A STUDY OF THE PATTERNS, STOICHIOMETRY, AND KINETICS OF MICROBIAL BTX DEGRADATION UNDER DENITRIFYING CONDITIONS BY AN ACTIVATED SLUDGE CONSORTIUM RECEIVING A MIXED WASTE

by,

James D. Fettig

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Dr. Nancy G. Love, Chair

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Dr. John T. Novak Dr. John C. Little

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(ABSTRACT)

The patterns, stoichiometry, and kinetics of microbial benzene, toluene, *p*-xylene, *m*-xylene, and *o*-xylene degradation by a denitrifying activated sludge consortium was investigated in a sequencing batch reactor (SBR) receiving a mixed waste. After six months of acclimation, toluene and *m*-xylene were routinely degraded to below detection. Both toluene and m-xylene could serve as sole carbon and energy sources. The removal of *o*-xylene was also possible; however, its transformation was dependent upon gratuitous metabolism during toluene degradation. Benzene and *p*-xylene were recalcitrant throughout the study. The first order decay coefficient (b) of the denitrifying biomass was determined to be 0.016 ± 0.006 h^{-1} on a theoretical oxygen demand (thOD) basis. The true growth yields (Y) for the biogenic and toluene/*m*-xylene components of the mixed waste were determined to be 0.41 ± 0.02 and 0.35 ± 0.04 mg thOD biomass per mg thOD substrate, respectively. The Monod parameters, q_{max} and K_S, for toluene ranged from 0.059 to 0.14 mg toluene/mg protein/h and 0.84 to 6.9 mg/L, respectively. For *m*-xylene, the q_{max} and K_S parameters ranged from 0.034 to 0.041 mg *m*-xylene/mg protein/h and 0.28 to 3.7 mg/L, respectively. Some of the variation observed between kinetic experiments was attributed to the different accumulation levels of the denitrification intermediate nitrite (NO_2^-) and the inhibitory effects of its conjugate acid, nitrous acid (HNO_2). Other evidence suggested that part of the variation was also due to a continuous acclimation and refinement towards higher affinity toluene- and *m*-xylene-degrading enzyme systems within the biomass.

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LITERATURE REVIEW

Background

The monoaromatic hydrocarbons benzene, toluene, *o*-xylene, *m*-xylene, and *p*-xylene (BTX) are common components of petroleum and are often encountered as contaminates in the aqueous environment. Selected physical and chemical properties of these compounds are given in Table 1. The BTX fractions, along with ethylbenzene, comprise the more water-soluble petroleum hydrocarbons. Because of this property, aqueous dissolution of these hydrocarbons commonly occurs when petroleum comes in contact with water, either through the inadvertent spilling of petroleum, the leaking of petroleum tanks, or the petroleum refining process. The contamination of waters by BTX is of environmental concern because they have been shown to exhibit acute toxic and chronic mutagenic properties (Dean, 1985, EPA, 1998). The environmental significance of these compounds is further supported by their relatively high emissions to the environment. In the 1995 EPA Toxics Release Inventory (TRI) report, toluene and the mixed xylene isomers ranked third and fifth of the 643 listed compounds, representing 6.6 and 4.3 % of the total toxic emissions, respectively (Hanson, 1997).

Physicochemical Treatment Methods

The Clean Water Act (CWA) of 1972 gave the EPA the regulatory authority to establish the "National Pollutant Discharge Elimination System" (NPDES). The NPDES permit system requires the petroleum refining industry to treat its wastewater to control the discharge of toxics to receiving waters, a large component of which are the BTX compounds. Conventional treatment of BTX-contaminated wastewaters typically involves the physical process of gas stripping (Dold, 1989). The volatility of the BTX compounds, as defined by their Henry's Law constants (see Table 1), makes this treatment process very effective in removing these compounds from solution to below regulatory concentrations. The stripping process, however, merely transfers the BTX compounds from water to air, where they may still pose an environmental threat (Chin, 1994).

In 1992, the EPA promulgated the "National Emissions Standards for Hazardous Air Pollutants", or NESHAP, which require certain chemical and petroleum industries to employ maximum achievable control technologies (MACT's) to prevent the release of hazardous air pollutants (HAP's) to the atmosphere (EPA, 1997). Industries that have loadings greater than 10 Mg/yr of benzene, have wastestreams with annual average concentrations of benzene greater than 10 ppmw, and a flow rate greater than 0.02 L/min, fall under this new regulation (EPA, 1997). For industries that are subject to the NESHAP regulations, gas stripping of BTX and other toxic volatiles is no longer sufficient as the only treatment method. Instead, refineries must employ additional technologies to prevent their emission to the atmosphere. Specifically, NESHAP regulations require a 99% reduction in benzene mass emissions through the use of suppression followed by steam stripping, biotreatment, or some other treatment process (EPA, 1997).

Table 1. Physical properties of the BTX hydrocarbons.

Fraction	Specific gravity, γ (20°C) (dimensionless)	Aqueous Solubility (mg/L) [†]	Henry's Law constant, m (dimensionless) [‡]
Benzene	0.879	1780 (20 °C)	0.225 (25 °C)
Toluene	0.866	470 (16 °C)	0.276 (25 °C)
o-Xylene	0.880	175 (20 °C)	0.210 (25 °C)
m-Xylene	0.864	35 (20 °C)	0.22 (20 °C) [*]
p-Xylene	0.861	198 (25 °C)	0.283 (25 °C)

[†] Verscheuer (1983)
[‡] Schwarzenback *et. al.* (1993)
[*] Selleck *et. al.* (1988)

Several alternate physicochemical methods have been employed to remove BTX from wastewater and to prevent their release to the atmosphere. For example, Wong *et al.* (1992) used powdered activated carbon (PAC) according to the PACT® process to sorb toxics from a petroleum wastewater.

The produced solids from the system were regenerated using a wet air oxidation system. Although BTX was not tested for specifically in this study, the sorption of BTX to PAC would likely occur under such a treatment system. In a full-scale petroleum refinery wastewater treatment plant, VOC-containing offgas from API-separators and aeration basins was channeled through thermal oxidation units to degrade the hazardous air pollutants (Al Tell and Leuders, 1994). Given these examples and the recommendations set forth by the EPA, physicochemical methods are currently the treatment technologies of choice for removing and preventing emission of BTX and other hazardous volatiles from petroleum industry wastewater.

Biodegradation of BTX Under Aerobic Conditions

The extent of biological treatment of petroleum wastewaters is typically limited to aerobic treatment for soluble organic contaminates after API separators and dissolved-air-flotation units have removed most of the free and emulsified oil (Al-Tell and Leuders, 1994; Dold, 1989; Wong *et al.*, 1992). Biological treatment of BTX contaminants is often overlooked as a potential treatment technology. The biodegradability of all of the BTX compounds under aerobic conditions is well established (Alvarez *et al.*, 1991; Hutchins *et al.*, 1991), and the pathways of degradation have been elucidated. Under aerobic conditions, benzene is initially converted to catechol and the aromatic ring is subsequently cleaved by one of two pathways: the *ortho*-cleavage, in which catechol 1,2 dioxygenase enzymes are utilized; or the *meta*-cleavage, which employs catechol 2,3-dioxygenase enzymes. Initial reactions of aerobic toluene degradation involve either the direct formation of 3-methyl catechol, or by methyl group hydroxylation to catechol. The xylenes are initially degraded to their corresponding methyl catechols by hydroxylation of one of the substituent methyl groups. The ring structure is then attacked via the *meta*-pathway (Smith, 1990). Unfortunately, complete aerobic biodegradation of BTX in conventional aeration basins is difficult due to the volatility of these compounds. The diffused- or mechanical-aeration systems employed in aerobic basins serve the duplicate purpose of stripping these

volatile compounds out of solution before they become completely available for microbial uptake. Conversely, the potential for biodegradation may be realized in anoxic basins, where such gas-liquid mass transfer systems are not employed, and hence, the stripping potential is minimized.

BTX Biodegradation Under Anoxic and Methanogenic Conditions

Extensive research in the field of groundwater remediation has been conducted to investigate the ability of microorganisms to degrade BTX under alternate electron acceptor environments. Environments such as nitrate-reducing (denitrifying), sulfate-reducing, iron (III)-reducing, and methanogenic have been shown to be effective in treating at least some of the BTX compounds. Under sulfate-reducing conditions, toluene has been shown to be amenable to degradation under pure culture (Beller *et al.*, 1996; Rabus *et al.*, 1993), by in-situ indigenous microbes in a unconfined aquifer under sulfate-enhanced conditions (Reinhard *et al.*, 1997), and by cultures derived from sediment microbes of a semi-confined aquifer (Chapelle *et al.*, 1996). Beller *et al.* (1996) found that *o*-xylene and *p*-xylene were transformed under sulfate-reducing conditions, but that their transformation was temporally linked to periods of toluene degradation and could not be sustained over time. Reinhard *et al.* (1997) also reported degradation of *m*-xylene and *o*-xylene under sulfate-reducing conditions that was temporally linked to toluene degradation; however, it was unclear whether their degradation was dependent upon toluene degradation. Under iron (III)-reducing conditions, degradation of toluene by a pure culture has been reported (Lovley *et al.*, 1990). Under methanogenic conditions, benzene, toluene, and *o*-xylene mineralization by a mixed culture derived from aquifer microorganisms has been reported (Edwards *et al.*, 1994; Grbic-Galic *et al.*, 1987). In a contradictory study, toluene was recalcitrant under methanogenic conditions (Chapelle *et al.*, 1996); however, it was postulated that the microbial community was not sufficiently acclimated to methanogenic conditions prior to the experiments.

As discussed above, several of the BTX fractions are amenable to biodegradation under sulfate-reducing, iron (III)-reducing, and methanogenic environments. The majority of research, however, has been conducted under anoxic, denitrifying environments, where nitrate is used as the terminal electron acceptor. Under denitrifying conditions, toluene is reported as the most commonly degraded BTX compound. In one study, enrichments from a pristine and three previously contaminated aquifers had the capacity to degrade toluene (Alvarez *et al.*, 1995). In the same study, *m*-xylene and *p*-xylene were degraded in enrichments from one of the contaminated aquifers only. Also in the same study, *o*-xylene was transformed in all of the aquifer samples. However its transformation was incomplete and was temporally linked to and dependent upon toluene degradation. Evans *et al.* (1991a) studied denitrifying enrichment cultures derived from a variety of sources, including anaerobic digester sludge, and found that toluene and *m*-xylene were amenable to biodegradation. It was also statistically shown that *o*-xylene was transformed during the degradation of toluene; however, the relationship was not entirely clear in the data. In another study by Evans *et al.* (1991b), it was shown that the transformation of *o*-xylene was dependent upon the concurrent degradation of toluene, and that *o*-xylene could not serve as a sole carbon and energy source. In a study involving the in situ degradation of BTX under enhanced-nitrate reducing conditions, indigenous microbes had the capacity to degrade toluene, *m*-xylene, and *o*-xylene to below detection after sufficient incubation time (Rienhard *et al.*, 1997). In another study, *p*-xylene enrichment cultures derived from a diesel fuel-contaminated aquifer were capable of using *p*-xylene as a sole carbon and energy source (Häner *et al.*, 1995). Further study of the p-xylene enrichment cultures indicated that toluene and *m*-xylene could also serve as sole carbon and energy sources. It was found during the same study that when the BTX compounds and ethylbenzene (BTEX) were added to the enrichment culture, toluene and *m*-xylene were preferentially degraded, followed by *p*-xylene. Benzene, *o*-xylene, and ethylbenzene were recalcitrant during the 37 day period of incubation. In another study, four different bacterial strains isolated from freshwater mud and closely related to *Thauera selenatis* (EbN1, PbN1, ToN1, and mXyN1) were used to investigate the

degradative capacity of the BTX compounds as well as ethylbenzene, propylbenzene, and butylbenzene under denitrifying conditions (Rabus and Widdel, 1995). Of the eight aromatic hydrocarbons tested, strain EbN1 could only degrade toluene and ethylbenzene, strain PbN1 could only degrade ethylbenzene and propylbenzene, strain ToN1 could only degrade toluene, and strain mXyN1 could only degrade toluene and *m*-xylene. In a subsequent study, Rabus and Widdel (1996) demonstrated that the bacterial strains could grow on crude oil, and in doing so, selectively degraded specific alkylbenzenes from the crude oil according to the degradative patterns described above, except that strain PbN1 did not grow well on crude oil. Biodegradation of benzene under denitrifying conditions has been reported (Major *et al.*, 1988); however, most of the current research suggest that benzene is recalcitrant under strictly denitrifying conditions. In summary, the trend in the literature on BTX degradation under denitrifying conditions is that the degradation of toluene is most commonly encountered, followed by the degradation of *m*-xylene, the transformation of *o*-xylene, and the degradation *p*-xylene, with benzene typically reported as resistant to biodegradation.

Denitrification

Denitrification is classified as anaerobic (anoxic) respiration, with nitrate serving as the terminal electron acceptor instead of oxygen. The denitrification process involves the dissimilatory reduction of nitrate (NO_3^-), in which nitrate is ultimately reduced to dinitrogen gas ($N_{2(g)}$). The pathways of both dissimilatory and assimilatory nitrate reduction, where nitrate is reduced to the oxidation state of ammonia (NH_3) for anabolic purposes, are presented in Figure 2. The first step of denitrification, the reduction of nitrate to nitrite (NO_2^-), involves a two electron transfer and takes nitrogen from the +5 to the +3 state. Subsequent reductions take nitrite to nitric oxide ($NO_{(g)}$), nitric oxide to nitrous oxide ($N_2O_{(g)}$), and ultimately nitrous oxide to dinitrogen. The accumulation of the various nitrogen oxides has been observed and is of concern because they have been shown to be inhibitory under certain circumstances (Glass *et al.*, 1997).

Nitrite accumulation can occur under nitrate-limited conditions; however, this accumulation is transitory, as nitrite undergoes further reduction to the gaseous nitrogenous oxides and ultimately dinitrogen gas. For example, Kornaros *et al.* (1996) found a transitory accumulation of approximately 20 mg/L nitrite-N by a batch culture of *Pseudomonas denitrificans* (ATCC 13867) when receiving approximately 35 mg N/L under nitrate-limited conditions. Nitrite accumulation has been witnessed under carbon-limited conditions (Kornaros *et al.*,1996; Elmén *et al.*, 1996); however, the accumulation of nitrite is not transient under such conditions because there is insufficient electron donor to fully reduce the electron acceptor.

Nitrite accumulation occurs in a system on kinetic grounds when the rate of nitrate reduction exceeds the rate of nitrite reduction. Several factors that can lead to this imbalance have been determined. Barak *et al.* (1997) found that exposure to light, especially the green spectra, inhibited nitrite reduction and led to greater nitrite accumulation. Glass *et al.* (1997) found that increasing pH values above pH 7.5 led to increasing degrees of nitrite accumulation during denitrification of a high strength nitrate brine by an activated sludge culture. Rijn *et al.* (1996) reported that the type of carbon source can also be a factor. It was reported that intermediate nitrite accumulation occurred when a *Pseudomonas stutzeri* strain was given acetate or propionate as a carbon and electron donor. Conversely, nitrate accumulation was not observed when the bacterial strain was grown on butyrate, valerate, or caproate. It was proposed that under growth on acetate, the respiration rate was insufficient for both maximal nitrate and nitrite reduction, and that under this condition, nitrate reductases held a competitive advantage over nitrite reductases for electrons, which led to the accumulation of nitrite. Under growth on butyrate, the rate of nitrite reduction was greater than the rate of nitrate reduction. Rijn *et al.* (1996) postulated that this might be due to butyrate donating electrons to the respiratory chain in an area closer to the vicinity of cytochrome *c*, where nitrite reductase accepts electrons.

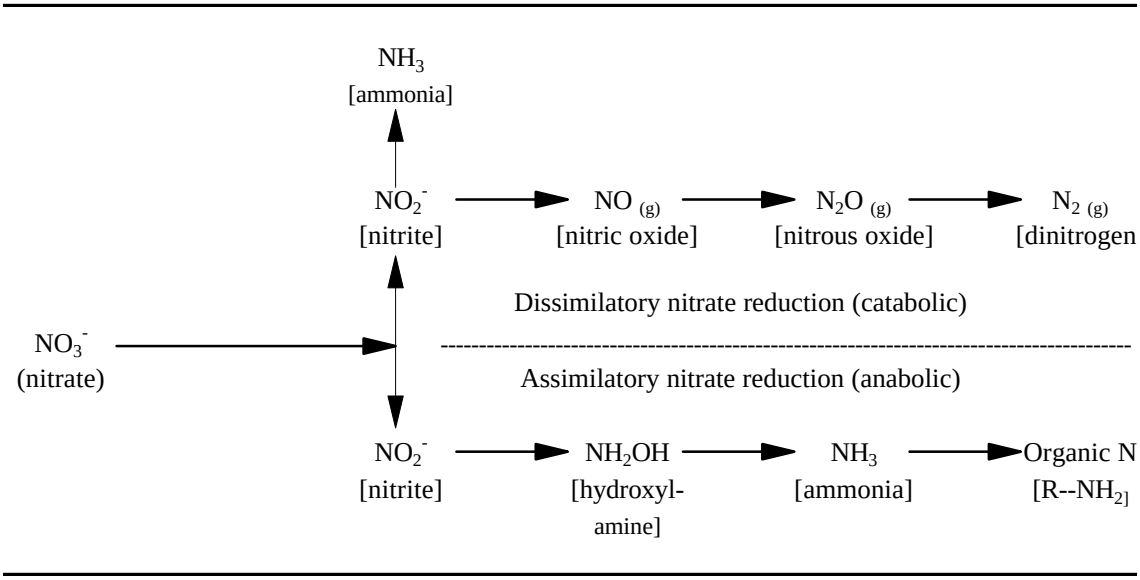

Figure 1. Assimilatory and dissimilatory nitrate reduction pathways.

Kinetic Models

Modeling the biodegradation of organic substrates by bacteria can be approached by one of two ways. One method is to model from the perspective of biomass growth, where the growth of biomass results in the simultaneous consumption of substrate. Alternatively, biodegradation can be modeled from the perspective of substrate uptake, where the consumption of substrate leads to the simultaneous growth of biomass. Biomass growth and substrate utilization can be made equivalent through the true growth yield coefficient. When considering the biodegradation of specific xenobiotic compounds, the latter approach is most often employed. This is because measuring the disappearance of substrate is usually more accurate and sensitive than measuring the production of biomass. The rate of substrate consumption, or degradation, is defined by the following relationship:

$$\frac{dS}{dt} = -q * X, \qquad (1)$$

where S is the substrate concentration, mg/L; t represents time, h; q is the specific uptake rate, h^{-1}; and X represents the active biomass concentration, mg/L. The specific uptake rate parameter, q, is dependent upon the concentration of substrate, S. The Monod equation is widely used to predict the relation between q and S for non-inhibitory substrates and is defined by the following:

$$\frac{dS}{dt} = -q_{max} * \left[\frac{S}{K_S + S} \right] * X, \qquad (2)$$

where q_{max} is the maximum specific uptake rate, h^{-1}; and K_S is the half saturation constant, mg/L. For inhibitory substrates, the Andrews equation is often employed to predict the relationship between q and S, and is defined as follows:

$$\frac{dS}{dt} = -q_{max} * \left[\frac{S}{K_S + S + \left(S^2 / K_I \right)} \right] * X, \qquad (3)$$

where K_I is the inhibition constant, mg/L. As mentioned above, substrate uptake and biomass growth are related through the yield coefficient (Y). The change of biomass is therefore defined by the following differential equation, which incorporates a term for biomass loss due to decay according to the traditional model for biomass growth:

$$\frac{dX}{dt} = (Y * q * X) - (b * X), \qquad (4)$$

where the term (Y*q) is also defined as the specific growth rate (m), h^{-1}.

Estimation of X

When modeling the biodegradation of organic substrates, accurate estimation of the biomass actively involved in substrate degradation is vital to the determination of accurate kinetic parameters. This arises from the fact that, for a given set of conditions, the term q_{max}*X is constant. When modeling a wastewater composed entirely of readily biodegradable substrates, the biomass is usually assumed to be comprised of substrate generalists, with the entire viable biomass involved in the degradation of all of the compounds. Therefore, the entire viable biomass in the system is usually reported as X. However, when modeling specific, xenobiotic substrates within a heterogeneous waste, the estimation of X is complicated by the potential existence of bacterial specialists within the system biomass. If a population of bacterial specialists is present, it can be expected to selectively use the xenobiotic substrate as its carbon and energy source. Under such circumstances, reporting the entire viable biomass in the system as X would lead to an underestimation of q_{max}. Accurate determination of q_{max} is therefore dependent upon the ability to reliably estimation the fraction of these specialists to the entire biomass in the system. Enumeration techniques such as the MPN technique as reported by Silverstein *et al.* (1994) and various molecular techniques such as whole cell and in-situ hybridization with specific nucleotide probes (Neef *et al.*, 1996; Schofield *et al.*, 1996; Tyagi *et al.*, 1995), can serve to experimentally determine the fraction of bacterial specialists within the system. In lieu of these techniques, however, estimations of the specialist population can be made. Preliminary results by Ellis *et al.* (1996) suggested that the fraction of bacteria selectively degrading a xenobiotic compound can be estimated by relating it to that substrate's fraction of chemical oxygen demand (COD) in the influent. Although not yet reported, this estimation could be refined by taking into account the different growth yields of the specific xenobiotic compound and the bulk wastewater.

Intrinsic and Extant Kinetics

The physiologic state of a biomass--in terms of the levels of nucleic acids, enzymes, and other macromolecules--can have a significant impact on the kinetic parameters determined for that biomass. The physiological state is largely determined by the growth history of a biomass; that is, factors such as the culture type, reactor configuration, and the growth rate (m) under which a biomass was cultured all affect the macromolecular constitution of the biomass. Intrinsic parameters describe the degradation kinetics of a biomass under an optimum physiological state. Alternatively, extant parameters describe the degradation kinetics of a biomass under its existing physiological state. Intrinsic parameters are determined under conditions where the initial substrate to biomass ratio (S_0/X_0) is sufficiently high to allow unrestricted, optimal growth. Under such conditions, the growth history and previous physiologic state of a biomass is not important; the high S_0/X_0 ratio allows the biomass to adjust its internal composition to achieve maximal growth. Extant kinetic parameters are determined under conditions where the S_0/X_0 is sufficiently low. Under low S_0/X_0 conditions, the biomass is not afforded the energy to alter its internal constituents, and therefore the kinetic parameters obtained under these conditions are reflective of the existing physiological state in that biomass.

Stoichiometric and Kinetic Parameters

Although many studies have been conducted to determine the degradability of BTX under denitrifying conditions, relatively few have investigated the degradation kinetics. Jørgensen *et al.* (1995) used an enrichment culture reared on a mixture of alkylbenzenes and methylated phenols for over a year to determine the stoichiometry and kinetics of toluene degradation. The initial toluene concentration for the kinetic experiments was 10.8 mg/L. The uptake of toluene was modeled using Monod kinetics and yielded values of 0.71 ± 0.04 mg toluene/(mg protein*h) and 0.4 ± 0.2 mg/L for q_{max} and K_S, respectively. Subsequent experiments conducted suggested that a more precise estimation of K_S was 0.20 ± 0.01 mg/L. A summary of the values reported by Jørgensen *et al.* (1995) for q_{max}, K_S,

Y, b, and m_{max} is presented in Table 2. In another study, the kinetics of toluene degradation by a pure culture of an *Azoarcus tolulyticus* strain was determined (Elmén *et al.*, 1996). Kinetic parameters were determined using different initial toluene concentrations in the range of 0 to 184 mg/L and by measuring the initial uptake rates. Various inhibition models were used to model the data, including the Andrews model. The determined values for q_{max}, K_S, and K_I of the Andrews' model were 0.70 mmol/(g dry cell weight*h), 0.38 mM, and 1.5 mM, respectively. A summary of these values, including the experimentally determined yield values, can also be found in Table 2. In both studies, the entire biomass was used to represent X, because they were either pure culture (Elmén *et al.*, 1996) or enrichment culture receiving a single substrate (Jørgensen *et al.*, 1995).

Table 2. Reported kinetic parameters for toluene degradation under denitrifying conditions.

Author	q_{max} (mg/mg protein/h)	K_S (mg/L)	K_I (mg/L)	Y (mg protein/mg)	b (h^{-1})
Jørgensen *et. al.* (1995)	0.71 ± 0.04	0.4 ± 0.2	NA	0.14 ± 0.02	~0
		0.20 ± 0.01 †	NA		
Elmén *et. al.* (1997) ‡	0.035	35	140	2.8	ND

† Separate experiments by Jørgensen *et. al.* (1995) suggested 0.20 ± 0.01 as a more precise estimate of K_S

‡ Values converted from reported units assuming protein content of dry cell weight is 55% (Neidhart, 1987)

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BTX DEGRADATION BY AN ACTIVATED SLUDGE CONSORTIUM UNDER DENITRIFYING CONDITIONS

James D. Fettig and Nancy G. Love, Ph.D.

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ABSTRACT

BTX biodegradation patterns were observed in an anoxic activated sludge culture maintained in a sequencing batch reactor for over 22 months. In order to maintain a diverse microbial community, and to emulate a heterogeneous wastestream contaminated with these priority pollutants, the reactor was fed a mixed waste containing readily biodegradable substrates and approximately 5 mg/L of each of the BTX hydrocarbons. After approximately eight months of reactor operation and biomass acclimation, toluene and *m*-xylene were degraded to below detection on a routine basis. Both toluene and *m*-xylene were found to serve as sole carbon and energy sources. Removal of *o*-xylene was also possible; however, its transformation was dependent upon the simultaneous degradation of toluene. Benzene and *p*-xylene were recalcitrant throughout the study. The results described herein indicate that complete biological treatment of toluene, *m*-xylene, and *o*-xylene can occur in anoxic basins within a wastewater treatment system, but that *p*-xylene and benzene are likely to be recalcitrant

INTRODUCTION

Benzene, toluene, *o*-xylene, *m*-xylene, and *p*-xylene (BTX) are of environmental significance because they represent the more water-soluble components of petroleum, and because they have been shown to elicit adverse health effects in mammalian systems (Dean, 1985; EPA, 1998). Toluene and the xylene isomers ranked third and fifth of the 643 compounds in the 1995 EPA's Toxic Release Inventory (TRI) report, representing 6.6 and 4.3 % of the total release of listed chemicals, respectively (Hanson, 1997). All of the BTX compounds have been shown to be amenable to biodegradation under aerobic conditions (Alvarez and Vogel, 1991; Hutchins *et al.*, 1992; Smith, 1990). However, aerobic biologic treatment of BTX in wastewaters contaminated with these compounds is difficult to achieve. The gas-liquid mass transfer systems employed in conventional aeration basins tend to strip the volatile BTX compounds out of solution before they become completely available for microbial uptake. Indeed, past treatment of BTX specifically took advantage of their volatility and involved the physical process of gas stripping (Dold *et al.*, 1989). Although effective, the stripping process simply transferred the BTX hydrocarbons from wastewater to the atmosphere, where they still posed an environmental threat (Chin, 1994; EPA, 1998). In order to comply with the 1993 NESHAP regulations promulgated by the EPA, additional technologies must now be employed to prevent the emission of BTX and other hazardous volatiles to the atmosphere. Specifically, maximum achievable control technologies (MACT's) in the form of various physicochemical techniques are now required (Al-Tell and Leuders, 1994; EPA, 1997; Wong *et al.*, 1992).

As discussed above, complete aerobic biological treatment of BTX in wastewater is difficult to realize in practice. Anoxic biological treatment, however, presents itself as an alternative biotreatment technology. Anoxic environments utilize terminal electron acceptors of greater aqueous solubility than oxygen and, therefore, do not require the gas-liquid mass transfer systems of aeration basins that lead

to stripping. This advantage of anoxic systems suggests that emissions of BTX from anoxic basins within a wastewater treatment system would be at a minimum and caused only by the natural volatility of these hydrocarbons.

Extensive research has been conducted in the realm of soil and groundwater remediation to evaluate the biodegradability of the BTX compounds under a variety of anoxic and methanogenic environments. Under sulfate-reducing conditions, toluene has been shown to be amenable to biodegradation under pure culture (Beller *et al.*, 1996; Rabus *et al.*, 1993), by indigenous microbes in an unconfined aquifer (Reinhard *et al.*, 1997), and by enrichment cultures derived from sediment microbes (Chapelle *et al.*, 1996). Beller *et al.* (1996) also observed the transformation of *o*-xylene and *p*-xylene under sulfate-reducing conditions, although the transformation was incomplete and could not be sustained. In addition to toluene, Reinhard *et al.* (1997) reported the complete degradation of *o*-xylene and *m*-xylene. Toluene biodegradation has been reported under iron (III)-reducing conditions (Lovley *et al.*, 1990). More recently, benzene oxidation coupled to iron (III)-reduction has been reported in situ in a petroleum contaminated aquifer (Anderson *et al.*, 1998). Under methanogenic conditions, benzene, toluene, and *o*-xylene mineralization by a mixed culture has been reported (Edwards *et al.*, 1994; Grbic-Galic *et al.*, 1987). The majority of anoxic research has been conducted under denitrifying conditions, where nitrate serves as the terminal electron acceptor. Under denitrifying conditions, biodegradation of toluene and *m*-xylene has been reported (Alvarez and Vogel, 1995; Hutchins, 1991; Rabus and Widdel, 1995; Rabus and Widdel, 1996; Reinhard *et al.*, 1997). Toluene has been shown to serve as a sole carbon and energy source under pure culture (Evans *et al.*, 1991b) and enrichment culture (Evans *et al.*, 1991a). The degradation of *p*-xylene has also been observed under denitrifying conditions (Alvarez and Vogel, 1995; Häner et al., 1995). It has been demonstrated that *o*-xylene can be transformed under denitrifying conditions, either in conjunction with toluene degradation (Evans *et al.*, 1991a; Evans *et al.*, 1991b), in conjunction with *m*-xylene degradation (Hutchins, 1991), or by itself (Reinhard *et al.*, 1997). During toluene-dependent transformation, it was

reported that *o*-xylene could not serve as a sole carbon and energy source (Evans *et al.*, 1991b). Although Reinhard *et al.* (1997) achieved *o*-xylene transformation without requiring concomitant toluene degradation, the potential for *o*-xylene to serve as a sole carbon and energy source was not investigated. Benzene degradation has been observed under denitrifying conditions (Major, 1988), however, most of the current research suggests that benzene is recalcitrant under strictly anoxic, denitrifying conditions.

Most of the current research suggests that microorganisms have the capacity to either degrade or transform toluene, *m*-xylene, *p*-xylene, and *o*-xylene under denitrifying conditions. However, a single microbial culture--whether pure, enrichment, or consortium--has yet to be reported that can degrade all of these compounds. Nonetheless, anoxic biological treatment may be a viable alternative to physicochemical methods in a treatment scheme designed for the destruction of the BTX hydrocarbons. This paper presents the results of a two year study in which the propensity of a denitrifying activated sludge consortium to degrade BTX was investigated.

MATERIALS AND METHODS

Reactor Setup and Operation

A sealed, bench-scale, denitrifying sequencing batch reactor (SBR) with a liquid volume of five liters and a headspace volume of 1.75 liters was operated for approximately 24 months. The jacketed glass reactor (B. Braun Biotech) was mixed at 100 rpm and maintained initially at room temperature and subsequently at 25 °C. The reactor was initially seeded with innoculum derived from the activated sludge basin of a chemical process industry wastewater treatment plant and was later augmented with activated sludge from a domestic wastewater treatment plant. Both biomass seeding and augmentation were performed prior to intensive study of the system.

The batch operations of the reactor were electronically controlled using timers (ChronTrol Corp., San Diego, CA), and included fill, react, settle, and draw phases over a 24-hour cycle time. Peristaltic pumps (Cole Parmer Instrument Co., Chicago, IL) were used to control the flow rate of the feed solutions and effluent to yield an effective hydraulic retention time (HRT) of 4 days. An effective solids retention time (SRT) of 15 days was maintained by wasting daily. Reactor pH was monitored and controlled between 7.0 and 8.5 with a pH probe and an analog controller (Cole Parmer Instrument Co., Chicago, IL). Reactor offgas was stored in a tedlar air sampling bag (SKC Inc., Eighty Four, PA) during reaction and vented to a fume hood during reactor fill. In order to ensure anoxic conditions and to remove any residual BTX from the previous cycle, the reactor was purged with purified N_2 gas during each fill phase. The reactor was also purged prior to settling in order to strip entrained gas bubbles in the flocs that may have formed during reaction and would have led to a floating sludge. Because of this, the residence time for volatile compounds was 24 hours. Introduction of N_2 gas to the reactor was done using a sparger and was controlled with solenoid valves and a flow meter.

The denitrifying reactor received a mixed synthetic wastewater containing the five BTX

compounds and readily biodegradable (biogenic) substrates. This was done in order to maintain a robust, diverse microbial consortium in the reactor. The biogenic substrate feed comprised the majority of influent carbon and was composed of the following constituents: 256 mg/L beef extract, 136 mg/L casitone, 136 mg/L peptone, 416 mg/L yeast extract, 156 mg/L glucose, 156 mg/L fructose, 156 mg/L galactose, 1.03 g/L acetic acid, and 177 mg/L glycerol. Mineral salt media was prepared to provide essential nutrients and to form the base matrix of the synthetic wastewater. Mineral salts were amended to tap water and contained the following nutrients: 21.2 mg/L $CaCl_2$, 12.0 mg/L $FeCl_3 \cdot 6H_2O$, 1.20 mg/L $CoCl_2 \cdot 6H_2O$, 1.20 mg/L $ZnCl_2$, 0.400 mg/L $CuCl_2 \cdot 2H_2O$, 0.120 mg/L H_3BO_3, 75.6 mg/L $MgCl_2 \cdot 6H_2O$, 28.0 mg/L $MgSO_4 \cdot 7H_2O$, 3.60 mg/L $MnSO_4 \cdot H_2O$, 0.400 mg/L $NaMoO_4 \cdot 2H_2O$, 348 mg/L K_2HPO_4, and 272 mg/L KH_2PO_4. The mineral salt media was acidified with 2 mL/L concentrated nitric acid (HNO_3) to ensure solubility, and was augmented with a sodium nitrate stock to a final nitrate level of 800 mg/L NO_3-N to provide sufficient nitrate and prevent electron acceptor limited conditions. The influent feed was diluted 1:4 in the reactor. Benzene, toluene, *o*-xylene, *m*-xylene, and *p*-xylene (Aldrich Chemical Co., Inc., Milwaukee, WI) were added neat to the reactor to yield approximate dissolved concentrations of 5 mg/L for each compound.

Data Collection and Analysis

Reactor samples for monitoring effluent quality (BTX, anions, and soluble COD (sCOD) concentrations) were collected just prior to settling and before N_2 sparging. Solids samples for mixed-liquor-suspended-solids (MLSS) and mixed-liquor-volatile-suspended solids (MLVSS) were also collected at this time and effluent total suspended solids (TSS) and volatile suspended solids (VSS) were taken from the drawn supernatant. Samples to be analyzed for anions and sCOD were centrifuged at 2,800xg for five minutes and the centrate was filtered through 0.2 *m*m prewashed Supor® filters (Gelman Sciences, Ann Arbor, MI). Filtered sCOD samples were acidified to pH<2 with concentrated

sulfuric acid, and all soluble samples were stored at 4 °C prior to analysis. *Standard methods* (APHA, 1995) were used to analyze suspended solids, sCOD (closed reflux titrimetric method), and anions. Anions were analyzed using suppressed ion chromatography with conductivity detection using an IONPAC AS4A-SC column (Dionex Corp., Sunnyvale, CA). External standards for chloride (Cl^-), phosphate (PO_4^{3-}), nitrite (NO_2^-), nitrate (NO_3^-), and sulfate (SO_4^{2-}) were used. Samples were diluted (1:10) prior to injection.

Nitrite is converted to nitrate under the conditions of the COD test, and exerts a theoretical interference of 1.14 mg COD per mg NO_2-N. According to *Standard Methods* (APHA, 1995), nitrite interference of the COD test can be overcome by adding sulfamic acid to COD samples. However, this method proved to be unreliable. It was found that preserving COD samples by acidification to pH<2 reduced much of the nitrite interference and allowed for a reliable correlation between nitrite concentration and COD interference. The reduction in nitrite interference is presumed to be a result of the conversion of nitrite to the volatile nitrous acid form ($HNO_{2\,(g)}$) and oxidation to nitrate during storage. The correlation was experimentally determined ($R^2 = 0.99$) and allowed for the following conversion between measured COD values and corrected COD values:

corrected COD (mg/L) = measured COD (mg/L) - 0.2353 * NO_2-N (mg/L).

BTX was analyzed by gas chromatography using a Hewlett Packard model 5890 GC with flame ionization detection and a PAG capillary column (Supelco Inc., Bellefonte, PA). Injector and detector port temperatures were 250 and 260 °C, respectively. The oven temperature was maintained isothermal at 70 °C. Samples for BTX analysis were collected in 16 mL clear glass vials with teflon-lined caps and extracted in hexane for 2 hours in a rotary mixer at a ratio of 14 mL mixed-liquor sample to 2 mL hexane. After extraction, the hexane layer was transferred to two mL amber glass vials equipped with teflon-lined caps and stored at 4°C until GC analysis. External standards for benzene, toluene, *o*-xylene, *m*-xylene, and *p*-xylene were prepared gravimetrically in hexane and stored in amber glass vials with teflon-lined caps at 4 °C. Preliminary experiments suggested that certain BTX fractions partially

sorbed to biomass. The sorption losses were recoverable only when extractions were conducted using mixed-liquor samples with biomass intact. The method detection limit (MDL) for the BTX compounds was 0.1 mg/L. Analysis of samples spiked with BTX indicated that extraction efficiencies of greater than 99% for all of the BTX compounds were achieved with this method.

Total protein was analyzed using the bicinchoninic acid protein assay (Sigma Chemicals, St. Louis, MO). Aliquots (2 to 15 mL) of biomass were washed by centrifuging at 2,800xg for 5 minutes at room temperature. The supernatant was discarded and the biomass pellet was washed once in 100 mM phosphate buffer. The final biomass pellet was resuspended in (200 to 1000 *m*L) 1.0 N sodium hydroxide (NaOH), transferred to 2 mL microfuge tubes, and frozen prior to analysis. Frozen samples were thawed, then digested at 100 °C for 5 minutes to extract protein from the biomass. After cooling, the samples were centrifuged at 15,000xg in a microfuge for 5 minutes and supernatant aliquots of 100 *m*L were incubated at 37 °C with 2 mL of a fifty to one, bicinchoninic acid to $CuSO_4 \cdot 5H_2O$ (4%) protein determination reagent (Sigma Chemicals, St. Louis, MO) for 30 minutes. Absorbances were analyzed at a wavelength of 562 nm with a spectrophotometer (Beckman Instr., Inc. Fullerton, CA) and referenced against bovine serum albumin (BSA) standards obtained from Sigma Chemicals (St. Louis, MO).

Reactor Profile Studies

Profile experiments were conducted to better understand the interactions between substrate, biomass, and terminal electron acceptor during the reaction phase of the batch cycle. Sample were collected for BTX, anions, and sCOD every thirty minutes for the first hour, every hour for the next five hours, and every two hours from then until the end of the phase. Unlike normal daily operation, biogenic substrate was not added until after the first sample in order to capture the initial values for nitrate and nitrite. BTX was also added neat to the reactor after the first sample.

Sole Carbon and Energy Source Studies

Separate anaerobic batch studies (1 L) were conducted in a glove box (N_2 atmosphere) using biomass from the main reactor to determine if toluene and *m*-xylene could serve as sole carbon and energy sources. Toluene and *m*-xylene were added aseptically to a 50% sterile mineral salts solution (pH 7.0) amended with 50 mg/L NH_4Cl and 55 mg/L NO_3-N in one liter amber glass reactors equipped with teflon-lined caps. The reactors were sealed and the aromatic hydrocarbons were allowed to equilibrate for two hours to achieve an initial target concentration of 20 mg/L each. Biomass was removed from the main reactor two hours after BTX addition to ensure that the biomass was in a physiological state conducive to toluene and *m*-xylene uptake. Biomass aliquots were gently purged for five minutes with N_2 gas to remove residual BTX and washed twice in 100 mM phosphate buffer by centrifuging at 2,800xg for five minutes. Washed biomass was added to the batch reactors at a final dilution of 1:500 of the MLSS concentration in the main reactor to initiate the experiment. Samples were analyzed in triplicate for toluene, *m*-xylene, and total protein, which was used as an indicator of biomass. Analysis of the biomass in the main reactor indicated a total protein to VSS mass ratio of 0.76 ($R^2 = 0.97$).

In order to estimate abiotic losses of toluene and *m*-xylene from the experimental flasks, abiotic control reactors were employed using the same media except that ammonia, phosphate buffer, and biomass were not added. It was assumed that volatility losses occurred only during sampling periods, during which time each bottle was exposed to the glove box atmosphere for approximately 15 seconds. The loss of toluene and *m*-xylene in the control flasks were then used to back out the volatility loss in the experimental reactors. This was done by using loss ratios between each sampling point. The independence of this loss ratio to time supported the assumption that loss only occurred during sampling periods.

o-Xylene Transformation Studies

Additional profile studies were conducted with multiple toluene spikes to determine if *o*-xylene was transformed during the biodegradation of toluene. Duplicate experiments were performed on the main reactor approximately two weeks apart. In both experiments, samples for BTX were taken every hour for the first twelve hours, with a final sample taken at the end of the reaction phase. Biogenic substrate, BTX, nitrate, and mineral salts were added at time zero in the same fashion as described above. During the first experiment, toluene was spiked at the same target concentration (5 mg/L) to the reactor neat at four, eight, and twelve hours after the start of reaction. This was modified for the second experiment in that toluene was added at six and twelve hours after reaction.

RESULTS AND DISCUSSION

Toluene and m-Xylene Degradation by Denitrifying Activated Sludge

Intensive monitoring of the SBR system did not commence until approximately six months after reactor startup. Initially, benzene, toluene, *o*-xylene, *m*-xylene, and *p*-xylene (BTX) were added at a concentration of 10 mg/L each. Six months later, this concentration was decreased to 5 mg/L because inhibitory effects were occasionally observed at 10 mg/L. A four month period of acclimation after startup was required before toluene exhibited degradation (data not shown). The degradation of *m*-xylene occurred approximately two months later (data not shown). However, eight months were required before consistent removal of both of these compounds to below detection was achieved. A time-line of the effluent BTX concentrations after acclimation for both toluene and *m*-xylene is shown in Figure 1. The reactor cycled, sometimes on a day-to-day basis, between periods of degradation and non-degradation with respect to toluene and *m*-xylene for a period of approximately two months until it settled down and degraded these compounds on a routine basis. After the reactor stabilized, toluene and *m*-xylene would occasionally only partially degrade; however, these periods were rather anomalous. Benzene, *p*-xylene, and *o*-xylene were consistently present in the effluent at concentrations near their influent levels.

Experiments were conducted to better understand the interactions between substrate, biomass, and terminal electron acceptor during the reaction cycle. Representative profiles of BTX, nitrate, nitrite, sulfate, and sCOD are shown in Figure 2a through 2c. Soluble COD, which represents the biogenic substrate concentration, is plotted in Figure 2a. The dual points at time zero represent the soluble COD before substrate addition (~ 66 mg/L) and the theoretical soluble COD after substrate addition and dilution (~ 675 mg/L). Soluble COD uptake was rapid, with approximately 95% of the total COD removal occurring within the first hour of reaction.

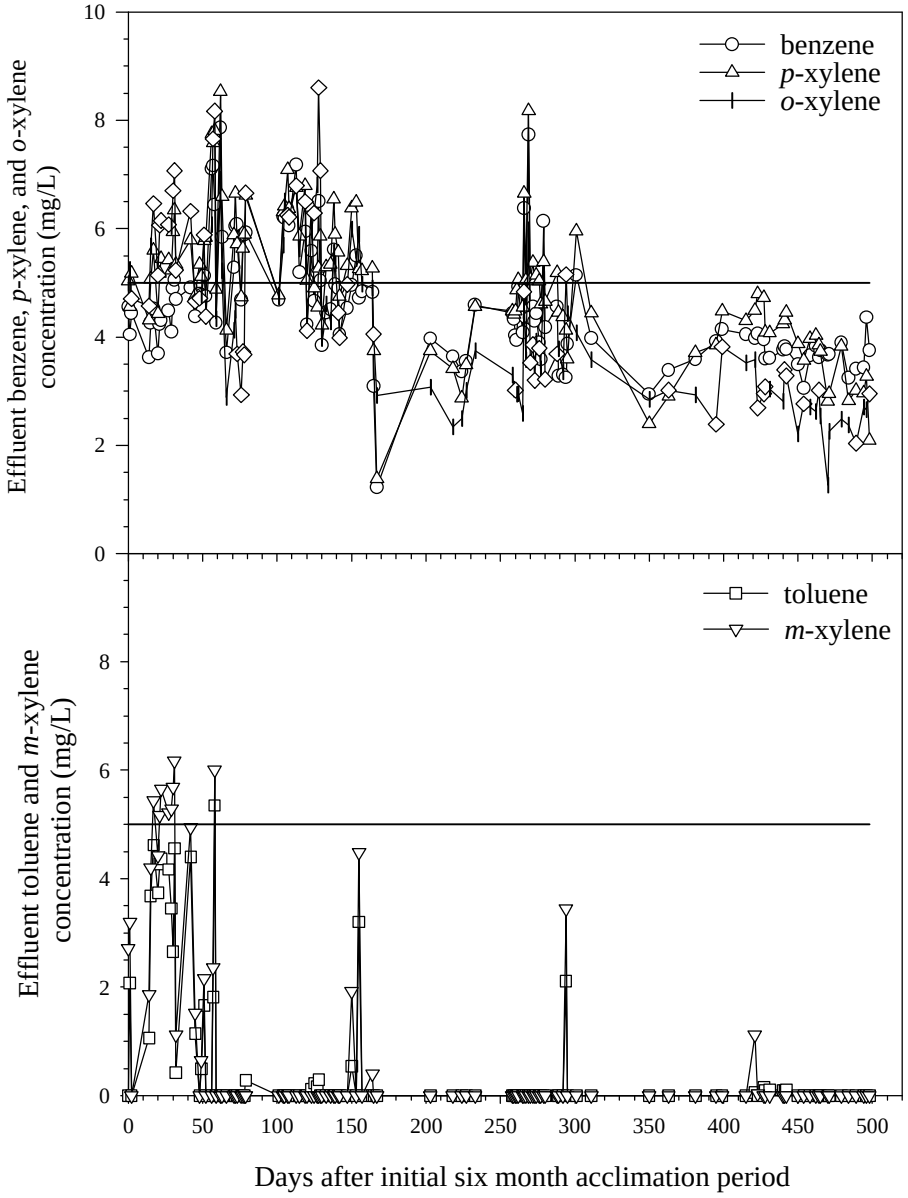

Figure 2. Effluent BTX concentrations from the denitrifying reactor after the initial six month acclimation period. The solid line at 5 mg/L represents the target initial concentration for all of the BTX compounds.

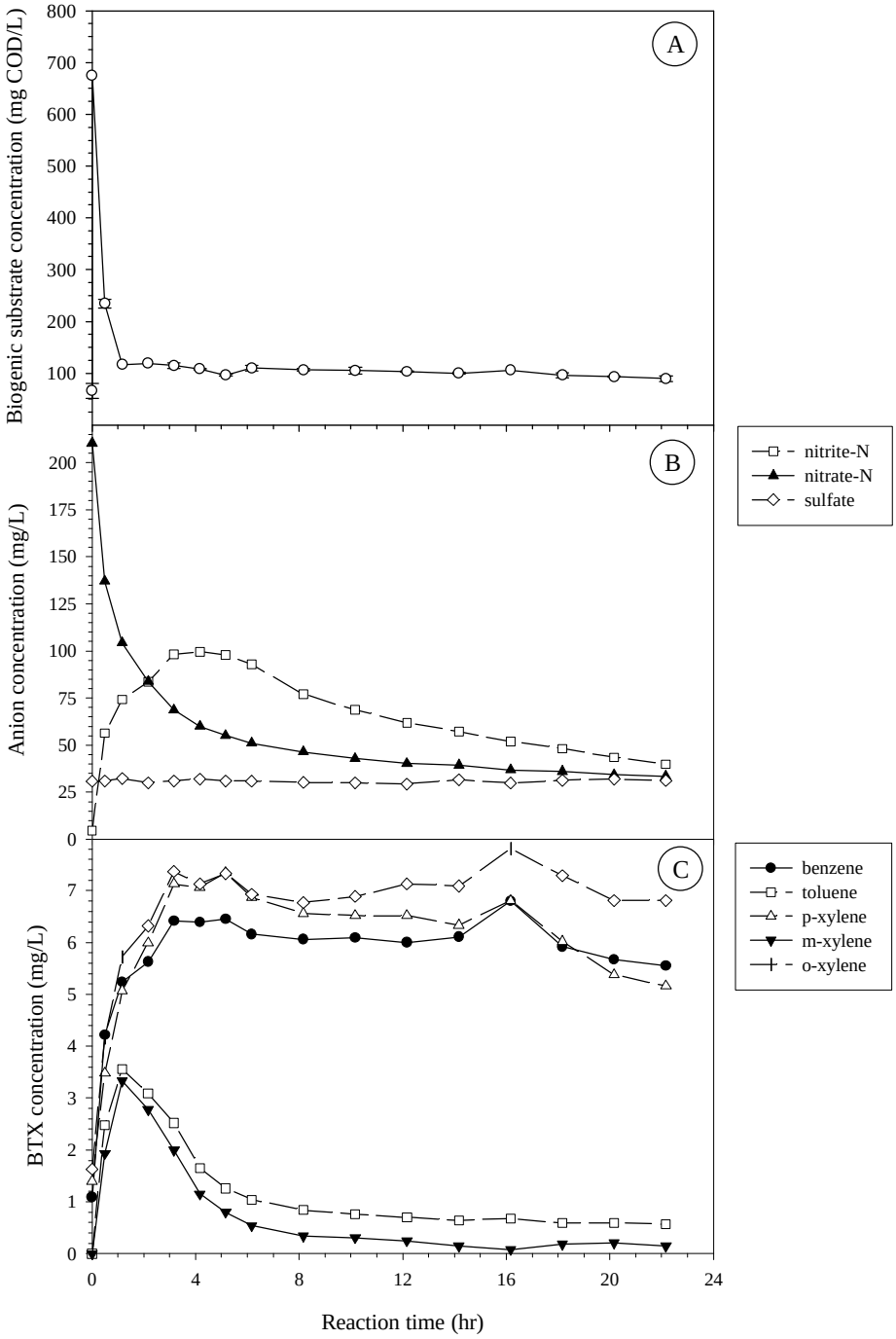

Figure 2. Interactions of biogenic substrate (A), anions (B), and BTX (C) during the course of reaction (day 105 after initial acclimation period). Error bars for COD samples represent one standard deviation of duplicate samples.

Nitrate, nitrite, and sulfate profiles are illustrated in Figure 2b. Nitrate was consumed as the reaction progressed with a concomitant production and accumulation of nitrite. Several phenomena that lead to nitrite build up during denitrification have been identified and include the type of carbon source (van Rijn *et al.*, 1996), initial COD:$NO3^-$ ratio (Phillips and Love, 1998), exposure to light in the green spectrum (Barak *et al.*, 1998), and pH (Glass *et al.*, 1997 in press). Both nitrate and nitrite were not completely removed by the end of the cycle, which showed that the provided terminal electron acceptor was sufficient for the oxidation of both the biogenic and BTX substrates. This was supported by the constant concentration of sulfate in the reactor, which showed that at no point during the reaction did the system turn to sulfate-reducing conditions.

Profiles for benzene, toluene, *o*-xylene, *m*-xylene, and *p*-xylene are shown in Figure 2c. Because the BTX compounds were added in their pure form at the start of the reaction, a dissolution period, of approximately three hours in length, was required before the compounds became completely dissolved in the reactor. The subsequent minor losses of benzene, *o*-xylene, and *p*-xylene are assumed to be due to volatility that was enhanced by the production of N_2 gas within the reactor during denitrification. Using the ideal gas law and the assumption that all NO_X that was consumed was reduced to N_2 gas, it was estimated that the consumption of nitrate led to the evolution of approximately 0.6 liters of N_2 gas. The net losses of benzene, *o*-xylene, and *p*-xylene were 14.6, 6.5, and 28.1 %, respectively. The trend in volatility losses of these compounds can be explained according to their Henry's Law constants (m, dimensionless), which are 0.225, 0.210, and 0.283 at 25 °C, respectively (Schwarzenback *et al.*, 1993). Toluene and *m*-xylene exhibited completely different responses than benzene, *p*-xylene, and *o*-xylene. They underwent a similar period of dissolution of about one hour, after which their concentrations began to decrease markedly. It appeared that two phenomena were occurring with toluene and *m*-xylene. Initially, they were subjected to dissolution in much the same manner as benzene, *p*-xylene, and *o*-xylene. They were, however, also subject to biodegradation during this period of dissolution. The two competing rates of dissolution and biodegradation led to the curves shown in

Figure 2c. Toluene and *m*-xylene were not completely degraded during the reaction time for the profile shown; however, effluent data and other profile experiments showed that toluene and *m*-xylene were typically degraded to below detection.

Toluene and m-Xylene as Sole Carbon and Energy Sources

Reactor effluent and profile data showed that toluene and *m*-xylene were amenable to biodegradation under the anoxic, denitrifying conditions imposed on the reactor. However, the presence of biogenic substrate in the main reactor feed precluded the determination of whether or not toluene and/or *m*-xylene could serve as sole carbon and energy sources. Therefore, anoxic side batch experiments were conducted to determine if the biomass could utilize toluene and *m*-xylene in this manner. Representative responses of toluene, *m*-xylene, and total protein are shown in Figure 3. There was an initial lag period of approximately two days before changes in constituent concentrations occurred despite the fact that preacclimated biomass was used. The lag may have been due to the sudden exposure to elevated levels of toluene and *m*-xylene, which had been shown to cause decreased levels of biodegradation in the main reactor. The consumption of toluene was rapid after the lag period and was coupled with a marked increase in total protein levels. *m*-Xylene was consumed slowly at first after the lag period, but underwent a rapid decline after approximately 4 days of incubation. The consumption pattern of *m*-xylene was coupled with a similar gradual, then sharp, rise in total protein levels. The coupling of toluene and *m*-xylene consumption to the production of total protein indicated that the biomass was able to use toluene and *m*-xylene as sole carbon and energy sources.

The sole carbon and energy source experiments were also conducted to determine if there was any substrate competition between toluene and *m*-xylene. Substrate competition between

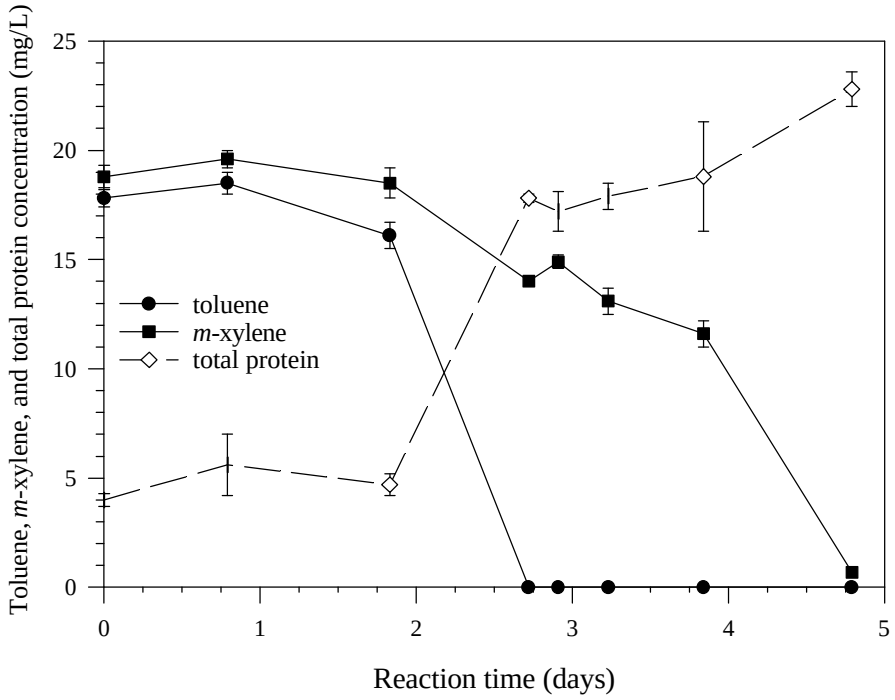

Figure 3. Total protein increase coupled with toluene and *m*-xylene degradation. Error bars represent one standard deviation of triplicate measurements.

toluene and *m*-xylene was difficult to determine using data from the main reactor due to the complicating presence of biogenic substrate. In the above experiment, it was evident that toluene was utilized prior to *m*-xylene. In fact, *m*-xylene was scarcely utilized until toluene was completely degraded. This suggests that toluene was the favored substrate for the biomass, and that its presence may have prevented the consumption of *m*-xylene. It should be noted, and is demonstrated in Figure 2c, that this phenomenon was not witnessed in the main reactor. Experiments on the main reactor showed that toluene and *m*-xylene were degraded concurrently in the presence of other biogenic substrates, with no apparent competition between the two aromatic hydrocarbons.

o-Xylene Transformation Studies

Effluent and profile data taken towards the end of the study suggested that *o*-xylene depletion occurred to a greater extent than it did earlier in the study when it responded in a manner similar to benzene and *p*-xylene. It also appeared that this depletion was linked temporally to periods of toluene degradation. Therefore, the propensity of the system to transform *o*-xylene while degrading toluene was investigated.

Duplicate profile experiments were conducted on the main reactor in which toluene was spiked neat to the reactor multiple times. The responses of benzene, toluene, *o*-xylene, *m*-xylene, and *p*-xylene from these experiments are shown in Figure 4. Figure 4a depicts the transformation experiment in which toluene was fed like usual at the start of reaction and spiked subsequently at six and twelve hours. In the transformation experiment depicted in Figure 4b, toluene was fed initially as usual and subsequently spiked at four, eight, and twelve hours. Each spike delivered 33.4 mg of toluene to the system. The initial feed spikes of toluene and *m*-xylene responded in a similar fashion to previous experiments, in that there were competing rates of dissolution and degradation. In this case, however, it should be noted that both *m*-xylene and toluene were degraded completely which was more typical based on effluent data (Figure 1). The maximum concentration achieved by toluene dissolution

decreased with each subsequent spike. For example, in Figure 4b, toluene dissolved to 1.82, 0.90, and 0.41 mg/L after the initial, the fourth hour, and the eighth hour spike, respectively. This trend suggested that the rate of toluene degradation increased with each additional toluene spike. Previous experiments showed that the MLSS concentration was relatively constant across the reaction cycle (data not shown). However, it is unclear whether the increase in the rate of toluene biodegradation was due to an increase in the population of toluene degraders or an increase in the affinity for toluene of a constant toluene degrading population.

Benzene exhibited a similar response to previous profile experiments in that there was a two to three hour period of dissolution followed by a slow depletion, presumably due to volatility facilitated by N_2 gas production during the denitrifying reaction. The average net loss of benzene between the two transformation experiments was 24.1 %. The loss of *p*-xylene, however, was markedly greater than in previous experiments. The average *p*-xylene loss between the two experiments was 57.4 %. In the represented profile study described in Figure 2c, benzene and *p*-xylene losses were only 14.6 and 28.1 %, respectively. In another profile experiment (data not shown), the losses of benzene and *p*-xylene were 12.1 and 40.6 %, respectively. Although the differences between the benzene and *p*-xylene losses in each case followed their Henry's Law constants, it is difficult to attribute a 57.4 % loss in *p*-xylene to volatility alone. However, direct evidence of biodegradation is inconclusive at this point.

The response of *o*-xylene was completely different from previous profiles. In both experiments, *o*-xylene underwent significant depletion even after the initial toluene feed. In Figure 4a, the *o*-xylene depletion rate slowed just prior to the first toluene spike at six hours. After the first toluene spike was added and toluene began to degrade, the depletion rate of *o*-xylene increased

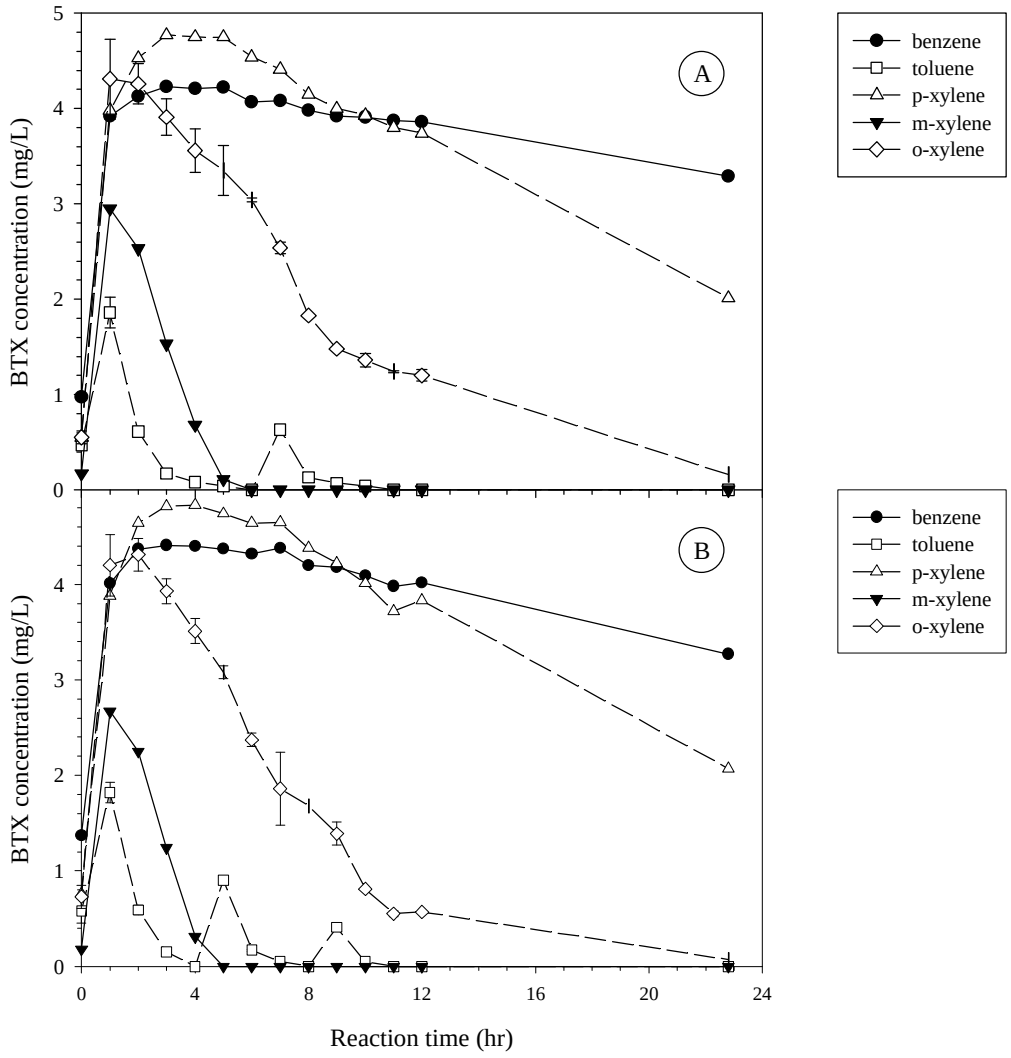

Figure 4. Concomitant depletion of *o*-xylene during toluene degradation. Day 485 and 472 transformation experiments shown in (A) and (B), respectively. Arrows represent toluene additions. Error bars for toluene and *o*-xylene represent one standard deviation of duplicate samples. For clarity, error bars are not shown for benzene, *m*-xylene, or *p*-xylene.

again. As toluene was consumed to completion, the rate of *o*-xylene depletion leveled off dramatically. The same trend is evident in Figure 4b. The rate of *o*-xylene transformation clearly corresponds with periods when toluene is rapidly biodegraded.

The parallel between toluene degradation and *o*-xylene depletion suggests that *o*-xylene was transformed during the degradation of toluene. In neither experiment was *o*-xylene degraded to completion. Although one additional toluene spike was added during the transformation experiment depicted in Figure 4b, the effluent concentration of *o*-xylene was only slightly lower that the profile shown in Figure 4a (0.16 mg/L versus 0.07 mg/L). The average net mass of o-xylene transformed per mass of toluene degraded was 0.28 mg/mg for the experiment in Figure 4a and 0.16 mg/mg for the experiment in Figure 4b. This may suggest that the mass of *o*-xylene transformed is not solely dependent on the mass of toluene degraded. Except for the time periods following the initial feed of BTX, where the exact initial concentration of *o*-xylene could not be precisely determined, the plots seem to suggest that the mass of *o*-xylene degraded per mass of toluene degraded decreased with each subsequent toluene spike. In Figure 4a, the transformation ratio was 0.36 milligrams of *o*-xylene transformed per milligram of toluene degraded for the period after the first spike (six hours) and the transformation ratio was 0.18 mg/mg for the period after the second spike (twelve hours). In Figure 4b, the transformation ratios were 0.36, 0.21, and 0.09 mg/mg for the periods after the first, second, and third spikes, respectively. As discussed above, the rate of toluene degradation increased with each additional toluene spike. Given the above, it is postulated that the mass of *o*-xylene transformed is more dependent upon the length of time toluene degradation is occurring rather than upon the mass of toluene degraded. This strongly suggests that *o*-xylene is not involved with inducing anaerobic toluene degradation genes, that the enzymes responsible for biodegrading toluene and transforming *o*-xylene are rapidly inactivated (or labile) in the absence of toluene, and that *o*-xylene degradation in the presence of toluene is strictly gratuitous metabolism when capable enzymes are present.

CONCLUSIONS

The studies presented herein show that biological treatment of toluene, *m*-xylene, and *o*-xylene can occur in anoxic, denitrifying reactors receiving wastes of varied composition including biogenic substrates and the BTX hydrocarbons. The results indicate that anoxic zones may be used in a wastewater treatment system as an alternative treatment technology for the destruction of selected BTX components. Specifically, for wastewaters contaminated with toluene and *m*-xylene, anoxic zones staged upstream of conventional aeration basins can be used to biodegrade these compounds before entering an aerobic basin, where they would otherwise be stripped to the atmosphere. In addition to toluene and *m*-xylene, the results indicate that *o*-xylene transformation could occur in such a system. However, complete removal of *o*-xylene would be possible only if toluene degradation occurred for a sufficient amount of time for its complete transformation. The studies also indicate that anoxic zones would not be effective in treating wastewaters containing benzene and *p*-xylene.

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STOICHIOMETRY AND KINETICS OF BTX DEGRADATION BY A DENITRIFYING ACTIVATED SLUDGE CONSORTIUM RECEIVING A MIXED WASTE

James D. Fettig and Nancy G. Love, Ph.D.

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ABSTRACT

Studies were conducted under denitrifying conditions in a sequencing batch reactor (SBR) containing an activated sludge consortium which received a mixed waste of readily biodegradable, biogenic substrates and the five BTX compounds. It was found that toluene and *m*-xylene were biodegradable to below detection under such conditions, whereas benzene, *p*-xylene, and *o*-xylene were usually found near their influent concentrations. An evaluation of the stoichiometric coefficients and kinetic parameters for toluene and *m*-xylene degradation was performed. The first order decay coefficient (b, h^{-1}) was found to be 0.016 ± 0.006 h^{-1} on a theoretical oxygen demand (thOD) basis. The true growth yield (Y) for toluene and *m*-xylene was 0.35 ± 0.04 mg thOD biomass per mg thOD substrate. The yield for the biogenic substrate component of the mixed waste was found to be 0.41 ± 0.02 mg thOD biomass per mg thOD substrate. Based on the different substrate yields and the fraction of toluene and *m*-xylene carbon in the influent, the toluene- and *m*-xylene-degrading fraction of the biomass was estimated to be 0.04. Zero-order initial uptake rates in the SBR for toluene and *m*-xylene ranged from 0.031 to 0.047 and 0.015 and 0.029 mg substrate/mg protein/h, respectively. The maximum specific utilization rate (q_{max}) and half saturation constant (K_S) values for toluene and m-xylene were determined for the denitrifying activated sludge consortium using nonlinear curve fitting techniques based on the Monod model. For toluene, q_{max} and K_S ranged from 0.059 to 0.14 mg toluene/mg protein/h and 0.84 to 6.9 mg/L, respectively. The q_{max} and K_S values for *m*-xylene ranged from 0.034 to 0.041 mg toluene/mg protein/h and 0.28 to 3.7 mg/L, respectively. The values of the toluene and *m*-xylene kinetic parameters were varied based on the test date and the accumulation of nitrite. Using the toluene and *m*-xylene yield value, the estimated maximum specific growth rates (μ_{max}) ranged from 0.12 to 0.28 h^{-1} and 0.067 to 0.082 h^{-1} for toluene and *m*-xylene, respectively.

INTRODUCTION

The aromatic hydrocarbons benzene, toluene, *o*-xylene, *m*-xylene, and *p*-xylene (BTX) are of environmental significance because they represent the more water-soluble fractions of petroleum and have been shown to exhibit acute toxic and chronic mutagenic properties (Dean, 1985; EPA, 1998). Because they are relatively soluble, the BTX compounds are often encountered in aqueous environments that have been exposed to petroleum, such as wastewaters produced from the petroleum refining process. It has been demonstrated that all of the BTX compounds are amenable to biodegradation under aerobic conditions (Alvarez *et al.*, 1991; Hutchins *et al.*, 1991), and the pathways for aerobic biodegradation have been elucidated (Smith, 1990). However, complete mineralization of the BTX compounds in wastewaters by aerobic biological treatment is difficult to achieve. The volatile nature of the BTX compounds causes them to be stripped from solution before they become completely available for microbial uptake and transformation. Indeed, past treatment of wastewaters contaminated with BTX has specifically taken advantage of this volatility and involved the physical process of gas stripping, in which the BTX compounds are transferred from the wastewater to the atmosphere (Chin, 1994; Dold, 1989). In order to comply with current NESHAP regulations, however, gas stripping alone is no longer sufficient as the sole treatment method for these hazardous air pollutants. Instead, a variety of physicochemical treatment methods are now required to prevent the emission of BTX to the atmosphere (EPA, 1997; Wong *et al.*, 1992; Al-Tell and Leuders, 1994).

Although the complete degradation of BTX in wastewater is difficult to realize by aerobic biological treatment due to the complication of stripping, complete biological treatment may be possible under anoxic environments, where gas-liquid mass transfer systems are not required. Extensive research has been conducted in the field of groundwater remediation to investigate the ability of bacteria to degrade the BTX hydrocarbons under such alternate electron acceptor environments.

Under sulfate-reducing conditions, the biodegradation of toluene and the transformation of *o*-xylene and *p*-xylene has been reported (Beller *et al.*, 1996; Chapelle *et al.*, 1996; Rabus *et al.*, 1993; Reinhard *et al.*, 1997). Lovley and Lonergan (1990) reported that toluene was amenable to biodegradation under iron (III)-reducing conditions. Under methanogenic conditions, benzene, toluene, and *o*-xylene mineralization has been observed (Edwards and Grbic-Galic, 1994; Grbic-Galic and Vogel, 1987). The majority of anoxic research has been conducted under denitrifying conditions, where nitrate serves as the terminal electron acceptor. Under denitrifying conditions, toluene and *m*-xylene have been shown to be amenable to biodegradation (Alvarez *et al.*, 1995; Evans *et al.*, 1991a; Hutchins *et al.*, 1991; Rabus and Widdel, 1995; Rabus and Widdel, 1996; Reinhard *et al.*, 1997). In addition, toluene has been shown to serve as a sole carbon and energy source under pure culture (Evans *et al.*, 1991b) and enrichment culture (Evans *et al.*, 1991a) conditions. It has also been shown that *o*-xylene can be transformed under denitrifying conditions, either in conjunction with toluene degradation (Evans *et al.*, 1991a; Evans *et al.*, 1991b), in conjunction with *m*-xylene degradation (Hutchins, 1991), or by itself (Reinhard *et al.*, 1997). The biodegradation of *p*-xylene has also been reported under denitrifying conditions (Alvarez and Vogel, 1995; Häner *et al.*, 1995). Biodegradation of benzene under denitrifying conditions has been observed (Major *et al.*, 1988); however, most of the current research suggests that benzene is recalcitrant under strictly anoxic conditions.

The ability of bacteria to degrade BTX under denitrifying conditions indicates that the potential exists for using biological treatment to remove selected BTX constituents from wastewater in lieu of physicochemical techniques. If denitrifying zones are going to be employed for such a purpose, an understanding of the stoichiometry and kinetics of BTX degradation is necessary. In addition, if the results of kinetic evaluations are going to be applicable to real world systems, the evaluations should be conducted in such a manner that is reflective of such systems. Therefore, this study was conducted to determine the stoichiometry and kinetics of BTX degradation by a diverse, activated sludge microbial consortium under denitrifying conditions receiving a mixed waste.

MATERIALS AND METHODS

Reactor setup and operation

A bench-scale, denitrifying sequencing batch reactor (SBR) with a workable volume of five liters and a headspace volume of 1.75 liters was operated for approximately 24 months. The jacketed-glass reactor (B. Braun Biotech) was maintained at 25 °C and was mixed at 100 rpm. The SBR was initially inoculated with biomass derived from the activated sludge basin of a chemical process industry wastewater treatment plant and was subsequently augmented with activated sludge from a domestic wastewater treatment plant. Both biomass seeding and augmentation were performed prior to intensive study of the system.

The batch operations of the reactor were electronically controlled using timers (ChronTrol Corp., San Diego, CA), and included fill, react, settle, and draw phases over a 24-hour cycle time, with over 22 hours of reaction. Peristaltic pumps (Cole Parmer Instrument Co., Chicago, IL) were used to control the flow rate of the feed solutions and the effluent to yield an effective hydraulic retention time of four days. An effective solids retention time (SRT) was maintained at 15 days by wasting daily. Reactor pH was monitored and controlled between 7 and 8.5 with a pH probe and an analog pH controller (Cole Parmer Instrument Co., Chicago, IL). Reactor offgas was stored in a tedlar air sampling bag (SKC Inc., Eighty Four, PA) during reaction and vented to a fume hood during reactor fill. The tedlar air bag was also used to store a reservoir of N_2 gas so as to retain positive pressure in the reactor and prevent oxygen entrainment during experiments requiring vigorous sampling across the reaction cycle. Introduction of N_2 gas was controlled with solenoid valves and a flow meter and introduced to the reactor through a ring sparger. In order to ensure anoxic conditions, the reactor was purged with purified N_2 gas during each fill phase. The reactor was also purged prior to settling in order to strip entrained gas bubbles in the flocs that may have formed during the denitrifying reaction and would have led to a floating sludge. Because of this, the residence time of BTX and other volatiles

was 24 hours.

The denitrifying batch reactor was fed a synthetic wastewater composed of the five BTX compounds and readily biodegradable (biogenic) substrates. This was done in order to maintain a robust, diverse microbial consortium in the reactor. Benzene, toluene, *o*-xylene, *m*-xylene, and *p*-xylene (Aldrich Chemical CO., Inc., Milwaukee, WI) were added neat directly to the reactor at the start of reaction during normal daily operation to concentrations of approximately 5 mg/L of each compound. The biogenic substrate feed comprised the majority of influent carbon and was comprised of the following: 256 mg/L beef extract, 136 mg/L casitone, 136 mg/L peptone, 416 mg/L yeast extract, 156 mg/L glucose, 156 mg/L fructose, and 156 mg/L galactose, 1.03 g/L acetic acid, and 177 mg/L glycerol. Mineral salt media was prepared to provide essential nutrients and to form the base matrix of the synthetic wastewater. Mineral salts were amended to tap water and contained the following nutrients: 21.2 mg/L $CaCl_2$, 12.0 mg/L $FeCl_3 \cdot 6H_2O$, 1.20 mg/L $CoCl_2 \cdot 6H_2O$, 1.20 mg/L $ZnCl_2$, 0.400 mg/L $CuCl_2 \cdot 2H_2O$, 0.120 mg/L H_3BO_3, 75.6 mg/L $MgCl_2 \cdot 6H_2O$, 28.0 mg/L $MgSO_4 \cdot 7H_2O$, 3.60 mg/L $MnSO_4 \cdot H_2O$, 0.400 mg/L $NaMoO_4 \cdot 2H_2O$, 348 mg/L K_2HPO_4, and 272 mg/L KH_2PO_4. The mineral salt media was acidified with 2 mL/L concentrated nitric acid (HNO_3) to ensure solubility, and was augmented with a sodium nitrate stock to a final nitrate level of 800 mg/L NO_3-N to provide sufficient nitrate and prevent electron acceptor limited conditions. The influent feed was diluted was diluted 1:4 in the reactor.

Data Collection and Analysis

Total suspended solids (TSS) and volatile suspended solids (VSS) were analyzed according to *Standard Methods* (APHA, 1995) using previously washed and dried 1.2 *m*m filter membranes (Gelman Sciences, Ann Arbor, MI). Soluble COD (sCOD) samples and anion samples were centrifuged at 2,800xg for five minutes and the centrate was filtered through prewashed 0.2 *m*m Supor®

filters (Gelman Sciences, Ann Arbor, MI). Filtered sCOD samples were acidified to pH<2 with concentrated sulfuric acid, and all soluble samples were stored at 4 °C prior to analysis. Chemical oxygen demand (COD) was analyzed using the closed-reflux titrimetric method as described in *Standard Methods* (APHA, 1995). Anions, including nitrate and nitrite, were analyzed using suppressed ion chromatography with conductivity detection using an IONPAC AS4A-SC column (Dionex Corp., Sunnyvale, CA). External standards for chloride (Cl^-), phosphate (PO_4^{3-}), nitrite (NO_2^-), nitrate (NO_3^-), and sulfate (SO_4^{2-}) were used. Samples were diluted (1:10) prior to injection.

Nitrite (NO_2^-) is converted to nitrate (NO_3^-) under the conditions of the test, and in doing so exerts a theoretical interference of 1.14 mg COD per mg NO_2-N. According to *Standard Methods* (APHA, 1995), nitrite interference of the COD test can be overcome by adding sulfamic acid to COD samples. However, this method proved to be unreliable. It was found that preserving COD samples with sulfuric acid reduced much of the nitrite interference and allowed for a reliable correlation between nitrite concentration and COD interference. The reduction in nitrite interference is presumed to be a result of the conversion of nitrite to the volatile nitrous acid form ($HNO_{2\,(g)}$) and subsequent oxidation to nitrate. The correlation was experimentally determined ($R^2 = 0.99$) and allowed for the following conversion between measured COD values and corrected COD values:

corrected COD (mg/L) = measured COD (mg/L) - 0.2353 * NO_2-N (mg/L)

BTX was analyzed by gas chromatography using a Hewlett Packard model 5890 GC with flame ionization detection and a PAG capillary column (Supelco Inc., Bellefonte, PA). Injector and detector port temperatures were 250 and 260 °C, respectively. The oven temperature was maintained isothermal at 70 °C. Samples for BTX analysis were collected in 16 mL glass vials equipped w/ teflon-lined caps and extracted in hexane for 2 hours in a rotary mixer at a ratio of fourteen mL mixed liquor sample to two mL hexane in 16 mL glass vials equipped with teflon-lined caps. Preliminary experiments suggested that certain BTX fractions partially sorbed to biomass. The sorption losses were recoverable only when extractions were conducted using mixed liquor samples with biomass present. After

extraction, the hexane layer was transferred to two mL amber glass vials equipped with teflon-lined caps and stored at 4 °C until analysis. External standards for benzene, toluene, *o*-xylene, *m*-xylene, and *p*-xylene were prepared gravimetrically in hexane and stored in amber glass vials with teflon-lined caps at 4 °C. The method detection limit (MDL) for the BTX compounds was 0.1 mg/L. Analysis of samples spiked with BTX indicated that extraction efficiencies of greater than 99% for all of the BTX compounds were achieved with this method.

Total protein was analyzed using the Bicinchoninic Acid Protein Assay (Sigma Procedure No. TPRO-562, Sigma Chemicals, St. Louis, MO). Aliquots of biomass were washed by centrifuging at 2,800xg in a Centrific® 225 centrifuge (Fisher Scientific, Pittsburgh, PA) for 5 minutes at room temperature. The supernatant was discarded and biomass was resuspended in 2 mL of 100 mM phosphate buffer. The biomass was centrifuged again and the supernatant discarded. The biomass pellet was resuspended in (200 to 1000 *m*L) 1.0 N sodium hydroxide (NaOH) and transferred to 2 mL microfuge tubes and frozen prior to analysis. The volumes of biomass and NaOH used were varied for each sample in order to get total protein concentrations within the linear range of the test. Samples were digested at 100 °C for 5 minutes to extract protein from the biomass. After cooling, the samples were centrifuged at 15,000 g in a microfuge (Baxter Scientific Products, McGaw Park, IL) for 5 minutes and supernatant aliquots of 100 *m*L were incubated at 37 °C with 2 mL of a fifty to one, bicinchoninic acid to $CuSO_4 \cdot 5H_2O$ (4%) protein determination reagent (Sigma Chemicals, St. Louis, MO) for 30 minutes. Absorbances were analyzed at a wavelength of 562 nm with a spectrophotometer (Beckman Instruments, Inc. Fullerton, CA) and referenced against bovine serum albumin (BSA) standards obtained from Sigma Chemicals (St. Louis, MO).

Kinetic modeling

The kinetics of biodegradation was modeled using the Monod model according to the following

equation for a batch reactor:

$$\frac{dS}{dt} = -q_{max}\left[\frac{S}{K_S + S}\right]X, \qquad (1)$$

where q_{max} represents the maximum specific uptake rate, h^{-1}; K_S represents the half saturation constant , mg/L; S represents the substrate concentration, mg/L; t represents time, h; and X is the active biomass, mg/L. The integrated form of equation 3, with $S = S_0$ at $t = t_0$ and assuming that the active biomass concentration is constant during the course of reaction is defined by the following equation:

$$K_S \ln(S) + S = K_S \ln(S_0) + S_0 - \left(q_{max} X t\right). \qquad (2)$$

A nonlinear, least squares estimation of q_{max} and K_S was performed according to the method reported by Robinson *et al.* (1983) using the Solver® program in Microsoft Excel® version 5.0. The maximum specific growth rates (m_{max}, h^{-1}) were determined from the values obtained for q_{max} and the true growth yield for toluene and *m*-xylene (Y) according to the following relationship:

$$m_{max} = q_{max} Y. \qquad (3)$$

Biomass decay studies

During the course of biodegradation, the concentration of biomass can either increase through growth or decrease through decay. Typically, biomass decay is considered negligible, and unless growth is evident, the concentration of biomass is assumed to be constant. The rate of biomass decay typically follows first order kinetics, which is represented by the following relationship:

$$r_d = -\frac{dX}{dt} = -bX, \quad (4)$$

where X represents the biomass concentration, mg/L; t represents time, h; and b is the first order decay coefficient, h^{-1}.

Experiments were conducted to determine the first order decay coefficient (b, h^{-1}) of the denitrifying biomass. The decay experiments were run for approximately 8 days in an anaerobic glove box under a 100% purified N_2 gas atmosphere. Nitrate and nitrite were used for anoxic decay in an analogous manner to oxygen in aerobic decay studies, and were converted to equivalent theoretical oxygen demand (thOD) units by the factors 2.86 and 1.71 mg thOD per mg nitrogen, respectively (Grady and Daigger, 1998). At each sample time, the combined thOD of nitrate and nitrite were added to create a sum of the thOD value of the available terminal electron acceptors (NO_X). The NO_X electron acceptor uptake rate (NUR) was calculated between sampling periods, and the rate of change in the NUR was then used to evaluate the rate of change of biomass decay, and thus the decay coefficient (b, h^{-1}). The NUR data was linearized by natural logarithm transformation, with the slope of the least squares line representing the value of the decay coefficient.

Biogenic Yield Studies

Experiments were conducted to determine the true growth yield (Y) of the biomass growing solely on the biogenic substrate. The biogenic yield studies were conducted for approximately 24 hours in an anaerobic glove box under a 100% purified N_2 gas atmosphere. Biogenic substrate, nitrate, mineral salts, and phosphate buffer were used at the same concentrations as in the main reactor after dilution. The pH of the solution was adjusted to pH 7 using sodium hydroxide. Media was prepared, sterilized by autoclaving, and aseptically transferred to 0.5 liter amber glass reactors using a laminar

flow hood (Envirco Corp., Albuquerque, NM). The reactors were allowed to degas in the glove box overnight. Biomass aliqouts were drawn from the main reactor and washed twice by centrifuging at 2,800xg. Supernatants were discarded and the pellets were resuspended in 100 mM phosphate buffer. The washed biomass aliquots were transferred to the glove box, allowed to degas, and added to the reactors to start the experiment.

Samples were taken over a 24 hour period for total protein and soluble COD. Total protein was used as an indicator of biomass. A correlation between total protein and VSS was experimentally determined on the reactor biomass to be 0.762 mg total protein per mg VSS (R^2 =0.93). VSS was converted to a thOD basis assuming an empirical formula for the biomass of $C_5H_7O_2N$, which leads to a 1.42 mg thOD/mg VSS ratio (Grady and Daigger, 1998). The true growth yields were determined by plotting the thOD biomass concentrations versus the sCOD concentrations. Least squares analysis was performed on the data, with the absolute value of the slope of the best fit line representing the value of the yield on a mg thOD biomass per mg COD substrate basis.

Toluene and m-Xylene Yield Studies

Experiments were conducted to determine the true growth yield of the biomass growing on toluene and *m*-xylene. The growth studies were conducted for approximately five days in an anaerobic glove box under a 100% purified nitrogen atmosphere. Samples were taken for toluene, *m*-xylene, and total protein. Total protein was used as an indicator of biomass as described above. Nitrate (NO_3^-) and nitrite (NO_2^-) were also analyzed to ensure the system was denitrifying.

The toluene and *m*-xylene yield studies were conducted using biomass taken from the main reactor two hours after BTX addition to ensure that the biomass was in a physiological state conducive to toluene and *m*-xylene uptake. Biomass aliquots were gently purged with N_2 gas to strip residual BTX and then washed twice by centrifuging at 2,800xg and resuspending the pellets in a 100 mM

phosphate buffer. Biomass concentrations were diluted 1:500 from the main reactor to the yield reactors. Toluene and *m*-xylene were added at approximately 20 mg/L each. Biomass dilution and elevated levels of toluene and *m*-xylene were used to achieve initial substrate to biomass (S_0:X_0) values high enough (greater than 20 on a thOD basis) for an intrinsic, true growth yield determination (Grady and Daigger, 1998). Mineral salts were amended to tap water at concentrations half of that for the main reactor feed with 0.94 mM of NH_4Cl added because biogenic substrate was lacking. Nitrate was added at an initial concentration of 55 mg/L as N. The media was buffered at pH 7 using a phosphate buffer. Media lacking toluene, *m*-xylene, and NH_4Cl was sterilized by autoclaving and aseptically transferred to one liter amber glass reactors equipped with teflon-lined caps using a laminar flow hood (Envirco Corp., Albuquerque, NM). The reactors were allowed to degas overnight in the anaerobic glove box. Toluene and *m*-xylene were added neat to the reactors and the reactors were sealed and mixed for over two hours to allow for dissolution. Biomass and NH_4Cl were added to the reactors after dissolution to start the experiments.

In order to estimate the volatility losses of toluene and *m*-xylene incurred during each sampling period in the experimental reactors, abiotic control reactors were employed using the same media except that ammonia, phosphate, and biomass were not added. The volatility losses of toluene and *m*-xylene in the control reactors were used to back out the volatility losses in the experimental reactors. This was done by calculating loss ratios between each sampling period for the control reactors and then applying these loss ratios to the corresponding sampling periods for the experimental reactors.

Estimation of X

Accurate estimation of the biomass fraction actively involved in substrate degradation is vital to the determination of accurate kinetic parameters. This arises from the fact that q_{max} and K_S are correlated in term $q_{max} \cdot X$ (see equation 2) such that an overestimation of X leads to an underestimation

of q_{max}. For example, if the fraction of biomass involved in the degradation of a substrate is overestimated by a factor of 2, the determined q_{max} value would be erroneously underestimated by one half. When modeling a wastewater composed entirely of readily biodegradable substrates, the biomass is usually assumed to be comprised of substrate generalists, with the entire biomass involved in the degradation of all the compounds. However, when modeling specific xenobiotic substrates within a heterogeneous waste, the estimation of X is complicated by the potential existence of bacterial specialists. If a population of bacterial specialists is present, it can be expected to selectively use the xenobiotic substrate as its carbon and energy source. Under such circumstances, reporting the entire viable biomass within the system would lead to an underestimation of q_{max}. Accurate determination of q_{max} is therefore dependent upon the ability to reliably estimate the fraction of these specialists to the entire biomass in the system. Enumeration techniques such as the MPN technique as described by Silverstein *et al.* (1994) and various molecular techniques such as hybridization with specific nucleotide probes (Neef *et al.*, 1996; Schofield *et al.*, 1996; Tyagi *et al.*, 1995) can serve to experimentally determine this fraction. In lieu of these techniques, however, estimation of the specialist population can be made. Preliminary results by Ellis *et al.* (1996) suggested that the fraction of bacteria selectively degrading a xenobiotic compound can be estimated by relating it to that substrate's fraction of COD in the influent. Further refinement of this estimation would include the different growth yields of the specific compound and the bulk wastewater according to the following equation:

$$f_{specialist} = \left[\frac{COD_{in,xenobiotic}}{COD_{in,bulkwaste}}\right]\left[\frac{Y_{xenobiotic}}{Y_{bulk}}\right]. \qquad (5)$$

Toluene and m-Xylene Kinetic studies

Experiments were conducted on the main reactor to evaluate the kinetic parameters of maximum specific substrate utilization rate (q_{max}, h^{-1}) and the half saturation constant (K_S, mg/L) parameters of

the Monod model for toluene and *m*-xylene. Reactor feed was prepared in a 4-liter amber glass jar equipped with a teflon-lined cap and sterilized by autoclaving and allowed to go anaerobic overnight in an anaerobic glove box under a 100% purified nitrogen atmosphere. Benzene, toluene, *o*-xylene, *m*-xylene, and *p*-xylene were added neat to the feed jar, the feed jar was sealed, and the BTX compounds were allowed to dissolve for over two hours. After the dissolution period, the feed was introduced anaerobically to the reactor at the start of the cycle. This was accomplished by using a purified N_2 gas pressure displacement system where gas was introduced to the headspace of the feed jar, which forced the feed solution through tubing into the reactor. In order to minimize contamination and prevent BTX volatility and sorption during transfer, all tubing was composed of either glass or teflon and all junctions were made of stainless steel.

Rigorous sampling of the reactor was performed for the first four hours of reaction in order to establish a sufficient number of points during the K_S portion of the substrate uptake curve for reliable modeling. Specifically, samples for BTX were taken at least every ten minutes during this time. Samples for anions and sCOD were likewise taken every fifteen minutes during this period. Samples for all of the constituents were subsequently staggered for the remainder of the reaction cycle.

RESULTS AND DISCUSSION

Degradation Studies

Approximately eight months after reactor startup, the microbial consortium within the denitrifying batch reactor completely degraded toluene and *m*-xylene on a routine basis (Fettig and Love, 1998). The degradation of these compounds was evident from both reactor effluent quality and batch profile data. Benzene, o-xylene, and p-xylene were not completely degraded during the course of a normal batch cycle, and were typically found in the effluent at concentrations near their influent levels. Experiments were therefore conducted to determine the stoichiometric coefficients and kinetic parameters of degradation for toluene and *m*-xylene only.

Biomass Decay Studies

The results from one of the three decay experiments is represented in Figure 1. Both the logarithm transformed NUR data and total protein are plotted versus the time of reaction. The value of the decay coefficient from the shown experiment was 0.0130 h^{-1}. The poor R^2 value in this case (0.77) is attributable to the two data points at approximately days 1 and 6. Although these points cause the data to deviate from a line form, they do not significantly affect the decay coefficient value--analyzing the data without these points resulted in a decay coefficient of 0.0121 h^{-1} and an R^2 value of 0.99. The overall results from the decay studies are shown in Table 1. The average value of the first order decay coefficient from the three decay experiments was 0.016 ± 0.006 h^{-1}. This value compares well to the typical value of 0.01 h^{-1} for an anoxic, heterotrophic biomass (Grady and Daigger, 1998).

Recent investigation into the affects of xenobiotic chemicals on the rate of microbial death and lysis suggest that relatively high concentrations (>250 mg COD/L) are required before

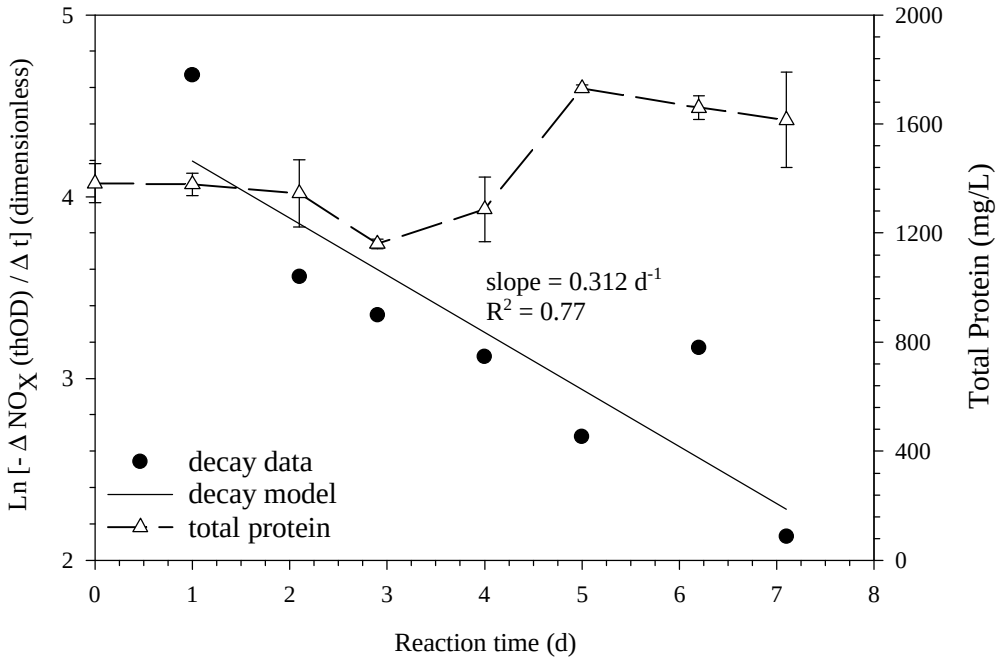

Figure 1. Natural logarithm transformed NUR data for the determination of the biomass decay rate. Total protein concentration during the course of decay is also shown. Error bars on total protein samples represent one standard deviation of duplicate samples.

stimulation in the decay rate is observed (Perez-Padilla and Grady, 1997). Therefore, although these experiments were conducted in the absence of BTX, they can be expected to be representative of the decay kinetics in the main reactor. Although the consumption of terminal electron acceptor and the absence of substrate indicate that biomass decay was occurring, the decay of biomass was not evident in the total protein data as illustrated in Figure 1. This illustrates the need to evaluate anoxic decay based on the responses of nitrate and nitrite, in an analogous manner to using oxygen in aerobic decay, and not on biomass indicators such as total protein. For example, Jørgensen *et al.* (1995) estimated the decay coefficient of their denitrifying BTX enrichment culture to be ~0 h^{-1} based on the lack of a significant decrease in total protein levels.

True Growth Yields and Estimation of X

The influent carbon of the denitrifying batch reactor was divided into two distinct groups: the biogenic substrate and the BTX compounds. Therefore, true growth yield experiments were conducted on both the biogenic substrate and the BTX compounds that were amenable to degradation, namely toluene and *m*-xylene. The true growth yield for toluene and *m*-xylene was needed to calculate m_{max} according to equation (4) and the true growth yield was needed to estimate the specialist fraction of the biomass according to equation (2).

The results of an experiment in which the true growth yield of the biogenic substrate was studied is presented in Figure 2. The consumption of sCOD led to a linear increase in the biomass concentration. The least squares regression line is presented and had a slope of -0.396 and an R^2 value of 0.93. This led to a yield value of 0.40 mg thOD biomass per mg COD substrate for this experiment. The average of duplicate true growth yield experiments on the biogenic substrate was 0.41 ± 0.02 mg thOD biomass per mg COD substrate, as shown in Table 1.

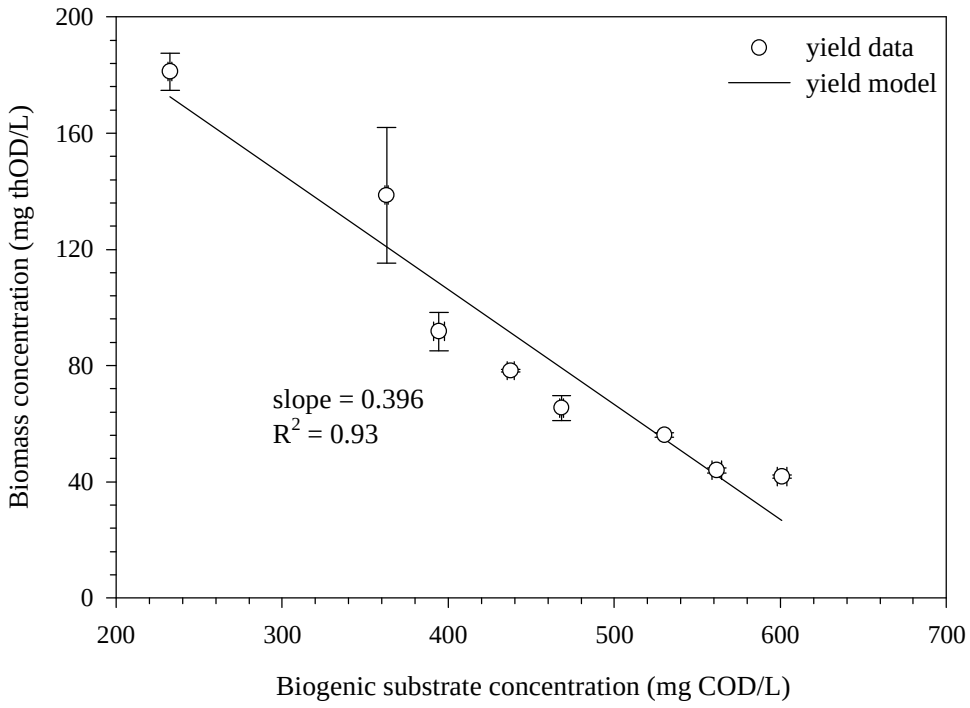

Figure 2. True growth yield determination for biogenic substrate. Error bars represent one standard deviation of duplicate measurements for substrate COD and triplicate measurements for biomass.

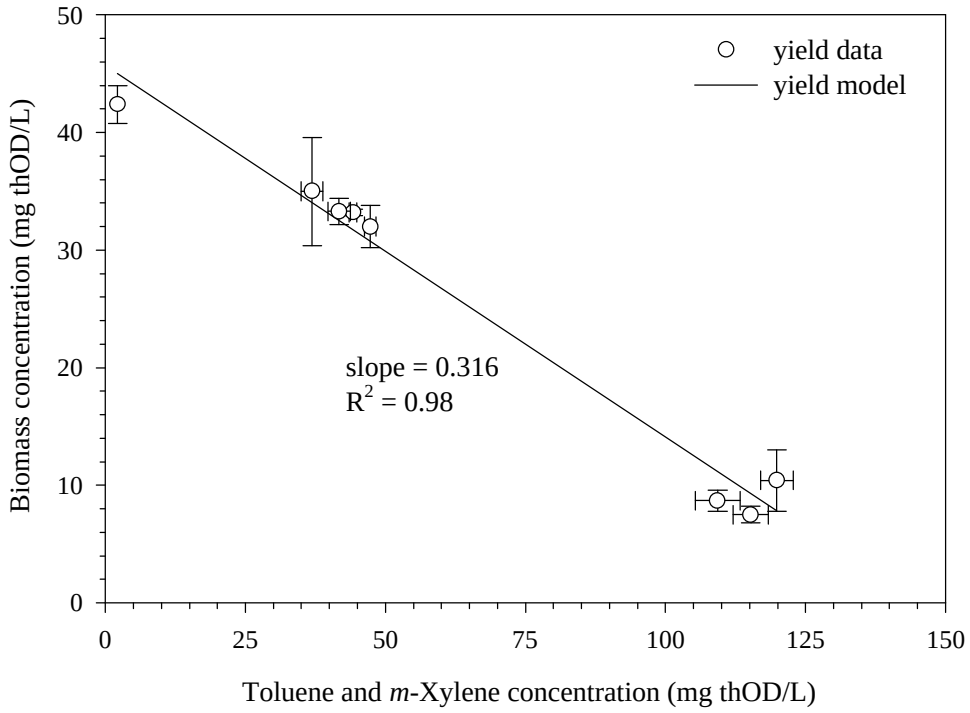

Figure 3. True growth yield determination for toluene and *m*-xylene. Error bars represent one standard deviation of triplicate measurements.

Experiments were conducted to determine the true growth yield for toluene and *m*-xylene. In order to determine a true growth yield, the initial substrate to biomass concentration (S_0:X_0) must be sufficiently high to allow for unfettered growth and negligible decay. Typically, an S_0:X_0 ratio of greater than 20, when both are expressed on a thOD basis, is sufficient for such an evaluation (Grady and Daigger, 1998). However, achieving this ratio with compounds that are inhibitory and have low solubilities can be difficult. The S_0:X_0 ratio for each of the true growth yield experiments was between 12 and 15, which is slightly lower than this benchmark. However, the X_0 value applies to the active fraction of bacteria, which will be significantly less than the total thOD reported based on total protein levels. For example, if we assume that because toluene and *m*-xylene represented 5% of the influent COD that 5% of the active biomass was toluene and *m*-xylene degraders, as was discussed above, the S_0/X_0 ratios would increase twenty-fold to between 240 and 300. Therefore, although the bulk biomass thOD led to S_0:X_0 ratios less than 20, the actual S_0:X_0 ratio was much greater than the ratio of 20 required for intrinsic parameter determination.

The results from a representative toluene and *m*-xylene true growth yield experiment is presented in Figure 3. Toluene and *m*-xylene were converted to a thOD basis by using the factors 3.13 and 3.17 mg thOD per mg compound, respectively. The consumption of toluene and *m*-xylene was coupled to a linear increase in biomass concentration. The least squares regression line through the data had a slope of -0.316 and an R^2 value of 0.98. This led to a yield value of 0.32 mg thOD biomass per mg thOD toluene and *m*-xylene for this experiment. The average true growth yield of triplicate experiments was 0.34 ± 0.03 mg thOD biomass per mg thOD toluene and *m*-xylene, as shown in Table 1. On a total protein basis, the growth yield was 0.17 ± 0.04 mg protein per mg toluene and *m*-xylene. This value is in good agreement with the value reported by Jørgensen *et al.* (1995) of 0.14 ± 0.02 mg protein per mg toluene. The results are also in reasonably good agreement with the results by Evans *et al.* (1991b). It was reported therein that 29% of toluene carbon was assimilated into cellular carbon. Assuming that cellular carbon represents 53% of the dry cell weight (based on $C_5H_7O_2N$ as an empirical formula) and

that 55% of the dry cell weight is protein (Neidhart *et al.*, 1987), this value converts to 0.25 mg protein per mg toluene. Elmén *et al.* (1996) reported a value of 140 g dry cell weight (DCW) per mol of toluene and 76 g DCW per mol *m*-xylene. Using the assumptions above, these yield values convert to 0.84 mg protein per mg toluene and 0.39 mg protein per mg *m*-xylene. Although substantially higher than the results by Jørgensen *et al.* (1995), Evans *et al.* (1991b), and the those described herein, they are not thermodynamically impossible.

Preliminary experiments on the biomass in the system using the Most-Probable-Number (MPN) technique as described by Silverstein *et al.* (1994) suggested that toluene degrading bacteria were only a fraction of the total active bacteria in the system (data not shown). Therefore, the system was assumed to be composed of substrate specialists with respect to the degradation of toluene and *m*-xylene. However, reliable estimates of the fraction of specialists to the entire biomass could not be made with this technique. Therefore, an estimation of the specialist fraction was made according to equation (2). The average yields for the biogenic and xenobiotic substrates were 0.41 ± 0.02 mg thOD biomass per mg thOD substrate and 0.34 ± 0.03 mg thOD biomass per mg COD substrate, respectively. The fraction of influent xenobiotic thOD to the reactor was 0.05 (32 mg thOD to 657 mg thOD total). Therefore, the fraction of biomass in the system involved in the degradation of toluene and *m*-xylene was estimated to be 0.04.

Extant Kinetic Parameters qmax and K_S

Kinetic experiments were conducted on days 280, 416, and 466 to determine the Monod parameters q_{max} and K_S for toluene and *m*-xylene degradation in the denitrifying activated sludge sequencing batch reactor. The responses of toluene and *m*-xylene during these tests are presented in Figures 4 and 5, respectively. The data show that a period of linear decrease in concentrations existed for each test day during approximately the first 30 minutes of reaction. After this period, there was a

distinct discontinuity caused by an abrupt increase in the rate of uptake. The

Table 1. True growth yields and decay parameter for the denitrifying reactor.

Statistic	b (h^{-1})	Y, xenobiotic (mg thOD/mg thOD)	Y, biogenic (mg thOD/mg COD)
average	0.016	0.34	0.41
standard deviation	0.006	0.03	0.02
observations	3	3	2

discontinuity is most evident in the day 280 and day 466 profiles, but is also observed to a lesser extent in the day 416 profile. The discontinuity is temporally linked to the period of rapid biogenic substrate consumption which was typically complete within the first 30 minutes of each cycle. Kinetic parameters were estimated based on fits was performed on the toluene and *m*-xylene data after this discontinuity to prevent complication between these two events. A nonlinear, least squares approach was used to fit the integrated form of the Monod model (equation 2) to the data. The q_{max} and K_S parameter values determined for the Monod model as well as the zero-order initial uptake rates (k, mg substrate/mg protein/h) for toluene and *m*-xylene are presented in Tables 2 and 3, respectively. The best fit curves that are described by the Monod parameters are plotted against the data in Figures 4 and 5.

As can be seen in Figures 5 and 6, the behavior of toluene and *m*-xylene degradation differed between tests. As is evident from the mean sum of squares error values (MSSE) presented in Tables 2 and 3, the Monod model describes the data quite well for days 280 and 466, but not as

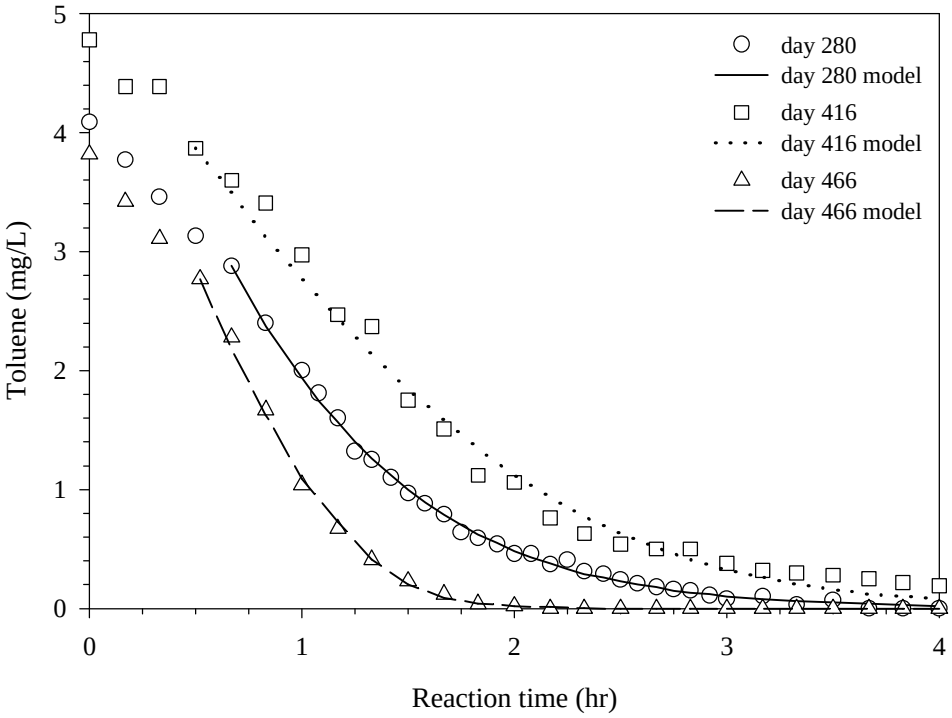

Figure 4. Toluene uptake by the denitrifying activated sludge consortium. Curves are fitted against the data used in nonlinear parameter estimation, which only included those points after the apparent lag period. The lag and subsequent discontinuity coincided with the period of rapid biogenic substrate uptake and depletion.

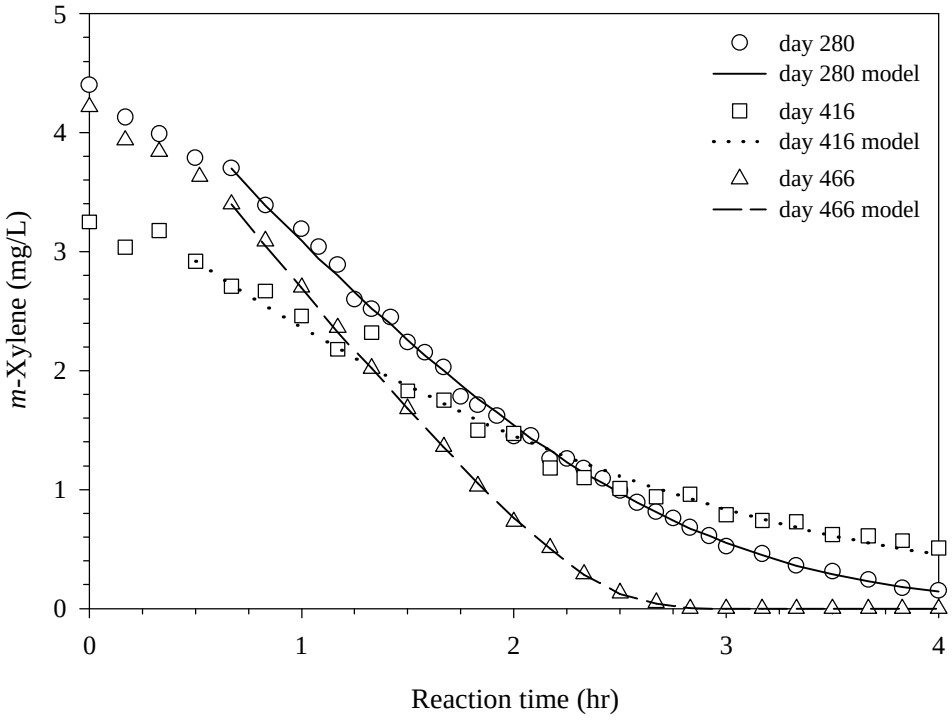

Figure 5. *m*-Xylene uptake by the denitrifying activated sludge consortium. Curves are fitted against the data used in the nonlinear parameter estimations, which included only those points after the apparent lag period. The lag and subsequent discontinuity coincided with the period of rapid biogenic substrate uptake and depletion.

Table 2. Initial uptake rates and Monod parameters for toluene degradation.

Test	$k^{\dagger}$; (R^2 value)	$q_{max}{}^{\dagger}$	$K_S{}^{\ddagger}$	MSSE*	$\mu_{max}{}^{**}$
day 280	0.032 (0.99)	0.139	6.92	0.0012	0.278
day 416	0.031 (0.97)	0.0591	2.49	0.0200	0.118
day 466	0.047 (0.99)	0.0694	0.839	0.0024	0.139

† k and q_{max} in units of mg toluene/mg protein/h

‡ K_S in units of mg/L toluene

* MSSE = mean sum of square error; goodness of fit descriptor for Monod parameters

** μ_{max} in units of h^{-1}, calculated according to equation (2) and a protein:VSS ratio of 0.76

Table 3. Initial uptake rates and Monod parameters for *m*-xylene degradation.

Test	$k^{\dagger}$; (R^2 value)	$q_{max}{}^{\dagger}$	$K_S{}^{\ddagger}$	MSSE	$\mu_{max}{}^{*}$
day 280	0.022 (0.97)	0.035	1.73	0.0023	0.069
day 416	0.015 (0.95)	0.041	3.69	0.0095	0.082
day 466	0.029 (0.99)	0.034	0.281	0.0004	0.068

† k and q_{max} in units of mg *m*-xylene/mg protein/h

‡ K_S in units of mg/L *m*-xylene

* MSSE = mean sum of square error; goodness of fit descriptor for Monod parameters

** μ_{max} in units of h^{-1}, calculated according to equation (2) and a protein:VSS ratio of 0.76

well for day 416. Nitrite accumulation on days 280 and 466 reached approximately the same maximum (140 and 133 mg/L NO_2-N, respectively), but the accumulation on day 416 was much greater (220 mg/L NO_2-N). As alkalinity was produced from the denitrifying reaction, the reactor pH increased from 7.0 to approximately 7.5 during the first four hours of reaction. Using a pK_a for nitrous acid of 3.17 (*Lange's Handbook of Chemistry*, 1987), this led nitrous acid concentration ranges for days 280, 416, and 466 of 0.021 to 0.007, 0.033 to 0.010, and 0.020 to 0.006 mg/L HNO_2-N, respectively. The

onset of significant inhibition in activated sludge has been observed at nitrous acid at concentrations as low as 0.04 mg/L HNO_2-N at pH 7 (Glass *et al.*, 1997). Although 0.04 mg/L HNO_2-N was not reached on any of the test days, the nitrous acid level on day 416 approached this benchmark and may have resulted in moderate inhibition. This would explain the substantial decrease in the initial uptake rates for *m*-xylene on day 416. The initial uptake rate for toluene was relatively unaffected compared to day 280, however. Substantially greater accumulation of nitrous acid may also be responsible for the poorer fits of the Monod model to the day 416 data, as the model does not account for the presence of inhibitory substrates.

The accumulated nitrous acid concentrations on test days 280 and 466 were almost identical and therefore can not explain the differences in initial uptake rates for toluene and *m*-xylene. As seen in Tables 2 and 3, the initial uptake rates were greater on day 466 for both toluene and *m*-xylene. In addition, the best fit Monod parameters followed a trend in that both the q_{max} and K_S parameters were higher on day 280 than they were on day 466 for both of the aromatic hydrocarbons. The only differences between these two tests were that they were conducted six months apart and that the initial concentrations of *p*-xylene and *o*-xylene were slightly greater in test date 280 (*p*-xylene, 5.66 vs. 4.33 mg/L; *o*-xylene, 5.60 vs. 4.46 mg/L). Slightly elevated levels of these non-degrading BTX compounds could have exerted a slight inhibitory effect on the biomass, resulting in the lower initial uptake rates observed for day 280. However, the differences between the *p*-xylene and *o*-xylene concentrations is slight compared to the time difference between the two tests. Evidence suggested that although the reactor was operated for a long duration, and should have been at a steady-state, the physiological capacity of the biomass was continuously being refined. For example, the onset of *o*-xylene transformation was witnessed after over a year of incubation (Fettig and Love, 1998). It is postulated that during the course of the study the toluene and *m*-xylene degradative enzyme systems may have trended towards higher affinity systems, which would have resulted in the lower q_{max} and K_S values observed at the later test date.

Jørgensen *et al.* (1995) evaluated the stoichiometry and kinetics of toluene degradation under denitrifying conditions by an enrichment culture and reported values of 0.71 ± 0.04 mg toluene/mg protein/h and 0.4 ± 0.2 mg/L for the maximum specific uptake rate, q_{max}, and half saturation constant, K_S, respectively. The values reported herein for q_{max} are significantly lower by roughly an order of magnitude. The kinetic experiments conducted by Jørgensen *et al.* (1995) used initial toluene concentrations of 10.8 mg/L and initial biomass concentrations of 0.5 mg/L. Assuming a dry cell protein content of 55% and a cell formula of $C_5H_7O_2N$ as described above, the theoretical S_0:X_0 ratio for their experiments was approximately 86. The high S_0:X_0 value for these experiments probably enabled alterations in the bacterial physiological state by increasing the concentration of internal macromolecules (RNA, enzymes, etc.) and led to the determination of intrinsic kinetic parameters. Conversely, the S_0:X_0 ratio of the kinetic experiments reported herein was approximately 0.1, assuming toluene and *m*-xylene bacterial specialists comprised approximately 4% of the biomass as discussed above. The low S_0:X_0 ratio in these experiments did not afford the bacteria sufficient energy to alter their internal macromolecular composition (RNA, enzymes, etc.), and hence led to the determination of extant kinetic parameters. Because of this fundamental difference in how the two experiments were conducted, it is expected that the q_{max} values reported by Jørgensen *et al.* (1995) should be greater because they represent toluene degradation under an optimum physiological state; whereas the extant values reported herein are representative of the existing physiological state of the biomass in the reactor.

CONCLUSIONS

The studies presented illustrate that biological treatment of toluene and *m*-xylene would occur in anoxic, denitrifying zones within activated sludge systems treating wastewaters contaminated with these compounds. The low S_0:X_0 ratios used during the kinetic evaluations led to the determination of extant parameter values for the maximum substrate uptake rates (q_{max}) and half saturation constants (K_S). Extant kinetic parameters tend to be more representative of real world systems and are therefore important for accurate modeling of the in situ degradation rates in wastewater treatment systems. Zero order initial uptake rates (k) showed that the rate of toluene degradation was greater than the rate of *m*-xylene degradation in all experiments. Zero-order initial uptake rates in the SBR for toluene and *m*-xylene ranged from 0.031 to 0.047 and 0.015 and 0.029 mg substrate/mg protein/h, respectively. The maximum specific utilization rate (q_{max}) and half saturation constant (K_S) values for toluene and *m*-xylene were varied according to the test date with a temporal trend towards higher affinity enzyme systems, suggesting that even after approximately a year and a half, the system was not at steady-state. For toluene, q_{max} and K_S ranged from 0.059 to 0.14 mg toluene/mg protein/h and 0.84 to 6.9 mg/L, respectively. The q_{max} and K_S values for *m*-xylene ranged from 0.034 to 0.041 mg toluene/mg protein/h and 0.28 to 3.7 mg/L, respectively. The studies also suggested that a population of toluene- and *m*-xylene-degrading specialists developed within the activated sludge consortium receiving a mixed waste. Therefore, the kinetic parameters determined herein must be applied using estimates of such a fraction. A reasonable estimation method for this biomass fraction, outside of direct enumeration methods, was used and is based on the different substrate concentrations of the influent on a theoretical oxygen demand basis in conjunction with the true growth yield values. The presented initial uptake rates and Monod parameters indicate that even with a majority of influent carbon in the form of biogenic

substrates, toluene and *m*-xylene degradation is relatively rapid and would be feasible in a properly designed system.

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ENGINEERING SIGNIFICANCE

Benzene, toluene, *p*-xylene, *m*-xylene, and *o*-xylene (BTX) are of environmental significance because they are the more water-soluble petroleum hydrocarbons and they have been shown to induce acute toxic and chronic mutagenic effects in humans and other forms of life. Because of these properties, the Environmental Protection Agency (EPA) has set strict regulatory levels on the permitted wastewater effluent concentrations of these compounds. Specific industrial wastewaters can contain concentrations of these compounds well in exceedance of their regulatory limits; and therefore, treatment technologies are needed to remove them to below their regulated levels. Historically, aerobic biological treatment was used for this purpose, although stripping of the volatile BTX compounds was the primary removal mechanism. The National Emissions Standards for Hazardous Air Pollutants (NESHAP), promulgated by the EPA in 1992, requires chemical and petroleum industries to employ Maximum Achievable Control Technologies (MACTs) to achieve 99% reduction in mass emissions of the Hazardous Air Pollutants (HAPs) to the atmosphere. Due to this, aerobic biological treatment of BTX in wastewaters has become a seldom-used technology, as the gas-liquid mass transfer systems employed by these systems tend to strip the BTX compounds out of the wastewater and into the atmosphere. Current practice has turned away from biological treatment and now involves using a variety of physicochemical techniques, such as air stripping and carbon adsorption.

Anoxic, denitrifying environments utilize nitrate (NO_3^-) instead of oxygen (O_2) as a terminal electron acceptor, and therefore do not employ the gas-liquid mass transfer systems required by aerobic environments. Because of this, anoxic biological treatment may be a viable technology for removing BTX from wastewater while minimizing air emissions to those resulting from the natural physical process of volatilization. The results of this research have shown that toluene and *m*-xylene are indeed amenable to biological treatment under denitrifying conditions in a properly designed anoxic system

after sufficient acclimation time. Additionally, this research has shown that transformation of *o*-xylene is also possible, with its transformation resulting from gratuitous metabolism during toluene degradation. The findings of this research also have shown that benzene and *p*-xylene will most likely not be effectively treated biologically in a denitrifying environment. Therefore, the findings of this research suggest that for wastewaters contaminated only with toluene and *m*-xylene, or wastewaters also contaminated with *o*-xylene and with sufficient toluene for its complete gratuitous metabolism, denitrifying biological treatment systems may be used as a viable treatment alternative. Atmospheric emissions of toluene, *m*-xylene, and *o*-xylene from these systems would be limited to volatilization alone. The findings of this research also suggest that for wastewaters contaminated with benzene and *p*-xylene, denitrifying biological systems may not be effective. Microaerophilic environments, however, hold promise for the biological degradation of these recalcitrant compounds, but further investigation is needed in that area.

The stoichiometric coefficients and kinetic parameters determined by this research offer a starting point to describe the biodegradation rates of toluene and *m*-xylene in a denitrifying activated sludge system. Because the kinetic parameters were determined from a microbial consortium receiving a mixed waste of readily biodegradable substrates and the BTX hydrocarbons, they are more indicative of real world systems than values determined from either enrichment or pure culture. It should be emphasized that biodegradation rates will vary from wastewater to wastewater; and therefore, kinetic parameters should be obtained for a specific wastewater through pilot- or bench-scale testing.

APPENDIX A:

SOURCE DATA FOR MANUSCRIPT 1

"BTX DEGRADATION BY AN ACTIVATED SLUDE CONSORTIUM

UNDER DENITRIFYING CONDITIONS"

Table A.1. Reactor Effluent BTX Concentrations After Six Month Acclimation Period.

	Averages					Standard Deviations				
Days after acclimation	benz. (mg/L)	tol. (mg/L)	*p* -xyl. (mg/L)	*m* -xyl. (mg/L)	*o* -xyl. (mg/L)	benz. (mg/L)	tol. (mg/L)	*p* -xyl. (mg/L)	*m* -xyl. (mg/L)	*o* -xyl. (mg/L)
0	4.55	ND	5.03	2.71	4.99	**	**	**	**	**
1	4.04	2.08	4.56	3.20	5.25	**	**	**	**	**
2	4.44	ND	5.18	ND	4.71	**	**	**	**	**
14	3.62	1.06	4.31	1.86	4.57	**	**	**	**	**
15	4.26	3.68	5.07	4.20	5.68	**	**	**	**	**
17	4.52	4.62	5.60	5.44	6.46	**	**	**	**	**
20	3.69	3.74	4.43	4.42	5.14	**	**	**	**	**
21	4.24	4.27	5.18	5.17	6.06	**	**	**	**	**
22	4.30	4.37	5.45	5.65	6.15	**	**	**	**	**
27	4.48	4.17	5.42	5.22	6.07	**	**	**	**	**
29	4.09	3.45	5.23	5.29	6.04	**	**	**	**	**
30	4.90	2.65	5.94	5.69	6.70	**	**	**	**	**
31	5.05	4.56	6.34	6.17	7.07	**	**	**	**	**
32	4.70	0.42	5.34	1.12	5.23	**	**	**	**	**
42	4.91	4.40	5.79	4.95	6.32	**	**	**	**	**
45	0.22	ND	0.62	ND	0.68	**	**	**	**	**
48	4.38	1.14	4.76	1.52	4.66	**	**	**	**	**
49	4.80	ND	5.34	ND	4.72	**	**	**	**	**
51	4.77	0.49	5.12	0.65	4.76	**	**	**	**	**
52	5.13	1.66	5.78	2.16	5.88	**	**	**	**	**
56	4.75	ND	5.84	ND	4.38	**	**	**	**	**
57	7.10	ND	7.75	ND	6.07	**	**	**	**	**
58	7.16	1.82	7.58	2.36	7.66	**	**	**	**	**
59	6.44	5.35	7.81	6.01	8.17	**	**	**	**	**
62	4.26	ND	4.88	ND	4.34	**	**	**	**	**
63	7.86	ND	8.53	ND	6.91	**	**	**	**	**
66	5.84	ND	6.59	ND	5.22	**	**	**	**	**
71	3.71	ND	4.12	ND	2.88	**	**	**	**	**
72	5.28	ND	5.88	ND	4.13	**	**	**	**	**
73	6.04	ND	6.65	ND	4.49	**	**	**	**	**
76	6.08	ND	5.72	ND	3.69	**	**	**	**	**
77	4.68	ND	4.74	ND	2.93	**	**	**	**	**
78	5.88	ND	5.64	ND	3.73	**	**	**	**	**
79	5.89	ND	5.89	ND	3.67	**	**	**	**	**
101	5.93	0.28	6.61	ND	6.66	**	**	**	**	**
104	4.68	ND	4.77	ND	4.74	**	**	**	**	**
105	6.22	ND	6.31	ND	5.70	**	**	**	**	**
107	6.20	ND	6.41	ND	6.51	**	**	**	**	**
108	6.36	ND	7.09	ND	6.25	**	**	**	**	**
113	6.06	ND	6.37	ND	6.21	**	**	**	**	**
115	7.18	ND	6.76	ND	6.79	**	**	**	**	**
119	5.19	ND	5.86	ND	5.62	**	**	**	**	**
120	5.94	ND	6.80	ND	6.50	**	**	**	**	**
123	4.23	ND	5.04	ND	4.12	**	**	**	**	**
125	0.65	ND	1.22	ND	0.88	**	**	**	**	**
127	5.59	0.12	6.39	ND	5.97	**	**	**	**	**
128	4.66	0.23	4.91	ND	6.29	**	**	**	**	**
129	5.18	0.17	4.55	ND	6.81	**	**	**	**	**
130	6.50	0.29	5.27	ND	8.60	**	**	**	**	**
133	5.04	ND	5.86	ND	7.07	**	**	**	**	**
136	3.85	ND	4.22	ND	3.97	**	**	**	**	**

Table A.1. Continued...

	Averages					Standard Deviations				
Days after acclimation	benz. (mg/L)	tol. (mg/L)	*p* -xyl. (mg/L)	*m* -xyl. (mg/L)	*o* -xyl. (mg/L)	benz. (mg/L)	tol. (mg/L)	*p* -xyl. (mg/L)	*m* -xyl. (mg/L)	*o* -xyl. (mg/L)
138	4.45	ND	5.29	ND	4.61	**	**	**	**	**
139	4.51	ND	5.34	ND	4.28	**	**	**	**	**
141	5.61	ND	6.54	ND	5.57	**	**	**	**	**
142	4.97	ND	5.90	ND	5.35	**	**	**	**	**
147	4.90	ND	5.56	ND	4.45	**	**	**	**	**
150	4.05	ND	4.75	ND	3.99	**	**	**	**	**
153	4.53	ND	5.32	ND	4.97	**	**	**	**	**
155	4.96	0.54	6.38	1.92	5.99	0.02	0.02	0.07	0.02	0.07
157	5.50	ND	6.48	ND	5.45	0.04	***	0.09	***	0.13
164	4.72	3.20	5.26	4.49	5.90	0.04	0.04	0.04	0.03	0.08
165	4.77	0.10	5.09	0.10	4.88	0.07	0.00	0.10	0.00	0.02
167	4.86	0.20	5.31	0.40	4.91	0.05	0.00	0.09	0.01	0.12
203	3.09	ND	3.74	ND	4.05	0.06	***	0.02	***	0.00
218	1.22	ND	1.39	ND	2.92	0.02	***	0.00	***	0.05
224	3.97	ND	3.74	ND	3.07	**	**	**	**	**
227	3.64	ND	3.41	ND	2.34	0.04	***	0.01	***	0.06
233	3.36	ND	2.87	ND	2.49	0.02	***	0.12	***	0.03
258	3.55	ND	3.49	ND	3.01	0.04	***	0.01	***	0.08
259	4.59	ND	4.56	ND	3.75	0.03	***	0.08	***	0.07
260	4.44	ND	4.48	ND	3.30	0.16	***	0.10	***	0.13
261	4.33	ND	4.42	ND	3.04	0.11	***	0.02	***	0.12
262	4.03	ND	4.48	ND	3.02	0.02	***	0.03	***	0.05
265	3.94	ND	4.87	ND	2.94	0.08	***	0.06	***	0.05
266	4.93	ND	5.06	ND	2.96	0.07	***	0.11	***	0.06
267	4.08	ND	4.55	ND	2.59	0.05	***	0.05	***	0.04
269	6.37	ND	6.65	ND	4.84	0.08	***	0.09	***	0.02
270	4.37	ND	5.05	ND	3.58	0.03	***	0.09	***	0.03
272	7.73	ND	8.18	ND	5.98	0.10	***	0.07	***	0.01
273	4.35	ND	5.06	ND	3.53	0.08	***	0.07	***	0.08
274	4.55	ND	5.38	ND	3.86	0.06	***	0.00	***	0.07
276	4.29	ND	5.03	ND	3.20	0.09	***	0.24	***	0.10
277	4.43	ND	5.14	ND	3.93	0.00	***	0.02	***	0.13
279	3.89	ND	5.03	ND	3.79	0.00	***	0.01	***	0.01
280	3.59	ND	4.61	ND	3.42	0.04	***	0.03	***	0.03
288	6.14	ND	5.39	ND	5.07	0.51	***	0.23	***	0.22
289	4.18	ND	4.66	ND	3.22	0.02	***	0.01	***	0.15
292	4.56	ND	5.19	ND	3.69	0.05	***	0.10	***	0.04
294	3.28	ND	4.45	ND	3.78	**	**	**	**	**
295	3.30	ND	4.37	ND	3.36	0.06	***	0.07	***	0.08
301	3.25	2.11	4.12	3.44	5.15	0.78	0.48	0.93	0.77	1.08
311	3.87	ND	3.60	ND	4.43	0.52	***	0.46	***	0.55
350	5.14	ND	5.96	ND	4.08	0.59	***	0.65	***	0.43
363	3.98	ND	4.44	ND	3.58	0.06	***	0.03	***	0.00
381	2.94	ND	2.40	ND	2.85	0.01	***	0.03	***	0.02
395	3.39	ND	2.91	ND	3.02	0.00	***	0.02	***	0.04
399	3.59	ND	3.71	ND	2.93	0.05	***	0.07	***	0.04
415	3.92	ND	3.85	ND	2.39	0.33	***	0.37	***	0.25
421	4.14	ND	4.48	ND	3.82	0.05	***	0.32	***	0.29
423	4.05	ND	4.30	ND	3.52	0.36	***	0.34	***	0.34
427	3.98	0.06	4.49	1.12	3.58	0.05	0.00	0.07	0.00	0.05
428	4.06	ND	4.80	0.04	2.69	0.11	***	0.14	0.00	0.07

Table A.1. Continued...

	Averages					Standard Deviations				
Days after acclimation	benz. (mg/L)	tol. (mg/L)	*p* -xyl. (mg/L)	*m* -xyl. (mg/L)	*o* -xyl. (mg/L)	benz. (mg/L)	tol. (mg/L)	*p* -xyl. (mg/L)	*m* -xyl. (mg/L)	*o* -xyl. (mg/L)
431	3.95	0.15	4.73	ND	2.95	0.05	0.01	0.06	***	0.03
440	3.60	0.10	4.09	ND	3.08	0.28	0.01	0.39	***	0.28
441	3.61	0.11	4.07	ND	3.04	0.29	0.01	0.34	***	0.25
442	3.76	0.10	4.24	ND	2.81	0.02	0.00	0.02	***	0.03
450	3.82	0.10	4.31	ND	3.40	0.08	0.02	0.03	***	0.03
454	3.77	0.11	4.46	ND	3.28	0.03	0.00	0.02	***	0.01
458	3.49	ND	3.87	ND	2.21	0.05	***	0.09	***	0.00
462	3.06	ND	3.57	ND	2.77	0.03	***	0.01	***	0.02
464	3.66	ND	3.97	ND	2.71	0.05	***	0.05	***	0.07
465	3.83	ND	4.03	ND	2.62	0.01	***	0.01	***	0.05
470	3.62	ND	3.85	ND	3.02	0.07	***	0.07	***	0.05
471	3.75	ND	3.73	ND	2.54	0.01	***	0.03	***	0.00
479	3.67	ND	2.81	ND	1.27	0.05	***	0.06	***	0.01
484	3.68	ND	2.95	ND	2.26	0.10	***	0.10	***	0.07
489	3.89	ND	3.83	ND	2.48	0.00	***	0.02	***	0.02
494	3.24	ND	2.83	ND	2.38	0.02	***	0.02	***	0.01
496	3.41	ND	3.01	ND	2.04	0.00	***	0.02	***	0.00
498	3.43	ND	2.97	ND	2.71	0.01	***	0.03	***	0.01
505	4.36	ND	3.27	ND	2.66	0.00	***	0.02	***	0.01
512	3.75	ND	2.09	ND	2.95	0.01	***	1.56	***	0.02

** -- Duplicate samples were not analyzed on days 1 through 153, therefore standard deviations could not be calculated. The average values presented represent the average value of replicate analyses of the same sample.
*** -- Compound not detected, therefore standard deviations could not be calculated.
ND -- Not Detected.

Table A.2. BTX, Nitrate-N, Nitrite-N, and sCOD Profiles Across Reaction Cycle on Day 105 (March 4, 1997).

Time (hr)	benzene (mg/L)	toluene (mg/L)	*p*-xylene (mg/L)	*m*-xylene (mg/L)	*o*-xylene (mg/L)	nitrite-N (mg/L)	nitrate-N (mg/L)	sulfate (mg/L)	ave. sCOD (mg/L)	s.d. sCOD (mg/L)
0.00	1.09	ND	1.40	ND	1.63	4.6	210.4	30.8	65.9	14.5
0.50	4.22	2.48	3.48	1.93	4.16	56.4	137.1	31.1	234.6	8.4
1.17	5.24	3.56	5.07	3.33	5.72	74.3	104.3	32.3	116.5	**
2.17	5.63	3.09	5.99	2.78	6.32	83.6	83.8	30.0	119.1	**
3.17	6.42	2.52	7.13	2.00	7.37	98.3	68.8	31.1	114.8	5.6
4.17	6.39	1.65	7.06	1.15	7.13	99.7	60.0	31.9	108.2	1.1
5.17	6.45	1.26	7.35	0.80	7.33	97.8	55.1	31.0	96.4	1.7
6.17	6.16	1.04	6.87	0.54	6.93	92.8	51.0	30.9	109.8	5.6
8.17	6.06	0.84	6.56	0.34	6.77	77.1	46.4	30.1	106.4	2.2
10.17	6.09	0.76	6.52	0.31	6.89	68.7	42.9	30.0	105.2	6.7
12.17	6.00	0.70	6.52	0.25	7.13	61.8	40.3	29.4	102.9	1.1
14.17	6.11	0.64	6.33	0.15	7.09	57.2	39.4	31.7	100.0	2.2
16.17	6.80	0.68	6.81	0.08	7.81	51.9	36.8	29.9	106.0	0.0
18.17	5.92	0.59	6.02	0.18	7.29	48.1	36.1	31.4	95.9	4.5
20.17	5.67	0.59	5.38	0.21	6.81	43.5	34.4	31.9	93.0	1.1
22.17	5.55	0.57	5.16	0.15	6.81	39.9	33.5	31.2	89.5	5.0

BTX added neat to reactor at time zero.
sCOD samples were analyzed in duplicate (N = 2)
Other data from single analyses (N = 1).
** -- Duplicate sample lost, therefore standard deviations could not be computed.
ND -- Not Detected.

Table A.3. Toluene and *m*-Xylene Sole Carbon and Energy Source Experimental Data.

Sample	ave. toluene (mg/L)	ave. *m*-xylene (mg/L)	$\Phi^{\dagger}$, toluene	Φ, m-xylene	adj. toluene (mg/L)	adj. *m*-xylene (mg/L)	st. dev. toluene (mg/L)	st. dev. *m*-xylene (mg/L)	ave. protein (mg/L)	st. dev. protein (mg/L)
0	17.81	18.76	***	***	17.81	18.76	0.44	0.55	4.0	0.3
1	17.61	18.51	0.05	0.06	18.49	19.56	0.51	0.40	5.6	1.4
2	15.28	17.50	0.06	0.06	16.14	18.53	0.58	0.69	4.7	0.5
3	ND	14.32	-0.03	-0.03	ND	13.95	***	0.23	17.8	0.2
4	ND	13.51	0.09	0.10	ND	14.92	***	0.30	17.2	0.9
5	ND	11.67	0.12	0.13	ND	13.15	***	0.63	17.9	0.6
6	ND	11.28	0.03	0.03	ND	11.64	***	0.61	18.8	2.5
7	ND	0.63	0.08	0.08	ND	0.69	***	0.02	22.8	0.8

† F is the abiotic loss ratio computed from sterile controls.
All data from triplicate analyses (N = 3).
ND -- Not Detected.
*** -- Compound not detected, therefore standard deviations could not be computed.

Table A.4. BTX Response During *o*-Xylene Transformation Experiment on Day 472 (March 6, 1998).

	benzene (mg/L)		toluene (mg/L)		*p* -xylene (mg/L)		*m* -xylene (mg/L)		*o* -xylene (mg/L)	
Time (h)	average	st.dev.	average	st.dev.	average	st.dev.	average	st.dev.	average	st.dev.
0.0	1.37	0.11	0.58	0.13	0.76	0.16	0.18	0.11	0.73	0.12
1.0	4.01	0.18	1.82	0.11	3.88	0.21	2.67	0.17	4.20	0.32
2.0	4.37	0.11	0.59	0.05	4.64	0.16	2.25	0.09	4.31	0.17
3.0	4.41	0.13	0.15	0.00	4.82	0.15	1.24	0.00	3.93	0.13
4.0	4.40	0.10	ND	***	4.83	0.19	0.31	0.04	3.51	0.13
5.0	4.37	0.07	0.90	0.03	4.74	0.11	ND	***	3.08	0.07
6.0	4.32	0.07	0.17	0.01	4.64	0.13	ND	***	2.37	0.07
7.0	4.38	0.18	0.05	0.00	4.65	0.25	ND	***	1.86	0.38
8.0	4.20	0.05	ND	***	4.38	0.08	ND	***	1.68	0.00
9.0	4.18	0.09	0.41	0.03	4.22	0.11	ND	***	1.39	0.12
10.0	4.09	0.12	0.05	0.00	4.01	0.09	ND	***	0.81	0.00
11.0	3.98	0.00	ND	***	3.72	0.04	ND	***	0.55	0.03
12.0	4.02	**	ND	***	3.83	**	ND	***	0.57	**
22.8	3.27	0.05	ND	***	2.07	0.03	ND	***	0.07	0.00

All data from duplicate analyses (N = 2).
ND -- Not Detected.
** -- Duplicate sample lost, therefore standard deviations could not be computed.
*** -- Compounds not detected, therefore standard deviations could not be computed.

Table A.5. BTX Response During *o*-Xylene Transformation Experiment on Day 485 (March 19, 1998).

	benzene (mg/L)		toluene (mg/L)		*p* -xylene (mg/L)		*m* -xylene (mg/L)		*o* -xylene (mg/L)	
Time (h)	average	st.dev.	average	st.dev.	average	st.dev.	average	st.dev.	average	st.dev.
0.0	0.97	0.07	0.47	0.02	0.55	0.05	0.17	0.02	0.55	0.07
1.0	3.92	0.32	1.86	0.16	3.98	0.42	2.95	0.29	4.31	0.42
2.0	4.13	0.25	0.61	0.02	4.53	0.25	2.53	0.15	4.26	0.21
3.0	4.23	0.26	0.17	0.00	4.77	0.32	1.53	0.09	3.91	0.19
4.0	4.21	0.35	0.08	0.01	4.75	0.39	0.68	0.04	3.56	0.23
5.0	4.22	0.42	0.04	0.01	4.75	0.42	0.11	0.01	3.35	0.26
6.0	4.07	0.00	ND	***	4.54	0.07	ND	***	3.04	0.02
7.0	4.08	0.03	0.63	0.01	4.41	0.05	ND	***	2.54	0.06
8.0	3.98	0.07	0.13	0.02	4.15	0.08	ND	***	1.83	0.01
9.0	3.92	0.00	0.07	0.01	4.00	0.02	ND	***	1.48	0.02
10.0	3.91	0.12	0.04	0.01	3.93	0.11	ND	***	1.36	0.07
11.0	3.87	0.02	ND	***	3.80	0.00	ND	***	1.24	0.01
12.0	3.86	0.22	ND	***	3.74	0.18	ND	***	1.20	0.06
22.8	3.29	**	ND	***	2.01	**	ND	***	0.16	**

All data from duplicate analyses (N = 2).
ND -- Not Detected.
** -- Duplicate sample lost, therefore standard deviations could not be computed.
*** -- Compounds not detected, therefore standard deviations could not be computed.

Table A.7. Analysis of *o*-Xylene Transformation/Toluene Degradation Data from Day 472 (March 6, 1998).

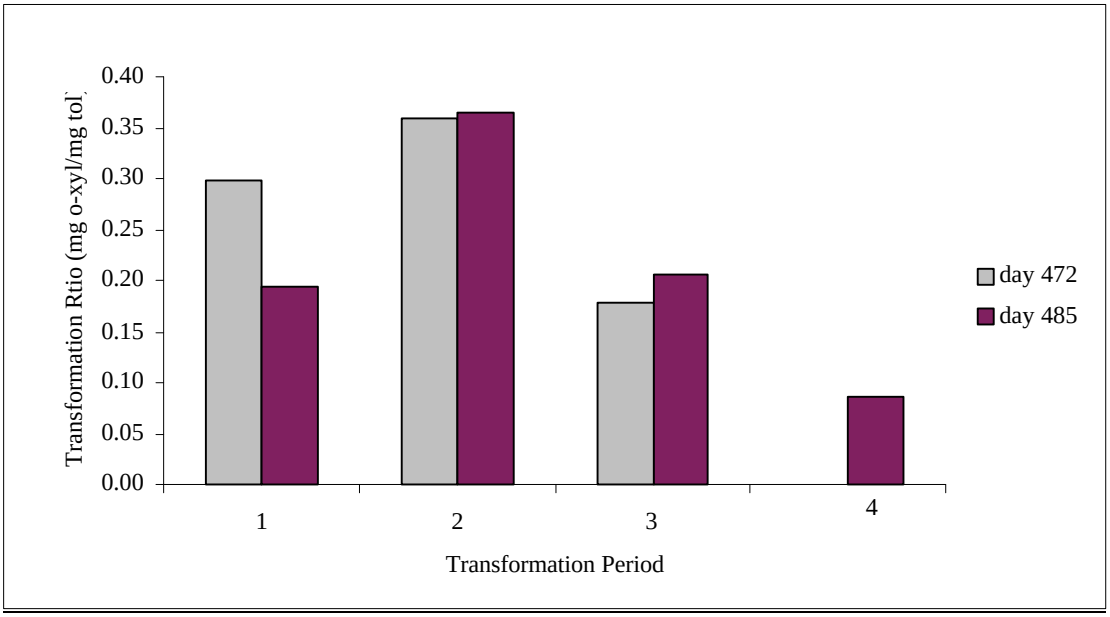
Reaction time (hr)	Period	*o* -xylene (mg/L)	Reactor volume (L)	*o* -xylene mass transformed (mg)	Toluene mass degraded (mg)	Transformation ratio (mg *o* -xyl/mg tol)
0		4.31	5.00			
6	1	3.04	4.64	7.4	25.0	0.30
12	2	1.20	4.28	9.0	25.0	0.36
final	3	0.16	4.22	4.5	25.0	0.18
Total *o* -xylene mass transformed; mg			20.9			
Total toluene mass degraded; mg			75.0			
Bulk ratio; mg *o* -xyl/mg tol			0.28			

Table A.8. Analysis of *o*-Xylene Transformation/Toluene Degradation Data from Day 485 (March 19, 1998).

Reaction time (hr)	Period	*o* -xylene (mg/L)	Reactor volume (L)	*o* -xylene mass transformed (mg)	Toluene mass degraded (mg)	Transformation ratio (mg *o* -xyl/mg tol)
0		4.31	5.00			
4	1	3.51	4.76	4.8	25.0	0.19
8	2	1.68	4.52	9.1	25.0	0.36
12	3	0.57	4.28	5.2	25.0	0.21
final	4	0.07	4.22	2.1	25.0	0.09
Total *o* -xylene mass transformed; mg			16.4			
Total toluene mass degraded; mg			100			
Bulk ratio; mg *o* -xyl/mg tol			0.16			

Table A.6. and A.7. Notes: The *o*-xylene concentration at time zero was taken as the maximum dissolved concentration between time zero and the first toluene spike. Additionally, the mass of toluene added was based on the total volume added, 28.9 µL. Headspace losses were neglected because toluene was completely degraded and reverse flux was possible. Initial *o*-xylene concentrations of 4.91 and 5.31 mg/L would equate period 1 and 2 transformation ratios in the experiments on days 472 and 485, respectively. These initial values could be possible in the system, but couldn't be precisely determined because of the dissolution process.

Figure A.1. Comparison of *o*-Xylene Transformation/Toluene Degradation Ratios Across the Reaction Cycle.



APPENDIX B:

SOURCE DATA FOR MANUSCRIPT 2

"STOICHIOMETRY AND KINETICS OF BTX DEGRADATION BY A DENITRIFYING ACTIVATED SLUDGE CONSORTIUM RECEIVING A MIXED WASTE"

Table B. 1. Nitrate-N, Nitrite-N, and Sulfate Data for Decay Experiment 1 on Days 324 to 331 (October 9 to 16, 1997).

Time (d)	nitrite-N (mg/L)	nitrate-N (mg/L)	sulfate (mg/L)	thOD NO_2-N (mg/L)	thOD NO_3-N (mg/L)	Σ NO_X-N (mg thOD/L)	Δ time (d)	-Δ thOD (mg/L)	-NUR (thOD/L/d)	ln (-NUR)
0.0	42.9	178	29.2	73.3	508	582	***	***	***	***
1.0	11.7	151	30.4	20.0	432	452	1.01	129.6	128.3	4.9
2.0	2.3	132	31.8	3.9	379	383	1.01	69.6	68.9	4.2
4.0	2.2	104	34.2	3.8	298	302	2.01	80.6	40.1	3.7
7.1	-1.5	85.5	36.8	-2.5	245	242	3.07	59.8	19.5	3.0

Table B. 2. Nitrate-N, Nitrite-N, and Sulfate Data for Decay Experiment 2 on Days 324 to 331 (October 9 to 16, 1997).

Time (d)	nitrite-N (mg/L)	nitrate-N (mg/L)	sulfate (mg/L)	thOD NO_2-N (mg/L)	thOD NO_3-N (mg/L)	Σ NO_X-N (mg thOD/L)	Δ time (d)	-Δ thOD (mg/L)	-NUR (thOD/L/d)	ln (-NUR)
0.0	42.6	177	28.8	72.9	507	580	***	***	***	***
1.0	12.9	143	30.6	22.0	409	431	1.01	149	147.2	4.99
2.0	2.7	115	32.5	4.5	330	335	1.01	96.4	95.4	4.56
4.0	5.0	97.7	35.4	8.5	279	288	2.01	46.6	23.2	3.14
7.1	9.4	89.2	47.2	16.1	255	271	3.07	16.7	5.46	1.70

Table B.3. Nitrate-N, Nitrite-N, Sulfate, Protein, and sCOD Data for Decay Experiment 3 on Days 428 to 435 (January 21 to 28, 1998).

Time (d)	nitrite-N (mg/L)	nitrate-N (mg/L)	sulfate (mg/L)	thOD NO_2-N (mg/L)	thOD NO_3-N (mg/L)	Σ NO_X-N (mg thOD/L)	Δ time (d)	-Δ thOD (mg/L)	-NUR (thOD/L/d)	ln (-NUR)	ave. protein (mg/L)	s.d. protein (mg/L)	ave. sCOD (mg/L)	s.d. sCOD (mg/L)
0.0	11.0	81.1	28.6	18.8	232	251	***	***	***	***	1382	72	43.9	3.3
1.0	1.5	49.5	27.0	2.5	142	144	1.00	107	107	4.67	1378	40	36.8	8.9
2.1	0.6	37.1	27.3	1.1	106	107	1.05	36.9	35.1	3.56	1345	123	38.4	6.7
2.9	0.5	28.2	28.2	0.9	80.7	81.6	0.90	25.6	28.5	3.35	1160	18	33.7	0.0
4.0	nd	20.0	28.1	ND	57.1	57.1	1.08	24.5	22.6	3.12	1287	118	32.9	1.1
5.0	0.5	14.9	27.3	0.8	42.6	43.4	0.94	13.7	14.6	2.68	1730	13	33.7	***
6.2	nd	4.6	27.9	ND	13.2	13.2	1.27	30.2	23.8	3.17	1659	43	36.1	1.1
7.1	nd	2.1	28.4	ND	6.0	6.0	0.85	7.2	8.4	2.13	1614	175	42.3	1.1

Table B.4. Biogenic True Growth Yield Data, Experiment 1, Day 395 (December 19, 1997).

Sample	ave. sCOD (mg/L)	st.dev. sCOD (mg/L)	ave. protein (mg/L)	st.dev. protein (mg/L)	ave. thOD protein (mg/L)	st.dev. thOD protein (mg/L)
1	601.0	2.8	22.4	0.3	41.8	0.5
2	561.7	2.8	23.5	0.5	43.9	0.9
3	530.3	0.0	30.1	0.4	56.1	0.7
4	468.2	1.1	35.1	2.3	65.4	4.3
5	437.6	2.2	42.0	0.2	78.2	0.4
6	394.4	3.3	49.2	3.6	91.7	6.6
7	362.9	1.1	74.4	12.5	138.6	23.3
8	232.5	1.1	97.3	3.4	181.2	6.4

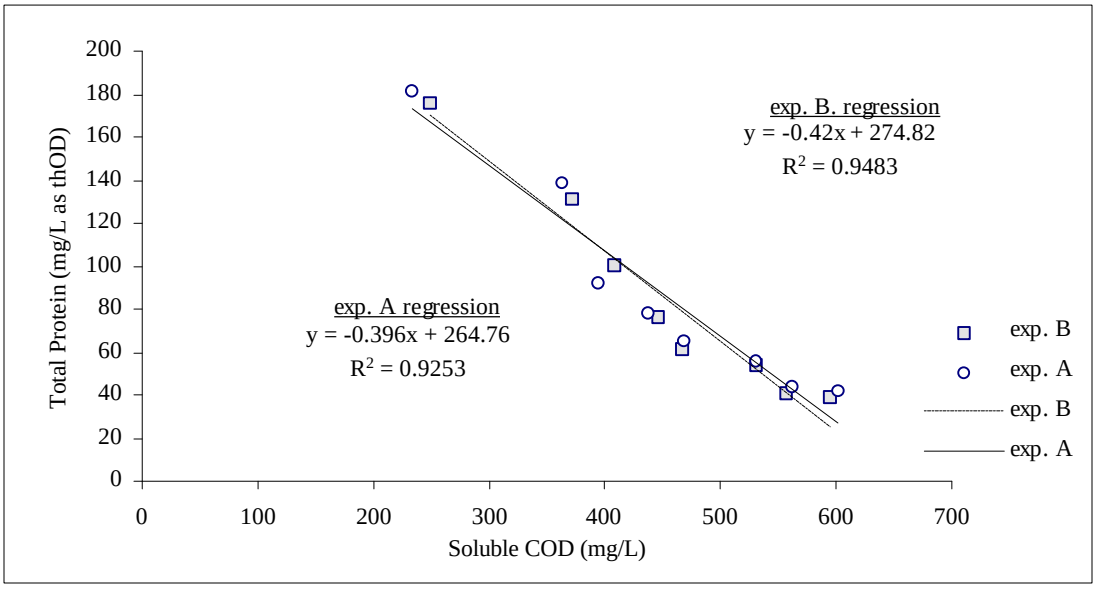
All samples analyzed in duplicate (N = 2).

Table B.5. Biogenic True Growth Yield Data, Experiment 2, Day 395 (December 19, 1997).

Sample	ave. sCOD (mg/L)	st.dev. sCOD (mg/L)	ave. protein (mg/L)	st.dev. protein (mg/L)	ave. thOD protein (mg/L)	st.dev. thOD protein (mg/L)
1	595.1	5.6	20.9	2.3	38.9	4.2
2	555.8	5.6	22.1	3.2	41.1	6.0
3	530.3	0.0	28.7	0.1	53.5	0.2
4	465.9	2.2	32.7	0.1	61.0	0.2
5	445.4	2.2	41.0	0.0	76.3	0.1
6	407.7	***	54.1	1.1	100.8	2.1
7	370.8	1.1	70.5	3.2	131.4	6.0
8	248.2	1.1	94.2	0.3	175.6	0.5

All samples analyzed in duplicate (N =2).
*** -- Duplicate sample was lost, therefore standard deviations could not be computed.

Figure B.1. Biogenic True Growth Yield Plots for Experiments A and B on Day 395 (December 19, 1997).



Tables B.6. Toluene and *m*-Xylene True Growth Yield Data for Experiment 1, Days 497 to 503 (March 31 to April 6, 1998).

sample	ave. tol. (mg/L)	ave. *m*-xyl. (mg/L)	Φ, toluene	Φ, *m*-xylene	adj. tol. (mg/L)	adj. *m*-xyl. (mg/L)	st. dev. tol. (mg/L)	st.dev. *m*-xyl. (mg/L)
0	17.8	18.8	***	***	17.8	18.8	0.4	0.5
1	17.6	18.5	0.051	0.057	18.5	19.6	0.5	0.4
2	15.3	17.5	0.057	0.059	16.1	18.5	0.6	0.7
3	ND	14.3	-0.030	-0.026	ND	14.0	***	0.2
4	ND	13.5	0.092	0.104	ND	14.9	***	0.3
5	ND	11.7	0.121	0.127	ND	13.1	***	0.6
6	ND	11.3	0.029	0.032	ND	11.6	***	0.6
7	ND	0.6	0.081	0.082	ND	0.7	***	0.0

sample	thOD, tol. (mg/L)	thOD, *m*-xyl. (mg/L)	st.dev. thOD tol. (mg/L)	st.dev. thOD *m*-xyl. (mg/L)	Σ thOD tol./*m*-xyl. (mg/L)	Σ thOD st. dev. (mg/L)	ave. protein (mg/L)	ave. thOD protein (mg/L)	st. dev. protein (mg/L)	st. dev. thOD protein
0	55.7	59.5	1.4	1.7	115.2	3.1	4.0	7.5	0.3	0.7
1	57.9	62.0	1.6	1.3	119.9	2.9	5.6	10.4	1.4	2.6
2	50.5	58.8	1.8	2.2	109.3	4.0	4.7	8.7	0.5	0.9
3	***	44.2	***	0.7	44.2	0.7	17.8	33.2	0.2	0.3
4	***	47.3	***	1.0	47.3	1.0	17.2	32.0	0.9	1.8
5	***	41.7	***	2.0	41.7	2.0	17.9	33.3	0.6	1.1
6	***	36.9	***	1.9	36.9	1.9	18.8	35.0	2.5	4.6
7	***	2.2	***	0.0	2.2	0.0	22.8	42.4	0.8	1.6

All samples analyzed in triplicate (N = 3); Φ is the abiotic loss ratio determined from sterile controls.
ND -- Not Detected
*** -- Compound not detected, therefore standard deviation could not be computed.

Tables B.7. Toluene and *m*-Xylene True Growth Yield Data for Experiment 2, Days 497 to 503 (March 31 to April 6, 1998).

sample	ave. tol. (mg/L)	ave. *m*-xyl. (mg/L)	Φ, toluene	Φ, *m*-xylene	adj. tol. (mg/L)	adj. *m*-xyl. (mg/L)	st. dev. tol. (mg/L)	st.dev. *m*-xyl. (mg/L)
0	17.6	18.9	***	***	17.6	18.9	0.2	0.2
1	17.7	18.8	0.051	0.057	18.5	19.9	0.6	0.7
2	14.6	17.2	0.057	0.059	15.5	18.3	0.6	0.7
3	***	13.9	-0.030	-0.026	***	13.5	***	0.4
4	***	12.7	0.092	0.104	***	14.0	***	0.3
5	***	11.2	0.121	0.127	***	12.6	***	0.1
6	***	10.4	0.029	0.032	***	10.7	***	0.3
7	***	***	0.081	0.082	***	***	***	***

sample	thOD, tol. (mg/L)	thOD, *m*-xyl. (mg/L)	st.dev. thOD tol. (mg/L)	st.dev. thOD *m*-xyl. (mg/L)	Σ thOD tol./*m*-xyl. (mg/L)	Σ thOD st.dev. (mg/L)	ave. protein (mg/L)	ave. thOD protein (mg/L)	st. dev. protein (mg/L)	st.dev. thOD protein
0	55.1	59.8	0.7	0.7	114.9	1.4	5.3	9.9	0.1	0.1
1	58.1	63.0	1.9	2.1	121.1	4.0	5.2	9.7	1.4	2.6
2	48.4	57.9	2.0	2.3	106.3	4.3	5.0	9.2	1.1	2.0
3	***	42.8	***	1.3	42.8	1.3	20.1	37.4	3.6	6.6
4	***	44.5	***	0.8	44.5	0.8	22.5	42.0	0.9	1.6
5	***	39.9	***	0.2	39.9	0.2	15.5	28.9	2.1	3.9
6	***	34.0	***	1.1	34.0	1.1	18.0	33.5	1.3	2.3
7	***	***	***	***	0.0	0.0	30.7	57.3	3.9	7.3

All samples analyzed in triplicate (N = 3); Φ is the abiotic loss ratio determined from sterile controls.
*** -- Compound not detected, therefore standard deviation could not be computed.

Table B.8. Toluene and m-Xylene True Growth Yield Data for Days 451 to 456 (February 13 to 18, 1998).

sample	ave. tol. (mg/L)	ave. *m*-xyl. (mg/L)	Φ, toluene	Φ, *m*-xylene	adj. tol. (mg/L)	adj. *m*-xyl. (mg/L)	st. dev. tol. (mg/L)	st. dev. *m*-xyl. (mg/L)
0	18.0	19.0	***	***	18.0	19.0	0.1	0.2
1	16.6	17.5	0.08	0.08	17.9	18.9	0.4	0.4
2	16.0	16.8	0.03	0.04	16.6	17.4	0.3	0.3
3	15.2	15.9	0.10	0.11	16.8	17.6	0.5	0.6
4	13.6	14.2	0.10	0.10	14.9	15.7	0.3	0.3
5	9.33	12.1	0.10	0.10	10.2	13.3	0.3	0.4
6	ND	6.33	0.17	0.18	ND	7.5	***	0.1

sample	thOD, tol. (mg/L)	thOD, *m*-xyl. (mg/L)	st. dev. thOD tol. (mg/L)	st. dev. thOD *m*-xyl. (mg/L)	Σ thOD tol./*m*-xyl. (mg/L)	Σ thOD st.dev. (mg/L)	ave. protein (mg/L)	ave. thOD protein (mg/L)	st. dev. protein (mg/L)	st. dev. thOD protein
0	56.3	60.3	0.5	0.5	115.2	0.9	6.9		0.8	1.6
1	56.0	59.9	1.3	1.4	119.9	2.7	8.2		0.4	0.8
2	51.9	55.1	0.8	0.9	109.3	1.7	7.8	14.6	0.8	1.5
3	52.5	55.8	1.7	1.9	44.2	3.6	8.0	14.9	0.1	0.3
4	46.5	49.8	0.8	0.9	47.3	1.7	7.0	13.0	0.5	0.9
5	32.0	42.2	1.0	1.3	41.7	2.3	8.8	16.5	0.6	1.1
6	***	23.8	***	0.3	36.9	0.3	19.6	36.5	0.4	0.7

All samples analyzed in triplicate (N = 3); Φ is the abiotic loss ratio determined from sterile controls.
ND -- Not Detected
*** -- Compound not detected, therefore standard deviation could not be computed.

Figure B.2. Toluene and m-Xylene True Growth Yield Experimental Data.

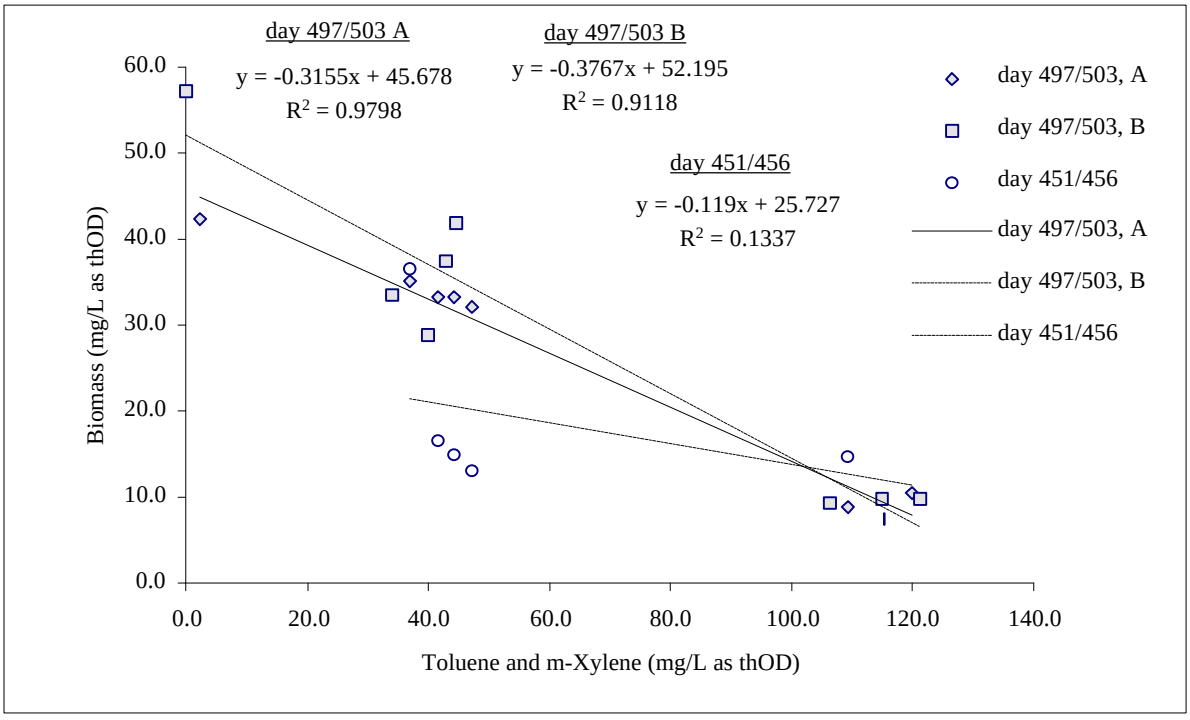


Table B.9. Toluene Kinetic Evaluation of Day 280 (August 26, 1997) Batch Profile Data Set. Nonlinear Parameter Estimation for q_{max} and K_S of Monod Model using Microsoft Excel® Solver (version 5.0) Algorithm.

Input	X_A	79.6	Estimated active, toluene-degrading fraction of biomass; mg protein/L
Constants	S_0	2.88	Initial substrate concentration after lag; mg/L
	Y_{XENO}	0.31	True growth yield for toluene and *m* -xylene; COD/COD
Output	q_{max}	0.139	Maximum specific uptake rate; mg toluene/mg protein/h
Values	K_S	6.92	Monod half saturation constant; mg/L
	μ_{max}	0.251	Maximum specific growth rate; h^{-1}, COD basis
Fit	RSS	0.034	Regression Sum of Squares
Statistics	MSE	0.0012	Mean Square Error

Batch Sample	Sample Time (h)	Time after lag (h)	Tol. obs. (mg/L)	LHS of Monod	RHS of Monod	LHS-RHS	Tol. pred. (mg/L)	Residuals (mg/L)	Residuals squared
0	0.00	***	4.09	***	***	***	***	***	***
1	0.17	***	3.77	***	***	***	***	***	***
2	0.33	***	3.46	***	***	***	***	***	***
3	0.50	***	3.13	***	***	***	***	***	***
4	0.67	0.00	2.88	10.20	10.20	0.00	2.88	-8.2E-14	6.7E-27
5	0.83	0.17	2.40	8.36	8.36	0.00	2.37	3.0E-02	8.9E-04
6	1.00	0.33	2.00	6.52	6.52	0.00	1.94	6.1E-02	3.7E-03
7	1.08	0.42	1.81	5.60	5.60	0.00	1.74	6.2E-02	3.8E-03
8	1.17	0.50	1.60	4.68	4.68	0.00	1.57	3.6E-02	1.3E-03
9	1.25	0.58	1.32	3.76	3.76	0.00	1.40	-8.0E-02	6.5E-03
10	1.33	0.67	1.25	2.84	2.84	0.00	1.26	-9.0E-03	8.1E-05
11	1.42	0.75	1.10	1.91	1.91	0.00	1.12	-2.5E-02	6.0E-04
12	1.50	0.83	0.97	0.99	0.99	0.00	1.00	-2.6E-02	7.0E-04
13	1.58	0.92	0.88	0.07	0.07	0.00	0.89	-4.8E-03	2.3E-05
14	1.67	1.00	0.79	-0.85	-0.85	0.00	0.79	-2.8E-03	8.0E-06
15	1.75	1.08	0.64	-1.77	-1.77	0.00	0.70	-5.5E-02	3.1E-03
16	1.83	1.17	0.59	-2.69	-2.69	0.00	0.62	-3.3E-02	1.1E-03
17	1.92	1.25	0.54	-3.61	-3.61	0.00	0.55	-8.1E-03	6.6E-05
18	2.00	1.33	0.46	-4.53	-4.53	0.00	0.48	-2.7E-02	7.2E-04
19	2.08	1.42	0.46	-5.45	-5.45	0.00	0.43	3.6E-02	1.3E-03
20	2.17	1.50	0.37	-6.37	-6.37	0.00	0.38	-1.1E-02	1.1E-04
21	2.25	1.58	0.41	-7.29	-7.29	0.00	0.33	7.3E-02	5.3E-03
22	2.33	1.67	0.31	-8.21	-8.21	0.00	0.29	1.5E-02	2.4E-04
23	2.42	1.75	0.29	-9.13	-9.13	0.00	0.26	3.3E-02	1.1E-03
24	2.50	1.83	0.24	-10.05	-10.05	0.00	0.23	1.1E-02	1.1E-04
25	2.58	1.92	0.21	-10.97	-10.97	0.00	0.20	1.6E-02	2.5E-04
26	2.67	2.00	0.18	-11.89	-11.89	0.00	0.18	9.2E-03	8.4E-05
27	2.75	2.08	0.16	-12.81	-12.81	0.00	0.15	9.0E-03	8.2E-05
28	2.83	2.17	0.15	-13.73	-13.73	0.00	0.13	1.4E-02	1.9E-04
29	2.92	2.25	0.11	-14.65	-14.65	0.00	0.12	-5.1E-03	2.6E-05
30	3.00	2.33	0.08	-15.57	-15.57	0.00	0.10	-2.6E-02	6.8E-04
31	3.17	2.50	0.10	-17.41	-17.41	0.00	0.08	1.6E-02	2.5E-04
32	3.33	2.67	0.03	-19.25	-19.25	0.00	0.06	-2.9E-02	8.7E-04
33	3.50	2.83	0.07	-21.10	-21.10	0.00	0.05	2.2E-02	4.8E-04

Table B.10. *m*-Xylene Kinetic Evaluation of Day 280 (August 26, 1997) Batch Profile Data Set. Nonlinear Parameter Estimation for q_{max} and K_S of Monod Model using Microsoft Excel® Solver (version 5.0) Algorithm.

Input	X_A	79.6	Estimated active, *m*-xylene-degrading fraction of biomass; mg protein/L
Constants	S_0	3.70	Initial substrate concentration after lag; mg/L
	Y_{XENO}	0.31	True growth yield for toluene and *m*-xylene; COD/COD
Output	q_{max}	0.0346	Maximum specific uptake rate; mg *m*-xylene/mg protein/h
Values	K_S	1.723	Monod half saturation constant; mg/L
	μ_{max}	0.0625	Maximum specific growth rate; h^{-1}, COD basis
Fit	RSS	0.0720	Regression Sum of Squares
Statistics	MSE	0.0023	Mean Square Error

Batch Sample	Sample Time (h)	Time after lag (h)	*m*-Xyl $_{obs.}$ (mg/L)	LHS of Monod	RHS of Monod	LHS-RHS	*m*-Xyl $_{pred.}$ (mg/L)	Residuals (mg/L)	Residuals squared
0	0.00	***	4.40	***	***	***	***	***	***
1	0.17	***	4.13	***	***	***	***	***	***
2	0.33	***	3.99	***	***	***	***	***	***
3	0.50	***	3.79	***	***	***	***	***	***
4	0.67	0.00	3.70	5.95	5.95	0.00	3.70	0.0E+00	0.0E+00
5	0.83	0.17	3.39	5.49	5.49	0.00	3.39	-9.7E-04	9.4E-07
6	1.00	0.33	3.19	5.03	5.03	0.00	3.09	1.0E-01	1.1E-02
7	1.08	0.42	3.04	4.80	4.80	0.00	2.94	9.9E-02	9.9E-03
8	1.17	0.50	2.89	4.57	4.57	0.00	2.80	8.9E-02	7.9E-03
9	1.25	0.58	2.60	4.35	4.35	0.00	2.66	-5.7E-02	3.3E-03
10	1.33	0.67	2.52	4.12	4.12	0.00	2.52	-1.6E-03	2.7E-06
11	1.42	0.75	2.45	3.89	3.89	0.00	2.39	6.4E-02	4.2E-03
12	1.50	0.83	2.24	3.66	3.66	0.00	2.26	-1.6E-02	2.6E-04
13	1.58	0.92	2.15	3.43	3.43	0.00	2.13	2.0E-02	3.8E-04
14	1.67	1.00	2.03	3.20	3.20	0.00	2.00	2.5E-02	6.3E-04
15	1.75	1.08	1.78	2.97	2.97	0.00	1.88	-1.1E-01	1.1E-02
16	1.83	1.17	1.71	2.74	2.74	0.00	1.76	-5.5E-02	3.0E-03
17	1.92	1.25	1.62	2.51	2.51	0.00	1.65	-3.3E-02	1.1E-03
18	2.00	1.33	1.45	2.28	2.28	0.00	1.54	-8.4E-02	7.1E-03
19	2.08	1.42	1.45	2.05	2.05	0.00	1.43	2.0E-02	4.0E-04
20	2.17	1.50	1.26	1.82	1.82	0.00	1.33	-7.0E-02	5.0E-03
21	2.25	1.58	1.26	1.59	1.59	0.00	1.23	2.6E-02	6.7E-04
22	2.33	1.67	1.18	1.36	1.36	0.00	1.14	4.0E-02	1.6E-03
23	2.42	1.75	1.09	1.14	1.14	0.00	1.05	3.6E-02	1.3E-03
24	2.50	1.83	0.99	0.91	0.91	0.00	0.97	2.8E-02	7.7E-04
25	2.58	1.92	0.89	0.68	0.68	0.00	0.89	7.5E-03	5.7E-05
26	2.67	2.00	0.81	0.45	0.45	0.00	0.81	-2.1E-03	4.4E-06
27	2.75	2.08	0.76	0.22	0.22	0.00	0.74	2.1E-02	4.2E-04
28	2.83	2.17	0.68	-0.01	-0.01	0.00	0.67	3.0E-03	9.1E-06
29	2.92	2.25	0.61	-0.24	-0.24	0.00	0.61	-1.1E-03	1.2E-06
30	3.00	2.33	0.52	-0.47	-0.47	0.00	0.55	-3.6E-02	1.3E-03
31	3.17	2.50	0.46	-0.93	-0.93	0.00	0.45	1.4E-02	1.9E-04
32	3.33	2.67	0.36	-1.39	-1.39	0.00	0.36	4.7E-05	2.2E-09
33	3.50	2.83	0.31	-1.85	-1.85	0.00	0.29	2.1E-02	4.6E-04
34	3.67	3.00	0.24	-2.30	-2.30	0.00	0.23	8.2E-03	6.7E-05
35	3.83	3.17	0.17	-2.76	-2.76	0.00	0.18	-9.4E-03	8.9E-05
36	4.00	3.33	0.15	-3.22	-3.22	0.00	0.14	3.2E-03	1.0E-05

Table B.11. Toluene Kinetic Evaluation of Day 416 (January 9, 1998) Batch Profile Data Set. Nonlinear Parameter Estimation for q_{max} and K_S of Monod Model using Microsoft Excel® Solver (version 5.0) Algorithm.

Input	X_A	65.8	Estimated active, toluene-degrading fraction of biomass; mg protein/L
Constants	S_0	3.87	Initial substrate concentration after lag; mg/L
	Y_{XENO}	0.31	True growth yield for toluene and *m* -xylene; COD/COD
Output	q_{max}	0.0591	Maximum specific uptake rate; mg toluene/mg protein/h
Values	K_S	2.49	Monod half saturation constant; mg/L
	μ_{max}	0.107	Maximum specific growth rate; h^{-1}, COD basis
Fit	RSS	0.40	Regression Sum of Squares
Statistics	MSE	0.020	Mean Square Error

Batch Sample	Sample Time (h)	Time after lag (h)	Tol. $_{obs.}$ (mg/L)	LHS of Monod	RHS of Monod	LHS-RHS	Tol. $_{pred.}$ (mg/L)	Residuals (mg/L)	Residuals squared
0	0.00	***	4.78	***	***	***	***	***	***
1	0.17	***	4.39	***	***	***	***	***	***
2	0.33	***	4.39	***	***	***	***	***	***
3	0.50	0.00	3.87	7.24	7.24	0.00	3.87	0.0E+00	0.0E+00
4	0.67	0.17	3.60	6.60	6.60	0.00	3.49	1.1E-01	1.2E-02
5	0.83	0.33	3.41	5.95	5.95	0.00	3.12	2.9E-01	8.4E-02
6	1.00	0.50	2.97	5.30	5.30	0.00	2.77	2.0E-01	4.0E-02
7	1.17	0.67	2.47	4.65	4.65	0.00	2.44	2.9E-02	8.4E-04
8	1.33	0.83	2.37	4.01	4.01	0.00	2.13	2.4E-01	6.0E-02
9	1.50	1.00	1.75	3.36	3.36	0.00	1.84	-8.8E-02	7.7E-03
10	1.67	1.17	1.51	2.71	2.71	0.00	1.58	-6.2E-02	3.8E-03
11	1.83	1.33	1.12	2.06	2.06	0.00	1.34	-2.1E-01	4.5E-02
12	2.00	1.50	1.06	1.41	1.41	0.00	1.12	-6.1E-02	3.7E-03
13	2.17	1.67	0.76	0.77	0.77	0.00	0.93	-1.7E-01	2.9E-02
14	2.33	1.83	0.63	0.12	0.12	0.00	0.77	-1.4E-01	1.9E-02
15	2.50	2.00	0.54	-0.53	-0.53	0.00	0.63	-8.9E-02	7.9E-03
16	2.67	2.17	0.50	-1.18	-1.18	0.00	0.51	-1.3E-02	1.6E-04
17	2.83	2.33	0.50	-1.83	-1.83	0.00	0.41	8.8E-02	7.8E-03
18	3.00	2.50	0.38	-2.47	-2.47	0.00	0.32	5.8E-02	3.4E-03
19	3.17	2.67	0.32	-3.12	-3.12	0.00	0.26	6.0E-02	3.5E-03
20	3.33	2.83	0.30	-3.77	-3.77	0.00	0.20	9.6E-02	9.3E-03
21	3.50	3.00	0.28	-4.42	-4.42	0.00	0.16	1.2E-01	1.5E-02
22	3.67	3.17	0.25	-5.07	-5.07	0.00	0.12	1.3E-01	1.7E-02
23	3.83	3.33	0.22	-5.71	-5.71	0.00	0.10	1.3E-01	1.6E-02
24	4.00	3.50	0.19	-6.36	-6.36	0.00	0.08	1.1E-01	1.3E-02

Table B.12. *m*-Xylene Kinetic Evaluation of Day 416 (January 9, 1998) Batch Profile Data Set. Nonlinear Parameter Estimation for q_{max} and K_S of Monod Model using Microsoft Excel® Solver (version 5.0) Algorithm.

Input	X_A	65.8	Estimated active, *m*-xylene-degrading fraction of biomass; mg protein/L
Constants	S_0	2.92	Initial substrate concentration after lag; mg/L
	Y_{XENO}	0.31	True growth yield for toluene and *m*-xylene; COD/COD
Output	q_{max}	0.0409	Maximum specific uptake rate; mg *m*-xylene/mg protein/h
Values	K_S	3.69	Monod half saturation constant; mg/L
	μ_{max}	0.0748	Maximum specific growth rate; h^{-1}, COD basis
Fit	RSS	0.19	Regression Sum of Squares
Statistics	MSE	0.0097	Mean Square Error

Batch Sample	Sample Time (h)	Time after lag (h)	*m*-Xyl $_{obs.}$ (mg/L)	LHS of Monod	RHS of Monod	LHS-RHS	*m*-Xyl $_{pred.}$ (mg/L)	Residuals (mg/L)	Residuals squared
0	0.00	***	3.25	***	***	***	***	***	***
1	0.17	***	3.04	***	***	***	***	***	***
2	0.33	***	3.18	***	***	***	***	***	***
3	0.50	0.00	2.92	6.86	6.86	0.00	2.92	0.0E+00	0.0E+00
4	0.67	0.17	2.71	6.42	6.42	0.00	2.72	-1.2E-02	1.5E-04
5	0.83	0.33	2.67	5.97	5.97	0.00	2.54	1.4E-01	1.9E-02
6	1.00	0.50	2.46	5.52	5.52	0.00	2.36	1.0E-01	1.1E-02
7	1.17	0.67	2.18	5.07	5.07	0.00	2.19	-2.6E-03	6.6E-06
8	1.33	0.83	2.32	4.62	4.62	0.00	2.02	2.9E-01	8.7E-02
9	1.50	1.00	1.83	4.18	4.18	0.00	1.87	-3.7E-02	1.3E-03
10	1.67	1.17	1.75	3.73	3.73	0.00	1.72	2.4E-02	5.6E-04
11	1.83	1.33	1.50	3.28	3.28	0.00	1.58	-8.5E-02	7.3E-03
12	2.00	1.50	1.47	2.83	2.83	0.00	1.45	1.5E-02	2.3E-04
13	2.17	1.67	1.18	2.39	2.39	0.00	1.33	-1.5E-01	2.2E-02
14	2.33	1.83	1.10	1.94	1.94	0.00	1.22	-1.1E-01	1.3E-02
15	2.50	2.00	1.01	1.49	1.49	0.00	1.11	-1.0E-01	1.0E-02
16	2.67	2.17	0.94	1.04	1.04	0.00	1.01	-6.5E-02	4.2E-03
17	2.83	2.33	0.96	0.59	0.59	0.00	0.92	4.7E-02	2.2E-03
18	3.00	2.50	0.79	0.15	0.15	0.00	0.83	-3.6E-02	1.3E-03
19	3.17	2.67	0.74	-0.30	-0.30	0.00	0.75	-1.6E-02	2.7E-04
20	3.33	2.83	0.73	-0.75	-0.75	0.00	0.68	4.8E-02	2.3E-03
21	3.50	3.00	0.62	-1.20	-1.20	0.00	0.61	9.9E-03	9.8E-05
22	3.67	3.17	0.61	-1.65	-1.65	0.00	0.55	6.2E-02	3.8E-03
23	3.83	3.33	0.57	-2.09	-2.09	0.00	0.50	7.0E-02	4.9E-03
24	4.00	3.50	0.51	-2.54	-2.54	0.00	0.45	6.4E-02	4.1E-03

Table B.13. Toluene Kinetic Evaluation of Day 466 (February 28, 1998) Batch Profile Data Set. Nonlinear Parameter Estimation for q_{max} and K_S of Monod Model using Microsoft Excel® Solver (version 5.0) Algorithm.

Input	X_A	68.4	Estimated active, toluene-degrading fraction of biomass; mg protein/L
Constants	S_0	2.77	Initial substrate concentration after lag; mg/L
	Y_{XENO}	0.31	True growth yield for toluene and *m* -xylene; COD/COD
Output	q_{max}	0.0694	Maximum specific uptake rate; mg toluene/mg protein/h
Values	K_S	0.839	Monod half saturation constant; mg/L
	μ_{max}	0.126	Maximum specific growth rate; h^{-1}, COD basis
Fit	RSS	0.019	Regression Sum of Squares
Statistics	MSE	0.0024	Mean Square Error

Batch Sample	Sample Time (h)	Time after lag (h)	Tol. $_{obs.}$ (mg/L)	LHS of Monod	RHS of Monod	LHS-RHS	Tol. $_{pred.}$ (mg/L)	Residuals (mg/L)	Residuals squared
0	0.00	0.00	3.82	***	***	***	***	***	***
1	0.17	0.17	3.42	***	***	***	***	***	***
2	0.33	0.33	3.11	***	***	***	***	***	***
3	0.52	0.00	2.77	3.62	3.62	0.00	2.77	0.0E+00	0.0E+00
4	0.67	0.17	2.28	2.83	2.83	0.00	2.18	1.0E-01	1.1E-02
5	0.83	0.33	1.67	2.04	2.04	0.00	1.63	3.9E-02	1.5E-03
6	1.00	0.52	1.04	1.17	1.17	0.00	1.09	-5.1E-02	2.6E-03
7	1.17	0.67	0.67	0.46	0.46	0.00	0.73	-5.3E-02	2.8E-03
8	1.33	0.83	0.41	-0.33	-0.33	0.00	0.41	3.0E-03	8.8E-06
9	1.50	1.00	0.23	-1.13	-1.13	0.00	0.20	2.8E-02	7.9E-04
10	1.67	1.17	0.12	-1.92	-1.92	0.00	0.09	3.1E-02	9.9E-04
11	1.83	1.33	0.04	-2.71	-2.71	0.00	0.04	7.0E-03	4.9E-05
12	2.00	1.50	0.02	-3.50	-3.50	0.00	0.02	3.8E-03	1.5E-05

Table B.14. *m*-Xylene Kinetic Evaluation of Day 466 (February 28, 1998) Batch Profile Data Set. Nonlinear Parameter Estimation for q_{max} and K_S of Monod Model using Microsoft Excel® Solver (version 5.0) Algorithm.

Input Values	X_A	68.4	Estimated active, *m*-xylene-degrading fraction of biomass; mg protein/L
	S_0	3.40	Initial substrate concentration after lag; mg/L
	Y_{XENO}	0.31	True growth yield for toluene and *m*-xylene; COD/COD
Output Values	q_{max}	0.0336	Maximum specific uptake rate; mg *m*-xylene/mg protein/h
	K_S	0.281	Monod half saturation constant; mg/L
	μ_{max}	0.0615	Maximum specific growth rate; h^{-1}, COD basis
Fit Statistics	RSS	0.0046	Regression Sum of Squares
	MSE	0.0004	Mean Square Error

Batch Sample	Sample Time (h)	Time after lag (h)	*m*-Xyl $_{obs.}$ (mg/L)	LHS of Monod	RHS of Monod	LHS-RHS	*m*-Xyl $_{pred.}$ (mg/L)	Residuals (mg/L)	Residuals squared
0	*0.00*	*0.00*	*4.22*	***	***	***	***	***	***
1	*0.17*	*0.17*	*3.94*	***	***	***	***	***	***
2	*0.33*	*0.33*	*3.84*	***	***	***	***	***	***
3	*0.50*	*0.00*	*3.63*	***	***	***	***	***	***
4	0.67	0.00	3.40	3.74	3.74	0.00	3.40	1.6E-08	2.4E-16
5	0.83	0.17	3.09	3.36	3.36	0.00	3.05	4.4E-02	1.9E-03
6	1.00	0.33	2.70	2.98	2.98	0.00	2.70	-1.5E-03	2.3E-06
7	1.17	0.52	2.36	2.56	2.56	0.00	2.32	3.4E-02	1.2E-03
8	1.33	0.67	2.02	2.21	2.21	0.00	2.02	7.1E-03	5.0E-05
9	1.50	0.83	1.68	1.83	1.83	0.00	1.68	-1.2E-03	1.4E-06
10	1.67	1.00	1.36	1.45	1.45	0.00	1.36	-1.1E-03	1.2E-06
11	1.83	1.17	1.03	1.06	1.06	0.00	1.05	-2.3E-02	5.1E-04
12	2.00	1.33	0.73	0.68	0.68	0.00	0.76	-2.6E-02	6.8E-04
13	2.17	1.50	0.51	0.30	0.30	0.00	0.50	9.4E-03	8.9E-05
14	2.33	1.67	0.29	-0.08	-0.08	0.00	0.28	1.0E-02	1.1E-04
15	2.50	1.83	0.13	-0.47	-0.47	0.00	0.12	3.0E-03	9.1E-06
16	2.67	2.00	0.05	-0.85	-0.85	0.00	0.04	8.5E-03	7.3E-05

APPENDIX C:

ANALYTICAL STANDARDS AND CORRELATIONS

Table C.1. Typical BTX Standard Concentrations Gravimetrically Prepared in Hexane.

BTX standard	Benzene (mg/L)	Toluene (mg/L)	*p*-Xylene (mg/L)	*m*-Xylene (mg/L)	*o*-Xylene (mg/L)
blank	0	0	0	0	0
6	0.65	0.84	0.85	0.89	0.85
30	3.23	4.21	4.23	4.43	4.26
50	5.39	7.01	7.05	7.38	7.10

Table C.2. Typical Standard Response to GC/FID Analysis (Peak Area and Peak Height).

	Integrated peak areas (mV*s)					Measured peak heights (64ths of an inch)				
BTX standard	Benz.	Tol.	*p*-Xyl.	*m*-Xyl.	*o*-Xyl.	Benz.	Tol.	*p*-Xyl.	*m*-Xyl.	*o*-Xyl.
blank	0	0	0	0	0	0.00	0.00	0.00	0.00	0.00
6	983	1165	1202	1262	1189	12.75	12.25	8.50	8.50	7.00
30	4368	5679	5780	6120	5830	56.25	57.00	40.75	41.25	32.25
50	6845	8760	8783	9250	8738	101.25	98.50	68.50	69.25	53.00

Table C.3. Typical Regression Output for Benzene Standards (peak area).

Regression Statistics--benzene	
Multiple R	0.999
R Square	0.998
Adjusted R Square	0.997
Standard Error	0.126
Observations	4

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	18.4	18.4	1161	0.000860
Residual	2	0.0317	0.0159		
Total	3	18.4			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Y intercept	-0.083	0.094	-0.876	0.474	-0.489	0.324	-0.489	0.324
benzene area	0.000787	2.31E-05	34.1	8.60E-04	0.000688	0.000886	0.000688	0.000886

Table C.4. Typical Regression Output for Toluene Standards (peak area).

Regression Statistics--toluene	
Multiple R	0.999
R Square	0.998
Adjusted R Square	0.997
Standard Error	0.190
Observations	4

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	31.1	31.1	858	0.00116
Residual	2	0.0726	0.0363		
Total	3	31.2			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Y intercept	-0.0791	0.142	-0.556	0.634	-0.691	0.533	-0.691	0.533
toluene area	0.000793	2.71E-05	29.3	0.00116	0.000677	0.000910	0.000677	0.000910

Table C.5. Typical Regression Output for *p*-Xylene Standards (peak area).

Regression Statistics--p-xylene	
Multiple R	0.998
R Square	0.997
Adjusted R Square	0.995
Standard Error	0.233
Observations	4

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	31.4	31.4	580	0.00172
Residual	2	0.108	0.0542		
Total	3	31.5			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Y intercept	-0.0978	0.175	-0.560	0.632	-0.849	0.653	-0.849	0.653
p -xylene area	0.000794	3.30E-05	24.1	0.00172	0.000652	0.000936	0.000652	0.000936

Table C.6. Typical Regression Output for *m*-Xylene Standards (peak area).

Regression Statistics--m-xylene	
Multiple R	0.998
R Square	0.996
Adjusted R Square	0.994
Standard Error	0.257
Observations	4

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	34.4	34.4	521	0.00191
Residual	2	0.132	0.0661		
Total	3	34.6			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Y intercept	-0.103	0.193	-0.534	0.647	-0.932	0.726	-0.932	0.726
m -xylene area	0.000788	3.45E-05	22.8	0.00191	0.000640	0.000937	0.000640	0.000937

Table C.7. Typical Regression Output for *o*-Xylene Standards (peak area).

Regression Statistics--o-xylene	
Multiple R	0.998
R Square	0.995
Adjusted R Square	0.993
Standard Error	0.271
Observations	4

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	31.9	31.9	435	0.00229
Residual	2	0.146	0.0732		
Total	3	32.0			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Y intercept	-0.101	0.203	-0.499	0.667	-0.974	0.772	-0.974	0.772
o -xylene area	0.000801	3.84E-05	20.9	0.00229	0.000636	0.000966	0.000636	0.000966

Table C.8. Typical Regression Output for Benzene Standards (peak height).

Regression Statistics--benzene	
Multiple R	0.999
R Square	0.998
Adjusted R Square	0.996
Standard Error	0.147
Observations	4

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	18.4	18.4	848	0.00118
Residual	2	0.0434	0.0217		
Total	3	18.4			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Y intercept	0.0229	0.108	0.213	0.851	-0.441	0.487	-0.441	0.487
benzene height	0.0539	0.00185	29.1	0.00118	0.0459	0.0619	0.0459	0.0619

Table C.9. Typical Regression Output for Toluene Standards (peak height).

Regression Statistics--toluene	
Multiple R	1.000
R Square	0.999
Adjusted R Square	0.999
Standard Error	0.0975
Observations	4

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	31.2	31.2	3279	0.000305
Residual	2	0.0190	0.00951		
Total	3	31.2			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Y intercept	0.00836	0.0717	0.117	0.918	-0.300	0.317	-0.300	0.317
toluene height	0.0717	0.00125	57.3	0.000305	0.0663	0.0771	0.0663	0.0771

Table C.10. Typical Regression Output for *p*-Xylene Standards (peak height).

Regression Statistics--p-xylene	
Multiple R	1.00
R Square	1.00
Adjusted R Square	1.00
Standard Error	0.0294
Observations	4

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	31.5	31.5	36609	2.73E-05
Residual	2	0.00172	0.000862		
Total	3	31.5			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Y intercept	-0.00780	0.0216	-0.361	0.753	-0.101	0.0853	-0.101	0.0853
p -xylene height	0.103	0.000540	191	2.73E-05	0.101	0.106	0.101	0.106

Table C.11. Typical Regression Output for *m*-Xylene Standards (peak height).

Regression Statistics--m-xylene	
Multiple R	1.00
R Square	1.00
Adjusted R Square	1.00
Standard Error	0.0253
Observations	4

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	34.6	34.6	54015	1.85E-05
Residual	2	0.00128	0.000640		
Total	3	34.6			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Y intercept	-0.00445	0.0186	-0.239	0.834	-0.0846	0.0757	-0.0846	0.0757
m -xylene height	0.107	0.000460	232	1.85E-05	0.105	0.109	0.105	0.109

Table C.12. Typical Regression Output for *o*-Xylene Standards (peak height).

Regression Statistics--o-xylene	
Multiple R	1.00
R Square	1.00
Adjusted R Square	1.00
Standard Error	0.0513
Observations	4

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	32.0	32.0	12139	8.24E-05
Residual	2	0.00527	0.00264		
Total	3	32.0			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Y intercept	-0.0472	0.0381	-1.24	0.341	-0.211	0.117	-0.211	0.117
o -xylene height	0.134	0.00122	110	8.24E-05	0.129	0.140	0.129	0.140

Table C.13. Typical Anion Concentrations and IC Response.

IC standard	Standard conc. (mg/L)	chloride (peak area)	nitrite-N (peak area)	nitrate-N (peak area)	phosphate-P (peak area)	sulfate (peak area)
Blank	0	0	0	0	0	0
std 6	10	991006	1473873	1568811	433608	441642
std 4	50	3533718	6554016	9489299	2702080	2211029
std 2	150	11360288	19436208	33768352	9899354	7514112

Table C.14. Typical Regression Statistics for Chloride Standards.

Regression Statistics--chloride	
Multiple R	0.999
R^2	0.999
Adjusted R^2	0.998
Standard Error	3.16
Observations	4

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	14055	14055	1412	0.000708
Residual	2	19.9	9.96		
Total	3	14075			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Y intercept	-0.323	2.11	-0.153	0.893	-9.41	8.77	-9.41	8.77
chloride area	1.33E-05	3.54E-07	37.6	0.000708	1.18E-05	1.48E-05	1.18E-05	1.48E-05

Table C.15. Typical Regression Statistics for Nitrite-N Standards.

Regression Statistics--nitrite-N	
Multiple R	1.00
R^2	1.00
Adjusted R^2	1.00
Standard Error	0.716
Observations	4

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	14074	14074	27460	3.64E-05
Residual	2	1.03	0.513		
Total	3	14075			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Y intercept	-0.711	0.481	-1.48E+00	0.277	-2.78	1.36	-2.78	1.36
nitrite-N area	7.75E-06	4.68E-08	166	3.64E-05	7.55E-06	7.95E-06	7.55E-06	7.95E-06

Table C.16. Typical Regression Statistics for Nitrate-N Standards.

Regression Statistics--nitrate-N	
Multiple R	0.999
R^2	0.997
Adjusted R^2	0.996
Standard Error	4.40
Observations	4

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	14036	14036	725	0.00138
Residual	2	38.7	19.3		
Total	3	14075			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Y intercept	3.37	2.86	1.18	0.359	-8.92	15.7	-8.92	15.7
nitrate-N area	4.38E-06	1.63E-07	26.9	0.00138	3.68E-06	5.08E-06	3.68E-06	5.08E-06

Table C.17. Typical Regression Statistics for Phosphate-P Standards.

Regression Statistics--phosphate-P	
Multiple R	0.998
R^2	0.996
Adjusted R^2	0.995
Standard Error	5.07
Observations	4

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	14024	14024	546	0.00183
Residual	2	51.4	25.7		
Total	3	14075			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Y intercept	3.88	3.28	1.18	0.358	-10.2	18.0	-10.2	18.0
phosph.-P area	1.49E-05	6.38E-07	23.4	0.00183	1.22E-05	1.77E-05	1.22E-05	1.77E-05

Table C.18. Typical Regression Statistics for Sulfate Standards.

Regression Statistics--sulfate	
Multiple R	0.999
R^2	0.998
Adjusted R^2	0.998
Standard Error	3.38
Observations	4

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	14052	14052	1232	0.000811
Residual	2	22.8	11.4		
Total	3	14075			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Y intercept	2.08	2.22	0.937	0.448	-7.46	11.6	-7.46	11.6
sulfate area	1.98E-05	5.65E-07	35.1	0.000811	1.74E-05	2.23E-05	1.74E-05	2.23E-05

Table C.19. Typical Absorbance Response at 562 nm of Total Protein (TP) Standards Analysis.

TP standard (ug/100ul)	Absorbance 1 (562 nm)	Absorbance 2 (562 nm)	Average absorbance	Corrected absorbance
0	0.0922	0.0862	0.0892	0.0000
20	0.1188	0.1202	0.1195	0.0303
40	0.1742	0.1725	0.1734	0.0842
60	0.2098	0.2062	0.2080	0.1188
80	0.2388	0.2434	0.2411	0.1519
100	0.2893	0.2871	0.2882	0.1990

Table C.20. Typical Regression Statistics of Total Protein Standards.

Regression Statistics	
Multiple R	0.998
R^2	0.995
Adjusted R^2	0.994
Standard Error	2.93
Observations	6

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	6966	6966	811	9.05E-06
Residual	4	34.4	8.59		
Total	5	7000			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Y intercept	1.37	2.09	0.655	0.548	-4.42	7.16	-4.42	7.16
Absorbance	500	17.5	28.5	9.05E-06	451	548	451	548

Table C.21. Method Detection Limit (MDL) Data Derived from 1.0 mg/L and 0.5 mg/L Aqueous BTX Standards, Analyzed at a 1:20 GC Split Ratio.

	1.0 mg/L standard					0.5 mg/L standard				
Replicate analysis	benzene (mg/L)	toluene (mg/L)	*p*-xylene (mg/L)	*m*-xylene (mg/L)	*o*-xylene (mg/L)	benzene (mg/L)	toluene (mg/L)	*p*-xylene (mg/L)	*m*-xylene (mg/L)	*o*-xylene (mg/L)
1	0.97	1.01	1.05	1.01	1.00	0.50	0.49	0.50	0.48	0.48
2	0.97	1.00	1.03	1.00	0.97	0.45	0.49	0.51	0.49	0.48
3	0.98	0.96	1.03	1.00	0.99	0.40	0.50	0.52	0.50	0.48
4	1.00	1.00	1.03	0.99	1.02	0.47	0.49	0.51	0.49	0.48
5	0.98	1.00	1.04	1.00	0.97	0.50	0.49	0.51	0.49	0.49
6	0.98	1.01	1.03	0.99	1.02	0.50	0.48	0.50	0.49	0.50
7	0.99	1.01	1.04	1.00	0.99	0.50	0.50	0.52	0.50	0.49
8	0.99	1.02	1.05	1.01	0.99	0.50	0.50	0.53	0.51	0.48
9	0.97	0.99	1.04	1.00	0.97	0.50	0.51	0.51	0.50	0.50
10	0.98	0.99	1.03	1.00	0.88	0.50	0.49	0.54	0.52	0.48
mean	0.98	1.00	1.04	1.00	0.98	0.48	0.49	0.52	0.50	0.49
st. dev.	0.009	0.015	0.008	0.007	0.040	0.032	0.008	0.013	0.012	0.007

Table C.22. Method Detection Limit (MDL) Determination for 1:20 GC Split Ratio Data presented in Table C.21.

BTX fraction	1.0 mg/L data set	0.5 mg/L data set	pooled st. dev.'s	t value ($\alpha = 0.01$)	MDL (mg/L)
benzene	0.009	0.032	0.023	2.55	0.06
toluene	0.015	0.008	0.012	2.55	0.03
p -xylene	0.008	0.013	0.010	2.55	0.03
m -xylene	0.007	0.012	0.010	2.55	0.03
o -xylene	0.040	0.007	0.028	2.55	0.07

Table C.23. Method Detection Limit (MDL) Data Derived from 1.0 mg/L and 0.5 mg/L Aqueous BTX Standards, Analyzed at a 1:40 Split Ratio.

	1.0 mg/L standard					0.5 mg/L standard				
Replicate analysis	benzene (mg/L)	toluene (mg/L)	*p* -xylene (mg/L)	*m* -xylene (mg/L)	*o* -xylene (mg/L)	benzene (mg/L)	toluene (mg/L)	*p* -xylene (mg/L)	*m* -xylene (mg/L)	*o* -xylene (mg/L)
1	0.95	0.98	0.93	0.95	1.06	0.48	0.52	0.45	0.47	0.48
2	0.96	1.00	0.97	0.88	1.00	0.48	0.50	0.48	0.48	0.48
3	0.97	0.99	0.99	0.97	0.96	0.41	0.49	0.45	0.51	0.48
4	0.94	1.00	1.01	0.98	0.98	0.48	0.49	0.46	0.47	0.50
5	0.96	1.01	0.97	0.96	0.97	0.48	0.53	0.51	0.49	0.53
6	0.97	0.99	0.95	0.96	0.98	0.48	0.52	0.48	0.49	0.51
7	0.96	1.01	0.96	0.94	0.99	0.49	0.50	0.46	0.50	0.55
8	0.98	0.99	0.95	0.95	1.00	0.49	0.39	0.45	0.45	0.51
9	0.97	1.01	0.96	0.96	0.96	0.52	0.53	0.48	0.47	0.57
10	0.99	1.01	1.08	1.00	1.00	0.49	0.38	0.47	0.50	0.52
mean	0.97	1.00	0.98	0.95	0.99	0.48	0.49	0.47	0.48	0.51
st. dev.	0.015	0.011	0.041	0.033	0.028	0.027	0.054	0.017	0.019	0.030

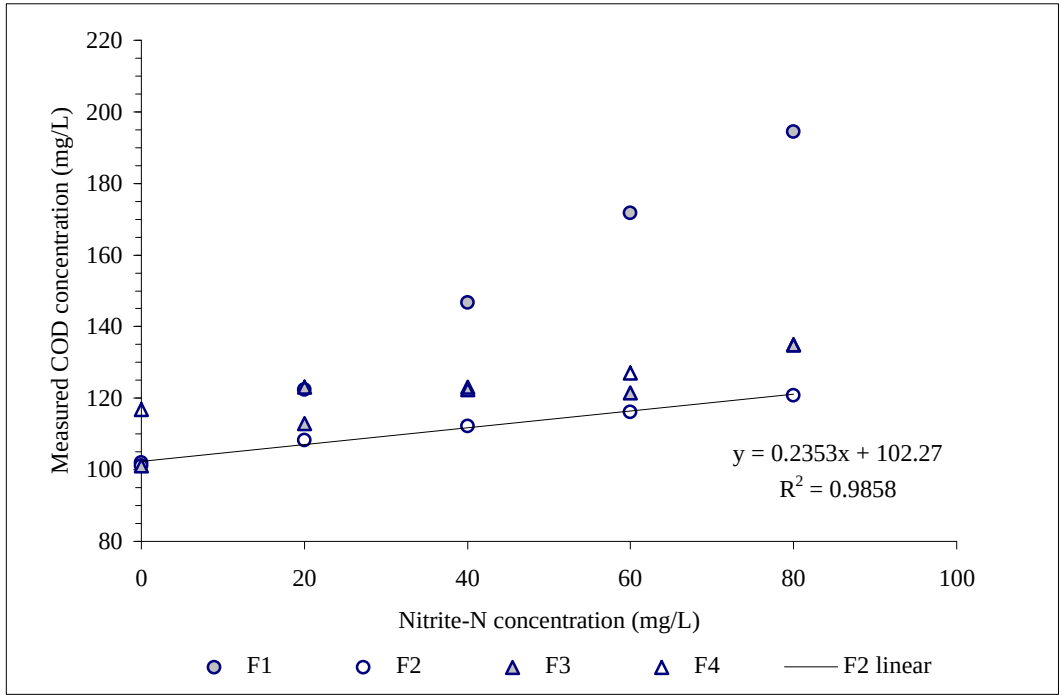
Table C.24. Method Detection Limit (MDL) Determination for 1:40 GC Split Ratio Data Presented in Table C.7.

BTX fraction	1.0 mg/L data set	0.5 mg/L data set	pooled std. dev.'s	t value ($\alpha = 0.01$)	MDL (mg/L)
benzene	0.015	0.027	0.022	2.55	0.06
toluene	0.011	0.054	0.039	2.55	0.10
p -xylene	0.041	0.017	0.031	2.55	0.08
m -xylene	0.033	0.019	0.027	2.55	0.07
o -xylene	0.028	0.030	0.029	2.55	0.07

Table C.25. Experimental Data from Nitrite-N Interference on COD Test.

Sample group	Nitrite-N (mg/L)	average COD (mg/L)	st. dev. COD (mg/L)
F1 1	0	102.0	0.0
F1 2	20	122.4	***
F1 3	40	146.7	1.1
F1 4	60	171.8	5.5
F1 5	80	194.5	4.4
F2 1	0	101.2	3.3
F2 2	20	108.2	2.2
F2 3	40	112.2	1.1
F2 4	60	116.1	4.4
F2 5	80	120.8	0.0
F3 1	0	101.2	3.3
F3 2	20	112.9	2.2
F3 3	40	123.1	1.1
F3 4	60	121.6	1.1
F3 5	80	134.9	0.0
F4 1	0	116.9	3.3
F4 2	20	123.1	7.8
F4 3	40	122.4	4.4
F4 4	60	127.1	***
F4 5	80	134.9	0.0

Figure C.1. Nitrite Interference on COD Correlation Determination.



Notes for Table C.25. and Figure C.1.:

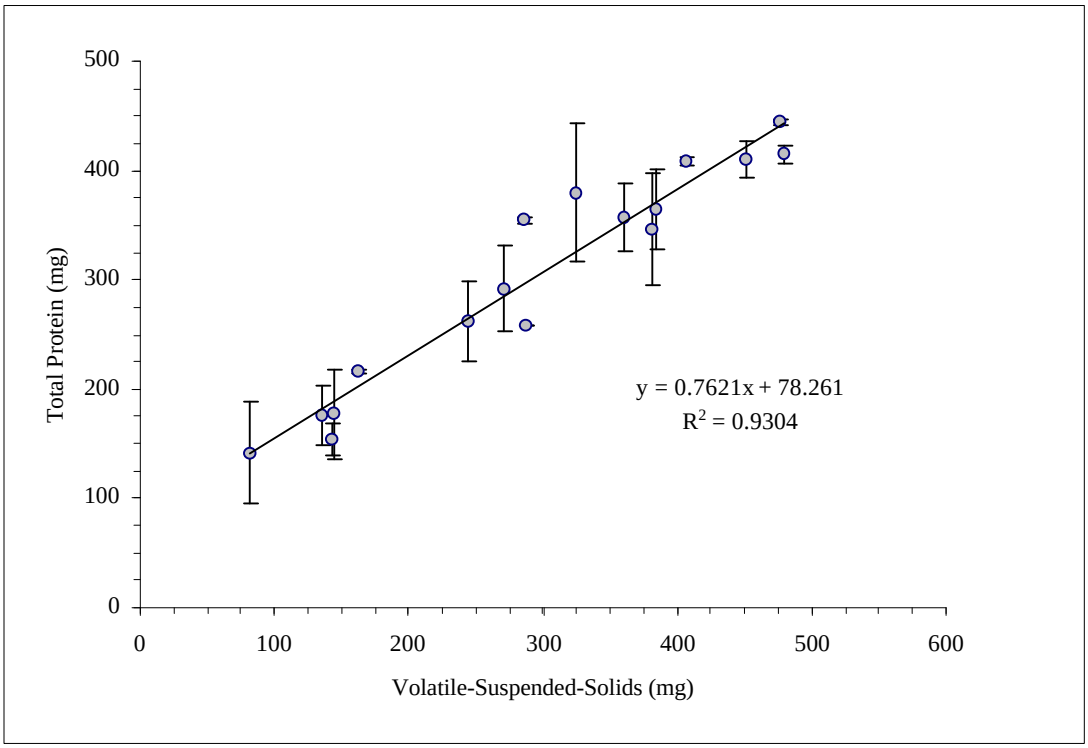
1) Four series in test matrix: **F1**--no treatment (control); **F2**--sulfuric acid preservation (pH<2) only; **F3**--sulfuric acid (pH<2) and 10 mg sulfamic acid/mg nitrite; **F4**--sulfuric acid (pH<2) and sulfamic acid for 80 mg nitrite.
2) Five nitrite-N concentrations in each series: **1** = 0 mg/L (control), **2** = 20 mg/L, **3** = 40 mg/L, **4** = 60 mg/L, **5** = 80 mg/L.
3) All samples spiked with 100 mg/L as COD of Potassium Hydrogen Phthalate.
4) Normality of Ferrous Ammonium Sulfate (FAS) solution was 0.0490; hot blank titrant volume was 6.04 mL.
5) All samples performed in duplicate (N = 2).
6) *** -- Duplicate samples were defective and were not included in overall analysis, therefore standard deviations could not be computed.

Table C.26. Total Protein to Volatile-Suspended-Solids Determination Experimental Data Set.

Sample date	VSS (mg/L)	Sample volume (mL)	VSS (µg)	average TP (µg)	st. dev. TP (µg)
Day 511	27.1	3	81.3	141.4	47.1
Day 511	27.1	6	162.6	216.7	1.7
Day 511	27.1	9	243.9	262.8	36.6
Day 511	27.1	12	325.2	379.9	63.1
Day 511	27.1	15	406.5	408.6	4.1
Day 513	48.0	3	144	176.7	41.1
Day 513	48.0	6	288	258.1	0.2
Day 513	48.0	8	384	363.9	36.5
Day 513	48.0	10	480	415.5	8.5
Day 517	47.7	3	143.1	153.7	14.5
Day 517	47.7	6	286.2	354.6	2.1
Day 517	47.7	8	381.6	345.7	51.6
Day 517	47.7	10	477.0	444.3	3.4
Day 518	45.1	3	135.3	175.9	26.8
Day 518	45.1	6	270.5	291.9	38.7
Day 518	45.1	8	360.7	357.2	31.9
Day 518	45.1	10	450.8	410.0	16.1

All data from duplicate analyses (N = 2).

Figure C.3. Total Protein to Volatile-Suspended-Solids Ratio Analysis. Error Bars Represent One Standard Deviation of Duplicate Samples.



APPENDIX D:

INFLUENT COMPOSITION

Table D.1. Composition of Influent Biogenic Substrate.

C-source	g/L in stock	thOD/g	Stock thOD (g/L)	Measured COD (g/L)
Protein feed stock (use 200 mL to 1 L ddH $_2$ O, dilute 1:80 in reactor)				
Beef extract	25.6	1.0	25.6	
Casitone	13.6	1.0	13.6	
Peptone	13.6	1.0	13.6	
Yeast extract	41.6	1.0	41.6	
Protein stock totals			94.4	94.0 ± 0.7
Sugar feed stock (use 200mL to 1 L ddH $_2$ O, dilute 1:80 in reactor)				
Glucose	15.6	1.07	16.6	
Fructose	15.6	1.07	16.6	
Galactose	15.6	1.07	16.6	
Sugar stock totals			49.8	48.9 ± 0.9
Organic Acids feed stock (use 200mL to 1 L ddH $_2$ O, dilute 1:80 in reactor)				
	(mL/L)			
Acetic acid (γ = 1.053)	98	1.07	110	
Glycerol (γ = 1.264)	14.0	1.21	21.5	
Organic acid stock total			132	128 ± 2

Table D.2. Composition of Influent BTX Substrate.

BTX fraction	γ	uL/L reactor	thOD (mg/mg)	thOD (mg/L)
benzene	0.879	5.70	3.077	15.4
toluene	0.866	5.78	3.183	15.9
o -xylene	0.880	5.68	3.556	17.8
m -xylene	0.868	5.80	3.556	17.8
p -xylene	0.861	5.82	3.556	17.8

Table D.3. Stability in Terms of sCOD of a Typical Batch of Biogenic Feed.

Batch life cycle	COD in feed jar (g/L)	COD as diluted in reactor at t=0 (mg/L)
as prepared (t = 0 days)	53.9 ± 1.7	673 ± 22
at end of batch (t = ~15 days)	53.7 ± 0.9	671 ± 11

Table D.4. Composition of Mineral Salts Media.

Compound	Formula weight (g/mol)	Stock conc. (g/L)	Stock conc. (mM)	MSM conc. (mM)	MSM conc. (mg/L)
Calcium Chloride Stock Solution (dilution ratio: 4 ml stock per L tap water)					
$CaCl_2$	111.0	5.3	47.8	0.191	21.2
Chloride Stock Solution (dilution ratio: 4 mL stock per L tap water)					
$FeCl_3*6H_2O$	270.3	3.00	11.1	0.044	12.0
$CoCl_2*6H_2O$	237.9	0.30	1.26	0.0050	1.20
$ZnCl_2$	136.3	0.30	2.20	0.0088	1.20
$CuCl_2*2H_2O$	170.5	0.10	0.59	0.0023	0.400
H_3BO_3	61.83	0.03	0.49	0.0019	0.120
$MgCl_2*6H_2O$	203.3	18.9	93.0	0.37	75.6
Sulfate Stock Solution (dilution ratio: 4 mL stock per L tap water)					
$MgSO_4*7H_2O$	246.5	7.00	28.4	0.114	28.0
$MnSO_4*H_2O$	169.0	0.90	5.32	0.0213	3.60
$Na_2MoO_4*2H_2O$	241.9	0.10	0.413	0.00165	0.400
Phosphate Stock Solution (dilution ratio: 4 mL stock per L tap water)					
K_2HPO_4	174.2	87.0	499	2.00	348
KH_2PO_4	136.1	68.0	500	2.00	272

APPENDIX E:

PROFILE DATA

Table E.1. BTX Data for Reactor Profile Experiment Conducted on Day 85 (February 12, 1997).

Reactor sample	Sample time (hr)	benzene (mg/L)	toluene (mg/L)	*p* -xylene (mg/L)	*m* -xylene (mg/L)	*o* -xylene (mg/L)
0	0.00	0.78	ND	0.86	*ND*	1.00
1	0.50	2.98	1.68	2.63	1.41	3.09
2	1.17	4.16	1.68	4.33	1.39	4.92
3	2.17	4.27	0.11	4.50	ND	4.46
4	3.17	4.28	ND	4.56	ND	4.04
5	4.17	4.30	ND	4.54	ND	3.91
6	5.17	4.24	ND	4.54	ND	3.83
7	6.17	4.25	ND	4.33	ND	3.66
8	8.17	4.36	ND	4.25	ND	3.68
9	10.17	4.12	ND	3.90	ND	3.52
10	12.17	4.06	ND	3.63	ND	3.44
11	14.17	4.49	ND	3.64	ND	3.69
12	16.17	3.92	ND	3.19	ND	3.41
13	18.17	3.89	ND	3.00	ND	3.33
14	20.17	3.76	ND	2.73	ND	3.28
15	22.17	3.76	ND	2.69	ND	3.39

All BTX data from analysis of single samples (N = 1).
Samples were analyzed on GC in replicate with the concentrations presented representing the average of the replicate analyses.
ND -- Not Detected

Table E.2. Anion and sCOD Data for Reactor Profile Experiment Conducted on Day 85 (February 12, 1997).

Reactor sample	Sample time (hr)	chloride (mg/L)	nitrite-N (mg/L)	nitrate-N (mg/L)	phosphate-P (mg/L)	sulfate (mg/L)	average COD (mg/L)	st. dev. COD (mg/L)
0	0.00	64.7	ND	138.9	108.3	28.8	57.3	10.9
1	0.50	66.4	37.6	49.8	102.4	27.9	134.4	24.1
2	1.17	61.5	42.9	35.3	106.3	28.9	98.3	0.0
3	2.17	61.5	46.4	18.9	103.8	28.8	75.0	38.3
4	3.17	61.5	35.0	13.2	105.3	28.5	106.4	13.1
5	4.17	62.2	25.6	10.0	105.8	28.4	98.5	5.5
6	5.17	62.0	16.0	7.5	103.1	28.2	86.1	4.4
7	6.17	62.3	7.1	5.7	104.3	28.1	90.5	1.1
8	8.17	63.9	ND	ND	105.8	28.3	93.7	5.5
9	10.17	64.7	ND	ND	103.1	28.8	81.3	7.7
10	12.17	65.4	ND	ND	104.1	26.8	96.0	6.6
11	14.17	67.1	ND	ND	103.7	27.0	91.4	0.0
12	16.17	63.3	ND	ND	103.9	27.1	84.4	16.4
13	18.17	62.2	ND	ND	101.0	26.5	97.6	4.4
14	20.17	63.1	ND	ND	105.0	26.6	99.9	12.0
15	22.17	63.4	ND	ND	104.1	26.5	89.8	6.6

Anion data from analysis of single samples (N = 1).
sCOD data from analysis of duplicate samples (N = 2).
ND -- Not Detected

Table E.3. BTX Data for Reactor Profile Experiment Conducted on Day 105 (March 4, 1997).

Reactor sample	Sample time (hr)	benzene (mg/L)	toluene (mg/L)	*p* -xylene (mg/L)	*m* -xylene (mg/L)	*o* -xylene (mg/L)
0	0.00	1.09	ND	1.40	ND	1.63
1	0.50	4.22	2.48	3.48	1.93	4.16
2	1.17	5.24	3.56	5.07	3.33	5.72
3	2.17	5.63	3.09	5.99	2.78	6.32
4	3.17	6.42	2.52	7.13	2.00	7.37
5	4.17	6.39	1.65	7.06	1.15	7.13
6	5.17	6.45	1.26	7.35	0.80	7.33
7	6.17	6.16	1.04	6.87	0.54	6.93
8	8.17	6.06	0.84	6.56	0.34	6.77
9	10.17	6.09	0.76	6.52	0.31	6.89
10	12.17	6.00	0.70	6.52	0.25	7.13
11	14.17	6.11	0.64	6.33	0.15	7.09
12	16.17	6.80	0.68	6.81	0.08	7.81
13	18.17	5.92	0.59	6.02	0.18	7.29
14	20.17	5.67	0.59	5.38	0.21	6.81
15	22.17	5.55	0.57	5.16	0.15	6.81

All BTX data from analysis of single samples (N = 1).
Samples were analyzed on GC in replicate with the concentrations presented representing the average of the replicate analyses.
ND -- Not Detected

Table E.4. Anion and sCOD Data for Reactor Profile Experiment Conducted on Day 105 (March 4, 1997)

Reactor sample	Sample time (hr)	chloride (mg/L)	nitrite-N (mg/L)	nitrate-N (mg/L)	phosphate-P (mg/L)	sulfate (mg/L)	average COD (mg/L)	st. dev. COD (mg/L)
0	0.00	62.6	4.6	210.4	98.3	30.8	65.9	14.5
1	0.50	66.8	56.4	137.1	96.8	31.1	234.6	8.4
2	1.17	61.8	74.3	104.3	94.4	32.3	116.5	***
3	2.17	67.9	83.6	83.8	95.4	30.0	119.1	***
4	3.17	63.0	98.3	68.8	95.3	31.1	114.8	5.6
5	4.17	62.6	99.7	60.0	94.4	31.9	108.2	1.1
6	5.17	63.8	97.8	55.1	96.1	31.0	96.4	1.7
7	6.17	62.9	92.8	51.0	95.5	30.9	109.8	5.6
8	8.17	67.7	77.1	46.4	96.6	30.1	106.4	2.2
9	10.17	67.6	68.7	42.9	93.8	30.0	105.2	6.7
10	12.17	65.9	61.8	40.3	97.3	29.4	102.9	1.1
11	14.17	71.0	57.2	39.4	97.5	31.7	100.0	2.2
12	16.17	67.1	51.9	36.8	95.8	29.9	106.0	0.0
13	18.17	63.7	48.1	36.1	95.6	31.4	95.9	4.5
14	20.17	62.9	43.5	34.4	98.0	31.9	93.0	1.1
15	22.17	63.2	39.9	33.5	96.6	31.2	89.5	5.0

Anion data from analysis of single samples (N = 1).
sCOD data from analysis of duplicate samples (N = 2).
ND -- Not Detected

Table E.5. BTX Data for Reactor Profile Experiment Without Biogenic Substrate Conducted on Day 160 (April 28, 1997).

		Duplicate averages					Duplicate standard deviations				
Reactor sample	Sample time (hr)	benzene (mg/L)	toluene (mg/L)	*p*-xylene (mg/L)	*m*-xylene (mg/L)	*o*-xylene (mg/L)	benzene (mg/L)	toluene (mg/L)	*p*-xylene (mg/L)	*m*-xylene (mg/L)	*o*-xylene (mg/L)
0	0.00	4.89	4.96	5.91	5.15	6.38	0.05	0.07	0.10	0.09	0.09
1	0.25	4.70	4.72	5.62	4.88	6.17	0.10	0.06	0.10	0.12	0.18
2	0.50	4.52	4.48	5.39	4.66	5.90	0.04	0.01	0.05	0.05	0.02
3	0.75	4.32	4.27	5.15	4.49	5.73	0.01	0.02	0.02	0.02	0.00
4	1.00	4.33	4.22	5.19	4.41	5.70	0.00	0.02	0.00	0.02	0.00
5	1.17	4.40	4.25	5.31	4.56	5.84	0.01	0.01	0.00	0.02	0.02
6	1.33	4.31	4.15	5.14	4.39	5.72	0.01	0.03	0.00	0.05	0.02
7	1.50	4.70	4.50	5.55	4.80	6.23	0.42	0.39	0.34	0.39	0.53
8	1.67	4.51	4.30	5.45	4.56	6.00	0.02	0.05	0.07	0.05	0.15
9	1.83	4.42	4.31	5.52	4.65	6.10	0.06	0.08	0.14	0.10	0.13
10	2.00	4.54	4.12	5.26	4.41	5.86	0.05	0.12	0.20	0.19	0.31
11	2.25	4.12	4.02	5.10	4.27	5.67	0.35	0.11	0.19	0.16	0.19
12	2.50	4.32	4.12	5.28	4.43	5.89	0.23	0.14	0.17	0.14	0.22
13	2.75	4.43	4.13	5.18	4.38	5.81	0.55	0.48	0.50	0.46	0.50
14	3.00	3.95	3.72	4.71	3.96	5.28	0.03	0.01	0.07	0.00	0.07
15	4.00	4.08	3.76	4.86	4.05	5.49	0.19	0.19	0.30	0.22	0.34
16	5.03	4.11	3.78	4.87	4.07	5.55	0.15	0.09	0.12	0.05	0.13
17	6.00	4.18	3.89	5.05	4.18	5.73	0.18	0.21	0.27	0.24	0.35
18	9.00	3.83	3.50	4.43	3.71	5.05	0.05	0.07	0.08	0.00	0.11
19	17.17	4.19	3.87	5.02	4.17	5.89	0.02	0.03	0.03	0.05	0.09
20	22.17	4.02	3.65	4.57	3.99	5.69	0.04	0.00	0.30	0.02	0.02

All data from duplicate analyses (N = 2).

Table E.6. Anion Data for Reactor Profile Experiment Without Biogenic Substrate Conducted on Day 160 (April 28, 1997).

Reactor sample	Sample time (hr)	chloride (mg/L)	nitrite-N (mg/L)	nitrate-N (mg/L)	phosphate-P (mg/L)	sulfate (mg/L)
0	0.00	62.5	64.0	197.5	109.6	22.8
1	0.25	61.8	63.3	193.9	109.1	22.2
2	0.50	63.4	64.0	194.7	111.3	22.6
3	0.75	62.8	64.2	194.8	110.6	22.6
4	1.00	62.1	63.6	194.0	110.1	23.6
5	1.25	62.0	63.5	194.0	110.4	24.9
6	1.50	62.0	63.1	193.2	110.9	23.0
7	1.75	62.3	62.7	194.0	110.5	23.2
8	2.00	62.1	62.3	192.3	111.0	23.5
9	2.25	61.8	61.8	192.8	110.8	22.1
10	2.50	61.6	61.6	191.2	109.2	21.8
11	2.75	61.5	61.2	191.4	109.7	22.5
12	3.00	62.5	61.8	192.5	110.7	22.4
13	4.00	61.7	61.1	189.9	110.7	22.2
14	5.00	62.6	60.4	189.0	110.2	22.1
15	6.00	61.4	60.1	188.4	108.9	22.1
16	9.00	61.2	59.8	186.3	110.0	23.0
17	17.17	67.6	58.7	185.0	110.5	22.0
18	22.17	61.1	57.5	183.0	109.4	22.2

All data from single analyses (N = 1).

Table E.7. BTX Data for Reactor Profile Experiment Conducted on Day 202 (June 9, 1997).

		Duplicate averages					Duplicate standard deviations				
Reactor sample	Sample time (hr)	benzene (mg/L)	toluene (mg/L)	*p* -xylene (mg/L)	*m* -xylene (mg/L)	*o* -xylene (mg/L)	benzene (mg/L)	toluene (mg/L)	*p* -xylene (mg/L)	*m* -xylene (mg/L)	*o* -xylene (mg/L)
0	0.00	5.05	4.28	5.66	4.42	5.85	0.25	0.09	0.12	0.06	0.05
1	0.17	4.98	4.15	5.49	4.25	5.69	0.14	0.15	0.23	0.18	0.22
2	0.33	4.83	3.85	5.34	4.05	5.55	0.07	0.05	0.13	0.10	0.12
3	0.50	4.82	3.73	5.38	4.07	5.62	0.06	0.09	0.15	0.12	0.17
4	0.67	4.77	3.53	5.28	3.95	5.50	0.10	0.08	0.19	0.12	0.15
5	0.83	4.68	3.25	5.19	3.79	5.37	0.03	0.04	0.04	0.04	0.07
6	1.00	4.75	2.97	5.30	3.70	5.46	**	**	**	**	**
7	1.08	4.71	2.83	5.21	3.65	5.37	0.03	0.01	0.06	0.04	0.07
8	1.17	4.68	2.62	5.22	3.56	5.34	0.12	0.07	0.19	0.16	0.17
9	1.25	4.65	2.46	5.14	3.41	5.23	0.08	0.05	0.15	0.10	0.12
10	1.33	4.62	2.32	5.10	3.34	5.21	0.06	0.05	0.17	0.12	0.15
11	1.42	4.60	2.21	5.07	3.24	5.18	0.08	0.05	0.25	0.14	0.20
12	1.50	4.60	2.03	5.07	3.18	5.16	0.09	0.03	0.13	0.10	0.17
13	1.58	4.58	1.94	5.10	3.18	5.23	0.09	0.05	0.21	0.10	0.22
14	1.67	4.56	1.82	4.99	2.99	5.11	0.00	0.00	0.10	0.02	0.05
15	1.75	4.47	1.65	4.94	2.92	5.02	0.06	0.03	0.10	0.08	0.12
16	1.83	4.39	1.57	4.92	2.86	4.99	0.07	0.01	0.04	0.04	0.12
17	1.92	4.39	1.49	4.98	2.86	5.04	0.09	0.01	0.15	0.08	0.10
18	2.00	4.46	1.40	4.98	2.74	5.00	0.09	0.03	0.15	0.10	0.15
19	2.17	4.47	1.26	4.90	2.64	4.95	0.02	0.01	0.04	0.00	0.02
20	2.33	4.39	1.10	4.84	2.47	4.90	0.09	0.05	0.15	0.12	0.15
21	2.50	4.43	1.03	4.98	2.44	5.02	0.02	0.01	0.12	0.04	0.07
22	2.67	4.44	0.94	5.04	2.39	5.06	0.08	0.03	0.17	0.04	0.17
23	2.83	4.34	0.82	4.85	2.23	4.84	0.05	0.04	0.10	0.06	0.12
24	3.00	4.35	0.66	4.84	1.97	4.69	**	**	**	**	**
25	6.00	4.10	0.31	4.62	1.17	4.49	0.10	0.00	0.23	0.06	0.22
26	9.00	3.86	0.09	4.39	0.51	4.24	0.02	0.01	0.13	0.00	0.12
27	21.25	3.09	ND	3.74	ND	4.05	0.06	***	0.02	***	0.00

All data from duplicate analyses (N = 2).
** -- Duplicate sample lost, therefore standard deviations could not be computed.
*** -- Compound not detected, therefore standard deviations could not be computed.
ND -- Not Detected.

Table E.8. Anion and sCOD Data for Reactor Profile Experiment Conducted on Day 202 (June 9, 1997).

Reactor sample	Sample time (hr)	chloride (mg/L)	nitrite-N (mg/L)	nitrate-N (mg/L)	phosphate-P (mg/L)	sulfate (mg/L)	average COD (mg/L)	st. dev. COD (mg/L)
0	0.00	68.8	81.2	132.1	106.4	23.0	450.0	**
1	0.25	70.6	129.6	85.7	104.8	23.3	229.7	5.5
2	0.50	68.9	162.2	46.7	104.5	18.6	91.5	3.3
3	0.75	69.0	166.0	40.7	107.0	22.6	94.5	6.6
4	1.00	***	***	***	***	***	***	***
5	1.25	68.8	175.2	26.9	104.7	21.1	89.2	15.4
6	1.50	68.0	177.2	21.0	102.9	20.5	76.4	2.2
7	1.75	***	***	***	***	***	***	***
8	2.00	67.8	179.8	14.0	104.3	20.7	119.2	0.0
9	2.25	68.0	179.7	11.6	102.8	20.8	38.7	9.1
10	2.50	68.9	180.3	9.6	103.7	20.5	63.2	28.6
11	2.75	68.8	180.3	8.3	105.0	20.6	62.4	5.5
12	3.00	68.4	179.9	7.3	102.6	21.6	51.1	8.5
13	6.00	69.3	166.8	3.0	99.7	20.7	58.6	4.4
14	9.00	69.3	150.6	2.8	101.5	19.6	55.4	3.3
15	21.25	70.0	107.4	2.8	102.5	18.4	64.0	5.5

Anion data from single analyses (N =1).
sCOD data from duplicate analyses (N = 2).
** -- Duplicate sample lost, therefore standard deviations could not be computed.
*** -- Samples during these times lost.

Table E.9. BTX Data for Reactor Profile Experiment Without Biogenic Substrate Conducted on Day 217 (June 24, 1997).

		Duplicate averages					Duplicate standard deviations				
Reactor sample	Sample time (hr)	benzene (mg/L)	toluene (mg/L)	*p* -xylene (mg/L)	*m* -xylene (mg/L)	*o* -xylene (mg/L)	benzene (mg/L)	toluene (mg/L)	*p* -xylene (mg/L)	*m* -xylene (mg/L)	*o* -xylene (mg/L)
0	0.00	4.11	4.22	4.96	4.29	5.11	0.02	0.04	0.02	0.08	0.05
1	0.17	3.88	3.86	4.73	4.05	4.88	0.08	0.07	0.08	0.06	0.02
2	0.33	3.85	3.66	4.68	3.95	4.83	0.04	0.03	0.04	0.04	0.05
3	0.50	3.82	3.36	4.64	3.90	4.77	0.08	0.09	0.13	0.12	0.12
4	0.67	3.78	3.04	4.53	3.81	4.70	0.07	0.09	0.10	0.08	0.07
5	0.83	3.70	2.61	4.50	3.79	4.60	0.02	0.03	0.06	0.04	0.02
6	1.00	3.68	2.19	4.46	3.65	4.54	0.01	0.00	0.15	0.12	0.15
7	1.08	3.64	2.02	4.37	3.60	4.42	0.06	0.03	0.02	0.02	0.07
8	1.17	3.63	1.85	4.37	3.53	4.42	0.00	0.04	0.10	0.04	0.12
9	1.25	3.61	1.63	4.37	3.48	4.42	0.08	0.03	0.06	0.04	0.02
10	1.33	3.57	1.47	4.30	3.41	4.33	0.01	0.01	0.15	0.14	0.10
11	1.42	3.56	1.30	4.27	3.37	4.35	0.01	0.01	0.04	0.00	0.02
12	1.50	3.60	1.17	4.39	3.41	4.33	0.04	0.05	0.17	0.10	0.10
13	1.58	3.57	1.04	4.37	3.34	4.33	0.01	0.03	0.06	0.04	0.05
14	1.67	3.49	0.87	4.23	3.20	4.14	0.10	0.05	0.17	0.16	0.12
15	1.75	3.65	0.83	4.46	3.25	4.33	0.03	0.03	0.08	0.04	0.05
16	1.83	3.52	0.70	4.24	3.04	4.19	0.07	0.03	0.15	0.10	0.30
17	1.92	3.47	0.61	4.16	2.93	4.00	0.09	0.01	0.00	0.06	0.02
18	2.00	3.43	0.51	4.12	2.82	3.89	0.05	0.01	0.02	0.02	0.02
19	2.17	3.40	0.43	4.05	2.69	3.86	0.04	0.03	0.12	0.04	0.07
20	2.33	3.38	0.40	4.03	2.54	3.77	0.07	0.01	0.15	0.10	0.15
21	2.50	3.31	0.36	3.92	2.37	3.68	0.01	0.01	0.12	0.06	0.12
22	2.67	3.21	0.32	3.81	2.28	3.61	0.06	0.01	0.12	0.04	0.07
23	2.83	3.18	0.33	3.77	2.22	3.61	0.04	0.00	0.02	0.04	0.02
24	3.00	3.14	0.34	3.71	2.29	3.63	0.04	0.04	0.02	0.14	0.00
25	4.00	3.01	0.33	3.65	2.26	3.63	0.02	0.00	0.08	0.18	0.05
26	5.00	2.91	0.30	3.51	2.08	3.66	0.07	0.01	0.00	0.04	0.05
27	6.00	2.74	0.30	3.35	1.94	3.59	0.02	0.01	0.00	0.00	0.00
28	9.00	2.36	0.16	2.90	1.32	3.38	0.03	0.00	0.06	0.00	0.00
29	12.00	2.22	0.10	2.80	0.76	3.50	0.17	0.00	0.19	0.04	0.22
30	22.00	1.22	ND	1.39	ND	2.92	0.02	***	0.00	***	0.05

All data from duplicate analyses (N = 2).
*** -- Compound not detected, therefore standard deviations could not be computed.
ND -- Not Detected.

Table E.10. Anion and sCOD Data for Reactor Profile Without Biogenic Substrate Conducted on Day 217 (June 24, 1997).

Reactor sample	Sample time (hr)	chloride (mg/L)	nitrite-N (mg/L)	nitrate-N (mg/L)	phosphate-P (mg/L)	sulfate (mg/L)	average COD (mg/L)	st. dev. COD (mg/L)
0	0.00	42.9	ND	134.6	339.5	16.8	77.4	1.1
1	0.25	44.4	ND	131.1	338.3	16.5	80.5	16.6
2	0.50	45.3	ND	130.0	338.9	17.9	76.6	0.0
3	0.75	45.2	3.2	128.2	340.3	17.0	77.4	0.0
4	1.00	44.5	5.1	126.1	339.1	17.2	77.0	4.4
5	1.25	43.8	5.2	124.1	338.4	16.5	62.9	4.4
6	1.50	43.0	6.1	122.1	339.0	16.9	64.2	2.2
7	1.75	44.2	7.7	121.0	336.7	16.5	70.9	1.1
8	2.00	44.0	8.0	119.7	337.9	16.8	73.9	1.1
9	2.25	43.8	8.0	118.1	336.7	16.5	73.2	4.4
10	2.50	43.3	8.0	117.0	333.9	18.2	65.3	2.2
11	2.75	43.7	8.9	116.6	343.7	18.3	63.6	2.2
12	3.00	43.9	10.4	117.0	340.6	17.6	78.1	5.5
13	4.00	43.8	11.0	115.4	337.3	16.8	73.2	3.3
14	5.00	43.0	11.4	115.6	339.8	16.2	72.4	0.0
15	6.00	43.6	11.3	112.4	338.8	16.1	66.9	1.1
16	9.00	44.3	10.9	109.4	336.0	18.1	71.7	1.1
17	12.00	43.0	7.9	102.3	337.8	16.1	66.9	2.2
18	22.00	42.8	ND	92.5	337.2	16.0	57.9	0.0

Anion data from single analyses (N = 1).
sCOD data from duplicate analyses (N = 2).
ND -- Not Detected.

Table E.11. BTX Data for Reactor Profile Experiment Conducted for Kinetics Determination on Day 280 (August 26, 1997).

		Duplicate averages					Duplicate standard deviations				
Reactor sample	Sample time (hr)	benzene (mg/L)	toluene (mg/L)	*p* -xylene (mg/L)	*m* -xylene (mg/L)	*o* -xylene (mg/L)	benzene (mg/L)	toluene (mg/L)	*p* -xylene (mg/L)	*m* -xylene (mg/L)	*o* -xylene (mg/L)
0	0.00	4.47	4.09	5.66	4.40	5.60	0.03	0.00	0.02	0.02	0.02
1	0.17	4.28	3.77	5.48	4.13	5.39	0.12	0.06	0.13	0.03	0.08
2	0.33	4.17	3.46	5.38	3.99	5.28	0.01	0.01	0.05	0.00	0.05
3	0.50	4.14	3.13	5.27	3.79	5.18	0.11	0.10	0.08	0.09	0.10
4	0.67	4.16	2.88	5.28	3.70	5.26	0.04	0.02	0.01	0.05	0.08
5	0.83	4.19	2.40	5.23	3.39	5.14	0.02	0.04	0.19	0.10	0.23
6	1.00	4.14	2.00	5.26	3.19	5.17	0.10	0.06	0.12	0.09	0.15
7	1.08	4.07	1.81	5.20	3.04	5.08	0.12	0.06	0.20	0.05	0.27
8	1.17	4.06	1.60	5.19	2.89	5.05	0.11	0.05	0.10	0.11	0.11
9	1.25	3.98	1.32	5.10	2.60	4.90	0.07	0.01	0.07	0.05	0.09
10	1.33	3.97	1.25	5.08	2.52	4.88	0.07	0.04	0.15	0.11	0.13
11	1.42	4.01	1.10	5.16	2.45	4.90	0.09	0.03	0.11	0.04	0.07
12	1.50	3.98	0.97	5.13	2.24	4.89	0.03	0.05	0.04	0.04	0.05
13	1.58	3.89	0.88	5.04	2.15	4.84	0.10	0.00	0.09	0.02	0.07
14	1.67	3.96	0.79	5.16	2.03	4.89	0.07	0.01	0.15	0.07	0.19
15	1.75	3.81	0.64	4.87	1.78	4.62	0.09	0.01	0.07	0.02	0.09
16	1.83	3.86	0.59	4.97	1.71	4.64	0.07	0.01	0.08	0.04	0.09
17	1.92	3.84	0.54	4.91	1.62	4.58	0.13	0.03	0.08	0.01	0.07
18	2.00	3.81	0.46	4.78	1.45	4.49	0.03	0.03	0.01	0.03	0.05
19	2.08	3.90	0.46	5.00	1.45	4.64	0.11	0.01	0.30	0.10	0.27
20	2.17	3.71	0.37	4.76	1.26	4.40	0.05	0.02	0.01	0.04	0.01
21	2.25	3.66	0.41	4.74	1.26	4.39	0.08	0.03	0.11	0.04	0.09
22	2.33	3.66	0.31	4.72	1.18	4.37	0.11	0.01	0.11	0.04	0.12
23	2.42	3.61	0.29	4.67	1.09	4.26	0.11	0.01	0.11	0.02	0.09
24	2.50	3.59	0.24	4.60	0.99	4.26	0.07	0.01	0.09	0.04	0.09
25	2.58	3.55	0.21	4.55	0.89	4.15	0.07	0.01	0.09	0.01	0.11
26	2.67	3.51	0.18	4.59	0.81	4.17	0.08	0.01	0.15	0.00	0.07
27	2.75	3.51	0.16	4.51	0.76	4.09	0.11	0.01	0.09	0.03	0.08
28	2.83	3.42	0.15	4.50	0.68	3.99	0.03	0.01	0.02	0.04	0.00
29	2.92	3.40	0.11	4.43	0.61	3.91	0.13	0.01	0.15	0.02	0.07
30	3.00	3.41	0.08	4.38	0.52	3.88	0.10	0.01	0.15	0.04	0.16
31	3.17	3.39	0.10	4.38	0.46	3.81	0.03	0.01	0.11	0.00	0.07
32	3.33	3.40	0.03	4.33	0.36	3.77	0.02	***	0.03	0.03	0.01
33	3.50	3.34	0.07	4.32	0.31	3.72	0.05	***	0.05	0.01	0.01
34	3.67	3.27	ND	4.30	0.24	3.65	0.04	***	0.04	0.02	0.02
35	3.83	3.25	ND	4.21	0.17	3.58	0.00	***	0.05	0.00	0.07
36	4.00	3.19	ND	4.14	0.15	3.48	0.05	***	0.16	0.00	0.12
37	4.50	3.11	ND	4.03	ND	3.33	0.03	***	0.10	***	0.05
38	5.00	3.01	ND	3.92	ND	3.22	0.09	***	0.07	***	0.07
39	6.00	2.96	ND	3.92	ND	3.05	0.03	***	0.07	***	0.02
40	8.00	2.77	ND	3.68	ND	2.98	0.02	***	0.07	***	0.02
41	10.00	2.58	ND	3.59	ND	2.95	0.06	***	0.05	***	0.07
42	12.00	2.46	ND	3.54	ND	2.93	0.06	***	0.09	***	0.09
43	14.00	2.31	ND	3.36	ND	2.82	0.06	***	0.09	***	0.12
44	18.00	2.04	ND	3.16	ND	2.75	0.09	***	0.09	***	0.02
45	22.17	1.76	ND	2.97	ND	2.72	0.02	***	0.05	***	0.02

All data from duplicate analyses (N = 2).
*** -- Compound not detected, therefore standard deviations could not be computed.
ND -- Not Detected.

Table E.12. Anion and sCOD Data for Reactor Profile Experiment for Kinetics Determination Conducted on Day 280 (August 26, 1997)>

Reactor sample	Sample time (hr)	chloride (mg/L)	nitrite-N (mg/L)	nitrate-N (mg/L)	phosphate-P (mg/L)	sulfate (mg/L)	average COD (mg/L)	st. dev. COD (mg/L)
0	0.0	81.7	43.2	161.6	92.3	35.4	427.3	10.9
1	0.3	78.9	92.0	101.4	91.3	35.9	200.3	3.8
2	0.5	81.8	117.5	64.0	91.7	35.8	92.5	2.6
3	0.8	81.9	131.3	41.6	93.9	36.8	81.8	0.4
4	1.0	78.3	122.7	52.5	88.5	35.2	86.5	15.4
5	1.3	79.6	134.1	37.7	92.8	35.3	41.0	40.6
6	1.5	80.6	136.3	32.3	91.3	34.4	70.0	1.1
7	1.8	79.6	137.6	27.6	90.4	34.4	89.6	0.0
8	2.0	79.9	138.0	23.8	89.8	34.3	83.9	10.1
9	2.3	81.2	139.3	21.6	91.8	34.1	70.9	5.6
10	2.5	79.9	138.9	18.9	91.8	34.1	75.0	***
11	2.8	80.9	138.6	17.0	93.9	33.6	86.2	***
12	3.0	81.1	137.4	15.3	92.2	33.0	84.1	10.1
13	3.3	81.9	136.9	13.9	93.3	33.5	61.9	3.4
14	3.5	82.9	135.2	12.6	91.4	33.8	***	***
15	3.8	82.0	134.8	11.9	91.2	33.3	43.2	3.4
16	4.0	81.4	133.4	10.8	91.9	32.9	57.9	3.4
17	4.5	80.8	130.8	9.5	92.2	32.6	49.0	3.4
18	5.0	78.8	128.1	8.5	91.9	32.3	43.2	1.1
19	6.0	81.2	123.4	6.9	93.2	33.2	72.2	40.6
20	8.0	80.1	113.7	4.9	91.3	32.1	49.8	5.6
21	10.0	81.3	105.8	3.8	91.9	31.5	66.8	0.0
22	12.0	83.0	97.6	3.8	91.9	31.6	57.6	2.3
23	14.0	81.6	89.1	3.8	93.5	31.5	62.0	1.1
24	18.0	81.5	75.6	3.8	91.4	31.7	54.0	3.4
25	22.2	83.9	63.5	3.8	92.3	32.4	56.0	4.5

Anion data from single analyses (N = 1).
sCOD data from duplicate analyses (N = 2).
*** -- One or both of the duplicate samples lost. If only one sample was lost, standard deviations could not be computed, but the value from the single analysis is presented.

Table E.13. BTX Data for Reactor Profile Experiment for Kinetics Determination Conducted on Day 416 (January 9, 1998).

		Duplicate averages					Duplicate standard deviations				
Reactor sample	Sample time (hr)	benzene (mg/L)	toluene (mg/L)	*p*-xylene (mg/L)	*m*-xylene (mg/L)	*o*-xylene (mg/L)	benzene (mg/L)	toluene (mg/L)	*p*-xylene (mg/L)	*m*-xylene (mg/L)	*o*-xylene (mg/L)
0	0.00	4.67	4.78	4.02	3.25	4.22	0.06	0.14	0.24	0.19	0.23
1	0.17	4.42	4.39	3.77	3.04	4.71	0.12	0.27	0.26	0.24	0.68
2	0.33	4.70	4.39	4.36	3.18	4.80	0.20	0.03	0.49	0.02	0.80
3	0.50	4.31	3.87	3.82	2.92	4.01	0.10	0.34	0.30	0.30	0.31
4	0.67	4.20	3.60	3.65	2.71	3.85	0.15	0.33	0.36	0.31	0.38
5	0.83	4.21	3.41	3.69	2.67	3.96	0.19	0.45	0.45	0.37	0.45
6	1.00	4.19	2.97	3.66	2.46	3.87	0.19	0.29	0.38	0.27	0.36
7	1.17	3.88	2.47	3.39	2.18	3.53	0.13	0.05	0.09	0.09	0.09
8	1.33	4.39	2.37	3.95	2.32	4.06	0.14	0.11	0.07	0.14	0.17
9	1.50	3.84	1.75	3.20	1.83	3.47	0.12	0.05	0.16	0.04	0.06
10	1.67	4.01	1.51	3.44	1.75	3.57	0.22	0.24	0.37	0.19	0.37
11	1.83	3.74	1.12	3.24	1.50	3.36	0.13	0.04	0.11	0.11	0.07
12	2.00	3.99	1.06	3.47	1.47	3.55	0.19	0.17	0.47	0.28	0.47
13	2.17	3.49	0.76	3.03	1.18	3.13	0.36	0.06	0.15	0.01	0.13
14	2.33	3.35	0.63	3.00	1.10	3.05	0.49	0.02	0.12	0.03	0.08
15	2.50	3.69	0.54	3.16	1.01	3.25	0.05	0.03	0.14	0.14	0.16
16	2.67	3.64	0.50	3.17	0.94	3.25	0.74	0.05	0.39	0.02	0.38
17	2.83	3.92	0.50	3.33	0.96	3.41	1.07	0.08	0.70	0.16	0.67
18	3.00	3.42	0.38	2.94	0.79	2.99	0.33	0.02	0.15	0.05	0.12
19	3.17	3.38	0.32	2.90	0.74	2.96	0.29	0.02	0.10	0.10	0.08
20	3.33	3.32	0.30	2.84	0.73	2.87	0.29	0.00	0.15	0.02	0.13
21	3.50	3.33	0.28	2.86	0.62	2.89	0.26	0.02	0.16	0.08	0.13
22	3.67	3.36	0.25	2.89	0.61	2.92	0.28	0.01	0.17	0.03	0.17
23	3.83	3.34	0.22	2.89	0.57	2.88	0.31	0.00	0.21	0.06	0.15
24	4.00	3.28	0.19	2.81	0.51	2.84	0.32	0.00	0.15	0.05	0.09
25	4.50	3.28	0.18	2.80	0.44	2.82	0.28	0.01	0.13	0.09	0.10
26	5.00	3.18	0.11	2.76	0.32	2.78	0.26	0.01	0.13	0.13	0.12
27	5.50	3.11	0.10	2.69	0.31	2.71	0.28	0.00	0.16	0.09	0.10
28	6.00	3.08	0.08	2.67	0.28	2.71	0.26	0.04	0.12	0.07	0.10
29	7.00	2.97	0.05	2.60	0.22	2.63	0.25	0.03	0.09	0.13	0.09
30	8.00	2.88	0.04	2.53	0.20	2.62	0.25	0.01	0.08	0.13	0.05
31	10.00	2.71	-0.03	2.43	0.15	2.57	0.19	0.03	0.06	0.10	0.04
32	12.00	2.53	-0.03	2.25	0.15	2.46	0.20	0.03	0.07	0.10	0.07
33	14.00	2.45	-0.03	2.20	0.12	2.48	0.17	0.03	0.04	0.12	0.04
34	22.00	2.08	ND	1.89	ND	2.34	0.17	***	0.04	***	0.06

All data from duplicate analyses (N = 2).
ND -- Not Detected.
*** -- Compound not detected, therefore standard deviations could not be computed.

Table E.14. Anion and sCOD Data for Reactor Profile Experiment for Kinetics Determination Conducted on Day 416 (January 9, 1998).

Reactor sample	Sample time (hr)	chloride (mg/L)	nitrite-N (mg/L)	nitrate-N (mg/L)	phosphate-P (mg/L)	sulfate (mg/L)	average COD (mg/L)	st. dev. COD (mg/L)
0	0.00	76.8	107.6	166.6	112.4	37.2	555.0	5.6
1	0.25	74.1	156.3	115.1	111.6	36.2	343.5	5.6
2	0.50	75.0	192.3	75.3	111.9	37.1	216.8	0.0
3	0.75	74.5	197.2	65.6	111.9	36.7	203.8	1.1
4	1.00	75.3	203.8	56.8	112.2	37.8	184.1	0.0
5	1.25	74.0	206.5	49.8	112.1	35.4	185.9	3.3
6	1.50	73.0	209.5	44.1	111.7	34.9	193.0	3.3
7	1.75	74.9	211.5	39.7	111.3	36.2	180.8	***
8	2.00	74.3	213.9	34.8	111.1	36.2	190.4	3.3
9	2.25	75.4	216.6	31.5	111.5	37.0	191.3	5.6
10	2.50	73.1	217.7	27.5	111.7	34.2	184.8	3.3
11	2.75	73.2	217.8	24.3	111.9	43.1	183.2	5.6
12	3.00	72.9	218.9	22.0	111.3	35.7	195.5	1.1
13	3.25	73.7	218.5	19.9	111.5	35.1	217.7	1.1
14	3.50	74.1	219.5	18.0	111.1	34.7	186.7	0.0
15	3.75	73.5	219.8	16.7	111.7	41.6	186.7	2.2
16	4.00	73.3	219.6	14.6	111.1	33.6	186.7	***
17	4.50	73.6	219.5	12.2	111.5	33.4	192.2	1.1
18	5.00	74.0	217.9	10.3	111.0	33.2	193.4	2.2
19	5.50	74.7	216.8	8.9	111.6	33.4	191.3	1.1
20	6.00	76.3	213.8	7.4	111.4	35.1	188.1	0.0
21	7.00	73.6	211.2	5.7	110.5	32.5	190.8	2.2
22	8.00	73.9	206.9	4.3	110.7	32.1	130.3	0.0
23	10.00	75.7	199.5	2.3	110.2	32.5	115.5	3.3
24	12.00	76.7	193.7	1.3	111.3	34.2	128.7	0.0
25	14.00	76.3	185.5	1.0	110.4	34.5	126.7	1.1
26	22.00	77.2	156.0	1.0	111.1	32.6	157.3	1.1

Anion data from single analyses (N = 1).
sCOD data from duplicate analyses (N = 2).
*** -- Duplicate sample lost, therefore standard deviations could not be computed.

Table E.15. BTX Data for Reactor Profile Experiment for Kinetics Determination Conducted on Day 466 (February 28, 1998).

		Duplicate averages					Duplicate standard deviations				
Reactor sample	Sample time (hr)	benzene (mg/L)	toluene (mg/L)	*p*-xylene (mg/L)	*m*-xylene (mg/L)	*o*-xylene (mg/L)	benzene (mg/L)	toluene (mg/L)	*p*-xylene (mg/L)	*m*-xylene (mg/L)	*o*-xylene (mg/L)
0	0.00	4.29	3.82	4.33	4.22	4.46	0.15	0.13	0.09	0.06	0.08
1	0.17	4.11	3.42	4.06	3.94	4.25	0.10	0.11	0.09	0.10	0.07
2	0.33	4.11	3.11	4.09	3.84	4.20	0.19	0.18	0.12	0.16	0.14
3	0.52	4.02	2.77	4.04	3.63	4.17	0.13	0.12	0.06	0.05	0.08
4	0.67	3.99	2.28	4.00	3.40	4.09	0.10	0.08	0.09	0.05	0.05
5	0.83	3.90	1.67	3.93	3.09	4.00	0.07	0.08	0.02	0.05	0.02
6	1.00	3.87	1.04	3.85	2.70	3.82	0.06	0.01	0.01	0.02	0.01
7	1.17	3.80	0.67	3.75	2.36	3.67	0.11	0.08	0.07	0.07	0.13
8	1.33	3.77	0.41	3.76	2.02	3.55	0.12	0.05	0.08	0.09	0.07
9	1.50	3.83	0.23	3.78	1.68	3.48	0.01	0.03	0.06	0.01	0.03
10	1.67	3.74	0.12	3.70	1.36	3.30	0.03	0.04	0.00	0.02	0.02
11	1.83	3.73	0.04	3.66	1.03	3.16	0.01	0.04	0.03	0.01	0.08
12	2.00	3.77	0.02	3.69	0.73	3.10	0.10	0.04	0.13	0.04	0.08
13	2.17	3.73	-0.01	3.63	0.51	2.94	0.01	0.04	0.01	0.04	0.00
14	2.33	3.68	ND	3.57	0.29	2.84	0.04	***	0.01	0.04	0.02
15	2.50	3.74	ND	3.58	0.13	2.75	0.11	***	0.07	0.05	0.03
16	2.67	3.65	ND	3.47	0.05	2.65	0.17	***	0.14	0.03	0.09
17	2.83	3.67	ND	3.50	ND	2.57	0.15	***	0.13	***	0.05
18	3.00	3.66	ND	3.46	ND	2.48	0.09	***	0.09	***	0.09
19	3.17	3.61	ND	3.41	ND	2.42	0.15	***	0.13	***	0.14
20	3.33	3.59	ND	3.38	ND	2.33	0.07	***	0.04	***	0.01
21	3.50	3.60	ND	3.41	ND	2.33	0.08	***	0.08	***	0.04
22	3.67	3.56	ND	3.34	ND	2.25	0.01	***	0.01	***	0.01
23	3.83	3.55	ND	3.35	ND	2.18	0.09	***	0.05	***	0.05
24	4.00	3.54	ND	3.31	ND	2.14	0.05	***	0.06	***	0.05
25	4.50	3.56	ND	3.28	ND	2.04	0.00	***	0.02	***	0.05
26	5.00	3.56	ND	3.25	ND	1.96	0.03	***	0.01	***	0.03
27	5.50	3.53	ND	3.21	ND	1.85	0.00	***	0.06	***	0.05
28	6.00	3.50	ND	3.19	ND	1.83	0.02	***	0.02	***	0.02
29	7.00	3.46	ND	3.17	ND	1.73	0.02	***	0.02	***	0.01
30	9.00	3.41	ND	3.03	ND	1.66	0.00	***	0.00	***	0.02
31	12.50	3.18	ND	2.68	ND	1.57	0.00	***	0.02	***	0.01
32	16.00	3.02	ND	2.39	ND	1.52	0.01	***	0.02	***	0.01
33	20.17	2.68	ND	1.82	ND	1.53	0.02	***	0.02	***	0.02

All data from duplicate analyses (N = 2).
ND -- Not Detected.
*** -- Compound not detected, therefore standard deviations could not be computed.

Table E.16. Anion and sCOD Data for Reactor Profile Experiment for Kinetics Determination Conducted on Day 466 (February 28, 1998).

Reactor sample	Sample time (hr)	chloride (mg/L)	nitrite-N (mg/L)	nitrate-N (mg/L)	phosphate-P (mg/L)	sulfate (mg/L)	average COD (mg/L)	st. dev. COD (mg/L)
0	0.00	81.8	40.6	154.8	103.2	21.8	413.7	0.0
1	0.25	79.8	94.6	105.9	103.1	22.4	175.0	5.8
2	0.50	80.1	115.6	80.0	103.7	22.7	119.1	1.2
3	1.00	80.0	126.1	60.1	104.0	22.0	92.0	3.5
4	1.50	81.4	131.0	48.5	102.8	21.7	77.7	8.1
5	2.00	80.4	132.4	40.7	103.3	21.4	77.3	5.8
6	2.50	81.3	132.8	34.6	102.3	21.1	74.0	1.2
7	3.00	79.5	130.2	30.5	99.0	20.8	75.4	4.6
8	3.50	80.7	130.4	28.1	100.7	20.8	77.0	2.3
9	4.00	81.5	128.7	26.4	103.2	21.0	77.4	2.3
10	4.50	81.9	126.9	24.5	104.2	20.3	81.1	0.0
11	5.00	81.1	124.6	22.5	101.1	20.2	70.9	5.8
12	5.50	81.3	123.2	21.3	102.1	20.6	77.0	2.3
13	6.00	82.4	120.7	19.9	102.1	20.1	76.0	0.0
14	7.00	80.9	116.7	18.1	101.7	19.4	69.5	1.2
15	9.00	82.6	111.1	14.9	101.6	19.2	76.6	2.3
16	12.50	81.3	101.2	10.1	100.3	18.8	72.2	8.9
17	16.00	82.5	93.9	7.0	101.1	18.8	74.7	1.1
18	20.17	82.9	81.4	2.8	101.2	18.7	68.2	1.1

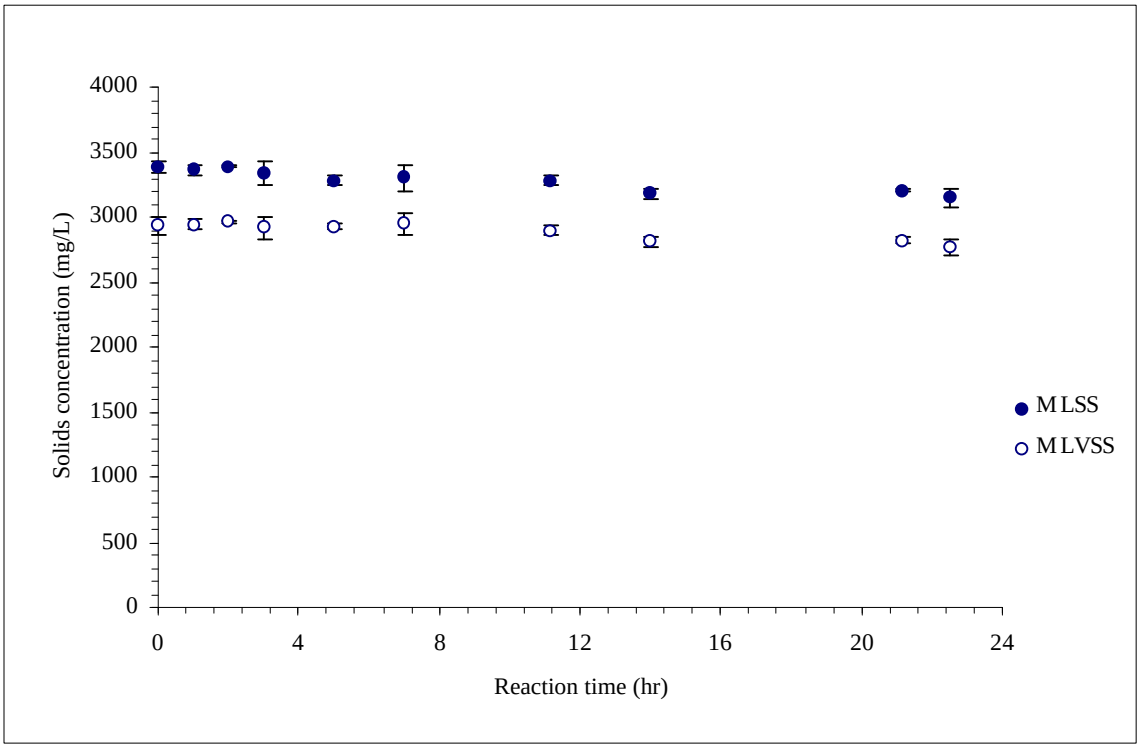
Anion data from single analyses (N = 1).
sCOD data from duplicate analyses (N = 2).

Table E.17. Mixed-Liquor-Suspended-Solids (MLSS) and Mixed-Liquor-Volatile-Suspended-Solids (MLVSS) Profiles Across the Reaction Cycle on Day 383 (December 7, 1997).

Sample time (hr)	average MLSS (mg/L)	st. dev. MLSS (mg/L)	average MLVSS (mg/L)	st. dev. MLVSS (mg/L)
0.00	3383	49	2937	70
1.00	3363	35	2943	35
2.00	3390	10	2967	6
3.00	3340	95	2920	82
5.00	3283	35	2930	26
7.00	3307	101	2947	86
11.17	3283	35	2897	38
14.00	3180	36	2813	38
21.17	3207	6	2817	23
22.50	3147	70	2767	65

All data from triplicate analyses (N = 3).

Figure E.1. Mixed-Liquor-Suspended-Solids (MLSS) and Mixed-Liquor-Volatile-Suspended-Solids (MLVSS) Concentration Profiles Across the Reaction Cycle on Day 383 (December 7, 1997). Error Bars Represent one standard deviation of Triplicate Samples.



APPENDIX F:

ANCILLARY REACTOR HISTORICAL DATA

Table F.1. Reactor Total and Volatile (TSS and VSS) History.

Sample ID	Sample date	Day after acclimation	Sample type	TSS (mg/L)	VSS (mg/L)	average TSS (mg/L)	std. dev. TSS (mg/L)	average VSS (mg/L)	std. dev. VSS (mg/L)	Wastage for 15 day SRT (mL)
B	11/19/96	0	MLSS	2510	***					
D	11/19/96	0	MLSS	2380	***	2445	92	***	***	
EF1	11/19/96	0	effluent TSS	14.3	***					
I	11/19/96	0	effluent TSS	16.0	***	15.2	1.2	***	***	326
K1	11/20/96	1	MLSS	2750	***					
2	11/20/96	1	MLSS	3090	***	2920	240	***	***	
1	11/20/96	1	effluent TSS	24.0	***	24.0	***	***	***	323
B	11/21/96	2	MLSS	3300	***					
K2	11/21/96	2	MLSS	2980	***	3140	226	***	***	
D	11/21/96	2	effluent TSS	25.3	***					
K5	11/21/96	2	effluent TSS	23.8	***	24.6	1.1	***	***	324
F	12/2/96	13	MLSS	2950	***					
J	12/2/96	13	MLSS	3350	***					
I	12/2/96	13	MLSS	3320	***	3207	223	***	***	
3	12/2/96	13	effluent TSS	70.0	***					
E	12/2/96	13	effluent TSS	58.0	***	64.0	8.5	***	***	308
K6	12/4/96	15	MLSS	2920	***					
K4	12/4/96	15	MLSS	2905	***	2913	11	***	***	
EF1	12/4/96	15	effluent TSS	28.8	***					
G	12/4/96	15	effluent TSS	28.5	***	28.7	0.2	***	***	321
EF3	12/10/96	21	MLSS	3035	***					
A	12/10/96	21	MLSS	3175	***	3105	99	***	***	
EF2	12/10/96	21	effluent TSS	53.8	***					
C	12/10/96	21	effluent TSS	46.9	***	50.4	4.9	***	***	313
H	12/19/96	30	MLSS	2980	***					
B	12/19/96	30	MLSS	3140	***	3060	113	***	***	
E	12/19/96	30	effluent TSS	41.0	***					
EF2	12/19/96	30	effluent TSS	39.0	***	40.0	1.4	***	***	317
K4	12/22/96	33	MLSS	3345	***					
I	12/22/96	33	MLSS	2865	***	3105	339	***	***	
3	12/22/96	33	effluent TSS	26.0	***					
K1	12/22/96	33	effluent TSS	23.3	***	24.7	1.9	***	***	323
A	12/28/96	39	MLSS	2800	***					
C	12/28/96	39	MLSS	2715	***	2758	60	***	***	
D	12/28/96	39	effluent TSS	34.0	***					
J	12/28/96	39	effluent TSS	47.0	***	40.5	9.2	***	***	315
K6	12/30/96	41	MLSS	2600	***					
F	12/30/96	41	MLSS	2805	***	2703	145	***	***	
K2	12/30/96	41	effluent TSS	36.0	***					
EF3	12/30/96	41	effluent TSS	24.0	***	30.0	8.5	***	***	319
K5	1/3/97	45	MLSS	3065	***					
1	1/3/97	45	MLSS	2855	***	2960	148	***	***	
2	1/3/97	45	effluent TSS	14.5	***					
K3	1/3/97	45	effluent TSS	13.0	***	13.8	1.1	***	***	328

Table F.1. Continued...

Sample ID	Sample date	Day after acclimation	Sample type	TSS (mg/L)	VSS (mg/L)	average TSS (mg/L)	std. dev. TSS (mg/L)	average VSS (mg/L)	std. dev. VSS (mg/L)	Wastage for 15 day SRT (mL)
EF1	1/7/97	49	MLSS	2875	***					
K3	1/7/97	49	MLSS	3075	***	2975	141	***	***	
G	1/7/97	49	effluent TSS	17.8	***					
K2	1/7/97	49	effluent TSS	17.5	***	17.7	0.2	***	***	326
H	1/9/97	51	MLSS	2930	***					
K1	1/9/97	51	MLSS	2940	***	2935	7	***	***	
EF2	1/9/97	51	effluent TSS	18.8	***					
EF3	1/9/97	51	effluent TSS	20.0	***	19.4	0.8	***	***	325
2	1/13/97	55	MLSS	2935	***					
3	1/13/97	55	MLSS	2965	***	2950	21	***	***	
K6	1/13/97	55	effluent TSS	16.8	***					
D	1/13/97	55	effluent TSS	16.8	***	16.8	0.0	***	***	326
K1	1/17/97	59	MLSS	2695	***					
B	1/17/97	59	MLSS	3040	***	2868	244	***	***	
C	1/17/97	59	effluent TSS	22.5	***					
E	1/17/97	59	effluent TSS	22.8	***	22.7	0.2	***	***	323
A	1/20/97	62	MLSS	3390	***					
K5	1/20/97	62	MLSS	3025	***	3208	258	***	***	
F	1/20/97	62	effluent TSS	26.0	***					
J	1/20/97	62	effluent TSS	25.5	***	25.8	0.4	***	***	323
1	1/24/97	66	MLSS	3040	***					
I	1/24/97	66	MLSS	3210	***	3125	120	***	***	
A	1/24/97	66	effluent TSS	12.3	***					
K6	1/24/97	66	effluent TSS	17.5	***	14.9	3.7	***	***	327
J	1/29/97	71	MLSS	3545	***					
E	1/29/97	71	MLSS	3355	***	3450	134	***	***	
G	1/29/97	71	effluent TSS	13.0	***					
B	1/29/97	71	effluent TSS	16.3	***	14.7	2.3	***	***	328
EF3	2/3/97	76	MLSS	3430	***					
H	2/3/97	76	MLSS	3205	***	3318	159	***	***	
2	2/3/97	76	effluent TSS	13.5	***					
D	2/3/97	76	effluent TSS	16.2	***	14.9	1.9	***	***	328
EF2	2/11/97	84	MLSS	3335	***					
K5	2/11/97	84	MLSS	3295	***	3315	28	***	***	
EF1	2/11/97	84	effluent TSS	6.7	***					
K2	2/11/97	84	effluent TSS	6.0	***	6.4	0.5	***	***	331
F	2/18/97	91	MLSS	3150	***					
3	2/18/97	91	MLSS	3245	***	3198	67	***	***	
K4	2/18/97	91	effluent TSS	15.7	***					
K3	2/18/97	91	effluent TSS	23.2	***	19.5	5.3	***	***	326
K2	3/4/97	105	MLSS	2710	***					
C	3/4/97	105	MLSS	2660	***	2685	35	***	***	
K5	3/4/97	105	effluent TSS	20.7	***					
EF1	3/4/97	105	effluent TSS	21.0	***	20.9	0.2	***	***	324

Table F.1. Continued...

Sample ID	Sample date	Day after acclimation	Sample type	TSS (mg/L)	VSS (mg/L)	average TSS (mg/L)	std. dev. TSS (mg/L)	average VSS (mg/L)	std. dev. VSS (mg/L)	Wastage for 15 day SRT (mL)
I	3/11/97	112	MLSS	2535	***					
EF3	3/11/97	112	MLSS	2690	***	2613	110	***	***	
J	3/11/97	112	effluent TSS	15.0	***					
1	3/11/97	112	effluent TSS	17.5	***	16.2	1.8	***	***	326
B	3/14/97	115	MLSS	2655	***					
EF2	3/14/97	115	MLSS	2905	***	2780	177	***	***	
K6	3/17/97	118	MLSS	3010	***					
K1	3/17/97	118	MLSS	2935	***	2973	53	***	***	
D	3/17/97	118	effluent TSS	15.5	***					
E	3/17/97	118	effluent TSS	9.8	***	12.6	4.1	***	***	328
G	3/21/97	122	MLSS	2995	***					
H	3/21/97	122	MLSS	2915	***	2955	57	***	***	
2	3/21/97	122	effluent TSS	15.3	***					
A	3/21/97	122	effluent TSS	15.3	***	15.3	0.0	***	***	327
D	3/24/97	125	MLSS	3030	2770					
A	3/24/97	125	MLSS	3060	2805	3045	21	2788	25	
K1	3/24/97	125	effluent TSS	14.8	11.8					
EF3	3/24/97	125	effluent TSS	15.3	13.5	15.0	0.4	12.6	1.2	327
EF2	3/27/97	128	MLSS	2985	2735					
E	3/27/97	128	MLSS	3100	2815	3043	81	2775	57	
K3	3/27/97	128	effluent TSS	10.5	9.3					
2	3/27/97	128	effluent TSS	10.5	9.5	10.5	0.0	9.4	0.2	329
K6	3/31/97	132	MLSS	3015	2745					
K2	3/31/97	132	MLSS	3070	2810	3043	39	2777	46	
G	3/31/97	132	effluent TSS	15.7	14.3					
1	3/31/97	132	effluent TSS	15.0	13.7	15.4	0.5	14.0	0.4	327
A3	4/3/97	135	MLSS	3715	2875					
A4	4/3/97	135	MLSS	3730	2860	3723	11	2868	11	
3	4/3/97	135	effluent TSS	15.7	15.3					
I	4/3/97	135	effluent TSS	18.8	17.3	17.3	2.1	16.3	1.4	328
F	4/8/97	140	MLSS	3490	3200					
2	4/8/97	140	MLSS	3810	3195	3650	226	3198	4	
2	4/11/97	143	MLSS	3365	3085	3365	***	3085	***	
E	4/15/97	147	MLSS	3140	2860	3140	***	2860	***	
2	4/18/97	150	MLSS	2890	2595					
A13	4/18/97	150	MLSS	2950	2650	2920	42	2623	39	
B3	4/18/97	150	effluent TSS	17.2	14.0					
B8	4/18/97	150	effluent TSS	22.0	19.0	19.6	3.4	16.5	3.5	325
H	4/21/97	153	MLSS	2950	2670					
K6	4/21/97	153	MLSS	3060	2775	3005	78	2723	74	
K3	4/21/97	153	effluent TSS	23.8	20.0					
K2	4/21/97	153	effluent TSS	19.5	18.0	21.6	3.0	19.0	1.4	324

Table F.1. Continued...

Sample ID	Sample date	Day after acclimation	Sample type	TSS (mg/L)	VSS (mg/L)	average TSS (mg/L)	std. dev. TSS (mg/L)	average VSS (mg/L)	std. dev. VSS (mg/L)	Wastage for 15 day SRT (mL)
A	4/28/97	160	MLSS	3180	2915					
EF3	4/28/97	160	MLSS	3175	2905	3177	4	2910	7	
F	4/28/97	160	effluent TSS	20.5	20.0					
B5	4/28/97	160	effluent TSS	22.5	20.5	21.5	1.4	20.3	0.4	325
B7	5/2/97	164	MLSS	2670	2250					
I	5/2/97	164	MLSS	2715	2435	2693	32	2343	131	
B1	5/2/97	164	effluent TSS	6.7	4.8					
C	5/2/97	164	effluent TSS	9.2	8.0	8.0	1.8	6.4	2.3	330
EF1	5/9/97	171	MLSS	3165	2860					
B	5/9/97	171	MLSS	3100	2810	3133	46	2835	35	
K4	5/9/97	171	effluent TSS	11.7	11.5					
J	5/9/97	171	effluent TSS	12.5	12.2	12.1	0.5	11.9	0.5	328
3	5/16/97	178	MLSS	3185	2875					
B6	5/16/97	178	MLSS	3025	2745	3105	113	2810	92	
2	5/16/97	178	effluent TSS	25.5	23.3	25.5	***	23.3	***	323
B	5/27/97	189	MLSS	3370	3025					
A	5/27/97	189	MLSS	3305	2985	3338	46	3005	28	
B7	5/29/97	191	MLSS	3375	3035					
J	5/29/97	191	MLSS	3370	3020	3372	4	3028	11	
A13	5/29/97	191	effluent TSS	28.8	25.7					
D	5/29/97	191	effluent TSS	31.5	27.0	30.1	1.9	26.4	0.9	322
K5	6/4/97	197	MLSS	3200	2860					
B5	6/4/97	197	MLSS	3255	2925	3227	39	2893	46	
B3	6/4/97	197	effluent TSS	15.0	12.7					
B8	6/4/97	197	effluent TSS	14.0	11.5	14.5	0.7	12.1	0.9	328
F	6/6/97	199	MLSS	3340	3010					
E	6/6/97	199	MLSS	3295	2940	3318	32	2975	49	
EF3	6/6/97	199	effluent TSS	20.7	18.8					
EF1	6/6/97	199	effluent TSS	21.0	19.5	20.9	0.2	19.1	0.5	325
K1	6/9/97	202	MLSS	3110	2795					
B1	6/9/97	202	MLSS	3215	2905	3163	74	2850	78	
I	6/19/97	212	MLSS	3055	2710					
A0	6/19/97	212	MLSS	3060	2720	3058	4	2715	7	
2	6/19/97	212	effluent TSS	45.8	38.2					
K4	6/19/97	212	effluent TSS	43.5	36.0	44.6	1.6	37.1	1.6	315
EF1	6/21/97	214	MLSS	3260	2940					
C	6/21/97	214	MLSS	3360	2985	3310	71	2963	32	
G	6/21/97	214	effluent TSS	21.3	6.5					
1	6/21/97	214	effluent TSS	22.8	29.5	22.0	1.1	18.0	16.3	325
K1	7/1/97	224	MLSS	2930	2590					
K5	7/1/97	224	MLSS	2995	2655	2963	46	2623	46	
B3	7/1/97	224	effluent TSS	31.5	26.5					
B4	7/1/97	224	effluent TSS	31.0	23.0	31.2	0.4	24.7	2.5	320

Table F.1. Continued...

Sample ID	Sample date	Day after acclimation	Sample type	TSS (mg/L)	VSS (mg/L)	average TSS (mg/L)	std. dev. TSS (mg/L)	average VSS (mg/L)	std. dev. VSS (mg/L)	Wastage for 15 day SRT (mL)
F	7/7/97	230	MLSS	3335	2910					
B	7/7/97	230	MLSS	3110	2715	3223	159	2813	138	
K2	7/7/97	230	effluent TSS	20.3	16.5					
K3	7/7/97	230	effluent TSS	20.7	17.2	20.5	0.4	16.9	0.5	325
B5	7/28/97	251	MLSS	3035	***					
1	7/28/97	251	MLSS	2900	***	2968	95	***	***	
A0	7/28/97	251	effluent TSS	21.0	***					
I	7/28/97	251	effluent TSS	20.7	***	20.9	0.2	***	***	325
EF3	8/2/97	256	MLSS	3270	2835					
2	8/2/97	256	MLSS	3120	2850	3195	106	2843	11	
K2	8/2/97	256	effluent TSS	28.5	25.0					
EF1	8/2/97	256	effluent TSS	27.0	24.0	27.8	1.1	24.5	0.7	322
B6	8/4/97	258	MLSS	3560	3125					
B1	8/4/97	258	MLSS	3440	3055	3500	85	3090	49	
2	8/4/97	258	effluent TSS	18.2	16.0					
K3	8/4/97	258	effluent TSS	18.5	16.0	18.4	0.2	16.0	0.0	327
K5	8/6/97	260	MLSS	3205	2830					
F	8/6/97	260	MLSS	3250	2875	3228	32	2853	32	
B	8/6/97	260	effluent TSS	18.8	17.0					
K6	8/6/97	260	effluent TSS	14.5	14.0	16.6	3.0	15.5	2.1	327
A	8/8/97	262	MLSS	3230	2870					
B2	8/8/97	262	MLSS	3185	2855	3208	32	2863	11	
B3	8/8/97	262	effluent TSS	14.0	13.0					
C	8/8/97	262	effluent TSS	15.0	13.2	14.5	0.7	13.1	0.2	328
H	8/11/97	265	MLSS	3280	2950					
K5	8/11/97	265	MLSS	3350	3035	3315	49	2993	60	
A0	8/11/97	265	effluent TSS	26.3	25.0					
I	8/11/97	265	effluent TSS	29.0	26.0	27.7	1.9	25.5	0.7	323
D	8/13/97	267	MLSS	3275	2890					
B5	8/13/97	267	MLSS	3175	2810	3225	71	2850	57	
F	8/13/97	267	effluent TSS	30.0	25.8					
1	8/13/97	267	effluent TSS	31.7	27.0	30.9	1.2	26.4	0.9	321
2	8/15/97	269	MLSS	3450	3090					
K1	8/15/97	269	MLSS	3495	3130	3473	32	3110	28	
B8	8/15/97	269	effluent TSS	21.7	18.7					
J	8/15/97	269	effluent TSS	20.0	18.7	20.8	1.2	18.7	0.0	326
B6	8/18/97	272	MLSS	3060	2730					
K2	8/18/97	272	MLSS	3055	2700	3058	4	2715	21	
B	8/18/97	272	effluent TSS	27.0	22.3					
B1	8/18/97	272	effluent TSS	25.7	23.5	26.4	0.9	22.9	0.9	323
K6	8/20/97	274	MLSS	2925	2610					
B7	8/20/97	274	MLSS	3075	2745	3000	106	2678	95	
K3	8/20/97	274	effluent TSS	22.5	19.8					
E	8/20/97	274	effluent TSS	24.0	20.3	23.2	1.1	20.0	0.4	324

Table F.1. Continued...

Sample ID	Sample date	Day after acclimation	Sample type	TSS (mg/L)	VSS (mg/L)	average TSS (mg/L)	std. dev. TSS (mg/L)	average VSS (mg/L)	std. dev. VSS (mg/L)	Wastage for 15 day SRT (mL)
K3	8/22/97	276	MLSS	3050	2730					
J	8/22/97	276	MLSS	3135	2780	3093	60	2755	35	
K1	8/22/97	276	effluent TSS	32.0	27.3					
E	8/22/97	276	effluent TSS	33.2	29.3	32.6	0.9	28.3	1.4	320
B7	8/25/97	279	MLSS	3260	2925					
K6	8/25/97	279	MLSS	3390	3065	3325	92	2995	99	
B2	8/25/97	279	effluent TSS	27.7	24.7					
B1	8/25/97	279	effluent TSS	26.5	24.2	27.1	0.9	24.5	0.4	323
2	8/26/97	280	MLSS	3240	2920					
B8	8/26/97	280	MLSS	3310	2965	3275	49	2943	32	
C	8/26/97	280	effluent TSS	29.0	25.3					
B3	8/26/97	280	effluent TSS	28.5	25.3	28.7	0.4	25.3	0.0	322
K4	9/3/97	288	MLSS	3413	3013					
K2	9/3/97	288	MLSS	3460	3087	3437	33	3050	52	
B6	9/3/97	288	effluent TSS	24.4	22.0					
I	9/3/97	288	effluent TSS	28.4	24.4	26.4	2.8	23.2	1.7	324
A0	9/6/97	291	MLSS	3430	3030					
3	9/6/97	291	MLSS	3555	3130	3493	88	3080	71	
EF1	9/6/97	291	effluent TSS	20.5	17.8					
H	9/6/97	291	effluent TSS	23.0	19.3	21.8	1.8	18.5	1.1	326
B7	9/8/97	293	MLSS	3425	3040					
G	9/8/97	293	MLSS	3690	3300	3558	187	3170	184	
D	9/8/97	293	effluent TSS	18.0	16.2					
K5	9/8/97	293	effluent TSS	18.2	17.2	18.1	0.2	16.7	0.7	327
B1	9/10/97	295	MLSS	3530	3150					
I	9/10/97	295	MLSS	3540	3180	3535	7	3165	21	
C	9/10/97	295	effluent TSS	19.5	16.2					
B	9/10/97	295	effluent TSS	20.0	16.7	19.8	0.4	16.5	0.4	326
B3	9/16/97	301	MLSS	4330	3810					
E	9/16/97	301	MLSS	4060	3620	4195	191	3715	134	
K4	9/16/97	301	effluent TSS	46.5	37.5					
B5	9/16/97	301	effluent TSS	45.0	36.5	45.8	1.1	37.0	0.7	320
A	9/25/97	310	MLSS	4760	4230					
K2	9/25/97	310	MLSS	4720	4210	4740	28	4220	14	
F	9/25/97	310	effluent TSS	17.0	13.7					
1	9/25/97	310	effluent TSS	16.0	12.7	16.5	0.7	13.2	0.7	329
EF2	10/8/97	323	MLSS	4720	4130					
K1	10/8/97	323	MLSS	4840	4220	4780	85	4175	64	
B6	10/9/97	324	MLSS	4670	4090					
K3	10/9/97	324	MLSS	4690	4110	4680	14	4100	14	
H	10/23/97	338	MLSS	3620	3130					
C	10/23/97	338	MLSS	3740	3250	3680	85	3190	85	
D	10/23/97	338	effluent TSS	18.2	13.7					
3	10/23/97	338	effluent TSS	18.0	14.5	18.1	0.2	14.1	0.5	327

Table F.1. Continued...

Sample ID	Sample date	Day after acclimation	Sample type	TSS (mg/L)	VSS (mg/L)	average TSS (mg/L)	std. dev. TSS (mg/L)	average VSS (mg/L)	std. dev. VSS (mg/L)	Wastage for 15 day SRT (mL)
B	10/28/97	343	MLSS	3860	3370					
E	10/28/97	343	MLSS	3960	3410	3910	71	3390	28	
J	10/31/97	346	MLSS	3580	3080					
A	10/31/97	346	MLSS	3140	2850	3360	311	2965	163	
A0	10/31/97	346	effluent TSS	27.6	24.0					
F	10/31/97	346	effluent TSS	26.0	23.2	26.8	1.1	23.6	0.6	323
K6	11/2/97	348	MLSS	3240	2870					
B1	11/2/97	348	MLSS	3290	2910	3265	35	2890	28	
B8	11/2/97	348	effluent TSS	18.7	16.5					
I	11/2/97	348	effluent TSS	18.0	16.0	18.4	0.5	16.2	0.4	326
EF1	11/5/97	351	MLSS	3160	2850					
2	11/5/97	351	MLSS	3200	2760	3180	28	2805	64	
K3	11/5/97	351	effluent TSS	29.3	30.5					
G	11/5/97	351	effluent TSS	32.8	23.8	31.0	2.5	27.1	4.8	321
K5	11/7/97	353	MLSS	3230	2950					
B6	11/7/97	353	MLSS	3220	2940	3225	7	2945	7	
B5	11/7/97	353	effluent TSS	25.7	23.5					
B7	11/7/97	353	effluent TSS	26.5	23.8	26.1	0.5	23.6	0.2	323
K1	11/12/97	358	MLSS	3920	3570					
K2	11/12/97	358	MLSS	3570	3320	3745	247	3445	177	
EF2	11/17/97	363	MLSS	3650	3280					
B3	11/17/97	363	MLSS	3630	3270	3640	14	3275	7	
I	11/17/97	363	effluent TSS	19.8	16.2					
B1	11/17/97	363	effluent TSS	17.3	15.0	18.5	1.8	15.6	0.9	327
F	12/4/97	380	MLSS	3800	3400					
D	12/4/97	380	MLSS	3830	3400					
C	12/4/97	380	MLSS	3790	3320	3807	21	3373	46	
B8	1/9/98	416	MLSS	2640	2410					
B2	1/9/98	416	MLSS	2710	2460					
G	1/9/98	416	MLSS	2730	2430	2693	47	2433	25	
B6	1/14/98	421	MLSS	2320	2040					
2	1/14/98	421	MLSS	2180	1960	2250	99	2000	57	
A13	1/14/98	421	effluent TSS	15.3	13.1					
A0	1/14/98	421	effluent TSS	14.8	12.8	15.0	0.4	12.9	0.2	325
EF2	1/16/98	423	MLSS	2060	1830					
I	1/16/98	423	MLSS	1960	1730	2010	71	1780	71	
K6	1/16/98	423	effluent TSS	11.3	9.8					
J	1/16/98	423	effluent TSS	11.2	10.0	11.3	0.0	9.9	0.2	326
K4	1/17/98	424	MLSS	2190	1910					
I	1/17/98	424	MLSS	2200	1930	2195	7	1920	14	
F	1/17/98	424	effluent TSS	11.7	9.8					
1	1/17/98	424	effluent TSS	11.2	9.8	11.5	0.4	9.8	0.0	327

Table F.1. Continued...

Sample ID	Sample date	Day after acclimation	Sample type	TSS (mg/L)	VSS (mg/L)	average TSS (mg/L)	std. dev. TSS (mg/L)	average VSS (mg/L)	std. dev. VSS (mg/L)	Wastage for 15 day SRT (mL)
A	1/20/98	427	MLSS	2780	2490					
3	1/20/98	427	MLSS	2810	2500	2795	21	2495	7	
K3	1/20/98	427	effluent TSS	28.0	24.5					
B1	1/20/98	427	effluent TSS	28.0	25.5	28.0	0.0	25.0	0.7	321
EF1	1/21/98	428	MLSS	2730	2450					
2	1/21/98	428	MLSS	2630	2380	2680	71	2415	49	
F	1/24/98	431	MLSS	2580	2330					
EF2	1/24/98	431	MLSS	2840	2540	2710	184	2435	148	
K4	1/24/98	431	effluent TSS	11.5	10.3					
B8	1/24/98	431	effluent TSS	11.2	10.0	11.4	0.2	10.1	0.2	328
K6	1/26/98	433	MLSS	2900	2560					
B5	1/26/98	433	MLSS	2980	2610	2940	57	2585	35	
K2	1/26/98	433	effluent TSS	12.0	10.7					
H	1/26/98	433	effluent TSS	13.7	11.7	12.8	1.2	11.2	0.7	328
1	2/2/98	440	MLSS	2270	2010					
I	2/2/98	440	MLSS	2260	1980	2265	7	1995	21	
B7	2/2/98	440	effluent TSS	17.7	14.2					
J	2/2/98	440	effluent TSS	17.8	14.5	17.8	0.0	14.4	0.2	324
K1	2/3/98	441	MLSS	2270	2000					
B2	2/3/98	441	MLSS	2270	2010	2270	0	2005	7	
A13	2/3/98	441	effluent TSS	15.7	12.2					
G	2/3/98	441	effluent TSS	14.8	13.5	15.3	0.7	12.9	0.9	325
A0	2/4/98	442	MLSS	2380	2110					
C	2/4/98	442	MLSS	2350	2070	2365	21	2090	28	
K5	2/4/98	442	effluent TSS	12.8	10.7					
D	2/4/98	442	effluent TSS	12.5	11.0	12.6	0.2	10.9	0.2	327
B6	2/12/98	450	MLSS	1840	1560					
B3	2/12/98	450	MLSS	3460	3130	2650	1146	2345	1110	
B	2/12/98	450	effluent TSS	19.3	15.7					
E	2/12/98	450	effluent TSS	19.3	16.8	19.3	0.0	16.3	0.7	324
D	2/19/98	457	MLSS	2940	2610					
K5	2/19/98	457	MLSS	2880	2540	2910	42	2575	49	
B	2/19/98	457	effluent TSS	23.5	20.3					
3	2/19/98	457	effluent TSS	22.8	19.5	23.1	0.5	19.9	0.5	323
B3	2/24/98	462	MLSS	2820	2670					
K3	2/24/98	462	MLSS	2960	2550	2890	99	2610	85	
H	2/24/98	462	effluent TSS	22.8	19.3					
B1	2/24/98	462	effluent TSS	22.5	18.5	22.6	0.2	18.9	0.5	324
G	2/25/98	463	MLSS	3060	2700					
B6	2/25/98	463	MLSS	2890	2520	2975	120	2610	127	
A13	2/25/98	463	effluent TSS	21.5	18.3					
B7	2/25/98	463	effluent TSS	21.5	18.2	21.5	0.0	18.2	0.0	324
B2	2/26/98	464	MLSS	2990	2640					
C	2/26/98	464	MLSS	2900	2540	2945	64	2590	71	
A	2/26/98	464	effluent TSS	20.7	17.7					
K1	2/26/98	464	effluent TSS	21.7	18.2	21.2	0.7	18.0	0.4	324

Table F.1. Continued...

Sample ID	Sample date	Day after acclimation	Sample type	TSS (mg/L)	VSS (mg/L)	average TSS (mg/L)	std. dev. TSS (mg/L)	average VSS (mg/L)	std. dev. VSS (mg/L)	Wastage for 15 day SRT (mL)
H	2/28/98	466	MLSS	2910	2550					
K4	2/28/98	466	MLSS	2830	2510	2870	57	2530	28	
B8	3/4/98	470	MLSS	2790	2490					
K3	3/4/98	470	MLSS	2790	2480	2790	0	2485	7	
J	3/4/98	470	effluent TSS	8.0	7.0					
F	3/4/98	470	effluent TSS	9.3	8.0	8.7	0.9	7.5	0.7	329
B8	3/6/98	472	MLSS	2700	2380					
B3	3/6/98	472	MLSS	2700	2370	2700	0	2375	7	
K3	3/6/98	472	effluent TSS	12.7	11.0					
J	3/6/98	472	effluent TSS	14.3	11.5	13.5	1.1	11.2	0.4	327
B	3/10/98	476	MLSS	2780	2490					
E	3/10/98	476	MLSS	2780	2500	2780	0	2495	7	
K4	3/10/98	476	effluent TSS	17.5	15.7					
EF2	3/10/98	476	effluent TSS	16.5	14.8	17.0	0.7	15.3	0.7	326
B1	3/14/98	480	MLSS	2820	2500					
F	3/14/98	480	MLSS	2760	2430	2790	42	2465	49	
I	3/14/98	480	effluent TSS	22.0	19.5					
K5	3/14/98	480	effluent TSS	20.3	18.5	21.1	1.2	19.0	0.7	324
2	3/16/98	482	MLSS	2870	2560					
B5	3/16/98	482	MLSS	2820	2510	2845	35	2535	35	
D	3/16/98	482	effluent TSS	15.3	15.5					
I	3/16/98	482	effluent TSS	15.5	14.0	15.4	0.2	14.8	1.1	327
K2	3/18/98	484	MLSS	3040	2720					
K5	3/18/98	484	MLSS	3040	2710	3040	0	2715	7	
EF2	3/18/98	484	effluent TSS	15.7	14.3					
K6	3/18/98	484	effluent TSS	15.3	14.0	15.5	0.4	14.1	0.2	327
H	3/23/98	489	MLSS	2820	2470					
K4	3/23/98	489	MLSS	2930	2610	2875	78	2540	99	
B3	3/23/98	489	effluent TSS	15.8	13.8					
K3	3/23/98	489	effluent TSS	15.7	13.7	15.8	0.0	13.7	0.0	326
B7	3/28/98	494	MLSS	2940	2610					
EF1	3/28/98	494	MLSS	2970	2640	2955	21	2625	21	
1	3/28/98	494	effluent TSS	17.5	16.0					
A0	3/28/98	494	effluent TSS	17.5	15.5	17.5	0.0	15.7	0.4	326
B6	3/30/98	496	MLSS	3120	2750					
K6	3/30/98	496	MLSS	3210	2820	3165	64	2785	49	
G	3/30/98	496	effluent TSS	16.2	13.5					
A	3/30/98	496	effluent TSS	17.3	15.0	16.8	0.7	14.3	1.1	327
1	3/31/98	497	MLSS	3000	2660					
K2	3/31/98	497	MLSS	2950	2610	2975	35	2635	35	
B3	3/31/98	497	effluent TSS	21.2	18.8					
H	3/31/98	497	effluent TSS	19.5	17.0	20.4	1.2	17.9	1.2	325
K4	4/1/98	498	MLSS	3030	2690					
A0	4/1/98	498	MLSS	3060	2700	3045	21	2695	7	
EF2	4/1/98	498	effluent TSS	15.1	13.4					
B2	4/1/98	498	effluent TSS	14.9	13.1	15.0	0.2	13.3	0.2	327

Table F.1. Continued...

Sample ID	Sample date	Day after acclimation	Sample type	TSS (mg/L)	VSS (mg/L)	average TSS (mg/L)	std. dev. TSS (mg/L)	average VSS (mg/L)	std. dev. VSS (mg/L)	Wastage for 15 day SRT (mL)
K5	4/4/98	501	MLSS	2820	2480					
B7	4/4/98	501	MLSS	2810	2470	2815	7	2475	7	
C	4/4/98	501	effluent TSS	15.0	13.0					
EF1	4/4/98	501	effluent TSS	14.5	12.7	14.8	0.4	12.9	0.2	327
A	4/8/98	505	MLSS	3040	2610					
D	4/8/98	505	MLSS	2940	2470	2990	71	2540	99	
B	4/8/98	505	effluent TSS	16.0	13.3					
B6	4/8/98	505	effluent TSS	16.0	13.5	16.0	0.0	13.4	0.2	327
B8	4/13/98	510	MLSS	3010	2680					
K6	4/13/98	510	MLSS	3100	2730	3055	64	2705	35	
G	4/15/98	512	MLSS	3110	2730					
E	4/15/98	512	MLSS	3100	3040					
K3	4/15/98	512	MLSS	3150	2800					
F	4/15/98	512	MLSS	3150	2480	3128	26	2763	230	
B1	4/16/98	513	MLSS	3330	2920					
A13	4/16/98	513	MLSS	3350	2840	3340	14	2880	57	
K1	4/20/98	517	MLSS	3190	2840					
I	4/20/98	517	MLSS	3320	2880					
2	4/20/98	517	MLSS	3290	2870	3267	68	2863	21	
B1	4/20/98	517	effluent TSS	12.5	10.3					
K3	4/20/98	517	effluent TSS	12.0	9.8	12.3	0.4	10.0	0.4	329
G	4/21/98	518	MLSS	3100	2720					
3	4/21/98	518	MLSS	3050	2690	3075	35	2705	21	

Table F.2. Reactor Effluent Soluble Chemical Oxygen Demand (sCOD) History.

Sample date	Days after acclimation	FAS vol. 1 (mL)	FAS vol. 2 (mL)	sCOD 1 (mg/L)	sCOD 2 (mg/L)	average COD (mg/L)	st. dev. COD (mg/L)
12/16/96	27	4.10	**	131.9	**	131.9	**
12/18/96	29	4.24	**	121.1	**	121.1	**
12/19/96	30	4.30	**	116.4	**	116.4	**
12/20/96	31	4.66	**	88.5	**	88.5	**
12/21/96	32	4.90	**	69.8	**	69.8	**
12/22/96	33	4.92	**	68.3	**	68.3	**
12/28/96	39	4.98	**	63.6	**	63.6	**
12/31/96	42	4.50	**	100.9	**	100.9	**
1/3/97	45	5.36	**	34.1	**	34.1	**
1/6/97	48	4.84	**	74.5	**	74.5	**
1/7/97	49	5.20	**	46.6	**	46.6	**
1/9/97	51	5.12	**	52.8	**	52.8	**
1/10/97	52	5.08	**	55.9	**	55.9	**
1/14/97	56	5.28	**	72.5	**	72.5	**
1/15/97	57	5.02	**	92.5	**	92.5	**
1/16/97	58	3.20	**	233.0	**	233.0	**
1/17/97	59	3.00	**	248.0	**	248.0	**
1/20/97	62	4.58	**	126.0	**	126.0	**
1/22/97	64	4.22	**	154.0	**	154.0	**
1/24/97	66	4.76	**	113.0	**	113.0	**
1/29/97	71	5.00	**	94.1	**	94.1	**
1/30/97	72	4.80	**	110.0	**	110.0	**
1/31/97	73	4.70	**	117.0	**	117.0	**
2/3/97	76	4.66	**	120.0	**	120.0	**
2/4/97	77	4.72	**	116.0	**	116.0	**
2/5/97	78	4.60	**	125.0	**	125.0	**
2/6/97	79	4.72	**	116.0	**	116.0	**
2/18/97	91	4.82	5.02	98.6	82.8	90.7	11.2
2/19/97	92	4.94	***	89.1	***	89.1	***
2/20/97	93	4.94	4.98	89.1	86.0	87.6	2.2
2/28/97	101	5.04	4.92	81.2	90.7	86.0	6.7
3/3/97	104	5.00	4.94	84.4	89.1	86.8	3.3
3/4/97	105	4.88	4.80	93.9	100.2	97.0	4.5
3/6/97	107	4.90	3.68	92.3	188.5	140.4	68.0
3/7/97	108	***	4.54	***	120.7	120.7	***
3/12/97	113	5.00	4.94	87.3	92.0	89.6	3.3
3/14/97	115	5.04	5.02	84.2	85.8	85.0	1.1
3/15/97	116	4.94	5.02	92.0	85.8	88.9	4.4
3/19/97	120	4.94	5.00	92.0	87.3	89.6	3.3
3/22/97	123	5.10	5.06	79.6	82.7	81.1	2.2
3/24/97	125	5.16	5.18	75.0	73.4	74.2	1.1
3/26/97	127	5.04	5.04	84.2	84.2	84.2	0.0
3/27/97	128	4.94	5.02	92.0	85.8	88.9	4.4
3/28/97	129	5.02	4.92	85.8	93.5	89.6	5.5
3/29/97	130	4.64	4.84	115.1	99.7	107.4	10.9
4/1/97	133	4.92	4.94	93.5	92.0	92.7	1.1
4/4/97	136	5.00	5.08	87.3	81.1	84.2	4.4
4/6/97	138	5.00	4.98	87.3	88.9	88.1	1.1

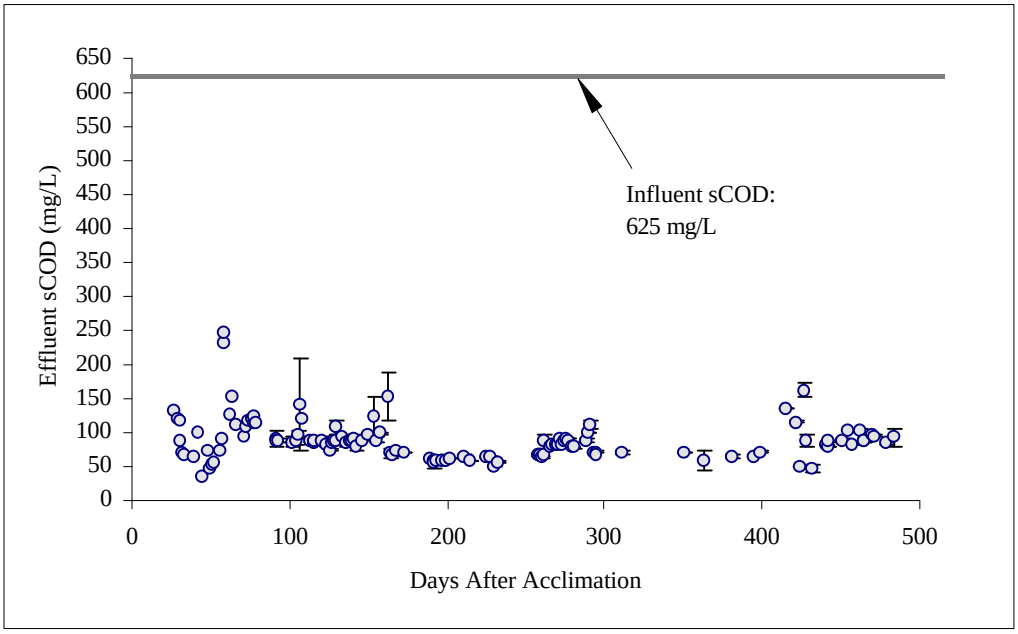
Table F.2. Continued...

Sample date	Days after acclimation	FAS vol. 1 (mL)	FAS vol. 2 (mL)	sCOD 1 (mg/L)	sCOD 2 (mg/L)	average COD (mg/L)	st. dev. COD (mg/L)
4/7/97	139	4.92	5.02	93.5	85.8	89.6	5.5
4/9/97	141	4.90	4.98	95.1	88.9	92.0	4.4
4/10/97	142	5.16	5.02	75.0	85.8	80.4	7.7
4/14/97	146	4.98	5.00	88.9	87.3	88.1	1.1
4/18/97	150	4.94	4.84	92.0	99.7	95.8	5.5
4/21/97	153	4.82	4.28	101.2	143.0	122.1	29.5
4/23/97	155	5.00	4.94	87.3	92.0	89.6	3.3
4/25/97	157	4.84	4.86	99.7	98.1	98.9	1.1
4/30/97	162	4.46	3.84	129.1	177.0	153.0	33.9
5/2/97	164	5.08	4.94	64.4	75.3	69.8	7.7
5/3/97	165	5.06	5.02	66.0	69.1	67.5	2.2
5/5/97	167	4.94	5.02	75.3	69.1	72.2	4.4
5/10/97	172	5.00	5.00	70.6	70.6	70.6	0.0
5/27/97	189	5.26	5.26	63.1	63.1	63.1	0.0
5/29/97	191	5.30	5.30	59.9	59.9	59.9	0.0
5/30/97	192	5.42	5.30	50.5	59.9	55.2	6.7
5/31/97	193	5.34	5.30	56.8	59.9	58.4	2.2
6/4/97	197	5.32	5.30	58.4	59.9	59.2	1.1
6/6/97	199	5.32	5.30	58.4	59.9	59.2	1.1
6/9/97	202	5.28	5.28	61.5	61.5	61.5	0.0
6/18/97	211	5.26	5.22	63.1	66.3	64.7	2.2
6/22/97	215	5.30	5.30	59.9	59.9	59.9	0.0
7/1/97	224	5.26	5.24	63.1	64.7	63.9	1.1
7/4/97	227	5.26	5.24	63.1	64.7	63.9	1.1
7/7/97	230	5.40	5.42	52.1	50.5	51.3	1.1
7/9/97	232	5.36	5.32	55.2	58.4	56.8	2.2
8/4/97	258	5.22	5.20	66.3	67.8	67.0	1.1
8/5/97	259	5.20	5.22	67.8	66.3	67.0	1.1
8/6/97	260	5.24	5.26	64.7	63.1	63.9	1.1
8/7/97	261	***	5.22	***	66.3	66.3	***
8/8/97	262	4.86	5.04	94.8	80.5	87.6	10.1
8/11/97	265	5.04	5.06	80.5	78.9	79.7	1.1
8/12/97	266	5.04	5.00	80.5	83.7	82.1	2.3
8/15/97	269	4.98	5.08	85.3	77.3	81.3	5.6
8/16/97	270	5.04	5.00	80.5	83.7	82.1	2.3
8/18/97	272	4.90	4.94	91.6	88.4	90.0	2.3
8/19/97	273	5.00	5.00	83.7	83.7	83.7	0.0
8/20/97	274	4.96	4.94	86.9	88.4	87.6	1.1
8/22/97	276	4.90	4.90	91.6	91.6	91.6	0.0
8/23/97	277	4.96	4.92	86.9	90.0	88.4	2.3
8/25/97	279	5.04	5.06	80.5	78.9	79.7	1.1
8/26/97	280	5.08	5.00	77.3	83.7	80.5	4.5
9/3/97	288	4.90	4.96	91.6	86.9	89.2	3.4
9/4/97	289	4.80	4.80	99.6	99.6	99.6	0.0
9/6/97	291	4.70	4.60	107.6	115.5	111.6	5.6
9/8/97	293	5.18	5.18	70.3	70.3	70.3	0.0
9/9/97	294	5.16	5.18	71.8	70.3	71.0	1.1
9/10/97	295	***	5.20	***	68.7	68.7	***

Table F.2. Continued...

Sample date	Days after acclimation	FAS vol. 1 (mL)	FAS vol. 2 (mL)	sCOD 1 (mg/L)	sCOD 2 (mg/L)	average COD (mg/L)	st. dev. COD (mg/L)
9/26/97	311	5.16	5.20	71.8	68.7	70.3	2.2
11/4/97	350	5.18	5.18	70.3	70.3	70.3	0.0
11/17/97	363	5.20	5.46	68.7	48.2	58.4	14.5
12/5/97	381	5.22	5.28	67.1	62.4	64.7	3.3
12/19/97	395	5.22	5.26	67.1	63.9	65.5	2.2
12/23/97	399	5.18	5.14	70.3	73.4	71.8	2.2
1/8/98	415	4.34	4.34	136.6	136.6	136.6	0.0
1/14/98	421	4.62	4.60	114.5	116.0	115.3	1.1
1/17/98	424	5.00	5.02	51.1	49.5	50.3	1.1
1/20/98	427	3.68	3.48	154.8	170.5	162.6	11.1
1/21/98	428	4.60	4.46	82.5	93.5	88.0	7.8
1/24/98	431	5.00	5.10	51.1	43.2	47.1	5.6
2/2/98	440	4.98	4.98	82.0	82.0	82.0	0.0
2/3/98	441	5.00	5.00	80.5	80.5	80.5	0.0
2/4/98	442	4.90	4.94	88.3	85.2	86.8	2.2
2/12/98	450	4.88	4.94	89.9	85.2	87.6	3.3
2/16/98	454	***	4.72	***	102.5	102.5	***
2/19/98	457	4.96	4.96	83.6	83.6	83.6	0.0
2/24/98	462	4.68	4.74	105.7	101.0	103.3	3.3
2/26/98	464	4.94	4.90	85.2	88.3	86.8	2.2
2/27/98	465	4.90	4.92	88.3	86.8	87.6	1.1
3/4/98	470	4.78	4.80	97.8	96.2	97.0	1.1
3/5/98	471	4.82	4.80	94.7	96.2	95.4	1.1
3/13/98	479	4.74	4.70	82.7	86.0	84.4	2.3
3/18/98	484	4.50	4.72	102.4	84.4	93.4	12.7

Figure F.1. Plot of Reactor Effluent sCOD Concentrations. Error Bars Represent One Standard Deviation of Duplicate Samples.



VITA

James D. Fettig (Jamie) was born on August 20, 1973 in Red Bank, NJ to Douglas and Elaine Fettig, who originate from Queens, NY. He graduated from Raritan High School in 1991 and went to Virgina Polytechnic Institute and State University (Virginia Tech) where he graduated Summa Cum Laude with a Bachelor of Science degree in Honors. Jamie then went on to pursue a Master of Science degree from Virginia Tech in Environmental Engineering. Jamie finished and defended his graduate work in the Fall of 1998. Currently he is residing in Syracuse, NY working as a project enginner for an environmental consulting firm.