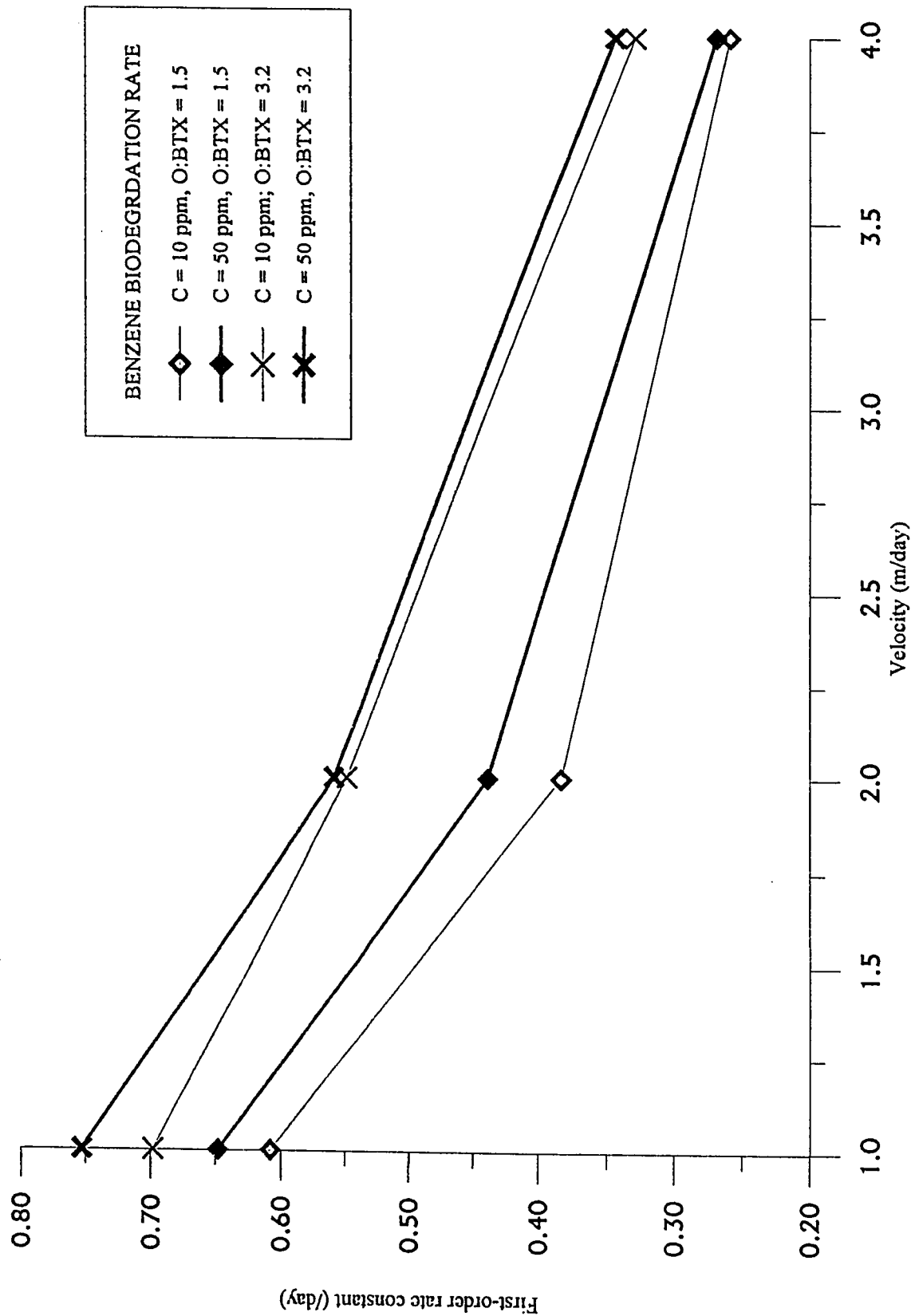


Figure 6.37 Variation of first-order rate constant of benzene with Concentration (C) and DO (O:BTX ratio)

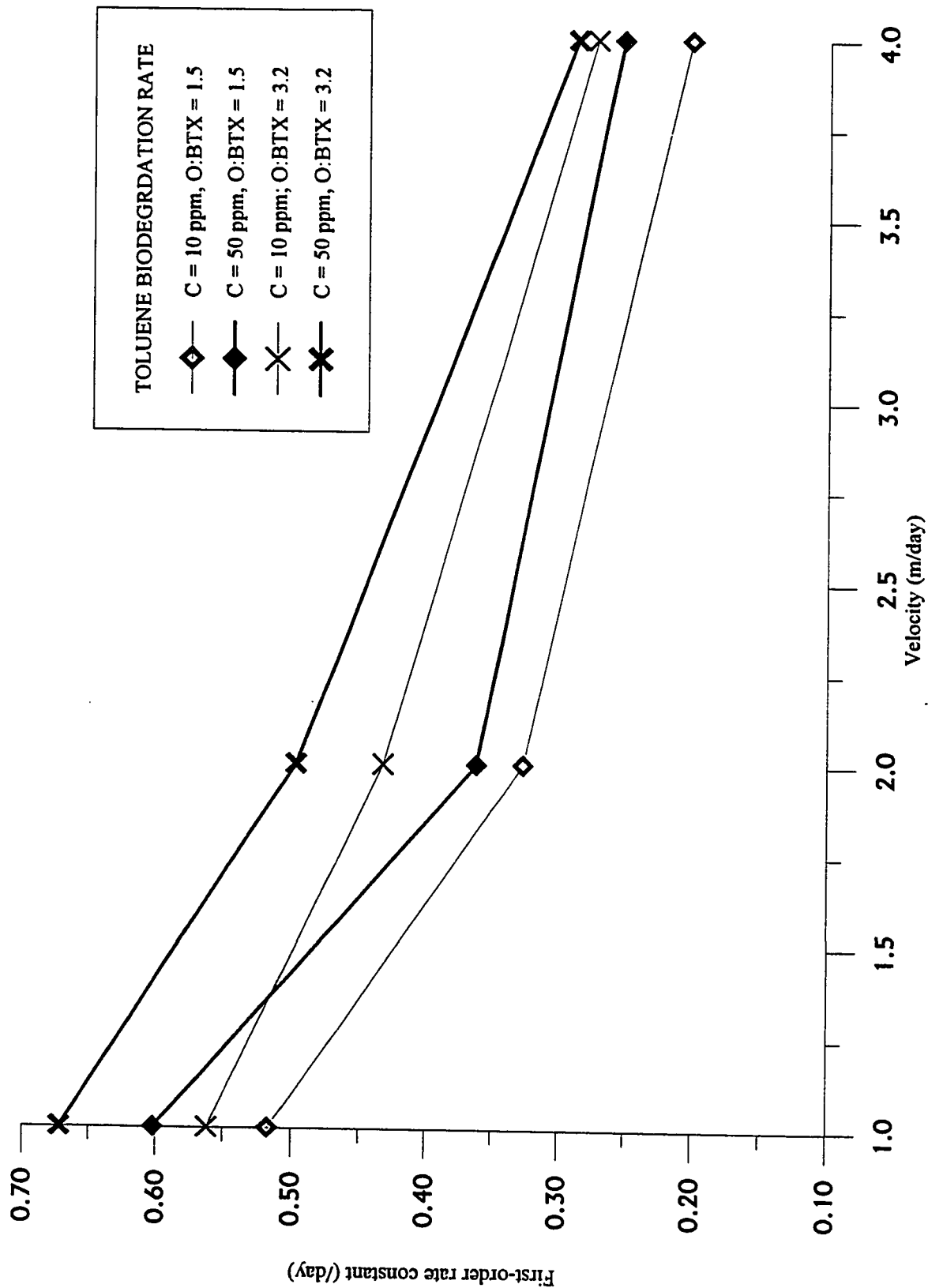


Figure 6.38 Variation of first-order rate constant of toluene with Concentration (C) and DO (O:BTX ratio)

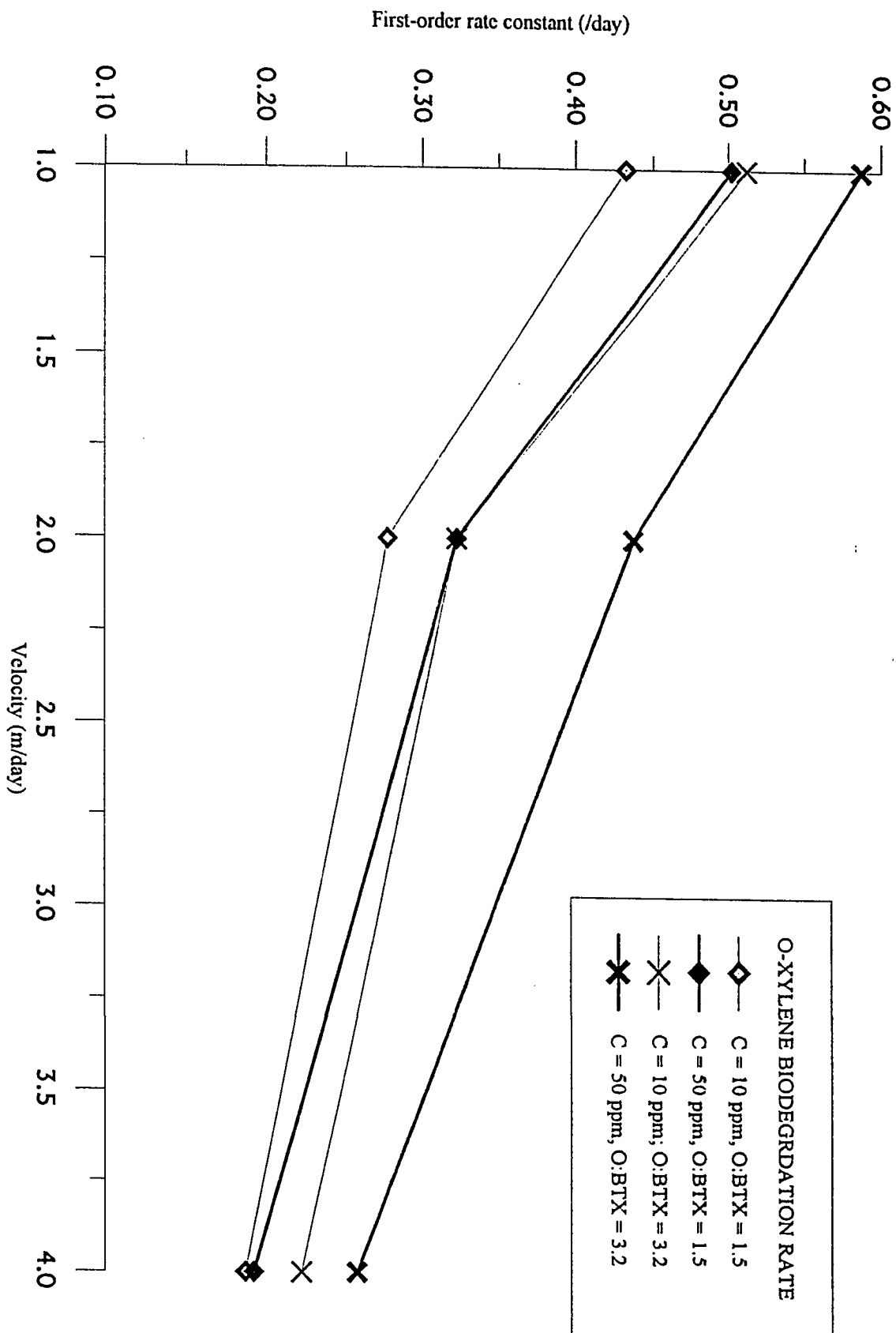


Figure 6.39 Variation of first-order rate constant of o-xylene with Concentration (C) and DO (O:BTX ratio)

Since all the factors (V, O, C) are numeric, attempt was made to find linear models of  $\mu$  as a function of V, O, and C. Table 6.12 shows that linear model with the corresponding values of  $R^2$  computed with the available data. The values of  $R^2$  did not improve including the cross products (VC, VO, OC, and VOC) with the models, however, it improved remarkably adding the square of the velocity in the models as shown in Table 6.12.

Table 6.12 Equations for the biodegradation rate ( $\mu$ ) of BTX compounds

Compound	Model Equations	$R^2$ yielded
Benzene	$\mu = 0.60099 - 0.12071V + 0.06078O + 0.00075C$	94.22 %
	$\mu = 0.78987 - 0.31500V + 0.03777V^2 + 0.06078O + 0.00075C$	98.64 %
Toluene	$\mu = 0.51768 - 0.10833V + 0.04509O + 0.00150C$	93.31 %
	$\mu = 0.69268 - 0.28833V + 0.03500V^2 + 0.04509O + 0.00150C$	97.89 %
O-xylene	$\mu = 0.43179 - 0.09250V + 0.04166O + 0.00144C$	92.04 %
	$\mu = 0.59707 - 0.26250V + 0.03305V^2 + 0.04166O + 0.00144C$	97.41 %

### 6.6.3 Retardation Constant (R)

The analysis of variance of the retardation constant, R for all benzene, toluene, and xylene shows that none of the factors are significant event at 70% confidence level. Results for benzene is shown in Table 6.13. Low value of  $R^2$  also implies that about half of the data is unaccounted by the linear model (3.1).



Table 6.13 Analysis of variance of retardation factor (R) of benzene  
[Factors: V (velocity), O (O:BTX ratio), C (concentration)]

	Factor	Degree of Freedom	Sum of Square	Mean sum of square	F value	Significance (%)
<i>Main factors</i>	<b>V</b>	2	0.001905	0.000953	1.41	68.55
	<b>O</b>	1	0.00000278	0.00000278	0.00	5.00
	<b>C</b>	1	0.00000278	0.00000278	0.00	5.00
<i>Interactions</i>	<b>VO</b>	2	0.001172	0.000586	0.87	53.36
	<b>VC</b>	2	0.0013722	0.000686	1.02	52.33
	<b>OC</b>	1	0.0003361	0.0003361	0.50	49.31
	<b>VOC</b>	2	0.0001055	0.0000527	0.08	7.43
<i>Error</i>		6	0.00405	0.000675		
<i>Total</i>		17	0.008894			

Mean of $\mu$	Standard Deviation	Coefficient of variation	R Square (%)
1.0694	0.02598	2.4293	54.4660

#### 6.6.4 Plate Count

The analysis of variance of the cell count is given in Table 6.14 from where it can be inferred that the population of microorganisms is dependent on all main factors as well as their interactions. The F values implies the relative significance, higher F value implying higher significance. Thus concentration is the most important factor for the population of the biodegrading microorganisms. Velocity, that effects the shear loss of the microorganisms is the second important factor.

Table 6.14 Analysis of variance of mean cell count ( $\times 10^7$ )  
[Factors: V (velocity), O (O:BTX ratio), C (concentration)]

	Factors	Degree of Freedom	Sum of Square	Mean sum of square	F value	Significance (%)
<i>Main factor</i>	V	2	706.0311	353.0158	200.58	99.99
	O	1	53.2900	53.2900	30.28	99.85
	C	1	778.4100	778.4100	442.28	99.99
<i>Interactions</i>	VO	2	21.7116	10.8558	6.17	96.50
	VC	2	319.07116	159.5358	90.65	99.99
	OC	1	18.7777	18.7777	10.67	98.29
	VOC	2	29.0405	14.5203	8.25	98.10
<i>Error</i>		6	10.5600	1.7600		
<i>Total</i>		17	2493.6827			

Mean of $\mu$	Standard Deviation	Coefficient of variation	R Square (%)
$15.36 \times 10^7$	1.3266	8.6364	99.5765

### 6.6.5 Porosity ( $\Phi$ )

Although many other factors such as initial porous media concentration, initial plate count, sequence of experiment, etc. affect the porosity of the media, analysis of variance of porosity was performed as the function of the three factors (V, O, C) and their interaction.

The variance of the porosity has also been analyzed as a function of cell count. The results are shown in Tables (6.15) and (6.16). From the analysis of variance, it can be concluded that porosity is dependent to some degree on all the main factors as well as few interactions. Although cell count is also a significant factor at about 95% confidence limit, very low value of  $R^2$  implies that porosity cannot be modeled with cell count only.

Table 6.15 Analysis of variance of effective porosity ( $\Phi$ ) medium  
[Factors: V (velocity), O (O:BTX ratio), C (concentration)]

	Factor	Degree of Freedom	Sum of Square	Mean sum of square	F value	Significance (%)
<i>Main factors</i>	<b>V</b>	2	0.00570	0.00285	4.98	94.70
	<b>O</b>	1	0.00230	0.00230	4.03	90.85
	<b>C</b>	1	0.01712	0.01712	29.94	99.84
<i>Interactions</i>	<b>VO</b>	2	0.00065	0.00032	0.57	40.73
	<b>VC</b>	2	0.00452	0.00226	3.95	91.97
	<b>OC</b>	1	0.00236	0.00236	4.13	91.16
	<b>VOC</b>	2	0.00058	0.00029	0.51	37.47
<i>Error</i>		6	0.00343	0.000572		
<i>Total</i>		17	0.04621			

Mean of $\mu$	Standard Deviation	Coefficient of variation	R Square (%)
<b>0.2074</b>	<b>0.02391</b>	<b>11.5305</b>	<b>92.57</b>

Table 6.16 Analysis of variance of medium porosity ( $\Phi$ ) as a function of plate count  
[Factor: Plate count ( $\times 10^7$ ): Low (3.9-10), Medium (10-15), High (15-20), Very high (>20)]

	Factor	Degree of Freedom	Sum of Square	Mean sum of square	F value	Significance (%)
<i>plate count</i>	<b>COUNT</b>	3	0.01982	0.00660	3.51	95.61
<i>Error</i>		14	0.0263	0.00188		
<i>Total</i>		17	0.04619			

Mean of $\mu$	Standard Deviation	Coefficient of variation	R Square (%)
<b>0.2074</b>	<b>0.04340</b>	<b>20.92565</b>	<b>42.90</b>

## 6.6 Discussions

As mentioned in Chapter 4, biodegradation of BTX compounds has been reported to be given by first-order [Chiang et al., 1989; Hutchins et al., 1991; Lyman et al., 1982; and Major et al., 1988], zero-order [Barker et al., 1987; Hutchins et al., 1991], Monod kinetics [Alvarez et al., 1991, Arcangeli & Ervin, 1994, Chu and Jewell, 1994, Coreseuil and Weber, 1994, and Mihelcic and Luthy, 1991, Lodaya et al., 1991], and Michaelis-Menten Kinetics [Barrio Lage et al., 1987, Jones, 1972]. Haldane kinetics were used by Brown et al. [1990] and Zarooq et al. [1993]. The Monod kinetics used by Lodaya et al. [1991] is a non-growth associated model since they ignored bacterial growth and decay. The biodegradation kinetics have been reported to depend on the type of microbial culture. Probably this is the main reason of large variation of biodegradation kinetics of BTX compounds. Table 6.17 shows the biodegradation kinetics used by a number of authors to model the removal of BTX compounds. Table 6.18 shows that parameters of Monod and Michaelis-Menten model. The results of the present study are within the range of literature values.

Table 6.17 Biodegradation removal kinetics of BTX compounds

Authors	Compound	Concentration	Electron donor	Removal Kinetics	Parameter values
Barket et al., 1987	BTX	2 to 6 ppm each	1 to 3.3 mg/L DO	Zero-order (first-order worse)	B (33 µg/l/day) to X(43 µg/l/day)
Anid et al., 1993	BTEX	20 ppm each	110 mg/L of H <sub>2</sub> O <sub>2</sub>	T (84% in 3 days) B, X (80%, 70%) in 3 days	Not reported
Hutchins et al., 1991	BTEX	about 5 ppm each	39 ppm nitrate	First-order	upto -0.38 /day
Lodaya et al., 1991	BTX	150+100+255	1 mL/H of H <sub>2</sub> O <sub>2</sub> (> 2 ppm DO)	Non-growth Monod	K <sub>s</sub> = 3.3-40 mg/l, k = 3.3-7.5 mg/l
Alvarez and Vogel, 1991	BTX	50+50+50	Oxygen gas in the headspace	Zero-order removal	25, 23, 6 mg/l/day upto 52 mg/l/day (bioaugmentation)
Alvarez et al., 1991	B, T	250 + 250	Air and oxygen	Non-growth Monod	Removed upto 100 ppm each, K <sub>s</sub> = 12.2 (B), 17.4 (T), k= 8.3 (B), 9.9(T)
Arcangeli & Ervin, 1994	BTEX	60 ppm total	Nitrate	1st order for Co=2-3 mg/L, Monod for higher (K <sub>s</sub> =0.4-0.85), zero order for Co =8-30 mg/L, Only TEX removed	
Coreseul and Weber, 1994	BTX	2 + 2 + 4	Nitrate	Monod	K <sub>s</sub> = 0.1 - 0.7 mg/L k = 2.5 - 3.11, Y= 0.65 - 0.67, b = 0.11-0.14

Table 6.18 Biokinetic constants for BTX biodegradation

Authors	k	K <sub>s</sub> (mg/L)	Y	b	k/K <sub>s</sub>
<b>BENZENE</b>					
Grady et al., 1989	4.7	10.8	0.39	-	1.33
Corseuil & Weber, 1994	2.58	Small	0.65	0.11	-
Alvarez et al., 1991	8.3	12.2	0.0	0.0	0.65
Lodaya et al., 1991	3.3-7.5	3.3-40.0	0.0	0.0	
<b>TOLUENE</b>					
Vetch et al., 1988	2.97	-	1.42		
Button, 1985	0.004	0.33	0.01	-	0.01
Button, 1985	11.0	0.43	0.28	-	25.5
Robertson & Button, 1987	0.013	0.034	0.1	-	0.38
Robertson & Button, 1987	0.33	0.044	0.1	-	7.7
MacQuarry et al., 1990	0.49	0.65	0.43	-	0.75
Jorgensen et al., 1990	4.32	0.15	-	-	28.8
Alvarez et al., 1991	9.9	17.4	0.0	0.0	0.57
Corseuil & Weber, 1994	2.73	Small	0.66	0.11	-
<b>O-XYLENE</b>					
Corseuil & Weber, 1994	3.03-3.18	0.1-0.8	0.67	0.11	3.8-30.0

Although commonly ignored, few authors including Bauer and Capone [1988], Arvin et al. [1989], Alvarez and Vogel [1991], and Oh et al. [1994] reported substrate interaction of benzene, toluene, and xylene in a mixture. Bauer and Capone reported that interaction effects in the degradation of one aromatic hydrocarbon by microbial populations grown on another are not well understood. Arvin et al. [1989] observed that toluene and xylene degraders are able to degrade benzene if either toluene or xylene are present. Reasons for this behavior were not determined. Alvarez and Vogel [1991] examined such interactions and concluded that, despite similar chemical structure of these compounds, some microorganisms may be capable of metabolizing more than one compounds but not necessarily all of them. Interaction effects of benzene, toluene and xylene in the mixer has not been revealed in the present study.

The intermediate products of biodegradation products was not detected in the Gas Chromatograph. This might be due to the programming of the GC. Lack of proper GC

columns made it impossible to study the intermediates. The doses of hydrogen peroxide used is the theoretical oxygen requirement and with this dose conflicting results about oxygen limitations has been reported. Lu [1994] reported that requirement of  $H_2O_2$  for the complete biodegradation of benzene was twice that theoretically calculated from stoichiometric equation. Anid et al. [1993] reported 90-95% removal of benzene and toluene (20 mg/l each) with a  $H_2O_2$  dose of only 110 mg/l which is much below the theoretical need. In their study, the DO at the outlet of the soil columns never dropped below 2 mg/l. Lodaya et al. [1991] was able to maintain the aerobic condition with a very low dose of  $H_2O_2$ . In the present study the DO profile at the outlet of the sand tank is shown in Figure 6.33. The minimum DO was 2.56 mg/l and the maximum DO was 4.03 mg/l. Therefore, it is extremely unlikely that there was an oxygen limitation in the sand tank during the course of the experiment.

## **Chapter 7**

# **CONCLUSIONS & RECOMMENDATIONS**



## 7 CONCLUSIONS AND RECOMMENDATIONS

### 7.1 Conclusions

- [1] A finite difference model has been developed for simulating one dimensional BTX transport allowing equilibrium sorption given by linear isotherm and biodegradation given by first-order and/or zero-order kinetics under a variety of initial and boundary condition. The model considers time-dependent groundwater velocity and spatially variable initial concentration in addition to constant velocity and concentration. The model has been tested for high accuracy comparing with the analytical solutions for especial cases.
- [2] An optimization routine has been developed using a modified Gauss Newton method to compute the transport parameters by nonlinear least square fit to the above model. The least square fitting procedure was found to converge rapidly regardless of the sign and magnitude of the initial guesses.
- [3] A generalized model has been developed using orthogonal collocation for simulating one dimensional transport allowing equilibrium sorption given by linear isotherm and biodegradation given by a variety of biodegradation kinetics including or excluding microbial growth. Two models using Michaelis Menten and Monod Kineics have been used for analyzing the data collected in the present data. These models also consider time-dependent groundwater velocity and spatially variable initial concentration in addition to constant velocity and concentration and has been tested for accuracy.
- [4] Models using Michelis Menten and Monod kinetics have also been inverted using a modified Gauss-Levenberg-Marquardt's method. The objective was to study the suitability of these models, being frequently used in literature, to predict BTX fate in saturated sandy soil.

- [5] The results of this study confirm that microorganisms present in raw sewage can be acclimatized to get a mixed culture capable of biodegrading benzene, toluene and xylene (BTX) in a mixture.
- [6] Biodegradation of benzene, toluene and xylene in a mixture can be satisfactorily represented by first-order kinetics for the range of concentration studied (10 to 50 ppm each).
- [7] Michaelis-Menten kinetics can be used to model BTX biodegradation in a mixture. Observed data corresponding to the input concentration of 50 mg/l has been found to fit better to this model than the first-order/zero-order model.
- [8] Monod Kinetics with microbial growth and decay can also be used to model BTX biodegradation in a mixture. However with two more parameters ( $Y$  and  $b$ ), the goodness of fit judged with the value of  $R^2$  did not improve in the present study. Therefore caution should be exercised for using Monod kinetics for biodegradation of BTX compounds in a sand tank model or a packed bed reactor. Plate count confirms that in a porous medium growth does not continue as long as  $\frac{dx}{dt} = 0$  (or  $S_{min} = \frac{bK_s}{Yk - b}$ ) and reaches a steady state earlier. Moreover the decay coefficient,  $b$  is strongly dependent on shear loss or pore water velocity, and in cases where  $v$  keeps on changing,  $b$  should be also changed.
- [9] The rate of biodegradation of BTX compounds in a mixture is strongly dependent on velocity, DO and concentration. The factor of highest significance is velocity and that of least significance is concentration. However this conclusion is valid for the range of factors studied. If any of these factors are selected outside the range studied, the factorial experiment should be repeated. the interaction of the factors are all found to be insignificant. Few of the second-order interactions are found to significant at a very low confidence limit (75%).

- [10] Sorption of BTX compounds in sand of low organic content is very low and can be represented by a linear isotherm. The capacity of sorption was found in the order of toluene > benzene > o-xylene. This finding supports the results of Zytner [1994].
- [11] Sorption of BTX compounds is statistically independent of velocity, DO and concentration.
- [12] Considerable change of permeability and effective porosity was observed during the course of the experiment. Slight changes were also observed in the control runs meaning that changes in the permeability attributed to BTX volatilization,  $H_2O_2$  breakdown, and  $CO_2$  production. More study is needed to quantify the changes due to each of these factors independently.

## **7.2 Recommendations**

The study can be extended with various types of soil and more levels the factors studied to cover all the practical problems and with more factors such as nutrients, temperature. Anaerobic treatment with different doses of nitrate and corresponding advantages and disadvantages with regard to time of treatment, clogging of the media, cost, etc. can be studied. Very few studies has been conducted to study the interaction of each compound when biodegraded in a mixture. Biodegradation of each of the BTX compounds and the mixture can be separately studied to have a clear understanding of the interaction effects.

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## Appendix A

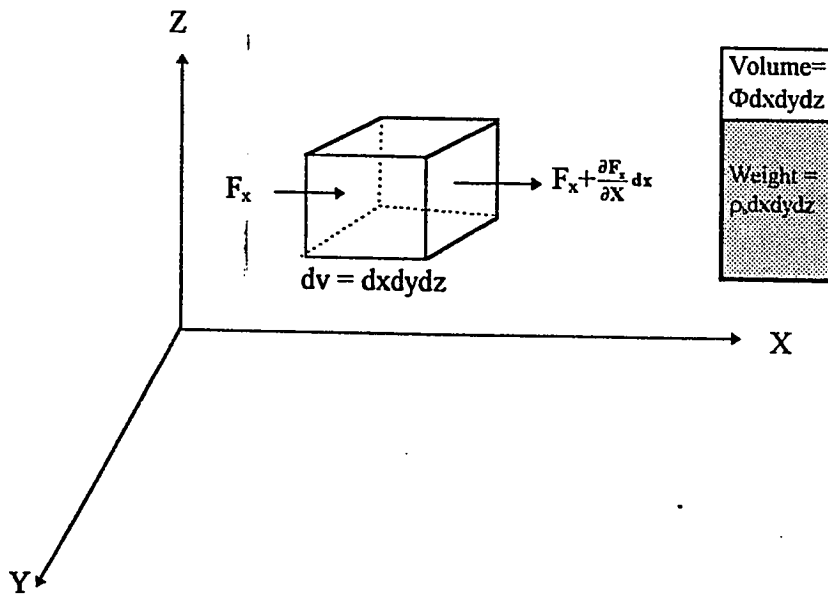
# **DERIVATION OF THE ADVECTION DISPERSION EQUATION**

## APPENDIX A

### Derivation of the 1D Advection-Dispersion Equation

The advection-dispersion equation is derived from the law of conservation of mass. The derivation is based on following assumptions:

1. porous medium is homogeneous and isotropic
2. the medium is saturated
3. the flow is steady
4. Darcy's law applies



Considering an elementary volume of porous medium,  $dv = dx dy dz$  as shown above, if  $C$  is the solute mass per unit volume of the liquid phase and  $S$  is the solute mass per unit weight of the solid phase, then the total solute mass contained in the liquid phase of the elementary volume is  $C(\Phi dx dy dz)$  and the solid phase is  $S(\rho_b dx dy dz)$  where  $\rho_b$  is the unit weight of solid. Considering flow in  $x$  direction and letting  $v$  be the average linear velocity ( $q/\Phi$ ), the solute mass (per unit area) entering by means of advection is,

$$qC = v\Phi C$$

and the solute mass (per unit area) leaving by means of dispersion is,

$$\Phi D \frac{\partial C}{\partial x}$$

Where D is the coefficient of hydrodynamic dispersion. If  $F_x$  represents the total mass of solute per unit cross section transported in the x direction per unit time, then

$$F_x = \Phi v C - \Phi D \frac{\partial C}{\partial x} \dots\dots\dots (1)$$

The total amount of solute entering the cubic element is ( $F_x dydz$ ) and the total amount of solute leaving the element is  $\left(F_x + \frac{\partial F}{\partial x} dx\right) dydz$ . The difference in the amount entering and leaving is the amount of accumulation  $\left(-\frac{\partial F_x}{\partial x} dx dydz\right)$

Now, if the rate of change of mass per unit volume of liquid phase is  $\left(-\frac{\partial C}{\partial t}\right)$  then the rate of change of mass in the liquid phase of the element is  $\left(-\Phi \frac{\partial C}{\partial t} dx dydz\right)$ . Also, if the rate of change of mass of solute per unit mass of the solid phase is  $\left(-\frac{\partial S}{\partial t}\right)$  then the rate of change of mass in the solid phase of the element is  $\left(-\rho_b \frac{\partial S}{\partial t} dx dydz\right)$ .

### *Chemical reactions*

Let both first-order and zero-order reactions causes solute production in both the liquid and solid phase. If  $\mu_w$  and  $\mu_s$  are the rate constants for first-order production in the liquid and solid phase of the soil respectively; and  $\gamma_w$  and  $\gamma_s$  as the the corresponding zero-order production coefficients, the rate of production of solute in the liquid phase due to first-order reaction is  $(-\mu_w C(\Phi dx dydz))$ .

The rate of production in the solid phase due to first-order reaction is  $(-\mu_s S(\rho_b dx dydz))$

In the liquid phase, the rate of production due to zero-order reaction is  $(-\gamma_w(\Phi dx dydz))$  and



in the solid phase, the rate of production due to zero-order reaction is  $(-\gamma\rho_b dx dy dz)$ , the minus sign is included in the terms above because C decreases with time and reactions are productive (not decay). Compiling all terms of advection, dispersion and reactions, the rate of change of solute mass in the control volume becomes

$$-\Phi \frac{\partial C}{\partial t} dx dy dz - \rho_b \frac{\partial S}{\partial t} dx dy dz =$$

$$-\frac{\partial F_x}{\partial x} dx dy dz - \mu_w C \Phi dx dy dz - \mu_s S \rho_b dx dy dz - \gamma_w \Phi dx dy dz - \gamma_s \rho_b dx dy dz$$

Substituting for  $F_x$  from (1) and dividing by  $\Phi dx dy dz$  we get

$$\frac{\rho_b}{\Phi} \frac{\partial S}{\partial t} + \frac{\partial C}{\partial t} = D \frac{\partial^2 C}{\partial x^2} - v \frac{\partial C}{\partial x} + \mu_w C + \mu_s \frac{\rho_b}{\Phi} S + \gamma_w + \gamma_s \frac{\rho_b}{\Phi} \dots \dots \dots (2)$$

which is the general form of the one dimensional advection dispersion equation for first-order and/or zero-order reactions. The reactions terms can be easily modified for other reaction kinetics such as Monod, Haldane, and other kinetics as shown in Chapter 4.

## Appendix B

# **DATA SHEETS**

## APPENDIX B-1

Expt. # BIO-1.1.1(1)  
Date: 1/August/1994

Initial parameters:

Q = 129.6 L/day	v = 4 m/day	BTX = (10+10+10) mg/l	Oxygen:BTX = 1.5
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Change of hydraulic conductivity and velocity:

T (day) →	0	2	4	6
ΔH (cm)	4.40	5.55	6.05	6.50
K (m/day)	261.82	207.56	190.41	177.23
v (m/day)	4.00	4.31	4.44	4.56
Φ	0.355	0.329	0.319	0.312

Observed Concentration [C(x,t)] at different space and time

t (day) ↓	X (m) →	0	1	2	3	4	5	6	7	8
2	Benzene	10.00	8.65	9.65	9.25	7.96	5.67	6.02	2.96	2.56
	Toluene	10.00	9.32	9.00	7.76	8.56	7.25	5.50	1.59	2.42
	Xylene	10.00	9.82	9.50	8.00	8.23	6.50	5.78	4.05	2.09
4	Benzene	10.00	9.53	9.05	7.56	8.12	7.23	5.50	5.79	4.87
	Toluene	10.00	10.00	9.67	8.02	8.56	7.03	8.10	6.02	7.00
	Xylene	10.00	9.83	8.95	9.35	8.77	7.45	8.45	6.23	6.78
6	Benzene	10.00	9.89	8.78	9.06	8.34	8.01	7.03	5.23	4.05
	Toluene	10.00	10.00	9.12	8.67	8.15	7.02	8.08	5.78	6.59
	Xylene	10.00	9.58	10.00	8.79	8.90	7.46	7.56	6.78	7.05

Parameters Computed: (fitting the observed concentration by nonlinear least square fit)

Compound	R	μ	γ
Benzene	1.08	0.30	0.00
Toluene	1.15	0.25	0.00
Xylene	1.05	0.21	0.00

## APPENDIX B-2

Expt. # BIO-1.1.1(2)

Date 06/October/1994

Initial parameters:

Q = 69.84 L/day	v = 4 m/day	BTX = (10+10+10) mg/l	Oxygen:BTX = 1.5
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Change of hydraulic conductivity and velocity:

T (day) →	0	2	4	6
ΔH (cm)	8.50	8.40	8.55	8.65
K (m/day)	73.04	73.90	72.60	71.77
v (m/day)	4.00	3.98	4.01	4.03
Φ	0.194	.195	.194	.192

Observed Concentration [C(x,t)] at different space and time

t (day) ↓	X (m) →	0	1	2	3	4	5	6	7	8
2	Benzene	10.00	9.22	9.00	8.56	7.56	6.22	5.87	5.01	4.67
	Toluene	10.00	9.66	9.23	8.23	7.40	7.32	6.66	6.00	4.00
	Xylene	10.00	9.87	8.56	8.00	8.23	6.78	6.56	6.23	4.98
4	Benzene	10.00	9.55	8.89	8.00	7.99	7.32	5.89	6.25	5.86
	Toluene	10.00	9.89	9.23	8.65	8.01	7.37	7.11	6.37	5.89
	Xylene	10.00	10.00	9.24	8.76	8.00	7.50	7.99	7.34	6.95
6	Benzene	10.00	9.45	8.99	8.34	7.45	7.50	7.11	6.35	5.50
	Toluene	10.00	9.78	9.07	8.00	7.70	7.59	7.00	6.03	6.00
	Xylene	10.00	9.15	9.34	8.45	8.02	7.89	7.65	6.89	6.77

Parameters Computed: (fitting the observed concentration by nonlinear least square fit)

Compound	R	μ	γ
Benzene	1.03	0.32	0.00
Toluene	1.08	0.24	0.00
Xylene	1.02	0.22	0.00

## APPENDIX B-3

Expt. # BIO-1.1.2

Date 07/August/1994

Initial parameters:

$Q = 112.3 \text{ L/day}$	$v = 4 \text{ m/day}$	$\text{BTX} = (10+10+10) \text{ mg/l}$	$\text{Oxygen:BTX} = 3.2$
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Change of hydraulic conductivity and velocity:

T (day) →	0	2	4	6
$\Delta H \text{ (cm)}$	5.8	6.2	6.80	
$K \text{ (m/day)}$	178.28	161.03	146.82	
$v \text{ (m/day)}$	4.00	4.14	4.28	
$\Phi$	0.312	0.301	0.291	

Observed Concentration  $[C(x,t)]$  at different space and time

t (day) ↓	X (m) →	0	1	2	3	4	5	6	7	8
2	Benzene	10.00	9.01	8.02	8.25	6.78	6.13	6.04	4.50	3.12
	Toluene	10.00	9.35	8.24	7.82	8.02	6.50	5.35	4.78	3.25
	Xylene	10.00	9.85	8.23	7.99	7.78	7.54	5.67	5.24	3.89
4	Benzene	10.00	9.00	8.56	7.79	7.34	6.00	6.23	5.57	5.05
	Toluene	10.00	9.65	8.00	8.02	7.42	7.56	6.52	6.00	5.60
	Xylene	10.00	9.95	9.26	8.02	7.44	7.22	7.00	6.65	5.78
6	Benzene									
	Toluene									
	Xylene									

Parameters Computed: (fitting the observed concentration by nonlinear least square fit)

Compound	R	$\mu$	$\gamma$
Benzene	1.04	0.39	0.00
Toluene	1.11	0.31	0.00
Xylene	1.06	0.25	0.00

## APPENDIX B-4

Expt. # BIO-1.2.1(1)

Date 11/August/1994

Initial parameters:

$Q = 52.4 \text{ L/day}$	$v = 2 \text{ m/day}$	$\text{BTX} = (10+10+10) \text{ mg/l}$	$\text{Oxygen:BTX} = 1.5$
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Change of hydraulic conductivity and velocity:

T (day) →	0	4	8	12
$\Delta H \text{ (cm)}$	3.15	3.65	4.05	
$K \text{ (m/day)}$	147.81	127.56	114.96	
$v \text{ (m/day)}$	2.00	2.11	2.19	
$\Phi$	0.291	0.276	0.266	

Observed Concentration [C(x,t)] at different space and time

t (day) ↓	X (m) →	0	1	2	3	4	5	6	7	8
	Benzene	10.00	8.01	6.78	5.05	4.78	3.43	2.04	2.50	1.87
4	Toluene	10.00	8.23	7.43	5.59	5.78	4.00	3.67	2.44	1.78
	Xylene	10.00	8.84	7.27	6.82	5.34	5.08	3.86	3.31	2.44
	Benzene	10.00	8.00	7.06	6.19	4.64	4.22	2.54	2.07	2.11
8	Toluene	10.00	8.95	7.01	6.02	5.24	4.22	3.92	2.65	2.88
	Xylene	10.00	8.58	7.88	6.32	6.08	4.76	4.55	3.69	3.39
	Benzene									
12	Toluene									
	Xylene									

Parameters Computed: (fitting the observed concentration by nonlinear least square fit)

Compound	R	$\mu$	$\gamma$
Benzene	1.06	0.42	0.00
Toluene	1.10	0.36	0.00
Xylene	1.07	0.30	0.00

## APPENDIX B-5

Expt. # BIO-1.2.1(2)

Date 28/September/1994

Initial parameters:

$Q = 36.0$ L/day	$v = 2$ m/day	BTX = (10+10+10) mg/l	Oxygen:BTX = 1.5
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Change of hydraulic conductivity and velocity:

T (day) →	0	4	8	12
$\Delta H$ (cm)	4.10	4.25	4.40	
K (m/day)	78.00	75.29	72.73	
v (m/day)	2.00	1.107	2.06	
$\Phi$	0.20	.197	.194	

Observed Concentration [C(x,t)] at different space and time

t (day) ↓	X (m) →	0	1	2	3	4	5	6	7	8
4	Benzene	10.00	8.23	6.98	4.78	4.01	3.42	1.98	1.50	1.15
	Toluene	10.00	8.78	6.43	5.99	4.78	4.01	2.67	2.24	.89
	Xylene	10.00	8.75	7.14	6.65	5.15	4.80	3.39	2.40	1.11
8	Benzene	10.00	7.50	6.98	5.25	3.78	3.54	2.68	2.35	1.29
	Toluene	10.00	8.65	6.26	6.02	5.24	4.22	3.00	3.03	1.78
	Xylene	10.00	8.46	7.67	6.09	5.78	4.48	4.24	3.41	3.20
12	Benzene									
	Toluene									
	Xylene									

Parameters Computed: (fitting the observed concentration by nonlinear least square fit)

Compound	R	$\mu$	$\gamma$
Benzene	1.08	0.45	0.00
Toluene	1.05	0.37	0.00
Xylene	1.06	0.31	0.00

## APPENDIX B-6

Expt. # BIO-1.2.2

Date 19/August/1994

Initial parameters:

$Q = 47.9 \text{ L/day}$	$v = 2 \text{ m/day}$	$\text{BTX} = (10+10+10) \text{ mg/l}$	$\text{Oxygen:BTX} = 3.2$
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Change of hydraulic conductivity and velocity:

T (day) →	0	4	8	12
$\Delta H \text{ (cm)}$	3.70	4.00	4.35	
$K \text{ (m/day)}$	115.03	106.4	97.84	
$v \text{ (m/day)}$	2.00	2.06	2.12	
$\Phi$	0.266	0.258	0.25	

Observed Concentration  $[C(x,t)]$  at different space and time

t (day) ↓	X (m) →	0	1	2	3	4	5	6	7	8
4	Benzene	10.00	6.56	6.55	4.26	3.77	2.41	2.39	1.05	1.01
	Toluene	10.00	8.37	6.01	5.55	3.64	3.57	1.75	1.47	1.23
	Xylene	10.00	8.94	6.71	6.46	4.64	4.42	3.09	2.13	1.68
8	Benzene	10.00	7.12	6.75	4.12	3.98	2.25	2.59	1.47	1.55
	Toluene	10.00	7.66	6.84	4.84	4.43	3.06	2.88	1.92	1.70
	Xylene	10.00	8.09	7.71	5.64	5.66	3.82	3.92	2.89	2.44
12	Benzene									
	Toluene									
	Xylene									

Parameters Computed: (fitting the observed concentration by nonlinear least square fit)

Compound	R	$\mu$	$\gamma$
Benzene	1.05	0.60	0.00
Toluene	1.09	0.47	0.00
Xylene	1.02	0.35	0.00



## APPENDIX B-7

Expt. # BIO-1.3.1 (1)  
Date 27/August/1994

Initial parameters:

Q = 22.5 L/day	v = 1 m/day	BTX = (10+10+10) mg/l	Oxygen:BTX = 1.5
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Change of hydraulic conductivity and velocity:

T (day) →	0	8	16	24
ΔH (cm)	2.05	2.35	2.55	
K (m/day)	77.56	85.11	78.43	
v (m/day)	1.00	1.05	1.08	
Φ	0.250	0.24	0.23	

Observed Concentration [C(x,t)] at different space and time

t (day) ↓	X (m) →	0	1	2	3	4	5	6	7	8
8	Benzene	10.00	5.98	2.51	2.03	1.00	.51	.43	.22	.00
	Toluene	10.00	6.77	3.41	2.42	1.09	.92	.32	.34	.10
	Xylene	10.00	6.70	4.06	3.15	1.55	1.42	.57	.49	.21
16	Benzene	10.00	5.00	3.63	1.78	.87	.77	.00	.26	.00
	Toluene	10.00	6.01	3.96	2.02	1.61	.69	.57	.26	.21
	Xylene	10.00	6.55	4.70	2.61	2.26	1.05	1.00	.49	.40
24	Benzene									
	Toluene									
	Xylene									

Parameters Computed: (fitting the observed concentration by nonlinear least square fit)

Compound	R	μ	γ
Benzene	1.08	0.65	0.00
Toluene	1.08	0.55	0.00
Xylene	1.04	0.45	0.00

## APPENDIX B-8

Expt. # BIO-1.3.1(2)

Date 12/October/1994

Initial parameters:

Q = 17.28 L/day	v = 1 m/day	BTX = (10+10+10) mg/l	Oxygen:BTX = 1.5
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Change of hydraulic conductivity and velocity:

T (day) →	0	8	16	24
ΔH (cm)	2.15	2.45	2.60	
K (m/day)	71.44	62.69	59.07	
v (m/day)	1.00	1.05	1.08	
Φ	0.192	0.182	0.178	

Observed Concentration [C(x,t)] at different space and time

t (day) ↓	X (m) →	0	1	2	3	4	5	6	7	8
8	Benzene	10.00	5.01	3.11	1.15	.98	.35	.30	.20	.00
	Toluene	10.00	5.98	3.24	2.25	1.00	.83	.30	.35	.12
	Xylene	10.00	6.50	3.82	2.88	1.38	1.24	.54	.47	.27
16	Benzene	10.00	5.77	2.52	1.89	.75	.50	.41	.17	.00
	Toluene	10.00	5.81	3.69	1.82	1.41	.58	.51	.17	.21
	Xylene	10.00	6.30	4.35	2.33	1.94	.87	.85	.33	.33
24	Benzene									
	Toluene									
	Xylene									

Parameters Computed: (fitting the observed concentration by nonlinear least square fit)

Compound	R	μ	γ
Benzene	1.10	0.67	0.00
Toluene	1.12	0.56	0.00
Xylene	1.08	0.47	0.00

## APPENDIX B-9

Expt. # BIO-1.3.2

Date 12/September/1994

Initial parameters:

$Q = 20.7 \text{ L/day}$	$v = 1 \text{ m/day}$	$\text{BTX} = (10+10+10) \text{ mg/l}$	$\text{Oxygen:BTX} = 3.2$
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Change of hydraulic conductivity and velocity:

T (day) →	0	8	16	24
$\Delta H \text{ (cm)}$	2.35	3.05	3.50	
$K \text{ (m/day)}$	78.29	60.32	52.57	
$v \text{ (m/day)}$	1.00	1.107	1.17	
$\Phi$	0.23	0.21	0.197	

Observed Concentration  $[C(x,t)]$  at different space and time

t (day) ↓	X (m) →	0	1	2	3	4	5	6	7	8
8	Benzene	10.00	5.53	2.02	1.10	.89	.50	.20	.10	.01
	Toluene	10.00	6.11	3.03	2.01	1.11	.58	.48	.15	.17
	Xylene	10.00	6.45	3.14	2.52	1.02	.93	.48	.25	.00
16	Benzene	10.00	5.12	3.25	1.01	.50	.20	.01	.01	.01
	Toluene	10.00	5.87	2.88	2.31	1.00	.78	.67	.20	.00
	Xylene	10.00	5.67	3.89	1.98	1.45	.71	.50	.17	.25
24	Benzene									
	Toluene									
	Xylene									

Parameters Computed: (fitting the observed concentration by nonlinear least square fit)

Compound	R	$\mu$	$\gamma$
Benzene	1.10	0.75	0.00
Toluene	1.15	0.60	0.00
Xylene	1.07	0.54	0.00

## APPENDIX B-10

Expt. # BIO-2.1.1

Date: 27/October/1994

Initial parameters:

$Q = 68.08 \text{ L/day}$	$v = 4 \text{ m/day}$	$\text{BTX} = (50+50+50) \text{ mg/l}$	$\text{Oxygen:BTX} = 1.5$
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Change of hydraulic conductivity and velocity:

T (day) →	0	2	4	6
$\Delta H \text{ (cm)}$	9.60	9.25	9.20	9.20
$K \text{ (m/day)}$	59.33	61.57	61.91	61.91
$v \text{ (m/day)}$	4.00	3.94	3.94	3.94
$\Phi$	0.178	0.180	0.180	0.180

Observed Concentration  $[C(x,t)]$  at different space and time

t (day) ↓	X (m) →	0	1	2	3	4	5	6	7	8
2	Benzene	50.00	47.03	42.02	40.02	36.20	32.95	26.22	17.96	9.58
	Toluene	50.00	48.08	42.43	41.02	36.06	33.36	27.04	18.83	10.21
	Xylene	50.00	48.62	43.50	43.01	39.66	36.46	29.85	21.04	11.74
4	Benzene	50.00	44.91	43.42	38.52	36.45	33.05	31.51	28.69	26.91
	Toluene	50.00	45.24	43.66	39.97	38.05	34.99	32.93	30.28	28.32
	Xylene	50.00	45.71	45.32	42.05	41.07	37.57	36.49	34.05	32.66
6	Benzene	50.00	47.85	41.46	40.51	35.81	34.29	30.92	29.02	26.97
	Toluene	50.00	48.20	42.07	41.41	36.87	35.57	32.31	30.54	28.17
	Xylene	50.00	49.03	43.53	43.58	39.48	38.74	35.80	34.43	32.31

Parameters Computed: (fitting the observed concentration by nonlinear least square fit)

Compound	R	$\mu$	$\gamma$
Benzene	1.07	0.32	0.00
Toluene	1.10	0.29	0.00
Xylene	1.05	0.22	0.00

## APPENDIX B-11

Expt. # BIO-2.1.2(1)

Date 12/December/1994

Initial parameters:

$Q = 58.32 \text{ L/day}$	$v = 4 \text{ m/day}$	$\text{BTX} = (50+50+50) \text{ mg/l}$	$\text{Oxygen:BTX} = 3.2$
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Change of hydraulic conductivity and velocity:

T (day) →	0	2	4	6
$\Delta H \text{ (cm)}$	11.30	10.50	10.30	10.00
$K \text{ (m/day)}$	45.87	49.37	50.33	51.84
$v \text{ (m/day)}$	4.00	3.88	3.85	3.80
$\Phi$	0.162	0.167	0.168	0.170

Observed Concentration  $[C(x,t)]$  at different space and time

t (day) ↓	X (m) →	0	1	2	3	4	5	6	7	8
2	Benzene	50.00	46.99	40.74	39.21	32.06	30.76	22.14	16.35	8.48
	Toluene	50.00	47.92	42.30	40.66	35.60	33.00	24.80	18.56	9.55
	Xylene	50.00	48.76	43.05	42.83	38.34	35.97	28.54	20.14	10.82
4	Benzene	50.00	43.81	42.09	36.62	34.70	29.54	28.69	24.54	23.71
	Toluene	50.00	44.68	43.45	38.72	37.21	33.49	31.42	28.67	27.01
	Xylene	50.00	45.46	45.71	40.77	40.18	36.56	35.32	32.58	31.69
6	Benzene	50.00	46.98	40.73	38.21	33.24	31.14	26.91	25.60	22.85
	Toluene	50.00	47.92	42.29	40.66	36.81	33.58	31.61	30.19	26.89
	Xylene	50.00	48.75	43.04	42.84	38.59	37.65	34.60	33.09	30.58

Parameters Computed: (fitting the observed concentration by nonlinear least square fit)

Compound	R	$\mu$	$\gamma$
Benzene	1.04	0.39	0.00
Toluene	1.07	0.33	0.00
Xylene	1.06	0.29	0.00

## APPENDIX B-12

Expt. # BIO-2.1.2 (2)

Date 23/January/1995

Initial parameters:

$Q = 59.76 \text{ L/day}$	$v = 4 \text{ m/day}$	$\text{BTX} = (50+50+50) \text{ mg/l}$	$\text{Oxygen:BTX} = 3.2$
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Change of hydraulic conductivity and velocity:

T (day) →	0	2	4	6
$\Delta H \text{ (cm)}$	11.00	10.80	10.70	10.70
$K \text{ (m/day)}$	48.30	49.2	49.6	49.6
$v \text{ (m/day)}$	4.00	3.97	3.96	3.96
$\Phi$	0.166	0.167	0.168	0.168

Observed Concentration [C(x,t)] at different space and time

t (day) ↓	X (m) →	0	1	2	3	4	5	6	7	8
2	Benzene	50.00	45.69	40.15	38.34	32.11	30.50	22.64	16.52	9.37
	Toluene	50.00	47.53	43.44	43.15	37.58	37.04	28.40	21.30	12.35
	Xylene	50.00	40.26	30.90	26.86	18.64	17.45	10.87	8.36	4.32
4	Benzene	50.00	44.90	41.90	35.95	35.00	29.20	28.62	24.43	22.72
	Toluene	50.00	46.70	45.33	41.46	40.98	36.57	35.26	32.19	31.10
	Xylene	50.00	39.57	32.82	24.01	21.94	14.70	14.12	9.71	8.32
6	Benzene	50.00	45.08	41.84	36.34	35.29	30.74	26.57	24.96	22.00
	Toluene	50.00	46.52	45.44	41.16	39.81	36.66	35.67	31.70	31.09
	Xylene	50.00	39.23	32.95	23.78	22.03	15.67	13.54	9.93	8.03

Parameters Computed: (fitting the observed concentration by nonlinear least square fit)

Compound	R	$\mu$	$\gamma$
Benzene	1.08	0.40	0.00
Toluene	1.10	0.32	0.00
Xylene	1.03	0.28	0.00

## APPENDIX B-13

Expt. # BIO-2.2.1

Date: 18/November/1994

Initial parameters:

Q = 30.96 L/day	v = 2 m/day	BTX = (50+50+50) mg/l	Oxygen:BTX = 1.5
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Change of hydraulic conductivity and velocity:

T (day) →	0	4	8	12
ΔH (cm)	5.25	5.35	5.25	
K (m/day)	52.41	51.44	52.41	
v (m/day)	2.00	2.01	2.00	
Φ	0.172	0.171	0.172	

Observed Concentration [C(x,t)] at different space and time

t (day) ↓	X (m) →	0	1	2	3	4	5	6	7	8
8	Benzene	50.00	40.28	29.98	24.91	18.74	15.47	11.71	9.56	7.08
	Toluene	50.00	42.29	32.71	28.39	22.42	18.98	15.21	12.99	10.14
	Xylene	50.00	44.66	36.06	32.96	27.00	24.41	20.07	18.23	15.08
16	Benzene	50.00	37.57	31.26	23.09	18.87	14.26	11.71	8.51	7.23
	Toluene	50.00	39.32	34.22	26.44	22.59	17.53	15.26	11.83	10.24
	Xylene	50.00	41.36	37.86	30.56	27.93	22.63	20.61	16.72	15.18
24	Benzene									
	Toluene									
	Xylene									

Parameters Computed: (fitting the observed concentration by nonlinear least square fit)

Compound	R	μ	γ
Benzene	1.07	0.49	0.00
Toluene	1.09	0.40	0.00
Xylene	1.06	0.35	0.00

## APPENDIX B-14

Expt. # BIO-2.2.2 (1)

Date 12/December/1994

Initial parameters:

Q = 30.60 L/day	v = 2 m/day	BTX = (50+50+50) mg/l	Oxygen:BTX = 1.5
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Change of hydraulic conductivity and velocity:

T (day) →	0	4	8	12
ΔH (cm)	5.25	5.35	5.40	5.40
K (m/day)	51.80	50.84	50.37	50.37
v (m/day)	2.00	2.02	2.02	2.02
Φ	0.170	0.169	0.169	0.169

Observed Concentration [C(x,t)] at different space and time

t (day) ↓	X (m) →	0	1	2	3	4	5	6	7	8
2	Benzene	50.00	38.75	26.96	20.42	11.89	9.32	7.22	7.53	7.18
	Toluene	50.00	40.05	28.33	22.63	13.67	10.49	8.50	8.66	8.20
	Xylene	50.00	41.41	30.47	24.91	15.38	11.92	9.67	9.96	9.38
4	Benzene	50.00	36.15	28.84	20.11	16.56	11.08	8.82	5.01	4.66
	Toluene	50.00	37.36	31.27	22.00	18.68	13.19	10.66	7.23	5.63
	Xylene	50.00	38.62	33.03	24.31	21.34	15.57	12.97	9.03	6.96
6	Benzene	50.00	38.72	27.10	21.80	15.11	12.67	7.97	6.42	5.27
	Toluene	50.00	40.03	28.50	24.18	17.01	14.33	10.36	8.29	6.21
	Xylene	50.00	41.38	30.47	26.73	19.44	16.93	12.64	10.68	8.06

Parameters Computed: (fitting the observed concentration by nonlinear least square fit)

Compound	R	μ	γ
Benzene	1.09	0.60	0.00
Toluene	1.10	0.53	0.00
Xylene	1.06	0.46	0.00



## APPENDIX B-15

Expt. # BIO-2.2.2 (2)

Date 11/January/1995

Initial parameters:

$Q = 29.7$ L/day	$v = 2$ m/day	BTX = (50+50+50) mg/l	Oxygen:BTX = 3.2
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Change of hydraulic conductivity and velocity:

T (day) →	0	2	4	6
$\Delta H$ (cm)	5.55	5.50	5.50	
K (m/day)	47.56	48.00	48.00	
$v$ (m/day)	2.00	1.99	1.99	
$\Phi$	0.165	0.166	0.166	

Observed Concentration [C(x,t)] at different space and time

t (day) ↓	X (m) →	0	1	2	3	4	5	6	7	8
4	Benzene	50.00	37.46	26.75	21.63	13.97	12.19	7.11	5.17	2.55
	Toluene	50.00	38.74	28.61	23.92	15.98	14.41	8.67	6.48	3.27
	Xylene	50.00	40.26	30.90	26.86	18.64	17.45	10.87	8.36	4.32
8	Benzene	50.00	36.82	28.42	19.34	16.44	10.25	9.05	5.85	4.67
	Toluene	50.00	38.07	30.39	21.39	18.81	12.13	11.20	7.41	6.12
	Xylene	50.00	39.57	32.82	24.01	21.94	14.70	14.12	9.71	8.32
12	Benzene	50.00	36.42	28.70	19.57	16.35	10.31	9.60	5.58	4.35
	Toluene	50.00	38.70	29.96	22.87	17.56	12.57	10.31	7.44	5.89
	Xylene	50.00	39.23	32.95	23.78	22.03	15.67	13.54	9.93	8.03

Parameters Computed: (fitting the observed concentration by nonlinear least square fit)

Compound	R	$\mu$	$\gamma$
Benzene	1.05	0.61	0.00
Toluene	1.08	0.54	0.00
Xylene	1.04	0.47	0.00

## APPENDIX B-16

Expt. # BIO-2.3.1

Date: 2/November/1994

Initial parameters:

$Q = 16.2 \text{ L/day}$	$v = 1 \text{ m/day}$	$\text{BTX} = (50+50+50) \text{ mg/l}$	$\text{Oxygen:BTX} = 1.5$
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Change of hydraulic conductivity and velocity:

T (day) →	0	8	16	24
$\Delta H \text{ (cm)}$	2.30	2.60	2.75	
$K \text{ (m/day)}$	62.60	55.38	52.36	
$v \text{ (m/day)}$	1.00	1.08	1.13	
$\Phi$	0.18	0.175	0.172	

Observed Concentration  $[C(x,t)]$  at different space and time

t (day) ↓	X (m) →	0	1	2	3	4	5	6	7	8
8	Benzene	50.00	28.09	14.03	7.97	3.85	2.26	1.21	.73	.69
	Toluene	50.00	29.85	15.00	9.72	4.05	2.89	1.98	1.26	.86
	Xylene	50.00	32.94	18.52	12.32	7.01	4.57	2.71	1.70	1.15
16	Benzene	50.00	26.93	15.55	8.23	4.77	2.57	1.50	.86	.44
	Toluene	50.00	28.07	17.76	9.52	5.78	2.65	1.96	1.16	1.00
	Xylene	50.00	30.11	20.99	12.47	8.26	5.05	3.69	1.96	1.25
24	Benzene									
	Toluene									
	Xylene									

Parameters Computed: (fitting the observed concentration by nonlinear least square fit)

Compound	R	$\mu$	$\gamma$
Benzene	1.05	0.70	0.00
Toluene	1.09	0.64	0.00
Xylene	1.04	0.53	0.00

## APPENDIX B-17

Expt. # BIO-2.3.2(1)

Date 26/November/199

4

Initial parameters:

Q = 15.48 L/day	v = 1 m/day	BTX = (50+50+50) mg/l	Oxygen:BTX = 3.2
-----------------	-------------	-----------------------	------------------

Change of hydraulic conductivity and velocity:

T (day) →	0	2	4	6
ΔH (cm)	2.60	2.80	2.90	3.0
K (m/day)	52.92	49.14	47.45	45.87
v (m/day)	1.00	1.03	1.04	1.06
Φ	0.172	0.167	.0165	0.162

Observed Concentration [C(x,t)] at different space and time

t (day) ↓	X (m) →	0	1	2	3	4	5	6	7	8
2	Benzene	50.00	25.54	11.76	6.18	2.65	1.44	.59	.44	.20
	Toluene	50.00	27.89	14.03	7.97	3.85	2.26	.89	.73	.29
	Xylene	50.00	30.27	16.01	10.62	5.25	3.52	1.73	1.01	.49
4	Benzene	50.00	24.57	13.32	6.48	3.50	1.48	.89	.64	.17
	Toluene	50.00	26.73	15.55	8.23	4.77	2.27	1.50	.56	.44
	Xylene	50.00	28.91	18.98	9.88	6.87	3.50	2.39	1.29	.81
6	Benzene									
	Toluene									
	Xylene									

Parameters Computed: (fitting the observed concentration by nonlinear least square fit)

Compound	R	μ	γ
Benzene	1.11	0.80	0.00
Toluene	1.10	0.70	0.00
Xylene	1.07	0.60	0.00

## APPENDIX B-18

Expt. # BIO-2.3.2 (2)

Date 26/December/1994

Initial parameters:

Q = 15.21 L/day	v = 1 m/day	BTX = (50+50+50) mg/l	Oxygen:BTX = 3.2
-----------------	-------------	-----------------------	------------------

Change of hydraulic conductivity and velocity:

T (day) →	0	2	4	6
ΔH (cm)	2.70	2.80	2.85	
K (m/day)	50.07	48.29	47.44	
v (m/day)	1.00	1.01	1.02	
Φ	0.169	0.166	0.165	

Observed Concentration [C(x,t)] at different space and time

t (day) ↓	X (m) →	0	1	2	3	4	5	6	7	8
2	Benzene	50.00	25.32	11.20	6.32	2.28	1.66	.42	.43	.16
	Toluene	50.00	26.25	11.91	7.58	2.55	2.31	.54	.51	.22
	Xylene	50.00	28.47	13.81	8.68	3.71	3.44	.86	.84	.32
4	Benzene	50.00	24.37	13.48	5.62	3.78	1.21	1.01	.72	.20
	Toluene	50.00	24.63	14.01	5.65	4.08	1.09	1.23	.36	.26
	Xylene	50.00	26.70	16.83	7.41	6.17	1.62	1.96	.61	.33
6	Benzene									
	Toluene									
	Xylene									

Parameters Computed: (fitting the observed concentration by nonlinear least square fit)

Compound	R	μ	γ
Benzene	1.07	0.81	0.00
Toluene	1.13	0.72	0.00
Xylene	1.07	0.63	0.00

## Appendix C

# **SAS OUTPUTS**

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# APPENDIX C - 1

Analysis of Benzene Biodegradation Data with SAS procedure GLM 14:59 Sunday, April 9, 1995 1  
3 X 2 X 2 Unbalanced Factorial Design

## General Linear Models Procedure Class Level Information

Class	Levels	Values
V	3	1 2 4
O	2	1.5 3.2
C	2	10 50

Number of observations in data set = 18

Dependent Variable: First-order Rate Constant of Benzene

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	0.49720000	0.04520000	271.20	0.0001
Error	6	0.00100000	0.00016667		
Corrected Total	17	0.49820000			
R-Square					Y Mean
0.997993			Root MSE		0.48466667
		2.663675	0.01290994		

Source	DF	Type III SS	Mean Square	F Value	Pr > F
V	2	0.38021667	0.19010833	1140.65	0.0001
O	1	0.04271111	0.04271111	256.27	0.0001
C	1	0.00360000	0.00360000	21.60	0.0035
V*O	2	0.00310556	0.00155278	9.32	0.0145
V*C	2	0.00081667	0.00040833	2.45	0.1668
O*C	1	0.00010000	0.00010000	0.60	0.4680
V*O*C	2	0.00081667	0.00040833	2.45	0.1668

Means of the First-order Constant Used in the Regression Model 14:59 Sunday, April 9, 1995 3

# General Linear Models Procedure

Level of V	N	Mean	SD
------------	---	------	----

1	6	0.67800000	0.06723095
2	6	0.47633333	0.08518607
4	6	0.29966667	0.04308906

Level of O	N	Mean	SD
------------	---	------	----

1.5	9	0.42800000	0.15874508
3.2	9	0.54133333	0.17277153

Level of C	N	Mean	SD
------------	---	------	----

10	9	0.45244444	0.16561334
50	9	0.51688889	0.18030838

Level of V	Level of O	N	Mean	SD
------------	------------	---	------	----

1	1.5	3	0.62133333	0.02516611
1	3.2	3	0.73466667	0.03214550
2	1.5	3	0.40133333	0.03511885

2	3.2	3	0.55133333	0.00577350
4	1.5	3	0.26133333	0.01154701
4	3.2	3	0.33800000	0.01000000

Level of V	Level of C	N	Mean	SD
1	10	3	0.63800000	0.05291503
1	50	3	0.71800000	0.06082763
2	10	3	0.43800000	0.09643651
2	50	3	0.51466667	0.06658328
4	10	3	0.28133333	0.04163332
4	50	3	0.31800000	0.04358899

Level of O	Level of C	N	Mean	SD
1.5	10	6	0.41633333	0.15917496
1.5	50	3	0.45133333	0.19035055
3.2	10	3	0.52466667	0.18610033
3.2	50	6	0.54966667	0.18345753

Level of V	Level of O	Level of C	N	Mean	SD
1	1.5	10	2	0.60800000	0.01414214
1	1.5	50	1	0.64800000	.
1	3.2	10	1	0.69800000	.
1	3.2	50	2	0.75300000	0.00707107
2	1.5	10	2	0.38300000	0.02121320
2	1.5	50	1	0.43800000	.
2	3.2	10	1	0.54800000	.
2	3.2	50	2	0.55300000	0.00707107
4	1.5	10	2	0.25800000	0.01414214
4	1.5	50	1	0.26800000	.
4	3.2	10	1	0.32800000	.
4	3.2	50	2	0.34300000	0.00707107



# APPENDIX C-2

Analysis of Toluene Biodegradation Data with SAS procedure GLM 14:58 Sunday, April 9, 1995 1  
3 X 2 X 2 Unbalanced Factorial Design

General Linear Models Procedure  
Class Level Information

Class	Levels	Values
V	3	1 2 4
O	2	1.5 3.2
C	2	10 50

Number of observations in data set = 18

Dependent Variable: First-order Rate Constant of Toluene

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	0.41202778	0.03745707	374.57	0.0001
Error	6	0.00060000	0.00010000		
Corrected Total	17	0.41262778			
R-Square					
C.V.			Root MSE		Y Mean
0.998546		2.40488	0.01000000		0.41588889

Source	DF	Type III SS	Mean Square	F Value	Pr > F
V	2	0.30020556	0.15010278	1501.03	0.0001
O	1	0.02351111	0.02351111	235.11	0.0001
C	1	0.01440000	0.01440000	144.00	0.0001
V*O	2	0.00377222	0.00188611	18.86	0.0026
V*C	2	0.00301667	0.00150833	15.08	0.0046
O*C	1	0.00004444	0.00004444	0.44	0.5298
V*O*C	2	0.00087222	0.00043611	4.36	0.0677

Means of the First-order Constant Used in the Regression Model 14:58 Sunday, April 9, 1995 3

# General Linear Model's Procedure

Level of V .....Y.....  
N Mean SD

1 6 0.59033333 0.07111024  
2 6 0.40700000 0.07968689  
4 6 0.25033333 0.04020779

Level of O .....Y.....  
N Mean SD

1.5 9 0.36755556 0.14638230  
3.2 9 0.46422222 0.15777973

Level of C .....Y.....  
N Mean SD

10 9 0.37311111 0.13869431  
50 9 0.45866667 0.16800298

Level of V Level of O .....Y.....  
N Mean SD

1 1.5 3 0.54533333 0.04932883  
1 3.2 3 0.63533333 0.06429101

2	1.5	3	0.33866667	0.02081666
2	3.2	3	0.47533333	0.03785939
4	1.5	3	0.21866667	0.03055050
4	3.2	3	0.28200000	0.01000000

Level of V	Level of C	N	Mean	SD
1	10	3	0.53200000	0.02645751
1	50	3	0.64866667	0.04163332
2	10	3	0.36200000	0.06082763
2	50	3	0.45200000	0.07810250
4	10	3	0.22533333	0.04163332
4	50	3	0.27533333	0.02081666

Level of V	Level of C	N	Mean	SD
0				
1.5	10	6	0.34866667	0.14207979
1.5	50	3	0.40533333	0.17897858
3.2	10	3	0.42200000	0.14525839
3.2	50	6	0.48533333	0.17258814

Level of V	Level of O	Level of C	N	Mean	SD
1	1.5	10	2	0.51700000	0.00707107
1	1.5	50	1	0.60200000	.
1	3.2	10	1	0.56200000	.
1	3.2	50	2	0.67200000	0.01414214
2	1.5	10	2	0.32700000	0.00707107
2	1.5	50	1	0.36200000	.
2	3.2	10	1	0.43200000	.
2	3.2	50	2	0.49700000	0.00707107
4	1.5	10	2	0.20200000	0.01414214
4	1.5	50	1	0.25200000	.
4	3.2	10	1	0.27200000	.
4	3.2	50	2	0.28700000	0.00707107

# APPENDIX C-3

Analysis of Xylene Biodegradation Data with SAS procedure GLM 15:05 Sunday, April 9, 1995 1  
3 X 2 X 2 Unbalanced Factorial Design

## General Linear Models Procedure Class Level Information

Class	Levels	Values
V	3	1 2 4
O	2	1.5 3.2
C	2	10 50

Number of observations in data set = 18

Dependent Variable: First-order rate Constant of Xylene

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	0.31340000	0.02849091	201.11	0.0001
Error	6	0.00085000	0.00014167		
Corrected Total	17	0.31425000			
R-Square					Y Mean
0.997295			Root MSE		0.35700000
		3.334000	0.01190238		

Source	DF	Type III SS	Mean Square	F Value	Pr > F
V	2	0.23180556	0.11590278	818.14	0.0001
O	1	0.02006944	0.02006944	141.67	0.0001
C	1	0.01322500	0.01322500	93.35	0.0001
V*O	2	0.00087222	0.00043611	3.08	0.1202
V*C	2	0.00285000	0.00142500	10.06	0.0121
O*C	1	0.00122500	0.00122500	8.65	0.0259
V*O*C	2	0.00071667	0.00035833	2.53	0.1597

Means of the First-order Constant Used in the Regression Model 15:05 Sunday, April 9, 1995 3

# General Linear Models Procedure

Level of V	N	Mean	SD
------------	---	------	----

1	6	0.50866667	0.07033254
2	6	0.34533333	0.07393691
4	6	0.21700000	0.03391165

Level of O	N	Mean	SD
------------	---	------	----

1.5	9	0.31200000	0.11905881
3.2	9	0.40200000	0.14335271

Level of C	N	Mean	SD
------------	---	------	----

10	9	0.31644444	0.11769782
50	9	0.39755556	0.14740345

Level of V	Level of O	N	Mean	SD
------------	------------	---	------	----

1	1.5	3	0.45533333	0.04163332
1	3.2	3	0.56200000	0.04582576

2	1.5	3	0.29200000	0.02645751
2	3.2	3	0.39866667	0.06658328
4	1.5	3	0.18866667	0.00577350
4	3.2	3	0.24533333	0.02081666

Level of V	Level of C	N	Mean	SD
1	10	3	0.45866667	0.04725816
1	50	3	0.55866667	0.05131601
2	10	3	0.29200000	0.02645751
2	50	3	0.39866667	0.06658328
4	10	3	0.19866667	0.02081666
4	50	3	0.23533333	0.03785939

Level of O	Level of C	N	Mean	SD
1.5	10	6	0.29866667	0.11111556
1.5	50	3	0.33866667	0.15567059
3.2	10	3	0.35200000	0.14730920
3.2	50	6	0.42700000	0.14815532

Level of V	Level of O	Level of C	N	Mean	SD
1	1.5	10	2	0.43200000	0.01414214
1	1.5	50	1	0.50200000	.
1	3.2	10	1	0.51200000	.
1	3.2	50	2	0.58700000	0.02121320
2	1.5	10	2	0.27700000	0.00707107
2	1.5	50	1	0.32200000	.
2	3.2	10	1	0.32200000	.
2	3.2	50	2	0.43700000	0.00707107
4	1.5	10	2	0.18700000	0.00707107
4	1.5	50	1	0.19200000	.
4	3.2	10	1	0.22200000	.
4	3.2	50	2	0.25700000	0.00707107

# APPENDIX C-4

Analysis of Benzene Biodegradation Data with SAS procedure GLM  
3 X 2 X 2 Unbalanced Factorial Design

13:50 Tuesday, May 2, 1995 1

## General Linear Models Procedure Class Level Information

Class	Levels	Values
V	3	1 2 4
O	2	1.5 3.2
C	2	10 50

Number of observations in data set = 18

Dependent Variable: First-order Rate Constant of Benzene

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	0.49628333	0.06203542	291.30	0.0001
Error	9	0.00191667	0.00021296		
Corrected Total	17	0.49820000			

R-Square	C.V.	Root MSE	Y Mean
0.996153	3.010987	0.01459325	0.48466667

Source	DF	Type III SS	Mean Square	F Value	Pr > F
V	2	0.43003333	0.21501667	1009.64	0.0001
O	1	0.04271111	0.04271111	200.56	0.0001
C	1	0.00360000	0.00360000	16.90	0.0026
V*O	2	0.00310556	0.00155278	7.29	0.0131
V*C	2	0.00081667	0.00040833	1.92	0.2025

# APPENDIX C-5

Analysis of Benzene Biodegradation Data with SAS procedure GLM 10:56 Wednesday, May 3, 1995 1  
3 X 2 X 2 Unbalanced Factorial Design

## General Linear Models Procedure Class Level Information

Class	Levels	Values
V	3	1 2 4
O	2	1.5 3.2
C	2	10 50

Number of observations in data set = 18

## Dependent Variable: First-order Rate Constant of Benzene

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.49143333	0.12285833	236.03	0.0001
Error	13	0.00676667	0.00052051		
Corrected Total	17	0.49820000			

R-Square		C.V.	Root MSE	Y Mean
0.986418	4.707307		0.02281475	0.48466667

Source	DF	Type III SS	Mean Square	F Value	Pr > F
V	2	0.43003333	0.21501667	413.09	0.0001
O	1	0.04271111	0.04271111	82.06	0.0001
C	1	0.00360000	0.00360000	6.92	0.0208



# APPENDIX C-6

Analysis of Benzene Biodegradation Data with SAS procedure GLM 12:24 Wednesday, May 3, 1995 1  
3 X 2 X 2 Unbalanced Factorial Design

## General Linear Models Procedure Class Level Information

Class	Levels	Values
V	3	1 2 4
O	2	1.5 3.2
C	2	10 50

Number of observations in data set = 18

## General Linear Models Procedure

Dependent Variable: Biodegradation Rate Of Benzene

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	0.43003333	0.21501667	47.31	0.0001
Error	15	0.06816667	0.00454444		
Corrected Total	17	0.49820000			

R-Square	C.V.	Root MSE	Y Mean
0.863174	13.90904	0.06741249	0.48466667

Source	DF	Type III SS	Mean Square	F Value	Pr > F
V	2	0.43003333	0.21501667	47.31	0.0001

# APPENDIX C-7

Analysis of Benzene Biodegradation Data with SAS procedure GLM 13:35 Saturday, May 6, 1995 1  
Linear Biodegradation rate model as  $\mu = f(V, O, C)$

## General Linear Models Procedure

Number of observations in data set = 18

Dependent Variable: Biodegradation Rate Of Benzene

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	0.46941429	0.15647143	76.10	0.0001
Error	14	0.02878571	0.00205612		
Corrected Total	17	0.49820000			
R-Square					
C.V.					
Root MSE					
		9.355809	0.04534449		Y Mean
		0.942221			0.48466667

Source	DF	Type I SS	Mean Square	F Value	Pr > F
V	1	0.40801429	0.40801429	198.44	0.0001
O	1	0.05780000	0.05780000	28.11	0.0001
C	1	0.00360000	0.00360000	1.75	0.2070
Source	DF	Type III SS	Mean Square	F Value	Pr > F
V	1	0.40801429	0.40801429	198.44	0.0001
O	1	0.04271111	0.04271111	20.77	0.0004
C	1	0.00360000	0.00360000	1.75	0.2070

Parameter	Estimate	T for H0: Parameter=0	Pr >  T	Std Error of Estimate
-----------	----------	--------------------------	---------	--------------------------

INTERCEPT	0.6009901961	15.89	0.0001	0.03781730
V	-.1207142857	-14.09	0.0001	0.00856930
O	0.0607843137	4.56	0.0004	0.01333661
C	0.0007500000	1.32	0.2070	0.00056681

Observation	Observed Value	Predicted Value	Residual
1	0.59800000	0.57895238	0.01904762
2	0.61800000	0.57895238	0.03904762
3	0.69800000	0.68228571	0.01571429
4	0.36800000	0.45823810	-0.09023810
5	0.39800000	0.45823810	-0.06023810
6	0.54800000	0.56157143	-0.01357143
7	0.24800000	0.21680952	0.03119048
8	0.26800000	0.21680952	0.05119048
9	0.32800000	0.32014286	0.00785714
10	0.64800000	0.60895238	0.03904762
11	0.74800000	0.71228571	0.03571429
12	0.75800000	0.71228571	0.04571429
13	0.43800000	0.48823810	-0.05023810
14	0.54800000	0.59157143	-0.04357143
15	0.55800000	0.59157143	-0.03357143
16	0.26800000	0.24680952	0.02119048
17	0.33800000	0.35014286	-0.01214286
18	0.34800000	0.35014286	-0.00214286

Sum of Residuals  
 Sum of Squared Residuals  
 Sum of Squared Residuals - Error SS  
 First Order Autocorrelation  
 Durbin-Watson D

0.00000000  
 0.02878571  
 -0.00000000  
 0.40001182  
 1.18721297

# APPENDIX C-8

Analysis of Benzene Biodegradation Data with SAS procedure GLM 14:27 Thursday, May 18, 1995 1  
Linear Biodegradation rate model as  $\mu = f(V, O, C, V*V)$

## General Linear Models Procedure

Number of observations in data set = 18

Dependent Variable: Biodegradation rate of benzene

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.49143333	0.12285833	236.03	0.0001
Error	13	0.00676667	0.00052051		
Corrected Total	17	0.49820000			
R-Square		C.V.	Root MSE		Y Mean
0.986418		4.707307	0.02281475		0.48466667

Source	DF	Type I SS	Mean Square	F Value	Pr > F
V	1	0.40801429	0.40801429	783.87	0.0001
O	1	0.05780000	0.05780000	111.04	0.0001
C	1	0.00360000	0.00360000	6.92	0.0208
V*V	1	0.02201905	0.02201905	42.30	0.0001
Source	DF	Type III SS	Mean Square	F Value	Pr > F
V	1	0.05670000	0.05670000	108.93	0.0001
O	1	0.04271111	0.04271111	82.06	0.0001
C	1	0.00360000	0.00360000	6.92	0.0208
V*V	1	0.02201905	0.02201905	42.30	0.0001

T for H0: Pr > [T] Std Error of

Parameter	Estimate	Parameter=0	Estimate
INTERCEPT	0.7898790850	22.75	0.03471987
V	-.3150000000	-10.44	0.03018108
O	0.0607843137	9.06	0.00671022
C	0.0007500000	2.63	0.00028518
V*V	0.0377777778	6.50	0.00580835

Observation	Observed Value	Predicted Value	Residual
1	0.59800000	0.61133333	-0.01333333
2	0.61800000	0.61133333	0.00666667
3	0.69800000	0.71466667	-0.01666667
4	0.36800000	0.40966667	-0.04166667
5	0.39800000	0.40966667	-0.01166667
6	0.54800000	0.51300000	0.03500000
7	0.24800000	0.23300000	0.01500000
8	0.26800000	0.23300000	0.03500000
9	0.32800000	0.33633333	-0.00833333
10	0.64800000	0.64133333	0.00666667
11	0.74800000	0.74466667	0.00333333
12	0.75800000	0.74466667	0.01333333
13	0.43800000	0.43966667	-0.00166667
14	0.54800000	0.54300000	0.00500000
15	0.55800000	0.54300000	0.01500000
16	0.26800000	0.26300000	0.00500000
17	0.33800000	0.36633333	-0.02833333
18	0.34800000	0.36633333	-0.01833333

Sum of Residuals  
Sum of Squared Residuals  
Sum of Squared Residuals - Error SS  
First Order Autocorrelation  
Durbin-Watson D

0.00000000  
0.00676667  
-0.00000000  
0.27175698  
1.38054187

# APPENDIX C-9

Analysis of Benzene Retardation Constant by SAS procedure GLM 16:11 Sunday, April 9, 1995 1  
3 X 2 X 2 Unbalanced Factorial Design

## General Linear Models Procedure Class Level Information

Class	Levels	Values
V	3	1 2 4
O	2	1.5 3.2
C	2	10 50

Number of observations in data set = 18

## Dependent Variable: Retardation Constant of Benzene

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	0.00484444	0.00044040	0.65	0.7449
Error	6	0.00405000	0.00067500		
Corrected Total	17	0.00889444			
R-Square		C.V.	Root MSE		R Mean
0.544660		2.429370	0.02598076		1.06944444

Source	DF	Type III SS	Mean Square	F Value	Pr > F
V	2	0.00190556	0.00095278	1.41	0.3145
O	1	0.00000278	0.00000278	0.00	0.9509
C	1	0.00000278	0.00000278	0.00	0.9509
V*O	2	0.00117222	0.00058611	0.87	0.4664
V*C	2	0.00137222	0.00068611	1.02	0.4167
O*C	1	0.00033611	0.00033611	0.50	0.5069
V*O*C	2	0.00010556	0.00005278	0.08	0.9257

# APPENDIX C-10

Analysis of Variance of Plate Count Data with SAS procedure GLM 15:01 Sunday, April 9, 1995 1  
3 X 2 X 2 unbalanced Factorial Design

## General Linear Models Procedure Class Level Information

Class	Levels	Values
V	3	1 2 4
O	2	1.5 3.2
C	2	10 50

Number of observations in data set = 18

## Dependent Variable: Cell Count of the Mixed Species

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	2483.1227778	225.73843434	128.26	0.0001
Error	6	10.56000000	1.76000000		
Corrected Total	17	2493.6827778			
R-Square					
C.V.			Root MSE		Y Mean
0.995765		8.636419	1.32664992		15.36111111



Source	DF	Type III SS	Mean Square	F Value	Pr > F
V	2	706.03166667	353.01583333	200.58	0.0001
O	1	53.29000000	53.29000000	30.28	0.0015
C	1	778.41000000	778.41000000	442.28	0.0001
V*O	2	21.71166667	10.85583333	6.17	0.0350
V*C	2	319.07166667	159.53583333	90.65	0.0001
O*C	1	18.77777778	18.77777778	10.67	0.0171
V*O*C	2	29.04055556	14.52027778	8.25	0.0190

Means of the Plate Count Data Used in the Regression Model 15:01 Sunday, April 9, 1995 3

General Linear Models Procedure

Level of V .....Y.....  
N Mean SD

1 6 24.9333333 16.4414922  
2 6 13.1000000 5.8134327  
4 6 8.0500000 3.7930199

Level of O .....Y.....  
N Mean SD

1.5 9 11.2111111 8.1948832  
3.2 9 19.5111111 14.3458569

Level of C .....Y.....  
N Mean SD

10 9 7.7777778 2.6484796  
50 9 22.9444444 13.2402899

Level of V Level of O .....Y.....  
N Mean SD

1 1.5 3 17.0666667 11.8077658  
1 3.2 3 32.8000000 18.7277868  
2 1.5 3 10.3666667 5.2443621

2	3.2	3	15.8333333	5.879093
4	1.5	3	6.2000000	3.3955854
4	3.2	3	9.9000000	3.7643060

Level of V	Level of C	N	Mean	SD
1	10	3	10.5666667	0.56862407
1	50	3	39.3000000	7.50199973
2	10	3	8.0000000	1.21243557
2	50	3	18.2000000	2.23383079
4	10	3	4.7666667	0.96090235
4	50	3	11.3333333	1.64418166

Level of V	Level of C	N	Mean	SD
0				
1.5	10	6	7.2833333	2.7095510
1.5	50	3	19.0666667	10.5557251
3.2	10	3	8.7666667	2.7392213
3.2	50	6	24.8833333	14.9126009

Level of V	Level of O	Level of C	N	Mean	SD
1	1.5	10	2	10.2500000	0.21213203
1	1.5	50	1	30.7000000	.
1	3.2	10	1	11.2000000	.
1	3.2	50	2	43.6000000	1.27279221
2	1.5	10	2	7.3500000	0.63639610
2	1.5	50	1	16.4000000	.
2	3.2	10	1	9.3000000	.
2	3.2	50	2	19.1000000	2.26274170
4	1.5	10	2	4.2500000	0.49497475
4	1.5	50	1	10.1000000	.
4	3.2	10	1	5.8000000	.
4	3.2	50	2	11.9500000	1.76776695

# APPENDIX C-11

Analysis of Effective Porosity Data with SAS procedure GLM 15:08 Sunday, April 9, 1995 1  
 3 X 2 X 2 Unbalanced Factorial Design

## General Linear Models Procedure Class Level Information

Class	Levels	Values
V	3	1 2 4
O	2	1.5 3.2
C	2	10 50

Number of observations in data set = 18

## Dependent Variable: Effective Porosity of the Medium

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	0.04278035	0.00388912	6.80	0.0142
Error	6	0.00343194	0.00057199		
Corrected Total	17	0.04621229			
R-Square			Root MSE		Y Mean
0.925735			0.02391631		0.20741667

Source	DF	Type III SS	Mean Square	F Value	Pr > F
V	2	0.00570204	0.00285102	4.98	0.0530
O	1	0.00230400	0.00230400	4.03	0.0915
C	1	0.01712608	0.01712608	29.94	0.0016
V*O	2	0.00065367	0.00032684	0.57	0.5927
V*C	2	0.00452196	0.00226098	3.95	0.0803
O*C	1	0.00236196	0.00236196	4.13	0.0884
V*O*C	2	0.00058136	0.00029068	0.51	0.6253

# APPENDIX C-12

Analysis of Porosity and Plate Count with SAS procedure GLM 15:14 Sunday, April 9, 1995 1

## General Linear Models Procedure Class Level Information

Class	Levels	Values
COUNT	4	HIGH LOW MEDIUM VERYHIGH

Number of observations in data set = 18

## Dependent Variable: Effective Porosity of the Sand

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	0.01982178	0.00660726	3.51	0.0439
Error	14	0.02637666	0.00188405		
Corrected Total	17	0.04619844			

R-Square	C.V.	Root MSE	PHI Mean
0.429057	20.92565	0.04340561	0.20742778

Source	DF	Type III SS	Mean Square	F Value	Pr > F
COUNT	3	0.01982178	0.00660726	3.51	0.0439