DENITRIFICATION OF RECIRCULATING AQUACULTURE SYSTEM WATERS USING AN UPFLOW BIOFILTER AND A FERMENTED SUBSTRATE

by

Jennifer B. Phillips

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APPROVED:

Dr. Nancy G. Love, Chair
Dr. Gregory D. Boardman
Dr. George S. Libey
Dr. John T. Novak

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Dr. Nancy G. Love, Chair

Department of Environmental Engineering

(ABSTRACT)

The ability of an upflow, denitrifying biofilter using a fermentation generated carbon source to treat the high nitrate concentrations typically seen in recirculating aquaculture systems was studied using a synthetic nitrate wastewater supplied at two nitrate loadings, 1.13 and 2.52 kg NO_3-N/m^3/day. A supplemental carbon source was provided primarily through the fermentation of fish food which generated volatile fatty acids (VFA) in the form of acetic, propionic, isobutyric, *n*-butyric, 2-methylbutyric, 3-methylbutyric, and *n*-valeric acids. Acetic and propionic acids were the predominant constituents generated, while lower concentrations of the longer carbon chain butyric and valeric acids were produced. The VFAs proved to be a viable carbon source for the denitrification process as indicated by the ability of the biofilm to assimilate all of the constituents generated. Carbon limiting the system resulted in an increase in effluent nitrite and incomplete nitrate removal. During the low nitrate loading condition, influent COD to NO_3-N ratios greater than 5 typically achieved high total nitrogen removals greater than 95%. This influent ratio corresponded with a COD to NO_x-N consumption ratio of 4.62 ± 0.28 mg/L as COD per mg/L as N for complete nitrogen removal. Under the high nitrate loading condition,

influent COD to NO_3-N ratios achieving high nitrogen removals showed great variability and did not correspond to a distinct value. The COD to NO_x-N consumption ratios were often below stoichiometric values, which was attributed to the hydrolysis of influent fermentation solids captured within the column to generate a COD source not measured by filtered samples. The column biofilm kinetics were modeled using a half-order reaction rate and denitrification coefficients (k) of 0.70 ± 0.02 (mg NO_x-N/L)$^{1/2}$ / min and 1.18 ± 0.12 (NO_x-N /L)$^{1/2}$ / min were determined for the low and high nitrate loading phases, respectively.

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EXECUTIVE SUMMARY

Problem

The demand for seafood continues to rise while the natural resources that have historically supplied this commodity are being depleted. In the wake of this decreasing availability of fish from natural waters, the aquaculture industry has flourished by providing an alternative source through the controlled propagation of seafood. Fish production has traditionally occurred in ponds and raceways, which require large volumes of water that are discharged to nearby waterways. However, the application of water reclamation and conservation techniques is becoming necessary in the era of decreasing availability of clean water and the increasing stringency of effluent discharge regulations (Stickney, 1994). In order to address these issues, the industry developed the recirculating aquaculture system, which rear high densities of fish while continuously recycling the culture water, thereby employing water conservation techniques.

Since fresh water addition is minimized, the quality of the culture water can deteriorate quite rapidly from the accumulation of ammonia and particulate waste generated from the metabolism of feed. Aquaculturists employ common wastewater treatment techniques in recirculating production systems to yield an environment that is conducive to rearing aquatic organisms. Solids removal is typically achieved through clarification or filtration, while nitrification is employed to convert ammonia to nitrate, via nitrite, in order to prevent free ammonia toxicity (Lucchetti and Gray, 1988). The combined implementation of the nitrification process and decreased water exchanges leads to the accumulation of nitrates over time in recirculating aquaculture systems. Easter (1992) reported nitrate concentrations as high as 170 mg/L as N while rearing hybrid striped bass stocked at a density of 132 fish/m^3 in a recirculating

system that received a maximum weekly water exchange of one system volume. Chronic toxicity to certain fish species (Hrubec et al., 1996), as well as tightening water regulations with regard to nutrient discharge, have led to concern over the accumulation of nitrates in recirculating systems.

Biological denitrification can be used to remove nitrates from recirculating aquaculture system waters. Denitrification is the dissimilative reduction of nitrate (NO_3^-) to nitrogen gas (N_2), through the production of nitrite (NO_2^-) and gaseous nitric oxide (NO) and nitrous oxide (N_2O) intermediates.

$$NO_3^- \longrightarrow NO_2^- \longrightarrow NO_{(g)} \longrightarrow N_2O_{(g)} \longrightarrow N_{2\,(g)}$$

This process is performed by heterotrophic bacteria under anoxic conditions and uses nitrate as a terminal electron acceptor in the presence of a carbon and energy source (Madigan et al., 1997).

An electron donor is required as a carbon and energy source to fuel the denitrification process. Dissolved organic compounds accumulate in recirculating aquaculture systems as a result of the introduction of feed and the extent of accumulation is greatly affected by fish stocking densities and feeding rates (Hirayama et al., 1988). However, these systems typically possess relatively low concentrations of dissolved organic carbon (DOC) as shown by Easter (1992) who reported that DOC concentrations remained lower than 50 mg/L over a 225 day growth trial of hybrid striped bass stocked at a density of 132 fish/m^3. Wastewater treatment plants often add an exogenous carbon source, such as methanol or acetate, when a carbon deficiency exists (de Mendonca et al., 1992; Sadick et al., 1996), though the associated cost does not make this an attractive option for aquaculturists. Growing interest has been expressed for using biosolids as a carbon supplement in the denitrification process. Fermented municipal sludge and swine waste have been shown to be good electron donors, effecting enhanced denitrification

rates over methanol and acetate alone (Lee et al., 1995). Fish waste and uneaten feed constitute a source of organic matter produced within the fish culture unit that can be used to generate a suitable carbon source for the denitrification process (Arbiv and van Rijn, 1995). Since this organic matter is in the particulate form and not readily available for microbial use, hydrolysis and fermentation can be applied to convert these substances into volatile fatty acids, which can be more easily consumed by denitrifying microorganisms (Eastman and Ferguson, 1981; Lee et al., 1995). The use of VFAs as electron donors in the denitrification process has gained increasing interest (Fass et al, 1994; Aboutboul et al., 1995) because they possess high amounts of stored energy, making them suitable as carbon and energy sources. The use of an organic substrate that is prevalent in the system is aimed towards the development of a self-sustaining treatment process. In addition, the amount of particulate waste requiring disposal is reduced by converting a fraction of the particulate matter into a soluble form that is consumed by the denitrification process.

Biofilters are an attached growth process in which a biofilm is generated from the propagation of microorganisms on an inert surface. Biofilters maintain a higher active fraction of biomass, as compared to suspended growth environments, which enables the use of a smaller reactor (M'Coy, 1997). The efficient operation and compact size makes biofilters an attractive treatment device for the aquaculture industry, as is illustrated by their wide scale use in the performance of nitrification. Complete nitrogen removal can be achieved in recirculating aquaculture systems through the implementation of a coupled biofiltration treatment scheme employing nitrification and denitrification (Metcalf and Eddy, Inc., 1991).

Purpose

This study was designed to investigate the removal of nitrates from recirculating aquaculture system waters using a denitrifying biofilter to reduce nitrate to nitrogen gas and a supplemental carbon source provided through the fermentation of fish food. The original design of this system incorporated the fermentation of fish waste to provide a carbon source for the denitrification process, thereby using organic matter prevalent in the recirculating aquaculture system. Fish waste was to be collected from on-site pilot-scale recirculating aquaculture systems used to research closed system fish production. However, at the inception of this study renovations had just been completed at the Aquaculture Center and the rearing tanks were lightly stocked with fish prior to the start of intensive studies. The low stocking densities (< 12 g/L, 0.1 lb/gal) resulted in fish waste production that was insufficient to provide a consistent carbon source for the denitrification system. Therefore, fish food was fermented in place of the preferred fish waste. The specific objectives of this study were to:

1. determine the relationship between the applied carbon to nitrogen ratio and the subsequent denitrification efficiency achieved,
2. characterize the nature of the organic matter produced from the fermentation of fish waste and food,
3. determine the fermentation product(s) preferred by the denitrification process, and
4. demonstrate the overall performance of these biological processes and their application in the treatment of recirculating aquaculture system waters.

Approach

A pilot-scale biological denitrification system comprised of an upflow, fixed film column 4.0 m x 15.2 cm) and two fermentation units (450 L each) was operated at the Virginia Tech Aquaculture Center (Blacksburg, VA) to examine the removal of nitrates from a synthetic wastewater simulating nitrate concentrations expected in recirculating aquaculture system waters. The column was packed with 0.044 m^3 of 2-3 mm floating, polystyrene media (Biostyr®, Krüger, Cary, North Carolina) possessing a specific surface area of 1000 m^2/m^3. Low (1.13 kg NO_3-N/m^3/day) and high (2.52 kg NO_3-N/m^3/day) nitrate loading conditions were studied at a hydraulic loading rate of 3.0 m^3/m^2/hr.

Results and Conclusions

The pilot-scale system employed the fermentation of fish food, while batch-scale tests were performed to analyze the fermentation of fish waste. The fermentation of fish food and fish waste generated volatile fatty acids (VFA) in the form of acetic, propionic, isobutyric, *n*-butyric, 2-methylbutyric, 3-methylbutyric, and *n*-valeric acids. The 2-methylbutyric and 3-methylbutyric compounds are isomer forms of valeric acid. For the overall fermentation process, acetic and propionic acids were produced in the greatest quantities, while smaller amounts of the longer carbon chain butyric and valeric acid compounds were generated. The average calculated VFA fraction of the total measured COD for the fermentation of fish waste and the fermentation of fish food during the low and high nitrate loading phases was 70.1% ± 3.72, 86.3% ± 3.58, and 106% ± 3.90, respectively. Although these values exhibited great variability, with some values exceeding 100%, it was evident that VFAs accounted for a majority of the soluble COD produced. In addition, the VFA constituents accounted for more of the soluble COD produced

from the fermentation of fish food than that generated from fish waste fermentation which was as expected since the fish metabolize part of the stored energy in the food. The non VFA fraction was not characterized, but showed to be not readily available to the denitrifying organisms as an average concentration of 22.5 ± 3.02 mg sCOD/L was detected in effluent samples even when total nitrogen removal was incomplete. The denitrifying biofilm was able to assimilate all of the VFAs produced, yet certain constituents were preferred. Under conditions in which there were no VFAs in the effluent and high nitrogen removals (> 95%) were achieved, the acetic acid consumption rate was faster than the rate for propionic acid. When VFAs were supplied in excess, it was apparent that the normal structures of both butyric and valeric acids were preferred over their isomer forms, yet propionic acid was consumed nearly twice as fast as acetic acid. The relative VFA constituent concentrations and VFA fraction of the total COD generated from the fermentation process varied slightly in response to changes in pH, as well as the solids and hydraulic retention times, suggesting that the fermentation process may be manipulated through operational control to preferentially produce desired acids.

Available carbon limiting conditions prevailed for influent COD to NO_3-N ratios less than 5 and resulted in incomplete nitrate removal and measurable effluent nitrite concentrations as high as 12 mg NO_2-N/L. For influent COD to NO_3-N ratios above 5, high total nitrogen removals greater than 95% were consistently achieved and corresponded with a COD to NO_x-N consumption ratio of 4.62 ± 0.28 mg/L as COD per mg/L as N calculated for complete nitrogen removal. Upon increasing the influent nitrate concentration to simulate a high loading of 2.52 kg NO_3-N/m^3/day, nitrate removal efficiencies greater than 99% were regularly seen. However, nitrite was detected in the column effluent at concentrations as high as 34 mg NO_2-N/L. The

presence of measurable COD in the effluent suggested that carbon limitation was not the problem. In order to remedy the high effluent nitrite problem, the hydraulic loading was decreased by 50%, thereby doubling the column retention time. After observing several days of complete nitrogen removal during the low hydraulic loading period, operation was returned to the original flowrate. At this point, nitrate removal efficiencies greater than 95% were achieved and effluent nitrite remained at or below 1 mg NO_2-N /L. During the low nitrate loading phase, visual inspection revealed that biomass growth was concentrated at the bottom of the column where a majority of the nitrogen removal occurred, while sparse microbial colonization prevailed at the top. The improved efficiency that resulted from an increased hydraulic loading was attributed to an acclimation period during which biomass growth occurred throughout the entire column as a result of increased contact time with substrates. This increase in biofilm density enabled the removal of higher nitrate concentrations. Complete nitrogen removal was achieved for a COD to NO_x-N consumption ratio of 3.07 ± 0.58 mg/L as COD per mg/L as N during the high nitrate loading, but did not correspond to a distinct influent COD to NO_3-N ratio and exhibited great variability. This was attributed to the hydrolysis of influent fermentation solids captured within the column to generate a COD source not measured by filtered samples. The column biofilm kinetics were modeled using a half-order reaction rate and denitrification coefficients (k) of 0.70 ± 0.02 (mg NO_x-N/L)$^{1/2}$/min and 1.18 ± 0.12 (NO_x-N /L)$^{1/2}$/min were determined for the low and high nitrate loading phases, respectively. The increase in the denitrification rate with the loading was expected as a higher bulk concentration of nitrate and COD would have encouraged a faster rate of mass transfer and biological reaction within the biofilm.

The results of this study demonstrated the feasibility of using a fermentation generated carbon source in the denitrification of high nitrate recirculating aquaculture system waters. The nitrate loadings examined in this study were lower than the maximum nitrate concentrations observed in nitrifying closed recirculating aquaculture systems not employing denitrification. However, nitrate concentrations in the fish rearing tanks increase gradually over the span of a growth period and it may be possible to maintain concentrations at manageable levels by applying denitrification as a sidestream process so that extreme concentrations do not result. In order to evaluate the efficiency and self-sustainability of this denitrification system at increased nitrate concentrations, additional studies are recommended. It is anticipated that a full-scale recirculating aquaculture facility would generally have several culture tanks containing fish at all stages of growth and would be able to provide a more consistent source of fish waste for the fermentation process. However, this aspect of the treatment system must be evaluated further to determine if complete self-sustainability is possible, or whether an external carbon source must be partially supplemented.

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LITERATURE REVIEW

Aquaculture

Aquaculture is the propagation of aquatic species within a controlled or semi-controlled environment. It has become a thriving industry as a result of the depletion of natural sources of seafood. Traditionally, fish production has occurred primarily in water intensive systems such as ponds and raceways. These systems require large volumes of water which are discharged to nearby waterways. However, the decreasing availability of clean water and the increasing stringency of effluent discharge regulations have encouraged the industry to migrate towards the implementation of water reclamation and conservation techniques (Stickney, 1994). One result of these efforts was the development of the recirculating aquaculture system.

Recirculating aquaculture systems are used to rear high densities of fish while employing water conservation techniques by continuously recycling the culture water. The percentage of the total system volume that is recycled varies with system operation, but for completely closed systems water replacement compensates mainly for evaporation and splash out losses. Since fresh water addition is minimized, water quality can deteriorate rapidly from the accumulation of nitrogenous compounds and suspended solids in the form of fish waste and uneaten feed. As a result, treatment of the culture water is necessary to yield an environment that is conducive to growing aquatic organisms.

Aquaculturists employ common wastewater treatment techniques in closed or semi-closed production systems to remove suspended solids and control dissolved nitrogenous compounds (Lucchetti and Gray, 1988). Historically, the nitrogenous compound of greatest concern has been ammonia, specifically the unionized form (NH_3), because of its toxicity to fish at small

concentrations (Colt and Tchobanoglous, 1976). Ammonia is generated from the metabolism of protein contained in commercial fish feed and is excreted primarily across the gills (Stickney, 1994). Once in the water column, ammonia establishes a dynamic equilibrium between its ionized and unionized (NH_4^+) forms:

$$NH_4^+ \rightleftharpoons NH_3 + H^+ \qquad (pK_a = 9.3, \text{ at } 25^oC) \qquad (1)$$

(Snoeyink and Jenkins, 1980). Ammonia is removed in recirculating aquaculture systems through nitrification, which is the oxidation of ammonia to nitrate (NO_3^-) via nitrite (NO_2^-). Biofilters are predominantly used by aquaculturists to achieve nitrification and include such devices as the rotating biological contactor, submerged bed, and trickling filter (Lucchetti and Gray, 1988; Landau, 1992). The combined implementation of the nitrification process and decreased water exchanges leads to the accumulation of nitrates over time in recirculating aquaculture systems. The extent of nitrate accumulation depends on the amount of system water replaced, as influenced by the fish stocking density and feeding regime. Easter (1992) reported nitrate concentrations as high as 170 mg/L as N while rearing hybrid striped bass stocked at a density of 132 fish/m^3 in a recirculating system that received a maximum weekly water exchange of one system volume.

Nitrification

The nitrification process is comprised of two steps in which ammonia-oxidizing bacteria oxidize ammonia to nitrite and nitrite-oxidizing bacteria convert nitrite to nitrate. Recent phylogenetic studies have indicated that *Nitrosomonas* (Mobarry et al., 1996) and *Nitrosococcus mobilis* (Wagner et al., 1997) are common ammonia-oxidizing organisms. However, in situ analysis conducted by Wagner et al. (1996) on biomass collected from nitrifying wastewater

treatment plants revealed that *Nitrobacter*, long thought to be the predominant organism responsible for nitrite oxidation, may not be the primary genus. Citing earlier research studies concerning nitrite oxidation, Wagner et al. (1996) suggested that other nitrite-oxidizers, such as *Nitrospira* (Watson et al., 1986) or *Nitrospina* and *Nitrococcus* (Watson and Waterbury, 1971), may be more common.

Nitrate Concerns

The introduction of vast amounts of nutrients, specifically nitrogen and phosphorous, leads to increased algal growth. The decomposition of algae consumes dissolved oxygen supplies and enhances the eutrophication of receiving waters (Vesilind et al., 1990). Tightening water regulations with regard to nutrient discharge have been targeted to mitigate this heightened degradation. The high nitrogen concentration of recirculating aquaculture system waters makes effluent discharge a concern. In addition to nutrient effluent concerns, nitrate has been shown to cause chronic toxicity in certain fish species. Hrubec et al. (1996) studied the effect of extended exposure to 200 mg NO_3-N/L on hybrid striped bass. Results suggested that high nitrate levels compromised the immune system, thereby increasing the chance for mortality.

Reduction of Nitrate

Biological denitrification can be used to remove nitrates from recirculating aquaculture system waters. Denitrification is the dissimilative reduction of nitrate to nitrogen gas (N_2) through the production of nitrite and gaseous nitric and nitrous oxide intermediates. This process is performed by heterotrophic bacteria under anoxic conditions and uses nitrate as a terminal electron acceptor in the presence of a carbon and energy source (Madigan et al., 1997). Denitrification generally transpires in the absence of oxygen, though it can occur in the presence

of low oxygen concentrations if sufficient nitrate is available (Kukor and Olsen, 1996). Dissimilative means can also reduce nitrate to ammonia, although this mechanism is considered to be less significant. If ammonia is unavailable, assimilative reduction of nitrate to ammonia occurs in order to provide a nitrogen nutrient source for cell synthesis. This process may occur in the presence or absence of oxygen (Madigan et al., 1997). Figure 1 illustrates the assimilative and dissimilative reduction of nitrate.

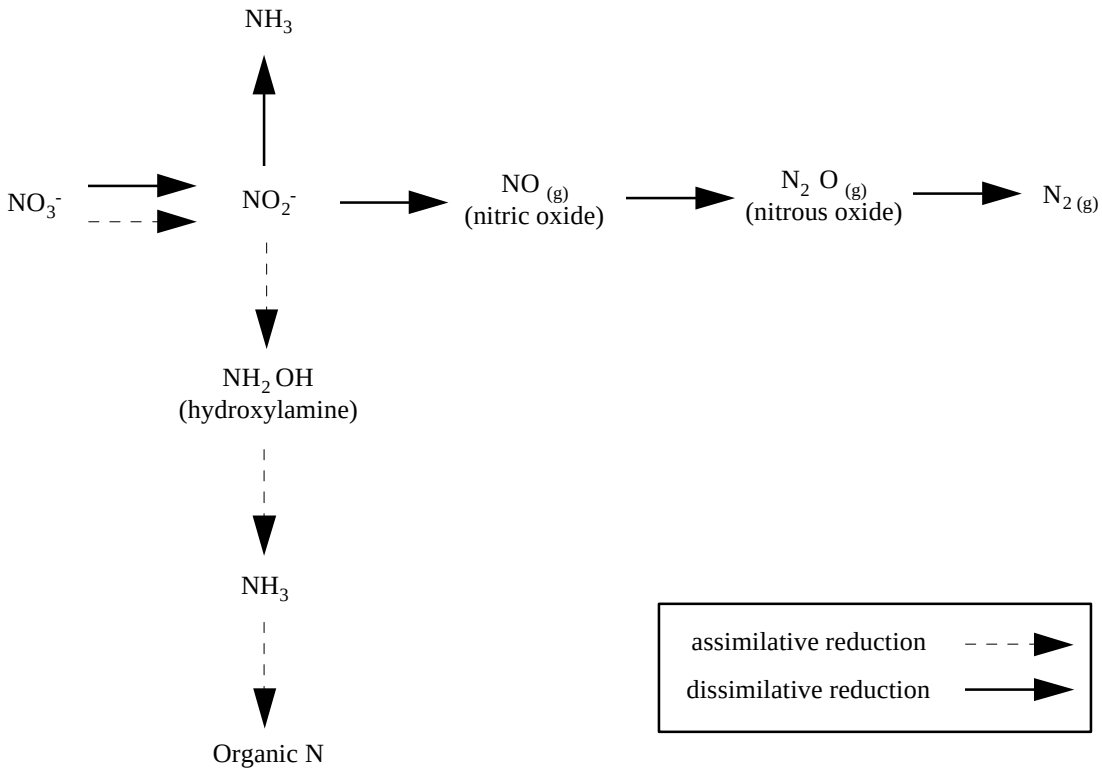


Figure 1. Reduction of nitrate through assimilative and dissimilative pathways (adapted from Madigan et al., 1997).

Denitrification pH

The optimum pH for denitrification typically lies within the range of 7 to 8, depending on the microbial community (Metcalf and Eddy, 1991). Glass and Silverstein (1997) reported an inhibition of denitrification in activated sludge for a high nitrogenous wastewater (2700 mg/L NO_3-N) at pH values of 6.5 and 7.0. However, for pH values of 7.5, 8.5, and 9 denitrification occurred and the nitrate reduction rate increased with pH. Beccari et al. (1983) reported decreased nitrite reduction rates at pH values below 7.5 and suggested that nitrous acid (HNO_2) created an inhibition effect. The acid-base reaction for nitrous acid and nitrite is represented by the following equation:

$$HNO_2 \rightleftharpoons NO_2^- + H^+ \qquad (pK_a = 4.5, \text{ at } 25^oC) \qquad (2)$$

(Snoeyink and Jenkins, 1980). Glass et al. (1997) attributed decreased denitrification rates at low pH (≤ 7.0) to HNO_2, which can be toxic to denitrifiers at low concentrations.

Nitrite Accumulation

Traditionally, the production of nitrite during the denitrification process in domestic wastewater treatment has been insignificant because nitrate concentrations are relatively low. However, the accumulation of nitrite has become an issue of concern for wastewaters containing significantly higher nitrate levels (Glass and Silverstein, 1997; Martienssen and Schöps, 1997). Proposed explanations include a difference in nitrate and nitrite reduction rates (Betlach and Tiedje, 1981), heightened activity of nitrate reductases over nitrite reductases (van Rijn and Sich, 1992), and a competition between the two reductase enzymes for electrons (Glass and Silverstein, 1997; Almeida et al., 1995; van Rijn et al., 1996). The extent of nitrite accumulation is also affected by the denitrifying strain, whereby *Pseudomonas* sp (van Rijn and Sich, 1992) and

Pseudomonas fluorescens (Betlach and Tiedje, 1981) have been shown to accumulate nitrite, while both studies reported little or no accumulation for *Flavobacterium.*

Carbon Source

An electron donor is required as a carbon and energy source to fuel the denitrification process. Dissolved organic compounds accumulate in recirculating aquaculture systems as a result of the introduction of feed and the extent of accumulation is greatly affected by fish stocking densities and feeding rates (Hirayama et al., 1988). However, these systems typically possess relatively low concentrations of dissolved organic carbon (DOC) as shown by Easter (1992) who reported that DOC concentrations remained lower than 50 mg/L over a 225 day growth trial of hybrid striped bass stocked at 132 fish/m^3.

Wastewater treatment plants often add an exogenous carbon source when a carbon deficiency exists. The most widely used carbon sources include methanol and acetate (de Mendonca et al., 1992; Sadick et al., 1996), though the associated cost does not make this an attractive option for aquaculturists. In addition, the explosive nature of methanol makes this compound a potential hazard. Growing interest has been expressed for using biosolids as a carbon supplementation in the denitrification process. Fermented municipal sludge and swine waste have been shown to be good electron donors, effecting enhanced denitrification rates over methanol and acetate alone (Lee et al., 1995). The fish waste and uneaten feed accumulating in recirculating aquaculture systems is a prevalent source of organic matter that can be transformed through fermentation into a readily available substrate for use in the denitrification process (Arbiv and van Rijn, 1995).

The fermentation process uses organic matter as both the electron donor and acceptor and is the intermediate step in anaerobic digestion. Enzymes hydrolyze both biodegradable particulate matter and high weight soluble organic compounds into smaller components that are more easily assimilated by microorganisms performing fermentation and anaerobic oxidation. The resulting carbohydrates and proteins are converted into volatile fatty acids through fermentation, while lipids are transformed into hydrogen gas (H_2) through anaerobic oxidation (Figure 2) (Eastman and Ferguson, 1981; Grady and Daigger, 1998).

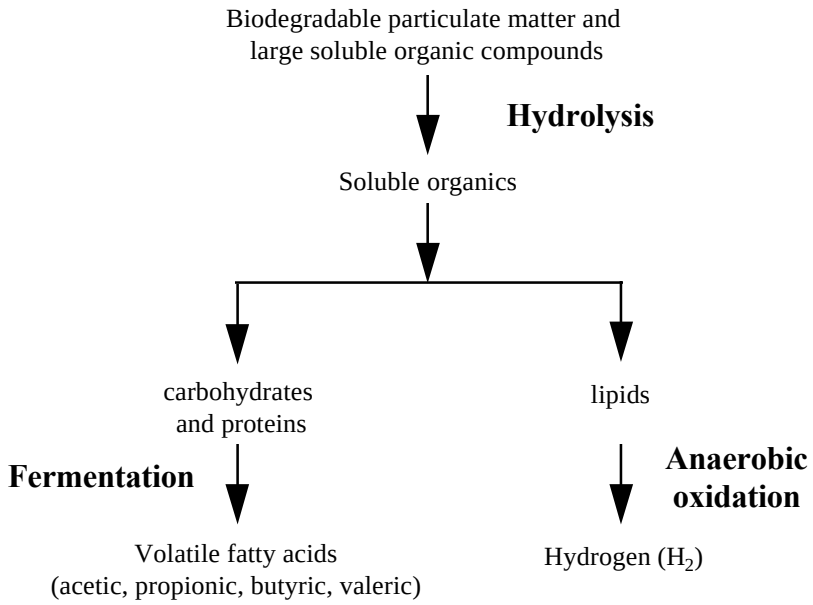


Figure 2. Substrates and products of the hydrolysis, fermentation, and anaerobic oxidation processes (adapted from Eastman and Ferguson, 1981; Grady and Daigger ,1998).

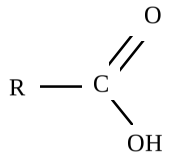
Eastman and Ferguson (1981) reported that the principal soluble constituents generated from the fermentation of primary domestic sludge were volatile fatty acids (VFAs), comprising 85 to 95% of the soluble COD measured. Specific constituents detected were acetic and propionic acids, as well as the isomer and normal forms of butyric and valeric acids.

VFAs are carboxylic acids comprised of the carboxyl group (- COOH) attached to an alkyl (R) or aryl group (Ar) (Morrison and Boyd, 1966). Figure 3 illustrates the general chemical structure for carboxylic acids, as well as the specific structures for the typical volatile fatty acids generated from the acid fermentation process. The use of VFAs as electron donors in the denitrification process has gained increasing interest in recent years (Fass et al., 1994; Aboutboul et al., 1995; Lee et al., 1995). VFAs possess high amounts of stored energy, making them suitable as carbon and energy sources. The amount of stored energy depends on the number and oxidation state of the carbon atoms in the compound (Grady and Daigger, 1998; Madigan et al., 1997). Among the VFAs generated through fermentation, acetic acid possesses the least amount of stored potential energy, while the valeric acid compounds exhibit the highest (Table 1).

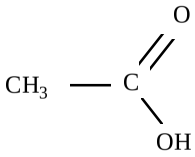
Aquaculture Denitrification Studies

Several researchers have studied the application of denitrifying biofilters to achieve nitrate removal in recirculating aquaculture systems (Balderston and Sieburth, 1976; Whitson et al., 1993; Arbiv and van Rijn, 1995; Abeysinghe et al., 1996). While most researchers have employed methanol as the carbon and energy source, van Rijn and Rivera (1990) used endogenous organic matter generated within the fish culture unit. Culture water containing particulate organic matter, in the form of fish waste and uneaten feed, was metered directly from the rearing tank into a fluidized bed reactor. This resulted in great variability in the removal of nitrate, as well as an accumulation of nitrite. In a subsequent study, denitrification was enhanced by diverting the culture water to a sedimentation basin, with a hydraulic residence time of 5 hours, prior to entering the fluidized bed reactor (Arbiv and van Rijn, 1995).

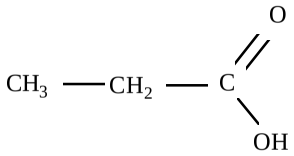
General structure



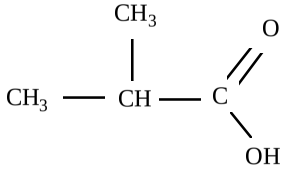
Acetic acid



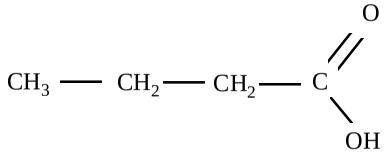
Propionic acid



Isobutyric acid

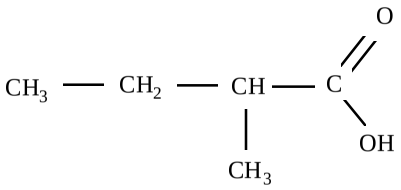


n-Butyric acid

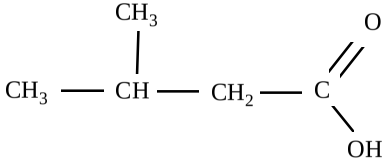


Isovaleric acids

2-Methylbutyric acid



(3-Methylbutyric acid)



n-valeric acid

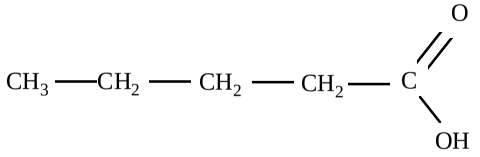


Figure 3. General and specific chemical structures for volatile fatty acids generated from the fermentation process (adapted from Morrison and Boyd, 1966).

Table 1. Stored potential energy for volatile fatty acids expressed as oxidation states and COD values for carbon atom and total compound.

Volatile Fatty Acid (VFA)	Average oxidation state of carbon atom	g COD / g VFA	g COD/g C
Acetic acid CH_3COOH (MW = 60.0)	0	1.07	2.67
Propionic acid CH_3CH_2COOH (MW = 74.1)	-0.67	1.51	3.11
Isobutyric acid $(CH_3)_2CHCOOH$ (MW = 88.1)	-1	1.82	3.33
n-Butyric acid $CH_3(CH_2)_2COOH$ (MW = 88.1)	-1	1.82	3.33
2-Methylbutyric acid $(CH_3)_2CH_2CHCOOH$ (MW = 102)	-1.2	2.04	3.47
3-Methylbutyric acid $(CH_3)_2CHCH_2COOH$ (MW = 102)	-1.2	2.04	3.47
n-Valeric acid $CH_3(CH_2)_3COOH$ (MW = 102)	-1.2	2.04	3.47

High nitrogen removal was consistently achieved, with only minor concentrations of nitrite (<1 mg NO_2-N/L) detected. The improved performance was attributed to the production of more easily assimilated substrates through the hydrolysis and fermentation of the organic matter.

Biofiltration

Biofilters employ the attached growth process in which a biofilm is generated from the propagation of microorganisms on an inert surface. Pollutant removal through biofiltration is governed by liquid film diffusion, biofilm diffusion, and metabolic reactions. Liquid film diffusion involves the transfer of substrate from the turbulent bulk liquid phase to the stagnant layer adjacent to the biofilm. Molecular diffusion then transports the substrates into the biofilm where metabolic reactions transpire and the resulting products are transferred back to the liquid phase (Harremoës, 1978). Figure 4 illustrates the concept of a functioning biofilm, where S_b is the substrate concentration in the bulk liquid and S_s is the substrate concentration at the surface of the biofilm.

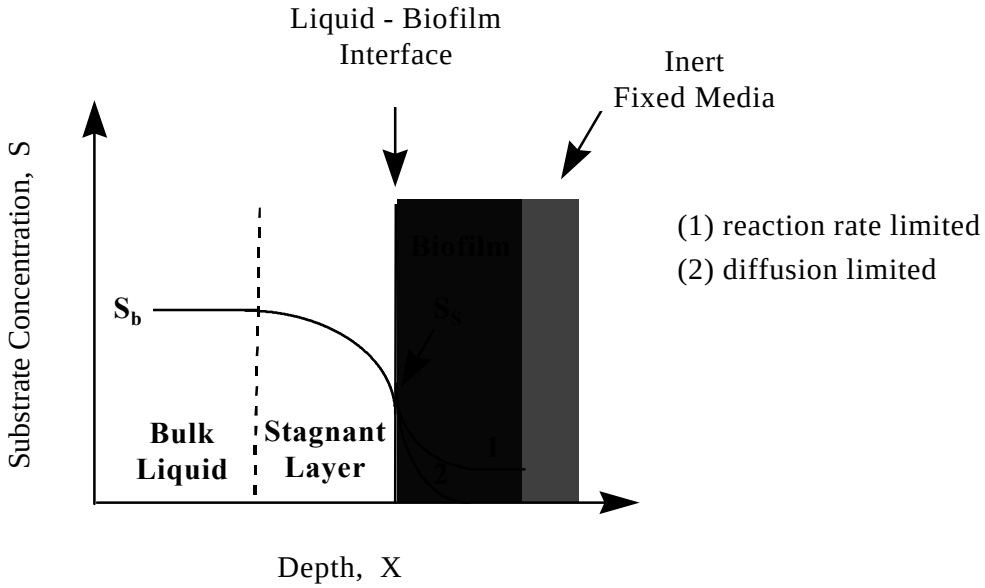


Figure 4. Biofilm kinetic model illustrating reaction rate and diffusion limitations. (adapted from Williamson and McCarty, 1976; Harremoës, 1978).

Filter performance and reaction kinetics are affected by limitations relating to reaction rates and diffusion conditions in the presence of a growth rate limiting substrate. When the substrates are able to fully permeate the biofilm, substrate utilization is controlled by the rate of reaction (Figure 4, condition 1). A diffusion limitation exists when metabolism of the growth rate limiting substrate in the biofilm is much faster than diffusion into the biofilm (Figure 4, condition 2) (Williamson and McCarty, 1976; Harremoës, 1978). Limitations with respect to liquid film diffusion are generally considered to be insignificant. Christiansen et al. (1995) reported that the denitrification reaction rate for a downflow, submerged bed was not notably affected by liquid film diffusion when the superficial velocity ranged from 1.3-10.9 m/hr.

The Monod equation is an empirical growth model that can be used to explain the metabolic reactions that occur within the biofilm:

$$\mu = \mu_{max} S_s / (K_s + S_s) \qquad (3)$$

where μ = specific growth rate coefficient, μ_{max} = maximum specific growth rate, K_s = half saturation coefficient, and S_s = growth rate limiting substrate concentration. The reaction is zero order when S_s is significantly greater than K_s, transforming the equation into $\mu \approx \mu_{max}$. When S_s is considerably less than K_s , the equation may be approximated by $\mu = (\mu_{max}/ K_s) * S_s$ and represents a first-order reaction rate. As a result, zero-order kinetics reign for high substrate concentrations while first-order kinetics govern for low substrate concentrations, with regard to the growth rate limiting substrate (Grady and Daigger, 1998).

Biofilm kinetics have been characterized using zero, first, and half-order equations with respect to the limiting substrate (Harremoës, 1978), as represented by the following equations:

General equation	$r = dC/dT = - k C^n$	(4)
Zero-order	$r = - k C^0$	(5)
Half-order	$r = - k C^{1/2}$	(6)
First-order	$r = - k C^1$	(7)

where C = substrate concentration, k = denitrification reaction coefficient, and n = order of reaction. The reaction order within a filter is greatly affected by the degree to which the reaction rate limiting substrate penetrates the biofilm. Half-order, and sometimes first-order (Green et al., 1995), reaction rates are seen for partial penetration of the substrate into the biofilm, representing diffusion limitations, while zero-order reaction rates are found for situations in which the substrate fully permeates the biofilm, exhibiting reaction rate limiting conditions (Williamson and McCarty, 1976; Harremoës and Reimer, 1977; Harremoës, 1978). Permeation of substrates is influenced by the bulk liquid substrate loading, as well as biofilm thickness (Green et al., 1995), which is controlled by sloughing off excess biomass through backwashing (Williamson and McCarty, 1976). Denitrification rates throughout the entire filter are often depicted by an overall half-order model (de Mendonca et al., 1992) which combines a zero-order reaction rate governing the first portion of the filter and a half or first-order reaction depicting activity in the second region (Green et al., 1995). Table 2 provides half-order denitrification coefficients for biofilter systems reported in the literature.

Biofilters maintain a greater active fraction of biomass, as compared to suspended-growth environments, which enables the use of a smaller treatment unit (M'Coy, 1997). The efficient operation and compact size makes biofilters an attractive treatment device for the aquaculture industry, as is illustrated by their widespread use in the performance of nitrification. Complete

nitrogen removal can be achieved in recirculating aquaculture systems through the implementation of a coupled biofiltration treatment scheme employing both nitrification and denitrification.

Table 2. Literature reported half-order denitrification coefficients (k).

Conditions	Half-order denitrification coefficient (k)	Reference
upflow, fixed film column using acetate	0.0161 ± 0.0056 (mg NO_x /L)$^{1/2}$/min (hydraulic loading - 3.63 $m^3/hr/m^2$)	de Mendonca et al., 1992
	0.0437 ± 0.0078 (mg NO_x /L)$^{1/2}$/min (hydraulic loading - 7.24 $m^3/hr/m^2$)	
downflow, submerged column using methanol	0.13 - 0.21(mg NO_3-N/L)$^{1/2}$/day* (hydraulic loading - 4.05 $m^3/hr/m^2$)	Janning et al, 1995

*Units were converted from (g NO_3-N/m^3)$^{1/2}$/day.

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DENITRIFICATION OF RECIRCULATING AQUACULTURE SYSTEM WATERS USING AN UPFLOW BIOFILTER AND A FERMENTED SUBSTRATE

Jennifer B. Phillips and Nancy G. Love, Ph.D.

Submitted to Water Research

ABSTRACT

The ability of an upflow, denitrifying biofilter using a fermentation generated carbon source to treat the high nitrate concentrations typically seen in recirculating aquaculture systems was studied using a synthetic nitrate wastewater supplied at two nitrate loadings, 1.13 and 2.52 kg NO_3-N/m^3/day. A supplemental carbon source was provided primarily through the fermentation of fish food which generated volatile fatty acids (VFA) in the form of acetic, propionic, isobutyric, *n*-butyric, 2-methylbutyric, 3-methylbutyric, and *n*-valeric acids. Acetic and propionic acids were the predominant constituents generated, while lower concentrations of the longer carbon chain butyric and valeric acids were produced. The VFAs proved to be a viable carbon source for the denitrification process as indicated by the ability of the biofilm to assimilate all of the constituents generated. Carbon limiting the system resulted in an increase in effluent nitrite and incomplete nitrate removal. During the low nitrate loading condition, influent COD to NO_3-N ratios greater than 5 typically achieved high total nitrogen removals greater than 95%. This influent ratio corresponded with a COD to NO_x-N consumption ratio of 4.62 ± 0.28 mg/L as COD per mg/L as N for complete nitrogen removal. Under the high nitrate loading condition, influent COD to NO_3-N ratios achieving high nitrogen removals showed great variability and did not correspond to a distinct value. The COD to NO_x-N consumption ratios were often below stoichiometric values, which was attributed to the hydrolysis of influent fermentation solids captured within the column to generate a COD source not measured by filtered samples. The column biofilm kinetics were modeled using a half-order reaction rate and denitrification coefficients (k) of 0.70 ± 0.02 (mg NO_x-N/L)$^{1/2}$ / min and 1.18 ± 0.12 (NO_x-N /L)$^{1/2}$ / min were determined for the low and high nitrate loading phases, respectively.

INTRODUCTION

The demand for seafood continues to rise while the natural resources that have historically supplied this commodity are being depleted. In the wake of this decreasing availability of fish from natural waters, the aquaculture industry has flourished by providing an alternative source through the controlled propagation of seafood. Traditional methods of fish production have been water intensive, but as the availability of clean water sources becomes more limited the application of water reclamation and conservation techniques will become essential (Stickney, 1994). Water conservation techniques are employed in recirculating aquaculture systems, which are used to rear high densities of fish while continuously recycling the water. Since fresh water addition is minimized, the accumulation of ammonia and particulate waste generated from the metabolism of feed causes the rapid deterioration of the culture water if treatment techniques are not applied. Aquaculturists employ common wastewater treatment techniques in recirculating production systems to yield an environment that is conducive to rearing aquatic organisms. Solids removal is typically achieved through clarification, while nitrification is employed to convert ammonia to nitrate in order to prevent free ammonia toxicity (Lucchetti and Gray, 1988). The high concentrations of nitrate that can accumulate from the application of the nitrification process result in nitrogen rich discharges from such systems. Chronic toxicity to certain fish species (Hrubec et al., 1996), as well as tightening water regulations with regard to nutrient discharge have generated concern over the accumulation of nitrates in recirculating systems.

Biological denitrification has been shown to be an efficient process for the removal of nitrates in wastewater treatment (Metcalf and Eddy, 1991). Since recirculating aquaculture systems already employ nitrification, complete nitrogen removal can be achieved through the

addition of denitrification technologies. Denitrification requires an electron donor as a carbon and energy source to fuel the process. Typically recirculating aquaculture systems possess relatively low concentrations of dissolved organic compounds (Easter, 1992), thus a supplemental carbon source is required. Wastewater treatment plants usually add methanol or acetate (de Mendonca et al, 1992; Sadick et al., 1996) to fuel denitrification reactions when an available carbon deficiency exists. While other researchers have used methanol in denitrification systems treating aquaculture system waters, (Balderston and Sieburth, 1976; Whitson et al., 1993; Abeysinghe et al., 1996) the associated cost generally does not make this an attractive option.

Fish waste constitutes a source of organic matter produced within the fish culture unit that can be used to generate a suitable carbon source for the denitrification process (Arbiv and van Rijn, 1995). Since this organic matter is in the particulate form, hydrolysis and fermentation can be applied to convert these substances into soluble substrates which are more readily available for microbial use (Eastman and Ferguson, 1981; Lee et al., 1995). The use of an organic substrate that is prevalent in the system is aimed towards the development of a partially or fully self-sustaining treatment process. In addition, the amount of particulate waste requiring disposal is reduced by converting a fraction of the particulate matter into a soluble form that is consumed by the denitrification process.

Several researchers have studied the application of denitrifying biofilters to achieve nitrate removal in recirculating aquaculture systems (Balderston and Sieburth, 1976; Whitson et al., 1993; Arbiv and van Rijn, 1995; Abeysinghe et al., 1996). While most researchers have employed methanol as the carbon and energy source, van Rijn and Rivera (1990) used endogenous organic matter generated within the fish culture unit. Culture water containing particulate organic matter

in the form of fish waste and uneaten food was metered directly from the rearing tank into a fluidized bed reactor. This resulted in great variability in the removal of nitrate, as well as an accumulation of nitrite. In a subsequent study, denitrification was enhanced by directing the culture water to a sedimentation basin having a hydraulic residence time of 5 hours prior to entering the fluidized bed reactor (Arbiv and van Rijn, 1995). High nitrogen removal was consistently achieved, with only minor concentrations of nitrite detected. The improved performance was attributed to the production of more easily assimilated substrates through the hydrolysis and fermentation of the organic matter into a predominantly soluble form.

The efficient operation and compact size of biofilters makes them an attractive treatment device for the aquaculture industry, as is evident by their widespread use in the performance of nitrification. Biofiltration is an attached growth process in which a biofilm is generated from the propagation of microorganisms on an inert surface. Filter performance and reaction kinetics are affected by limitations relating to reaction rates and diffusion conditions in the presence of one or more growth rate limiting substrates. When the substrates are able to fully permeate the biofilm, substrate utilization is controlled by the metabolic reaction rate, while a diffusion limitation exists when metabolism of the growth rate limiting substrate(s) within the biofilm is much faster than diffusion into the biofilm (Williamson and McCarty, 1976; Harremoës, 1978).

Denitrification biofilm kinetics are often depicted by an overall half-order model (Harremoës, 1978) which reflects the combination of a zero-order reaction rate and first-order diffusion rate in the first portion of a filter, while a first-order reaction rate depicts both metabolism and diffusion in the second region of a filter (Grady and Lim, 1980). The half-order model is represented by the following equation:

$$r = - k C^{1/2} \quad (8)$$

where r = the change in substrate concentration over time, C = substrate concentration, and k = denitrification reaction coefficient. Use of a half-order rate expression does not explicitly reflect the different phenomena occurring within biofilters; rather, it implies that a combination of first and zero-order rate controlling reactions are occurring throughout the filter. Additionally, it provides a basis for comparing the overall performance of different biofilters in terms of substrate depletion and will be used for that purpose here.

The purpose of this study was to investigate the removal of nitrates from recirculating aquaculture system waters through denitrification at various nitrate loadings. A denitrifying biofilter was used to reduce nitrate to nitrogen gas and a supplemental carbon source was provided through the fermentation of fish food. The specific objectives of this study were to:

1. determine the relationship between the applied carbon to nitrogen ratio and the subsequent denitrification efficiency achieved,
2. characterize the nature of the organic matter produced from the fermentation of fish waste and food,
3. determine the fermentation product(s) preferred by the denitrification process, and
4. demonstrate the overall performance of these biological processes and their application in the treatment of recirculating aquaculture system waters.

MATERIALS AND METHODS

A pilot-scale biological denitrification system comprised of an upflow, fixed film column and two fermentation units (Figure A.1, Appendix A) was operated at the Virginia Tech Aquaculture Center (Blacksburg, VA). Low and high nitrate loading conditions (1.13 kg NO_3-N/m^3/day and 2.52 kg NO_3-N/m^3/day, respectively), simulating anticipated influent nitrate concentrations in recirculating systems, were studied at a hydraulic loading rate of 3.0 m^3/m^2/hr.

The original design of this system incorporated the fermentation of fish waste to provide a carbon source for the denitrification process, thereby using organic matter prevalent in the recirculating aquaculture system. Fish waste was to be collected from on-site pilot-scale recirculating aquaculture systems used to research closed system fish production. However, at the inception of this study renovations had just been completed at the Aquaculture Center and the rearing tanks were lightly stocked with fish prior to the start of intensive studies. The low stocking densities (< 12 g/L, 0.1 lb/gal) resulted in fish waste production that was insufficient to provide a consistent carbon source for the denitrification system. Therefore, fish food was fermented in place of the preferred fish waste.

Denitrification Column

A fixed film biological denitrifying column (4.0 m x 15.2 cm) was constructed with schedule 40 clear PVC pipe connected by schedule 80 PVC couplings. Six sample ports were spaced 48.3 cm apart along the height of the column. The column was packed with 0.044 m^3 of 2-3 mm floating, polystyrene media (Biostyr®, Krüger, Cary, North Carolina) possessing a specific surface area of 1000 m^2/m^3. A steel screen located directly above the highest sample port

retained the media within the column. The column was seeded with activated sludge obtained from a denitrification basin at a local municipal wastewater treatment plant (Blacksburg, VA).

Fermenter Operation

Commercial fish food (Southern States Cooperative, Inc., Richmond, VA) containing minimum crude protein levels ranging from 36 - 42% and minimum fat concentrations between 4.5 - 7% was provided as the fermentation source. The feed was ground prior to addition in order to facilitate hydrolysis. Sodium bicarbonate ($NaHCO_3$) was added as a buffer to maintain a pH between 6.5 to 7.5 and prevent pickling of the fermentation process. Mixing was achieved in each fermenter with one submersible pump (1/6 hp).

Fermentation was conducted under two operational regimes during this study using 450 L, black polyethylene tanks. From days 1 – 101 fermentation was conducted in one tank, F1, operated as a sequencing batch reactor (SBR) with a solids retention time (SRT) and hydraulic retention time (HRT) equaling 3 days. The daily wastage from the fermenter was collected and settled in plastic buckets from which the supernatant was transferred to a 380 L, white polyethylene storage container. Fermented supernatant was then pumped from the storage container into the denitrifying column. The complete SBR operational cycle consisted of a waste and fill phase, reaction phase, settle phase, and decant phase that spanned 24 hours (Figure 5).

On day 102, fermenter operation was modified to allow for the continuous pumping of supernatant directly from the fermentation tank into the column during the SBR decant phase. This was accomplished by increasing the complete fermentation cycle to a 48 hour period and adding a second fermenter, F2, so that operations for each fermenter were offset from each other by 24 hours, as shown in Figure 5. During this second operational regime, the two fermenters

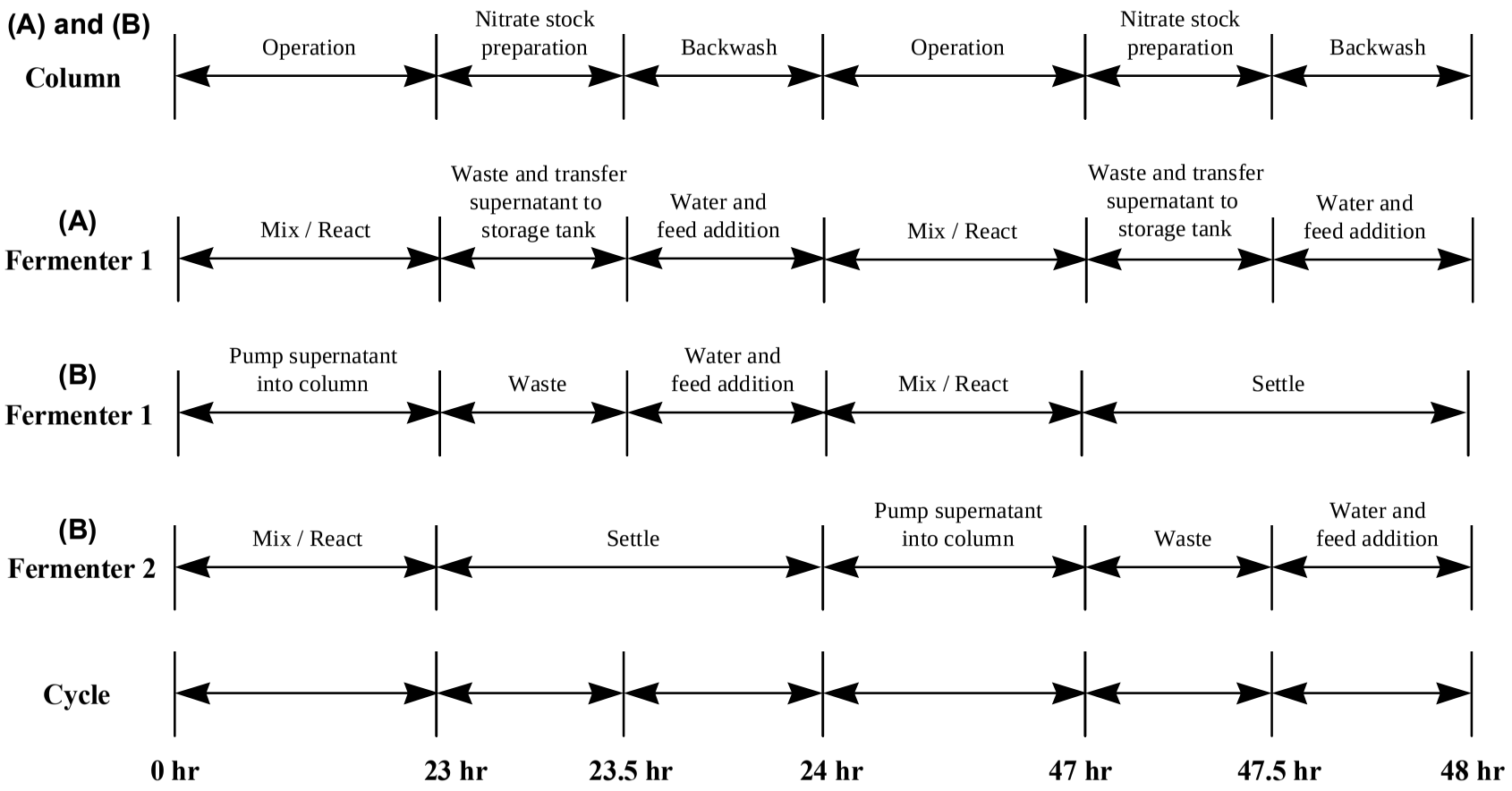

Figure 5. Operational schedules for the denitrifying column and fermentation units during the periods in which (A) one fermenter was operated and (B) two fermenters were operated.

were operated as modified sequencing batch reactors on a 6 day SRT and 3.5 day HRT.

Fish waste was supplied as the sole source of organic matter to F2 upon start-up because fish waste production in the aquaculture tanks appeared to approach sufficient levels. However, it was discovered that waste production was still insufficient to provide adequate quantities of carbon similar to those provided by the fish food alone. Therefore, the use of fish waste in F2 was discontinued on day 116 and fish food was added instead. Fish food addition to the fermenters on days for which wastage and food addition were performed ranged from 300 - 800 g during the low nitrate loading phase and 1200 - 3500 g during the high nitrate loading phase.

System Operation

A synthetic nitrate wastewater was prepared daily in two fiberglass tanks (800 L each) using industrial grade sodium nitrate ($NaNO_3$) (Chilean Nitrate Corporation, Norfolk, VA) and tap water. Nitrate solutions were well mixed for 30 minutes with a submersible pump. The nitrate feed and fermented supernatant were delivered by Masterflex® peristaltic pumps (Cole Parmer Instrument Co., Chicago, IL) and mixed in-line prior to entering the column. The column was operated in an upflow manner, with untreated influent entering the bottom of the column and exiting the top. The system was operated on a 24 hour cycle, with maintenance occurring in the last hour (Figure 5). After 23 hours of operation, the column was shutdown and backwashed to remove excess biomass. In order to effect media bed turnover for efficient biomass sloughing, backwashing was achieved by draining the column and then adding pressurized tap water in a downflow manner. At the same time, the fermenters were either settled or wasted and fed.

Biomass Yield Studies

During the low nitrate loading phase, sampling was conducted over a 24 hour column cycle to determine the observed biomass yield of the denitrifying organisms. At the end of the cycle the backwash water was collected and mixed well in a plastic container, from which a subsample was obtained for solids concentration analysis. It was assumed that the amount of biomass wasted through each backwash represented the net amount of biomass growth that occurred during the 24 hour period. Several influent and effluent samples were collected over the cycle to determine an average value for COD consumption. The observed yield value determined by this method was used to estimate biomass production for COD balance calculations for column samples collected during the low nitrate loading using the following equation (Grady and Daigger, 1998):

COD consumed = terminal electron acceptor consumed + α (biomass produced) (9)

where α = 1.0 when NH_4^+ serves as the nitrogen nutrient source. All components in this equation are expressed in COD units. The amount of terminal electron acceptor consumed was calculated from measured concentrations of nitrate and nitrite and assumed that nitrate was reduced to nitrogen gas (N_2), thereby excluding gaseous intermediates. Terminal electron acceptor consumption values provided in terms of COD were calculated by applying the theoretical COD demand, as follows: 2.86 mg O_2/mg NO_3-N for the reduction of NO_3^- to N_2; 1.14 mg O_2/mg NO_3-N for the reduction of NO_3^- to NO_2^-; and 1.71 mg O_2/mg NO_2-N for the reduction of NO_2^- to N_2.

Batch Studies

Fish food was fermented under batch conditions to determine an appropriate solids residence time for the prevention of methanogenesis, while achieving optimum fermentation as indicated by soluble COD production. Fermentation was conducted for 18 days in a completely mixed, two liter glass bottle sealed with a stopper containing an off-gas vent hole. The solution was purged with nitrogen gas for 45 minutes prior to beginning the test in order to create an anaerobic environment. Samples were collected on a daily basis. To compare the nature of the fermentation products generated by fish food and fish waste, fish waste was fermented in a two liter glass SBR operated in the same manner as the pilot-scale fermenters, with respect to wasting and fish waste addition, to ensure a 3.5 day HRT and 6 day SRT. Fish waste was obtained on a weekly basis from a multitube clarifier operating on a fish culture unit rearing Tilapia and stored in a plastic bucket prior to addition to the SBR. The batch reactor was buffered with $NaHCO_3$ for pH control to prevent pickling of the fermentation process. The test was conducted over 48 days and samples were collected daily.

During the high nitrate loading, batch kinetics tests were conducted using biomass collected from the column backwash water. Tests were performed in duplicate for initial concentrations of 20, 50, and 100 mg/L NO_3-N and NO_2-N. Tests were conducted in 500 mL glass bottles under a nitrogen gas ($N_{2\ (g)}$) headspace. Samples were collected from a submerged port located at the bottom of the bottle. A mineral salts medium was added in the following quantities to provide nutrients for the microorganisms: 0.036 mM $CaCl_2$, 0.011 mM $FeCl_3 \cdot 6H_20$, 0.0012 mM $CoCl_2 \cdot 6H_20$, 0.0022 mM $ZnCl_2$, 0.0006 mM $CuCl_2 \cdot 2H_20$, 0.0004 mM H_3BO_3, 0.0122 mM $MgSO_4 \cdot 7H_20$, 0.0054 mM $MnSO_4 \cdot H_20$, 0.0004 mM $Na_2MoO_4 \cdot 2H_20$, 5.33 mM

NH_4Cl, and 1 mM KH_2PO_4. Prior to initiating a test, biomass samples were purged with nitrogen gas for 30 minutes to remove oxygen from the mixture. The top of the vessel was sealed with a rubber stopper containing an influent nitrogen gas line and an effluent line with a balloon attached to allow for the expulsion of nitrogen gas formed during the denitrification process, while preventing the introduction of oxygen. After purging, nitrate or nitrite was added to initiate a test. Sodium acetate ($NaCH_3COOH$) was added in excess as a carbon and energy source to provide an initial concentration of 500 mg/L as COD. Samples were collected over time and immediately filtered. To prevent the formation of a vacuum within the bottle, nitrogen gas was administered prior to each sampling event.

Data collection and analytical procedures

Column influent and effluent samples were routinely collected on a weekly basis. Additionally, samples were collected along the length of the column at least biweekly. All samples, except those analyzed for ammonia, were centrifuged for 5-10 minutes at 4000 rpm x g and subsequently filtered using 0.45 μm Supor membrane filters (Gelman Sciences, Ann Arbor, MI).

Nitrate (NO_3^-), nitrite (NO_2^-), sulfate (SO_4^{2-}), and chloride (Cl^-) anions were measured according to Standard Method 4110 B (APHA, 1995) using a Dionex 2010I ion chromatograph equipped with an HP 3395 Integrator (Sunnyvale, CA). Samples were analyzed upon collection, or frozen immediately for later analysis. Ammonia was analyzed immediately according to Standard Method 4500-NH_3 C (APHA, 1995).

Dissolved organic carbon (DOC) was measured using a total organic carbon analyzer (Dohrmann Model DC 80, Santa Clara, CA). Chemical oxygen demand (COD) was measured

according to Standard Method 5220 C (APHA, 1995). Samples were acidified with concentrated sulfuric acid (pH < 2) and either stored at 4 ^{o}C for analysis within 24 hours or frozen for later analysis. Nitrite has an oxygen demand of 1.1 mg O_2/mg NO_2-N which, according to Standard Methods, can typically be ignored as wastewaters rarely have nitrite concentrations above 1 or 2 mg/L as N. For samples containing higher nitrite concentrations, the addition of 10 mg sulfamic acid is recommended for each mg of NO_2-N present to correct for this interference. High nitrite concentrations were measured during this study, thereby creating a positive interference in COD measurements. An experiment was conducted to evaluate the effect sulfuric acid preservation had on nitrite interference and determine if sulfamic acid addition was necessary (Figure A.2, Appendix A). Five nitrite concentrations ranging from 0 to 80 mg NO_2-N /L were evaluated using a 100 mg/L as COD potassium hydrogen phthalate standard spike. Results indicated that analysis of sulfuric acid preserved samples had fewer unexplainable variations when sulfamic acid was omitted than when it was added. Therefore, a correction factor for nitrite interference was determined based on sulfuric acid preservation alone:

$$\text{Corrected COD} = \text{measured COD} - 0.2353 * NO_2\text{-N concentration} \quad (10)$$

Volatile fatty acids (VFA) were measured using a Hewlett Packard 5880 A Series gas chromatograph equipped with a flame ionization detector (FID) and a 30” x 1/4” x 4 mm ID packed column made of 60/80 carbopack C, 30% Carbowax® 20 M, 10 % H_3PO_4 (Supelco, Inc. Bellefonte, PA). Nitrogen served as the carrier gas and flowed at 50 mL/min. Hydrogen and air pressures were maintained at 40 psi. Injector and detector temperatures were set at 200 ^{o}C. The oven temperature was maintained at an initial value of 120 ^{o}C for 3 minutes, increased to a final temperature of 130 ^{o}C at a rate of 0.5 ^{o}C/min, and then held at the final temperature for 1 minute.

Standards and samples were prepared with formic acid (50 μL formic acid per 5 mL sample) prior to injection. Acid concentrations provided in terms of COD were calculated by applying the theoretical COD demand to the measured concentration. The following theoretical COD values were used, expressed as mg COD/mg acid: acetic acid - 1.07; propionic acid - 1.51; isobutyric and *n*-butyric acids - 1.82; 2-methylbutyric, 3-methylbutyric, and *n*-valeric acid - 2.04.

Total suspended solids (TSS) and volatile suspended solids (VSS) were analyzed according to Standard Methods 2540 D and 2540 E, respectively. Values provided on a COD basis were calculated by applying the conversion factors of 1.2 g COD/g dry weight and 1.42 g COD/g ash-free dry weight to TSS and VSS measurements, respectively. Dissolved oxygen and temperature were measured using a dissolved oxygen meter (YSI Digital Model 58). A pHep stick meter (Fisher Scientific and Hach) was used for on-site pH determinations of pilot-scale denitrification system samples and a pH/mV meter (Fisher Scientific, Accumet model) was employed during bench-scale batch tests.

Kinetics Calculations

Nitrate and nitrite reduction rates determined during the suspended growth batch kinetic tests were calculated using a zero-order regression model, as represented by the following equation:

$$\mathbf{r = dC/dT = - kC^0 = -k} \qquad \mathbf{(11)}$$

where r = the change in nitrate concentration over time and k = the coefficient of denitrification. The rate of nitrite reduction in the presence of nitrate was calculated from the difference between the rate of nitrate reduction and nitrite accumulation (Glass and Silverstein, 1997).

Biofilm kinetics for denitrification and VFA consumption were depicted using a half-order model, as shown previously in equation 8. The rate change in nitrogen (NO_x) or VFA as COD concentration per unit time was evaluated using this model. A non-linear curve fitting routine (Marquardt-Levenberg algorithm) was applied to column profile data sets in order to obtain estimates for k (Sigma-Plot, Version 4.0). The k values were calculated using data collected from sample ports along the column and the effluent. The influent value was excluded because the influent had not been deoxygenated prior to entering the column. The first column port was located at a depth 7.6 cm from the bottom of the media bed and it was assumed that the ambient oxygen had been removed from solution by this point through carbonaceous oxidation; consequently, only denitrification metabolism was presumed. Kinetic constants were calculated for the section of column that contained media and demonstrated active metabolism through substrate consumption profiles. In some cases where substrate consumption was complete prior to the end of the column, only data representing active metabolism were used to estimate k.

Statistical analysis methods

Statistical data analyses were conducted using Number Cruncher Statistical System (August 1997), Sigma-Plot Version 4.0, and Excel Version 5.0. These software packages were used to perform one-tailed student t-tests, descriptive statistics, and linear and non-linear regression curve fits. Average values are provided with the standard error of the mean.

RESULTS

Fixed film denitrification was investigated under two different nitrate loading conditions. During the high nitrate loading period, the flowrate was decreased from 3.0 $m^3/m^2/hr$ to 1.5 $m^3/m^2/hr$ from day 283 through 306 in order to address high nitrite concentrations detected in the column effluent. Unless otherwise specified, data presented for the high nitrate loading phase were collected during the regular hydraulic loading condition (3.0 $m^3/m^2/hr$). Table 3 outlines the loading conditions for each phase of this study.

Table 3. Nitrate and hydraulic loading conditions for the denitrification system. Average values are provided ± standard error.

Parameter	Low nitrate loading	High nitrate loading	
Period of study (days)	1 - 200	201 - 282, 307 – 346	283 - 306
Influent concentration* (mg NO_3-N/L)	38.5 ± 1.0	85.3 ± 1.7	64.8 ± 6.4
(mg NO_2-N/L)	0.65 ± 0.31	2.94 ± 0.46	14.6 ± 5.6
Mass loading (kg NO_3-N/m^3/day)	1.13	2.52	0.96
(kg NO_2-N/m^3/day)	0.02	0.09	0.22
Mass removal (kg NO_x-N/m^3/day)	0.81	2.21	1.08
Hydraulic loading (m^3 /m^2/hr)	3.0	3.0	1.5

*Measurable influent nitrite concentrations were detected only during the high nitrate loading phase under the low hydraulic loading. Standard errors for other parameters provided in this table were insignificant and therefore not provided.

The pH conditions for the denitrification column and the fermentation process are provided in Table 4. Data collected for both fermenters (F1 and F2) were combined to provide a representative value for the entire fermentation process.

Table 4. Conditions for the denitrification system. Average values are provided ± standard error of the mean.

Phase of study	Column		Fermenters
	Influent pH	Effluent pH	pH
Low nitrate loading	7.08 ± 0.07	7.87 ± 0.12	6.31 ± 0.06
High nitrate loading*	7.33 ± 0.03	8.59 ± 0.05	7.41 ± 0.02

*Values provided are comprised of data from both hydraulic loading conditions.

Fermentation

The use of a separate storage container from days 1 through 101 to hold the daily reserve of fermented supernatant resulted in significant COD degradation within the container over the 24 hour column cycle (Figure A.3 Part A, Appendix A). This loss of COD prompted a modification of the pilot-scale fermentation system on day 102. Supernatant was pumped directly from the fermentation unit, rather than stored, and the problem of declining COD concentration over time was eliminated (Figure A.3 Part B, Appendix A). Although the fermenter SRT was increased from 3 to 6 days to accommodate this operational change, batch studies conducted with fish food suggested that no significant difference in soluble carbon production would result from this increase (Figure A.4, Appendix A).

Figure 6 illustrates soluble COD (sCOD) production from the pilot-scale fermenters over the course of this study. In order to determine an appropriate influent COD to NO_3-N ratio for the denitrification system, the soluble COD production in the fermenters was varied by adding a range of fish food amounts. The average soluble COD production during the high nitrate loading phase (2352 ± 128 mg/L) was much greater than that measured during the low nitrate loading

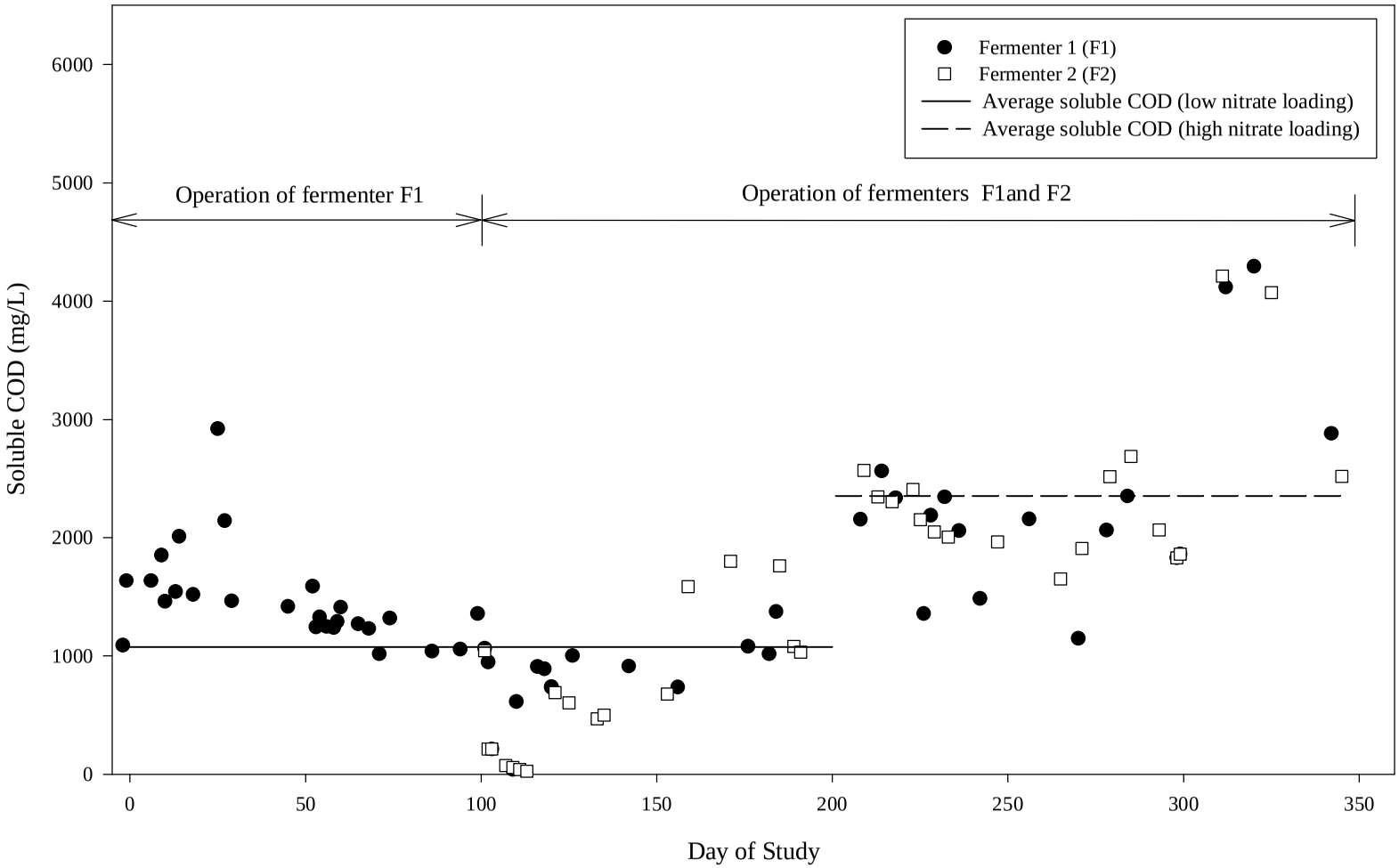

Figure 6. Soluble COD production from the pilot-scale fermentation of fish food. Fish waste was added to F2 for days 101 to 115. Lines represent average soluble COD production during the low and high nitrate loading phases based on data collected from both fermenters (F1 and F2). Average values are provided with standard error of the mean.

phase (1078 ± 76 mg/L) as a result of increased amounts of fish food added to the fermenters to generate greater soluble COD concentrations to accommodate the increase in nitrate loading. The carbon source for the denitrification system was primarily generated from the fermentation of fish food, except for the addition of fish waste to F2 during its first 13 days of operation. During this start-up period, COD concentrations below 213 mg/L were measured in F2. This COD production was significantly lower than that achieved from the fermentation of fish food as a result of insufficient fish waste production in the fish rearing tanks due to low stocking densities and light feeding rates. Consequently, fish food was initiated as the carbon source in F2.

The fermentation of fish food and fish waste generated VFAs in the form of acetic, propionic, isobutyric, *n*-butyric, isovaleric as 2-methylbutyric and 3-methylbutyric, and *n*-valeric acids. Figure 7 illustrates the relative concentrations of each VFA generated, as a percentage of the total VFA fraction of soluble COD, from the pilot-scale fermentation of fish food and the bench-scale batch reactor fermentation of fish waste. In all cases, acetic and propionic acids were produced in the greatest quantities while smaller amounts of the longer carbon chain butyric and valeric acid compounds were generated. When comparing the production of butyric and valeric acids by the two sources, the normal structures were detected in higher concentrations than the isomer forms on average when using fish food, while the fermentation of fish waste produced greater concentrations of the isomer forms. Acid production from the fermentation of fish food during the low and high nitrate loading phases yielded different concentrations of the organic constituents. Lower concentrations of the normal structures of butyric and valeric acids were produced during the high nitrate loading phase than was observed during the low nitrate

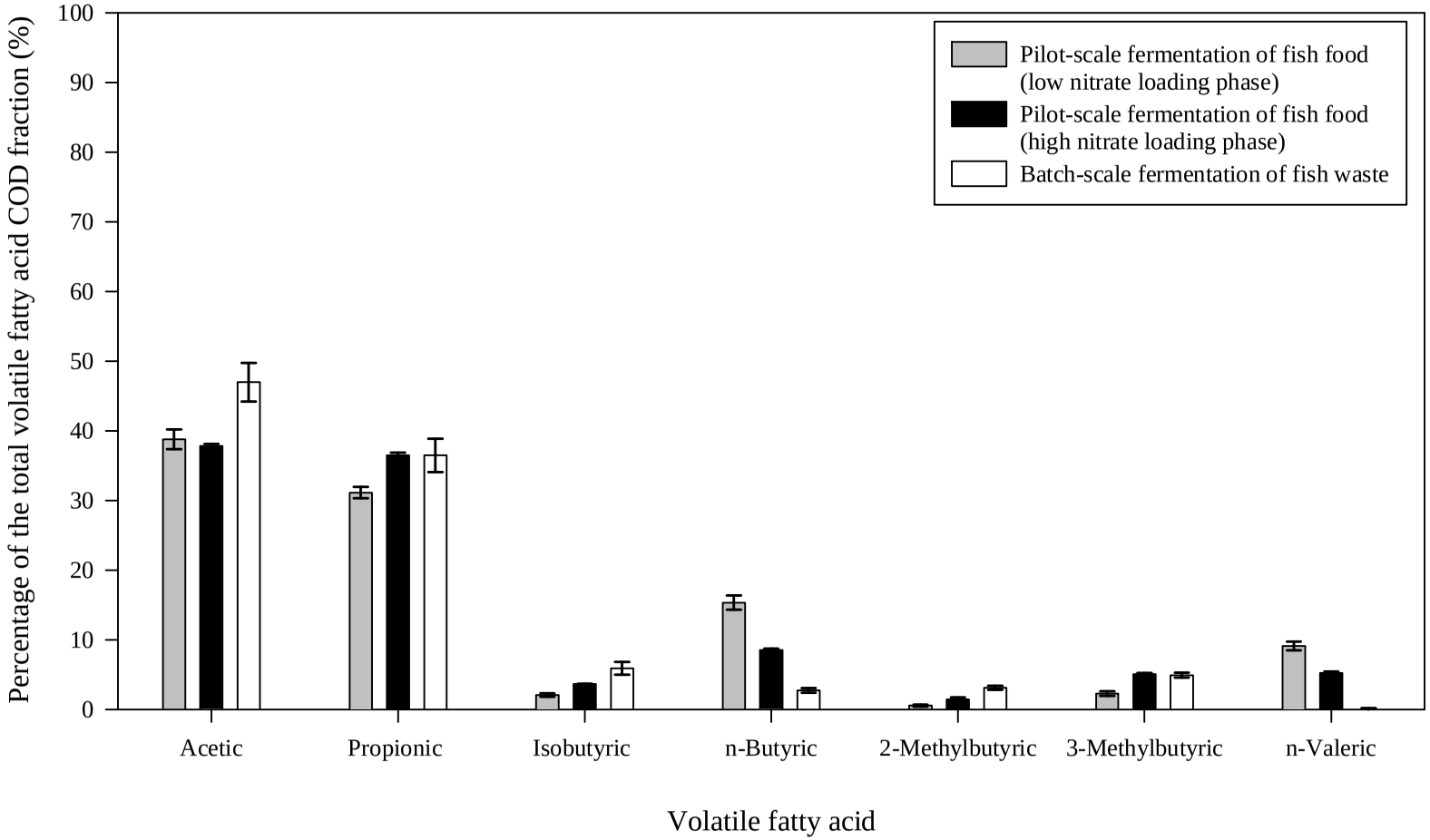

Figure 7. Volatile fatty acid (VFA) production from the pilot-scale fermentation of fish food and the batch-scale fermentation of fish waste. Relative constituent concentrations are expressed as a percentage of the total VFA fraction of COD. The fish food values are based on data combined from both fermenters (F1 and F2). Error bars represent the standard error of the mean.

loading phase. However, generation of propionic and isomer forms of butyric and valeric acids increased during the high nitrate loading phase relative to the low nitrate loading phase. Acetic acid concentrations were comparable during the two loading conditions.

The average calculated VFA fraction of the total measured COD for the fermentation of fish waste and the fermentation of fish food during the low and high nitrate loading phases was 70.1% ± 3.72, 86.3% ± 3.58, and 106% ± 3.90, respectively (Figure A.5, Appendix A). Despite a high degree of variability within each fermentation treatment, it was evident that VFAs accounted for a majority of the soluble COD produced. In addition, the VFA constituents accounted for more of the soluble COD produced from the fermentation of fish food than that generated from fish waste fermentation, on average. The non VFA fraction was not characterized.

The suspended solids concentration in the fermenter units was evaluated to determine if this parameter could be used as an indicator of soluble COD production. However, no correlation was found between soluble COD production and VSS concentration in the fish waste fermentation batch reactor or the fish food fermentation pilot-scale units (Figure A.6, Appendix A). Based on the amount of fish food added to the fermenters, 334 ± 18.7 mg sCOD was generated/mg food added in F1 during the portion of the study in which only one fermenter was operated. During the period in which two fermenters were operated, 159 ± 6.8 mg sCOD was generated/mg food added.

COD to NO_3-N Ratio

In order to prevent the reduction of sulfate (SO_4^{-2}) to hydrogen sulfide (H_2S), the denitrification column was originally run under carbon limiting conditions during the low nitrate loading phase. Available carbon limiting conditions prevailed for influent COD to NO_3-N ratios

less than 5 and resulted in incomplete nitrate removal (Figure 8a) and measurable effluent nitrite concentrations as high as 12 mg NO_2-N/L (Figure 8b). For influent COD to NO_3-N ratios above 5, high total nitrogen removals greater than 95% were consistently achieved, except in one case where the influent pH fell below 7.0. This influent ratio corresponded with a COD to NO_x-N consumption ratio of 4.62 ± 0.28 mg/L as COD per mg/L as N calculated for complete nitrogen removal (NO_2^- and NO_3^- < 1 mg/L as N) (Table A.1, Appendix A). COD was detected in all effluent samples (Figure 8c) with an average concentration of 22.5 ± 3.02 mg sCOD/L even when NO_x removal was incomplete.

Upon increasing the influent nitrate concentration on day 201 to yield a high loading of 2.52 kg NO_3-N/m^3/day, nitrate removal efficiencies greater than 99% were regularly seen (Figure 9a). Effluent nitrite concentrations were greater than those measured during the lower nitrate loading, reaching values as high as 34 mg NO_2-N/L (Figure 9b). The presence of measurable COD in the effluent suggested that carbon limitation was not the problem (Figure 9c). In an effort to remedy the high effluent nitrite problem, the hydraulic loading was decreased by 50% in order to increase the column retention time from 32 minutes to 64 minutes. After observing several days of complete nitrogen removal during the low hydraulic loading period, the flow was returned to the original rate of 3.0 m^3/m^2/hr. At this point, nitrate removal efficiencies greater than 95% were achieved (Figure 10a) and effluent nitrite remained at or below 1 mg/L as NO_2-N (Figure 10b). Complete nitrogen removal (NO_2^- and NO_3^- < 1 mg/L as N) was achieved for a COD to NO_x-N consumption ratio of 3.07 ± 0.58 mg/L as COD per mg/L as N during the high nitrate loading phase (Table A.2, Appendix A). However, complete nitrogen removal did not

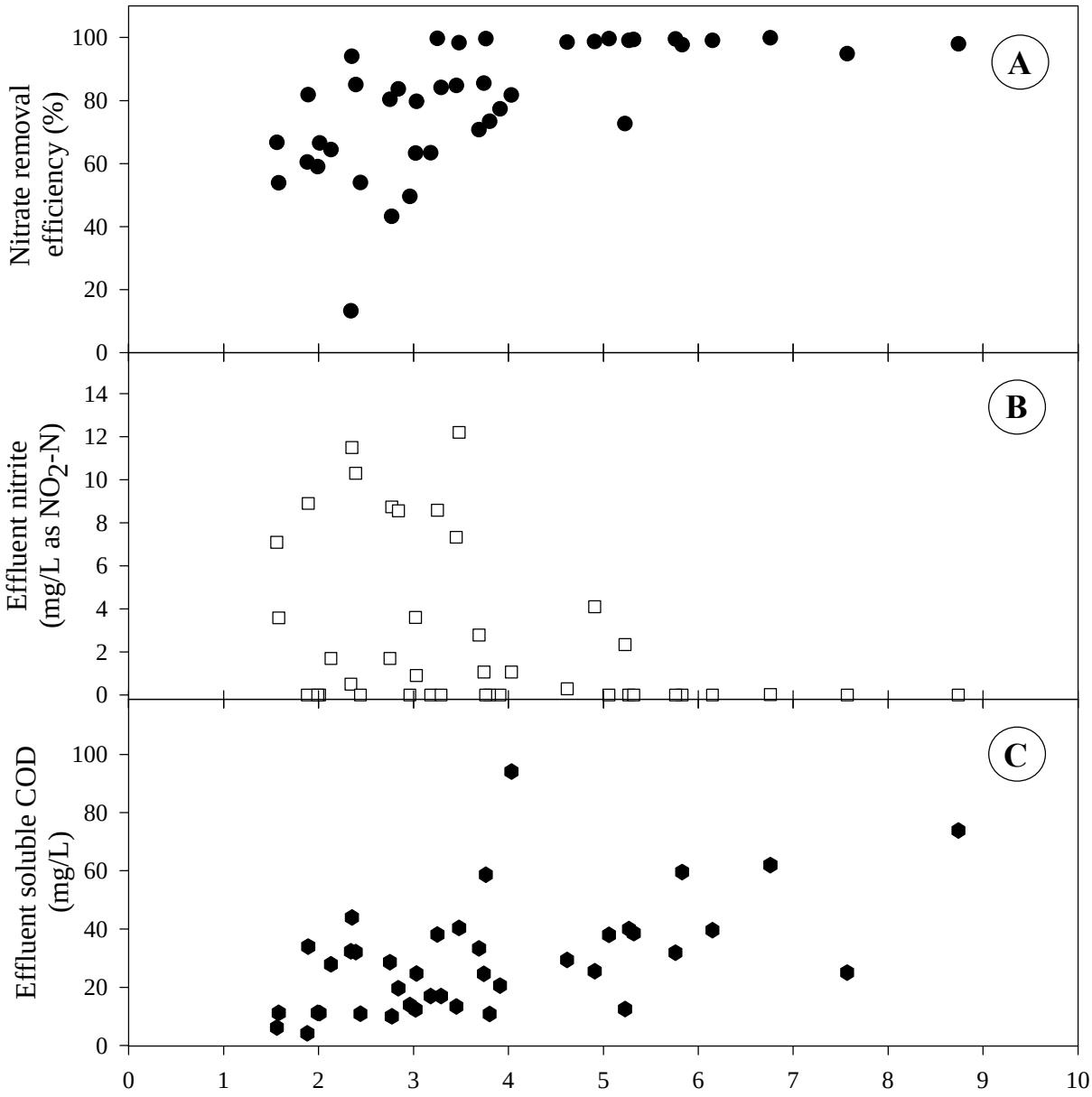

Figure 8. Denitrification column performance during the low nitrate loading phase with respect to (A) nitrate removal, (B) effluent nitrite concentration, and (C) effluent soluble COD concentration as a function of the influent COD to influent NO_3-N ratio.

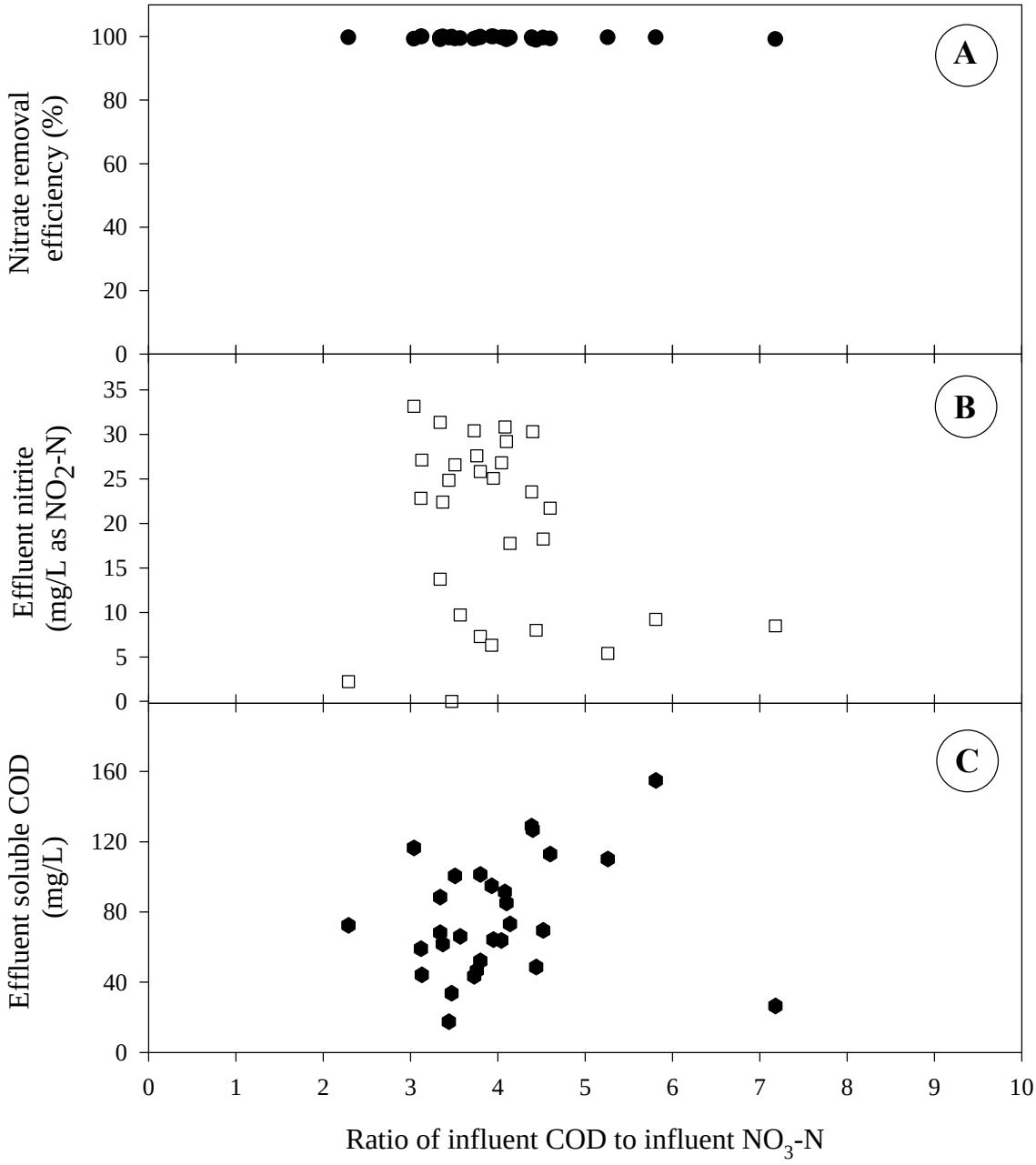

Figure 9. Denitrification column performance during high nitrate loading phase with respect to (A) nitrate removal, (B) effluent nitrite concentration, and (C) effluent soluble COD concentration, as a function of influent COD to influent NO_3-N ratio. This data is presented for days 201 through 282, prior to lowering the flowrate.

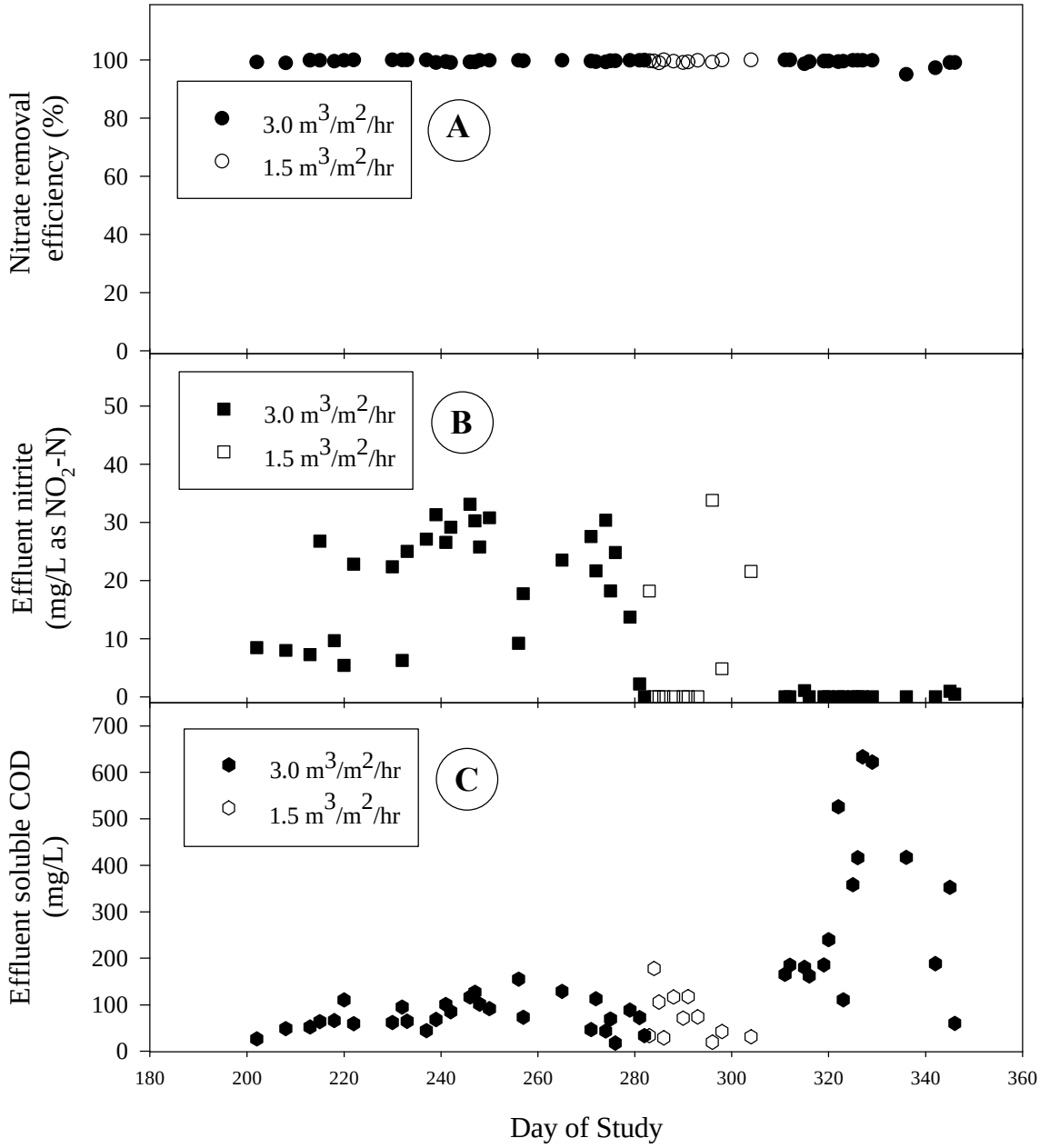

Figure 10. (A) Nitrate removal, (B) effluent nitrite concentration, and (C) effluent soluble COD concentration measured during the high nitrate loading phase as a function of hydraulic loading (1.5 or 3.0 $m^3/m^2/hr$). The flowrate was lowered during days 283 through 306.

correspond to a distinct influent COD to NO_3-N ratio and instead exhibited great variability, with ratios ranging from 3.39 to 10.4. In addition, effluent COD increased dramatically once the flowrate was returned to the original value (Figure 10c). This resulted from the application of increased influent COD to NO_3-N ratios to determine if the required ratio had shifted upward because effluent nitrite was being detected at influent ratios that achieved complete nitrogen removal during the lower nitrate loading condition.

COD Balance

COD balances calculated for the low nitrate loading phase revealed an average difference of 8.6% (ranging from 1 to 22%) between the measured and calculated COD consumption values (Table A.3, Appendix A). These calculations used an experimentally determined observed yield value of 0.35 ± 0.15 mg biomass formed as COD/mg sCOD consumed (Table A.4, Appendix A). Ammonia was detected in column effluent samples during low and high nitrate loading conditions, indicating that sufficient ammonia was available as the nitrogen source for cell growth. Analysis of the fish waste batch reactor also revealed the presence of ammonia. COD balances were not performed for data collected during the high nitrate loading phase.

Denitrification Profiles

Figure 11a presents nitrate and nitrite concentration profiles throughout the column in which high total nitrogen (NO_x) removals, greater than 95%, were achieved during the low nitrate loading condition. The nitrite concentration increased within the column and did not decline until nearly all of the nitrate was consumed. Additionally, nitrate and nitrite reduction occurred in the first 2.3 m of the column, while the remaining 1 m of the column showed

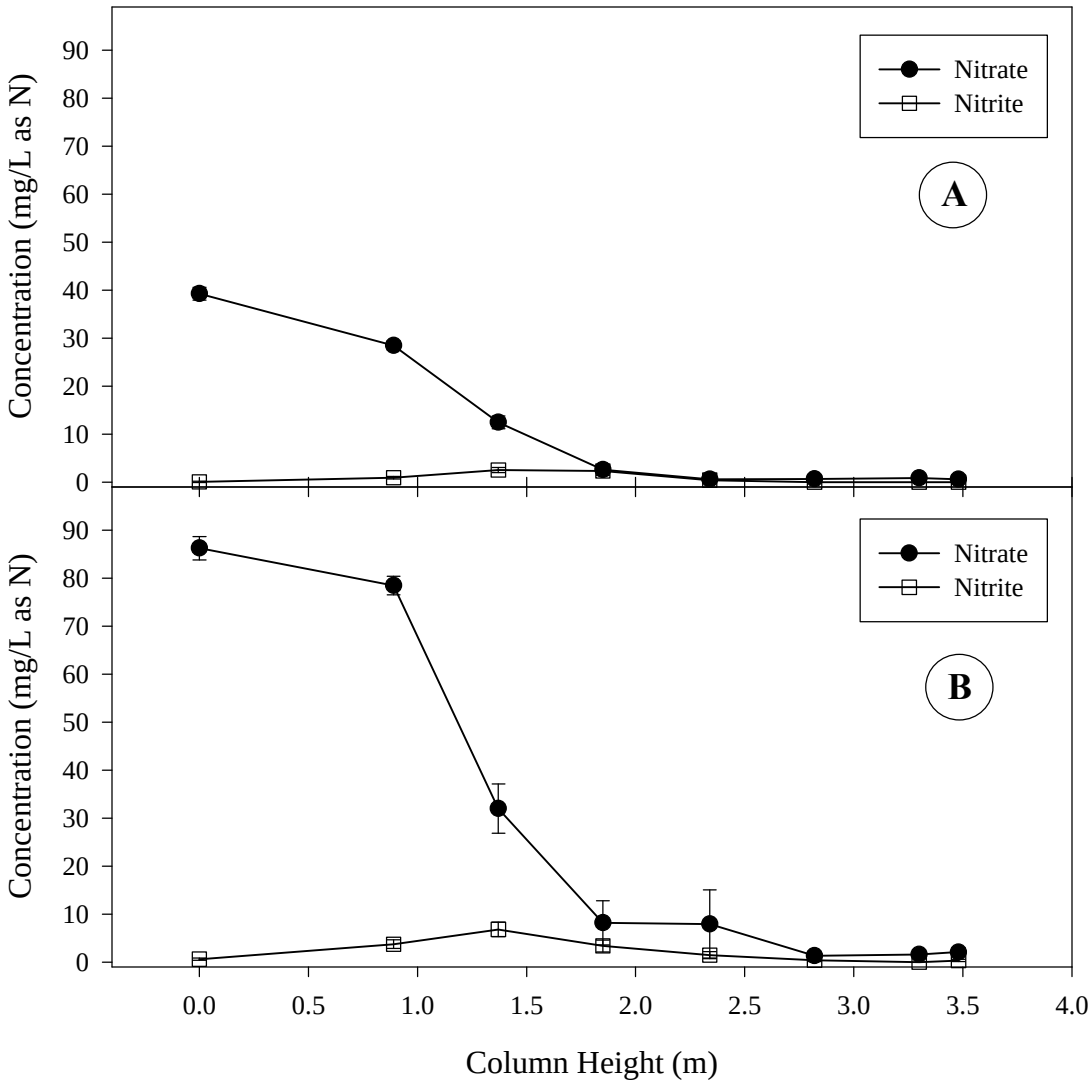

Figure 11. Column profiles taken during the (A) low nitrate (n=5) and (B) high nitrate (n=4) loading phases. Average values are based on column profiles during which nitrogen (NO_X) removal greater than 95% was achieved across the column. Error bars represent the standard error of the mean.

minimal activity. Visual inspection showed a higher biomass concentration in the lower portion of the column relative to the sparse microbial colonization at the top. Figure 11b provides a column profile for nitrate and nitrite consumption under the high nitrate loading condition for which high total nitrogen removals (NO_x) greater than 95% were obtained. Nitrite accumulated within the column reaching levels as high as 6.8 mg NO_2-N/L, which was nearly three times the peak concentration of 2.5 mg NO_2-N/L seen at the lower nitrate loading condition. In addition, 2.8 m of the column length was being used for denitrification, which was a 22% increase in column length usage from the low nitrate loading. Note that the peak nitrite concentrations measured within the column do not correspond to the peak effluent nitrite concentrations given in Figures 8 and 9. When high nitrogen removal efficiencies were achieved, the nitrite concentrations that accumulated within the column were lower than the peak nitrite concentrations detected in the effluent when nitrogen removal was incomplete.

VFA Consumption

Figure 12 illustrates VFA and COD consumption throughout the denitrifying column for profiles taken during the low nitrate phase in which nitrogen removals greater than 95% were achieved and influent COD to NO_3 ratios were sufficient to result in low effluent COD concentrations. The absence of VFAs in the effluent indicates that the denitrifying microbial population was able to assimilate all of the VFAs produced. In addition, a difference existed between the initial measured soluble COD and VFA COD fraction and seemed to increase throughout the column. The nature of this non VFA fraction was not characterized.

Under the high nitrate loading condition, the application of increased influent COD to NO_3^- ratios resulted in an increased effluent COD with measurable VFA concentrations.

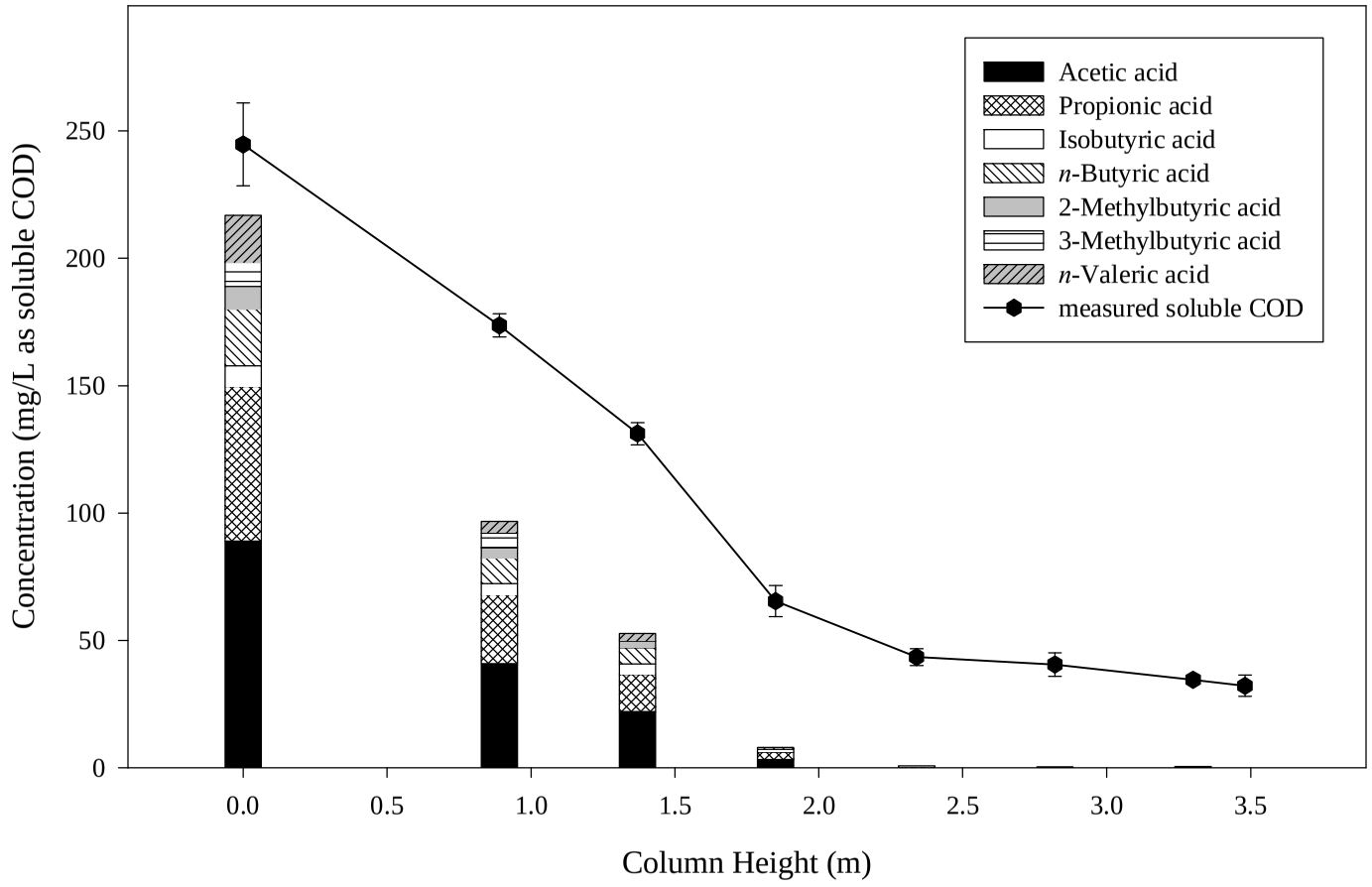

Figure 12. Column profiles taken during the low nitrate loading phase (n=3). The average values are based on column profiles in which complete volatile fatty acid removal and total nitrogen (NO_x) removal greater than 95% was achieved. Stacked bars represent the absolute concentrations measured for each VFA constituent. Error bars represent the standard error of the mean.

Although not desirable in actual operation, these excess COD conditions revealed a preference for VFAs by the denitrifying organisms. It was shown that propionic acid and the normal structures of both butyric and valeric acids were consumed most rapidly, while a slower decrease in the acetic acid concentration and less significant changes in the isomer structures of butyric and valeric acids were observed (Figure 13).

A half-order model was applied to determine the consumption rates for acetic and propionic acids throughout the column, as these two constituents supplied in the greatest concentrations to the denitrification column. The half-order model was not applied to the remaining VFAs because insufficient data points were available within a column profile to perform a valid fit. Consumption rates were determined for column profiles in which high nitrogen removals were achieved. The consumption rates for acetic and propionic acids were 0.96 and 0.81 (mg sCOD/L)$^{1/2}$ /min, respectively, during the low nitrate loading phase for column profiles in which complete VFA removal occurred, illustrating a faster consumption rate for acetic acid over propionic acid. During the high nitrate loading phase in which excess VFA conditions prevailed, the consumption rates for acetic and propionic acids were 0.64 and 1.12 (mg sCOD/L)$^{1/2}$ /min, respectively. Under these conditions, the consumption of propionic acid was nearly double that for acetic acid.

Biomass and Effluent TSS

The average biomass concentration measured in the column backwash during the high nitrate loading was not greater than that measured during the low nitrate loading ($p>0.05$, t-test, Table A.5, Appendix A) (Figure A.7.a, Appendix A). Although the biomass concentration within the column cannot be estimated from the backwash sample, this parameter was assumed to

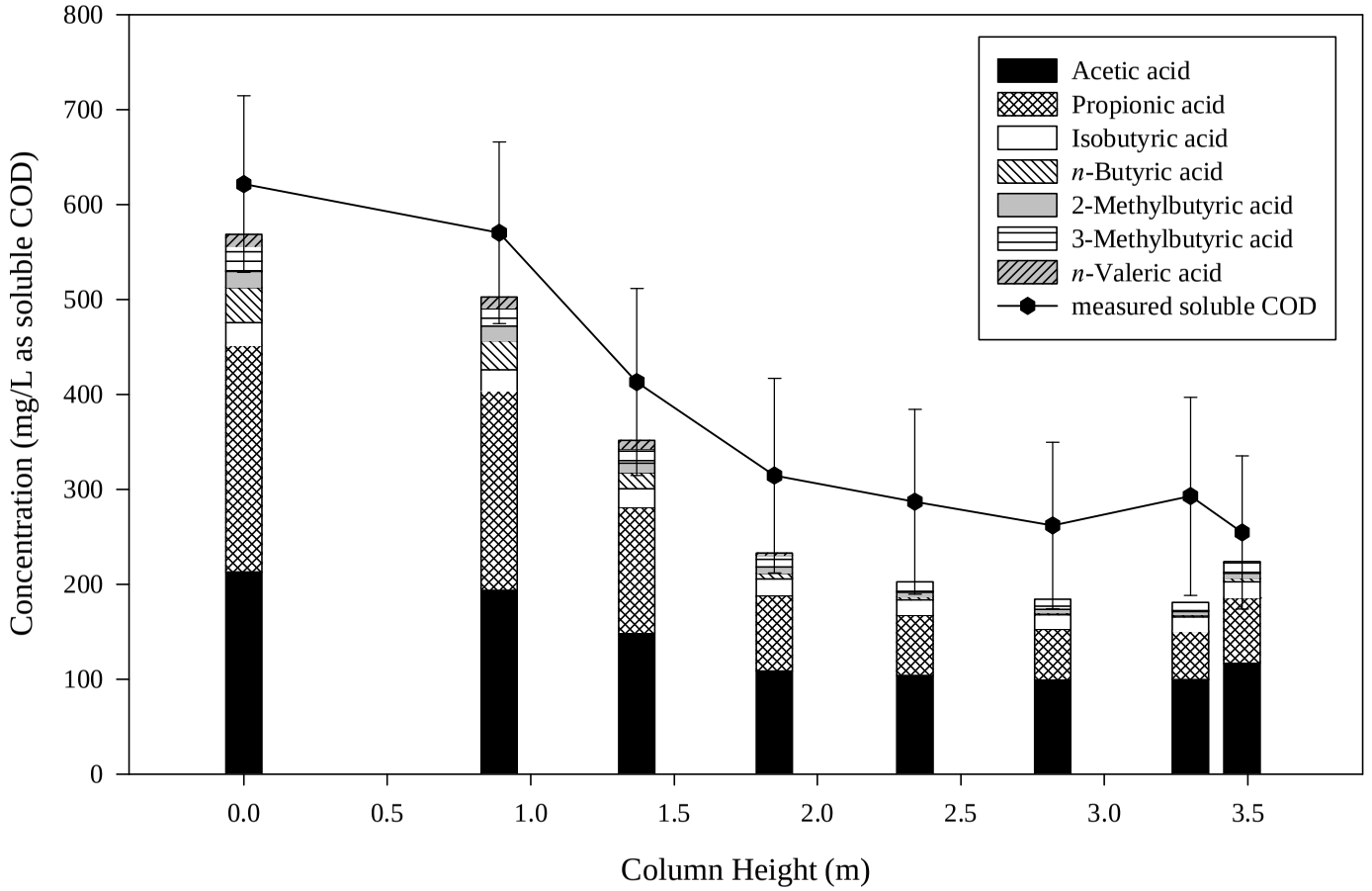

Figure 13. Column profiles taken during the high nitrate loading phase (n=4). The average values are based on column profiles in which total nitrogen (NO_x) removal greater than 95% was achieved. Stacked bars represent the absolute concentrations measured for each VFA constituent. Error bars represent the standard error of the mean.

reflect the amount of biomass growth that occurred during an operational cycle and can be used as an indicator of biofilm development. An increase in effluent solids was seen from the low nitrate loading (14 ± 3 mg/L TSS) to the high nitrate loading (54 ± 12 mg/L TSS) ($p<0.05$, t-test, Table A.6, Appendix A) (Figure A.7.b, Appendix A).

Kinetics

Suspended culture batch tests conducted with backwash biosolids during the high nitrate loading phase revealed that the microbial community was able to completely reduce both nitrate and nitrite (Figure A.8, Appendix A), even though high nitrite concentrations were being detected in the column effluent. For tests conducted at initial concentrations of 50 and 100 mg NO_3-N and NO_2-N/L, the reduction rate of nitrite decreased when nitrate was present (Table A.7, Appendix A). No nitrite accumulation was detected at the low concentration of 20 mg NO_3-N /L.

Although denitrification was represented by zero-order kinetics in the batch tests, denitrification throughout the column was governed by a half-order reaction rate (Figure 14). The denitrification coefficients (k) for the low and high nitrate loading phases were 0.70 ± 0.02 (mg NO_x-N/L)$^{1/2}$/min and 1.18 ± 0.12 (NO_x-N /L)$^{1/2}$/min, respectively.

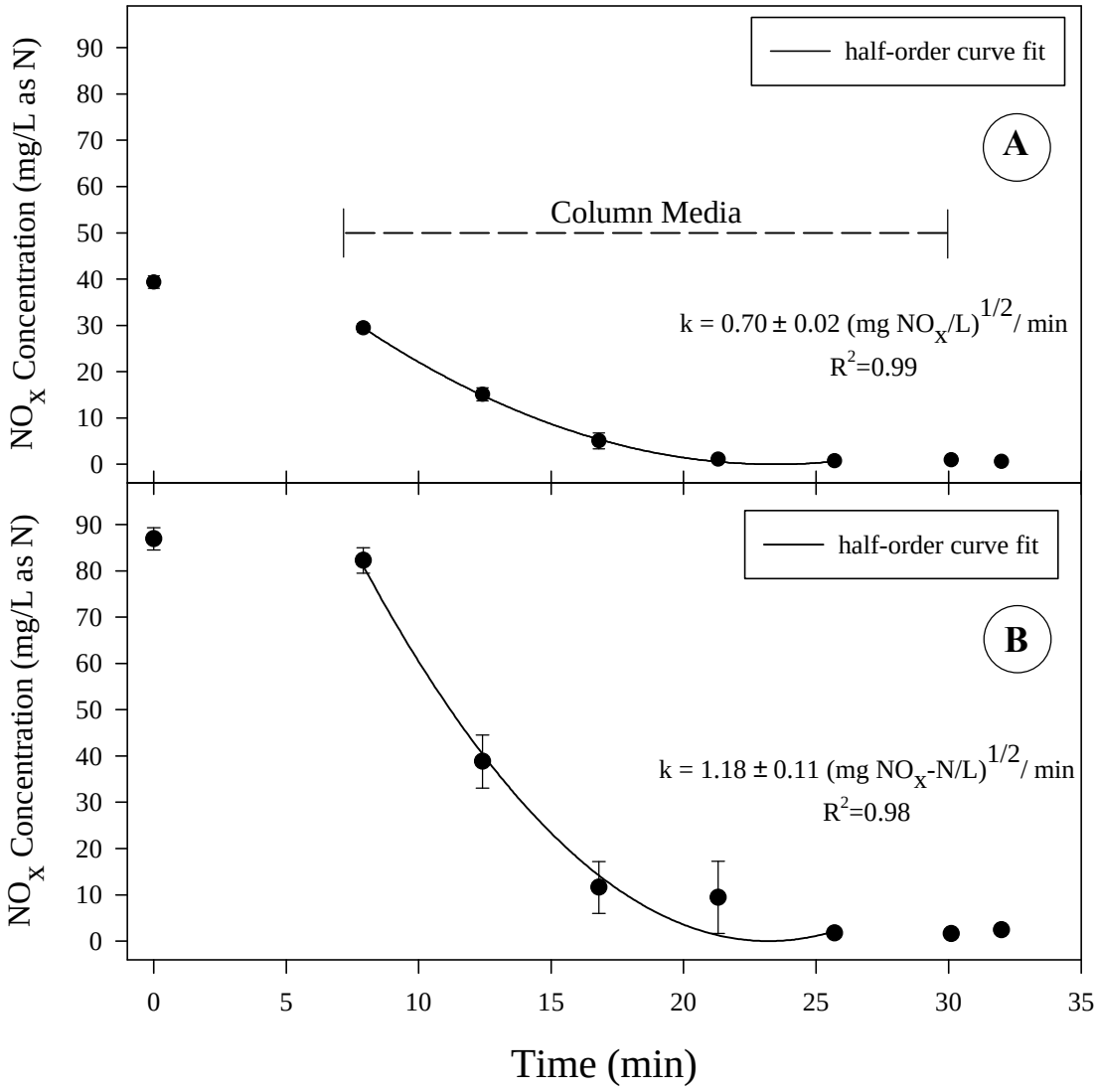

Figure 14. Denitrification profiles during the (A) low (n=5) and (C) high (n=4) nitrate loading phases. Half-order model fits were performed for the section of the column in which media was located, as indicated by the dashed line, and active metabolism occurred. Denitrification rate constants (k) are provided with standard error of the mean.

DISCUSSION

A sCOD to NO_x consumption ratio of 4.62 ± 0.28 corresponded with complete denitrification under the low nitrate loading conditions studied. This value is comparable to the 4.62 ± 1.81 mg COD/mg NO_x consumption ratio (± SD) reported by Aboutboul et al. (1995) for complete nitrogen reduction, using fish food fermentation generated volatile fatty acids as the carbon source. A lower sCOD to NO_x consumption ratio (3.07 ± 0.58) was obtained during the high nitrate loading phase and showed greater variability, with some values falling below the theoretical chemical oxygen demand of 2.86 mg O_2/mg NO_3-N required for complete reduction of NO_3^- to N_2. Abufayed and Schroeder (1986) reported lower than theoretical filtered carbon consumption values for denitrification in a sequencing batch reactor using hydrolyzed primary sludge. They attributed the discrepancy to the use of soluble products generated from the continued hydrolysis of particulate matter, which would not have been originally measured in the influent because the samples were filtered. When the nitrate loading was increased in this study, the amount of feed added to the fermenters was elevated to raise the soluble COD production. Even though the fermenters were settled prior to supernatant removal, a greater amount of suspended solids were visually detected in the carbon source line leading to the filter. It is possible that a portion of these fermentation solids were retained within the column because biofilters are efficient in the removal of particulate matter (M'Coy, 1997). As a result, the hydrolysis of influent particulate COD may have occurred to a greater degree within the column under the high nitrate loading conditions and could account for the lower than theoretical sCOD to NO_x consumption ratios measured. The consumption ratios reported only reflect the contribution of readily biodegradable, soluble substrate to denitrification, as only soluble COD

concentrations were measured based on the analysis of filtered samples. As a result, a representative sCOD to NO_x consumption ratio could not be provided for the high nitrate loading phase. In order to determine accurate influent and consumptive COD to NO_x ratios for biofilter systems fed a fermented carbon source, it is recommended that soluble and total CODs be measured to characterize the contribution from both readily and slowly biodegradable substrates.

Nitrite Accumulation

Notable concentrations of nitrite were detected in the column effluent under carbon limiting conditions and within the column even when complete nitrogen removal was achieved. The peak concentrations of nitrite measured in the column profiles were lower than the stoichiometric amounts expected to be generated from the reduction of the influent nitrate, indicating that both nitrate and nitrite were being reduced at the same time, though at different rates (Betlach and Tiedje, 1981; Almeida et al., 1995). It has been suggested that the greater reduction rate of nitrate as compared to nitrite is governed by the heightened activity of nitrate reductase over that of nitrite reductase (van Rijn and Sich, 1992), as well as the relative concentrations of these two reductase enzymes (Her and Huang, 1995).

Ambient nitrate concentrations seemed to affect the reduction rate of nitrite as well. Batch kinetic tests revealed that the rate of nitrite reduction was decreased when nitrate was present. It has been suggested that the presence of nitrate suppresses the rate of nitrite reduction because a competition for available electrons exists between the two reductases, with nitrate reductase being the preferred electron recipient (Almeida et al., 1995; van Rijn et al., 1996). Van Rijn et al. (1996) found that the nitrite reduction rate for *Pseudomonas stutzeri* grown on acetate and nitrite alone was nearly twice that measured when nitrate was present. Kornaros et al. (1996)

observed a 62.5% decrease in the rate of nitrite reduction by *Pseudomonas denitrificans* after the application of a 33 mg NO_3-N/L spike. Upon removal of the added nitrate, nitrite reduction returned to the initial rate.

In this study, the peak nitrite concentration exhibited in column profiles taken during the high nitrate loading was nearly three times the concentration measured at the low loading. In addition, the peak effluent nitrite concentrations measured when nitrogen removal was incomplete were greater during the high nitrate loading phase (34 mg NO_2-N/L) than those detected during the low nitrate loading conditions (12 mg NO_2-N/L). This suggests that the degree of nitrite accumulation may be related to the initial nitrate concentration, which is consistent with observations made by other researchers (Bernet et al., 1995; van Rijn and Sich, 1992). At the same time, the change in pH seen between the column influent and effluent increased from 0.79 ± 0.10 under the low nitrate loading condition to 1.25 ± 0.03 during the high nitrate loading condition. This increased change in pH was probably due to an increase in the influent nitrate concentration, as alkalinity production in the denitrification process is a function of the amount of nitrate reduced. Glass and Silverstein (1997) reported that when nitrate was present, the suppression of nitrite reduction and subsequent nitrite accumulation increased significantly with pH for values ranging from 7.5 to 9.0 in activated sludge treating a high nitrogenous wastewater (1350 mg NO_3-N/L). With respect to cell microbiology, reducing power in the form of electrons is transferred from the electron donor to the terminal electron acceptor via the electron transport chain and this reduction process consumes a proton (Neidhardt et al., 1990). While the reduction of nitrate to nitrite and then nitric oxide consumes electrons in the electron transport chain, Glass and Silverstein (1997) explained that nitrate reductase consumes protons located in the cytoplasm,

while nitrite reductase consumes protons from the periplasmic space. They suggested that the competition for available electrons, as well as a limited number of protons in the periplasm of the cell at high pH, increased the suppression of nitrite reduction and resulted in the increase of nitrite accumulation. Consequently, the increase in intermediate nitrite concentration with increased nitrate loadings seen in this study may be due, in part, to an increase in pH throughout the system.

During the high nitrate loading phase of this study, high effluent nitrite concentrations were detected even though effluent COD concentrations and complete removal of nitrate suggested there was no carbon limitation. This performance was thought to result from a shift in the microbial population from complete denitrifiers, which perform both nitrate and nitrite reduction, to organisms which only reduce nitrate to nitrite. However, the use of glucose as a carbon source has been found to result in the selection of microorganisms that are not complete denitrifiers, while acetic acid has been shown to support denitrification (Wilderer et al., 1987; Akunna et al., 1993). The use of acetic acid as one of the primary VFA constituents supplied as the carbon source in this study suggested that such a population shift was unlikely. Batch kinetic tests performed during the high nitrate loading period confirmed this assertion by demonstrating the ability of the microorganisms to reduce both nitrate and nitrite, indicating that a loss of nitrite reducing bacteria did not occur.

Another possible explanation for the high nitrite build-up was thought to relate to the fact that the nitrate loading was significantly higher than the recommended treatment capacity (1.5 kg NO_3-N/m^3/day) of this media. However, Jepsen and Jansen (1993) reported consistently high nitrate removal rates by the Biostyr® media for loadings up to 4 kg NO_3-N/m^3/day, which exceed the 2.52 kg NO_3-N/m^3/day loading applied during this study. The analysis of nitrite was not

indicated in their study. Once the hydraulic loading was decreased and subsequently returned to the original flowrate in this study, complete nitrogen removal was attained which suggests that another factor was contributing to the high effluent nitrite concentrations.

Threshold nitrite concentrations exist at which point the compound becomes inhibitory to the denitrification process; however, Beccari et al. (1983) suggested that denitrifiers may be acclimated by slowly increasing the nitrite concentration. In this study, the increase in influent nitrate would have resulted in a stoichiometric increase in nitrite production. Since the increase in nitrate concentration was performed instantaneously without an acclimation period, the increase in nitrite production may have been inhibitory to the biofilm in this study. Beccari et al. (1983) found that greater biomass concentrations endured higher nitrite values before showing the effects of inhibition. Based on the backwash biomass from the column, there seemed to be no significant increase in the biomass concentration between the lower and higher nitrate loadings. Therefore, the biofilm density may have not been sufficient to accommodate the rise in nitrite. The increase in retention time would have allowed the microorganisms more time for utilization of the electron acceptors and donors, leading to the development of biomass in the upper portion of the column. As was seen in Figure10b, nitrite was not detected in the effluent after the flowrate was returned to the original loading. This indicated that the low flowrate phase during the high nitrate loading condition may have served as an acclimation period for the biomass in which the capacity of the biofilm was increased by enhancing growth throughout the entire column.

Volatile fatty acids

The carbon source supplied to the column in this study was comprised of seven different VFA constituents. Column profiles during the low nitrate loading phase in which influent soluble

COD concentrations were sufficient to achieve complete nitrate reduction revealed that the biofilm population was able to assimilate all of the VFAs generated from the fermentation process. However, when VFAs were supplied in abundance, as was the case during the high nitrate loading phase, it appeared that propionic acid was preferred over acetic acid and the normal structures of both butyric and valeric acids were preferred over the isomer forms. This suggests that although all of the VFA constituents appear to be readily assimilable by the denitrifying organisms, certain compounds may be favored. It has been shown that the type of carbon source affects denitrification efficiency (Fass et al., 1994; Aboutboul et al., 1995; van Rijn et al., 1996), though there is a lack of agreement between researchers regarding which forms are preferred. For example, Aboutboul et al. (1995) reported that propionate facilitated nitrate reduction rates were greater than for acetate or butyrate, while others (Eilersen et al., 1995; Takai et al., 1997) suggested that propionic acid is not a viable carbon source due to slow biodegradability and that acetate is a preferred denitrification substrate. The preference of the normal forms of butyric and valeric acid over the isomer forms seen in this study may have resulted from the difference in chemical structure (Eilersen et al., 1995), suggesting that it is easier for the microorganisms to assimilate the linear forms than the branched structures.

Since the carbon source supplied to the column in this study was comprised of multiple VFA constituents, the results may have differed from the previously discussed studies where individual VFA components were used as the single carbon source. However, Fass et al. (1994) reported equivalent denitrification rates when a combination of volatile fatty acids (acetate, propionate, butyrate, and valerate) were used as the carbon source and when acetate, butyrate, and valerate were supplied as the lone carbon sources. In addition, they found that the rate of

denitrification was significantly decreased when propionic acid was used compared to acetate, butyrate, and valerate. In this study, propionic acid appeared to disappear more quickly than acetic acid when VFAs were provided in excess. At the same time, the apparent decrease in acetic acid consumption may be explained if, in fact, it was simultaneously being produced within the column through the degradation of propionic and butyric acids (Aboutboul et al., 1995).

As well as affecting the reduction rate, the carbon source has also been shown to contribute to the extent of nitrite accumulation. Studies with *Pseudomonas stutzeri* revealed an accumulation of nitrite when acetate and propionate were used as the carbon source, but not for butyrate and valerate *(*van Rijn et al., 1996). Therefore, the type of VFA constituents consumed may also have affected the accumulation of nitrite seen in the denitrifying column, as discussed earlier.

In order to accommodate the shift from the low to high nitrate loading in the column, larger amounts of fish food were fed to the fermentation system to increase the soluble COD production. At the same time, the VFA fraction of the total COD increased and the relative concentrations of VFA constituents generated changed slightly, which may have resulted from the increase in pH of the fermentation process from 6.31 ± 0.06 during the low nitrate loading phase to 7.41 ± 0.02 in the high nitrate loading phase (Eastman and Ferguson, 1981). The production of soluble COD per unit weight of fish food decreased from 334 ± 18.7 to 159 ± 6.8 mg sCOD/mg food when the fermenter SRT and HRT were increased, suggesting that these parameters may also affect optimum fermentation. Additionally, it was evident that the VFA fraction of soluble COD produced was greater from the fermentation of fish food than for fish waste. Discrepancies in VFA production between these two substrates could possibly be

explained by the operation of the fish waste reactor. Since fish waste was collected from a recirculating aquaculture system once a week and not daily, the waste added to the reactor was not fresh. This resulted in a different SRT for the fish waste reactor than for the fish food fermenters and may have contributed to the difference in COD production. In addition, since fish waste is a byproduct of metabolized feed, fermentation products may be different. Van Rijn et al. (1995) surmised that the VFA production from the fermentation of fish waste would probably be less per unit weight than that for fish food since a portion of the energy is consumed by the fish.

Biomass Yield

The type of substrate affects the observed yield of microorganisms. Since biomass synthesis involves the use of organic compounds containing three carbons or greater, additional energy is directed to synthesis reactions when organic compounds containing less than three carbon atoms have to be converted into microbial building blocks (Grady and Daigger, 1998). Grabinska-Loniewska (1991) found that both carbon consumption and biomass growth rates for denitrifying cultures increased with the number of carbon atoms present in single organic acid substrates. Therefore, it is expected that different growth yield values will result from utilizing different VFAs, with biomass production being lowest for acetic acid and highest for valeric acid. The biomass observed yield reported here reflects biomass production for a range of organic acids.

COD Balance

COD balances performed on the column influent and effluent during the low nitrate loading phase showed a close relationship between the measured and calculated COD consumption values, indicating that denitrification accounted for the removal of the electron

donors and acceptors. Slight discrepancies may be explained by the presence of small oxygen concentrations in the influent (<2 mg/L) since nitrogen purging was not performed to ensure completely anoxic conditions, as well as the use of an average observed yield value in the calculations. The observed yield value is affected by the type of carbon source and since multiple VFA constituents were available for cell synthesis, this value may have varied slightly on a daily basis depending on the amounts of the various VFAs consumed. COD balances were not performed for data obtained during the high nitrate loading condition, as COD may have been generated from the hydrolysis of solids retained in the column. Influent solids concentrations would be required to determine the total influent COD and calculate an accurate balance, but these data were not collected.

Recalcitrant Organic Compounds

The monitored VFA constituents accounted for most, but not all, of the soluble COD generated by the fermentation of fish food and fish waste, indicating that other compounds were created as well. Eastman and Ferguson (1981) found that while VFAs constituted the majority (85 to 95%) of the measured soluble COD generated from the hydrolysis and fermentation of primary municipal sludge, a small fraction of additional compounds were generated in the process. Detection of soluble COD in the column effluent in this study, even when the system was unable to achieve complete nitrogen removal, suggests that the residual COD was comprised of components that were not readily available to the denitrifying organisms. The inability of the biofilm to assimilate this COD fraction may indicate the production of soluble microbial products (SMP) in the fermentation process. SMPs are microorganism components related to cell decay and lysis that are slowly degraded due to their high molecular weight (Grady and Daigger, 1998).

SMP production within the column also appears to have contributed to this recalcitrant fraction of COD, as noted in the column profile illustrating COD and VFA consumption (Figures 12) during the low nitrate loading, whereby the difference between the measured soluble COD and the VFA fraction increased throughout the column. This assertion is supported by Fass et al. (1994) who attributed the detection of COD in the effluent of a VFA facilitated denitrification system to SMP since complete consumption of influent VFAs was achieved.

Kinetics

The zero order reaction has long been used to describe denitrification reduction rates (Beccari et al., 1983). However, fixed film denitrification is often represented by an overall half-order rate as a result of reaction rate limitations in the first part of the biofilter and diffusion limitations in the second part (Harremoës and Reimer, 1977; Green et al., 1995). The overall denitrification rate observed in the biofilter in this study appeared to follow a half-order reaction rate, which increased with increasing nitrate loading. This trend was also observed in an upflow, denitrifying biofilter by Janning et al. (1995). These results suggest that the nitrate loading affects the value of the denitrification coefficient in a biofilter, with higher nitrate loadings generating a faster rate. This is as would be expected since a higher bulk concentration of nitrate and COD will encourage faster rates of both mass transfer and biological reaction. Green et al. (1995) observed that in a fluidized-bed reactor, zero-order reactions existed in the first part of the bed because high bulk fluid substrate concentrations eliminated mass transfer limitations and allowed for the full permeation of substrates into the biofilm. In the second part of the fluidized-bed reactor, substrate utilization conformed to a half or first-order model because diffusion limitations resulted from a reduction in the mass transfer driving force in the presence of lower substrate

concentrations. This variation in reaction order is also a function of biomass growth, which increases with the bulk substrate loading (van Loosdrecht et al., 1995). As a result of the decreasing substrate concentration gradient applied throughout the column, biofilm growth is often not uniform. Through visual inspection of the denitrification column operated in this study, it was apparent that biomass growth was concentrated in the lower portion of the column where the bulk substrate loading was the greatest, while the upper portion was less densely populated as a result of being exposed to a decreased substrate concentration.

CONCLUSIONS

The results of this study demonstrated the feasibility of using a fermentation generated carbon source in the denitrification of high nitrate recirculating aquaculture system waters. The fermentation of both fish waste and fish food generated volatile fatty acids that were assimilated by denitrifying organisms, though it was evident that certain VFA constituents were preferred. The slight variation in the relative concentrations generated for each compound in response to changes in pH, as well as the solids and hydraulic retention times, suggests that the fermentation process may be manipulated through operational control to produce desired acids.

In addition, limiting the amount of available carbon supplied to the denitrification system resulted in an increase in effluent nitrite and incomplete nitrate removal, while influent COD to NO_3-N ratios greater than 5 typically achieved high total nitrogen removals greater than 95%. To prevent the problem of nitrite accumulation, as well as the discharge of significant amounts of carbon that would return to the fish culture tank, a denitrification system using fermented fish waste or food as the carbon source to produce volatile fatty acids should be operated closely to this ratio.

The nitrate loadings examined in this study were lower than the maximum nitrate concentrations observed in nitrifying closed recirculating aquaculture systems not employing denitrification. However, nitrate concentrations in fish rearing tanks increase gradually over the span of a growth period and it may be possible to maintain concentrations at manageable levels by applying denitrification as a sidestream process so that extreme concentrations do not result. In order to evaluate the efficiency and self-sustainability of this denitrification system at increased nitrate concentrations, additional studies are recommended. It is anticipated that a full-scale

recirculating aquaculture facility would generally have several culture tanks containing fish at all stages of growth and would be able to provide a more consistent source of fish waste for the fermentation process. However, this aspect of the treatment system must be evaluated to determine if complete self-sustainability is possible, or whether an external carbon source must be partially supplemented.

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ENGINEERING SIGNIFICANCE

Recirculating aquaculture systems have traditionally employed the nitrification process to convert ammonia to nitrate in order to prevent free ammonia toxicity (Lucchetti and Gray, 1988). This results in the accumulation of nitrates since water replacement is minimized in these systems. Biological denitrification has been proven to be an economical and effective way to remove nitrates by converting them to nitrogen gas. A coupled treatment scheme employing nitrification and denitrification can be used to attain total nitrogen removal within a system (Metcalf and Eddy, 1991). Since nitrification is already incorporated into the design of most recirculating systems, the system can be easily retrofitted to include the denitrification process. Figure 15 depicts the components of a recirculating aquaculture system operating at the Virginia Tech Aquaculture facility. The system consists of (1) a rearing tank for the cultivation of fish, (2) a multi-tube clarifier for the removal of solids, and (3) a rotating biological contactor to perform nitrification. This diagram provides the typical components found in currently operating recirculating systems, though the actual technologies employed for each task may differ within the industry. The flow of water moves from the rearing tank to the solids removal device, at which point it enters the nitrifying biofilter and upon exiting is recycled back to the rearing tank. The logical placement of a denitrifying biofilter would be at point (4), with a fraction of the effluent from the nitrifying biofilter acting as the influent, and the denitrified effluent being directed to the solids removal device for removal of any biomass that may slough off the filter. Its placement after the nitrification process would treat the water at its highest nitrate concentration. The fermented supernatant would be metered into the stream right as it enters the denitrifying biofilter. The purpose of using organic matter already found in the recirculating

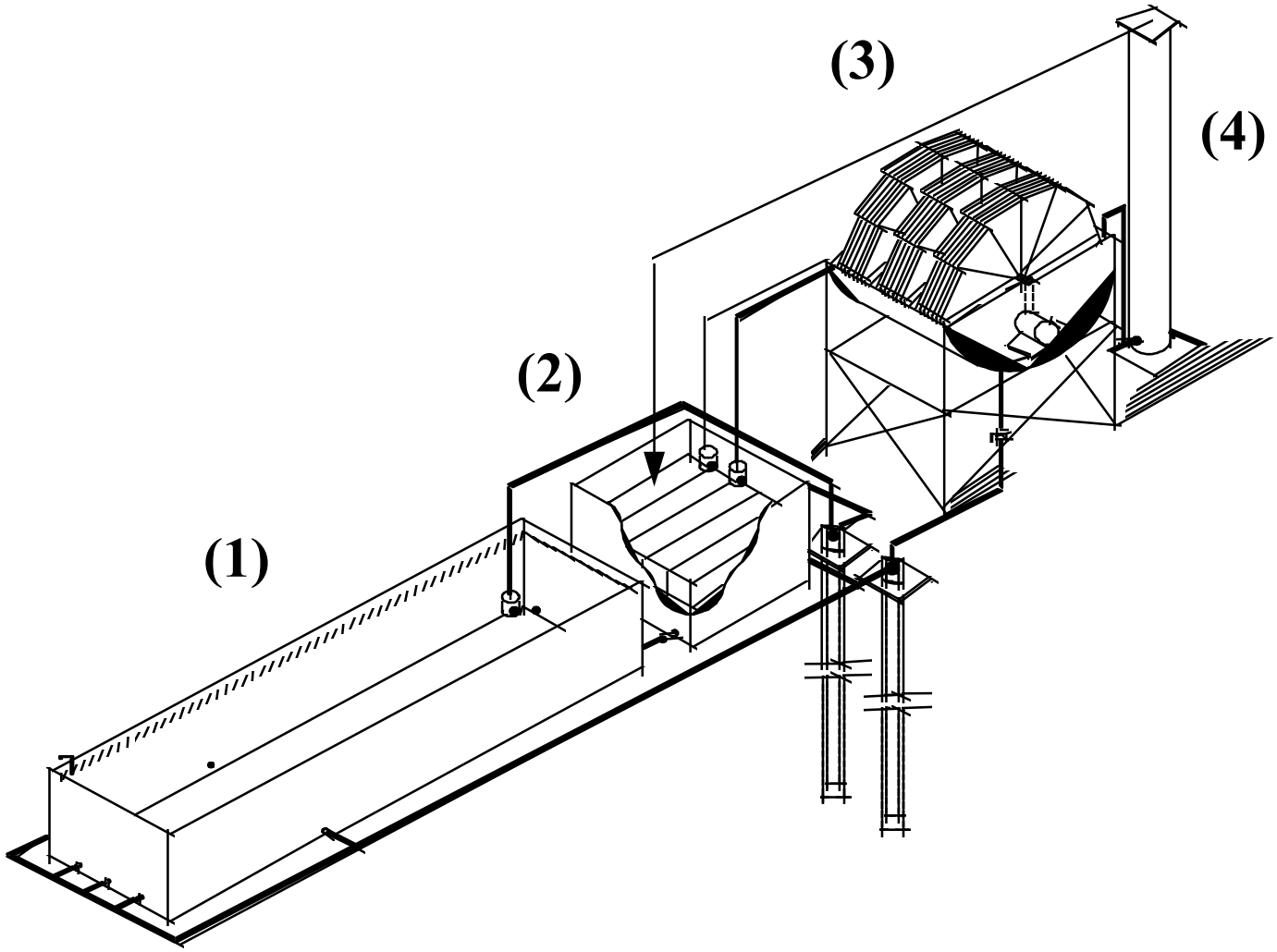

Figure 15. Pilot-scale recirculating aquaculture system employed at the Virginia Tech Aquaculture Center. The system consisted of a (1) rearing tank, (2) solids removal device (multi-tube clarifier), (3) nitrifying biofilter (rotating biological contactor). The proposed placement of the denitrifying biofilter (4) is after the nitrifying biofilter, with effluent being returned to the solids removal device.

system as the carbon source was to design a self-sustainable treatment process. Since the fermentation process converts particulate matter into soluble forms, the fermentation of fish waste would potentially reduce the amount of solid waste requiring disposal. Therefore, both reduction of nitrates and waste solids can potentially be achieved with this suggested design.

The results of this study demonstrated that nitrate reduction to nitrogen gas through denitrification is possible using volatile fatty acids generated from the fermentation of fish food. Although the fermentation of fish waste would be preferred over that of fish food in treating actual recirculating aquaculture systems, batch tests revealed the fermentation of both sources yielded the same VFA constituents. Therefore, the use of fish food in this study was a valid substitution for fish waste and the results can be used in the design of a system incorporating the fermentation of fish waste.

The reduction of nitrate concentrations in recirculating aquaculture systems using the biological treatment methods examined in this study have two main advantages. First, extended exposure to high nitrate concentrations of 200 mg NO_3-N/L have been shown to compromise the immune system of hybrid striped bass (Hrubec et al., 1996), suggesting that nitrate can be chronically toxic to certain fish species. Therefore, reducing the accumulated nitrates in recirculating systems would benefit the general health of growing fish. Second, the high nitrogen concentration of the system effluent would be decreased, which is a concern in the wake of tightening water regulations with regard to nutrient discharge.

Recommendations

This research has raised several questions with regard to the fermentation and denitrification processes. The varying COD and VFA generation patterns in the fermenters

indicates that HRT, SRT, and pH affect this process. Initial batch tests were performed to optimize total COD production, but it is apparent that specific VFA constituents may be selected by controlling other operating parameters. Further tests concerning the optimization of the fermentation process would be valuable in order to select for those constituents that are preferred by denitrifying organisms. Although the accumulation of nitrite in the denitrification process is beginning to receive more focus as the treatment of high nitrate concentrations becomes more common, the mechanism is still not clear. Studies to analyze the production of nitrite and inhibitory effects may help to control the accumulation of this anion in denitrification systems.

A synthetic nitrate wastewater was treated in this study to prevent possible toxic effects to the fish. Now that this treatment scheme, consisting of a denitrifying biofilter supplemented with a fermentation generated carbon source, has been shown to effectively treat nitrate concentrations in recirculating aquaculture systems, the next step would be to apply this biological treatment system to an actual recirculating aquaculture system. Recirculating systems operate in a dynamic fashion and employ other treatment processes such as nitrification and solids removal. It would be important to evaluate the performance of this coupled denitrification and fermentation technology within the entire system and determine the effects it has on the other components.

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APPENDIX A

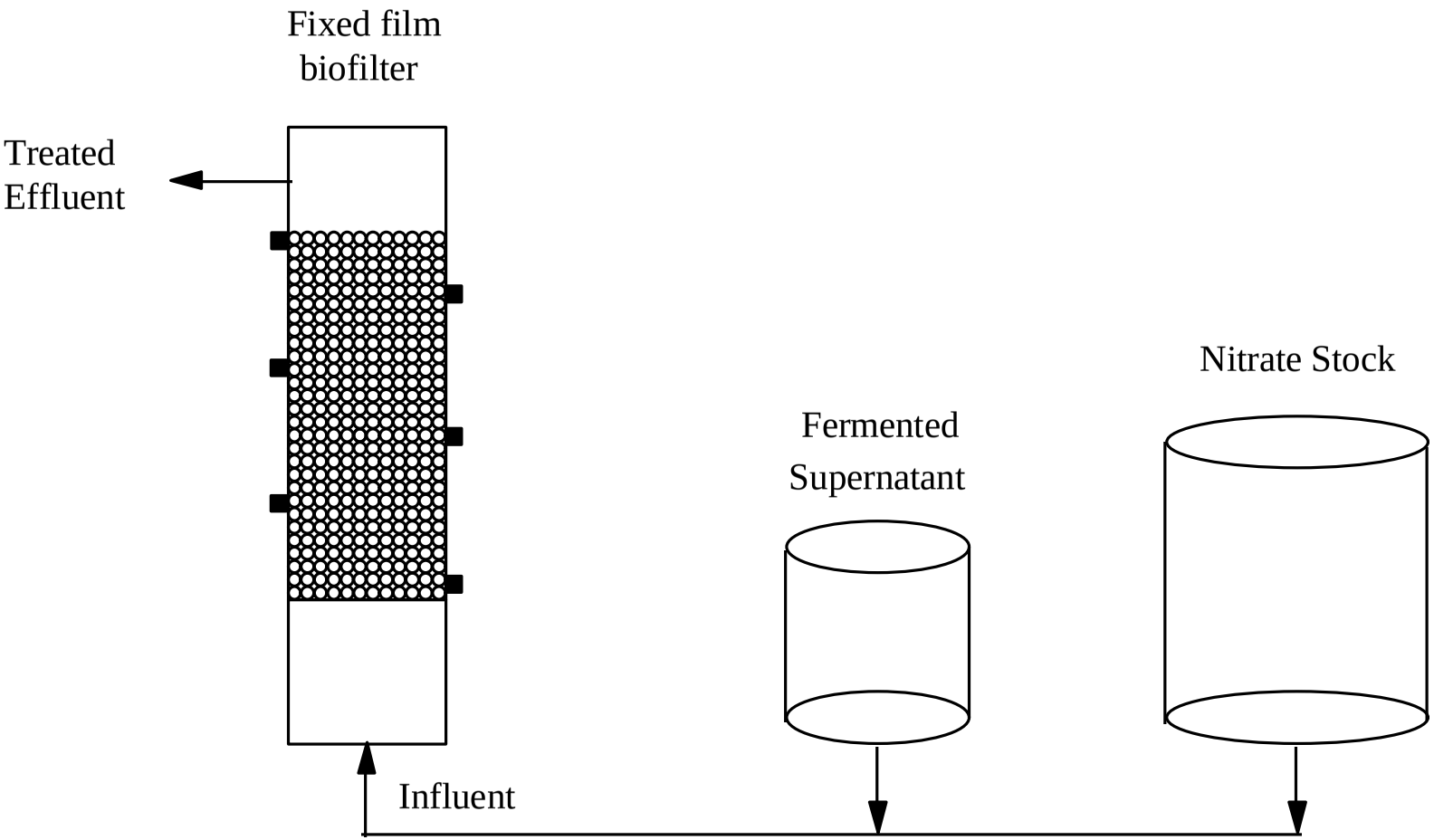


Figure A.1. Pilot-scale denitrification system comprised of an upflow biofilter and a fermentation unit.

Test for nitrite interference of COD measurements.

Test performed by Jamie Fettig, ENE Graduate Student, Virginia Tech

Treatment 1: no treatment

All samples had 100 mg/L as KHP.

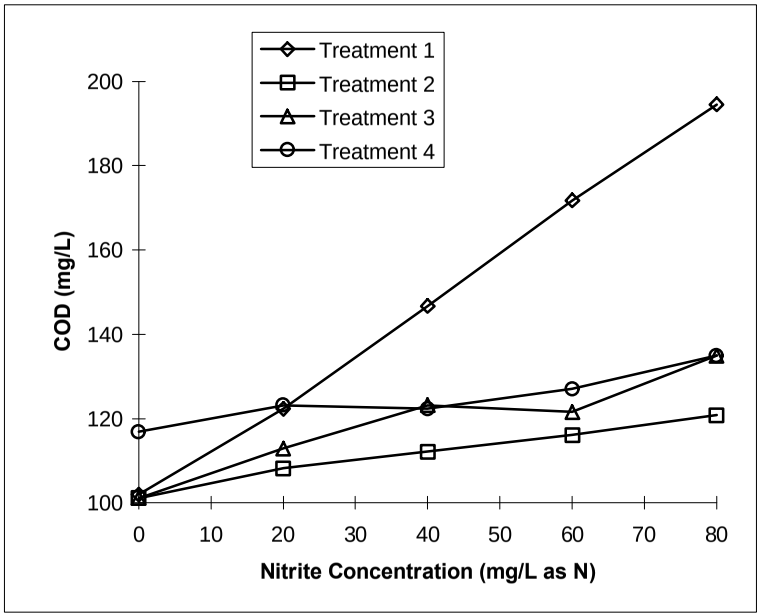
Treatment 2: sulfuric acid addition

Treatment 3: sulfuric acid plus 10 mg sulfamic acid/mg nitrite

Treatment 4: sulfuric acid plus 80 mg sulfamic acid/mg nitrite

Treatment of sample	Nitrite (mg/L as N)	FAS 1 (mL)	FAS 2 (mL)	Average FAS (mL)	COD (mg/L)
1	0	4.74	4.74	4.74	102
1	20	1.04	4.48	4.48	122
1	40	4.16	4.18	4.17	147
1	60	3.9	3.8	3.85	172
1	80	3.6	3.52	3.56	195
2	0	4.78	4.72	4.75	101
2	20	4.68	4.64	4.66	108
2	40	4.62	4.6	4.61	112
2	60	4.6	4.52	4.56	116
2	80	4.5	4.5	4.5	121
3	0	4.78	4.72	4.75	101
3	20	4.58	4.62	4.60	113
3	40	4.46	4.48	4.47	123
3	60	4.5	4.48	4.49	122
3	80	4.32	4.32	4.32	135
4	0	4.52	4.58	4.55	117
4	20	4.54	4.4	4.47	123
4	40	4.52	4.44	4.48	122
4	60	4.42	3.48*	4.42	127
4	80	4.32	4.32	4.32	135
	Cold blank		6.12	6.12	Norm FAS
	Hot blank 0	6.06	6.06	6.06	0.049
	Hot blank 20		6.02	6.02	
	Hot blank 40		6	6	Avg HB
	Hot blank 60		6.04	6.04	6.04
	Hot blank 80		6.08	6.08	
	*leaking cap				

Figure A.2. Test for nitrite interference of COD measurements.



Linear Regression Equations

Treatment 1	y = 1.1725x + 100.55	R^2 = 0.9989
Treatment 2	y = 0.2353x + 102.27	R^2 = 0.9858
Treatment 3	y = 0.3804x + 103.53	R^2 = 0.9178
Treatment 4	y = 0.2x + 116.86	R^2 = 0.8944

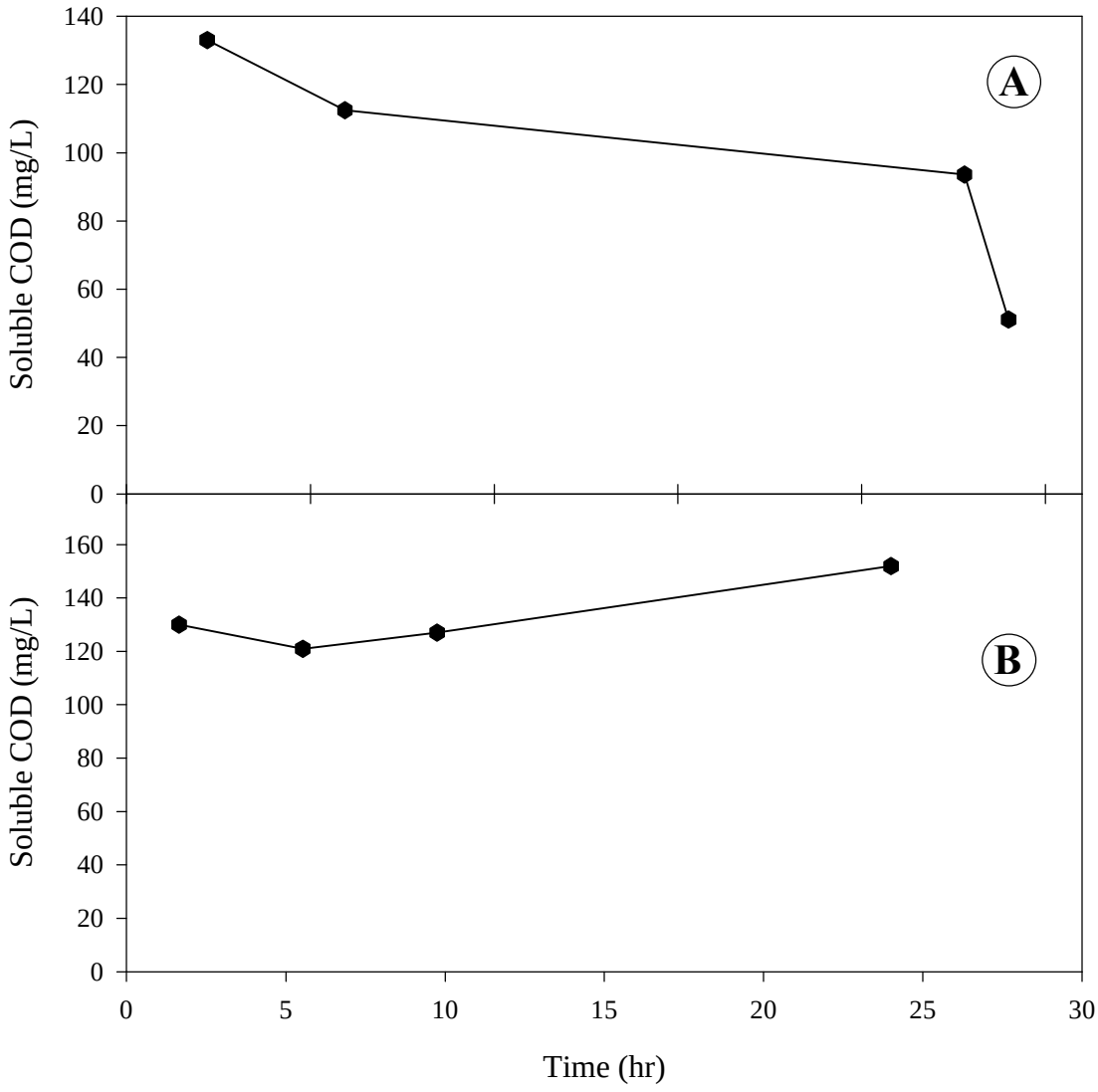


Figure A.3. Profile of column influent soluble COD over a 24 hr operational cycle during the period when (A) carbon was supplied from the storage container and (B) carbon was pumped directly from the fermenter.

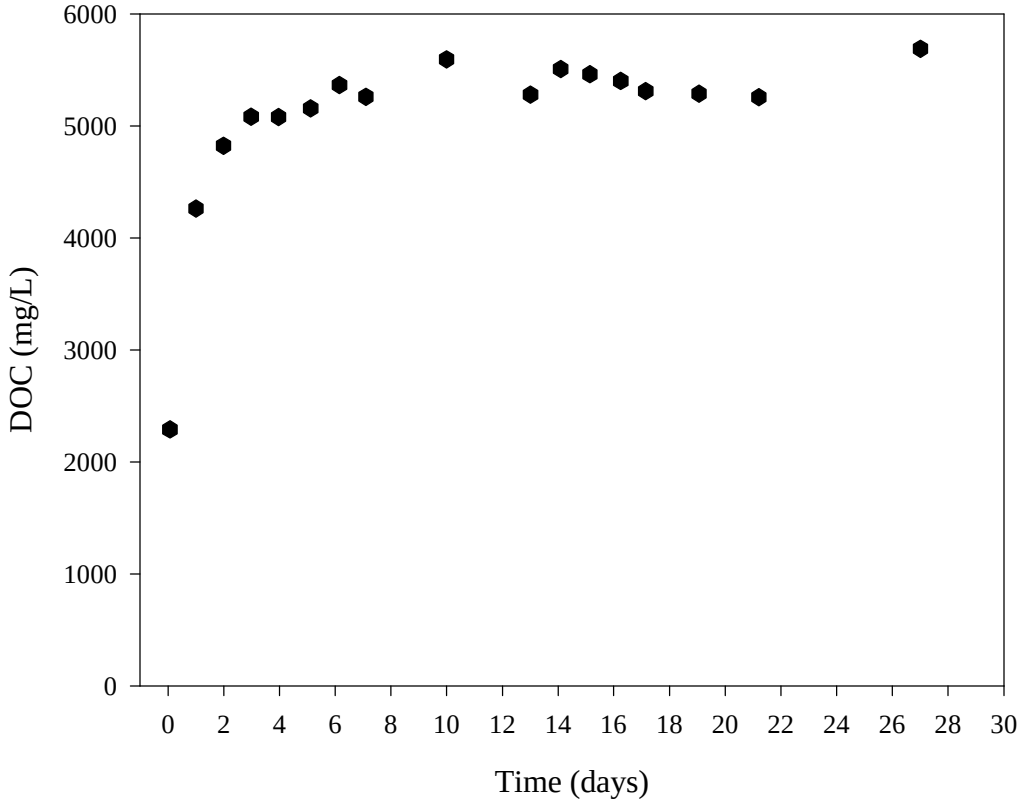


Figure A.4. DOC production measured over time from the fermentation of fish food conducted in a 2 L batch reactor without wasting or feed addition.

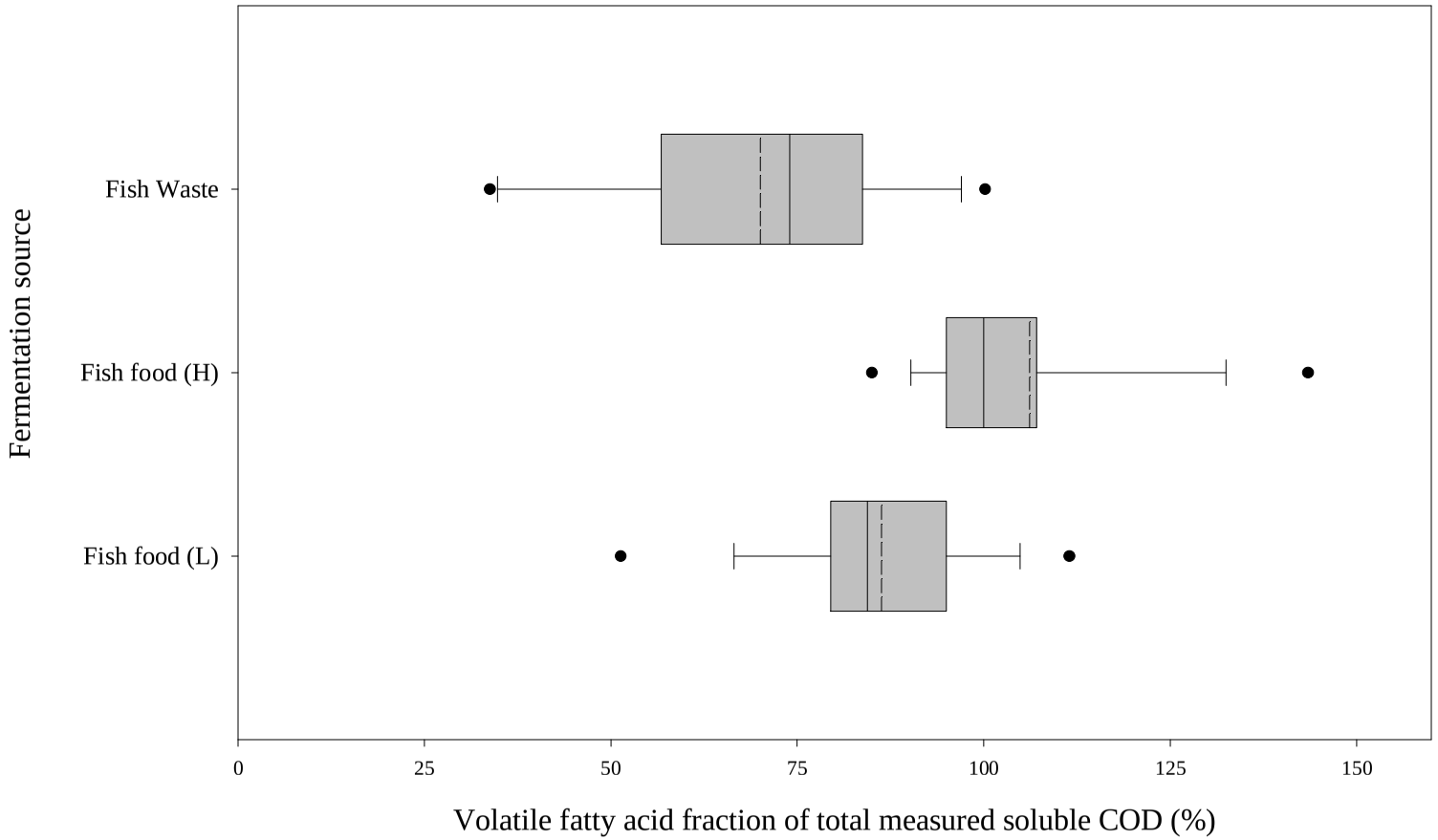


Figure A.5. Volatile fatty acid fraction of total measured soluble COD. Plots provide data for the fermentation of fish waste and fish food during the low (L) and high (H) nitrate loadings. Black dots represent $5^{th}/95^{th}$ percentiles, error bars represent $10^{th}/90^{th}$ percentiles, box represents $25^{th}/75^{th}$ percentiles, solid line represents the median, and dashed line represents the mean.

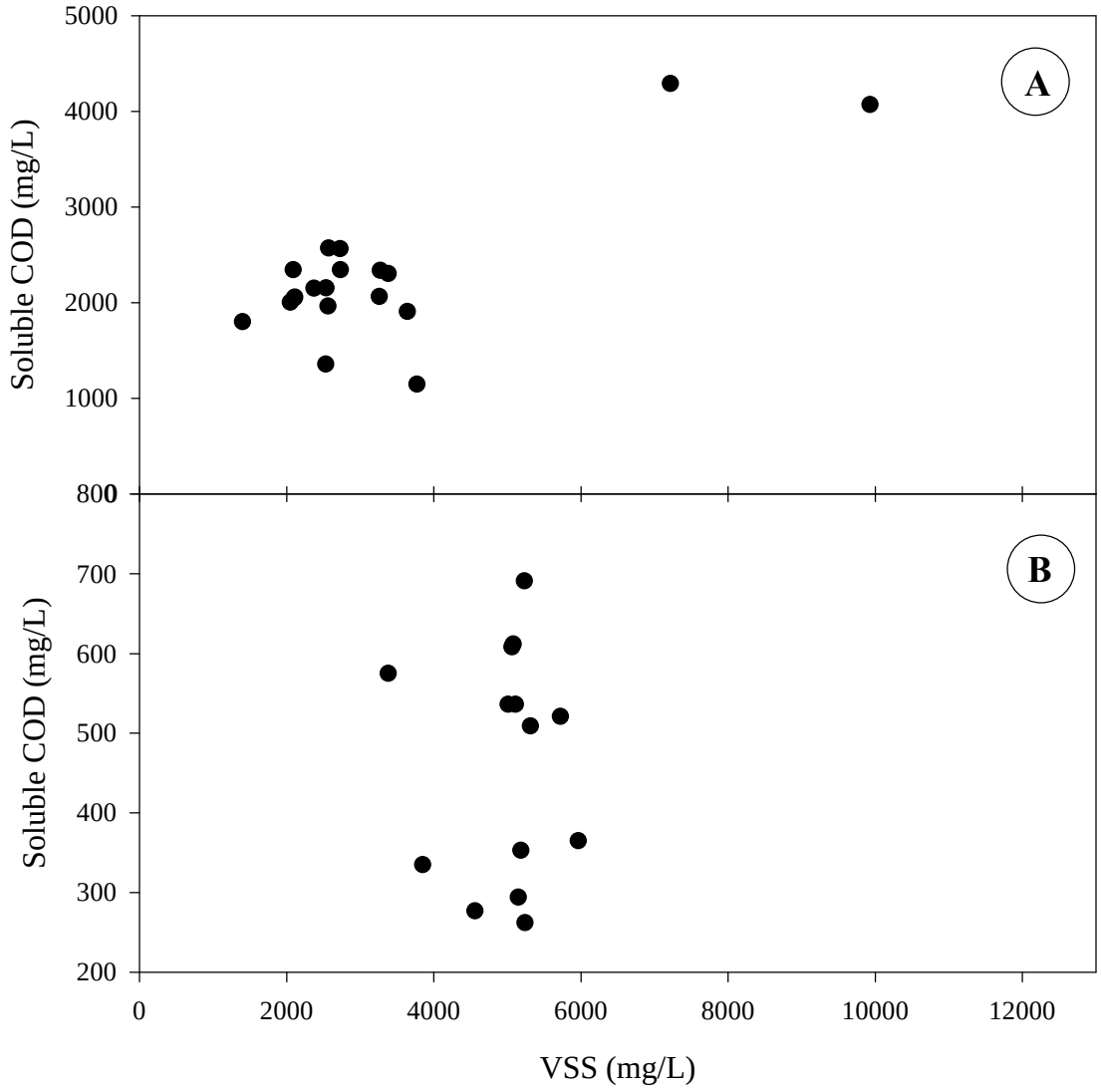


Figure A.6. Relationship between soluble COD and VSS for the (A) pilot-scale fermentation of fish food (data were combined from both fermenters, F1 and F2) and (B) batch reactor fermentation of fish waste.

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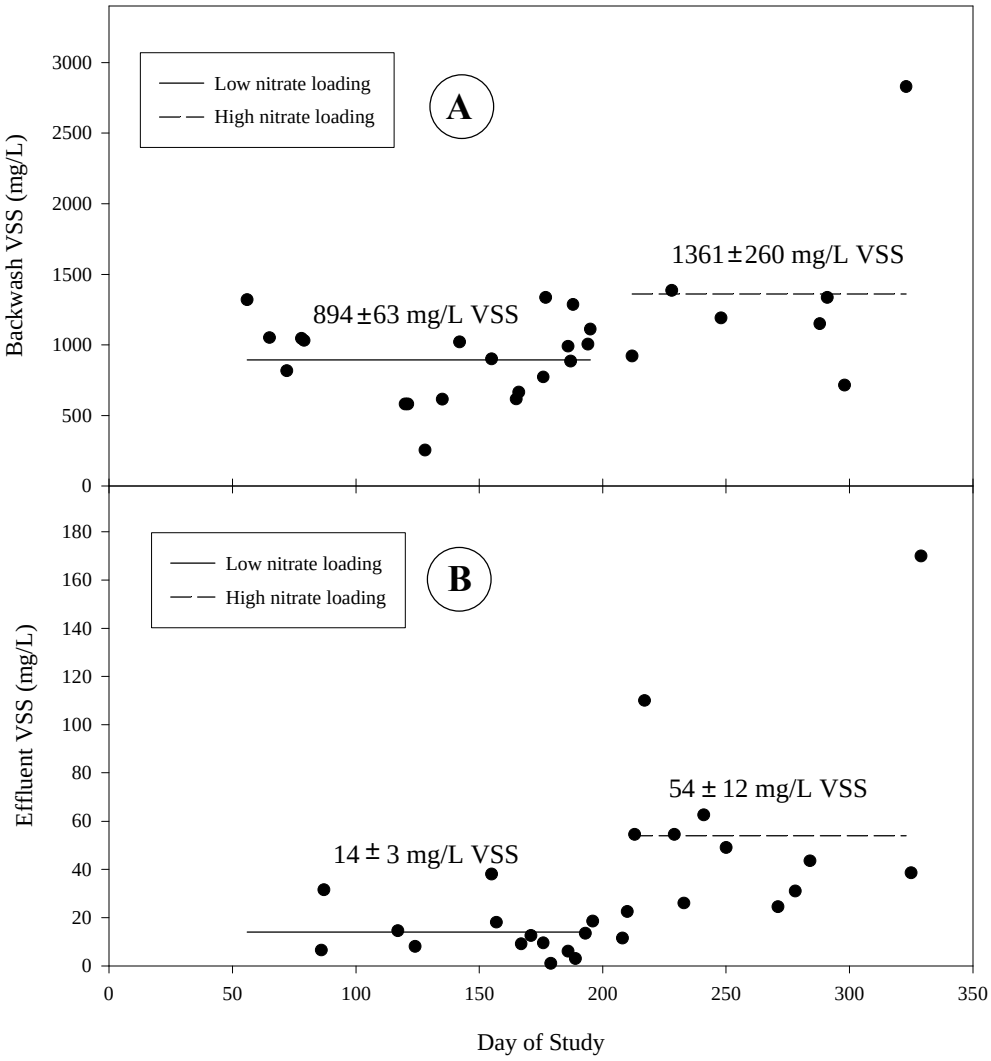

Figure A.7. VSS concentrations for (A) column backwash and (B) effluent solids over the course of the study. Averages values and standard error of the mean are provided for the low and high nitrate loading portions of the study. Lines represent the average VSS for each phase.

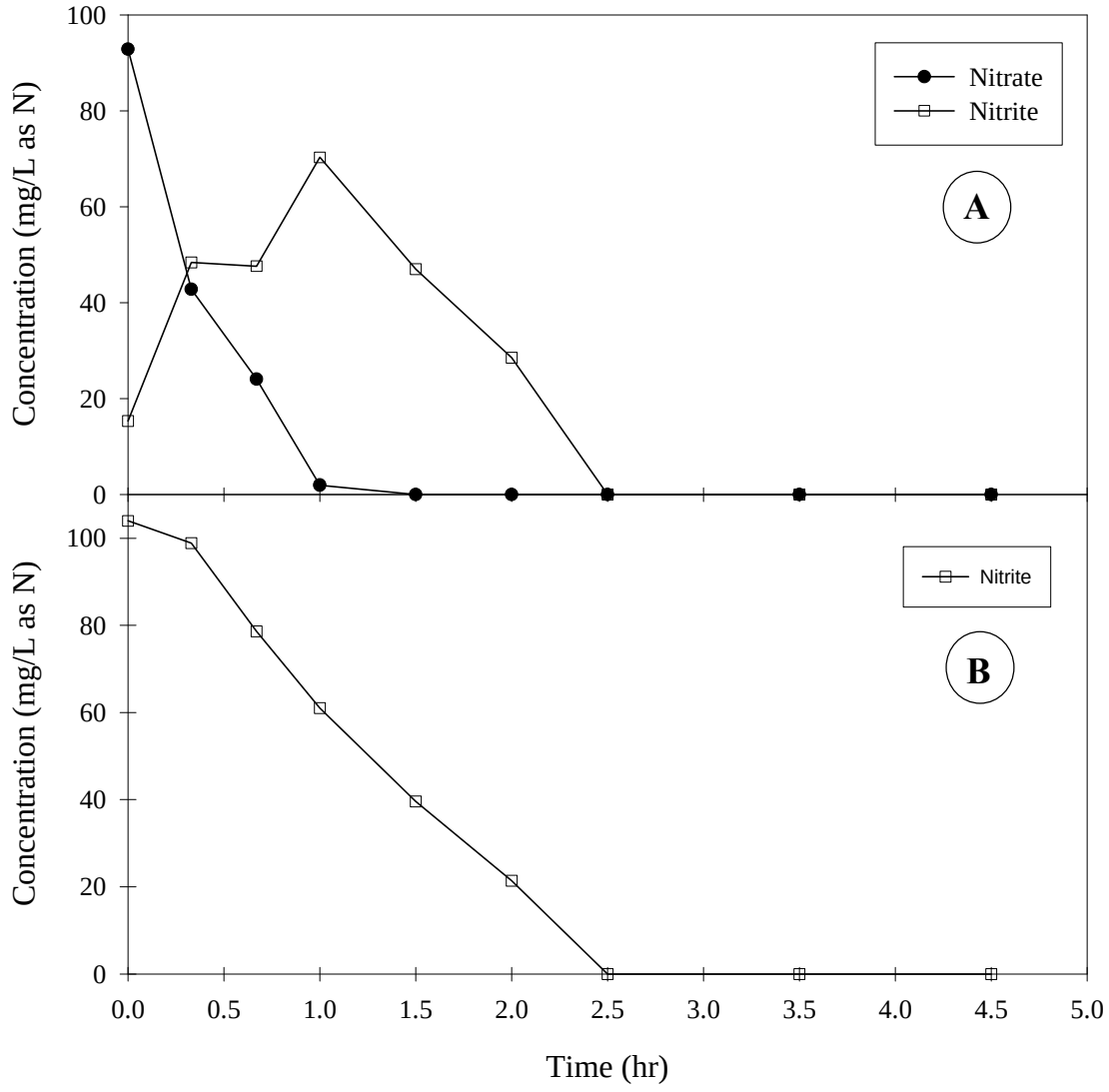

Figure A.8. Batch test performed on day 288 to determine if biofilm could assimilate nitrite. Tests were conducted using initial concentrations of (A) 100 mg/L as NO3-N and (B) 100 mg/L as NO2-N.

Table A.1. Calculation of COD to NO_x ratio for influent COD:NO_3-N ratios greater than 5 and complete nitrogen removal during the low nitrate loading phase. Complete removal is characterized by an effluent having NO_3^- and NO_2^- concentrations < 1 mg/L as N. NO_x represents the total forms of oxidized nitrogen, $NO_3^- + NO_2^-$.

Day of Study	COD Corrected for NO_2^- Interference		Nitrate (mg/L as N)		Nitrite (mg/L as N)		$COD:NO_3^-$ (mg/L per mg N/L)	$COD:NO_x^-$ (mg/L per mg N/L)
	Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Consumed
22	139	59.6	23.9	0.56	1.73	0.00	5.83	3.18
162	223	39.6	36.3	0.37	0.00	0.00	6.15	5.11
166	265	62.0	39.2	0.02	0.48	0.02	6.76	5.13
176	208	40.0	39.4	0.36	0.00	0.00	5.27	4.29
184	220	38.0	43.6	0.17	0.00	0.00	5.06	4.20
187	234	31.9	40.6	0.19	0.00	0.00	5.75	4.99
193	210	38.6	39.4	0.28	0.70	0.00	5.32	4.30
200	229	73.8	26.2	0.52	1.33	0.00	8.74	5.74
						Average =	6.11	4.62
						Standard error =	0.42	0.28

Table A.2. Calculation of COD to NO_x ratio for complete nitrogen removal during the high nitrate loading phase. Complete removal is characterized by an effluent having NO_3^- and NO_2^- concentrations < 1 mg/L as N. NO_x represents the total forms of oxidized nitrogen, $NO_3^- + NO_2^-$.

Day of Study	COD Corrected for NO_2^- Interference		Nitrate (mg/L as N)		Nitrite (mg/L as N)		$COD:NO_3^-$ (mg/L per mg N/L)	$COD:NO_x^-$ (mg/L per mg N/L)
	Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Consumed
282	343	34	98.9	0.14	2.73	0.00	3.47	3.05
311	504	165	72.2	0.00	9.44	0.00	6.97	4.14
312	294	185	86.7	0.00	7.95	0.00	3.39	1.16
316	540	162	126	0.79	3.19	0.00	4.30	2.95
319	376	186	84.1	0.33	0.21	0.00	4.48	2.27
320	296	240	86.4	0.31	0.00	0.00	3.43	0.65
322	674	526	84.7	0.48	0.00	0.00	7.96	1.76
323	881	111	84.9	0.40	0.00	0.00	10.4	9.12
325	755	358	91.2	0.17	2.66	0.00	8.28	4.24
326	704	416	87.5	0.16	0.00	0.00	8.04	3.29
327	740	633	86.1	0.18	3.72	0.00	8.59	1.19
329	711	622	82.6	0.19	1.36	0.00	8.61	1.06
345	679	353	81.8	0.75	0.91	0.96	8.30	4.03
346	389	60	82.5	0.73	0.29	0.45	4.72	4.04
						Average =	6.49	3.07
						Standard error =	0.64	0.58

Table A.3. Calculation of COD balances using column influent and effluent data and the experimentally determined observed biomass yield value.

Day of Study	Measured COD Consumed (mg/L)	Nitrate Consumed (mg/L as N)	Nitrite Consumed (mg/L as N)	Measured COD Consumed (mg/L as COD)	1 Electron Acceptor Consumed (mg/L as COD)	2 Estimated Biomass Formed (mg/L as COD)	1 + 2 Calculated Total Electron Donor Consumed (mg/L as COD)	Measured COD - Calculated COD (mg/L as COD)	Difference Between Measured & Calculated % difference
22	80	23	1.73	80	70	28	97	-18	-22
25	162	41	-0.29	162	116	57	172	-11	-7
162	184	36	0.00	184	103	64	167	17	9
166	203	39	0.46	203	113	71	184	19	10
176	168	39	0.00	168	112	59	170	-3	-2
184	182	43	0.00	182	124	64	188	-6	-3
187	202	40	0.00	202	116	71	186	16	8
193	171	39	0.70	171	113	60	173	-2	-1
200	155	26	1.33	155	76	54	130	25	16
								Average =	8.60

Table A.4. Determination of observed biomass yield during the low nitrate loading phase.

Date	Backwash Volume (L)	Biomass Formed as VSS (mg/L as COD)	Biomass formed (mg as COD)	Total Volume Treated (L)	Average COD Consumed (mg/L)	Total COD Consumed (mg)	Observed Yield (mg biomass formed as COD/ mg COD consumed)
1/28/97 - 1/29/97	45	944	42493	1336	222	296721	0.14
2/8/97 - 2/9/97	45	1896	85307	1329	123	163128	0.52
2/18/97 - 2/19/97	45	1257	56552	1362	156	213064	0.27
2/19/97 - 2/20/97	51.4	1825	93790	1313	181	237890	0.39
2/26/97 - 2/27/97	45	1576	70929	1221	133	162825	0.44
						Average =	0.35
						Standard error =	0.15

Table A.5. Student's one-tailed t-Test for column backwash measured for the low and high nitrate loading conditions. Assumed unequal variances.

	Low nitrate loading	High nitrate loading
Mean	894	1360
Variance	79743	474128
Observations	20	7
Hypothesized mean difference	0	
df	7	
t stat	-1.75	
P(T<=t) one-tail	0.062	
t critical one-tail	1.90	

Null hypothesis: Backwash produced during low nitrate loading is equivalent to or less than that generated during the high nitrate loading. Since $p>0.05$, the null hypothesis is accepted.

Table A.6. Student's one-tailed t-Test for column effluent suspended solids measured for the low and high nitrate loading conditions. Assumed unequal variances.

	Low nitrate loading	High nitrate loading
Mean	13.5	53.7
Variance	108	1830
Observations	14	13
Hypothesized mean difference	0	
df	13	
t stat	-3.29	
P(T<=t) one-tail	0.003	
t critical one-tail	1.77	

Null hypothesis: Effluent solids produced during low nitrate loading is equivalent to or less than that generated during the high nitrate loading. Since $p<0.05$, the null hypothesis is rejected and it can be inferred that the solids measured in the effluent increased with the nitrate loading.

Table A.7. Batch kinetic tests conducted during the high nitrate loading phase of this study. Reduction rates were determined using a zero order model fit and are provided for nitrate and nitrite in the presence and absence of nitrate. Average values ± standard deviation are provided (n=2).

Parameter	Initial Concentration (mg/L as NO_3-N or NO_2-N)		
	20	50	100
Nitrate Test			
Nitrate reduction (mg NO_3-N/g VSS/hr)	97 ± 3	79 ± 2	59 ± 5
Nitrite reduction in presence of nitrate (mg NO_3-N/g VSS/hr)	ND*	59 ± 18	48 ± 16
Initial pH	6.60 ± 0.01	6.35 ± 0.06	7.29 ± 0.39
Final pH	8.31 ± 0.01	8.47 ± 0.02	8.79 ± 0.12
Change in pH	1.71 ± 0.01	2.12 ± 0.04	1.50 ± 0.51
mg VSS/L	189 ± 8.54	246 ± 97.2	1253 ± 668
Nitrite Test			
Nitrite reduction (mg NO_3-N/g VSS/hr)	57 ± 7	98 ± 3	57 ± 5
Initial pH	6.14 ± 0.03	6.39 ± 0.01	7.31 ± 0.41
Final pH	7.89 ± 0.03	8.76 ± 0.09	8.95 ± 0.18
Change in pH	1.75 ± .05	2.37 ± 0.10	1.64 ± 0.59
mg VSS/L	171 ± 31	314 ± 5.3	1291 ± 489

*Nitrite was not detected during this test.

APPENDIX B

Table B.1. Operating conditions throughout study.

Condition	Date
Fermenter 1 start-up	8/12/96
Fermenter 2 start-up	11/24/96
Column start-up	8/16/96
Low nitrate loading phase	8/16/96 - 3/4/97
High nitrate loading phase	3/5/97 - 7/28/97
High nitrate loading phase, low hydraulic loading condition	5/26/97 - 6/18/97
High nitrate loading phase, high hydraulic loading condition	3/5/97 - 5/25/97, 6/19/97 - 7/28/97

Table B.2. Anion column data for low nitrate loading phase.

	Day of	Nitrate (mg/L as N)		Nitrite (mg/L as N)		Sulfate (mg/L)		Chloride (mg/L)	
Date	Study	Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent
8/16/96	0	38.3		0.00		8.40			
8/18/96	2	44.0		0.00		11.7			
8/21/96	5	39.2	2.87	2.06	1.02	9.20	13.9	20.8	18.1
8/26/96	10	34.6		1.41		10.1			
9/3/96	18	25.9	0.43	5.77	0.22	8.32	15.0	16.2	15.5
9/7/96	22	23.9	0.56	1.73	0.00	8.39	6.83	15.1	14.0
9/10/96	25	41.4	0.62	0.00	0.29	7.53	7.99	16.1	16.5
9/15/96	30	41.2	8.37	0.00	0.90	7.37	8.08	14.7	15.2
9/17/96	32	41.4	6.01	0.00	1.07	7.45	7.56	16.6	15.0
9/24/96	39	40.2	7.36	0.00	1.07	8.12	11.3	15.2	15.6
10/2/96	47	43.9	12.9	0.00	2.79	7.92	7.25	16.0	14.3
10/6/96	51	44.7	8.13	0.00	8.92	6.61	6.94	12.6	15.9
10/7/96	52	39.4	5.91	0.00	10.3	6.06	7.96	12.7	18.2
10/8/96	53	40.7	2.44	0.53	11.5	14.4	8.23	11.9	38.6
10/12/96	57	35.5	12.6	0.00	1.68	6.62	7.59	13.4	14.5
10/23/96	68	42.0	8.27	0.00	1.68	7.69	8.03	17.7	15.2
10/26/96	71	37.1	0.50	0.36	4.07	7.67	7.99	16.4	15.6
10/29/96	74	40.0	13.3	0.00	7.06	7.21	7.49	15.0	13.9
11/7/96	83	42.5	24.1	0.00	8.74	6.08	22.7	10.5	19.3
11/10/96	86	46.0	21.3	0.00	3.59	6.86	7.80	12.3	16.4
11/23/96	99	40.7	9.84	0.00	6.56	6.95	10.3	13.2	18.5
12/1/96	107	30.6	6.95	0.00	0.00	34.2	35.1	47.1	34.5
12/3/96	109	27.5	10.9	0.00	0.00	17.5	15.6	25.1	22.7
12/4/96	110	45.0	47.0	0.00	0.00	28.1	13.7	51.6	58.2
12/5/96	111	40.6	13.6	0.00	0.00	14.8	8.6	15.5	15.5
12/6/96	112	43.6	44.4	0.00	0.00	8.93	9.26	40.1	39.9
12/19/96	125	41.5	11.0	0.00	0.00	6.67	5.90	14.2	14.8
12/21/96	127	25.5	12.9	0.00	0.00	8.07	8.22	14.5	13.7
12/23/96	129	18.5	16.0	0.00	0.50	39.0		57.3	114.8
12/27/96	133	53.7	14.7	0.00	2.35	17.4	16.1	27.4	23.7
12/29/96	135	36.0	10.4	0.00	0.00	19.5	15.7	10.7	8.8
1/5/97	142	35.4	13.0	0.00	3.60	6.65	8.80	21.2	23.7
1/18/97	155	40.5	18.6	2.12	0.00	6.16	5.68	16.2	15.7
1/19/97	156	45.3	6.87	0.00	7.33	7.40	5.34	24.1	16.2
1/22/97	159	36.9	15.1	0.00	0.00	7.56	8.00	14.1	14.8
1/25/97	162	36.3	0.37	0.00	0.00	5.20	6.64	14.4	15.2
1/29/97	166	39.2	0.02	0.48	0.02				
2/7/97	175	42.0	15.4	0.00	0.00	7.79	10.1	16.0	18.0
2/8/97	176	39.4	0.36	0.00	0.00	7.57	8.43	8.42	16.9
2/11/97	179	40.6	6.48	0.00	0.00	7.92	9.93	15.6	19.4
2/16/97	184	43.6	0.17	0.00	0.00	21.6	24.0	26.6	26.9
2/17/97	185	36.1	0.14	12.90	0.00	74.6		23.7	37.9
2/18/97	186	36.8		0.00		12.9		19.2	
2/19/97	187	40.6	0.19	0.00	0.00				
2/20/97	188	36.6	1.91	0.27	0.00		7.91		18.7
2/21/97	189	39.2	6.41	0.00	8.56	5.66	8.37	12.9	18.0
2/23/97	191	47.0	0.14	0.00	8.59	9.01	6.75	20.7	15.1
2/25/97	193	39.4	0.28	0.70	0.00	6.36	9.41	16.7	22.6
2/28/97	196	32.3		0.98		6.52		14.2	
3/2/97	198	36.3	0.61	1.81	12.20	12.2	16.6	22.0	30.1
3/4/97	200	26.2	0.52	1.33	0.00	4.78	7.27	15.3	19.0

Table B.3. Anion column data for high nitrate loading phase and hydraulic loading = 3.0 $m^3/m^2/hr$.

	Day of	Nitrate (mg/L as N)		Nitrite (mg/L as N)		Sulfate (mg/L)		Chloride (mg/L)	
Date	Study	Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent
3/5/97	201	61.5	5.91	2.19	19.7	6.30	6.91	19.7	19.4
3/6/97	202	52.7	0.44	1.25	8.52	5.30	8.18	17.3	21.4
3/12/97	208	68.3	0.74	3.46	7.98	6.83	8.66	19.8	21.1
3/17/97	213	93.3	0.10	0.00	7.31	41.9	55.2	59.4	44.0
3/19/97	215	96.8	0.17	8.92	26.8	8.74	10.3	24.2	22.8
3/22/97	218	85.7	0.43	2.88	9.67	7.99	9.33	26.3	25.2
3/24/97	220	84.3	0.13	1.93	5.37	7.63	9.35	22.6	20.7
3/26/97	222	93.3	0.00	2.60	22.8	7.87	8.44	23.0	21.0
4/1/97	228	98.9		0.00		8.39		22.1	
4/3/97	230	94.7	0.00	0.00	22.4	7.53	7.94	24.6	20.8
4/5/97	232	71.9	0.00	14.28	6.31	36.5	36.0	36.4	36.4
4/6/97	233	93.2	0.00	0.00	25.0	12.4	11.4	31.5	26.2
4/10/97	237	90.4	0.00	3.31	27.1	6.94	7.03	22.0	19.2
4/12/97	239	92.0	0.82	0.00	31.4	6.60	7.35	24.7	8.1
4/14/97	241	93.4	0.60	0.00	26.6	7.07	8.01	27.6	8.5
4/15/97	242	91.7	0.80	0.00	29.2	7.32	9.32	23.3	10.4
4/19/97	246	94.7	0.63	0.00	33.2	6.33	7.23	22.6	18.6
4/20/97	247	93.3	0.64	3.29	30.3	56.0	6.99	19.7	19.9
4/21/97	248	88.7	0.19	5.45	25.8	7.46	7.02	20.9	17.3
4/23/97	250	78.7	0.18	3.71	30.8	9.83	7.44	18.8	19.0
4/29/97	256	70.4	0.17	6.13	9.23	8.46	7.66	21.2	19.5
4/30/97	257	61.9	0.17	7.38	17.75	5.84	7.47	17.9	17.2
5/8/97	265	88.2	0.17	3.87	23.5	6.48	6.85	23.4	19.4
5/14/97	271	86.9	0.36	3.55	27.6	6.55	15.0	24.8	19.6
5/15/97	272	80.8	0.49	4.18	21.7	4.92	6.63	17.9	17.5
5/16/97	273		0.41		20.1		8.97		20.8
5/17/97	274	81.6	0.54	0.77	30.4	6.09	7.19	17.61	20.2
5/18/97	275	86.2	0.28	4.30	18.2	6.08	6.57	18.6	17.9
5/19/97	276	81.5	0.20	4.78	24.8	5.36	6.64	17.3	17.6
5/20/97	277								
5/21/97	278	74.1	0.20	1.80	28.6	4.37	8.07	14.7	20.2
5/22/97	279	88.6	0.19	3.23	13.74	5.74	5.55	18.0	15.4
5/24/97	281	94.7	0.19	8.54	2.23	6.91	4.75	19.9	12.6
5/25/97	282	98.9	0.14	2.73	0.00	7.75	6.76	20.4	19.2
6/20/97	308				20.8		7.11		22.3
6/23/97	311	72.2	0.00	9.44	0.00	6.05	6.05	21.9	23.5
6/24/97	312	86.7	0.00	7.95	0.00	6.30	6.09	19.2	24.8
6/27/97	315	80.6	1.13	0.81	1.09	15.7	92.0	21.3	23.2
6/28/97	316	125.6	0.79	3.19	0.00	6.59	6.02	33.3	23.8
7/1/97	319	84.1	0.33	0.21	0.00	4.33	9.07	22.6	25.2
7/2/97	320	86.4	0.31	0.00	0.00	4.73	6.42	23.1	23.2
7/4/97	322	84.7	0.48	0.00	0.00	4.09	6.87	45.9	26.9
7/5/97	323	84.9	0.40	0.00	0.00	4.63	7.24	37.9	25.7
7/7/97	325	91.2	0.17	2.66	0.00	4.80	6.92	22.1	24.5
7/8/97	326	87.5	0.16	0.00	0.00	4.33	6.29	42.0	27.6
7/9/97	327	86.1	0.18	3.72	0.00	7.23	5.91	22.9	23.0
7/11/97	329	82.6	0.19	1.36	0.00	7.03	6.97	37.0	25.2
7/18/97	336	90.1	4.47	1.14	0.00	41.1	7.46	21.7	23.9
7/24/97	342	90.8	2.49	0.12	0.00	10.1	7.87	20.1	20.5
7/27/97	345	81.8	0.75	0.91	0.96	6.51	8.52	22.2	19.4
7/28/97	346	82.5	0.73	0.29	0.45	6.72	7.47	18.7	19.0

Table B.4. Anion column data for high nitrate loading phase and hydraulic loading = 1.5 $m^3/m^2/hr$.

Date	Day of Study	Nitrate (mg/L as N)		Nitrite (mg/L as N)		Sulfate (mg/L)		Chloride (mg/L)	
		Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent
5/26/97	283	71.3	0.18	3.54	18.21	4.4	11.9	14.4	18.4
5/27/97	284	44.6	0.16	7.89	0.00	3.5	7.1	13.2	42.8
5/28/97	285	46.0	0.47	10.12	0.00	4.4	7.0	10.5	18.7
5/29/97	286	72.1	0.00	16.76	0.00	6.3	6.9	15.5	18.0
5/31/97	288	82.8	0.44	3.83	0.00	7.3	9.9	17.2	18.8
6/2/97	290	60.1	0.52	14.00	0.00	6.6	13.6	14.9	18.5
6/3/97	291	76.5	0.52	8.13	0.00	6.8	7.3	16.9	18.4
6/5/97	293	84.5	0.15	6.74	0.00	7.6	4.7	17.8	14.6
6/8/97	296	24.1	0.20	64.08	33.8	6.59	7.27	16.9	18.3
6/10/97	298	78.3	0.00	5.94	4.83	6.68	11.00	18.7	17.9
6/11/97	299	60.8	0.36	23.87	20.4	9.08	6.73	24.0	21.9
6/16/97	304	79.0	0.00	8.90	21.6	5.94	8.41	29.0	18.0

Table B.5. COD column data for low nitrate loading phase. Values corrected for nitrite interference using an experimentally determined correction factor [corrected COD (mg/L) = measured COD (mg/L) - 0.2353*NO2-N(mg/L)].

Date	Day of Study	COD (mg/L)		Nitrite (mg/L as N)		Corrected for Nitrite Interference COD (mg/L)	
		Influent	Effluent	Influent	Effluent	Influent	Effluent
8/26/96	10	102	47.5	1.41		102	47.5
9/3/96	18	111	98.1	5.77	0.22	110	98.1
9/7/96	22	140	59.6	1.73	0.00	139	59.6
9/10/96	25	191	29.4	0.00	0.29	191	29.4
9/15/96	30	125	24.9	0.00	0.90	125	24.7
9/17/96	32	155	24.9	0.00	1.07	155	24.7
9/24/96	39	162	94.3	0.00	1.07	162	94.1
10/2/96	47	162	34.0	0.00	2.79	162	33.3
10/6/96	51	84.7	36.1	0.00	8.9	84.7	34.0
10/7/96	52	94.1	34.5	0.00	10.3	94.1	32.1
10/8/96	53	95.7	46.7	0.53	11.5	95.6	44.0
10/12/96	57	75.3	28.2	0.00	1.7	75.3	27.8
10/23/96	68	115	29.0	0.00	1.7	115	28.6
10/26/96	71	182	26.5	0.36	4.1	182	25.5
10/29/96	74	62	7.79	0.00	7.1	62.3	6.13
11/7/96	83	118	12	0.00	8.74	118	9.94
11/10/96	86	72.8	12.0	0.00	3.59	72.8	11.2
11/27/96	103	86.7	23.2				
11/28/96	104	8.57	19.5				
11/30/96	106	8.57					
12/1/96	107	120	20.6	0.00	0.00	120	20.6
12/3/96	109	51.7	4.13	0.00	0.00	51.7	4.13
12/4/96	110	10.1	5.45	0.00	0.00	10.1	5.45
12/5/96	111	81.5	11.1	0.00	0.00	81.5	11.1
12/6/96	112	9.55	8.00	0.00	0.00	9.55	8.00
12/19/96	125	157	10.8	0.00	0.00	157	10.8
12/21/96	127	76	13.9	0.00	0.00	75.6	13.9
12/23/96	129	43	32.4	0.00	0.50	43.2	32.3
12/27/96	133	281	13.0	0.00	2.35	281	12.4
12/29/96	135		27.8	0.00	0.00		27.8
1/5/97	142	107	13.2	0.00	3.60	107	12.4
1/18/97	155	99	10.9	2.12	0.00	98.9	10.9
1/19/97	156	156	15.1	0.00	7.33	156	13.3
1/22/97	159	73.5	11.2	0.00	0.00	73.5	11.2
1/25/97	162	223	39.6	0.00	0.00	223	39.6
1/29/97	166	265	62		0.02		62.0
2/7/97	175	134	16.9	0.00	0	134	16.9
2/8/97	176	208	40.0	0.00	0	208	40.0
2/11/97	179	134	16.9	0.00	0	134	16.9
2/16/97	184	220	38.0	0.00	0	220	38.0
2/17/97	185	139	58.6	12.9	0	136	58.6
2/19/97	187	234	31.9		0		31.9
2/20/97	188	277	25.0		0.00		25.0
2/21/97	189	111	21.6	0.00	8.56	111	19.6
2/23/97	191	153	40.1	0.00	8.59	153	38.1
2/25/97	193	210	38.6	0.70	0.00	210	38.6
2/28/97	196	165	38.6	0.98		165	
3/2/97	198	127	43.2	1.81	12.2	126	40.3
3/4/97	200	229	73.8	1.33	0.00	229	73.8

Table B.6. COD column data for high nitrate loading and hydraulic loading = 3.0 $m^3/m^2/hr$. Values corrected for nitrite interference using an experimentally determined correction factor [corrected COD (mg/L) = measured COD (mg/L) - 0.2353*NO2-N(mg/L)].

Date	Day of Study	COD (mg/L)		Nitrite (mg/L as N)		Corrected for Nitrite Interference COD (mg/L)	
		Influent	Effluent	Influent	Effluent	Influent	Effluent
3/5/97	201	314	20.5	2.19	19.7	314	16
3/6/97	202	379	28.4	1.25	8.52	379	26
3/12/97	208	304	50.5	3.46	7.98	303	49
3/17/97	213	355	53.8	0.00	7.31	355	52
3/19/97	215	394	70.1	8.92	26.8	391	64
3/22/97	218	307	68.3	2.88	9.67	306	66
3/24/97	220	444	111	1.93	5.37	444	110
3/26/97	222	291	64.5	2.60	22.8	291	59
4/1/97	228	318	117	0.00		318	117
4/3/97	230	319	67.0	0.00	22.4	319	62
4/5/97	232	286	96.3	14.3	6.31	282	95
4/6/97	233	368	70.3	0.00	25.0	368	64
4/10/97	237	284	50.6	3.31	27.1	283	44
4/12/97	239	308	75.6	0.00	31.4	308	68
4/14/97	241	328	107	0.00	26.6	328	101
4/15/97	242	376	91.9	0.00	29.2	376	85
4/19/97	246	288	124	0.00	33.2	288	117
4/20/97	247	411	134	3.29	30.3	410	127
4/21/97	248	338	108	5.45	25.8	337	101
4/23/97	250	322	98.7	3.71	30.8	321	91
4/29/97	256	411	157	6.13	9.23	409	155
4/30/97	257	258	77.4	7.38	17.8	256	73
5/8/97	265	388	134	3.87	23.5	387	129
5/14/97	271	328	53.2	3.55	27.6	327	47
5/15/97	272	373	114	4.18	21.7	372	109
5/16/97	273	361	85.1		20.1	361	80
5/17/97	274	305	50.6	0.77	30.4	304	43
5/18/97	275	390	73.8	4.30	18.2	389	70
5/19/97	276	281	23.3	4.78	24.8	280	17
5/20/97	277	334	40.1			334	40
5/21/97	278			1.80	28.6	0	-7
5/22/97	279	296	91.8	3.23	13.7	296	89
5/24/97	281	219	72.9	8.54	2.23	217	72
5/25/97	282	344	33.7	2.73	0.00	343	34
6/20/97	308	565	202		20.8	565	197
6/23/97	311	506	165	9.44	0.00	504	165
6/24/97	312	296	185	7.95	0.00	294	185
6/27/97	315	526	181	0.81	1.09	526	181
6/28/97	316	541	162	3.19	0.00	540	162
7/1/97	319	376	186	0.21	0.00	376	186
7/2/97	320	296	240	0.00	0.00	296	240
7/4/97	322	674	526	0.00	0.00	674	526
7/5/97	323	881	111	0.00	0.00	881	111
7/7/97	325	756	358	2.66	0.00	755	358
7/8/97	326	704	416	0.00	0.00	704	416
7/9/97	327	741	633	3.72	0.00	740	633
7/11/97	329	711	622	1.36	0.00	711	622
7/18/97	336	834	417	1.14	0.00	834	417
7/24/97	342	584	188	0.12	0.00	584	188
7/27/97	345	679	353	0.91	0.96	679	353
7/28/97	346	389	60.0	0.29	0.45	389	60

Table B.7. COD column data for high nitrate loading and hydraulic loading = 1.5 $m^3/m^2/hr$. Values corrected for nitrite interference using an experimentally determined correction factor [corrected COD (mg/L) = measured COD (mg/L) - 0.2353*NO2-N(mg/L)].

Date	Day of Study	COD (mg/L)		Nitrite (mg/L as N)		Corrected for Nitrite Interference COD (mg/L)	
		Influent	Effluent	Influent	Effluent	Influent	Effluent
5/26/97	283	277	37.6	3.54	18.2	276	33.4
5/27/97	284	184	178	7.89	0.00	182	178
5/28/97	285	348	106	10.1	0.00	346	106
5/29/97	286	297	29.0	16.8	0.00	293	29.0
5/31/97	288	222	117	3.83	0.00	221	117
6/2/97	290	282	71.4	14.0	0.00	278	71.4
6/3/97	291	439	118	8.13	0.00	437	118
6/5/97	293	340	73.7	6.74	0.00	339	73.7
6/8/97	296	267	27.5	64.1	33.8	252	19.5
6/10/97	298	342	43.6	5.94	4.83	340	42.5
6/11/97	299			23.9	20.4		
6/16/97	304	315	36.4	8.90	21.6	313	31.3

Table B.8. Anion and COD column profile data for 12/19/96, low nitrate loading.

Date: 12/19/96 Column Port	Column Height (ft)	Column Height (m)	NO_3^- (mg/L as N)	NO_2^- (mg/L as N)	SO_4^{2-} (mg/L)	Cl^- (mg/L)	COD (mg/L)	COD Corrected for Nitrite Interference*
NO_3 stock			48.3	0.0	7.7	11.7		
Influent	0.00	0.00	41.5	0.0	6.7	14.2	157	157
Port 1	2.92	0.89	36.9	1.4	6.1	12.6	117	116
Port 2	4.50	1.37	27.6	1.6	6.3	12.6	78.7	78.3
Port 3	6.08	1.85	16.3	0.0	6.9	13.8	27.8	27.8
Port 4	7.67	2.34	12.1	0.0	8.0	13.1	10.0	10.0
Port 5	9.25	2.82	13.0	0.0	7.3	13.1	4.63	4.63
Port 6	10.83	3.30	13.2	0.0	6.9	13.0	8.49	8.49
Effluent	11.42	3.48	11.0	0.0	5.9	14.8	10.8	10.8

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

Table B.9. Anion and COD column profile data for 12/27/96, low nitrate loading.

Date: 12/27/96 Column Port	Column Height (m)	Column Height (m)	NO_3^- (mg/L as N)	NO_2^- (mg/L as N)	SO_4^{2-} (mg/L)	Cl^- (mg/L)	COD (mg/L)	COD Corrected for Nitrite Interference*
NO_3 stock								
Influent	0.00	0.00	53.67	0.00	17.41	27.45	281	281
Port 1	2.92	0.89	24.61	0.49	9.05	16.65	154	154
Port 2	4.50	1.37	21.96	1.21	12.34	18.07	84.1	83.8
Port 3	6.08	1.85	12.48	1.58	8.15	14.46	45.1	44.7
Port 4	7.67	2.34	9.20	1.45	7.46	8.28	20.6	20.3
Port 5	9.25	2.82	6.88	1.57	8.25	9.02	10.7	10.3
Port 6	10.83	3.30	8.32	2.02	9.58	10.49	10.7	10.2
Effluent	11.42	3.48	7.96	1.76	9.71	9.94	13.0	12.6

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

Table B.10. Anion and COD column profile data for 1/5/97, low nitrate loading.

Date: 1/5/97 Column Port	Column Height (m)	Column Height (m)	NO_3^- (mg/L as N)	NO_2^- (mg/L as N)	SO_4^{2-} (mg/L)	Cl^- (mg/L)	COD (mg/L)	COD Corrected for Nitrite Interference*
NO_3 stock			46.4	0.0	9.2	15.5		
Influent	0.00	0.00	35.4	0.0	6.6	21.2	107	107
Port 1	2.92	0.89	26.9	-0.3	4.6	18.3	108	108
Port 2	4.50	1.37	22.7	3.4	8.4	23.2	46.6	45.8
Port 3	6.08	1.85	18.2	3.9	8.6	24.0	39.6	38.7
Port 4	7.67	2.34	13.1	4.1	7.9	23.0	43.5	42.5
Port 5	9.25	2.82	13.3	4.0	9.2	24.4	48.2	47.2
Port 6	10.83	3.30	16.3	3.5	8.8	23.1	15.5	14.7
Effluent	11.42	3.48	13.0	3.6	8.8	23.7	13.2	12.4

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

Table B.11. Anion and COD column profile data for 1/18/97, low nitrate loading.

Date:1/18/96 Column Port	Column Height (m)	Column Height (m)	NO_3^- (mg/L as N)	NO_2^- (mg/L as N)	SO_4^{2-} (mg/L)	Cl^- (mg/L)	COD (mg/L)	COD Corrected for Nitrite Interference*
NO_3 stock			47.60	-0.58	6.97	14.15		
Influent	0.00	0.00	40.47	0.00	6.16	16.21	99.4	99.4
Port 1	2.92	0.89	39.38	0.00	5.80	15.20	90.1	90.1
Port 2	4.50	1.37	28.59	0.18	5.90	15.53	51.3	51.2
Port 3	6.08	1.85	22.14	1.87	7.52	15.44	24.9	24.4
Port 4	7.67	2.34	17.31	2.17	5.83	15.71	17.1	16.6
Port 5	9.25	2.82	18.48	2.12	5.82	15.74	9.32	8.82
Port 6	10.83	3.30	18.08	1.98	5.80	15.72	14.8	14.3
Effluent	11.42	3.48	18.60	2.12	5.68	15.66	10.9	10.4

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

Table B.12. Anion and COD column profile data for 1/25/97, low nitrate loading.

Date: 1/25/97 Column Port	Column Height (ft)	Column Height (m)	NO_3^- (mg/L as N)	NO_2^- (mg/L as N)	SO_4^{2-} (mg/L)	Cl^- (mg/L)	COD (mg/L)	COD Corrected for Nitrite Interference*
NO_3 stock			46.58		7.15	14.71		
Influent	0.00	0.00	36.33	0.00	5.20	14.39	223	223
Port 1	2.92	0.89	25.09	0.98	4.96	14.16	173	173
Port 2	4.50	1.37	14.93	3.66	5.46	13.45	140	139
Port 3	6.08	1.85	2.68	3.76	6.73	14.36	57.8	56.9
Port 4	7.67	2.34	0.46	0.15	6.24	15.70	43.6	43.5
Port 5	9.25	2.82	0.44	0.00	7.00	16.83	42.0	42.0
Port 6	10.83	3.30	0.41	0.00	6.69	15.51	34.9	34.9
Effluent	11.42	3.48	0.37	0.00	6.64	15.16	39.6	39.6

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

Table B.13. VFA column profile data for 1/25/97, low nitrate loading.

Date: 1/25/97 Column Port	Column Height (m)	Acetic acid (mg/L as COD)	Propionic acid (mg/L as COD)	Isobutyric acid (mg/L as COD)	*n* -Butyric acid (mg/L as COD)	2-Methylbutyric acid (mg/L as COD)	3-Methylbutyric acid (mg/L as COD)	*n* -Valeric acid (mg/L as COD)
Influent	0.00	89.87	58.55	7.32	28.51	5.51	7.21	20.51
Port 1	0.89	62.76	43.16	6.57	19.75	4.20	7.40	13.94
Port 2	1.37	38.70	27.28	4.97	12.92	0.00	0.00	8.80
Port 3	1.85	9.80	6.66	2.13	2.24	0.00	0.00	0.00
Port 4	2.34	1.56	0.76	0.89	0.00	0.00	0.00	0.00
Port 5	2.82	2.04	0.15	0.00	0.00	0.00	0.00	0.00
Port 6	3.30	1.99	0.46	0.00	0.00	0.00	0.00	0.00
Effluent	3.48	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table B.14. Anion and COD column profile data for 1/29/97, low nitrate loading.

Date: 1/29/97 Column Port	Column Height (ft)	Column Height (m)	NO_3^- (mg/L as N)	NO_2^- (mg/L as N)	SO_4^{2-} (mg/L)	Cl^- (mg/L)	COD (mg/L)	COD Corrected for Nitrite Interference*
NO_3 stock								
Influent	0.00	0.00	39.2	0.48	6.84	15.0	265	265
Port 1	2.92	0.89	31.0	0.52	5.03	14.2	242	241
Port 2	4.50	1.37	11.8	2.21	6.72	15.2	192	192
Port 3	6.08	1.85	2.44	1.63	6.20	15.3	151	150
Port 4	7.67	2.34	1.16	0.17	8.84	15.4	126	126
Port 5	9.25	2.82	1.16	0.15	6.64	15.2	120	120
Port 6	10.83	3.30	1.29	0.14	6.57	15.4	128	128
Effluent	11.42	3.48	0.39	0.00	6.69	15.9	62.0	62.0

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

Table B.15. VFA column profile data for 1/29/97, low nitrate loading.

Date: 1/29/97 Column Port	Column Height (m)	Acetic acid (mg/L as COD)	Propionic acid (mg/L as COD)	Isobutyric acid (mg/L as COD)	*n*-Butyric acid (mg/L as COD)	2-Methylbutyric acid (mg/L as COD)	3-Methylbutyric acid (mg/L as COD)	*n*-Valeric acid (mg/L as COD)
Influent	0.00	84.82	66.80	7.03	29.87	9.41	10.53	25.47
Port 1	0.89	72.14	52.44	6.65	23.64	8.65	10.09	19.16
Port 2	1.37	37.20	24.03	4.12	11.02	6.15	7.80	0.00
Port 3	1.85	23.79	9.42	2.77	4.22	7.06	0.00	0.00
Port 4	2.34	18.88	5.53	2.35	1.63	0.00	0.00	0.00
Port 5	2.82	11.63	3.20	1.31	0.00	0.00	0.00	0.00
Port 6	3.30	23.92	4.88	1.76	0.00	0.00	0.00	0.00
Effluent	3.48	17.56	5.56	2.17	0.00	0.00	0.00	0.00

Table B.16. Anion and COD column profile data for 2/16/97, low nitrate loading.

Date: 2/16/97 Column Port	Column Height (m)	Column Height (m)	NO_3^- (mg/L as N)	NO_2^- (mg/L as N)	SO_4^{2-} (mg/L)	Cl^- (mg/L)	COD (mg/L)	COD Corrected for Nitrite Interference*
NO_3 stock			48.7	0.42	24.1	26.5		
Influent	0	0.00	43.6	0.00	21.6	26.6	220	220
Port 1	2.92	0.89	28.4	0.76	18.2	25.4	117	117
Port 2	4.50	1.37	7.88	2.42	22.5	28.4	108	107
Port 3	6.08	1.85	0.31	0.08	22.5	25.6	57.7	57.7
Port 4	7.67	2.34	0.15	0.00	23.4	26.1	39.5	39.5
Port 5	9.25	2.82	0.14	0.00	23.0	26.1	39.5	39.5
Port 6	10.83	3.30	0.74	0.00	22.2	25.9	39.5	39.5
Effluent	11.42	3.48	0.17	0.00	24.0	26.9	38.0	38.0

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

Table B.17. VFA column profile data for 2/16/97, low nitrate loading.

Date: 2/16/97 Column Port	Column Height (m)	Acetic acid (mg/L as COD)	Propionic acid (mg/L as COD)	Isobutyric acid (mg/L as COD)	*n* -Butyric acid (mg/L as COD)	2-Methylbutyric acid (mg/L as COD)	3-Methylbutyric acid (mg/L as COD)	*n* -Valeric acid (mg/L as COD)
Influent	0	80.42	54.17	9.39	17.23	13.79	7.27	0.00
Port 1	0.89	36.52	29.12	4.41	11.67	6.60	3.30	0.00
Port 2	1.37	30.98	17.47	5.07	5.11	8.37	0.00	0.00
Port 3	1.85	10.67	4.28	2.54	0.00	0.00	0.00	0.00
Port 4	2.34	1.44	0.95	0.00	0.00	0.00	0.00	0.00
Port 5	2.82	1.24	0.79	0.00	0.00	0.00	0.00	0.00
Port 6	3.30	0.97	0.00	0.00	0.00	0.00	0.00	0.00
Effluent	3.48	12.17	9.16	1.46	0.00	0.00	0.00	0.00

Table B.18. Anion and COD column profile data for 2/19/97, low nitrate loading.

Date: 2/19/97 Column Port	Column Height (ft)	Column Height (m)	NO_3^- (mg/L as N)	NO_2^- (mg/L as N)	SO_4^{2-} (mg/L)	Cl^- (mg/L)	COD (mg/L)	COD Corrected for Nitrite Interference*
NO_3 stock								
Influent	0.00	0.00	40.63	0.00	10.09	18.01	234	234
Port 1	2.92	0.89	28.54	2.01	7.91	19.06	182	182
Port 2	4.50	1.37	15.30	0.86	10.11	20.26	125	124
Port 3	6.08	1.85	2.41	1.07	9.63	19.69	62	62
Port 4	7.67	2.34	0.23	1.01	11.30	19.86	38	38
Port 5	9.25	2.82	0.42	0.00	10.46	19.85	32	32
Port 6	10.83	3.30	0.14	0.00	9.78	19.75	33	33
Effluent	11.42	3.48	0.19	0.00	9.30	20.29	32	32

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

Table B.19. VFA column profile data for 2/19/97, low nitrate loading.

Date: 2/19/97 Column Port	Column Height (m)	Acetic acid (mg/L as COD)	Propionic acid (mg/L as COD)	Isobutyric acid (mg/L as COD)	*n*-Butyric acid (mg/L as COD)	2-Methylbutyric acid (mg/L as COD)	3-Methylbutyric acid (mg/L as COD)	*n*-Valeric acid (mg/L as COD)
Influent	0.00	64.24	45.50	6.74	14.73	8.79	10.73	12.80
Port 1	0.89	59.34	31.98	6.36	10.47	7.97	9.55	0.00
Port 2	1.37	28.13	15.91	3.95	5.81	8.26	0.00	0.00
Port 3	1.85	1.83	0.21	1.41	0.00	0.00	0.00	0.00
Port 4	2.34	1.09	0.00	0.00	0.00	0.00	0.00	0.00
Port 5	2.82	0.90	0.00	0.00	0.00	0.00	0.00	0.00
Port 6	3.30	0.99	0.00	0.00	0.00	0.00	0.00	0.00
Effluent	3.48	1.92	0.00	0.00	0.00	0.00	0.00	0.00

Table B.20. Anion and COD column profile data for 2/20/97, low nitrate loading.

Date: 2/20/97 Column Port	Column Height (ft)	Column Height (m)	NO_3^- (mg/L as N)	NO_2^- (mg/L as N)	SO_4^{2-} (mg/L)	Cl^- (mg/L)	COD (mg/L)	COD Corrected for Nitrite Interference*
NO_3 stock			47.79	0.00	8.01	14.6		
Influent	0.00	0.00	36.58	0.27	6.61	26.2	277	277
Port 1	2.92	0.89	29.34	0.59	6.44	17.0	166	166
Port 2	4.50	1.37	12.75	3.63	6.10	17.6	131	130
Port 3	6.08	1.85	5.35	5.42	6.85	17.5	78.5	77.2
Port 4	7.67	2.34	1.18	0.92	8.46	17.9	49.2	49.0
Port 5	9.25	2.82	1.22	0.00	8.13	17.4	47.7	47.7
Port 6	10.83	3.30	1.83	0.00	6.75	17.0	35.4	35.4
Effluent	11.42	3.48	1.91	0.00	7.27	17.7	25.0	25.0

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

Table B.21. VFA column profile data for 2/20/97, low nitrate loading.

Date: 2/20/97 Column Port	Column Height (m)	Acetic acid (mg/L as COD)	Propionic acid (mg/L as COD)	Isobutyric acid (mg/L as COD)	*n* -Butyric acid (mg/L as COD)	2-Methylbutyric acid (mg/L as COD)	3-Methylbutyric acid (mg/L as COD)	*n* -Valeric acid (mg/L as COD)
Influent	0.00	112.70	78.12	10.57	23.56	12.00	10.56	22.09
Port 1	0.89	0.42	6.17	0.00	0.00	0.00	0.00	0.00
Port 2	1.37	0.00	0.77	3.54	0.00	0.00	0.00	0.00
Port 3	1.85	0.00	1.84	0.00	0.00	0.00	0.00	0.00
Port 4	2.34	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Port 5	2.82	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Port 6	3.30	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Effluent	3.48	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table B.22. Anion and COD column profile data for 4/15/97, high nitrate loading and normal hyrdaulic loading = 3.0 $m^3/m^2/hr$.

Date: 4/15/97 Column Port	Column Height (ft)	Column Height (m)	NO_3^- (mg/L as N)	NO_2^- (mg/L as N)	SO_4^{2-} (mg/L)	Cl^- (mg/L)	COD (mg/L)	COD Corrected for Nitrite Interference*
NO_3 stock								
Influent	0.00	0.00	91.7	0.00	7.32	23.3	376	376
Port 1	2.92	0.89	79.5	6.64	6.72	20.3	360	358
Port 2	4.50	1.37	36.4	23.14	7.19	15.6	263	258
Port 3	6.08	1.85	19.8	35.99	7.47	13.7	323	314
Port 4	7.67	2.34	4.54	35.66	36.29	12.3	238	230
Port 5	9.25	2.82	1.41	31.66	8.09	18.0	182	175
Port 6	10.83	3.30	0.63	29.31	12.87	18.6	132	126
Effluent	11.42	3.48	0.80	29.20	9.32	10.4	147	140

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

Table B.23. Anion and COD column profile data for 5/15/97, high nitrate loading and normal hyrdaulic loading = 3.0 $m^3/m^2/hr$.

Date: 5/15/97 Column Port	Column Height (m)	Column Height (m)	NO_3^- (mg/L as N)	NO_2^- (mg/L as N)	SO_4^{2-} (mg/L)	Cl^- (mg/L)	COD (mg/L)	COD Corrected for Nitrite Interference*
NO_3 stock								
Influent	0.00	0.00	80.8	4.18	4.92	17.9	373	354
Port 1	2.92	0.89	80.8	3.47	4.42	17.9	356	337
Port 2	4.50	1.37	21.3	48.3	5.94	13.7	308	303
Port 3	6.08	1.85	20.7	34.3	6.15	11.9	236	231
Port 4	7.67	2.34	5.66	31.7	5.66	13.0	209	207
Port 5	9.25	2.82	2.31	34.7	6.61	10.8	173	172
Port 6	10.83	3.30	0.55	27.8	6.63	18.1	141	141
Effluent	11.42	3.48	0.49	21.7	6.63	17.5	114	114

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

Table B.24. Anion and COD column profile data for 7/18/97, high nitrate loading and normal hyrdaulic loading = 3.0 $m^3/m^2/hr$.

Date: 7/18/97 Column Port	Column Height (ft)	Column Height (m)	NO_3^- (mg/L as N)	NO_2^- (mg/L as N)	SO_4^{2-} (mg/L)	Cl^- (mg/L)	COD (mg/L)	COD Corrected for Nitrite Interference*
NO_3 stock								
Influent	0.00	0.00	90.08	1.14	41.13	21.69	834	834
Port 1	2.92	0.89	83.05	5.95	8.13	21.71	795	793
Port 2	4.50	1.37	46.99	8.24	16.21	21.32	660	658
Port 3	6.08	1.85	21.70	6.59	11.80	21.10	564	563
Port 4	7.67	2.34	29.27	3.56	13.65	21.51	521	520
Port 5	9.25	2.82	3.23	1.21	11.41	21.71	465	465
Port 6	10.83	3.30	1.21	0.00	17.82	23.78	477	477
Effluent	11.42	3.48	4.47	0.00	7.46	23.90	417	417

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

Table B.25. VFA column profile data for 7/18/97, high nitrate loading and normal hyrdaulic loading = 3.0 $m^3/m^2/hr$.

Date: 7/18/97 Column Port	Column Height (m)	Acetic acid (mg/L as COD)	Propionic acid (mg/L as COD)	Isobutyric acid (mg/L as COD)	*n*-Butyric acid (mg/L as COD)	2-Methylbutyric acid (mg/L as COD)	3-Methylbutyric acid (mg/L as COD)	*n*-Valeric acid (mg/L as COD)
Influent	0.00	294	313	33.1	52.5	23.3	36.9	25.9
Port 1	0.89	270	286	31.7	44.8	22.4	33.1	26.2
Port 2	1.37	248	230	32.7	32.5	19.7	30.5	24.2
Port 3	1.85	224	168	30.8	17.7	14.0	26.6	10.1
Port 4	2.34	208	136	29.2	10.3	9.62	24.8	0.0
Port 5	2.82	196	107	27.9	4.28	8.01	23.4	0.0
Port 6	3.30	201	103	27.8	3.79	6.84	21.6	0.0
Effluent	3.48	213	108	28.7	3.27	5.99	24.7	0.0

Table B.26. Anion and COD column profile data for 7/24/97, high nitrate loading and normal hyrdaulic loading = 3.0 $m^3/m^2/hr$.

Date: 7/24/97 Column Port	Column Height (ft)	Column Height (m)	NO_3^- (mg/L as N)	NO_2^- (mg/L as N)	SO_4^{2-} (mg/L)	Cl^- (mg/L)	COD (mg/L)	COD Corrected for Nitrite Interference
NO_3 stock								
Influent	0.00	0.00	90.8	0.12	10.1	20.1	584	584
Port 1	2.92	0.89	79.2	2.79	10.1	20.6	505	504
Port 2	4.50	1.37	25.1	3.63	14.5	20.8	297	296
Port 3	6.08	1.85	6.34	1.18	15.2	18.4	223	223
Port 4	7.67	2.34	0.86	0.67	8.84	17.8	200	200
Port 5	9.25	2.82	0.69	0.00	7.91	19.3	184	184
Port 6	10.83	3.30	3.86	0.00	10.3	20.8	184	184
Effluent	11.42	3.48	2.49	0.00	7.87	20.5	188	188

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

Table B.27. VFA column profile data for 7/24/97, high nitrate loading and normal hyrdaulic loading = 3.0 $m^3/m^2/hr$.

Date: 7/24/97 Column Port	Column Height (m)	Acetic acid (mg/L as COD)	Propionic acid (mg/L as COD)	Isobutyric acid (mg/L as COD)	*n* -Butyric acid (mg/L as COD)	2-Methylbutyric acid (mg/L as COD)	3-Methylbutyric acid (mg/L as COD)	*n* -Valeric acid (mg/L as COD)
Influent	0	209	243	22.8	36.8	14.7	23.4	0.00
Port 1	0.89	168	189	18.4	27.5	10.6	0.00	0.00
Port 2	1.37	91.2	89.4	15.1	10.1	0.00	0.00	0.00
Port 3	1.85	56.1	43.2	14.0	0.00	4.21	3.55	0.00
Port 4	2.34	52.1	31.2	13.4	0.00	0.00	0.00	0.00
Port 5	2.82	51.3	25.4	12.4	0.00	0.00	0.00	0.00
Port 6	3.30	52.1	25.8	12.5	0.00	0.00	0.00	0.00
Effluent	3.48	50.3	24.2	12.1	0.00	0.00	0.00	0.00

Table B.28. Anion and COD column profile data for 7/27/97, high nitrate loading and normal hyrdaulic loading = 3.0 $m^3/m^2/hr$.

Date: 7/27/97 Column Port	Column Height (m)	Column Height (m)	NO_3^- (mg/L as N)	NO_2^- (mg/L as N)	SO_4^{2-} (mg/L)	Cl^- (mg/L)	COD (mg/L)	COD Corrected for Nitrite Interference*
NO_3 stock								
Influent	0.00	0.00	81.77	0.91	6.51	22.2	679	679
Port 1	2.92	0.89	73.68	1.88	7.38	20.5	639	639
Port 2	4.50	1.37	30.18	5.11	7.83	20.0	482	480
Port 3	6.08	1.85	2.55	1.73	7.22	19.1	385	385
Port 4	7.67	2.34	0.76	1.13	7.02	18.5	358	358
Port 5	9.25	2.82	0.68	0.00	6.93	20.0	336	336
Port 6	10.83	3.30	0.74	0.00	7.31	21.3	458	458
Effluent	11.42	3.48	0.75	0.96	8.52	19.4	353	353

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

Table B.29. VFA column profile data for 7/27/97, high nitrate loading and normal hyrdaulic loading = 3.0 $m^3/m^2/hr$.

Date: 7/27/97 Column Port	Column Height (m)	Acetic acid (mg/L as COD)	Propionic acid (mg/L as COD)	Isobutyric acid (mg/L as COD)	*n*-Butyric acid (mg/L as COD)	2-Methylbutyric acid (mg/L as COD)	3-Methylbutyric acid (mg/L as COD)	*n*-Valeric acid (mg/L as COD)
Influent	0.00	208	245	26.4	42.5	20.3	31.3	24.2
Port 1	0.89	211	223	24.6	34.6	20.1	28.4	22.2
Port 2	1.37	180	151	22.9	20.6	15.9	23.1	12.5
Port 3	1.85	142	96.0	21.2	5.49	9.31	19.1	0.00
Port 4	2.34	151	82.4	21.4	3.22	7.92	19.4	0.00
Port 5	2.82	149	80.9	21.5	3.09	7.31	18.9	0.00
Port 6	3.30	144	75.9	19.9	3.22	7.98	16.9	0.00
Effluent	3.48	202	144	26.9	13.0	13.3	25.2	0.00

Table B.30. Anion and COD column profile data for 7/28/97, high nitrate loading and normal hyrdaulic loading = 3.0 $m^3/m^2/hr$.

Date: 7/28/97 Column Port	Column Height (ft)	Column Height (m)	NO_3^- (mg/L as N)	NO_2^- (mg/L as N)	SO_4^{2-} (mg/L)	Cl^- (mg/L)	COD (mg/L)	COD Corrected for Nitrite Interference*
NO_3 stock			103	-0.17	8.70	15.9		
Influent	0.00	0.00	82.5	0.29	6.72	18.7	389	389
Port 1	2.92	0.89	78.1	4.37	6.93	16.9	346	345
Port 2	4.50	1.37	25.7	10.3	6.05	19.5	220	218
Port 3	6.08	1.85	2.32	3.96	7.19	18.0	88	87
Port 4	7.67	2.34	0.91	0.54	7.03	18.5	69	69
Port 5	9.25	2.82	0.74	0.40	7.27	18.7	62	62
Port 6	10.83	3.30	0.71	0.00	8.37	21.0	51	51
Effluent	11.42	3.48	0.73	0.45	7.47	19.0	60	60

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

Table B.31. VFA column profile data for 7/28/97, high nitrate loading and normal hyrdaulic loading = 3.0 $m^3/m^2/hr$.

Date: 7/28/97 Column Port	Column Height (m)	Acetic acid (mg/L as COD)	Propionic acid (mg/L as COD)	Isobutyric acid (mg/L as COD)	*n* -Butyric acid (mg/L as COD)	2-Methylbutyric acid (mg/L as COD)	3-Methylbutyric acid (mg/L as COD)	*n* -Valeric acid (mg/L as COD)
Influent	0.00	140	156	13.3	16.5	9.50	13.1	0.00
Port 1	0.89	125	143	13.0	16.0	9.04	11.5	0.00
Port 2	1.37	71.4	60.9	8.44	5.07	4.16	6.38	0.00
Port 3	1.85	11.6	10.6	3.89	0.00	0.00	0.00	0.00
Port 4	2.34	4.03	3.09	1.98	0.00	0.00	0.00	0.00
Port 5	2.82	0.36	0.00	0.00	0.00	0.00	0.00	0.00
Port 6	3.30	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Effluent	3.48	0.79	0.00	0.00	0.00	0.00	0.00	0.00

Table B.32. Anion and COD column profile data for 6/3/97, high nitrate loading and low hyrdaulic loading = 1.5 $m^3/m^2/hr$.

Date: 6/3/97 Column Port	Column Height (ft)	Column Height (m)	NO_3^- (mg/L as N)	NO_2^- (mg/L as N)	SO_4^{2-} (mg/L)	Cl^- (mg/L)	COD (mg/L)	COD Corrected for Nitrite Interference*
NO_3 stock			118	0.00	16.4	14.8		
Influent	0.00	0.00	76.5	8.13	6.81	16.9	439	437
Port 1	2.92	0.89	70.9	6.46	6.92	17.3	351	350
Port 2	4.50	1.37	8.01	26.01	9.07	18.1	181	174
Port 3	6.08	1.85	0.58	5.92	8.47	17.9	147	145
Port 4	7.67	2.34	0.50	0.00	Problem	19.0	148	148
Port 5	9.25	2.82	0.58	0.00	7.82	19.8	88	88
Port 6	10.83	3.30	0.54	0.00	7.37	20.1	114	114
Effluent	11.42	3.48	0.52	0.00	7.27	18.4	118	118

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

Table B.33. Anion and COD column profile data for 6/14/97, high nitrate loading and normal hyrdaulic loading = 1.5 $m^3/m^2/hr$.

Date: 6/14/97 Column Port	Column Height (m)	Column Height (m)	NO_3^- (mg/L as N)	NO_2^- (mg/L as N)	SO_4^{2-} (mg/L)	Cl^- (mg/L)	COD (mg/L)	COD Corrected for Nitrite Interference*
NO_3 stock								
Influent	0.00	0.00	60.9	19.4	5.48	16.8	331	326
Port 1	2.92	0.89	72.7	13.7	22.6	17.4	302	299
Port 2	4.50	1.37	7.09	28.6	6.82	17.6	123	116
Port 3	6.08	1.85	1.14	16.7	7.96	18.8	75	71
Port 4	7.67	2.34	0.45	0.00	7.06	18.7	56	56
Port 5	9.25	2.82	0.48	0.00	7.03	18.4	56	56
Port 6	10.83	3.30	0.73	0.00	7.10	19.1	53	53
Effluent	11.42	3.48	0.53	0.00	7.67	18.4	53	53

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

Table B.34. Anion and COD column profile data for 6/16/97, high nitrate loading and low hyrdaulic loading = 1.5 $m^3/m^2/hr$.

Date: 6/16/97 Column Port	Column Height (ft)	Column Height (m)	NO_3^- (mg/L as N)	NO_2^- (mg/L as N)	SO_4^{2-} (mg/L)	Cl^- (mg/L)	COD (mg/L)	COD Corrected for Nitrite Interference*
NO_3 stock								
Influent	0.00	0.00	79.0	8.9	5.94	29.0	315	313
Port 1	2.92	0.89	71.7	15.2	6.02	19.6	293	290
Port 2	4.50	1.37					118	118
Port 3	6.08	1.85	0.00	35.4	6.49	17.6	64	56
Port 4	7.67	2.34	0.12	25.3	7.38	17.3	49	43
Port 5	9.25	2.82	0.09	24.8	7.47	18.0	40	35
Port 6	10.83	3.30	0.00	21.9	7.39	17.9	38	33
Effluent	11.42	3.48	0.00	21.6	8.41	18.0	36	31

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

Table B.35. Column effluent suspended solids during low nitrate loading phase.

Low nitrate loading phase			
Date	Day of Study	TSS (mg/L)	VSS (mg/L)
11/10/96	86	7.00	6.50
11/11/96	87	9.50	31.5
12/11/96	117	14.5	14.5
12/18/96	124	8.00	8.00
1/18/97	155	46.5	38.0
1/20/97	157	20.7	18.0
1/30/97	167	9.00	9.00
2/3/97	171	13.0	12.5
2/8/97	176	11.0	9.50
2/11/97	179	6.50	1.00
2/18/97	186	6.00	6.00
2/21/97	189	5.00	3.00
2/25/97	193	16.0	13.5
2/28/97	196	18.5	18.5

Table B.36. Column effluent suspended solids during high nitrate loading phase.

High nitrate loading phase			
Date	Day of Study	TSS (mg/L)	VSS (mg/L)
3/12/97	208	15.0	11.5
3/14/97	210	22.5	22.5
3/17/97	213	54.5	54.5
3/21/97	217	115	110
4/2/97	229	55.5	54.5
4/6/97	233	26.5	26.0
4/14/97	241	65.5	62.5
4/23/97	250	51.5	49.0
5/14/97	271	28.0	24.5
5/21/97	278	49.0	31.0
5/27/97	284	55.5	43.5
7/7/97	325	50.0	38.5
7/11/97	329	180	170

Table B.37. Column backwash suspended solids during low nitrate loading phase.

Low nitrate loading phase				
Date	Day of Study	TSS (mg/L)	VSS (mg/L)	Backwash Volume (L)
10/11/96	56	1390	1320	-
10/20/96	65	1185	1050	45
10/27/96	72	935	815	51.5
11/2/96	78	1130	1045	45
11/3/96	79	1160	1030	45
12/14/96	120	628	580	45
12/15/96	121	634	580	45
12/22/96	128	286	254	45
12/29/96	135	650	614	45
1/5/97	142	1045	1020	45
1/18/97	155	1000	900	45
1/28/97	165	685	615	45
1/29/97	166	745	665	45
2/8/97	176	900	772	45
2/9/97	177	1430	1335	45
2/18/97	186	1000	990	45
2/19/97	187	935	885	45
2/20/97	188	1380	1285	45
2/26/97	194	1100	1005	45
2/27/97	195	1190	1110	45

Table B.38. Column backwash suspended solids during high nitrate loading phase.

High nitrate loading phase				
Date	Day of Study	TSS (mg/L)	VSS (mg/L)	Backwash Volume (L)
3/16/97	212	1015	920	45
4/1/97	228	1505	1385	45
4/21/97	248	1280	1190	45
5/31/97	288	1190	1150	45
6/3/97	291	1505	1335	45
6/10/97	298	830	715	45
7/5/97	323	3100	2830	45

Table B.39. Ammonia concentrations for column samples and fish waste fermentation batch reactor.

Condition	Date	Column Influent NH_3 (mg/L as N)	Column Effluent NH_3 (mg/L as N)	Consumption NH_3 (mg/L as N)	Batch Reactor NH_3 (mg/L as N)
Low nitrate loading	2/19/97	13.9	7.98	5.90	
	2/20/97	13.4	8.09	5.32	
	2/21/97	10.4	4.86	5.55	
High nitrate loading	4/30/97	25.7	14.2	11.6	
	7/27/97	31.4	28.7	2.69	
	7/28/97	26.0	13.0	13.0	
Fish waste batch study	4/30/97				29.0

Table B.40. Observed biomass yield test performed 11/2 through 11/3/96 during low nitrate loading phase.

Date	Time (hr)	Column Sample	COD (mg/L)	COD Consumed (mg/L)	Backwash TSS (mg/L)	Backwash VSS (mg/L)	Backwash Volume (L)
11/2/96	0.00	Influent	38.6	-17.3	1130	1045	45
		Effluent	55.9				
	2.19	Influent	133	104			
		Effluent	29.1				
	5.94	Influent	113				
11/3/96	22.8	Influent	93.6	58.2			
		Effluent	35.4				
	24.0	Influent	51.1	22.0	1160	1030	45
		Effluent	29.1				
			Average =	61.4			
			Standard deviation =	41.0			

Table B.41. Observed biomass yield test performed 1/28 through 1/29/97 during low nitrate loading phase.

Date	Time (hr)	Effluent NO_2^- (mg/L as N)	Cabon line COD (mg/L)	Influent COD (mg/L)	Effluent COD (mg/L)	Effluent COD (mg/L)*	Consumed COD (mg/L)	Influent pH	Effluent pH	Backwash TSS (mg/L)	Backwash VSS (mg/L)	Backwash Volume (L)
1/28/97	0.00									685	615	45
	1.65	9.72	1518	275	17	15	260	7.34	8.63			
	2.83	10.1	1502	256	19	17	239	7.42	8.83			
	3.67	9.52	1462	226	14	12	214	7.08	8.73			
	4.75	8.60	1311	242	19	17	225	7.12	8.95			
	8.75	3.58	1661	202	24	23	179	7.13	9.06			
1/29/97	20.6	10.6	1669	285	54	52	233	7.09	8.99			
	23.8	9.73	1677	265	62	60	206	7.05	8.77	745	665	45
* effluent COD corrected for nitrite interference						Average =	222					
corrected COD (mg/L) = measured COD (mg/L) - 0.2353*NO_2-N(mg/L)						Standard error =	9.81					

Table B.42. Calculations for observed biomass yield test performed 1/28 through 1/29/97 during low nitrate loading phase.

Observed Biomass Yield Calculations

Fermenter 2 (F2)			TSS	1.2	g COD / g dry weight
Fermenter volume consumed (L) =		179	VSS	1.42	g COD / g ash-free dry weight
NO_3 water volume consumed (L) =		1157			
Total Volume (L) =	1336				
Flow (L / day) =	1336				
Average TSS (mg/L) formed=		745			
Average VSS (mg/L) formed =		665			
Average TSS (mg/L as COD) formed =		894	Average TSS (mg as COD) formed =		40230
Average VSS (mg/L as COD) formed =		944	Average TVSS (mg as COD) formed =		42493
Yield = mg biomass COD formed / mg COD consumed					
Average COD consumed (mg/L) =		222	Yield =	0.136	Using TSS
Volume consumed (L) =		1336	Yield =	0.143	Using VSS
Total COD consumed (mg) =		296721			

Table B.43. Observed biomass yield test performed 2/8 through 2/9/97 during low nitrate loading phase.

Date	Time (hr)	Effluent NO_2^- (mg/L as N)	Cabon line COD (mg/L)	Influent COD (mg/L)	Effluent COD (mg/L)	Effluent COD (mg/L)*	COD Consumed (mg/L)	Backwash TSS (mg/L)	Backwash VSS (mg/L)	Backwash Volume (L)
2/8/97	0.00							900	772	45
	1.65	0.00	923	130	11	11	119			
	5.53	0.00	904	121	9	9	111			
	9.75	0.00	1004	127	11	11	116			
2/9/97	24.0	0.00	1192	152	8	8	144	1430	1335	45
* effluent COD corrected for nitrite interference						Average =	123			
corrected COD (mg/L) = measured COD (mg/L) - 0.2353*NO_2-N(mg/L)						Standard error =	7.4			

Table B.44. Calculations for observed biomass yield test performed 2/8 through 2/9/97 during low nitrate loading phase.

Observed Biomass Yield Calculations					
Fermenter 1 (F1)			TSS	1.2 g COD / g dry weight	
Fermenter volume consumed (L) =	169		TVSS	1.42 g COD / g ash-free dry weight	
NO_3 water volume consumed (L) =	1160				
Total Volume (L) =	1329				
Flow (L / day) =	1329				
Average TSS (mg/L) formed=	1430				
Average VSS (mg/L) formed =	1335				
Average TSS (mg/L as COD) formed =	1716			Average TSS (mg as COD) formed =	77220
Average VSS (mg/L as COD) formed =	1896			Average VSS (mg as COD) formed =	85307
Yield = mg biomass COD formed / mg COD consumed					
Average COD consumed (mg/L) =	123	Yield =	0.473	Using TSS	
Volume consumed (L) =	1329	Yield =	0.523	Using VSS	
Total COD consumed (mg) =	163128				

Table B.45. Observed biomass yield test performed 2/18 through 2/19/97 during low nitrate loading phase.

Date	Time (hr)	Effluent NO_2^- (mg/L as N)	Influent COD (mg/L)	Effluent COD (mg/L)	Effluent COD (mg/L)*	COD Consumed (mg/L)	Influent pH	Effluent pH	Backwash TSS (mg/L)	Backwash VSS (mg/L)	Backwash Volume (L)
2/18/97	0.00								1000	990	45
	0.97	10.5	156	31	29	125	7.60	8.50			
	10.45	11.8	159	22	19	138	7.70	8.70			
2/19/97	22.75	0.0	234	32	32	202	-	8.90			
	24.00	0.0	184	23	23	161	7.60	8.90	935	885	45
* effluent COD corrected for nitrite interference						156	(Average)				
corrected COD (mg/L) = measured COD (mg/L) - 0.2353*NO_2-N(mg/L)						16.9	(Standard error)				

Table B.46. Calculations for observed biomass yield test performed 2/18 through 2/19/97 during low nitrate loading phase.

Observed Biomass Yield Calculations

Fermenter 1 (F1)		TSS	1.2 g COD / g dry weight	
Fermenter volume consumed (L) =	174	TVSS	1.42 g COD / g ash-free dry weight	
NO_3 water volume consumed (L) =	1188			
Total Volume (L) =	1362			
Flow (L / day) =	1362			
Average TSS (mg/L) formed=	935			
Average VSS (mg/L) formed =	885			
Average TSS (mg/L as COD) formed =	1122	Average TSS (mg as COD) formed =		50490
Average VSS (mg/L as COD) formed =	1257	Average VSS (mg as COD) formed =		56552
Yield = mg biomass COD formed / mg COD consumed				
Average COD consumed (mg/L) =	156	Yield =	0.237 Using TSS	
Volume consumed (L) =	1362	Yield =	0.265 Using VSS	
Total COD consumed (mg) =	213064			

Table B.47. Observed biomass yield test performed 2/19 through 2/20/97 during low nitrate loading phase.

Date	Time (hr)	Effluent NO_2^- (mg/L as N)	Influent COD (mg/L)	Effluent COD (mg/L)	Effluent COD (mg/L)*	COD Consumed (mg/L)	Influent pH	Effluent pH	Backwash TSS (mg/L)	Backwash VSS (mg/L)	Backwash Volume (L)
2/19/97	0.00								935	885	45
	1.58	11.6	155	26.6	23.9	131	7.7	8.5			
	10.1	11.7	213	25.0	22.3	190	7.5	8.7			
2/20/97	19.8	0	277	25.0	25.0	252	7.3	8.8			
	24.0		184	32.8	32.8	152	7.3	8.7	1380	1285	51.4
* effluent COD corrected for nitrite interference						181	(Average)				
corrected COD (mg/L) = measured COD (mg/L) - 0.2353*NO_2-N(mg/L)						26.5	(Standard error)				

Table B.48. Calculations for observed biomass yield test performed 2/19 through 2/20/97 during low nitrate loading phase.

Observed Biomass Yield Calculations

Fermenter 2 (F2)		TSS	1.2 g COD / g dry weight		
Fermenter volume consumed (L) =	168	TVSS	1.42 g COD / g ash-free dry weight		
NO_3 water volume consumed (L) =	1145				
Total Volume (L) =	1313				
Flow (L / day) =	1313				
Average TSS (mg/L) formed=	1380				
Average VSS (mg/L) formed =	1285				
Average TSS (mg/L as COD) formed =	1656	Average TSS (mg as COD) formed =		85118	
Average VSS (mg/L as COD) formed =	1825	Average VSS (mg as COD) formed =		93790	
Yield = mg biomass COD formed / mg COD consumed					
Average COD consumed (mg/L) =	181	Yield =	0.358	Using TSS	
Volume consumed (L) =	1313	Yield =	0.394	Using VSS	
Total COD consumed (mg) =	237890				

Table B.49. Observed biomass yield test performed 2/26 through 2/27/97 during low nitrate loading phase.

Date	Time (hr)	Effluent NO_2^- (mg/L as N)	Influent COD (mg/L)	Effluent COD (mg/L)	Effluent COD (mg/L)*	COD Consumed (mg/L)	Influent pH	Effluent pH	Backwash TSS (mg/L)	Backwash VSS (mg/L)	Backwash Volume (L)
2/26/97	0.00								1100	1005	45
	2.07	18.3	136	20.3	16.0	120	7.6	8.3			
	7.95		150	18.8	18.8	131	7.5	8.4			
2/27/97	21.8	2.68	169	25.0	24.4	144	7.5	8.9			
	24.0	0.00	166	28.1	28.1	138	7.5	9	1190	1110	45
* effluent COD corrected for nitrite interference						133	(Average)				
corrected COD (mg/L) = measured COD (mg/L) - 0.2353*NO_2-N(mg/L)						5.2	(Standard error)				

Table B.50. Calculations for observed biomass yield test performed 2/19 through 2/20/97 during low nitrate loading phase.

Observed Biomass Yield Calculations

Fermenter 1 (F1)		TSS	1.2 g COD / g dry weight		
Fermenter volume consumed (L) =	162	TVSS	1.42 g COD / g ash-free dry weight		
NO_3 water volume consumed (L) =	1059				
Total Volume (L) =	1221				
Flow (L / day) =	1221				
Average TSS (mg/L) formed=	1190				
Average VSS (mg/L) formed =	1110				
Average TSS (mg/L as COD) formed =	1428	Average TSS (mg as COD) formed =		64260	
Average VSS (mg/L as COD) formed =	1576	Average VSS (mg as COD) formed =		70929	
Yield = mg biomass COD formed / mg COD consumed					
Average COD consumed (mg/L) =	133	Yield =	0.395	Using TSS	
Volume consumed (L) =	1221	Yield =	0.436	Using VSS	
Total COD consumed (mg) =	162825				

Table B.51. Soluble COD production from the pilot-scale fermenters during the low nitrate loading

Fermenter 1(F1)			Fermenter 2 (F2)		
Date	Day of Study*	COD (mg/L)	Date	Day of Study	COD (mg/L)
8/14/96	-2	1091	11/25/96	101	1045
8/15/96	-1	1636	11/26/96	102	213
8/22/96	6	1636	11/27/96	103	213
8/25/96	9	1851	12/1/96	107	73.9
8/26/96	10	1461	12/3/96	109	57.2
8/29/96	13	1543	12/5/96	111	40.3
8/30/96	14	2010	12/7/96	113	24.6
9/3/96	18	1519	12/15/96	121	691
9/10/96	25	2922	12/19/96	125	604
9/12/96	27	2143	12/27/96	133	469
9/14/96	29	1465	12/29/96	135	501
9/30/96	45	1418	1/16/97	153	679
10/7/96	52	1590	1/22/97	159	1587
10/8/96	53	1245	2/3/97	171	1800
10/9/96	54	1330	2/17/97	185	1762
10/11/96	56	1249	2/21/97	189	1080
10/13/96	58	1240	2/23/97	191	1034
10/14/96	59	1291			
10/15/96	60	1412			
10/20/96	65	1272			
10/23/96	68	1232			
10/26/96	71	1017			
10/29/96	74	1319			
11/10/96	86	1041			
11/18/96	94	1057			
11/23/96	99	1359			
11/25/96	101	1065			
11/26/96	102	948			
11/27/96	103	213			
12/3/96	109	40			
12/4/96	110	615			
12/10/96	116	911			
12/12/96	118	891			
12/14/96	120	741			
12/20/96	126	1003			
1/5/97	142	913			
1/19/97	156	738			
2/8/97	176	1081			
2/14/97	182	1018			
2/16/97	184	1374			

* Negative numbers resulted because the day of study was based on column start-up and fermenter 1 was placed into operation prior to this date.

Table B.52. Soluble COD production from the pilot-scale fermenters during the high nitrate loadi

Fermenter 1 (F1)			Fermenter 2 (F2)		
Date	Day of Study	COD (mg/L)	Date	Day of Study	COD (mg/L)
3/12/97	208	2155	3/13/97	209	2571
3/18/97	214	2564	3/17/97	213	2345
3/22/97	218	2338	3/21/97	217	2306
3/30/97	226	1359	3/27/97	223	2408
4/1/97	228	2189	3/29/97	225	2151
4/5/97	232	2345	4/2/97	229	2048
4/9/97	236	2059	4/6/97	233	2004
4/15/97	242	1485	4/20/97	247	1964
4/29/97	256	2158	5/8/97	265	1650
5/13/97	270	1149	5/14/97	271	1909
5/21/97	278	2064	5/22/97	279	2517
5/27/97	284	2352	5/28/97	285	2689
6/10/97	298	1830	6/5/97	293	2067
6/11/97	299	1861	6/10/97	298	1830
6/24/97	312	4119	6/11/97	299	1861
7/2/97	320	4293	6/23/97	311	4212
7/24/97	342	2883	7/7/97	325	4071
			7/27/97	345	2519

Table B.53. Suspended solids measured in the pilot-scale fermenters.

Low nitrate loading phase					
Date	Day of Study	TSS (mg/L)	VSS (mg/L)	Source	Fermenter
11/24/96	100	1007.5	910	Fish waste & food	F1
12/20/96	126	1450	1340	Fish waste & food	F1
2/17/97	185	1060	925	Fish food	F1
11/24/96	100	420	360	Fish waste	F2
12/3/96	109	537.5	432	Fish waste	F2
12/8/96	114	1380	1055	Fish waste	F2
12/19/96	125	1790	1480	Fish waste	F2
12/29/96	135	1305	1135	Fish waste	F2
2/3/97	171	1530	1400	Fish food	F2
High nitrate loading phase					
Date	Day of Study	TSS (mg/L)	VSS (mg/L)	Source	Fermenter
3/12/97	208	3100	2535	Fish food	F1
3/18/97	214	3285	2725	Fish food	F1
3/22/97	218	4080	3270	Fish food	F1
3/30/97	226	3150	2530	Fish food	F1
4/5/97	232	2530	2090	Fish food	F1
4/9/97	236	2660	2110	Fish food	F1
4/19/97	246	4000	3110	Fish food	F1
5/13/97	270	4340	3770	Fish food	F1
5/21/97	278	4020	3260	Fish food	F1
7/2/97	320	8190	7220	Fish food	F1
3/13/97	209	3060	2570	Fish food	F2
3/17/97	213	3240	2730	Fish food	F2
3/21/97	217	4230	3380	Fish food	F2
3/29/97	225	2870	2370	Fish food	F2
4/2/97	229	2610	2100	Fish food	F2
4/6/97	233	2420	2050	Fish food	F2
4/10/97	237	2760	2260	Fish food	F2
4/20/97	247	3090	2560	Fish food	F2
5/14/97	271	4410	3640	Fish food	F2
7/1/97	319	9550	8400	Fish food	F2
7/7/97	325	11300	9930	Fish food	F2

Table B.54. Logsheet for the batch-scale fermentation of fish waste in a 2 L gla

Date	Sample Time	pH	$NaHCO_3$ added (g)	Temperature ^oC
3/20/97	-			
3/21/97	8:00 AM	6.8	1.5	-
3/22/97	9:15 AM	6.8	3.5	-
3/23/97	8:12 AM	7.1		22
3/24/97	8:45 AM	7		-
3/25/97	12:16 PM	7		-
3/26/97	8:51 AM	6.9		-
3/27/97		-		-
3/28/97		-		-
3/29/97	9:30 AM	6.9	3	24.2
3/30/97	9:30 AM	7.2		-
3/31/97	10:10 AM	7		22
4/1/97	10:30 AM	6.7		23
4/2/97	9:58 AM	6.8	3	21.8
4/3/97	8:36 AM	8.1		24.8
4/4/97				
4/5/97	10:10 AM	6.9		25.3
4/6/97	10:00 AM	7		23.2
4/7/97	9:43 AM	-	3	25.3
4/8/97	10:10 AM	-		21.7
4/9/97	10:30 AM	-		24
4/10/97	10:15 AM	6.6	3	21.3
4/11/97	-	-		-
4/12/97	9:15 AM	-	3	23.9
4/13/97	10:05 AM	6.8	3	24.2
4/14/97	10:10 AM	7.2		22
4/15/97	10:10 AM	6.6	4	23.4
4/16/97	-			
4/17/97	-			
4/18/97	-			
4/19/97	9:45 AM	-		24
4/20/97	10:00 AM	6.6	7	-
4/21/97	10:05 AM	7.2		22.9
4/22/97	10:15 AM	7.2	7	22
4/23/97	10:15 AM	7.2		27.6
4/24/97	10:15 AM	7.2	7	23
4/25/97	10:00 AM	7.2		22.1
4/26/97	10:30 AM	7.4		22.5
4/27/97	9:50 AM	7		-
4/28/97	-	-		-
4/29/97	9:50 AM	7		-
4/30/97	9:30 AM	7		-
5/1/97	9:45 AM	6.8	5.3	23.9
5/2/97		6.7	7	25.3
5/3/97		7.2		24.4
5/4/97	8:30 AM			
5/5/97				
5/6/97				

Table B.55. Soluble COD and dissolved organic carbon produ
fish waste in a 2 L glass reactor.

Date	Day of Study	COD (mg/L)	DOC (mg/L)
3/21/97	1	210	64.0
3/22/97	2	350	126
3/23/97	3	545	169
3/24/97	4	623	192
3/25/97	5	528	133
3/26/97	6	536	222
3/27/97	7	575	178
3/29/97	9	485	124
3/30/97	10	536	192
4/1/97	13	849	239
4/2/97	14	636	224
4/3/97	16	608	228
4/5/97	17	612	229
4/6/97	18	407	155
4/7/97	19	521	208
4/8/97	20	651	241
4/9/97	21	536	199
4/10/97	23	437	160
4/12/97	24	505	185
4/13/97	25	596	214
4/14/97	26	691	209
4/15/97	30	604	224
4/19/97	31	365	141
4/20/97	32	353	136
4/21/97	33	509	172
4/22/97	34	184	68.1
4/23/97	35	262	101
4/24/97	36	225	84.9
4/25/97	37	264	101
4/26/97	40	246	96.1
4/29/97	42	353	132
5/1/97	44	277	97.2
5/3/97	45	294	104
5/4/97	46	353	122
5/5/97	47	335	126
5/6/97	48	205	77.9

Table. B.57. VFA production from the batch-scale fermentation of fish waste in a 2 L glass reactor.

Date	Calculated COD (mg/L)	Acetic mg/L as COD	Acetic % of calc. COD	Propionic mg/L as COD	Propionic % of calc. COD	Isobutyric mg/L as COD	Isobutyric % of calc. COD	*n*-Butyric mg/L as COD	*n*-Butyric % of calc. COD	2-methylbutyric mg/L as COD	2-methylbutyric % of calc. COD	3-methylbutyric mg/L as COD	3-methylbutyric % of calc. COD	*n*-valeric mg/L as COD	*n*-valeric % of calc. COD
3/21/97	61.5	35.4	57.6	26.4	42.9	-0.3	-0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3/22/97	337	138	40.8	144	42.6	7.3	2.2	19.1	5.7	6.1	1.8	13.3	3.9	10.0	3.0
3/24/97	348	148	42.6	138	39.8	14.4	4.1	17.6	5.1	12.2	3.5	17.1	4.9	0.0	0.0
3/25/97	390	178	45.6	147	37.6	18.2	4.7	13.1	3.3	15.7	4.0	18.6	4.8	0.0	0.0
3/26/97	555	254	45.8	193	34.9	27.3	4.9	25.4	4.6	22.7	4.1	32.1	5.8	0.0	0.0
3/27/97	442	237	53.7	119	26.9	25.9	5.9	5.6	1.3	22.8	5.2	31.2	7.1	0.0	0.0
3/29/97	278	148	53.1	80.3	28.9	12.5	4.5	13.6	4.9	10.3	3.7	14.0	5.0	0.0	0.0
3/30/97	472	235	49.7	149	31.5	22.0	4.7	26.7	5.6	18.3	3.9	21.6	4.6	0.0	0.0
4/1/97	471	228	48.3	166	35.2	17.8	3.8	26.8	5.7	15.0	3.2	18.2	3.9	0.0	0.0
4/2/97	481	236	49.1	162	33.8	20.7	4.3	24.0	5.0	15.0	3.1	22.6	4.7	0.0	0.0
4/3/97	451	269	59.7	112	24.7	21.5	4.8	12.2	2.7	12.0	2.7	24.3	5.4	0.0	0.0
4/5/97	421	224	53.2	128	30.4	22.3	5.3	10.3	2.4	12.4	3.0	24.0	5.7	0.0	0.0
4/6/97	261	151	57.9	70.1	26.8	12.6	4.8	5.3	2.0	7.4	2.8	14.8	5.7	0.0	0.0
4/7/97	175	27	15.2	112	63.9	17.9	10.2	0.0	0.0	10.1	5.7	8.6	4.9	0.0	0.0
4/8/97	544	276	50.7	182	33.4	23.5	4.3	17.1	3.1	17.9	3.3	27.8	5.1	0.0	0.0
4/9/97	348	156	44.9	142	40.8	17.5	5.0	6.8	1.9	12.2	3.5	13.5	3.9	0.0	0.0
4/10/97	157	25	15.8	97.1	61.8	15.2	9.7	0.9	0.6	11.8	7.5	7.1	4.5	0.0	0.0
4/12/97	392	186	47.4	142	36.2	18.6	4.7	14.5	3.7	11.6	3.0	19.8	5.1	0.0	0.0
4/14/97	499	237	47.5	176	35.2	27.2	5.5	15.7	3.2	16.6	3.3	26.9	5.4	0.0	0.0
4/15/97	520	249	47.9	179	34.4	23.7	4.6	24.7	4.7	17.9	3.4	25.3	4.9	0.0	0.0
4/19/97	354	186	52.6	108	30.5	18.8	5.3	8.6	2.4	8.8	2.5	23.7	6.7	0.0	0.0
4/20/97	264	139	52.7	79.7	30.2	16.0	6.0	4.6	1.7	6.2	2.4	18.6	7.0	0.0	0.0
4/21/97	308	169	54.9	86.7	28.2	15.9	5.2	5.5	1.8	9.4	3.0	21.1	6.9	0.0	0.0
4/22/97	184	100	54.4	50.5	27.5	10.7	5.8	2.2	1.2	6.6	3.6	13.9	7.5	0.0	0.0
4/25/97	228	129	56.6	60.9	26.8	12.0	5.3	3.2	1.4	7.7	3.4	15.0	6.6	0.0	0.0
4/26/97	170	98.8	58.0	42.4	24.9	8.6	5.0	2.2	1.3	6.2	3.6	12.3	7.2	0.0	0.0
4/29/97	194	110	56.9	56.2	29.0	6.5	3.3	4.4	2.3	4.8	2.5	11.7	6.0	0.0	0.0
5/3/97	93.2	30.2	32.4	51.7	55.5	8.8	9.5	2.5	2.6	0.0	0.0	0.0	0.0	0.0	0.0
5/5/97	226	144	63.9	50.5	22.3	11.5	5.1	4.2	1.8	5.1	2.2	10.4	4.6	0.0	0.0
Average		164	48.6	112	35.0	16.4	5.1	10.9	2.8	11.1	3.2	17.5	5.1	0.3	0.1
Standard deviation		74.7	11.2	48.4	10.3	6.7	2.0	8.7	1.7	5.9	1.4	8.1	1.8	1.9	0.6
Standard error		13.9	2.1	9.0	1.9	1.2	0.4	1.6	0.3	1.1	0.3	1.5	0.3	0.3	0.1

Table B.58. COD and VFA production from the batch-scale fermentation of fish
VFA concentrations provided as COD were based on seven VFA constituents ar
by converting measured VFA concentrations to COD values using theoretical ox

Date	Day of Study	Measured COD (mg/L)	VFA as COD (mg/L)	VFA % of Measured COD (%)
3/21/97	1	210	62	29.2
3/22/97	2	350	337	96.6
3/24/97	4	623	348	55.8
3/25/97	5	528	390	73.8
3/26/97	6	536	555	104
3/27/97	7	575	442	76.8
3/29/97	9	485	278	57.3
3/30/97	10	536	472	88.1
4/1/97	12	849	471	55.5
4/2/97	13	636	481	75.7
4/3/97	14	608	451	74.2
4/5/97	16	612	421	68.8
4/6/97	17	407	261	64.3
4/7/97	18	521	175	33.6
4/8/97	19	651	544	83.5
4/9/97	20	536	348	64.8
4/10/97	21	437	157	35.9
4/12/97	23	505	392	77.8
4/14/97	25	691	499	72.2
4/15/97	26	604	520	86.1
4/19/97	30	365	354	97.1
4/20/97	31	353	264	74.9
4/21/97	32	509	308	60.5
4/22/97	33	184	184	100
4/25/97	36	264	228	86.2
4/26/97	37	246	170	69.2
4/29/97	40	353	194	54.9
5/3/97	44	278	93	33.6
5/5/97	46	279	226	81.2
Average		473	332	70.0
Standard deviation		163	140	20.1
Standard error		30.2	26.0	3.72

Table B.57. Nitrate and nitrite batch kinetic tests conducted using backwash biomass collected from the denitrifying column (5/31/97, high nitrate loading, hydraulic loading = 1.5 $m^3/m^2/hr$ Sampling from the batch reactor was conducted from a submerged side port using a syringe.

100 mg NO_3-N/L						100 mg NO_2-N/L				
Time (hr)	Nitrate (mg/L as N)	Nitrite (mg/L as N)	TSS (mg/L)	VSS (mg/L)	pH	Time (hr)	Nitrite (mg/L as N)	TSS (mg/L)	VSS (mg/L)	pH
0.00	92.9	15.4	1190	1150	7.6	0.00	104	1100	1020	7.63
0.33	42.8	48.4				0.33	98.9			
0.67	24.1	47.7				0.67	78.6			
1.00	1.98	70.3				1.00	61.0			
1.50	0.00	47.0				1.50	39.6			
2.00	0.00	28.6				2.00	21.4			
2.50	0.00	0.00				2.50	0.00			
3.50	0.00	0.00				3.50	0.00			
4.50	0.00	0.00	870	810	8.46	4.50	0.00	770	780	8.57

Table B.58. Nitrate and nitrite batch kinetic tests conducted using backwash biomass collected from the denitrifying column (6/3/97, high nitrate loading, hydraulic loading = 1.5 $m^3/m^2/hr$). Samples were taken from the batch reactor by inserting a pipette.

100 mg NO_3-N/L						100 mg NO_2-N/L					
Time (hr)	Nitrate (mg/L as N)	Nitrite (mg/L as N)	TSS (mg/L)	VSS (mg/L)	pH	Time (hr)	Nitrite (mg/L as N)	DOC (mg/L)	TSS (mg/L)	VSS (mg/L)	pH
0.00	81.7	4.90	1505	1335		0.00	99.2	128	1550	1360	
0.33	94.6	22.3				0.33	79.2	116			
0.67	49.1	33.0				0.67	44.8	105			
1.00	23.2	39.4				1.00	42.4	97.0			
1.33	2.94	41.9				1.33	8.32	93.4			
1.67	2.18	35.0				1.67	7.36	96.7			
2.00	1.25	27.9				2.00	0.00	82.3			
2.33	1.17	24.4				2.33	0.00	85.7			
2.67	1.18	5.39				2.67	0.00	87.6			
3.00	1.04	0.00				3.00	0.00	95.6			
24.0	1.19	0.00	1145	990	8.02	24.0	0.00	189	1140	915	8.51

Table B.59. Nitrate and nitrite batch kinetic tests conducted using backwash biomass collected from the denitrifying column (6/10/97, high nitrate loading, hydraulic loading = 1.5 $m^3/m^2/hr$
Samples were taken from the batch reactor via a submerged side port sealed with a rubber stopper.

100 mg NO_3-N/L						100 mg NO_2-N/L				
Time (hr)	Nitrate (mg/L as N)	Nitrite (mg/L as N)	TSS (mg/L)	VSS (mg/L)	pH	Time (hr)	Nitrite (mg/L as N)	TSS (mg/L)	VSS (mg/L)	pH
0.02	95.2	0.38	830	715	7.65	0.02	100	870	770	7.63
0.33	93.7	14.1				0.33	102			
0.50	97.1	17.9				0.50	81.0			
0.75	68.2	21.4				0.75	88.4			
1.00	54.0	20.8				1.00	87.5			
1.25	44.3	26.2				1.25	79.6			
1.50	40.3	40.7				1.50	75.0			
1.75	22.4	53.3				1.75	82.8			
2.00	12.9	39.7				2.00	70.3			
2.50	5.02	49.0				2.50	56.8			
3.00	1.71	44.2				3.00	62.4			
3.50	1.63	43.4	795	730		3.50	36.5	775	635	

Table B.60. Nitrate and nitrite batch kinetic tests conducted using backwash biomass collected from the denitrifying column (6/27/97, high nitrate loading, hydraulic loading = 3.0 $m^3/m^2/hr$). Samples were taken from the batch reactor via a submerged side port sealed with a rubber stopper.

100 mg NO_3-N/L						100 mg NO_2-N/L				
Time (hr)	Nitrate (mg/L as N)	Nitrite (mg/L as N)	Sulfate (mg/L as N)	COD (mg/L)	COD corrected for nitrite interference*	Time (hr)	Nitrite (mg/L as N)	Sulfate (mg/L as N)	COD (mg/L)	COD corrected for nitrite interference*
0.01	96.2	1.38	12.3	572	572	0.01	99	10.1	576	553
0.25	71.7	8.8	12.4	486	483	0.25	74	11.3	529	511
0.50	52.0	17.9	12.7	430	426	0.50	59.6	10.8	466	452
0.75	17.5	25.1	11.1	292	286	0.75	26.6	9.94	412	405
1.00	3.4	20.8	11.4	241	236	1.00	5.1	9.65	395	394
1.25	0.9	0.0	12.8	249	249	1.25	0.0	10.0	403	403
1.50	0.9	0.0	10.4	276	276	1.50	0.0	8.88	391	391
1.75	-	-	-			1.75	0.0	9.16	411	411
3.00	0.87	0.0	12.1	233	233	3.00	0.0	10.5	407	407

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

Test	Time (hr)	TSS (mg/L)	VSS (mg/L)
100 mg NO_3-N/L	0.00	1890	1800
	3.00	1910	1650
100 mg NO_2-N/L	0.00	2090	1950
	3.00	1830	1610

Table B.61. Nitrate and nitrite batch kinetic tests conducted using backwash biomass collected from the denitrifying column (7/5/97, high nitrate loading, hydraulic loading = 3.0 $m^3/m^2/hr$). Samples were taken from the batch reactor via a submerged side port sealed with a rubber stopper.

100 mg NO_3-N/L						100 mg NO_2-N/L				
Time (hr)	Nitrate (mg/L as N)	Nitrite (mg/L as N)	Sulfate (mg/L as N)	COD (mg/L)	COD corrected for nitrite interference*	Time (hr)	Nitrite (mg/L as N)	Sulfate (mg/L as N)	COD (mg/L)	COD corrected for nitrite interference*
0.01	79.5	3.33	8.4	516	515	0.01	83.6	7.17	559	539
0.25	62.9	3.9	6.9	457	456	0.25	74.3	7.08	481	463
0.50	48.1	5.0	8.1	461	460	0.50	46.2	9.91	493	482
0.75	41.3	6.5	7.7	375	374	0.75	39.4	5.43	410	401
1.00	23.1	6.5	10.2	340	339	1.00	26.4	41.4	461	455
1.25	13.2	6.9	5.4	242	241	1.25	12.8	6.07	360	357
1.50	4.5	7.0	6.9	278	276	1.50	3.60	8.53	430	429
1.75	2.4	0.0	7.5	250	250	1.75	0.00	6.10	-	-
2.00	2.11	0.0	9.6	235	235	2.00	0.00	6.35	418	418
2.50	2.14	0.0	7.5	246	246					

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

Test	Time (hr)	TSS (mg/L)	VSS (mg/L)	pH
100 mg NO_3-N/L	0.00	875	785	7.01
	2.50	870	775	8.87
100 mg NO_2-N/L	0.00	850	770	7.02
	2.00	930	835	9.08

Table B.62. Nitrate and nitrite batch kinetic tests conducted using backwash biomass collected from the denitrifying column (7/8/97, high nitrate loading, hydraulic loading = 3.0 $m^3/m^2/hr$). Samples were taken from the batch reactor via a submerged side port sealed with a rubber stopper.

50 mg NO_3-N/L						50 mg NO_2-N/L				
Time (hr)	Nitrate (mg/L as N)	Nitrite (mg/L as N)	Sulfate (mg/L as N)	COD (mg/L)	COD corrected for nitrite interference*	Time (hr)	Nitrite (mg/L as N)	Sulfate (mg/L as N)	COD (mg/L)	COD corrected for nitrite interference*
0.02	43.9	0.00	6.94	285	285	0.02	49.2	3.76	281	270
0.25	39.8	0.00	3.40	253	253	0.25	46.1	6.37	269	258
0.50	35.6	0.00	5.98	246	246	0.50	45.9	6.59	253	243
0.75	30.8	0.00	5.08	234	234	0.75	36.5	8.69	250	241
1.00	25.4	0.00	4.81	198	198	1.00	25.0	4.06	218	212
1.25	20.0	0.00	4.06	166	166	1.25	13.4	3.83	190	187
1.50	15.3	0.00	14.9	170	170	1.50	4.61	4.35	230	229
1.75	9.39	2.16	16.8	143	142	1.75	0.00	6.21	158	158
2.00	4.33	2.84	4.86	95.0	94					
2.50	1.32	0.00	4.76	95.0	95					
3.00	0.00	0.00	3.62	75.2	75					

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

Test	Time (hr)	TSS (mg/L)	VSS (mg/L)	pH
50 mg NO_3-N/L	0.00	265	265	6.39
	3.00	415	365	8.48
50 mg NO_2-N/L	0.00	300	300	6.39
	1.75	350	335	8.69

Table B.63. Nitrate and nitrite batch kinetic tests conducted using backwash biomass collected from the denitrifying column (7/13/97, high nitrate loading, hydraulic loading = 3.0 $m^3/m^2/hr$). Samples were taken from the batch reactor via a submerged side port sealed with a rubber stopper.

50 mg NO_3-N/L						50 mg NO_2-N/L		
Time (hr)	Nitrate (mg/L as N)	Nitrite (mg/L as N)	Sulfate (mg/L as N)	COD (mg/L)	COD corrected for nitrite interference*	Time (hr)	Nitrite (mg/L as N)	Sulfate (mg/L as N)
0.02	46.1	0.00	6.17	294	294	0.02	88.2	5.18
0.25	43.3	0.00	728	318	318	0.25	61.4	103
0.50	41.6	0.00	4.72	278	278	0.50	56.9	5.31
0.75	36.7	0.00	4.44	267	267	0.75	51.1	7.37
1.00	32.7	0.00	3.26	259	259	1.00	40.9	12.11
1.25	27.8	0.85	4.44	231	231	1.25	37.2	69.2
1.50	24.3	1.39	4.2	231	231	1.50	33.7	57.7
1.75	18.2	2.12	295	192	192	1.75	26.7	34.5
2.00	12.6	3.20	103	180	180	2.00	21.5	4.27
2.50	4.94	6.07	6.65	122	120	2.50	8.47	53.1
3.00	0.77	0.78	67.3	98.0	98	3.00	0.00	9.63
3.50	0.93	0.00	5.98	86.3	86			

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

Test	Time (hr)	TSS (mg/L)	VSS (mg/L)	pH
50 mg NO_3-N/L	0.00	140	140	6.31
	3.50	215	215	8.45
50 mg NO_2-N/L	0.00	175	175	6.32
	3.00	230	230	8.63

Table B.64. Nitrite batch kinetic tests conducted using backwash biomass collected from the denitrifying column (7/26/97, high nitrate loading, hydraulic loading = 3.0 m^3/m
Samples were taken from the batch reactor via a submerged side port sealed with a rubber stopper.

20 mg NO_2-N/L					50 mg NO_2-N/L				
Time (hr)	Nitrite (mg/L as N)	Sulfate (mg/L as N)	COD (mg/L)	COD corrected for nitrite interference*	Time (hr)	Nitrite (mg/L as N)	Sulfate (mg/L as N)	COD (mg/L)	COD corrected for nitrite interference*
0.02	20.4	46.7	275	271	0.02	43.7	16.3	252	242
0.25	20.1	14.5	252	247	0.25	42.2	14.2	268	258
0.50	14.8	14.2	252	248	0.50	37.4	82.9	224	215
0.75	15.4	14.6	240	236	0.75	26.6	14.8	240	234
1.00	12.3	15.5	244	241	1.00	17.8	14.9	209	204
1.25	9.93	14.0	232	230	1.25	9.33	13.4	201	198
1.50	7.75	14.0	228	226	1.50	3.23	12.8	216	216
1.75	5.19	24.4	232	231	1.75	0.00	15.1	185	185
2.00	3.10	22.0	197	196					
2.25	0.00	13.5	193	193					

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

Test	Time (hr)	TSS (mg/L)	VSS (mg/L)	pH
20 mg NO_2-N/L	0.00	167	140	6.11
	2.25	160	133	7.92
50 mg NO_2-N/L	0.00	357	327	6.38
	1.75	330	293	8.82

Table B.65. Nitrite batch kinetic tests conducted using backwash biomass collected from the denitrifying column (7/22/97, high nitrate loading, hydraulic loading = 3.0 m^3/m
Samples were taken from the batch reactor via a submerged side port sealed with a rubber stopper.

20 mg NO_2-N/L, #1					20 mg NO_2-N/L, #2				
Time (hr)	Nitrite (mg/L as N)	Sulfate (mg/L as N)	COD (mg/L)	COD corrected for nitrite interference*	Time (hr)	Nitrite (mg/L as N)	Sulfate (mg/L as N)	COD (mg/L)	COD corrected for nitrite interference*
0.02	17.5	9.60	272	268	0.02	18.2	3.97	264	260
0.25	12.7	3.97	272	269	0.25	13.0	7.03	284	281
0.50	11.9	2.16	284	281	0.50	11.8	6.92	276	273
0.75	12.4	4.65	276	273	0.75	9.62	4.15	236	234
1.00	6.34	79.2	316	315	1.00	6.93	1.51	232	230
1.25	4.16	3.59	248	247	1.25	3.94	5.12	240	239
1.50	2.03	6.63	244	244	1.50	2.19	3.46	228	227
1.75	0.00	3.73	244	244	1.75	0.00	5.89	232	232

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

Test	Time (hr)	TSS (mg/L)	VSS (mg/L)	pH
20 mg NO_2-N/L, #1	0.00	183	173	6.15
	1.75	203	193	7.86
20 mg NO_2-N/L, #2	0.00	213	200	6.17
	1.75	190	190	7.90

Table B.66. Nitrate batch kinetic tests conducted using backwash biomass collected from the denitrifying column (7/21/97, high nitrate loading, hydraulic loading = 3.0 $m^3/m^2/hr$). Samples were taken from the batch reactor via a submerged side port sealed with a rubber stopper.

20 mg NO_3-N/L, #1*				20 mg NO_3-N/L, #2*			
Time (hr)	Nitrate (mg/L as N)	Sulfate (mg/L as N)	COD (mg/L)	Time (hr)	Nitrate (mg/L as N)	Sulfate (mg/L as N)	COD (mg/L)
0.02	20.1	5.57	329	0.02	19.7	7.23	349
0.25	15.1	5.09	290	0.25	14.9	6.19	255
0.50	10.3	15.9	314	0.50	9.80	6.54	290
0.75	5.97	11.2	290	0.75	5.35	4.96	243
1.00	2.24	5.32	271	1.00	1.44	10.9	227
1.25	0.83	5.59	247	1.25	0.75	3.83	239

* nitrite was not detected

Test	Time (hr)	TSS (mg/L)	VSS (mg/L)	pH
20 mg NO_3-N/L, #1	0.00	183	173	6.59
	1.25	203	193	8.3
20 mg NO_3-N/L, #2	0.00	213	200	6.61
	1.25	190	190	8.31

APPENDIX C

Table. C.1. Data used to generate Figure 6.

Low nitrate loading phase						High nitrate loading phase					
Date	Day of study	F1 COD (mg/L)	Date	Day of study	F2 COD (mg/L)	Date	Day of study	F1 COD (mg/L)	Date	Day of study	F2 COD (mg/L)
8/14/96	-2	1091	11/25/96	101	1045	3/12/97	208	2155	3/13/97	209	2571
8/15/96	-1	1636	11/26/96	102	213	3/18/97	214	2564	3/17/97	213	2345
8/22/96	6	1636	11/27/96	103	213	3/22/97	218	2338	3/21/97	217	2306
8/25/96	9	1851	12/1/96	107	73.9	3/30/97	226	1359	3/27/97	223	2408
8/26/96	10	1461	12/3/96	109	57.2	4/1/97	228	2189	3/29/97	225	2151
8/29/96	13	1543	12/5/96	111	40.3	4/5/97	232	2345	4/2/97	229	2048
8/30/96	14	2010	12/7/96	113	24.6	4/9/97	236	2059	4/6/97	233	2004
9/3/96	18	1519	12/15/96	121	691	4/15/97	242	1485	4/20/97	247	1964
9/10/96	25	2922	12/19/96	125	604	4/29/97	256	2158	5/8/97	265	1650
9/12/96	27	2143	12/27/96	133	469	5/13/97	270	1149	5/14/97	271	1909
9/14/96	29	1465	12/29/96	135	501	5/21/97	278	2064	5/22/97	279	2517
9/30/96	45	1418	1/16/97	153	679	5/27/97	284	2352	5/28/97	285	2689
10/7/96	52	1590	1/22/97	159	1587	6/10/97	298	1830	6/5/97	293	2067
10/8/96	53	1245	2/3/97	171	1800	6/11/97	299	1861	6/10/97	298	1830
10/9/96	54	1330	2/17/97	185	1762	6/24/97	312	4119	6/11/97	299	1861
10/11/96	56	1249	2/21/97	189	1080	7/2/97	320	4293	6/23/97	311	4212
10/13/96	58	1240	2/23/97	191	1034	7/24/97	342	2883	7/7/97	325	4071
10/14/96	59	1291							7/27/97	345	2519
10/15/96	60	1412									
10/20/96	65	1272	Low nitrate loading phase (Fermenters 1 and 2)					High nitrate loading phase (Fermenters 1 and 2)			
10/23/96	68	1232									
10/26/96	71	1017	Soluble COD average =		1078			Soluble COD average =			2352
10/29/96	74	1319	n =		58			n =			35
11/10/96	86	1041	Standard error =		76			Standard error =			128
11/18/96	94	1057									
11/23/96	99	1359									
11/25/96	101	1065									
11/26/96	102	948									
11/27/96	103	213									
12/3/96	109	40									
12/4/96	110	615									
12/10/96	116	911									
12/12/96	118	891									
12/14/96	120	733									
12/20/96	126	1003									
12/14/96	120	741									
1/5/97	142	913									
1/19/97	156	738									
2/8/97	176	1081									
2/14/97	182	1018									
2/16/97	184	1374									

Table. C.2. Data used to generate Figure 7.

	Low nitrate loading phase		High nitrate loading phase		Fish waste batch reactor	
Volatile Fatty Acid (VFA)	Constituent fraction of VFA COD (%)	Standard error	Constituent fraction of VFA COD (%)	Standard error	Constituent fraction of VFA COD (%)	Standard error
Acetic	38.8	1.4	37.9	0.26	47.0	2.79
Propionic	31.2	0.83	36.5	0.38	36.5	2.38
Isobutyric	2.08	0.24	3.66	0.06	5.92	0.90
n -Butyric	15.3	1.02	8.53	0.22	2.74	0.32
2-Methylbutyric	0.56	0.14	1.45	0.29	3.13	0.28
3-Methylbutyric	2.29	0.35	5.09	0.14	4.92	0.36
n -Valeric	9.14	0.62	5.26	0.2	0.10	0.10

Table. C.3. Data used to generate Figure 8.

Date	Day of Study	Ratio of Influent COD to NO_3-N	Nitrate Removal (%)	Effluent Nitrite	Effluent COD
9/7/96	22	5.83	97.7	0.0	59.6
9/10/96	25	4.62	98.5	0.3	29.4
9/15/96	30	3.03	79.7	0.9	24.7
9/17/96	32	3.74	85.5	1.1	24.7
9/24/96	39	4.03	81.7	1.1	94.1
10/2/96	47	3.69	70.7	2.8	33.3
10/6/96	51	1.89	81.8	8.9	34.0
10/7/96	52	2.39	85.0	10.3	32.1
10/8/96	53	2.35	94.0	11.5	44.0
10/12/96	57	2.13	64.4	1.7	27.8
10/23/96	68	2.75	80.3	1.7	28.6
10/26/96	71	4.91	98.7	4.1	25.5
10/29/96	74	1.56	66.7	7.1	6.1
11/7/96	83	2.77	43.2	8.7	9.9
11/10/96	86	1.58	53.8	3.6	11.2
12/1/96	107	3.91	77.3	0.0	20.6
12/3/96	109	1.88	60.4	0.0	4.1
12/5/96	111	2.01	66.5	0.0	11.1
12/19/96	125	3.8	73.4	0.0	10.8
12/21/96	127	2.96	49.6	0.0	13.9
12/23/96	129	2.34	13.2	0.5	32.3
12/27/96	133	5.23	72.6	2.4	12.4
1/5/97	142	3.02	63.3	3.6	12.4
1/18/97	155	2.44	54.0	0.0	10.9
1/19/97	156	3.45	84.8	7.3	13.3
1/22/97	159	1.99	59.0	0.0	11.2
1/25/97	162	6.15	99.0	0.0	39.6
1/29/97	166	6.76	99.9	0.0	62.0
2/7/97	175	3.18	63.4	0.0	16.9
2/8/97	176	5.27	99.1	0.0	40.0
2/11/97	179	3.29	84.1	0.0	16.9
2/16/97	184	5.06	99.6	0.0	38.0
2/17/97	185	3.76	99.6	0.0	58.7
2/19/97	187	5.76	99.5	0.0	31.9
2/20/97	188	7.57	94.8	0.0	25.0
2/21/97	189	2.84	83.6	8.6	19.6
2/23/97	191	3.25	99.7	8.6	38.1
2/25/97	193	5.32	99.3	0.0	38.6
3/2/97	198	3.48	98.3	12.2	40.3
3/4/97	200	8.74	98.0	0.0	73.9

Table. C.4. Data used to generate Figure 9.

Date	Day of Study	Ratio of Influent COD to NO_3-N	Nitrate Removal (%)	Effluent Nitrite	Effluent COD
3/6/97	202	7.18	99.2	8.5	26.4
3/12/97	208	4.44	98.9	8.0	48.7
3/17/97	213	3.80	99.9	7.3	52.1
3/19/97	215	4.04	99.8	26.8	63.8
3/22/97	218	3.57	99.5	9.7	66.1
3/24/97	220	5.26	99.8	5.4	110
3/26/97	222	3.12	100	22.8	59.1
4/3/97	230	3.37	100	22.4	61.8
4/5/97	232	3.93	100	6.3	94.8
4/6/97	233	3.95	100	25.1	64.4
4/10/97	237	3.13	100	27.1	44.2
4/12/97	239	3.34	99.1	31.4	68.2
4/14/97	241	3.51	99.4	26.6	101
4/15/97	242	4.10	99.1	29.2	85.1
4/19/97	246	3.04	99.3	33.2	117
4/20/97	247	4.40	99.3	30.3	127
4/21/97	248	3.80	99.8	25.8	101.5
4/23/97	250	4.08	99.8	30.8	91.5
4/29/97	256	5.81	99.8	9.2	155
4/30/97	257	4.14	99.7	17.8	73.2
5/8/97	265	4.39	99.8	23.6	129
5/14/97	271	3.76	99.6	27.6	46.7
5/15/97	272	4.6	99.4	21.7	113
5/17/97	274	3.73	99.3	30.4	43.4
5/18/97	275	4.52	99.7	18.2	69.6
5/19/97	276	3.44	99.7	24.8	17.4
5/22/97	279	3.34	99.8	13.7	88.5
5/24/97	281	2.29	99.8	2.2	72.4
5/25/97	282	3.47	99.9	0.0	33.7

Table. C.5. Data used to generate Figure 10.

Date	Day of Study	Nitrate Removal (%)	Effluent Nitrite	Effluent COD
High nitrate loading phase, hydraulic loading = 3.0 $m^3/m^2/hr$				
3/6/97	202	99.2	8.5	26.4
3/12/97	208	98.9	8.0	48.7
3/17/97	213	99.9	7.3	52.1
3/19/97	215	99.8	26.8	63.8
3/22/97	218	99.5	9.7	66.1
3/24/97	220	99.8	5.4	110
3/26/97	222	100	22.8	59.1
4/3/97	230	100	22.4	61.8
4/5/97	232	100	6.3	94.8
4/6/97	233	100	25.1	64.4
4/10/97	237	100	27.1	44.2
4/12/97	239	99.1	31.4	68.2
4/14/97	241	99.4	26.6	101
4/15/97	242	99.1	29.2	85.1
4/19/97	246	99.3	33.2	117
4/20/97	247	99.3	30.3	127
4/21/97	248	99.8	25.8	101
4/23/97	250	99.8	30.8	91.5
4/29/97	256	99.8	9.2	155
4/30/97	257	99.7	17.8	73.2
5/8/97	265	99.8	23.6	129
5/14/97	271	99.6	27.6	46.7
5/15/97	272	99.4	21.7	113
5/17/97	274	99.3	30.4	43.4
5/18/97	275	99.7	18.2	69.6
5/19/97	276	99.7	24.8	17.4
5/22/97	279	99.8	13.7	88.5
5/24/97	281	99.8	2.2	72.4
5/25/97	282	99.9	0.0	33.7
6/23/97	311	100	0.0	165
6/24/97	312	100	0.0	185
6/27/97	315	98.6	1.1	181
6/28/97	316	99.4	0.0	162
7/1/97	319	99.6	0.0	186
7/2/97	320	99.6	0.0	240
7/4/97	322	99.4	0.0	526
7/5/97	323	99.5	0.0	111
7/7/97	325	99.8	0.0	358
7/8/97	326	99.8	0.0	416
7/9/97	327	99.8	0.0	633
7/11/97	329	99.8	0.0	622
7/18/97	336	95	0.0	417
7/24/97	342	97.3	0.0	188
7/27/97	345	99.1	1.0	353
7/28/97	346	99.1	0.5	59.9
High nitrate loading phase, hydraulic loading = 1.5 $m^3/m^2/hr$				
5/26/97	283	99.7	18.2	33.4
5/27/97	284	99.6	0.0	178
5/28/97	285	99.0	0.0	106
5/29/97	286	100	0.0	29.0
5/31/97	288	99.5	0.0	117
6/2/97	290	99.1	0.0	71.4
6/3/97	291	99.3	0.0	118
6/5/97	293	99.8	0.0	73.7
6/8/97	296	99.2	33.8	19.5
6/10/97	298	100	4.8	42.5
6/16/97	304	100	21.6	31.3

Table. C.6. Data used to generate Figure 11.A.

Nitrate - Low nitrate loading phase							
Column Height (m)	1/25/97 NO_3^- (mg/L as N)	2/16/97 NO_3^- (mg/L as N)	1/29/97 NO_3^- (mg/L as N)	2/19/97 NO_3^- (mg/L as N)	2/20/97 NO_3^- (mg/L as N)	Average NO_3^- (mg/L as N)	Standard Error
0.00	36.3	43.6	39.2	40.6	36.6	39.3	1.35
0.89	25.1	28.4	31.0	28.5	29.3	28.5	0.97
1.37	14.9	7.88	11.8	15.3	12.7	12.5	1.33
1.85	2.68	0.31	2.44	2.41	5.35	2.64	0.80
2.34	0.46	0.15	1.16	0.23	1.18	0.64	0.22
2.82	0.44	0.14	1.16	0.42	1.22	0.68	0.22
3.30	0.41	0.74	1.29	0.14	1.83	0.88	0.30
3.48	0.37	0.17	0.39	0.19	1.91	0.60	0.33
Nitrite - Low nitrate loading phase							
Column Height (m)	1/25/97 NO_2^- (mg/L as N)	2/16/97 NO_2^- (mg/L as N)	1/29/97 NO_2^- (mg/L as N)	2/19/97 NO_2^- (mg/L as N)	2/20/97 NO_2^- (mg/L as N)	Average NO_2^- (mg/L as N)	Standard Error
0.00	0.00	0.00	0.48	0.00	0.27	0.15	0.10
0.89	0.98	0.76	0.52	2.01	0.59	0.97	0.27
1.37	3.66	2.42	2.21	0.86	3.63	2.56	0.52
1.85	3.76	0.08	1.63	1.07	5.42	2.39	0.97
2.34	0.15	0.00	0.17	1.01	0.92	0.45	0.21
2.82	0.00	0.00	0.15	0.00	0.00	0.03	0.03
3.30	0.00	0.00	0.14	0.00	0.00	0.03	0.03
3.48	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table. C.7. Data used to generate Figure 11.B.

Nitrate - High nitrate loading phase						
Column Height (m)	7/18/97 NO_3^- (mg/L as N)	7/24/97 NO_3^- (mg/L as N)	7/27/97 NO_3^- (mg/L as N)	7/28/97 NO_3^- (mg/L as N)	Average NO_3^- (mg/L as N)	Standard Error
0.00	90.1	90.8	81.8	82.5	86.28	2.41
0.89	83.1	79.2	73.7	78.1	78.51	1.72
1.37	47.0	25.1	30.2	25.7	31.98	4.59
1.85	21.7	6.34	2.55	2.32	8.23	4.10
2.34	29.3	0.86	0.76	0.91	7.95	6.35
2.82	3.23	0.69	0.68	0.74	1.34	0.57
3.30	1.21	3.86	0.74	0.71	1.63	0.67
3.48	4.47	2.49	0.75	0.73	2.11	0.79
Nitrite - High nitrate loading phase						
Column Height (m)	7/18/97 NO_2^- (mg/L as N)	7/24/97 NO_2^- (mg/L as N)	7/27/97 NO_2^- (mg/L as N)	7/28/97 NO_2^- (mg/L as N)	Average NO_2^- (mg/L as N)	Standard Error
0.00	1.14	0.12	0.91	0.29	0.61	0.24
0.89	5.95	2.79	1.88	4.37	3.75	0.80
1.37	8.24	3.63	5.11	10.3	6.82	1.35
1.85	6.59	1.18	1.73	3.96	3.37	1.10
2.34	3.56	0.67	1.13	0.54	1.47	0.63
2.82	1.21	0.00	0.00	0.40	0.40	0.25
3.30	0.00	0.00	0.00	0.00	0.00	0.00
3.48	0.00	0.00	0.96	0.45	0.35	0.20

Table. C.8. Data used to generate Figure 12.

Low nitrate loading phase - Average values based on three profiles (1/25/97, 2/19/97, 2/20/97)									
Column Height (m)	Average COD (mg/L)	Standard Error	Acetic Acid	Propionic Acid	Isobutyric Acid	*n*-Butyric Acid	2-Methylbutyric Acid	3-Methylbutyric Acid	*n*-Valeric Acid
0.00	245	16.3	88.9	60.7	8.21	22.3	8.77	9.50	18.5
0.89	174	4.56	40.8	27.1	4.31	10.1	4.06	5.65	4.65
1.37	131	4.37	22.0	14.7	4.15	6.24	2.75	0.00	2.93
1.85	65.4	6.08	3.23	2.91	1.18	0.75	0.00	0.00	0.00
2.34	43.4	3.26	0.16	0.25	0.30	0.00	0.00	0.00	0.00
2.82	40.5	4.62	0.39	0.05	0.00	0.00	0.00	0.00	0.00
3.30	34.6	0.59	0.31	0.15	0.00	0.00	0.00	0.00	0.00
3.48	32.2	4.21	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table. C.9. Data used to generate Figure 13.

High nitrate loading phase - Average values based on four profiles (7/18/97, 7/24/97, 7/27/97, 7/28/97)									
Column Height (m)	Average COD (mg/L)	Standard Error	Acetic Acid	Propionic Acid	Isobutyric Acid	*n*-Butyric Acid	2-Methylbutyric Acid	3-Methylbutyric Acid	*n*-Valeric Acid
0.00	622	93.0	213	239	23.9	37.1	16.9	26.2	12.5
0.89	570	95.6	194	210	21.9	30.7	15.5	18.2	12.1
1.37	413	98.4	148	133	19.8	17.0	9.92	15.0	9.18
1.85	315	103	108	79.5	17.5	5.80	6.88	12.3	2.53
2.34	287	97.5	104	63.3	16.5	3.38	4.38	11.1	0.00
2.82	262	87.8	99.0	53.4	15.4	1.84	3.83	10.6	0.00
3.30	293	105	99.3	51.2	15.1	1.75	3.71	9.63	0.00
3.48	255	80.8	117	68.9	16.9	4.07	4.81	12.5	0.00

Table. C.10. Data used to generate Figure 14.A.

$NO_x = (NO_3^- + NO_2^-)$ - Low nitrate loading phase							
Time in Column (min)	1/25/97 NO_x^- (mg/L as N)	2/16/97 NO_x^- (mg/L as N)	1/29/97 NO_x^- (mg/L as N)	2/19/97 NO_x^- (mg/L as N)	2/20/97 NO_x^- (mg/L as N)	Average NO_x^- (mg/L as N)	Standard Error
0.00	36.3	43.6	39.7	40.6	36.8	39.4	1.32
7.9*	26.1	29.1	31.5	30.5	29.9	29.4	0.93
12.4*	18.6	10.3	14.1	16.2	16.4	15.1	1.40
16.8*	6.44	0.39	4.07	3.48	10.8	5.03	1.73
21.3*	0.61	0.15	1.33	1.24	2.10	1.09	0.33
25.7*	0.44	0.14	1.31	0.42	1.22	0.71	0.23
30.1	0.41	0.74	1.43	0.14	1.83	0.91	0.31
32.0	0.37	0.17	0.39	0.19	1.91	0.60	0.33
*data points used to fit half-order curve							
Nitrate - Low nitrate loading phase							
Time in Column (min)	1/25/97 NO_3^- (mg/L as N)	2/16/97 NO_3^- (mg/L as N)	1/29/97 NO_3^- (mg/L as N)	2/19/97 NO_3^- (mg/L as N)	2/20/97 NO_3^- (mg/L as N)	Average NO_3^- (mg/L as N)	Standard Error
0.00	36.3	43.6	39.2	40.6	36.6	39.3	1.35
7.90	25.1	28.4	31.0	28.5	29.3	28.5	0.97
12.4	14.9	7.88	11.8	15.3	12.7	12.5	1.33
16.8	2.68	0.31	2.44	2.41	5.35	2.64	0.80
21.3	0.46	0.15	1.16	0.23	1.18	0.64	0.22
25.7	0.44	0.14	1.16	0.42	1.22	0.68	0.22
30.1	0.41	0.74	1.29	0.14	1.83	0.88	0.30
32.0	0.37	0.17	0.39	0.19	1.91	0.60	0.33
Nitrite - Low nitrate loading phase							
Time in Column (min)	1/25/97 NO_2^- (mg/L as N)	2/16/97 NO_2^- (mg/L as N)	1/29/97 NO_2^- (mg/L as N)	2/19/97 NO_2^- (mg/L as N)	2/20/97 NO_2^- (mg/L as N)	Average NO_2^- (mg/L as N)	Standard Error
0.00	0.00	0.00	0.48	0.00	0.27	0.15	0.10
7.90	0.98	0.76	0.52	2.01	0.59	0.97	0.27
12.4	3.66	2.42	2.21	0.86	3.63	2.56	0.52
16.8	3.76	0.08	1.63	1.07	5.42	2.39	0.97
21.3	0.15	0.00	0.17	1.01	0.92	0.45	0.21
25.7	0.00	0.00	0.15	0.00	0.00	0.03	0.03
30.1	0.00	0.00	0.14	0.00	0.00	0.03	0.03
32.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table. C.11. Data used to generate Figure 14.B.

$NO_x = (NO_3^- + NO_2^-)$ - High nitrate loading phase						
Time in Column (min)	7/18/97 NO_x^- (mg/L as N)	7/24/97 NO_x^- (mg/L as N)	7/27/97 NO_x^- (mg/L as N)	7/28/97 NO_x^- (mg/L as N)	Average NO_x^- (mg/L as N)	Standard Error
0.00	91.2	90.9	82.7	82.8	86.9	2.41
7.9*	89.0	82.0	75.6	82.5	82.3	2.75
12.4*	55.2	28.7	35.3	36.0	38.8	5.72
16.8*	28.3	7.52	4.28	6.27	11.6	5.61
21.3*	32.8	1.53	1.90	1.45	9.43	7.80
25.7*	4.44	0.69	0.68	1.13	1.74	0.91
30.1	1.21	3.86	0.74	0.71	1.63	0.75
32.0	4.47	2.49	1.71	1.18	2.46	0.72
*data points used to fit half-order curve						
Nitrate - High nitrate loading phase						
Time in Column (min)	7/18/97 NO_3^- (mg/L as N)	7/24/97 NO_3^- (mg/L as N)	7/27/97 NO_3^- (mg/L as N)	7/28/97 NO_3^- (mg/L as N)	Average NO_3^- (mg/L as N)	Standard Error
0.00	90.1	90.8	81.8	82.5	86.3	2.41
7.90	83.1	79.2	73.7	78.1	78.5	1.72
12.4	47.0	25.1	30.2	25.7	32.0	4.59
16.8	21.7	6.34	2.55	2.32	8.23	4.10
21.3	29.3	0.86	0.76	0.91	7.95	6.35
25.7	3.23	0.69	0.68	0.74	1.34	0.57
30.1	1.21	3.86	0.74	0.71	1.63	0.67
32.0	4.47	2.49	0.75	0.73	2.11	0.79
Nitrite - High nitrate loading phase						
Time in Column (min)	7/18/97 NO_2^- (mg/L as N)	7/24/97 NO_2^- (mg/L as N)	7/27/97 NO_2^- (mg/L as N)	7/28/97 NO_2^- (mg/L as N)	Average NO_2^- (mg/L as N)	Standard Error
0.00	1.14	0.12	0.91	0.29	0.61	0.24
7.90	5.95	2.79	1.88	4.37	3.75	0.80
12.4	8.24	3.63	5.11	10.3	6.82	1.35
16.8	6.59	1.18	1.73	3.96	3.37	1.10
21.3	3.56	0.67	1.13	0.54	1.47	0.63
25.7	1.21	0.00	0.00	0.40	0.40	0.25
30.1	0.00	0.00	0.00	0.00	0.00	0.00
32.0	0.00	0.00	0.96	0.45	0.35	0.20

Table. C.12. Data used to generate Figure A.3.

Figure	Date	Time (hr)	COD (mg/L)
A	11/2-3, 1996	2.19	133
		5.94	113
		22.8	93.6
		24.0	51.1
B	2/8-9, 1997	1.65	130
		5.53	121
		9.75	127
		24.0	152

Table. C.13. Data used to generate Figure A.4.

Day	DOC (mg/L) (mg/L)
0.07	2289
1.01	4264
1.99	4824
2.99	5084
3.97	5080
5.12	5158
6.16	5366
7.10	5262
10.0	5596
13.0	5280
14.1	5509
15.1	5462
16.3	5402
17.1	5311
19.1	5290
21.2	5259
27.0	5690

Table. C.14. Data used to generate Figure A.5.

Low nitrate loading phase			High nitrate loading phase			Fish waste batch reactor	
Date	Fermenter	VFA fraction of measured COD (%)	Date	Fermenter	VFA fraction of measured COD (%)	Date	VFA fraction of measured COD (%)
8/25/96	F1	51	3/6/97	F1	85	3/21/97	29
8/29/96		95	3/12/97		100	3/22/97	97
8/30/96		79	3/18/97		84	3/24/97	56
9/30/96		82	3/22/97		105	3/25/97	74
10/8/96		84	3/29/97		141	3/26/97	104
10/9/96		90	4/1/97		105	3/27/97	77
10/13/96		92	4/5/97		98	3/29/97	57
10/14/96		95	4/9/97		98	3/30/97	88
10/20/96		91	4/19/97		124	4/1/97	56
10/26/96		74	5/13/97		192	4/2/97	76
10/29/96		87	5/21/97		121	4/3/97	74
11/18/96		107	6/24/97		90	4/5/97	69
11/23/96		99	7/2/97		99	4/6/97	64
12/4/96		83	7/24/97		100	4/7/97	34
12/10/96		80	3/13/97	F2	95	4/8/97	83
12/12/96		104	3/17/97		92	4/9/97	65
12/14/96		161	3/21/97		97	4/10/97	36
12/20/96		54	3/27/97		105	4/12/97	78
1/5/97		95	3/29/97		107	4/14/97	72
1/19/97		91	4/2/97		96	4/15/97	86
2/8/97		84	4/6/97		105	4/19/97	97
2/14/97		112	4/20/97		112	4/20/97	75
2/16/97		82	5/8/97		104	4/21/97	60
12/15/96	F2	86	5/14/97		144	4/22/97	100
12/19/96		97	5/22/97		98	4/25/97	86
12/27/96		43	5/28/97		91	4/26/97	69
12/29/96		72	6/5/97		102	4/29/97	55
1/16/97		82	6/11/97		109	5/3/97	34
1/22/97		72	6/23/97		91	5/5/97	81
2/3/97		81	7/7/97		95		
2/17/97		73					
2/23/97		85					
Average =		86.3	Average =		106	Average =	70.1
Standard deviation =		20.2	Standard deviation =		21.4	Standard deviation =	20.0
Standard error =		3.58	Standard error =		3.90	Standard error =	3.72

Table. C.15. Data used to generate Figure A.6.

Pilot-scale fermenters				Fish waste batch reactor		
Date	Fermenter	COD (mg/L)	VSS (mg/L)	Date	COD (mg/L)	VSS (mg/L)
2/3/97	F2	1800	1400	3/27/97	575	3380
3/12/97	F1	2155	2535	3/30/97	536	5010
3/13/97	F2	2571	2570	4/3/97	608	5060
3/17/97	F2	2345	2730	4/5/97	612	5080
3/18/97	F1	2564	2725	4/7/97	521	5720
3/21/97	F2	2306	3380	4/9/97	536	5110
3/22/97	F1	2338	3270	4/14/97	691	5230
3/29/97	F2	2151	2370	4/19/97	365	5960
3/30/97	F1	1359	2530	4/21/97	509	5310
4/2/97	F2	2048	2100	4/23/97	262	5240
4/5/97	F1	2345	2090	4/29/97	353	5180
4/6/97	F2	2004	2050	5/1/97	277	4560
4/9/97	F1	2059	2110	5/3/97	294	5150
4/20/97	F2	1964	2560	5/5/97	335	3850
5/13/97	F1	1149	3770			
5/14/97	F2	1909	3640			
5/21/97	F1	2064	3260			
7/2/97	F1	4293	7220			
7/7/97	F2	4071	9930			

Table. C.16. Data used to generate Figure A.7.A.

Low nitrate loading phase		
Date	Day of Study	VSS (mg/L)
10/11/96	56	1320
10/20/96	65	1050
10/27/96	72	815
11/2/96	78	1045
11/3/96	79	1030
12/14/96	120	580
12/15/96	121	580
12/22/96	128	254
12/29/96	135	614
1/5/97	142	1020
1/18/97	155	900
1/28/97	165	615
1/29/97	166	665
2/8/97	176	772
2/9/97	177	1335
2/18/97	186	990
2/19/97	187	885
2/20/97	188	1285
2/26/97	194	1005
2/27/97	195	1110
Average =		894
Standard error =		63
High nitrate loading phase		
Date	Day of Study	VSS (mg/L)
3/16/97	212	920
4/1/97	228	1385
4/21/97	248	1190
5/31/97	288	1150
6/3/97	291	1335
6/10/97	298	715
7/5/97	323	2830
Average =		1361
Standard error =		260

Table. C.17. Data used to generate Figu

Low nitrate loading phase		
Date	Day of Study	VSS (mg/L)
11/10/96	86	6.50
11/11/96	87	31.5
12/11/96	117	14.5
12/18/96	124	8.00
1/18/97	155	38.0
1/20/97	157	18.0
1/30/97	167	9.00
2/3/97	171	12.5
2/8/97	176	9.50
2/11/97	179	1.00
2/18/97	186	6.00
2/21/97	189	3.00
2/25/97	193	13.5
2/28/97	196	18.5
Average =		14
Standard error =		3
High nitrate loading phase		
Date	Day of Study	VSS (mg/L)
3/12/97	208	11.5
3/14/97	210	22.5
3/17/97	213	54.5
3/21/97	217	110
4/2/97	229	54.5
4/6/97	233	26.0
4/14/97	241	62.5
4/23/97	250	49.0
5/14/97	271	24.5
5/21/97	278	31.0
5/27/97	284	43.5
7/7/97	325	38.5
7/11/97	329	170
Average =		54
Standard error =		12

Table. C.18. Data used to generate Figure A.8.

100 mg/L as NO_3-N Test			100 mg/L as NO_2-N Test	
Time (hr)	NO_3^- (mg/L as N)	NO_2^- (mg/L as N)	Time (hr)	NO_2^- (mg/L as N)
0.00	92.9	15.4	0.00	104
0.33	42.8	48.4	0.33	98.9
0.67	24.1	47.7	0.67	78.6
1.00	1.98	70.3	1.00	61.0
1.50	0.00	47.0	1.50	39.6
2.00	0.00	28.6	2.00	21.4
2.50	0.00	0.00	2.50	0.00
3.50	0.00	0.00	3.50	0.00
4.50	0.00	0.00	4.50	0.00

Table C.19. Values used to determine the hydraulic loading during the low nitrate loading phase.

Date	Time (hr)	TIme (day)	Nitrate Volume (L)	Nitrate Flow (L/day)	Cabon Volume (L)	Cabon Flow (L/day)	Total Volume (L)	Total Flow (L/day)	Hydraulic Loading $m^3/hr/m^2$
12/2-12/3	24.8	1.03	1200	1161	179	173	1379	1335	3.05
12/3-12/4	22.9	0.95	1085	1136	174	182	1259	1319	3.01
12/5-12/6	22.0	0.92	1090	1187	166	181	1256	1368	3.12
12/6-12/7	22.8	0.95	1145	1208	166	175	1311	1383	3.16
12/7-12/8	22.8	0.95	1115	1172	166	174	1281	1346	3.08
12/8-12/9	23.3	0.97	1120	1154	166	171	1286	1325	3.03
12/9-12/10	22.8	0.95	995	1049	179	189	1174	1238	2.83
12/12-12/13	22.8	0.95	980	1034	174	184	1154	1217	2.78
12/13-12/14	22.0	0.92	990	1080	166	181	1156	1261	2.88
12/15-12/16	21.8	0.91	1050	1154	168	185	1218	1339	3.06
12/18-12/19	23.4	0.98	1010	1035	171	175	1181	1210	2.76
12/19-12/20	21.3	0.89	925	1045	166	187	1091	1232	2.81
12/20-12/21	24.8	1.03	1150	1115	166	161	1316	1276	2.91
12/21-12/22	21.8	0.91	1025	1127	166	182	1191	1309	2.99
12/26-12/27	15.5	0.65	725.0	1123	117	181	842	1304	2.98
12/27-12/28	22.9	0.95	950	995	174	182	1124	1177	2.69
12/28-12/29	23.7	0.99	1099	1111	174	176	1273	1287	2.94
12/29-12/30	18.8	0.78	880	1126	150	192	1030	1318	3.01
12/31 - 1/1	23.4	0.98	1145	1174	182	187	1327	1361	3.11
1/2 - 1/3	20.8	0.87	965	1112	157	181	1122	1293	2.95
1/3 - 1/4	23.3	0.97	1065	1097	174	179	1239	1276	2.92
1/4 - 1/5	22.0	0.92	1050	1145	165	180	1215	1324	3.03
1/5 - 1/6	23.2	0.97	1105	1145	239	248	1344	1392	3.18
1/6 - 1/7	22.4	0.93	1020	1092	187	200	1207	1292	2.95
1/7 - 1/8	24.5	1.02	1160	1136	174	170	1334	1307	2.98
1/8 - 1/9	21.8	0.91	1028	1130	167	184	1195	1314	3.00
1/9 - 1/10	23.0	0.96	1075	1122	169	176	1244	1298	2.97
1/10 - 1/11	22.4	0.93	1025	1099	162	174	1187	1273	2.91
1/11 - 1/12	23.4	0.97	1080	1108	166	170	1246	1279	2.92
1/12 - 1/13	23.1	0.96	1060	1102	166	173	1226	1275	2.91
1/16 - 1/17	21.6	0.90	1110	1236	157	175	1267	1411	3.22
1/17 - 1/18	23.3	0.97	1207	1242	157	162	1364	1404	3.21
1/18 - 1/19	22.0	0.92	1105	1205	157	171	1262	1377	3.14
1/19 - 1/20	22.0	0.92	1075	1173	125	136	1200	1309	2.99
1/28-1/29	24.0	1.00	1157	1157	179	179	1336	1336	3.05
2/8-2/9	24.0	1.00	1160	1160	169	169	1329	1329	3.04
2/10-2/11	20.4	0.85	1005	1183	150	177	1155	1360	3.11
2/15-2/16	22.1	0.92	1132	1232	150	163	1282	1395	3.19
2/16-2/17	21.4	0.89	1087	1217	158	177	1245	1394	3.18
2/17-2/18	24.3	1.01	1235	1220	166	164	1401	1384	3.16
2/18-2/19	24.1	1.00	1188	1185	174	174	1362	1358	3.10
2/19-2/20	24.0	1.00	1145	1145	168	168	1313	1313	3.00
2/20-2/21	19.9	0.83	915	1104	125	151	1040	1254	2.86
2/21-2/22	22.5	0.94	1065	1135	150	160	1215	1295	2.96
2/22-2/23	23.2	0.97	1086	1125	125	129	1211	1255	2.87
2/23-2/24	23.6	0.98	1120	1141	168	171	1288	1312	3.00
2/24-2/25	22.3	0.93	1078	1158	158	170	1236	1328	3.03
2/25-2/26	24.0	1.00	1167	1167	174	174	1341	1341	3.06
2/26-2/27	24.0	1.00	1059	1059	162	162	1221	1221	2.79
2/27-2/28	21.8	0.91	1005	1106	150	165	1155	1271	2.90
2/28-3/1	24.0	1.00	1110	1110	114	114	1224	1224	2.80
3/1-3/2	24.0	1.00	1110	1110	120	120	1230	1230	2.81
3/2-3/3	20.6	0.86	930	1083	141	164	1071	1247	2.85
3/3-3/4	23.3	0.97	1113	1149	166	171	1279	1320	3.02
							Average	1307	2.99
							Standard deviation	55.3	0.13
							Standard error	7.5	0.02

Table C.20. Values used to determine mass loading and removal during the low nitrate loading phase.

	NO_3			NO_2		NO_x		
Date	Influent mg/L as N	Effluent mg/L as N	Removed mg/L as N	Influent mg/L as N	Effluent mg/L as N	Influent mg/L as N	Effluent mg/L as N	Removed mg/L as N
8/21/96	39.2	2.9	36.3	2.1	1.0	41.2	3.9	37.3
9/3/96	25.9	0.4	25.5	5.8	0.2	31.7	0.6	31.0
9/7/96	23.9	0.6	23.3	1.7	0.0	25.6	0.6	25.1
9/10/96	41.4	0.6	40.7	0.0	0.3	41.4	0.9	40.5
9/15/96	41.2	8.4	32.8	0.0	0.9	41.2	9.3	31.9
9/17/96	41.4	6.0	35.4	0.0	1.1	41.4	7.1	34.3
9/24/96	40.2	7.4	32.9	0.0	1.1	40.2	8.4	31.8
10/2/96	43.9	12.9	31.1	0.0	2.8	43.9	15.7	28.3
10/6/96	44.7	8.1	36.6	0.0	8.9	44.7	17.0	27.7
10/7/96	39.4	5.9	33.5	0.0	10.3	39.4	16.2	23.2
10/8/96	40.7	2.4	38.2	0.5	11.5	41.2	14.0	27.2
10/12/96	35.5	12.6	22.8	0.0	1.7	35.5	14.3	21.1
10/23/96	42.0	8.3	33.7	0.0	1.7	42.0	9.9	32.1
10/26/96	37.1	0.5	36.6	0.4	4.1	37.5	4.6	32.9
10/29/96	40.0	13.3	26.7	0.0	7.1	40.0	20.4	19.6
11/7/96	42.5	24.1	18.4	0.0	8.7	42.5	32.8	9.6
11/10/96	46.0	21.3	24.8	0.0	3.6	46.0	24.9	21.2
11/23/96	40.7	9.8	30.9	0.0	6.6	40.7	16.4	24.3
12/1/96	30.6	7.0	23.7	0.0	0.0	30.6	7.0	23.7
12/3/96	27.5	10.9	16.6	0.0	0.0	27.5	10.9	16.6
12/4/96	45.0	47.0	-2.0	0.0	0.0	45.0	47.0	-2.0
12/5/96	40.6	13.6	27.0	0.0	0.0	40.6	13.6	27.0
12/6/96	43.6	44.4	-0.8	0.0	0.0	43.6	44.4	-0.8
12/19/96	41.5	11.0	30.4	0.0	0.0	41.5	11.0	30.4
12/21/96	25.5	12.9	12.7	0.0	0.0	25.5	12.9	12.7
12/23/96	18.5	16.0	2.4	0.0	0.5	18.5	16.5	1.9
12/27/96	53.7	14.7	39.0	0.0	2.4	53.7	17.1	36.6
12/29/96	36.0	10.4	25.6	0.0	0.0	36.0	10.4	25.6
1/5/97	35.4	13.0	22.4	0.0	3.6	35.4	16.6	18.8
1/18/97	40.5	18.6	21.9	2.1	0.0	42.6	18.6	24.0
1/19/97	45.3	6.9	38.4	0.0	7.3	45.3	14.2	31.1
1/22/97	36.9	15.1	21.8	0.0	0.0	36.9	15.1	21.8
1/25/97	36.3	0.4	36.0	0.0	0.0	36.3	0.4	36.0
1/29/97	39.2	0.0	39.2	0.5	0.0	39.7	0.0	39.7
2/7/97	42.0	15.4	26.6	0.0	0.0	42.0	15.4	26.6
2/8/97	39.4	0.4	39.1	0.0	0.0	39.4	0.4	39.1
2/11/97	40.6	6.5	34.2	0.0	0.0	40.6	6.5	34.2
2/16/97	43.6	0.2	43.4	0.0	0.0	43.6	0.2	43.4
2/17/97	36.1	0.1	35.9	12.9	0.0	49.0	0.1	48.8
2/19/97	40.6	0.2	40.4	0.0	0.0	40.6	0.2	40.4
2/20/97	36.6	1.9	34.7	0.3	0.0	36.8	1.9	34.9
2/21/97	39.2	6.4	32.8	0.0	8.6	39.2	15.0	24.2
2/23/97	47.0	0.1	46.9	0.0	8.6	47.0	8.7	38.3
2/25/97	39.4	0.3	39.1	0.7	0.0	40.1	0.3	39.8
3/2/97	36.3	0.6	35.7	1.8	12.2	38.1	12.8	25.3
3/4/97	26.2	0.5	25.7	1.3	0.0	27.5	0.5	27.0
Average	38.5	9.1	29.3	0.7	2.5	39.1	11.6	27.5
Std. deviation	6.7	10.2	10.8	2.1	3.7	6.5	10.5	11.0
Std. Error	1.0	1.5	1.6	0.3	0.5	1.0	1.6	1.6

Table C.21. Calculations for mass loading and removal values during the low nitrate loading phase.

Column Crossectional area (m^2) =		0.0182		Flow (L/day)		1307
Media (m^3) =		0.04		Flow (m3/day)		1.307
Nitrate Loadings				**NO_x Loadings**		
Average (mg/L)=		38.5		Average (mg/L)=		39.1
Mass (mg/day) =		50270		Mass (mg/day) =		51125
Mass (kg/day) =		0.05		Mass (kg/day) =		0.051
Nitrate Effluent				**NO_x Effluent**		
Average (mg/L) =		9.13		Average (mg/L) =		11.62
Nitrite Loadings				**NO_x Removal**		
Average (mg/L)=		0.65		Average (mg/L) =		27.5
Mass (mg/day) =		854.86		Average (mg/day) =		35929
Mass (kg/day) =		0.00085		Average (kg/day) =		0.036
Nitrate Removal						
Average (mg/L) =		29.3		Total nitrogen	Mass Loading	Mass Removal
Average (mg/day) =		38332			$kg/m^3/day$	$kg/m^3/day$
Average (kg/day) =		0.04			1.15	0.81
Nitrate	Mass Loading $kg/m^3/day$	Mass Removal $kg/m^3/day$		Nitrite Loading $kg/m^3/day$		
	1.13	0.86		0.02		

Table C.22. Values used to determine the hydraulic loading during the high nitrate loading phase (high hydraulic loading

Date	Time (hr)	TIme (day)	Nitrate Volume (L)	Nitrate Flow (L/day)	Cabon Volume (L)	Cabon Flow (L/day)	Total Volume (L)	Total Flow (L/day)	Hydraulic Loading $m^3/hr/m^2$
3/4-3/5	23.4	0.98	1099	1127	174	178	1273	1306	2.98
3/5-3/6	23.2	0.97	1085	1122	166	172	1251	1293	2.95
3/6-3/7	23.2	0.97	1079	1115	166	172	1245	1287	2.94
3/7-3/8	23.2	0.97	1127	1165	150	155	1277	1320	3.02
3/8-3/9	23.3	0.97	1110	1146	158	163	1268	1309	2.99
3/9-3/10	23.3	0.97	1119	1155	166	171	1285	1326	3.03
3/10-3/11	25.7	1.07	1310	1225	190	178	1500	1403	3.20
3/11-3/12	20.5	0.86	1005	1175	150	175	1155	1350	3.08
3/12-3/13	23.2	0.97	1130	1167	169	175	1299	1342	3.07
3/13-3/14	23.3	0.97	1125	1159	166	171	1291	1330	3.04
3/14-3/15	23.3	0.97	1103	1139	158	163	1261	1302	2.97
3/15-3/16	23.2	0.97	1090	1129	166	172	1256	1301	2.97
3/16-3/17	23.2	0.97	1134	1172	158	163	1292	1336	3.05
3/17-3/18	23.2	0.97	1120	1157	158	163	1278	1320	3.02
3/18-3/19	23.2	0.97	1077	1113	166	172	1243	1285	2.94
3/19-3/20	23.2	0.97	1055	1091	166	172	1221	1263	2.89
3/20-3/21	23.3	0.97	1060	1094	166	171	1226	1266	2.89
3/21-3/22	23.4	0.97	1060	1089	166	170	1226	1259	2.88
3/22-3/23	22.9	0.95	1045	1094	164	172	1209	1266	2.89
3/23-3/24	23.7	0.99	1070	1084	166	168	1236	1252	2.86
3/24-3/25	25.4	1.06	1170	1108	182	172	1352	1280	2.92
3/25-3/26	19.9	0.83	970	1172	142	172	1112	1343	3.07
3/26-3/27	21.6	0.90	1020	1132	158	175	1178	1308	2.99
3/27-3/28	23.4	0.98	1090	1116	141	144	1231	1261	2.88
3/28-3/29	23.3	0.97	1080	1115	158	163	1238	1278	2.92
3/29-3/30	23.4	0.97	1073	1103	158	162	1231	1265	2.89
3/30-3/31	23.0	0.96	1075	1120	158	165	1233	1285	2.93
3/31-4/1	23.1	0.96	1060	1100	125	130	1185	1229	2.81
4/1-4/2	23.1	0.96	1095	1136	158	164	1253	1300	2.97
4/2-4/3	23.2	0.97	1095	1132	150	155	1245	1287	2.94
4/3-4/4	23.3	0.97	1091	1126	158	163	1249	1289	2.94
4/4-4/5	23.1	0.96	1090	1132	158	164	1248	1297	2.96
4/5-4/6	25.1	1.05	1110	1061	158	151	1268	1212	2.77
4/6-4/7	21.3	0.89	880	993	147	166	1027	1159	2.65
4/7-4/8	23.3	0.97	1063	1096	158	163	1221	1259	2.87
4/8-4/9	23.2	0.96	1077	1117	158	164	1235	1280	2.92
4/9-4/10	23.2	0.97	1105	1141	158	163	1263	1305	2.98
4/10-4/11	23.1	0.96	1123	1166	158	164	1281	1330	3.04
4/11-4/12	23.2	0.97	1099	1135	158	163	1257	1298	2.97
4/14-4/15	23.2	0.97	1088	1127	158	164	1246	1291	2.95
4/15-4/16	23.3	0.97	1092	1127	158	163	1250	1290	2.95
4/16-4/17	23.6	0.98	1110	1128	165	168	1275	1296	2.96
4/17-4/18	24.2	1.01	1145	1136	173.5	172	1318.5	1308	2.99
4/18-4/19	22.4	0.93	1063	1141	158	170	1221	1311	2.99
4/19-4/20	23.2	0.96	1110	1151	173.5	180	1283.5	1331	3.04
4/20-4/21	23.2	0.97	1105	1145	168	174	1273	1319	3.01
4/21-4/22	23.2	0.97	1078	1117	168	174	1246	1291	2.95
4/22-4/23	23.0	0.96	1047	1092	158	165	1205	1256	2.87
4/23-4/24	23.2	0.96	1052	1091	158	164	1210	1254	2.87
4/24-4/25	23.5	0.98	1065	1090	158	162	1223	1252	2.86

Table C.22. Continued.

Date	Time (hr)	TIme (day)	Nitrate Volume (L)	Nitrate Flow (L/day)	Cabon Volume (L)	Cabon Flow (L/day)	Total Volume (L)	Total Flow (L/day)	Hydraulic Loading $m^3/hr/m^2$
4/25-4/26	23.8	0.99	1100	1108	165.5	167	1265.5	1274	2.91
4/26-4/27	22.6	0.94	1072	1139	158	168	1230	1307	2.99
4/27-4/28	23.8	0.99	1134	1144	165.5	167	1299.5	1311	3.00
4/28-4/29	22.4	0.93	1035	1111	158	170	1193	1280	2.92
4/29-4/30	23.3	0.97	1097	1132	165.5	171	1262.5	1302	2.97
4/30-5/1	22.6	0.94	1045	1110	158	168	1203	1278	2.92
5/1-5/2	23.6	0.98	1010	1028	168	171	1178	1199	2.74
5/2-5/3	25.6	1.06	1171	1100	168	158	1339	1258	2.87
5/3-5/4	20.9	0.87	970	1112	147	169	1117	1281	2.93
5/4-5/5	23.9	0.99	1110	1116	158	159	1268	1275	2.91
5/5-5/6	22.8	0.95	1115	1176	158	167	1273	1343	3.07
5/6-5/7	24.2	1.01	1188	1178	165	164	1353	1342	3.06
5/7-5/8	22.7	0.95	1125	1189	158	167	1283	1356	3.10
5/8-5/9			1150						
5/9-5/10			1140						
5/10-5/11			1110						
5/11-5/12			1273						
5/12-5/13			857		117				
5/13-5/14	22.9	0.95	1070	1123	150	157	1220	1280	2.92
5/14-5/15	23.2	0.97	1071	1106	158	163	1229	1270	2.90
5/15-5/16	23.2	0.97	1055	1093	158	164	1213	1257	2.87
5/16-5/17	23.5	0.98	1096	1118	158	161	1254	1279	2.92
5/17-5/18	23.1	0.96	1070	1114	158	165	1228	1279	2.92
5/18-5/19	22.3	0.93	1073	1157	158	170	1231	1328	3.03
5/19-5/20	23.1	0.96	1063	1107	158	165	1221	1271	2.90
5/20-5/21	23.3	0.97	1070	1105	158	163	1228	1268	2.90
5/21-5/22	23.3	0.97	1100	1135	158	163	1258	1299	2.97
5/22-5/23	23.3	0.97	1133	1168	158	163	1291	1331	3.04
5/23-5/24	23.1	0.96	1085	1126	158	164	1243	1290	2.95
5/24-5/25	23.6	0.98	1087	1106	158	161	1245	1267	2.89
5/25-5/26	22.8	0.95	1060	1115	125	131	1185	1246	2.85
6/18-6/19	23.3	0.97	1150	1187	158	163	1308	1350	3.08
6/19-6/20	23.3	0.97	920	950	158	163	1078	1113	2.54
6/20-6/21	23.0	0.96	1150	1198	165	172	1315	1370	3.13
6/21-6/22	23.2	0.97	1155	1194	158	163	1313	1357	3.10
6/22-6/23	23.3	0.97	1160	1193	165	170	1325	1363	3.11
6/23-6/24	23.1	0.96	1170	1216	158	164	1328	1381	3.15
6/24-6/25	23.1	0.96	1140	1185	158	164	1298	1350	3.08
6/25-6/26	22.8	0.95	1120	1182	158	167	1278	1348	3.08
6/26-6/27	22.8	0.95	1120	1177	158	166	1278	1343	3.07
6/27-6/28	22.3	0.93	1098	1184	150	162	1248	1346	3.07
6/28-6/29	23.5	0.98	1140	1163	158	161	1298	1324	3.02
6/29-6/30	22.8	0.95	1125	1183	158	166	1283	1350	3.08
6/30-7/1	23.3	0.97	1150	1185	158	163	1308	1348	3.08
7/1-7/2	23.3	0.97	1145	1182	158	163	1303	1345	3.07
7/2-7/3	23.3	0.97	1140	1176	158	163	1298	1339	3.06
7/3-7/4	23.3	0.97	1140	1177	223	230	1363	1407	3.21
7/4-7/5	23.3	0.97	1140	1177	223	230	1363	1407	3.21
7/5-7/6	23.2	0.97			158	164			0.00
7/6-7/7	23.0	0.96	1125	1174	158	165	1283	1339	3.06
7/7-7/8	23.0	0.96	1120	1170	190	199	1310	1369	3.13
7/8-7/9	23.2	0.97	1130	1169	223	231	1353	1400	3.20

Table C.22. Continued.

Date	Time (hr)	TIme (day)	Nitrate Volume (L)	Nitrate Flow (L/day)	Cabon Volume (L)	Cabon Flow (L/day)	Total Volume (L)	Total Flow (L/day)	Hydraulic Loading m3/hr/m2
7/9-7/10	23.3	0.97	1135	1172	223	230	1358	1402	3.20
7/10-7/11	23.2	0.97	1130	1171	223	231	1353	1402	3.20
7/11-7/12	23.2	0.97	1140	1179	223	231	1363	1410	3.22
7/12-7/13	23.2	0.97	1120	1158	223	231	1343	1388	3.17
7/13-7/14	23.3	0.97	1135	1172	223	230	1358	1402	3.20
7/14-7/15	23.2	0.97	1130	1170	223	231	1353	1401	3.20
7/15-7/16	23.2	0.97	1140	1179	223	231	1363	1410	3.22
7/16-7/17	23.2	0.97	1120	1160	223	231	1343	1391	3.18
7/17-7/18	23.1	0.96	1120	1163	223	232	1343	1394	3.18
7/18-7/19	23.1	0.96	1125	1171	223	232	1348	1404	3.21
7/19-7/20	23.0	0.96			223	233			
7/20-7/21	23.1	0.96			223	231			
7/21-7/22	23.0	0.96	1155	1204	223	233	1378	1437	3.28
7/22-7/23	23.3	0.97	1165	1203	223	230	1388	1433	3.27
7/23-7/24	23.5	0.98	1155	1181	223	228	1378	1409	3.22
7/247/25	22.8	0.95	1120	1177	223	234	1343	1412	3.22
7/25-7/26	23.1	0.96	1125	1170	223	232	1348	1402	3.20
7/26-7/27	26.6	1.11	1080	973	223	201	1303	1174	2.68
7/27-7/28	25.1	1.05			223	213			
							Average =	1315	3.0
							Standard deviation =	58.97	0.31
							Standard error =	5.57	0.03

Table C.23. Values used to determine hydraulic loading during the high nitrate loading phase (low hydraulic loading).

Date	Time (hr)	TIme (day)	Nitrate Volume (L)	Nitrate Flow (L/day)	Cabon Volume (L)	Cabon Flow (L/day)	Total Volume (L)	Total Flow (L/day)	Hydraulic Loading $m^3/hr/m^2$
5/26-5/27	23.0	0.96	500	522	103	107	603	629	1.44
5/27-5/28	23.4	0.97	555	570	82	84	637	654	1.49
5/28-5/29	23.2	0.97	590	611	71	74	661	685	1.56
5/29-5/30	23.3	0.97	495	510	92	95	587	605	1.38
5/30-5/31	23.3	0.97	540	557	103	106	643	664	1.52
5/31-6/1	24.6	1.02	650	635	103	101	753	735	1.68
6/1-6/2	21.9	0.91	510	558	92	101	602	659	1.51
6/2-6/3	23.3	0.97	510	526	103	106	613	633	1.45
6/3-6/4	23.3	0.97	555	573	92	95	647	668	1.53
6/4-6/5	23.3	0.97	517	534	92	95	609	629	1.44
6/5-6/6	23.3	0.97	580	599	92	95	672	694	1.58
6/6-6/7	23.5	0.98	495	505	103	105	598	610	1.39
6/7-6/8	22.6	0.94	537	570	92	98	629	667	1.52
6/8-6/9	23.1	0.96	513	534	92	96	605	629	1.44
6/9-6/10	22.6	0.94	530	562	103	109	633	672	1.53
6/10-6/11	23.1	0.96	550	571	103	107	653	677	1.55
6/11-6/12	23.2	0.97	555	573	103	106	658	680	1.55
6/12-6/13	23.1	0.96	550	572	103	107	653	679	1.55
6/13-6/14	23.5	0.98	528	538	92	94	620	632	1.44
6/14-6/15	22.7	0.95	536	566	103	109	639	675	1.54
6/15-6/16	23.3	0.97	535	550	92	95	627	645	1.47
6/16-6/17	23.0	0.96	550	574	103	107	653	681	1.56
6/17-6/18	23.3	0.97	530	547	81	84	611	631	1.44
							Average =	658	1.50
							Standard deviation =	30.38	0.07
							Standard error =	6.33	0.01

Table C.24. Values used to determine mass loading and removal during the high nitrate loading phase (high hydraulic loading).

	NO_3			NO_2		NO_x		
Date	Influent mg/L as N	Effluent mg/L as N	Removed mg/L as N	Influent mg/L as N	Effluent mg/L as N	Influent mg/L as N	Effluent mg/L as N	Removed mg/L as N
3/5/97	61.5	5.9	55.6	2.2	19.7	63.7	25.6	38.1
3/6/97	52.7	0.4	52.3	1.3	8.5	54.0	9.0	45.0
3/12/97	68.3	0.7	67.6	3.5	8.0	71.8	8.7	63.1
3/17/97	93.3	0.1	93.2	0.0	7.3	93.3	7.4	85.9
3/19/97	96.8	0.2	96.7	8.9	26.8	105.8	27.0	78.8
3/22/97	85.7	0.4	85.3	2.9	9.7	88.6	10.1	78.5
3/24/97	84.3	0.1	84.2	1.9	5.4	86.2	5.5	80.7
3/26/97	93.3	0.0	93.3	2.6	22.8	95.9	22.8	73.1
4/3/97	94.7	0.0	94.7	0.0	22.4	94.7	22.4	72.3
4/5/97	71.9	0.0	71.9	14.3	6.3	86.2	6.3	79.9
4/6/97	93.2	0.0	93.2	0.0	25.0	93.2	25.0	68.2
4/10/97	90.4	0.0	90.4	3.3	27.1	93.7	27.1	66.6
4/12/97	92.0	0.8	91.2	0.0	31.4	92.0	32.2	59.9
4/14/97	93.4	0.6	92.8	0.0	26.6	93.4	27.2	66.2
4/15/97	91.7	0.8	90.9	0.0	29.2	91.7	30.0	61.7
4/19/97	94.7	0.6	94.1	0.0	33.2	94.7	33.8	61.0
4/20/97	93.3	0.6	92.6	3.3	30.3	96.6	30.9	65.7
4/21/97	88.7	0.2	88.5	5.4	25.8	94.1	26.0	68.1
4/23/97	78.7	0.2	78.5	3.7	30.8	82.4	31.0	51.4
4/29/97	70.4	0.2	70.2	6.1	9.2	76.5	9.4	67.1
4/30/97	61.9	0.2	61.7	7.4	17.8	69.3	17.9	51.3
5/8/97	88.2	0.2	88.0	3.9	23.5	92.1	23.7	68.3
5/14/97	86.9	0.4	86.6	3.5	27.6	90.5	27.9	62.5
5/15/97	80.8	0.5	80.3	4.2	21.7	85.0	22.2	62.8
5/17/97	81.6	0.5	81.1	0.8	30.4	82.4	30.9	51.5
5/18/97	86.2	0.3	85.9	4.3	18.2	90.5	18.5	71.9
5/19/97	81.5	0.2	81.3	4.8	24.8	86.2	25.0	61.2
5/22/97	88.6	0.2	88.4	3.2	13.7	91.8	13.9	77.9
5/24/97	94.7	0.2	94.5	8.5	2.2	103.3	2.4	100.8
5/25/97	98.9	0.1	98.8	2.7	0.0	101.6	0.1	101.5
5/26/97	71.3	0.2	71.2	3.5	18.2	74.9	18.4	56.5
6/23/97	72.2	0.0	72.2	9.4	0.0	81.7	0.0	81.7
6/24/97	86.7	0.0	86.7	8.0	0.0	94.7	0.0	94.7
6/27/97	80.6	1.1	79.4	0.8	1.1	81.4	2.2	79.1
6/28/97	125.6	0.8	124.8	3.2	0.0	128.8	0.8	128.0
7/1/97	84.1	0.3	83.8	0.2	0.0	84.3	0.3	84.0
7/2/97	86.4	0.3	86.1	0.0	0.0	86.4	0.3	86.1
7/4/97	84.7	0.5	84.2	0.0	0.0	84.7	0.5	84.2
7/5/97	84.9	0.4	84.5	0.0	0.0	84.9	0.4	84.5
7/7/97	91.2	0.2	91.0	2.7	0.0	93.8	0.2	93.7
7/8/97	87.5	0.2	87.3	0.0	0.0	87.5	0.2	87.3
7/9/97	86.1	0.2	86.0	3.7	0.0	89.9	0.2	89.7
7/11/97	82.6	0.2	82.4	1.4	0.0	83.9	0.2	83.8
7/18/97	90.1	4.5	85.6	1.1	0.0	91.2	4.5	86.7
7/24/97	90.8	2.5	88.3	0.1	0.0	90.9	2.5	88.4
7/27/97	81.8	0.8	81.0	0.9	1.0	82.7	1.7	81.0
7/28/97	82.5	0.7	81.7	0.3	0.4	82.8	1.2	81.6
Average	85.3	0.6	84.7	2.9	12.9	88.2	13.5	74.7
Std. Dev.	11.6	1.1	11.8	3.1	12.1	11.3	12.1	16.4
Std. Error	1.7	0.2	1.7	0.5	1.8	1.7	1.8	2.4

Table C.24. Values used to determine mass loading and removal during the high nitrate loading phase (low hydraulic loading).

	NO_3			NO_2		NO_x		
Date	Influent mg/L as N	Effluent mg/L as N	Removed mg/L as N	Influent mg/L as N	Effluent mg/L as N	Influent mg/L as N	Effluent mg/L as N	Removed mg/L as N
5/27/97	44.6	0.2	44.4	7.9	0.0	52.5	0.2	52.3
5/28/97	46.0	0.5	45.5	10.1	0.0	56.1	0.5	55.6
5/29/97	72.1	0.0	72.1	16.8	0.0	88.9	0.0	88.9
5/31/97	82.8	0.4	82.3	3.8	0.0	86.6	0.4	86.2
6/2/97	60.1	0.5	59.6	14.0	0.0	74.1	0.5	73.6
6/3/97	76.5	0.5	75.9	8.1	0.0	84.6	0.5	84.1
6/5/97	84.5	0.2	84.4	6.7	0.0	91.3	0.2	91.1
6/8/97	24.1	0.2	23.9	64.1	33.8	88.2	34.0	54.2
6/10/97	78.3	0.0	78.3	5.9	4.8	84.2	4.8	79.4
6/16/97	79.0	0.0	79.0	8.9	21.6	87.9	21.6	66.4
Average	64.8	0.2	64.6	14.6	6.0	79.4	6.3	73.2
Std. Dev.	20.3	0.2	20.4	17.8	11.9	14.1	11.8	15.1
Std. Error	6.4	0.1	6.4	5.6	3.8	4.4	3.7	4.8

Table C.25. Calculations for mass loading and removal values during the low nitrate loading phase (high hydraulic loading).

Crossectional area (m^2) =	0.0182					
Media (m^3) =	0.04					
Flow (L/day)	1315					
Flow (m3/day)	1.315					
Nitrate Loadings		Nitrate		**NO_x Loadings**		
Average (mg/L)=	85.3	Mass	Mass	Average (mg/L)=		88.2
Mass (mg/day) =	112121	Loading	Removal	Mass (mg/day) =		115984
Mass (kg/day) =	0.11	kg/m3/day	kg/m3/day	Mass (kg/day) =		0.116
Nitrate Effluent		2.52	2.50	**NO_x Effluent**		
Average (mg/L) =	0.58			Average (mg/L) =		13.48
Nitrate Removal		Nitrite		**NO_x Removal**		
Average (mg/L) =	84.7	Mass		Average (mg/L) =		74.7
Average (mg/day) =	111352	Loading		Average (mg/day) =		98
Average (kg/day) =	0.11	kg/m3/day		Average (kg/day) =		0.000
Nitrite Loadings		0.09				
Average (mg/L)=	2.9			Nitrogen	Mass	Mass
Mass (mg/day) =	3862.50				Loading	Removal
Mass (kg/day) =	0.0039				kg/m3/day	kg/m3/day
					2.61	0.00

Table C.26. Calculations for mass loading and removal values during the low nitrate loading phase (low hydraulic loading).

Crossectional area (m^2) =	0.0182			Flow (L/day)		658
Media (m^3) =	0.04			Flow (m3/day)		0.658
Nitrate Loadings				**NO_x Loadings**		
Average (mg/L)=	64.8			Average (mg/L)=		79.4
Mass (mg/day) =	42639			Mass (mg/day) =		52271
Mass (kg/day) =	0.04			Mass (kg/day) =		0.052
Nitrate Effluent				**NO_x Effluent**		
Average (mg/L) =	0.25			Average (mg/L) =		6.27
Nitrate Removal				**NO_x Removal**		
Average (mg/L) =	64.6			Average (mg/L) =		73.2
Average (mg/day) =	42477			Average (mg/day) =		48148
Average (kg/day) =	0.04			Average (kg/day) =		0.048
Nitrite Loadings						
Average (mg/L)=	14.6			Total	Mass	Mass
Mass (mg/day) =	9632			nitrogen	Loading	Removal
Mass (kg/day) =	0.010				kg/m3/day	kg/m3/day
Nitrate					1.18	1.08
Mass Loading kg/m3/day	Mass Removal kg/m3/day	Nitrite Loading kg/m3/day				
0.96	0.95	0.22				

Table C.27. Determination of average pH for fermentation process during the low nitrate loading phas

Date	pH - F1	Date	pH - F1	pH - F2	
8/12/96	7.5	11/17/96	6		Average pH
8/13/96	5.3	11/18/96	6.2		6.31
8/14/96	5.8	11/19/96	6		
8/15/96	5.8	11/20/96	6.04		Std. err.
8/17/96	5.8	11/25/96	6.12	6.74	0.06
8/18/96	5.8	11/26/96	6.28	6.87	
8/19/96	5.7	11/30/96	6.00		
8/20/96	5.8	12/1/96		6.88	
8/21/96	5.7	12/2/96	5.88		
8/28/96	5.3	12/3/96		6.99	
8/29/96	6	12/4/96	5.91		
8/30/96	5.5	12/5/96		7.16	
9/1/96	5.4	12/6/96		7.32	
9/2/96	5.5	12/7/96	5.74		
9/4/96	4.5	12/8/96	5.99		
9/5/96	4.7	12/9/96		7.37	
9/6/96	5.2	12/10/96	6.06		
9/10/96	6	12/11/96		6.77	
9/12/96	6	12/12/96	5.93		
9/13/96	5.8	12/13/96		6.88	
9/16/96	5.6	12/14/96		6.99	
9/26/96	5.7	12/15/96	6.14	6.96	
10/2/96	5.7	12/18/96	6.40		
10/3/96	5.8	12/19/96	6.51	6.86	
10/4/96	6	12/20/96	6.42		
10/5/96	6	12/21/96	6.50	6.60	
10/6/96	6.2	12/22/96	6.69		
10/7/96	6.2	12/27/96	6.54	7.01	
10/8/96	6.2	12/29/96		7.10	
10/9/96	6.1	12/30/96		6.83	
10/12/96	6	12/31/96		6.78	
10/14/96	6	1/1/97	6.45		
10/15/96	6.2	1/2/97		6.65	
10/16/96	6.1	1/5/97		6.31	
10/17/96	6.3	1/6/97		6.47	
10/21/96	6.2	1/19/97	6.33		
10/24/96	6.07	1/20/97		7.11	
10/25/96	6.07	2/7/97		7.00	
10/29/96	6.01	2/10/97	6.60		
10/31/96	6.15	2/12/97	7.13		
11/2/96	6.18	2/16/97	7.50		
11/3/96	6.19	2/17/97		7.00	
11/7/96	6.1	2/18/97	7.30		
11/8/96	5.87	2/19/97		7.20	
11/9/96	5.73	2/21/97	7.30	7.00	
11/10/96	5.8	2/22/97	7.20		
11/11/96	5.91	2/23/97	7.40	7.30	
11/14/96	6.04	2/25/97		7.30	
		2/27/97		7.30	

Table C.28. Determination of average pH for fermentation process during the high nitrate loading phase.

Date	pH - F1	pH - F2	Date	pH - F1	pH - F2	
3/4/97	7.30		5/22/97			
3/5/97		7.00	5/23/97	7.5	7.5	Average pH
3/6/97	7.20		5/24/97			7.41
3/7/97		7.30	5/28/97		7	
3/8/97	7.30		5/30/97		7.4	Std. err.
3/9/97		7.30	5/31/97	7.5	7.5	0.02
3/10/97	7.30		6/2/97	7.6		
3/12/97	7.30	7.30	6/4/97	7.6		
3/13/97	7.30	7.30	6/5/97			
3/14/97	7.50		6/10/97	7.8	7.8	
3/15/97		7.30	6/11/97			
3/17/97	7.30	7.40	6/15/97		7.6	
3/18/97	7.40		6/17/97		7.6	
3/19/97		7.40	6/19/97		7.6	
3/21/97		7.4	6/23/97		7.5	
3/22/97	7.2		6/24/97	7.77	7.6	
3/23/97		7.4	6/26/97	7.7		
3/24/97	7.4		6/28/97	7.5		
3/26/97	7		7/1/97		7.5	
3/29/97		7.3	7/3/97		7.4	
3/30/97	7.4		7/4/97	7.4	7.3	
3/31/97		7.2	7/5/97			
4/1/97	7.5		7/7/97		7.4	
4/2/97		7.5	7/10/97	7.4	7.4	
4/3/97	7.5		7/11/97			
4/5/97	7.3		7/12/97	7.5	7.4	
4/6/97		7.5	7/14/97	7.6		
4/9/97	7.6		7/16/97	7.4		
4/10/97		7.4	7/20/97	7.5		
4/12/97		7.4	7/21/97			
4/13/97	7.3		7/23/97		7.6	
4/14/97		7.3	7/24/97		7.5	
4/15/97	7.4	7.2	7/26/97	7.6	7.6	
4/16/97			7/27/97			
4/17/97	7.4				7.4	
4/20/97		7.3				
4/21/97	7.4					
4/22/97		7.3				
4/23/97	7.3					
4/27/97	7.3					
4/29/97	7.5					
5/8/97	7.3					
5/14/97		7.1				
5/15/97	7.2					
5/16/97		7.3				
5/17/97	7.5					
5/18/97		7.2				
5/21/97	7.5					

Table C.29. Determination of soluble COD produced per unit weight of food when only one fermenter was in operation.

Date	Day of Study	Cumulative[a] feed (kg)	COD[b] mg/L	COD mg	Cumulative[c] feed (kg)	COD/food mg/L per g	COD/food mg/g
8/12/96	-4	2.00			2		
8/13/96	-3	2.00			2		
8/14/96	-2	2.00	1091	490909	2		
8/15/96	-1	2.00	1636	736364	Started wasting		368
8/22/96	6	7.00	1636	736364	2.04	0.80	361
8/25/96	9	8.99	1851	832792	2.01	0.92	414
8/26/96	10	9.66	1461	657468	2.01	0.73	328
8/29/96	13	11.66	1543	694286	2.00	0.77	347
8/30/96	14	12.32	2010	904675	2.00	1.01	452
9/3/96	18	14.99	1519	683766	2.00	0.76	342
9/10/96	25	19.65	2922	1314935	2.00	1.46	658
9/12/96	27	20.98	2143	964286	2.00	1.07	483
9/14/96	29	22.31	1465	659221	2.00	0.73	330
9/30/96	45	32.97	1418	638182	2.00	0.71	319
10/7/96	52	37.63	1590	715325	2.00	0.80	358
10/8/96	53	38.30	1245	560136	2.00	0.62	280
10/9/96	54	38.96	1330	598576	2.00	0.67	300
10/11/96	56	40.30	1249	561966	2.00	0.63	281
10/13/96	58	41.63	1240	557822	2.00	0.62	279
10/14/96	59	42.29	1291	580990	2.00	0.65	291
10/15/96	60	42.96	1412	635186	2.00	0.71	318
10/20/96	65	46.29	1272	572185	2.00	0.64	286
10/23/96	68	48.29	1232	554400	2.00	0.62	277
10/26/96	71	50.29	1017	457650	2.00	0.51	229
10/29/96	74	52.28	1319	593550	2.00	0.66	297
11/10/96	86	60.28	1041	468450	2.00	0.52	234
11/18/96	94	65.60	1057	475650	2.00	0.53	238
11/23/96	99	69.03	1359	611550	2.02	0.67	303
		Average =	1475			0.74	334
		Standard deviation =	406			0.21	91.4
		Standard error =	78			0.0	18.7

[a]Weight of feed added to fermenter, disregarding wasting.

[b]COD measured prior to fish food addition and wasting on given day

[c]Weight of feed in fermenter, incorporating wasting.

Table C.30. Determination of soluble COD produced per unit weight of food when both fermenters were in operation.

Date	Cumulative[a] feed (kg)	COD[b] mg/L	COD mg	Cumulative[c] feed (kg)	COD/food mg/L per g	COD/food mg/g
Fermenter 1						
11/26/96	71.03	948	426774	2.00	0.47	212.977
11/27/96	71.03	213	95806			
12/3/96	73.33	40	18000			
12/4/96	74.10	615	276835	3.16	0.19	88
12/10/96	76.27	911	410127	3.29	0.28	124
12/12/96	77.03	891	401042	3.37	0.26	119
12/14/96	77.79	741	333376	3.44	0.22	97
12/20/96	80.01	1003	451139	3.47	0.29	130
1/5/97	85.38	913	410844	3.24	0.28	127
1/19/97	90.98	738	331948	2.89	0.26	115
2/8/97	97.38	1081	486346	3.44	0.31	141
2/14/97	99.73	1018	457975	3.38	0.30	135
2/16/97	100.53	1374	618135	3.45	0.40	179
3/12/97	113.13	2155	969868	5.46	0.39	178
3/18/97	117.33	2564	1153636	6.00	0.43	192
3/22/97	120.13	2338	1052039	6.31	0.37	167
3/30/97	125.33	1359	611650	6.59	0.21	93
4/1/97	126.59	2189	985016	6.89	0.32	143
4/5/97	129.04	2345	1055375	6.39	0.37	165
4/9/97	131.54	2059	926733	6.15	0.33	151
4/15/97	135.74	1485	668404	6.29	0.24	106
4/29/97	145.74	2158	970945	6.75	0.32	144
5/13/97	156.24	1149	516832	6.88	0.17	75
5/21/97	162.24	2064	928730	6.87	0.30	135
5/27/97	167.04	2352	1058614	7.46	0.32	142
6/10/97	175.74	1830	823366	4.94	0.37	167
6/24/97	189.54	4119	1853465	9.10	0.45	204
7/2/97	199.54	4293	1931881	10.44	0.41	185
7/24/97	231.54	2883	1297426	14.81	0.19	88
Fermenter 2						
12/15/96	1183	1	691	0.95	0.72	326
12/19/96	1933	2	604	1.26	0.48	215
12/27/96	2583	3	469	0.98	0.48	215
12/29/96	2883	3	501	1.08	0.46	209
1/6/97	4583	5	679	1.66	0.41	185
1/22/97	10166	10	1587	3.23	0.49	221
2/3/97	14966	15	1800	3.68	0.49	220
2/17/97	19766	20	1762	2.84	0.62	279
2/21/97	21366	21	1080	3.21	0.34	152
2/23/97	22166	22	1034	3.03	0.34	154
3/13/97	32366	32	2571	5.73	0.45	202
3/17/97	35166	35	2345	6.00	0.39	176
3/21/97	37966	38	2306	6.32	0.36	164
3/27/97	42166	42	2408	7.10	0.34	153
3/29/97	43416	43	2151	6.72	0.32	144
4/2/97	45916	46	2048	6.18	0.33	149
4/6/97	48316	48	2004	5.90	0.34	153
4/20/97	57816	58	1964	6.28	0.31	141
5/8/97	71016	71	1650	5.86	0.28	127
5/14/97	75516	76	1909	5.55	0.34	155
5/22/97	81516	82	2517	6.39	0.39	177
5/28/97	86316	86	2689	6.61	0.41	183
6/5/97	91116	91	2067	5.24	0.39	177
6/11/97	94716	95	1861	5.23	0.36	160
6/23/97	107316	107	4212	8.23	0.51	230
7/7/97	131316	131	4071	16.66	0.24	110
7/27/97	157816	158	2519	18.70	0.13	61
low nitrate loading					Average	172
					Standard error	14
high nitrate loading					Average	151
					Standard error	6.8
low and high nitrate loading					Average	159
					Standard error	6.8

[a]Weight of feed added to fermenter, disregarding wasting.

[b]COD measured prior to fish food addition and wasting on given day

[c]Weight of feed in fermenter, incorporating wasting.

Table C.30. Determination of column pH.

Low nitrate loading phase				High nitrate loading phase			
	pH				pH		
Date	Influent	Effluent	Change	Date	Influent	Effluent	Change
10/7/96	7.00	7.60	0.60	3/5/97	7.30	8.40	1.10
10/8/96	7.00	7.70	0.70	3/17/97	7.10	8.50	1.40
10/26/96	7.22	8.45	1.23	3/19/97	7.40	8.70	1.30
10/29/96	7.07	7.70	0.63	3/22/97	7.20	8.70	1.50
11/7/96	7.28	7.76	0.48	3/24/97	7.40	8.80	1.40
12/1/96	6.74	7.23	0.49	4/3/97	7.20	8.80	1.60
12/3/96	6.54	7.05	0.51	4/5/97	7.40	8.40	1.00
12/4/96	6.86	7.00	0.14	4/6/97	7.40	8.70	1.30
12/5/96	6.36	7.11	0.75	4/10/97	7.10	8.40	1.30
12/6/96	7.44	6.97	-0.47	4/12/97	7.20	8.40	1.20
12/19/96	6.57	7.30	0.73	4/14/97	7.20	8.30	1.10
12/21/96	6.94	7.42	0.48	4/15/97	7.10	8.20	1.10
12/27/96	6.72	7.25	0.53	4/20/97	7.30	8.20	0.90
1/5/97	7.00	7.91	0.91	4/21/97	7.20	8.10	0.90
1/18/97	7.49	8.03	0.54	4/23/97	7.20	8.10	0.90
1/19/97	6.81	8.42	1.61	4/29/97	7.50	8.50	1.00
1/29/97	7.05	8.77	1.72	4/30/97	7.20	8.60	1.40
2/7/97	6.96	7.81	0.85	5/14/97	7.20	8.20	1.00
2/16/97	7.60	8.60	1.00	5/15/97	7.10	8.50	1.40
2/17/97	7.30	8.10	0.80	5/17/97	7.20	8.30	1.10
2/20/97	7.30	8.80	1.50	5/18/97	7.40	8.70	1.30
2/21/97	7.40	8.50	1.10	5/19/97	7.30	8.40	1.10
2/23/97	7.50	8.50	1.00	5/22/97	7.40	9.00	1.60
2/25/97	7.40	8.80	1.40	5/24/97	7.20	8.80	1.60
3/2/97	7.40	8.00	0.60	5/25/97	6.80	8.00	1.20
Average	7.08	7.87	0.79	5/26/97	7.00	8.40	1.40
Std err.	0.07	0.12	0.10	5/28/97	7.50	8.90	1.40
				5/31/97	7.40	8.70	1.30
				6/2/97	7.40	8.80	1.40
				6/8/97	7.40	8.40	1.00
				6/10/97	7.60	9.10	1.50
				6/23/97	7.50	8.90	1.40
				6/24/97	7.60	9.00	1.40
				6/27/97	7.50	8.90	1.40
				6/28/97	7.50	9.00	1.50
				7/2/97	7.70	8.90	1.20
				7/4/97	7.30	8.40	1.10
				7/7/97	7.90	8.70	0.80
				7/8/97	7.50	8.60	1.10
				7/11/97	7.20	8.30	1.10
				7/27/97	7.40	8.70	1.30
				7/28/97	7.60	9.20	1.60
				Average	7.33	8.59	1.25
				Std err.	0.20	0.30	0.22

VITA

Jennifer Brooke Phillips was born in Washington, D.C. on September 22, 1969. She attended Yorktown High School in Arlington, Virginia and graduated in June 1987. She then pursued a bachelor of science degree in Civil Engineering at the University of Virginia and graduated in May 1992. After working at VíGYAN Incorporated until 1995, she enrolled in the graduate program at the Virginia Polytechnic Institute to pursue a Master of Science degree in Environmental Engineering. She successfully defended her thesis on December 17, 1997.