

**NITROGEN REMOVAL FROM DAIRY MANURE WASTEWATER
USING SEQUENCING BATCH REACTORS**

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(ABSTRACT)

The purpose of this research was to characterize a flushed dairy manure wastewater and to develop the kinetic and stoichiometric parameters associated with nitrogen removal from the wastewater, as well as to demonstrate experimental and simulated nitrogen removal from the wastewater. The characterization showed that all the wastewaters had carbon to nitrogen ratios large enough for biological nitrogen removal. Analysis of carbon to phosphorus ratios showed that enough carbon is available for phosphorus removal but enough may not be available for both nitrogen and phosphorous removal in anaerobically pretreated wastewater. In addition, kinetic and stoichiometric parameters were determined for the biological nitrogen removal in sequencing batch reactors for the dairy manure wastewater. Results showed that many parameters are similar to those of municipal wastewater treatment systems. This characterization and the derived kinetic and stoichiometric parameters provided some of the information necessary for development of a nitrogen removal process in a sequencing batch reactor. Lab scale treatment of a 1:2 dilution of the anaerobically pretreated wastewater was demonstrated. Treatment was able to achieve between 89 and 93% removal of soluble inorganic nitrogen as well as up to 98% removal of biodegradable soluble and colloidal COD. In addition, a solids removal efficiency of between 79 and 94% was achieved. The lab scale treatment study demonstrated that sequencing batch reactors are capable of achieving high nitrogen removal on wastewaters with the carbon

to nitrogen ratios of the dairy manure wastewater. Model simulations of the treatment process were used to develop a sensitivity analysis of the reactor feed configuration as well as the kinetic and stoichiometric parameters. The analysis of the feed configuration demonstrated the advantage of decreasing the amount of feed that is fed in the last feed period so that the effluent nitrate will be minimized. The analysis indicated that the autotrophic growth rate is one of the most important parameters to measure while error in the heterotrophic decay or yield values can lead to miscalculations of oxygen required for treatment.

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LIST OF ABBREVIATIONS

Ammonia oxidizing bacteria	AOB
Activated sludge model	ASM
Confined animal feeding operations	CAFOs
Chemical oxygen demand	COD
Continuous flow stirred tank reactor	CSTR
Dissolved oxygen	DO
Dissolved organic carbon	DOC
Environmental Protection Agency	EPA
Feed to microbe ratio	F:M
Free ammonia	FA
Fixed suspended solids	FSS
Hydraulic retention time	HRT
Inert suspended solids	ISS
Modified Ludzack-Ettinger	MLE
Mixed liquor suspended solids	MLSS
Nitrite oxidizing bacteria	NOB
Oxygen uptake rate	OUR
Sequencing batch reactor	SBR
Simultaneous nitrification-denitrification	SND
Solids retention time	SRT
Total suspended solids	TSS
United States Department of Agriculture	USDA
Volatile suspended solids	VSS

I. INTRODUCTION

Historically, the major emphasis of environmental regulation and cleanup has been focused on municipal and industrial wastes. Now, agricultural wastes are increasingly being recognized as a major source of pollution. In recent years the U.S. Environmental Protection Agency (EPA) has developed several programs and regulations dealing directly with strategies associated with the control of agricultural wastes. Some of these include the Unified National Strategy for Animal Feeding Operations cosponsored by the EPA and the USDA, as well as the EPA's Strategy for Addressing Environmental Public Health Impacts from Concentrated Animal Feeding Operations (Shepard, 2000). The U.S. is not alone in its concern about agricultural wastes. Other countries including Canada and the Netherlands are conducting research and implementing regulations targeted at agricultural wastes (Zebarth *et al.*, 1999; Oenema and Roest, 1998).

Among the most significant pollutants in agricultural wastes are the nutrients nitrogen and phosphorous. Nitrogen can be lost into the environment from agricultural areas in three main ways. These are nitrate leaching into groundwater, surface runoff of nitrogen compounds into waterways, and gaseous losses of ammonia and nitrous oxide (Schmitt *et al.*, 1999). A main source of the nitrogen that is lost from agricultural wastes is animal manure. Nutrients from manure are used as a fertilizer by spreading the manure on cropland. Application of manure in excess of crop needs can lead to nitrogen losses. A trend towards the larger and more concentrated animal production facilities contributes to the problem. Confined animal feeding operations (CAFOs) pose a problem in this area. When CAFOs are placed in locations where there is little land available for crop production, then few options exist for reusing the manure

on-site. Relying on imported feed rather than producing feed from on-site crops can lead to an overapplication of manure nutrients on the crops that are produced.

In Virginia, animal wastes are a significant source of pollution. In 1985, Virginia and other states along the Chesapeake Bay committed to lowering controllable nutrient losses to the Bay by 40% by the year 2000 (Randall and Cokgor, 2000). This is because Chesapeake Bay has been significantly impacted by pollution from nutrients. Dairy is one of the major agricultural industries in Virginia. Virginia dairy farmers maintain about 119,000 dairy cows (USDA-NASS, 2001) which produce a total of more than 2.7 billion kilograms of manure waste per year, including more than 12 million kilograms of nitrogen (based on data from Van Horn *et al.*, 1994). Given the concern with agricultural wastes in Virginia and around the world, it is likely that the dairy industry and other livestock industries will face increasingly stringent environmental legislation regulating nutrient applications and losses in the near future.

The new Virginia Tech Dairy Center presented an opportunity to develop treatment strategies for removing nitrogen from manure wastewater. The new dairy will incorporate advanced methods for managing the manure wastes, and will include a manure flushing and collection system, screening of the flushed manure to remove some of the solids, gravity settling, and currently undefined biological treatment. The treated water will be recycled and used to supplement flushing water needs in the barn.

Nitrogen removal systems have been used in municipal and industrial wastewater treatment systems for a number of years. Most of these systems are large, with several reactors required for treatment. It is unlikely that a dairy farm would be in a position to support a large treatment system with many reactors, based both on land limitations and cost. Research has been done recently on biological nitrogen removal in piggery wastes with sequencing batch reactors

(SBR) (Andreottola *et al.*, 1996; Edgerton *et al.*, 2000; Fernandes *et al.*, 1991). SBRs may be feasible for animal waste treatment because they require decreased land area and cost. In addition, the discontinuous nature of dairy manure wastewater collection at a farm lends itself to SBR treatment. It should be noted that design of such a biological nitrogen removal system would be specific to a particular farm because of the high variability in the parameters that are involved in developing effluent guidelines for the reactor. Some of these parameters include the soil characteristics where the wastewater is to be applied, the type and amount of crops grown on the farm, number of animals contributing to the wastewater, and the diet of the animals on the farm.

There is little information on treatment methods incorporating nitrogen removal that have been developed specifically for treating dairy manure. In addition, when computer simulations were used to develop optimum treatment configurations for the piggery wastes, kinetic and stoichiometric parameters developed from municipal wastewater treatment were used. Finally, little has been done to characterize flushed dairy manure in the literature. Therefore, this research effort was undertaken to address the following objectives:

1. Characterize flushed and screened dairy manure wastewaters based on their pollutant concentrations, including solids, ammonia, organic nitrogen, phosphorus, and chemical oxygen demand,
2. determine kinetic and stoichiometric parameters associated with biological nitrogen removal from dairy wastewater,
3. demonstrate lab-scale treatment of the dairy wastewater in SBRs and determine the efficiency of treatment, and
4. use simulations to determine the sensitivity of nitrogen removal in SBRs to the kinetic

and stoichiometric parameters and the reactor configuration.

II. LITERATURE REVIEW

Introduction

Characterization of wastewater is an important part of the initial work in the design of a treatment process. Therefore, a short review of dairy manure wastewater characterization studies has been included. In addition, the basics of biological nitrogen removal are discussed and include the actual biological transformations of nitrogen as well as the strategies used in wastewater treatment. Because of the importance of sequencing batch reactors to this research, an in depth review of the SBR has been included. Also, a brief synopsis of nitrification inhibition is included because of the impact it can have on treatment schemes and nitrogen removal efficiency. Finally, computer modeling of biological processes used in wastewater treatment is reviewed to provide background for the simulation and design of the treatment process studied in this research project.

Dairy Manure Wastewater Characteristics

Wastewater characterization is essential to the design of a treatment process. Generally, domestic wastewater has similar concentrations of pollutant constituents in different municipalities and only varies due to industrial content. However, the organic and nutrient content of dairy manure wastewaters depends on the size, lactation cycle, and diet of the cow. In addition, dairy wastewater composition is significantly influenced by the waste collection method and any solids removal methods that are used. Most of the data in the literature is based on manure “as excreted.” The American Society of Agricultural Engineers (1990) has developed a table of “as excreted” data that is frequently used to predict dairy manure composition for developing plans for waste application to land. However, “as excreted” data is specific to the diet of the cow and does not consider any solids removal. “As excreted” data

presented by Van Horn et al. (1994) varies up to 20% for nitrogen and up to 100% for phosphorus. Because the excreted nutrients are equal to the intake minus the nutrients produced in the milk, the diet of the animals has a major effect on the excreted values.

Some research has been done on the effect of screening animal manures. Holmberg *et al.* (1983) separated flushed swine manure using different screen diameters and flow rates, which resulted in widely varying effluents. Organic solids removal varied from 14 to 70%, nitrogen removal from 2.5 to 50.9%, and phosphorus removal from 2.5 to 58.9%. Powers (1993) presented data on the removal of solids through settling for flushed dairy manures showing removal of 65% of the solids and 40% of the nitrogen. Thus, even when using the same “as excreted” manure, the wastewater characteristics are very specific to the particular methods of collection and separation as well as the operation of those devices. Given the many methods available to collect wastes and separate solids, there is limited data on the composition of flushed dairy wastewaters exposed to sequential screening and settling, which pertains to this research. Therefore, it is important to characterize the particular wastewater that is going to be used as influent in the treatment process.

Biological Nitrogen Removal

The two most widely used methods for removing nitrogen from wastewater are physical and biological. The screening and settling processes mentioned earlier are physical ways to remove organic nitrogen bound in suspended solids. While this has the potential to remove a significant fraction of the nitrogen in wastewaters, Van Horn *et al.* (1994) point out several studies which show that much of the nutrients including nitrogen remain in the wastewater even after screening and/or settling. A screening study by Powers (1993) showed less than 10% removal of nitrogen and less than 5% removal of phosphorous through screening of dairy

manure. While solids removal can remove some nutrients, it cannot remove most of the nutrients including the large fraction of nitrogen that is soluble. This leaves biological treatment as the next choice in nitrogen removal.

Biological Transformations of Nitrogen

Three major biological processes directly involved with biological nitrogen removal in wastewater treatment are ammonification, nitrification, and denitrification. Figure 1 shows the interaction between the three processes in what is known as the Nitrogen Cycle.

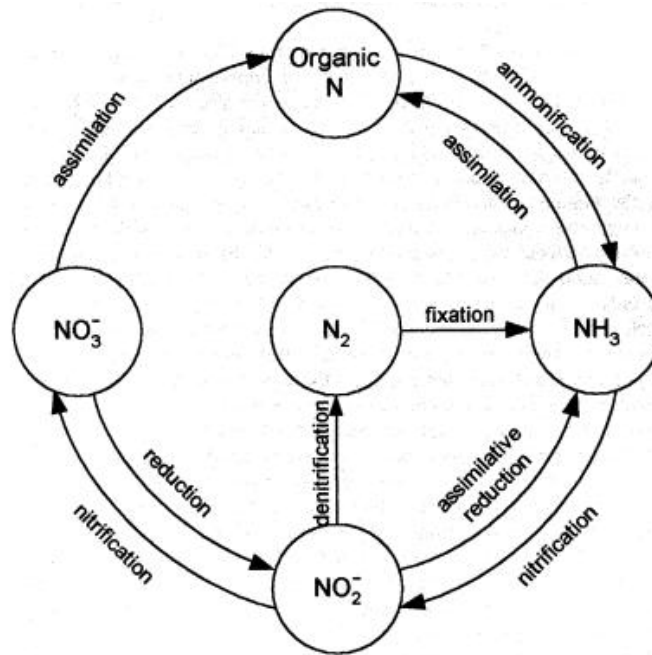


Figure 1. The Nitrogen Cycle in Wastewater Treatment (Grady *et al.*, 1999)

Ammonification occurs when organic nitrogen is converted to ammonia. It is an important mechanism that ultimately allows organic nitrogen to be removed from wastewaters through hydrolysis to amino acids, which are broken down to produce ammonium or directly incorporated into biosynthetic pathways in support of bacterial growth.

Nitrogen as ammonia or nitrate can be assimilated by bacteria to form cellular mass. Although this assimilation of nitrogen does result in a net loss of nitrogen from the soluble

phase, it is not one of the major transformations of nitrogen that leads to removal. In most domestic and high strength agricultural wastewaters requiring nitrogen removal, the initial level of nitrogen is high enough that high levels remain even after the bacteria use what they need for growth.

Nitrification is the biological oxidation of ammonium nitrogen and is shown in Figure 2. Ammonium nitrogen is oxidized to nitrite by ammonia oxidizing bacteria (AOB) and then to nitrate by nitrite oxidizing bacteria (NOB). Many AOBs and NOBs are autotrophic, although heterotrophic bacteria are known to function as nitrifiers (Painter, 1977). In most situations, very little nitrite exists in a system at any one time because the conversion of ammonium to nitrite by AOBs is generally the rate-limiting step (Antoniou *et al.*, 1990). Consequently, nitrite oxidation follows quickly. The nitrate formed can then be used as a nitrogen source or as an electron acceptor. Many domestic wastewater treatment systems in the USA end treatment at this stage. However, nitrate can also have some detrimental effects on the environment. Therefore, nitrogen removal systems that incorporate denitrification are becoming more common in regions where surface water eutrophication is occurring.

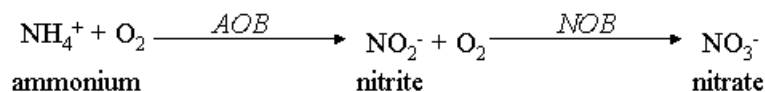


Figure 2. Nitrification Process (adapted from US EPA, 1993)

Denitrification is the key process involved in removing nitrogen from wastewaters. It occurs when the oxygen concentration in the wastewater becomes low enough that the bacteria begin to utilize nitrate as an electron acceptor under anoxic conditions. Nitrate is reduced by

heterotrophic bacteria to the intermediate nitrite and then to nitrogen gas. The nitrogen is then able to leave the wastewater as inert nitrogen gas.

Biological Nitrogen Removal Systems

Many types of biological nitrogen removal systems have been developed. The common theme between them is the involvement of sequential aerobic and anoxic zones, which are required to produce conditions in which nitrification and denitrification can occur. Some treatment schemes conduct nitrification and denitrification in separate systems, and are known as a dual sludge processes. One disadvantage to this approach is the involvement of more equipment, such as additional clarifiers and piping. Single sludge processes are also used, where nitrification and denitrification occur in one system but in different zones. These can involve any number of tanks.

Some of the first single sludge treatment processes developed for nitrogen removal were the Modified Ludzack-Ettinger (MLE) (Ludzack and Ettinger, 1962) and the Bardenpho (Barnard, 1975) (Figures 3 and 4 respectively). These processes work by operating separate aerated and non-aerated tanks in series. In the MLE, a mixed liquor recycle runs from the aerobic reactor back to the anoxic reactor. The Bardenpho adds two additional reactors (one anoxic, the other aerobic) after the first anoxic and aerobic reactors that allow more denitrification to occur in the second anoxic reactor by using endogenous and slowly degradable substrate as a carbon source for denitrification.

Other work has been done on closed loop bioreactors and oxidation ditches. By manipulating the feed, aeration, and flow, both anoxic and aerobic conditions can be

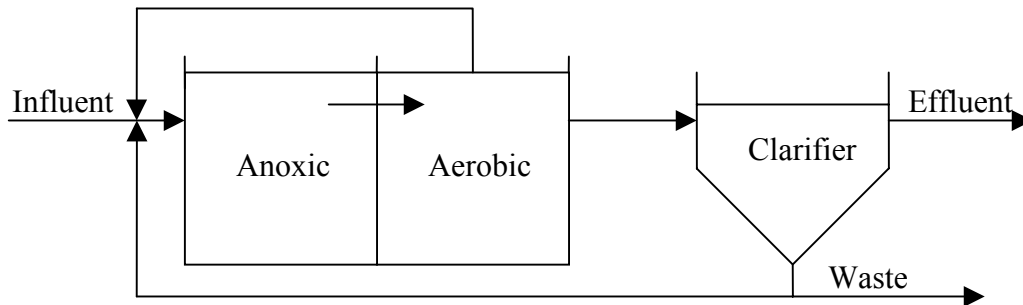


Figure 3. Modified Ludzack-Ettinger (MLE) process for biological nitrogen removal

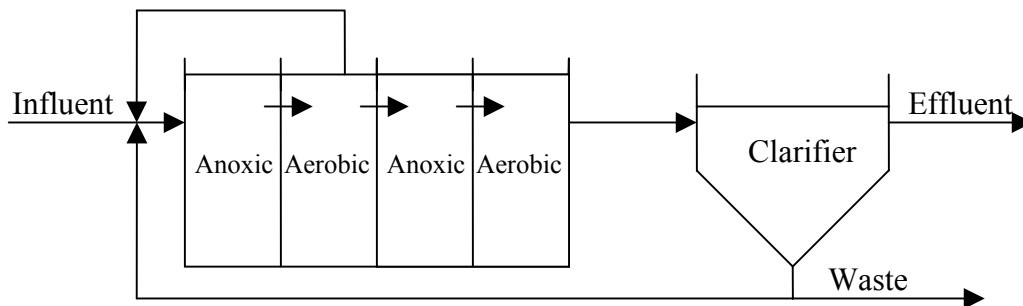


Figure 4. Bardenpho process for biological nitrogen removal

created in a single ditch or looped bioreactor. Daigger and Littleton (2000) analyzed nitrogen removal in a multi-channel oxidation system. Potter *et al.* (1996) used phased isolation ditch technology to remove nitrogen. By adjusting the time spent in the nitrification and denitrification phases, nitrogen removal was accomplished.

Some of the most recent research on nitrogen removal has involved using a sequencing batch reactor. This type of reactor does not operate continuously as the previously mentioned reactors do, but as a series of operations. By using different aeration and feeding strategies it is

possible to develop the same processes previously mentioned in a single tank. The operation of a sequencing batch reactor for nitrogen removal is discussed in detail in a later section.

Operational Conditions and Problems in Biological Nitrogen Removal

While many parameters such as solids retention time and hydraulic retention time affect the operation of wastewater treatment systems, some conditions are particularly important to biological nitrogen removal systems. A few of these include the carbon to nitrogen ratio of the wastewater, temperature and pH in the reactor, and inhibition of nitrification.

The carbon to nitrogen ratio (C:N) is a very important parameter when it comes to determining how easy it will be to remove the nitrogen from the wastewater. Grady *et al.* (1999) suggest that wastewaters with a COD:N ratio less than 5 will be inefficient in biological nitrogen removal and wastewaters with COD:N > 9 will achieve excellent nitrogen removal. A higher organic carbon content increases the nitrogen removal efficiency because it provides more electron donor (or fuel) for denitrification.

Nitrification and denitrification processes are also most efficient over a certain range of temperature and pH. Antoniou *et al.* (1990) showed that the growth rate of AOB reached a maximum at a pH of 7.8. Therefore, nitrification should be optimum in this range as well. Denitrification is most efficient at a neutral pH (Metcalf and Eddy, 1991). Both nitrification and denitrification rates decrease with decreasing temperature over a normal range of operating temperatures (5 – 30°C), and, therefore, optimum rates are achieved at higher temperatures (US EPA, 1993).

Nitrification inhibition is not usually a problem with domestic wastewaters or in wastewaters containing a low amount of ammonia. Nitrification can be inhibited by many means, including low temperature, low dissolved oxygen (DO), organic inhibitors, free

ammonia, and free nitrous acid. Anthonisen *et al.* (1976) determined the levels of free ammonia and free nitrous acid that are inhibitory to AOB and NOB. AOB became inhibited at free ammonia levels of between 10 and 150 mg/L as N, while NOB became inhibited at free ammonia concentrations between 0.1 and 1.0 mg/L as N. Free nitrous acid (unionized nitrite) became inhibiting to NOB between 0.22 and 2.8 mg/L as N.

From the levels of free ammonia that become inhibiting to AOB and NOB, it should be noted that NOB can become inhibited without the inhibition of AOB. This results in an accumulation of nitrite. This has been demonstrated and reported in the literature (Alleman, 1984). Since SBRs are operated so that a high concentration of ammonia can be in the reactor just after feeding, inhibition of nitrification by free ammonia is a real possibility. In fact, several SBRs have demonstrated an accumulation of nitrite because of inhibition of the nitrite oxidizers (Alleman and Irvine, 1980; Ng *et al.*, 2000). This is not to say that other sources of nitrification inhibition do not occur in SBRs. Bailey and Love (1999) showed NOB inhibition due to low DO in SBRs.

Sequencing Batch Reactors

A sequencing batch reactor consists of a batch reactor that operates under a series of periods which constitutes a cycle. The cycle generally consists of fill, react, settle, decant, and idle periods. The use of these periods allows a single reactor to act as a train of reactors and a clarifier. By manipulating these periods within a single cycle, an SBR can accomplish most of what a continuous flow plant can accomplish with several reactors, each operating under a different condition.

SBR Operation

Irvine and Ketchum (1989) describe the SBR and its periods in detail. During the “fill”

period, influent wastewater is added to the biomass that was left in the tank from the previous cycle. The length of the “fill” period depends on the number of SBRs, the volume of the SBRs, and the nature of the flow of the wastewater source, which can be intermittent or continuous. The reactor may or may not be mixed during this period. Filling ends when the wastewater has reached the maximum water level or at some fraction of that if multiple fill periods are used during a cycle. The “react” period occurs after a fill period. In most cases the reactor is mixed during this period. Aeration may or may not be used depending on the reactor’s objective and operation. In addition, the react period may be interrupted with fill periods and/or sludge wastage. During the react period many reactions can take place such as nitrification, denitrification, COD removal, phosphorous removal, and many others. After the react period, a “settle” period takes place. During the settle period, the SBR acts as a clarifier. The solids, including biomass and particulate substrate, settle and leave the relatively clear effluent on top. The settle period normally lasts between 0.5 and 1.5 hours so that the solids blanket does not begin to float due to gas buildup. “Decant” occurs at the end of the settle period and is the time when the effluent is drawn off. This may take place with a pipe or weir. The decant level should be adjustable so as to make the SBR more adaptable to changes. Finally, some systems include an “idle period.” This period is most necessary when several SBRs are being used with a continuous wastewater source. This allows a small amount of leeway when trying to match the cycles of the SBRs so that one SBR is always on the fill period.

Advantages of SBRs

Arora *et al.* (1985) notes many of the advantages of using an SBR. First, since an SBR is a batch process, the effluent can be held in the reactor until it is treated if there is somewhere for the influent to be stored. This can minimize the deterioration of effluent quality sometimes

associated with influent spikes. Also, biomass will not be washed out of an SBR because of flow surges. In addition, simplification of SBRs compared to flow-through activated sludge systems negates the need for return activated sludge to be pumped from the clarifier. Another advantage over conventional systems is that settling occurs when there is no inflow or outflow. Therefore, short-circuiting of the “clarifier” cannot occur.

In addition, the nature of an SBR leaves a lot of room for changes to the system based solely on operation and do not require construction. Edgerton *et al.* (2000) studied piggery wastewater treatment with SBRs and pointed out that cycle timing can be optimized according to changes in livestock feed cycles. As previously mention, the diet of livestock is a main determining factor in the makeup of agricultural wastewaters. Therefore, as diets change on a farm, the SBR can be optimized for the changing conditions in the wastewater.

Another example of the flexibility of SBR treatment relates to the effect of temperature. Fernandes *et al.* (1994) demonstrated how temperature affected SBRs treating screened swine-manure. They showed that the temperature of the wastewater could play a major role in the efficiency of treatment. Low temperatures decreased the efficiency of the process. By adjusting cycle times, seasonal temperature effects could be compensated for without losing efficiency. Creating a reactor that can nitrify, denitrify, oxidize substrate, and clarify in one vessel saves space and cost and may make wastewater treatment feasible for smaller farms that would have difficulty dealing with a multi-unit treatment train (Edgerton *et al.*, 2000).

SBR Nitrogen Removal

In general, all SBRs designed with the goal of nitrogen removal have both anoxic and aerobic periods in the cycle. The characteristics that are manipulated in a nitrogen removal SBR are hydraulic retention time (HRT), solids retention time (SRT), anoxic/aerobic ratio, number of

anoxic/aerobic periods, and fill strategy. Much research has been done in the last decade to determine optimum conditions for different wastewaters. Two different scenarios can be used in completing nitrogen removal with SBRs. One scenario is to have two separate nitrification and denitrification phases and the other is to create conditions such that nitrification and denitrification take place under the same macroscopic conditions in the reactor.

Figure 5 shows a profile of the major soluble nitrogen species and the soluble chemical oxygen demand (COD) in a reactor, as well as the stages taking place during the cycle. While this is just one cycle sequence, all nitrogen removal cycles involve these basic parts. During the fill and first anoxic period, any nitrate/nitrite that is left in the reactor from the previous cycle is denitrified. Once the denitrification is complete, aeration begins. During the aerated react stage, carbon is oxidized and nitrification takes place. The aerated react can last as long as it takes for the carbon or ammonia to become oxidized. After aeration ceases, an anoxic react period begins. During this stage, the oxidized nitrogen species are denitrified by heterotrophs that use endogenous or slowly degradable COD for the carbon and energy source due to the lack of bioavailable soluble COD. The total amount of oxidized nitrogen may or may not be denitrified, depending on the amount of biodegradable COD available. Usually, a short aeration phase is inserted at the end of the anoxic react phase to assist in removing nitrogen gas formed during denitrification. Once the react phase is completed, a settling phase begins. Some researchers have noted that denitrification can take place during the settling period (Kazmi and Furumai, 2000). Finally, after settling, a decant phase occurs.

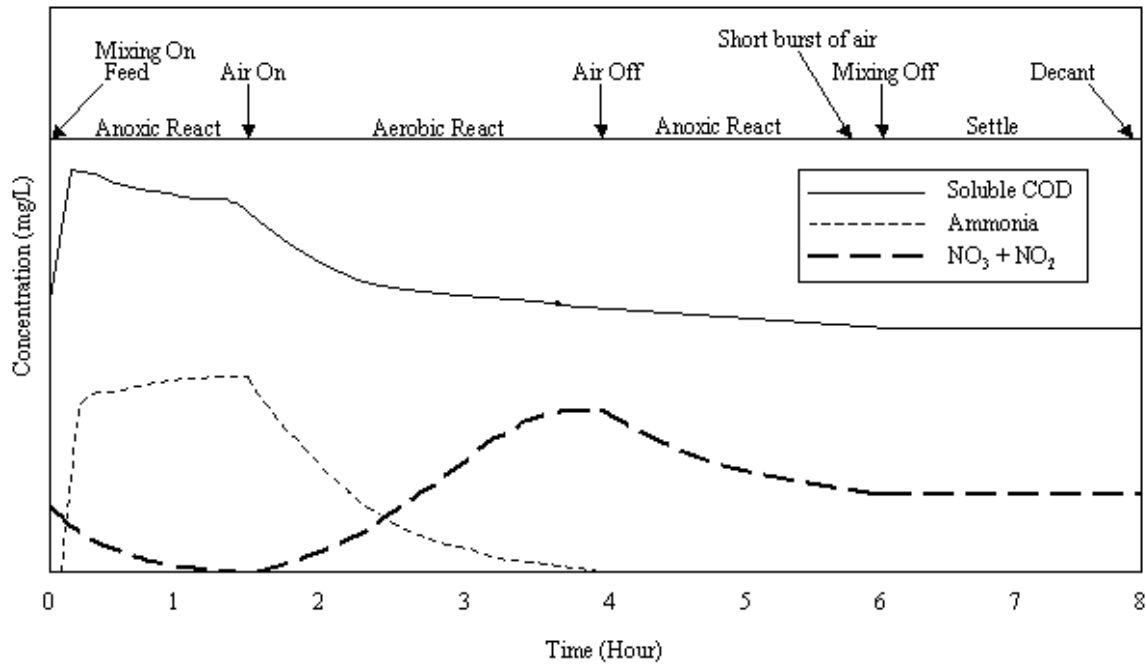


Figure 5. Typical SBR cycle incorporating partial nitrogen removal.

Some of the first published research on using SBRs for nitrogen removal was that of Alleman and Irvine (1980). A study was completed showing the use of stored carbon as the electron donor source for denitrification. Using a high-strength influent waste stream, they removed 92% of the nitrogen using a 10-day SRT. Their cycle consisted of a two hour mixed fill, three hour mixed aerated react, three hour mixed anoxic react, 0.33 hour mix/aeration react, one hour settle, and a 0.17 hour decant. Carbon was stored in the cells as glycogen during the aerobic period and consumed to fuel denitrification during the anoxic period.

Fernandes *et al.* (1991) used a similar cycle strategy to remove nitrogen from high strength screened swine manure. Using a 20-day SRT and a 6 or 9 day HRT, they varied the length of the aerobic and anoxic periods. The total length of the fill and reactor period was maintained at 22 hours. The three-hour fill period was anoxic. The react period consisted of between nine and eighteen hours of aeration, between three and nine hours of anoxic mix, and a

one-hour aeration period before settling. The wastewater served as carbon source and electron donor for denitrification during the fill period while endogenous carbon was assumed to serve this role during the second anoxic period, which was attributed to the different donors being used for denitrification. Their results showed that it is not recommended to operate anoxically for more than 8 hours during each 24 hour cycle because the process performance including sludge settling and odor characteristics deteriorates.

Bortone *et al.* (1992) investigated the use of multiple anoxic and aerobic periods similar to that of earlier work done by Alleman and Irvine (1980) and Fernandes *et al.* (1991). Bortone *et al.* increased the length of the anoxic period after filling and lengthened the final aeration at the end of the react period. One reason for increasing the length of the first anoxic period was to allow anaerobic conditions to develop to aid in phosphorus removal. The biological removal of excess phosphorus did appear to take place. Two SBRs were run with the cycle, with one SBR having a fill period at the beginning of the second anoxic period as well. By adding a second fill, the SBR mimicked a step-feed activated sludge system. The second dose of feed served to increase the denitrification rate in the second anoxic period as compared to the reactor without the second feed.

Andreottola *et al.* (1997) used piggery waste and a cycle similar to Bortone's (1992) to experimentally validate a nitrogen removal model. Their cycle was a series of three subcycles. Each subcycle contained an anoxic period (3 h and 15 min) and an aerobic phase (4 h and 15 min). There was also a feed phase at the beginning of each subcycle. The impact of using more subcycles and including feed at the beginning of each one is that more of the carbon source can be used for denitrification rather than just being oxidized during the aerobic period. This strategy enhanced nitrogen removal in the piggery wastes that had a low COD:N ratio.

As can be seen from the literature presented, there are many ways to set up a cycle in an SBR for nitrogen removal through separate nitrification and denitrification periods. Most of the differences in the setups involve the type of carbon and energy source used for denitrification.

Simultaneous Nitrification-Denitrification

Nitrification and denitrification can take place in the same environment. Normally nitrification systems are operated at high DO concentrations because bulk liquid DO concentrations below 0.5 to 2 mg/L inhibit nitrification (US EPA, 1993). Also, denitrification systems are usually operated in the absence of measurable DO because it can inhibit denitrification. In simultaneous nitrification-denitrification (SND), both nitrification and denitrification take place under the same macroscopic conditions, usually at an average DO concentration between 0.5 and 1.0 mg/L. One advantage to using SND in SBRs rather than alternating nitrification and denitrification periods is that SND could decrease the time necessary to remove the nitrogen (Munch *et al.*, 1996). A potential disadvantage is the selection for low DO filaments that interfere with settling and can cause sludge bulking (Lukasse *et al.*, 1998).

There are three mechanisms that are possibly responsible for SND (Daigger and Littleton, 2000). They are: anoxic and aerobic zones developing within the same reactor as a result of mixing patterns, anoxic and aerobic zones developing at different positions inside a floc, and novel microorganisms including aerobic denitrifiers and heterotrophic nitrifiers participating in SND. While much work has been done on simultaneous nitrification-denitrification recently (Pochana and Keller, 1999; Munch *et al.*, 1996; Daigger and Littleton, 2000), it is still a relatively new treatment method and little is known about how to operate a reactor under these conditions most efficiently.

Modeling of Biological Wastewater Treatment

A matrix of kinetic and stoichiometric equations have been developed to describe the biological processes generally used in biological wastewater treatment. The most well known mathematical model for describing the performance of single sludge processes is the Activated Sludge Model (ASM) No. 1 (Henze *et al.*, 1987), which incorporates carbon oxidation, nitrification, and denitrification. The model consists of a matrix of equations describing the processes that occur in a biological reactor. To successfully use the model to describe and design wastewater treatment processes, many parameters and influent values must be known. Methods have been developed for determining many of these kinetic and stoichiometric parameters.

Activated Sludge Model No. 1

The Activated Sludge Model incorporates many previous models of carbon oxidation, nitrification, and denitrification. The major kinetic equations include growth and decay of heterotrophs and autotrophs, hydrolysis of particulate material, and ammonification of organic nitrogen. The model contains many parameters that must be known for the model to be useful for design purposes. While many of these parameters are constant for most wastewaters and treatment processes, some of the most important parameters must be determined through experimentation or calibration to existing experimental data. The next section describes typical values of key parameters as well as methods that have been used to measure them.

Kinetic and Stoichiometric Parameters

Table 1 presents some of the most commonly measured parameters as well as typical values determined for systems treating municipal wastewater. Most of the values in the table were adapted from values chosen as typical by the task group that developed ASM No. 1.

Experimental Methods for Determining Parameters

The parameters that are most often experimentally determined include $\mu_{\max,H}$, K_S , b_H , Y_H , and $\mu_{\max,A}$. These parameters are most likely to affect the concentrations of organics and nutrients in the reactor and effluent. Many methods have been developed for measuring these parameters.

The heterotrophic maximum specific growth rate ($\mu_{\max,H}$) and half-saturation constant (K_S) can affect the mixed liquor concentration, carbon oxidation rate, and the denitrification rate. Many methods have been developed for measuring these parameters. There are two main differences in the methods. One is the method with which growth is measured. Different protocols have been developed that measure substrate uptake, biomass production, or oxygen consumption (Grady *et al.*, 1989). Since substrate consumption, biomass production, and oxygen consumption are all related by the kinetic equations in the model, any of these can be measured to determine the kinetic growth parameters. The following equations describe the relationship of substrate consumption, biomass production, and oxygen consumption.

Equation 1 describes the COD balance of heterotrophic growth with ammonia as the nitrogen source for bacterial production.



where S_S is the substrate in terms of COD, Y_H is the true growth yield with units of mass of biomass COD produced per mass of substrate COD consumed, S_O is oxygen, and $X_{B,H}$ is the active heterotrophic biomass in terms of COD. Rearrangement and conversion of the equation to a COD balance produces Equation 2.

$$\left(\frac{-1}{Y_H}\right) S_S + (-1) [-(1-Y_H)/Y_H] S_O + X_{B,H} = 0 \quad (2)$$

Table 1. Typical Parameter Values for Activated Sludge Model No. 1

Symbol	Parameter	Unit	Typical Value (Range)*
Y_A	Autotrophic yield	g cell COD formed (g N oxidized) ⁻¹	0.24 (0.07-0.28)
Y_H	Heterotrophic yield	g cell COD formed (g COD oxidized) ⁻¹	0.67 (0.46-0.69)
f_P	Fraction of biomass as inert products in decay	dimensionless	0.08
i_{xB}	Nitrogen per mass of COD in biomass	g N(g COD) ⁻¹ in biomass	0.086
i_{XE}	Nitrogen per mass of COD in inerts	g N(g COD) ⁻¹ in endogenous mass	0.06
$\mu_{max, H}$	Specific heterotrophic maximum growth rate constant	day ⁻¹	6.0 (3-13.2)
K_S	Heterotrophic half-saturation constant for growth	mg COD L ⁻¹	20.0 (10-180)
$K_{O,H}$	Heterotrophic half-saturation constant for DO utilization	mg O ₂ L ⁻¹	0.20
K_{NO}	Heterotrophic half-saturation constant for nitrate	mg NO ₃ -N L ⁻¹	0.50 (0.1-0.5)
b_H	Heterotrophic decay rate	day ⁻¹	0.62 (0.05-1.6)
η_g	Anoxic growth correction factor	Dimensionless	0.8 (0.6-1.0)
η_h	Anoxic hydrolysis correction factor	Dimensionless	0.4
K_X	Hydrolysis half-saturation constant	g COD (g cell COD day) ⁻¹	3.0
$\mu_{max, A}$	Autotrophic maximum specific growth rate constant	g COD (g cell COD) ⁻¹	0.8 (0.14 – 1.44)
K_{NH}	Autotrophic half-saturation constant for ammonium utilization	mg NH ₃ -N L ⁻¹	1.0 (0.06-5.6)
$K_{O,A}$	Autotrophic half-saturation constant for oxygen utilization	mg O ₂ L ⁻¹	0.4 (0.5-2.0)
b_A	Autotrophic decay rate	day ⁻¹	0.072 (0.05-0.15)
K_a	Ammonification rate	L COD (mg day) ⁻¹	0.08

*Typical values and ranges adapted from Henze *et al.*(1987) and Grady *et al.* (1999)

The equation can be translated through a rate term, r , with units of mg COD/(L*hr) to:

$$\frac{r_{SS}}{\left(\frac{-1}{Y_H}\right)} = \frac{r_{SO}}{\left\{-1\left[\frac{-(1-Y_H)}{Y_H}\right]\right\}} = \frac{r_{XB}}{1} \quad (3)$$

Equation 3 shows the relationship between substrate consumption (r_{SS}), oxygen consumption (r_{SO}), and biomass production (r_{XB}). The overall rate of biomass production has been expressed as a product of a specific biomass growth rate (μ), with units of hr^{-1} and the active biomass concentration (X_B).

$$r_{XB} = \mu X_B \quad (4)$$

Combining Equations 3 and 4, an equation describing the substrate uptake rate in terms of the specific biomass growth rate can be developed.

$$r_{SS} = -\left(\frac{\mu}{Y_H}\right)X_B \quad (5)$$

The Monod Equation (Equation 6), which was developed empirically, describes how the specific growth rate is affected by the substrate concentration.

$$\mu = \mu_{\max} \frac{S_S}{K_S + S_S} \quad (6)$$

where μ_{\max} is the maximum specific growth rate and K_S is the half saturation coefficient.

The most common version of the equation that describes substrate uptake is

$$r_{SS} = -\frac{\mu_{\max}}{Y_H} \frac{S_S}{K_S + S_S} X_B \quad (7)$$

As previously mentioned, the relationship of substrate uptake, biomass production, and oxygen consumption described in Equation 3 provides three different methods for determining rates. Either substrate, biomass, or oxygen can be measured in the kinetic experiments. In

general, respirometry (oxygen consumption) is the most widely used method because of the ease at which measurements can be made. Strides were made in developing batch respirometric techniques for determining the maximum specific growth rate by Ellis *et al.* (1996). They developed a method for determining the extant (defined below) growth parameters from a batch reactor by measuring the DO and comparing the oxygen uptake during substrate consumption to endogenous oxygen uptake.

Another method that has been used to determine μ_{\max} and K_S is to measure substrate levels during a batch test and to calculate growth rates from a substrate uptake curve. The specific substrate removal rate, q_{\max} , with units of mass of substrate COD consumed/hr can be related to μ_{\max} through Equation 8.

$$q_{\max} = \frac{\mu_{\max}}{Y_H} \quad (8)$$

Combining Equations 7 and 8 and integrating the rate, r_{SS} , with respect to time results in Equation 9.

$$K_S \ln\left(\frac{S_O}{S_S}\right) + (S_O - S_S) = q_{\max} X t \quad (9)$$

Once substrate COD values are available from the batch substrate uptake experiment, q_{\max} can be determined from the initial rate of substrate uptake when S_S is much greater than K_S , and K_S can be determined from the slope of a plot of $\ln(S_O/S_S)$ versus $q_{\max} X t$. In this experiment X is all of the biomass in the reactor capable of degrading the organic compounds. In the case of heterotrophic growth on simple organics, most of the viable biomass is capable of this.

An important factor to consider when measuring kinetic growth parameters is whether the parameters are measured under intrinsic or extant growth. Intrinsic parameters are defined when the biomass in which the growth rate is being measured is allowed to grow at the fastest possible

rate at the given environmental conditions. This is usually a longer term test, which occurs over several hours or days and allows the physiological state of the biomass to change during the test. Extant kinetics are measured when the physiological state of the biomass is not allowed to change significantly during the parameter estimation experiment. These tests are much shorter so that the biomass remains in the same state of growth. Generally, most of the parameters reported in the literature are intrinsic. This is because intrinsic parameters can be compared from one substrate to another. This allows the biodegradability of one substrate to be compared with another. Extant parameters, on the other hand, are useful when modeling the performance of a particular bioreactor configuration with a particular substrate (Grady *et al.*, 1996).

Measurement of decay rate and yield are much easier and fewer methods are used to determine these parameters. First, equation 10 shows the oxygen consumption associated with decay.

$$\text{Oxygen Uptake Rate} = \text{OUR} = -r_{\text{SO}} = (1-f_{\text{D}})b_{\text{H}}X_{\text{B,H}} \quad (10)$$

where f_{D} is the fraction of active biomass contributing to biomass debris from the traditional approach to decay and b_{H} is the traditional decay constant. In a batch reactor with no added soluble substrate, where only decay contributes to oxygen uptake, Equation 11 describes the mass balance for active biomass.

$$\frac{dX_{\text{BH}}}{dt} = -b_{\text{H}}X_{\text{BH}} \quad (11)$$

When Equation 11 is integrated with respect to time and substituted into Equation 10, the linearized result is Equation 12.

$$\ln(\text{OUR})_t = \ln[(1-f_{\text{D}})b_{\text{H}}X_{\text{BHO}}] - b_{\text{H}}t \quad (12)$$

This equation and the batch reactor it is based on allows for determination of the traditional decay rate (b_{H}). A batch reactor is operated with no added substrate, and the oxygen uptake rate

(OUR) is repeatedly measured over time as decay progresses. A plot of the natural log of the OUR versus time gives a slope equal to the decay rate, as shown in Equation 12. The traditional decay rate can be converted to the lysis:regrowth decay rate through Equation 13.

$$b_{L,H} = \left[\frac{b_H}{1 - Y_H (1 - f_D')} \right] \quad (13)$$

where $b_{L,H}$ is the lysis:regrowth decay rate and f_D' is the active biomass contributing to debris in the lysis:regrowth approach.

True growth yield can be measured by plotting the biomass concentration versus the substrate concentration in a batch experiment. Yield, which is the mass of biomass formed/mass of substrate consumed, comes from the slope of the plot. One thing that should be noted is that a very high substrate to biomass ratio (100) (on a COD basis) should be used when determining true growth yield so that little decay will take place and affect the measurement (Grady *et al.*, 1999). If decay is allowed to take place in the reactor, the yield measured is an “observed yield.”

Autotrophic maximum specific growth rate is a very important parameter in nitrifying and nitrogen removal systems. The reason for this is that $\mu_{\max,A}$ defines the solids retention time (SRT) at which the nitrifiers are washed-out. The wash out SRT for nitrifiers is when the wastage rate becomes larger than the growth rate. As the growth rate decreases below the wastage rate, more autotrophic biomass is wasted than is produced and all of the autotrophic biomass washes out. Autotrophic maximum specific growth rate can be determined from a batch reactor containing biomass and sufficient ammonia by measuring the production of oxidized nitrogen species (nitrate/nitrite) over time. The slope of nitrate/nitrite production equals the specific rate of growth minus decay. An assumed value for decay is often used to determine the maximum specific rate constant ($\mu_{\max,A}$) (Grady *et al.*, 1999).

III. MATERIALS AND METHODS

The purpose of this research was to characterize a flushed dairy manure wastewater, determine kinetic and stoichiometric parameters associated with the treatment of the wastewater, demonstrate lab-scale treatment of the waste, and examine the sensitivity of the kinetic and stoichiometric parameters in a simulated environment. This section provides the details to the methods used to accomplish these objectives. The experimental approach for this research is explained first. Secondly, the materials used in this research including the different wastewaters are described. Finally, the actual experiments and analyses used in this research are described. Included in this section are descriptions of experiments as well as methods of simulations used.

Experimental Approach

To reach the goals set forth in this research, wastewater characterization was completed, lab-scale reactors were operated, and simulations were run. The first step involved a detailed analysis of the chemical and physical characteristics of the wastewater. These characteristics provided information that allowed the development of a treatment process for the wastewater. Lab-scale reactors were run to acclimate biomass to the wastewater being used. Biomass from these reactors was used to determine the kinetic and stoichiometric parameters associated with the wastewater treatment. These reactors were also used to demonstrate the treatability of the wastewater. Finally, the experimentally determined parameters and characterized dairy wastewater were used to run a number of computer simulations. These simulations were used to show the sensitivity of the model to both experimentally determined and assumed parameter values.

Materials

Seed Biomass

The initial biomass used in the different acclimation and treatment reactors was obtained from the Blacksburg/VPI Wastewater Treatment Plant located near Blacksburg, VA. The source treatment plant employs single sludge nitrification and denitrification. The biomass was obtained from an aeration basin and immediately transported to the lab. The biomass was settled before being added to the laboratory scale SBRs (described below) in order to provide a biomass concentration closer to that expected to exist in the reactor at steady-state.

Dairy Manure Wastewater

The emphasis of the research was dairy manure wastewater treatment. Since the research was also designed to support the development of a treatment scheme that could be used at the new Virginia Tech Dairy Facility, it was desired that the wastewater used in the research be similar to the flushed and screened wastewater that will come from the Virginia Tech Dairy once the new facility is constructed. A dual stage roller press separator is to be used at the new facility and waste is to be flushed at a dilution ratio of 10.75 gallons of flush water per gallon of dairy manure. Since the target wastewater was not available at the time this research was conducted, a wastewater that would be similar to that of the Virginia Tech Dairy was obtained and manipulated to represent the future wastewater.

Wastewater was obtained from two different fully operational dairy farms during this study. The preferred wastewater would come from a dairy that scrapes their manure (no flushing) and also screens their waste through a screening system similar to that to be used at the Virginia Tech Dairy Facility. The wastewater was to be diluted at a ratio of flush water to manure (1.844:1) which would provide wastewater of similar solids content to that of the new

Virginia Tech Dairy Facility. The first batch of wastewater (PA1) was collected in July 2000 in Cumberland County, PA and met the desired criteria. The farm was operating with about 500 cows and used a screen size of 3-mm (0.12 in.) for the wastewater screening. Approximately 90 gallons of wastewater was obtained from this farm and stored in 5-gallon plastic buckets. About half of the wastewater was stored at 4°C and about half was frozen at -15°C.

Towards the end of the study (April 2001), more wastewater was needed and a different dairy facility had to be used due to problems associated with the screening device at the first. The second farm, located in Lancaster County, PA, was partly scraped and partly flushed. The farm operated with approximately 2500 cows and used the same screen size (3-mm) for wastewater screening. About 30% of the wastewater was scraped and 70% was flushed using decant liquid from the top of the anaerobic lagoon. About 80 gallons of wastewater (PA2) was obtained and stored at 4°C in 5-gallon plastic buckets. Despite the fact that the second wastewater source was partially flushed, it was diluted at the same ratio as the first wastewater source. It was assumed that the flushwater from the anaerobic lagoon decant likely had about the same concentration of soluble contaminants as the wastewater. Once characterized, it was determined that the diluted wastewaters from the two farms had similar concentrations of soluble components (see Results and Discussion).

Methods

Operation of Reactors

Six different reactors were used during this research. They included an acclimation continuous flow stirred tank reactor (CSTR) used for determining inert soluble organic nitrogen, two aerobic SBRs used for determining an autotrophic growth rate and heterotrophic decay rate and yield, an anaerobic SBR for pretreatment of the wastewater, and two nitrogen removal SBRs

operated under either anoxic, anaerobic, and aerobic conditions or low DO to achieve SND. The latter SBRs were used to demonstrate treatability, determine autotrophic and heterotrophic growth rates, and determine the heterotrophic decay and the heterotrophic half saturation constant.

Operation of Acclimation CSTR. During the summer of 2000, a CSTR was started and operated to acclimate biomass to the manure wastewater. Biomass from this reactor was used in the determination of the inert soluble organic nitrogen. The reactor was operated for approximately 7 weeks.

The CSTR was a 10 L plexiglass reactor setup as an Eckenfelder reactor as shown in Figure 6 (with part of the reactor used as a clarifier). Feed to the reactor was from the first batch of dairy manure wastewater, well-mixed and diluted 1:100 with tap water. The feed was refrigerated during operation to minimize biological activity in the influent bucket. The volume of the reactor was maintained at 9.72 L. Feed was pumped into the reactor using a Masterflex L/S pump drive and head (Cole Parmer, Vernon Hills, IL). The influent flow rate was maintained at about 540 ml/hr, which gave a hydraulic retention time (HRT) of about 18 hours. Samples were taken approximately three and two times per week for mixed liquor suspended solids (MLSS) and effluent total suspended solids (TSS), respectively. Wastage of biomass from the reactor was done manually and was varied according to the effluent TSS concentration to

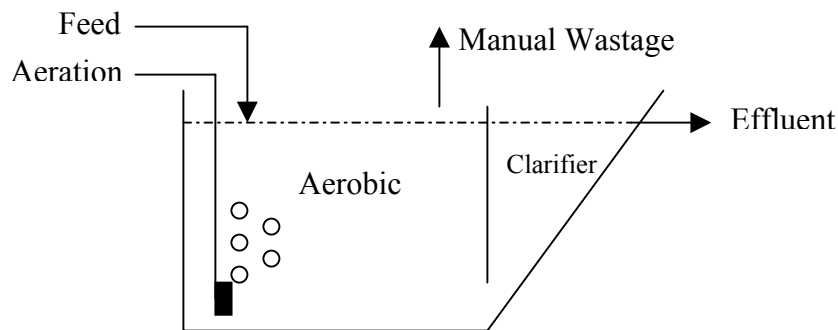


Figure 6. Schematic of laboratory-scale Eckenfelder-type CSTR

maintain a solids retention time (SRT) near 8 days. Air was provided continuously to the reactor by a Tetratec AP200 aquarium pump (Blacksburg, VA).

Operation of Aerobic SBRs. Two aerobic SBRs were operated for approximately eleven weeks. These SBRs were used to acclimate biomass to the dairy manure wastewater so that autotrophic maximum specific growth rate, heterotrophic yield, and heterotrophic decay experiments could be completed. The reactors were seeded with biomass from the CSTR and from the Blacksburg/VPI Wastewater Treatment Plant (Blacksburg, VA). A switch was made from the CSTR to the SBR because reactor configuration (e.g. SBR versus CSTR) can affect the physiological state of biomass and therefore affect the kinetics and stoichiometry of the biomass.

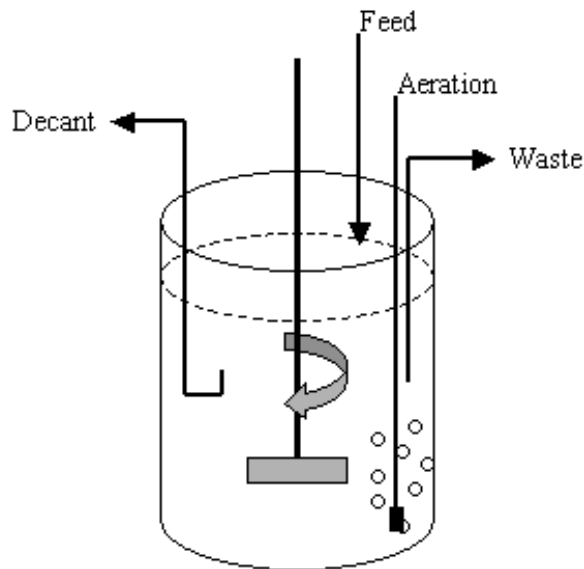


Figure 7. Schematic of laboratory-scale SBR.

Two 4-L glass graduated flasks were used as the reactors. Figure 7 shows the reactor setup. The reactor volume was maintained at 3.5-L. Each reactor was mixed with a horizontal paddle attached to a rod that was rotated at 104 rpm by an electric motor. A Tetratec AP200

(Blacksburg, VA) aquarium pump provided continuous aeration during the react period.

Decanting was facilitated through a J-tube as shown in the figure. The point that the effluent entered the J-tube was located at the level to which the reactor was decanted. This minimized loss of settled solids during decant and provided for the same level of decant each day. Feeding, wastage, and decant pumping was performed with 1 to 100 rpm variable speed Masterflex L/S pump drives and heads (Cole Parmer, Vernon Hills, IL). The pumps were controlled to turn on and off at set times by a ChronTrol XT timer (ChronTrol Corporation, San Diego, CA). Feed to the reactors consisted of well-mixed dairy wastewater from the first farm which was diluted 1:100 with tap water. The feed was kept in a refrigerator during operation and was changed every other day.

The reactor cycle provided for an effective HRT of 18 hours in both reactors, and for effective SRTs of about 2 and 8 days. The reactors operated with 3 cycles per day and 8 hours per cycle. Aerated filling and react lasted 6 hours and settling and decant occurred during the last two hours. Table 2 provides a summary of the operating parameters for each reactor.

Suspended solids in the reactor and in the effluent were analyzed two to three times a week. Volumes of the decant and wastage were recorded on the same days as suspended solids analysis was performed. This allowed for calculation of the HRT and SRT.

Table 2. Operation of Aerobic SBRs

3 cycles / day

6 hours of aerated and mixed react per cycle

~1 min wastage per cycle

1 hr 47 min settling per cycle

12 min decant per cycle

Total Volume: 3.5 liters

Influent: 1:100 dilution of mixed raw wastewater

Effective HRT: 18 hours

Effective SRT: 2 days and 8 days

Operation of Anaerobic SBR. An anaerobic pretreatment reactor was operated to mimic the type of pretreatment that might occur during manure storage at a farm and to determine the extent to which changes in the characterization of the wastewater might take place. The reactor was operated for a period of about three and a half months. It was seeded with sludge from the anaerobic lagoon at the Virginia Tech Dairy Facility (Blacksburg, VA). The reactor was setup and maintained by Krissy Yanosek.

Feed to the anaerobic reactor consisted of settled and diluted wastewater. The wastewater obtained from Pennsylvania was mixed well and then allowed to settle quiescently for 24 hours. The wastewater was then decanted down to the settled solids. The settled solids were disposed of and the decanted wastewater was diluted 1:2.844 with tap water (one part wastewater and 1.844 parts tap water). The dilution ratio was calculated so that the solids concentration in diluted wastewater from the first farm would be similar to the solids concentration expected at the new Virginia Tech Dairy Facility. Feed wastewater to the anaerobic reactor was stored in a refrigerator during operation and changed every other day.

The anaerobic reactor was a covered 55 gallon plastic drum. The operating volume of the reactor was 150 L. The HRT and SRT were maintained at 15 and 30 days, respectively. Mixing was intermittently provided to the reactor four times per day for five minutes. The reactor was mixed using a horizontal paddle similar to the ones in the aerobic SBRs. The reactor operated with one cycle per day. Feeding and decanting of the reactor was done with Masterflex L/S pump drives and heads (Cole Parmer, Vernon Hills, IL). The feed and decant pumps were controlled by a ChronTrol XT timer (ChronTrol Corporation, San Diego, CA). A three hour idle period was inserted at the end of the decant period to allow leeway for the manual wastage that

occurred each day. Table 3 provides a summary of the operation parameters of the anaerobic reactor. Decant from the anaerobic reactor was refrigerated immediately and was used as influent to the anoxic/aerobic SBRs.

Several types of analyses were performed on the anaerobic reactor and its effluent. Ammonia, MLSS, effluent TSS, and total and soluble COD were monitored approximately three times per week to check the operation of the reactor. Total phosphorus and soluble orthophosphate in the anaerobic effluent was measured once each week.

Table 3. Operation of Anaerobic SBR

Timing Sequence:
0:00 Feed 10L
0:15 Mix 5 min
6:15 Mix 5 min
12:15 Mix 5 min
18:15 Mix 5 min
21:00 Decant 5 L
Mix and Waste 5 L manually

Total Volume: 150 liters
Influent: 1:2.844 dilution of settled wastewater
HRT: 15 days
SRT: 30 days

Operation of Anoxic/Aerobic and Simultaneous Nitrification/Denitrification SBRs. The anoxic/aerobic and SND SBRs were operated to demonstrate the treatability of the wastewater under a biological nitrogen removal sequence and to provide acclimated biomass for testing of several kinetic parameters. The reactors were operated for approximately three and one half months. The reactors were seeded with biomass from the Blacksburg/VPI Wastewater Treatment Plant (Blacksburg, VA). Biomass had to be added to the reactors several times during the course of experimentation because of nitrification failures. The details of those dates and reasons for addition of biomass are provided in the Results and Discussion.

The anoxic/aerobic SBRs were operated under a cycle adapted from Andreottola *et al.* (1996), who successfully removed nitrogen from pre-screened piggery wastewater. While operating at different residence times (HRT and SRT) because of different wastewater strengths, the basic idea of multiple feed and aerobic periods during one cycle is consistent.

The influent to the anoxic/aerobic and SND SBRs was the effluent from the anaerobic SBR, diluted 50%. The reason for diluting the effluent from the pretreatment reactor was strictly a lab scale issue. Considering the high strength of the effluent from the anaerobic reactor and the decision to run at a 3 day HRT, it is doubtful that enough air could be supplied to the anoxic/aerobic reactor to create aerobic conditions without causing sheering and settling problems. As will be shown in the Results and Discussion, even with the diluted influent, it took almost an hour of aeration for the reactor to achieve a DO greater than 2 mg/L. The aquarium pump could only provide between 0.23 and 0.29 standard cubic feet per hour (SCFH) per liter of reactor volume. In addition, the lack of sufficient freeboard in the reactor to contain the slight foaming that occurred during aeration prohibited adding more pumps or using compressors. Therefore, the influent was diluted so that aerobic conditions could be obtained. It is likely that a full-scale system could provide the larger amount of air necessary for the same HRT and with an undiluted influent.

The physical setup of the anoxic/aerobic and SND SBRs is the same as that of the aerobic SBRs shown previously in Figure 7. Two four-liter glass graduated flasks were used as the reactors. A J-tube (described previously) was used to decant supernatant after the settling period. Masterflex L/S pump drives and heads (Cole Parmer, Vernon Hills, IL) were used to feed, waste, and decant, and were controlled by a ChronTrol XT timer (ChronTrol Corporation, San Diego, CA). Mixing was provided through a horizontal paddle attached to a paddle spinning at 104

rpm. Influent was made from diluted anaerobic effluent every day and stored in a refrigerator during operation. The pH in the reactors was maintained below 7.0 during part of the experimental period by using pH controllers. The controllers used were Cole Parmer Model 5797-29 pH-mV controllers (Cole Parmer, Vernon Hills, IL). The two pH probes used were an Accumet standard size liquid filled polymer body single junction combination probe with a Ag/AgCl reference (Cole Parmer, Vernon Hills, IL) and an autoclavable, glass, sealed, gel filled, double junction probe (Cole Parmer, Vernon Hills, IL). A 25% sulfuric acid solution was titrated into the reactors by Masterflex L/S pump drives and heads (Cole Parmer, Vernon Hills, IL) controlled by the pH controllers.

Table 4. Operation of Anoxic/Aerobic and SND Reactors

Timing Sequence:
0:00 Feed / Mixing on
6:00 Feed
12:00 Feed
21:45 Waste
22:00 Mixing off / Settling
23:50 Decant
Total Volume after third feed: 3.5 liters
Volume Fed per subcycle: 389 ml
Volume Wasted per cycle: ~220 ml
Volume Decanted per cycle: ~947 ml
HRT: ~3 days
SRT: ~14 days

The anoxic/aerobic and SND cycles are summarized in Table 4. They consist of a 24-hour cycle with 3 six hour sub-cycles per cycle. Each subcycle involves a feeding period in both reactors and in a 2-hour anoxic/anaerobic period and a four-hour aerobic period in the anoxic/aerobic SBR only (see Table 5). The SND aeration sequence provided for a low amount of air flow (0.3 to 0.4 SCFH), thereby establishing low (<1 mg/L) DO conditions during the entire react period. The step feeding strategy allowed for use of the readily degradable influent carbon during the denitrification process. The last four hours of the react phase (Hour 18 – 22)

in the anoxic/aerobic reactor were operated under two different aeration sequences. Each aeration cycle used in the anoxic/aerobic reactors is shown in Table 5. One reactor (R2) operated under the SND cycle from Day 24 to Day 72 and on the Anoxic/Aerobic cycle from Day 72 until they were shut down on Day 104. R1 operated under the Anoxic/Aerobic Cycle from Day 1 to Day 104. Both reactors were run with an HRT of about 3 days and an SRT of about 14 days.

Table 5. Aeration Sequence for Anoxic/Aerobic SBRs

Time	R1 (Day 1 – 41)	R1 (Day 42 – 104) and R2 (Day 72 – 104)
0:00		Aeration off / ~4min Feed
2:00		Aeration on
6:00		Aeration off / ~4min Feed
8:00		Aeration on
12:00		Aeration off / ~4min Feed
14:00		Aeration on
18:00		Aeration off
19:00	Aeration off	Aeration on
20:00	Aeration on	Aeration on
21:00	Aeration on	Aeration off
21:40	Aeration on	Aeration on
21:50	Aeration on	Aeration off

The same analyses run on the anaerobic SBR were run on the Anoxic/Aerobic and SND SBRs and included MLSS and effluent TSS, ammonium, and soluble COD. These analyses were performed two to three times per week. Also, total phosphorus and soluble orthophosphate were analyzed on the reactor effluents about once each week. In addition, nitrate and nitrite analyses were monitored in the effluents for three weeks.

Influent Characterization Experiments

Analytical methods discussed later in this section were used to characterize the wastewater in detail because of the impact the characterization has on treatment. Several special experiments and analyses were done that are not normally done during a wastewater

characterization, and these included the determination of colloidal COD, truly soluble COD, inert soluble nitrogen, and inert soluble COD. The values of the characterized wastewater are listed in the Results and Discussion. There was significant variability in wastewater quality during the study, due to both variability in the settling and variability between buckets. Given this variability and the limited number of replicates, calculated averages were used to represent wastewater quality. Calculations of the averages, the data used, and standard deviations of many concentrations listed in the Results and Discussion are shown in Appendix A. Unless otherwise stated, all 1.5 μm filters were Whatman 934AH (Clifton, NJ), 0.45 μm filters were 0.45 MCE - 25mm membrane filters (Fisher, Pittsburgh, PA) and Supor 45 – 47mm membrane filters, and 0.025 μm filters were 0.025 μm white VSWP – 25mm membrane filters (Millipore).

COD Breakdown. Chemical oxygen demand (COD) is normally broken down into particulate and soluble COD for wastewaters. The reason for this is that bacteria degrade the particulate COD more slowly than the soluble COD. The simulation model used in this study also includes colloidal COD. Equations are included in the model that allow for the sorption of colloidal COD to biomass flocs. Therefore it was necessary to define particulate, colloidal, and truly soluble COD. Since suspended solids are defined to be greater than 1.5 μm , that size filter was chosen for the definition of particulate COD. Therefore, particulate COD was measured as the difference between total COD and the COD of the filtrate through a 1.5 μm filter. Colloidal COD was defined to exist in the range between 0.025 μm and 1.5 μm . Choosing a value of 0.025 μm as a lower bound for colloidal matter is somewhat arbitrary, but it is not unreasonable. Colloidal is often defined as particles sized between 1 and 0.001 μm (Levine *et al.*, 1991). A filter with the pore size of 0.025 μm is the smallest available that can feasibly be used with manually operated syringe filters. Many researchers define soluble as that COD passing through

a 0.45 or 0.2 μm filter, but this is most often a definition of convenience and it was felt that the particles between 0.025 and 0.45 μm act as a colloid as well. In this research the term “truly soluble” means that the COD sample has gone through a 0.025 μm filter. If “colloidal” is used, it is defined as those particles between 0.025 and 1.5 μm . Particulate is that which is larger than 1.5 μm . Finally, most of the COD and organic nitrogen results presented in this research were determined from filtrate passing through a 1.5 μm filter, and are defined as “soluble”; these results include colloidal plus truly soluble components. This was done for ease of measurement, since it is very difficult to filter enough sample volume for multiple analyses through 0.025 μm membranes.

Inert Soluble Organic Nitrogen. An experiment was performed to determine the inert soluble organic nitrogen in the wastewater. This experiment was performed on the first batch of wastewater without anaerobic treatment. The experiment was based on information from Henze *et al.* (1987). Biomass was taken from the acclimation CSTR which was operating with an SRT of 8 days. Two, one gallon glass reactors were filled with 3-L each of mixed liquor from the CSTR. The reactors were aerated with humidified air for six weeks. Samples were taken approximately twice a week, filtered through 1.5 μm filters, and analyzed in duplicate for soluble organic nitrogen. Measurement of a stable residual soluble organic nitrogen indicates the presence of inert soluble organic nitrogen components.

Inert Soluble COD. This experiment was run in the same manner as the inert soluble organic nitrogen experiment described previously. Two hundred ml of biomass was taken from R1 on Day 70 and diluted 50%. The biomass was aerated with humidified air for one month. Samples of soluble COD (1.5 μm) were taken once per week until a reasonable steady state ensued. A final measurement of truly soluble COD was made and defined as the inert soluble

COD. This experimental approach includes soluble microbial product in the measurement of inert soluble COD. Since the models do not create soluble microbial product in the simulations, the inclusion of it in the inert soluble COD is appropriate.

Inert Suspended Solids and Inert Particulate/Colloidal COD. Inert suspended solids (ISS) were assumed to be equal to the fixed suspended solids (FSS). This value was measured by subtracting the VSS from the TSS. Since some volatile suspended solids can also be inert in biological treatment, the value of ISS assumed in this research is most likely less than the true inert value.

The determined values of ISS were used in the calculation of inert particulate and colloidal COD as shown in Equation 14:

$$\text{Inert Particulate/Colloidal COD} = (\text{Total COD} - 0.025 \text{ mm COD})(\text{ISS}/\text{TSS}) \quad (14)$$

The concept behind Equation 14 is that the fraction of particulate and colloidal COD that is inert is the same as the fraction of TSS that is inert. Again, since the ISS values calculated in this research are lower than the actual values, the inert particulate and colloidal COD determined is also most likely lower than the true values.

Inert particulate COD is normally determined by a method suggested by Grady *et al.* (1999). In this method, several reactors are operated at different SRTs greater than 5 days. By operating at greater than 5 days the biodegradable soluble and particulate substrate in the reactor is much less than in the influent wastewater. Therefore, simplifications in the model describing the MLSS in the reactor can be made. Equation 15 has been developed to model the reactors and determine the inert particulate COD.

$$X_M = (\text{SRT}/\text{HRT})[X_{IO} + (1 + f_D b_H \text{SRT})Y_H(\text{COD}_{TO} - S_{IO} - X_{IO}) / (1 + b_H \text{SRT})] \quad (15)$$

where X_M is the MLSS, X_{IO} is the particulate inert COD, COD_{TO} is the total COD, and S_{IO} is the

inert soluble COD. Using the steady state MLSS concentrations for several reactors operating at SRTs greater than 5 days, X_{IO} can be calculated from Equation 15 as it is the only unknown.

Kinetic and Stoichiometric Parameter Experiments

Several different kinetic and stoichiometric parameters were determined through experimentation. Biomass was acclimated in the different SBRs for use in these experiments. The parameters measured were heterotrophic yield, heterotrophic decay, autotrophic growth, and heterotrophic growth.

Heterotrophic Yield. Heterotrophic yield was determined because of its importance in predicting the value of the mixed liquor suspended solids and oxygen demand. It was determined in duplicate using an experiment provided by Grady *et al.* (1999). The general principle behind the yield experiment is to grow biomass at a very high feed COD to microbe COD ratio (F:M), and measure the amount of biomass produced per unit substrate consumed. Biomass can be measured in terms of VSS or COD. A high F:M is used because it is desirable to maintain decay at a low level compared to growth. This experiment, with minimal decay, will provide a true growth yield that can be used in the activated sludge model.

Raw wastewater (1500 ml) from the first farm, diluted one part wastewater to thirty-four parts tap water, was centrifuged at 1200 x g for 25 minutes, and filtered through a TSS filter (1.5 μm). This wastewater was used as the soluble substrate for the yield experiment. Two, 1-L glass reactors were filled with either 700 ml or 650 ml of soluble wastewater, respectively. Mixed liquor was taken from the 2-day SRT aerobic SBR, and the MLSS was 230 mg/L. A volume of 8.6 mL MLSS was added to the reactor containing 700 ml of wastewater, and 3 ml of MLSS was added to the reactor containing 650 ml of wastewater. The measured values of the feed to microbe ratio are given in the Results and Discussion.

The two batch reactors were aerated with humidified air, and 6 samples were taken from each reactor over 15 hours. These samples were analyzed in triplicate for total and soluble (1.5 μm) COD. Biomass COD was calculated as the difference between the total and soluble COD. A plot was made of substrate COD versus biomass COD for each reactor. The slope of the line on the plot reflects the yield in mass biomass COD created/ mass substrate COD consumed.

Heterotrophic Decay. The heterotrophic decay rate was also measured because of the effect it has on the biomass concentration and, therefore, the organic carbon oxidation rate and denitrification rate. The decay experiment was adapted from Henze *et al.* (1987) and Grady *et al.* (1999). The details of the theoretical background behind this experiment were presented in the Literature Review.

The heterotrophic decay (b_H) experiment was performed twice during the experimentation period. The first time the experiment was run, 800 ml of biomass from the 2-day SRT aerobic SBR was placed in a 1-gallon glass reactor. The short SRT of the biomass likely provided for little nitrification, although this was not confirmed with analyses of nitrate/nitrite. The biomass was aerated with humidified air for several days. Distilled water was added to make up for evaporation. The reactor was maintained at 20°C. Oxygen uptake rate was measured by filling a BOD bottle with biomass from the decay reactor and monitoring DO once per minute for 13 minutes. The DO stabilized during the first three minutes, and only the last 10 minutes was used to determine the OUR. The OUR was measured in duplicate at each time period. Measurements of the OUR were made approximately once every six to twelve hours for three days. A plot of $\ln(\text{OUR})$ versus time gave a slope equal to the traditional decay rate.

A second estimate for heterotrophic decay was measured using 200 mls of biomass

(diluted 50%) from the anoxic/aerobic SBR, R1. Dilution should not affect the decay rate.

Because of the high SRT and nitrification occurring in this biomass, nitrification was inhibited with 2 mg/L allylthiourea. The OUR was measured several times, as previously described, over six days, and a plot of $\ln(\text{OUR})$ versus time gave a slope equal to the traditional decay rate, as described by equation 12 in the Literature Review section.

Autotrophic Maximum Specific Growth Rate ($\mu_{\text{max,A}}$). Autotrophic maximum specific growth rate is extremely important in the design of biological nitrogen removal reactors. The $\mu_{\text{max,A}}$ experiment was adapted from Antoniou *et al.* (1990). The procedure involves measuring nitrate/nitrite production over time in a batch reactor seeded with a small amount of biomass. Rate of nitrate/nitrite production indicates the nitrifier growth rate. A plot of the natural log of the sum of nitrate and nitrite concentration versus time gives a slope equal to the autotrophic maximum specific growth rate minus autotrophic decay rate. Once the decay rate is assumed, the growth rate can be calculated.

The autotrophic growth rate experiment was run in triplicate on both the 8-day SRT aerobic SBR and the anoxic/aerobic SBR R2. In the first experiment on the 8-day SRT aerobic SBR, a one liter flask was filled with 600 mls of effluent and 300 mls of MLSS from the SBR resulting in a MLSS concentration in the batch reactor of 300 mg/L. The reactor was spiked with 10 mls of an ammonium stock solution containing 3.256 g/L $(\text{NH}_4)_2\text{SO}_4$. The ammonia level was monitored throughout the experiment with an electrode and maintained between 10 and 20 mg/L $\text{NH}_4\text{-N}$. This allowed for maximum growth of nitrifiers to occur without substrate inhibition of nitrification, which can occur at higher ammonia levels. Sodium hydroxide was used to manually maintain the pH between 7.6 and 8.6 during the experiment. The reactor was aerated with humidified air and operated in a constant temperature room at 20°C. Samples were

taken approximately once every twelve hours for 4 days and were analyzed for nitrate and nitrite.

The second experiment conducted with anoxic/aerobic SBR R2 was run in approximately the same manner. The effluent from R2 had to be diluted to 10% of its original concentration because the ammonia concentration was much greater than 20 mg/L. Although the experiment was run in triplicate, only two of the reactors were used to calculate $\mu_{\max,A}$ because nitrification did not occur in one reactor for unknown reasons. The actual nitrification rate was slower relative to the first experiment, and ammonia stock did not have to be added during the experiment since the ammonia concentration remained above 10 mg/L. The pH was monitored and remained between 6.5 and 6.9. A lower pH was maintained in this experiment to mimic the experimental SBRs as much as possible. Samples were taken twice a day for three days and analyzed for nitrate and nitrite.

Heterotrophic Maximum Specific Growth Rate ($\mu_{\max,H}$) and Half Saturation Constant (K_S). A batch experiment was run to determine q_{\max} and K_S , using biomass (188 ml) from anoxic/aerobic reactor R2 to yield a MLSS in the reactor of 4181 mg/L. Diluted (50%) anaerobic effluent was filtered through a 1.5 μm membrane filter, and 22.2 ml was added to the biomass. Samples (1.5 ml) were taken of the diluted and filtered anaerobic effluent as well as the seed biomass before the two were added together, then samples were taken approximately every 3 minutes for 71 minutes after the experiment was initiated. Each sample was immediately centrifuged at 8000xg for one minute, then 1 ml of the centrate was diluted with 9 ml of nanopure water and filtered through a 0.45 μm membrane filter. The samples were stored at -15°C and later analyzed for DOC. Acidified subsamples kept at 4°C were also analyzed for COD. This allowed for a COD: DOC ratio to be determined. Measurement of this ratio was

necessary because the COD data was more variable than the DOC data. The ratio was used to translate the DOCs into CODs. A plot of calculated COD versus time yielded a curve, and the maximum initial slope on the curve divided by the capable initial biomass concentration equals q_{\max} . Next, a plot of $q_{\max}Xt$ vs. $\ln(S_0/S)$ gives a slope equal to K_S (see equation 9, Literature Review section).

SBR Profiles

Ammonia, nitrate, nitrite, DOC, and DO concentrations were measured on anoxic/aerobic SBR R1 on days 103 and 104. Samples (approximately 20 ml) were taken at the beginning of each stage (anoxic/aerobic) and more frequently between hours 6 and 12 of the cycle. Samples were immediately centrifuged at about 4500xg for 5 minutes, then filtered through a 1.5 μm filter. About 15 mls of the filtrate was stored at -15°C , and later analyzed for ammonia, nitrite, and nitrate. The remaining 5 ml was filtered again through a 0.45 μm filter, acidified with H_3PO_4 and stored at 4°C for DOC analysis at a later time.

Simulations and Sensitivity Analysis

Several different simulations were performed using a mixture of measured and assumed values for the influent wastewater composition and stoichiometric and kinetic parameters. Simulations were used to determine the sensitivity of the model to the different kinetic and stoichiometric parameters. *BioWin32 Process Simulator* by EnviroSim (Oakville, Ontario) was used for the simulations. The models in the software are very similar to the Activated Sludge Model No. 1 (Henze *et al.*, 1987). The only significant difference is the addition of a colloidal term and sorption equations, as mentioned previously. One assumption made in using the Activated Sludge Model No. 1 is that the model is applicable to dairy manure wastewater. As the model was developed from activated sludge treatment of municipal wastes, there may be

differences in the kinetics and stoichiometry associated with dairy manure wastewater. The following sections describe the parameters and influent values used in the simulations as well as the protocol for conducting the sensitivity analysis.

A simulated SBR was configured for nitrogen removal in BioWin, and is shown in Table 6. The SBR configuration used the same sequences as the experimental anoxic/aerobic SBR R1 (see Tables 4 and 5) except that a feed period was added at Hour 18 for a total of four feeds per cycle. The aeration was set to replicate a gradual increase in DO with time, as measured in the experimental anoxic/aerobic reactors, although only the on/off times are shown in Table 6 to indicate where anoxic and aerobic periods begin and end. The final six hours of operation were the same as in the experimental anoxic/aerobic reactors after Day 42. An influent wastewater similar to that characterized from the anaerobic effluent (and presented in Results and Discussion) was used. The values for the inputs to the simulated influent wastewater are shown in Table 7. The computer model was run 5 times using a set of median values for the kinetic and stoichiometric parameters (shown in Results and Discussion) to analyze the effect of feeding more of the influent early in the cycle as opposed to feeding the same amount during each feed period. The same total influent was fed during each cycle in the 5 simulations, but the fraction of the total influent fed during each feed period differed for the different simulations. Four of the five simulations used an incremented step feed while one simulation used the same quantity of feed during each period. The percentages of the influent that was fed each period during the simulations are shown in Table 8.

Simulations were also run using different values for the kinetic and stoichiometric parameters. The configuration used for these simulations was the same as the above simulations and included the “incremented 40” step feed from Table 8. Twenty-three different simulations

were run with manipulations of 11 different parameters. A base simulation was run using median values for all the parameters. Then other simulations were run by changing one parameter and maintaining constant values for the other parameters. One assumption made in the modeling is that the other parameters that were not varied, including the anoxic growth correction factor (η_g), have values for dairy manure wastewater treatment similar to values for municipal wastewater treatment. The indices considered in evaluating model sensitivity were time periods required for nearly complete nitrification, denitrification, and soluble COD removal, effluent quality, and total oxygen consumption. A table displaying the parameters and values used is located in the Results and Discussion section.

Table 6. SBR Configuration for Sensitivity Analysis

Total Volume: 3.5 L	
SRT: 14 days	
HRT: 3 days	
<hr/>	
Aeration and Feed Sequence:	
0:00	Feed (Air Off)
2:00	Air On
6:00	Feed (Air Off)
8:00	Air On
12:00	Feed (Air Off)
14:00	Air On
18:00	Feed (Air Off)

Table 7. Influent Wastewater Characteristics for Sensitivity Simulation

Parameter	Units	Value
Particulate COD (Degradable)	mg/L as COD	1,112
Colloidal COD (Degradable)	mg/L as COD	1,298
Soluble COD (Degradable)	mg/L as COD	3,176
Inert Particulate COD	mg/L as COD	773
Inert Soluble COD	mg/L as COD	374
Particulate Organic Nitrogen (Degradable)	mg/L as N	47
Soluble Organic Nitrogen (Degradable)	mg/L as N	50
Soluble Inert Organic Nitrogen	mg/L as N	0
Ammonia	mg/L as N	454
Total Phosphorous	mg/L as P	87.6
Inert Suspended Solids	mg/L	800
Alkalinity	mmol/L as CaCO ₃	57

Table 8. Percentage of Wastewater Fed during Each Period for Sensitivity Analysis

Simulation	Feed Period 1 (0:00)	Feed Period 2 (6:00)	Feed Period 3 (12:00)	Feed Period 4 (18:00)
Non-incremented	25%	25%	25%	25%
Incremented 30	30%	26.7%	23.3%	20%
Incremented 35	35%	28.3%	21.7%	15%
Incremented 40	40%	30%	20%	10%
Incremented 45	45%	31.7%	18.3%	5%

Analytical Methods

Analytical methods from Standard Methods for the Examination of Water and Wastewater (1998) were used in most cases, and method numbers listed are from that source. A description of the storage procedures used is also provided.

Chemical Oxygen Demand. The chemical oxygen demand (COD) was determined by Method 5220 C, the closed reflux titrimetric method. In most cases, a 5 ml sample and a 0.05 M ferrous ammonium sulfate titrant solution was used. Samples were stored by acidification with sulfuric acid and held at 4°C. Samples were generally analyzed in triplicate.

Total and Volatile Suspended Solids. Total and volatile suspended solids were measured using Methods 2540 D and 2540 E, respectively. The membrane filter used for filtration of samples was a Whatman 934AH (Clifton, NJ) filter with a pore size of 1.5 µm. Samples were normally analyzed in duplicate.

Ammonium. Ammonium samples were analyzed by one of three methods. The first method used was Method 4500NH₃ C, the distillation method. This method was used for the majority of samples, including analysis of reactor effluent samples as well as characterization of the feed wastewaters. Samples were stored by acidifying with sulfuric acid and held at 4°C.

Analyses were generally done in duplicate.

The second method used was Method 4500NH₃ D, the ammonium electrode. An ammonium selective electrode produced by Fisher Scientific (Pittsburgh, PA) was used with a Fisher Accumet pH Meter Model 610. This method was only used for samples collected during the autotrophic growth rate experiments because an accurate value was not necessary and the method is more rapid than the distillation method.

Finally, because of the small sample volume available for determination of ammonia during the profile analysis (5 ml), ion chromatography was invoked as a third method. Duplicate samples of about 1.5 ml were hand injected along with standards. A Dionex DX-120 Ion Chromatograph (Dionex Corp., Sunnyvale, CA) was used with a 20 mM methanesulfonic acid eluent solution flowing at 1 ml/min. Samples were stored by freezing, and were thawed, centrifuged, and filtered through a 0.45 µm filter before analysis.

Total Kjeldahl Nitrogen (TKN). TKN was measured with Method 4500 Norg B, the macro Kjeldahl method. Soluble samples were filtered through a 1.5 µm filter. Samples were acidified with sulfuric acid and stored at 4°C. Samples were generally analyzed in duplicate.

Nitrate. Nitrate was analyzed by ion chromatography (Method 4110) using a Dionex DX 300 with an AS40 autosampler (Dionex Corp., Sunnyvale, CA) and eluent (1.0 mM NaHCO₂ and 3.5 mM Na₃CO₂) flowing at 1 ml/min. Samples were stored by freezing, and then were thawed and filtered through a 0.45 µm filter before analysis. Samples were generally analyzed in duplicate.

Nitrite. Nitrite could not be measured by ion chromatography because of interference with the chloride peak. Therefore, nitrite was analyzed using Method 4500NO₂- B, a colorimetric method. It was necessary to dilute the samples for analysis to stay within the linear

response range. A Beckman DU-640 Spectrophotometer (Beckman Instruments, Fullerton, CA) was used for the colorimetric analysis.

Total Phosphorus. The total phosphorus content of a sample was measured with Method 4500P 5 and Method 4500P E. The first method describes a persulfate digestion procedure for solublizing bound phosphorus to orthophosphate. The second procedure is the ascorbic acid method for colorimetric analysis of orthophosphate. Standards were processed through the entire procedure along with the samples. A Beckman DU-640 Spectrophotometer (Beckman Instruments, Fullerton, CA) was used for colorimetric analysis.

Soluble Orthophosphate. The soluble orthophosphate was analyzed on samples that had been filtered through a 1.5 μm filter. Method 4500P E., the ascorbic acid method described above, was used for analysis.

Dissolved Organic Carbon. Dissolved organic carbon (DOC) was measured with a Dohrmann DC-80 TOC Analyzer (Xertex Corp., Santa Clara, CA) using Method 5310. Samples were filtered through a 0.45 μm filter before analysis. An output range of 10 mg/L was used for the samples because of the low DOC (<100 mg/L) in the samples.

Dissolved Oxygen. The dissolved oxygen was measured using DO probes. For measurement of the DO in the reactors during operation, a YSI 5739 DO probe was used with a YSI Model 57 DO meter (Yellow Springs Instrument Co., Yellow Springs, OH). During the decay experiment, a YSI 5905 BOD Probe was used with a YSI Model 58 DO meter (Yellow Springs, OH) for measurement of DO.

IV. RESULTS AND DISCUSSION

Wastewater/Pretreatment Characterization

An important part in the design of a treatment strategy for a wastewater is characterizing the potential pollutants in the wastewater. By determining the concentrations of wastewater constituents, it is possible to analyze the feasibility of specific treatment options. The analysis provides the basis for decisions of treatment strategy including aeration cycles, feeding strategy, solids retention time (SRT), and hydraulic retention time (HRT).

A characterization of several of the wastewaters used in this study was performed. The wastewaters studied include two raw wastewaters from different dairy farms in Pennsylvania (PA1 and PA2), the same two wastewaters after one day of settling and flush water dilution (PA1sd and PA2sd), and effluent from the anaerobic reactor using each settled and diluted wastewater as an influent (AE1 and AE2). Table 9 shows an overview of all the characteristics measured for each wastewater. The data, methods of calculation, and uncertainties for the values in Table 9 are provided in Appendix A. Statistics (t tests) supporting the conclusions drawn in this section are in Appendix E.

Figure 8 shows a comparison of total suspended solids (TSS) content of the different wastewaters. The values presented for PA1 and PA2 consider the dilutions applied (1 part wastewater to 1.844 parts tap water) so that they can be directly compared to show the effects of settling (PA#sd) and anaerobic (AE#) treatment. The figure shows that TSS decreased with both the presettling and the settling that occurred with the anaerobic digester. However, the two different wastewaters had different settling characteristics. Settling of PA1 removed approximately 64% of the solids while only 37% of the solids were removed when PA2 was allowed to settle. Although the solids removal efficiencies during settling were different

Table 9. Average Characteristics for Various Wastewater Types

	PA1 ³	PA2 ³	PA1sd	PA2sd	AE1	AE2
TSS (mg/L)	27,817	18,400	3,505	4,080	964	3,037
VSS (mg/L)	24,427	16,079	3,006	3,388	908	2,440
Total COD (mg/L)	34,529	34,743	9,891	8,821	5,921	7,101
1.5 COD ¹ (mg/L)	12,544	14,753	4,999	5,187	4,434	5,193
0.025 COD ² (mg/L)	7,919	9,081	2,784	3,193	ND	2,596
Inert Particulate and Colloidal COD (mg/L)	ND	3191	ND	955	ND	996
Inert Soluble COD (mg/L)	ND	2,127	ND	748	ND	748
Total TKN (mg/L as N)	1,736	1,911	443	609	449	613
Soluble TKN (mg/L as N)	846	1,425	297	501	ND	554
NH ₃ -N (mg/L as N)	728	1,115	256	456	363	498
Total P (mg/L as P)	325	441	ND	110	84	78
Ortho P (mg/L as P)	213	196	75	67	48	65

¹ 1.5 COD = the COD of filtrate through a 1.5 µm filter (colloidal + truly soluble)

² 0.025 COD = the COD of filtrate through a 0.025 µm filter (truly soluble)

³ Values given are for undiluted wastewater. A dilution of 1 part wastewater to 1.844 parts tap water was applied before settling (PA#sd) and digestion (AE#)

* Numbers of replicates for each characteristic are given in detail in Appendix A

** PA1 = raw waste 1; PA1sd = settled waste 1; AE1 = anaerobic effluent 1; PA2 = raw waste 2; PA2sd = settled waste 2; AE2 = anaerobic effluent 2

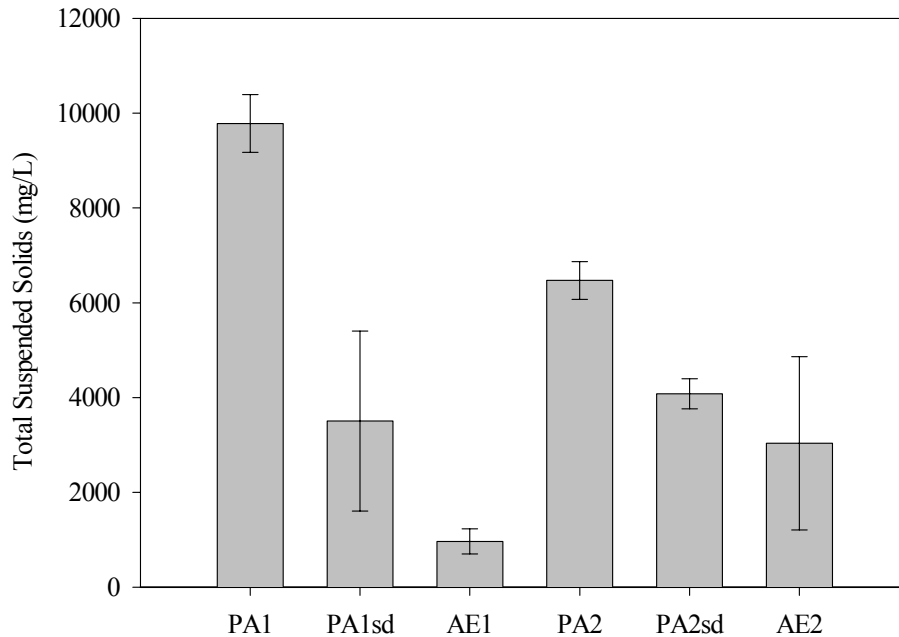


Figure 8. Impact of settling and anaerobic pretreatment processes on suspended solids.
(PA1 - raw wastewater 1; PA1sd - settled wastewater 1; AE1 - anaerobic effluent 1; PA2 - raw wastewater 2; PA2sd -settled wastewater 2; AE2 - anaerobic effluent 2)

for the two different wastewaters, the TSS for the two settled wastewaters (PA1sd and PA2sd) were statistically the same. Wastewaters PA1sd and PA2sd had TSS values of 3505 ± 1897 mg/L and 4080 ± 318 mg/L, respectively. Also, although the effluent TSS of the anaerobic SBR for each wastewater source was highly variable the average values were statistically different. One possible explanation for the poorer settling for the second batch of wastewater is that more gas may have been produced during the period when AE2 was measured and this disrupted the settling.

Figure 9 compares the COD fractions for the two batches of wastewater (diluted 1 part wastewater to 1.844 parts tap water), and the settled and anaerobically treated effluents associated with each of them. The two wastewaters responded very similarly to pretreatment. Measurements were not made of the truly soluble or colloidal COD for AE1.

The most obvious result of the COD analysis is that settling and anaerobic pretreatment had a much larger effect on the particulate COD than on the colloidal and truly soluble COD. For the second batch of wastewater, particulate COD decreased 73% while the colloidal and truly soluble COD change was statistically insignificant. This resulted in a total COD removal of 42%. Therefore the pretreatment was effective at removing much of the particulate COD but ineffective at treating the soluble and colloidal COD. This may be similar to what was occurring at the second farm where wastewater was collected. While the raw scraped waste soluble COD is unknown, dilution with decant from the anaerobic lagoon did not lower the soluble COD below that of PA1. Therefore, it is likely that the decant had a high soluble COD and that little degradation of the soluble COD was occurring in the anaerobic lagoon.

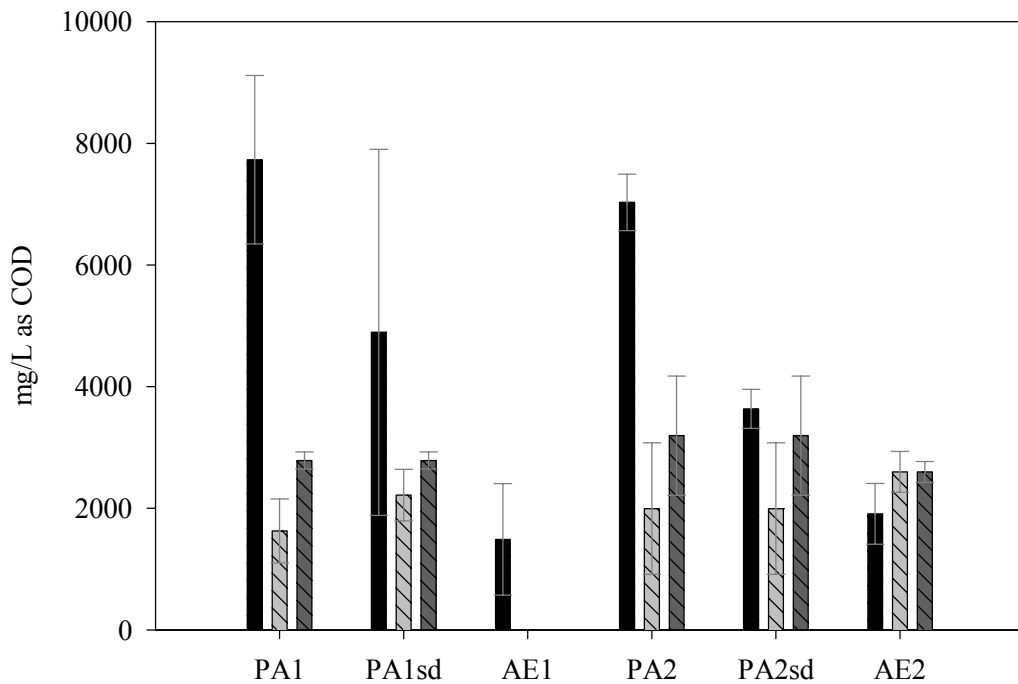


Figure 9. Comparison of COD fractions in diluted raw wastewaters and pretreated wastewaters.

(PA1 - raw wastewater 1; PA1sd - settled wastewater 1; AE1 - anaerobic effluent 1; PA2 - raw wastewater 2; PA2sd - settled wastewater 2; AE2 - anaerobic effluent 2)

Figure 10 shows the nitrogen fractions for the diluted raw and pretreated wastewaters. While it appears from the figure that the presettling and settling plus digestion in the anaerobic reactor resulted in removal of particulate organic nitrogen, the removal is statistically insignificant because of the high variability of the samples measured. The anaerobic treatment process may have converted some of the organic nitrogen to ammonia. The average particulate organic nitrogen decreased by 111 mg/L during pretreatment and the ammonia level increased slightly by 42 mg/L.

Although some of the particulate organic nitrogen was likely removed through settling, hydrolysis and ammonification probably played a part by converting some of the particulate organic nitrogen to soluble organic nitrogen and then to ammonia. It must also be noted that ammonification was likely occurring during raw wastewater storage prior to use as influent to the anaerobic reactor. Therefore, some of this increase in ammonia probably occurred before the wastewater was added to the anaerobic reactor. While the ammonia value shown for the settled and diluted feed to the anaerobic reactor was an average of samples taken over the course of the experimental period, the ammonia in the samples increased over time. Noting that any change in the nitrogen concentrations was mostly a conversion from one nitrogen species to another, there was no statistically significant change in the TKN.

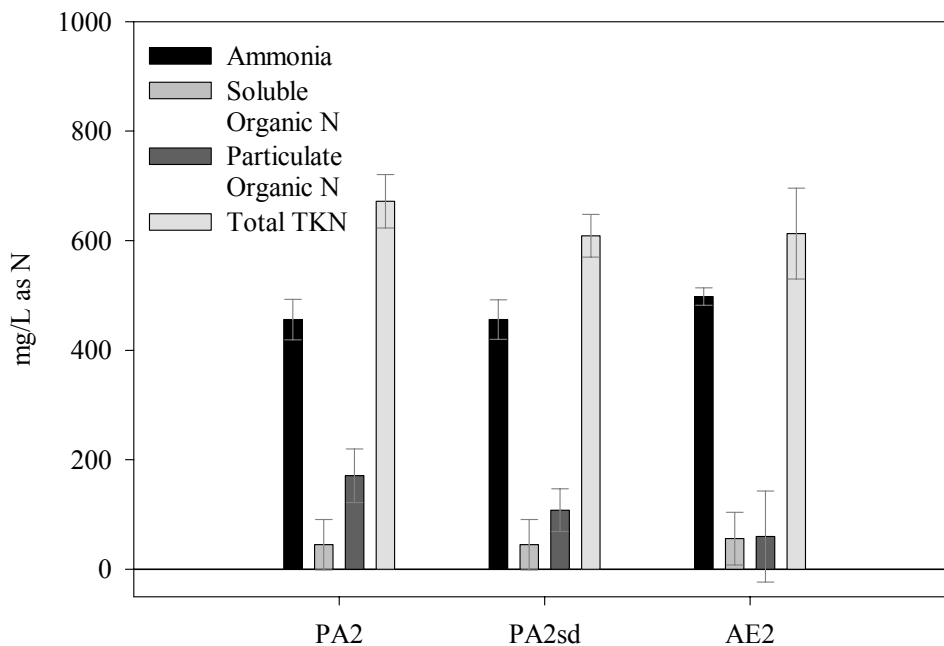


Figure 10. Comparison of nitrogen fractions in diluted raw wastewater and pretreated wastewater for wastewater batch number 2. (PA2-raw wastewater 2; PA2sd - settled wastewater 2; AE2 - anaerobic effluent 2)

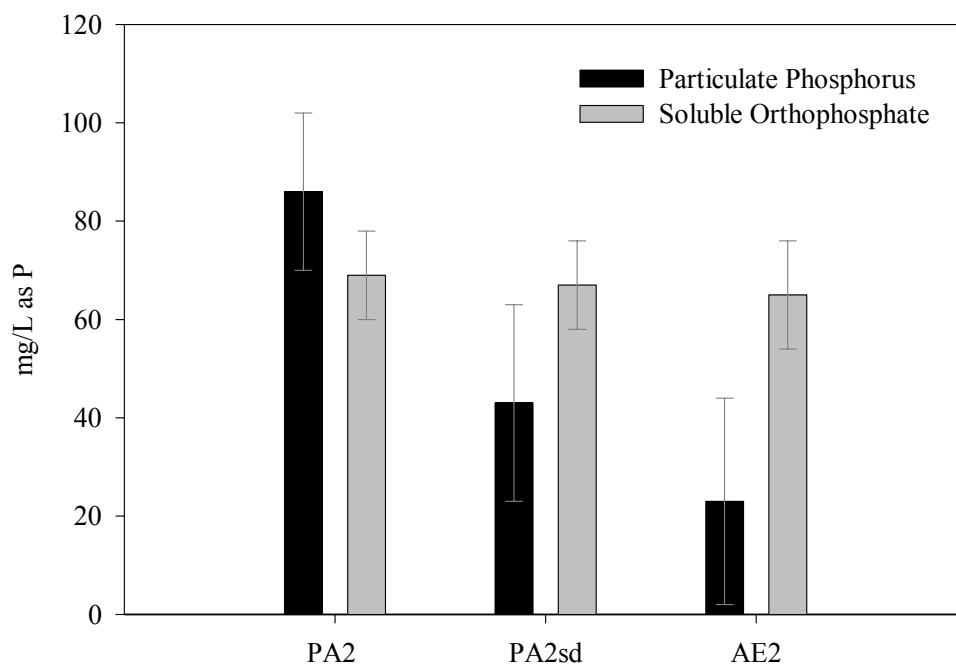


Figure 11. Comparison of phosphorus fractions in diluted raw wastewater and pretreated wastewater for wastewater batch number 2.
 (PA2 - raw wastewater 2, PA2sd - settled wastewater 2; AE2 - anaerobic effluent 2)

Figure 11 shows the phosphorus composition of the wastewater fractions. There is little evidence of any removal method other than settling removing phosphorus during the pretreatment processes for the second batch of wastewater. Particulate phosphorus (total phosphorus minus soluble orthophosphate) decreased by a statistically insignificant 50% during the initial settling period and then by a statistically significant total of 85% after anaerobic treatment and settling. The concentration of orthophosphate did not change during pretreatment.

Inert soluble organic nitrogen were determined for the first batch of wastewater while the inert soluble COD was determined for the second batch of wastewater. The inert soluble COD

for PA2sd was determined to be 748 mg/L for PA2. Inert soluble organic nitrogen in PA1 was below the limit of detection at the end of the batch experiment used to estimate it. Based on an ammonia detection limit of 0.06 mg/L and a wastewater dilution of 1 part wastewater to 99 parts tap water in the feed of the acclimation CSTR used to produce the mixed liquor for the batch experiment, it was determined that the inert soluble organic nitrogen was less than 6 mg/L.

The inert particulate/colloidal COD was 12, 17, and 22 percent of the total particulate/colloidal COD for PA2, PA2sd, and AE2, respectively. The actual values shown in Table 9 were calculated using Equation 14 (from the Literature Review). Some MLSS data was available for the aerobic SBRs at three different SRTs (2, 3.8, 8.4 days). This data was used to compare the two different methods for determining inert particulate COD using values calculated for AE1. The values determined using the two different methods varied by about 15%. The method using the SBRs at different SRTs did include an assumption of the inert soluble COD in AE1 being equal to that of AE2. Also, it is noted that the SRTs in two of the SBRs did not meet the criteria of being greater than 5 days (Grady *et al.*, 1999). Regardless of these discrepancies, the ISS/TSS ratio overestimated the inert COD compared with the reactor method. Therefore, since the main question about the ISS/TSS method is the volatile organic carbon that is inert, picking the larger value in this case is warranted. This lends credibility to the method used.

Carbon to nitrogen ratios are helpful in determining the ease with which biological nitrogen removal can take place. Excellent removal efficiency can be expected for a COD/TKN ratio greater than 9 (Grady *et al.*, 1999). Figure 12 shows the COD:TKN ratio based on the biodegradable fractions of nitrogen and COD for the second batch of wastewater before and after each stage of pretreatment. Not enough data was available to analyze the first batch of wastewater in this method. Since a large fraction of the total nitrogen was soluble, settling and

anaerobic treatment reduced the COD:TKN ratio greatly by removing more particulate COD than total nitrogen. The ratio dropped to between 7 and 11 after the last stage of pretreatment. The pretreated wastewater is, therefore, close to being carbon limited for nitrogen removal, but the carbon content will most likely be sufficient from removing most of the nitrogen as long as the reactor setup uses the carbon efficiently. Other researchers working with slaughterhouse waste (Subramaniam et al., 1994) have also noted that too much pretreatment can cause carbon limitations for nitrogen removal.

Carbon to phosphorus ratios are also used to determine the efficiency at which biological phosphorus removal might occur in a wastewater. Low efficiency phosphorus removal processes

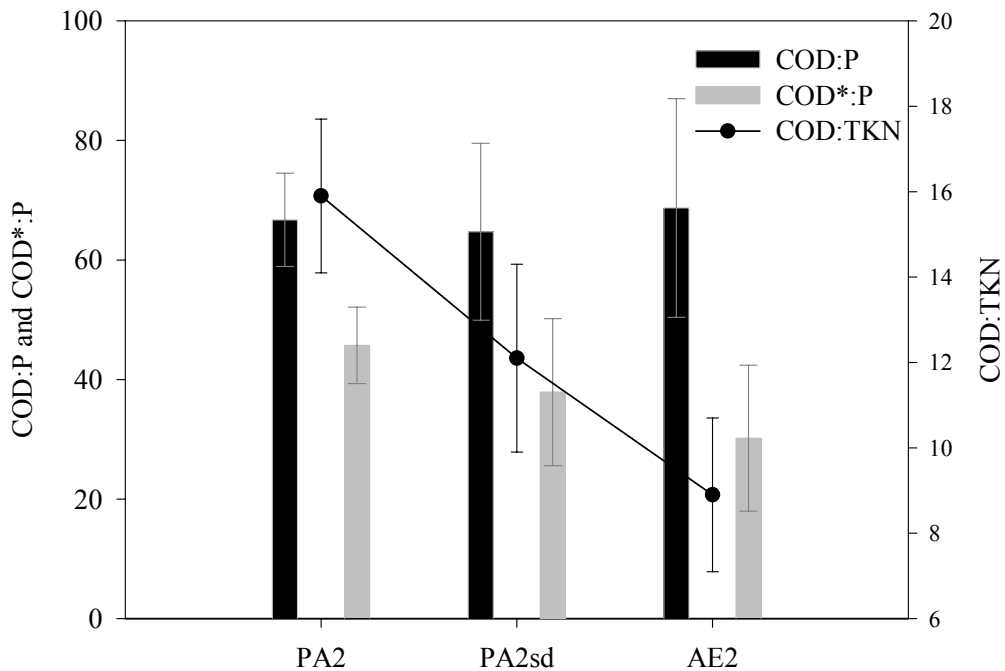


Figure 12. Effect of settling and anaerobic pretreatment on the nutrient ratios in wastewater batch number 2.

(PA2 - raw wastewater 2; PA2sd - settled wastewater 2; AE2 - anaerobic effluent 2; COD* - COD available for P removal after subtracting COD used for N removal)

need a COD:P ratio of greater than 43 (Grady *et al.*, 1999). Figure 12 shows that the COD:P ratios for the wastewaters meet this requirement if all the COD is allowed to be used for phosphorus removal.

A second set of COD*:P ratios were calculated to show what the ratio would be if carbon was preferentially used on a 5:1 (COD:TKN) basis to remove nitrogen, and only the remaining carbon would be available strictly for phosphorus removal. This reduced the COD*:P ratio to 30.2 ± 12.2 . The wastewater will, therefore, become carbon limited if the goal is phosphorus removal as well as nitrogen removal. A slight change in the particulate COD or phosphorus removal efficiencies during pretreatment could change this ratio. In addition, it should be noted that these ratios depend on the values that were determined for the inert particulate and colloidal COD, which were lower than the actual values because of the methods used. This causes the calculated nutrient ratios to be higher than the true ratios are.

Kinetic/Stoichiometric Parameter Determination

Kinetic and stoichiometric parameters define the rate and extent of degradation of substrate and growth of biomass in treatment of a particular wastewater. The parameters are generally consistent for a particular type of wastewater but very greatly between different wastewater types. Parameters determined for treatment of an industrial wastewater, for instance, could not be used in design of a process for treatment of a municipal wastewater. Because of this specificity, the following key parameters were determined for dairy manure wastewater: heterotrophic true growth yield (Y_H), heterotrophic decay (b_H), and autotrophic maximum specific growth rate ($\mu_{A,max}$). An attempt was also made to determine heterotrophic maximum specific growth rate as well as the heterotrophic half-saturation constant (K_S). Other parameters necessary for design were assumed based on typical values for domestic wastewater.

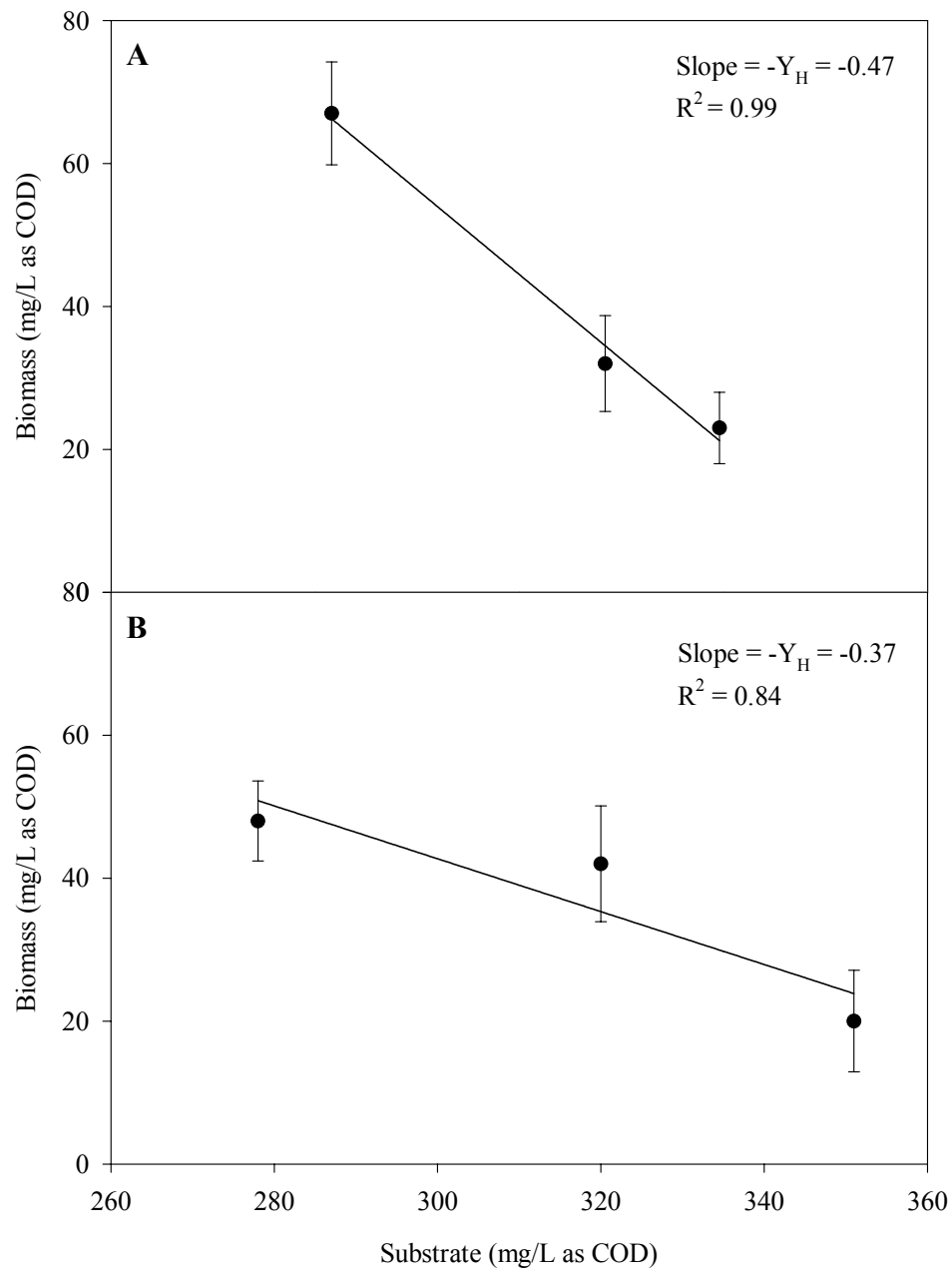


Figure 13. Experimental determination of heterotrophic true growth yield (Y_H) for dairy manure wastewater.

Figure 13 shows the results from the heterotrophic true growth yield experiment. The experiment resulted in an average value of $Y_H = 0.42 \pm 0.07$ mg biomass COD formed per mg substrate COD consumed. This value (0.42) is lower than most literature values for domestic wastewaters, which typically are between 0.46 and 0.69 (Grady *et al.*, 1999). One reason for this may be that the yield experiment was run at a slightly lower substrate to biomass ratio than is normally used. Because the F:M cannot be known exactly until after the experiment, in this experiment, the biomass initially accounted for 5 or 6% of the total COD. It is generally recommended that the initial biomass concentration in the experiment be only 1% of the total COD (Grady *et al.*, 1999). This insures that decay is relatively small compared to the large amount of growth taking place and the result of the experiment is a true growth yield, rather than an observed growth yield that includes decay. Therefore, a small amount of decay may have occurred in the yield experiment, which may have artificially lowered the yield measured. Nevertheless, the value of Y_H determined does lie on the low end of the range found in the literature and may be indicative of dairy wastewaters.

Heterotrophic decay was determined from the data shown in Figure 14. The values of heterotrophic decay determined were 0.250 d^{-1} for an all aerobic 2-day SRT SBR and 0.238 d^{-1} for an anoxic/aerobic 14-day SRT SBR. The average of these two values gives a decay constant (b_H) of $0.244 \pm 0.008 \text{ d}^{-1}$. These values are in the range of values reported in the literature for municipal wastewaters. Traditional decay rates for treatment on municipal wastes in the literature vary from 0.05 d^{-1} to 1.6 d^{-1} (Henze *et al.*, 1987), with 0.24 d^{-1} viewed as typical (Dold *et al.*, 1986). Translation of this traditional decay rate through Equation 13 (from the Literature Review) to a lysis:regrowth approach

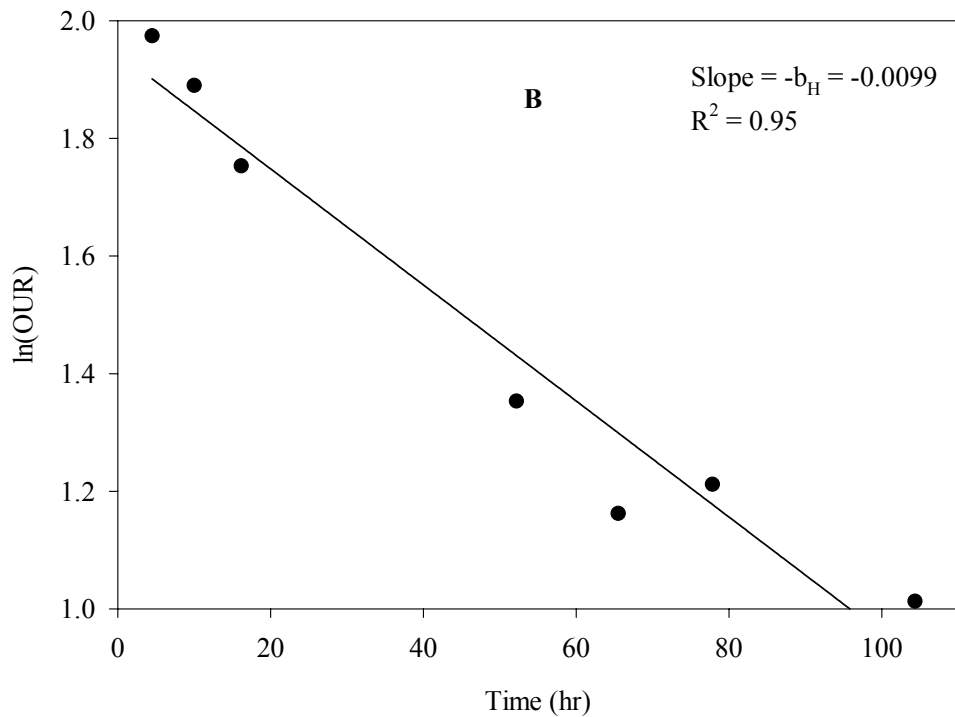
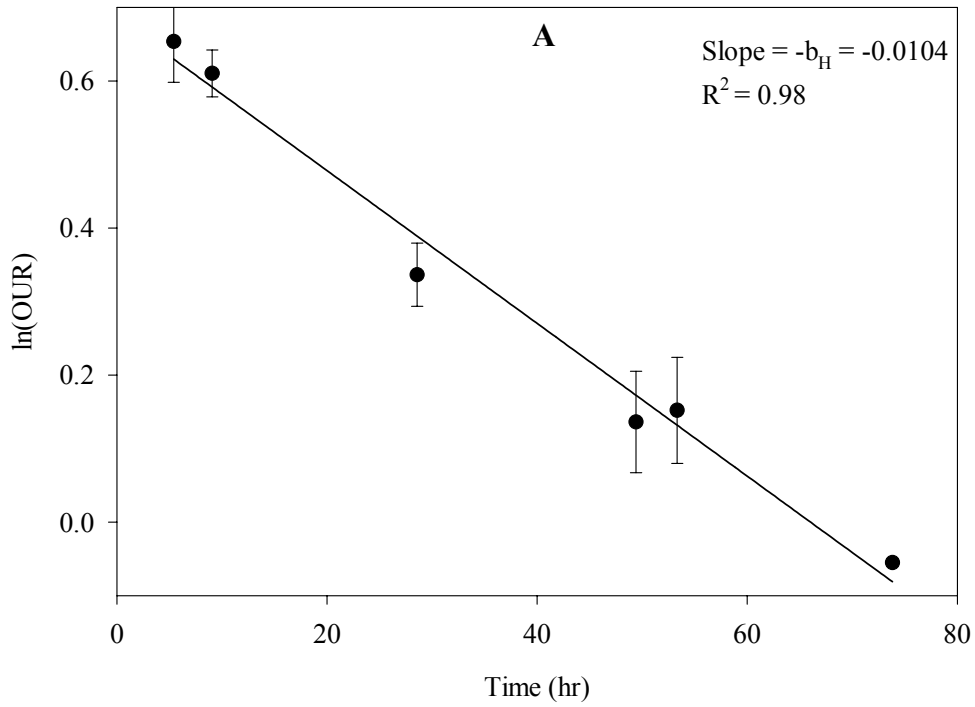


Figure 14. Experimental determination of heterotrophic decay rate (b_H) for dairy manure wastewater in (A) an aerobic SBR with a 2-day SRT, and (B) an anoxic/aerobic SBR, R2.

decay rate ($b_{L,H}$) results in a value of 0.17 d^{-1} assuming a value of 0.42 for yield.

The autotrophic/nitrifier maximum specific growth rate was also determined for two different biomass cultures. A plot of the natural log of nitrate/nitrite concentration versus time from the batch experiment is shown in Figure 15. The slope of this figure is equal to the growth rate minus the decay rate and a decay rate of 0.072 d^{-1} was assumed (Grady *et al*, 1999). The autotrophic maximum specific growth rate was determined to be $0.75 \pm 0.05 \text{ d}^{-1}$ for the 14 day SRT anoxic/aerobic SBR, and $0.72 \pm 0.02 \text{ d}^{-1}$ for the 8 day SRT aerobic SBR. The growth rates in the literature for *Nitrosomonas*, a common nitrifying autotroph, are between 0.34 and 2.21 d^{-1} , with 0.77 d^{-1} being considered typical (Grady *et al.*, 1999). Therefore, the autotrophic growth rates measured for dairy manure wastewater fall within the range of typical values observed with municipal wastewaters.

An attempt was made to determine the extant heterotrophic growth rate in Anoxic/Aerobic SBR R2. A substrate uptake curve, measured in a batch experiment using biomass from the reactor, was used to calculate maximum substrate uptake rate, q_{\max} , and the heterotrophic half-saturation constant for aerobic growth, K_S , based on Equation 9 (from Literature Review). If the initial substrate level in the batch reactor is significantly greater than K_S , q_{\max} can be calculated from the slope of the first few points of the substrate uptake curve. The linear form of Equation 9 (from Literature Review) was plotted in Figure 16 as $q_{\max}Xt$ versus $\ln(S_0/S)$ and the slope gives the value of K_S .

The values determined for q_{\max} and K_S were $4.39 \text{ mg substrate COD (mg biomass COD*day)}^{-1}$ and 234 mg/L COD respectively. Based on the biomass concentration in the batch reactor and a heterotrophic yield of 0.42, the value of q_{\max} corresponds to a heterotrophic maximum specific growth rate ($\mu_{\max,H}$) of 1.84 d^{-1} . Unfortunately the high estimate for K_S causes

a problem in the estimation procedure. This experiment was run with an initial substrate value of only 283 mg/L biodegradable COD because the batch experiment was setup to mimic the experimental SBRs biomass to substrate ratio. When determining q_{\max} from the first few points of the graph, it is essential that the substrate concentration be much higher than K_S so that the biomass is actually growing at the maximum rate. Therefore, the estimated values for q_{\max} and K_S are not the true values. The true value for the specific maximum growth rate ($\mu_{\max,H}$) is most likely higher because it is known that the growth rate is 1.84 d^{-1} at a substrate level of 283 mg/L. Therefore, since for non-inhibiting wastewaters the growth rate increases with substrate concentration, the maximum specific growth rate has to be at least that high. K_S is most likely in the range of hundreds of mg/L as well. If K_S was low, then the results from this experiment should have reflected that.

The kinetic and stoichiometric experiments yielded some interesting results. Several of the parameters measured including heterotrophic yield, autotrophic maximum specific growth rate, and heterotrophic decay rate are very similar to values measured for biomass growing on municipal wastes. This may mean that dairy manures and municipal wastewater can be treated with similar methods. However, two parameters that cause concern when using typical values from domestic wastewater treatment are the heterotrophic maximum specific growth rate and half-saturation constant. While uncertainty exists about the actual values of the parameters, this study suggests that while $\mu_{\max,H}$ may be similar to values for municipal wastewater treatment, K_S is likely much higher than typical municipal values. If K_S is significantly higher, methods used in the treatment of wastewaters with high K_S values (i.e. industrial wastewaters), such as higher SRTs, may be necessary.

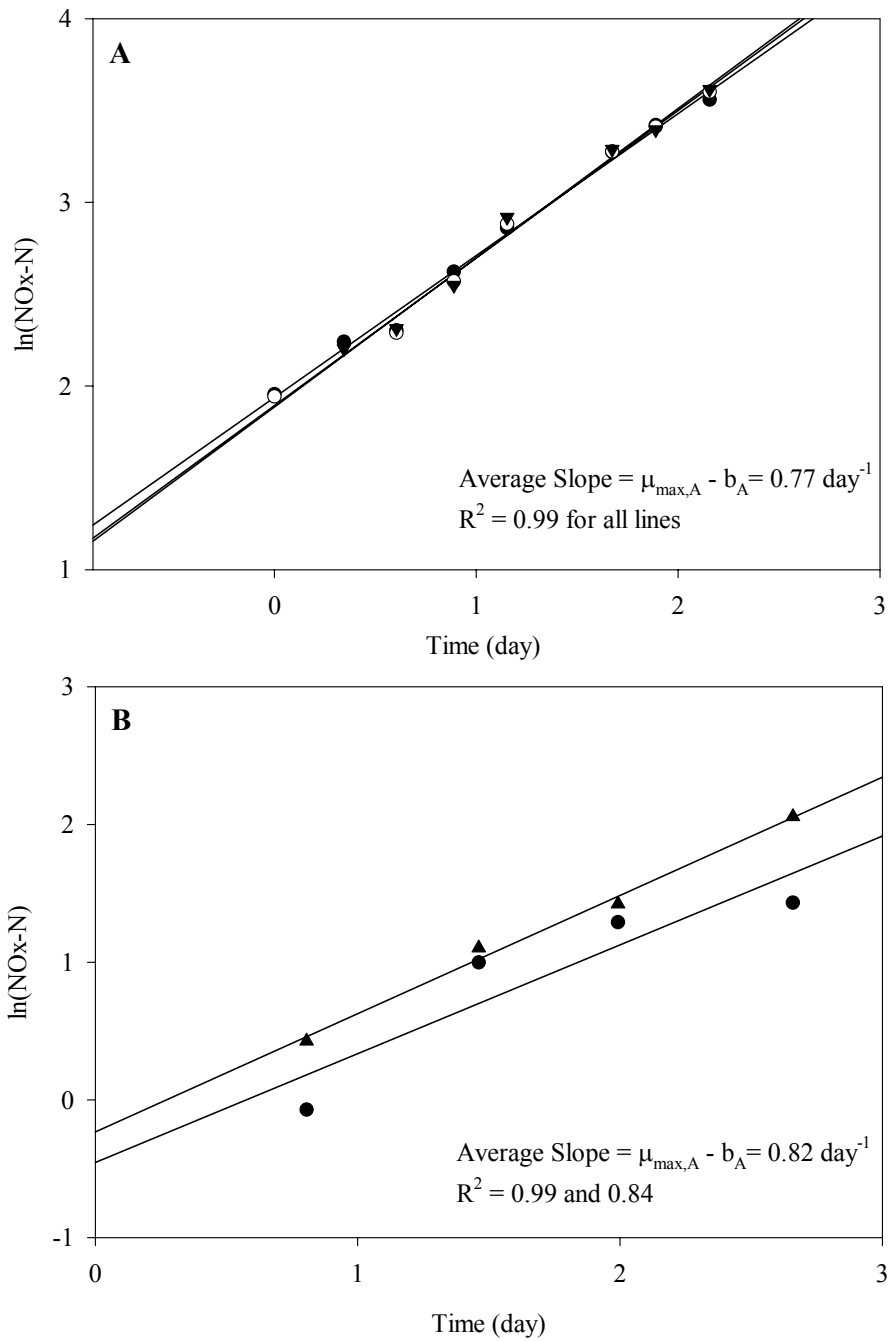


Figure 15. Experimental determination of maximum autotrophic growth rate for dairy manure wastewater in (A) an aerobic SBR operation with an 8 day SRT, and (B) anoxic/aerobic SBR R2. (Each data point type represents a separate replicate experiment)

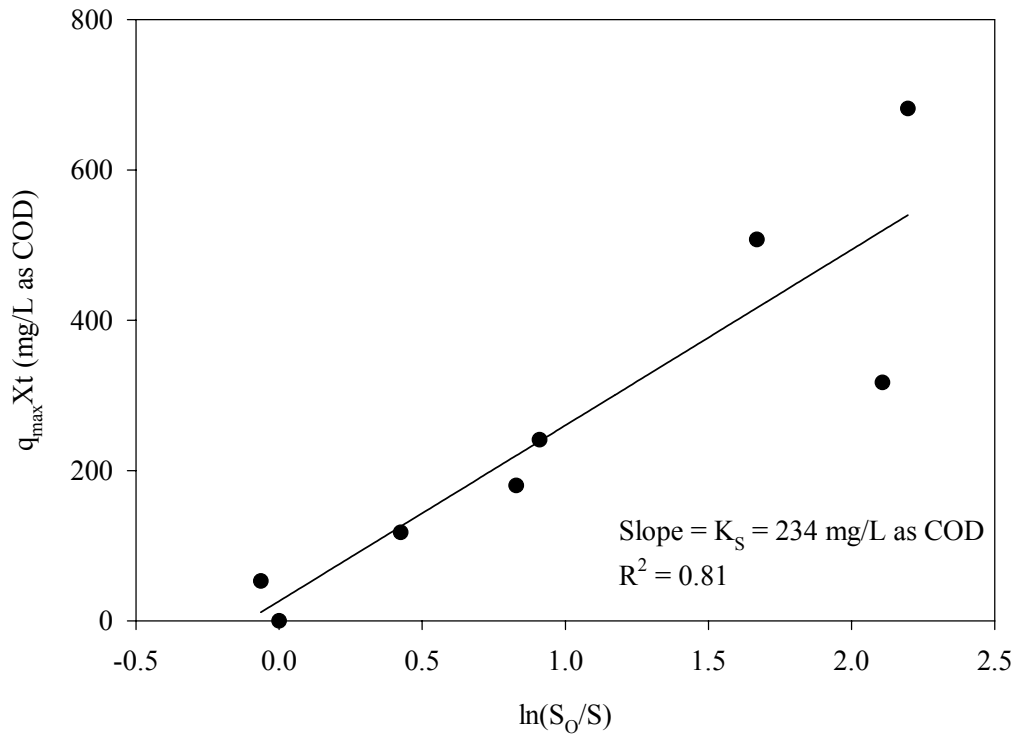


Figure 16. Experimental determination of the heterotrophic half saturation constant (K_s) for dairy manure wastewater.

Nitrogen Removal in Sequencing Batch Reactors: Experimental

Two SBRs were run for approximately three months to remove nitrogen from the wastewater. One reactor, R1, was run for the entire time as an anoxic/aerobic SBR while the other reactor R2 was run for a period of time as a reactor with low DO in an attempt to achieve simultaneous nitrification-denitrification (SND), and for a final period as an anoxic/aerobic SBR.

General Operation

Figure 17 shows the ammonia removal efficiency of the two reactors, R1 and R2, over the operating period. This figure is useful in noting the effects of the different events that

occurred during the operational period. Reactor 1 was started on Day 1 and was fed a 50% dilution of the anaerobic effluent and ran on the anoxic/aerobic cycle by Day 15. Reactor 2 was started on the 50% diluted effluent and was operated at a constant low DO beginning on Day 24. A pH controller was installed on each reactor on Day 42 to keep the level of free ammonia to a minimum so that nitrification inhibition did not persist. On the same day, new nitrifying activated sludge biomass was added to the reactors because nitrification was not taking place.

On Day 62 the new settled and diluted wastewater, PA2sd was begun as the influent to the anaerobic reactor. On Day 66 and 67, when steady state nitrogen removal was occurring in R1, the acid in the pH controllers ran out and the pH spiked to at least 8.2 for two days. Based on the known ammonia concentration in the reactors at that time, it is believed that free ammonia concentration reached inhibitory levels, as estimated using the method of Anthoniesen *et al.*

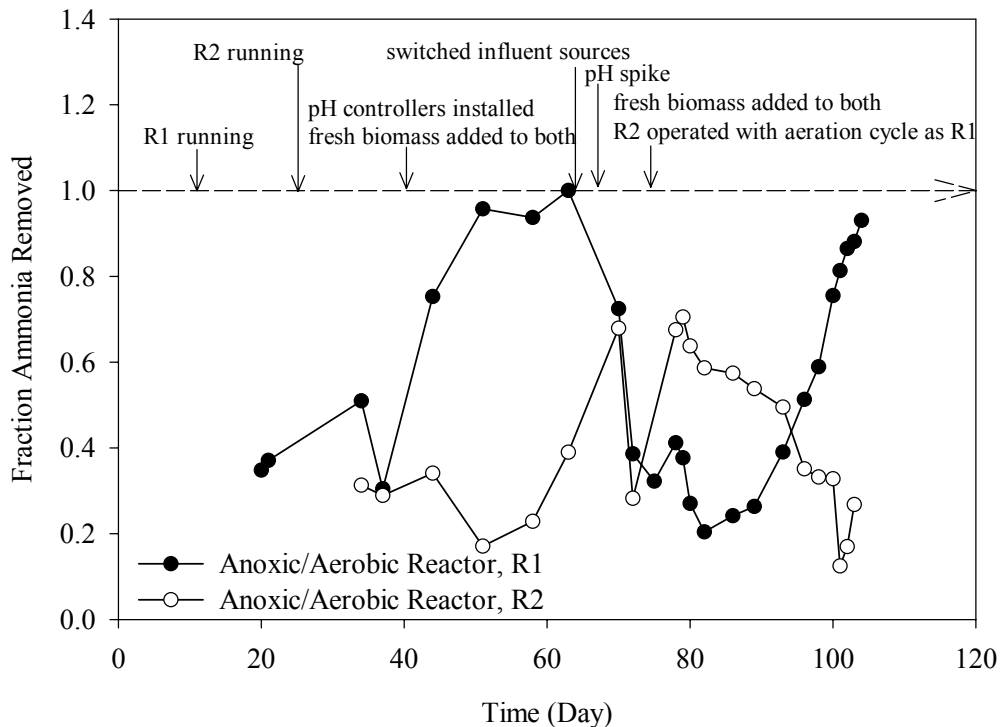


Figure 17. Ammonia removal efficiency in anoxic/aerobic SBRs.

(1976), and discussed below. New seed sludge was again added on Day 72 and 75 to replace the inhibited nitrifiers. In addition, the cycle operation of R2 was changed to that of R1. Ammonia removal was approaching 100% in R1 when sampling ended on day 104. The dates of these events are intended to provide a context for the data presented in the following sections.

Startup Problems and Upsets: Nitrification Inhibition

One major issue that developed while running the two SBRs was inhibition of nitrification during the aerobic part of the cycle. As was discussed previously, many factors can contribute to nitrification inhibition, but inhibition by free ammonia levels is considered to be most significant. As pH increases, the fraction of ammonia in the unionized state increases. A significant increase in pH with a high concentration of ammonia in the reactor can lead to level of free ammonia well above inhibitory levels.

It was noted that the influent for the two SBRs was the anaerobic effluent that had been diluted 50% with tap water. Anaerobic treatment leads to a production of carbon dioxide. It is believed that the covered anaerobic reactor used in this study led to high levels of dissolved carbon dioxide in the wastewater. Two conditions in this reactor most likely led to this. One is that the anaerobic reactor was not continuously mixed. Therefore, there was little outside disturbance to encourage dissipation of the carbon dioxide. Second, the cover over the reactor likely led to a higher than normal partial pressure of carbon dioxide in the headspace of the reactor. This would have slowed the mass transfer of the carbon dioxide from the liquid phase as well. The carbon dioxide is not in itself a problem to nitrification. In fact, nitrifying autotrophs use carbon dioxide as a carbon source. It was believed that carbon dioxide stripping through aeration and mixing in the SBR led to problems.

Experiments supported the notions that carbon dioxide stripping caused an increase in the

pH of the reactor. This was apparently a chemical effect rather than an interaction with the biomass. Effluent was taken from the anaerobic reactor and aerated in a batch reactor without any addition of biomass. The pH rose rapidly over the period of 45 minutes to an hour from approximately 7.2 to near 8.2. Again, this pH would not be harmful to nitrification on its own because nitrification actually approaches a maximum near pH 8 (US EPA, 1993). However, when this high pH is associated with high values of ammonia, the free ammonia levels can rise to inhibitory levels.

Effluent ammonia concentrations near 150 mg/L $\text{NH}_4\text{-N}$ were measured in the two reactors soon after startup. Assuming a pH of between 8.15 and 8.4 (values measured during the first few weeks of startup), the free ammonia (FA) concentration in the reactors varied between 12 and 20 mg/L as N. This is well above the 10 mg/L necessary for inhibition of both NOB and AOB, and this most likely led to inhibition of all nitrification in the reactors. Once pH controllers were installed to maintain the pH between 6.0 and 7.0, free ammonia concentrations stayed below 1.0 mg/L as N and nitrification was reestablished with the addition of new nitrifying biomass.

A pH upset on Day 66 and 67 led to pH values near 8.2 for two days. This created free ammonia concentrations between 4 and 5 mg/L as N. While this level less than 10mg/L, it is high enough to inhibit NOB and still may have been sufficient to inhibit AOB. The higher free ammonia concentrations along with the shock of rapidly raising the pH from less than 7 to more than 8 likely led to inhibition of both AOB and NOB as seen from the increasing effluent ammonia concentration during the week after the pH upset. Again, control of the pH and reseeded resulted in a resumption of nitrification.

Low DO Reactor: R2, Day 24 to 67

Reactor 2 was operated under a constant low DO condition from day 24 to day 67. This operation was meant to encourage simultaneous nitrification-denitrification. The DO in the reactor was approximately 1.0 mg/L at the end of a cycle. Therefore, the DO was never greater than 1.0 and was likely near 0.3 to 0.5 most of the time because of the step feed setup. This does match the DO condition of reactors that have shown simultaneous nitrification denitrification in the literature (Munch *et al.*, 1996).

Despite the similarity in DO concentration to the literature, nitrogen removal was not significant in this reactor nitrogen removal was only $29 \pm 8\%$ (Day 34 to 63), less than the $38 \pm 9\%$ removal during a nonnitrifying period in aerobic/anaerobic SBR R1 (Day 20 to 37). The most likely reason for the lack of nitrogen removal is the inhibition of nitrifiers by free ammonia during startup discussed previously. Inhibited nitrifiers cannot grow well, and would have been washed out of the system, and no nitrification could take place. Simultaneous nitrification-denitrification may be possible with this wastewater and setup, but suspected problems with free ammonia inhibition may have interfered with the process and prevented evaluation of SND.

Anoxic/Aerobic Reactor, R1

R1 was operated as an anoxic/aerobic SBR for the entire operating period. As noted in the Materials and Methods section, a small change was made in the last four hours of the aeration sequence on Day 69. It is unlikely that this change had any major effect on the effluent. The influent to the reactor was a 50% dilution of the anaerobic effluent. The reason for this dilution was noted in the Materials and Methods section. Steady state was attained from Day 55 to 62 with AE1 as the influent, after 1.5 SRTs since the most recent sludge amendment. The influent shifted to AE2 on Day 62. R1 was approaching steady state with AE2 as the influent (2 SRTs

after the most recent sludge amendment) when sampling ended on Day 105.

Removal Efficiencies. Ammonia and nitrate/nitrite nitrogen removal on Day 62 during the first steady state period was 93% corresponding to an effluent containing no ammonia or nitrite and only 9.8 mg/L NO_x-N. Based on the ammonia effluent values for R1 during the last period it was operated (Day 75 to 104, see Figure 18), it seems reasonable to assume that all the ammonia in the influent would be nitrified at steady state. Assuming a worse case scenario, that all the ammonia in the third feed period of a cycle is nitrified and none of the oxidized nitrogen is denitrified during the last anoxic period, the worst ammonia and nitrate/nitrite nitrogen removal efficiency possible using AE2 as the influent is 89%. It should be noted that the removal will be better than this because some of the ammonia nitrogen goes to producing cell mass rather than being oxidized.

Soluble and colloidal COD removal was also extremely efficient (as shown in Figure 19). Based on influent and effluent COD samples filtered through a 1.5 µm filter (colloidal plus soluble), the removal efficiency from Day 51 to 62 (operating with 50% diluted AE1 as influent) was $88 \pm 6 \%$. From Day 93 to 105 (operating with 50% diluted AE2 as influent), a period in which the effluent COD was consistent, the removal efficiency was $73 \pm 4 \%$. When it is taken into account that approximately 25% of the colloidal and soluble COD in AE2 was inert and if the inert COD is subtracted from the influent and effluent COD concentrations, the removal efficiency of biodegradable colloidal plus soluble COD between Day 93 and 105 was greater than 98%.

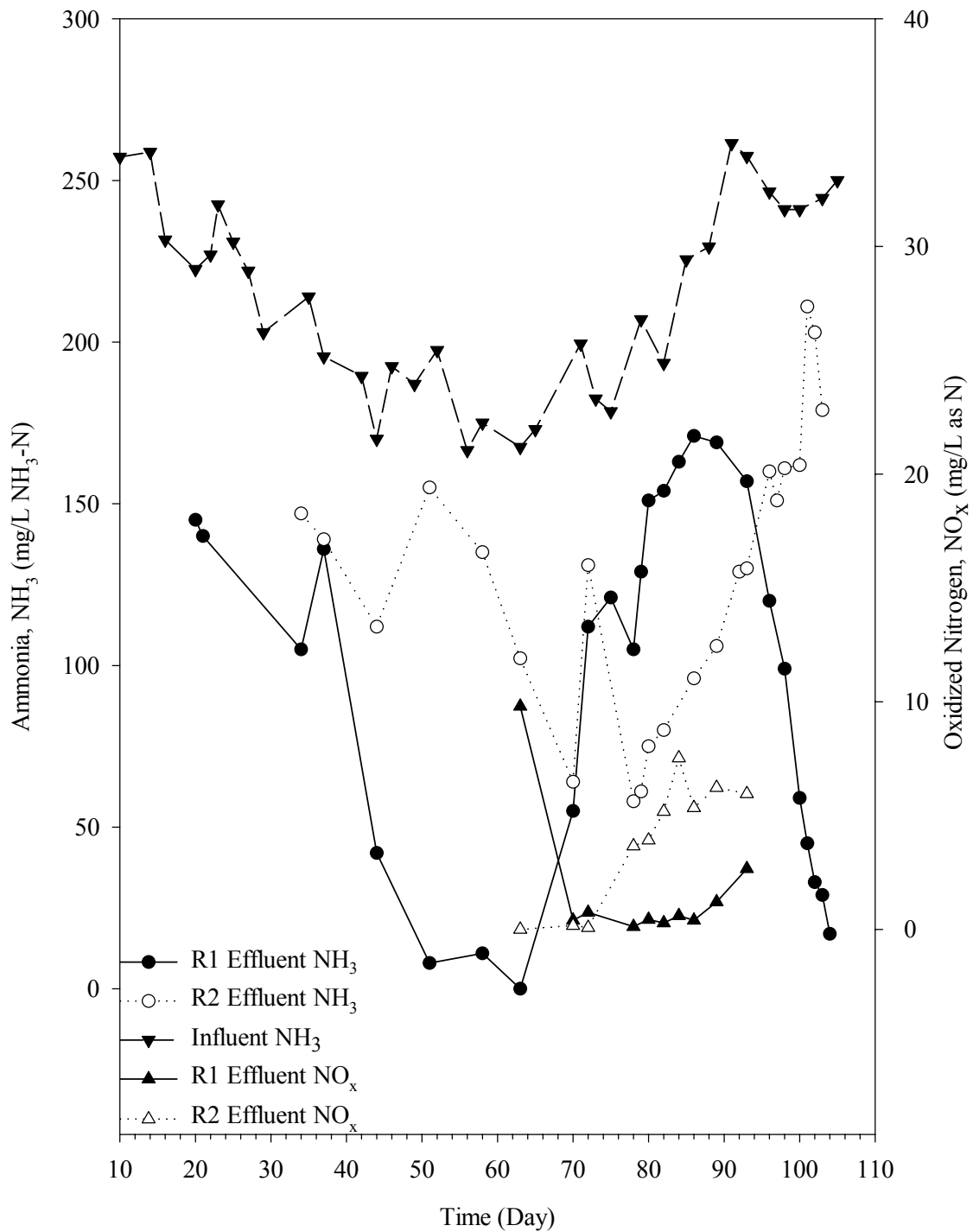


Figure 18. Influent and effluent ammonia and oxidized nitrogen for anoxic/aerobic SBRs.

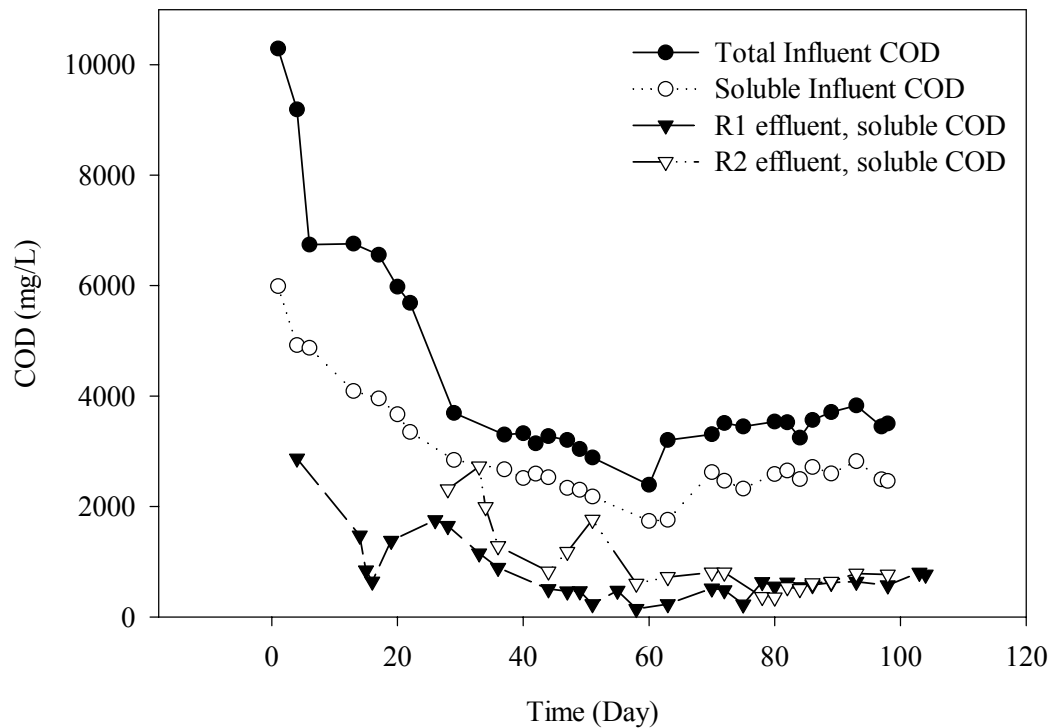


Figure 19. Influent and effluent COD for the anoxic/aerobic SBRs.

Finally, solids removal was $79 \pm 8\%$ from Day 55 to 63 in R1. The removal efficiency increased during the AE2 feed period from Day 84 to 103, to $94 \pm 1\%$. The increase in the removal efficiency was mainly due to an increase in the influent TSS rather than a decrease in the effluent TSS, as the average effluent TSS was 204 ± 78 mg/L and 192 ± 31 mg/L for the first and second feed periods, respectively. It should be noted here that the setup of the decant tube as a J-tube actually artificially inflates these values. The effluent TSS (as well as all the other effluent data) was taken on the decanted effluent. During settling, some of the solids settled into the end of the J-tube. This caused a plug of very high solids material to be decanted every cycle and, therefore, inflated the effluent TSS. The actual value of the TSS in the decant inside the reactor would be much lower. Regardless, for the purposes this reactor would be used for, the effluent TSS values are acceptable. Given that the effluent from the reactor would eventually be

applied to land, is unlikely that discharge would be directly to a waterway and where suspended solids content would pertain.

Profile Analysis. A profile of the major soluble constituents in the reactor was run on Days 103 and 104. Measurements of ammonia, nitrate, nitrite, DOC, and DO were made. This section describes the behavior of the reactor during the second subcycle (hours six through twelve). Measurements during the profile allowed for determination of true anoxic/anaerobic/aerobic time periods, nitrification and denitrification rates, and determination of when the organics were degraded during the cycle.

Table 10 and Figure 20 show the results from the profile measured in the reactor from Hour 6 to Hour 12 (second subcycle) of the cycle on Day 103. From the curves of DO and the $\text{NO}_x\text{-N}$ species, it is evident that the reactor does not go immediately aerobic when aeration begins at hour 8. Anaerobic conditions do develop during the first few minutes of the unaerated phase. From hour 6 to approximately hour 7, the reactor is anoxic (DO is less than 0.3 mg/L and nitrate/nitrite is present). After all the $\text{NO}_x\text{-N}$ is denitrified, the reactor becomes anaerobic from hour 7 to hour 8 until aeration begins. From hour 8 to about hour 8.75, there is a slow increase in DO as the reactor transitions from anaerobic to aerobic. It seems likely that both aerobic and anaerobic conditions occur in the reactor during this period, with aerobic conditions present near the diffuser

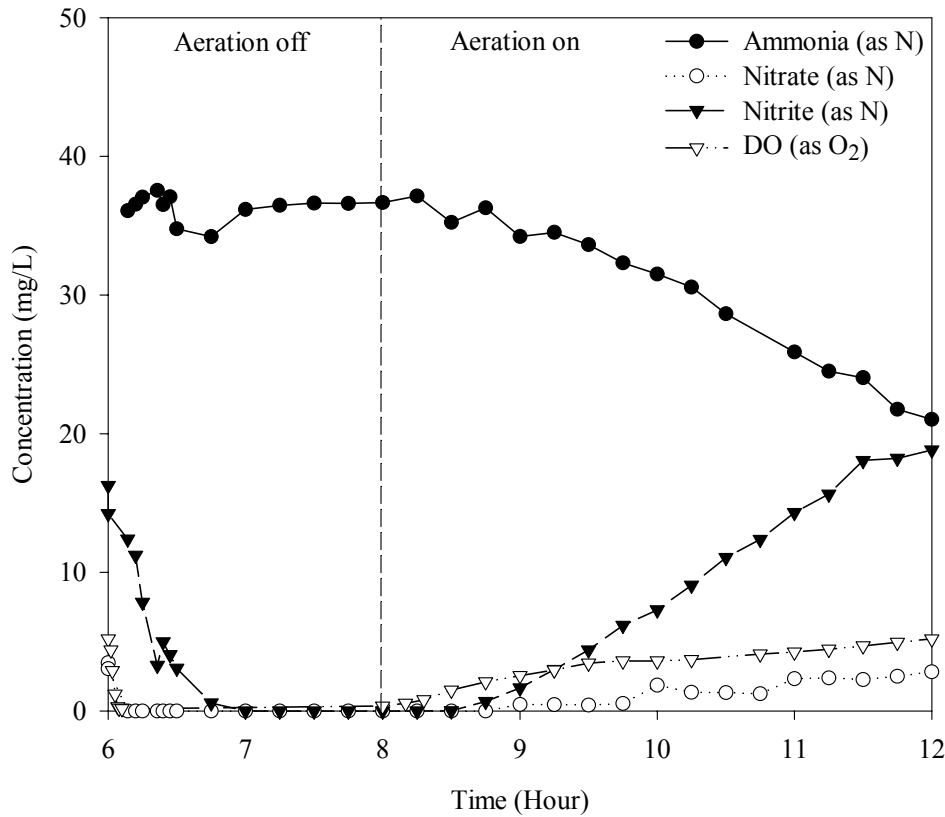


Figure 20. Profile of anoxic/aerobic SBR R1 subcycle on Day 103

Table 10: A Profile Analysis of Soluble Species during Subcycle 2 on Day 103 in Anoxic/Aerobic SBR R1

Hour	Ammonia (mg/L as N)	Nitrate (mg/L as N)	Nitrite (mg/L as N)	DOC (mg/L)	DO (mg/L)
6	36.0	3.0	12.4	127.5	5.2 → 0
8	36.6	0	0	89.8	< 0.3
12	21.0	2.8	18.8	82.8	5.2

and anaerobic conditions located in the rest of the bulk fluid. Finally, truly aerobic conditions persist during the final 3.25 hours of aeration, where the average DO concentration was 3.8 mg/L.

Significant nitrification began taking place after hour 8.5 when the DO reached 2.55

mg/L and continued through hour 12. It should be noted that since this reactor had not yet reached a steady state value for effluent ammonia, nitrification might or might not last the entire aeration period in the steady state reactor. Even though a steady state effluent concentration had not been reached, the nitrification rate was likely steady on a specific basis of oxidized nitrogen species produced per biomass per time. The rate of nitrification should have been at the maximum specific rate because the ammonia concentrations were much higher than the typical half saturation constant for nitrification, 1 mg/L as N. The biomass had been acclimating for a period of about 3 SRTs since the last addition of biomass and should have been acclimated to the wastewater.

Under these conditions, a nitrification rate can be calculated based on the profile measured. This value was calculated as $1.04 \text{ mg NO}_x\text{-N (g MLSS*hr)}^{-1}$. This is lower than the $1.5 \text{ to } 4.0 \text{ mg NH}_4\text{-N (g MLSS*hr)}^{-1}$ reported for an aerobic/anoxic activated sludge process (Panswad *et al.*, 1998). One possible reason for the lower specific nitrification rate with dairy manure wastewater is that the reactor was operating at a pH between 6.0 and 7.0. This low pH caused nitrification to occur at less than the maximum nitrification rate, which would occur closer to a pH of 8.0.

One interesting point is that the majority of the oxidized nitrogen is in the form of nitrite, not nitrate. Nitrification inhibition of the nitrite oxidizing bacteria is likely the reason for this. This is similar to the ammonia inhibition that caused startup problems, but on a lesser scale. Ammonia levels in the reactor averaged between 13 and 37 mg/L during the profile. For a pH of 7 and an ammonia concentration of 37 mg/L, the free ammonia concentration is 0.22 mg/L $\text{NH}_3\text{-N}$. That is on the low end of the 0.1 to 1 mg/L range that causes inhibition of NOB. However, just one SRT before the profile measurements were made, the ammonia level in the effluent was

169 mg/L NH₃-N. The corresponding free ammonia level at that concentration and a pH of 7.0 is 1.0 mg/L NH₃-N. Therefore, it is likely that the NOB were inhibited when the free ammonia was on the high end of the inhibition range and were beginning to recover when the samples were analyzed.

Denitrification of the oxidized nitrogen produced during the previous subcycle took place during the first hour of the profile. A denitrification rate was measured at 5.59 mg NO_x-N (g MLVSS*hr)⁻¹. This is higher than values of denitrification rates at noncarbon-limiting conditions cited by Metcalf and Eddy (1991) of 3.125 to 4.79 mg NO_x-N (g MLVSS*hr)⁻¹. As mentioned previously, denitrification rates are affected by the COD:NO_x-N ratio in the anoxic zone. The fast rate of denitrification in this subcycle is encouraged because of the feed period at the beginning of the anoxic zone. The COD in the influent serves as a carbon and energy source for denitrification. If the reactor was forced to use endogenous or slowly degradable carbon as its carbon source for denitrification, then the denitrification rate would be much slower. Reported denitrification rates for denitrification under endogenous carbon sources are several times slower than rates using fresh wastewater as a carbon and energy source (Metcalf and Eddy, 1991). There is, therefore, a significant benefit of using a step feed strategy in the SBR.

Finally, Figure 21 shows the DOC profile during the second subcycle on Day 103. While the data is somewhat variable, the majority of the DOC that is removed is removed during the anoxic and anaerobic periods. This shows that the DOC (and therefore the COD) in the effluent is generally either very slowly biodegradable or inert. This is in agreement with the 98% COD removal efficiency for R1 presented previously.

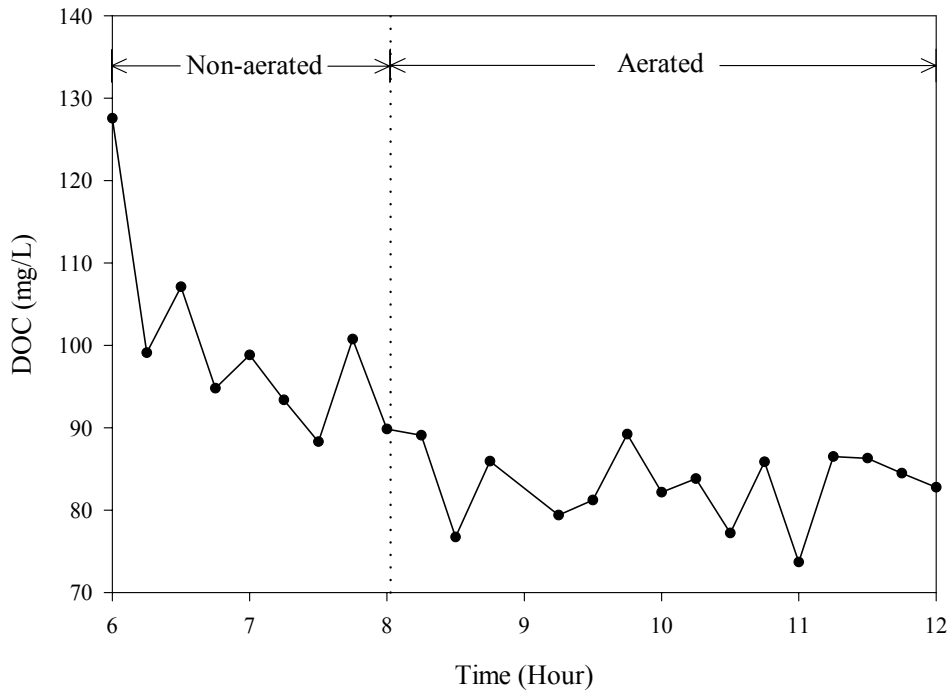


Figure 21. Profile of DOC in Anoxic/Aerobic SBR R1 on Day 103.

It should be noted that computer simulations were not used with the parameters previously measured to develop profiles to be compared with Figures 20 and 21. This is because more validation of the Activated Sludge Model for use with wastewaters other than municipal wastewaters, as the Activated Sludge Model was developed using municipal wastewater treatment as a basis. More data on wastewater characterization, further kinetic and stoichiometric parameter determination, and additional experimental treatment of dairy manure wastewater is necessary before the Activated Sludge Model can be successfully applied to the treatment of dairy manure wastewater.

The lab-scale treatment of the 50% diluted anaerobic effluent was successful at achieving high removal efficiencies of COD and soluble inorganic nitrogen. While this experimentation did not use full strength influent because of oxygen requirements that could not be met in the lab, it does suggest that dairy wastewater can be treated successfully for the removal of nitrogen

given the fractions of nitrogen and carbon that exist in the wastewater. It should be noted that while the effluent concentrations possible in the lab scale reactors are low relative to the influent and treatment is efficient, these concentrations may not be low enough to meet surface water discharge permit limits as the limits for TSS can be very low (i.e. Blacksburg/VPI Wastewater Treatment Plant's limit of ~30mg/L TSS).

Nitrogen Removal in Sequencing Batch Reactors: Simulated Sensitivity Analysis

Incremented Step Feed versus Non-incremented Step Feed

One part of the nitrogen removal SBR configuration that affects the reactor performance is the feeding. As noted previously, a step feed can enhance denitrification in an anoxic/aerobic reactor by using the carbon in the wastewater as the carbon source for denitrification. This allows for rapid denitrification during the fill/anoxic period. Still, there is nitrate/nitrite formed during the aeration period after the last feed. This nitrate/nitrite is not as easily denitrified because of the lack of carbon available for denitrification. Because denitrification of the nitrate/nitrite formed from the nitrogen in the last feed, the last feed volume fed should be as small as possible. This reduces the amount of nitrate/nitrite formed in the last aeration period and therefore lowers the effluent nitrate/nitrite. This cannot be done simply by decreasing the volume of the last feed period and leaving the other periods constant, because the last feed period is the source of carbon for denitrification of the nitrate/nitrite formed in the previous period.

One way to reduce the volume in the last feed period is to increase the number of subcycles and feed periods. This will decrease the amount of feed input during each subcycle. One problem with this is that an increase in the DO during the aeration period can be slow in a reactor with a high load and solids content (as seen from the DO data presented for the

anoxic/aerobic SBR profile in Figure 20). There is a minimum length of time necessary for the reactor to become aerobic, and, therefore, a minimum number of subcycles available during a cycle.

Another method to reduce the volume in the last feed period is to vary the amount of influent that is fed during each feed period. This method was analyzed in a sensitivity analysis. As previously described in Materials and Methods, five simulations were run using the same reactor setup with differing amounts of influent fed during each feed period. One simulation fed the same amounts during each subcycle, while the other four simulations fed decreasing quantities of influent in each subcycle. The percentages of feed in each subcycle were presented in Table 7.

Each of the reactors in the simulations had effluent ammonia and soluble COD close to zero. As suggested previously, the difference in the effluents was the nitrate concentration. Figure 22 shows the nitrate in the reactor during the cycle for each simulated reactor. As more of the influent is fed in the earlier subcycles, the effluent nitrate decreases. The effluent reaches an optimum in the simulation with 40% of the total influent fed in the first subcycle.

Upon moving even more of the influent to the earlier subcycles, the feed in the last subcycle becomes insufficient for providing the carbon necessary to denitrify the nitrate produced in the third subcycle. These simulations indicate that for a reactor that is limited in how short the subcycles can be because of oxygen demand and transfer, a step feed in which the feed amount decreases in each subcycle can increase denitrification and lower the effluent nitrate.

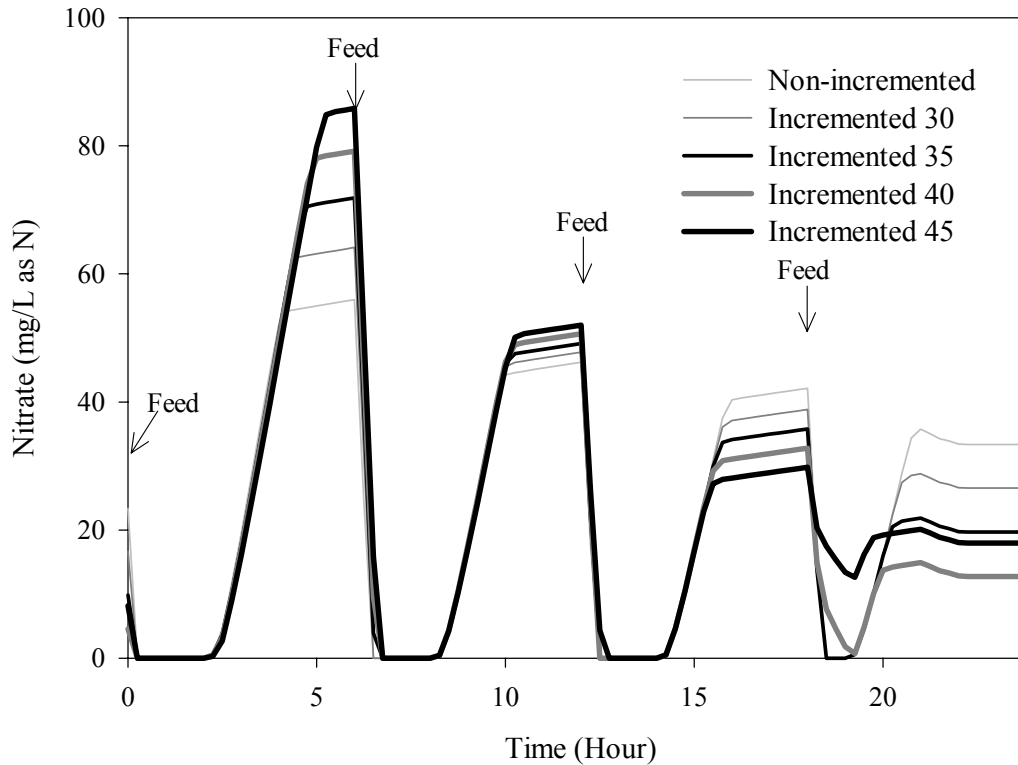


Figure 22. The effect increasing the amount of feed added early in the cycle has on effluent nitrate.

Sensitivity of the Anoxic/Aerobic SBR to Kinetic and Stoichiometric Parameters

Uncertainty in kinetic and stoichiometric experiments can create problems in simulating wastewater treatment. In an SBR that contains both anoxic and aerobic periods, the number of parameters is doubled compared to just an anoxic reactor as are the potential sources of parameter error associated with the estimates. For example, error in heterotrophic growth and decay rates can cause the simulated substrate uptake rate to be artificially high or low. The effect of this error can be quite complex depending on the actual setup of the reactor. Since the parameters differ in their contribution to the model, error in each parameter will affect the simulation in a different way. Also, since much time and effort is involved in determining all of the parameters for a given wastewater, it would be useful to know which parameters affect the effluent values of highest interest, and the degree to which the parameters affect the values.

A sensitivity analysis was performed, therefore, on the effect of varying several kinetic and stoichiometric parameters on a simulated anoxic/aerobic SBR using BioWin. The simulated reactor used was the incremented step-feed SBR with a 40:30:20:10 incremented feed outlined in the previous section.

Several of the points used as indices of sensitivity are shown in Figure 23. The amount of time to degrade a given compound was used to show the effect of changing parameters. Effluent quality may remain relatively consistent between different simulations using different parameters for a particular configuration. However, a change in a single parameter may show that the actual time to achieve satisfactory levels of degradation is less. This scenario suggests that acceptable effluent quality may be achieved with either a smaller reactor or with greater contaminant mass loadings, and demonstrates the value behind evaluating the impact of kinetic and stoichiometric parameters on simulated reactor performance. Point DN1 in Figure 23 is the time it takes for the nitrate created in the previous subcycle to be reduced below 1 mg/L through denitrification. This time value is measured from hour 6 to DN1 and is inversely proportional to the denitrification rate. The effect of each the parameter on the nitrification rate is measured relative to point N2. This point represents the time required during the aerobic period to nitrify the ammonia to a value less than 1 mg/L. The time is measured from the beginning of aeration at hour 2 until N2. The third point, S3, represents the time required for the soluble COD to decrease below 380 mg/L (about 8 mg/L degradable COD). This point shows the effect of the parameters on COD substrate uptake. This time is measured from the beginning of the subcycle when the reactor is fed at hour 0 until S3. With the given reactor configuration and influent, little of the soluble COD is actually degraded aerobically for many of the parameter combinations. Most of the initial soluble COD is degraded anoxically and anaerobically during

the first two hours as shown previously in Figure 18. In most cases, the remaining soluble COD degraded during the first hour of aeration while the DO in the reactor increased from near zero to approximately 2.5 mg/L.

In addition to these three sensitivity indices, other criteria were considered when evaluating simulations, including total oxygen consumption for a complete cycle, effluent particulate COD, effluent nitrate, effluent ammonia, and effluent soluble COD. Table 11 shows a summary of the parameters measured, the values used for each parameter, and the results for each point in units of time or concentration. To compare the relative effects of each parameter on a particular sensitivity index, Table 12 was developed and shows the relative sensitivities determined for each parameter. The relative sensitivities were calculated by dividing the percent change in the sensitivity index by the percent change made in the parameter (from the median

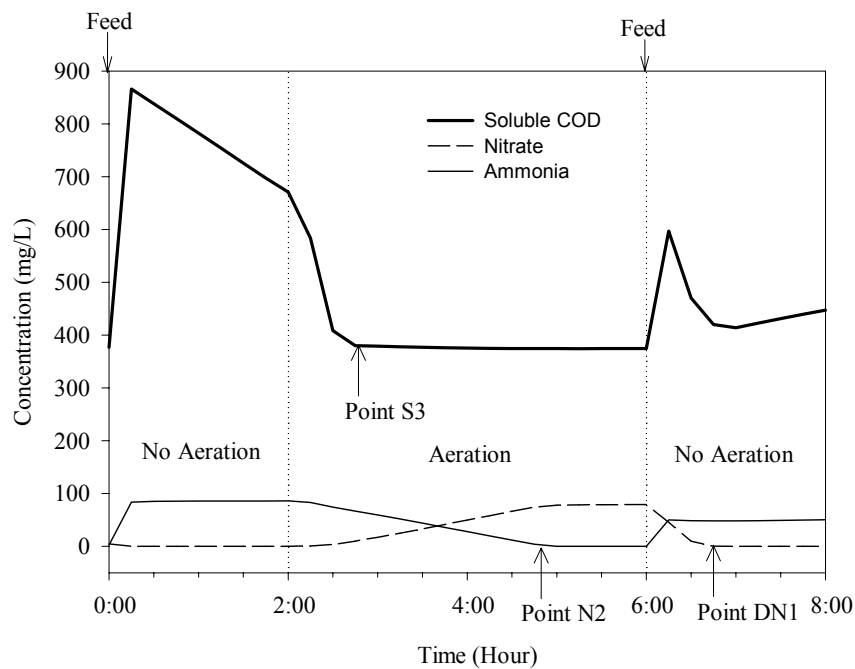


Figure 23. Location of the sensitivity indices with respect to the reactor cycle.

value to the upper value simulated). Equation 16 shows how the relative sensitivities were calculated.

$$\text{Relative Sensitivity} = \frac{\Delta I / I}{\Delta P / P} \quad (16)$$

where I is the sensitivity index value and P is the kinetic or stoichiometric parameter value that is varied. The values highlighted in the sensitivity index columns in Table 12 are relative sensitivities for which the sensitivity index changed more than 25% for a 100% change in the kinetic or stoichiometric parameter. Because the effluent concentrations desired and achieved were very low, small changes in the absolute effluent concentrations caused large relative sensitivities. Therefore, the nitrification, denitrification, and soluble COD removal rates are better descriptors of the actual effect of a change in a parameter.

Heterotrophic maximum specific growth rate ($\mu_{\max,H}$) did not have a significant effect on any of the indices as long as the growth rate was large enough to prevent washout of the biomass. While the extremely low value of 0.3 d^{-1} did cause the biomass to washout and disrupt the system, the parameter did not begin to affect the denitrification rate and the soluble COD removal until it was less than 2 d^{-1} . Values measured for heterotrophic growth on domestic wastes have ranged from 3.0 to 13.2 d^{-1} and the results from an extant growth experiment presented earlier in this report suggested that the heterotrophic maximum specific growth rate for the biomass treating diluted dairy waste was greater than 1.84 d^{-1} . Given this, $\mu_{\max,H}$ is not a parameter that will drastically affect the effluent values from a reactor using a similar cycle configuration. Design of a similar reactor using values of $\mu_{\max,H}$ in the range of 2 to 3 d^{-1} should be sufficient and will likely provide a safety factor for insurance of adequate COD removal and denitrification.

The heterotrophic half-saturation coefficient, K_S , had little effect on the effluent composition as well. However, a significant increase in K_S can lengthen the time required to remove the soluble COD. A change in K_S from 20 mg/L as COD to 200 mg/L results in an increase in the time required to remove soluble COD from two hours forty-five minutes to four hours forty-five minutes. The extant tests in this research suggested that K_S is high and therefore 200 mg/L may be more representative of the dairy manure wastewater. Interestingly, it takes approximately the same amount of time to remove the COD as it does to nitrify the ammonia when K_S is 200 mg/L. If the COD:TKN ratio is low enough to prevent nitrification inhibition, a K_S of 200 versus a K_S of 2 should not affect the efficiency of the design. In addition, as was mentioned previously, incorporating a longer SRT can minimize the effect of the high K_S that may be associated with dairy manure wastewater.

The heterotrophic decay coefficient, b_H , does have significant effect on several of the indices. First, a change in the decay coefficient caused large changes in the required oxygen. This is because the process of decay utilizes oxygen. A change in the decay rate across the range of typical values resulted in a change in required oxygen required by more than 30%. This much of an increase in oxygen requirement can be very expensive or even cause some designs to become unfeasible. In addition, an increase in decay rate from 0.24 d^{-1} to 1.2 d^{-1} caused significant increases in the time needed for satisfactory denitrification and soluble COD removal. The increased decay rate also caused a buildup in the particulate COD because of the reduced heterotroph population that would be in the reactor at such a high decay rate as well as an increase in the debris generated by a higher decay rate. Therefore, the decay rate must be measured precisely, as it has such a large affect on the treatment process. Fortunately, b_H is a relatively easy parameter to measure.

Heterotrophic yield also had a significant effect on the oxygen required as well. An increase in heterotrophic yield from 0.2 to 0.67 mg biomass/mg substrate decreased oxygen required by more than 50%. This occurs because a decrease in yield directs less of the electrons from the donor into biomass and more to the electron acceptor, oxygen. Therefore, a simulation with a low value of yield relative to typical values and near the experimentally determined value of 0.42 should provide an overestimation of the oxygen required for design purposes. While some of the other indices did have relative sensitivities greater than 0.25, since the typical range for heterotrophic yield is only from 0.4 to 0.7, it is unlikely that an error of 100% will be made in the estimation of yield. Therefore, the actual error in the indices should be less than that shown in Table 12. In addition, as the desired values for effluent ammonia and nitrate are likely to be very low, a relative error greater than 0.25 will not likely increase or decrease the magnitude of the effluent values significantly.

The autotrophic maximum specific growth and decay rates both have similar effects on the indices. They act in opposite manners, as an increase in the growth rate has a similar effect as a decrease in decay rate. These parameters determine both the SRT at which washout of the nitrifiers occurs and the amount of nitrifying biomass in the reactor, and are, therefore, very important. A change in the value of decay or growth across the typical range can cause a reactor to go from nitrifying all the ammonia in one and a half hours to washing out the nitrifiers and eliminating nitrification altogether.

The experiment for determining $\mu_{A,max}$ discussed earlier allowed for the determination of the specific growth rate and an assumed value for the decay rate. Since the decay rate in essence subtracts from the specific growth rate, the growth rate measured can be used in combination with the decay rate that is assumed for modeling and design. The autotrophic maximum specific

Table 11: Data from Sensitivity Analysis of Kinetic and Stoichiometric Parameters

Parameter	Median Value	Value	O ₂ consumed (mg)	DN1 (min)	N2 (min)	S3 (min)	COD _P (mg/L)	COD _S (mg/L)	NH ₃ (mg/L as N)	NO ₃ (mg/L as N)
All medians			5,117	45	180	165	9	374	0.3	12.8
$\mu_{\max,H}$ (d ⁻¹)	3	0.3	1,938	NA	NA	NA	3,465	4,820	66	417
		10	5,105	60	180	150	9.1	374	0.4	19.9
K _S (mg/L as COD)	20	2	5,185	45	180	150	8.8	379	0.3	15.9
		200	4,976	60	180	285	8.9	374	0.4	12.4
b _H (d ⁻¹)	0.24	0.048	4,390	30	215	150	3.2	379	0.1	13.0
		1.2	5,824	120	180	360+	2,339	374	0.5	11.0
Y _H	0.31	0.2	5,716	45	180	150	7.3	374	0.3	16.9
		0.67	3,721	30	180	180	14.7	375	0.6	9.0
$\mu_{\max,A}$ (d ⁻¹)	0.792	0.24	3,275	NA	NA	165	6.7	375	508	0
		2.16	4,861	45	90	165	9.0	374	0.2	10.5
b _A (d ⁻¹)	0.072	0.0072	5,063	45	120	180	8.5	374	0.3	11.8
		0.168	5,242	45	NA	165	9.2	374	0.5	13.2
Y _A	0.24	0.07	5,316	45	180	165	8.7	374	0.3	13.0
		0.35	5,032	45	180	180	9.1	374	0.4	12.6
k _H (d ⁻¹)	3	0.3	3,220	60	240	180	5,797	374	0.1	17.7
		10	4,758	45	180	165	2.4	374	0.4	12.8
K _H (mg/L)	0.03	0.003	4,960	45	180	180	1.2	374	0.4	12.3
		0.3	5,340	45	180	165	132	374	0.3	17.6
K _{O,A} (mg/L O ₂)	0.6	0.3	5,112	45	165	180	9.0	374	0.3	12.7
		1.3	5,165	45	210	165	9.0	374	0.5	12.9
K _{NH} (mg/L as NH ₃ -N)	1	0.1	5,181	45	165	180	8.9	374	0.2	12.6
		5	5,112	45	210	180	8.9	374	0.7	13.1

Headings: O₂ consumed, total oxygen consumed during a complete cycle; DN1, time for denitrification; N2, time for nitrification; S3, time for soluble COD uptake; COD_P, effluent particulate COD, COD_S, effluent soluble COD; NH₃, effluent ammonia; NO₃, effluent nitrate.

NA: Not applicable. Cells containing NA indicate that the time required for nitrification, denitrification, or COD removal exceeded the time allowed in the cycle or did not occur at all.

Table 12: Relative Sensitivity Analysis for Kinetic and Stoichiometric Parameters^a

Parameter	O ₂ consumed	DN1	N2	S3	COD _p	COD _s	NH ₃	NO ₃
$\mu_{\max,H}$ (d ⁻¹)	0.00	0.14	0.00	0.04	0.00	0.00	0.14	0.24
K _S (mg/L as COD)	0.00	0.04	0.00	0.08	0.00	0.00	0.04	0.00
b _H (d ⁻¹)	0.03	0.42	0.00	0.30	64.72	0.00	0.17	0.04
Y _H	0.23	0.29	0.00	0.08	0.55	0.00	0.86	0.26
$\mu_{\max,A}$ (d ⁻¹)	0.03	0.00	0.29	0.00	0.00	0.00	0.19	0.10
b _A (d ⁻¹)	0.02	0.00	did not nitrify	0.00	0.02	0.00	0.50	0.02
Y _A	0.04	0.00	0.00	0.20	0.02	0.00	0.73	0.03
k _H (d ⁻¹)	0.03	0.00	0.00	0.00	0.31	0.00	0.14	0.00
K _H (mg/L)	0.00	0.00	0.00	0.00	1.52	0.00	0.00	0.04
K _{O,A} (mg/L O ₂)	0.01	0.00	0.14	0.00	0.00	0.00	0.57	0.01
K _{NH} (mg/L as NH ₃ -N)	0.00	0.00	0.04	0.02	0.00	0.00	0.33	0.01

^a The highlighted values are relative sensitivities for which the sensitivity index changed more than 25% for a 100% change in the kinetic or stoichiometric parameter

growth rate should be measured when designing systems similar to the one here since it has such a large effect on the nitrification rate and the effluent ammonia.

Autotrophic yield, Y_A , had little or no effect on the overall system. The indices in the sensitivity analysis changed very little over a broad range of Y_A . Only effluent ammonia had a relative sensitivity greater than 0.25, which, as previously mentioned, is not necessarily descriptive of the actual effect the parameter has on nitrification. Values for Y_A have been reported to range between 0.07 and 0.28 mg biomass/mg nitrogen oxidized (Henze *et al.*, 1987). Choosing the theoretical value of 0.24 should be sufficient for design purposes.

Hydrolysis can affect the efficiency of the reactor in several ways, especially in terms of the rate and extent of particulate COD removal. A low hydrolysis rate, k_H , can cause slow particulate COD breakdown and accumulation of particulate COD in the reactor. A high value of the hydrolysis half saturation constant, K_H , can also slow particulate COD removal. A low hydrolysis rate can also artificially slow down the nitrification rate (as shown in Table 11) by slowing down the rate at which the particulate nitrogen gets converted to organic nitrogen, which is subsequently ammonified to ammonia and then oxidized to nitrate. Although, both k_H and K_H had an effect on the indices, the simulated reactor was not extremely sensitive to them. The only index which had a relative sensitivity greater than 0.25 was the effluent particulate COD. If efficient settling occurs in the reactor, particulate COD may not be a major concern in the effluent. Therefore, as long as the actual value of k_H is close to the typical value of 2.2 d^{-1} (Grady *et al.*, 1999), there will be little change in the efficiency of the treatment process. In addition, if K_H is not outside of the range analyzed there will be little effect on the reactor as a result of changes in K_H .

The half-saturation coefficient for autotrophic growth, K_{NH} , and the half saturation constant for oxygen affinity used in the autotrophic growth switching function, K_{OA} , are values that are normally assumed in modeling. Measured values for K_{OA} have been reported to lie between 0.5 and 2.0 mg O₂/L (Henze et al., 1987). Effluent ammonia was the only index for which the relative sensitivity was greater than 0.25, and, as mentioned previously several times, this index is not a good descriptor of the actual effect of the parameter. K_{OA} will have less impact on a reactor that operates at DO concentrations greater than the K_{OA} value. The slow rise from zero to 2 mg/L in this reactor setup caused K_{OA} to have a measurable impact on the nitrification rate. To be conservative, a value on the order of 1.3 mg/L should be chosen so as to provide enough time for nitrification. The autotrophic half-saturation constant for ammonia, K_{NH} , can have a similar effect on the nitrification rate. A low value (0.1 mg/L) for K_{NH} can cause nitrification to be very fast. Higher values (5 mg/L) slow the nitrification and require that longer times be used for nitrification. Normally, it is very common to use $K_{NH} = 1$ mg/L as N (Henze et al., 1987). Actual values should not vary widely from this as long as the biomass is not exposed to nitrification inhibitors, and will only cause problems if an extremely low effluent value for ammonia is required.

The sensitivity analysis indicates that the particular values of many of the parameters may not be extremely important as long as they fall in a range of common values. One exception to that is the autotrophic maximum specific growth rate ($\mu_{max,A}$). It can affect nitrification rates, concentrations of autotrophic biomass, and the SRT at which nitrifiers are washed out. In addition, heterotrophic yield can have a major effect on the total oxygen consumption in the reactor. Finally, the heterotrophic decay rate can have a significant impact on the time period required for denitrification because of the effect it has on the biomass concentration.

V. SUMMARY AND CONCLUSIONS

Summary

Research was conducted to develop a biological nitrogen removal process for dairy wastewater. Flushed and screened dairy wastewater was characterized by its concentrations of pollutants. In addition, kinetic and stoichiometric parameters were determined for the biological nitrogen removal processes operating on dairy wastewater. An SBR cycle based on previous success with piggery wastewater was used to demonstrate lab-scale treatment and nitrogen removal from the dairy manure wastewater. Finally, using the wastewater characterization and parameters measured, the sensitivity of the SBR sequence to selected kinetic and stoichiometric parameters as well as the feed configuration was determined.

Conclusions

The following conclusions can be drawn from the wastewater characterization, determination of kinetic/stoichiometric parameters, lab-scale treatment, and mathematical model simulations.

Wastewater Characterization

1. Settling and anaerobic pretreatment of the flushed/screened wastewater can result in good removal of particulate. This reduces the quantity of COD, organic nitrogen, and phosphorus in the wastewater. Therefore, pretreatment through settling and anaerobic treatment is an important part of the treatment process.
2. The carbon to nitrogen ratio for the raw wastewater is more than enough to operate efficient biological nitrogen removal. The same is true for the carbon to phosphorus ratio and phosphorus removal. Pretreatment reduces the COD:TKN ratio, but not to the level at which

inefficiency would result. On the other hand, a pretreated wastewater may not have enough carbon to be efficient at removing both nitrogen and phosphorus.

Kinetic and Stoichiometric Parameter Determination

1. Heterotrophic yield was determined to be at the low end of the range reported for municipal systems. Heterotrophic decay autotrophic maximum specific rates were very similar to typical values reported in the literature. Therefore, when modeling treatment of dairy manure wastewater, it is appropriate to use reported typical municipal values for these three parameters.
2. The heterotrophic maximum specific growth rate was not easily determined, but appears to be in the range of typical values found with biomasses treating municipal wastes. It is possible that the half-saturation constant for heterotrophic growth on dairy manure wastewater may be significantly higher than values found for municipal wastewaters. Further tests are needed to provide adequate support for these conclusions.

Lab-Scale Biological Nitrogen Removal

1. Biological nitrogen removal is effective using a step feed anoxic/aerobic SBR. Ammonia removal efficiencies of near 100% were achieved for the 50% diluted wastewater and reactor configuration used. Soluble inorganic nitrogen (ammonia and oxidized nitrogen) removal efficiencies of 93% and 89% were possible as well.
2. The step feed provided for very high specific denitrification rates. This configuration is also able to denitrify without adding an external carbon and energy source.

Modeling and Simulations

1. Analysis of the feeding method showed that, under the same aeration conditions, using an anoxic/aerobic SBR with a step feed in which the feed quantity decreases during each

subcycle can lower the effluent nitrate/nitrite concentration compared with that of a step feed that uses an equal proportion of the total feed during each subcycle.

2. The sensitivity analysis indicated that for the reactor configuration and wastewater used in the simulations, many of the kinetic and stoichiometric parameters had little effect on the treatment processes as long as they were in the range of values reported in the literature. The parameters measured that had minor impact on the results were heterotrophic maximum specific growth rate and half-saturation constant, autotrophic yield, autotrophic half-saturation constant, and the oxygen concentration used in the autotrophic switching function. In addition, hydrolysis only has an effect on particulate COD in the effluent that can be insignificant if efficient settling occurs in the reactor.
3. The sensitivity analysis indicated that heterotrophic yield, heterotrophic decay, and the autotrophic maximum specific growth rate can affect the reactor performance. Heterotrophic yield had little impact on the effluent quality or degradation rates, but did have a large impact on the amount of oxygen required for treatment. Heterotrophic decay had a significant effect on the time period required for denitrification. Autotrophic growth rate is the most important parameter and determines the SRT that nitrifiers wash out as well as the nitrification rate.

Engineering Significance

Recently there has been an increase in concern over pollution from animal manures. Much of the research centered on dealing with this has been on removing excess nutrients from the diet of the animals. Some research has been conducted on nutrient removal from piggery wastes by biological means, but little research has been done on dairy manure treatment. Results from this study provide some of the initial information necessary for developing a successful biological nitrogen removal system for dairy manure. This study does not provide the

information necessary to develop a nitrogen removal sequence in an SBR for all farms. As was mentioned in the Introduction, there are a large number of parameters associated with the individual farm that go into developing effluent criteria from the reactors including the soil characteristics where the wastewater is to be applied, the type and amount of crops grown on the farm, number of animals contributing to the wastewater, and the diet of the animals on the farm as well as many others. This study should provide a basis for further research in nitrogen removal from dairy wastewater, which can then be incorporated with effluent guidelines developed for individual farms to design treatment systems.

First, the wastewater characterization provided detailed information of the nutrient components of two flushed/screened dairy manure wastewaters. In addition, the pretreatment analysis showed that pretreatment is helpful in removing particulate matter but may make it difficult to remove both nitrogen and phosphorous in the same system. As more dairies begin treating their wastes, this information may be helpful in determining whether pretreatment should be used or not and will provide characterization data for comparison with their wastes.

The kinetic and stoichiometric parameters determined in this study indicate that most of the parameters have values similar to that of municipal wastes. This shows that the assumption of typical municipal parameters used for modeling in previous research is reasonable. The parameters determined also provide values for parameters not previously determined for dairy manure wastewater treatment in the literature.

Finally, the sensitivity analysis gives information as to the most important parameters to be experimentally determined for this treatment sequence. This can save future researchers time and effort when performing parameter estimations on this type system.

Recommendations

This study demonstrated the large variability that occurs in dairy manure wastewaters. There is variability between wastewater from different farms as well as variability in the wastewater from a single farm. Before the initiation of any treatment process design, the wastewater to be treated at a particular farm should be characterized in detail for nitrogen, phosphorous, and carbon species.

The study provided insight into some of the kinetic and stoichiometric parameters associated with the dairy manure nitrogen removal. The values of heterotrophic growth rate and half-saturation constant were not determined with certainty but questions were raised as to the magnitude of the half-saturation constant. An attempt should be made for determining if the half-saturation constant is actually as high as this research suggests. While the sensitivity analysis shows that K_S has little impact on this particular treatment configuration, it may have a larger impact on a sequence with less leeway for error in design.

The study also suggested that there is enough carbon available in the wastewater for removal of nitrogen and phosphorus in the same system. It also suggested that pretreatment may make removal of both nutrients more difficult. Research should be undertaken to attempt removals of nitrogen and phosphorus in the same system. This may be done through experimentation or simulation.

Lastly, while the lab scale reactors did possibly have periods of free ammonia inhibition of nitrification, this was not studied in depth. As free ammonia inhibition may be a problem that is typical of dairy manure wastewater, it is recommended that research be completed to determine whether it will occur in the full-scale systems in the field or if the inhibition was just

an artifact of the anaerobic pretreatment SBR and the lab scale systems. Control of pH in the field could be a difficult and expensive part of the treatment for a farm and should be investigated further.

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APPENDIX A: Calculations of Wastewater Characterization Values

Table A1: Wastewater PA1 Calculation Explanation

Parameter	Method of Determination	Value	Std. Dev.
TSS	Composite from 3 storage buckets/ 5 measurements	27,817	1,729
VSS	Composite from 3 storage buckets/ 5 measurements	24,427	1,229
Total COD	3 samples (including a composite)	34,529	3,666
1.5 mm COD	4 samples (including a composite)	12,544	1,443
0.025 mm COD	2 samples	7,919	394
Total TKN	2 samples (including a composite)	1,736	8
Soluble TKN	1 sample	846	ND
Ammonia	2 samples (including a composite)	728	24
Total P	1 sample/ 4 measurements	325	66
Sol. Ortho P	1 sample/ 6 measurements	213	37

Table A2: Wastewater PA1sd Calculation Explanation

Parameter	Method of Determination	Value	Std. Dev.
TSS	4 samples	3,505	1,897
VSS	4 samples	3,006	1,494
Total COD	2 samples	9,891	2,983
1.5 mm COD	2 samples	4,999	400
0.025 mm COD	Value for PA1 diluted 1:2.844	2,784	139
Total TKN	1 sample	443	32
Soluble TKN	Value for PA1 diluted 1:2.844	297	ND
Ammonia	Value for PA1 diluted 1:2.844	256	8
Total P	ND	ND	ND
Sol. Ortho P	Value for PA1 diluted 1:2.844	75	13

Table A3: Wastewater AE1 Calculation Explanation

Parameter	Method of Determination	Value	Std. Dev.
TSS	9 samples taken from March 11th through April 4th	964	264
VSS	9 samples taken from March 11th through April 4th	908	239
Total COD	5 samples taken from March 23rd through April 8th	5,921	701
1.5 mm COD	5 samples taken from March 23rd through April 8th	4,434	591
0.025 mm COD	ND	ND	ND
Total TKN	2 samples	449	10
Soluble TKN	ND	ND	ND
Ammonia	6 samples taken from March 23rd through April 6th	363	25
Total P	4 samples from March 15th through April 3rd	84	26
Sol. Ortho P	5 samples from March 15th through April 3rd	48	7

Table A4: Wastewater PA2 Calculation Explanation

Parameter	Method of Determination	Value	Std. Dev.
TSS	Average of 2 composite samples from 3 buckets each	18,400	1,131
VSS	Average of 2 composite samples from 3 buckets each	16,079	76
Total COD	Average of 2 composite samples from 3 buckets each	34,743	233
1.5 mm COD	Average of 2 composite samples from 3 buckets each	14,753	1,300
0.025 mm COD	Determined a ratio of the 0.025 COD to 1.5 COD measured in the June composite and multiplied that ratio by the PA2 1.5 COD average	9,081	2,786
Total TKN	Sum of particulate and soluble TKN from below	1,911	140
Particulate TKN	Determined a ratio of the particulate TKN to TSS measured in the April composite and multiplied that ratio by the PA2 TSS average	486	115
Soluble TKN	Determined by measuring the soluble TKN in a 3 day composite sample of anaerobic feed and multiplying by 2.844 to account for the dilution	1,425	80
Ammonia	Average of 6 samples to of the anaerobic feed (including one 3 day composite) multiplied by 2.844 to account for the dilution	1297	102
Total P	Composite sample of three buckets in April	441	44
Sol. Ortho P	Composite sample of three buckets in April	196	27

Table A5: Wastewater PA2sd Calculation Explanation

Parameter	Method of Determination	Value	Std. Dev.
TSS	Once multiplied by 2.844 to account for dilution, samples from the feed to the anaerobic reactor gave larger values of TSS/VSS than were in the unsettled wastewater. This is most likely because the feed buckets were not totally mixed when the samples were drawn. Therefore, a settling test was run separately on a composite sample of raw wastewater. The ratio of settled wastewater TSS divided by the raw wastewater TSS was multiplied by the PA2 TSS average to get PA2sd TSS. The same was done for VSS.	4,080	318
VSS		3,388	228
Total COD	Particulate COD plus 1.5 COD from below	8,821	558
Particulate COD	Determined by measuring a particulate COD to TSS ratio in the settling experiment and multiplying by the PA2sd TSS	3,633	321
1.5 mm COD	value for PA2 divided by 2.844	5,187	457
0.025 mm COD	value for PA2 divided by 2.844	3,193	980
Total TKN	Sum of particulate and soluble TKN from below	609	39
Particulate TKN	Ratio of PA2sd TSS to PA2 TSS was multiplied by the PA2 particulate TKN	108	28
Soluble TKN	Composite sample of three days of anaerobic feed	501	28
Ammonia	value for PA2 divided by 2.844	1296	104
Total P	Composite sample of three days of anaerobic feed	110	18
Sol. Ortho P	Composite sample of three days of anaerobic feed	67	9

Table A6: Wastewater AE2 Calculation Explanation

Parameter	Method of Determination	Value	Std. Dev.
TSS	4 samples taken from May 14th through May 23rd	3,037	1,828
VSS	4 samples taken from May 14th through May 23rd	2,440	1,300
Total COD	6 samples taken from May 2nd through May 16th	7,101	409
1.5 mm COD	6 samples taken from May 2nd through May 16th	5,193	288
0.025 mm COD	Ratio of 0.025 COD to 1.5 COD from one sample multiplied by the AE2 1.5 COD value	2,596	171
Total TKN	Sum of particulate and soluble TKN from below	613	83
Particulate TKN	The total TKN, soluble TKN, and ammonia was measured for a three day composite of SBR feed. The values were multiplied by 2 to account for the dilution. Particulate TKN and soluble organic nitrogen was	60	66
Soluble Organic N	determined from the values.	56	48
Ammonia	7 samples taken from May 9th through May 23rd	498	16
Soluble TKN	Added values of AE2 soluble organic nitrogen and ammonia	554	51
Total P	3 samples from April 20th to May 3rd	78	18
Sol. Ortho P	4 samples from April 20th to May 3rd	65	11

Table A7: TSS/VSS Data for PA1

	TSS (mg/L)	VSS (mg/L)	ISS (mg/L)
Composite	30,650	26,400	4,250
	27,350	24,600	2,750
	26,400	23,450	2,950
	26,550	23,350	3,200
	28,133	24,333	3,800
Average	27,817	24,427	3,390
Std Dev	1,729	1,229	622

Table A8: COD Data for PA1

	Total COD (mg/L) (mg/L)	1.5 μ m COD (mg/L) (mg/L)	0.025 μ m COD (mg/L) (mg/L)
Composite	35,416	10,441	-
21-Sep	37,670	12,870	-
3-Oct	30,500	13,163	8,197
9-Nov	-	13,700	7,640
Average	34,529	12,544	7,919
Std Dev	3,666	1,443	394

Table A8: Nitrogen Data for PA1

	Total TKN (mg/L as N)	Soluble TKN (mg/L as N)	Ammonia (mg/L as N)
Composite	1,730	-	711
14-Sep	1,742	846	745
Average	1,736	846	728
Std Dev	8		24

Table A10: Phosphorous Data for PA1

	Total P (mg/L as P)	Soluble Ortho-P (mg/L as P)	Particulate P (mg/L as P)
Composite	281	182	
	321	212	
	324	282	
	272	177	
	370	209	
	259	217	
	448		
Average	325	213	112
Std Dev	66	37	76

Table A11: TSS/VSS Data for PA1sd

	TSS (mg/L)	VSS (mg/L)	ISS (mg/L)	ISS/TSS
9-Feb	1,110	1,000	110	0.10
10-Feb	5,400	4,286	1,114	0.21
2-Mar	2,933	2,750	183	0.06
2-Apr	4,575	3,988	587	0.13
Average	3,505	3,006	499	0.12
Std Dev	1,897	1,494	461	0.06

Table A12: COD Data for PA1sd

	Total COD (mg/L)	1.5 μ m COD (mg/L)
9-Feb	12,000	5,282
19-Mar	7,782	4,716
Average	9,891	4,999
Std Dev	2,983	400

Table A13: Nitrogen Data for PA1sd

	Total TKN (mg/L as N)
19-Mar	420
	465
Average	443
Std Dev	32

Table A14: TSS/VSS Data for AE1

Date	TSS (mg/L)	VSS (mg/L)	ISS (mg/L)	ISS/TSS
11-Mar	1,300	1,325	-25	-0.019
13-Mar	1,263	1,125	138	0.109
14-Mar	788	675	113	0.143
16-Mar	675	675	0	0.000
18-Mar	800	800	0	0.000
21-Mar	787	775	12	0.015
23-Mar	963	875	88	0.091
25-Mar	1,338	1,175	163	0.122
4-Apr	762	750	12	0.016
Average	964	908	56	0.053
Std Dev	264	239	70	0.062

Table A15: COD Data for AE1

Date	Total COD (mg/L)	1.5 μ m COD (mg/L)
23-Mar	6,549	5,058
26-Mar	6,409	4,672
28-Mar	6,083	4,605
30-Mar	5,776	4,358
8-Apr	4,786	3,477
Average	5,921	4,434
Std Dev	701	591

Table A16: Nitrogen Data for AE1

Date	Total TKN (mg/L)	Date	Ammonia (mg/L)
3/16	456	3/23	340
3/22	442	3/25	385
		3/28	374
		3/31	395
		4/4	333
		4/6	350
Average	449	Average	363
Std Dev	10	Std Dev	25

Table A18: Phosphorous Data for AE1

	Total P (mg/L as P)	Soluble Ortho-P (mg/L as P)	Particulate P (mg/L as P)
15-Mar	71	53	18
20-Mar	79	51	28
30-Mar	121	50	71
3-Apr	63	38	25
Average	84	48	36
Std Dev	26	7	24

Table A18: TSS/VSS Data for PA2

Date	TSS (mg/L) (mg/L)	VSS (mg/L) (mg/L)	ISS (mg/L) (mg/L)	ISS/TSS (mg/L)
April composite	19,200	16,025	3,175	0.165
June composite	17,600	16,133	1,467	0.083
Average	18,400	16,079	2,321	0.124
Std Dev	1,131	76	1,208	0.058

Table A19: Total and 1.5 µm COD Data for PA2

Date	Total COD (mg/L) (mg/L)	1.5µm COD (mg/L) (mg/L)
April composite	34,907	13,834
June composite	34,578	15,672
Average	34,743	14,753
Std Dev	233	1300

Table A20: 0.025µm COD Data for PA2

	0.025µm COD (from June composite) (mg/L)	1.5µm COD (from June composite) (mg/L)	0.025 COD/1.5 COD (from June composite)	0.025µm COD Ratio from June composite*1.5µm PA2 (mg/L)
Average	9,647	15,672	0.62	9,081
Std Dev	90	1,207	0.05	2,786

Table A21: TKN Data for PA2

	Soluble TKN PA2 (mg/L as N)	Total TKN (from April composite) (mg/L as N)	Particulate TKN (from April composite) (mg/L as N)
Average	1,425	1,932	507
Std Dev	80	76	110

	TSS (mg/L) (from April composite) (mg/L as N)	Part. TKN:TSS ratio (from April composite) (mg/L as N)	Particulate TKN PA2 (mg/L as N)	Total TKN PA2 (mg/L as N)
Average	19,200	0.0264	486	1,911
Std Dev	1,378	0.0060	115	140

Table A22: Ammonia Data for PA2

Date	Ammonia (mg/L as N)
April composite	995
5/6	1,368
5/11	1,138
5/15	1,311
5/23	1,402
5/25	1,263
Average	1,296
Std Dev	104

Table A23: Phosphorous Data for PA2

	Total P (mg/L as P)	Soluble Ortho-P (mg/L as P)	Particulate P (mg/L as P)
	497	170	
	425	228	
	391	221	
	450	172	
		220	
		194	
		166	
Average	441	196	245
Std Dev	44	27	52

Table A24: TSS/VSS Data for PA2sd

	Raw TSS (mg/L)	Settled TSS (mg/L) (from settling experiment (non-diluted))	Raw VSS (mg/L)	Settled VSS (mg/L)	Ratio of settle to Raw TSS	VSS	TSS PA2sd (mg/L)	VSS PA2sd (mg/L)
Average	17,600	11,100	16,133	9,667	0.63	0.60	4,080	3,388
Std Dev	693	300	231	635	0.03	0.04	318	228

Table A25: COD Data for PA2sd

	Total COD (mg/L)	1.5 µm COD (mg/L) (from settling expt. (non-diluted))	Part COD (mg/L)	Part COD/TSS ratio (mg/L)	Part COD PA2sd (mg/L)	1.5 COD PA2sd (mg/L)	0.025 COD PA2sd (mg/L)	Total COD PA2sd (mg/L)
Average	31,112	15,440	15,672	0.89	3,633	5,187	3,193	8,821
Std Dev	219	0	219	0.04	321	457	980	558

Table A27: Nitrogen Data for PA2sd

	PA2sd TSS /PA2 TSS	Part TKN PA2sd (mg/L as N)	Soluble TKN (from 3 day composite) (mg/L as N)	Total TKN PA2sd (mg/L as N)	Ammonia PA2sd (mg/L as N)
			482		
			521		
Average	0.631	108	501	609	456
Std Dev	0.063	28	28	39	36

Table A28: Phosphorous Data for PA2sd

	Total P (mg/L as P)	Soluble Ortho-P (from 3 day composite) (mg/L as P)	Particulate P (mg/L as P)
	89	58	
	122	68	
	119	76	
Average	110	67	43
Std Dev	18	9	21

Table A29: TSS/VSS Data for AE2

Date	TSS (mg/L)	VSS (mg/L)	ISS (mg/L)	ISS/TSS
5/14	1,138	1,183		
5/16	2,635	2,115	520	0.20
5/18	5,450	4,263	1,187	0.22
5/23	2,925	2,200	725	0.25
Average	3,037	2,440	811	0.22
Std Dev	1,789	1,300	342	0.03

Table A30: Total and 1.5 µm COD Data for AE2

Date	Total COD (mg/L)	1.5µm COD (mg/L)
5/2	6,488	4,989
5/4	7,134	5,428
5/7	7,418	5,195
5/11	7,657	5,641
5/15	6,900	4,979
5/16	7,008	4,924
Average	7,101	5,193
Std Dev	409	288

Table A31: 0.025µm COD Data for AE2

	0.025µm COD (from single sample) (mg/L)	1.5µm COD (from single sample) (mg/L)	Ratio of 0.025COD:1.5COD (from single sample)	0.025µm COD AE2 (mg/L)
Average	1,920	3,841	0.50	2,596
Std Dev	0	137	0.02	171

Table A32: Nitrogen Data for AE2

	Total TKN (mg/L as N)	Soluble TKN (from 3 day composite of SBR feed x 2) (mg/L as N)	Ammonia (mg/L as N)
	605	470	454
	571	538	442
	515		
Average	564	504	448
Std Dev	45	48	8

	Part TKN AE2 (mg/L as N)	Sol Org N AE2 (mg/L as N)	Ammonia AE2 (mg/L as N)	Sol TKN AE2 (mg/L as N)	Total TKN AE2 (mg/L as N)
			9-May	523	
			11-May	515	
			14-May	493	
			16-May	482	
			18-May	482	
			21-May	489	
			23-May	500	
Average	60	56	Average	498	613
Std Dev	66	48	Std Dev	16	83

Table A34: Phosphorous Data for AE2

Date	Total P (mg/L as P)	Soluble Ortho-P (mg/L as P)	Part P (mg/L as P)
4/20	57	56	1
4/27	89	62	27
5/3	88	78	10
Average	78	65	13
Std Dev	18	11	13

Table A35: Alkalinity Data

	PA1 (mg/L as CaCO ₃)	PA2 (mg/L as CaCO ₃)	AE2 (May 25) (mg/L as CaCO ₃)	SBR Effluent (May 25) (mg/L as CaCO ₃)
	6,600	8,113	2,850	65
	6,510			
Average	6,555	8,113	2,850	65
Std Dev	64			

APPENDIX B: Raw Data for Kinetic/Stoichiometric Experiments

Table B1. Heterotrophic Yield (Y_H) Experiment Data

Experiment, Sample	Total COD (mg/L)	Error (mg/L)	Soluble COD (mg/L)	Error (mg/L)	Biomass COD (mg/L)	Error (mg/L)
A,1	372	1.1	349	3.9	23	5.0
A,2	354	4.2	321	2.5	32	6.7
A,3	321	5.6	254	1.6	67	7.2
A,4	240	3.4	144	4.6	97	8.0
B,1	371	0.0	351	7.1	20	7.1
B,2	362	4.9	320	3.2	42	8.1
B,3	326	2.8	278	2.8	48	5.6
B,4	243	4.1	172	0.9	71	5.0

* only data points 1 through 3 were used in the calculation of Y_H ; error is one standard deviation

Table B2. Heterotrophic Decay (b_H) Experiment Data

Data for A				
Hour	OUR	Error	ln(OUR)	Error
0.0	2.397	0.096	0.874	0.041
5.4	1.923	0.104	0.654	0.056
9.1	1.841	0.058	0.610	0.032
28.6	1.400	0.059	0.337	0.043
49.4	1.146	0.076	0.136	0.069
53.3	1.164	0.081	0.152	0.072
73.8	0.947	0.004	-0.055	0.004

Data for B		
Hour	OUR	ln(OUR)
0.0	10.1604	2.3185
4.5	7.2	1.9741
10.0	6.618	1.8898
16.2	5.772	1.7530
52.2	3.87	1.3533
65.5	3.198	1.1625
77.8	3.36	1.2119
104.3	2.754	1.0131

Table B3. Autotrophic Maximum Specific Growth Rate Data for 8-d SRT Aerobic SBR

Time (day)	Replicate 1				Replicate 2				Replicate 3			
	NO ₃	NO ₂	NO ₃ +NO ₂	ln(NO _x -N)	NO ₃	NO ₂	NO ₃ +NO ₂	ln(NO _x -N)	NO ₃	NO ₂	NO ₃ +NO ₂	ln(NO _x -N)
0.00	0.26	6.80	7.07	1.96	0.12	6.86	6.98	1.94				
0.34	1.06	8.35	9.41	2.24	0.93	3.58	4.51	1.51	0.90	8.16	9.06	2.20
0.60	0.49	9.52	10.01	2.30	0.32	9.55	9.87	2.29	0.47	9.63	10.10	2.31
0.89	1.86	11.91	13.77	2.62	1.56	11.52	13.08	2.57	1.56	11.23	12.79	2.55
1.15	3.34	14.12	17.47	2.86	3.55	14.31	17.86	2.88	3.85	14.67	18.52	2.92
1.67	8.17	18.25	26.42	3.27	7.75	18.75	26.51	3.28	8.11	18.70	26.81	3.29
1.89	9.76	20.83	30.59	3.42	9.59	20.74	30.33	3.41	9.27	20.55	29.82	3.40
2.16	10.93	24.21	35.13	3.56	12.35	24.32	36.67	3.60	12.94	24.21	37.15	3.61
2.62	18.07	28.53	46.60	3.84					16.54	27.12	43.66	3.78
2.99	20.81	30.80	51.61	3.94	19.78	31.99	51.77	3.95	20.14	32.08	52.22	3.96
3.47	25.79	29.24	55.04	4.01	21.60	37.67	59.27	4.08				

Table B4. Autotrophic Maximum Specific Growth Rate Data for 14-d SRT Anoxic/Aerobic SBR

Time (day)	Replicate 1				Replicate 2			
	NO ₃	NO ₂	NO ₃ +NO ₂	ln(NO _x -N)	NO ₃	NO ₂	NO ₃ +NO ₂	ln(NO _x -N)
0.00	0	-	0		0.00	-	0.00	
0.28	0	-	0		0.00	-	0.00	
0.81	0.931	-	0.93089	-0.07	1.53	-	1.53	0.43
1.46	2.71	-	2.71171	1.00	3.01	-	3.01	1.10
1.99	3.633	-	3.63318	1.29	4.14	-	4.14	1.42
2.66	4.186	-	4.185947	1.43	7.82	-	7.82	2.06

Table B5. Heterotrophic Maximum Specific Growth Rate and Half Saturation Constant Data

Sample or time (min)	DOC (mg/L)	COD (mg/L)	COD/DOC	DOC*COD/DOC = COD (mg/L)	(COD - inert) (mg/L)
feed wastewater	614.9	1933.3	3.1	2767	
seed biomass	121.9	511.1	4.2	549	
0.00	174.0			783	282.9
2.25	178.1	659.3	3.7	801	301.5
5.00	152.2	629.6	4.1	685	184.9
7.66	138.6	688.9	5.0	623	123.5
10.25	136.4	703.7	5.2	614	113.8
13.50	118.8	629.6	5.3	534	34.4
21.58	123.0	600.0	4.9	553	53.3
25.00	110.5	511.1	4.6	497	
29.00	118.1	525.9	4.5	531	31.4
35.00	111.9	525.9	4.7	503	3.3
40.75	112.0	525.9	4.7	504	4.0
46.00	113.1	496.3	4.4	509	8.7
52.00	114.0	466.7	4.1	513	12.8
60.58	114.4	525.9	4.6	515	14.8

*Ratio of COD/DOC used for calculation of COD values in figure was 4.5 (the average of the COD/DOC ratio from time 25min to 60.58 min)

*Values used to calculate the initial maximum substrate uptake (q_{max}) were from time 0min to 7.66min

*MLSS used in calculation of q_{max} was 7704 mg/L as COD (measured from total and soluble CODs)

*"Inert" is defined as the steady state soluble COD at the end of the experiment (~500 mg/L as COD)

APPENDIX C: Raw Data for Operation of Reactors

Table C1. Operation Data for "2 day SRT" Aerobic SBR

Date	Wastage (ml/cycle)	Decant (ml/cycle)	MLSS (mg/L)	MLVSS (mg/L)	TSS (mg/L)	HRT (hr)	SRT (day)
5-Oct			1468	1278			
9-Oct			448				
12-Oct			366	272			
16-Oct			345	310			
22-Oct			230	204			
26-Oct	450	670				18.8	
29-Oct	508	570				19.5	
31-Oct	410	643	206	184	24	19.9	1.8
1-Nov	393	665	154		25	19.8	1.7
3-Nov	413	672	154		25	19.4	1.7
7-Nov	419	656	151		27	19.5	1.6
10-Nov			224				
12-Nov	365	705	360		28	19.6	2.1
14-Nov	369	708	312		30	19.5	2.0
16-Nov	369	708	318	296	28	19.5	2.0
19-Nov	375	721	254		32	19.2	1.9
22-Nov	381	818	362		34	17.5	1.9
26-Nov	362	784	380		14	18.3	2.2
28-Nov	369	777	276		38	18.3	1.8
30-Nov	375	822	404		25	17.5	2.1
3-Dec	371	816	270		25	17.7	2.0
6-Dec	371	803	266		28	17.9	1.9
12-Dec	371	803	390		28	17.9	2.0
18-Dec	211	975	424		22	17.7	3.3
21-Dec	208	977	510		22	17.7	3.5
22-Dec	210	976	482			17.7	4.2
23-Dec	210	1027	424			17.0	4.2

* SBR wastage rate was changed on Dec 13 so a 3.8 day SRT could be achieved for use in the particulate inert calculations

Table C2. Operation Data for "8 day SRT" Aerobic SBR

Date	Wastage (ml/cycle)	Decant (ml/cycle)	MLSS (mg/L)	MLVSS (mg/L)	TSS (mg/L)	HRT (hr)	SRT (day)
5-Oct			1468	1278			
9-Oct			1424				
12-Oct			1477	1258			
16-Oct			1240	1121			
22-Oct			987.5	832.5			
26-Oct							
29-Oct							
31-Oct	85.0	970.0	746	676	35	19.9	6.7
1-Nov	86.7	965.0	768		43	20.0	6.2
3-Nov	88.3	963.3	768		43	20.0	6.2
7-Nov	91.7	955.6	553		51	20.1	4.9
10-Nov			582				
12-Nov	84.2	925.0	848		48	20.8	6.4
14-Nov	85.0	948.0	868		63	20.3	5.7
16-Nov	75.6	957.5	828		28	20.3	8.1
19-Nov	70.0	968.7	950		29	20.2	8.8
22-Nov	83.3	1020.0	796		32	19.0	7.0
26-Nov	55.5	1040.0	858		19	19.2	11.1
28-Nov	84.2	1038.3	1172		24	18.7	8.3
30-Nov	82.2	1036.1	1004		18	18.8	8.7
3-Dec	81.9	1042.5	1026		24	18.7	8.3
6-Dec	82.8	1039.0	926		22	18.7	8.2
12-Dec	82.8	1039.0	988		22	18.7	8.3
18-Dec	70.3	1039.0	902		22	18.9	9.2

Table C3. Operational Data for Anaerobic SBR (30d SRT/ 15d HRT)

Date	Nth Day	MLSS (%)	MLVSS (%)	TSS (%)	VSS (%)	Effluent NH ₃ (mg/L as N)	Total COD (mg/L)	Soluble COD (mg/L)
2/8/01	1					663	20580	11980
2/9/01	2							
2/10/01	3			0.42	0.36	654		
2/11/01	4						18385	9846
2/12/01	5	1.44	1.42	0.43	0.44	651		
2/13/01	6						13487	9744
2/14/01	7			0.76	0.74	558		
2/15/01	8							
2/16/01	9							
2/17/01	10	0.91	0.86	0.29	0.27	514		
2/18/01	11							
2/19/01	12							
2/20/01	13			0.46	0.41		13515	8179
2/21/01	14	0.74	0.60	0.38	0.33	518		
2/22/01	15							
2/23/01	16					463		
2/24/01	17						13113	7911
2/25/01	18							
2/26/01	19							
2/27/01	20			0.41	0.45	445	11960	7336
2/28/01	21	1.52	1.46					
3/1/01	22	0.71	0.72	0.28	0.28	454	11375	6697
3/2/01	23					485		
3/3/01	24							
3/4/01	25	0.70	0.61			462		
3/5/01	26							6113
3/6/01	27	3.12	3.03	0.42	0.38	444		
3/7/01	28							
3/8/01	29					406	7389	5688
3/9/01	30	2.07	1.94	0.15	0.14			
3/10/01	31							
3/11/01	32	2.47	2.45	0.13	0.13			
3/12/01	33							
3/13/01	34			0.13	0.11			
3/14/01	35	0.61	0.58	0.08	0.07	428		
3/15/01	36							
3/16/01	37	0.54	0.49	0.07	0.07	391	6603	5345

Table C3. (continued)

Date	Nth Day	MLSS (%)	MLVSS (%)	TSS (%)	VSS (%)	Effluent NH ₃ (mg/L as N)	Total COD (mg/L)	Soluble COD (mg/L)
3/17/01	38							
3/18/01	39	0.54	0.52	0.08	0.08			
3/19/01	40	0.46	0.43				6655	5031
3/20/01	41							
3/21/01	42	0.32	0.32	0.08	0.08	379	6288	5188
3/22/01	43							
3/23/01	44			0.10	0.09	340	6549	5058
3/24/01	45							
3/25/01	46	1.02	0.88	0.13	0.12	385		
3/26/01	47						6409	4672
3/27/01	48							
3/28/01	49	0.33	0.33			374	6083	4605
3/29/01	50							
3/30/01	51						5776	4358
3/31/01	52					395		
4/1/01	53							
4/2/01	54	0.31	0.31					
4/3/01	55						5285	
4/4/01	56			0.08	0.08	333		
4/5/01	57							
4/6/01	58	0.42	0.42			350		
4/7/01	59							
4/8/01	60						4786	3477
4/9/01	61							
4/10/01	62							
4/11/01	63			0.10	0.10	335	6407	3518
4/12/01	64							
4/13/01	65			0.03	0.03	346		
4/14/01	66							
4/15/01	67							
4/16/01	68	0.43	0.41	0.08	0.08			
4/17/01	69							
4/18/01	70	0.44	0.43				6614	5244
4/19/01	71			0.12	0.11	399		
4/20/01	72						7027	4934
4/21/01	73			0.07	0.07	365		
4/22/01	74							
4/23/01	75	0.54	0.52	0.09	0.08	357	6898	4648

Table C3. (continued)

Date	Nth Day	MLSS (%)	MLVSS (%)	TSS (%)	VSS (%)	Effluent NH ₃ (mg/L as N)	Total COD (mg/L)	Soluble COD (mg/L)
4/24/01	76							
4/25/01	77	0.50	0.46	0.08	0.07			
4/26/01	78							
4/27/01	79			0.08	0.07	414		
4/28/01	80						7073	5174
4/29/01	81							
4/30/01	82	0.62	0.48	0.11	0.08	387	7047	5304
5/1/01	83							
5/2/01	84	0.74	0.57				6488	4989
5/3/01	85			0.14	0.12	451		
5/4/01	86	0.67	0.51				7134	5428
5/5/01	87							
5/6/01	88	0.75	0.75	0.11	0.11	459		
5/7/01	89						7418	5195
5/8/01	90							
5/9/01	91	0.76	0.84	0.11	0.12	523		
5/10/01	92							
5/11/01	93	0.92	0.91			515	7657	5641
5/12/01	94							
5/13/01	95							
5/14/01	96	0.91	0.84	0.11	0.12	493		
5/15/01	97						6900	4979
5/16/01	98	1.23	1.04	0.26	0.21	482	7008	4924
5/17/01	99							
5/18/01	100	1.04	0.91	0.55	0.43	482		
5/19/01	101							
5/20/01	102							
5/21/01	103	0.68	0.62			489		
5/22/01	104							
5/23/01	105			0.29	0.22	500		
5/24/01								
5/25/01		1.06	0.96					

Table C4. Operational Data for Anoxic/Aerobic SBR (R1)

	MLSS (mg/L)	TSS (mg/L)	Waste (ml/d)	Decant (ml/d)	SRT (day)	HRT (day)	Soluble COD (mg/L)	NH ₃ (mg/L as N)	NO ₃	NO ₂
2/8	1									
2/9	2									
2/10	3									
2/11	4						2872			
2/12	5									
2/13	6									
2/14	7									
2/15	8									
2/16	9									
2/17	10									
2/18	11									
2/19	12									
2/20	13									
2/21	14						1475			
2/22	15						845			
2/23	16						644			
2/24	17									
2/25	18									
2/26	19						1382			
2/27	20							145		
2/28	21							140		
3/1	22									
3/2	23									
3/3	24		222	948		2.99				0.3
3/4	25	4650	222	948		2.99				0.1
3/5	26		230	955		2.95	1754			
3/6	27		230	1025		2.79				
3/7	28	4200	375	230	1100	10.7	2.63	1648		
3/8	29		230	950		2.97				
3/9	30		230	900		3.1				
3/10	31									
3/11	32									
3/12	33		223	900		3.12	1153			
3/13	34						3249	105		
3/14	35		200	850		3.33				
3/15	36	5938	280	230	980	12.7	2.89	891		
3/16	37		230	1030		2.78			136	
3/17	38	5500	276	205	950	13.9	3.03			

Table C4. (continued)

	MLSS (mg/L)	TSS (mg/L)	Waste (ml/d)	Decant (ml/d)	SRT (day)	HRT (day)	Soluble COD (mg/L)	NH ₃ (mg/L as N)	NO ₃	NO ₂	
3/18	39		210	960		2.99					
3/19	40		220	950		2.99					
3/20	41		210	950		3.02					
3/21	42										
3/22	43		215	920		3.08					
3/23	44	4367					506	42			
3/24	45										
3/25	46										
3/26	47	4883	180	200	955	14.9	3.03	463			
3/27	48		225	955		2.97					
3/28	49	5183	230	930		3.02	466				
3/29	50		215	900		3.14					
3/30	51	5017	215	970		2.95	235	8			
3/31	52										
4/1	53										
4/2	54										
4/3	55	4833	272	215	920	13.1	3.08	475			
4/4	56										
4/5	57										
4/6	58	4742	221				147	11			
4/7	59										
4/8	60										
4/9	61										
4/10	62										
4/11	63	4733	118	213	995	14.7	2.9	235	0	9.8	0
4/12	64										
4/13	65								402		
4/14	66										
4/15	67										
4/16	68										
4/17	69										
4/18	70	5216.7	172.5	230	890	13.5	3.13	517	55	0.1	0.31
4/19	71		200	940		3.07					
4/20	72	5600	112	230	940	14.1	2.99	486	112	0.14	0.61
4/21	73			940							
4/22	74			965							
4/23	75						229	121			
4/24	76										
4/25	77										

Table C4. (continued)

		MLSS (mg/L)	TSS (mg/L)	Waste (ml/d)	Decant (ml/d)	SRT (day)	HRT (day)	Soluble COD (mg/L)	NH3 (mg/L as N)	NO3	NO2
4/26	78	4283		220	890	15.9	3.15	634	105	0.08	0.05
4/27	79								129		
4/28	80	4450	132	210	880	14.8	3.21	551	151	0.2	0.24
4/29	81										
4/30	82	4983	244	200	940	14.2	3.07	624	154	0.12	0.17
5/1	83										
5/2	84	5333	212	215	940	13.9	3.03	538	163	0.38	0.22
5/3	85										
5/4	86	5567	184	220	940	13.9	3.02	579	171	0.23	0.18
5/5	87										
5/6	88										
5/7	89	4917	204	220	920	13.6	3.07	620	169	0.8	0.41
5/8	90										
5/9	91										
5/10	92										
5/11	93	5617	202	220	945	13.8	3	639	157	1.65	1.02
5/12	94										
5/13	95										
5/14	96	5600	134	220	945	14.4	3		120		
5/15	97										
5/17	98										
5/17	99										
5/18	100	5733							59		
5/19	101								45		
5/20	102								33		
5/21	103	5950	218	220	940	13.8	3.02	803	29		
5/22	104										770

Table C5. Operational Data for Anoxic/Aerobic SBR (R2)

	MLSS (mg/L)	TSS (mg/L)	Waste (ml/d)	Decant (ml/d)	SRT (day)	HRT (day)	Soluble COD (mg/L)	NH ₃ (mg/L as N)	NO ₃ (mg/L as N)	NO ₂ (mg/L as N)
2/8	1									
2/9	2									
2/10	3									
2/11	4									
2/12	5									
2/13	6									
2/14	7									
2/15	8									
2/16	9									
2/17	10									
2/18	11									
2/19	12									
2/20	13									
2/21	14									
2/22	15									
2/23	16									
2/24	17									
2/25	18									
2/26	19									
2/27	20									
2/28	21									
3/1	22									
3/2	23									
3/3	24		110						0.4	
3/4	25	3250	110	950		3.30			0.1	
3/5	26		110	910		3.43				
3/6	27		210	950		3.02				
3/7	28	3250	587	210	950	9.17	3.02	2316		
3/8	29		220	890		3.15				
3/9	30		210	860		3.27				
3/10	31									
3/11	32									
3/12	33		173	850		3.42	2725			
3/13	34						1991	147		
3/14	35		180	770		3.68				
3/15	36	3300	230	210	970	12.61	2.97	1284		
3/16	37		215	880		3.20			139	
3/17	38	2350	204	150	910	15.28	3.30			

Table C5. (continued)

	MLSS (mg/L)	TSS (mg/L)	Waste (ml/d)	Decant (ml/d)	SRT (day)	HRT (day)	Soluble COD (mg/L)	NH ₃ (mg/L as N)	NO ₃	NO ₂
3/18	39		210	970		2.97				
3/19	40		190	910		3.18				
3/20	41		200	940		3.07				
3/21	42									
3/22	43		200	940		3.07				
3/23	44	3133					825	112		
3/24	45									
3/25	46									
3/26	47	3533	331	200	960	12.07	3.02	1180		
3/27	48		205	965		2.99				
3/28	49	4000	205	930		3.08				
3/29	50		210	885		3.20				
3/30	51	3667	210	960		2.99	1762	155		
3/31	52									
4/1	53									
4/2	54									
4/3	55	2750	725	215	970	7.44	2.95	3911		
4/4	56									
4/5	57									
4/6	58	3183	670				605	135		
4/7	59									
4/8	60									
4/9	61									
4/10	62									
4/11	63	2767	915	192	1060	6.45	2.80	725	102.2	0
4/12	64									
4/13	65									
4/14	66									
4/15	67									
4/16	68									
4/17	69									
4/18	70		275	0	1090		3.21	806	64	0
4/19	71	3367		10	1220		2.85			0.18
4/20	72	4300	292	10	1160	39.43	2.99	806	131	0
4/21	73			0	1330					0.08
4/22	74									
4/23	75									
4/24	76									
4/25	77									

Table C5. (continued)

	MLSS (mg/L)	TSS (mg/L)	Waste (ml/d)	Decant (ml/d)	SRT (day)	HRT (day)	Soluble COD (mg/L)	NH ₃ (mg/L as N)	NO ₃	NO ₂
4/26 78	3967		215	905	16.28	3.13	364	58	3.45	0.21
4/27 79								61		
4/28 80	4600	100	200	890	15.96	3.21	354	75	3.87	0.05
4/29 81										
4/30 82	5267	90	190	940	16.99	3.10	541	80	5.07	0.11
5/1 83										
5/2 84	5317	120	210	930	15.15	3.07	507		7.5	0.03
5/3 85										
5/4 86	5800	170	210	900	14.81	3.15	610	96	5.35	0.00
5/5 87										
5/6 88										
5/7 89	4883	114	210	880	15.18	3.21	641	106	6.2	0.03
5/8 90										
5/9 91										
5/10 92								129		
5/11 93	4467	278	210	885	13.20	3.20	786	130	5.9	0.06
5/12 94										
5/13 95										
5/14 96	4500	242	210	885	13.59	3.20		160		
5/15 97								151		
5/16 98							770	161		
5/17 99										
5/18 100								162		
5/19 101								211		
5/20 102								203		
5/21 103	3800							179		
5/22 104										

Appendix D: Data Points in Figures

Table D1. Data for Figure 8

Wastewater	TSS (mg/L)	Error Bar (mg/L)
PA1	9781	608
PA1sd	3505	1897
AE1	964	264
PA2	6470	398
PA2sd	4080	318
AE2	3037	1828

Table D2. Data for Figure 9

Wastewater	Part. COD (mg/L)	Error Bar (mg/L)	Colloidal COD (mg/L)	Error Bar (mg/L)	Sol. COD (mg/L)	Error Bar (mg/L)
PA1	7730	1385	1626	526	2784	139
PA1sd	4892	3010	2215	423	2784	139
AE1	1487	917				
PA2	7029	464	1994	1081	3193	980
PA2sd	3633	321	1994	1081	3193	980
AE2	1908	500	2597	335	2596	171

Table D3. Data for Figure 10

Wastewater	Ammonia (mg/L as N)	Error Bar (mg/L as N)	Sol. Org. N (mg/L as N)	Error Bar (mg/L as N)	Part. Org. N (mg/L as N)	Error Bar (mg/L as N)	Total TKN (mg/L as N)	Error Bar (mg/L as N)
PA2	456	37	45	46	171	40	672	49
PA2sd	456	36	45	46	108	28	609	39
AE2	498	16	56	48	60	66	613	83

Table D4. Data for Figure 11

Wastewater	Particulate P (mg/L as P)	Error Bar (mg/L as P)	Ortho P (mg/L as P)	Error Bar (mg/L as P)	Total P (mg/L as P)	Error Bar (mg/L as P)
PA2	86	16	69	9	155	13
PA2sd	43	20	67	9	110	18
AE2	23	21	65	11	78	18

Table D5. Data for Figure 12

Wastewater	COD:TKN	Error Bar	COD:P	Error Bar	COD*:P	Error Bar
PA2	15.9	1.8	66.7	7.8	45.7	6.4
PA2sd	12.1	2.2	64.7	14.8	37.9	12.3
AE2	8.9	1.8	68.7	18.3	30.2	12.2

Table D6. Data for Figure 13A

Biomass COD (mg/L)	Soluble COD (mg/L)	Error Bar (mg/L)
23	349	5
32	321	6.7
67	254	7.2

Table D7. Data for Figure 13B

Biomass COD (mg/L)	Soluble COD (mg/L)
20	351
42	320
48	278

Table D8. Data for Figure 14b

Time (hr)	ln(OUR)	Error Bar
5.417	0.6537	0.0556
9.083	0.6102	0.0318
28.583	0.3365	0.043
49.417	0.1363	0.069
53.333	0.1521	0.0721
73.833	-0.055	0.0043

Table D9. Data for Figure 14b

Time (hr)	ln(OUR)
4.5	1.9741
10	1.8898
16.167	1.753
52.167	1.3533
65.5	1.1625
77.833	1.2119
104.333	1.0131

Table D10. Data for Figure 15A

Time (day)	ln(NO _x -N) (1)	ln(NO _x -N) (2)	ln(NO _x -N) (3)
0.000	1.955	1.944	
0.344	2.242		2.203
0.604	2.304	2.289	2.313
0.889	2.623	2.571	2.548
1.153	2.860	2.883	2.919
1.674	3.274	3.277	3.289
1.889	3.421	3.412	3.395
2.156	3.559	3.602	3.615

Table D11. Data for Figure 15B

Time (day)	ln(NO _x -N) (1)	ln(NO _x -N) (2)
0.806	0.427	-0.071613732
1.462	1.103	0.997579285
1.993	1.422	1.290108298
2.660	2.057	1.43173301

Table D12. Data for Figure 16

ln(S _o /S)	q _{max} X _t (mg/L as COD)
0.00	0.00
-0.06	52.88
0.43	117.50
0.83	180.01
0.91	240.88
2.11	317.25
1.67	507.21
2.20	681.50

Table D13. Data for Figure 17

Day	R1	R2
20	0.35	
21	0.37	
34	0.51	0.31
37	0.30	0.29
44	0.75	0.34
51	0.96	0.17
58	0.94	0.23
63	1.00	0.39
70	0.72	0.68
72	0.39	0.28
75	0.32	
78	0.41	0.68
79	0.38	0.71
80	0.27	0.64
82	0.20	0.59
86	0.24	0.57
89	0.26	0.54
93	0.39	0.50
96	0.51	0.35
98	0.59	0.33
100	0.76	0.33
101	0.81	0.12
102	0.87	0.17
103	0.88	0.27
104	0.93	

Table D14. Data for Figure 18

Day	R1 NH3	R2 NH3	R1 NOx	R2 NOx	Day	Influent NH3
20	145				1	332
21	140				3	327
34	105	147			5	326
37	136	139			7	279
44	42	112			10	257
51	8	155			14	259
58	11	135			16	232
63	0	102.2	9.80	0.00	20	223
70	55	64	0.41	0.18	22	227
72	112	131	0.75	0.08	23	243
75	121				25	231
78	105	58	0.13	3.66	27	222
79	129	61			29	203
80	151	75	0.44	3.92	35	214
82	154	80	0.29	5.18	37	196
84	163		0.60	7.53	42	190
86	171	96	0.41	5.35	44	170
89	169	106	1.21	6.23	46	193
92		129			49	187
93	157	130	2.67	5.96	52	198
96	120	160			56	167
97		151			58	175
98	99	161			63	168
100	59	162			65	173
101	45	211			71	200
102	33	203			73	183
103	29	179			75	179
104	17				79	207
					82	194
					85	226
					88	230
					91	262
					93	258
					96	247
					98	241
					100	241
					103	245
					105	250

Table D15. Data for Figure 21

Time (hr)	DOC (mg/L)
6.00	127.55
6.25	99.09
6.50	107.10
6.75	94.79
7.00	98.83
7.25	93.37
7.50	88.30
7.75	100.75
8.00	89.83
8.25	89.07
8.50	76.73
8.75	85.93
9.25	79.40
9.50	81.21
9.75	89.22
10.00	82.18
10.25	83.81
10.50	77.22
10.75	85.86
11.00	73.69
11.25	86.50
11.50	86.30
11.75	84.47
12.00	82.78

Table D16. Data for Figure 19

Day	Feed COD _T	Feed COD _S	Day	R1 COD _S	R2 COD _S
1	10290	5990	4	2872	
4	9192.5	4923	14	1475	
6	6743.5	4872	15	845	
13	6757.5	4089.5	16	644	
17	6556.5	3955.5	19	1382	
20	5980	3668	26	1754	
22	5687.5	3348.5	28	1648	2316
29	3694.5	2844	33	1153	2725
37	3301.5	2672.5			1991
40	3327.5	2515.5	36	891	1284
42	3144	2594	44	506	825
44	3274.5	2529	47	463	1180
47	3204.5	2336	49	466	
49	3041.5	2302.5	51	235	1762
51	2888	2179	55	475	
60	2393	1738.5	58	147	605
63	3203.5	1759	63	235	725
70	3307	2622	70	517	806
72	3513.5	2467	72	486	806
75	3449	2324	75	229	
80	3536.5	2587	78	634	364
82	3523.5	2652	80	551	354
84	3244	2494.5	82	624	541
86	3567	2714	84	538	507
89	3709	2597.5	86	579	610
93	3828.5	2820.5	89	620	641
97	3450	2489.5	93	639	786
98	3504	2462	98	573	770
			103	803	
			104	770	

Table D17. Data for Figure 20

Time (hr)	Ammonia (mg/L as N)	Nitrate (mg/L as N)	Nitrite (mg/L as N)	Time for DO (hr)	DO (mg/L as O2)
6.00		3.48	16.26	6.00	5.2
6.00		3.04	14.23	6.02	4.4
6.14	36.07	0.00	12.40	6.03	2.9
6.20	36.54	0.00	11.23	6.05	1.2
6.25	37.06	0.00	7.84	6.07	0.3
6.36	37.54	0.00	3.29	6.08	0.2
6.40	36.52	0.00	4.99	6.10	0.16
6.45	37.08	0.00	4.06	8.00	0.35
6.50	34.77	0.00	3.08	8.17	0.55
6.75	34.21	0.00	0.57	8.30	0.8
7.00	36.16	0.00	0.00	8.50	1.5
7.25	36.46	0.00	0.00	8.75	2.1
7.50	36.63	0.00	0.00	9.00	2.55
7.75	36.60	0.00	0.00	9.25	2.95
8.00	36.66	0.00	0.00	9.50	3.45
8.25	37.14	0.00	0.00	9.75	3.6
8.50	35.24	0.00	0.01	10.00	3.6
8.75	36.29	0.00	0.68	10.25	3.7
9.00	34.22	0.47	1.66	10.75	4.1
9.25	34.52	0.46	2.99	11.00	4.25
9.50	33.63	0.43	4.40	11.25	4.45
9.75	32.32	0.54	6.17	11.50	4.7
10.00	31.51	1.86	7.29	11.75	4.95
10.25	30.57	1.35	9.07	12.00	5.2
10.50	28.65	1.33	11.07		
10.75		1.24	12.38		
11.00	25.89	2.33	14.30		
11.25	24.50	2.38	15.65		
11.50	24.03	2.26	18.06		
11.75	21.76	2.50	18.21		
12.00	21.03	2.82	18.83		

APPENDIX E: Statistical Analysis for Comparison of Wastewater Characterization Values

Note: n = number of samples (assumed 2 for values that were calculated to be conservative); df = degrees of freedom, T_{exp} = experimental t statistic; T = t distribution statistic for $\alpha = 0.05$ (95% confidence); if absolute value of T_{exp} is greater than T then there is a statistical difference

Table E1. Statistical Analysis of TSS Comparison

	Average	Std. Dev.	Variance (S^2)	n	$(n-1)S^2$
PA1	9781	608	3.70E+05	1	0.00E+00
PA1sd	3505	1897	3.60E+06	2	3.60E+06
AE1	964	264	6.97E+04	9	5.58E+05
PA2	6470	398	1.58E+05	2	1.58E+05
PA2sd	4080	318	1.01E+05	2	1.01E+05
AE2	3037	1828	3.34E+06	4	1.00E+07

Comparing	df	S^2_{pooled}	T_{exp}	T	Statistically different?
PA1 - PA1sd	1	3.60E+06	2.701	2.015	y
PA1sd - AE1	9	4.62E+05	4.783	1.833	y
PA1 - AE1	8	6.97E+04	31.684	1.782	y
PA2 - PA2sd	2	1.30E+05	6.637	2.92	y
PA2sd - AE2	4	2.53E+06	0.757	2.132	n
PA2 - AE2	4	2.55E+06	2.484	2.132	y
PA1sd - PA2sd	2	1.85E+06	-0.423	2.92	n
AE1-AE2	11	9.62E+05	-3.517	1.796	y

Table E2. Statistical Analysis of Particulate COD Comparison

	Average	Std. Dev.	Variance (S^2)	n	$(n-1)S^2$
PA1	7730	1385	1.92E+06	2	1.92E+06
PA1sd	4892	3010	9.06E+06	2	9.06E+06
AE1	1487	917	8.41E+05	2	8.41E+05
PA2	7029	464	2.16E+05	2	2.16E+05
PA2sd	3633	321	1.03E+05	2	1.03E+05
AE2	1908	500	2.50E+05	2	2.50E+05

Comparing	df	S^2_{pooled}	T_{exp}	T	Statistically different?
PA1 - PA1sd	2	5.49E+06	1.212	2.92	n
PA1sd - AE1	2	4.95E+06	1.531	2.92	n
PA1 - AE1	2	1.38E+06	5.315	2.92	y
PA2 - PA2sd	2	1.59E+05	8.507	2.92	y
PA2sd - AE2	2	1.77E+05	4.104	2.92	y
PA2 - AE2	2	2.33E+05	10.610	2.92	y

Table E3. Statistical Analysis of Colloidal COD Comparison

	Average	Std. Dev.	Variance (S ²)	n	(n-1)S ²
PA1	1626	526	2.77E+05	2	2.77E+05
PA1sd	2215	423	1.79E+05	2	1.79E+05
PA2	1994	1081	1.17E+06	2	1.17E+06
PA2sd	1994	1081	1.17E+06	2	1.17E+06
AE2	2597	335	1.12E+05	2	1.12E+05

Comparing	df	S ² _{pooled}	T _{exp}	T	Statistically different?
PA1 - PA1sd	2	2.28E+05	-1.233	2.92	n
PA2 - PA2sd	2	1.17E+06	0.000	2.92	n
PA2sd - AE2	2	6.41E+05	-0.753	2.92	n
PA2 - AE2	2	6.40E+05	-0.753	2.92	n

Table E4. Statistical Analysis of Truly Soluble COD Comparison

	Average	Std. Dev.	Variance (S ²)	n	(n-1)S ²
PA1	2784	139	1.92E+04	2	1.92E+04
PA1sd	2784	139	1.93E+04	2	1.93E+04
PA2	3193	980	9.60E+05	2	9.60E+05
PA2sd	3193	980	9.60E+05	2	9.60E+05
AE2	2596	171	2.92E+04	2	2.92E+04

Comparing	df	S ² _{pooled}	T _{exp}	T	Statistically different?
PA1 - PA1sd	2	1.93E+04	0.003	2.92	n
PA2 - PA2sd	2	9.60E+05	0.000	2.92	n
PA2sd - AE2	2	4.95E+05	0.849	2.92	n
PA2 - AE2	2	4.94E+05	0.849	2.92	n

Table E5. Statistical Analysis of Ammonia Comparison

	Average	Std. Dev.	Variance (S ²)	n	(n-1)S ²
PA2	456	36	1.30E+03	6	6.48E+03
PA2sd	456	36	1.32E+03	6	6.62E+03
AE2	498	16	2.56E+02	7	1.54E+03

Comparing	df	S ² _{pooled}	T _{exp}	T	Statistically different?
PA2 - PA2sd	10	1.31E+03	0.010	2.228	n
PA2sd - AE2	11	7.42E+02	-2.785	2.201	y
PA2 - AE2	11	7.29E+02	-2.797	2.201	y

Table E6. Statistical Analysis of Soluble Organic N Comparison

	Average	Std. Dev.	Variance (S ²)	n	(n-1)S ²
PA2	45	46	2.13E+03	2	2.13E+03
PA2sd	45	46	2.08E+03	2	2.08E+03
AE2	56	48	2.30E+03	2	2.30E+03

Comparing	df	S ² _{pooled}	T _{exp}	T	Statistically different?
PA2 - PA2sd	2	2.10E+03	0.008	2.92	n
PA2sd - AE2	2	2.19E+03	-0.235	2.92	n
PA2 - AE2	2	2.22E+03	-0.226	2.92	n

Table E7. Statistical Analysis of Particulate Organic N Comparison

	Average	Std. Dev.	Variance (S ²)	n	(n-1)S ²
PA2	171	40	1.64E+03	2	1.64E+03
PA2sd	108	28	7.84E+02	2	7.84E+02
AE2	60	66	4.36E+03	2	4.36E+03

Comparing	df	S ² _{pooled}	T _{exp}	T	Statistically different?
PA2 - PA2sd	2	1.21E+03	1.808	2.92	n
PA2sd - AE2	2	2.57E+03	0.947	2.92	n
PA2 - AE2	2	3.00E+03	2.026	2.92	n

Table E8. Statistical Analysis of TKN Comparison

	Average	Std. Dev.	Variance (S ²)	n	(n-1)S ²
PA2	672	49	2.42E+03	2	2.42E+03
PA2sd	609	39	1.52E+03	2	1.52E+03
AE2	613	83	6.89E+03	2	6.89E+03

Comparing	df	S ² _{pooled}	T _{exp}	T	Statistically different?
PA2 - PA2sd	2	1.97E+03	1.417	2.92	n
PA2sd - AE2	2	4.21E+03	-0.062	2.92	n
PA2 - AE2	2	4.66E+03	0.864	2.92	n

Table E9. Statistical Analysis of Total Phosphorus Comparison

	Average	Std. Dev.	Variance (S ²)	n	(n-1)S ²
PA2	155	15	2.39E+02	2	2.39E+02
PA2sd	110	18	3.24E+02	2	3.24E+02
AE2	78	19	3.61E+02	3	7.22E+02

Comparing	df	S ² _{pooled}	T _{exp}	T	Statistically different?
PA2 - PA2sd	2	2.82E+02	2.685	2.92	n
PA2sd - AE2	3	3.49E+02	1.877	2.92	n
PA2 - AE2	3	3.20E+02	4.716	2.353	y

Table E10. Statistical Analysis of Orthophosphate Comparison

	Average	Std. Dev.	Variance (S ²)	n	(n-1)S ²
PA2	69	9	9.01E+01	2	9.01E+01
PA2sd	67	9	8.10E+01	2	8.10E+01
AE2	65	11	1.21E+02	4	3.63E+02

Comparing	df	S ² _{pooled}	T _{exp}	T	Statistically different?
PA2 - PA2sd	2	8.56E+01	0.207	2.92	n
PA2sd - AE2	4	1.11E+02	0.219	2.92	n
PA2 - AE2	4	1.13E+02	0.425	2.132	n

Table E10. Statistical Analysis of Orthophosphate Comparison

	Average	Std. Dev.	Variance (S ²)	n	(n-1)S ²
PA2	86	18	3.34E+02	2	3.34E+02
PA2sd	43	21	4.41E+02	2	4.41E+02
AE2	13	13	1.69E+02	2	1.69E+02

Comparing	df	S ² _{pooled}	T _{exp}	T	Statistically different?
PA2 - PA2sd	2	3.88E+02	2.191	2.92	n
PA2sd - AE2	2	3.05E+02	1.718	2.92	n
PA2 - AE2	2	2.52E+02	4.611	2.92	y

VITA

David Whichard was born on May 11, 1977 in Hartsville, South Carolina. He attended Hartsville High School and graduated in June 1995. He then pursued a Bachelor of Science degree in Chemical Engineering at Clemson University (Go Tigers!). After completing his work at Clemson in May 1999, he attended Virginia Polytechnic and State University and sought a Master of Science degree in Environmental Engineering. Upon completion of his graduate program David began work as an Environmental Engineer for International Paper in Eastover, SC.