# MEASUREMENT OF MAXIMUM NITRIFIER SPECIFIC GROWTH RATE FOR USE IN ACTIVATED SLUDGE MODELING

by

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# ABSTRACT

## Measurement of Maximum Nitrifier Specific Growth Rate For Use in Activated Sludge Modeling

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**Dimitrios Katehis**

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The objective of this study was to measure the maximum specific growth rate of nitrifying bacteria active in municipal wastewater treatment plants. The operation of the plants was simulated using bench scale sequencing batch reactors (SBRs) operated at solids retention times ranging from eight to 20 days at 20°C. A total of four reactors were operated over the course of two years using settled wastewater from two plants located in New York City. Once steady nitrification of ammonia was achieved in the SBRs, nitrification rates were measured and used to compute the maximum specific nitrifier growth rate, $\mu_A^{MAX}$. The value of $\mu_A^{MAX}$ was also determined using a second method that is commonly known as the "High F/M Method".

The measured nitrifier decay rates, $b_A$ ranged from 0.09 to 0.11 $d^{-1}$, more than twice the value of 0.04 $d^{-1}$ typically used in engineering calculations. When calculated using the experimentally derived nitrifier decay rates, $\mu_A^{MAX}$ averaged 43% and 49% greater than the values calculated using the decay rate of 0.04 $d^{-1}$. The experiments demonstrated that the value of $b_A$ was critical in calculating the associated $\mu_A^{MAX}$. Thus, the maximum

specific growth rate and decay rate must be treated as a pair, and always reported as such. The maximum specific nitrifier growth rates determined from the exponential growth batch experiments were always higher than the values derived from methods using the sequencing batch reactors. For example, the rates determined from exponential growth tests were 5% higher than SBR derived values for one facility, whereas for a second facility the exponential growth tests were 37% higher. Though higher values of $\mu_A^{MAX}$ may require a higher safety factor for design purposes, the high F/M method is less labor intensive, of shorter duration, and is less costly, making it more appealing to municipalities.

For the two wastewaters tested the corresponding values of $\mu_A^{MAX}$ and decay rate were 0.55 $d^{-1}$, 0.11 $d^{-1}$, and 0.83 $d^{-1}$, 0.09 $d^{-1}$, respectively. The findings suggest that varied between facilities, and must be measured prior to utilizing the parameter for determination of the nitrification capacity of the facility. Furthermore, the nitrifier decay rate should be measured in tandem with $\mu_A^{MAX}$ in order to assure proper design of the biological nitrification process at a cost effective basis.

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# 1. INTRODUCTION

The basic tenets of nitrogen removal relate to the kinetics of nitrification, the first step in nitrogen removal, whereby ammonia is sequentially converted to nitrite and then nitrate under aerobic conditions.

In nitrification, the critical parameter dictating the design of a biological reactor, the associated clarification facilities and to a lesser extent the solids processing facilities, is considered by many to be the maximum specific nitrifier growth rate, $\mu_A^{MAX}$. The specific nitrifier growth rate represents the rate of growth of the nitrifying bacterial population, per unit time per unit mass of the nitrifying bacteria present. Growth rate is the basis for plant design as it defines the solids retention time, which determines the reactor volumes, aeration energy requirements and solids process facility sizing. However the body of literature developed between 1965 and the present has shown a very wide variability on the value of this parameter. To cope with this level of uncertainty, designers have historically used safety factors of up to 3. This, in turn, has resulted in very conservative designs of nitrifying facilities. Whereas such an approach was acceptable during the construction grants era (1970’s and early 1980’s) this is no longer the case as the cost of improvements has been transferred directly to ratepayers.

Most practices to measure the specific nitrifier growth rate were based mainly on short duration exponential growth batch tests. These studies essentially used a wastewater to which an acclimated seed was added and the exponential increase over time in nitrite and nitrate concentrations was monitored. A curve fit was conducted on the resulting exponential curve to obtain the maximum specific growth rate.

Another approach to obtain $\mu_A^{MAX}$ was to use sequencing batch reactors to develop and acclimate the nitrifying biomass. Due to the expense associated with operating these reactors over an extended period of time (2 to 3 months at a minimum) this method has not been used extensively in industry.

Because of the critical nature of $\mu_A^{MAX}$ in the design process, the propagation of increasingly stringent effluent limitations and the availability of improved tools, such as the sequencing batch reactor methodology, re-application of the methodology for measurement of $\mu_A^{MAX}$ was warranted.

The objective of this study is to carry out a series of experiments using sequencing batch reactors as well as exponential growth batch reactors to better define the maximum specific nitrifier growth rate and thus improve their utilization as a design tool. Key elements of this study will include:

- Assessment of the experimental protocols utilized for measurement of the specific nitrifier growth rate
- Examination of the assumptions incorporated into the analysis of the data used for growth rate measurements
- Experimental evaluation of the methods for measurement of the specific nitrifier growth rate

# 2. LITERATURE REVIEW

The nitrification process has been extensively studied at both the micro and macro scale. An overview of the bacterial species believed to be responsible for nitrification in WWTPs, kinetic equations describing their growth and the effect of environmental variables such as temperature, pH and dissolved oxygen on their growth rate are presented in this chapter. A summary of methods for determination of $\mu_A^{MAX}$ is presented and the experimental conditions and data analysis techniques used are discussed.

## 2.1. Nitrification in Wastewater Treatment Plants

Bacteria capable of converting ammonia to nitrite and then to nitrate are prevalent in the environment. Originally believed to be mediated by two independent species of autotrophic bacteria, with the *Nitrosomonas spp*. responsible for the conversion of ammonia to nitrite and *Nitrobacter spp.* converting the nitrite to nitrate, currently there are complete taxonomic trees with dozens of species of nitrifying autotrophs as shown in **Figure 2.1.** (Ward et al, 1985; Teske et al, 1994; Purkhold et al, 2000). Nitrifying bacteria have been identified in environments ranging from activated sludge processes and waste oil, to oceans and acidic soils.

The diversity of nitrifiers in nature as well as wastewater treatment plants has been articulated recently with the advance of analytical procedures. Lazarova et al. (1999) used rRNA oligonucleotide probes to characterize biofilm biomass from a circulating bed reactor and concluded that different species of nitrifiers were present when the reactor was operated as a teritiary nitrification unit vs. a secondary nitrification unit vs. a denitrification/nitrification reactor. The effect of pH, dissolved oxygen and ammonia concentration as ecological selectors was studied for nitrifying bacteria (Princic, 1998) by the use of a combination of phylogenetic oligonucleotide probes, small subunit ribosomal DNA (rDNA) sequencing, rDNA restriction analysis and cellular fatty acid analysis. Whereas dissolved oxygen levels did not significantly effect the nitrifying ecology, shifting the pH from 7.0 to 6.0 or to 8.2 resulted in irreversible modification of the ecology (Princic, 1998). At high ammonia concentrations (500-3000 mg/L-N) a single nitrifying bacterium dominated the sludge, but the ecology was returned to it's normal state upon reduction of the ammonia to 50 mg/L-N. Thus diversity can be seen as an inversely proportional function of deviation from natural environmental conditions, with maximum diversity expected to occur at near neutral pH, and ambient temperatures, with only a few specialized species capable of tolerating environmental extremes.

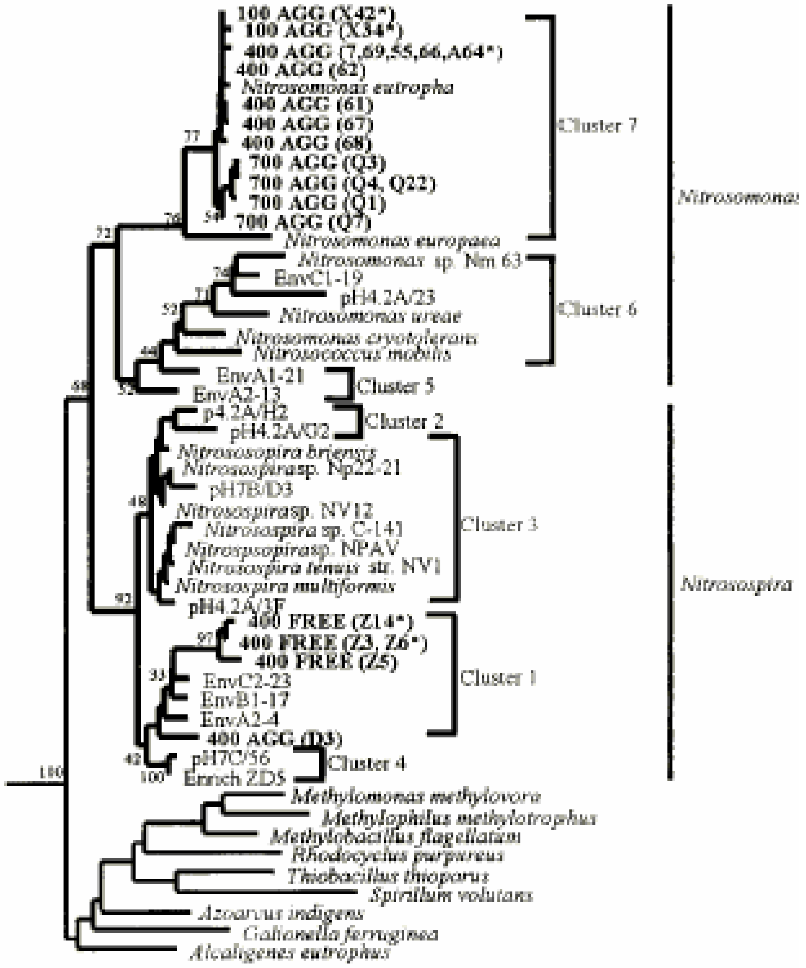

**Figure 2.1. Phylogenetic tree showing the position of b-protobacterial ammonia oxidizers' DNA sequences extracted by Phillips et al. (1999).**

Another class of bacteria that convert ammonia to nitrite, and in certain species nitrate, are heterotrophic. Many papers have been devoted to them, partly because they are characterized by much higher growth rates than autotrophs. However, it has been found that these heterotrophs (which in many instances also aerobically denitrify) tend to lose their nitrifying ability during culturing - when the environmental stresses that the bacteria would normally be exposed to are removed. The very low nitrifying activity exhibited by heterotrophic nitrifiers, relative to autotrophic nitrifiers (lower by 2 or more orders of magnitude), has caused interest in these bacteria to wane in recent years as their net effect in activated sludge nitrification is deemed negligible (Randall et al, 1992).

Thus autotrophic organisms are still believed to be the primary catalysts for nitrification in activated sludge wastewater treatment plants, and this work will focus on the determination of the autotrophic nitrifier maximum growth rate.

## 2.2. Autotrophic Nitrification Stoichiometry and Kinetics

In autotrophic nitrifiers, as typified by *Nitrosomonas spp.*, ammonia (Suzuki, 1974; Alleman, 1985)) is oxidized in a three-step process to nitrite:

$$NH_3 \rightarrow NH_2OH \rightarrow NOH \rightarrow NO_2^- \qquad (2.1)$$

The oxidation state of nitrogen is sequentially increased from (-3) in ammonia to (-1) in hydroxylamine to (+1) in nitroxyl and finally to (+3) in nitrite. The oxidation reaction for the first stage of nitrification is generally accepted to be:

$$2NH_4^+ + 3O_2 \rightarrow 2NO_2^- + 4H^+ + 2H_2O \qquad (2.2)$$

The above equation shows that 3.43 gr oxygen are required per gram of $NH_3$-N oxidized. Oxidation of ammonia will also increase the acidity of the matrix as protons will be liberated, resulting in a reduction in alkalinity of approximately 7.14 mg alkalinity as $CaCO_3$ per mg $NH_3$-N nitrified.

Nitrite is further oxidized to nitrate by autotrophic nitratifiers (Alleman, 1985), as exemplified by *Nitrobacter spp.* :

$$2NO_2^- + O_2 \rightarrow 2NO_3^- \qquad (2.3)$$

Based on the above equation an additional 1.14 gr DO per gr oxidized NO2-N is required, for a total of 4.57 gr DO per gr $NH_3$-N for the complete oxidation of ammonia to nitrate. No alkalinity is consumed in this step. The generally accepted net equation (Nitrogen Control Manual, 1993):

$$NH_4^+ + 1.83O_2 + 1.98HCO_3^- \rightarrow 0.98NO_3^- + 1.88H_2CO_3 + 0.021C_5H_7O_2N + 2H_2O \qquad (2.4)$$

was developed using yield coefficients of 0.15 and 0.02 gr cell mass per gr N oxidized for the *Nitrosomonas* and *Nitrobacter spp.*, respectively. With new cell synthesis incorporated into the reaction the net oxygen demand is reduced to 4.2 gr DO/gr N oxidized. The net oxygen requirement will vary depending on the yield values utilized in the calculation (Wezernak and Gannon, 1967; Poduska, 1973; Nitrogen Control Manual, 1993)

In typical wastewater treatment plant operations, the conversion of ammonia to nitrite is the limiting step in the process. This is due to the higher growth rate associated with the nitratifiers, as typified by *Nitrobacter* species. The conversion of nitrite to nitrate has been found to be limiting in instances where an inhibitor is present in the wastewater, and in reactors with ammonia or nitrite concentrations exceeding a few hundred milligrams per liter as nitrogen (Randall et al, 1992). Nitratifiers are more sensitive than nitrifiers to ammonia and nitrous acid inhibition (Anthonisen, 1976).

A significant quantity of information is reported in the literature with respect to the optimal environmental conditions for growth of nitrifiers. Parametric-type studies established the general trends with respect to applicable kinetic models (Knowles, 1965, Shieh, 1979 and Charley, 1980), effect of dissolved oxygen and substrate concentrations (Wild, 1971 and Chudoba, 1985), pH (Painter, 1983 and Loveless, 1968, Antoniou, 1990) and temperature (Knowles, 1965 and Painter, 1983). In most studies, the effect of environmental variables such as pH, DO and temperature is reported as the offset of the nitrification rate from some benchmark value as each parameter was varied.

### 2.2.1. Temperature

Initial studies indicated high nitrifier sensitivity to decreasing temperature (Knowles, 1965; Antoniou, 1990; Barker and Dold, 1997). A temperature dependency relationship that has found wide acceptance (Nitrogen Control Manual, 1993) in design is:

$$\mu_A = \mathbf{0.47} \cdot e^{\mathbf{0.098(}T\mathbf{-15)}} \qquad (2.5)$$

where T is in degrees Celsius. In activated sludge process modeling temperature correlations of the form:

$$\mu_A(T) = \mu_A(20^o) \cdot \vartheta^{(T-20)} \qquad (2.6)$$

are used. The severity of the temperature correction is a function of the value of the temperature correction coefficient $\vartheta$. Theta values in the range of 1.02 (Randall et al, 1992b) to 1.127 (Barker and Dold, 1997) have been reported. Recent studies, showing a reduced effect of temperature (Randall et al,1992b and Sears et al, 1997) used biological reactors that were operated to acclimate the biomass to the lower temperatures. Thus it appears that acclimating the biomass and reactor operating conditions may significantly affect the reported temperature dependencies. The temperature dependency is generally believed to be exponential (Figure 2.2.), but for the lower values of the temperature correction factor (1.03 in Figure 2.2.) the relationship is essentially linear.

At temperatures above 35°C the growth rate has been reported to rapidly decrease (Alleman,1985; Nitrogen Control Manual, 1993) though nitrification has been observed to occur at temperatures as high as 50ºC(Monib, 1979).

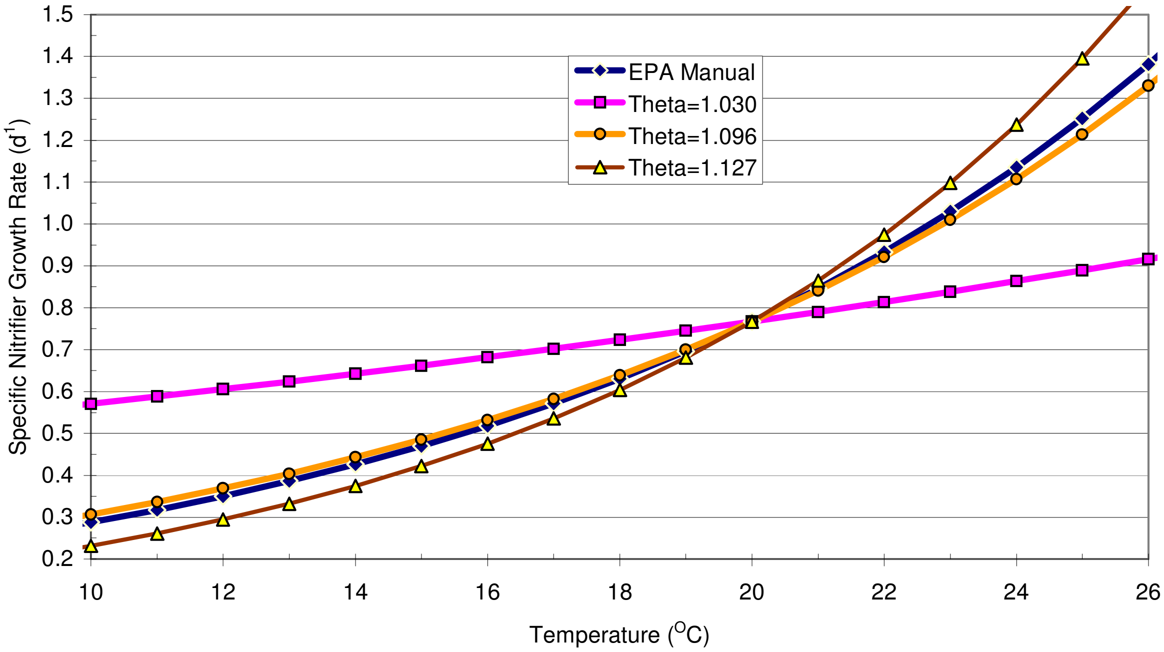

**Figure 2.2. Temperature Dependency on Nitrifier Specific Growth Rate**

Although little is known on the effect of temperature on the diversity of nitrifier species in the activated sludge system, studies using oligonucleotide rRNA techniques have shown that diversity is reduced as environmental conditions increasingly deviate from natural systems (Princic et al, 1998). Thus, whereas reducing the temperature of an activated sludge culture from 20ºC to 10ºC and then measuring the nitrification rate will provide a "value", this measurement does not account for the fact that at the lower temperature, over time, the species of nitrifiers dominating the sludge may change. This can result in a culture that has significantly different activity characteristics –and thus nitrification rates- from the original sludge.

### 2.2.2. Effect of pH

The other significant environmental parameter is pH, since nitrification is a biological reaction during which protons are liberated and thus depression of pH occurs. Whereas there are multiple pH dependencies reported in the literature (Antoniou, 1990, Painter, 1983 and Knowles, 1965) the optimum ranges from approximately pH 6.5 to pH 8 (Nitrogen Control Manual, 1993). In acclimated cultures a reduction of approximately 50% in the nitrification rate was observed when the pH was reduced to 6.0-6.1(Forster, 1974; Stratta, 1982; Nitrogen Control Manual, 1993). Conversely, Stankewich (1972) and Haug and McCarty (1972), as quoted by Randall et al. (1992), saw reestablishment of maximum nitrification rates after re-acclimation of the culture to lower pH levels (6.0 from 7.0). Recently, Sears et al, (1998) found that at lower temperature, longer acclimation periods and higher solids retention times (SRT's) were required in order fully nitrify when a pure oxygen system was operated at pH 5.0 to 6.3. However, in design the following equation has found widespread use for predicting nitrifier growth rate at pH below 7.2 (Nitrogen Control Manual, 1993 and Metcalf & Eddy,1991):

$$\mu_A = \mu_A^{MAX}\left[\mathbf{1 - 0.833(7.2} - pH\mathbf{)}\right] \qquad (2.7)$$

which results in a linear decrease in nitrifier growth rate with decreasing pH, as pH drops below 7.2.

The exact mechanism by which the pH effects nitrification is not fully understood. It has been theorized that at low pH the gaseous ammonia, which is believed to be the substrate (Suzuki,1974), is depleted as ammonium is the predominant species at lower pH. Other possibilities include inhibition of the monoxygenase, and/or increased toxicity of the intermediary, hydroxylamine, to the bacterial cell. The monooxygenase is highly unstable and there has been significant difficulty in characterizing it's effectiveness in vitro (Hyman, 1988).

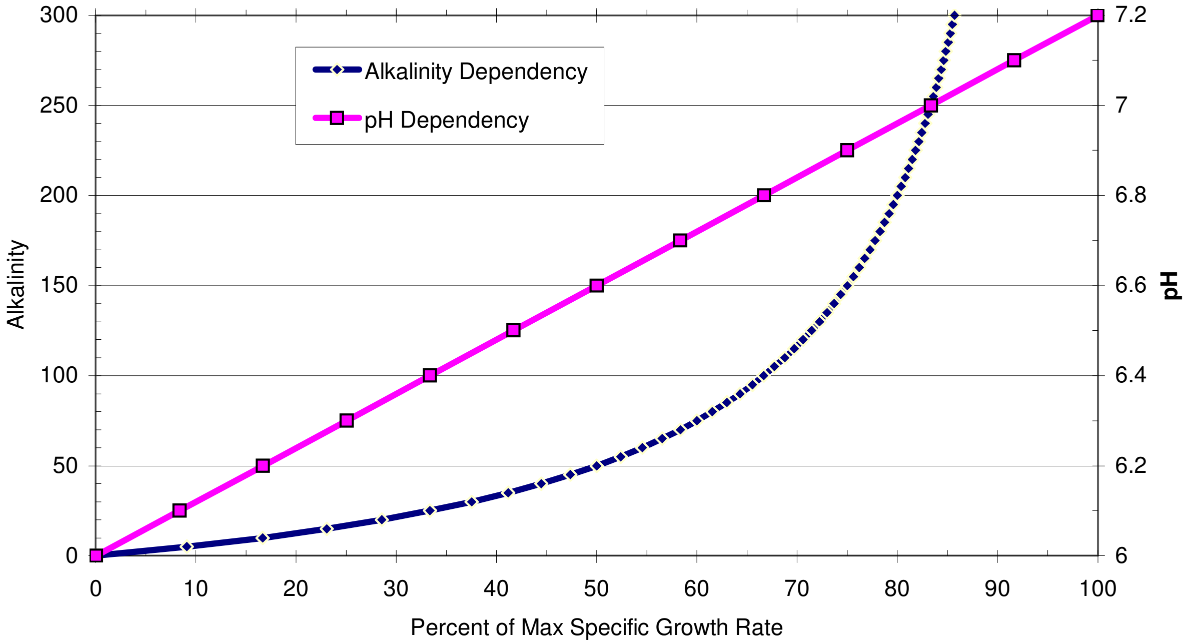

**Figure 2.3. Using pH versus Alkalinity to Correct for Nitrification Rate Reduction**

The effect of pH on the nitrification process is not modeled by IAWQ's ASM 1 or it's derivatives. The interdependency of pH on operating conditions such as OUR, aeration intensity, aeration efficiency and wastewater characteristics make modeling of pH in aeration tanks an insurmountable challenge. An attempt at modeling the effect of decreased pH has been made in some commercially available ASM1 derivatives by including a Monod-type half-saturation term for alkalinity in the nitrification process equation. Difficulty exists in the application of this methodology, as this approach utilizes alkalinity as a surrogate to pH and assumes a reproducible correlation between pH and alkalinity throughout the range of conditions (aeration intensity, DO, biomass respiration rate) found in activated sludge wastewater treatment plants. On the other hand, the usage of the alkalinity half-saturation term allows modeling of the transient depression in nitrification under conditions of limited inorganic carbon concentrations. This is theoretically correct since inorganic carbon from the carbonate buffer system is the substrate believed to be used by nitrifying autotrophs for biomass synthesis. However any attempt at correlating alkalinity depletion with pH depression must be purely site specific.

A far more significant problem is that attempting to predict alkalinity-limited nitrification performance with a Monod term applied to the alkalinity will result in significant under-prediction of process performance as shown in Figure 2.3. Using a Monod half saturation coefficient of 50 mg/L, at an alkalinity concentration of 50-60 mg/L, with a typical process pH of 6.5-6.6, both the model and the EPA equation will provide the expected 50% reduction in nitrifier growth rate. When the alkalinity is greater than 100 mg/L as $CaCO_3$, typically resulting in a activated sludge pH of greater than 7.1-7.2, no suppression of the nitrification rate is expected. However, the model using the Monod alkalinity term would predict a nitrification rate of only 70% of the maximum.

### 2.2.3. Dissolved Oxygen Concentration

Oxygen is required for the oxidation of the ammonia to nitrite and finally nitrate. Dissolved oxygen levels of greater than 2.5 mg/L are considered non-limiting (Nitrogen Control Manual, 1993) on nitrifier growth, although it has been reported that the growth rate of *Nitrosomonas spp* is not limited by dissolved oxygen concentrations above 1 mg/L (Hanaki, 1990 and Loveless, 1968). The actual dissolved oxygen (DO) value at which nitrification is affected is a complex function of parameters such as solids retention time (SRT), floc size, relative distribution of heterotrophs and nitrifiers in the floc, and mixing intensity and is thus highly site and reactor specific. As oxygen levels decrease below the limiting value for a particular system, nitrification performance is significantly eroded (Hanaki, 1990).

Dissolved oxygen (DO) effects are incorporated in nitrification kinetics and modeling by inclusion of a Monod half saturation term for DO. The selected value of the half saturation coefficient attempts to lump together the effect of the site and reactor specific DO diffusion limitations. Typical values for the half saturation coefficient for dissolved oxygen range from 0.25 to 1 mg/L.

### 2.2.4. Nitrification Kinetics

The above relationships are incorporated into an equation describing the net growth rate of ammonia oxidizing bacteria (*Nitrosomonas spp.*), $r_g$:,

$$r_g = \frac{dX_A}{dt} = (\mu_A - b_A)\cdot X_A \tag{2.8}$$

with

$$\mu_A = \mu_A^{MAX}\frac{S_N}{K_N + S_N}\frac{DO}{K_{DO} + DO}\frac{ALK}{K_{ALK} + ALK} \tag{2.9}$$

where $\mu_A$ and $\mu_A^{MAX}$ are the adjusted and maximum nitrifier specific growth rates ($d^{-1}$), respectively, $X_A$ is the nitrifier concentration, and $b_A$ is the decay rate ($d^{-1}$). $S_N$ and $K_N$ are the combined ammonia and ammonium concentration (the substrate) and substrate half saturation coefficient, respectively. DO and $K_{DO}$ are the dissolved oxygen concentration and half saturation coefficient, and ALK and $K_{ALK}$ are the total alkalinity and associated half saturation coefficient. All concentrations and half saturation coefficients are typically in mg/L. The above equation incorporates the primary variables affecting nitrifier growth rate at a given temperature. Activated sludge models may also include other Monod terms in order to have the capability to simulate a reduction in nitrification due to lack of a necessary nutrient such as phosphorus.

With respect to the decay rate, $b_A$, it should also be noted that whereas activated sludge models typically include a decay term for nitrifiers, in a significant portion of the literature, nitrifier decay was not accounted for (Randall et al, 1992; Nitrogen Control Manual, 1993; Daigger and Sadick, 1999), primarily due to the assumption that it was negligible.

Integration of equation 2.8 provides the nitrifier concentration as a function of time:

$$X_A(t) = X_A^{INI} \cdot e^{(\mu_A - b_A)t} \tag{2.10}$$

where $X_A^{INI}$ is the initial nitrifier concentration at time zero.

Realizing that some or all of the nitrite produced by the *Nitrosomonas spp.* will be converted to nitrate, the rate of substrate utilization can be expressed in terms of the combined nitrate and nitrite, $NO_X$-N:

$$r_N = \frac{d[NH_3 - N]}{dt} = \frac{d\{[NO_2 - N] + [NO_3 - N]\}}{dt} = \frac{d[NO_X - N]}{dt} = \mu_A \cdot \frac{X_A(t)}{Y_A} \tag{2.11}$$

where $Y_A$ is nitrifier yield (mg biomass/mg $NH_3$-N oxidized).

If the substrate, dissolved oxygen and alkalinity concentrations are not limiting ($S_N >> K_N$, $DO >> K_{DO}$, $ALK >> K_{ALK}$) $\mu_A$ will be equal to $\mu_A^{MAX}$.

As will be seen in Section 2.4.1, manipulation of equations 2.8 thru 2.11 will provide the basis for the design of the nitrifier growth rate measurement experiments.

## 2.3. Nitrification Inhibition

Nitrifiers have historically been considered very susceptible to chemical inhibitors (Nitrogen Control Manual, 1993 and Randall et al, 1992). This high level of perceived sensitivity is one of the driving forces for classifying the nitrifier specific growth rate as a site specific parameter.

It should be noted though that most of the studies conducted to assess the effect of inhibitors did not use acclimated cultures. Thus the reported results would be applicable when a slug of the suspected inhibitor is introduced into the activated sludge process. However, if the inhibitor is typically present in the wastewater the nitrifying bacteria may acclimate to it, and the inhibition effect may be reduced or disappear altogether (Stover, 1980).

Due to the difficulty in identification and characterization of the effects of inhibitory compounds in wastewaters, and their effect on nitrifiers, it has been proposed that the net effect of inhibitors drives the observed variability of the maximum nitrifier growth rates, and thus makes it a wastewater specific value (Barker and Dold, 1997b). Thus design of all but the smallest nitrifying WWTPs would require that a pilot be constructed and operated in order to obtain nitrification rates.

As an indicator of the level of sensitivity of nitrifiers to inhibitors, organic compounds such as chloroform and phenol have been reported to be inhibitory at concentrations of less than 6 mg/L, whereas cyanide is inhibitory at concentrations greater than 0.06 mg/L, with complete inhibition occurring at concentrations as low as 0.2 mg/L (Daigger and Sadick, 1999) at neutral pH. Heavy metals are considered inhibitory at concentrations above 10-20 mg/L (Painter, 1970; Blum, 1991). Plaza (1990) and Gujer (1977) also indicated that anaerobic digestion supernatant is inhibitory to the main plant’s flow's nitrification process, with growth rate reductions at the 20% level.

Free ammonia (FA) and free nitrous acid (FNA) have been reported to be inhibitory to nitrification at concentrations of 1-150 mg/L FA and 0.22-2.8 mg/L FNA. This corresponds roughly to ammonia and nitrite concentrations of 100-15,000 and 280-2800 mg/L-N, respectively (Randall et al, 1992 and Nitrogen Control Manual, 1993), and would only be of significance in the treatment of high ammonia solids processing wastewaters and in industrial wastewater nitrification processes.

## 2.4. Measurement of Nitrifier Maximum Specific Growth Rate

Henze (1987), Gibson (1991) and Barker and Dold (1997), among others, proposed that the $\mu_A^{MAX}$ is an intrinsic wastewater characteristic and should be treated as such during the wastewater characterization process. The varying levels of industrial input, the differences in wastewater pH, and biochemical reactions in the sewer system are cited as possible causes for the observed variation in $\mu_A^{MAX}$.

Experimentally determined maximum nitrifier specific growth rates are typically used to predict nitrification process performance at different operating conditions, typically at different SRTs, anaerobic/anoxic/oxic volume ratios, aeration tank volumes and hydraulic retention times (HRTs).

Whereas the NOx-N production rate can be measured with relative ease, neither the nitrifier concentration, nor the yield can be directly measured with a high degree of confidence. A number of protocols have been devised to estimate the nitrifier concentration and yield (Hall, 1980 and Schramm, 1999), but none have found widespread use primarily due to the high variability in experimental results, and in some instances the high level of complexity. A number of molecular probe methodologies have been developed, but at this stage these enumeration methods do not lend themselves for the purpose of wastewater treatment plant performance analysis and design (Schramm, 1999; Sakano,1998; Hastings, 1998; Holben, 1998) as they are essentially qualitative, rather than quantitative.

In order to obtain an accurate estimate of the nitrifier biomass the most widely accepted method is the meticulous operation of a reactor at a constant SRT for a period exceeding 3 SRT's. The 3 SRT period allows the system to achieve greater than 90% turnover of the solids inventory. This would produce biomass that, at least in theory, would be acclimated to the particular wastewater. Assuming a typical SRT of 10-15 days, this requires operation of the reactor for a period of 30 to 45 days prior to collecting any meaningful data. After the stabilization period, steady state operation is assumed to have been achieved, and the nitrifier concentration and specific growth rate can be calculated (Randall et al, 1992, and Daigger, 1999).

Application of this type of methodology to full scale facilities is difficult or impossible without operating a dedicated bench or pilot scale reactor, due to uncertainties arising from the need to accurately measure time varying process parameters, (i.e. flow, TKN, ammonia and COD). To circumvent these problems short duration batch experiments for growth rate determination have been devised such that the need to directly determine nitrifier concentration and yield, and thus avoid the time and expense associated with long term operation of activated sludge reactors is avoided.

The $\mu_A^{MAX}$ measurement methods available in the literature can be broadly placed into four categories:

a. Exponential Growth Batch Test at Low Biomass Concentrations

b. Exponential Growth Batch Test at High Biomass Concentrations
c. Nitrification Rate Measurement in a Pseudo-Steady State Reactor
d. Differential Nitrification Rate Measurement with and without Nitrifier Addition

The fourth method uses the novel concept of measurement of the nitrification rate prior to and after addition of a know quantity, of kinetically defined pure culture *Nitrosomonas* into the batch reactor. The difference in rates permits comparative calculation of the activated sludge nitrifiers' specific growth rate using the pure culture's kinetics as the frame of reference. Originally developed by Srinath et al (1976) and refined by Hall (1980) it has not found use within the engineering community most probably due to the variability in the pure culture's kinetics (growth rate) and acclimation effects that would not allow usage of the measured growth rates for the purposes of engineering calculations (i.e. extrapolation to different SRT, operating conditions). Thus no further time will be devoted to this method.

### 2.4.1. Exponential Growth Batch Tests at Low Biomass Concentrations

Knowles (1965) estimated $\mu_A^{MAX}$, $K_N$, and described the effect of pH and temperature on the nitrification of Thames River water by incubating river water samples and measuring the ammonia, nitrate and nitrite concentrations over a period of 3-11 days. Temperature was controlled, and in most experiments, pH ranged between 7.3 and 7.8. DO was maintained at greater than 2 mg/L by agitation and/or reaeration. The majority of the experiments were run without biomass seeding.

For his data analysis, Knowles solved the differential equation:

$$\frac{d[NO_X - N]}{dt} = \mu_A^{MAX} \frac{S_N}{K_N + S_N} \frac{X_A^{INI}}{Y_A} \cdot e^{\mu_A^{MAX} \frac{S_N}{K_N + S_N} t} \tag{2.12}$$

which is obtained by substituting equations (2.9) and (2.10) into equation (2.11) and neglecting nitrifier decay, and dissolved oxygen and alkalinity limitations. Thus in essence, the reported values are maximum specific net growth rates ($\mu_A^{MAX} - b_A$) rather than maximum specific growth rates ($\mu_A^{MAX}$). The concentration-time profiles that were obtained experimentally were fitted by trial and error into the solution of the above equation as presented by Knowles (1965):

$$\mu_A^{MAX} t = \left(\frac{1}{A}\right)\left[Y_A K_N \ln\left(\frac{S_N^{INI}}{S_N}\right) + (A + Y_A K_N)\ln\left(\frac{A - Y_A K_N}{X_A^{INI}}\right)\right] \tag{2.13}$$

$$\text{where} \quad A = X_A^{INI} + Y_A S_N^{INI}$$

The combination of $\mu_A^{MAX}$, $K_N$ and $X_A^{INI}$, that produced the "best fit" through the experimentally measured nitrate/nitrite time profile was reported. Numerical criteria for the quality of the fit to the data were not used. The data set contains growth rates for a temperature range of 8.3-30.8 ºC, and pH primarily in the range of 7.3-7.6. It should be noted that Knowles did not incorporate nitrifier decay into his data analysis, he did recognize and experimentally verify that *Nitrosomonas* decayed when exposed to oxygen in the absence of ammonia. He also confirmed the work of Buswell et al (1954) who indicated that for ammonia starvation periods of less than 12 days, nitrifier growth rate was not altered when ammonia was re-introduced.

Antoniou et. al (1990) utilized an aerobic batch method where the batch reactor was operated with an initial nitrifying biomass concentration of 150-300 mg/L as VSS in secondary effluent and the nitrite and nitrate ($NO_X$-N) concentration was tracked over a period of 3-4 days during which exponential growth occurred. The net specific growth rate was determined at 15, 20 and 25 C with pH ranging from 6.8 to 8.5. The pH was controlled by varying the percentage of $CO_2$ provided in the aeration air supply.

Antoniou dropped the substrate Monod term in equation (2.12) and integrated to obtain the equation describing the time-concentration profile of the oxidation products:

$$[NO_X - N](t) = [NO_X - N]_{INI} - \frac{\mu_A^{MAX} X_A^{INI}}{(\mu_A^{MAX} - b_A)Y_A} + \frac{\mu_A^{MAX} X_A^{INI}}{(\mu_A^{MAX} - b_A)Y_A} \cdot e^{(\mu_A^{MAX} - b_A)t} \qquad (2.14)$$

Antoniou then assumed that the sum of the first and second terms of the right hand side (RHS) of equation (2.14) would be negligible relative to the third term, and simplified the equation by dropping those two terms. Taking the natural log of the simplified equation then yielded:

$$\ln[NO_X - N](t) = \ln\frac{\mu_A^{MAX} X_A^{INI}}{(\mu_A^{MAX} - b_A)Y_A} + (\mu_A^{MAX} - b_A)t \qquad (2.15)$$

The net specific growth rate ($\mu_A^{MAX}$ - $b_A$) can then be determined from a plot of the natural logarithm of the oxidized product concentration [$NO_X$-N] versus time. The maximum specific growth rate was then calculated using an assumed decay value of $0.04d^{-1}$.

Antoniou also recognized the possible inhibitory implications of using a large initial ammonium dose and thus proposed that the ammonia be fed to the batch reactor intermittently, so as to avoid atypically high ammonia concentrations. The author attributed inconsistent results to the effects of bacterial predation and recommended limiting the test to 3-4 days (Antoniou, 1990).

Sozen et al. (1996) attempted to utilize Antoniou's protocol using biomass concentrations of 50-100 mg/L and primary effluent, but he did not obtain a linear plot of the natural logarithm of the [$NO_X$-N] versus time. Rather a curved plot was generated, as was a poor correlation coefficient for the ensuing "linear" slope. Sozen estimated the values of the first and second term on the RHS of equation (2.14), which Antoniou (1990) dropped as negligible, and concluded that these terms, although small relatively to the last term, were not even of the same magnitude relative to each other in his experiments. Utilizing the complete equation, in it's non-linear form, he applied a one dimensional search technique to find the value of the reciprocal of the second term of the RHS of equation (2.14) that would minimize the error between the experimental [$NO_X$-N] profile and the calculated profile. This is essentially similar to the technique used by Knowles (1965) with the addition of a formalized mathematical search methodology for obtaining the optimal value of the net growth rate.

Painter and Loveless (1983), studied the effect of pH and temperature in activated sludge cultures developed in sequencing batch reactors fed settled domestic wastewater. No further details on the SBR operation were given other than that intentional wasting was not practiced but solids were lost to the effluent. A retention time of 8-11 hours is described as being used for the experiments. Nitrifier $\mu_A^{MAX}$ was determined by pipeting 2 ml of mixed liquor into a 250 ml flask containing wastewater and buffering solution so as to maintain a target pH and an initial ammonia concentration of 58 mg/L-N. The ensuing mixture was aerated by shaking for at least 24 hours and the initial and final $NO_X$-N production rates were determined. These values were corrected for a blank (i.e. aerated wastewater sample with no sludge added) and the growth rate was calculated from the slope of the log-activity versus time plot for a non-limiting substrate.

A wide scatter in the data was observed by the authors, who indicated that this may have been caused by inhibitory substances in the wastewater. As in the work of Knowles(1965) nitrifier decay was neglected.

Similar batch nitrifier exponential growth experiments that utilize oxygen uptake rates in place of combined nitrite and nitrate (NOx-N) measurements have been proposed (Stensel, 1992). The analytical effort is significantly reduced, but usage of literature values or measurement of nitrifier yield, and thus the conversion factor from aerobic respiration rate to a NOx-N production rate, is necessary.

The basic assumption of the exponential growth experiment at low biomass concentrations is that the nitrifiers will grow at a constant rate. The interaction between heterotrophic and autotrophic bacteria, as well as protozoa, which are typically completely neglected in modeling, may be significant. It has also been postulated that growth stimulants may be present in activated sludge, impacting the bacterial growth rate (Van Loosdrecht and Henze, 1998 and Ratsak et. al, 1996) and increasing nitrifier activity (Hockenbury, 1977, Hall, 1985, Batchelor et al, 1997). It is uncertain if these type of interactions can be duplicated in a short-term batch experiment with a possibly significantly different microbial ecology owing to the very low MLVSS and high F/M typically used in the nitrification batch tests described in the literature (Knowles, 1965; Antoniou, 1992; Sozen,1996).

This is particularly important as the work of Shammas (1986) and Batchelor (1997) showed that there may be a significant effect of biomass concentration on the nitrifier growth and decay dynamics at both, the overall activity level (Shammas, 1986) as well as the molecular level (Batchelor, 1997) via the accumulation of growth signaling molecules such as *N*-acyl homoserine lactones (Salmond et al, 1995 and Swift et al, 1996) that impact bacterial dormancy and decay.

Rastak (1996) also indicated that the effects of predation are a function of biomass concentration, with the growth of higher organisms in sludge being favored by increased solids concentrations, and the existence of a minimum threshold number, below which minimal protozoan growth is expected. Thus this brings into question whether the net specific growth rate calculated by these methods would apply to an activated sludge system, where decay via predation would be far more significant, and cell density would be greater by at least one or two orders of magnitude.

The pH control method employed in the test may also influence the result. Antoniou (1992), realizing that inorganic carbon is required for autotrophic growth, used $CO_2$ to control the pH. Earlier investigations used combined phosphate and carbonate buffers. The effect of possible depletion of the carbonate buffer system cannot be ascertained, as the pH would have been maintained by the phosphate buffer system.

The great advantage of this protocol is the relative ease and low cost associated with it. Over a period of just a few days the experiment can be carried out, samples collected, analyzed and the net nitrifier growth rate calculated. If (1)appropriate experimental conditions are defined, (2) the nitrifier decay rate is negligible in both activated sludge, as well as the low MLSS system this protocol utilizes, and (3) the seed sludge nitrifiers are acclimated to the wastewater, this method would theoretically be able to provide a good estimate for modeling purposes.

However this protocol cannot replicate floc-level interactions, as flocs are not formed at the low MLVSS concentrations required for development of an exponential growth. curve. It is uncertain what effect these type of interactions will have and how the nitrifier growth rate measured using this protocol would compare to actual values.

### 2.4.2. Exponential Growth Batch Test at High Biomass Concentrations

Lesouef et al. (1992) explored a modified exponential growth batch test at solids concentrations that reflect WWTP operating conditions. Lesouef (1992) proposed that $\mu_A^{MAX}$ can be estimated by taking a sample of the WWTP sludge, measuring the initial maximum nitrification rate (substrate utilization rate), $r_N$, under non-limiting conditions (DO, pH, ammonia concentration), then exposing the sludge to a high ammonia concentration for a period of half to one day and re-measuring the maximum nitrification rate.

At the beginning of the test, the nitrifier concentration will be $X_A^{INI}$, and the nitrification rate $r_N^{INI}$ will be:

$$r_N^{INI} = \frac{d[NH_3 - N]}{dt} = \frac{d[NO_X - N]}{dt} = \mu_A^{MAX} \cdot \frac{X_A^{INI}}{Y_A} \quad (2.16a)$$

At the end ($t=t_G$) the nitrifiers, having grown exponentially under non-limiting conditions, will have increased in concentration to:

$$X_A(t=t_G)=X_A^{INI}\cdot e^{\left(\mu_A^{MAX}-b_A\right)t_G} \qquad (2.16b)$$

resulting in an increased nitrification rate $r_N^{FIN}$:

$$r_N^{FIN}=\frac{d[NH_3-N]}{dt}=\frac{d[NO_X-N]}{dt}=\mu_A^{MAX}\cdot\frac{X_A^{INI}\cdot e^{\left(\mu_A^{MAX}-b_A\right)t_G}}{Y_A} \qquad (2.16c)$$

Taking the ratio of the initial to final nitrification rate ($r_N^{INI}/r_N^{FIN}$), and linearizing, the maximum nitrifier growth rate is (Lesouef, 1992):

$$\mu_A^{MAX}=\frac{\ln r_N^{FIN}-\ln r_N^{INI}}{t_G}+b_A \qquad (2.16)$$

where $t_G$ is the time allowed for growth of the nitrifiers. The decay rate $b_A$, must also be determined in a similar manner to $\mu_A^{MAX}$, by measuring an initial nitrification rate and then re-measuring the rate after a period of half to one day ($t_D$), during which the sludge is exposed to ammonia starvation conditions. The nitrifier decay rate is:

$$b_A=\frac{\ln r_N^{FIN}-\ln r_N^{INI}}{t_D} \qquad (2.17)$$

The above set of equations is identical to the equation set in the work of Painter (1983), with the addition of the incorporation of the decay rate into the analysis.

Lesouef's method, as the method of Painter (1983), relies on two data points (the initial and final nitrification rates) making data confidence a concern. However, the problem of a "drifting" substrate utilization rate due to nitrifier growth during the course of the batch experiment as may have been experienced in the low biomass experiments of Painter and Loveless (1983), is circumvented by utilizing a high mixed liquor concentration in Lesouef's batch test, thus making the effect of nitrifier growth during the course of the nitrification rate measurement negligible.

Of interest is whether the assumption of exponential growth for the "growth" period $t_G$ holds at high biomass concentrations. No data has been presented to indicate whether the initially high ammonia concentrations employed in this experiment, or the high nitrate, and possibly nitrite, concentrations that would occur in the latter portion of it, would have an inhibitory effect on nitrifier growth.

The choice of pH control method would not be as critical in this test as in the low biomass tests, as the significant concentrations of heterotrophic bacteria, are respiring and producing $CO_2$, thus replenishing the carbonate buffer system. This would need to be experimentally confirmed.

### 2.4.3. Nitrification Rate Measurement in a Pseudo-Steady State Reactor

Long-term operation (greater than 3-4 SRTs) of a single reactor at a constant HRT and SRT would permit development of a well acclimated culture, and a condition approaching steady state with respect to nitrifier concentration in the reactor. However, due to the fluctuations associated with wastewater strength and characteristics, this operation is termed pseudo-steady state, as true steady state cannot be achieved with a variable strength influent, as is the norm for wastewater systems.

Initially continuous flow stirred (CSTR) or plug flow (PFR) reactors, similar to small pilot plants, have been used (Chudoba et al , 1985; Ekama, 1986; Randall et al, 1992). With the advent of SBR systems in the early 90's protocols using SBRs rather than continuous flow systems were devised. In Dold's (1996) SBR based protocol for wastewater characterization, measurement of the sludge $\mu_A^{MAX}$ was an integral part. Theoretically an SBR can be used to simulate the kinetics of an ideal PFR. Thus utilization of SBRs for growth and acclimatization of the nitrifying biomass was ideal for measurement of typical PF aeration tank kinetics.

Data analysis of pseudo steady state reactors typically entails use of the mass balance approach (Randall et al, 1992; Dold, 1996; Daigger and Saddick, 1999). This method utilizes the nitrogen mass balance for determining the nitrifier concentration at an assumed yield, and then uses the $NO_X$-N production rate from an in-situ rate measurement, at non-limiting conditions, to directly calculate the maximum specific nitrifier growth rate. (Dold, 1995).

If steady state operation is assumed, the nitrifier concentration in the reactor is:

$$X_A = \frac{Y_A \cdot C_N \cdot V_{ww} \cdot SRT}{(1 + b_A \cdot SRT) \cdot V_R} \qquad (2.18)$$

where $C_N$ is the concentration of ammonia oxidized to NOx-N, $V_{WW}$ is the volume of wastewater added per day and $V_R$ is the reactor volume. Note that usage of this equation assumes that ammonia is not a limiting nutrient at any point in the reactor's cycle. If ammonia is fully expended, NOx will be utilized by the biomass, resulting in reduction in the measured effluent NOx-N concentration (Dold, 1997a). When ammonia limitations occur a more general form of the equation must be used (Dold, 1996) which fully accounts for nitrogen used in synthesis, irrespectively of speciation.

The calculated nitrifier concentration, $X_A$, is used in conjunction with the nitrification rate obtained under non-limiting conditions to calculate the maximum specific nitrifier growth rate:

$$\mu_A^{MAX} = \frac{dNO_x - N}{dt} \cdot \frac{Y_A}{X_A} \qquad (2.19)$$

Substituting equation 2.19 into 2.18:

$$\mu_A^{MAX} = \frac{dNO_x - N}{dt} \cdot \frac{Y_A}{\frac{Y_A \cdot C_N \cdot V_{ww} \cdot SRT}{(1 + b_A \cdot SRT) \cdot V_R}} = \frac{dNO_x - N}{dt} \cdot \frac{(1 + b_A \cdot SRT) \cdot V_R}{C_N \cdot V_{ww} \cdot SRT} \qquad (2.20)$$

Note that in this approach the nitrifier yield is not used in the calculation as substitution of equation (2.18) into (2.19) results in cancellation of the nitrifier yield. Thus $\mu_A^{MAX}$ is reduced to a function of variables that can be accurately determined, with the exception of $b_A$, a parameter whose value is increasingly significant at higher sludge ages, and as shall be shown, significantly impacts the data analysis process, the reported growth rates and our ability to use the growth rates to extrapolate process performance for different operating scenarios.

Steady state or dynamic modeling of the reactor's operation can also be used for data analysis of an SBR (Parker et al, 1996). Models such as the IAWQ ASM 1 or it's derivatives can be used as a tool, to calculate nitrifier concentrations and simulate the rate measurement experiments.

The significant advantage of operating a reactor over an extended period of time rather than just conducting a short term batch test is that a well acclimated culture will be developed, and the nitrification reaction will progress under conditions similar, if not identical to the ones within the WWTP's activated sludge reactor.

### 2.4.4. Research Needs

Available methods for nitrifier growth rate measurement assume that measured growth rates can be extrapolated to significantly different operating conditions. Some factors that may impact nitrifier growth and compromise the measured growth rates usefulness for real-world activated sludge process optimization include:

a. *Interactions between heterotrophic and autotrophic bacteria*. It has been postulated that growth stimulants may be present in activated sludge, impacting the bacterial growth rate (Van Loosdrecht and Henze, 1998 and Ratsak et. al,

1996) and increasing nitrifier activity (Hockenbury, 1977 and Hall, 1985). Classes of enzymes, that are believed to be produced by both heterotrophs and nitrifiers, and can impact nitrifier decay in high biomass concentration systems have been identified to date (Batchelor,et al, 1997). The level of decay in the test reactor will impact data analysis and the actual decay rate in the aeration tank, if different from the test reactor, will result in erroneous model predictions. It is uncertain if these type of interactions can be duplicated in the low MLSS batch growth experiments (Antoniou, 1992) with possibly significantly different microbial growth dynamics owing to the very low MLVSS and high F/M typically used.

b. *The need for long term acclimation of the nitrifying biomass to a wastewater*. A significant dataset on nitrification exists in the literature, however there is significant variation in the reported growth rates due to among other factors, the degree of acclimation and the contribution of industrial streams to the wastewater (EPA, 1993). The realization that continuous operation for a time equal to several SRTs may be required to achieve acclimatization of nitrifying biomass, and that wastewater treatment plants may need more than a year of operation to develop a fully acclimated nitrifier biomass (Harremoës, 1998) may question the results obtained from low MLSS batch experiments (Antoniou, 1992) using unacclimated biomass.

c. *The effect of operating SRT on the nitrifier growth rate exhibited by the biomass.* Using a synthetic high ammonia concentration (80 mg/L-N) solution fed to CSTRs, Hanaki (1990) concluded that influent COD concentration had no significant effect on $\mu_A^{MAX}$. However, he did observe a reduction in the overall nitrifier growth rate with lower effluent ammonia concentrations, which the author attributed to the half saturation coefficient for ammonia, as had Chudoba (1985) before him.

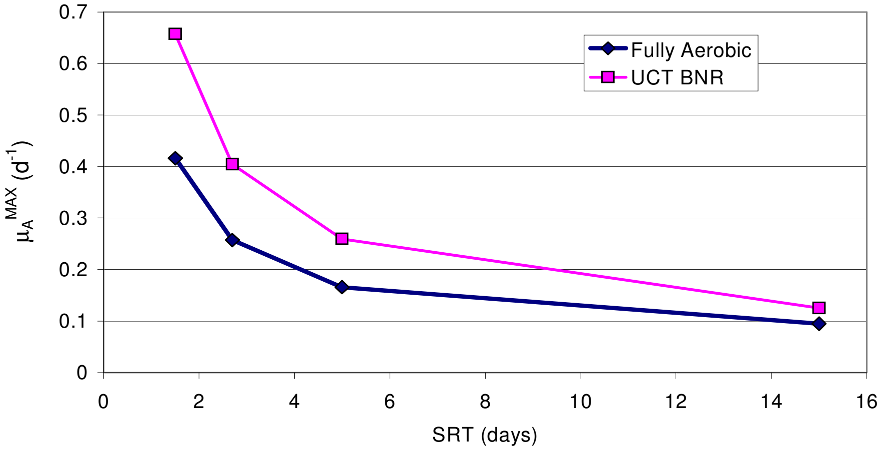

**Figure 2.2. Effect of SRT and Operating Mode on Maximum Nitrifier Growth Rate (from the results of Randall et al, 1992)**

In Hanaki's study, because the reactors were operated as chemostats, SRT was equivalent to HRT, and the effluent ammonia concentration was equal to the reactor bulk concentration. The lower effluent ammonia concentrations and the lower $\mu_A$

values were observed at the higher HRTs employed. This may indicate an effect of SRT and/or substrate levels in the CSTRs on the nitrifier growth rate that the author did not recognize. Analysis of the data of Randall (1992) where a continuous flow conventional aerobic and UCT type pilot plant were run in tandem, showed a significant variation in the nitrifier growth rate with both SRT and process configuration. As shown in **Figure 2.2**, higher SRT's resulted in a significant decrease in nitrifier growth rates, while the anaerobic/anoxic/aerobic UCT process configuration's nitrifier growth rates were 32 to 58% higher than in the conventional fully aerobic system. Randall theorized that the higher nitrification rates were due to “reduced competition for DO and nutrients in the BNR aerobic zone” versus the fully aerobic reactors. Other possible causes may be a higher intrinsic nitrifier growth rate in the low SRT reactors, due to selection of the faster growing nitrifying species.

d. *Effect of process flow sheet on nitrifier biomass growth rate.* Recent work by Sears (1998) may confirm the higher nitrifier growth rates in the BNR configuration that were observed by Randall (1992). Sears (1998) observed a 130% increase in the $\mu_A$ values for an anoxic/aerobic pure oxygen system versus a fully aerobic pure oxygen system. However, as stated by the researcher, the pH in sections of the fully aerobic reactor may have been lower than the BNR reactor, possibly accounting for the difference in measured $\mu_A$ values. A pH range of 6.2-6.4 is given for the BNR reactor with a pH of 5.0-6.1 in the fully aerobic reactor. An extended acclimation period ( greater than 80 days) was necessary in order to achieve above 80% ammonia removal at 12ºC and pH below 6.3. Chudoba

(1985) and indirectly, Hanaki (1990), demonstrated that nitrifiers grown at very low ammonia concentrations (0.1-1 mgN/L) exhibited a lower growth rate than their counterparts that were grown at higher concentrations (Hanaki, 1990) or that were exposed to significant ammonia gradients (10->1 mgN/L), such as in a plug flow reactor (Chudoba, 1985). This indicates that the nitrifier growth rate may be not only site specific, but also process specific.

e. *Data analysis technique utilized- Steady state versus dynamic.* In the cases where activated sludge reactors were operated over a relatively long period of time, the pseudo-steady state assumption (Equations 2.18-19) was typically utilized to permit averaging of performance data over extended periods (2-3 SRTs) and thereby calculation of $\mu_A^{MAX}$(Daigger and Saddick, 1999; Randall et al, 1992; Lesouef et al, 1992; Nowak et al, 1994). Dynamic modeling, where the influent data is comprised of daily concentrations, rather than averages, is currently available and has been used (Parker and Dold-Milwaukee) for data analysis. Whereas for reactors receiving synthetic feed, the steady state assumption may be valid, it is not true for wastewater systems where the variability of the wastewater characteristics is pronounced and consequently results in an oscillating nitrifier concentration.

f. *Impact of nitrifier decay rate on data analysis and extrapolation.* With reported decay rates in the range of negligible to 0.43 $d^{-1}$ (EPA, 1993; Randall, 1992; Leenen, 1997; Siegrist, 1999) the value of the decay rate used in Equations 2.18-

19 will result in significantly different nitrifier concentrations –particularly when the process analysis is conducted on high SRT units. This will in turn affect the value of $\mu_A^{MAX}$ that is calculated and possibly have significant practical impacts on the assessment of the nitrification capacity of the WWTP, as the choice of the combination of specific growth and decay rate may result in a different volumetric nitrification rate in the plant. The selection of decay rate used in the data analysis and subsequent activated sludge process calculations needs to be confirmed separately.

A comprehensive methodology for measurement of the nitrifier maximum growth rate will thus need to be defined, such that the value obtained can be extrapolated to different SRT and process configurations. The operating envelope that one can extrapolate to, using activated sludge modeling will need to be evaluated. Two methodologies for nitrifier growth rate measurement will be experimentally evaluated at 20°C:

i. Exponential Growth Batch Test at Low Biomass Concentrations
ii. Nitrification Rate Measurements in a Sequencing Batch Reactor

The literature review showed that it is generally accepted that there is a change in specific nitrifier growth rates as a function of the environmental variables encountered in wastewater treatment. The primary factors impacting growth rate are temperature, pH and dissolved oxygen levels:

- Temperature - Specific nitrifier growth rates are generally considered temperature sensitive, with nitrifiers being more sensitive to reduction in temperature than heterotrophic bacteria.
- pH- Nitrification progresses at it's maximum rate at pH values of 7 to 8. The borderlines are not well defined and appear to be specific to the process configuration. Nitrification activity subsides as pH drops below 6.0 or increases above 9.
- Dissolved oxygen – it is generally accepted that nitrification activity is reduced as dissolved oxygen levels drop below 2 mg/L in activated sludge systems, with effective cessation of activity at levels below 0.2-0.5 mg/L. In fixed film system higher dissolved oxygen values are typically required to achieve maximum nitrification activity, as there are mass transfer limitations that typically need to be overcome. Monod type kinetics are used to describe the effects of dissolved oxygen in activated sludge systems, where mass transfer limitations are negligible.

Whereas there is significant agreement on the qualitative effects of environmental conditions, the literature review showed a wide disparity in the reported specific nitrifier growth rates when they were normalized to standard conditions (20ºC, non-limiting pH and DO). The cause of the significant variation in values was routinely attributed to the perceived sensitivity of the nitrifying bacteria to inhibitors in a given wastewater. This has resulted in the development of the hypothesis that the nitrifier growth rate is a wastewater specific value, much like the volatile content of the influent suspended solids, and thus needs to be directly measured for a given plant (Barker and Dold, 1997).

However, examination of the experiments conducted, the data analysis procedures and inherent assumptions utilized, it was also observed that there were significant differences in both the experimental and analytical approaches utilized by the different researchers and engineers in the application of the specific nitrifier growth rate measurements.

Specifically, the following areas of concern were noted:

- It was observed that simplifications to the equation set describing the experiments were typically applied. In some work, influent wastewater characteristics and reactor operating parameters were averaged over an operating period, effectively resulting in a steady state approximation, and allowing for simpler calculations (Hanaki,1996). Whereas this appeared to be acceptable for bench scale experiments using synthetic wastewater, it would not be for actual wastewater treatment facilities.

- Another simplification typically encountered was the assumption that the nitrifier decay rate was negligible (Knowles, 1965). This allowed researchers to simplify the differential equations describing the nitrifier growth experiments. Antoniou (1990) assumed that terms in the differential equation representing growth were equal and could be eliminated. This simplified the analysis, permitting usage of a simplified linearization technique to estimate the net nitrifier growth rate. In both instances, the effects of these assumptions may affect the analysis of the experimental results, generally resulting in lower than actual specific growth rates.

- The nitrifier growth rate was frequently reported as a stand-alone value. However, in the few instances were raw data was provided in the literature, re-analysis of literature data using a different decay rate resulted in a significant change (typically greater than 80%) in the specific growth rate.

- Rates were reported for biological systems where the activated sludge seed was not acclimated to the environmental conditions being applied during the test. This was the case for example in the results presented by Hall (1980) and Painter and Loveless (1983). Care should be exercised when attempting to generalize the results of this type of testing.

- An increase in the specific nitrifier growth rate with decreasing SRT was reported in one study in particular (Randall, 1993). The cause of this effect was not determined.

It was thus necessary to assess measurement techniques for specific nitrifier growth rate so that guidance on the test methods specifics (environmental conditions for the test, sludge of the seed organisms, data analysis technique) could be developed.

# 3. MATERIALS AND METHODS

Three primary types of reactors were operated for this study – (1) batch exponential growth reactors, (2) sequencing batch reactors, and (3) batch decay reactors. Wastewater sampling, analytical procedures, and details on the operation of each of the reactor types are provided in this section.

## 3.1. Wastewater Sampling

Wastewater was obtained from hourly composite primary settling tank effluent samples which were collected three times a week using large volume (60 Liter) ISCO 6700 refrigerated automatic samplers (ISCO, Lincoln, Nebraska). Wastewater used in this study was collected from two large wastewater treatment plants in the metropolitan New York Area. The plants from which wastewater was obtained serve a combined sewer system, and are thus subject to influent flow, concentration, and thus loading variations. Primary settling tanks did operate within expected levels of efficiency (Fillos, 2003), removing settleable solid particles. The WWTPs from which the wastewater was obtained do not have grit removal facilities upstream of the primary tanks.

Each reactor was operated using wastewater from a single plant. The feed lot samples were trucked to CCNY and stored at 3-4$^{o}$C. Prior to utilizing the sample for an experiment, the appropriate volume was removed and equilibrated to the desired temperature. Each feed lot sample was analyzed for the conventional wastewater parameters shown in **Table 3.1.**

**Table 3.1** also provides the analytical methods used for each of the parameters. All analyses were conducted in the CCNY Environmental Laboratory, which is certified under the National Environmental Laboratory Accreditation Program.

**Table 3.1. Parameters and Analytical Methods**

| PARAMETER | ANALYTICAL METHOD [1] |
|---|---|
| Total and Soluble [2] COD<br>($COD_t$ & $COD_s$) | Reactor Digestion Method, Procedure codes 0.9 and 0.10<br>(DR/3000 HACH manual) [3] |
| Readily Biodegradable COD (RBCOD) | Flocculation-Filtration Method[4]<br>(Mamais et al., 1993) |
| Total and Soluble [2] TKN<br>($TKN_t$ &$TKN_s$) | Semi-Micro Kjeldahl, 4500-$N_{Org}$ C [5] |
| Total Ammonia Nitrogen (TAN) | Automated Phenate, 4500-$NH_3$ G |
| Oxidized Inorganic Nitrogen ($NO_x$-N) | Automated Hydrazine Reduction, 4500 $NO_3^-$ H |
| Total Suspended Solid (TSS) | Total Suspended Solids Dried at 103-105°C, 2540 D |
| Volatile Suspended Solid (VSS) | Fixed and Volatile Suspended Solids Ignited at 500°C, 2540 E |
| Inert Suspended Solid (ISS) | Fixed and Volatile Suspended Solids Ignited at 500°C, 2540 E |
| pH | Electrometric, 4500-$H^+$ B |
| Alkalinity | Titration, 2320 B |

1. Standard Methods for the Examination of Water and Wastewater, 20th Ed. (1998)
2. Soluble: sample is filtered through a 0.45 :m membrane filter.[2]
3. Federal Register, April 21, 1980, 45 (78) 26811-26812.
4. A Rapid Physical-Chemical Method for the Determination of of Readily biodegradable Soluble COD in Municipal Wastewater, Daniel Mamais et al, Water Research, Vol, 27, No. 1, pp 195-197, 1993 . Federal Register, April 21, 1980, 45 (78) 26811-26812.
5. Without previous ammonia removal.

## 3.2. Sequencing Batch Reactors

Sequencing batch reactors, (SBRs) were used to measure $\mu_{A,MAX}$ for specific wastewaters. Sequencing batch reactors operate as "fill and draw" units. The procedure involves operating the SBRs for a sufficient time to acclimate the biomass to the wastewater and achieve pseudo steady-state conditions. Subsequently, kinetic studies were performed to measure the rate of nitrification which is then used to calculate $\mu_{A,MAX}$, in concert with the nitrifier mass balance. **Figure 3.1** is a schematic of an SBR system used in this study to measure $\mu_{A,MAX}$.

In total, four SBRs were set up using return activated sludge as seed from the respective wastewater treatment plants. The SBRs were located in a constant temperature environmental chamber set at $20^oC$. All the SBRs were operated in accordance to the parameters outlined in **Table 3.2.** The testing was conducted in two phases. In the first phase two SBRs (noted as SBR 1 and SBR 2) were operated in a fully aerobic mode, but at different SRTs (8 and 20 days). In the second phase two SBRs (noted as SBR 3 and SBR 4) were operated at the same SRT (15 days) but one was fully aerobic, whereas the second one had both an anoxic (2 hours per cycle) and an aerobic (9 hours per cycle) operating period.

DO was controlled at 5.0 ± 0.3 mg/L with automated intermittent air addition. A 1 gr/L $NaHCO_3$ solution was utilized to maintain the pH at 7.3±0.1. Whereas dissolved oxygen levels and pH were controlled using identical setups with those utilized for the exponential growth batch reactors, addition of $CO_2$ was not required in the SBRs. In the SBRs $CO_2$ was continuously generated by the heterotrophic biomass in the SBRs through respiration, thus preventing the rapid upswings in pH, that had initially been observed in the exponential growth batch reactors.

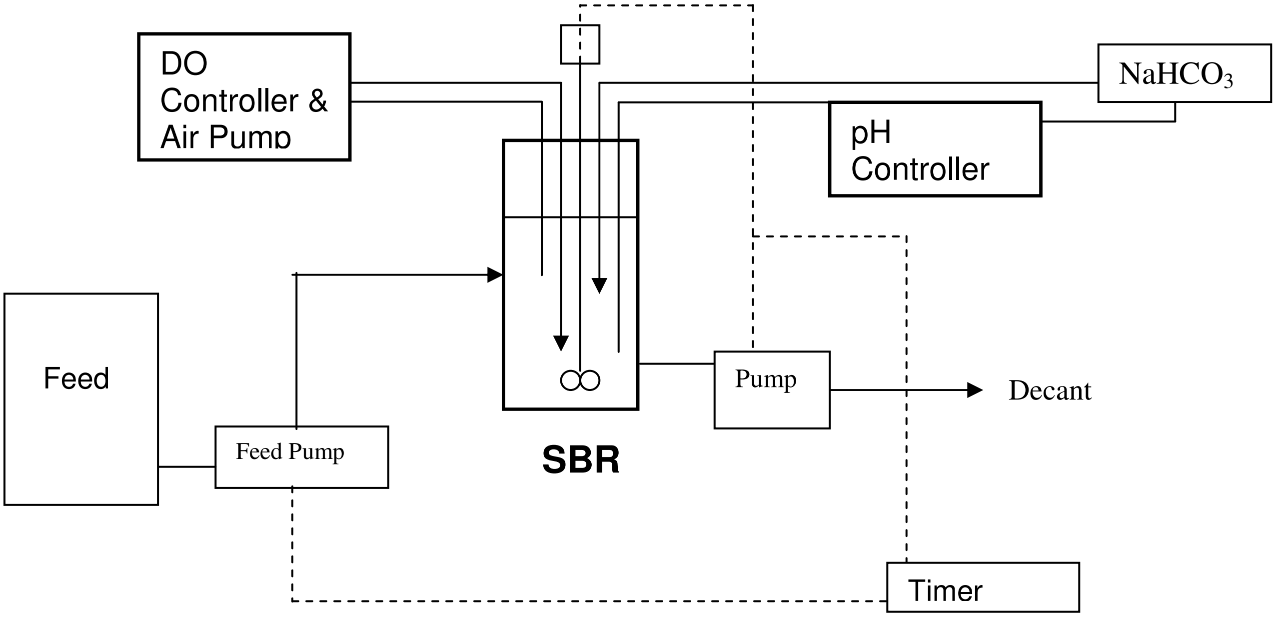

**Figure 3.1. Schematic diagram of laboratory-scale SBR system**

As deduced from **Table 3.2**, the SBRs were fed twice per day with primary effluent from the respective wastewater treatment plant, resulting in two operating cycles per day. Process control, in term of maintaining the target SRT was achieved by measuring the suspended solids in the reactor effluent on a daily basis, and then calculating the additional wastage volume required to maintain the targeted SRT.

**Table 3.2. SBR Operating Parameters**

| Reactor Configuration | | Operating Mode | |
|---|---|---|---|
| Reactor Volume: | 6.0 L | Aeration Cycle: | Fully Aerobic- 11 hrs |
| Feed Volume: | 4.5 L | | Anox/Aerob.- 9 hrs |
| SBR Cycle Time: | 12 hrs | Aerobic Stage DO: | 5.0 mg/L |
| React Period: | 11 hrs | pH: | 7.2-7.4 |
| Settling Period: | 0.667 hrs (40 min) | Alkalinity Source: | 5 gr/L $NaHCO_3$ |
| Decant Period: | 0.250 hrs (15 min) | Mixing: | Continuously Stirred |
| Idle Period: | 0.083 hrs ( 5 min) | SRT: | 100% Aerobic: 8, 15 & 20 days |
| Feed Period: | 0.083 hrs ( 5 min) | | Anoxic/Oxic: 15 days |
| | | SRT Control: | Eff. TSS + Manual Wasting Once Every Second Cycle |

Pseudo steady-state conditions were identified when full nitrification of the ammonia was established and a relatively steady concentration of MLSS was achieved. These conditions are typically achieved after continuous operation for a minimum period of approximately three SRTs, barring any major disruptions. During the stabilization period, influent and effluent samples were analyzed three times a week for $COD_T$, TSS & VSS, $NH_3$-N & $NO_X$-N and $TKN_T$. Alkalinity and RBCOD in the influent were also measured once a week. In addition, effluent $NO_X$-N and MLSS samples were analyzed on a daily basis.

Once the SBRs reached pseudo steady-state conditions, a period of intensive sampling and analysis was initiated in order to perform a nitrogen balance and collect sufficient data to determine the nitrification rates. During the intensive sampling period, daily additional analysis of $COD_T$ & $COD_S$, RBCOD, $TKN_T$ & $TKN_S$, $NH_3$-N & $NO_X$-N, on the influent and effluent were carried out to facilitate the nitrogen mass balance. The TKN concentration in the MLVSS was also periodically measured.

Kinetic rate measurements to determine the nitrification rate were conducted. Each of the kinetic experiments was conducted over a 3-day period covering six consecutive cycles. One batch of approximately 60 liters of PSTE was used from each of the respective WPCPs during this 3-day period to ensure that the feed for the 6 cycles was from the same sample batch. Three profiles of $NH_3$-N and $NO_X$-N were carried out on alternate cycles. Each of the profile measurements was initiated at the start of the cycle and consisted of collecting 20 ml samples from the SBRs, which were immediately filtered and acidified for later analysis of $NH_3$-N and $NO_X$-N using flow injection analysis. Samples were collected at 20-minute intervals for the first two hours and at 30-minute intervals for the third hour. DO, pH, and temperature were recorded during the sampling interval and alkalinity was measured at the start and end of the profile.

The end of the profile was identified as the point when the ammonia nitrogen concentration reached approximately 4 mg/L. Utilizing data collected at concentrations below this level would potentially result in the growth rate measurement being affected by the ammonia concentration, as the half saturation coefficient for nitrifying bacteria has been reported in the range of 0.5-1 mg/L for the environmental conditions encountered in this study's SBRs.

### 3.2.1. Data Analysis – Sequencing Batch Reactors

The fundamental equation, on which the calculation of the nitrifier specific growth rate was determined using the SBR is:

$$\mu_A^{MAX} = \frac{dNO_x - N}{dt} \cdot \frac{Y_A}{X_A} = \frac{dNO_x - N}{dt} \cdot \frac{(1 + b_A \cdot SRT) \cdot V_R}{C_N \cdot V_{ww} \cdot SRT} \qquad (2.20)$$

In the above equation, the critical parameters, shown on the right hand side, are:

- the operating characteristics (reactor and feed wastewater volumes & SRT)
- the concentration of ammonia oxidized by the nitrifying organisms, $C_N$
- the nitrifier decay rate, $b_A$ and
- the rate of the nitrification reaction, dN/dt.

The first three elements are critical in determining the concentration of nitrifier's present in the reactor, and thus correlating the reaction rate with the specific growth rate.

The overall approach for data analysis consisted of:

- Verification of the operating data
- Determination of the concentration of nitrifying bacteria

- Calculation of the maximum nitrifier growth rate from the kinetic rate measurements and the concentration of nitrifying bacteria

### 3.2.2. Operating Data Verification – Sequencing Batch Reactors

An accurate knowledge of the operating SRT is critical in the data analysis of the nitrogen removal system, as the concentration of the slower growing organisms responsible for nutrient removal and nitrifiers in particular will vary significantly with SRT. Although the SRT was controlled in the SBRs during the study period, it was also necessary to ensure that the mass balance was accurate, and that any inadvertent solids losses were being accounted for.

The inert, inorganic, suspended solids (ISS) are conserved in the activated sludge system, and have been used for the development of mass balances for verification or determination of the operating SRT (Melcer, 1998, Fillos, 2000). Care should be exercised in that inert solids are not conserved (ISS is produced) in biological phosphorus removal systems, due to polyphosphate accumulation in the Bio-P sludge. This was not of concern in the systems operated under this study, as the reactors were either fully aerobic, or included the use of relatively small anoxic operating periods, precluding complete removal of the nitrates present, and thus the potential for induction of biological phosphorus removal.

The ISS balance was conducted by measuring the inert, inorganic, suspended solids entering the system via the primary effluent feed, and the inert suspended solids exiting via the reactor effluent and the mixed liquor wasting volume. The balance was checked by tracking the cumulative influent and effluent ISS mass for each SBR over the operating period. The cumulative influent ISS was plotted against the cumulative effluent ISS. Ideally, the average mass of inert solids entering the system would be removed that same day. This would be represented by a plot where the data coincides with the diagonal.

In reality, due to the dynamic nature of the loadings, small deviations are expected from day to day. This may be compounded by to measurement/experimental error, affecting the precision of the balance, however there should be no bias in the balance; i.e. the ISS entering the system should not consistently be more than the ISS being removed, or vice-versa. This ensures that there are no consistent, underlying errors in the operation of the reactors that would affect the results of the analysis.

### 3.2.3. Determination of Nitrifier Growth Rate – Sequence Batch Reactors

Two distinct methodologies were used to develop the calculation for the maximum nitrifier growth rate:

a. Manual calculations using the WRC steady-state equation set
b. Using a dynamic activated sludge process model

The fundamental difference between the two methods lies in the approach towards development of the mass balance for the nitrifying bacteria. This difference will be illustrated as each method is described in detail.

**Manual nitrifier concentration calculation using the WRC Equation Set.** In order to be able to use Equation 2.20 to calculate the specific growth rate, the nitrifier concentration must be calculated:

$$X_A = \frac{Y_A \cdot C_N \cdot V_{ww} \cdot SRT}{(1 + b_A \cdot SRT) \cdot V_R} \qquad (2.18)$$

A yield of 0.15 mg biomass COD per mg $NH_3$-N nitrified was used in the calculation. The fundamental, underlying assumption when using this equation set is that the reactor is at a steady state. i.e. the nitrogen and COD loadings during the 3-day intensive sampling interval prevailed for a period of 2-3 SRTs. The form of equation (2.20) presented also assumes that none of produced nitrite or nitrate is being utilized for biomass synthesis. If a portion of the produced nitrate is being used for biomass synthesis, it would not be present in the effluent, and would thus not be accounted for in the calculation of the nitrifier concentration.

**Calculation of nitrifier concentration using a dynamic activated sludge process model.** Using a dynamic activated sludge model permitted accounting of the variations in influent COD and nitrogen concentrations that were observed during the course of the study. The General Model (Barker, 1997a, 1997b) was used, running on the BioWin process simulation platform. The General Model equation set is presented in the

Appendix. Although there is a large number of equations present in the General Model, the nitrifier concentration calculated using the model is still a function of the parameters shown in Equation 2.18. The only advantages that the dynamic model will potentially provide is that a more accurate estimate of the concentration of ammonia nitrified can be obtained, and the variation in the nitrifier concentration due to influent variability can be accounted for. This should provide a better estimate of the nitrifier concentration at any given time in the reactor.

**Figure 3.2** summarizes the process modeling approach. In order to determine the nitrifier concentration, $X_A$, each SBR was modeled for it's complete operating period. The daily influent concentrations were inputted into the model, along with the reactors' operating conditions. The first goal is to obtain a good correlation for the mass of ammonia nitrified. This is achieved by correctly estimating the mass of ammonia consumed in the reactor for biological assimilation, the production of bacterial cell mass. **Figure 3.3** shows the model output was compared to the actual operating data and the non-biodegradable particulate fraction of COD was varied as the primary calibration parameter until a good correlation between actual and model mixed liquor concentrations was obtained. The effluent model $NO_3$-N was then compared to the actual effluent $NO_X$-N data. The calibration was accepted if no bias in either the MLSS or NOx-N concentrations were observed.

The time variable concentration of the sludge components, from the model output, was retained and used with the corresponding nitrification profiles conducted during the intensive sampling events to determine $\mu_A^{MAX}$. **Figure 3.3** describes the steps followed for the Modeling of Nitrification Profile for Determination of $\mu_A^{MAX}$. In some profiles, sufficient data was collected to include the first order portion of the nitrification curve, in which case, the half saturation coefficient for ammonia, $K_N$, was also determined.

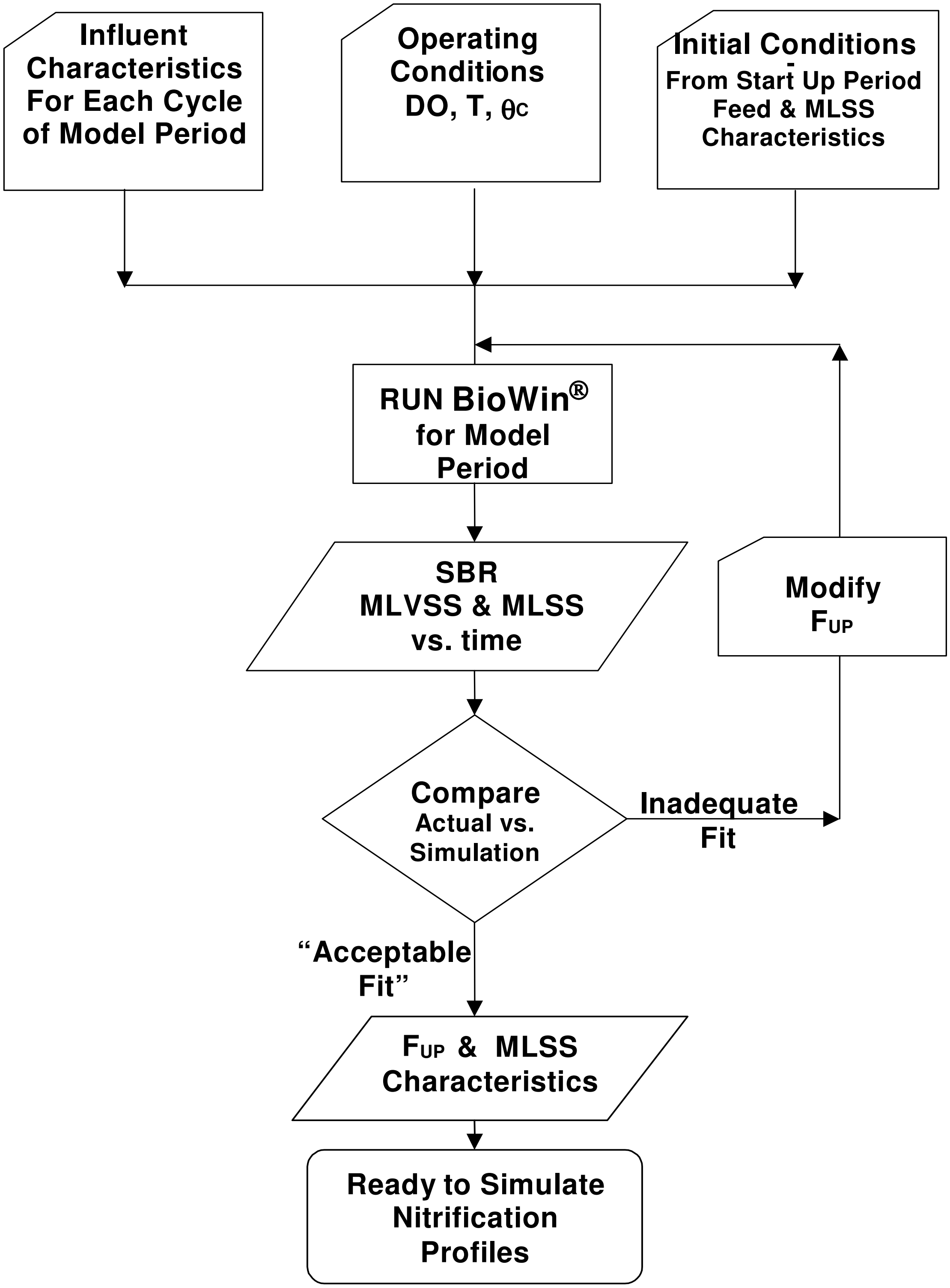

Figure 3.2. Approach Used for Solids Production Calibration

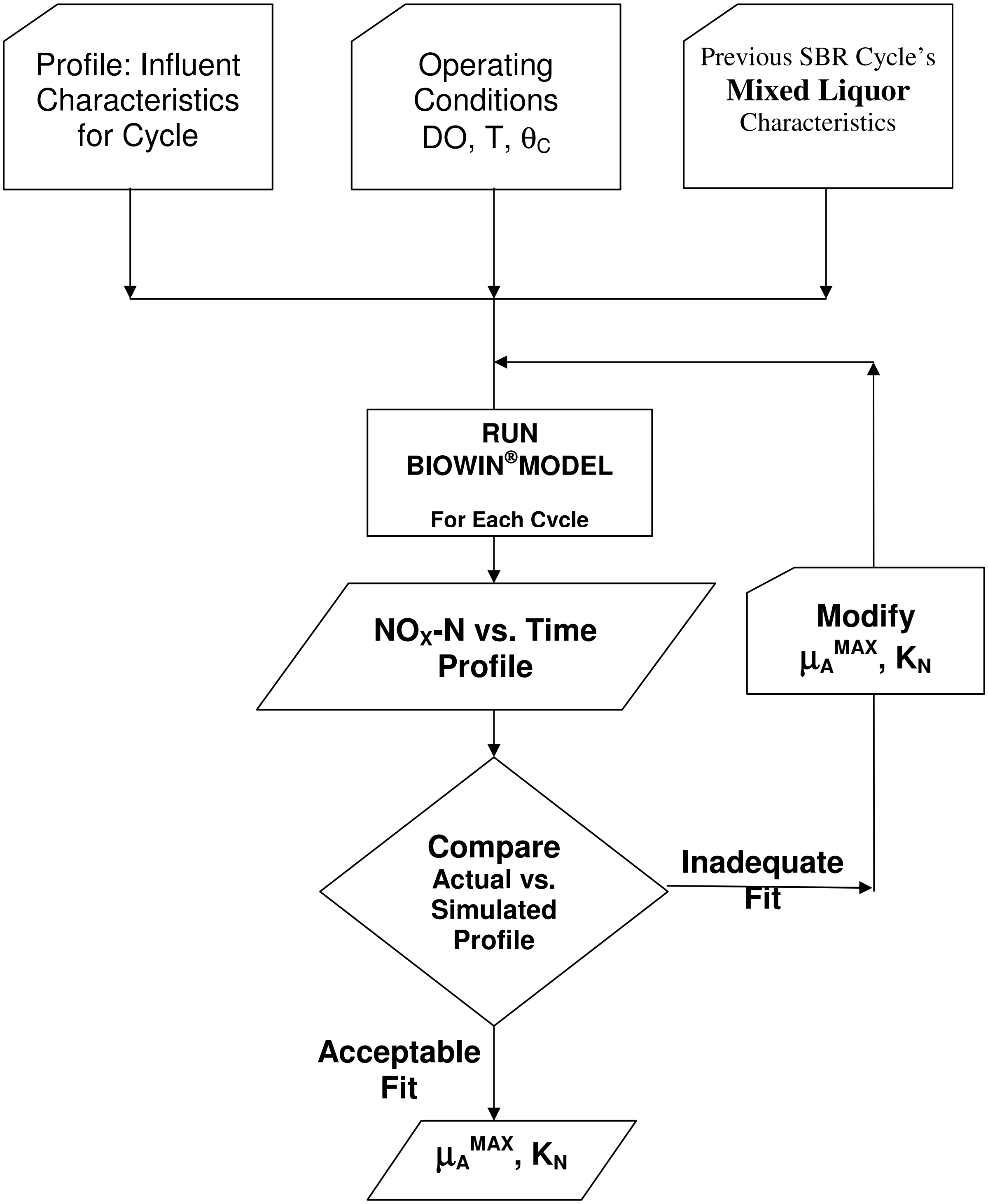

**Figure 3.3. Modeling of Nitrification Profile for Determination of $\mu_A^{MAX}$**

## 3.3. Nitrifier Decay Rate Measurement

The nitrifier decay rate, $b_A$, was measured in each reactor at least twice after pseudo-steady state (operation > 3 SRTs) was achieved. The protocol of Siegrist et al (1999) was utilized, with the modification of direct $NH_3$-N and NOx-N measurement rather than reliance on OUR data. In order to conduct the decay rate measurement wasting was suspended for 2 days, resulting in an increase of the SBR MLSS (Reactor 1 in **Figure 3.4**). The aggregate wasting volume (~400-1000 ml typically) was removed from the SBR and placed in a new reactor, termed the Decay Reactor. The DO and pH was controlled in the Decay Reactor, just as in the SBR. The only exception is that $CO_2$ was utilized to decrease pH, as necessary. No feed or ammonia source is added to the Decay Reactor after this point. Every 18-24 hours a 50-80 ml subsample was removed from the decay reactor and placed in a 100 ml beaker (Rate Reactor in Figure 3.4). An ammonia source ($NH_4CO_3$) was added to provide a concentration of approximately 8 mgN/L, and the nitrification rate was measured.

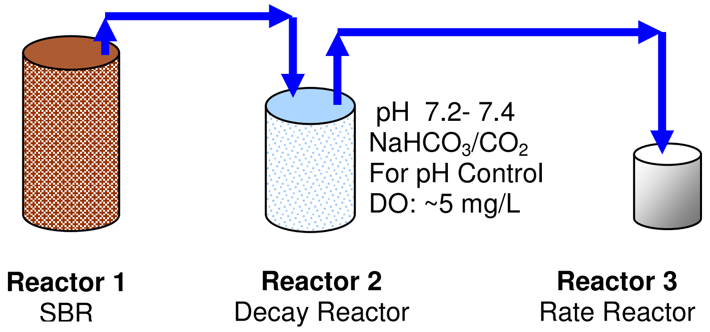

**Figure 3.4. Nitrifier Decay Rate Reactor Configuration**

### 3.3.1. Data Analysis – Nitrifier Decay Rate Measurement

A reduction in nitrifier activity was thus expected with the passage of time. The batch rate measurement on the subsample of the Decay Reactor biomass was repeated 4-6 times over a three to five day period, permitting development of a decay curve in accordance with the equation:

$$R_N(T) = \frac{d(NOx-N)}{dt}(T) = \mu_A^{MAX}\frac{X_A(T)}{Y_A} = \mu_A^{MAX}\frac{X_A^{INI}e^{(-b_A T)}}{Y_A} = \mu_A^{MAX}\frac{X_A^{INI}}{Y_A}e^{(-b_A T)} \quad (3.1)$$

Linearization of the above equation results in:

$$\ln R_N(T) = \ln\left(\mu_A^{MAX}\frac{X_A^{INI}}{Y_A}\right) - b_A T \quad (3.2)$$

Thus the nitrifier decay rate is obtained as the slope of the natural log of the nitrification rate versus time.

A certain amount of ammonia was liberated during the course of the decay experiment due to endogenous biomass activity and the hydrolysis of particulate TKN to soluble TKN and finally ammonia. This was reflected by the initially rapid, and then slow increase in $NO_X$-N concentrations in the Decay Reactor during the course of the experiment. Thus, a better estimation of the decay rate may be obtained by modeling the decay experiment using a model such as ASM1 or the General Model (Barker and Dold, 1997a). This will allow accounting of the change in nitrifying biomass due to the nitrification of the small quantities of ammonia liberated from the particulate matter.

Whether this effect will be significant or not must be judged on a case by case basis as the initial nitrifier concentration and the amount of ammonia nitrified during the course of the decay experiment will vary. For example, if the sludge is from a low-organic load, high SRT system, such as the systems used in this study, the initial nitrifier concentration relative to the heterotrophic biomass concentration will be high, and the added growth due to nitrification during the course of the decay experiment will be negligible (<10%). However, if sludge from a heavily loaded, low SRT system is used, it's low initial nitrifier concentration, along with high level of organic particulates and heterotrophic biomass that will be decaying and releasing ammonia, will make this effect significant necessitating the usage of modeling for accurate determination of the nitrifier decay rate

## 3.4. Exponential Growth Batch Reactors

Exponential growth batch tests were conducted using biomass from the fully aerobic sequencing batch reactors as seed. The low VSS batch test was conducted using the procedure guidelines outlined in Antoniou (1990) and Sozen (1996). Two liters of filtered (0.45 micron) primary effluent were added to a small volume of mixed liquor of known VSS concentration, such that the mixture had an initial MLVSS of 40-80 mg/L. Filtration of the primary effluent was deemed necessary to avoid seeding from nitrifiers that may be present in it. The pH and DO was controlled in the batch reactor. DO was controlled at 5.0 ± 0.3 mg/L with automated intermittent air addition. A 1 gr/L $NaHCO_3$ solution was utilized to maintain the pH at 7.3±0.1. In initial experiments a sulfuric acid solution (0.05 N) was used to decrease pH when levels above 7.4 were reached. However, this affected the nitrifier growth rate. The pH control system was converted after a series of four experiments, such that addition of $CO_2$ was provided when the pH increased above the maximum allowable of 7.4. The aeration rate was also reduced to approximately 0.3 L/hr, such that the DO targets were met, without excessive aeration energy, and thus $CO_2$ stripping imparted. The reactors were placed in an environmental chamber, where temperature is controlled at 20±0.2°C. A 10 grN/L $NH_4Cl$ stock solution was used to intermittently spike the reactor with ammonia, such that the ammonia concentration was in the range of 10-20 mgN/L. Continuous mixing was provided by a magnetic stirrer. A 1 cm thick piece of foam between the bottom of the beaker and the magnetic stirrer eliminated heat transfer from the stirring mechanism to the batch reactor contents. Each experiment was typically conducted in duplicate.

Each reactor was sampled at 1 to 3 hour intervals for $NH_3$-N and NOx-N, over a 55 to 72 hour period in order to develop a well defined exponential growth curve. The time, reactor temperature and pH was recorded at each sampling interval. This was a modification of the method as applied by Antoniou and Sozen who sampled the batch reactor once or twice per day for 3-5 days. The purpose of this modification was to permit better definition of the exponential growth curve, and allow calculation of the initial nitrifier concentration using the refined data analysis procedures that will be described. The MLSS and MLVSS were measured in the first and last sample.

### 3.4.1. Data Analysis – Exponential Growth Batch Reactors

Data analysis was conducted using the non-linear parameter estimation method first utilized by Sozen (1996). A spreadsheet was developed that used a one dimensional search technique to find the value of the reciprocal of the second term of the RHS of equation (2.14) such that the error between the experimental [$NO_X$-N] profile and the calculated profile was minimized.

$$[NO_X - N](t) = [NO_X - N]_{INI} - \frac{\mu_A^{MAX} X_A^{INI}}{(\mu_A^{MAX} - b_A)Y_A} + \frac{\mu_A^{MAX} X_A^{INI}}{(\mu_A^{MAX} - b_A)Y_A} \cdot e^{(\mu_A^{MAX} - b_A)t} \qquad (2.14)$$

In addition to using conventional measures, such as the residual of the least squares parameter estimation procedure, data quality was evaluated by examining subsets (one day in length) of each experiment's data set and comparing the net growth rate between the different data subsets. This permitted checking of the data to assess whether the growth rates were remaining stable through the experiment. Reproducibility of the method was evaluated by conducting simultaneous duplicate measurements using the same primary effluent and equal volumes of the same seed sludge.

The increased sampling, relative to the original protocols, as defined by Antoniou and Sozen, served a dual purpose: (1) to check that the growth rate was stable, as described previously, and (2) to allow for estimation of the initial nitrifier concentration, $X_A^{INI}$ in the batch reactor. This is obtained by solving equation 2.14 for $X_A^{INI}$:

$$X_A^{INI} = \frac{[NO_X - N](t) - [NO_X - N]_{INI}}{\left[e^{(\mu_A^{MAX} - b_A)t} - 1\right]} \cdot \frac{(\mu_A^{MAX} - b_A)Y_A}{\mu_A^{MAX}} \qquad (3.3.)$$

Using literature values for nitrifier yield, $Y_A$, and assuming a typical decay rate of 0.1 $d^{-1}$, the initial nitrifier concentration was estimated. After completion of the decay rate measurements, the nitrifier decay rate value was modified to the experimentally determine value and the data was re-analyzed.

# 4. EXPERIMENTAL RESULTS

The experimental results are presented in four parts: (1) the characteristics of the wastewater used during the course of the study, (2) the sequencing batch reactor operation and the nitrification kinetic rates measured, (3) the determination of nitrifier decay rates using biomass from the SBRs and (4) the specific growth rates measured using the exponential growth batch tests.

## 4.1. Wastewater Characteristics

Wastewater was obtained from two plants for this study. The wastewater from both plants was typical of relatively weak, primarily domestic, wastewater. During the first phase of the study (Stage I) wastewater was obtained from treatment plant “W”. Wastewater obtained from the second plant, “WI” for Stage II was of similar characteristics but had a COD to nitrogen ratio of 8.2, slightly lower than the 10.4 COD/N ratio in the wastewater from plant “W”.

The wastewater did exhibit some variability as shown in the case of plant “W” in **Figures 4.1** and **4.2,** which was used to feed the Stage I reactors. This is particularly evident in the first 30 days of the reactor’s operation, when raw influent wastewater was being used. After primary settling was instituted (by allowing the raw wastewater to settle quiescently for 30 minutes prior to decanting the supernatant) the particulate levels in the wastewater stabilized, **Figure 4.3**, resulting in stabilization of the total COD as shown in **Figure 4.1**.

Soluble COD levels were observed to be relatively constant throughout the operating period, with no significant changes in characteristics, **Figure 4.1** and **4.2**. Although the concentration of the soluble COD varied between 50 and 100 mg/L during the operating period, it typically ranged between 30 and 40% of the total COD. The observed variation in absolute concentrations was primarily due to the combined sewerage system feeding the wastewater treatment plant, which resulted in dilution of the wastewater by wet weather flows, diluting the concentration of the constituents.

This also resulted in the variability in the inorganic suspended solids carried into the system -however this variability was significantly dampened after primary settling was provided, as shown in **Figure 4.3**. The overall volatile content of the influent wastewater suspended solids was typically in the range of 80-90%, and did not markedly change after primary settling was instituted, indicating that the inorganic suspended solids in the sample had similar settling characteristics to that of the organics with which they were enmeshed, and would thus not be expected to behave differently from the organics in the reactor with respect to settling.

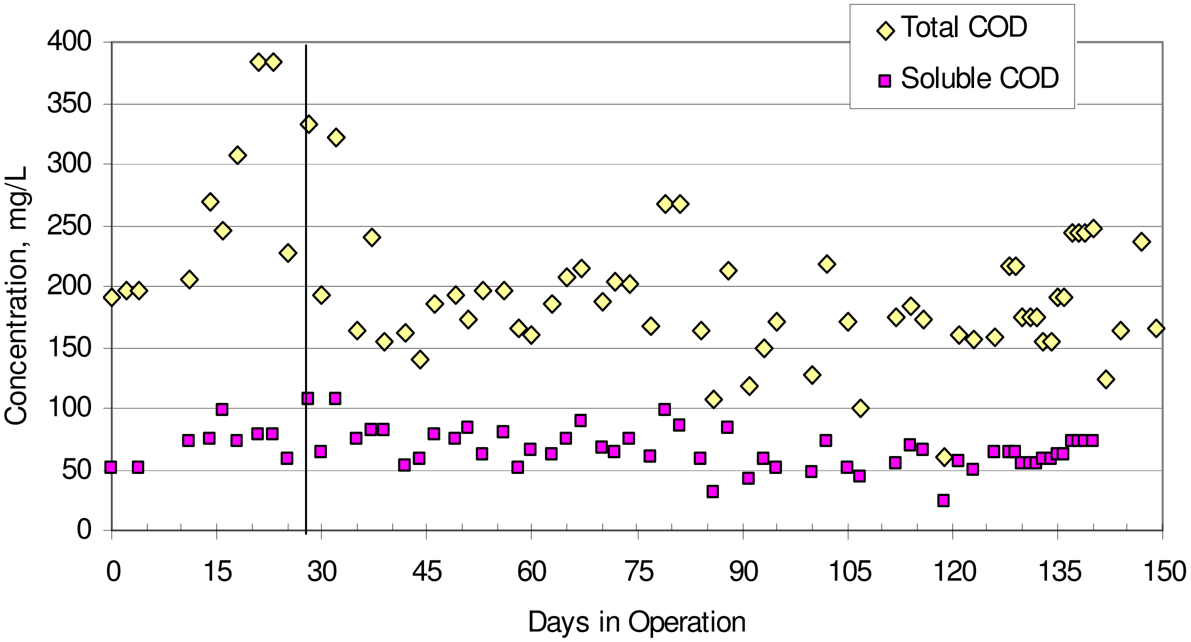

Figure 4.1. Total and Soluble COD Concentration in SBR W1 and SBR W2 Influent

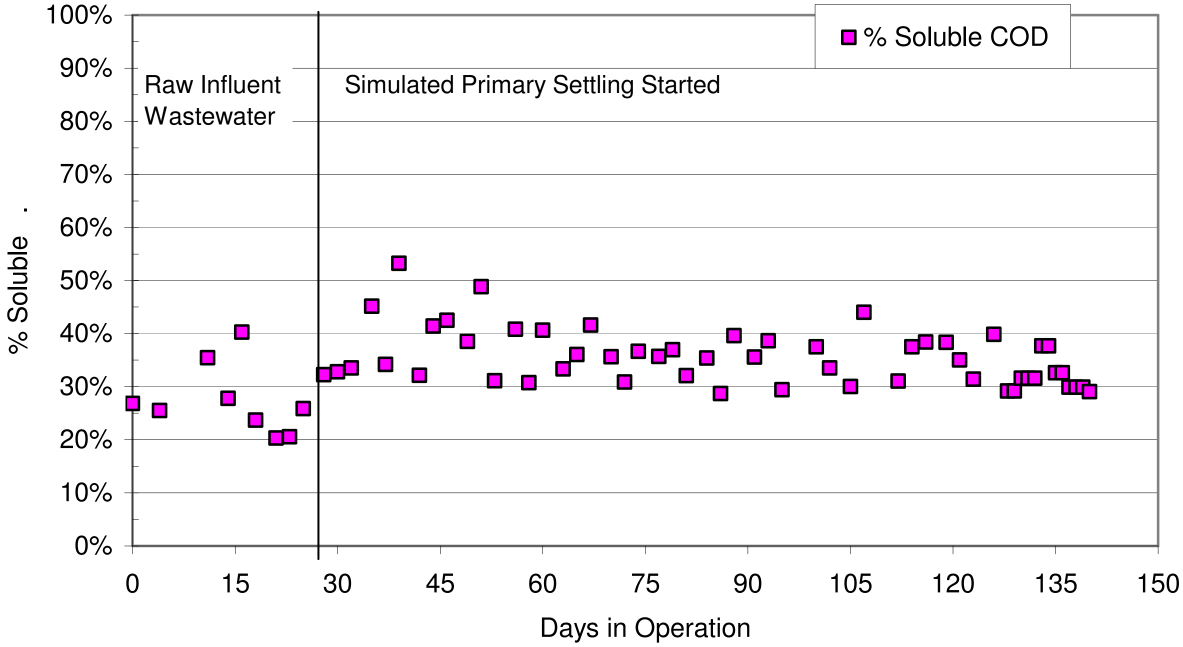

Figure 4.2. Percentage of Soluble COD in SBR W1 and SBR W2 Influent. Note Stabilization in Percentage After Simulated Primary Settling Initiated

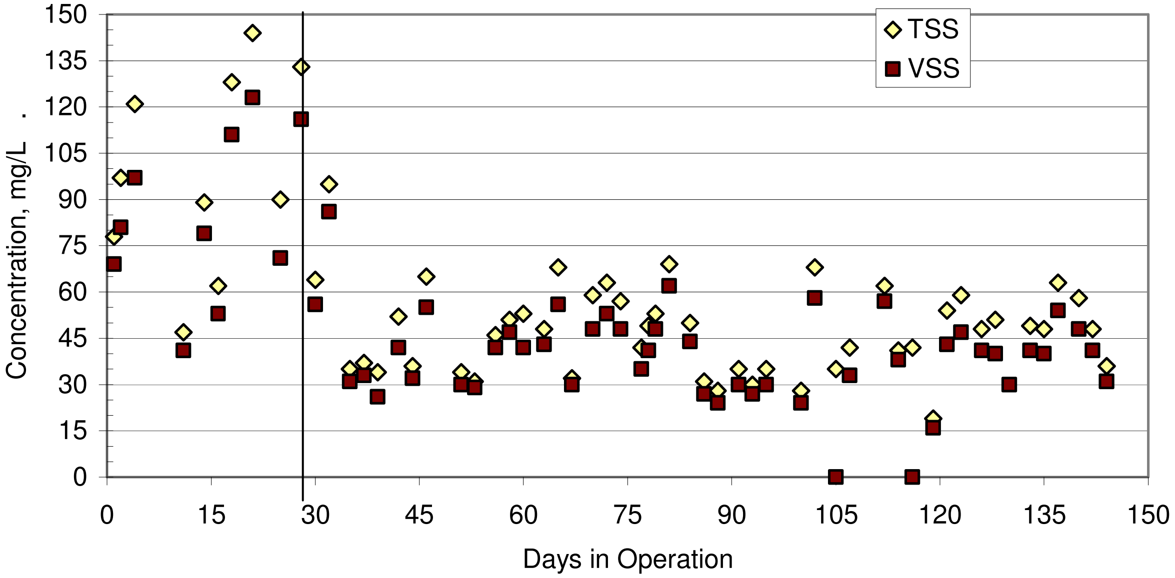

Figure 4.3. Total Suspended and Volatile Solids in SBR W1 and SBR W2 Influent

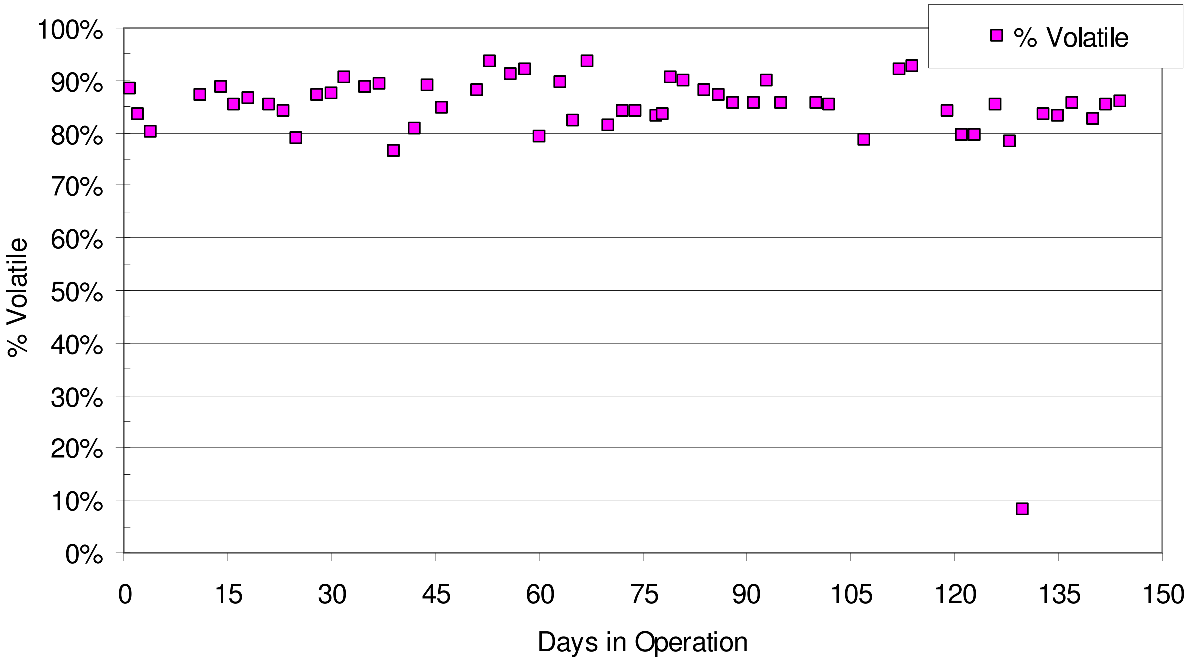

Figure 4.4. Percentage of Organic Suspended Solids in SBR W1 and SBR W2 Influent

Kjeldahl nitrogen levels typically ranged between 15 and 20 mgN/L, **Figure 4.5**, with approximately 76% of the Kjeldahl nitrogen being soluble. Ammonia concentrations typically ranged from 10 to 14 mgN/L, **Figure 4.6**, typically comprising 65-75% of the influent nitrogen and greater than 90% of the influent soluble Kjeldahl nitrogen. The observed concentrations resulted in nitrogen loadings on the order of 22-30 mgN per L reactor volume per day. This is on the lower end of the loading rates typically encountered in full scale systems (30-80 mgN per L reactor volume per day).

Alkalinity levels in the influent were periodically monitored. Typical of a rainwater derived water source, alkalinity ranged from 80 to 130 mg/L as $CaCO_3$.

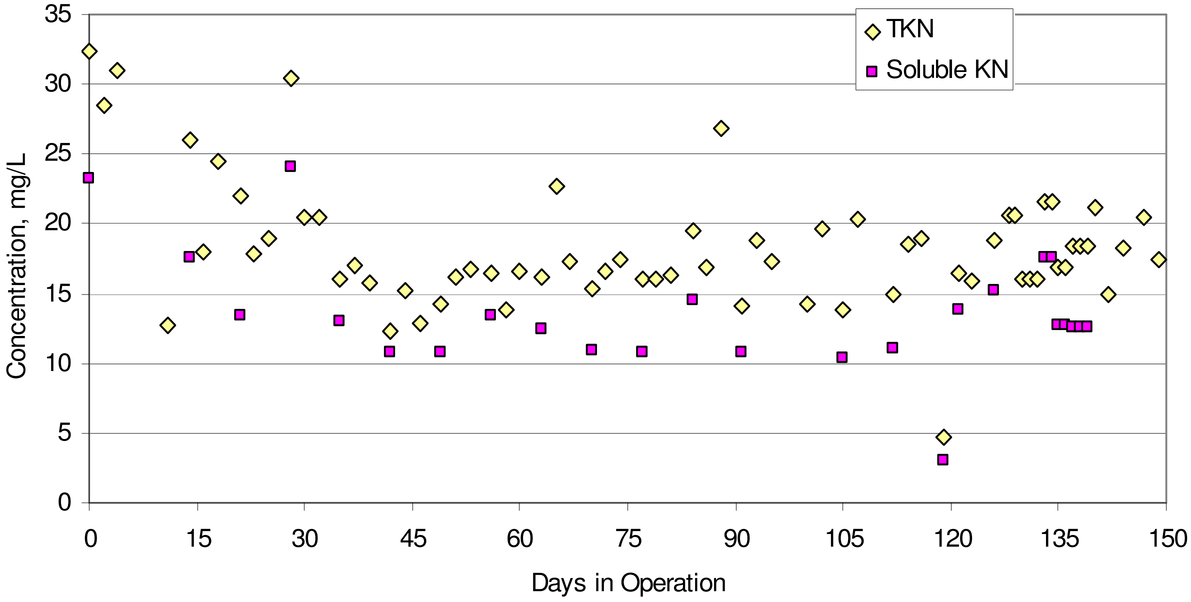

**Figure 4.5. Total and Soluble Kjeldahl Nitrogen Concentrations in SBR W1 and SBR W2 Influent**

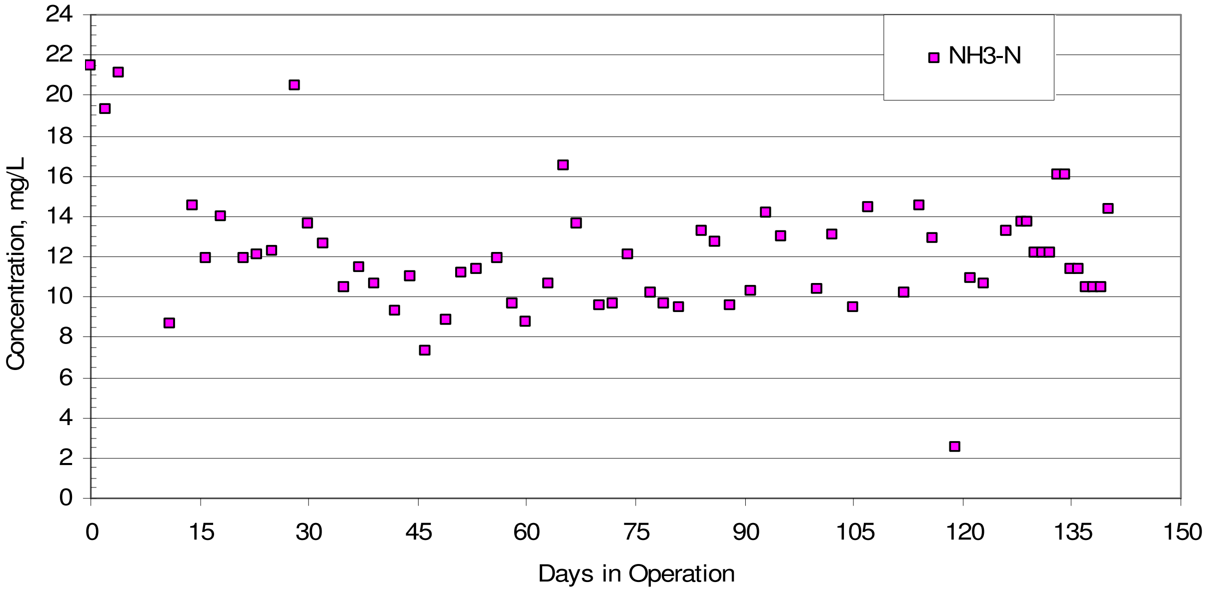

**Figure 4.6. Ammonia-Nitrogen Concentrations in SBR W1 and SBR W2 Influent**

## 4.2. Sequencing Batch Reactor Data

The experimental phase was conducted in two stages. As previously described, in each stage a pair of bench scale sequencing batch reactors were set up, receiving primary effluent from a New York City wastewater treatment plant. **Table 4.1** summarizes the operating conditions of the reactors for each stage. The primary effluent was fully characterized, and deemed to be a weak domestic wastewater (COD~150-200 mg/L; TKN~15-22 mg/L).

In the first stage both reactors were operated in a fully aerobic react mode, with one reactor running at a high sludge age, 20 days, while the second was operated at a low age of 8 days.

**Table 4.1.** SBR Mode and SRT Summary

| Stage | Reactor | Operating Mode | SRT (days) |
|---|---|---|---|
| Stage I | SBR W1 | Fully Aerobic | 20 |
| | SBR W2 | Fully Aerobic | 8 |
| Stage II | SBR WI1 | Fully Aerobic | 15 |
| | SBR WI2 | Anoxic/Aerobic | 15 |

The reactors were operated for a period of three to four sludge ages (SRT), permitting the reactor to reach a pseudo-steady state condition, termed as such to signify that acclimation of the biomass had occurred and the level of active biomass was deemed to have stabilized within the reactor. Solids concentrations in each reactor stabilized after two to three SRT's as shown in **Figure 4.7**. Reactor SRT was maintained constant by measuring solids inventory in the reactor and losses to the effluent and then wasting the appropriate volume on a daily basis, **Figure 4.8**. The reactors typically fully nitrified the nitrogen loadings applied, however there was significant variability in the effluent NOx-N concentration as shown in **Figure 4.9**. This in part was due to the variable loading of ammonia to the SBR as shown in **Figure 4.6.**

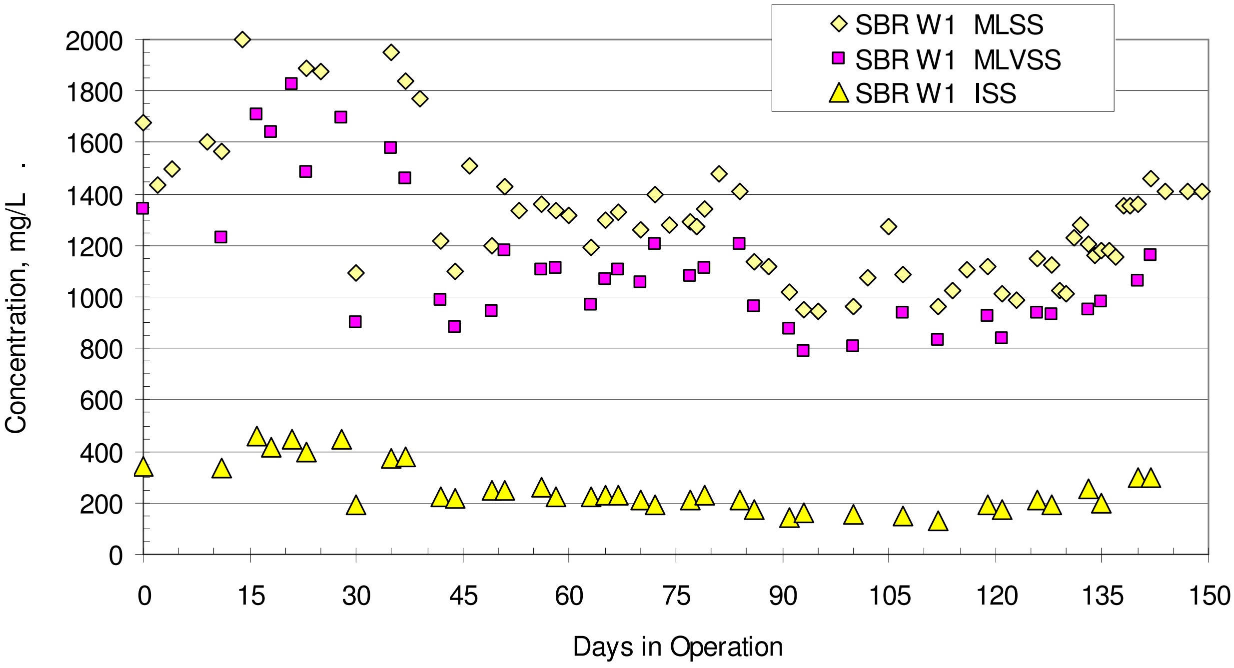

Figure 4.7. SBR W1 Mixed Liquor Solids Concentrations

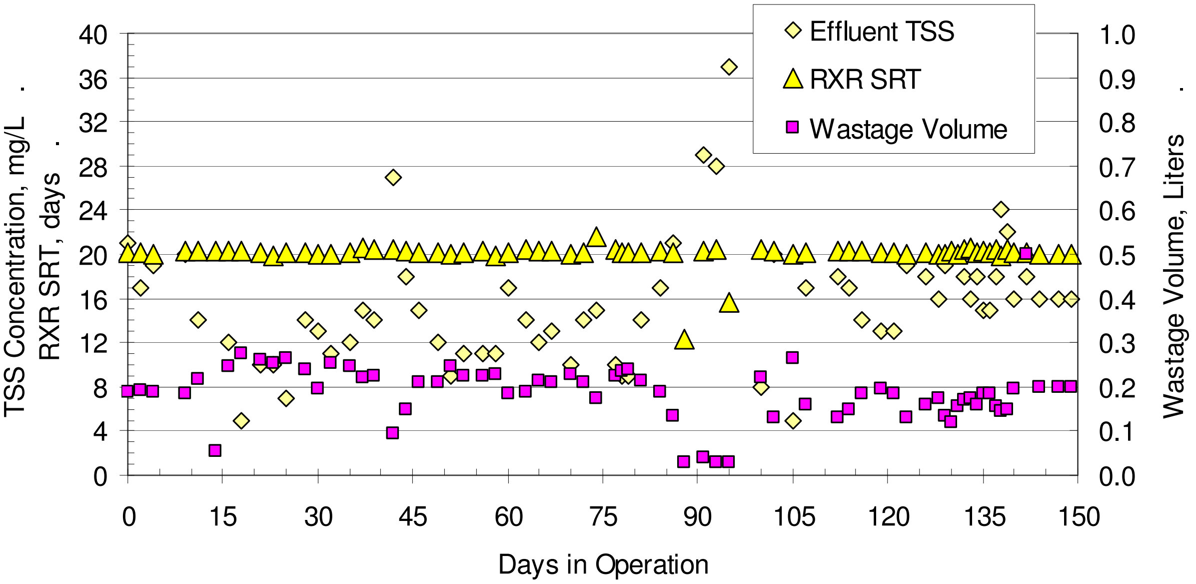

Figure 4.8. SBR W1 Effluent Suspended Solids, Wastage Volumes and Ensuing Operating SRT

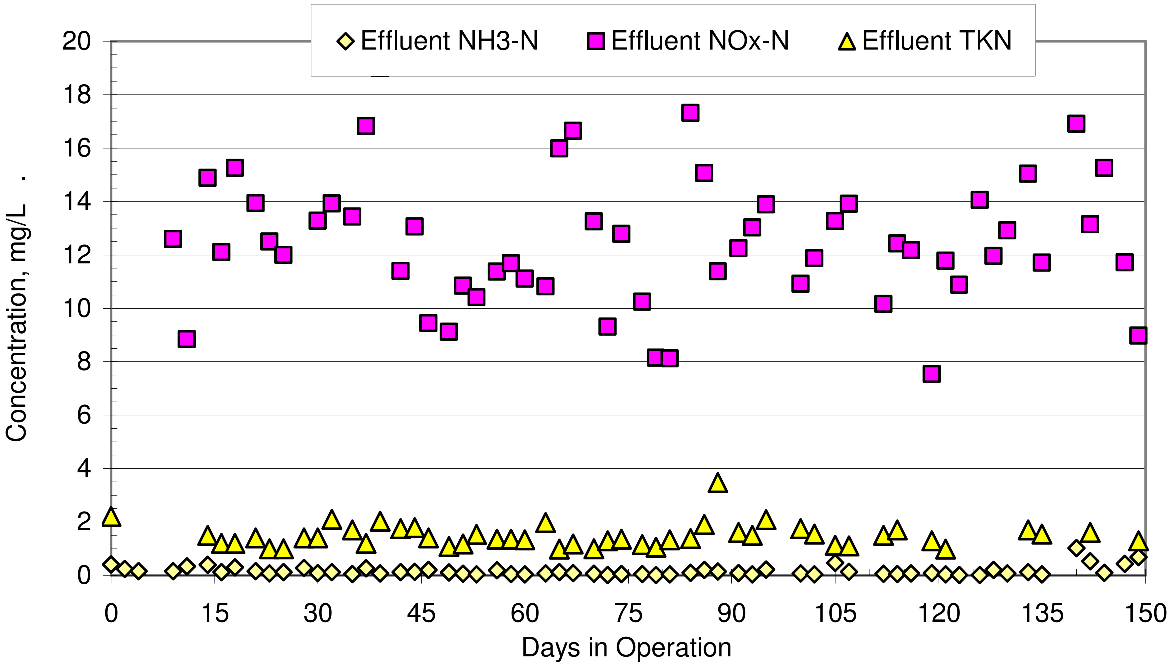

**Figure 4.9. SBR W1 Effluent NH3-N, NOx-N and TKN Levels**

### 4.2.1. Sequencing Batch Reactor Kinetic Testing Results

After the conclusion of the stabilization period, the volumetric and specific nitrification rates were measured in each reactor under the conditions shown in **Table 4.1**. The operating conditions in **Table 4.1** resulted in measurement of the maximum nitrification rate during the initial phases of the SBR's daily operating cycle, immediately after the feed phase. A sample nitrification rate measurement is shown in **Figure 4.10**.

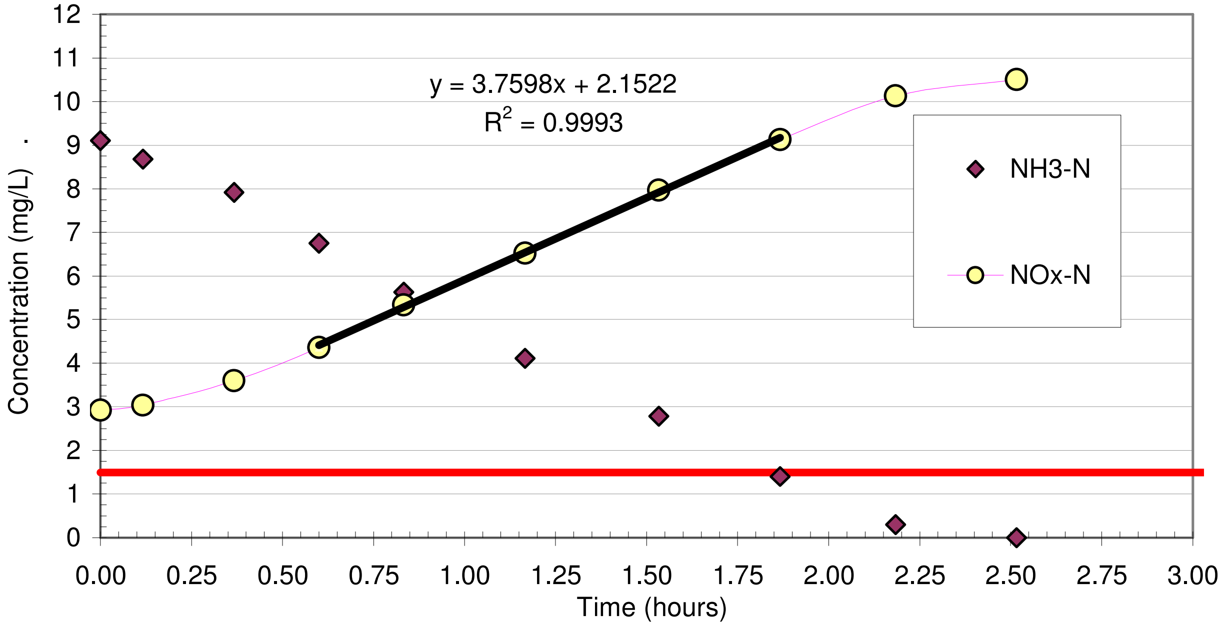

**Figure 4.10. Typical Nitrification Rate Measurement in SBR**

The maximum slope of the nitrate production curve was used to determine the volumetric nitrification rate, such that ammonia concentration would not be limiting. The initial points in the measurement were typically not considered, as the nitrification rate achieved it's maximum value approximately half hour after the completion of the feed phase. The primary cause of this delay was that during the first few minutes of the SBR cycle, the DO levels were depressed, as the readily biodegradable organic carbon was being consumed.

The results of the nitrification rate measurements for the four sequencing batch reactors operated during both stages of the experimental phase of the work are summarized in **Tables 4.2** through **4.5**.

The specific nitrification rates ranged between 0.07 and 0.09 mgN/mgVSS-d for the Stage I reactors and 0.10 to 0.18 mgN/mgVSS-d in the Stage II reactors. The increased specific nitrification rate in the Stage II reactors was in part due to the lower carbon to nitrogen ratio in the Stage II reactors' wastewater feed. The influent wastewater COD to TKN ratio averaged 10.4 in the Stage I influent versus 8.2 in the Stage II influent.

The volumetric nitrate production rates exhibited differences among reactors of each stage. The 8 day SRT Stage I reactor provided nitrate production rates of approximately 50 mgN/L-d, whereas the 20 day reactor provided nitrate production rates of 70 to 100 mgN/L-d. This is expected given the increased SRT of SBR W1 versus W2.

Full interpretation of the acquired nitrification kinetic data requires conversion of the presented raw data to specific nitrifier growth rates.

### Table 4.2. Summary of Observed Nitrification Rates – Stage I - SBR W1 – 20d SRT

| Operating Day | MLSS (mg/L) | MLVSS (mg/L) | pH | Temp ($^{o}$C) | Nitrate Production Rate (mg/L-d) | Regression Correlation Coefficient $R^2$ | Specific Nitrification Rate (mgN/mgVSS-d) |
|---|---|---|---|---|---|---|---|
| 36 | | | 7.3-7.5 | 20.0 | 104 | 0.9989 | 0.0691 |
| 37 | 1850 | 1500 | 7.3-7.4 | 19.3 | 108 | 0.9981 | 0.0717 |
| 38 | | | 7.3-7.5 | 20.1 | 105 | 0.9987 | 0.0698 |
| **Average** | | | | **19.8** | **105** | | **0.0702** |
| **St. Deviation** | | | | | ***2.0*** | | ***0.0013*** |
| 64 | | | 7.2-7.3 | 20.1 | 70 | 0.9976 | 0.0657 |
| 65 | 1300 | 1070 | 7.1-7.3 | 19.9 | 71 | 0.9979 | 0.0664 |
| 66 | | | 7.1-7.3 | 19.7 | 81 | 0.9979 | 0.0758 |
| **Average** | | | | **19.9** | **74** | | **0.0693** |
| **St. Deviation** | | | | | ***6.0*** | | ***0.0056*** |
| 78 | | | 7.3-7.5 | 20.5 | 91 | 0.9977 | 0.0823 |
| 79 | 1340 | 1100 | 7.3-7.5 | 19.7 | 93 | 0.9991 | 0.0850 |
| 80 | | | 7.3-7.5 | 19.8 | 83 | 0.9982 | 0.0755 |
| **Average** | | | | **20.0** | **89** | | **0.0809** |
| **St. Deviation** | | | | | ***5.4*** | | ***0.0049*** |
| 106 | | | 7.1-7.3 | 19.4 | 74 | 0.9975 | 0.0760 |
| 107 | 1150 | 980 | 7.1-7.3 | 19.3 | 82 | 0.9998 | 0.0839 |
| 108 | | | 7.3-7.5 | 20.2 | 88 | 0.9996 | 0.0898 |
| **Average** | | | | **19.6** | **82** | | **0.0832** |
| **St. Deviation** | | | | | ***6.8*** | | ***0.0069*** |
| 128 | | | 7.2-7.4 | 20.7 | 78 | 0.9983 | 0.0829 |
| 129 | 1080 | 940 | 7.2-7.3 | 20.4 | 77 | 0.9978 | 0.0817 |
| 130 | | | 7.1-7.3 | 19.3 | 68 | 0.9990 | 0.0720 |
| **Average** | | | | **20.1** | **74** | | **0.0789** |
| **St. Deviation** | | | | | ***5.6*** | | ***0.0060*** |
| 138 | | | 7.1-7.3 | 19.2 | 67 | 0.974 | 0.0623 |
| 139 | 1350 | 1080 | 7.2-7.3 | 19.6 | 79 | 0.9958 | 0.0731 |
| 139 | | | 7.0-7.3 | 20 | 83 | 0.9931 | 0.0767 |
| **Average** | | | | **19.6** | **76** | | **0.0707** |
| **St. Deviation** | | | | | ***8.1*** | | ***0.0075*** |
| 145 | | | 7.2-7.3 | 20.5 | 87 | 0.9935 | 0.0783 |
| 145 | 1410 | 1110 | 7.2-7.4 | 19.2 | 76 | 0.9907 | 0.0680 |
| 146 | | | 7.1-7.3 | 19.2 | 80 | 0.9998 | 0.0722 |
| **Average** | | | | **19.6** | **81** | | **0.0728** |
| **St. Deviation** | | | | | ***5.8*** | | ***0.0052*** |
| 161 | | | 7.0-7.3 | 19.4 | 86 | 0.9996 | 0.0784 |
| 162 | 1440 | 1100 | 7.2-7.3 | 19.6 | 89 | 0.9968 | 0.0807 |
| 162 | | | 7.1-7.3 | 19.2 | 78 | 0.9992 | 0.0710 |
| **Average** | | | | **19.4** | **84** | | **0.0767** |
| **St. Deviation** | | | | | **5.6** | | **0.0051** |

**Table 4.3. Summary of Observed Nitrification Rates – Stage I - SBR W2 – 8d SRT**

| Operating Day | MLSS (mg/L) | MLVSS (mg/L) | pH | Temp (oC) | Nitrate Production Rate (mg/L-d) | Regression Correlation Coefficient R2 | Specific Nitrification Rate (mgN/mgVSS-d) |
|---|---|---|---|---|---|---|---|
| 70 | | | 7.1-7.2 | 20.0 | 43.7 | 0.9953 | 0.0721 |
| 71 | 700 | 600 | 7.2-7.3 | 19.5 | 45.6 | 0.9999 | 0.0785 |
| 72 | | | 7.1-7.3 | 20.4 | 48.7 | 0.9977 | 0.0752 |
| **Average** | | | | **19.4** | **46.0** | | **0.0753** |
| **St. Deviation** | | | | | **2.5** | | **0.0032** |
| 80 | | | 7.1-7.2 | 19.1 | 40.8 | 0.9963 | 0.0648 |
| 81 | 740 | 630 | 7.2-7.3 | 19.4 | 45.6 | 0.9997 | 0.0724 |
| 81 | | | 7.1-7.3 | 19.8 | 48.7 | 0.9927 | 0.0773 |
| **Average** | | | | **19.4** | **45.1** | | **0.0715** |
| **St. Deviation** | | | | | **4.0** | | **0.0063** |
| 87 | | | 7.2-7.3 | 20.1 | 51.3 | 0.9944 | 0.0884 |
| 87 | 650 | 580 | 7.2-7.4 | 19.1 | 47.3 | 0.9996 | 0.0816 |
| 88 | | | 7.1-7.5 | 18.9 | 50.5 | 0.9949 | 0.0871 |
| **Average** | | | | **19.4** | **49.7** | | **0.0857** |
| **St. Deviation** | | | | | **2.1** | | **0.0036** |
| 103 | | | 7.1-7.5 | 19.2 | 52.2 | 0.9992 | 0.0870 |
| 104 | 710 | 600 | 7.2-7.3 | 18.9 | 49.4 | 0.9931 | 0.0823 |
| 104 | | | 7.1-7.3 | 19.2 | 52.8 | 0.9993 | 0.0880 |
| **Average** | | | | **19.1** | **51.5** | | **0.0858** |
| **St. Deviation** | | | | | **1.8** | | **0.0030** |

# Table 4.4. Summary of Observed Nitrification Rates-Stage II - SBR WI1- 15d SRT

| Operating Day | MLSS (mg/L) | MLVSS (mg/L) | pH | Temp ($^oC$) | Nitrate Production Rate (mg/L-d) | Regression Correlation Coefficient $R^2$ | Specific Nitrification Rate (mgN/mgVSS-d) |
|---|---|---|---|---|---|---|---|
| 81 | | | 7.2-7.4 | 20.4 | 90 | 0.9973 | 0.129 |
| 82 | 875 | 700 | 7.1-7.3 | 20.0 | 99 | 0.9991 | 0.141 |
| 83 | | | 7.1-7.3 | 20.5 | 96 | 0.9989 | 0.137 |
| **Average** | | | | **20.3** | **95** | | **0.136** |
| **St. Deviation** | | | | | ***4.3*** | | ***0.006*** |
| | | | | | | | |
| 102 | | | 7.1-7.3 | 20.3 | 102 | 0.9994 | 0.145 |
| 103 | 875 | 700 | 7.1-7.3 | 20.0 | 72 | 0.9717 | 0.103 |
| 104 | | | 7.1-7.3 | 20.6 | 99 | 0.9926 | 0.142 |
| **Average** | | | | **20.3** | **91** | | **0.130** |
| **St. Deviation** | | | | | ***16.4*** | | ***0.023*** |
| | | | | | | | |
| 116 | | | 7.1-7.3 | 20.3 | 105 | 0.9932 | 0.142 |
| 117 | 925 | 740 | 7.1-7.3 | 20.0 | 109 | 0.999 | 0.147 |
| 118 | | | 7.1-7.3 | 21.0 | 112 | 0.9919 | 0.151 |
| **Average** | | | | **20.4** | **109** | | **0.147** |
| **St. Deviation** | | | | | ***3.5*** | | ***0.005*** |
| | | | | | | | |
| 141 | | | 7.1-7.3 | 19.5 | 82 | 0.9989 | 0.105 |
| 142 | 975 | 780 | 7.1-7.3 | 19.4 | 90 | 0.9982 | 0.115 |
| 143 | | | 7.1-7.3 | 19.1 | 78 | 0.9925 | 0.100 |
| **Average** | | | | **19.3** | **83** | | **0.106** |
| **St. Deviation** | | | | | ***6.0*** | | ***0.008*** |

**Table 4.5. Summary of Observed Nitrification Rates-Stage II - SBR WI2- 15d SRT**

| Operating Day | MLSS (mg/L) | MLVSS (mg/L) | pH | Temp ($^oC$) | Nitrate Production Rate (mg/L-d) | Regression Correlation Coefficient $R^2$ | Specific Nitrification Rate (mgN/mgVSS-d) |
|---|---|---|---|---|---|---|---|
| 81 | | | 7.1-7.3 | 20.3 | 100 | 0.9955 | 0.154 |
| 82 | 815 | 650 | 7.1-7.3 | 20.0 | 129 | 0.9914 | 0.198 |
| 83 | | | 7.1-7.3 | 20.4 | 117 | 0.9968 | 0.180 |
| **Average** | | | | **20.2** | **115** | | **0.177** |
| **St. Deviation** | | | | | **14.4** | | **0.022** |
| | | | | | | | |
| 102 | | | 7.1-7.3 | 20.1 | 105 | 0.9969 | 0.135 |
| 103 | 975 | 780 | 7.1-7.3 | 20.0 | 83 | 0.9637 | 0.107 |
| 104 | | | 7.1-7.3 | 20.3 | 105 | 0.9988 | 0.135 |
| **Average** | | | | **20.1** | **98** | | **0.125** |
| **St. Deviation** | | | | | **12.5** | | **0.016** |
| | | | | | | | |
| 116 | | | 7.1-7.3 | 20.0 | 99 | 0.993 | 0.124 |
| 117 | 1000 | 800 | 7.1-7.3 | 20.0 | 104 | 0.9976 | 0.130 |
| 118 | | | 7.1-7.3 | 20.1 | 99 | 0.9945 | 0.124 |
| **Average** | | | | **20.0** | **101** | | **0.126** |
| **St. Deviation** | | | | | **2.4** | | **0.003** |
| | | | | | | | |
| 141 | | | 7.1-7.3 | 19.1 | 83 | 0.9903 | 0.095 |
| 142 | 1090 | 870 | 7.1-7.3 | 19.2 | 94 | 0.9926 | 0.108 |
| 143 | | | 7.1-7.3 | 18.9 | - | - | - |
| **Average** | | | | **19.1** | **88** | | **0.101** |
| **St. Deviation** | | | | | **7.9** | | **0.009** |

An initial assessment was conducted using the General Model's default decay rate of 0.04 $d^{-1}$. The calculated values are presented in Tables 4.6 and 4.7. A key observation is that the specific growth rates calculated for during the first stage (Plant W) were lower than the values calculated for the Stage II plant (Plant WI). The calculated values, presented in **Tables 4.6** and **4.7** were recalculated using the nitrifier decay rate measured for each wastewater source.

**Table 4.6. Calculated maximum specific nitrifier growth rates from fully aerobic SBRs operated at 20°C and SRT of 20 and 8 days ($b_A$=0.04 $d^{-1}$)**

| | SBR W1 - 20 day SRT | SBR W2 – 8 day SRT |
|---|---|---|
| Sampling Event | Model Default Decay | Model Default Decay |
| E1 | 0.32 | 0.38 |
| E2 | 0.33 | 0.42 |
| E3 | 0.34 | 0.40 |
| E4 | 0.27 | 0.32 |
| **Aver. & 95%CI** | **0.32±0.03** | **0.38±0.04** |

**Table 4.7. Calculated maximum specific nitrifier growth rates from fully aerobic and anoxic/oxic SBR operated at 20°C and SRT of 15 days ($b_A$=0.04 $d^{-1}$)**

| | SBR WI1-Aerobic | SBR WI2 – Anoxic/Aerobic |
|---|---|---|
| Sampling Event | Model Default Decay | Model Default Decay |
| WI1 | 0.48 | 0.56 |
| WI2 | 0.45 | 0.55 |
| WI3 | 0.60 | 0.62 |
| WI4 | 0.42 | 0.51 |
| **Aver. & 95% CI** | **0.49±0.08** | **0.56±0.04** |

## 4.3. Nitrifier Decay Rate Measurement

The nitrifier decay rate was measured for the SBR mixed liquor using the protocol outlined by Lesouef et al (1992). A portion of the SBR mixed liquor was removed from the SBR and placed in a separate reactor (Decay Reactor) within the environmental chamber (20°C±0.5°C). The nitrifying sludge was maintained without an external ammonia source for 2-5 days. During this period mixed liquor subsamples were removed from the Decay Reactor, spiked with ammonia to a concentration of approximately 10 mgN/L and the nitrification rate was measured. The protocol was modified in that multiple measurements of the decaying biomass's nitrification rate were obtained over the course of 2-5 days (Siegrist et al, 1999) and direct measurement of NOx-N and $NH_3$-N was used instead of oxygen uptake rates. The pH and dissolved oxygen was controlled at 7.3±0.1 and 5.0±0.3 mg/L, respectively, in both the Decay Reactor, as well as the nitrification rate measurement reactor using a setup similar to that used for the SBRs. A combination of $CO_2$ and $NaHCO_3$ was used to maintain a stable pH, as it was found during method development that the specific nitrifier growth rate would drop off after subjecting the bacteria to extended period of very low carbonate alkalinity.

Assuming exponential decay with negligible nitrifier growth in the decay reactor, the nitrification rate, $R_N$, as a function of time, will follow the equation:

$$R_N(T) = \frac{d(NOx-N)}{dt}(T) = \mu_A^{MAX} \frac{X_A(T)}{Y_A} = \mu_A^{MAX} \frac{X_{A0} \cdot e^{(-b_A T)}}{Y_A} = \mu_A^{MAX} \frac{X_{A0}}{Y_A} e^{(-b_A T)} \quad (4.1)$$

where $b_A$ is the nitrifier decay rate ($d^{-1}$), $X_A$ is the nitrifier concentration (mgVSS/L), and $Y_A$ is the nitrifier yield (mg VSS/mg N utilized). Linearizing the above equation:

$$\ln R_N(T) = \ln\left(\mu_A^{MAX} \frac{X_{A0}}{Y_A}\right) - b_A T \quad (4.2)$$

the nitrifier decay rate was thus obtained as the slope of the natural log of the nitrification rate versus time, **Figure 4.11**.

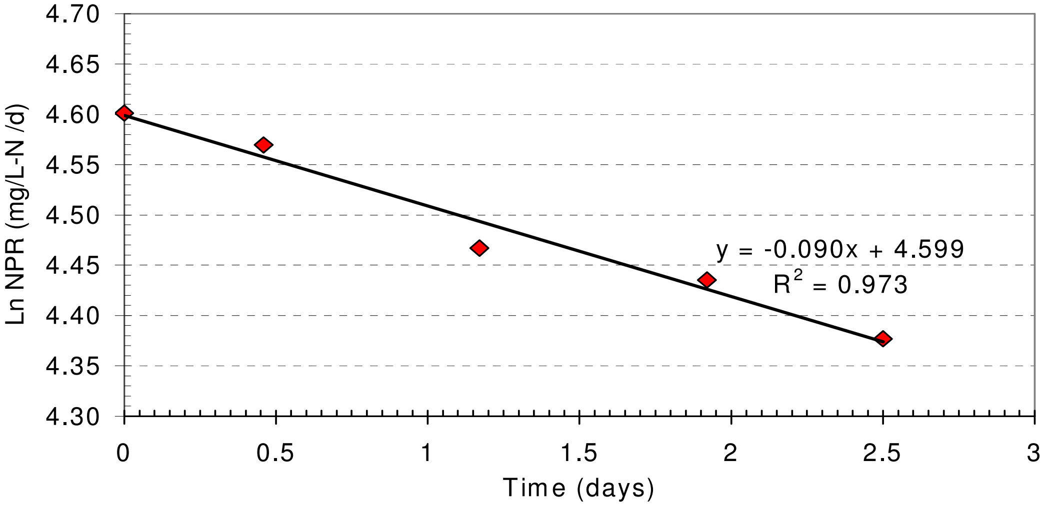

**Figure 4.11. Sample Nitrifier Decay Rate Measurement**

A certain amount of ammonia is liberated during the course of the decay experiment due to endogenous biomass activity and the hydrolysis of particulate TKN to soluble TKN and finally ammonia. This is reflected by the slow increase in $NO_X$-N concentrations in the Decay Reactor during the course of the experiment. Where necessary, the decay rate can be obtained by modeling the decay experiment using the General Model (Barker and Dold, 1997). This allows accounting for the change in nitrifying biomass due to the nitrification of the small quantities of ammonia liberated from the hydrolysis of the particulate matter. The sensitivity analysis showed that the re-growth of nitrifiers in the Decay Reactor nitrifying sludge needed to be accounted for in the calculation when (1) the influent wastewater had a high organic content and/or (2) the reactor that was the source of the nitrifying sludge was operated at a low SRT. Under the first case there would be a significant heterotrophic biomass component in the activated sludge, which would decay liberating ammonia, whereas in the second case because the initial concentration of nitrifiers in the decay reactor would be relatively low, the nitrifiers that would re-grow in the reactor would represent a significant percentage of the initial nitrifier concentration.

To quantify the variability in this complex experiment, and thus provide a measure of the confidence in the results a sensitivity analysis on the results was conducted, resulting in the development of 95% confidence intervals. Sensitivity coefficients were determined by taking the partial derivative of each of the varied parameters from the non-linear regression analysis of the equation describing the decay experiment (Dold, Personal Communication, 2000):

$$R_N(t) = \mu \cdot \left( \frac{X_{A0}}{Y_A} - \frac{f_N \cdot (1-f) \cdot b_H \cdot X_{H0}}{b_A - b_H} \right) \cdot e^{-b_A t} + \mu \cdot \left( \frac{f_N \cdot (1-f) \cdot b_H \cdot X_{H0}}{b_A - b_H} \right) \cdot e^{-b_H t} \quad (4.3)$$

The sensitivity coefficients for the two varied parameters, the initial nitrifier concentration, $Z_{AO}$, and the nitrifier decay rate, $b_A$ were:

$$X_{Z_{AO}} = \frac{\partial R_N(t)}{\partial Z_{AO}} = \frac{\mu \cdot e^{-b_A \cdot t}}{Y_A} \quad (4.4)$$

$$X_{b_A} = \frac{\partial R_N(t)}{\partial b_A} = \frac{-t \cdot \mu \cdot X_{AO} \cdot e^{-b_A \cdot t}}{Y_A} + \frac{e^{-b_A \cdot t} \{[(b_A - b_H) \cdot \mu \cdot f_N \cdot (1-f) \cdot b_H \cdot t] + 1\} - \mu \cdot f_N \cdot (1-f) \cdot e^{-b_H \cdot t}}{(b_A - b_H)^2} \quad (4.5)$$

The covariance matrix was developed using the above sensitivity coefficients and the 95% confidence intervals were estimated.

**Table 4.8** summarizes the results of this component of the work. The results of the decay experiment analyses, using both the linearization and the modeling/non-linear regression technique are presented, along with the associated confidence intervals are presented in Table 4.6.

**Table 4.8.** Nitrifier Decay Rate Measurement Summary

| Reactor | Linearization Results* | Non-Linear Regression Results | (95%) Confidence Interval | Comments |
|---|---|---|---|---|
| W1 | 0.22 | 0.27 | ±0.07 | pH Controller Failure on Day 1 and Day 2 |
| W1 | 0.08 | 0.10 | ±0.02 | |
| W2 | 0.08 | 0.11 | ±0.01 | |
| W2 | 0.09 | 0.11 | ±0.03 | |
| WI1 | 0.06 | 0.08 | ±0.02 | Decay Rxr Accidentally fed ~4 mg/L $NH_4$-N |
| WI1 | 0.09 | 0.10 | ±0.02 | |
| WI2 | 0.07 | 0.09 | ±0.01 | |
| WI2 | 0.08 | 0.09 | ±0.01 | |

*Units in $d^{-1}$.

The observed values were significantly higher than proposed in some of the literature (Dold, 1995; EPA 1993). The above values were utilized in the calculation of the specific nitrifier growth rate and the results compared with calculated growth rates assuming literature values for the nitrifer decay rate.

## 4.4. Exponential Growth Batch Test Data

Exponential growth batch tests were conducted using biomass from the fully aerobic SBRs and wastewater from the treatment plants. The low mass batch test, or as referred by some as the high F/M test, was conducted using the procedure guidelines outlined in Antoniou (1990) and Sozen (1996). A sample experiment is shown in **Figure 4.12**. A sensitivity analysis was conducted to assess effect of the sampling interval on the measurement. The analysis showed that frequent sampling (every 3 hours) with an experiment that lasted 3-4 days would provide the optimal accuracy in results. Sampling more frequently did not materially enhance the accuracy of the results, but would have required significantly more effort. Extending the duration of the run beyond 3-4 days would result in the accumulation of significant levels of nitrite and nitrate leading to (1) the potential for increased analytical errors due to the need to conduct serial dilutions and (2) the potential for nitric or nitrous acid inhibition of the nitrifiers significantly affecting the experimental results.

Using the General Model default value for nitrifier yield, $Y_A$, and the experimentally determined decay rate for the seed source SBR, the initial nitrifier concentration in the batch reactor, and thus the concentration in the seed source was estimated. However, using this technique for estimation of the initial nitrifier concentration proved difficult, as the NOx-N data must be of excellent quality with minimal noise. Scatter in the data, particularly in the first 24 hours of the batch experiment significantly impacted the ability to confidently calculate $X_A^{INI}$.

Confidence intervals at the 95% level, are shown in **Tables 4.9** and **4.10** for the exponential growth tests. The confidence intervals were calculated in the same manner that was utilized in the nitrifier decay measurements.

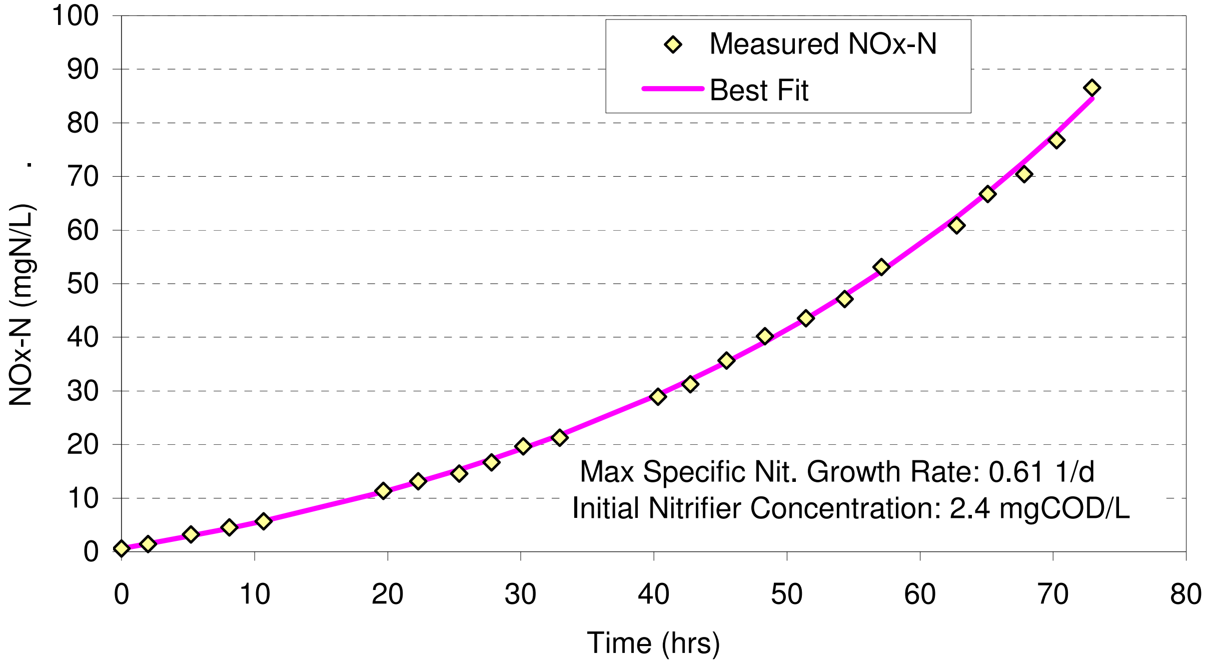

**Figure 4.12.** Exponential Batch Growth Experiment – SBR W1

**Table 4.9.** Maximum Specific Nitrifier Growth Rate Determined from Exponential Growth Batch Test Results – SBRs W1 and W2

| Test No. | Seed Source | Maximum Nitrifier Growth Rate ($d^{-1}$) |
|---|---|---|
| A1 | W1 | 0.61±0.03 |
| A2 | W1 | 0.66±0.04 |
| A3 | W1 | 0.72±0.03 |
| A4 | W1 | 0.64±0.05 |
| A5 | W2 | 0.80±0.21 |
| A6 | W2 | 0.81±0.16 |
| A7 | W2 | 0.63±0.04 |
| A8 | W2 | 0.57±0.06 |

**Table 4.10.** Maximum Specific Nitrifier Growth Rate Determined from Exponential Growth Batch Test Results – SBRs WI1 and WI2

| Test No. | Seed Source | Maximum Nitrifier Growth Rate ($d^{-1}$) |
|---|---|---|
| B1 | WI1 | 0.81±0.05 |
| B2 | WI1 | 0.83±0.02 |
| B3 | WI1 | 0.45±0.11* |
| B4 | WI1 | 0.75±0.06 |
| B5 | WI2 | 0.82±0.04 |
| B6 | WI2 | 0.81±0.09 |
| B7 | WI2 | - |
| B8 | WI2 | 0.91±0.12 |

*$CO_2$ Addition Failure – Data Not Used

# 5. DISCUSSION

The experimental results presented in the previous section were utilized to:

- assess the applicability of a steady state approximation for the calculation of the specific nitrifier growth rate using a sequencing batch reactor
- compare the specific nitrifier growth rate calculated using the experimentally determined nitrifier decay rate versus the General Model's default decay rate of 0.04 $d^{-1}$
- assess the impacts of using the experimentally derived pair of specific nitrifier growth and decay rates on treatment plant plant design
- assess the potential of using the exponential growth batch experiment for determination of the specific nitrifier growth rate

## 5.1. Use of a Steady State Approximation in Growth Rate Determination

The maximum specific nitrifier growth rate was calculated using (1) dynamic simulation of the SBR and (2) the steady state WRC equation set. The General Model default nitrifier decay rate was used in the calculation of the maximum nitrifier growth rates for SBR W1. The results of this analysis, summarized in **Figure 5.1**, show that utilizing the dynamic process simulator, which permits accounting of daily variation in influent loadings enhances both the accuracy and the precision of the overall measurement. Whereas using the simulator netted a maximum specific growth rate of 0.42 $d^{-1}$ ±0.04 (95% confidence interval), the steady state approximation resulted in a maximum specific nitrifier growth rate of 0.43 $d^{-1}$ ±0.11. One cause of the offsets observed when the steady state approximation was used was the inability of the steady state approximation to account for changes in the nitrifier concentrations during periods immediately preceding the sampling events. Thus by averaging operating conditions over a period of time (in this particular instance the period of one SRT, 20 days), the "memory" of the sludge characteristics was lost and as a result the resolution of the measurement was affected.

Based on these results the use of dynamic simulation to fully account for the operation of the reactors and variability of the influent wastewater is deemed appropriate.

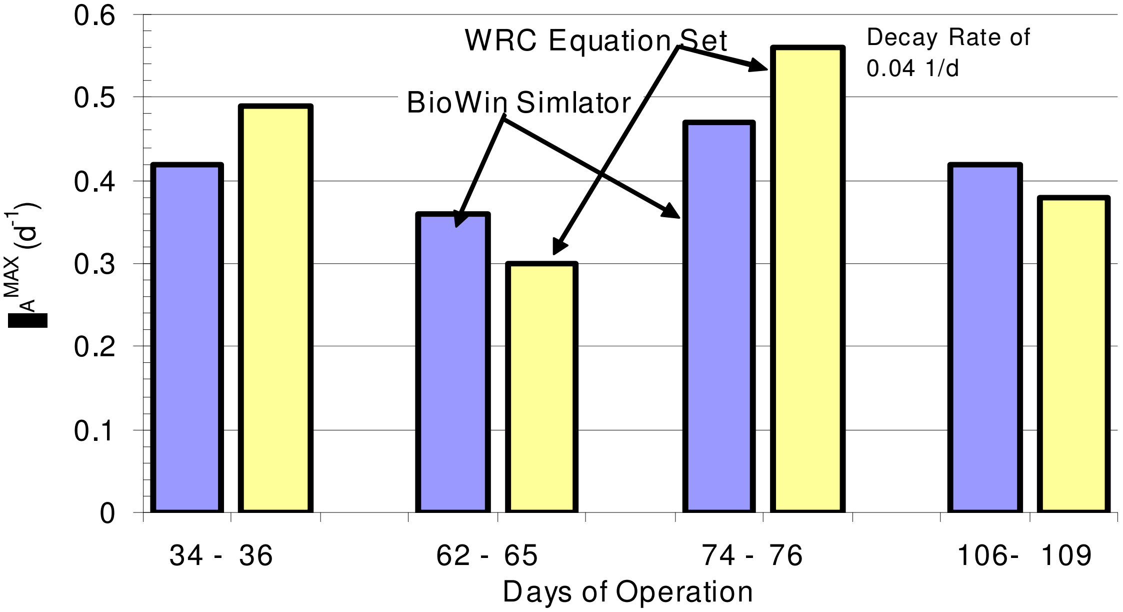

**Figure 5.1. Comparison of $\mu_A^{MAX}$ Values Obtained Using BioWin vs. WRC Equation Set**

## 5.2. Determination of Maximum Nitrifier Growth Rates from SBR Data

The specific growth rates calculated from the four SBRs operated in this study are summarized in **Table 5.1** for the Stage I SBRs (SBRs W1 and W2) and in **Table 5.2.** for the Stage II SBRs (WI1 and WI2).

Inspection of Tables 5.1 and 5.2 indicates that the specific nitrifier growth rates measured in the reactors receiving wastewater from the first plant (SBRs W1 and W2) were significantly lower than the values observed in reactors SBR WI1 and WI2, which were fed wastewater from a different wastewater treatment plant. This is expected based on the lower C/N ratio of the WI influent.

However there appear to be differences in the specific growth rates measured in individual reactors receiving wastewater from the same plant as well. SBR W1 (**Table 5.1**) consistently exhibited a lower specific growth rate (at $\alpha$=.05, t=2.45, $t_{STAT}$=1.94) than SBR W2. The primary difference in the reactor's operation was that the sludge age of biomass was held at 20 days in W1, whereas it was maintained at 8 days in W2.

**Table 5.1.** Maximum specific nitrifier growth rates from Stage I SBRs: fully aerobic SBRs at 20°C and SRT of 8 and 20 days

| | Maximum Specific Nitrifier Growth Rate @ 20°C ($d^{-1}$) | |
|---|---|---|
| | SBR W1 - 20 day SRT | SBR W2 – 8 day SRT |
| Samp. Event | Measured Decay ($b_A$=0.10 $d^{-1}$) | Measured Decay ($b_A$=0.11 $d^{-1}$) |
| E1 | 0.45 | 0.56 |
| E2 | 0.46 | 0.61 |
| E3 | 0.48 | 0.56 |
| E4 | 0.40 | 0.45 |
| **Aver. & 95%CI** | **0.45±0.03** | **0.55±0.06** |

**Table 5.2.** Maximum specific nitrifier growth rates from Stage II SBRs fully aerobic and anoxic/oxic SBR at 20°C and SRT of 15 days

| | Maximum Specific Nitrifier Growth Rate @ 20°C ($d^{-1}$) | |
|---|---|---|
| | SBR WI1-Aerobic | SBR WI2 – Anoxic/Aerobic |
| Samp. Event | Measured Decay ($b_A$=0.09 $d^{-1}$) | Measured Decay ($b_A$=0.09 $d^{-1}$) |
| F1 | 0.75 | 0.85 |
| F2 | 0.66 | 0.79 |
| F3 | 0.90 | 0.93 |
| F4 | 0.62 | 0.73 |
| **Aver. & 95% CI** | **0.73±0.12** | **0.83±0.08** |

Whereas the specific growth rates observed in reactors WI2 appears to be 14% higher than that observed in reactor WI1,the difference is not statistically significant (at $\alpha$=.05, t=1.23, $t_{STAT}$=1.94). Eliminating the F3 sampling event from both reactors results in a statistically significant difference in growth rates(at $\alpha$=.05, t=2.13, $t_{STAT}$=2.20) between reactors WI1 and WI2, although it would also result in a reduction in the average reported specific growth rates to 0.68 and 0.79 $d^{-1}$ for SBR WI1 and WI2, respectively.

The difference in the calculated specific growth rates in reactors WI1 and WI2 would indicate that there is an underlying difference in the nitrifying biology in the two reactors. However, the difference in specific growth rates may be attributed to a reduced decay rate under the anoxic portion of the reactor's operating cycle. Preliminary data from other researchers would indicate that biomass decay rates are reduced under anaerobic and anoxic conditions (Siegrist, 1999). This would result in a larger nitrifier population in SBR WI2 than what was accounted for in the calculations used to generate the rates shown in **Table 5.2** Definitive data does not exist at this time on the effects of anoxic operation (i.e. effects of duration of anoxic period, SRT of reactor, etc.) to be able to accurately calculate the effects of the anoxic operating period on the nitrifying biomass. Furthermore, the significant reduction in biomass decay rates, particularly with respect to the heterotrophic biomass, observed by Siegrist (1999) are contrary to field observations (i.e. significant ammonia releases observed in secondary anoxic zones in four and five stage Bardenpho systems). Additional studies, where the nitrifier decay rate is measured in reactors with varying anoxic detention periods would need to be considered prior to providing a conclusive assessment on the effects of anoxic conditions on biomass decay rates, and nitrifier decay rates specifically.

Inspection of the individual specific growth rate measurements indicates that the measurements trended together. For example, the measurements from sampling events E1, E2 and E3 where higher in both reactors than the measurement from sampling event E4. A similar trend is observed in the data from reactors WI1 and WI2 in **Table 5.2**.

During this study, SBRs W1 and W2 were fed wastewater from one NYC WWTP, whereas SBRs WI1 and WI2 were fed wastewater from a second plant. The specific nitrifier growth rates measured using the Sequencing Batch Reactor protocol materially differed between the two datasets. Using the experimentally determined nitrifier decay rates the average specific nitrifier growth rates in SBRs W1 and W2 ranged between 0.45 and 0.55 $d^{-1}$, whereas the average specific growth rates in SBRs WI1 and WI2 ranged between 0.73 and 0.83 $d^{-1}$. Based on the results obtained from this study, the use of different nitrifier growth rates in the two plants in question would be indicated.

The range of nitrifier decay rates observed during the course of this study were higher than the values utilized in the literature. To gauge the effect of the elevated nitrifier decay rate on the results obtained from this study, the specific nitrifier growth rate was calculated using two decay rates:

- The default value of 0.04 $d^{-1}$ utilized in the General Model (Barker and Dold, 1997). Note that a value of 0.05 $d^{-1}$ is routinely used in nitrification design calculations (Metcalf & Eddy, 1993)
- The measured decay rate for each SBR, which ranged from 0.09 for the SBRs WI1 and WI2, to 0.11 for SBR W2.

**Tables 5.3** and **5.4** show the comparison of the calculated values of the specific nitrifier growth rate based on the model default and measured nitrifier decay rates. With the measured decay rates at levels that are two to three times higher than the generally accepted values, the ensuing specific nitrifier growth rates were 40 to 50% greater than what would have been calculated if the decay rates had not been measured during the course of this study.

**Table 5.3.** Comparison of calculated maximum specific nitrifier growth rates from fully aerobic SBRs operated at 20°C and SRT of 8 and 20 days

| | Maximum Specific Nitrifier Growth Rate @ 20°C ($d^{-1}$) | | | |
|---|---|---|---|---|
| | SBR W1 - 20 day SRT | | SBR W2 – 8 day SRT | |
| Samp. Event | Model Default Decay ($b_A$=0.04 $d^{-1}$) | Measured Decay ($b_A$=0.10 $d^{-1}$) | Model Default Decay ($b_A$=0.04 $d^{-1}$) | Measured Decay ($b_A$=0.11 $d^{-1}$) |
| E1 | 0.32 | 0.45 | 0.38 | 0.56 |
| E2 | 0.33 | 0.46 | 0.42 | 0.61 |
| E3 | 0.34 | 0.48 | 0.40 | 0.56 |
| E4 | 0.27 | 0.40 | 0.32 | 0.45 |
| **Aver. & 95%CI** | **0.32±0.03** | **0.45±0.03** | **0.38±0.04** | **0.55±0.06** |

**Table 5.4.** Comparison of calculated maximum specific nitrifier growth rates from fully aerobic versus anoxic/oxic SBR operated at 20°C and SRT of 15 days

| | Maximum Specific Nitrifier Growth Rate @ 20°C ($d^{-1}$) | | | |
|---|---|---|---|---|
| | SBR WI1-Aerobic | | SBR WI2 – Anoxic/Aerobic | |
| Samp. Event | Model Default Decay ($b_A$=0.04 $d^{-1}$) | Measured Decay ($b_A$=0.09 $d^{-1}$) | Model Default Decay ($b_A$=0.04 $d^{-1}$) | Measured Decay ($b_A$=0.09 $d^{-1}$) |
| WI1 | 0.48 | 0.75 | 0.56 | 0.85 |
| WI2 | 0.45 | 0.66 | 0.55 | 0.79 |
| WI3 | 0.60 | 0.90 | 0.62 | 0.93 |
| WI4 | 0.42 | 0.62 | 0.51 | 0.73 |
| **Aver. & 95% CI** | **0.49±0.08** | **0.73±0.12** | **0.56±0.04** | **0.83±0.08** |

The direct effect of the decay rate measurement was the increase in the specific nitrifier growth rate determined for the two plants. This in turn directly impacts the sizing and operation of the facility. To illustrate these effects, **Figure 5.2** compares the reactor volumes required to achieve the typical effluent ammonia criterion for fully nitrifying plants of 1 mgN/L. In this example a 100 MGD flow is used, with typical North American wastewater characteristics (BOD=150 mg/L, TSS=150 mg/L, TKN=40 mg/L). The mixed liquor concentration was maintained constant at 2,500 mg/L, rendering the reactor volume a surrogate for the age of the sludge (SRT). **Figure 5.2** shows that the nitrification goal can be met with an activated sludge reactor volume of 24 million gallons, whereas if the kinetics developed without measuring the decay rate were used, the reactor volume would have been increased to 33 million gallons. At typical costs for activated sludge reactors, including appurtenances, of \$3 per gallon installed, this translates to a capital cost savings of \$27,000,000 in this illustration.

However when optimized designs are implemented, savings can continue with the operation of the facility. In **Figure 5.3**, the oxygen requirements and digester gas production are plotted as a function of the reactor volume used in the design of the facility. By allowing the facility to operate at a lower sludge age, aeration requirements, expressed as pounds of oxygen used per day, are reduced by 20%. Digester gas production would be increased by 10%, as volatiles that would have been oxidized in the aeration tank would now be anaerobically processed, contributing to gas production.

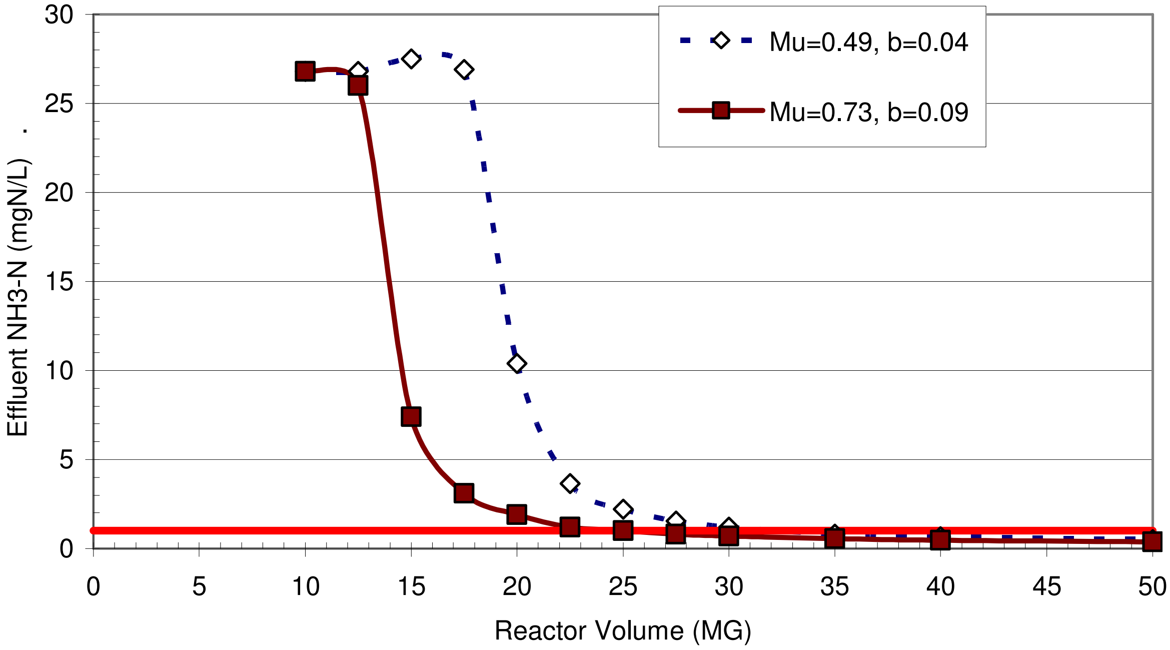

**Figure 5.2.** Reactor Volume Required as a Function of Nitrification Kinetics Applied

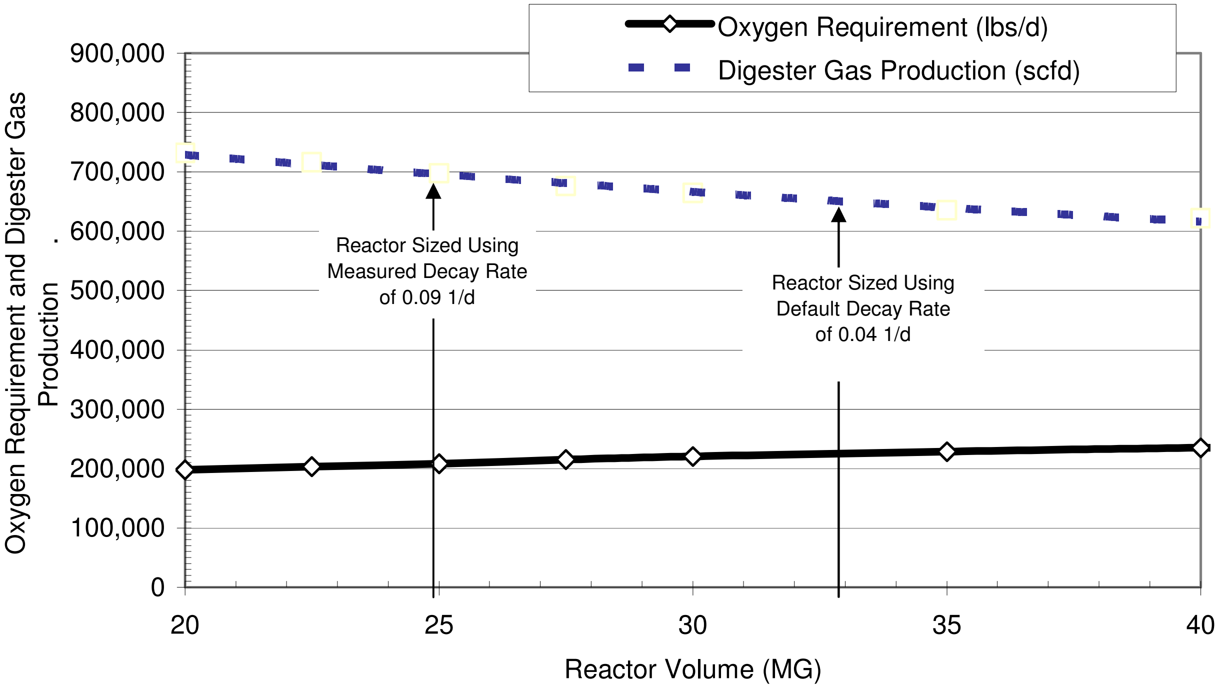

**Figure 5.3.** Reduction in Oxygen Requirements and Increased Digester Gas Production Possible by Using Kinetics Based on Measured Decay Rate

Some of the cost savings indicated above would be tempered by the need to provide for greater solids processing capacity, as sludge production would be expected to increase on the order of 10%, since the operating sludge age of the facility would be reduced.

From the preceding discussion it is apparent that all facets of the design of the wastewater treatment plant, including the solids processing facilities and the energy recovery systems would be impacted by the selection of the values of $\mu_{A,MAX}$ and the decay rate, $b_A$.

The higher decay rate measured also impacts the application of other nitrification process technologies. Consideration to the effect of a higher decay rate will need to be given when designing nitrifier bio-augmentation processes (commonly referred to as nitrifier seeding processes), and when developing procedures for recovery of nitrification from activated sludge facilities that have been compromised. In both examples, key to the success of the process (whether it is seeding nitrifiers or building up a robust population) is developing a significant mass of nitrifying bacteria – and then maintaining them in the system. The higher decay rates observed in this study would indicate that seeding of nitrifiers may not be as beneficial as initially assumed (Lee, 1997; Hultman, 1998). Direct measurement of nitrifier decay rates in these systems would help elucidate the true benefits of bio-augmentation, and optimize strategies for re-seeding wastewater treatment plants with nitrifiers.

Another area that would be impacted is the design and operation of facilities achieving Limit of Technology (LOT) nitrogen discharges (i.e. effluent total nitrogen levels of 2.5-3 mgN/L). One of the problems faced in LOT systems designed on published recommendations is that larger aerobic tankage volumes are typically selected, beyond what is required to reliably nitrify the influent load. This results in reduced overall performance for the facility and drives up operating costs. Specifically, the larger aerobic volumes result in increased decay of biomass, which in turn liberates ammonia. The ammonia is nitrified, effectively altering the BOD to nitrogen ratio of the influent. The net result is that the load of nitrate that needs to be denitrified becomes larger, as the aerobic volume increases. By measuring the actual decay rate in the system, the tankage volumes can be optimized, minimizing denitrification requirements.

As the database of measured decay rates increases over time, augmented by measurements from treatment plants with different operating modes and applied loadings, it may be possible to redefine a new “default” value for use in the kinetic equations describing nitrification. Although it is a labor intensive experiment to conduct, the majority of the decay rates measured in this study ranged from 0.09 to 0.11 $d^{-1}$. This is a relatively narrow band given that the confidence intervals where on the range of ± 0.01 to 0.03 $d^{-1}$ for most of the decay measurements. However, even higher decay rates have been reported in the literature (Leenan, 1997, Siegrist, 1999), indicating the possibility that the decay rate is a function of the biomass developed in the reactor, or the operating conditions under which it is grown. These questions can only be addressed as the database of nitrifier decay rate measurements is expanded over time.

## 5.3. Exponential Growth Batch Experiment

The second type of experiment evaluated during the course of this work was the exponential growth batch test for measurement of the specific nitrifier growth rate. The potential of using the exponential batch growth experiment for measurement of the specific nitrifier growth rate, instead of operation of the sequencing batch reactor (effectively an activated sludge pilot plant) would reduce the cost of the measurement. It would thus provide an attractive option to permit development of a large database of specific growth rates.

In **Figure 5.4**, the specific nitrifier growth rates obtained from the exponential growth batch experiments that were conducted in parallel to the SBR sampling events are shown, along with the $\mu_A^{MAX}$ values calculated for the SBRs, using the measured nitrifier decay rates. For the exponential batch tests, the reported maximum specific growth rates were calculated by adding the measured decay rate to the net specific growth rate obtained from the batch test.

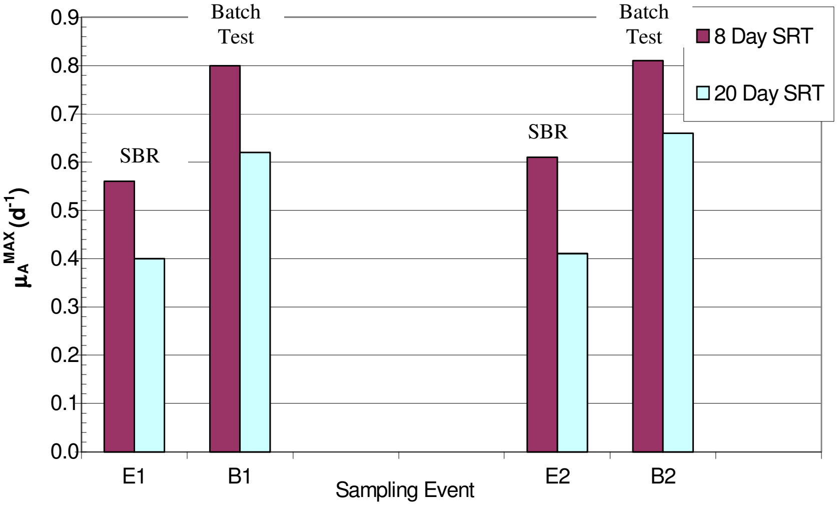

**Figure 5.4**. Comparison of $\mu_A^{MAX}$ values measured during SBR Sampling Events E1 & E2 and the exponential growth batch experiments (B1 & B2) using the respective SBR's sludge for seed

In general, the specific growth rates determined from the exponential growth experiments were typically higher than the values obtained from the long term reactors. The cause of this effect is not known, however it is possible that the exponential growth test provides a ideal condition for growth of the nitrifying organisms (elevated carbonate levels from the combination of $CO_2$ and NaOH addition, no competition for micronutrients and DO due to the very high F/M and very low concentrations of heterotrophs) that can not be duplicated in an activated sludge system operating at mixed liquor levels of greater than 1000 mg/L. The optimal conditions provided in the test may also result in lower nitrifier decay rates in the exponential batch test reactor. Unfortunately there is no direct method of measuring the decay rate in the exponential growth batch reactor due to the very low nitrifier concentrations present.

# 6. CONCLUSIONS AND RECOMMENDATIONS

The nitrifier decay was found to be significant in the activated sludge systems evaluated in this study. The nitrifier decay rates measured in this study ranged from 0.09 to 0.11 $d^{-1}$, and were significantly higher than the values typically used in design practice (0.04 $d^{-1}$), and quoted in the bulk of the literature. As a result, the validity of a significant subset of the literature, which uses the assumption that nitrifier decay rate was small or negligible must be questioned. The process optimization that can be brought forth by having an accurate measurement of the nitrifier decay rate necessitates that this parameter be measured as part of any effort that targets characterization of the nitrification kinetics in a facility. Furthermore, it is necessary to report the specific nitrifier growth rate in conjunction with the nitrifier decay rate used in the calculation.

When actual wastewater is used, as would be the case for any actual application of a nitrifier growth rate measurement for a wastewater treatment plant, it is necessary to account for the variability in loadings applied to the reactors, and the inherent variability in the biological reactor’s performance. This necessitates the use of a dynamic process model, capable of tracking the variability in the sludge composition. Using a steady state approximation risks losing the “memory” of the sludge composition, and thus losing resolution in the calculation of the growth rate that is dependent upon it.

The nitrifier growth rate for the two facilities where the protocols were executed appears to differ significantly. Both test methods used (the long term sequencing batch reactor operation and the exponential growth batch test) trended in the same manner. It would thus be expected that different nitrifier specific growth rates would need to be used in the design of each of these facilities. Thus based on this study, the nitrifier growth rate remains a wastewater specific parameter that should be measured on a case by case basis.

Although the exponential growth batch test would provide a less costly alternative to the long term operation of the sequencing batch reactors and the associated kinetic testing, the results of this study indicated that the growth rate measured in the exponential growth batch were generally higher than the values obtained from the sequencing batch reactor experiments. Furthermore, it is not possible to measure the nitrifier decay rate in the exponential growth batch reactor. Given these results, usage of the exponential growth batch test is not a replacement of the sequencing batch reactor protocol. However, because of it's lower cost it is more attractive to municipalities and may be used as a comparative screening tool.

# 7. REFERENCES

Alleman, J.E. (1985). Elevated nitrite occurrence in biological wastewater treatment. Wat. Sci. Tech., 17(1), 409-412.

Allison, S. M., and J. I. Prosser. (1993) Ammonia oxidation at low pH by attached populations of nitrifying bacteria. Soil Biol. Biochem. 25:935–941.

Andreadakis, A., Hatziconstantinou, G.J. (2002) Differences in nitrification potential between fully aerobic and nitrogen removal activated sludge systems. Wat. Sci. Tech., 46(1-2), 297-304.

Anthonisen, A.C. (1976) Inhibition of nitrification by ammonia and nitrous acid. J Wat. Poll. Con. Fed., 48, 835.

Antoniou P., Hamilton J., Koopman B., Jain R., Holloway B., Lyberatos G., and Svoronos S.A. (1990) Effect of temperature and pH on the effective maximum specific growth rate of nitrifying bacteria. Wat. Res., 24(1), 97-101.

Ballinger S.J., Head I.M., Curtis T.P., Godley A.R. (2002) The effect of C/N ratio on ammonia oxidizing bacteria community structure in a laboratory nitrification-denitrification reactor. Wat. Sci. Tech., 46(1-2), 543-550.

Barker P.S. and Dold, P.L. (1997a) General model for biological nutrient removal activated-sludge systems: model presentation. Water Environ. Res. 69(5), 969-984.

Barker P.S. and Dold, P.L. (1997b) General model for biological nutrient removal activated-sludge systems: model application. Water Environ. Res. 69(5), 985-991.

Batchelor, S. E., M. Cooper, S. R. Chabra, L. A. Glover, G. S. A. B. Stewart, P. Williams, and J. I. Prosser. (1997) Cell density-regulated recovery of starved biofilm populations of ammonia-oxidizing bacteria. Appl. Environ. Microbiol. 63:2281–2286.

Bernardes, R. S., and Klapwijk, A. (1996). ‘‘Biological nutrient removal in a sequencing batch reactor treating domestic wastewater.’’ *Water Sci. Techno*l., 33(3), 29–38.

Blum, D.J.W. and Speece, R.E. (1991) A database of chemical toxicity to environmental bacteria and its use in interspiecies comparison and correlations. Water Environ. Res. 63(1), 198-202.

Buswell A.M., Shiota T.,Lawrence N. and Van Meter I.(1954) Laboratory studies on the kinetics of growth of Nitrosomonas with relation to the nitrification phase of the BOD test. App. Microbiol. 2, 21-25.

Chudoba J., ech J.S., and Chudoba P.(1985) The effect of aeration tank configuration on nitrification kinetics. J. Water Pollut. Control Fed., 57(11), 1078-1083.

Dokianakis S.N., Kornaros M.E., Lyberatos G. (2004) On the effect of pharmaceuticals on bacterial nitrite oxidation. Wat. Sci Tech., 50(5), 341-346.

Eum, Y., Choi, E. (2002) Optimization of nitrogen removal from piggery waste by nitrite nitrification. Wat. Sci. Tech., 45(12), 89-96.

Fillos, J., Ramalingam K., Katehis, D., Carrio, L. and Fragias J. (1999) Full scale evaluation of step feed biological nitrogen removal at the Tallman Island WPCP. Proceedings 6th International Conference on Environmental Science and Technology, Greece. Vol. A, p.498-507.

Fillos, J., Katehis, D., Ramalingam K., Carrio L. (2000) Measurement of nitrifier growth rate in New York City wastewater treatment plants. Proceedings WEFTEC 2000, Anaheim,CA. Water Environment Federation, Virginia, USA.

Hall E.R., and Murphy K.L. (1980) Estimation of nitrifying biomass and kinetics in wastewater. Wat. Res., 14, 297-304.

Hall E.R., and Murphy K.L. (1985) Sludge age and substrate effects on nitrification kinetics. J. Water Pollut. Control Fed., 57(5), 413-418.

Harremoës P., Haarbo A., Winther-Nielsen M. and Thirsing C. (1998) Six years of pilot plant studies for design of treatment plants for nutrient removal. Wat. Sci. Tech., 38(1), 219-226.

Head, I. M., W. D. Hiorns, T. M. Embley, A. J. McCarthy, and J. R.Saunders. (1993) The phylogeny of autotrophic ammonia-oxidising bacteriaas determined by analysis of 16S ribosomal gene sequences. J. Gen. Micro-biol. 139:1147–1153

Hiorns, W. D., R. C. Hastings, I. M. Head, A. J. McCarthy, J. R. Saunders, R. W. Pickup, and G. H. Hall. 1995. Amplification of 16S ribosomal RNA genes of autotrophic ammonia-oxidising bacteria. Microbiology 141:2793–2800.

Hockenbury M.R., Daigger G.T., and Grady C.P.L. Jr. (1977) Factors affecting nitrification. Jour. Env. Engng. Div. Amer. Soc. Civil Engrs. 103, 9-19.

Holben, W.E., Noto, K., Sumino, T., Suwa, Y. (1998) Molecular analysis of bacterial communities in a three-compartment granular activated sludge system inicates community-level control by incompatible nitrification processes. Appl. Environ. Microbiol. 64(7), 2528-2532.

Hultman, B. (1998). Seeding of nitrifying bacteria to the activated sludge process - role of separate nitrification of supernatant from dewatering of digested sludge. *Poster* *presented at the 19th Biennal Conference of the International Association on Water Quality (IAWQ), Vancouver, Canada.*

Hyman, M.R., Murton, IB, Arp, D.J. (1988) Interaction of ammonia monooxygenase from Nitrosomonas europaea with alkanes, alkenes and alkynes. Appl. Environ. Microbiol. 24(10), 3187-3193.

Hyman, M. R., and D. J. Arp. (1995) Effects of ammonia on the de novo synthesis of polypeptides in cells of *Nitrosomonas europaea* denied ammonia as an energy source. J. Bacteriol. 177:4974–4979.

Johnstone, B. H., and R. D. Jones. (1988) Physiological effects of long-term energy-source deprivation on the survival of a marine chemolithotrophic ammonium-oxidizing bacterium. Mar. Ecol. Prog. Ser. 49:295–303.

Johnstone, B. H., and R. D. Jones. (1988) Recovery of a marine chemolitho-trophic ammonium-oxidising bacterium from long term energy source de-privation. Can. J. Microbiol. 34:1347–1350.

Katsogiannis AN, Kornaros M, Lyberatos G. (2003) Enhanced nitrogen removal in SBRs bypassing nitrate generation accomplished by multiple aerobic/anoxic phase pairs. Wat. Sci Tech. 47(11):53-59

Kim, D.J., Chang J.S., Lee, D.I., Han D.W. (2003) Nitrification of high strength ammonia wastewater and nitrite accumulation characteristics. Wat. Sci. Tech., 47(11), 45-51.

Knowles G., Downing A.L., and Barrett M.J. (1965) Determination of kinetic constants for nitrifying bacteria in mixed culture, with the aid of an electronic computer. J. Gen. Microbiol. 38, 263-278.

Kolter, R., D. A. Siegele, and A. Tormo. (1993) The stationary phase of the bacterial life cycle. Annu. Rev. Microbiol. 47:855–874.

Lee, C.-Y. (1997). Discussion of: How input active biomass affects sludge age and process stability. J. Env.Eng., ASCE, 123 (1), 101-103.

Lesouef A., Payraudeau M., Rogalla F. and Kleiber B. (1992) Optimizing nitrogen removal reactor configurations by on-site calibration of the IAWPRC activated sludge model. Wat. Sci. Tech., 25(6), 105-123.

Leenen E.J.T.M., Boogert A.A., van Lammeren A.A.M., Tramper J., and Wijfells R.H. (1997) Dynamics of artificially immobilized Nitrosomonas europaea: Effect of biomass decay. Biotechnol. Bioeng., 55(4), 630-641.

Monib M.,Hosny I., El-Hadidy T.T., El-Shahawy R. (1979) Temperature adaptability of nitrifying bacteria in soils in Egypt. Zentralbl Bakteriol, 134(6), 528-535.

Mobarry, B. K., M. Wagner, V. Urbain, B. E. Rittman, and D. A. Stahl. (1996) Phylogenetic probes for analyzing abundance and spatial organization of nitrifying bacteria. Appl. Environ. Microbiol. 62:2156–2162.

Nowak O., Schweighofer P. and Svardal K. (1994) Nitrification inhibition - A method for the estimation of actual maximum autotrophic growth rates in activated sludge systems. Wat. Sci. Tech., 30(2), 9-19.

Painter, H.A. (1970) A review of literature on inorganic nitrogen metabolism in microorganisms. Wat. Res. 4(6):393.

Painter, H.A. and Loveless, J.E. (1983). Effect of temperature and pH value on the growth rate constants of nitrifying bacteria in the activated sludge process. Wat. Res. 17(3), 237-248.

Park, H.D., Regan J.M., Noguera, D.R (2002) Molecular analysis of ammonia oxidizing bacterial populations in aerated-anoxic Orbal processes. Wat. Sci. Tech., 46(1-2), 273-280.

Pedersen J. and Sinkjaer O. (1992) Test of the activated sludge model's capabilities as a prognostic tool on a pilot scale wastewater treatment plant. Wat. Sci. Tech., 25(6), 185-194.

Pratt, S., Gapes, D., Yuan, Z., Keller, J. (2004) Rate of nitrate production during as two stage nitrification batch reaction. Wat. Sci. Tech., 50(10), 81-87.

Princic A.., Mahne I., Megusar F., Paul E.A. and Tiedge J.M. (1998) Effects of pH, oxygen and ammonium concentrations on the community structure of nitrifying bacteria from wastewater. Appl. Environ. Microbiol. 64(10), 3584-3590.

Prosser, J. I. (1989) Autotrophic nitrification in bacteria. Adv. Microb. Physiol. 30:125–177.

Purkhold U., Pommerening-Roser A., Juretschko S., Schmid M.C., Koops H., Wagner M. (2000) Phylogeny of all recognized species of ammonia oxidizers based on comparative 16S rRNA and amoA sequence analysis: Implications for molecular diversity surveys. Appl. Environ. Microbiol. 66(12), 5368-5382.

Randall C.W., Pattarkine V.M. and McClintock S.A. (1992) Nitrification kinetics in single sludge biolgical nutrient removal activated sludge systems. Wat. Sci. Tech., 25(6), 195-214.

Rastak C.H., Maarsen K.A., and Kooijman S.A.L.M. (1996) Effects of protozoa on carbon mineralization in activated sludge. Wat. Res. 30(1), 1-12.

Sakano, Y., Kerkhof, L. (1998) Assessment of changes in microbial community structure during operation of an ammonia biofilter with molecular tools. Appl. Environ. Microbiol. 64(12), 4877-4882.

Salmond, G. P. C., B. W. Bycroft, G. S. A. B. Stewart, and P. Williams. 1995. The bacterial ’enigma‘: cracking the code of cell-cell communication. Mol. Microbiol. 16:615–624.

Schramm, A., de Beer, D., Heuvel, J.C., Ottengraf, S., Amann, R. (1999) Microscale distribution of populations and activities of Nitrosospira and Nitrospira spp. along a macroscale gradient in a nitrifying bioreactor: Quantification by in situ hybridization and the use of microsensors. Appl. Environ. Microbiol. 65(8), 3690-3696.

Sears K., Oleszkiewicz J.A., Takach T., and Lagasse P. (1997) Pushing the limits of nitrification - Low temperature, pH and hydraulic retention time. Proceedings 1997 WEFTEC. p 269-279. Water Environment Federation, Virginia, USA.

Siegrist H., Brunner I., Koch G., Phan L.C., and Le V.C. (1999) Reduction of biomass decay rate under anoxic and anaerobic conditions. Wat. Sci. Tech., 39(1), 129-137.

Skinner, F. A., and N. Walker. (1961) Growth of *Nitrosomonas europaea* in batch and continuous culture. Arch. Mikrobiol. 38:339–349.

Sollfrank U., Kappeler J., and Gujer W. (1992) Temperature effects on wastewater characterization and the release of soluble inert organic material. Wat. Sci. Tech., 25(6), 33-41.

Stein, L.Y. and Arp, D.J. (1998) Ammonium limitation results in the loss of ammonia oxidizing activity in Nitrosomonas europaea. Appl. Environ. Microbiol. 64(4), 1514-1521.

Stein, L. Y., D. J. Arp, and M. R. Hyman. (1997) Regulation of the synthesis and activity of ammonia monooxygenase in *Nitrosomonas europaea* by altering pH to affect $NH_3$ availability. Appl. Environ. Microbiol. 63:4588–4592.

Suzuki, I., Kwok, S.C., Dular, U. (1974) Ammonia or ammonium ion as substrate for oxidation by Nitrosomonas europaea cells and extracts. J. Bacteriology. 120, 556

Swift, S., J. P. Throup, G. P. C. Salmond, and G. S. A. B. Stewart. (1996) Quorum sensing: a population density component in the determination of bacterial phenotype. Trends Biochem. Sci. 21:214–219.

Teske, A., E. Alm, J. M. Regan, B. E. Rittman, and D. A. Stahl. (1994) Evolutionary relationships among ammonia- and nitrite-oxidizing bacteria. J. Bacteriol. 176:6623–6630.

Utåker, J. B., L. Bakken, Q. Q. Jiang, and I. F. Nes. (1996) Phylogenetic analysis of seven new isolates of ammonia-oxidising bacteria based on 16S rRNA sequences. Syst. Appl. Microbiol. 18:549–559.

Votyakova, T. V., A. S. Kaprelyants, and D. B. Kell. (1994) Influence of viable cells on the resuscitation of dormant cells in *Micrococcus luteus* cultures held in an extended stationary phase: the population effect. Appl. Environ. Mi-crobiol. 60:3284–3291.

Ward, B. B., and A. F. Carlucci. (1985) Marine ammonia and nitrite-oxidizing bacteria: a serological diversity determined by immunofluorescence in culture and in the environment. Appl. Environ. Microbiol. 50:194–201.

Wijffels, R.H., De Gooijer, C.D., Scherpers, A.W., Beuling E.E., Mallee, L.F., Tramper, J.(1995) Dynamic modeling of immobilized Nitrosomonas europaea: Implementation of diffusion limitations over expanding microcolonies. Enz. Microb. Technol. 17, 462-471.

Zeng, R.J., Lemaire, R., Yuan, Z., Keller, J. (2004) A novel wastewater treatment process: simultaneous nitrification, denitrification and phosphorus removal. Wat. Sci. Tech., 50(10), 163-170.