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**Biodegradation of BTEX under Electron Acceptor and Nutrient
Limiting Conditions**

by

Lorie Cummings

A thesis submitted under the supervision of Dr. Wayne Parker

Department of Civil and Environmental Engineering

Carleton University,

Ottawa, Ontario

Presented to Ottawa-Carleton Institute for Environmental Engineering

in partial fulfillment of the requirements for the degree of

Master of Applied Science in Environmental Engineering

December 2001

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Department of Civil and Environmental Engineering

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**Biodegradation of BTEX under Electron Acceptor and Nutrient
Limiting Conditions**

Submitted by

Lorie Cummings

in partial fulfillment of the requirements for the degree of
Master of Applied Science in Environmental Engineering

A handwritten signature in black ink, appearing to read "Wayne Parker", is written over a solid horizontal line.

Chair, Department of Civil and Environmental Engineering

Supervisor, Dr. Wayne Parker

Carleton University,

Ottawa, Ontario

December 2001

Abstract

The biodegradation of BTEX compounds under aerobic, nitrate-reducing and mixed aerobic/nitrate-reducing conditions was investigated in a laboratory scale batch reactor. Ammonium-nitrogen limitation within the feed/nutrient solution led to the dual role of nitrate as an electron acceptor and as a source of assimilative nitrogen.

The lack of ammonium-nitrogen hindered both growth and BTEX removal when nitrate was unavailable for assimilation. Where nitrate was added, under high oxygen conditions, it was utilized primarily for synthesis. Evidence of nitrate utilization as an electron acceptor was observed at low oxygen levels.

A model to simulate BTEX biodegradation and to evaluate microbial activity was developed using Monod kinetics. Multiple saturation and inhibition terms were added to address the effects of electron acceptor and nutrient concentrations on growth.

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1. INTRODUCTION

1.1 Background

Petroleum hydrocarbons in groundwater systems is a widespread problem resulting from spills due to leaking fuel tanks, transportation, storage, and handling of fuels. A portion of the fuel is made up of monoaromatic compounds: benzene, toluene, ethylbenzene and three isomers *meta*-, *ortho*-, and *para*-xylene, otherwise known as BTEX. These compounds are of particular concern due to their recalcitrance to decay in the environment and their toxicity to human life. Benzene, specifically, is considered a human carcinogen (Chapelle, 1992).

Due to their high solubility compared to other hydrocarbons, BTEX have been found to account for most of the dissolved fraction of hydrocarbons in the water (Chapelle, 1992). As a result, their impact on groundwater and, hence, drinking water may be significant.

BTEX compounds are known to have a very stable structure, which is difficult for some microorganisms to utilize for metabolism. However, in recent research bacteria have demonstrated the ability to degrade BTEX compounds (Chapelle, 1992). This has led to the development of remediation methods that capitalize on this biological process.

Currently, there are two main bioremediation strategies to treat contaminated groundwater: enhanced remediation where the addition of nutrients and electron acceptors stimulates biodegradation processes or natural attenuation where the natural process is documented and monitored. To evaluate the potential for either of these

processes at a given site, it is important to develop a model to characterize the biodegradation of BTEX contaminants.

1.2 Objectives and Scope of Study

The main objective of this study was to investigate the biodegradation of BTEX compounds under aerobic, nitrate-reducing and mixed aerobic/nitrate-reducing conditions. A series of bench scale experiments were performed under a range of electron acceptor conditions. Due to a lack of ammonium-nitrogen within the nutrient solution, nitrate was a potential source of nitrogen for synthesis. A portion of this study focuses on nitrogen limitation and the dual role of nitrate as electron acceptor and assimilative nitrogen.

Initially, it was intended to develop a model that described the impact of electron acceptor conditions on the kinetics of BTEX biodegradation. However, nitrogen limitation made the system somewhat more complex. As a result, a model was developed to describe the input of nitrogen limitations and electron acceptor concentrations on microbial activity and BTEX biodegradation.

2. LITERATURE REVIEW

2.1 Microbial Growth

2.1.1 Energy Requirements

Oxidation-Reduction Reactions

Microorganisms obtain chemical energy for metabolism through oxidation-reduction (redox) reactions. This process involves the transfer of electrons between two substances: an electron donor and an electron acceptor. The electron donor undergoes oxidation, which is defined as the removal of an electron or electrons from a substance. The electron acceptor undergoes reduction, which is defined as the addition of an electron or electrons to a substance (Madigan et al., 1996).

The potential energy available from the redox reaction is dependent upon the particular electron donor and electron acceptor and their tendency to donate or accept electrons. The reduction potential, E_o , is an expression of this tendency. It is measured electrically in reference to a standard substance, H_2 . Values of E_o , which are more negative, indicate a preference to donate electrons, while a more positive value of E_o indicates a preference to accept electrons. The difference in reduction potentials, ΔE_o , between electron acceptor-donor pairs is proportional to the free energy of the redox reaction. Hence, the greater the difference between the values of E_o of the two substances, the greater the energy released from their reaction (Madigan et al., 1996).

Electron Donors in the Subsurface

In soil environments, typical substances that act as electron donors are low molecular weight organic compounds. These carbon-containing substances are present in reduced forms. Through the biodegradation process they are converted to an oxidized state, typically carbon dioxide (CO_2). Trace contaminants may also participate in redox reactions as electron donors.

Electron Acceptors in the Subsurface

In natural environments, inorganic compounds such as oxygen (O_2), nitrate (NO_3^-), and sulfate (SO_4^{2-}) are potential electron acceptors. While several of these substances may be present, microorganisms tend to utilize them in order of decreasing energy. O_2 is a widely used electron acceptor due to its high energy yield. Its reduction potential is more negative in comparison with other possible electron acceptors. An environment with substantial amounts of O_2 present is said to be aerobic. If O_2 is depleted, anaerobic conditions prevail. Nitrate is energetically the best electron acceptor under anaerobic conditions. Microorganisms will preferentially use NO_3^- until its supply is almost, or completely, depleted. A NO_3^- enhanced environment is specifically known as anoxic. The use of NO_3^- is followed by ferric iron (Fe^{3+}), manganese (Mg^{4+}), sulfate (SO_4^{2-}) and, finally CO_2 (Krumholz et al., 1996).

Due to this sequential consumption of electron acceptors, discrete redox zones are formed within the subsurface (Baedecker et al., 1993). Potential electron acceptors and their corresponding redox zones are presented in Table 2-1.

Table 2-1: Electron acceptors within the subsurface.

Decreasing Order of Energy Yield (Less negative reduction potential, E_0) ↓	Electron Acceptor	Reduced Product	Redox Zone	
	O_2	H_2O	Aerobic	
	NO_3^-	N_2	Anaerobic	Nitrate- Reducing (Anoxic)
	Fe^{3+}	Fe^{2+}		Iron- Reducing
	Mn^{4+}	Mn^{2+}		Manganese- Reducing
	SO_4^{2-}	S^{2-}		Sulfate- Reducing
	CO_2	CH_4		Methanogenic

2.1.2 Nutrient Requirements

Microorganisms require nutrients to build new cells. These nutrients are obtained from the environment and transformed into cell constituents. The two most abundant elements in a bacterial cell are carbon and nitrogen. In the subsurface, carbon dioxide or organic compounds, which may also act as electron donors, provide sources of carbon. Nitrogen may also be found in organic compounds. However, inorganic forms of nitrogen, such as ammonium (NH_4^+) and nitrate (NO_3^-), are more prevalent (Madigan et al., 1996). Many bacteria can utilize either substance as a source for nitrogen. However, microorganisms preferentially use NH_4^+ . Nitrogen is required to form the amino group of amino acids in proteins. The nitrogen within these amino groups ($R-NH_2$) is in a reduced state. Ammonium is already in a reduced state and, hence, easily incorporated into newly synthesized proteins. Nitrate, in contrast, is in a more oxidized state. Energy would be

spent reducing it, prior to synthesis (Widdowson et al., 1988).

Other nutrients required in trace amounts include phosphorus, sulfur, magnesium, calcium, sodium and iron (Madigan et al., 1996).

2.1.3 Microbial Diversity

In the natural environment, a large number of microbial species are present. These species are categorized according to the substances that are utilized to meet their energy and nutritional requirements.

Heterotrophic bacteria utilize organic compounds as their source of carbon and energy. Heterotrophic growth utilizing O_2 as the electron acceptor is known as aerobic respiration. Bacteria, which utilize this method for growth, are known as aerobes. Heterotrophs may also use NO_3^- as an electron acceptor. This anaerobic process is called denitrification. Denitrifying bacteria reduce NO_3^- to N_2O and then to N_2 . Facultative aerobes are microorganisms that can utilize both O_2 and NO_3^- as electron acceptors (Madigan et al., 1996). Other types of heterotrophs utilize ferric iron (Fe^{3+}), manganese (Mg^{4+}), sulfate (SO_4^{2-}) and CO_2 as electron acceptors in conjunction with the organic compound for carbon and energy.

Autotrophs utilize inorganic compounds as electron donors. Typically CO_2 is their sole source of carbon. Autotrophic growth generally occurs under aerobic conditions with O_2 acting as an electron donor. Nitrification is one example of autotrophic growth. This is a

two step process in which ammonia (NH_3) is oxidized to nitrite (NO_2^-) and subsequently nitrate (NO_3^-) (Madigan et al., 1996).

2.2 BTEX Biodegradation

2.2.1 Target Compounds

Benzene, toluene, ethylbenzene and the three isomers *meta*-, *ortho*- and *para*-xylene, otherwise known as BTEX, are the targeted compounds of this research. These monoaromatic compounds are characterized by the presence of a ring of 6 carbon atoms joined by 3 single and 3 double bonds, with and without side groups. This forms a very stable structure, which is difficult for some microorganisms to utilize as a carbon and energy source. However, research has demonstrated that several bacteria have developed pathways for degrading BTEX compounds (Chapelle, 1992).

The biodegradation of BTEX compounds varies within the different redox zones of the natural environment. It is dependent upon the microorganisms' ability to oxidize BTEX with the available electron acceptors. In order to understand the nature of biodegradation within the subsurface, it is necessary to examine the degradation of each BTEX compound within each redox zone, as well as in the transitional stage between zones.

2.2.2 Aerobic Biodegradation

Aerobic biodegradation of BTEX has been well established over the years. Numerous field and laboratory studies (Alvarez et al., 1991, Alvarez and Vogel, 1991, Bayly and Barbour, 1984, Deutz et al., 1994, Durant et al., 1995, Haigler et al., 1992, and Mikesell et al., 1993) have demonstrated the removal of BTEX under aerobic conditions. In addition, several bacteria have been isolated which grow on one or more of the BTEX compounds in the presence of oxygen. These are outlined in Table 2-2.

Table 2-2: Microbial species which have been reported to biodegrade BTEX compounds under aerobic conditions (Riser-Roberts, 1998).

Compound	Microbial Species
Benzene	<i>Pseudomonas putida</i> , <i>P. aeruginosa</i> , <i>P. gladioli</i> , <i>Methylosinus trichosporium</i> OB3b, <i>Nocardia</i> sp, <i>Flavobacterium</i> DS-711, and <i>Selanastrum capricornatu</i>
Toluene	<i>Bacillus</i> sp., <i>Pseudomonas putida</i> , <i>Cunninghamella elegans</i> , <i>P. Aeruginosa</i> , <i>P. mildenbergii</i> , <i>Methylosinus trichosporium</i> OB3b, <i>P. anaerooleophila</i> , <i>Nocardia corallina</i> , <i>P. gladioli</i> and <i>Phanerochaete chrysosporium</i>
Ethylbenzene	<i>Pseudonomas putida</i> and <i>Nocardia</i> sp.
m-, o- and p-Xylenes	<i>Pseudomonas putida</i> , <i>P. aeruginosa</i> , <i>Phanerochaete chrysosporium</i> , and <i>Nocardia</i> sp

2.2.3 Anaerobic Biodegradation

It was originally believed that BTEX compounds were recalcitrant to anaerobic degradation due to the lack of molecular oxygen in their structures. Recently, however, several studies have successfully demonstrated the biodegradation of the BTEX compounds under anaerobic conditions.

Benzene

Benzene is the most recalcitrant of the BTEX compounds in the absence of oxygen. Most research to date, including field (Gillham et al, 1990, Reinhard et al., 1984, Acton and Barker, 1992, Barbaro et al., 1992) and laboratory (Hutchins et al., 1991, Rueter et al., 1994, Kao and Borden, 1997, and Alvarez and Vogel, 1995) studies, has

demonstrated no reduction of benzene under anaerobic conditions. Until recently, it was believed to be completely resistant to anaerobic biodegradation. However, a few reports have suggested that it is possible to degrade benzene with electron acceptors other than oxygen.

Morgan et al. (1993) reported benzene removal from groundwater samples under nitrate-reducing conditions. However, oxygen within the groundwater was not removed prior to the tests and hence, the conclusions of anaerobic degradation are suspect. This is a common source of contention in investigations that suggest anaerobic degradation has occurred (Krumholz et al., 1996). However, Major et al. (1988) demonstrated the biodegradation of benzene using nitrate as the electron acceptor. Within 30 days, benzene was removed by a microcosm from an aquifer in Borden, Ontario. In this study strict attention was given to preventing oxygen contamination within the experiment.

Under iron (III) reducing conditions, Lovley et al. (1996) found that benzene could be quickly degraded in sediments containing iron oxides if a metal chelator was added. The fast degradation was attributed to solubilization of the iron, which made it more readily available to the microorganisms. Baedeker et al. (1993) have shown a 98% reduction of benzene coupled with an increase in the level of dissolved Fe(II) without the addition of any metal chelators. Anderson et al. (1998) were less successful at demonstrating benzene degradation under iron-reducing conditions although the importance of microbial populations was indicated in their work. They found benzene degradation in sediments from only one of three petroleum-contaminated aquifers evaluated. While all

three sites contained iron-reducing microorganisms related to *Geothrix fermentans*, the site where benzene degradation was observed contained a large number of microorganisms from the *Geobacteraceae* family.

Lovley et al. (1994) provided one of the first examples of anaerobic benzene oxidation in a natural sediment. Cultures taken from San Diego Bay, California sediments biodegraded a ^{14}C -benzene tracer to $^{14}\text{CO}_2$ under sulfate-reducing conditions. The sulfate reduction was stoichiometrically consistent with the depletion of benzene indicating that sulfate was the primary electron acceptor. Edwards and Grbic-Galic (1992) and Caldwell and Suflita (1995) also demonstrated benzene degradation using sediments from Seal Beach, California. However, both groups had difficulty linking degradation to the consumption of sulfate.

In heavily contaminated aquifers, alternative electron acceptors may become depleted and the degradation of benzene under methanogenic conditions can be important. Weiner and Lovley (1998) provided one of the first reports of conversion of ^{14}C benzene to $^{14}\text{CH}_4$ and $^{14}\text{CO}_2$ in methanogenic sediments. No lag period during their investigations suggested that the microorganisms were already adapted to benzene. The site had a long history of benzene contamination, which may be an important factor in understanding its methanogenic degradation.

Toluene

Unlike benzene, toluene is known to be biodegradable under a wide variety of anaerobic conditions (Krumholz et al., 1996). The ability of microorganisms to degrade toluene under nitrate-reducing conditions was first shown in laboratory columns of aquifer material (Kuhn et al., 1985, Zeyer et al., 1986). Since then, nitrate has been used to stimulate toluene removal at a number of contaminated areas (Aelion and Bradley, 1991, Barbaro et al., 1992, and Morgan et al., 1993). It appears that toluene-degrading, nitrate-reducing microorganisms are prevalent in a wide variety of soils, sediments and groundwaters (Krumholz et al., 1996).

Toluene degradation has also been observed in aquifer sediments under iron-reducing conditions (Lovley et al., 1989). Similar to benzene, toluene oxidation coupled to iron reduction could be enhanced with the addition of chelators (Lovley et al., 1994).

Berwanger and Barker (1988) were one of the first groups to demonstrate toluene degradation under sulfate-reducing conditions. Sediments impacted with landfill leachate from Gloucester, Ontario demonstrated the biodegradation of toluene over 159 days at which time, the sulfate had depleted or hydrogen sulfide had reached inhibitory levels. The oxidation of toluene by sulfate has also been reported in soils contaminated with aviation fuel (Beller et al., 1991) and gasoline (Edwards et al., 1992).

Under methanogenic conditions, evidence of the degradation of toluene has been less consistent. Wilson et al. (1986b) reported the disappearance of toluene in aquifer slurries

from Norman, Oklahoma, although rates of decay were relatively slow. Others (Nielsen et al., 1992 and Reinhard et al., 1984) have not observed toluene degradation in methanogenic areas.

Ethylbenzene

Evidence for the removal of ethylbenzene under anaerobic conditions has been inconsistent. In the presence of nitrate, ethylbenzene degradation has been reported in tests with aquifer material (Hutchins, 1991, Hutchins, 1992, Jensen and Arvin, 1994, and Morgan et al., 1993). In all of these cases, the initial concentration of ethylbenzene was very small (less than 200 μM) and complete depletion was extremely rapid. In contrast, Barbaro et al. (1992) saw only a 30% removal of the substrate over 1.5 years using microcosms from a Borden aquifer. Also, Alvarez and Vogel (1994) witnessed no ethylbenzene degradation within 11 weeks from four different aquifer sediments under denitrifying conditions.

No laboratory studies illustrating ethylbenzene removal with sulfate as an electron acceptor were found in the literature. A few field studies (Thierrin et al., 1993, Acton and Barker, 1992, and Piet and Smeenk, 1985), have reported its biodegradation under sulfate-reducing conditions. However, it is difficult to assess redox zones in the field and it is possible that other electron acceptors may be active within a site. Therefore, without substantial confirmation from laboratory experimentation, it is difficult to verify that ethylbenzene will be degraded in a sulfate-reducing environment.

Similarly, the biodegradation of ethylbenzene under methanogenic conditions has not been fully proven. Of the few studies found in the literature addressing this situation, evidence from laboratory and field work has been both for (Wilson et al., 1936b, Grbic-Galic, 1986) and against (Berwanger and Barker, 1988, Reinhard et al., 1984) ethylbenzene biodegradation in methanogenic environments.

m-, o- and p-Xylenes

One of the first studies illustrating the anaerobic degradation of aromatic hydrocarbons involved the removal of xylenes from a contaminated groundwater system (Reinhard et al., 1984). This study demonstrated that xylene reduction could not be attributed to other hydrodynamic processes such as mixing or dispersion but was instead caused by microbial degradation.

Several laboratory (Alvarez and Vogel, 1994, Barbaro et al., 1992, Hutchins, 1991, 1992, Kuhn et al., 1985, and Zeyer et al., 1986) and field (Barlaz et al., 1993, and Barbaro et al., 1992) studies have reported m-, o-, and p-xylene removal under nitrate-reducing conditions as sole substrates or in combinations of the three isomers with and without other BTEX compounds. Typically, removal of all of the xylene isomers has been successful with rates of approximately 1µM/day. However, a few studies have shown xylene to be recalcitrant with nitrate reduction. Using creosote-contaminated groundwater as an inoculum, Flyvbjerg et al. (1993) did not see xylene degradation during their experiment. In this case, it was possible that Na₂SO₃, which was added to remove residual oxygen, may have inhibited xylene removal. In addition, there was very

little biomass available since only groundwater was used. In a study by Acton and Barker (1992), acetylene, which was present at the contaminated site, may have been the inhibiting factor that restricted xylene removal.

Under nitrate-reducing conditions, several studies (Evans et al., 1991, and Hutchins, 1993) have reported the cometabolic degradation of o-xylene by toluene-degrading bacteria, usually in the presence of toluene. In the study by Evans et al. (1991), o-xylene removal ceased when the toluene supply was depleted. This will have further implications when modeling the interactions between the two.

There has been limited study of xylene degradation under iron-reducing conditions in the literature. One field study by Cozzarelli et al. (1990) provides evidence of this type of xylene removal. Oxidized products of all three xylenes were present in the groundwater at Bemidji, Minnesota where Fe(III) was determined to be the electron acceptor.

Under sulfate-reducing conditions, Edwards et al. (1992) reported that all xylenes were degraded only after the removal of toluene. Such selective removal of BTEX compounds may explain why xylene biodegradation has not been evident in other studies using sulfate as an electron acceptor (Barbaro et al., 1992, Flyvbjerg et al., 1993). However, there have been several investigations using different aquifer materials that have successfully demonstrated xylene removal with sulfate (Beller et al., 1995, Acton and Barker, 1992, and Thierrin et al., 1993).

Evidence for the methanogenic degradation of xylene isomers is also limited. Only a few studies have provided positive results. Wilson et al. (1990) observed degradation of all three isomers under methanogenic conditions. In studies by Wilson et al. (1986b) and Edwards and Grbic-Galic (1994), o-xylene behaved in a similar manner as in nitrate-reducing conditions. As such, the degradation of toluene occurred much sooner than that of xylene. However, this lag period for o-xylene was not observed when Edwards and Grbic-Galic (1994) amended the microcosm with toluene and o-xylene.

2.2.4 Mixed Microaerophilic and Denitrifying Zones

Of recent interest are the effects of biodegradation under mixed oxygen and denitrifying conditions. As discussed in Section 2.2.3, researchers have had limited success at degrading benzene in anaerobic environments. Some studies have suggested that benzene and other hydrocarbons may be less recalcitrant in an anaerobic environment with a limited oxygen supply. A second reason for interest in this area is that oxygen may be difficult to supply to the subsurface in an enhanced bioremediation process since it has a low solubility in water (10 mg/L at 15°C) (Wilson and Bouwer, 1997). In addition, it may be lost before reaching the contaminated zone by reacting with inorganic species to precipitate oxides, or by degassing (Hutchins et al., 1991). In contrast, nitrate is highly water-soluble (660 g/L), does not precipitate oxides, is inexpensive, has a similar yield to oxygen, and is non-toxic to bacteria below 500 mg/L. Potential problems with nitrate are its role in eutrophication and its effects on infants due to nitrate in the drinking water (Wilson and Bouwer, 1997).

It has been demonstrated that the presence of oxygen can inhibit denitrification at levels as low as 0.08 mg/L (Wilson and Bouwer, 1997). However, several researchers (Bonin et al, 1989, Krul and Veeningen, 1977, and Nakajima et al., 1984) have observed denitrification in the presence of low oxygen concentrations and others (Thomas and Ward, 1989 and Trudell et al., 1986) have evidence for denitrification processes in aerobic aquifers.

There are few studies of BTEX degradation, specifically, under mixed nitrate and microaerophilic reducing conditions. Major et al. (1988) observed benzene, toluene and m-xylene biodegradation in the presence of both oxygen and nitrate. The removal of all three components was greater under mixed conditions than in strictly aerobic and anaerobic environments. Alvarez and Vogel (1995) found similar results for benzene. In column tests using contaminated aquifer material, Hutchins et al. (1992) demonstrated that TEX removal was much greater under denitrifying conditions than in an aerobic environment. However, the biodegradation was not affected when small amounts of oxygen were added. Finally, it has been shown in the field (Hutchins et al, 1991), that the addition of nitrate to microaerophilic environments has enhanced removal of toluene, ethylbenzene, and m- and p-xylene.

Currently, it is unclear what mechanisms regulate oxygen and nitrate use during biodegradation of BTEX compounds. It has been proposed that denitrifying bacteria incorporate oxygen into the ring structure of an aromatic hydrocarbon with oxygenases. Further degradation of the oxygenated aromatic intermediaries occurs via denitrification.

Since facultative bacteria are able to use both oxygen and nitrate as electron acceptors, it is thought that the presence of oxygen may initially increase the size of the microbial population which may then degrade hydrocarbons via denitrification once the oxygen has been depleted (Wilson and Bouwer, 1997).

2.2.5 Nitrogen-Limited Conditions

It has typically been assumed that nutrient availability has been sufficient for hydrocarbon biodegradation in subsurface treatment. However, Wilson et al. (1986) and Batterman (1986) demonstrated that levels of nutrients, such as nitrogen, may not be optimum and that nutrient supplementation is an essential step for enhanced biodegradation of polluted aquifers. Only one study was found, which included the effect of nitrogen-limitation on BTEX biodegradation. Allen-King et al. (1996) used a column infiltration experiment to evaluate the biodegradation of toluene in unsaturated sandy soil under nitrogen-limited conditions. One result of the limitation was the incomplete mineralization of toluene, which was determined from the low carbon dioxide production compared to the rate of oxygen consumption.

As discussed in Section 2.1.2, nitrate may be utilized for synthesis in place of ammonium. No studies were found specific to BTEX biodegradation or more general hydrocarbon biodegradation, where nutrient limitations were overcome by the availability of nitrate.

2.3 Kinetics

2.3.1 Modeling Biodegradation Kinetics

Determining the rates of biodegradation is critical for predicting the fate of contaminants in the subsurface. Numerous field and laboratory studies have been attempted to obtain values for BTEX biodegradation rates under different redox conditions. Several mathematical models have been developed to describe this process. Most of these are based on Monod, first- or zero-order kinetic models.

Monod Kinetic Model

The Monod kinetic model describes the utilization of a substrate and the corresponding microbial growth.

$$\frac{dS}{dt} = -\frac{\mu_{\max} X}{Y} \left(\frac{S}{K_s + S} \right) \quad \text{Eqn. 2-1}$$

$$\frac{dX}{dt} = \mu_{\max} X \left(\frac{S}{K_s + S} \right) \quad \text{Eqn. 2-2}$$

where	S	substrate concentration (mass volume ⁻¹)
	t	time
	$\mu_{\max}$	maximum specific growth rate (time ⁻¹)
	Y	biomass yield (mass biomass mass substrate ⁻¹)
	X	biomass concentration (mass volume ⁻¹)
	K_s	half-saturation constant (mass volume ⁻¹)

The relationship between the substrate and the growth of the microorganisms is presented in Figure 2-1. The specific growth rate, μ is defined as: $\mu_{\text{MAX}} \frac{S}{K_s + S}$. For a single limiting substrate concentration, the specific growth rate of the microorganisms initially increases rapidly as the substrate concentration increases. Eventually, it asymptotically approaches the maximum specific growth rate. The half-saturation constant defines how quickly μ approaches μ_{MAX} .

Figure 2-1: Typical plot of a Monod function.

Rate data for modeling BTEX biodegradation according to Monod kinetics have been studied in the laboratory by several investigators under a variety of conditions. Examples of Monod kinetic parameters are presented in Tables 2-3, 2-4 and 2-5.

Table 2-3: Monod rate constants for benzene biodegradation from published studies.

Substrate	Redox	$\mu_{\max}$ (d ⁻¹)	K _s (mg/L)	Conditions	Source
Benzene	Aerobic	8.28	12.20	Batch slurries, mixed culture	Alvarez et al., 1991
		8.05	3.17	Suspended cell lab microcosms	Chang et al., 1993
		8.3	12.20	Batch, mixed culture	Chen et al., 1992
		3.84	20.31	Liquid-batch reactors, mixed culture	Goldsmith and Balderson, 1988
		1.83	10.8	Respirometry	Grady et al., 1989
		0.89-5.26		Column, mixed culture	Kelly et al., 1996
		1.18	0.31	Batch, mixed culture	
		16.32	12.22	Batch, mixed culture	
		10.56	3.36	Batch, <i>Pseudomonas</i> strain PPO1	Oh et al., 1994
		12.96	6.00	Batch, mixed culture	
		6.77	6.57	Laboratory, sludge from wastewater	Tabak et al., 1991

Note: 1. Modified from Suarez and Rifai, 1999.

Table 2-4: Monod rate constants for toluene biodegradation from published studies.

Substrate	Redox	$\mu_{\max}$ (d ⁻¹)	K _s (mg/L)	Conditions	Source
Toluene	Aerobic	0.65-0.60	<0.1	Batch, Mixed culture	Allen-King et al., 1994
		9.91	17.40	Batch slurries, mixed culture	Alvarez et al., 1991
		0.00	0.33	Batch, <i>Pseudomonas</i> sp. T2 (uninduced cells)	Button, 1985
		3.08	0.43	Batch, <i>Pseudomonas</i> sp. T2 (induced cells)	
		13.03	1.22	Laboratory, <i>Pseudomonas</i> sp. B2	Chang et al., 1993
		10.84	1.88	Laboratory, <i>Pseudomonas</i> sp. X1	
		9.90	17.40	Batch, mixed culture	Chen et al., 1992
		6.24	20.27	Batch, mixed culture	Goldsmith and Balderson, 1988
		2.09-12.48	1.57	Column, mixed culture	Kelley et al., 1996
		1.10	0.28	Batch, mixed culture	
		0.21	0.65	Column, mixed culture	Macquarrie et al., 1990

Table 2-4: continued.

Substrate	Redox	μ_{max} (d ⁻¹)	K _s (mg/L)	Conditions	Source
Toluene	Aerobic	37.44	15.07	Batch, <i>Pseudomonas</i> sp. PPO2	Oh et al., 1994
		36.00	11.03	Batch, mixed culture	
		18.96	10.00	Batch, mixed culture	Park and Cowan, 1997
		0.00	0.03	Batch, <i>Pseudomonas</i> sp. T2 (uninduced cells)	Robertson and Button, 1987
		0.03	0.04	Batch, <i>Pseudomonas</i> sp. T2 (induced cells)	
		12.55	7.75	Laboratory, sludge from wastewater	Tabak et al., 1991
		4.22		Chemostat, <i>Pseudomonas putida</i>	Vecht et al., 1988
	Nitrate-Reducing	4.8	0.11-0.15	Batch, mixed culture	Jorgensen et al., 1991
		4.8	0.11	Biofilm, mixed culture	Arcangeli and Arvin, 1995

Note: 1. Modified from Suarez and Rifai, 1999.

Table 2-5: Monod rate constants for ethylbenzene and m-, o- and p-Xylene biodegradation from published studies.

Substrate	Redox	$\mu_{\max}$ (d ⁻¹)	K_s (mg/L)	Conditions	Source
Ethylbenzene	Aerobic	5.18	10.07	Laboratory, sludge from wastewater	Tabak et al., 1991
m-Xylene	Aerobic	2.95	0.75	Laboratory, sludge from wastewater	Tabak et al., 1991
o-Xylene	Aerobic	2.03		Batch, mixed culture	Corseuil and Weber, 1994
p-Xylene	Aerobic	3.12	15.93	Batch, mixed culture	Goldsmith and Balderson, 1988
		3.36	2.47	Laboratory, sludge from wastewater	Tabak et al., 1991
Xylenes	Aerobic	12.85	4.55	Laboratory, <i>Pseudomonas</i> sp. X2	Chang et al., 1993
		1.49-9	0.85	Column, mixed culture	Kelly et al., 1996
		9.19	13.27	Batch, mixed culture	
		6.67	5.52	Batch, mixed culture	

Note: 1. Modified from Suarez and Rifai, 1999.

Most of the studies were performed under aerobic conditions. Studies determining values for Monod kinetics under anaerobic conditions were not found, with the exception of two reports (Jorgensen et al., 1991 and Arcangeli and Arvin, 1995) in which toluene degradation was observed under nitrate-reducing conditions. There were several studies determining Monod kinetics under aerobic conditions for both benzene and toluene. Values for $\mu_{\max}$ range between 0.89 and 16.32 d⁻¹ for benzene and 0.03 and 37.44 d⁻¹ for

toluene. These values cover a range of three orders of magnitude for benzene, and four orders of magnitude for toluene. Values for K_S ranged between 0.31 and 20.31 mg/L for benzene and 0.04 and 20.27 mg/L for toluene. This constitutes a range of two orders of magnitude for benzene and three orders of magnitude for toluene. The variability within the results presented demonstrates the difficulty in choosing parameter values for estimating biodegradation in an aquifer where unique values are required. This variability may lead to difficulties when applying these rates to future analyses.

For ethylbenzene and the xylenes, there is limited experimental research determining Monod rates. This lack of data also leads to difficulties in applying rates to new situations.

First-Order Model

If $S \ll K_s$, the Monod model may be approximated by a first-order equation. In this case, the disappearance rate of the substrate is proportional to the remaining concentration:

$$\frac{dS}{dt} = -k_1 S \quad \text{Eqn. 2-3}$$

where k_1 rate constant (time^{-1})

Table 2-6 presents first order rate constants for BTEX biodegradation found in the literature. Patterson et al. (1993) obtained first-order kinetic rates for BTEX removal in column experiments containing indigenous organisms from an uncontaminated aquifer

beneath an urban area in Western Australia. The study modeled benzene degradation under aerobic conditions as well as the degradation of toluene and ethylbenzene under denitrifying conditions and toluene under sulfate-reducing conditions. Hutchins et al. (1991) estimated slightly lower first order rate constants for toluene and ethylbenzene under denitrifying conditions.

Chapelle et al. (1996) determined rate constants with data from both field and laboratory experiments. Toluene decay rates measured in the laboratory corresponded roughly with those measured along aquifer flow paths in the field. Rates for benzene showed a similar correspondence. However, the benzene field data had a great deal of variability.

Table 2-6: First order rate parameters for BTEX biodegradation.

Substrate	Redox	k (d ⁻¹)	Conditions	Source
Benzene	Aerobic	>9.50	Column	Patterson et al., 1993
	Sulphate-reducing	.003	Batch	Chapelle et al., 1996
Toluene	Nitrate-reducing	0.151	Batch	Hutchins et al. (1991)
		0.345	Column	Patterson et al., 1993
	Sulphate-reducing	.010	Batch	Chapelle et al., 1996
		.0075 to .03	Field	
		0.345	Column	Patterson et al., 1993
Ethylbenzene	Nitrate-reducing	0.0652	Microcosm	Hutchins et al. (1991)
		0.345	Column	Patterson et al. (1993)

Zero-Order Model

A zero-order model can be developed from the Monod equation if $S \gg K_s$. In this model, the rate of degradation is completely independent of the substrate or biomass concentrations.

$$\frac{dS}{dt} = -k_0 \quad \text{Eqn. 2-4}$$

where k_0 rate constant (mass volume⁻¹ time⁻¹)

Reinhard et al. (1997) examined the removal of BTEX under both nitrate- and sulfate-reducing conditions by incubating a test zone of an aquifer with slugs of groundwater contaminated with BTEX. The rate constant was used as a fitting parameter for a two-dimensional flow and transport equation. Under nitrate-reducing conditions, the removal of toluene, ethylbenzene, m-xylene and o-xylene were fit with zero order kinetics. Rate constants were determined to have values of: 4.5 µg /L.hr for toluene and ethylbenzene, 2.8 µg/L.hr for m-xylene, and 0.1 µg/L.hr for o-xylene. Under sulfate-reducing conditions, toluene and m- and o-xylene again could be fit to a zero order kinetic model although the rate constants were not provided.

2.3.2 Comparison of Biodegradation Models

There are several issues with the use of these three models for estimating degradation rates, which need to be considered. None of these models include mechanisms to account

for the effects of other environmental factors. Toxic effects of contaminants, inhibition from other competing substrates, depletion of, and competition between, different electron acceptors are some of the factors which need to be considered when combining the biodegradation with a transport model to estimate bioremediation processes. In addition, none of the models account for lag periods. Each model assumes that biodegradation is instantaneous and occurring at all times.

An advantage of first- and zero-order kinetics is that only one parameter is required. The rate constants, k_1 , and k_0 , can be estimated using field data, while it is not possible to find unique values of K_s and $\mu_{\max}$ for Monod kinetics from field data alone (Chapelle et al., 1996). However, while the first- and zero-order models are convenient, they may not be suitable for estimating and/or predicting biodegradation rates. The main issue is that these models may not be valid over the entire concentration range of BTEX compounds present at a contaminated site. In contrast, the legitimacy of the Monod model has been established over a wide range of substrate concentrations and conditions (Grady and Lim, 1980). Bekins et al. (1998) have illustrated the potential problems which may arise if the first-order rate model is applied without first verifying that the maximum concentration of the rate-limiting species is much less than the half-saturation constant, K_s . First-order rate constants should not be applied to concentrations above or below the range upon which they were originally established. Extrapolation to higher concentrations may over-predict biodegradation and extrapolation to lower concentrations may under-predict it (Bekins et al., 1998).

In addition, the first- and zero-order models do not account for biomass growth. The microbial population may be increasing or decreasing over time and may have substantial effects on the rate of substrate degradation.

2.3.3 Interactions Among Substrates

Typically, contaminated waste streams contain BTEX together as a mixture as opposed to single components. Clearly, understanding the interactions among these components are important for modeling biodegradation processes in the environment. Substrate interactions may be beneficial or detrimental to the degradation process.

Cometabolism is one example of a beneficial interaction. This involves the transformation of an organic compound by microorganisms that are unable to derive any nutritional benefit from the compound or its transformation products. The microorganism utilizes another (primary) substrate for energy and growth. The biodegradation of this primary substrate stimulates microbial growth on the non-growth substrate by inducing the required catabolic enzymes (Grady et al., 1999).

Detrimental interactions include the presence of a BTEX compound that may limit the degradation of another by inhibition, depletion of electron acceptors, catabolite repression, or exerting toxicity (Alvarez and Vogel, 1991). Inhibition occurs when the presence of one substrate reduces the activity of an enzyme required by a second substrate. Inhibition may be competitive or non-competitive. During competitive inhibition both substrates compete for the same catalytic sites on an enzyme. The

inhibitor binds on the same location of the enzyme where the substrate would bind. Competitive inhibition may be overcome by increasing the substrate concentration. During non-competitive inhibition, the inhibitor disables the catalytic capability of the enzyme for the desired reaction with the substrate. It cannot be overcome with an increased substrate concentration.

Experimental Work

Understanding the degradation of BTEX compounds in combination with one another has been attempted in four main studies under aerobic conditions (Alvarez and Vogel, 1991, Chang et al., 1993, Oh et al., 1994, and Bielefeldt and Stensel, 1999). In these studies, both cometabolism and competitive inhibition have been observed for different combinations of BTEX compounds by single strains and microbial consortiums. Observations from these studies are summarized in Table 2-7.

Monod kinetics has been used to develop models for cometabolism (Chang et al., 1993) and competitive inhibition (Chang et al., 1993, Oh et al., 1994, and Bielefeldt and Stensel, 1999). However, further research is required in this area for different combinations of BTEX and for other redox conditions.

Table 2-7: Observed degradation patterns for different BTEX combinations.

Substrates	Observations	Conditions	Source
B and T	Competitive inhibition of B by T	<i>Pseudomonas</i> strain B1	Chang et al. , 1993
	T not affected and B not degraded	<i>Pseudomonas</i> strain X1	
	Competitive inhibition	Mixed culture and <i>Pseudomonas</i> strain PPO1	Oh et al., 1994
	No effect	Mixed culture	Alvarez and Vogel, 1991
	Enhanced B degradation	<i>Pseudomonas</i> strain CFS-215 isolated with toluene	
	Cometabolism of T	<i>Anthrobacter</i> strain HCB isolated with benzene	
T and p-X	Cometabolism of X	<i>Pseudomonas</i> strain B1	Chang et al., 1993
	Competitive inhibition of X by T	<i>Pseudomonas</i> strain X1	
	Cometabolism of X (degradation not to completion)	Mixed culture and <i>Pseudomonas</i> strain PPO1	Oh et al., 1994
	Cometabolism of X	Mixed culture	Alvarez and Vogel, 1991
	Enhanced X degradation	<i>Pseudomonas</i> strain CFS-215 isolated with toluene	

Table 2-7: continued.

Substrates	Observations	Conditions	Source
B and p-X	Cometabolism of X	<i>Pseudomonas</i> strain B1	Chang et al., 1993
	X not affected and B not degraded	<i>Pseudomonas</i> strain X1	
	Cometabolism of X (degradation not to completion)	Mixed culture and <i>Pseudomonas</i> strain PPO1	Oh et al., 1994
	Increased lag time, but similar degradation rate for B as alone	Mixed culture	Alvarez and Vogel, 1991
	Cometabolism of X	<i>Anthrobacter</i> strain HCB isolated with benzene	
B, T and p-X	Cometabolism of X (degradation not to completion)	Mixed culture and <i>Pseudomonas</i> strain PPO1	Oh et al., 1994
	Increased lag time, but similar degradation rate for B as alone	Mixed culture	Alvarez and Vogel, 1991
	Decreased degradation for T		
B, T, E and o-X	Competitive inhibition	Mixed culture	Bielefeldt and Stensel, 1999

Note: 1. B = benzene, T = toluene, E = ethylbenzene, and X = xylene

2.3.4 Modeling the Effects of Multiple Electron Acceptors and Nutrients

The effects of electron acceptor concentration were first described by Rich (1973) who

modified the Monod equation by multiplying it by a saturation term $\frac{S_{\text{oxygen}}}{K_{S,\text{oxygen}} + S_{\text{oxygen}}}$ to

represent the rate-limiting effect of oxygen on substrate degradation.

If the oxygen concentration is very small, this term will be small and hence, substrate degradation will be decreased. If the concentration of oxygen is large, this term will tend towards one and will have no effect on biodegradation.

Waddill and Widdowson (1998) and Salvage and Yeh (1998) have used a similar approach for describing microbial degradation within the subsurface. Additional saturation terms corresponding to other electron acceptors have been added to the initial Monod reaction kinetics to describe their availability and effects on substrate degradation as follows:

$$\frac{dS}{dt} = -\frac{\mu_{\max}X}{Y} \left(\frac{S}{K_s + S} \right) \left(\frac{S_o}{K_o + S_o} \right) \left(\frac{S_n}{K_n + S_n} \right) \cdots \left(\frac{S_i}{K_i + S_i} \right) \quad \text{Eqn. 2-4}$$

where subscript o kinetic terms for oxygen

subscript n kinetic terms for nitrate

subscript i kinetic terms for the i^{th} electron acceptor

However, these models were not applied strictly to BTEX compounds but were intended for general 'subsurface pollutants'.

The multiple Monod equation can also be extended to nutrients such as ammonia or phosphorous which are necessary for microbial growth. Prommer et al. (1999) developed a one-dimensional transport model that incorporated multiple saturation terms for both electron acceptors and nutrients. It was applied to a synthetic case where BTEX compounds were degraded by sequential reduction of electron acceptors. The model was found to be comparable to an existing model used for one-dimensional redox situations.

The presence of electron acceptors may also have a detrimental effect on microbial growth. For example, growth under anoxic conditions would be inhibited by high oxygen concentrations. In this case, the Monod equation can be supplemented with an

inhibition term $\frac{K_{I, \text{oxygen}}}{K_{I, \text{oxygen}} + S_{\text{oxygen}}}$. The parameter, $K_{I, \text{oxygen}}$, is the inhibition coefficient

for oxygen and the term acts as a switching function. When the concentration of oxygen is large, the function goes to zero and anoxic growth is diminished. When the concentration of oxygen is small, the function goes to one, and anoxic growth is unhindered (Grady et al., 1999).

Widdowson et al. (1988) developed a one-dimensional transport model utilizing the modified Monod equation with multiple saturation and inhibition terms. This study simulated the aerobic and anoxic biodegradation of hydrocarbons with potential nitrogen-nutrient limitation. The microbial population was simulated as predominantly sediment-

bound clusters of bacteria. Thus, it was assumed that materials for metabolism were required to cross a boundary layer via intrapore scale diffusion for use in metabolism. Widdowson et al. concluded that electron acceptor and nutrient availability were important factors in the simulation of organic carbon transport within the subsurface.

No field or laboratory studies were found applying Monod models with multiple saturation and inhibition terms specifically to BTEX compounds.

For the project described in this thesis, it was necessary to find a model that could be applied to a bench scale batch experiment for BTEX biodegradation. The Activated Sludge Model No. 2 (ASM2) (Henze et al., 1995) of the International Association on Water Quality (IAWQ) was developed to simulate biological processes involved in wastewater treatment. This model incorporates both saturation terms for electron acceptors and nutrients, and inhibition terms for electron acceptors.

None of the models discussed above addresses the potential role of nitrate as a source of nitrogen for synthesis. While the depletion of ammonium-nitrogen was incorporated into the Widdowson model and the ASM2 model, neither included the ability of microorganisms to assimilate nitrate when the ammonium supply had been depleted.

2.3.5 Experimental Design for Modeling BTEX Biodegradation

Experimental design is an important consideration for determining biodegradation rate data. Typically, there are two different methods utilized for estimating biodegradation

model parameters for subsurface environments. These include suspended growth (batch) reactors and flow through columns, containing some type of packing or soil.

One of the limitations of column experiments is the inability to ensure steady state conditions throughout the length of the system. Microenvironments, having different redox conditions, may develop within the column. Hence, estimated kinetic parameters may not be attributable to a single redox condition. In contrast, conditions within a batch reactor are fairly constant throughout the system

The use of batch reactors, as opposed to flow through columns, has been questioned for determining applicable biodegradation rates for field situations. Columns offer the advantage of providing more realistic simulations of natural environments. Batch systems have a much lower soil-to-solution ratio than a flow through column, which more closely models a groundwater scenario. Since bacteria prefer solid surfaces (Atlas and Bartha, 1987), this ratio is an important component for microbial growth. In addition, diffusion into small pores and sorption can also compete for the substrate resulting in a lower substrate utilization rate than in suspended liquid systems (Alexander and Scow, 1989). However, Kelly et al. (1996) evaluated the degradation of benzene, toluene and xylene under aerobic conditions in both batch and column systems. Similar rate parameters were estimated by both systems. The difference in soil-to-solution ratios had no significant effect on the results and there were no indications of mass-transport limitations.

2.4 Conclusions

Several laboratory investigations have demonstrated the ability of microorganisms to degrade BTEX compounds under a variety of aerobic and anaerobic conditions. There is specific interest in BTEX biodegradation under mixed aerobic and anoxic conditions. Nitrate, which is the next most energy-efficient electron acceptor after oxygen, is a highly soluble anion, and may play an important role in biological aquifer restoration.

Nitrate is also a potential source of nitrogen for synthesis. Due to its oxidized state, it is not as efficiently transformed into proteins, as is ammonium. However, in the absence of ammonium, it may be a valuable nutrient for growth. This dual role of nitrate requires further investigation.

A review of the literature supports the modeling of BTEX biodegradation with a form of the Monod equation. The simple Monod model is ubiquitous in the literature for biodegradation in general. However, there is a lack of studies that evaluate Monod kinetic parameters for BTEX compounds. With the exception of benzene and toluene under aerobic conditions, the BTEX compounds under different redox conditions have not been fully examined with respect to Monod kinetics.

Several transport models for hydrocarbon pollutants in the subsurface have been developed with the Monod model as a base. Often the model has been modified by the addition of multiple terms that express the effects of electron acceptor and nutrient concentrations on microbial growth. An inhibition term has also been applied to the

Monod model. This function describes the negative effects on growth, which may be caused by the presence of electron acceptors.

The application of Monod kinetics and its accompanying saturation/inhibition terms to BTEX biodegradation has not been fully investigated. While several models are developed for general hydrocarbon application, only one study of BTEX biodegradation with a multiple term Monod model was found.

The use of a batch reactor to assess the microbial biodegradation of BTEX has been shown to be an effective method for determining Monod kinetic parameters.

3. EXPERIMENTAL AND ANALYTICAL METHODS

3.1 Experimental Methods

3.1.1 Experimental Plan

The study consisted of 12 experiments during which oxygen (O_2) and nitrate (NO_3^-) loading rates were varied under nitrogen-limited conditions. The experimental plan was designed to examine steady-state BTEX biodegradation under conditions of O_2 addition alone, NO_3^- addition alone, and various combinations of high, medium and low additions of each. Acetic acid and trace amounts of BTEX contaminants acted as primary and secondary substrates, respectively.

The system was operated in a fashion similar to a CSTR, with the exception of a short settling period every hour, prior to effluent removal. This was intended to maximize the amount of biomass (volatile suspended solids (VSS)) within the reactor. As a result, it was assumed that all measurements of the bulk parameter concentrations within the reactor, excluding the VSS concentration, were equivalent to the effluent stream concentrations. The total influent and effluent flows were 3 L/day, resulting in an HRT of 1 d.

Two reactor inputs were maintained constant, not only within each experiment, but also over the entire study period. These were the mass loading rates of acetic acid and BTEX to the reactor. The acetic acid concentration in the feed was adjusted with the influent flow rate to maintain a COD mass loading rate of 3000 mg/day under all operating conditions.

Initially the mass loading rate of each BTEX compound was set at 600 $\mu\text{g/day}$ with a total BTEX loading rate of 3,600 $\mu\text{g/day}$. Experiments 1 to 3 were performed with this approach. The system and the preparation and sampling techniques were modified several times prior to the experimental period to improve the measured influent BTEX concentrations. Despite these adjustments, low influent concentrations were measured for these initial experiments. It is most likely that high volatilization rates were the cause of BTEX losses. As a result, it was decided to increase the mass loading rate to ensure a substantial amount of BTEX would be available for biodegradation. Therefore, a more concentrated solution was prepared to allow for a maximum mass loading rate of 1,200 $\mu\text{g/day}$ for each compound, resulting in a total BTEX mass loading rate of 7,200 $\mu\text{g/day}$. All remaining experiments used this rate.

The target mass loading rates and average flows of the input streams are summarized for each of the 12 experiments in Tables 3-1 and 3-2, respectively.

Table 3-1: Summary of mass loading rates for input parameters.

Experiment	Feed Mass Loading Rate (mg COD/day)	H₂O₂ Mass Loading Rate (mg O₂/day)	NO₃⁻ Mass Loading Rate (mg NO₃⁻/day)	BTEX Mass Loading Rate (µg/day)
1	3,000	1,100		3,600
2	3,000	880		3,600
3	3,000	1,000		3,600
4	3,000	2,300	4,600	7,200
5	3,000	1,200	4,600	7,200
6	3,000	80	4,600	7,200
7	3,000		4,600	7,200
8	3,000		900	7,200
9	3,000		400	7,200
10	3,000	310	400	7,200
11	3,000	150	400	7,200
12	3,000	150	700	7,200

Table 3-2: Summary of influent flow rates for input parameters.

Experiment	Feed Solution Flow Rate (mL/day)	H₂O₂ Solution Flow Rate (mL/day)	NO₃⁻ Solution Flow Rate (mL/day)	BTEX Solution Flow Rate (mL/day)
1	2,352	636		12
2	2,352	636		12
3	2,352	636		12
4	2,000	500	488	12
5	2,000	500	488	12
6	2,000	500	488	12
7	2,000		988	12
8	2,000		988	12
9	2,000		988	12
10	2,000	500	488	12
11	2,000	500	488	12
12	2,000	500	488	12

3.1.2 Materials and Schematic

A schematic of the system is provided in Figure 3-1.

Reactor

A 4 L glass reactor, with an inside diameter of 13 cm and a height of 28 cm, was seeded with 90 mL of waste activated sludge (WAS) from the City of Ottawa sewage treatment plant (ROPEC). It was believed that this sludge would be acclimatized to BTEX compounds from previous exposure. The reactor was filled with distilled water for a total volume of 3 L.

The contents of the reactor were mixed with a magnetic stir bar that was rotated by a magnetic stir plate on which the reactor sat. The system was operated via a computer on a one-hour cycle. Mixing occurred for 52.5 minutes of every hour. This was followed by a 5 minute settling period, and finally, effluent removal over the last 2.5 minutes.

Feed Solution

A solution containing the primary substrate, acetic acid (CH_3COOH), and nutrients was pumped continuously into the reactor with a Masterflex Easy-load variable speed pump. The composition of this feed stream is presented in Table 3.3. The solution had a COD:N:P mass ratio of 91:1:1 and was a modification of that employed by Puhakka and Jarvinen (1992).

Figure 3-1: Experimental system.

Table 3-3: Composition of feed solution.

Compound	Concentration for Experiments 1 to 3 (mg/L) <i>flow rate: 2352 mL/day</i>	Concentration for Experiments 4 to 12 (mg/L) <i>flow rate: 2000 mL/day</i>
CH ₃ COOH	1196	1407
K ₂ HPO ₄	49	58
KH ₂ PO ₄	26	31
NH ₄ Cl	52	62
NaHCO ₃	162	192
FeCl ₂	15	18
MgCl ₂	50	59
CaCO ₃	32	39
ZnCl ₂	7.6	9.0
Yeast extract	6.4	7.6

The feed solution was prepared every two days in volumes of 6 L (experiments 1, 2 and 3) or 5 L (experiments 4 to 12). As the feed solution was made-up, additional sodium bicarbonate, ranging from 1 to 3 g/L, was added in response to pH changes within the reactor. The specific amount of bicarbonate was dependent upon the value of the most recent pH measurement.

To minimize biomass growth in the feed tank and transfer lines, the feed solution was refrigerated. The feed tubing and the stainless steel connectors were either cleaned with bleach and hot water, or replaced, prior to the start of a new experiment. With this

regular maintenance, no biomass growth was visible within these components. Standard laboratory-grade tygon tubing was utilized for this stream up until the connection with the BTEX solution. The remainder, i.e. from the BTEX/feed solution connection to the reactor, was Viton tubing.

Oxygen was removed from the feed solution with nitrogen gas during experiments 6 through 12. This was done primarily to ensure that the feed solution was not a source of O₂ into the reactor. The nitrogen gas was continuously bubbled through the feed solution at a rate of approximately 700 mL/min.

Oxygen

In experiments where O₂ was added to the reactor, hydrogen peroxide (H₂O₂) was utilized. Hydrogen peroxide has been employed as a source of O₂ during remediation of aquifers (Lee et al., 1988) since it dissociates into O₂ and H₂O as per:

A 1.5 L (experiments 4, 5, 6, 10, 11 and 12) or 2 L (experiments 1, 2 and 3) solution of 30% w/w (H₂O₂) in distilled water was prepared every two days. A Masterflex Easy-load variable speed pump, controlled by a ChronTrol® timer, pumped the solution into the reactor. The timer turned the pump on for 2 minutes 47 seconds every 5 minutes. This cycle repeated continuously to ensure the desired average influent flow. Standard laboratory-grade tygon tubing was utilized for this stream.

Typically, bacteria naturally contain the enzyme catalase needed to convert H_2O_2 into O_2 . However, to ensure that the reaction would proceed, 2 μL of catalase from *Aspergillus niger* was injected daily into the reactor via a port on the T connector of the feed stream. This is a long-acting, stable form of catalase, which is active between a pH of 2 to 12. It had approximately 70,000 activity units/mL.

Nitrate

Nitrate addition was accomplished with a solution made from NaNO_3 and distilled water. Volumes of 1.4 L (experiments 4, 5, 6, 10, 11 and 12) and 3L (experiments 7, 8 and 9) were prepared every two days. A Masterflex Easy-load variable speed pump, which was controlled by a ChronTrol® timer, was employed to pump the solution into the reactor. A 5 minute cycle, in which the pump was powered for 2 minutes and 47 seconds, was used to produce the desired average influent NO_3^- flow. Standard laboratory-grade tygon tubing was utilized for this stream.

Oxygen was removed from the nitrate solution with nitrogen gas during experiments 6 through 12. This was done primarily to ensure that the nitrate solution was not a source of O_2 into the reactor. The nitrogen gas was continuously bubbled through the nitrate solution at a rate of approximately 700 mL/min.

BTEX

The BTEX solution was prepared by injecting approximately 15 μL (experiments 1 to 3) and 30 μL (experiments 4 to 7) of all six compounds into distilled water in a 254 mL

glass bottle which was filled to the top to minimize BTEX volatilization into the headspace. The bottle was placed on a shaker at room temperature overnight. Five 40 mL glass vials were filled with the solution, immediately sealed with Teflon-coated septa and capped. The vials were refrigerated until required.

Every two days, a 25 mL GasTight® glass syringe was filled with the BTEX solution from one of the glass vials. The syringe was inserted into a Cole-Parmer® single syringe infusion pump that was attached to the feed tubing via a stainless steel T section approximately 12 inches from the reactor. At this point, the BTEX solution was introduced into the feed stream to allow mixing prior to the reactor as well as the influent sample port.

All tubing, which came in contact with the BTEX compounds, was Viton. The syringe containing the BTEX, as well as all sampling syringes, were composed of glass, with Teflon-coated plungers. All connectors were made of either glass or stainless steel. Rubber stoppers, located at the top 5 ports of the reactor, were covered with aluminum foil. Septa were also coated with Teflon.

Sodium Hydroxide Trap

Due to the volatile nature of the BTEX compounds, the headspace of the reactor was contained to prevent losses of VOCs from the system. However, it was recognized that the biomass in the system would be continuously generating gases. Sodium hydroxide (NaOH) pellets, covered with water, were contained in a 250 mL Erlenmeyer flask which

was connected with tubing to the headspace of the reactor through a port at the top. This acted as a trap for carbon dioxide (CO₂) gases produced from biological activity. As the acid-base reaction between NaOH and CO₂ consumed the NaOH, supplementary pellets were added to the flask. A 0.5 L Tedlar bag was connected to the same tubing to collect any remaining gases emitted by the system.

3.1.3 Sampling Procedure

Three sampling events were performed for each experiment in order to collect 3 sets of data. For each experiment, a minimum of three HRTs was allowed to elapse before sampling was performed. It was assumed that this would ensure that steady-state conditions had been attained.

All effluent and reactor samples were collected from the outlet sample port during the mixing stage of the reactor cycle. With the exception of VSS, it was assumed that there was no difference in bulk parameter quality between samples taken during the mixing stage and the effluent removal stage which occurred after the settling process and therefore, contained less biomass. Henceforth, all bulk parameters, except VSS, sampled from the outlet port will be referred to as 'effluent' parameters, although their corresponding 'reactor' values would be equivalent. VSS, in contrast, will be referred to as 'reactor' VSS. The corresponding 'effluent' VSS concentration would be substantially lower. However, 'effluent' VSS was not measured during this study.

Influent and Effluent COD

For each sampling event, two influent and two effluent samples, with a volume of approximately 10 mL, were obtained from their respective sample ports for COD analysis. To remove particulate matter, the samples were filtered into a beaker in a vacuum vessel through a Buchner filtration funnel, seated with a filter paper (Whatman 934-AH 70 mm GMF). From each sample, 1 mL of the influent and 2.5 mL of the effluent filtrates were each placed in glass test tubes. An additional 1.5 mL of distilled water was added to each of the influent samples for a total volume of 2.5 mL. Samples were acidified with 2 drops of sulfuric acid and stored in a refrigerator until the COD analysis was performed.

Reactor VSS

In each sampling event, two effluent samples, each with a volume ranging from 20 to 25 mL, were collected from the outlet sample port for VSS analysis.

Effluent O₂

The effluent O₂ concentration was measured at the beginning and end of each sampling round. A Mark 21 Martek Dissolved Oxygen Analyzer was used to measure O₂ concentrations within the system. The meter has an accuracy level of ± 0.01 ppm in the 0 to 2.00 ppm range and an accuracy of ± 2.00 ppb in the 0 to 200.0 ppb range. The probe associated with the analyzer contains a galvanic (platinum/lead) sensor. A flow chamber was attached to the system via a short recycle stream of Viton tubing that was clamped shut between O₂ concentration measurements. When analysis was required, the probe

was inserted into the flow chamber, the clamps were opened and the recycle stream was pumped with a Masterflex Easy-load variable speed pump until the value for O_2 concentration stabilized (approximately 3 to 4 minutes). The flow rate within the recycle stream was approximately 100 mL/min. Viton tubing was utilized to minimize O_2 diffusion into the stream.

Influent and Effluent NO_3^-

The influent NO_3^- concentration was measured once during each sampling event for experiments 7 to 12. The effluent NO_3^- concentration was measured at the beginning and end of each sampling event. This procedure involved collecting a 50 mL sample from the outlet port in a 100 mL beaker. One mL of nitrate ionic strength adjuster (ISA) was added to the sample and the beaker was placed on a magnetic stir plate. An Orion Model 97-07 ionplus nitrate electrode, attached to an Orion Model 420A pH/mV meter, was inserted into the liquid to measure the NO_3^- concentration expressed as millivolts (mV). Measurements were converted to mg/L NO_3^- via calibration curves described in Section 3.1.4.

Oxidation-Reduction Potential

To determine the oxidation-reduction potential (ORP) of the system, a sample, with an approximate volume of 40 mL, was collected in a 100 mL glass beaker from the effluent port. The beaker was placed on a magnetic stir plate and a Cole Parmer electrode with a Sealed Ag/AgCl salt bridge and a platinum sensing tip, attached to an Orion 420A

pH/mV meter, was immersed into the sample to record the ORP. The oxidation-reduction potential, E_h , of the sample was calculated according to the following formula.

$$E_h = E + E_R \quad \text{Eqn. 3-1}$$

where E observed voltage

E_R standard potential of the reference electrode

At 25°C, E_R is equal to 202 mV.

Active Cells

The concentration of active cells within the system was determined from an iodonitrotetrazolium (INT) analysis. A sample with a volume of 7.8 mL was collected from the effluent port in a 10 mL glass test tube. Initial steps of the INT analysis, outlined in Section 3.2.3, were performed immediately.

pH and Temperature

To determine pH, an Accumet polymer body, gel-filled pH probe, attached to a Corning model 340 pH meter was immersed in an effluent sample of approximately 40 mL. The temperature of the system was measured by the Mark 21 Martek dissolved oxygen analyzer in conjunction with the O_2 measurement.

Influent and Effluent BTEX

For all experiments, the influent BTEX loading rate was measured by sampling the feed stream just prior to the reactor entrance. The combined influent BTEX/feed solution stream was allowed to drip from the inlet sample port into a 10 mL glass test tube. From

this, two 1.5 mL samples were drawn and placed in 2 mL glass vials. For experiments 4 to 12, a second method was also employed to determine the influent BTEX loading rate. Approximately 2 mL of the BTEX solution contained in the 25 mL syringe was squirted into a glass test tube. Two samples of 1.5 μ L were drawn and inserted into two 2 mL glass vials, which had been filled with 1.485 mL of distilled water, for a total volume of 1.5 mL.

To measure the effluent BTEX concentration, a 40 mL glass vial was filled with a sample from the effluent port, sealed with a Teflon-lined septum and immediately capped. The contents were allowed to settle for approximately 5 minutes. From the supernatant, two 1.5 mL samples were collected.

All BTEX samples were collected in 2 mL glass vials. The volume of each sample was 1.5 mL. While it was desirable to minimize the headspace within the vial to prevent volatilization of the BTEX compounds, it was necessary for some headspace to be present for proper agitation to occur during the absorption phase of the solid phase microextraction (SPME) sample technique described in Section 3.2.4 (Caron, 1999).

One drop of an 80 g/L copper sulfate solution was added to each vial, prior to sample collection, to prevent further biological activity. After collection of the sample, the vials were immediately sealed with Teflon-lined septa, capped and refrigerated until GC analysis.

3.1.4 Procedures for Standards and Calibration Curves

COD

For every experiment, Chemical Oxygen Demand (COD) calibration curves were developed for measuring influent and effluent COD concentrations. These were based on standards of 0, 200, 400, 600, 800 and 1,000 mg COD/L which were prepared according to Method 5220 D. Closed Reflux Colorimetric Method (APHA, AWWA, WEF, 1992).

Nitrate

Standard solutions of 1, 10, 100, 1,000 and 10,000 mg/L NO_3^- were prepared by dissolving NaNO_3 in distilled water. These solutions were made up every three experiments, i.e. at the beginning of experiments 4, 7 and 10. For each sampling event, calibration curves were constructed by measuring samples of the 5 standards following the same procedure as the measurement of the influent and effluent samples as outlined in Section 3.1.3.

BTEX

BTEX standards for GC analysis were prepared for every three experiments, i.e. prior to experiments 1, 4, 7 and 10. Methanol was utilized, at a concentration of 0.1% by volume, to aid in the dissolution of the BTEX compounds. Preparation of the standards involved the following steps.

- A 0.1% solution of methanol in distilled water was prepared. Five 40 mL glass vials were almost completely filled with this solution.
- A 20,000 mg/L solution of BTEX in methanol was prepared in a 10 mL volumetric glass flask which was filled to the base of the stopper to minimize BTEX volatilization into the headspace.
- Utilizing the BTEX/methanol solution of the previous step, a 20 mg/L solution in distilled water was prepared in a 100 mL volumetric flask, also filled to the base of the stopper to minimize volatilization. This had a concentration of 0.1% by volume methanol.
- The five 40 mL glass vials, partially filled with a solution of 0.1% methanol in water, were each spiked with different amounts of the 20 mg/L solution to make up concentrations of 50, 100, 250, 500 and 1,000 mg BTEX/L. The vials were immediately topped up with the methanol solution, sealed with Teflon septa and capped.
- For each standard, five 2 mL glass vials were filled with 1.5 mL of standard, sealed with Teflon septa and capped. All standards were refrigerated until GC analysis.

For each experiment, new standards were analyzed with the GC prior to feed-stream, syringe and effluent sample analysis. In addition, samples of distilled water were passed through the GC between each standard and experimental sample, to flush out any residual BTEX remaining in the system.

3.2 Analytical Methods

3.2.1 Chemical Oxygen Demand

The Chemical Oxygen Demand (COD) analysis was performed according to Method 5220 D. Chemical Oxygen Demand (COD) Closed Reflux Colorimetric Method (APHA, AWWA, WEF, 1992).

3.2.2 Volatile Suspended Solids (VSS)

For each of the two effluent samples, a crucible with filter paper (Whatman 934-Ah 70 mm GMF), stored in a dessicator, was weighed. The paper was placed in a Buchner filtration funnel attached to a vacuum vessel and pump. The volume of the sample was measured and then the sample was filtered through the funnel. The paper was returned to the crucible and set in a Fisher Scientific model 718F Isotemp oven at 105°C for 24 hours. The sample was dessicated and weighed and then combusted in a Ney model 2-160 Series 2 muffle furnace at 500°C for 1 hour. Again, it was dessicated and weighed. The mass difference between the sample after drying and the sample after being combusted was the mass of the reactor VSS.

3.2.3 Iodonitrotetrazolium (INT) Analysis

This analysis, modified from Sprouse and Rittmann (1991), employed a microscopic counting technique to measure biological activity. With the addition of a tetrazolium salt to the sample, the electron transport systems of the microorganisms are interrupted. The result is a reddish crystal formed within the active cells, versus inactive cells, which can

then be identified and counted. After collecting a 7.8 mL effluent sample in a 10 mL test tube, the following additions were made:

- 1 mL of an iodonitrotetrazolium salt solution (2 g/L)
- 0.1 mL of nalidixic acid (4 g/L)
- 1 mL of yeast extract (30 g/L)

The mixture was stirred with a vortex and allowed to sit overnight at room temperature. Following this incubation period, 1 mL of 37 % (w/w) formaldehyde was added to prevent further biological activity. Clumps within the mixture were removed by suctioning it through a syringe approximately 10 times. One mL of a malachite green stain, with a concentration of 10 g/L, was added to the mixture which then sat for 5 minutes. A sample was drawn and active cells were enumerated with a counting chamber on a microscope. For each sample, 20 squares were counted and averaged.

3.2.4 BTEX Measurement/GC Method

A Varian Chrompack 8200 autosampler, utilizing a 50/30 μm DVB/Carboxen/PDMS Stableflex solid phase micro extraction (SPME) fiber assembly, was used to inject BTEX compounds from liquid samples into a Varian Chrompack CP 3800 gas chromatograph (GC). The GC was equipped with a flame ionization detector (FID) and a J&W Scientific DB-1 capillary column with a length of 15 m and an inner diameter of 0.32 mm.

The Autosampler and GC operated according to the following conditions:

Absorb Time	1 minute (with agitation)
Desorb Time	5 minutes
Injector Temperature	250°C
Initial Oven Temperature	30°C
Initial Hold Time	5 minutes
Rate Increase	10°C/minute
Final Temperature	80°C
Final Hold Time	1 minute
Detector Temperature	300°C
Column Gases	Helium (carrier) at 2.0 mL/min
Detector Gases	Hydrogen (flame) at 30 mL/min Air (flame) at 300 mL/min Nitrogen (makeup) at 28 mL/min

Both *meta*- and *para*-Xylenes were eluted at the same time according to this program. As a result, they will be referred to as a m&p-Xylenes combination in the remainder of this analysis. Approximate elution times for the BTEX compounds were:

Benzene	1.6 minutes
Toluene	3.0 minutes
Ethylbenzene	5.9 minutes
m&p-Xylenes	6.2 minutes
o-Xylene	6.8 minutes

Distilled water samples were injected between each BTEX standard and experimental sample.

4. EXPERIMENTAL RESULTS & ANALYSIS

Raw data for each of the experiments is found in Tables A-1 to A-12 of Appendix A.

4.1 Data Quality

Measures were taken to ensure the highest possible data quality. However, due to the properties of the compounds, experimental error, and sampling and measurement techniques, variability within the data was observed. The following section describes the quality of the data and steps taken to organize the data for further analysis in succeeding sections.

4.1.1 Calibration

Several meters were used for measuring parameters. The dissolved oxygen (DO) and pH meters were calibrated daily. The Oxidation-Reduction Potential (ORP) meter was checked against optimum values with a solution of quinhydrone several times throughout the experimental period.

NO₃⁻, COD and BTEX concentrations were estimated from calibration curves based on their respective standards as outlined in Section 3.1.4. The coefficient of determination (R²) values for these curves were very high indicating that nearly linear calibration curves had been achieved which would provide acceptable estimation of the desired concentrations. Table 4-1 presents the range of R² values for each of the parameters over the study period.

Table 4-1: Coefficient of determination (R^2) values for calibration curves.

Parameter	R^2 Range
COD	0.976 – 0.999
NO_3^-	0.993 – 1
BTEX	0.960 – 0.999

4.1.2 Duplicate Sample Analysis

During each sampling event, several measured parameters were duplicated. These included effluent O_2 , effluent NO_3^- , influent and effluent COD, reactor VSS and feed stream, syringe and effluent BTEX concentrations. Average values were calculated for each sampling event and were used to represent the parameters for that sampling event. An analysis of the variability between the two samples indicates the error caused by sample collection and measurement techniques, not from changes to the system. This is simply from a time perspective. Changes to the system might have occurred over the period that it took to measure three sampling event of an experiment (2 to 3 days). However, it is highly unlikely that the system would have changed during sample collection of a single sampling event. Several of the duplicate samples (COD, VSS and BTEX) were obtained within seconds of each other, while others (DO and NO_3^-) were taken at the beginning and end of the sample period (approximately 2 hours). Therefore, only sample collection and analysis should have been the cause of any variability between duplicates.

To assess the variability between duplicate samples of a parameter, the following formula was developed:

$$\frac{|s_1 - s_2|}{\bar{x}} \quad \text{Eqn. 4-1}$$

where s_1, s_2 sample values
 $\bar{x}$ mean of the two samples

Due to the nature of this calculation, smaller values displayed a greater variability than larger values with the same absolute difference between the two values. For example, the variability of duplicates with values of 5 and 15 is 1.00 while the variability of duplicates with larger values such as 305 and 315 is 0.03. Despite a difference of 10 between each pair of duplicates, there is a large difference in values of variability. This will be recognized when discussing large variability between duplicates of small magnitude.

Results of this analysis are found in Appendix B in Tables B-1 to B-20.

Effluent O₂

The majority of the duplicate effluent O₂ measurements showed little variability, less than 0.35. Of exception was sampling event 1 of experiment 2, which had a variability of 0.46. The first O₂ measurement (1.01 mg O₂/L) was much higher than the second (0.632 mg O₂/L) of that sampling event as well as higher than all other O₂ measurements for that experiment which ranged from 0.416 to 0.646 mg O₂/L. The third sampling events of experiments 8, 9 and 10 also exhibited high variability, 0.68, 0.51 and 0.71 respectively.

The second measurement (0.692 mg O₂/L) for experiment 8 was more than double the first (0.341 mg O₂/L) of that sampling event and was also larger than all other measurements for that experiment, ranging from 0.145 to 0.408. During experiment 9, despite the large value for variability, both values (0.140 and 0.235 mg O₂/L) of the third sampling event were not endpoints of the range of all values (0.124 to 0.326 mg O₂/L) within this experiment and fall among other measurements taken. For experiment 10, the first O₂ measurement (0.650 mg O₂/L) was low compared to the second (1.366 mg O₂/L) and to other measurements within the three sampling events.

Effluent NO₃⁻

None of the duplicate samples of effluent NO₃⁻ concentration displayed appreciable variability, less than 0.30.

Influent and Effluent COD

Results for both influent and effluent COD exhibited little variability with respect to duplicate samples. There were no noticeable exceptions for the influent COD. All measurements of variability were less than 0.17. For the effluent COD, a large variability, 0.57, was calculated for sampling event 3 of experiment 1. With values of 562 and 312 mg COD/L, this sampling event contained the opposing limits of the range of effluent COD for this experiment. Sampling event 1 of experiment 4 also had duplicates with large variability, 0.80. These samples had values of 14 and 6 mg COD/L. As discussed previously, the high variability was caused by the small magnitude of the values and did not play a large role in the final analysis.

Reactor VSS

The majority of duplicate samples of reactor VSS had little variability between them. The most noticeable variability, 0.80, between duplicate samples was for the third sampling event of experiment 5 where 559 and 1,300 mg VSS/L represented the samples collected. In comparison with other samples from the same operating condition, these values marked opposing ends of the range of values.

Other sample duplicates with high variability were from sampling event 3 of experiment 1 and from sampling event 3 of experiment 6. In the former, a variability of 0.40 was calculated for duplicate samples with values of 244 and 365 mg VSS/L. In the latter, a variability of 0.65 was calculated for duplicate samples of 348 and 177 mg VSS/L.

Influent BTEX: Feed Stream Data

The data set had 157 cases, 87%, where variability was low, less than 0.50. Of these, 153 had variability less than 0.40.

There were four cases in which the variability between duplicate feed stream samples was high (0.55 to 1.34) for most, or all, of the BTEX components within the same run. For the first sampling event of experiment 1 and the first sampling event of experiment 2, one set of samples was significantly higher than the other, for all BTEX concentrations. In the third sampling event of experiment 3 and the second sampling event of experiment 7, four of the five BTEX concentrations had one set of samples significantly higher than

the other. The exceptions in these two data sets were ethylbenzene in experiment 3, and benzene in experiment 7. While both compounds exhibited the same trend as the other compounds (i.e. the same sample is higher than the other), there was much less variability, 0.12 and 0.16 respectively, between the duplicate values.

There were other duplicate samples with large variability, greater than 0.50, among the feed stream BTEX data. However, these did not have a shared trend among all of the BTEX compounds. Instead, these were occasions where one of the five BTEX concentrations showed a high degree of variability. In four of the five cases, ethylbenzene was the compound of concern as indicated in Table 4-2.

Table 4-2: Single instances of high variability between duplicate samples of feed stream influent BTEX concentrations.

Experiment	Sampling Event	Component	Variability
1	2	Ethylbenzene	0.71
3	2	Ethylbenzene	0.50
4	2	Toluene	0.52
9	3	Ethylbenzene	0.62
10	2	Ethylbenzene	1.00

Influent BTEX: Syringe Data

There was very little variability, less than 0.50, between duplicate samples measuring the contents of the syringe containing BTEX. The one noticeable exception was the third sampling event of experiment 6, where there was a large difference in the values (123 and 72 $\mu\text{g/L}$) for ethylbenzene concentration. This resulted in a variability of 0.52.

Effluent BTEX

Similar to the feed stream BTEX data, there was one occasion where all BTEX compounds had a high variability (0.67 to 1.14) between duplicates. For all compounds, the first sample was lower than the second sample of the sampling event. This occurred in the third sampling event of experiment 3.

There were several cases of a single compound with duplicates having high variability. Due to the small magnitude of effluent concentrations, the large variability may not be of great significance, as discussed earlier. However, in a few instances, there were sample concentrations which were not only much different from the corresponding duplicate of that sampling event, but also varied substantially from all other values in the same experiment. These are presented in Table 4-3.

Table 4-3: Single instances of high variability between duplicate samples of effluent BTEX concentrations.

Experiment	Sampling Event	Component	Variability
4	2	Benzene, Ethylbenzene	0.98, 0.84
7	2	Toluene	1.75
8	1	o-Xylene	0.87
9	3	m&p-Xylene	0.78
10	2	Toluene	0.57

Representative Values

All duplicates, analyzed in this section, were averaged to obtain representative values, for their given parameters, for each sampling event. As a result, the data has been refined to consist of 3 data sets for each experiment.

4.1.3 Steady State

To assess whether steady state conditions had been achieved during each experiment, the data set was examined for increasing or decreasing values with time. The appearance of these trends within the three sampling events, which occurred over a period of 2 or 3 days, would indicate that steady state conditions may not have been achieved.

Experiments, where three or more of the parameter values exhibited increasing or decreasing trends with time, were examined for evidence of non-steady state conditions. These trends are outlined in Table 4-4.

During experiment 2, the observed increasing reactor VSS concentration was consistent with the decreasing effluent COD concentration. These trends indicate that microbial activity was increasing within the system. In contrast, however, the increasing effluent o-Xylene concentration suggests that microbial activity had decreased.

Table 4-4: Experiments with indicators of non-steady state conditions.

Experiment	Parameter	Range of Values*	Trend
2	Effluent COD	351 to 445 mg COD/L	Increasing
	Reactor VSS	497 to 395 mg VSS/L	Decreasing
	Effluent o-Xylene	36 to 24 µg o-Xylene/L	Decreasing
3	Effluent O ₂	1.59 to 3.35 mg O ₂ /L	Increasing
	Effluent Toluene	5 to 12 µg toluene/L	Increasing
	Effluent m&p-Xylenes	16 to 33 µg m&p-Xylene/L	Increasing
	Effluent o-Xylene	17 to 46 µg o-Xylene/L	Increasing
5	Effluent NO ₃ ⁻	1,563 to 1,348 mg NO ₃ ⁻ /L	Decreasing
	Reactor VSS	1,223 to 930 mg VSS/L	Decreasing
	Effluent m&p-Xylene	39 to 45 µg m&p-Xylene/L	Increasing
8	Effluent NO ₃ ⁻	252 to 245 mg NO ₃ ⁻ /L	Decreasing
	Reactor VSS	125 to 86 mg VSS/L	Decreasing
	Effluent o-Xylene	30 to 13 µg o-Xylene/L	Decreasing
11	Effluent O ₂	0.495 to 0.286 mg O ₂ /L	Decreasing
	Effluent COD	1,004 to 805 mg COD/L	Decreasing
	Reactor VSS	308 to 237 mg VSS/L	Decreasing
	Effluent Benzene	201 to 178 µg benzene/L	Decreasing
12	Effluent O ₂	0.453 to 0.797 mg O ₂ /L	Increasing
	Effluent NO ₃ ⁻	157 to 180 mg NO ₃ ⁻ /L	Increasing
	Reactor VSS	186 to 123 mg VSS/L	Decreasing
	Effluent Toluene	140 to 171 µg toluene/L	Increasing

The increasing effluent O_2 concentration, in combination with the increasing effluent toluene, m&p-Xylenes and o-Xylene concentrations, indicate that microbial activity was decreasing during experiment 3. However, it is recognized that the effluent BTEX concentrations are extremely small (parts per billion) and the concentration ranges are minimal.

Experiments 5 and 8 exhibit similar trends for the bulk parameters. For both experiments, there is a decrease in effluent NO_3^- concentration combined with a decrease in reactor VSS concentration. The decrease in NO_3^- suggests that NO_3^- utilization was increasing with time and, hence, biomass growth should also increase. However, the corresponding reactor VSS concentration was decreasing. For experiment 5, an increase in effluent m&p-xylenes was observed. However, this was extremely small, with a range of 39 to 45 $\mu g/L$. For experiment 8, effluent o-xylene concentration was observed to decrease with time from 30 to 13 $\mu g/L$. This is consistent with the effluent NO_3^- trend but not the reactor VSS trend. Due to the conflicting trends, it is difficult to determine if non-steady state conditions were obtained.

During experiment 11, the observed decreasing effluent O_2 , effluent COD and effluent benzene concentrations suggest that microbial activity was increasing during the experiment. However, these are not consistent with the observed decreasing reactor VSS concentration. An increase in reactor VSS concentration would be expected with these trends. For this experiment, three of the four indicators suggest that the system may not have achieved steady state.

For experiment 12, all four indicators suggest that steady state was not achieved. Increases in effluent O_2 , NO_3^- and toluene concentrations suggest that there was a decrease in biological activity during the course of the experiment. The decrease in reactor VSS concentration agrees with this conclusion. Although the trend of decreasing biological activity was not visible among the remaining parameters (effluent COD concentration and the effluent concentrations of the other BTEX compounds), there is evidence to suggest that steady state conditions were not achieved for this experiment.

In general, there were increasing or decreasing concentrations with time for several experiments. However, in all of the experiments, except experiment 12, the trends of the parameters did not all agree on an overall trend of decreasing or increasing biological activity within the system. It is likely that most of these trends were caused by variability within the data. It was concluded that, with the exception of experiment 12, little evidence was found to indicate that steady state conditions had not been achieved.

During this analysis, it was noticed that reactor VSS concentrations declined considerably in several experiments. The large ranges of concentration are addressed in the discussion of variability for reactor VSS (Section 4.1.5). It is possible that these declines may also have had an impact on data variability of other parameters, specifically BTEX concentrations. However, since corresponding trends were not apparent within the effluent BTEX concentrations, this was not considered to be a major source of variability within these measurements.

4.1.4 Outlier Analysis

Upon further examination of the data, there were several cases, within the BTEX concentrations, where one of the three values for an experiment appeared to be substantially different from the others. As a result, an outlier analysis was performed on these data sets. This test (Davies and Goldsmith, 1972) is based on the ratio:

$$\frac{|\text{Extreme Value} - \text{Overall Mean}|}{\text{Overall Standard Deviation}} \quad \text{Eqn. 4-2}$$

The extreme value, or outlier, was rejected if this ratio exceeded a critical level of 1.15. This was based on an α value of 0.05 for a data set containing 3 values. Where outliers were rejected, further analysis of the data was based on the 2 remaining values. Appendix C contains the results of this analysis. The occurrences of outliers in the data set are presented in Table 4-5.

Table 4-5: Occurences of outliers.

Parameter	Experiment	Component
Feed Stream BTEX	7	Benzene,
Feed Stream BTEX	8	Benzene, Toluene, Ethylbenzene, m&p-Xylenes, and o-Xylene
Syringe BTEX	8	Benzene
Effluent BTEX	1	m&p-Xylene
Effluent BTEX	4	Benzene and Toluene

All of the outliers were found in the BTEX data. Most noticeable was the feed stream BTEX data from experiment 8 where outliers occurred for all 5 compounds. All of these outliers were from the first sampling event of that experiment. In addition, the values of the outliers for benzene, toluene and ethylbenzene (1,291, 1,002 and 676 $\mu\text{g/L}$) were all higher than the target maximum of 600 $\mu\text{g/L}$ (based on a combined BTEX/feed solution flow rate of 2,012 mL/day at the inlet port) . Values for m&p-Xylenes and o-Xylene were also high for this sampling event. This suggests that there may have been some residual BTEX contaminating the sample, resulting in a high measurement. Values for the second and third sampling events of experiment 8 for all of the BTEX compounds were similar to one another as well as being substantially lower than values for sampling event 1.

4.1.5 Variability within Experiments

For each parameter, the 3 values obtained from each of the 3 sampling events (2 values where outliers were identified) were used to obtain a mean and standard deviation for the given operating condition. Column plots, illustrating the mean and 2 standard deviations, of the bulk parameters are presented in Figures 4-1 to 4-8.

Effluent O₂

The variability within experiments for effluent O₂ concentrations (Figure 4-1), was most noticeable for experiment 10. In this case, the O₂ values ranged between 0.70 and 2.0 mg/L. The fluctuation in the effluent O₂ concentration at this critical level suggest that steady state conditions were not reached and it may be difficult to assess the activity of

Figure 4-1: Effluent O_2 concentration ($\bar{x} \pm 2s$).

Figure 4-2: Influent NO_3^- concentration ($\bar{x} \pm 2s$).

- Notes:
1. Concentrations for experiments 4, 5 and 6 were estimated (Appendix E).
 2. Influent concentrations correspond to actual influent flow rates (Table 3-2).

Figure 4-3: Effluent NO_3^- concentration ($\bar{x} \pm 2s$).

Figure 4-4: Influent COD mass loading rate ($\bar{x} \pm 2s$).

Figure 4-5: Effluent COD concentration ($\bar{x} \pm 2s$). **Figure 4-6: Reactor VSS concentration ($\bar{x} \pm 2s$).**

Figure 4-7: Active cell concentration ($\bar{x} \pm 2s$). **Figure 4-8: Oxidation Reduction Potential ($\bar{x} \pm 2s$).**

the bacteria at this point. For experiment 4, a value of 20 mg/L was measured during each sampling event. This was the maximum value that the DO probe would allow. During this time, the reactor was being heavily dosed with hydrogen peroxide such that the O_2 concentration was higher than the solubility limit. It is possible that the reactor was not in equilibrium with atmospheric conditions during this time. In contrast, this high O_2 concentration may be explained by a high partial pressure of O_2 in the gas phase resulting from an elevated O_2 concentration in the headspace. This would increase the solubility limit of the liquid within the reactor.

Influent and Effluent NO_3^-

Note that the influent NO_3^- concentrations for experiments 4, 5 and 6 were not measured. These values were estimated using calculations described in Appendix E. Both influent and effluent NO_3^- concentrations (Figures 4-2 and 4-3) exhibited some variability within operating conditions at high concentrations. For experiment 7, a standard deviation of 222 mg NO_3^- /L was calculated for an average of 4,808 mg NO_3^- /L. At lower concentrations, the variability was reduced. For the remaining experiments, standard deviations were less than 52 mg NO_3^- /L.

Influent COD

The mass loading rate of primary substrate, acetic acid, was intended to remain constant at 3,000 mg COD/day. COD measurements were taken to establish actual mass loading rates during each run. Figure 4-4 presents the mean and 2 standard deviations for influent

COD. Little variability was exhibited among sampling events during each experiment. Standard deviations ranged from 35 to 230 mg COD/day.

For the entire experimental period, sampling event values ranged from 1,694 to 2,827 mg COD/day. The mean value of all influent COD loading rates was 2,399 mg COD/day with a standard deviation of 309 mg COD/day.

Effluent COD

With the exception of experiment 3, the variability of effluent COD concentrations (Figure 4-5) among sampling events was small. Standard deviations were less than 68 mg COD/L, with the exception of experiment 3, which had a standard deviation of 120 mg COD/L for a mean of 424 mg COD/L.

Reactor VSS

Variability within reactor VSS concentrations (Figure 4-6) was fairly small (standard deviations less than 71 mg VSS/L) with the exception of experiments 4, 5 and 6. These latter experiments produced standard deviations of 222, 149 and 159 respectively.

Active Cells

Results from the INT test (Figure 4-7), were highly variable for several of the experiments (2, 4, 6, 7, 9 and 12). Average values ranged from 3.48×10^7 to 1.58×10^8 cells/mL. Standard deviations were usually an order of magnitude lower, ranging from 3.80×10^6 to 3.94×10^7 cells/mL. The difficulty in counting cells, some of which were still

mobile, under the microscope may have caused this discrepancy among sampling events. Therefore, conclusions regarding the biological activity of the system may not be strong.

ORP

ORP measurements (Figure 4-8) for all experiments were fairly consistent. However, other shortcomings of the ORP data were problematic. It was not clear why the ORP values for experiments 4 and 5 were lower than experiments 1 to 3. Not only was the O_2 loading rate greater for experiments 4 and 5, but also there was an input of NO_3^- . The additional electron acceptor loading should clearly have increased the ORP readings. A second implausible situation arose in experiments 10, 11 and 12. Following the low NO_3^- loading of experiment 9, a small amount of O_2 was added to the system for experiment 10. Two further combinations of O_2 and NO_3^- loading at low levels followed this. However, the difference in the ORP values between experiment 9 and experiments 10 to 12 was extreme with values of -54 mV to 299 mV. The ORP values for these last three experiments were similar to, if not greater than, those of experiments 1, 2 and 3 where the O_2 loading rate was substantially greater. The reasons for these anomalies were not understood. As a result of these issues, the ORP data was not included in further analysis of the data.

Influent BTEX

The loading rates, rather than concentrations, have been plotted for influent BTEX to compare the two methods for estimating this parameter. Figures 4-9 to 4-13 depict the loading rates (mean and 2 standard deviations) for BTEX estimated via the feed stream

Figure 4-9: Benzene influent loading rate ($\bar{x} \pm 2s$). **Figure 4-10: Toluene influent loading rate ($\bar{x} \pm 2s$).**

Figure 4-11: Ethylbenzene influent loading rate ($\bar{x} \pm 2s$). **Figure 4-12: m&p-Xylenes influent loading rate ($\bar{x} \pm 2s$).**

Figure 4-13: o-Xylene influent loading rate ($\bar{x} \pm 2s$).

and the syringe methods. It was apparent that the mean influent loading rates varied substantially over the entire experimental period for all BTEX components. In addition, the loading rates did not increase substantially for experiments 4 to 12 despite a doubling of the influent BTEX concentration as discussed in Section 3.1.1.

For all BTEX components, the variability among each sampling events of experiments 1, 2 and 3 was extremely high in comparison with other experiments (with the exception of experiment 10 for benzene, toluene and ethylbenzene). Also, for these experiments, there was no syringe data to compare or replace the feed stream data. As a result, an average of all sampling events for the first three experiments was used to estimate the influent loading rates. Table 4-6 presents the final results of this analysis.

In a comparison of the feed stream data and the syringe data, for the experiments, both sets of data appeared to follow the same general trends among experiments. The data sets were tested for differences between their means via a two sample t test with a two tailed hypothesis (Zar, 1984). Appendix D contains results of the analysis. Using an α value of 0.05, 78 % of the pairs of means were found to be statistically identical. However, the variability within each experiment was greater for the feed stream data 60% of the time. The variability between duplicate samples of each sampling event (Section 4.1.2) was also much greater for the feed stream data. In addition, it was felt that there was less opportunity for error, technically, with the sampling method of the syringe data. As a result, the syringe data appeared to be a more reliable indicator of the loading rate.

Table 4-6: Estimate of average BTEX influent loading rates for experiments 1 to 3.

Experiment	Sampling Event	Benzene Mass Loading Rate ($\mu\text{g/L}\cdot\text{day}$)	Toluene Mass Loading Rate ($\mu\text{g/L}\cdot\text{day}$)	Ethylbenzene Mass Loading Rate ($\mu\text{g/L}\cdot\text{day}$)	m&p-Xylenes Mass Loading Rate ($\mu\text{g/L}\cdot\text{day}$)	o-Xylene Mass Loading Rate ($\mu\text{g/L}\cdot\text{day}$)
1	1	146	167	92	171	129
	2	35	35	40	46	30
	3	174	191	128	240	169
2	1	232	256	191	372	275
	2	67	53	46	84	58
	3	129	93	70	132	87
3	1	62	75	61	118	148
	2	13	17	16	45	52
	3	57	117	199	365	325
$\bar{x}$		102	111	94	175	141
s		73	79	66	126	102

Note: 1. Loading rate calculations were based on an influent flow rate of 2364 mL/day.

Therefore, further analysis of the BTEX data was based on the influent loading rate estimated with the syringe data for experiments 4 to 12.

The variability between sampling events for the syringe data was still considerable. Most noticeable were experiments 4 and 6, for all BTEX compounds, and experiments 10 for benzene, toluene and ethylbenzene.

Effluent BTEX

The effluent BTEX concentrations have been converted to effluent rates for ease of comparison with the corresponding influent values. The mean and 2 standard deviations for effluent BTEX rates are depicted in Figures 4-14 to 4-18. Two experiments exhibited significant variability among several BTEX components. Ethylbenzene, m&p-Xylene and o-Xylene had high standard deviations with respect to their means for experiment 4. For experiment 10, all BTEX compounds showed high variability. For this latter experiment, the variability was caused by the second sample of sampling event 2, for all BTEX compounds, where values were higher than the other 5 sample values. Since the first sample of that sampling event was comparable to the other values, it is unlikely that there was a change within the reactor over the experiment sampling time. It is more likely that an error, such as contamination, occurred during the sampling or measurement process. For the high variability of experiment 4, no such trend was visible within the data.

Figure 4-14: Effluent benzene rate ($\bar{x} \pm 2s$). **Figure 4-15: Effluent toluene rate ($\bar{x} \pm 2s$).**

Figure 4-16: Effluent ethylbenzene rate ($\bar{x} \pm 2s$). **Figure 4-17: Effluent m&p-xylenes rate ($\bar{x} \pm 2s$).**

Figure 4-18: Effluent o-xylene rate ($\bar{x} \pm 2s$).

4.2 Data Analysis

4.2.1 Bulk Parameters

Data

The averaged bulk parameter data, which was used for analysis, is presented in Table 4-7. To facilitate comparisons with subsequent model calculations in the following sections, several parameters have been converted to $\text{g O}_2/\text{m}^3$ and $\text{g N}/\text{m}^3$. Also, all influent concentrations were converted to values based on a total flow of 3 L/day.

The influent O_2 concentrations are not measured values but were calculated from the hydrogen peroxide loading rates. Also, the influent concentrations of NO_3^- for experiments 4 to 6, were not measured. The average difference between target and measured influent NO_3^- concentrations for experiments 7 to 12 was determined to be an increase of 5%. Therefore, the expected inlet concentrations for experiments 4 to 6 were also increased by 5% to estimate actual NO_3^- concentrations. See Appendix E for this analysis.

Nitrogen Limitation

It was determined early in the data analysis that nitrogen, utilized for microbial synthesis, was limiting. As a result, it was suspected that NO_3^- , during some experiments, was being employed as a source of nitrogen for synthesis instead of, or as well as, an electron acceptor. This made the system much more complex. The nutrient limitation became a potential cause of system responses. Further analysis was approached with the focus adjusted to include this element.

Table 4-7: Summary of bulk parameter concentrations.

Experiment	Influent O ₂ Concentration (g O ₂ /m ³)	Effluent O ₂ Concentration (g O ₂ /m ³)		Influent NO ₃ ⁻ Concentration (g N/m ³)		Effluent NO ₃ ⁻ Concentration (g N/m ³)	
		$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s
1	367	10.11	0.48				
2	293	0.630	0.173				
3	333	2.31	0.92				
4	767	20	0	365		358	20
5	400	1.45	0.10	365		330	24
6	27	0.528	0.065	365		304	20
7	0	0.310	0.077	358	17	363	21
8	0	0.360	0.190	77	3	56	1
9	0	0.207	0.079	28	4	1	0
10	103	1.25	0.71	31	1	9	1
11	50	0.382	0.106	30	1	25	0
12	50	0.659	0.182	63	0	38	3

Notes:

1. All influent values were converted to concentrations based on a total influent flow of 3 L/day.
2. Influent O₂ concentrations were calculated from peroxide addition.
3. Influent NO₃⁻ concentrations for experiments 4, 5 and 6 were estimated. See Appendix E.

Table 4-7: continued.

Experiment	Influent COD Concentration (g COD/m ³)		Effluent COD Concentration (g COD/m ³)		Reactor VSS Concentration (g O ₂ /m ³)		ORP (mV)		INT Concentration (cells/mL)	
	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s
1	846	47	402	64	531	85	228	3	6.64E+07	9.85E+06
2	625	32	399	47	619	77	232	5	1.52E+08	3.93E+07
3	854	27	424	120	846	101	244	4	5.51E+07	6.69E+06
4	673	77	34	21	2198	315	179	7	1.36E+08	2.74E+07
5	852	51	532	35	1505	212	173	3	9.03E+07	7.33E+06
6	876	12	682	17	602	226	163	8	1.35E+08	3.94E+07
7	730	58	636	16	305	101	131	24	5.18E+07	1.90E+07
8	948	14	748	8	142	31	83	2	3.48E+07	3.80E+06
9	972	29	842	20	65	3	-54.2	1.6	8.36E+07	1.74E+07
10	806	38	486	34	435	47	299	7	1.17E+08	6.56E+06
11	767	47	523	68	376	54	238	5	8.00E+07	1.09E+07
12	765	46	486	29	219	45	239	3	9.80E+07	2.43E+07

Notes:

1. All influent values were converted to concentrations based on a total influent flow of 3 L/day.
2. Reactor VSS concentrations were converted to g O₂/m³ by assuming a typical biomass composition of C₅H₇NO₂, which results in a ratio of 1.42 g O₂/g VSS (Metcalf and Eddy, 1991).

An assessment of which experiments were limited by nitrogen was performed via a comparison of the nitrogen available from the nutrient mixture added to the reactor (33 mg N/day) and the nitrogen required as estimated from the biomass production of each experiment. This assumed that the biomass had a typical composition of $C_5H_7NO_2$ (Metcalf and Eddy, 1991). The analysis also took into consideration the potential nitrogen available from decaying bacterium. A typical decay rate of 0.05 d^{-1} (Metcalf and Eddy, 1991) was assumed.

The required nitrogen for synthesis was estimated by:

$$\frac{\text{g VSS}}{\text{day}} \times \frac{1 \text{ mole VSS}}{113 \text{ g VSS}} \times \frac{1 \text{ mole N}}{\text{mole VSS}} \times \frac{14 \text{ g N}}{\text{mole N}} \quad \text{Eqn. 4-3}$$

The nitrogen available from decayed VSS was estimated by:

$$\frac{\text{g VSS}}{\text{L}} \times \frac{0.05}{\text{day}} \times 3 \text{ L} \times \frac{1 \text{ mole VSS}}{113 \text{ g VSS}} \times \frac{1 \text{ mole N}}{\text{mole VSS}} \times \frac{14 \text{ g N}}{\text{mole N}} \quad \text{Eqn. 4-4}$$

As illustrated in Table 4-8, all experiments, with the exception of experiment 9, were deficient in nitrogen from the nutrient mixture for synthesis. The inclusion of nitrogen from the breakdown of the biomass had little effect on the outcome of the analysis.

With the addition of the electron acceptor NO_3^- , during experiments 3 to 12, there was the potential to overcome this limitation by assimilative NO_3^- use. Evidence of the nutrient limitation and of the possible dual role of NO_3^- was reflected in the bulk parameter data. This will be addressed as each parameter is discussed in this section.

Table 4-8: Nitrogen deficiency analysis.

Experiment	Reactor VSS Concentration (mg VSS/L)	Reactor VSS Rate (mg VSS/day)	Required N (mg N/day)	N from nutrient mix (mg N/day)	Result	N from decaying VSS (mg N/day)	Total N (Nutrient mix and VSS) (mg N/day)	Result
1	374	1122	139	33	deficient	7	40	deficient
2	436	1308	162	33	deficient	8	41	deficient
3	596	1788	222	33	deficient	11	44	deficient
4	1548	4644	575	33	deficient	29	62	deficient
5	1060	3180	394	33	deficient	20	53	deficient
6	424	1272	158	33	deficient	8	41	deficient
7	215	645	80	33	deficient	4	37	deficient
8	100	300	37	33	deficient	2	35	deficient
9	46	138	17	33	OK	1	34	OK
10	306	918	114	33	deficient	6	39	deficient
11	265	795	98	33	deficient	5	38	deficient
12	154	462	57	33	deficient	3	36	deficient

Electron Acceptor Balance

An electron acceptor balance was performed using the experimental data. Equation 4-5 illustrates the potential sources and sinks for electron acceptors.

$$\begin{array}{ccccccc} \text{Oxygen} & \text{Oxygen} & \text{Nitrate} & \text{Nitrate} & \text{COD} & \text{COD} & \text{VSS} \\ \text{Influent} & - \text{Effluent} & + \text{Influent} & - \text{Effluent} & = \text{Influent} & - \text{Effluent} & - \text{Effluent} \\ \text{Rate} & \text{Rate} & \text{Rate} & \text{Rate} & \text{Rate} & \text{Rate} & \text{Rate} \end{array} \quad \text{Eqn. 4-5}$$

All components of the equation were converted to mg O₂/day for a consistent basis. Several assumptions were made to do this.

- NO₃⁻ was converted to O₂ by assuming all of it was employed as an electron acceptor.

This was achieved by comparing the redox reactions of the primary substrate, acetic acid, coupled with the electron acceptors O₂ and NO₃⁻:

The resulting equation for conversion of NO₃⁻ to O₂ is:

$$\frac{\text{g N}}{\text{L}} \times \frac{3 \text{ L}}{\text{day}} \times \frac{1 \text{ mole N}}{14 \text{ g N}} \times \frac{2 \text{ moles O}_2}{\frac{8}{5} \text{ moles N}} \times \frac{32 \text{ g O}_2}{\text{mole O}_2} \quad \text{Eqn. 4-6}$$

- The biomass composition was assumed to be C₅H₇NO₂ (Metcalf and Eddy, 1991), which resulted in a ratio of 1.42 g O₂/g biomass for converting VSS to O₂.
- Due to the settling process, the reactor VSS that was measured was not the same as the effluent VSS. Twenty percent of the measured VSS was assumed to leave the reactor in the effluent. This number was obtained from the subsequent modeling effort discussed in Section 5.1.1.

Results of this analysis are presented in Table 4-9. The last column of this table presents the percent difference between the left and right sides of Equation 4-5. Results of the electron acceptor balance were fair. Where O_2 addition was high (experiments 1 to 3) and where NO_3^- addition was high, but also included some O_2 (experiments 4 to 6), positive values were obtained. This indicates that less than the amount of O_2 available was utilized for COD or growth. In contrast, for NO_3^- addition alone (7 to 9) or where NO_3^- and O_2 additions were low (experiments 10 to 12), negative values were obtained in the analysis. In these cases, more O_2 was utilized than was available. Some of these differences may be a result of the variability within the data.

The most noticeable discrepancies were observed in experiments 4 and 5. This is most likely a result of estimating the effluent VSS with a single value of 20 %. Where the reactor VSS concentrations were very high, as in experiments 4 and 5, the effluent VSS was likely overestimated. Due to the substantial settling of biomass within the reactor prior to effluent removal, a more appropriate percentage for the effluent concentration may be closer to 1 % for these experiments.

The first assumption may also be a cause of problems with the O_2 balance. For experiments 4, 5 and 6, where the differences between the two sides of Equation 4-5 are positive, it is likely that not all of the NO_3^- was used as an electron acceptor but assimilated for synthesis. This might have been true in other cases where NO_3^- addition occurred (experiments 7 to 12). However, the variability of other parameters rendered this effect immaterial with respect to the electron acceptor analysis.

Table 4-9: Electron acceptor balance.

Experiment	O ₂ Influent Rate (mg O ₂ / day)	O ₂ Effluent Rate (mg O ₂ / day)	NO ₃ ⁻ Influent Rate (mg O ₂ / day)	NO ₃ ⁻ Effluent Rate (mg O ₂ / day)	COD Influent Rate (mg COD/ day)	COD Effluent Rate (mg COD/ day)	VSS Effluent Rate (mg O ₂ / day)	Left side- Right side of Eqn. 4-5 (mg O ₂ / day)	% Difference
1	1100	30			2537	1206	319	57	5
2	880	2			1875	1197	371	572	65
3	1000	7			2563	1272	508	210	21
4	2300	60	3129	3069	2019	102	1319	1702	74
5	1200	4	3129	2828	2556	1596	903	1440	96
6	80	2	3129	2609	2627	2046	361	378	63
7	0	1	3065	3110	2191	1908	183	-146	314
8	0	1	664	482	2845	2244	85	-335	-185
9	0	1	243	5	2916	2526	39	-114	-48
10	310	4	264	75	2418	1458	261	-204	-41
11	150	1	258	211	2301	1569	226	-310	-158
12	150	2	537	329	2295	1458	131	-350	-98

It should also be recognized that the effluent O_2 concentrations were very low with respect to their corresponding influent concentrations. Differences among these effluent values had little effect on the electron acceptor balance.

Electron Acceptor Uptake Rates

Oxygen and nitrate uptake rates are presented in Table 4-10. The low O_2 effluent rates result in uptake rates that nearly match their corresponding influent rates. In general, most of the O_2 was consumed under all experiments. In contrast, the microbial utilization of NO_3^- was less apparent. There was a substantial amount of residual NO_3^- within the effluent for several experiments.

For experiment 1, some O_2 was present within the reactor at steady state. It is likely that this was the result of the nitrogen-limited environment. Not all of the O_2 was consumed as a result of the lack of nitrogen for synthesis.

Nitrate was first introduced to the system in experiment 4. With the addition of NO_3^- , a source of nitrogen for synthesis was available. It is doubtful that the bacteria would have used NO_3^- as an electron acceptor within this highly aerobic environment. Although the NO_3^- uptake rate was small, 21 mg N/day, it is most likely that the bacteria utilized this amount for synthesis.

Nitrate uptake rates were substantial during experiments 5 and 6. For experiment 5, with a moderate O_2 input, it is likely that most of the NO_3^- was being used as nitrogen for

Table 4-10: Oxygen and nitrate uptake rates.

Experiment	Influent O ₂ Rate (mg O ₂ /day)	Effluent O ₂ Rate (mg O ₂ /day)	O ₂ Uptake Rate (mg O ₂ /day)	Influent NO ₃ ⁻ Rate (mg N/day)	Effluent NO ₃ ⁻ Rate (mg N/day)	NO ₃ ⁻ Uptake Rate (mg N/day)
1	1100	30	1070			
2	880	2	878			
3	1000	7	993			
4	2300	60	2240	1095	1074	21
5	1200	4	1196	1095	990	105
6	80	2	78	1095	913	182
7	0	1	-1	1073	1089	-16
8	0	1	-1	232	169	64
9	0	1	-1	85	2	83
10	310	4	306	92	26	66
11	150	1	149	90	74	17
12	150	2	148	188	115	73

synthesis. For experiment 6, where O_2 loading was low, a more substantial amount of the NO_3^- was probably being utilized as an electron acceptor.

During experiments 7 through 9, some residual O_2 was measured within the reactor despite no loading. The system was checked for leaks on several occasions, yet the source of O_2 was not found. Possibly, this was caused by errors within the measurement technique or equipment.

During experiment 7, there appeared to be zero NO_3^- uptake. Since there was no peroxide addition, some NO_3^- utilization was expected. In contrast, experiments 8 and 9, also without O_2 loadings, exhibited some small NO_3^- usage despite lower NO_3^- loadings. Data variability may be a cause of the negative uptake rate of experiment 7.

During experiments 10 through 12, small NO_3^- uptake rates were also exhibited. This occurred in conjunction with low O_2 loading rates. However, while nearly all the O_2 was consumed, much of the NO_3^- remained unused.

The data suggests that both O_2 and NO_3^- were utilized as electron acceptors during different experiments. However, as expected, O_2 was preferred when both were available. When denitrification took place, NO_3^- was not used to its capacity. Also, the limitation of nitrogen from NH_4 , for synthesis, most likely resulted in the assimilative uptake of NO_3^- in some cases.

To characterize the system under different electron acceptor conditions, the responses of other bulk parameters in terms of electron acceptor utilization were evaluated. Effluent COD, reactor VSS and active cell concentrations were plotted with O_2 and NO_3^- uptake rates (Figures 4-19 to 4-21). It was expected that microbial activity would increase with greater O_2 and or NO_3^- utilization. Corresponding responses of the bulk parameters would be: decreased effluent COD, increased VSS and increased number of active cells. However, due to the nutrient limitation and, hence, the dual role of NO_3^- , the responses of these three indicators were more intricate.

Effluent COD

Effluent COD concentrations are presented as a function of O_2 and NO_3^- uptake rates (Figure 4-19). The lowest effluent COD concentration of 34 g COD/m³ occurred when the reactor was dosed with O_2 during experiment 4. The large amount of O_2 available produced an aerobic environment in which growth could occur unhindered, provided all necessary nutrients were available, as was the case, with the addition of NO_3^- .

The effluent COD concentrations for experiments 1 to 3 (402, 399 and 424 g COD/m³) were substantially higher than experiment 4. These conditions also were characterized by high O_2 uptake rates. However, the smaller utilization of COD likely reflects the limitation of nitrogen available for synthesis.

During experiment 5, a high effluent COD concentration of 532 g COD/m³ was observed. This was unexpected due to the high O_2 and NO_3^- uptake rates. In addition, it is inconsistent with the trends of experiments 1, 2, 3 and 4.

Figure 4-19: Effluent COD concentration versus electron acceptor uptake rates.

Figure 4-20: Reactor VSS concentration versus electron acceptor uptake rates.

Figure 4-21: Active cell concentration versus electron acceptor uptake rates.

The highest effluent COD concentrations (682, 636, 748 and 842 g COD/m³) were measured during experiments with little or no O₂ present (experiments 6, 7, 8 and 9 respectively). For experiment 7, where the NO₃⁻ uptake was negligible, this is understood. However, the moderate NO₃⁻ utilization present in experiments 6, 8 and 9, were expected to produce a more substantial COD removal. The presence of only NO₃⁻, appears to have had only a small effect on COD utilization by the bacteria.

During experiments 10 to 12, effluent COD concentrations are slightly lower than the previous conditions. The additional utilization of O₂, even at low levels, increased the utilization of COD.

Reactor VSS

Reactor VSS concentrations are presented as a function of O₂ and NO₃⁻ uptake rates in Figure 4-20. The most substantial growth occurred during experiment 4 and 5 producing 2198 and 1505 g O₂/m³ of VSS. These were also conditions of the highest O₂ uptake rates. It should be noted that the NO₃⁻ uptake rate for experiment 5, was also substantial at 168 mg N/m³. However, the role of NO₃⁻ during this experiment was difficult to ascertain. Experiments 1, 2 and 3 also produced fairly high reactor VSS at 531, 619 and 846 g O₂/m³. The lack of nitrogen from NO₃⁻ in these cases, might have been a limitation causing the smaller growth compared to experiments 4 and 5, which were exposed to the NO₃⁻ and higher O₂ loading rates.

Experiment 6 also had a high reactor VSS value of $602 \text{ g O}_2/\text{m}^3$. The corresponding high NO_3^- uptake rate of $245 \text{ mg N}/\text{m}^3$, yet a low O_2 uptake rate, provided evidence of the use of NO_3^- as an electron acceptor.

All other experiments (7 to 12) had much less biomass growth with reactor VSS values ranging from 65 to $435 \text{ g O}_2/\text{m}^3$. The O_2 utilization for these remaining cases was also small, less than $306 \text{ mg O}_2/\text{m}^3$.

Active Cells

The concentration of active cells within the reactor was plotted against O_2 and NO_3^- uptake rates in Figure 4-21. No general trends were evident. Due to the small range of the values and the high variability for several of the experiments (Section 4.1.4), results of this analysis were not considered in the succeeding analysis.

4.2.2 BTEX

Data

The averaged influent and effluent BTEX rates, which were used for analysis, are presented in Tables 4-11 and 4-12.

Variability of Removal Rates

Removal rates for each of the BTEX compounds were calculated for each sampling event. From the three sampling event values of each experiment, the average removal rates were estimated as the difference between the influent and effluent mass flow rates.

Table 4-11: Summary of influent BTEX loading rates.

Experiment	Influent Benzene Loading Rate (mg/L.day)		Influent Toluene Loading Rate (mg/L.day)		Influent Ethylbenzene Loading Rate (mg/L.day)		Influent m&p-Xylenes Loading Rate (mg/L.day)		Influent o-Xylene Loading Rate (mg/L.day)	
	x	s	x	s	x	s	x	s	x	s
1	102	73	111	79	94	66	175	126	141	102
2	102	73	111	79	94	66	175	126	141	102
3	102	73	111	79	94	66	175	126	141	102
4	234	27	261	41	163	21	204	38	85	11
5	173	10	147	6	98	9	91	3	52	5
6	94	12	129	39	48	11	88	28	24	8
7	127	0	112	9	72	34	82	9	48	27
8	186	19	118	2	128	19	96	23	86	8
9	136	37	83	9	88	19	56	6	48	12
10	175	76	247	113	107	59	169	90	80	73
11	269	42	266	18	186	27	199	16	101	15
12	153	81	290	16	111	62	223	19	67	33

Table 4-12: Summary of effluent BTEX rates.

Experiment	Effluent Benzene Rate (mg/L.day)		Effluent Toluene Rate (mg/L.day)		Effluent Ethylbenzene Rate (mg/L.day)		Effluent m&p-Xylenes Rate (mg/L.day)		Effluent o-Xylene Rate (mg/L.day)	
	x	s	x	s	x	s	x	s	x	s
1	19	3	8	1	8	5	13	1	16	6
2	20	2	10	2	10	1	22	4	30	6
3	13	4	8	3	11	6	22	9	32	15
4	147	1	84	1	29	14	85	12	66	24
5	77	9	34	4	13	1	41	3	30	1
6	37	9	14	6	4	2	12	6	10	4
7	77	3	38	12	12	3	45	13	32	9
8	87	5	52	5	16	4	28	5	20	9
9	72	6	31	6	12	2	39	9	26	5
10	150	68	75	44	19	12	84	17	51	10
11	187	12	114	15	37	2	94	4	60	5
12	213	8	152	16	43	6	130	4	81	6

The means and two standard deviations for the rates are presented in column plots (Figures 4-22 to 4-26) to illustrate variability within and between the experiments.

For experiments 1 to 3, the variability in removal rates for all BTEX components was low. This was partially a result of the averaged influent BTEX concentrations for each sampling event as presented in Section 4.1.4. By removing the variation among influent values, the difference among calculated removal rates, based on influent concentrations, decreased.

For experiment 4, the large variability for all compounds, was due to the large variability within both the influent and effluent BTEX concentrations as described in Section 4.1.4. Similarly, all of the compounds exhibited high variability in experiments 6 and 10, as well as 11 and 12, although to a lesser extent. There are several other instances of high variability for removal efficiency for single BTEX components. Most noticeable are: benzene during experiment 9, toluene for experiment 8, m&p-Xylenes for experiments 8 and 9, and o-Xylene for experiment 8.

Analysis of Variance (ANOVA)

Single factor analyses of variance (ANOVAs) were performed with the BTEX data to determine if there was a statistical difference among experiments (Zar, 1984). For each BTEX component, the effluent mass flow rates and the removal rates were evaluated. Results of this analysis are found in Appendix F. These results showed that both parameters for all compounds were statistically different. It was concluded that varying

Figure 4-22: Benzene removal rate ($\bar{x} \pm 2s$). **Figure 4-23: Toluene removal rate ($\bar{x} \pm 2s$).**

Figure 4-24: Ethylbenzene removal rate ($\bar{x} \pm 2s$). **Figure 4-25: m&p-Xylenes removal rate ($\bar{x} \pm 2s$).**

Figure 4-26: o-Xylene removal rate ($\bar{x} \pm 2s$).

electron acceptor loading rates on the system had a statistically significant effect on BTEX removal. This occurred despite the substantial variability of the data, as discussed in Section 4.1. Pairwise testing (via Tukey analyses) of the effluent mass flow rates and the removal rates for each compound would have determined where statistical differences occurred. However due to the multitude of pairs involved, no further statistical testing was performed.

BTEX Removal Rates versus Oxygen and Nitrate Uptake Rates

The BTEX removal rates were plotted with O_2 and NO_3^- uptake rates in Figures 4-27 to 4-31 to compare the effects of electron acceptor utilization on BTEX removal. The removal rates for all BTEX compounds appeared to follow the same general trends. For the first three experiments, where the O_2 uptake rate ranged from 878 to 1070 mg O_2 /day and NO_3^- addition was zero, removal rates were high, ranging from 82 to 161 $\mu\text{g/L}\cdot\text{day}$. These were aerobic environments and high removal rates were expected.

With the exception of the xylenes, the removal rates increased during experiment 4 where the O_2 uptake rate was highest, at 2,240 mg O_2 /day, and NO_3^- was added. Unexpectedly, for experiment 5, where the O_2 uptake rate was still substantial (1196 mg O_2 /day) and there was a greater NO_3^- uptake rate (168 mg N/day) than experiment 4, the removal rates dropped significantly for all BTEX components, except o-xylene which remained at an already low level.

Figure 4-27: Benzene removal rate versus electron acceptor uptake rates.

Figure 4-28: Toluene removal rate versus electron acceptor uptake rates.

Figure 4-29: Ethylbenzene removal rate versus electron acceptor uptake rates.

Figure 4-30: m&p-Xylene removal rate versus electron acceptor uptake rates.

Figure 4-31: o-Xylene removal rate versus electron acceptor uptake rates.

With respect to experiments 1 to 3, the trend for experiment 5 was also inconsistent. Removal rates for experiment 5 were less than those of the first 3 experiments. This was unexpected since the O_2 uptake rate was similar, yet the nitrogen-limited environment for experiments 1 to 3 was not an issue for experiment 5 due to the NO_3^- loading.

At the highest NO_3^- uptake rate (experiment 6), where the O_2 utilization was small, the BTEX removal rates were relatively high, with the exception of o-Xylene again. It is possible that the combination of microaerobic and high anoxic conditions was favourable for BTEX biodegradation. It may be that some of the NO_3^- was employed for synthesis as well.

For experiments 7, 8 and 9, where O_2 addition, and hence uptake rates, were zero, the smallest removal rates were observed, with the exception of experiment 9 for m&p-xylenes. As discussed in Section 4.1.4, the variability for this observed rate was extremely large and hence, the value may not be an anomaly.

During experiments 10, 11 and 12, with combinations of low O_2 and NO_3^- uptake rates, the trends of the removal rates varied for each BTEX compound. However, in general, the average values were similar for these three experiments for benzene, toluene, ethylbenzene and o-xylene. In comparison with other experiments, removal rates were higher for these three conditions with both O_2 and NO_3^- utilization, than the rates of experiments 7, 8 and 9, where O_2 was not available. This was observed for all BTEX compounds, with the exception of experiment 10 for m&p-xylenes. When compared to

experiments 1 to 3, the removal rates for experiments 10 to 12 were approximately the same for benzene and ethylbenzene, but lower for toluene, m&p-xylenes and o-xylene.

4.2.3 Discussion and Conclusions

The responses of each parameter to electron acceptor loading act as indicators to the biological activity within the system. With the combination of all parameters, a reasonable interpretation of the system during each experiment is possible.

The indicators suggest that, microbial activity was highest during experiment 4. For the largest O_2 uptake rate, the lowest effluent COD and highest BTEX removal rates were observed. The reactor VSS concentration was also significant, but not the highest value observed during the study. As expected, the highly aerobic environment allowed for substantial biological activity. The utilization of NO_3^- under these conditions was most likely for synthesis.

During experiments 1 to 3, a substantial O_2 uptake rate was also observed. However, it was less than the rate of experiment 4. The higher effluent COD concentration, lower reactor VSS concentration and lower BTEX removal rates were consistent with this result. Without NO_3^- addition during these experiments, the lack of nitrogen from the nutrient solution likely imposed a limitation on microbial growth.

For experiment 5, three of the five indicators suggest high microbial activity. The O_2 uptake rate was substantial (1196 mg O_2 /day) and there was a greater NO_3^- uptake rate

(168 mg N/day) than experiment 4. This was consistent with the extremely high reactor VSS concentration. However, the removal rates dropped significantly for all BTEX components, except o-Xylene, which remained at an already low level. Also, the high effluent COD concentration showed little COD utilization. The conditions within the reactor were still aerobic and hence, it is likely that most of the NO_3^- was used for synthesis. The low BTEX removal rates and effluent COD concentration are not understood.

During experiment 6, the highest NO_3^- uptake rate was observed in conjunction with a low O_2 uptake rate. It is likely that most of the NO_3^- was being utilized as an electron acceptor. It was expected that microbial activity would decrease somewhat if denitrification occurred due to its reduced energy availability. Most of the indicators were consistent with this expectation. The reactor VSS concentration had decreased from experiments 4 and 5, but was still high, comparable to experiments 1 to 3. BTEX removal was also fairly substantial, with the exception of o-Xylene. However, the effluent COD concentration was extremely high in comparison with experiments 1 to 4.

The indicators suggest that the lowest growth occurred during experiments 7 to 9 where there was no O_2 loading. Low NO_3^- uptake rates, high effluent COD concentrations, low reactor VSS concentrations and low BTEX removal rates characterized these experiments. The data suggests that the amount of facultative bacteria within the system was extremely small.

During experiments 10, 11 and 12, all indicators suggest an increase in microbial activity from experiments 7 to 9. The presence of O_2 , even at low concentrations appeared to have a significant effect. As exhibited by the electron acceptor utilization, in which O_2 was almost completely consumed but substantial NO_3^- remained, O_2 was the preferred electron acceptor. However, it is possible that some NO_3^- was utilized as an electron acceptor while the remainder was a source of nitrogen for synthesis.

When compared to experiments 1 to 3, the removal rates for experiments 10 to 12 were similar for benzene and ethylbenzene, but lower for toluene, m&p-Xylenes and o-Xylene. Effluent COD and reactor VSS concentrations displayed somewhat similar results. While effluent COD values were similar for experiments 1 to 3 and 10 to 12, reactor VSS values showed slightly less biomass growth for the last three experiments.

With the exception of experiment 5, the measured and calculated parameters were consistent with one another as indicators of the microbial activity within the system. The data suggests that microbial growth occurred utilizing both O_2 and NO_3^- as electron acceptors during different experiments. However, as expected, O_2 was preferred when both were available. When denitrification took place, NO_3^- was not used to its capacity and growth was substantially less than in the presence of O_2 . Even small O_2 concentrations had a significant effect on the system. It is likely that the number of facultative bacteria was limited within the consortium.

The lack of nitrogen from NH_4^+ available for synthesis had two impacts. When NO_3^- was unavailable, microbial growth was limited by the lack of nitrogen. Secondly, NO_3^- acted as both an electron acceptor, during conditions with minimal or no O_2 loading, and as a source of nitrogen for synthesis during aerobic conditions.

5 MODEL DEVELOPMENT & APPLICATION

5.1 Model Description

One of the initial objectives of this study was to estimate biodegradation kinetics for BTEX removal. Due to the nitrogen limitation present during the experiments, the responses of the system were much more complex than anticipated. As a result, it was necessary to model the bulk parameters, prior to modeling the BTEX compounds, in an effort to understand the system.

5.1.1 Modeling the Bulk Parameters

A model of the experimental system that was based on the Activated Sludge Model No.2 (ASM2) developed by the International Association on Water Quality (IAWQ) (Henze et al., 1995) was developed. This model, which uses Monod kinetics, predicts COD and nitrogen removal. It is based on a set of interrelated biological processes and uses a matrix notation to describe kinetics and stoichiometry for the system components.

Components

The components, C , in the system are characterized as either substrates, S , or particulates, X . Substrates are soluble components that are transported with the water and may carry ionic charges. Particulates are non-soluble components associated with the biomass and are typically referred to as suspended solids. Table 5–1 describes the different components within the system.

Table 5-1: Substrate and particulate components within the model.

Symbol	Component	Description
S_F	Fermentable, readily biodegradable organic substances	<ul style="list-style-type: none"> the fraction of the soluble COD that is readily available for biodegradation. may act as a substrate for fermentation. expressed as g COD/m³.
S_{NH4}	Ammonium and ammonia nitrogen	<ul style="list-style-type: none"> assumed to be all NH₄⁺. expressed as g N/m³.
S_A	Fermentation products	<ul style="list-style-type: none"> the end-products of fermentation. a mixture of low molecular weight organics and hydrogen. includes the primary substrate, acetic acid expressed as g COD/m³.
S_I	Inert dissolved organic material	<ul style="list-style-type: none"> dissolved organic matter not biodegradable. a product of hydrolysis. expressed as g COD/m³.
S_{O2}	Oxygen	<ul style="list-style-type: none"> dissolved oxygen that may be subject to gas exchange. expressed as g O₂/m³.
S_{NO3}	Nitrate and nitrite nitrogen	<ul style="list-style-type: none"> assumed to be all NO₃⁻. expressed as g N/m³.
X_H	Heterotrophic microorganisms	<ul style="list-style-type: none"> are able to grow aerobically, anoxically (denitrification) and anaerobically (fermentation). responsible for hydrolysis of particulate substrates (X_S). expressed as g COD/m³.
X_A	Nitrifying microorganisms	<ul style="list-style-type: none"> autotrophic organisms responsible for nitrification in the presence of oxygen. expressed as g COD/m³.
X_S	Slowly biodegradable solids	<ul style="list-style-type: none"> high molecular weight, colloidal and particulate organic substrates which must undergo hydrolysis before they are available for biodegradation. may be present from the influent stream or as a result of cell lysis. expressed as g COD/m³.
X_I	Inert particulate organic material	<ul style="list-style-type: none"> material not degraded in the system. may be present from the influent stream or as a result of cell lysis. expressed as g COD/m³.

Mass Balance Approach

A mass balance on each component i , in the reactor determines the fate of that component.

$$V \frac{dC_i}{dt} = Q(C_{i,initial} - C_i) \pm V r_i \quad \text{Eqn. 5-1}$$

Several transformation processes, ρ_j , within the system may affect a component, C_i .

These are combined with expressions based on stoichiometry, v_{ji} , and summed to describe the overall rate, r_i , of generation or consumption of component i .

$$r_i = v_{1i}\rho_1 + v_{2i}\rho_2 + v_{3i}\rho_3 + \dots + v_{ni}\rho_n \quad \text{Eqn. 5-2}$$

Processes

Equations for the different processes, ρ , occurring within the system are presented in Table 5-2. These include hydrolysis, heterotrophic growth, fermentation and lysis, and autotrophic growth and lysis.

A significant modification of the ASM2 model was required to address NO_3^- utilization for synthesis. Due to the nitrogen limitation within the system, heterotrophic growth on both ammonium (NH_4^{++}) and NO_3^- for synthesis was included. This was accomplished with the addition of four new processes (5, 7, 9 and 11) to the original ASM2 model. These duplicate processes (4, 6, 8 and 10) for aerobic and anoxic growth on S_F and S_A , with the exception that a Monod function for NO_3^- , instead of NH_4^{++} , represents the limiting nutrient for synthesis.

Table 5-2: Process rate equations.

Hydrolysis processes:	
1. Aerobic hydrolysis	$K_h \cdot \frac{S_{O_2}}{K_{O_2} + S_{O_2}} \cdot \frac{X_S / X_H}{K_X + X_S / X_H} \cdot X_H$
2. Anoxic hydrolysis	$K_h \cdot \eta_{NO_3} \cdot \frac{K_{O_2}}{K_{O_2} + S_{O_2}} \cdot \frac{S_{NO_3}}{K_{NO_3} + S_{NO_3}} \cdot \frac{X_S / X_H}{K_X + X_S / X_H} \cdot X_H$
3. Anaerobic hydrolysis	$K_h \cdot \eta_{fe} \cdot \frac{K_{O_2}}{K_{O_2} + S_{O_2}} \cdot \frac{K_{NO_3}}{K_{NO_3} + S_{NO_3}} \cdot \frac{X_S / X_H}{K_X + X_S / X_H} \cdot X_H$
Heterotrophic processes:	
4. Growth on fermentable substrates, S_f using NH_4 N	$\mu_H \cdot \frac{S_{O_2}}{K_{O_2} + S_{O_2}} \cdot \frac{S_F}{K_F + S_F} \cdot \frac{S_F}{S_F + S_A} \cdot \frac{S_{NH_4}}{K_{NH_4} + S_{NH_4}} \cdot X_H$
5. Growth on fermentable substrates, S_f using NO_3 N	$\mu_H \cdot \frac{S_{O_2}}{K_{O_2} + S_{O_2}} \cdot \frac{S_F}{K_F + S_F} \cdot \frac{S_F}{S_F + S_A} \cdot \frac{S_{NO_3}}{K_{NO_3_N} + S_{NO_3}} \cdot X_H$
6. Growth on fermentation products, S_A using NH_4 N	$\mu_H \cdot \frac{S_{O_2}}{K_{O_2} + S_{O_2}} \cdot \frac{S_A}{K_A + S_A} \cdot \frac{S_A}{S_F + S_A} \cdot \frac{S_{NH_4}}{K_{NH_4} + S_{NH_4}} \cdot X_H$
7. Growth on fermentation products, S_A using NO_3 N	$\mu_H \cdot \frac{S_{O_2}}{K_{O_2} + S_{O_2}} \cdot \frac{S_A}{K_A + S_A} \cdot \frac{S_A}{S_F + S_A} \cdot \frac{S_{NO_3}}{K_{NO_3_N} + S_{NO_3}} \cdot X_H$
8. Denitrification on fermentable substrates, S_f using NH_4 N	$\mu_H \cdot \eta_{NO_3} \cdot \frac{K_{O_2}}{K_{O_2} + S_{O_2}} \cdot \frac{S_F}{K_F + S_F} \cdot \frac{S_F}{S_F + S_A} \cdot \frac{S_{NH_4}}{K_{NH_4} + S_{NH_4}} \cdot \frac{S_{NO_3}}{K_{NO_3} + S_{NO_3}} \cdot X_H$
9. Denitrification on fermentable substrates, S_f using NO_3 N	$\mu_H \cdot \eta_{NO_3} \cdot \frac{K_{O_2}}{K_{O_2} + S_{O_2}} \cdot \frac{S_F}{K_F + S_F} \cdot \frac{S_F}{S_F + S_A} \cdot \frac{S_{NO_3}}{K_{NO_3_D} + S_{NO_3}} \cdot X_H$
10. Denitrification on fermentable products, S_A using NH_4 N	$\mu_H \cdot \eta_{NO_3} \cdot \frac{K_{O_2}}{K_{O_2} + S_{O_2}} \cdot \frac{S_A}{K_A + S_A} \cdot \frac{S_A}{S_F + S_A} \cdot \frac{S_{NH_4}}{K_{NH_4} + S_{NH_4}} \cdot \frac{S_{NO_3}}{K_{NO_3} + S_{NO_3}} \cdot X_H$
11. Denitrification on fermentable products, S_A using NO_3 N	$\mu_H \cdot \eta_{NO_3} \cdot \frac{K_{O_2}}{K_{O_2} + S_{O_2}} \cdot \frac{S_A}{K_A + S_A} \cdot \frac{S_A}{S_F + S_A} \cdot \frac{S_{NO_3}}{K_{NO_3_D} + S_{NO_3}} \cdot X_H$
12. Fermentation	$q_{fe} \cdot \frac{K_{O_2}}{K_{O_2} + S_{O_2}} \cdot \frac{S_{NO_3}}{K_{NO_3} + S_{NO_3}} \cdot \frac{S_F}{K_{fe} + S_F} \cdot X_H$
13. Lysis	$b_H \cdot X_H$

Table 5-2: continued.

Autotrophic processes:	
14. Growth	$\mu_{AUT} \cdot \frac{S_{O_2}}{K_{O_2} + S_{O_2}} \cdot \frac{S_{NH_4}}{K_{NH_4} + S_{NH_4}} \cdot X_{AUT}$
15. Lysis	$b_{AUT} \cdot X_{AUT}$

Where NO_3^- plays a dual role of electron acceptor and nutrient for heterotrophic growth (processes 9 and 11), NO_3^- limitation is modeled by a single Monod function. This is consistent with NH_4^+ under autotrophic growth where it plays a dual role as nutrient source and electron donor. For this process (14), NH_4^+ is also modeled with a single Monod function.

Table 5-3 summarizes the three Monod expressions representing the different roles of nitrate within the system. It provides the process in which each occurs.

Table 5-3: Summary of NO_3^- Monod expressions.

Expression	Description	Process
$\frac{S_{NO_3}}{K_{NO_3} + S_{NO_3}}$	Nitrate acts as an electron acceptor.	2, 3, 8, 10, 12
$\frac{S_{NO_3}}{K_{NO_3_N} + S_{NO_3}}$	Nitrate acts as a source of nitrogen for synthesis.	5, 7
$\frac{S_{NO_3}}{K_{NO_3_D} + S_{NO_3}}$	Nitrate acts as both an electron acceptor and a source of nitrogen for synthesis.	9, 11

Nitrate was not considered as an alternate source of synthesis nitrogen for autotrophic growth. Since NH_4^+ is required as an electron donor as well as for synthesis, the presence of NO_3^- in a system limited by NH_4^+ would be immaterial.

Stoichiometry

Table 5-4 presents the expressions of stoichiometry, v , relating the generation and consumption of different components for each process. These are based on continuity equations in which elements, electrons and net electrical charges may neither be formed nor destroyed.

Values for Stoichiometric and Rate Coefficients

Values for most of the stoichiometric and rate coefficients were obtained from the ASM2 model for a temperature of 20°C. These represent typical values found in the literature. However, some modifications were required to calibrate the model. This included the adjustment of the following coefficients: μ_{H} , η_{NO_3} , f_{XI} , and the addition of $K_{\text{NO}_3\text{-N}}$ and $K_{\text{NO}_3\text{-D}}$ to describe the dual roles of NO_3^- within the system. A discussion of these changes follows in section 5.2.1. Table 5-5 defines each of the stoichiometric and rate coefficients used in the model, and presents the final values obtained for the calibrated model.

Table 5-4: Component stoichiometry according to each process.

Hydrolysis processes:								
Process	S _F	S _I	S _{NH4}			X _S		
1. Aerobic hydrolysis	1-f _{SI}	f _{SI}	i _{NXS} -(i _{NSF} X (1-f _{SI})+i _{NSI} X f _{SI})			-1		
2. Anoxic hydrolysis	1-f _{SI}	f _{SI}	i _{NXS} -(i _{NSF} X (1-f _{SI})+i _{NSI} X f _{SI})			-1		
3. Anaerobic hydrolysis	1-f _{SI}	f _{SI}	i _{NXS} -(i _{NSF} X (1-f _{SI})+i _{NSI} X f _{SI})			-1		
Heterotrophic processes:								
Process	S _{O2}	S _F	S _A	S _{NO3}	S _{NH4}	X _I	X _S	X _H
4. Growth on fermentable substrates, S _f using NH ₄ N	$1 - \frac{1}{Y_H}$	$-\frac{1}{Y_H}$			-i _{NBM}			1
5. Growth on fermentable substrates, S _f using NO ₃ _N	$1 - \frac{1}{Y_H}$	$-\frac{1}{Y_H}$		-i _{NBM}				1
6. Growth on fermentation products, S _A using NH ₄ N	$1 - \frac{1}{Y_H}$		$-\frac{1}{Y_H}$		-i _{NBM}			1
7. Growth on fermentation products, S _A using NO ₃ N	$1 - \frac{1}{Y_H}$		$-\frac{1}{Y_H}$	-i _{NBM}				1
8. Denitrification on fermentable substrates, S _f using NH ₄ N		$-\frac{1}{Y_H}$		$-\frac{1-Y_H}{2.86Y_H}$	-i _{NBM}			1
9. Denitrification on fermentable substrates, S _f using NO ₃ N		$-\frac{1}{Y_H}$		$-\frac{1-Y_H}{2.86Y_H} - i_{NBM}$				1
10. Denitrification on fermentable products, S _A using NH ₄ N			$-\frac{1}{Y_H}$	$-\frac{1-Y_H}{2.86Y_H}$	-i _{NBM}			1
11. Denitrification on fermentable products, S _A using NO ₃ N			$-\frac{1}{Y_H}$	$-\frac{1-Y_H}{2.86Y_H} - i_N$				1
12. Fermentation		-1	1					
13. Lysis						f _{XI}	1-f _{XI}	-1

Table 5-4: continued.

Autotrophic processes:						
Process	S_{O2}	S_{NH4}	S_{NO3}	X_I	X_S	X_A
14. Growth	$-\frac{4.57 - Y_A}{Y_A}$	$-i_{NBM} - \frac{1}{Y_A}$	$\frac{1}{Y_A}$			1
15. Lysis				f _{XI}	1-f _{XI}	-1

Note: 1. Parameter definitions are given in Table 5-5.

Table 5-5: Stoichiometric and rate coefficients.

Symbol	Value	Units	Description
Hydrolysis Processes:			
K_h	3.00	1/d	Hydrolysis rate constant
η_{NO_3}	0.60	-	Anoxic hydrolysis reduction factor
η_{fe}	0.10	-	Anaerobic hydrolysis reduction factor
K_{O_2}	0.20	g O ₂ /m ³	Saturation/inhibition coefficient for oxygen
K_{NO_3}	0.50	g N/m ³	Saturation/inhibition coefficient for nitrate
K_X	0.10	g COD/g COD	Saturation coefficient for particulate COD
f_{SI}	0.00	g COD/g COD	Fraction of inert COD in particulate substrate
i_{NXS}	0.04	g N/g COD	N content of particulate substrate X_S
i_{NSF}	0.03	g N/g COD	N content of soluble substrate S_F
i_{NSI}	0.01	g N/g COD	N content of inert soluble S_I
Heterotrophic Processes:			
μ_H	2.50	1/d	Maximum growth rate on substrate
q_{fe}	3.00	g COD/g COD.d	Maximum rate for fermentation
η_{NO_3}	0.10	-	Reduction factor for denitrification
b_H	0.40	1/d	Rate constant for lysis
K_{O_2}	0.20	g O ₂ /m ³	Saturation/inhibition coefficient for oxygen
K_F	4.00	g COD/m ³	Saturation coefficient for growth on S_F
K_{fe}	20.00	g COD/m ³	Saturation coefficient for fermentation on S_F
K_A	4.00	g COD/m ³	Saturation coefficient for growth on S_A
K_{NO_3}	0.50	g N/m ³	Saturation/inhibition coefficient for nitrate as an electron acceptor
$K_{NO_3\ N}$	5.00	g N/m ³	Saturation coefficient for nitrate as a nutrient
$K_{NO_3_D}$	5.00	g N/m ³	Saturation coefficient for nitrate as both electron acceptor and nutrient
K_{NH_4}	0.05	g N/m ³	Saturation coefficient for ammonium (nutrient)
Y_H	0.63	g COD/g COD	Yield coefficient
i_{NBM}	0.07	g N/COD	N content of biomass X_H
f_{XI}	0.01	g COD/g COD	Fraction of inert COD generated in biomass lysis
Autotrophic processes:			
μ_{AUT}	1.00	1/d	Maximum growth rate
b_{AUT}	0.15	1/d	Decay rate
K_{O_2}	0.50	g O ₂ /m ³	Saturation coefficient for oxygen
K_{NH_4}	1.00	g N/m ³	Saturation coefficient for ammonium
Y_A	0.24	g COD/g N	Yield coefficient
i_{NBM}	0.07	g N/g COD	N content of biomass X_H
f_{XI}	0.01	g COD/g COD	Fraction of inert COD generated in biomass lysis

Solving the System

The program Aquasim 2.0 (Reichert, 1998) was used as a platform to solve the differential equations of the modified ASM2 model. This program provided a dynamic solution that was run for a period long enough that steady state solutions were obtained.

The initial values for the substrates S_A (the primary substrate acetic acid), SO_2 , S_{NO_3} and S_{NH_4} were obtained from measured or calculated data. These varied according to each operating condition. Initial values for the substrates S_I and S_F were set to 5 g COD/m³. The particulates X_H , X_A , X_S and X_I were initially set to 20 g COD/m³. Initial values for both these last two substrates and all of the particulates remained constant under all operating conditions within the model.

In order to simulate the settling process, and hence the retainment of VSS, an 80% recycle of all particulates (X_H , X_A , X_S and X_I) was incorporated into the program. It is understood that a single value for recycle does not accurately describe the experimental system. Where the production of VSS within the reactor was high, it is unlikely that 20% was removed in the effluent after the settling process. In these cases, the recycle rate was probably much higher. Conversely, at times of low VSS, the recycle rate was probably lower. Attempts were made to incorporate into the model a variable recycle rate dependent upon the level of VSS in the reactor, however, this was not possible using the Aquasim platform. As a result, an average value of 80% was used.

5.1.2 Modeling BTEX Biodegradation

BTEX degradation was incorporated into the model by the addition of a substrate, S_{VOC} , which could be used as another source for bacterial growth. Each BTEX compound was modeled separately, except for m&p-xylenes due to the combined experimental results.

In order to model the BTEX degradation, it was assumed that the ratio of bacteria using the primary substrate (acetic acid) to those using the secondary substrate (BTEX) was equal to the ratio of the concentrations of the two substrates present or entering the system. This is consistent with the approach used by Moos et al. (1983) and Hutchinson and Robinson (1988). To simulate this growth, four additional processes (16 to 19) were incorporated into the model. These processes, outlined in Table 5-6, represent growth under aerobic and anoxic conditions with either NH_4^+ or NO_3^- as a source of nitrogen for synthesis.

Similar to the primary substrate, acetic acid, a Monod expression $\frac{S_{\text{VOC}}}{S_{\text{VOC}} + K_{\text{VOC}}}$ is used to represent the limitations on growth caused by the BTEX compounds.

Table 5-6: Process rate equations for BTEX biodegradation.

16. Growth on VOC using NH ₄ _N	$\mu_{\text{VOC}} \cdot \frac{S_{\text{O}_2}}{K_{\text{O}_2} + S_{\text{O}_2}} \cdot \frac{S_{\text{VOC}}}{K_{\text{VOC}} + S_{\text{VOC}}} \cdot \frac{S_{\text{NH}_4}}{K_{\text{NH}_4} + S_{\text{NH}_4}} \cdot X_H$
17. Growth on VOC using NO ₃ _N	$\mu_{\text{VOC}} \cdot \frac{S_{\text{O}_2}}{K_{\text{O}_2} + S_{\text{O}_2}} \cdot \frac{S_{\text{VOC}}}{K_{\text{VOC}} + S_{\text{VOC}}} \cdot \frac{S_{\text{NO}_3}}{K_{\text{NO}_3_N} + S_{\text{NO}_3}} \cdot X_H$
18. Denitrification on VOC using NH ₄ _N	$\mu_{\text{VOC}} \cdot \eta_{\text{NO}_3} \cdot \frac{K_{\text{O}_2}}{K_{\text{O}_2} + S_{\text{O}_2}} \cdot \frac{S_{\text{VOC}}}{K_{\text{VOC}} + S_{\text{VOC}}} \cdot \frac{S_{\text{NH}_4}}{K_{\text{NH}_4} + S_{\text{NH}_4}} \cdot \frac{S_{\text{NO}_3}}{K_{\text{NO}_3} + S_{\text{NO}_3}} \cdot X_H$
19. Denitrification on VOC using NO ₃ _N	$\mu_{\text{VOC}} \cdot \eta_{\text{NO}_3} \cdot \frac{K_{\text{O}_2}}{K_{\text{O}_2} + S_{\text{O}_2}} \cdot \frac{S_{\text{VOC}}}{K_{\text{VOC}} + S_{\text{VOC}}} \cdot \frac{S_{\text{NO}_3}}{K_{\text{NO}_3_N} + S_{\text{NO}_3}} \cdot \frac{S_{\text{NO}_3}}{K_{\text{NO}_3} + S_{\text{NO}_3}} \cdot X_H$

The expressions for stoichiometry for each component participating in the BTEX degradation processes are presented in Table 5-7.

Table 5-7: Component stoichiometry according to each process for BTEX degradation.

Process	S _{O2}	S _{VOC}	S _{NO3}	S _{NH4}	X _H
16. Growth on VOC using NH ₄ _N	$1 - \frac{1}{Y_H}$	$-\frac{1}{Y_H}$		$-i_{\text{NBM}}$	1
17. Growth on VOC using NO ₃ _N	$1 - \frac{1}{Y_H}$	$-\frac{1}{Y_H}$	$-i_{\text{NBM}}$		1
18. Denitrification on VOC using NH ₄ _N		$-\frac{1}{Y_H}$	$-\frac{1 - Y_H}{2.86 Y_H}$	$-i_{\text{NBM}}$	1
19. Denitrification on VOC using NO ₃ _N		$-\frac{1}{Y_H}$	$-\frac{1 - Y_H}{2.86 Y_H} - i_{\text{NBM}}$		1

Two new rate coefficients were introduced for the addition of BTEX degradation. These are the maximum growth rate on the BTEX substrate, μ_{VOC} , and the half-saturation constant for BTEX, K_{VOC} . The model was calibrated using these two coefficients. Initial

values of S_{VOC} for the program were taken from experimental data. Note that the subscript VOC has been used to avoid confusion with maximum growth rates and half-saturation constants of other substrates. However, these are equivalent to the Monod kinetic parameters μ_{MAX} and K_S .

The final values for μ_{VOC} and K_{VOC} , as presented in Table 5-8, were obtained by comparing the experimental and model effluent BTEX concentrations at steady state. The model was executed using μ_{VOC} values ranging between 0.05 and 0.15 d^{-1} . The final μ_{VOC} and K_{VOC} values were those that provided the smallest sum of squared differences between the experimental and model data sets for all operating conditions.

Table 5-8: Final model values for μ_{VOC} and K_{VOC} .

Coefficient	Benzene	Toluene	Ethylbenzene	m&p-Xylene	o-Xylene
$\mu_{VOC} (d^{-1})$	0.07	0.10	0.13	0.12	0.14
$K_{VOC} (g\ COD/m^3)$	20	11	4	7	9

It is recognized that μ_{VOC} and K_{VOC} are highly correlated. In determining these values, there was little difference in the sum of the squared differences between measured and predicted values within the range of μ_{VOC} examined. Given the nature of the data, a sophisticated statistical estimate was not possible.

5.2 Model Analysis

5.2.1 Analysis of the Bulk Parameter Model

Model Calibration

As discussed in Section 5.1.1, calibration of the model was attempted by varying the following coefficients: μ_H , η_{NO_3} , and f_{XI} , as well as the saturation coefficients for NO_3^- : $K_{NO_3_N}$ and $K_{NO_3_D}$. It was assumed that values for all other coefficients, as given in the ASM2 model, were applicable to the experimental data. The initial step to the calibration process focused on experiments with highly aerobic environments (1 to 5). Using these experiments, μ_H , f_{XI} and $K_{NO_3_N}$ were varied until reasonable results were achieved for all of these experiments. Based on effluent COD and reactor VSS concentrations, it appeared early on that μ_H would be significantly higher than its corresponding ASM2 value. Varying the value for f_{XI} had a minimal effect on all of the parameters except reactor VSS concentration. It was decreased to minimize the amount of VSS produced within the system. Changing the value for $K_{NO_3_N}$ was reflected in the effluent NO_3^- concentrations only. Using these concentrations as a response, the $K_{NO_3_N}$ value was adjusted until these measured values were approximated.

The second portion of the bulk parameter modeling effort involved the determination of $K_{NO_3_D}$ and η_{NO_3} . For this part, data from all experiments was utilized. It was decided that $K_{NO_3_D}$, where NO_3^- acts as both electron acceptor and assimilative nitrogen, would be equivalent to the value for $K_{NO_3_N}$, where NO_3^- acts solely as assimilative nitrogen. Rather than using two Monod functions for the different roles of NO_3^- , a single Monod

function was utilized. Since the use of NO_3^- for synthesis has a greater limiting effect than NO_3^- as an electron acceptor, i.e. a larger saturation coefficient ($K_{\text{NO}_3_N}$), this value would also be employed for $K_{\text{NO}_3_D}$. The reduction factor for denitrification, η_{NO_3} , had a significant effect on effluent COD, reactor VSS and effluent NO_3^- concentrations for the denitrification processes (8 to 11). This value was varied until the observed responses were simulated.

Slight modifications were then made to all of the coefficients to refine the calibration. However, it is recognized that this is not a robust calibration. Final values were chosen when the data set appeared to be reasonably well approximated and general trends were duplicated.

Model Calibration Results and Interpretation

The final values for the coefficients, as presented in Table 5-5 of Section 5.1.2, may be used to interpret the microbial activity within the experiment.

The maximum heterotrophic growth rate, μ_H , at a final value of 2.5 d^{-1} , was less than half the typical value of 6.0 d^{-1} presented in the ASM2 model. This limited, to a greater extent, the formation of heterotrophic microorganisms (X_H) as well as the utilization of oxygen (O_2), substrate (S_F or S_A), ammonium (NH_4^+) and/or nitrate (NO_3^-) (processes 4 to 11 in Table 5-2). The decrease in μ_H indicated that the bacteria were not as active as has been observed elsewhere.

The final value for the reduction factor for denitrification was 0.1; a decrease from 0.8 in the ASM2 model. As a result, this limited the growth of X_H and utilization of S_F , S_A , S_{NH4} and S_{NO3} even further under anoxic conditions (processes 8 to 11). In terms of microbial activity, the growth under anoxic conditions was much slower than under aerobic conditions. This indicates that there were few facultative heterotrophic bacteria in the consortia.

The change in value for f_{XI} from 0.1, of the ASM2 model, to 0.01 limited the amount of inert particulates (X_I) and, hence, increased the slowly biodegradable particulates (X_S) during lysis (processes 13 and 15). Since the X_S was then used as a source of substrate for growth via hydrolysis (processes 1 to 3), the total particulate matter at steady state was reduced. This was done to simulate the reactor VSS values, and had little effect on other parameters.

Values for the saturation coefficients for NO_3^- are indicative of the bacteria's ability to utilize NO_3^- as an electron acceptor and/or as a source of nitrogen for synthesis. Where NO_3^- acted solely as an electron acceptor (processes 2, 3, 8, 10 and 12), the saturation coefficient, K_{NO3} , had a value of 0.5 g N/m^3 . This value was greater than the saturation coefficient for oxygen, K_{O2} , which constrained growth via denitrification more quickly than aerobic growth, if the electron acceptor concentrations were limited.

The value of 5.0 g N/m^3 for K_{NO3_N} and K_{NO3_D} was much greater than the coefficient of nitrate's competitor ammonium, K_{NH4} , which had a value of 0.05 g N/m^3 . With respect to

the model, growth using NO_3^- for synthesis (processes 5, 7, 9 and 11) was much more limited than that using NH_4^+ (processes 4, 8, 10 and 12). It is indicative of the bacteria's ability to use NH_4^+ as a source of nitrogen more easily than NO_3^- . There is a greater expenditure of energy for the bacteria to reduce NO_3^- for synthesis as opposed to using NH_4^+ .

Comparison of Experimental and Model Bulk Parameters

The experimental and model-predicted data for effluent and reactor concentrations at steady state are presented in Table 5-9. Also included in this table is the effluent NH_4^+ concentration predicted by the model. This was not a measured parameter and, hence, there is no experimental data for comparison.

The general trends observed between experiments, as discussed in Section 4.2.1, appear to be replicated by the model. It adequately predicted the effluent and reactor concentrations of the bulk parameters, with the exception of effluent O_2 concentrations for all experiments and the effluent COD concentration for experiment 5, specifically.

The discrepancies between model and experimental effluent O_2 concentration are problematic. However, other predicted responses from the model were not based on these values. The model was driven by electron acceptor addition and not by conditions within the reactor at steady state. As described in Section 5.1.1, electron acceptor loading rates, via O_2 and NO_3^- influent concentrations, were the initial values utilized to run the model. (It is recognized that the influent COD concentration was also a parameter that

Table 5-9: Experimental and model-predicted bulk parameters.

Experiment	Effluent O ₂ (g O ₂ /m ³)		Effluent NO ₃ ⁻ (g N/m ³)		Effluent COD (g COD/m ³)		Reactor VSS (g O ₂ /m ³)		Effluent NH ₄ ⁺ (g N/m ³)
	Exp.	Model	Exp.	Model	Exp.	Model	Exp.	Model	
1	10.11	252	-	0.23	402	610	531	497	0.01
2	0.63	178	-	0.22	399	402	619	497	0.01
3	2.31	218	-	0.22	424	617	846	497	0.01
4	20	294	365	347	34	1	2198	1521	0.02
5	1.45	0.051	330	281	532	7.5	1505	1836	0.03
6	0.528	0.003	304	332	682	545	602	646	2.8
7	0.31	0	363	336	636	607	305	347	8.9
8	0.36	0	56	59	748	841	142	324	9.25
9	0.207	0	1	16	842	903	65	283	9.8
10	1.25	0	9	3.4	486	536	435	669	0.1
11	0.382	0	25	6.5	523	577	376	496	1.8
12	0.659	0	38	17	486	457	219	653	1.2

varied with experiment. However, influent COD was intended to remain constant over the experimental period and did not fluctuate substantially. As a result, it did not have a major influence on the general trends among experiments predicted by the model).

Model-Predicted Growth Rates

Nearly all of the microbial growth under all experiments was heterotrophic. Growth rates from each of the 8 heterotrophic processes (4 to 11 in Table 5-2) have been separated according to aerobic and anoxic conditions, and are plotted in Figures 5-1 and 5-2 respectively. Figure 5-3 is a plot of the total heterotrophic growth during each experiment.

Interpretation of Experimental Data from Model-Predicted Bulk Parameters and Growth Rates

During the first three experiments, a substantial portion of the growth was on S_A using NH_4^+ for synthesis. Growth on S_F using NH_4^+ and on S_A using NO_3^- for nitrogen were much less, but still contributed to the total heterotrophic growth. The presence of S_F within the system was from microbial decay and hydrolysis and hence, it was only expected to be present at low levels. While there was no NO_3^- addition during these experiments, there was some formation of NO_3^- as a result of NH_4^+ utilization during autotrophic growth (process 14). The model predicted some residual NO_3^- in the effluent (Table 5-9). However, comparison with experimental data was not possible since effluent NO_3^- was not measured during these experiments. Anoxic rates for these experiments were negligible.

Figure 5-1: Aerobic growth based on model simulations.

Note: 1. Corresponding process numbers are in parentheses.

Figure 5-2: Anoxic growth based on model simulations.

Note: 1. Corresponding process numbers are in parentheses.

Figure 5-3: Total heterotrophic growth based on model simulations.

In contrast to experiments 1 to 3, the model predicted that the growth for experiments 4 and 5 was based largely on the use of NO_3^- for synthesis on both S_A and S_F . In addition, there was an overall increase in total heterotrophic growth. The high O_2 loading resulted in mostly aerobic growth with some anoxic growth during experiment 5. Also, the model predicted lower effluent COD and higher reactor VSS concentrations in contrast to the first 3 experiments. These indicators are consistent with most of the experimental data. This suggests that the first three experiments were limited by the lack of nitrogen for synthesis, as discussed in Section 4.2.1. The only discrepancy between model and experimental data was the experimental effluent COD concentration during experiment 5, which was relatively high despite a high reactor VSS concentration.

The differences between experiments 4 and 5, according to the model, were slightly lower aerobic and slightly greater anoxic growth rates for experiment 5. Oxygen, which had a reduced availability for growth, during experiment 5, was completely consumed as indicated by the negligible O_2 effluent concentration. This caused a limitation on aerobic growth and hence, forced the model to utilize NO_3^- as an electron acceptor.

For experiment 6, where O_2 loading was minimal, the major process for growth was on S_A using NH_4^+ for synthesis under anoxic conditions. However, due to the low η_{NO_3} and K_{NO_3} values, denitrification, and hence total growth, decreased from the previous two experiments, despite the high NO_3^- loading rate. This was reflected in the high effluent NO_3^- concentration. The corresponding effluent COD and reactor VSS were also indicative of the decreased growth.

In experiments 7 to 9, where there was no O_2 loading, the model predicted zero aerobic growth, as expected. The anoxic growth was equally distributed between growth on S_A using NH_4^+ and NO_3^- as sources of nitrogen for synthesis. As the NO_3^- loading was decreased, the total heterotrophic growth also decreased. However, for all three experiments, the utilization of NO_3^- was not complete. The observed effluent NO_3^- concentrations, predicted by the model, were relatively high in conjunction with their corresponding influent concentrations. Experimental values for experiments 7 and 8 were consistent with this result. The effluent NO_3^- concentration for experiment 9 was less than its corresponding model value. Both model and experimental values for effluent COD and reactor VSS concentrations agreed with the decreased growth for these three experiments. COD utilization decreased resulting in higher effluent concentrations. At the same time, reactor VSS concentrations also decreased indicating less microbial growth.

Where low O_2 addition was combined with low NO_3^- addition (experiments 10 to 12) the total heterotrophic growth increased as compared to conditions of no O_2 addition. The model attributed this to both aerobic and anoxic growth. In a comparison between experiments 9 and 10, which had similar NO_3^- loading rates, the presence of O_2 , during experiment 10, not only caused aerobic growth, which was non-existent previously, but also caused an increase in anoxic growth. The model predicted nearly twice as much growth for each of the anoxic processes (8, 9, 10 and 11) of experiment 10, when compared with experiment 9. This was partially a result of aerobic hydrolysis (process 1), in which X_H is degraded into S_F , allowing for increased growth. An increase in the

amount of NO_3^- availability through the utilization of NH_4^+ for autotrophic growth (process 14) was also partially responsible for this effect. Bulk parameters were consistent with this trend of increased growth between experiment 9 and 10. The effluent COD concentration decreased by nearly half, and the reactor VSS concentration increased substantially.

The trends in aerobic and anoxic growth rates corresponded to the trends in O_2 and NO_3^- loading rates for experiments 10, 11 and 12. Aerobic growth, for experiment 10, was approximately double that of 11 and 12, similar to the O_2 loading rates. Anoxic growth remained constant for experiments 10 and 11, and increased for experiment 12, as did the NO_3^- loading rates.

The use of NO_3^- as a source of nitrogen for synthesis was predicted in the model for these last three experiments. Clearly, NH_4^+ was utilized to a greater extent under both aerobic and anoxic conditions. However, the availability of NH_4^+ might have limited growth, had NO_3^- not been present. Growth rates on S_A using NO_3^- for nitrogen were only one third less than growth rates using NH_4^+ . The lower rates were a result of the higher saturation coefficients, $K_{\text{NO}_3_N}$ and $K_{\text{NO}_3_D}$, for NO_3^- .

The bulk parameters, again, followed the growth trends for experiments 10, 11 and 12, with the exception of the reactor VSS concentration for experiment 12. In this instance, the concentration was much lower than predicted by the model.

5.2.2 Analysis of BTEX Biodegradation Model

Model Calibration Results

Results of the model calibration for BTEX biodegradation are presented in Table 5-8 of Section 5.1.2. Maximum growth rates, μ_{VOC} , ranged from 0.07 to 0.13 d^{-1} for the BTEX compounds. The smallest maximum growth rate of 0.07 d^{-1} described benzene biodegradation. Toluene, m&p-Xylenes, ethylbenzene and o-Xylene with larger values followed this. It suggests that microbial growth was slowest when utilizing benzene as a substrate. In contrast, ethylbenzene, m&p-Xylenes and o-Xylene, with similar maximum growth rates of 0.13, 0.12 and 0.14 d^{-1} , provided substrates that were more easily utilized by the bacteria.

The half-saturation constants, K_s , ranged from 4 to 20 $\text{g O}_2/\text{m}^3$. With the largest value, growth on benzene was limited at higher concentrations than the other BTEX compounds. The half-saturation constant estimated for ethylbenzene was the smallest, suggesting that, even at low concentrations of ethylbenzene, the bacteria were able to efficiently use the substrate.

The magnitudes of the K_s values, for all BTEX compounds, were substantially greater than effluent BTEX concentrations by one or two orders of magnitude. This suggests that the data may have been adequately modeled by a first order approximation.

Comparison of Model and Experimental Effluent BTEX Rates

Final values for the effluent BTEX rates, predicted by the model, are presented in Table 5-10. These are compared with measured values from the experiment. The sums of the squared differences between model and experimental effluent rates were greatest for benzene, followed by m&p-Xylenes, toluene, o-Xylene and ethylbenzene, which indicated that ethylbenzene biodegradation was modeled the best. The model over-predicted effluent rates of all BTEX compounds for experiments 1, 2, 3 and 9. This means that, for the given electron acceptor loading rates, the model expected less growth utilizing BTEX for these than experimental data suggested. Values were under-predicted for all BTEX compounds for experiments 4, 5 and 12. In these cases, the model predicted greater growth than was observed, on the BTEX compounds. The remaining experiments contained both over- and under-predicted experimental rates.

Comparison of Model and Experimental Removal Rates

The BTEX removal rates from both model and experimental data were plotted in Figures 5-4 to 5-8. It is important to note that the experimental removal rates in these figures were calculated from average influent and effluent BTEX concentrations to be consistent with the calculations for the model removal rates. There are slight discrepancies between the experimental removal rates from this section and those from Section 4.2.2 where experimental removal rates are average values calculated from three (or in a few cases two) sets of influent and effluent data points. These discrepancies occur where the outlier analysis, described in Section 4.1.4, had resulted in the availability of only two data

Table 5-10: Experimental and model-predicted effluent BTEX rates.

Experiment	Benzene (mg/L.day)		Toluene (mg/L.day)		Ethylbenzene (mg/L.day)		m&p-Xylenes (mg/L.day)		o-Xylene (mg/L.day)	
	Exp.	Model	Exp.	Model	Exp.	Model	Exp.	Model	Exp.	Model
1	19	73	8	56	8	20	14	61	16	52
2	20	73	10	55	10	20	22	61	30	52
3	13	73	8	56	11	20	22	61	32	52
4	147	26	84	8	29	2	85	4	66	2
5	77	34	34	10	13	2	41	6	30	3
6	37	82	14	35	4	9	12	21	10	9
7	77	94	38	55	12	19	45	47	32	25
8	87	101	52	67	16	24	28	22	20	13
9	72	74	31	43	12	18	39	72	26	30
10	150	154	75	66	19	14	84	42	51	23
11	187	194	114	101	37	27	94	69	60	39
12	213	184	152	89	43	22	130	60	81	33
Sum of Squares	29,279		18,264		1,961		21,454		10,591	

Figure 5-4: Experimental and model removal rates for benzene. **Figure 5-5: Experimental and model removal rates for toluene.**

Figure 5-6: Experimental and model removal rates for ethylbenzene.

Figure 5-7: Experimental and model removal rates for m&p-Xylenes.

Figure 5-8: Experimental and model removal rates for o-Xylene.

points for further analysis. For the most part, the model followed the general trends of the data. Further discussion of these figures follows in the next section.

BTEX Removal Rates during Model-predicted Aerobic and Anoxic Conditions

The contributions of aerobic and anoxic conditions to the total removal rates are presented in Figures 5-9 to 5-13. This analysis was used to confirm conclusions, regarding O_2 and NO_3^- uptake rates and experimental BTEX removal, made in Sections 4.2.1 and 4.2.2.

During experiments 1, 2 and 3, aerobic growth using NH_4^+ for synthesis was predicted to be the major source of BTEX utilization, as discussed previously. Some aerobic growth using NO_3^- occurred, despite a lack of NO_3^- addition. As described in the model bulk parameter analysis (Section 5.2.1). This could be attributed to autotrophic growth (process 14).

Corresponding to the microbial growth predicted by the model (Section 5.2.1), there was a substantial increase in the utilization of BTEX compounds, with the exception of o-Xylene, during experiment 4. The decreased removal for o-Xylene was most likely a result of the low influent concentration. The model indicated that the increased removal was a result of considerable aerobic growth using NO_3^- as a source of nitrogen for synthesis. This suggests that the previous experiments (1 to 3) were limited by the lack of nitrogen. The trends exhibited by the model were consistent with experimental BTEX

Figure 5-9: Model removal rates for benzene under aerobic and anoxic conditions.

Note: 1. Corresponding process numbers are in parentheses.

Figure 5-10: Model removal rates for toluene under aerobic and anoxic conditions.

Note: 1. Corresponding process numbers are in parentheses.

Figure 5-11: Model removal rates for ethylbenzene under aerobic and anoxic conditions.

Note: 1. Corresponding process numbers are in parentheses.

Figure 5-12: Model removal rates for m&p-Xylenes under aerobic and anoxic conditions.

Note: 1. Corresponding process numbers are in parentheses.

Figure 5-13: Model removal rates for o-Xylene under aerobic and anoxic conditions.

Note: 1. Corresponding process numbers are in parentheses.

removal, with the exception of m&p-xylenes, which did not show an increase in removal rate during experiment 4.

During experiment 5, aerobic growth using NO_3^- continued to be the major cause of BTEX removal. However, the model indicated some growth on BTEX, using NO_3^- as an electron acceptor.

In a comparison of experiments 4 and 5, which both demonstrated substantial heterotrophic aerobic growth on S_A and S_F (Figure 5-1), there was a large difference between removal rates of the two conditions for both experimental and model data (Figures 5-4 to 5-8); those of experiment 4 being much higher. The larger influent BTEX concentrations were partially responsible for this effect. By using the Monod function to describe microbial growth, the lower influent concentrations of experiment 5 for all BTEX compounds were more limiting to growth than the higher influent concentrations of experiment 4. In addition, there was much more O_2 available for growth during experiment 4 due to the higher O_2 loading rate. While anoxic growth played a greater role during experiment 5 than 4, the combination of aerobic and anoxic growth on the BTEX compounds was not as great as the aerobic growth of experiment 4. The low reduction factor for denitrification, η_{NO_3} , limited the anoxic growth to a certain extent.

The model predicted that anoxic growth on NH_4^+ and NO_3^- were the two major causes of BTEX removal during experiment 6. However, the behaviour between the experimental and model data, as well as among the BTEX compounds was not consistent. The

experimental data for all compounds, except o-xylene, exhibited an increase in removal as compared to experiment 5. In contrast, the model data depicted a decrease in removal rates, when compared to experiment 5, for all compounds except ethylbenzene, which remained fairly constant. For the model, this was a result of varying influent concentrations and different values for μ_{VOC} and K_{VOC} , which were specific to the compounds. The total heterotrophic growth on S_A and S_F , discussed in Section 5.1.1, was reduced from experiment 5 due to the substantially decreased O_2 loading rate and the low rate of denitrification caused by η_{NO_3} . As specified, this same trend was exhibited in the model data of all BTEX compounds, except ethylbenzene, which remained unchanged.

For experiments 7, 8 and 9, very low removal rates were predicted by the model. Due to the lack of O_2 addition, strictly anoxic growth on BTEX occurred. For m&p-Xylenes and o-Xylene, the removal rates for experiment 9, were higher than those of experiment 8, despite a decrease in NO_3^- loading. The greater influent concentrations, of experiment 9 were responsible for this effect. In general, the trends displayed by experimental removal rates, were replicated well by the model for these experiments.

For all BTEX compounds, the model and experimental removal rates for experiments 10, 11 and 12 were greater than those of 7, 8 and 9. The aerobic growth resulting from the O_2 addition was a direct cause of this result. In addition, the influent BTEX concentrations were greater for these last three experiments. For experiment 10, aerobic growth on NH_4^+ was predominant among all BTEX compounds. For all compounds, anoxic growth on NH_4^+ was predicted to be the greatest for both experiments 11 and 12.

The only exception was benzene, which had a greater removal rate for anoxic growth on NO_3^- for experiment 12.

Model-predicted removal rates followed the trends of the experimental data for experiment 10 and 11. For experiment 12, the model over-predicted removal rates for all BTEX compounds.

One problem with the data was the variability of influent BTEX concentrations among experiments. Since concentration has an effect on growth via its presence in the Monod function, the variable influent BTEX concentrations would be reflected in the responses of the other measured parameters. At low concentrations, growth is inhibited by the Monod function. During experiments 6 to 9, with extremely low influent BTEX concentrations, system responses would be strongly affected. This was most evident for o-Xylene, which had very low influent concentrations. While the general trends of the bulk parameter experimental data were reflected with the model, trends in removal rates with respect to O_2 and NO_3^- loading may not be as evident as under more constant influent BTEX concentrations.

5.3 Comparison of Results with Literature

5.3.1 Monod Rate Constants

The Monod rate constants estimated within this report are compared with literature values in Tables 5-11 and 5-12. Note that the values for K_s presented in Table 5-8 of Section 5.1.2 have been converted to mg BTEX/L

The μ_{MAX} values from this study appear to be low when compared with published values, although not unrealistic. There is considerable variability amongst μ_{MAX} values reported within the literature. For benzene and toluene, μ_{MAX} values range up to 3 and 4 orders of magnitude, respectively. The μ_{MAX} for benzene, from this report, falls outside the range of published data by one order of magnitude. Toluene, however, is well within the range of literature values. The results of this analysis for ethylbenzene and the xylenes are one order of magnitude below published values. Unfortunately, there is limited data of Monod rate constants for these compounds. Since, the comparisons for ethylbenzene and the xylenes are against one or two other studies, the results of this report cannot be considered inappropriate.

The published values of the half-saturation constant, K_s , also vary substantially, over 3 and 4 orders of magnitude for benzene, and toluene respectively. The results presented in this report for all BTEX compounds are within the range of values from these studies. (Note that the values of K_s , from this analysis (Section 5.2.2), have been converted to mg BTEX/L).

Table 5-11: Comparison of model μ_{MAX} with literature values.

Substrate	Present Analysis	Documented Values			
		Aerobic (d^{-1})	Number of Studies	Anoxic (d^{-1})	Number of Studies
Benzene	0.07	0.89 - 16.32	9	4.8	2
Toluene	0.10	0.00-37.44	13		
Ethylbenzene	0.13	5.18	1		
m-Xylene		2.95	1		
o-Xylene	0.14	2.03	1		
p-Xylene		3.12 - 3.36	2		
m&p-Xylenes	0.12				
Xylenes		1.5 - 12.85	2		

Note: 1. Based on Tables 2-4, 2-5 and 2-6 of Section 2.3.2.

Table 5-12: Comparison of model K_s (mg BTEX/L) with literature values.

Substrate	Present Analysis	Documented Values			
		Aerobic (mg/L)	Number of Studies	Anoxic (mg/L)	Number of Studies
Benzene	6.5	0.31 - 20.31	9	0.11 - 0.15	2
Toluene	3.5	0.04 - 20.27	13		
Ethylbenzene	1.3	10.07	1		
m-Xylene		0.75	1		
o-Xylene	2.2				
p-Xylene		2.47 - 15.93	2		
m&p-Xylenes	2.8				
Xylenes		0.85 - 13.27	2		

Note: 1. Based on Tables 2-4, 2-5 and 2-6 of Section 2.3.2.

It is also recognized that this study modeled the data with one set of Monod rate constants to describe the biodegradation under aerobic, mixed aerobic/anoxic and anoxic redox zones. Typically, these constants have been analyzed according to a single redox zone, for the most part, aerobic. Evidence of other studies estimating Monod parameters for changing redox zones was not found.

5.3.2 Mixed Redox Zones

As observed in other studies (Bonin et al, 1989, Krul and Veeningen, 1977, and Nakajima et al., 1984) results obtained in this study indicated that BTEX degradation did occur under mixed low O_2 and NO_3^- conditions. During experiments 6, 10, 11 and 12, with combinations of low O_2 and high, medium and low NO_3^- concentrations, some of the BTEX degradation was attributed to anoxic microbial growth using both NO_3^- and NH_4^+ as sources of nitrogen.

Major et al. (1988) and Alvarez and Vogel (1995) observed that some of the BTEX compounds were biodegraded more quickly under mixed O_2 and NO_3^- conditions than either electron acceptor alone. In contrast to the experiments without O_2 addition (7, 8 and 9), BTEX removal rates were higher for experiments of low O_2 mixed with NO_3^- addition. However, it is unknown if this was a result of influent BTEX concentrations that varied among experiments.

Where O_2 was the sole electron acceptor, experiments 1, 2 and 3, the observed removal rates were higher than all other experiments, with the exception of experiment 4.

However, the apparent increase in biodegradation, for this latter case, was a direct result of the lack of nitrogen for synthesis. The NO_3^- addition allowed for a substantial increase in aerobic growth by providing a source of nitrogen. According to the model analysis, anoxic growth did not contribute to biodegradation during this time.

6. CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

Biodegradation of BTEX compounds was investigated under aerobic, nitrate-reducing and mixed aerobic/nitrate-reducing conditions. Nitrogen limitation was also a factor that influenced microbial activity within the system. It resulted in the dual role of NO_3^- as an electron acceptor and as a nutrient for synthesis.

Responses of the bulk parameters and BTEX removal rates to electron acceptor loading and nitrogen-limitation were examined to evaluate biological activity and the ability of the microorganisms to degrade BTEX under different operating conditions.

The experimental data was successfully modeled with a modified version of the ASM2 model for biological wastewater processes. This model uses Monod kinetics to simulate microbial growth. Multiple saturation and inhibition terms supplemented the Monod equation to address the effects of electron acceptor and nutrient concentrations on growth. Significant modifications to the ASM2 model were made by the addition of eight new processes. Four of these processes were added to simulate the utilization of NO_3^- for synthesis. The remaining four processes represented growth on the BTEX compounds under different conditions.

Results of the data analysis, combined with the model predictions, were used to evaluate the system under different redox conditions and potential nutrient limitation. Removal of BTEX compounds was predominantly under aerobic conditions, when substantial

amounts of O_2 were available. The lack of ammonium-nitrogen hindered both growth and BTEX removal when NO_3^- was unavailable for assimilation. Where NO_3^- was added, under high O_2 conditions, NO_3^- was utilized mainly as a source of nitrogen for synthesis. However, based on saturation constants determined for the model, there was a preference for NH_4^+ over NO_3^- for assimilation when NH_4^+ was available.

There was some evidence of NO_3^- utilization as an electron acceptor at low O_2 levels. However, as expected, O_2 was preferred when both were available. When denitrification took place, NO_3^- was not used to its capacity and growth was substantially less than in the presence of O_2 . BTEX removal under anoxic conditions was also fairly limited. This indicates that the number of facultative bacteria within the consortium was small.

Monod rate constants were estimated for each of the BTEX compounds. The maximum specific growth rates appear to be slightly lower than those determined in previous studies. The values for the half-saturation constant are within the range of values from other studies. However, there is a wide range of variability within the literature for Monod rate constants for benzene and toluene under aerobic conditions. For the remaining BTEX compounds under aerobic conditions and for all BTEX under anaerobic conditions, there is a significant lack of data for comparison.

The modified ASM2 model developed herein is novel in its application. It attempts to simulate the biodegradation of both primary (acetic acid) and trace secondary (BTEX) compounds as found in subsurface environments via a batch reactor system. It has the

ability to address the affects of both electron acceptor and nutrient concentrations through a multiple term Monod equation, not seen in other reports concerning BTEX, or more general hydrocarbon, biodegradation.

6.2 Recommendations

6.2.1 Experimental Design

Variability within the BTEX data needs to be decreased. More consistent influent data would have been beneficial to examining responses to electron acceptor loading. Preparation of the influent BTEX solution and/or sampling technique may be contributors to this variability. These need to be improved in future analyses.

A detailed examination of the breakdown products of BTEX would be useful. Measurement of these products would allow for a mass balance to be approximated. With this data, the validity of the observed removal rates could be assessed.

6.2.2 Experimental and Model Analysis

As discussed in Section 2.3.4, interactions among BTEX compounds may have significant effects on the biodegradation of each of the compounds. Analysis of substrate interactions was beyond the scope of this study and was not addressed. However, further examination of this phenomenon is necessary. This should include an examination of the compounds as single substrates, as well as in combination with one another

Calibration of the model was fairly relaxed. More rigorous calibration of the model is required.

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Appendix A: Raw Data Tables

Table A-1: Experiment 1 raw data.

Sampling Event	Sample	Effluent O ₂ (mg O ₂ /L)	Influent NO ₃ ⁻ (mg NO ₃ ⁻ /L)	Effluent NO ₃ ⁻ (mg NO ₃ ⁻ /L)	Influent COD (mg COD/L)	Effluent COD (mg COD/L)	Reactor VSS (mg VSS/L)	ORP (mV)	Active Cells (cells/mL)
1	1	9.55			1,010	329	373		6.78E+09
	2	9.98			1,023	327	438		
2	1	9.18			1,079	449	434	226	7.89E+09
	2	10.62			1,191	432	387		
3	1	11.87			1,123	562	244	230	4.44E+09
	2	9.43			1,010	312	365		

BTEX																
Sampling Event	Sample	Feed Stream Concentration (µg/L)					Syringe Concentration (µg/L)					Effluent Concentration (µg/L)				
		B	T	E	m&p-X	o-X	B	T	E	m&p-X	o-X	B	T	E	m&p-X	o-X
1	1	242	270	149	283	217						19	7	4	11	12
	2	127	153	84	151	111						24	11	6	16	16
2	1	43	47	33	54	41						13	6	4	12	10
	2	45	41	69	61	34						20	8	5	14	12
3	1	184	196	134	240	160						20	3	17	29	28
	2	258	290	192	368	267						13	12	11	23	16

Notes: 1. B = benzene, T = toluene, E = ethylbenzene, and X = xylene.

2. Influent concentrations correspond to actual influent flow rates (Table 3-2). Effluent concentrations are based on effluent flow rate of 3 L/day.

3. Syringe concentration diluted according to sampling procedure of Section 3.1.3.

Table A-2: Experiment 2 raw data.

Sampling Event	Sample	Effluent O_2 (mg O_2 /L)	Influent NO_3^- (mg NO_3^- /L)	Effluent NO_3^- (mg NO_3^- /L)	Influent COD (mg COD/L)	Effluent COD (mg COD/L)	Reactor VSS (mg VSS/L)	ORP (mV)	Active Cells (cells/mL)
1	1	1.01			693	357	504	232	4.21E+09
	2	0.632			799	345	489		
2	1	0.536			824	375	404	238	4.32E+09
	2	0.416			805	427	425		
3	1	0.542			799	450	366	228	4.14E+09
	2	0.646			836	440	424		

Sampling Event	Sample	BTEx															
		Feed Stream Concentration (µg/L)					Syringe Concentration (µg/L)					Effluent Concentration (µg/L)					
		B	T	E	m&p-X	o-X	B	T	E	m&p-X	o-X	B	T	E	m&p-X	o-X	
1	1	417	470	351	689	505							25	7	12	29	29
	2	171	179	132	254	193							16	12	8	18	43
2	1	80	62	53	97	66							17	14	14	32	26
	2	89	71	62	114	79							17	7	8	18	33
3	1	136	98	73	136	91							22	11	9	21	16
	2	191	137	104	198	131							19	4	9	15	32

Notes: 1. B = benzene, T = toluene, E = ethylbenzene, and X = xylene.

2. Influent concentrations correspond to actual influent flow rates (Table 3-2). Effluent concentrations are based on effluent flow rate of 3 L/day.

3. Syringe concentration diluted according to sampling procedure of Section 3.1.3.

Table A-3: Experiment 3 raw data.

Sampling Event	Sample	Effluent O ₂ (mg O ₂ /L)	Influent NO ₃ ⁻ (mg NO ₃ ⁻ /L)	Effluent NO ₃ ⁻ (mg NO ₃ ⁻ /L)	Influent COD (mg COD/L)	Effluent COD (mg COD/L)	Reactor VSS (mg VSS/L)	ORP (mV)	Active Cells (cells/mL)
1	1	1.637			1,045	513	537	249	6.27E+07
	2	1.548			1,045	548	545		
2	1	2.001			1,089	273	742	241	5.00E+07
	2	1.97			1,114	316	610		
3	1	3.51			1,126	401	530	243	5.27E+07
	2	3.18			1,083	493	614		

Sampling Event	Sample	BTX															
		Feed Stream Concentration (µg/L)						Syringe Concentration (µg/L)						Effluent Concentration (µg/L)			
		B	T	E	m&p-X	o-X	B	T	E	m&p-X	o-X	B	T	E	m&p-X	o-X	
1	1	71	84	70	136	164						9	7	24	18	17	
	2	87	106	86	164	211						9	5	4	13	17	
2	1	17	21	25	56	67						13	5	4	11	24	
	2	15	20	15	57	65						12	8	6	25	41	
3	1	29	49	237	326	141						9	5	10	14	22	
	2	114	247	265	599	682						24	18	20	51	70	

Notes: 1. B = benzene, T = toluene, E = ethylbenzene, and X = xylene.

2. Influent concentrations correspond to actual influent flow rates (Table 3-2). Effluent concentrations are based on effluent flow rate of 3 L/day.

3. Syringe concentration diluted according to sampling procedure of Section 3.1.3.

Table A-4: Experiment 4 raw data.

Sampling Event	Sample	Effluent O ₂ (mg O ₂ /L)	Influent NO ₃ ⁻ (mg NO ₃ ⁻ /L)	Effluent NO ₃ ⁻ (mg NO ₃ ⁻ /L)	Influent COD (mg COD/L)	Effluent COD (mg COD/L)	Reactor VSS (mg VSS/L)	ORP (mV)	Active Cells (cells/mL)
1	1	20		1,520	1,034	14	1,479	181	3.29E+09
	2	20		1,630	922	6	1,545		
2	1	20		1,501	891	39	1,819	170	3.10E+09
	2	20			916	46	1,752		
3	1	20		1,680	1,103	49	1,304	185	3.35E+09
	2	20			1,153	49	1,389		

Sampling Event	Sample	BTEx														
		Feed Stream Concentration (µg/L)					Syringe Concentration (µg/L)					Effluent Concentration (µg/L)				
		B	T	E	m&p-X	o-X	B	T	E	m&p-X	o-X	B	T	E	m&p-X	o-X
1	1	377	250	129	211	97	722	539	306	489	218	134	68	28	76	44
	2	397	280	140	226	103	735	657	480	762	300	159	102	58	103	59
2	1	371	262	119	227	108	615	458	260	414	194	41	45	9	59	37
	2	245	154	96	284	76	765	589	334	565	245	119	64	22	84	149
3	1	298	215	123	202	93	460	356	198	351	145	142	81	27	96	51
	2	403	296	154	261	123	611	464	263	445	196	149	84	28	91	53

Notes:

1. B = benzene, T = toluene, E = ethylbenzene, and X = xylene.
2. Influent concentrations correspond to actual influent flow rates (Table 3-2). Effluent concentrations are based on effluent flow rate of 3 L/day.
3. Syringe concentration diluted according to sampling procedure of Section 3.1.3.

Table A-5: Experiment 5 raw data.

Sampling Event	Sample	Effluent O ₂ (mg O ₂ /L)	Influent NO ₃ ⁻ (mg NO ₃ ⁻ /L)	Effluent NO ₃ ⁻ (mg NO ₃ ⁻ /L)	Influent COD (mg COD/L)	Effluent COD (mg COD/L)	Reactor VSS (mg VSS/L)	ORP (mV)	Active Cells (cells/mL)
1	1	1.30		1,566	1,373	542	1,213	169	3.08E+09
	2	1.38		1,559	1,342	484	1,232		
2	1	1.32		1,653	1,254	462	875	175	3.19E+09
	2	1.71		1,288	1,217	559	1,178		
3	1	1.30		1,348	1,198	579	559	175	3.17E+09
	2	1.70			1,236	564	1,300		

Sampling Event	Sample	BTEx														
		Feed Stream Concentration (µg/L)					Syringe Concentration (µg/L)					Effluent Concentration (µg/L)				
		B	T	E	m&p-X	o-X	B	T	E	m&p-X	o-X	B	T	E	m&p-X	o-X
1	1	262	138	68	136	78	423	258	139	279	157	84	36	11	36	28
	2	219	124	70	125	72	298	184	101	202	117	91	40	13	41	32
2	1	274	153	76	154	85	346	213	132	250	133	69	31	14	42	33
	2	264	155	87	163	88	417	256	137	286	155	71	31	11	38	28
3	1	254	151	83	166	89	402	251	137	284	153	78	35	15	44	31
	2	275	154	81	166	87	309	195	116	225	122	69	31	11	45	28

Notes: 1. B = benzene, T = toluene, E = ethylbenzene, and X = xylene.

2. Influent concentrations correspond to actual influent flow rates (Table 3-2). Effluent concentrations are based on effluent flow rate of 3 L/day.

3. Syringe concentration diluted according to sampling procedure of Section 3.1.3.

Table A-6: Experiment 6 raw data.

Sampling Event	Sample	Effluent O ₂ (mg O ₂ /L)	Influent NO ₃ ⁻ (mg NO ₃ ⁻ /L)	Effluent NO ₃ ⁻ (mg NO ₃ ⁻ /L)	Influent COD (mg COD/L)	Effluent COD (mg COD/L)	Reactor VSS (mg VSS/L)	ORP (mV)	Active Cells (cells/mL)
1	1			1,451	1,325	688	622	154	1.78E+08
2	2					680	358		
2	1	0.421		1,333	1,000	703	456	169	1.25E+08
3	2	0.542		1,267	1,200	625	404		
	1	0.57		1,282	969	703	348	167	1.01E+08
	2	0.577		1,302	1,050	690	177		

Sampling Event	Sample	BTX															
		Feed Stream Concentration (µg/L)						Syringe Concentration (µg/L)						Effluent Concentration (µg/L)			
		B	T	E	m&p-X	o-X	B	T	E	m&p-X	o-X	B	T	E	m&p-X	o-X	
1	1	133	71	37	56	37	456	314	214	323	141	45	18	6	16	13	
	2	135	72	40	63	33	387	270	181	270	122	46	19	2	19	14	
2	1	151	64	27	36	18	349	231	147	184	76	29	8	2	7	7	
	2	103	44	19	25	13	291	197	139	162	66	29	8	2	7	7	
3	1	153	83	43	54	26	275	192	123	156	62	39	20	5	11	9	
	2	167	92	49	62	28	175	116	72	100	42	35	12	5	10	9	

Notes: 1. B = benzene, T = toluene, E = ethylbenzene, and X = xylene.

2. Influent concentrations correspond to actual influent flow rates (Table 3-2). Effluent concentrations are based on effluent flow rate of 3 L/day.

3. Syringe concentration diluted according to sampling procedure of Section 3.1.3.

Table A-7: Experiment 7 raw data.

Sampling Event	Sample	Effluent O ₂ (mg O ₂ /L)	Influent NO ₃ ⁻ (mg NO ₃ ⁻ /L)	Effluent NO ₃ ⁻ (mg NO ₃ ⁻ /L)	Influent COD (mg COD/L)	Effluent COD (mg COD/L)	Reactor VSS (mg VSS/L)	ORP (mV)	Active Cells (cells/mL)
1	1	0.216	5,056	1,579	984	647	296	150	7.27E+07
	2	0.264		1,421	969	631	297		
2	1	0.424	4,739	1,685	854	639	183	139	4.73E+07
	2	0.360		1,659	829	597	185		
3	1	0.279	4,629	1,659	939	655	167	104	3.55E+07
	2	0.314		1,640	984	645	163		

Sampling Event	Sample	BTEX														
		Feed Stream Concentration (µg/L)						Syringe Concentration (µg/L)								
		B	T	E	m&p-X	o-X	B	T	E	m&p-X	o-X	B	T	E	m&p-X	o-X
1	1	90	52	29	36	18	271	193	126	164	83	85	47	12	38	24
	2	91	50	28	36	18	239	169	112	146	75	63	45	5	26	
2	1	173	100	76	104	55	295	210	134	272	131	81	45	10	45	29
	2	204	190	141	247	110	287	214	132	246	130	70	3	16	69	31
3	1	202	138	82	146	77	287	214	136	255	134	76	42	15	46	43
	2	176	118	76	126	66	305	231	157	291	143	76	43	15	47	40

Table A-9: Experiment 9 raw data.

Sampling Event	Sample	Effluent O ₂ (mg O ₂ /L)	Influent NO ₃ ⁻ (mg NO ₃ ⁻ /L)	Effluent NO ₃ ⁻ (mg NO ₃ ⁻ /L)	Influent COD (mg COD/L)	Effluent COD (mg COD/L)	Reactor VSS (mg VSS/L)	ORP (mV)	Active Cells (cells/mL)
1	1	0.261	322	4.4	1,268	911	54	-53	9.55E+07
	2	0.326		4.2	1,118	811	39		
2	1	0.153	401	1.67	1,268	831	49	-56	6.36E+07
	2	0.124		1.66	1,260	854	44		
3	1	0.140	420	1.82	1,243	787	45	-54	9.18E+07
	2	0.235		2.46		857	43		

Sampling Event	Sample	BTEx														
		Feed Stream Concentration (µg/L)					Syringe Concentration (µg/L)					Effluent Concentration (µg/L)				
		B	T	E	m&p-X	o-X	B	T	E	m&p-X	o-X	B	T	E	m&p-X	o-X
1	1	191	161	67	135	64	200	131	85	238	107	75	35	12	38	21
	2	199	141	74	145	69	226	150	102	270	118	83	38	13	37	21
2	1	143	87	51	135	57	194	128	91	269	93	67	27	11	57	23
	2	160	111	57	133	66	170	123	77	216	101	69	25	9	38	36
3	1	300	173	118	215	109	175	121	73	332	96	66	28	14	41	24
	2	223	117	62	150	76	272	188	120	338	150	70	29	11	18	27

- Notes:
1. B = benzene, T = toluene, E = ethylbenzene, and X = xylene.
 2. Influent concentrations correspond to actual influent flow rates (Table 3-2). Effluent concentrations are based on effluent flow rate of 3 L/day.
 3. Syringe concentration diluted according to sampling procedure of Section 3.1.3.

Table A-11: Experiment 11 raw data.

Sampling Event	Sample	Effluent O ₂ (mg O ₂ /L)	Influent NO ₃ ⁻ (mg NO ₃ ⁻ /L)	Effluent NO ₃ ⁻ (mg NO ₃ ⁻ /L)	Influent COD (mg COD/L)	Effluent COD (mg COD/L)	Reactor VSS (mg VSS/L)	ORP (mV)	Active Cells (cells/mL)
1	1	0.435	848	109	1,258	627	283	234	9.00E+07
	2	0.555		107	1,125	577	333		
2	1	0.373	815	107	1,075	480	250	237	6.91E+07
	2	0.355		114	1,275	487			
3	1	0.336	798	99	1,083	467	237	243	8.00E+07
	2	0.235		115	1,042	500			

Sampling Event	Sample	BTX														
		Feed Stream Concentration (µg/L)					Syringe Concentration (µg/L)					Effluent Concentration (µg/L)				
		B	T	E	m&p-X	o-X	B	T	E	m&p-X	o-X	B	T	E	m&p-X	o-X
1	1	480	331	168	253	146	581	420	258	406	226	211	123	39	102	74
	2	420	279	143	221	126	688	513	313	498	273	190	139	33	88	55
2	1	431	290	167	256	146	636	457	292	464	251	182	111	37	94	59
	2	414	298	169	256	148	651	506	326	488	263	182	99	34	84	51
3	1	335	229	123	195	110	743	550	338	547	291	211	116	46	111	67
	2	325	232	129	201	112	694	532	314	506	270	145	93	31	82	53

Appendix B: Duplicate Sample Analysis

Table B-1: Duplicate analysis for effluent O₂.

Exp.	Sampling Event	Sample	Effluent Concentration (mg/L)	Mean of Duplicates (mg/L)	Variability of Duplicates
1	1	1	9.55	9.77	0.04
		2	9.98		
	2	1	9.18	9.90	0.15
		2	10.62		
	3	1	11.87	10.65	0.23
		2	9.43		
2	1	1	1.01	0.821	0.46
		2	0.632		
	2	1	0.536	0.476	0.25
		2	0.416		
	3	1	0.542	0.594	0.18
		2	0.646		
3	1	1	1.64	1.59	0.06
		2	1.55		
	2	1	2.00	1.99	0.02
		2	1.97		
	3	1	3.51	3.35	0.10
		2	3.18		
4	1	1	20	20	0.00
		2	20		
	2	1	20	20	0.00
		2	20		
	3	1	20	20	0.00
		2	20		
5	1	1	1.30	1.34	0.06
		2	1.38		
	2	1	1.32	1.52	0.26
		2	1.71		
	3	1	1.30	1.50	0.27
		2	1.70		
6	1	1			
		2			
	2	1	0.421	0.482	0.25
		2	0.542		
	3	1	0.570	0.574	0.01
		2	0.577		

Table B-1: continued.

Exp.	Sampling Event	Sample	Effluent Concentration (mg/L)	Mean of Duplicates (mg/L)	Variability of Duplicates
7	1	1	0.216	0.240	0.20
		2	0.264		
	2	1	0.424	0.392	0.16
		2	0.360		
	3	1	0.279	0.297	0.12
		2	0.314		
8	1	1	0.408	0.415	0.03
		2	0.422		
	2	1	0.153	0.149	0.05
		2	0.145		
	3	1	0.341	0.517	0.68
		2	0.692		
9	1	1	0.261	0.294	0.22
		2	0.326		
	2	1	0.153	0.139	0.21
		2	0.124		
	3	1	0.140	0.188	0.51
		2	0.235		
10	1	1	2.00	2.045	0.04
		2	2.09		
	2	1	0.653	0.699	0.13
		2	0.744		
	3	1	0.650	1.010	0.71
		2	1.37		
11	1	1	0.435	0.495	0.24
		2	0.555		
	2	1	0.373	0.364	0.05
		2	0.355		
	3	1	0.336	0.286	0.35
		2	0.235		
12	1	1	0.380	0.453	0.32
		2	0.525		
	2	1	0.632	0.728	0.26
		2	0.824		
	3	1	0.657	0.797	0.35
		2	0.937		

Table B-2: Duplicate analysis for effluent NO₃⁻.

Exp.	Sampling Event	Sample	Effluent Concentration (mg/L)	Mean of Duplicates (mg/L)	Variability of Duplicates
1	1	1			
		2			
	2	1			
		2			
	3	1			
2	2				
2	1	1			
		2			
	2	1			
		2			
	3	1			
2					
3	1	1			
		2			
	2	1			
		2			
	3	1			
2					
4	1	1	1,825	1,764	0.07
		2	1,703		
	2	1	1,689		
		2			
	3	1	1,585		
2					
5	1	1	1,566	1,563	0.00
		2	1,559		
	2	1	1,653	1,471	0.25
		2	1,288		
	3	1	1,348		
2					
6	1	1	1,451		
		2			
	2	1	1,333	1,300	0.05
		2	1,267		
	3	1	1,282	1,292	0.02
2	1,302				

Table B-2: continued.

Exp.	Sampling Event	Sample	Effluent Concentration (mg/L)	Mean of Duplicates (mg/L)	Variability of Duplicates
7	1	1	1,579	1,500	0.11
		2	1,421		
	2	1	1,685	1,672	0.02
		2	1,659		
	3	1	1,659	1,650	0.01
		2	1,640		
8	1	1	271	252	0.16
		2	232		
	2	1	261	250	0.09
		2	238		
	3	1	241	245	0.03
		2	248		
9	1	1	4.4	4.3	0.05
		2	4.2		
	2	1	1.7	1.7	0.01
		2	1.7		
	3	1	1.8	2.1	0.30
		2	2.5		
10	1	1	37	38	0.03
		2	38		
	2	1	33	35	0.09
		2	36		
	3	1	46	45	0.04
		2	44		
11	1	1	109	108	0.02
		2	107		
	2	1	107	111	0.06
		2	114		
	3	1	99	107	0.15
		2	115		
12	1	1	153	157	0.05
		2	161		
	2	1	156	172	0.19
		2	188		
	3	1	176	180	0.04
		2	184		

Table B-3: Duplicate analysis for influent COD.

Exp.	Sampling Event	Sample	Influent Concentration (mg COD/L)	Mean of Duplicates (mg COD/L)	Variability of Duplicates
1	1	1	1,010	1,017	0.01
		2	1,023		
	2	1	1,079	1,135	0.10
		2	1,191		
	3	1	1,123	1,067	0.11
		2	1,010		
2	1	1	693	746	0.14
		2	799		
	2	1	824	815	0.02
		2	805		
	3	1	799	818	0.05
		2	836		
3	1	1	1,045	1,045	0.00
		2	1,045		
	2	1	1,089	1,102	0.02
		2	1,114		
	3	1	1,126	1,105	0.04
		2	1,083		
4	1	1	1,034	978	0.11
		2	922		
	2	1	891	904	0.03
		2	916		
	3	1	1,103	1,128	0.04
		2	1,153		
5	1	1	1,373	1,358	0.02
		2	1,342		
	2	1	1,254	1,236	0.03
		2	1,217		
	3	1	1,198	1,217	0.03
		2	1,236		
6	1	1	1,325		
		2			
	2	1	1,333	1,300	0.05
		2	1,267		
	3	1	1,282	1,292	0.02
		2	1,302		

Note: 1. Concentrations correspond to actual influent flow rates (Table 3-2).

Table B-3: continued.

Exp.	Sampling Event	Sample	Influent Concentration (mg COD/L)	Mean of Duplicates (mg COD/L)	Variability of Duplicates
7	1	1	984	977	0.02
		2	969		
	2	1	854	842	0.03
		2	829		
	3	1	939	962	0.05
		2	984		
8	1	1	1,274	1,218	0.09
		2	1,161		
	2	1	1,274	1,209	0.11
		2	1,143		
	3	1	1,168	1,184	0.03
		2	1,199		
9	1	1	1,268	1,193	0.13
		2	1,118		
	2	1	1,268	1,264	0.01
		2	1,260		
	3	1	1,243	1,243	
		2			
10	1	1	1,242	1,162	0.14
		2	1,082		
	2	1	1,242	1,175	0.11
		2	1,108		
	3	1	1,258	1,267	0.01
		2	1,275		
11	1	1	1,258	1,192	0.11
		2	1,125		
	2	1	1,075	1,175	0.17
		2	1,275		
	3	1	1,083	1,063	0.04
		2	1,042		
12	1	1	1,058	1,067	0.02
		2	1,075		
	2	1	1,192	1,150	0.07
		2	1,108		
	3	1	1,225	1,204	0.03
		2	1,183		

Note: 1. Concentrations correspond to actual influent flow rates (Table 3-2).

Table B-4: Duplicate analysis for effluent COD.

Exp.	Sampling Event	Sample	Effluent Concentration (mg COD/L)	Mean of Duplicates (mg COD/L)	Variability of Duplicates
1	1	1	329	328	0.01
		2	327		
	2	1	449	441	0.04
		2	432		
	3	1	562	437	0.57
		2	312		
2	1	1	357	351	0.03
		2	345		
	2	1	375	401	0.13
		2	427		
	3	1	450	445	0.02
		2	440		
3	1	1	513	531	0.07
		2	548		
	2	1	273	295	0.15
		2	316		
	3	1	401	447	0.21
		2	493		
4	1	1	14	10	0.80
		2	6		
	2	1	39	43	0.16
		2	46		
	3	1	49	49	0.00
		2	49		
5	1	1	542	513	0.11
		2	484		
	2	1	462	511	0.19
		2	559		
	3	1	579	572	0.03
		2	564		
6	1	1	688	684	0.01
		2	680		
	2	1	703	664	0.12
		2	625		
	3	1	703	697	0.02
		2	690		

Table B-4: continued.

Exp.	Sampling Event	Sample	Effluent Concentration (mg COD/L)	Mean of Duplicates (mg COD/L)	Variability of Duplicates
7	1	1	647	639	0.03
		2	631		
	2	1	639	618	0.07
		2	597		
	3	1	655	650	0.02
		2	645		
8	1	1	772	749	0.06
		2	725		
	2	1	727	756	0.08
		2	785		
	3	1	755	740	0.04
		2	725		
9	1	1	911	861	0.12
		2	811		
	2	1	831	843	0.03
		2	854		
	3	1	787	822	0.09
		2	857		
10	1	1	467	477	0.04
		2	487		
	2	1	443	457	0.06
		2	470		
	3	1	537	524	0.05
		2	510		
11	1	1	627	602	0.08
		2	577		
	2	1	480	484	0.01
		2	487		
	3	1	467	484	0.07
		2	500		
12	1	1	483	477	0.03
		2	470		
	2	1	467	462	0.02
		2	457		
	3	1	463	518	0.21
		2	573		

Table B-5: Duplicate analysis for reactor VSS.

Exp.	Sampling Event	Sample	Reactor Concentration (mg VSS/L)	Mean of Duplicates (mg VSS/L)	Variability of Duplicates
1	1	1	373	406	0.16
		2	438		
	2	1	434	411	0.11
		2	387		
	3	1	244	305	0.40
		2	365		
2	1	1	504	497	0.03
		2	489		
	2	1	404	415	0.05
		2	425		
	3	1	366	395	0.15
		2	424		
3	1	1	537	541	0.01
		2	545		
	2	1	742	676	0.20
		2	610		
	3	1	530	572	0.15
		2	614		
4	1	1	1,479	1,512	0.04
		2	1,545		
	2	1	1,819	1,786	0.04
		2	1,752		
	3	1	1,304	1,347	0.06
		2	1,389		
5	1	1	1,213	1,223	0.02
		2	1,232		
	2	1	875	1,027	0.30
		2	1,178		
	3	1	559	930	0.80
		2	1,300		
6	1	1	622	580	0.14
		2	538		
	2	1	456	430	0.12
		2	404		
	3	1	348	263	0.65
		2	177		

Table B-5: continued.

Exp.	Sampling Event	Sample	Reactor Concentration (mg VSS/L)	Mean of Duplicates (mg VSS/L)	Variability of Duplicates
7	1	1	296	297	0.00
		2	297		
	2	1	183	184	0.01
		2	185		
	3	1	167	165	0.02
		2	163		
8	1	1	136	125	0.18
		2	114		
	2	1	89	89	0.00
		2	89		
	3	1	82	86	0.08
		2	89		
9	1	1	54	47	0.32
		2	39		
	2	1	49	47	0.11
		2	44		
	3	1	45	44	0.05
		2	43		
10	1	1	357	305	0.34
		2	252		
	2	1	317	340	0.14
		2	363		
	3	1	248	274	0.19
		2	300		
11	1	1	283	308	0.16
		2	333		
	2	1	250		
		2			
	3	1	237		
		2			
12	1	1	176	186	0.10
		2	195		
	2	1	146	152	0.07
		2	157		
	3	1	135	123	0.20
		2	110		

Table B-6: Duplicate analysis for feed-stream benzene.

Exp.	Sampling Event	Sample	Feed-Stream Concentration (µg/L)	Mean of Duplicates (µg/L)	Variability of Duplicates
1	1	1	242	185	0.62
		2	127		
	2	1	43	44	0.05
		2	45		
	3	1	184	221	0.33
		2	258		
2	1	1	417	294	0.84
		2	171		
	2	1	80	85	0.11
		2	89		
	3	1	136	164	0.34
		2	191		
3	1	1	71	79	0.20
		2	87		
	2	1	17	16	0.13
		2	15		
	3	1	29	72	1.19
		2	114		
4	1	1	377	387	0.05
		2	397		
	2	1	371	308	0.41
		2	245		
	3	1	298	351	0.30
		2	403		
5	1	1	262	241	0.18
		2	219		
	2	1	274	269	0.04
		2	264		
	3	1	254	265	0.08
		2	275		
6	1	1	133	134	0.01
		2	135		
	2	1	151	127	0.38
		2	103		
	3	1	153	160	0.09
		2	167		

Note: 1. Concentrations correspond to actual influent flow rates (Table 3-2).

Table B-6: continued.

Exp.	Sampling Event	Sample	Feed-Stream Concentration (µg/L)	Mean of Duplicates (µg/L)	Variability of Duplicates
7	1	1	90	91	0.01
		2	91		
	2	1	173	189	0.16
		2	204		
	3	1	202	189	0.14
		2	176		
8	1	1	1,344	1,291	0.08
		2	1,237		
	2	1	246	257	0.08
		2	267		
	3	1	251	297	0.31
		2	342		
9	1	1	191	195	0.04
		2	199		
	2	1	143	152	0.11
		2	160		
	3	1	300	262	0.29
		2	223		
10	1	1	151	139	0.17
		2	127		
	2	1	327	363	0.20
		2	399		
	3	1	298	283	0.11
		2	267		
11	1	1	480	450	0.13
		2	420		
	2	1	431	423	0.04
		2	414		
	3	1	335	330	0.03
		2	325		
12	1	1	217	214	0.03
		2	210		
	2	1	388	355	0.19
		2	321		
	3	1	127	116	0.19
		2	105		

Note: 1. Concentrations correspond to actual influent flow rates (Table 3-2).

Table B-7: Duplicate analysis for feed-stream toluene.

Exp.	Sampling Event	Sample	Feed-Stream Concentration (µg/L)	Mean of Duplicates (µg/L)	Variability of Duplicates
1	1	1	270	212	0.55
		2	153		
	2	1	47	44	0.14
		2	41		
	3	1	196	243	0.39
		2	290		
2	1	1	470	325	0.90
		2	179		
	2	1	62	67	0.14
		2	71		
	3	1	98	118	0.33
		2	137		
3	1	1	84	95	0.23
		2	106		
	2	1	21	21	0.05
		2	20		
	3	1	49	148	1.34
		2	247		
4	1	1	250	265	0.11
		2	280		
	2	1	262	208	0.52
		2	154		
	3	1	215	256	0.32
		2	296		
5	1	1	138	131	0.11
		2	124		
	2	1	153	154	0.01
		2	155		
	3	1	151	153	0.02
		2	154		
6	1	1	71	72	0.01
		2	72		
	2	1	64	54	0.37
		2	44		
	3	1	83	88	0.10
		2	92		

Note: 1. Concentrations correspond to actual influent flow rates (Table 3-2).

Table B-7: continued.

Exp.	Sampling Event	Sample	Feed-Stream Concentration (µg/L)	Mean of Duplicates (µg/L)	Variability of Duplicates
7	1	1	52	51	0.04
		2	50		
	2	1	100	145	0.62
		2	190		
	3	1	138	128	0.16
		2	118		
8	1	1	1,058	1,002	0.11
		2	946		
	2	1	161	171	0.12
		2	181		
	3	1	182	212	0.28
		2	242		
9	1	1	161	151	0.13
		2	141		
	2	1	87	99	0.24
		2	111		
	3	1	173	145	0.39
		2	117		
10	1	1	65	64	0.05
		2	62		
	2	1	203	235	0.27
		2	267		
	3	1	201	180	0.23
		2	159		
11	1	1	331	305	0.17
		2	279		
	2	1	290	294	0.03
		2	298		
	3	1	229	231	0.01
		2	232		
12	1	1	167	156	0.14
		2	145		
	2	1	299	262	0.28
		2	225		
	3	1	85	78	0.19
		2	70		

Note: 1. Concentrations correspond to actual influent flow rates (Table 3-2).

Table B-8: Duplicate analysis for feed-stream ethylbenzene.

Exp.	Sampling Event	Sample	Feed-Stream Concentration (µg/L)	Mean of Duplicates (µg/L)	Variability of Duplicates
1	1	1	149	117	0.56
		2	84		
	2	1	33	51	0.71
		2	69		
	3	1	134	163	0.36
		2	192		
2	1	1	351	242	0.91
		2	132		
	2	1	53	58	0.16
		2	62		
	3	1	73	89	0.35
		2	104		
3	1	1	70	78	0.21
		2	86		
	2	1	25	20	0.50
		2	15		
	3	1	237	252	0.12
		2	266		
4	1	1	129	135	0.08
		2	140		
	2	1	119	108	0.21
		2	96		
	3	1	123	139	0.22
		2	154		
5	1	1	68	69	0.03
		2	70		
	2	1	76	82	0.13
		2	87		
	3	1	83	82	0.02
		2	81		
6	1	1	37	39	0.08
		2	40		
	2	1	27	23	0.35
		2	19		
	3	1	43	46	0.13
		2	49		

Note: 1. Concentrations correspond to actual influent flow rates (Table 3-2).

Table B-8: continued.

Exp.	Sampling Event	Sample	Feed-Stream Concentration (µg/L)	Mean of Duplicates (µg/L)	Variability of Duplicates
7	1	1	28	28	0.00
		2	28		
	2	1	76	109	0.60
		2	141		
	3	1	82	79	0.08
		2	76		
8	1	1	722	676	0.14
		2	630		
	2	1	93	119	0.43
		2	144		
	3	1	114	136	0.32
		2	157		
9	1	1	67	71	0.10
		2	74		
	2	1	51	54	0.11
		2	57		
	3	1	118	90	0.62
		2	62		
10	1	1	26	25	0.08
		2	24		
	2	1	358	239	1.00
		2	119		
	3	1	94	92	0.04
		2	90		
11	1	1	168	156	0.16
		2	143		
	2	1	167	168	0.01
		2	169		
	3	1	123	126	0.05
		2	129		
12	1	1	108	97	0.23
		2	86		
	2	1	156	150	0.09
		2	143		
	3	1	56	52	0.15
		2	48		

Note: 1. Concentrations correspond to actual influent flow rates (Table 3-2).

Table B-9: Duplicate analysis for feed-stream m&p-Xylenes.

Exp.	Sampling Event	Sample	Feed-Stream Concentration (µg/L)	Mean of Duplicates (µg/L)	Variability of Duplicates
1	1	1	283	217	0.61
		2	151		
	2	1	54	58	0.12
		2	61		
	3	1	240	304	0.42
		2	368		
2	1	1	689	472	0.92
		2	254		
	2	1	97	106	0.16
		2	114		
	3	1	136	167	0.37
		2	198		
3	1	1	136	150	0.19
		2	164		
	2	1	56	57	0.02
		2	57		
	3	1	326	463	0.59
		2	599		
4	1	1	211	219	0.07
		2	226		
	2	1	227	256	0.22
		2	284		
	3	1	202	232	0.25
		2	261		
5	1	1	136	131	0.08
		2	125		
	2	1	154	159	0.06
		2	163		
	3	1	166	166	0.00
		2	166		
6	1	1	56	60	0.12
		2	63		
	2	1	36	31	0.36
		2	25		
	3	1	54	58	0.14
		2	62		

Note: 1. Concentrations correspond to actual influent flow rates (Table 3-2).

Table B-9: continued.

Exp.	Sampling Event	Sample	Feed-Stream Concentration (µg/L)	Mean of Duplicates (µg/L)	Variability of Duplicates
7	1	1	36	36	0.00
		2	36		
	2	1	104	176	0.81
		2	247		
	3	1	146	136	0.15
		2	126		
8	1	1	757	620	0.44
		2	482		
	2	1	60	64	0.11
		2	67		
	3	1	72	86	0.33
		2	100		
9 10	1	1	135	140	0.07
		2	145		
	2	1	135	134	0.01
		2	133		
	3	1	215	183	0.36
		2	150		
	1	1	107	106	0.03
		2	104		
	2	1	151	164	0.15
		2	176		
	3	1	134	131	0.05
		2	127		
11	1	1	253	237	0.14
		2	221		
	2	1	256	256	0.00
		2	256		
	3	1	195	198	0.03
		2	201		
12	1	1	170	155	0.20
		2	139		
	2	1	251	236	0.13
		2	221		
	3	1	91	82	0.22
		2	73		

Note: 1. Concentrations correspond to actual influent flow rates (Table 3-2).

Table B-10: Duplicate analysis for feed-stream o-Xylene.

Exp.	Sampling Event	Sample	Feed-Stream Concentration ($\mu\text{g/L}$)	Mean of Duplicates ($\mu\text{g/L}$)	Variability of Duplicates
1	1	1	217	164	0.65
		2	111		
	2	1	41	38	0.19
		2	34		
	3	1	160	214	0.50
		2	267		
2	1	1	505	349	0.89
		2	193		
	2	1	66	73	0.18
		2	79		
	3	1	91	111	0.36
		2	131		
3	1	1	164	188	0.25
		2	211		
	2	1	67	66	0.03
		2	65		
	3	1	141	412	1.31
		2	682		
4	1	1	97	100	0.06
		2	103		
	2	1	108	92	0.35
		2	76		
	3	1	93	108	0.28
		2	123		
5	1	1	78	75	0.08
		2	72		
	2	1	85	87	0.03
		2	88		
	3	1	89	88	0.02
		2	87		
6	1	1	37	35	0.11
		2	33		
	2	1	18	16	0.32
		2	13		
	3	1	26	27	0.07
		2	28		

Note: 1. Concentrations correspond to actual influent flow rates (Table 3-2).

Table B-10: continued.

Exp.	Sampling Event	Sample	Feed-Stream Concentration (µg/L)	Mean of Duplicates (µg/L)	Variability of Duplicates
7	1	1	18	18	0.00
		2	18		
	2	1	55	83	0.67
		2	110		
	3	1	77	72	0.15
		2	66		
8	1	1	249	234	0.13
		2	218		
	2	1	27	29	0.11
		2	30		
	3	1	35	40	0.25
		2	45		
9	1	1	64	67	0.08
		2	69		
	2	1	57	62	0.15
		2	66		
	3	1	109	93	0.36
		2	76		
10	1	1	62	61	0.03
		2	60		
	2	1	84	90	0.12
		2	95		
	3	1	72	71	0.03
		2	70		
11	1	1	146	136	0.15
		2	126		
	2	1	146	147	0.01
		2	148		
	3	1	110	111	0.02
		2	112		
12	1	1	92	85	0.16
		2	78		
	2	1	138	130	0.13
		2	121		
	3	1	50	45	0.22
		2	40		

Note: 1. Concentrations correspond to actual influent flow rates (Table 3-2).

Table B-11: Duplicate analysis for syringe benzene.

Exp.	Sampling Event	Sample	Syringe Concentration (µg/L)	Mean of Duplicates (µg/L)	Variability of Duplicates
1	1	1			
		2			
	2	1			
		2			
	3	1			
		2			
2	1	1			
		2			
	2	1			
		2			
	3	1			
		2			
3	1	1			
		2			
	2	1			
		2			
	3	1			
		2			
4	1	1	722	729	0.02
		2	735		
	2	1	615	690	0.22
		2	765		
	3	1	460	536	0.28
		2	611		
5	1	1	423	361	0.35
		2	298		
	2	1	346	382	0.19
		2	417		
	3	1	402	356	0.26
		2	309		
6	1	1	456	422	0.16
		2	387		
	2	1	349	320	0.18
		2	291		
	3	1	275	225	0.44
		2	175		

Note: 1. Concentration diluted according to sampling procedure of Section 3.1.3.

Table B-11: continued.

Exp.	Sampling Event	Sample	Syringe Concentration (µg/L)	Mean of Duplicates (µg/L)	Variability of Duplicates
7	1	1	271	255	0.13
		2	239		
	2	1	295	291	0.03
		2	287		
	3	1	287	296	0.06
		2	305		
8	1	1	430	423	0.03
		2	416		
	2	1	294	291	0.02
		2	288		
	3	1	366	299	0.45
		2	232		
9	1	1	200	213	0.12
		2	226		
	2	1	194	182	0.13
		2	170		
	3	1	175	224	0.43
		2	272		
10	1	1	307	324	0.10
		2	340		
	2	1	849	888	0.09
		2	926		
	3	1	586	639	0.17
		2	692		
11	1	1	581	635	0.17
		2	688		
	2	1	636	644	0.02
		2	651		
	3	1	743	719	0.07
		2	694		
12	1	1	623	688	0.19
		2	753		
	2	1	739	766	0.07
		2	793		
	3	1	687	720	0.09
		2	752		

Note: 1. Concentration diluted according to sampling procedure of Section 3.1.3.

Table B-12: Duplicate analysis for syringe toluene.

Exp.	Sampling Event	Sample	Syringe Concentration (µg/L)	Mean of Duplicates (µg/L)	Variability of Duplicates
1	1	1			
		2			
	2	1			
		2			
	3	1			
		2			
2	1	1			
		2			
	2	1			
		2			
	3	1			
		2			
3	1	1			
		2			
	2	1			
		2			
	3	1			
		2			
4	1	1	539	598	0.20
		2	657		
	2	1	458	524	0.25
		2	589		
	3	1	356	410	0.26
		2	464		
5	1	1	258	221	0.33
		2	184		
	2	1	213	235	0.18
		2	256		
	3	1	251	223	0.25
		2	195		
6	1	1	314	292	0.15
		2	270		
	2	1	231	214	0.16
		2	197		
	3	1	192	154	0.49
		2	116		

Note: 1. Concentration diluted according to sampling procedure of Section 3.1.3.

Table B-12: continued.

Exp.	Sampling Event	Sample	Syringe Concentration (µg/L)	Mean of Duplicates (µg/L)	Variability of Duplicates
7	1	1	193	181	0.13
		2	169		
	2	1	210	212	0.02
		2	214		
	3	1	214	223	0.08
		2	231		
8	1	1	310	307	0.02
		2	304		
	2	1	215	214	0.01
		2	213		
	3	1	229	201	0.28
		2	172		
9	1	1	131	141	0.14
		2	150		
	2	1	128	126	0.04
		2	123		
	3	1	121	155	0.43
		2	188		
10	1	1	169	180	0.12
		2	190		
	2	1	591	627	0.11
		2	662		
	3	1	426	462	0.15
		2	497		
11	1	1	420	467	0.20
		2	513		
	2	1	457	482	0.10
		2	506		
	3	1	550	541	0.03
		2	532		
12	1	1	507	541	0.13
		2	575		
	2	1	569	610	0.13
		2	651		
	3	1	452	519	0.26
		2	585		

Note: 1. Concentration diluted according to sampling procedure of Section 3.1.3.

Table B-13: Duplicate analysis for syringe ethylbenzene.

Exp.	Sampling Event	Sample	Syringe Concentration ($\mu\text{g/L}$)	Mean of Duplicates ($\mu\text{g/L}$)	Variability of Duplicates
1	1	1			
		2			
	2	1			
		2			
	3	1			
		2			
2	1	1			
		2			
	2	1			
		2			
	3	1			
		2			
3	1	1			
		2			
	2	1			
		2			
	3	1			
		2			
4	1	1	306	393	0.44
		2	480		
	2	1	260	297	0.25
		2	334		
	3	1	198	231	0.28
		2	263		
5	1	1	139	120	0.32
		2	101		
	2	1	132	135	0.04
		2	137		
	3	1	137	127	0.17
		2	116		
6	1	1	214	198	0.17
		2	181		
	2	1	147	142	0.07
		2	137		
	3	1	123	98	0.52
		2	72		

Note: 1. Concentration diluted according to sampling procedure of Section 3.1.3.

Table B-13: continued.

Exp.	Sampling Event	Sample	Syringe Concentration (µg/L)	Mean of Duplicates (µg/L)	Variability of Duplicates
7	1	1	126	119	0.12
		2	112		
	2	1	134	133	0.02
		2	132		
	3	1	136	147	0.14
		2	157		
8	1	1	203	199	0.04
		2	195		
	2	1	130	128	0.03
		2	126		
	3	1	158	132	0.40
		2	105		
9	1	1	85	94	0.18
		2	102		
	2	1	91	84	0.17
		2	77		
	3	1	73	97	0.49
		2	120		
10	1	1	79	83	0.10
		2	87		
	2	1	334	357	0.13
		2	380		
	3	1	242	267	0.18
		2	291		
11	1	1	258	286	0.19
		2	313		
	2	1	292	309	0.11
		2	326		
	3	1	338	326	0.07
		2	314		
12	1	1	304	340	0.21
		2	376		
	2	1	361	374	0.07
		2	387		
	3	1	321	338	0.10
		2	355		

Note: 1. Concentration diluted according to sampling procedure of Section 3.1.3.

Table B-14: Duplicate analysis for syringe m&p-Xylenes.

Exp.	Sampling Event	Sample	Syringe Concentration (µg/L)	Mean of Duplicates (µg/L)	Variability of Duplicates
1	1	1			
		2			
	2	1			
		2			
	3	1			
		2			
2	1	1			
		2			
	2	1			
		2			
	3	1			
		2			
3	1	1			
		2			
	2	1			
		2			
	3	1			
		2			
4	1	1	489	626	0.44
		2	762		
	2	1	414	490	0.31
		2	565		
	3	1	351	398	0.24
		2	445		
5	1	1	279	241	0.32
		2	202		
	2	1	250	268	0.13
		2	286		
	3	1	284	255	0.23
		2	225		
6	1	1	323	297	0.18
		2	270		
	2	1	184	173	0.13
		2	162		
	3	1	156	128	0.44
		2	100		

Note: 1. Concentration diluted according to sampling procedure of Section 3.1.3.

Table B-14: continued.

Exp.	Sampling Event	Sample	Syringe Concentration (µg/L)	Mean of Duplicates (µg/L)	Variability of Duplicates
7	1	1	164	155	0.12
		2	146		
	2	1	272	259	0.10
		2	246		
	3	1	255	273	0.13
		2	291		
8	1	1	143	140	0.05
		2	136		
	2	1	74	83	0.21
		2	91		
	3	1	95	79	0.42
		2	62		
9	1	1	238	254	0.13
		2	270		
	2	1	269	243	0.22
		2	216		
	3	1	332	335	0.02
		2	338		
10	1	1	363	382	0.10
		2	401		
	2	1	459	492	0.13
		2	524		
	3	1	340	376	0.19
		2	412		
11	1	1	406	452	0.20
		2	498		
	2	1	464	476	0.05
		2	488		
	3	1	547	527	0.08
		2	506		
12	1	1	498	560	0.22
		2	622		
	2	1	599	625	0.08
		2	650		
	3	1	496	523	0.10
		2	550		

Note: 1. Concentration diluted according to sampling procedure of Section 3.1.3.

Table B-15: Duplicate analysis for syringe o-Xylene.

Exp.	Sampling Event	Sample	Syringe Concentration (µg/L)	Mean of Duplicates (µg/L)	Variability of Duplicates
1	1	1			
		2			
	2	1			
		2			
	3	1			
		2			
2	1	1			
		2			
	2	1			
		2			
	3	1			
		2			
3	1	1			
		2			
	2	1			
		2			
	3	1			
		2			
4	1	1	218	259	0.32
		2	300		
	2	1	194	220	0.23
		2	245		
	3	1	145	171	0.30
		2	196		
5	1	1	157	137	0.29
		2	117		
	2	1	133	144	0.15
		2	155		
	3	1	153	138	0.23
		2	122		
6	1	1	141	132	0.14
		2	122		
	2	1	76	71	0.14
		2	66		
	3	1	62	52	0.38
		2	42		

Note: 1. Concentration diluted according to sampling procedure of Section 3.1.3.

Table B-15: continued.

Exp.	Sampling Event	Sample	Syringe Concentration (µg/L)	Mean of Duplicates (µg/L)	Variability of Duplicates
7	1	1	83	79	0.10
		2	75		
	2	1	131	131	0.01
		2	130		
	3	1	134	139	0.06
		2	143		
8	1	1	66	65	0.05
		2	63		
	2	1	81	66	0.45
		2	51		
	3	1	42	36	0.33
		2	30		
9	1	1	107	113	0.10
		2	118		
	2	1	93	97	0.08
		2	101		
	3	1	96	123	0.44
		2	150		
10	1	1	196	208	0.11
		2	219		
	2	1	228	244	0.13
		2	260		
	3	1	174	189	0.16
		2	204		
11	1	1	226	250	0.19
		2	273		
	2	1	251	257	0.05
		2	263		
	3	1	291	281	0.07
		2	270		
12	1	1	258	287	0.20
		2	316		
	2	1	306	320	0.09
		2	334		
	3	1	261	275	0.10
		2	288		

Note: 1. Concentration diluted according to sampling procedure of Section 3.1.3.

Table B-16: Duplicate analysis for effluent benzene.

Exp.	Sampling Event	Sample	Effluent Concentration (mg/L)	Mean of Duplicates (mg/L)	Variability of Duplicates
1	1	1	19	22	0.23
		2	24		
	2	1	13	17	0.42
		2	20		
	3	1	20	17	0.42
		2	13		
2	1	1	25	21	0.44
		2	16		
	2	1	17	17	0.00
		2	17		
	3	1	22	21	0.15
		2	19		
3	1	1	9	9	0.00
		2	9		
	2	1	13	13	0.08
		2	12		
	3	1	9	17	0.91
		2	24		
4	1	1	134	147	0.17
		2	159		
	2	1	41	80	0.98
		2	119		
	3	1	142	146	0.05
		2	149		
5	1	1	84	88	0.08
		2	91		
	2	1	69	70	0.03
		2	71		
	3	1	78	74	0.12
		2	69		
6	1	1	45	46	0.02
		2	46		
	2	1	29	29	0.00
		2	29		
	3	1	39	37	0.11
		2	35		

Table B-16: continued.

Exp.	Sampling Event	Sample	Effluent Concentration (mg/L)	Mean of Duplicates (mg/L)	Variability of Duplicates
7	1	1	85	74	0.30
		2	63		
	2	1	81	80	0.03
		2	79		
	3	1	76	76	0.00
		2	76		
8	1	1	97	93	0.09
		2	89		
	2	1	83	84	0.01
		2	84		
	3	1	84	85	0.01
		2	85		
9	1	1	75	79	0.10
		2	83		
	2	1	67	68	0.03
		2	69		
	3	1	66	68	0.06
		2	70		
10	1	1	66	78	0.30
		2	89		
	2	1	161	212	0.48
		2	263		
	3	1	154	160	0.07
		2	165		
11	1	1	211	201	0.10
		2	190		
	2	1	182	182	0.00
		2	182		
	3	1	211	178	0.37
		2	145		
12	1	1	211	222	0.09
		2	232		
	2	1	184	207	0.22
		2	229		
	3	1	212	209	0.03
		2	206		

Table B-17: Duplicate analysis for effluent toluene.

Exp.	Sampling Event	Sample	Effluent Concentration (mg/L)	Mean of Duplicates (mg/L)	Variability of Duplicates
1	1	1	7	9	0.44
		2	11		
	2	1	6	7	0.29
		2	8		
	3	1	3	8	1.20
		2	12		
2	1	1	7	10	0.53
		2	12		
	2	1	14	11	0.67
		2	7		
	3	1	11	8	0.93
		2	4		
3	1	1	7	6	0.33
		2	5		
	2	1	5	7	0.46
		2	8		
	3	1	5	12	1.13
		2	18		
4	1	1	68	85	0.40
		2	102		
	2	1	45	55	0.35
		2	64		
	3	1	81	83	0.04
		2	84		
5	1	1	36	38	0.11
		2	40		
	2	1	31	31	0.00
		2	31		
	3	1	35	33	0.12
		2	31		
6	1	1	18	19	0.05
		2	19		
	2	1	8	8	0.00
		2	8		
	3	1	20	16	0.50
		2	12		

Table B-17: continued.

Exp.	Sampling Event	Sample	Effluent Concentration (mg/L)	Mean of Duplicates (mg/L)	Variability of Duplicates
7	1	1	47	46	0.04
		2	45		
	2	1	45	24	1.75
		2	3		
	3	1	42	43	0.02
		2	43		
8	1	1	59	54	0.19
		2	49		
	2	1	61	55	0.22
		2	49		
	3	1	43	46	0.11
		2	48		
9	1	1	35	37	0.08
		2	38		
	2	1	27	26	0.08
		2	25		
	3	1	28	29	0.04
		2	29		
10	1	1	26	31	0.32
		2	36		
	2	1	84	118	0.57
		2	151		
	3	1	70	75	0.13
		2	80		
11	1	1	123	131	0.12
		2	139		
	2	1	111	105	0.11
		2	99		
	3	1	116	105	0.22
		2	93		
12	1	1	129	140	0.16
		2	151		
	2	1	114	146	0.44
		2	178		
	3	1	176	171	0.06
		2	166		

Table B-18: Duplicate analysis for effluent ethylbenzene.

Exp.	Sampling Event	Sample	Effluent Concentration (mg/L)	Mean of Duplicates (mg/L)	Variability of Duplicates
1	1	1	4	5	0.40
		2	6		
	2	1	4	5	0.22
		2	5		
	3	1	17	14	0.43
		2	11		
2	1	1	12	10	0.40
		2	8		
	2	1	14	11	0.55
		2	8		
	3	1	9	9	0.00
		2	9		
3	1	1	24	14	1.43
		2	4		
	2	1	4	5	0.40
		2	6		
	3	1	10	15	0.67
		2	20		
4	1	1	28	43	0.70
		2	58		
	2	1	9	16	0.84
		2	22		
	3	1	27	28	0.04
		2	28		
5	1	1	11	12	0.17
		2	13		
	2	1	14	13	0.24
		2	11		
	3	1	15	13	0.31
		2	11		
6	1	1	6	4	1.00
		2	2		
	2	1	2	2	0.00
		2	2		
	3	1	5	5	0.00
		2	5		

Table B-18: continued.

Exp.	Sampling Event	Sample	Effluent Concentration (mg/L)	Mean of Duplicates (mg/L)	Variability of Duplicates
7	1	1	12	9	0.82
		2	5		
	2	1	10	13	0.46
		2	16		
	3	1	15	15	0.00
		2	15		
8	1	1	21	20	0.10
		2	19		
	2	1	15	16	0.06
		2	16		
	3	1	7	12	0.78
		2	16		
9	1	1	12	13	0.08
		2	13		
	2	1	11	10	0.20
		2	9		
	3	1	14	13	0.24
		2	11		
10	1	1	8	8	
		2			
	2	1	24	31	0.45
		2	38		
	3	1	17	19	0.16
		2	20		
11	1	1	39	36	0.17
		2	33		
	2	1	37	36	0.08
		2	34		
	3	1	46	39	0.39
		2	31		
12	1	1	42	45	0.13
		2	48		
	2	1	34	37	0.16
		2	40		
	3	1	46	48	0.08
		2	50		

Table B-19: Duplicate analysis for effluent m&p-Xylenes.

Exp.	Sampling Event	Sample	Effluent Concentration (mg/L)	Mean of Duplicates (mg/L)	Variability of Duplicates
1	1	1	11	14	0.37
		2	16		
	2	1	12	13	0.15
		2	14		
	3	1	29	26	0.23
		2	23		
2	1	1	29	24	0.47
		2	18		
	2	1	32	25	0.56
		2	18		
	3	1	21	18	0.33
		2	15		
3	1	1	18	16	0.32
		2	13		
	2	1	11	18	0.78
		2	25		
	3	1	14	33	1.14
		2	51		
4	1	1	76	90	0.30
		2	103		
	2	1	59	72	0.35
		2	84		
	3	1	96	94	0.05
		2	91		
5	1	1	36	39	0.13
		2	41		
	2	1	42	40	0.10
		2	38		
	3	1	44	45	0.02
		2	45		
6	1	1	16	18	0.17
		2	19		
	2	1	7	7	0.00
		2	7		
	3	1	11	11	0.10
		2	10		

Table B-19: continued.

Exp.	Sampling Event	Sample	Effluent Concentration (mg/L)	Mean of Duplicates (mg/L)	Variability of Duplicates
7	1	1	38	32	0.38
		2	26		
	2	1	45	57	0.42
		2	69		
	3	1	46	47	0.02
		2	47		
8	1	1	30	29	0.07
		2	28		
	2	1	31	32	0.06
		2	33		
	3	1	20	22	0.14
		2	23		
9	1	1	38	38	0.03
		2	37		
	2	1	57	48	0.40
		2	38		
	3	1	41	30	0.78
		2	18		
10	1	1	68	82	0.33
		2	95		
	2	1	77	102	0.49
		2	127		
	3	1	63	68	0.15
		2	73		
11	1	1	102	95	0.15
		2	88		
	2	1	94	89	0.11
		2	84		
	3	1	111	97	0.30
		2	82		
12	1	1	123	134	0.16
		2	145		
	2	1	103	126	0.36
		2	148		
	3	1	132	130	0.04
		2	127		

Table B-20: Duplicate analysis for effluent o-Xylene.

Exp.	Sampling Event	Sample	Effluent Concentration (mg/L)	Mean of Duplicates (mg/L)	Variability of Duplicates
1	1	1	12	14	0.29
		2	16		
	2	1	10	11	0.18
		2	12		
	3	1	28	22	0.55
		2	16		
2	1	1	29	36	0.39
		2	43		
	2	1	26	30	0.24
		2	33		
	3	1	16	24	0.67
		2	32		
3	1	1	17	17	0.00
		2	17		
	2	1	24	33	0.52
		2	41		
	3	1	22	46	1.04
		2	70		
4	1	1	44	52	0.29
		2	59		
	2	1	37	93	1.20
		2	149		
	3	1	51	52	0.04
		2	53		
5	1	1	28	30	0.13
		2	32		
	2	1	33	31	0.16
		2	28		
	3	1	31	30	0.10
		2	28		
6	1	1	13	14	0.07
		2	14		
	2	1	7	7	0.00
		2	7		
	3	1	9	9	0.00
		2	9		

Table B-20: continued.

Exp.	Sampling Event	Sample	Effluent Concentration (mg/L)	Mean of Duplicates (mg/L)	Variability of Duplicates
7	1	1	24	24	
		2			
	2	1	29	30	0.07
		2	31		
	3	1	43	42	0.07
		2	40		
8	1	1	43	30	0.87
		2	17		
	2	1	17	17	0.00
		2	17		
	3	1	13	13	0.00
		2	13		
9	1	1	21	21	0.00
		2	21		
	2	1	23	30	0.44
		2	36		
	3	1	24	26	0.12
		2	27		
10	1	1	43	50	0.28
		2	57		
	2	1	47	61	0.46
		2	75		
	3	1	40	42	0.10
		2	44		
11	1	1	74	65	0.29
		2	55		
	2	1	59	55	0.15
		2	51		
	3	1	67	60	0.23
		2	53		
12	1	1	86	88	0.03
		2	89		
	2	1	67	77	0.25
		2	86		
	3	1	80	78	0.06
		2	75		

Appendix C: Outlier Analysis

Table C-1: Outlier analysis.

Parameter	Experiment	Compound	Sample Values (mg/L)			Average (mg/L)	Standard Deviation (mg/L)	Potential Outlier	I test	Result
Feed Stream BTEx	7	Benzene	91	189	189	156	57	91	1.1547	Outlier
	8	Benzene	1291	257	297	615	586	1291	1.15403	Outlier
		Toluene	1002	171	212	462	468	1002	1.15359	Outlier
		Ethylbenzene	676	119	136	310	317	676	1.15428	Outlier
		m&p-Xylene	620	64	86	257	315	620	1.154	Outlier
		o-Xylene	234	29	40	101	115	234	1.15339	Outlier
Syringe BTEx	8	Benzene	423	291	299	338	74	423	1.15301	Outlier
Effluent BTEx	1	m&p-Xylene	14	13	26	18	7	26	1.15194	Outlier
	4	Benzene	147	80	146	124	38	80	1.1546	Outlier
		Toluene	85	55	83	74	17	55	1.15265	Outlier

Note: 1. Value of concern is considered an outlier if greater than I value.
(For 3 numbers: I value ($\alpha=0.05$) = 1.15)

Appendix D: t-Test Analysis to Compare Feed Stream and Syringe Methods for Measuring Influent BTEX Loading Rates

Table D-1: t-Test to compare benzene feed stream and syringe values.

Experiment	Parameter	Sampling Event Values ($\mu\text{g/L.day}$)			Average ($\mu\text{g/L.day}$)	T-test	Result
4	Feed Stream Syringe	260	207	235	234	0.94274358	no difference
		292	276	214	261		
5	Feed Stream Syringe	162	180	178	173	4.0755523	difference
		144	153	142	146		
6	Feed Stream Syringe	90	85	107	94	1.47289561	no difference
		169	128	90	129		
7	Feed Stream Syringe		127	127	127	2.30845129	no difference
		102	116	118	112		
8	Feed Stream Syringe		172	199	186	4.94601731	difference
			116	120	118		
9	Feed Stream Syringe	131	102	176	136	2.42716632	no difference
		85	73	90	83		
10	Feed Stream Syringe	93	243	190	175	0.91254197	no difference
		130	355	256	247		
11	Feed Stream Syringe	302	284	221	269	0.08707249	no difference
		254	258	288	267		
12	Feed Stream Syringe	144	238	78	153	2.88321444	difference
		275	306	288	290		

Notes: 1. For comparison, values have been converted to influent loading rate ($\mu\text{g/L.day}$)

2. Averages of feed stream and syringe loading rates are considered to be different if t statistic is greater than the critical value t.

(Critical values: $t_{0.05(2),4} = 2.776$, $t_{0.05(2),3} = 3.182$, $t_{0.05(2),2} = 4.303$)

Table D-2: t-Test to compare toluene feed stream and syringe values.

Experiment	Parameter	Sampling Event Values ($\mu\text{g/L}\cdot\text{day}$)				Average ($\mu\text{g/L}\cdot\text{day}$)	T-test	Result
4	Feed Stream Syringe	178	139	172		163	1.6549523	no difference
		239	210	164		204		
5	Feed Stream Syringe	88	103	103		98	1.4375	no difference
		88	94	89		90		
6	Feed Stream Syringe	48	36	59		48	2.35751543	no difference
		117	86	62		88		
7	Feed Stream Syringe	34	97	86		72	0.48106697	no difference
		72	85	89		82		
8	Feed Stream Syringe	123	115	142		129	1.60323853	no difference
			86	80		96		
9	Feed Stream Syringe	101	66	97		88	2.76094708	no difference
		56	50	62		56		
10	Feed Stream Syringe	43	158	121		107	0.99532151	no difference
		72	251	185		169		
11	Feed Stream Syringe	205	197	155		186	0.72836784	no difference
		187	193	216		199		
12	Feed Stream Syringe	105	176	52		111	2.97440136	difference
		216	244	208		223		

Notes: 1. For comparison, values have been converted to influent loading rate ($\mu\text{g/L}\cdot\text{day}$)

2. Averages of feed stream and syringe loading rates are considered to be different if t statistic is greater than the critical value t.

(Critical values: $t_{0.05(2),4} = 2.776$, $t_{0.05(2),3} = 3.182$, $t_{0.05(2),2} = 4.303$)

Table D-3: t-Test to compare ethylbenzene feed stream and syringe values.

Experiment	Parameter	Sampling Event Values ($\mu\text{g/L}\cdot\text{day}$)			Average ($\mu\text{g/L}\cdot\text{day}$)	T-test	Result
4	Feed Stream Syringe	91 157	72 119	93 92	85 123	1.86614836	no difference
5	Feed Stream Syringe	46 48	55 54	55 51	52 51	0.28867513	no difference
6	Feed Stream Syringe	26 79	15 57	31 39	24 58	2.74788822	no difference
7	Feed Stream Syringe	19 48	73 53	53 59	48 53	0.31095179	no difference
8	Feed Stream Syringe	80 80	80 51	91 53	86 61	1.89551931	no difference
9	Feed Stream Syringe	48 38	36 34	60 39	48 37	1.55047525	no difference
10	Feed Stream Syringe	17 33	160 143	62 107	80 94	0.27567386	no difference
11	Feed Stream Syringe	105 114	113 124	85 130	101 123	2.26989846	no difference
12	Feed Stream Syringe	65 136	101 150	35 135	67 140	3.72560414	difference

Notes: 1. For comparison, values have been converted to influent loading rate ($\mu\text{g/L}\cdot\text{day}$)

2. Averages of feed stream and syringe loading rates are considered to be different if t statistic is greater than the critical value t.

(Critical values: $t_{0.05(2),4} = 2.776$, $t_{0.05(2),3} = 3.182$, $t_{0.05(2),2} = 4.303$)

Table D-4: t-Test to compare m&p-Xylenes feed stream and syringe values.

Experiment	Parameter	Sampling Event Values ($\mu\text{g/L.day}$)				Average ($\mu\text{g/L.day}$)	T-test	Result
4	Feed Stream Syringe	147	172	156		158	1.58067125	no difference
		250	196	159		202		
5	Feed Stream Syringe	88	107	111		102	0.04287465	no difference
		96	107	102		102		
6	Feed Stream Syringe	40	21	39		33	2.17960459	no difference
		119	69	51		80		
7	Feed Stream Syringe	24	118	91		78	0.44208077	no difference
		62	104	109		92		
8	Feed Stream Syringe	56	43	58		51	0.8793911	no difference
		94	33	32		40		
9	Feed Stream Syringe	102	90	123		102	0.55660355	no difference
		102	97	134		111		
10	Feed Stream Syringe	71	110	88		90	4.06831398	difference
		153	197	150		167		
11	Feed Stream Syringe	159	172	133		155	2.71139019	no difference
		181	190	211		194		
12	Feed Stream Syringe	104	158	55		106	3.80466425	difference
		224	250	209		228		

Notes: 1. For comparison, values have been converted to influent loading rate ($\mu\text{g/L.day}$)

2. Averages of feed stream and syringe loading rates are considered to be different if t statistic is greater than the critical value t.

(Critical values: $t_{0.05(2),4} = 2.776$, $t_{0.05(2),3} = 3.182$, $t_{0.05(2),2} = 4.303$)

Table D-5: t-Test to compare o-Xylene feed stream and syringe values.

Experiment	Parameter	Sampling Event Values ($\mu\text{g/L}\cdot\text{day}$)				Average ($\mu\text{g/L}\cdot\text{day}$)	T-test	Result
4	Feed Stream	67	62	72		67	1.81991311	no difference
	Syringe	104	88	68		87		
5	Feed Stream	50	58	59		56	0.11043153	no difference
	Syringe	55	58	55		56		
6	Feed Stream	23	11	18		17	1.61542667	no difference
	Syringe	53	28	21		34		
7	Feed Stream	12	56	48		39	0.51832106	no difference
	Syringe	32	52	56		47		
8	Feed Stream		19	27		23	0.1677051	no difference
	Syringe	26	26	14		22		
9	Feed Stream	45	42	62		50	0.776114	no difference
	Syringe	45	39	49		44		
10	Feed Stream	41	60	48		50	4.21669157	difference
	Syringe	83	98	76		86		
11	Feed Stream	91	99	74		88	2.07173338	no difference
	Syringe	100	103	112		105		
12	Feed Stream	57	87	30		58	3.44613319	difference
	Syringe	115	128	110		118		

Notes: 1. For comparison, values have been converted to influent loading rate ($\mu\text{g/L}\cdot\text{day}$)
 2. Averages of feed stream and syringe loading rates are considered to be different if t statistic is greater than the critical value t.

(Critical values: $t_{0.05(2),4} = 2.776$, $t_{0.05(2),3} = 3.182$, $t_{0.05(2),2} = 4.303$)

Appendix E: Estimation of Influent Nitrate Concentrations for Experiments 4, 5 and 6

Table E-1: Estimation of influent nitrate concentrations for experiments 4, 5 and 6.

Experiment	Target Mass Loading Rate (mg NO₃⁻/day)	Flow Rate (mL/day)	Target Concentration (mg NO₃⁻/L)	Measured Concentration (mg NO₃⁻/L)	Difference Between Target and Measured Concentrations (%)	Estimated Concentration (mg NO₃⁻/L)
1						
2						
3						
4	4600	488	9426			9945
5	4600	488	9426			9945
6	4600	488	9426			9945
7	4600	988	4656	4808	3.3	
8	900	988	911	1042	14.4	
9	400	988	405	381	-5.9	
10	400	488	820	839	2.4	
11	400	488	820	820	0.0	
12	700	488	1434	1705	18.9	
Average:						5.5

Appendix F: Analysis of Variance of BTEX Effluent Mass Rates and Removal Rates

Table F-1: Anova analysis of benzene mass effluent rates ($\mu\text{g/L}\cdot\text{day}$).

Experiment	$\bar{x}$	s	$(n-1)s^2$	$n\bar{x}^2$	$n\bar{x}$	n
1	19	3	18	1045	56	3
2	20	2	10.8889	1160	59	3
3	13	4	32	507	39	3
4	147	1	0.44444	43022	293	2
5	77	9	174.222	17941	232	3
6	37	9	150.222	4181	112	3
7	77	3	18	17633	230	3
8	87	5	50	22881	262	3
9	72	6	80.2222	15408	215	3
10	150	68	9157.56	67500	450	3
11	187	12	304.222	104907	561	3
12	213	8	128	135681	638	3
Total			10123.8	431869	3147	35

	SS	DF	MS
Total	158,972	34	
Group	148,849	11	13532
Error	10,124	23	440
F:			30.74
Critical Value of $F_{0.05(2),11,23}$:			2.62

Table F-2: Anova analysis of toluene mass effluent rates ($\mu\text{g/L}\cdot\text{day}$).

Experiment	$\bar{x}$	s	$(n-1)s^2$	$n\bar{x}^2$	$n\bar{x}$	n
1	8	1	2	192	24	3
2	10	2	8	300	30	3
3	8	3	18	192	24	3
4	84	1	1	14112	168	2
5	34	4	32	3468	102	3
6	14	6	72	588	42	3
7	38	12	288	4332	114	3
8	52	5	50	8112	156	3
9	31	6	72	2883	93	3
10	75	44	3872	16875	225	3
11	114	15	450	38988	342	3
12	152	16	512	69312	456	3
Total			5377	159354	1776	35

Total	SS	DF	MS
Group	74,612	34	
Error	69,235	11	6294
	5,377	23	234
F:			26.92
Critical Value of $F_{0.05(2),11,23}$:			2.62

Table F-3: Anova analysis of ethylbenzene mass effluent rates ($\mu\text{g/L}\cdot\text{day}$).

Experiment	$\bar{x}$	s	(n-1)s ²	n $\bar{x}^2$	n $\bar{x}$	n
1	8	5	50	192	24	3
2	10	1	2	300	30	3
3	11	6	72	363	33	3
4	29	14	196	1682	58	2
5	13	1	2	507	39	3
6	4	2	8	48	12	3
7	12	3	18	432	36	3
8	16	4	32	768	48	3
9	12	2	8	432	36	3
10	19	12	288	1083	57	3
11	37	2	8	4107	111	3
12	43	6	72	5547	129	3
Total			756	15461	613	35

	SS	DF	MS
Total	5,481	34	
Group	4,725	11	429.5
Error	756	23	32.9
F:			13.07
Critical Value of F_{0.05(2),11,23}:			2.62

Table F-4: Anova analysis of m&p-xylenes mass effluent rates ($\mu\text{g/L}\cdot\text{day}$).

Experiment	$\bar{x}$	s	$(n-1)s^2$	$n\bar{x}^2$	$n\bar{x}$	n
1	13	1	1	338	26	2
2	22	4	32	1452	66	3
3	22	9	162	1452	66	3
4	85	12	288	21675	255	3
5	41	3	18	5043	123	3
6	12	6	72	432	36	3
7	45	13	338	6075	135	3
8	28	5	50	2352	84	3
9	39	9	162	4563	117	3
10	84	17	578	21168	252	3
11	94	4	32	26508	282	3
12	130	4	32	50700	390	3
Total			1765	141758	1832	35

Total	SS	DF	MS
Group	47,631	34	
Error	45,866	11	4170
	1,765	23	77
F:			54.34
Critical Value of $F_{0.05(2),11,23}$:			
			2.62

Table F-5: Anova analysis of o-xylene mass effluent rates ($\mu\text{g/L}\cdot\text{day}$).

Experiment	$\bar{x}$	s	(n-1)s ²	n $\bar{x}^2$	n $\bar{x}$	n
1	16	6	72	768	48	3
2	30	6	72	2700	90	3
3	32	15	450	3072	96	3
4	66	24	1152	13068	198	3
5	30	1	2	2700	90	3
6	10	4	32	300	30	3
7	32	9	162	3072	96	3
8	20	9	162	1200	60	3
9	26	5	50	2028	78	3
10	51	10	200	7803	153	3
11	60	5	50	10800	180	3
12	81	6	72	19683	243	3
Total			2476	67194	1362	36

Total	SS	DF	MS
18,141	18,141	35	
Group	15,665	11	1424
Error	2,476	24	103
F:			13.80
Critical Value of $F_{0.05(2),11,24}$:			2.59

Table F-6: Anova analysis of benzene removal rates ($\mu\text{g/L}\cdot\text{day}$).

Experiment	$\bar{x}$	s	(n-1)s ²	$n\bar{x}^2$	$n\bar{x}$	n
1	83	3	18	20667	249	3
2	82	2	8	20172	246	3
3	89	4	32	23763	267	3
4	107	54	2916	22898	214	2
5	69	13	338	14283	207	3
6	92	35	2450	25392	276	3
7	36	7	98	3888	108	3
8	34	2	4	2312	68	2
9	11	9	162	363	33	3
10	97	46	4232	28227	291	3
11	79	28	1568	18723	237	3
12	77	23	1058	17787	231	3
Total			12884	198475	2427	34

	SS	DF	MS
Total	38,114	33	
Group	25,230	11	2294
Error	12,884	22	586
F:			3.92
Critical Value of $F_{0.05(2),11,22}$:			2.65

Table F-7: Anova analysis of toluene removal rates ($\mu\text{g/L.day}$).

Experiment	$\bar{x}$	s	(n-1)s ²	$n\bar{x}^2$	$n\bar{x}$	n
1	103	1	2	31827	309	3
2	102	2	8	31212	306	3
3	103	3	18	31827	309	3
4	118	52	2704	27848	236	2
5	57	6	72	9747	171	3
6	74	26	1352	16428	222	3
7	44	17	578	5808	132	3
8	45	21	882	6075	135	3
9	26	7	98	2028	78	3
10	95	48	4608	27075	285	3
11	85	28	1568	21675	255	3
12	70	31	1922	14700	210	3
Total			13812	226250	2648	35

Total	SS	DF	MS
39,722		34	
Group	25,910	11	2355
Error	13,812	23	601
F:			3.92
Critical Value of $F_{0.05(2),11,23}$:			2.62

Table F-8: Anova analysis of ethylbenzene removal rates ($\mu\text{g/L.day}$).

Experiment	$\bar{x}$	s	(n-1)s ²	$n\bar{x}^2$	$n\bar{x}$	n
1	86	5	50	22188	258	3
2	84	1	2	21168	252	3
3	82	6	72	20172	246	3
4	94	26	1352	26508	282	3
5	38	3	18	4332	114	3
6	55	21	882	9075	165	3
7	41	3	18	5043	123	3
8	45	13	338	6075	135	3
9	25	1	2	1875	75	3
10	75	45	4050	16875	225	3
11	86	7	98	22188	258	3
12	97	14	392	28227	291	3
Total			7274	183726	2424	36

Total	SS	DF	MS
27,784	27,784	35	
Group	20,510	11	1865
Error	7,274	24	303
F:			6.15
Critical Value of $F_{0.05(2),11,24}$:			2.59

Table F-9: Anova analysis of m&p-xylenes removal rates ($\mu\text{g/L.day}$).

Experiment	$\bar{x}$	s	(n-1)s ²	n $\bar{x}^2$	n $\bar{x}$	n
1	161	1	2	77763	483	3
2	152	4	32	69312	456	3
3	152	9	162	69312	456	3
4	117	48	4608	41067	351	3
5	61	6	72	11163	183	3
6	68	31	1922	13872	204	3
7	46	16	512	6348	138	3
8	13	13	338	507	39	3
9	72	28	1568	15552	216	3
10	8	12	288	192	24	3
11	100	14	392	30000	300	3
12	98	23	1058	28812	294	3
Total			10954	363900	3144	36

Total	100,278	DF	MS
Group	89,324	11	8120
Error	10,954	24	456
F:			17.79
Critical Value of $F_{0.05(2),11,24}$:			
			2.59

Table F-10: Anova analysis of o-xylene removal rates ($\mu\text{g/L}\cdot\text{day}$).

Experiment	$\bar{x}$	s	$(n-1)s^2$	$n\bar{x}^2$	$n\bar{x}$	n
1	126	6	72	47628	378	3
2	111	6	72	36963	333	3
3	109	15	450	35643	327	3
4	23	26	1352	1587	69	3
5	26	1	2	2028	78	3
6	24	14	392	1728	72	3
7	15	7	98	675	45	3
8	4	5	50	48	12	3
9	19	9	162	1083	57	3
10	34	2	8	3468	102	3
11	45	9	162	6075	135	3
12	37	13	338	4107	111	3
Total			3158	141033	1719	36

Total	SS	DF	MS
62,109	62,109	35	
Group	58,951	11	5359
Error	3,158	24	132
F:			40.73
Critical Value of $F_{0.05(2),11,24}$:			
			2.59